Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Fully Automated radiosynthesis of [^{18}F]Fluoro-*C*-glyco-c(RGDfC): Exploiting all the abilities of the AllInOne synthesizer

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1. Experimental section

Synthesis of 4,8-anhydro-1-azido-1,2,3-trideoxy-9-*O*-trifluoromethylsulfonate-5,6,7-tri-*O*-acetyl-D-*glycero*-D-*gulo*-nonitol (2)[1]

To a solution of **1**[9] (300 mg, 0.80 mmol) diluted in 6,4 ml of dichloromethane and 0,2 ml of pyridine, was added drop by drop at -25 °C under inert atmosphere 0.2 ml of Tf_2O (2.3 eq, 1.85 mmol). After stirring 10 minutes at -25 °C then 25 minutes at room temperature, the reaction mixture was diluted with CH_2Cl_2 and hydrolyzed with water. The organic layer was isolated and washed with a saturated aqueous solution of $NaHCO_3$ and with water. The organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by flash column chromatography on silica gel (eluent: cyclohexane/EtOAc 90/10 to 20/80) to give **2** as a white solid (303 mg, 0.6 mmol, 80%).

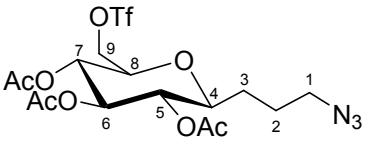

Fig S1

mp: 84°C. $[\alpha]_D^{20}$ -4.8 (c 0.50, $CHCl_3$). IR (ν, cm^{-1}) 2943, 2099, 1755, 1414, 1371, 1241, 1212. ^1H NMR (400 MHz, $CDCl_3$) δ 1.49-1.56 (m, 1H, H3a), 1.58-1.72 (m, 2H, H2a and H3b), 1.77-1.87 (m, 1H, H2b), 2.01 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 3.24-3.36 (m, 2H, H1), 3.49 (app td, 1H, $J_{4,5} = J_{4,3a} = 9.5$ Hz, $J_{4,3b} = 2.5$ Hz, H4), 3.77 (dt, 1H, $J_{8,7} = 9.5$ Hz, $J_{8,9} = 4.5$ Hz, H8), 4.48 (d, 2H, H9), 4.89 (app t, 1H, $J_{5,6} = J_{5,4} = 9.5$ Hz, H5), 4.95 (app t, 1H, $J_{7,6} = J_{7,8} = 9.5$ Hz, H7), 5.20 (app t, 1H, H6). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.7 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 24.4 (C2), 28.5 (C3), 51.2 (C1), 68.7 (C7), 71.5 (C5), 73.9 (C6), 73.9 (C9), 75.2 (C8), 77.4 (C4), 118.7 (q, $J_{C,F} = 320.0$ Hz, C_{Tf}) 169.7 (2C=O), 170.4 (C=O). ^{19}F NMR (235 MHz, $CDCl_3$) δ -74.5. HRMS (ESI): calcd for $C_{16}H_{22}F_3N_3O_{10}SNa$ $[M+Na]^+$: 528.0876; found: 528.0847.

Synthesis of 4,8-anhydro-1-azido-5-fluoro-1,2,3-trideoxy-5,6,7,9-tri-*O*-acetyl-D-*glycero*-D-*gulo*-nonitol (3)[1]

To a solution of **1**[1] (189 mg, 0.51 mmol) in 1.26 mL of diglyme was added 180 µL of DAST (2.8 eq., 1.47 mmol) at room temperature under argon atmosphere. The reaction mixture was stirred 8 min at 110°C. MeOH (0.25 mL) was added at 0°C. The solution was diluted with CH_2Cl_2 (5 mL), washed with a saturated solution of $NaHCO_3$ (2x10 mL) and water (2x10 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, cyclohexane/AcOEt 9/1 then 7/3) to afford **3**.

Yield 61%. White solid. mp 95-97°C. $[\alpha]_D$: -6.3 (*c* 0.75; $CHCl_3$). IR (ν, cm^{-1}): 2957, 2099, 1755, 1375, 1220. ^1H NMR (400 MHz, $CDCl_3$): δ 1.47-1.55 (m, 1H, H3a), 1.59-1.71 (m, 2H, H2a and H3b), 1.79-1.89 (m, 1H, H2b), 2.00 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.24-3.38 (m, 2H, H1), 3.46 (app td, 1H, $J_{4,5} = J_{4,3a} = 9.5$ Hz, $J_{4,3b} = 2.5$ Hz, H4), 3.66 (dddd, 1H, $J_{H8,F} = 21.0$ Hz, $J_{8,7} = 9.5$ Hz, $J_{8,9a} = 5.0$ Hz, $J_{8,9b} = 2.5$ Hz, H8), 4.41 (ddd, 1H, $J_{H9a,F} = 47.5$ Hz, $J_{9a,9b} = 10.5$ Hz, H9a), 4.45 (ddd, 1H, H9b), 4.88 (app t, 1H, $J_{5,6} = J_{5,4} = 9.5$ Hz, H5), 5.01 (app t, 1H, $J_{6,7} = J_{6,5} = 9.5$ Hz, H7), 5.20 (app t, 1H, H6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.8 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 24.7 (C2), 28.5 (C3), 51.3 (C1), 68.5 (d, $J_{C,F} = 7.0$ Hz, C7), 71.8 (C5), 74.3 (C6), 76.5 (d, $J_{C-F} = 19$ Hz, C8), 77.4 (C4), 81.8 (d, $J_{C-F} = 175.0$ Hz, C9), 169.6 (C=O), 169.8 (C=O), 170.6 (C=O). ^{19}F NMR (235 MHz, $CDCl_3$): δ -231.5 (dt, $J_{H9,F} = 47.0$ Hz, $J_{H8,F} = 21.0$ Hz). HRMS (ESI, $C_{15}H_{22}FN_3O_7Na$: $[M + Na]^+$) calcd 398.1339, found 398.1368. HPLC purity: 98.6% (230 nm).

Synthesis of 4,8-anhydro-1-azido-5-fluoro-5,1,2,3-trideoxy-D-*glycero*-D-*gulo*-nonitol (4)[1]

To a solution of **3** (1.11 mmol, 420 mg) in 8 mL of anhydrous methanol, was added a catalytic amount of sodium. The reaction was stirred 2h then neutralized by a resin (Amberlite® IR-120). The reaction mixture was filtered of and the solvent was removed under reduced pressure to quantitatively give **4**.

Quantitative yield. White solid. mp 43-45°C. $[\alpha]_D$: -7.1 (*c* 0.1; H_2O). IR (ν, cm^{-1}): 3345, 2953, 2920, 2857, 2091, 1630, 1560, 1451, 1350, 1254. ^1H NMR (400 MHz, D_2O): δ 1.48-1.58 (m, 1H, H3a), 1.68-1.79 (m, 1H, H2a), 1.80-1.90 (m, 1H, H2b), 1.92-2.01 (m, 1H, H3b), 3.24 (app t, 1H, $J_{5,4} = J_{5,6} = 9.0$ Hz, H5), 3.37-3.44 (m, 3H, H1 and H4), 3.47-3.62 (m, 3H, H6, H7 and H8), 4.72 (bd, 2H, $J_{H,F} = 47.0$ Hz, H9). ^{13}C NMR (100.6 MHz, D_2O): δ 24.2 (C2), 28.0 (C3), 50.9 (C1), 68.7 (d, $J_{C,F} = 7.0$ Hz, C7), 73.4 (C5), 77.2 (C6), 78.1 (d, $J_{C,F} = 18.0$ Hz, C8), 79.2 (C4), 82.3 (d, $J_{C,F} = 168.0$ Hz, C9). ^{19}F NMR (235 MHz, D_2O): δ - 234.9. HRMS (ESI, $C_9H_{16}FN_3O_4Na$: $[M + Na]^+$) calcd 272.1023, found 272.1039. HPLC purity: 99.4% (230 nm).

Synthesis of cyclo[L-arginylglycyl-L-α-aspartyl-D-phenylalanyl-S-[[1-[1-(4,8-anhydro-5-fluoro-5,1,2,3-trideoxy-D-*glycero*-D-*gulo*-nonitol)]-1H-1,2,3-triazol-4-yl]methyl]-L-cysteinyl] (6)[1]

To a solution of S-propargyl derivative **5**[2] (0.02 mmol) and azido derivatives **4** (0.005 mmol) diluted in water (0.1 mL), was added at room temperature sodium ascorbate (0.001 mmol) in water (0.1 mL) and a solution of $Cu(OAc)_2$ (0.0005 mmol) in water (0.011 mL). The solution

turned immediately pale green. The reaction was stopped when the intense blue color reappeared (2h of stirring). Chelex® resin (50 mg) was then added to the blue solution and the suspension was stirred until the solution turned colorless. The resin was filtered off and the resulting solution was freeze-dried. Purification was achieved on Sephadex LH20 resin. Elution with water provided pure fluoro-*C*-glycopeptide **6**.

Yield 69%. White foam. ^{1}H NMR (400 MHz, D_2O): δ 1.42-1.64 (m, 3H, H3a, H2a and ½CH_2), 1.65-1.77 (m, 1H, H2b), 1.81-1.93 (m, 2H, H3b, ½CH_2), 2.00-2.23 (m, 2H, CH_2), 2.55 (dd, 1H, J = 16.0 Hz, J = 7.0 Hz, ½CH_2), 2.68 (dd, 1H, J = 16.0 Hz, J = 7.0 Hz, ½CH_2), 2.75 (bd, 1H, J = 7.0 Hz, ½CH_2), 3.00-3.13 (m, 1H, ½CH_2), 3.16-3.26 (m, 3H, H5 and CH_2), 3.33 (app td, 1H, $J_{4,5}$ = 9.5 Hz, $J_{4,3}$ = 3.0 Hz, H4), 3.45-3.56 (m, 4H, H6, H7, H8 and CH), 3.71 (s, 2H, CH_2), 4.19-4.27 (m, 2H, CH_2), 4.41 (dd, 1H, J = 9.0 Hz, J = 6.0 Hz, CH), 4.48-4.52 (m, 2H, H1), 4.61-4.77 (m, 6H, CH_{2benz}, H9 and 2CH), 7.26-7.39 (m, 5H, H_{Ar}), 7.91 (s, 1H, H-triazole). ^{13}C NMR (100.6 MHz, D_2O): δ 24.5 (C2), 25.2 (CH_2), 25.5 (CH_2), 27.1(CH_2), 27.7 (C3), 36.4 (2CH_2), 40.5 (2CH_2), 43.7 (CH_2), 50.2 (C1), 50.8 (CH), 52.7 (CH), 54.3 (CH), 55.2 (CH), 68.6 (d, $J_{C,F}$ = 7.0 Hz, C7), 73.3 (C5), 77.2 (C6), 78.1 (d, $J_{C,F}$ = 17.0 Hz, C8), 78.9 (C4), 82.4 (d, $J_{C,F}$ = 168.0 Hz, C9), 124.0 ($CH_{triazole}$), 127.1(C_{Ar}), 128.8 (2C_{Ar}), 129.2 (2C_{Ar}), 136.1(C_{qAr}), 144.6 ($C_{triazole}$), 156.8 (C=N), 171.2 (2C=O), 172.3 (C=O), 172.6 (C=O),173.3 (C=O), 177.9 (C=O). ^{19}F NMR (235 MHz, D_2O): δ - 234.6. HRMS (ESI, $C_{36}H_{53}FN_{11}O_{11}SH$: $[M + H]^+$) calcd 866.3631, found 866.3680. HPLC purity: 99.2% (230 nm).

3. HPLC Chromatograms

HPLC analyses were carried out on analytical HPLC with UV, radioactive and ELSD series detectors arranged in the mentioned order. HPLC purifications were carried out on semi-preparative HPLC with radioactive and UV series detectors arranged in the mentioned order. Retention times were based on UV chromatograms for non-radioactive compounds and on radioactive chromatograms for radioactive compounds

Analytical HPLC of 2

(t_R = 15.7 min, Alltima C8, 5µm, 150x4.6 mm, 55/45 ACN/H_2O in isocratic condition, 1.0 mL/min)

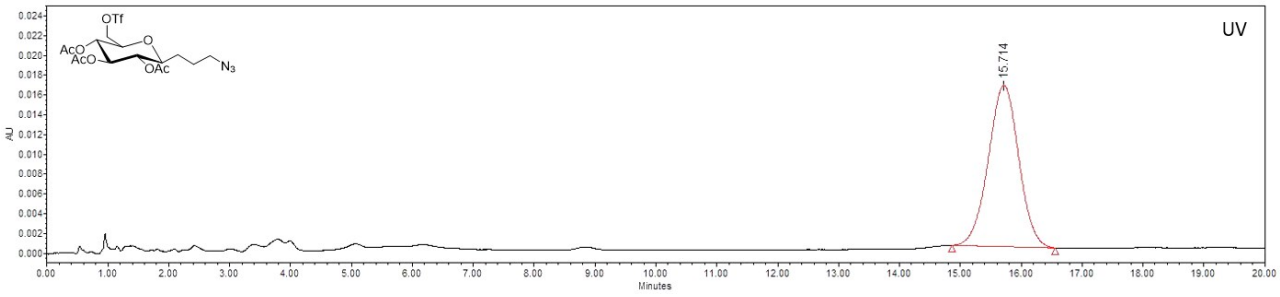

Figure S2. UV chromatogram of **2** (230 nm)

Analytical HPLC of 3

(t_R = 7.5 min, Alltima C8, 5µm, 150x4.6 mm, 55/45 ACN/H_2O in isocratic condition, 1.0 mL/min)

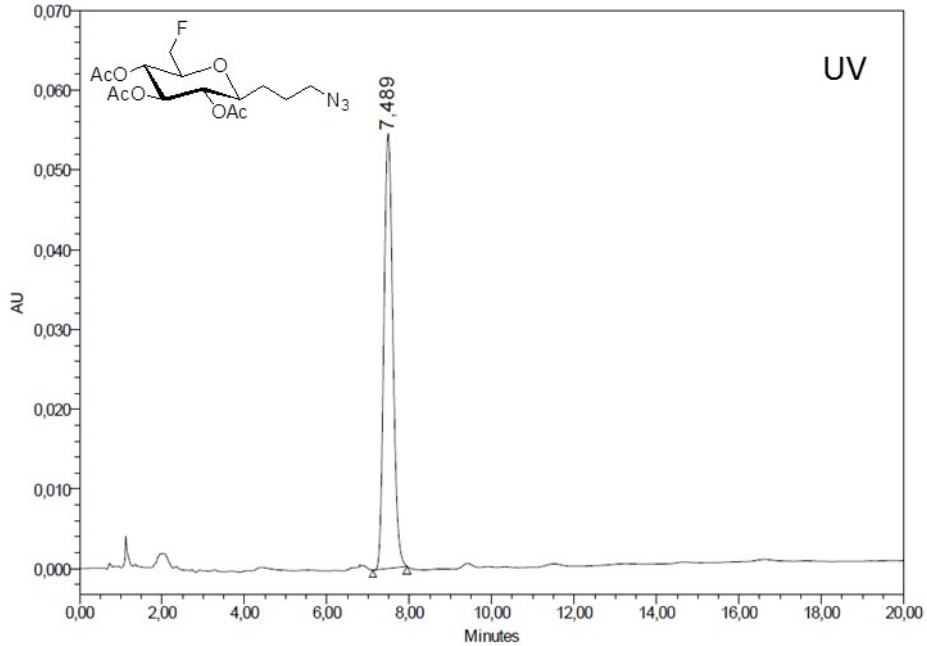

Figure S3. UV chromatogram of **3** (230 nm)

Analytical HPLC of 4

(t_R = 4.7 min, Vydac 218TP C18, 5µm, 250x4.6mm, ACN/H_2O 17/83 (v/v) with 0.1% of TFA in isocratic condition, 1.0 mL/min)

Given that **4** do not contain any chromophore groups no signal was observed with UV detection, only ELSD chromatogram is presented.

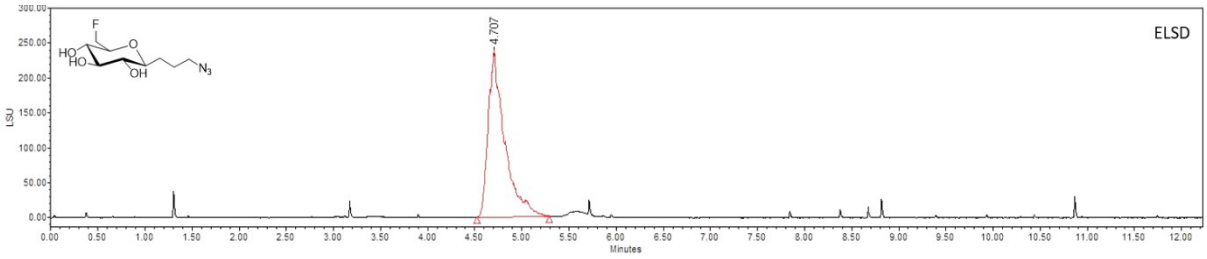

Figure S4. ELSD chromatogram of **4** (T°C nebulisation: cooling, T°C drift tub: 50°C, 50 psi)

Analytical HPLC of 5

(t_R = 13.2 min, Vydac 218TP C18, 5µm, 250x4.6mm, ACN/H_2O 17/83 (v/v) with 0.1% of TFA in isocratic condition, 1.0 mL/min)

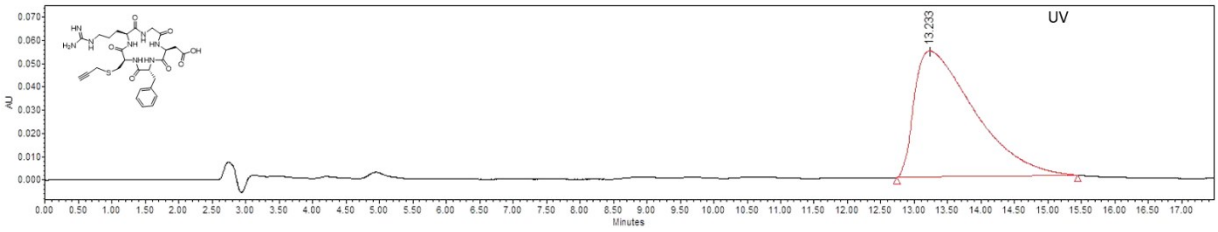

Figure S5. UV chromatogram of **5** (230 nm)

Analytical HPLC of 6

(t_R = 7.3 min, Vydac 218TP C18, 5µm, 250x4.6mm, ACN/H_2O 17/83 (v/v) with 0.1% of TFA in isocratic condition, 1.0 mL/min)

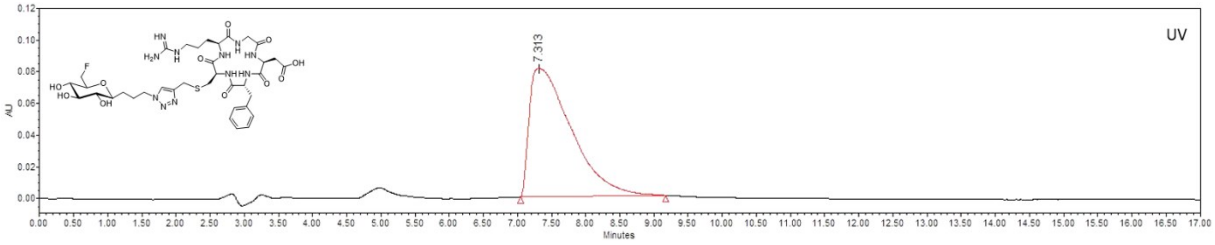

Figure S6. UV chromatogram of **6** (230 nm)

4. Radioactivity trending

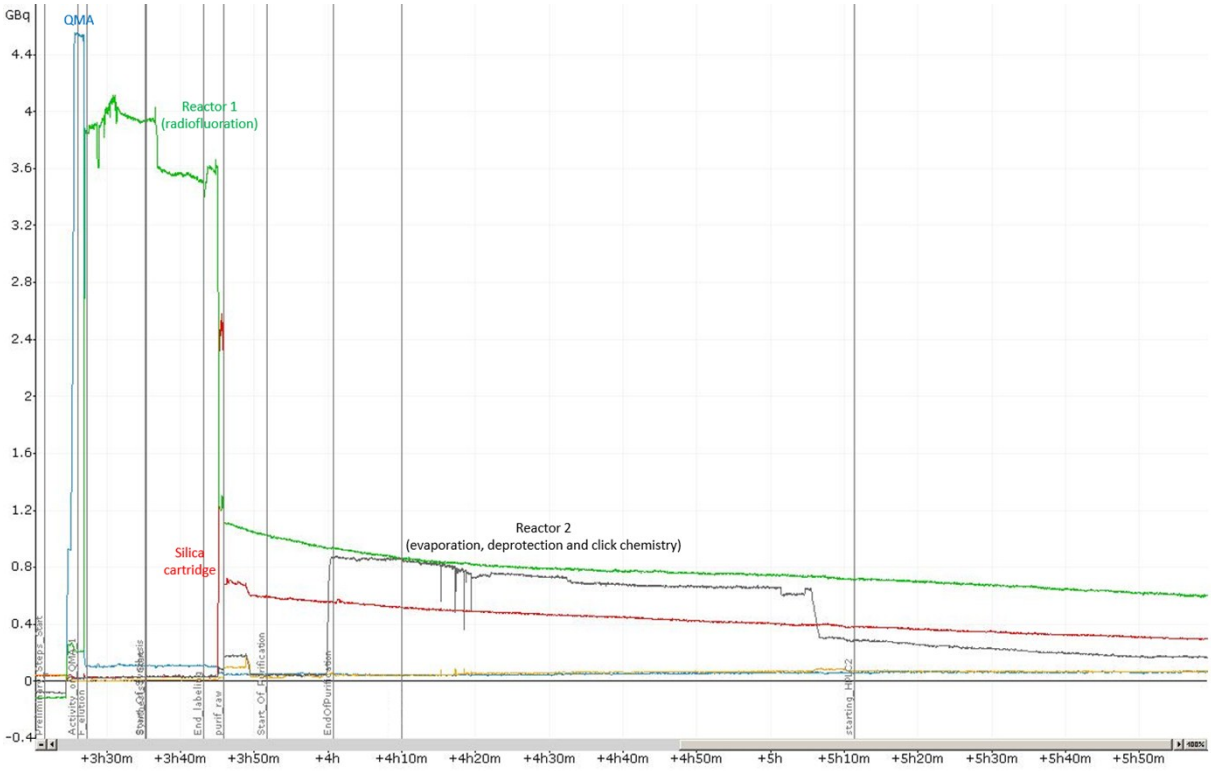

Figure S7. Radioactivity trending of **[^{18}F]6** automated radiosynthesis.

5. References

1. T. Vucko, N. Pétry, F. Dehez, A. Lambert, A. Monari, C. Lakomy, P. Lacolley, V. Regnault, C. Collet, G. Karcher, S. Lamandé-Langle, *Bioorg. Med. Chem*., **2019**, ASAP, DOI: 10.1016/j.bmc.2019.07.039.

2. S. Lamandé-Langle, C. Collet, R. Hensienne, C. Vala, F. Chrétien, Y. Chapleur, A. Mohamadi, P. Lacolley, V. Regnault, *Bioorg. Med. Chem*., 2014, **22**, 6672-6683.