A Valuable Advance in the Synthesis of Carbohydrate Based Anticancer Vaccines Through Extended Cycloaddition Chemistry

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General Procedure for HPLC and LCMass

All separations involved a mobile phase of 0.05% TFA in water (solvent A)/.00425% TFA in acetonitrile (solvent B).

Compound 3: FmocHN-Tn(OAc)₃-CONH-(CH₂)₃-N₃

C₃₅H₄₂N₆O₁₂
 Exact Mass: 738.29
 Mol. Wt.: 738.74

To a solution of Tn-acid (**1**, 100.0 mg, 0.15 mmol) in DMF-CH₂Cl₂ (1:1, 1.5 mL) at -10 °C was added HOBT (32.4 mg, 0.24 mmol) followed by EDCI (38.0 mg, 0.20 mmol). The reaction mixture was stirred at 0 °C for 30 min and then H₂N-(CH₂)₃-N₃ (15.3 mg, 16.0 μL, 0.15 mmol) was added. After stirring for overnight, the reaction mixture was diluted with EtOAc, washed with brine-H₂O (1:1). The organic phase was dried with Na₂SO₄, concentrated in vacuo. The residue was purified by silica gel column chromatography (3-5% MeOH in CH₂Cl₂) to give the desired amide (**3**, 103.6 mg, 0.14 mmol, 92%) as a white foam. $[\alpha]_D^{23} = 63.8$ (c 1.16, CHCl₃); IR 3318, 2929, 2099, 1748, 1660, 1537, 1371, 1236, 1051, 761, and 741 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.75(d, 2 H, *J* = 7.5 Hz), 7.57 (d, 2 H, *J* = 7.2 Hz), 7.39 (t, 2 H, *J* = 7.4 Hz), 7.30 (t, 2 H, *J* = 7.4 Hz), 6.52 (br, 1 H), 6.01 (d, 1 H, *J* = 9.5 Hz), 5.72 (m, 1 H), 5.32 (d, 1 H, *J* = 2.7 Hz), 5.03 (dd, 1 H, *J* = 3.0, 11.3 Hz), 4.83 (d, 1 H, *J* = 2.8 Hz), 4.60-4.40 (m, 3 H), 4.32 (br, 1 H), 4.20 (t, 1 H, *J* = 6.4 Hz), 4.09 (t, 1 H, *J* = 6.4 Hz), 4.02 (d, 2 H, *J* = 6.3 Hz), 3.86 (m, 2 H), 3.41-3.28 (m, 4 H), 2.14 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.94 (s, 3 H). ¹³C NMR (125 MHz, CDCl₃): δ 170.9, 170.6, 170.4, 170.3, 169.3, 143.6, 143.5, 141.3, 127.9, 127.1, 124.9, 120.1, 99.1, 69.5, 68.3, 67.2, 67.1, 61.8, 54.7, 49.4, 47.6, 47.1, 37.5, 28.4, 23.1, 20.73, 20.71 and 20.66; MS (EI) *m/z* 761.5 [M+ Na]⁺; HRMS calcd for C₃₅H₄₃N₆O₁₂ [M+ H]⁺ 739.2939, found 739.2935.

Compound 4: FmocHN-STn(OAc)₅-CONH-(CH₂)₃-N₃

C₅₃H₆₇N₇O₂₃
 Exact Mass: 1169.43
 Mol. Wt.: 1170.13

To a solution of STn-acid **2** (22.0 mg, 20.2 μmol) in DMF-CH₂Cl₂ (1:1, 1.0 mL) at 0 °C was added HOBT (4.1 mg, 30.3 μmol) followed by EDCI (5.8 mg, 30.3 μmol). The reaction mixture was stirred at 0 °C for 30 min and then H₂N-(CH₂)₃-N₃ (2.2 mg, 20.2 μmol) was added. The reaction mixture was warmed to rt and stirred at rt for 2 h before it

was concentrated *in vacuo*. The resultant reaction residue was purified by silica gel column chromatography (3-5% MeOH in CH₂Cl₂) to give the desired amide **4** (18.5 mg, 15.8 μ mol, 78%) as a white foam. $[\alpha]_D^{23} = 31.4$ (*c* 0.88, CHCl₃); IR 3317, 2903, 2097, 1723, 1643, 1386, 1250, 1101 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.77 (d, 2 H, *J* = 7.5 Hz), 7.60 (d, 2 H, *J* = 7.3 Hz), 7.41 (t, 2 H, *J* = 7.4 Hz), 7.32 (t, 2 H, *J* = 7.3 Hz), 5.44 (d, 1 H, *J* = 2.1 Hz), 5.30 (m, 2 H), 5.09 (m, 2 H), 4.87 (m, 2 H), 4.56 (ddd, 1 H, *J* = 3.6, 9.7, 11.5 Hz), 4.48 (m, 1 H), 4.44 (m, 1 H), 4.35 (m, 1 H), 4.22 (t, 1 H, *J* = 6.8 Hz), 4.17 (t, 1 H, *J* = 6.6 Hz), 4.11 (dd, 1 H, *J* = 4.6, 12.5 Hz), 4.03 (m, 2 H), 3.96 (m, 1 H), 3.92 (m, 1 H), 3.77 (s, 3 H), 3.74 (dd, 1 H, *J* = 6.6, 10.5 Hz), 3.39 (m, 5 H), 2.52 (dd, 1 H, *J* = 4.7, 12.9 Hz), 2.17 (s, 3 H), 2.11 (s, 6 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.80 (m, 2 H), 1.89 (s, 3 H), 1.21 (m, 2 H); ¹³C NMR (125 MHz, CDCl₃): δ 171.3, 171.1, 170.9, 170.7, 170.6, 170.6, 170.0, 168.2, 163.0, 144.0, 141.72, 128.3, 127.5, 125.4, 120.5, 99.7, 99.3, 73.0, 70.4, 69.2, 69.0, 68.9, 67.8, 67.6, 67.6, 63.7, 62.6, 53.3, 49.8, 49.7, 48.1, 47.6, 37.9, 37.7, 36.9, 31.9, 29.0, 23.7 and 23.6; MS (EI) *m/z* 1192.6 [M+ Na]⁺.

Compound 6: AcHN-STn(OAc)₆-CONH-(CH₂)₃-N₃

To a solution of compound **4** (18.0 mg, 15.4 μ mol) in DMF (2.0 mL) was added Et₂NH (8.0 μ L). The resultant reaction mixture was stirred at rt for 1 h before acetic anhydride (10.0 μ L) and Et₃N (8.0 μ L) were added. After stirring at rt for 2 h, the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography (EtOAc 100% to 10% MeOH in CH₂Cl₂) to give the desired azide (**6**, 15.0 mg, 15.1 μ mol, 98% over 2 steps) as a white foam. ¹H NMR (500 MHz, CDCl₃): δ 6.85 (t, 1 H, *J* = 5.8 Hz), 6.78 (d, 1 H, *J* = 7.7 Hz), 6.19 (d, 1 H, *J* = 9.7 Hz), 5.42 (d, 1 H, *J* = 2.2 Hz), 5.31 (m, 2 H), 5.13 (m, 1 H), 5.09 (dd, 1 H, *J* = 3.2, 11.3 Hz), 4.90 (m, 1 H), 4.84 (d, 1 H, *J* = 3.6 Hz), 4.68 (m, 1 H), 4.56 (m, 1 H), 4.37 (dd, 1 H, *J* = 2.6, 12.3 Hz), 4.13 (m, 3 H), 4.08 (m, 2 H), 3.96 (dd, 1 H, *J* = 3.6, 10.5 Hz), 3.88 (dd, 1 H, *J* = 5.2, 10.5 Hz), 3.79 (s, 3 H), 3.76 (dd, 1 H, *J* = 6.6, 10.4 Hz), 3.35 (m, 6 H), 2.52 (dd, 1 H, *J* = 4.8, 12.7 Hz), 2.17 (s, 3 H), 2.15 (s, 3 H), 2.14 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 1.98 (s, 3 H), 1.89 (s, 3 H), 1.80 (m, 2 H), 1.21 (m, 2 H); MS (EI) *m/z* 1012.5 [M+ Na]⁺.

Compound 8: AcHN-STn-CONH-(CH₂)₃-N₃

To a solution of compound **AcHN-STn(OAc)₆-CONH-(CH₂)₃-N₃** (15.0 mg, 15.1 μ mol) in MeOH (1.1 mL) was added aqueous NaOH (0.1 N, 1.1 mL) at 0 °C. The resultant reaction mixture was warmed to rt and stirred at rt overnight before it was neutralized with Dowex and filtered. The crude reaction mixture was purified by RP-HPLC (5-25 % solvent B/solvent A over 20 min.) to give the desired product **8** (10.5 mg, 14.6 μ mol, 96%) as a white foam. ¹H NMR (500 MHz, D₂O): δ 4.78 (d, 1 H, J = 3.7 Hz), 4.44 (m, 1 H), 4.06 (dd, 1 H, J = 3.7, 10.9 Hz), 4.05 (dd, 1 H, J = 3.7, 11.1 Hz), 3.88 (m, 4 H), 3.79 (m, 4 H), 3.69 (m, 3 H), 3.57 (m, 2 H), 3.46 (d, 1 H, J = 8.5 Hz), 3.23 (m, 4 H), 2.61 (dd, 1 H, J = 4.7, 12.5 Hz), 1.98 (s, 3 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.69 (m, 3 H); ¹³C NMR (125 MHz, D₂O): δ 175.3, 174.8, 174.6, 172.2, 171.7, 99.7, 98.1, 73.1, 71.5, 70.1, 68.7, 68.7, 68.0, 67.8, 67.7, 64.1, 63.2, 54.4, 52.1, 50.1, 49.0, 39.9, 37.2, 28.0, 22.4, 22.4 and 22.1; MS (EI) m/z 724.3 [M+ H]⁺.

Compound 9: FmocHN-[Tn(OAc)₃]₂-CONH-(CH₂)₃-N₃

To a solution of **FmocHN-Tn(OAc)₃-CONH-(CH₂)₃-N₃** (**3**, 66.7 mg, 90.0 μ mol) in DMF (1.5 mL) was added diethylamine (20.0 μ L). The reaction mixture was stirred at room temperature for 3 h, the solvents were removed by high vacuum. To a solution of Tn-acid (**1**, 62.3mg, 94.0 μ mol) in DMF-CH₂Cl₂ (1:1, 1.0 mL) at -10 °C was added HOBT (19.2

mg, 0.14 mmol) followed by EDCI (22.4 mg, 0.12 mmol). The reaction mixture was stirred at 0 °C for 30 min and then was transferred to the solution of **H₂N-Tn(OAc)₃-CONH-(CH₂)₃-N₃** in DMF-CH₂Cl₂ (1:1, 1.0 mL) at room temperature. After stirring for 3 h, the reaction mixture was diluted with EtOAc, washed with brine-H₂O (1:1). The organic phase was dried with Na₂SO₄, concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3-5% MeOH in CH₂Cl₂) to give the desired dimer (**9**, 92.0 mg, 80.0 μmol, 88%) as a white foam. $[\alpha]_D^{23} = 75.7$ (*c* 1.12, CHCl₃); IR 3312, 3069, 2937, 2099, 1748, 1652, 1538, 1450, 1371, 1249 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 7.74 (d, 2 H, *J* = 7.6 Hz), 7.55 (m, 2 H), 7.39 (m, 2 H), 7.29 (m, 2 H), 7.15 (d, 1 H, *J* = 7.3 Hz), 6.74 (m, 1 H), 6.60 (d, 1 H, *J* = 8.3 Hz), 6.16 (d, 1 H, *J* = 9.2 Hz), 6.01 (d, 1 H, *J* = 6.1 Hz), 5.32 (d, 2 H, *J* = 1.4 Hz), 5.08 (dd, 1 H, *J* = 3.2, 11.7 Hz), 5.05 (dd, 1 H, *J* = 3.2, 11.7 Hz), 4.94 (m, 1 H), 4.85 (d, 1 H, *J* = 3.4 Hz), 4.67 (m, 1 H), 4.53 (m, 3 H), 4.39 (dd, 1 H, *J* = 6.4, 10.1 Hz), 4.27 (m, 1 H), 4.18 (t, 1 H, *J* = 6.3 Hz), 4.11 (t, 1 H, *J* = 6.4 Hz), 4.10-3.90 (m, 7 H), 3.70 (m, 2 H), 3.37 (m, 4 H), 2.14 (t, 2 H, *J* = 3.4 Hz), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.01 (m, 1 H), 1.99 (s, 3 H), 1.98 (s, 1 H), 1.96 (s, 3 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.92 (s, 3 H) and 1.80 (m, 2 H). ¹³C NMR (125 MHz, CDCl₃): δ 170.9, 170.9, 170.85, 170.80, 170.7, 170.5, 170.3, 169.5, 168.6, 156.4, 143.6, 143.4, 141.3, 127.8, 127.1, 127.1, 124.8, 124.7, 120.1, 99.5, 98.6, 69.5, 69.4, 68.4, 68.2, 67.6, 67.2, 67.0, 66.9, 61.8, 61.7, 54.8, 53.8, 53.2, 49.3, 47.9, 47.5, 47.0, 31.7, 30.9, 29.3, 28.3, 23.1, 23.0, 20.7 and 20.7. MS (EI) *m/z* 1177.6 [M+ Na]⁺, 1189.6 [M+ Cl]⁻.

Compound 10: FmocHN-[Tn(OAc)₃]₃-CONH-(CH₂)₃-N₃

To a solution of **FmocHN-[Tn(OAc)₃]₂-CONH-(CH₂)₃-N₃** (**9**, 90.0 mg, 78.0 μmol, 1) in DMF (1.5 mL) was added diethylamine (20.0 μL). The reaction mixture was stirred at room temperature for 1 h, the solvents were removed by high vacuum. To a solution of Tn-acid (**1**, 53.7mg, 82.0 μmol) in DMF-CH₂Cl₂ (1:1, 1.0 mL) at -10 °C was added HOBt (16.6 mg, 0.12 mmol) followed by EDCI (19.4 mg, 0.10 mmol). The reaction mixture was stirred at 0 °C for 30 min and then was transferred to the solution of **H₂N-[Tn(OAc)₃]₂-CONH-(CH₂)₃-N₃** in DMF-CH₂Cl₂ (1:1, 1.0 mL) at room temperature. After stirring for 3 h, the reaction mixture was diluted with EtOAc, washed with brine-H₂O (1:1). The organic phase was dried with Na₂SO₄, concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3-5% MeOH in CH₂Cl₂) to give the desired trimer (**10**, 111.0 mg, 0.07 mmol, 90% over 2 steps) as a white foam. $[\alpha]_D^{24} = 103.3$ (*c* 1.08, CHCl₃); IR 3327, 3070, 2934, 2100, 1747, 1664, 1530, 1445, 1372, 1233,

1132, 1051, 915 and 734 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 7.72 (d, 2 H, $J = 7.5$ Hz), 7.54 (d, 2 H, $J = 7.4$ Hz), 7.37 (t, 2 H, $J = 7.4$ Hz), 7.27 (t, 2 H, $J = 7.4$ Hz), 7.14 (m, 1 H), 7.04 (m, 2 H), 6.95 (d, 1 H, $J = 8.6$ Hz), 6.56 (d, 1 H, $J = 8.9$ Hz), 6.40 (d, 1 H, $J = 8.7$ Hz), 5.96 (d, 1 H, $J = 6.9$ Hz), 5.35 (d, 1 H, $J = 2.5$ Hz), 5.33 (s, 2 H), 5.14 (dd, 1 H, $J = 3.1, 11.4$ Hz), 5.09 (dd, 1 H, $J = 3.1, 11.6$ Hz), 5.05 (dd, 1 H, $J = 3.0, 11.5$ Hz), 4.93 (d, 2 H, $J = 3.1$ Hz), 4.89 (d, 1 H, $J = 2.8$ Hz), 4.56-4.45 (m, 5 H), 4.45-4.30 (m, 3 H), 4.21-4.15 (m, 4 H), 4.10-4.03 (m, 3 H), 4.03-4.0 (m, 2 H), 4.0-3.85 (m, 3 H), 3.83-3.80 (m, 3 H), 3.73 (t, 1 H, $J = 8.6$ Hz), 3.68 (t, 1 H, $J = 8.5$), 3.40-3.15 (m, 4 H), 2.12 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.01-1.97 (s x 6, 18 H), 1.94 (s, 3 H), 1.93 (s, 3 H), 1.92 (s, 3 H), 1.89 (s, 3 H) and 1.75 (m, 2 H). ^{13}C NMR (125 MHz, CDCl_3): δ 171.6, 171.2, 171.1, 171.0, 170.8, 170.6, 170.6, 170.5, 170.3, 169.8, 168.9, 155.9, 143.6, 143.4, 141.3, 127.8, 127.0, 124.9, 124.8, 120.0, 98.6, 98.2, 98.1, 69.5, 68.8, 68.4, 67.9, 67.8, 67.3, 67.1, 67.1, 66.7, 62.7, 61.8, 61.7, 60.3, 54.1, 53.9, 53.8, 53.0, 49.1, 47.7, 47.5, 47.4, 47.0, 31.7, 29.2, 28.3, 23.2, 22.9, 22.9, 21.0, 20.9, 20.8, and 20.7. MS (EI) m/z 1593.7 $[\text{M} + \text{Na}]^+$, 1605.6 $[\text{M} + \text{Cl}]^-$, HRMS calcd for $\text{C}_{69}\text{H}_{90}\text{N}_{10}\text{O}_{32}$ $[\text{M} + \text{Na}]^+$ 1593.5620, found 1593.5592.

Compound 11: AcHN-[Tn(OAc)₃]₃-CONH-(CH₂)₃-N₃

To the solution of **FmocHN-[Tn(OAc)₃]₃-CONH-(CH₂)₃-N₃ (10, 109.0 mg, 69.0 μmol) in DMF (1.5 mL), was added diethylamine (30.0 μL). The reaction mixture was stirred at room temperature for overnight then Ac_2O (60.0 μL) was added. After stirring for 3 h, the reaction mixture was diluted with EtOAc , washed with brine- H_2O (1:1). The organic phase was dried with Na_2SO_4 , concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3-5% MeOH in CH_2Cl_2) to give the desired cluster (**11**, 92.0 mg, 66.0 μmol , 96%) as a white foam. $[\alpha]_D^{21} = 117.7$ (c 1.13, CHCl_3); IR 3316, 3075, 2937, 2100, 1748, 1660, 1539, 1434, 1372, 1236, 1330, 1051, 917 and 732 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 7.47 (br, 1 H), 7.15 (br, 1 H), 7.08 (d, 1 H, $J = 8.1$ Hz), 6.83 (br, 1 H), 6.59 (d, 1 H, $J = 9.1$ Hz), 5.34 (d, 1 H, $J = 2.1$ Hz), 5.33 (d, 1 H, $J = 2.9$ Hz), 5.31 (d, 1 H, $J = 2.4$ Hz), 5.15 (m, 1 H), 5.11 (dd, 1 H, $J = 11.5, 3.0$ Hz), 5.08 (dd, 1 H, $J = 3.0, 11.4$ Hz), 4.94 (d, 1 H, $J = 3.2$ Hz), 4.91 (d, 1 H, $J = 3.4$ Hz), 4.87 (d, 1 H, $J = 3.5$ Hz), 4.75-4.45 (m, 5 H), 4.21-4.10 (m, 3 H), 4.10-3.96 (m, 5 H), 3.94 (dd, 1 H, $J = 7.4, 11.3$ Hz), 3.89 (dd, 1 H, $J = 4.7, 9.9$ Hz), 3.81-3.72 (m, 4 H), 3.68 (dd, 1 H, $J = 6.7, 9.7$ Hz), 3.41-3.10 (m, 2 H), 3.11-3.02 (m, 2 H), 2.13 (m, 1 H), 2.12 (s, 3 H), 2.11 (s, 6 H), 2.03 (s,**

3 H), 2.02 (s, 3 H), 2.01 (s, 6 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.96 (s, 3 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.92 (s, 3 H) and 1.76 (m, 2 H). ^{13}C NMR (125 MHz, CDCl_3): δ 171.3, 171.2, 171.1, 170.9, 170.8, 170.7, 170.6, 170.5, 170.3, 169.8, 169.2, 169.1, 98.6, 98.3, 98.2, 69.5, 68.3, 68.2, 67.7, 67.5, 67.2, 66.2, 67.1, 66.8, 66.8, 66.7, 62.5, 61.9, 61.7, 53.8, 53.7, 53.1, 52.6, 49.1, 47.7, 47.6, 47.5, 37.4, 31.7, 30.9, 29.3, 28.3, 23.1, 23.0, 22.93, 22.89, 20.87, 20.80, 20.75 and 20.66. MS (EI) m/z 1413.7 $[\text{M} + \text{Na}]^+$, 1425.6 $[\text{M} + \text{Cl}]^-$, HRMS calcd for $\text{C}_{56}\text{H}_{83}\text{N}_{10}\text{O}_{31}$ $[\text{M} + \text{H}]^+$ 1391.5226, found 1391.5227.

Compound 12: AcHN-[Tn]₃-CONH-(CH₂)₃-N₃

AcHN-[Tn(OAc)₃]₃-CONH-(CH₂)₃-N₃ (11, 90.0 mg, 64.7 μmol) was dissolved in the solution of NH_3 in MeOH (2.0 mL, 7 N). The reaction mixture was stirred at room temperature for overnight and concentrated *in vacuo*. The residue was purified by HPLC (0-25% solvent B/solvent A over 20 min) gave desired compound (**12**, 50.0 mg, 49.0 μmol , 76%) as a white foam. ^1H NMR (500 MHz, D_2O): δ 4.91 (m, 3 H), 4.72 (t, 1 H, J = 5.6 Hz), 4.66 (t, 1 H, J = 5.6 Hz), 4.59 (t, 1 H, J = 5.5 Hz), 4.19 (m, 3 H), 4.02-3.91 (m, 6 H), 3.91-3.85 (m, 6 H), 3.83-3.71 (m, 9 H), 3.35 (t, 2 H, J = 6.6 Hz), 3.10 (m, 2 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.06 (s, 3 H), 2.01 (s, 3 H) and 1.78 (ddd, 2 H, J = 6.7, 13.4 Hz). ^{13}C NMR (125 MHz, D_2O + trace of acetone): 175.3, 174.9, 174.9, 174.7, 171.9, 171.3, 171.3, 98.6, 98.5, 98.3, 71.9, 71.9, 68.9, 68.9, 68.3, 68.2, 67.8, 67.8, 67.7, 61.74, 61.67, 54.2, 54.0, 50.3, 50.2, 49.2, 37.5, 28.3, 22.6 and 22.2. MS (EI) m/z 1013.29 $[\text{M} + \text{H}]^+$.

Compound 13: AcNH-Ala-Lys-Arg-Tyr-Lys-Phe-Ala-Lys-Ser-Ala-NH₂

The peptide was prepared by standard solid phase synthesis with PAL-PEG-PS amine resin. The peptide was cleaved from resin by the use of 95% TFA with triethylsilane (2.5%) and water (2.5%). All the acid sensitive protecting groups on the side chain were removed too. And the peptide was purified with RP-HPLC (15-45% solvent B / solvent A over 20 min) to give the desired peptide **13**.

Compound 14:

To the solution of **AcNH-Ala-Lys-Arg-Tyr-Lys-Phe-Ala-Lys-Ser-Ala-NH₂** (**13**, 10.0 mg, 8.26 μ mol) in H₂O (1.0 mL), was added the 0.4 mL of the solution of sodium bicarbonate (5.0 mg/1.0 mL) in H₂O at room temperature. *N*-succinimido 4-pentynonate (11.6 mg) was dissolved in DMF (0.2 mL) at room temperature. To the solution of peptide was added the solution of *N*-succinimido 4-pentynonate (29.7 μ mol, 100.0 μ L) in DMF at room temperature. The reaction mixture was stirred for 1 h, and then 100.0 μ L of the solution of NaHCO₃ was added. 19 h later, LC-MS (5-60%, solvent B/solvent A over 20 min) showed the reaction completed. To the reaction mixture, 1.5 mL of 0.04% TFA in water and 1.0 mL of CH₃CN were added. The residue was purified by HPLC (0-25% solvent B/solvent A over 20 min, semi prep C18 column) gave desired compound (**14**, 8.2 mg, 68%) as a white foam. Selected ¹H NMR (500 MHz, CD₃OD): δ 7.30-7.19 (m, 5 H), 7.08 (d, 1 H, *J* = 8.4 Hz), 7.03 (d, 1 H, *J* = 8.4 Hz), 6.72 (d, 1 H, *J* = 8.4 Hz), 6.71 (d, 1 H, *J* = 8.4 Hz). MS (EI) *m/z* 1450.91 [*M*+ H]⁺.

Figure 1: UV and MS traces of LC-Mass analysis of synthesis of compound **14**.

Figure 2: UV and MS traces of LC-Mass analysis of compound **14** after purification by HPLC

Compound 15:

Figure 3: UV and MS traces of LC-Mass analysis of synthesis of compound 15.

Figure 4: UV and MS traces of LC-Mass analysis of compound **15** after purification by HPLC

Figure 5: UV and MS traces of LC-Mass analysis of synthesis of compound **15**.

Compound 16:

To a vial with peptide **14** (1.1 mg) and azide **8** (2.4 mg, 4.5 eq) was added a solution of triethylamine hydrochloride (2.5 mg in 2.5 mL, pick up 0.15 mL) and nanosized copper (0.8 mg). After stirring at rt for 1 h, analysis of LC-MS showed that all the three alkyne moieties were converted into triazoles. The reaction mixture was filtered and purified by RP-HPLC (5-60% solvent B/solvent A over 20 min, semi prep C18 column) to give the desired product (**16**, 2.1 mg, 78 %).

Figure 6: UV and MS traces of LC-Mass analysis of synthesis of compound **16**.

Compound 17:

To a mixture of **AcHN-[Tn]₃-CONH-(CH₂)₃-N₃** (**12**, 4.7 mg, 4.65 μ mol) and **14** (1.5 mg, 1.03 μ mol), were added water (200.0 μ L), *t*-BuOH (50 μ L) and Cu(0) nanosize powder (1.5 mg) at room temperature. The reaction mixture was stirred for 2 days, and then 0.5 mg of Cu(0) nanosize powder was added. The reaction mixture was stirred for one more day, and then centrifuged for 5 min. The aqueous phase was removed, the Cu(0) was washed with 500.0 μ L of water and centrifuged. The aqueous phases were combined, and passed through 0.2 μ m filter. The light blue solution was purified by HPLC (5-50% solvent B/solvent A over 20 min, semi prep C18 column) gave desired compound (**17**, 3.0 mg, 0.67 μ mol, 65%) as a white powder. Selected ¹H NMR (500 MHz, D₂O): δ 7.78 (s, 2 H), 7.77 (s, 1 H), 7.31 (m, 2 H), 7.23 (m, 3 H), 7.06 (d, 2 H, *J* = 8.4 Hz), 7.79 (d, 1 H, *J* = 8.4 Hz) and 7.77 (d, 1 H, *J* = 8.4 Hz). MS (EI) *m/z* 2245.26 [*M*+ 2*H*]⁺⁺, 1497.27 [*M*+ 3*H*]⁺⁺⁺.

Figure 7: UV and MS traces of LC-Mass analysis of synthesis of compound 17.

Figure 8: UV and MS traces of LC-Mass analysis of synthesis of compound 17.

Figure 9: UV and MS traces of LC-Mass analysis of compound **17** after purification by HPLC

Compound 19:

To a solution of phenylacetylene (78.5 μ L, 0.72 mmol) and azido-Leu (75.0 mg, 0.48 mmol), **18**, in *t*-BuOH (0.1 mL) and PBS buffer (0.1 mL) was added Cu (0) nano powder (3.0 mg, 0.1 eq). The reaction mixture was stirred at room temperature for overnight. The reaction mixture was diluted with EtOAc, and filtered by silical gel. The organic phase was dried with Na₂SO₄, concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% MeOH and 0.05% AcOH in CH₂Cl₂) to give the desired trimer (**19**, 112.0 mg, 0.47 mmol, 98%) as a white solid. IR 2960, 1735, 1463, 1240, 1198, 1087, 977, 765 and 697 cm⁻¹; ¹H NMR (500 MHz, CD₃OD): δ 8.49 (s, 1 H), 7.84 (d, 2 H, *J* = 7.7 Hz), 7.43 (t, 2 H, *J* = 7.7 Hz), 7.34 (t, 1 H, *J* = 7.7 Hz), 5.52 (dd, 1 H, *J* = 4.3, 11.0 Hz), 2.30 (m, 1 H), 2.12 (m, 1 H), 1.39 (m, 1 H), 1.04 (d, 3 H, *J* = 6.5 Hz), 0.95 (d, 3 H, *J* = 6.5 Hz). ¹³C NMR (125 MHz, CD₃OD): δ 172.2, 149.1, 131.8, 130.1, 129.5, 126.8, 122.3, 63.1, 41.7, 26.2, 23.2 and 21.6. MS (EI) *m/z* 260.1 [M+H]⁺, 257.8 [M-H]⁻; HRMS calcd for C₁₄H₁₇N₃O₂ [M+ H]⁺ 260.1399, found 260.1402.