Heterocycles in Biomolecules

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Peptide-Embedded Heterocycles by Mild Single and Multiple Aza-Wittig Ring Closures**

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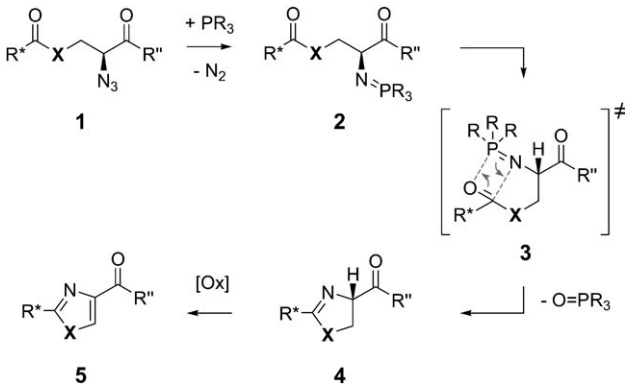
In a large number of bioactive natural products and microbial secondary metabolites, 1,3-azolines and 1,3-azoles as well as oligomers thereof are found as key structural features.[1] These heterocycles rigidify otherwise flexible backbones, contribute to lipophilicity, and their presence frequently correlates with DNA, RNA, and protein binding properties. Naturally occurring azolines and azoles are biosynthesized by dehydration and oxidation of Ser-, Thr-, or Cys-containing peptide precursors.[1b] For the chemical synthesis of 1,3-azolines and 1,3-azoles, a considerable spectrum of methodologies has been devised.[2,3] In an ideal synthetic approach, easily available building blocks should be assembled and transformed in a fashion that is tolerant towards the cornucopia of functional groups typical for nonribosomal peptide metabolites. Most current methodologies,[3] however, utilize strong dehydrating reagents, which limits their applicability to complex substrates. Furthermore, anhydrous reaction conditions that are not easily compatible with complex molecules are often required. A general, mild, chemoselective, and stereoretentive procedure would be very desirable.

We envisioned that the (conceptual) intramolecular dehydration might be the key to overcome limitations associated with current methods. A suitable azide[4] precursor **1** could be used to localize the cyclization site (Scheme 1). The azide **1** could easily be transformed into an iminophosphorane **2** (Staudinger reaction),[5] which could attack a proximal carbonyl in an "aza-Wittig" reaction. The putative transition state **3** would benefit both from a favorable *exo* geometry as well as the attractive antiparallel arrangement of the P=N and C=O dipoles. Driven by the extrusion of phosphine oxide, the condensation product **4** would be regioselectively formed. Further oxidation[6] could then provide the unsaturated heterocycles **5**.

Reactions of this type are well documented for aldehydes and ketones and are typically referred to as aza-Wittig reactions.[7] Less electrophilic esters and thioesters have been found to undergo aza-Wittig cyclizations in select cases.[8] On the other hand, the treatment of an azido peptide with phosphanes was reported to lead to fragmentation of the peptide chain,[9] and amide carbonyls tend to display low reactivity.[10]



Scheme 1. General mechanistic proposition for heterocycle synthesis by intramolecular aza-Wittig condensation(s); X = $(CR_2)_n$, S, O, NR, etc.

α-Azido acids would be the most general building blocks for the realization of aza-Wittig ring closures on peptides. Accordingly, α-azido derivatives of cysteine (**6**), serine (**7**), and threonine (**8**) were generated on a multigram scale through diazo transfer[11] and the introduction of suitable protecting groups.[12,13]

The thioesters **10**, which were easily obtained from azido thiol **6** and protected amino acids **9**, were initially investigated (Table 1). Gratifyingly, treatment of the thioesters **10** with phosphanes induced their clean cyclization to the thiazolines **11**. Of all the tested phosphanes (PPh_3, PBu_3, PMe_3, $P(OMe)_3$), PPh_3 gave by far the best results. Both aliphatic as well as aromatic side chains delivered the desired thiazoline in high yields as did the problematic Cys (**9e**) and the sterically encumbered substrates. The following oxidation[6d] proceeded cleanly to give the corresponding thiazoles **12**. Notably, the *ee* values of the final products remained high throughout the series of substrates. The magnitude of the *ee* values reflects the amount of epimerization during thioester formation. The *de* values of the respective thioesters **10** and the thiazolines **11** were identical, and a resubmission of the thiazoles **12** to the oxidation conditions did not lead to any reduction in the *ee* value.

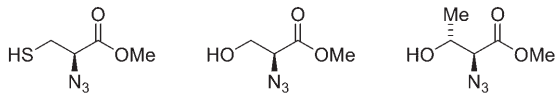


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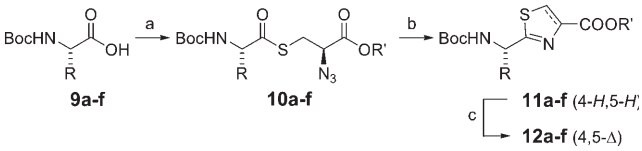
Table 1: Formation of thiazoles through thiazolines from amino acid thioester azides.[a]



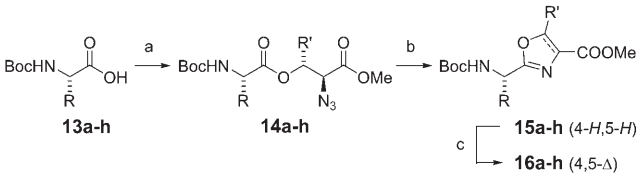
	Amino acid 9	R′	Yield [%][b] 10	11	12	*ee* [%]
a	Gly	Me	75	96	80	–
b	Ala	Me	76	98	94	94[c,d]
c	Phe	Me	74	82	90	94[c]
d	Val	Me	66[e]	78	87	95[c]
e	Cys(Tr)	Me	68	82	78	88[d]
f	Thr(*t*Bu)	Allyl	76[f]	98	96	>96[g]

[a] Conditions: a) diisopropylcarbodiimide (DIC), 1 mol% 4-*N*,*N*-dimethylaminopyridine (DMAP), CH_2Cl_2, 0→20°C, 0.5–8 h. b) PPh_3, THF, −20→20°C, 2–6 h. c) $BrCCl_3$, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), CH_2Cl_2, 0→20°C, 2–4 h. Boc = *tert*-butyloxycarbonyl, Tr = trityl. [b] Yield of isolated product. [c] Determined by ^{1}H NMR spectroscopy after derivatization. [d] Determined by chiral HPLC (Daicel AD). [e] DIC, 1 mol% DMAP, CH_2Cl_2, −20→20°C, 4 h. [f] 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), NEt_3, CH_2Cl_2, 0°C. [g] Determined to be diastereomerically pure by ^{1}H NMR spectroscopy.

Our attention then shifted towards the oxazoles, for which most known preparations necessitate harsh reaction conditions.[3,8] We were pleased to find that azido esters **14**, which are easily available from suitably protected amino acids **13** and the azido alcohols **7** and **8**, could be generally engaged in aza-Wittig transformations under mild conditions (Table 2). The rather acid-labile oxazolines **15** were obtained in good to excellent yields for a large variety of side-chain substituents. Exceptions are electron-withdrawing side chains and/or β-position branching side chains, both of which led to substantial retardation (Table 2, entries **g** and **h**). The oxidation to oxazoles **16** proceeded smoothly. The resulting products **16** were obtained with minimal loss of stereochemical integrity at the C2 exomethine carbon center, with the exception of Cys, for which we found that the epimerization during esterification was difficult to suppress.

The ease of this aza-Wittig reaction prompted us to apply this method to the synthesis of oligomeric azole motifs, which frequently occur in bioactive natural products.[1d] The (thio)-ester oligomers **17** were assembled from suitable building blocks in good yields (Table 3)[12] and could be converted into the desired bis-heterocycles upon exposure to PPh_3. In all cases investigated, the bis-azolines **18** were found as the exclusive products, which clearly indicates the preference for five-membered ring closure in all cases. However, the bis-thiazoline **18b** was found to be very prone to autoxidation (atmospheric O_2), whereas the thiazolino-oxazoline **18c** was isolated as a mixture of tautomers featuring a 4–2′ double bond. Final oxidation[6d] cleanly gave the bis-azoles **19** in all cases, but conversion of the bis-oxazoline **18a** was slow. Although the *ee* values of the final products were indicative of some incidental stereochemical erosion (especially **19b**), improvements are expected with further optimization of the esterification conditions. The proof of concept of these mild

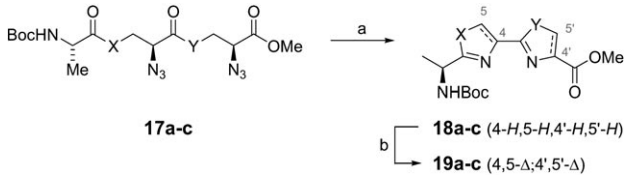
Table 2: Formation of oxazoles through oxazolines from amino acid ester azides.[a]



	Amino acid 13	R′	Yield [%][b] 14	15	16	*ee* [%]
a	Ala	H	99	84	62	89–98[c,d]
b	Ala	Me	86	78	79	>96[c]
c	Phe	H	88	78	78	95[c]
d	Cys(Tr)	H	87	97	62	23[d]
e	Glu(Cy)	H	99	65	75	>98[d]
f	His(Ts)	H	83	74	31	92[e]
g	Thr(Bn)	H	92	18	56	>96[c]
h	Val	H	95	18	43	94[d]

[a] Conditions: a) DIC, 5 mol% DMAP, CH_2Cl_2, 0→20°C, 2–16 h. b) PPh_3, THF, −20°C→40°C, 4–18 h. c) $BrCCl_3$, DBU, CH_2Cl_2, 0→20°C, 2–4 h. Cy = cyclohexyl, Ts = toluene-*p*-sulfonyl, Bn = benzyl. [b] Yield of isolated product. [c] Determined by ^{1}H NMR spectroscopy after derivatization. [d] Determined by chiral HPLC (Daicel AD). [e] Determined by HPLC after derivatization.

Table 3: Multiple aza-Wittig ring closures on amino acid bis-(thio)-esters.[a]

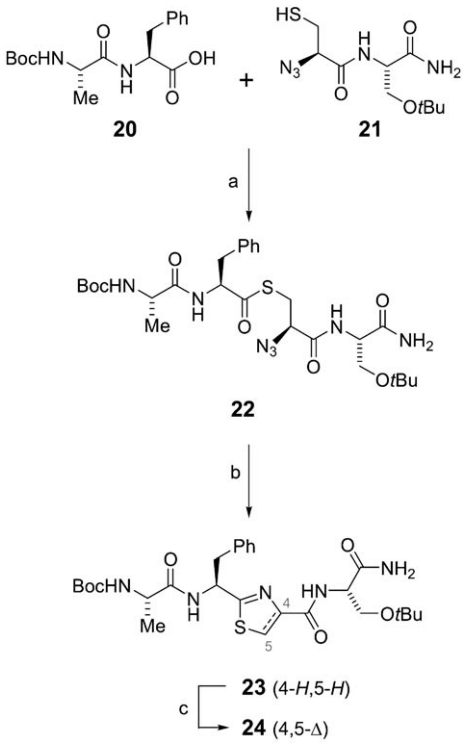


	X	Y	Yield [%][b] 17	18	19	*ee* [%]
a	O	O	64	83	30	82[c]
b	S	S	51	n.d.[d]	60[e]	60[f]
c	S	O	47	n.d.[d]	64[e]	>96[c]

[a] Conditions: a) PPh_3, THF, 0→20°C, 4–18 h. b) $BrCCl_3$, DBU, CH_2Cl_2, 0→20°C, 2–4 h. [b] Yield of isolated product. [c] Determined by ^{1}H NMR spectroscopy after derivatization. [d] n.d. = not determined. [e] Combined yield for two steps. [f] Determined by HPLC after derivatization.

and practical multiple aza-Wittig cyclizations nevertheless opens up a multitude of future applications.

To test if the reaction conditions of these aza-Wittig cyclizations are mild enough, the two dipeptides **20** and **21** were assembled from the respective amino acid building blocks[12] and bound together to give the stable peptide thioester **22** (Scheme 2). Despite the presence of acid-, base-, and dehydration-sensitive functionalities, compound **22** smoothly cyclized under the action of PPh_3 to deliver thiazoline **23** as the sole product. Most notably, no regioisomeric condensation products were observed, and the presence of aqueous solvent (25% v/v water) did not change the reaction outcome. Products of premature hydrolysis of the putative iminophosphorane intermediate were not detected, nor was fragmentation of the thioester or any other major side products seen (<5% by HPLC). Oxidation of **23** then cleanly delivered the thiazole peptide **24**. This example demonstrates



Scheme 2. Thiazole synthesis on a peptidic oligomer. a) Dicyclohexylcarbodiimde (DCC), HOBt, CH_2Cl_2, 0 °C, 2 h; b) PPh_3, THF, 90%, or PPh_3, THF/H_2O 4:1, 69%; c) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C→RT.

the profound potential and applicability of the aza-Wittig cyclization on highly functionalized linear peptidic precursors.

In conclusion, we have shown that the aza-Wittig reaction can be employed on peptidic azidoesters and peptidic thioesters to yield oxazoles, thiazoles, and azole dimers in a clean, mild, and selective fashion. The outlined procedures were found to be compatible with a wide range of relevant functional groups and are capable of providing complex, multiheterocyclic structures directly from simpler, linear precursors. Both the building blocks as well as the transformations are amenable to iterative as well as multiple ring-closure strategies. Notably, this intramolecular aza-Wittig reaction does tolerate the presence of water. In light of these experimental findings, we anticipate that the aza-Wittig cyclization may become a broadly applicable tool for the synthesis of complex, bioactive molecules from peptidic as well as nonpeptidic origin.

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[13] Although we have not witnessed any single indication for instabilities of the azides reported in this work, we **strongly caution the use of appropriate protection measures** during handling, especially when heating and/or concentrating azides of low molecular weight. See also reference [4].

