Pergamon

Tetrahedron Letters 42 (2001) 7261–7263

TETRAHEDRON LETTERS

A useful bicyclic topological decapeptide template for solution-phase combinatorial synthesis of tetrapodal libraries

Qingchai Xu,[a] Frans Borremans[a,*] and Bart Devreese[b]

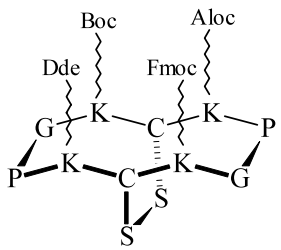
[a]*Department of Organic Chemistry, University of Ghent, Krijgslaan 281, 9000 Ghent, Belgium*
[b]*Department of Biochemistry, University of Ghent, Ledeganckstraat 35, 9000 Ghent, Belgium*

Received 3 May 2001; revised 14 August 2001; accepted 21 August 2001

Abstract—An orthogonally protected bicyclic decapeptide has been constructed and evaluated as a template for combinatorial synthesis. This template is found most useful for *solution*-phase combinatorial synthesis of tetrapodal libraries. © 2001 Elsevier Science Ltd. All rights reserved.

The use of a topological structure as a template for construction of combinatorial libraries is one of the recent advances in combinatorial chemistry.[1–4] We have developed a class of bicyclic decapeptide molecules bicyclo-(KCKPGKCKPG) as templates for the construction of tetrapodal libraries.[5] The most interesting protected template **1** has been synthesized by SPPS approach in gram-scale.[6] This peptide has four (quasi)-orthogonal protecting groups (Fmoc, Boc, Aloc and Dde), allowing site-selective assembly of building blocks (Fig. 1).

The merits of solution-phase versus solid-phase combinatorial synthesis have been emphasized by Boger et al.[7] Using different scaffolds, these authors demonstrated[8] that if simple and efficient isolation and purification of the reaction products are implemented, solution-phase combinatorial synthesis is a most attractive alternative. Solution-phase combinatorial synthesis has several advantages because of the homogeneous reaction conditions. Typical shortcomings of solid-phase approach do not exist in solution synthesis. The scale of solid-phase approach is restricted by the amount of required solid support and its loading capacity, and the production of multi-milligram quantities of each library member can be cumbersome and expensive. The idea of the application of peptide template **1** to solution-phase synthesis of libraries was inspired by our observation that these bicyclic peptides appear to readily give quantitative precipitation in diethyl ether. This offers the possibility of easy purification of intermediate products in solution synthesis.



1

Figure 1. Schematic representation of the orthogonally protected peptide template.

In this communication, we report and demonstrate the practical convenience of this bicyclic peptide template in solution-phase combinatorial synthesis. We performed a solution synthesis of a model library starting with 75 mg (47 μmol) of **1**, using divide–couple–mix cycles (split-synthesis). The four attachment sites are labeled A, B, D and F referring to the protecting groups of the side-chain amino groups of the four lysine residues Aloc, Boc, Dde and Fmoc, respectively. Assembly of building blocks followed the following order: first the B-site (after selective deprotection of Boc),[9a] then the F-site (after selective removal of Fmoc),[9b] the A-site (after removal of Aloc)[9c] and finally the D-site (after removal of Dde).[9d] Four groups (B_i, F_i, A_i and D_i) of three different amino acids were respectively used for the corresponding four diversity points (Fig. 2). In each growth point, after splitting into three portions, three different amino acids, one for each

Keywords: template; bicyclic peptide; solution-phase; combinatorial synthesis.

* Corresponding author. Fax: 32-9-2644972; e-mail: frans.borremans@rug.ac.be

PII: S0040-4039(01)01528-3

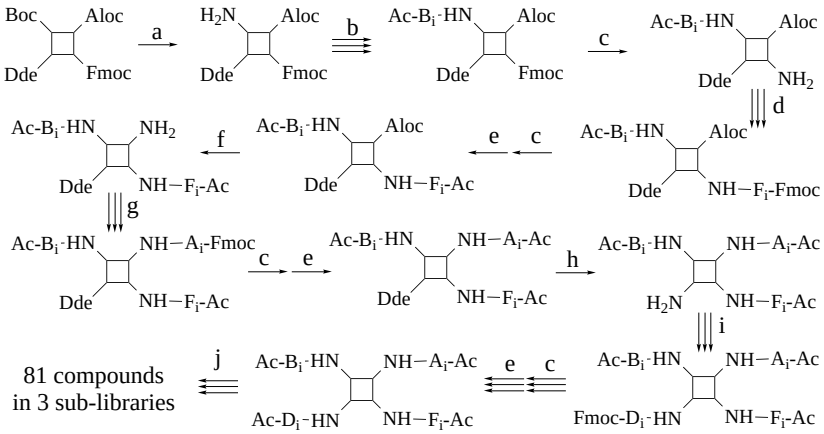


Figure 2. Strategy for the solution-phase combinatorial (split) synthesis of template **1**-based libraries. *Building blocks*: B_i: Phe, Ala or Asp(O*t*Bu); F_i: Tyr(*t*Bu), Ile or Glu(O*t*Bu); A_i: (D)-4-Cpa, Ser(*t*Bu) or (D)-Asp(O*t*Bu), with Cpa = chlorophenylalanine; D_i: Nal, Pro or Glu(O*t*Bu), with Nal = (L)-3-(2-naphtyl)alanine). *Reaction conditions*: (a) 50% TFA/DCM; (b) three parallel couplings of Ac-B_i with HBTU/DIEA; (c) 10% piperidine in DMF; (d) three parallel couplings of Fmoc-F_i with HBTU/DIEA in DMF; (e) Ac_2O/DCM; (f) $Pd[PPh_3]_4/PhSiH_3$ in DCM; (g) three parallel couplings of Fmoc-A_i with HBTU/DIEA; (h) 2% NH_2NH_2 in DMF; (i) three parallel couplings of Fmoc-D_i with HBTU/DIEA in DMF; (j) TFA.

portion, were introduced by three parallel HBTU-mediated couplings. For the first three positions (B-, F- and A-sites), after each coupling, the corresponding three sub-mixtures were combined for subsequent deprotection. However, after incorporation of building blocks at the last position (D-site), the resulting three portions were not combined, but were *separately* subjected to deprotection, acetylation and total cleavage reactions, thus finally yielding three sub-libraries each having a defined residue (Nal, Pro or Glu) at the D-site, hereafter referred to as the Nal-, Pro- and Glu-sub-libraries, respectively.

All reactions[10] were monitored by RP-HPLC. Most reactions could be well controlled by RP-HPLC. Difficulty was only met with the acetylation reaction because the retention times of acetylated and unacetylated products are very similar. Therefore, the ninhydrin test was employed to monitor the acetylation reaction.[11] After each reaction, excessive reactants and byproducts formed can be simply removed by multi-precipitation with diethyl ether.[12] By this synthesis, the Nal-, Pro- and Glu-sub-libraries each containing the expected 27 library members were obtained in crude yields of 73, 75 and 80%, respectively.[13]

As shown in Fig. 3, RP-HPLC traces of these three mixtures indicate the presence of a reasonable number of peaks in near equimolar ratio. The three sub-libraries were subjected to MALDI-TOF mass analysis. Most of the expected library compounds were apparently detected as $[M+Na^+]$ signals within the expected lowest and highest mass region. Signals out of this region were also observed and were identified as due to side products. Such undesired signals were more pronounced in the Nal-sub-library, which implies more side-products in this sub-library.

Since several compounds in each mixture have close molecular weights, these compounds appear as overlapping clusters in the MALDI-TOF MS spectra. To prove the presence of *all* library members, liquid chromatography coupled with electrospray mass spectrometry (LC/ES–MS) was applied to the three sublibraries. By these techniques, all theoretically expected 27×3 library compounds were detected and appeared as major components.[5]

By the combination of MALDI-TOF-MS and LC/ES–MS data, we were also able to assign the majority of undesired signals. Side products observed in the Pro-

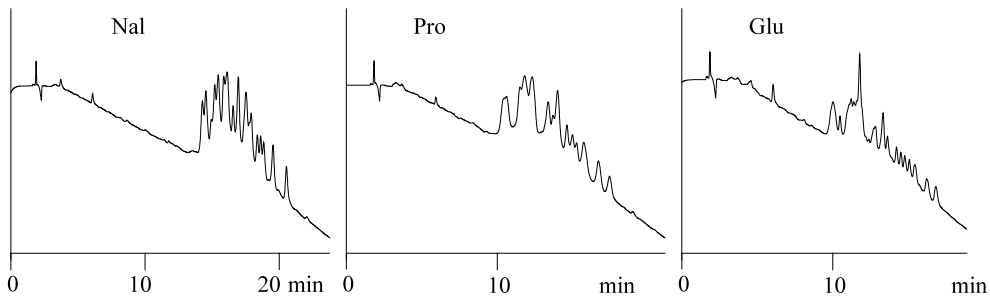


Figure 3. RP-HPLC traces (absorbance at 214 nm) of Nal-, Pro- and Glu-sub-libraries.

and Glu-sub-libraries were mainly from incomplete incorporation of building blocks on the A-site. For the Nal-sub-library, besides these side products, several side products from incomplete acetylation of the Nal residue were also observed.

These analytical results validate the three synthetic mixtures and also indicate that the solution approach to combinatorial synthesis with peptide template **1** is reliable. It is reasonable to consider that this template is suitable for convenient construction of tetrapodal libraries for specific biological purposes. Similarly, the homogeneously protected peptide templates such as, bicyclo-(K(Boc)CK(Boc)PGK(Boc)CK(Boc)PG) or the Fmoc-protected analogue can be very useful for solution-phase combinatorial synthesis. Their application involves the use of a set of pre-determined mixtures of building blocks for coupling. As a result, it will easily lead to the generation of a complete combinatorial library.

In summary, our studies show that the bicyclic decapeptide template **1** is useful for easy and fast generation of combinatorial libraries by the solution-phase approach.

Acknowledgements

The authors are indebted to the GOA program (GOA96009) and to the FWO-Vlaanderen (G.O422.98) for financial support. Q.X. thanks the University of Ghent for a grant and is grateful to C. Becu, F. Becu and F. Fant for technical assistance. B.D. is a postdoctoral fellow of the Funds for Scientific Research, Flanders.

References

1. Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385–1401.
2. Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Smith, A. B. *J. Am. Chem. Soc.* **1992**, *114*, 9699–9701.
3. Hauske, J. R.; Julin, S. M. *Tetrahedron Lett.* **1993**, *34*, 4909–4912.
4. Barry, J. F.; Davis, A. P.; Pérez-Payan, M. N. *Tetrahedron Lett.* **1999**, *40*, 2849–2852.
5. Xu, Q. *Ph.D. Thesis*; University of Ghent, 2000.
6. Manuscript in preparation.
7. Boger, D. L.; Tarby, C. M.; Myers, P. L.; Caporale, L. H. *J. Am. Chem. Soc.* **1996**, *118*, 2109–2110.
8. Cheng, S.; Tarby, C. M.; Comer, D. D.; Williams, J. P.; Caporale, L. H.; Myers, P. L.; Boger, D. L. *Bioorg. Med. Chem.* **1996**, *4*, 727–737.
9. Experimental conditions for deprotection of (a) Boc: 50% TFA/DCM (30 min); (b) Fmoc: 10% piperidine/DMF (10 min); (c) Aloc: $Pd(PPh_3)_4$ (0.8 μmol) and $PhSiH_3$ (80 μmol) in 5 mL DCM under argon (20 min); (d) Dde: 2% hydrazine/DMF (10 min).
10. Experimental conditions for coupling: 5.0 equiv. (relative to reactive amine-site on template) of protected amino acid and 5.0 equiv. of HBTU/DIEA in DMF (1 mL), reaction time: 1–2 h; for acetylation: 5.0 equiv. of acetic anhydride in DCM (1 mL) for 30 min.
11. Samples for the ninhydrin test were taken from the solid product after precipitation with ether.
12. Precipitation work-ups: reaction mixtures or solutions of crude product (1–2 mL) are diluted with 30 mL diethyl ether and after 30 min filtered to collect solid product. This procedure is repeated. For work-ups after each coupling, extractive washing with DCM (30 mL)/water(2×20 mL) is necessary.
13. Crude yield was calculated based on weight of a dry sub-mixture and its average molecular weight.