Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting information

Synthesis of 1,4-triazole linked zanamivir dimers as highly potent inhibitors of influenza A and B

Benjamin H. Fraser[a]*, Stephanie Hamilton[b], Anwen M. Krause-Heuer[a], Philip J. Wright[b], Ivan Greguric[a], Simon P. Tucker[b], Alistair G. Draffan[b], Valery V. Fokin[c], K. Barry Sharpless[c]

[a]*Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, New South Wales, 2232, Australia. Fax:+61 2 9717 9262 Tel:+61 2 9717 3887 E-mail: benjamin.fraser@ansto.gov.au*

[b]*Biota Holdings Limited, Unit 10, 585 Blackburn Road, Notting Hill, Victoria, 3168, Australia.*

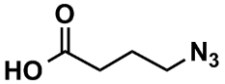
[c]*The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California, 92037, United States of America.*

1. Chemistry general

All reagents were purchased from commercial suppliers and were used without further purification. All solvents used (dichloromethane, ethyl acetate, hexanes, toluene) were HPLC grade and were dried using a Braun MB SPS-800 (Solvent Purification System). All reactions were performed under an atmosphere of dry nitrogen unless specified otherwise. Infra-red spectra were collected on a Bruker Alpha-P FTIR spectrometer (diamond ATR) and the main peaks are reported in wavenumbers (cm^{-1}) with the suffixes s = strong, w = weak, m = medium. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR); chemical shifts (δ) were recorded in ppm and spectra were referenced using the residual internal solvent signal or tetramethylsilane (TMS); multiplicities are reported as s = singlet, d = doublet, t = triplet, q = quartet with prefixes b = broad and/or a = apparent; coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were performed on a Waters Micromass ZQ mass spectrometer using chemical ionization. HPLC analysis was performed on a Phenomonex Kinetex C18 3 x 50 mm column at 40°C and absorbance was measured at 230 nm. Samples were prepared at 1 mg/mL concentration, injection volumes were 5.0 µL and the flow rate was 1 mL/min. Solvent gradient conditions are specified for each sample where solvent A = 5 mM NH_4OAc (pH unadjusted) and B = Methanol. Flash chromatography was performed on a Grace Reveleris® or Biotage Isolera® automated chromatography system using normal and reverse phase cartridges. Reaction progress was monitored using thin layer chromatography (Sigma-Aldrich® TLC plates Z122785).

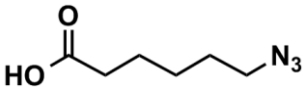
2. Procedures

4-azidobutanoic acid.



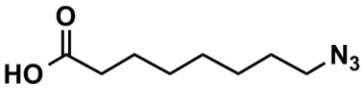
To a stirred solution of ethyl-4-bromobutyrate (5.00 g, 25.6 mmol) in 1:1 acetone/water (30 mL) was added sodium azide (2.50 g, 38.4 mmol). The solution was heated to reflux for 18 hrs after which ethyl acetate (50 mL) was added and the organic phase separated from the aqueous phase. The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the organic phase and extractions were combined, washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give a colourless crude oil (3.87 g). The crude oil was dissolved in THF/water (1:1) and to this solution was added potassium hydroxide (4.20 g, 75 mmol). The solution was stirred at rt o/n after which it was acidified to pH 1 by addition hydrochloric acid. The solution was diluted with ethyl acetate (50 mL) and the organic phase separated from the aqueous phase. The aqueous phase was extracted with ethyl acetate (3 × 30 mL) and the organic phase and extractions were combined, washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the *title compound* as a colourless oil (2.77 g, 84%). The spectroscopic data for the compound is consistent with that reported previously.[1] ^{1}H NMR ($CDCl_3$) δ 3.366 (t, 2H, *J* = 6.6 Hz), 2.466 (t, 2H, *J* = 7.2 Hz), 1.902 (ap, 2H, *J* = 6.9 Hz). ^{13}C NMR ($CDCl_3$) δ 179.50, 50.51, 31.02, 23.98. IR (ATR) 3041w, 2939m, 2882w, 2668w, 2093s, 1703s, 1446m, 1413m, 1350m, 1240s, 1165m, 1078w, 1002w, 924m, 858m, 673w.

6-azidohexanoic acid.



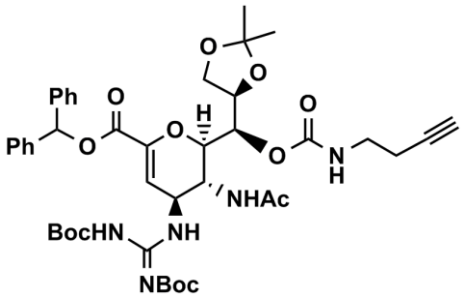
To a stirred solution of 6-bromohexanoic acid (3.00 g, 15.4 mmol) in 1:1 acetone/water (10 mL) was added sodium azide (2.0 g, 30.8 mmol). The solution was heated to 40 °C and stirred for 24 hrs. After this time ethyl acetate (20 mL) was added and the organic phase separated from the aqueous phase. The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the organic phase and extractions were combined, washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the *title compound* as a colourless oil (2.20 g, 91%). The spectroscopic data for the compound is consistent with that reported previously.[2] ^{1}H NMR ($CDCl_3$) δ 3.351 (t, 2H, *J* = 6.7 Hz), 2.461 (t, 2H, *J* = 7.1 Hz), 1.622-1.751 (m, 4H), 1.350-1.511 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 179.88, 51.29, 33.92, 28.64, 26.25, 24.25. IR (ATR) 3271m, 3101m, 2941m, 2867m, 2671w, 2092s, 1704s, 1563w, 1437m, 1456m, 1412m, 1370m, 1252s, 1184s, 1097m, 1005s, 924m, 860m, 769s.

8-azidooctanoic acid.



To a stirred solution of 8-bromooctanoic acid (2.00 g, 8.96 mmol) in 1:1 acetone/water (5 mL) was added sodium azide (1.16 g, 17.8 mmol). The solution was heated to 40 °C and stirred for 18 hrs. After this time ethyl acetate (10 mL) was added and the organic phase separated from the aqueous phase. The aqueous phase was extracted with ethyl acetate (3 × 10 mL) and the organic phase and extractions were combined, washed with water (5 mL) and brine (5 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the *title compound* as a colourless oil (1.44 g, 87%). The spectroscopic data for the compound is consistent with that reported previously.[3] ^{1}H NMR ($CDCl_3$) δ 3.213 (t, 2H, *J* = 6.8 Hz), 2.303 (t, 2H, *J* = 7.4 Hz), 1.617-1.748 (m, 4H), 1.325-1.293 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 179.73, 51.387, 33.953, 28.913, 28.869, 28.811, 26.551, 24.634. IR (ATR) 3273m, 3094m, 2939m, 2864m, 2670w, 2091s, 1702s, 1561w, 1434m, 1410m, 1369m, 1248s, 1183s, 1003s, 922m, 855m, 772s.

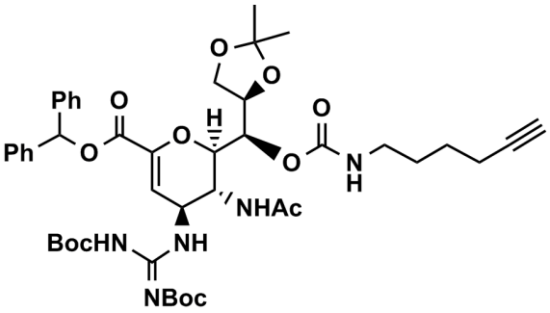
(2*R*, 3*R*, 4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-2-((*S*)-(but-3-ynylcarbamoyloxy)((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3,4-dihydro-2H-pyran-6-carboxylate (14)



To a flask containing pent-4-ynoic acid (1.01 g, 10.3 mmol) was added thionyl chloride (3.68 g, 2.24 mL, 30.9 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (10 mL) and

trimethylsilyl azide (2.37 g, 2.71 mL, 20.6 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (10 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (3.80 g, 5.15 mmol) and DMAP (1.26 g, 10.3 mmol) in DCM (10 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (6 mL) and purified directly by reverse phase chromatography (Biotage® 60g SNAP cartridge KP-C18-HS) using a gradient elution of 5% MeCN/water to 100% MeCN/water over 15 column volumes. The *title compound* was obtained as a white solid (4.29 g, 83%). ^{1}H NMR ($CDCl_3$) δ 11.437 (s, 1H), 8.45 (d, 1H, *J* = 8.3 Hz), 7.261-7.502 (m, 10H), 6.969 (s, 1H), 6.075 (d, 1H, *J* = 7.6 Hz), 5.968 (at, 1H, *J* = 1.8 Hz), 5.007-5.304 (m, 4H), 4.312-4.443 (m, 2H), 3.951-4.207 (m, 4H), 3.632-3.781 (m, 2H), 3.238-3.400 (m, 3H), 2.303-2.508 (m, 4H), 1.906 (s, 3H), 1.502 (s, 18H), 1.362 (s, 3H), 1.288 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.80, 163.15, 160.60, 155.54, 152.79, 145.37, 128.663, 128.663, 128.218, 127.215, 110.460, 109.043, 83.797, 79.692, 78.109, 77.832, 74.547, 70.211, 69.930, 68.050, 66.291, 48.989, 48.267, 28.352, 28.219, 26.643, 23.512, 21.398. IR (ATR) 3301m, 2933m, 1725s, 1639s, 1607s, 1541s, 1432s, 1408s, 1358s, 1302s, 1243s, 1137s, 1052s, 1032s, 970s, 844m, 805m, 757s, 697s. LRMS predicted for $C_{43}H_{55}N_5O_{12}$ = 833.4. Found 834.4 ($M+H^+$). HRMS predicted for $C_{43}H_{55}N_5O_{12}+H^+$ = 834.3925. Found 834.3928 ($M+H^+$).

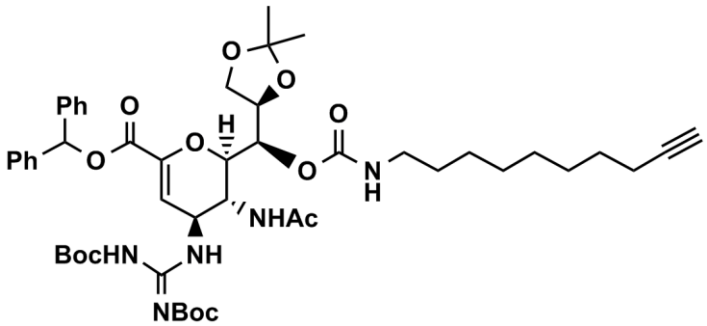
(2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hex-5-ynylcarbamoyloxy)methyl)-3,4-dihydro-2H-pyran-6-carboxylate (15)



To a flask containing hept-6-ynoic acid (0.76 g, 6.02 mmol) was added thionyl chloride (2.15 g, 1.31 mL, 18.1 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (5 mL) and trimethylsilyl azide (1.38 g, 1.58 mL, 12.0 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (5 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (2.22 g, 3.01 mmol) and DMAP (0.74 g, 6.02 mmol) in DCM (5 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (4 mL) and purified directly by reverse phase chromatography

(Biotage® 30g SNAP cartridge KP-C18-HS) using a gradient elution of 5% MeCN/water to 100% MeCN/water over 15 column volumes. The *title compound* was obtained as a white solid (2.05 g, 79%).^{1}H NMR ($CDCl_3$) δ 8.461 (d, 1H, *J* = 8.2 Hz), 7.312-7.441 (m, 10H), 6.962 (s, 1H), 5.988 (d, 1H, *J* = 3.1 Hz), 5.838 (d, 1H, *J* = 9.2 Hz), 5.248 (dt, 1H, *J* = 3, 8.6 Hz), 5.132 (d, 1H, *J* 7.2 Hz), 4.848 (at, 1H, *J* = 6 Hz), 4.288-4.429 (m, 2H), 3.958-4.220 (m, 4H), 3.044-3.307 (m, 2H), 2.124-2.265 (m, 2H), 1.508-1.712 (m, 5H), 1.472 (s, 18H), 1.396 (s, 3H), 1.272 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.75, 163.09, 160.62, 157.06, 155.66, 152.78, 145.41, 139.77, 128.66, 128.64, 128.22, 127.32, 127.22, 110.40, 108.99, 104.89, 83.80, 79.740, 78.095, 77.746, 74.632, 70.097, 68.734, 66.338, 49.065, 48.261, 40.802, 28.836, 28.355, 28.156, 26.645, 25.625, 25.3678, 23.218, 18.128. IR (ATR) 3300m, 2935m, 1726s, 1638s, 1607s, 1543s, 1455s, 1412s, 1367s, 1303s, 1243s, 1137s, 1055s, 1029s, 971s, 848m, 805m, 759s, 698s. LRMS predicted for $C_{45}H_{59}N_5O_{12}$ = 861.4. Found 862.5 ($M+H^+$). HRMS predicted for $C_{45}H_{59}N_5O_{12}+H^+$ = 862.4238. Found 862.4241 ($M+H^+$).

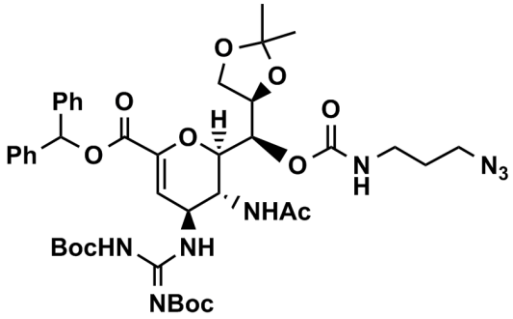
(2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-2-((*S*)-(dec-9-ynylcarbamoyloxy)((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3,4-dihydro-2H-pyran-6-carboxylate (16)



To a flask containing undec-10-ynoic acid (1.02 g, 5.54 mmol) was added thionyl chloride (1.97 g, 1.21 mL, 16.6 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (10 mL) and trimethylsilyl azide (1.28 g, 1.46 mL, 11.1 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (10 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (2.05 g, 2.77 mmol) and DMAP (0.68 g, 5.54 mmol) in DCM (10 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (6 mL) and purified directly by reverse phase chromatography (Biotage® 60g SNAP cartridge KP-C18-HS) using a gradient elution of 5% MeCN/water to 100% MeCN/water over 15 column volumes. The *title compound* was obtained as a white solid (2.16 g, 85%).^{1}H NMR ($CDCl_3$) δ 11.381 (s, 1H), 8.443 (d, 1H, *J* = 8.3 Hz), 7.238-7.476 (m, 10H), 6.930 (s, 1H), 5.962 (d, 1H, *J* = 3.0 Hz), 5.220 (dt, 1H, *J* = 2.8, 8.3 Hz), 5.143 (d, 1H, *J* = 7.1 Hz), 4.833 (at, 1H, *J* = 5.9 Hz), 4.523 (t, 1H, *J* = 6.0 Hz), 4.305-4.419 (m, 2H), 3.942-4.214 (m, 4H), 3.948-4.286 (m, 4H), 2.067-2.195 (m, 4H), 1.930 (s, 3H), 1.486 (s, 18H), 1.472 (s, 18H), 1.381 (s, 3H), 1.295 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.69, 163.99, 160.61, 157.02, 155.60, 152.76, 145.33, 139.64, 128.61,

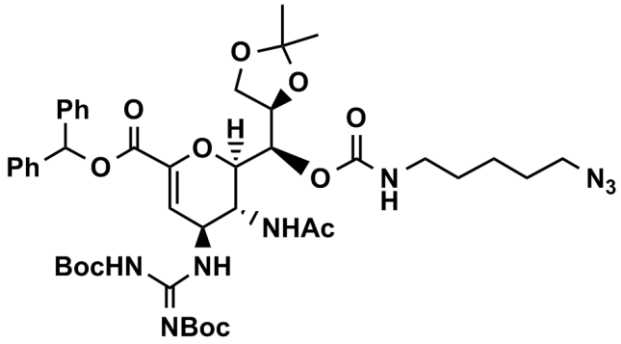
128.18, 127.30, 127.19, 110.47, 108.96, 104.86, 83.682, 79.583, 78.052, 77.679, 74.617, 70.031, 68.267, 66.312, 49.026, 48.228, 41.361, 40.643, 30.349, 29.723, 29.278, 26.202, 29.166, 29.092, 29.042, 28.709, 28.488, 28.341, 28.131, 26.927, 26.875, 25.18, 23.482, 18.211. IR (ATR) 3310m, 3284m, 2982m, 2931m, 2857m, 1726s, 1680s, 1640m, 1609m, 1562m, 1454m, 1415m, 1369m, 1302m, 1247m, 1228m, 1139m, 1057m, 1029m, 790w, 907s, 851w, 807w, 759m, 728s, 698s. LRMS predicted for $C_{49}H_{67}N_5O_{12}$ = 917.5 Found 918.5 ($M+H^+$). HRMS predicted for $C_{49}H_{67}N_5O_{12}+H^+$ = 918.4864. Found 918.4863 ($M+H^+$).

(2*R*, 3*R*, 4*S*)-benzhydryl 3-acetamido-2-((*S*)-(3-azidopropylcarbamoyloxy)((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-3,4-dihydro-2H-pyran-6-carboxylate (23)



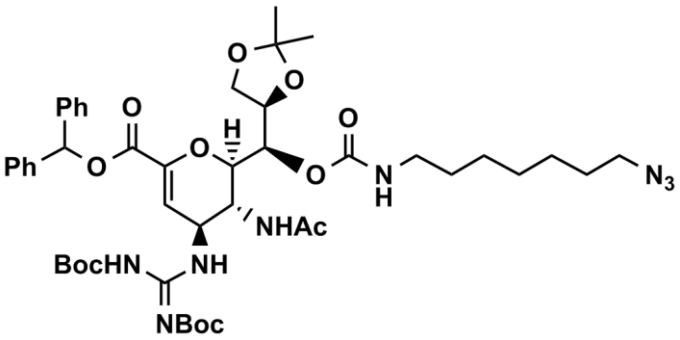
To a flask containing 4-azidobutanoic acid (0.72 g, 5.58 mmol) was added thionyl chloride (2.99 g, 1.21 mL, 16.7 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (5 mL) and trimethylsilyl azide (1.29 g, 1.47 mL, 11.2 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (5 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (2.05 g, 2.77 mmol) and DMAP (0.68 g, 5.58 mmol) in DCM (5 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (4 mL) and purified directly by reverse phase chromatography (Biotage® 30g SNAP cartridge KP-C18-HS) using a gradient elution of 5% MeCN/water to 100% MeRTCN/water over 15 column volumes. The *title compound* was obtained as a white solid (1.77 g, 82%). ^{1}H NMR ($CDCl_3$) δ 11.388 (s, 1H), 8.451 (d, 1H, *J* = 8.4 Hz), 7.258-7.495 (m, 10H), 6.931 (s, 1H), 5.982-6.031 (m, 2H), 5.177 (bs, 2H), 5.003 (bs, 1H), 4.209-4.360 (m, 3H), 3.958-4.105 (m, 2H), 3.051-3.487 (m, 4H), 1.894 (s, 3H), 1.763 (at, 2H, *J* = 6 Hz), 1.743 (bs, 18H), 1.411 (s, 3H), 1.350 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.68, 163.12, 160.51, 157.02, 155.64. 152.69, 145.27, 139.57, 128.59, 128.16, 127.25, 110.43, 108.91, 83.659, 79.558, 78.040, 77.786, 74.657, 70.106, 66.191, 49.185, 48.914, 47.929, 38.623, 28.939, 28.291, 28.070, 26.553, 25.267, 23.136. IR (ATR) 3313m, 2981m, 2935m, 2097s, 1725s, 1685m, 1638m, 1607m, 1551m, 1498m, 1477m, 1454s, 1413s, 1367s, 1322s, 1300s, 1245s, 1227s, 1136s, 1055s, 1028s, 970m, 911m, 876m, 849m, 804m, 759s, 734s, 698s. LRMS predicted for $C_{42}H_{56}N_8O_{12}$ = 864.4. Found 865.5 ($M+H^+$). HRMS predicted for $C_{42}H_{56}N_8O_{12}+H^+$ = 865.4096. Found 865.4091 ($M+H^+$).

(2*R*, 3*R*, 4*S*)-benzhydryl 3-acetamido-2-((*S*)-(5-azidopentylcarbamoyloxy)((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-3,4-dihydro-2H-pyran-6-carboxylate (24)



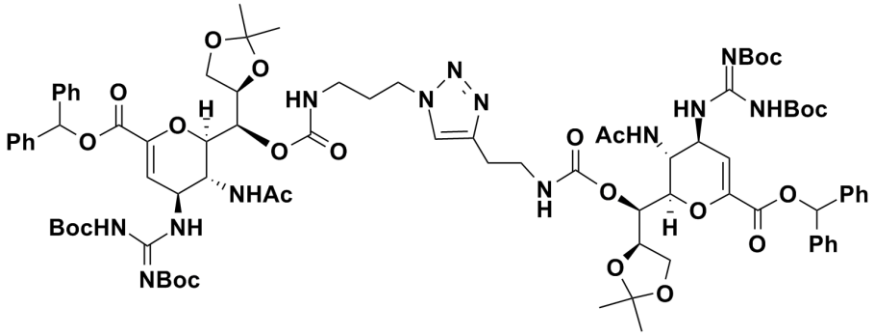
To a flask containing 6-azidohexanoic acid (0.41 g, 2.61 mmol) was added thionyl chloride (0.93 g, 0.57 mL, 7.83 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (4 mL) and trimethylsilyl azide (0.6 g, 0.79 mL, 5.22 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (4 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (0.97 g, 1.31 mmol) and DMAP (0.32 g, 2.62 mmol) in DCM (4 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (4 mL) and purified directly by reverse phase chromatography (Biotage® 30g SNAP cartridge KP-C18-HS) using a gradient elution of 5% MeCN/water to 100% MeCN/water over 15 column volumes. The *title compound* was obtained as a yellow/white solid (0.97 g, 83%). ^{1}H NMR ($CDCl_3$) δ 8.443 (d, 1H), 7.249-7.445 (m, 10H), 6.923 (s, 1H), 5.941 (bs, 1H), 5.828 (d, 1H, *J* = 8.3 Hz), 5.221 (at, 1H, *J* = 8.1 Hz), 5.138 (d, 1H, *J* = 7.6 Hz), 4.795 (at, 1H, *J* = 3.2 Hz), 4.321-4.439 (m, 2H), 3.965-4.243 (m, 5H), 3.084-3.325 (m, 5H), 1.921 (s, 3H), 1.235-1.660 (m, 4H), 1.505 (s, 18H), 1.403 (s, 3H), 1.339 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.54, 163.20, 160.41, 157.02, 155.63, 152.71, 145.31, 139.55, 128.64, 128.17, 110.34, 108.91, 83.663, 79.559, 78.041, 77.782, 74.632, 70.089, 66.190, 49.185, 48.927, 47.935, 38.620, 29.614, 28.934, 28.290, 27.991, 26.559, 25.267, 23.786, 23.134. IR (ATR) 3312m, 3066w, 2980m, 2935m, 2096s, 1726s, 1686m, 1638m, 1607m, 1550m, 1477m, 1454m, 1414m, 1367m, 1301m, 1423s, 1227s, 1136s, 1055s, 1028m, 971m, 876w, 848m, 804m, 759s, 698s. LRMS predicted for $C_{44}H_{60}N_8O_{12}$ = 892.4. Found 893.5 ($M+H^+$). HRMS predicted for $C_{44}H_{60}N_8O_{12}+H^+$ = 893.4409. Found 893.4407 ($M+H^+$).

(2*R*, 3*R*, 4*S*)-benzhydryl 3-acetamido-2-((*S*)-(7-azidoheptylcarbamoyloxy)((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-(2,3-bis(*tert*-butoxycarbonyl)guanidino)-3,4-dihydro-2H-pyran-6-carboxylate (25)



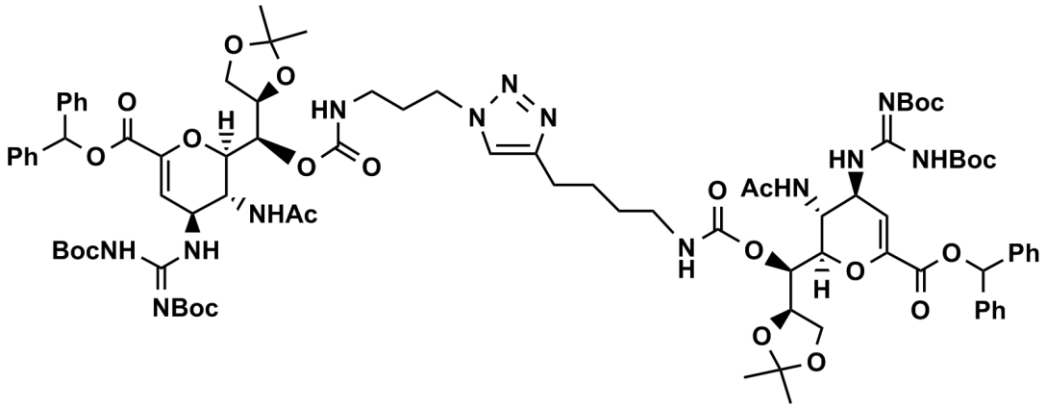
To a flask containing 8-azidooctanoic acid (0.65 g, 3.51 mmol) was added thionyl chloride (1.25 g, 0.76 mL, 10.5 mmol) and 1 drop of *N*,*N*-dimethylformamide. The solution was stirred at rt for 3 hr after which the excess thionyl chloride was removed by distillation under reduced pressure. The residual yellow oil was dissolved in toluene (4 mL) and trimethylsilyl azide (0.81 g, 0.92 mL, 7.02 mmol) was added. The solution was heated to reflux for 2 hr after which the excess trimethylsilyl azide and toluene were removed by distillation under reduced pressure. The residual yellow oil was dissolved in DCM (4 mL) and to this was added a suspension of (2*R*,3*R*,4*S*)-benzhydryl 3-acetamido-4-(2,3-bis(*tert*-butoxycarbonyl) guanidino)-2-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy) methyl)-3,4-dihydro-2H-pyran-6-carboxylate (1.30 g, 1.76 mmol) and DMAP (0.43 g, 3.52 mmol) in DCM (4 mL). The suspension was stirred for 18 hr at rt after which the DCM was removed by distillation under reduced pressure. The residual was dissolved in DMF (4 mL) and purified directly by reverse phase chromatography (Biotage® 30g SNAP cartridge KP-C18-HS) using a gradient elution of 5%MeCN/water to 100% MeCN/water over 15 column volumes. The *title compound* was obtained as a white solid (1.39 g, 86%). ^{1}H NMR ($CDCl_3$) δ 11.42 (s, 1H), 8.476 (d, 1H, *J* = 8.2 Hz), 7.250-7.444 (m, 10H), 6.952 (s, 1H), 5.975 (at, 1H, *J* = 2.8 Hz), 5.904 (bs, 1H), 5.262 (at, 1H, *J* = 7.8 Hz), 5.184 (d, 1H, *J* = 7.5 Hz), 4.833 (at, 1H, *J* = 3.1 Hz). 4.285-4.426 (m, 3H), 3.951-4.214 (m, 3H), 2.930-3.286 (m, 5H), 1.904 (s, 3H), 1.238-1.691 (m, 8H), 1.505 (s, 18H), 1.381 (s, 3H) 1.331 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.72, 160.63, 157.06, 155.64, 152.81, 145.48, 139.68, 128.68. 128.66, 127.34, 109.03, 83.850, 77.751, 77.584, 77.160, 76.737, 70.098, 66.424, 51.513, 41.337, 40.653, 30.291, 28.952, 28.872, 28.378, 28.175, 26.843, 26.746, 26.717, 26.675, 25.399, 23.272. IR (ATR) 3311m, 2979m, 2932m, 2859m, 2094s, 1727s, 1685m, 1637s, 1347s, 1322s, 1302s, 1243s, 1227s, 1136s, 1055s, 1029m, 970m, 913w, 877w, 848m, 804m, 759s, 742s, 698s. LRMS predicted for $C_{46}H_{64}N_8O_{12}$ = 920.5 Found 921.6 ($M+H^+$). HRMS predicted for $C_{46}H_{64}N_8O_{12}+H^+$ = 921.4722. Found 921.4719 ($M+H^+$).

Protected zanamivir dimer from coupling alkyne 14 and azide 23.



To a flask containing alkyne **14** (42 mg, 0.05 mmol) and azide **23** (43 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a white semi-solid (70 mg, 82%). ^{1}H NMR ($CDCl_3$) δ 8.501 (d, 1H, *J* = 8.3 Hz), 8.429 (1H, *J* = 8.2 Hz), 7.714 (s, 1H), 7.208-7.411 (m, 20H), 6.956 (s, 1H), 6.882 (s, 1H), 6.308 (d, 1H, *J* = 8.8 Hz), 6.322 (d, 1H, *J* = 8.4 Hz), bs (5.994, 2H), 5.004-5.043 (m, 6H), 3.908-4.556 (m, 12H), 3.288-3.581 (m, 2H), 3.088-3.292 (m, 2H), 2.805-3.084 (m, 4H), 1.803-2.188 (m, 4H), 1.920 (s, 3H), 1.882 (s, 3H), 1.432 (s, 36H), 1.356 (s, 6H), 1.292 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 171.12, 163.28, 163.14, 160.66, 157.08, 156.97, 152.77, 152.73, 145.36, 145.34, 139.68, 139.63, 128.63, 128.21, 128.16, 127.30, 127.28, 127.21, 127.17, 108.90, 83.700, 83.080, 79.643, 79.502, 78.117, 78.068, 77.730, 74.706, 74.516, 70.102, 70.080, 66.164, 60.477, 49.092, 48.693, 32.588, 28.334, 28.115, 26.665, 26.617, 23.461. IR (ATR) 3312m, 2981m, 2934m, 1725s, 1684m, 1638s, 1607s, 1551s, 1498m, 1478m, 1454m, 1367s, 1323s, 1244s, 1227s, 1055s, 1029s, 967m, 911m, 876m, 848m, 804m, 759s, 730s, 697s. LRMS predicted for $(C_{85}H_{111}N_{13}O_{24}+2H^+)/2$ = 849.9. Found 850.2 $(M+2H^+)/2$. HRMS predicted for $(C_{85}H_{111}N_{13}O_{24}+2H^+)/2$ = 849.9011. Found 849.9013 $(M+2H^+)/2$.

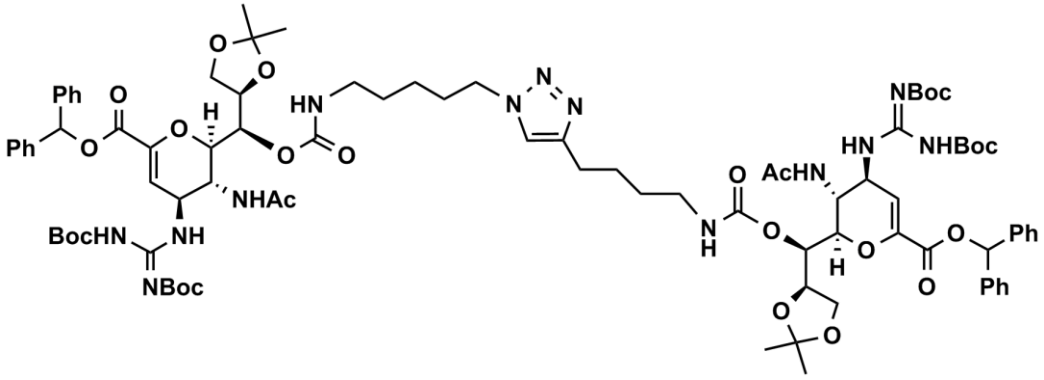
Protected zanamivir dimer from coupling alkyne 15 and azide 23.



To a flask containing alkyne **15** (43 mg, 0.05 mmol) and azide **23** (43 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a

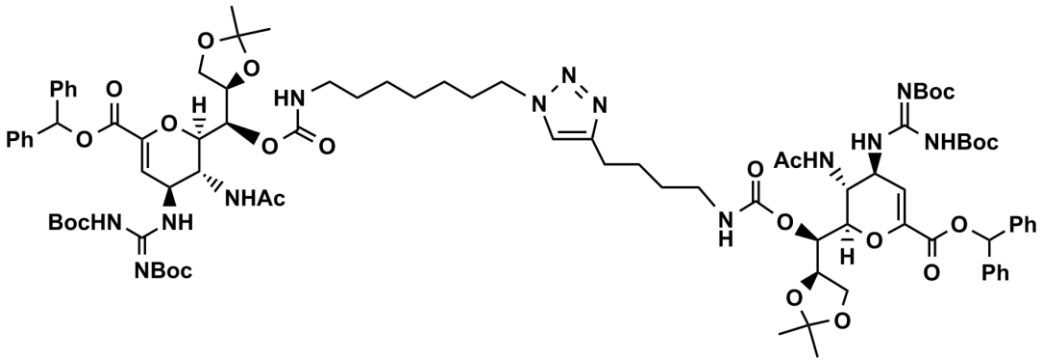
gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a pale yellow solid (68 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 11.42 (bs, 2H), 8.365-8.553 (m, 2H), 7.194-7.623 (m, 20H), 6.933 (s, 1H), 6.922 (s, 1H), 6.135-6.364 (m, 2H), 5.984 (bs, 2H), 5.088-5.376 (m, 6H), 3.985-4.501 (m, 12H), 2.835-3.306 (m, 6H), 2.605-2.765 (m, 2H), 1.558-2.350 (m, 6H), 1.882 (bs, 6H), 1.441 (s, 36H), 1.353 (s, 6H), 1.294 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.91, 170.72, 163.24, 163.14, 160.68, 160.59, 157.12, 157.03, 155.85, 152.77, 145.34, 139.68, 139.62, 128.68, 128.66, 128.21, 127.31, 127.24, 127.21, 108.98, 83.783, 83.629, 79.707, 79.561, 78.894, 74.210, 70.877, 66.273, 66.244, 60.702, 48.246, 47.456, 41.070, 28.370, 28.138, 26.659, 23.226. IR (ATR) 3274m, 2980m, 2934m, 1725s, 1680m, 1637s, 1607s, 1551m, 1497m, 1476m, 1454m, 1413s, 1367s, 1324s, 1227s, 1245s, 1136s, 1054s, 1028s, 971m, 951m, 913s, 876s, 849m, 804m, 759s, 738s, 698s. LRMS predicted for ($C_{87}H_{115}N_{13}O_{24}$+2H$^+$)/2 = 863.9. Found 863.9 (M+2H$^+$)/2. HRMS predicted for ($C_{87}H_{115}N_{13}O_{24}$+2H$^+$)/2 = 863.9167. Found 863.9169 (M+2H$^+$)/2.

Protected zanamivir dimer from coupling alkyne 15 and azide 24.



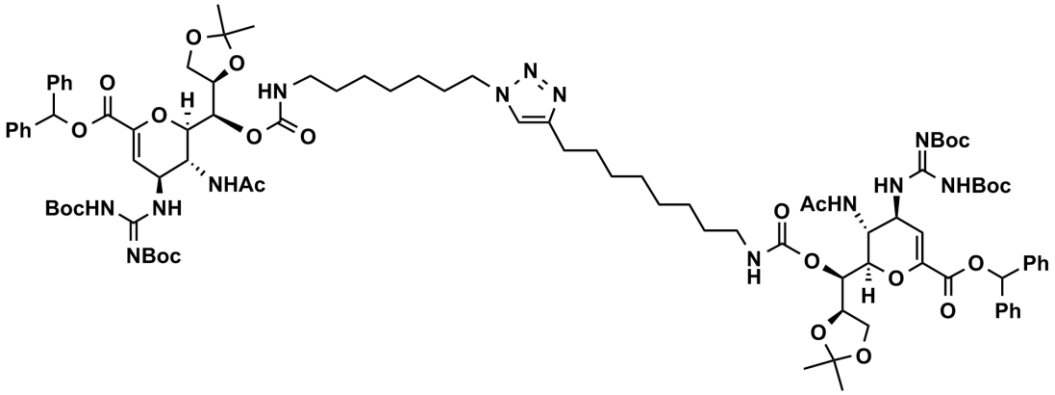
To a flask containing alkyne **15** (43 mg, 0.05 mmol) and azide **24** (45 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a pale yellow solid (75 mg, 85%). ^{1}H NMR ($CDCl_3$) δ 11.38 (bs, 2H), 8.342-8.576 (m, 2H), 7.189-7.641 (m, 20H), 6.928 (s, 1H), 6.917 (s, 1H), 6.148-6.337 (m, 2H), 6.005 (bs, 2H), 5.078-5.365 (m, 6H), 3.971-4.456 (m, 12H), 2.841-3.308 (m, 6H), 2.600-2.762 (m, 2H), 1.561-2.348 (m, 8H), 1.881 (bs, 6H), 1.438 (s, 36H), 1.351 (s, 6H), 1.289 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.87, 170.71, 163.14, 163.03, 160.64, 160.61, 157.09, 157.05, 155.84, 152.79, 145.36, 139.70, 139.62, 128.59, 128.65, 128.24, 127.32, 127.26, 127.17, 109.01, 83.780, 83.630, 79.7011, 79.562, 78.892, 74.208, 70.879, 66.274, 66.250, 60.704, 48.251, 47.462, 41.071, 28.365, 28.140, 26.953, 26.661, 24.785, 23.225. IR (ATR) 3288m, 2979m, 2932m, 1726s, 1684m, 1610m, 1551m, 1455m, 1412m, 1368m, 1323m, 1302m, 1247m, 1140s, 1056s 1028s, 962s, 848s, 803s, 759s, 698s. LRMS predicted for ($C_{89}H_{119}N_{13}O_{24}$+2H$^+$)/2 = 877.9. Found 877.9 (M+2H$^+$)/2. HRMS predicted for ($C_{89}H_{119}N_{13}O_{24}$+2H$^+$)/2 = 877.9324. Found 877.9320 (M+2H$^+$)/2.

Protected zanamivir dimer from coupling alkyne 15 and azide 25.



To a flask containing alkyne **15** (43 mg, 0.05 mmol) and azide **25** (46 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a white semi-solid (75 mg, 84%). ^{1}H NMR ($CDCl_3$) δ 11.37 (bs, 2H), 8.30-8.573 (m, 2H), 7.195-7.482 (m, 20H), 6.930 (s, 2H), 6.023-6.332 (m, 2H), 5.978 (bs, 2H), 5.072-5.303 (m, 4H), 4.784-4.992 (m, 2H), 3.981-4.449 (m, 12H), 2.838-3.311 (m, 6H), 2.651-2.759 (m, 2H), 1.481-2.335 (m, 10H), 1.879 (bs, 6H), 1.435 (s, 36H), 1.348 (s, 6H), 1.279 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.882, 170.171, 163.20, 169.20, 163.19, 163.15, 160.65, 160.61, 157.05, 155.70, 155.67, 155.63, 152.76, 147.78, 145.34, 139.65, 128.74, 128.65, 128.21, 127.30, 127.20, 110.55, 110.51, 108.95, 104.91, 83.743, 83.642, 79.661, 79.587, 78.068, 78.023, 77.756, 74.663, 74.626, 70.032, 66.308, 66.275, 50.145, 49.058, 41.282, 40.959, 40.925, 30.332, 30.136, 29.651, 28.692, 28.452, 28.438, 28.420, 28.352, 28.135, 26.637, 26.594, 24.779, 23.191. IR (ATR) 3311m, 2980m, 2934m, 2863m, 1725s, 1681s, 1637s, 1607s, 1551s, 1498m, 1477m, 1413m, 1367s, 1324s, 1301s, 1245s, 1228s, 1137s, 1055s, 1029s, 971m, 913m, 875m, 848m, 803m, 759m, 742m, 698s. LRMS predicted for $(C_{91}H_{121}N_{13}O_{24}+2H^+)/2$ = 891.9. Found 892.3 $(M+2H^+)/2$. HRMS predicted for $(C_{91}H_{121}N_{13}O_{24}+2H^+)/2$ = 891.9480. Found 891.9469 $(M+2H^+)/2$.

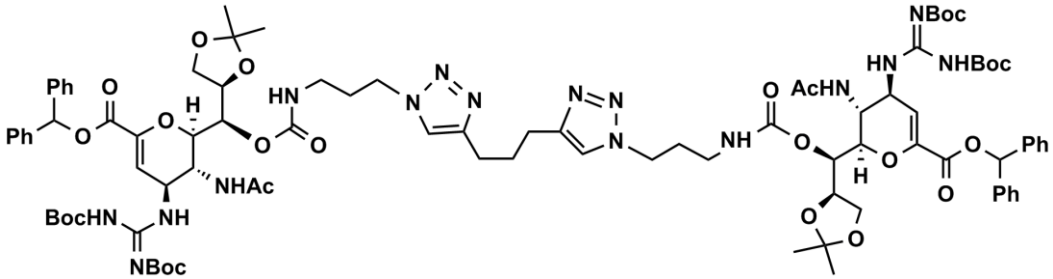
Protected zanamivir dimer from coupling alkyne 16 and azide 25.



To a flask containing alkyne **16** (46 mg, 0.05 mmol) and azide **25** (46 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by

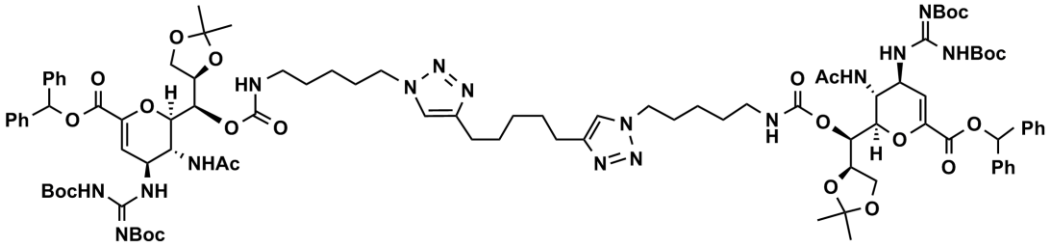
reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a white solid (69 mg, 75%). ^{1}H NMR ($CDCl_3$) δ 11.34 (bs, 2H), 8.403 (d, *J* = 8.3 Hz, 2H), 7.171-7.445 (m, 20H), 6.857 (bs, 2H), 6.114 (d, *J* = 7.9 Hz, 2H), 5.988 (bd, 2H), 5.091-5.246 (m, 4H), 4.886-5.001 (m, 2H), 3.943-4.429 (m, 18H), 3.015-3.257 (m, 6H), 2.686 (bt, *J* = 7.1 Hz, 4H), 1.481-2.055 (m, 14H), 1.857 (s, 6H), 1.457 (s, 36H), 1.354 (s, 6H), 1.286 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.71, 170.68, 163.17, 160.61, 156.95, 155.59, 152.70, 145.19, 145.15, 139.61, 128.61, 128.16, 127.24, 127.16, 110.54, 110.51, 108.89, 83.637, 79.533, 78.019, 77.696, 77.667, 74.691, 69.944, 66.226, 50.153, 49.051, 41.227, 40.999, 30.293, 30.184, 29.719, 29.440, 29.312, 29.252, 29.189, 29.135, 28.654, 28.315, 28.097, 26.779, 26.572, 26.456, 24.778. IR (ATR) 3309m, 2982m, 2930m, 2860m, 1723s, 1681s, 1635s, 1604s, 1550s, 1454m, 1411m, 1367s, 1323s, 1302s, 1245s, 1228s, 1136s, 1054s, 1028s, 970m, 911m, 851m, 803m, 756m, 742m, 698s. LRMS predicted for $(C_{95}H_{131}N_{13}O_{24}+2H^+)/2$ = 920.0. Found 920.3 $(M+2H^+)/2$. HRMS predicted for $(C_{95}H_{131}N_{13}O_{24}+2H^+)/2$ = 919.9793. Found 919.9789 $(M+2H^+)/2$.

Protected zanamivir dimer from coupling hepta-1,6-diyne and azide 23



To a flask containing hepta-1,6-diyne (4.6 mg, 0.05 mmol) and azide **23** (43 mg, 0.05 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a white solid (71 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 11.32 (bs, 2H), 8.461 (d, *J* = 8.2 Hz, 2H), 7.561 (bs, 2H), 7.220-7.439 (m, 20H), 6.928 (bs, 2H), 6.204 (bs, 2H), 5.983 (bs, 2H), 5.003-5.279 (m, 6H), 4.178-4.532 (m, 10H), 3.960-4.121 (m, 4H), 3.161-3.342 (m, 2H), 2.925-3.052 (m, 2H), 2.680-2.837 (m, 4H), 1.745-2.333 (m, 10H), 1.884 (s, 6H), 1.464 (s, 36H), 1.345 (s, 6H), 1.284 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.82, 163.16, 160.58, 157.13, 155.91, 152.79, 147.37, 145.92, 139.64, 128.80, 128.71, 128.69, 128.28, 127.37, 127.25, 110.56, 109.03, 104.93, 83.825, 81.722, 79.721, 78.201, 77.950, 76.736, 75.037, 70.168, 66.130, 49.380, 47.744, 47.717, 47.071, 30.668, 28.482, 28.390, 28.158, 26.618, 25.091, 24.629, 22.778. IR (ATR) 3312m, 2980m, 2933m, 1725m, 1679m, 1639m, 1607m, 1551m, 1497m, 1477m, 1454m, 1413m, 1368s, 1324s, 1303m, 1246s, 1227s, 1138s, 1056s, 1029m, 971m, 912m, 876w, 806m, 760m, 732s, 699s. LRMS predicted for $(C_{91}H_{120}N_{16}O_{24}+2H^+)/2$ = 911.4. Found 912.0 $(M+2H^+)/2$. HRMS predicted for $(C_{91}H_{120}N_{16}O_{24}+2H^+)/2$ = 911.4409. Found 911.4415 $(M+2H^+)/2$.

Protected zanamivir dimer from coupling nona-1,8-diyne and azide 24.

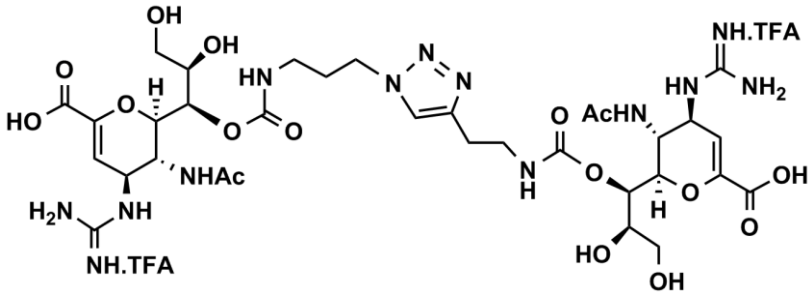


To a flask containing nona-1,8-diyne (6.0 mg, 0.05 mmol) and azide **24** (135 mg, 0.15 mmol) was added 1:2 water/*tert*-butanol (1 mL), $CuSO_4$ (0.015 mmol, 3.8 mg, 30 mol%) and sodium ascorbate (0.075 mmol, 15 mg, 150 mol%). The flask was sealed and stirred at rt for 18 hr after which DMSO (1 mL) was added. The solution was purified directly by reverse phase chromatography (Biotage® 12g SNAP cartridge KP-C18-HS) using a gradient elution of 30% MeCN/water to 100% MeCN/water over 15 column volumes. The protected dimer was obtained as a white solid (70 mg, 73%). ^{1}H NMR ($CDCl_3$) δ 11.45 (s, 2H), 8.454 (d, *J* = 8.2 Hz, 2H), 7.216-7.442 (m, 20H), 6.909 (s, 2H), 6.061 (bd, *J* = 7.8 Hz, 2H), 5.970 (s, 2H), 5.121-5.321 (m, 4H), 4.939 (bt, *J* = 4.2 Hz, 2H), 3.939-4.440 (m, 16H), 3.133 (aq, *J* = 7.1 Hz, 4H), 2.678 (t, *J* = 7.6 Hz, 4H), 1.412-2.361 (m, 16H), 1.909 (s, 6H), 1.473 (s, 36H), 1.364 (s, 6H), 1.273 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170.81, 163.18, 160.53, 157.21, 155.88, 152.77, 147.47, 145.81, 139.52, 128.81, 128.70, 128.64, 128.23, 127.33, 127.20, 122.16, 110.46, 108.93, 104.91, 83.816, 81.719, 79.715, 78.191, 77.948, 76.728, 75.029, 70.153, 66.126, 49.370, 48.744, 47.704, 42.185, 41.057, 40.006, 30.657, 28.472, 28.381, 28.152, 26.520, 25.085, 24.619, 22.759. IR (ATR) 3312m, 2979m, 2934m, 1725m, 1680m, 1680m, 1637m, 1608m, 1550m, 1454m, 1413m, 1367m, 1324m, 1301s, 1245s, 1227s, 1136s, 1054s, 1028s, 970m, 912m, 849m, 803m, 757s, 698s. LRMS predicted for $(C_{97}H_{132}N_{16}O_{24}+2H^+)/2$ = 953.5. Found 953.9 $(M+2H^+)/2$. HRMS predicted for $(C_{97}H_{132}N_{16}O_{24}+2H^+)/2$ = 953.4878. Found 953.4892 $(M+2H^+)/2$.

General method for TFA de-protection of protected zanamivir dimers

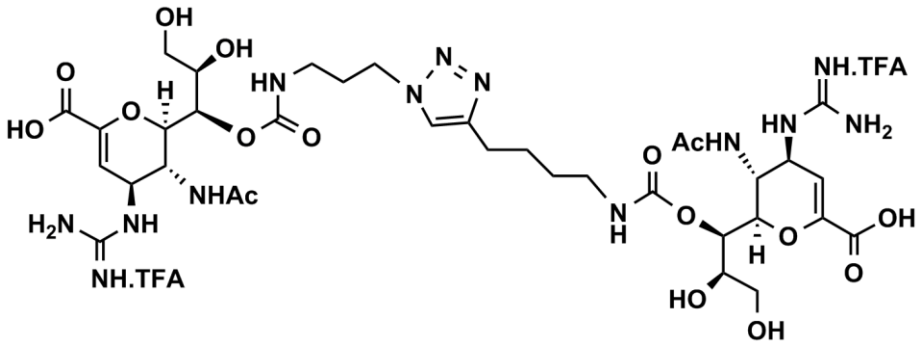
To a flask containing the appropriate protected zanamivir dimer (0.05 mmol) was added DCM (1 mL) and dry TFA (1 mL). The reaction was stirred at rt for 24 hr after which the DCM and TFA were removed by distillation under reduced pressure. The residual was dissolved in 1:1 DMSO/water (2 mL) and purified by reverse phase chromatography (Biotage® 4g SNAP cartridge KP-C18-HS) using a gradient elution of 2% MeCN/water to 40% MeCN/water over 15 column volumes. The protected dimers were obtained as white solids after freeze drying.

Zanamivir dimer 1



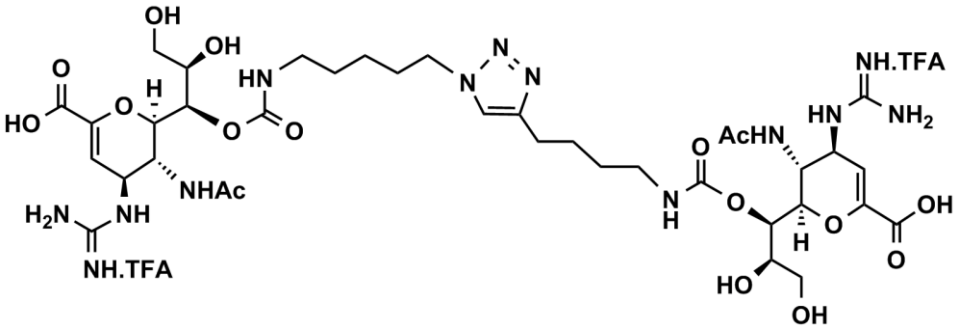
The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of alkyne **14** and azide **23**. The double TFA salt was obtained – after freeze drying – as a white solid (51 mg, 91%). ^{1}H NMR (D_2O) δ 7.692 (s, 1H), 5.538-5.632 (m, 2H), 4.518-4.869 (m, 2H), 4.198-4.435 (m, 6H), 3.810-4.050 (m, 4H), 3.095-3.593 (m, 5H), 2.632-2.994 (m, 4H), 1.850-2.047 (m, 2H), 1.822 (s, 3H), 1.791 (s, 3H). ^{13}C NMR (D_2O) δ 170.91, 163.64, 160.45, 157.09, 156.91, 152.55, 145.45, 110.23, 108.55, 83.909, 79.991, 78.182, 77.637, 63.364, 48.818, 48.182, 38.545, 28.913, 27.925, 26.545, 24.889. IR (ATR) 3305s, 1636s, 1406m, 1376w, 1327w, 1261w, 1193w, 1143w, 1039w. HPLC purity (solvent gradient profile: 90%A10%B 0.2 mins, 90%A10%B to 20%A80%B 16.8 mins, 20%A80%B to 90%A10%B 3.0 mins): 95.8%, 3.956 min; LRMS predicted for $(C_{33}H_{51}N_{13}O_{16}+2H^{+})/2$ = 443.7. Found 443.9 $(M+2H^{+})/2$. HRMS predicted for $(C_{33}H_{51}N_{13}O_{16}+2H^{+})/2$ = 443.6865. Found 443.6861 $(M+2H^{+})/2$.

Zanamivir dimer 2



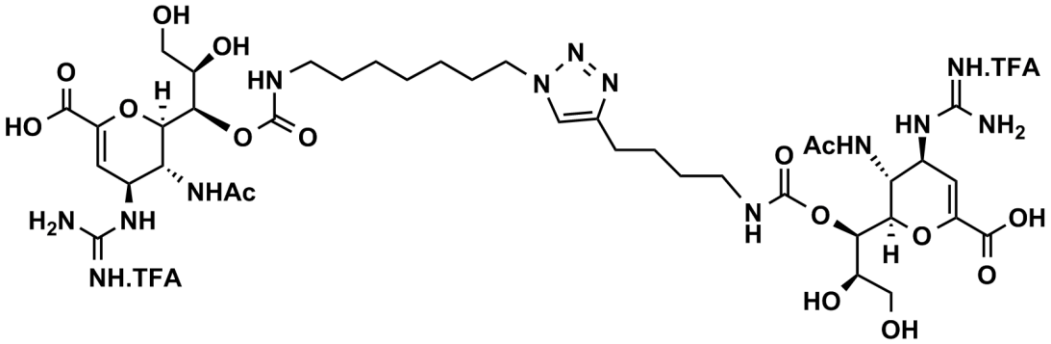
The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of alkyne **15** and azide **23**. The double TFA salt was obtained – after freeze drying – as a white solid (51 mg, 90%). ^{1}H NMR (D_2O) δ 8.086 (s, 1H), 5.912-5.924 (bs, 2H), 4.818-4.855 (m, 2H), 4.441-4.460 (bd, 4H) 4.320-4.342 (m, 2H), 4.011-4.067 (m, 2H), 3.581-3.724 (m, 2H), 3.879-3.917 (m, 2H), 3.513-3.565 (m, 4H), 3.344-3.407 (m, 2H), 2.948-3.063 (m, 4H), 2.713-2.750 (m, 2H), 2.015-2.049 (m, 2H), 1.837 (s, 6H), 1.578-1.615 (m, 2H), 1.406-1.441 (m, 2H). ^{13}C NMR (D_2O) δ 170.89, 163.59, 160.35, 156.98, 156.90, 152.45, 145.39, 110.19, 108.53, 83.910, 79.989, 78.180, 77.631, 63.355, 48.814, 48.179, 38.551, 29.405, 28.901, 28.802, 27.919, 26.544, 24.868. IR (ATR) 3302s, 1633s, 1402m, 1372w, 1326w, 1260w, 1193w, 1139w. HPLC purity (solvent gradient profile: 90%A10%B 0.2 mins, 90%A10%B to 20%A80%B 16.8 mins, 20%A80%B to 90%A10%B 3.0 mins): 97.2%, 4.156 min; LRMS predicted for $(C_{35}H_{57}N_{13}O_{16}+2H^{+})/2$ = 458.7. Found 457.9 $(M+2H^{+})/2$. HRMS predicted for $(C_{35}H_{57}N_{13}O_{16}+2H^{+})/2$ = 458.7101. Found 458.7115 $(M+2H^{+})/2$.

Zanamivir dimer 3



The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of alkyne **15** and azide **24**. The double TFA salt was obtained – after freeze drying – as a white solid (54 mg, 92%). ^{1}H NMR (D_2O) δ 7.785 (s, 1H), 5.627 (bs, 2H), 4.712-5.017 (m, 2H), 4.325-4.532 (m, 6H), 3.985-4.183 (m, 4H), 3.458-3.682 (m, 2H), 3.401-3.504 (m, 2H), 2.951-3.197 (m, 4H), 2.698 (bt, *J* = 7.7 Hz, 2H), 1.945 (s, 3H), 1.851 (s, 3H), 1.844-1.850 (m, 2H), 1.496-1.743 (m, 6H), 1.183-1.302 (m, 2H). ^{13}C NMR (D_2O) δ 170.93, 163.71, 160.52, 157.19, 156.98, 152.67, 145.62, 110.21, 108.43, 83.916, 79.986, 78.179, 77.642, 63.373, 48.820, 48.211, 38.539, 28.912, 27.931, 26.537, 24.904, 24.884, 22.118. IR (ATR) 3264s, 2944s, 1655s, 1663s, 1586s, 1618s, 1560s, 1401s, 1370s, 1325s, 1283s, 1255s, 1202s, 1179s, 1137s, 1087s, 1069s, 1038s, 997s, 943s, 883s, 770s, 670s. HPLC purity (solvent gradient profile: 95%A5%B 0.2 mins, 95%A5%B to 50%A50%B 16.8 mins, 50%A50%B to 95%A5%B 3.0 mins): 98.6%, 4.416 min; LRMS predicted for ($C_{37}H_{61}N_{13}O_{16}$+2H$^+$)/2 = 471.7. Found 471.9 (M+2H$^+$)/2. HRMS predicted for ($C_{37}H_{61}N_{13}O_{16}$+2H$^+$)/2 = 471.7180. Found 471.7184 (M+2H$^+$)/2.

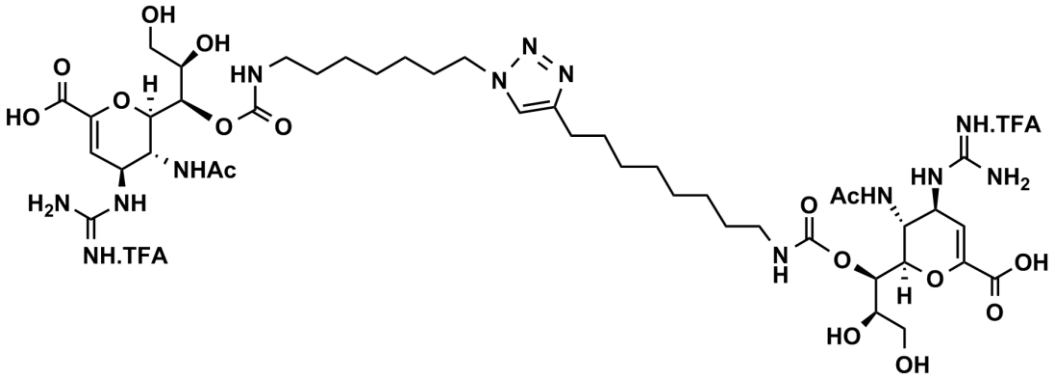
Zanamivir dimer 4



The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of alkyne **15** and azide **25**. The double TFA salt was obtained – after freeze drying – as a white solid (53 mg, 90%). ^{1}H NMR (D_2O) δ 7.821 (bs, 1H), 5.682 (bs, 2H), 4.672-4.993 (m, 2H), 4.496 (d, *J* = 8.4 Hz, 2H), 4.319-4.438 (m, 4H), 3.990-4.172 (m, 4H), 3.642 (d, *J* = 8.4 Hz, 2H), 3.398-3.526 (m, 2H), 2.943-3.207 (m, 4H), 2.632-2.818 (m, 2H), 1.956 (s, 3H), 1.921 (s, 3H), 1.760-1.892 (m, 2H), 1.583-1.761 (m, 2H), 1.118-1.548 (m, 10H). ^{13}C NMR (D_2O) δ 170.84, 163.58, 160.02, 157.93, 156.80, 152.55, 145.28, 123.01, 110.23, 105.26, 83.816, 79.987, 78.14, 75.430, 69.759, 69.728, 68.705, 62.669, 55.069, 51.993, 51.905, 50.463, 47.528, 47.487, 47.472, 40.598, 40.335, 39.285, 29.278, 25.481, 21.975. IR (ATR) 3303s, 1635s, 1403m, 1373w, 1326w, 1260w, 1191w, 1139w, 1038w. HPLC purity (solvent gradient profile: 95%A5%B 0.2 mins, 95%A5%B to 50%A50%B 16.8 mins, 50%A50%B to 95%A5%B 3.0 mins):

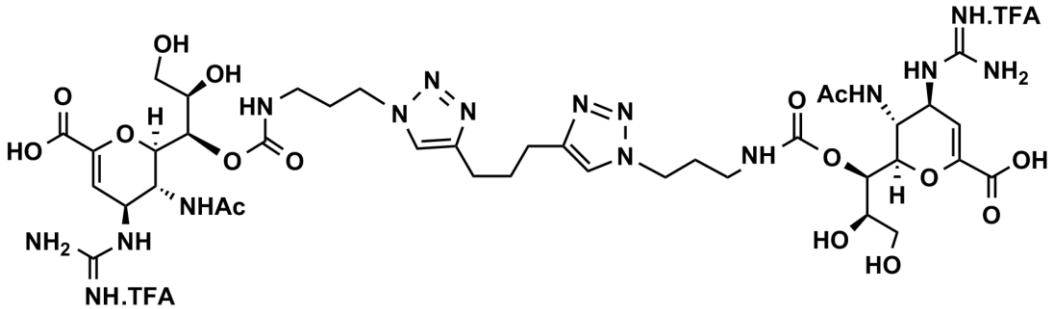
96.8%, 4.448 min; LRMS predicted for ($C_{39}H_{63}N_{13}O_{16}$+2H^+)/2 = 485.7. Found 486.0 (M+2H^+)/2. HRMS predicted for ($C_{39}H_{63}N_{13}O_{16}$+2H^+)/2 = 485.7336. Found 485.7327 (M+2H^+)/2.

Zanamivir dimer 5



The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of alkyne **16** and azide **25**. The double TFA salt was obtained – after freeze drying – as a white solid (56 mg, 89%). ^{1}H NMR (D_2O) δ 7.796 (bs, 1H), 5.669 (bs, 2H), 4.713-4.957 (m, 2H), 4.492 (d, *J* = 8.3 Hz, 2H), 4.323-4.441 (m, 4H), 4.004-4.157 (m, 4H), 3.630 (d, *J* = 8.3 Hz, 2H), 3.387-3.518 (m, 2H), 2.913-3.151 (m, 4H), 2.628-2.813 (m, 2H), 1.943 (s, 3H), 1.932 (s, 3H), 1.760-1.905 (m, 2H), 1.581-1.758 (m, 2H), 1.094-1.492 (m, 18H). ^{13}C NMR (D_2O) δ 170.81, 163.52, 159.98, 157.90, 156.77, 152.52, 145.27, 122.99, 110.21, 105.23, 83.806, 79.969, 78.12, 75.427, 69.760, 69.719, 68.711, 62.670, 55.058, 51.997, 51.901, 50.468, 47.536, 47.477, 47.471, 40.591, 40.330, 39.280, 29.275, 25.477, 22.403, 21.969, 20.689, 20.006, 19.832. IR (ATR) 3269s, 1662s, 1560s, 1401s, 1372s, 1280s, 1325w, 1256s, 1190s, 1139s, 1038s, 997m, 942m, 880m, 774s. HPLC purity (solvent gradient profile: 90%A10%B 0.2 mins, 90%A10%B to 20%A80%B 16.8 mins, 20%A80%B to 90%A10%B 3.0 mins): 93.4%, 8.246 min; LRMS predicted for ($C_{43}H_{71}N_{13}O_{16}$+2H^+)/2 = 513.8. Found 513.8 (M+2H^+)/2. HRMS predicted for ($C_{43}H_{71}N_{13}O_{16}$+2H^+)/2 = 513.7649. Found 513.7647 (M+2H^+)/2.

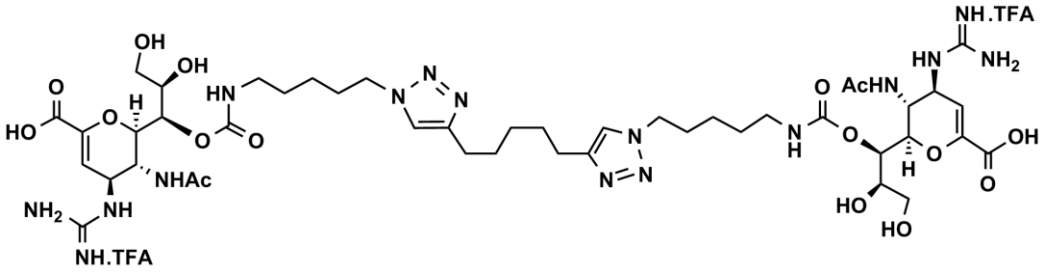
Zanamivir dimer 6



The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of hepta-1,6-diyne and azide **23**. The double TFA salt was obtained – after freeze drying – as a white solid (57 mg, 92%). ^{1}H NMR (D_2O) δ 7.846 (s, 2H), 5.719 (bs, 2H), 4.679-4.997 (m, 10H), 4.316-4.553 (m, 6H), 3.933-4.158 (m, 4H), 3.237-3.711 (m, 6H), 2.771-3.103 (m 4H), 2.004-2.198 (m, 2H), 1.960 (s, 3H), 1.941 (s, 3H). ^{13}C NMR (D_2O) δ 170.82, 163.19, 160.56, 157.08, 152.64, 139.45, 128.64, 104.89, 76.735, 75.168, 70.068, 66.122, 49.382, 47.644 47.021, 39.312, 30.668, 28.384, 28.142, 23.614. IR (ATR) 3264s, 1659s, 1557s, 1400s, 1371s, 1279s, 1323w, 1255s,

1188s, 1038s, 993m, 937m, 880m, 771s. HPLC purity (solvent gradient profile 95%A5%B 0.2 mins, 95%A5%B to 50%A50%B 16.8 mins, 50%A50%B to 95%A5%B 3.0 mins): 97.2%, 3.748 min; LRMS predicted for ($C_{39}H_{62}N_{16}O_{16}$+2H$^+$)/2 = 506.2. Found 506.2 (M+2H$^+$)/2. HRMS predicted for ($C_{39}H_{62}N_{16}O_{16}$+2H$^+$)/2 = 506.2343. Found 506.2335 (M+2H$^+$)/2.

Zanamivir dimer 7



The general method for acid de-protection was applied to the protected zanamivir dimer obtained from the CuAAC coupling of nona-1,8-diyne and azide **24**. The double TFA salt was obtained – after freeze drying – as a white solid (59 mg, 89%). ^{1}H NMR (D_2O) δ 7.732 (bs, 2H), 5.681 (bs, 2H), 4.661-4.981 (m, 10H), 4.475 (d, *J* = 11.7 Hz, 2H), 4.350 (aq, *J* = 11.7 Hz, 6H), 4.003-4.156 (m, 4H), 3.611 (dd, *J* = 11.6, 2.8 Hz, 2H), 3.436 (dd, *J* = 11.7, 7.4 Hz, 2H), 2.545-2.720 (m, 4H), 1.942 (s, 3H), 1.778-1.952 (m, 10H), 1.564-1.720 (m, 4H), 1.366-1.537 (m, 4H), 1.097-1.331 (m, 4H). ^{13}C NMR (D_2O) δ 170.79, 163.12, 160.67, 157.32, 152.44, 139.21, 128.29, 105.04, 76.439, 74.878, 70.225, 66.269, 49.380, 47.582, 47.067, 39.218, 30.587, 30.213, 28.347, 28.045, 27.872, 24.389, 23.589. IR (ATR) 3303s, 1633s, 1405m, 1377w, 1328w, 1260w, 1191w, 1140w, 1039w. HPLC purity (solvent gradient profile: 90%A10%B 0.2 mins, 90%A10%B to 20%A80%B 16.8 mins, 20%A80%B to 90%A10%B 3.0 mins): 94.4 %, 5.896 min; LRMS predicted for ($C_{45}H_{74}N_{16}O_{16}$+2H$^+$)/2 548.3 = 547.3. Found 547.3 (M+2H$^+$)/2. HRMS predicted for ($C_{45}H_{74}N_{16}O_{16}$+2H$^+$)/2 = 547.2735. Found 547.2734 (M+2H$^+$)/2.

3. Biology

Cytopathic effect (CPE) assays were performed using a previously published method.[4] MDCK cells were infected with either 25 PFU/well of A/Sydney/5/97 or 50 PFU/well of B/Harbin/7/94 in the presence of triplicate serial (9 × half $\log_{10}$) dilutions of the compounds in 96 well plates. Cultures were incubated for 72 h at 37 °C in a 5% CO_2 atmosphere in modified Eagle's medium supplemented with 0.2% bovine serum albumin (BSA), 1.2 μg/mL of TPCK-trypsin, 1% w/v L-glutamine and 1× insulin-transferrin-selenium mixture. The extent of the CPE and, hence viral replication, was determined by measurement of the metabolism of the vital dye (MTT) by published methods. The compound concentration that inhibited the CPE by 50% (EC_{50}) was calculated by non-linear regression.

4. References

1. Khoukhi, N. *Tetrahedron* **1987**, *43*, 1811.
2. Budhathoki-Uprety, J.; Novak, B. M. *Macromolecules* **2011**, *44*, 5947-5954.

3. Pirali, T.; Pagliai, F.; Mercurio, C.; Boggio, R.; Canonico, P. L.; Sorba, G.; Tron, G. C.; Genazzani, A. A. *J. Comb. Chem.* **2008**, *10*, 624-627.
4. Wantanabe, W.; K. Konno, K.; Ijichi, H. Inoue, T.; Yokota, S.; Shigeta, S. *J. Virol. Methods* **48**, 257-265.