Supplementary Material for Chemical Communications

Supplementary data

Efficient microwave-assisted synthesis of multivalent dendrimeric peptides using cycloaddition reaction (click) chemistry

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Experimental Section

Instruments and methods: The peptides were synthesized on an Applied Biosystems 433A Peptide Synthesizer. Analytical HPLC runs were carried out on a Shimadzu HPLC system and preparative HPLC runs were performed on a Gilson HPLC workstation. Analytical HPLC runs were performed on Alltech Adsorbosphere XL C18 and Alltech Prosphere C4 columns (250 × 4.6 mm, pore size 300Å, particle size: 5 μm) or on a Merck LiChroCART CN column (250 × 4.6 mm, pore size 100Å, particle size: 5 μm) at a flow rate of 1.0 mL/min using a linear gradient of buffer B (0 – 100% in 25 min) in buffer A (buffer A: 0.1% TFA in H_2O, buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 v/v). Preparative HPLC runs were performed on an Alltech Prosphere C4 column (250 × 22 mm, pore size 300Å, particle size: 10 μm) or on a Merck LiChroCART CN column (250 × 10 mm, pore size 100Å, particle size: 10 μm) at a flow rate of 4.0 mL/min using a linear gradient of buffer B (0 – 100% in 50 min) in buffer A (buffer A: 0.1% TFA in H_2O, buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 v/v). Liquid chromatography electrospray ionization mass spectrometry was measured on a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode. MALDI-TOF analysis was performed on a Kratos Axima CFR apparatus with bradykinin(1-7) (monoisotopic $[M + H]^+$ 757.399), human ACTH(18-39) (monoisotopic $[M + H]^+$ 2465.198), bovine insulin oxidized B chain (monoisotopic $[M + H]^+$ 3494.651), bovine insulin (monoisotopic $[M + H]^+$ 5730.609) and equine cyotchrome c (average $[M + H]^+$ 12361.96) as external references and α-cyano-4-hydroxycinnamic acid or sinapic acid as matrices. ^1H NMR spectra were recorded on a Varian G-300 (300 MHz) spectrometer and chemical shifts are given in ppm (δ) relative to TMS. ^{13}C NMR spectra were recorded on a Varian G-300 (75.5 MHz) spectrometer and chemical shifts are given in ppm relative to $CDCl_3$ (77.0 ppm). The ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence. R_f values were determined by thin layer chromatography (TLC) on Merck precoated silicagel

60F254 plates. Spots were visualized by UV-quenching, ninhydrin or Cl_2/TDM.[1] Elemental analyses were done by Kolbe Mikroanalytisches Labor (Mülheim an der Ruhr, Germany).

Syntheses:

Compound **1**: 3,5-dihydroxymethylbenzoate (21.4 g, 130 mmol) was dissolved in dry DMF (250 mL) and anhydrous K_2CO_3 (45 g, 330 mmol, 2.5 equiv) was added. To this suspension, a solution of propargylbromide in toluene (35 mL, 314 mmol, 2.5 equiv) was added dropwise. The reaction mixture was stirred for 48 h at room temperature. Then, DMF was removed by evaporation and the residue was redissolved in EtOAc (400 mL) and the organic phase was washed with H_2O (3 × 100 mL), 1N $KHSO_4$ (3 × 100 mL) and brine (3 × 100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was recrystallized from EtOAc/hexane to obtain **1** as off-white crystals in 81% yield (25.2 g). R_f(EtOAc/hexane 4:1 v/v): 0.76; R_f(DCM/MeOH 98:2 v/v): 0.87; R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.83; ^{1}H-NMR ($CDCl_3$) δ 2.55 (t (*J* 2.47 Hz), 2H), 3.91 (s, 3H), 4.72 (d (*J* 2.47 Hz), 4H), 6.81 (t (*J* 2.20 Hz), 1H), 7.29 (d (*J* 2.20 Hz), 2H); ^{13}C-NMR ($CDCl_3$) δ 52.4, 56.0, 76.0, 77.9, 107.5, 108.8, 132.0, 157.8, 158.4; Elemental analysis: calcd for $C_{14}H_{12}O_4$ C 68.83, H 4.95, found C 68.76, H 4.95.

Compound **2**: Methyl ester **1** was dissolved in dioxane/MeOH (114 mL, 14:5 v/v) and 4N NaOH (15 mL, 2.5 equiv) was added in one portion. The obtained reaction mixture was stirred for 5 h at room temperature. Then, the reaction mixture was neutralized by the addition of 1N HCl and the solvent were removed by evaporation. The residue was redissolved in EtOAc (100 mL) and the organic phase was washed with 1N $KHSO_4$ (3 × 50 mL) and brine (3 × 50 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residual solid was obtained in 96% yield (5.13 g) and used without further purification in the next synthesis steps. ^{1}H-NMR (DMSO-d_6) δ 2.50 (broad s, 2H), 4.85 (d (*J* 2.20 Hz), 4H), 6.86 (t (*J* 2.47 Hz), 1H), 7.17 (d (*J* 2.47 Hz), 2H).

The synthesis of dendrimers **3**, **4** and **5** were synthesized using the protocols as described previously.[2]

Compound **3**: R_f(EtOAc/hexane 4:1 v/v): 0.03; R_f(DCM/MeOH 98:2 v/v): 0.13; R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.80; ^{1}H-NMR ($CDCl_3$) δ 2.55 (t (*J* 2.47 Hz), 4H), 3.82 (m, 4H), 3.88 (s, 3H), 4.07 (t (*J* 4.94 Hz), 4H), 4.68 (d (*J* 2.47 Hz), 8H), 6.54 (t (*J* 2.20 Hz), 1H), 6.72 (t (*J* 2.20 Hz), 2H), 6.93 (t (*J* 5.77 Hz), 2H), 7.05 (d (*J* 2.47 Hz), 4H), 7.09 (d (*J* 2.47 Hz) 2H); ^{13}C-NMR ($CDCl_3$) δ 40.4, 53.2, 56.9, 57.6, 77.0, 78.8, 106.3, 107.1, 107.6, 109.0 132.9, 137.4, 159.6, 160.3, 167.4, 168.1; MS analysis: calcd for $C_{38}H_{34}N_2O_{10}$ 678.22, found ES-MS 679.40 $[M + H]^+$, 701.45 $[M + Na]^+$; MALDI-TOF 679.298 $[M + H]^+$, 701.245 $[M + Na]^+$.

Compound **4**: R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.73; ^{1}H-NMR (DMSO-d_6) δ 2.50 (broad s, 8H), 3.59 (m, 12H), 3.61 (s, 3H), 4.14 (m, 12H), 4.83 (d, 8H), 6.78 (m, 7H), 7.12 (m, 14H), 8.68 (m, 6H); ^{13}C-NMR

(DMSO-d_6) δ 37.8, 49.8, 53.3, 63.7, 76.0, 76.4, 102.5, 103.5, 104.2, 105.1, 129.1, 133.8, 155.7, 156.9, 157.1, 163.3; MS analysis: calcd for $C_{86}H_{78}N_6O_{22}$ 1546.52, found MALDI-TOF 1547.490 $[M + H]^+$, 1569.496 $[M + Na]^+$.

Compound **5**: ^{1}H-NMR (DMSO-d_6) δ 2.50 (broad s, 16H), 3.58 (m, 28H), 3.81 (s, 3H), 4.13 (m, 28H), 4.82 (d, 32H), 6.88 (m, 15H), 7.11 (m, 30H), 8.70 (m, 14H); MS analysis: calcd for $C_{182}H_{166}N_{14}O_{46}$ 3282.33, found MALDI-TOF 3321.467 $[M + K]^+$.

Azide **6** was prepared according to: S.G. Alvarez and M.T. Alvarez, *Synthesis*, 1997, 413; azides **7** – **10**, **13** and **14** were synthesized by diazotransfer in solution according to: J.T. Lundquist, IV and J.C. Pelletier, *Org. Lett.*, 2001, **3**, 781; azido peptides **11** and **12** were synthesized by diazotransfer on the solid support according to: D.T.S. Rijkers, H.H.R. van Vugt, H.J.F. Jacobs and R.M.J. Liskamp, *Tetrahedron Lett.*, 2002, **43**, 3657.

Compound **6**: ^{1}H-NMR ($CDCl_3$) δ 1.32 (t (*J* 7.14 Hz), 3H), 3.88 (s, 2H), 4.26 (q (*J* 7.14 Hz), 2H); ^{13}C-NMR ($CDCl_3$) δ 14.0, 50.2, 61.8, 168.2.

Compound **7**: ^{1}H-NMR ($CDCl_3$) δ 1.01/1.03-1.06/1.08 (dd (*J* 15. 11 Hz, *J* 6.88 Hz), 6H), 2.23 (m, 1H), 3.79 (d (*J* 5.49 Hz), 1H).

Compound **8**: ^{1}H-NMR ($CDCl_3$) δ 2.09/2.23 (double m, 2 × 1H), 2.59 (m, 2H), 4.13 (m, 1H).

Compound **9**: ^{1}H-NMR ($CDCl_3$) δ 0.85/0.88 (dd (*J* 6.59 Hz, *J* 1.10 Hz), 6H), 1.26-1.57 (broad m, 3H), 3.02/3.05-3.07/3.09 (dd (*J* 14.1 Hz, *J* 7.5 Hz), 1H), 3.28/3.28-3.32/3.34 (dd (*J* 14.1 Hz, *J* 4.2 Hz), 1H), 3.73 (s, 3H), 4.28 (m, 1H), 4.52 (m, 1H), 6.21 (broad s, 1H), 6.65 (d (*J* 8.52 Hz), 1H), 7.29 (m, 5H); ^{13}C-NMR ($CDCl_3$) δ 22.0, 23.0, 24.8, 38.5, 41.5, 50.8, 52.6, 65.5, 127.5, 128.8, 129.8, 136.1, 168.4, 173.1.

Compound **10**: ^{1}H-NMR ($CDCl_3$) δ 0.96/0.97-0.98/0.99 (dd (*J* 6.4 Hz, *J* 2.3 Hz), 6H), 1.42 (d (*J* 7.14 Hz), 3H), 1.67-1.85 (broad m, 3H), 3.78 (s, 3H), 3.97 (m, 1H), 4.57 (m, 1H), 6.89 (d, 1H).

Compound **11**: Synthesized as described, see reference 3. $[M + H]^+$: calcd: 1338.70, found 1338.72 (ES-MS).

Compound **12**: R_t: 17.88 min (C4); R_t: 19.66 min (C18); MS analysis: calcd for $C_{28}H_{36}N_8O_6$ 580.27, found ES-MS 581.55 $[M + H]^+$, 603.55 $[M + Na]^+$.

Compound **13**: R_t: 12.89 min (C4); R_t: 15.57 min (C18); MS analysis: calcd for $C_{21}H_{37}N_{11}O_8$ 571.28, found 572.55 $[M + H]^+$.

Compound **14**: R_t: 16.81 min (C4); MS analysis: calcd for $C_{27}H_{39}N_{11}O_7$ 629.30, found 630.55 $[M + H]^+$, 652.70 $[M + Na]^+$, 668.25 $[M + K]^+$.

General procedure for the microwave-assisted click reaction: the alkyne (1 equiv) and the azide (1.3 equiv per arm) were dissolved in 3 mL DMF/H_2O 1:1 v/v or THF/H_2O 1:1 v/v. To this solution, $CuSO_4.5H_2O$ (0.05 equiv) and Na-ascorbate (0.50 equiv) were added. The reaction mixture was placed in a microwave reactor (Biotage) and irradiated during 5 – 30 min at 100°C. The cycloaddition reaction was monitored on TLC for completion of the reaction.

Compound **15**: R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.73; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t (*J* 7.14 Hz), 6H), 3.89 (s, 3H), 4.24 (q (*J* 7.14 Hz), 4H), 5.19 (s, 4H), 5.21 (s, 4H), 6.81 (m, 1H), 7.27 (m, 2H); 7.81 (s, 2H); ^{13}C-NMR ($CDCl_3$) δ 14.0, 33.8, 50.8, 52.3, 62.0, 62.4, 106.9, 108.6, 124.3, 132.1, 143.9, 159.1, 166.6; MS analysis: calcd for $C_{22}H_{26}N_6O_8$ 502.48, found ES-MS 503.30 $[M + H]^+$, 525.30 $[M + Na]^+$; MALDI-TOF 503.259 $[M + H]^+$.

Compound **16**: R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.68; ^{1}H-NMR (DMSO-d_6): δ 1.21 (t (*J* 7.14 Hz), 12H), 3.59 (m, 4H), 3.81 (s, 3H), 4.17 (q (*J* 7.14 Hz), 8H,), 4.21 (m, 4H), 5.21 (s, 8H), 5.42 (s, 8H), 6.82 (m, 1H), 6.91 (m, 2H), 7.09 (m, 2H), 7.15 (m, 4H), 8.24 (s, 4H), 8.63 (t, 2H); ^{13}C-NMR (DMSO-d_6): δ 14.2, 40.5, 50.6, 52.5, 61.5, 61.7, 66.6, 104.5, 106.6, 107.8, 126.3, 131.8, 136.5, 142.7, 159.2, 159.9, 166.1, 167.4; MS analysis: calcd for $C_{54}H_{62}N_{14}O_{18}$, 1194.437, found MALDI-TOF 1195.597 $[M + H]^+$, 1217.578 $[M + Na]^+$; Elemental analysis: calcd for $C_{54}H_{62}N_{14}O_{18}$ C 54.27%, H 5.23%, N 16.41%, found C 54.16%, H 5.17%, N 16.22%.

Compound **17**: ^{1}H-NMR (DMSO-d_6): δ 1.20 (t (*J* 7.14 Hz) 24H), 3.60 (broad m, 12H), 3.79 (s, 3H), 4.18 (m, 28 H), 5.21 (s, 16H), 5.41 (s, 16H), 6.72 (m, 2H), 6.81 (m, 1H), 6.93 (m, 4H) 7.04 (m, 6H), 7.18 (m 8H), 8.23 (s, 8H), 8.68 (m, 6H); MS analysis: calcd for $C_{118}H_{134}N_{30}O_{38}$, 2580.50, found MALDI-TOF 2581.012 $[M + H]^+$, 2603.116 $[M + Na]^+$; Elemental analysis: calcd for $C_{118}H_{134}N_{30}O_{38}$ C 54.87%, H 5.37%, N 16.00%, found C 54.88%, H 5.17%, N 16.19%.

Compound **18**: MS analysis: calcd for $C_{246}H_{278}N_{62}O_{78}$, 5347.969, found MALDI-TOF 5389.460 $[(M+CH_3CN) + H]^+$.

Compound **19**: R_t: 19.05 min (C4); R_t: 20.84 min (C18); ^{1}H-NMR (DMSO-d_6): δ 0.74 (d (*J* 6.59 Hz), 6H), 0.94 (d (*J* 6.59 Hz), 6H), 1.24 (m, 2H), 3.84 (s, 3H), 4.10 (broad s, 2H), 5.03 (m, 2H), 5.21 (s, 4H), 7.07 (m, 1H), 7.18 (m, 2H), 8.30 (s, 2H); ^{13}C-NMR (DMSO-d_6): δ 18.5, 19.4, 31.0, 52.6, 61.8, 107.0, 108.3, 124.9, 131.8, 134.2, 142.2, 159.4, 166.1, 170.3; MS analysis: calcd for $C_{16}H_{30}N_6O_8$ 530.21, found; MALDI-TOF

531.339 $[M + H]^+$, 553.308 $[M + Na]^+$.

Compound **20**: R_t: 15.98 min (CN); MS analysis: calcd for $C_{24}H_{26}N_6O_{12}$ 590.16, found; ES-MS 591.29 $[M + H]^+$.

Compound **21**: ^{1}H NMR ($CDCl_3$): δ 0.85 (d (*J* 5.49 Hz), 12H), 1.55 (m, 6H), 3.38 (m, 2H), 3.57 (m, 2H), 3.67 (s, 6H), 3.90 (s, 3H), 4.49 (m, 2H), 5.13 (s, 4H), 5.57 (m, 2H), 6.77 (m, 1H), 6.99 (m, 4H), 7.27 (m, 5H), 7.33 (m, 5H), 7.86 (s, 2H); ^{13}C-NMR ($CDCl_3$): δ 21.6, 22.5, 24.6, 39.5, 40.8, 51.1, 52.2, 61.9, 65.4, 106.8, 108.4, 123.7, 127.2, 128.5, 128.7, 132.0, 134.9, 143.4, 159.0, 166.2, 167.2, 172.3; MS analysis: calcd for $C_{46}H_{56}N_8O_{10}$ 880, found ES-MS 881.50 $[M + H]^+$, 903.30 $[M + Na]^+$; MALDI-TOF 881.288 $[M + H]^+$, 903.244 $[M + Na]^+$; Elemental analysis: calcd for $C_{46}H_{56}N_8O_{10}$ C 62.71%, H 6.41%, N 12.72%, found C 62.64%, H 6.37%, N 12.64%.

Compound **22**: ^{1}H NMR (DMSO-d_6): δ 0.89 (m, 12H), 1.24 (m, 2H), 1.30 (d (*J* 7.42 Hz), 6H), 1.89 (broad m, 4H), 3.64 (s, 6H), 3.84 (s, 3H), 4.25 (m, 2H), 5.19 (s, 4H), 5.47 (m, 2H), 7.05 (m, 1H), 7.19 (m, 2H), 8.35 (s, 2H), 9.03 (d (*J* 6.59 Hz), 2H); ^{13}C NMR (DMSO-d_6): δ 17.3, 22.2, 23.0, 24.8, 41.3, 48.5, 52.7, 53.0, 61.4, 62.2, 107.4, 108.7, 132.3, 143.0, 159.9, 166.5, 168.7, 173.3; MS analysis: calcd for $C_{34}H_{48}N_8O_{10}$ 728.35, found ES-MS 729.55 $[M + H]^+$, 751.45 $[M + Na]^+$; MALDI-TOF 729.417 $[M + H]^+$, 751.359 $[M + Na]^+$; Elemental analysis: calcd for $C_{34}H_{41}N_4O_7$ C 56.03%, H 6.64%, N 15.38%, found C 56.10%, H 6.60%, N 15.28%.

Compound **23**: ^{1}H NMR ($CDCl_3$): δ 0.87/0.90 (d (*J* 6.59 Hz), 24H), 1.29/1.31 (d (*J* 7.14 Hz), 12H), 1.39 (m, 4H), 2.04 (m, 8H), 3.72 (s, 12H), 3.79 (s, 3H), 3.84 (m, 4H), 4.17 (m, 4H), 4.51 (m, 4H), 5.02 (s, 8H), 5.49 (m, 4H), 6.63 (m, 2H), 6.75 (m, 1H), 6.98 (m, 4H), 7.16 (m, 2H), 7.42 (d (J 7.78 Hz), 4H), 7.68 (m, 2H), 8.07 (s, 4H); MS analysis: calcd for $C_{78}H_{106}N_{18}O_{22}$ 1646.77, found MALDI-TOF 1647.730 $[M + H]^+$, 1669.732 $[M + Na]^+$.

Compound **24**: ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 48H), 1.21 (m, 8H), 1.28 (d (7.14 Hz), 24H), 1.98 (m, 16H), 3.58 (overlapping signals, 36H), 3.80 (overlapping signals, 15H), 4.13 (m, 8H), 4.82 (broad s, 16H), 5.19 (m, 8H), 6.77 (m, 21H), 7.05 (m, 14H), 8.19 (s, 8H); ^{13}C NMR (DMSO-d_6): δ16.8, 21.7, 22.5, 24.4, 40.5, 48.0, 52.2, 56.0, 66.4, 78.7, 79.1, 99.7, 104.2, 105.2, 106.2, 107.8, 131.8, 136.5, 142.5, 158.4, 159.2, 159.6, 166.0, 166.1, 168.2, 172.7.

Compound **25**: R_t: 19.1 min (C4); MS analysis: calcd for $C_{136}H_{202}N_{34}O_{36}S_2$ 2952.872, found; MALDI-TOF 2953.310 $[M + H]^+$.

Compound **26**: MS analysis: calcd for $C_{70}H_{84}N_{16}O_{16}$, 1404.625, found MALDI-TOF 1427.822 $[M + Na]^+$.

Compound **27**: MS analysis: calcd for $C_{150}H_{178}N_{34}O_{24}$, 2999.325, found MALDI-TOF 3021.827 $[M + H]^+$.

Compound **28**: MS analysis: calcd for $C_{56}H_{86}N_{22}O_{20}$, 1386.639, found MALDI-TOF 1386.638 $[M + H]^+$.

Compound **29**: R_t: 16.78 min (CN); MS analysis: calcd for $C_{122}H_{182}N_{46}O_{42}$, 2963.352, found MALDI-TOF 2963.805 $[M + H]^+$.

Compound **30**: MS analysis: calcd for $C_{68}H_{90}N_{22}O_{18}$, 1502.680, found MALDI-TOF 1503.913 $[M + H]^+$.

Compound **31**: R_t: 21.23 min (CN); MS analysis: calcd for $C_{146}H_{190}N_{46}O_{38}$, 3195.435, found MALDI-TOF 3195.730 $[M + H]^+$.

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