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Synthesis of conformationally constrained peptides via solid-phase incorporation of the constraints

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Abstract

A simple solid-phase method has been developed, in which Pictet–Spengler cyclization is applied on resin for the synthesis of some conformationally constrained tripeptides. This methodology was also used for the synthesis of oxytocin antagonist analogues. © 2000 Elsevier Science Ltd. All rights reserved.

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Small peptides are usually highly flexible molecules and their structures in solution depend greatly on the environment. In order to restrict the conformational freedom of the parent peptide and to stabilize the desired bioactive conformation, local constraints can be introduced into the molecule. Modulation of the flexibility of a peptide backbone from an extended conformation to a β-turn structure is an important breakthrough in the rational design of highly selective and active peptides or peptidomimetic drugs.[1,2]

The recent developments in combinatorial solid-phase chemistry[3–5] have made feasible the parallel (or one-pot) synthesis of peptides which contain different constrained amino acids, e.g. 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), 4,5,6,7-tetrahydroimidazo[4,5-*c*]pyridine-6-carboxylic acid (spinacin) or 3-carboxy-1,2,3,4-tetrahydro-2-carboline (Tcc).[1] In recent years, the Pictet–Spengler reaction has been widely applied in the solid phase in heterocyclic chemistry.[6–11] Although solid-phase chemistry originated in the peptide field, as far as we are aware the Pictet–Spengler reaction has not utilized for peptide synthesis. The aim of the present work was to introduce the Pictet–Spengler reaction in the solid phase in the process of oligopeptide synthesis, with a view to the synthesis of conformationally constrained peptides.

The initial goal was the synthesis of tripeptides with the general structure H–Lys–Xxx–Ala–NH_2, where Xxx is methyl-substituted Tic (MeTic), methyl-substituted 7-

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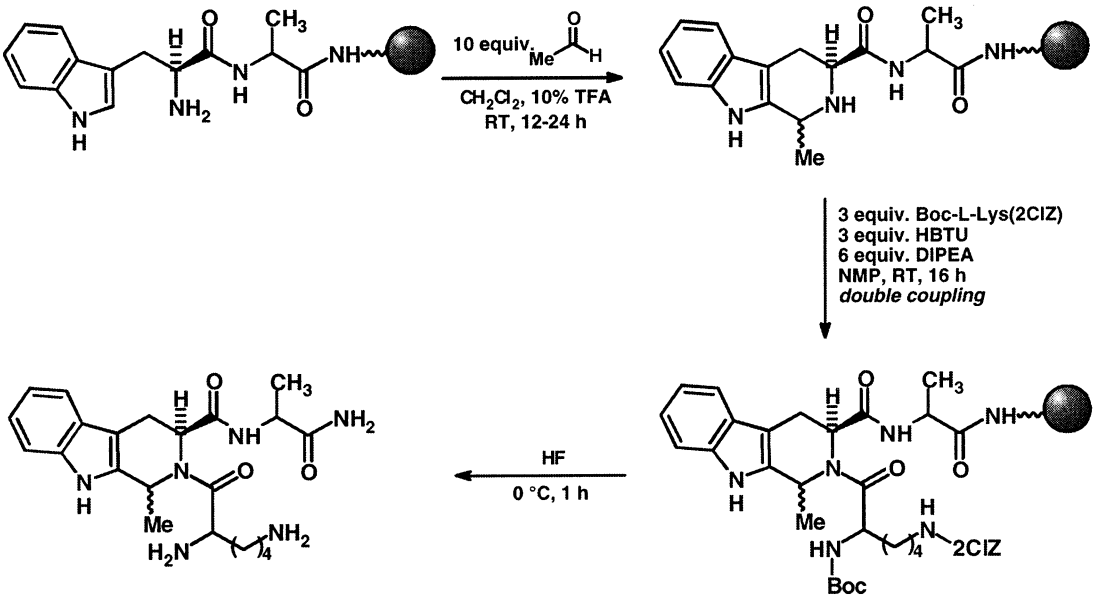
hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (MeHat), or methyl-substituted Tcc (MeTcc), derived from Phe, Tyr or Trp. The syntheses were carried out on *p*-methylbenzhydrylamine resin, using *t*-Boc chemistry[12] (see Scheme[13] below).

After the incorporation of the appropriate aromatic amino acids, the resulting dipeptidyl resin was subjected to reaction with acetaldehyde. Under the conditions applied, ring closure with formaldehyde failed. Following completion of the peptide chain, the resulting constrained tripeptide was detached from the resin by means of the standard liquid HF method. Even in the case of Trp, this method led to no serious side-reaction, although the resulting MeTcc, containing a β-carboline ring, is acid-sensitive in an aqueous environment. The use of standard peptide coupling revealed the presence of a deletion peptide, i.e. the lack of the Lys residue, from one peak in the HPLC trace. The amount of such deletion sequences varied from a few percent to the majority.[14] The acylation was especially difficult in the case of MeTcc, and the amount of non-acylated dipeptide was high.



This difficulty in acylation seemed to be the major barrier to the application of the above solid-phase method (in another experiment, we successfully acylated sterically hindered amino acid derivatives, e.g. β-carboline derivatives, in the liquid phase). Gilon et al.[15] recently published a new bis(trichloromethyl) carbonate (triphosgene, BTC)-mediated coupling for the incorporation of amino acid derivatives into sterically hindered molecules. The main advantage of this method is the in situ formation of the highly reactive acid chloride from the protected amino acid with BTC. Our experiments demonstrated that, even in the case of MeTcc, BTC coupling resulted in the desired tripeptide derivatives in acceptable yields[16] (75–80%, based on HPLC results).

During ring closure, the substituent incorporated introduces a new chiral center. The crude cleavage mixture therefore contains two diastereomeric peptides; in most cases, these were easily separable by RP-HPLC. The ratio of the diastereomers differed somewhat from 1:1 (68:32 for **I**:**II**, 31:69 for **III**:**IV**, and 35:65 for **V**:**VI**, based on HPLC analysis).

After the successful synthesis of the model tripeptides, this solid-phase method was used to synthesize two oxytocin antagonist analogues containing conformationally constrained amino acids (MeTic or MeTcc) in position 2:[17] Mpa–Xxx–Ile–Gln–Asn–Cys–Sar–Arg–Gly–NH_2, where Xxx is MeTic or MeTcc and Mpa is β-mercaptopropionic acid. The linear peptide was folded without isolation by stirring with $K_3[Fe(CN)_6]$. After the ring closure only one main product was isolated, the diastereomers were not separable in the applied HPLC conditions (Table 1).

Table 1
Characterization of the synthesized peptides

Peptide [a]	MW calc.	M+1 [b]	Rt	Gradient [c]
Lys–MeTcc–Ala–NH_2 (**I**)	428.54	429	7.13	a
Lys–MeTcc–Ala–NH_2 (**II**)	428.54	429	7.28	a
Lys–MeTic–Ala–NH_2 (**III**)	389.50	390	10.37	b
Lys–MeTic–Ala–NH_2 (**IV**)	389.50	390	10.62	b
Lys–MeHat–Ala–NH_2 (**V**)	405.50	406	32.8	c
Lys–MeHat–Ala–NH_2 (**VI**)	405.50	406	33.9	c
Mpa–MeTic–Ile–Gln–Asn–Cys–Sar–Arg–Gly–NH_2	1019.23	1019.5	9.83	e
Mpa–MeTcc–Ile–Gln–Asn–Cys–Sar–Arg–Gly–NH_2	1058.26	1058.5	8.21	f

[a] The absolute configuration of the peptides were not determined, the roman numerals **I**–**VI** indicate the individual enantiomers.

[b] Measured on a Finnigan Mat TSQ 7000 instrument by electrospray ionization.

[c] Solvents: $A = 0.1\%$ TFA in water, $B = 0.1\%$ TFA in an 8:2 acetonitrile water mixture; gradients (a) 14→20% of B in 12 min, flow 1.5 ml min^{-1}, Lichrosorb 10 RP-18; (b) 10→30% of B in 20 min, flow 0.8 ml min^{-1}, Nucleosil 5 C-18; (c) 0→30% in 60 min flow: 0.15 ml min^{-1}, Alltech 5 C-18 (d) 24→34% of B in 15 min, flow: 1.5 ml min^{-1}, Lichrosorb 10 RP-18; (e) 30→45% of B in 15 min, flow: 1.2 ml min^{-1}, Lichrosorb 10 RP-18; (f) 26→41% of B in 15 min, flow: 1.2 ml min^{-1}, Lichrosorb 10 RP-18.

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12. The peptides were synthesized by a standard *t*-Boc solid-phase procedure. After incorporation of the appropriate aromatic amino acid (Phe, Trp or Tyr(2BrZ)), the free amino group-containing polymer was shaken in 10% TFA/CH_2Cl_2 with 10 equivalents of freshly distilled acetaldehyde for 12–24 h at ambient temperature. After washing of the resin, the elongation of the peptide chain was completed. The peptides were detached from the resin by standard HF cleavage, and the products were characterized by mass spectrometry and RP HPLC.
13. Abbreviations: 2-chlorobenzyloxycarbonyl (2ClZ), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), *N*,*N*-diisopropylethylamine (DIPEA).
14. The yield of this coupling step could be improved by prolonging the reaction time of the acylation.
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16. 1.25 mmol protected amino acid was dissolved in dry THF, and 0.41 mmol (122 mg) BTC and 3.5 mmol (461 μl) collidine were added to the solution. After stirring for 1 min, the resulting suspension was poured into 0.25 mmol free amino or imino group-containing peptide resin and the mixture was shaken for 6 h at RT. The whole procedure was repeated, and the resin was then washed with MeOH and CH_2Cl_2, dried and subjected to HF cleavage.
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