Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

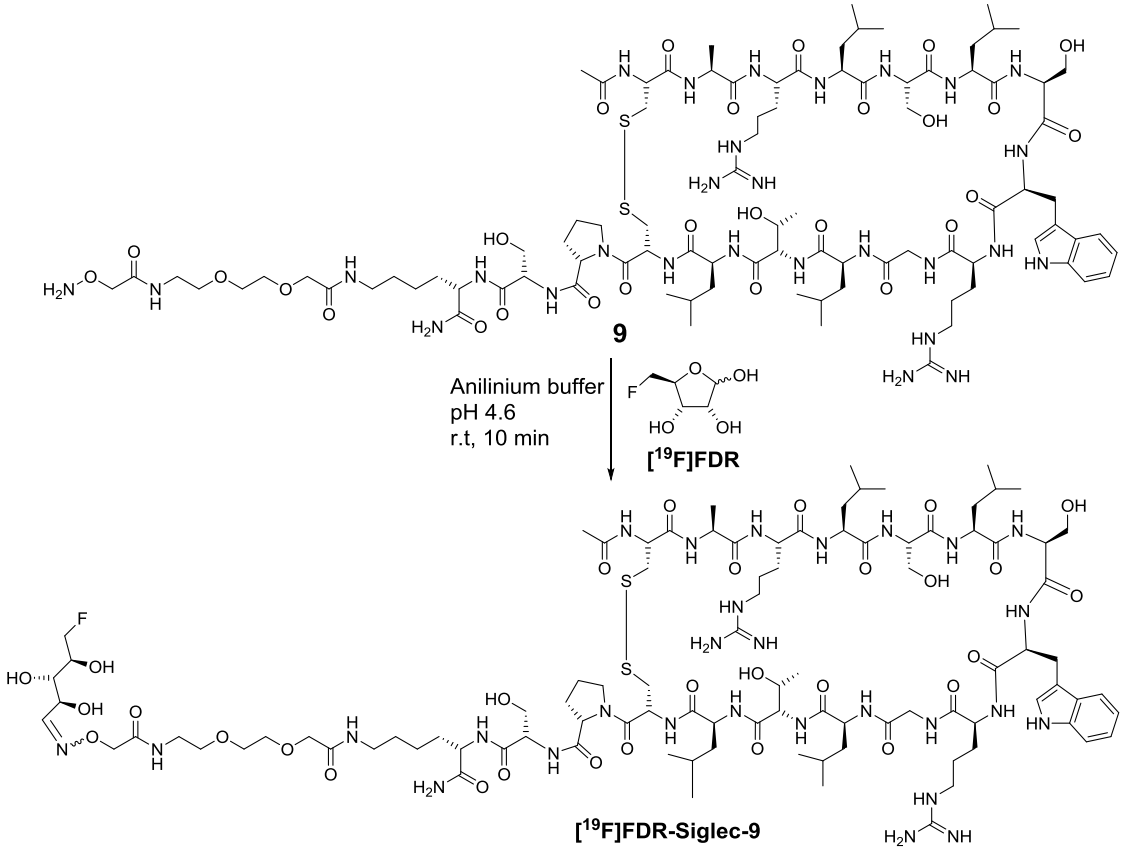
Translating the concept of peptide labeling with 5-deoxy-5-[^{18}F]fluororibose into preclinical practice: ^{18}F-labeling of Siglec-9 peptide for PET imaging of inflammation

Xiang-Guo Li, Anu Autio, Helena Ahtinen, Kerttuli Helariutta, Heidi Liljenbäck, Sirpa Jalkanen, Anne Roivainen and Anu J. Airaksinen

Material and methods

Compounds **6** and 5-deoxy-5-fluororibose ([^{19}F]FDR) were prepared according to previously described procedures.[1] Peptide **9** was purchased from PolyPeptide Group. Compound **10** and other reagents were from commercial suppliers. The radiosynthesis was carried out in a DM-Automation device and ^{18}F-fluoride was produced on an IBA Cyclone 10/5 cyclotron in a ^{18}O(p,n)^{18}F reaction. All studies were conducted with approval from the Lab-Animal Care & Use Committee of the State Provincial Office of Southern Finland and in compliance with the Finnish laws relating to the conduct of animal experimentation.

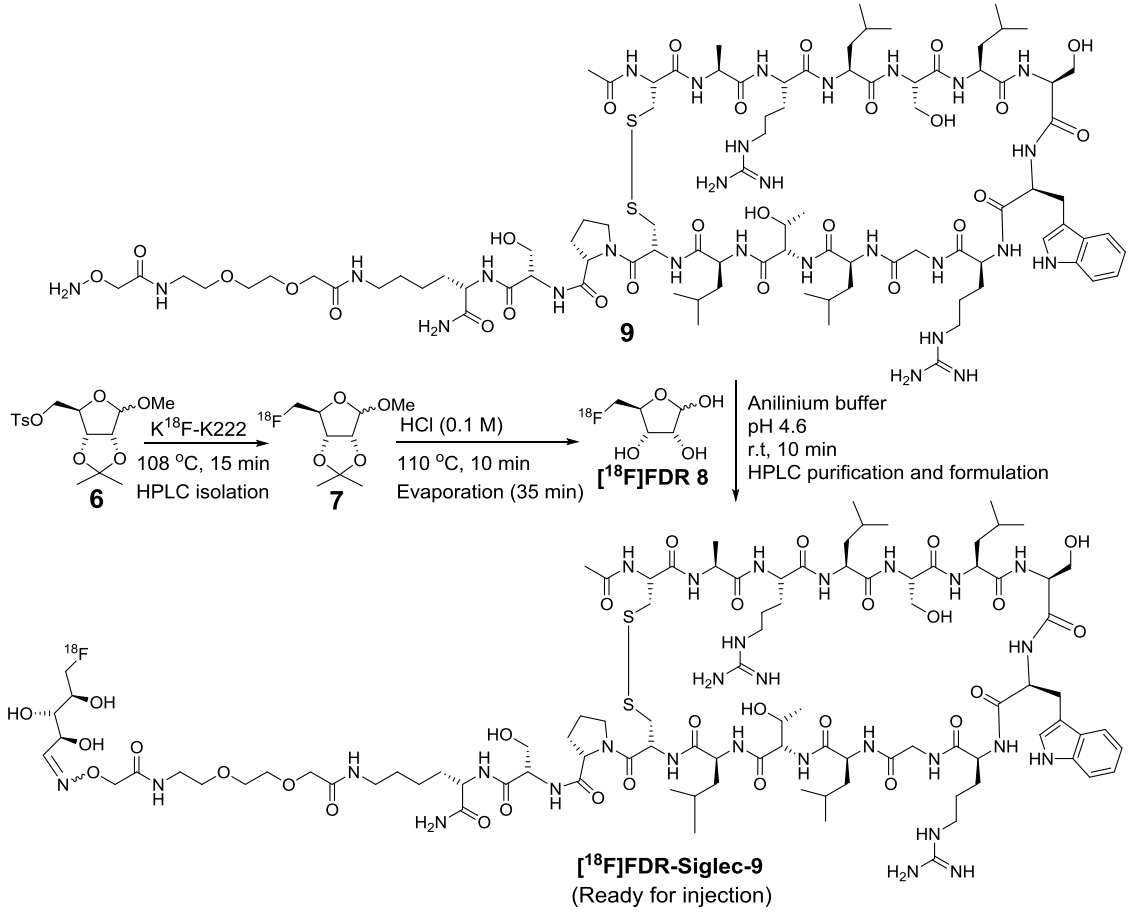
Preparation of reference compound [^{19}F]FDR-Siglec-9



Peptide **9** (2.0 mg, 931 nmol) was added to a solution of 5-deoxy-5-fluororibose ([^{19}F]FDR, 152 µg, 1 µmol) in anilinium buffer (0.2 M, 3.3 mL). The reaction mixture was kept standing for 10 min at

r.t. The reaction mixture was loaded onto a HLB cartridge (Waters). The cartridge was washed with water (10 mL) and [^{19}F]FDR-Siglec-9 was eluted from the cartridge with ethanol (0.5 mL, containing 2.7 mM HCl). [^{19}F]FDR-Siglec-9 (1.9 mg, 838 nmol) was obtained in 90% isolated yield after evaporation off the solvents. HRMS ES$^+$ (*m*/*z*) calculated for $C_{97}H_{161}FN_{28}O_{30}S_2Na$, 2303.1282; found 2303.1372.

Radiosynthesis of [^{18}F]FDR-Siglec-9



The dried K^{18}F-K222 complex was prepared according to the known procedure.[2] At the end of bombardment (EOB), $^{18}F^-$ was trapped in an anion exchange cartridge (Sep-Pak QMA Light Plus, Waters Corporation) and was subsequently eluted as K^{18}F-K222 complex into a reaction vessel. The initial radioactivity at the EOB was in the range of 0.5-10 GBq. K^{18}F-K222 was dried with acetonitrile azeotropically at 120 °C with argon flow and cooled down to r.t. The preparation of K^{18}F-K222 took typically 15 minutes from EOB.

A solution of compound **6** (5.0 mg, 14.0 µmol) in acetonitrile (0.5 mL) was added into dried K^{18}F-K222 and the mixture was kept without stirring at 108 °C for 15 min. Then the reaction mixture was cooled to 28 °C and diluted with acetonitrile in water (25%, 1 mL). The mixture was injected into a semi-preparative HPLC column (µBondapak C18, 300 × 7.8 mm, Waters). Intermediate **7** (RT 7 min) was eluted with acetonitrile in water (42%, containing 0.1% trifluoroacetic acid, flow rate 4 mL/min) and collected into a reaction vessel which was pre-loaded with a solution of HCl (5 M, 60

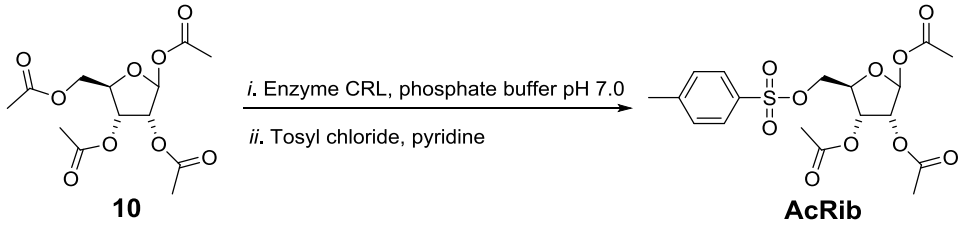
µL). The reaction mixture was kept at 110 °C without stirring for 10 min before it was cooled down to r.t. A solution of NaOH (2 M, 150 µL) was added to the reaction mixture. The obtained [^{18}F]FDR **8** solution was concentrated to 0.5 mL by evaporating off the solvents at 110 °C with argon flow (35 min) and was used for the next step without any need for further purification.

A solution of peptide **9** (0.6 mg, 279 nmol) in anilinium buffer (0.5 mL, 0.45 M, pH 4.6) was added into [^{18}F]FDR **8** (0.5 mL). The reaction mixture was kept without stirring at r.t for 10 min and was subsequently injected into a semi-preparative HPLC column (Jupiter Proteo, 250 × 10 mm, Phenomenex). [^{18}F]FDR-Siglec-9 (RT 12 min) was eluted with acetonitrile in water (23%, containing 0.1% trifluoroacetic acid, flow rate 4 mL/min). For *in vivo* PET imaging in rats, [^{18}F]FDR-Siglec-9 was isolated with a HLB cartridge (Waters) and formulated in phosphate-buffered saline (PBS) for intravenous injection. Total synthesis time of [^{18}F]FDR-Siglec-9 was typically 120 min starting from the EOB (35 min starting from [^{18}F]FDR **8**). Decay-corrected radiochemical yield was 27% starting from the EOB. Radiochemical purity was >98% by HPLC analyses and >95% by reverse phase radio-TLC analyses. Specific radioactivity of [^{18}F]FDR-Siglec-9 was 36-43 GBq/µmol at the EOS.

In vivo PET imaging of rats

Sprague-Dawley rats (n=8) were subcutaneously injected with turpentine oil (Sigma-Aldrich) into the shoulder area, and inflammation was allowed to develop for 24 hours before the PET study. The whole-body distribution kinetics and inflammation imaging of intravenously administered tracer (18.3 ± 5.1 MBq) were evaluated using a High Resolution Research Tomograph (Siemens Medical Solutions). Two rats were imaged at the same time and they were kept on a warm pallet during the imaging procedure. Data acquired for 60 minutes in a list mode, starting at the same as the injection, were iteratively reconstructed with the ordered-subsets expectation maximization 3D algorithm. Quantitative analysis was performed by defining regions of interest (ROI) in the inflammation focus and healthy muscle with Carimas 2.6 software (Turku PET Centre, Turku, Finland). The average radioactivity concentration in the ROI (kBq/mL) was used for further analyses. The uptake was reported as standardized uptake value (SUV), which was calculated as the radioactivity concentration of the ROI divided by the relative injected radioactivity expressed per animal body weight. The radioactivity remaining in the injection site (tail) was also compensated.

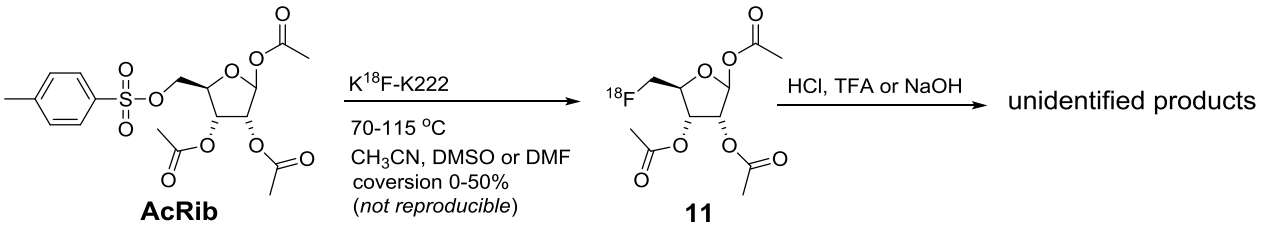
Preparation of 1,2,3-tri-*O*-acetyl-5-*O*-(*p*-toluenesulfonyl)-β-*D*-ribofuranose (AcRib)



According to the previously described procedures,[3,4] an enzyme preparation of *Candida rugosa* lipase (CRL, 750 mg) was added to a solution of compound **10** (509 mg, 1.6 mmol) in phosphate buffer/dimethylformamide (DMF) (9:1, pH 7.0, 40 mL). The reaction mixture was incubated with shaking (132 rpm) for 24 h at r.t before the enzyme was filtered off. Subsequently, the reaction

mixture was extracted with ethyl acetate (3 × 40 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. After filtering off the Na_2SO_4, the filtrate was concentrated to dryness. The residue was dissolved in pyridine (4 mL) and *p*-toluenesulfonyl chloride (286 mg, 1.5 mmol) was added at 0 ^oC. The reaction mixture was kept with stirring for 2 h at 0 ^oC and was poured into ice-cold water (20 mL). After routine work up, AcRib (344 mg, 0.8 mmol, yield 50%) was isolated with a silica-gel column. ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 6.09 (s, 1H), 5.35-5.31 (m, 2H, *overlapping signals*), 4.33 (m, 1H), 4.22 (dd, *J* = 11.0, 3.5 Hz, 1H), 4.10 (dd, *J* = 11.0, 4.5 Hz, 1H), 2.45 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.54, 169.36, 169.05, 145.17, 132.53, 129.95, 128.00, 97.84, 78.79, 73.96, 70.14, 68.06, 21.66, 20.90, 20.49, 20.39.

Testing of AcRib in the production of [^{18}F]FDR



AcRib was subjected to the ^{18}F-fluorination reaction in CH_3CN, DMF or dimethyl sulfoxide (DMSO) at different temperatures (70-115 ^oC). Intermediate **11** was obtained in 0-50% yields and the reactions were not reproducible. Compound **11** was rapidly decomposed upon acid- or base-catalyzed hydrolysis.

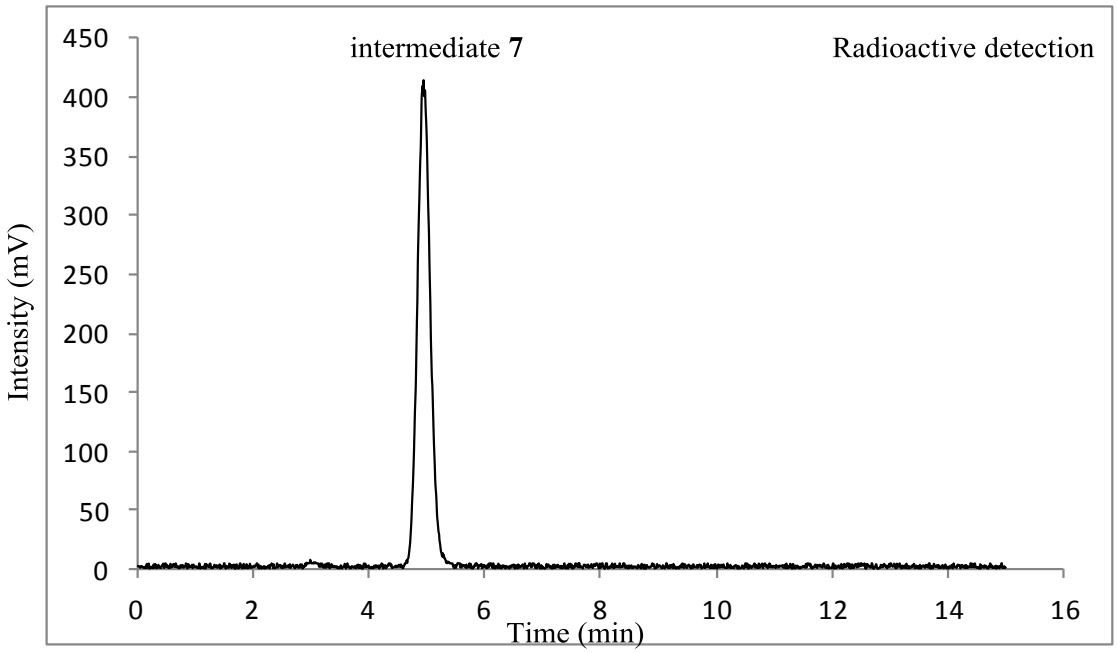
References:

1. X.-G. Li, S. Dall'Angelo, L. F. Schweiger, M. Zanda and D. O'Hagan, *Chem. Commun*., 2012, **48**, 5247-5249.
2. M. Sarparanta, E. Mäkilä, T. Heikkilä, J. Salonen, E. Kukk, V.-P. Lehto, H. A. Santos, J. Hirvonen and A. J. Airaksinen, *Mol. Pharmaceutics*, 2011, **8**, 1799-1806.
3. W. J. Hennen, H. M. Sweers, Y.-F. Wang and C.-H. Wong, *J. Org. Chem*., 1988, **53**, 4939-4945.
4. S. J. Jun, M. S. Moon, S. H. Lee, C. S. Cheong and K. S. Kim, *Tetrahedron Lett*., 2005, **46**, 5063-5065.

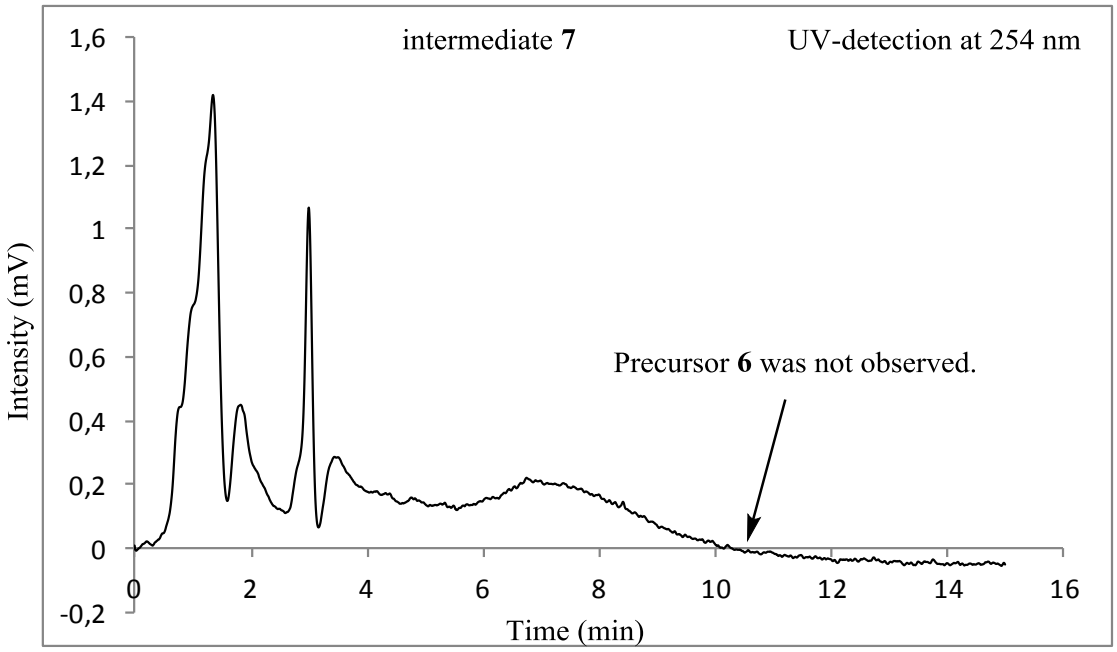
HPLC and TLC analyses

Figure 1. [a] Radioactivity chromatogram of intermediate **7** (>99% radiochemical purity). HPLC conditions: µBondapak C18 column (250 × 4.6 mm), 43% CH_3CN and 0.1% TFA in water, flow rate 1 mL/min. [b] UV-detection of intermediate **7**. [c] As a reference, precursor **6** was analyzed with UV detection under the same HPLC conditions as for the analysis of **7**. [d] Radio-TLC analysis of intermediate **7** (>99% radiochemical purity).

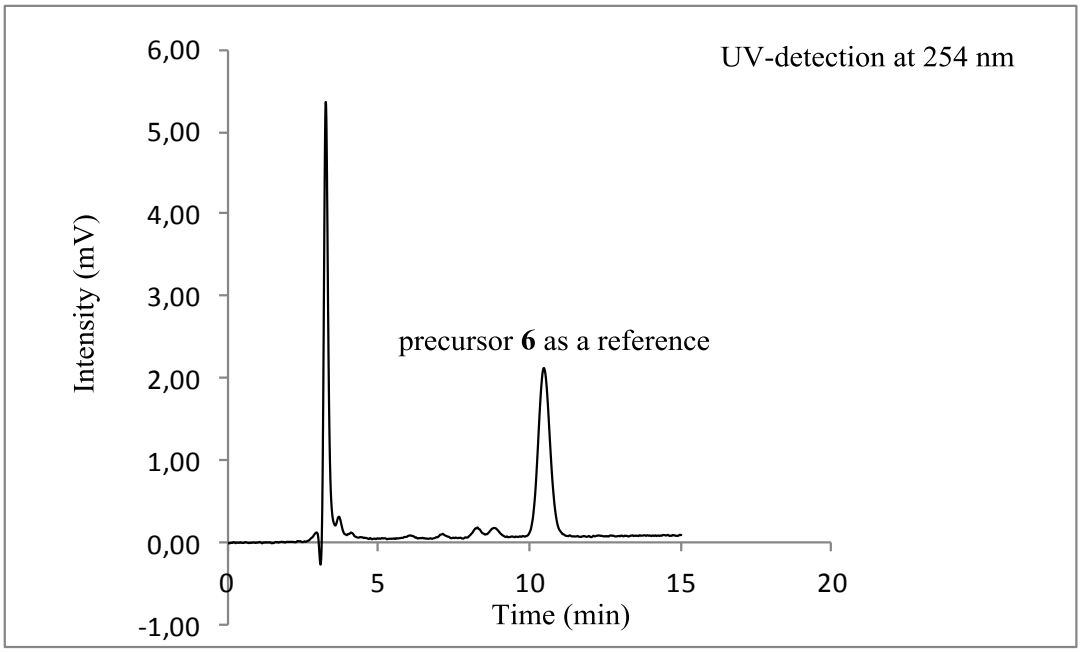
[a].



[b].



[c].


UV-detection at 254 nm
Intensity (mV)
6,00
5,00
4,00
3,00
2,00
1,00
0,00
-1,00
precursor 6 as a reference
0
5
10
15
20
Time (min)

[d].

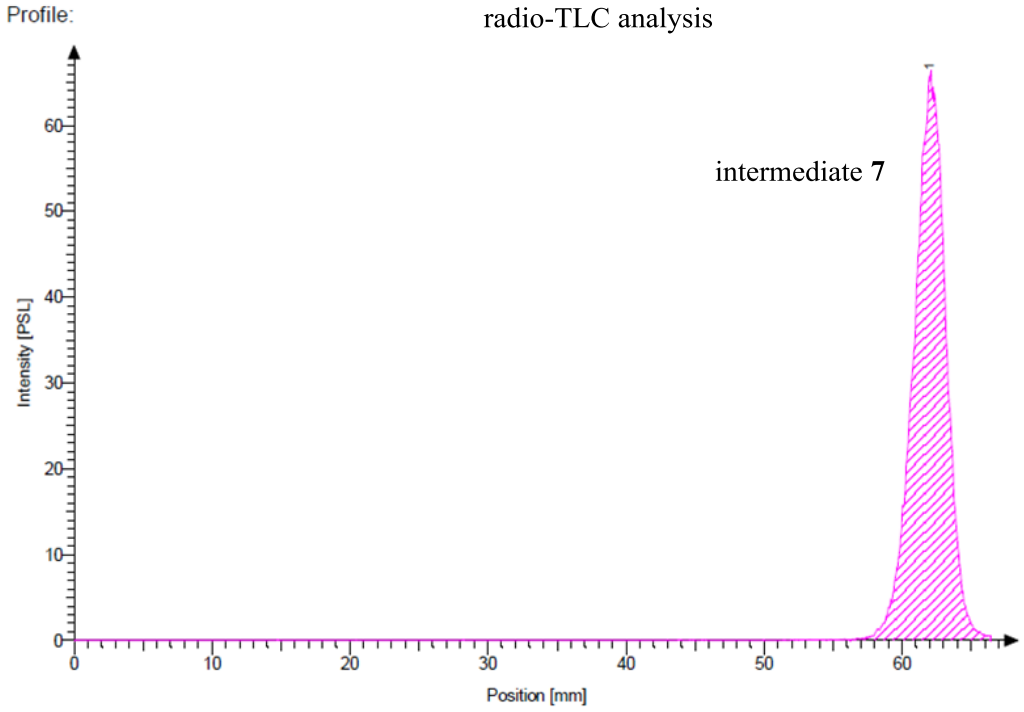

Profile:
radio-TLC analysis
intermediate 7
Intensity [PSL]
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Position [mm]

Figure 2. Radioactivity chromatogram of [^{18}F]FDR **8** (>99% radiochemical purity). HPLC conditions: Jupiter Proteo column (250 × 4.6 mm), 30% CH_3CN and 0.1% TFA in water, flow rate 1 mL/min.

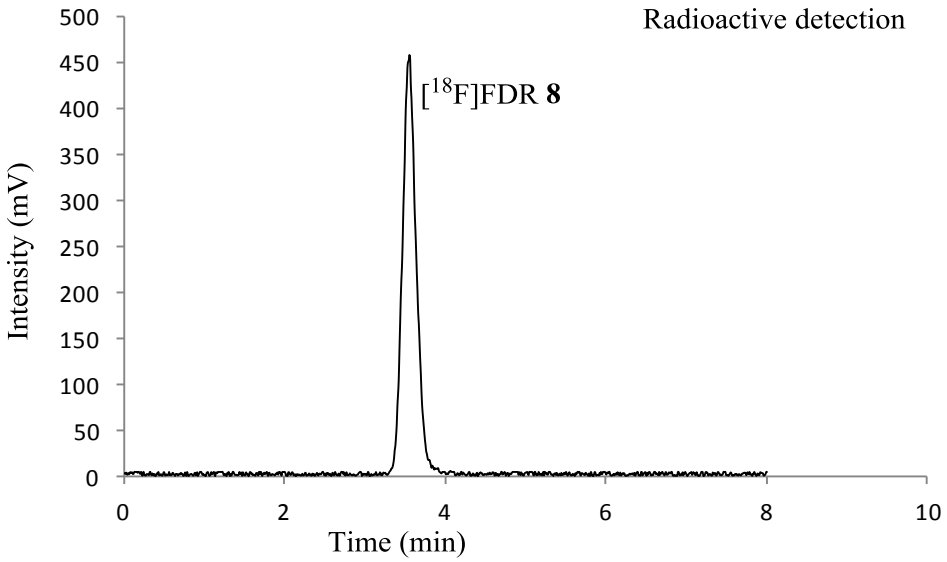
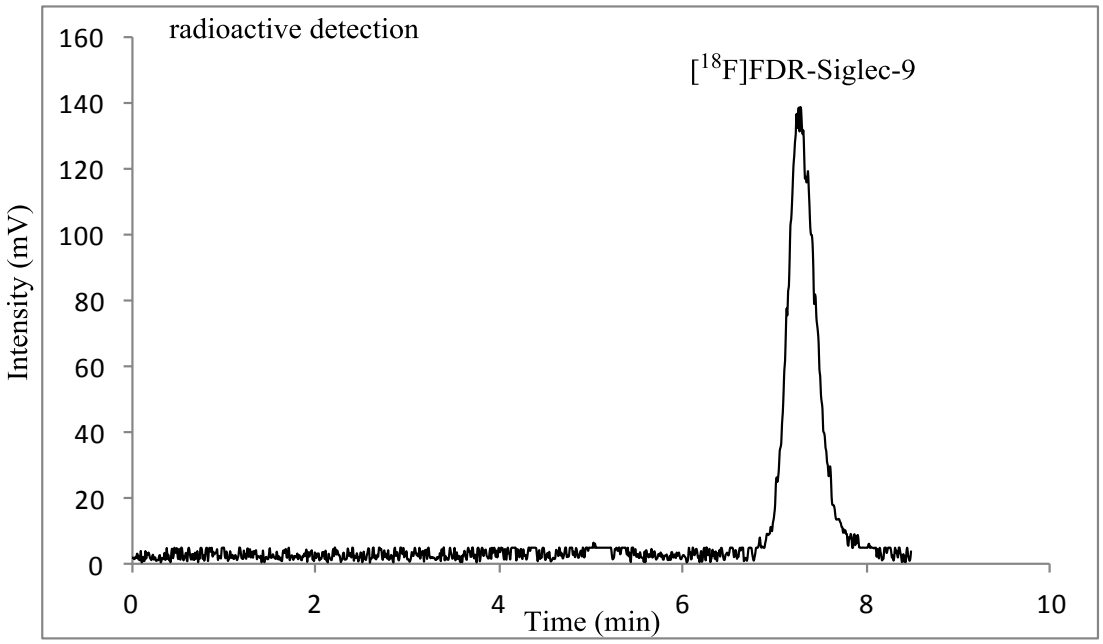
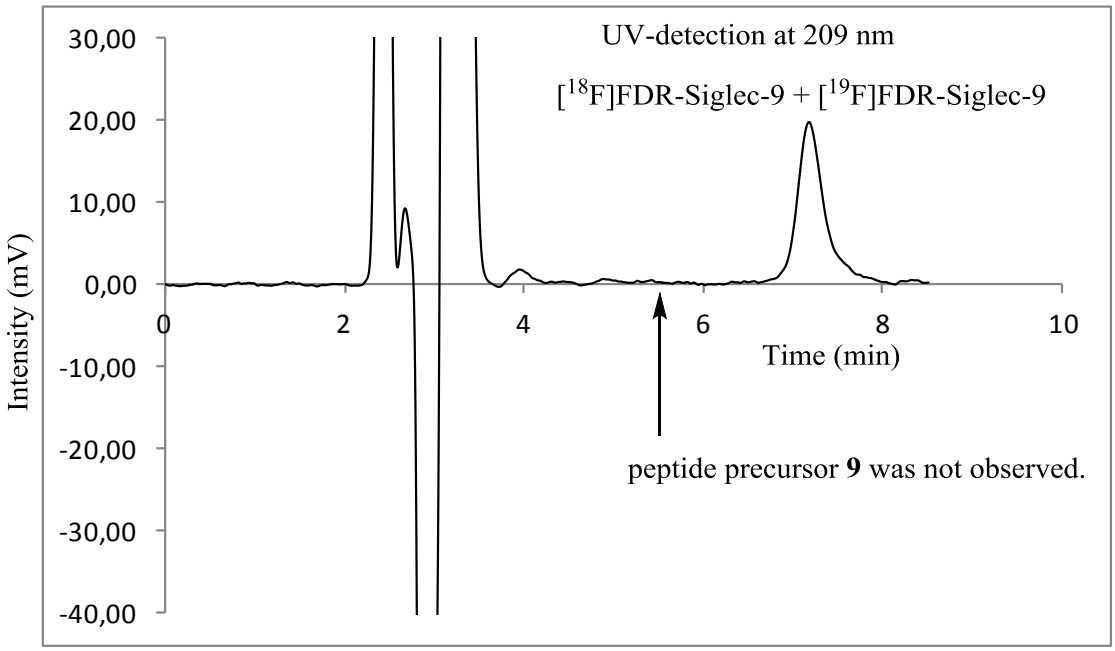


Figure 3. [a] Radioactivity chromatogram of [^{18}F]FDR-Siglec-9 (>98% radiochemical purity). HPLC conditions: Jupiter Proteo column (250 × 4.6 mm), 30% CH_3CN and 0.1% TFA in water, flow rate 1 mL/min. [b] Co-injection of [^{18}F]FDR-Siglec-9 and [^{19}F]FDR-Siglec-9 to the HPLC and the sample was analyzed under the same conditions except UV (209 nm)-detection was used. [c] Compound **9** as a reference was analyzed under the same HPLC conditions as in [b].

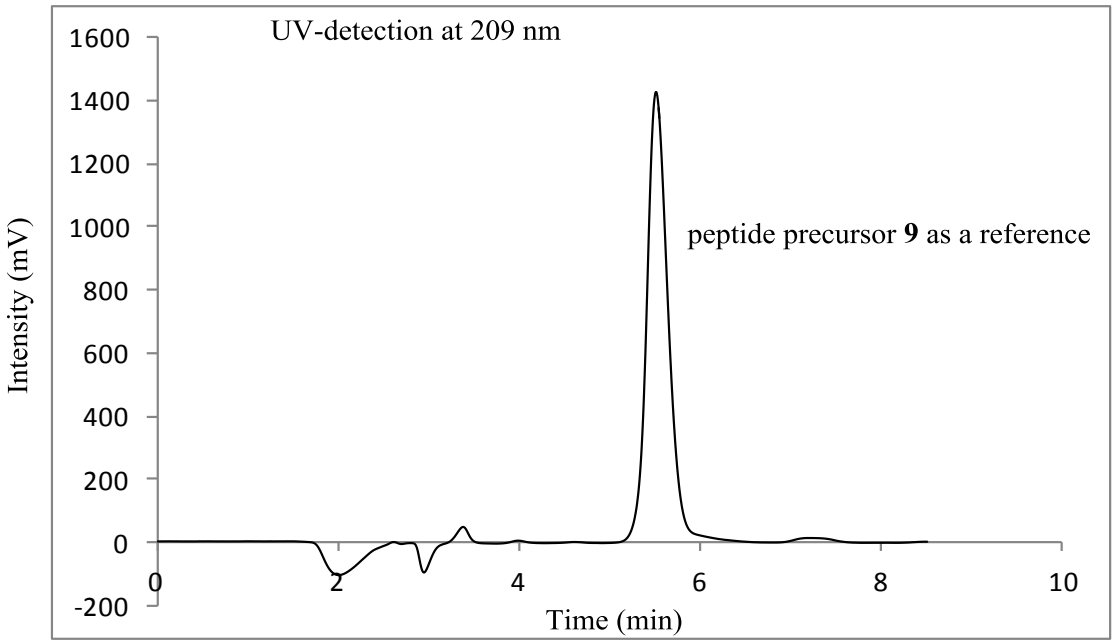
[a].



[b].


UV-detection at 209 nm
[18F]FDR-Siglec-9 + [19F]FDR-Siglec-9
Intensity (mV)
30,00
20,00
10,00
0,00
-10,00
-20,00
-30,00
-40,00
0
2
4
6
8
10
Time (min)
peptide precursor 9 was not observed.

[c].


UV-detection at 209 nm
Intensity (mV)
1600
1400
1200
1000
800
600
400
200
0
-200
0
2
4
6
8
10
Time (min)
peptide precursor 9 as a reference