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A NOVEL, CONVENIENT, THREE-DIMENSIONAL ORTHOGONAL STRATEGY FOR SOLID-PHASE SYNTHESIS OF CYCLIC PEPTIDES[1-3]

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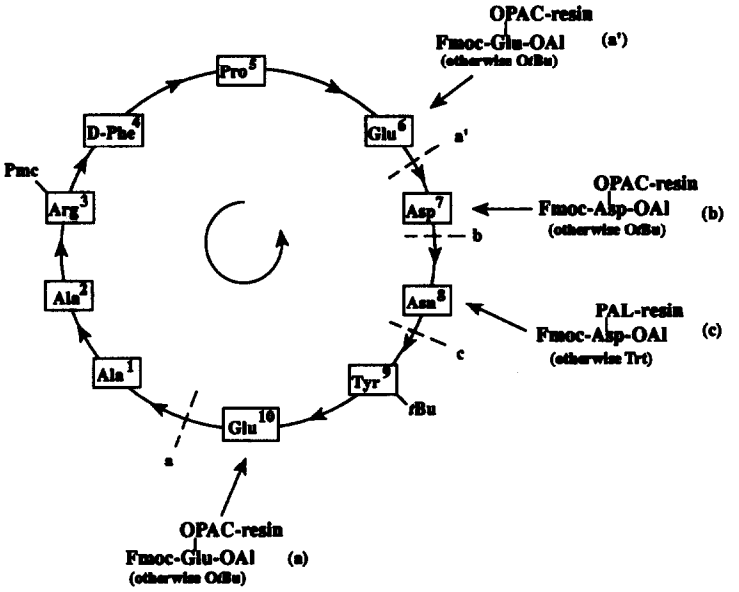
Abstract: Head-to-tail cyclic peptides are made by an efficient three-dimensional orthogonal solid-phase strategy (Fmoc/*t*Bu/allyl), featuring side-chain anchoring to PAC or PAL supports, selective palladium (0)-catalyzed allyl removal, and resin-bound cyclization mediated by BOP/HOBt/DIEA.

A number of cyclic peptides, generally comprising 4 to 12 residues and of either natural or designed origin, have interesting biological activities and hence are compelling targets for total chemical synthesis and analogue work.[4, 5] Literature methods rely on suitably protected linear precursors, which are selectively activated and cyclized in solution under highly dilute conditions. Cyclodimerizations and cyclo-oligomerizations can also occur, usually as unwanted side reactions but occasionally to benefit for certain symmetrical targets.[4, 6] The present communication describes an alternative approach with the following general features: (i) *side-chain anchoring*[7] of an initial partially protected amino acid residue to a polymeric support; (ii) *stepwise solid-phase*[8] assembly of the linear sequence; (iii) *orthogonal*[9] deprotection to liberate selectively a free C^{α}-carboxyl group for the subsequent cyclization step; (iv) efficient *activation* of the C^{α}-carboxyl group and its *condensation* with a free N^{α}-amino group to close the desired "head-to-tail" ring, taking advantage of the *pseudo-dilution* phenomenon[8, 10, 11] which favors intramolecular resin-bound reactions; and (v) final *deprotection* and *cleavage* to release the required free cyclic peptide into solution.

Specific tactics that are applied to this strategy (retaining the same organization) include: (i) esterification, mediated by *N,N'*-diisopropylcarbodiimide (DIPCDI) in the presence of 4-dimethylaminopyridine (DMAP), of ω-carboxyls of Asp or Glu to *p*-alkoxybenzyl alcohol (PAC) supports,[12] or coupling of these same ω-carboxyls to form tris(alkoxy)benzylamide (PAL) linkages[13] that ultimately cleave to generate the ω-carboxamide of Asn or Gln[7b,c]; (ii) temporary N^{α}-amino group protection provided by the base-labile 9-fluorenylmethyloxycarbonyl (Fmoc) function,[14] and *tert*-butyl and related acid-labile groups for side-chain protection; (iii) application of allyl chemistry[15] to provide the third dimension of orthogonality, i.e., the initially anchored Asp or Glu contains a C^{α}-allyl ester[16] which at the appropiate stage is deblocked with palladium (0) under nearly neutral conditions; (iv) after the usual Fmoc removal step to generate the free N^{α}-amino end group,[17] application of Castro's benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) reagents[18] to effect cyclization,[5, 11] and (v) treatment with moderate strength acid, i.e., trifluoroacetic acid (TFA) in the presence of suitable scavengers, to achieve high yield cleavage/deprotection and release the free cyclic peptide into solution.

As a model to illustrate the title strategy, the decapeptide *cyclo*(Ala-Ala-Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu), originally described by McMurray,[5c] was selected. Note that this target can be assembled by four different variations, as specified by the starting resins (Scheme 1): (a) Fmoc-Glu(OPAC-PEG-PS)-OAl at Glu^{10}; (a') the same for Glu^{6}; (b) Fmoc-Asp(OPAC-PEG-PS)-OAl at Asp^{7}; and (c) Fmoc-Asp(PAL-PEG-PS)-OAl at Asn^{8}. Note that in strategy c, the plan was to derive Asn^{8} from an Asp with its β-carboxyl function bound to the resin *via* the PAL linker. Linear syntheses were carried out in the $C \rightarrow N$ direction on a Millipore 9050-Plus continuous-flow synthesizer,[19] followed by deprotection of the allyl esters with $Pd(PPh_3)_4$ in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1), for 2 h at 25 °C, then N^{α}-Fmoc removal, BOP/HOBt/DIEA-mediated cyclization,[20,21] and final cleavage of the anchoring linkage and *t*Bu, Trt, and Pmc side-chain protecting groups with reagent R (TFA–thioanisole–β–mercaptoethanol–anisole, 90:5:3:2)[13] for 1 h at 25 °C. All four strategies gave the desired peptide; the best yield and purity was with the Asn^{8} strategy, and the Glu^{10} strategy gave the highest level of by-products (Figure 1). The crude peptide product obtained from the Asn^{8} strategy included 71% of the desired *monomeric* cyclized product,[22] as ascertained by comparison of HPLC peak areas with those from an authentic standard of known concentration. These and other[23] results encourage us to conclude that the simple, effective, and automatable approach of this paper will have wide generality for the preparation of cyclic peptides containing Asp, Asn, Glu, and Gln.



Scheme 1. Structure of target cyclic decapeptide (ref. 5c), and four synthetic strategies (this work). The counter-clockwise arrow in the center of the diagram shows the direction of linear chain growth by Fmoc chemistry. Arrowheads between successive residues in the sequence are oriented in the $N \rightarrow C$ direction. The starting resins are drawn alongside the target structure, and the final bond which must be formed to achieve cyclization in each strategy is indicated by a perpendicular dashed line.

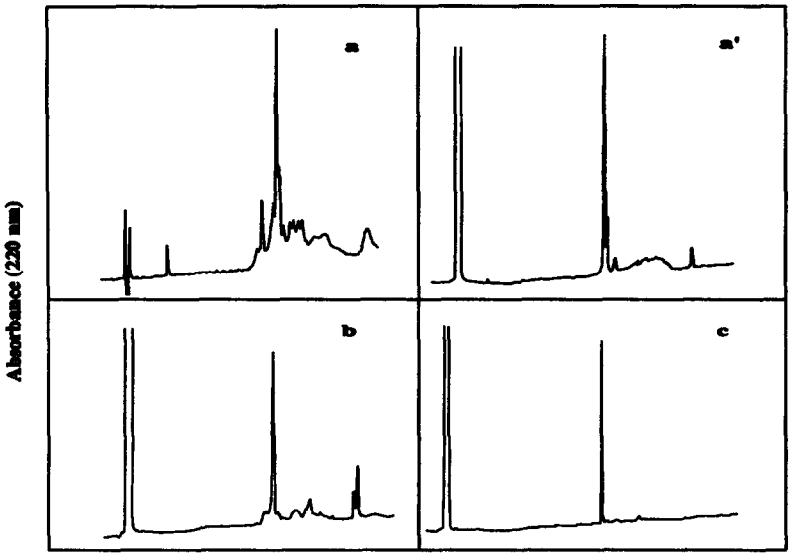


Figure 1. HPLC chromatograms of crude peptides directly after cleavage . Synthetic strategies **a**, **a'**, **b**, and **c** were as specified in the text and Scheme 1. Reversed-phase C-18 columns were eluted with a linear gradient over 20 min of 0.1% TFA in CH_3CN and 0.1% aqueous TFA from 1:9 to 1:1, flow rate 1.0 mL/min; the major peaks in each case were at 14.5 min and corresponded to an authentic standard of the desired cyclic peptide.

References and Notes

1. A preliminary report of portions of this work was presented at the Twenty-second European Peptide Symposium, September 13-19, 1992, Interlaken, Switzerland. As our experimental studies were being completed, we became aware of a similar approach exploiting side-chain anchoring and allyl chemistry (ref. 5d).
2. Amino acids and peptides are abbreviated and designated following rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **247**, 977-983 (1972). Additionally, the following abbreviations are used: Al, allyl; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DBU, 1,8-diazobicyclo[5,4,0]-undec-7-ene; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; FABMS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; Nva, norvaline; PAC, *p*-alkoxybenzyl alcohol handle; PAL, 5-(4-(Fmoc)-aminomethyl-3,5-dimethoxyphenoxy)valeric acid handle; PDMS, plasma desorption mass spectrometry; PEG-PS, polyethylene glycol-polystyrene graft; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulphonyl, PyBOP, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; *t*Bu, *tert*-butyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Trt, trityl. Amino acid symbols denote the L-configuration unless indicated otherwise.
3. This investigation was supported in part by NIH GM 28934 and 42722.
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9. An *orthogonal* system is defined as one using two or more independent classes of protecting groups that are removable by differing chemical mechanisms. See ref. 8a, p. 12, and G. Barany and R.B. Merrifield, *J. Am. Chem. Soc.* **99**, 7363-7365 (1977).
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13. F. Albericio, N. Kneib-Cordonier, S. Biancalana, L. Gera, I. Maseda, D. Hudson, and G. Barany, *J. Org. Chem.* **55**, 3730-3743 (1990), and references cited therein.
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16. Fmoc-Asp(OH)-OAl and Fmoc-Glu(OH)-OAl were obtained by refluxing commercially available Fmoc-Asp/Glu(O*t*Bu)-OH in neat allyl bromide (5 mL/g of Fmoc-derivative) in the presence of DIEA (2 equiv. with respect to Fmoc-derivative) for 30 min. The reaction mixture was diluted with EtOAc, extracted successively with 0.1 N aqueous HCl, pH 9.5 carbonate buffer [J.P. Tam, S.B. Kent, T.W. Wong, and R.B. Merrifield, *Synthesis*, pp. 955-957 (1979)], and saturated aqueous NaCl, followed by drying ($MgSO_4$), and concentration *in vacuo*. The resultant solid was dissolved in TFA–CH_2Cl_2 (1:1), and after 30 min, the reaction mixture was evaporated to dryness to provide solid title product which was recrystallized from ethyl ether–pentane. Fmoc-Asp(OH)-OAl (84% overall yield), mp 92-93 °C; Fmoc-Glu(OH)-OAl (66% overall yield), mp 120-121 °C. Alternatively, the desired derivatives were formed by overnight coupling of allyl alcohol (large excess; part of solvent) to Fmoc-Asp/Glu(O*t*Bu)-OH with DCC (1 equiv.) in allyl alcohol–THF (1:2; 7 mL/g of Fmoc-derivative); remainder of procedure the same, overall yields ~ 90%.
17. When deprotection was carried out with piperidine–DMF (1:4), an extra brief wash with 0.4% (v/v) concentrated aqueous HCl in DMF prior to cyclization was helpful. This expedient ensured complete removal of piperidine, and was reflected by the avoidance of *C*-terminal piperidylamides [otherwise detected by PDMS] as by-products in the cyclization step. When the DBU–piperidine–DMF (1:1:48) deprotecting reagent was used (ref. 19), an acid wash step was not necessary. We have also shown that the order of Fmoc and allyl removal steps can be reversed with equivalent results in the cyclization.
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19. Syntheses were carried out on polyethylene glycol-polystyrene (PEG-PS) graft supports [G. Barany, F. Albericio, N.A. Solé, G.W. Griffin, S.A. Kates, and D. Hudson. *In* "Peptides 1992, Proceedings of the 22nd European Peptide Symposium" (C.H. Schneider and A.N. Eberle, eds.), ESCOM, Leiden, 1993, in press]; using PyBOP/DIEA/HOBt (5 equiv.) coupling for 30 min and DBU–piperidine–DMF (1:1:48) deprotection for 6 min.
20. D. Hudson, *J. Org. Chem.* **53**, 617-624 (1988).
21. A typical cyclization experiment was carried out as follows: Peptide-resin (~ 100 mg) was suspended in DMF (1 mL), following which BOP (5 equiv.), HOBt (5 equiv.), and DIEA (10 equiv.) in DMF (1 mL) were added. After 2-5 h, ninhydrin tests [E. Kaiser, R.L. Colescott, C.D. Bossinger, and P.I. Cook, *Anal Biochem.* **34**, 595-598 (1970)] were negative or very slightly positive.
22. As further evidence for the correct structure, FABMS analysis showed MH^+ 1193.5 (calcd. 1193.3). For the crude peptide, a small peak was noted at 2386.9, corresponding to the cyclic dimer (calcd. 2386.5).
23. The same general strategy was also used to prepare a cyclic heptapeptide tachykinin antagonist (sequence from ref. 5b); MH^+ 1092.3 (FABMS), HPLC purity ~90%, overall yield not determined; *cyclo*(Gly-Gly-Gly-Pro-Nva-Arg-Ala-Phe-Ala-Gln); MH^+ 941 (PDMS), absolute yield 45%; and *cyclo*(Gly-Gly-Gly-Gly-Gly-Pro-Nva-Arg-Ala-Phe-Ala-Gln); MH^+ 1054 (PDMS), absolute yield 35%. Cyclizations for the examples in this footnote were all mediated by PyBOP/HOBt/NMM (compare to text examples).

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