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A Selectively Deprotectable Triazacyclophane Scaffold for the Construction of Artificial Receptors

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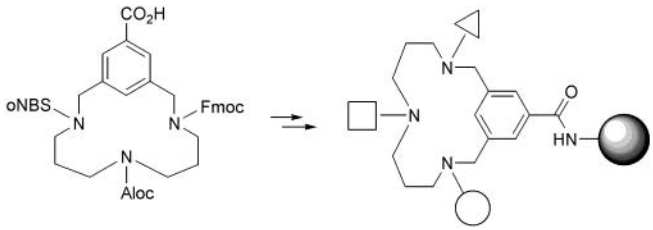
ABSTRACT



The synthesis of a triazacylclophane scaffold bearing a set of selectively removable protecting groups is described. This versatile scaffold, which can be linked to a solid support, allows the attachment of three different side chains and can therefore be used for the combinatorial synthesis of libraries of artificial receptor molecules of high structural diversity.

The construction of artificial receptors capable of selectively binding small peptides or other biologically relevant molecules is an interesting approach for uncovering new molecules with antibacterial or antifungal properties. Especially the use of combinatorial methods for the generation of large libraries of synthetic receptor molecules and subsequent screening of the binding affinity to a particular ligand may be a powerful strategy. This approach somewhat resembles the unsurpassed way the immune system fights infections by screening antigenic structures with the antibodies present on lymphocytes which constantly roam the body.

In a recent publication we demonstrated the use of a triazacyclophane scaffold for the combinatorial construction of vancomycin mimetics.[1] These artificial receptors, like the glycopeptide antibiotics vancomycin and teicoplanin, are able to bind the C-terminal D-Ala-D-Ala sequence of bacterial peptidoglycan.[2] In addition to mimicking the binding properties of the naturally occurring glycopeptides, their artificial counterparts can be designed to bind the mutant D-Ala-D-Lac sequence found in bacteria that have become resistant to these valuable drugs which are often referred to as the last resort of current antibacterial chemotherapy.[3]

In a library of our recently described tripodal receptor molecules, compound **1** (Figure 1) was able to bind both the D-Ala-D-Ala and the D-Ala-D-Lac sequences. In this

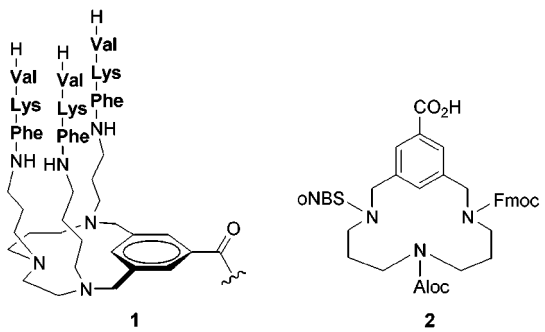


Figure 1. Triazacyclophanes.

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synthetic receptor, the central triazacyclophane ring carries three peptide chains that may form a cavity into which the cognate ligand fits.

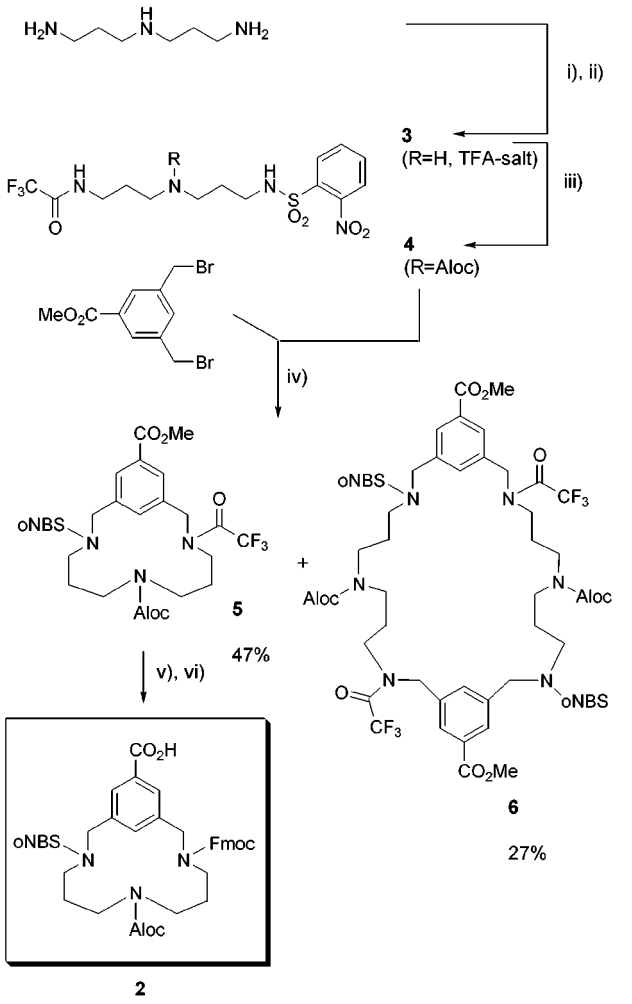
Previous investigations have shown the amino acid sequence of the receptor arms to be crucial for the selective recognition of the ligand molecules. Consequently, combinatorial solid phase synthesis and on-bead screening techniques are the methods of choice in the search for receptors with superior affinity and/or selectivity.[1,4]

Although having a large number of potential applications, only a limited number of selectively deprotectable scaffolds have been described so far. In particular, templates that in addition allow a parallel alignment of attached side chains are scarce.[5,6] Herein, we report the synthesis of **2**, a protected triazacyclophane scaffold which—upon attachment to a solid support—allows for selective introduction of a wide variety of substituents and elongation of three different peptidic or peptidomimetic chains, thus giving access to libraries of artificial receptors with a very high structural diversity. For the protection of the three secondary amines in the triazacyclophane, a combination of the Fmoc, the Aloc, and the *o*-nitrobenzenesulfonyl (oNBS) group was chosen. In contrast to the Boc-/Fmoc-/Aloc triad employed by Savage et al. for the protection of a steroid-derived triamine scaffold,[5] our protective group pattern allows the introduction of amino acids with acid labile side chain protection at any position as well as their elongation to form oligopeptide side chains following the protocols of standard Fmoc peptide chemistry.

The synthesis of **2** started with the reaction of bis(3-aminopropyl)amine (10 equiv) with *o*-nitrobenzenesulfonyl chloride. The crude product was then trifluoroacetylated using ethyl trifluoroacetate (3 equiv) in the presence of water (1 equiv) leading to trifluoroacetate salt **3**.[7,8]

This salt can be crystallized from the reaction mixture (yield: 52% over two steps), but analysis by mass spectrometry still revealed the presence of small amounts of the bis(*o*-nitrobenzenesulfonyl)amine as an impurity which was preferrably removed after introduction of the Aloc-group in the next step. This furnished the triply protected triamine **4** which was easily purified by column chromatography and obtained in 85% yield.

Scheme 1. Synthesis of **2**[a]



[a] Reagents and conditions: (i) oNBS-Cl, CH_2Cl_2, rt; then (ii) CF_3CO_2Et, H_2O, MeCN, reflux; 52% over two steps; (iii) Aloc-Cl, $NaHCO_3$, H_2O/dioxane, rt; 85%; (iv) Cs_2CO_3, Bu_4NBr, MeCN, reflux; (v) Tesser's base (neat), then (vi) Fmoc-OSu, DIPEA, H_2O/MeCN; 95% over two steps.

Subsequently, compound **4** was reacted with 3,5-bis(bromomethyl)benzoic acid methyl ester[9] under basic conditions to give the crystalline triazacyclophane **5** in an acceptable yield of 47%.[8,10] The yield of this macrocyclization reaction was significantly higher when both termini of the linear triamine precursor carried the same protecting group. Thus, the symmetrical bis(*o*-nitrobenzenesulfonyl)—as well as bis(trifluoroacetyl)—derivatives cyclized smoothly under similar conditions. The lower yield of **5** was caused by formation of considerable amounts of the dimeric byproduct **6**. Dilution of the heterogeneous reaction mixture or changing solvents or base did not prevent the formation of this remarkable 28-membered hexaazacycle. Unfortunately, desymmetrization of the above-mentioned more readily accessible symmetrical triazacyclophanes by means of double deprotection followed by mono(re)protection was unsatisfactory so that desymmetrization in the first synthetic step was preferable.

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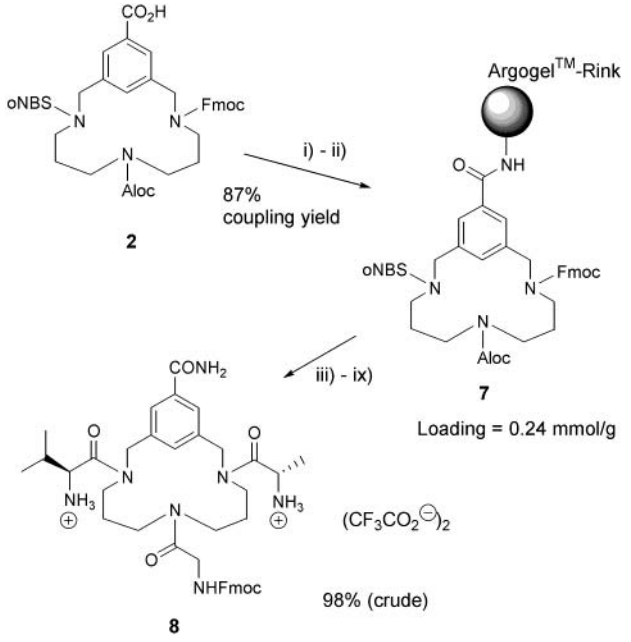
Compond **5** was crystalline, only poorly soluble in nonhalogenated solvents, and readily purified. Simultaneous cleavage of the trifluoroacetyl group and the methyl ester with Tesser's base (4 N NaOH/methanol/dioxane 1:5:14 v/v) yielded an amino carboxylic acid which without isolation was reacted with Fmoc-*O*-hydroxysuccinimide. After chromatography, triprotected carboxylic acid **2** was obtained in a yield of 95%.

As a test of its suitability for the eventual construction of artificial receptors with three different binding arms, **2** was attached via a Rink linker[11] to Argogel resin (PEG-grafted polystyrene devoid of PEG benzyl ether moieties for improved chemical stability) and the remaining amino groups on the resin were acetylated (Scheme 2). After cleavage of the Fmoc-group, Boc-L-alanine was introduced using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate[12] (HBTU) as the coupling reagent in the presence of 1-hydroxybenzotriazole (HOBt) as an additive. Despite possible steric hindrance of the ring secondary amines, fast and clean reactions were observed when a 5-fold excess of amino acid, coupling reagent, and coupling additive was used.

Cleavage of the oNBS group was achieved by treatment with 2-mercaptoethanol and DBU in dry DMF.[13] It proved to be important that no dichloromethane was present during this deprotection reaction which involves highly nucleophilic thiolate anions.[13c]

Scheme 2. Solid Phase Synthesis of **8** as a Model for Complete Functionalization[a]



[a] Reagents and conditions: (i) Argogel-Rink-NH_2, HBTU, HOBt, DIPEA, NMP; (ii) Ac_2O, pyridine, dioxane; (iii) 20% piperidine, NMP; (iv) Boc-L-Ala, HBTU, HOBt, DIPEA, DMF; (v) HS-$(CH_2)_2$OH, DBU, NMP; (vi) Boc-L-Val, HBTU, HOBt, DIPEA, DMF; (vii) *p*-TolSO_2H, morpholine, cat. $Pd(PPh_3)_4$, NMP; (viii) Fmoc-Gly, HBTU, HOBt, DIPEA, DMF; (ix) TFA, *i*Pr_3SiH, H_2O.

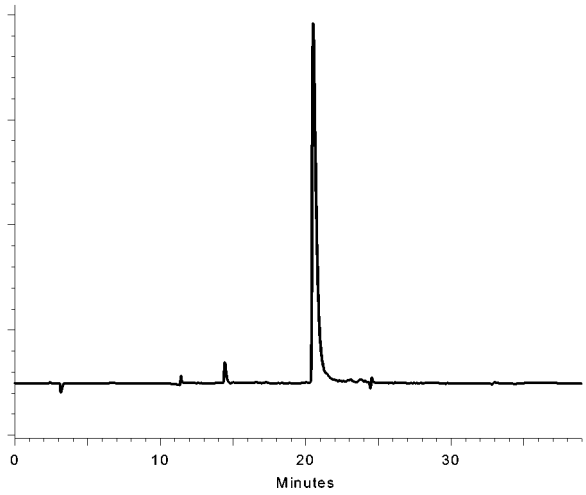


Figure 2. RP-HPLC of **8** (UV detection at 220 nm).

The second amino acid (Boc-L-valine) was then introduced. Removal of the Aloc group was carried out by Pd^0-catalyzed allyl transfer to *p*-toluenesulfinate as the trapping nucleophile.[14] Provided that a sufficiently high concentration and amount of this nucleophile (20 equiv) was used, the undesired reallylation of the liberated amine by the cationic allylpalladium intermediate could be almost completely suppressed. In addition, N-formylation during Aloc cleavage in DMF, which has recently been described by Fernández-Forner and co-workers,[15] could be prevented by using NMP as the solvent. A mixture of *p*-toluenesulfinic acid and morpholine and a solution of the stable and crystalline anilinium *p*-toluenesulfinate performed equally well in this step. Introduction of Fmoc-glycine as the third amino acid was then accomplished followed by cleavage of the Rink linker to furnish the product **8** in a yield of 98% (based on determination of the resin loading by Fmoc quantification).

The RP-HPLC and ESI-MS analyses of the crude product are shown in Figures 2 and 3.

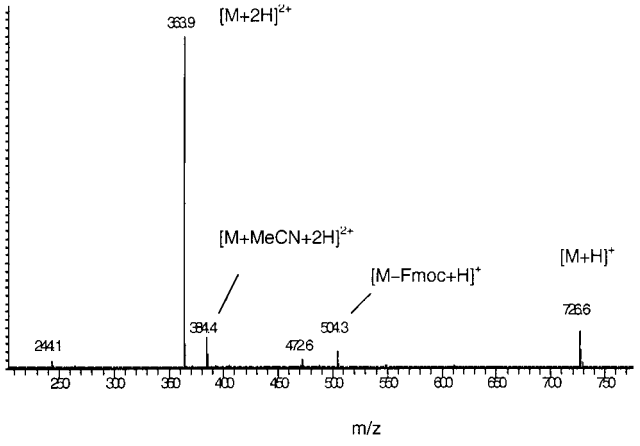


Figure 3. ESI-MS of **8** (crude product).

In conclusion, the obtained results clearly demonstrate that **2** is a convenient, selectively deprotectable scaffold which is well suited for the combinatorial synthesis of tripodal receptor molecules on the solid phase. In addition, the use of a sufficiently acid stable linker should allow the preparation of resin-bound tripods in their protected as well as in their fully deprotected form. Besides application as a scaffold for artificial receptors, under present investigation is the use of **2** as a template for construction of enzyme mimics or discontinuos epitopes.

Acknowledgment. This work was supported by the European Commission (Marie Curie Individual Fellowship, Contract No. HPMFCT-2000-00609).

Supporting Information Available: Experimental procedures, spectral data of compounds **2** to **5**, spectra of compound **8** (crude product and a purified sample) as well as combustion analysis of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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