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Solid-phase synthesis of peptide–heterocycle hybrids containing a tripeptide-derived 6,6-fused bicyclic subunit

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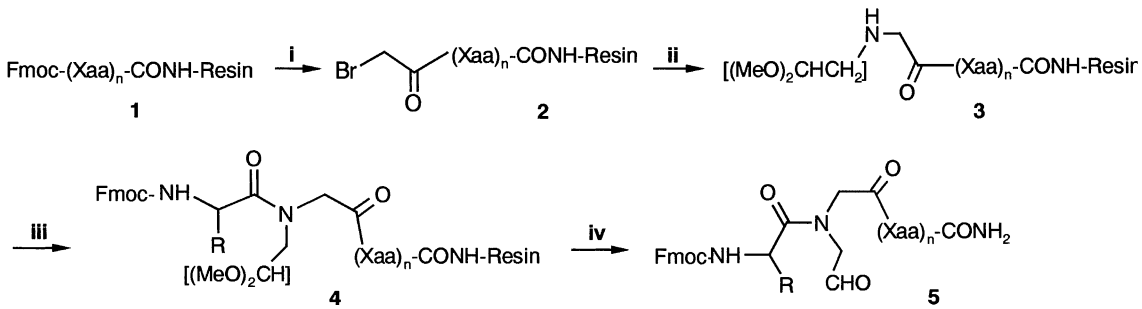
Abstract—The solid-phase synthesis of peptides incorporating a tripeptide-derived 6,6-fused bicyclic subunit is described. The bicyclic moiety (3,8,10-trisubstituted 2,9-dioxo-5-thia-1,8-diazabicyclo[4.4.0]decane) can be incorporated anywhere in a peptide sequence and is formed spontaneously upon TFA cleavage through condensation of an aldehyde with a backbone amide nitrogen and side-chain thiol, resulting in a thiazinone ring. The reaction is regio- and stereoselective and also allows for the incorporation of the bicyclic moiety, which may represent a constrained β-turn mimic, into a macrocyclic peptide. © 2001 Elsevier Science Ltd. All rights reserved.

The design and synthesis of conformationally constrained peptidomimetics for drug discovery has recently been an active field of research.[1] Often, these molecules are based on scaffolds containing one or more heterocyclic rings.[2] Amino acids and linear peptide precursors have proven to be particularly useful in the synthesis of both monocyclic and bicyclic heterocycle-containing peptidomimetics largely due to their well-defined protection/deprotection and activation chemistries.[3] A number of reports have shown the design and synthesis of constrained, dipeptide-derived, fused bicyclic scaffolds, particularly those incorporating lactams.[4] Several recent studies illustrated the synthesis of tripeptide-derived, fused bicyclic subunits through bicyclization of a latent aldehyde precursor, which occurs spontaneously upon acid-catalyzed cleavage from solid support.[3a–d] We report here a related strategy in which a tripeptide-derived 6,6-fused bicyclic moiety (3,8,10-trisubstituted 2,9-dioxo-5-thia-1,8-diazabicyclo[4.4.0]decane) can be incorporated anywhere in a peptide sequence via the use of a novel peptoid-like, aldehyde-containing subunit. The bicyclic structure has also been incorporated in macrocyclic molecules.

The key to our synthetic approach is the incorporation of an acetal-containing side chain, which yields the corresponding aldehyde upon TFA treatment (Scheme 1). The acetal-containing side chain is readily introduced via a two-step procedure, which was previously exploited for synthesis of polypeptoids (oligomers of



Scheme 1. Incorporation of aldehyde-containing 'peptoid' residue. *Reagents*: (i) (a) 20% piperidine/DMF, (b) $BrCH_2CO_2H$/DIC 2:1 in DMF; (ii) $NH_2CH_2CH(OMe)_2$, DIEA, DMF; (iii) Fmoc-AA-OH, PyAOP, DIEA, DMF; (iv) TFA:thioanisole:H_2O 90:5:5.

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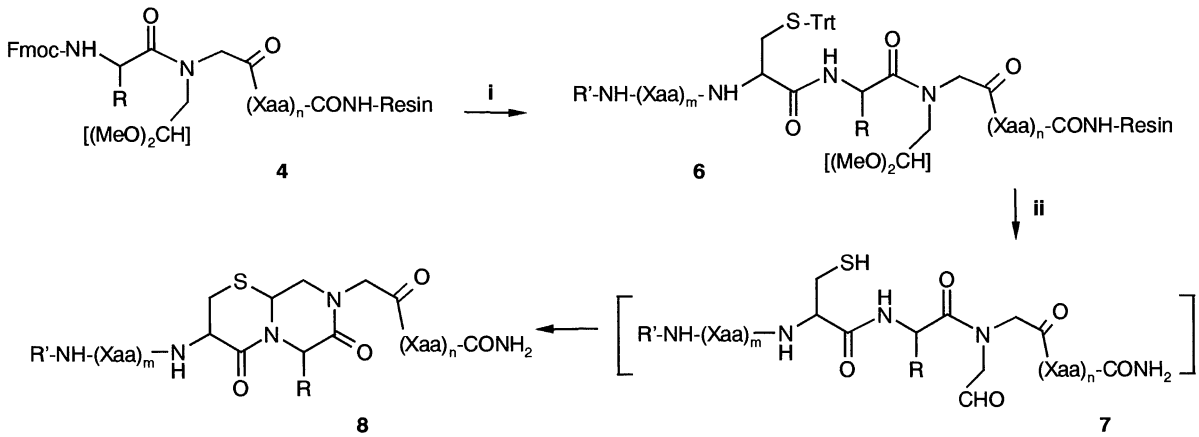
N-substituted glycines).[5] Acylation with bromoacetic anhydride is followed by an S_N2 reaction of aminoacetaldehyde dimethylacetal with the resin, resulting in a 'peptoid-like' residue. In contrast, other methods for incorporation of an aldehyde moiety to peptides have required more complex chemistry such as conversion of a side-chain acid to aldehyde prior to chain assembly[3c] or attachment via an acetal linker requiring a transacetalization step.[3a,b,d] The latter approach is further limited by the fact that the aldehyde group can be incorporated only at the C-terminus.

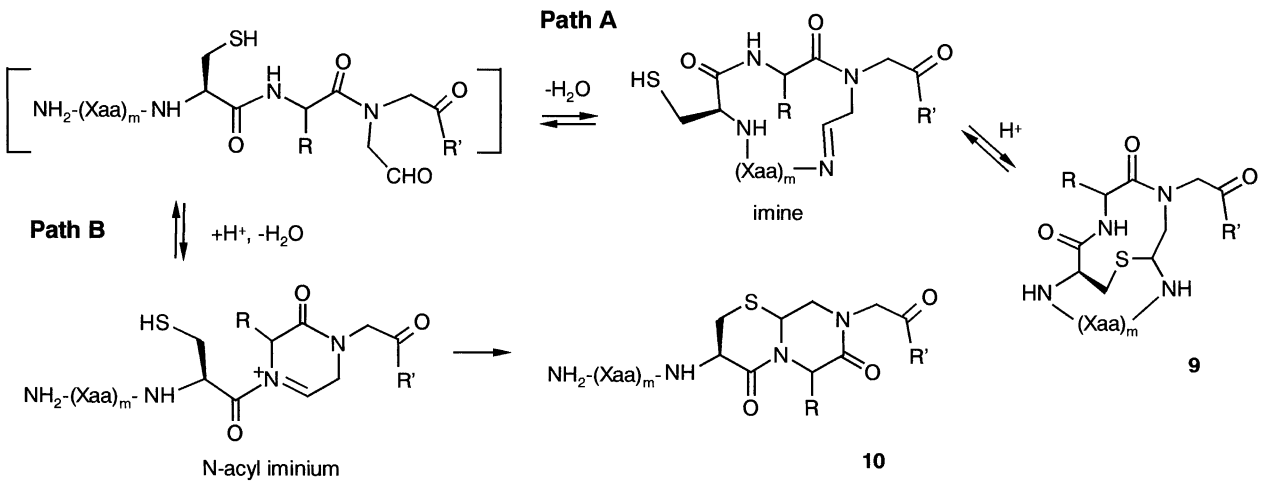
Syntheses of compounds containing the 6,6-fused bicyclic moiety (Scheme 2) involve elongation of **4** with cysteine and subsequent amino acid residues indicated by $(Xaa)_m$. Upon cleavage from the polymer support, the aldehyde containing intermediate **7** is generated and undergoes further reaction to the predicted final product **8** as indicated by a difference of 18 a.m.u. between the observed mass and the mass of **7** as well as a lack of free thiol in the final product as determined by use of Ellman's reagent.[6]

Theoretically, reaction of the aldehyde could follow two distinct paths. In one case, the aldehyde carbon condenses with the amino terminus to create an imine intermediate, which would undergo subsequent capture by the thiol group of Cys, resulting in a thiaza-macrocyclic structure (Path A in Scheme 3). Alternatively, the aldehyde could condense with the amide nitrogen one residue toward the amino terminus to form an *N*-acyliminium six-membered ring intermediate followed by nucleophilic attack from the thiol group (Path B in Scheme 3). Two experiments provided evidence against Path A. First, **6** yielded the same final product whether it was *N*-acetylated then cleaved or cleaved with a free amino terminus and acetylated subsequently. Further, treatment of **8a** with trypsin, causing cleavage of the peptide bond on the C-terminal side of Arg, led to two fragments, which should not occur if the N-terminus is involved in cyclization.

Diverse variants of **8** were synthesized in good purity (Table 1).[7] In all cases, only one peak with the correct molecular weight was observed by reversed-phase HPLC–MS, indicating that the bicyclization is highly stereoselective. A single diastereomer was also obtained in previous bicyclization studies[3a–d] and was experimentally determined in all cases to have a *trans* relationship



Scheme 2. Synthesis of tripeptide-derived, 6,6-fused bicyclic scaffold-containing compounds. *Reagents*: (i) (a) 20% piperidine/DMF, (b) Fmoc-Cys(trt)-OH, HOBt, DIC, DMF, (c) solid-phase peptide synthesis using Fmoc-amino acids and HOBt/DIC activation, (d) final Fmoc deprotection and acetylation for R′ = Ac; (ii) TFA:thioanisole:H_2O 90:5:5.



Scheme 3. Possible cyclization mechanisms involving aldehyde condensation.

Table 1. Examples of peptide–heterocycle hybrids that were synthesized

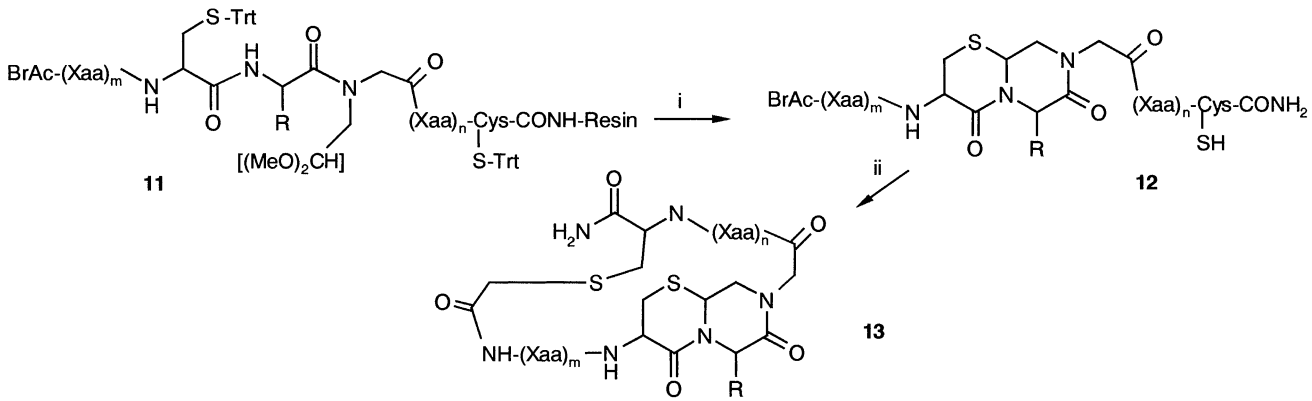
Cmpd	R′	R[a]	Nucl.[b]	$(Xaa)_m$	$(Xaa)_n$	Purity[c]	Cmpd[d]	$(Xaa)_m$	$(Xaa)_n$	Purity[c]
8a	H	Leu	Cys	W-R-Q	P-Q	75	**13a**	W-Q-R	P-F-L	71
8b	CH_3CO	Leu	Cys	W-R-Q	P-Q	75	**13b**	W-Q-R	P-L	62
8c	H	Leu	Cys	W-F-R	P-L	79	**13c**	W-Q-R	F-L	63
8d	H	Leu	Cys	W-R-Q	Q	82	**13d**	W-Q-R	dF-L	67
8e	H	Leu	Cys	W-R	L	81	**13e**	L-W-Q-R	P-F-L	72
8f	H	Leu	Cys	Q-L-Q	L	83	**13f**	L-W-Q-R	P-L	58
8g	H	Arg	Cys	W-L-E	P-Y	75	**13g**	L-W-Q-R	F-L	56
8h	H	Arg	Cys	W-L-F	P-S	75	**13h**	L-W-Q-R	dF-L	66
8i	CH_3CO	Asn	Cys	R-W	R	77	**13i**	W-R	P-F-L	75
8j	CH_3CO	Asn	dCys	R-W	R	82	**13j**	W-R	P-L	59
8k	CH_3CO	dAsn	Cys	R-W	R	75	**13k**	W-R	F-L	62
8l	CH_3CO	dAsn	dCys	R-W	R	73	**13l**	W-R	dF-L	68
8m	CH_3CO	Asn	Pen	R-W	R	76				
8n	CH_3CO	Asn	HoCys	R-W	R	77				

[a] R refers to the amino acid residue immediately adjacent (C-terminal side) to Cys or other nucleophilic residue.
[b] The residue present at the position occupied by Cys in Scheme 2.
[c] Determined by reversed-phase HPLC using UV detection at 220 nm.
[d] For **13a–l** the R group is $-CH_2CO_2H$ (Asp side chain).

between the hydrogen at the bicyclic junction and the α-hydrogen of the amino acid corresponding to R in **8** (Scheme 2). The stereochemistry at the R-substituted position in the *N*-acyliminium intermediate (Scheme 3) may direct attack of the nucleophile from the opposite side of the ring. All four combinations of Cys/dCys at the nucleophilic amino acid position and Asn/dAsn at the R position (**8i–l**) resulted in a single peak and very close retention times on reversed-phase HPLC, suggesting similar overall structures, regardless of the stereochemistry at different positions in the bicyclic ring system. Two additional side chains, penicillamine (Pen; $-C(CH_3)_2$-SH) in **8m** and homocysteine (HoCys; $-CH_2CH_2$-SH) in **8n** were substituted successfully for Cys at the nucleophilic amino acid position. In **8n** the resulting product has a 6,7-bicyclic structure due to the extra methylene group in the side chain of HoCys. Overall yields, based on the claimed substitution level of the Fmoc-resin, were in the range 40–50%.

Limitations of the method were identified upon attempts to alter the positioning of the Cys residue. Increasing the number of intervening residues between Cys and the aldehyde-containing peptoid residue from one to two or three dramatically diminished the purity to less than 10%. Formation of a bicyclic system via placement of Cys two residues C-terminal to the aldehyde-containing peptoid residue (potentially leading to a 6,8-bicyclic moiety) was also unsuccessful. In contrast, Johannsson et al.[3c] observed formation of a 5,8-bicyclic structure upon placement of Cys two residues C-terminal to an aldehyde derivative of Asp.

Impurities were primarily side products generated during the treatment with TFA solution, during which cleavage, side-chain deprotection, and heterocycle formation occur concomitantly. These impurities almost always eluted on reversed-phase HPLC several minutes after the desired product, and displayed higher molecular weights than the desired product. Generally, cleaved protecting groups, such as trityl, are problematic due to reattachment (particularly to Cys) and other side reactions unless strong scavenging agents are included. 1,2-Ethanedithiol (EDT) is an effective scavenger but reacted quantitatively with the aldehyde-containing peptides, likely due to the formation of a dithioacetal



Scheme 4. Synthesis of macrocyclic compounds containing a tripeptide-derived, 6,6-fused bicyclic moiety. *Reagents*: (i) TFA:thioanisole:H_2O 90:5:5, 1 h, followed by TFA:thioanisole:H_2O:TIPS 88.2:4.9:4.9:2, 0.5 h; (ii) 50 mM NH_4HCO_3 in H_2O/CH_3CN 50:50.

adduct (a highly favored five-membered ring formation). Cleavage in the presence of EDT was useful for assessing the purity prior to cyclization (efficiency of chain assembly), which in all cases was >95%. Triisopropylsilane (TIPS) is also an effective scavenger but was found to partially reduce the aldehyde to an alcohol during cleavage. Thus, cleavage had to be carried out with less effective scavengers and required the formation of the heterocyclic ring system to be highly favorable to overcome side reactions.

The methodology was expanded to the synthesis of macrocyclic peptide–heterocycle hybrids (Scheme 4), which involved incorporation of a second Cys residue at the C-terminus of the linear precursor and acylation of the amino terminus with BrAcOH. Upon TFA treatment the bicyclic heterocycle structure **12** is formed. Dissolution in pH 8 aqueous solution resulted in reaction of the C-terminal Cys thiol and the N-terminal bromoacetyl moiety to form a thioether bond. Macrocyclic peptides with varying ring sizes (**13a**–**l**) were synthesized in good purity (Table 1).[7] Tuning of the cleavage conditions was critical in order to obtain the macrocyclic products. Cleavage with the conditions used for the linear product (**8**) led to a derivatized C-terminal Cys side chain due to improper scavenging. Thus, the TFA reaction was carried out in TFA/thioanisole/H_2O for 1 h (during which time the 6,6-fused bicyclic system forms) followed by addition of TIPS for 0.5 h to effectively scavenge trityl and other cations away from the C-terminal thiol. Yields were similar to those of the non-macrocyclic molecules, in the range 40–50%.

In summary, a method has been developed for the solid-phase synthesis of linear or macrocyclic peptide–heterocycle hybrids containing a tripeptide-derived, 6,6-fused bicyclic moiety, through condensation of an aldehyde to form a thiazinone ring. The bicyclization reaction occurs during TFA cleavage from the solid phase with high regio- and stereoselectivity, and peptide macrocyclization is subsequently performed in aqueous solution. Similar bicyclic structures have been studied as potential β-turn mimetics,[3a–c] and in general, compounds incorporating such conformationally constrained moieties may prove to have utility in drug discovery applications.

Acknowledgements

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7. General procedure for the synthesis of compounds **8** and **13**: Synthesis was carried out in fritted 12 mL polypropylene tubes on Fmoc-AM RAM amide resin (0.65–0.76 mmol/g) (Rapp Polymere; Tübingen, Germany). Chain assembly was carried out on a 0.1 mmol scale using standard 9-fluorenyl methoxycarbonyl (Fmoc) protocols. Side-chain protecting groups were: tBu (Ser, Tyr); trityl (Gln, Asn, dAsn, Cys, dCys, HoCys); Xan (Pen, Cys); Pbf (Arg); and tBoc (Trp). Fmoc deprotection was carried out with a 15 min treatment of 20% piperidine/DMF, and couplings were allowed to go for 1.5–2 h. Amino acids (1 mmol, 10-fold excess) were activated in situ with equal amounts of *N*,*N*-diisopropylcarbodiimide (DIC) and 1-hydroxy benzotriazole (HOBt). BrAcOH was coupled as a symmetric anhydride by addition of 20 equivalents of BrAcOH to DIC in a 2:1 ratio in DMF and a 15 min preactivation followed by a 1 h coupling. Coupling of aminoacetaldehyde–dimethylacetal to the BrAcetyl resin was carried out in DMF with a 20-fold excess in the presence of 20 equiv. of *N*,*N*-diisopropylethylamine (DIEA) and was complete after 2 h at rt. Coupling to the secondary amine resulting from aminoacetaldehyde–dimethylacetal coupling was carried out with a 10-fold excess of amino acid activated with PyAOP and DIEA in DMF for 2 h. Cleavage was carried out with 25–30 mg of peptide resin in 2 mL of 90:5:5 TFA:thioanisole:H_2O for 1.5 h. The TFA solution was then filtered away from the resin and concentrated to <300 μL in a Genevac HT-4

evaporator. Compounds were precipitated with diethyl ether and collected by centrifugation and decanting of the ether. For **13**, cleavage was carried out in 2 mL of 90:5:5 TFA:thioanisole:H_2O for 1 h followed by addition of 200 μL triisopropylsilane for 30 min and work-up as above to give linear intermediate **12**. Cyclization of **12** to give **13** was carried out overnight at pH 8 in 50/50 H_2O/CH_3CN containing 0.05 M NH_4HCO_3 with a peptide concentration of about 1 mg/mL. All products were found to have the correct molecular weight by ESI-MS (positive mode) within ±1 a.m.u. For example, for **13k**: observed $(MH)^+ = 1062.9$ a.m.u.; calculated $(MH)^+ = 1063.2$ a.m.u. Selected ^1H NMR data of **13k** (DMSO-d_6, 300 MHz, 21°C): δ 0.83–0.88 (br dd, 6H), 1.22–1.36 (m, 2H), 1.44–1.64 (m, 4H), 1.74–1.87 (m, 1H), 2.61–2.95 (m, 7H), 2.97–3.25 (m, 8H), 3.30–3.43 (m, 2H), 3.93–3.98 (m, 1H), 4.22–4.29 (m, 2H), 4.35–4.42 (m, 2H), 4.50–4.62 (m, 2H), 5.10–5.22 (m, 2H), 6.97 (t, $J = 7.5$ Hz, 1H), 7.06 (t, $J = 7.2$ Hz, 1H), 7.12–7.34 (m, 6H), 7.56 (d, $J = 7.8$ Hz, 1H), 7.71 (d, $J = 8.4$ Hz, 1H), 8.04–8.10 (m, 3H), 8.27 (d, $J = 6.9$ Hz, 1H), 8.32 (d, $J = 7.5$ Hz, 1H), 10.80 (s, 1H), 12.30 (br s, 1H).