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Four-dimensional orthogonal solid-phase synthesis of new scaffolds based on cyclic tetra-β-peptides†

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Abstract—A four-dimensional orthogonal protecting scheme that involves the acid-labile BAL linker in conjunction with Fmoc, Alloc and *p*Nb protecting groups, which can be removed by β-elimination, allyl transfer, and reductive hydrolysis, respectively, allows the solid-phase preparation of scaffolds based on a cyclic tetra-β-peptide with free amino side chains ready for further elaboration. © 2002 Published by Elsevier Science Ltd.

In medicinal chemistry, small cyclic peptides are excellent scaffolds for the incorporation of functional groups that can interact with the corresponding receptor.[1] Although the vast majority of cyclic peptides described in the literature are based on α-amino acids, there is increasing interest in the design and synthesis of cyclic peptides derived from β-amino acids. For example, Seebach[2] and Gellman[3] have independently demonstrated that peptides based on β-amino acids have a well-defined secondary structure as well as excellent stability against degradation by proteases. Furthermore, Seebach has also shown that cyclic β-peptides are arranged in the solid state as tubular structures with a tight net of pleated-sheet-type hydrogen bonds.[4] That supposes that those type of cyclic peptides present secondary structure. Given the interest in these systems, it would be advantageous to have at our disposal a versatile method for the preparation of cyclic β-peptides with protected functionalized side-chains that, after deprotection, could be further manipulated for the preparation of libraries based on the cyclic β-peptide scaffold. In order to gain maximum advantage from the synthetic effort, the ideal method would involve anchoring the cyclic peptide on a solid support.[5,6] We describe here the preparation of a solid-phase anchored cyclic β-peptide with protected side-chains and also report some examples of the modified scaffold.

The only valid strategy for the solid-phase preparation of cyclic peptides with protected side-chains is to anchor the peptide through the backbone amide nitrogen. In order to obtain a completely flexible system it is necessary to have at least a four-dimensional orthogonal protection scheme (Fig. 1).[7,8] More specifically, the scheme requires the following: a permanent protecting group to anchor the first amino acid to the support; two semi-permanent protecting groups, one for the protection of the *C*-terminal carboxylic acid group and the other for the side-chains; and finally a temporary protecting group for the protection of the β-amino function.

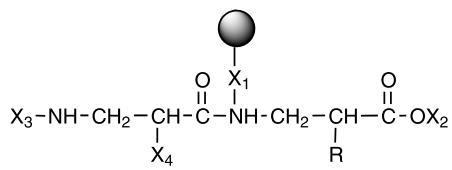

Figure 1. General protecting strategy for the solid-phase preparation of scaffolds based on cyclic tetra-β-peptides.

Abbreviations: Alloc, allyloxycarbonyl; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; EtOAc, ethyl acetate; HOAc, acetic acid; HOBt, hydroxybenzotriazole; IRAA, internal reference amino acid; PS, polystyrene; PyAOP, 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)-phosphonium hexafluorophosphate; SPOS, solid-phase organic synthesis; SPPS, solid-phase peptide synthesis; TBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide; TFA, trifluoroacetic acid. Amino acid symbols denote L-configuration unless otherwise noted.

Keywords: β-amino acid; combinatorial chemistry; high-throughput synthesis; libraries; protecting groups.

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† This paper is dedicated to Professor Joan A. Subirana on the occasion of his 65th birthday.

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For the anchoring of the backbone, a backbone amide linker (BAL)[9] resin (X_1) was chosen, which at the end of the process will liberate the peptide by treatment with acid. The protection of the β-amino function was performed with the fluorenylmethoxycarbonyl (Fmoc)[10] group (X_3), which is removed by a β-elimination reaction. The side-chains of the amino acids were protected with allyl-based groups[11] (X_4), which can be removed by an allyl transfer reaction in the presence of Pd(0). Finally, the *p*-nitrobenzyl group (*p*Nb)[12,13] (X_2), which is removed by reductive hydrolysis, was used for the protection of the carboxylic acid group.

As a key trifunctional β-amino acid, *N*α-Alloc-*N*β-Fmoc-L-diaminopropionic acid (Dapa) was chosen and this was elongated through the β-amino function.[14] The first cyclic peptide to be synthesized was *c*(L-Dapa-βAla-L-Dapa-βAla), which has as side-chains the two α-amino functions of the two Dapa residues.

Our strategy started with the addition of 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid (PALdehyde) to an amino-functionalized PS solid support through TBTU/DIEA coupling. This step was followed by on-resin reductive amination using the appropriate amino acid (usually β-alanine), *p*Nb ester hydrochloride salt and $NaBH_3CN$ in DMF (Fig. 2). In the case where the β-amino group is attached to a BAL resin, the acylation of the secondary amine was found to be faster than the comparable reaction with α-amino acids anchored to the same BAL resin.[9] Quantitative yields were obtained in the acylation of *N*α-Alloc-*N*β-Fmoc-L-Dapa by using PyAOP/DIEA[15] in DMF. Deprotection of the β-amino function of the Dapa residue with piperidine was followed by incorporation of Fmoc-β-alanine and *N*α-Alloc-*N*β-Fmoc-L-Dapa. After incorporation of the last residue, the removal of the *p*Nb group was accomplished by treatment with $SnCl_2$/HOAc/phenol/DMF.[12,13] Subsequent removal of the Fmoc group was followed by cyclization with PyAOP/DIEA[16] to give the cyclic β-tetrapeptide in which the two α-amino functions of the two Dapa residues were protected with the Alloc group. Removal of the Alloc group with $Pd(PPh_3)_4$[17] in the presence $PhSiH_3$ left the two amino functions free for further manipulation.[18]

After removal of the Alloc group, different aliquots of the resin were derivatized separately. For example, phenylacetic acid (PhAcOH) was incorporated using DIPCDI/HOBt and the Arg-Gly-Asp (RGD)[19] receptor recognition motif was constructed using a stepwise Fmoc/*t*Bu strategy.

The same scheme was used to prepare BAL-*c*[L-Dapa(Boc)-βAla-L-Dapa(Boc)-βAla], which after treatment with TFA gave *c*(L-Dapa-βAla-L-Dapa-βAla) with the side-chain amino function of the Dapa residues free for subsequent functionalization in solution. Finally, the simplest cyclic tetra-β-peptide, *c*$(\beta Ala)_4$, was also prepared in good yield and with excellent purity.[20]

In order to confirm any advantages inherent in our cyclization on-resin method, the linear peptide was prepared on a chloro–trityl-(Cl–Trt-)[21] resin by following the scheme shown in Fig. 3. Assembly of the peptide chain was followed by removal of the Alloc groups and PhAcOH was then incorporated as

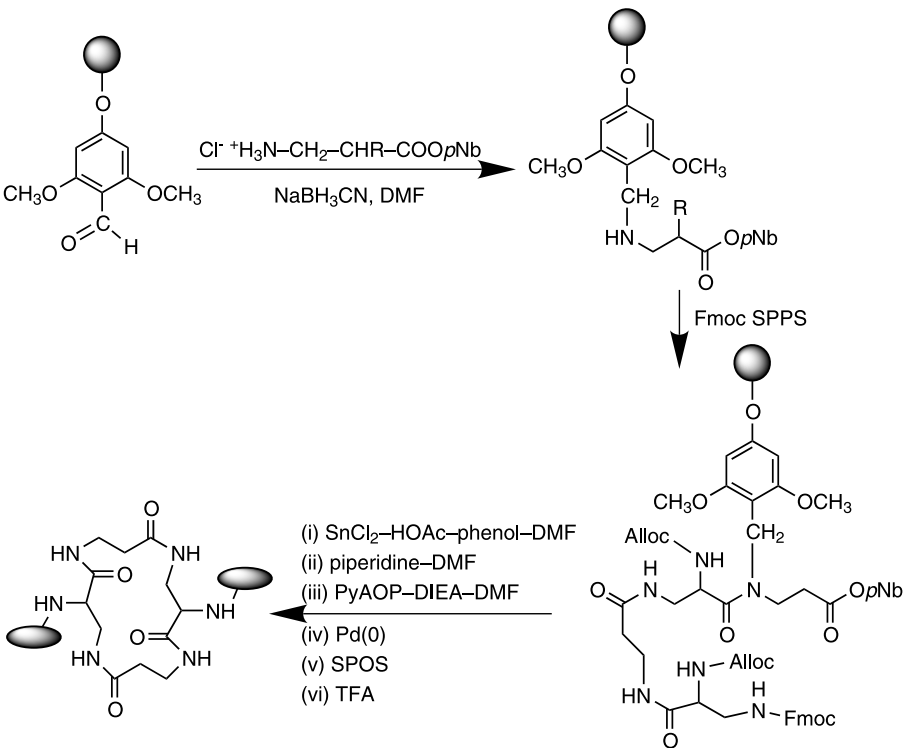

Figure 2. General strategy for the solid-phase preparation of scaffolds based on cyclic tetra-β-peptides containing Dapa residues.

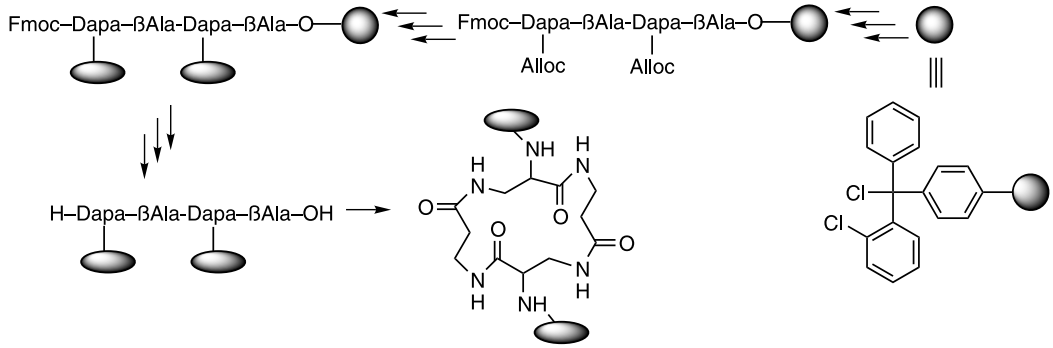

Figure 3. General strategy for the preparation of scaffolds based on cyclic tetra-β-peptides using a Cl–Trt-resin.

described above. Cleavage of the peptide with TFA and subsequent cyclization in solution gave *c*[L-Dapa-(PhAc)-βAla-L-Dapa(PhAc)-βAla]. Comparison of the two methods shows that cyclization on solid-phase using a BAL resin, where the whole process was performed on the solid-phase, gave better results than cyclization in solution after synthesis of the linear peptide on a Cl–Trt-resin. Thus, while the linear peptide was never detected by HPLC in the crude cyclic peptides obtained in solid-phase after 2 h of reaction, in solution large amounts of the linear peptide were detected after 16 h of reaction (the ratio cyclic versus linear was 1:4).[22]

In conclusion, a solid-phase strategy has been developed that uses a tetra-orthogonal protecting scheme and allows the preparation of cyclic tetra-β-peptides with free amino side chains ready for further elaboration. Cyclic tetra-β-peptide libraries prepared in this way will be suitable for both on- and off-resin screening.

1. Experimental

SPPS on BAL-resin. PALdehyde (5 equiv.) and TBTU (5 equiv.) were dissolved in DMF and then DIEA (10 equiv.) was added. After 1 min preactivation, this solution was added to the amino-functionalized PS. Coupling was allowed to proceed at 25°C for 2 h, after which time the resin was negative to the Kaiser ninhydrin test.

Mixtures of β-alanine *p*Nb ester hydrochloride[23] (10 equiv.) and $NaBH_3CN$ (10 equiv.) in DMF were added to the PALdehyde–IRAA-resins (1 equiv.). The reactions were allowed to proceed for 1 h at 25°C. The resins were then washed with CH_2Cl_2 and MeOH and finally dried.

*N*α-Alloc-*N*β-Fmoc-L-Dapa (5 equiv.), PyAOP (5 equiv.), and DIEA (10 equiv.) in DMF were added to amino acyl ester BAL-resins and the mixtures were allowed to react for 2 h at 25°C. After washing with DMF and CH_2Cl_2, the couplings were repeated for a further 2 h with fresh reagents.

The Fmoc group was removed using piperidine/DMF (1:4) (2×15 min). Incorporation of the rest of the protected amino acids and acids (10 equiv.) was performed with DIPCDI (10 equiv.) and HOBt (10 equiv.) in DMF for 2 h.

Removal of the *p*Nb group was carried out with 8 M $SnCl_2$ in DMF containing 1.6 mM HOAc and 0.2% phenol (2×30 min, 60°C).

Solid-phase cyclization was carried out with PyAOP (5 equiv.) and DIEA (10 equiv.) in DMF for 2 h at 25°C.

Removal of the Alloc group was achieved with $Pd(PPh_3)_4$ (0.1 equiv.) in the presence of $PhSiH_3$ (24 equiv.) in CH_2Cl_2 under Ar (2×20 min, 25°C).

Cleavage of the cyclic peptide from the resin was carried out with TFA/CH_2Cl_2 (9:1) for 2 h at 25°C.

SPPS on ClTrt-resin. Fmoc-βAla-OH (0.5 equiv.)[24] and DIEA (2 equiv.) in CH_2Cl_2 were added to a Cl–TrtCl-resin (1.35 mmol/g) for 2 h at 25°C. The reaction was terminated by addition of MeOH and stirring for 15 min.

The rest of the manipulations were carried out in a similar way to those described above.

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