PART 4

Application in Bioorganic Chemistry

Organic Azides: Syntheses and Applications Edited by Stefan Bräse and Klaus Banert
 ISBN: 978-0-470-51998-1

15

Aza-Wittig Reaction in Natural Product Syntheses

Francisco Palacios, Concepción Alonso, Domitila Aparicio, Gloria Rubiales and Jesús M. de los Santos

Departamento de Química Orgánica I, Facultad de Farmacia, Universidad del País Vasco. Apartado 450. 01080 Vitoria, Spain

15.1 Introduction

Staudinger and Meyers reported in 1919 the first example of an aza-Wittig reagent (Scheme 15.1).[1] These phosphorus reagents are named λ^5-phosphazenes, iminophosphoranes or phosphine imines although, in this account, we will use the general term, phosphazenes. Phosphazenes were first prepared at the beginning of the last century,[1] but it was not until Wittig's work, more than 30 years later, that the aza-Wittig reaction became accepted practice. In an analogous manner to phosphorus ylides in the Wittig reaction, phosphazenes can also react with carbonyl compounds to afford an excellent method for the construction of C=N double bonds (Scheme 15.1).[2]

Since then, the Wittig and aza-Wittig reactions have undergone tremendous development and have become a powerful tool in organic synthetic strategies directed towards the construction of acyclic and cyclic compounds, mainly because the reaction is conducted in neutral solvents in the absence of catalysts, generally at mild temperatures, and usually proceeds high yields.

Numerous research papers and several reviews[3] have appeared describing the general use of phosphazenes as reagents and intermediates in organic synthesis. This account describes the use of the aza-Wittig reaction for the preparation of natural products by means of intermolecular and intramolecular processes. Despite the aza-Wittig strategy

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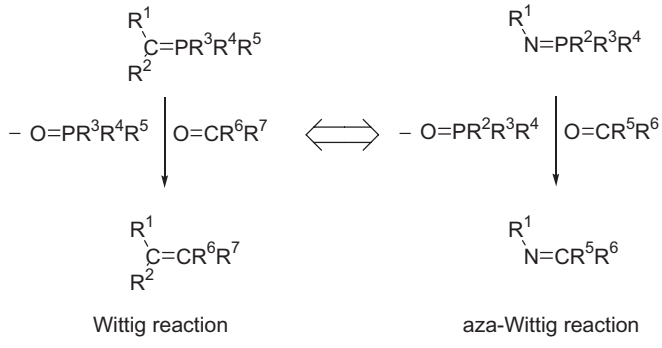


Scheme 15.1

has been developed for the synthesis of a wide type of heterocycles and their analogues, in this review we present those syntheses that exclusively lead to the preparation of natural products.

15.2 Intermolecular Aza-Wittig Reaction

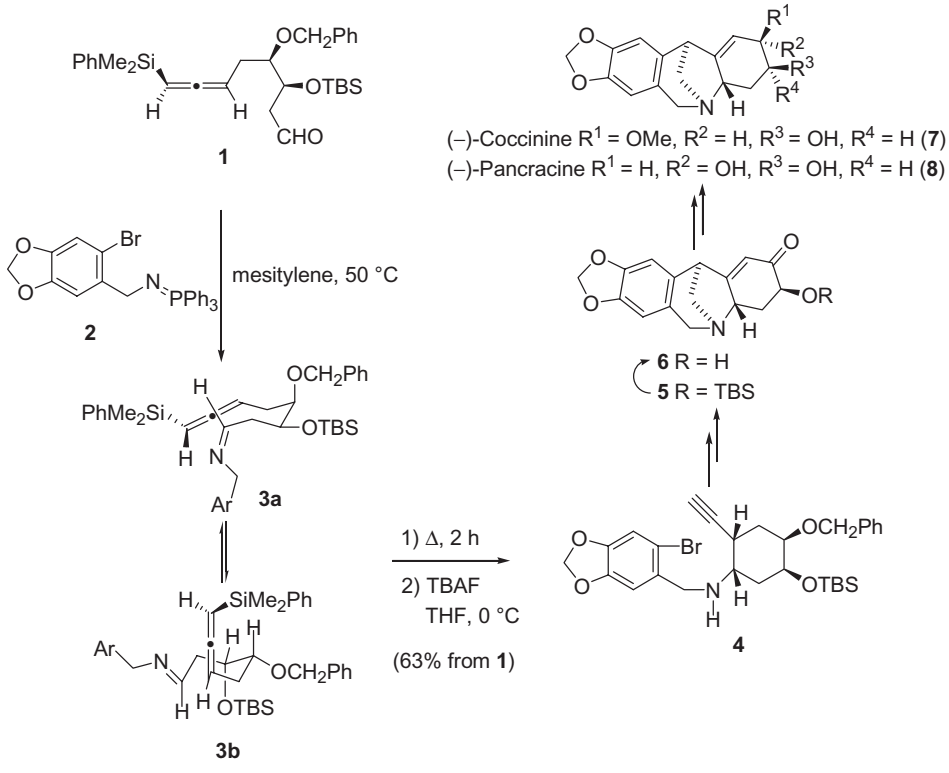
15.2.1 Reaction with Carbonyl Compounds

The reaction of phosphazenes with carbonyl compounds is an excellent tool for the formation of carbon-nitrogen double bonds and this reaction seems to be one of the most efficient methods for the creation of the imine group under mild reaction conditions.[4]

15.2.1.1 Reaction of Phosphazenes with Aldehydes

The 5,11-methanomorphanthridine alkaloids are a small subclass of compounds of the *Amaryllidaceae* type first isolated by Wildman *et al.*[5] These natural products, produced by plants of various *Pancratium*, *Narcissus*, and *Brunsvigia* species, have a unique pentacyclic structural framework exemplified by the alkaloids (–)-pancracine, and (–)-coccinine. A very elegant synthesis of (–)-coccinine (**7**) and (–)-pancracine (**8**) has been described by Weinreb *et al.*[6] The corresponding imines were prepared by reaction between *N*-phenyl phosphazene **2** and functionalized aldehyde **1** (Scheme 15.2). Stereospecific cyclization was accomplished upon heating this imine/allenylsilane **3** in mesitylene at 162 °C by subsequent alkyne desilylation to afford amino acetylene **4**. Subsequent transformations led to the formation of the pentacyclic structural framework **6**, which can be reduced to (–)-pancracine **8** with sodium triacetoxyborohydride. In addition, enone **5** could be transformed into (–)-coccinine **7** after conversion of ketone group to the dimethyl ketal, followed by treatment with DIBALH (diisobutylaluminium hydride) for the reduction of the hydroxy ketal and cleavage of the TBS (tributylsilyl) group.

Conjugated phosphazenes have been widely used for the preparation of azadienes.[7] An important extension of the aza-Wittig/intramolecular electrocyclic ring closure (AW-IEC) methodology has been used in the construction of β-carboline alkaloids, which contain a

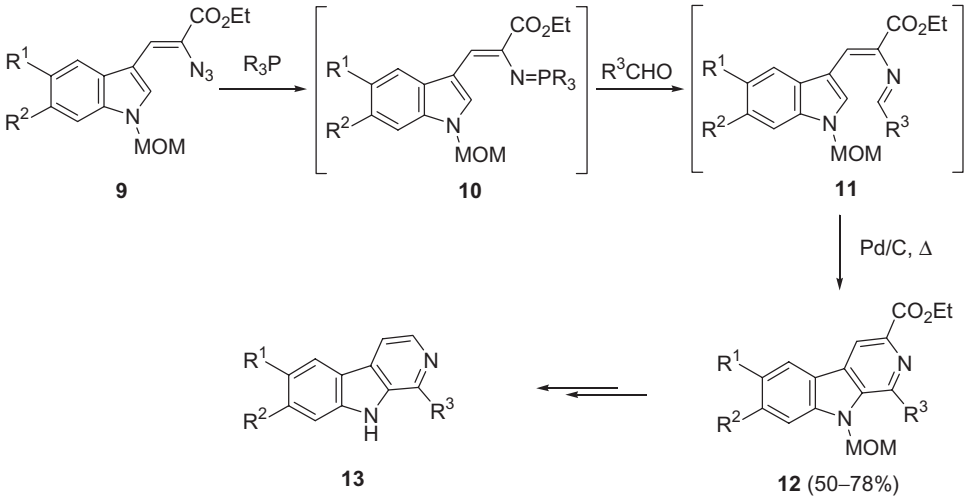


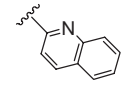
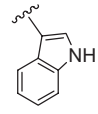
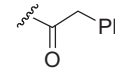
Scheme 15.2

phenyl acetyl or heteroaryl substituent at C-1. The reaction involves an initial formation of phosphazenes **10** by treatment of functionalized azides **9** with phosphine followed by aza-Wittig reaction with heterocyclic aldehyde to give the imine **11** (Scheme 15.3). Thermal treatment in the presence of Pd/C provides the corresponding 1-heteroarylsubstituted β-carbolines **12**. Finally, deprotection of the *N*-MOM (methoxymethyl) group, hydrolysis of the ester group and thermal decarboxylation afforded eudistomins A (**13a**) and M (**13b**),[8a] eudistomin U (**13c**),[8b] and nitramarine (**13d**).[8c] When this methodology is allowed using benzylglyoxal, the corresponding 1-phenylacetyl-β-carboline **12** was converted into eudistomins T (**13e**) and S (**13f**).[8d] The same approach has been extensively used for the synthesis of other alkaloids such as xestomanzamine A (**14**),[8d] fascaplysin (**15**),[8c] the unusual group of azafluoranthrene alkaloids rufescine (**16**),[8e] imeluteine (**17**),[8e] which are biosynthetically related to the tropoloisoquinolines imerubine and grandirubine and lavendamycin (**18**)[8a,8f] (Figure 15.1).

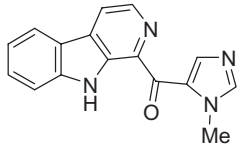
15.2.1.2 Reaction of Phosphazenes with Ketones

The preparation of a variety of vinylogous amides, precursors for a facile entry to the tetracyclic core of selaginoidine (**26**) (Scheme 15.4), was first attempted in different

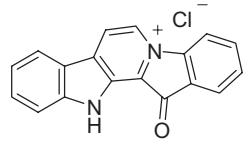


	R^1	R^2	R^3		R^1	R^2	R^3
13a Eudistomin A	OH	Br		**13d** Nitramarine	H	H	
13b Eudistomin M	OH	H					
13c Eudistomin U	H	H		**13e** Eudistomin T	H	H	
				13f Eudistomin S	Br	H	

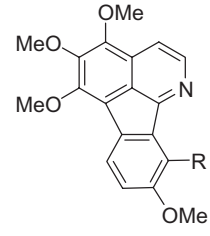
Scheme 15.3



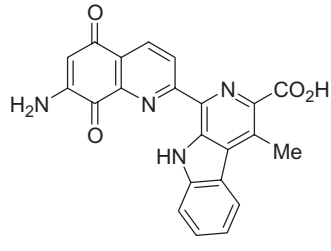
Xestomanzamine A (**14**)



Fascaplysin (**15**)

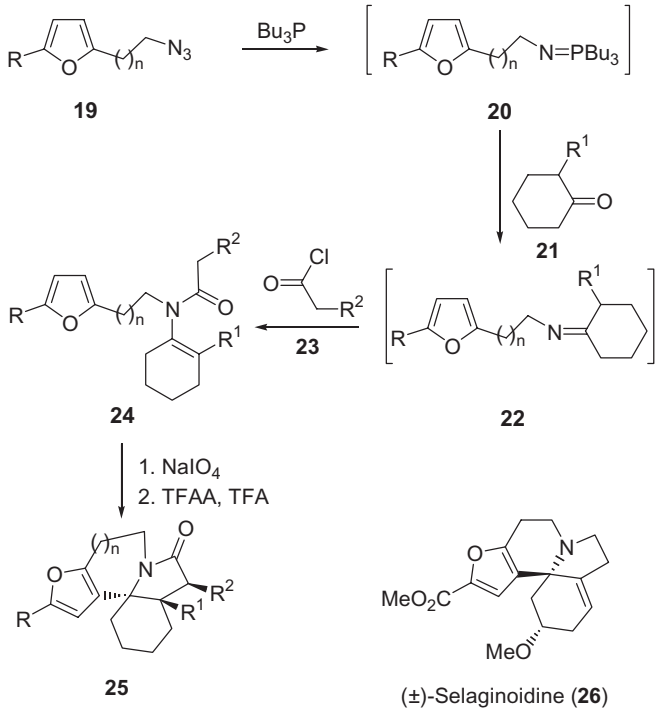


Rufescine R = H (**16**)
Imeluteine R = OMe (**17**)



Lavendamycin (**18**)

Figure 15.1



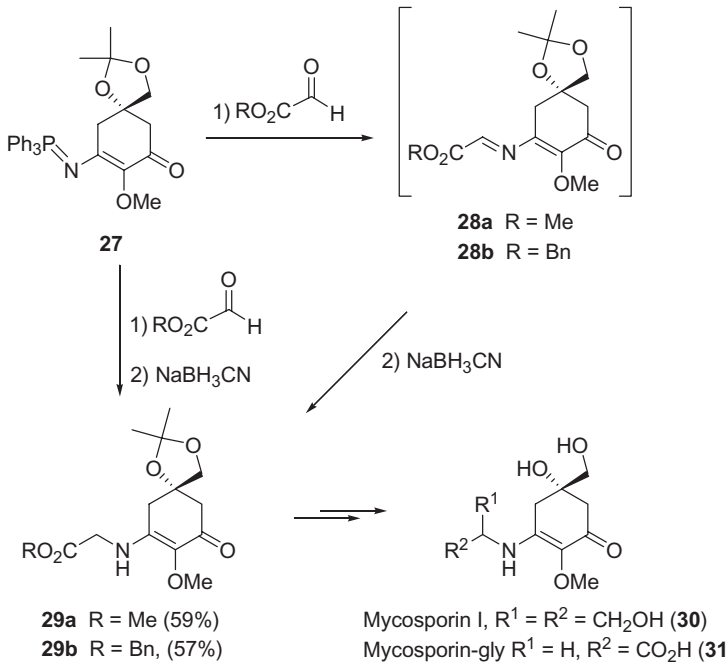
Scheme 15.4

ways.[9] However, only the aza-Wittig reaction gave the expected results, improved by the use of microwave technology. Reaction of phosphazenes **20**, derived from azides **19**, with cyclohexanone derivatives **21** gives the corresponding imines **22** (Scheme 15.4). Subsequent condensation with carboxylic acid derivatives **23** and rapid cyclization furnished the desired hexahydroindolinone systems **25**. By the moment, with a subsequent intramolecular electrophilic substitution of furanyl derivative, related homoerythrina alkaloids can be prepared.

Aza-Wittig reaction of the phosphazene **27** with methyl or benzyl glyoxalate, followed by *in situ* reduction of the intermediate aldimine **28a**,**b** with sodium cyanoborohydride, gives the cyclic enaminones **29a** or **29b**, which are the starting materials for the asymmetric synthesis of mycosporin I (**30**) and mycosporin-gly (**31**) (Scheme 15.5).[10]

15.2.1.3 Reaction of Phosphazenes with Carboxylic Acid Derivatives

The reaction of acyl halides with phosphazenes gives an mild method for the preparation of 2,5-disustituted oxazoles.[11] This method has been used in the preparation of naturally occurring oxazole alkaloids, such as pimprinine analogues **35a**,[12] *O*-methylhalfordinol (**35b**) and annuloline (**35c**).[13] The preparation of five pimprinine analogues through

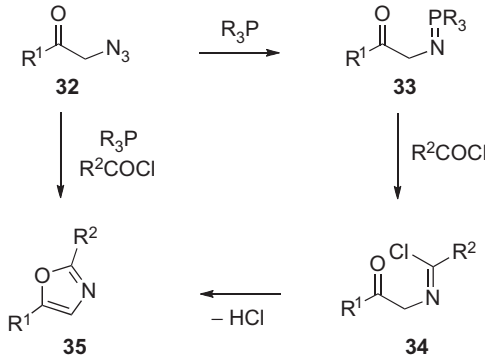


Scheme 15.5

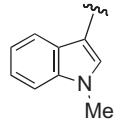
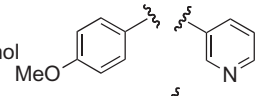
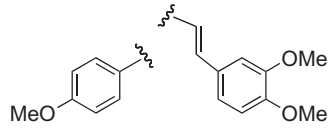
aza-Wittig type reaction of phosphazenes with acyl chlorides has been reported. The one-pot reaction between an α-azidoketone **32**, trialkylphosphine and an acyl halide leads to oxazoles **35** (Scheme 15.6). The aza-Wittig reaction between the initially formed phosphazene **33** and the acyl chloride gives an imidoyl chloride **34** which cyclizes across the enol form of the carbonyl function to give the five-membered ring.

The first synthesis of the bis(indole) alkaloid rhopaladin D (**40**) isolated from the marine Okinawan tunicate *Rhopalaea sp*. was achieved.[14] Alkaloids rhopaladins A-D showed antibacterial activity against *Sarcina lutea* and *Corynebacterium xerosis* and inhibitory activity against cyclin dependent kinase 4 and *c-erb*β-2-kinase. The key step, construction of the central imidazolinone ring, is based on the aza-Wittig reaction of the phosphazene **37** derived from the α-azido-β-(3-indolyl)propenamide **36**, and indol-3-ylglyoxylyl chloride in the presence of a base such as polymer-supported BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine) as a base (Scheme 15.7). Thus, the aza-Wittig reaction followed by intramolecular cyclization of the resulting imidoyl chloride **38** proceeded smoothly to afford the cyclized product **39** as a 6:4 mixture of *E*/*Z* isomers. The *N*-SEM ([β-(trimethylsilyl)ethox] methyl) deprotection of **39** with TBAF (tetrabutylammonium fluoride) in THF at reflux gave the rhopaladin D (**40**).

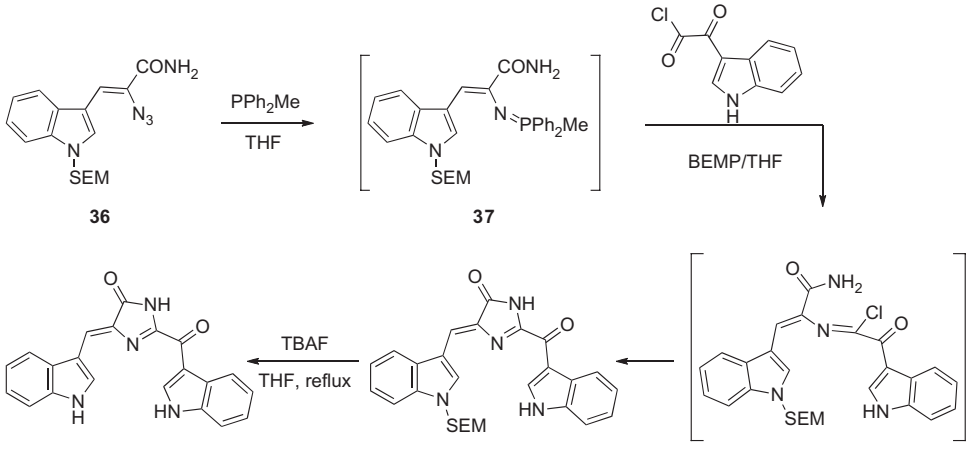
The same strategy has been reported for the synthesis of the marine alkaloid almazole C, isolated from the red seaweed *Heraldiophylum sp*., which showed antibacterial activity against Gram-negative pathogens. The formation of the central 2,5-disubstituted oxazole



32 **33** **35** **34**

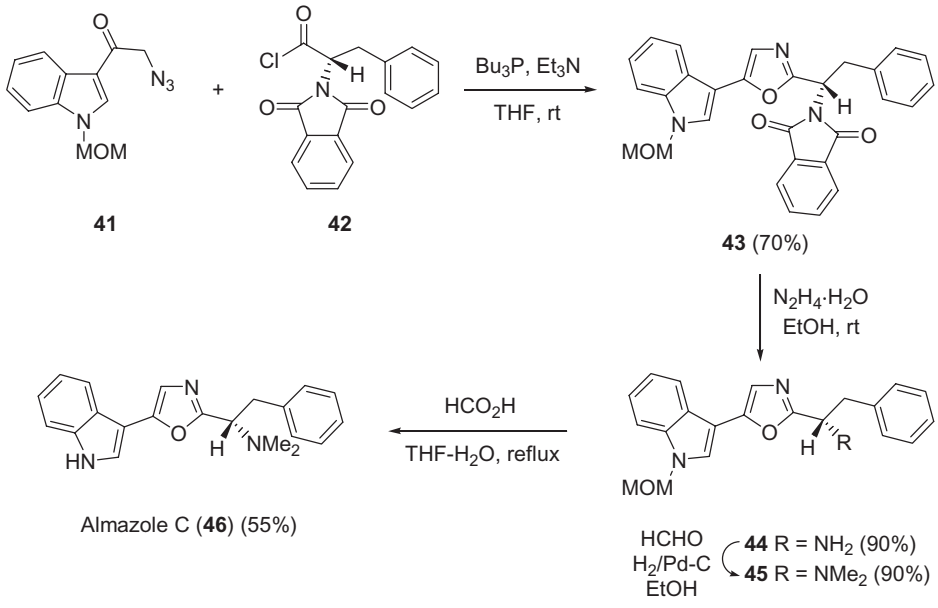
	R^1	R^2	Yield
35a Pimprinine		Me	(60%)
35b *O*-methyhalfordinol			(56%)
35c Annuloline			(54%)

Scheme 15.6



36 **37** **38** **39** (56%) Rhopaladin D (**40**) (60%)

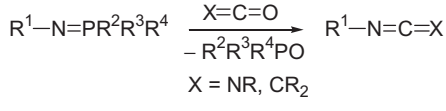
Scheme 15.7



Scheme 15.8

ring was achieved using the phosphazene methodology (Scheme 15.8).[15] The key intermediate **43** was prepared in 70% yield by reaction of α-azidoacetyl indole **41** with tributylphosphine in THF and subsequent addition of (*S*)-*N*-phthaloylphenylalanyl chloride **42**, followed by treatment with Et_3N. The *N*-phthaloyl group was removed with hydrazine to give amine **44**, which was dimethylated by reductive amination with hydrogen in the presence of formaldehyde and palladium as catalysis, to give compound **45** in 90% yield. Finally, compound **45** was converted into almazole C (**46**) by deprotection of the *N*-methoxymethyl substituent with formic acid in THF (Scheme 15.8).

15.2.2 Reaction with Heterocumulene Derivatives

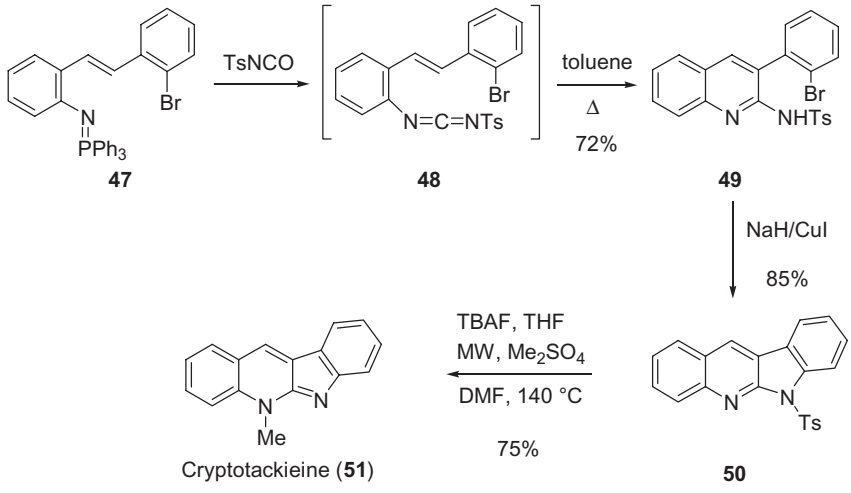


Scheme 15.9

The aza-Wittig reaction of phosphazenes with heterocumulenes[3a,16] such as isocyanates (X = NR) and ketenes (X = CR_2) has been extensively used for the formation of carbodiimides and ketenimines, respectively (Scheme 15.9).

15.2.2.1 Reaction of Phosphazenes with Isocyanates

From the range of general methods available for the construction of the carbodiimide functionality, the intermolecular aza-Wittig-type reaction of phosphazenes and isocya-



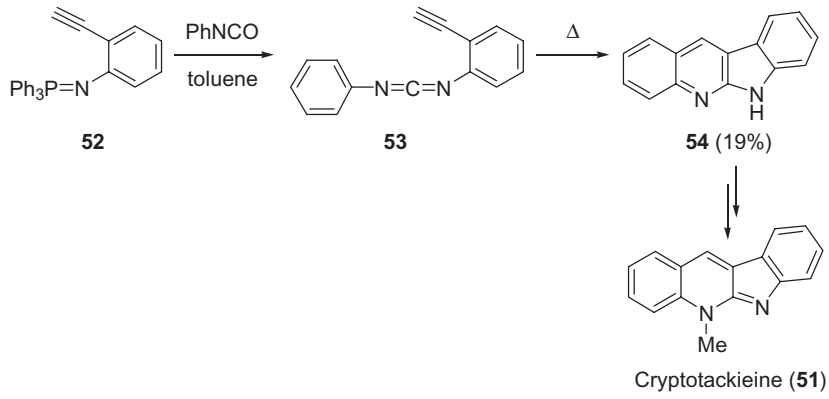
Scheme 15.10

nates seems to be an attractive method for preparation of these compounds, since it takes place under neutral conditions. In addition, this type of compounds can be subsequently used for the preparation of natural products by means of tandem or domino reactions. Aromatic phosphazenes have also been used in the synthesis of nitrogenated six-membered ring systems following the strategy of the aza-Wittig reaction and subsequent electrocyclic ring closure. The protocol has been used for the synthesis of indoloquinoline alkaloid cryptotackieine (**51**).[17] Thus, the phosphazenes **47**, containing an unsaturated side chain at the *ortho*-position (Scheme 15.10), participate in an aza-Wittig/electrocyclic ring closure, allowing the preparation of indolo[2,3-*b*]quinoline **50** through an electrocyclic process of **48** followed by the ring closure of **49**. Conversion of compound **50** into cryptotackieine **51** was achieved by deprotection of *N*-sulfonyl group with TBAF and subsequent microwave-promoted methylation with dimethyl sulfate followed by deprotonation.

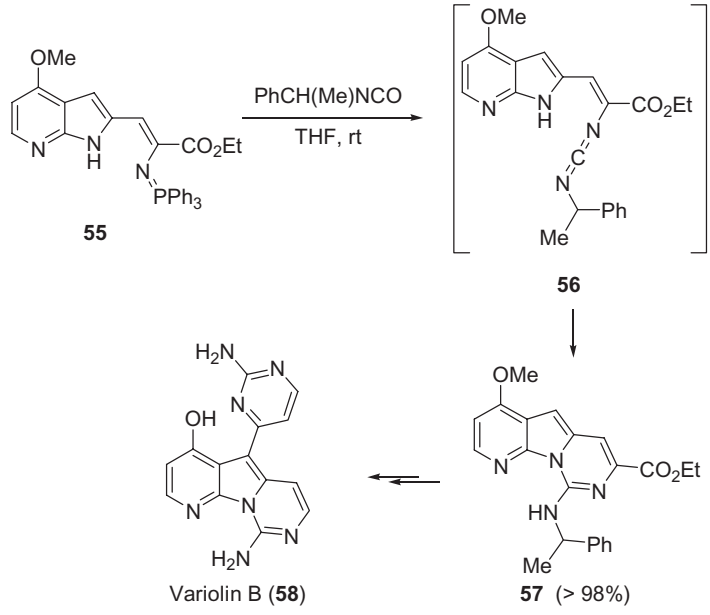
N-Aromatic phosphazenes containing a triple bond reacted with heterocumulenes to give carbodiimides which can give heteropolycyclic compounds through an intramolecular [4+2] cycloaddition reaction. Thus, initial aza-Wittig reaction of phosphazene **52** (Scheme 15.11) with phenyl isocyanate and subsequent intramolecular cycloaddition of the formed carbodiimide **53** gave the quinindoline heterocycle **54**,[18] which was used in a straightforward formal total synthesis of cryptotackieine **51**[19] (neocryptolepine)[20] after methylation and subsequent deprotonation of the quinindoline **54**.

Usually, carbodiimides obtained by an aza-Wittig reaction of *N*-vinylic phosphazenes with isocyanates cannot be isolated.[3a,21] Therefore, the very reactive carbodiimides can be used as synthetic intermediates of polyheterocyclic natural products by domino processes involving aza-Wittig/intramolecular cyclization (AW-IC). In the synthesis of variolin B (**58**), the formation of the annulated 2-aminopyrimidine ring **57** is achieved from phosphazene **56** by a tandem aza-Wittig/carbodiimide-mediated intramolecular cyclization process[22] (Scheme 15.12).

Five-membered heterocycles were obtained when ammonia or amine nucleophiles reacted with carbodiimides, generated *in situ* from phosphazenes by a cascade process

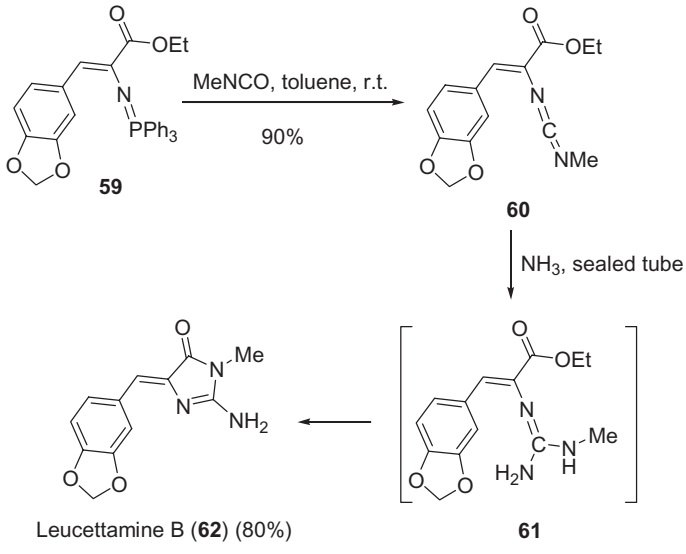


Scheme 15.11

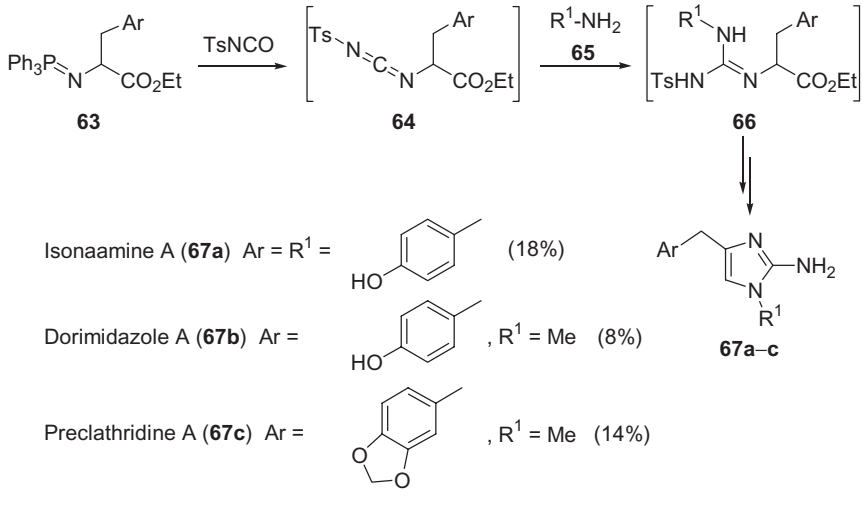


Scheme 15.12

involving aza-Wittig/intermolecular nucleophilic addition/intramolecular cyclization (AW-NA-IC). The strategy has also proved to be suitable for the preparation of leucettamine B (**62**).[23] Aza-Wittig reaction of phosphazene **59** with methyl isocyanate furnished the carbodiimide **60** in almost quantitative yield. Posterior treatment with ammonia yielded leucettamine B (**62**) through a guanidine-substituted intermediate **61**, which undergoes regioselective imidazole ring-formation across the ester and methyl amino functionality (Scheme 15.13).



Scheme 15.13

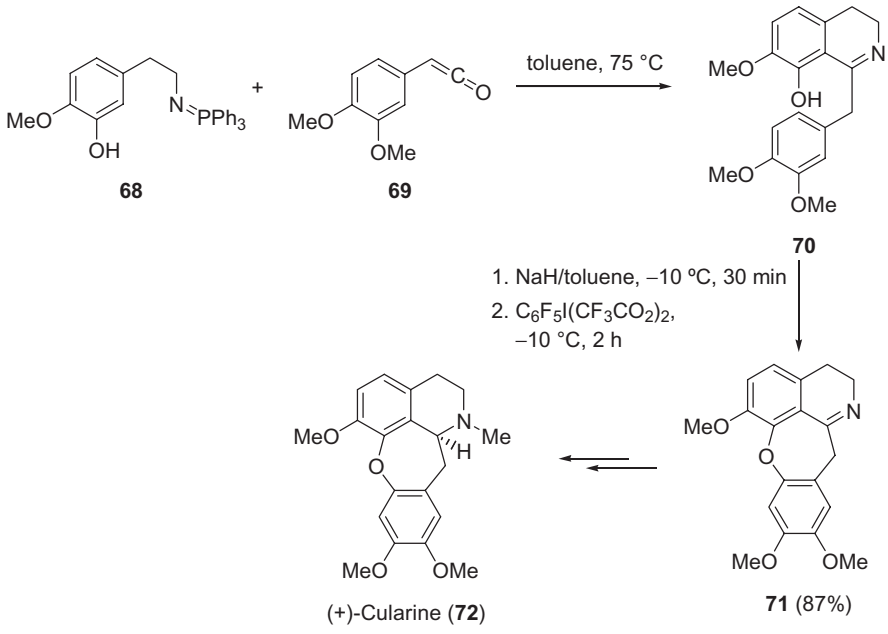


Scheme 15.14

This methodology has also been used for the preparation of several alkaloids (Scheme 15.14). Syntheses of the marine alkaloids, isonaamine A (**67a**), dorimidazole A (**67b**) and preclathridine A (**67c**),[24] have been described by aza-Wittig reactions of phosphazenes **63** with tosyl isocyanate, followed by addition of amine **65** to the carbodiimide intermediate **64** and subsequent intramolecular cyclization of compound **66**.

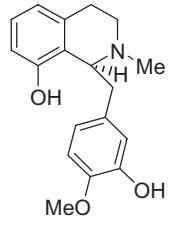
15.2.2.2 Reaction of Phosphazenes with Ketenes

The alkaloid (+)-cularine (**72**) was isolated by Manske in 1938 from plants belonging to the genera *Dicentra* and *Corydalis*.[25] Recently, Rodrigues *et al.* reported a diastereoselec-

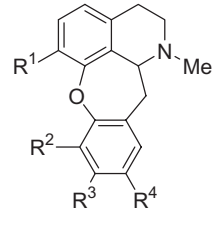


Scheme 15.15

tive synthesis of cularine alkaloids demonstrating the versatility of the aza-Wittig reaction for the construction of the isoquinoline core of the cularine alkaloids.[26] Thus, as shown in Scheme 15.15, the cascade aza-Wittig reaction/intramolecular cyclization (AW-IC) between phosphazene **68** and ketene **69** gave the 3,4-dihydroisoquinoline **70**. The corresponding aryloxenium or oxeniumoid was generated using a 1:1 ratio of the sodium salt of **70** and $C_6F_5I(CF_3CO_2)_2$, and cyclization product **71** (87%) was formed. Methylation of **71** followed by reduction with sodium borohydride at 0 °C for 4 h, yielded (+)-cularine (**72**) in 94% yield (Scheme 15.15). The synthesis of (+)-crassifoline (**73**), (+)-*O*-demethylcularine (**74**), (+)-sarcocapnine (**75**), and (+)-sarcocapnidine (**76**) has been reported by using the same approach (Figure 15.2).[26]

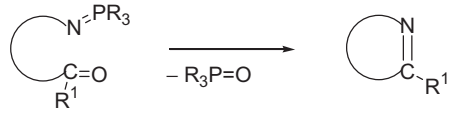


(+)-Crassifoline (**73**)



(+)-*O*-Demethylcularine (**74**) $R^1 = R^3 = OMe$, $R^2 = H$, $R^4 = OH$
(+)-Sarcocapnine (**75**) $R^1 = R^2 = R^3 = OMe$, $R^4 = H$
(+)-Sarcocapnidine (**76**) $R^1 = R^3 = OMe$, $R^2 = OH$, $R^4 = H$

Figure 15.2



77 78

R^1 = H, Alk, Ar, OR^2, NR^3

Scheme 15.16

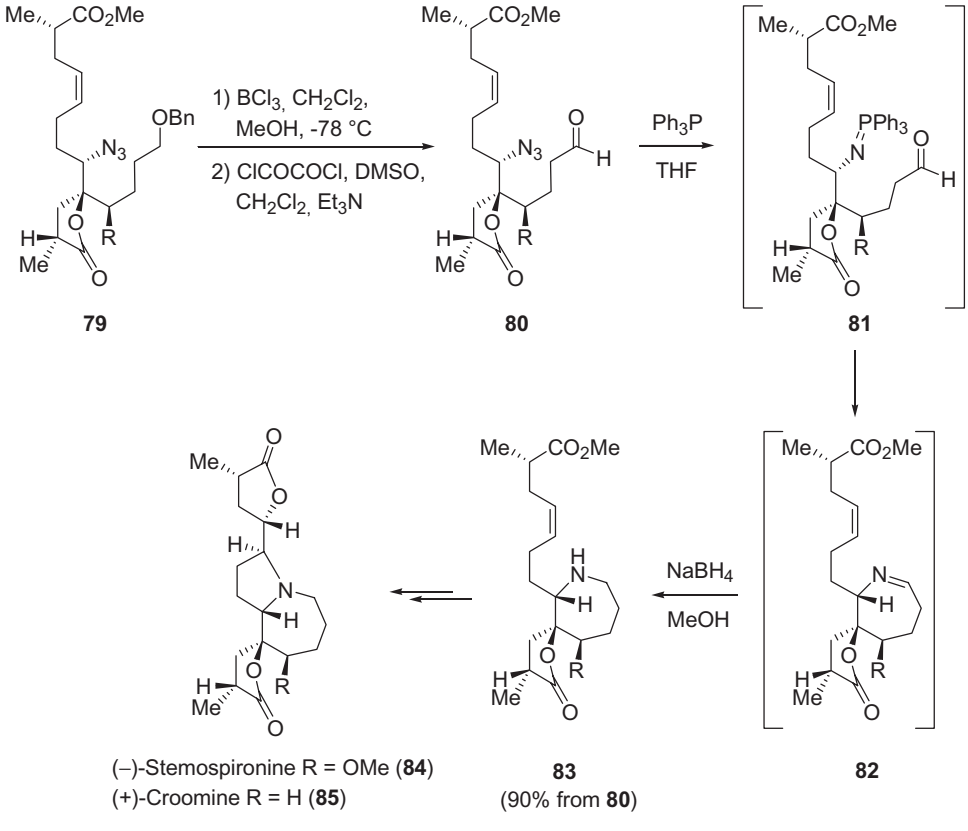
15.3 Intramolecular Aza-Wittig Reaction

Special interest has been focused on those aza-Wittig reactions of compounds **77** (Scheme 15.16) where both the phosphazene, moiety and the carbon-oxygen double bond (C=O) (aldehydes, ketones, esters and amides) are found within one molecule.[3a,27] This strategy involving intramolecular aza-Wittig reactions allows a method for the preparation of five- to higher-membered heterocyclic compounds **78** under very mild reaction conditions, that are a structural feature in the skeleton of natural products.

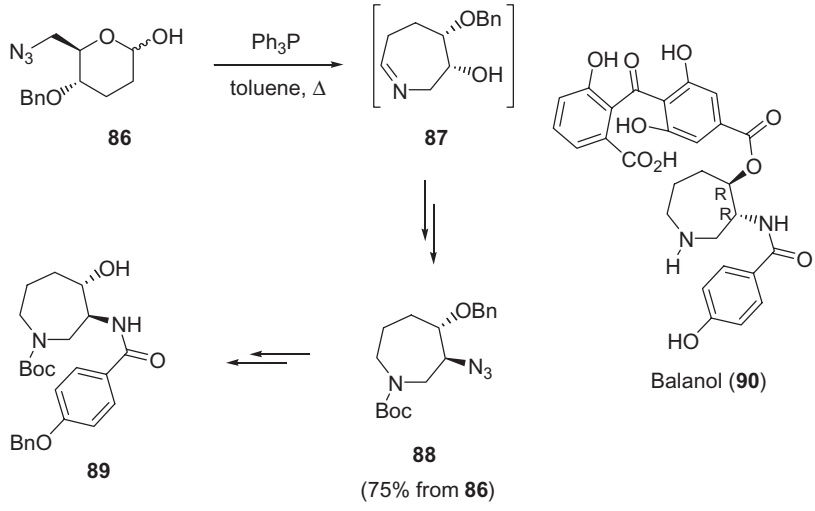
15.3.1 Functionalized Phosphazenes Containing an Aldehyde Group

An intramolecular aza-Wittig reaction with an aldehyde function allowed the stereocontrolled total synthesis of the polycyclic stemona alkaloid which characteristically contains the 1-azabicyclo[5.3.0.]decane nucleous, such as (−)-stemospironine (**84**)[28] and (+)-croomine (**85**).[29] The required aldehyde **80** was prepared from the starting azide **79** by cleavage of benzyl ether and Dess-Martin oxidation of the obtained primary alcohol (Scheme 15.17). Subsequent addition of triphenylphosphine to give phosphazene **81** and *in situ* reduction of the formed imine bond of **82** in the intramolecular aza-Wittig reaction gave a seven-membered ring precursor **83** of the expected alkaloids **84** and **85**.

Phosphazenes containing an aldehyde group have been used by Yadav *et al.*[30] for the synthesis of the optically active (3*S*,4*S*)-hexahydroazepine core of balanol (**90**) and ophiocordin by ring expansion to the seven-membered azepine through an intramolecular aza-Wittig process as the key step. As shown in Scheme 15.18, treatment of functionalized azide **86** with triphenylphosphine in toluene at reflux temperature gave the crude imine **87** which was subjected to reduction with $NaBH_4$ in methanol followed by *in situ* protection with Boc_2O and TEA (triethylamine) to give the azepine segment, which was converted into the azide **88** *via* treatment with Tf_2O and 2,6-lutidine followed by NaN_3 displacement (Scheme 15.18). The final elaboration of the targeted hexahydroazepine moiety **89** of balanol is very straightforward. Hydrogenation of **88** in AcOEt liberated the amino group from its azido surrogate and simultaneously deprotected the benzyl ether function with PtO_2. Acylation of the resulting amino alcohol delivers the product **89**, which is directly amenable to the total synthesis of (3*S*,4*S*)-balanol.

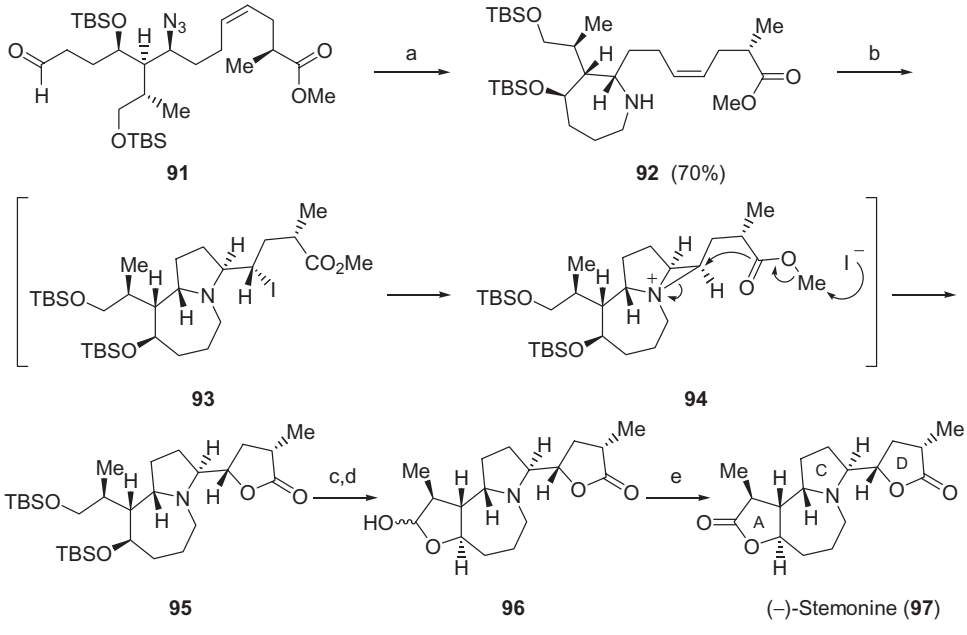


Scheme 15.17



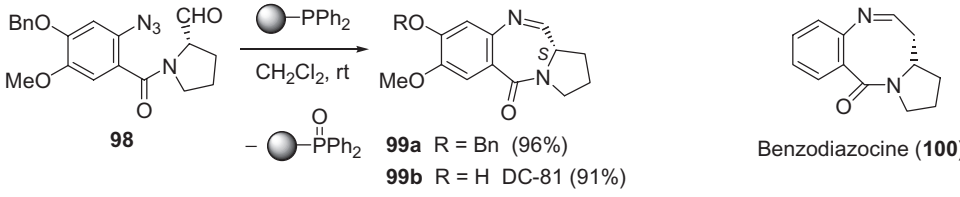
Scheme 15.18

The intramolecular aza-Wittig reaction has been successfully used for an elegant synthesis of the seven-membered nitrogen ring of (–)-stemonine (**97**) (Scheme 15.19). *Stemona* alkaloids represent a class of approximately 50 structurally novel, polycyclic metabolites isolated from monocotyledonous plants comprising the genera of *Stemona*, *Croomia*, and *Stichoneuron*. The total synthesis of (–)-stemonine (**97**) was reported for the first time by Williams *et al.*[31] nearly 75 years after its initial discovery. Cyclization to give perhydroazepine **92** *via* the Staudinger reaction of aldehydic azide **91** with ethyldiphenylphosphine generated the seven-membered imine by an intramolecular aza-Wittig process (Scheme 15.19). After imine formation, a reductive quench with $NaBH_4$ gave the amine **92** in 70% yield. Stereocontrolled formation of the pyrrolidinobutyrolactone C–D ring system occurred in a single step as a consequence of an iodine-induced cyclization. Thus, treatment of **92** with I_2 led to formation of **95** in reproducible yields of 42%. The synthesis of **97** was completed by simultaneous deprotection of TBS ethers followed by Dess–Martin oxidation, which resulted in isolation of the stable lactol **96**. Brief exposure to Jones reagent gave synthetic (–)-stemonine (**97**).



Reaction conditions: (a) $EtPPh_2$, benzene, rt, 18 h; the mixture was then concentrated in vacuo, and THF, $NaBH_4$, and MeOH were added, 70%. (b) I_2, CH_2Cl_2/Et_2O (2.5:1), rt, 48 h, 42%. (c) TBAF, THF, rt, 77%. (d) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 69%. (e) CrO_3, aq H_2SO_4, acetone, THF, rt, 68%.

Scheme 15.19



Scheme 15.20

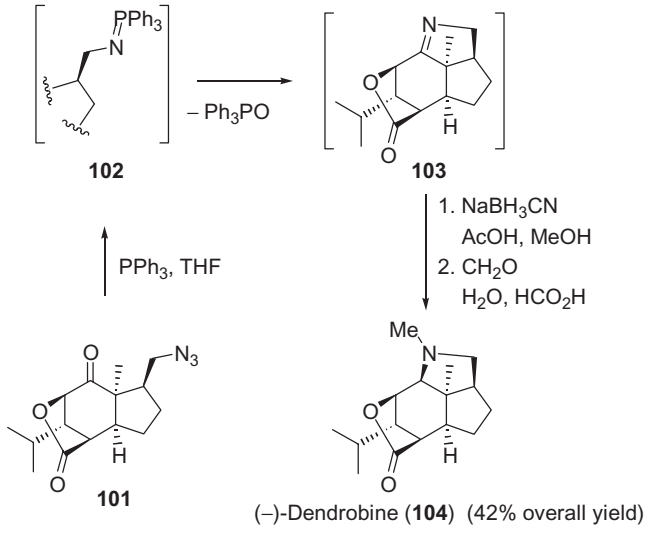
Likewise, the antibiotic DC-81 (**99b**) can be synthesized by using the same strategy through an intramolecular reductive cyclization with polymer-supported triphenylphosphine.[32] Treatment of the azide **98** this polymer at room temperature afforded the intramolecular aza-Wittig compound **99a**, which, in turn, could be converted into the natural product DC-81 (**99b**) in a straightforward manner (Scheme 15.20). This strategy has also been used by Molina *et al.*[33] for the preparation of optically active (*R*)-enantiomer of antibiotic DC-81. A similar approach has been used for the synthesis of benzodiazocine (**100**), a novel eight-membered ring analogue to the anthramicin family of antibiotics, synthetized by O′Neil *et al.*[34]

15.3.2 Functionalized Phosphazenes Containing a Ketone Group

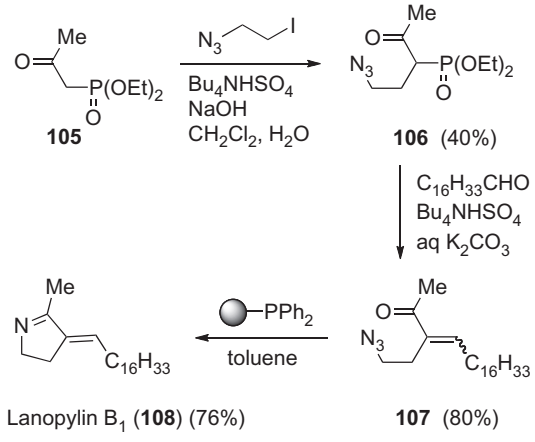
Phosphazenes having a ketone substitution cyclize by an intramolecular aza-Wittig reaction to give ring systems of 5- or 6-membered heterocycles.[3a] In the total synthesis of (−)-dendrobine (**104**), the 5-membered nitrogen heterocycle can be formed by an intramolecular aza-Wittig reaction of the azido ketone **101** (Scheme 15.21).[35] Thus, treatment of **101** with triphenylphosphine gave polycyclic imine **103** *via* **102**. Reduction of the imine moiety with sodium cyanoborohydride from the less hindered α-face, followed by reductive methylation of the amine with paraformaldehyde and formic acid, afforded the enantiomerically pure (−)-dendrobine (**104**) (Scheme 15.21). In this synthesis, six stereogenic centers were induced, each in a stereoselective fashion, from a single chiral center of the starting material.

The formation of five-membered cyclic imines through a Staudinger/intramolecular aza-Wittig reaction can also be performed by solid-phase synthesis and has been applied for the first synthesis of lanopylin B_1 (**108**).[36] The total synthesis, which takes only four steps, starts with a phase-transfer alkylation of diethyl 2-oxopropylphosphonate **105** with a 2-iodoethyl azide, affording the azido phosphonate **106**, which undergoes a phase-transfer Horner-Emmons Wittig reaction with heptadecanal to provide the azido enone **107**. An intramolecular aza-Wittig reaction of the enone **107** with polymer-supported triphenylphosphine in toluene completed the first total synthesis of lanopylin B_1 (**108**) in 76% yield (Scheme 15.22).

A similar approach has been reported for the total synthesis of the marine cyanobacterial apratoxin A (**111**).[37] Apratoxin A, isolated from *Lyngbya spp*. cyanobacteria, is representative of a growing class of marine cyanobacterial cyclodepsipeptides wherein discrete polypeptide and polyketide domains are merged by ester and amide or amide-derived linkages. For the preparation of apratoxin A, the reaction of the azide **109** with



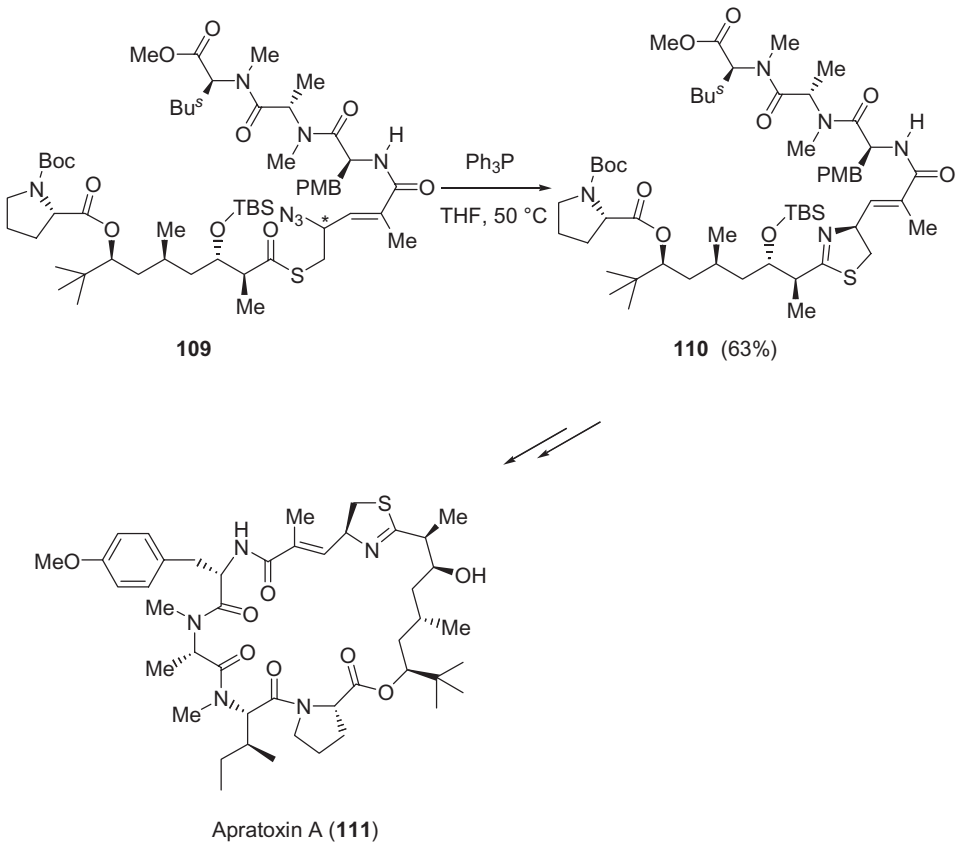
Scheme 15.21



Scheme 15.22

Ph_3P in anhydrous THF effected thiazoline formation by the intramolecular Staudinger-aza-Wittig process to deliver C35 *O*-TBS ether **110**. Completion of the total synthesis of the cyclodepsipeptide apratoxin A (**111**) from thiazoline **110** would have required only two further operations: amide formation between proline amine and *iso*-leucine carboxylate residues and removal of the C35 *O*-TBS-protecting group (Scheme 15.23).

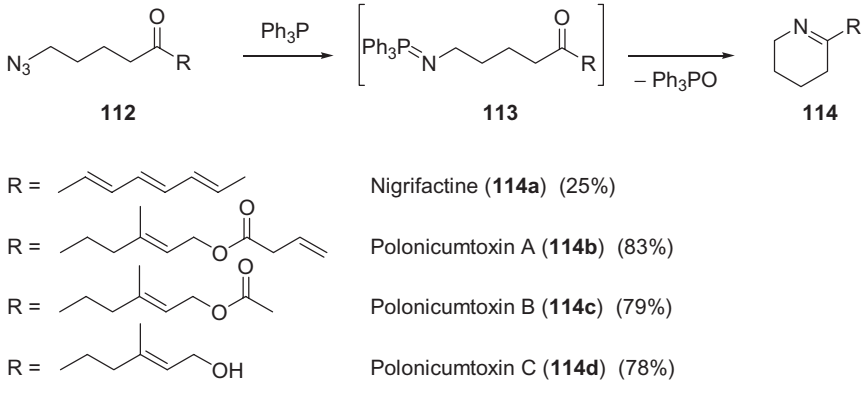
The formation of a tetrahydropyridine ring by means of the Staudinger reaction of azido-ketones and phosphines followed by intramolecular Aza-Witig reaction was applied



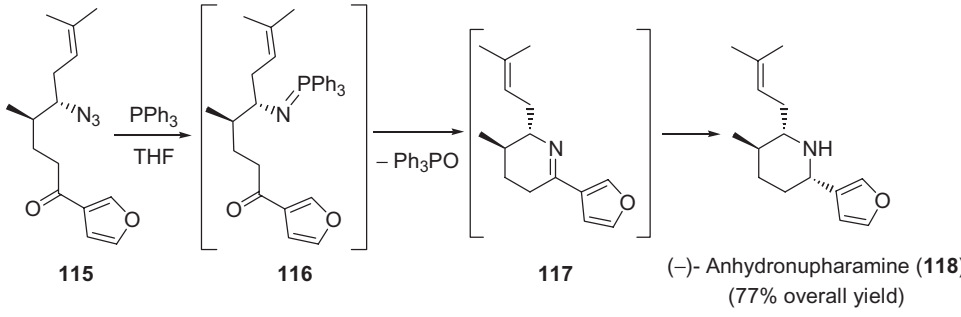
Scheme 15.23

for the synthesis of the alkaloid nigrifactine (**114a**) (Scheme 15.24), and represents the first example reported for the use of this synthetic strategy for the preparation of a natural product.[38] Polonicumtoxins A (**114b**), B (**114c**), and C (**114d**) are cyclic ketimine toxins isolated from the freshwater dinoflagellate *Peridinium polonicum*, which occasionally blooms in lakes and drinking water reservoirs. They exhibit extremely potent toxicity toward fish, making the dinoflagellate blooms a serious environmental problem. Yasumoto *et al.*[39] have reported the preparation of these compounds through a similar strategy involving an aza-Wittig procedure for the construction of the ketimine unit (Scheme 15.24). A microwave-assisted intramolecular aza-Wittig reaction was used by De Kimpe *et al.*[40] for the synthesis of the principal bread flavour component, 6-acetyl-1,2,3,4-tetrahydropyridine.

An optically active piperidine ring has been constructed by an intramolecular aza-Wittig reaction allowing a concise enantiospecific synthesis of nuphar piperidine alkaloids, among them (−)-anhydronupharamine (**118**), which has a sesquiterpenoid structure



Scheme 15.24

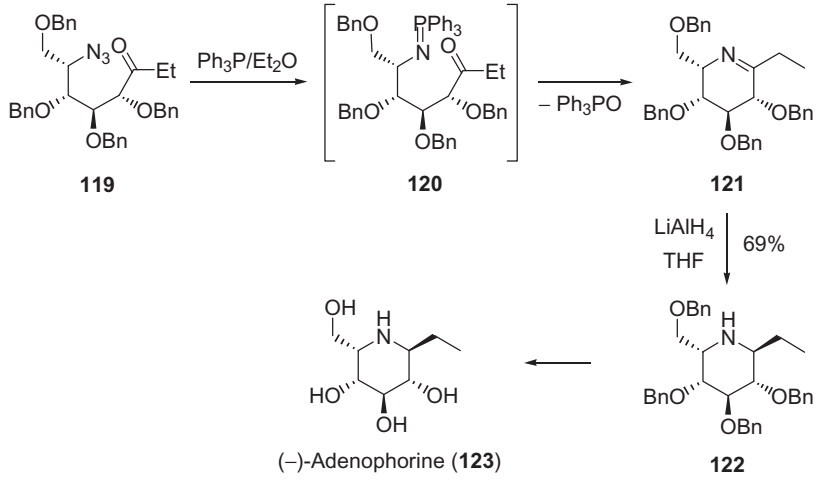


Scheme 15.25

with a furane and piperidine rings. Reaction of the phosphazene **116**, obtained by a Staudinger reaction of azide **115** with triphenylphosphine, in refluxing THF yielded the imine **117**. The non-isolated imine **117** was reduced with sodium borohydride in ethanol to give (–)-anhydronupharamine (**118**) stereoselectively (Scheme 15.25).[41]

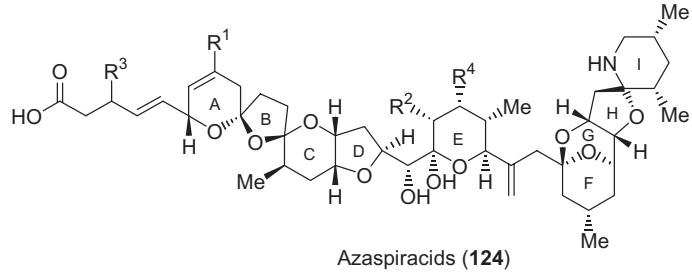
A similar strategy has been applied for the preparation of tetrahydropyridine precursors in the synthesis of (–)-adenophorine (**123**), a rare example of a naturally occurring aza-sugar with hydrophobic susbtituents, 1-*epi*-adenophorine or deoxynojirimycin (DNJ) variants.[42] Ethyl ketimine **121** was synthesized through the Staudinger/aza-Wittig sequence from compound **119**, as shown in Scheme 15.26. The use of $LiAlH_4$ yielded tetrabenzyladenophorine **122**. Deprotection of **122** yielded (–)-adenophorine (**123**).

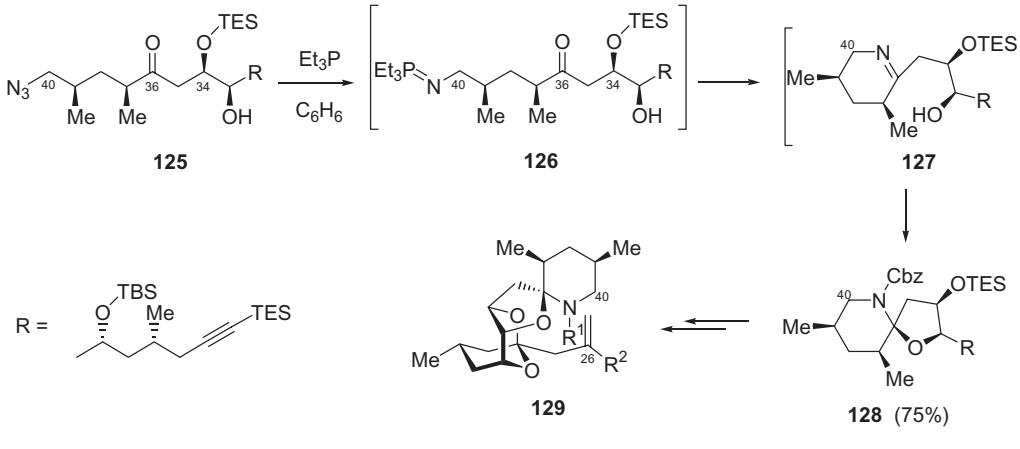
The azaspiracids **124** (Scheme 15.27) are the causative agents of a recently defined class of human poisoning resulting from consumption of tainted shellfish. The archetypal member of this novel class of marine toxins, azaspiracid-1 (AZA1, $R^1 = R^3 = H$, $R^2 = R^4 = Me$, Scheme 15.27), was reported by Yasumoto *et al.* as an isolated compound from the cultivated Irish mussel *Mytilus edulis*.[43] The azaspiracid natural products display



119 120 121 122

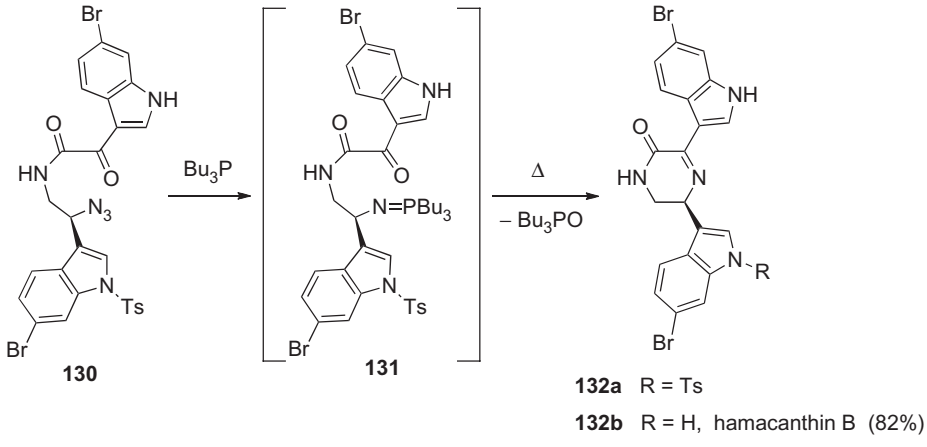
Scheme 15.26





125 126 127 128 (75%) 129

Scheme 15.27



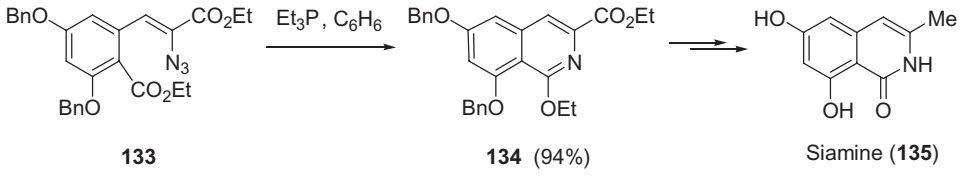
Scheme 15.28

a common spiroaminal-containing terminal domain that has inspired the development of its synthesis through a Staudinger reduction-aza-Wittig process.[44] The anticipated spiroaminal moiety in **128** was initially formed stereoselectively in 75% yield upon treatment of azide **125** with Et_3P in benzene (Scheme 15.27). The generated phosphazene **126** undergoes an intramolecular aza-Wittig reaction with the C36 ketone to form the six-membered cyclic imine **127**. Addition of the C33 hydroxyl group to the imine completes the cascade. Compound **128** represents the fully functionalized C27-C40 azaspiracid intermediate that is amenable to elaboration into the complete F-G-H-I ring domain.

This approach has been also used as the key step for the enantioselective synthesis of the marine indole alkaloid, hamacanthin B (**132b**)[45] (Scheme 15.28), and the antipode of hamacanthin A.[46] The central pyrazinone ring was achieved by the reaction of azide **130** with tributylphosphine in toluene at room temperature, to afford phosphazene intermediate **131**, followed by heating, to provide the expected cyclized product **132a**. Deprotection of **132a** led to the formation of hamacanthin B (**132b**) in 82% yield and keeping the configuration of the *C*–α of the starting azide.

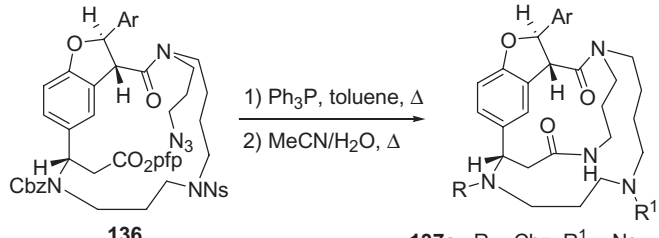
15.3.3 Functionalized Phosphazenes Containing an Ester Group

It is well known that the carbonyl group of esters is less reactive than that of aldehydes and ketones in an aza-Wittig reaction. However, in recent years, some reports of the intramolecular aza-Wittig reaction of phosphazenes containing ester derivatives in the molecule for the preparation of heterocyclic systems[3a] have appeared. In the synthesis of siamine (**135**) the treatment of α-azidocinnamate **133** with triethylphosphine in benzene at room temperature gave the 1-ethoxyisoquinoline **134**.[47] The ester substituent of **134** was reduced in high yielding indirect sequence and finally treatment with boron tribromide resulted in simultaneous cleavage of ethyl and benzyl ethers to give siamine (**135**) (Scheme 15.29).



133 **134** (94%) Siamine (**135**)

Scheme 15.29



136

137a R = Cbz, R^1 = Ns
Ar = *p*-BnO-C_6H_4
(73% from **136**)

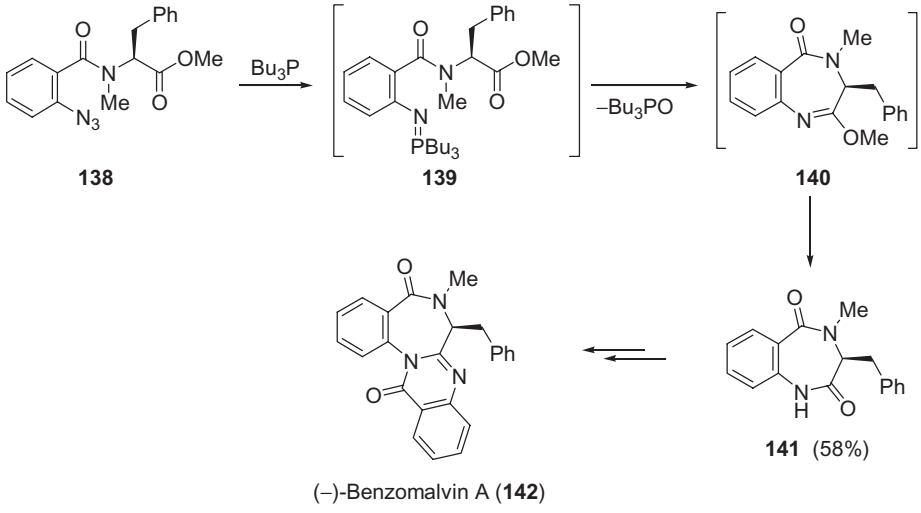
pfp: pentafluorophenyl
Ns: 2-nitrobenzenesulfonyl

137b R = R^1 = H,
Ar = *p*-OH-C_6H_4
(–)-Ephedradine A
(75% in two steps)

Scheme 15.30

The formation of complex 13-membered macrocycles through a Staudinger/intramolecular aza-Wittig reaction has been applied to the total synthesis of (–)-ephedradine A (orantine) (**137b**) (Scheme 15.30).[48,49] (–)-Ephedradine A is a complex macrocyclic spermine alkaloid, whose one of its two macrocycles can be constructed by an intramolecular aza-Wittig strategy. In this way the formation of the 13-membered iminoether **137a** was successfully obtained by treatment of the azide **136** (Ar = *p*-BnO-C_6H_4) with Ph_3P in refluxing toluene under high-dilution conditions (Scheme 15.30). Subsequent hydrolysis, removal of the Ns (2-nitrobenzenesulfonyl) group and simultaneous cleavage of the Cbz group and benzyl ether yielded (–)-ephedradine A (**137b**).

Methods for the preparation of seven-membered nitrogen-ring systems by the use of the intramolecular aza-Wittig reaction have increased in the last decade. This heterocycle is quite common in benzodiazepine derived alkaloids. This methodology has been applied for the first total synthesis of (–)-benzomalvin A (**142**) (Scheme 15.31).[50,51] Reaction of the starting azide **138** with tributylphosphine leads to the formation of the phosphazene intermediate **139**, which under the reaction conditions affords the benzodiazepine **141** in 58% yield *via* compound **140**. Benzodiazepine **141** suffered subsequent transformations to afford (–)-benzomalvin A (**142**).



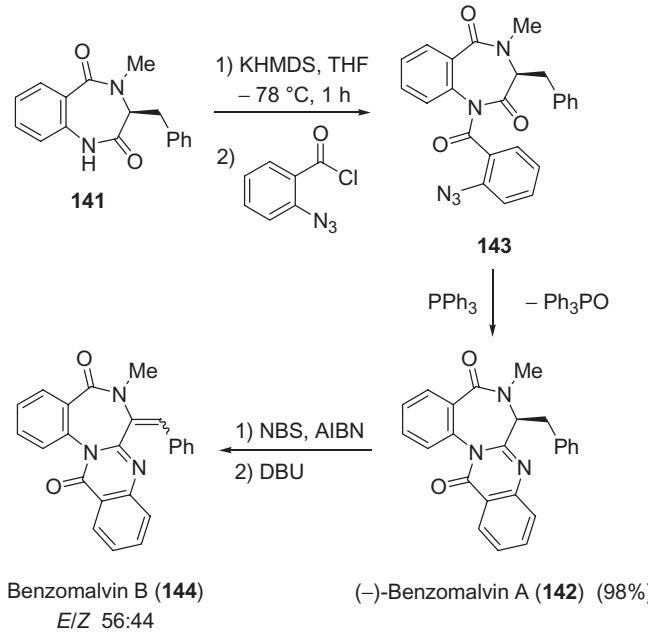
Scheme 15.31

15.3.4 Functionalized Phosphazenes Containing an Amide Group

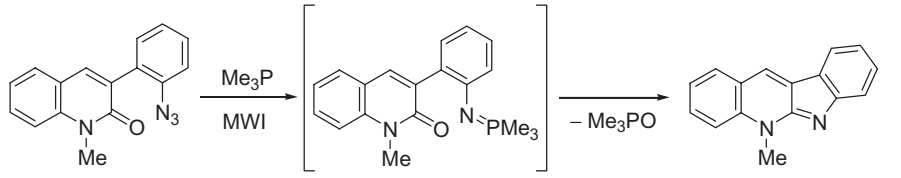
Intramolecular aza-Wittig imination reactions involving less reactive amide carbonyl groups, which are known as the Eguchi protocol, have been reported, usually suffering from low yields. As previously reported (Section 15.3.3), intramolecular aza-Wittig reaction with ester substituents allowed the preparation of benzomalvin A. The last step in this total synthesis involves an intramolecular aza-Wittig reaction of a functionalized phosphazene containing an amide moiety. Thus, the azide **143** was treated with triphenylphosphine to generate the corresponding phosphazene, which reacted with the amide function to afford (–)-benzomalvin A (**142**) in 98% yield. Benzomalvin B (**144**) can also be obtained in two steps from (–)-benzomalvin A (Scheme 15.32).[51]

On the other hand a convenient combination of intramolecular aza-Wittig strategy and microwave technology for the preparation of the alkaloid, cryptotackieine, which has an indolo[2,3-*b*]quinoline core, has been described.[52,53] Thus, treatment of 3-(*o*-azidophenyl) quinolin-2-one **145** with trimethylphosphine in nitrobenzene under microwave irradiation between 150–180 °C, after five-membered ring construction, afforded cryptotackieine (**51**) in 40% yield *via* **146** (Scheme 15.33).

The intramolecular aza-Wittig reaction of functionalized phosphazenes containing an amide moiety has been used as the key-step for the preparation of the natural product deoxyvasicinone.[54] Azide **149** (R = H) was obtained from **148** and pyrrolidone **147** (R = H) in the presence of sodium hydride as a base at room temperature (Scheme 15.34). Then azide **149** (R = H) was treated with tributylphosphine and the natural product deoxyvasicinone (**150**) was successfully obtained in 99% yield even at room temperature for 2 h. More recently, fused [2,1-*b*]quinazolinones, namely vasicinone, deoxyvacisinone and related heterocycles have been prepared by solid-phase methods using the intramolecular aza-Wittig reaction of a phosphazene with an amide moiety.[55,56] Similarly, this



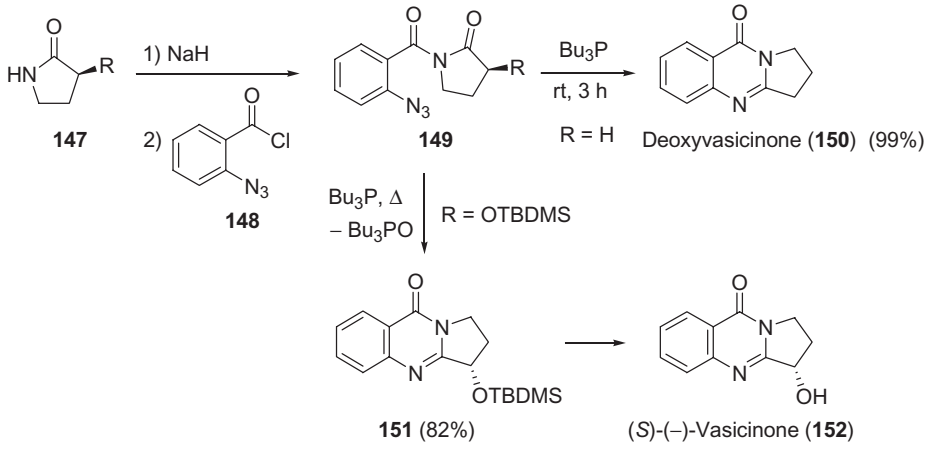
Scheme 15.32



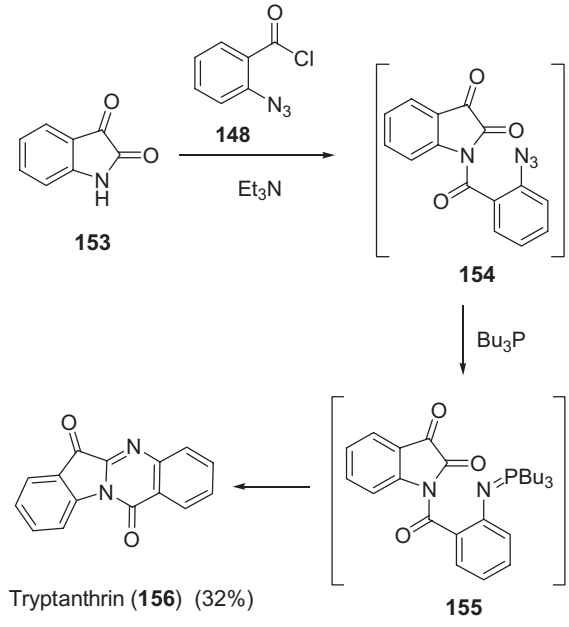
Scheme 15.33

strategy has also been used for the synthesis of optically active pyrrolo[2,1-*b*]-quinazoline alkaloid, (*S*)-(−)-vasicinone (**152**).[57] Fortunately, by this synthesis the authors have clarified that natural *L*-vasicinone has the (*S*)-configuration. After *O*-TBDMS (*O*-*tert*-butyldimethylsilyl) protection, *o*-azidobenzoylation followed by treatment of compound **149** (R = OTBDMS) with tributylphosphine afforded (*S*)-(−)-vasicinone (**152**) *via* the tandem Staudinger/intramolecular aza-Wittig reaction followed by TBDMS deprotection of **151** (Scheme 15.34).

Quinazoline alkaloids containing the indole skeleton such as tryptanthrin (**156**)[58] have been constructed *via* intramolecular aza-Wittig reaction of amide derivatives (Scheme 15.35). The fused quinazoline ring in tryptanthrin (**156**) could be synthesized efficiently in a one-pot procedure *via* the consecutive Staudinger/intramolecular aza-Wittig reaction of the corresponding azide **154** with tributylphosphine (Eguchi protocol).

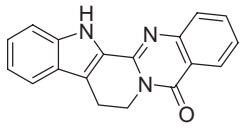


Scheme 15.34

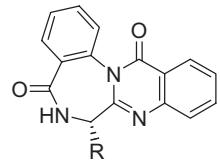


Scheme 15.35

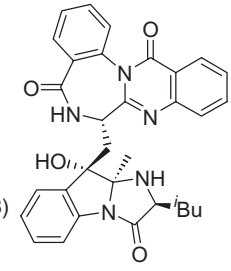
Analogously, rutercarpine (**157**)[58] and alkaloids such as (–)-asperlicin C (**158**),[59] circumdatin F (**159**),[60] sclerotigenin (**160**),[60] and (–)-asperlicin (**161**)[59] (Figure 15.3) containing a quinazolino[3.2-*a*][1,4]benzodiazepinedione nucleus have been prepared through an intramolecular aza-Wittig procedure involving an amide moiety in very mild reaction conditions. The modified Eguchi protocol using polymer-supported phosphine-mediated intramolecular aza-Wittig reaction relied on an efficient formation of the fused



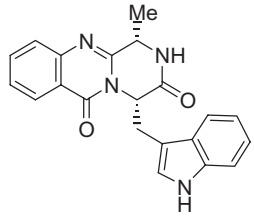
Rutercarpine (**157**)



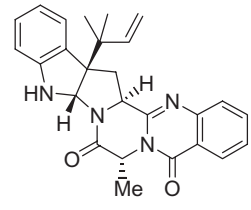
(–)-Asperlicin C R = Indol-3-ylmethyl (**158**)
Circumdatin F R = Me (**159**)
Sclerotigenin R = H (**160**)



(–)-Asperlicin (**161**)



(–)-Fumiquinazoline G (**162**)



Ardeemin (**163**)

Figure 15.3

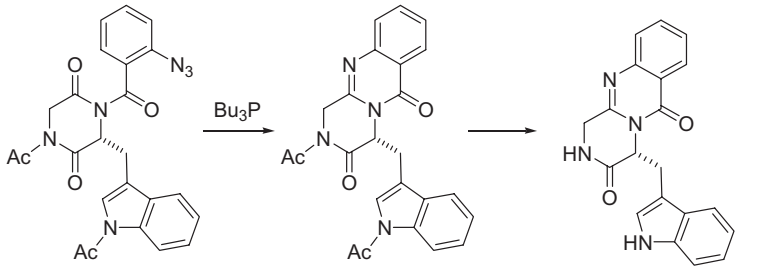
quinazoline ring system[61] yielding the simplest member of the benzodiazepine-quinazolinone family, sclerotigenin (**160**). In this manner, a multi-arrayed library generation strategy has been developed for the preparation of benzodiazepine-quinazolinone alkaloid structure of the circumdatin family of natural products. Also the key step in the synthesis of pyrazino[2,1-*b*]quinazoline core, found in the (–)-fumiquinazoline G (**162**)[60,62] and ardeemin (**163**)[63] (Figure 15.3), involves annulation of a quinazolin-4-one onto an amide by reaction with tributylphosphine following the Eguchi procedure.

This route has been adapted to the synthesis of both enantiomers of the alkaloid glyantrypine (**166**). In this case, the intramolecular aza-Wittig strategy allowed the preparation of pyrazino[2,1-*b*]quinazoline ring system present in this alkaloid and many others which exhibit very interesting biological properties.[64] The cyclization to pyrazino[2,1-*b*] quinazoline-3,6-dione derivatives was carried out through a Staudinger/aza-Wittig sequence by treatment of compound **164** with Bu_3P, which afforded compound **165** in 66% yield (Scheme 15.36). Subsequent deacetylation by addition of hydrazine hydrate gave compounds **166a**,**b** in good yields.

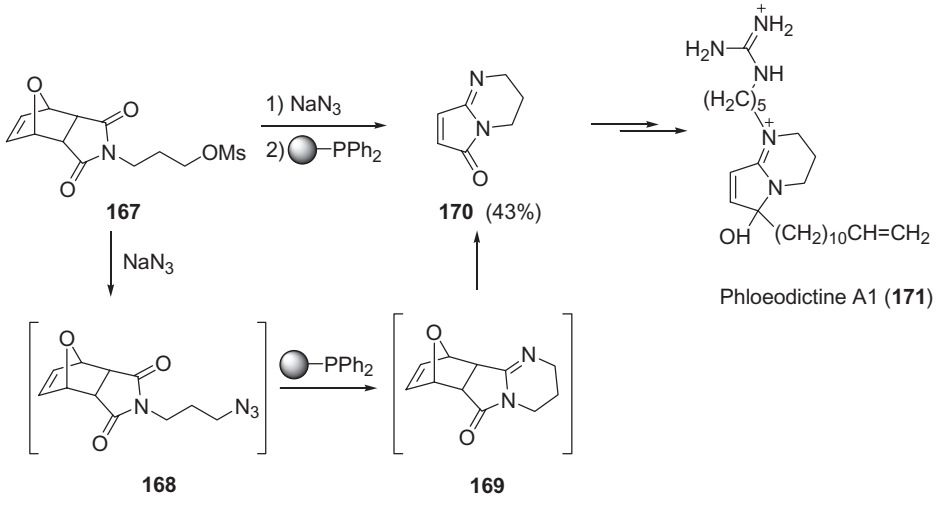
The antitumor antibiotic phloeodictine A1 (**171**) has been synthesized by Snider's group[65] (Scheme 15.37). The unstable azide derived from **167** was subjected to a polymer supported tandem Staudinger-aza-Wittig followed by a retro Diels-Alder reaction to afford intermediate **170**. Addition of 11-dodecenyl magnesium bromide followed by alkylation reaction and deprotection completes an efficient synthesis of phloeodictine A1 (**171**).

15.4 Conclusions

In summary, this review presents recent progress in the synthesis of some natural products based on the intermolecular and intramolecular aza-Wittig reaction of phosphazenes with



Scheme 15.36



Scheme 15.37

carbonyl compounds. These results indicate the importance and utility of these phosphazenes as versatile building blocks for the construction of C-N double bonds in very mild conditions, not only in the preparation of acyclic compounds, but also for heterocycle construction, ranging from simple monocyclic compounds to complex polycyclic and macrocyclic systems. In many cases, the synthesis is carried out stereoselectively and the resulting compounds are physiologically active or are potential intermediates in the synthesis of physiologically active compounds including analogues of natural products.

Acknowledgments

The present work has been supported by the *Dirección General de Investigación del Ministerio de Ciencia e Innovación* (MICINN, Madrid DGI, CTQ2006/09323) and *Departamento de Educación Universidades e Investigación (Gobierno Vasco) – Universidad del País Vasco* (GV, IT277-07; UPV, GIU06/51).

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16

Azides in Carbohydrate Chemistry

Henning S.G. Beckmann and Valentin Wittmann

Fachbereich Chemie, Universität Konstanz, Universitätsstr. 10, D-78457 Konstanz, Germany

16.1 Introduction

The first azide-containing sugar, a glycosyl azide, was reported in 1930 by Bertho.[1] Since that time various methods have been developed for the introduction of azides at different positions of sugars. A survey of available methods is given in Section 16.2. Until the late 1970s, azides contained in carbohydrate derivatives were simply used as accessible synthons for amines because of their easily performed reduction to amines. Due to their stability against a variety of reaction conditions, azides often can serve as masked amines during the course of carbohydrate synthesis. The development of the diazo transfer reaction facilitated the use of azides also as temporary protecting group for amines. This was extensively applied during the preparation of aminoglycoside derivatives (Section 16.3).

Over the last three decades, azides became an important tool especially for the synthesis of glycopeptides and -proteins. In 1978 Paulsen *et al.* developed the 'azide method' for the preparation of 1,2-*cis*-glycosides of glycosamine derivatives using 2-azido-2-deoxy-donors (Section 16.4). This reaction is widely used for the synthesis of *O*-linked glycosyl amino acid building blocks. In *N*-glycoproteins, the glycan chains are attached to the protein via a β-glycosyl amide. Staudinger-type reactions offer a convenient access to such structures and are applied since the 1990s for the synthesis of α- and β-glycosyl amides directly from glycosyl azides (Section 16.5).

An enormous impact on the field of glycobiology during the last decade had the development of two bioorthogonal reactions based on azides: the copper-catalyzed azide-alkyne [3+2] cycloaddition and the Staudinger ligation. Together with the possibility of *in vivo* incorporation of azide and alkyne tags into glycans and proteins, these reactions offer new options for selective labeling and manipulation of biomolecules even within

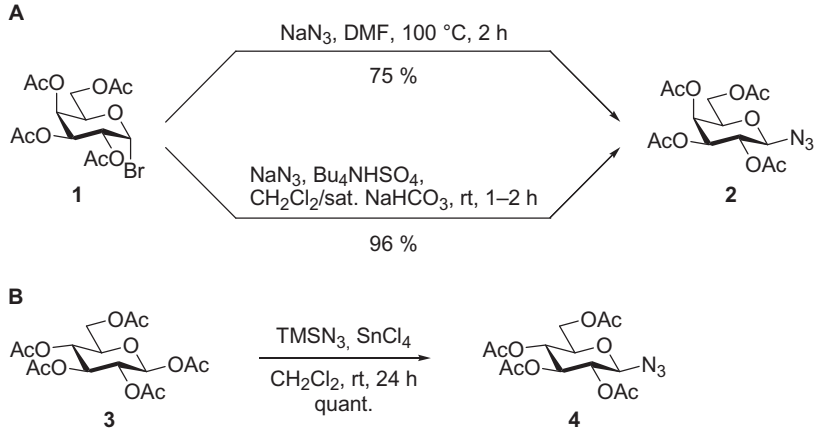
Organic Azides: Syntheses and Applications Edited by Stefan Bräse and Klaus Banert
 ISBN: 978-0-470-51998-1

living cells. Especially the azide-alkyne cycloaddition has been extensively applied for the chemical synthesis of neoglycoconjugates such as glycopeptide and glycoprotein mimics or multivalent glycoclusters (Section 16.6). Metabolic oligosaccharide engineering uses the biosynthetic pathways for the introduction of azide- (and alkyne-)tagged sugar moieties into the glycans of cells that can subsequently be labeled by a detectable probe. This approach is discussed in Section 16.7.

16.2 Synthesis of Azide-Containing Carbohydrates

A common way for the introduction of azides into carbohydrates is the nucleophilic replacement of leaving groups by the azide ion. These reactions can be divided into three groups: substitutions at the anomeric center leading to glycosyl azides, substitutions at primary, and substitutions at secondary carbon atoms.

A widely used method for the preparation of glycosyl azides[2–4] is the conversion of acetylated halogenoses, such as **1**, by treatment with sodium azide based on Bertho's initial work (Scheme 16.1A).[1] While homogeneous one-phase reactions in DMF often require elevated temperatures,[5] phase-transfer catalysis enables milder conditions.[6] One limitation of this methodology is the instability of glycosyl halides. Thus, sequential one-pot procedures have been developed that avoid the isolation of glycosyl halides.[7] An alternative, which circumvents the preparation of glycosyl halides completely, is the direct conversion of glycosyl acetates into the corresponding glycosyl azides using trimethylsilyl azide under Lewis acid catalysis (Scheme 16.1B).[8] Glycosyl azides with 1,2-*trans*-configuration are easily obtained by the described methods using acyl protecting groups due to their neighboring group participation. Glycosyl azides with 1,2-*cis*-configuration can be prepared from 1,2-*trans*-glycosyl halides in an S_N2-type reaction or from ether-protected glycosyl acetates by treatment with trimethylsilyl azide.[2–4]



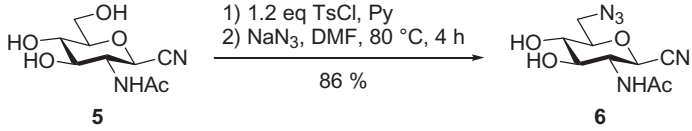
Scheme 16.1 *Preparation of glycosyl azides from (A) peracetylated glycosyl halides under classical homogeneous conditions[5] and under mild phase-transfer catalysis[6] and (B) from peracetylated sugars[9]*

The introduction of azides at the primary carbon of carbohydrates is conveniently carried out by an S_N2 reaction. The generation of a good leaving group, such as a sulfonate, is often possible in a selective way without need for protection of the secondary hydroxy groups as was shown for GlcNAc derivative **5** (Scheme 16.2).[10] Subsequent substitution with sodium azide usually proceeds at elevated temperatures with good yields.

In contrast, S_N2 reactions at secondary carbons of the sugar ring system are more complex. The success of such reactions is strongly dependent on the type of sugar (stereochemistry), the position at which the S_N2 reaction is carried out, anomeric configuration, and used protecting groups. Nevertheless, this approach is widely applied for the introduction of azido groups at the ring system. For instance, the mesylate of glucoside **7** was substituted yielding 4-azido galactoside **8** under inversion of configuration (Scheme 16.3).[11]

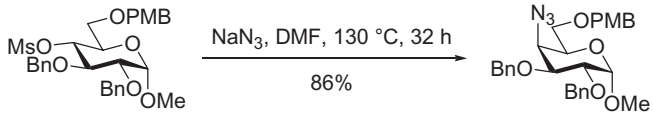
Epoxides are also useful precursors for the incorporation of azido groups by nucleophilic attack. According to the Fürst-Plattner rule,[12] ring opening of sugar epoxides by azide ions preferentially leads to the diaxial product. For instance, 2-azido compound **10** is obtained regioselectively by opening of Cerny epoxide **9** with sodium azide (Scheme 16.4).[13] **10** was further converted into the suitably protected glycosyl donor **11**, which was applied in the synthesis of a heparan sulfate synthon by 1,2-*cis*-glycosylation (cf. Section 16.4).

Azides can also be introduced by radical addition to glycals. The classical azidonitration, developed by Lemieux *et al.* in 1979, is a powerful method for the preparation of



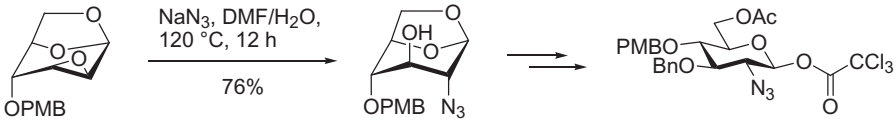
5 **6**

Scheme 16.2 *Regioselective introduction of an azido group at the primary carbon of* ***5*** *via nucleophilic replacement of a sulfonate intermediate*[10] *Py = pyridine*



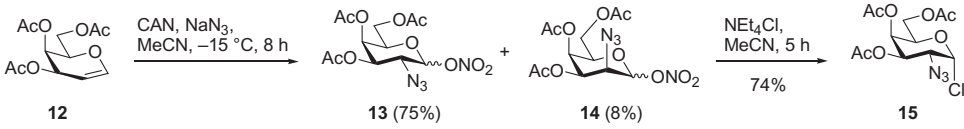
7 **8**

Scheme 16.3 *Replacement of a mesylate by an azido group under inversion of configuration at a secondary center of the sugar ring*[11]

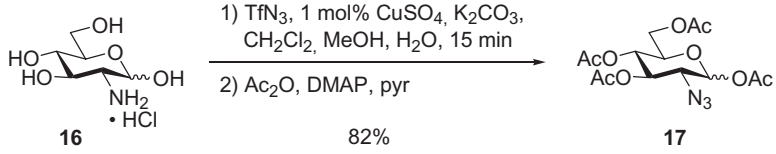


9 **10** **11**

Scheme 16.4 *Regio- and stereoselective opening of Cerny epoxide* ***9*** *leads to 2-azido compound* ***10****, which can be further converted into glycosyl donor* ***11***[13]



Scheme 16.5 *Azidonitration of galactal* ***12*** *leads to an epimeric mixture of the 2-azido-1-nitro-pyranoses* ***13*** *and* ***14*** *from which glycosyl donor* ***15*** *can be prepared directly.*[14] *CAN = cerium(IV) ammonium nitrate*



Scheme 16.6 *Typical procedure for the Cu(II)-catalyzed diazo transfer.*[26] *DMAP = 4-(dimethylamino)pyridine*

2-azido sugars that is still frequently used (Scheme 16.5).[14] It is especially useful for the synthesis of those 2-azido derivatives, whose corresponding glycosamines lack accessibility from natural sources as in the case of galactosamine. However, while the reaction is highly regioselective, in most cases epimeric mixtures of the 2-azido compounds are formed. The ratio of the epimers strongly depends on the employed glycal substrate.[15] The obtained 1-nitro-pyranoses can easily be converted into glycosyl donors, such as glycosyl halides,[14] trichloroacetimidates,[16] *n*-pentenyl glycosides,[17] or thioglycosides,[18–20] which are valuable building blocks for the preparation of 1,2-*cis* glycosides of *N*-acetyl-glycosamines (cf. Section 16.4). Similar methods for the synthesis of 2-azido sugars using radical addition to glycals are the azidochlorination[21] and the azidophenylselenation.[22,23]

Another possibility for the synthesis of organic azides is the diazo transfer using triflyl azide.[24] In contrast to the methods described above, not the entire azido group is incorporated into a molecule but an N_2 moiety is transferred onto an existing amine under retention of configuration. The first diazo transfer onto amino sugars was reported in 1991 by Vasella *et al.*[25] They treated different unprotected glycosamines with freshly prepared triflyl azide under basic conditions. After subsequent acetylation, the 2-azido sugars were isolated in good yields. This methodology was further improved by the addition of catalytic amounts of copper sulfate which leads to a much faster and more reliable reaction (Scheme 16.6).[26,27] Using the diazo transfer, it is possible to employ azides not only as amine synthons but also as temporary protecting groups for amines. This has been applied for example to the synthesis of aminoglycosides (Section 16.3), heparan sulfate fragments,[28] heparin fragments,[29,30] hyaluronan neoglycopolymers,[31] and *N*-acetyl-neuraminic acid derivatives.[32]

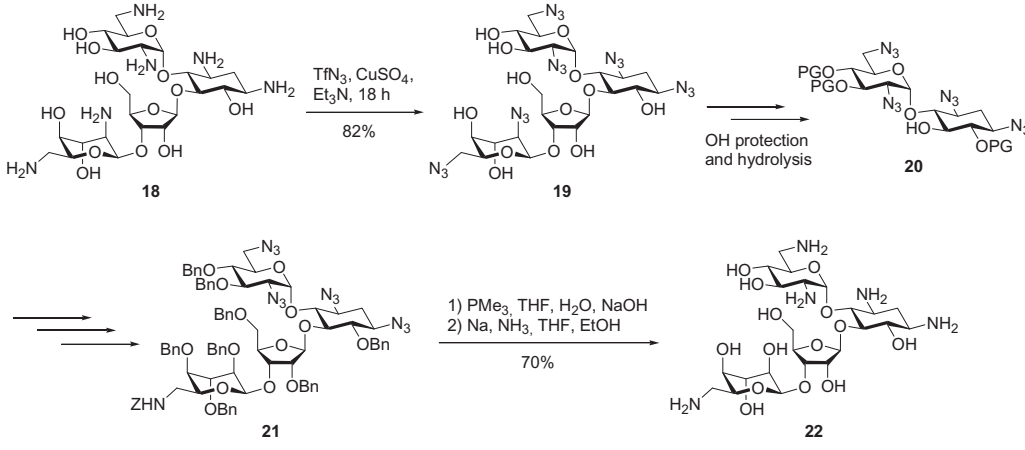
16.3 Azides as Protecting Groups during Aminoglycoside Synthesis

Protecting groups commonly employed for masking amino groups include alkyl carbamates such as benzyl-, *tert*-butyl-, and 9-fluorenylmethyl carbamates. If used for the

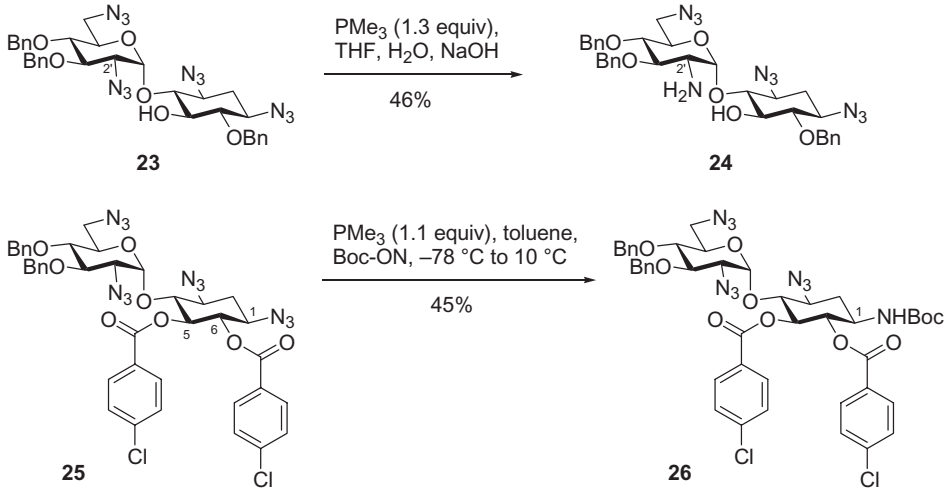
protection of molecules containing multiple amino groups, however, carbamate protecting groups can seriously complicate the interpretation of NMR spectra. This is due to the occurrence of *E/Z* rotamers that are in slow interconversion leading to multiple sets of signals. The use of azides as protecting groups circumvents this problem. Azides are easily reduced to amines, for example by catalytic hydrogenation or by reaction with thiols or complex hydrides.[4,33,34] A widely applied method in carbohydrate chemistry is the Staudinger reduction using triaryl- or trialkylphosphines.[35] This mild procedure enables the selective reduction of azides in the presence of esters and benzyl ethers which are frequently used as OH-protecting groups. Furthermore, azides can be directly converted into carbamate-protected amines using a variant of the Staudinger reaction (cf. Section 16.5).[27,36,37]

Aminoglycosides are highly potent, broad-spectrum antibiotics, containing several amino groups presented on an oligosaccharide-like core.[38–40] Due to the appearance of bacterial strains resistant to these drugs and due to their relatively high toxicity, the synthesis of aminoglycoside derivatives with improved properties is of great interest.[41] Several syntheses of aminoglycoside derivatives using azides as amine protecting groups were reported,[42,43] for instance the preparation of analogs of neomycin B as shown in Scheme 16.7.[27,44,45] Starting from commercially available neomycin B (**18**), all six amino groups were converted into azides by diazo transfer. After chemical derivatization of the structure, amines were regenerated by Staudinger reduction.

In the course of these studies, it was observed that the regioselective reduction of a single azide of multiple azide-containing molecules is feasible if only one equivalent of phosphine is used.[27] Reduction of neamine derivative **23**, for example, gave mono-amine **24** in a yield of 46 % (Scheme 16.8). Strong evidence was presented that the selectivity is primarily determined by electronic factors with electron-deficient azides being reduced more rapidly and efficiently than electron-rich azides. In compound **23** this is the case for



Scheme 16.7 *Synthesis of aminoglycoside derivative* ***22*** *using azides as protecting groups for amines. First, the amino groups of* ***18*** *were converted into azides by diazo transfer.*[27] *After chemical remodeling of the aminoglycoside (one amino group was replaced by a hydroxy group), the amines were regenerated by Staudinger reduction*[44]

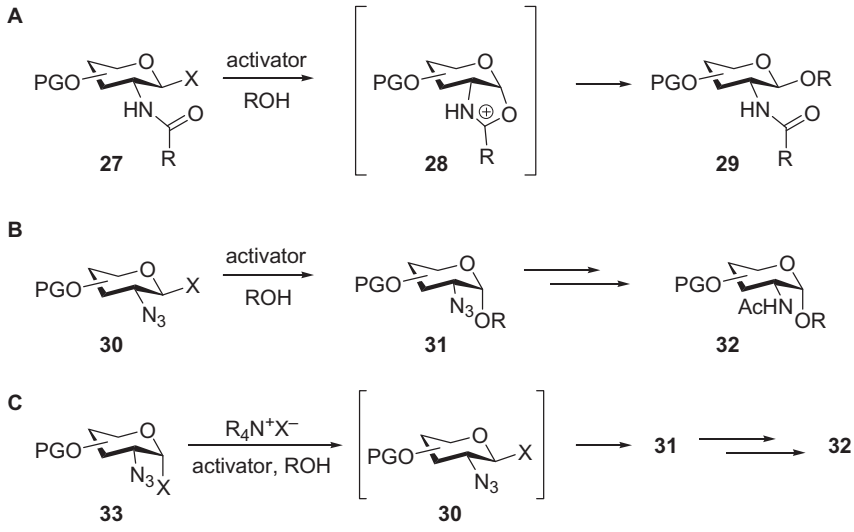


Scheme 16.8 *Regioselective reduction of tetra-azides* ***23***[27] *and* ***25***.[46,47] *Boc-ON = 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile*

the 2′-azide adjacent to the anomeric center. It was shown that the regioselectivity can be predicted on the basis of ^{15}N and, to some extent, ^{1}H NMR chemical shifts. Consequently, by introduction of electron withdrawing 4-chlorobenzoyl protecting groups in the 5- and 6-position, the selectivity can be tuned in favor for reduction of the 1-azide (**25** → **26**).[46,47]

16.4 Azides as Non-Participating Neighboring Groups in Glycosylations

Although 1,2-*cis* glycosides of 2-amino-2-deoxysugars are less frequently found in natural products compared to their 1,2-*trans* isomers, they are a common motive in important structures. In mucin-type *O*-glycoproteins, e.g. the glycan chains are attached to protein via an α-glycosidic linkage of *N*-acetyl-D-galactosamine to the β-hydroxy group of either serine or threonine, and α-glycosides of 2-acetamido-2-deoxy-D-glucose are found in the glycosaminoglycan heparan sulphate.[48–51] For the preparation of these 1,2-*cis* glycosides, the commonly employed *N*-acyl protecting groups are not suited because they lead to 1,2-*trans* products **29** via neighboring group participation (Scheme 16.9A).[15,52,53] In 1978 Paulsen *et al.* showed that 2-azido-2-deoxy-glycosyl halides are suitable donors in 1,2-*cis* glycosylations. This approach preferentially leads to α-glycosides **32** either directly from β-glycosyl halides **30** (Scheme 16.9B)[54,55] or by *in situ* anomerization[56] of α-glycosyl halides **33** (Scheme 16.9C).[57] Since then, the azide method has been widely used[15,52,58–62] and expanded by use of other glycosyl donors, such as trichloroacetimidates,[16] *n*-pentenyl glycosides,[17] and thioglycosides[18–20] just to name a few. The required 2-azido-2-deoxy sugars are usually prepared by azidonitration of glycals or by diazo transfer reaction of the corresponding glycosamines as described above. After glycosylation, the azide can



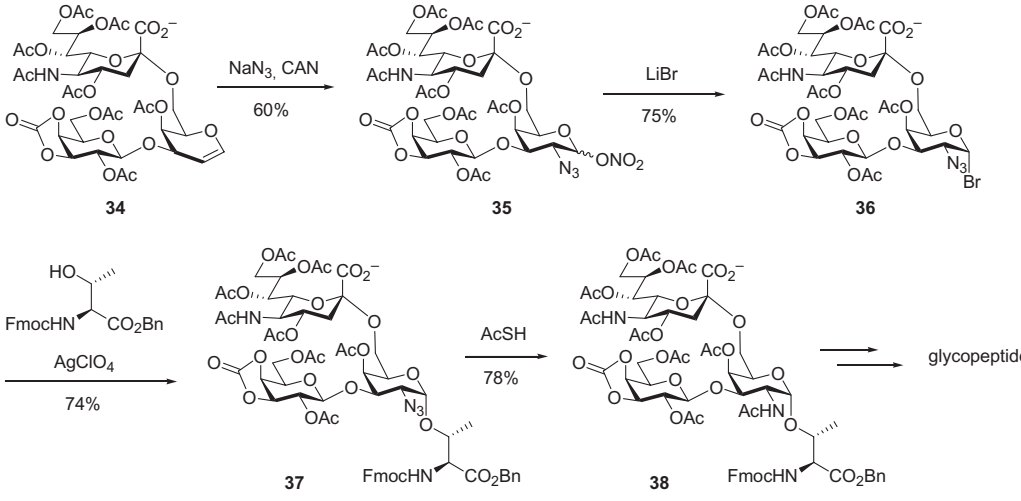
Scheme 16.9 *Preparation of O-glycosides of 2-amino-2-deoxysugars. (A) Use of N-acyl-protected donors* ***27*** *results in 1,2-trans glycosylation due to neighboring group participation. (B) 1,2-cis glycosylation products* ***32*** *from β-glycosyl halides* ***30***[54,55] *or (C) by in situ anomerization*[56] *of α-glycosyl halides* ***33***[57]

be transformed to the natural acetamido function either in two steps by reduction of the azide and subsequent acetylation or in one step by reductive acetylation using thioacetic acid.[63,64]

A successful approach for the synthesis of *O*-glycopeptides is the assembly of preformed, more or less complex glycosyl amino acid building blocks by solid phase peptide synthesis (SPPS).[60–62,65–67] Based on initial work of Ferrari[68] and Paulsen,[69] the azide method is extensively used for the preparation of such glycosyl amino acid building blocks. Especially the synthesis of complex glycosyl amino acids is challenging. Usually, glycosylation is performed with monosaccharides followed by attachment of further sugar residues because glycosylation reactions with oligosaccharide donors and serine or threonine acceptors often proceed with unpredictable stereochemistry. Nevertheless, oligosaccharides have been successfully used in many glycosylations as illustrated by the synthesis of glycosyl threonine building block **38** reported by Danishefsky and coworkers (Scheme 16.10).[64]

16.5 Glycosyl Azides as Precursors for Glycosyl Amides

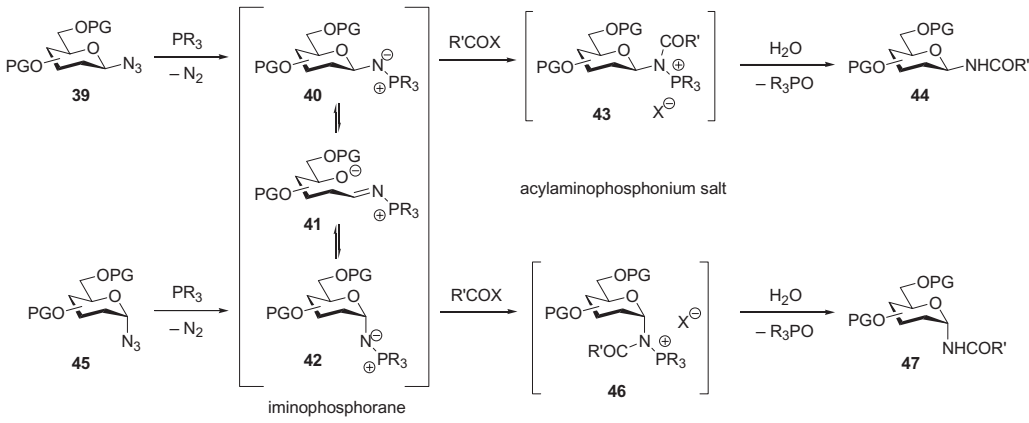
Beside the *O*-linked glycoproteins, the more prevalent form of glycosylation of proteins is *N*-linked glycosylation.[48,70,71] *N*-Glycoproteins are characterized by a *β*-*N*-glycosidic linkage of the terminal *N*-acetylglucosamine of the pentasaccharide core structure to the amide nitrogen of asparagine. The conventional synthetic strategy for the preparation of such glycosyl amides starts from glycosyl amines which are reacted with activated and



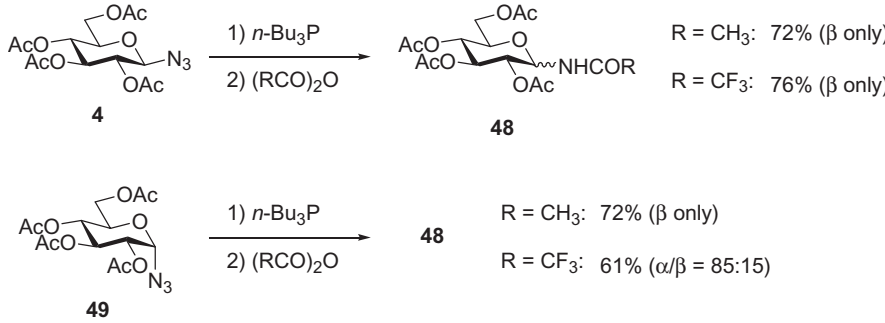
Scheme 16.10 *Synthesis of glycosyl threonine building block **38** using the azide method.*[64] *The 2-azido group is introduced by azidonitration of **34** followed by preparation of donor **36**. Glycosylation using threonine as acceptor leads to 1,2-cis glycoside **37**. After conversion of the azide group to an N-acetyl group by reductive acetylation, **38** was used as building block in glycopeptide synthesis*

suitably protected aspartic acid derivatives to form the amide linkage.[60–62,65–67] Glycosyl amines are commonly prepared either by reduction of glycosyl azides (cf. Section 16.3) or by amination of unprotected reducing sugars with saturated aqueous ammonium bicarbonate.[72] Recently, improved variants of the latter procedure employing microwave irradiation[73,74] and ammonium carbamate,[75,76] respectively, have been published. Drawbacks of this method are the instability of glycosyl amines and their propensity for dimerization and anomerization. Also, the preparation of α-glycosyl amides is a synthetic challenge.

While the classical Staudinger reaction[35] leads to iminophosphoranes which can be hydrolyzed to amines under aqueous conditions (Staudinger reduction, cf. Section 16.3), the addition of acyl donors under dry conditions results in amide formation.[77,78] This procedure was repeatedly applied for the synthesis of glycosyl amides, thus circumventing the preparation of glycosyl amines. Initially, three-component reactions employing glycosyl azide, activated carboxyl derivative and phosphine were reported (Scheme 16.11). The reaction starts from the β- (**39**) or α-glycoside **45** with the formation of an iminophosphorane (**40** and **42**, respectively), which is then trapped by an acylating agent in the second step. The resulting acylaminophosphonium salt (**43/46**) yields the corresponding glycosyl amide (**44/47**) upon hydrolysis. The intermediate iminophosphorane can undergo anomerization via open-chain form **41** preferring β-configuration. The degree of isomerization is dependent on the efficiency of iminophosphorane trapping by the acylating agent. Differently activated carboxylic acids, such as carboxylic halides,[79,80] anhydrides,[80,81] and carbodiimide-activated acids,[82,83] have been employed as acylating agents. While β-glycosyl amides **44** can be obtained easily from β-glycosyl azides **39**, the stereospecific



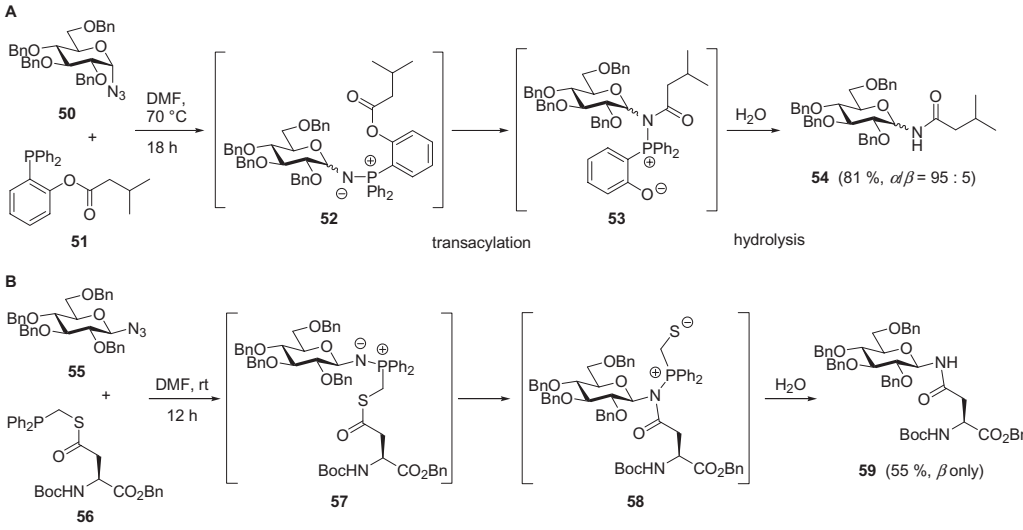
Scheme 16.11 *Mechanism of the Staudinger reaction with glycosyl azides*



Scheme 16.12 *Three-component Staudinger-type reaction with β-glycosyl azide* ***4*** *stereoselectively leads to the β-glycosyl amides* ***48****. α-Glycosyl amides can only be obtained from α-glycosyl azide* ***49*** *with strong acylating agents to prevent complete anomerization of the intermediate iminophosphorane*[80]

synthesis of α-glycosyl amides **47** starting from α-glycosyl azides **45** is only possible with strong acylating agents which trap the intermediate iminophosphorane **42** before anomerization can take place.[80] Representative examples for the three-component Staudinger reaction are shown in Scheme 16.12. Rarely, the Staudinger reaction with reactive alkylphosphines and free carboxylic acids has been reported.[84,85] In this case, amide-bond formation is assumed to proceed in a concerted reaction without generation of an iminophosphorane intermediate.

Recently, the synthesis of glycosyl amides has also been achieved employing the traceless two-component Staudinger ligation[9,86,87] developed in the laboratories of Bertozzi[88] and Raines[89,90] (Scheme 16.13). Starting from glycosyl azides **50** and **55**, respectively, the initially formed iminophosphorane **52/57** reacts with an intramolecular (thio)ester group to form the acylaminophosphonium salt **53/58** from which the phosphine moiety is removed by hydrolysis with water. Using benzyl protected α-glycosyl azides such as **50**



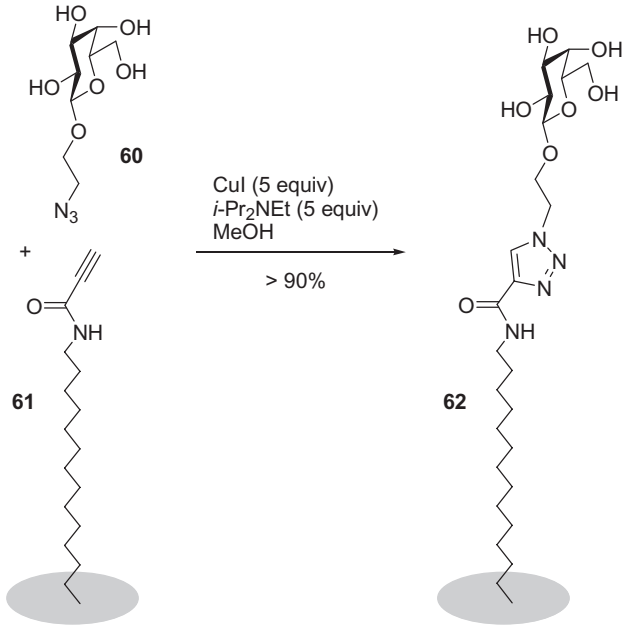
Scheme 16.13 *Two-component traceless Staudinger ligations using phosphine-derivatized ester* ***51*** *(A)*[9] *or thioester* ***56*** *(B)*[87]

and stable phosphine **51** or similar esters in polar aprotic solvents such as DMF, the reaction proceeded stereo conservatively to yield predominantly α-glycosyl amides (Scheme 16.13A).[9] The use of acetyl protected α-glycosyl azides, however, resulted only in β-glycosyl amides due to isomerization of the less reactive iminophosphorane.

All methods described above have been used for the preparation of the β-glycosyl amide linkage between *N*-acetylglucosamine and the side chain of asparagine in both three-component reactions using free[84,85] or activated[82,83] carboxylic acids and two-component reactions as shown in Scheme 16.13.[9,87] The obtained protected glycosyl amino acids can be used as building blocks in SPPS of *N*-linked glycopeptides.[91,92] It was also shown that deprotected sugars can be attached to amino acids and whole peptides using the three-component reaction.[92] Beside Staudinger-type reactions, another route towards the synthesis of glycosyl amides is the reaction of glycosyl azides with thiocarboxylic acids.[93]

16.6 Synthesis of Glycoconjugates via Azide-Alkyne [3+2] Cycloaddition

Although the azide-alkyne [3+2] cycloaddition[94] (cf. Chapter 9) is known in carbohydrate chemistry for more than 50 years,[95] its application for the preparation of glycoconjugates became particularly attractive with the development of the copper(I)-catalyzed variant by Meldal[96] and Sharpless.[97] The copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)[98,99] enables the regioselective formation of 1,4-disubstituted 1,2,3-triazoles under very mild conditions even in a biological context. However, the cellular toxicity of the copper catalyst precludes applications wherein cells must remain viable. Therefore, as an alternative



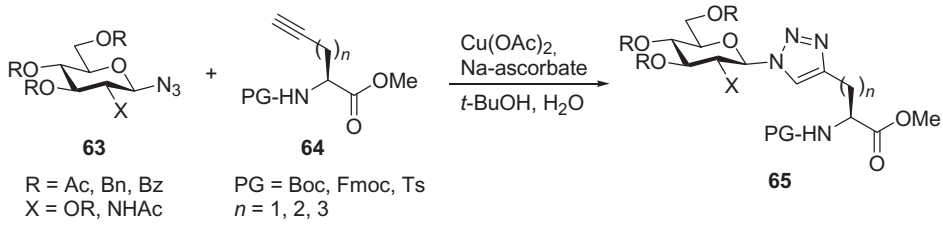
Scheme 16.14 *Coupling of azide-substituted galactoside* ***60*** *to alkyne-modified* C_{14} *hydrocarbon* ***61*** *noncovalently bound to the microtiter well surface*[108]

to CuAAC, strain-promoted azide-alkyne [3+2] cycloadditions have been developed that proceed at room temperature without the need for a catalyst.[100,101] These reactions are discussed in the next section dealing with metabolic oligosaccharide engineering. Another example of metal-free triazole formation by a tandem [3+2] cycloaddition-retro-Diels-Alder reaction has been developed by van Berkel *et al.* although no carbohydrate-related application was reported.[102]

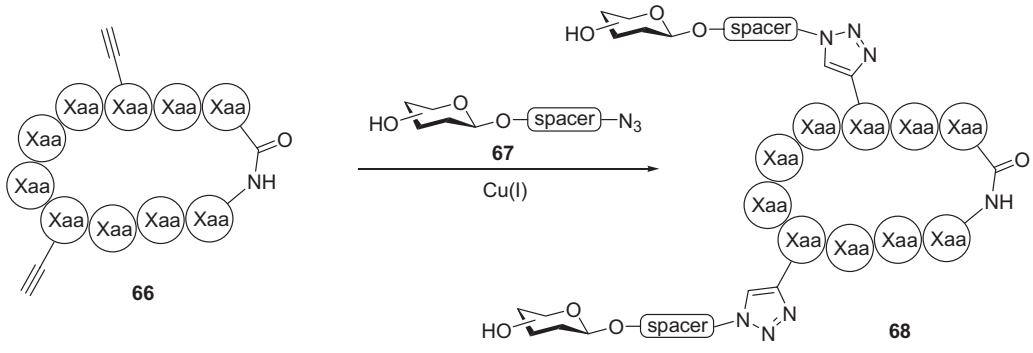
CuAAC reactions have been extensively applied in carbohydrate chemistry including the synthesis of simple glycoside and oligosaccharide mimetics, glyco-macrocycles, glycoconjugates, glycoclusters, and for the attachment of carbohydrates to surfaces. The field has been thoroughly reviewed[98,103–107] and, therefore, we will focus on a few selected examples which are of special interest for glycobiology.

One of the first applications of CuAAC in carbohydrate chemistry was – beside the one in the seminal paper of Meldal[96] – the immobilization of azide-substituted sugars on microtiter plates (Scheme 16.14).[108] The surface-bound sugars such as **62** were screened with various lectins and could be elongated by glycosyltransferase-catalyzed fucosylation. The technique was later on improved by incorporation of a cleavable disulfide bond in the linker allowing mass spectrometric characterization of the carbohydrate array.[109]

Neoglycopeptides and -proteins[110] differ from naturally occurring structures by replacement of the natural carbohydrate-peptide linkage with a non-natural one. This not only allows studying the influence of distinct structural elements on biological activity, but has many practical applications as well. Use of chemoselective ligation reactions such as



Scheme 16.15 *Application of CuAAC for the preparation of triazole-linked glycosyl amino acids* ***65***[111]



Scheme 16.16 *Preparation of tyrocidine derivatives* ***68*** *by CuAAC of propargylglycine-containing cyclic peptides* ***66*** *and azido-functionalized sugars* ***67***[113]

CuAAC makes glycoconjugates accessible to a broader community. Furthermore, the non-natural linkage often is more stable (both chemically and with respect to enzymatic degradation) which can lead to an increased half life of a glycoconjugate within a biological system. Scheme 16.15 depicts the synthesis of triazole-linked glycosyl amino acids **65** starting from glycosyl azides **63** and different alkyne-containing amino acids **64** which can be used as building blocks in peptide synthesis.[111,112]

Lin and Walsh applied CuAAC for the attachment of 21 different azido-functionalized monosaccharides **67** to 13 derivatives **66** of the cyclic decapeptide tyrocidine containing one to three propargylglycine residues at positions 3–8 (Scheme 16.16).[113] Head-to-tail cyclization of the peptides was accomplished using a thioesterase domain from tyrocidine synthetase. Antibacterial and hemolytic assays showed that the two best glycopeptide mimetics had a 6-fold better therapeutic index than the natural tyrocidine. CuAAC has further been used to attach carbohydrates to whole virus particles[114,115] and DNA.[116]

More challenging is the modification of bacterially expressed proteins by site-specific attachment of carbohydrates. Crucial step is the introduction of a chemical tag, which can be chemoselectively modified, into the protein. It has been shown that alkyne- and azido-modified amino acids, such as 2-amino-5-hexynoic acid (homopropargylglycine, Hpg),[117] 4-azidohomoalanine (Aha),[118,119] and with less efficiency also 3-azidoalanine, 5-azidonorvaline, and 6-azidonorleucine,[120] act as methionine surrogates that are acti-

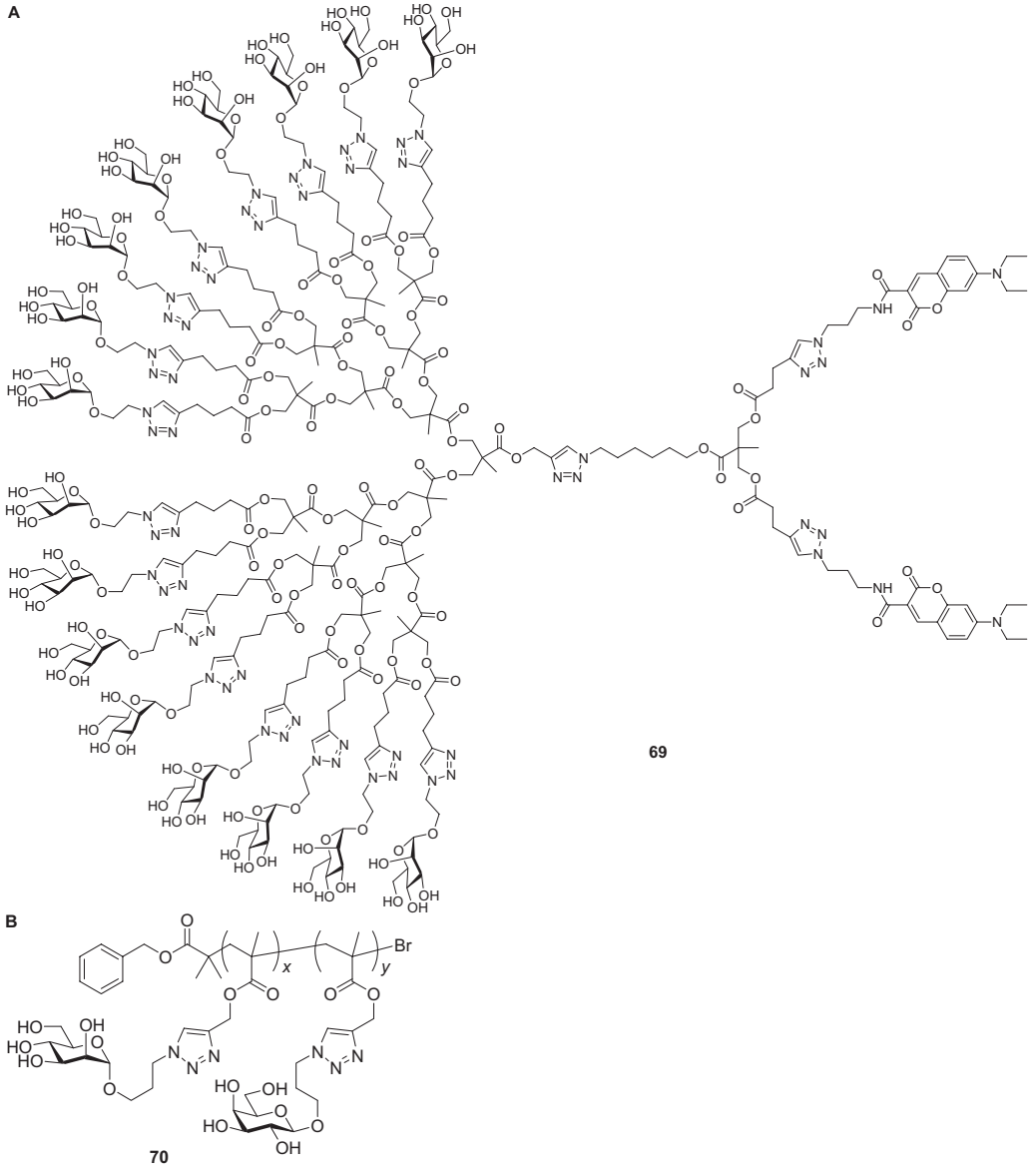
vated by the methionyl-tRNA synthetase of *E. coli* and replace methionine in proteins expressed in methionine-depleted bacterial cultures. This, together with other methods for the incorporation of non-canonical amino acids into proteins,[121–123] offers the possibility to use azide-alkyne cycloaddition (and also Staudinger ligation[124–126]) not only for protein labeling within cells or on cell surfaces[119,120] but also for the preparation of neoglycoproteins.

Davis and coworkers expanded the diversity of chemical protein modification by a combination of this CuAAC-based labeling and disulfide bond formation via genetically engineered cysteine (Cys) residues.[127] Aha and Hpg, respectively, were introduced into engineered proteins by the auxotrophy-based residue-specific method. Subsequent CuAAC reactions with alkyne- and azide-substituted carbohydrates, respectively, resulted in homogeneous protein glycoconjugates. As second modification reaction, conjugation of Cys residues with substituted methanethiosulfonates was chosen. Applying these two orthogonal protein modification reactions, derivatives of the LacZ reporter enzyme carrying the tetrasaccharide sialyl Lewis X and a sulfotyrosine mimic were created that allowed detection of mammalian brain inflammation.

Recently, Merkel *et al.* reported efficient N-terminal glycoconjugation of proteins by the N-end rule.[128] Bulky amino acids at the second and third sequence position of the barnase inhibitor barstar efficiently prevent excision of N-terminal methionine analogue Aha introduced by the auxotrophy-based residue-specific method. The created azide tag at the protein's N-terminus was subsequently conjugated to propargyl glycosides of *N*-acetylglucosamine and *N*,*N*′-diacetylchitobiose, respectively, by CuAAC. The obtained glycoprotein mimetics show binding affinity to the lectin wheat germ agglutinin whereas the natural activity of barstar is conserved.

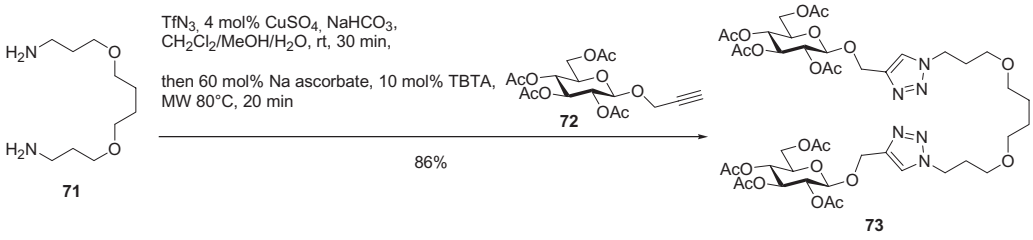
Lectins are carbohydrate-binding proteins other than immunoglobulins without enzymatic activity towards the recognized sugars.[129–131] Carbohydrate-lectin interactions are involved in numerous intra- and intercellular events during development, inflammation, immune response and cancer metastasis.[132–136] Multivalency appears to play an important role in lectin-mediated interactions,[137–140] and many lectins are found to recognize individual carbohydrate epitopes only with low affinity. Preparation of carbohydrate clusters, therefore, is a common strategy to obtain high-affinity lectin ligands.[141–144] Because of its robustness, CuAAC is excellently suited for the simultaneous attachment of several carbohydrate epitopes to a scaffold. Initially, Santoyo-Gonzáles and coworkers prepared different multivalent mannose clusters starting from propargyl mannosides and azide-containing scaffolds.[145] This strategy as well as the opposite approach based on azide-containing carbohydrates and alkyne-bearing scaffolds have been used intensively for the preparation of glycoclusters.[98,103–107] Glycosyl azides are easily produced, however, the direct attachment of a triazole to the sugar may interfere with the recognition of the carbohydrate by the protein and, therefore, linkers of varying length have been introduced between the sugar and the triazole moiety. It would be far beyond the scope of this chapter to mention all applications. Exemplarily, the asymmetrical, bifunctional dendrimer **69** containing 16 mannose units and two coumarin chromophores[146] and poly(methacrylate)-based glycopolymer **70**[147] are depicted in Scheme 16.17.

Although organic azides are stable against most reaction conditions, compounds containing multiple azide residues (like multivalent scaffolds) are potentially explosive. Therefore, several one-pot procedures to generate azides *in situ* followed by CuAAC have



Scheme 16.17 *(A) Asymmetrical, bifunctional dendrimer* ***69*** *containing 16 mannose units and two coumarin chromophores*[146] *and (B) poly(methacrylate)-based glycopolymer* ***70***[147] *prepared by CuAAC and used for lectin binding studies with concanavalin A*

been reported.[148–154] While the azides in most of these procedures are introduced by a nucleophilic substitution of a leaving group in allyl, benzyl, glycosyl, or similar position,[148–152] aliphatic[154] and aromatic[153] amino groups may also serve as precursors. We reported, for example a one-pot procedure for diazo transfer and subsequent CuAAC which allows the preparation of multivalent structures starting from commercially avail-



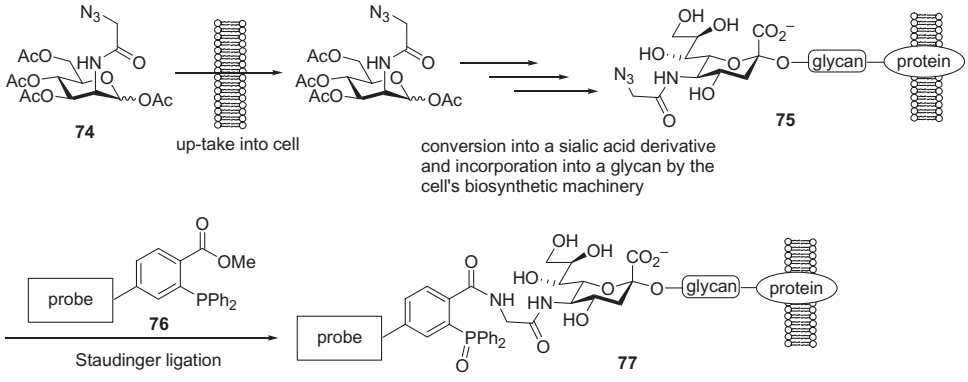
Scheme 16.18 *Sequential one-pot procedure for diazo transfer and CuAAC.*[154] *First, diamine* ***71*** *is transformed to the corresponding diazide by Cu(II)-catalyzed diazo transfer. After completion, Cu(I) required for subsequent CuAAC with* ***72*** *is generated by addition of reducing agent Na ascorbate. MW = microwave; TBTA = tris(benzyltriazolylmethyl)amine*

able amine scaffolds without need for isolation of the azide-containing intermediates.[154] As an example, divalent glycoconjugate **73** was synthesized from diamine **71** and propargyl glycoside **72** as shown in Scheme 16.18.

Azides can also undergo [3+2] cycloaddition reactions with nitriles giving access to 1,5-disubstituted tetrazoles. *Inter*molecular reactions, however, require electron deficient nitriles and very forcing conditions to occur with sufficiently high reaction rates.[155–157] High yields have been reported for the reaction of sulfonyl and acyl cyanides with unhindered aliphatic azides by neat, thermal fusion.[158,159] *Intra*molecular [3+2] cycloaddition reactions of organic azides to nitriles occur more readily.[160–164] Still, they require high reaction temperatures and yields are with few exceptions[165] not satisfactory. When precisely positioned on a rigid carbohydrate scaffold, however, azides can undergo cycloaddition reactions with nitriles under exceptionally mild conditions. Thus, 3-azido-1,2-*O*-cyanoethylidene-3-deoxy-allopyranose was shown to form a tetrazole embedded in a bridged tetracyclic ring system even at room temperature.[166]

16.7 Metabolic Oligosaccharide Engineering

Glycosylation of proteins is an important co- and posttranslational event that has been estimated to occur on more than 50% of eukaryotic proteins.[167] The glycan chains of cell-surface glycoproteins are involved in numerous recognition processes such as cell adhesion and attachment of bacteria or viruses. Inside cells, glycans direct protein trafficking and they modulate structure and activity of proteins.[132–136] Hence, in vivo monitoring of glycosylation processes is of utmost interest.[168] While fluorescent fusion proteins and other genetically encoded tags provide a means for labeling specific proteins in live cells, analogous techniques are not available for secondary gene products including glycans. Metabolic oligosaccharide engineering offers the possibility to introduce carbohydrates with unnatural structural elements into the glycans without genetic manipulation making use of the cell's biosynthetic machinery.[169] If suitable chemical reporter groups are introduced, subsequent addition of an exogenously delivered detectable probe allows for tagging of the glycans by a chemoselective ligation reaction. Examples for such reporter groups include ketones[170,171] and thiols.[172] However, the azido group is much more

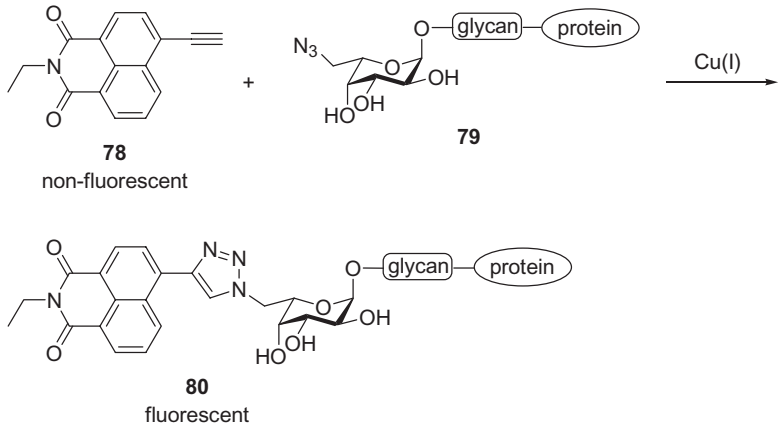


Scheme 16.19 *Metabolic oligosaccharide engineering: peracetylated ManNAz* ***74*** *is taken up by mammalian cells and converted into an azide-containing sialic acid derivative which is incorporated into sialic acid-bearing glycans* ***75****. In the next step, a detectable probe* ***76*** *can be attached to* ***75*** *via Staudinger ligation*[124,173]

suited for this approach because azides can take part in two important bioorthogonal ligation reactions, Staudinger ligation[124] (cf. Section 16.5) and azide-alkyne [3+2] cycloaddition (cf. Section 16.6 and Chapter 9).

Azide derivatization of monosaccharides represents a subtle structural change that is accepted by several metabolic pathways. Thus, azide derivatives of *N*-acetylmannosamine (i.e. *N*-(azidoacetyl)mannosamine, ManNAz), *N*-acetylgalactosamine (i.e. *N*-(azidoacetyl) galactosamine, GalNAz), *N*-acetylglucosamine (i.e. *N*-(azidoacetyl)glucosamine, GlcNAz), and L-fucose (i.e. 6-azido-L-fucose) have been explored.[168,169] Initially, ManNAz was employed to tag sialylated cell surface glycans of mammalian cells in vitro (Scheme 16.19).[124,173] Cells are grown in the presence of peracetylated ManNAz **74** which can be taken up by the cells more easily than ManNAz. After de-*O*-acetylation by cellular esterases, resulting ManNAz is metabolized similarly to its native counterpart *N*-acetylmannosamine and integrated into cellular glycans. Finally, the azide-labeled glycans are reacted with a detectable probe by Staudinger ligation. GalNAz can be metabolically introduced at the core position of mucin-type *O*-linked glycoproteins.[174] Thus, a selective labeling of mucin-type glycoproteins is possible. Both, the metabolic labeling of sialylated glycans with ManNAz[175] and labeling of mucin-type *O*-glycoproteins with GalNAz[176] can be carried out *in vivo*. Analogously, GlcNAz has been used for the labeling of *O*-GlcNAc glycosylated proteins.[177] Recently, cells were labeled simultaneously with an azide- and a ketone-containing sugar.[178] Using orthogonal ligation reactions, glycans bearing these sugar residues can be visualized in parallel on the same cells.

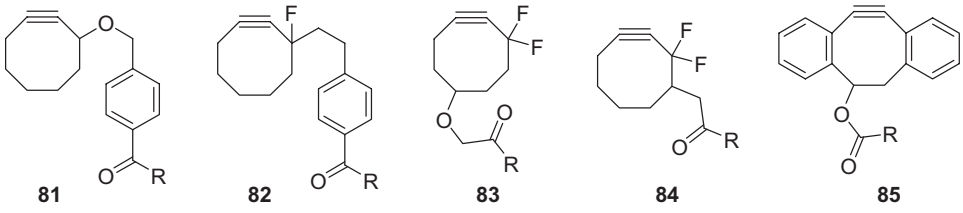
In the cases mentioned so far, fluorescence labeling has been achieved by a two-step procedure. First, a biotin label[124] or FLAG tag (octapeptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys)[173,174,177] is covalently attached to the azide-containing glycan by Staudinger ligation at high concentration. In a second step, a fluorescently labeled receptor (avidin and anti-FLAG antibody, respectively) is added at lower concentration. To avoid the problem of high background fluorescence caused by the application of fluorescent dyes,



Scheme 16.20 *Generation of fluorescent triazole* ***80*** *by CuAAC of fluorogenic ethynylnaphthalimide* ***78*** *and azide-labeled glycoproteins* ***79*** *applicable for intracellular localization of fucosylated glycoconjugates*[179]

Wong and coworkers developed a one-step labeling method based on CuAAC ligation using fluorogenic dyes (Scheme 16.20).[179] 6-Azido-L-fucose was applied for tagging of fucosylated proteins by metabolic oligosaccharide engineering. Reaction of alkyne-substituted naphthalimide **78** and azide-modified glycoprotein **79** results in formation of fluorescent triazole **80**. Since **78** is not fluorescent, it can be applied at high concentrations without producing a background signal. The method was used for cell surface glycoprotein analysis and intracellular localization of fucosylated glycoconjugates by using fluorescence microscopy.

Other examples for the application of CuAAC for labeling and visualization of glycoproteins in cells have been published by the research groups of Bertozzi[180] and Wong.[181] The main advantage of CuAAC over Staudinger ligation is its much faster reaction kinetics. However, the use of CuAAC for applications *in vivo* is limited due to the cellular toxicity of copper ions. This led to the development of copper-free variants of this cycloaddition. Based on observations made by Wittig who described the exothermic cycloaddition of cyclooctyne with phenyl azide,[182] Bertozzi and coworkers reported the copper-free, strain-promoted cycloaddition between azides and substituted cyclooctyne **81** for covalent modification of biomolecules in living systems (Scheme 16.21).[100] The reaction rates were lower than those of CuAAC but comparable to those of Staudinger ligation.[183] The validity of the approach was demonstrated by functionalization of modified Jurkat cells with a biotin derivative of **81**.[100] Reaction rates of the strain-promoted azide-alkyne cycloaddition could be dramatically improved by introduction of electron-withdrawing fluorine substituents in α position of the triple bond (Scheme 16.21, **82**–**84**) with the difluorinated cyclooctyne (DIFO) derivatives **83** and **84** possessing comparable kinetics to those of CuAAC.[183–185] Similar reaction rates were observed with dibenzocyclooctyne derivative **85**.[101] These reactions are not regioselective but proceed chemoselectively within minutes on live cells with no apparent toxicity.[101,184,185] Latest application of DIFO derivative **83** is the *in vivo* imaging of membrane-associated glycans in live



81 **82** **83** **84** **85**

Scheme 16.21 *Cyclooctyne derivatives for use in copper-free, strain-promoted azide-alkyne [3+2] cycloadditions designed by Bertozzi (**81**,[100] **82**,[183] **83**,[184] **84**[185]) and Boons (**85**[101]). The second-generation difluorinated derivative **84** is easier to synthesize than **83***

developing zebrafish.[186] Using two derivatives **83** with different fluorophores attached, it was possible to perform a spatiotemporal analysis of glycan expression and trafficking.

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Organic Azides: Syntheses and Applications Edited by Stefan Bräse and Klaus Banert

ISBN: 978-0-470-51998-1