Functionalized Cyclotriproline – A Bowl-Shaped Tripodal Scaffold

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Abstract: The synthesis and conformational analysis of C_3-symmetric cyclotri[(4*S*)-aminoproline] as a bowl-shaped scaffold for three-armed receptors is described.

Key words: cyclic peptides, scaffolds, proline, cyclization, receptors

Synthetic receptors are typically based on a template that is functionalized with recognition elements.[1] While the recognition elements can be regarded as the selectivity determining modules, the template is responsible for directing the recognition elements into a conformation that allows for intermolecular interactions. Thus, the choice of the template is crucial for the binding properties of synthetic receptors. Generally, templates with a defined curvature and attachment sites for the recognition elements that point into the same direction have proven superior to flexible, conformationally undefined templates.[2,3] Over the last decade, receptors based on templates that allow for the attachment of two peptides as recognition elements have shown good to excellent binding selectivities towards peptidic guest molecules.[2–5] Receptors with an additional third recognition element can be expected to exhibit even higher binding specificities and particularly affinities due to a three-point rather than a two-point binding motif. However, examples of peptide receptors with a parallel alignment of three recognition elements are still rare.[6]

Here we describe the synthesis of the cyclotriproline derivative **1** that allows for the attachment of three recognition elements after reduction of the azide functionalities. The acetylated cyclotriproline derivative **2** served as a minimal fragment of three-armed receptors for the conformational analysis of the tripodal scaffold.

For the synthesis of **1** we envisioned the linear triprolines **3** and **4** as cyclization precursors (Figure 1). Their synthesis starts from *N*-Boc-(4*S*)-N_3-Pro-OCH_3 **5** that was on one hand *N*-Boc deprotected to the HCl-salt **6** and on the other hand hydrolyzed to acid **7**, which then was transformed to pentafluorophenyl (Pfp) ester **8**. Mixing of **6** with **8** in the presence of Hünig's base provided dipeptide **9**. The tripeptide **10** was obtained after methylester hydrolysis of **9** followed by coupling of the resulting acid and **6** with HATU.[7] Hydrolysis of the methylester **10** provided the acid **11** that was partially converted into the Pfp-ester **12**. *N*-Boc deprotection of **11** and **12** with HCl in dioxane yielded the cyclization precursors **3** and **4** (Scheme 1).

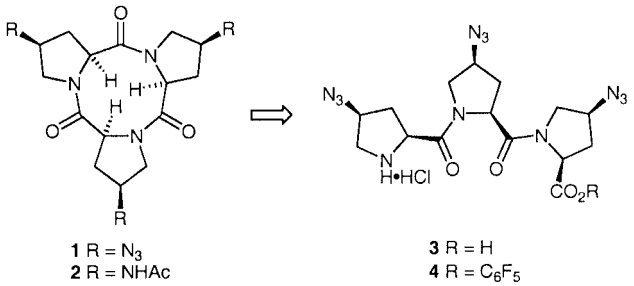
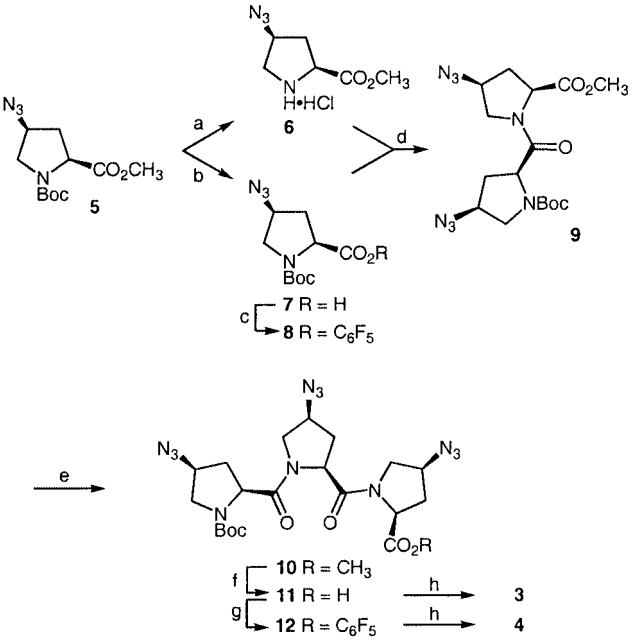


Figure 1 Cyclotriproline derivatives **1** and **2**



Scheme 1 Synthesis of the linear triprolines **3** and **4**. *Reagents and conditions*: (a) 4 M HCl in dioxane, quant; (b) NaOH, quant.; (c) C_6F_5OH, EDC, 93%; (d) *i*-$PrNEt_2$, 90%; (e) i. NaOH, ii. **5a**, HATU, *i*-$PrNEt_2$, 73%; (f) same as (b), 97%; (g) same as (c), 93%; (h) same as (a), quant.

The formation of **1** was on one hand examined by the cyclization of the Pfp-ester **4** in pyridine and on the other hand by cyclizing the acid **3** in the presence of different coupling reagents. For the cyclization of the Pfp-ester, a solution of **4** in DMF containing 1% acetic acid was slowly added to a heated solution of pyridine to a final peptide

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concentration of 1 mM. These high dilution conditions that were developed for the synthesis of non-functionalized cyclotriproline[8] provided **1** in a yield of 20% (Table 1).

The cyclization of **3** was tested with TBTU, PyBop and HATU as coupling reagents.[7,9] Solutions of **3** in DMF were dropped into the solution of the coupling reagent and Hünig's base in DMF such that a final peptide concentration of 8 mM was reached (Table 1). Under these conditions **1** was obtained in significantly higher yields. HATU proved to be the best coupling reagent, yielding 71% of **1**.[10,11]

Table 1 Cyclization of **3** and **4** to **1** with Different Coupling Reagents[a]

Entry	Reactant	Conditions	Yield [%]
1	**4**	Pyridine	20
2	**3**	PyBop	38
3	**3**	TBTU	31
4	**3**	HATU	71[b]

[a] Reaction conditions for the cyclization of **3**: slow addition of **3** to the coupling reagent (5 equiv) and *i*-Pr_2NEt (5 equiv) in DMF, 2 h, r.t.
[b] The same yield was obtained with 3 equiv of HATU and 6 equiv of *i*-Pr_2NEt.

For the conformational analysis of the tripodal scaffold we used the acetylated cyclotriproline derivative **2** since it can be regarded as a minimal fragment of a three-armed receptor with peptides as recognition elements. Compound **2** was prepared by catalytic hydrogenation of the azide **1** with Pd/C in a hydrogen atmosphere followed by reaction of the resulting triamine with acetic anhydride.

The NMR spectra of **2** show only one six-spin system for the pyrrolidine protons indicating that on the time scale of the NMR measurement **2** is C_3-symmetric. In the ^{1}H NMR spectra most vicinal coupling constants are in the range of 7–10 Hz indicating torsion angles that are either around 30–60° or 120–150° as judged by the Karplus curve.[12] Marked exceptions are the coupling constants ^3J(Hα-Hβ′) and ^3J(Hβ′-Hγ) that are close to 0 Hz thereby indicating that the torsion angles between these protons are close to 90° (Table 2).[13]

As for the positions of the NH-acetyl groups, two main conformations are conceivable: one with the NH-acetyl groups in pseudo-equatorial positions and another one with pseudo-axial NH-acetyl groups at the pyrrolidine rings. The coupling constants of close to 0 Hz between Hα and Hβ′ as well as between Hγ and Hβ′ are only in agreement with a conformation where the NH-acetyl groups occupy the pseudo-axial positions. As a result, the NH-acetyl groups point into the same direction and form a cavity with the cyclotriproline skeleton.

Table 2 ^3J(H,H) Coupling Constants (Hz, ±0.2) of **2** in $CDCl_3$ and CD_3OD[a]

Entry	(H,H)	^3J(H,H) in $CDCl_3$	^3J(H,H) in CD_3OD
1	Hα-Hβ	7.6	7.6
2	Hα-Hβ′	<1	<1
3	Hβ-Hγ	9.9	9.9
4	Hβ′-Hγ	<1	2.5
5	Hγ-Hδ	8.7	8.4
6	Hγ-Hδ′	4.2	5.5

[a] Hα, Hβ, Hγ and Hδ are on one face, Hβ′ and Hδ′ are on the opposite face of the pyrrolidine ring.

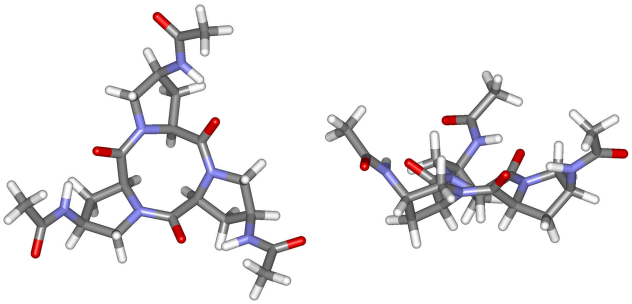

Figure 2 Lowest energy structures of the cyclotriproline **2** as calculated by MarcoModel 7.1. left: top view, right: side view.

To further support the conformational analysis we performed conformational searches using MacroModel 7.1 (Figure 2). The calculations used the OPLS-AA[14] and the AMBER force fields[15] and the GB/SA model for chloroform.[16] Searching was performed using the MCMM method in blocks of 20000 steps. The conformational searches yielded, regardless of the force field used, low-energy structures that support the conformations found for **2** by the ^{1}H NMR experiments. In the lowest energy structure the pyrrolidine rings possess envelope ($^{C\beta}E$) conformations. The NH-acetyl groups occupy the pseudo-axial positions and are in a distance of 7–8 Å away from each other.[17] The overall conformation resembles a bowl-shape with the attachment sites for the recognition elements pointing into the same direction.

Thus, cyclotri[(4*S*)-azidoproline] possesses all characteristics of a useful tripodal scaffold for three-armed receptors. We are currently employing this tridodal scaffold for the development of three-armed peptide receptors.

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References

(1) For reviews, see: (a) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perk. Trans. 1* **2002**, 841. (b) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **2001**, *40*, 3118. (c) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479.

(2) (a) Gennari, C.; Nestler, H. P.; Salom, B.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1765. (b) Löwik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwet, A. J.; Still, W. C.; Liskamp, R. M. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1846.

(3) Wennemers, H.; Nold, M. C.; Conza, M. M.; Kulicke, K. J.; Neuburger, M. *Chem.–Eur. J.* **2003**, *9*, 442.

(4) For examples, see: (a) Jensen, K. B.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem.–Eur. J.* **2002**, *8*, 1300. (b) Arienzo, R.; Kilburn, J. D. *Tetrahedron* **2002**, *58*, 711. (c) Botana, E.; Ongeri, S.; Arienzo, R.; Demarcus, M.; Frey, J. G.; Piarulli, U.; Potenza, D.; Kilburn, J. D.; Gennari, C. *Eur. J. Org. Chem.* **2001**, 4625. (d) Kyne, G. M.; Light, M. E.; Hursthouse, M. B.; de Mendoza, J.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1258. (e) Braxmeier, T.; Demarcus, M.; Fessmann, T.; McAteer, S.; Kilburn, J. D. *Chem.–Eur. J.* **2001**, *7*, 1889. (f) Fessmann, T.; Kilburn, J. D. *Angew. Chem. Int. Ed.* **1999**, *38*, 1993. (g) Davies, M.; Bonnat, M.; Guillier, F.; Kilburn, J. D.; Bradley, M. *J. Org. Chem.* **1998**, *63*, 8696.

(5) (a) Conza, M.; Wennemers, H. *J. Org. Chem.* **2002**, *67*, 2696. (b) Wennemers, H.; Conza, M.; Nold, M.; Krattiger, P. *Chem.–Eur. J.* **2001**, *7*, 3342.

(6) (a) Chamorra, C.; Liskamp, R. M. J. *J. Comb. Chem.* **2003**, *5*, 794. (b) Opatz, T.; Liskamp, R. M. J. *J. Comb. Chem.* **2002**, *4*, 275. (c) Opatz, T.; Liskamp, R. M. J. *Org. Lett.* **2001**, 3499.

(7) HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate; TBTU: *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazole-1-yl)-uronium-tetrafluoroborate; PyBop: benzotriazole-1-yloxy-tripyrrolidino-phosphonium hexafluorophosphate.

(8) Rothe, M.; Steffen, K.-D.; Rothe, I. *Angew. Chem. Int. Ed. Engl.* **1965**, *4*, 356.

(9) For the formation of cyclo[Pro-Thr($\Psi^{Me,Me}$pro)-Pro] using PyBop, see: Rückle, T.; de Lavallaz, P.; Keller, M.; Dumy, P.; Mutter, M. *Tetrahedron* **1999**, *55*, 11281.

(10) Preparation of **1a**: The HCl salt **5a** (440 mg, 0.94 mmol) was dissolved in anhyd DMF (17 mL) and added within 1 h via syringe pump to a stirred solution of HATU (1.07 g, 2.81 mmol) and Hünig's base (1.44 mL, 8.43 mmol) in anhyd DMF (90 mL). The reaction mixture was stirred for an additional hour before removal of all volatiles at reduced pressure. The remaining oil was extracted with CH_2Cl_2 (100 mL) and 2 M HCl (50 mL). The aqueous layers were extracted with CH_2Cl_2 (2 × 50 mL), the organic layers were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent at reduced pressure followed by flash chromatography on silica gel (EtOAc) yielded the cyclotripeptide **1a** (285 mg, 0.69 mmol, 73%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 5.07 (dd, *J* = 8.1 Hz, 2.1 Hz, 3 H; Hα), 4.52 (dd, *J* = 12.5 Hz, 8.1 Hz, 3 H; Hδ), 4.04 (dtd, *J* = 9.9 Hz, 8.1 Hz, 5.3 Hz, 3 H; Hγ), 3.14 (dd, *J* = 12.5 Hz, 8.2 Hz, 3 H; Hδ), 2.64 (ddd, *J* = 13.9 Hz, 5.2 Hz, 2.1 Hz, 3 H; Hβ), 2.54 (ddd, *J* = 13.8 Hz, 10.0 Hz, 8.2 Hz, 3 H; Hβ). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 165.9, 56.3, 55.7, 49.3, 34.1. FT-IR (NaCl): 2108, 1646, 1441, 1362, 1263, 1211 cm^{-1}. ESI-MS: *m/z* Calcd for $C_{15}H_{18}N_{12}O_3$ $[M + Na]^+$ 437. Found: 437 (100%). Elemental analysis calcd for $C_{15}H_{18}N_{12}O_3$ (414.16): C, 43.48; H, 4.38; N, 40.56. Found: C, 43.41; H, 4.34; N, 40.50.

(11) Performing the cyclization at higher or lower dilution did not improve the yield any further.

(12) Roberts, G. C. K. *NMR of Macromolecules – A Practical Approach*; Oxford University Press: Oxford, **1993**, 362.

(13) The coupling constants are similar to the ones observed for non-substituted cyclotriproline. See: Kessler, H.; Friedrich, A.; Hull, W. E. *J. Org. Chem.* **1981**, *46*, 3892.

(14) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.

(15) (a) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S. Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765. (b) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.

(16) Qiu, D.; Shenkin, P. S.; Hollinger, F. P.; Still, W. C. *J. Phys. Chem. A* **1997**, *101*, 3005.

(17) The distance of 7–8 Å has proven to be an ideal distance between the attachment sites of the recognition elements of two-armed receptors, see ref 3.