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Solid-Phase Synthesis of "Head-to-Tail" Cyclic Peptides *via* Lysine Side-Chain Anchoring[1]

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Abstract: The *N,N'*-disuccinimidyl carbonate (DSC) has been successfully used for the efficient conversion of conventional hydroxymethyl resins into active carbonate resins, which are suitable for the incorporation of protected amino acids *via* an amino function, allowing the preparation of "head-to-tail" cyclic lysine containing peptides.

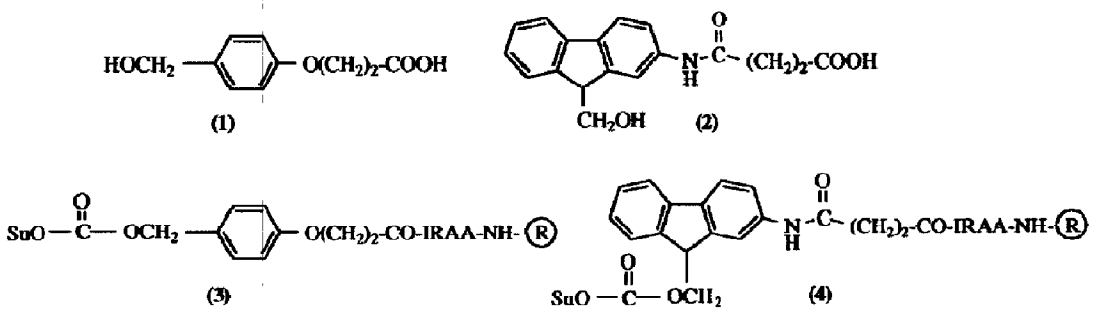
Although the interest of cyclic peptides dates back almost half a century to the discovery that the antibiotic gramicidin S is a cyclic peptide,[3] there has recently been an increase interest in both the isolation and the synthesis for this class of biomolecules.[4] The constrained geometry imposed by the cycle may construct a peptide with potential therapeutic properties. Furthermore, cyclic peptides are excellent models for conformational studies.[5] Classical methods to prepare cyclic peptides involve synthesis of the partially protected linear precursor in solution or by solid-phase, and cyclization in solution under high dilution. An attractive alternative includes the solid-phase chain assembly of the linear sequence, followed by cyclization while the peptide still remains anchored to the polymeric support.[6,7] This method may appropriate the advantage of the pseudo-dilution phenomenun attributed to the solid-phase, that favors intramolecular reactions over intermolecular side-reactions.[8] Recently, several groups have reported orthogonal strategies for the preparation of "head-to-tail" peptides *via* side-chain anchoring of protected Asp or Glu to hydroxymethyl- or aminomethyl resins,[7f-i, l, o] which after cyclization and cleavage of the peptide from the resin will afford the desired Asp/Asn or Glu/Gln containing peptides.[9]

Herein, the extension of the approach discussed above for the preparation of "head-to-tail cyclic" Lys containing peptides is described. The general features of this new strategy involves: (i) preparation of a *functionalized resin*, which allows the incorporation of an amino function through a bond that is stable during all the chain assembly, but cleaves at the end of the process; (ii) *side-chain anchoring*[10] of a partially protected lysine derivative; (iii) *stepwise solid-phase* assembly of the linear sequence; (iv) *orthogonal* [11] deprotection of the C^{α}-carboxyl group of the lysine; (v) *activation* of the C^{α}-carboxyl group and its condensation with the free N^{α}-amino group to close the desired ring; and (vi) final *deprotection* and *cleavage* to release the unprotected cyclic peptide into solution.

In all solid-phase peptide approaches, the linker between the first amino acid and the resin can be considered as a *permanent* protecting group[12] of the corresponding function involved, usually the C^{α}-carboxyl group. Since in SPPS amino functions are most commonly protected as carbamates,[13] resins that allow the attachment of amino group should be based on this concept. Carbamate-based resins have been used by several groups in SPPS approaches for chain elongation in both stepwise[14] and convergent[15] $N \rightarrow C$ terminal direction

strategies. These resins were prepared from the hydroxymethyl derivative of the classical Merrifield-resin, by reaction with phosgene, followed by incorporation of the corresponding amino component. The main drawbacks of this method are: (i) the use of phosgene is dangerous and not recommended; (ii) functionalization of the resin decreases, possibly due to formation of internal carbonates; and (iii) phosgene is not compatible with the more acid labile *p*-alkoxy benzyl alcohol resins required for an orthogonal scheme. On the other hand, *N,N'*-disuccinimidyl carbonate (DSC)[16] has been recently proposed as a mild method for alkoxycarbonylation of amines from the corresponding alcohols in solution.[17]

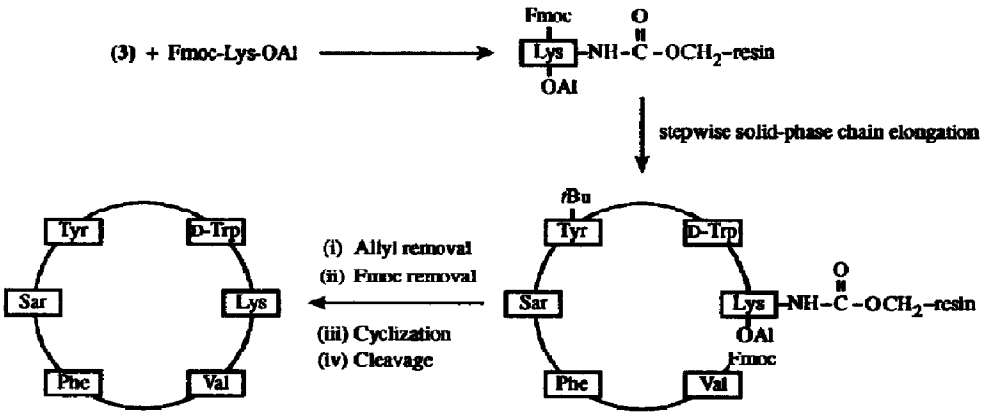
The present study focused on the use of resins derived from hydroxymethylphenoxypropionic acid[18] (TFA labile) (1) and *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid (secondary amine labile) (HMFS)[19] (2) handles, which with the appropriate N^{α}-amino protecting groups (Fmoc and Boc, respectively), C^{α}-carboxyl protecting group (Allyl) for Lys, and side-chain protecting groups (*t*Bu and Bzl, respectively) allow the application of the proposed scheme.



The key steps of this strategy involve the formation of the active carbonate resins (3) and (4) and the posterior reaction with the amino component. A survey was carried out to determine the best conditions. For resin derived of (1), optimal formation of the carbonate was achieved with the use of 10 equiv of DSC in DMF in the presence of 1 equiv of DMAP for 2 h at 25 °C, under Ar atmosphere. Incorporation of the amino acid was accomplished by dissolution of the amino derivative as a salt (10 equiv) in DMF followed by addition to (3) in the presence of DIEA (20 equiv) for 4 h at 25 °C. With these conditions, a quantitative overall yield was achieved.[20] The use of extended reaction times in the first step lead to a decrease of the level of incorporation. For resins derived of (2), control of both reactions is more critical since the presence of a good leaving group provides a favorable β-elimination side-reaction. Application of the identical conditions used for (1) lead to a very low level of substitution (5 %). Superior results for the preparation of (4) were acquired when pyridinium hydrochloride (10 equiv) replaced DMAP and the reaction proceeded in DMF for 24 h at 25 °C. For the second reaction, the amino derivative (10 equiv) was dissolved in DMF and added to resin (4) in the presence of DIEA (10 equiv) for 4 h at 25°C to give an overall yield of 75%.

As a model to illustrate this strategy, the hexapeptide *cyclo*(Val-Phe-Sar-Tyr-D-Trp-Lys), an analog of the peptide described by Veber and co-workers,[21] was selected. Fmoc-Lys-OAl[22] was incorporated to resin (3) according to the method described above, followed by manual stepwise synthesis in the $C \rightarrow N$ direction according to a standard Fmoc/*t*Bu protocol: Fmoc group removal with piperidine–DMF (1:4) (2 x 5 min), DMF washing (5 x 30 sec), amino acid coupling (30 min) with Fmoc-amino acids (10 equiv), HOAt (10 equiv)[23], and DIPCDI (10 equiv) in DMF, and DMF washing (5 x 30 sec). Allyl removal was carried out with $Pd(PPh_3)_4$ in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1)[24], for 150 min at 25 °C. Following N^{α}-Fmoc removal, BOP/HOAt/DIEA (5:5:10)-mediated cyclization was carried out for 2h (the ninhydrin test

was negative) at 25 °C. Final cleavage of the anchoring linkage and *t*Bu side-chain protecting group of Tyr were performed with TFA–thioanisole–ethanedithiol–anisole (90:5:3:2)[25] for 2 h at 25 °C (61% cleavage yield). The crude product obtained showed a major peak by HPLC (71% purity), and a correct FAB-MS (nitrobenzyl alcohol matrix): 795.7 [M + H$^+$] and amino acid analysis: Val, 1.00; Phe, 1.00; Tyr, 0.94; Lys, 1.05; Sar and Trp, N.D.



In conclusion, "head-to-tail" lysine containing cyclic peptides can be efficiently synthesized by a three-dimensional orthogonal solid-phase strategy (Fmoc/*t*Bu/allyl), featuring lysine side-chain anchoring on a novel active carbonate resin, selective palladium (0)-catalyzed allyl removal, and resin-bound cyclization. The use of these carbonate resins for the preparation of hydrazide peptides and other peptide derivatives is currently under investigation.

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References and Notes

1. Abbreviations used are: Al, allyl; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate; Bzl, benzyl; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; DSC, *N,N'*-disuccinimidyl carbonate; FAB-MS, Fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethyloxycarbonyl; HMFS, *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid; HOAc, acetic acid; HOAt, 7-aza-1-hydroxybenzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; IRAA, internal reference amino acid; SPPS, solid-phase peptide synthesis; Su, succinimidyl; *t*Bu, *tert.*-butyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran. Amino acid symbols denote L-configuration unless indicate otherwise.
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