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Fully symmetrical functionalization of multivalent scaffold molecules on solid support

Cristian Guarise, Leonard J. Prins* and Paolo Scrimin*

Department of Chemical Sciences and ITM-CNR, University of Padua, Via Marzolo 1, 35131 Padua, Italy

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Abstract—A new methodology for the functionalization of scaffold molecules on solid support is described, which does not require (partial) protection of the scaffold or a special functional group arrangement on it, while maintaining scaffold symmetry in the final product. As an illustration of the versatility of this approach, three scaffold molecules (1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene, tris(2-aminoethyl)-amine, and triazacyclononane) were functionalized with different functional groups in a moderate to high yield and purity. The developed protocol is a valuable tool for an easy access to multivalent molecules.

1. Introduction

Multivalent molecules, i.e., molecules containing multiple copies of a chemical function, are of increasing interest, because of their ability to efficiently interfere in biological processes.[1–4] Most recognition and catalytic processes in nature are governed by cooperative interactions between chemical functions present on the surface or in the active site of proteins. Approaches toward analogous artificial structures very frequently involve a scaffold molecule containing multiple sites for the introduction of functionalities.[5] In this way numerous artificial receptors,[6–10] catalysts,[11–13] and other biomimetic structures[14,15] have been prepared. Importantly, the activity of this kind of biomimetic structure depends critically on the scaffold, as it controls the spatial orientation of the functional groups.[16,17] Remarkably however, in virtually all reported studies the scaffold molecule is never taken as a variable; structural variations normally occur in the attached functionalities.[18] To our opinion, this limitation arises from the absence of a synthetic methodology that permits an easy variation of the scaffold molecule. Currently, scaffold functionalization occurs both in solution and, more frequently, on solid support. Intrinsically, solution-based strategies are less amendable to combinatorial chemistry, and, specifically for these kinds of structures, are often hampered by a difficult purification. The synthesis on solid phase relies on the use of AB_3-type scaffolds, in which functionality A is used for attachment to the resin and functionalities B for chemical diversification.[19–24] Although this permits the combinatorial synthesis of large libraries of biomimetic structures, the low commercial and synthetic accessibility of scaffold molecules with the required AB_3 functionalization pattern is a general limitation. Furthermore, the presence of chemical functionality A generally disrupts the symmetry of the scaffold, with the consequence that the functionalities B are not totally identical. Here, we describe a new synthetic protocol for the solid-phase functionalization of A_3-type scaffold molecules, maintaining C_3-symmetry in the final products. This approach is a valuable tool for an easy synthetic access to multivalent molecules.

2. Results and discussion

The synthetic scheme relies on conventional Fmoc-based peptide chemistry and uses, as starting material, Fmoc-Asp(OH)-OAll connected to Wang resin via the aspartic acid side chain, either commercially available or easily synthesizable following conditions reported in the literature.[25] The crucial steps are outlined in Scheme 1. Initially, the Fmoc-protecting group is replaced by Mmt (4-methoxytrityl), because of its higher stability against the nucleophilic/basic properties of the amino groups present in scaffold **1** added in the next steps. Subsequently, the allyl-group is removed under reported reductive conditions[26] and the resulting free acid activated with PyBOP (step i, Scheme 1). At this point, addition of a scaffold molecule **1** with three amino groups results in the linkage of the scaffold to the resin via 1, 2 or 3 amino groups.[27] For example, addition of 1,3,5-tris-(aminomethyl)-2,4,6-triethylbenzene **1a**, a frequently used scaffold molecule,[28] results in the formation of three products

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* Corresponding authors. Tel.: +39 049 8275256; fax: +39 049 8275239; e-mail addresses: leonard.prins@unipd.it; paolo.scrimin@unipd.it

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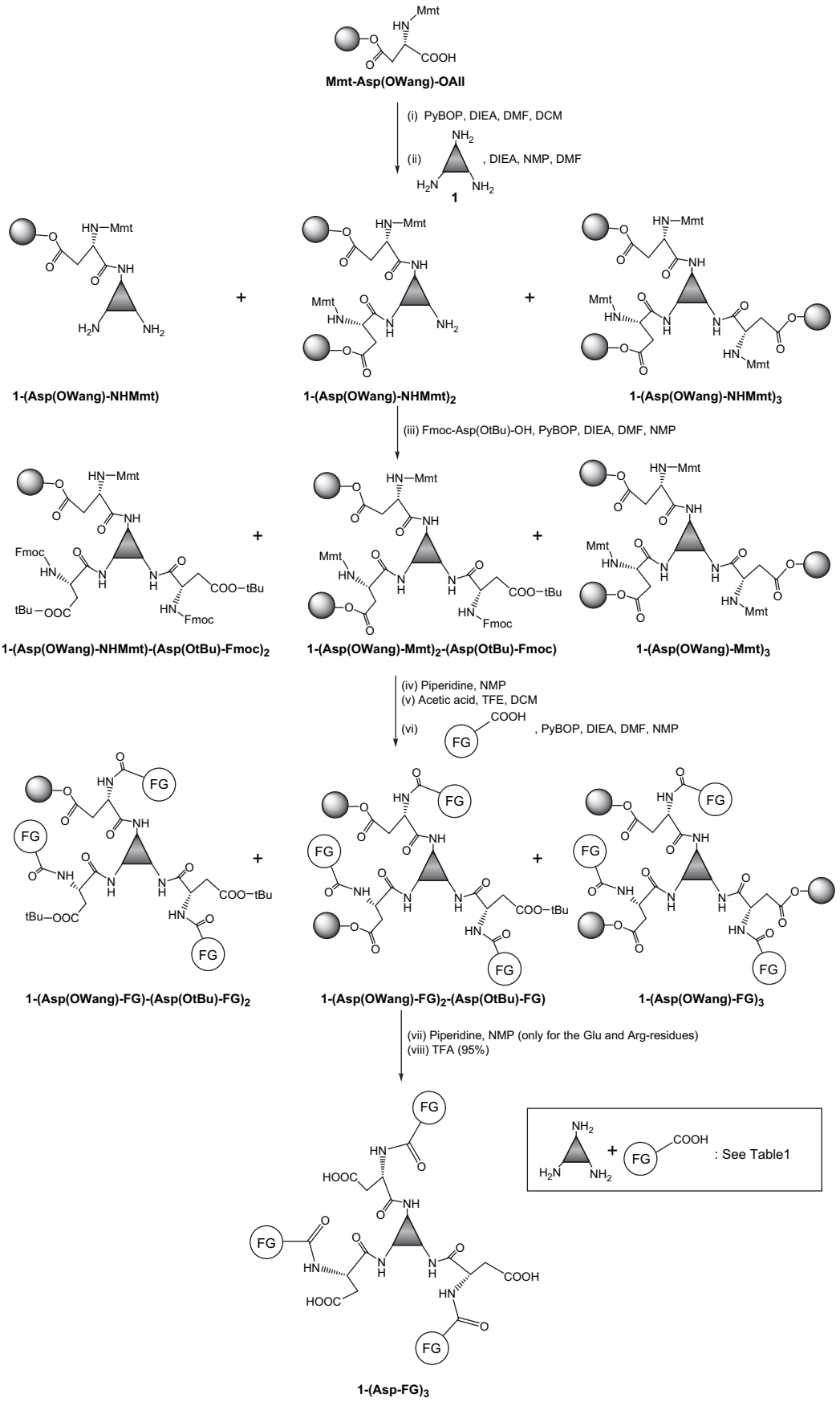


Scheme 1. Synthetic procedure for the synthesis of C_3-symmetrically functionalized scaffold molecules on solid support.

(step ii, Scheme 1, **1a**-(Asp(OWang)-NHMmt), **1a**-(Asp(OWang)-NHMmt)$_2$, and **1a**-(Asp(OWang)-NHMmt)$_3$). This is evidenced by the presence of three peaks in the HPLC spectrum after cleavage (Fig. 1a) corresponding to compounds **1a**-(Asp-NH$_2$), **1a**-(Asp-NH$_2$)$_2$, and **1a**-(Asp-NH$_2$)$_3$, as confirmed by ESI-MS. The mono-, di-, and tri-adducts are formed in a ratio of 48:35:17 based on integration of the HPLC signals. In the next step, *pseudo*-C_3-symmetry is restored by the addition of an excess of Fmoc-Asp(OtBu)-OH (activated with PyBOP) to the resin, which reacts with the remaining free amino groups of **1a** (step iii, Scheme 1). At this point, all three amino groups of **1a** are substituted with an Asp-residue, with an Mmt- or Fmoc-protected terminal amino group and with the side chains either connected to the Wang resin or protected as a *tert*-butyl ester. Next, removal of both the Mmt- and Fmoc-protecting groups renders scaffold molecule, **1a**-(Asp-NH$_2$)$_3$, accessible for further functionalization (steps iv and v, Scheme 1) with any carboxylic acid-containing moiety (FG). As an example, Fmoc-Glu(O*t*-Bu)-OH was added as the next residue, giving compound **1a**-(Asp-Glu-NH$_2$)$_3$ with a very high purity (>95%) after cleavage from the resin (Fig. 1b and step vi, Scheme 1). Upon cleavage from the resin (95% TFA) C_3-symmetry is completely restored since the Asp-side chains are obtained as the free carboxylic acid, regardless whether they were connected to the resin or protected as a *tert*-butyl ester. The product was fully characterized by ^{1}H NMR, HR ESI-MS, and HPLC. In a similar way, other functionalities, i.e., a basic amino acid residue (arginine) and an azacrown derivative for metal-binding ((1,4,7-triazacyclonon-1-yl)-acetic acid, AA$_{TACN}$), were connected to the scaffold molecule giving compounds **1a**-(Asp-Arg-NH$_2$)$_3$ and **1a**-(Asp-AA$_{TACN}$)$_3$ with purities of 88 and 83%, respectively (Table 1).

From the synthetic scheme just described it is evident that this approach is not restricted to the use of AB$_3$-type scaffold molecules, neither does it require the (partial) protection of the scaffold molecule. To illustrate its versatility, the synthetic procedure was repeated using two other scaffold molecules, **1b** and **1c** that are very frequently used in the literature, and which, in addition, have different characteristics with respect to scaffold **1a**. Tris(2-aminoethyl)amine (**1b**, Tren) has a much higher conformational freedom compared to **1a**, whereas the macrocycle triazacyclononane (**1c**, Tacn) has secondary amines as attachment points. Both scaffolds **1b** and **1c** were used in the same manner as discussed for **1a** and functionalized with the same functional groups

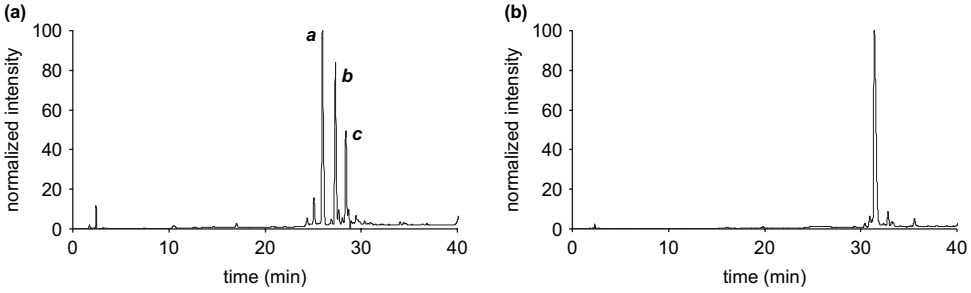
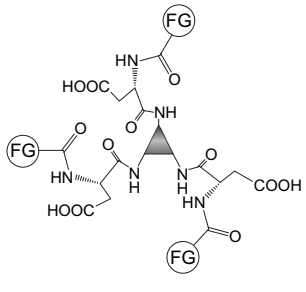


Figure 1. HPLC chromatograms of (a) the products: compounds **1a**-(Asp-NH$_2$) (peak a, 48%), **1a**-(Asp-NH$_2$)$_2$ (peak b, 35%), and **1a**-(Asp-NH$_2$)$_3$ (peak c, 17%) obtained after cleavage from resin after the addition of scaffold **1a** to the resin (step ii, Scheme 1) and (b) the final product **1a**-(Asp-Glu-NH$_2$)$_3$ after cleavage from resin (step viii, Scheme 1).

Table 1. Summary of the nine compounds synthesized using the described synthetic protocol with purities[a] and yields[b]



1-(Asp-FG)$_3$

scaffold / FG	**1a**	**1b**	**1c**
H$_2$N-Glu-OH	**1a-(Asp-Glu)$_3$** *Purity:>95%* *Yield: 91%*	**1b-(Asp-Glu)$_3$** *Purity:61%* *Yield: 57%*	**1c-(Asp-Glu)$_3$** *Purity:73%* *Yield: 66%*
H$_2$N-Arg-OH	**1a-(Asp-Arg)$_3$** *Purity: 88%* *Yield: 80%*	**1b-(Asp-Arg)$_3$** *Purity:48%* *Yield: 45%*	**1c-(Asp-Arg)$_3$** *Purity:78%* *Yield: 70%*
AA$_{TACN}$	**1a-(Asp-AA$_{TACN}$)$_3$** *Purity:83%* *Yield: 75%*	**1b-(Asp-AA$_{TACN}$)$_3$** *Purity:50%* *Yield: 45%*	**1c-(Asp-AA$_{TACN}$)$_3$** *Purity:80%* *Yield: 72%*

[a] Purities based on the ratio between peaks present in the HPLC spectra after cleavage from resin.
[b] Yields based on the initial loading of Fmoc-Asp(OH)-OAll to the resin (for **1a** compounds after precipitation from diethyl ether and for **1b/1c** compounds after purification with RP-HPLC).

i.e., Glu, Arg, and AA_{TACN}, obtaining a total of six additional products (Table 1). Purities of the crude mixtures were typically in the order of 50–80%, somewhat lower when compared to the analogous compounds based on scaffold **1a** but still very satisfactory. Especially the use of Tren as a scaffold causes the formation of one major side-product (30–40%) with a mass difference of −18 amu with respect to the desired product. We hypothesize that this side-product forms via an intramolecular cyclization between a terminal amine and one of the ester linkages between the scaffold and the resin. The occurrence of this reaction seems to be related to the conformational flexibility of the scaffold, because the formation of this side-product for the more rigid scaffolds **1a** and **1c** typically does not exceed 5%. However, even in cases where this side-product is present, all compounds could be easily purified using preparative RP-HPLC.

3. Conclusion

In conclusion, we have described a new methodology that permits an easy functionalization of scaffold molecules on solid support. No special requirements in terms of (partial) protecting groups on the scaffold molecule or functional group arrangements (for instance AB_3-type) are necessary, while the symmetry of the scaffold is maintained in the final products. The versatility of this approach was illustrated by the functionalization of three diverse scaffolds with a variety of functions. This methodology offers a valuable tool for an easy synthetic access to multivalent molecules.

4. Experimental

4.1. General

All starting materials, solvents, and resins were obtained from commercial sources and used without further purification. ^{1}H NMR spectra were recorded on a Bruker AC-300 (300, 13 MHz) spectrometer at 301 K. HPLC spectra were measured using a Shimadzu LC-10AT dual pump system and a Shimadzu SPD-10A UV–vis detector. HR ESI-mass spectra were obtained using a Perspective Biosystem Mariner spectrometer, equipped with a TOF-analyzer.

4.2. General procedure for the synthesis of compounds 1-(Asp-AA-NH_2)$_3$

Following a literature procedure,[25] Fmoc-Asp(OH)-OAll (6 equiv) was attached to Wang resin (100 mg, 0.093 mmol endgroups) using DIC (*N*,*N*-diisopropylcarbodiimide, 6 equiv) and DMAP (*N*,*N*-dimethylaminopyridine, 0.2 equiv) activation in DMF with subsequent acetylation of the non-reacted hydroxyl-moieties. The Fmoc-group was replaced by Mmt using 20% piperidine in NMP and subsequent capping with Mmt-Cl (4-methoxytriphenylchloromethane, 6 equiv) in the presence of DIEA (*N*,*N*-diisopropylethylamine, 15 equiv) in dichloromethane. Next, the allyl-group was removed under reported reductive conditions[26] (0.2 equiv tetrakis(triphenylphosphine)palladium(0) and 10 equiv phenylsilane in DCM, 1.5 h at rt under an inert N_2 atmosphere) and the resulting free acid activated with PyBOP (6 equiv) in NMP/DCM in the presence of DIEA (15 equiv). At this point, the scaffold molecule (3 equiv) was added to NMP/DMF containing DIEA (15 equiv), after which the resin was treated with Fmoc-Asp(O*t*-Bu)-OH activated prior with PyBOP under basic conditions. Finally, the Fmoc and Mmt functional groups were removed in two subsequent deprotection steps (first, 20% piperidine in NMP, and second, a mixture of acetic acid/TFE/DCM (1:2:6)). At this point, conventional peptide chemistry (PyBOP activation in the presence of DIPEA) was used to further functionalize the scaffold molecules. After the syntheses, the final compounds were cleaved from the resin with simultaneous side-chain deprotection using 95% TFA in DCM. After evaporation of volatiles, the crude products were precipitated from cold ether and purified by RP-HPLC when necessary. All compounds were characterized by RP-HPLC, high resolution ESI-MS, and ^{1}H NMR spectroscopy.

4.2.1. 1a-(Asp-Glu-NH_2)$_3$. Isolated yield: 20 mg, 91% after precipitation with diethyl ether, colorless solid. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 3 to 30% in 30 min, UV-detection 226 nm): 31.4 min (>95%). ^{1}H NMR (CD_3OD, 250 MHz) δ 4.71 (t, J=5.8 Hz, 3H), 4.45 (s, 6H), 3.95 (t, J=6.4 Hz, 3H), 2.75 (q, J=7.3 Hz, 6H,), 2.51 (t, J=8.0 Hz, 6H), 2.2–2.0 (m, 12H), 1.15 (t, J=7.3 Hz, 9H); ESI-MS $[M+H]^+$ requires 982.4291; found 982.4308.

4.2.2. 1a-(Asp-Arg-NH_2)$_3$. Isolated yield: 19 mg, 80% after precipitation with diethyl ether, yellowish solid. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 3 to 30% in 30 min, UV-detection 226 nm): 19.9 min (88%). ^{1}H NMR (CD_3OD, 250 MHz) δ 4.71 (t, J=5.8 Hz, 3H), 4.43 (s, 6H), 4.00 (t, J=6.4 Hz, 3H), 3.25 (t, J=6.4 Hz, 6H), 2.8–2.6 (m, 12H), 2.1–1.9 (m, 6H), 1.8–1.6 (m, 6H), 1.15 (t, J=7.3 Hz, 9H); ESI-MS $[M+H]^+$ requires 1063.6047; found 1063.6095.

4.2.3. 1a-(Asp-AA_{TACN})$_3$. Isolated yield: 18 mg, 75% after precipitation with diethyl ether, pale yellow solid. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 3 to 30% in 30 min, UV-detection 226 nm): 19.8 min (83%). ^{1}H NMR (CD_3OD, 300 MHz) δ 4.74 (t, J=6.0 Hz, 3H), 4.47 (m, 6H), 3.66 (br s, 12H), 3.53 (d, J=3.7 Hz, 6H), 3.34 (br s, 12H), 3.05 (br s, 12H), 2.8–2.6 (m, 12H), 1.14 (t, J=7.3 Hz, 9H); ESI-MS $[M+H]^+$ requires 1102.6659; found 1102.6691.

4.2.4. 1b-(Asp-Glu-NH_2)$_3$. Isolated yield: 12 mg, 57% after purification with RP-HPLC, yellowish thick oil. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 3 to 30% in 30 min, UV-detection 226 nm): 13.8 min (100%). ^{1}H NMR (CD_3OD, 300 MHz) δ 4.72 (t, J=6.0 Hz, 3H), 4.01 (t, J=6.3 Hz, 3H), 3.61 (br s, 6H), 3.46 (br s, 6H), 3.0–2.8 (m, 6H), 2.54 (t, J=6.6 Hz, 6H), 2.2–2.1 (m, 6H); ESI-MS $[M+H]^+$ requires 879.3618; found 879.3598.

4.2.5. 1b-(Asp-Arg-NH_2)$_3$. Isolated yield: 10 mg, 45% after purification with RP-HPLC, colorless thick oil. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 8 to 30% in 25 min, UV-detection 226 nm): 24.2 min (100%). ^{1}H NMR (CD_3OD, 250 MHz) δ 4.78 (t, J=5.8 Hz, 3H), 4.00 (t, J=6.4 Hz, 3H), 3.6–3.4

(m, 12H), 3.25 (t, J=6.4 Hz, 6H), 2.9–2.7 (m, 6H), 2.0–1.9 (m, 6H), 1.8–1.7 (m, 6H); ESI-MS $[M+H]^+$ requires 960.5373; found 960.5401.

4.2.6. 1b-(Asp-AA$_{TACN}$)$_3$. Isolated yield: 10 mg, 45% after purification with RP-HPLC, colorless oil that solidifies on standing. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 8 to 30% in 30 min, UV-detection 226 nm): 21.6 min (100%). ^{1}H NMR (CD_3OD, 250 MHz) δ 4.81 (t, J=5.8 Hz, 3H), 3.67 (br s, 12H), 3.6–3.4 (m, 18H), 3.3 (br s, 12H), 3.1 (br s, 12H), 2.9–2.8 (m, 6H); ESI-MS $[M+H]^+$ requires 999.5985; found 999.6009.

4.2.7. 1c-(Asp-Glu-NH$_2$)$_3$. Isolated yield: 13 mg, 66% after purification with RP-HPLC, colorless solid. HPLC (Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 3 to 30% in 30 min, UV-detection 226 nm): 10.9 min (100%). ^{1}H NMR (CD_3OD, 250 MHz) δ 4.72 (t, J=5.8 Hz, 3H), 4.01 (t, J=6.4 Hz, 3H), 3.8–3.4 (m, 12H), 2.9–2.8 (m, 6H), 2.54 (t, J=7.0 Hz, 6H), 2.2–2.0 (m, 6H); ESI-MS $[M+H]^+$ requires 862.3352; found 862.3398.

4.2.8. 1c-(Asp-Arg-NH$_2$)$_3$. Isolated yield: 18 mg, 70% after purification with RP-HPLC, pale yellow solid. HPLC (Jupiter Proteo 90 Å, 4 μ; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 5 to 25% in 30 min, UV-detection 226 nm): 13.1 min (100%). ^{1}H NMR (CD_3OD, 250 MHz) δ 5.01 (t, J=5.8 Hz, 3H), 3.95 (t, J=6.4 Hz, 3H), 3.8–3.4 (m, 12H), 3.25 (t, J=6.4 Hz, 6H), 3.0–2.8 (m, 6H), 2.1–2.0 (m, 6H), 1.8–1.7 (m, 6H); ESI-MS $[M+H]^+$ requires 942.5108; found 942.5196.

4.2.9. 1c-(Asp-AA$_{TACN}$)$_3$. Isolated yield: 16 mg, 72% after purification with RP-HPLC, colorless solid. HPLC (Jupiter Proteo 90 Å, 4 μ; gradient: H_2O/TFA (0.1%)–CH_3CN/TFA (0.1%) from 10 to 30% in 30 min, UV-detection 226 nm): 9.3 min (100%). ^{1}H NMR (CD_3OD, 250 MHz) δ 5.09 (t, J=5.8 Hz, 3H), 3.8–3.5 (m, 30H), 3.3 (br s, 12H), 3.1 (br s, 12H), 2.7–2.6 (m, 6H); ESI-MS $[M+H]^+$ requires 982.5720, found 982.5801.

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