Chemical Communications

Electronic Supplementary Information (ESI)

Enabling [^{18}F]-bicyclo[6.1.0]nonyne for oligonucleotide conjugation for positron emission tomography applications: [^{18}F]-anti-microRNA-21 as an example

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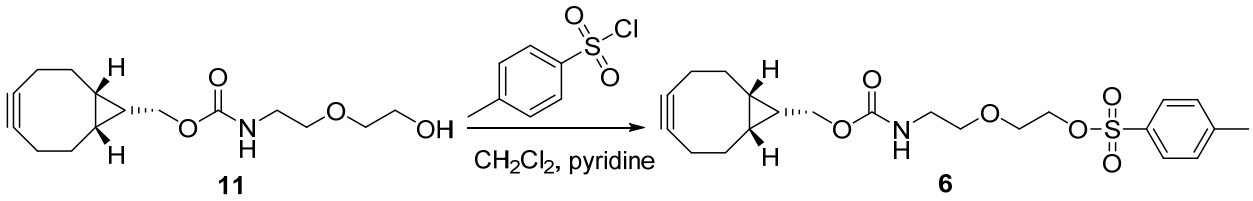
General remarks, material and methods

All radiation work complies with the regulations of the Radiation and Nuclear Safety Authority of Finland (STUK). Compound **6** was prepared in our lab or custom synthesized by SynAffix, The Netherlands. Oligonucleotide **8** was custom synthesized by Sigma and the sequence of **8** was 5′-mUmCmAmAmCmAmUmCmAmGmUmCmUmGmAmUmAmAmGmCmUmA-3′ (m denotes backbone modification with 2′-*O*-methylation). Compound **11** was purchased from SynAffix, The Netherlands. Other chemicals (including compound **9**) and reagents were purchased from Sigma-Aldrich-Fluka. Petroleum ether fraction 40–60 ºC (Sigma-Aldrich catalogue number 32299-2.5 L) was used for silica gel purification. Triethylammonium acetate (TEAA) buffer was prepared in our lab. Flash chromatography was performed using silica gel (60 Å, 230-400 mesh, enriched with 0.1% Ca, Merck). Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 500 MHz spectrometer. High resolution mass spectrometry (HRMS) were performed by using QSTAR Elite quadrupole/time-of-flight mass spectrometer (Applied Biosystems/MDS Sciex, Canada) equipped with a nano-electrospray ionization source (Proxeon, Odense, Denmark). The samples were loaded into offline nanospray emitters (Thermo Scientific) and analyzed in a negative or positive electrospray ionisation mass spectrometry (ESI-MS) mode. An Analyst 2.0 software (Applied Biosystems/MDS Sciex, Canada) was used as a mass spectrometer control and data acquisition software. The mass spectra of oligonucleotides were deconvoluted by using both charge

state and isotopic deconvolution in order to confirm an average mass of oligonucleotide samples. For small molecule samples, monoisotopic masses were measured.

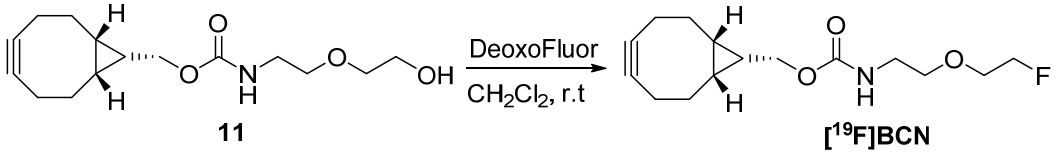
Preparation of compound 2-(2-(((((1*R*, 8*S*, 9*s*)-bicyclo[6.1.0]non-4-yn-9-yl)methoxy)carbonyl)amino)ethoxy)ethyl 4-methylbenzenesulfonate (6)



11 6

To a solution of compound **11** (50 mg, 0.18 mmol) in dry pyridine (120 µL) was added 4-toluenesulfonic chloride (85 mg, 0.45 mmol) at 0 ºC. The reaction mixture was stirred for 40 min at 0 ºC. Subsequently, the solvent was evaporated off under vacuum and the residue was applied onto a silica gel column for purification. A mixture of ethyl acetate in petroleum ether (33% by volume) was used as the eluent. The tosylated compound **6** (21 mg, 0.05 mmol) was isolated as an oil in 27% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 8.3 Hz, 2H), 4.93 (broad s, 1H), 4.08 (m, 4H), 3.57 (t, *J* = 4.5 Hz, 2H), 3.41 (t, *J* = 5.3 Hz, 2H), 3.24 (q, *J* = 5.2 Hz, 2H), 2.39 (s, 3H), 2.18 (m, 6H), 1.53 (m, 2H), 1.30 (m, 1H), 0.88 (m, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 156.73, 144.95, 133.02, 129.87, 127.98, 98.84, 70.24, 69.07, 68.43, 62.83, 40.66, 29.07, 21.67, 21.44, 20.13, 17.79. HRMS (ESI) for $[M + H]^+$, $C_{22}H_{30}NO_6S$ 436.1595 (Observed), 436.1794 (Calculated).

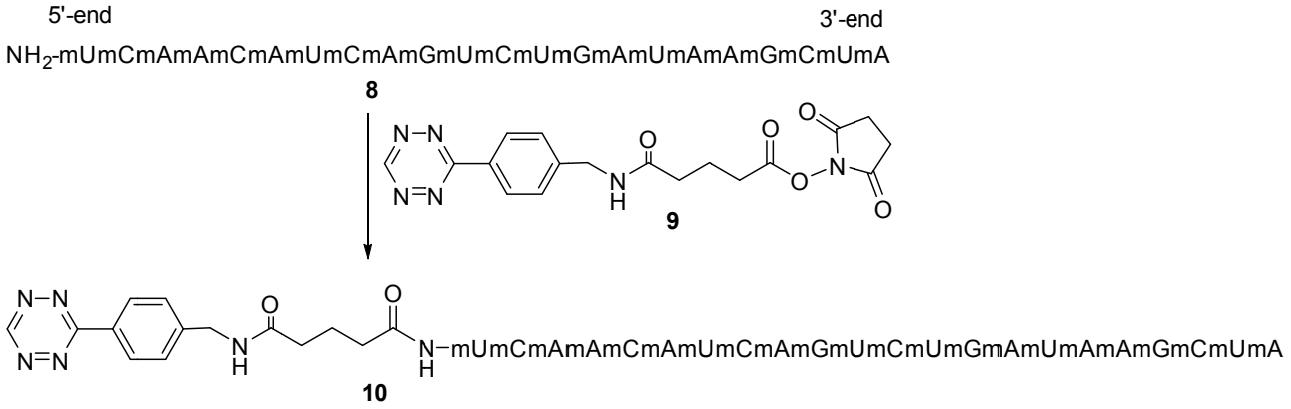
Preparation of compound ((1*R*, 8*S*, 9*s*)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-fluoroethoxy)ethyl)carbamate ([^{19}F]BCN)



11 [^{19}F]BCN

To a solution of **11** (56 mg, 0.20 mmol) in dry CH_2Cl_2 (200 µL) at 0 ºC, Deoxofluor (66 mg, 0.30 mmol) in toluene (111 µL) was added. Then the reaction mixture was kept for 16 hours at r.t. Silica gel column purification was performed with a mixture of ethyl acetate in petroleum ether (40% by volume) as the eluent, affording [^{19}F]BCN (6 mg, 0.02 mmol) in 10% yield. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.08 (broad s, 1H), 4.60 (t, *J* = 4.0 Hz, 1H), 4.50 (t, *J* = 4.0 Hz, 1H), 4.13 (d, *J* = 8.0 Hz, 2H), 3.72 (t, *J* = 4.0 Hz, 1H), 3.66 (t, *J* = 4.0 Hz, 1H), 3.57 (t, *J* = 5.0 Hz, 2H), 3.37 (m, 2H), 2.20 (m, 5H), 1.56 (m, 2H), 1.33 (m, 1H), 1.20 (m, 1H), 0.93 (m, 2H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ 156.76, 98.83, 83.64, 82.30, 70.32, 70.22, 70.06, 62.81, 40.76, 29.05, 21.42, 20.11, 17.75. HRMS (ESI) for $[M + K]^+$, $C_{15}H_{22}FKNO_3$ 322.1278 (Observed), 322.1216 (Calculated).

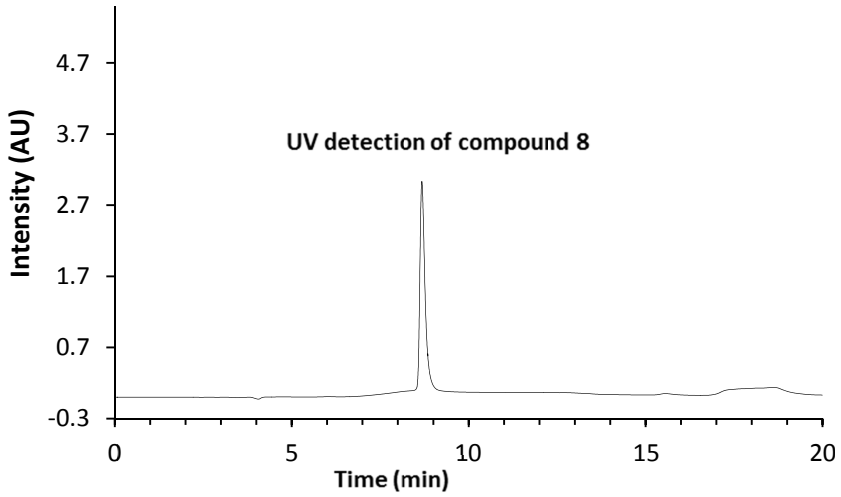
Preparation of compound 10



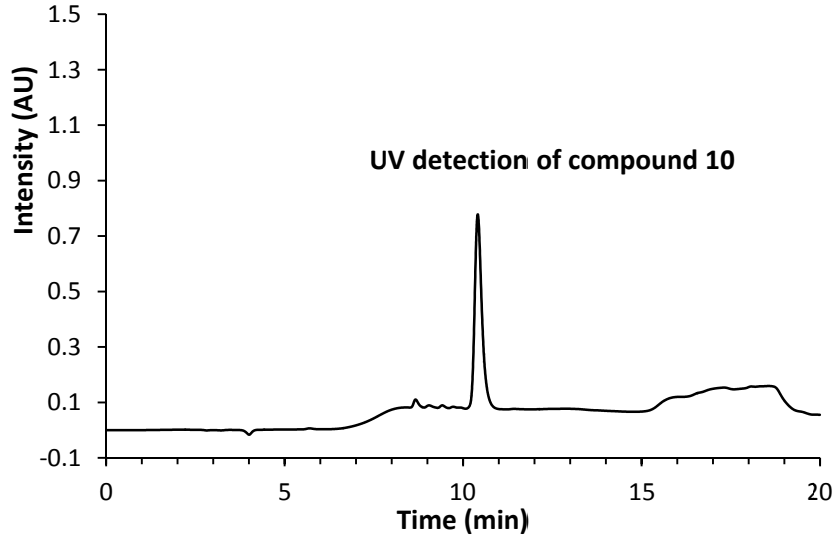
A solution of tetrazine-NHS (compound **9**, 1 mg, 2.51 μmol) in dry DMSO (100 μL) was freshly prepared and added to a solution of compound **8** (1 mg, 143 nmol) in aqueous $NaHCO_3$ (150 μL, 0.6 M) at 0 ºC. After 10 min, a $NaHCO_3$ solution (250 μL, 1.0 M) was added and the reaction mixture was kept at 4 ºC for 60 min. Then the reaction mixture was loaded onto a NAP-5 size exclusion column. The product **10** (906 μg, 117 nmol, isolated yield 82%) was obtained in sterile water (700 μL) as the eluent. The concentration of **10** was measured by a Biospec-nano photometer and the purity was 97% as measured by HPLC analyses. HRMS (ES**I** negative mode) for $C_{251}H_{330}N_{88}O_{155}P_{22}$: The theoretical molecular weight is 7741.2848. The measured average mass is 7740.8634 based on a charge state deconvolution and 7741.2420 based on an isotopic deconvolution of the most intense isotopic series.

Supplementary Figure 1. (a) HPLC analysis of starting material **8**. (b) HPLC analysis of product **10**. (c). Blank sample as a control. HPLC conditions: Phenomenex Jupiter Proteo C18 column (250 × 4.6 mm), UV detection at 260 nm, flow rate 1 mL/min, solvent A is TEAA (0.1 M, pH 7.0) and solvent B is CH_3CN, gradient elution from 10% B to 60% of B during 20 min.

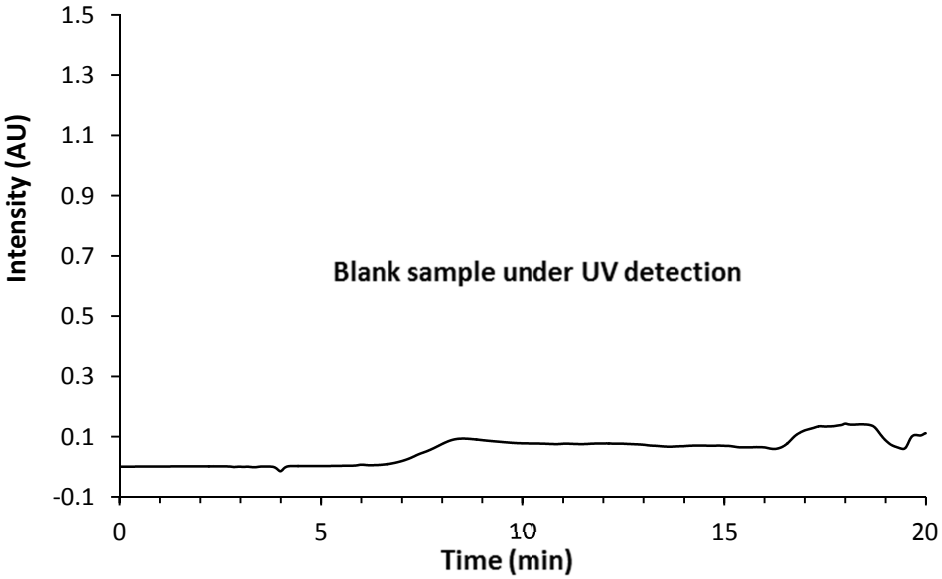
(a).



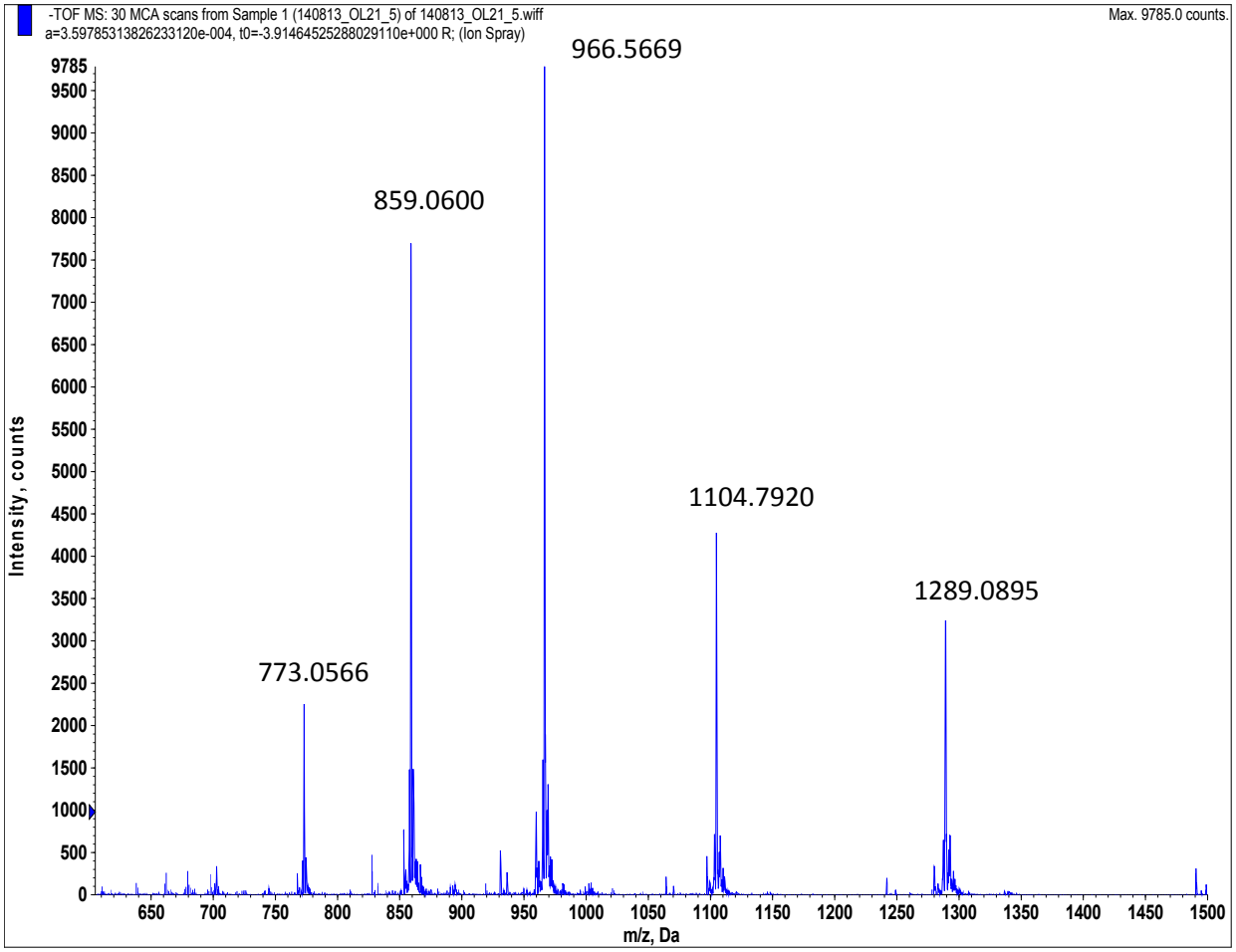
(b).


UV detection of compound 10
Intensity (AU)
Time (min)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0
5
10
15
20

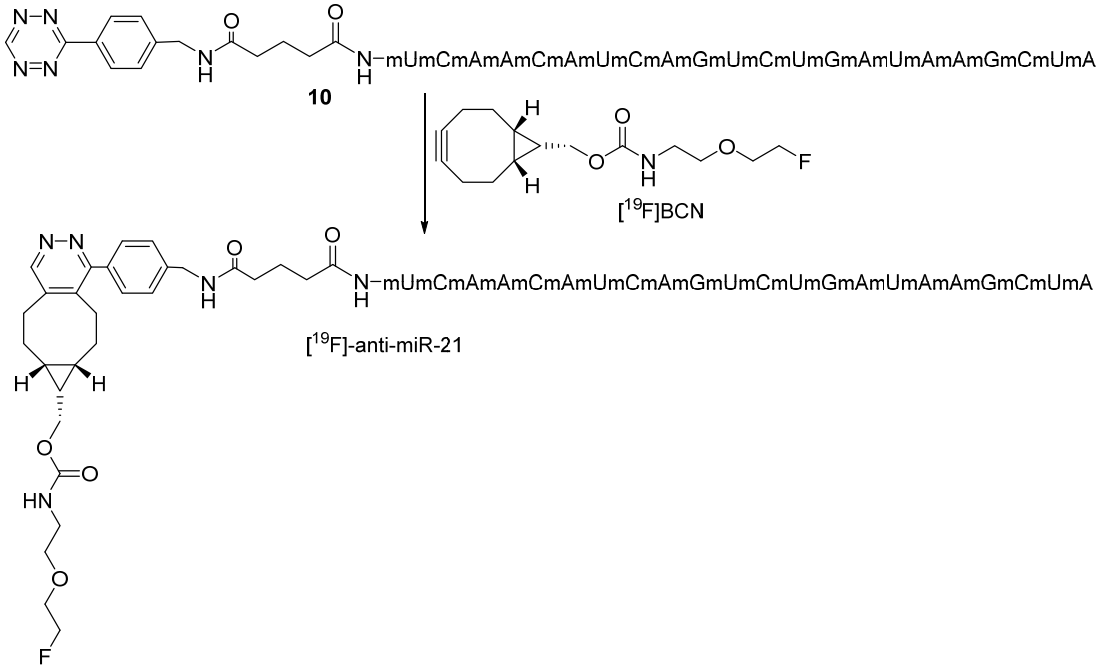
(c).


Blank sample under UV detection
Intensity (AU)
Time (min)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0
5
10
15
20

Supplimentary Figure 2. HRMS analysis of compound **10**.

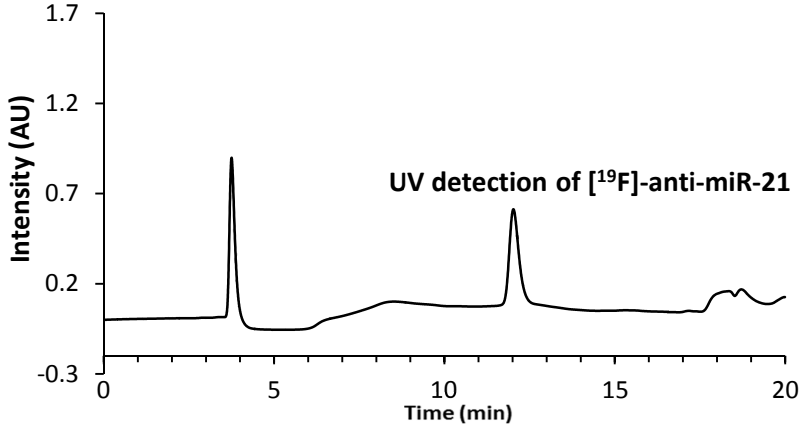


Preparation of [^{19}F]-anti-miR-21

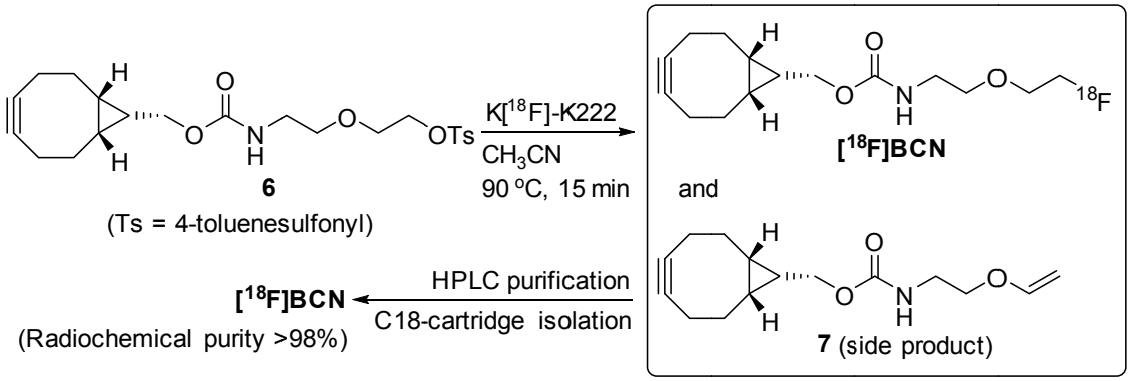


To a solution of compound **10** (6 nmol) in PBS (288 µL, pH 7.4) was added a solution of [^{19}F]BCN (6 nmol) in sterile water (12 µL) at r.t. After 3 min the conjugation reaction completed as indicated by HPLC analyses. The purity of obtained [^{19}F]-anti-miR-21 was > 95%. When necessary, [^{19}F]-anti-miR-21 was purified by HPLC. HRMS (ESI negative mode) for $C_{266}H_{352}FN_{87}O_{158}P_{22}$: The theoretical molecular weight is 7996.5538. The measured average mass is 7996.5154 based on a charge state deconvolution and 7996.7144 based on an isotopic deconvolution of the most intense isotopic series.

Supplementary Figure 3. HPLC analysis of [^{19}F]-anti-miR-21. HPLC conditions: Phenomenex Jupiter Proteo column (250 × 4.6 mm), UV detection at 260 nm, flow rate 1 mL/min, solvent A is TEAA (0.1 M, pH 7.0) and solvent B is CH_3CN, gradient elution from 10% B to 60% of B during 20 min.



Radiosynthesis of [^{18}F]BCN



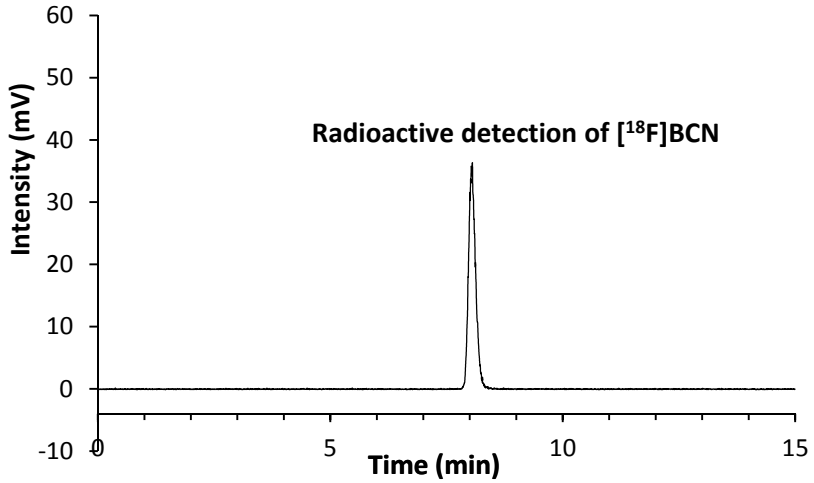
The ^{18}F-fluorination of compound **6** was performed according to the similar protocols as previously published.[1,2] At the end of bombardment (EOB), ^{18}F-fluoride was trapped in an anion exchange cartridge (Sep-Pak QMA Light Plus conditioned with 10 mL 0.5 M K_2CO_3 and 15 mL water, Waters Corporation) and subsequently eluted as K[^{18}F]-K222 complex into a 5-mL reaction vessel. The eluent was acetonitrile (2.0 mL) containing water (80 uL), Kryptofix 2.2.2 (9.5 mg) and K_2CO_3 (1.7 mg). The initial radioactivity at EOB was about 12.6 GBq. K^{18}F-K222 was dried with acetonitrile azeotropically at 118 ºC with nitrogen flow and then cooled down to 55 ºC. The

acetonitrile (2.0 mL) for drying was from the elution of QMA cartridge, and it was not necessary to add additional acetonitrile for drying the fluoride.[1]

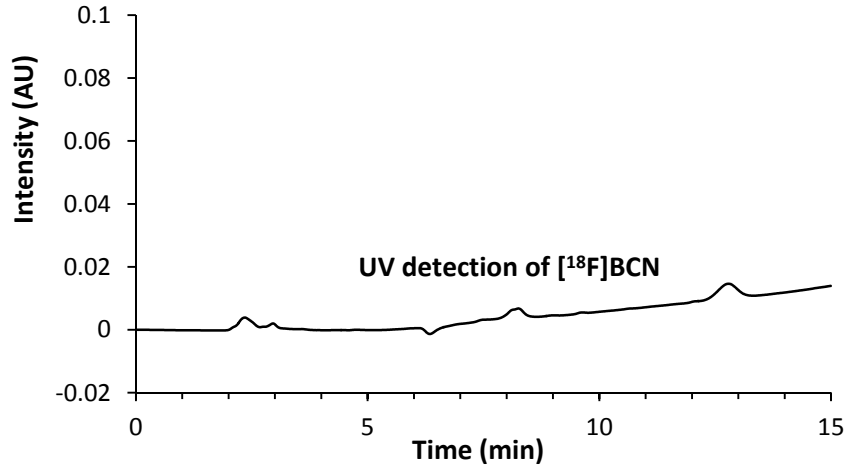
A solution of compound **6** (4.7 mg, 10.8 μmol) in acetonitrile (0.5 mL) was added to the dried $K^{18}F$-K222 complex. The mixture was kept without stirring at 90 ºC for 15 min in a sealed reaction vessel. Subsequently, the reaction mixture was cooled down to 30 ºC and diluted with acetonitrile in water (40% by volume, 0.8 mL). The resulted mixture was injected into a semi-preparative HPLC column (Phenomenex Jupiter Proteo C18, 250 × 10 mm) for purification. [^{18}F]BCN was eluted with acetonitrile in water (gradient 40-50% during 20 min, flow rate 4 mL/min) and collected into a Falcon tube which was pre-loaded with water (25 mL). The solution in the Falcon tube was passed through C18 cartridges (two 360 mg cartridges connected together). The C18 cartridges were washed with clinical grade ethanol (1.1 mL) and the eluent was discarded. [^{18}F]BCN was eluted with clinical grade ethanol (1.0 mL) into a sterile vessel. The total preparation time of [^{18}F]BCN was about 85 min and the decay-corrected radiochemical yield (RCY) starting from end of bombardment (EOB) was 20 ± 3% (n = 6). The radiochemical purity of [^{18}F]BCN was higher than 98%. The obtained [^{18}F]BCN was stable for 6 hours (longer time was not tested) and ready for next step conjugation reactions.

Supplementary Figure 4. Quality control of [^{18}F]BCN. HPLC conditions: Phenomenex Jupiter Proteo C18 column (250 × 4.6 mm), UV detection at 215 nm, flow rate 1 mL/min, solvent A was water and solvent B was CH_3CN, gradient elution from 50% B to 80% of B during 15 min. (a) Radioactive detection of [^{18}F]BCN. (b) The HPLC trace of a [^{18}F]BCN sample under UV detection. (c) [^{19}F]BCN as a reference. (d) Radioactive detection of a [^{18}F]BCN sample spiked with [^{19}F]BCN. (e) UV detection of a [^{18}F]BCN sample spiked with [^{19}F]BCN.

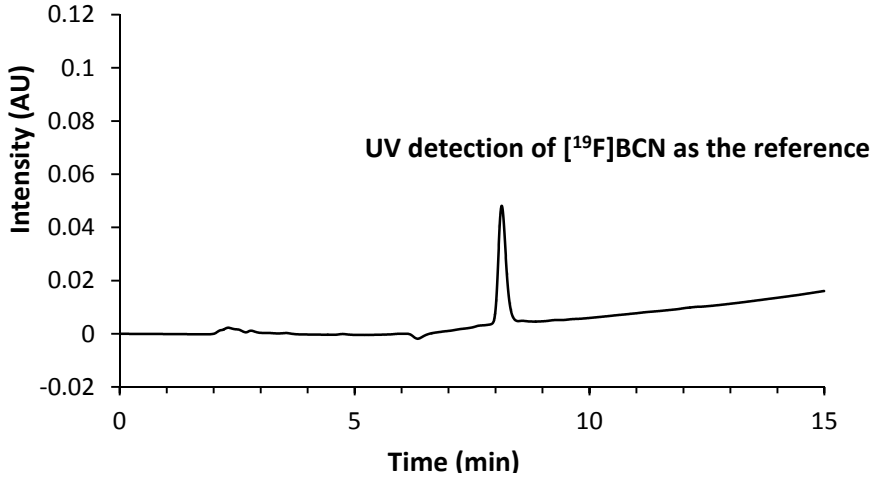
(a).



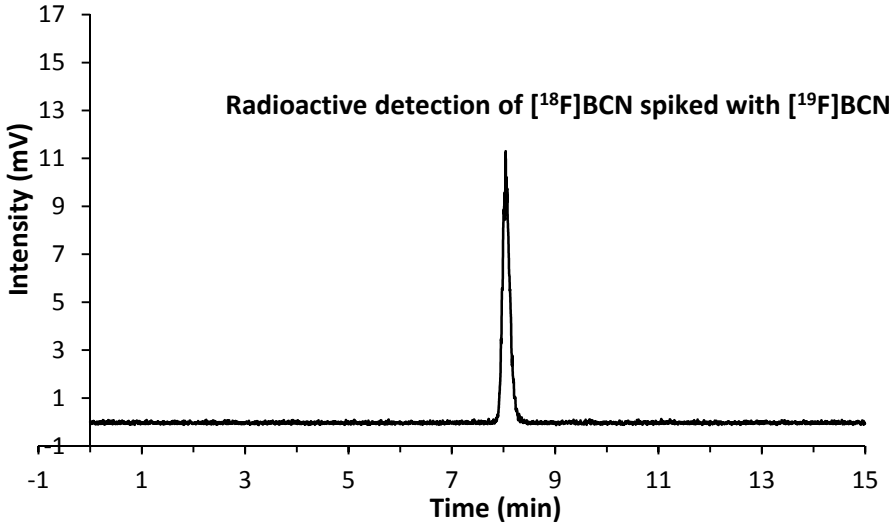
(b).


0.1
0.08
0.06
0.04
0.02
0
-0.02
Intensity (AU)
UV detection of [18F]BCN
0
5
10
15
Time (min)

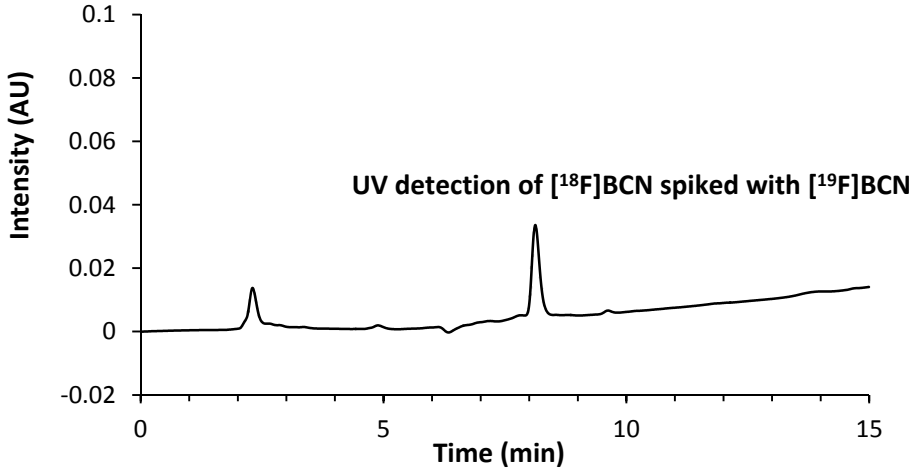
(c).


0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
Intensity (AU)
UV detection of [19F]BCN as the reference
0
5
10
15
Time (min)

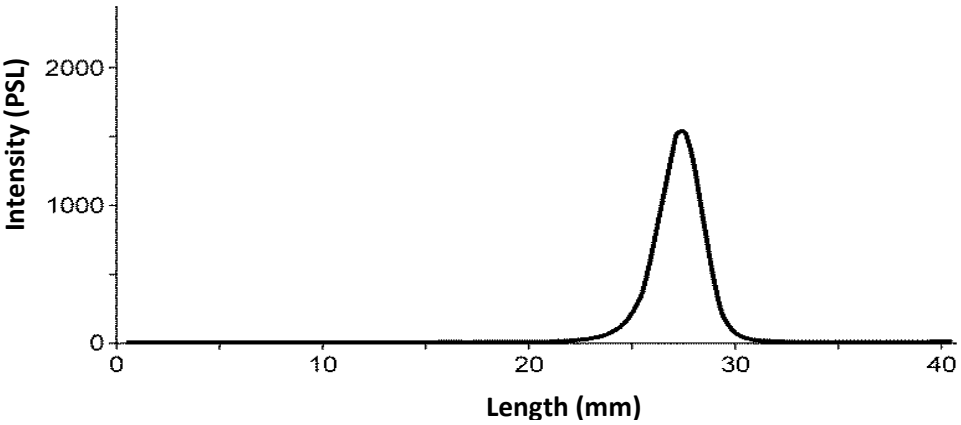
(d).


17
15
13
11
9
7
5
3
1
-1
Intensity (mV)
Radioactive detection of [18F]BCN spiked with [19F]BCN
-1
1
3
5
7
9
11
13
15
Time (min)

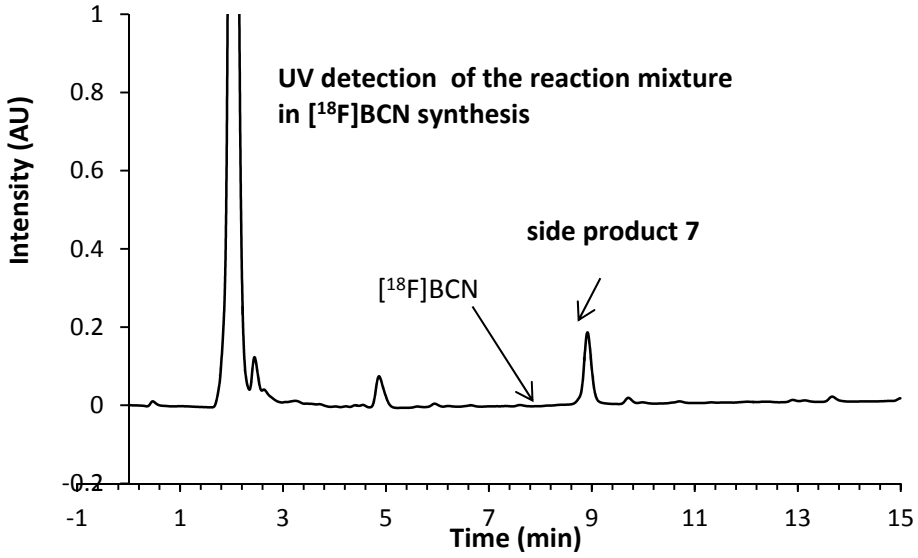
(e).



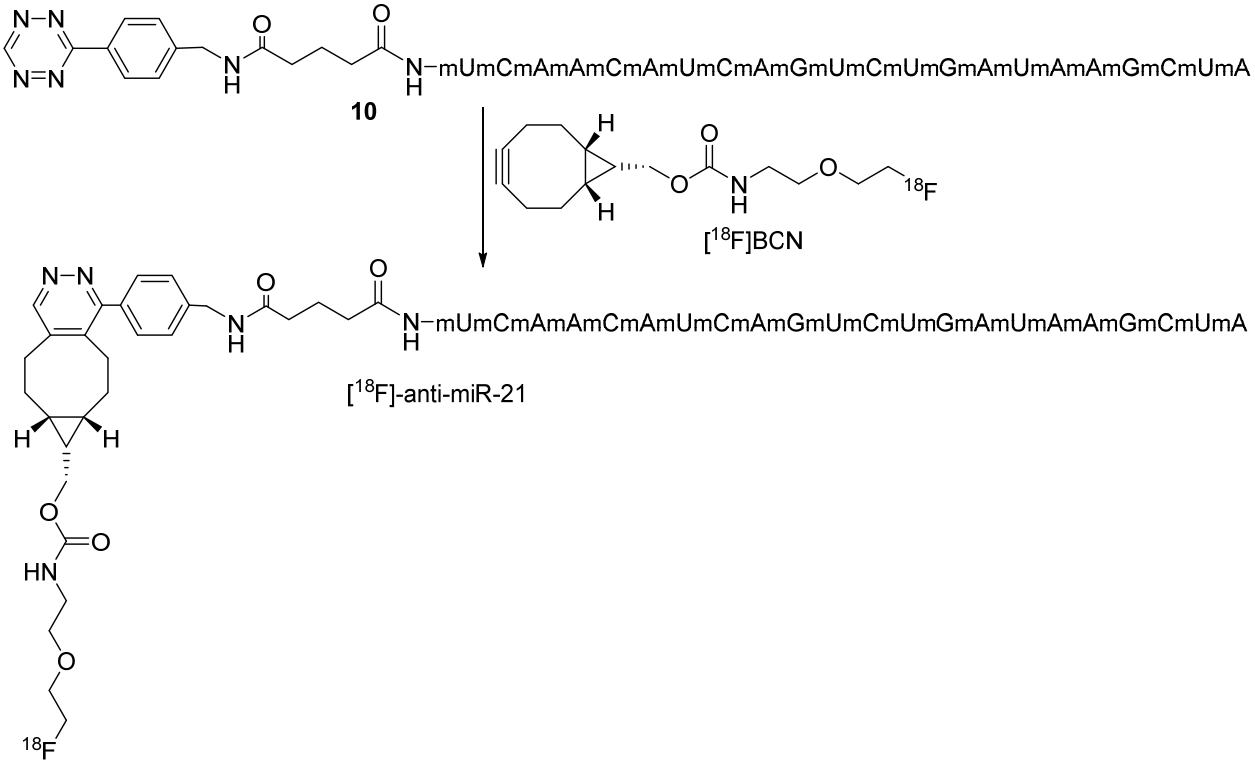
Supplementary Figure 5. Radio-TLC analysis of [^{18}F]BCN. TLC conditions: normal phase silica gel TLC plates, eluent for TLC development was water in acetonitrile (2% by volume).



Supplementary Figure 6. HPLC analysis of reaction mixture in [^{18}F]BCN synthesis. HPLC conditions: Phenomenex Jupiter Proteo C18 column (250 × 4.6 mm), UV detection at 215 nm, flow rate 1 mL/min, solvent A was water and solvent B was CH_3CN, gradient elution from 50% B to 80% of B during 15 min.



Radiosynthesis of [^{18}F]-anti-miR-21

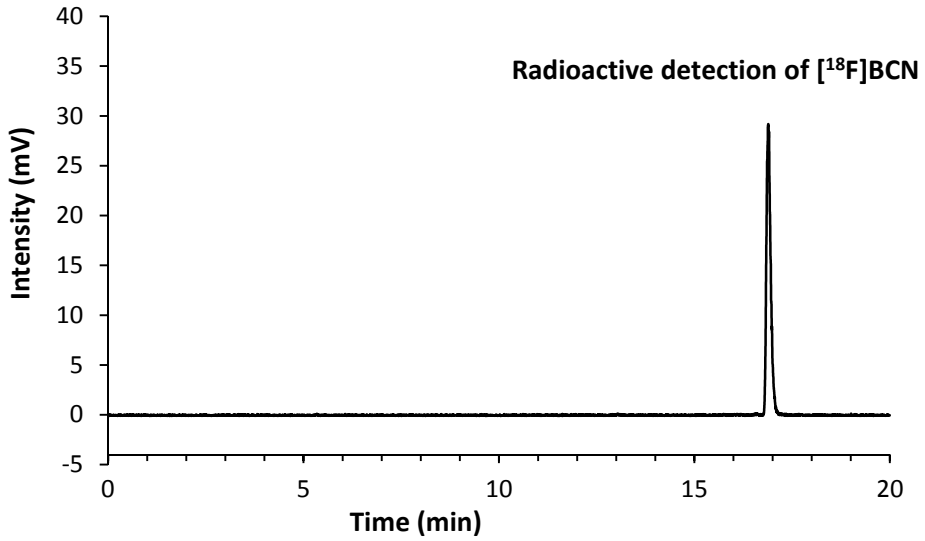


To above obtained [^{18}F]BCN solution (1 mL, 1.2 – 1.4 GBq) was added a solution of compound **10** (10-23 nmol) in PBS (1.3 mL, pH 7.4) containing PPG (175 µL). After 3 min at r.t, the reaction mixture was diluted with PBS (7.5 mL, pH 7.4) containing PPG (7%). [^{18}F]-anti-miR-21 was obtained in quantitative yield in this step. Starting from ^{18}F-fluoride at EOB, [^{18}F]-anti-miR-21was obtained in 90-95 min and the radiochemical purity was >95%. The specific radioactivity was 52–

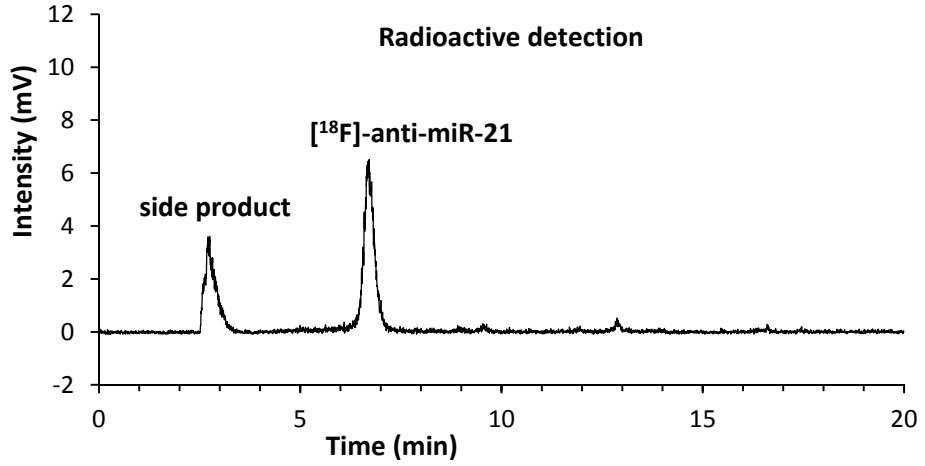
61 GBq/μmol (n = 6). Specific radioactivity was calculated by dividing the amount of radioactivity with the molar amount of **10** added to the reactions. The amount of **10** was measured with a Biospec-nano photometer.

Supplementary Figure 7. HPLC monitoring of the conjugation reaction between [^{18}F]BCN and compound **10**, and quality control of [^{18}F]-anti-miR-21. HPLC conditions: Phenomenex Jupiter Proteo C18 column (250 × 4.6 mm), UV detection at 260 nm and radioactive detection, flow rate 1 mL/min, solvent A was TEAA (0.1 M, pH 7.0) and solvent B was CH_3CN, the gradient was 20-50% B during 0-10 min and 50-95% B during 10-20 min. (a) Radioactive detection of [^{18}F]BCN. (b) [^{18}F]BCN conjugation with compound **10** in the absence of PPG. (c) [^{18}F]BCN conjugation with compound **10** in the presence of PPG (7 % by volume). (d) HPLC trace of [^{18}F]-anti-miR-21 under UV detection. (e) [^{19}F]-anti-miR-21 as the reference.

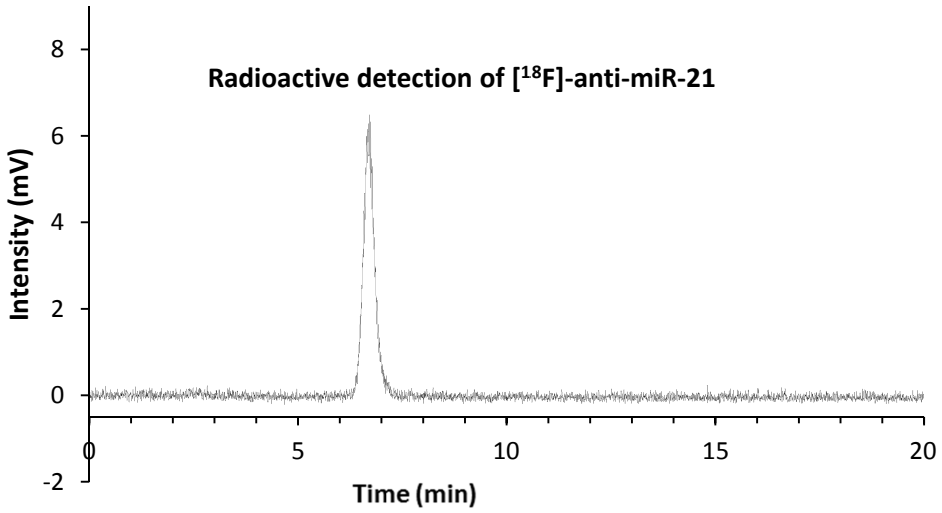
(a).



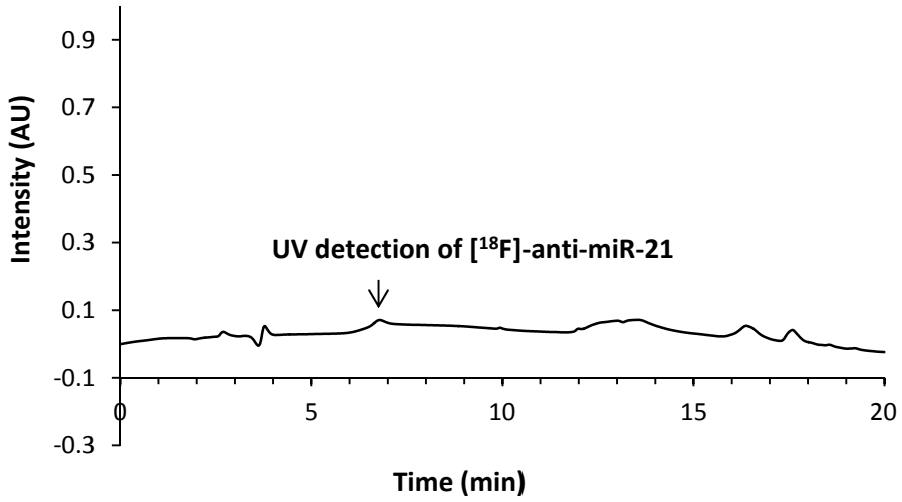
(b).



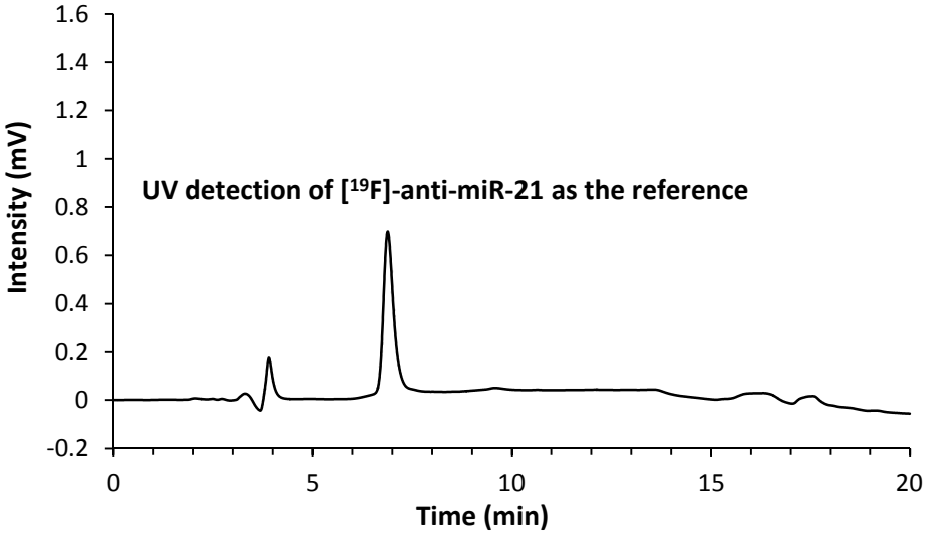
(c).


Radioactive detection of [18F]-anti-miR-21
Intensity (mV)
Time (min)
8
6
4
2
0
-2
0
5
10
15
20

(d).


UV detection of [18F]-anti-miR-21
Intensity (AU)
Time (min)
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
0
5
10
15
20

(e).


UV detection of [19F]-anti-miR-21 as the reference
Intensity (mV)
Time (min)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
5
10
15
20

References:

1. X.-G. Li, K. Helariutta, A. Roivainen, S. Jalkanen, J. Knuuti and A. J. Airaksinen, *Nat. Protoc*., 2014, **9**, 138-145.
2. X.-G. Li, A. Autio, H. Ahtinen, K. Helariutta, H. Liljenbäck, S. Jalkanen, A. Roivainen and A. J. Airaksinen, *Chem. Commun*., 2013, **49**, 3682-3684.