Electronic Supplementary Material (ESI) for MedChemComm.

Electronic supplementary information

Titel: A ^{18}F-labeled Dibenzocyclooctyne(DBCO)-Derivative for Copper-free Click Labeling of Biomolecules

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Content

I. Organic Syntheses of Dibenzocyclooctyne(DBCO)-Derivatives

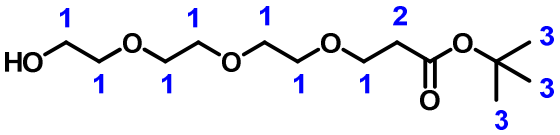
I.1. General

All regents were purchased from Acros Organics, Bachem, Deutero, Fisher-Scientific, Fluka, Jena Bioscience, Lancester, Merck AG, Sigma-Aldrich, Solvay-Organics and VWR and used without further purification. Reactions were monitored using thin layer chromatography (performed in Merck silica gel 60 F254) or high-performance liquid chromatography (HPLC). ^{1}H NMR spectra and ^{19}F NMR spectra were recorded using an AC-300-Spektrometer (300-MHz-T-NMR-spectrometer AC 300, Bruker Analytik GmbH) in $DCCl_3$ or DMSO-d_6. ^{13}C NMR spectra were measured on an Avance II-400-Spectrometer (400 MHz). Chemical shifts for ^{1}H NMR and ^{13}C NMR were referenced to tetramethylsilane (0.00 ppm) and ^{19}F NMR were referenced to trichloro-fluoro-methane (0.00 ppm). FD and ESI mass spectrometry were performed on a MAT 95-MS 7500 CE and a HP 4500 (Agilent Technologies and Hewlett-Packard, respectively, both Santa Clara, CA, USA).

Radiosyntheses were performed manually (starting activities < 4 GBq) using conventional heating. Analytical HPLC analysis and radio-HPLC were performed on a Dionex P680A pump, a Raytest NaI scintillation counter (Gabi) and a Dionex UVD 170U (254 nm) absorbance detector. Dionex Chromeleon software was used for UV-data analysis and Raytest Gina star software for radioactivity detection.

I. 2. Synthesis of DBCO labeling precursor

***Tert*-butyl-3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propanoate (3)**

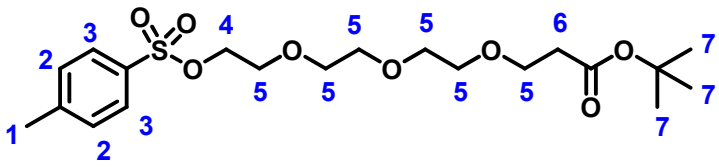


To a solution of triethylene glycol **1** (5.8 g, 38.6 mmol) in dry tetrahydrofuran (60 mL) sodium (12.8 mg, 0.56 mmol) was added and stirred for 30 min at rt. Then *tert*-butyl acrylate (2.28 g, 17.8 mmol) was added and the reaction was stirred for 24 h at rt. 400 µL 1 M hydrochloric acid were added to neutralize the reaction solution. Ethyl acetate (20 mL) was added and the organic layer was extracted 3-times (10 mL) with water and brine. The organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure obtaining **3** as colorless oil (3.65 mg, 13.12 mmol, 74%).

^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.42 (s, 9H, 3-H), 2.48 (2H, t, 6.5 Hz, 2-H), 3.61 – 3.71 (14H, m, 1-H).

MS (ESI positive): m/z 301.13 ($[M+Na]^+$, 73.12%), 317.13 ($[M+K]^+$, 62.19%), calculated for $C_{13}H_{26}O_6$: 278.34.

***Tert*-butyl-3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)propanoate (4)**

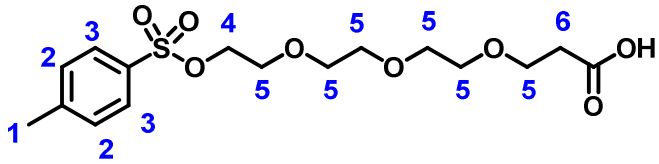


Compound **3** (1.02 g, 3.65 mmol) and triethylamine (0.87 mL, 0.63 mg, 6.24 mmol) were dissolved in dichloromethane (20 mL) and cooled to 0°C. Then toluenesulfonyl chloride (720 mg, 4.2 mmol) was added and the reaction mixture was stirred for 10 minutes at 0°C. The ice bath was removed and the reaction was stirred for an additional hour at rt. The solvent was removed under reduced pressure and the product was purified by column chromatography (n-hexane:ethyl acetate/2:1, R_f = 0.24) obtaining **4** as colorless oil (1.05 g, 2.43 mmol, 67%).

^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.44 (9H, s, 7-H), 2.44 (3H, s, 1-H), 2.49 (2H, t, 6.5 Hz, 6-H), 3.59 – 3.68 (12H, m, 5-H), 4.15 (2H, t, 4.9 Hz, 4-H), 7.32 (2H, d, 8.8 Hz, 2-H), 7.78 (2H, d, 8.2 Hz, 3-H).

MS (ESI positive): m/z 455.20 ($[M+Na]^+$), 471.17 ($[M+K]^+$), calculated for $C_{34}H_{38}N_2O_8S$: 432.53.

3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)propanoate (5)

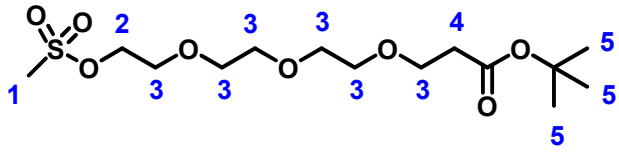


Compound **4** (100 mg, 0.32 mmol) was dissolved in dichloromethane chloride (1.3 mL) and the carboxyl acid function was deprotected with trifluoroacetic acid (1.3 mL, 1.94 g, 17 mmol) for 4 h. The solvent was removed under reduced pressure and the trifluoroacetic acid was removed by codestillation with toluene (4-times, 3 mL each), obtaining **5** as colorless oil (86 mg, 0.22 mmol, 99%).

^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 2.44 (3H, s, 1-H), 2.63 (2H, t, 6.2 Hz, 6-H), 3.59 (8H, m, 5-H), 3.69 (2H, t, 5 Hz, 5-H) 3.76 (2H, t, 6.1 Hz, 5-H), 4.15 (2H, t, 4.7 Hz, 4-H), 7.33 (2H, d, 8.4 Hz, 2-H), 7.78 (2H, d, 8.5 Hz, 3-H).

MS (ESI positive): m/z 399.13 ($[M+Na]^+$), 415.11 ($[M+K]^+$), calculated for $C_{16}H_{24}O_8S$: 376.12.

***Tert*-butyl-3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)propanoate (6)**

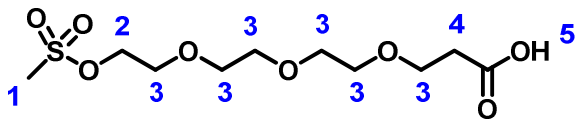


Compound **3** (500 mg, 1.8 mmol) and triethylamine (0.42mL, 0.31 mg, 3.1 mmol) were dissolved in dichloromethane (10 mL) and cooled to 0°C. Then methanesulfonyl chloride (266 mg, 1.97 mmol) was added and the reaction mixture was stirred for 10 minutes at 0°C. The ice bath was removed and the reaction was stirred for an additional hour at rt. The solvent was removed under reduced pressure and the product was purified by column chromatography (n-hexane:ethyl acetate/1:1, R_f = 0.2) obtaining **6** as colorless oil (567 mg, 1.6 mmol, 88%).

^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.44 (9H, s, 5-H), 2.49 (2H, t, 6.5 Hz, 4-H), 3.07 (3H, s, 1-H), 3.56 – 3.77 (12H, m, 3-H), 4.37 (2H, t, 4.6 Hz, 2-H).

MS (ESI positive): m/z 379.12 ([M+Na], 100%), 395.11 ([M+K], 78.67%), calculated for $C_{10}H_{20}O_8S$: 356.16.

3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)propanoate (7)

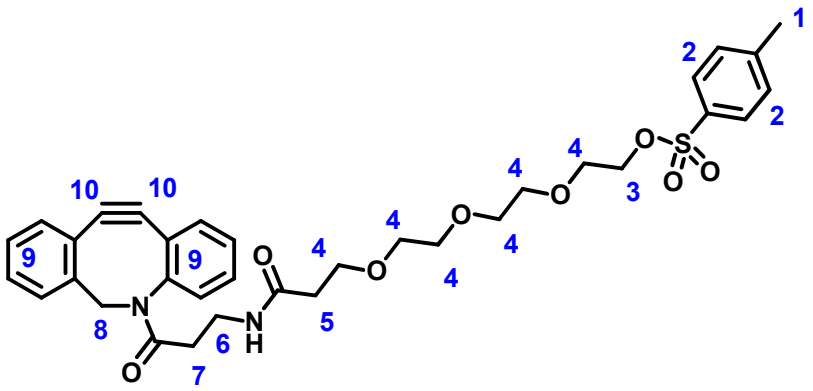


Compound **6** (100 mg, 0.27 mmol) was dissolved in dichloromethane chloride (1.3 mL) and the carboxic acid function was deprotected with trifluoroacetic acid (1.3 mL, 1.94 g, 17 mmol) for 4 h. The solvent was removed under reduced pressure and the trifluoroacetic acid was removed by codestillation with toluene (4-times, 3 mL each), obtaining **7** as colorless oil (80 mg, 0.31 mmol, 98%).

^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 2.60 (2H, t, 6.2 Hz, 4-H), 3.04 (3H, s, 1-H), 3.60 – 3.73 (12H, m, 3-H), 4.34 (2H, t, 4.5 Hz, 2-H), 9.15 (1H, s, 5-H).

MS (ESI positive): m/z 301.15($[M]^+$, 40.30%, 323.06 ($[M+Na]^+$, 100%), 339.06 ($[M+K]^+$, 63.77%) calculated for $C_{10}H_{20}O_8S$: 300.09.

3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (9)



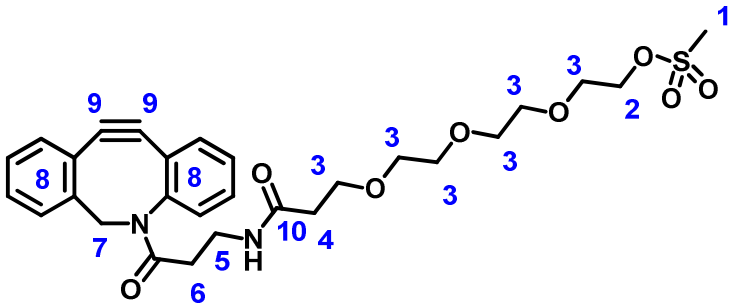
A solution of compound **5** (28 mg, 0.072 mmol), *N,N,N´,N´´*-Tetramethyl-*O*-(1-*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) (41 mg, 0.11 mmol) and *N,N*-Diisopropylethylamine (100 µL, 0,58 mmol) in dry dimethylformamide (1 mL) was stirred for 30 min at rt. Then Dibenzocyclooctyne-amine (DBCO-amine) (20 mg, 0.072 mmol) was dissolved in dry dimethylformamide (1 mL) and added to the reaction solution. After stirring the reaction mixture at rt for 12 h, the solvent was removed under reduced pressure and the product was purified by column chromatography (dichloromethane:methanol/30:1, R_f = 0.22) obtaining **9** as yellow oil (25 mg, 0.04 mmol, 56%).

^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.98 (1H, m, 7-H) und 2.58 (1H, m, 7-H), 2.38 (2H, d, 6 Hz, 5-H), 2.45 (3H, s, 1-H), 3.26 – 3.39 (2H, m, 6-H), 3.49 – 3.75 (13 H, m, 4-H und 8-H), 4.15 (2H, t, 4.7 Hz, 3-H), 5.15 (1H, dd, 8 Hz & 14.5 Hz, 8-H), 7.25 – 7.44 (9H, m, 9-H und 2-H), 7.66 (1H, d, 7.7 Hz, 9-H), 7.78 (2H, d, 9.0 Hz, 2-H).

^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] =21.69 (1-C), 34.34 (7-C), 35.39 (6-C), 36.32 (5-C), 55.45 (8-C), 66.69 – 70.68 (4-C), 107.83 (10-*C*), 114.62 – 150.85 (9-C).

MS (ESI positive): m/z 635.22 ($[M]^+$, 100%), 657.21 ($[M+Na]^+$, 25.92%), 673.19 ($[M+K]^+$, 3.99%), m/z (high resolution) 657.2259 ($[M+Na]^+$, 100%), calculated for $C_{34}H_{38}N_2O_8S$: 634.2349.

3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (10)



A solution of compound **7** (22 mg, 0.072 mmol), *N,N,N´,N´´*-Tetramethyl-*O*-(1-*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) (41 mg, 0.11 mmol) and *N,N*-Diisopropylethylamine (100 µL, 0,58 mmol) in dry dimethylformamide (1 mL) was stirred for 30 min at RT. Then Dibenzocyclooctyne-amine (DBCO-amine) (20 mg, 0.072 mmol) was dissolved in dry dimethylformamide (1 mL) and added to the reaction solution. After stirring the reaction mixture at RT for 12 h, the solvent was removed under reduced pressure and the product was purified by column chromatography (dichloromethane:methanol/20:1, R_f = 0.27) obtaining **10** as yellow oil (30 mg, 0.05 mmol, 87%).

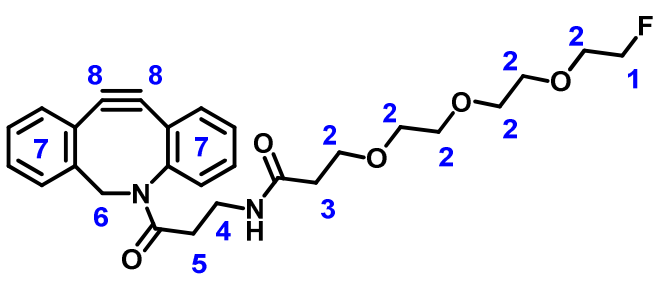
^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.97 – 2.02 (1H, m, 6-H) und 2.52. – 2.54 (1H, m, 6-H), 2.37 (2H, dd, 5.6 HZ & 6.6 Hz, 4-H), 3.07 (3H, s, 1-H), 3.3 (2H, m, 5-H), 3.52 – 3.64 (10 H, m, 3-H), 3.75 (3 H, m, 3-H und 7-H), 4.36 (2H, t, 4.6 Hz, 2-H), 5.13 – 5.16 (1H, d, 13.7 Hz, 7-H), 7.32 – 7.42 (7H, m, 8-H), 7.67 – 7.69 (1H, d, 7.5 Hz, 8-H).

^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 34.54 (6-C), 35.41 (5-C), 36.56 (4-C), 37.67 (1-C), 55.55 (7-C), 66.92 – 70.62 (3-C), 107.77 (9-C), 114.68 – 150.97 (8-C), 171.92 (10-C).

MS (ESI positive): m/z 581.15 ($[M+Na]^+$, 100%), 597.17 ($[M+K]^+$, 11.74%); calculated for $C_{28}H_{34}N_2O_8S$: 558.2036.

I. 2. Synthesis of DBCO reference compound

3-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (11)



A solution of compound **9** (29.5 mg, 0,047 mmol) and tetrabutylammonium fluoride (93.5 µL, 0,093 mmol) in dry tetrahydrofuran (15 mL) was heated to reflux for 2 h. The solvent was removed under reduced pressure. The product was purified by column chromatography (dichloromethane:methanol/30:2, R_f = 0.38) obtaining **11** as a colorless oil (18.4 mg, 0.038 mmol, 82%)

^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.95 – 2.02 (1H, m, 5-H), 2.35 (2H, dd, 5.6 Hz und 6.7 Hz, 3-H), 2.50 (1H, dd, 2.8 Hz und 4.7 Hz, 1-H), 3.24 – 3.40 (2H, m, 4-H), 3.61 – 3.72 (12H, m, 2-H), 3.77 (1H, t, 4.2 Hz, 6-H), 4.50 (1H, t, 4.1 Hz, 1-H), 4.62 (1H, t, 4.4 Hz, 1-H), 5.11 (1H, d, 14.4 Hz, 6-H), 7.29 – 7.44 (7 H, m, 7-H), 7.68 (1H, d, 7.2 Hz, 7-H)

^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 34.76 (5-C), 35.17 (4-C), 36.86 (3-C), 55.49 (6-C), 67.11 – 70.82 (2-C), 107.56 (8-C), 125.70 – 132.37 (7-C)

^{19}F-NMR (400 MHz, $CDCl_3$, CCl_3F): δ [ppm] = -77.20

MS (ESI positive): m/z 483.24 ($[M]^+$, 100%), 505.22 ($[M + Na]^+$, 40.82%), 521.20 ($[M+K]^+$, 4.59%); m/z (high resolution) 483.2306 ($[M]^+$, 100%); 505.2107 ($[M+Na]^+$, 100%); calculated for $C_{27}H_{31}FN_2O_5$: 482.2217

II. ^{18}F-labeling of DBCO precursor

II.1. General radiolabeling methods

N.c.a. [^{18}F]fluoride ion was produced using the ^{18}O(p,n)^{18}F nuclear reaction. The aqueous ^{18}F-solution was then trapped on an anion exchange resin (Sep Pak light Waters Accell Plus QMA cartridge), which was pre-conditioned with 1 M potassium carbonate solution (10 mL) and rinsed with millipore water (10 mL). For the elution of the QMA either tetrabutylammonium hydroxide solution or tetraethylammonium bicarbonate solution was used. The reaction kinetic was screened via radio-TLC by taking aliquots after 1, 3, 5, 10, 15, 30 min after addition of the activity to the precursor. The labeling reaction was optimized due to the amount of precursor, use of different bases and base concentrations and reaction time.

II.1.1.Tetrabutylammonium hydroxide solution (TBA-OH)

Elution of the ^{18}F-fluoride ion from the QMA cartridge was performed using 900 µl of a methanolic tetrabutylammoniumhydroxide solution (TBA-OH x 30 H_2O in 2 mL methanol). The azeotropic drying was performed using a helium stream and heating to 85°C for 20 min under reduced pressure (250 mbar). Within this time, dry acetonitrile (3 x 1 mL) was added and evaporated to yield final dry [^{18}F]fluoride-base mixture.

II.1.2. Tetraethylammonium bicarbonate solution ($Et_4N{\cdot}HCO_3$)

Elution of the ^{18}F-fluoride ion from the QMA cartridge was performed using a solution (450 µL or more), containing tetraethylammonium bicarbonate (3.4 mg, 17 µmol) dissolved in acetonitrile (405 µL) and water (45 µL). The azeotropic drying was performed using a helium stream and heating to 85°C for 20 min under reduced pressure (250 mbar). Within this time dry acetonitrile (4 x 1 mL) was added and evaporated to yield final dry [^{18}F]fluoride-base mixture.

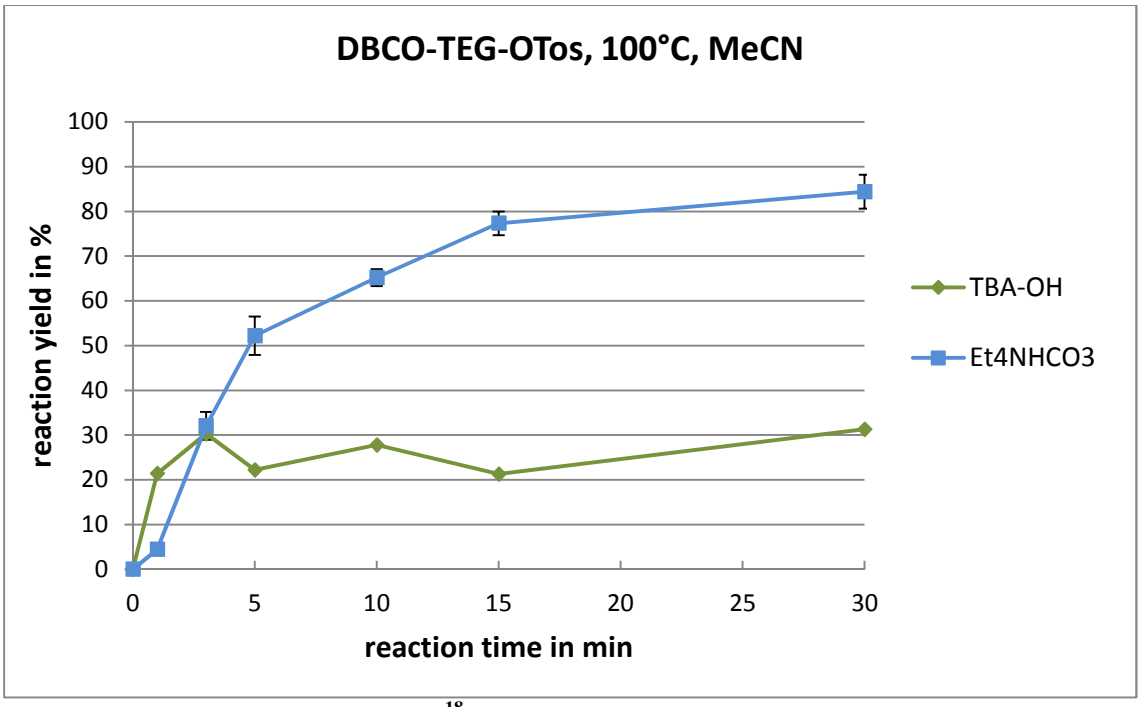
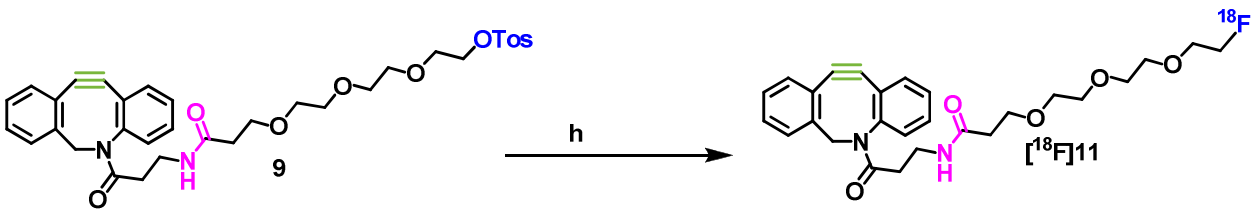


Figure 1: Radiolabeling kinetics of **[^{18}F]11** at 100°C in dependence on the use of two different bases (Tetrabutylammonium hydroxide and Tetraethylammonium bicarbonate).

II.2. Synthesis of [^{18}F]11

After azeotropic drying of the [^{18}F]fluoride ion, the [^{18}F]fluoride-base mixture was dissolved in acetonitrile (0.5 mL) and transferred into a 5-mL sealed reaction vial containing the DBCO-precursor **9** dissolved in acetonitrile (0.5 mL). The reaction mixture was heated to 100°C for 15 min (30 min for determining the kinetics) followed by quenching the labeling reaction with water (1 mL) and injection into a semi-preperative HPLC system at a flow of 2.5 mL/min, whereas **A** is water and **B** is acetonitrile. The following method was used: 50% **A** (isocratic).After purification using semi-preparativ HPLC (figure 2), the fraction was diluted with water (10 mL) and fixed on a Sep-Pak light SPE cartridge (Sep Pak Light tC18). The fixed ^{18}F-prosthetic group was eluted with acetonitrile (1 mL) into a reaction vessel and the acetonitrile was removed using helium steam at 85°C under reduced pressure (500 mbar) within 15 min. The dried prosthetic group was solved in the preferred reaction solvent to perform the click reaction. The ^{18}F-prosthetic group was prepared in an overall reaction time of 60 min in one step with an overall yield (n.d.c.) of (34±5)%.



Scheme 1: Radiolabeling of prosthetic group [^{18}F]**11**. Regents and conditions: h.) $Et_4N{\cdot}HCO_3/^{18}F^-$, acetonitrile, 100°C, 15 min.

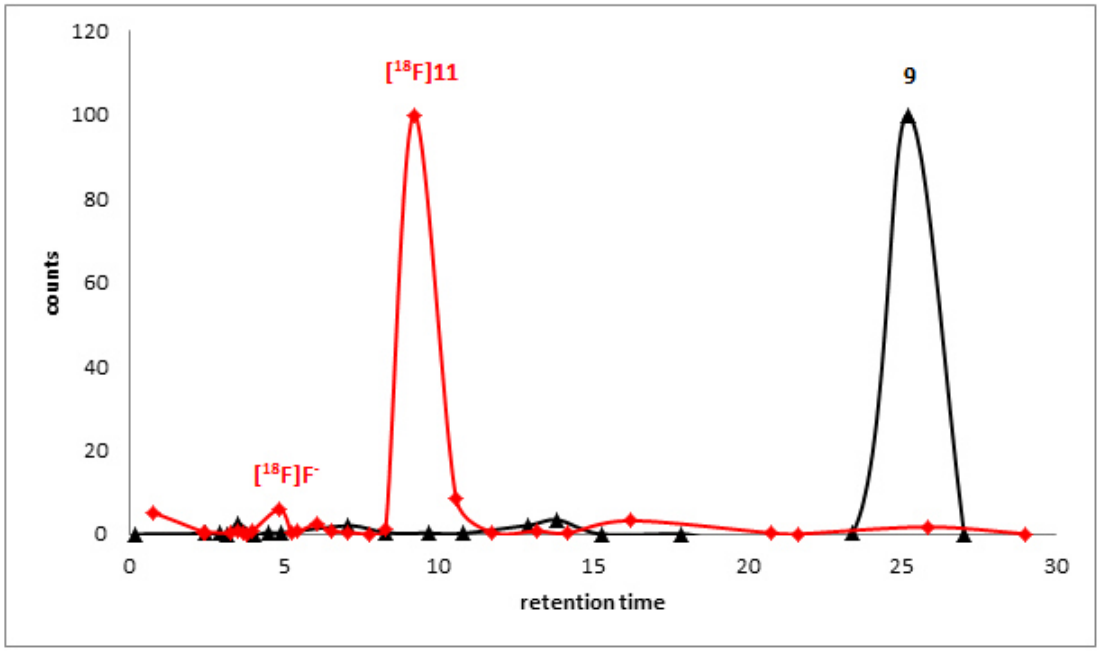


Figure 2: Analytical radio-HPLC chromatogram of the crude reaction mixture of the radiosynthesis of **[^{18}F]11**. Peak intensities are normalized to the maximum intensity. Analytical (radio-)HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x10 mm) with the following conditions: Flow of 0.7 mL min-1, with an isocratic eluent of water/acetonitrile (1:1) for 30 min. Red is the radioactive trace and black is the UV trace.

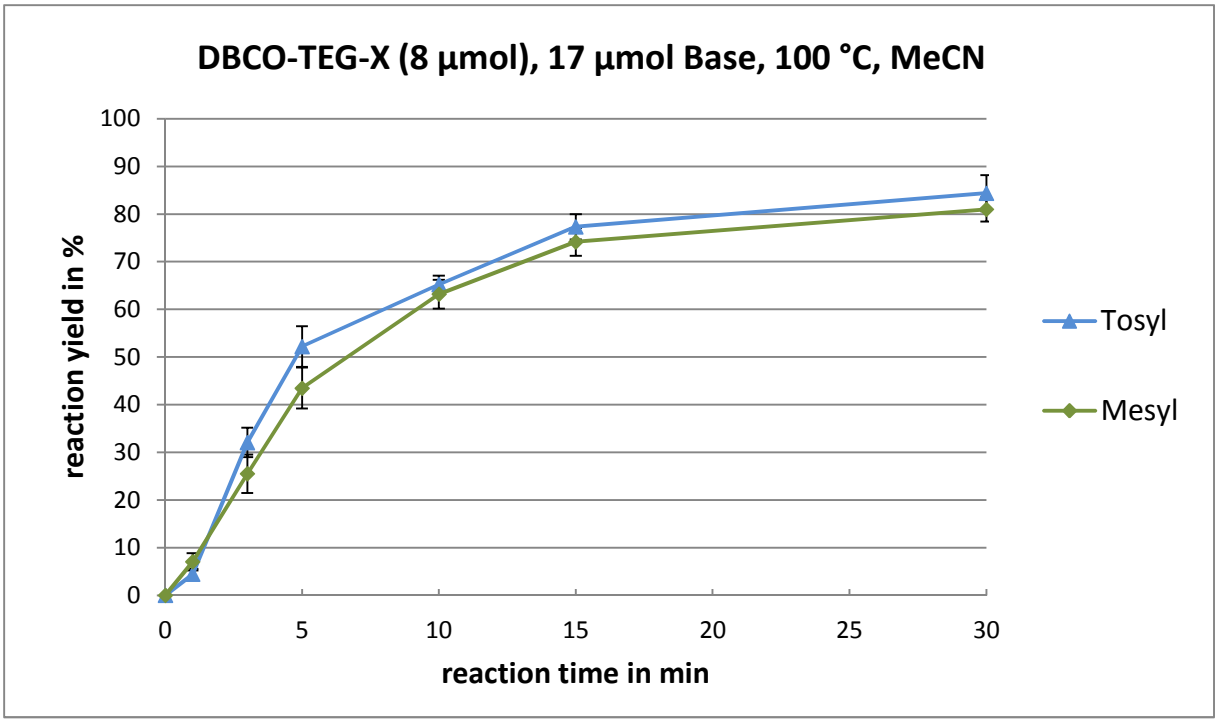


Figure 3: Radiolabeling kinetics of **[^{18}F]11** at 100°C in dependence on different leaving groups (tosyl and mesyl).

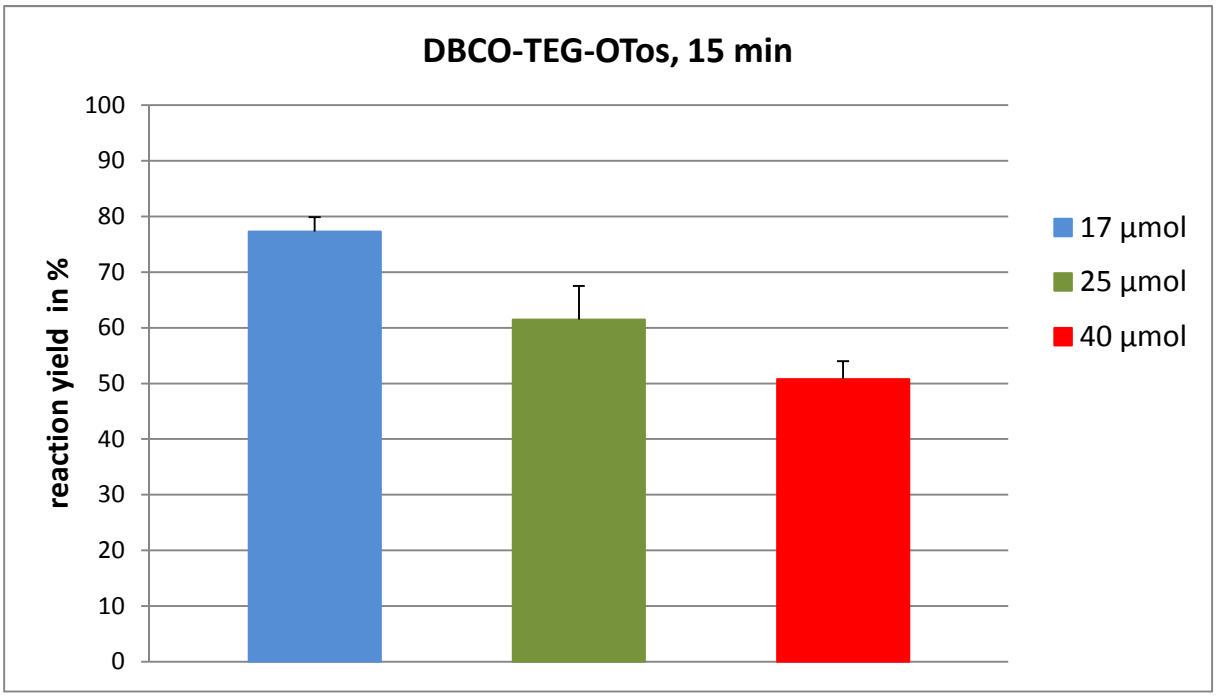


Figure 4: Radiolabeling kinetics of **[^{18}F]11** at 100°C in dependence on the amount of tetraethylammonium bicarbonate.

Figure 5: Radiolabeling kinetics of **[^{18}F]11** at 100°C in dependence on the amount of precursor **9**.

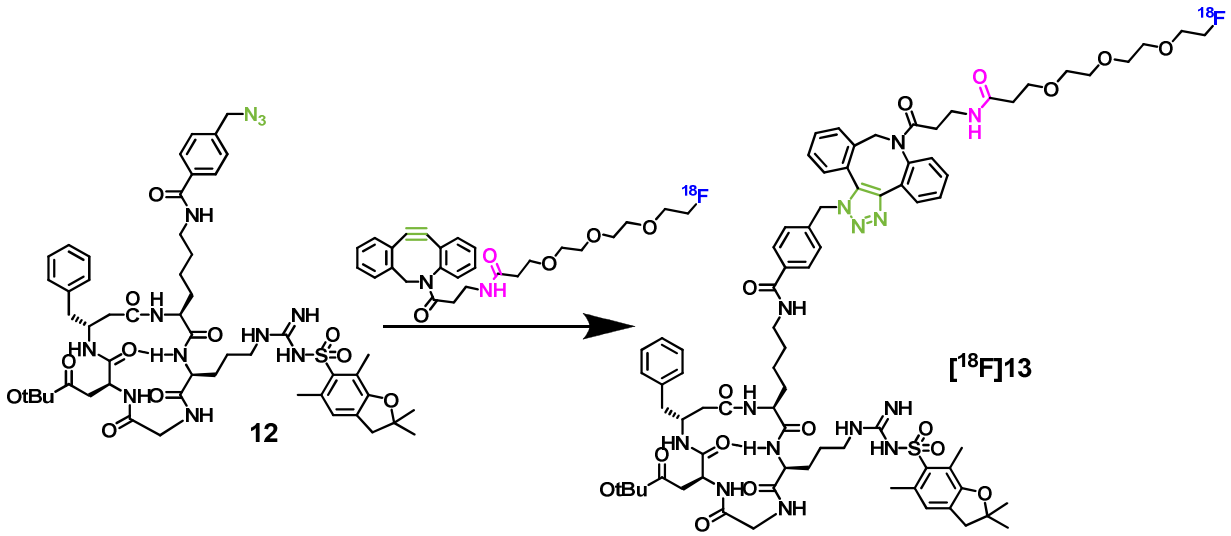
II.3. Octanol-water partition coefficient (logD octanol/water)

To determine the lipophilicity of the radiolabeled ^{18}F-prostehtic group, approximately 0.55 mCi of the radiolabeled prosthetic group were diluted in 0.7 mL phosphate-buffered saline (PBS). An equal volume of 1-octanole was added to obtain a binary phase system. After stirring the samples at 1.500 1/min for 2 min, the two layers were separated by centrifuge (12.000 U/min for 2 min). 300-µL samples were taken from each layer and radioactivity was measured using a curimeter. Besides activity was also determined using a TLC plate.

III. Labeling of Biomolecules

III.1. Copper-free cycloaddition of [^{18}F]11 and azido-functionalized cRDG as model system

The prosthetic group **[^{18}F]11** was dissolved in PBS buffer (200 µL) and acetonitrile (100 µL) and cRDG **12** (1 mg, 1.1 µmol) in 50 µL acetonitrile/water (1:1) was added. Independent from temperature (25°C and 40°C) RCY of ≥ 80% were observed after 5 min. Decreased precursor amount leaded to slower kinetics and an overall lower RCY of only 75%.



Scheme 2: SPAAC of protected azido-functionalized cRDG **12** and the new prosthetic group **[^{18}F]11**. Click reaction conditions: PBS buffer/acetonitrile (1:1), 25 °C or 40 °C, 30 min.

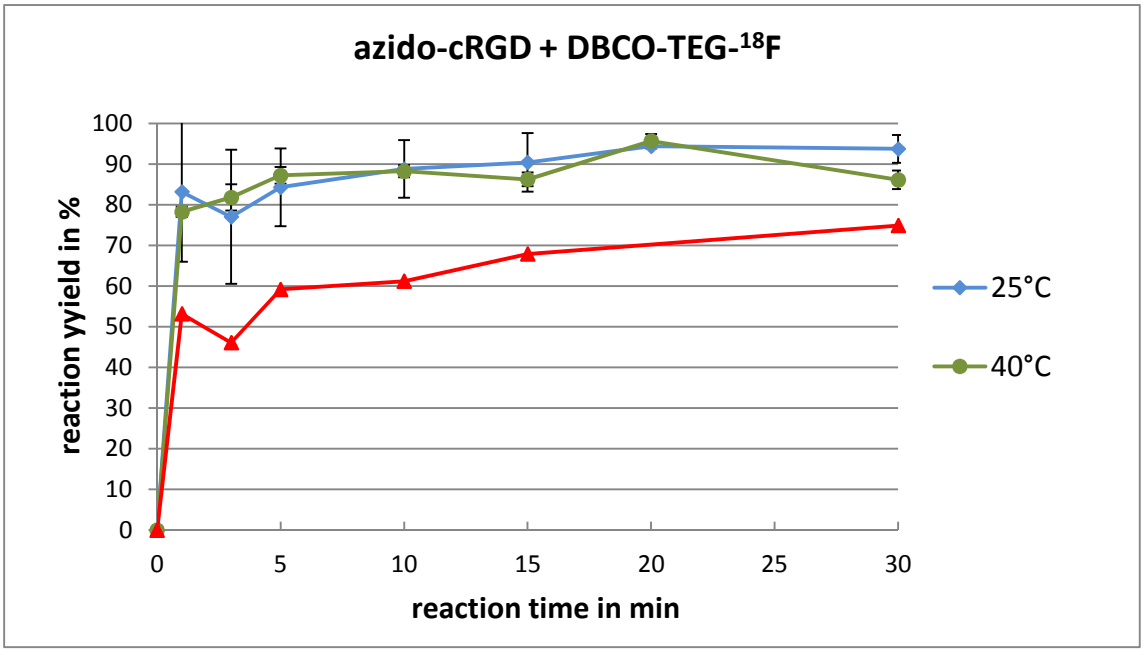


Figure 6: Radiolabeling kinetics of cRGD **12** with **[^{18}F]11** in dependence on temperature and precursor amount.

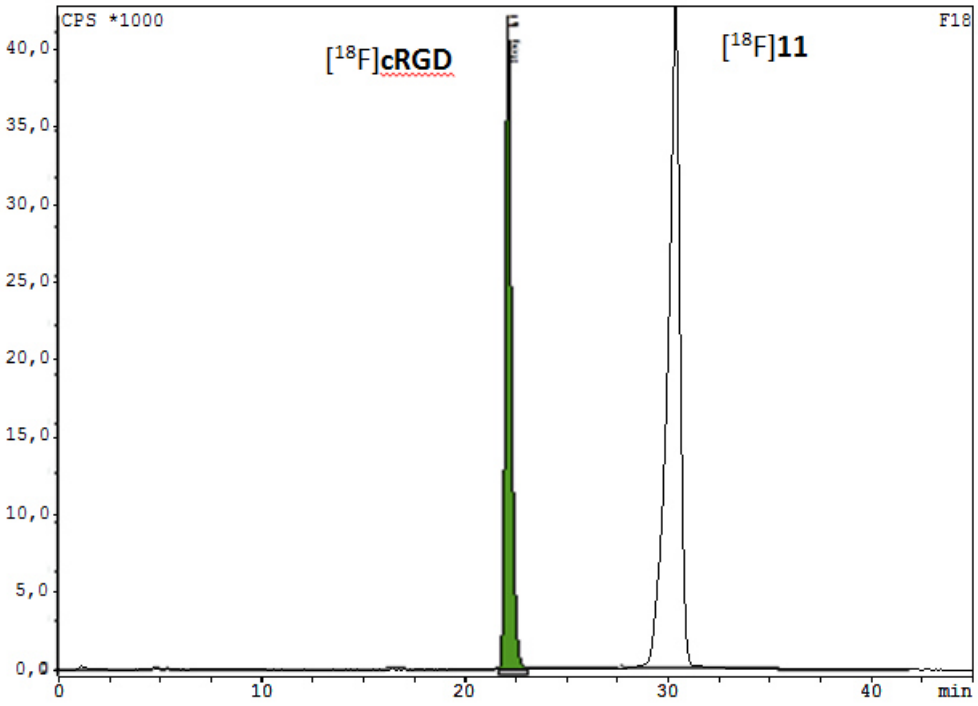
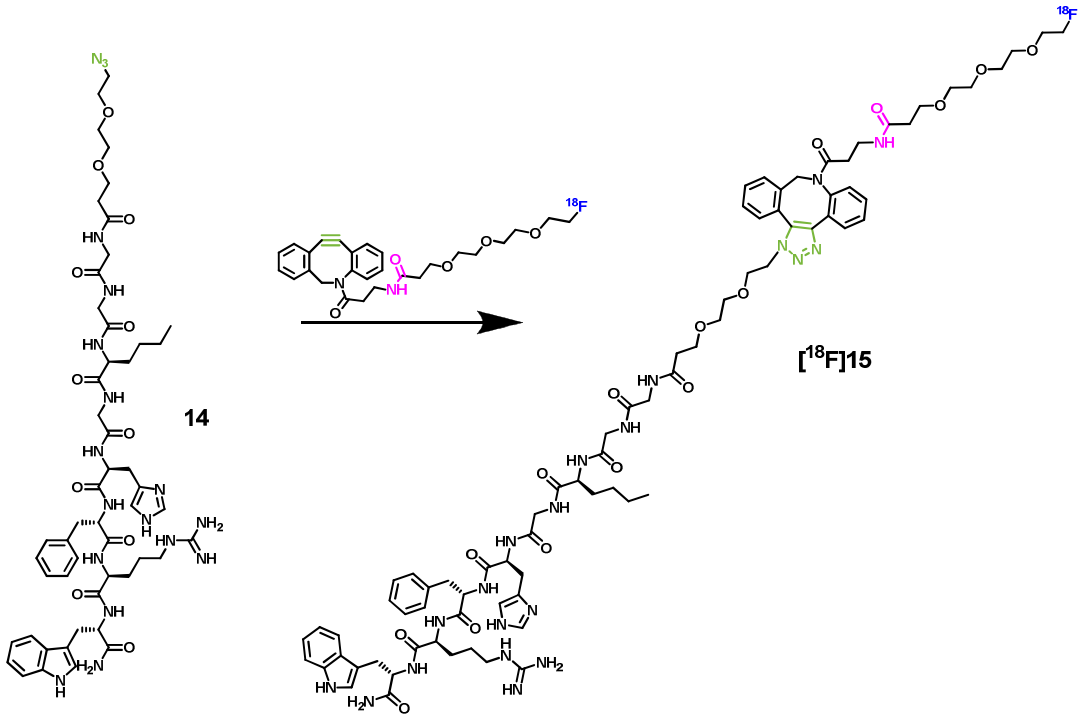


Figure 7: Analytical radio-HPLC chromatogram of the click radiosynthesis of **[^{18}F]RGD**. Analytical radio-HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x20 mm) using the following conditions: Flow 0.7 ml min-1, with eluent A was water with 0.1% TFA (trifluoroacetic acid) and eluent B was acetonitrile with 0.1% TFA. The following method was used: 0 - 40 min, 5 – 95% eluent B (gradient).

III.2. Copper-free cycloaddition of [^{18}F]11 and azido-functionalized α-MSH analogue Peptide

The prosthetic group **[^{18}F]11** was dissolved in PBS buffer (200 µL) and α-MSH Peptide **14** (0.5 mg, 0.4 µmol and 0.125 mg, 0.1 µmol) in 200 µL PBS was added. RCYs varied between 44% and 79% depending a lot on precursor amount (0.1 µmol and 0.4 µmol) and temperature (25°C and 40°C).



Scheme 3: SPAAC of α-MSH Peptide **14** and prosthetic group [^{18}F]**11**. Click reaction conditions: PBS buffer 25°C and 40°C, 30 min.

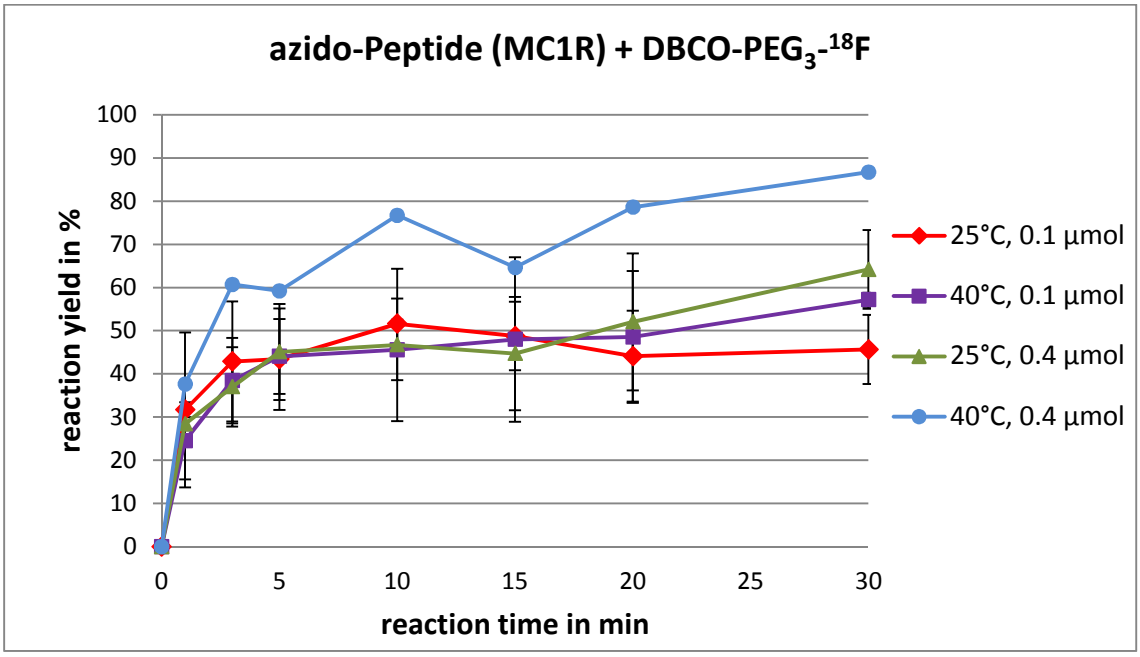
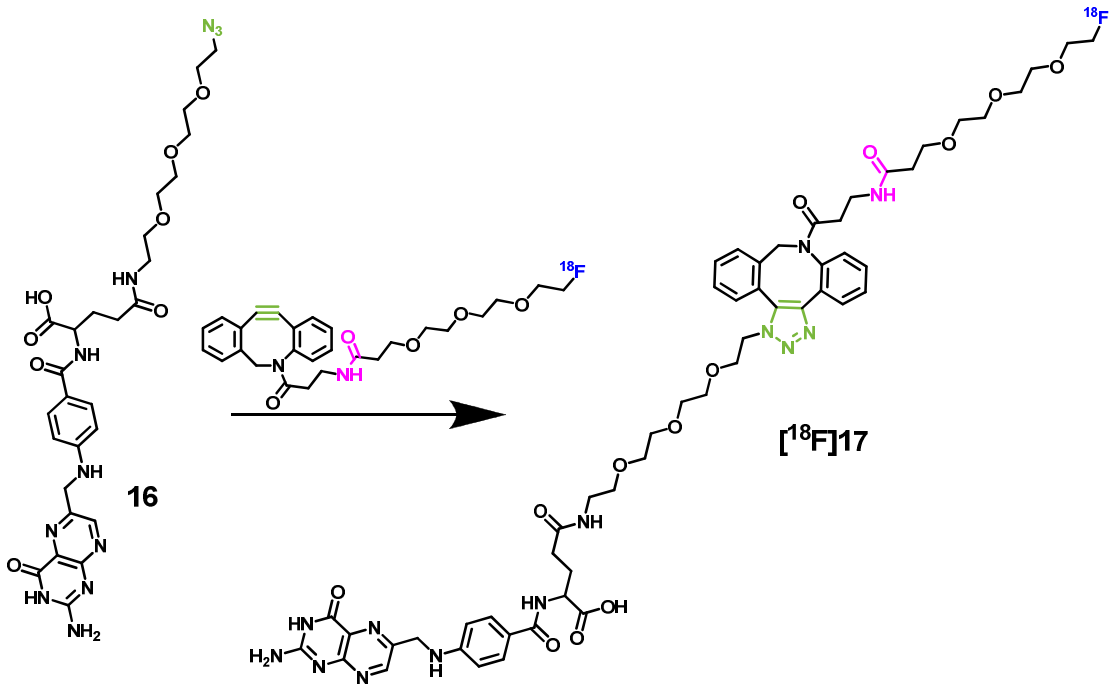


Figure 8: Radiolabeling kinetics of α-MSH Peptide **14** with **[^{18}F]11** in dependence on temperature and precursor amount.

III.3. Copper-free cycloaddition of [^{18}F]11 and azido-functionalized folate-derivative

The prosthetic group **[^{18}F]11** was dissolved in PBS buffer (200 µL) and azide-funktionalized folate-derivative **16** (0.5 mg, 1 µmol) in 200 µL PBS was added. Quantitative labeling was observed after 3 min for 25°C and 40°C.



Scheme 4: SPAAC of azide-functionalized folate-derivative **16** and prosthetic group [^{18}F]**11**. Click reaction conditions: PBS buffer 25°C and 40°C, 30 min.

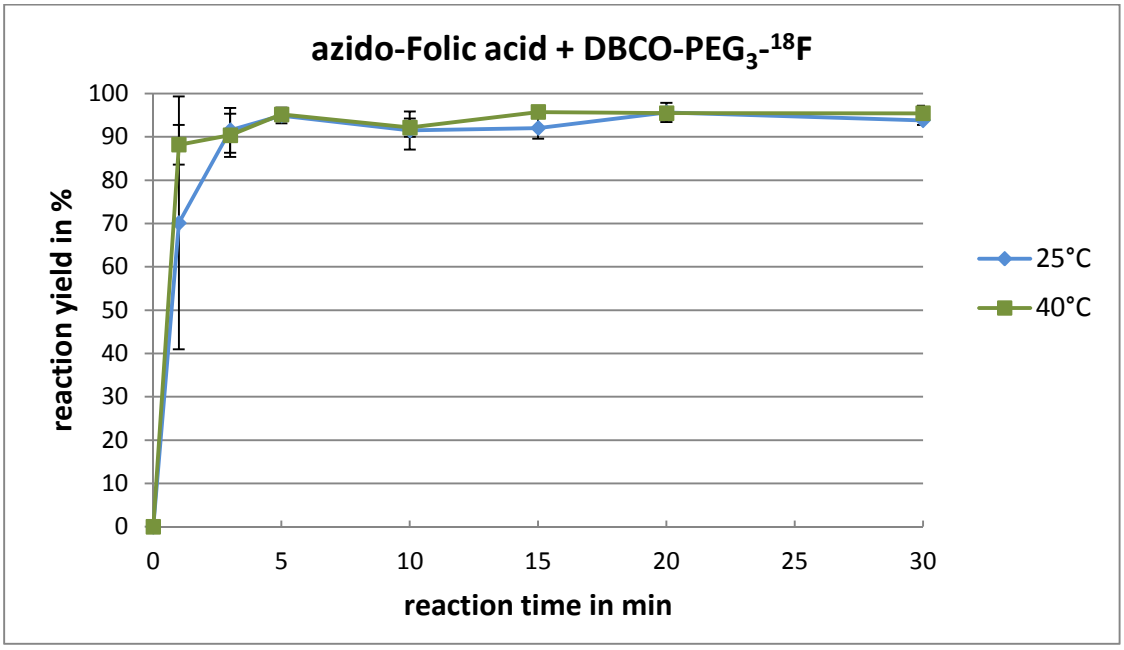


Figure 9: Radiolabeling kinetics of azido-folic acid **16** with **[^{18}F]11** in dependence on temperature.

Table 1: Screening of copper-free cycloaddition reactions with various biomolecule-azides. [a] Conversion was determined by analytical radio TLC. Errors are given as standard deviation representing n = 3.

Compound	**M_W [g/mol]**	**Solvent**	**Amount**	**Temp.**	**Click time**	**RCY[a] [%]**
cRGD	997.4	H_2O:MeCN (1:1)	1 µmol	25°C	20 min	94.4 ± 0.6
				40°C		95.7 ± 1.7
			0.1 µmol	40°C		74,.2
Folate-azide	641.3	PBS	0.5 µmol	25°C		95.7 ± 2.2
				40°C		95.5 ± 1.3
MC1R-Peptide (linear)	1112.6		0.1 µmol	25°C		44.1 ± 10.5
			0.4 µmol	40°C		48.5 ± 15.3
				25°C		52 ± 15.9
				40°C		78.6 ± 2.7
MC1R-Peptide (cyclic)	1316.6		0.2 µmol	40°C		92.2 ± 3.2

Ethical statement

All experiments with commercial available human serum (Sigma Aldrich, H4522, from human male AB plasma) were conducted in accordance with the local law and national and institutional guidelines and ethics. The officer for biological safety has approved the performed experiments and informed consent was obtained for any experiment with commercial available human serum.

Acknowledgments

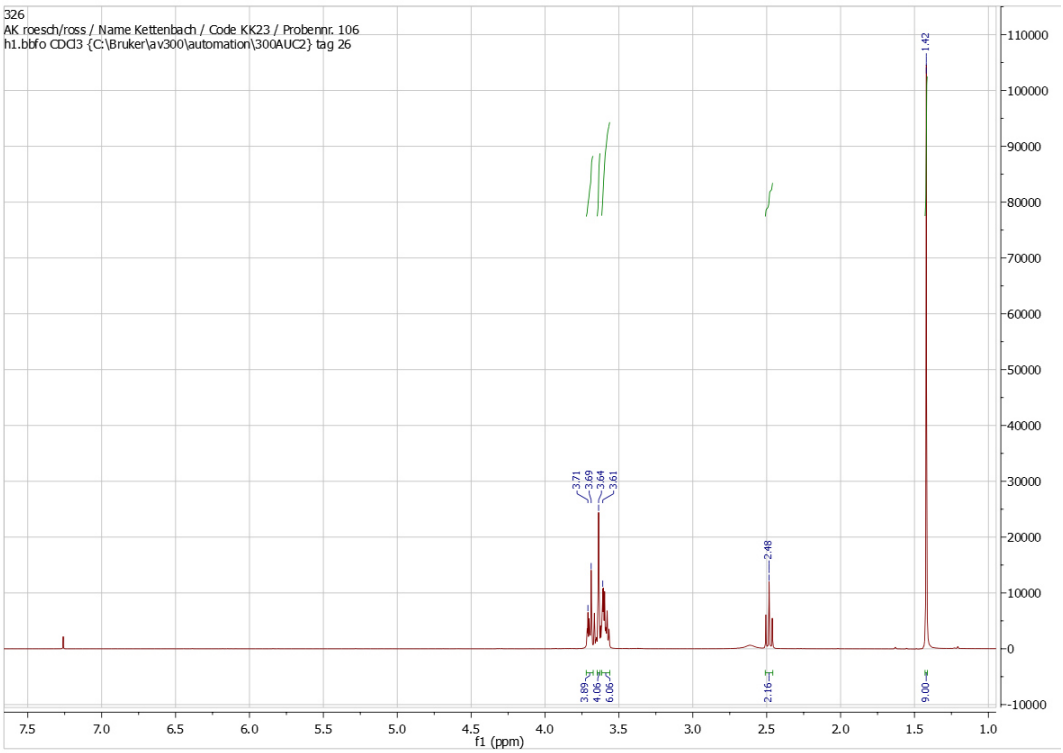
The authors thank the Max Planck Graduate Center for supporting Kathrin Kettenbach.

IV. Analytics

IV.I. NMR data

***Tert*-butyl-3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propanoate (3)**

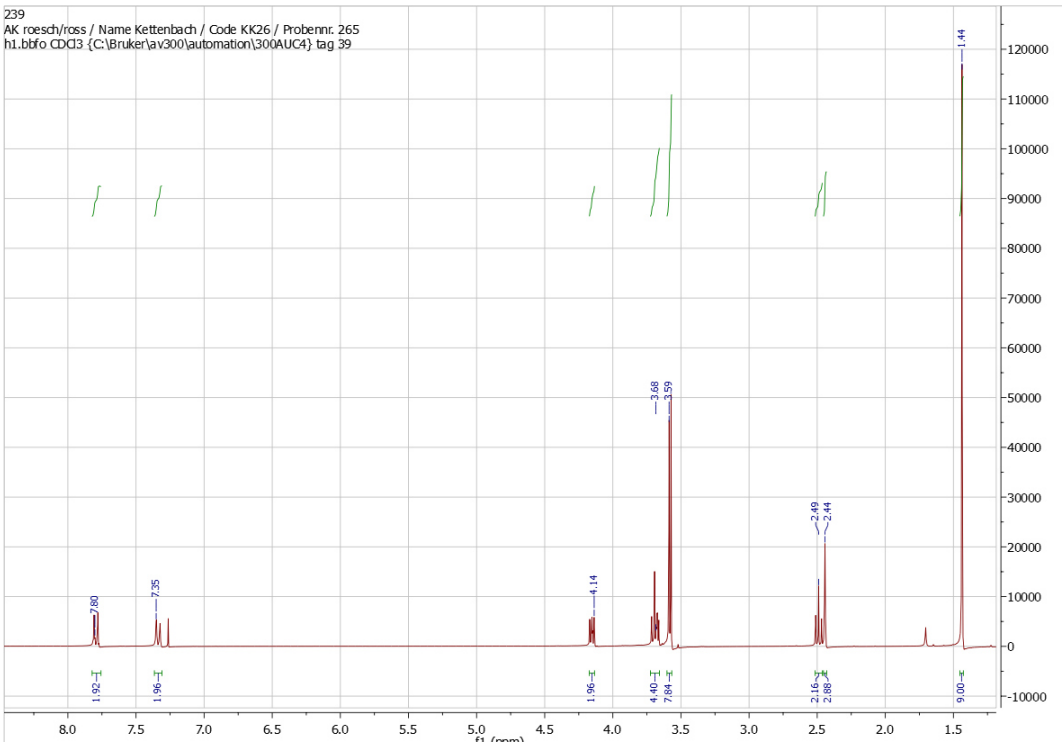
^{1}H-NMR



^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.42 (s, 9H, COOC*H*$_3$), 2.48 (2H, t, 6.5 Hz, C*H*$_2$COOCH$_3$), 3.61 – 3.71 (14H, m, PEG-C*H*$_2$).

***Tert*-butyl-3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)propanoate (4)**

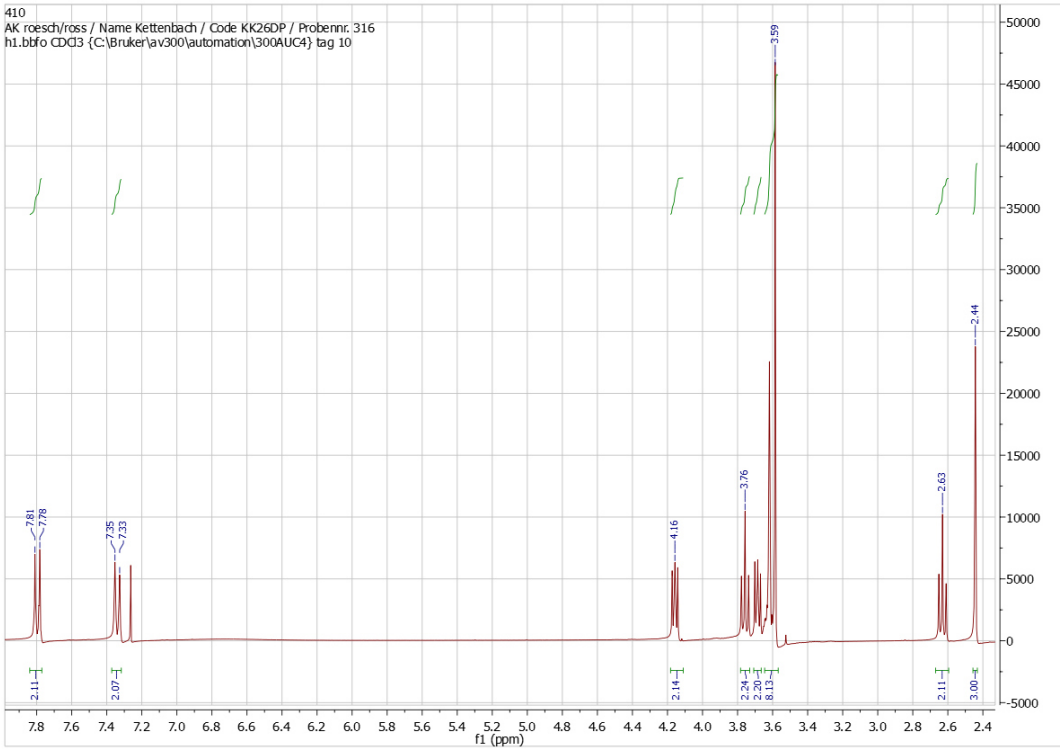
^{1}H-NMR



^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.44 (9H, s, COOC*H*$_3$), 2.44 (3H, s, Tosyl-C*H*$_3$), 2.49 (2H, t, 6.5 Hz, CH_3OOCCH_2), 3.59 – 3.68 (12H, m, PEG-C*H*$_2$), 4.15 (2H, t, 4.9 Hz, Tosyl-O-C*H*$_2$), 7.32 (2H, d, 8.8 Hz, Tosyl-C*H*), 7.78 (2H, d, 8.2 Hz, Tosyl-C*H*).

3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)propanoate (5)

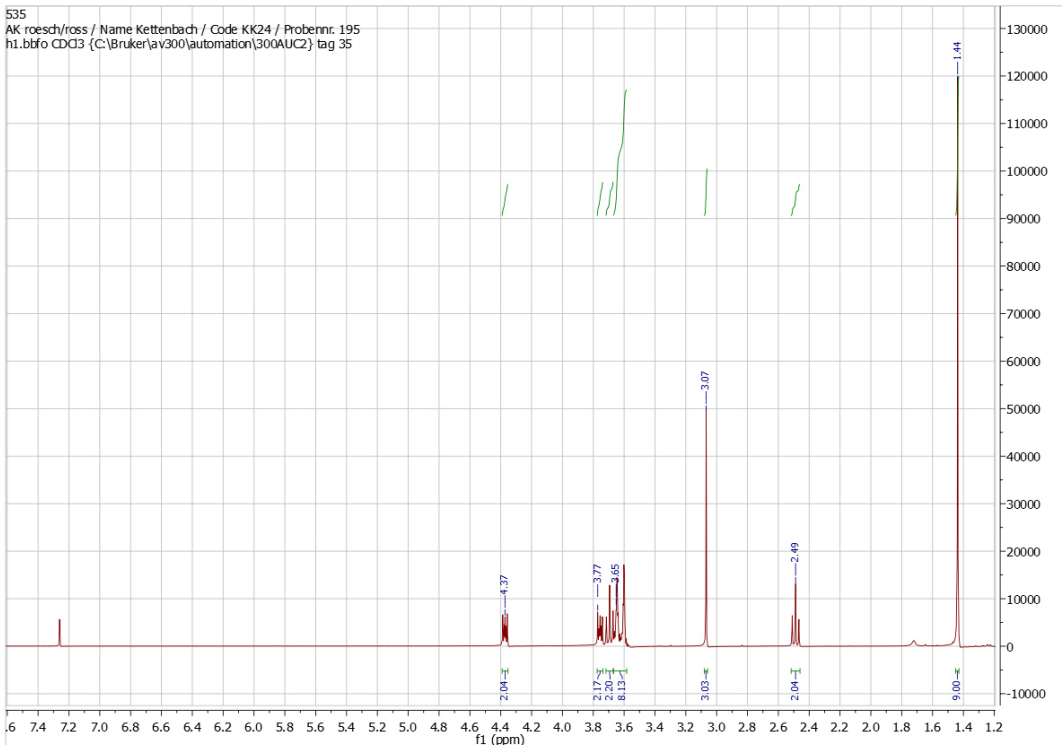
^{1}H-NMR



^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 2.44 (3H, s, Tosyl-C*H*$_3$), 2.63 (2H, t, 6.2 Hz, CH_3OOCC*H*$_2$), 3.59 (8H, m, PEG-C*H*$_2$), 3.69 (2H, t, 5 Hz, PEG-C*H*$_2$) 3.76 (2H, t, 6.1 Hz, PEG-C*H*$_2$), 4.15 (2H, t, 4.7 Hz, Tosyl-O-C*H*$_2$), 7.33 (2H, d, 8.4 Hz, Tosyl-C*H*), 7.78 (2H, d, 8.5 Hz, Tosyl-C*H*).

***Tert*-butyl-3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)propanoate (6)**

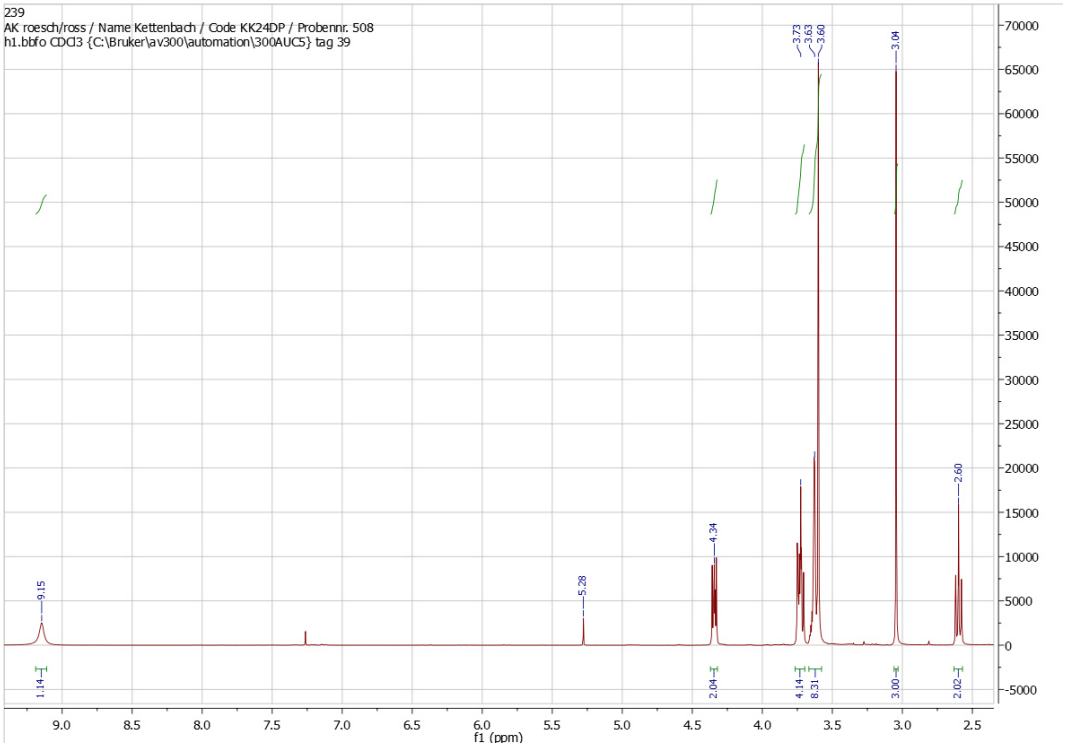
^{1}H-NMR



^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.44 (9H, s, COOCH_3), 2.49 (2H, t, 6.5 Hz, CH_3COOCH_2), 3.07 (3H, s, Mesyl-CH_3), 3.56 – 3.77 (12H, m, PEG-CH_2), 4.37 (2H, t, 4.6 Hz, Mesyl-CH_2).

3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)propanoate (7)

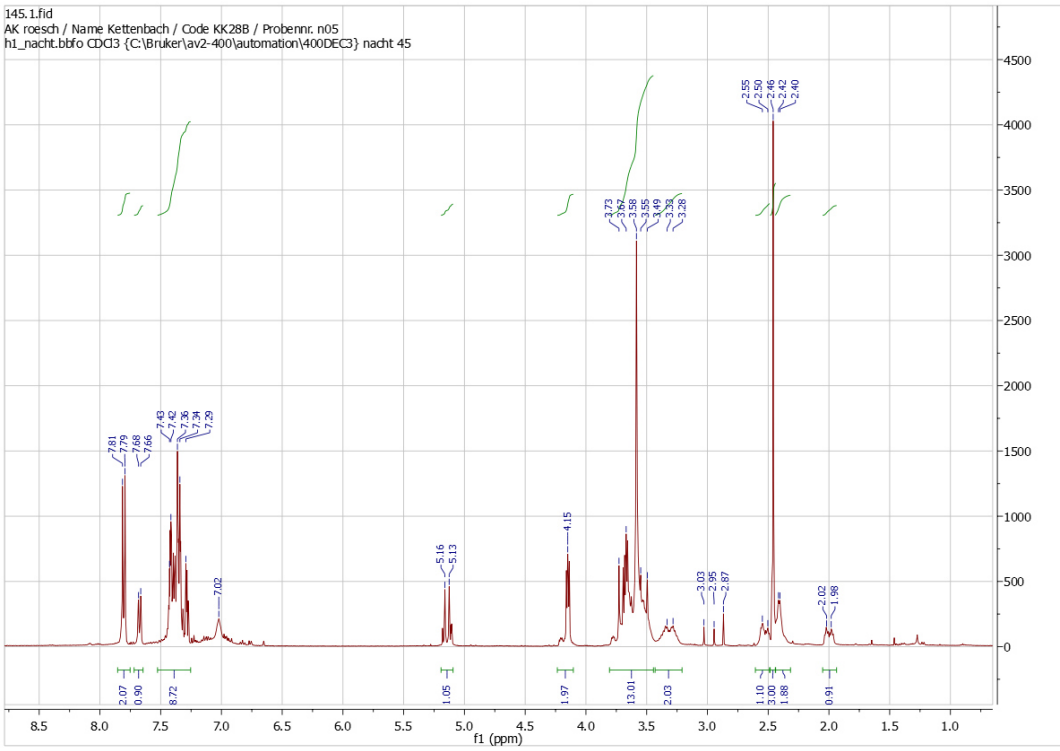
^{1}H-NMR



^{1}H-NMR (300 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 2.60 (2H, t, 6.2 Hz, CH_3COOCH_2), 3.04 (3H, s, Mesyl-CH_3), 3.60 – 3.73 (12H, m, PEG-CH_2), 4.34 (2H, t, 4.5 Hz, Mesyl-CH_2), 9.15 (1H, s, O*H*).

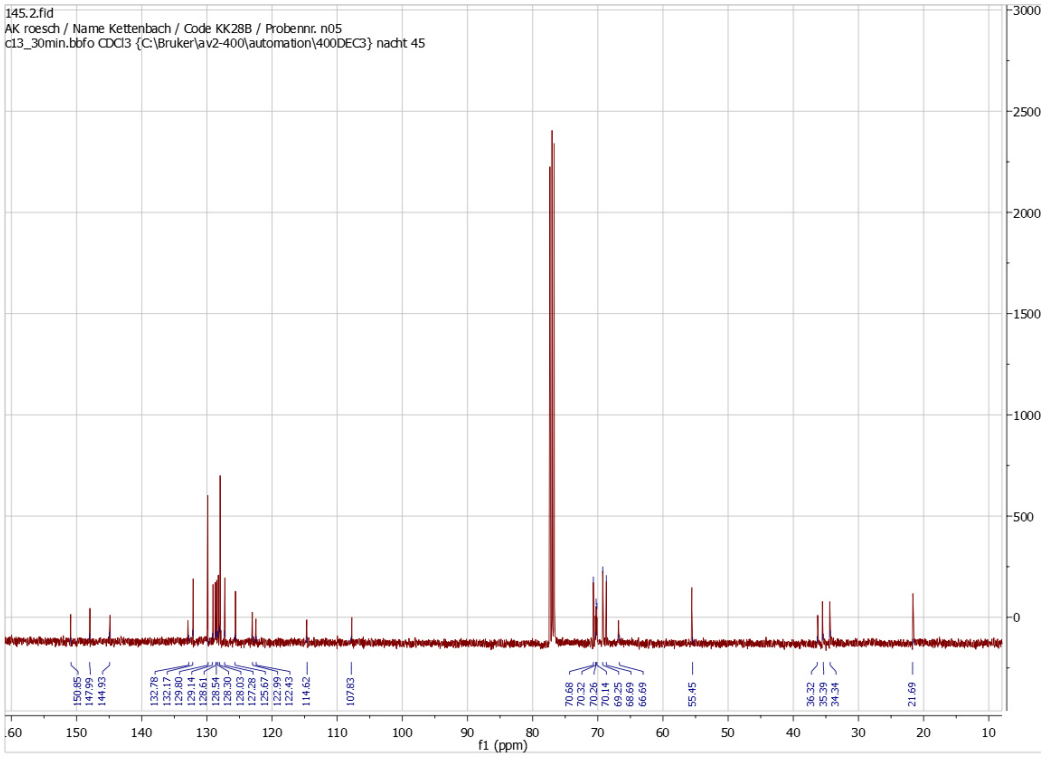
3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (9)

^{1}H-NMR



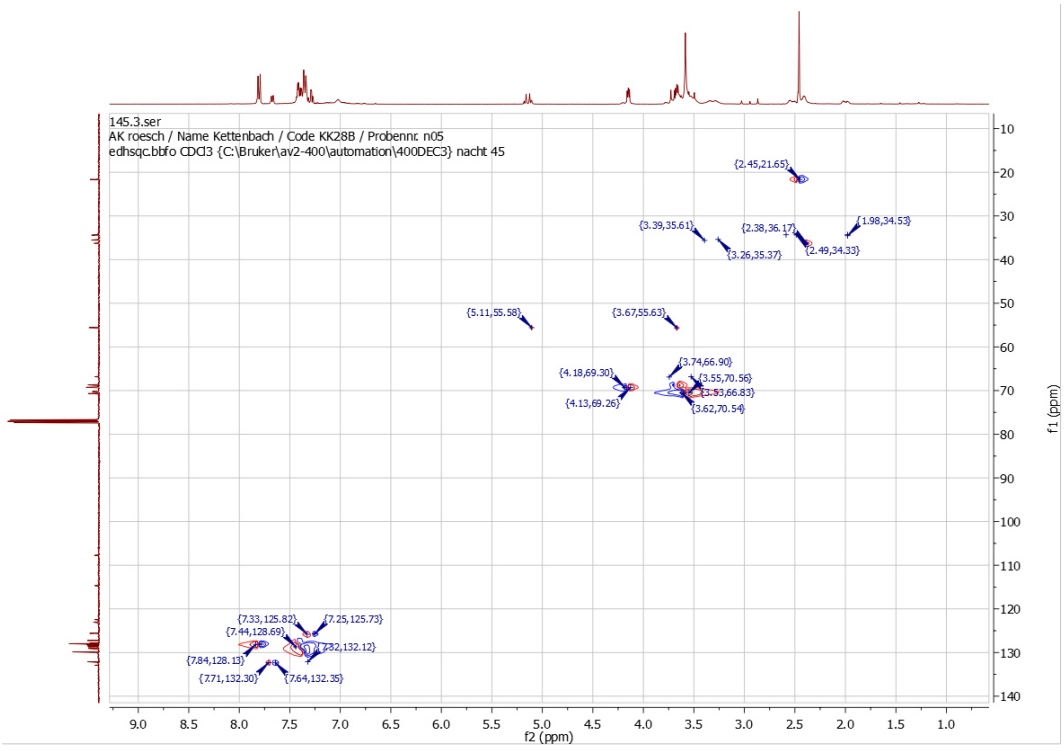
^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.98 (1H, m, N-CO-C*H*$_2$-CH_2-NH-CO) und 2.58 (1H, m, N-CO-C*H*$_2$-CH_2-NH-CO), 2.38 (2H, d, 6 Hz, PEG-C*H*$_2$-CO-NH), 2.45 (3H, s, Tosyl-C*H*$_3$), 3.26 – 3.39 (2H, m, N-CO-CH_2-C*H*$_2$-NH-CO), 3.49 – 3.75 (13 H, m, PEG-C*H*$_2$ und DBCO-C*H*$_2$), 4.15 (2H, t, 4.7 Hz, Tosyl-SO_2-O-C*H*$_2$), 5.15 (1H, dd, 8 Hz & 14.5 Hz, DBCO-C*H*$_2$), 7.25 – 7.44 (9H, m, DBCO- und Tos-C*H*), 7.66 (1H, d, 7.7 Hz, DBCO-C*H*), 7.78 (2H, d, 9.0 Hz, Tos-C*H*).

^{13}C-NMR

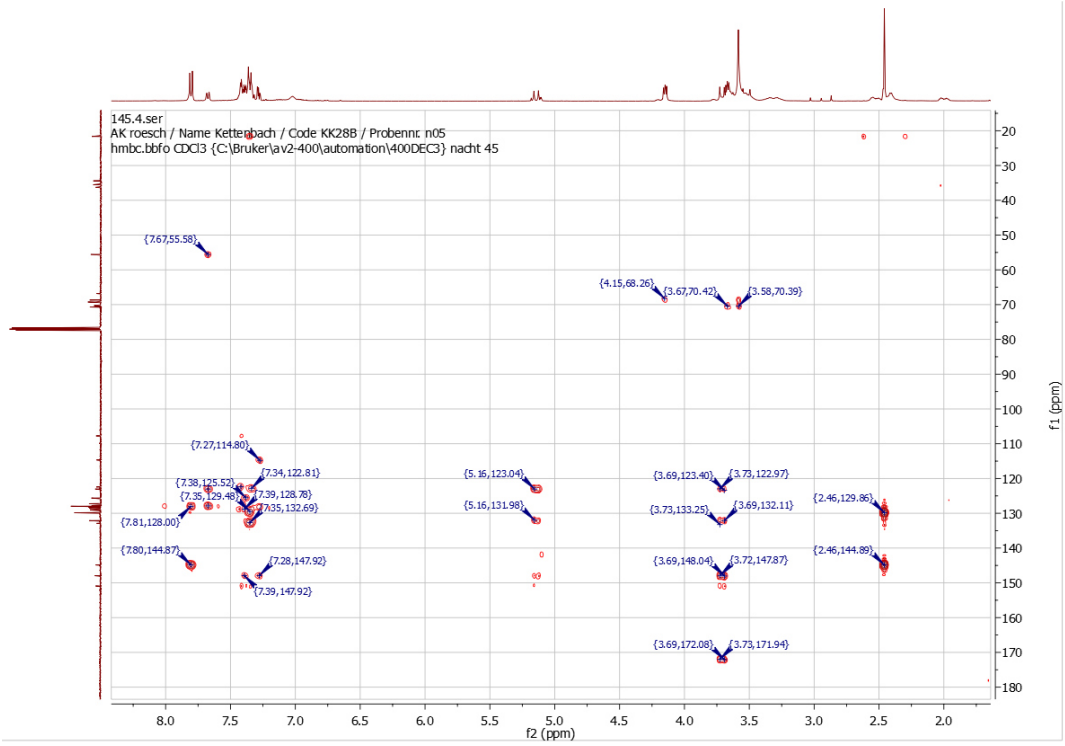


^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] =21.69 (Tosyl-*C*H_3), 34.34 (DBCO-CO-*C*H_2-CH_2-NH-CO), 35.39 (DBCO-CO-CH_2-*C*H_2-NH-CO), 36.32 (NH-CO-*C*H_2-CH_2-PEG-Tosyl), 55.45 (DBCO-*C*H_2), 66.69 – 70.68 (PEG-*C*H_2), 107.83 (Alkyne-*C*), 114.62 – 150.85 (aromatic *C*-atoms).

HSQC-2D-NMR



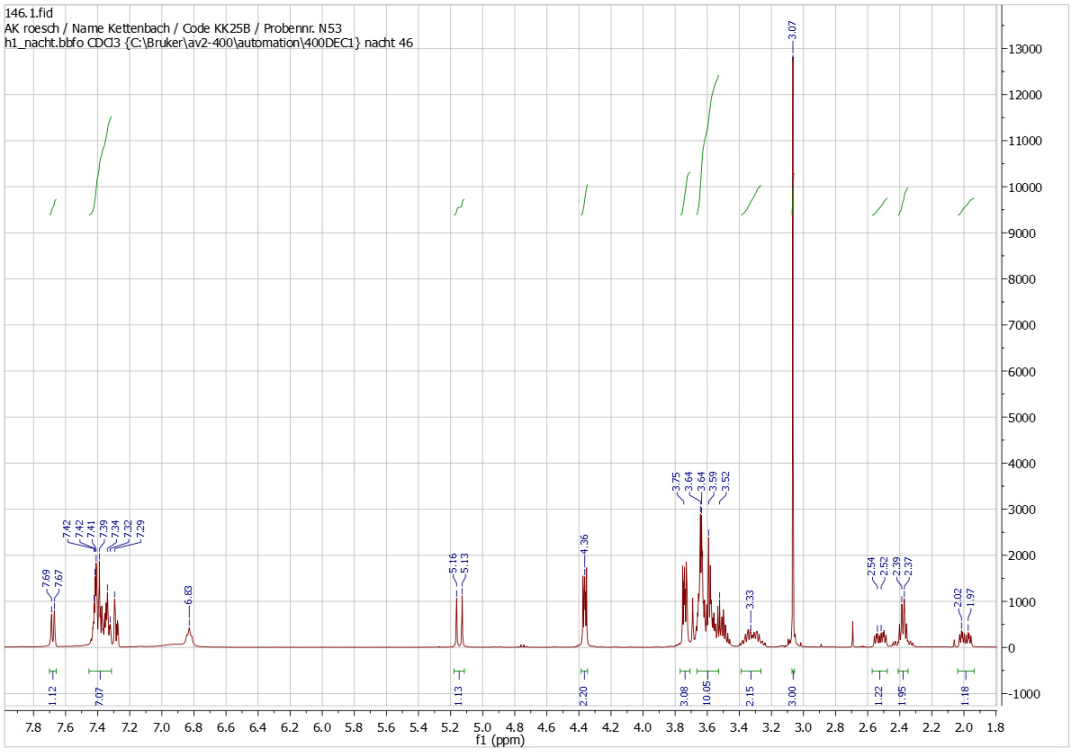
HMBC-2D-NMR



COSY-2D-NMR

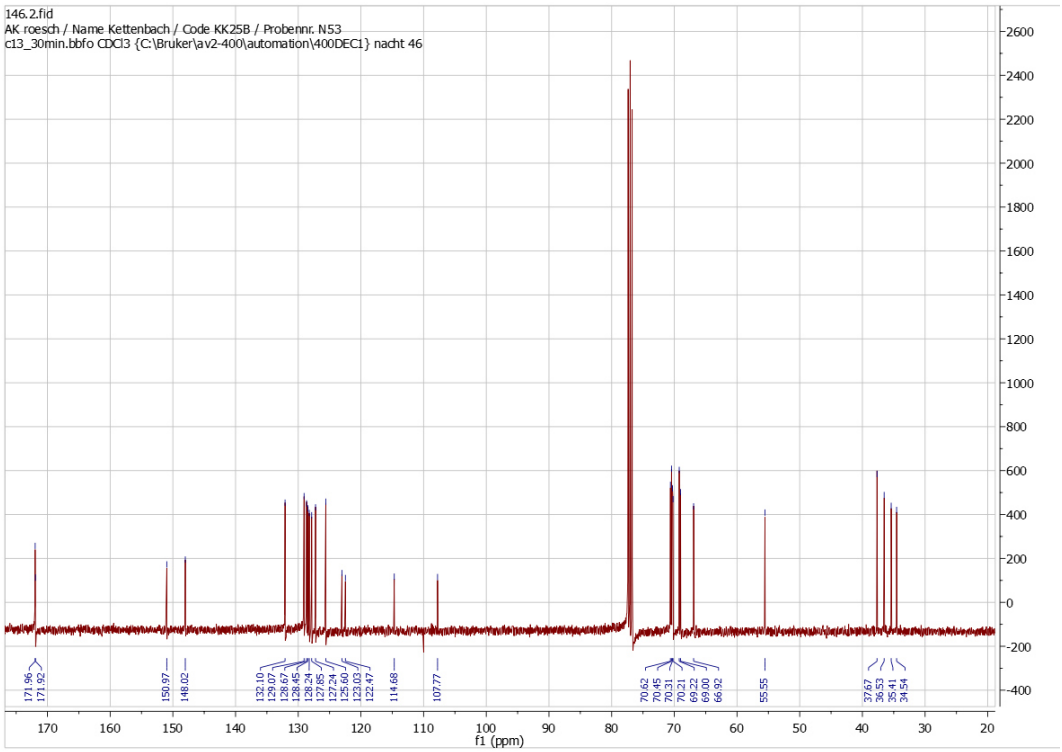
3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (10)

^{1}H-NMR



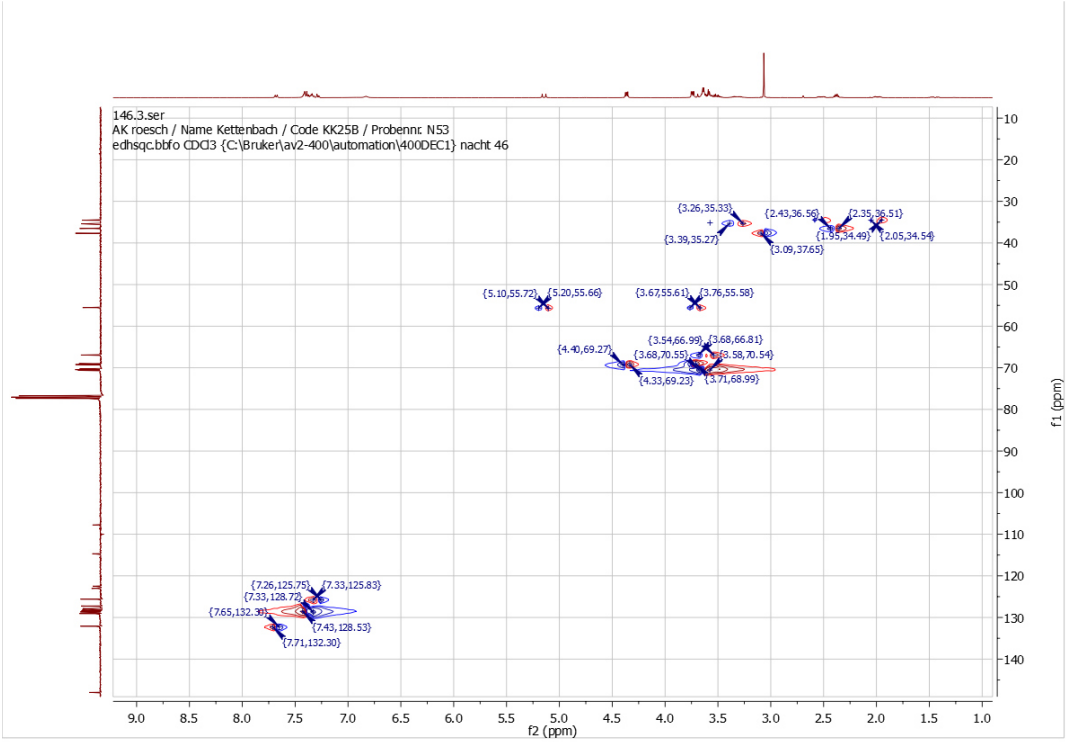
^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.97 – 2.02 (1H, m, N-CO-C*H*$_2$-CH_2-NH-CO) und 2.52. – 2.54 (1H, m, N-CO-C*H*$_2$-CH_2-NH-CO), 2.37 (2H, dd, 5.6 HZ & 6.6 Hz, PEG-C*H*$_2$-CO-N), 3.07 (3H, s, Mesyl-CH_3), 3.3 (2H, m, DBCO-CO-CH_2-C*H*$_2$), 3.52 – 3.64 (10 H, m, PEG-C*H*$_2$), 3.75 (3 H, m, Mesyl-PEG-C*H*$_2$-CH_2-CO und DBCO-C*H*$_2$), 4.36 (2H, t, 4.6 Hz, N-CO-CH_2-C*H*$_2$-N-CO), 5.13 – 5.16 (1H, d, 13.7 Hz, DBCO-C*H*$_2$), 7.32 – 7.42 (7H, m, DBCO- und Tos-C*H*), 7.67 – 7.69 (1H, d, 7.5 Hz, DBCO-C*H*).

^{13}C-NMR

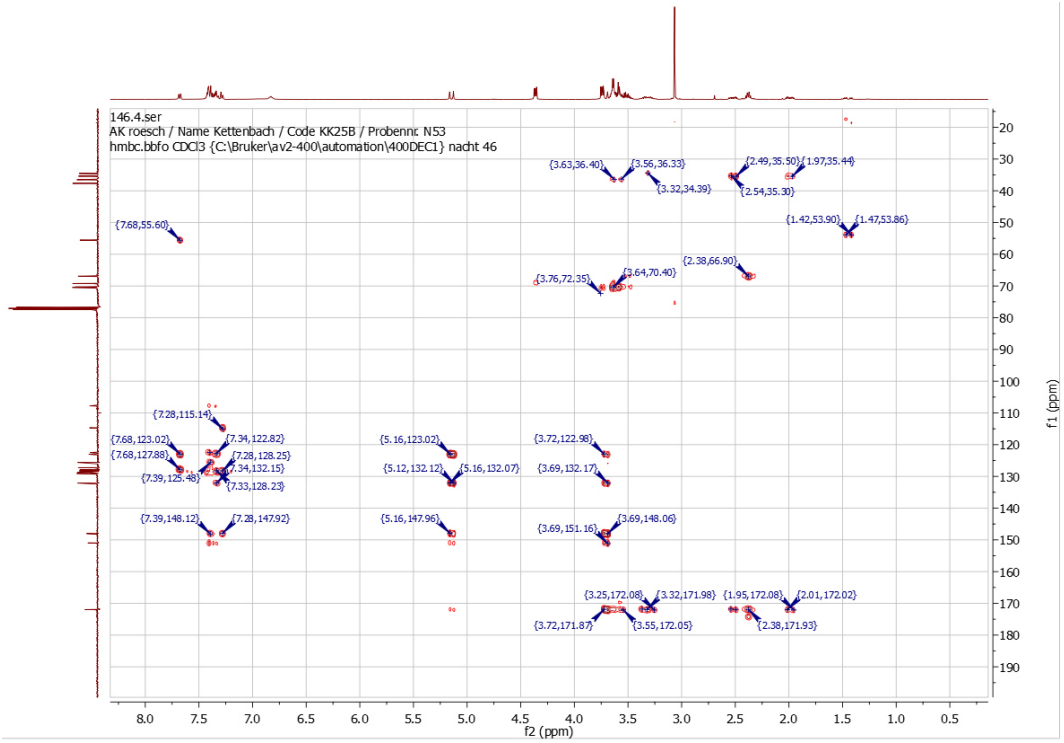


^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 34.54 (DBCO-CO-*C*H_2-CH_2-N-CO), 35.41 (DBCO-CO-CH_2-*C*H_2-N-CO), 36.56 (N-CO-*C*H_2-CH_2-PEG-Mesyl), 37.67 (Mesyl-*C*H_3), 55.55 (DBCO-*C*H_2), 66.92 – 70.62 (PEG-*C*H_2), 107.77 (Alkyne-C), 114.68 – 150.97 (aromatic C-atoms), 171.92 (*C*O).

HSQC-2D-NMR

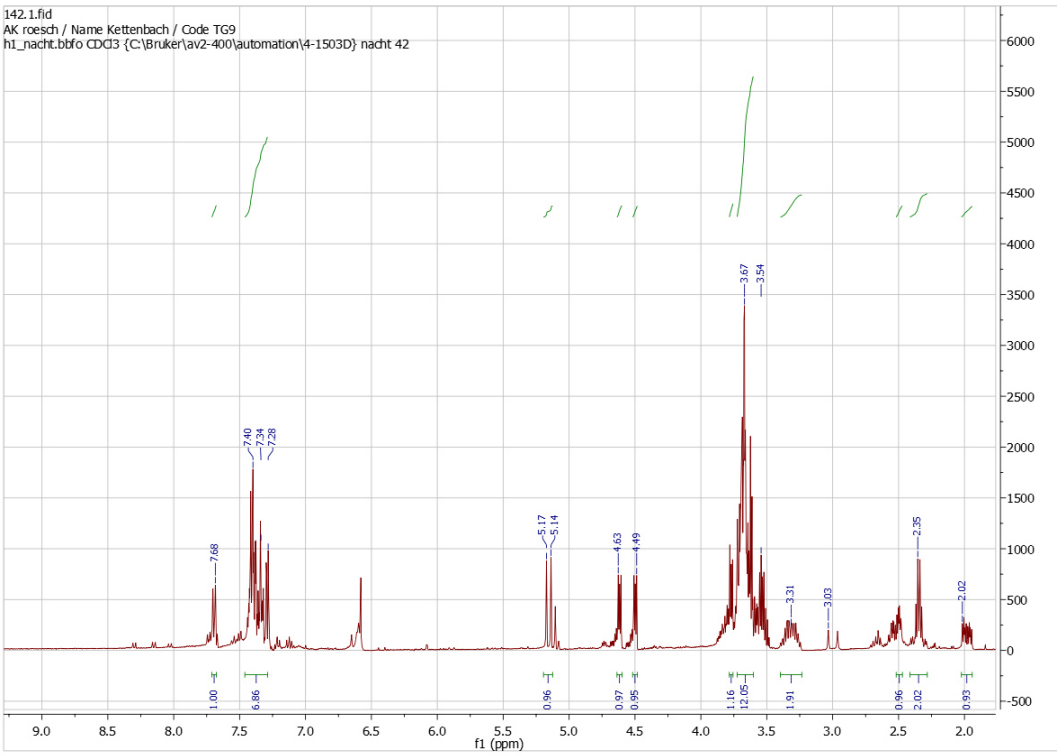


HMBC-2D-NMR



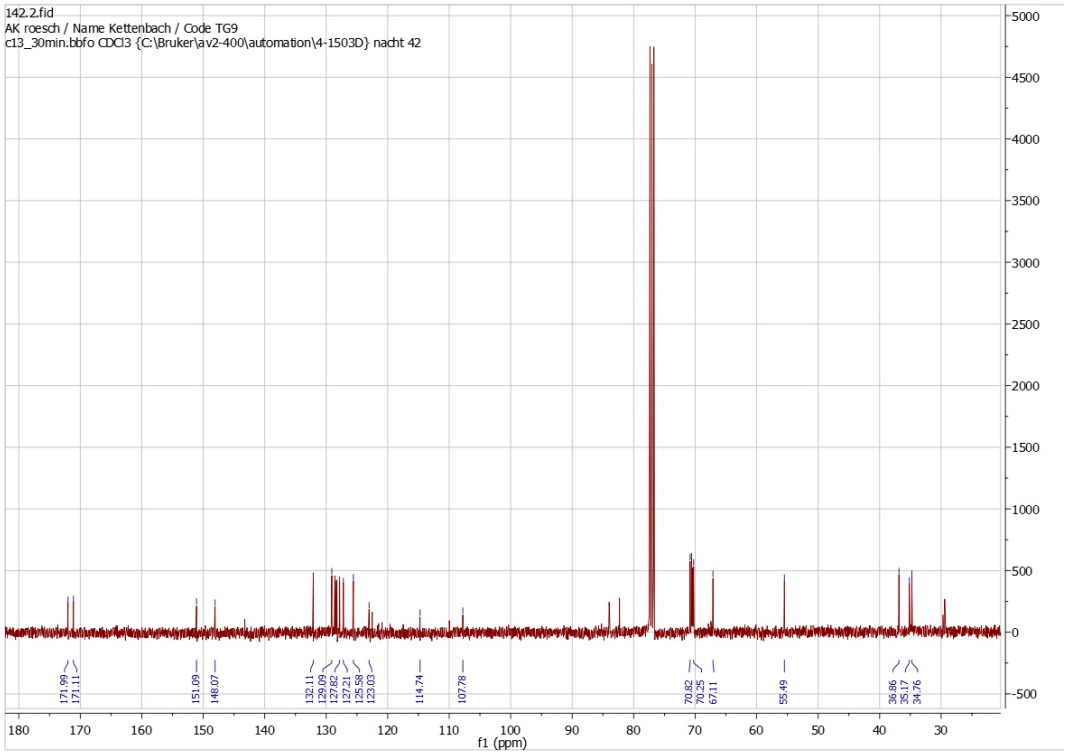
3-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (11)

^{1}H-NMR



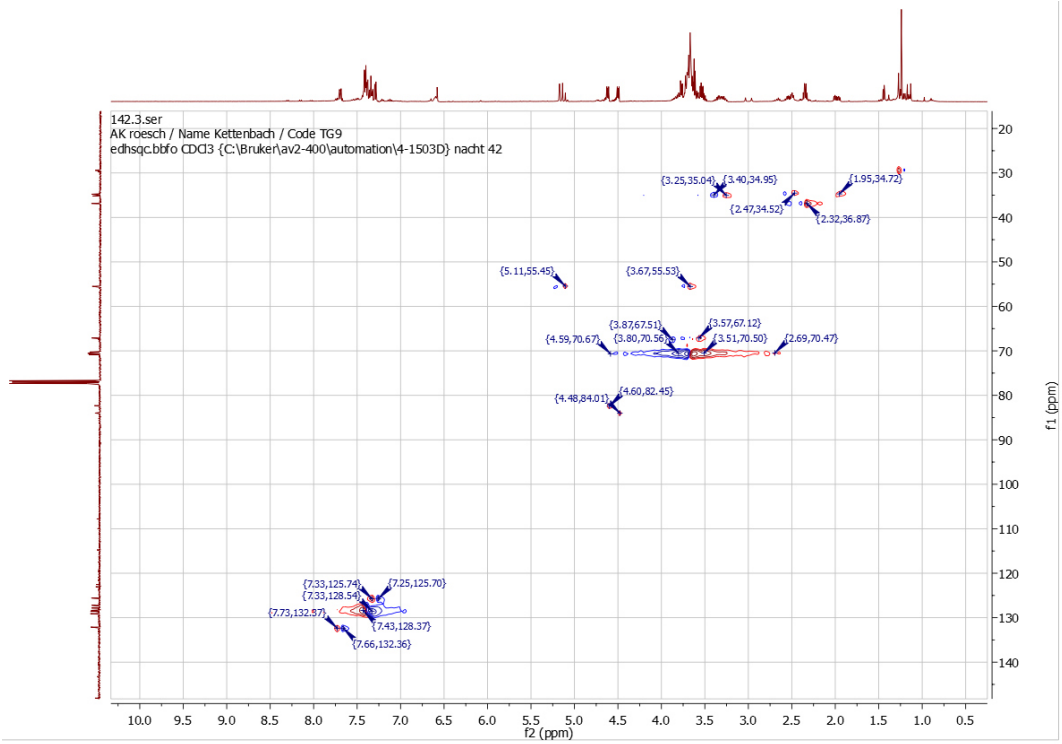
^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 1.95 – 2.02 (1H, m, N-CO-C*H*$_2$-CH_2-NH-CO), 2.35 (2H, dd, 5.6 Hz und 6.7 Hz, PEG-C*H*$_2$-CO-NH), 2.50 (1H, dd, 2.8 Hz und 4.7 Hz, N-CO-C*H*$_2$-CH_2-NH-CO), 3.24 – 3.40 (2H, m, N-CO-CH_2-C*H*$_2$-NH-CO), 3.61 – 3.72 (12H, m, PEG-CH_2), 3.77 (1H, t, 4.2 Hz, DBCO-C*H*$_2$), 4.50 (1H, t, 4.1 Hz, PEG-C*H*$_2$-F), 4.62 (1H, t, 4.4 Hz, PEG-C*H*$_2$-F), 5.11 (1H, d, 14.4 Hz, DBCO-C*H*$_2$), 7.29 – 7.44 (7 H, m, DBCO-C*H*), 7.68 (1H, d, 7.2 Hz, DBCO-C*H*)

^{13}C-NMR

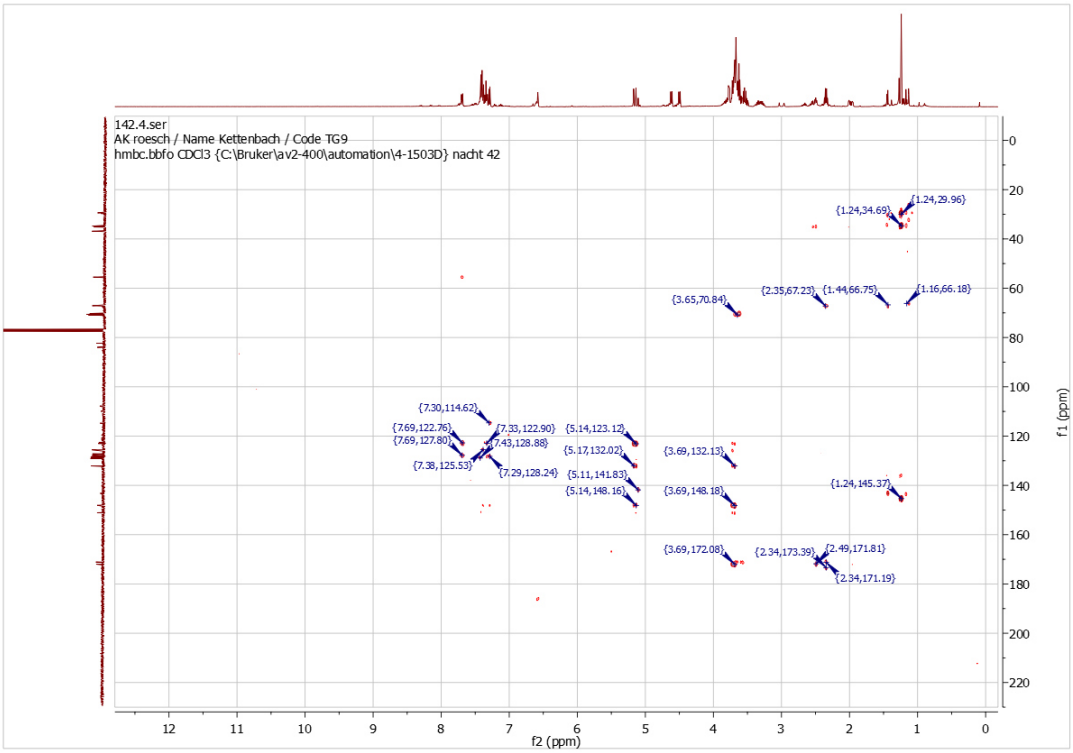


^{13}C-NMR (400 MHz, $CDCl_3$, Me_4Si): δ [ppm] = 34.76 (DBCO-CO-*C*H_2-CH_2-NH-CO), 35.17 (DBCO-CO-CH_2-*C*H_2-NH-CO), 36.86 (NH-CO-*C*H_2-CH_2-PEG), 55.49 (DBCO-*C*H_2), 67.11 – 70.82 (PEG-*C*H_2), 107.56 (Alkyne-*C*), 125.70 – 132.37 (aromatic *C*-atoms)

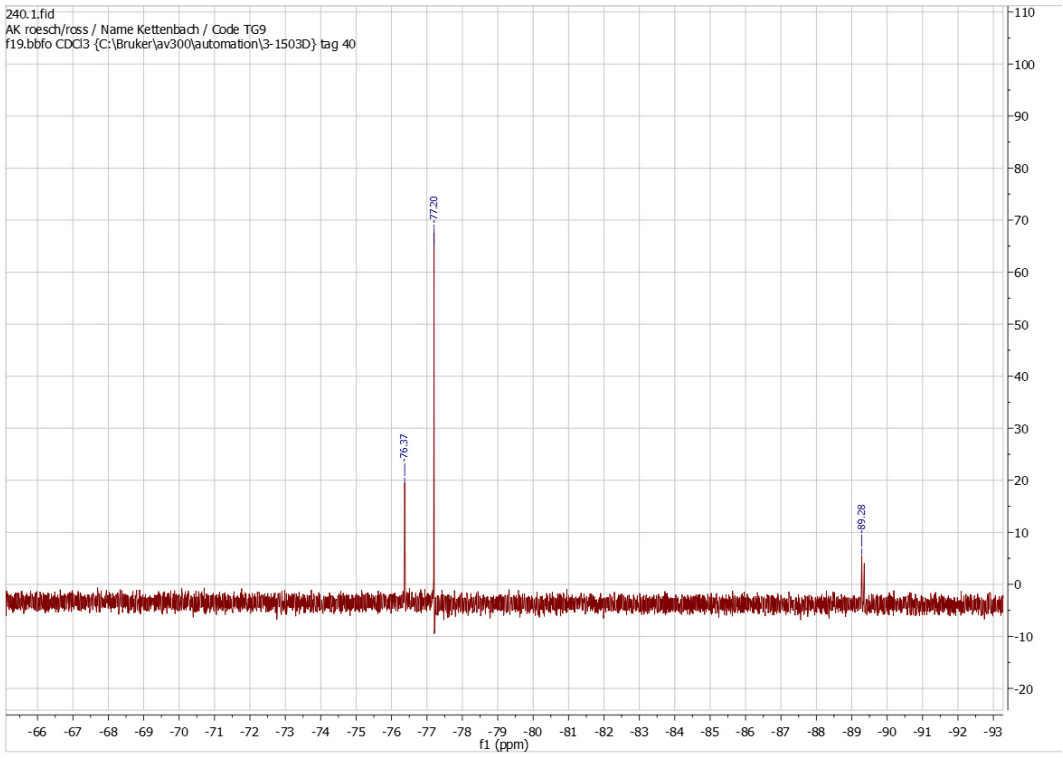
HSQC-2D-NMR



HMBC-2D-NMR



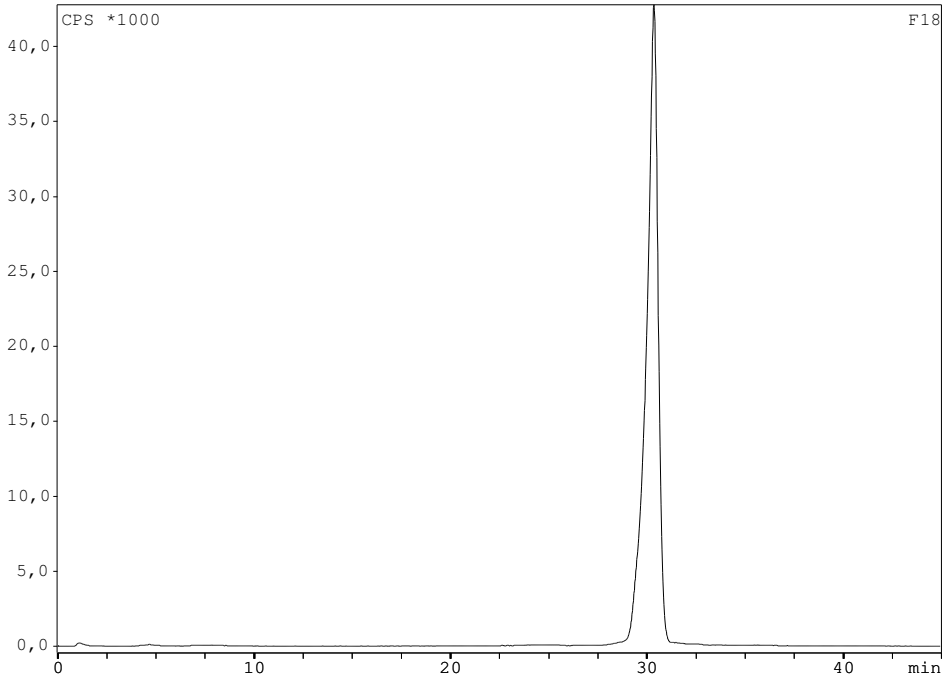
^{19}F-NMR



^{19}F-NMR (400 MHz, $CDCl_3$, CCl_3F): δ [ppm] = -77.20

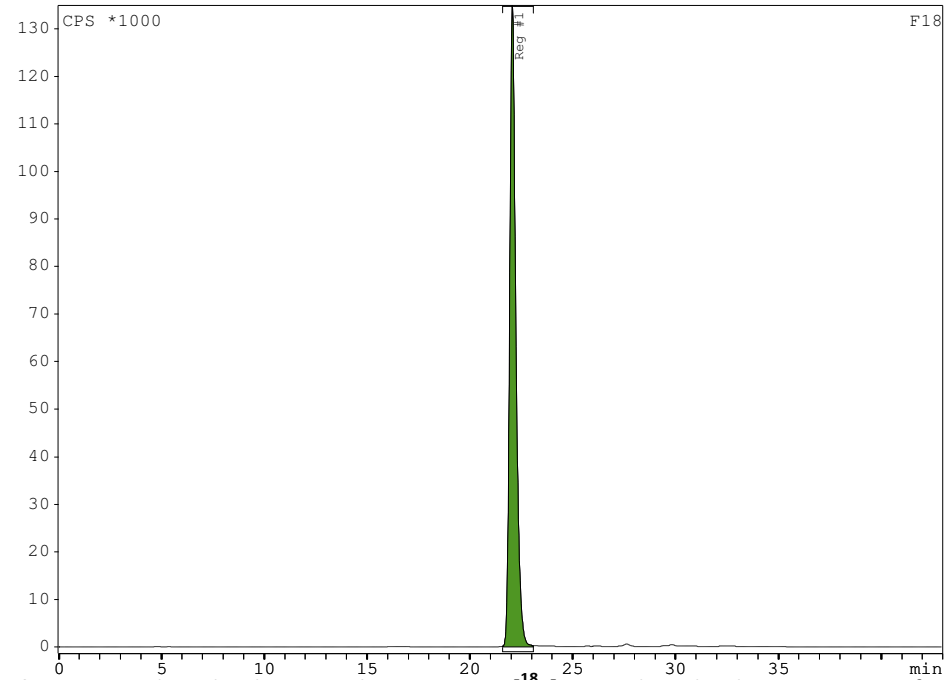
IV.II. Radio-HPLC data

[^{18}F]11



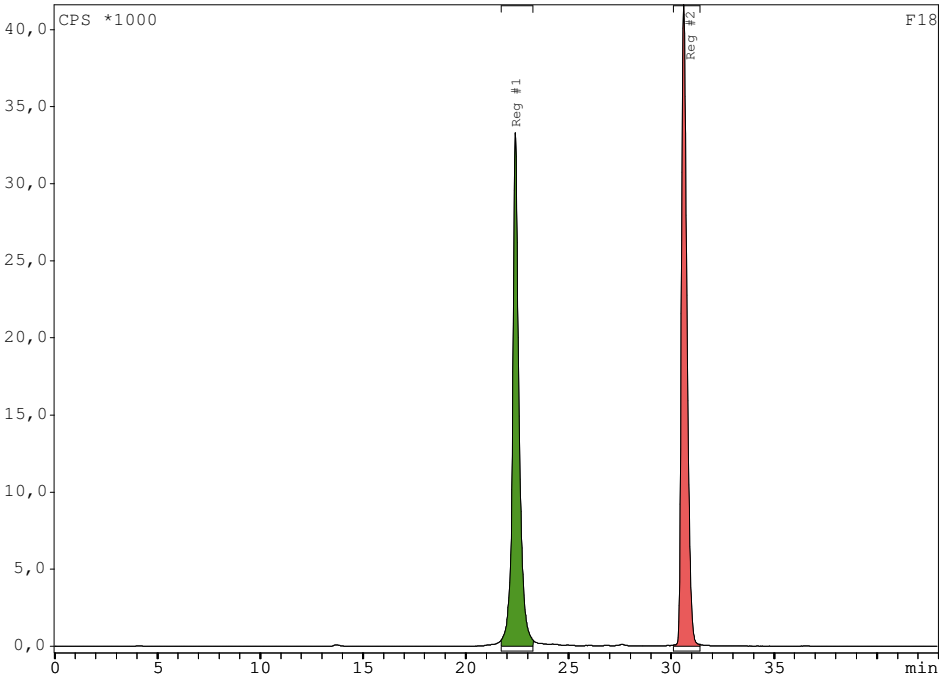
Scheme 1: Analytical radio-HPLC chromatogram **[^{18}F]11**. Analytical radio-HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x20 mm) using the following conditions: Flow 0.7 ml min-1, with eluent A was water with 0.1% TFA (trifluoroacetic acid) and eluent B was acetonitrile with 0.1% TFA. The following method was used: 0 - 40 min, 5 – 95% eluent B (gradient).

[^{18}F]13



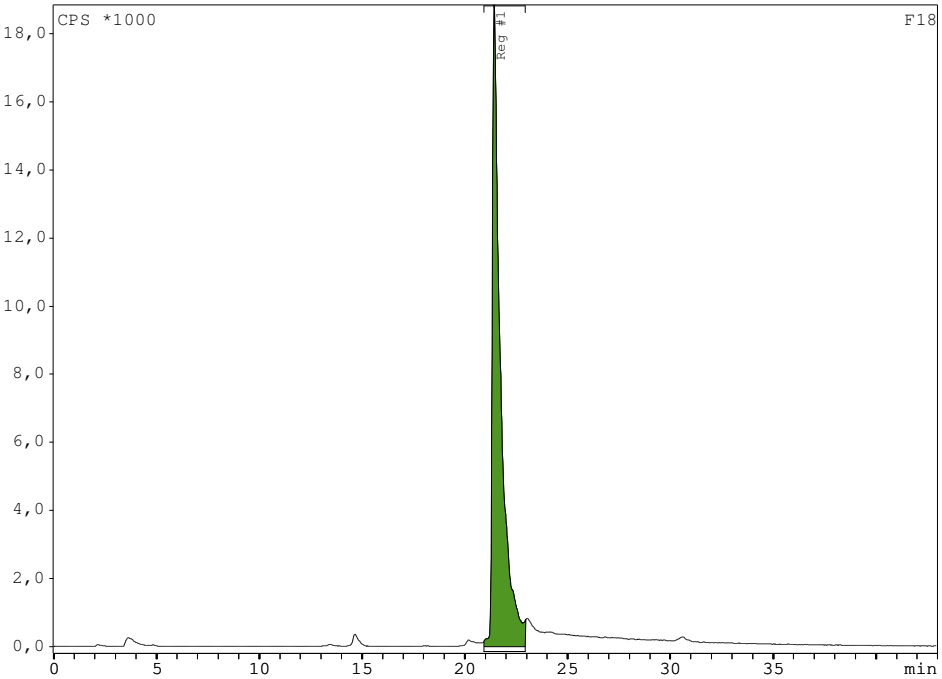
Scheme 2: Analytical radio-HPLC chromatogram **[^{18}F]13**. Analytical radio-HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x20 mm) using the following conditions: Flow 0.7 ml min-1, with eluent A was water with 0.1% TFA (trifluoroacetic acid) and eluent B was acetonitrile with 0.1% TFA. The following method was used: 0 - 40 min, 5 – 95% eluent B (gradient).

[^{18}F]15



Scheme 3: Analytical radio-HPLC chromatogram **[^{18}F]15**. Analytical radio-HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x20 mm) using the following conditions: Flow 0.7 ml min-1, with eluent A was water with 0.1% TFA (trifluoroacetic acid) and eluent B was acetonitrile with 0.1% TFA. The following method was used: 0 - 40 min, 5 – 95% eluent B (gradient).

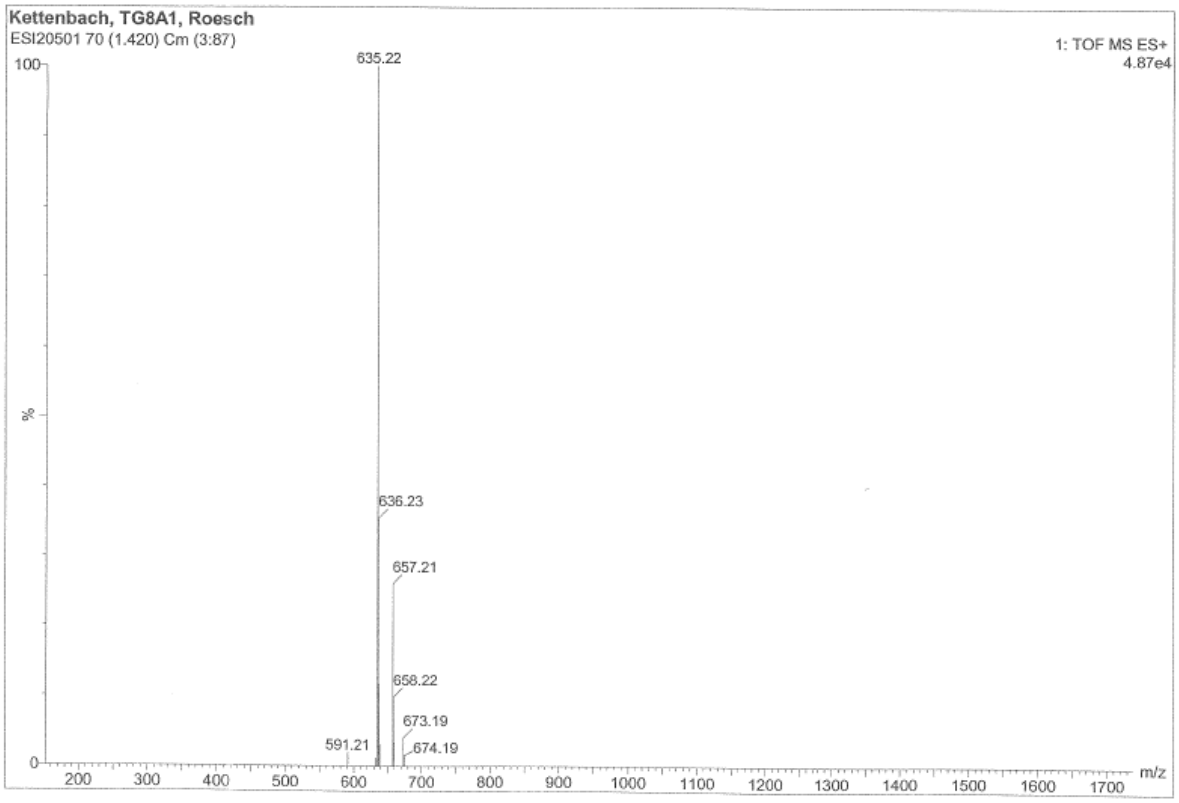
[^{18}F]17



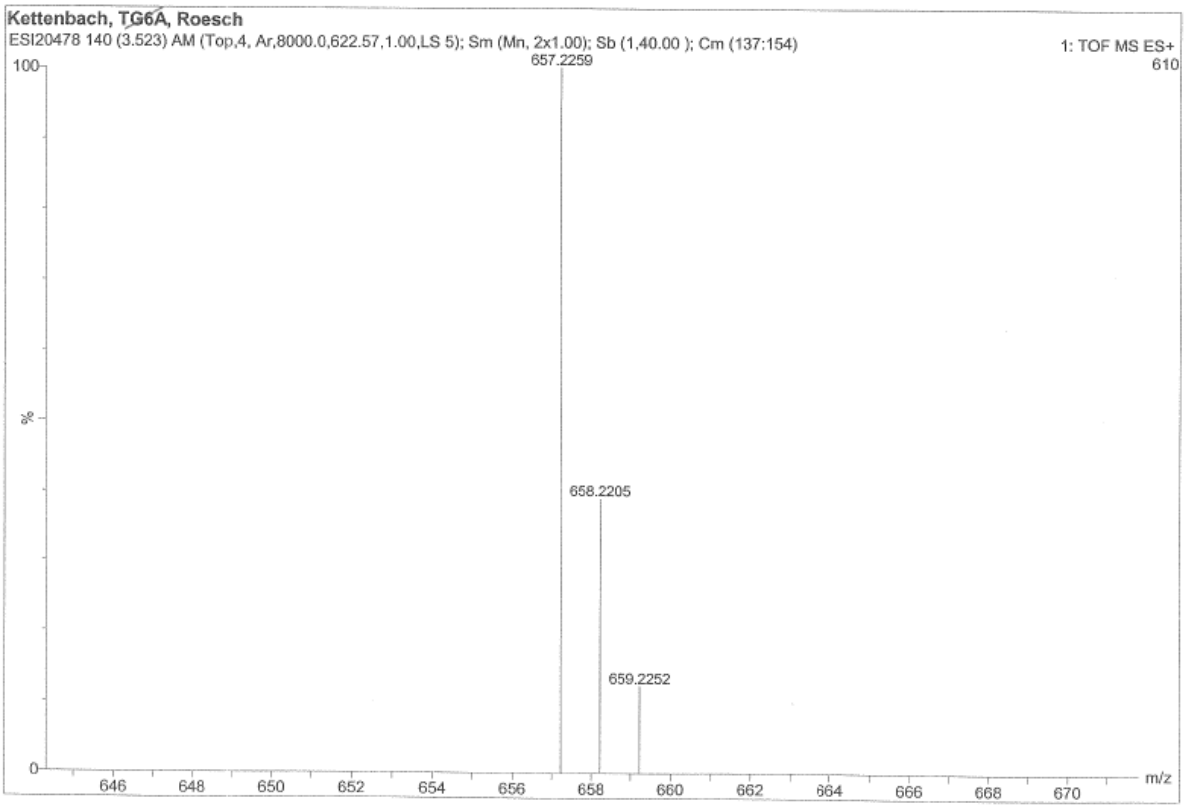
Scheme 4: Analytical radio-HPLC chromatogram **[^{18}F]17**. Analytical radio-HPLC was performed with a Phenomenex Luna C18 column (5 µm, 250x20 mm) using the following conditions: Flow 0.7 ml min-1, with eluent A was water with 0.1% TFA (trifluoroacetic acid) and eluent B was acetonitrile with 0.1% TFA. The following method was used: 0 - 40 min, 5 – 95% eluent B (gradient).

IV.III. Mass spectrometry

3-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (9)



MS (ESI positive): m/z 635.22 ($[M]^+$, 100%), 657.21 ($[M+Na]^+$, %), 673.19 ($[M+K]^+$, %); m/z (high resolution) 657.2259 ($[M+Na]^+$, 100%); calculated for $C_{34}H_{38}N_2O_8S$: 634.23.

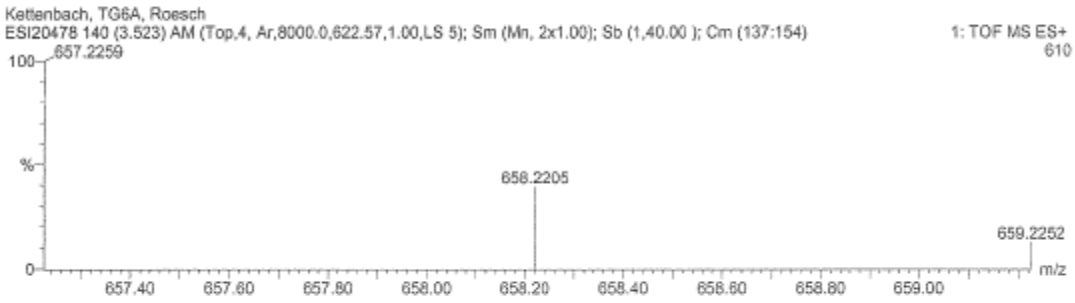


Elemental Composition Report

Single Mass Analysis

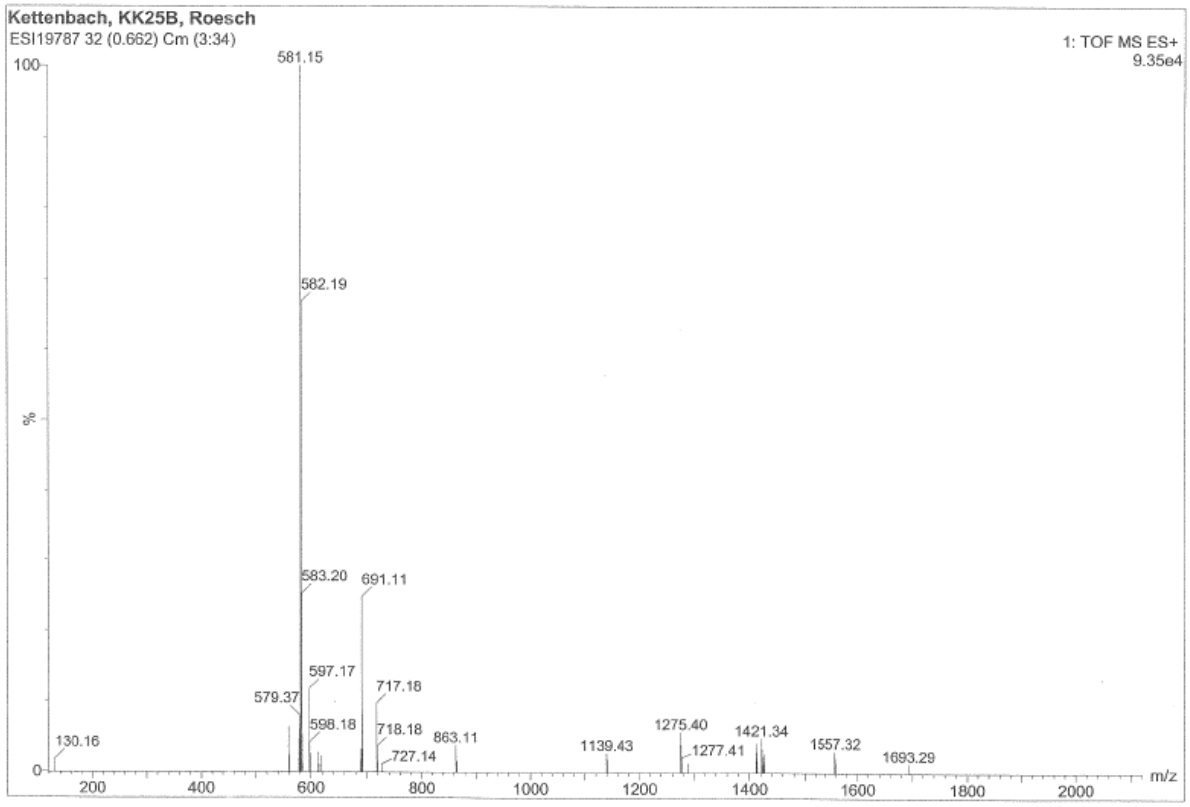
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 200.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
308 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)



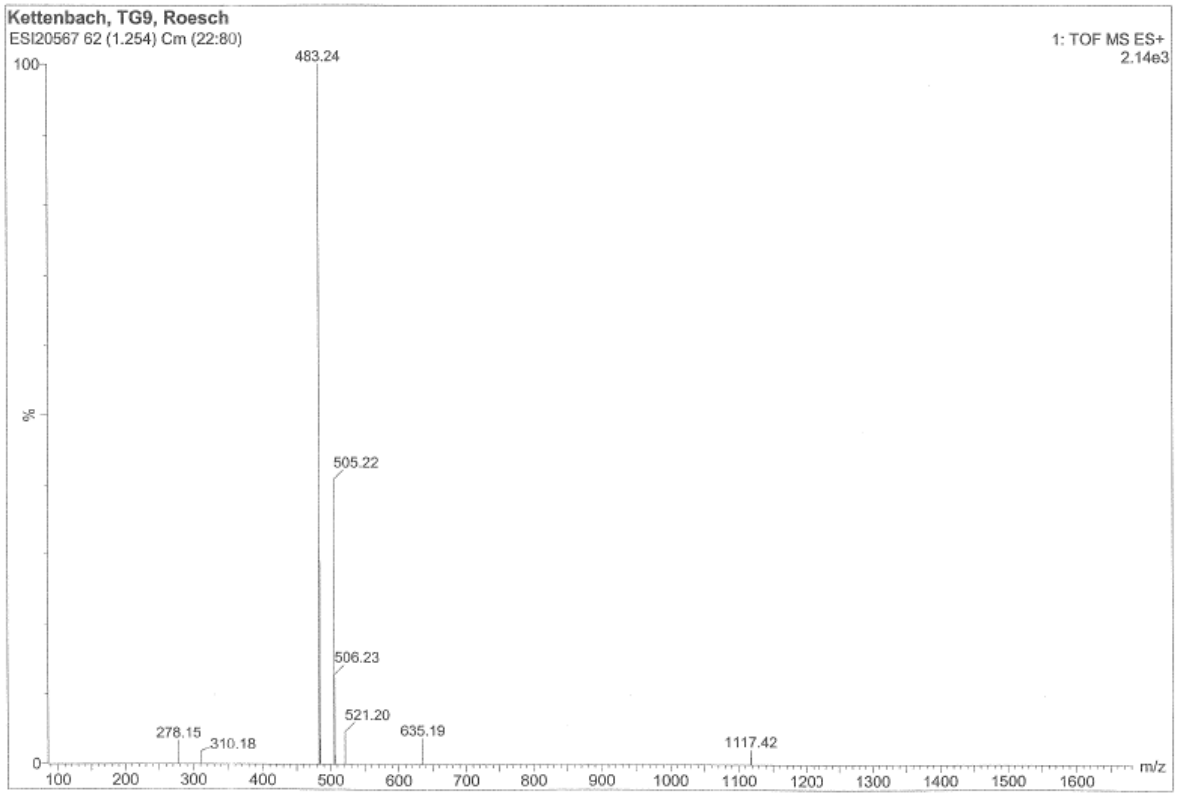
Minimum:				-1.5		
Maximum:		200.0	10.0	200.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
657.2259	657.2247	1.2	1.9	16.5	1	C34 H38 N2 O8 23Na S

3-(2-(2-(2-mesyloxyethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (10)

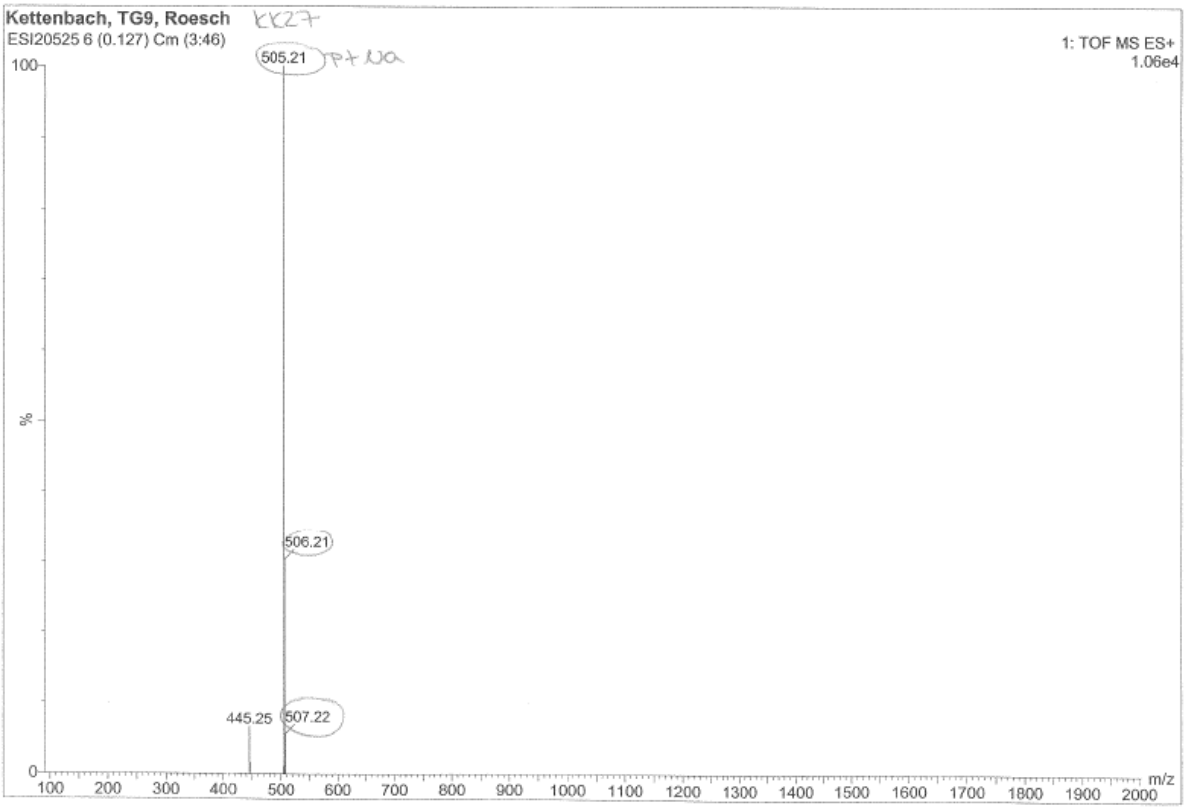


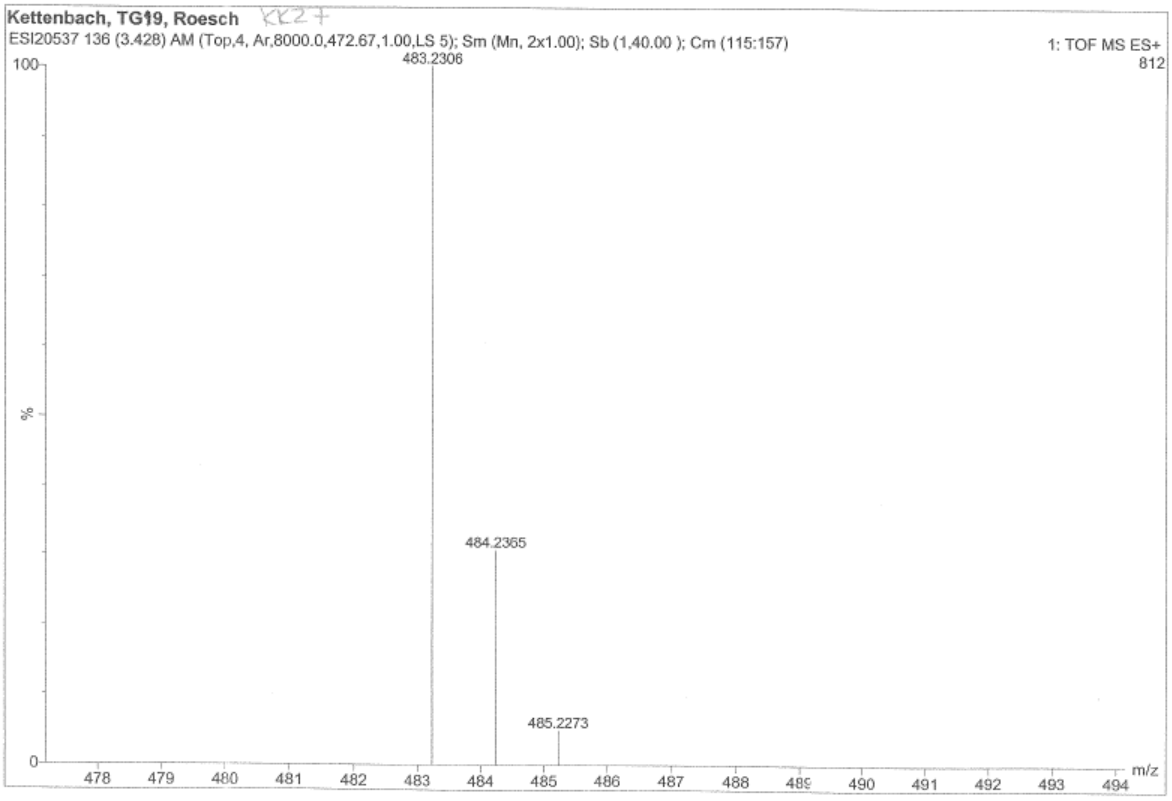
MS (ESI positive): m/z 581.15 ($[M+Na]^+$, 100%); calculated for $C_{28}H_{34}N_2O_8S$: 558.20.

3-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-N-[3-oxo-N-(DBCO)propyl]propanamid (11)



MS (ESI positive): m/z 483.24 ($[M]^+$, 100%), 505.22 ($[M + Na]^+$, %); m/z (high resolution) 483.2306 ($[M]^+$, 100%); 505.2107 ($[M+Na]^+$, 100%); calculated for $C_{27}H_{31}FN_2O_5$: 482.22



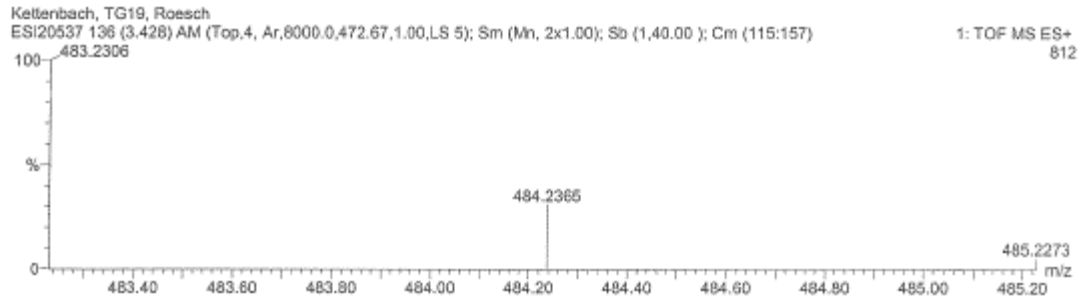


Elemental Composition Report

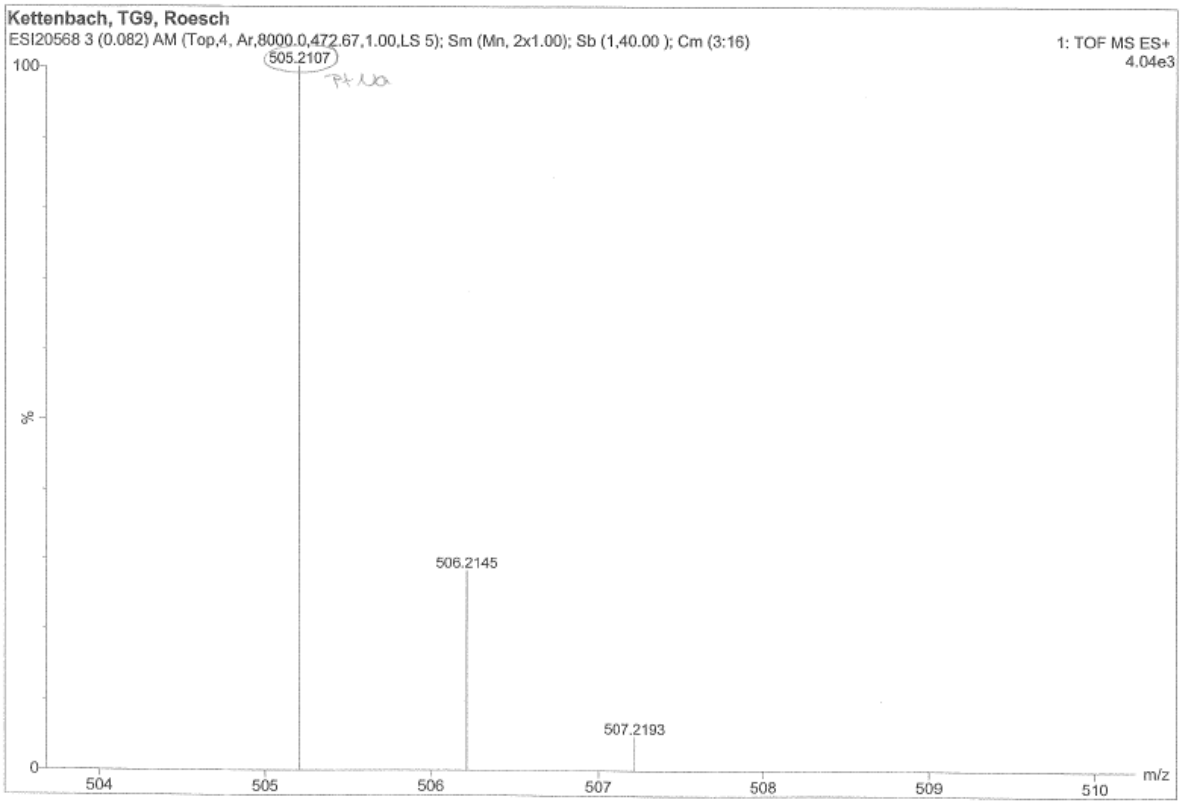
Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 200.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
171 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)



Minimum:				-1.5		
Maximum:		200.0	10.0	200.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
483.2306	483.2295	1.1	2.2	12.5	1	C27 H32 N2 O5 F
	483.2333	-2.7	-5.7	13.0	2	C27 H31 N3 O3 F2

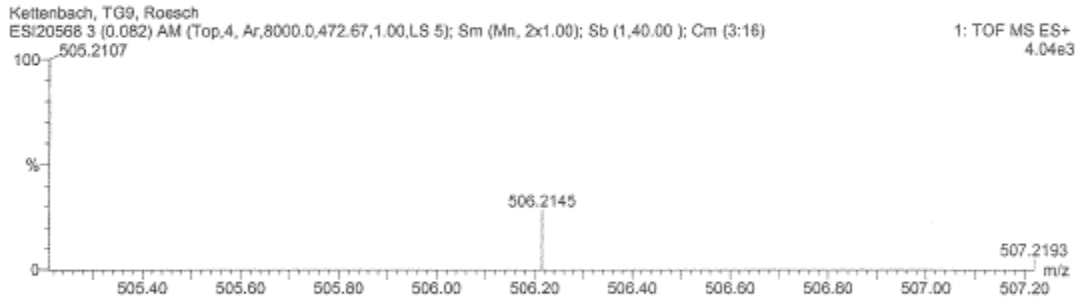


Elemental Composition Report

Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -1.5, max = 200.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
54 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)



Minimum:				-1.5		
Maximum:		200.0	20.0	200.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
505.2107	505.2115	-0.8	-1.5	12.5	3	C27 H31 N2 O5 23Na F
	505.2076	3.1	6.1	12.0	1	C27 H30 N O5 F3
	505.2153	-4.6	-9.1	13.0	2	C27 H30 N3 O3 23Na F2