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Tetrahedron 63 (2007) 4918–4928
Tetrahedron

Solid-phase synthesis of metal-complex containing peptides

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Received 2 March 2007; accepted 22 March 2007
Available online 27 March 2007

Abstract—We report the synthesis of Fmoc protected single amino acid chelates (SAAC) and their metal complexes. The modified amino acids are suitable for solid-phase peptide synthesis. The use of 4-hydroxymethylbenzoic acid AM (HMBA-AM) resin allows the nucleophilic cleavage of the peptide–metal complexes from the resin without decomplexation.

1. Introduction

Peptides with metal complexes in their side chains and peptide–metal complex conjugates have been used to enhance or control binding affinities and peptide conformations. Banerjee and Stephenson et al. have reported the solid-phase synthesis of peptides bearing 2,2′-dipicolylamine single amino acid chelate (dpa-SAAC) for peptide based technetium and rhenium radiopharmaceuticals.[1] Metzler-Nolte and co-workers prepared N-terminally modified bioconjugates by a series of dpa based copper or zinc complexes for potential applications as artificial metallochaperones.[2] Recent reports on dpa-SAAC describe a more flexible preparation of peptide–Re and peptide–Tc ligand conjugates with non-terminal SAAC.[3] However, the reported synthesis of peptide–metal complex conjugates on solid phase is either limited to conjugation of the metal complex to the N-terminus of the resin-bound peptide or focus on the coordination of $Tc(CO)_3$ and $Re(CO)_3$.[4] To extend the scope of solid-phase synthesis of peptide—metal complex conjugates, we report here their preparation from modified amino acids bearing metal complex ligands or metal complexes in their side chains. The modified amino acids can be incorporated at any position into the peptide during synthesis and the use of 4-hydroxymethylbenzoic acid AM (HMBA-AM) resin allows nucleophilic cleavage of the peptide from the resin avoiding decomplexation.

2. Results and discussion

2.1. Metal chelates

We have selected the iminodiacetic acid (IDA) motif as metal complex binding sites, known for its ability to bind imidazole residues and N-terminal His, and triazen-bis-zinc–cyclen, dipyridylmethyl amine (dpa) and bis-diypridylmethyl amine (bis-dpa) zinc complexes, which show affinity to phosphate groups. First we will discuss the synthesis of the modified amino acids bearing the respective ligand or complex, and then describe their use in solid-phase peptide synthesis.

2.2. Synthesis of Fmoc-amino acids with ligand or metal complex side chain

The synthesis of Fmoc protected IDA amino acid **1** (Fig. 1) has been described by reductive amination of *tert*-butyl 2-oxoacetate,[5] the starting material for this synthesis is rather expensive, or the nucleophilic substitution of the lysine side chain as reported by Tampe et al.[6] After optimization of the reaction conditions of hydrogenolytic cleavage of the *Z*-protecting group and Fmoc protection using the more reactive Fmoc–OSu instead of Fmoc–Cl, **1** was obtained in good yield from the latter method. The ligand

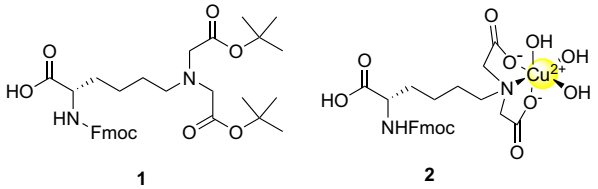

Figure 1. Fmoc protected IDA amino acid **1** and its copper(II) complex **2**.

Keywords: Solid-phase peptide synthesis; Metal complex; Single amino acid chelate.
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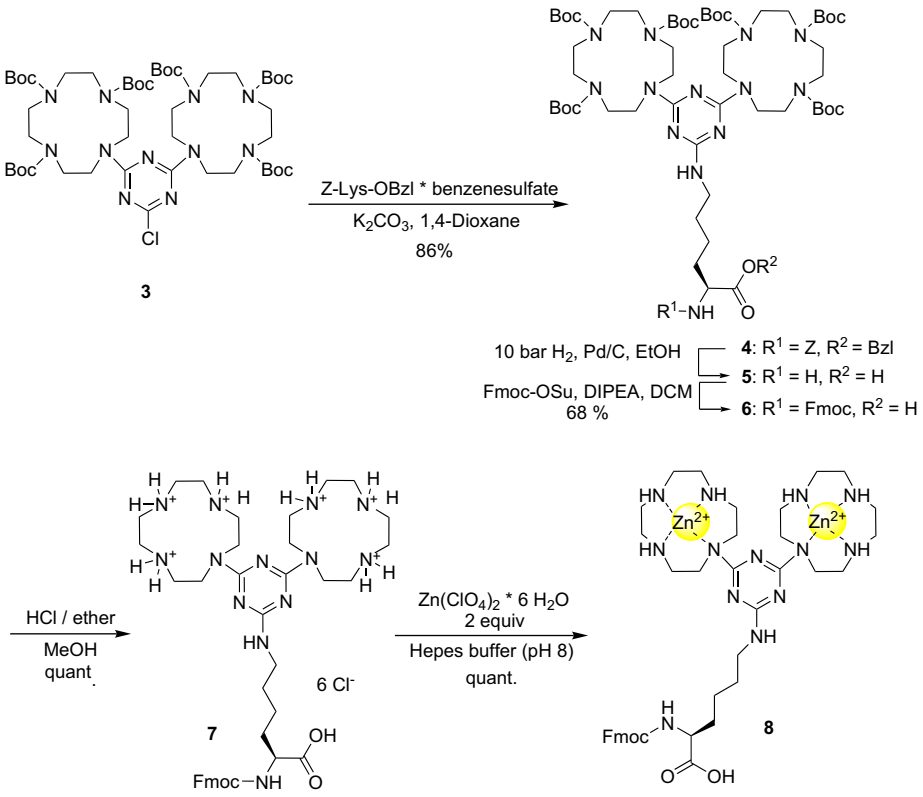
doi:10.1016/j.tet.2007.03.147



Scheme 1. Preparation of Fmoc protected amino acid **6** and the bis(Zn^{II}–cyclen) compound **8**.

was converted into its copper complex using either 2 equiv of $Cu_2(OH)_2CO_3$ or a stoichiometric amount of $CuCl_2$ with 1 equiv of base. In both cases, mass spectrometric analysis (ESMS) confirmed the complete formation of complex **2**.

Amino acid complex **8** was prepared from the previously reported triazene-bis-cyclen **3**[7] by reaction with α-amino Z-protected L-Lys-OBn (Scheme 1). The nucleophilic aromatic substitution gave compound **4** in 86% yield and Z and benzyl protecting groups were simultaneously removed by hydrogenation using 10% palladium on charcoal as catalyst. Fmoc protection gave compound **6**. The complexation of the cyclen ligands with Zn(II) requires careful control of the reaction conditions. After Boc deprotection with HCl saturated ether the hydrochloride salt **7** must be neutralized by base for metal ion complexation and the complexation step typically requires elevated temperatures,[8] conditions which may cleave the Fmoc group. Ion exchange chromatography for deprotonation of the hydrochloride salt was therefore not applicable and a buffered solution was used for complexation. A clean twofold Zn(II)-complexation was achieved in Hepes buffer (pH 8) with $Zn(ClO_4)_2 \cdot 6H_2O$ yielding the protected amino acid **8**.

Amino acid **9** with a tridentate donor side chain was prepared according to a literature procedure[9] from Fmoc–lysine hydrochloride pyridine-2-carboxyaldehyde by reductive amination with $NaBH(OAc)_3$.[10] The dpa complexes **10** are obtained quantitatively using stoichiometric amounts of the appropriate metal salts in a water/methanol solution (Fig. 2).

The synthesis of amino acids **15** with binuclear Zn(II) dpa complex starts from Boc-L-tyrosine methyl ester (Scheme 2). The Mannich reaction with dpa and paraformaldehyde afforded ligand **11**.[11] Saponification and subsequent Fmoc-protection resulted in the desired amino acid **14**. The complexation of **14** with 2 equiv of either $Zn(NO)_3 \cdot 6H_2O$ or $ZnCl_2$ gave dpa metal complexes **15**.

2.3. Solid phase peptide synthesis

2.3.1. Synthesis of dpa containing peptides. As a first peptide compound **16** was prepared (Scheme 3). After complete characterization by two-dimensional NMR, peptide **16** was complexed in solution. This approach allowed the use of the versatile Rink amide MBHA resin, a resin based on MBHA with a modified Rink amide linker as an ideal tool for Fmoc

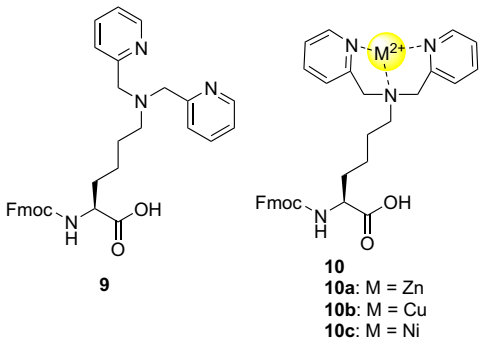
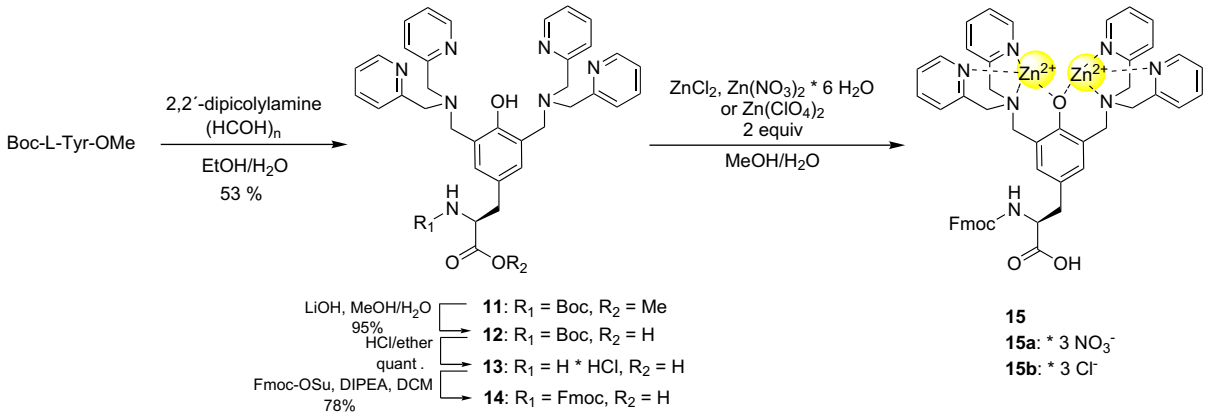
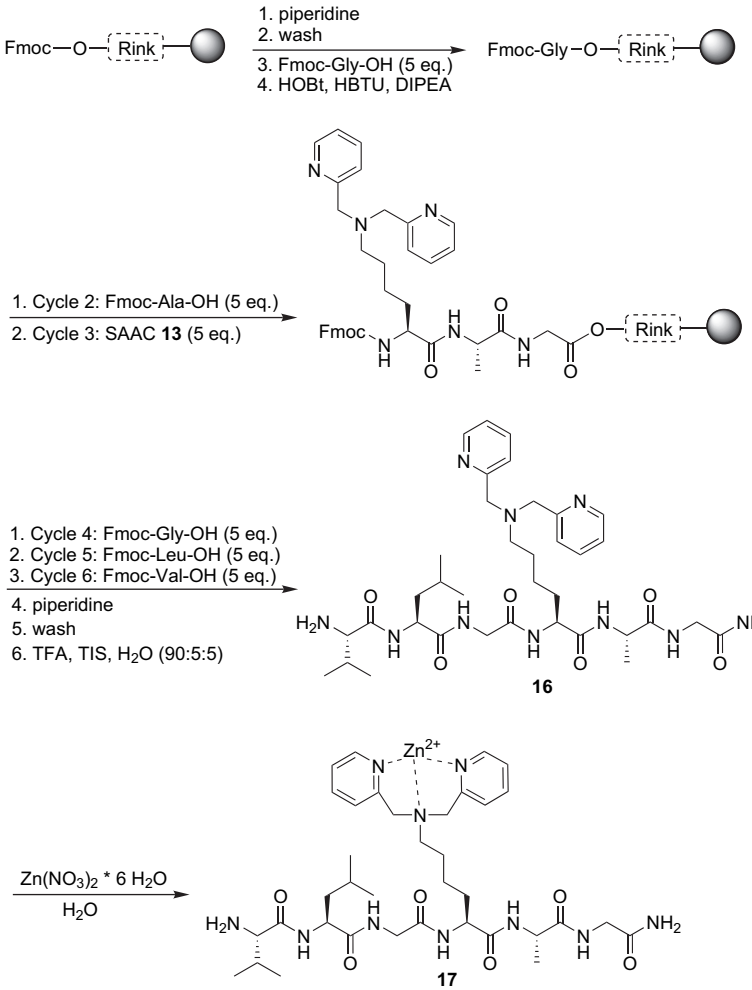


Figure 2. Dpa amino acid **9** and dpa metal complexes **10** (M=Zn, Cu and Ni).

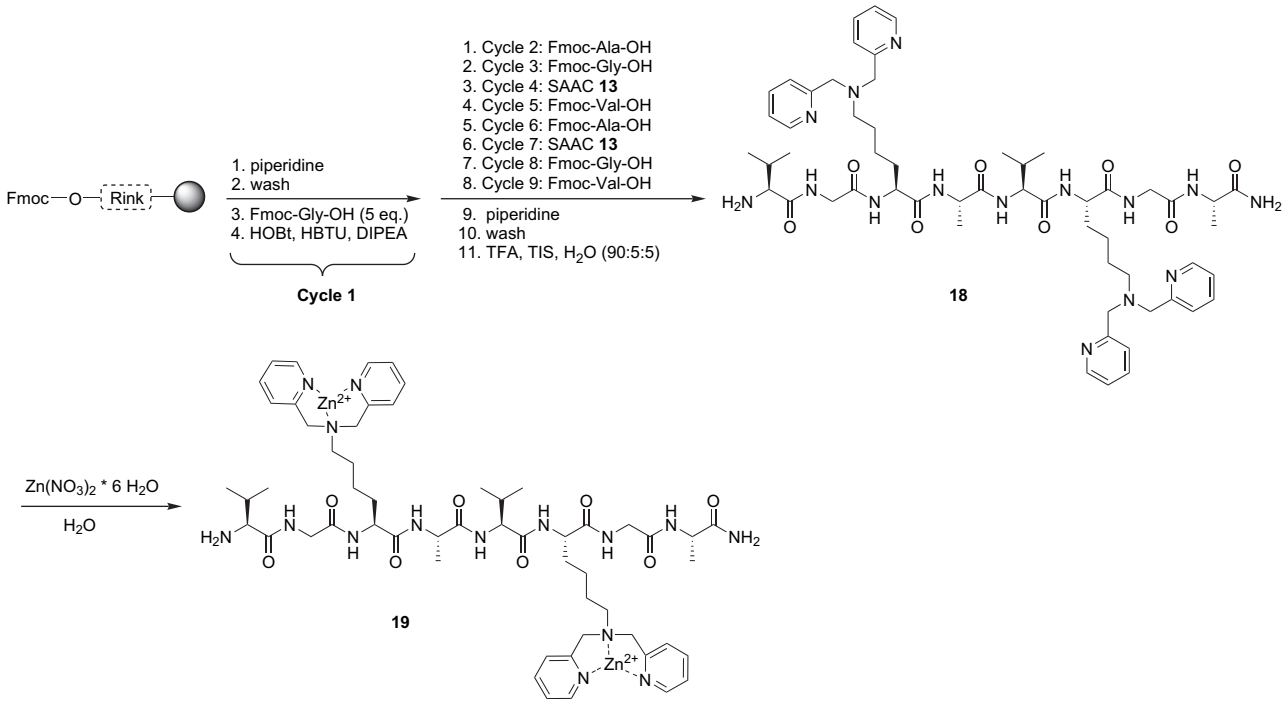


Scheme 2. Synthesis of bis-dpa amino acid **14** and bis-dpa metal complexes **15**.

SPPS, providing peptide amides in high yields and purities.[12] After Fmoc deprotection, the Fmoc protected aliphatic amino acids, glycine and alanine were coupled with HBTU, HOBt and DIPEA in NMP/DMF using the conventional frit-equipped syringe technique. All coupling steps were carried out only once. Standard deprotection and washing cycles as outlined in Scheme 3 were performed after each coupling step. The SAAC **9** was then coupled using the same coupling reagents and equivalents of reagents. The reaction was completed after 3 h indicated by a negative Kaiser test[13] and the deprotected N-terminus was subsequently coupled to Fmoc protected amino acids glycine, leucine and valine. Final Fmoc deprotection completes the solid-phase synthesis and gave peptide **16** after cleavage from the resin with



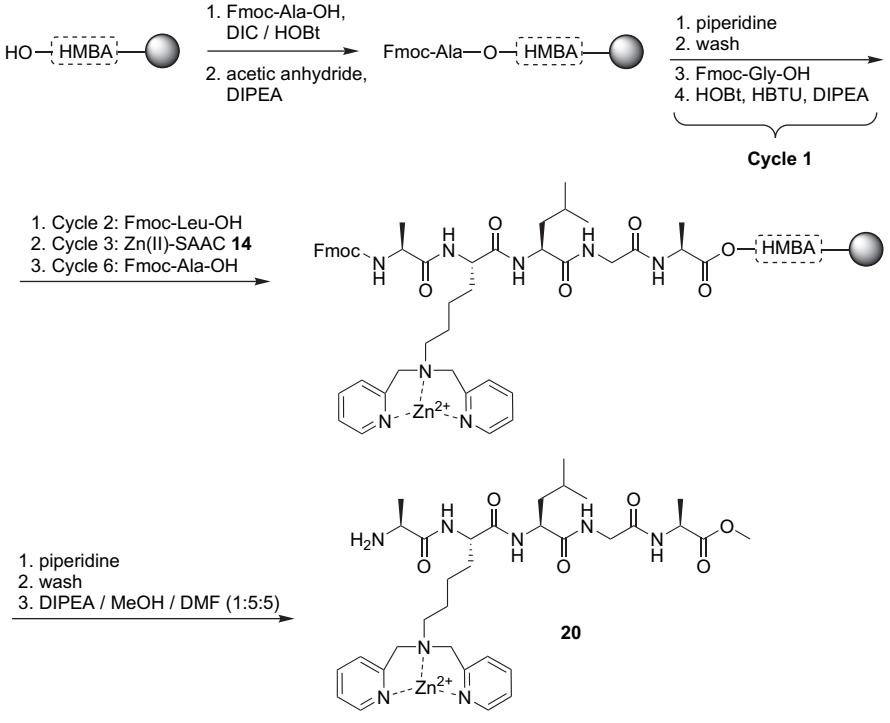
Scheme 3. Solid-phase synthesis of peptide conjugate **16** and peptide metal complex **17**.



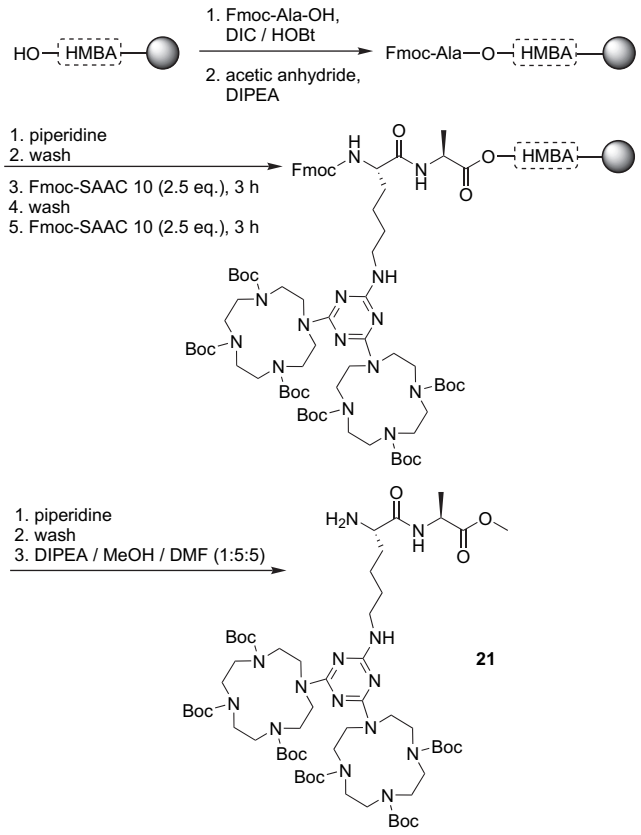
Scheme 4. SPRS of dinuclear peptide metal complex **19**.

90% TFA, 5% TIS and 5% H_2O. After subsequent precipitation using cold diethyl ether and centrifugation (see Section 4 for details), the peptide conjugate was dissolved in water, lyophilized and characterized by ESIMS and two-dimensional NMR. Twenty-six milligrams of the target peptide sequence **16** were obtained analytically pure. Thus, peptide **16** was treated with $Zn(NO_3)_2 \cdot 6H_2O$ (1 equiv) to obtain exclusively metal bound peptide conjugate **17** exclusively.

The same approach was used to prepare the dinuclear peptide receptor **19** (Scheme 4). Peptide **18** was first synthesized on Rink amide MBHA resin. After cleavage from the resin,



Scheme 5. Direct SPRS of **20** on HMBA-AM resin.



Scheme 6. Solid-phase synthesis of dipeptide conjugate **21**.

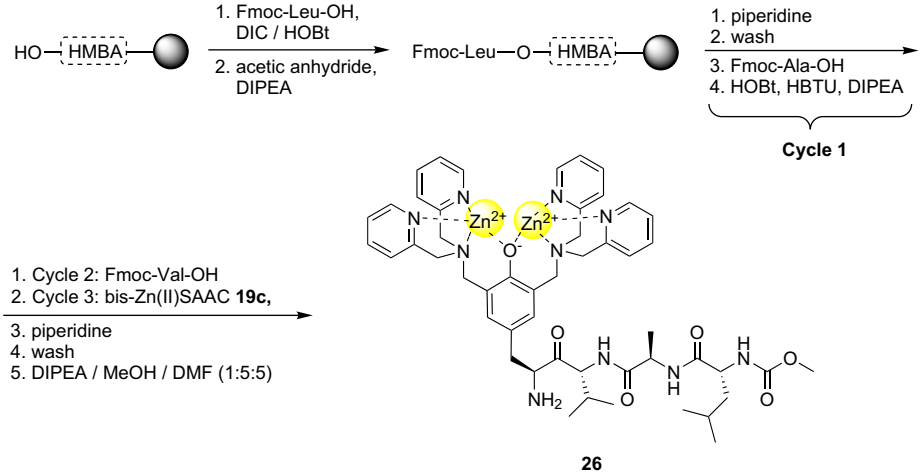
16 mg of the target peptide sequence **18** was obtained in >95% purity as verified by NMR. Two-dimensional NMR allowed the complete assignment of all resonances. Thus, peptide **18** was treated with $Zn(NO_3)_2 \cdot 6H_2O$ (1 equiv) to obtain metal bound dinuclear peptide receptor **19**.

In the synthesis of peptide **20**, the already metal coordinated Fmoc–dpa complex **10a** was incorporated into the peptide chain (Scheme 5). To avoid the loss of metal ions under acidic conditions, which are necessary to cleave from Rink amide resin, HMBA-AM was used as the resin as it allows nucleophilic cleavage of the peptide from the resin. Otherwise the same procedure as above was used yielding peptide **20** in >95% purity as verified via NMR.

2.3.2. Synthesis of peptides containing bis-zinc–cyclen amino acid 8. First attempt to couple amino acid **8** or **6** in solid-phase protocols to aliphatic amino acids using HBTU,[14] TBTU and DIPEA as coupling reagents failed. Therefore the more efficient reagent HOAt was used instead of HOBt together with the onium salt HATU. DIPEA was exchanged by collidine,[15] a more suitable base for the HOAt reagent. By using HOAt (2.5 equiv), HATU (2.5 equiv) and collidine (5 equiv) the coupling of **6** (2×2.5 equiv) using two coupling cycles gave dipeptide **21** (Scheme 6). A more extended peptide **22** was obtained on an Fmoc–Ala loaded SASRIN resin using the same coupling conditions (Scheme 7). In both cases the solely observed molecular ions in electro-spray mass spectrometry were only consistent with the mass of the desired compounds. In the following step, the Boc groups were cleaved with HCl saturated ether and the neutralized compound **23** was subsequently treated with $Zn(ClO_4)_2$ salt to obtain the peptide complex **24**.

2.3.3. Synthesis of peptides containing IDA amino acid 2. To illustrate the incorporation of the IDA amino acid metal complex **2** into a short peptide sequence by a solid-phase protocol, peptide **25** was prepared on HMBA-AM resin using the standard coupling conditions as outlined in examples of the dpa-chelate (Scheme 8). Electro-spray mass analysis confirmed the formation of the desired compound.

2.3.4. Synthesis of peptides containing bis-dpa-zinc amino acid 15. Bis(Zn^{II}–nitrate)-SAAC **15a**, derived from complexation of **14** with $Zn(NO)_3 \cdot 6H_2O$, was first tried for incorporation into a peptide backbone. Due to the poor solubility of the metal complexes in NMP or DMF, the coupling was not efficient enough to give pure products. Fortunately, bis-Zn^{II}–SAAC **15b**, having chloride counter ions, was more soluble in NMP and the synthesis of **26** on HMBA-AM resin was successful using standard HOBt/TBTU conditions. Electro-spray mass analysis confirmed the clean formation of the desired compound.



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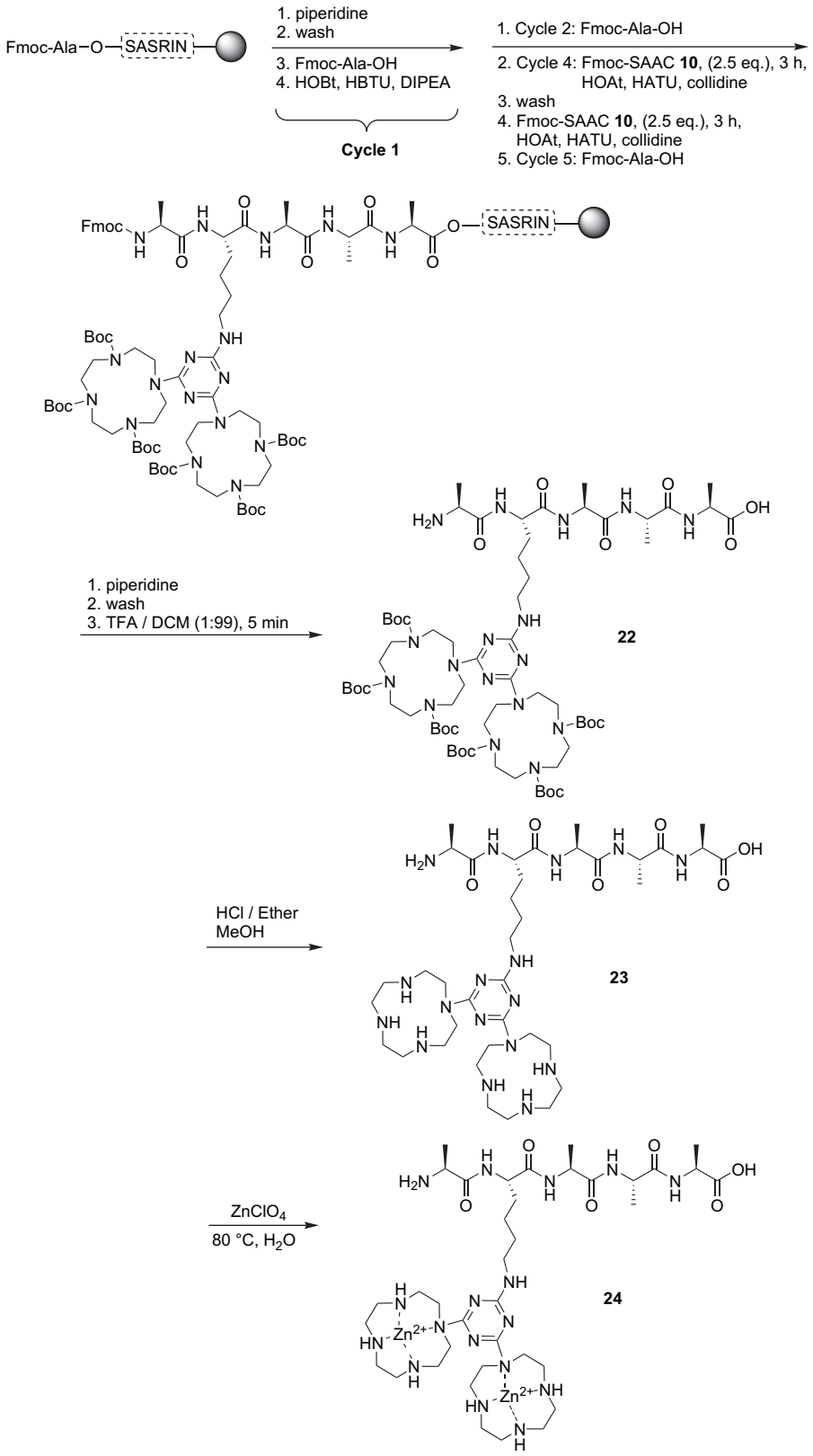
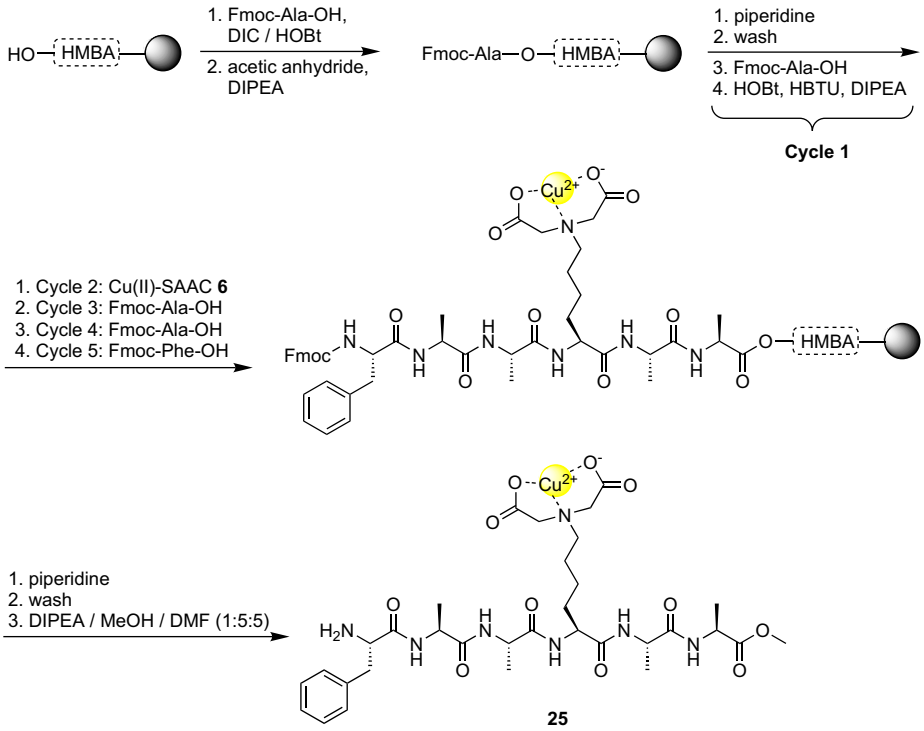


Scheme 7. Solid-phase synthesis of peptide **22** and peptide complex **24**.

3. Conclusion

In conclusion, we have reported the synthesis of Fmoc protected SAAC and their use in solid-phase synthesis. Peptide–metal complex conjugates were either obtained by incorporation of the metal coordinated SAAC followed by mild nucleophilic resin-cleavage or by complexation in metal salt solution after cleavage from the resin. Our



Scheme 8. SPRS of peptide metal complex **25**.

reported solid-phase peptide synthesis protocols are suitable for automation and the position and number of the modified amino acid within the peptide chain may vary. This allows the synthesis of libraries of modified peptides with metal complexes as binding sites in synthetic receptors or as paramagnetic labels.

4. Experimental

4.1. Cu–IDA-complex 2

Compound **1** was first treated with HCl saturated ether to obtain the free iminodiacetic acid functionality: Compound **1** (1.62 g, 2.72 mmol) was dissolved in DCM and cooled to 0 °C using an ice bath. To this mixture 30 mL of HCl saturated ether was added. The mixture was allowed to warm to room temperature and stirred over night. The reaction progress was controlled by ^{1}H NMR. The mixture was concentrated under reduced pressure and dried under high vacuum to obtain product **1a** as colourless solid in quantitative yield and was subsequently used for complexation. Mp: 135 °C; $[\alpha]_D^{20}$ −13.9 (*c* 0.13 in MeOH); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}]=3419, 2953, 2619, 1735, 1528, 1250, 740; MS (ESI, MeOH+10 mmol/L NH_4OAc): *m*/*z* (%)=485 (100) [MH$^+$], 619.4 (7) [MNa$^+$]. EA ($C_{25}H_{28}N_2O_8\cdot 3H_2O$) calculated (%): C 55.74, H 6.37, N 5.20. Found: C 55.18, H 6.21, N 4.96.

Fmoc–IDA–OH **1a** (930 g, 1.79 mmol) and $Cu_2(OH)_2CO_3$ (395 mg, 1.79 mmol) were suspended in H_2O/MeOH solution (30 mL, 1:1). The mixture was stirred for 3 h at 70 °C and the resulting blue solution was decanted from the residual $Cu_2(OH)_2CO_3$. MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **2** as a blue solid in quantitative yield. Mp: decompostition at 135 °C; IR (KBr disk): $\tilde{\nu}$ [cm^{-1}]=3414, 2945, 1707, 1622, 1583, 1400, 740; MS (ESI, H_2O/MeCN/MeOH+ 10 mmol/L NH_4Ac): *m*/*z* (%)=544 (100) [M−H$^+$]$^-$, 580 (35) [M+Cl$^-$]$^-$, 1091 (8) [2M−H$^+$]$^-$.

4.2. 1,4,7-Tri-*tert*-butyl 10,10′-(6-(6-(benzyloxy)-5-(benzyloxycarbonylamino)-6-oxohexylamino)-1,3,5-triazine-2,4-diyl)-bis-(1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate) 4

To a solution of Z-Lys–OBzl benzenesulfate (1.96 g, 371 mmol) and K_2CO_3 (1.03 g, 7.42 mmol) was added bis-Boc–cyclen triazine **3**[7] (3.73 g, 3.53 mmol) and the reaction mixture was refluxed for 3 d. The filtrate was concentrated under reduced pressure and the crude compound was purified by silica gel column (eluent: $CHCl_3$/MeOH=95:5; R_f=0.73) to give **4** as a white solid (4.19 g, 3.01 mmol, 85%). Mp: 140 °C; $[\alpha]_D^{20}$ −3.4 (*c* 0.01 in $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6): δ=1.13–1.55 (m, 58H, Boc–CH_3, Lys–$CHCH_2CH_2$, $CHCH_2CH_2CH_2$), 1.57–1.74 (m, 2H, Lys–$CHCH_2$), 3.06–3.78 (m, 34H, cyclen–CH_2, $CHCH_2CH_2CH_2CH_2$), 4.00–4.08 (m, 1H, CH), 4.98–5.07 (m, 2H, H-Bzl), 5.10 (s, 2H, H-Z), 6.64 (br s, 1H, NH), 7.27–7.38 (m, 10H, H–Aryl), 7.73 (d, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ=22.9 (−, C-3), 27.9 (+, 12C, Boc), 28.0 (+, 6C, Boc), 28.9 (−, C-4), 30.5 (−, C-2), 40.0 (−, C-5), 49.4 (−, 16C, cyclen), 54.0 (+, CH), 65.5 (−, CH_2Bzl), 66.8 (−, CH_2Z), 78.9 (C_{quat}, Boc), 79.0 (C_{quat}, Boc), 79.1 (C_{quat}, 2C, Boc), 79.3 (C_{quat}, Boc), 79.4 (C_{quat}, Boc), 127.7 (C_{quat}, 2C, Aryl), 127.7 (C_{quat}, 2C, Aryl), 127.8 (C_{quat}, Aryl), 127.9 (C_{quat}, Aryl), 128.3 (C_{quat}, 2C, Aryl), 128.3 (C_{quat}, 2C, Aryl), 155.5 (C_{quat}, 3C, triazin), 156.1 (C_{quat}, ester), 172.2 (C_{quat}, carbamate); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}]=3441, 2974, 2932, 2360, 2342, 1560, 1542, 1410,

1366, 1250, 1166; MS (ESI, DCM/MeOH+10 mmol/L NH_4OAc): *m/z* (%)=1391.2 (100) $[MH^+]$, 646.1 (26) $[M+2H^+-Boc]^{2+}$, 704.7 (7) $[MH^++NH_4^+]^{2+}$.

4.3. 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-6-(4,6-bis(4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl)-1,3,5-triazin-2-ylamino)hexanoic acid 6

Fmoc–OSuc (405 mg, 1.20 mmol) and DIPEA (155 μL, 1.20 mmol) were added in succession to a suspension of **5** (1.54 g, 1.10 mmol) in DCM (100 mL). The reaction mixture was stirred at room temperature for 12 h. It was extracted with an aqueous solution of NaH_2PO_4 (100 mM, 3×40 mL, pH 5.0) and the organic phase was washed with water, then dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The crude product was purified by silica column chromatography (eluent: $CHCl_3$/MeOH=95:5; R_f=0.38) to give **6** (1052 mg, 0.76 mmol, 68%) as a white solid solid. Mp: 102 °C; $[\alpha]_D^{20}$ +25.0 (*c* 0.008 in $CDCl_3$); ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC, HMBC): δ=1.35–1.48 (m, 54H, Boc), 1.49–165 (m, 4H, H-3, H-4), 1.76–1.98 (m, 2H, H-2), 2.99–3.95 (m, 34H, H–cyclen, H-5), 4.13–4.23 (m, 1H, H-10), 4.26–4.44 (m, 3H, H-1, H-9), 6.07 (br s, 1H, NH), 7.21–7.28 (m, 2H, H-B), 7.31–7.38 (m, 2H, H-C), 7.54–7.63 (m, 2H, H-A), 7.71 (d, 2H, ^3J=7.3 Hz, H-D); ^{13}C NMR (100 MHz, $CDCl_3$, HSQC, HMBC): δ=22.0 (−, C-3/4), 28.5 (+, 12C, Boc), 28.5 (+, 6C, Boc), 30.9 (−, C-3/4), 31.9 (−, C-2), 40.1 (−, C-5), 47.2 (+, C-10), 50.2 (−, 16C, cyclen), 54.3 (+, C-1), 66.9 (−, C-9), 80.0 (C_{quat}, 6C, Boc), 119.9 (C_{quat}, 2C, C-D), 125.3 (C_{quat}, 2C, C-A), 127.0 (C_{quat}, 2C, C-B), 127.6 (C_{quat}, 2C, C-C), 141.2 (C_{quat}, 4C, Fmoc), 143.9 (C_{quat}, 2C, Fmoc), 144.1 (C_{quat}, 2C, Fmoc), 156.1 (C_{quat}, triazin), 156.8 (C_{quat}, 2C, triazin), 156.8 (C_{quat}, ester), 175.2 (C_{quat}, acid); IR (KBr disk): $\tilde{\nu}$ $[cm^{-1}]$=3433, 2974, 2932, 1693, 1542, 1411, 1366, 1250, 1165; MS (ESI, DCM/MeOH+10 mmol/L NH_4OAc): *m/z* (%)=703.7 (100) $[MH^++NH_4^+]^{2+}$, 1389.1 (33) $[MH^+]$, 645.2 (21) $[M+2H^+-Boc]^{2+}$, 617.1 (21) $[MH^++2H^+-Boc-\Delta C_4H_8]^{2+}$, 695 (10) $[(MH^++2H^+)]^{2+}$. EA ($C_{70}H_{109}N_{13}O_{16}\cdot 5H_2O$) calculated (%): C 56.84, H 8.12, N 12.32: Found: C 56.80, H 7.86, N 12.03.

4.4. Bis-Zn–cyclen-complex 8

Hepes buffer (10 mM, pH 8) was prepared and 10 mL was heated to 80 °C in a round-bottom flask. L-Tyr(tri-Boc-bis-Cyc)–OH **7** (500 mg, 0.54 mmol) and $Zn(ClO_4)_2$ (402 mg, 1.08 mmol) were each dissolved in 10 mL of Hepes buffer and added dropwise and simultaneously under stirring. The reaction mixture was stirred for further 3 h at 80 °C and at room temperature over night. Compound **8** was obtained as a white solid after lyophilization.

MS (ESI, H_2O/MeCN/MeOH+10 mmol/L NH_4OAc): *m/z* (%)=456.8 (100) $[K^{4+}-2H^+]^{2+}$, 476.4 (24) $[K^{4+}+Cl^--H^+]^{2+}$.

4.5. Fmoc–bpa–Zn$(NO_3)_2$ 10a

To the SAAC **9** (850 mg, 1.54 mmol) in 80 mL MeOH was added a solution of $Zn(NO_3)_2\cdot 6H_2O$ (458 mg, 1.54 mmol) in 20 mL H_2O and the resulting solution was stirred for 1 h. The MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **10a** as a white solid in quantitative yield. Mp: decomposition at 150 °C; $[\alpha]_D^{20}$ −27.2 (*c* 0.007 in MeOH); IR (KBr disk): $\tilde{\nu}$ $[cm^{-1}]$=3416, 3066, 2944, 1711, 1609, 1415, 1383, 1312, 1026, 763; MS (ESI, H_2O/MeCN): *m/z* (%)=613 (100) [Fmoc–BPA–$Zn^{2+}-H^+]^+$, 1229 (10) [2(Fmoc–BPA–Zn^{2+})−$3H^+]^+$.

4.6. 2-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}-3-{3,5-bis[(bis-pyridin-2-ylmethyl-amino)methyl]-4-hydroxyphenyl}propanoic acid 14

DIPEA (0.68 mL, 4.0 mmol) and Fmoc–OSuc (236 mg, 0.70 mmol) were added successively to a suspension of **13** (520 mg, 0.48 mmol) in DCM (50 mL). The reaction mixture was stirred at room temperature for 12 h. The mixture was extracted with an aqueous solution of NaH_2PO_4 (100 mM, 3×20 mL, pH 5.0) and the organic phase was washed with water, then dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The crude product was purified by silica column chromatography (eluent: $CHCl_3$/MeOH=95:5; R_f=0.05) to give **14** (309 mg, 0.37 mmol, 78%) as a pale yellow solid. Mp: decomposition 88 °C; $[\alpha]_D^{20}$ +41.5 (*c* 0.02 in $CHCl_3$); IR (KBr disk): $\tilde{\nu}$ $[cm^{-1}]$=3414, 3252, 3057, 3057, 2924, 2822, 2362, 1714, 1592. ^1H NMR (600 MHz, $CDCl_3$, COSY, HSQC, HMBC): δ=2.97 (dd, 1H, ^2J=13.2 Hz, ^3J=5.0 Hz, TyrCHCH_2), 3.11 (dd, 1H, ^2J=12.7 Hz, ^3J=2.5 Hz, TyrCHCH_2), 3.48–3.50 (m, 2H, TyrCH_2N), 3.76–3.86 (m, 10H, Pyr–CH_2–N, TyrCH_2N), 4.11–4.16 (m, 1H, Fmoc–CH), 4.17–4.22 (m, 1H, Fmoc–CH_2), 4.26–4.31 (m, 1H, TyrCH), 4.32–4.37 (m, 1H, Fmoc–CH_2), 5.95 (d, 1H, ^3J=5.1 Hz, NH), 6.90 (br s, 2H, CH–phenol), 7.10–7.14 (m, 4H, pyr), 7.19–7.27 (m, 6H, pyr, aryl), 7.34–7.39 (m, 2H, aryl), 7.52–7.60 (m, 6H, pyr, aryl), 7.74 (d, 2H, ^3J=7.5 Hz, aryl), 8.61–8.70 (m, 4H, pyr), 10.96 (br s, 1H, Tyr–OH); ^{13}C NMR (151 MHz, $CDCl_3$, HSQC, HMBC): δ=37.6 (−, CH_2), 47.4 (+, CH), 54.8 (−, 2C, CH_2), 57.4 (+, CH), 59.6 (−, 4C, CH_2), 66.2 (−, CH_2), 119.8 (+, 2C, CH), 122.3 (+, 4C, CH), 122.9 (C_{quat}, 2C), 123.5 (+, 4C, CH), 125.3 (+, 2C, CH), 127.0 (+, 2C, CH), 127.5 (+, 2C, CH), 129.2 (C_{quat}, 1C), 131.3 (+, 2C, CH), 136.7 (+, 4C, CH), 141.2 (C_{quat}, 2C), 144.3 (C_{quat}, 1C), 149.8 (+, 4C, CH), 154.2 (C_{quat}, 1C), 155.3 (C_{quat}, carbamate), 158.3 (C_{quat}, 4C), 176.6 (C_{quat}, acid); MS (ESI, DCM/MeCN/H_2O+10 mmol/L TFA): *m/z* (%)=413 (100) $[M+2H^+]$, 826.4 (30) $[MH^+]$. HRMS calculated for $C_{50}H_{48}N_7O_5$ $[MH^+]$: 826.3765; found: 826.3765±5.8 ppm.

4.7. Bis-bpa–Zn(Cl)-complex 15b

The free ligand **14** (320 mg, 0.39 mmol) was dissolved in methanol (25 mL). To this solution, $ZnCl_2$ (52.8 mg, 0.39 mmol) in 5 mL H_2O was added and the mixture was stirred at room temperature for 3 h. Methanol was then removed under reduced pressure and the remaining aqueous solution was lyophilized to give the desired zinc coordinated complex as a white solid in quantitative yield. Mp: decomposition >170 °C; $[\alpha]_D^{20}$ +45.0 (*c* 0.01 in $CHCl_3$); IR (KBr disk): $\tilde{\nu}$ $[cm^{-1}]$=3422, 3055, 2921, 1713, 1606, 1526; MS (ESI, DCM/MeOH+10 mmol/L NH_4OAc): *m/z*

(%)=1070.4 (100) $[K^{3+}+2CH_3COO^-]^+$, 1010.3 (45) $[K^{3+}-H^++CH_3COO^-]^+$, 505.4 (6) $[K^{3+}-H^+]^{2+}$.

4.8. Peptide 16

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using Fmoc protected Rink amide resin (100 mg, subst.: 0.7 mmol/g). SPPS of Fmoc protected aliphatic amino acids and SAAC **9** according to the specific stated procedure provided peptide **16** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ=0.85 (d, 3H, ^3J=6.7 Hz, H-29a), 0.83 (d, 3H, ^3J=6.9 Hz, H-24a), 0.81 (d, 3H, ^3J=6.7 Hz, H-29b), 0.80 (d, 3H, ^3J=6.9 Hz, H-24b), 1.22 (d, 3H, ^3J=7.06 Hz, H-11), 1.23–1.32 (m, 2H, H-3), 1.42–1.46 (m, 2H, H-22), 1.47–1.52 (m, 1H, H-2a), 1.54–1.59 (m, 1H, H-23), 1.60–1.67 (m, 1H, H-2b), 1.69–1.76 (m, 2H, H-4), 1.90–1.96 (m, 1H, H-28), 3.06–3.13 (m, 2H, H-5), 3.52–3.62 (m, 2H, H-14), 3.68 (dd, 1H, ^3J=5.8 Hz, ^2J=16.7 Hz, H-18a), 3.74 (dd, 1H, ^3J=5.6 Hz, ^2J=16.7 Hz, H-18a), 4.06 (dd, 1H, $^3J_1$=6.9 Hz, $^3J_2$=8.9 Hz, H-27), 4.18–4.22 (m, 1H, H-1), 4.31–4.40 (m, 2H, H-21, H-10), 4.50 (br s, 7H, H-7), 7.00 (s, 1H, H-31a), 7.33 (s, 1H, H-31b), 7.41–7.45 (m, 2H, H-B), 7.51 (d, 2H, ^3J=7.9 Hz, H-D), 7.67 (d, 1H, NH-30), 7.87 (dd, 1H, $^3J_1$=7.7 Hz, $^3J_2$=7.7 Hz, H-Ca), 7.87 (dd, 1H, $^3J_1$=7.7 Hz, $^3J_2$=7.7 Hz, H-Cb), 7.96 (d, 1H, NH-25), 8.00–8.08 (m, 2H, NH-13, NH-19), 8.19 (d, 1H, ^3J=7.7 Hz, NH-16), 8.55 (d, 1H, ^3J=7.3 Hz), 8.61–8.63 (m, 2H, H-A); ^{13}C NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ=18.0 (+, C-29a), 18.4 (+, C-11), 19.2 (+, C-29b), 21.5 (+, C-24a), 22.4 (−, C-3), 23.1 (+, C-24b), 23.1 (−, C-4), 24.1 (+, C-23), 30.4 (+, C-28), 31.2 (−, C-2), 40.0 (−, C-14), 40.1 (−, C-22), 41.8 (−, C-18), 48.3 (+, C-10), 51.1 (+, C-21), 52.5 (+, C-1), 53.8 (−, C-5), 56.9 (−, 4C, C-7), 57.5 (+, C-27), 123.9 (+, 2C, C-B), 124.7 (+, 2C, C-D), 137.6 (+, 2C, C–C), 149.1 (+, 2C, C-A), 150.1 (C_{quat}, 2C, C-E), 165.5 (C_{quat}, C-8), 168.4 (C_{quat}, C-20), 171.4 (C_{quat}, C-17), 171.7 (C_{quat}, C-26), 171.8 (C_{quat}, C-12), 172.8 (C_{quat}, C-15); MS (ESI, DCM/MeOH+NH_4OAc): *m*/*z* (%)=363.2 (100) $[M+2H^+]^{2+}$, 725.6 (71) $[MH^+]$.

4.9. Peptide complex 17

To a solution of peptide **16** (20 mg, 0.03 mmol) in 10 mL H_2O was added $Zn(NO_3)_2\cdot 6H_2O$ (8.4 mg, 0.03 mmol). After the mixture was stirred over night a white solid was obtained after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ=0.80–0.86 (m, 12H, H-29, H-24), 1.08–1.19 (m, 2H, H-4), 1.12 (d, ^3J=7.0 Hz, 3H, H-11), 1.42–1.48 (m, 5H, H-22, H-2a, H-3), 1.53–1.61 (m, 2H, H-2b, H-23), 1.91–1.97 (m, 1H, H-28), 2.56–2.62 (m, 2H, H-5), 3.53–3.75 (m, 4H, H-14, H-18), 4.00 (d, 2H, ^2J=16.0 Hz, H-7a), 4.07 (dd, ^3J=9.0 Hz, ^3J=6.8 Hz, 1H, H-27), 4.17 (m, 1H, H-1), 4.24 (dd, ^2J=16.0 Hz, ^4J=2.3 Hz, 2H, H-7b), 4.30–4.40 (m, 2H, H-10, H-21), 7.02 (s, 1H, N-H), 7.34 (s, 1H, N-H), 7.59–7.66 (m, 2H, H-B, H-D), 7.93–7.99 (m, 1H, N-H), 8.05 (t, ^3J=5.5 Hz, 1H, N-H), 8.08–8.12 (m, 2H, H-C), 8.15 (d, 1H, ^3J=7.7 Hz, N-H), 8.50 (d, ^3J=7.2 Hz, 1H, NH), 8.71 (d, ^3J=4.8 Hz, 2H, H-A); ^{13}C NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ=18.0 (+, C-29a), 18.5 (+, C-11), 19.2 (+, C-29b), 21.5 (+, C-24a), 22.8 (−, C-3), 23.1 (+, C-24b), 23.1 (−, C-4), 24.1 (+, C-23), 30.4 (+, C-28), 31.5 (−, C-2), 40.1 (−, C-14), 40.8 (−, C-22), 41.8 (−, C-18), 48.3 (+, C-10), 51.1 (+, C-21), 52.6 (+, C-1), 54.9 (−, C-5), 56.7 (−, C-7), 57.4 (+, C-27), 124.2 (+, 2C, C-B), 124.6 (+, 2C, C-D), 140.7 (+, 2C, C-C), 147.8 (+, 2C, C-A), 155.1 (C_{quat}, 2C, C-E), 165.4 (C_{quat}, C-8), 168.4 (C_{quat}, C-20), 171.4 (C_{quat}, C-17), 171.7 (C_{quat}, C-26), 171.7 (C_{quat}, C-12), 172.7 (C_{quat}, C-15); MS (ESI, H_2O/NH_4OAc): *m*/*z* (%)=394.3 (100) $[M]^{2+}$, 901.4 (14) $[M^{2+}+TFA]^+$, 787.4 (8) $[M^{2+}-H^+]^+$, 850.4 (3) $[M^{2+}+NO_3^-]^+$.

4.10. Peptide 18

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using Fmoc protected Rink amide resin (50 mg, subst.: 0.7 mmol/g). SPPS of Fmoc protected amino acid monomers and SAAC **9** according to the specific stated procedure provided compound **18** as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ=0.75–0.83 (dd, 6H, $^3J_1$=6.7 Hz, $^3J_2$=6.9 Hz, H-21, H-21′), 0.94 (d, 6H, ^3J=6.7 Hz, H-35, H-35′), 1.17 (d, 3H, ^3J=7.2 Hz, H-11), 1.20 (d, 3H, ^3J=7.2 Hz, H-25), 1.20–1.32 (m, 4H, H-3), 1.55–1.42 (m, 2H, H-2′a, H-2′b), 1.55–1.67 (m, 2H, H-2a, H-2′a), 1.69–1.78 (m, 4H, H-4), 1.82 (s, 1H, H-36), 1.85 (s, 1H, H-36), 1.92–1.99 (m, 1H, H-20), 2.01–2.07 (m, 1H, H-34), 3.04–3.12 (m, 4H, H-5), 3.62–3.66 (m, 1H, H-30b), 3.67–3.76 (m, 2H, H-14b, H-30a), 3.94 (dd, 1H, ^3J=16.6 Hz, ^2J=6.1 Hz, H-14a), 4.11–4.23 (m, 3H, H-33, H-10, H-19), 4.28–4.34 (m, 2H, H-24, H-1), 4.49 (m, 8H, H-7), 7.00 (s, 1H, H-8), 7.29–7.33 (m, 1H, H-8), 7.42–7.45 (m, 4H, H-B), 7.53 (d, 4H, ^3J=7.9 Hz, H-D), 7.67 (dd, 1H, ^3J=27.7 Hz, ^4J=8.7 Hz, H-22), 7.86–7.90 (m, 4H, H-C), 7.91 (d, 1H, ^3J=7.4 Hz, H-12), 8.08–8.21 (m, 4H, H-15, H-26, H-28, H-31), 8.61–8.66 (m, 4H, H-A); ^{13}C NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ=17.7 (+, ^{i}Pr), 17.7 (+, C-11), 18.2 (+, ^{i}Pr), 18.2 (+, C-25), 19.1 (+, ^{i}Pr), 22.3 (−, 2C, C-3), 23.1 (−, C-4), 29.7 (+, C-34), 30.5 (+, C-20), 31.3 (−, C-2), 31.7 (−, C-2′), 41.6 (−, C-14), 41.9 (−, C-30), 48.0 (+, C-10), 48.2 (+, C-24), 52.0 (+, C-1), 52.5 (+, C-33), 53.7 (−, C-5), 56.9 (−, C-7), 57.3 (+, C-19), 123.9 (+, C-B), 124.7 (+, C-D), 137.6 (+, C-C), 149.1 (+, C-A), 151.4 (C_{quat}, C-E), 174.1 (C_{quat}, C-9), 167.9 (C_{quat}, C-16), 168.1 (C_{quat}, C-13), 168.1 (C_{quat}, C-29), 170.8 (C_{quat}, C-18), 171.0 (C_{quat}, C-27), 171.5 (C_{quat}, C-32), 171.7 (C_{quat}, C-23), 174.1 (C_{quat}, C-9); MS (ESI, H_2O/NH_4OAc): *m*/*z* (%)=365 (100) $[M+3H^+]^{3+}$, 547 (24) $[M+2H^+]^{2+}$, 1092.7 (0.3) $[MH^+]$.

4.11. Peptide complex 19

To a solution of peptide **18** (10 mg, 0.01 mmol) in 10 mL H_2O was added $Zn(NO_3)_2\cdot 6H_2O$ (5.6 mg, 0.02 mmol). After the mixture was stirred over night a white solid was obtained after lyophilization. MS (ESI, H_2O/MeOH+NH_4OAc): *m*/*z* (%)=407.3 (100) $[M^{4+}-H^+]^{3+}$, 608.9 (74) $[M^{4+}-2H^+]^{2+}$, 365.8 (40) $[(M^{4+}-2Zn^{2+}+3H^+)]^{3+}$.

4.12. Peptide complex 20

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 0.83 mmol/g). SPPS of Fmoc protected amino acids and SAAR **10a** according to the specific stated procedure provided compound **20** as a white solid. ^{1}H NMR

(600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ=0.80 (d, 3H, ^3J=6.7 Hz, H-18′), 0.85 (d, 3H, ^3J=6.5 Hz, H-18), 1.08 (d, 3H, ^3J=6.9 Hz, H-11), 1.15–1.28 (m, 2H, H-3), 1.25 (d, 3H, ^3J=7.3 Hz, H-25), 1.38–1.50 (m, 5H, H-2a, H-4, H-21), 1.52–1.62 (m, 2H, H-2b, H-17), 2.39 (t, 2H, ^3J=7.2 Hz, H-5), 3.21–3.40 (m, 1H, H-10), 3.60 (s, 3H, OMe), 3.66–3.71 (m, 4H, H-7, H-16), 4.18–4.29 (m, 3H, H-24, H-15, H-1), 7.20–7.23 (m, 2H, H-B), 7.50 (d, 2H, ^3J=7.7 Hz, H-D), 7.74 (dd, 1H, $^3J_1$=6.7 Hz, $^3J_2$=7.7 Hz, H-Ca), 7.74 (dd, 1H, $^3J_1$=7.7 Hz, $^3J_2$=7.7 Hz, H-Cb), 7.81–7.96 (m, 1H, C-14), 8.05 (d, 1H, ^3J=7.1 Hz, NH-8), 8.11 (d, 1H, $^3J_1$=6.9 Hz, H-23), 8.16 (t, 1H, ^3J=5.9 Hz, H-20), 8.43–8.47 (m, 2H, H-A); ^{13}C NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ 17.0 (+, C-25), 21.5 (+, H-11), 21.6 (+, C-18′), 22.9 (+, C-18), 24.1 (−, C-17), 26.3 (−, C-4), 32.3 (−, C-2), 40.4 (−, C-21), 41.6 (−, C-16), 47.5 (+, C-24), 50.1 (+, C-10), 51.3 (+, C-1), 51.8 (+, C-15), 51.8 (+, OMe), 53.5 (−, 4C, C-7), 122.0 (+, 2C, C-B), 122.4 (+, 2C, C-D), 136.4 (+, 2C, C-C), 148.7 (+, 2C, C-A), 159.5 (C_{quat}, 2C, C-E), 168.5 (C_{quat}, C-22), 171.8 (C_{quat}, C-13), 172.1 (C_{quat}, C-19), 172.9 (C_{quat}, C-26), 175.5 (C_{quat}, C-9); MS (ESI, H_2O/MeOH+NH_4OAc): *m/z* (%)=359.1 (100) $[M]^{2+}$, 717.3 (14) $[M^{2+}-H^+]^+$, 352.1 (30) $[M-OMe+OH]^{2+}$, 703.3 (20) $[(M-OMe+OH)-H^+]^+$.

4.13. Dipeptide 21

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 1.1 mmol/g). SPPS of Fmoc protected amino acids and SAAC **6** along with HOAt and HATU as coupling reagents according to the specific stated procedure provided compound **21** as a white solid. MS (ESI, DCM/MeOH+10 mmol/L NH_4OAc): *m/z* (%)=626.6 (100) $[M+2H^+]^{2+}$, 1252.1 (29) $[MH^+]$, 1220.1 (4) $[MH^+-CH_3OH]$.

4.14. Peptide 22

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using SASRIN resin (100 mg). SPPS of Fmoc protected amino acids and SAAC **6** along with HOAt and HATU as coupling reagents according to the specific stated procedure provided compound **22** as a white solid. MS (ESI, DCM/MeOH+10 mmol/L NH_4OAc): *m/z* (%)=726.1 (100) $[M+2H^+]^{2+}$, 1451.4 (20) $[MH^+]$, 1463.2 (4) $[M+Na^+]$.

4.15. Peptide complex 25

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 0.83 mmol/g). SPPS of Fmoc protected amino acids and SAAR **2** according to the specific stated procedure provided compound **25** as a white solid. MS (ESI, H_2O/MeOH+NH_4OAc): *m/z* (%)=441 (8) $[(M+2H^+)]^{2+}$, 882 (8) $[MH^+]$, 904 (6) $[M+Na^+]$.

4.16. Peptide complex 26

The synthesis was performed manually in a 5-mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 1.1 mmol/g). SPPS of Fmoc protected amino acids and SAAR **15b** according to the specific stated procedure provided compound **26** as a white solid. MS (ESI, H_2O/MeOH+NH_4OAc): *m/z* (%)=543.4 (100) $[K^{3+}+CH_3COO^-]^{2+}$, 1145.6 (50) $[K^{3+}+2CH_3COO^-]^+$.

Acknowledgements

We thank the Fonds der Chemischen Industrie and the Universität Regensburg for support. R.K. thanks the German Academic Exchange Service (DAAD) for a fellowship within the ISAP programme.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.147.

References and notes

1. Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1–20; Stephenson, K. A.; Banerjee, S. R.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *Bioconjugate Chem.* **2005**, *16*, 1189–1195; Banerjee, S. R.; Maresca, K. P.; Stephenson, K. A.; Valliant, J. F.; Babich, J. W.; Graham, W. A.; Barzana, M.; Dong, Q.; Fischman, A. J.; Zubieta, J. *Bioconjugate Chem.* **2005**, *16*, 885–902; Stephenson, K. A.; Banerjee, S. R.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *Can. J. Chem.* **2005**, *83*, 2060–2066; Stephenson, K. A.; Zubieta, J.; Banerjee, S. R.; Levadala, M. K.; Taggart, L.; Ryan, L.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Valliant, J. F. *Bioconjugate Chem.* **2004**, *15*, 128–136; Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598–8599.
2. Kirin, S. I.; Duebon, P.; Weyhermueller, T.; Bill, E.; Metzler-Nolte, N. *Inorg. Chem.* **2005**, *44*, 5405–5415.
3. Stephenson, K. A.; Zubieta, J.; Banerjee, S. R.; Levadala, M. K.; Taggart, L.; Ryan, L.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Valliant, J. F. *Bioconjugate Chem.* **2004**, *15*, 128–136.
4. See Ref. 5 and: Banerjee, S. R.; Levadala, M. K.; Lazarova, N.; Wei, L.; Valliant, J. F.; Stephenson, K. A.; Babich, J. W.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **2002**, *41*, 6417–6425; Levadala, M. K.; Banerjee, S. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J. *Synthesis* **2004**, 1759–1766; Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598–8599; Wei, L.; Babich, J.; Eckelman, W. C.; Zubieta, J. *Inorg. Chem.* **2005**, *44*, 2198–2209; Banerjee, S. R.; Babich John, W.; Zubieta, J. *Chem. Commun.* **2005**, 1784–1786.
5. Levadala, M. K.; Banerjee, S. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J. *Synthesis* **2004**, 1759–1766.
6. Hutschenreiter, S.; Neumann, L.; Raedler, U.; Schmitt, L.; Tampe, R. *ChemBioChem* **2003**, *4*, 1340–1344.

7. Turygin, D. S.; Subat, M.; Raitman, O. A.; Arslanov, V. V.; Koenig, B.; Kalinina, M. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 5340–5344.
8. Kruppa, M. Ph.D. Thesis, University of Regensburg, 2006.
9. Compound was synthesized according to a similar, at that time unpublished, procedure of Dr. Kate Jolliffe, University of Sydney, Australia. See also Ref. 5.
10. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
11. Jiang, H.; O'Neil, E. J.; DiVittorio, K. M.; Smith, B. D. *Org. Lett.* **2005**, *7*, 3013–3016; Sun, L.; Burkitt, M.; Tamm, M.; Raymond, M. K.; Abrahamsson, M.; LeGourrierec, D.; Frapart, Y.; Magnuson, A.; Kenez, P. H.; Brandt, P.; Tran, A.; Hammarstroem, L.; Styring, S.; Aakermark, B. *J. Am. Chem. Soc.* **1999**, *121*, 6834–6842.
12. *Novabiochem Catalog*; Merck Bioscience: Schwalbach/Ts, 2006; p 224.
13. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.
14. Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561–3564; Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
15. Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695–698; Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279–2282.