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Solid-Phase Synthesis of Bicyclic Dipeptide Mimetics by Intramolecular Cyclization of Alcohols, Thiols, Amines, and Amides with *N*-Acyliminium Intermediates

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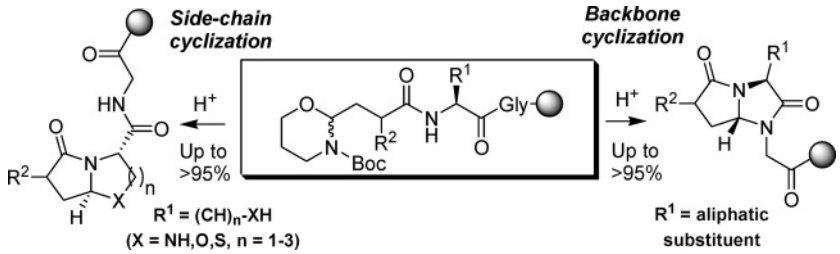
ABSTRACT



A general strategy for the solid-phase synthesis of structurally diverse bicyclic dipeptide mimetics is presented. Depending on the amino acid side-chain (R^1), peptide-derived *N*-acyliminium intermediates may undergo nucleophilic attack from either *side-chain* functional groups (–OH, –NH_2, –SH, and –$CONH_2$) or the amide *backbone* (–CONH–) of the peptide, thus affording a range of aza-, thia-, and oxabicycloalkanes in excellent purity and diastereoselectivity.

Peptides play a broad and decisive role in the regulation of biochemical processes in complex organisms.[1] Since more than 200 new peptide-based drugs are under different stages of development,[2] with approximately half being in clinical trials or prior to approval, the expectations are high for the rapidly growing market of therapeutic peptides.[3] Therefore, unraveling the relationship between conformation and biological activity of peptides represents a formidable task for peptide-based drug discovery of today. To meet the challenge of designing structural analogues of bioactive peptides with enhanced therapeutic properties, a combination of analytical tools comprising X-ray crystallography, NMR spectroscopy, and molecular modeling, is highly supportive. However, these techniques do not necessarily reveal the bioactive conformation of the peptide.

Reverse turns are common secondary structural elements important for the activity and molecular recognition of bioactive peptides.[4] Of the several types identified, the β-turn has most frequently been postulated to be essential for the interaction of linear peptides with receptors, enzymes, and antibodies. Consequently, the use of analogs imitating the conformation of a β-turn has emerged as a way to approach the problem.[5] This strategy has often been pursued by incorporating conformationally constrained dipeptide mimetics such as bicyclic amino acids into relevant regions of

(1) For an overview, consult: Sewald, N.; Jakubke, H.-D. *Peptides: Chemistry and Biology*; Wiley-VCH: Weinheim, 2002; Chapter 3.
(2) Bruckdorfer, T.; Marder, O.; Albericio, F. *Curr. Pharm. Biotechnol.* **2004**, *5*, 29−43.
(3) Loffet, A. *J. Peptide Sci.* **2002**, *8*, 1−7.
(4) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.
(5) (a) Kahn, M.; Eguchi, M. Synthesis of Peptides Incorporating β-Turn Inducers and Mimetics. In *Houben-Weyl, Methods of Organic Chemistry*; Felix, A., Moroder, L., Toniolo, C., Eds.; Thieme: Stuttgart, 2003; Vol. E22c, pp 695−740 and references therein. (b) Kee, K. S.; Jois, S. D. S. *Curr. Pharm. Des.* **2003**, *9*, 1209−1224.

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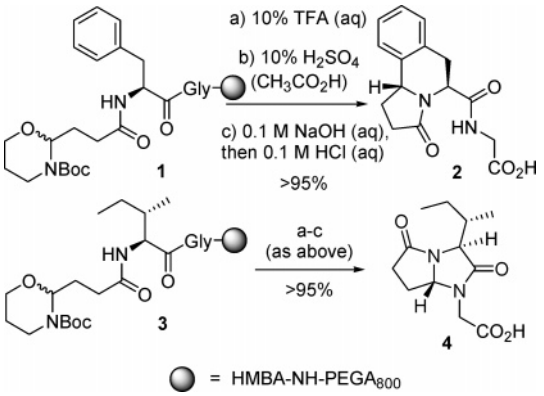
a peptide sequence. Since the classical work of Freidinger,[6] lactams and lactam-derived aza-, thia-, and oxabicycloalkane amino acids have proven to be highly successful in this context.[7] These building blocks are typically synthesized in solution as protected derivatives via lengthy synthetic routes, including several rounds of column chromatography for purification of intermediates and the final product. Although these building blocks are directly applicable and very convenient in standard solid-phase peptide synthesis protocols, it would be a more powerful approach to have a general synthetic route allowing the assembly of structurally diverse bicyclic skeletons *on* the solid support. However, to harness the benefits offered by solid-phase synthesis techniques and to render the synthetic methodology amenable for combinatorial library generation and on-bead biological assays, the generation of these scaffolds calls for a series of quantitative and stereoselective reactions on the solid support.

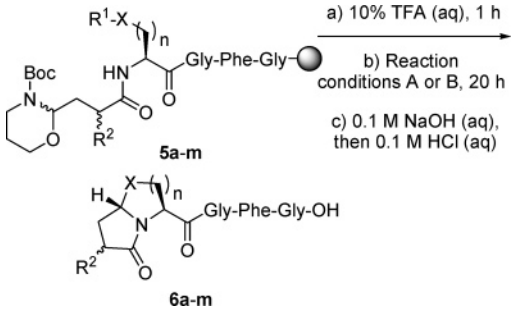
In connection with ongoing work dealing with the solid-phase intramolecular *N*-acyliminium Pictet−Spengler reaction,[8] highly efficient routes to cyclic *N*-acyliminium ions,[9] readily attacked by carbon-based π-nucleophiles (Scheme 1, **1** → **2**), were devised. A test substrate **3** not initially expected to permit a second cyclization was examined in parallel experiments in order to identify possible side-reactions of the intramolecular *N*-acyliminium Pictet−Spengler reaction. To our surprise, the amide nitrogen of the Ile residue was nucleophilic enough to react with the intermediate *N*-acyliminium ion under various sets of reaction conditions (Scheme 1, **3** → **4**).

Scheme 1. Different Amino Acid Side-Chains React Differently with *N*-Acyliminium Peptide Intermediates



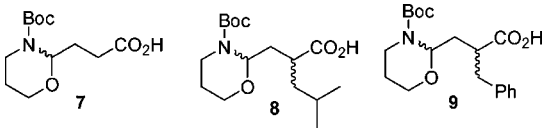
Bearing in mind the rich chemistry of *N*-acyliminium ions,[10] these findings prompted us to explore the *N*-acyliminium strategy as a general solid-phase entry to bicyclic ring-systems renowned as useful probes for mimicry of peptide secondary structural elements. In principle, a range of heteroatom-based nucleophiles should be within the scope of such methodology.[11] Accordingly, a series of substrates incorporating nucleophilic moieties were synthesized by standard solid-phase peptide synthesis protocols (see Supporting Information) from commercially available Fmoc amino acids and known racemic masked aldehyde building blocks **7**−**9** (Table 1, below).[9]

Table 1. Cyclization of Heteroatom−Nucleophilic Amino Acid Side-Chains with *N*-Acyliminium Peptide Intermediates[a,b]



entry	substrate, X	*n*	R^2	product, purity (%)[c]
1	**5a**, O[d]	1	H	**6a**, complex mixture
2	**5b**, O[d]	2	H	**6b**, >95
3	**5c**, O[d]	2	*i*-Bu	**6c**, >95[f]
4	**5d**, O[d]	2	Bn	**6d**, >95[f]
5	**5e**, S[d]	1	H	**6e**, 91
6	**5f**, S[d]	1	*i*-Bu	**6f**, 94[f]
7	**5g**, S[d]	1	Bn	**6g**, >95[f]
8	**5h**, NH[e]	1	H	**6h**, >95
9	**5i**, NH[e]	1	*i*-Bu	**6i**, 91[f]
10	**5j**, NH[e]	1	Bn	**6j**, 91[f]
11	**5k**, NH[e]	2	H	**6k**, >95
12	**5l**, NH[e]	3	H	**6l**, >95
13	**5m**, NH[e]	4	H	**6m**, complex mixture

[a] All reactions were run at 20 °C. [b] Reaction conditions: (A) TFA; (B) 10% H_2SO_4 (CH_3CO_2H). [c] Product purity was determined by RP-HPLC. [d] R^1 = Trt. [e] R^1 = Boc. [f] Product was formed as a 1:1 epimeric mixture.



Treatment of substrates **5a**−**m** with 10% TFA (aq) afforded 5-hydroxylactams, known to be in equilibrium with the corresponding *N*-acyliminium ions. Upon shifting to

(6) See, for example: (a) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656−658. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104−109.

(7) For reviews, see: (a) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854. (b) Hallab, L.; Gosselin, F.; Lubell, W. D. *Bioploymers* **2000**, *55*, 101−122. (c) Maison, W. In *Highlights in Bioorganic Chemistry*; Schmuck, C., Wennemers, H., Eds.; Wiley-VCH: Weinheim, 2004; Chapter 1.2, pp 18−29.

(8) For a review on the solid-phase Pictet−Spengler reaction, consult: Nielsen, T. E.; Diness, F.; Meldal, M. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 801−814.

(9) (a) Nielsen, T. E.; Meldal, M. *J. Org. Chem.* **2004**, *69*, 3765−3773. (b) Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2005**, in press.

(10) (a) Speckamp, W. N.; Hiemstra, H. *Tetrahedron* **1985**, *41*, 4367−4416. (b) Hiemstra, H.; Speckamp, W. N. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: San Diego, 1988; Vol. 32, pp 271−339. (c) Hiemstra, H.; Speckamp, W. N. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 2, pp 1047−1109. (d) Speckamp, W. N.; Moolenaar, M. J. *Tetrahedron* **2000**, *56*, 3817−3856. (e) Maryanoff, B.; Zhang, H.-C.; Cohen, J. H.; Turchi, I. J.; Maryanoff, C. A. *Chem. Rev.* **2004**, *104*, 1431−1628.

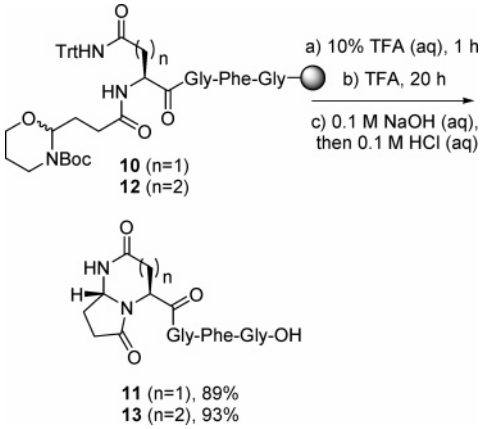
nonaqueous reaction conditions (A: TFA, or B: 10% H_2SO_4 (CH_3CO_2H)), residual protecting groups from serine, homoserine, cysteine, and diamino acid residues were removed, followed by cyclization to the *N*-acyliminium intermediate. In this event, both hydroxy, sulfhydryl, and amino groups reacted to provide a range of bicyclic products **6b**−**l** (Table 1, entries 2−12).

Curiously, the serine-derived product **6a** (as detected by MS) was only formed as part of a complex mixture displaying several overlapping peaks in RP-HPLC **6a**, whereas the homoserine-derived product **6b** was formed in excellent purity (>95%, Table 1, entry 2). Substituents in the masked aldehyde residue of the homoserine-derived substrates (**5c**, R^2 = *i*-Bu; **5d**, R^2 = Bn) were well tolerated, and the desired dipeptide mimetics **6c**,**d** were obtained as 1:1 epimeric mixtures in excellent purity (both >95%, Table 1, entries 3 and 4). The introduction of substituents (R^2) had no apparent effect on the high diastereoselectivity of the reaction. The resulting trans relationship between the proton at the ring junction and the α-proton of the homoserine residue is most likely governed by evolving steric interactions between the *N*-acyliminium carbonyl and the amide moiety of the homoserine residue, where the least hindered pathway accounts for the observed diastereoselectivity. The stereochemisty of **6b**−**d** was assigned by NOESY NMR, where the proton at the ring junction displayed a distinct NOE to the amide proton of the neighboring glycine residue and no NOE to the α-proton of the homoserine residue, which only correlates well with the anticipated trans stereochemistry. The stereochemistry of compounds **6c**−**l**, **13**, and **14** was assigned in analogy herewith.

As opposed to the serine-derived substrate **5a**, five-membered ring formation was highly feasible by attack of both the sulfhydryl moiety of cysteine derivatives **5e**−**g**[12] and the amino moiety of 1,3-diaminopropionic acid derivatives **5h**−**j** to the corresponding *N*-acyliminium intermediates, providing the corresponding thia- and azabicyclic dipeptide mimetics **6e**−**j** in 91 to >95% purity (Table 1, entries 5−10). For amino groups, this mode of cyclization was generally applicable to favorable ring sizes (n = 1−3), i.e., five- to seven-membered rings (Table 1, entries 8, 11, and 12), affording the (5,5)-, (5,6)-, and (5,7)-membered ring systems **6h**,**k**,**l**, respectively, in excellent purity (all >95%). A trace of the eight-membered derivative **6m** (n = 4) was detected by MS of the crude product, but the RP-HPLC chromatograms indicated formation of many products (Table 1, entry 13).

Given the feasibility of six- and seven-membered ring formation and the amide cyclization of Scheme 1 (**3**→**4**), it was investigated whether cyclization of the carbamyl side-chains of asparagine and glutamine derivatives **10** and **12** could be realized. Pleasingly, derivatives **11** and **13** were obtained in 89 and 93% purity, respectively (Scheme 2).

Scheme 2. Cyclization of Asparagine and Glutamine Carbamyl Side-Chains with *N*-Acyliminium Peptide Intermediates



Few examples have been reported on cyclization reactions of peptide amido groups with *N*-acyliminium ions. Although precursors for such transformations have been generated by solid-phase synthesis, they have normally been cleaved from the solid support in the event of aldehyde generation.[13] Following the general methodology of the present investigation, solid-phase synthesis of bicyclic bis-lactams was demonstrated to be feasible under strongly acidic reaction conditions such as 10% H_2SO_4 in CH_3CO_2H. In this approach, where reactive nucleophilic moieties in the side-chain (R) must be avoided and water is efficiently scavenged during the reaction, the amide backbone itself is forced to undergo a second reaction by amide-*N*-attack on the *N*-acyliminium intermediate (Table 2). For the seven aliphatic substituents listed in Table 2, the crude purity of the reaction products **4** and **15a**−**f** ranged from high to excellent (91→95%, Table 2, entries 1−7). In general, the stereochemistry was confirmed by NOESY NMR techniques, where distinct NOEs from the proton of the ring junction to protons present in the side-chain (R) were observed. The scope of this methodology is currently being investigated for more complex peptides.

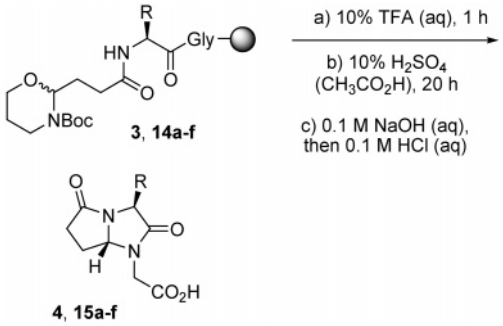
In summary, a range of bicyclic dipeptide mimetics may be obtained in excellent purity and diastereoselectivity via a general *N*-acyliminium strategy. Masked peptide aldehyde precursors, quantitatively generated by standard solid-phase

(11) For selected related solution-phase reactions: (a) Baldwin, J. E.; Freeman, R. T.; Schofield, C. *Tetrahedron Lett.* **1989**, *30*, 4019−4020. (b) Baldwin, J. E.; Hulme, C.; Edwards, A. J.; Schofield, C. J.; Parkes, K. E. B. *Tetrahedron Lett.* **1993**, *34*, 1665−1668. (c) Baldwin, J. E.; Hulme, C.; Schofield, C. J.; Edwards, A. J. *J. Chem. Soc., Chem. Comun.* **1993**, 935−936. (d) Qiu, W.; Gu, X.; Soloshonok, V. A.; Carducci, M. D.; Hruby, V. J. *Tetrahedron Lett.* **2001**, *42*, 145−148. (e) Sun, H.; Moeller, K. D. *Org. Lett.* **2002**, *4*, 1547−1550. (f) Mizutani, N.; Chiou, W.-H.; Ojima, I. *Org. Lett.* **2002**, *4*, 4575−4578. (g) Lewis, J. G.; Bartlett, P. A. *J. Comb. Chem.* **2003**, *5*, 278−284. (h) Ndungu, J. M.; Gu, X.; Gross, D. E.; Ying, J.; Hruby, V. J. *Tetrahedron Lett.* **2004**, *45*, 3245−3247.

(12) For related solid-phase aldehyde-amide backbone condensation/*N*-acyliminium ion formation, followed by attack from sulfhydryl residues, consult: (a) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Karlén, A.; Hallberg, A. *J. Med. Chem.* **1999**, *42*, 601−608. (b) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Synnergren, B.; Nyberg, F.; Karlén, A.; Hallberg, A. *J. Med. Chem.* **1999**, *42*, 4524−4537. (c) Johannesson, P.; Erdélyi, M.; Lindeberg, G.; Frändberg, P.-A.; Nyberg, F.; Karlén, A.; Hallberg, A. *J. Med. Chem.* **2004**, *47*, 6009−6019.

(13) (a) Vojkovský, T.; Weichsel, A.; Pátek, M. *J. Org. Chem.* **1998**, *63*, 3162−3163. (b) Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. *J. Am. Chem. Soc.* **1999**, *121*, 12204−12205.

Table 2. Cyclization of Amide Nitrogen Moieties with Cyclic *N*-Acyliminium Peptide Intermediates[a]



entry	R	product, purity (%)[b]
1	Pr	**15a**, 92
2	*i*-Pr	**15b**, 93
3	Bu	**15c**, 91
4	*sec*-Bu	**4**, >95
5	*i*-Bu	**15d**, >95
6	CH_2−Cy	**15e**, 94
7	CH_2CH_2−Cy	**15f**, 93

[a] All reactions were run at 20 °C. [b] Product purity was determined by RP-HPLC of the product cleaved off the resin by treatment with 0.1 M NaOH.

synthesis protocols, were treated with 10% TFA (aq) to liberate the aldehyde, and immediate reaction with the amide backbone formed the corresponding 5-hydroxylactam/*N*-acyliminium ion intermediates. Depending on the nature of the neighboring amino acid side-chain, exclusive modes of cyclization to the *N*-acyliminium intermediate were observed under strongly acidic reaction conditions: (i) *side-chain cyclization* occurred if a nucleophilic moiety (−NH_2, −OH, −SH, or −$CONH_2$) was present in the side chain, and (ii) *backbone cyclization* occurred in the absence of heteroatomic moieties in the side-chain. The latter event, where the carbonyl carbon of a newly formed peptide aldehyde ends up at two neighboring amide nitrogen atoms of the peptide sequence, represents, to the best of our knowledge, a novel approach for solid-phase synthesis.[14] Bearing in mind the course of reaction, when aromatic moieties are present in the side-chain,[9a,b] the picture of a unique approach for the generation of skeletal diversity via substrate-based folding processes is taking form,[15] and the generation of combinatorial libraries via such diversity-oriented synthesis[16] techniques is currently under investigation in our laboratories.

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Supporting Information Available: Analytical data (HPLC, MS, and NMR) for all compounds cleaved from the solid support. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) **General Procedure for Synthesis of Bicyclic Bis-lactams 4 and 15a−f.** The masked aldehyde substrate (**3**, **14a−f**) was treated with 10% TFA (aq) for 1 h at 20 °C in a plastic syringe fitted with a Teflon filter. The resin was washed with water (×6), DMF (×6), and CH_2Cl_2 (×6) and lyophilized for 20 h. Subsequently, the resin was treated with 10% H_2SO_4 (CH_3CO_2H) for 20 h at 20 °C. The resin was washed with CH_3CO_2H (×6), CH_2Cl_2 (×6), DMF (×6), water (×6), DMF (×6), and CH_2Cl_2 (×6) and then lyophilized to remove all traces of solvent. For release of material (**4**, **15a−f**) from the solid phase, beads were treated with 0.1 M NaOH (aq) for 2 h, then neutralized with an equimolar amount of 0.1 M HCl (aq), and finally diluted with CH_3CN. The resulting solution was filtered through a Teflon filter and analyzed by RP-HPLC and ESMS. When cleaved from the resin with 0.1 M NaOH (aq) and neutralized with 0.1 M HCl (aq), the crude compound (**4**, **15a−f**) was obtained as a mixture with solid NaCl. Corrected for the salt contents, crude yields were typically around 70−90%. When purified by preparative RP-HPLC, the yield of desalted, purified products generally dropped to 45−85% (consult Supporting Information).

(15) For a review of reagent-based differentiation and substrate-based folding in diversity-oriented synthesis, see: Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58.

(16) (a) Schreiber, S. L. *Chem. Eng. News* **2003**, *81*, 51−61. (b) Spring, D. R. *Org. Biomol. Chem.* **2003**, *1*, 3867−3870. (c) Strausberg, R. L.; Schreiber, S. L. *Science* **2003**, *300*, 294−295.