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Solution phase combinatorial chemistry using cyclotriveratrylene-based tripodal scaffolds

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Abstract

A library consisting of 40 cyclotriveratrylene-based tripodal scaffold molecules was constructed by *O*-alkylation of the CTV-triol followed by coupling of one or two amino acids; apart from the wash steps in the work-up the reaction products did not require additional purification. Preliminary screening experiments revealed that a dansylated receptor molecule selectively bound *N*-acylated dipeptides. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Recently, we found that tweezer-like receptor molecules were capable of selectively binding tripeptides from a combinatorial library.[1] We found that a more rigid hinge leading to a more preorganized tweezer structure resulted in a considerable increase of binding affinity.[2] In order to continue along these lines we are interested in developing tripodal C_3-receptor molecules containing a C_3 hinge with as much preorganisation as possible.

Although there are a number of C_3-molecules or scaffold molecules with three sites of attachment for peptides or peptidomimetics,[3–10] only a few have considerable preorganization, are capable of aligning the binding arms in a parallel way and are readily accessible on a gram scale.[7–9]

The cyclotriveratrylene core with three phenolic hydroxy groups (**1**) seemed to meet these requirements: it has a stable convex shape and the hydroxy groups position binding arms in a parallel way.† A Macromodel[11] minimized structure shows that the different handles of functionalized cyclotriveratrylene derivatives are 7–11 Å apart, whereas the distance to the centre of the tripod varies from 4–7 Å. These

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† The energy barrier for interconversion between the two crown conformations is ca. 26 kcal/mol (298 K).[13]

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distances are large enough to enable binding of a peptide but prevent intramolecular interactions between the arms, and hence collapse of the binding pocket.

2. Results and discussion

The central part of our hinge is derived from cyclotriveratrylene triol **1** which was synthesized in three steps, starting from veratryl alcohol, on a multigram scale without extensive purification procedures.[12] The phenolic hydroxyl groups enable the introduction of spacers with different lengths via simple *O*-alkylation. In order to allow solution phase chemistry it was decided to use Boc-protected bromo alkylamines **2**[14] and **3**, which were obtained from the corresponding bromo alkylamines. Tripodal scaffolds **4** and **5** were obtained in 70% yield by alkylation of triol **1** with bromides **2** and **3**, respectively, using Cs_2CO_3 as a base in DMF at 40°C (Scheme 1).‡ The Boc groups were readily removed using HCl/ether, and a Boc-protected amino acid was subsequently coupled to the free amine using benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) (3.5 equiv.) and Di-isopropylethylamine (DiPEA) (12 equiv.) in DCM (Scheme 1). After stirring overnight, the DCM was removed by evaporation and the crude product was redissolved in EtOAc. Products **6** were simply purified by subsequent alkaline (1 M Na_2CO_3) and acidic (1N $KHSO_4$) washings in order to remove excess amino acid and DiPEA/HMPA, respectively. The coupling step was carried out with a variety of amino acids using both **4** and **5** (see Scheme 1). Since the products with the propyl spacers appeared to be somewhat more soluble, the largest variety of amino acids was linked to this scaffold. In general reaction products were obtained in 68% to >95% yields, especially considering the threefold coupling per molecule. In addition, it should be emphasized that compounds resulting from incomplete coupling were not detected indicating the high efficiency of the coupling reactions.§

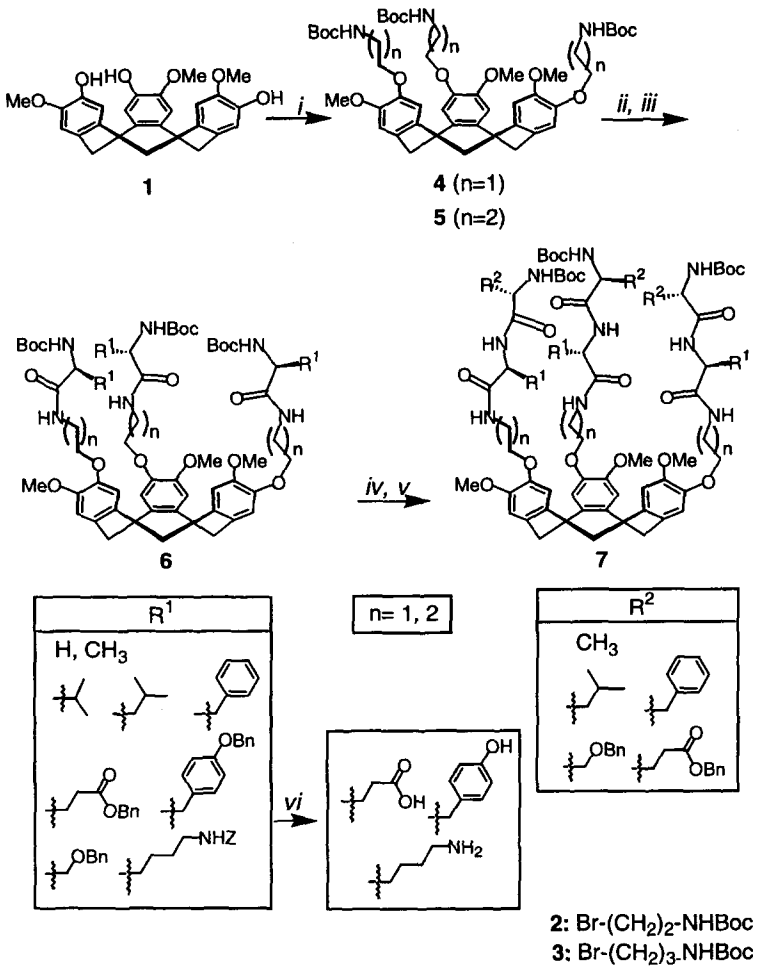
Products **6** now carrying one amino acid per spacer were obtained sufficiently pure for coupling with a second amino acid or any other functionalization (Table 1). To demonstrate this, a (small) library of CTV-scaffolds **7**, with two amino acids per handle, was synthesized (Scheme 1). Starting from compounds **6**, the Boc groups were removed and a second amino acid was coupled (vide supra). Using the convenient, simple work-up procedure described above, CTV-tripodal molecules **7** with two amino acid containing arms were obtained in 83 to >95% yield.‡ Molecules carrying up to five amino acids per arm have been synthesized, and the peptide chain can, in principle, be elongated as desired using the strategy outlined in this paper.

It was also possible to incorporate functional amino acids, e.g. Glu(OBn), Lys(Z), Ser(OBn), Tyr(OBn), into the arms of the CTV-scaffolds. The protecting groups were easily removed by hydrogenolysis. The only exceptions were the Ser(OBn) derivatives. In these cases the benzyl groups could not be removed by catalytic hydrogenation using Pd/C or $Pd(OH)_2$ and H_2 at 1 or 5 atm in a Parr apparatus.

In order to test the complexation properties of the tripodal receptor molecules, a dansylated receptor was synthesized with three propyl spacers carrying two leucine residues per arm (dansylated at the *N*-terminus) allowing screening by fluorescence microscopy. Preliminary screening results using an *N*-

‡ It should be noted that the CTV core exists as two enantiomers, viz. a P and M form.[12] The scaffolds carrying one amino acid residue show two signals on HPLC of equal intensity originating from both diastereomers. At this stage no effort was made to separate them.

§ Products **6** were characterized by ^1H (1D and 2D COSY, 300 MHz) and ^{13}C (APT) NMR spectroscopy and mass spectrometry, whereas bis-amino acid derivatives **7** were characterized by ^1H (1D and 2D COSY, 300 MHz) NMR spectroscopy and mass spectrometry. All products show one (major) spot on TLC (SiO_2, 7.5% MeOH/DCM).



Scheme 1. Reagents and conditions: (i) Cs_2CO_3, DMF, 40–50°C, **2** or **3**; (ii) HCl/ether, $CHCl_3$; (iii) Boc-amino acid, BOP, DiPEA, DCM; (iv) HCl/ether, $CHCl_3$; (v) Boc-amino acid, BOP, DiPEA, DCM; (vi) Pd/C, H_2

acylated dipeptide library[¶] on Argogel® resin, showed that this receptor selectively bound Ac-Ala-Ala compared to Ac-Ser(OBu^t)-Ala, and Ac-Leu-Ala with association constants of 55, 22 and 6 M^{-1} (in $CHCl_3$), respectively.[4]

Further extension of the combinatorial synthesis of CTV-tripodal scaffolds towards the synthesis of libraries of receptor molecules is currently under investigation and will be reported in due course.

3. Conclusions

In conclusion, we have demonstrated that a 40-member library of tripodal scaffold molecules using cyclotriveratrylene as a scaffold, is easily accessible by solution phase combinatorial chemistry methods. All products were obtained in high to excellent yields, and sufficiently pure for further functionalization. The use of orthogonal protecting groups such as Boc, Z, and benzyl groups, allows the introduction of a

[¶] 49-Member dipeptide library: Argogel-NH-Aa_1-Aa_2-NHAc; Aa_1, Aa_2: Gly, Ala, Leu, Phe, Ser(OBu^t), Lys(Boc), Glu(OBu^t).

Table 1
Summary of yields (Y/%) and R_f values[a] for a synthesized library of CTV-tripodal scaffold molecules

Spacer	R^1	R^2											
		-[b]		CH_3		$CH_2C_6H_5$		$CH_2CH(CH_3)_2$		CH_2OBn		$CH_2CH_2C(O)OBn$	
		Y	R_f	Y	R_f	Y	R_f	Y	R_f	Y	R_f	Y	R_f
Ethyl													
	CH_3	70	0.29	93	0.15	92	0.33			>95	0.33		
	CH_2OBn	>95	0.56	77	0.32					92	0.47		
	$CH_2C_6H_5$	>95	0.62	90	0.31	87	0.45			89	0.5/0.44 [c]		
Propyl													
	H	>95	0.27					84	0.30				
	CH_3	75	0.32	83	0.17	95	0.31	70	0.11 [d]	77	0.35		
	$CH_2CH(CH_3)_2$	70	0.53			90	0.44	86	0.46			87	0.41
	$CH_2C_6H_5$	68	0.68	97	0.31								
	CH_2OBn	88	0.51	>95	0.27					87	0.50		
	$(CH_2)_4NHZ$	>95	0.27	87	0.55								
	$(CH_2)_4NH_2$	>95	n.d.	>95	n.d.								
	$CH_2CH_2C(O)OBn$	>95	0.64	>95	0.46								
	$CH_2CH_2C(O)OH$	>95	n.d.	>95	0.12								
	$CH_2C_6H_4OBn$	>95	0.72	>95	0.46								
	$CH_2C_6H_4OH$	>95	0.33	>95	0.33								
	$CH(CH_3)_2$	[e]	0.50										

[a] SiO_2, 7.5% MeOH/DCM; [b] compounds **6** with only one amino acid per handle; [c] two spots with equal intensity were observed most likely corresponding to the two diastereoisomers. [d] SiO_2, 5% MeOH/DCM; [e] residual BocValOH could not be totally removed by basic washings.

variety of functional amino acids. Preliminary screening experiments revealed that a dansylated receptor molecule selectively bound *N*-acylated dipeptides.

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