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Solid-phase synthesis of hydroxyproline-based cyclic hexapeptides

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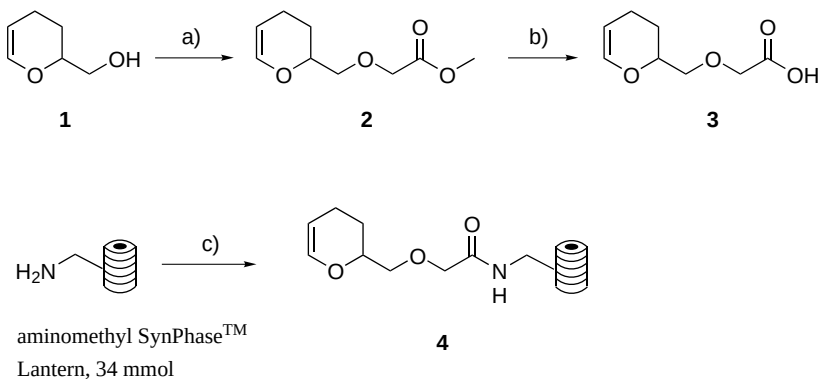
Abstract—Cyclic peptides are excellent tools to investigate the functional and spatial requirements for ligands to bind to a given target. In this paper we report the synthesis of a library of cyclic hexapeptides, designed to be Selectin antagonists. Based on molecular modelling calculations, these peptides contain a hydroxyproline building block that serves also as the point of attachment to the solid phase. A modified THP linker has been prepared to bind the hydroxy group of this amino acid to aminomethyl SynPhase™ Lanterns. Amino acids of the D- and L-series are used and their influence onto the cyclisation step is also investigated. © 2001 Elsevier Science Ltd. All rights reserved.

Cyclic peptides are molecules of interest both in chemistry and biology.[1] Due to their reduced conformational flexibility, they are excellent scaffolds for the presentation of diverse functionalities in a defined and predictable manner. For this reason, they are useful tools to explore the spatial requirements for pharmacophores binding to the biological target. Moreover, cyclic peptides are often more stable in vivo than their linear counterparts. Some examples of cyclic peptides used as drugs are present in the literature, such as Octreotide[2] and Cyclosporin A.[3]

In our search for new Selectin antagonists, mimicking the spatial presentation of the pharmacophores of the physiological ligand sialyl Lewis^x,[4] we became interested in cyclic peptides, since it has been shown that small peptides[5] can bind to E-Selectin in a calcium-dependent competitive manner. However, the examples reported in the literature use phage-displayed rather than chemically synthesised peptide libraries. Based on the X-ray structure of E-Selectin,[6] we examined, by the aim of molecular modelling (PrGen),[7] the requirements for cyclic peptides binding to the active site of the target protein. Our study was limited to hexapeptides containing a hydroxyproline building block. Hydroxyproline was chosen for various reasons: the proline ring could introduce additional rigidity into the macrocycle and the additional hydroxy group could either



Scheme 1. Synthesis of DHP-functionalised SynPhase™ Lanterns. *Reagents and conditions*: (a) NaH, methyl bromoacetate, THF, 0°C (85%); (b) NaOH (aq.), dioxane (quant.); (c) **3**, DIC, HOBt, DMF.

Keywords: solid-phase synthesis; cyclic peptides; Selectin-antagonists; DHP-linker.

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directly interact with the receptor or serve as a handle for the addition of functional groups or molecules (e.g. sugar moieties),[8] to increase the potency of the ligands.

Based on preliminary results of the molecular modelling studies, the solid-phase synthesis of a small library of cyclic hexapeptides was designed, starting from SynPhase™ Lanterns,[9] using the *mix & split* strategy and a radiofrequency tagging system.

Side-chain attachment was required to perform the cyclisation step onto the solid support. With hydroxyproline present in each member of the library, its hydroxy group offered an excellent point of attachment to the Lanterns. The THP linker[10] was chosen to bind the alcohol moiety to the solid support. To the best of our knowledge, this is the first example of solid-phase synthesis of cyclic peptides, involving *head to tail* cyclisation, in which hydroxyproline is used as point of attachment to the solid support.

In order to attach the dihydropyranyl moiety to commercially available aminomethyl Lanterns, 2-hydroxymethyl-3,4-dihydro-2*H*-pyran (**1**) was reacted with methyl bromoacetate in the presence of NaH.

After ester hydrolysis (→**3**, 85%), the modified DHP handle (**3**)[11] was readily attached to the solid support using a standard DIC/HOBt coupling procedure (Scheme 1). Completion of the coupling reaction could be easily monitored by a ninhydrin test.[12] In addition, the introduction of the acetic acid spacer between the linker and the polymeric backbone increases the accessibility of the active sites of the Lanterns.

In order to avoid diketopiperazine formation (likely to happen when a proline ester is the first amino acid)[13] and to introduce Hyp in a position supporting the final ring closure, the building blocks FmocHyp-Asp(*t*Bu)OAll (**5a**), FmocHypSer(*t*Bu)OAll (**5b**) and FmocHypLys(Boc)OAll (**5c**) were prepared in solution by coupling FmocHypOH with the respective allyl-protected amino acids (Scheme 2).

The dipeptides **5a**–**c** were coupled to the functionalised Lanterns **4** with PPTS in dichloroethane at 80°C for 48 h.[14] Fmoc quantification of the peptide bound to the solid support gave a loading of 28 μmol/Lantern, consistent with data reported in the literature for similar molecules.[10] Treatment of the Lanterns with TFA/H_2O 95:5, afforded the unprotected dipeptides as single peaks according to HPLC analysis.

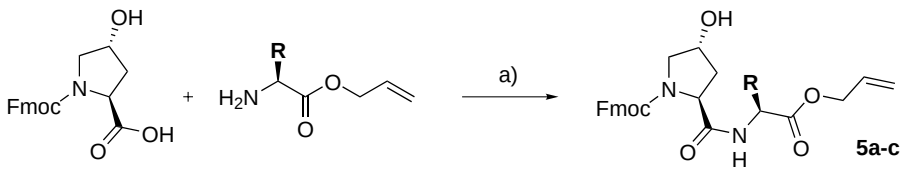
A small library of hexapeptides was then synthesised in which Glu and Lys were introduced in the third and fourth position, respectively. Both L- and D-configured amino acids in all four possible combinations were used, in order to study their influence onto the cyclisation step. Moreover, we expected the D- and L-side chains differently orientated in the resulting cyclic peptides, giving additional information for the SAR study.

By standard peptide synthesis using PyBOP/HOBt/DIPEA, the linear hexapeptides were obtained in high purity. For the final cyclisation, the allyl ester was deprotected with $Pd(PPh_3)_4$ in *N*-methylmorpholine/acetic acid buffer in CH_2Cl_2. After the following Fmoc deprotection (quantification confirmed the initial loading of 28 μmol/Lantern) the cyclisation step was conducted with PyBOP/HOAt.[15] The products were finally cleaved off the support with TFA/H_2O 95:5 (Scheme 3).

The results are summarised in Table 1. In all cases the product was the major component of the crude material and more interestingly the difference in cyclisation reactivity between hexapeptides from the LL-, DL-, LD- and DD-series was found to be negligible. On the other hand, the nature of the first amino acid seemed to have an important influence, where less hindered side-chains led to a higher purity of the final products.

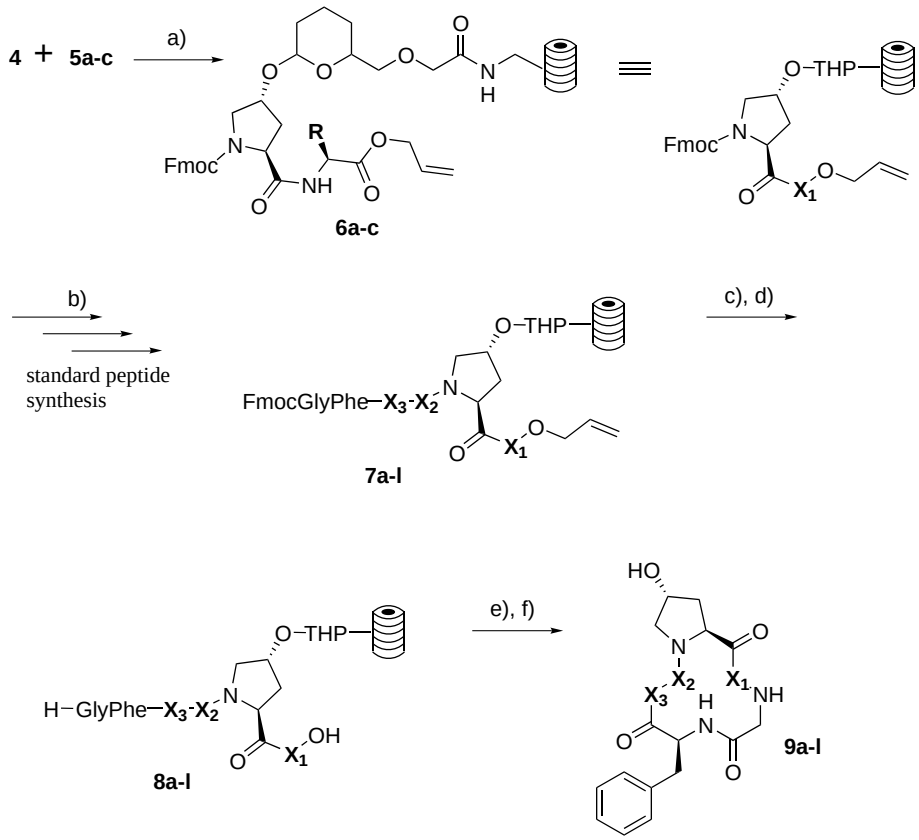
This approach was found to be very versatile: FmocThr allyl ester was also bound to the derivatised Lanterns (**4**), and cyclic hexapeptides were, again, assembled successfully. In principle this method could be used also with serine and unnatural amino acids bearing a hydroxy group in the side chain.

In conclusion, we have reported the solid-phase synthesis of a small library of cyclic hexapeptides, on derivatised SynPhase™ Lanterns using both natural and unnatural amino acids. Hydroxyproline was used for the first time as point of attachment to the solid support and a novel modified THP linker was successfully used and its coupling to the Lanterns could easily be monitored by a ninhydrin test. The members of this library and related compounds will be tested for their affinity to Selectins and the results will be reported in due course.



5a: **R** = CH_2CO_2tBu, 83%
5b: **R** = CH_2OtBu, 91%
5c: **R** = $(CH_2)_4NHBoc$, 84%

Scheme 2. Synthesis of dipeptide building blocks. *Reagents and conditions*: (a) WSC, HOBt, DIPEA, CH_2Cl_2.



Scheme 3. Synthesis of a small library of cyclic hexapeptides. *Reagents and conditions*: (a) PPTS, 1,2-dichloroethane, 80°C; (b) conditions for the standard peptide synthesis: amino acid, PyBOP, HOBt, DIPEA, DMF; then piperidine, DMF; (c) $Pd(PPh_3)_4$, *N*-methylmorpholine, acetic acid, CH_2Cl_2; (d) piperidine, DMF; (e) PyBOP, HOAt, DIPEA, DMF; (f) TFA, H_2O.

Table 1. Analytical results for the library of cyclic peptides c(Gly-Phe-$\mathbf{X_3}$-$\mathbf{X_2}$-Hyp-$\mathbf{X_1}$)

Compound	$\mathbf{X_1}$	$\mathbf{X_2}$	$\mathbf{X_3}$	HPLC purity (%)[a]	ES-MS[b]
9a	L-Asp	L-Glu	L-Lys	69	690.6
9b	L-Ser	L-Glu	L-Lys	47	662.3
9c	L-Lys	L-Glu	L-Lys	71	703.4
9d	L-Asp	D-Glu	L-Lys	54	690.6
9e	L-Ser	D-Glu	L-Lys	43	662.3
9f	L-Lys	D-Glu	L-Lys	82	703.4
9g	L-Asp	L-Glu	D-Lys	62	690.6
9h	L-Ser	L-Glu	D-Lys	42	662.3
9i	L-Lys	L-Glu	D-Lys	75	703.4
9j	L-Asp	D-Glu	D-Lys	55	690.6
9k	L-Ser	D-Glu	D-Lys	42	662.3
9l	L-Lys	D-Glu	D-Lys	77	703.4

[a] Detected at 220 nm.
[b] $(M+H)^+$ peak.

References

1. (a) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 1375–1389; (b) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans.1* **2001**, *5*, 471–484; (c) Davies, J. S. In *Cyclic Polymers*; Semlyen, J. A., Ed., 2nd ed.; Kluwer Academic: Dordrecht, The Netherlands, 2000; (d) Spatola, A. F.; Romanovskis, P. In *Amide Linkage*; Greenberg, A.; Breneman, C. M.; Liebman, J. F., Eds.; John Wiley & Sons: New York, NY, 2000; pp. 519–564.
2. Bauer, W.; Briner, U.; Doepfner, W.; Haller, R.; Huguenin, R.; Marbach, P.; Petcher, T. J. *Life Sci.* **1982**, *31*, 1133–1140.
3. Emmel, E. A.; Verweij, C. L.; Durand, D. B.; Higgins, K. M.; Lacy, E.; Crabtree, G. R. *Science* **1989**, *246*, 1617–1620.
4. (a) Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, 1571–1578; (b) Jahnke, W.; Kolb, H. C.; Blommers, M. J. J.; Magnani, J. L.; Ernst, B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2603–2607; (c) Kolb, H. C.; Ernst, B. *Pure Appl. Chem.* **1997**, *69*, 1879–1884.

5. (a) Fukuda, M. N.; Ohyama, C.; Lowitz, K.; Matsuo, O.; Pasqualini, R.; Ruoslahti, E.; Fukuda, M. *Cancer Res.* **2000**, *60*, 450–456; (b) Martens, C. L.; Cwirla, S. E.; Lee, Y.-W.; Whitehorn, E.; Chen, E. Y.-F.; Bakker, A.; Martin, E. L.; Wagstrom, C.; Gopalan, P.; Smith, W.; Tate, E.; Koler, K. J.; Schatz, P. J.; Dower, W. J.; Barrett, R. W. *J. Biol. Chem.* **1995**, *270*, 21129–21136; (c) O, I.; Kieber-Emmons, T.; Otvos, L.; Blaszczyk-Thurin, M. *Biochem. Biophys. Res. Comm.* **2000**, *268*, 106–111.
6. Graves, B. J.; Crowther, R. L.; Chandran, C.; Rumberger, J. M.; Li, S.; Huang, K. S.; Presky, D. H.; Familetti, P. C.; Wolitzky, B. A.; Burns, D. K. *Nature* **1994**, *367*, 532–538.
7. Zbinden, P.; Dobler, M.; Folkers, G.; Vedani, A. *Quant. Struct.-Act. Relat.* **1998**, *17*, 122–130.
8. Burger, K.; Kluge, M.; Fehn, S.; Koksch, B.; Hennig, L.; Mueller, G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1414–1416.
9. Rasoul, F.; Ercole, F.; Pham, Y.; Bui, C. T.; Wu, Z.; James, S. N.; Trainor, R. W.; Wickham, G.; Maeji, N. J. *Biopoly. (Pept. Sci.)* **2000**, *55*, 207–216.
10. Thompson, L. A.; Ellman, J. A. *Tetrahedron Lett.* **1994**, *35*, 9333–9336.
11. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.58–1.78 (1H, m), 1.74–1.82 (1H, m), 1.86–1.96 (1H, m), 2.00–2.09 (1H, m), 3.60 (1H, dd, $J=10$, 7), 3.65 (1H, dd, $J=10$, 3), 3.98 (1H, m), 4.14 (2H, s), 4.65 (1H, broad s), 6.32 (1H, d, $J=5$), 7.98–8.88 (1H, broad s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.5, 143.6, 101.2, 74.6, 74.3, 65.7, 60.9, 24.5, 19.6.
12. Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147–157.
13. (a) Bianco, A.; Sonksen, C. P.; Roepstorff, P.; Briand, J.-P. *J. Org. Chem.* **2000**, *65*, 2179–2187; (b) Pedroso, E.; Grandas, A.; de las Heras, X.; Eritja, R.; Girald, E. *Tetrahedron Lett.* **1986**, *27*, 743–746.
14. 5 equiv. of dipeptides were used, together with 2 equiv. of PPTS. The excess of peptide could be quantitatively recovered after the reaction, by removal of PPTS via water extraction.
15. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831–8838.