



Expedient synthesis of a novel asymmetric selectively deprotectable derivative of the ATAC scaffold



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ABSTRACT

An efficient multigram scale synthesis of a new asymmetric triazacyclophane scaffold, the ATAC (Asymmetric-TAC) scaffold, bearing three selectively removable groups is described. This scaffold is slightly more rigid than our frequently used TAC (TriAzaCyclophane) scaffold. The synthesis of the required triamine is very high yielding without difficult steps or purifications and was also applied to a much improved synthesis of our original TAC scaffold. Especially the tedious first reaction step, that is, mono-*o*NBS-protection of a triamine could be omitted. The rigidity of the triazacyclophane ring in both TAC- and ATAC scaffolds has also been investigated using variable temperature ¹H NMR experiments.

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1. Introduction

Several scaffolds have been described in the literature to which three or more identical ligands, such as peptides can be attached, but only relatively few scaffolds capable of selective attachment of three different ligands are available.¹ One of the first representatives of this class of selectively deprotectable scaffolds, originating from our group, is the TAC (TriAzaCyclophane) scaffold (**1**), which was originally developed for the preparation of synthetic receptors.² It consists of a disubstituted benzoic acid hinge connected to a symmetric cyclophane bridge containing three amine functions, which carry three different selectively removable protecting groups (Fig. 1).^{1g,3}

In addition to the use of the TAC scaffold in the preparation of synthetic receptors we have extended its applications greatly to the preparation of synthetic vaccines,⁴ involving mimicry of discontinuous epitopes,⁵ as well as combinatorial libraries,^{2,6} for example, toward artificial enzymes.⁶ So far our work has been mainly focused on selective introduction of different ligands with relatively defined distances between points of attachment, especially for peptide ligands.

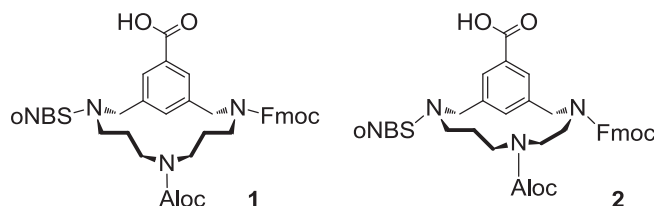


Fig. 1. Structures of the TAC (**1**) and ATAC (asymmetric-TAC) (**2**) scaffolds.

Since we have described several successful applications of site selective introduction of ligands onto the TAC scaffold, we are now increasingly directing our attention to the much more difficult issue of attempting to orient the ligands with respect to each other in space. Recently, we strongly varied the rigidity of the scaffold to influence the relative position in space of peptide loops.^{5c}

While the relatively rigid scaffolds, such as CTV,^{5c,7} calixarene⁸ and the steroid skeleton⁹ are strongly organizing, we were wondering whether we could effect directionality in space in a more subtle way by influencing the conformation of the cyclophane ring. One of the first things we wanted to investigate was reduction of cyclophane flexibility by removal of a rotatable single bond in the TAC scaffold (**1**), that is, removal of one carbon atom leading to ATAC (Asymmetric-TAC) scaffold **2** (Fig. 1). Relatively undemanding molecular modeling experiments (molecular mechanics energy minimization) showed that in the TAC scaffold the ligands are localized on average more above and below the plane of the

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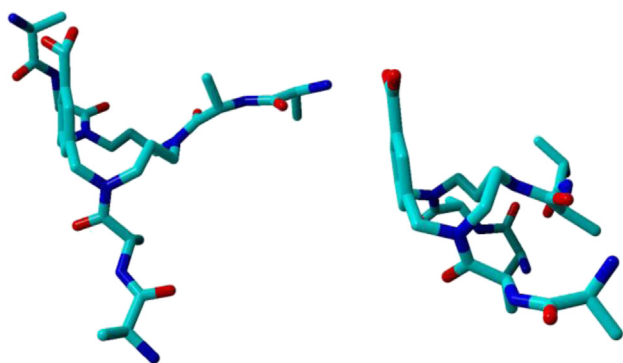


Fig. 2. Molecular modeling images for comparison of the spatial orientation of three attached peptides between the TAC (**18**, left) and ATAC (**19**, right) scaffolds. Both scaffolds contain three di-alanine peptides (see [Supplementary data](#) for both structures).

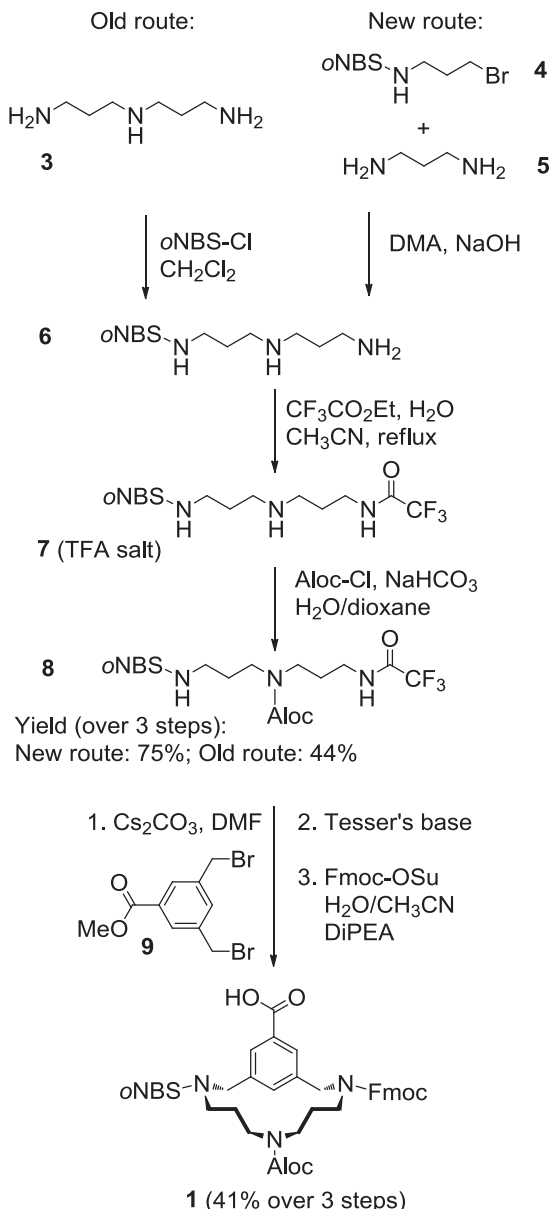
cyclophane ring as compared to being present within the plane. In the ATAC scaffold this is the other way around, that is, the ligands are predominantly located in the plane of the cyclophane ring ([Fig. 2](#)). This increased inclination towards a more defined position on the cyclophane ring might be favorable for a better orientation of ligands in a similar direction.

This asymmetric triazacyclophane (ATAC) scaffold (**2**) has been prepared and applied earlier by us, containing three identical peptide sequences.^{2a} However, for our applications it is essential that any future scaffold including the ATAC scaffold should be selectively deprotectable for introduction of at least three different ligands.

For synthesis of the ATAC scaffold with three selectively removable protecting groups on the amino functionalities, it was decided to adapt the original TAC synthetic route. The first step in this synthesis was a mono-*o*NBS-protection (*o*-nitrobenzenesulfonyl) of bis(3-aminopropyl) amine (**3**, [Scheme 1](#), left), which was a low yielding step due to the tedious workup.^{1g} This adaptation was also required for the synthesis of the ATAC scaffold, since the required triamine is not symmetrical and mono-*o*NBS-protection according to the previous route ([Scheme 1](#), left) will result in a complex mixture and an even further lower yield. Therefore, a stepwise and much more selective approach was chosen, starting with a reaction between *o*NBS-protected 3-bromopropylamine (**4**) and propyl or ethyl di-amine. This novel approach was first evaluated for an alternative synthesis of the TAC scaffold. An improvement of its synthesis was also desirable in view of our continuing demands for large amounts of the TAC scaffold.

2. Results and discussion

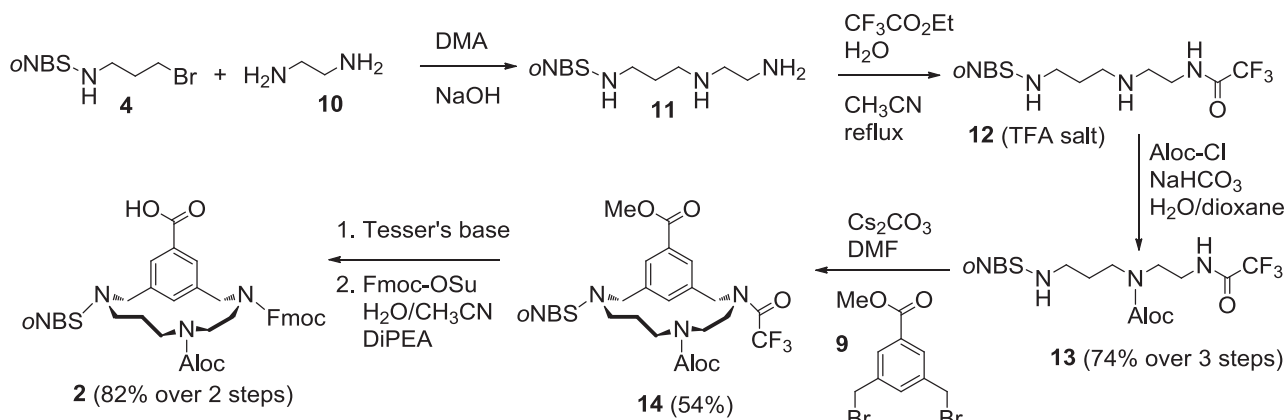
*o*NBS-protected 3-bromopropylamine (**4**)¹⁰ was reacted with 10 equiv of 1,3-diaminopropane (**5**) in DMA as a solvent ([Scheme 1](#)). After stirring overnight, 1 equiv of NaOH (4.0 M) was added to liberate all protonated amines, which was necessary for removal of excess diamine by evaporation. After co-evaporation with DMA until the receiving flask was pH neutral, the crude—almost pure—mono-protected triamine **6** was obtained. This was directly used for selective introduction of a trifluoroacetyl group on the free primary amine by treatment with ethyl trifluoroacetate. The workup was again only evaporation of the solvents and excess ethyl trifluoroacetate, leading to bis-protected triamine **7** in relatively high purity (based on TLC). Introduction of the last protecting group, the Aloc-group, took place by reaction with Aloc-chloride under Schotten Baumann conditions using NaHCO₃ as a base in a water/dioxane mixture. Purification by column chromatography afforded tri-protected amine **8** in a high yield of 75% (91% average per step), which was a huge improvement over the previous



Scheme 1. Synthesis routes of the TAC scaffold (left: original route; right: new route).

synthetic route with an overall yield of 44%. Subsequently, reaction of **8** with dibromide **9**¹¹ using cesium carbonate as a base gave the TAC scaffold. Treatment with Tesser's base¹² for saponification of the methyl ester and cleavage of the trifluoroacetyl group was followed by Fmoc-protection, which afforded the desired protected TAC scaffold **1** in 41% yield, containing the *o*NBS, Aloc and Fmoc protecting groups in addition to the carboxylic acid moiety, which, for example, can be used for attachment to the solid phase or other purposes.

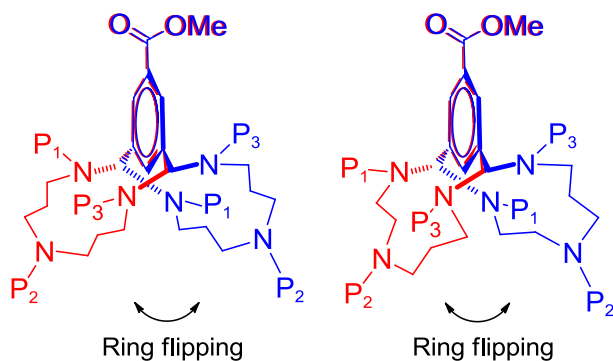
This approach was now applied to the synthesis of the ATAC scaffold ([Scheme 2](#)). Thus, *o*NBS-protected 3-bromopropylamine (**4**) was reacted with 10 equiv of 1,2-diaminoethane (**10**) in DMA, to give mono-*o*NBS-protected triamine **11**. Removal of excess of the more volatile diamine by co-evaporation with DMA was clearly easier than removal of 1,3-diaminopropane. The purity of crude **11** was very good (TLC) and comparable to **6** in the TAC synthesis. Trifluoroacetylation to afford bis-protected triamine **12**, was followed by introduction of the Aloc-group, yielding tri-protected amine **13** in high yield (74%, 90% per step), after column



Scheme 2. Synthesis of the ATAC scaffold (2).

chromatography. Cyclization with dibromide **9** in the presence of cesium carbonate afforded ATAC scaffold **14** in 54% yield. Treatment with Tesser's base,¹² followed by Fmoc-protection afforded the desired protected ATAC scaffold **2** in 82% yield.

After characterization of both ATAC scaffolds (**2** and **14**), we were wondering whether ring flipping would occur as easily as with the TAC scaffold (Scheme 3).

Scheme 3. Superimposed conformers resulting from-cyclophane ring flipping of TAC (**15**, left) and the ATAC (**16**, right) scaffolds (P_{1–3}=oNBS).^{2a}

Ring flipping is a phenomenon, which can occur with macrocycles, leading to different conformers, which can be observed in ¹H NMR spectra.¹³ The ease of ring flipping is proportional/correlated to the rigidity of the triazacyclophane ring.^{13d}

Thus, ring flipping properties of both oNBS-protected TAC and ATAC scaffolds (**15** and **16**, Scheme 3)^{2a} were studied by ¹H NMR. Since the ATAC triazacyclophane ring is one carbon atom smaller

than that of TAC, it was expected that ring flipping would be more difficult. Ring flipping was monitored using variable temperature ¹H NMR experiments (Fig. 3).¹³ At room temperature, the benzylic CH₂ groups in the scaffold were visible as a singlet for the TAC scaffold, and two slightly broader singlets for the ATAC scaffold, due to the asymmetry of this scaffold.

When measured at –40 °C, the TAC scaffold still showed a singlet (broader), but in the ¹H NMR spectrum of ATAC three very broad peaks were visible now instead of the earlier two singlets. This indicated that ATAC scaffold ring flipping at about –40 °C is very slow on the NMR timescale, in contrast to the TAC scaffold. The singlet of the TAC scaffold started to broaden significantly after further cooling to –80 °C, indicating a slower ring flipping, whereas the ATAC scaffold stopped ring flipping at about –80 °C, shown by the double AB system for the benzylic CH₂ protons. Determination of the coalescence temperatures gave –80 °C for the TAC scaffold, and –35 °C for the ATAC scaffold. These results showed that the ATAC scaffold represents a more constrained system, which might be beneficial for alignment of ligands attached to it.

3. Conclusions

In search of approaches for influencing the conformation of the cyclophane ring of the TAC scaffold, we have reduced the size of the cyclophane ring. To realize this, we have described an efficient method for the multigram scale synthesis of a novel asymmetric selectively deprotectable ATAC scaffold. Although this scaffold is very similar to our TAC scaffold, its cyclophane ring is only one CH₂-unit smaller, it is considerably less flexible. The same new synthetic approach was also very useful for a convenient large scale high

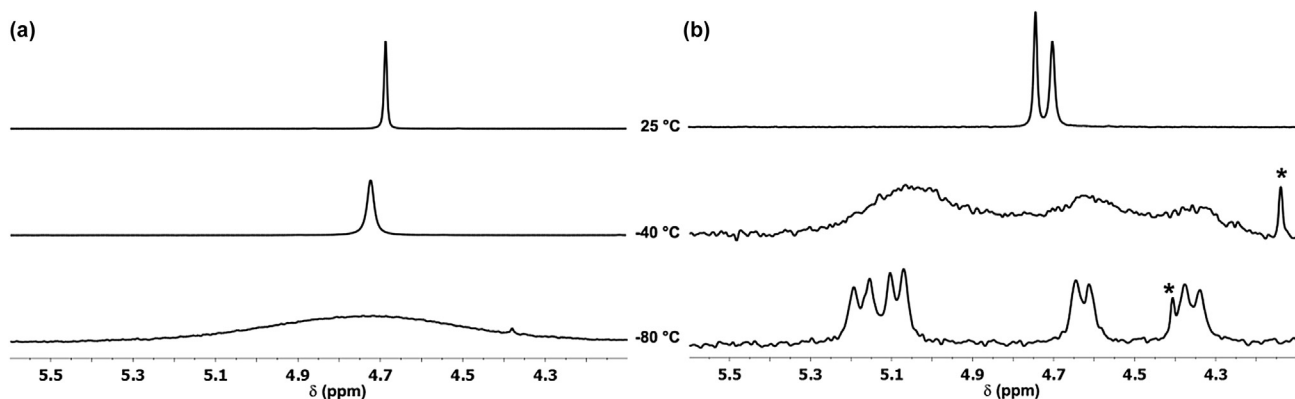


Fig. 3. Variable temperature ¹H NMR (acetone-*d*₆) signals for the diastereotopic benzylic CH₂ protons from TAC scaffold **15** (a) and ATAC scaffold **16** (b). * = A solvent peak, which shifts with temperature change.

yielding synthesis of the TAC scaffold. The crucial step in the synthesis of both scaffolds is the stepwise synthetic approach of a mono *o*NBS-protected triamine.

Investigation of the conformational behavior by ^1H NMR of both the TAC and ATAC scaffolds showed that ring flipping of the latter was more difficult. This increasing rigidity, due to the smaller size of the triazacyclophane ring, may affect the orientation of ligands to the ATAC scaffold and is therefore interesting for mimicry of discontinuous epitopes in protein mimics.

Under present investigation are additional structural modifications including those of the aromatic ring, which could provide additional pre-organization and therefore an increased capacity of the TAC-ring system to direct the positioning of substituents.¹⁴

4. Experimental section

4.1. Materials and methods

Chemicals were purchased from Aldrich. Peptide grade and HPLC grade solvents for synthesis and HPLC were purchased from Biosolve (The Netherlands). All solvent mixtures (eluent) are given in v/v. Reactions were carried out at ambient temperature unless stated otherwise. TLC analysis was performed on Merck pre-coated silica gel 60 F-254 (0.25 mm) plates. Spots were visualized with UV light, ninhydrin or Cl_2 -TDM.¹⁵ Solvents were evaporated under reduced pressure at 40 °C. Column chromatography was performed on Siliacflash P60 (40–63 μm) from Silicycle (Canada). Analytical HPLC was performed on a Shimadzu HPLC using a C18 Gemini column at a flow rate of 1.0 mL/min. Buffers used: buffer A (0.1% TFA in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 5/95) and buffer B (0.1% TFA in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 95/5). Runs started with an isocratic flow of buffer A (100%, 2 min), followed by a linear gradient of buffer B (100% in 48 min). Subsequently an isocratic flow of buffer B (100%, 2 min) was performed followed by a linear gradient to buffer A (100%, 5 min). The run ended with an isocratic flow of buffer A (100%, 5 min). Elemental analyses were carried out at Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany). High resolution mass spectrometry (HRMS) spectra were measured on an ESI-MS instrument. ^{13}C NMR (75 MHz), COSY and ^{19}F NMR (282 MHz) were recorded on a Varian M-300 spectrometer, ^1H NMR (500 MHz), TOCSY and HSQC spectra were recorded on a Varian Inova 500 spectrometer. Variable temperature ^1H NMR spectra (400 MHz) were recorded on a Varian S400 spectrometer. Chemical shifts (δ) are reported in parts per million relative to DMSO (2.50 ppm) for ^1H NMR, to DMSO- d_6 (39.52 ppm) for ^{13}C NMR and to α,α,α -trifluorotoluene (−63.72 ppm) for ^{19}F NMR as internal standards. Some of the ^{13}C NMR spectra were recorded using the attached proton test (apt) pulse sequence. The ^1H NMR spectra of **13** and scaffolds containing a TFA-group (**14**, **17** (see [Supplementary data](#))) contained many additional peaks, due the *cis/trans* isomers of the TFA-amide. The ^1H NMR spectra of scaffolds **1** and **2** also contained additional peaks due to the presence of Fmoc-rotamers. Some ^{13}C spectra contain an additional peak at 79.2 ppm, which was caused by an interference with the spectrometer. This peak was not visible in the ^{13}C spectra obtained using the attached proton sequence.

4.2. Synthesis

4.2.1. *N*-(3-Bromopropyl)-2-nitrobenzenesulfonamide (4). *N*-(3-Bromopropyl)-2-nitrobenzenesulfonamide (**4**) was prepared according to the literature.¹⁰ This procedure was slightly modified for use on a large scale.

To a cooled (ice bath) mixture of 3-bromopropylamine (113.3 g, 517.5 mmol), 2-nitrobenzenesulfonyl chloride (88.6 g, 388 mmol) and dichloromethane (800 mL) was added dropwise triethylamine (140 mL, 1.0 mol). During addition, 3-bromopropylamine dissolves

slowly. Directly a precipitate formed, which was probably $\text{Et}_3\text{N} \cdot \text{HBr}$. The mixture was stirred overnight at rt, affording a yellow suspension. After evaporation in vacuo, EtOAc (1.2 L) was added and the mixture was washed with a KHSO_4 solution (1.0 M, 600 mL, twice), a NaHCO_3 solution (5% w/w, 600 mL, twice) and brine. Drying (Na_2SO_4) followed by concentration in vacuo and by co-evaporation with CHCl_3 yielded **4** as an off-white solid (117.7 g, 92%). All spectroscopic data were in agreement with the literature.¹⁰

4.2.2. Allyl (3-(2-nitrophenylsulfonamido)propyl)(3-(2,2,2-trifluoroacetamido)propyl)carbamate (8). To a cooled (ice bath) solution of 1,3-diaminopropane (**5**) (50.5 mL, 600 mmol) in DMA (250 mL), was added dropwise a solution of *N*-(3-bromopropyl)-2-nitrobenzenesulfonamide (**4**) (19.5 g, 60 mmol) in DMA (180 mL). During addition the solution slowly turned green. After stirring overnight at rt, the reaction mixture turned yellow with a white precipitate. A solution of NaOH (4.0 M, 15 mL, 1.0 equiv) was added, after which the precipitate fully dissolved. The mixture was concentrated in vacuo until a third of the volume. DMA (200 mL) was added and again the mixture was concentrated until a third of the volume. This co-evaporation was repeated (usually three times) until the collected DMA was not basic anymore (pH indicator paper). After evaporation in vacuo of all the DMA, crude **6** was obtained as a yellow oil. To crude **6** was added acetonitrile (200 mL), water (1.08 mL, 60 mmol) and ethyl trifluoroacetate (28.6 mL, 240 mmol). After stirring overnight under reflux, the mixture was concentrated in vacuo to afford crude **7**. To a cooled (ice bath) solution of crude **7**, NaHCO_3 (20.2 g, 240 mmol), water (200 mL) and dioxane (200 mL), was added dropwise a solution of allyl chloroformate (7.68 mL, 72 mmol) in 100 mL dioxane. After stirring overnight, the dioxane was evaporated in vacuo and EtOAc (400 mL) was added. The organic layer was washed with a solution of NaHCO_3 (5%, w/w, 200 mL), KHSO_4 (1.0 M, 200 mL, twice) and brine. Drying (Na_2SO_4) and concentration afforded crude **8** as a yellow oil (30 g). Column chromatography (eluent: EtOAc/hexanes, 4/6 until first a yellow band was eluted, then 1/1 until the product started to elute, then 6/4 until all product had eluted) afforded **8** as a thick yellowish oil, which slowly solidified (22.3 g, 75%). R_f = 0.28 (acetone/ CH_2Cl_2 ; 5/95). ^1H NMR (500 MHz, DMSO- d_6) δ : 1.65 (m, 4H, $2 \times \text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.88 (t, J = 7.0 Hz, 2H, $\text{oNBSNHCH}_2\text{CH}_2\text{CH}_2\text{NAlOc}$), 3.16 (m, 6H, $\text{TFANHCH}_2\text{CH}_2\text{CH}_2\text{N}$, CH_2NHoNBS), 4.46 (d, J = 5.0 Hz, 2H, OCH_2 (AlOc)), 5.14, 5.22 (2d, J_{AX} = 10.4 Hz, J_{BX} = 16.25 Hz, 2H, $=\text{CH}_2$ (AlOc)), 5.86 (m, 1H, $=\text{CH}$ (AlOc)), 7.86, 7.98 (2m, 4H, Ar-CH (oNBS)). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 27.1, 27.6, 27.9, 28.5 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 37.0 (NCH_2), 40.5 ($\text{oNBSNHCH}_2\text{CH}_2\text{CH}_2\text{NAlOc}$), 44.1, 44.3 ($2 \times \text{NCH}_2$), 65.1 (OCH_2 (AlOc)), 110.2, 114.0, 117.9, 121.7 (q, J = 288 Hz, CF_3), 117.0 ($=\text{CH}_2$ (AlOc)), 124.4, 132.6, 133.4, 134.0, 147.8 (Ar-C (oNBS)), 129.5 ($=\text{CH}$ (AlOc)), 155.0 (C=O (AlOc)), 155.5, 156.0, 156.5, 156.9 (q, J = 36 Hz, C(O) CF_3). ^{19}F NMR (282 MHz, DMSO- d_6) δ : −77.0 (s). HRMS: calcd for $\text{C}_{18}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_7\text{S}$ [$\text{M}+\text{H}$] $^+$ 497.1318, found 497.1317. Anal. Calcd for $\text{C}_{18}\text{H}_{23}\text{F}_3\text{N}_4\text{O}_7\text{S}$: C, 43.55; H, 4.67; F, 11.48; N, 11.29. Found: C, 43.43; H, 4.41; F, 11.12; N, 11.00.

4.2.3. TAC scaffold 1. A suspension of triamine **8** (4.68 g, 10.0 mmol), dibromide **9**¹¹ (3.22 g, 10.0 mmol), and Cs_2CO_3 (13.0 g, 40.0 mmol) in DMF (1.0 L) was stirred at rt overnight (16 h). The color of the reaction mixture changed from yellow to light orange. After evaporation of the DMF in vacuo, EtOAc (500 mL) and water (300 mL) were added. The organic layer was washed with an aqueous solution of KHSO_4 (1 M, 250 mL) and with brine. Drying (Na_2SO_4) and concentration in vacuo afforded the crude product as an orange oil or foam, which was purified using column chromatography (eluent: acetone/ CH_2Cl_2 ; 2/98). The cyclic product (**17**, see [Supplementary data](#)) was obtained as a yellowish foam (3.37 g,

51%). R_f =0.30 (acetone/ CH_2Cl_2 ; 5/95). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 1.25 (m, 3H, $1\frac{1}{2}\times \text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.46 (m, 1H, $\frac{1}{2}\times \text{NCH}_2\text{CH}_2$), 2.81 (t, J =7.6 Hz, 1H, $\frac{1}{2}\times \text{NCH}_2\text{CH}_2$), 2.94 (m, 3H, $1\frac{1}{2}\times \text{NCH}_2\text{CH}_2$), 3.30 (m, 3H, $1\frac{1}{2}\times \text{NCH}_2\text{CH}_2$), 3.51 (t, J =6.4 Hz, 1H, $\frac{1}{2}\times \text{NCH}_2\text{CH}_2$), 3.86, 3.88 (2s, 3H, OCH_3), 4.41 (d, J =3.9 Hz, 2H, OCH_2 (Aloc)), 4.56, 4.59, 4.76, 4.82 (4s, 4H, PhCH_2), 5.12 (dd, J_{AX} =10.5 Hz, J_{AB} =1.3 Hz, 1H, $=\text{CH}^{\text{A}}$ (Aloc)), 5.17 (dd, J_{BX} =17.3 Hz, J_{AB} =1.3 Hz, 1H, $=\text{CH}^{\text{B}}$ (Aloc)), 5.83 (m, 1H, $=\text{CH}$ (Aloc)), 7.90–8.12 (2m, 7H, Ar–CH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 27.9, 28.3 ($\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 44.6, 45.1, 46.3, 46.7, 47.4, 47.6 (NCH_2), 51.0, 51.8, 52.4 (PhCH_2), 52.3 (OCH_3), 65.0 (OCH_2 (Aloc)), 110.7, 114.6, 118.4, 122.2 (q, J =288 Hz, CF_3), 116.6, 116.7 ($=\text{CH}_2$ (Aloc)), 124.5, 128.9, 129.1, 129.3, 129.5, 129.7, 130.8, 131.0, 132.7, 133.3, 133.9, 134.8, 136.9, 138.3, 138.9, 147.9 (Ar–C, $=\text{CH}$ (Aloc)), 154.7, 154.8 ($\text{C}=\text{O}$ (Aloc)), 155.5, 156.0, 156.4, 156.9 (q, J =35 Hz, $\text{C}(\text{O})\text{CF}_3$), 156.3, 156.8, 157.3, 157.7 (q, J =35 Hz, $\text{C}(\text{O})\text{CF}_3$), 165.5, 165.6 (CO_2Me). ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$) δ : –69.2 (s), –70.2 (s). HRMS: calcd for $\text{C}_{28}\text{H}_{32}\text{F}_3\text{N}_4\text{O}_9\text{S}$ $[\text{M}+\text{H}]^+$ 657.1842, found 657.1824.

A solution of cyclic product **17** (2.51 g, 4.0 mmol) in Tesser's base (140 mL, 28 mmol, dioxane/methanol/4 M NaOH; 15/4/1) was stirred overnight (16 h) at rt. The reaction mixture changed from clear yellow to turbid yellow. The pH of the mixture was adjusted to neutral (indicator paper) by addition of an aqueous solution of HCl (1.0 M, 24 mL), leading to a slight warming-up of the reaction mixture and a yellow solution. After addition of CH_3CN (65 mL) and water (65 mL), the pH was adjusted to approximately eight using DiPEA (using pH electrode). Then, a solution of Fmoc-OSu (1.48 g, 4.4 mmol) in CH_3CN (15 mL) was added, followed by dropwise addition of DiPEA to maintain the pH around 8. The reaction was considered complete when no more DiPEA was needed to maintain the pH above 7.5 during 10 min, under nitrogen atmosphere. Addition of an aqueous solution of HCl (1.0 M, 30 mL) and water (400 mL) was followed by extraction with EtOAc (twice; first with 250 mL, then with 150 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo affording a yellow oil or foam. Column chromatography (eluent: gradient from EtOAc/hexanes 2/1 to 3/1, and finally to EtOAc) afforded ATAC scaffold **1** as a yellowish foam (2.48 g, 81%). R_f =0.30 (EtOAc). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 0.82 (m, 1.3H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.17, 1.30 (2m, 2.7H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.32, 2.84, 3.27 (3m, 8H, $2\times \text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 4.31 (m, 1H, CH (Fmoc)), 4.42–4.66 (m, 8H, OCH_2 (Aloc), $2\times \text{PhCH}_2$, CH_2 (Fmoc)), 5.15 (m, 2H, $=\text{CH}_2$ (Aloc)), 5.84 (m, 1H, $=\text{CH}$ (Aloc)), 7.27–8.31 (m, 15H, Ar–CH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 27.7, 28.1 ($\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 44.5, 44.9, 45.7, 47.7 ($\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 46.8 (CH (Fmoc)), 51.5, 52.8 (PhCH_2), 64.9, 66.2, 66.9 (OCH_2 (Aloc), CH_2 (Fmoc)), 116.7 ($=\text{CH}_2$ (Aloc)), 120.0, 124.5, 124.9, 127.0, 127.4, 128.8, 129.1, 129.6, 130.9, 131.7, 132.1, 132.6, 134.7, 138.1, 140.3, 140.9, 144.0, 147.9 (Ar–C), 133.5 ($=\text{CH}$ (Aloc)), 154.7, 155.4, 156.0 ($\text{C}=\text{O}$ (Aloc, Fmoc)), 166.8 (CO_2H). HRMS: calcd for $\text{C}_{40}\text{H}_{41}\text{N}_4\text{O}_{10}\text{S}$ $[\text{M}+\text{H}]^+$ 769.2543, found 769.2564. Anal. Calcd for $\text{C}_{40}\text{H}_{40}\text{N}_4\text{O}_{10}\text{S}\cdot\text{H}_2\text{O}$: C, 61.06; H, 5.38; N, 7.12. Found: C, 61.20; H, 4.95; N, 7.00.

4.2.4. Allyl (3-(2-nitrophenylsulfonamido)propyl)(2-(2,2,2-trifluoroacetamido)ethyl)carbamate (13). Triamine **13** was prepared using the same procedure as for the synthesis of **8**. The synthesis was performed on 56.7 mmol scale, solvent volumes were the same as used for **8**. 1,2-Diaminoethane (**10**) was used as the diamine. Purification was performed using the same eluent for column chromatography as used for **8**, affording **13** as a yellowish solid (20.4 g, 74%). R_f =0.22 (acetone/ CH_2Cl_2 ; 5/95). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 1.71 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 2.90 (m, 2H, $\text{oNBSNHCH}_2\text{CH}_2\text{CH}_2\text{Naloc}$), 3.19 (m, 2H, CH_2NHonBS), 3.41 (m, 4H, $\text{HNCH}_2\text{CH}_2\text{NH}$), 4.46 (d, J =5.0 Hz, 2H, OCH_2 (Aloc)), 5.16 (2d, J =8.7 Hz ($2\times$), 1H, $=\text{CH}^{\text{A}}$ (Aloc)), 5.24 (2d, J =15.8 Hz ($2\times$), 1H, $=\text{CH}^{\text{B}}$ (Aloc)), 5.87 (m, 1H, $=\text{CH}$ (Aloc)), 7.86, 7.98 (2m, 4H, Ar–CH (oNBS)), 8.10 (br s, 1H, NH), 9.45 (br s, 1H, NH). ^{13}C

NMR (75 MHz, $\text{DMSO}-d_6$) δ : 27.7, 28.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 36.2, 36.9, 37.5, 37.8 ($\text{NCH}_2\text{CH}_2\text{N}$), 40.4 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{Naloc}$), 44.0, 44.4, 44.6, 45.0, 45.2, 45.6 (oNBSNHCH_2 , $\text{NCH}_2\text{CH}_2\text{N}$), 65.2 (OCH_2 (Aloc)), 110.2, 114.0, 117.8, 121.6 (q, J =288 Hz, CF_3), 116.8 ($=\text{CH}_2$ (Aloc)), 124.4, 129.5, 132.6, 133.2, 134.0, 147.8 (Ar–C (oNBS)), 129.5 ($=\text{CH}$ (Aloc)), 155.1, 155.3 ($\text{C}=\text{O}$ (Aloc)), 155.7, 156.2, 156.7, 157.2 (q, J =35.9 Hz, $\text{C}(\text{O})\text{CF}_3$). ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$) δ : –70.0 (s), –70.9 (s), –77.1 (s), –77.2 (s). The ^{19}F NMR spectrum showed four peaks, while only one or two peaks were expected (*cis/trans* isomers of the TFA-amide bond). Therefore, its purity was confirmed by analytical HPLC (retention time: 33.3 min, see [Supplementary data](#)), showing that the extra peaks were not from an impurity, but possibly from a conformer. This conformer was also visible in the ^1H NMR spectrum, which contained some small additional peaks with a similar pattern in the TOCSY spectrum as **13**. VT NMR up to 70 °C supported the presence of conformers. HRMS: calcd for $\text{C}_{17}\text{H}_{22}\text{F}_3\text{N}_4\text{O}_7\text{S}$ $[\text{M}+\text{H}]^+$ 483.1161, found 483.1117. Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{F}_3\text{N}_4\text{O}_7\text{S}\cdot\frac{1}{2}\text{H}_2\text{O}$: C, 41.55; H, 4.51; F, 11.60; N, 11.40. Found: C, 41.74; H, 4.15; F, 11.23; N, 11.40.

4.2.5. ATAC scaffold 14. Cyclization was performed as described for the synthesis of **1**, using triamine **13** (4.82 g, 10.0 mmol), dibromide **9**¹¹ (3.22 g, 10.0 mmol), and Cs_2CO_3 (13.0 g, 40.0 mmol) in DMF (1.0 L). The color of the reaction mixture changed overnight from yellow to light orange. After extraction, the crude product was obtained as an orange oil or foam, which was purified using column chromatography (eluent: acetone/ CH_2Cl_2 ; 2/98). ATAC scaffold **14** was obtained as a yellowish foam (3.48 g, 54%). R_f =0.31 (acetone/ CH_2Cl_2 ; 5/95). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 1.08 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.36, 2.50 (2m, 2H, $\text{NCH}_2\text{CH}_2\text{N}$), 3.13, 3.23, 3.49 (3m, 4H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.60 (m, 2H, $\text{NCH}_2\text{CH}_2\text{N}$), 3.87, 3.97 (2s, 3H, OCH_3), 4.46 (m, 2H, OCH_2 (Aloc)), 4.66, 4.81, 4.94 (3s, 4H, PhCH_2), 5.14, 5.16 (2d, J =8.3 Hz ($2\times$), $=\text{CH}^{\text{A}}$ (Aloc)), 5.20, 5.29 (2d, J =17.4 Hz ($2\times$), 1H, $=\text{CH}^{\text{B}}$ (Aloc)), 5.86 (m, 1H, $=\text{CH}$ (Aloc)), 7.86–8.08 (m, 7H, Ar–CH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 24.7, 25.2, 25.8 ($\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 40.8, 41.0, 42.5, 42.7, 43.2, 43.4, 45.7, 48.8, 48.9 (NCH_2), 51.4, 52.0, 53.1, 53.3 (PhCH_2), 52.5 ($2\times$) (OCH_3), 65.2, 65.4, 65.6 (OCH_2 (Aloc)), 110.7, 114.5, 118.3, 122.2 (q, J =288 Hz, CF_3), 110.8, 114.6, 118.5, 122.3 (q, J =288 Hz, CF_3), 116.3, 117.0, 117.2 ($=\text{CH}_2$ (Aloc)), 124.6, 128.4, 128.9, 129.3, 129.4, 131.2, 131.3, 131.5, 132.9, 133.0, 134.8, 136.6, 138.0, 139.9, 140.0, 148.1 (Ar–C), 133.4 ($=\text{CH}$ (Aloc)), 154.6, 154.7, 154.9 ($\text{C}=\text{O}$ (Aloc)), 154.9, 155.3, 155.8, 156.3 (q, J =35 Hz, $\text{C}(\text{O})\text{CF}_3$), 155.8, 156.3, 156.8, 157.2 (q, J =35 Hz, $\text{C}(\text{O})\text{CF}_3$), 155.9, 156.4, 156.8, 157.3 (q, J =36 Hz, $\text{C}(\text{O})\text{CF}_3$), 165.5, 165.6 (CO_2Me). ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$) δ : –69.2 (s), –70.2 (s). HRMS: calcd for $\text{C}_{27}\text{H}_{30}\text{F}_3\text{N}_4\text{O}_9\text{S}$ $[\text{M}+\text{H}]^+$ 643.1686, found 643.1665. Anal. Calcd for $\text{C}_{27}\text{H}_{29}\text{F}_3\text{N}_4\text{O}_9\text{S}\cdot\frac{1}{2}\text{H}_2\text{O}$: C, 49.77; H, 4.64; F, 8.75; N, 8.60. Found: C, 49.77; H, 4.43; F, 8.58; N, 8.38.

4.2.6. ATAC scaffold 2. TFA-removal and hydrolysis of the methyl ester was performed as was described for the synthesis of **1**, using the cyclic product (2.57 g, 4.0 mmol), and Tesser's base (140 mL, 28 mmol, dioxane/methanol/4 M NaOH; 15/4/1). The reaction mixture changed overnight from clear yellow to turbid yellow. Fmoc-protection was performed as described for the synthesis of **1**, using CH_3CN (65 mL) and water (65 mL), DiPEA as a base, and a solution of Fmoc-OSu (1.48 g, 4.4 mmol) in CH_3CN (15 mL). After workup, a yellow oil or foam was obtained. Column chromatography (eluent: gradient from EtOAc/hexanes 2/1 to 3/1, and finally to EtOAc) afforded ATAC scaffold **2** as an off-white foam (2.46 g, 82%). R_f =0.33 (EtOAc). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 1.07 (m, 2H, NCH_2CH_2), 2.36, 2.50 (m, 2H, $\text{NCH}_2\text{CH}_2\text{N}$), 3.18, 3.27, 3.51 (3m, 4H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.35, 3.58 (2m, 2H, $\text{NCH}_2\text{CH}_2\text{N}$), 4.22–4.67 (m, 9H, OCH_2 (Aloc), $2\times \text{PhCH}_2$, CHCH_2 (Fmoc)), 4.88–5.12 (m, 2H, $=\text{CH}_2$ (Aloc)), 5.68, 5.83 (2m, 1H, $=\text{CH}$ (Aloc)), 7.33–8.31 (m, 15H, Ar–CH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 24.7, 25.3 ($\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}$), 42.8, 45.3, 48.8 (NCH_2), 46.7 (CH (Fmoc)), 51.3, 51.6, 53.3 (PhCH_2),

65.1, 67.2, 67.8 (OCH₂ (Aloc), CH₂ (Fmoc)), 116.2, 116.5, 116.9 (=CH₂ (Aloc)), 120.8, 124.6, 125.0, 125.1, 125.4, 127.2, 127.7, 128.0, 129.2, 129.3, 131.1, 131.2, 132.3, 132.8, 134.7, 139.1, 139.4, 139.8, 140.6, 140.8, 143.7, 143.9, 148.0 (Ar–C), 133.2 (=CH (Aloc)), 154.7, 155.7 (C=O (Aloc, Fmoc)), 166.8 (CO₂H). HRMS: calcd for C₃₉H₃₉N₄O₁₀S [M+H]⁺ 755.2387, found 755.2344. Anal. Calcd for C₃₉H₃₈N₄O₁₀S·1½ H₂O: C, 59.91; H, 5.29; N, 7.17. Found: C, 60.16; H, 5.06; N, 6.72.

4.3. Molecular modeling

Molecular modeling was performed using the program Yasara Structure (version 8.5.29; www.yasara.org). Both the TAC and ATAC scaffolds (**18** and **19**) were drawn with three Ala–Ala peptides attached to each scaffold. The following settings were used: Yamber3 force field; Force cutoff at 7.86 Å; temperature control: simulated annealing; the simulation cell was 50×50×50 Å; no extra water molecules were added. The minimization was carried out starting at 300 K until it reached 0 K.

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Supplementary data

Structures of compounds **17**, **18**, and **19**, NMR spectra, and a HPLC trace of **13**. Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tet.2014.04.084>.

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