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Efficient Solid-Phase Synthesis of Peptides with Tripodal Side-Chain Bridges and Optimization of the Solvent Conditions for Solid-Phase Cyclizations.

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Abstract. Problems of cyclodimerization during the synthesis of two large-ring monocyclic peptides have allowed us to identify optimized solvent conditions for solid-phase cyclizations. These conditions combine high solvent polarity with excellent peptide-resin swelling. Using this newly optimized solvent system for the cyclizations, we have employed standard Boc/Benzyl methods and orthogonal Fmoc/OFm and Alloc/OAl protection to construct a novel type of bicyclic peptide with a tripodal side-chain bridge. The bridge, which links three amino-acid side chains to one trifunctional template, illustrates a new approach to peptide scaffolding for α-helix stabilization that might readily be applied to more complex structures.

Incorporation of conformational constraints into peptides or proteins is instructive for identifying the functional conformations of bioactive peptides, and guiding the design of peptidomimetic drugs or other pharmaceutical agents. We have been interested in developing the design and synthesis of multiple side-chain to side-chain lactam bridges as conformational constraints, initially with the goal of stabilizing discrete secondary structures. Ultimately, this approach could be extended to applications involving more complex (tertiary) peptide structures. Peptides with up to four such lactam bridges in separate segments of the peptide chain have been synthesized using solid-phase cyclization methods, either directly or by segment condensation.[1] Also, a combination of solid-phase and solution-phase cyclizations has been used to prepare a bicyclic peptide having lactam-bridged side chains in overlapping positions in the peptide chain,[2] and we have recently shown that these bicyclic peptides can be synthesized entirely by solid-phase methods.[3] The solid-phase approach has certain distinct advantages over solution-phase methods. First, the completeness of cyclization reactions can be enhanced by adding excess coupling reagents, which can easily be separated by filtration, therefore facilitating purification. Second, intermolecular side reactions can be reduced by the separation of the peptide chains linked to the solid support (the so-called pseudo-dilution effect).

Cyclic peptides incorporating side-chain bridges linking Lys and Asp residues in the i and i+4 positions in the peptide chain,[1] as well as peptides with flexible or rigid non-natural i to i+7 side-chain bridges[3b,4] have been reported to have increased α-helical structure. However, the success of this approach is limited by a significant dependence on the peptide sequence context of these bridges.[5] The aim of our present study is to initiate the development of a new type of multicyclic peptide, in which several side chains are covalently linked to a single multifunctional structure. Such structures might act as protein-mimetic scaffolds, or templates, favoring the formation of specific secondary or even tertiary peptide structures. We have chosen to illustrate this approach by using L-Ala or D-Ala as a bifunctional template structure linking Lys and Asp side-chains in the i and i+7 positions in a model monocyclic peptide (peptides **1** and **2**, respectively). We have then extended the approach by using *S*-1,3-diaminopropionic acid (Dap) as a trifunctional template linking Lys^{i}, Asp^{i+3} and Asp^{i+7} side-chains to form a bicyclic version of the same model peptide (peptide **3**). The structure of these bridges, including the new tripodal bridge in peptide **3**, were designed to be compatible with α-helix formation; the model peptide sequence used throughout was chosen to allow direct comparisons with our earlier lactam-bridged peptides.[1b] In the course of this work, we determined that optimization of the

solvent conditions for solid-phase lactam bridge formation was essential in order to obtain the desired peptides in reasonable yield. Peptides **1** and **2**, provided an excellent model system for this optimization process.

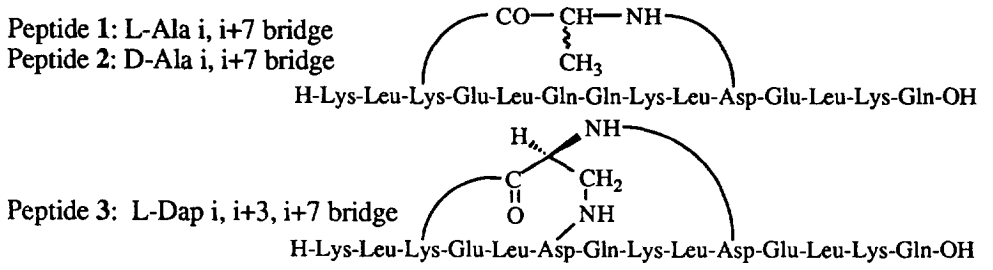
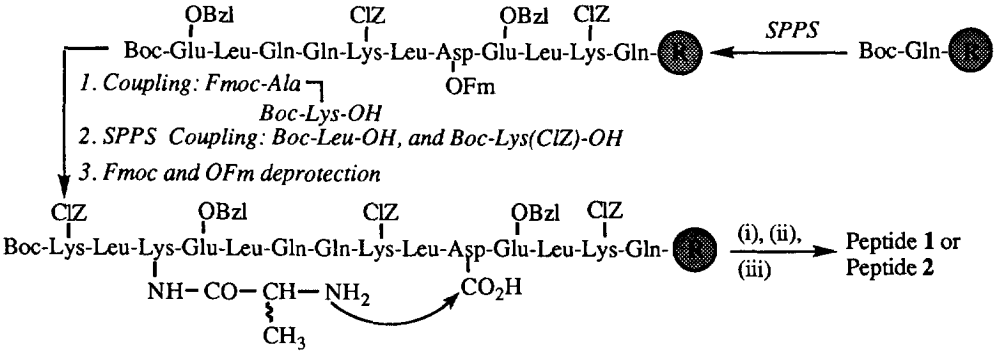


Figure 1. Structures of the monocyclic model peptides **1** and **2**, and the bicyclic model peptide **3**.

The syntheses of peptides **1** and **2** (Scheme 1) were performed manually on *p*-chloromethylbenzyl polystyrene (Merrifield) resin, starting with a substitution level of 0.09 mmol/g for the C-terminal amino-acid derivative, Boc-Gln. The peptide chains were then assembled by standard protocols, using the BOP coupling method.[1a] To simplify the protection strategy, Boc-Lys(Fmoc-L-Ala)-OH (peptide **1**) and Boc-Lys(Fmoc-D-Ala)-OH (peptide **2**) were coupled as the preformed pseudodipeptides. The completeness of each coupling was monitored by ninhydrin assays. Once the Fmoc and OFm groups had been selectively deprotected by 20% piperidine in DMF (v/v), the cyclization reaction was accomplished using 1.5eq BOP/DIEA.[1a] This reaction was initially performed in DCM/DMF (1/1), and its completion was also determined using ninhydrin assays. The last Boc group then was removed with TFA prior to HF cleavage.



Scheme 1. Solid-phase synthesis of peptides **1** and **2**. (i) Cyclization: BOP/DIEA (1.5 eq each), 2-4 h at RT in different solvent mixtures (Table 1); (ii) TFA:DCM (1:3), 30 min.; (iii) HF:anisole:DMS (9:1:1), 1 h, 0°C.

We were surprised by the remarkable result for the initial synthesis of Peptide **1** that the only major component in the crude peptide mixture was the undesired cyclodimerization product, identified by matrix-assisted laser desorption mass spectrometry (Table 1). The initial synthesis of peptide **2** gave two major products in good yield: the cyclodimer and the desired monocyclic peptide, in a 70:30 ratio, indicating a similar propensity for cyclodimerization. These results led us to change the solvent mixture used for the solid-phase cyclization reaction from the initial DCM/DMF (1/1) mixture and, ultimately, to investigate the solvent effect on these cyclization reactions in detail. The selection of solvent mixtures tested (Table 1) was based on earlier literature results.[6] The cyclization in each solvent mixture was performed on 200-500mg portions of the peptide resins, which had been assembled in one batch. After cyclization, the only additional reactions

that were performed separately for each resin portion (under identical conditions) were the Boc deprotection and the HF deprotection-cleavage. The lyophilized crude products were then analyzed by reverse-phase HPLC. All peptide monomers and cyclic dimers have been characterized by mass spectrometry and amino acid analyses. It is evident from Table 1 and Figure 2A that in the solvent mixture DMSO:NMP (1:4), the yield of the desired monomeric peptides is highly optimized. Based on our direct measurements on the solvents and the peptide-resin preparations, the essential solvent features appear to combine a high solvent polarity with excellent peptide-resin swelling characteristics.

Table 1. Solvent Effect on Solid-Phase Cyclization Reactions for Synthesis of Peptide **1** and **2**.

SOLVENT SYSTEM	$E_T(30)$[a] (kcal/mol)	Resin Volume[b] (mL/g)	Monomer:Dimer[c] Peptide **1**	Peptide **2**
DCM:DMF (96:4)	41.21	8.63		40:60
DCM:DMF (1:1)	43.15	6.43	0:100	70:30
DMF	43.40	6.05	25:75	70:30
THF:NMP (35:65)	43.38	7.15		86:14
DMSO:NMP (1:4)	43.52	7.56	65:35	94:6

[a]Solvent polarity parameter.

[b]Measured using the peptide-resin material obtained in the peptide **1** and **2** syntheses.

[c]Calculated as the ratio of the HPLC peak areas.

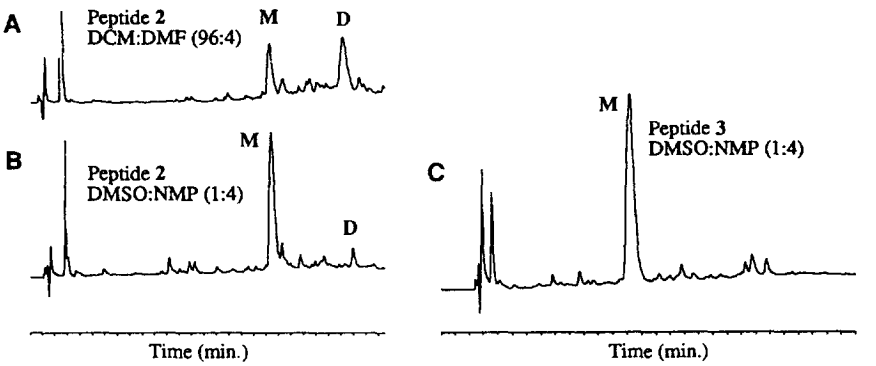
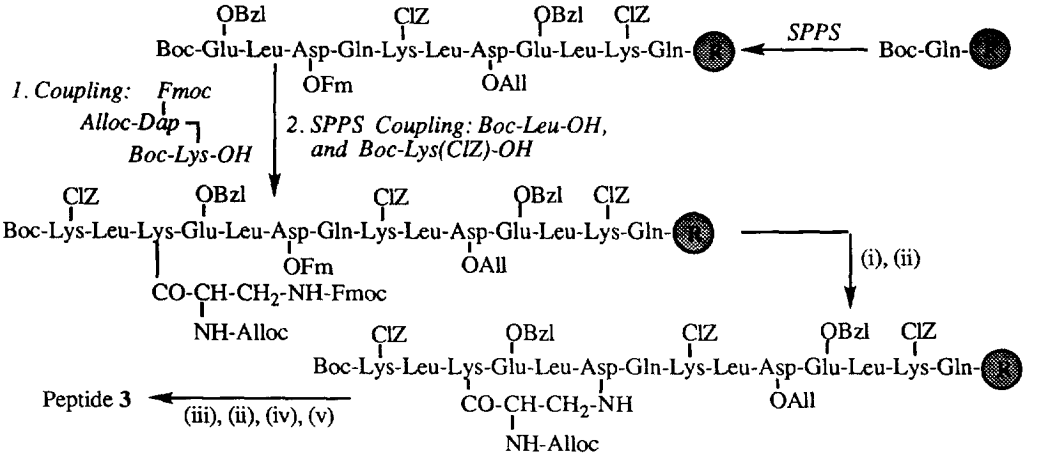


Figure 2. HPLC Profiles of the Crude Synthetic Products. (A) Peptide 2, after cyclization in DCM:DMF (96:4); (B) Peptide 2, after cyclization in DMSO:NMP (1:4); (C) Peptide 3, after cyclization in DMSO:NMP (1:4). (M = Monomer; D = Cyclodimer).

The optimized cyclization solvent described above was then incorporated into the synthesis of Peptide **3**, as outlined in Scheme 2. The substitution level of the C-terminal Boc-Gln residue on the Merrifield resin was, in this case, 0.2 mmol/g. Chain elongation was carried out using the same protocol as that used for the monocyclic peptides **1** and **2**. Boc-Lys(Alloc-DAP[Fmoc])-OH[7] was coupled to the peptide chain as the preformed pseudodipeptide. The standard Boc/Benzyl protection strategy was combined with orthogonal Fmoc/OFm and Alloc/OAl protection[8] of the two pairs of bridging functionalities, as described elsewhere for the solid-phase synthesis of bicyclic peptides.[3a] After selective Fmoc/OFm deprotection with 20% piperidine in DMF, the first cyclization (i to i+3) was completed with BOP/DIEA (1.5 eq each) in DMSO:NMP (1:4) within 2 hours. Then the Alloc/OAl groups were deprotected by $Pd(PPh_3)_4$ with N-methylaniline in THF:DMSO:1MHCl (4:4:1) in 4hrs, followed by a second cyclization under the same conditions as the first,

which was also completed within 2 hours. Each step was qualitatively monitored by Kaiser tests. After HF cleavage, the crude peptide product was precipitated with ethyl ether, extracted into 10% acetic acid, and lyophilized. HPLC analysis (Figure 2) shows only one major product, which was purified to homogeneity and confirmed as Peptide **3** by mass spectrometry (MW: Calc=1778.1, Found=1777.8) and amino-acid analysis.



Scheme 2. Synthesis of Peptide **3**. (i) 20% piperidine in DMF, 30min; (ii) cyclization with BOP/DIEA (1.5 eq each), 2 h at RT in DMSO:NMP (1:4); (iii) Alloc/OAl cleavage with $Pd(PPh_3)_4$/NMA, 2 h at RT in THF: DMSO:1M HCl (4:4:1); (iv) 25% TFA in DCM, 30min.; (v) HF:anisole:DMS (9:1:1), 1 h, 0°C.

References and Notes

1. (a) Felix, A.M.; Heimer, E.P.; Wang C.T.; Lambros, T.J.; Fournier, A.; Mowles, T.F.; Maines, S.; Campbell, R.M.; Wegrzynski, B.B.; Toome, V.; Fry, D.; Madison, V.S. *Int. J. Pept. Protein Res.* **1988**, *32*, 441-454. (b) Ösapay, G.; Taylor, J.W. *J. Am. Chem. Soc.*, **1992**, *114*, 6966-6973. (c) Ösapay, G.; Gulyás, J.; Profit, A. A.; Gulyás, E. S.; Taylor, J. W. *Proc. 12th Amer. Peptide Symp.*; *Peptides: Chemistry and Biology* Smith, J.A.; Rivier, J.E. Eds.; ESCOM, B.V., 1992, pp. 239-240.
2. Bracken, C.; Gulyas, J.; Taylor, J.W.; Baum, J. *J. Am. Chem. Soc.* **1994**, *116*, 6431-6432.
3. (a) Wu, B.; Taylor, J.W., manuscript submitted. (b) Yu, C.; Taylor, J.W., manuscript submitted.
4. Jackson, D.Y.; King, D.S.; Chmielewski, J.; Singh, S.; Schultz, P.G. *J. Am. Chem. Soc.* **1991**, *113*, 9391-9392.
5. Kapurniotu, A.; Taylor, J.W. *J. Med. Chem.* **1995**, *38*, 836-847.
6. Fields, G.B.; Fields, C.G. *J. Am. Chem. Soc.* **1991**, *113*, 4202-4207.
7. Boc-Lys(Alloc-DAP[Fmoc]) synthesis: First, asparagine was reacted with Alloc-Cl under basic conditions, (*J. Am. Chem. Soc.* **1950**, 72, 725-732). Pure Alloc-Asn-OH was obtained (75-80% yield) after recrystallizing from acidic aqueous solution. Alloc-Asn-OH was converted to Alloc-Dap-OH by reaction with bis(trifluoroacetoxy)-phenyliodine and DIEA (*Syns.* **1981**, 266-268). Without further purification, Alloc-Dap-OH was then reacted with Fmoc-OSu and DIEA to afford Alloc-Dap(Fmoc)-OH. (*Int. J. Peptide Protein Res.* **1986**, 398-400). After forming the active ester Alloc-Dap(Fmoc)-OSu, Boc-Lys and DIEA were added. The final product, Boc-Lys(Alloc-DAP[Fmoc])-OH, was recrystallized from dioxane/EtOAc/Et_2O in 63% yield, based on Alloc-Dap(Fmoc)-OH, and confirmed by NMR and mass spectrometry.
8. Kates, S.A.; Sole, N.A.; Johnson, C.R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549-1552.
9. All solvent mixtures are reported as volume ratios. Abbreviations: Alloc, allyloxycarbonyl; Boc, tert-butoxycarbonyl; BOP, benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate; DAP, diaminopropionic acid; DCM, dichloromethane DIEA, N,N-diisopropylethylamine; Fmoc, fluorenylmethyloxycarbonyl; NMP, 1-methyl-2-pyrrolidinone; OAl, allyl ester; OFm, fluorenylmethyl ester; TFA, trifluoroacetic acid. This research was supported by USPHS grant DA04197.

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