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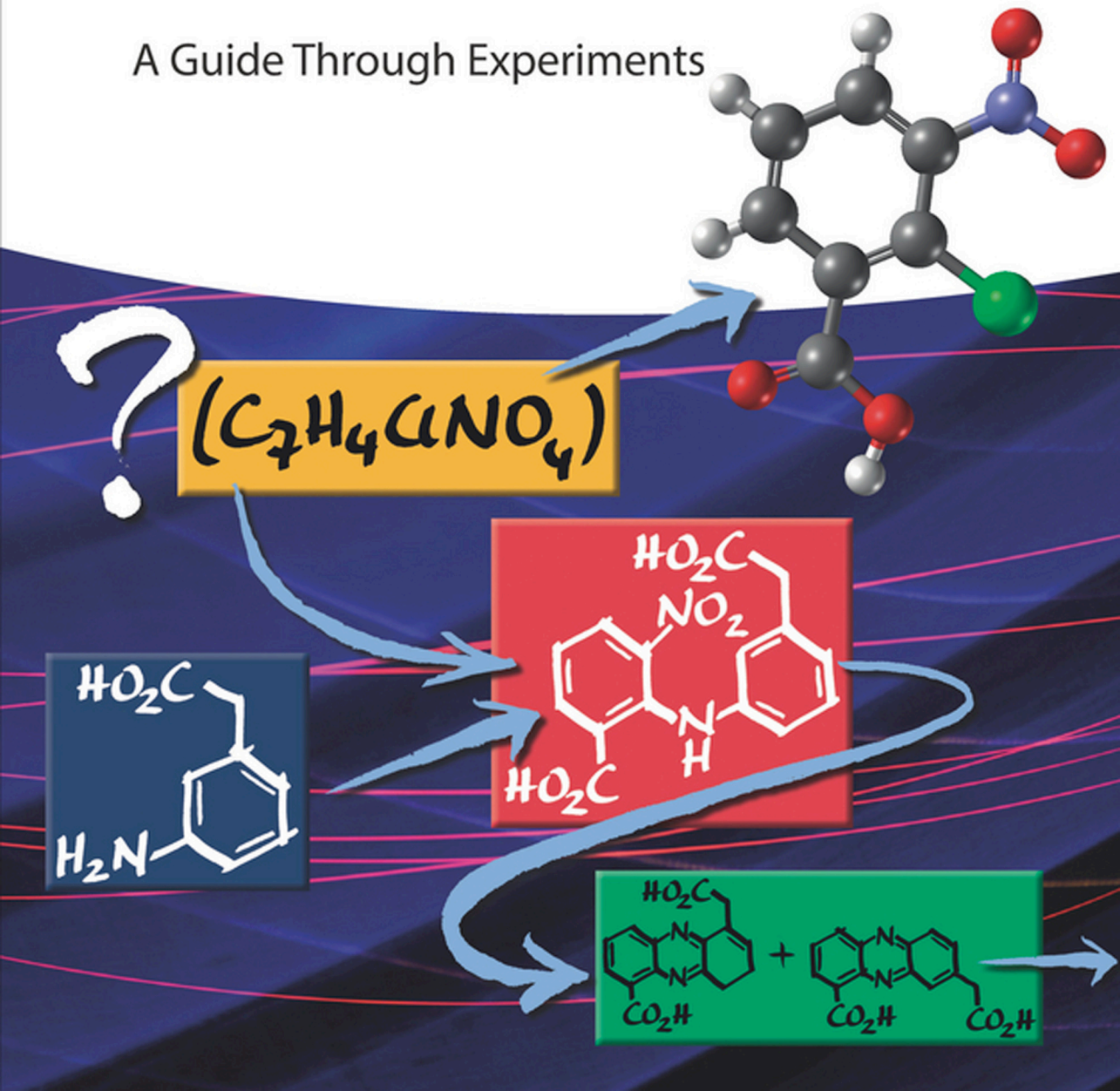
A Guide Through Experiments



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Dedicated to Lina and Juliette
In memory of Constant Bogliotti

Contents

Preface

This book is a collection of problems in organic chemistry finding its origin between 2010 and 2015 at École normale supérieure Paris-Saclay (at that time École normale supérieure de Cachan).

In the context of students' preparation for a competitive national examination in Chemistry (Agrégation de Sciences Physiques, option Chimie), giving access to teaching positions in French higher education institutions, a number of exercises dealing with multistep syntheses of natural products and active pharmaceutical ingredients were created from chemical research literature.

After extensive selection, adjustment, and modification, part of the original material is compiled in this volume. It is completed by exercises related to the field of chemical biology, which we consider an essential branch of chemical education, taught at Université Pierre et Marie Curie.

Besides its initial purpose, this work reflects to some extent a common practice in organic chemistry research laboratories, often on the occasion of group seminars, which is going through multistep synthesis with questions related to synthetic strategies, reaction conditions, and transformation mechanisms. In this respect, several excellent titles are available and are listed in the section "Further Reading."

While we tried to inject some of this *essence* in our book, our objective was also to provide a broad readership, not necessarily specialized in organic chemistry, an accessible set of problems in multistep synthesis, including experimental aspects, which are not extensively covered by current offers available on the market. The "self-studying" nature of this book indeed allows the reader to be assisted by a number of indications such as detailed textual description of the operating conditions (rate and order of reagents addition), macroscopic observations (color change, gas evolution, formation of a precipitate, increase in temperature, etc.), workup procedures (neutralization, extraction, etc.), as well as selected characteristic spectroscopic or spectrometric data of the products (infrared vibrations, ^{1}H-NMR and ^{13}C-NMR, mass spectrometry, etc.). Elucidation of molecular structure is thereby seen as a puzzle to be solved by aggregating available pieces. This vision of chemistry as essentially a game and a source of intellectual stimulation, shared by many of our colleagues, is worth being put forward, especially in the present troubled times when "societal impact" tend to constitute the quasi-exclusive input and justification for scientific research.

We stress that our book aims to be a practice medium *adapted* from published syntheses, not a strictly authentic description thereof. Indeed we chose to favor *pedagogy* over *authenticity* when we estimated that part of the original research article was not completely suited for teaching purposes. For example, while we enforced to keep intact the "spirit" of the initial work, we also took the freedom to slightly modify reaction conditions or synthetic routes and add expected characteristic spectroscopic data when missing in the original article, in order to create a story which, although not entirely *real,* remains mostly *plausible.* These modifications are listed as footnotes throughout the book. As teachers, we see such a choice as a requirement to render state-of-the-art syntheses overall accessible to nonexperts; while as researchers, we are convinced that students need to be in contact as early as possible with the practice of chemistry as it is performed in research laboratories.

In the first part, Chapters 1–5 describe short syntheses, with the longest linear sequences below five steps, which are well suited to emphasize the understanding of operating conditions and workup procedures. Process-scale syntheses of active pharmaceutical ingredients are especially represented, shedding light on common practices of the chemical industry that are often unknown (or unsuitable) to academic laboratories. Then, Chapter 6, presenting the total synthesis of a complex biologically active macrolide, might appear as uncommon in the sense that only a few chemical structures are mentioned (mostly starting materials, by-products, and target compounds). Rather, a number of indications are given in a textual form. Such a presentation, which somehow parallels the ability of some chemists to precisely define complex molecular structure by merely employing appropriate words, undoubtedly requires effort to maintain a sufficient level of mental representation. Chapters 7–10 deal with the synthesis of photochromic and fluorescent molecules, whose properties either allow the control of reactivity with light or the monitoring of enzyme activity in a biological context. Some general aspects of structure–property relation are included. Chapters 11–14 report synthetic approaches toward various natural products. Although slightly more "classical" in their form, as compared to other problems in the book, they highlight the detours, surprises, and dead ends commonly faced in total synthesis. Finally, given the growing interest for education at the chemistry/biology interface and the key role played by chemists in understanding living systems at the molecular scale, Chapters 15 and 16 are dedicated to the chemical synthesis of relevant bioactive compounds and study of their biological activities, with emphasis on the relation between tridimensional structure and function.

We express our warmest thanks to the reader paying attention to this book and our words, and also to our past and present students, colleagues, and mentors, for their input on this work.

Paris, France
January 2017

Nicolas Bogliotti
Roba Moumné

List of Abbreviations

AA	amino acid
Ac	acetyl
ACE-Cl	α-chloroethyl chloroformate
AIBN	azobisisobutyronitrile
All	allyl
aq.	aqueous
Ar or ar	aryl or aromatic
Arg	arginine
Asp	aspartic acid
atm	atmosphere
a.u.	arbitrary unit
9-BBN	9-borabicyclo[3.3.1]nonane
BINOL	1,1′-bi-2-naphtol
Bip	biphenylalanine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br	broad
C	cystein
ca.	circa
CAN	ceric ammonium nitrate
cat.	catalyst or catalytic
Cbz	carboxybenzyl
CBC	covalent bond classification
CDI	carbonyldiimidazole
Cha	cyclohexylalanine
ClF	4-chlorophenylalanine
COD	1,5-cyclooctadiene
conv.	conversion
Cy	cyclohexyl
D	aspartic acid
d	doublet
DAADH	D-amino acid dehydrogenase
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*-*N*′-dicyclohexylcarbodiimide
dd	doublet of doublets

de	diastereoisomeric excess
D-Glu	D-glucose
dr	diastereoisomeric ratio
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIC	*N*-*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-dimethylaminopyridine
DMBA	1,3-dimethylbarbituric acid
DMF	dimethylformamide
E	glutamic acid
F	phenylalanine
e^-	electron
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
equiv.	equivalent
G	glycine
GDH	glucose dehydrogenase
Gly	glycine
h	hour
5-HT	5-hydroxytryptamine
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HBTU	3-[bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate
Hfe	homophenylalanine
HFIP	hexafluoroisopropanol
HMPA	hexamethylphosphoramide
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	*N*-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
KHMDS	potassium bis(trimethylsilyl)amide
m	multiplet
IPAc	isopropyl acetate
K	lysine
L	leucine
Leu	leucine
LiHMDS	lithium bis(trimethylsilyl)amide
liq.	liquid
Lys	lysine
m-CPBA	meta-chloroperoxybenzoic acid
MEK	methyl ethyl ketone
Mes	mesityl
MIBK	methyl isobutyl ketone
MIC	minimal inhibitory concentration

MIO	4-methylideneimidazole-5-one
MS	molecular sieves
Ms	mesyl
1-Nal	1-naphtylalanine
2-Nal	2-naphtylalanine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NHS	*N*-hydroxysuccinimide
NIR	near infrared
Nle	norleucine
NMM	*N*-methylmorpholine
NMO	*N*-methylmorpholine *N*-oxide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
Np	naphtyl
P	proline
p	D-proline
Pro	proline
pro	D-proline
PAL	phenylalanine ammonia lyase
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PG	protecting group
PG-I	protegrin I
PLP	pyridoxal 5′-phosphate
PMB	4-methoxybenzyl
Pmc	2,2,5,7,8-pentamethyl-chromane-6-sulfonyl
P(*o*-Tol)$_3$	tri(*ortho*-tolyl)phosphine
Pp	2-phenyl-2-propyl
PTSA	*para*-toluenesulfonic acid
Ph	phenyl
Phe	phenylalanine
Phg	phenylglycine
Q	glutamine
q	quartet
quant.	quantitative
R	arginine
rt	room temperature
R_f	retardation factor
RFU	relative fluorescence unit
s	singlet or second
SMO	Smoothened
soln.	solution
Su	succinimide

t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBME	*tert*-butyl methyl ether
TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TEMPO	2,2,6,6-tetramethylpiperidinyloxy
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
Tol	tolyl
Ts	4-toluenesulfonyl
TSTU	2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate
Tyr	tyrosine
UV	ultraviolet
V	valine
Val	valine
vis	visible
vs	versus
v/v	volume by volume
W	tryptophane
w/w	weight by weight
Y	tyrosine

1

Atovaquone: An Antipneumocystic Agent

Atovaquone is a pharmaceutical compound marketed in the United States under different combinations to prevent and treat pneumocystosis and malaria. In a report from 2012, a team of researchers described a novel synthetic process scalable to 200 kg, starting from isochromandione **1** and aldehyde **2** (Scheme 1.1) [1, 2].

The route to **1** is described in Scheme 1.2. A mixture of phthalic anhydride **3** and Et_3N (1.07 equiv.) heated at 80 °C is treated over 4 h by portions of malonic acid (1.2 equiv.) and maintained at 80 °C for 10 h. Gas evolution was observed all along that period.[1] After adding an excess of aq. HCl solution and cooling the mixture to 25 °C, the solid is filtered off and dried to afford acid **5** in 67% yield. This transformation presumably occurs through intermediate **4**, having the molecular formula $C_{10}H_8O_5$ and containing two carboxylic acid groups [3, 4].

Question 1.1: Write the structure of **4** and suggest a plausible mechanism for its formation.

Question 1.2: Suggest a plausible mechanism for the formation of **5** from **4**.

A solution of **5** in chlorobenzene is reacted for 3 h at 30 °C in the presence of HBr (0.05 equiv.) and Br_2 (1 equiv.) in acetic acid. This reaction leads to the formation of intermediate **6** (molecular formula $C_9H_8O_3$) undergoing loss of a molecule of water to give intermediate **7**, transformed into lactones **8** and **9** under reaction conditions. Water is then added, and the mixture is refluxed for 3 h and cooled to 60 °C. The organic layer is removed, the aqueous layer is extracted with chlorobenzene, and the combined organic layers are concentrated under reduced pressure. Addition of *i*-PrOH followed by cooling to 0 °C results in the formation of a solid, which is filtered, washed with *i*-PrOH, and dried to afford **1** in 75% yield.

Question 1.3: Write the structure of **6** and suggest a plausible mechanism for its formation from **5** and its transformation into **7**.

1 This phenomenon was not reported in the original article, but was clearly observed under similar reaction conditions [3].

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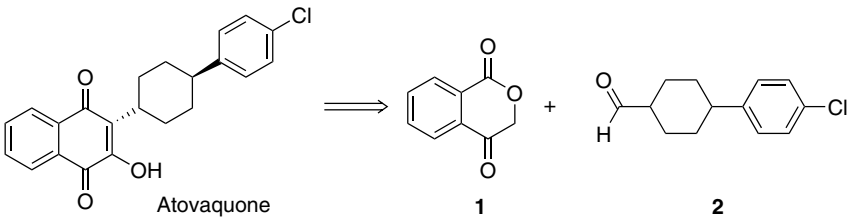
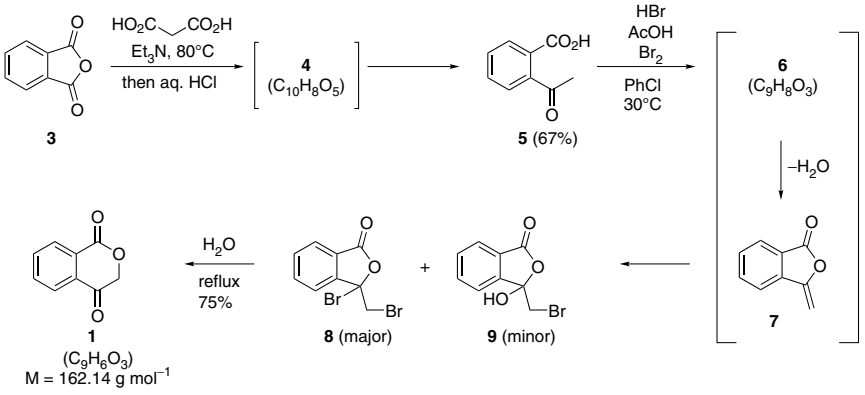


Scheme 1.1



Scheme 1.2

Question 1.4: Suggest a plausible mechanism for the formation of **1** from **8** and **9**.

Question 1.5: The ^{1}H-NMR spectra reported for compounds **1**, **3**, and **6** are described in the following table.[2] Assign characteristic signals for each compound and identify the corresponding spectrum (**A**, **B**, or **C**).

Spectrum	Description
A	**^{1}H-NMR** (400 MHz, $CDCl_3$): 8.30–8.28 (m, 1H), 8.10–8.08 (m, 1H), 7.91–7.82 (m, 2H), 5.14 (s, 2H) [2]
B	**^{1}H-NMR** (500 MHz, $CDCl_3$): 8.05–8.01 (m, 1H), 7.93–7.90 (m, 1H) [5]
C	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.86–7.84 (d, 1H), 7.73–7.69 (t, 1H), 7.63-7.52 (m, 2H), 4.13 (br. s, 1H), 1.97 (s, 3H) [2]

Compound **1** was found to be sensitive to basic conditions, undergoing unexpected transformation into a new product **10**. While HRMS analysis reveals a signal at $m/z = 161$ for **1** (negative mode chemical ionization), a signal at $m/z = 325$ (positive mode chemical ionization) was observed for **10**. ^{13}C-NMR spectra

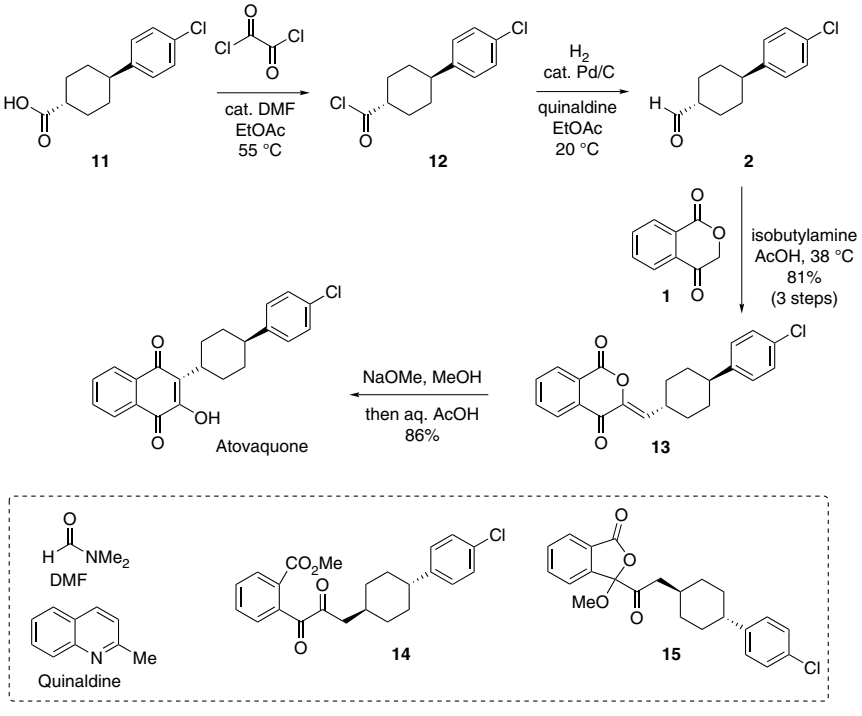
2 In $CDCl_3$ solution, compound **5** was found to spontaneously convert to **6**.

show peaks at 161.3 and 189.5 ppm for **1**, and at 161.5, 163.4 and 190.0 ppm for **10**. This latter compound also exhibits by ^{1}H-NMR spectroscopy (in DMSO-d_6) a broad signal at 6.57 ppm, exchangeable with D_2O.

Question 1.6: Suggest a plausible structure for the ion derived from **1** corresponding to signal at $m/z = 161$.

Question 1.7: Suggest a plausible structure for **10**, based on HRMS, ^{13}C-NMR, and ^{1}H-NMR analysis.

The end of synthesis is described in Scheme 1.3. A suspension of carboxylic acid **11** in ethyl acetate, in the presence of a catalytic amount of dimethylformamide (DMF), is warmed to 55 °C and treated with oxalyl chloride (1.1 equiv.) by slow addition over 30 min, to give acyl chloride **12**. The crude solution is concentrated, cooled to 20 °C, and quinaldine (1.4 equiv.) is added. The mixture is transferred into a hydrogenation vessel loaded with a catalytic amount of Pd/C, and stirred under hydrogen atmosphere until conversion to aldehyde **2** is complete. After removing the catalyst by filtration, **1**, acetic acid, and isobutylamine are successively added to the mixture; then, stirring at 38 °C until complete reaction results in the formation of **13**, isolated in 81% yield after filtration.



Scheme 1.3

Question 1.8: Suggest a plausible reaction mechanism for the formation of **12** from **11**. Clearly evidence the role played by DMF.

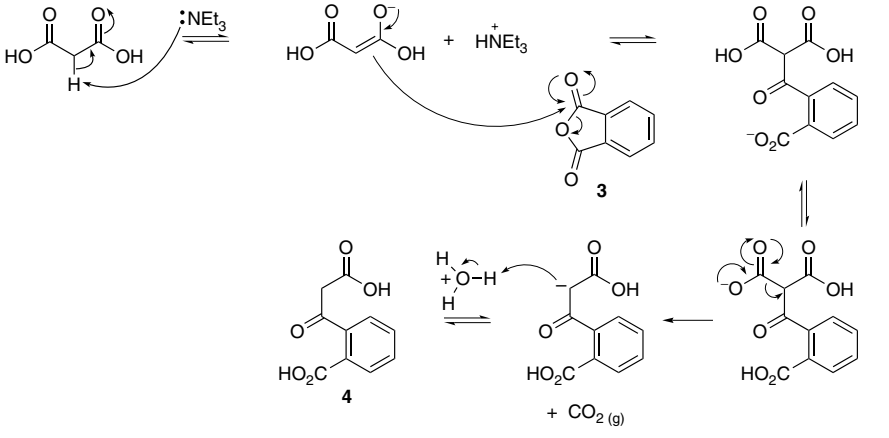
Question 1.9: What is the role of quinaldine during the hydrogenation step? Which other reagent is commonly used to perform such a transformation?

Finally, addition of a solution of sodium methoxide (1.2 equiv.) in methanol to a suspension of **13** in methanol at 20 °C followed by stirring for 18 h leads to the formation of a dark-red solution. Careful monitoring of the reaction reveals the rapid formation of methyl ester **14**, as well as lactone **15**. Treatment with aqueous acetic acid results in the precipitation of atovaquone as a bright-yellow solid collected by filtration in 86% yield.

Question 1.10: Suggest a plausible mechanism for the transformation of **13** into **14** and **15**, and their conversion into atovaquone.

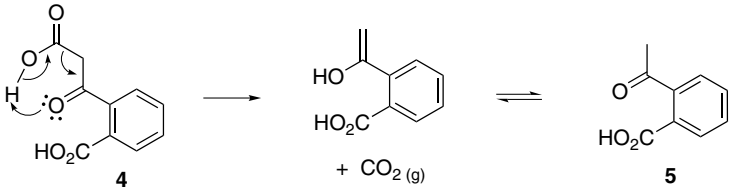
Answers

Question 1.1:

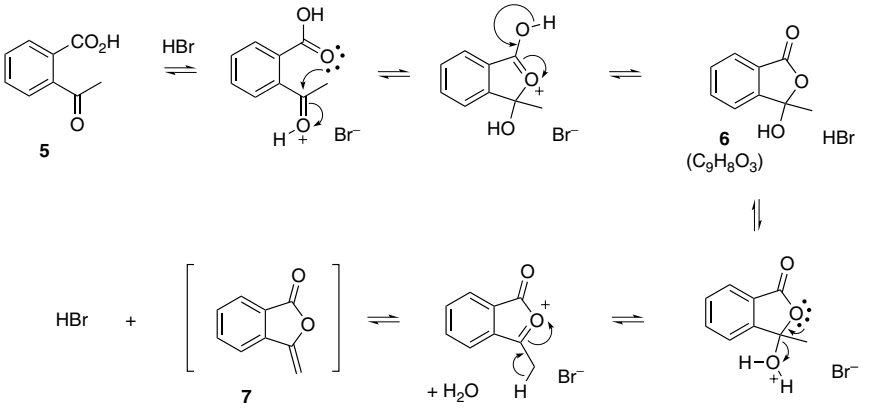


Remark: Hydrogen atoms in the malonic position are less acidic than those of the carboxylic acids and many acid/base exchanges can take place during the reaction. However, only deprotonation at this position allows C–C bond formation, finally leading to **4**, thus shifting all acid/base equilibria toward the desired compound.

Question 1.2:

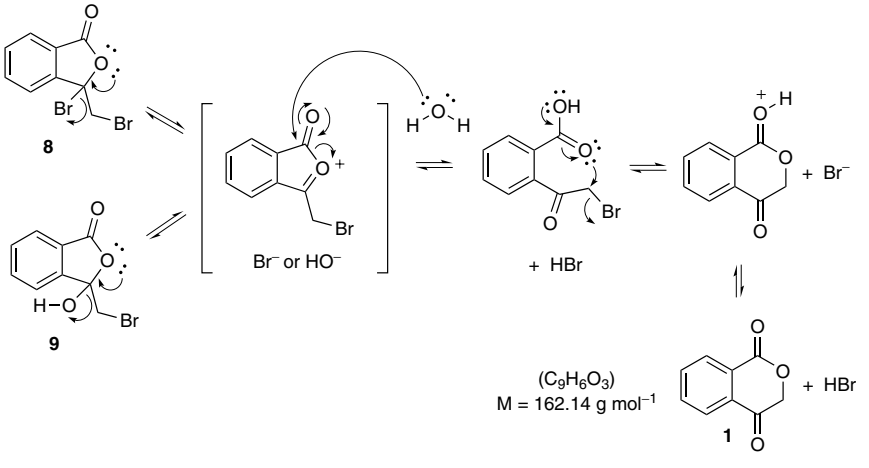


Question 1.3:



Question 1.4:

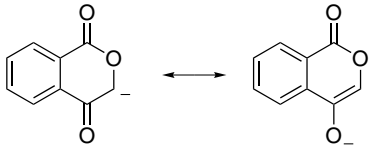
A common mechanism can be suggested for the formation of **1** from **8** or **9**:



Question 1.5:

Spectrum **A** corresponds to compound **1**: 4 aromatic CH, aliphatic CH_2 significantly up-fielded (α to both an oxygen atom and a carbonyl group).
Spectrum **B** corresponds to compound **3**: 4 aromatic CH.
Spectrum **C** corresponds to compound **6**: 4 aromatic CH, 1 exchangeable H (broad, typically OH), aliphatic CH_3.[3]

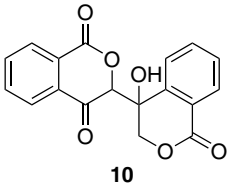
Question 1.6:



Ion derived from **1**: $[M–H]^-$

Question 1.7:

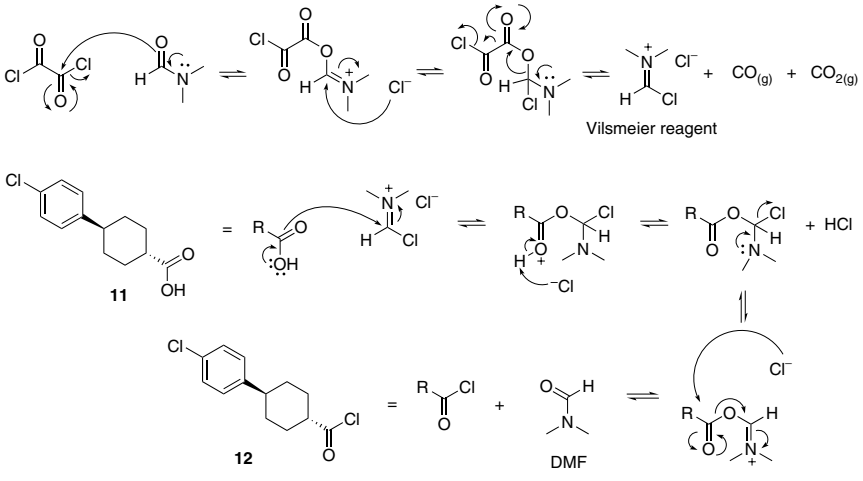
The mass spectrometry (MS) analysis of **10** in positive mode shows a signal at $m/z = 325$, likely corresponding to $[M + H]^+$ ion and thereby suggesting that **10** (M = 324) is a dimer of **1**. While the ^{13}C-NMR spectrum of **1** shows characteristic signals for ester (161.3 ppm) and ketone (189.5 ppm), **10** presumably contains two esters (161.5 and 163.4 ppm) and a ketone (190.0 ppm). The presence of a broad signal at 6.57 ppm (exchangeable with D_2O) in the ^{1}H-NMR spectrum of **10** reveals the presence of a hydroxyl group. Finally, since **1** contains both an enolizable H atom that could be easily deprotonated under basic conditions and an electrophilic ketone moiety, it could self-dimerize to the following compound **10**.



10

3 Although this spectrum was initially assigned to **5** [2], several studies evidenced an equilibrium in $CDCl_3$ solution favoring its existence as **6** [6, 7].

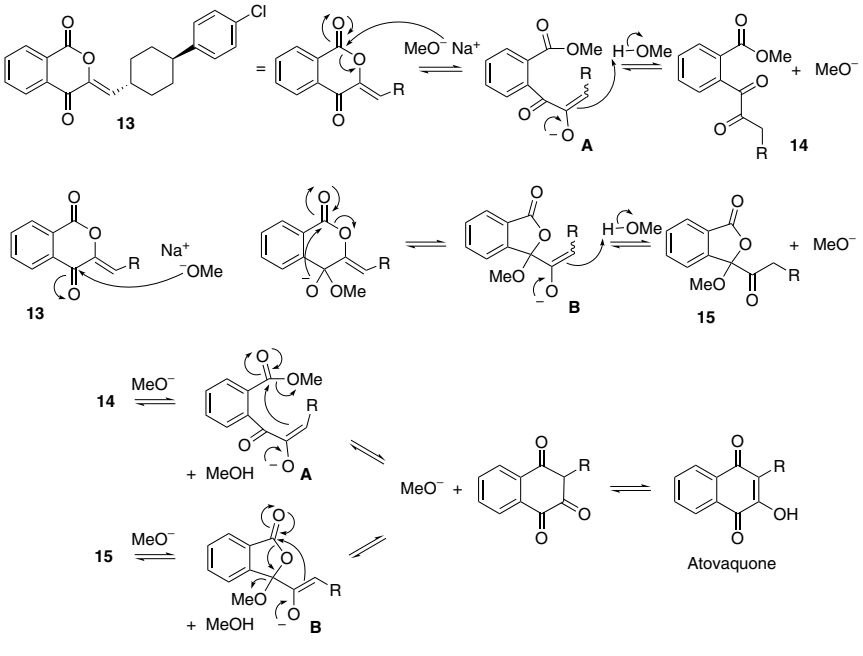
Question 1.8:



Question 1.9:

Quinaldine, like the commonly used quinoline (lacking the methyl substituent), adsorbs at the surface of palladium thus reducing catalyst activity ("poisoning" the catalyst) and avoiding further reduction of aldehyde function into alcohol.

Question 1.10:



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2

SEN794: An SMO Receptor Antagonist

In 2012, an optimized route to the brain-penetrant Smoothened (SMO) receptor antagonist SEN794, investigated for the treatment of tumors affecting the central nervous system, was reported [1, 2].

The first steps of the original medicinal chemistry route to the target compound are described in Scheme 2.1. A commercially available compound **1** is converted in two steps into compound **3**, which undergoes Negishi coupling with functionalized pyridine **4** to give **5**.

A mixture of 2-chloro-5-nitroaniline **1** and sulfuric acid (1.4 equiv.) in water, cooled to 0 °C, is treated dropwise with an aqueous solution of sodium nitrite (1.2 equiv.). After 30 min at 0 °C, a cationic intermediate is formed (compound **2**, molecular formula: $C_6H_3ClN_3O_2$), then an aqueous solution of KI (1.4 equiv.) is added dropwise while the temperature is maintained below 10 °C. After 2 h at rt, the mixture is extracted with EtOAc, the combined organic layers are washed with aq. $Na_2S_2O_5$ solution and brine, yielding a brown solid after drying over $MgSO_4$ and evaporation. Crystallization from *i*-PrOH affords **3** as a brown-red solid in 71% yield. Its infrared spectrum shows bands at 3086, 1522, 1342, 869, and 738 cm^{-1}.

Question 2.1: Write the structure of compounds **1** and **2**.

Question 2.2: Suggest a plausible reaction mechanism for the formation of **3** from **1**.

Question 2.3: Assign infrared absorption bands reported for compound **3**.

Compound **3** is dissolved in anhydrous dimethylacetamide (DMA) and treated with organozinc reagent **4** (1.4 equiv.), PPh_3 (0.2 equiv.), and $Pd(PPh_3)_4$ (0.05 equiv.). The solution is heated to 60 °C for 30 h, cooled to rt, and added to a mixture containing EtOAc, aq. NaOH (2M), and crushed ice. After stirring for 1 h and letting stand for 1 h 30 min, the suspension is filtered and the solid is washed with EtOAc. The filtrate is recovered and the layers are separated. The aqueous phase is extracted with EtOAc and concentrated to give a brown solid (**point 1**), which is taken up with aq. HCl (1M) and washed with EtOAc (**point 2**). The acidic

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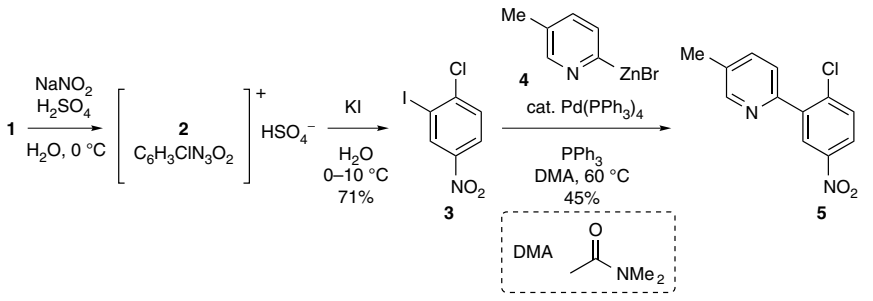


Scheme 2.1

aqueous phases are combined, cooled to 0 °C, and made basic with aq. NaOH (10M). This results in the formation of a brown solid (**point 3**), which is washed with water and dried to afford **5** in 41% yield.[1]

Question 2.4: Suggest a plausible reaction mechanism for the transformation of **3** into **5**.

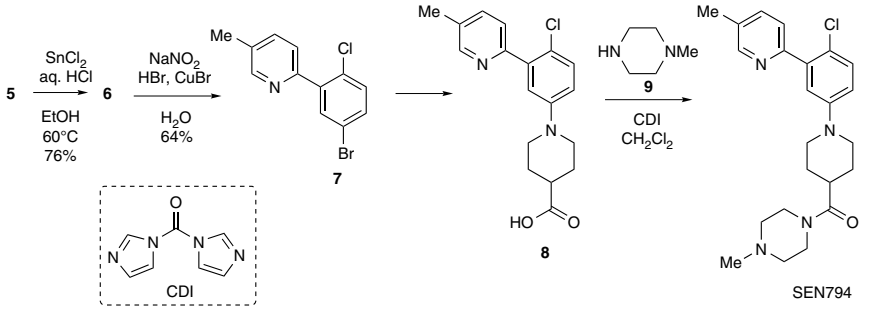
Question 2.5: What is the composition of the solid obtained at **point 1**?

Question 2.6: Indicate the repartition between organic and aqueous layers of DMA, compound **5**, and other organic by-products at **point 2**.

Question 2.7: What is the composition of the solid obtained at **point 3**?

The last steps leading to **SEN794** are shown in Scheme 2.2. They involve conversion of nitro **5** into bromide **7** followed by additional functionalization leading to carboxylic acid **8**, which undergoes final amide bond formation.

A suspension of pyridine **5** in EtOH is treated with $SnCl_2$ (3.6 equiv.) and aq. HCl (37%); then the resulting solution is heated to 60 °C for 3 h. Evaporation of the solvent leads to a residue (**point 1**), which is taken up with aq. HCl (1M) to give a suspension that is washed with EtOAc. The aqueous layer is cooled to 0 °C, made basic with aq. NaOH (10M) and extracted with EtOAc (**point 2**).



Scheme 2.2

1 The workup procedure has been slightly simplified as compared to that originally described [2].

The organic layers are combined, washed with aq. Na_2CO_3 solution, water, and brine, followed by drying over $MgSO_4$ and evaporation to give **6** in 76% yield.

Question 2.8: Give the structure of compound **6** and indicate its ionization state at **point 1**.

Question 2.9: Indicate the ionization state of **6** at **point 2** and its repartition between aqueous and organic layers.

Compound **6** is dissolved in aq. HBr (48%), cooled to 0 °C, treated dropwise with an aqueous solution of $NaNO_2$ (1.1 equiv.), and stirred for 30 min at rt. The mixture is cooled to −5 °C, treated dropwise with a solution of CuBr (1.1 equiv.) in aq. HBr (48%), left to warm to rt, and stirred for 1 h. After cooling to −5 °C, aq. NaOH (5M) is added and the resulting mixture is extracted with EtOAc. The combined organic layers are washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford a brown oil, which was purified by column chromatography to give **7** in 64% yield. The ^{1}H-NMR data for **7** are reported as follows:

^{1}H-NMR (400 MHz, DMSO-d_6) for **7**: 8.53 (m, 1H); 7.75–7.70 (m, 1H); 7.73 (d, J = 2.4 Hz, 1H); 7.64 (dd, J = 8.0 Hz, 2.4 Hz, 1H); 7.60 (d, J = 8.0 Hz, 1H); 7.53 (d, J = 7.8 Hz, 1H); 2.36 (s, 3H).

Question 2.10: Assign ^{1}H-NMR signals reported for compound **7** and justify their multiplicity.

Carboxylic acid **8**, obtained in two steps from **7**, is added by portions over 10 min to a suspension of carbonyldiimidazole (CDI) (1.2 equiv.) in CH_2Cl_2, resulting in intensive bubbling (**point 1**). The mixture is stirred for 1 h, followed by dropwise addition of a solution of amine **9** over 10 min. After stirring for 3 days at rt, the solution is washed with aq. NaOH (0.9M) (**point 2**), layers are separated, and organic phase is dried over Na_2SO_4 to afford SEN794.[2]

Question 2.11: Suggest a plausible mechanism for the transformation of **8** into SEN794.

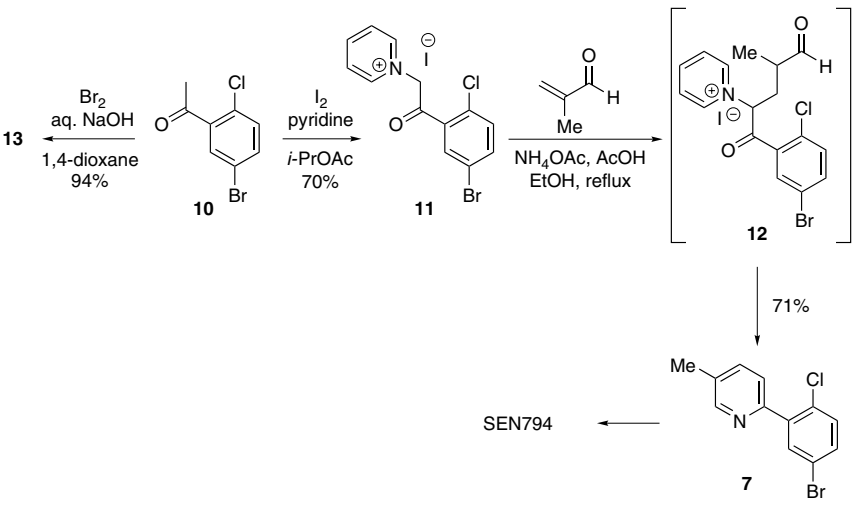
Question 2.12: What is the origin of bubbling observed at **point 1**?

Question 2.13: Why is the solution washed with a basic aqueous solution at **point 2**?

Question 2.14: Predict the relative polarity of compounds **8** and SEN794 observed by thin-layer chromatography (SiO_2).

In an optimized synthetic route, access to key pyridine **7** was redesigned starting from methyl-ketone **10** (Scheme 2.3). This strategy is based on the formation

2 The product obtained contains about 6% w/w CH_2Cl_2.



Scheme 2.3

of pyridinium salt **11**, which undergoes Kröhnke reaction through 1,5-dicarbonyl intermediate **12** [3] to give **7**.

Ketones such as **10** are well known to react with Br_2 in aqueous basic solution. For example, a mixture of **10** and 1,4-dioxane is treated dropwise with aq. NaOH (10 equiv.), followed by dropwise addition of Br_2 (3.1 equiv.). After vigorously stirring the biphasic solution for several hours, the reaction is concentrated under reduced pressure to give a pale yellow solid (**point 1**), which is taken up in water and acidified to pH 2 with aq. HCl. Upon stirring, the solution becomes cloudy (**point 2**) and a solid finally precipitates; it is filtered off, washed with water, and dried, to afford **13** in 94% yield.[3]

Question 2.15: Give the composition of the solid obtained at **point 1** and explain the formation of the products with a plausible mechanism.

Question 2.16: Explain the origin of the cloudy aspect observed at **point 2** and mention the structure of **13**.

A suspension of I_2 (1 equiv.) in *i*-PrOAc cooled to 10 °C is treated dropwise with pyridine (5.3 equiv.), followed by dropwise addition of a solution of **10**. The mixture is refluxed for 18 h, cooled to 15 °C, and the solid formed is filtered off. Washing with H_2O and EtOH followed by drying affords **11** in 70% yield.

Question 2.17: Suggest a plausible reaction mechanism for the formation of **11** from **10**.

A suspension of **11** in EtOH is treated with ammonium acetate (5 equiv.) by portions, then acetic acid (5 equiv.) and a solution of methacrolein (1.5 equiv.) in EtOH is added. The mixture is refluxed for 5 h, and the solvent is evaporated under reduced pressure. The residue is dissolved in CH_2Cl_2 and the organic

3 This reaction, not described in the original work, is adapted from Ref. [4].

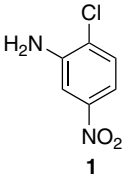
phase is washed with aq. saturated $NaHCO_3$ solution, aq. NaOH (15%), and water. Evaporation of the solvent gives a crude residue, which is dissolved in *i*-PrOH, heated to 45 °C, and slowly treated with water, thus leading to crystallization.[4] Filtration of the solid and washing with water affords pyridine **7** in 71% yield.

Question 2.18: Explain the formation of **12** and its transformation into **7** with a plausible reaction mechanism.

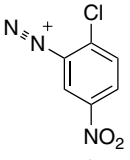
Question 2.19: Explain the procedure used for crystallization of **7**.

Answers

Question 2.1:



1

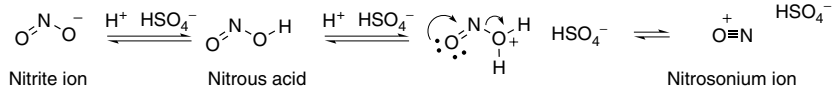


2

$(C_6H_3ClN_3O_2^+)$

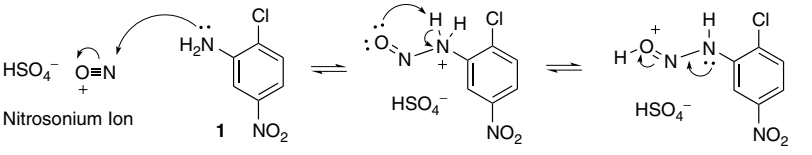
Question 2.2:

1. Nitrosonium ion formation:

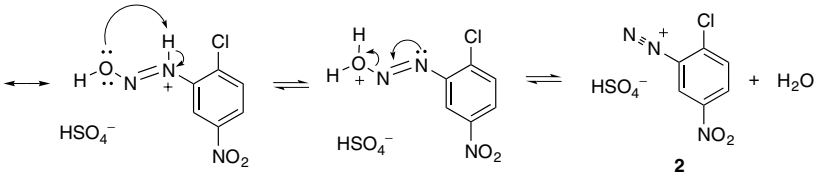


Nitrite ion — Nitrous acid — Nitrosonium ion

2. Diazonium salt formation:



Nitrosonium Ion — **1**

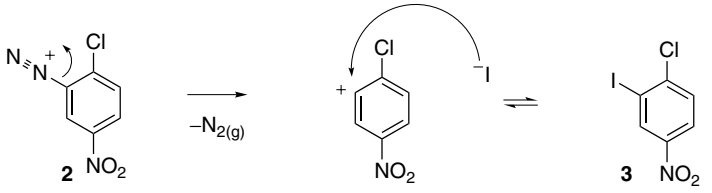


2

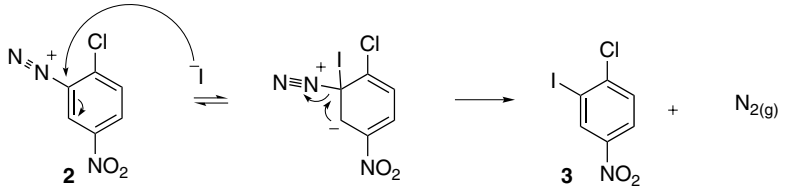
4 In original work, crystallization is triggered by addition of a crystal seed, upon careful control of internal temperature.

The most common pathways for substitution of N_2 with a nucleophile (here I^-) either involve SN_1 or SN_{Ar}-type mechanism. In the case of **2**, the electron-withdrawing nitro group reduces electron density on the aromatic ring and thus exerts a destabilizing effect on the carbocation intermediate formed in a SN_1 mechanism. Furthermore, addition of nucleophile followed by departure of the leaving group (SN_{Ar} mechanism) is not particularly favored because the negative charge developed is not stabilized by mesomeric effect of the nitro group located in meta position. Both mechanisms are thus plausible.

Nucleophilic substitution by SN_1 mechanism:



Nucleophilic substitution by SN_{Ar} mechanism:

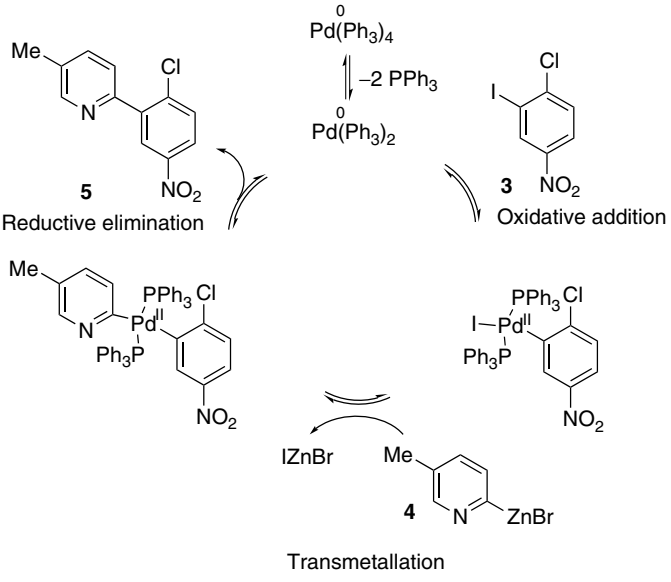


Question 2.3:

3086 cm^{-1}: aromatic C–H bond stretching
1522 cm^{-1}: asymmetric N–O bond stretching
1342 cm^{-1}: symmetric N–O bond stretching
869 and 738 cm^{-1}: aromatic C–H bond bending

Question 2.4:

Although not all elementary steps of Negishi are presently understood [5], the following mechanism is commonly accepted:

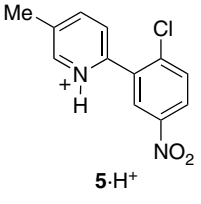


Question 2.5:

The brown solid at **point 1** corresponds to crude compound **5**.

Question 2.6:

DMA is miscible with both water and EtOAc and should therefore be partitioned between both phases. At **point 2**, compound **5** is protonated (exists as **5**·H^+) and is thus soluble in the aqueous layer. Other organic by-products (such as PPh_3) should be in the organic layer.



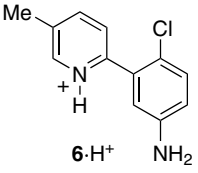
5·H^+

Question 2.7:

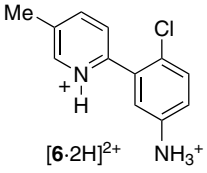
The solid at **point 3** corresponds to free pyridine **5** (insoluble in water).

Question 2.8:

Depending on the amount of aq. HCl (1M) added, compound **6** exists as **6**·H^+ or as $[\mathbf{6}\cdot 2H]^{2+}$ at **point 1**.



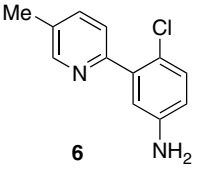
6·H^+



$[\mathbf{6}\cdot 2H]^{2+}$

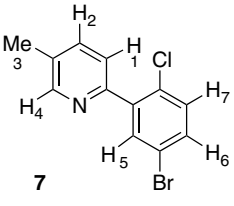
Question 2.9:

At **point 2**, compound **6** exists as a free base and is thus soluble in organic layer.



6

Question 2.10:



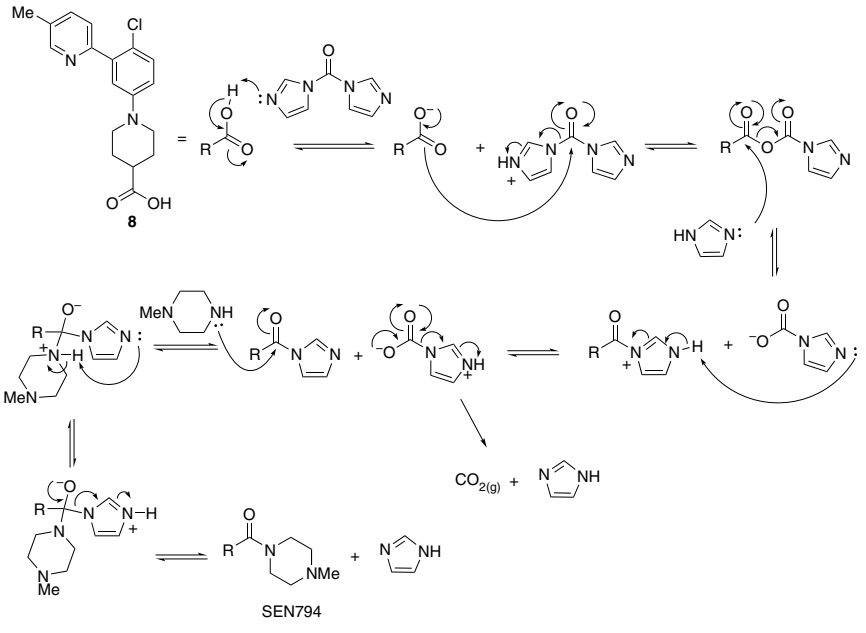
7

^{1}H-NMR signals can be assigned on the basis of their chemical shift and coupling constants using $^3J_{ortho} = 6–9$ Hz, $^4J_{meta} = 1–3$ Hz and $^5J_{para} = 0–1$ Hz as reference values.

^{1}H-NMR (400 MHz, DMSO-d_6): 8.53 (m, 1H, $\mathbf{H_4}$); 7.75–7.70 (m, 1H, $\mathbf{H_2}$); 7.73 (d, $\mathbf{J_{5,6\ meta} = 2.4\ Hz}$, 1H, $\mathbf{H_5}$); 7.64 (dd, $\mathbf{J_{6,7\ ortho} = 8.0\ Hz}$, $\mathbf{J_{6,5\ meta} = 2.4\ Hz}$, 1H, $\mathbf{H_6}$); 7.60 (d, $\mathbf{J_{7,6\ ortho} = 8.0\ Hz}$, 1H, $\mathbf{H_7}$); 7.53 (d, $\mathbf{J_{1,2\ ortho} = 7.8\ Hz}$, 1H, $\mathbf{H_1}$); 2.36 (s, 3H, $\mathbf{H_3}$).[5]

5 The originally reported value "7.53 (d, $J = 8.0$ Hz, 1H)" was replaced by "7.53 (d, $J = 7.8$ Hz, 1H)" to facilitate attribution based on coupling constants.

Question 2.11:



Additional information can be found in Ref. [6].

Question 2.12:

The bubbling corresponds to the formation of CO_2 (gas) during activation of **8** by CDI.

Question 2.13:

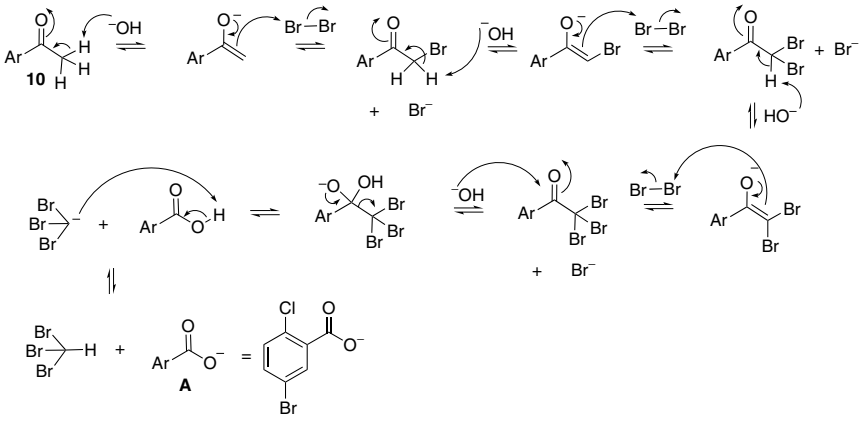
Washing with aq. basic solution allows the basic sites of SEN794 to remain unprotonated, thus conferring solubility in organic phases, while imidazole by-products will be removed in the aqueous layer.

Question 2.14:

Compound **8** contains a highly polar carboxylic acid group and should thus have a higher polarity than SEN794, and a lower Rf value on TLC (SiO_2).

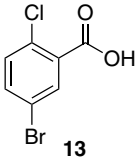
Question 2.15:

In the presence of bromine under basic conditions, methyl ketones undergo the so-called bromoform reaction. At **point 1**, the solid obtained corresponds to a mixture of carboxylate **A** and bromoform ($CHBr_3$).

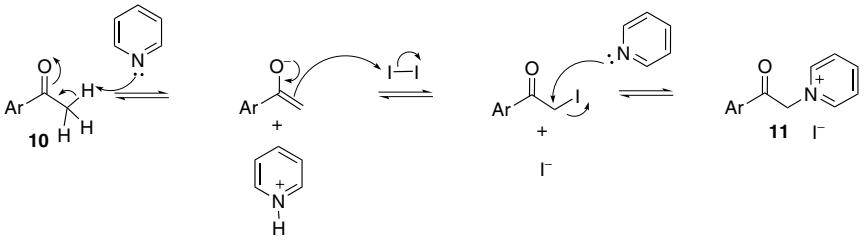


Question 2.16:

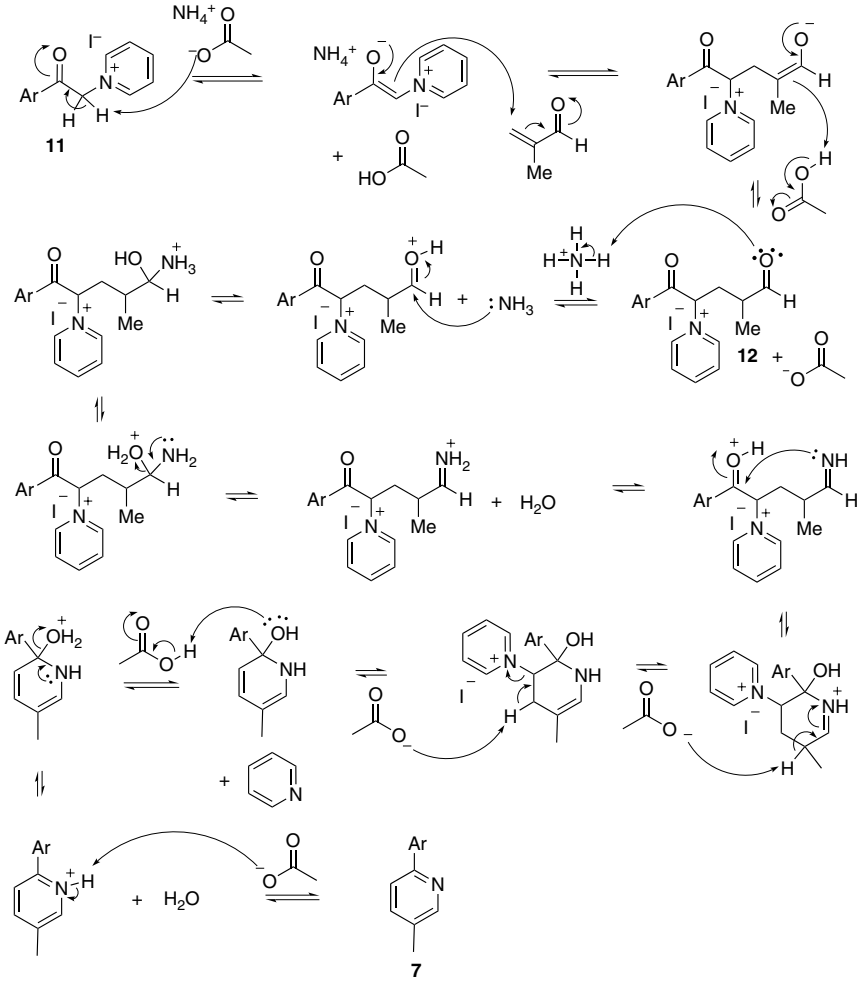
After addition of HCl, carboxylate **A** is protonated to give **13**, which is not soluble in water and therefore precipitates.



Question 2.17:



Question 2.18:



Additional information can be found in Ref. [3].

Question 2.19:

The crude residue composed of compound **7** and impurities is first dissolved in *i*-PrOH under heating. Upon addition of water, compound **7** (not soluble in water) crystallizes, while impurities remain in water.

References

1 Betti, M., Castagnoli, G., Panico, A., Sanna Coccone, S., and Wiedenau, P. (2012) Development of a scalable route to the SMO receptor antagonist SEN794. *Org. Process Res. Dev.*, **16** (11), 1739–1745.

2 Pericot, M.G.L., Thomas, R.J., Minetto, G., *et al.* (2012) Compound for the treatment of tumours and tumour metastases, WO Patent, 2012076413 A1, filed Dec. 2, 2011 and issued June 14, 2012.

3 Li, J.-J. and Corey, E.J. (eds) (2005) *Name Reaction in Heterocyclic Chemistry*, John Wiley & Sons, Inc.

4 Brough, P.A., Macias, A., Roughley, S.D., *et al.* (2015) Resorcinol N-aryl amide compounds, for use as pyruvate dehydrogenase kinase inhibitors, WO Patent 2015040425 A1, filed Sept. 22, 2014 and issued March 26, 2015.

5 Jin, L. and Lei, A. (2012) Insights into the elementary steps in Negishi coupling through kinetic investigations. *Org. Biomol. Chem.*, **10** (34), 6817–6825.

6 El-Faham, A. and Albericio, F. (2011) Peptide coupling reagents, more than a letter soup. *Chem. Rev.*, **111** (11), 6557–6602.

3

Synthesis of an H1–H3 Antagonist

Compound **1** is an H1–H3 antagonist developed for the oral treatment of allergic rhinitis. It is obtained from fragments **2** and **3** (Figure 3.1) [1].

3.1 Synthesis of Fragment 2

The synthetic strategy toward fragment **2** relies on the preparation of compound **6** according to the reaction sequence described in Scheme 3.1.

A suspension of diacid **4** and *p*-toluenesulphonic acid monohydrate (0.02 equiv.) in *o*-xylene is heated to reflux using Dean–Stark conditions. A solution of benzylamine (1 equiv.) in *o*-xylene is added over 2 h and reflux is maintained for about 24 h. During this period, two other portions of *p*-toluenesulphonic acid monohydrate (2 × 0.02 equiv.) are added to the reaction mixture. After cooling to 20 °C, the mixture is partitioned with aq. K_2CO_3 (11%) and the layers are separated (**point 1**). The organic layer is washed with aq. HCl (1M) (**point 2**) and concentrated under reduced pressure to afford **5** in 93% yield.

Question 3.1: Name compound **4** using systematic nomenclature.

Question 3.2: Explain the principle and utility of a Dean–Stark apparatus.

Question 3.3: Calculate the theoretical amount of water formed per mole of **4** upon completion of the reaction (the amount of water originating from hydrated *para*-toluenesulfonic acid (PTSA) is neglected).

Question 3.4: Indicate the structure of a plausible by-product formed during the preparation of **5**.

Question 3.5: Indicate the ionization state of **4**, **5**, and by-products at **point 1** and their repartition between aqueous and organic layers.

Question 3.6: Indicate the composition of aqueous and organic layers at **point 2**.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

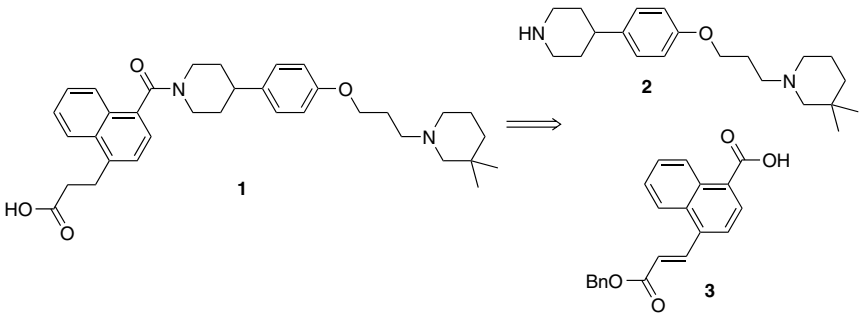
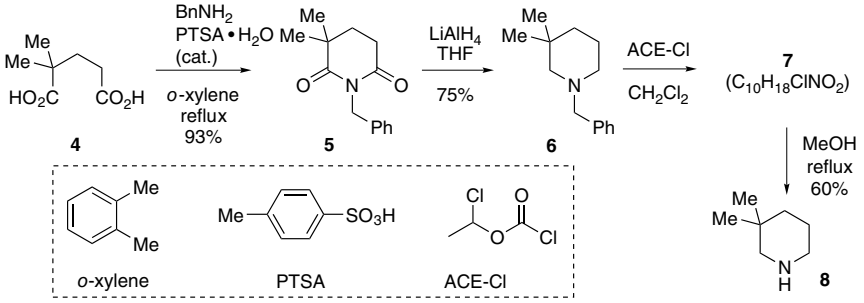


Figure 3.1 Retrosynthesis of **1**.



Scheme 3.1

A solution of **5**[1] in tetrahydrofuran (THF) is heated to 60 °C and treated dropwise with a solution of $LiAlH_4$ (2 equiv.) in THF while maintaining the temperature below 65 °C. After stirring for 2 h, aq. NaOH (32%) is slowly added, resulting in strong gas evolution. The resulting biphasic mixture is stirred for 1 h at 60 °C and the layers are separated.[2] The organic layer is concentrated under reduced pressure, yielding 75% of **6**.

Question 3.7: Suggest a plausible mechanism for the reduction of **5** with $LiAlH_4$ and explain the origin of gas evolution observed during workup.

A solution of **6** in CH_2Cl_2 cooled to 0 °C is treated dropwise with a solution of ACE-Cl (1.05 equiv.) in CH_2Cl_2.[3] The mixture is stirred for 1 h at 0 °C, refluxed for 1 h, and then the solvent is removed under reduced pressure to give crude product **7** (molecular formula $C_{10}H_{18}ClNO_2$), which presents an infrared absorption band at 1725 cm^{-1}.[4] This compound is dissolved in methanol, the solution is

1 In the original report, compound **5** is used as a mixture containing about 5% w/w toluene.
2 The original procedure involves additional separation steps.
3 The experimental details of such a reaction have not been reported in the original article. The procedure described here is adapted from Ref. [2].
4 The infrared data reported for several structurally related compounds indicate a strong absorption band in the range 1724–1731 cm^{-1} [3, 4].

refluxed for 1 h, and the solvent is evaporated under reduced pressure. The residue is purified by vacuum distillation to afford **8** in 60% yield.

Question 3.8: Give the structure of compound **7** and suggest a plausible mechanism for its formation from **6**.

Question 3.9: Suggest a plausible mechanism for the conversion of **7** into **8** accounting for the formation of CO_2 and $CH_3CH(OMe)_2$ as by-products.

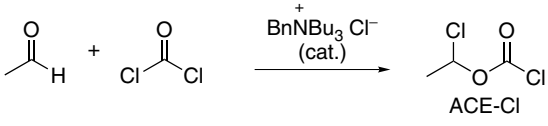
Question 3.10: Name compounds **5**, **6**, and **8** using systematic nomenclature.

Question 3.11: The ^{1}H-NMR spectra reported for compounds **5**, **6**, and **8** are described here. Assign characteristic signals for each compound and identify the corresponding spectrum (**A**, **B**, or **C**).

Spectrum	Description
A	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.35–7.00 (m, 5H), 3.41 (s, 2H), 2.35–2.26 (m, 2H), 1.99 (s, 2H), 1.61–1.54 (m, 2H), 1.25–1.15 (m, 2H), 0.92 (s, 6H).
B	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.32–7.20 (m, 3H), 7.14–7.06 (m, 2H), 4.93 (s, 2H), 2.71 (t, J = 7 Hz, 2H), 1.78 (t, J = 7 Hz, 2H), 1.25 (s, 6H).
C	**^{1}H-NMR** (400 MHz, $CDCl_3$): 2.77–2.69 (m, 2H), 2.47 (s, 2H), 1.52–1.46 (m, 2H), 1.39 (br s, 1H), 1.35–1.32(m, 2H), 0.91 (s, 6H).

Question 3.12: Which characteristic infrared vibration would you expect for compounds **5**, **6**, and **8**? Give an approximate value (in cm^{-1}) and indicate the corresponding vibration mode.

ACE-Cl is prepared in one step by slow addition of phosgene (1.1 equiv.) to a mixture of acetaldehyde and $BnNBu_3Cl$ (0.05 equiv.), as described in Scheme 3.2 [5].

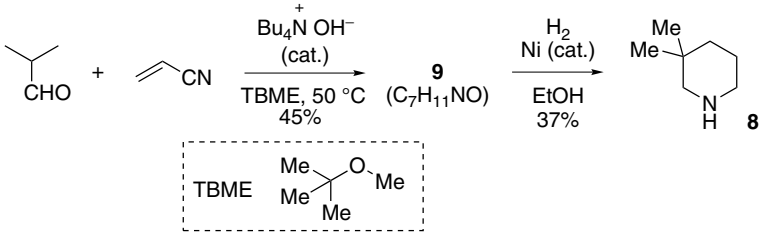


Scheme 3.2

Question 3.13: Suggest a plausible mechanism for this reaction.

An alternative route to produce compound **8** in two steps has also been studied (Scheme 3.3).

A solution of tetrabutylammonium hydroxide (0.03 equiv.) in *tert*-butyl methyl ether (TBME) is added over 2 h to a solution of isobutyraldehyde (1.1 equiv.) and acrylonitrile in TBME at 50 °C. When the reaction is completed, acetic acid (0.04 equiv.) is added, the solvent is removed under reduced pressure, and the residue is purified by vacuum distillation to afford **9** (molecular formula $C_7H_{11}NO$) in



Scheme 3.3

45% yield. This compound shows two infrared absorption bands at 2250 and 1730 cm^{-1}.[5]

Question 3.14: Write the structure of compound **9** and suggest a mechanism for its formation, accounting for utilization of tetrabutylammonium hydroxide in catalytic amount.

A vigorously stirred mixture of **9** and Raney Ni (33% w/w) in EtOH is treated with acetic acid (0.7 equiv.) and placed under hydrogen atmosphere. After 18 h, the catalyst is removed by filtration over Celite and the filtrate is evaporated under reduced pressure.[6] The residue is diluted with TBME, water and aq. NaOH (32%) are added, and the layers are separated. The organic phase is washed with aq. NaOH (40%) and concentrated under reduced pressure. The crude product is purified by vacuum distillation to afford **8** in 37% yield.

Question 3.15: Explain the transformation of **9** into **8** by providing a plausible mechanism accounting for the use of acetic acid.

A proposed manufacturing route to fragment **2** is described in Scheme 3.4.

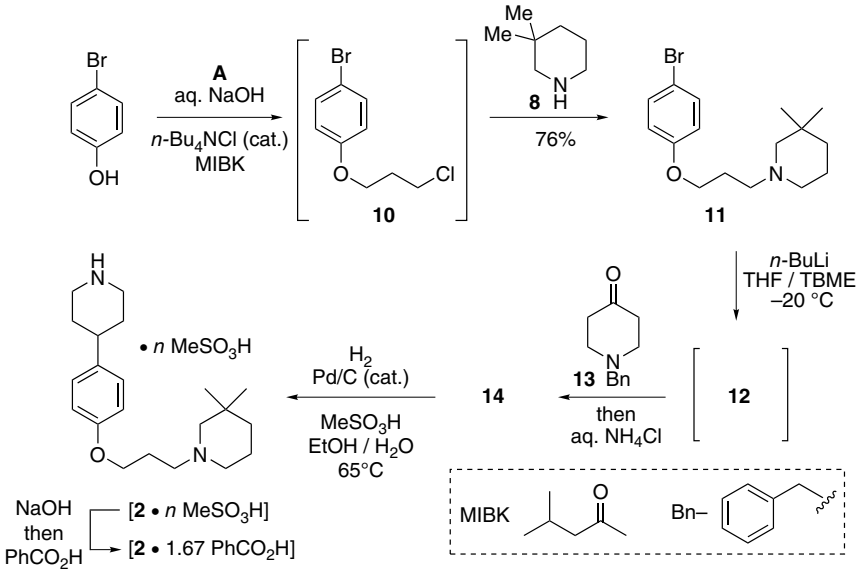
A mixture of aq. NaOH (3 equiv.), 4-bromophenol, tetrabutylammonium chloride (0.1 equiv.), and **A** (1.05 equiv.) in methyl isobutyl ketone (MIBK) is heated to 80 °C for 1 h and then cooled to 50 °C; compound **8** (1 equiv.) is added and the mixture is heated to 80 °C for 4 h. After cooling to 50 °C, TBME is added and the layers are separated. The organic phase is washed with water and aq. HCl (0.5M), dried by azeotropic evaporation with *i*-PrOH, concentrated to about a third of its volume by evaporation under reduced pressure, and cooled to 0 °C. After standing for 1 h, the solid is filtered off, washed with *i*-PrOH, and dried to afford **11** in 76% yield.

Question 3.16: Write the structure of **A**.

To a solution of *n*-BuLi (2.0 equiv.) in THF at −20 °C is added over 3 h a solution of **11** in TBME followed by ketone **13** (1.1 equiv.). Once reaction is completed, the mixture is warmed to 0 °C and quenched with saturated aq. NH_4Cl

5 These values were not reported in the original article.

6 The original experimental procedure involves additional steps, which are not detailed here.



Scheme 3.4

solution. After warming to 22 °C, the layers are separated, the organic phase is washed with water, and the solvent is removed under reduced pressure to afford tertiary alcohol **14**.[7]

Question 3.17: Write the structure of intermediate **12** formed upon reaction of **11** with *n*-BuLi. What do you call such type of reaction?

Question 3.18: Write the structure of compound **14**.

A mixture of **14**, 10% Pd/C (about 5% w/w), and $MeSO_3H$ (3 equiv.) in EtOH/H_2O (19:1) is stirred at 65 °C under 3 bar H_2 for 8 h. The catalyst is removed by filtration and the solvent is evaporated, yielding salt [**2** · *n* $MeSO_3H$] as a gum (**point 1**).[8] This product is taken up with a mixture of TBME and aq. NaOH (1.7M) (**point 2**), the layers are separated, and the organic layer is washed with water and dried and concentrated under reduced pressure. A solution of the residue in TBME is treated with benzoic acid (2 equiv.) and aged for 1 h at rt. The solid product is collected by filtration, washed with TBME, and dried to yield 65% of the benzoate salt of **2**, whose molecular formula corresponds to [**2** · 1.67 $PhCO_2H$].

Question 3.19: Draw the most plausible structure of [**2** · *n* $MeSO_3H$] (including the value of *n*) at **point 1**.

---

7 Compound **14** is not isolated in original procedure.

8 Although the precise nature of this salt is not mentioned in the original article, it is reported to be unsuitable for crystallization.

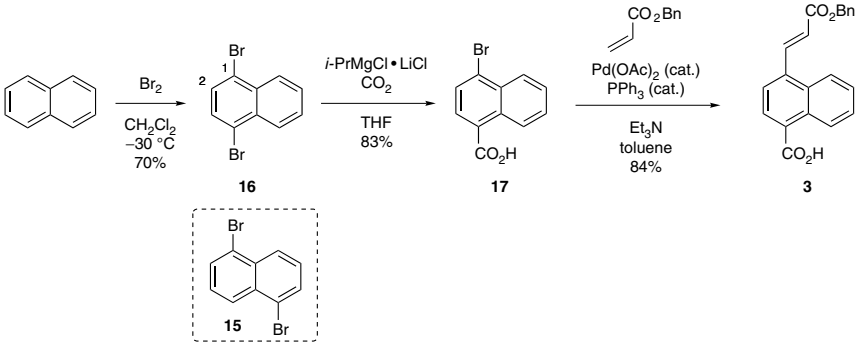
Question 3.20: Draw the various intermediates involved in the transformation of **14** into [**2** · *n* $MeSO_3H$] and explain their formation with a mechanism.

Question 3.21: Indicate the ionization state of **2** and other species at **point 2** as well as their repartition between aqueous and organic layers.

Question 3.22: The final product [**2** · 1.67 $PhCO_2H$] is, in fact, constituted by a mixture of two products. Mention their respective structure and relative amount.

3.2 Synthesis of Fragment 3

A solution of naphthalene in CH_2Cl_2 is cooled to −30 °C and treated dropwise with Br_2 (4 equiv.). After stirring for 72 h at rt, aq. $NaHSO_3$ (1M) is added and the layers are separated. The organic phase is dried over $MgSO_4$ and concentrated under reduced pressure. The residue is taken in CH_2Cl_2, cooled to −18 °C, and treated dropwise with hexanes, thus resulting in the formation of a solid which is collected by filtration and dried to afford **16** in 70% yield (Scheme 3.5) [6].



Scheme 3.5

Question 3.23: Justify the preferential formation of 1-bromonaphtalene over 2-bromonaphtalene during the first bromination step.

Question 3.24: Justify the preferential formation of **16** over **15** during the second bromination step.

Question 3.25: What is the role of aq. $NaHSO_3$ solution?

A solution of **16** in THF at 0 °C is treated with a solution of *i*-PrMgCl·LiCl (1.6 equiv.). The mixture is stirred for 30 min at 0 °C, warmed to 20 °C until complete formation of intermediate **A**, cooled to −10 °C, and bubbled with CO_2 gas for 1 h (**point 1**). The mixture is warmed to 20 °C, slowly added to water, and then THF is removed by evaporation. Addition of EtOAc and aq. HCl (5M) results in a biphasic mixture which is vigorously stirred at 45 °C and allowed to stand at rt (**point 2**). The layers are separated, the organic phase is washed with water,

diluted with heptane, and stored at 5 °C for 1 h and 30 min (**point 3**). The solid is filtered off, washed with heptane, and dried, yielding 83% of **17**.[9]

Question 3.26: Give the structure of intermediate **A**.

Question 3.27: What is the ionization state of **17** at **point 1**?

Question 3.28: Indicate the ionization state of **17** at **point 2** as well as the composition of aqueous and organic phases?

Question 3.29: What is the role of heptane at **point 3**?

A solution of **17** and Et_3N (5 equiv.) in toluene at 20 °C is treated with $Pd(OAc)_2$ (0.03 equiv.), triphenylphosphine (0.05 equiv.), and benzyl acrylate (1.5 equiv.). The mixture is stirred at 90 °C for 11 h and cooled to 20 °C. The precipitate is removed by filtration (**point 1**) and the filtrate is treated with aq. HCl (1M) (0.5 equiv. HCl), yielding a biphasic mixture which is vigorously stirred for 5 min (**point 2**). The organic phase is removed and 2-methyltetrahydrofuran and aq. HCl (1M) (1.5 equiv. HCl) are added to the aqueous phase, resulting in a biphasic mixture, which is stirred for 5 min. The aqueous phase (pH < 3) is removed (**point 3**) while the organic phase is concentrated under reduced pressure, slowly diluted with heptane, and allowed to stand at 20 °C for 5 h. The solid is filtered, washed with heptane, and dried to afford **3** in 84% yield.[9]

Question 3.30: What is the name of the palladium-catalyzed cross-coupling that allows transformation of **17** into **3**.

Question 3.31: Suggest a plausible catalytic cycle for this transformation, naming each step and mentioning the oxidation number and number of valence electrons for all metallic species involved.

Question 3.32: What is the nature of the precipitate observed at **point 1**?

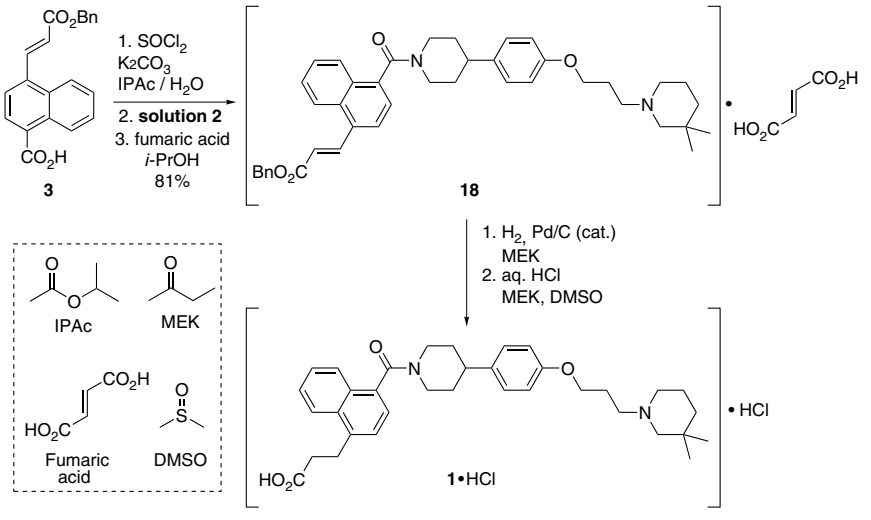
Question 3.33: Indicate the ionization state of **3** at **point 2** and mention in which phase it is present.

Question 3.34: Indicate the ionization state of **3** at **point 3** as well as the composition of aqueous and organic phases.

3.3 Fragment Assembly and End of Synthesis

A slurry of **3** (1.2 equiv.) in isopropyl acetate is heated to 79 °C, treated dropwise with $SOCl_2$ (1.4 equiv.), refluxed for 1 h, and cooled to 55 °C (**solution 1**)

9 The workup procedure described herein has been considerably simplified as compared to that reported in the original article.



Scheme 3.6

(Scheme 3.6). In parallel, a slurry of [**2** · 1.67 $PhCO_2H$] (Scheme 3.4) in IPAc is treated with aq. K_2CO_3 (20%) to give a biphasic mixture. After vigorously stirring for 10 min at 55 °C, the layers are separated and the organic phase is recovered (**solution 2**).[10] Addition of aq. K_2CO_3 (20%) to **solution 2** results in a biphasic mixture, which is heated to 55 °C and treated dropwise with **solution 1** while the temperature is maintained at 55 °C. After stirring for 15 min, the layers are separated, the organic layer is washed with water, and the solvent is evaporated under reduced pressure to produce a gum (**point 1**).[11] This gum is treated with *i*-PrOH and fumaric acid (1 equiv.), heated at 70 °C to obtain a solution, and cooled to 50 °C. After standing for several hours at 25 °C,[12] the solid is filtered, washed with a mixture of isopropyl acetate and *i*-PrOH, and dried to afford **18** in 81% yield.

Question 3.35: What is the main product present in **solution 1**? Write a mechanism to explain its formation.

Question 3.36: Indicate the composition of **solution 2**.

Question 3.37: Indicate the structure and ionization state of the main component of the gum obtained at **point 1**.

10 This process is repeated twice in the original procedure.

11 The details leading to isolation of this gum are not reported in the original article since solvent exchange from isopropyl acetate to *i*-PrOH is directly performed in the reactor by several cycles of dilution/evaporation.

12 The procedure described in the original article is more complex.

A slurry of **18** in methyl ethyl ketone (MEK) is treated with aq. K_2CO_3 (20%). The biphasic solution is stirred at 25 °C for 15 min and the layers are separated (**point 1**). The organic phase is recovered, 5% Pd/C (about 5% w/w) is added, and the vessel is placed under 3 bar H_2. After stirring vigorously at 40 °C for 8 h, the mixture is filtered, the filtrate is recovered, and the solvent is evaporated under reduced pressure to afford a glassy foam (**point 2**).[13] The residue is dissolved in a mixture of MEK and DMSO, heated to 70 °C, and treated with aq. concentrated HCl (1.05 equiv.) and MEK (slow addition over 2 h). After cooling to 20 °C and standing for a few hours, the solid is removed by filtration and washed with MEK to afford compound **1** · HCl in 80% yield.[14]

Question 3.38: Indicate the ionization state of the different species at **point 1** as well as the composition of the organic and aqueous phases.

Question 3.39: Indicate the composition and ionization state of the glassy foam obtained at **point 2**.

Question 3.40: What is the goal of final treatment with aq. concentrated HCl?

Answers

Question 3.1:

2,2-dimethylpentanedioic acid.

Question 3.2:

A Dean–Stark apparatus allows continuous removal of water during a reaction in order to shift equilibrium and bring a reversible reaction to completion. The principle is based on the use of a reaction solvent that is not miscible with water. The biphasic system is heated to reflux in order to evaporate both water and the solvent. The gases are condensed in a second flask and separated according to their density. Depending on the design of the apparatus, either the upper layer (used for solvents that have a lower density than that of water) or the lower layer (used for solvents that have a higher density than that of water) returns to the reaction flask while the other layer remains in the second flask.

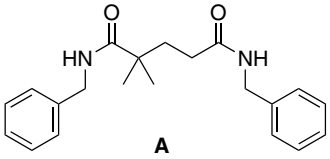
Question 3.3:

The reaction generates two equivalents of water relative to the starting material, which means 2 moles per mole of diacid and thus 36 mL of water per mole of **4**.

13 The details leading to isolation of this foam are not reported in the original article.
14 The entire procedure described here is significantly simplified as compared to that described in the original article.

Question 3.4:

Diamine **A** was found to be formed under reaction conditions.



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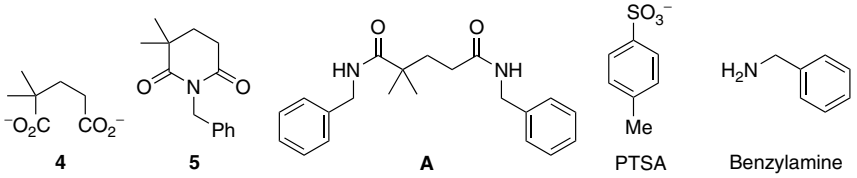
Question 3.5:

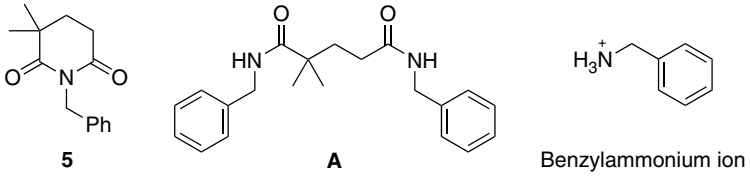
The ionization state of the various species at **point 1** is as follows:



4	**5**	**A**	PTSA	Benzylamine
Aqueous phase	Organic phase	Organic phase	Aqueous phase	Organic phase

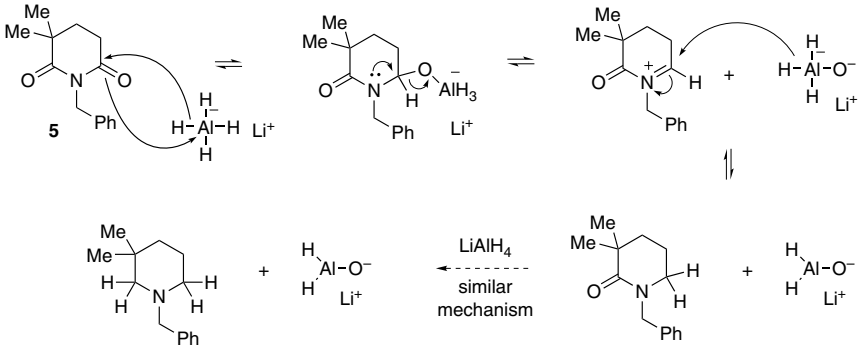
Question 3.6:

After acidification at **point 2**, the remaining species exist under the following ionization state:

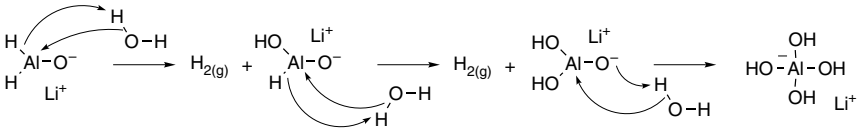


5	**A**	Benzylammonium ion
Organic phase	Organic phase	Aqueous phase

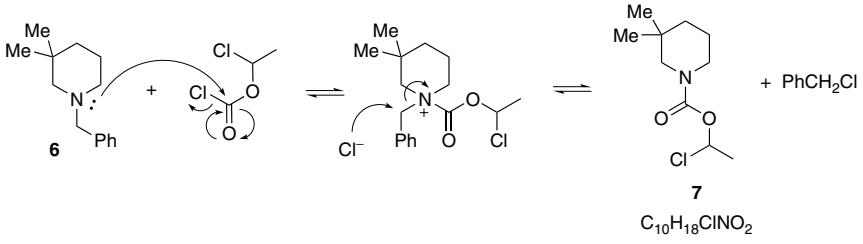
Question 3.7:



Gas evolution originates from the formation of H_2 upon neutralization in aq. basic media:

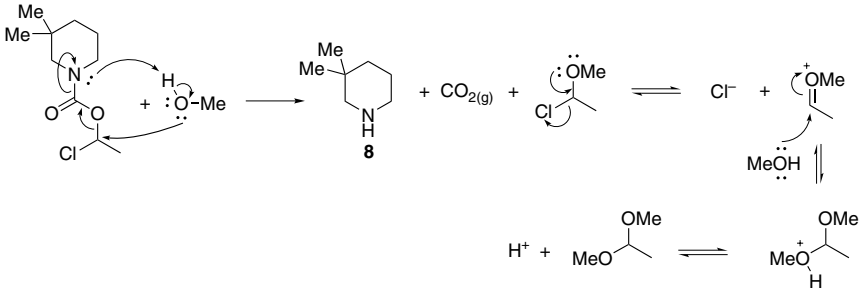


Question 3.8:



6 **7** $C_{10}H_{18}ClNO_2$

Question 3.9:



8

Remark: depending on its structure, the desired amine is sometimes isolated as the hydrochloride salt [5].

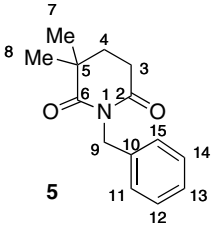
Question 3.10:

5 : 1-benzyl-3,3-dimethylpiperidine-2,6-dione
6 : 1-benzyl-3,3-dimethylpiperidine
8 : 3,3-dimethylpiperidine

Question 3.11:

Spectrum **A**, which contains eight aliphatic H and five aromatic H, corresponds to **6**.
Spectrum **B**, which contains six aliphatic H and five aromatic H, corresponds to **5**.
Spectrum **C**, which contains no aromatic H, corresponds to **8**.

See the following table for a tentative assignment.



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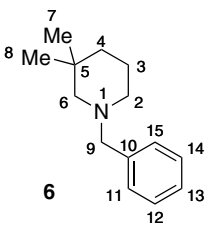

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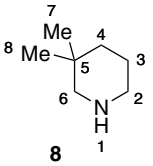


8

Spectra	Description
A: 6	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.35–7.00 (m, 5H, $\mathbf{H_{ar}}$), 3.41 (s, 2H, $\mathbf{H_9}$), 2.35–2.26 (m, 2H, $\mathbf{H_2}$), 1.99 (s, 2H, $\mathbf{H_6}$), 1.61–1.54 (m, 2H, $\mathbf{H_3}$), 1.15–1.25 (m, 2H, $\mathbf{H_4}$), 0.92 (s, 6H, $\mathbf{H_7}$ and $\mathbf{H_8}$)
B: 5	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.32–7.20 (m, 3H, $\mathbf{H_{ar}}$), 7.14–7.06 (m, 2H, $\mathbf{H_{ar}}$), 4.93 (s, 2H, $\mathbf{H_9}$), 2.71 (t, J = 7 Hz, 2H, $\mathbf{H_3}$), 1.78 (t, J = 7 Hz, 2H, $\mathbf{H_4}$), 1.25 (s, 6H, $\mathbf{H_7}$ and $\mathbf{H_8}$)
C: 8	**^{1}H-NMR** (400 MHz, $CDCl_3$): 2.77–2.69 (m, 2H, $\mathbf{H_2}$), 2.47 (s, 2H, $\mathbf{H_6}$), 1.52–1.46 (m, 2H, $\mathbf{H_3}$), 1.39 (br s, 1H, **NH**), 1.35–1.32 (m, 2H, $\mathbf{H_4}$), 0.91 (s, 6H, $\mathbf{H_7}$ and $\mathbf{H_8}$)

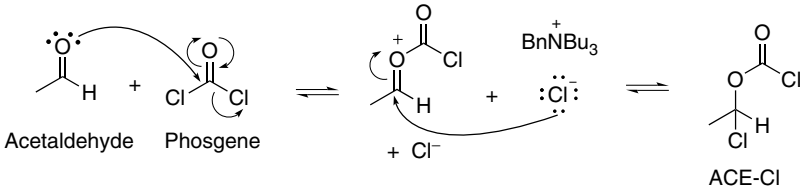
Question 3.12:

5: aromatic C–H stretching (3100–3000 cm^{-1}), alkyl C–H stretching (3000–2900 cm^{-1}), imide C=O stretching (about 1700 cm^{-1}), aromatic C=C stretching (about 1600 cm^{-1}), alkyl C–H bending (1500–1400 cm^{-1}), aromatic C–H bending (900–700 cm^{-1}).

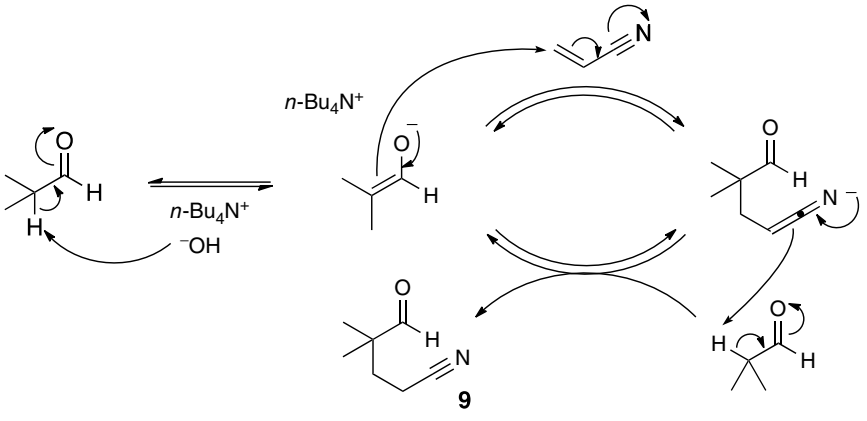
6: aromatic C–H stretching (3100–3000 cm^{-1}), alkyl C–H stretching (3000–2900 cm^{-1}), alkyl C–H bending (1500–1400 cm^{-1}), aromatic C=C stretching (about 1600 cm^{-1}), aromatic C–H bending (900–700 cm^{-1}).

8: N–H stretching (3500–3300 cm^{-1}), alkyl C–H stretching (3000–2900 cm^{-1}), alkyl C–H bending (1500–1400 cm^{-1}).

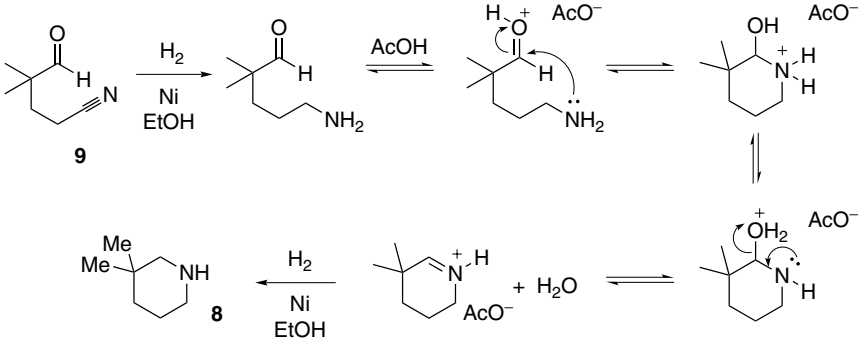
Question 3.13:



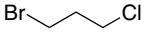
Question 3.14:



Question 3.15:



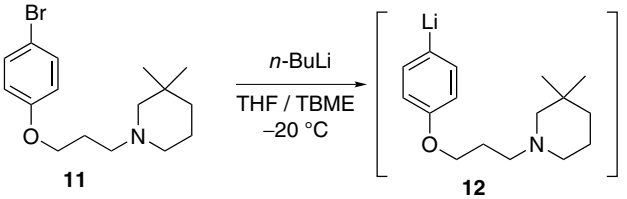
Question 3.16:



A

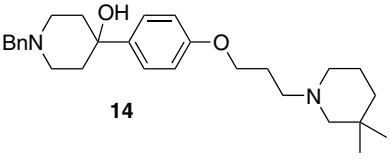
Question 3.17:

Lithium–bromide exchange between **11** and *n*-BuLi leads to **12** [7].



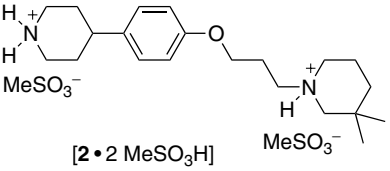
11 **12**

Question 3.18:



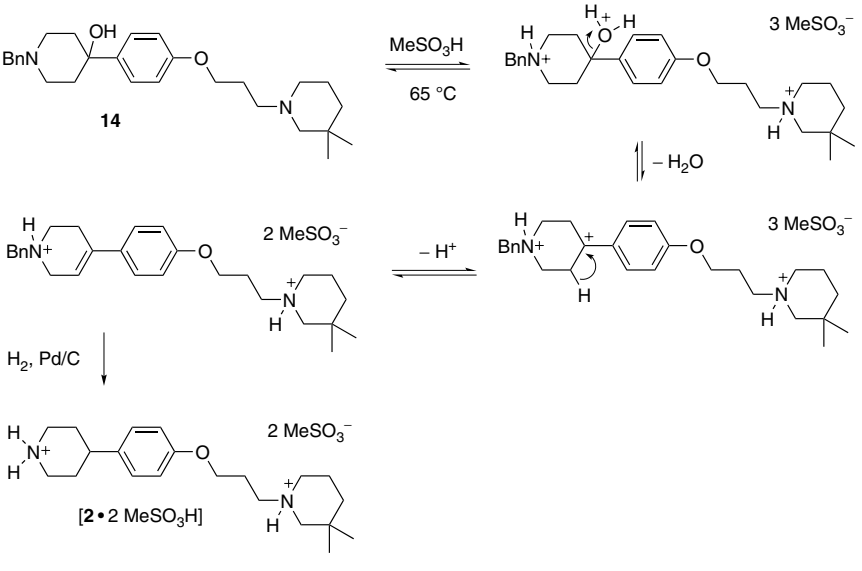
14

Question 3.19:



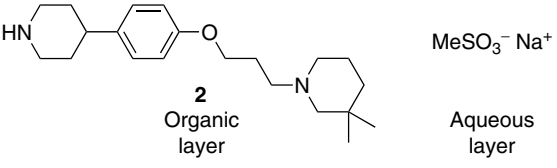
[**2** • 2 $MeSO_3H$]

Question 3.20:



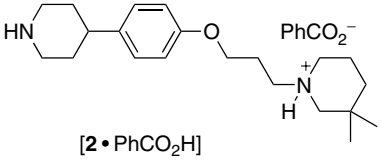
Question 3.21:

At **point 2**, the species exist in the following ionization state:

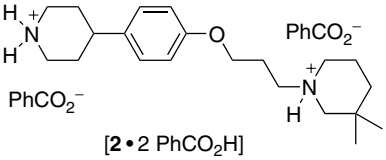


Question 3.22:

The final product is a mixture of [**2** · $PhCO_2H$] (33%) and [**2** · 2 $PhCO_2H$] (67%).



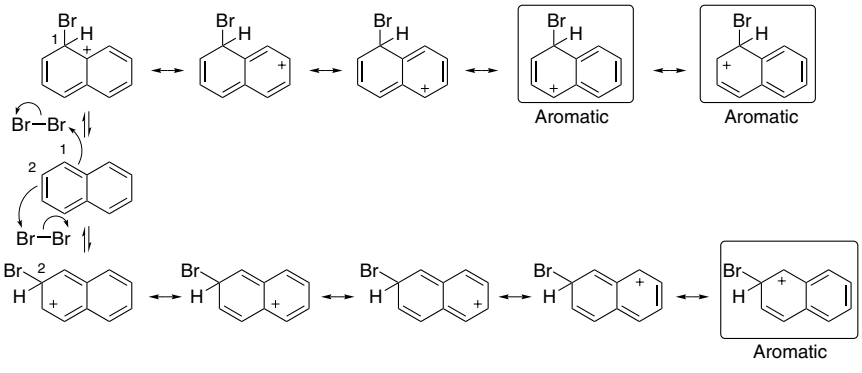
[**2** • $PhCO_2H$]



[**2** • 2 $PhCO_2H$]

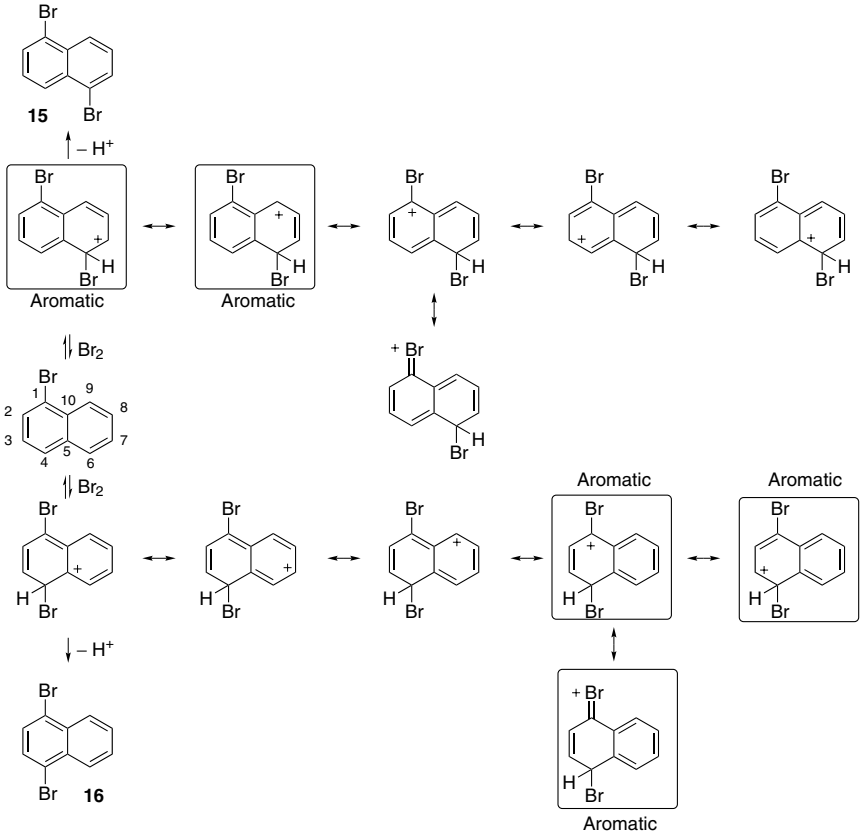
Question 3.23:

The regioselectivity of the reaction is dictated by the relative stability of the two possibly resulting carbocations (Wheland intermediates). While bromination at C(1) results in a carbocation existing as five resonance forms, two of which are aromatic, addition at C(2) also leads to five resonance structures albeit with only one aromatic form. Thus, the 1-bromo carbocation is preferentially formed and undergoes loss of H^+ to yield 1-bromonaphtalene.



Question 3.24:

Using similar reasoning as described, addition of bromide at C(6) results in the formation of a Wheland intermediate presenting six resonance structures (two of which are aromatic), while bromination at C(4) leads to the same number of resonance structures among which three are aromatic. Formation of the latter carbocation is thus favored and leads to **16** after loss of H^+.

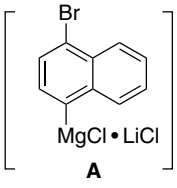


Question 3.25:

$NaHSO_3$ is used to reduce excess of Br_2 following the reaction:

$$Br_2 + HSO_3^- + 4\,H_2O \rightarrow SO_4^{2-} + 3\,H_3O^+ + 2\,Br^-$$

Question 3.26:



Additional information about this reaction can be found in Ref. [8].

Question 3.27:

The nucleophilic addition of intermediate **A** on CO_2 leads to **17** as its carboxylate salt.

Question 3.28:

After addition of aq. HCl, reprotonation of **17** leads to carboxylic acid, which is extracted in the organic phase.

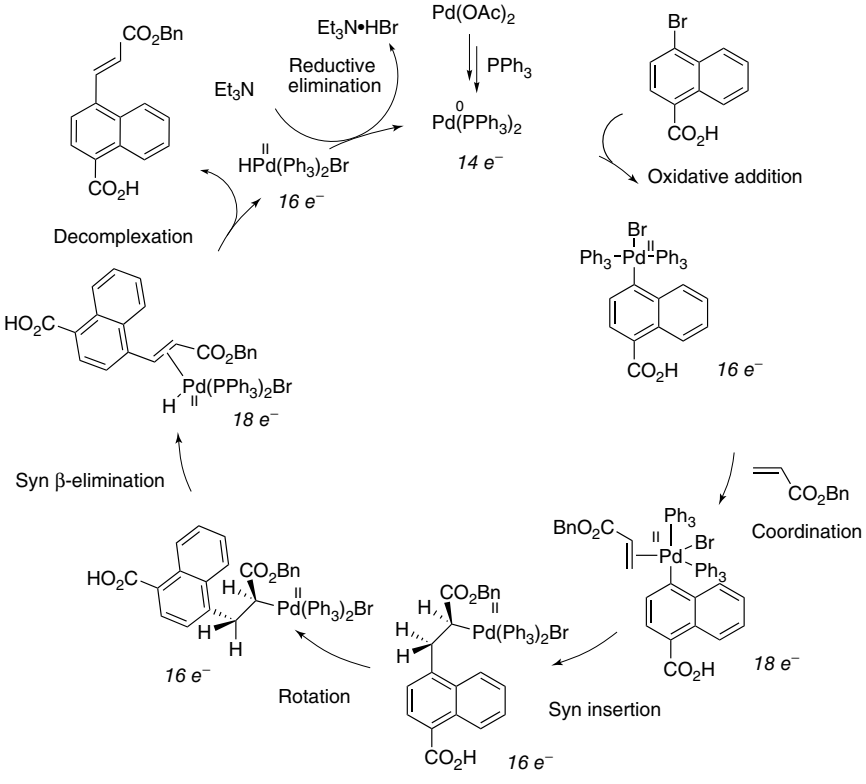
Question 3.29:

Heptane acts as a "non-solvent," inducing precipitation of the product.

Question 3.30:

Heck coupling.

Question 3.31:

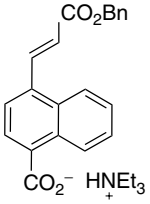


Question 3.32:

The precipitate corresponds to triethylammonium chloride ($Et_3N \cdot HCl$).

Question 3.33:

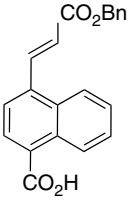
At **point 2**, compound **3** exists as



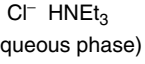
3
(aqueous phase)

Question 3.34:

At **point 3**, protonation of **3** occurs, thus allowing its extraction in the organic layer, while excess triethylamine is transformed in triethylammonium chloride and extracted in the aqueous layer.



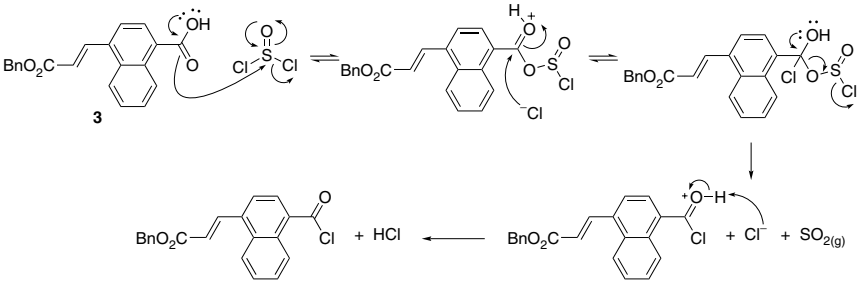
3
(organic phase)



(aqueous phase)

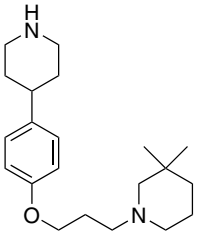
Question 3.35:

Solution 1 contains acyl chloride derived from acid **3**:



Question 3.36:

Solution 2 contains compound **2**, while potassium benzoate is eliminated in the aqueous layer.

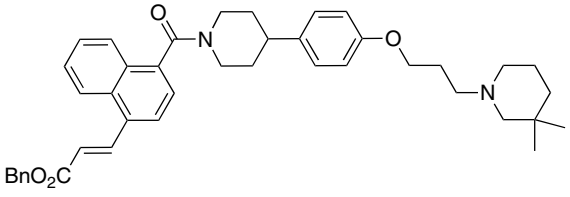


2
(in **solution 2**)

$PhCO_2^-\ K^+$
(removed in aqueous layer)

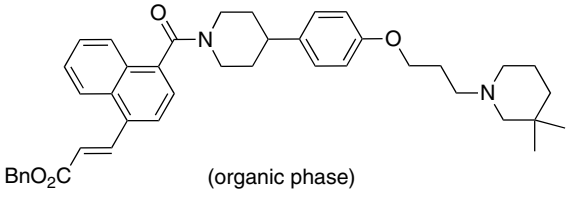
Question 3.37:

The gum obtained at **point 1** corresponds to the addition product of **2** on acyl chloride derived from **3**.

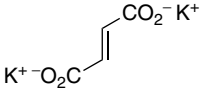


Question 3.38:

At **point 1**:



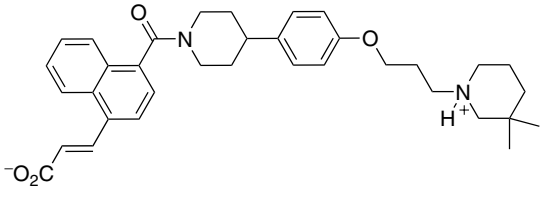
(organic phase)



(aqueous phase)

Question 3.39:

The glassy foam at **point 2** corresponds to **1** in its zwitterionic form:



Question 3.40:

The final treatment with aq. concentrated HCl leads to the formation of salt **1** · HCl, which is solid and can thus be isolated by crystallization.

References

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4

Synthesis of Eletriptan

Eletriptan **1** is a selective 5-HT agonist, approved in the United States in 2002 and commercialized for the treatment of migraine. Among the investigated manufacturing routes, those based on Fischer indole synthesis involving an acetal of type **2** and hydrazine **3** were found to be particularly attractive (Scheme 4.1) [1].

Question 4.1: Indicate the absolute configuration of the stereogenic center of eletriptan **1**.

The first strategy to access racemic pyrrolidine **10b** is described in Scheme 4.2. A mixture of acetal **4**, diol **5** (1.5 equiv.), and H_2SO_4 (1 equiv.) is refluxed for 16 h. After cooling to 4 °C, water is added and the resulting mixture is extracted twice with *tert*-butyl methyl ether (TBME); the combined organic layers are washed with water, dried with $MgSO_4$, and the solvent is removed under reduced pressure to afford compound **6**. The residue is dissolved in THF (tetrahydrofuran), a 1M solution of PEt_3 (1.5 equiv.) in THF is added, and the mixture is heated at 65 °C for 48 h. The solvent is evaporated under vacuum, yielding 90% of **7**.

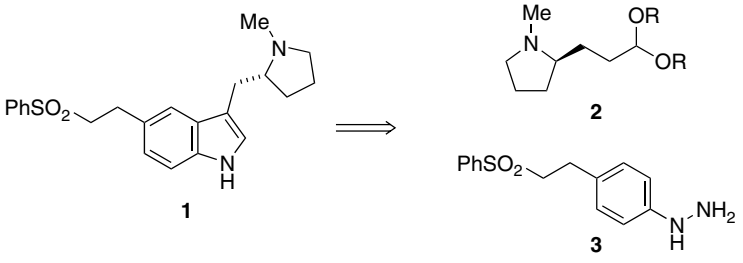
Question 4.2: Indicate the structure of **6** and write a mechanism explaining its formation.

Question 4.3: Indicate the structure of **7** and write a mechanism explaining its formation.

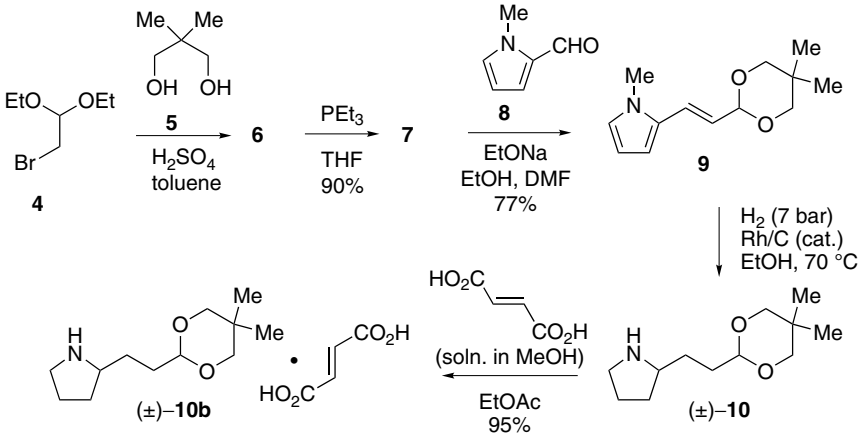
A solution of **7** in dimethylformamide (DMF) is treated with aldehyde **8** (1 equiv.) followed by a solution of EtONa (1.1 equiv.) in EtOH. After heating at 80 °C for 3 h and cooling to rt, water is added and the mixture is extracted twice with TBME (**point 1**). The combined organic layers are dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue is recrystallized from heptane to afford **9** in 77% yield.

Question 4.4: Write a mechanism explaining the formation of **9** (stereochemistry will not be discussed) and indicate the by-products obtained.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.



Scheme 4.1



Scheme 4.2

Question 4.5: Indicate the composition of both organic and aqueous phases at **point 1**.

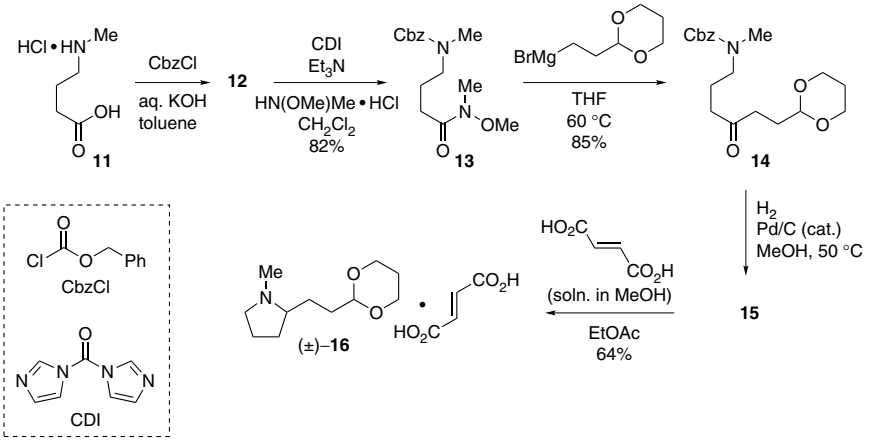
Final steps involve hydrogenation of **9** in EtOH using Rh/C as a catalyst to give **10**, followed by salt formation with fumaric acid, yielding **10b**.

Question 4.6: Suggest plausible experimental protocols allowing the preparation of **10b** from **9**.

Another route, allowing preparation of racemic pyrrolidine **16**, is described in Scheme 4.3. A solution of **11** in aq. KOH (4 equiv.) (**point 1**) is treated with CbzCl. After stirring for 2 h (**point 2**), aq. HCl (5 equiv.) is added (**point 3**) and the mixture is extracted twice with TBME. The combined organic phases are dried over $MgSO_4$ and filtered and the solvent is evaporated under reduced pressure to afford **12**.

Question 4.7: Indicate the ionization state of **11** at **point 1**.

Question 4.8: Indicate the structure of **12** and mention its ionization state at **point 2** and **point 3**.



Scheme 4.3

Question 4.9: Write a mechanism explaining the formation of **12**.

A solution of **12** in CH_2Cl_2 is treated with CDI (1 equiv.). After stirring for 1 h (**point 1**), Et_3N (1 equiv.) is added, followed by *N,O*-dimethylhydroxylamine hydrochloride (1 equiv.) and the resulting mixture is stirred for 16 h. Aq. HCl (2.1 equiv.) is added, the organic layer is washed with water, and the solvent is removed under reduced pressure to yield 82% of **13**.

Question 4.10: What is the structure of the intermediate formed at **point 1**?

Question 4.11: What is the role of Et_3N?

Question 4.12: What is the role of aq. HCl used during workup?

To a slurry of magnesium turnings (1.4 equiv.) in THF is added a crystal of iodine, followed by a solution of **A** (0.3 equiv.) in THF. The mixture is heated to 65 °C, a further solution of **A** (1.1 equiv.) in THF is added, and heating is maintained for another hour. This solution is added to a solution of **13** in THF cooled at 4 °C. The mixture is heated at 65 °C for 2 h (**point 1**), and then aq. citric acid (10%) is added (**point 2**). The organic layer is concentrated under reduced pressure, redissolved in TBME (**point 3**), washed with aq. citric acid (10%) and water, dried over $MgSO_4$, and filtered. The solvent is evaporated under reduced pressure to give ketone **14** in 85% yield.

Question 4.13: Write the structure of **A**.

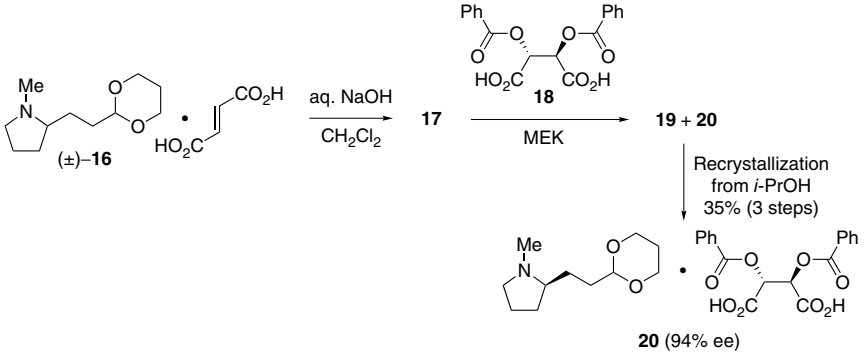
Question 4.14: Write the structure of intermediates obtained at **point 1** and **point 2**.

Question 4.15: Why is the THF phase concentrated and redissolved in TBME at **point 3**?

A mixture of **14** and 5% Pd/C (10% w/w) in MeOH at 50 °C is stirred under hydrogen pressure (4 bar) for 16 h. The catalyst is removed by filtration, the filtrate is recovered, and the solvent is evaporated to afford **15** as a clear oil. After dissolution in a mixture of EtOAc and MeOH, a solution of fumaric acid (1 equiv.) in MeOH is added. MeOH is removed by azeotropic distillation and replaced with EtOAc; after 16 h, the solid is collected by filtration and dried under vacuum to yield 64% of **16**.

Question 4.16: Write the structure of **15** and explain its formation with a plausible mechanism.

A third method, allowing access to enantiomerically enriched pyrrolidine acetal **20**, was developed (Scheme 4.4). Compound **16** is treated with a biphasic mixture of CH_2Cl_2 and aq. KOH (1M) (**point 1**); then the layers are separated and the aqueous layer is extracted twice with CH_2Cl_2. The combined organic phases are dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford **17**.



Scheme 4.4

Question 4.17: Indicate the composition of both aqueous and organic layers at **point 1**, as well as the structure of **17**.

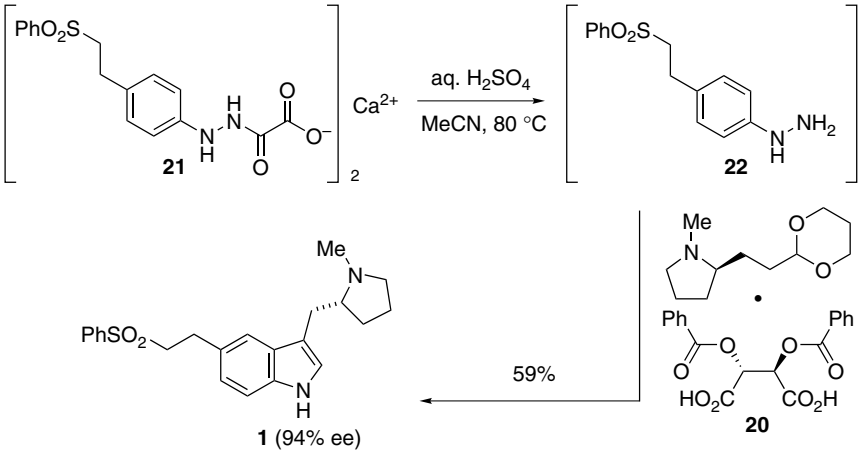
A solution of **17** in methyl ethyl ketone (MEK) at 75 °C is treated with a solution of enantiomerically pure **18** (1 equiv.) in MEK. The mixture is cooled to 0 °C, and after 16 h the solid is collected by filtration and dried under vacuum to afford a mixture of **19** and **20**. These compounds are dissolved in boiling *i*-PrOH, the solution is cooled to rt, the solid is collected by filtration (**point 2**), and dried to yield 35% of **20**, obtained with 94% *ee*.

Question 4.18: Write the structure of **19** and indicate its stereochemical relation to **20**.

Question 4.19: Indicate the composition of the filtrate obtained at **point 2**.

Question 4.20: What do you call such a process for enantiomer separation? Briefly explain its principle and indicate the maximum theoretical yield expected for **20**.

In a final step, a slurry of calcium salt **21** (0.5 equiv.) and tartrate salt **20** (Scheme 4.5) in MeCN is treated with aq. H_2SO_4 (5.6 equiv.) and heated to 80 °C for 16 h, leading to the transient formation of hydrazine **22** [2]. After quenching with aq. NaOH (2M), the mixture is extracted twice with EtOAc, washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by column chromatography gave **1** (94% ee) in 59% yield.

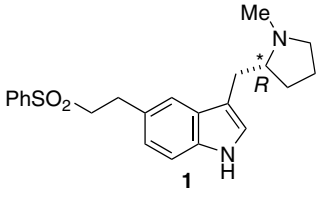


Scheme 4.5

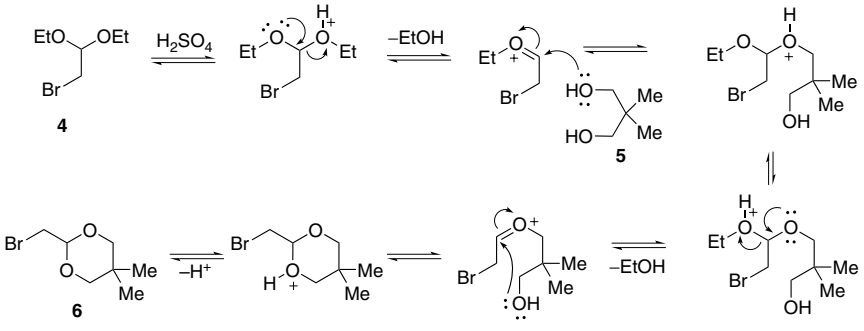
Question 4.21: Name the reaction leading to eletriptan **1** and suggest a plausible mechanism.

Answers

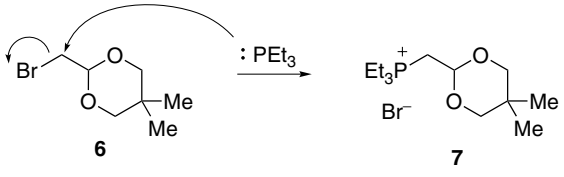
Question 4.1:



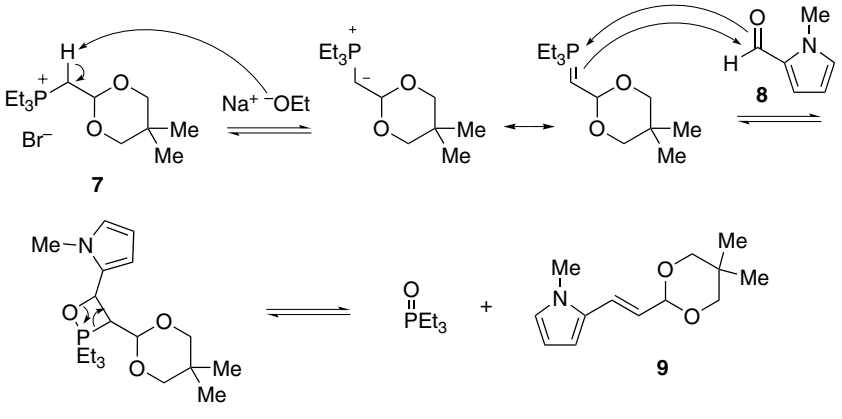
Question 4.2:



Question 4.3:



Question 4.4:



Question 4.5:

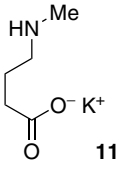
At **point 1**, compound **9** is in the organic layer while triethylphosphine oxide ($O{=}PEt_3$) is in the aqueous layer.

Question 4.6:

A mixture of **9** and Rh/C (cat.) in EtOH is vigorously stirred at 70 °C under hydrogen pressure (7 bar). Once the reaction is completed, the catalyst is removed by filtration, the filtrate is recovered, and the solvent evaporated under reduced pressure to afford **10**.

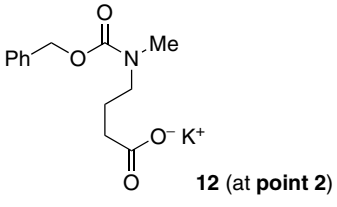
Compound **10** is dissolved in EtOAc and the solution is treated with a solution of fumaric acid (1 equiv.) in MeOH. After stirring for an appropriate time, the solid is collected by filtration and dried to give **10b**.

Question 4.7: At **point 1**, compound **11** exists as

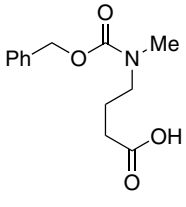


11 (at **point 1**)

Question 4.8: At **point 2** and **point 3**, compound **12** exists as

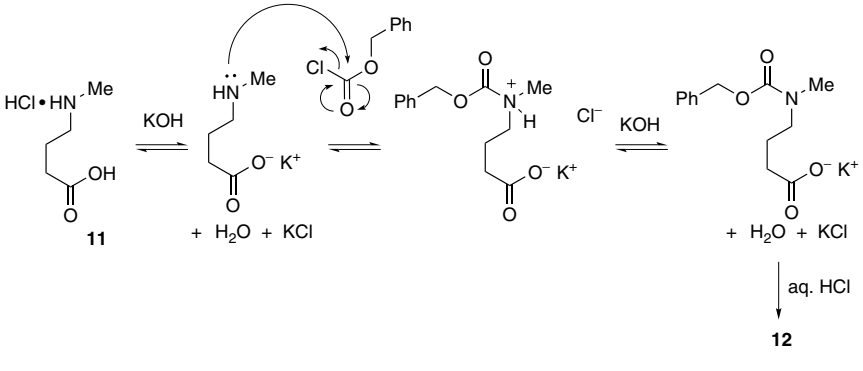


12 (at **point 2**)



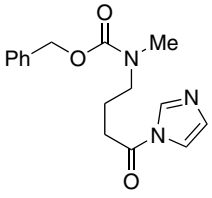
12 (at **point 3**)

Question 4.9:



Question 4.10:

At **point 1**:



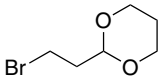
Question 4.11:

The role of Et_3N is to generate *N,O*-dimethylhydroxylamine from its corresponding hydrochloride salt.

Question 4.12:

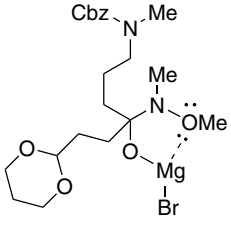
The role of HCl is to protonate imidazole and any residual traces of *N,O*-dimethylhydroxylamine to facilitate their extraction in water.

Question 4.13:

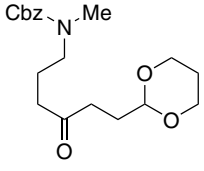


A

Question 4.14:



At **point 1**

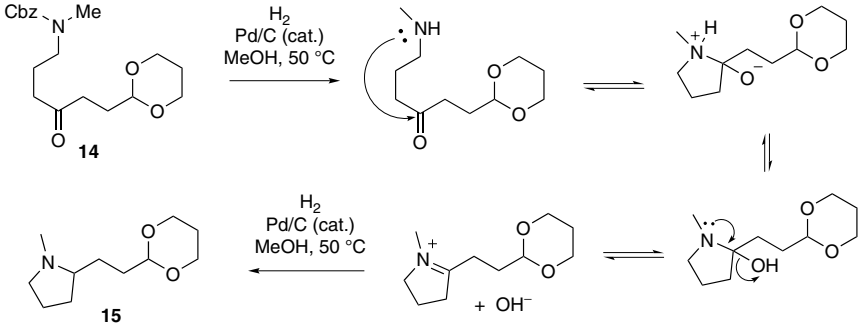


At **point 2**

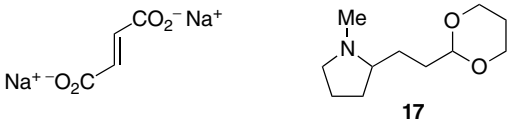
Question 4.15:

THF is miscible with water, so its presence does not allow proper phase separation. After evaporation of THF and redissolution with TBME, the organic and aqueous phases are clearly separated.

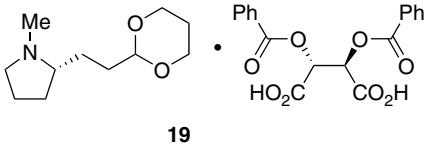
Question 4.16:



Question 4.17: At **point 1**:



Question 4.18: **19** and **20** are diastereoisomers.

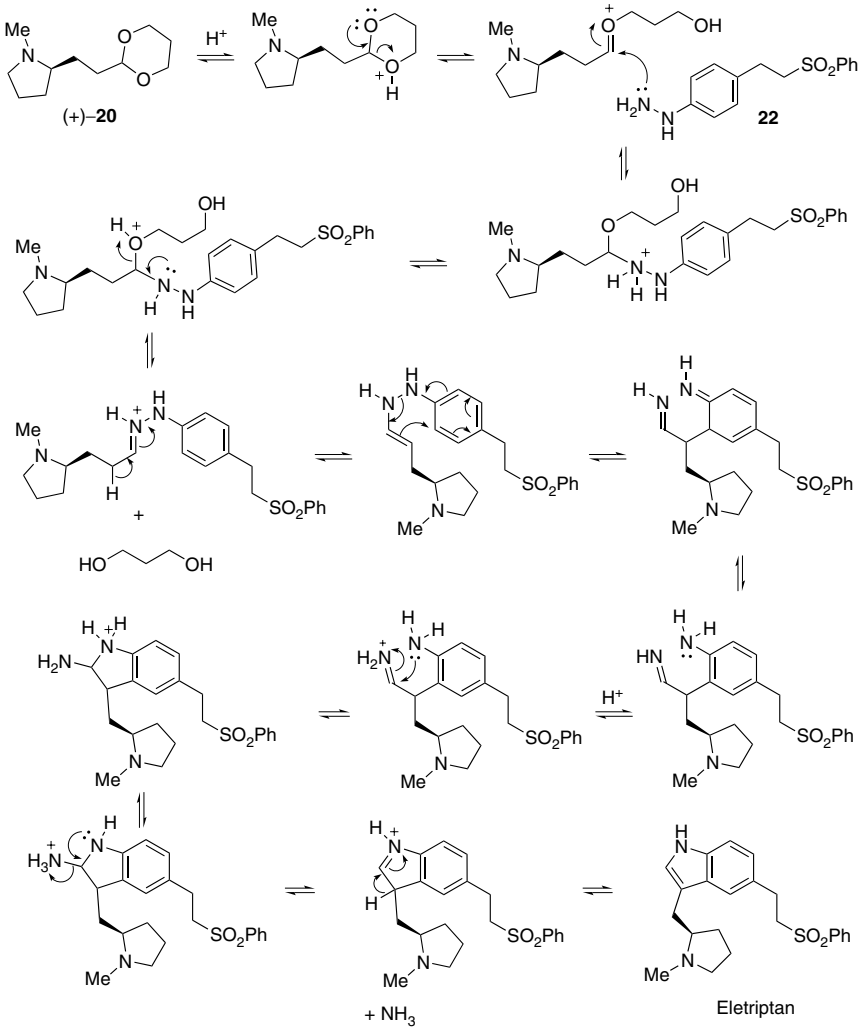


19

Question 4.19: At **point 2**, the filtrate is mainly composed of **19**.

Question 4.20: This process for separation of enantiomers is called chiral resolution. The principle is to transform a mixture of enantiomers into a mixture of diastereoisomers by formation of a covalent or noncovalent bond with an optically pure reagent. After separation of enantiomers, usually by crystallization or chromatography, the starting material is regenerated thus leading to both enantiomers as optically pure compounds. The maximum theoretical yield of such a process is 50%.

Question 4.21: Fischer indole synthesis.



References

1 Ashcroft, C.P., Hellier, P., Pettman, A., and Watkinson, S. (2011) Second-generation process research towards eletriptan: a Fischer indole approach. *Org. Proc. Res. Dev.*, **15** (1), 98–103.

2 Browne, D.L., Baxendale, I.R., and Ley, S.V. (2011) Piecing together the puzzle: understanding a mild, metal free reduction method for the large scale synthesis of hydrazines. *Tetrahedron*, **67** (52), 10296–10303.

5

Total Synthesis and Structure Revision of Streptophenazine A

In 2008, several compounds showing interesting antibiotic activity were isolated from a marine sponge of the Baltic Sea [1]. Based on various spectroscopic methods, structure **1** was suggested for streptophenazine A, though the stereochemistry of the side chain could not be determined (Figure 5.1). Interested by its promising biological activity, another team achieved in 2011 the total synthesis of **1**, and evidenced the original misassignment for the structure of streptophenazine [2]. Synthesis of compound **2** allowed identification of the correct structure.

The synthesis of **1**, the originally proposed structure for streptophenazine A, is described in Scheme 5.1. A mixture of compound **4** (molecular formula $C_7H_4ClNO_4$), K_2CO_3 (3 equiv.), and 1-pentanol is stirred at rt for 10 min. Amine **3** (1 equiv.) is added and the mixture is stirred at 150 °C overnight.[1]

After cooling to rt, water and EtOAc are added, yielding a biphasic mixture (**point 1**). The layers are separated and the organic phase is extracted twice with aq. NaOH (2M). The combined aqueous layers are acidified with aq. HCl (2M) to pH 4, and a precipitate appears upon stirring at rt (**point 2**). After 30 min, the solid is collected by filtration, washed with water, and dried under vacuum to afford **5** in 93% yield.

Question 5.1: Write the structure of **4**.

Question 5.2: Indicate the ionization state of **5** and the composition of both organic and aqueous phases at **point 1**.

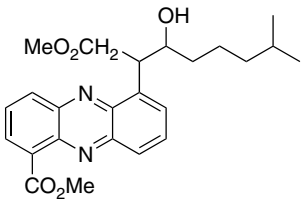
Question 5.3: Suggest a plausible mechanism for the formation of **5**.

Question 5.4: Explain the formation of a precipitate at **point 2**.

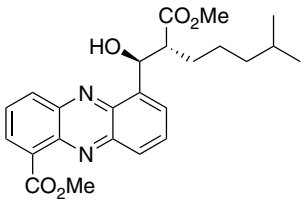
Reductive cyclization of **5** (procedure not detailed) leads to a mixture of compounds **6** and **7** in a 3 : 1 ratio. A slurry of **6** and **7** in MeOH is cooled to 0 °C and treated with a solution of Me_3SiCHN_2 (4 equiv.) in hexanes. After stirring for 1 h at 0 °C, the solvent is evaporated and the residue is purified by silica gel chromatography to yield 52% of **8** and 16% of **9**. Both compounds have the same

1 The original procedure describes the reaction with 2 equiv. K_2CO_3 followed by further addition of 1 equiv. K_2CO_3 and prolonged stirring.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

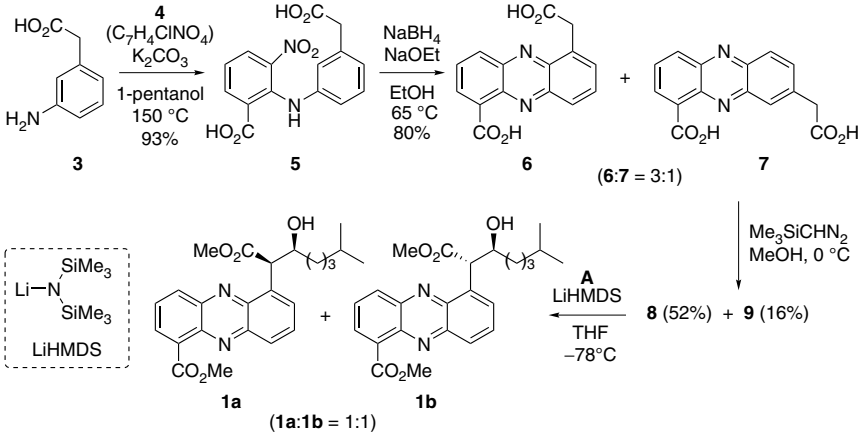


1 – Proposed structure of streptophenazine A



2 – Revised structure of streptophenazine A

Figure 5.1: Proposed and revised structure of streptophenazine A



Scheme 5.1

molecular formula ($C_{17}H_{14}N_2O_4$); their ^{1}H- and ^{13}C-NMR data are reported in the following table.

Compound	Spectrum description
8	**^{1}H-NMR** (500 MHz, $CDCl_3$): 8.38 (dd, J = 9.0, 1.5 Hz, 1H), 8.26 (dd, J = 8.5, 1.5 Hz, 1H), 8.24 (dd, J = 7.0, 1.5 Hz, 1H), 7.85–7.81 (m, 2H), 7.78 (d, J = 7.0 Hz, 1H), 4.40 (s, 2H), 4.11 (s, 3H), 3.71 (s, 3H)
	^{13}C-NMR (125 MHz, $CDCl_3$): 172.2, 167.1, 143.7, 142.2, 142.1, 140.9, 133.9 (2C), 132.1, 131.3 (2C), 130.7, 129.9, 128.8, 52.7, 52.1, 36.5
9	**^{1}H-NMR** (500 MHz, $CDCl_3$): 8.37 (dd, J = 9.0, 1.5 Hz, 1H), 8.23–8.20 (m, 3H), 7.85 (dd, J = 9.0, 7.0 Hz, 1H), 7.83 (dd, J = 8.0, 2.0 Hz, 1H), 4.11 (s, 3H), 3.93 (s, 2H), 3.75 (s, 3H)
	^{13}C-NMR (125 MHz, $CDCl_3$): 170.9, 167.1, 143.5, 142.8, 142.6, 141.2, 137.4, 133.4, 133.2, 132.0, 131.6, 130.1, 129.6, 129.0, 52.7, 52.4, 41.4

Question 5.5: Write the structure of **8** and **9**. Assign characteristic ^{1}H- and ^{13}C-NMR signals supporting your answer.

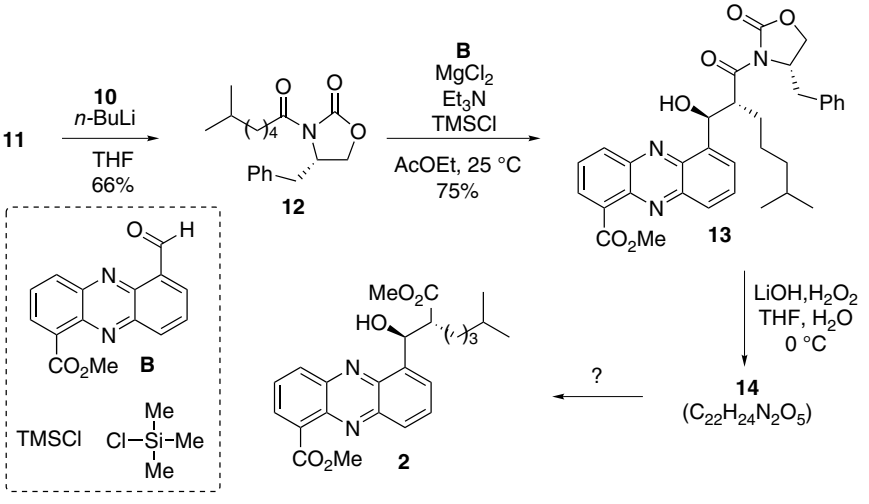
Question 5.6: Write the Lewis structure of Me_3SiCHN_2 and suggest a mechanism for the formation of **8** and **9**.

Finally, treatment of **8** with LiHMDS followed by addition of **A** affords, after appropriate workup, a separable mixture of **1a** and **1b**. Spectroscopic analysis reveals that none of these compounds is identical to those isolated from the marine sponge.

Question 5.7: Write the structure of reagent **A** and suggest a plausible reaction procedure for the conversion of **8** into **1a** and **1b**.

Question 5.8: What is the stereochemical relation between **1a** and **1b**? Does isolated **1a** or **1b** present any optical activity? Does a mixture of **1a** and **1b** present any optical activity? Justify your answer.

A synthetic route allowing access to the enantiomerically enriched compound **2** is described in Scheme 5.2. Reaction of oxazolidinone **10** with *n*-BuLi, followed by addition of acyl chloride **11** (procedure not detailed), leads to **12** in 66% yield after appropriate workup.



Scheme 5.2

Question 5.9: Write the structure of **10** and **11**.

A mixture of **12** (1.5 equiv.), anhydrous $MgCl_2$ (1 equiv.), Et_3N (3 equiv.), and EtOAc is stirred at rt for 15 min (**point 1**). Aldehyde **B** is added (**point 2**), followed by trimethylsilyl chloride (TMSCl) (2 equiv.), and the mixture is stirred at

rt (**point 3**). After 24 h, aq. saturated NH_4Cl solution is added and the mixture is extracted with EtOAc. The combined organic layers are washed with aq. saturated NaCl solution and the solvent is evaporated under reduced pressure. The residue is dissolved in MeOH, one drop of TFA is added, and the mixture is stirred for 10 min. Evaporation of the solvent and purification by silica gel chromatography affords **13** in 75% yield.

Question 5.10: Explain the transformation of **12** into **13** by writing the structure of intermediates formed at **point 1**, **point 2**, and **point 3**.

Question 5.11: Reasoning by analogy with the synthesis described in Scheme 5.1, suggest a plausible reaction sequence allowing preparation of **B**.

Question 5.12: Assign the stereochemical descriptor (using Cahn–Ingold–Prelog rules) of every asymmetric carbon atom in **13**.

A mixture of **13** in THF at 0 °C is treated with aq. H_2O_2 (30%) solution (10 equiv.) followed by LiOH (5 equiv.). After stirring at 0 °C for 5 h (**point 1**), aq. saturated $NaHSO_3$ solution is added; the solution is warmed to rt and stirred for 1 h (**point 2**). The mixture is diluted with aq. KH_2PO_4 (1M) and extracted with EtOAc. The combined organic phases are washed with aq. saturated NaCl solution, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford **14** (molecular formula $C_{22}H_{24}N_2O_5$). The crude product is transformed in a single step into compound **2**, whose optical rotation and spectroscopic data match those initially reported for streptophenazine A isolated from the marine sponge.

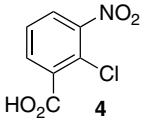
Question 5.13: Write the structure of the species formed at **point 1**.

Question 5.14: Explain the role of treatment with aq. saturated $NaHSO_3$ solution at **point 2** and write the structure of **14**.

Question 5.15: Suggest plausible reaction conditions for the preparation of **2** from **14**.

Answers

Question 5.1:



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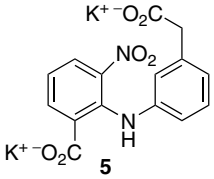
Question 5.2:
At **point 1**:



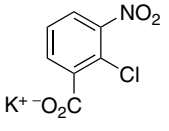
(aqueous phase)

Excess K_2CO_3 + $KHCO_3$

(aqueous phase)

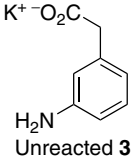
1-Pentanol

(organic phase)



Unreacted **4** if any

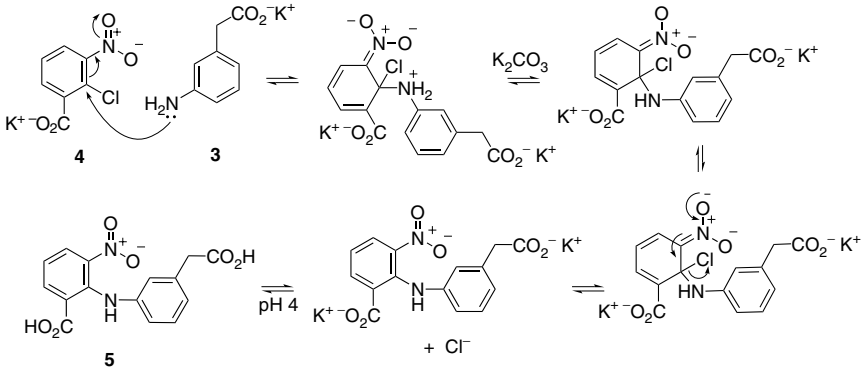
(aqueous phase)



Unreacted **3** if any

(aqueous phase)

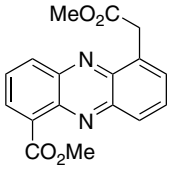
Question 5.3



Question 5.4:

Reducing pH to 4 results in protonation of the two carboxylic acid groups, leading to neutral compound **5**, insoluble in water. It is interesting to note that under such conditions, the diarylamine cannot be protonated (for example, the pK_a of the conjugate acid of diphenylamine is around 0.8).

Question 5.5:



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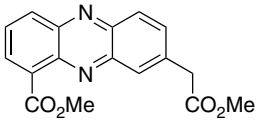
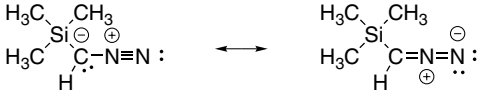


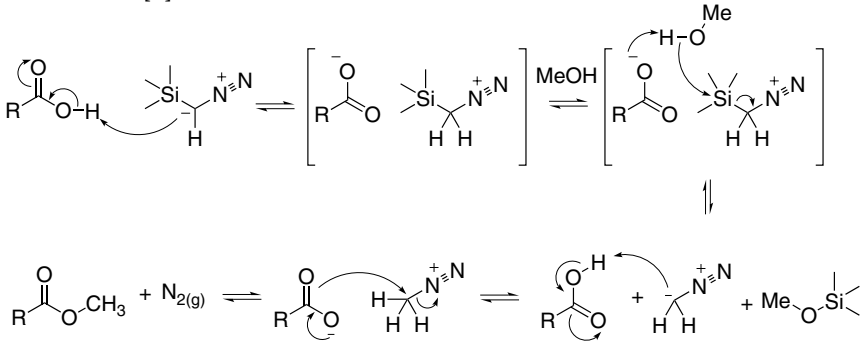
9

Treatment of a carboxylic acid with trimethylsilyldiazomethane allows its conversion into the corresponding methylester. This is corroborated by the presence of two singlets between 3.71 and 4.11 ppm integrating for 3H (CO_2**Me**) in the ^{1}H-NMR spectra, as well as two signals at 52.1–52.7 (CO_2**Me**) and 167.1–172.2 ppm (**C**O_2Me) in the ^{13}C-NMR spectra.

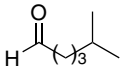
Question 5.6:



Mechanism [3]:



Question 5.7:



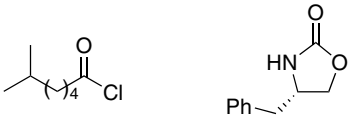
A

A mixture of **8** in THF is cooled to –78 °C and treated with a solution of LiHMDS (1 equiv.) in THF. After stirring for 10 min at –78 °C, **A** (1 equiv.) is added. Stirring is continued at –78 °C until consumption of the starting material, and then aq. saturated NH_4Cl solution is added. The mixture is warmed to rt and extracted three times with EtOAc. The combined organic layers are dried over $MgSO_4$ and filtered, and the solvent is evaporated under reduced pressure. If required, the residue is purified by chromatography on silica gel.

Question 5.8:

1a and **1b** are diastereoisomers. Each of these diastereoisomers is obtained as a racemic mixture, so neither isolated **1a** and **1b** nor their mixture present any optical activity.

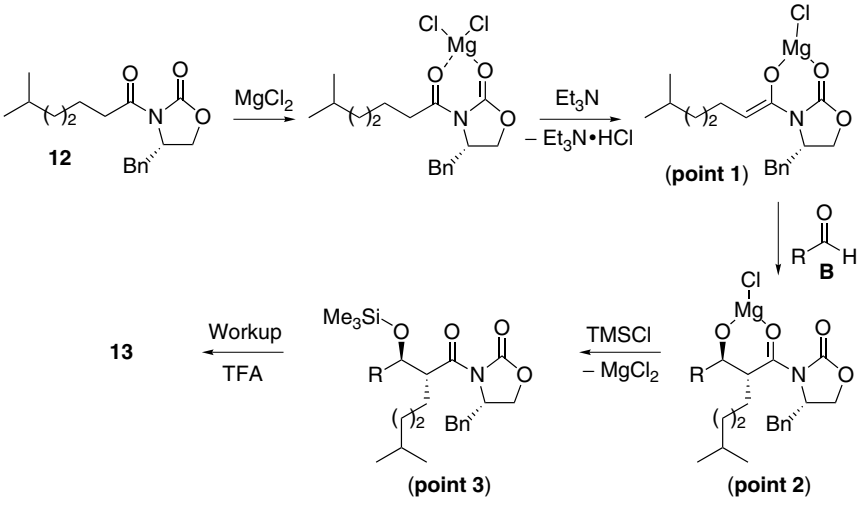
Question 5.9:



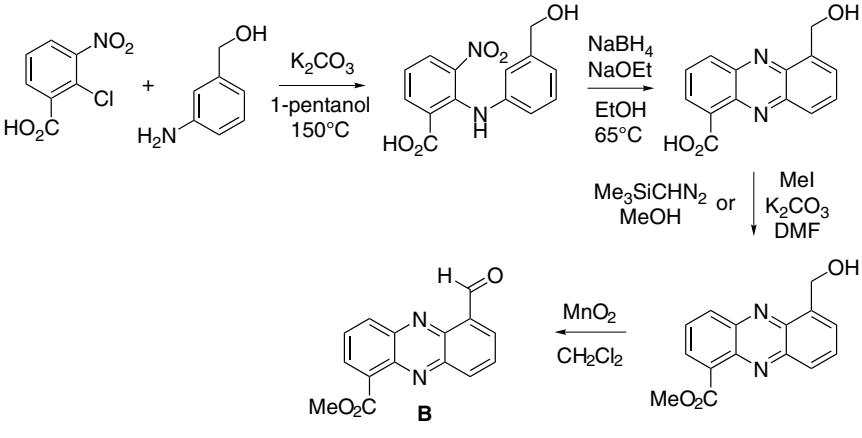
11 **10**

Question 5.10:

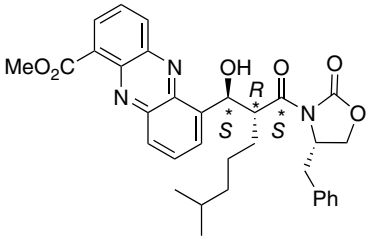
The mechanism of this reaction has been discussed in Ref. [4]:



Question 5.11:



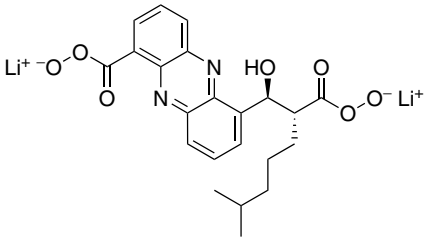
Question 5.12:



Question 5.13:

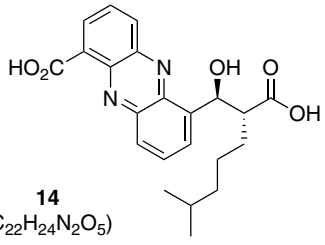
H_2O_2 reacts with LiOH to give LiOOH, which is the effective nucleophile in this reaction. Alkaline peroxide is less basic than LiOH and avoids epimerization of the stereogenic center α to the imide moiety. Moreover, it shows high selectivity and favors cleavage of the exocyclic carbonyl group of the oxazolidone-derived carboximide [5].

At **point 1**:



Question 5.14:

The role of $NaHSO_3$ is to neutralize unreacted H_2O_2 and reduce the lithium peracetate moieties, thus leading to **14** after workup.



14
($C_{22}H_{24}N_2O_5$)

Question 5.15:

AcCl, MeOH.

References

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6

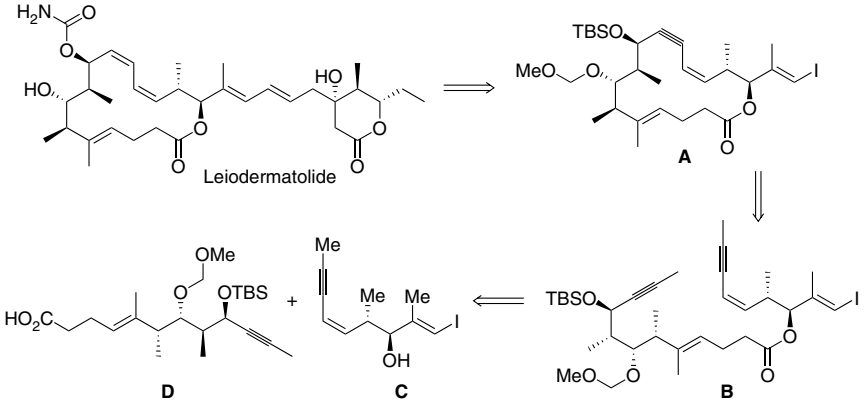
Synthesis of Leiodermatolide, A Biologically Active Macrolide

Leiodermatolide is a naturally occurring macrolide, identified through bioassay-guided fractionation of a marine sponge collected off the Florida coastline (Scheme 6.1) [1]. This compound exhibits an interesting biological activity on several cancer cell lines, with an original mode of action. In spite of the use of advanced NMR (nuclear magnetic resonance) techniques, the structure elucidation was quite challenging, especially regarding the relative stereochemical attribution of the macrocycle and δ-lactone moieties. Only recently, by total synthesis of several diastereoisomers and comparison with the natural product, could the absolute configuration of all stereogenic centers be ascertained. The following problems focus on a synthesis reported in 2014 [2].

Question 6.1: How would you define a macrolide?

Question 6.2: What is a δ-lactone? Write the structure of a β-, γ-, and ε-lactone.

Question 6.3: How would you explain the difficulty met in the attribution by NMR of relative configuration of macrocyclic and δ-lactone moieties?



Scheme 6.1

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

6.1 Access to Fragment C

6.1.1 Preparation of Compound 2

A solution of diethyl methylmalonate in anhydrous diethyl ether is slowly added (over about 30 min) to a suspension of sodium hydride (1.2 equiv.) in anhydrous diethyl ether (Figure 6.1). As addition proceeds, gas evolution is observed and solvent ebullition is reached. After complete addition of the reagent, the reaction mixture is allowed to cool slowly to room temperature. CHI_3 (1.0 equiv.) is added; then the solution is refluxed for 12 h. After workup and extraction, compound **1** is obtained as a yellow oil in 99% yield.

Question 6.4: Write a balanced chemical equation for the reaction of diethyl methylmalonate with sodium hydride. What is the gaseous species formed? Why is solvent ebullition reached?

Question 6.5: Justify the choice of an anhydrous solvent by giving at least two possible side reactions resulting from the presence of water.

Question 6.6: What is the common name for CHI_3?

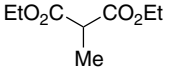
Question 6.7: Write the structure of **1** and explain its formation with a plausible mechanism.

To a solution of **1** in ethanol is added KOH (5 equiv.) and water; then the mixture is refluxed for 4 h. After acidification, treatment, and purification by silica gel chromatography, alkene (*E*)-**5**, exhibiting a broad infrared absorption band at 3079 cm^{-1} as well as a strong band at 1682 cm^{-1}, is obtained as a white solid in 72% yield. A plausible mechanism for the formation of **5** from **1** involves a first di-anionic intermediate **2**, which evolves by loss of a carbon dioxide molecule and elimination reaction into mono-anion **4**, furnishing **5** after acidic neutralization.

Question 6.8: Write the structure of **2**.

Question 6.9: Propose a structure for **5** and explain its formation from **2** with a plausible mechanism.

Question 6.10: Which valence vibration corresponds to the infrared absorption bands reported for compound **5**?



Diethyl methylmalonate

Figure 6.1 Structure of diethylmethylmalonate.

6.1.2 Preparation of Compound 7

A solution of **5** in tetrahydrofuran (THF) cooled at −15 °C is treated with a solution of BH_3·THF (1.5 equiv.) in THF and stirred for 2 h at room temperature.[1] Workup and purification by silica gel chromatography yield 60% of **6**. A solution of this compound in dichloromethane is then reacted with a large excess of manganese dioxide to afford, after filtration on a pad of celite, product **7** in quantitative yield.[2] ^{1}H-NMR signals observed for **6** and **7** are reported in the following table.

Compound	Spectrum description[a]
6	**^{1}H-NMR** (400 MHz, $CDCl_3$): 6.25 (s, 1H), 4.09 (d, 2H, J = 5.4 Hz), 3.65 (br t, 1H, J = 5.4 Hz)[b], 1.85 (s, 3H)
7	**^{1}H-NMR** (400 MHz, $CDCl_3$): 9.52 (s, 1H), 7.80 (q, 1H, J = 1.2 Hz), 1.92 (d, 3H, J = 1.2 Hz)

a) For **6**, the multiplicity of signals at 6.25 and 1.85 ppm was simplified, and the signal at 3.65 ppm was shifted as compared to the original description.
b) Signal disappears upon addition of D_2O.

Question 6.11: Suggest a structure for **6** and **7** based on the assignment of ^{1}H-NMR signals.

6.1.3 Preparation of Compound 12

A solution of ester **8** and triethylamine (1.2 equiv.) in dichloromethane cooled at −78 °C is treated with a solution of $(Cy)_2BOTf$ (1.2 equiv.) in pentane (Figure 6.2). Compound **8** contains two groups named "Group" whose structure is not detailed. After stirring for 5 h at −78 °C, a solution of compound **7** (2.3 equiv.) in dichloromethane is added slowly, and then the mixture is allowed to warm to room temperature. After treatment and purification by silica gel chromatography, compound **9** of molecular formula $C_{16}H_{20}INO_3(Group)_2$ is obtained as a single diastereoisomer in 76% yield. This compound is dissolved in dichloromethane and treated at 0 °C with 2,6-lutidine (2 equiv.) and TBSOTf (1.5 equiv.), leading to **10** after workup. Reaction of the crude product with a DIBAL-H solution (2.7 equiv.) in toluene at −78 °C, followed by treatment and purification by silica gel chromatography, affords compound **11**, which is directly engaged in the oxidation reaction using Dess–Martin periodinane (1.1 equiv.) in dichloromethane, leading to aldehyde **12**. Overall yield for these three steps is 80%. Selected data from infrared spectrum of compounds **9**, **10**, and **11** are reported in the following table.

1 In the original article, 1.2 equiv. BH_3·THF were added first, followed by another 0.25 equiv.
2 These conditions were adapted from another synthesis [3]; here, this step was more conveniently performed with the use of air oxidation catalyzed by copper and TEMPO.

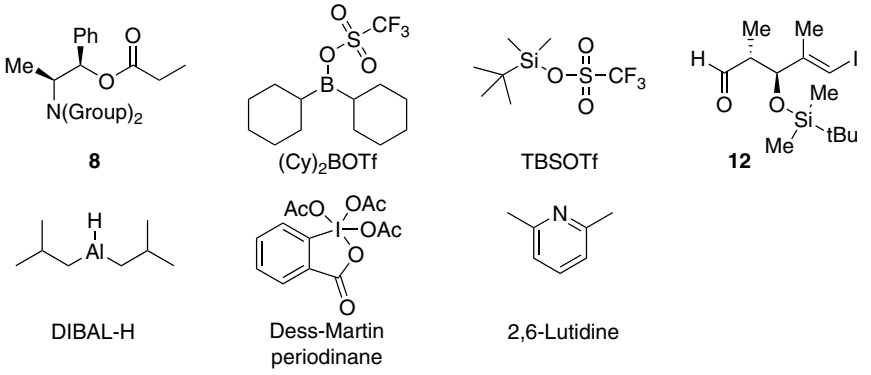


Figure 6.2 Reagents used for the synthesis of **12**.

Compound	Infrared spectrum description[a]
9	3496 cm^{-1} (br), 1741 cm^{-1} (strong)
10	No signal above 3100 cm^{-1}; 1743 (strong) cm^{-1}
11	3390 cm^{-1} (br); no band between 1615 cm^{-1} and 1850 cm^{-1}

a) "br" and "strong" are not mentioned in the original article.

Question 6.12: Write the structure of the two isomeric boron enolates that could be formed, in principle, upon reaction of **9**, triethylamine, and $(Cy)_2BOTf$.

Question 6.13: Assuming the reaction of the above-mentioned boron enolates with aldehyde **7** occurs through a cyclic six-membered chair-like transition state, write the structure of every possible transition state and the resulting product (obtained after workup).

Question 6.14: Based on the structure of **12** reported in Figure 6.2, suggest a structure for compound **9** and specify the absolute configuration of all stereogenic centers.

Question 6.15: From which transition states could compound **9** originate? Discuss their relative stability and deduce the configuration of boron enolate formed under the reaction conditions.

Question 6.16: Write the structure of **10** and **11**.

Question 6.17: Comment and interpret the infrared spectra reported for compounds **9**, **10**, and **11**.

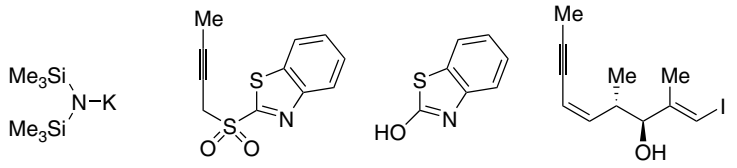


Figure 6.3 Structure of **C** and reagents used for its preparation.

6.1.4 Preparation of Fragment C

Construction of fragment **C** is based on Julia olefination. A solution of potassium hexamethyldisilazane (KHMDS) (2.3 equiv.) in THF (precooled at −78 °C) is added to a solution of sulfone **13** (2.5 equiv.) in THF at −55 °C, leading to intermediate **14** after 30 min (Figure 6.3). A solution of aldehyde **12** in THF (precooled at −78 °C) is added and the mixture is stirred for 13 h at −55 °C, yielding 56% of **18** after workup and purification by silica gel chromatography. This reaction leads to a first intermediate **15**, followed by a spirocycle intermediate **16**, which undergoes Smiles rearrangement to give sulfinate **17**. Loss of sulfur dioxide, followed by elimination, leads to **18** and **19**, which is transformed in **20** during workup. Silyl ether deprotection then leads to **C**.

Question 6.18: Explain the transformation of **13** into **18** with a mechanism, specifying the structure of **14**, **15**, **16**, **17**, and **19**.

Question 6.19: Suggest reaction conditions allowing transformation of **18** into **C**.

6.2 Access to Fragment D

6.2.1 Preparation of Compound 26

A solution of **21** in acetonitrile at −50 °C is slowly added to a solution of $Me_4NBH(OAc)_3$ (5 equiv.) in a mixture of acetonitrile and acetic acid to afford two diastereomeric diols **22** and **23** in a 92 : 8 ratio, respectively, and 98% overall yield (Figure 6.4). This reaction involves exchange of acetyloxy ligand of $Me_4NBH(OAc)_3$ by hydroxyl group of **21** and proceeds via a cyclic six-membered chair-like transition state. After some functional group interconversions,

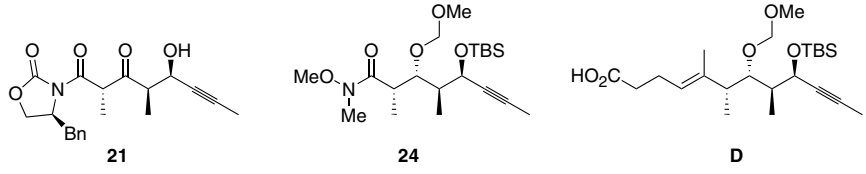


Figure 6.4 Structure of **21**, **24** and **D**.

24 is obtained. It is reacted with a solution of methylmagnesium chloride (3 equiv.) in diethyl ether at 0 °C for 2 h, followed by quenching with brine and extraction, to afford methyl ketone **25** in 97% yield.

Question 6.20: Calculate the diastereomeric excess observed in the reduction of **21**.

Question 6.21: Give the structure of **22** and **23**.

Question 6.22: Suggest a transition state model accounting for the diastereoselectivity of the reaction and the role of acetic acid.

Question 6.23: How do you name compounds such as **24**?

Question 6.24: Explain the transformation of **24** into **25** by providing a plausible mechanism. Explain why, in spite of the use of excess methylmagnesium chloride, only one methyl group is incorporated into the product.

6.2.2 Preparation of Fragment D

Addition of vinylmagnesium bromide (2 equiv.) to a solution of **25** in THF at −78 °C affords, after workup and purification, alcohol **26** as an inseparable mixture of two diastereoisomers. The latter is dissolved in diethyl ether and reacts with PBr_3 (2.4 equiv.) in the presence of pyridine (3 equiv.) to yield 83% (for two steps) of allyl bromide **27** after workup. ^{1}H-NMR analysis of this compound reveals the presence of a single ethylenic hydrogen atom. Reaction of **27** in THF at −60 °C with the lithiated enolate of ethyl acetate (19 equiv.) previously treated with CuI (36 equiv.), followed by workup, purification, ester saponification, and acidification, affords fragment **D** (Figure 6.4) in 62% yield for two steps.

Question 6.25: Write the structure of **26**.

Question 6.26: Write the structure of **27** and suggest a plausible mechanism accounting for its formation.

Question 6.27: Suggest plausible reaction conditions for the formation of the lithiated enolate of ethyl acetate.

Question 6.28: What is the role of CuI in the reaction involving transformation of **27** into **D**?

6.3 Final Steps

6.3.1 Assembly of B and Formation of A by Ring-Closing Alkyne Metathesis

A solution of alcohol **C** in dichloromethane at 0 °C is treated with EDC·HCl, and then a solution of acid **D** in dichloromethane is added (Scheme 6.1).[3] After stirring for 5 h at room temperature, workup, and purification by silica gel chromatography, ester **B** is obtained in 89% yield. A solution of **B** in dry toluene is then added to a solution of complex **Mo** (0.4 equiv., Figure 6.5) in toluene, and then the mixture is heated at 100 °C for 19 h, leading after treatment and purification by silica gel chromatography, to macrocycle **A** (Scheme 6.1) as a yellowish oil in 72% yield.[4] The mechanism involved in ring-closing alkyne metathesis reaction shares many similarities with that of alkene metathesis: here, pre-catalyst **Mo** is converted into catalytically active species **Mo2**.

Question 6.29: Write the mechanism of formation of **B** from **C** and **D**.

Question 6.30: What is the main advantage of using EDC·HCl instead of dicyclohexylcarbodiimide (DCC)?

Question 6.31: Suggest an experimental procedure for the workup of crude **B**.

Question 6.32: Using a simplified representation of catalyst **Mo2** and di-yne **B**, suggest a plausible catalytic cycle for macrocyclisation leading to **A**.

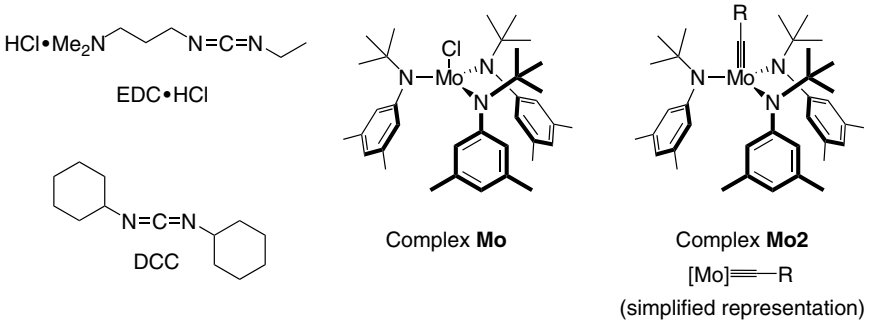
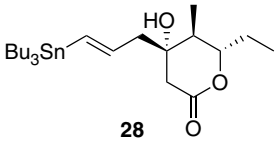


Figure 6.5 Structure of EDC•HCl, DCC, complex **Mo** and **Mo2**.

3 This reaction was originally performed in the presence of a stoichiometric amount of DMAP.
4 In the original article, a stock solution containing **Mo** as a major product is prepared from a molybdenum precursor. The reaction is driven to completion upon addition of a second aliquot of catalyst solution.



28

Figure 6.6 Structure of **28**.

6.3.2 Coupling of Sugar and Macrocycle

The final steps involve a palladium-catalyzed coupling between vinyl iodide **A** and stannylated derivative **28** (1.2 equiv.), in the presence of $Pd(PPh_3)_4$ (0.05 equiv.) in DMF at 50 °C (Figure 6.6).[5] Compound **29**, obtained in 78% yield after treatment and purification by silica gel chromatography, undergoes additional functional group transformations leading to the desired leiodermatolide.

Question 6.33: Write the structure of **29**.

Question 6.34: Name this Pd-catalyzed cross-coupling between **A** and **28**, and write its mechanism mentioning the number of electrons and the oxidation state of the metallic species involved.

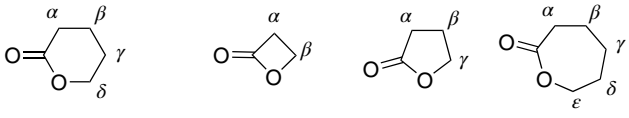
Answers

Question 6.1:

A macrolide is a biologically active compound, containing a macrocyclic lactone, one or several sugar moieties, and often a conjugated polyene chromophore [4].

Question 6.2:

A δ-lactone is a cyclic six-membered ester.



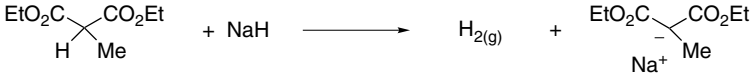
δ-Lactone β-Lactone γ-Lactone ε-Lactone

Question 6.3:

Both parts of the molecule (macrocycle and δ-lactone moieties) are quasi-independent because they are separated by a pentadienyl spacer, resulting in a weak correlation between stereoclusters.

5 Conditions reported in the original article involve additional use of tetra-*n*-butylammonium diphenylphosphinate and copper-thiophene carboxylate complex.

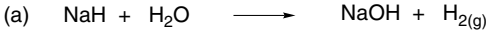
Question 6.4:

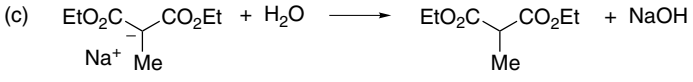


The gaseous species is H_2. Diethyl methylmalonate deprotonation is exothermic, resulting in solvent ebullition.

Question 6.5:

NaH is a strong base that can react with water (a), hydroxide ion formation could result in saponification (b), and water could also neutralize malonate carbanion (c).

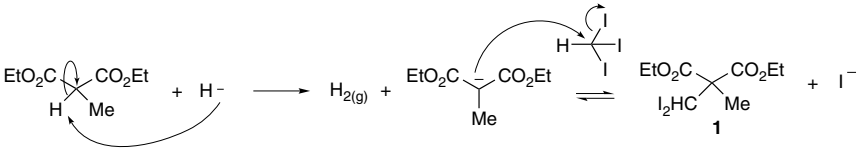




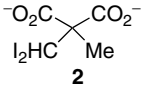
Question 6.6:

CHI_3 = Iodoform

Question 6.7:



Question 6.8:



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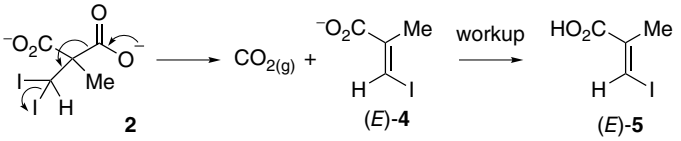
Question 6.9:

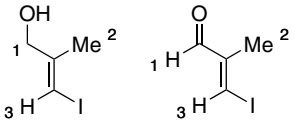


2 (*E*)-**4** (*E*)-**5**

Question 6.10:

3079 cm^{-1}: O—H stretching (carboxylic acid)
1682 cm^{-1}: C═O stretching (carboxylic acid)

Question 6.11:



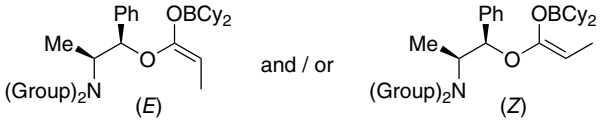
6 **7**

Compound	Spectrum description
6	**^{1}H-NMR** (400 MHz, $CDCl_3$): 6.25 (s, 1H, **H_3**), 4.09 (d, 2H, **$^3J_{1,OH}$ = 5.4 Hz, H_1**), 3.65 (br t, 1H, **$^3J_{OH,1}$ = 5.4 Hz, OH**)[a], 1.85 (s, 3H, **H_2**)
7	**^{1}H-NMR** (400 MHz, $CDCl_3$): 9.52 (s, 1H, **H_1**), 7.80 (q, 1H, **$^4J_{3,2}$ = 1.2 Hz**), 1.92 (d, 3H, **$^4J_{2,3}$ = 1.2 Hz**)

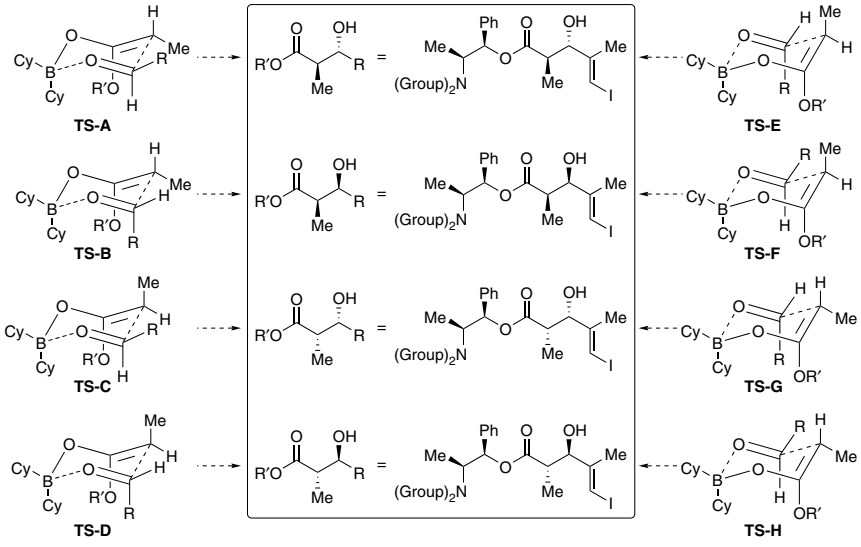
a) Signal disappears upon addition of D_2O.

Question 6.12:

In principle, the two following (*Z*) and (*E*)[6] boron enolates could be formed [5, 6].

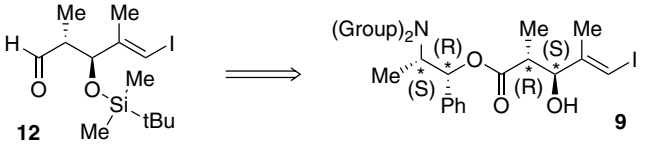


Question 6.13:



6 The stereochemical descriptors for boron enolates are defined on the assignment of highest priority to the OBR_2 group, as in Ref. [5].

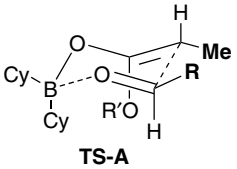
Question 6.14:



Question 6.15:

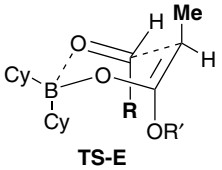
Compound **9** could, in principle, originate from the two transition states **TS-A** and **TS-E**. However, **TS-A** (R and Me in pseudo-equatorial position) is presumably the only transition state involved because it is strongly favored over **TS-E** (R and Me in pseudo-axial position).

This result suggests that (*E*)-boron enolate is formed under reaction conditions.



TS-A

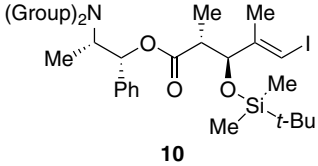
favored
(R and Me: pseudo-equatorial)



TS-E

unfavored
(R and Me: pseudo-axial)

Question 6.16:



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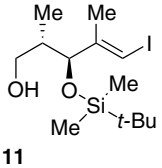

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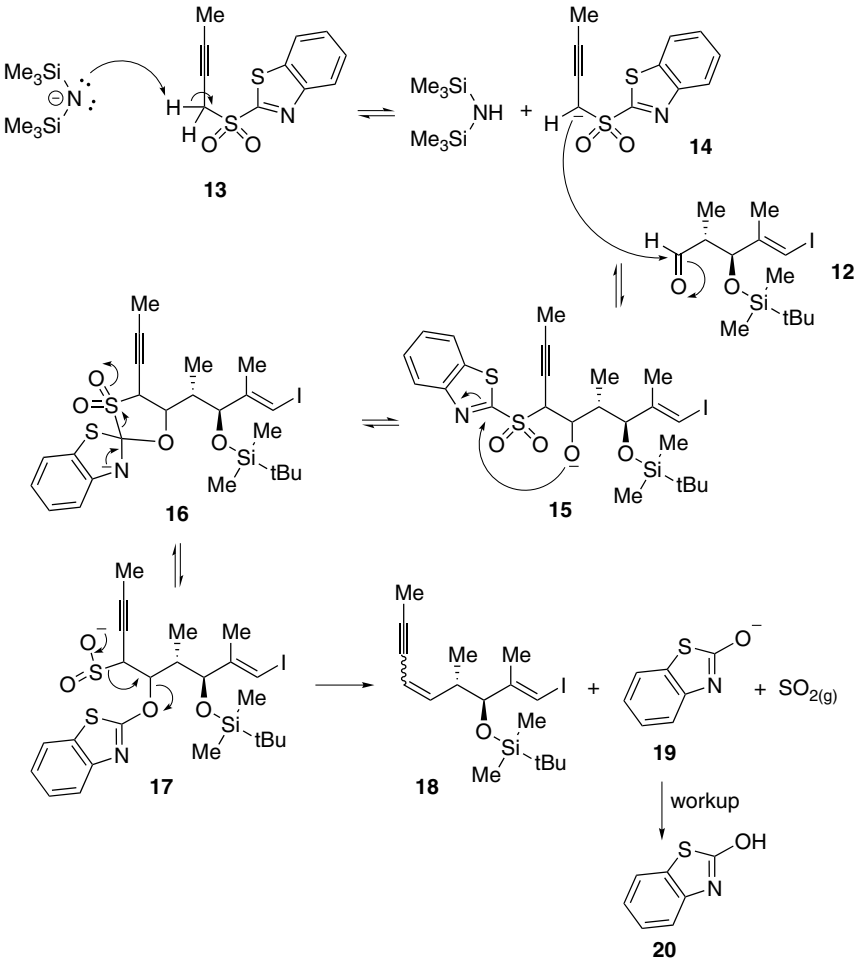
Question 6.17:

Compound	Infrared spectrum description
9	3496 cm^{-1} (broad band): **O—H stretching** 1741 cm^{-1} (strong): **C=O stretching (ester)**
10	No signal above 3100 cm^{-1}: **because OH is protected** 1743 cm^{-1} (strong): **C=O stretching (ester)**
11	3390 cm^{-1} (broad band): **O—H stretching** No band between 1615 and 1850 cm^{-1}: **because ester is reduced**

Question 6.18



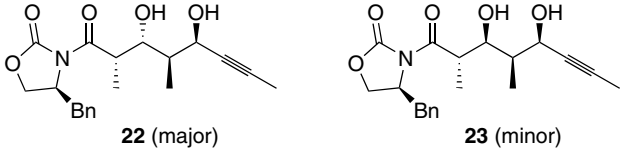
Question 6.19:

n-Bu_4NF, THF.

Question 6.20:

Diastereomeric excess: de = %(major dia) – %(minor dia) = 92 – 8 = 84%

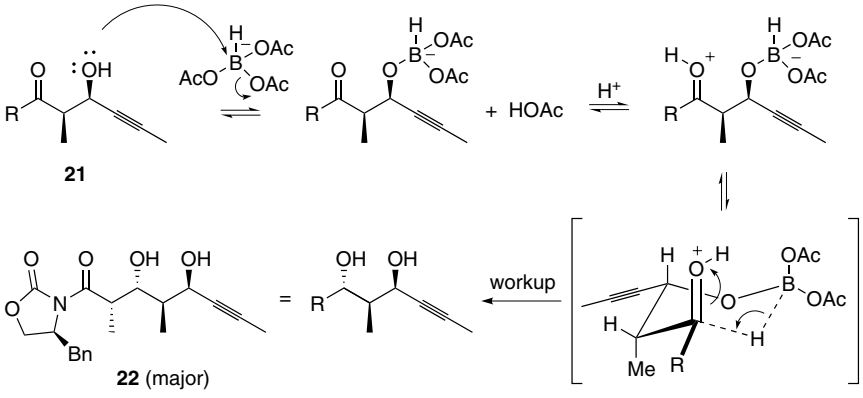
Question 6.21:



22 (major) **23** (minor)

Question 6.22:

Acetic acid allows electrophilic activation of C=O double bond.

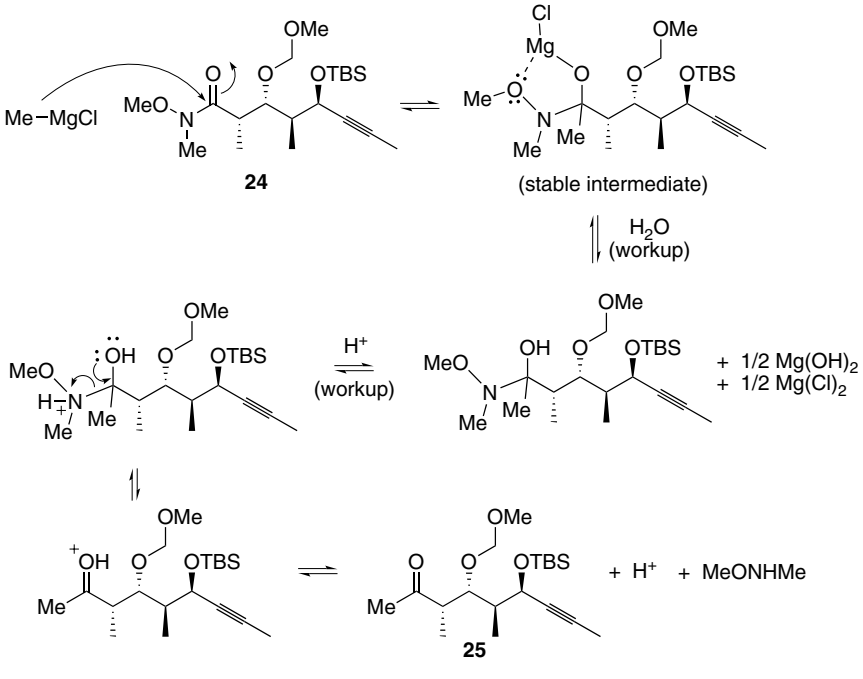


Question 6.23

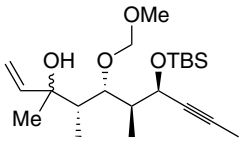
Weinreb amides.

Question 6.24:

Weinreb amides lead to stable metallic intermediates, thus preventing addition of a second nucleophile.



Question 6.25:



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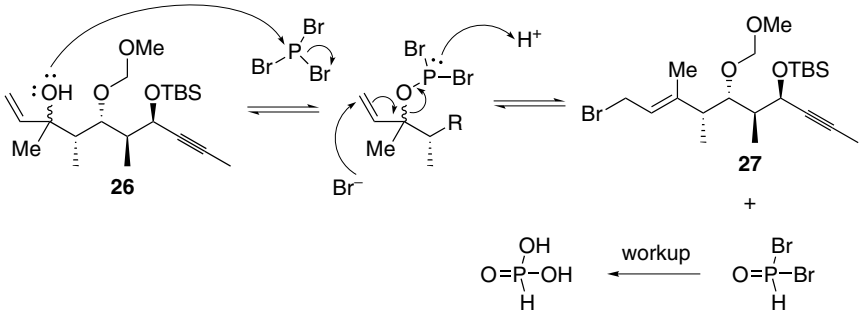
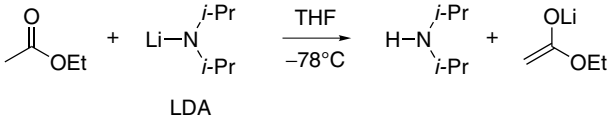
Question 6.26:



26

Question 6.27:



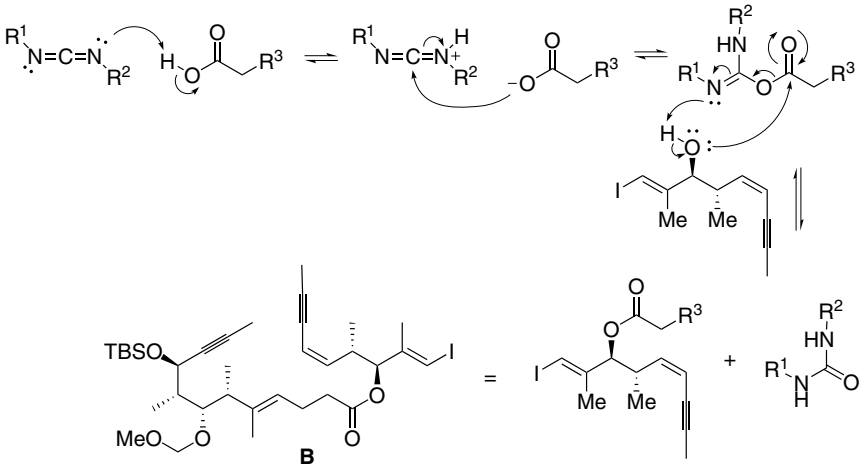
LDA

Question 6.28:

CuI leads to the formation of an organocopper derivative, which is less basic than its corresponding precursor and is thus more tolerant toward diverse functional groups.

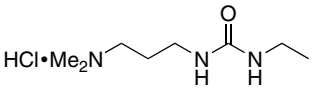
Question 6.29:

For a review on amide bond formation, see Ref. [7].

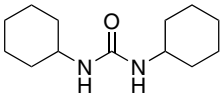


Question 6.30:

The urea by-product originating from the reaction with EDC is water soluble and can thus easily be extracted from the reaction mixture. This is not the case with DCC, which requires purification of the product. Moreover, EDC is often exploited as a water-soluble coupling agent.



Derived from EDC•HCl



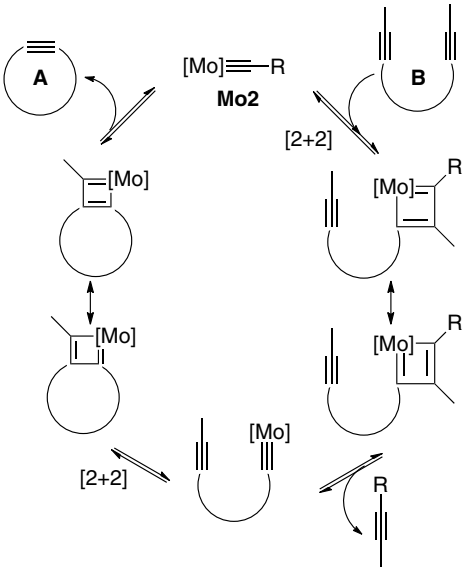
Derived from DCC

Question 6.31:

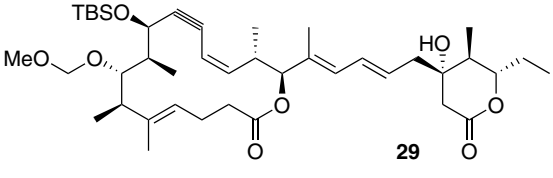
When the reaction is complete, water and dichloromethane are added and the layers are separated. The organic layer is washed with water (three times), dried with $MgSO_4$, filtered, and evaporated under reduce pressure.

Question 6.32:

For a review on alkyne metathesis, see Ref. [8].



Question 6.33:



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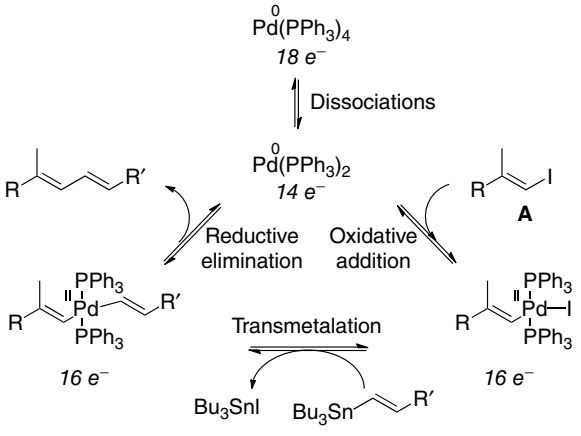
Question 6.34:

Stille coupling:



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7

Azobenzene-Thiourea Catalysts for the Control of Chemical Reactivity with Light

In 2014, a series of azobenzene-thiourea derivatives of type **I** and **II** were reported as potential catalysts whose reactivity could be controlled by light (Figure 7.1) [1].

7.1 Synthesis of Azobenzene-Thiourea Derivatives

The first steps of the synthetic route to azobenzene-thiourea **I** are described in Scheme 7.1. A solution of ethyl 2-aminobenzoate **1** in CH_2Cl_2 is treated with an aqueous solution of oxone (whose composition is $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$, 2 equiv.). The mixture is vigorously stirred for 20 h, and then the combined organic layers are washed with aq. HCl (1M) followed by aq. saturated $NaHCO_3$, water, and aq. saturated NaCl solution. Drying over $MgSO_4$ followed by filtration and evaporation under reduced pressure affords compound **2** (molecular formula $C_9H_9NO_3$) as a green solid. This material is dissolved in AcOH and treated with aniline **3**, obtained by reaction of 1,2-diaminobenzene with $(Boc)_2O$ (1 equiv.) in MeOH for 24 h. After stirring at rt overnight, the mixture is poured into aq. saturated $NaHCO_3$ solution, resulting in extensive bubbling (**point 1**),[1] and extracted three times with EtOAc. The combined organic layers are washed with aq. $NaHCO_3$, water, and aq. saturated NaCl solution; dried over $MgSO_4$; and evaporated to give a residue which is purified by silica gel chromatography to yield **4** as an orange oil. This compound is dissolved in CH_2Cl_2 and treated with CF_3CO_2H (32 equiv.) for 5 h. After appropriate workup, crude **5** is obtained with satisfactory purity.

Question 7.1: Explain why the reaction mixture should be stirred vigorously during the formation of **2** from **1**.

Question 7.2: Write the structure of compound **2**.

Question 7.3: Write the mechanism of formation of **3** from 1,2-diaminobenzene and $(Boc)_2O$.

1 Such bubbling is not mentioned in the original article.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

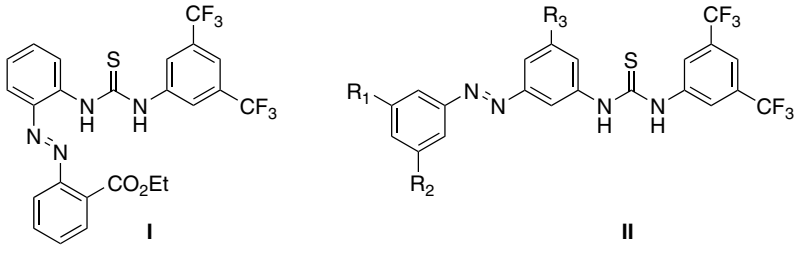
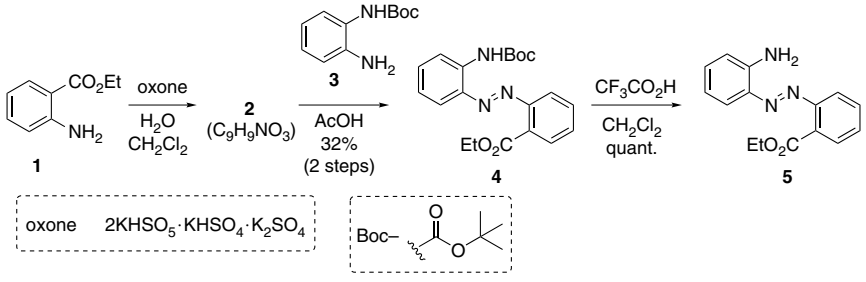


Figure 7.1 Structure of azobenzene-thiourea derivatives **I** and **II** investigated as potential catalysts.



Scheme 7.1

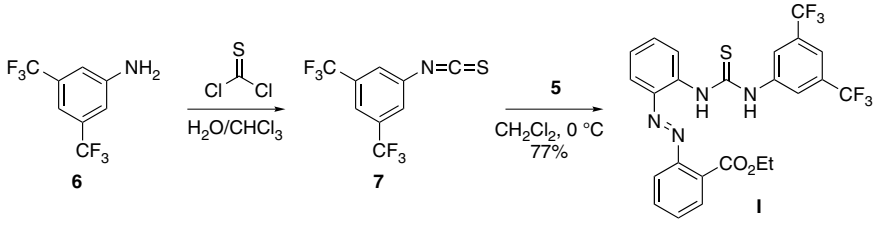
Question 7.4: Suggest a plausible mechanism for the formation of **4** from **2** and **3**.

Question 7.5: Explain the origin of bubbling observed at **point 1**.

Question 7.6: Write the mechanism of formation of **5** from **4**.

Question 7.7: Suggest plausible workup conditions for isolation of crude **5** following reaction with CF_3CO_2H.

A suspension of thiophosgene in water at 15 °C is treated with a solution of aniline **6** in $CHCl_3$ (Scheme 7.2) [2]. After stirring for 4 h, the mixture is poured into aq. HCl (10%) and extracted four times with CH_2Cl_2. The combined organic layers are dried over Na_2SO_4 and filtered, and the solvent is removed under reduced



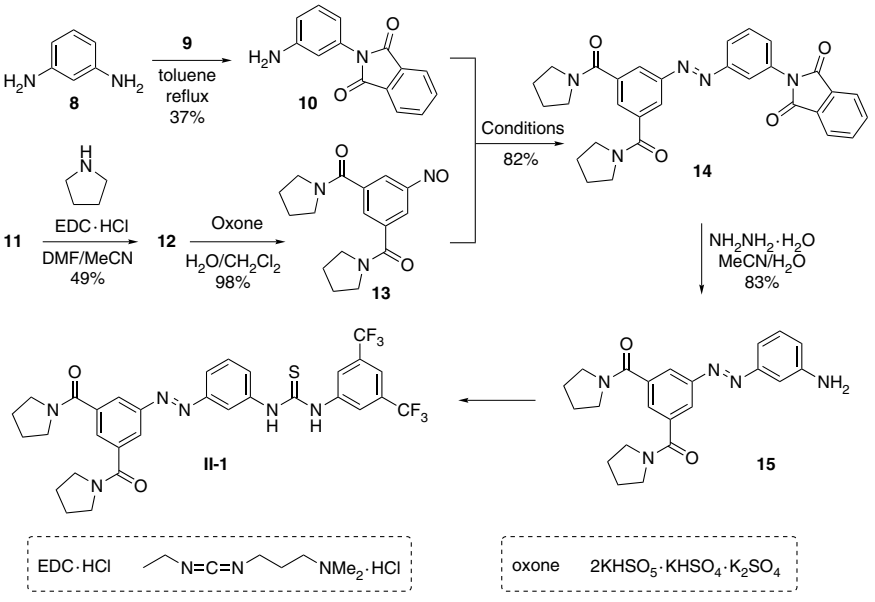
Scheme 7.2

pressure to afford isothiocyanate **7** in 47% yield. The latter is slowly added to a solution of **5** (prepared in Scheme 7.1) in CH_2Cl_2 at 0 °C; the mixture is stirred for 6 h and the solvent is removed under reduced pressure to yield a solid. Recrystallization from EtOAc and hexane affords **I** as orange crystals in 77% yield.

Question 7.8: Suggest a plausible mechanism for the formation of **7** from **6**.

Question 7.9: Suggest a plausible mechanism for the formation of **I** from **5** and **7**.

Question 7.10: Suggest an experimental procedure for recrystallization of **I**.



Scheme 7.3

The synthesis of azobenzene thiourea catalysts **II-1** is described in Scheme 7.3. A solution of diamine **8** in toluene is treated with **9** and the mixture is refluxed for 8 h under Dean–Stark conditions, affording after appropriate workup phthalimide **10** in 37 % yield. In parallel, to a solution of **11** in a mixture of dimethylformamide (DMF) and MeCN is added pyrrolidine (2 equiv.), followed by EDC·HCl (2.2 equiv.). After stirring for 16 h at rt, the solvent is evaporated under reduced pressure, and the crude residue (**point 1**) is purified by chromatography on silica gel (using CH_2Cl_2/MeOH 9/1 + 2.5% Et_3N as the eluent), to give **12** in 49% yield. The latter is transformed into nitroso **13** upon reaction with oxone in a biphasic mixture of H_2O and CH_2Cl_2, and reacted with aniline **10** to afford azobenzene **14** (procedures not detailed). This compound is dissolved in MeCN; water and

hydrazine monohydrate (10 equiv.) are added and the mixture is stirred for 30 min at rt (**point 2**). After addition of aq. NaOH, stirring is maintained for 2 h (**point 3**); then the mixture is diluted with water and extracted three times with EtOAc. The combined organic phases are dried over $MgSO_4$ and filtered and the solvent is evaporated under reduced pressure to give **15** in 83% yield. This compound is converted in one step into the desired azobenzene-thiourea derivative **II-1**.

Question 7.11: Write the structure of **9**.

Question 7.12: Which by-product is likely to be formed upon reaction of **8** with **9**, thereby accounting for the low yield observed for **10**?

Question 7.13: Write the structure of **11**.

Question 7.14: Write the structure of **12**.

Question 7.15: What is the composition of the residue obtained at **point 1**?

Question 7.16: Comment on the workup procedure used during transformation of **11** into **12**.

Question 7.17: What is the role of Et_3N added in the eluent used for column chromatography?

Question 7.18: Suggest plausible reaction conditions for the formation of **14** from **10** and **13**.

Question 7.19: Suggest a plausible mechanism for the formation of **15** from **14**, and specify the structure of intermediates obtained at **point 2** and **point 3**.

Question 7.20: Suggest plausible reaction conditions for the formation of **II-1** from **15**.

Using synthetic routes similar to those described in Scheme 7.3, azobenzene derivatives **16–19**, precursors to various azobenzene-thiourea catalysts of type **II**, were synthesized (Figure 7.2). The ^{1}H-NMR spectra of these compounds, as well as those of previously synthesized **4**, **5**, and **15** are reported in Figure 7.2.

Spectrum	Description
A	**^{1}H-NMR** (500 MHz, $CDCl_3$): 7.92 (ddd, J = 7.8, 1.5, 0.5 Hz, 1H), 7.89 (ddd, J = 8.0, 1.6, 0.4 Hz, 1H), 7.83 (ddd, J = 8.2, 1.3, 0.5 Hz, 1H), 7.59 (ddd, J = 8.1, 7.3, 1.5 Hz, 1H), 7.44 (ddd, J = 7.8, 7.3, 1.3 Hz, 1H), 7.20 (ddd, J = 8.2, 7.0, 1.6 Hz, 1H), 6.82 (ddd, J = 8.2, 7.0, 1.3 Hz, 1H), 6.74 (ddd, J = 8.3, 1.4, 0.4 Hz, 1H), 6.59 (br s, 2H), 4.38 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H)
B	**^{1}H-NMR** (400 MHz, $CDCl_3$): 8.09 (d, J = 1.6 Hz, 2H), 7.78 (t, J = 1.6 Hz, 1H), 7.35 (dt, J = 7.8, 1.6 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.21 (t, J = 1.6 Hz, 1H), 6.83 (ddd, J = 7.7, 2.4, 1.1 Hz, 1H), 3.90 (br s, 2H), 3.68 (t, J = 7.0 Hz, 4H), 3.49 (t, J = 6.6 Hz, 4H), 2.04–1.86 (m, 8H)
C	**^{1}H-NMR** (400 MHz, $CDCl_3$): 9.28 (s, 1H), 8.43 (dd, J = 8.5, 1.3 Hz, 1H), 7.87 – 7.83 (m, 1H), 7.82 (dd, J = 8.1, 1.6 Hz, 1H), 7.65 – 7.58 (m, 2H), 7.50 (ddd, J = 7.7, 6.0, 2.6 Hz, 1H), 7.46 (ddd, J = 8.7, 7.2, 1.7 Hz, 1H), 7.09 (ddd, J = 8.3, 7.2, 1.3 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.55 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H)
D	**^{1}H-NMR** (400 MHz, $CDCl_3$): 8.78 (t, J = 1.6 Hz, 1H), 8.70 (d, J = 1.6 Hz, 2H), 7.42 (dq, J = 7.8, 1.2 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.28 – 7.26 (m, 1H), 6.85 (ddd, J = 7.8, 2.4, 1.0 Hz, 1H), 4.46 (q, J = 7.0 Hz, 4H), 3.87 (br s, 2H), 1.45 (t, J = 7.0 Hz, 6H)
E	**^{1}H-NMR** (400 MHz, $CDCl_3$): 7.74–7.68 (m, 2H), 7.40 (t, J = 7.7 Hz, 1H), 7.35 (dt, J = 7.9, 1.3 Hz, 1H), 7.33–7.27 (m, 2H), 7.21 (t, J = 2.1 Hz, 1H), 6.80 (ddd, J = 7.6, 2.4, 1.2 Hz, 1H), 3.75 (br s, 2H), 2.46 (s, 3H)
F	**^{1}H-NMR** (300 MHz, $CDCl_3$): 8.04 (t, J = 1.9 Hz, 1H), 8.02–7.95 (m, 3H), 7.82 (dd, J = 5.5, 3.1 Hz, 2H), 7.77–7.71 (m, 2H), 7.67 (t, J = 7.9 Hz, 1H), 7.58 (ddd, J = 7.9, 2.1, 1.2 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.34–7.28 (m, 1H), 2.46 (s, 3H)
G	**^{1}H-NMR** (400 MHz, $CDCl_3$): 8.72 (t, J = 2.0 Hz, 1H), 8.32 (dd, J = 8.0, 2.0 Hz, 1H), 8.23 (dd, J = 8.0, 2.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.41 (dd, J = 7.8, 2.3 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.26 (t, J = 2.3 Hz, 1H), 6.86 (dd, J = 7.8, 2.3 Hz, 1H), 3.87 (s, 2H)

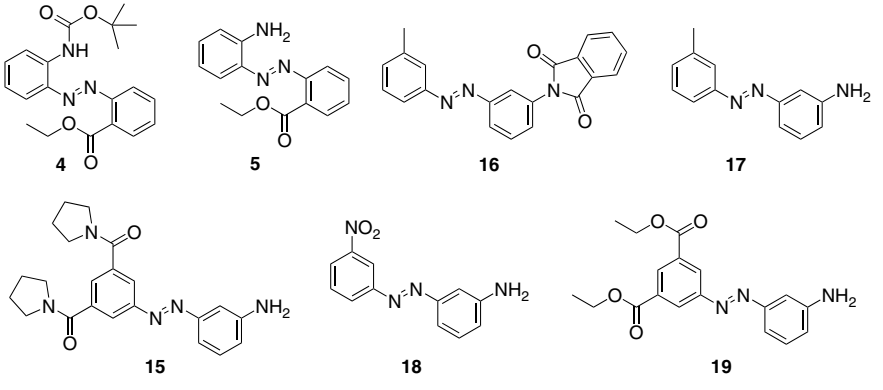
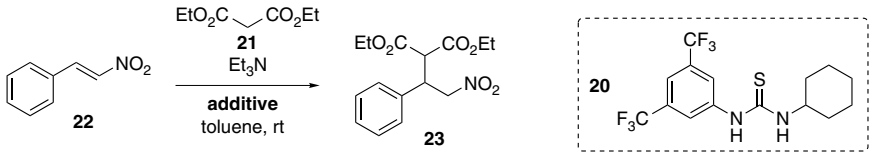


Figure 7.2 Structure of azobenzene derivatives precursors to **I** and **II**.



Scheme 7.4

Question 7.21: Assign *characteristic* signals for each compound of Figure 7.2 and identify the corresponding spectrum (**A**–**G**).

7.2 Investigation of Catalytic Properties

Thioureas such as **20** are well known to catalyze Michael addition of diethyl malonate **21** on nitrostyrene **22** leading to **23** (Scheme 7.4) [3]. The results obtained under two sets of reaction conditions are reported in the following table.

Entry	Additive	Time (h)	Percentage of yield
1	none	24	17
2	**20** (10 mol%)	24	57

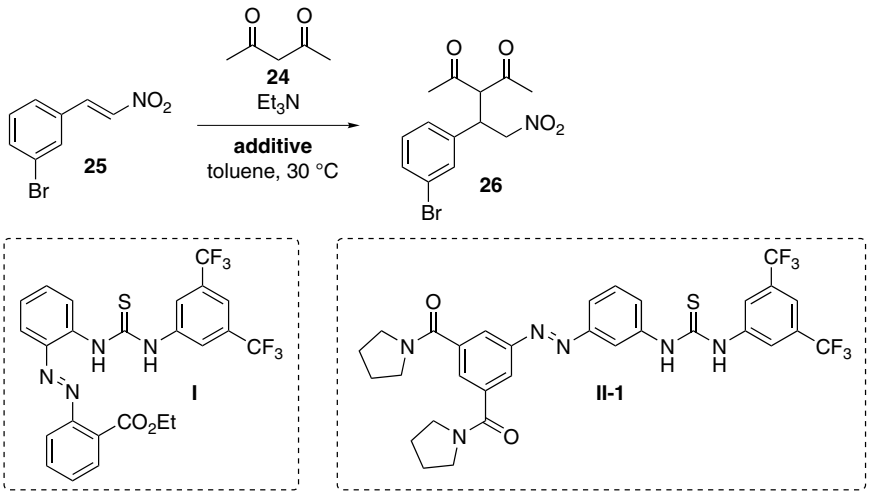
Question 7.22: Comment on the results reported for the reaction between **21** and **22**, under different reaction conditions.

Question 7.23: Write a plausible mechanism for the reaction performed under the conditions reported in entry 1 (without any additive).

Question 7.24: Which reagent (**21** or **22**) is more likely to interact with catalyst **20**? Justify your answer by drawing a schematic representation of the complex obtained, and mention the influence of such an interaction on reactivity.

A similar reaction was performed using acetylacetone **24** and 3-bromostyrene **25** in the presence of azobenzene-thiourea catalysts **I** or **II-1**, leading to **26** as reported in Scheme 7.5 and in the following table. The crystal structure of catalysts **I** and **II-1**, as determined by X-ray diffraction, is shown in Figure 7.3.[2]

2 Crystallographic data for **I** (CCDC 976348) and **II-1** (CCDC 976349) are available free of charge from The Cambridge Crystallographic Data Centre.



Scheme 7.5

Entry	Additive	Time (h)	Percentage of conversion
1	None	18	11
2	**I** (2 mol%)	18	11
3	**II-1** (2 mol%)	18	58

Question 7.25: Comment on the results obtained for the reaction of **24** and **25** under various conditions.

Question 7.26: Explain the difference of catalytic activity observed between **I** and **II-1**, on the basis of their structure reported in Figure 7.3.

Catalysts **II-2** and **II-3** were selected for further investigations (Scheme 7.6). Like the majority of azobenzene derivatives, these compounds undergo photoisomerization about the N=N bond upon irradiation at 365 nm, leading to ***Z*-II-2** and ***Z*-II-3**, respectively. The results obtained for the Michael addition of **24** to **25**, leading to **26**, are summarized in the following table.

Entry	Additive	Irradiation	Time (h)	Percentage of conversion
1	None	None	18	11
2	**II-2** (2 mol%)	None	18	96
3	**II-2** (2 mol%)	365 nm	18	21
4	**II-3** (2 mol%)	None	18	64
5	**II-3** (2 mol%)	365 nm	18	43

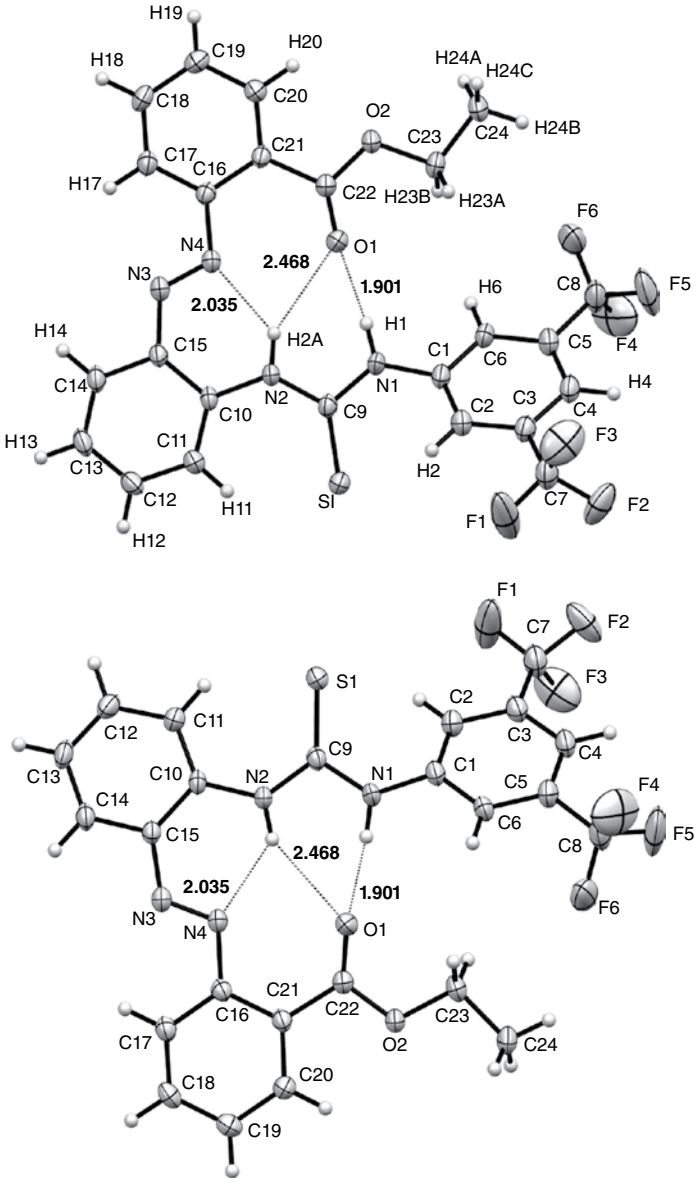
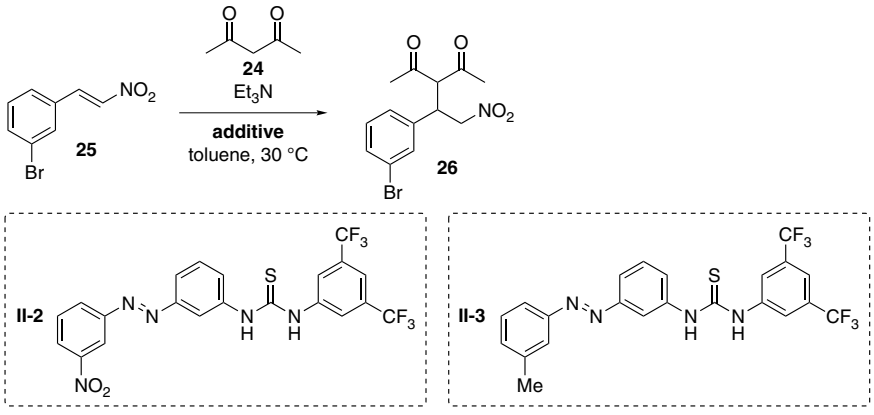


Figure 7.3 Crystal structure of azobenzene-thiourea **I** (top) and **II-1** (bottom).

Question 7.27: Write the structure of ***Z*-II-2** and ***Z*-II-3**.

Question 7.28: Comment on the results obtained for the reaction of **24** with **25** under various conditions, and suggest plausible explanations accounting for experimental observations.

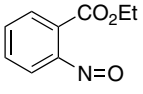


Scheme 7.6

Answers

Question 7.1:
The reaction mixture is biphasic (water/CH_2Cl_2), with oxone mostly present in the aqueous phase and compound **1** in the organic phase. Therefore, vigorous stirring must be applied to allow contact between reagents.

Question 7.2:



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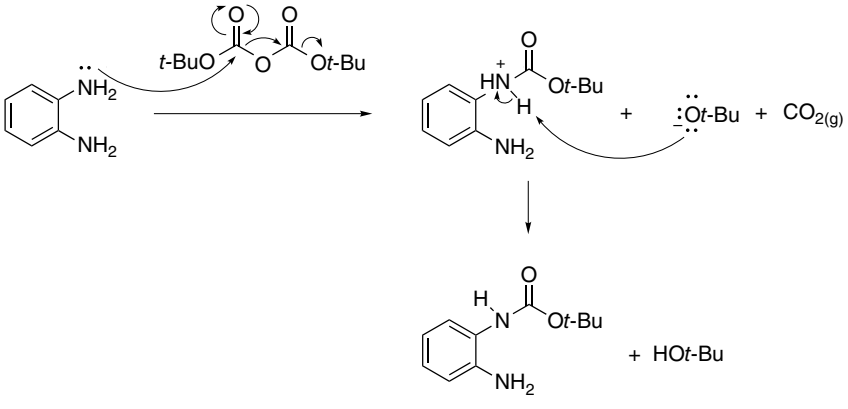
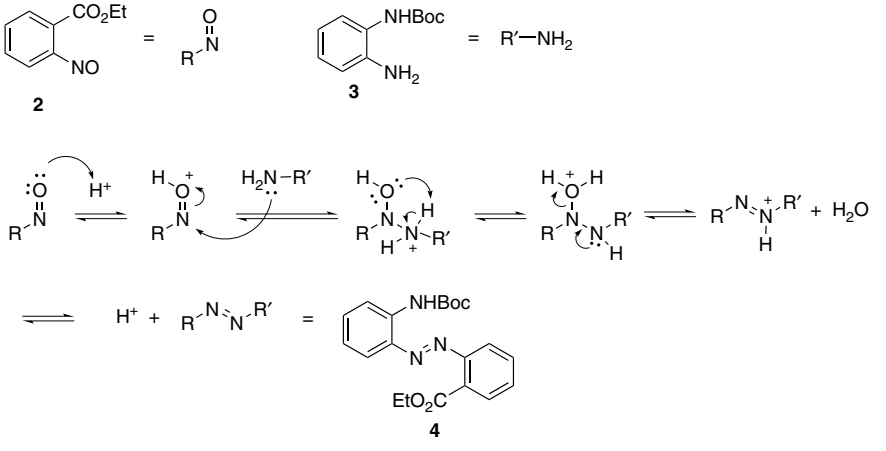
Question 7.3:



Question 7.4:

This transformation is known as the Mills reaction [4].



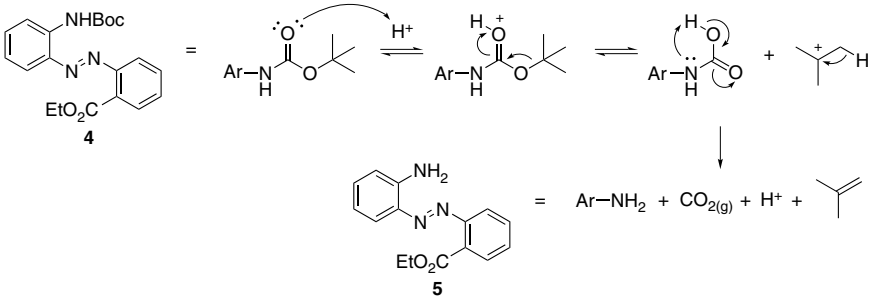
Question 7.5:

The bubbling observed at **point 1** originates from the formation of CO_2 upon neutralization of AcOH with $NaHCO_3$.

$$CH_3CO_2H + HCO_3^- \, Na^+ \rightarrow CH_3CO_2^- \, Na^+ + H_2CO_3$$

$$H_2CO_3 \rightarrow H_2O + CO_2(g)$$

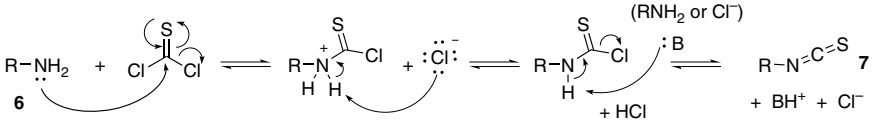
Question 7.6:



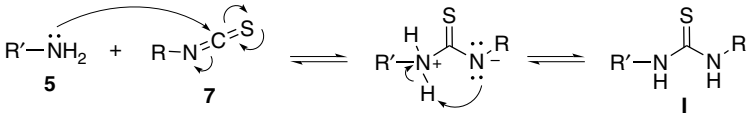
Question 7.7:

When the reaction is completed, the mixture is poured into the aq. $NaHCO_3$ solution. The layers are separated and the aqueous phase is extracted three times with CH_2Cl_2. The combined organic layers are dried over $MgSO_4$, and the solvent is evaporated under reduced pressure, to yield crude **5**.

Question 7.8:



Question 7.9:

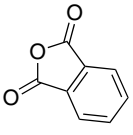


Question 7.10:

Suggest an experimental procedure for recrystallization of **I**.

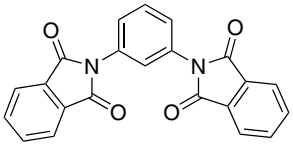
Crude **I** is dissolved in the minimum amount of EtOAc, and hexane is slowly added until the solution becomes cloudy. The mixture is left standing at low temperature until crystallization of **I**.

Question 7.11:

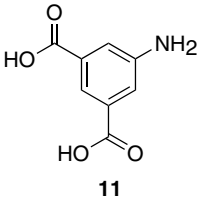


9
Phtalic anhydride

Question 7.12:

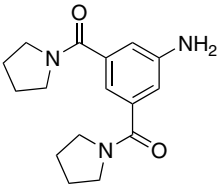


Question 7.13:



11

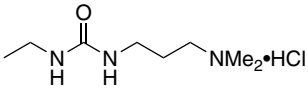
Question 7.14:



12

Question 7.15:

The residue obtained at **point 1** is mainly composed of **12** and urea derived from EDC·HCl (see subsequent text), together with unreacted EDC·HCl (in excess) and, possibly, traces of unreacted **11** and pyrrolidine.



Urea derived from EDC•HCl

Question 7.16:

Common workup procedure performed after EDC·HCl-mediated amide bond formation usually involves partitioning of urea-derived by-product and desired amide between water and organic solvent, respectively. Here, since the solvent used (mixture of DMF and MeCN) is miscible with water, evaporation under reduced pressure is performed and the crude residue is purified by column chromatography on silica gel.

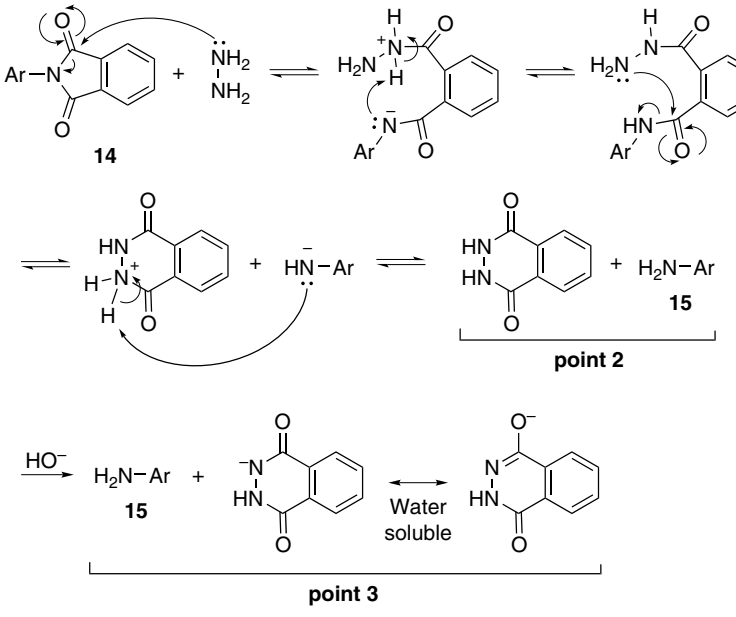
Question 7.17:

Because of their basic character, amines are often partially retained by the stationary phase upon chromatography on silica, which is slightly acidic. Addition of Et_3N to the eluent allows neutralization of silica and thus facilitates purification of amines.

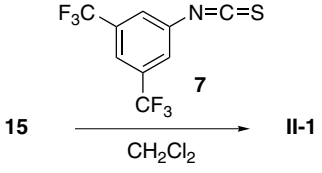
Question 7.18:

Compounds **3** and **9** are dissolved in AcOH (playing the role of both solvent and reagent), and the mixture is stirred at rt until completion of the reaction.

Question 7.19:



Question 7.20:



Question 7.21:

The most characteristic signals for **4**, **5**, and **15–19** are highlighted in bold in the following table.

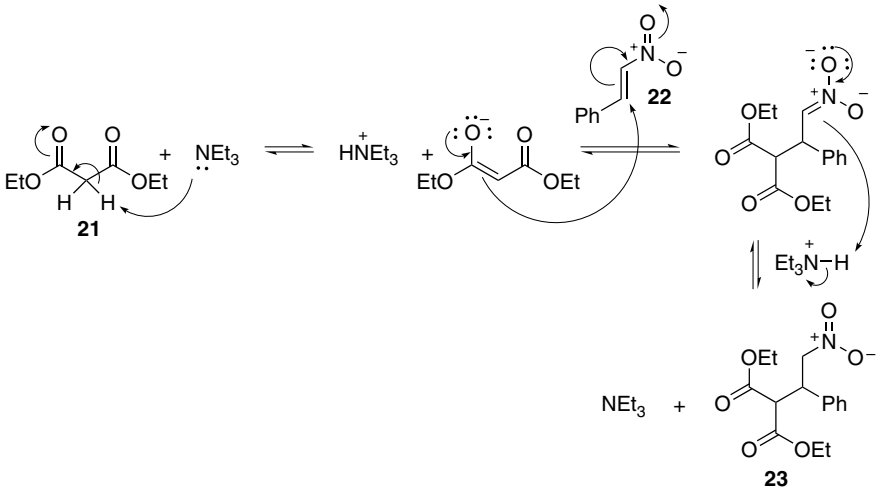
Spectrum	Description
A = 5	**^{1}H-NMR** (500 MHz, $CDCl_3$): δ 7.92 (ddd, *J* = 7.8, 1.5, 0.5 Hz, 1H), 7.89 (ddd, *J* = 8.0, 1.6, 0.4 Hz, 1H), 7.83 (ddd, *J* = 8.2, 1.3, 0.5 Hz, 1H), 7.59 (ddd, *J* = 8.1, 7.3, 1.5 Hz, 1H), 7.44 (ddd, *J* = 7.8, 7.3, 1.3 Hz, 1H), 7.20 (ddd, *J* = 8.2, 7.0, 1.6 Hz, 1H), 6.82 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 6.74 (ddd, *J* = 8.3, 1.4, 0.4 Hz, 1H), **6.59 (br s, 2H) –NH_2, 4.38 (q, *J* = 7.1 Hz, 2H) –OCH_2–, 1.37 (t, *J* = 7.1 Hz, 3H) –CH_3**
B = 15	**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 1.6 Hz, 2H), 7.78 (t, *J* = 1.6 Hz, 1H), 7.35 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 7.21 (t, *J* = 1.6 Hz, 1H), 6.83 (ddd, *J* = 7.7, 2.4, 1.1 Hz, 1H), **3.90 (br s, 2H) –NH_2, 3.68 (t, *J* = 7.0 Hz, 4H) –CH_2N, 3.49 (t, *J* = 6.6 Hz, 4H) –CH_2N, 2.04–1.86 (m, 8H) –CH_2**

Spectrum	Description
C = 4	**^{1}H-NMR** (400 MHz, $CDCl_3$): δ **9.28 (s, 1H) –NHBoc**, 8.43 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.87 – 7.83 (m, 1H), 7.82 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.65 – 7.58 (m, 2H), 7.50 (ddd, *J* = 7.7, 6.0, 2.6 Hz, 1H), 7.46 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H), 7.09 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H), **4.38 (q, *J* = 7.1 Hz, 2H) –OCH_2–, 1.55 (s, 9H) –$C(CH_3)_3$, 1.30 (t, *J* = 7.1 Hz, 3H) –CH_3**
D = 19	**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 8.78 (t, *J* = 1.6 Hz, 1H), 8.70 (d, *J* = 1.6 Hz, 2H), 7.42 (dq, *J* = 7.8, 1.2 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.28 – 7.26 (m, 1H), 6.85 (ddd, *J* = 7.8, 2.4, 1.0 Hz, 1H), **4.46 (q, *J* = 7.0 Hz, 4H) –OCH_2–, 3.87 (br s, 2H) –NH_2, 1.45 (t, *J* = 7.0 Hz, 6H) –CH_3**
E = 17	**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 7.74–7.68 (m, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.35 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.33–7.27 (m, 2H), 7.21 (t, *J* = 2.1 Hz, 1H), **3.75 (br s, 2H) –NH_2,** 6.80 (ddd, *J* = 7.6, 2.4, 1.2 Hz, 1H), **2.46 (s, 3H) –CH_3**
F = 16	**^{1}H-NMR** (300 MHz, $CDCl_3$): δ 8.04 (t, *J* = 1.9 Hz, 1H), 8.02–7.95 (m, 3H), 7.82 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.77–7.71 (m, 2H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.58 (ddd, *J* = 7.9, 2.1, 1.2 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.34–7.28 (m, 1H), **2.46 (s, 3H) –CH_3**
G = 18	**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 8.72 (t, *J* = 2.0 Hz, 1H), 8.32 (dd, *J* = 8.0, 2.0 Hz, 1H), 8.23 (ds, *J* = 8.0, 2.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.41 (dd, *J* = 7.8, 2.3 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.26 (t, *J* = 2.3 Hz 1H), 6.86 (dd, *J* = 7.8, 2.3 Hz, 1H), **3.87 (s, 2H) –NH_2**

Question 7.22:

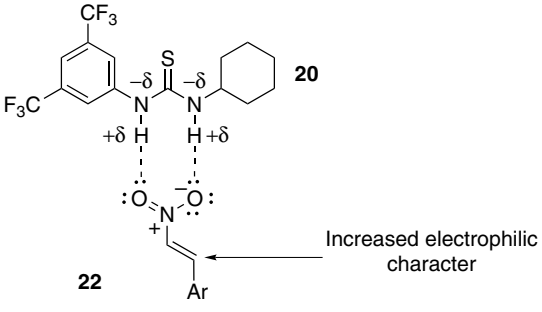
The reaction of **21** with **22** in the presence of Et_3N leads to **23** in 17% yield, while addition of a catalytic amount of **22** under otherwise identical conditions significantly increases the yield of **23** to 57%.

Question 7.23:



Question 7.24:

Catalyst **20** interacts with nitro-alkene **22** through H-bonding, resulting in an increase of the electrophilic character of the carbon atom in β-position.



Question 7.25:

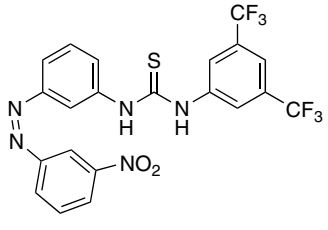
Reaction of **24** with **25** in the presence of Et_3N (without any additive) for 18 h leads to adduct **26** with 11% conversion (entry 1). The same result is observed when the reaction is performed in the presence of a catalytic amount of **I** (entry 2), while using catalyst **II-1** under otherwise identical conditions leads to **26** with a significantly higher conversion of 58% (entry 3). These results show that azobenzene-thiourea **I** has no significant effect on the reactivity of **24** and **25**, while **II-1** increases the rate of transformation.

Question 7.26:

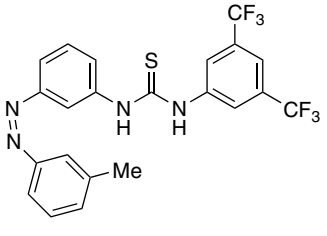
The crystal structure of **I** shows two intramolecular hydrogen bonds between the N—H groups of thiourea and the C=O group of ester moiety, as well as one of the N atom of the azo bond. Such interactions make the thiourea moiety unavailable for activation of the nitro group of **25**. In contrast, N—H groups of thiourea are accessible in **II-1**, thus allowing hydrogen bonding with the nitro group of **25** and electrophilic activation of the double bond (see preceding text).

Question 7.27:

***Z*-II-2**



***Z*-II-3**



Question 7.28:

In the absence of illumination, compound **II-2** shows high catalytic activity, leading to product **26** with 96% conversion after 18 h (entry 2), which is significantly higher than the uncatalyzed reaction (11% conversion, entry 1). Under identical reaction conditions, catalyst **II-3** exhibits significant activity, albeit reduced as compared to **II-2** (64% conversion, entry 4). These results show that the thiourea moieties in these two species are available for electrophilic activation of nitroalkene **25**; furthermore, the higher activity of **II-2** as compared to **II-3** could be explained by the electron-withdrawing effect of the nitro group, which would increase the acidity of the N—H groups.

The activity of **II-2** and **II-3** is significantly reduced upon illumination at 365 nm (entry 3 and 5), as a consequence of their conversion into ***Z*-II-2** and ***Z*-II-3**, resulting in the formation of **26** with 21% and 43% conversion, respectively. The overall decrease in catalytic activity of the *Z* isomers as compared to their *E* precursors could be the result of steric effects (making thiourea moieties less accessible). The stronger reduction of catalytic activity observed in the case of ***Z*-II-2** as compared to ***Z*-II-3** could also originate from intramolecular hydrogen bonding between the nitro and N—H groups of thiourea moiety.

References

1 Osorio-Planes, L., Rodríguez-Escrich, C., and Pericàs, M.A. (2014) Photoswitchable thioureas for the external manipulation of catalytic activity. *Org. Lett.*, **16** (6), 1704–1707.
2 Tárkányi, G., Király, P., Varga, S., Vakulya, B., and Soós, T. (2008) Edge-to-face CH/π aromatic interaction and molecular self-recognition in *epi*-cinchona-based bifunctional thiourea organocatalysis. *Chem. - Eur. J.*, **14** (20), 6078–6086.
3 Okino, T., Hoashi, Y., Furukawa, T., Xu, X., and Takemoto, Y. (2005) Enantio- and diastereoselective Michael reaction of 1,3-dicarbonyl compounds to nitroolefins catalyzed by a bifunctional thiourea. *J. Am. Chem. Soc.*, **127** (1), 119–125.
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8

Synthesis and Properties of a Photo-activatable Mimic of Pyridoxal 5′-Phosphate

Pyridoxal 5′-phosphate (PLP) is a coenzyme responsible for a variety of transformations involving amino acids, such as transamination, decarboxylation, and racemization. At physiological pH, PLP and alanine react to form *aldimine* **1** (molecular formula $C_{11}H_{13}N_2O_7P^{2-}$), which spontaneously evolves into the *quinonoid* structure **2** (molecular formula $C_{11}H_{12}N_2O_7P^{3-}$, Scheme 8.1).

Question 8.1: What is the ionization state of PLP and alanine at physiological pH?

Question 8.2: Write the structure of aldimine **1** and suggest a plausible reaction mechanism accounting for its formation.

Question 8.3: Write the structure of quinonoid structure **2** and suggest a plausible reaction mechanism accounting for its formation.

In 2012, dithienylethene **3o** was synthesized as a photoactivatable mimic of PLP, possibly allowing the control of racemization of amino acids with light (Figure 8.1) [1]. This compound is obtained from dithienylethene **7**, derived from 2-methylthiophene **4**.

The synthetic route to **7** is described in Scheme 8.2 [2]. To a 1 : 1 mixture of benzene and acetic acid (7 equiv.) is added 2-methylthiophene **4** and *N*-chlorosuccinimide (NCS) (1.1 equiv.). After stirring for 30 min at rt, the mixture is refluxed for 1 h, cooled to rt, and poured into aq. NaOH (3M). The layers are separated (**point 1**), the organic phase is washed three times with aq. NaOH (3M), dried over Na_2SO_4 and filtered, and the solvent is evaporated under reduced pressure. The residue is purified by vacuum distillation to afford **5** in 84% yield. This compound is dissolved in CS_2, glutaryl dichloride (0.5 equiv.) is added, and the mixture is cooled to 0 °C. Under vigorous stirring, $AlCl_3$ (1.2 equiv.) is slowly added by portions and the mixture is stirred at rt for 2 h. Then, ice-cold water is carefully added, the layers are separated, and the aqueous phase is extracted three times with Et_2O. The combined organic layers are washed with water, dried over Na_2SO_4 and filtered, and the solvent is evaporated under reduced pressure. The residue is purified by flash chromatography on silica gel to

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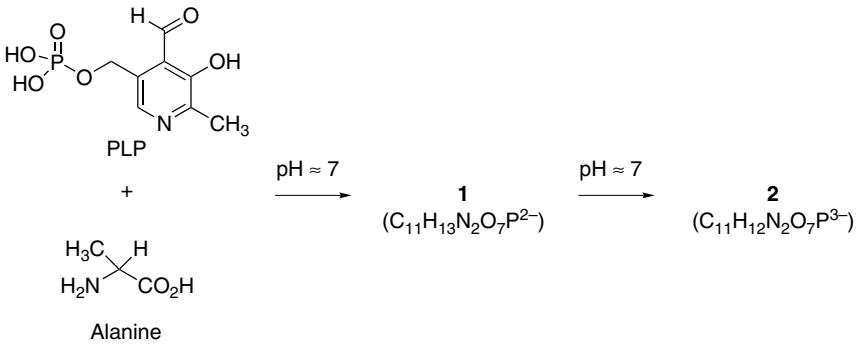


Scheme 8.1

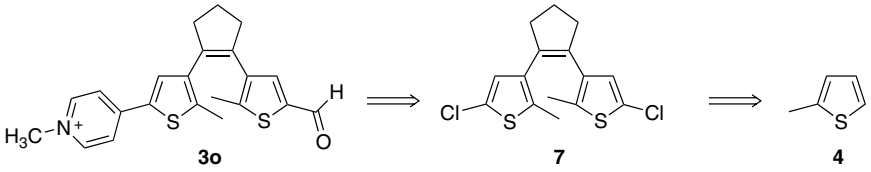
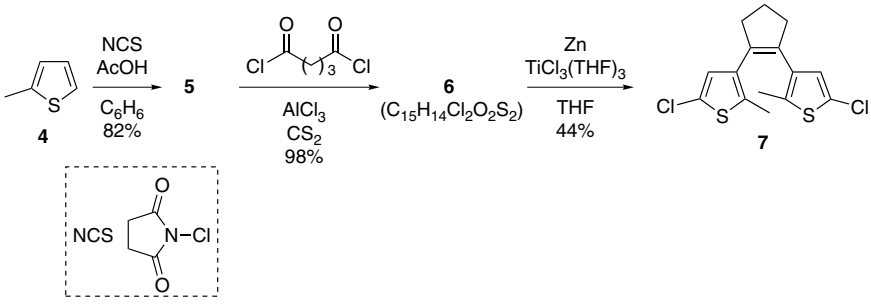


Figure 8.1 Retrosynthesis of compound **3o**.



Scheme 8.2

yield 48% of **6** (molecular formula $C_{15}H_{24}Cl_2O_2S_2$) as a white solid. Selected analytical data for this compound are reported here. Finally, reaction of **6** with $TiCl_3(THF)_3$ (2 equiv.) and Zn dust (2.5 equiv.) in tetrahydrofuran (THF) followed by appropriate workup (procedure not detailed) affords dithienylethene **7** in 44% yield.

Analytical data for **6**: ^{1}H-NMR (200 MHz, $CDCl_3$): 7.19 (s, 2H), 2.86 (t, J = 6.8 Hz, 4H), 2.66 (s, 6H), 2.12–1.98 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): 194.7 (s), 147.6 (s), 134.7 (s), 126.7 (s), 125.2 (d), 40.4 (t), 18.1 (t), 16.0 (q). IR (Nujol): 1675 cm^{-1}.

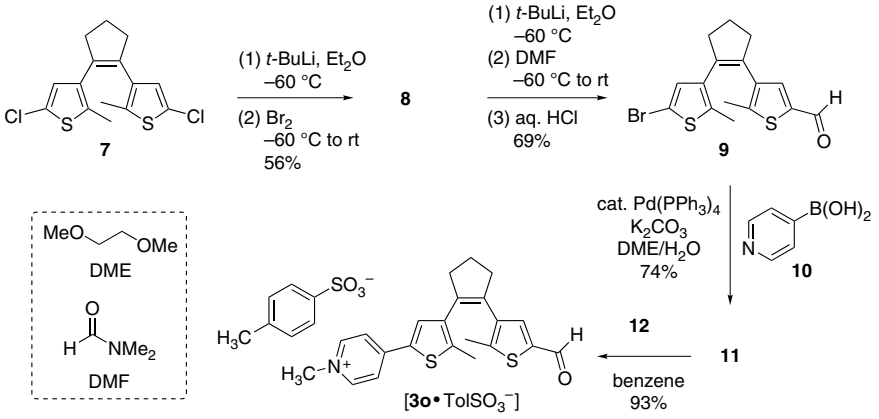
Question 8.4: Write the structure of compound **5** and suggest a plausible mechanism for its formation.

Question 8.5: Indicate the composition of aqueous and organic layer at **point 1**.

Question 8.6: Write the structure of compound **6** and suggest a plausible mechanism for its formation.

Question 8.7: Assign analytical data reported for **6**.

The synthesis of compound **3o** is described in Scheme 8.3. A solution of **7** in anhydrous Et_2O cooled to –60 °C is treated dropwise over 15 min with a solution of *t*-BuLi (2.1 equiv.) in pentane (**point 1**). The mixture is further stirred for 10 min at –60 °C, treated with a solution of Br_2 (2.2 equiv.) in anhydrous Et_2O, and stirring is maintained for 30 min at –60 °C. After warming to rt, the mixture is poured into water, the layers are separated (**point 2**), the organic layer is washed with brine, dried over $MgSO_4$ and filtered, and the solvent is removed under reduced pressure. The residue is purified by column chromatography on silica gel to give **8** in 56% yield, as a white crystalline solid. The latter is dissolved in anhydrous THF, cooled to –60 °C, and treated dropwise over 15 min with a solution of *t*-BuLi (1.0 equiv.) in pentane (**point 3**). After stirring for 10 min at –60 °C, DMF (2.0 equiv.) is added and stirring is maintained for 1 h (**point 4**). After warming to rt, the mixture is poured into aq. HCl (2M) and extracted with Et_2O. The combined organic layers are washed with aq. saturated $NaHCO_3$ solution and aq. saturated NaCl solution, dried over $MgSO_4$ and filtered, and the solvent is removed under reduced pressure yielding 69% of **7** after purification by silica gel chromatography.



Scheme 8.3

Question 8.8: Write the structure of the intermediate obtained at **point 1**.

Question 8.9: Write the structure of **8**.

Question 8.10: Indicate the composition of the aqueous and organic layers at **point 2**.

Question 8.11: Write the structure of the intermediate obtained at **point 3**.

Question 8.12: Write the structure of the intermediate obtained at **point 4**.

Question 8.13: Write a plausible mechanism for the formation of **9** from **point 4**.

To a mixture of K_2CO_3 (5.1 equiv.) in DME/water (3:2) is added **9** followed by boronic acid **10** (1.5 equiv.) and $Pd(PPh_3)_4$ (0.1 equiv.). After refluxing for 18 h, the mixture is poured into water and extracted twice with CH_2Cl_2, the combined organic layers are dried over $MgSO_4$, filtered, and concentrated under vacuum to an oil, which is purified by chromatography on silica gel leading to **11** in 74% yield. The latter is added to a solution of **12** (3.2 equiv.) in benzene, and the resulting solution is refluxed until completion of the reaction, as determined by thin-layer chromatography (TLC) (about 48 h). After evaporation of the solvent, a dark green solid is obtained (**point 1**), which is washed with Et_2O (**point 2**) to afford ionic compound [**3o**·$TolSO_3^-$] in 93% yield.

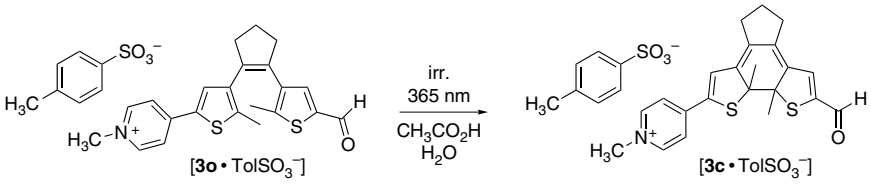
Question 8.14: Write the structure of **11** and suggest a mechanism for its formation from **9** and **10**.

Question 8.15: Write the structure of **12**.

Question 8.16: What is the composition of the solid obtained at **point 1**?

Question 8.17: What is the composition of the solid and filtrate at **point 2**?

A pale yellow solution of [**3o**·$TolSO_3^-$] in a 9:1 mixture of AcOH and water is irradiated at 365 nm for 30 min, leading to a deep-blue solution of [**3c**·$TolSO_3^-$], obtained with a purity >97% (Scheme 8.4). The evolution of UV/vis absorption spectra observed all along this process is shown in Figure 8.2. Conversely, illumination of a solution of [**3c**·$TolSO_3^-$] with visible light of wavelengths greater than 490 nm results in its discoloration.



Scheme 8.4

Question 8.18: Identify in Figure 8.2 the most characteristic absorption bands related to **3o** and **3c**, and justify your answer.

Question 8.19: How do you interpret the discoloration of a solution of [**3c**·$TolSO_3^-$] upon illumination at wavelengths higher than 490 nm?

Treatment of a solution of alanine in CH_3CO_2H/H_2O (9:1) at 40 °C with compound **3o** results in the formation of product **13o**, while under similar

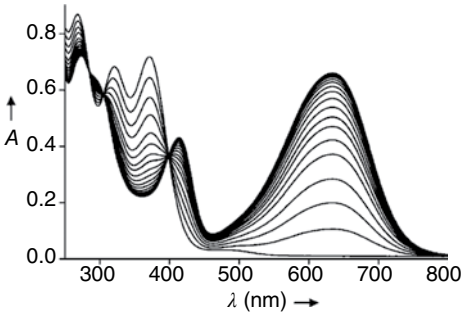
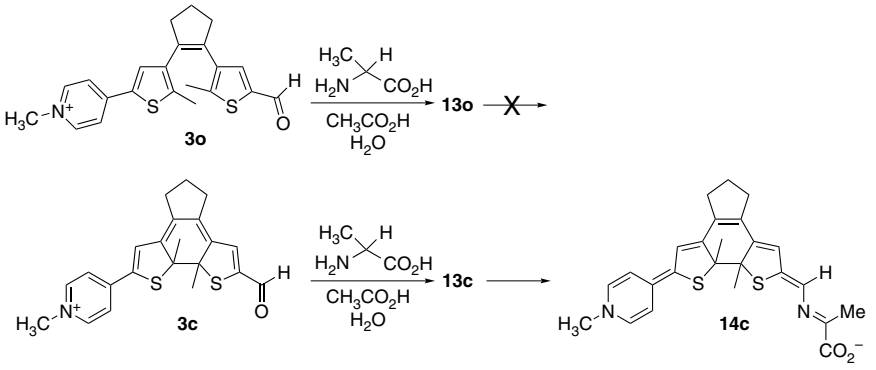


Figure 8.2 Change in the UV/vis absorption spectrum of a CH_3CO_2H/H_2O (9:1 v/v) solution of **3o** in response to irradiation at 365 nm.

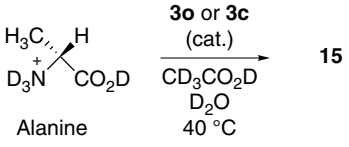
conditions compound **3c** affords **13c**, which spontaneously evolves into **14c** (Scheme 8.5).[1]



Scheme 8.5

Question 8.20: Write the structure of **13o** and **13c**, and explain why only the latter can evolve into a structure such as **14c**.

In other experiments, a solution of **13o** or **13c** (0.2 equiv.) in a 9:1 mixture of CD_3CO_2D and D_2O, is treated with alanine, and the mixture is heated to 40 °C (Scheme 8.6).[2] The evolution of ^{1}H-NMR with time is shown in Figure 8.3.



Scheme 8.6

1 The formation of **13o**, **13c**, and **14c** has not been explicitly reported in the original article.
2 The original experiments were performed with enantiomerically pure *L*-alanine.

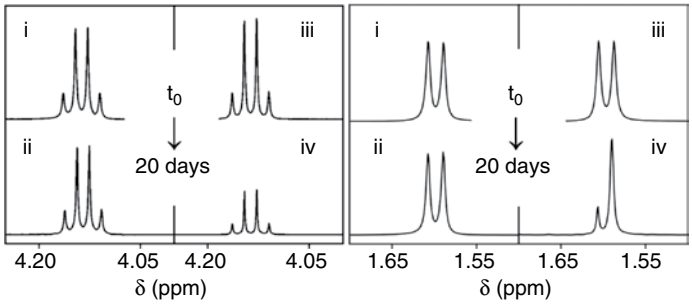


Figure 8.3 Portions of the ^{1}H-NMR spectrum of alanine (0.1M in CD_3CO_2D/D_2O 9:1 v/v) immediately after addition of a catalytic amount of **3o** (i) and **3c** (iii) and its evolution after 20 days at 40 °C (ii and iv, respectively).

Question 8.21: Assign the signals reported for alanine in Figure 8.3 (i and iii), describe their evolution upon standing at 40 °C (ii and iv), and deduce the structure of 15.

Question 8.22: Suggest a plausible mechanism accounting for experimental observations. What would be the enantiomeric excess of **15**, if enantiomerically pure L-alanine were used as a starting material?

Finally, a mixture of alanine and **13o** placed under the same conditions as described earlier, is submitted to alternating cycles of irradiation at 365 nm and with visible light while the percentage of hydrogen/deuterium (H/D) exchange is monitored (Figure 8.4).

Question 8.23: Describe and rationalize the result reported in Figure 8.4.

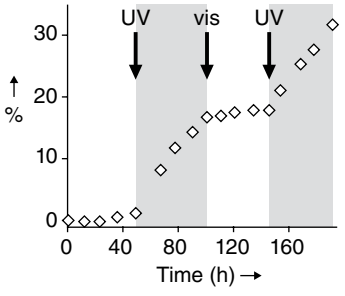
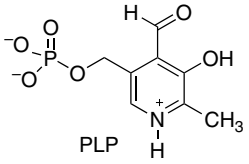


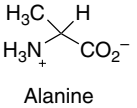
Figure 8.4 Evolution of the percentage of H/D exchange at α position of alanine (0.2M in CD_3CO_2D/D_2O 9:1 v/v) in the presence of a catalytic amount of **3o** in response to alternating cycles of irradiation with UV and visible light in CD_3CO_2D/D_2O (9:1 v/v).

Answers

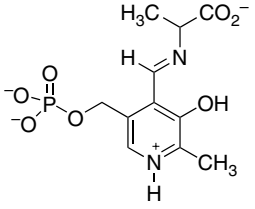
Question 8.1

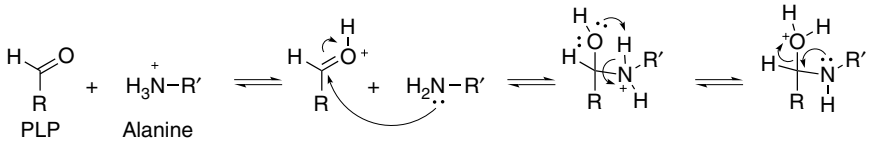
At physiological pH (≃7)

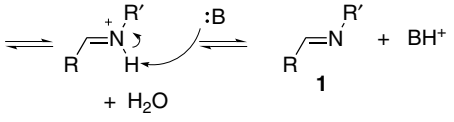




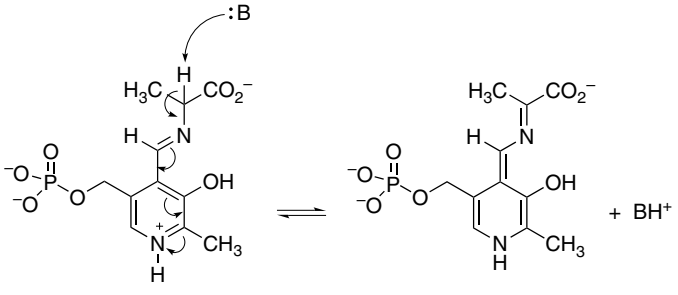
Question 8.2



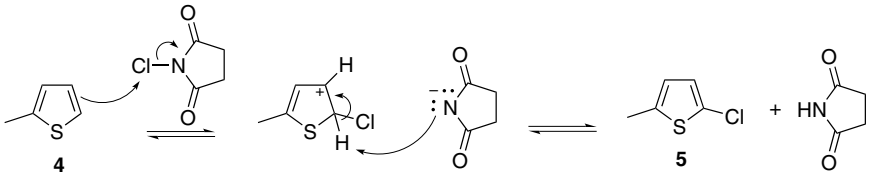




Question 8.3

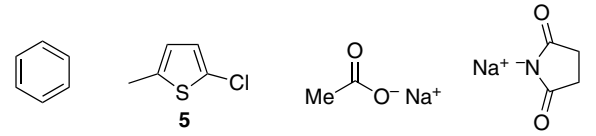


Question 8.4



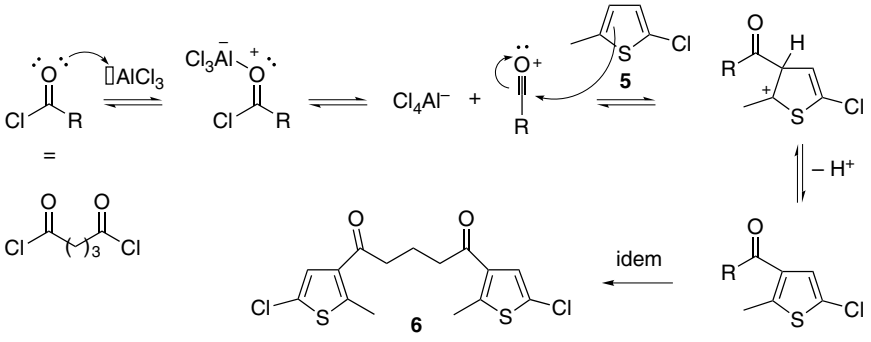
Question 8.5

At **point 1**:

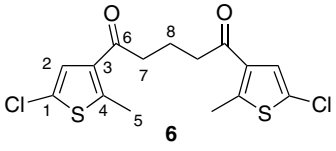


Organic phase	Organic phase	Aqueous phase	Aqueous phase

Question 8.6

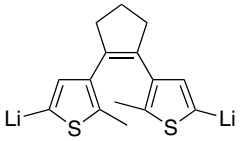


Question 8.7



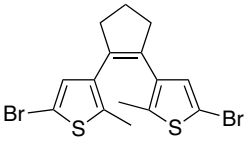
^{1}H-NMR (200 MHz, $CDCl_3$): 7.19 (s, 2H, **H$_2$**), 2.86 (t, J = 6.8 Hz, 4H, **H$_7$**), 2.66 (s, 6H, **H$_5$**), 2.12–1.98 (m, 2H, **H$_8$**). ^{13}C-NMR (125 MHz, $CDCl_3$): 194.7 (s, **C$_6$**), 147.6 (s, **C$_3$**), 134.7 (s, **C$_4$**), 126.7 (s, **C$_1$**), 125.2 (d, **C$_2$**), 40.4 (t, **C$_7$**), 18.1 (t, **C$_8$**), 16.0 (q, **C$_5$**). IR (Nujol): 1675 cm^{-1} (**C=O stretching**).

Question 8.8



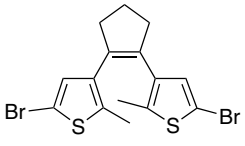
(at **point 1**)

Question 8.9



8

Question 8.10



8
Organic phase

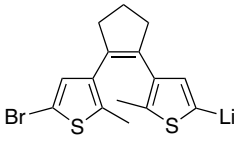
Br_2 (excess)

Organic phase

LiBr

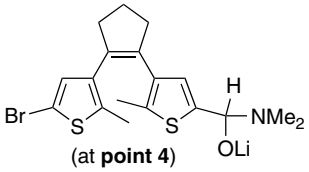
Aqueous phase

Question 8.11



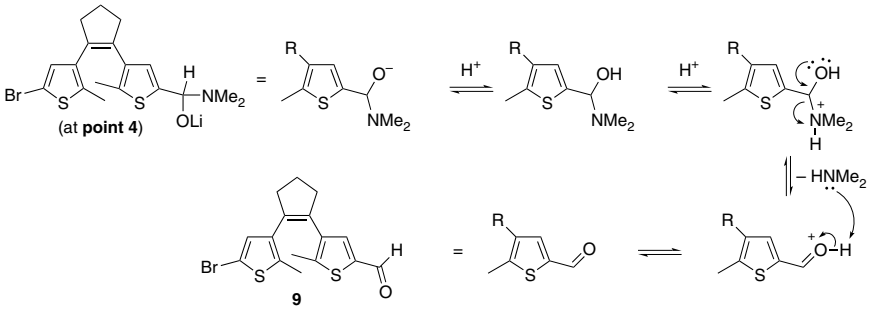
(at **point 3**)

Question 8.12



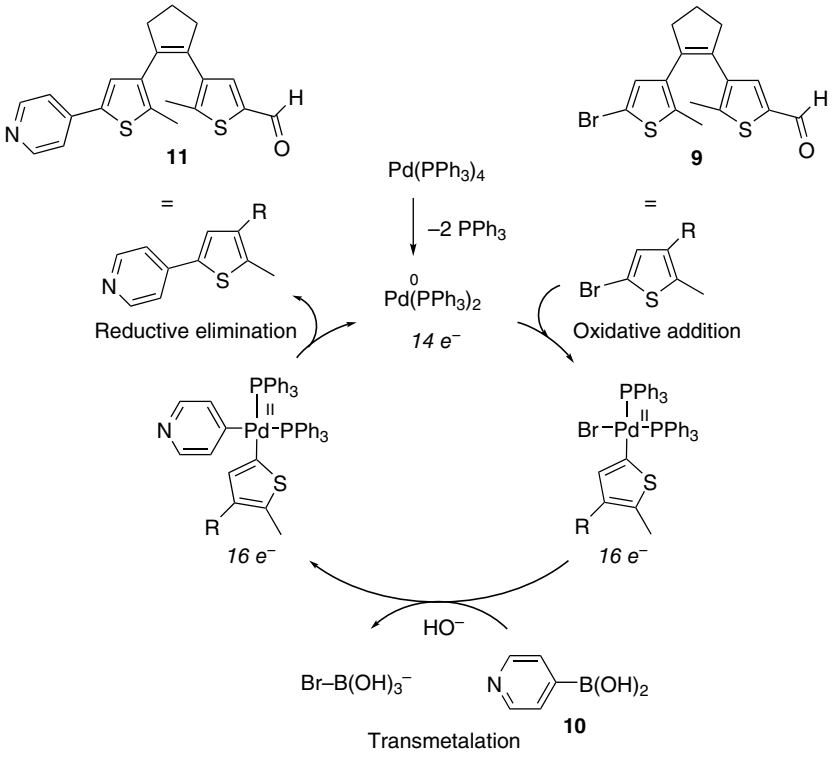
(at **point 4**)

Question 8.13

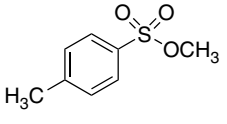


Question 8.14

Mechanism of Suzuki coupling (see Refs [3–5] for additional information):



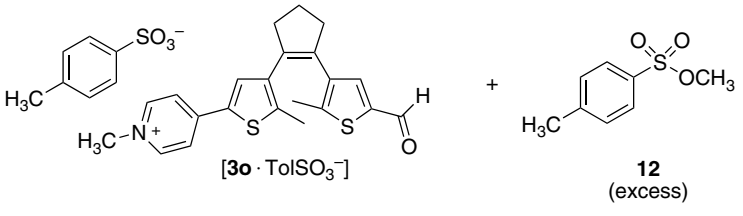
Question 8.15



12

Question 8.16

The solid obtained at **point 1** is mostly composed of [**3o** · $TolSO_3^-$] and unreacted **12** (used in excess).



Question 8.17

At **point 2**, the filtrate mostly contains **12**, while the solid corresponds to the desired product [**3o** · $TolSO_3^-$].

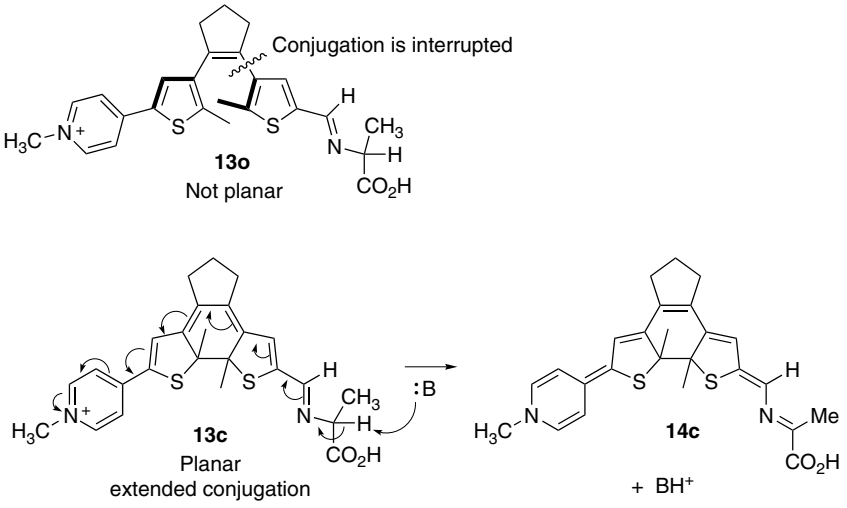
Question 8.18

From the indication that a solution of [**3c** · $TolSO_3^-$] exhibits a deep-blue color, one can deduce that this compound strongly absorbs visible light. This can also be inferred by the fact that conjugation is extended in the case of **3c**, which is not the case with **3o** (see Question 8.20). Consequently, the bands centered around 630 and 420 nm can be ascribed to [**3c** · $TolSO_3^-$], while those at around 380 and 320 nm correspond to [**3o** · $TolSO_3^-$].

Question 8.19

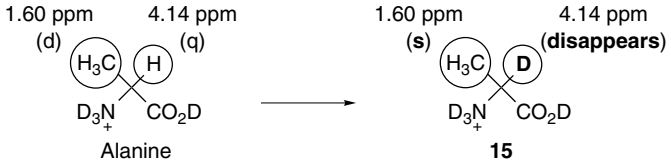
Illumination at wavelengths higher than 490 nm induces back conversion of [**3c**·$TolSO_3^-$] (highly colored) to [**3o**·$TolSO_3^-$] (weakly colored).

Question 8.20



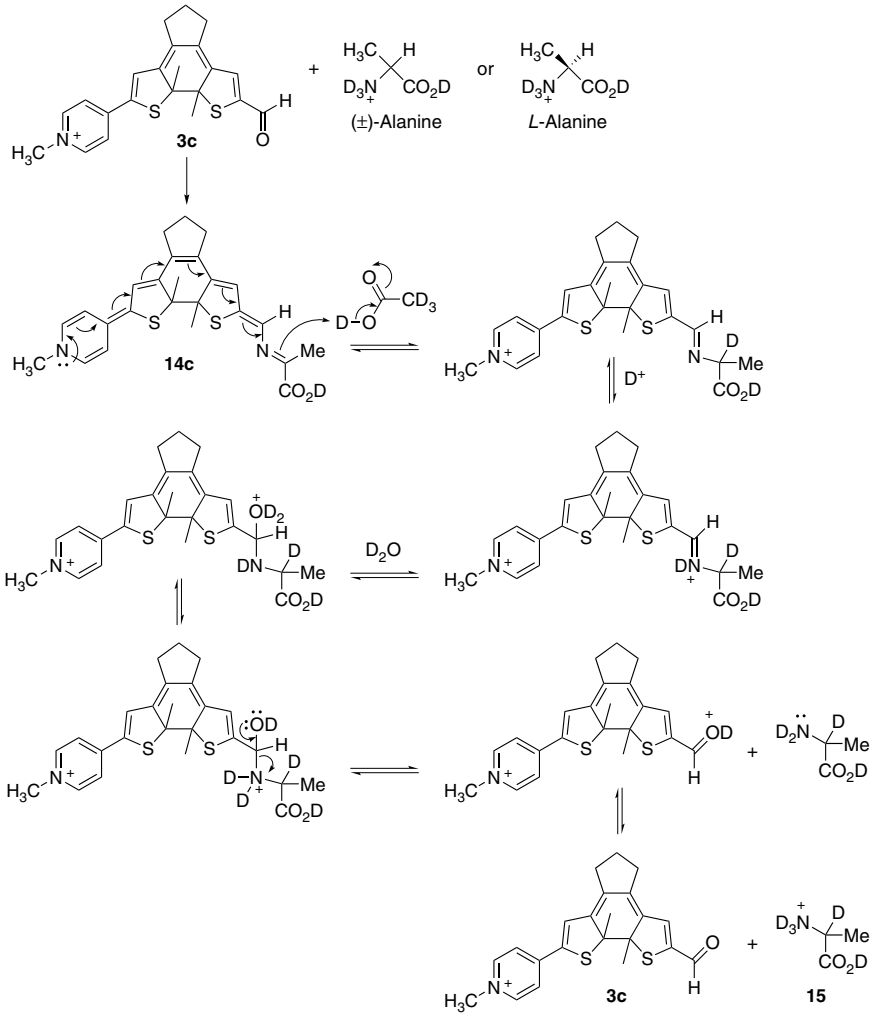
Question 8.21

The quadruplet observed at 4.14 ppm in the ^{1}H-NMR spectrum of alanine (in a 9:1 mixture of CD_3CO_2D and D_2O) corresponds to Hα, while the doublet at 1.60 ppm corresponds to the $-CH_3$ group. Upon standing at 40 °C with a catalytic amount of 3o, no change is observed in the ^{1}H-NMR spectrum, both in the region of Hα and in the $-CH_3$ region (compare i and ii). In contrast, when 3c is used, the intensity of the quadruplet at 4.14 ppm decreases significantly (as a consequence of the steady disappearance of this signal) and the doublet at 1.60 ppm transforms into a singlet (compare iii and iv). These results reveal an H/D exchange at α position of alanine, leading to 15 whose structure is described here.



Question 8.22

H/D exchange at α position of alanine can be explained by the mechanism described here. Starting from enantiomerically pure alanine, reaction with catalyst **3c** to form **14c** results in the loss of stereochemical information, leading to a racemic mixture.



Question 8.23

When alanine is treated with a catalytic amount of **3o** in a 9:1 mixture of CD_3CO_2D and D_2O, no significant amount of H/D exchange is observed after 50 h. Upon illumination with UV light, the extent of deuterium incorporation into alanine steadily increases as a consequence of the conversion of **3o** into **3c**, precursor to quinonoid structure **14c** (see preceding text). Then, illumination with visible light converts back **3c** into **3o**, thereby inhibiting H/D exchange; catalytic activity is finally restored by UV illumination (conversion of **3o** into **3c**) until reaction reaches completion.

References

1 Wilson, D. and Branda, N.R. (2012) Turning 'on' and 'off' a pyridoxal 5′-phosphate mimic using light. *Angew. Chem. Int. Ed.*, **51** (22), 5431–5434.

2 Lucas, L.N., de Jong, J.J., van Esch, J.H., Kellogg, R.M., and Feringa, B.L. (2003) Syntheses of dithienylcyclopentene optical molecular switches. *Eur. J. Org. Chem.*, **1**, 155–166.

3 Miyaura, N. and Suzuki, A. (1995) Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.*, **95** (7), 2457–2483.

4 Martin, R. and Buchwald, S.L. (2008) Palladium-catalyzed Suzuki–Miyaura cross-coupling reactions employing dialkylbiaryl phosphine ligands. *Acc. Chem. Res.*, **41** (11), 1461–1473.

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9

Fluorescent Peptides for Monitoring Activity of Autophagy-Initiating Enzyme

Autophagy is a regulated destructive cellular process allowing the degradation and recycling of dysfunctional components. On the basis of increasing evidence that inhibition of autophagy could limit tumor progression and increase efficacy of chemotherapeutic agents, several fluorogenic peptides were recently prepared in order to monitor the activity of enzyme ATG4B, exhibiting a key role in initiation of autophagy [1].

9.1 Solid-Phase Synthesis of a Putative Fluorogenic Peptide Substrate for ATG4B

In order to synthesize a series of fluorogenic peptides derived from 7-aminocoumarin (see subsequent text), functionalized resin **3** was synthesized following the route described in Figure 9.1.

A mixture of Rink amide resin and dimethylformamide (DMF) is agitated for 1 h and filtered (**point 1**). The resin is recovered and a 20% solution of piperidine in DMF is added, the mixture is agitated for 30 min, and the resin is filtered and washed three times with DMF (**point 2**). Compound **1** (3 equiv.), HOBt (3.2 equiv.), and DMF are added, followed by DIC (3.1 equiv.). The mixture is agitated for 24 h and the resin is filtered and washed with DMF (**point 3**), leading to **2**. A 20% solution of piperidine in DMF is added to the resin and the mixture is agitated for 30 min. The resin is filtered and washed three times with DMF, leading to **4**. In parallel, a solution containing Fmoc-Gly ($FmocNHCH_2CO_2H$), HOBt (1 equiv.), and DIC (1 equiv.) in DMF is prepared and agitated for 5 min (**point 4**), before being added to the suspension of resin **4**, and reacted for one night. Filtration of the resin and washing three times with DMF afford the desired resin precursor **3**.

Question 9.1: Why is the resin stirred for 1 h in DMF prior to reaction (**point 1**)?

Question 9.2: Write the structure of the resin at **point 2**.

Question 9.3: Write the structure of **2**.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

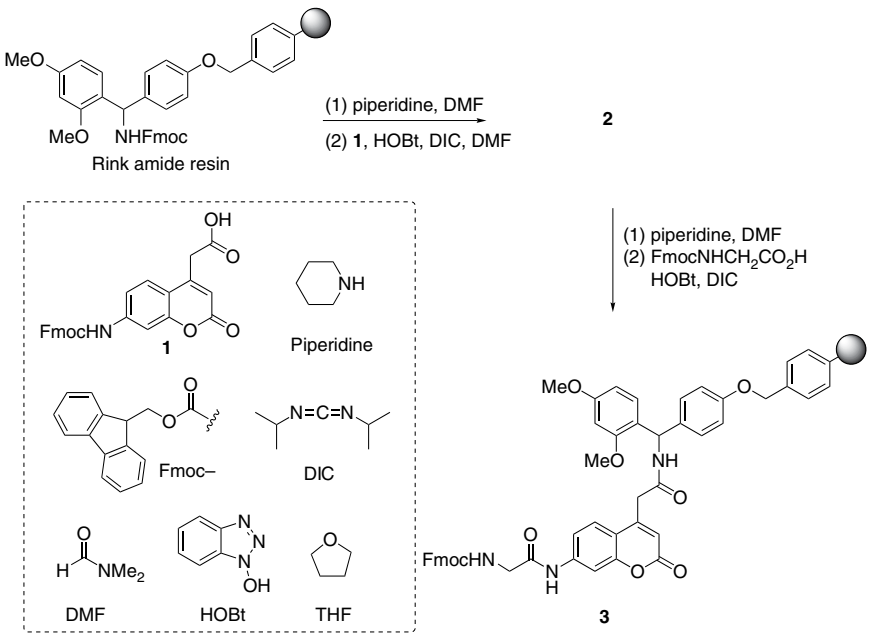


Figure 9.1 Synthesis of compound **3**.

Question 9.4: Indicate the composition of the filtrate at **point 3**?

Question 9.5: Write the structure of resin **4**.

Question 9.6: Write the structure of the reactive intermediate obtained at **point 4**.

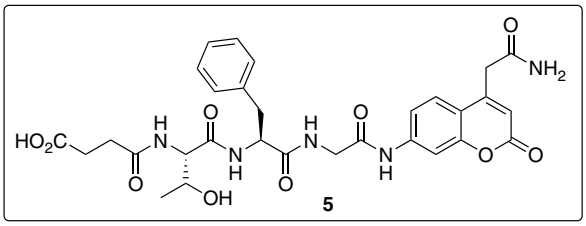
Peptide **5** is synthesized from **3** using the available reagents mentioned in Figure 9.2.

Question 9.7: Suggest an experimental protocol allowing the preparation of **5** from **3** using the available reagents mentioned in Figure 9.2.

9.2 Evaluation as Fluorogenic Substrates for ATG4B

The principle underlying the test for the monitoring activity of ATG4B is described here. Putative peptide substrates of type **A** (weakly fluorescent) undergo hydrolysis of the peptide–coumarine amide bond upon binding the enzyme (Figure 9.3). This process results in the formation of peptides of type **B** with concomitant liberation of aminocoumarine **9**, which exhibits strong fluorescence. Therefore, the rate of amide bond cleavage can be determined by monitoring the rate of fluorescence change, expressed as RFUs/s (relative fluorescence units per second), whose value increases with binding efficiency. The results obtained upon incubation of a series of peptides **5** and **10–20** (synthesized

Target peptide:



Available reagents and solvents:

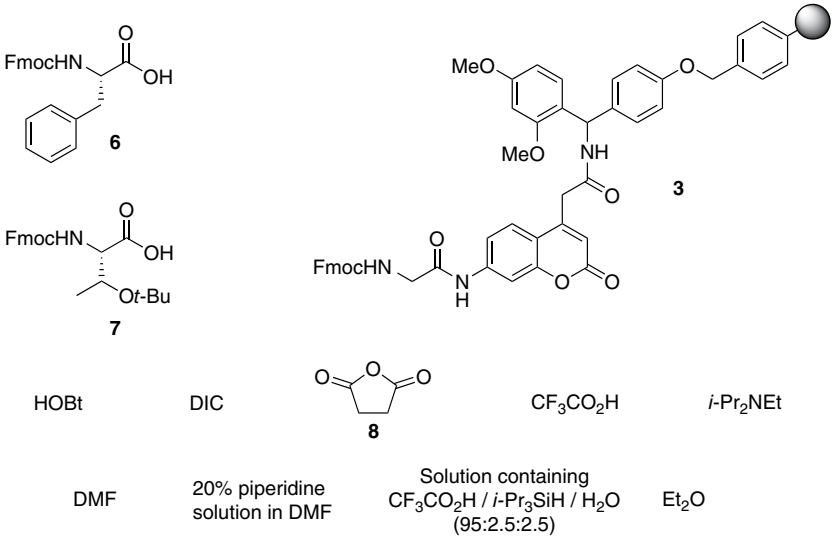


Figure 9.2 Structure of target peptide **5** and reagents available for its synthesis.

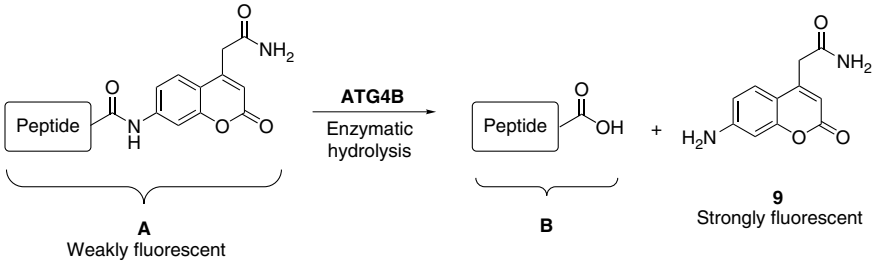


Figure 9.3 Principle underlying the activity assay for ATG4B.

according to a procedure similar to that described previously) with ATG4B are reported in Figures 9.4 and 9.5.

Question 9.8: Based on the results displayed in Figure 9.4, indicate which peptide shows the highest hydrolysis rate in the presence of ATG4B; and comment briefly on the structure–activity relation displayed by compounds **10–16**, especially the influence of the *N*-terminal group.

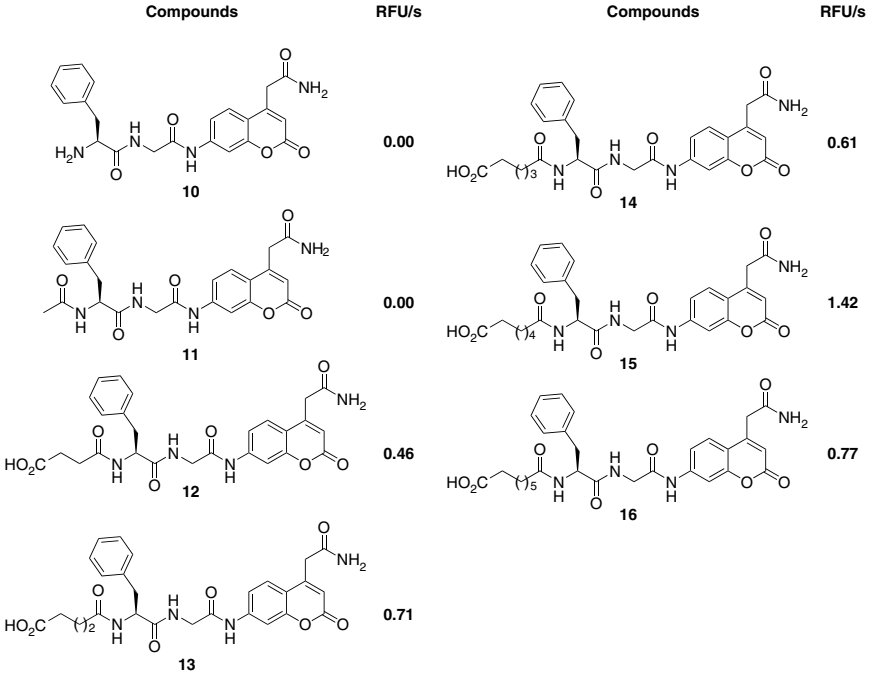


Figure 9.4 Rate of the ATG4B amide bond cleavage obtained for substrates **10**–**16**.

Question 9.9: Comment briefly on the structure–property relation reported in Figure 9.5.

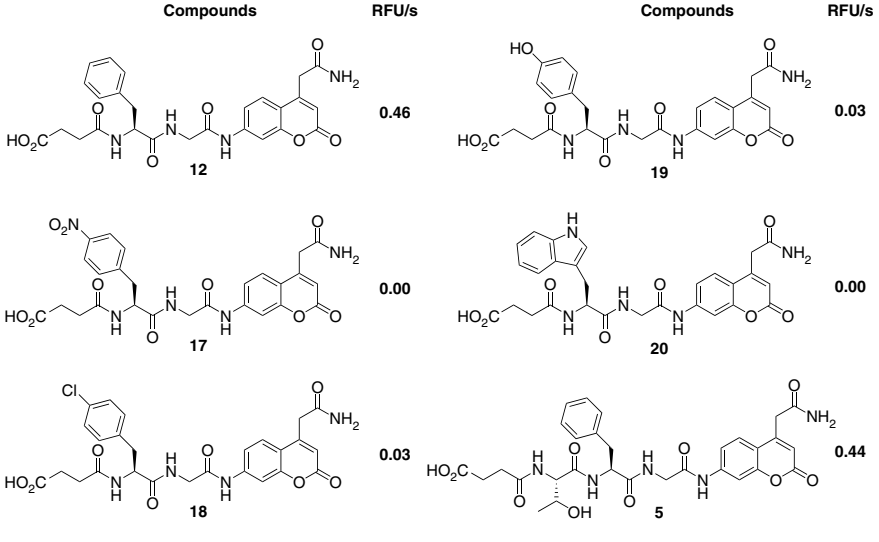


Figure 9.5 Rate of the ATG4B amide bond cleavage obtained for substrate **17**–**20**.

9.3 Solution-Phase Synthesis of a Fluorogenic Substrate Analog Containing a Self-Immolating Linker

In order to improve the interesting properties of compound **15**, analog **26** containing a self-immolating linker at the cleavage site was synthesized using a scalable solution-phase synthesis (Figures 9.6 and 9.7).

A solution of Boc-L-phenylalanine **21** in NMP is treated with NMM (4.5 equiv.) and HBTU (1 equiv.). After stirring for 5 min at rt, compound **22** (1.1 equiv.) is added and the mixture is stirred for 5 h. EtOAc is added, the layers are separated, and the organic phase is washed with saturated aq. NaCl and $NaHCO_3$ solution, aq. HCl (1M), and saturated aq. NaCl solution. The organic phase is dried over Na_2SO_4 and filtered, and the solvent is removed under reduced pressure to give **23** as a white solid in 80% yield. The latter is dissolved in a mixture of EtOH and DMF, 10% Pd/C is added (about 50% w/w) followed by ammonium formate (9 equiv.), and the mixture is stirred overnight at rt. The mixture is filtered on Celite, the filtrate is recovered, and EtOH is evaporated under reduced pressure. The remaining solution is diluted with EtOAc, aq. HCl (0.5M) is added, the phases are separated, and the organic layer is washed with saturated aq. NaCl, dried over Na_2SO_4 and filtered, and the solvent is removed under reduced pressure to give **24** as a white solid.

Question 9.10: Write the structure of compound **22**.

Question 9.11: Write a reaction mechanism accounting for the formation of **23** from **21** and **22**.

Question 9.12: Explain the role of reagents involved in the formation of **24** from **23**.

Compound **24** is then transformed in three steps into **25**, which is converted in two steps into the desired peptide **26** (Figure 9.7).

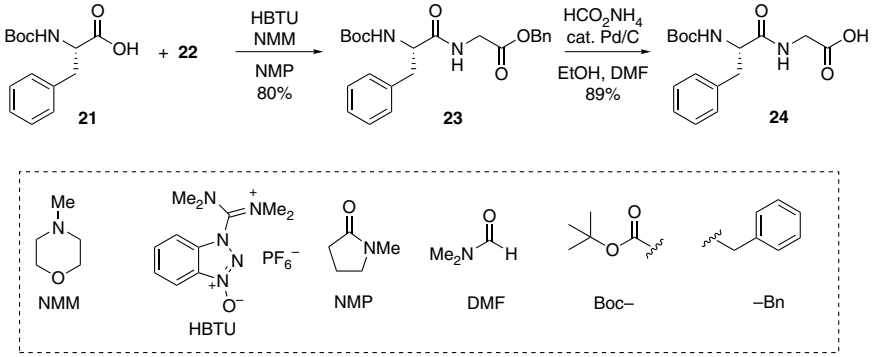


Figure 9.6 Synthesis of compound **24** from **21**.

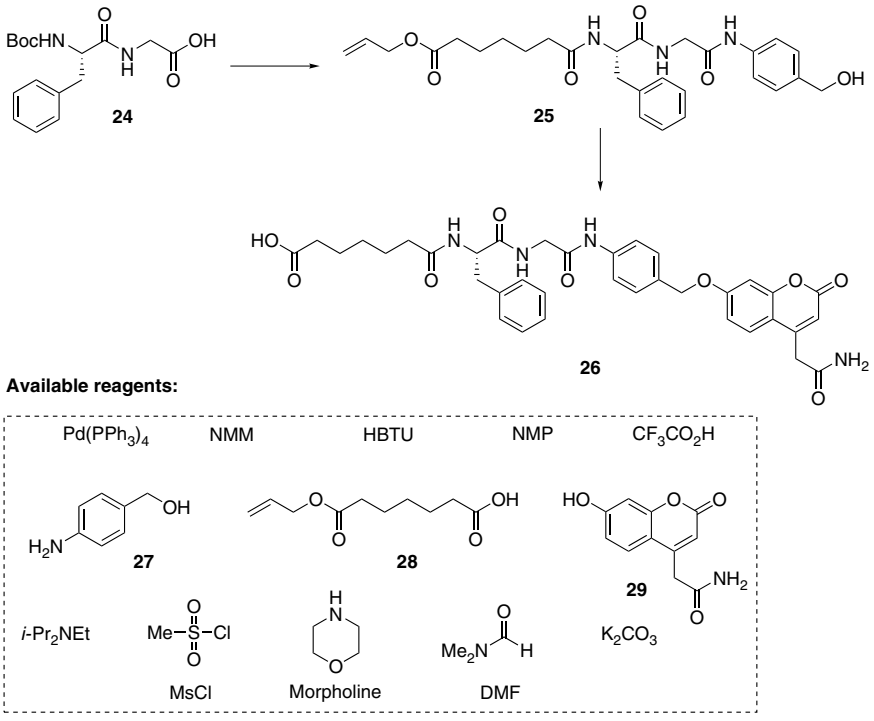


Figure 9.7 Available reagents for the synthesis of **26** from **24**.

Question 9.13: Suggest a plausible synthetic route to **25** and **26** using the available reagents mentioned in Figure 9.7.

Peptide substrate **26** proved to bind significantly better to enzyme ATG4B than its parent **15**, lacking a self-immolating linker, and undergoes amide bond hydrolysis leading to the formation of peptide **30** and hydroxy-coumarin **29**, together with by-product **31**.

Question 9.14: Write the structure of compound **31**, and suggest a plausible mechanism accounting for its formation, together with **29** and **30** (Figure 9.8).

Answers

Question 9.1:

Stirring in DMF for 1 h allows swelling of the resin, which is necessary for diffusion and accessibility to the reactive sites. The choice of solvent depends on the nature of the resin.

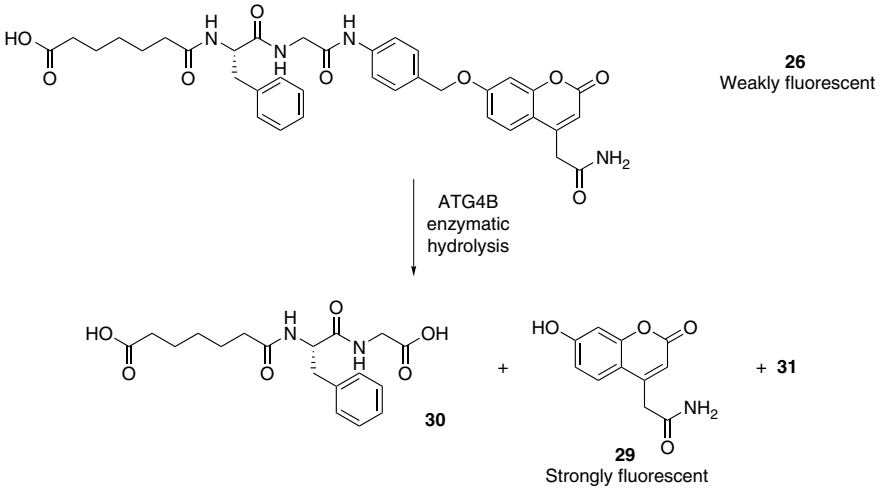
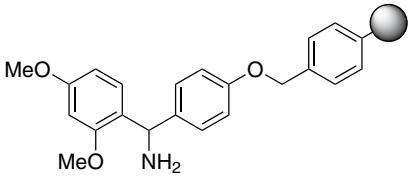


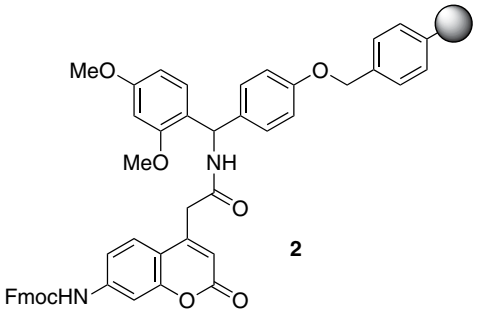
Figure 9.8 Enzymatic assay involving the self immolating substrate **26**.

Question 9.2:



at **point 2**

Question 9.3:

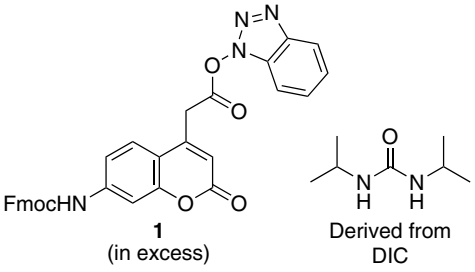


Question 9.4:

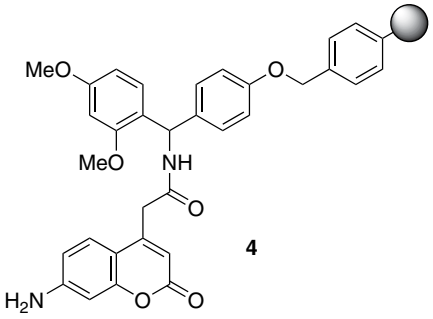
Composition of filtrate at **point 3**:

HOBt (in excess) DIC (in excess)

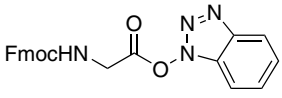
DMF THF MeOH



Question 9.5:



Question 9.6:



(at **point 4**)

Question 9.7:

1) *Swelling*: A mixture of resin **3** in DMF is agitated for 1 h and filtered.
2) *Fmoc deprotection*: The resin is treated with a 20% piperidine solution in DMF and the mixture is agitated for 10 min. The resin is filtered and washed three times with DMF. This step is repeated twice; then the deprotection is monitored using a colorimetric assay.
3) *Coupling with Fmoc-phenylalanine* **6**: In a separate vessel, a solution of **6** (5 equiv.) and HOBt (5 equiv.) in DMF is treated with DIC (5 equiv.). After 5 min, the entire mixture is added to the resin and agitated for 5 h. The resin is filtered and washed three times with DMF. The completeness of the reaction is monitored using a colorimetric assay and, if necessary, a second coupling cycle is performed.

4) *Fmoc deprotection*: The resin is treated with a 20% piperidine solution in DMF and the mixture is agitated for 10 min. The resin is filtered and washed three times with DMF. This step is repeated twice; then the deprotection is monitored using a colorimetric assay.
5) *Coupling with Fmoc-threonine* **7**: In a separate vessel, a solution of **7** (5 equiv.) and HOBt (5 equiv.) in DMF is treated with DIC (5 equiv.). After 5 min, the entire mixture is added to the resin and agitated for 5 h. The resin is filtered and washed three times with DMF. The completeness of the reaction is monitored using a colorimetric assay and, if necessary, a second coupling cycle is achieved.
6) *Fmoc deprotection*: The resin is treated with a 20% piperidine solution in DMF and the mixture is agitated for 30 min. The resin is filtered and washed three times with DMF. This step is repeated twice; then the deprotection is monitored using a colorimetric assay.
7) *Coupling with succinic anhydride* **8**: Succinic anhydride **8** (10 equiv.), DMF, and *i*-Pr_2NEt (9 equiv.) are added, and the mixture is agitated overnight. The resin is filtered and washed three times with DMF.
8) *Final deprotection and cleavage from the resin*: A solution of CF_3CO_2H/*i*-Pr_3SiH/H_2O (95 : 2.5 : 2.5) is added to the resin and the mixture is agitated overnight. The resin is filtered and washed twice with CF_3CO_2H. The combined filtrates are concentrated under reduced pressure. The crude peptide is precipitated from the mixture through addition of cold Et_2O, filtrated off, and washed with Et_2O. The resulting peptide **5** is purified by reverse-phase high-performance liquid chromatography (HPLC).

Question 9.8:

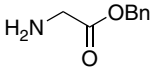
Inspection of the RFU/s values reported in Figure 9.4 indicates that the presence of a carboxylic acid moiety at the *N*-terminal position is required for recognition by the enzyme, as compounds **10** and **11** containing free amine and *N*-acetyl group, respectively, show RFU/s values of 0.00. The size of the linker significantly influences the rate of hydrolysis, with RFU/s ranging from 0.46 to 1.42 for compounds **12–16**. Peptide **15**, with an RFU/s value of 1.42, shows the highest hydrolysis rate.

Question 9.9:

In this series of peptides, the influence of the neighboring residue is studied by mutation of the phenylalanine to other aromatic residues, in order to try to establish additional interaction and improve the affinity of the peptide to the enzyme. As can be seen, the presence of a substituent on the phenyl ring (in **17**, **18**, and **19**) or its replacement with an indole ring (in **20**) results in significant reduction of the rate of hydrolysis (RFU/s values ranging between 0.00 and 0.03). This presumably reflects the fact that the active site of the enzyme is too small to accommodate additional substituent on the phenyl ring. Moreover, in peptide **5**, a threonine has been introduced between the succinate moiety and the

phenylalanine. This modification has no effect on substrate potency, which suggests that the nature of the side chain in this position does not influence substrate recognition, and probably that this side chain does not directly interact with the enzyme.

Question 9.10:



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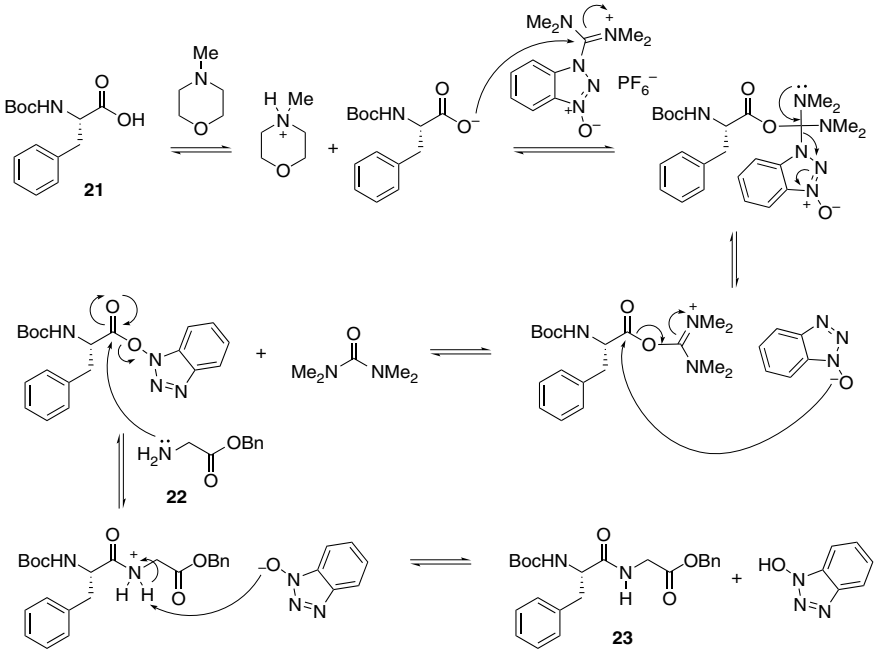
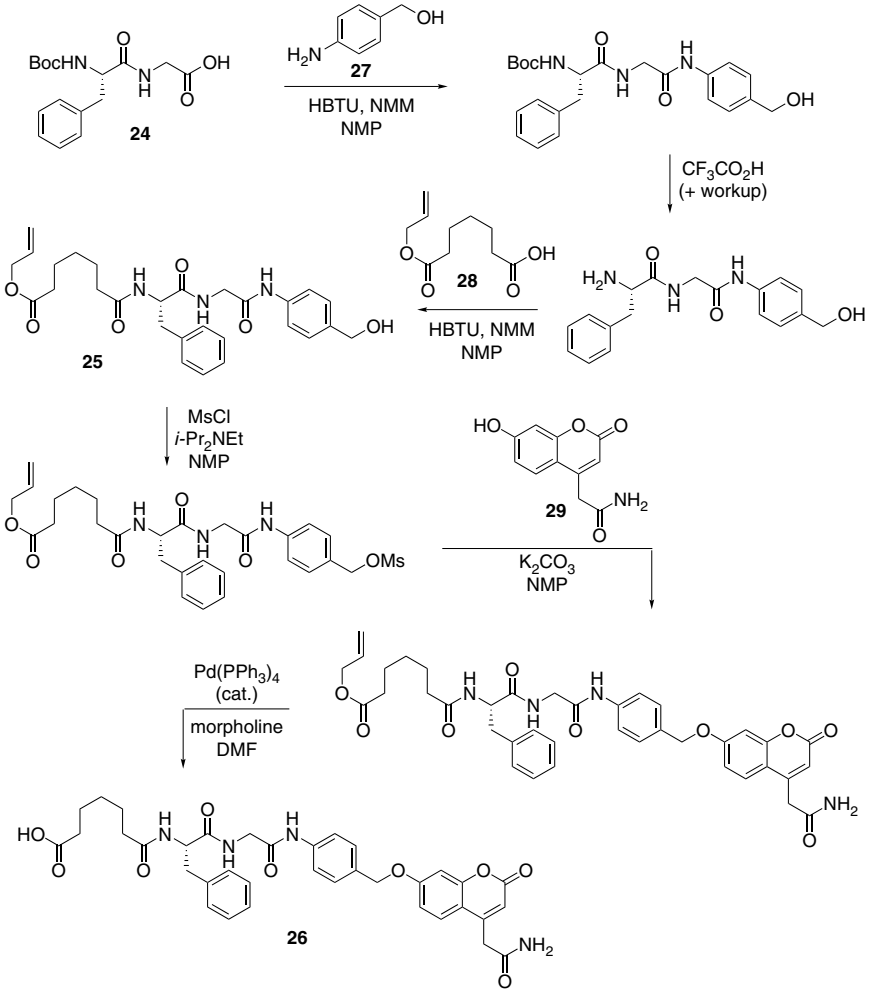
Question 9.11:



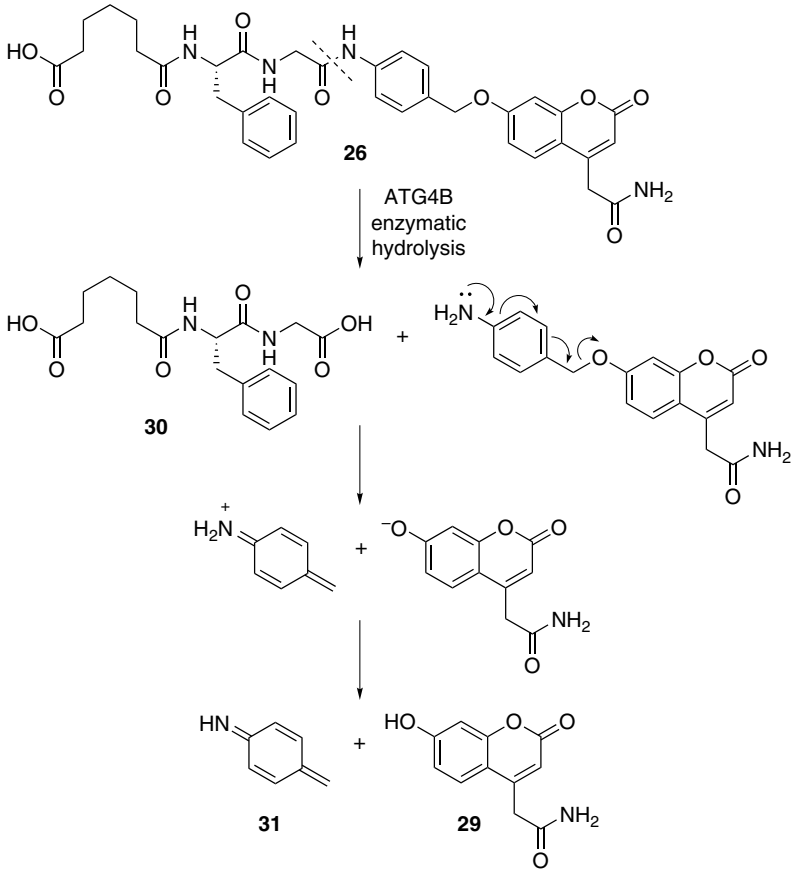
Question 9.12:

In the presence of Pd/C, ammonium formate (HCO_2NH_4) decomposes to carbon dioxide (CO_2), ammonia (NH_3), and hydrogen (H_2) [2]. The latter adsorbs at the surface of Pd, thus allowing hydrogenolysis of the benzyl ester. EtOH and DMF here play the role of the solvent.

Question 9.13:



Question 9.14:



References

1 Vezenkov, L., Honson, N.S., Kumar, N.S., Bosc, D., Kovacic, S., Nguyen, T.G., Pfeifer, T.A., and Young, R.N. (2015) Development of fluorescent peptide substrates and assays for the key autophagy-initiating cysteine protease enzyme, ATG4B. *Bioorg. Med. Chem.*, **23** (13), 3237–3247.

2 Rajagopal, S. and Spatola, A.F. (1995) Mechanism of palladium-catalyzed transfer hydrogenolysis of aryl chlorides by formate salts. *J. Org. Chem.*, **60** (5), 1347–1355.

10

Fluorescent Peptide Probes for Cathepsin B

Cathepsin B is a lysosomal cysteine protease involved in intracellular proteolysis, which is overexpressed in many tumors and surrounding cells. This protein is therefore an interesting target for cancer diagnosis and therapy, thus stimulating the development of fluorescent probes for optical imaging, especially within the near-infrared (NIR) window.

This chapter deals with the synthesis of NIR-fluorescent dyes suitable for cellular and *in vivo* imaging, and their incorporation into peptide probes for the monitoring of cathepsin B activity.

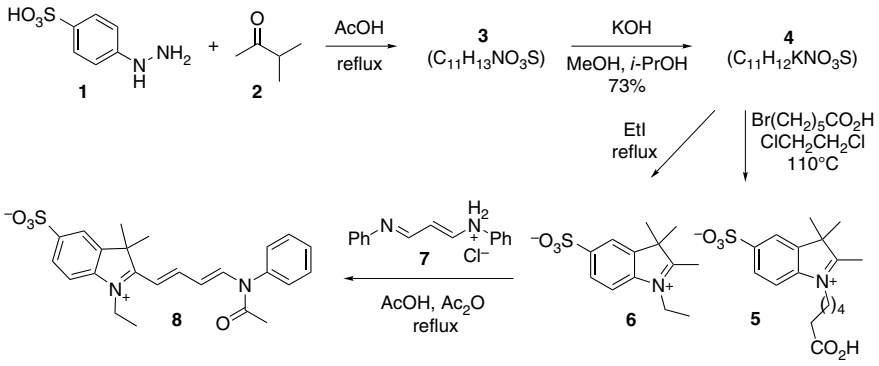
10.1 Solution Synthesis of a Water-Soluble Cyanine Fluorophore

The synthesis of compound **8**, precursor to cyanine dye **10**, is described in Scheme 10.1 [1].

A mixture of **1** and **2** (3 equiv.) in acetic acid is refluxed for 3 h and cooled to rt, leading to a pink solid **3** (molecular formula $C_{11}H_{13}NO_3S$). The latter is recovered by filtration, dissolved in MeOH, and treated with a saturated solution of KOH in *i*-PrOH, rapidly leading to a yellow solution and to the precipitation of a yellow solid **4** (molecular formula $C_{11}H_{12}KNO_3S$), obtained in 73% yield for the two steps after filtration and drying.[1] Compound **4** and 6-bromohexanoic acid (1.3 equiv.) are mixed in $ClCH_2CH_2Cl$ and heated to 110 °C for 12 h. After cooling to rt, the solid is recovered by filtration and triturated with *i*-PrOH (**point 1**), leading to **5** as a powder. In parallel, a mixture of **4** and a large excess of ethyl iodide is refluxed for 24 h, the reaction mixture is cooled, and the light purple solid is recovered by filtration and triturated with acetone (**point 2**). The resulting powder **6** is directly reacted with malonaldehyde dianilide hydrochloride **7** (1.1 equiv.) in a refluxing mixture of acetic acid and acetic anhydride. Monitoring the reaction by UV–vis absorption spectroscopy revealed the disappearance of the absorption band centered around 286 nm in the initial mixture of reagents with concomitant apparition of a band at about 445 nm corresponding to **8** in

1 The procedure is slightly modified as compared to the original article.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.



Scheme 10.1

about 4 h. Acetic acid and acetic anhydride are removed by evaporation under reduced pressure, and the residue is triturated with EtOAc (**point 3**) and dried to afford **8**, which is used without additional purification.[1]

Question 10.1: Write the structure of **3** and suggest a mechanism accounting for its formation from **1** and **2**.

Question 10.2: Write the structure of **4**.

Question 10.3: What is the role of trituration at **point 1**?

Question 10.4: What is the role of trituration at **point 2**?

Question 10.5: What is the role of trituration at **point 3**?

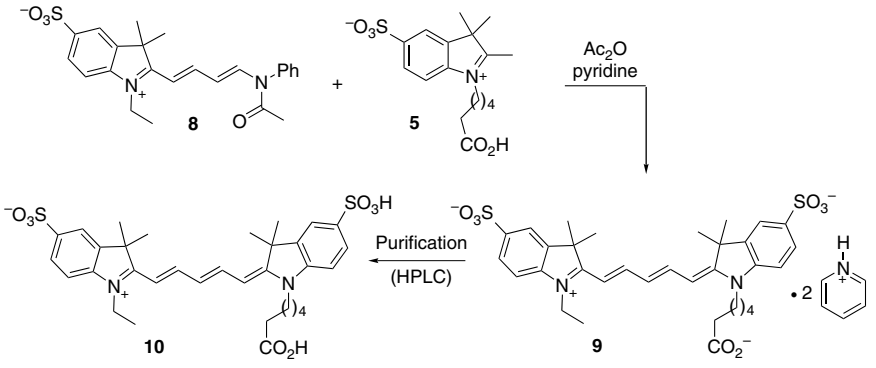
Question 10.6: How do you interpret the evolution of the UV–vis absorption spectrum of the reaction mixture leading to **8**.

Compound **8** is dissolved in a mixture of acetic anhydride and pyridine; then indolenine **5** (about 1 equiv) is added, and the mixture is heated at 110 °C until the absorption band at 445 nm disappears and a new band centered around 650 nm appears (Scheme 10.2). After cooling to rt, a large amount of EtOAc is added, resulting in the formation of a gummy product **9**, which is triturated with *i*-PrOH and purified by high-performance liquid chromatography (HPLC) (conditions not detailed) to afford product **10**.[2]

Question 10.7: Suggest a plausible mechanism accounting for the formation of **10** from **5** and **8**.

Question 10.8: How do you interpret the evolution of the UV–vis absorption spectrum of the reaction mixture?

2 The procedure is slightly modified as compared to the one initially reported.



Scheme 10.2

10.2 Synthesis of a Water-Soluble Cyanine Fluorophore Using a Polymeric Support

In 2007, an alternative strategy to the synthesis of water-soluble cyanine dyes such as **10**, based on soluble polymer-supported synthesis, was reported [2]. This technique aims at combining the high reactivity of homogeneous reactions and the easy isolation and purification of solid-phase synthesis.

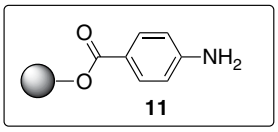
Polyethylene glycol with a molecular weight of 2000 g mol^{-1} (PEG 2000) was used as a soluble support, in combination with the reagents and solvents indicated in Scheme 10.3, to prepare the PEG-bound aniline **11**.

Question 10.9: Indicate the approximate number of ethylene glycol units (*n* value) in PEG 2000.

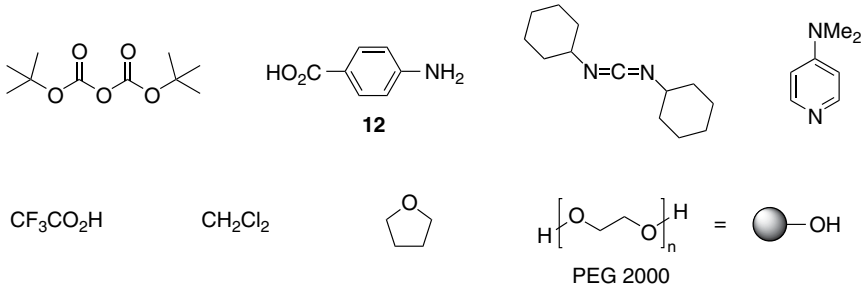
Question 10.10: Suggest a synthetic sequence allowing the preparation of supported aniline **11** from the available reagents and solvents indicated in Scheme 10.3.

A mixture of **11** and 1,1,3,3-tetramethoxypropane (25 equiv.) in acetic acid is stirred at 55 °C for 6 h (Scheme 10.4). After cooling to rt, cold Et_2O is added upon vigorous stirring to give a dark yellow precipitate, which is recovered by filtration, dissolved in CH_2Cl_2, and precipitated by addition of Et_2O, yielding 73% of **13**. The latter is mixed with **6** (1.0 equiv.), a large excess of acetic acid is added, and the resulting mixture is heated at 80 °C for 1 h. Addition of cold Et_2O upon vigorous stirring results in the formation of a brown precipitate, which is collected by filtration, dissolved in MeOH, and precipitated again by addition of Et_2O. The solid obtained at this stage mainly consists of **14**, contaminated with a small amount of **14b** (molecular formula $C_{29}H_{36}N_2O_6S_2$). Addition of EtOAc results in dissolution of most of the solid material, and the remaining particles are filtered off (**point 1**). The filtrate is concentrated under reduced pressure; then cold Et_2O is added upon vigorous stirring leading to the precipitation of **14**,

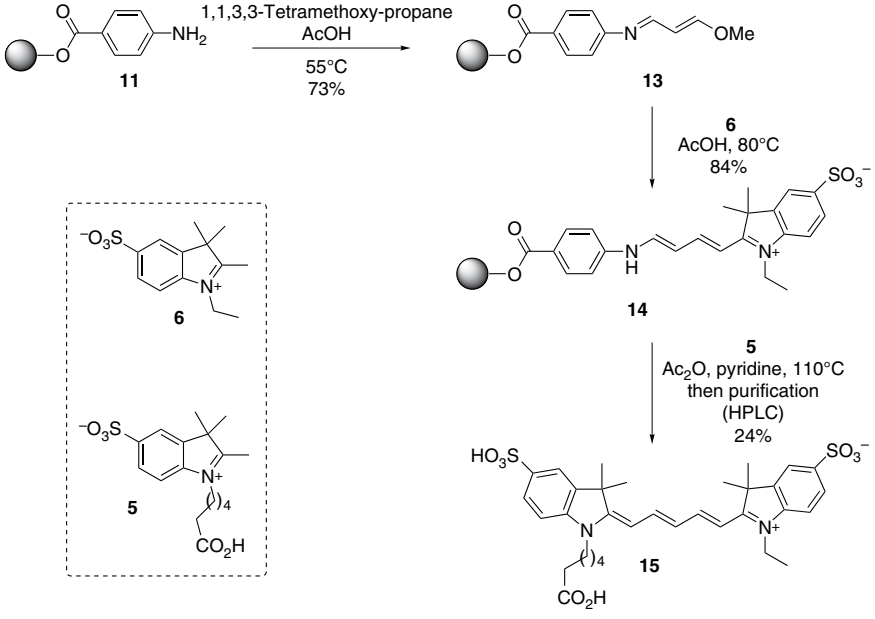
Target compound:
(supported aniline)



Available reagents and solvents:



Scheme 10.3



Scheme 10.4

which is isolated in 84% yield after filtration and drying. The latter is treated with **5** (0.5 equiv.) followed by Ac_2O and pyridine, and the resulting mixture is heated to 110 °C for 15 min. After cooling to rt, EtOAc is added, resulting in the formation of a gum (**point 2**) which is washed with CH_2Cl_2 until a blue powder is

obtained (**point 3**).[3] After purification by HPLC, compound **15** is obtained in high purity and 24% yield.[4]

Question 10.11: Write the structure of 1,1,3,3-tetramethoxy-propane.

Question 10.12: Write a plausible mechanism for the formation of **13** from **11**.

Question 10.13: Write the structure of **14b** and suggest a plausible mechanism accounting for its formation.

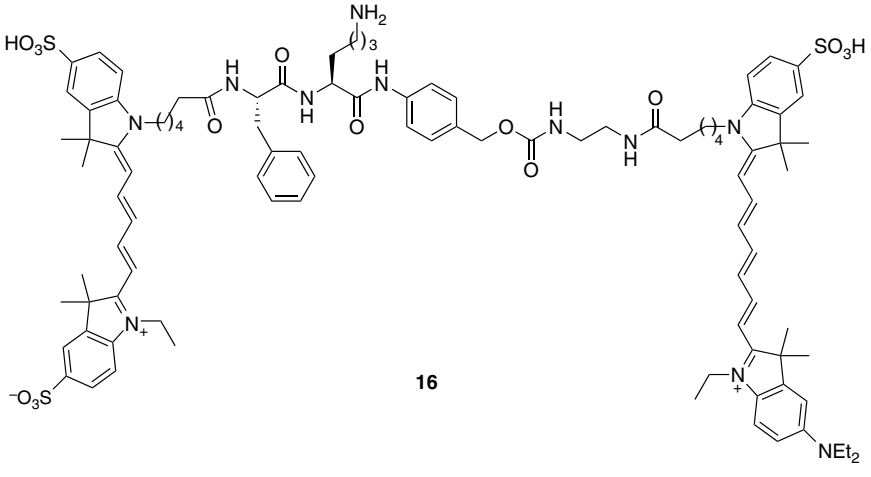
Question 10.14: Indicate the composition of solid particles and filtrate obtained at **point 1**.

Question 10.15: Indicate the composition of the gum at **point 2**.

Question 10.16: Indicate the composition of the blue powder obtained at **point 3**.

10.3 Synthesis and Evaluation of Cyanine-Based NIR Peptide Probes for Monitoring Cathepsin B Activity

Molecular construct **16**, combining a fluorescent dipeptide with a quencher through a suitable linker, was investigated as a NIR "turn-on" probe for monitoring cathepsin B activity (Scheme 10.5) [3]. Its preparation requires the synthesis of **17**, obtained from the reagents and solvents listed in Scheme 10.6.

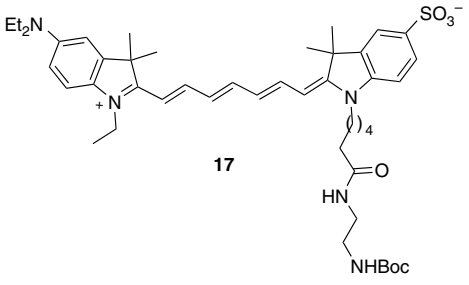


16

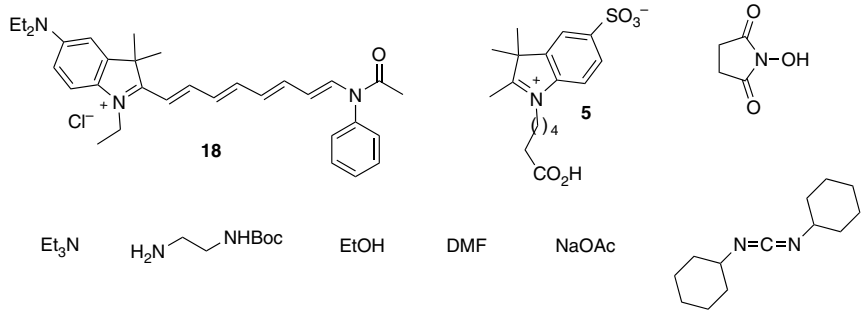
Scheme 10.5

3 The overall procedure is slightly modified as compared to the one originally described.
4 In the original article, compound **15** was obtained with sufficient purity without HPLC purification, although the nature of counterions is undermined (see Ref. [1] for details).

Target compound:



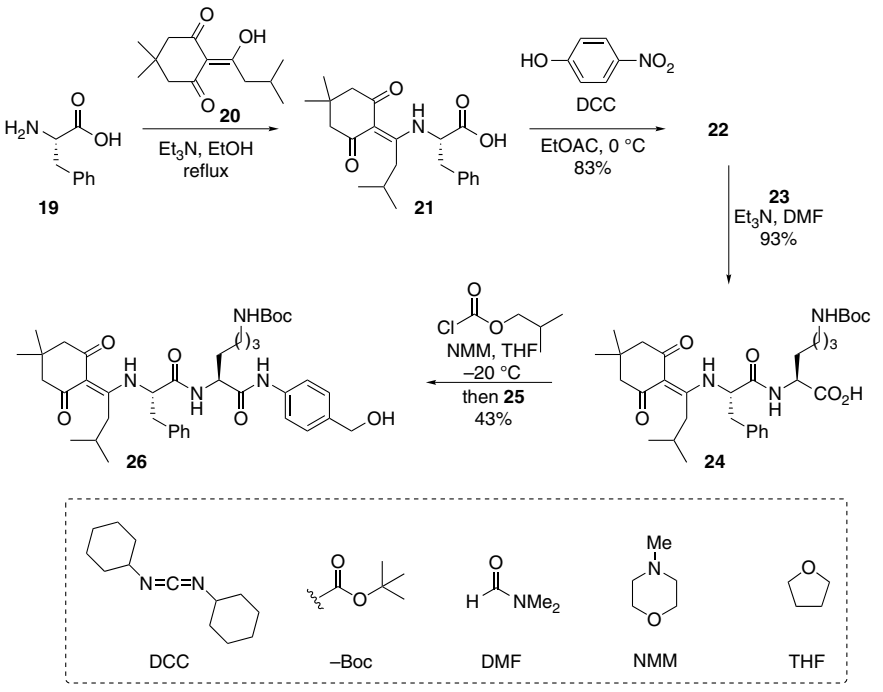
Available reagents and solvents:



Scheme 10.6

Question 10.17: Suggest a plausible reaction sequence allowing the preparation of **17** from **5** and **18** using the reagents and solvents described in Scheme 10.6.

The following steps toward probe **16** are described in Scheme 10.7. A solution of phenylalanine **19** in EtOH is treated with **20** (1.3 equiv.) followed by Et_3N (1.5 equiv.). After stirring to reflux overnight, the mixture is diluted with EtOAc and washed with aq. HCl (1M). The layers are separated and the organic layer is dried over $MgSO_4$ and filtered, and the solvent is evaporated under reduced pressure. Purification by chromatography on silica gel affords **21** in quantitative yield. The latter is dissolved in EtOAc and cooled to 0 °C; then 4-nitrophenol (1.2 equiv.) and DCC (1.2 equiv.) are added. The mixture is stirred at rt for 30 min, the solid is filtered off (**point 1**), and the filtrate is evaporated under reduced pressure. The crude product is purified by column chromatography on silica gel to yield 83% of **22**. A solution of the latter in demethylformamide (DMF) is treated with **23** (1 equiv.) followed by Et_3N (2 equiv.). After stirring at rt overnight, the solvent is evaporated under reduced pressure and the crude residue is purified by chromatography on silica gel, to afford **24** in 93% yield. This compound is dissolved in tetrahydrofuran (THF), cooled to –20 °C, and treated with NMM (1.5 equiv.) and with isobutyl chloroformate (2.0 equiv.). The mixture is stirred at –20 °C for 20 min (**point 2**), and then a solution of **25** (1.3 equiv.) in THF is added dropwise.



Scheme 10.7

After stirring at rt for 1 h, the solvent is removed by evaporation under reduced pressure and the crude product is purified by chromatography on silica gel to yield 43% of **26**.

Question 10.18: Write a plausible mechanism for the formation of **21** from **19** and **20**.

Question 10.19: Write the structure of **22**.

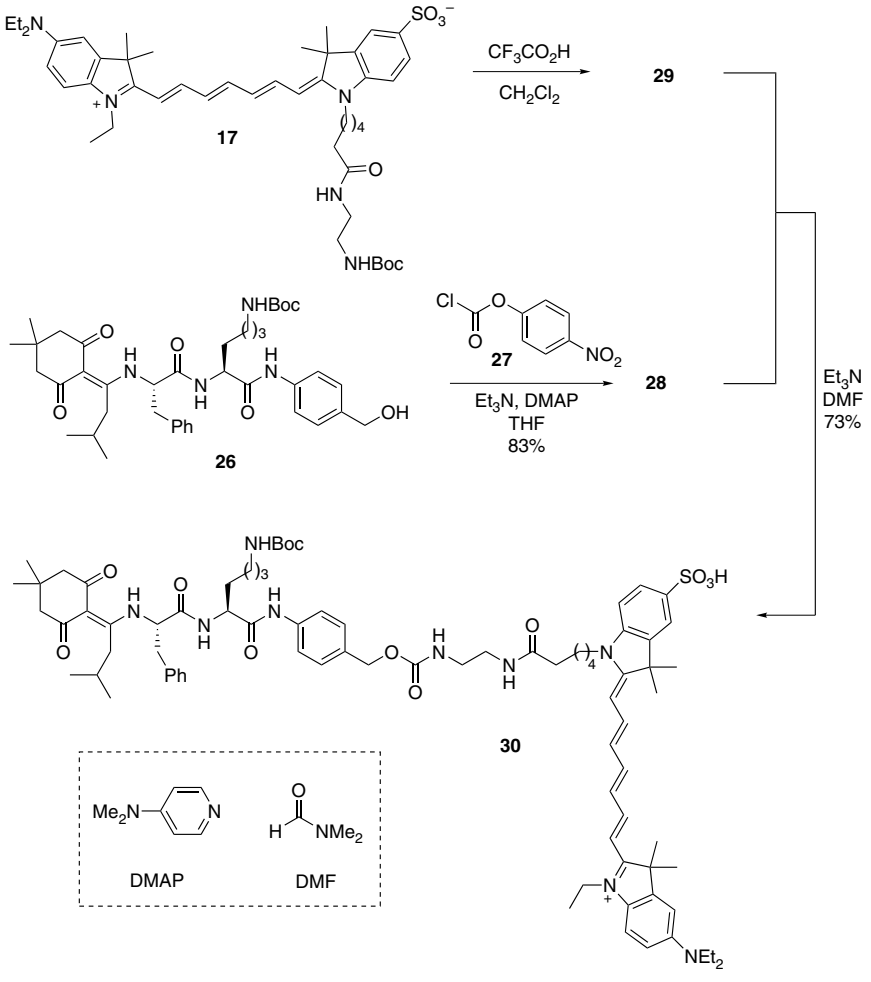
Question 10.20: Write the structure of **23**.

Question 10.21: Indicate the composition of the solid obtained at **point 1**.

Question 10.22: Write the structure of **25**.

Question 10.23: Write the mechanism of formation of **26** from **24** and **25** and mention the structure of the intermediate obtained at **point 2**.

A solution of **26** in THF is cooled to 0 °C and treated with Et_3N (1.5 equiv.), DMAP (0.1 equiv.), and a solution of **27** (1.5 equiv.) in THF (Scheme 10.8). After stirring at rt for 1 h, the mixture is diluted with EtOAc and washed with aq. saturated NH_4Cl and aq. saturated NaCl solutions. The layers are separated and the

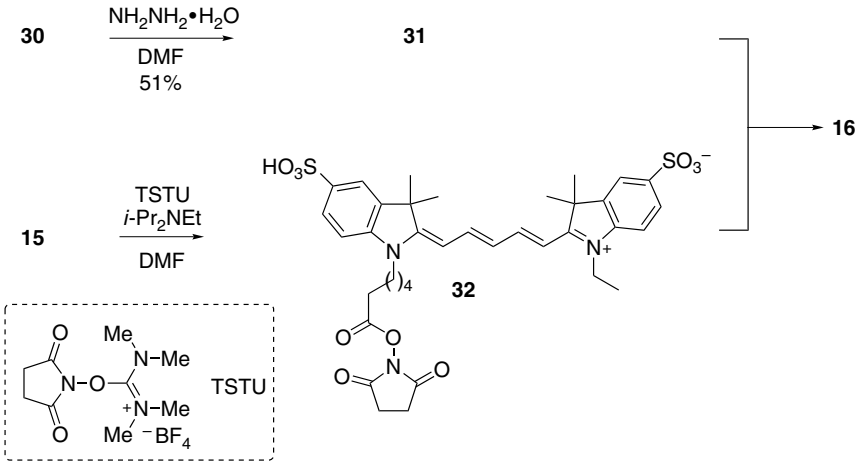


Scheme 10.8

organic phase is dried with $MgSO_4$ and filtered, and the solvent is removed under reduced pressure. The residue is purified by silica gel chromatography, affording **28** in 83% yield as a yellow solid. In parallel, a solution of **17** in CH_2Cl_2 is treated with a large excess of CF_3CO_2H at rt for 5 min. Evaporation of the volatiles under reduced pressure furnishes **29**, which is dissolved in DMF and reacted with **28** (1.0 equiv.), followed by Et_3N (1.9 equiv.). The mixture is stirred for 2 h at rt and the solvent is removed under reduced pressure, leading to a residue which is purified by chromatography on silica gel yielding 73% of **30** as a blue solid.

Question 10.24: Write the structure of **28**.

Question 10.25: Write the structure of **29**.



Scheme 10.9

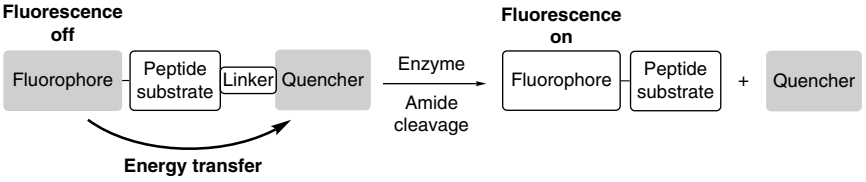
A solution of **30** in DMF (Scheme 10.9) is treated with an aqueous solution of hydrazine monohydrate (65% w/w, 3.8 equiv.). The mixture is stirred for 4 h at rt and the solvent is removed under reduced pressure to give a residue which is purified by chromatography on silica gel, leading to **31** as a blue solid in 51% yield. In parallel, dye **15** (Scheme 10.4) is dissolved in DMF and treated with *i*-Pr_2NEt (6 equiv.) followed by TSTU (1.5 equiv.). After stirring for 1 day at rt, the resulting solution containing **32** is treated with **31** (1.2 equiv.) followed by *i*-Pr_2NEt (5 equiv.) and the mixture is stirred for 2 h at rt.[5] Evaporation of the solvent under reduced pressure followed by purification by HPLC affords the desired probe **16** (Scheme 10.5) in 12% yield.

Question 10.26: Write the structure of **31** and suggest a plausible mechanism accounting for its formation from **30**.

Question 10.27: Write a plausible mechanism for the formation of **32** from **15**.

The general principle for monitoring enzymatic activity with a fluorescent "turn-on" probe is summarized in Scheme 10.10. The probe consists in a fluorophore moiety attached to a quencher through a suitable peptide substrate and linker. Upon excitation of the fluorophore with light, energy is transferred to the nearby quencher, which relaxes in a nonradiative manner, thereby switching off the fluorescence of the probe. In the presence of a target enzyme able to recognize the peptide substrate, an amide bond is hydrolyzed, thus resulting in the release of the quencher away from the probe with concomitant switching on of the fluorescence of the probe.

5 The procedure described here has been adapted from Ref. [4].



Scheme 10.10

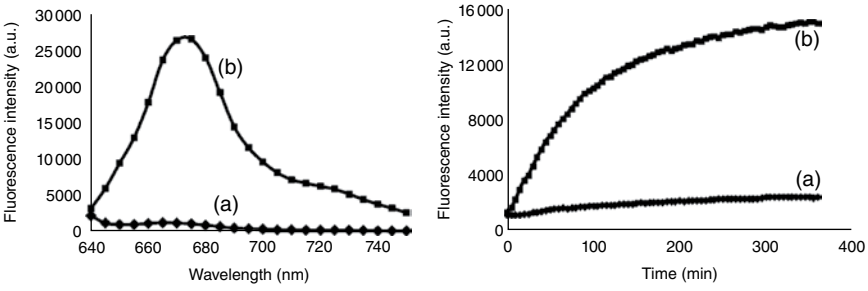


Figure 10.1 (Left) Fluorescence spectra (excitation at 620 nm) of probe **16** in PBS buffer (pH = 6.0) containing 10% DMSO (a) and dye **15** in PBS buffer (pH = 6.0) (b). (Right) Evolution of fluorescence intensity (a.u.) at 670 nm (excitation at 620 nm) with time upon incubation of probe **16** in the absence (a) or presence (b) of cathepsin B in activity buffer solution (pH = 6.0) containing 10% DMSO.[6]

In order to evaluate the ability of compound **16** to act as a "turn-on" probe for monitoring cathepsin B activity, its fluorescence properties were studied. Figure 10.1(A) shows a comparison between the fluorescence spectrum of molecular construct **16** and dye **15** (Scheme 10.4), while Figure 10.1(B) shows the evolution of fluorescence intensity of **16** with time in the absence or presence of cathepsin B. Noteworthy is the fact that enzyme processing of probe **16** results in the formation of CO_2, together with carboxylic acid **33**, amine **34**, and a product **35** (molecular formula C_7H_7N).

Question 10.28: Identify the fluorophore, quencher, peptide substrate, and linker in probe **16** (see Scheme 10.5).

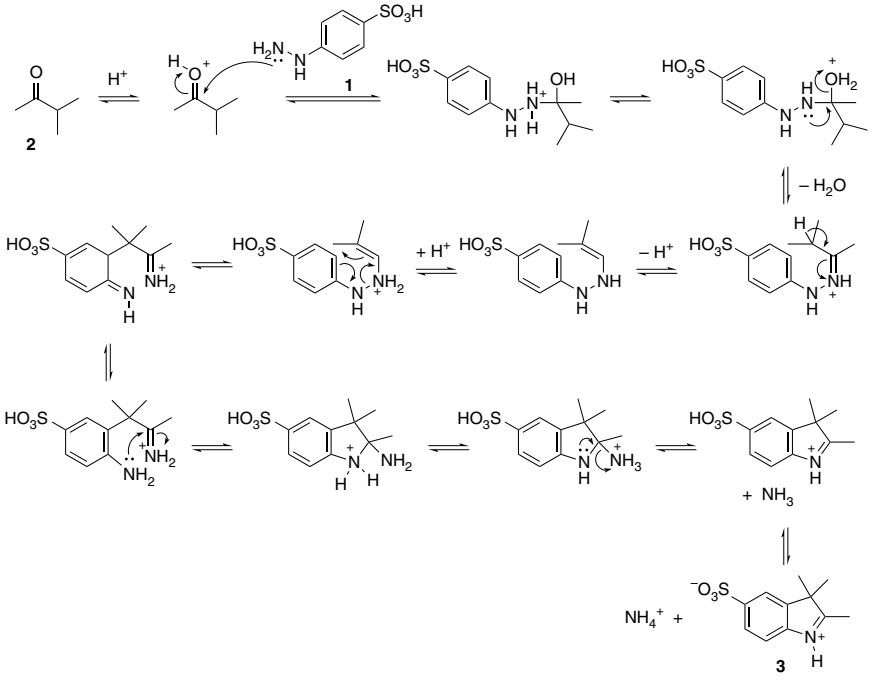
Question 10.29: Describe and rationalize the results reported in Figure 10.1.

Question 10.30: Write the structure of compounds **33**, **34**, and **35** and explain their formation with a mechanism.

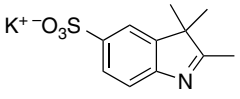
6 This figure is adapted from Ref. [3].

Answers

Question 10.1:



Question 10.2:



Question 10.3:

Trituration with *i*-PrOH at **point 1** allows removal of excess bromohexanoic acid and potassium bromide (KBr) formed during the reaction.

Question 10.4:

Trituration with acetone at **point 2** allows removal of potassium iodide (KI) formed during the reaction of **4** with EtI.

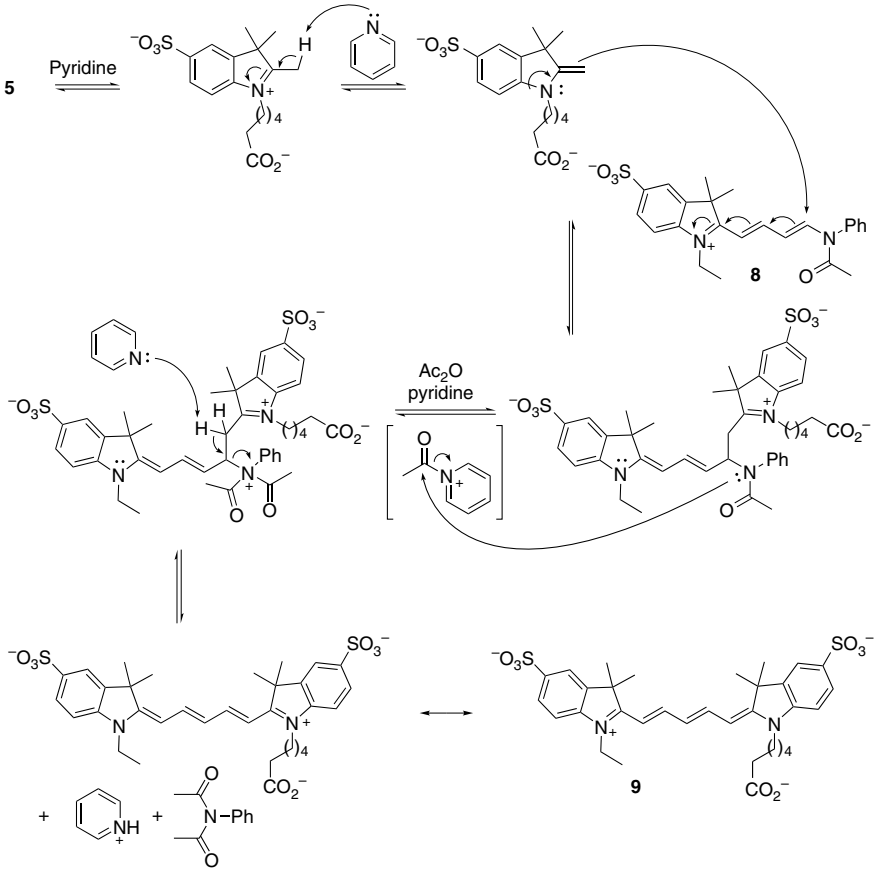
Question 10.5:

Trituration with EtOAc at **point 3** allows removal of excess malonaldehyde dianilide hydrochloride **7**.

Question 10.6:

The signal observed around 286 nm by UV–vis absorption spectroscopy presumably corresponds to the combination of absorption bands of both **6** and **7**, which disappear as the reaction proceeds. As the new product **8** is formed, a novel band centered at 445 nm appears, thus revealing extended conjugation.

Question 10.7:



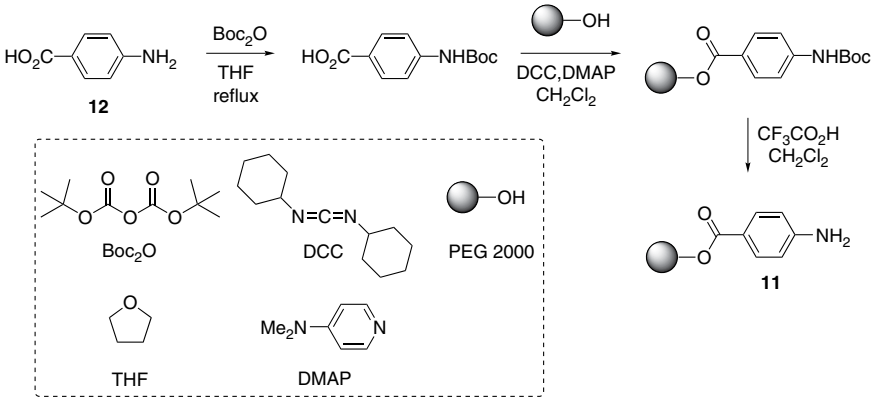
Question 10.8:

The absorption band at 445 nm, corresponding to **8**, disappears as this compound reacts and transforms into **9**, whose absorption band is redshifted to about 650 nm because of its extended conjugation.

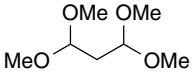
Question 10.9:

The molar mass of ethylene glycol unit $-OCH_2CH_2O-$ is $60\,g\,mol^{-1}$; therefore, PEG 2000 contains approximately 33 ethylene glycol units ($n \simeq 33$).

Question 10.10:

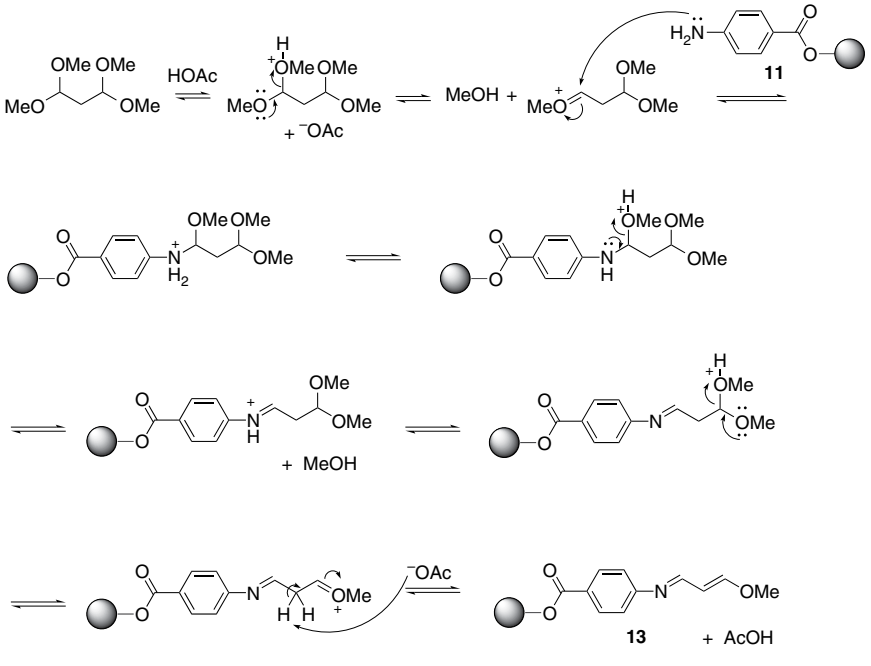


Question 10.11:

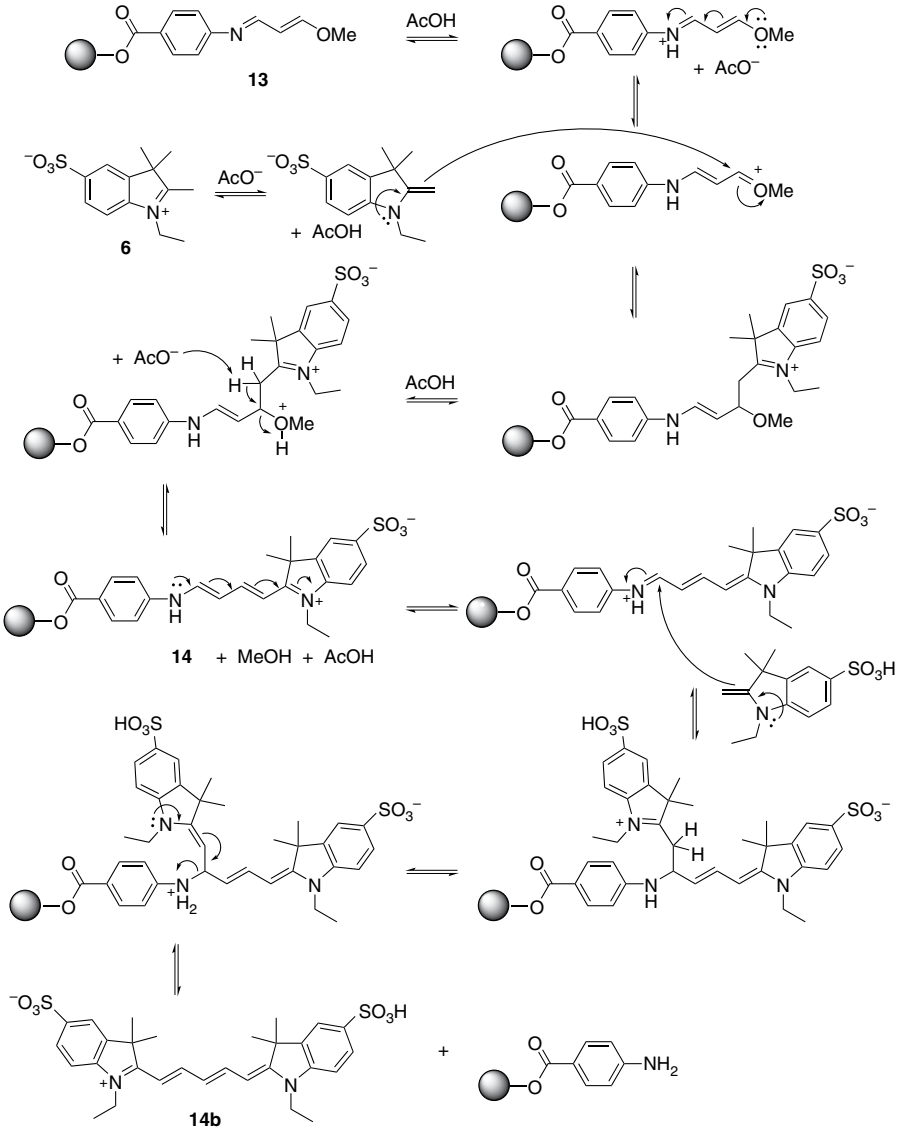


1,1,3,3-Tetramethoxypropane

Question 10.12:



Question 10.13:

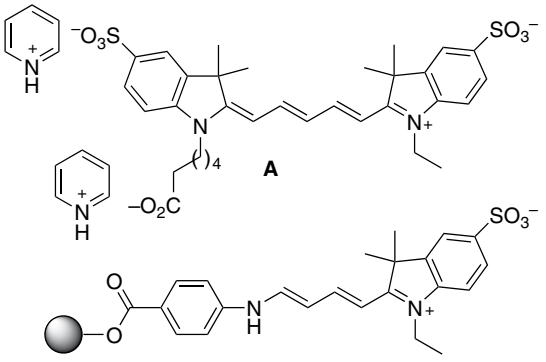


Question 10.14:

The solid particles correspond to dye **14b**, while the filtrate is composed of PEG-bound dye **14**, well soluble in EtOAc.

Question 10.15:

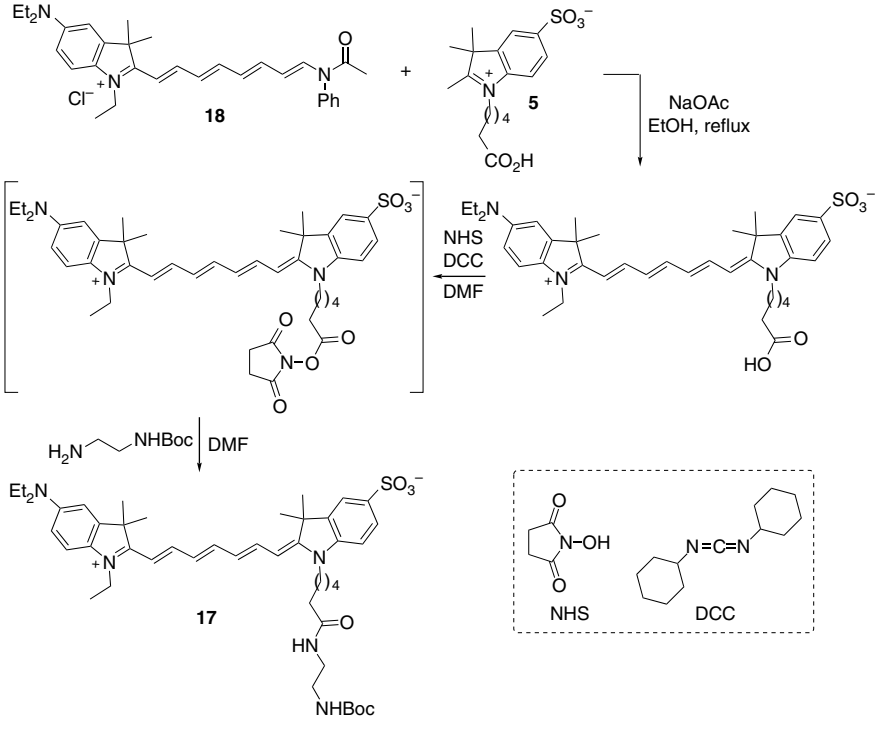
The gum at **point 2** mostly contains salt **A** (precursor to **15**), insoluble in EtOAc, presumably with some amount of excess **14**, Ac_2O, and pyridine trapped inside.



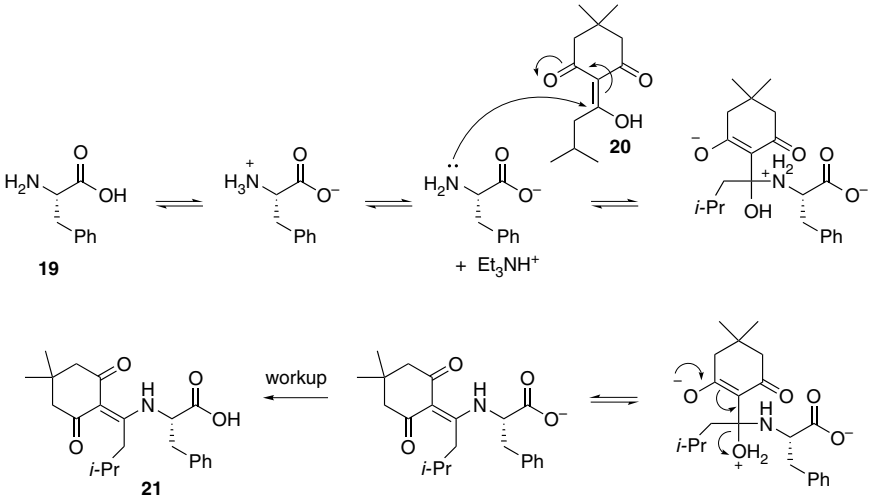
Question 10.16:

The blue powder at **point 3** mostly contains **A**, all other impurities having been washed out with CH_2Cl_2.

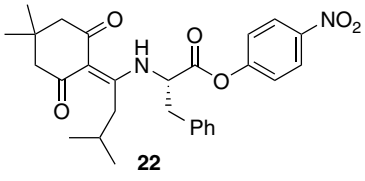
Question 10.17:



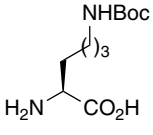
Question 10.18:



Question 10.19:

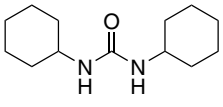


Question 10.20:

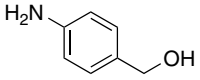


Question 10.21:

The solid obtained at **point 1** is the urea derived from DCC, formed during amide coupling.



Question 10.22:



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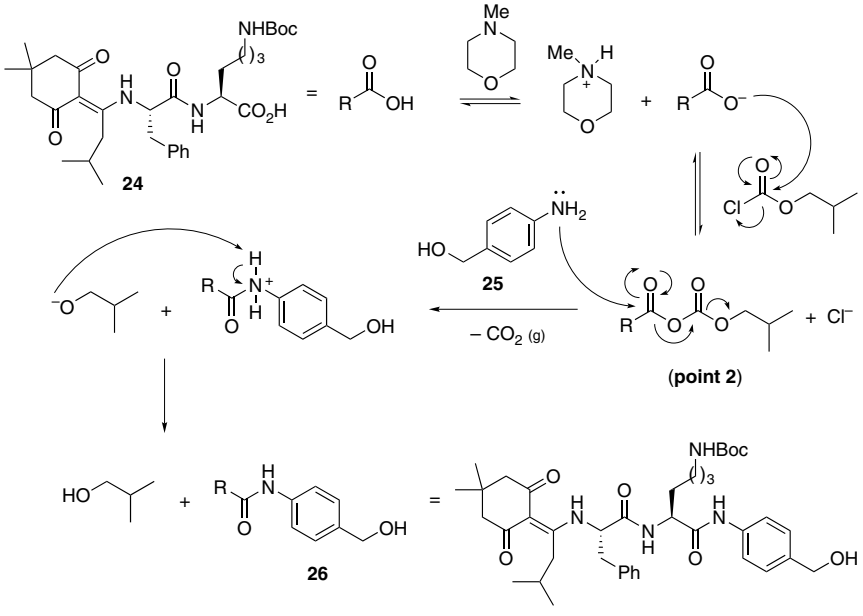

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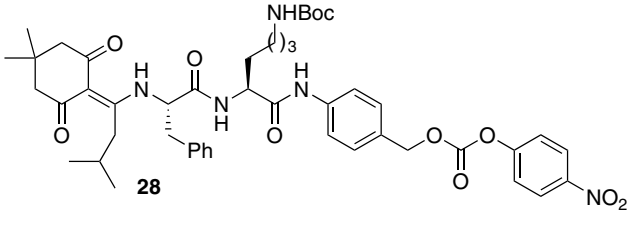

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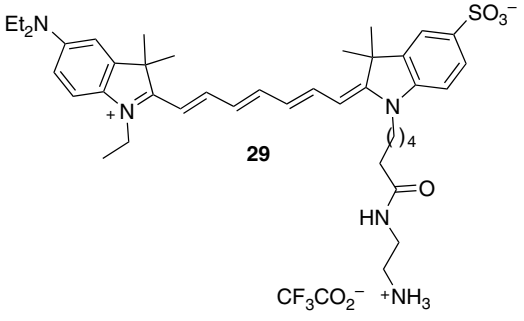

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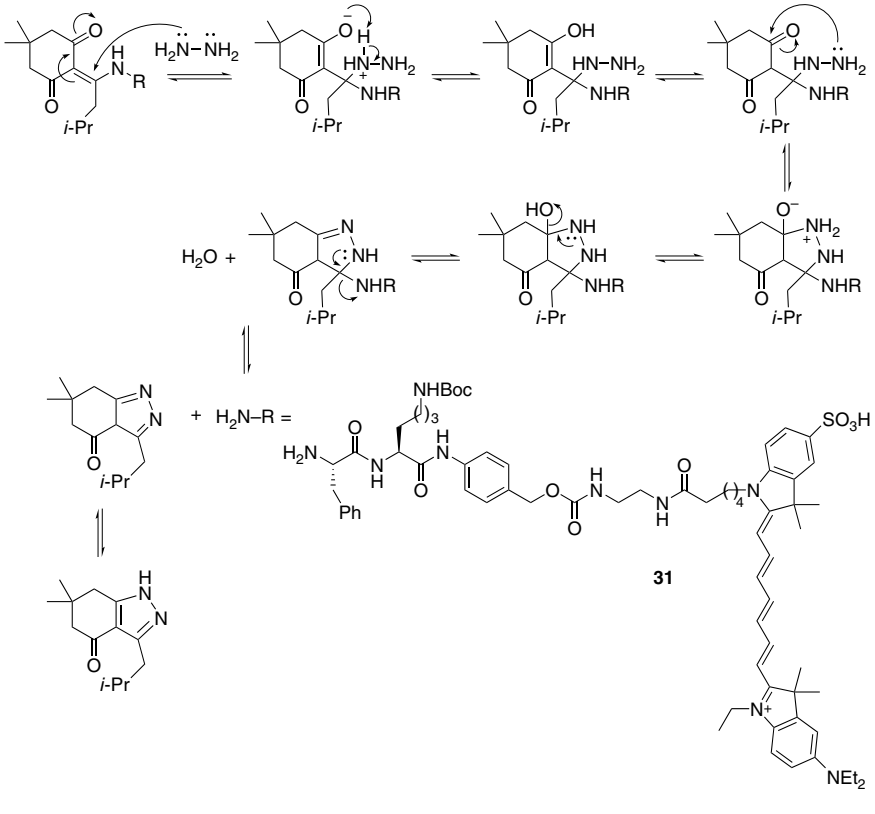

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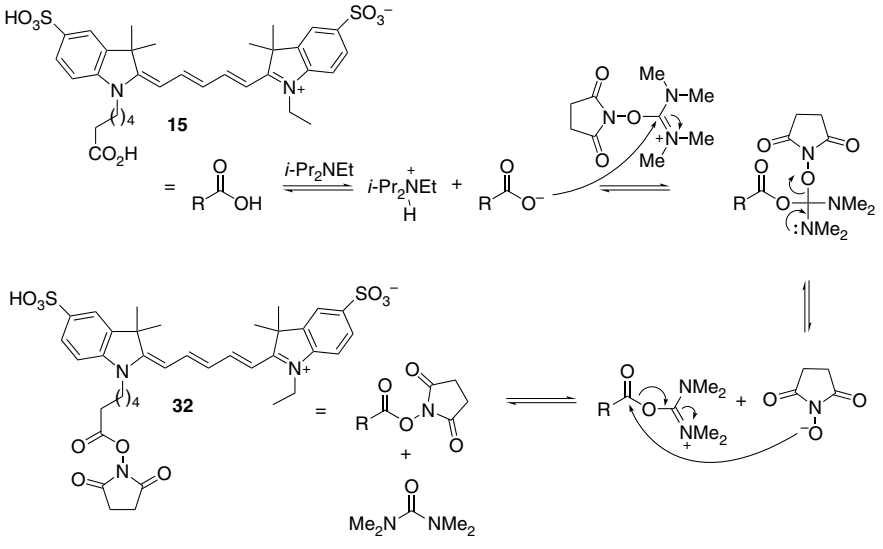
Question 10.27:



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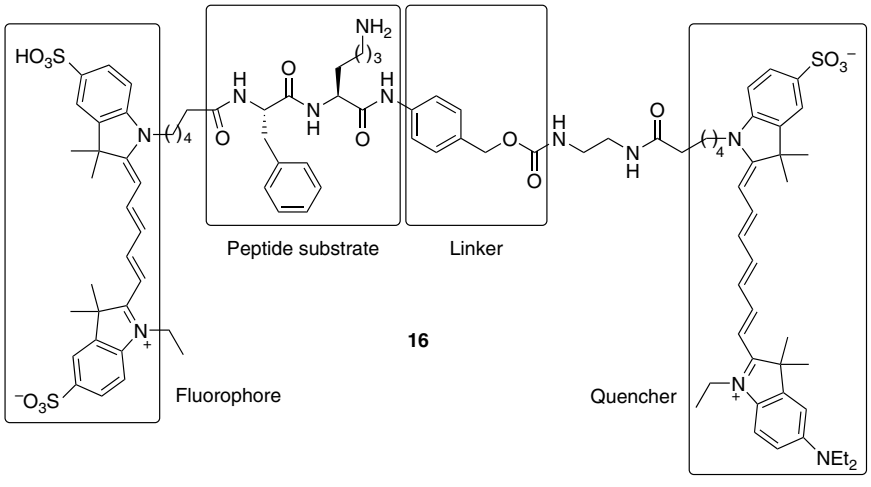


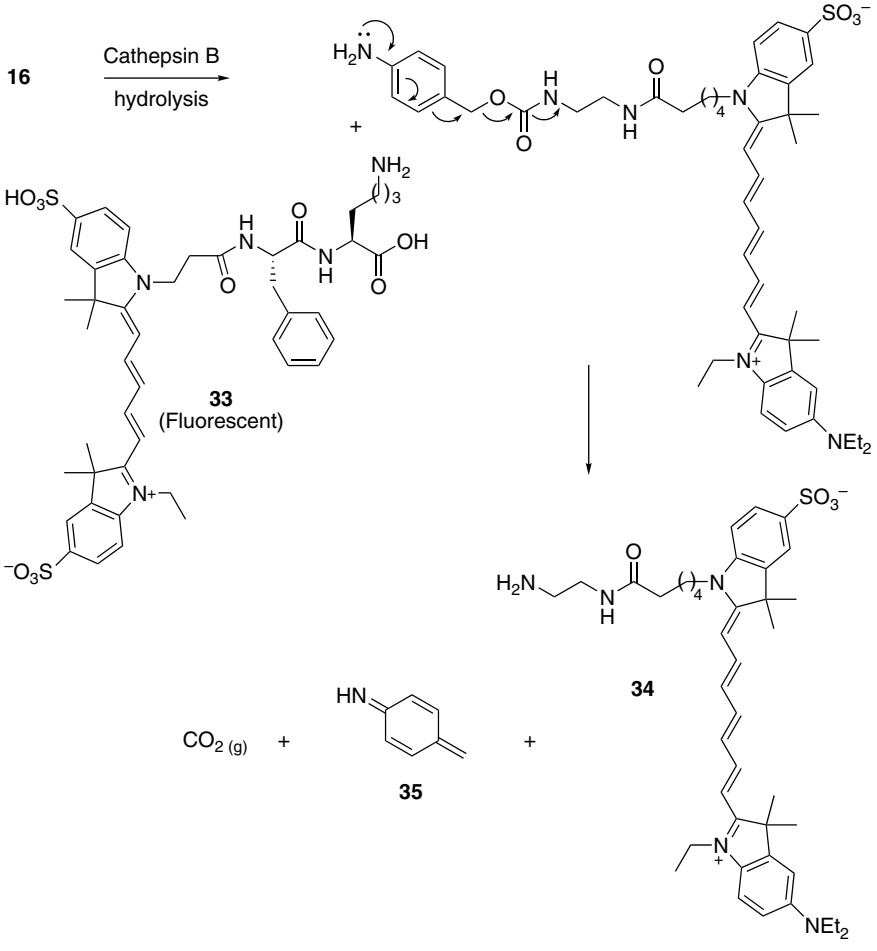
Question 10.29

Upon excitation at 620 nm, the fluorescence spectrum of dye 15 shows a strong emission band centered around 670 nm (Figure 10.1A, curve b), while probe 16 does not show any signal under similar conditions (Figure 10.1A, curve a) as a consequence of the proximity between the fluorophore and quencher moieties.

When standing in the activity buffer containing 10% DMSO for over 350 min, probe 16 remains nonfluorescent (Figure 10.1B, curve a). In contrast, its incubation

in the presence of cathepsin B under the same reaction conditions results in a steady increase of fluorescence intensity at 670 nm (Figure 10.1B, curve b). This observation evidences a separation between fluorophore and quencher moieties, through amide bond hydrolysis after recognition of dipeptide phenylalanine-arginine by the enzyme.

Question 10.30:



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4 Patrick, M.J., Janjic, J.M., Teng, H., O'Hear, M.R., Brown, C.W., Stokum, J.A., Schmidt, B.F., Ahrens, E.T., and Waggoner, A.S. (2013) Intracellular pH measurements using perfluorocarbon nanoemulsions. *J. Am. Chem. Soc.*, **135** (49), 18445–18457.

11

Total Synthesis of Stemoamide

Plants from the *Stemona* (Stemonaceae) family such as *Stemona tuberosa*, *Stemona japonica*, or *Stemona pierrei* are used in Chinese traditional medicine for the treatment of cough and as an insecticide. The roots and rhizomes of these plants contain a number of alkaloids, among which is (–)-stemoamide, isolated in 1992 from the roots of *Stemona tuberosa* (Figure 11.1) [1].

In addition to these interesting biological activities, the construction of the polycyclic structure of *stemona* alkaloids constitutes a synthetic challenge, and a number of groups have developed access to different members of this family. This chapter presents some synthetic approaches toward stemoamide.

Question 11.1: Assign the stereochemical descriptor (using Cahn–Ingold–Prelog rules) of every asymmetric carbon atom of (–)-stemoamide.

11.1 Radical Approach to the Construction of the Tricyclic Core of Stemoamide

This racemic approach begins with hydration of 2,3-dihydrofuran with aq. HCl (0.2M) to yield compounds **1** and **2** (Scheme 11.1). The crude mixture obtained after workup is directly reacted with vinyl magnesium bromide in tetrahydrofuran (THF), to afford a separable mixture of acetal **3** and diol **4** in 18% and 10% overall yield, respectively [2].

Question 11.2: Explain the formation of compounds **1**, **2**, **3**, and **4** with a plausible mechanism.

Succinimide **6** is synthesized from alcohol **5** (derived from **4**) in 91% yield by reaction with succinimide, diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (Scheme 11.2). Reduction with $LiBEt_3H$ leads to **7**, which is treated with PTSA in thiophenol to afford thio-aminocetal **8** with concomitant loss of the silyl protecting group. Esterification with methacrylic acid in the presence of DCC and DMAP gives **9**, which is transformed into **10** by ring-closing metathesis performed with ruthenium catalyst **GII**.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

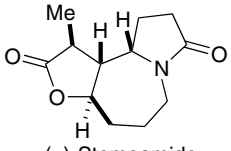
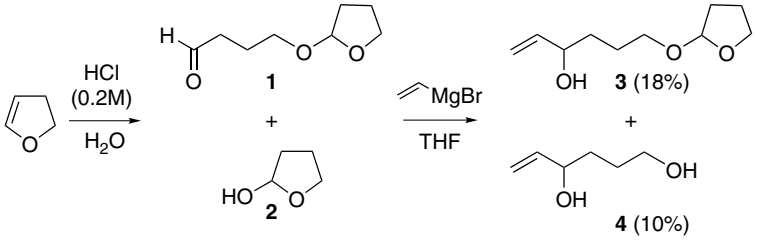
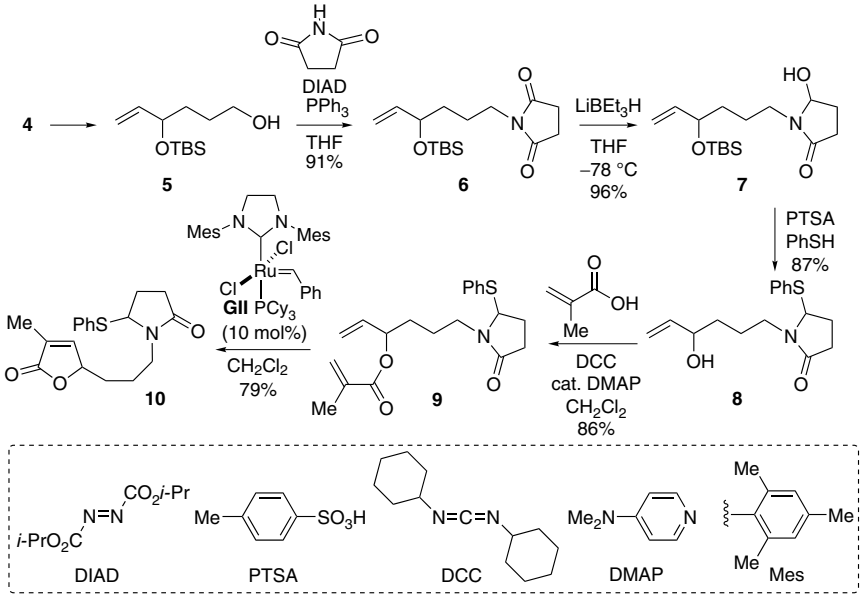


Figure 11.1 Chemical structure of (–)-Stemoamide.



Scheme 11.1



Scheme 11.2

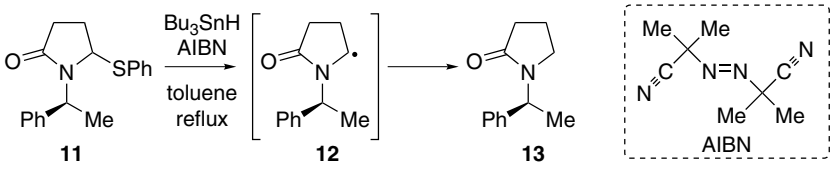
Question 11.3: Suggest a plausible reaction sequence allowing formation of **5** from **4**.

Question 11.4: Write a plausible mechanism for the formation of **6** from **5**.

Question 11.5: Write the mechanism of transformation of **7** into **8**.

Question 11.6: Write the mechanism of transformation of **9** into **10**.

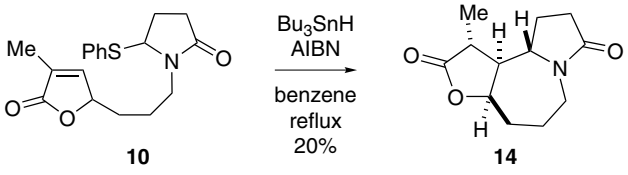
Phenylsulfanyl group is, under appropriate conditions, a good radical precursor. For example, when **11** is heated in refluxing toluene in the presence of tributyltinhydride[1] and a catalytic amount of AIBN, radical **12** is formed, and then reduced to afford **13** in 53% overall yield (Scheme 11.3) [3].



Scheme 11.3

Question 11.7: Detail the steps involved in the transformation of **11** into **13** (initiation, propagation, termination).

Based on this type of reactivity, compound **10** is refluxed in benzene under dilute conditions (C ≈ 6 mM) in the presence of a catalytic amount of AIBN, while a solution of Bu_3SnH is slowly added over 2 h with a syringe pump (Scheme 11.4). Tricyclic compound **14**, a diastereoisomer of stemoamide, is obtained in 20% yield.



Scheme 11.4

Question 11.8: Suggest a plausible reaction mechanism accounting for the formation of **14** (stereoselectivity of the cyclization will not be discussed).

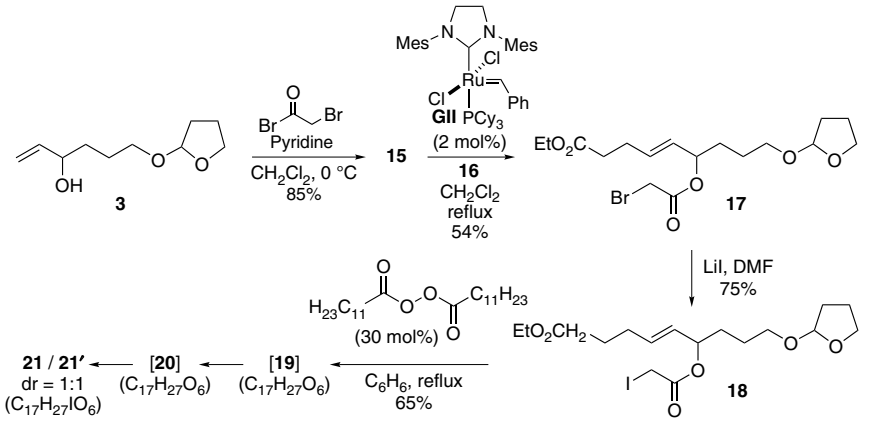
Question 11.9: Why is such a reaction performed under dilute conditions?

Question 11.10: Why are both rate and order of reagents addition, that is, slow introduction of a solution of Bu_3SnH into a solution of **10** and AIBN, crucial to the success of the reaction? Why should the solvent be degassed?

11.2 Formal Synthesis of (±)-Stemoamide

Previously synthesized allylic alcohol **3** (Scheme 11.1) is treated with bromoacetyl bromide in the presence of pyridine to afford compound **15** (Scheme 11.5).

1 The original procedure involves utilization of tributyltindeuteride (Bu_3SnD).



Scheme 11.5

The latter reacts with Grubbs second-generation catalyst **GII** and compound **16** in refluxing dichloromethane, yielding alkene **17**. After reaction with lithium iodide, **18** is obtained and reacted with dilauroyl peroxide in refluxing benzene. Under these conditions, a 1 : 1 mixture of diastereomeric lactones **21**/**21′** (molecular formula $C_{17}H_{27}IO_6$) is formed via radical intermediates **19** and **20** (both with molecular formula $C_{17}H_{27}O_6$) [4].

Question 11.11: Write the structure of compounds **15** and **16**.

Question 11.12: Which radical species are generated upon heating dilauroyl peroxide?

Question 11.13: Indicate the reactivity of these radicals with iodide **18** and give the structure of radical intermediate **19**.

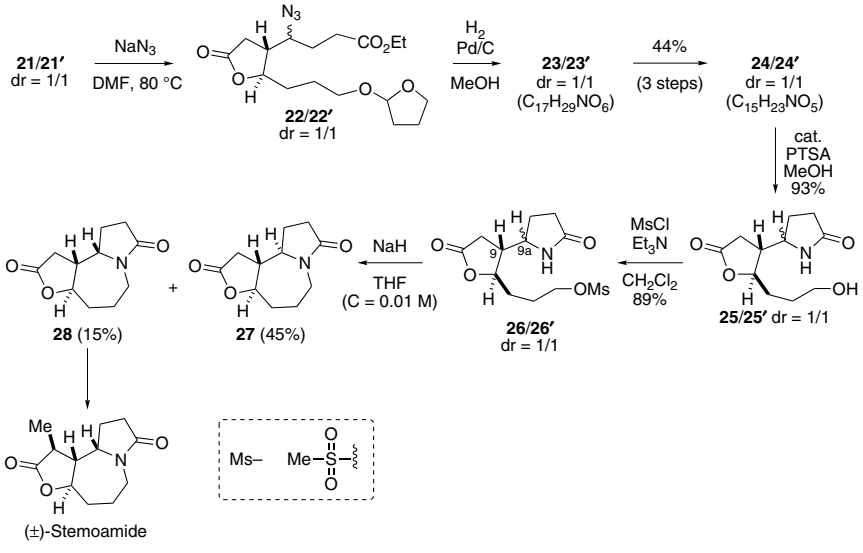
Question 11.14: Write the structure of radical intermediate **20** and compounds **21**/**21′**. Explain their formation with a plausible mechanism (stereoselectivity of the reaction will not be discussed).

Action of sodium azide on compounds **21**/**21′** leads to azido-esters **22**/**22′**, which undergo catalytic hydrogenation, to yield compounds **23**/**23′** (molecular formula $C_{17}H_{29}NO_6$) that spontaneously transform into compounds **24**/**24′** (molecular formula $C_{15}H_{23}NO_5$) (Scheme 11.6).[2] After a few steps of functional group interconversion, compound **26**/**26′** are obtained and treated with NaH in THF under dilute conditions, leading to two tricyclic diastereomeric compounds **27** and **28** in 45% and 15% yield, respectively.[3]

---

2 In the original article, azides **22**/**22′** were obtained together with diastereomeric alkenes originating from elimination of iodine. This mixture could only be separated after catalytic hydrogenation and spontaneous transformation of **23**/**23′** to **24**/**24′**.

3 The original work reports isolation of an inseparable 3 : 1 mixture of **27** and **28**, respectively, in 60% overall yield.



Scheme 11.6

Question 11.15: Write the structure of compounds **23**/**23′** and **24**/**24′**.

Question 11.16: Draw the presumably most stable conformation of compounds **26** and **26′** using Newman projection along C(9)-C(9a) axis.

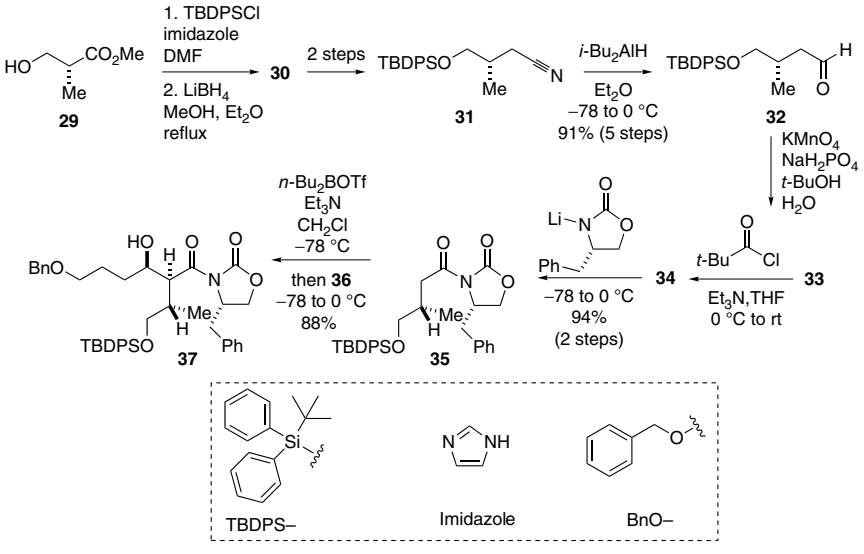
Question 11.17: From this result, justify the different ease of cyclizations observed for the two diastereoisomers **26** and **26′**.

Question 11.18: Suggest plausible reaction conditions allowing preparation of stemoamide from **28**. Justify the expected diastereoselectivity.

11.3 Enantioselective Total Synthesis of (–)-Stemoamide

Enantiomerically pure hydroxy ester **29** is treated with TBDPSCl and imidazole in DMF; the crude product is reacted with lithium borohydride and MeOH in refluxing diethyl ether to afford compound **30**, whose infrared spectrum exhibits a broad band centered around 3400 cm^{-1} (Scheme 11.7).[4] This compound is then transformed in two steps into cyanide **31**, and then into aldehyde **32** by reaction with diisobutylaluminium hydride. Action of $KMnO_4$ and NaH_2PO_4 then leads to compound **33**, which is converted by action of pivaloyl chloride into **34** (containing two C═O bonds) that is directly reacted with the lithiated anion of (*S*)-4-benzyl-oxazolidin-2-one to give **35**. The latter reacts first with *n*-Bu_2BOTf and triethylamine, and then with compound **36** to afford alcohol **37** in 88% overall yield [5].

4 Infrared spectrum is not reported in the original article.



Scheme 11.7

Question 11.19: Write the structure of **30** and assign the vibration band observed in its infrared spectrum.

Question 11.20: Suggest plausible reaction conditions allowing the transformation of **30** into **31**.

Question 11.21: Write the structure of **33** and **34** and suggest a mechanism accounting for the formation of **34**.

Question 11.22: Explain the formation of **35** from **34**.

Question 11.23: Write the structure of **36**.

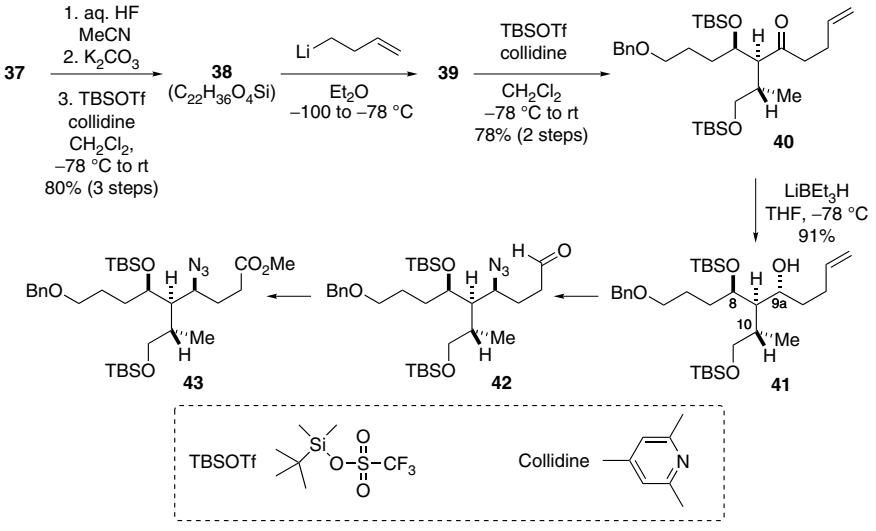
Question 11.24: Suggest a plausible mechanism and transition state accounting for the diastereoselectivity of the transformation of **35** in **37**.

Compound **37** reacts with dilute aq. HF in acetonitrile (**point 1**), followed by careful addition of excess K_2CO_3. After appropriate workup, the crude material (**point 2**) is reacted with TBSOTf (1.3 equiv.) and collidine (1.5 equiv.) in CH_2Cl_2 to afford compound **38** in 80% overall yield (Scheme 11.8).[5] The infrared spectrum of this product, whose molecular formula is $C_{22}H_{36}O_4Si$, shows only one characteristic band around 1770 cm^{-1}.[6]

Compound **38** is then reacted with 4-lithiobutene to afford, after neutralization, compound **39**, which is converted into **40** by TBSOTf (3.2 equiv.) in the presence of collidine (3.4 equiv.). Reduction with lithium triethyl borohydride in

5 The original article describes slightly different conditions for treatment with $NaHCO_3$ as well as isolation of the intermediate preceding reaction with TBSOTf.

6 Infrared spectrum is not reported in the original article.



Scheme 11.8

THF at low temperature gives **41** in 91% yield. Azido aldehyde **42** and azido ester **43** are prepared in a few steps through functional group interconversions.

Question 11.25: Write the structure of **38** as well as its precursors formed at **point 1** and **point 2**.

Question 11.26: Write the structure of **39**.

Question 11.27: Suggest plausible reaction conditions for the preparation of 4-lithiobutene from 4-iodobutene?

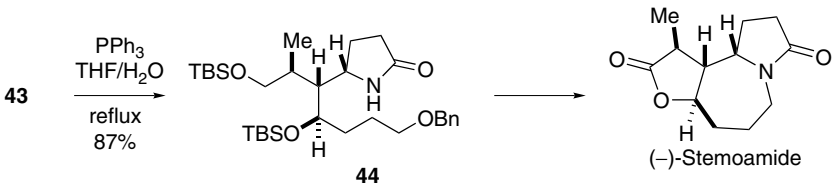
Question 11.28: Suggest an alternative nucleophilic species possibly allowing conversion of **38** to **39**. How could it be prepared?

Question 11.29: Suggest a plausible transition state for the reduction of **40** into **41** accounting for the diastereoselectivity?

Question 11.30: Discuss relative R_f values expected for compounds **40** and **41** on TLC (SiO_2 as the stationary phase).

Question 11.31: Suggest a synthetic sequence allowing transformation of **41** into **42**.

End of the synthesis involves reduction of azide **43** with triphenylphosphine in a refluxing mixture of THF and water to yield a gaseous by-product and lactame **44**, precursor to (–)-stemoamide (Scheme 11.9).

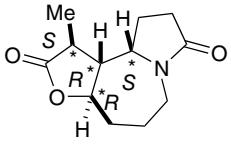


Scheme 11.9

Question 11.32: Suggest a plausible reaction mechanism accounting for the conversion of **43** into **44**.

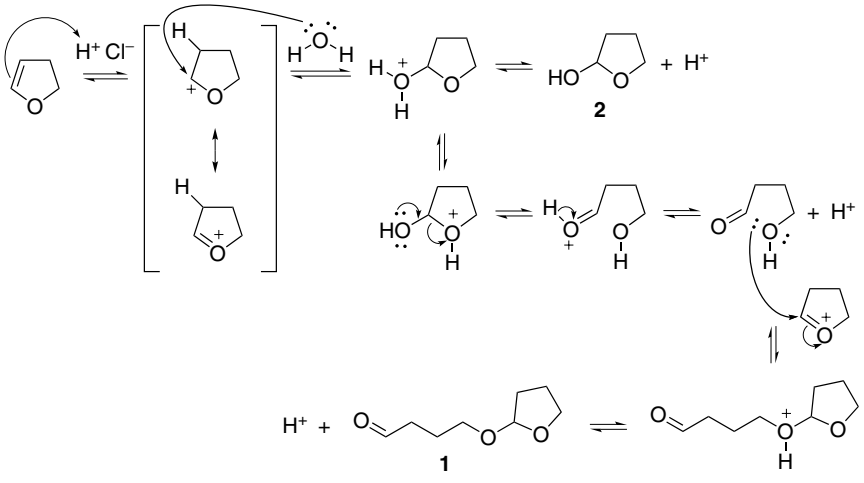
Answers

Question 11.1:

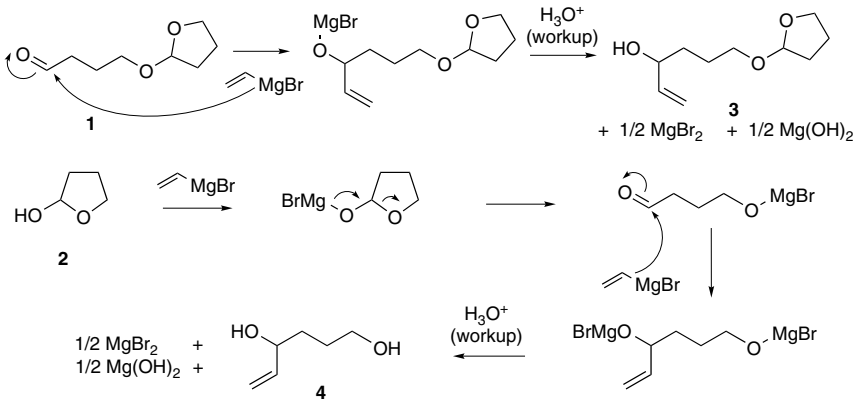


Question 11.2:

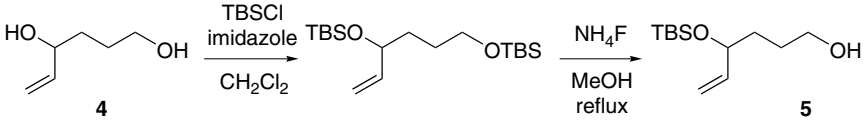
Formation of **1** and **2**:



Formation of **3** and **4**:

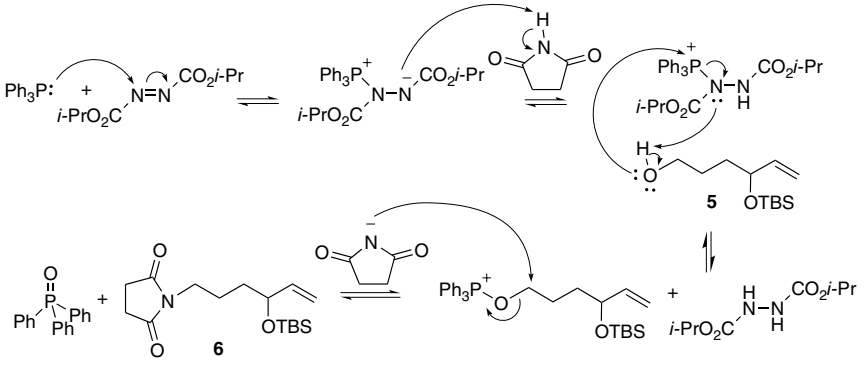


Question 11.3:



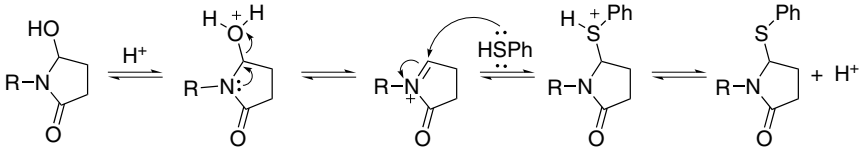
Question 11.4:

Although identification of all intermediates involved in the Mitsunobu reaction is still the subject of extensive studies, a mechanism such as described here accounts for most experimental outcomes [6, 7].

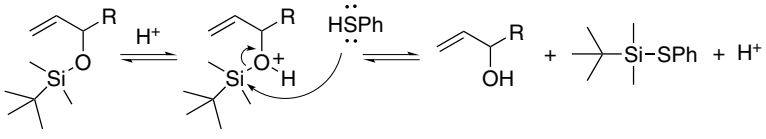


Question 11.5

Thio-aminocetal formation:

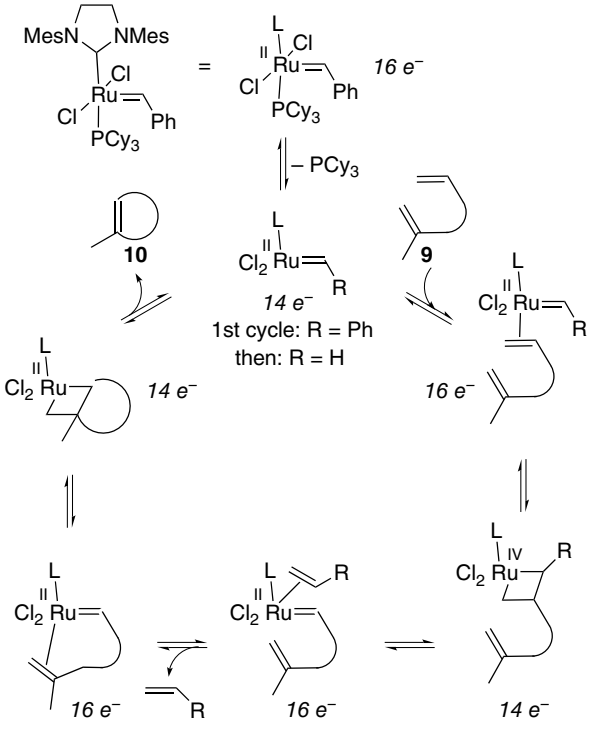


TBS group deprotection:



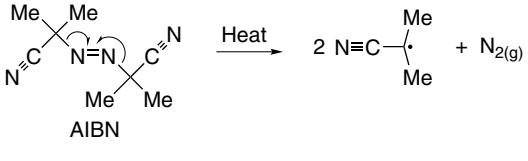
Question 11.6:

The mechanism of this reaction has been the subject of detailed investigations and is commonly described as follows [8, 9].

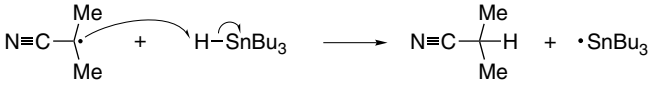


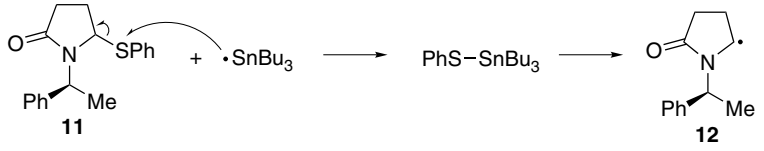
Question 11.7:

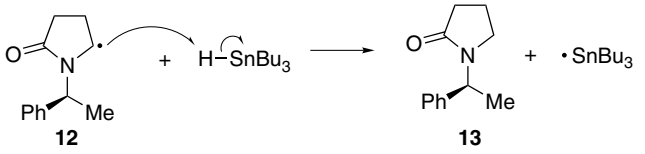
Initiation:



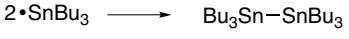
Propagation:





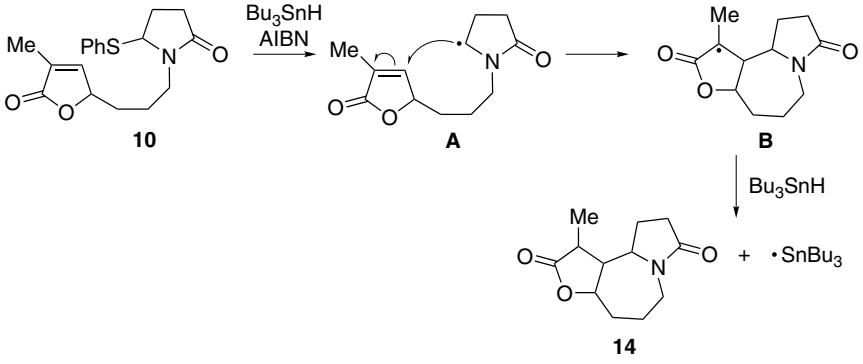


Termination:



Question 11.8:

Following the same sequence as detailed earlier, a radical can be generated on the carbon atom bearing thiophenyl group (**A**). A 5-exo-trig cyclization then leads to a new radical (**B**) that is finally trapped with Bu_3SnH to give **14**.



Question 11.9:

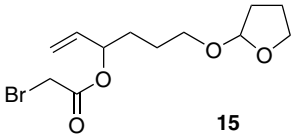
The reaction is performed under dilute conditions to favor intramolecular reaction (**A** → **B**) over intermolecular reduction of **A** with Bu_3SnH.

Question 11.10:

Slow addition of Bu_3SnH allows keeping its concentration as low as possible in order to decrease the rate of reduction of radical **A**. The solvent should be

degassed to remove traces of O_2 that would quickly react with any radical formed during the process.

Question 11.11:



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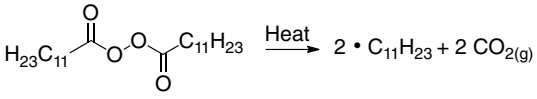
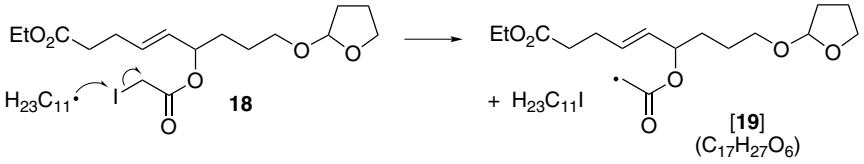
16

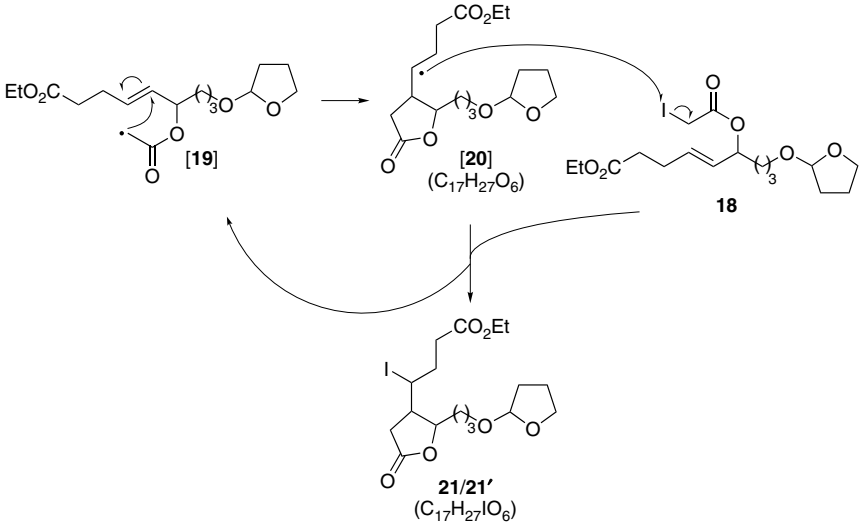
Question 11.12:



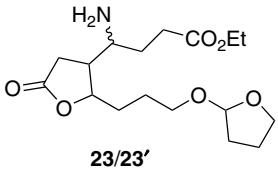
Question 11.13:



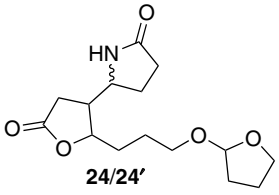
Question 11.14:



Question 11.15:



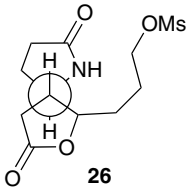
23/23′



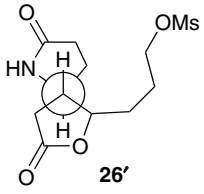
24/24′

Question 11.16:

The most stable conformation for each diastereoisomer is presumably the one that minimizes gauche interactions between the two 5-membered rings.



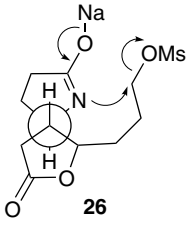
26



26′

Question 11.17:

In the most stable conformation of diastereoisomer **26**, amide function is in close proximity with the carbon atom bearing the mesylate group, thus allowing substitution reaction leading to the tricyclic core of **27**. In contrast, cyclization of **26′** requires reaching a higher energy conformation of type **A**, which significantly decreases the rate of formation of **28**.



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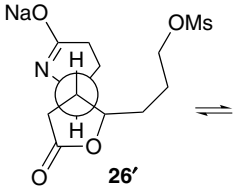
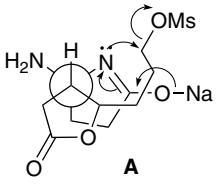
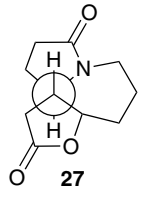


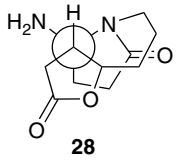
26′



A



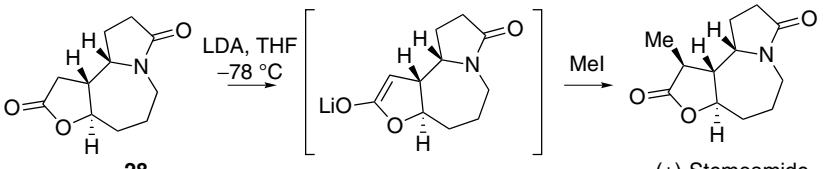
27



28

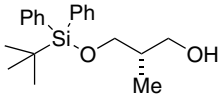
Question 11.18:

The following conditions were used in a former synthesis, and showed that alkylation of intermediate enolate occurs on the less hindered convex face of the tricyclic structure [10].



Question 11.19:

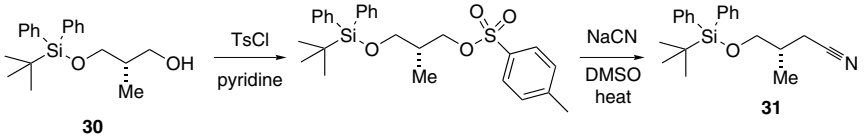
Compound **30** results from protection of the hydroxyl group as a silyl ether followed by reduction of ester moiety. The broad band centered around 3400 cm^{-1} is characteristic of the O—H bond stretching vibration.



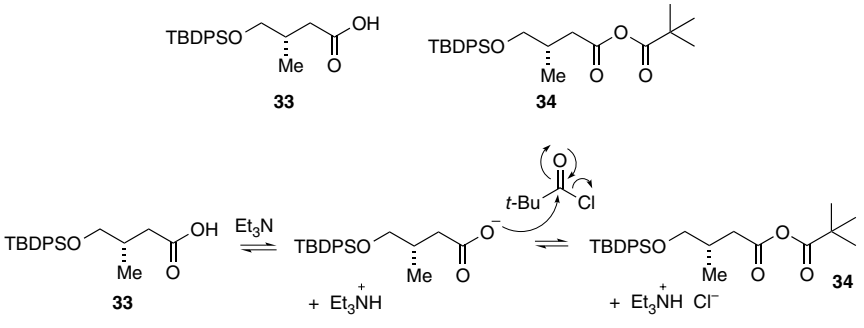
30

Question 11.20:

Transformation of **30** into **31** requires conversion of alcohol into a leaving group, for example, a tosylate, followed by nucleophilic substitution with a cyanide anion.



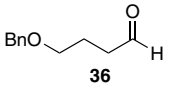
Question 11.21:



Question 11.22:

The nucleophilic addition of Evans' oxazolidinone on compound **34** occurs at the less sterically hindered carbonyl group, leading to compound **35**.

Question 11.23:

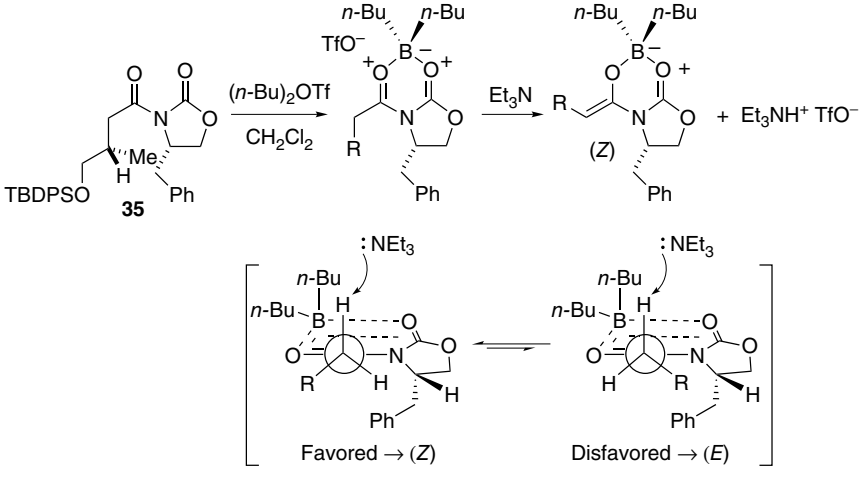


36

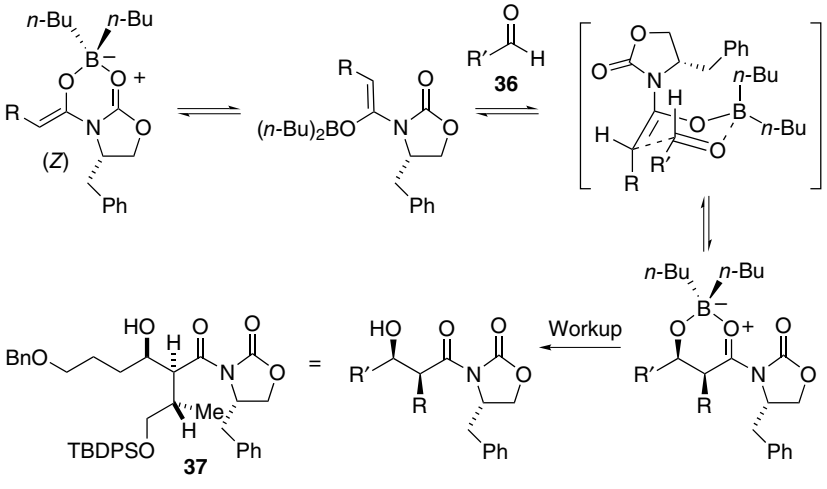
Question 11.24:

This reaction and the related processes are discussed in detail in Refs [11, 12].

1. Selective formation of boron enolate from acyl oxazolidinone:

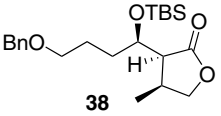


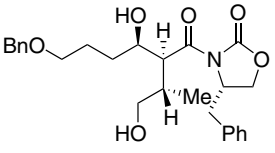
2. Selective aldol reaction of *Z*-boron enolate:



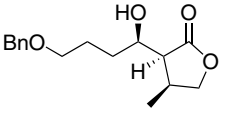
Question 11.25:

The IR band around 1770 cm^{-1} reported for **38** is characteristic of ester C=O stretching vibration.



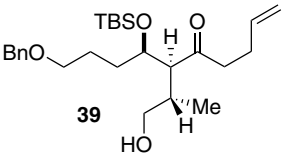


(at **point 1**)



(at **point 2**)

Question 11.26:

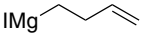


Question 11.27:

4-lithiobutene can be easily obtained from 4-iodobutene through a metal-halogen exchange reaction using *t*-BuLi, in Et_2O at low temperature (–78 °C).

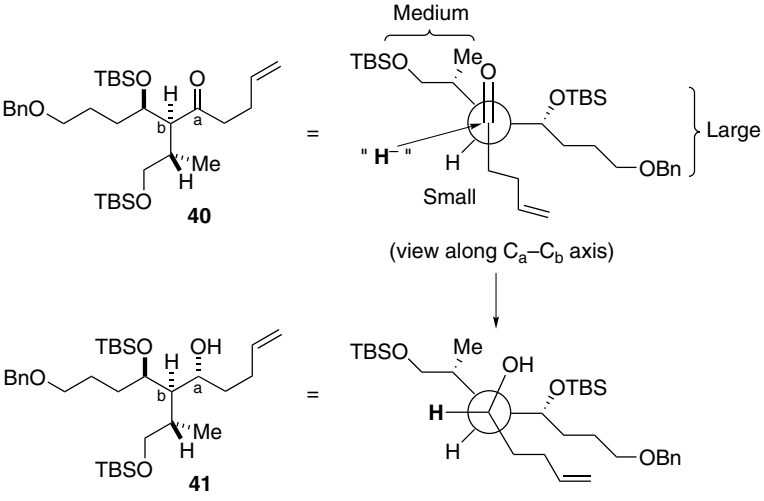
Question 11.28:

Compound **39** could be also obtained from **38** using the following organomagnesium derivative (Grignard reagent), easily prepared from 4-iodobutene using magnesium, in Et_2O.



Question 11.29:

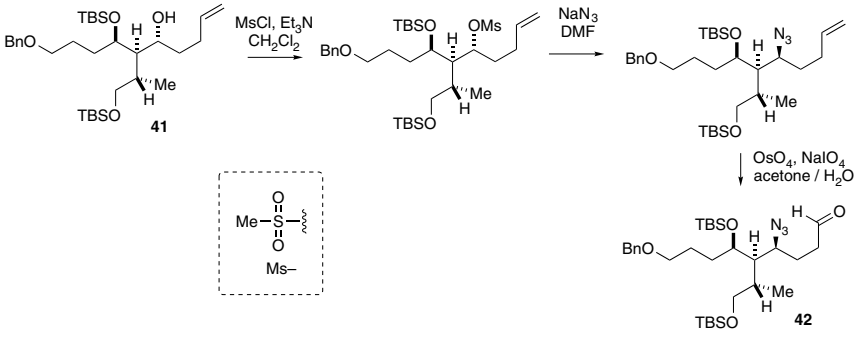
Diastereoselectivity in the reduction of **40** is controlled by the stereogenic center α to the carbonyl group and can be rationalized by the Felkin–Ahn model:



Question 11.30:

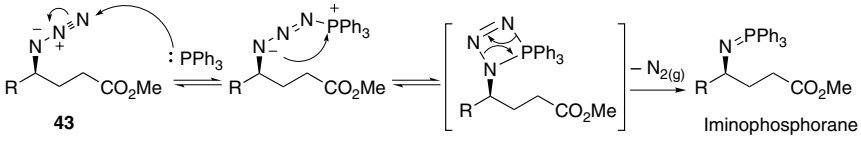
The alcohol obtained is more polar than the starting cetone; thus, it should be more retained by the polar stationary phase (SiO_2) on TLC and have a lower R_f value.

Question 11.31:[7]

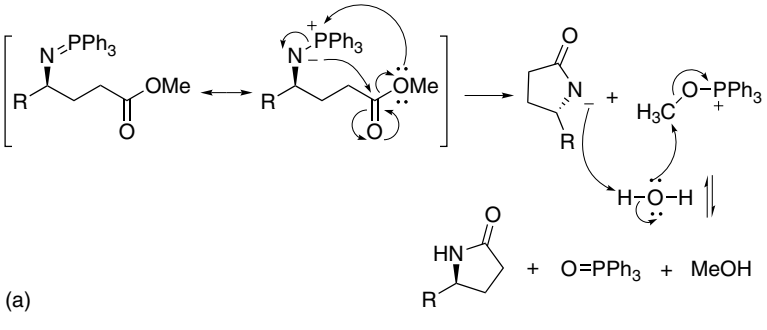


Question 11.32:

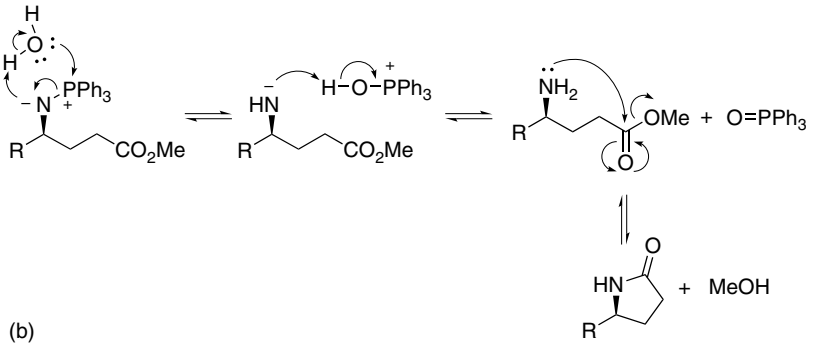
The Staudinger reaction has been the subject of mechanistic studies [13].



Lactam **44** could be formed from iminophosphorane following two plausible pathways:



7 The conditions used in the original article were (i) MsCl, pyridine; (ii) NaN_3, HMPA; (iii) O_3, CH_2Cl_2/MeOH, –78 °C, then Me_2S.



(b)

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12

Total Synthesis and Structure Revision of Caraphenol B

In 2001, several oligostilbenes were isolated from the roots of *Caragan sinica*, a plant used in Chinese folk medicine for the treatment of hypertension, asthenia, and contusion [1]. Based on advanced nuclear magnetic resonance (NMR) experiments, structure **1** was proposed for caraphenol B (Figure 12.1). On the basis of chemical etiology considerations, an alternative structure **2** was suggested, and confirmed in 2011 by total synthesis (Figure 12.1) [2]. The following problems deal with some aspects of the total synthesis of **1** and **2**.

12.1 Synthesis of the Proposed Structure of Caraphenol B

Reduction of aldehyde **3** with sodium borohydride in MeOH leads to **4**, which is treated with PBr_3 in the presence of a catalytic amount of pyridine in Et_2O to afford **5** (Scheme 12.1). Then, reaction with NBS in CH_2Cl_2 yields **6**, which reacts with a preformed solution of potassium hexamethyldisilazane (KHMDS) and diethylphosphite to afford phosphonate **7**. Finally, deprotonation with *t*-BuOK followed by addition of *p*-anisaldehyde gave stilbene derivative **8**, converted to **9** in a single step [2, 3].

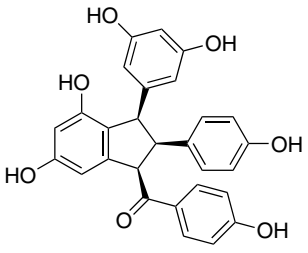
Question 12.1: Write the structure of compounds **4**, **5**, **6**, and **7**.

Question 12.2: Suggest plausible reaction conditions for the transformation of **8** into **9**.

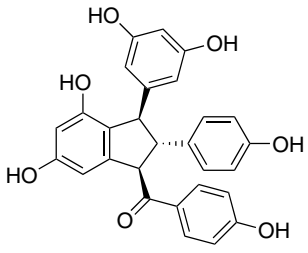
Reaction of **9** (Scheme 12.1) with PTSA and thiol **10** in dichloromethane yields, after appropriate workup, a mixture of compounds **11** and **12**, both of molecular formula ($C_{34}H_{36}O_8S$) (Scheme 12.2). This mixture is treated with *m*-CPBA in the presence of $NaHCO_3$ to afford **13** and **14** in 78% overall yield. Then, reaction with KOH in a mixture of CCl_4, *t*-BuOH and water gives compound **15**.

Question 12.3: Write the structure of **11** and **12**, and explain their formation with a plausible mechanism.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

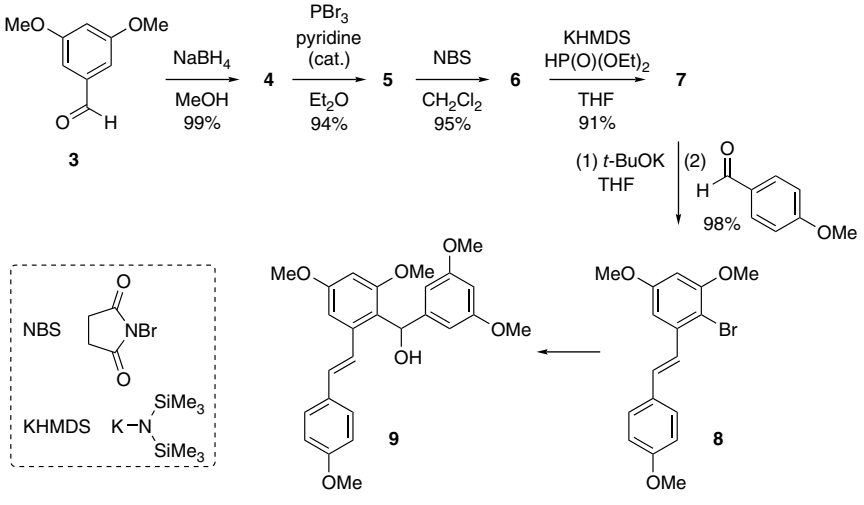


1 – Proposed structure of caraphenol B

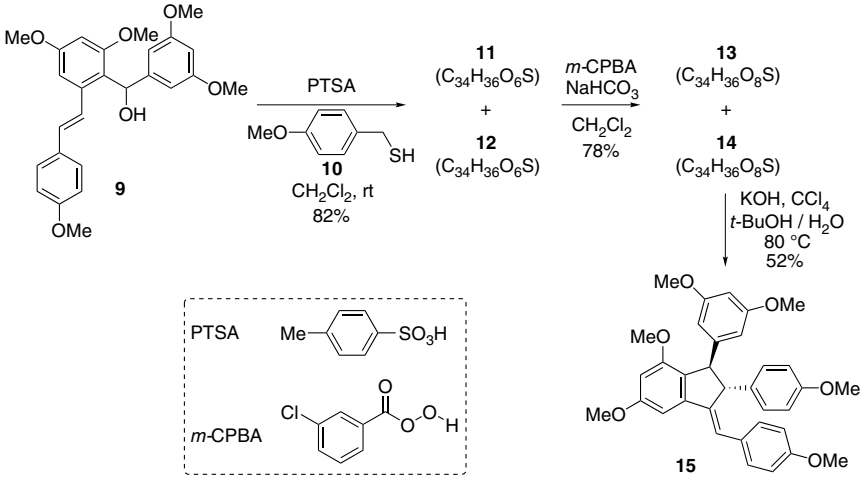


2 – Revised structure of caraphenol B

Figure 12.1 Proposed and revised structures of caraphenol B.



Scheme 12.1

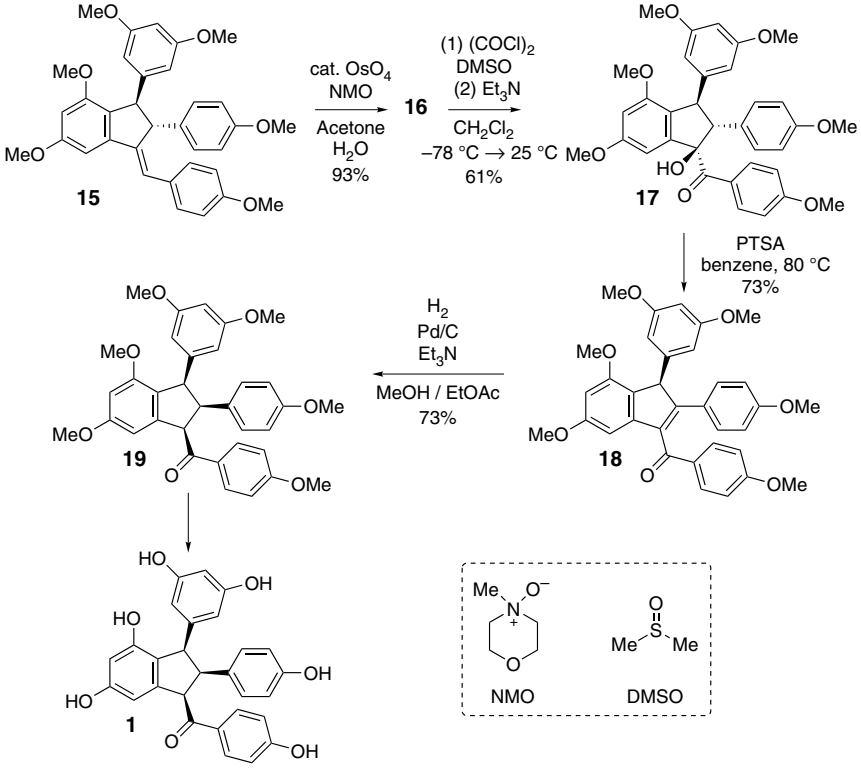


Scheme 12.2

Question 12.4: Write the structure of compounds **13** and **14**.

Question 12.5: Suggest a plausible mechanism for the formation of **15** from **13** and **14**.

Alkene **15** is converted to compound **16** by reaction with a catalytic amount of OsO_4 in the presence of NMO in aqueous acetone followed by appropriate reductive workup (Scheme 12.3).[1] A solution of this crude material in dichloromethane is then slowly added to a preformed mixture of DMSO and oxalyl chloride in CH_2Cl_2 at −78 °C. After stirring for 30 min, Et_3N is added and the mixture is warmed to 25 °C, affording **17** in 61% yield after workup and purification. Action of PTSA in refluxing benzene leads to enone **18**, which is reduced to compound **19** (73% yield) by catalytic hydrogenation in the presence of Et_3N in a mixture of MeOH and EtOAc. Noteworthy is the fact that in the absence of triethylamine a by-product **20** (molecular formula $C_{34}H_{36}O_7$) is formed in significant amounts.



Scheme 12.3

1 The original procedure involves the use of an additional catalytic amount of quinuclidine.

The infrared vibration bands for **19** and **20** are described in the following table.

Compound	Infrared vibration band frequencies (cm^{-1})
19	3000, 2936, 2836, 1674, 1598, 1511, 1461, 1426, 1308, 1251, 1203, 1153, 1064, 1034, 832
20[a]	3478, 2997, 2933, 2835, 1598, 1511, 1462, 1427, 1338, 1203, 1248, 1201, 1179, 1152, 1063, 1035, 830

a) Infrared spectrum is not reported in the original article.

Question 12.6: Write the structure of **16** and justify the observed selectivity.

Question 12.7: What is the name of the reaction involved in the transformation of **16** into **17**? Write a mechanism for this transformation.

Question 12.8: Which product of this reaction is responsible for the characteristic smell associated with this transformation? How do you get rid of it?

Question 12.9: Explain the formation of compound **19** from **18** and justify the diastereoselectivity of the reaction.

Question 12.10: Analyze the infrared vibration bands reported for compounds **19** and **20** and suggest a plausible structure for **20**.

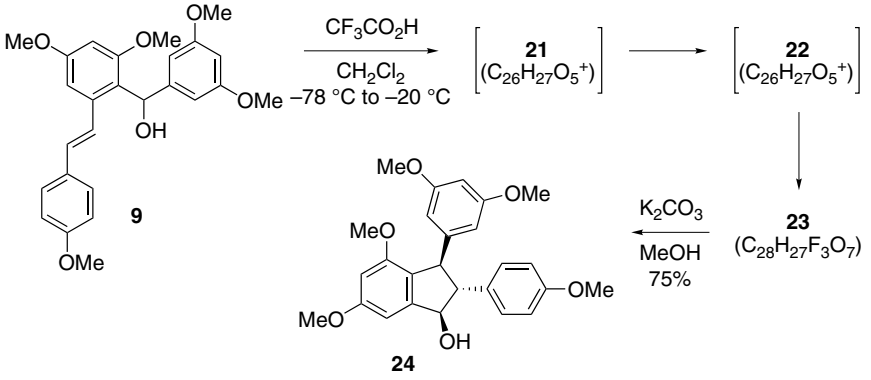
Question 12.11: Suggest plausible reaction conditions allowing the transformation of **19** into **1**.

12.2 Synthesis of the Revised Structure of Caraphenol B

Reaction of **9** with a stoichiometric amount of trifluoroacetic acid at low temperature leads to a first cationic intermediate **21** (molecular formula $C_{26}H_{27}O_5^+$), which rearranges to a second cationic intermediate **22** (molecular formula $C_{26}H_{27}O_5^+$), finally affording ester **23** (molecular formula $C_{28}H_{27}F_3O_7$) (Scheme 12.4).[2] Then, addition of excess K_2CO_3 and MeOH yielded 75% of **24** over the whole sequence [2, 3].

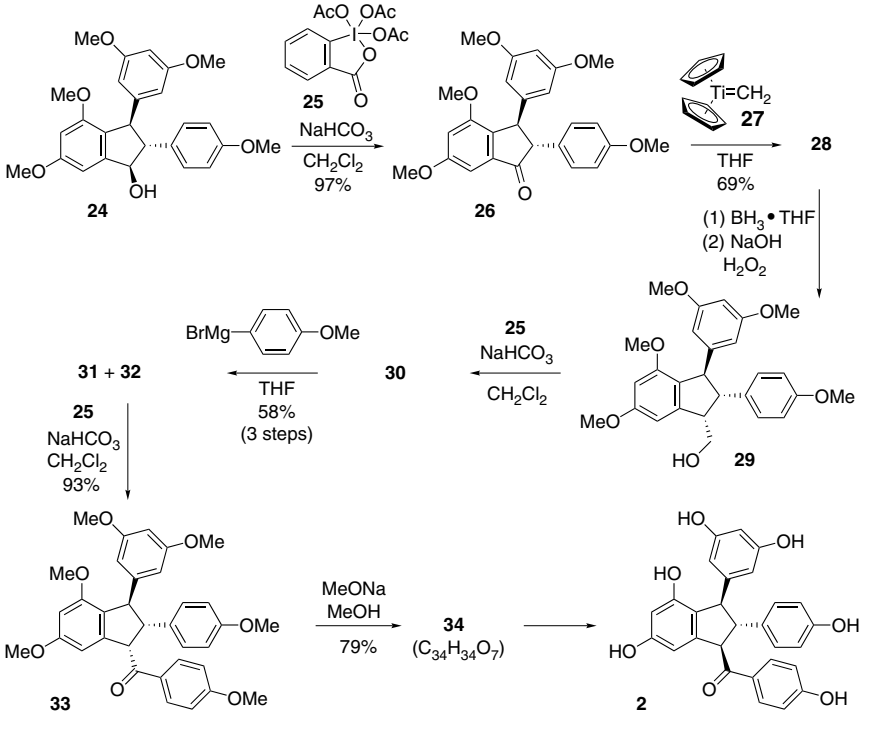
2 Isolation of ester **23** has not been reported in the original work. However, its formation is most likely as supported by the influence of the nature of nucleophilic counteranions on the outcome of the reaction (see Refs [2, 3]).

Question 12.12: Write the structure of compounds **21**, **22**, and **23** and suggest a plausible mechanism for their formation.



Scheme 12.4

Alcohol **24** is treated with periodinane **25** in the presence of $NaHCO_3$ to give ketone **26**, which is transformed into compound **28** upon reaction with titanium complex **27** (Scheme 12.5). After treatment with BH_3·THF followed by H_2O_2 in



Scheme 12.5

the presence of sodium hydroxide, alcohol **29** is isolated and reacted with periodinane **25**, yielding compound **31**. Addition of *p*-methoxyphenylmagnesium bromide furnishes a mixture of **32** and **33**, which are treated with **30** to give ketone **32**. The latter reacts with sodium methylate in methanol, yielding **35** (molecular formula $C_{34}H_{34}O_7$), precursor of **2**.

Question 12.13: What is the usual name of reagent **25**?

Question 12.14: Write the mechanism of transformation of **24** into **26**, and mention the oxidation state of iodine in all intermediates involved. What is the role of $NaHCO_3$?

Question 12.15: What is the usual name for reagent **27**?

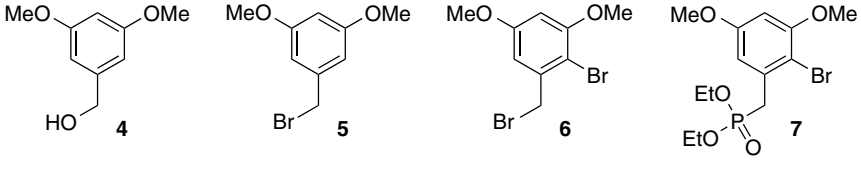
Question 12.16: Write the structure of **28** and suggest a mechanism accounting for its formation.

Question 12.17: Write the structure of compounds **30**, **31**, **32**, and **34**.

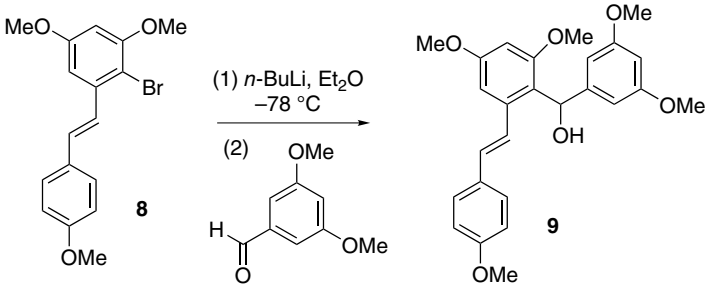
Question 12.18: Explain the formation of **34**.

Answers

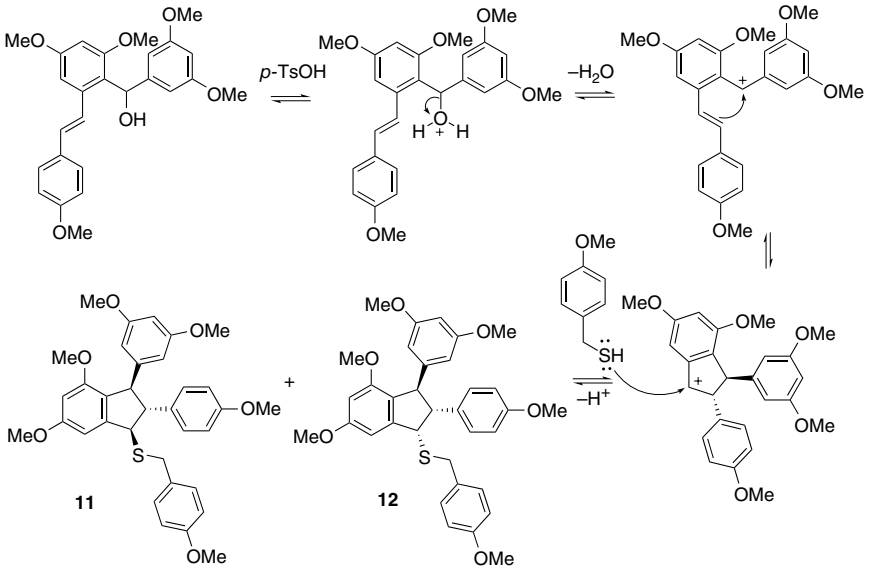
Question 12.1:



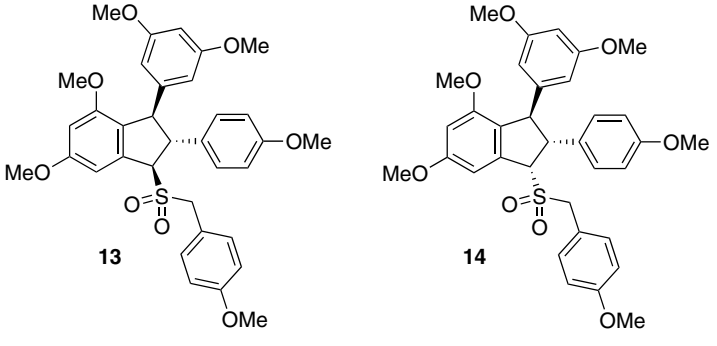
Question 12.2:



Question 12.3:

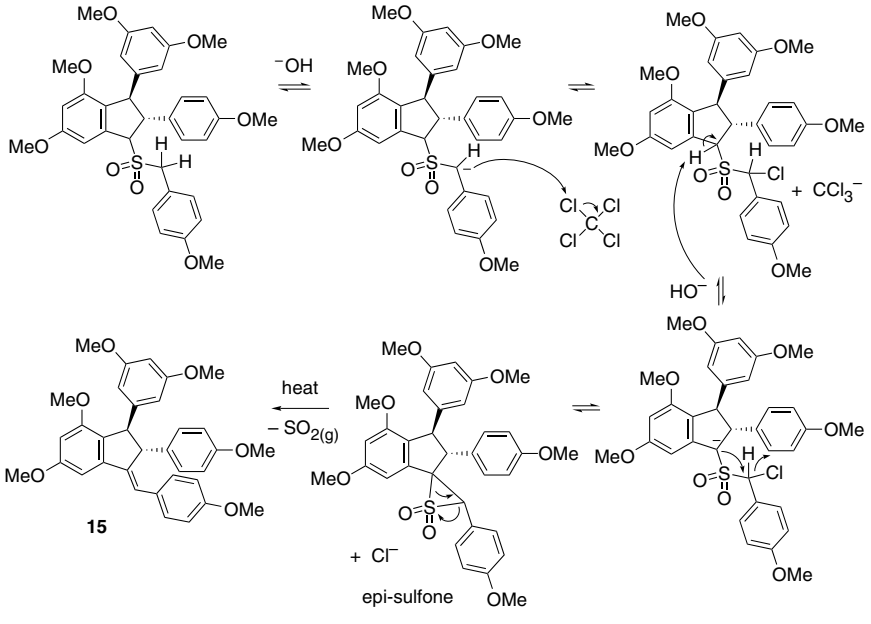


Question 12.4:

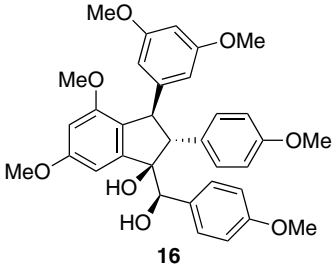


Question 12.5:

The formation of **15** from **13** and **14** is known as the Ramberg–Bäcklung reaction, which allows conversion of α-halosulfones into alkene *via* a base-mediated epi-sulfone formation followed by SO_2 extrusion [4, 5].



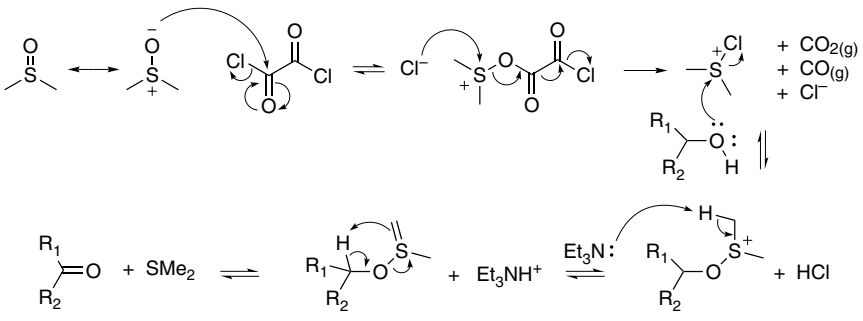
Question 12.6:



Bis-hydroxylation of **15** with OsO_4 is a *syn* addition and proceeds on the less hindered face of the alkene, opposite to the substituent attached to the neighboring carbon atom.

Question 12.7:

This reaction is known as Swern oxidation, and the currently accepted mechanism is described here [6]:



Question 12.8

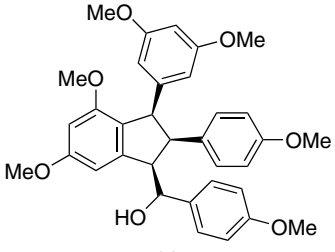
Dimethyl sulfide (SMe_2), a toxic and volatile compound, is responsible for the characteristic unpleasant smell associated with this reaction. To get rid of it, this volatile compound is usually trapped with bleach upon evaporation in order to oxidize it into DMSO and eliminate the smell. All glassware used for the reaction may also be rinsed with bleach.

Question 12.9:

Catalytic hydrogenation in the presence of Pd/C proceeds through a *syn* addition of H_2 on the alkene. Here, facial selectivity is controlled by the stereogenic center α to the double bond which guides H_2 addition on the less hindered face.

Question 12.10:

Compound **20** presents an infrared band at 3478 cm^{-1}, characteristic of O—H bond stretching vibration. Moreover, the band corresponding to C═O bond of **19** at 1674 cm^{-1} is absent in **20**. Thus, **20** is an alcohol originating from reduction of the ketone, in agreement with reported molecular formula.

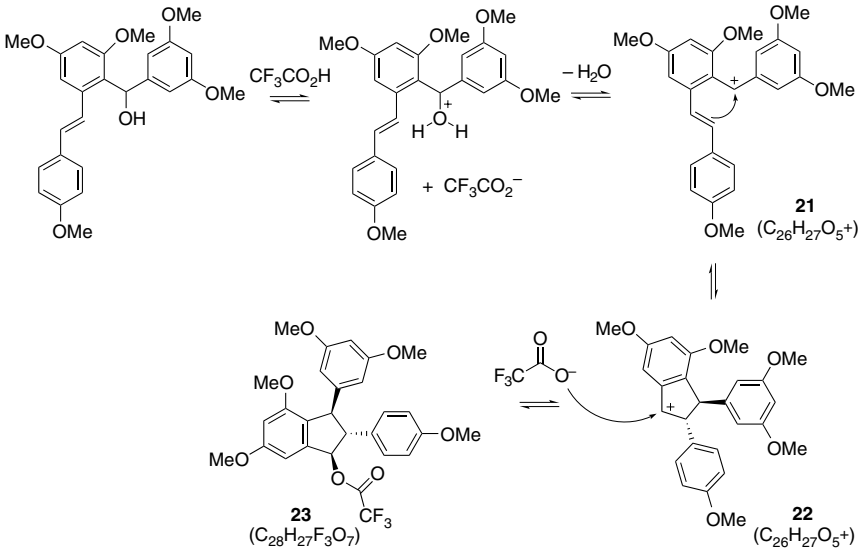


20

Question 12.11:

BBr_3, CH_2Cl_2, −78 °C

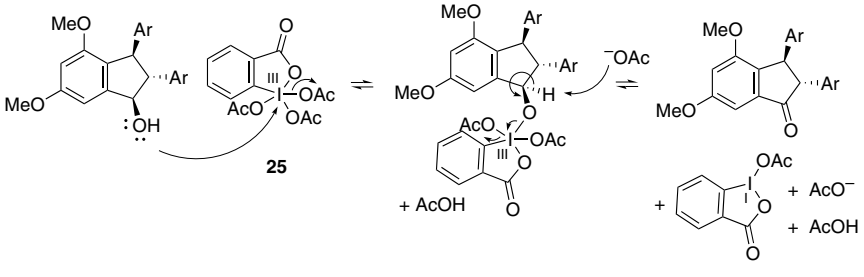
Question 12.12:



Question 12.13:

Dess–Martin periodinane.

Question 12.14:

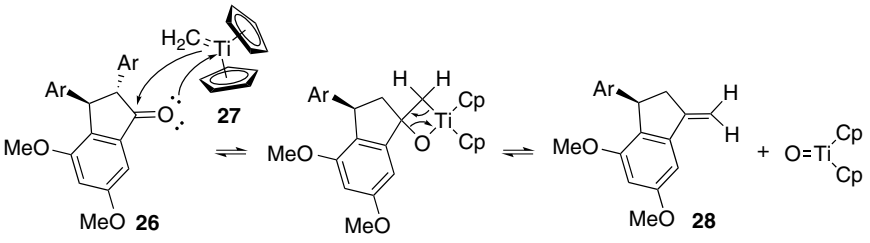


The role of $NaHCO_3$ is to neutralize the acetic acid generated during the reaction.

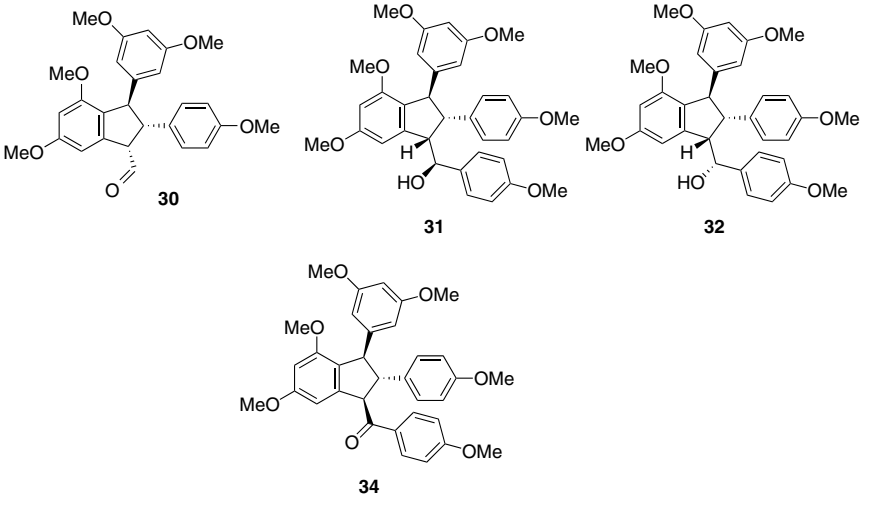
Question 12.15:

Tebbe reagent.

Question 12.16:

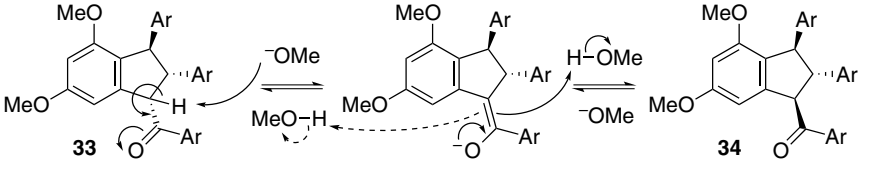


Question 12.17:



Question 12.18:

Transformation of **33** into **34** involves epimerization of the stereogenic center α to the ketone. The H atom in this position can indeed be abstracted by MeO^-, leading to the corresponding enolate. Reprotonation of this enolate could lead to either **33** or **34**; but under these thermodynamic conditions, the most stable *trans/trans* substituted five-membered ring **34** is formed.



References

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13

Synthetic Routes Toward Muricatacin and Analogs

Muricatacin **1** (Figure 13.1) is a naturally occurring molecule, isolated in the 1990s from the seeds of *Annona muricata*. This compound shows cytotoxic activity on various cancer cell lines.

Since its discovery, several synthetic approaches have been developed with the aim of elucidating the absolute configuration of stereocenters C-4 and C-5, studying possible biosynthetic pathways, and accessing novel analogs of therapeutic interest.

13.1 Synthesis of (+)-Muricatacin

L-Glutamic acid **2** is dissolved in concentrated aq. HCl and treated with $NaNO_2$ to give, *via* cationic intermediate **3** and neutral intermediate **4**, carboxylic acid **5** after appropriate treatment (Scheme 13.1).[1] This compound is converted into acyl chloride **6** by reaction with oxalyl chloride in the presence of a catalytic amount of **7** in CH_2Cl_2, followed by evaporation [1].

Question 13.1: Write the Lewis structure of ion NO_2^-.

Question 13.2: Which species is formed upon addition of $NaNO_2$ to aq. concentrated HCl?

Question 13.3: Suggest a plausible mechanism for the formation of **5** from **2**, mentioning the structure of **3** and **4**, including absolute configuration of their stereocenter.

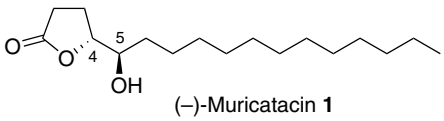
Question 13.4: Write the structure of **7**, involved in the conversion of **5** into **6**.

Reaction of **6** with dodecylmagnesium bromide leads, after treatment and purification by silica gel chromatography, to lactone **8** (63%) together with **9** and **10** in 10% and 8% yield, respectively, both of formula $C_{29}H_{56}O_3$ (Scheme 13.2).[2]

1 No mention of **3** and **4** is made in the original article.

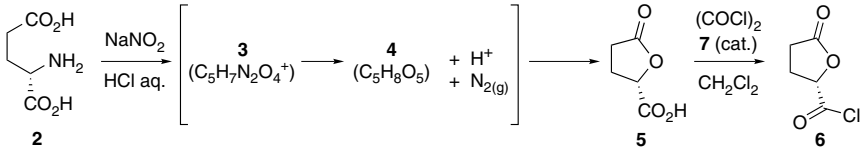
2 The formation of **9** and **10** is not reported in the original article.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

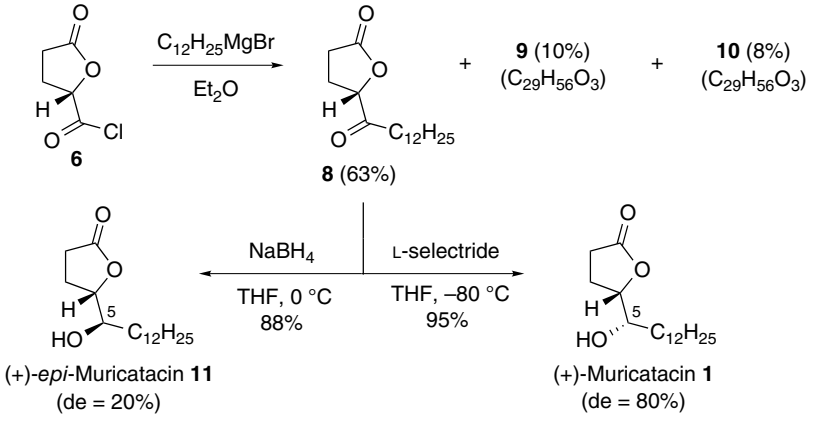


(–)-Muricatacin **1**

Figure 13.1 Chemical structure of (–)-Muricatacin **1**.



Scheme 13.1



Scheme 13.2

Characteristic infrared vibrations of compounds **8–10** are described here.[3] When **8** is treated with $NaBH_4$ in tetrahydrofuran (THF) at 0 °C, (+)-*epi*-muricatacin **11** is obtained as the major product in 95% yield, as a mixture of diastereoisomer at C-5 with 20% diastereoisomeric excess. In contrast, reduction of **8** with L-selectride in THF at –80 °C yields 88% (+)-muricatacin **1**, obtained as a mixture of diastereoisomers at C-5 in 80% diastereoisomeric excess.

Compound	Infrared vibration band frequencies (cm^{-1})
8	2950, 1770, 1706, 1453
9	3402, 2953, 1719, 1701, 1459, 1090
10	3450, 2960, 1765, 1457, 1125

3 These values are suggested on the basis of compounds with similar structure [2].

Question 13.5: Assign infrared vibration bands for compounds **8**, **9**, and **10**, and deduce the structure of **9** and **10**.

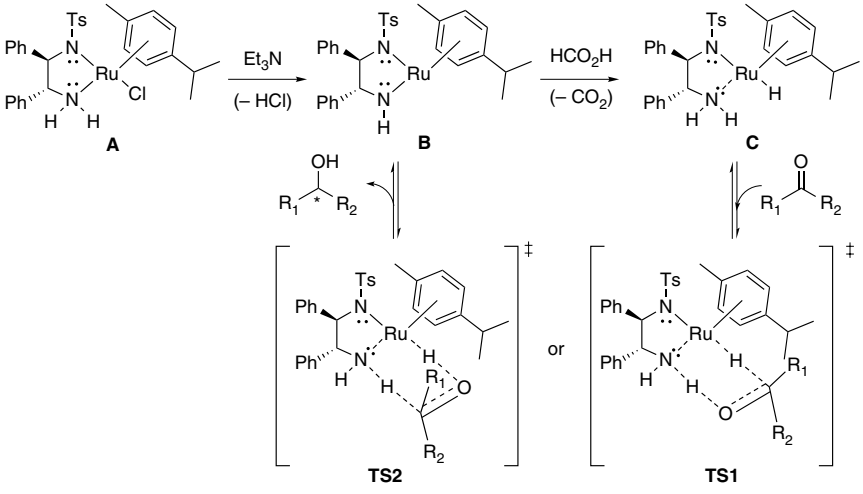
Question 13.6: Write the structure of the minor compound obtained during reduction of **8** into **11** with $NaBH_4$ and precise its relative amount with respect to **11**.

Question 13.7: Write the structure of the minor compound obtained during reduction of **8** into **1** with L-selectride and precise its relative amount with respect to **1**.

Question 13.8: Explain with an appropriate model the preferential formation of **11** (with $NaBH_4$) and **1** (with L-selectride) from **8.**

13.2 Synthesis of (+)-*epi*-Muricatacin by Enantioselective Ketone Reduction

This synthesis is based on the use of arene-ruthenium complex **A** (known as Noyori's catalyst) to control the absolute configuration of stereogenic centers (Scheme 13.3) [3–5].



Scheme 13.3

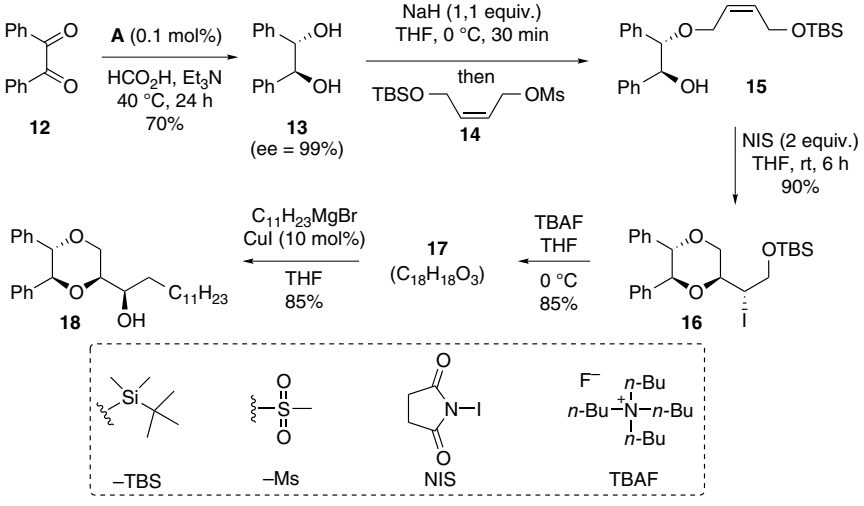
Under effect of triethylamine, species **A** is converted into **B**, which reacts with formic acid to form ruthenium-hydride **C**. This organometallic complex is able to reduce aromatic ketones into the corresponding alcohols with high enantioselectivity, *via* a six-membered transition state.

Question 13.9: Indicate the oxidation state and number of valence electrons for ruthenium in complexes **A**, **B**, and **C**. Specify the "X" or "L" character of ligands, as defined by the covalent bond classification (CBC) method.

Question 13.10: Comment on Ru—H and N—H bond polarization in complex **C**, and indicate partial charges (+δ, −δ) on the considered atoms.

Question 13.11: On the basis of your previous answer, indicate which transition state **TS1** or **TS2** is most likely to be involved.

Question 13.12: Assuming the conversion of **C** into **B** is a reversible process, which operational conditions should be applied in order to drive the reaction to completion?



Scheme 13.4

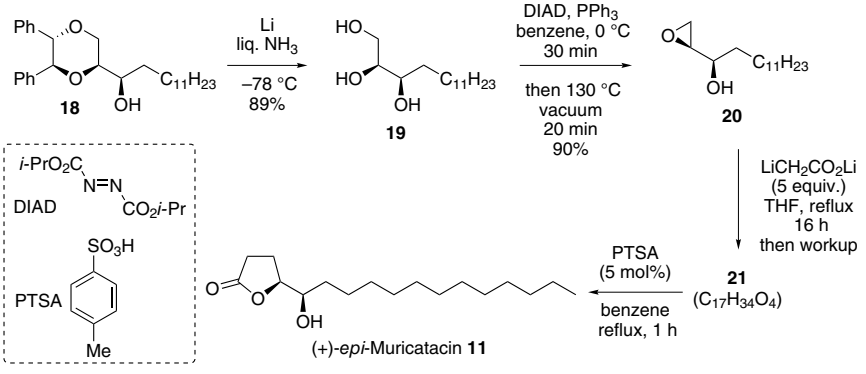
The first steps of the synthetic route are described in Scheme 13.4. Diketone **12** is reacted with a mixture of formic acid and triethylamine in the presence of a catalytic amount of complex **A** (Scheme 13.3) at 40 °C for 24 h, yielding 70% of diol **13** (99% enantiomeric excess) after evaporation. A solution of this compound in THF is then treated with NaH (1.1 equiv.), stirred for 30 min at 0 °C, and reacted with mesylate **14** in THF solution, to afford after workup alkene **15**. The latter undergoes highly diastereoselective cyclization, leading to **16**. Treatment with TBAF gives **17** (molecular formula $C_{18}H_{18}O_3$), which is converted into **18** by treatment with undecylmagnesium bromide in the presence of CuI, followed by appropriate workup.

Question 13.13: Can complex **A** be *rigorously* qualified as a catalyst? Justify your answer.

Question 13.14: Suggest a short reaction sequence allowing preparation of **14** from but-2-yne-1,4-diol.

Question 13.15: Suggest a plausible mechanism for the conversion of **15** into **16** (stereochemistry will not be discussed).

Question 13.16: Write the structure of **17** and explain its formation from **16**.



Scheme 13.5

Reduction of **18** with lithium in liquid NH_3 affords diol **19** (Scheme 13.5). A solution of this compound and PPh_3 in benzene at 0 °C is treated with diisopropyl azodicarboxylate (DIAD); after stirring for 2 h, solvent is evaporated and the residue is heated to 130 °C under vacuum to give epoxide **20**. Treatment with $LiCH_2CO_2Li$ followed by heating to reflux in THF for 16 h, leads after acidic workup and extraction to compound **21** (molecular formula $C_{17}H_{34}O_4$), which, upon reaction with a catalytic amount of PTSA in refluxing benzene, is converted into (+)-*epi*-muricatacin **11**.

Question 13.17: Write a plausible mechanism explaining the transformation of **19** into **20**, accounting for the observed regio- and stereoselectivity.

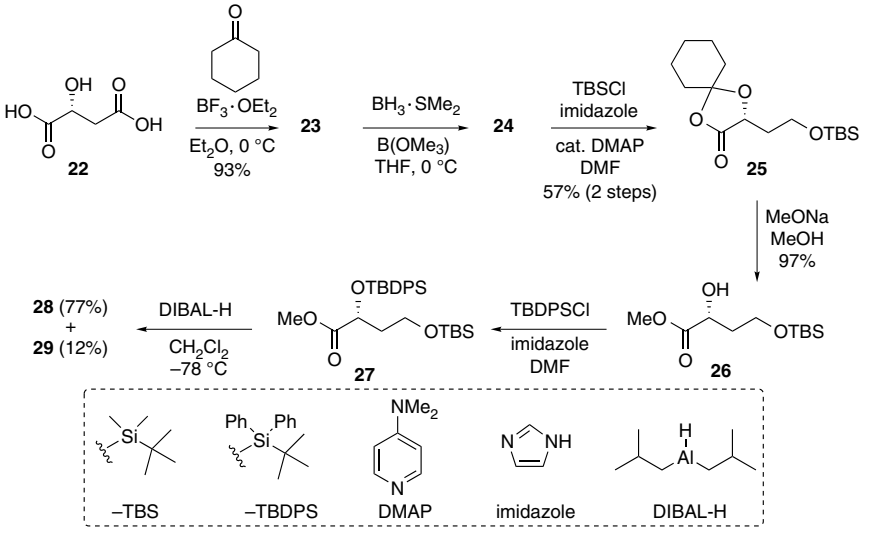
Question 13.18: Propose a simple method for preparation of $LiCH_2CO_2Li$.

Question 13.19: Write the structure of compound **21** and provide a mechanism accounting for its transformation. Why should $LiCH_2CO_2Li$ be used in excess?

Question 13.20: Suggest a plausible mechanism for the transformation of **21** into **11**.

13.3 Synthesis of (–)-Muricatacin

This synthetic route uses D-malic acid **22** as a starting material (Scheme 13.6). Reaction with cyclohexanone in the presence of $BF_3{\cdot}OEt_2$ leads to **23**, which is treated with borane-dimethylsulfide complex to give, after appropriate workup, compound **24**, converted to **25** by reaction with TBSCl in the presence of imidazole and catalytic amount of DMAP in dimethylformamide (DMF). Reaction with sodium methylate in methanol then affords **26**. Compound **27**, obtained by reaction with TBDPSCl and imidazole in DMF, is reduced with DIBAL-H in dichloromethane at –78 °C yielding after workup a mixture of **28** (77%) and **29** (12%). Infrared vibration band frequencies for these compounds are reported below [6].



Scheme 13.6

Compound	Infrared vibration band frequencies[a]
28	3425, 3066, 2939, 2889, 2862, 1442, 1078 cm^{-1}
29	3050 2956, 2859, 1738, 1427, 1113 cm^{-1}

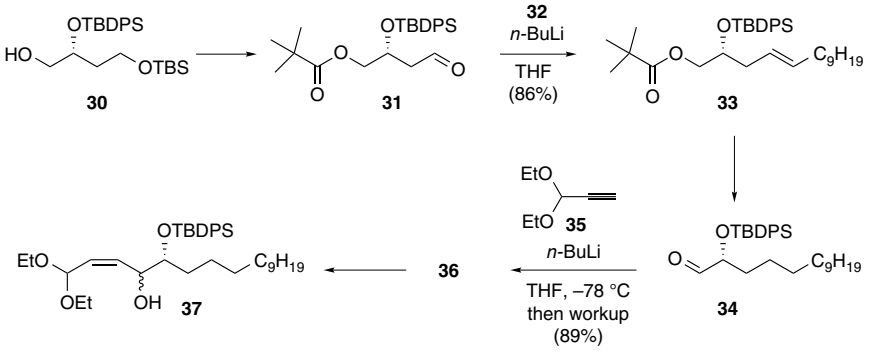
a) These values are adapted from those reported for **28** [6] and other compounds with similar structure [7].

Question 13.21: Write the structure of **23** and **24**.

Question 13.22: Write the structure of **26** and explain its formation with a plausible mechanism.

Question 13.23: Assign infrared vibration bands described for compounds **28** and **29** and deduce their structure.

The synthesis continues with conversion of alcohol **30** into aldehyde **31**, in a few steps (Scheme 13.7). A solution of a compound **32** in THF is treated with *n*-BuLi, followed by **31**, leading to alkene **33**. After a few steps, aldehyde **34** is isolated and treated with the lithiated anion of alkyne **35** to form compound **36**, precursor to allylic alcohol **37**.



Scheme 13.7

Question 13.24: Suggest a plausible reaction sequence allowing conversion of **30** into **31**.

Question 13.25: Write the structure of compound **32**.

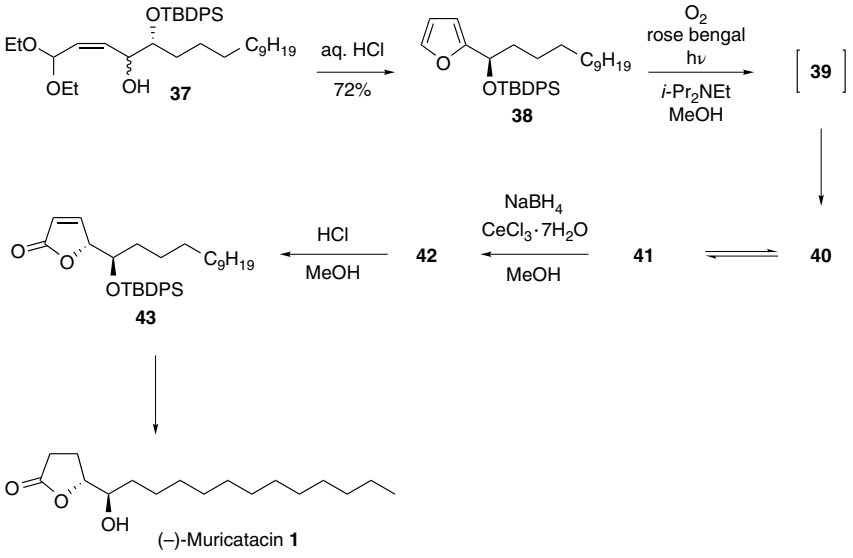
Question 13.26: Suggest a plausible reaction sequence allowing preparation of **34** from **33**.

Question 13.27: Write the structure of **36**.

Question 13.28: Suggest plausible reaction conditions allowing conversion of **36** into **37**.

Compound **37** exhibits low stability in acidic media; the mere washing of a solution of this compound in CH_2Cl_2 with aq. HCl (3M) indeed leads to furan **38** (Scheme 13.8). This compound reacts with singlet oxygen (generated photochemically) to afford a bicyclic intermediate **39** that rapidly rearranges into an equilibrating mixture of hydroxy-lactone **40** and keto-carboxylic acid **41**.[4] The latter reacts diastereoselectively with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$ in methanol to give **42**, which rapidly transforms into lactone **43** upon acidic treatment in methanol. The last steps leading to (–)-muricatacin involve classical functional group interconversions.

4 In the original article, no mention of compound **39** is made in the mechanistic proposal provided by the authors.



Scheme 13.8

Question 13.29: Explain the transformation of **37** into **38** by providing a plausible mechanism.

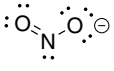
Question 13.30: Write the structure of **39**, **40**, and **41**, and explain their formation with a plausible mechanism.

Question 13.31: Write the structure of **42**, and justify the strereoselectivity of the reduction with a transition state model.

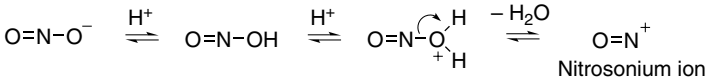
Question 13.32: Suggest a plausible reaction sequence allowing preparation of (–)-muricatacin **1** from **43**.

Answers

Question 13.1:

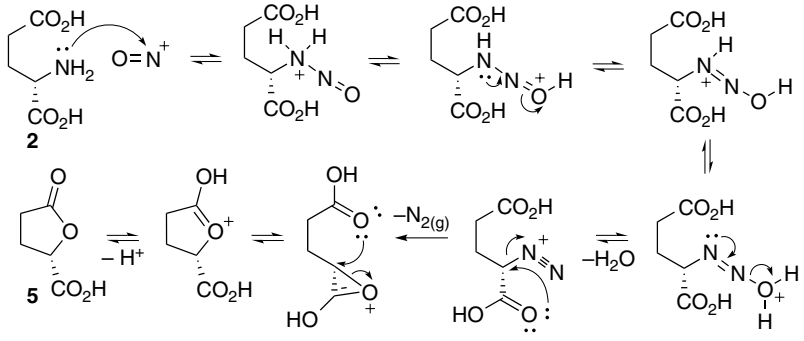


Question 13.2:



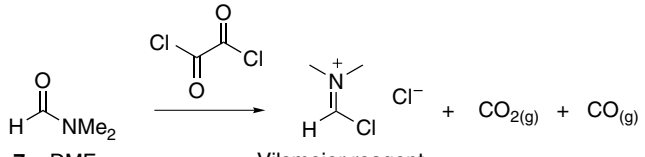
Question 13.3:

Mechanism adapted from Ref. [8]:



Question 13.4:

Compound **7** is DMF, leading to Vilsmeier reagent as the effective chlorinating agent.



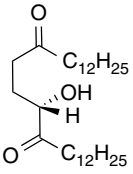
7 = DMF

Vilsmeier reagent

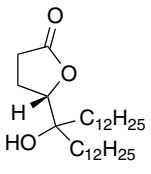
Question 13.5:

Compound	Infrared vibration band frequencies
8	2950 (Csp^3—H stretching), 1770 (C═O ester stretching), 1706 (C═O ketone stretching), 1453 cm^{-1} (C—H bending)
9	3402 (O—H stretching), 2953 (Csp^3—H stretching), 1719 (C═O ketone stretching), 1701 (C═O ketone stretching), 1459 (C—H bending), 1090 cm^{-1} (C—O stretching)
10	3450 (O—H stretching), 2960 (Csp^3—H stretching), 1765 (C═O ester stretching), 1457 (C—H bending), 1125 cm^{-1} (C—O stretching)

The infrared spectrum of **9** shows the presence of a hydroxyl group and two ketone moieties, while **10** contains a hydroxyl group and one ester moiety. Their molecular formula shows the presence of two dodecyl groups. Consequently, these compounds arise from the addition of a second equiv. of Grignard reagent on **8**, either on the ester moiety (leading to **9**) or on the ketone moiety (leading to **10**).



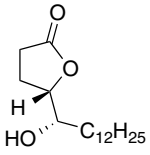
9 (10%)
($C_{29}H_{56}O_3$)



10 (8%)
($C_{29}H_{56}O_3$)

Question 13.6:

Reduction of **8** can occur by attack of the two diastereotopic faces of the ketone moiety, leading to **11** (major) and **1** (minor).



1

The relative amount of each compound can be calculated from the de value:

$$\text{de} = \left(\%\ \text{major dia}\right) - \left(\%\ \text{minor dia}\right) = 20\%$$

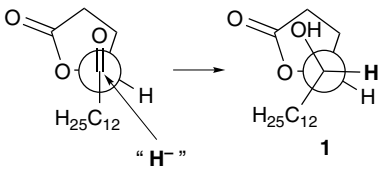
which gives 60% of **11** and 40% of **1**.

Question 13.7:

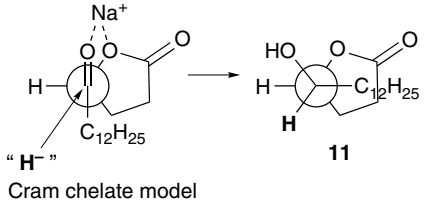
Similarly, during reduction of **8** using L-selectride, **11** is obtained as the minor product. The **1**/**11** ratio is 90:10 according to the 80% de value reported.

Question 13.8:

The formation of **1** as a major product using L-selectride as the reducing agent can be rationalized by the Felkin–Ahn model, while preferential formation of its diastereoisomer **11** when using $NaBH_4$ can be explained by the Cram-chelate model:



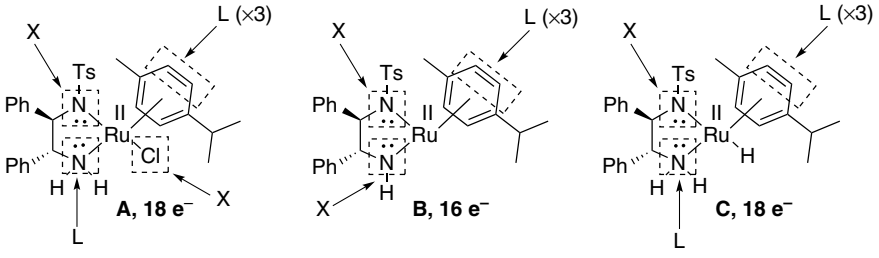
Felkin-Anh model



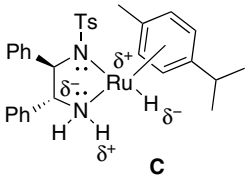
Cram chelate model

Question 13.9:

The CBC method classifies a ligand surrounding a transition metal, according to the type of interaction involved: L (two electrons), X (one electron), or Z (zero electron) [9].

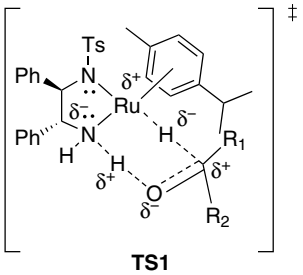


Question 13.10:



C

Question 13.11:



TS1

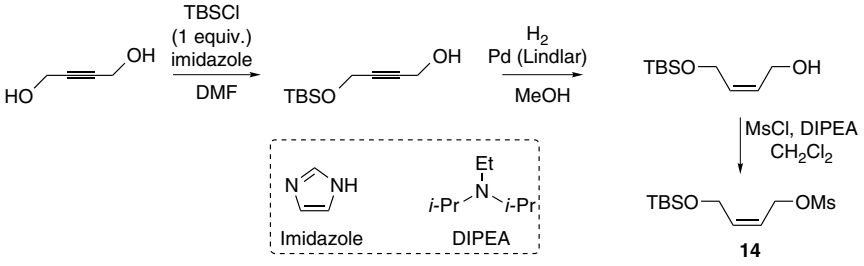
Question 13.12:

To shift the equilibrium toward **C**, an excess of HCO_2H should be used.

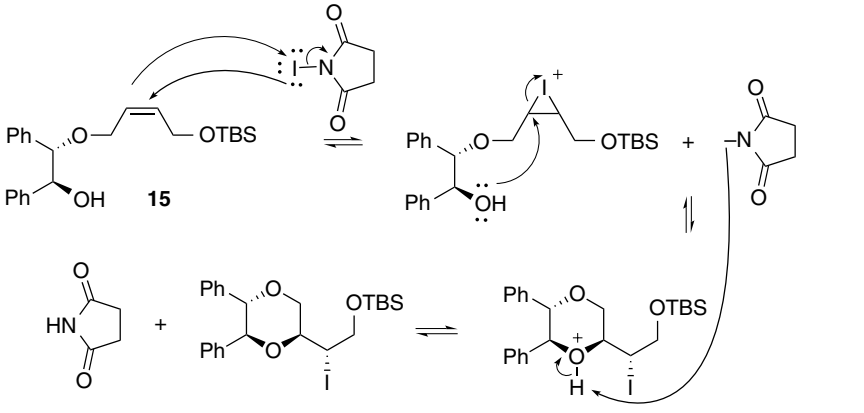
Question 13.13:

Compound **A** cannot be *rigorously* qualified as a catalyst since it is not regenerated during the catalytic cycle. It is a precatalyst, transformed into **B**, which is the effective catalytic species of the reaction.

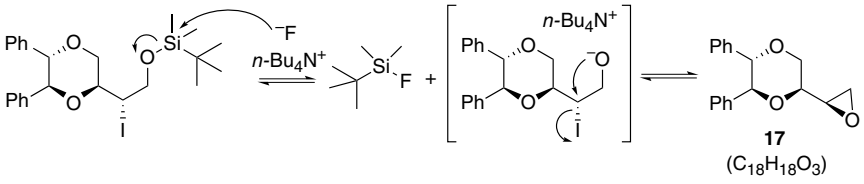
Question 13.14:



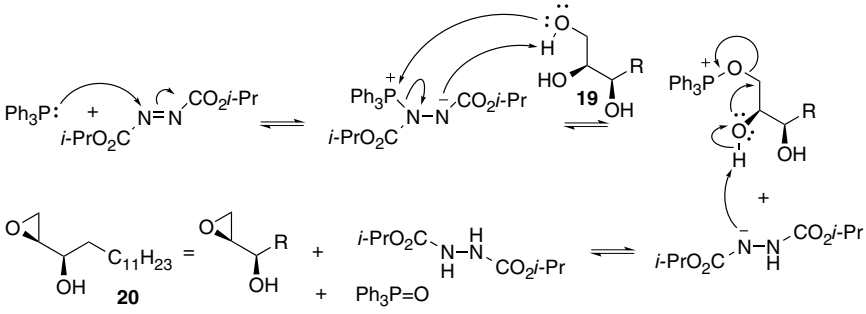
Question 13.15:



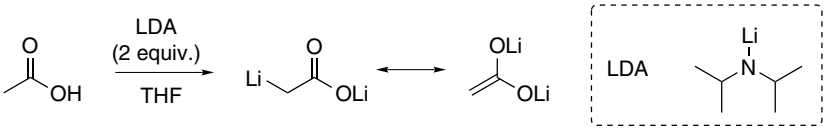
Question 13.16:



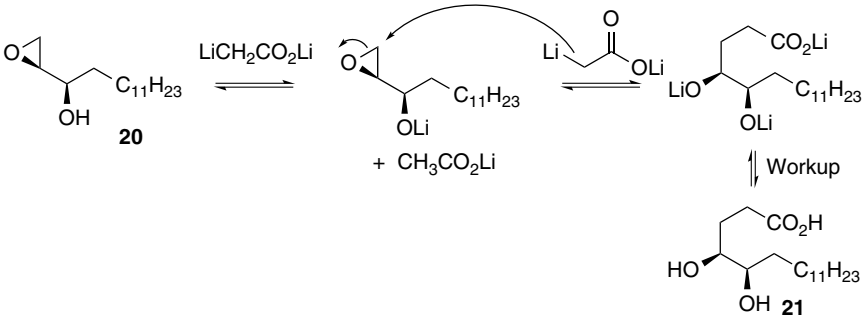
Question 13.17:



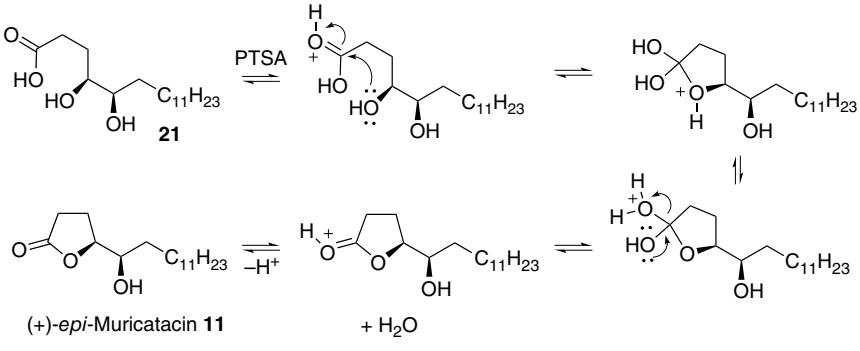
Question 13.18:



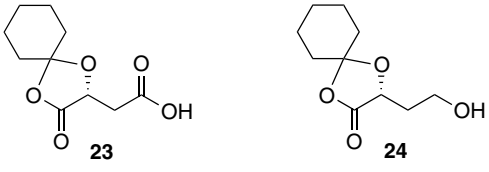
Question 13.19:



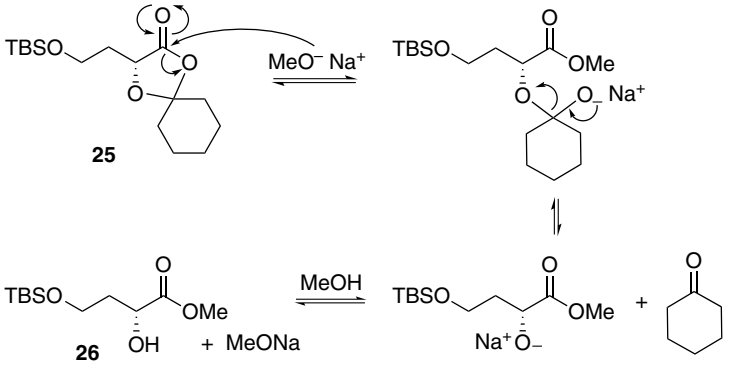
Question 13.20:



Question 13.21:

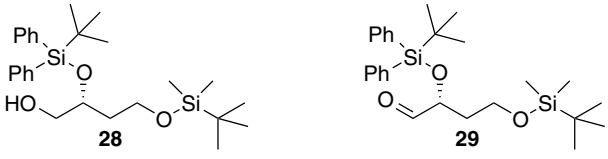


Question 13.22:

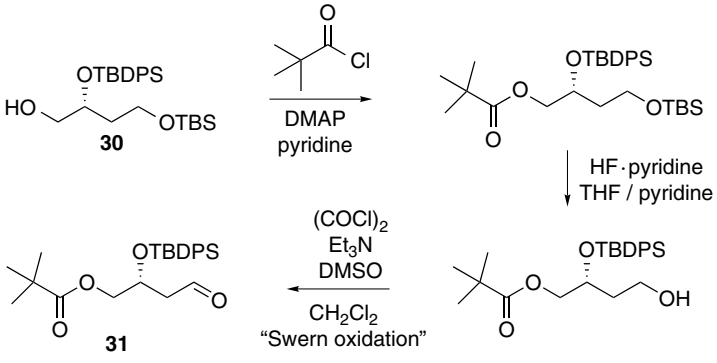


Question 13.23:

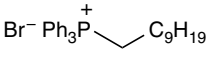
Compound	Infrared vibration band frequencies
28	3425 (O—H stretching), 3066 (Csp^2—H stretching) 2939, 2889, 2862 (Csp^3—H stretching), 1442 (C—H bending), 1078 (C—O stretching) cm^{-1}
29	3050 (Csp^2—H stretching), 2956, 2859 (Csp^3—H stretching), 1738 (C=O stretching), 1427 (C—H bending), 1113 (C—O stretching) cm^{-1}



Question 13.24:

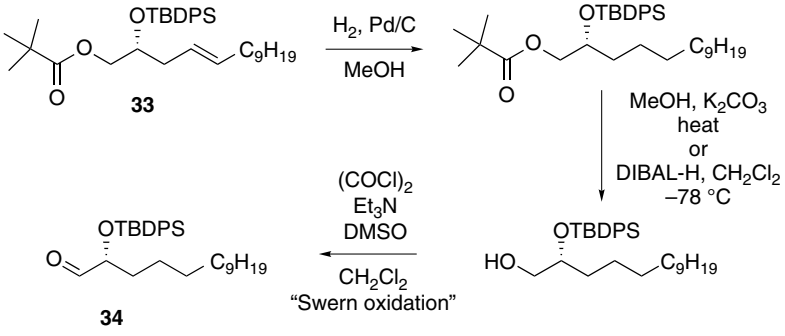


Question 13.25:

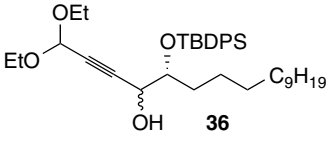


32

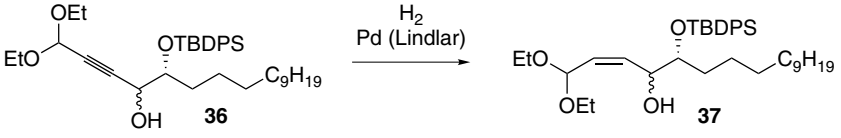
Question 13.26:



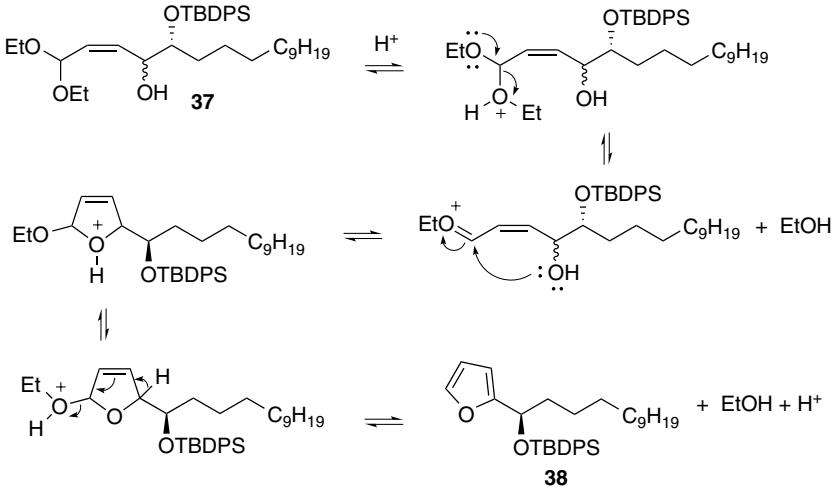
Question 13.27:



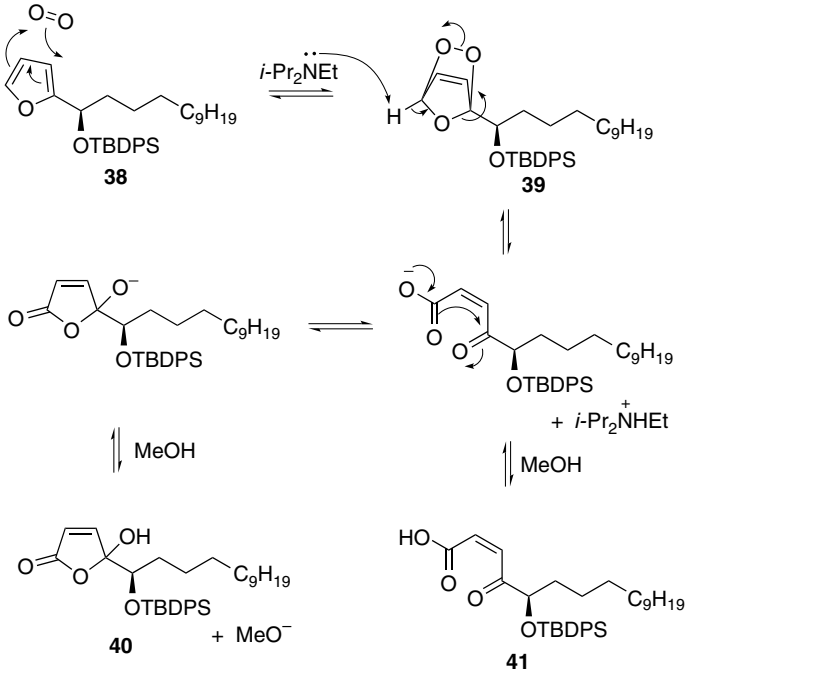
Question 13.28:



Question 13.29:

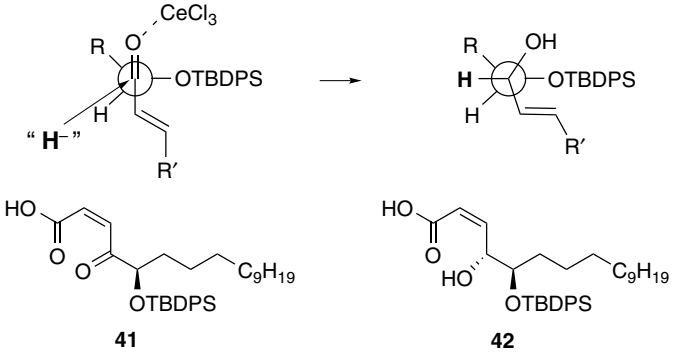


Question 13.30:

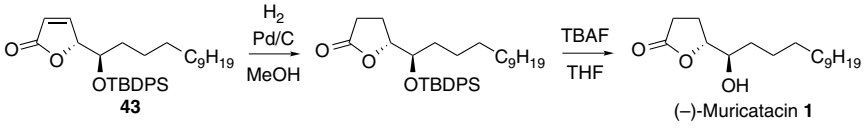


Question 13.31:

Compound **42** is formed by selective 1,2-reduction of α-β-unsaturated ketone **41** (Luche reduction) following the Felkin–Ahn model.



Question 13.32:



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14

Asymmetric Synthesis of (–)-Martinellic Acid

(–)-Martinellic acid **1** was isolated in 1995 from a plant, *Martinella iquitoensis*, used by South American tribes for the treatment of ocular disorders (Figure 14.1) [1].

In the following problems, some aspects of its total synthesis achieved in 2013 are presented [2]. The preparation of (–)-**1** was envisaged from intermediate **19**, which could be obtained by diastereoselective addition of enantiomerically pure lithium amide **6** and α,β-unsaturated ester **5** (Figure 14.1).

14.1 Preliminary Studies: Toward the Formation of a Model Tricyclic Compound

In order to validate their synthetic approach, the authors first focused on the synthesis of model tricyclic compound **2** (Figure 14.2).[1]

Palladium-catalyzed cross-coupling of 2-iodoaniline **3** with *tert*-butyl acrylate affords crude compound **4**, which is directly reacted with allyl iodide in the presence of K_3PO_4 in refluxing acetone to give **5**, obtained in 89% yield over the two steps (Scheme 14.1). Reaction of lithiated anion **6**, followed by aqueous workup and extraction, leads to a mixture of separable diastereoisomers **7** and **7′** in 96% and 2% yield, respectively.[2] Major diastereoisomer **7** reacts with LDA to give an intermediate that can be transformed into **8**, **9**, or **10** depending on the reagent added to the reaction mixture. *N*-deallylation of **8–10** into **11–13** is then performed by Tsuji–Trost reaction in the presence of $Pd(PPh_3)_4$ and excess DMBA. This reagent allows straightforward elimination of reaction side-products by simple washing of reaction media with basic aqueous solution.

Question 14.1: Write the structure of **4** and suggest a plausible mechanism for its formation.

Question 14.2: Write the structure of compounds **7** and **7′**.

Question 14.3: Determine the absolute configuration of asymmetric carbon atoms in compound **8**.

---

1 Compound **2** is not precisely a model target, but rather a potentially useful intermediate.

2 In the original article, **7** was obtained in 97% yield (dr > 99 : 1) and **7′** was not isolated.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

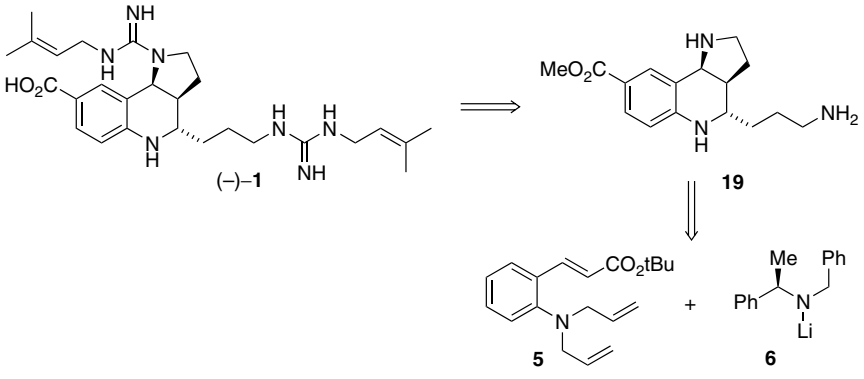


Figure 14.1 Retrosynthetic analysis of (–)-Martinellic Acid.

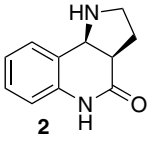
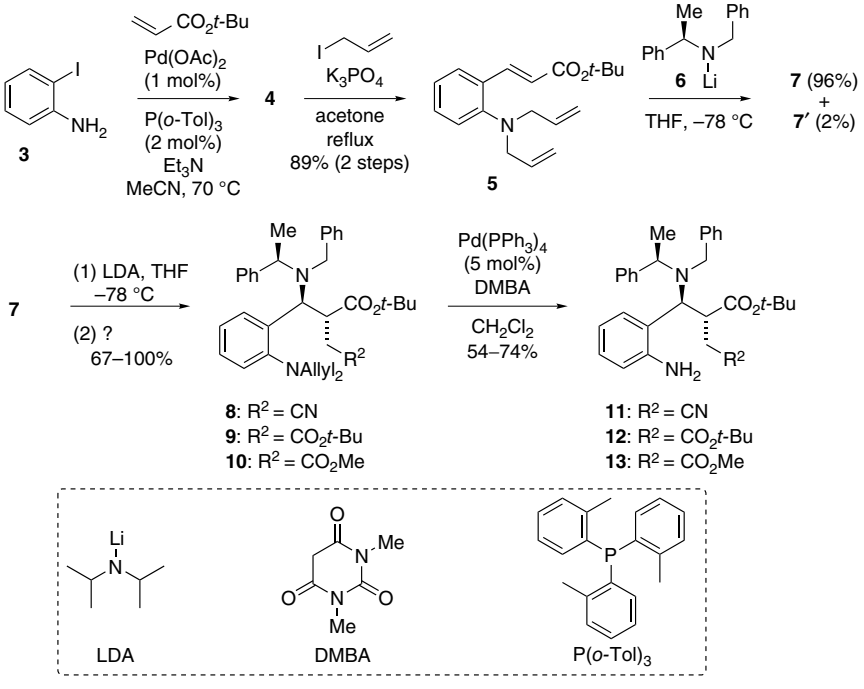


Figure 14.2 Chemical structure of model tricyclic compound **2**.



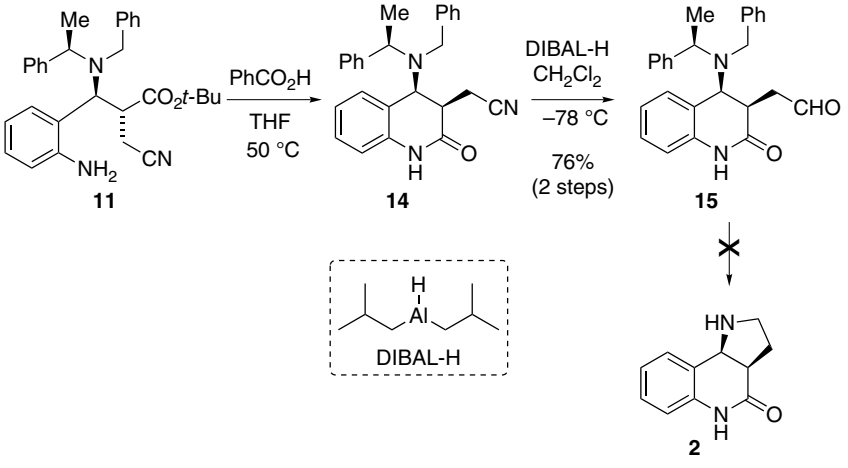
Scheme 14.1

Question 14.4: Indicate the reagents allowing preparation of **8**, **9**, and **10** from **7**.

Question 14.5: Explain the deprotection step of **8** into **11** with a plausible mechanism, emphasizing the role of DMBA.

Question 14.6: Justify why the by-products originating from action of DMBA are easily washed out from the reaction mixture with an aq. basic solution.

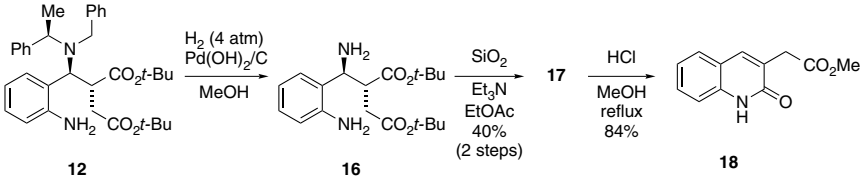
Compound **11** is reacted with 3 equiv. benzoic acid, yielding lactame **14**, whose nitrile group is reduced into aldehyde **15** by the action of DIBAL-H followed by appropriate aqueous workup and purification (Scheme 14.2). Unfortunately, in spite of several attempts, the desired product **2** has never been isolated pursuing this route.



Scheme 14.2

Question 14.7: Suggest a plausible mechanism for the formation of **14** from **11**.

Question 14.8: Suggest a plausible mechanism for the formation of **15** from **14.**



Scheme 14.3

In a novel attempt to access tricycle **2**, compound **12** is hydrogenated into diamine **16**, which spontaneously converts into **17** upon attempted silica gel chromatography using EtOAc/Et_3N: 100/1 as eluent (Scheme 14.3). After treatment with HCl in refluxing MeOH followed by concentration *in vacuo*, basic aqueous workup, and extraction, unexpected compound **18** is isolated in 84%

yield. A selection of infrared vibration bands for **16**, **17**, and **18** are presented in the following table.

Compound	Infrared vibration band frequencies
16	3429, 3383, 3311, 2978, 2932, 1728 cm^{-1}
17	3357, 3278, 3029, 2978, 2929, 1725, 1680 cm^{-1}
18	2952, 2857, 1736, 1665 cm^{-1}

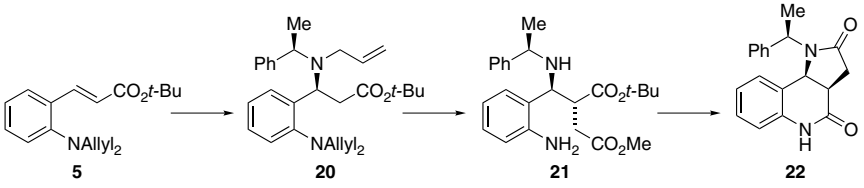
Question 14.9: Assign infrared vibration bands reported for compounds **16**, **17**, and **18**, and deduce the structure of **17**.

Question 14.10: Suggest a plausible reaction mechanism for the transformation of **17** into **18**.

14.2 Synthesis of an Advanced Intermediate

To overcome the unsuccessful attempts described, a new synthetic route toward key intermediate **19** (Figure 14.1) was devised.

Compound **22** is prepared in several steps from **5**, *via* intermediates **20** and **21** (Scheme 14.4).



Scheme 14.4

Question 14.11: Suggest plausible reaction conditions for the transformation of **5** into **20**.

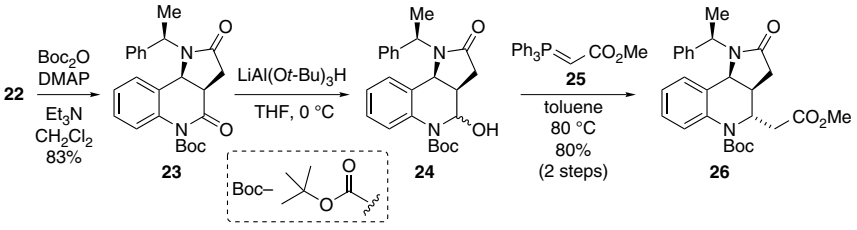
Question 14.12: Suggest a plausible reaction sequence allowing transformation of **20** into **21**.

Question 14.13: Suggest plausible reaction conditions for the transformation of **21** into **22**.

Reaction of amide **22** with Boc_2O in the presence of Et_3N and DMAP leads to **23**, which is reduced into hemi-aminal **24** (Scheme 14.5). The latter reacts with phosphorane **25**, leading to **26** through a series of intermediates.

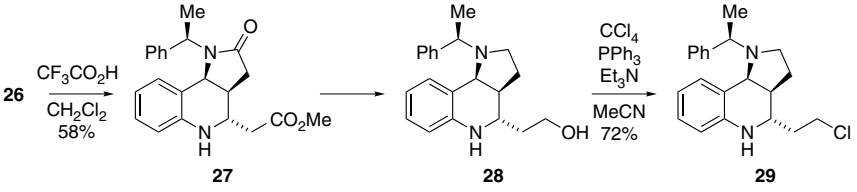
Question 14.14: Suggest plausible reaction conditions for the preparation of **25**.

Question 14.15: Explain the transformation of **24** into **26** by writing the structure of reaction intermediates.



Scheme 14.5

Deprotection of **26** is performed in the presence of trifluoroacetic acid, affording **27** after appropriate workup and purification (Scheme 14.6). This compound is converted into alcohol **28**, and then into the corresponding chloride **29** by reaction with PPh_3 and CCl_4 in the presence of triethylamine.



Scheme 14.6

Question 14.16: Suggest a plausible reaction procedure for the transformation of **26** into **27**, including workup conditions.

Question 14.17: Write the mechanism of transformation of **26** into **27**.

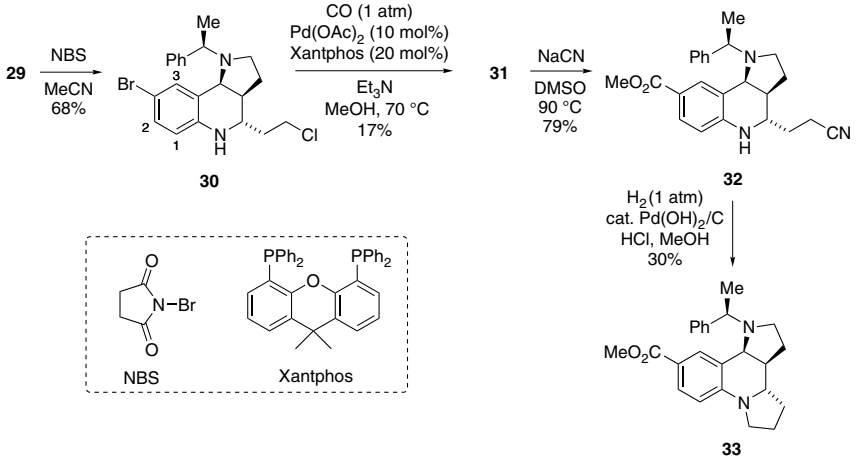
Question 14.18: Suggest plausible reaction conditions allowing the transformation of **27** into **28**.

Question 14.19: Suggest a plausible reaction mechanism for the transformation of **28** into **29**.

Bromination of **29** with NBS in acetonitrile yields a mixture of the expected compound **30** and a di-brominated compound **A**, obtained in 68% and 6% yield, respectively (Scheme 14.7). Analysis of ^{1}H-NMR signals corresponding to H(1), H(2), and H(3) of **30** in $CDCl_3$ reveals the presence of two doublets ($J = 2.3$ Hz and $J = 8.5$ Hz) and a doublet of doublet ($J = 8.5$, 2.3 Hz), while by-product **A** shows only two doublets ($J = 2.2$ Hz).

Reaction of **30** in the presence of a catalytic amount of $Pd(OAc)_2$ and Xantphos under CO atmosphere in MeOH at 70 °C yields compound **31**, which is treated with NaCN in DMSO at 90 °C to afford **32** in 79% yield. Finally, hydrogenation in the presence of $Pd(OH)_2$/C unfortunately leads to the undesired tetracyclic compound **33** as the major product, in 30% yield.

Question 14.20: Assign the ^{1}H-NMR signals reported for **30**.



Scheme 14.7

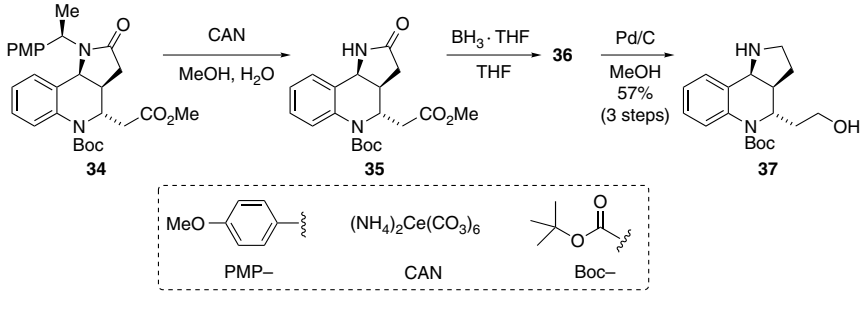
Question 14.21: Suggest two possible structures for by-product **A** based on spectroscopic data. Which one would you favor on the basis of expected reactivity of **29**?

Question 14.22: Write the structure of **31**.

Question 14.23: Explain the transformation of **32** into **33** by writing the structure of reaction intermediates.

14.3 Completion of the Synthesis

The total synthesis of martinellic acid could finally be achieved from **34**, prepared according to a method similar to that employed for **27** (Scheme 14.8). Reaction of **34** with CAN leads to **35**, which reacts with a large excess of $BH_3 \cdot THF$ complex to give **36**. The latter is transformed into the desired amino-alcohol **37** after treatment with Pd/C in MeOH.

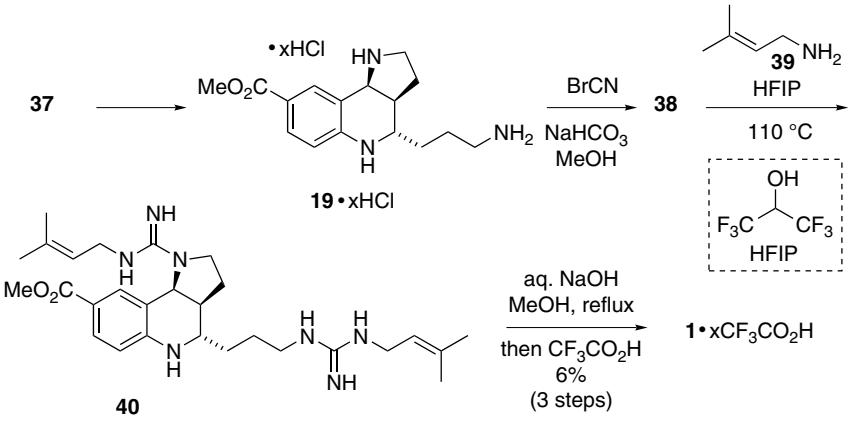


Scheme 14.8

Analysis by high-resolution mass spectrometry (positive mode electrospray ionization) reveals for compound **37** a signal at $m/z = 341.1837$ (corresponding to adduct $[\mathbf{37} + Na]^+$) and for compound **36** a signal at $m/z = 355.2162$ (corresponding to adduct $[\mathbf{36} + Na]^+$).

Question 14.24: Deduce the structure of **36** based on mass spectrometry analysis.

After a few steps of functional group interconversions, **37** is transformed into **19**, obtained as an HCl adduct (Scheme 14.9). Then, treatment with 2 equiv. BrCN in the presence of $NaHCO_3$ leads to compound **38**, which reacts with amine **39** in HFIP at 110 °C to give **40**. The final step leading to **1** is a basic hydrolysis of methyl ester followed by purification in the presence of trifluoroacetic acid.



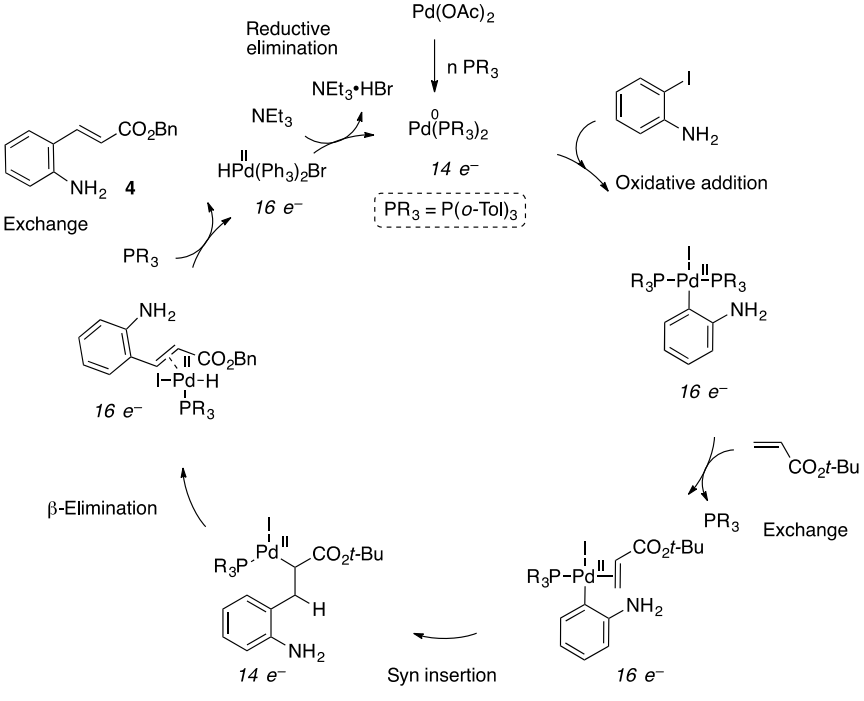
Scheme 14.9

Question 14.25: Write the Lewis structure of BrCN.

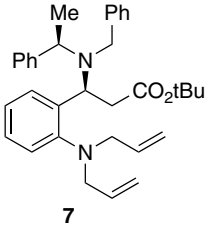
Question 14.26: Write the structure of compound **38**.

Answers

Question 14.1:



Question 14.2:



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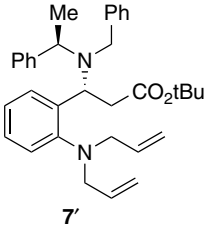

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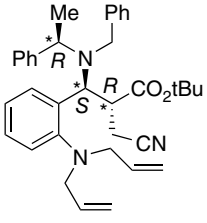
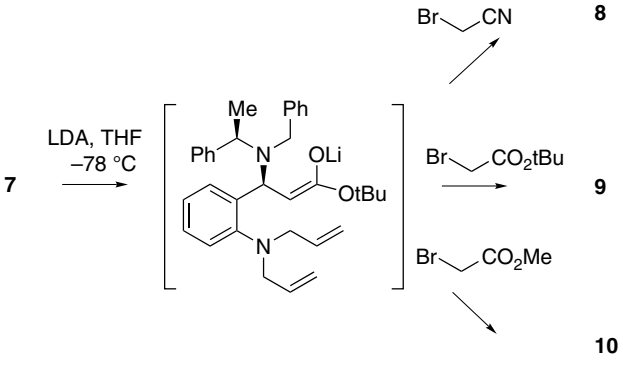
Question 14.3

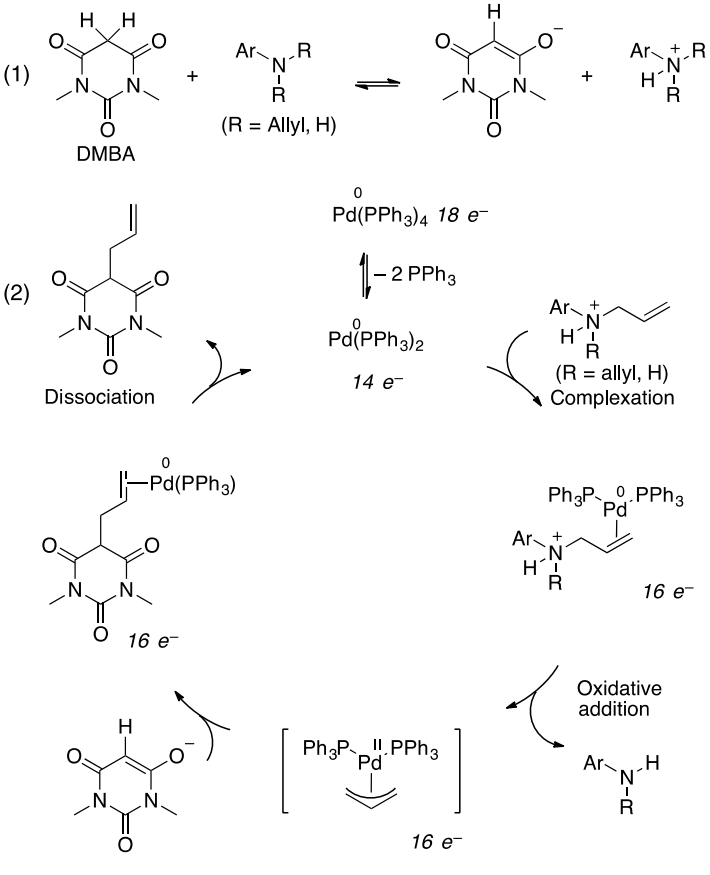


Question 14.4:



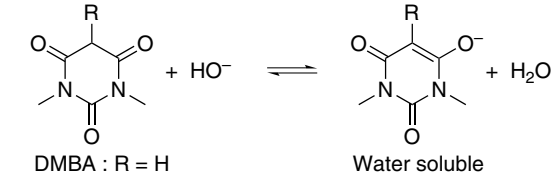
Question 14.5:

The deprotection step of **8** into **11** is a Tsuji–Trost reaction, and DMBA acts as a nucleophile to trap the allyl cation formed during the reaction [3].

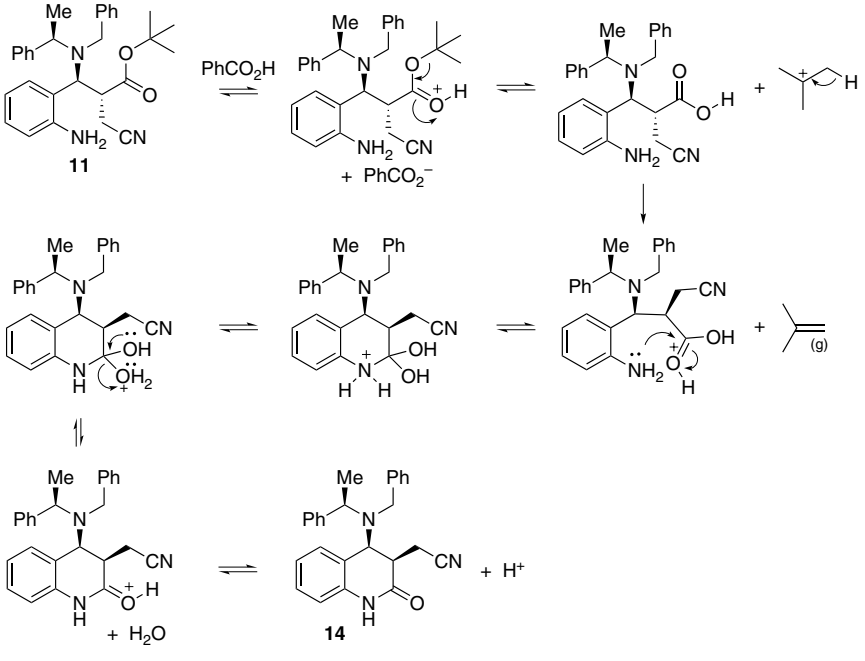


Question 14.6:

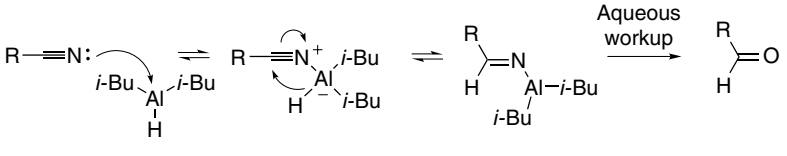
The labile proton α to the two carbonyl moieties in DMBA and allyl-DMBA is easily deprotonated at basic pH, resulting in a water-soluble anion.



Question 14.7:



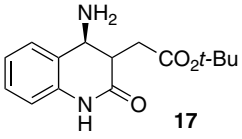
Question 14.8:



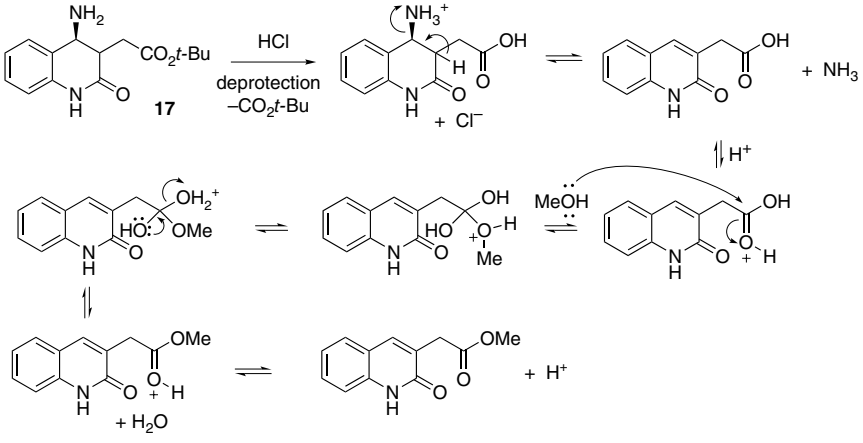
Question 14.9:

Compound	Infrared vibration band frequencies
16	3429, 3383, 3311 (NH stretching), 2978, 2932 (Csp^3–H stretching), 1728 (C═O ester stretching) cm^{-1}
17	3357, 3278, 3029 (NH stretching), 2978, 2929 (Csp^3–H stretching), 1725 (C═O ester stretching), 1680 (C═O amide stretching) cm^{-1}
18	2952, 2857 (Csp^3–H stretching), 1736 (C═O ester stretching), 1665 (C═O amide stretching) cm^{-1}

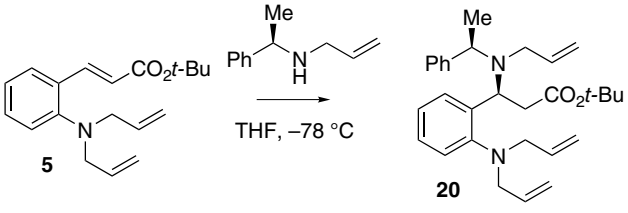
According to infrared data, **17** contains amine, ester, and amide moieties:



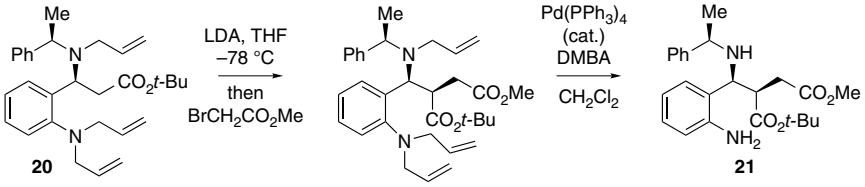
Question 14.10:



Question 14.11:

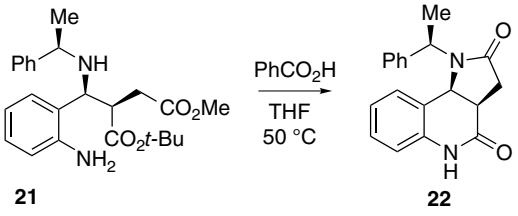


Question 14.12:

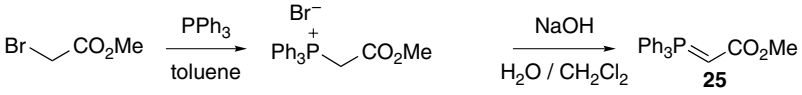


Question 14.13:

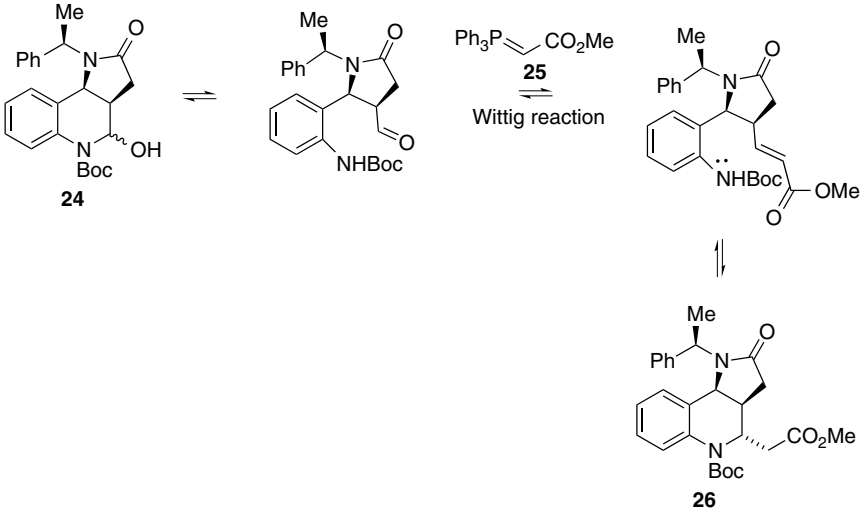
Conditions similar to those used for the conversion of **11** into **14** (Scheme 14.2) can be used:



Question 14.14:



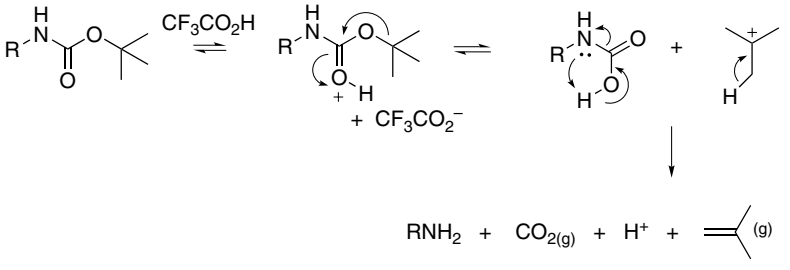
Question 14.15:



Question 14.16:

CF_3CO_2H is added dropwise to a solution of **26** in dichloromethane. A gas evolution (CO_2) is observed. After completion, the reaction mixture is neutralized by addition of aq. NaOH. The layers are separated, and the aqueous layer is extracted with CH_2Cl_2. The combined organic layers are dried over $MgSO_4$ and evaporated *in vacuo*.

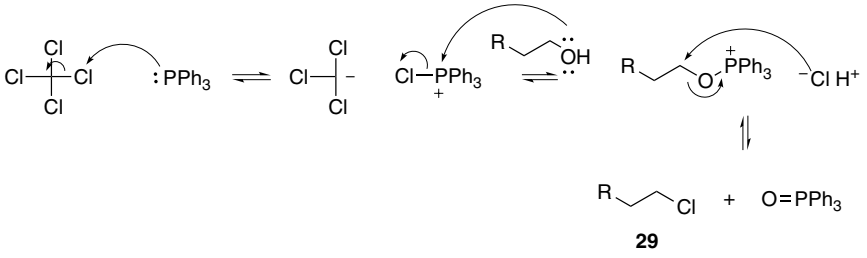
Question 14.17:



Question 14.18:

$LiAlH_4$, THF, reflux

Question 14.19:



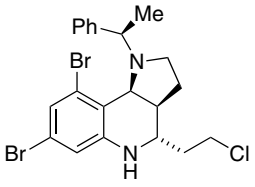
Question 14.20:

^{1}H-NMR signals:

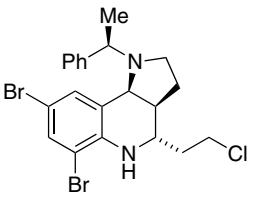
H(1): d ($J_{1,2}$ = 8.5 Hz)
H(2): dd ($J_{2,1}$ = 8.5 Hz; $J_{2,3}$ = 2.3 Hz)
H(3): d ($J_{3,2}$ = 2.3 Hz)

Question 14.21:

The coupling constants reported for **A** (J = 2.3 Hz) are typical of ^4J; thus, two plausible structures are



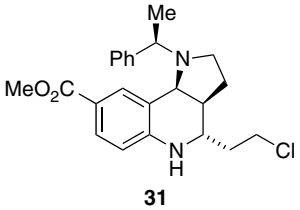
A$_1$



A$_2$

However, the presence of the *N* atom as an electron-donating group should direct electrophilic addition on *ortho* and *para* position, and thus preferentially lead to **A$_2$**.

Question 14.22:



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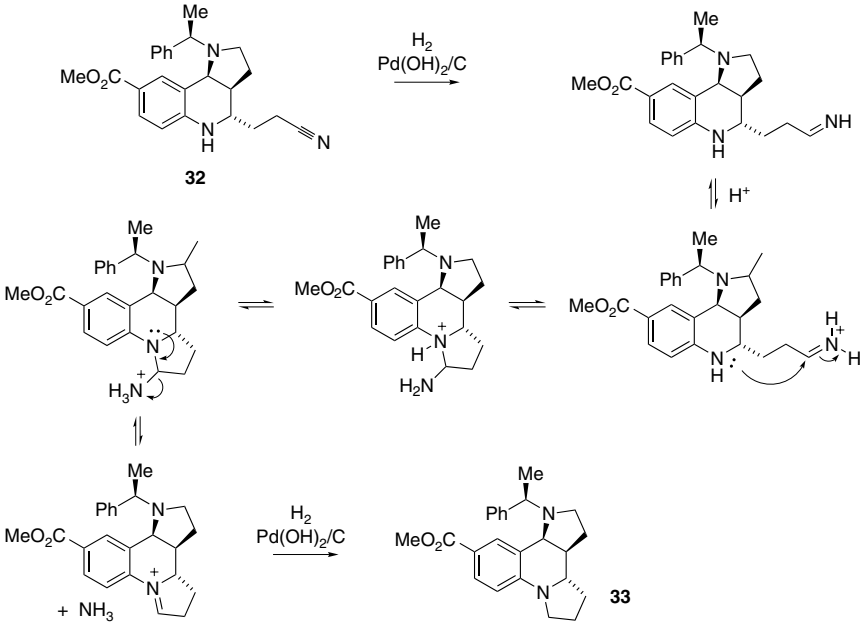
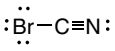
Question 14.23:



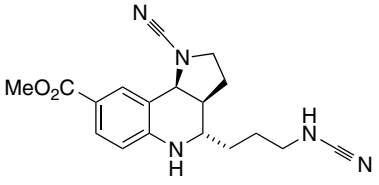
Question 14.24:

$\Delta M = M(\mathbf{36}) - M(\mathbf{37}) = 14$, corresponding to $M(BH_3)$.

Question 14.25:



Question 14.26:



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References

1 Witherup, K.M., Ransom, R.W., Graham, A.C., Bernard, A.M., Salvatore, M.J., Lumma, W.C., Anderson, P.S., Pitzenberger, S.M., and Varga, S.L. (1995) Martinelline and martinellic acid, novel G-protein linked receptor antagonists from the tropical plant Martinella iquitosensis (Bignoniaceae). *J. Am. Chem. Soc.*, **117** (25), 6682–6685.

2 Davies, S.G., Fletcher, A.M., Lee, J.A., Lorkin, T.J.A., Roberts, P.M., and Thomson, J.E. (2013) A diastereodivergent strategy for the asymmetric syntheses of (–)-martinellic acid and (–)-4-epi-martinellic acid. *Tetrahedron*, **69** (46), 9779–9803.

3 Garro-Helion, F., Merzouk, A., and Guibe, F. (1993) Mild and selective palladium(0)-catalyzed deallylation of allylic amines. Allylamine and diallylamine as very convenient ammonia equivalents for the synthesis of primary amines. *J. Org. Chem.*, **58** (22), 6109–6113.

15

Cyclic Pseudopeptides as Potent Integrin Antagonists

Integrins are α/β heterodimeric cell surface receptors which play a key role in cellular recognition and adhesion. The selective inhibition of a specific subtype of integrin receptor is a promising strategy for the development of new therapeutic agents in medicinal chemistry.

Many integrins recognize a common motif constituted of the tripeptide sequence Arg-Gly-Asp (RGD). The selectivity between the different families of integrin receptors lies in their specific recognition of different conformations of this tripeptide sequence, as well as secondary binding sites. In the aim of developing selective inhibitors for a particular family of integrin, an interesting strategy consists in introducing geometrical constraints into the tripeptide sequence in order to favor a given conformation. One example of this approach has been reported in the late 1990s, with the development of cyclopentapeptide **A** as a selective $\alpha_v\beta_3$ integrin inhibitor (Scheme 15.1) [1]. Following a similar strategy, a small library of cyclic RGD mimetics of type **B**, containing fused bicyclic lactam templates (**Temp**) with different ring size and stereochemistry, was also reported more recently [2]. The structure of amino acids constituting **A** and **B**, as well as the pK_a values of their functional groups, is reported in Scheme 15.1.

Question 15.1: Write the structure of peptides **A** and **B** ($n = 1$, undefined stereochemistry) and indicate their ionization state at pH 7.

15.1 Conformational Analysis

In order to favor specific conformations of the tripeptide sequence in **B**, a series of bicyclic lactam templates featuring various geometrical constraints due to different ring size and stereochemistry were considered (Scheme 15.2). The propensity of each diastereoisomer to adopt reverse-turn conformations was evaluated by molecular mechanics methods (Monte Carlo/energy minimization) on the model dipeptide analogs **Mod-Temp** [3].

The lowest energy conformers were analyzed and the following parameters determined: distance C(a)–C(f) (defined as $d\alpha$) and dihedral angle C(b)–C(c)–C(d)–C(e) (defined as virtual torsion angle β) (Scheme 15.3). The criteria commonly adopted as indicative of a turn are $|\beta| < 30°$ and $d\alpha < 7$ Å. This structural

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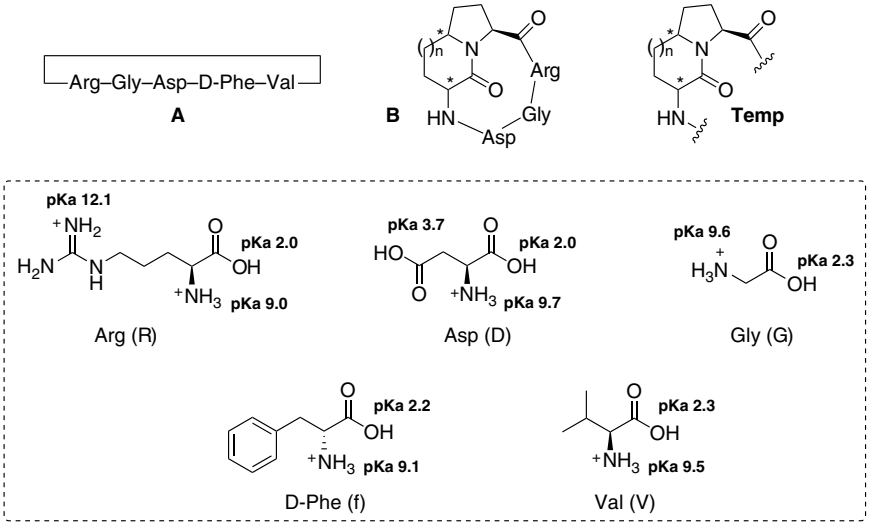
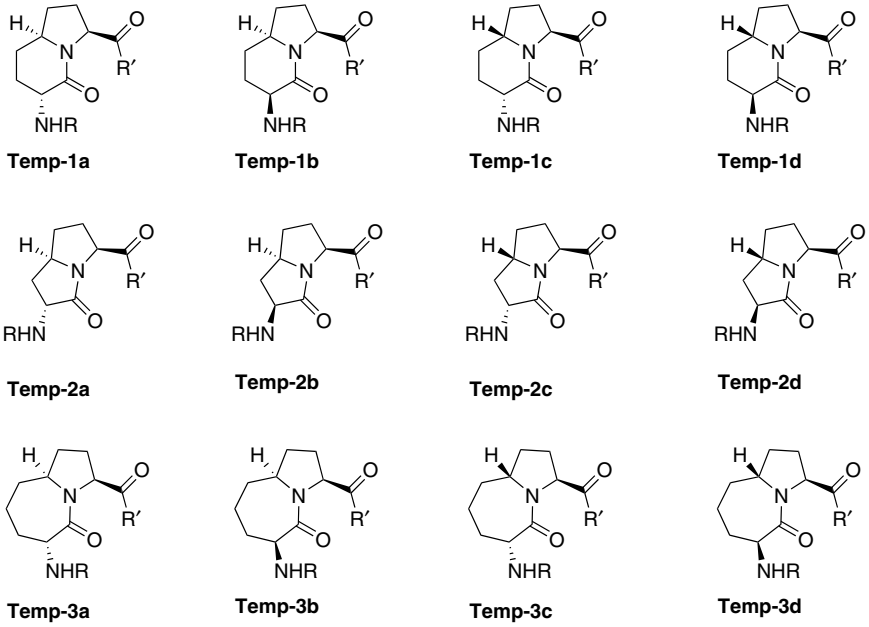
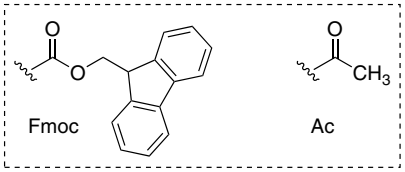
element is often stabilized by an intramolecular hydrogen (7- and 10-membered ring for γ- and β-turns, respectively), evidenced by H–O distance < 2.5 Å.



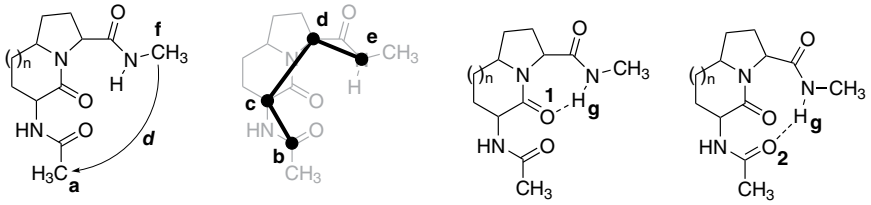
Scheme 15.1



Fmoc-Temp : R = Fmoc, R′ = OH
Mod-Temp : R = Ac, R′ = NHMe



Scheme 15.2



Scheme 15.3

Question 15.2: How would you define a turn?

Question 15.3: Justify the quantitative criteria cited for the comparison of turn-inducing properties of compounds **Mod-Temp**.

The computed percentage of the lowest energy conformers fulfilling the criteria stated is reported in the following table.

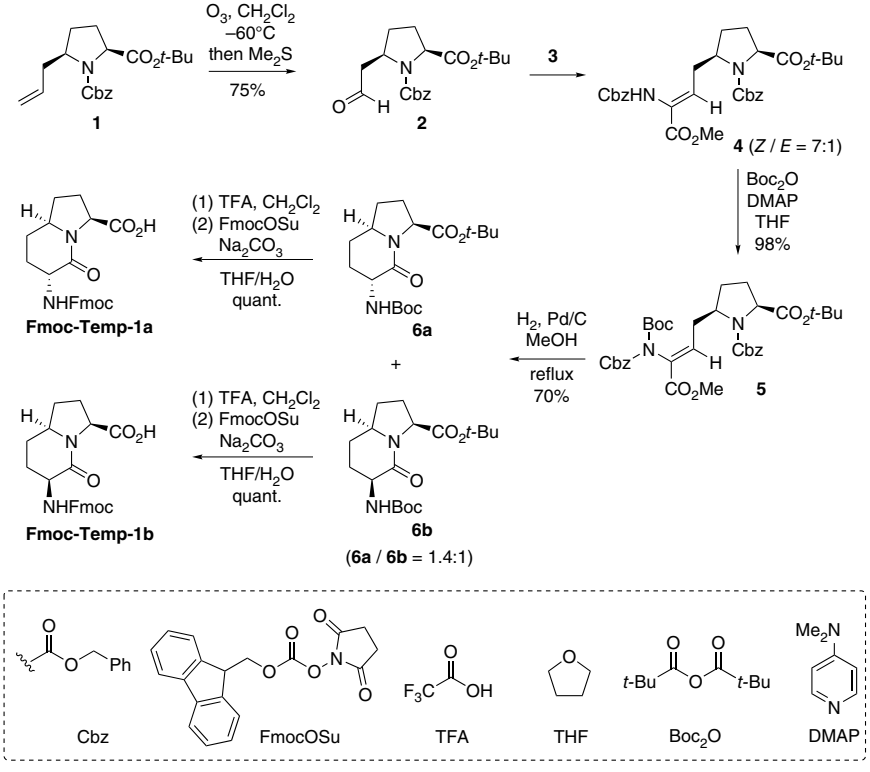
Compound	% $\lvert\beta\rvert < 30°$	% $d\alpha < 7$ Å	% O(1)–H(g) < 2.5 Å	% O(2)–H(g) < 2.5 Å
Mod-Temp-1a	0	0	50	0
Mod-Temp-2a	0	0	25	0
Mod-Temp-3a	8	0	63	0
Mod-Temp-1b	69	27	27	8
Mod-Temp-2b	21	42	21	11
Mod-Temp-3b	0	0	67	0
Mod-Temp-1c	0	0	71	0
Mod-Temp-2c	0	0	100	0
Mod-Temp-3c	80	20	53	0
Mod-Temp-1d	82	46	64	27
Mod-Temp-2d	44	22	44	22
Mod-Temp-3d	27	48	48	21

Question 15.4: On the basis of analysis of β and $d\alpha$ values, identify the compounds with highest propensity to induce turns, and indicate the structural relation in **Mod-Temp** responsible for such folding.

Question 15.5: On the basis of the previous answer and of O(1)–H(g) and O(2)–H(g) distances, indicate which compounds promote γ- or β-turn.

15.2 Synthesis of Bicyclic Lactam Templates

The synthesis of 6,5-fused lactams **Fmoc-Temp-1a** and **Fmoc-Temp-1b** is reported in Scheme 15.4 [4]. The required *cis*-aldehyde **2** is obtained from the known *cis*-5-allylproline derivative **1** [5] by treatment with ozone followed by addition of dimethylsulfide. Compound **2** is then reacted with **3** to give enamide **4** as a mixture of two diastereoisomers in a 7 : 1 ratio. The latter is reacted with Boc_2O in the presence of DMAP to afford **5**, which undergoes catalytic hydrogenation in the presence of Pd/C, followed by spontaneous *in situ* cyclization to give the two diastereoisomers **6a** and **6b** in a 1.4 : 1 ratio, which are separated by flash chromatography. Treatment of **6a** and **6b** with trifluoroacetic acid (TFA) leads to two intermediates, which are directly reacted with Fmoc-OSu without further purification, thus leading to the templates **FmocTemp-1a** and **FmocTemp-1b**.



Scheme 15.4

Question 15.6: Suggest a plausible reaction mechanism for the transformation of **1** into **2**.

Question 15.7: Indicate the structure of reagent **3** and the conditions required for the formation of **4** from **2.**

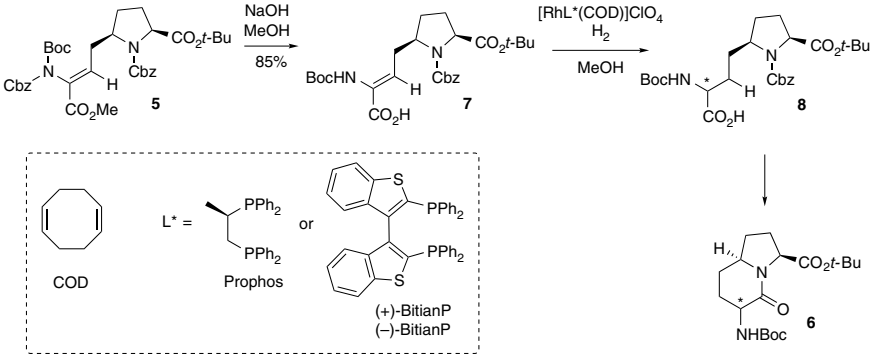
Question 15.8: Explain the diastereoselectivity observed for the formation of **4** by providing a reaction mechanism.

Question 15.9: What is the role of DMAP in the transformation of **4** into **5**?

Question 15.10: Explain the formation of **6a** and **6b** from **5** by writing the sequence of intermediates involved.

Question 15.11: Write the structure of intermediates obtained after treatment of **6a** and **6b**, with TFA. How could these products be isolated?

Question 15.12: Suggest a plausible reaction procedure for the transformation of **6a** and **6b** into **Fmoc-Temp-1a** and **Fmoc-Temp-1b**.



Scheme 15.5

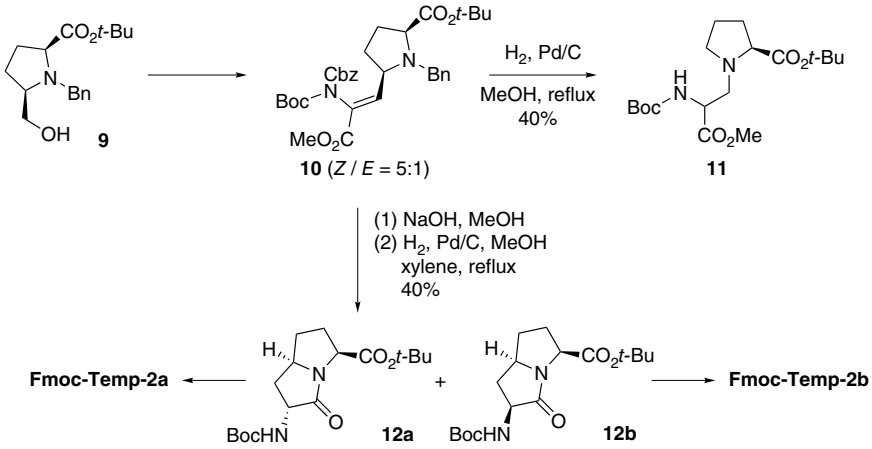
A stereoselective synthesis of both epimers **6a** and **6b** was also investigated, based on a well-established rhodium-catalyzed enantioselective hydrogenation of enamido acid (Scheme 15.5) [4, 6]. Treatment of methyl ester **5** with sodium hydroxide in methanol results in the deprotection of methyl ester with concomitant selective cleavage of one of the Cbz protecting groups, to give **7** in 85% yield. The latter undergoes catalytic hydrogenation in the presence of various chiral rhodium catalysts, thus affording enantiomerically enriched compound **8**, transformed into **6** in two steps. The results obtained for the catalytic reduction of **8** in the presence of various ligands L* are summarized in the following table.

L*	Configuration of the newly generated stereogenic center	de (%)
Prophos	R	72
(+)-BitianP	S	74
(–)-BitianP	R	80

Question 15.13: Given the fact that protection of the nitrogen atom of the enamide as a carbamate (or acetamide) is a requirement to achieve good level of enantioselectivity in the hydrogenation step reaction, suggest a plausible catalytic cycle.

Question 15.14: Write the structure of the starting material required for the preparation of **Fmoc-Temp-1c** and **Fmoc-Temp-1d**.

Other 5,5-fused bicycles **Fmoc-Temp-2a** and **Fmoc-Temp-2b** were obtained following the routes reported in Scheme 15.6. Alcohol **9** is transformed in a few steps into **10**, which, upon hydrogenation in the presence of Pd/C in refluxing MeOH, surprisingly leads to a complex mixture containing 1,2-diaminoester **11** as the major compound (Scheme 15.6). This problem could be circumvented by deprotection of the ester prior to hydrogenation, affording the two easily separable diastereoisomers **12a** and **12b** in 40% yield over the two steps.

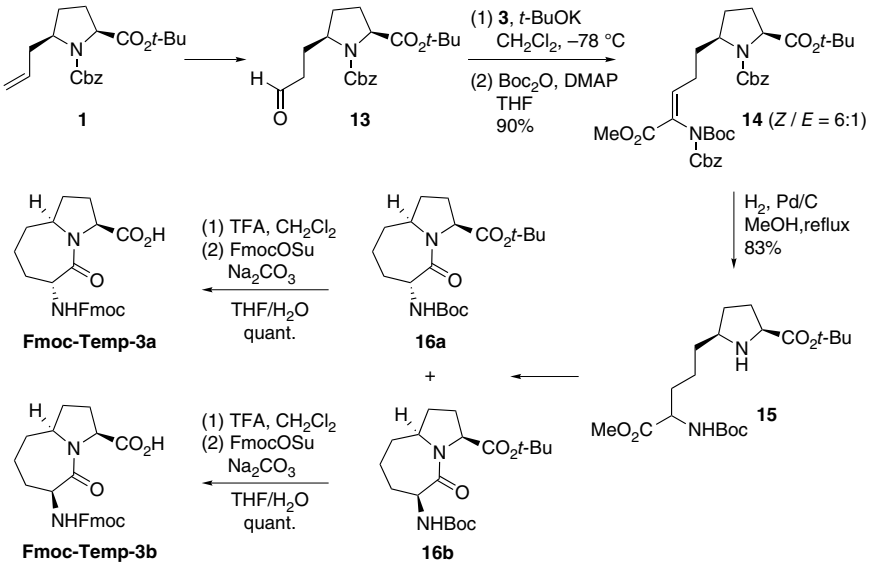


Scheme 15.6

Question 15.15: Suggest a plausible reaction sequence allowing the preparation of **10** from alcohol **9**.

Question 15.16: Suggest a plausible reaction mechanism accounting for the formation of **11**.

The corresponding 7,5-fused lactams **Fmoc-Temp-3a** and **Fmoc-Temp-3b** were obtained from 5-allyl-proline **1**, which is transformed into aldehyde **13** in two steps (Scheme 15.7). The latter reacts with **3** in the presence of *t*-BuOK, followed by Boc_2O/DMAP to give key enamide **14** in 90% yield for the two steps. Unlike previous cases, hydrogenation leads to saturated compound **15**, for which spontaneous thermal cyclization to **16** does not occur, thus requiring other reaction conditions. Finally, removal of the acid-labile protecting groups followed by



Scheme 15.7

Fmoc protection leads to the pseudodipeptides **Fmoc-Temp-3a** and **Fmoc-Temp-3b**, ready for use in peptide solid-phase synthesis.

Question 15.17: Suggest a plausible reaction sequence allowing the preparation of **13** starting from 4-allyl-proline derivative **1**.

Question 15.18: Suggest a plausible reaction sequence allowing the transformation of **15** into **16a** and **16b**.

15.3 Solid Phase Peptide Synthesis

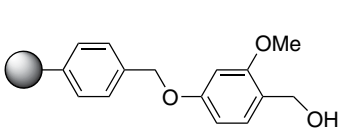
The synthesized fused bis-lactam templates were then introduced into peptide sequences via solid-phase synthesis in Fmoc strategy, using the highly acid-labile SASRIN or 2-chlorotrityl resins (Scheme 15.8).[1]

Question 15.19: What are the main advantages of solid-phase synthesis over liquid-phase synthesis?

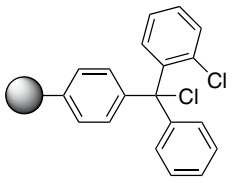
Question 15.20: Justify the qualification of "highly acid-labile resins."

The synthetic sequence allowing preparation of peptide **B** is described in Scheme 15.9. The resin is coupled to Fmoc-Gly-OH by reaction with DIC, HOAt[2],

1 In the original article, only SASRIN resin was reported for this synthesis.
2 In the original article, HOBt was used instead of HOAt.

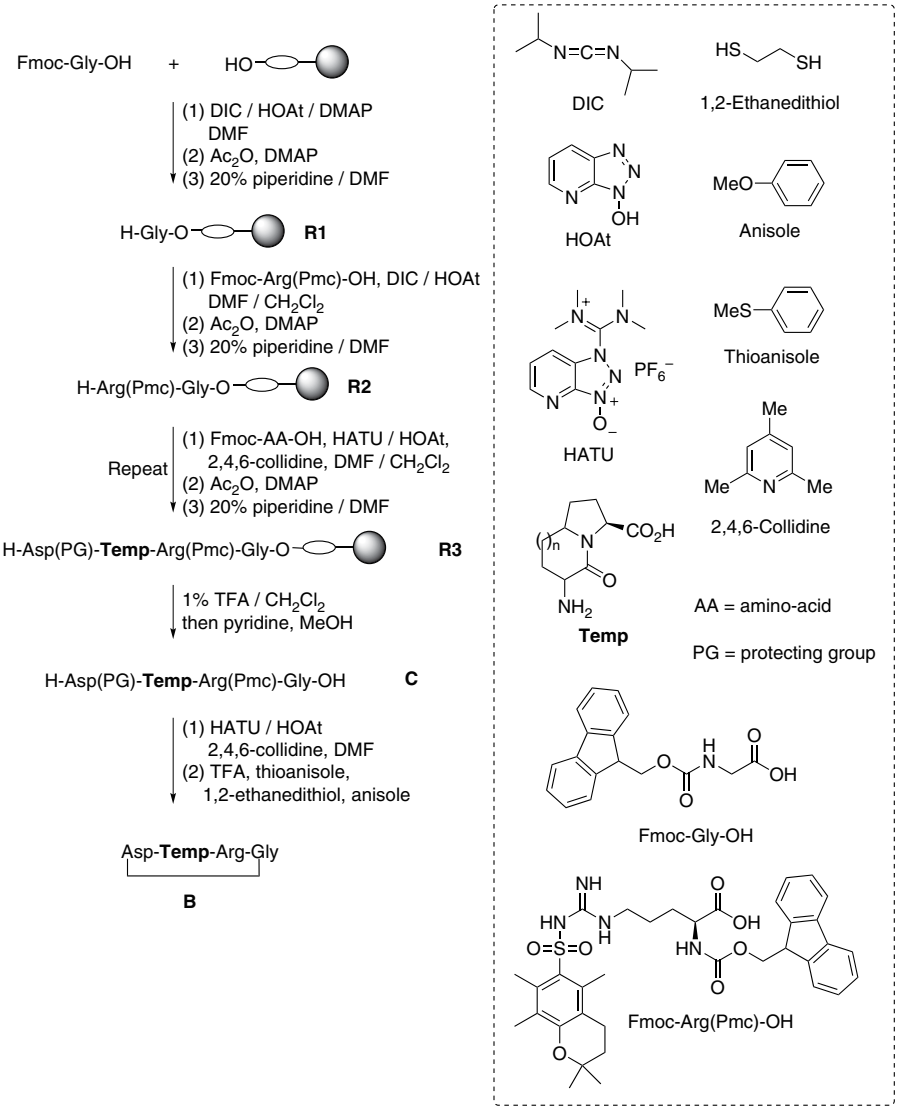


SASRIN resin



2-Chlorotrityl resin

Scheme 15.8



Scheme 15.9

and a catalytic amount of DMAP in dimethylformamide (DMF), followed by treatment with acetic anhydride and DMAP. Deprotection of the Fmoc group, affording functionalized resin **R1**, is achieved by reaction with a solution of piperidine in DMF. A solution of Fmoc-Arg(Pmc)-OH, pretreated with HOAt and DIC, is added, followed by treatment with acetic anhydride and DMAP,[3] and with a solution of piperidine in DMF to afford **R2**. Any **Fmoc-Temp** derivative described earlier (Scheme 15.2) could be introduced at this stage by reaction with HATU, HOAt, and 2,4,6-collidine, followed by treatment with acetic anhydride and DMAP,[3] and with a solution of piperidine in DMF. The same procedure involving coupling/treatment with acetic anhydride and DMAP/deprotection is repeated for the introduction of Asp residue, thus leading to a series of compounds of type **R3**.

Cleavage of the linear peptides from the resin is performed using a solution of TFA in CH_2Cl_2, followed by immediate neutralization with a solution of pyridine (large excess) in MeOH and evaporation under reduced pressure. The residue is separated from pyridinium salts by size-exclusion chromatography, using H_2O and MeOH as eluent, to afford **C** in 30–67% yield.

Macrocyclization of linear peptide **C** is performed by treatment with HATU, HOAt, and 2,4,6-collidine in highly diluted DMF solution (0.05M). After appropriate workup and purification, the resulting fully protected peptide is obtained in yields varying between 26% and 70% depending on the nature of the bis-lactam template (**Temp**) incorporated. Finally, reaction with TFA in the presence of thioanisole, 1,2-ethanedithiol, and anisole affords cyclopeptide **B** after workup and purification by ion-exchange chromatography. Its purity is checked by analytical high-performance liquid chromatography (HPLC).

Question 15.21: What is the role of treatment with Ac_2O and DMAP following each amino acid coupling step?

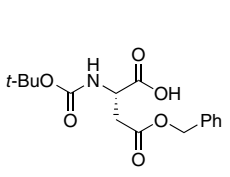
Question 15.22: Suggest a reaction mechanism for the deprotection of the Fmoc group with piperidine.

Question 15.23: Suggest a reaction mechanism for the coupling of Fmoc-Arg(Pmc)-OH to the glycine residue promoted by DIC/HOAt. What is the role of HOAt?

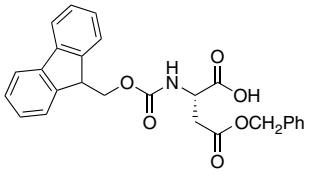
Question 15.24: What is the advantage of using HATU instead of DIC during conversion of **R2** to **R3**?

3 In the original article, acetylimidazole (10 equiv.) in CH_2Cl_2 was used.

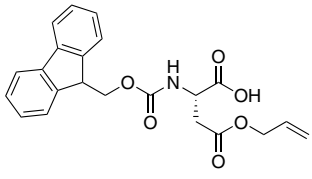
Question 15.25: Which of the following Asp derivatives are suitable for the synthesis described in Scheme 15.9? Justify your answer.



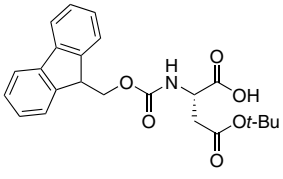
Boc-Asp(OBn)-OH



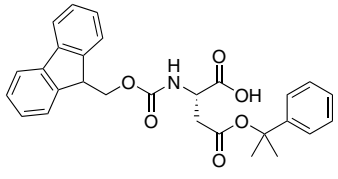
Fmoc-Asp(OBn)-OH



Fmoc-Asp(OAll)-OH



Fmoc-Asp(O*t*-Bu)-OH



Fmoc-Asp(OPp)-OH

Question 15.26: Why should the filtrate resulting from cleavage of peptide from the resin in **R3** be immediately neutralized?

Question 15.27: Briefly explain the principle of size-exclusion chromatography?

Question 15.28: Why should macrocyclization of **C** be performed under highly diluted conditions?

Question 15.29: What is the role of thioanisole, 1,2-ethanedithiol, and anisole during the final deprotection step leading to **B**?

Question 15.30: Briefly explain the principle of ion-exchange chromatography?

The yields of macrocyclization are highly heterogeneous, depending on the structure of the bis-lactam template (ring size and stereochemistry). Particularly with linear peptide **C** containing **Temp-1a,** no macrocyclization was observed even upon heating.

Question 15.31: Suggest a plausible explanation for the poor reactivity of the linear peptide in this particular case.

15.4 Pharmacological Study

The synthesized RGD cyclic pseudopeptides were examined *in vitro* for their ability to compete with radiolabeled ^{125}I-echistatin (entry 1), a known integrin $\alpha_v\beta_3$ receptor ligand. The results are presented in Table 15.1 as IC_{50} values (half maximal inhibitory concentration) [2]. The natural integrin ligands vitronectin

Table 15.1 IC_{50} values obtained for echistatin, vitronectin, fibronectin, and the synthesized cyclic peptides.

Entry	Compound	IC_{50} (nM)
1	Echistatin	0.28 ± 0.08
2	Vitronectin	44.1 ± 17.0
3	Fibronectin	835.4 ± 287.0
4	c[RGDfV]	195.9 ± 16.8
5	c[RGD**Temp-1b**]	206.9 ± 8.7
6	c[RGD**Temp-1c**]	14.3 ± 4.7
7	c[RGD**Temp-1d**]	97.3 ± 7.5
8	c[RGD**Temp-3a**]	2478.4 ± 373.2
9	c[RGD**Temp-3b**]	245.2 ± 43.0
10	c[RGD**Temp-3c**]	3.7 ± 0.6
11	c[RGD**Temp-3d**]	412.5 ± 94.1

(entry 2) and fibronectin (entry 3), as well as cyclic RGD sequence (entry 4), were also included in the screening.

Question 15.32: What is the aim of the experiments reported in entries 1 to 4?

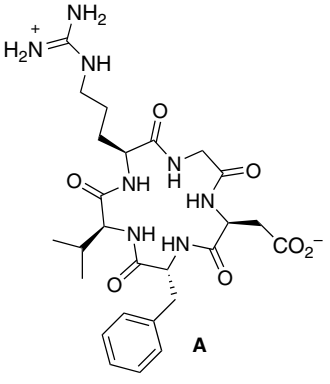
Question 15.33: Which biophysical technique is used in these experiments? What are the main advantages and drawbacks of this technique?

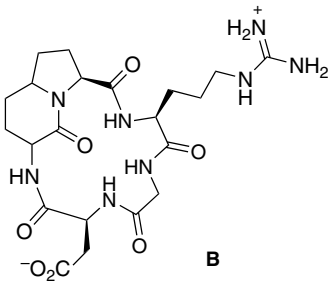
Question 15.34: What is IC_{50}? How is this value obtained?

Question 15.35: According to the results reported, which compounds are the best integrin $\alpha_v\beta_3$ ligands?

Answers

Question 15.1:





Question 15.2:

A turn is a site where the peptide backbone reverses the direction of propagation by adopting a U-shaped conformation.

Question 15.3:

If $|\beta| < 30°$, the peptide chains attached to C(b) and N(e) are roughly located in the same plan, while a distance $d\alpha < 7$ Å ensures that these two chains point toward the same direction, which is in agreement with the definition of a turn.

Question 15.4:

Analysis of the percentage of the lowest energy conformers with $|\beta|$ values below 30° and $d\alpha$ distances below 7 Å reveal that compounds **Mod-Temp-1b**, -**2b**, -**1d**, -**2d**, -**3d**, and -**3c** induce a reversal of direction in the peptide backbone, which is not the case with **Mod-Temp-3b**, -**1a**, -**2a**, -**3a**, -**1c**, and -**2c**.

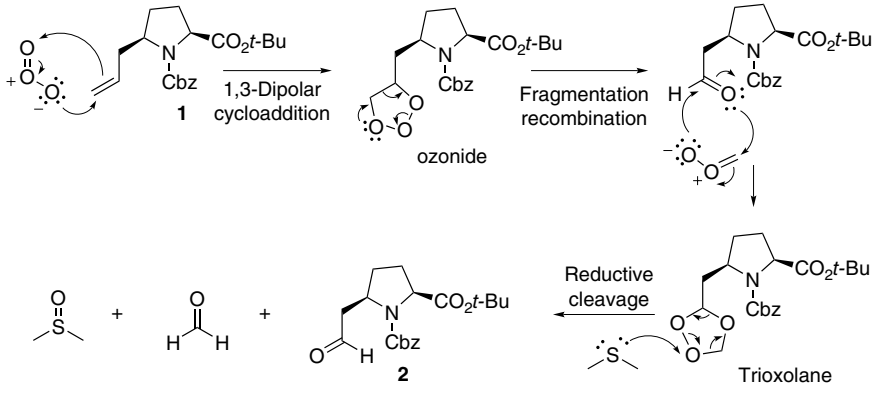
With the exception of **Mod-Temp-3b**, all compounds with a potential use as turn mimetics have a *cis* relation between the carboxy and amino groups attached to the bicyclic lactam system; in contrast, all compounds showing *trans* relation (excepting **Mod-Temp-3c**) do not adopt a geometry suitable for this purpose.

Question 15.5:

The bicyclic lactam templates **Mod-Temp-1b**, -**2b**, -**1d**, -**2d**, and -**3d** can promote both γ- and β-turn, as revealed by the significant population of conformers with suitable $|\beta|$ and $d\alpha$ values displaying intramolecular hydrogen bond between O(1)–H(g) and O(2)–H(g), respectively (Scheme 15.3).

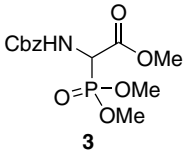
Among other bicyclic templates only **Mod-Temp-3c** exhibits geometries inducing a reversal of peptide direction, with a hydrogen pattern evidencing γ-turn folding.

Question 15.6:



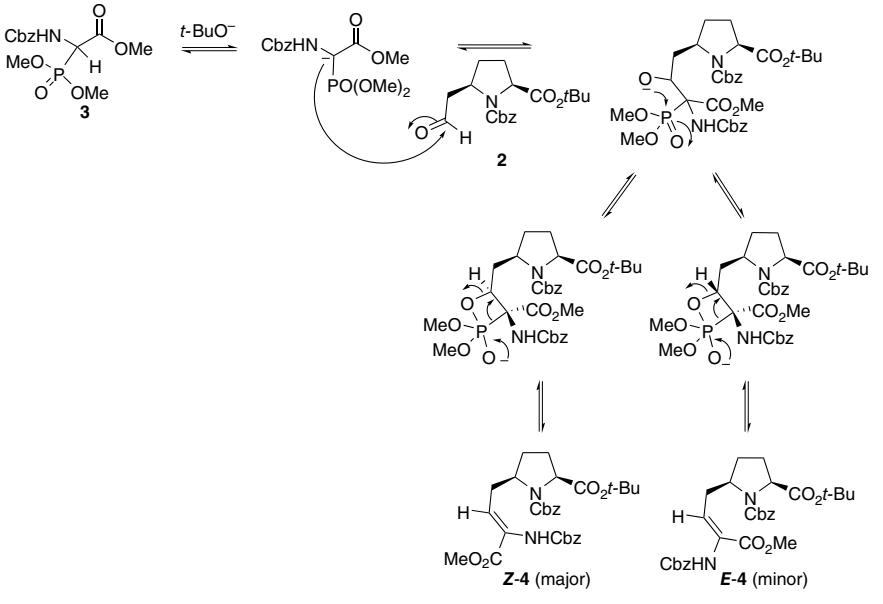
Question 15.7:

The transformation of **2** into **4** can be done through a Horner–Wadsworth–Emmons reaction, using a phosphonoglycine derivative **3** in the presence of a base such as *t*-BuOK.



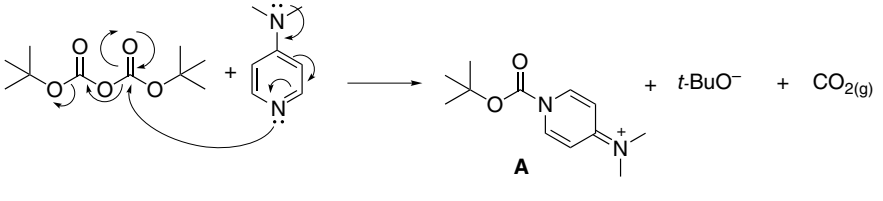
Question 15.8:

In the Horner–Wadsworth–Emmons reaction (also known as Wittig–Horner reaction), the reaction occurs according to the mechanism described here, in which all steps are reversible. The reaction is thus under thermodynamic control, and leads preferentially to the thermodynamic compound, *Z*-**4**

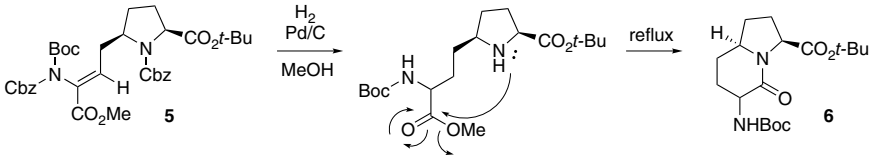


Question 15.9:

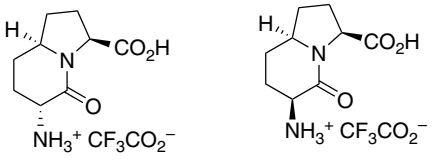
The role of DMAP is to activate Boc_2O by formation of species **A**, and thus favoring attack by the weakly nucleophilic carbamate protected amide in **4**.



Question 15.10:



Question 15.11:



Intermediates obtained from **6a** and **6b** contain both an amine and a carboxylic acid group, and are either positively or negatively charged depending on the pH of the solution. Because of their high polarity and water solubility they have to be isolated by reverse-phase chromatography or ion-exchange chromatography. Moreover, they could be precipitated in water at their exact isoelectric point, pH at which the positive charge of the ammonium balances the negative charge of the carboxylate. This form is called a zwiterrion.

In general, to avoid purification of the free amino acid, protection of the amine is performed directly on the crude material.

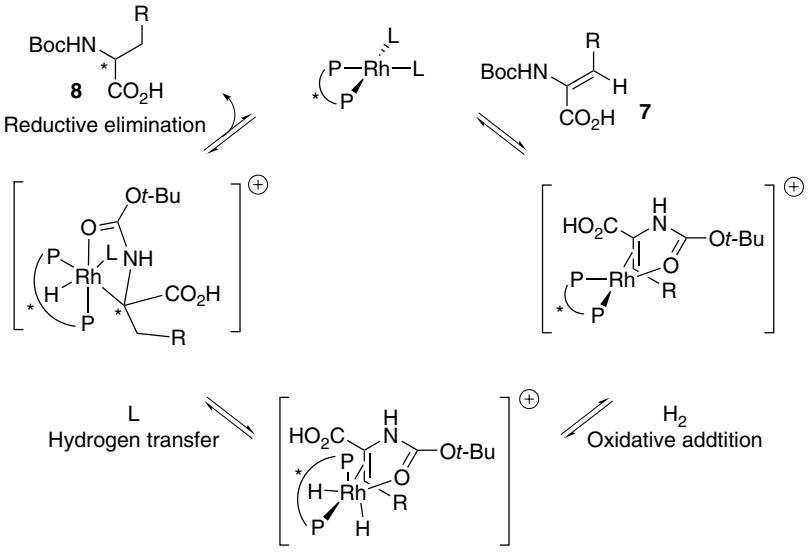
Question 15.12:

To a solution of **6a**/**b** (1 equiv.) in dichloromethane is added trifluoroacetic acid and the resulting mixture is stirred at room temperature. The solvents are evaporated under reduced pressure, the crude residue is dissolved in tetrahydrofuran (THF), and aq. Na_2CO_3 10 % solution is added, followed by Fmoc-OSu (1.2 equiv.). The resulting mixture is stirred at rt, THF is evaporated under reduced pressure, and the aqueous phase is washed with EtOAc. Aq. concentrated HCl is added until a pH value of 2 is reached, the solution is extracted three times with EtOAc, and the combined organic layers are dried over Na_2SO_4 and evaporated under reduced pressure to afford **Fmoc-Temp-1a**/**b**.

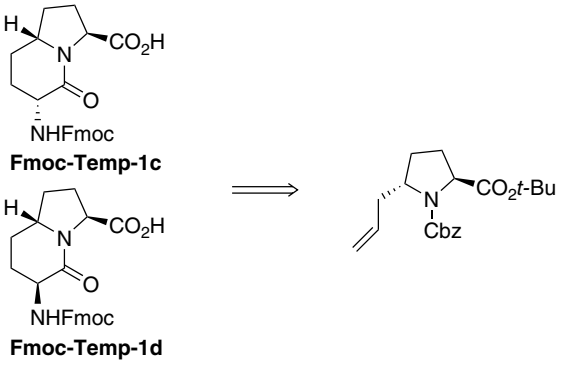
Question 15.13:

The fact that a C═O group attached to the nitrogen atom of enamide is required to achieve good levels of enantioselection suggests that coordination to the metal center of the catalyst occurs.

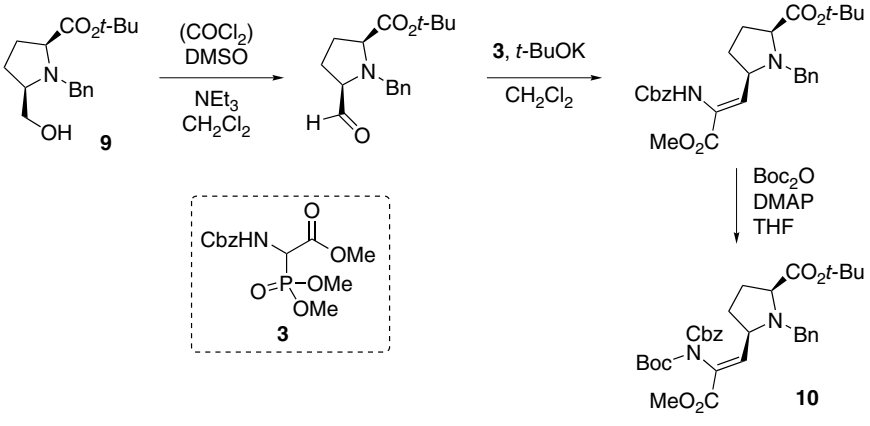
A commonly established mechanism for such type of reaction is reported here [6].



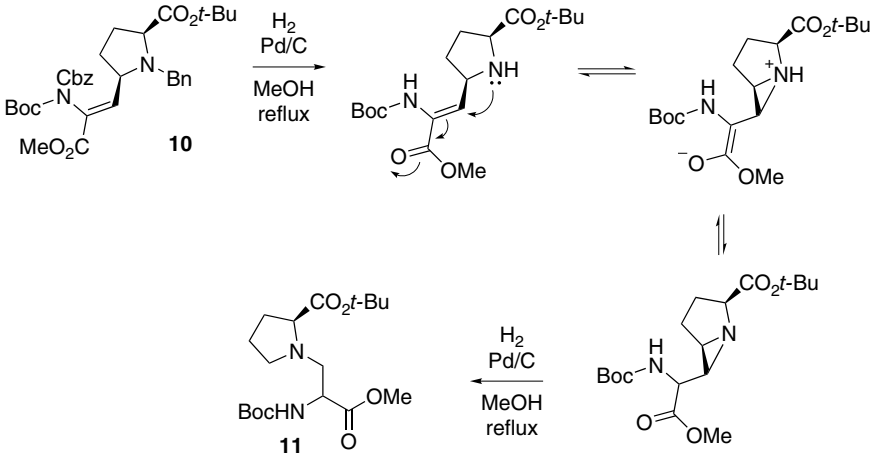
Question 15.14:



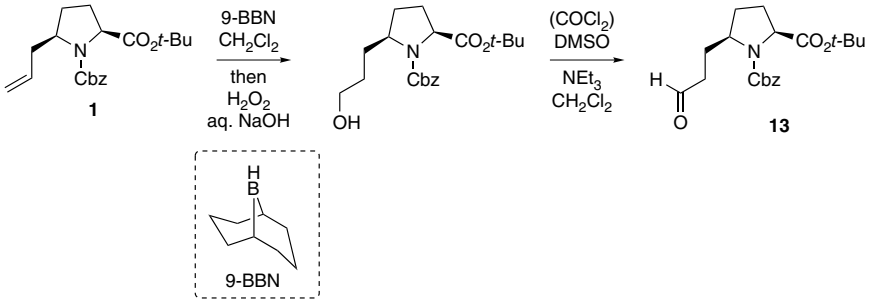
Question 15.15:



Question 15.16:

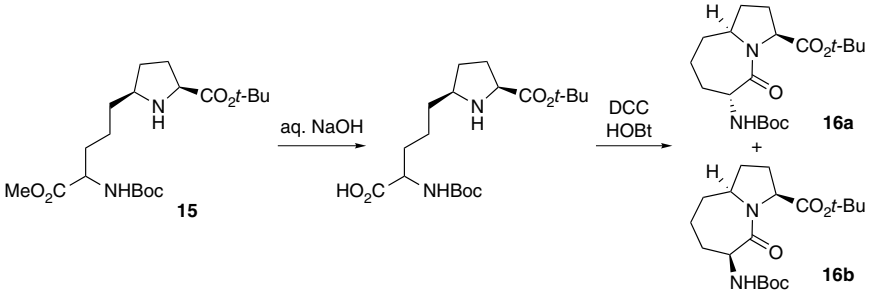


Question 15.17:



Question 15.18:

Cyclization of **15** requires the formation of carboxylic acid followed by lactamization.



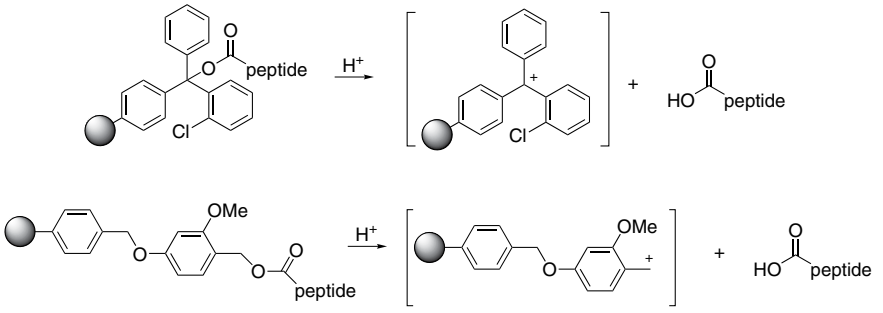
Question 15.19:

In solid-phase synthesis, the reactant is attached to an insoluble polymer that may be easily separated from excess reagents and by-products by simple filtration. The

main advantage of the technique is that there is no need to perform tedious and time-consuming isolation and purification of all intermediates; only the final product obtained after cleavage from the resin is purified. Furthermore, the simplicity of the technical operation allows for automation of peptide synthesis.

Question 15.20:

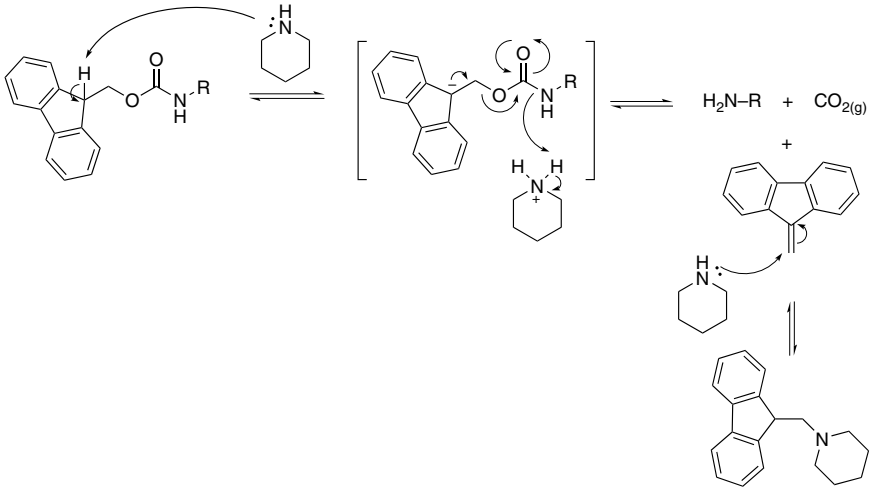
The high sensitivity of these resins to acidic conditions is due to the high stability of the intermediate benzylic carbocation generated by acidolysis of the peptide. Cleavage from such resins is typically performed in the presence of only 0.5–1% TFA, or by treatment with an acidic alcohol such as hexafluoroisopropanol, thus allowing access to fully protected peptides that could undergo further transformations.



Question 15.21:

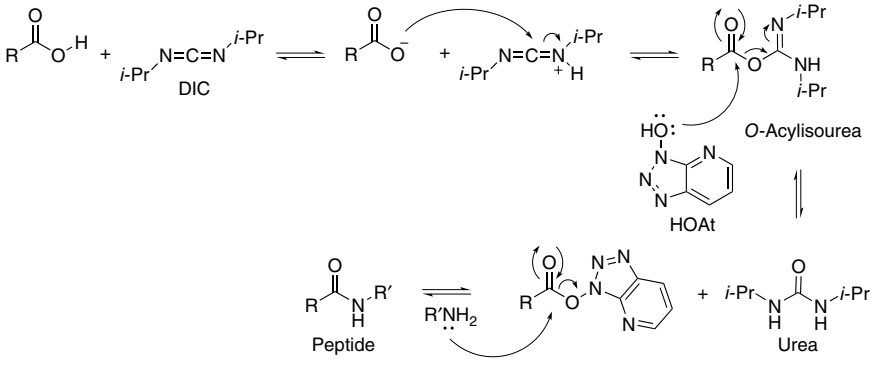
Treatment with Ac_2O/DMAP at the end of each amino acid coupling step aims at "capping" unreacted amines (transformed into the corresponding acetamides) to avoid further elongation of mismatch sequences during the next coupling steps. The capped sequences are usually easily separated from the final product.

Question 15.22:

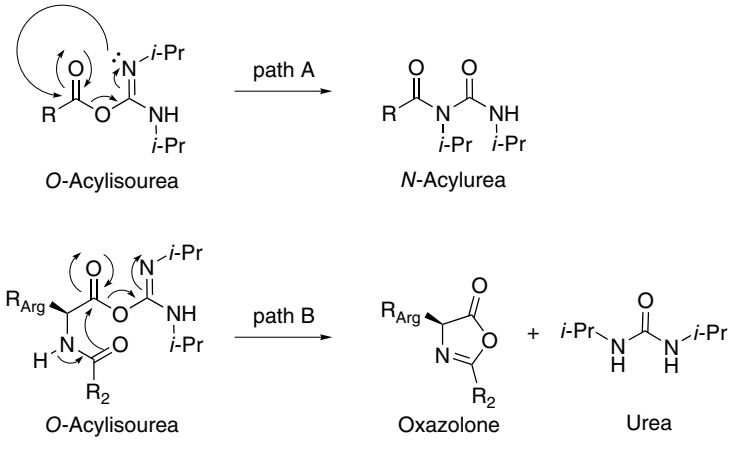


Question 15.23:

The coupling mechanism of an amine with a carboxylic acid to form an amide bond in the presence of DIC and HOAt is presented here.



The intermediate *O*-acylisourea can undergo two side reactions: a migration of the acyl group leading to the unreactive *N*-acylurea (path A) or a cyclization leading to an oxazolone (path B), which is prone to racemization. The role of HOAt is to intercept the highly reactive *O*-acylisourea, thus avoiding both *N*-acylurea formation and racemization.



Question 15.24

HATU is highly efficient for difficult couplings involving hindered substrates [7]. Its high reactivity is thought to arise from the neighboring effect of the pyridine nitrogen atom, which stabilizes the incoming amine through a hydrogen-bonded seven-membered cyclic transition state.

Question 15.25:

A suitable aspartate derivative for the synthesis of peptide **C** should contain a temporary amine (*N*α) protective group that can be removed from the resin after amino acid coupling and a semipermanent side chain carboxy protective group stable toward peptide elongation, *N*α deprotection, and cleavage from the resin. Since the synthesis is performed on a highly acid-labile resin, Boc-Asp(OBn)-OH derivative is excluded because deprotection of the backbone amine (Boc removal) would also lead to the cleavage from the resin. Phenylisopropyl ester in Fmoc-Asp(OPp)-OH is unsuitable because it would be removed during cleavage of the peptide from the resin (1% TFA in CH_2Cl_2).

In principle, Fmoc-Asp(OBn)-OH, Fmoc-Asp(OAll)-OH, and Fmoc-Asp(O*t*-Bu)-OH could lead to peptide **C**. However, only the *t*-Bu group is compatible with the conditions described in Scheme 15.9 (final deprotection with TFA), since Bn and All groups would require an additional deprotection step.

Question 15.26:

The filtrate needs to be immediately neutralized to avoid deprotection of the acid-labile Pcm and *t*-Bu side chains protective groups.

Question 15.27:

Size-exclusion chromatography, also known as gel-filtration chromatography, allows separation of molecules according to their size, and is especially useful to remove small organic compounds from large molecules such as peptides. The stationary phase contains pores able to trap small molecules, while larger ones (too large to enter the pores) simply flow through the column.

Question 15.28:

Macrocyclization is performed under highly diluted conditions to avoid intermolecular reaction leading to polymer formation.

Question 15.29:

Thioanisole, 1,2-ethanedithiol, and anisole (commonly defined as scavengers) react with electrophilic carbocations generated upon acid-catalyzed deprotection of the side chains, thus avoiding addition to the nucleophilic sites of the peptide.

Question 15.30:

Ion-exchange chromatography allows separation of molecules according to the difference in their net surface charge. It is based on the retention of a charged molecule on an oppositely charged stationary phase (electrostatic interactions). Elution is performed by changing the pH or ionic strength of the mobile phase.

Question 15.31:

The ease of cyclization of the peptide is dependent on the conformational constraints brought by the bicyclic lactam template. The latter can either preorganize the macrocycle by orienting the two chains in an optimal arrangement or position them away from each other, thus disfavoring the reaction. Such a geometrical effect accounts for the heterogeneity observed during the macrocyclization step.

Question 15.32:

Experiments 1–4 are positive controls aimed at testing the reliability of the assay with known ligands.

Question 15.33:

The experiments performed to measure IC_{50} are based on the displacement of a radiolabeled ligand (^{125}I-echistatin). The advantage of such a technique is its very high sensitivity and the weak structural impact of the label, as compared to fluorescent probes. However, it is highly limited by safety considerations and requires dedicated equipment.

Question 15.34:

IC_{50} (half maximal inhibitory concentration) is representative of the effectiveness of a compound to inhibit the binding of a competitor. It is measured by incubating a receptor with a fixed concentration of a radioligand, followed by addition in increasing concentration of a competitor. After removal of the free ligand, the remaining emission corresponding to the bound ligand is plotted against competitor concentration. The competitor concentration for which emission reaches 50% of its initial value corresponds to IC_{50}.

Question 15.35:

Compound c(RGD**Temp-3c**), showing IC_{50} value of 3.7 nM, is the best ligand of integrin $\alpha_v\beta_3$ reported in this study. According to structural calculations, bicyclic lactam template **Temp-3c** is the best γ-turn promoter, suggesting that this secondary structure is involved in the bioactive conformation of RGD peptide bound to this receptor.

It is interesting to note, however, that although **Temp-1c** was not anticipated by molecular modeling to induce reverse turns, peptide c(RGD**Temp-1c**) is also a good inhibitor of the target integrin (IC_{50} = 14.3 nM).

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16

Enantioselective Synthesis of Nonnatural Amino Acids for Incorporation in Antimicrobial Peptides

Protegrin I (PG-I) is an 18-amino acids peptide showing interesting antibacterial properties (Figure 16.1). Its structure reveals the presence of two disulfide bridges (C8/C13 and C6/C15), forcing the backbone to fold into a regular β-hairpin conformation, with a β-turn formed by residue 9–12.

In an attempt to improve the selectivity for bacterial versus mammalian cells, macrocyclic analogs of PG-I of general structure **I** with enhanced β-hairpin folding were reported in 2002 (Figure 16.1) [1].

Question 16.1: Define a peptidomimetic.

Question 16.2: Write the structure of PG-I and indicate its ionization state at pH 7.

Question 16.3: What is a β-hairpin conformation?

Question 16.4: Why should enhanced β-hairpin folding be expected for peptidomimetics of general structure **I** as compared to PG-I?

16.1 First Generation Mimetics: Synthesis and Biological Evaluation

Antimicrobial peptides are characterized by a specific segregation of cationic basic residues (arginine or lysine) and hydrophobic residues, resulting in an overall amphiphilic structure.

Question 16.5: What is an amphiphilic compound?

In a first study, a small library of 12-residue cyclic peptides of type **I**, with sequences inspired from PG-I, was prepared [1]. Their general topology is depicted in Scheme 16.1.

Multi-Step Organic Synthesis: A Guide Through Experiments, First Edition. Nicolas Bogliotti and Roba Moumné.

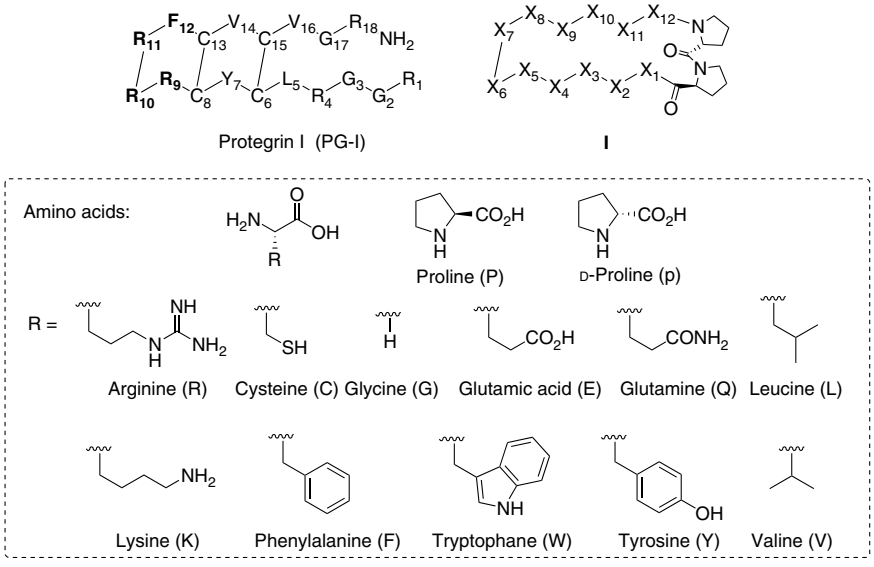
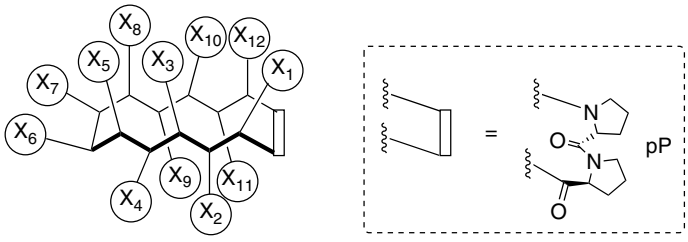


Figure16.1 Schematic structure of Protegrin I and its cyclic analog **I**.



Scheme 16.1

Antimicrobial activity was evaluated by measuring minimal inhibitory concentration (MIC) against different bacterial strains as well as hemolytic activity on human red blood cells. The results are given in the following table.

Peptide	Sequence	MIC ($\mu g\,mL^{-1}$)				Percentage of hemolysis (at 100 $\mu g\,mL^{-1}$)
		S. aureus	E. coli	P. aerug	C. albic	
PG-I	RGGRLCYCRRRFCVCVGRNH2	6	3	3	6	37
1	c[LRLKYRRFKYRVpP]	25	12	25	12	27
2	c[LRLKYRRFQYRVpP]	12	6	12	6	27
3	c[LRLEYRRFEYRVpP]	100	100	>100	50	14
4	c[LRLKKRRWKYRVpP]	12	12	6	12	1
5	c[LCLKKRRWKYCVpP]	25	6	25	12	3
6	c[LRCKKRRWKCRVpP]	25	25	50	50	1

Question 16.6: Suggest a general synthetic strategy for the synthesis of peptide **1** by solid-phase peptide synthesis (Fmoc/*t*-Bu), mentioning the nature of resin, building blocks, and coupling agents.

Question 16.7: What is a minimal inhibitory concentration?

Question 16.8: What is the aim of measuring hemolytic activity on human red blood cells?

Question 16.9: What is the aim of evaluating activity of compound **3** and what can be concluded from this experiment?

Question 16.10: Using the general topology depicted in Scheme 16.1, comment on the amphipathic nature of mimetics 1 and 4. Is there a direct relationship between the amphiphilic nature of these compounds and their antimicrobial activities and selectivities?

Question 16.11: What is the aim of evaluating the activity of compounds **5** and **6** and what can be concluded from this experiment?

Question 16.12: Comment on the results reported in the earlier table and indicate which compounds show best results in terms of both activity and selectivity? Justify your answer.

16.2 Structural Analysis and Mechanism of Action

Structural investigations by ^{1}H-NMR spectroscopy were performed on peptide **4** both in water and in a micellar medium mimicking membrane environment. Advanced experiments allowed the calculation of average structures reported in Figure 16.2.

Question 16.13: What conclusion can be drawn from the results reported in Figure 16.2?

The general mechanism of action of cationic antimicrobial peptides involves interaction of the positively charged residues with anionic outer microbial cell membrane leading to its disruption, leakage of cell content, and eventually cell death.

The interaction of peptide **4** with membranes was investigated. Nuclear magnetic resonance (NMR) experiments were performed in the presence of micelles doped with a paramagnetic probe, whose unpaired electron accelerates longitudinal and transversal relaxation of protons in close spatial proximity. The NH-Cα cross-peak region of the TOCSY spectrum of mimetic **4** performed in the presence of micelles with (right) and without (left) paramagnetic probe is shown in Figure 16.3.

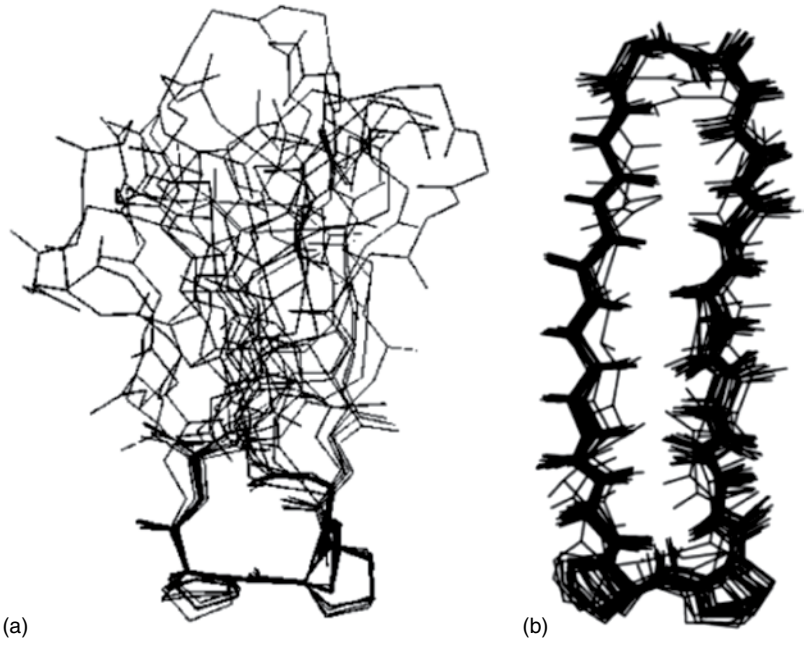


Figure16.2 Average structures for peptide **4** in water (a) and in the presence of micelles (b). *Source:* Shankaramma 2002 [1]. Reproduced with permission of Wiley.

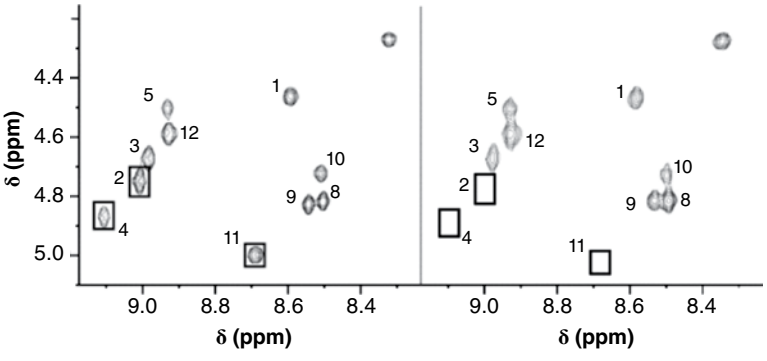


Figure16.3 TOCSY spectra of peptide **4** in the presence of micelles with (right) and without (left) paramagnetic probe. *Source:* Shankaramma 2002 [1]. Reproduced with permission of Wiley.

Other studies consisted in the synthesis of peptide ***ent*-4**, the enantiomer of **4**, and evaluation of its antimicrobial potency. The results revealed essentially similar or even slightly higher antimicrobial activities.

Question 16.14: What do the results reported in Figure 16.3 suggest?

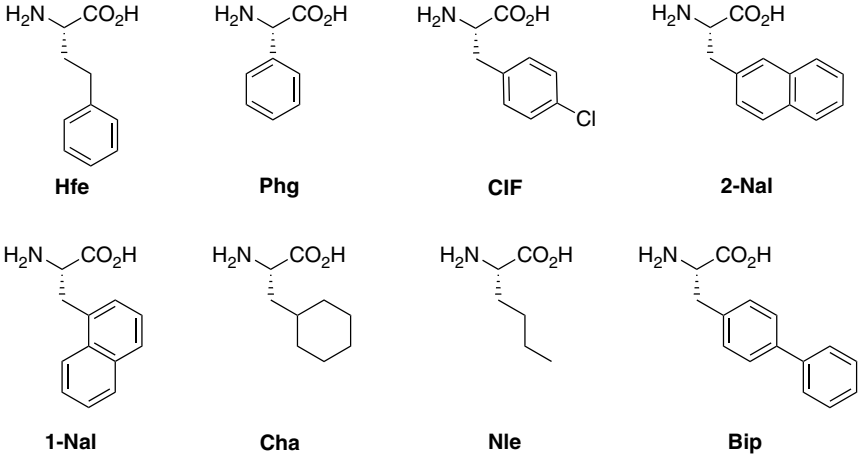
Question 16.15: How can ***ent*-4** be prepared?

Question 16.16: What conclusion can be drawn from the results concerning the mechanism of action of **4** and ***ent*-4**?

Question 16.17: How can the slight enhancement of activity of ***ent*-4** as compared to **4** be interpreted?

16.3 Sequence Optimization: Synthesis of Nonnatural Amino Acids

In order to further improve the antimicrobial activity of compound **4**, a library of analogs was synthesized, in which each hydrophobic residue at position 1, 3, 8, 10, and 12 was modified. To extend the repertoire of natural apolar residues, various nonnatural amino acids were incorporated: homophenylalanine (Hfe), phenylglycine (Phg),[1] 4-chlorophenylalanine (ClF), 2-naphtylalanine (2-Nal), 1-naphtylalanine (1-Nal), cyclohexylalanine (Cha), norleucine (Nle), and biphenylalanine (Bip) (Scheme 16.2). The enantioselective synthesis of these building blocks is presented here.

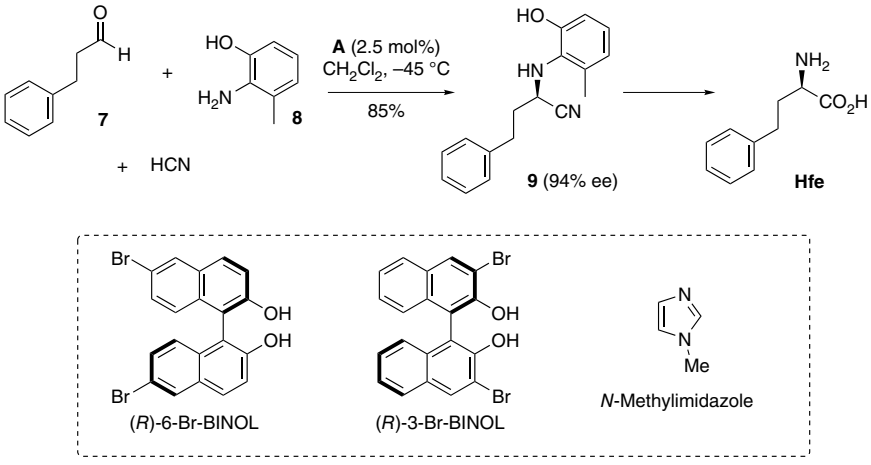


Scheme 16.2

16.3.1 Synthesis of Homophenylalanine (Hfe)

An example of catalytic enantioselective Strecker reaction providing access to **Hfe** is presented in Scheme 16.3 [2]. A chiral binuclear zirconium complex **A** is generated *in situ* by mixing 1 equiv. of $Zr(O\textit{t}\text{-}Bu)_4$, 1 equiv. of (*R*)-6-Br-BINOL, 0.5 equiv. of (*R*)-3-Br-BINOL, and 1.5 equiv. of *N*-methylimidazole in dichloromethane. Then, reaction in the presence of aldehyde **7**, aniline **8**, and HCN in CH_2Cl_2 at −45 °C affords aminonitrile **9** (85% yield, 94% *ee*), which is converted to **Hfe** in three steps.

1 Phenylglycine was not included in the structure–activity relationship study reported in the original article.



Scheme 16.3

^{1}H-NMR studies show that the active catalyst **A** generated *in situ* is a binuclear zirconium complex, containing two (*R*)-6-Br-BINOL, one (*R*)-3-Br-BINOL, and two *N*-methylimidazole units.

Question 16.18: Write the general mechanism of Strecker reaction (involving an aldehyde, NH_4Cl, and NaCN).

Question 16.19: Suggest a structure for binuclear complex **A** generated *in situ.*

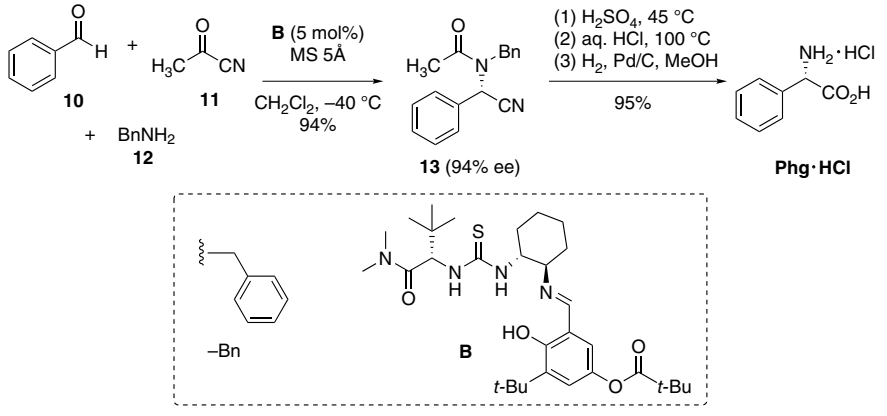
Question 16.20: Suggest a plausible reaction sequence allowing conversion of aminonitrile **9** into **Hfe**.

16.3.2 Synthesis of Phenylglycine (Phg)

Another example of Strecker reaction, catalyzed by chiral hydrogen bond donor thiourea **B**, has been reported for the enantioselective synthesis of phenylglycine. The one-pot three-component reaction of **10**, **11**, and **12** in the presence of catalyst **B** leads to acetamide **13**, precursor to **Phg**, isolated as the corresponding hydrochloride salt (Scheme 16.4) [3, 4].

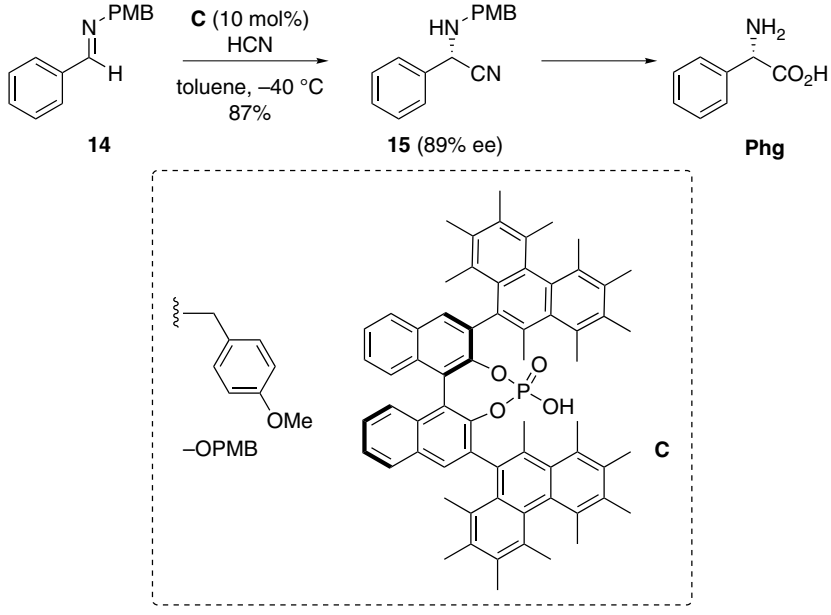
Question 16.21: What is the main advantage of this reaction as compared to the original Strecker reaction?

Question 16.22: Suggest a plausible reaction mechanism for the formation of **13**, accounting for the observed enantioselectivity.



Scheme 16.4

An alternative strategy, based on the reaction of imine **14** with HCN using a strong Brønsted acid derivative **C** as enantioselective organocatalyst, results in the formation of **15** (89% ee), precursor to phenyglycine **Phg** (Scheme 16.5) [5].



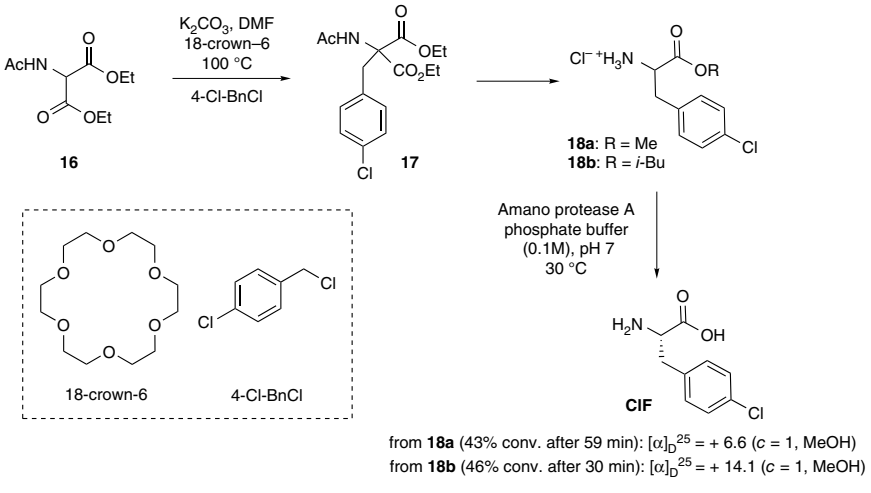
Scheme 16.5

Question 16.23: Suggest a synthetic sequence allowing the preparation of **C** from (*R*)-BINOL.

Question 16.24: Explain the main factors responsible for stereoinduction during formation of **15**.

16.3.3 Synthesis of 4-Chlorophenylalanine (ClF)

A common method to access various types of amino acid derivatives relies on the formation of nucleophilic glycine synthons. Reaction of diethyl acetamidomalonate **16** with electrophilic 4-Cl-Bn-Cl in the presence of K_2CO_3 and 18-crown-6 leads to racemic compound **17** (Scheme 16.6) [6]. After a few steps, racemic amino ester derivatives **18a** and **18b** are obtained, for which enantiomer separation is achieved by enzymatic kinetic resolution using microbial amano protease A, leading to enantiomerically enriched 4-chlorophenylalanine **ClF**.



Scheme 16.6

Question 16.25: What are the two main advantages of using diethyl acetamidomalonate **16** as glycine enolate precursor instead of glycine itself?

Question 16.26: Suggest a synthetic sequence allowing the transformation of **17** into **18a** and **18b**.

Question 16.27: Explain the principle underlying the transformation of **18a/b** into **ClF** in the presence of Amano protease A. What maximum conversion is expected for such a reaction? What happens upon prolonged reaction time?

Question 16.28: What is the main difficulty associated with the development of enzymatic resolution method for nonnatural amino acids?

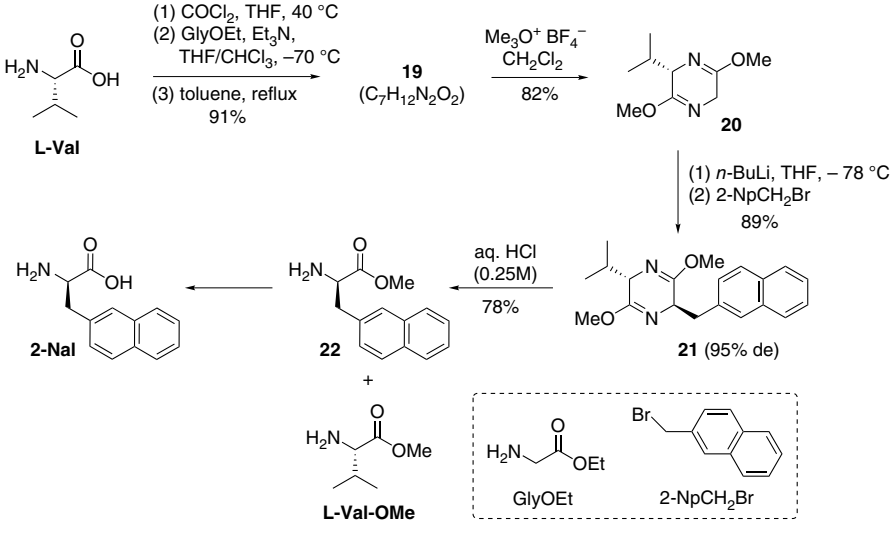
Question 16.29: Which other method is commonly used for the resolution of racemic mixtures of amino acids?

Question 16.30: Considering that the optical rotation of an authentic sample of enantiopure **ClF** is $[\alpha]_D^{25} = +14.4$ ($c = 1$, MeOH), calculate the enantiomeric excess of **ClF** obtained from **18a** and **18b**.

Question 16.31: Comment on the results obtained for the resolution of **18a** and **18b** and highlight the role of the ester group in the efficiency of this step.

16.3.4 Synthesis of 2-Naphtylalanine (2-Nal)

In order to avoid the use of resolution processes, chiral nucleophilic glycine synthons such as Schöllkopf bis-lactim ether **20** were also developed to achieve stereoselective reactions with electrophiles, thus leading to the efficient preparation of **2-Nal**, as reported in Scheme 16.7 [7].



Scheme 16.7

Starting from L-valine, diketopiperazine **19** (of molecular formula $C_7H_{12}N_2O_2$) is obtained and reacted with Meerwein's salt (Me_3OBF_4), to afford **20**. Deprotonation with *n*-BuLi followed by alkylation with 2-$NpCH_2Br$ stereoselectively leads to **21** (95% de), transformed by acidolysis into methyl esters **L-Val-OMe** and **22**, precursor to **2-Nal**.

Question 16.32: Write the structure of **19** and suggest a plausible mechanism for its formation.

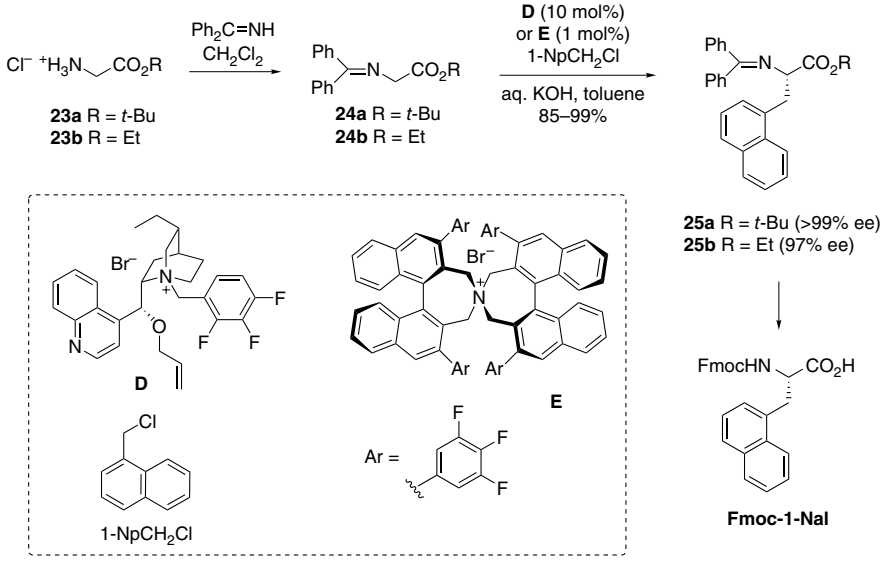
Question 16.33: Suggest a plausible mechanism for the transformation of diketopiperazine **19** into bis-lactim ether **20**.

Question 16.34: Explain the selectivity observed for the alkylation of **20**.

16.3.5 Synthesis of 1-Naphtylalanine (1-Nal)

Asymmetric phase-transfer catalysis is commonly employed for the enantioselective synthesis of α-amino acids [8]. The naturally occurring Cinchona-alkaloid derivative **D** or the synthetic chiral C_2-symmetric spiro ammonium salt **E** derived

from BINOL were both used for the preparation of Fmoc-protected 1-naphtylalanine **Fmoc-1-Nal**[2] (Scheme 16.8) [9, 10]. The sequence starts with amino esters **23a/b**, converted to the corresponding Schiff bases **24a/b**, which undergo asymmetric alkylation with 1-$NpCH_2Cl$ in the presence of catalyst **D** or **E** under basic conditions. The resulting **25a/b** is then transformed into **Fmoc-1-Nal** in a few steps.



Scheme 16.8

Question 16.35: Suggest a plausible mechanism for the formation of **24** from **23**.

Question 16.36: Suggest a plausible mechanism for the transformation of **24** into **25** and explain the origin of enantioselectivity observed.

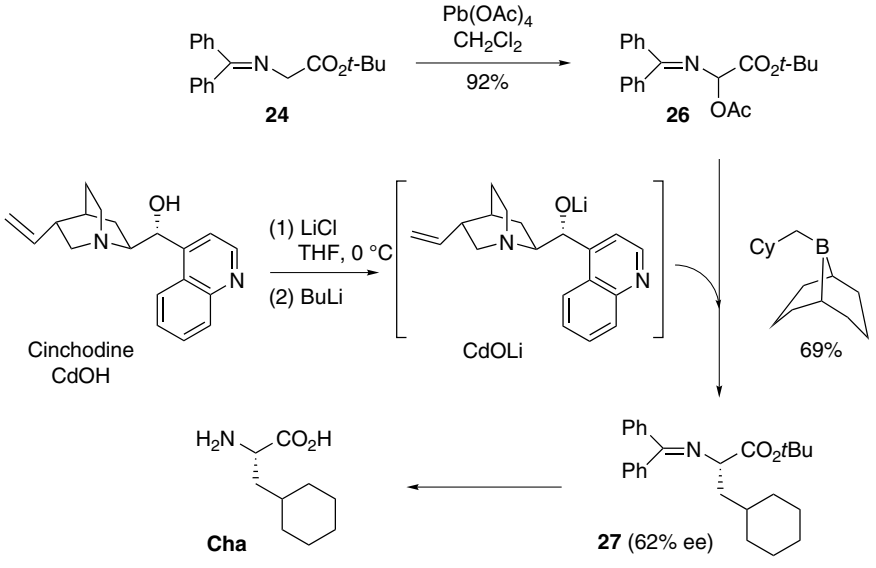
Question 16.37: Suggest a reaction sequence allowing the transformation of **25a** and **25b** into **Fmoc-1-Nal**.

Question 16.38: Compare the two methods (involving catalysts **D** or **E**) and highlight their respective advantage and drawback.

16.3.6 Synthesis of Cyclohexylalanine (Cha)

Electrophilic glycine synthons were also used for the stereoselective introduction of amino acid side chains. For example, electrophilic acetate **26** (derived from Schiff base **24**) reacts with an organoboron nucleophile in the presence of

2 In the original article, catalyst ***ent*-E** was used, leading to **Fmoc-*ent*-1-Nal**.



Scheme 16.9

the lithium alkoxide of cinchonidine (CdOLi), to afford enantiomerically enriched imine **27** obtained with 62% *ee* (Scheme 16.9) [11]. Although the mechanism of formation of **27** has not been fully understood, it has been shown to involve deprotonation of **26** by (CdOLi) to give the corresponding enolate, which adds to the organoboron reagent. The resulting ate complex undergoes alkyl group migration with loss of acetate ion, leading to an α-boryl ester that tautomerizes to a boron enolate. Final reprotonation of this intermediate leads to the corresponding derivative **27**. After a series of functional group interconversions, cyclohexylalanine **Cha** is obtained.

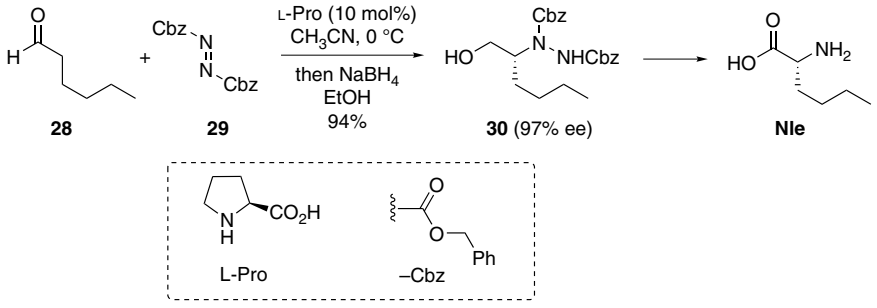
Question 16.39: Write the structure of the various intermediates involved in the transformation of **26** into **27**.

Question 16.40: Mention which step is responsible for stereo discrimination.

16.3.7 Synthesis of Norleucine (Nle)

The synthesis of norleucine was recently reported by enantioselective electrophilic α-amination catalyzed by L-proline, using azodicarboxylates as nitrogen electrophiles (Scheme 16.10) [12, 13]. Reaction of aldehyde **28** with azo **29** in the presence of a catalytic amount of L-proline results in the formation of compound **F**, which is directly reacted with $NaBH_4$ in EtOH to yield 94% of **30** with 97% *ee*, further transformed into norleucine **Nle**.

Question 16.41: Write the structure of **F** and suggest a catalytic cycle accounting for its stereoselective formation.

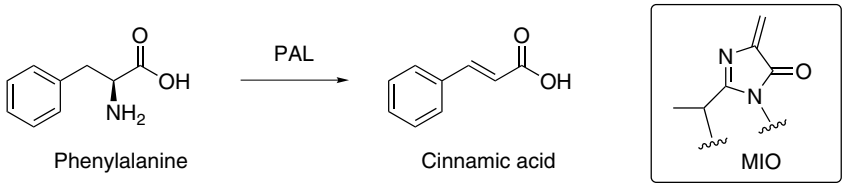


Scheme 16.10

Question 16.42: Give a short sequence allowing the preparation of **Nle** from **30**.

16.3.8 Synthesis of Biphenylalanine (Bip)

A chemoenzymatic approach to biphenylalanine **Bip** has been reported on the basis of the utilization of phenylalanine ammonia lyase (PAL). In nature, this enzyme catalyzes the deamination of phenylalanine to cinnamic acid (Scheme 16.11).

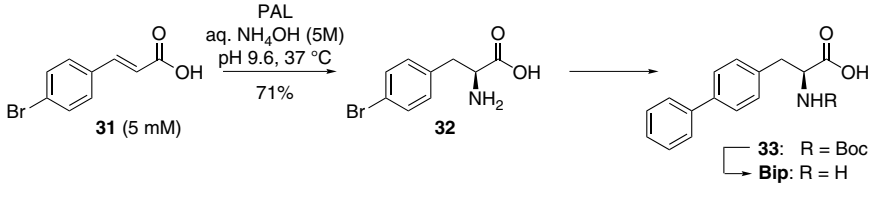


Scheme 16.11

The reaction mechanism involves a Friedel–Craft-type attack of the phenyl group of phenylalanine on the heterocyclic fragment MIO from the active site. The resulting intermediate undergoes β-elimination by a basic residue of the enzyme, finally leading to cinnamic acid.

Using excess NH_4OH, PAL can be used to perform reverse hydroamination reaction [14], such as the conversion of **31** to **32**, precursor to **Bip** (Scheme 16.12) [15].

Question 16.43: Why is hydroamination favored when working in the presence of NH_4OH?



Scheme 16.12

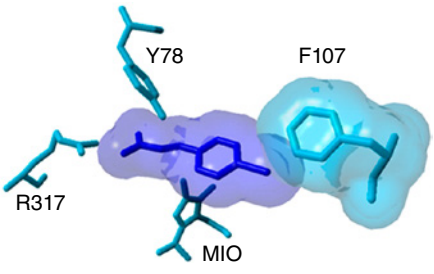


Figure16.4 Active site model of **31** bound to enzyme PAL. *Source:* Ahmed 2015 [15]. Reproduced with permission of American Chemical Society.

Question 16.44: Based on the indications given, suggest a plausible mechanism for the transformation of **31** into **32** catalyzed by PAL.

To optimize conversion, different mutants of the enzyme were prepared and tested. Mutant PAL-F107A (in which phenylalanine 107 is replaced by alanine) allowed to slightly improve the conversion to 80%. In the model structure of enzyme, the side chain of phenylalanine 107 is pointing toward the phenyl ring of substrate **31**. (Figure 16.4)

Question 16.45: How can the improvement of conversion observed with mutant PAL-F107A as compared to PAL be explained?

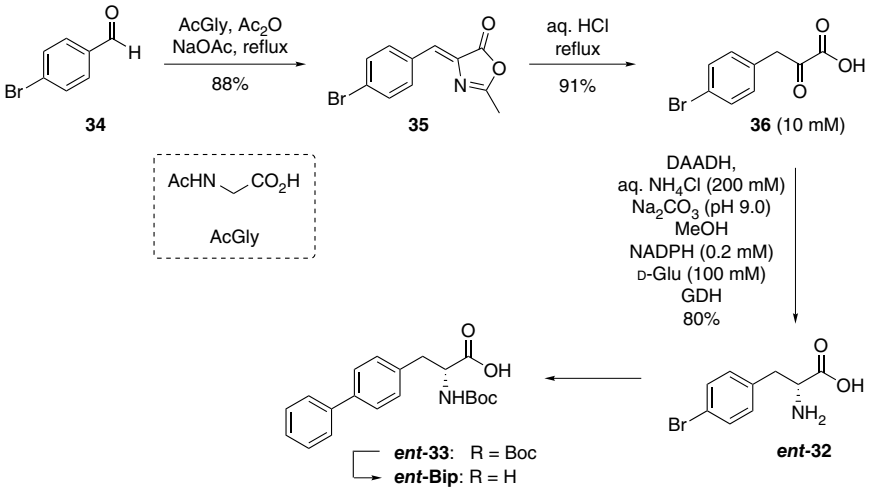
Question 16.46: Suggest a reaction sequence allowing the preparation of **33** from **32**.

To access ***ent*-Bip**, the enantiomer of **Bip**, another chemoenzymatic strategy was developed on the basis of the reductive amination, using the NADH-dependent D-amino acid dehydrogenase (DAADH). Treatment of 4-bromobenzaldehyde **34** with *N*-acetyl glycine in the presence of AcONa and Ac_2O leads to azlactone **35**, which undergoes acidic hydrolysis to yield 4-bromophenylpyruvic acid **36** (Scheme 16.13). Reductive amination was performed using DAADH, in the presence of aq. NH_4Cl solution (200 mM), carbonate buffer (pH 9.0), NaDPH cofactor, D-glucose (D-Glu), and glucose dehydrogenase (GDH), using methanol as a cosolvent. The resulting ***ent*-32** is converted into ***ent*-Bip** in a few steps.

Question 16.47: Why should a different strategy be developed to access ***ent*-Bip**?

Question 16.48: Suggest a plausible mechanism for the transformation of **34** into **36** through **35**.

Question 16.49: What is the role of NADPH in the enzymatic transformation of **36** into ***ent*-32**?



Scheme 16.13

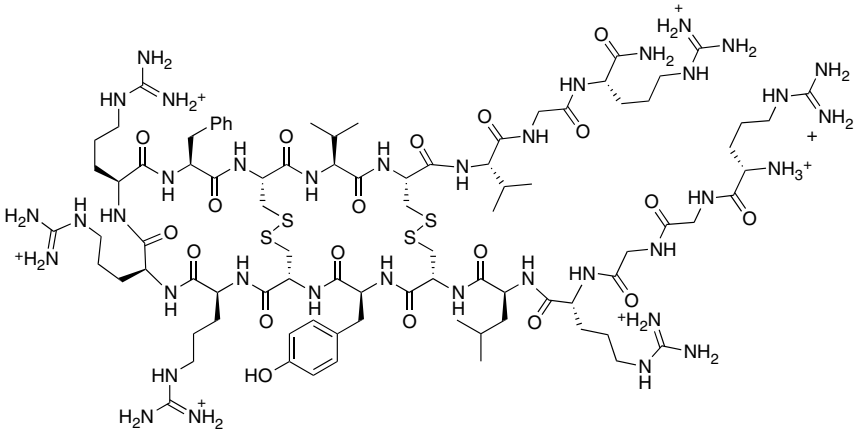
Question 16.50: What is the maximum conversion that can be obtained by treatment of **36** with DAADH and NADPH under the same reaction conditions, but in the absence of the D-Glu/GDH system? Justify your answer.

Answers

Question 16.1:

A peptidomimetic is a small compound designed to mimic a natural peptide or protein and recapitulate its biological function.

Question 16.2:

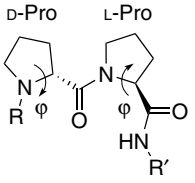


Question 16.3:

A β-hairpin conformation is a structural motif involving two consecutive hydrogen-bonded antiparallel β-strands connected by a short turn or loop sequence.

Question 16.4:

Because of its cyclic backbone, the dihedral angle φ of D-proline and L-proline is locked around +65° and −65°, respectively. Dipeptide D-Pro-L-Pro thus adopts a rigid β-turn and can promote a β-hairpin conformation.



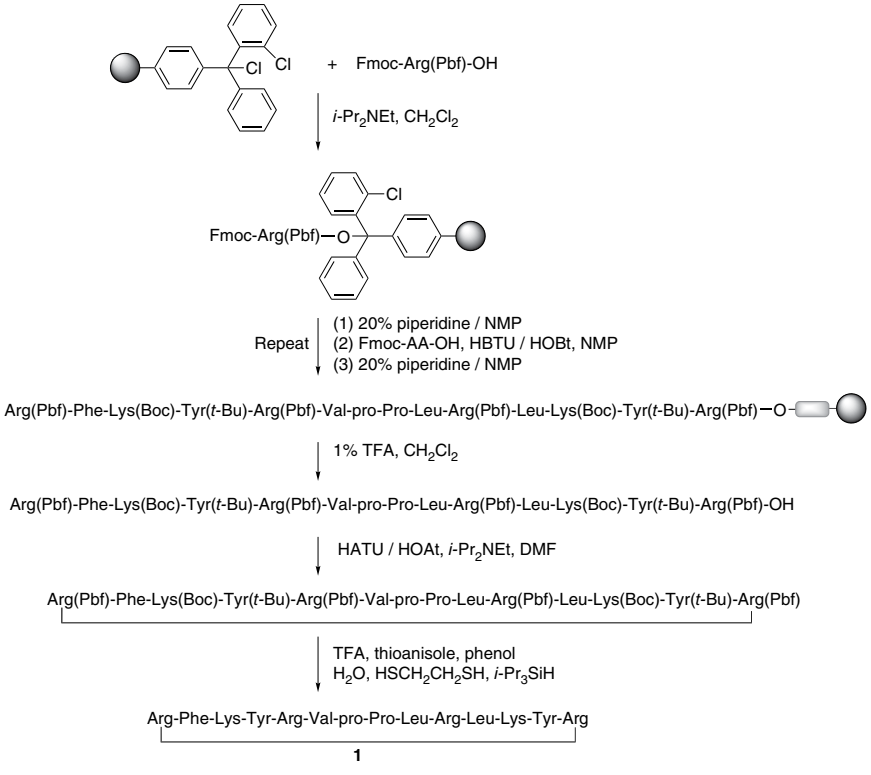
Question 16.5:

An amphiphilic compound possesses both a hydrophilic and a hydrophobic surface.

Question 16.6:

The synthesis of cyclic peptide by solid-phase synthesis can be performed using a highly acid-labile resin, such as 2-chlorotrityl. Fmoc amino acids bearing orthogonal protective groups on their side chains have to be used. After assembling the linear sequence on resin, the fully protected peptide is cleaved and macrocyclization is performed in solution under highly diluted conditions. In order to facilitate cyclization, the D-Pro-L-Pro template should be placed in the middle of the sequence, opposite to the cyclization site, to allow prestructuration of the peptide.

The sequence reported here was obtained as described:



Question 16.7:

The minimal inhibitory concentration (MIC) is the lowest concentration of compound that prevents visible growth of a bacterium.

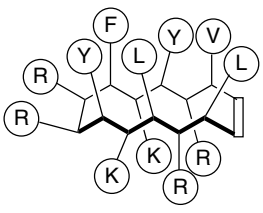
Question 16.8:

The aim of measuring hemolytic activity on human red blood cells is to evaluate the cytotoxicity on eucaryotic cell.

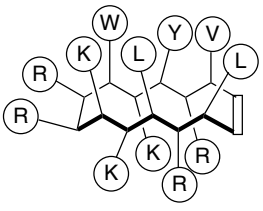
Question 16.9:

In compound **3** two cationic residues (K) were replaced by anionic glutamate (E), reducing the overall net charge of the peptide from +6 to +2. Since this compound shows significantly reduced antimicrobial activity, the highly cationic charge of the peptide seems to be a requisite for its activity.

Question 16.10:



1



4

In peptide **1** the upper face is covered with hydrophobic residues (L, Y, F, V) while the lower face contains only positively charged residues (K, R), resulting in an overall amphiphilic nature of the compound. Replacement of Y at position 5 with K in compound **4** breaks the apparent amphipathic character of the hairpin. However, significant improvement of selectivity is observed with this compound, with higher antimicrobial activity and a much lower hemolytic activity. Thus, the activity and selectivity of these compounds are not directly related to their amphiphilic nature.

Question 16.11:

In peptides **5** and **6**, two cysteine (C) residues leading to an additional disulfide bridge were introduced in order to further increase conformational stability. No improvement of activity and/or selectivity is however observed as compared to compound **4**. This suggests that introduction of additional constraints induces a distortion of the β-hairpin structure that deviates from active conformation.

Question 16.12:

Among peptides **1–6**, compound **4** appears as the most potent since it leads to both best antimicrobial activities (lowest MIC toward the different bacterial strains tested) and lowest hemolytic activity.

Question 16.13:

The results show that despite the presence of a D-Pro-**l**-Pro template, peptide **4** is largely unstructured in water, but clearly adopts a well-defined β-hairpin conformation through interaction with a lipid bilayer.

Question 16.14:

In peptide **4**, the disappearance of TOCSY signals associated with residues 2 (R), 4 (K), and 11(K) when the experiment is performed in the presence of a paramagnetic probe reveals a direct interaction of these residues with the micelles (membrane mimic). This suggests that compound **4** acts by insertion, at least partial, inside membranes.

Question 16.15:

Compound ***ent*-4** can be prepared following the same strategy as for **4**, using the enantiomer of each amino acid.

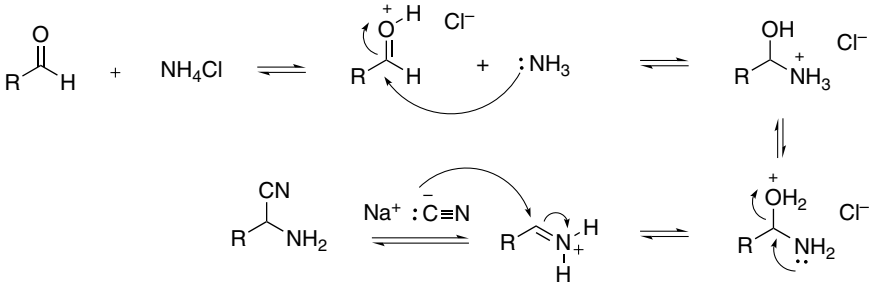
Question 16.16:

The activity similar to that observed for ***ent*-4** and **4** suggests that the mechanism of action does not involve binding to a receptor. The latter (which is chiral) would indeed exert a discrimination between the two enantiomers of a chiral compound.

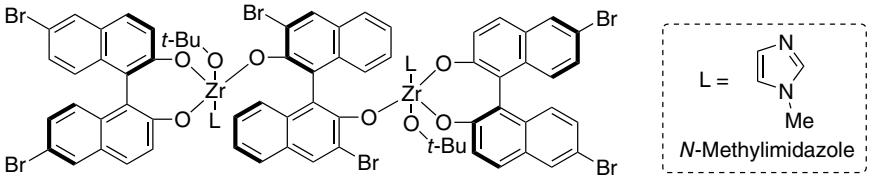
Question 16.17:

The slight enhancement of activity observed for ***ent*-4** as compared to **4** probably reflects a higher proteolytic stability of the peptide made from nonnatural amino acids in bacterial cultures.

Question 16.18:

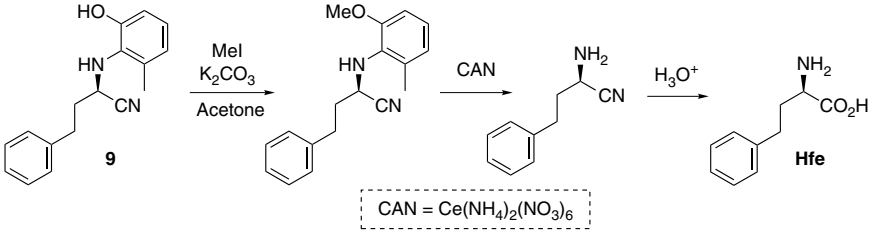


Question 16.19:



Question 16.20:

In order to deprotect nitrogen atom in **9** under mild conditions, methylation of the hydroxyl group has to be performed before oxidative cleavage using CAN.

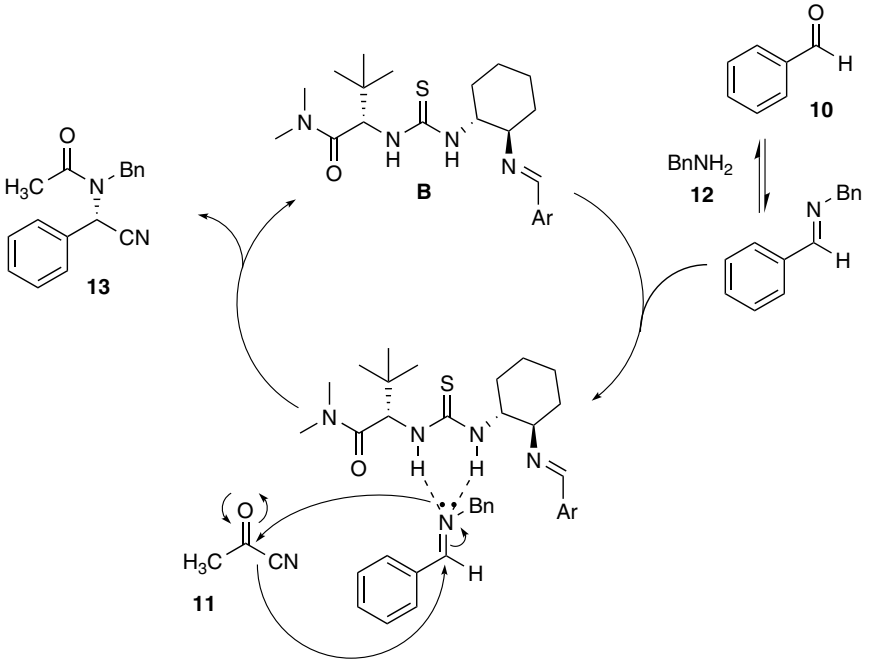


Question 16.21:

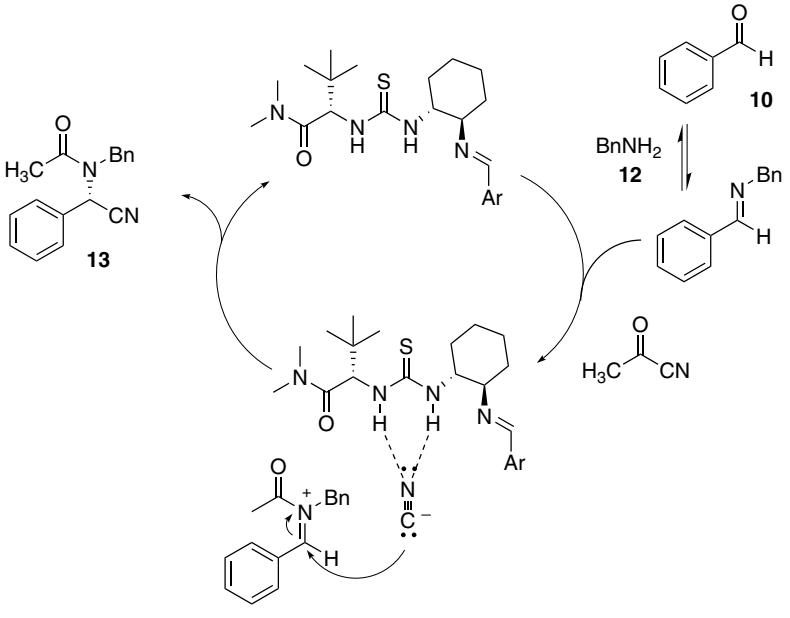
The first advantage is the formation of imine *in situ*, thus avoiding an additional isolation step. Moreover, the highly toxic CN^- ions have been replaced by safer acetyl cyanide.

Question 16.22:

A plausible mechanism for this reaction involves formation of the imine derived from **10** and **12** followed by activation with thiourea and simultaneous addition of cyanide and acylation of the nitrogen.

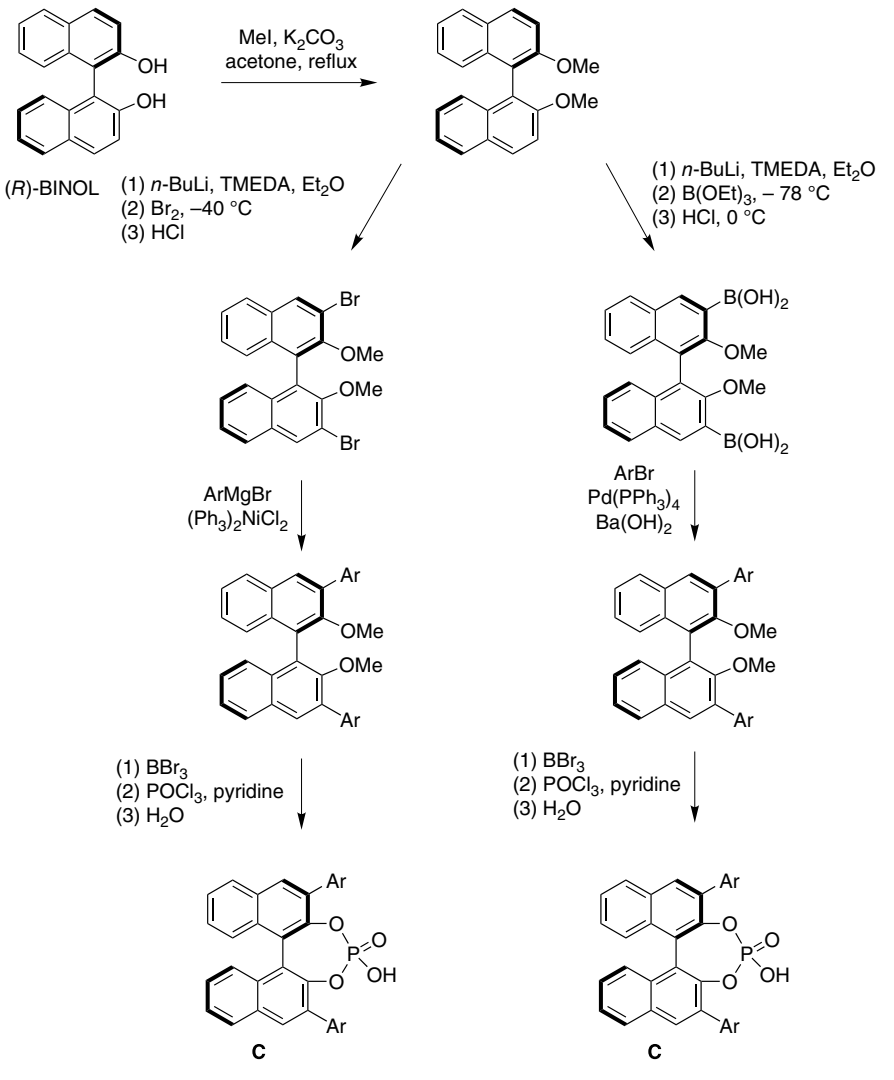


An alternative interesting mechanism has been suggested by the authors, involving the formation of *N*-acyl iminium ion, which undergoes enantioselective nucleophilic attack of cyanide ion bound to thiourea (acting as a chiral counteranion) [16].



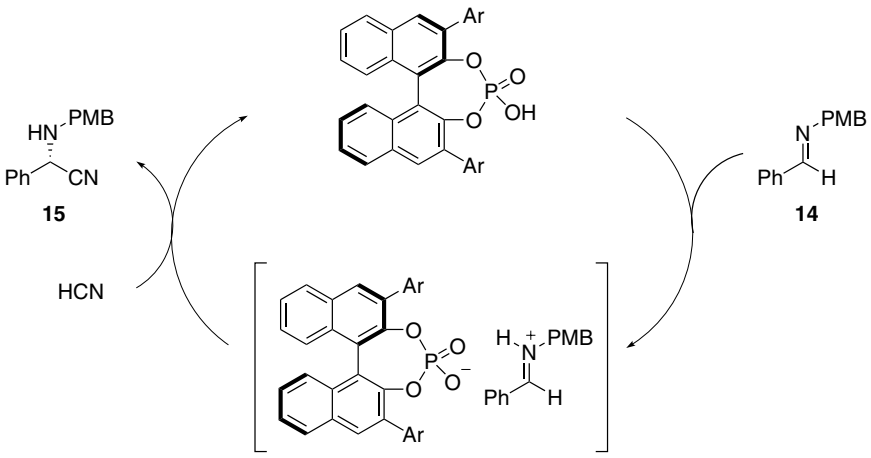
Question 16.23:

Two routes have been reported for the preparation of compounds of type **C**, either by palladium-catalyzed Suzuki-Miyaura coupling or nickel-catalyzed Kumada coupling. The latter strategy gave the best results [17].



Question 16.24:

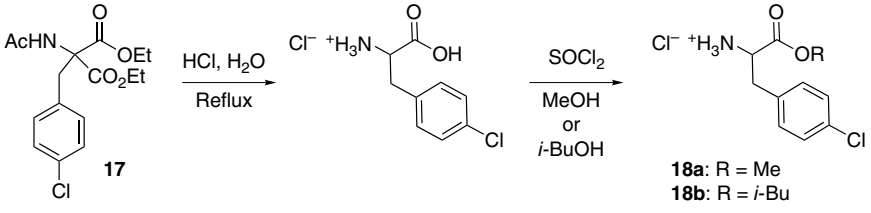
In this reaction, chiral phosphoric acid catalyst acts as Brønsted acid. Protonation of imine **14** leads to the formation of the corresponding iminium, together with a chiral phosphate counterion, responsible for the stereoselectivity observed upon cyanide addition.



Question 16.25:

The α proton of diethyl acetamidomalonate **16** is much more acidic than that of glycine, allowing the use of milder bases for its abstraction. Moreover, the formation of dialkylated product, which is a problem commonly met upon α-alkylation of enolate, is avoided when using **16** as a starting material.

Question 16.26:



Question 16.27:

The transformation of **18a/b** into **ClF** is a kinetic resolution, allowing separation of two enantiomers based on their different reaction rate with enzyme. As the reaction proceeds, unreacted starting material (**18a/b**) is enriched with the less reactive enantiomer, while the product (**ClF**) is preferentially formed from the most reactive enantiomer.

In an ideal system, only the most reactive enantiomer of the starting material is converted into the product, thus leading to a maximum of 50% conversion. In practice, prolonged reaction time can lead to higher conversion with erosion of enantiomeric purity of the product.

Question 16.28:

Enzymes usually show high specificity for a given substrate, and therefore do not recognize nonprotein amino acids.

Question 16.29:

Another common method for the resolution of racemic mixtures of amino acids is the formation of diastereoisomeric salts, which could be separated on the basis of their different physical properties.

Question 16.30:

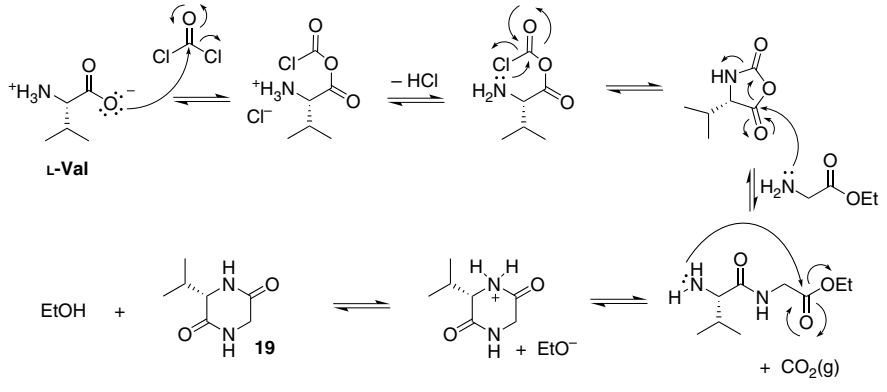
$$ee = \frac{[\alpha]_D^{exp}}{[\alpha]_D^{pure}}$$

thus, with R = Me: ee = 46% and with R = *i*-Pr: ee = 98%

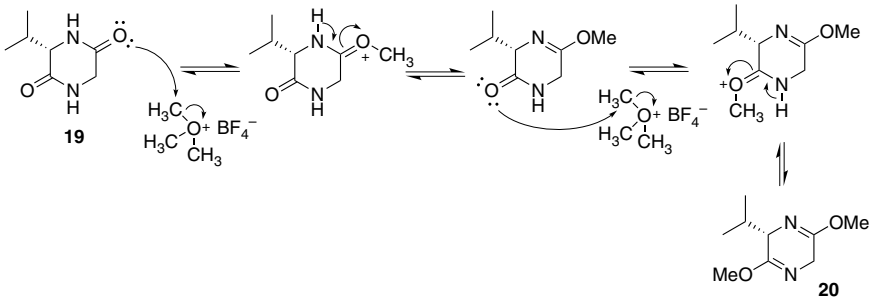
Question 16.31:

The results show that enantioselectivity of the reaction is greatly enhanced by employing isopropyl rather than methyl esters. This observation may reflect a better fit of bulkier substrates into the active site of the enzyme.

Question 16.32:

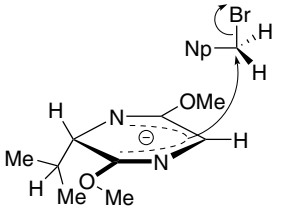


Question 16.33:

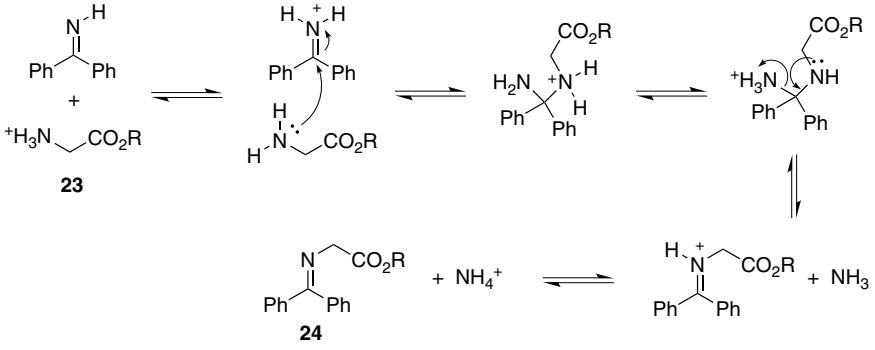


Question 16.34:

The enolate obtained from **20** has a planar geometry, and alkylation occurs at the face opposite to the sterically hindered isopropyl group [7].



Question 16.35:



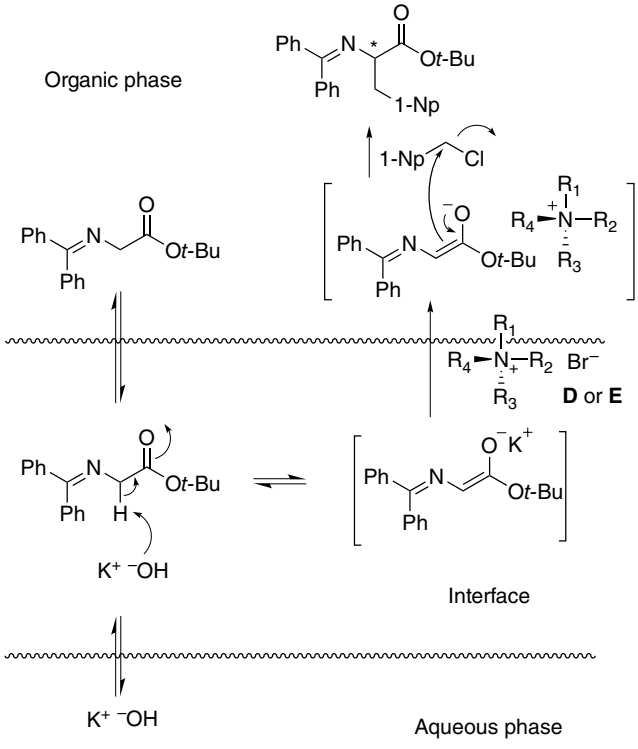
Question 16.36:

Enantioselective alkylation of **24** proceeds in a biphasic mixture. Potassium hydroxide is soluble in water and insoluble in toluene, while the opposite holds for both Schiff base **24** and 1-NpCH_2Cl.

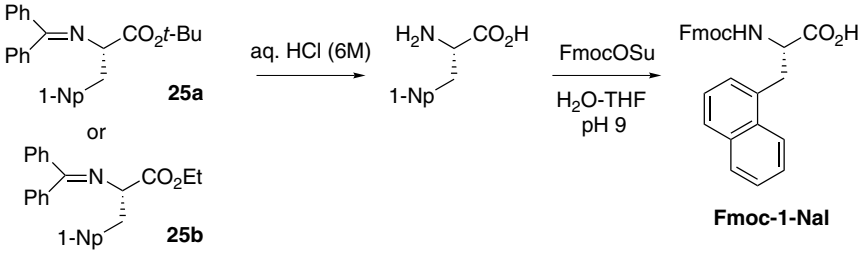
Deprotonation of **24** by KOH occurs at the interface between the two layers, leading to a potassium enolate. The latter undergoes counterion exchange with

chiral quaternary ammonium catalyst **D** or **E**, leading to a lipophilic enolate that is transferred to organic phase, where alkylation with 1-NpCH_2Cl occurs.

The enantioselectivity of the reaction can be thus explained by the presence of chiral counterion that allows discrimination of the two enantiotopic faces of the enolate during the alkylation step.



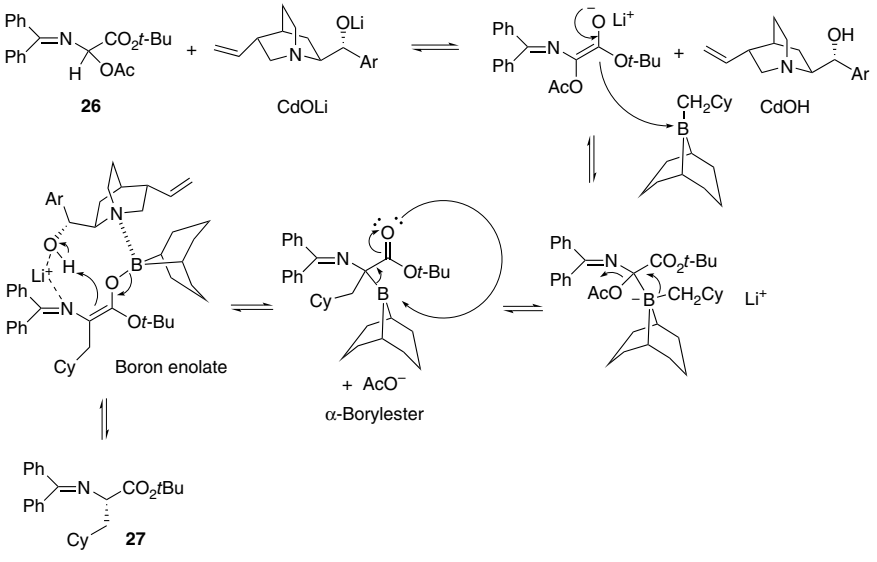
Question 16.37:



Question 16.38:

Catalyst **D** is used in a tenfold higher loading than **E**. However, while the former is readily accessed from cheap naturally occurring cinchona alkaloids, the latter is not derived from the chiral pool and is prepared in a multistep process.

Question 16.39:

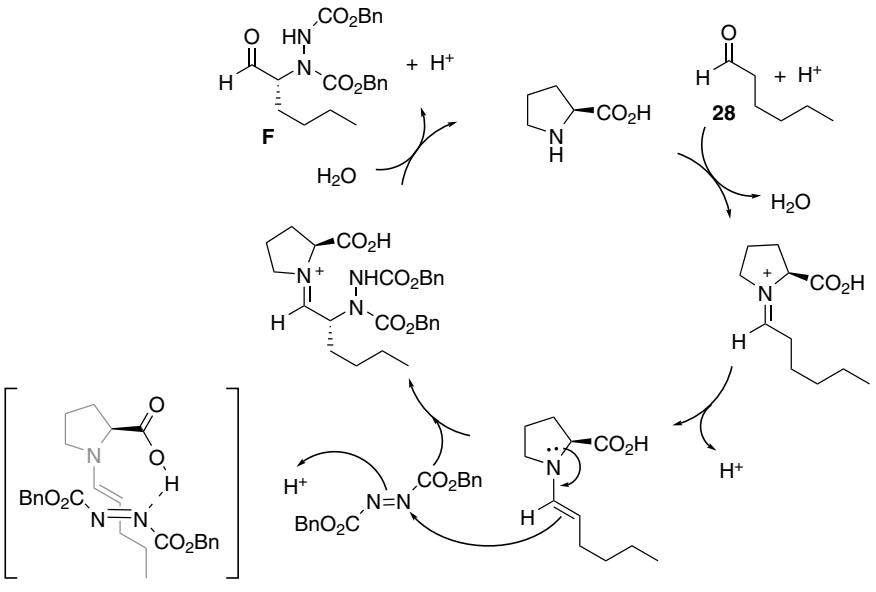


Question 16.40:

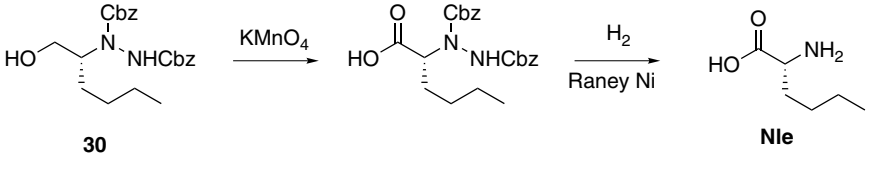
The stereoselective step is the protonation of boron enolate mediated by the parent alkaloid.

Question 16.41:

The mechanism of catalytic reaction leading to **F** is described here. The enantioselectivity observed during attack of enamine to azodicarboxylate is rationalized by (i) formation of the thermodynamically favored (*E*)-enamine and (ii) presence of an intermolecular H-bond between the two partners, directing the approach of the electrophile from the upper side of enamine.



Question 16.42:

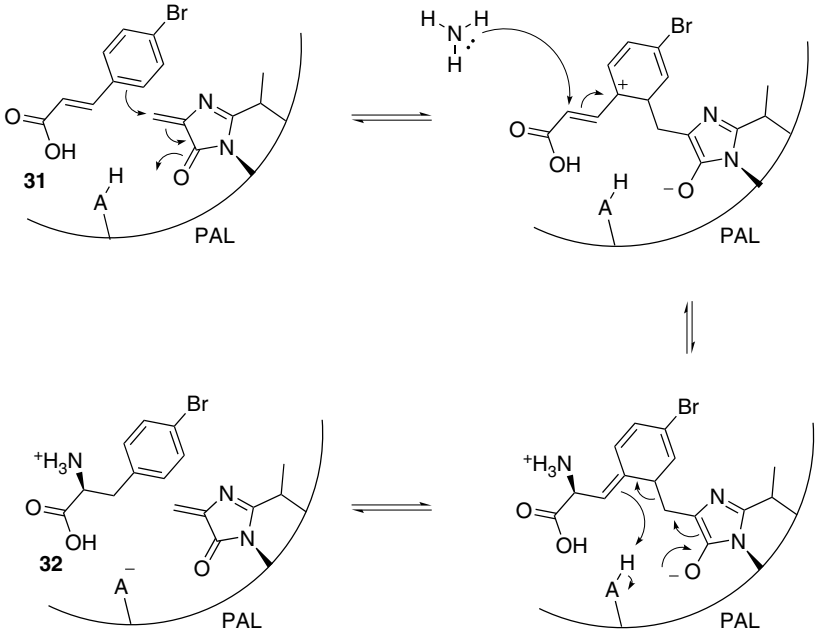


30 **Nle**

Question 16.43:

According to Le Chatelier's principle, a reversible reaction can be shifted in one or other direction by using an excess of appropriate reagent. Here, performing the reaction with **31** (5 mM) in the presence of a large excess of NH_4OH (5M) will drive the reaction toward formation of **32**.

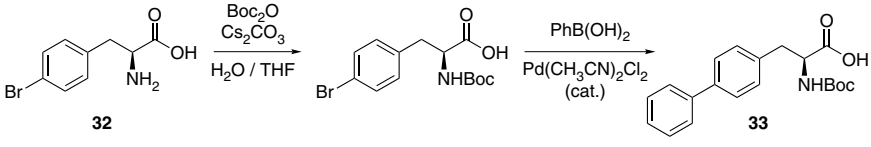
Question 16.44:



Question 16.45:

According to the active site model of **31** bound to PAL, the bromine atom of the substrate is pointing toward a phenylalanine residue (F107), resulting in destabilizing steric interactions. Replacement of phenylalanine by alanine in mutant PAL-F107A reduces steric hindrance and allows improvement of conversion.

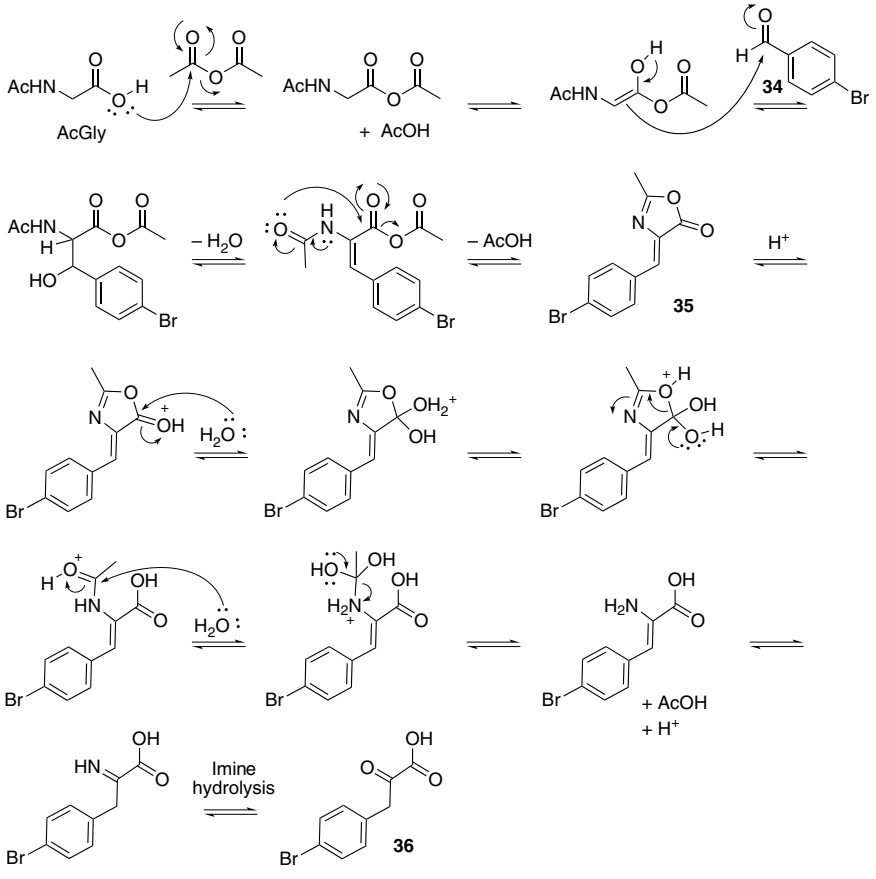
Question 16.46:



Question 16.47:

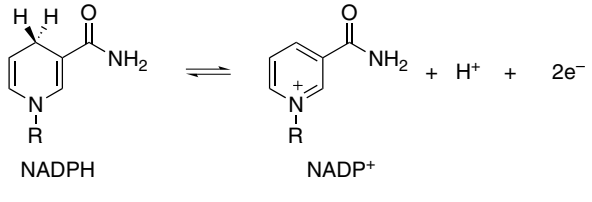
As a chiral biocatalyst, PAL specifically leads to the formation of L-amino acids. Access to enantiomers such as *ent*-**32** thus requires the use of an enzyme specific to D-amino acids.

Question 16.48:



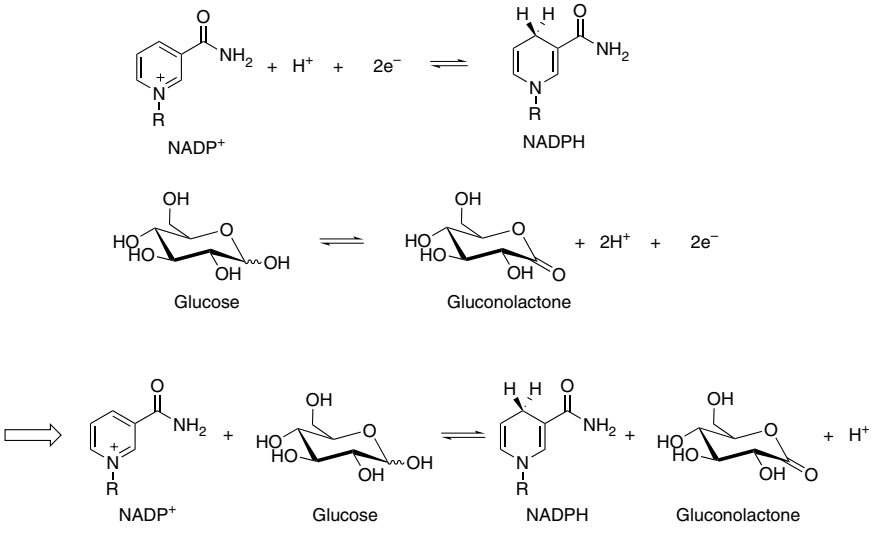
Question 16.49:

As co-factor of the enzyme, NADPH plays the role of a reducing agent, following the redox equation:



Question 16.50:

In the absence of D-Glu/GDH system, 1 equiv. of NADPH is required to completely reduce **36**. Since only 0.2 equiv. are used here, the maximum conversion would be 20%. However, in the presence of the D-Glu/GDH system, NADPH is regenerated by reduction of $NADP^+$ with concomitant oxidation of D-Glu, catalyzed by GDH:



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