Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Inhibitor-Conjugated Harmonic Nanoparticles Targeting Fibroblast Activation Protein

Raphaël De Matos,[1] Jérémy Vuilleumier,[1] Christophe Mas,[2] Samuel Constant,[2,3] Davide Staedler,[4] Sandrine Gerber-Lemaire[1]*

[1]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, Group for Functionalized Biomaterials, EPFL SB ISIC SCI-SB-SG, Station 6, CH-1015 Lausanne, Switzerland
[2]Oncotheis, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland
[3]Epithelix, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland
[4]Department of Pharmacology and Toxicology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, CH-1011, Switzerland

*E-mail: sandrine.gerber@epfl.ch

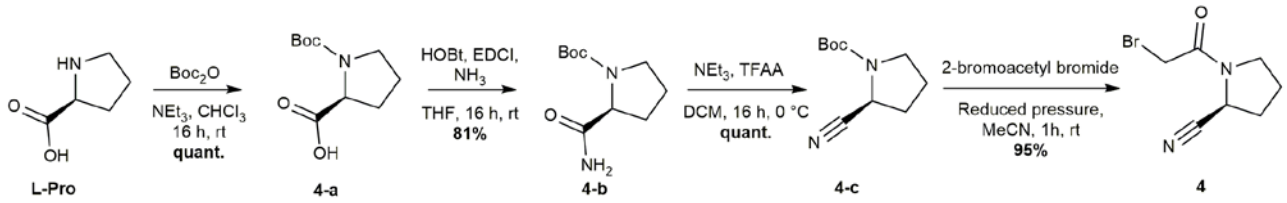
Table of contents

Preparation of (*S*)-1-(2-bromoacetyl)pyrrolidine-2-carbonitrile (4)

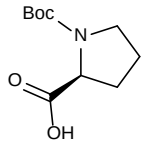
Adapted from protocols described in:

S. Passemard, D. Staedler, G. Sonego, T. Magouroux, G. S. Schneiter, L. Juillerat-Jeanneret, L. Bonacina and S. Gerber-Lemaire, *J. Nanoparticle Res.*, 2015, **17**, 414.

Scheme S1. Preparation of compound **4** from L-Proline



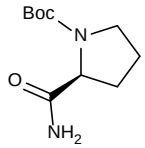
(*tert*-butoxycarbonyl)-L-proline (**4-a**)



To a suspension of L-proline (1 equiv, 43 mmol, 5 g) and Boc_2O (1.05 equiv, 45.2 mmol, 9.87 g) in $CHCl_3$ (86 mL), NEt_3 (1.3 equiv, 55.9 mmol, 7.8 mL) was added dropwise at 0 °C. The solution was warmed up to rt and stirred for 16 hr. The mixture was washed with an aqueous solution of 1 M HCl. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to afford **4-a** as a yellow oil (43 mmol, 9.3 g, quant.). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 1.49 (9H, s), 1.81 – 2.12 (4H, m), 2.37 (1H, dd, *J* = 6.0, 12.3 Hz), 3.27 – 3.51 (2H, m), 4.35 (1H, dd, *J* = 2.8, 8.4 Hz).

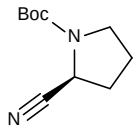
tert-butyl (*S*)-2-carbamoylpyrrolidine-1-carboxylate (**4-b**)



To a solution of **4-a** (1 equiv, 41.8 mmol, 9 g) in THF (527 mL) were added HOBt (1 equiv, 41.8 mmol, 6.4 g) and EDCI (1.15 equiv, 48.1 mmol, 7.47 g), and the mixture was stirred for 30 min at rt. Aqueous ammonia 25 % (2 equiv, 83.6 mmol, 5.7 mL) was added dropwise and the mixture was stirred for 16h at rt. The crude mixture was evaporated in vacuo. The residue was dissolved in AcOEt, and washed with sat. aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo to afford **4-b** as a white solid (33.96 mmol, 7.28 g, 81 %). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 1.47 (9H, s), 1.80 – 2.46 (4H, m), 3.39 (2H, d, *J* = 42.4 Hz) 4.26 (1H, d, *J* = 38.1 Hz).

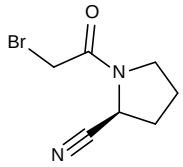
tert-butyl (*S*)-2-cyanopyrrolidine-1-carboxylate (**4-c**)



To a solution of **4-b** (1 equiv, 18.7 mmol, 4 g) and NEt_3 (4.5 equiv, 84.2 mmol, 11.7 mL) in DCM (120 mL), TFAA (2 equiv, 37.4 mmol, 5.2 mL) was added dropwise at 0 °C. The mixture was stirred for 16h at rt. The solution was washed with water (50 ml), an aqueous solution of 0.5 M HCl (50 mL) and sat. aqueous solution of $NaHCO_3$ (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo to afford **4-c** as a yellow oil (18.7 mmol, 3.67 g, quant.). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 1.51 (9H, s), 1.97 – 2.33 (4H, m), 3.21 – 3.63 (2H, m), 4.40 – 4.60 (1H, m).

(S)-1-(2-bromoacetyl)pyrrolidine-2-carbonitrile (**4**)

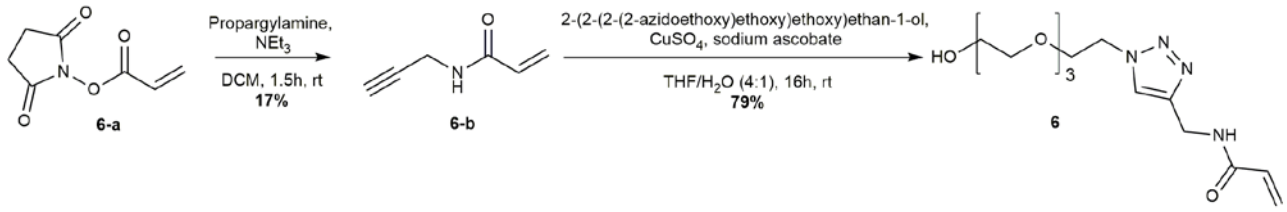


To a solution of **4-c** (1 equiv, 5.09 mmol, 1 g) in MeCN (10 mL), 2-bromoacetyl bromide (2 equiv, 10.2 mmol, 890 µL) was added dropwise and the reaction mixture was stirred under reduced pressure for 2.5 h at rt. The solvent was evaporated *in vacuo* and the crude product was purified by FCC (DCM/MeOH 75:1) to afford **4** as a red oil (4.84 mmol, 1.05 g, 95 %). The analytical data were in accordance with previously reported data.

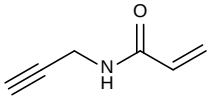
^{1}H NMR (400 MHz, Chloroform-d) δ 2.06 – 2.48 (4H, m), 3.58 – 3.77 (2H, m), 3.84 (2H, s) 4.76 (1H, dd, J = 1.6, 6.4 Hz).

Preparation of compound 6

Scheme S2. Preparation of PEG_3 spacer **6**



N-(prop-2-yn-1-yl)acrylamide (**6-b**)

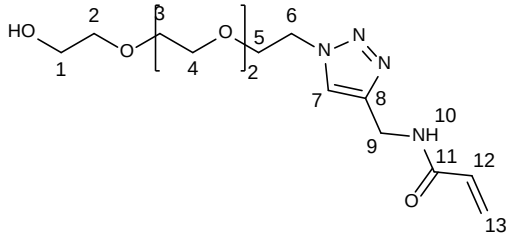


Adapted from protocol described in:
Y. Wang, R. Zhang, N. Xu, F.-S. Du, Y.-L. Wang, Y.-X. Tan, S.-P. Ji, D.-H. Liang and Z.-C. Li, *Biomacromolecules*, 2011, **12**, 66.

To a solution of **6-a** (1 equiv., 4.52 mmol, 764 mg) in anhydrous DCM (30 mL), NEt_3 (5 equiv., 23.05 mmol, 3.2 mL) and propargylamine (2 equiv., 9.22 mmol, 0.6 mL) were added dropwise and the mixture was stirred for 1.5 h at rt. The solution was washed with sat. aqueous NH_4Cl (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by FCC (PE/EA 1:1) to afford **6-b** as a white solid (0.78 mmol, 85 mg, 17%). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 4.15 (2H, dd, *J* = 2.5, 5.4 Hz), 5.70 (1H, d, *J* = 10.3 Hz), 6.07 (1H, s), 6.12 (1H, d, *J* = 10.3 Hz), 6.16 (1H, d, *J* = 10.3 Hz), 6.34 (1H, d, *J* = 17.0 Hz).

Compound **6**



To a solution of **6-b** (1 equiv., 1.3 mmol, 142 mg) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol (1 equiv., 1.3 mmol, 285 mg) in THF (5.2 mL), was added a solution of $CuSO_4$ (10 mol%, 0.13 mmol, 21 mg) and sodium ascorbate (30 mol%., 0.39 mmol, 77 mg) in water (1.3 mL). The mixture was stirred for 16 h at rt. The solvent was evaporated *in vacuo* and the crude product was purified by FCC (DCM/MeOH 10:1) to afford **6** as a yellow oil (1.3 mmol, 427 mg, quant.).

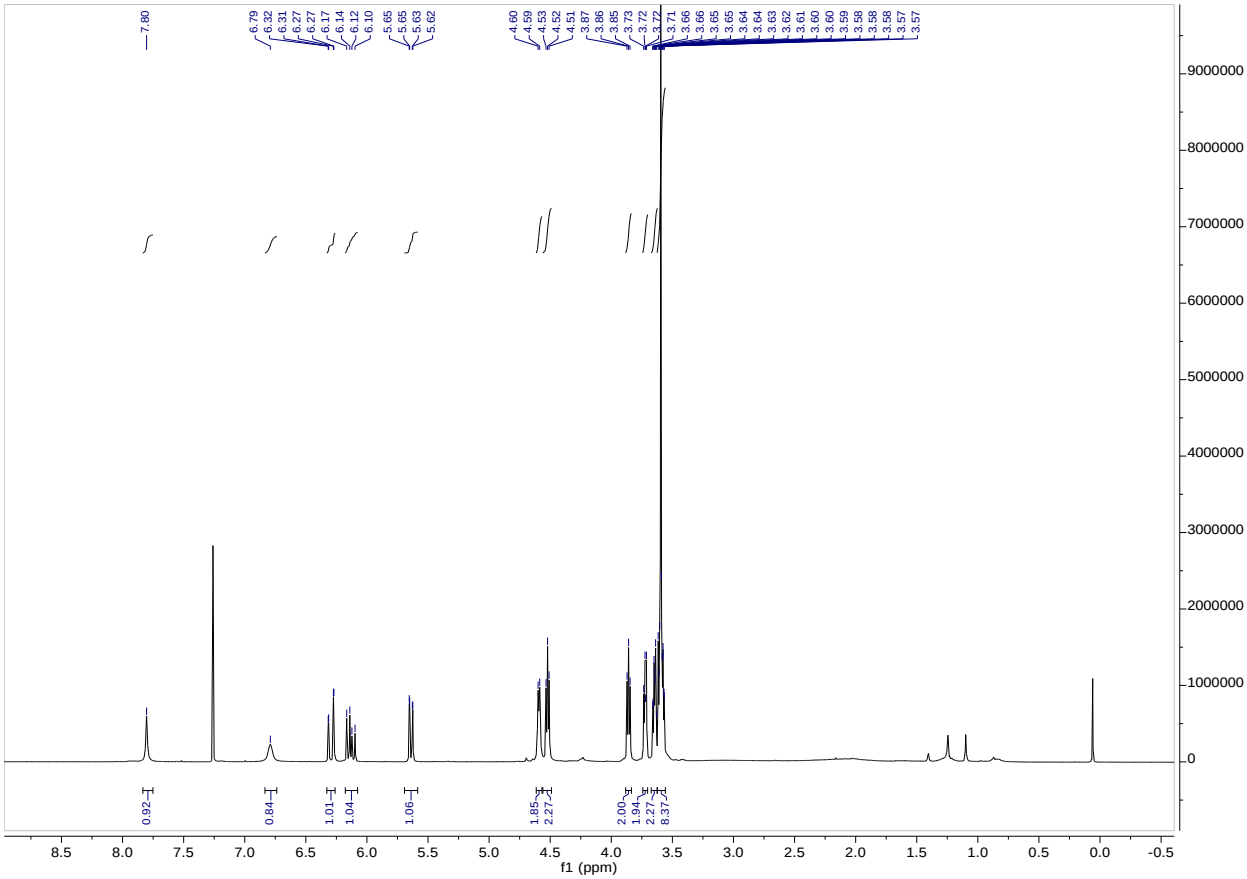
^{1}H NMR (400 MHz, Chloroform-d) δ 3.56 – 3.62 (8H, m, 3-H, 4-H), 3.63 – 3.67 (2H, m, 2-H), 3.69 – 3.75 (2H, m, 1-H), 3.86 (2H, t, *J* = 5.0 Hz, 5-H), 4.52 (2H, t, *J* = 5.0 Hz, 6-H), 4.59 (2H, d, *J* = 5.2 Hz, 9-H), 5.64 (1H, dd, *J* = 1.3, 10.2 Hz, 12-H), 6.13 (1H, dd, *J* = 10.2, 17.1 Hz, 13-H), 6.29 (1H, dd, *J* = 1.3, 17.1 Hz, 12-H), 6.79 (1H, s, 10-H), 7.80 (1H, s, 7-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 35.0 (C_9), 50.4 (C_6), 61.7 (C_1), 69.5 (C_5) , 70.4 (C_2, C_3, C_4) , 70.5 (C_2, C_3, C_4), 70.7 (2 x C_2, C_3, C_4), 72.7 (C_2, C_3, C_4), 123.8 (C_7), 126.9 (C_{12}), 130.7 (C_{13}), 144.2 (C_8), 165.7 (C_{11}).

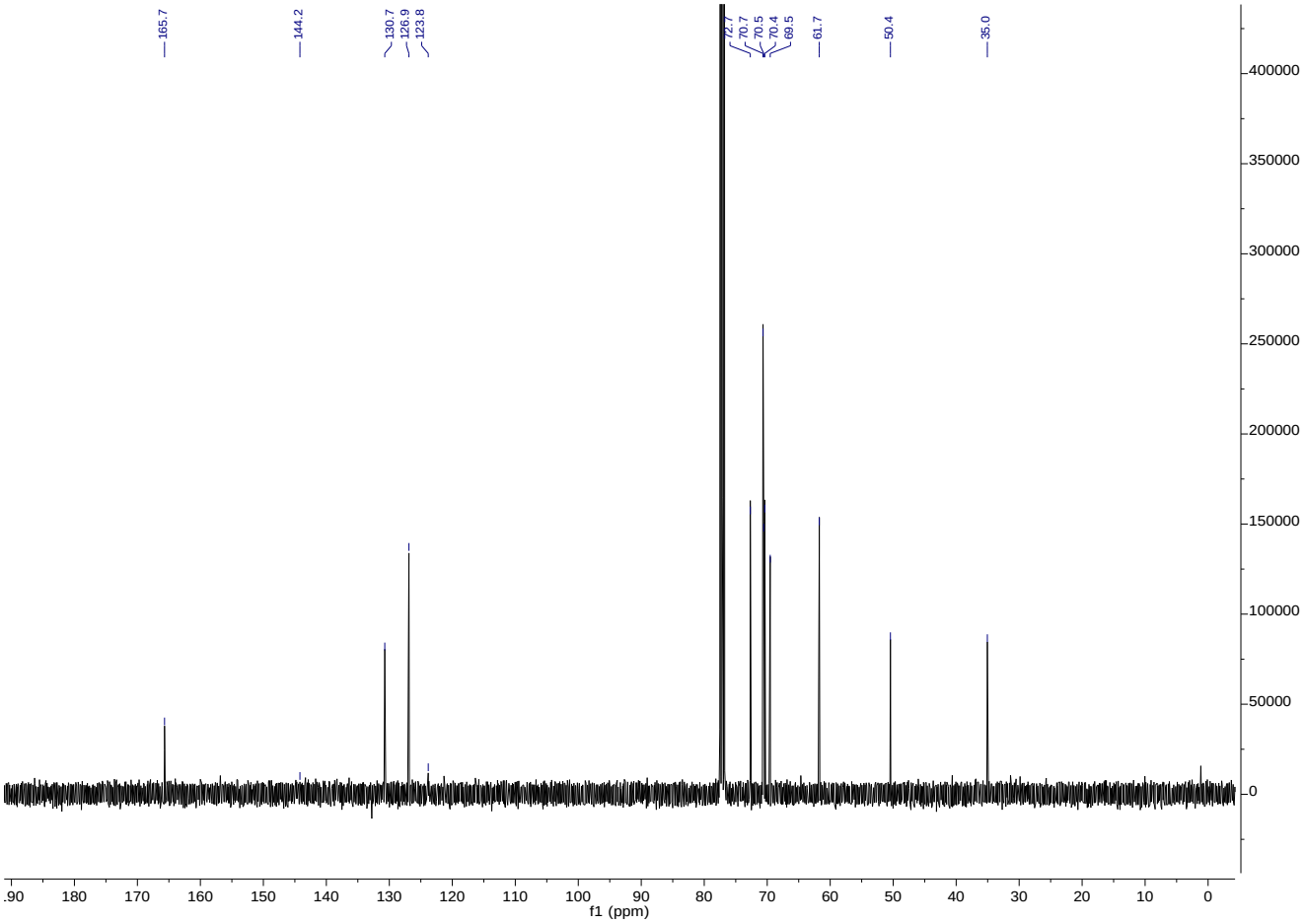
HRMS: *m/z* $[M + Na]^+$ calcd for $C_{14}H_{24}N_4NaO_5^+$ 351.1639; found 351.1641.

IR: (ν_{max}, cm^{-1}) 3314m, 2922m, 2877m, 1789w, 1663s, 1625m, 1542m, 1245m, 1466w, 1352w, 1118s, 1068s, 985w, 808w, 758m, 720w.

^{1}H NMR spectrum of **6**



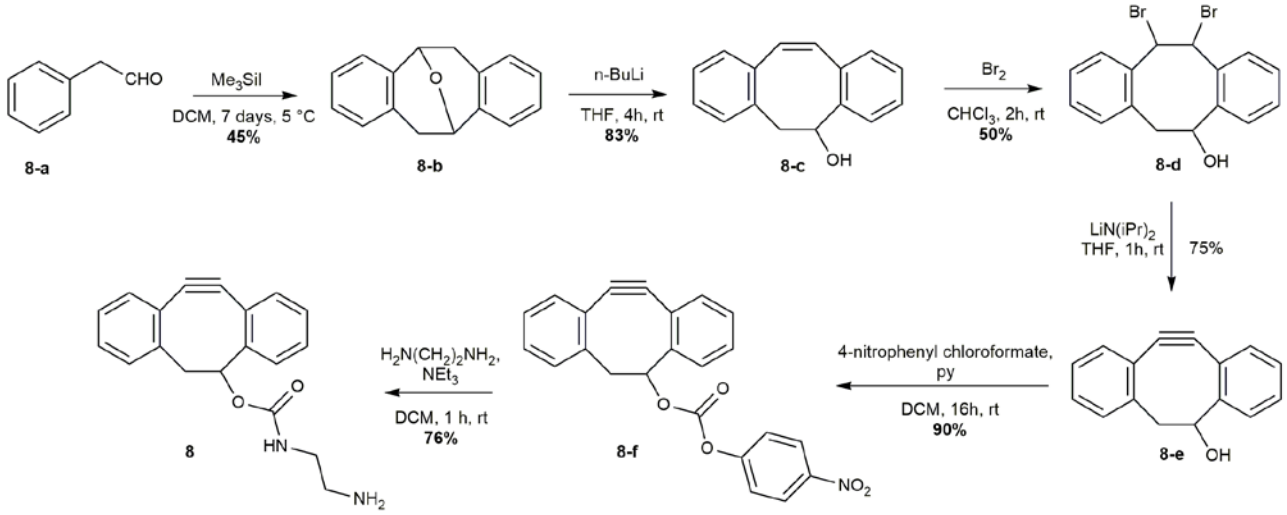
^{13}C NMR spectrum of **6**



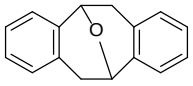
Preparation of DIBO derivative (8)

Adapted from protocols described in:
N. E. Mbua, J. Guo, M. A. Wolfert, R. Steet and G. J. Boons, *ChemBioChem*, 2011, **12**, 1912.
M. E. Jung and S. J. Miller, *J. Am. Chem. Soc*., 1981, **103**, 1984.
M. E. Jung, A. B. Mossman and M. A. Lyster, *J. Org. Chem*. 1978, **43**, 3698.

Scheme S3. Preparation of a DIBO derivative for click reaction at the surface of BFO HNPs



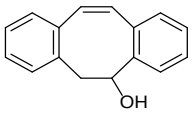
5,6,11,12-Tetrahydro-5,11-epoxydibenzo[a,e][8]annulene (**8-b**)



To a solution of 2-phenylacetaldehyde (10 mL, 90 mmol, 1.0 eq.) in DCM (45 mL), cooled to 0 °C under argon atmosphere, was added dropwise trimethylsilyl iodide (13 mL, 92 mmol, 1.02 eq.). The mixture was warmed to 5 °C and stirred for 7 days. A sat. aqueous solution of $Na_2S_2O_3$ (30 mL) and DCM (20 mL) were added and the reaction mixture was stirred until the iodine color vanished. The aqueous phase was extracted with DCM (2 x 25 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by FCC (PE / EtOAc 50:1 then 1:1) to afford **8-b** as a light brown solid (4.56 g, 20.5 mmol, 45%). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) 2.78 (2H, d, *J* = 16.2 Hz), 3.56 (2H, dd, *J* = 6.1, 16.2 Hz), 5.30 (2H, d, *J* = 6.0 Hz), 6.95 – 7.01 (2H, m), δ 7.05 – 7.17 (6H, m).

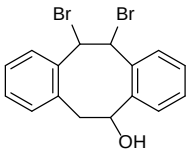
(*Z*)-5,6-dihydrodibenzo[a,e][8]annulen-5-ol (**8-c**)



To a solution of **8-b** (3.6 g, 16 mmol, 1 eq.) in THF (180 mL) under argon was added dropwise a 2.1 M solution of *n*-BuLi (15 mL, 32 mmol, 2 eq.) in pentane. The reaction mixture was stirred for 4h at rt. The reaction was quenched by addition of water and THF was removed under reduced pressure. The aqueous layer was extracted with DCM (3 x 25 mL) and the combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by FCC (PE / EtOAc 5:1) to afford **8-c** as a white solid (2.99 g, 13.5 mmol, 83%). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 3.28 – 3.52(2H, m), 5.28 (1H, dd, J = 6.0, 10.2 Hz), 6.79 – 6.93 (2H, m), 7.03 – 7.31 (6H, m), 7.39 – 7.53 (1H, m).

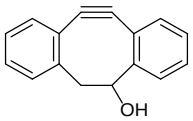
11,12-Dibromo-5,6,11,12-tetrahydrodibenzo[a,e][8]annulen-5-ol (**8-d**)



To a solution of **8-c** (843 mg, 3.8 mmol, 1 eq.) in $CHCl_3$ (11 mL) was added dropwise bromine (0.29 mL, 5.7 mmol, 1.5 eq.) and the reaction mixture was stirred for 2h at rt. The reaction was quenched by addition of a sat. aqueous solution of $Na_2S_2O_3$. The aqueous phase was extracted with $CHCl_3$ (3 x 5 mL) and the combined organic layers were washed with brine (5 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by FCC (PE / DCM 2:1) to afford **8-d** as a yellow oil (718 mg, 1.88 mmol, 50%). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 2.85 (1H, d, *J* = 16.4 Hz), 3.59 (1H, dd, *J* = 6.3, 16.4 Hz), 5.46 (1H, s), 5.79 (1H, d, *J* = 24.3 Hz), 5.87 (1H, d, *J* = 5.5 Hz), 6.82 – 7.23 (5H, m), 7.39 (1H, d, *J* = 7.0 Hz), 7.55 – 7.72 (m, 2H).

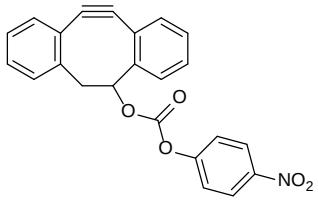
DIBO (**8-e**)



To a solution of **8-d** (518 mg, 1.4 mmol, 1 eq.) in THF (17 mL), freshly prepared $LiN(^iPr)_2$ (0.8 M in THF, 10 mL, 8.1 mmol, 5.8 eq.) was added dropwise at 0°C. The mixture was warmed up to rt and the reaction was stirred for 1h. The reaction was quenched with water (10 mL) and the volatiles were evaporated under reduced pressure. The aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were washed with water (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by FCC (DCM / PE 3:1) to afford **8-e** as a white solid (231 mg, 1.05 mmol, 75 %). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 2.94 (1H, dd, *J* = 3.8, 14.7 Hz), 3.11 (1H, dd, *J* = 2.2, 14.7 Hz), 4.65 (1H, d, *J* = 5.8 Hz), 7.27 – 7.47 (7H, m), 7.75 (1H, d, *J* = 7.8 Hz).

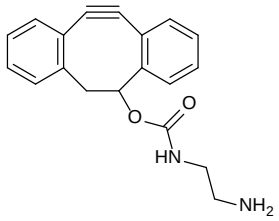
11,12-Didehydro-5,6-dihydrodibenzo[a,e][8]annulen-5-yl 4-nitrophenyl carbonate (**8-f**)



To a solution of **8-e** (500 mg, 2.3 mmol, 1 eq.) in DCM (66 mL) was added 4-nitrophenyl chloroformate (915 mg, 4.6 mmol, 2 eq.) and pyridine (0.92 mL, 11.4 mmol, 5 eq.) and the reaction mixture was stirred for 16 h at rt. The reaction mixture was washed with brine (2 x 10 mL) and the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by FCC (PE / EtOAc 5:1) to afford **8-f** as a white solid (784 mg, 2.03 mmol, 90 %). The analytical data were in accordance with previously reported data.

^{1}H NMR (400 MHz, Chloroform-d) δ 3.05 (1H, dd, *J* = 4.0, 15.4 Hz), 3.34 (1H, dd, *J* = 2.2, 15.4 Hz), 5.59 (1H, t, *J* = 3.1 Hz), 7.30 – 7.55 (9H, m), 7.62 (1H, dd, *J* = 1.1, 7.7 Hz), 8.28 (2H, d, *J* = 9.1 Hz).

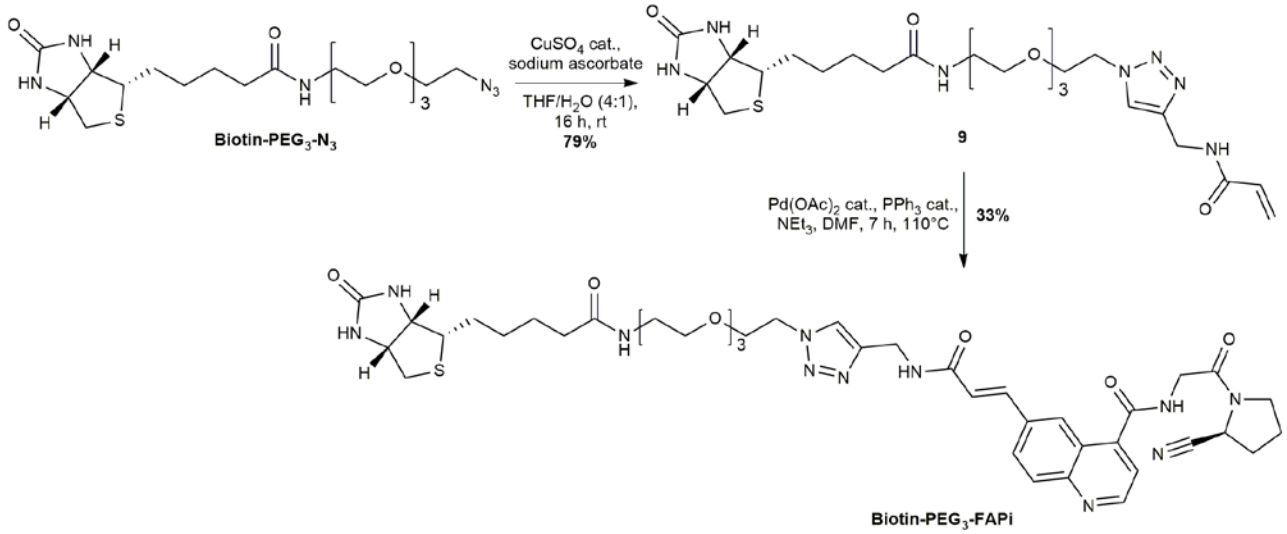
Compound **8**



To a solution of **8-f** (1 equiv, 0.26 mmol, 100 mg) in DCM (8 mL) were added NEt_3 (3 equiv, 0.78 mmol, 0.11 mL) and ethylenediamine (5 equiv, 1.3 mmol, 87 µL). The reaction mixture was stirred for 1 h at rt. The solution was concentrated *in vacuo* and the crude product was purified by FCC (PE/EtOAc 5:1) to afford **8** as a yellow solid (0.2 mmol, 61 mg, 76 %). The analytical data were in accordance with previously reported data.

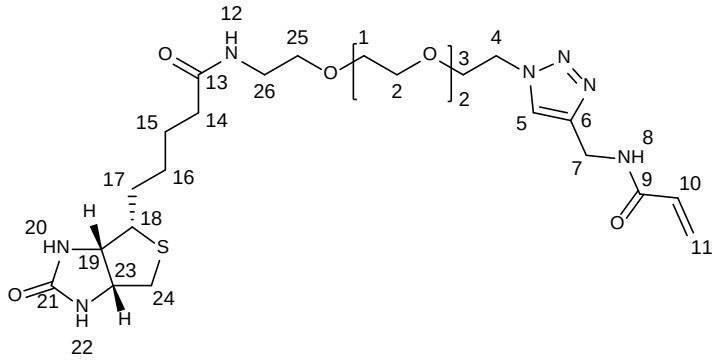
Preparation of Biotin-PEG$_3$-FAPi

Scheme S4. Preparation of **Biotin-PEG$_3$-FAPi**



N-(2-(2-(2-(2-(4-(acrylamidomethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**9**)

To a solution of **Biotin-PEG$_3$-N$_3$** (1 equiv., 0.46 mmol, 50 mg) and **6-b** (1 equi., 0.46 mmol, 205 mg) in THF (2 mL), was added a solution of $CuSO_4$ (10 mol%, 0.1 mmol, 7 mg) and sodium ascorbate (30 mol%., 0.3 mmol, 28 mg) in water (0.2 mL) and the mixture was stirred for 16h. The solvent was evaporated *in vacuo* and the crude product was purified by FCC (DCM/MeOH 10:1) to afford **9** as a white solid (0.36 mmol, 201 mg, 79%).



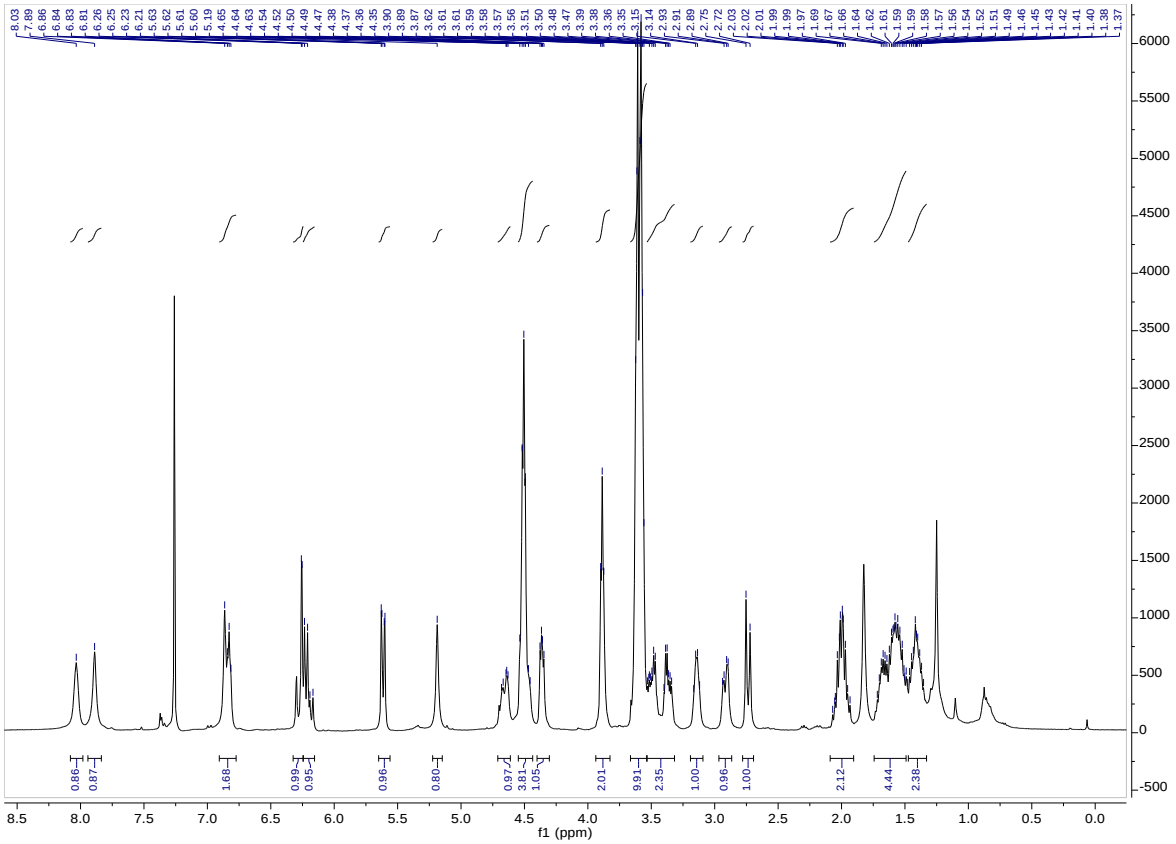
^{1}H NMR (400 MHz, Chloroform-d) δ 1.35 – 1.47 (2H, m, 15-H), 1.47 – 1.75 (4H, m, 16-H, 17-H), 1.91 – 2.10 (2H, m, 14-H), 2.74 (1H, d, *J* = 12.8 Hz, 24-H), 2.92 (1H, dd, *J* = 4.8, 12.9 Hz, 24-H), 3.14 (1H, q, *J* = 6.3 Hz, 18-H), 3.29 – 3.55 (2H, m, 26-H), 3.53 – 3.69 (10H, m, 1-H, 2-H, 25-H), 3.89 (2H, t, *J* = 5.0 Hz, 3-H), 4.36 (1H, dd, *J* = 4.7, 7.8 Hz, 19-H), 4.43 – 4.58 (4H, m, 24-H, 7-H), 4.61 – 4.72 (1H, m, 23-H), 5.19 (1H, s, N-H), 5.61 (1H, dd, *J* = 2.2, 9.5 Hz, 11-H), 6.20 (1H, dd, *J* = 9.6, 17.1 Hz, 10-H), 6.26 (1H, d, *J* = 2.3 Hz, 11-H), 6.70 – 6.93 (2H, m, 2 x N-H), 7.89 (1H, s, 5-H) 8.03 (1H, s, N-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 25.5 (C_{17}), 28.4 (C_{15} and C_{16}), 34.7 (C_{23}), 35.6 (C_{14}), 39.5 (C_{26}), 40.7 (C_{24}), 50.5 (C_9), 55.9 (C_{18}), 60.2 (C_4), 62.3 (C_{19}), 69.5 (C_3), 70.0, 70.3, 70.6, 70.7, 70.8 (CH_2O), 123.8 (C_5), 126.6 (C_{11}), 131.1 (C_{10}), 134.9 (C_q), 164.0 (C_q), 166.1 (C_q), (C_5), 173.4 (C_q).

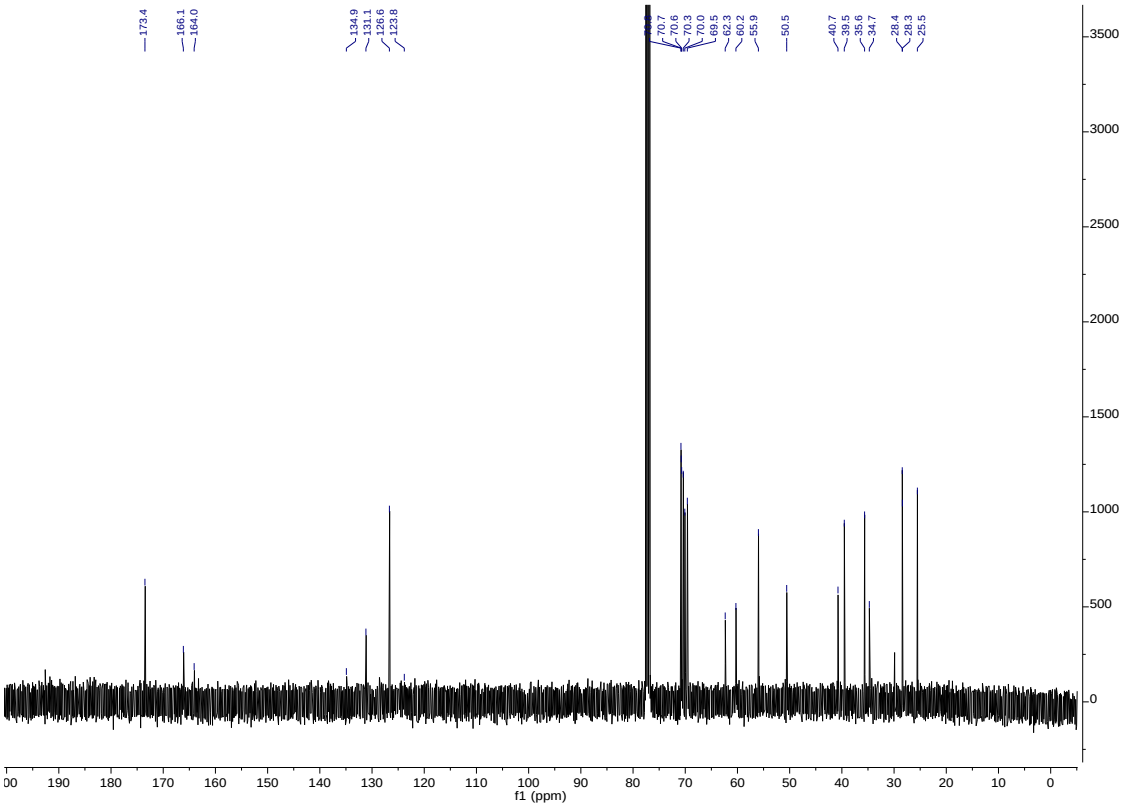
HRMS *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{39}N_7NaO_6S^+$ 576.2575; found 576.2588.

IR (ν_{max}, cm^{-1}): 3300m, 2925m, 2870m, 2110w, 2099w, 1940w, 1887w, 1801w, 1656s, 1544m, 1440m, 1323m, 1307m, 1265m, 1088m, 1037m, 976w, 920w, 867w, 834w, 760w.

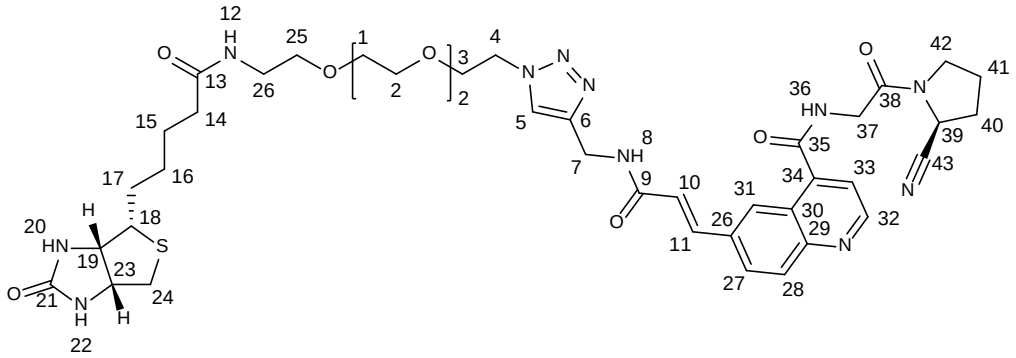
^{1}H NMR spectrum of **9**



^{13}C NMR spectrum of **9**



N-(2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-((E)-3-oxo-3-(((1-(13-oxo-17-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1H-1,2,3-triazol-4-yl)methyl)amino)prop-1-en-1-yl)quinoline-4-carboxamide (**Biotin-PEG$_3$-FAPi**)



To a solution of **5** (1 equiv, 0.1 mmol, 40 mg) and **9** (2 equiv., 0.2 mmol, 113 mg) in degassed DMF (0.4 mL) were added $Pd(OAc)_2$ (10 mol%, 0.01 mmol, 2 mg), PPh_3 (40 mol%, 0.04 mmol, 12 mg) and NEt_3 (5.5 equiv., 0.56 mmol). The solution was stirred in a Pyrex pressure resistant tube for 7 h at 110 °C. The solvent was evaporated *in vacuo* and the crude product was purified by FCC (DCM/MeOH 6:1) to afford **Biotin-PEG$_3$-FAPi** as a white solid (0.03 mmol, 28 mg, 33%).

^{1}H NMR (400 MHz, Chloroform-d) δ 1.42 – 1.67 (6H, m, 15-H, 16-H, 17-H), 1.93 – 2.07 (2H, m, 14-H), 2. 19 – 2.37 (4H, m, 40-H, 41-H), 2.49 (1H, d, *J* = 12.8 Hz, 24-H), 2.69 (1H, dd, *J* = 4.9, 13.0 Hz, 24-H), 2.86 – 2.93 (1H, m, 18-H), 3.30 – 3.51 (2H, m, 26-H), 3.50 – 3.67 (11H, m, 1-H, 2-H, 25-H, 42-H), 3. 74 – 3.82 (1H, m, 42-H), 3.81 – 3.92 (3H, m, 3-H, 19-H), 4.14 (1H, t, *J* = 6.4 Hz, 23-H), 4.32 (2H, dd, *J* =2.3, 5.5 Hz, 37-H), 4.47 – 4.54 (2H, m, 4-H), 4.58 (2H, t, *J* = 6.7 Hz, 7-H), 4.78 – 4.85 (1H, m, 39-H), 5.16 (1H, s, N-H), 6.63 (1H, s, N-H), 6.75

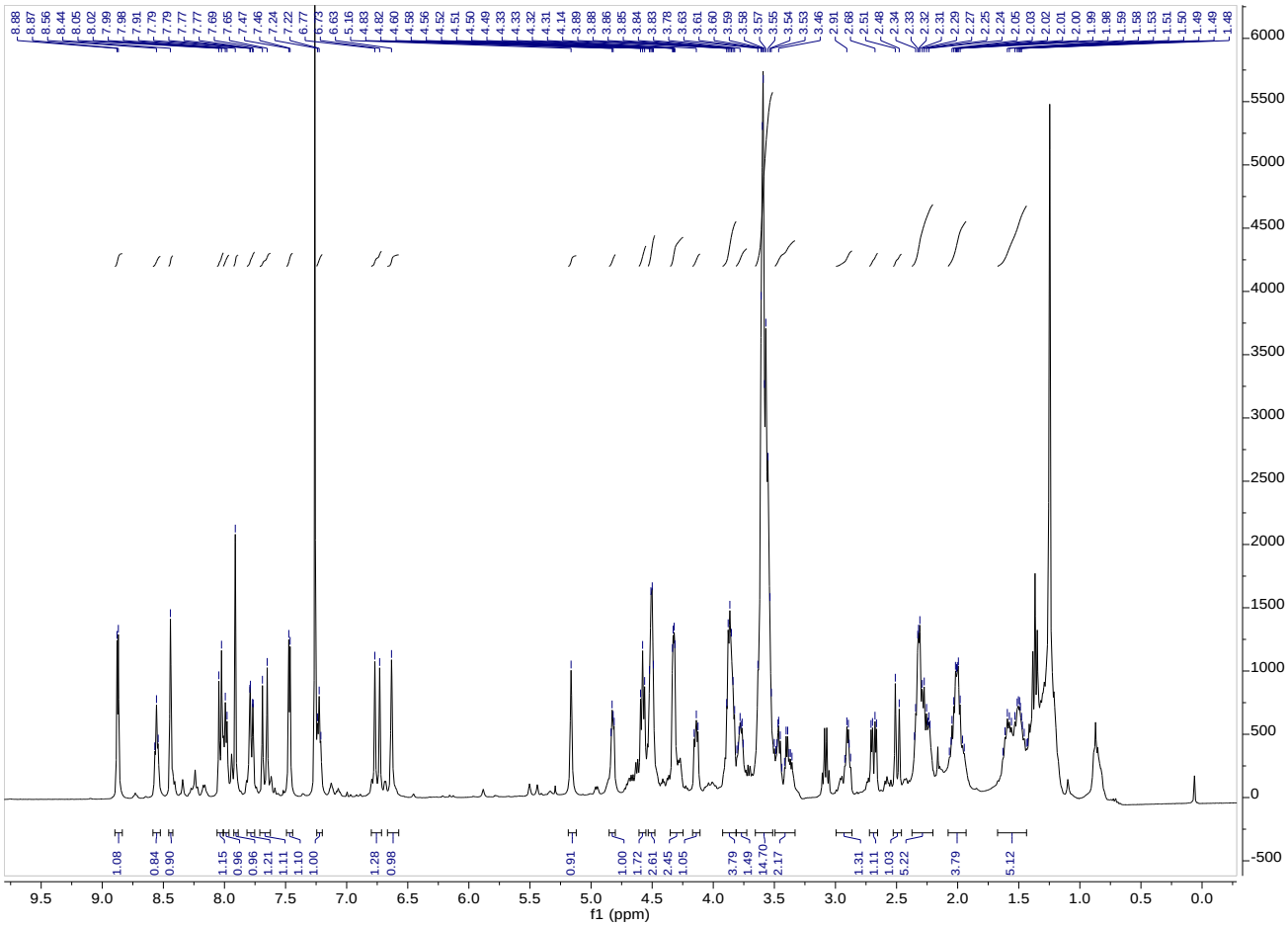
(1H, d, *J* = 15.7 Hz, 10-H), 7.22 (1H, t, *J* = 5.5 Hz, N-H), 7.47 (1H, d, *J* = 4.4 Hz, Ar-H), 7.67 (1H, d, *J* = 15.6 Hz, 11-H), 7.78 (1H, dd, *J* = 1.8, 8.9 Hz, Ar-H), 7.91 (1H, s, 5-H), 7.97 – 8.01 (1H, m, N-H), 8.03 (1H, d, *J* = 8.7 Hz, Ar-H), 8.44 (1H, s, Ar-H), 8.56 (1H, t, *J* = 5.5 Hz, N-H), 8.87 (1H, d, *J* = 4.3 Hz, Ar-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 25.4 (C_{40} or C_{41}), 25.6, 28.2, 28.4 (C_{15}, C_{16}, C_{17}), 29.8 (C_{40} or C_{41}), 34.9 (C_7), 35.8 (C_{14}), 39.5 (C_{26}), 40.6 (C_{24}), 42.4 (C_{37}), 46.1 (C_{42}), 47.1 (C_{39}), 50.4 (C_4), 55.6 (C_{18}), 60.0 (C_{23}), 61.9 (C_{19}), 69.4 (C_3), 70.0, 70.3, 70.6, 70.7 (CH_2O), 118.3 (C_q), 119.5 (C_{Ar}), 123.1 (C_{10}), 124.0 (C_5), 124.8 (C_{Ar}), 125.0 (C_q), 129.6 (C_{Ar}), 130.3 (C_{Ar}), 134.2 (C_q), 139.8 (C_{11}), 141.7 (C_q), 144.8 (C_q), 149.0 (C_q), 150.6 (C_{Ar}), 163.9 (C_q), 166.2 (C_q), 168.2 (C_q), 168.3 (C_q), 173.6 (C_q).

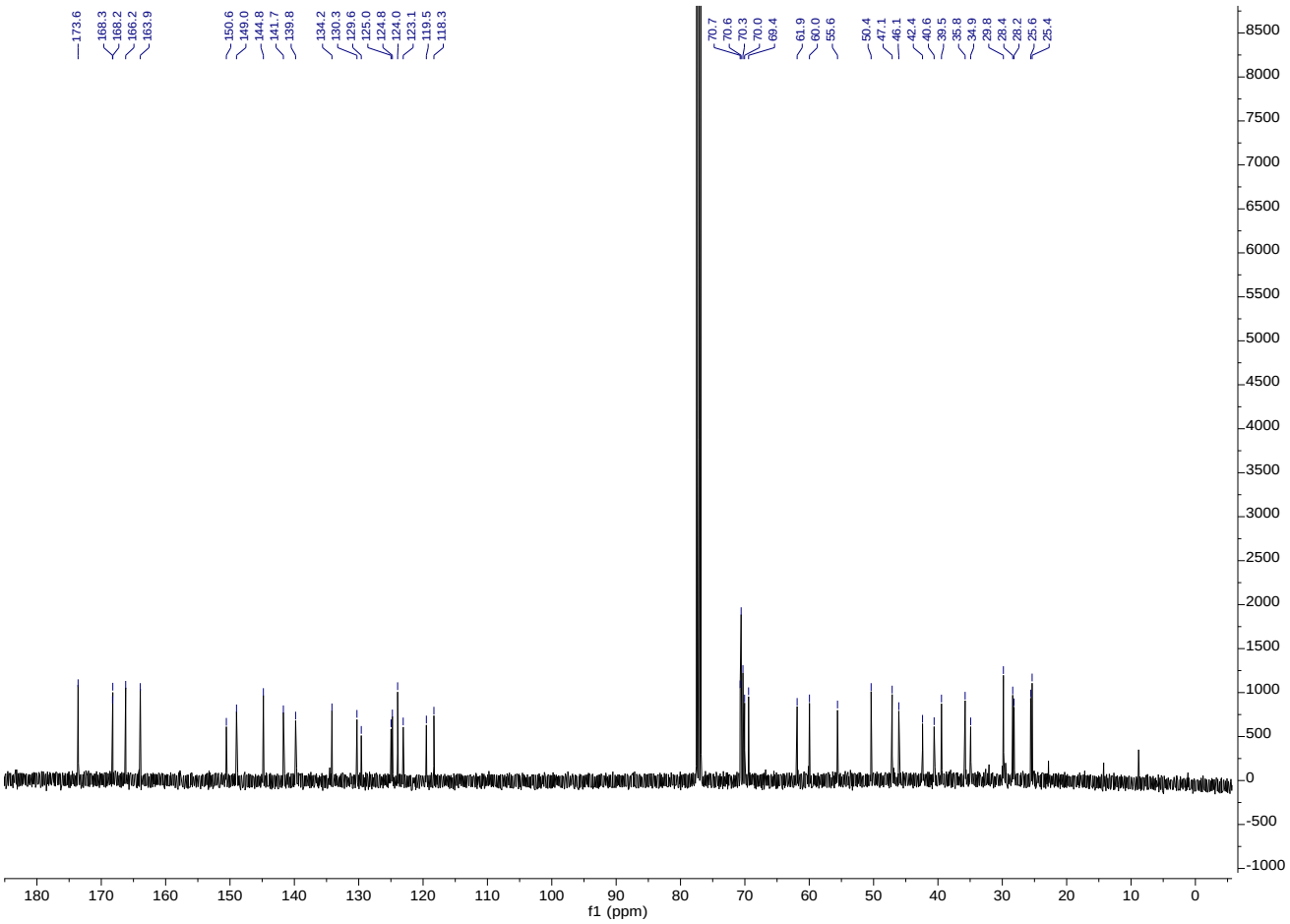
HRMS m/z: $[M + Na]^+$ calcd for $C_{41}H_{53}N_{11}NaO_8S^+$ 882.3691; found 882.3713.

IR: (ν_{max}, cm^{-1}) 3300m, 2925m, 2870m, 2110w, 2099w, 1940w, 1887w, 1801w, 1656s, 1544m, 1440m, 1323m, 1307m, 1265m, 1088m, 1037m, 976w, 920w, 867w, 834w, 760w

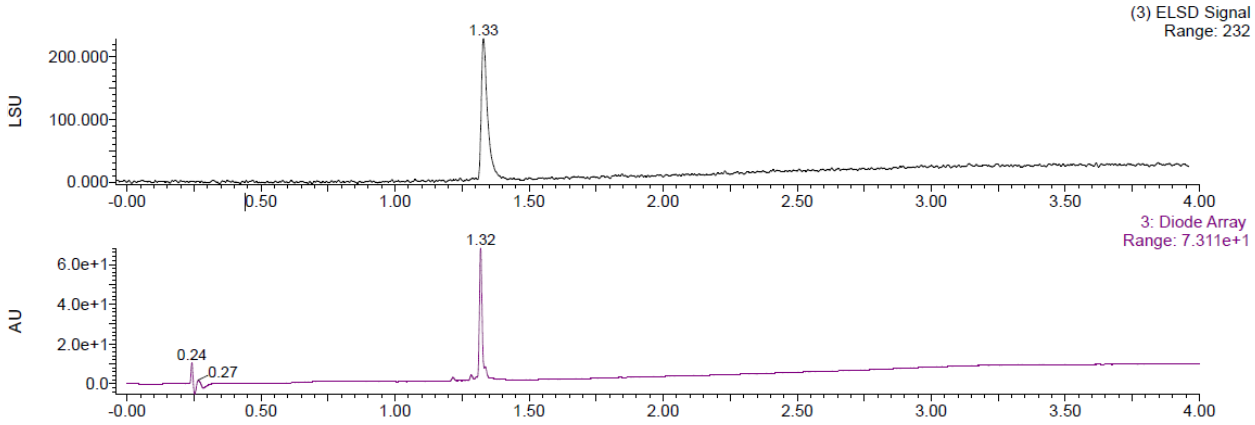
^{1}H NMR spectrum of **Biotin-PEG$_3$-FAPi**



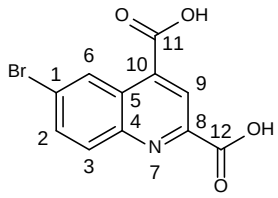
^{13}C NMR spectrum of **Biotin-PEG$_3$-FAPi**



Analytical UPLC trace of **Biotin-PEG$_3$-FAPi**



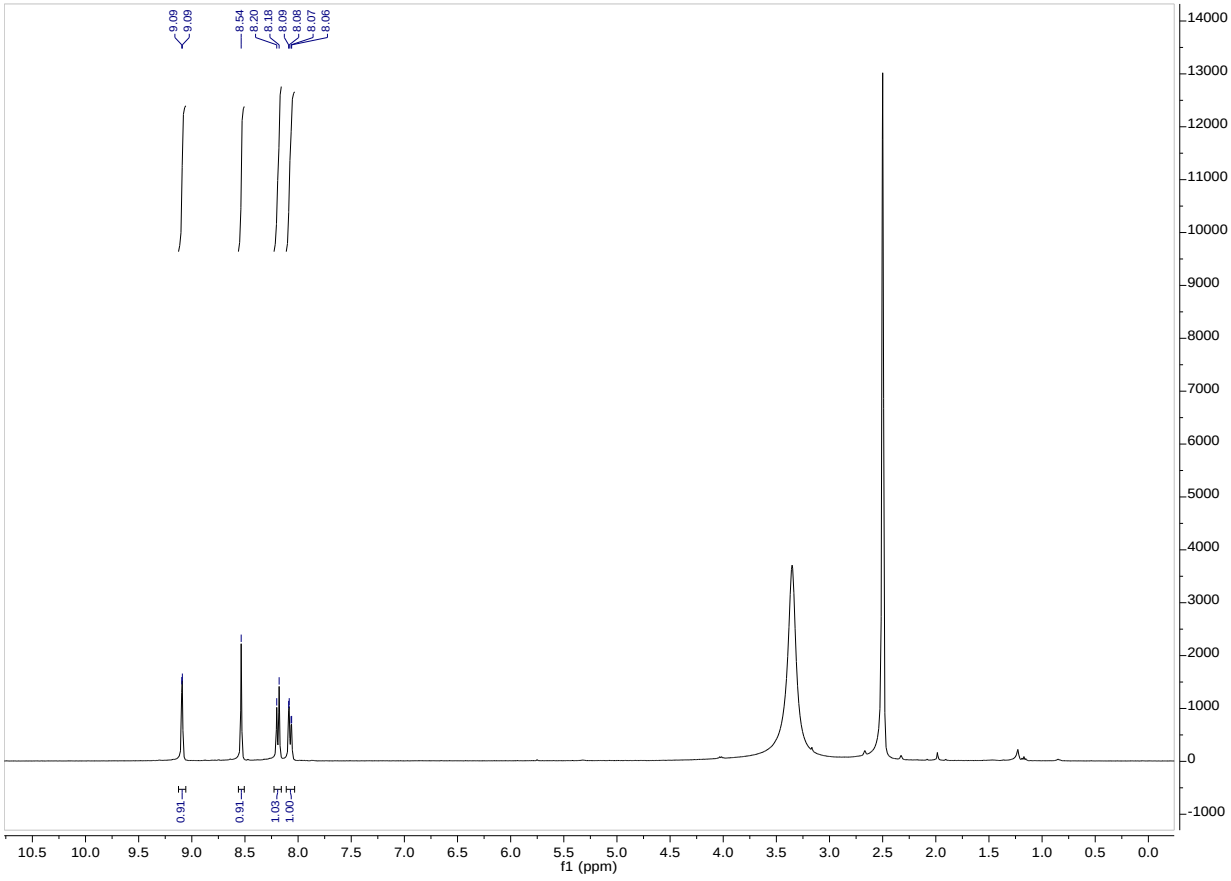
6-bromoquinoline-2,4-dicarboxylic acid (2)



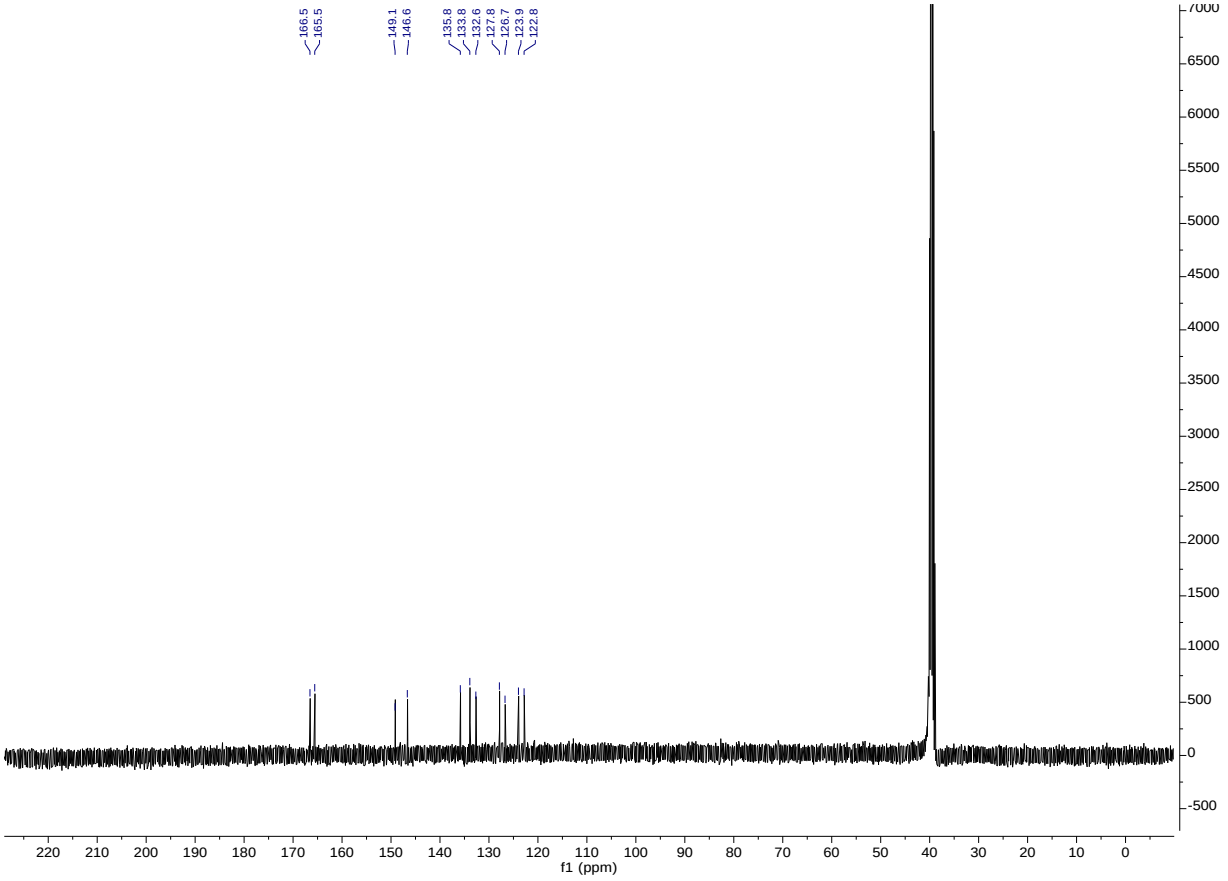
^{1}H NMR (400 MHz, DMSO-d6) δ 8.07 (1H, dd, *J* = 2.2, 9.0 Hz, 2-H), 8.19 (1H, d, *J* = 9.0 Hz, 3-H), 8.54 (1H, s, 9-H) 9.09 (1H, d, *J* = 2.2 Hz, 6-H).

^{13}C NMR (101 MHz, DMSO-d6) δ 122.8 (C_9), 123.9 (C_1). 126.7 (C_5), 127.8 (C_6), 132.6 (C_3), 133.8 (C_2), 135.8 (C_{10}), 146.6 (C_4), 149.1 (C_8), 165.5 (C_{11}), 166.5 (C_{12}).

^{1}H NMR spectrum of **2**

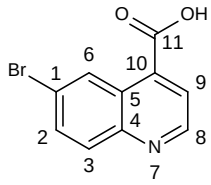


^{13}C NMR spectrum of **2**



6-bromoquinoline-4-carboxamide (3)

Intermediate 6-bromoquinoline-4-carboxylic acid



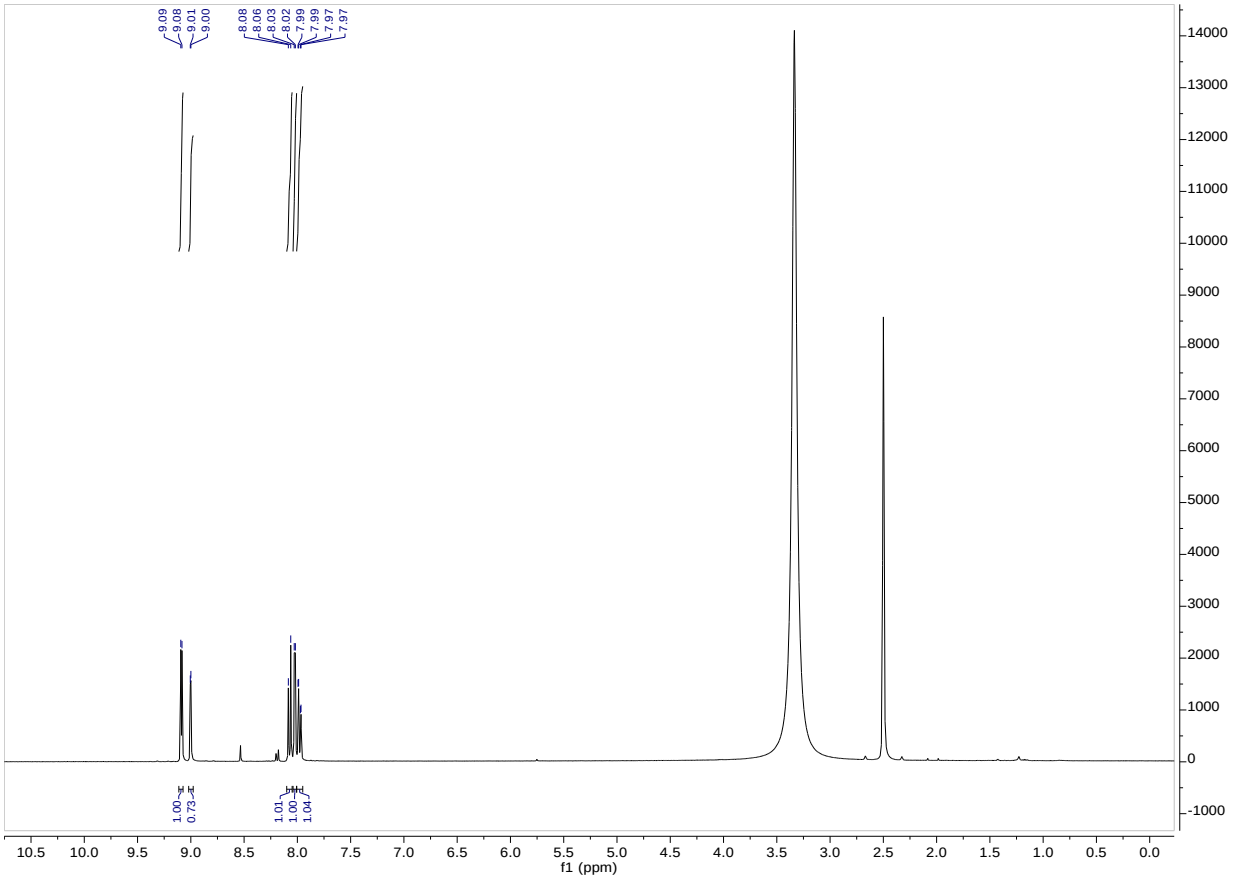
^{1}H NMR (400 MHz, DMSO-d6) δ 7.98 (1H, dd, *J* = 2.3, 9.0 Hz, 2-H), 8.02 (1H, d, *J* = 4.4 Hz, 9-H), 8.07 (1H, d, *J* = 9.0 Hz, 3-H), 9.00 (1H, d, *J* = 2.3 Hz, 6-H), 9.09 (1H, d, *J* = 4.4 Hz, 8-H).

^{13}C NMR (101 MHz, DMSO-d6) δ 121.6 (C_1), 123.3 (C_9), 125.7 (C_5), 127.7 (C_6), 131.8 (C_3), 132.9 (C_2), 134.5 (C_{10}), 147.1 (C_4), 151.2 (C_8), 167.0 (C_{11}).

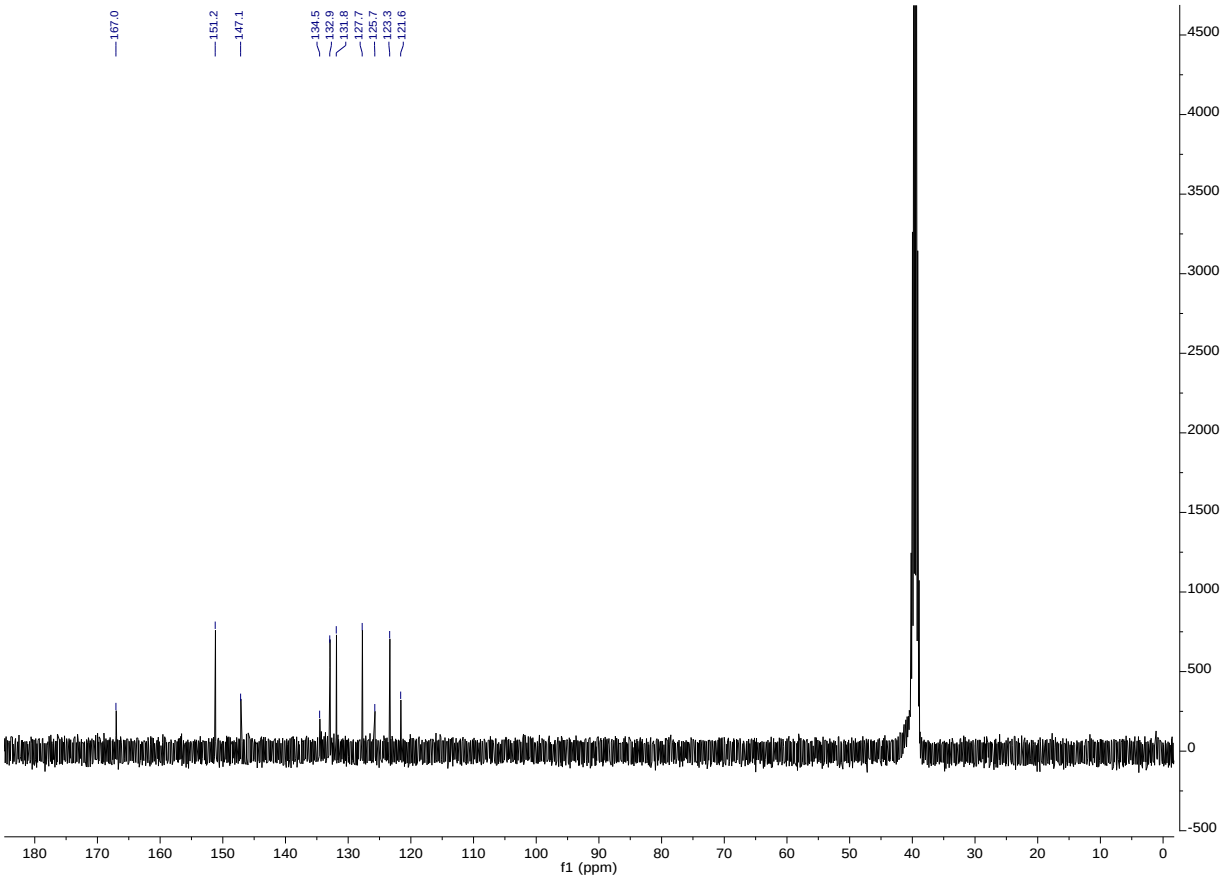
HRMS: *m/z* $[M + H\text{-}1]^-$ calcd for $C_{10}H_5BrNO_2^-$ 249.9509; found 249.9507.

IR: (ν_{max}, cm^{-1}): 2419w, 1948w, 1911w, 1707m, 1595m, 1495m, 1431w, 1414w, 1349m, 1322m, 1264m, 1196m, 1145w, 1076m, 1055m, 1018m, 987w, 925m, 876m, 839m, 820m, 788m, 746s, 723s.

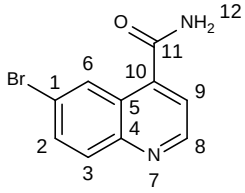
^{1}H NMR spectrum of 6-bromoquinoline-4-carboxylic acid



^{13}C NMR spectrum of 6-bromoquinoline-4-carboxylic acid



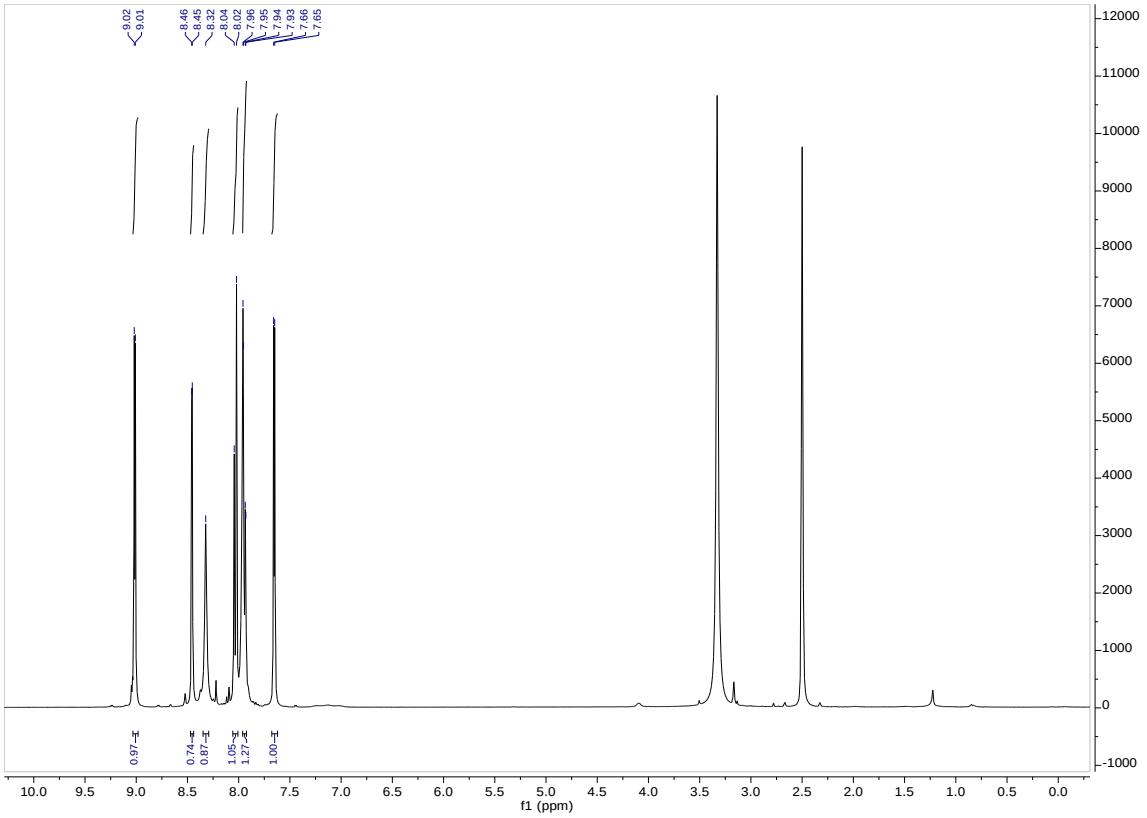
Compound **3**



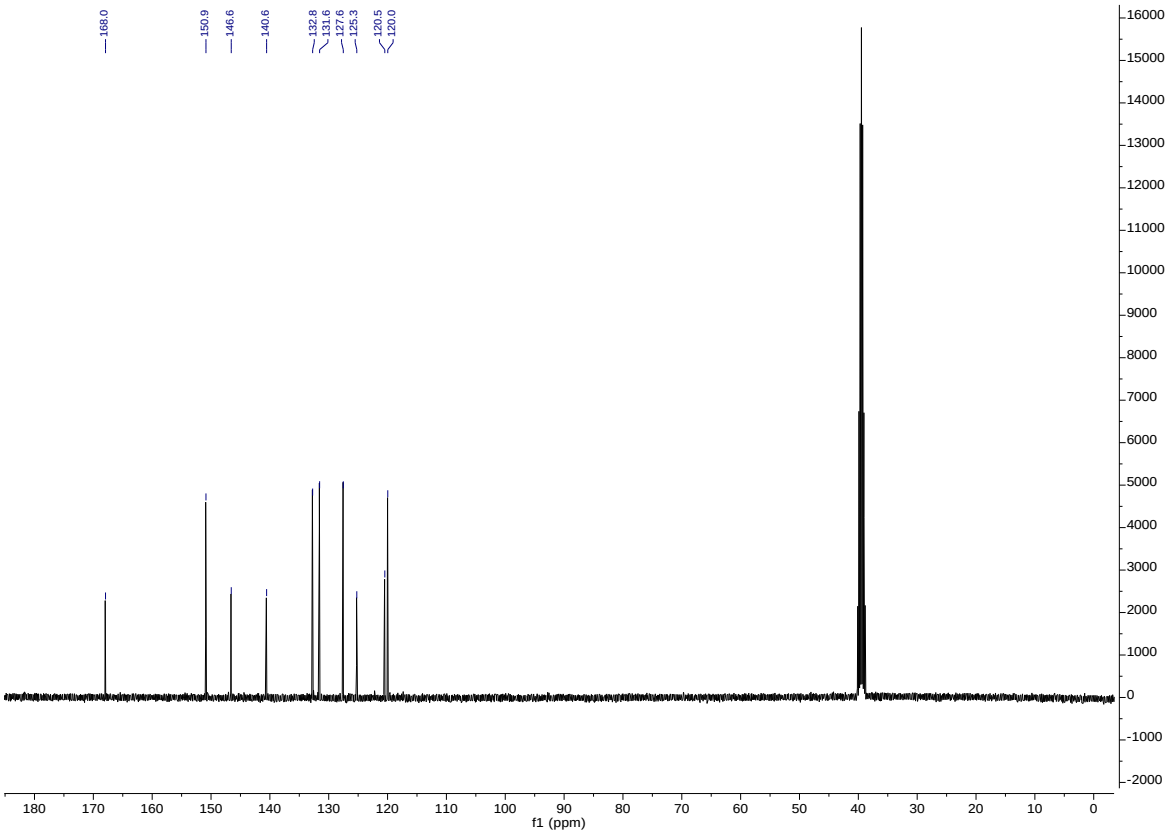
^{1}H NMR (400 MHz, DMSO-d6) δ 7.65 (1H, d, *J* = 4.3 Hz, 9-H), 7.94 (1H, dd, *J* = 2.3, 9.0 Hz, 2-H), 8.03 (1H, d, *J* = 9.0 Hz, 3-H), 8.32 (2H, s, 12-H), 8.46 (1H, d, *J* = 2.3 Hz, 6-H), 9.01 (1H, d, *J* = 4.4 Hz, 8-H).

^{13}C NMR (101 MHz, DMSO-d6) δ 120.0 (C_9), 120.5 (C_1), 125.3 (C_5), 127.6 (C_6), 131.6 (C_3), 132.8 (C_2), 140.6 (C_{10}), 146.6 (C_4), 150.9 (C_8), 168.0 (C_{11}).

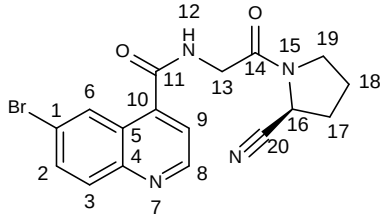
^{1}H NMR spectrum of **3**



^{13}C NMR spectrum of **3**



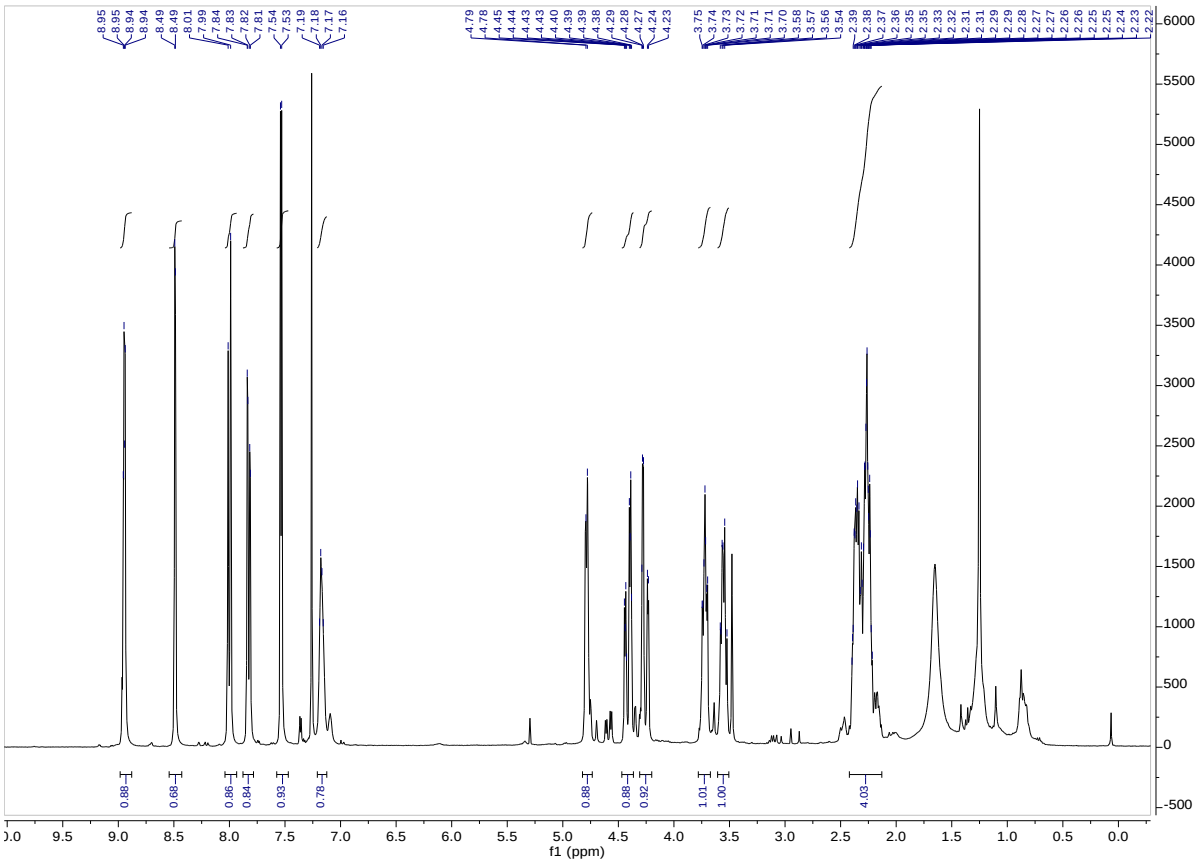
(*S*)-6-bromo-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (5)



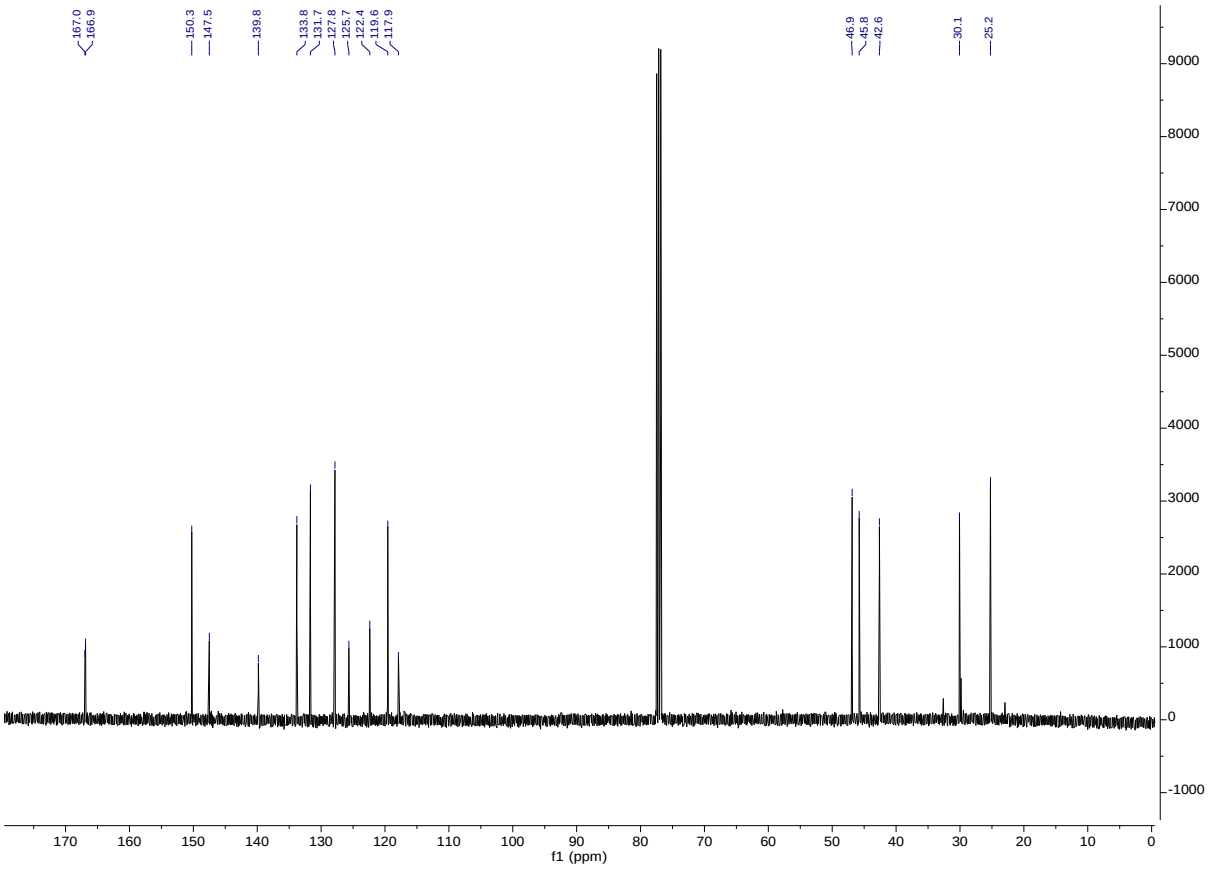
^{1}H NMR (400 MHz, Chloroform-d) δ 2.19 – 2.43 (4H, m, 17-H, 18-H), 3.51 – 3.59 (1H, m, 19-H), 3. 69 – 3.76 (1H, m, 19-H), 4.26 (1H, dd, *J* = 3.6, 17.8 Hz, 13-H), 4.41 (1H, ddd, *J* = 2.8, 4.9, 17.8 Hz, 13-H), 4.79 (1H, d, *J* = 5.2 Hz, 16-H), 7.18 (1H, s, 12-H), 7.54 (1H, d, *J* = 4.3 Hz, 9-H), 7.83 (1H, dd, *J* = 2.2, 9.0 Hz, 2-H), 8.00 (1H, d, *J* = 9.0 Hz, 3-H), 8.49 (1H, d, *J* = 2.2 Hz, 6-H) 8.95 (1H, dd, *J* = 2.3, 4.4 Hz, 8-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 25.2 (C_{18}), 30.1 (C_{17}), 42.6 (C_{13}), 45.8 (C_{19}), 46.9 (C_{16}), 117.9 (C_{20}), 119.6 (C_{9}), 122.4 (C_{1}), 125.7 (C_{5}), 127.8 (C_{6}), 131.7 (C_{3}), 133.8 (C_{2}), 139.8 (C_{10}), 147.5 (C_{4}), 150.3 (C_{8}), 166.9 (C_{11}), 167.0 (C_{14}).

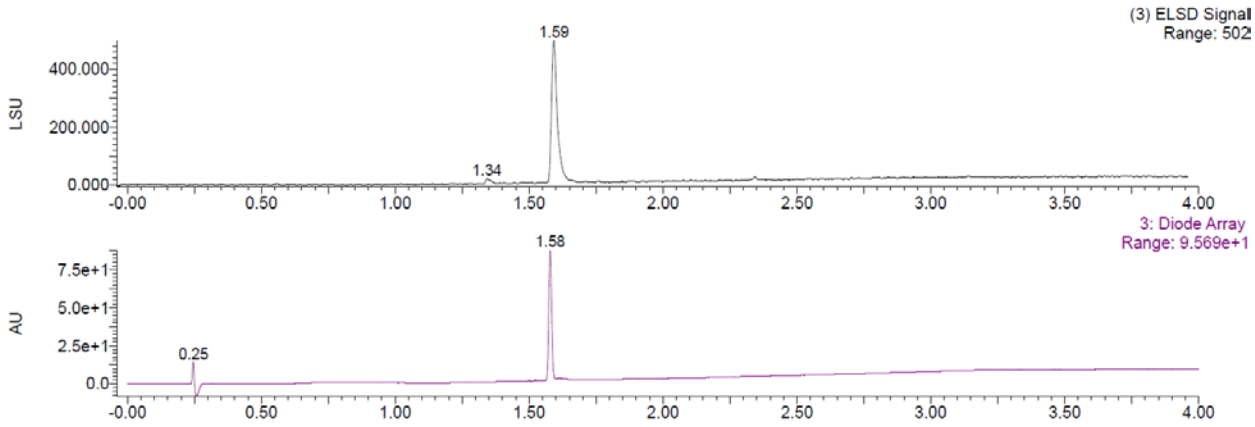
^{1}H NMR spectrum of **5**



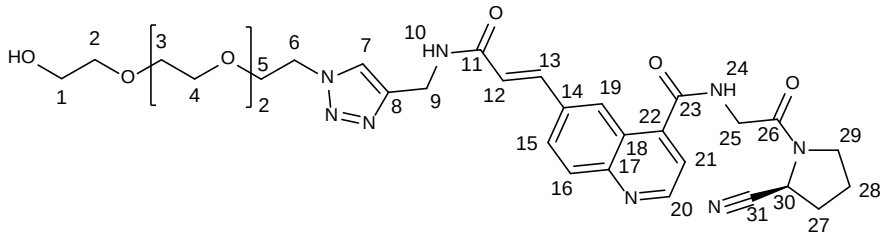
^{13}C NMR spectrum of **5**



Analytical UPLC trace of compound **5**



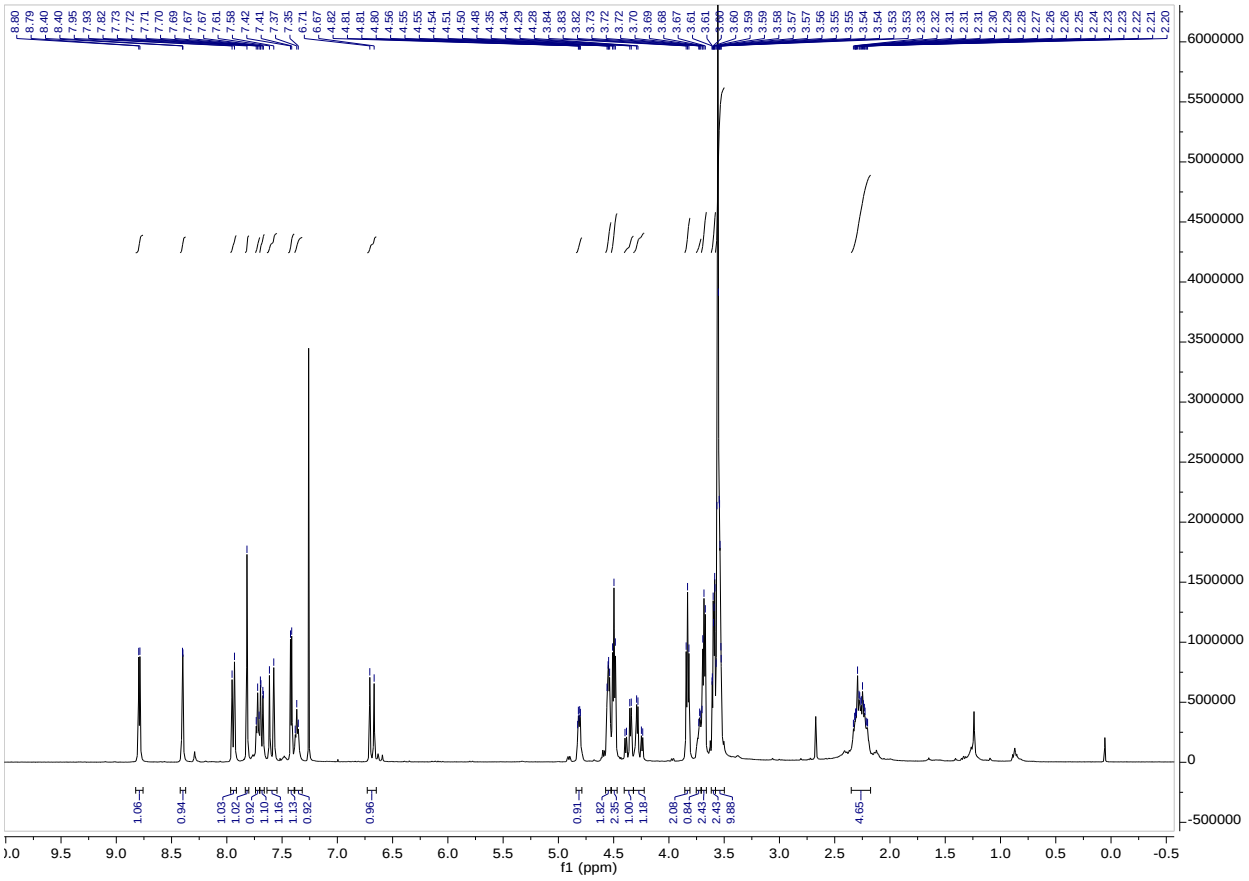
(*S*,*E*)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(3-(((1-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-3-oxoprop-1-en-1-yl)quinoline-4-carboxamide (7)



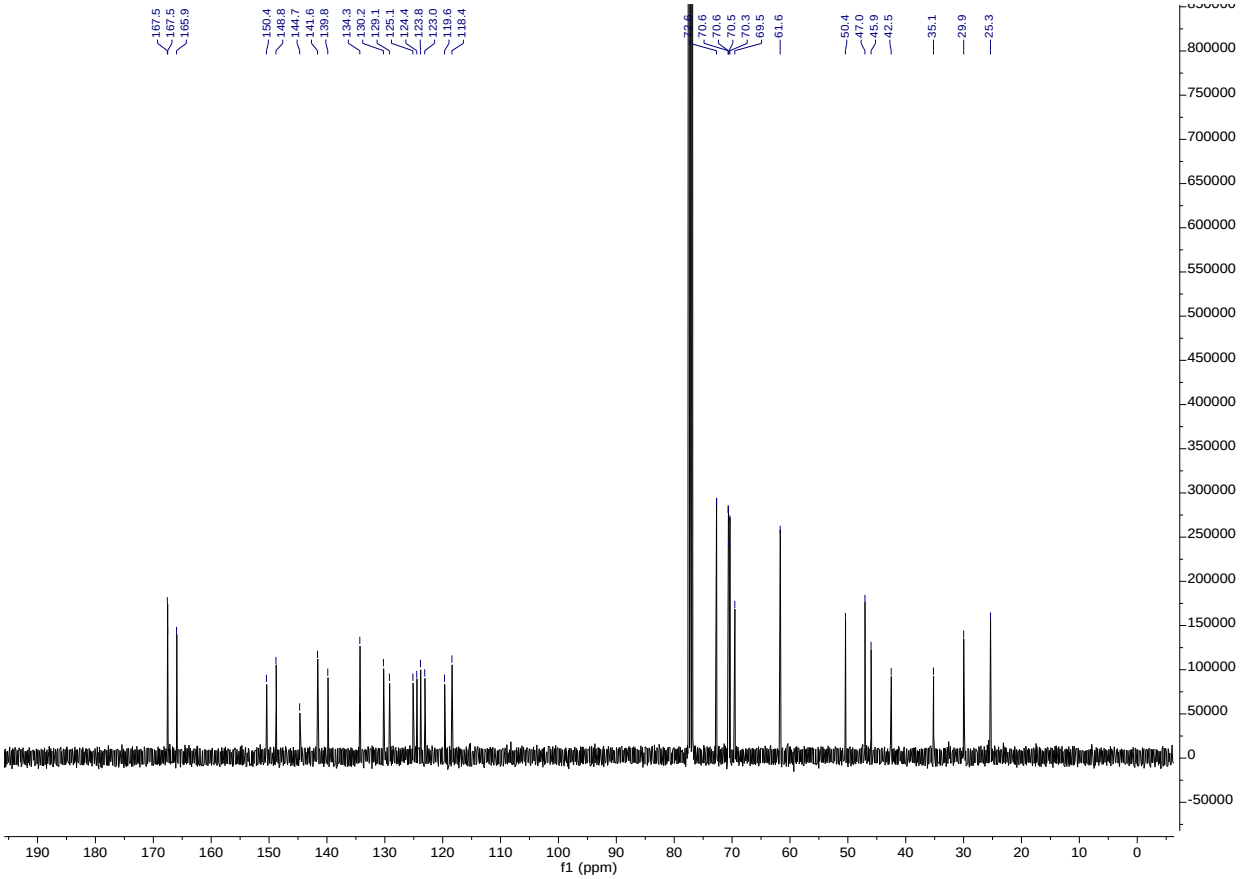
^{1}H NMR (400 MHz, Chloroform-d) δ 2.17 – 2.38 (4H, m, 27-H, 28-H), 3.48 – 3.62 (11H, m, 2-H, 3-H, 4-H, 29-H), 3. 64 – 3.72 (2H, m, 1-H), 3.70 – 3.76 (1H,m, 29-H), 3.83 (t, *J* = 5.0 Hz, 2H, 5-H), 4.26 (1H, dd, *J* = 4.8, 17.3 Hz, 25-H), 4.37 (1H, dd, *J* = 5.4, 17.3 Hz, 25-H), 4.50 (2H, t, *J* = 5.0 Hz, 6-H), 4.55 (2H, dd, *J* = 3.7, 5.6 Hz, 9-H), 4.79 – 4.84 (1H, m, 30-H), 6.69 (1H, d, *J* = 15.7 Hz, 12-H), 7.37 (1H, t, *J* = 5.6 Hz, N-H), 7.42 (1H, d, *J* = 4.3 Hz, Ar-H), 7.59 (1H, d, *J* = 15.6 Hz, 13-H), 7.68 (1H, dd, *J* = 1.8, 8.9 Hz, Ar-H), 7.72 (1H, t, *J* = 5.2 Hz, N-H), 7.82 (1H, s, 7-H), 7.94 (1H, d, *J* = 8.8 Hz, Ar-H), 8.40 (1H, d, *J* = 1.5 Hz, Ar-H), 8.79 (1H, d, *J* = 4.3 Hz, Ar-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 25.3, 29.9 (C_{27}, C_{28}), 35.1 (C_9), 42.5 (C_{25}), 45.9 (C_{29}), 47.0 (C_{30}), 50.4 (C_6), 61.6 (C_1), 69.5 (C_5), 70.3, 70.5, 70.60, 70.62, 72.6 (CH_2O), 118.4 (C_q), 119.6 (C_{Ar}), 123.0 (C_{12}), 123.8 (C_7), 124.4 (C_q), 125.1 (C_{Ar}), 129.1 (C_{Ar}), 130.2 (C_{Ar}), 134.3 (C_q), 139.8 (C_{13}), 141.6 (C_q), 144.7 (C_q), 148.8 (C_q), 150.4 (C_{Ar}), 165.9 (C_q), 167.46 (C_q), 167.51 (C_q).

^{1}H NMR spectrum of **7**

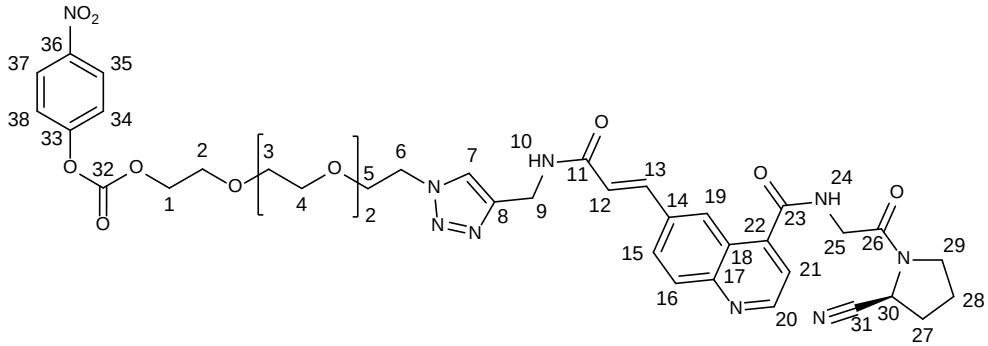


^{13}C NMR spectrum of **7**



DIBO-PEG$_3$-FAPi

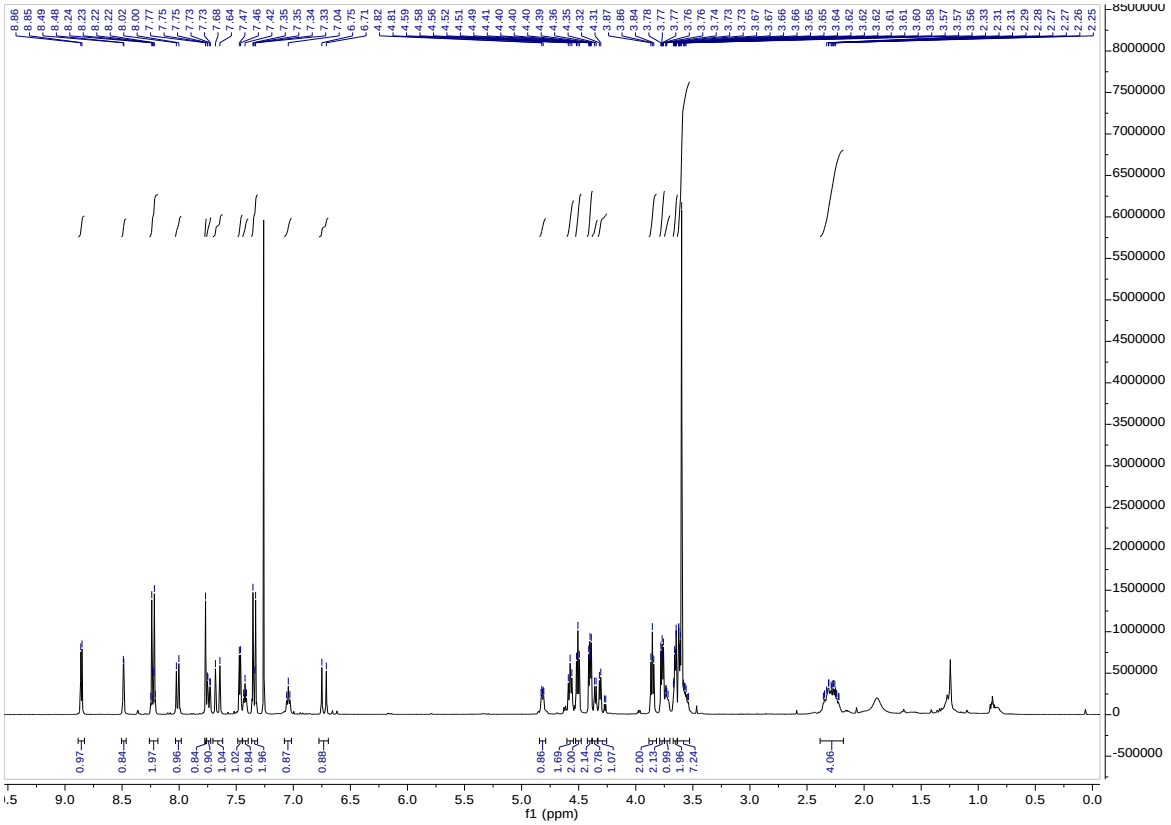
Intermediate (*S*,*E*)-2-(2-(2-(2-(4-((3-(4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)acrylamido)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl (4-nitrophenyl) carbonate



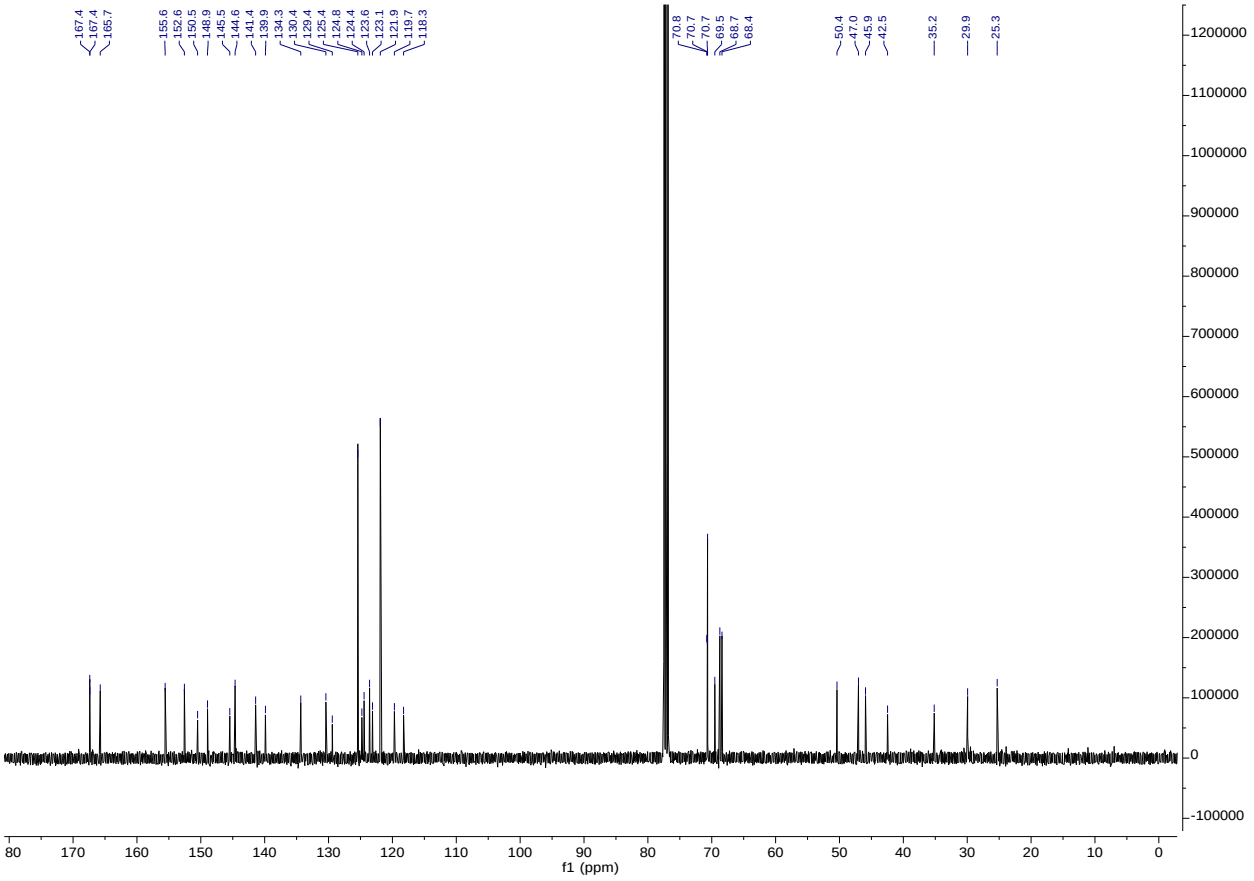
^{1}H NMR (400 MHz, Chloroform-d) δ 2.19 – 2.39 (4H, m, 27-H, 28-H), 3.52 – 3.63 (7H, m, 3-H, 4-H, 29-H), 3.64 – 3.68 (2H, m, 3-H, 4-H), 3.70 – 3.75 (1H, m, 29-H), 3.75 – 3.79 (2H, m, 2-H), 3.86 (2H, t, *J* = 5.1 Hz, 5-H), 4.29 (1H, dd, *J* = 4.9, 17.5 Hz, 25-H), 4.34 – 4.39 (1H, m, 25-H), 4.39 – 4.42 (2H, m, 1-H), 4.51 (2H, t, *J* = 5.1 Hz, 6-H), 4.58 (2H, t, *J* = 6.2 Hz, 9-H), 4.79 – 4.84 (1H,m, 30-H), 6.73 (1H, d, *J* = 15.7 Hz, 12-H), 7.04 (1H, t, *J* = 5.7 Hz, N-H), 7.32 – 7.36 (2H, m, Nitrophenyl-H), 7.42 (1H, t, *J* = 5.0 Hz, N-H), 7.47 (1H, d, *J* = 4.3 Hz, Quinoline-H), 7.66 (1H, d, *J* = 15.6 Hz, 13-H), 7.74 (1H, dd, *J* = 1.9, 8.8 Hz, Quinoline-H), 7.77 (1H, s, 7-H), 8.01 (1H, d, *J* = 8.7 Hz, Quinoline-H), 8.20 – 8.27 (2H, m, Nitrophenyl-H), 8.49 (1H, d, *J* = 1.9 Hz, Quinoline-H), 8.86 (1H, d, *J* = 4.3 Hz, Quinoline-H).

^{13}C NMR (101 MHz, Chloroform-d) δ 25.3 29.9, (C_{27}, C_{28}), 35.2 (C_9), 42.5 (C_{25}), 45.9 (C_{29}), 47.0 (C_{30}), 50.4 (C_6), 68.4 (C_2), 68.7 (C_1), 69.5 (C_5), 70.66, 70.69, 70.8 (CH_2O), 118.3 (C_q), 119.7 ($C_{Quinoline}$), 121.9 (2 x $C_{Nitrophenyl}$), 123.1 (C_{12}), 123.6 (C_7), 124.4 (C_q), 124.8 ($C_{Quinoline}$), 125.4 (2 x $C_{Nitrophenyl}$), 129.4 ($C_{Quinoline}$), 130.4 ($C_{Quinoline}$), 134.3 (C_q), 139.9 (C_{13}), 141.4 (C_q), 144.6 (C_q), 145.5 (C_q), 148.9 (C_q), 150.5 ($C_{Quinoline}$), 152.6 (C_q), 155.6 (C_q), 165.7 (C_q), 167.37 (C_q), 167.39 (C_q).

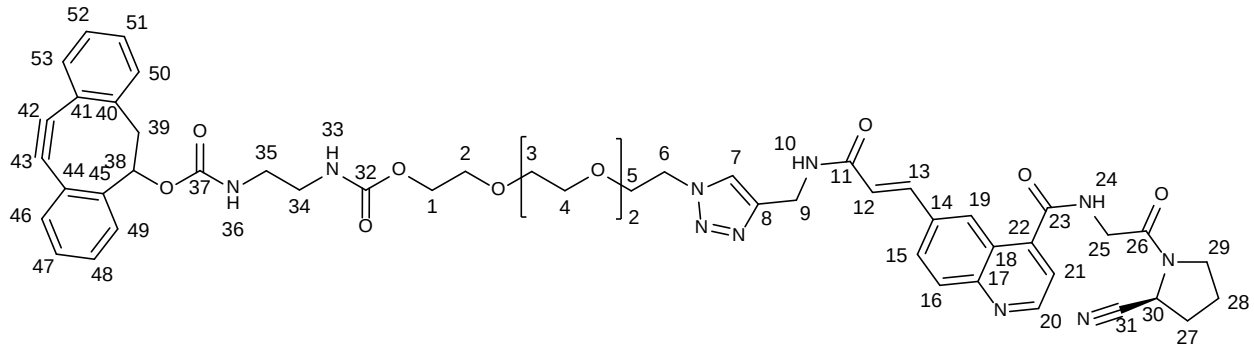
^{1}H NMR spectrum of intermediate



^{13}C NMR spectrum of intermediate



DIBO-PEG$_3$-FAPi

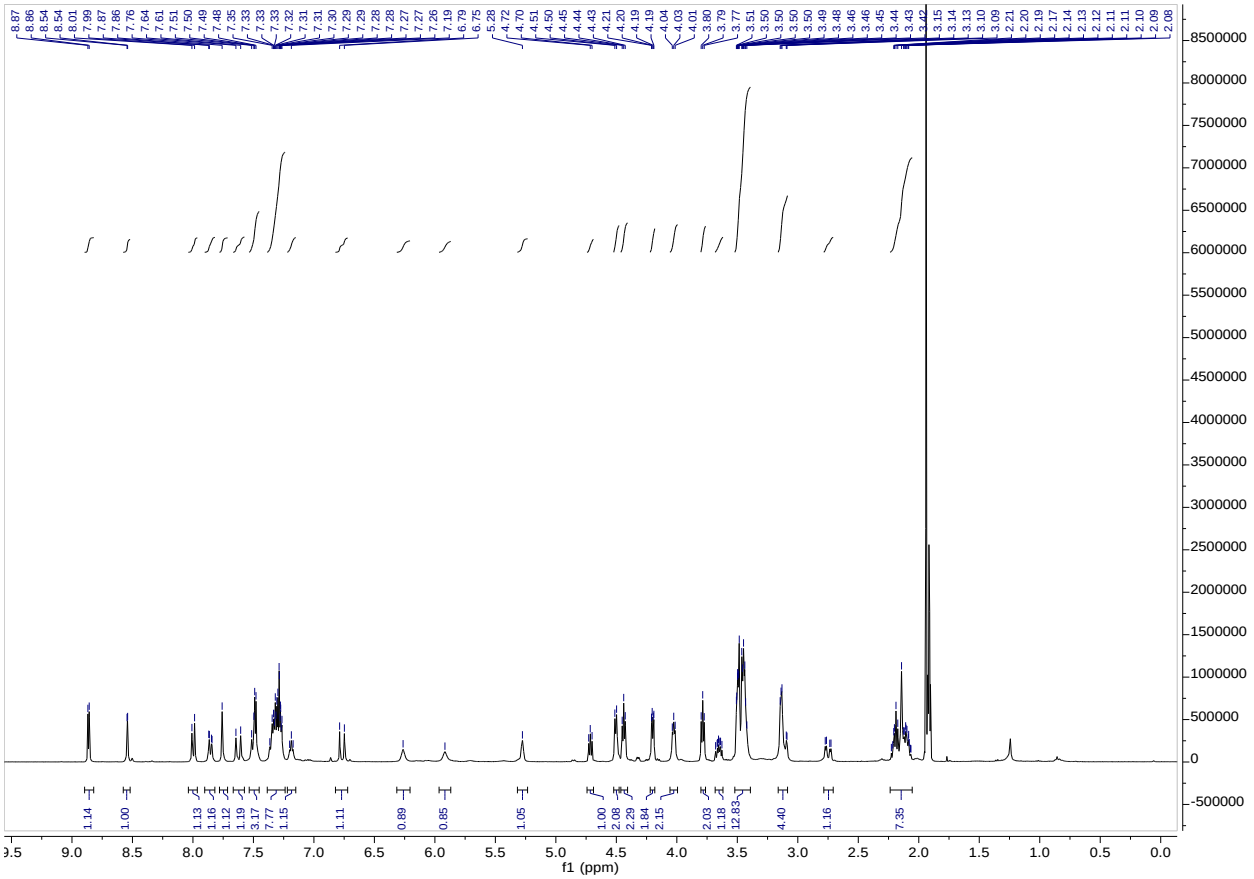


^{1}H NMR (400 MHz, Acetonitrile-d$_3$) δ 2.05 – 2.24 (4H, m, 27-H, 28-H), 2.75 (1H, dd, *J* = 4.0, 15.0 Hz, 39-H), 3.07 – 3.18 (5H, m, 34-H, 35-H, 39H), 3.39 – 3.53 (11H, m, 2-H, 3-H, 4-H, 29-H), 3.62 – 3.69 (1H, m, 29-H), 3.79 (2H, t, *J* = 5.1 Hz, 5-H), 4.03 (2H, t, *J* = 4.7 Hz, 1-H), 4.20 (2H, dd, *J* = 2.1, 5.7 Hz, 25-H), 4.44 (2H, t, *J* = 5.0 Hz, 6-H), 4.51 (2H, d, *J* = 5.8 Hz, 9-H), 4.68 – 4.75 (1H, m, 30-H), 5.28 (1H, s, 38-H), 5.92 (1H, s, Carbamate-H), 6.26 (1H, s, Carbamate-H), 6.77 (1H, d, *J* = 15.7 Hz, 12-H), 7.19 (1H, t, *J* = 5.8 Hz, Amide-H), 7.24 – 7.39 (7H, m, DIBO-H), 7.44 – 7.55 (3H, m, Quinoline-H, DIBO-H, Amide-H), 7.63 (1H, d, *J* = 15.7 Hz, 13-H), 7.76 (1H, s, 7-H), 7.86 (1H, dd, *J* = 1.9, 8.9 Hz, Quinoline-H), 8.00 (1H, d, *J* = 8.8 Hz, Quinoline-H), 8.54 (1H, d, *J* = 1.9 Hz, Quinoline-H), 8.86 (1H, d, *J* = 4.3 Hz, Quinoline-H).

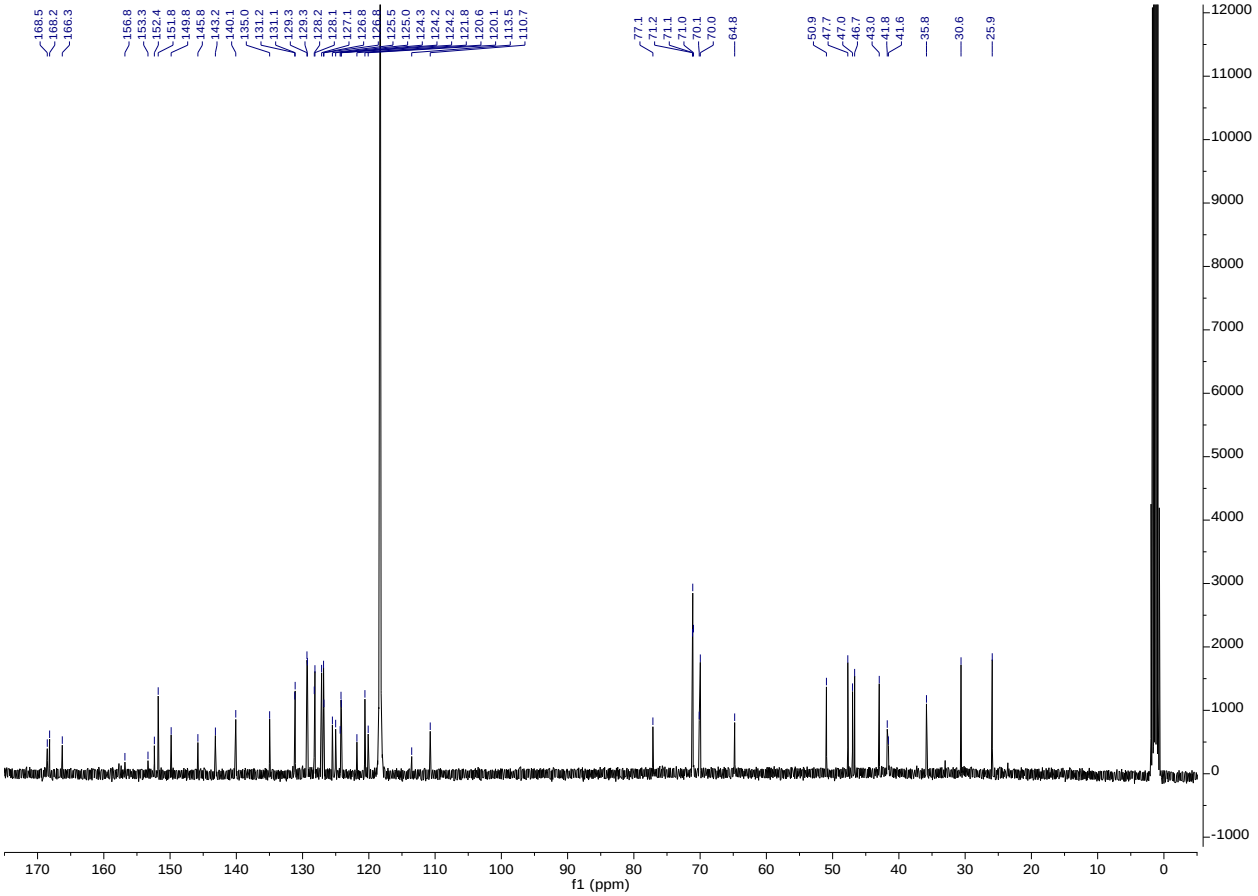
^{13}C NMR (101 MHz, Acetonitrile-d$_3$) δ 25.9, 30.6, (C_{27}, C_{28}), 35.8 (C_9), 41.6, 41.8 (C_{34}, C_{35}), 43.0 (C_{25}), 46.7 (C_{29}), 47.0 (C_{39}), 47.7 (C_{30}), 50.9 (C_6), 64.8 (C_1), 70.0 (C_5), 70.1, 71.0, 71.1, 71.2 (CH_2O), 77.1 (C_{38}), 110.7 (C_q), 113.5

(C_q), 120.1 (C_q), 120.6 ($C_{Quinoline}$), 121.8 (C_{12}), 124.15 (C_7), 124.20 (C_{DIBO}), 124.3 (C_q), 125.0 (C_q), 125.5 ($C_{Quinoline}$), 126.78 (C_{DIBO}), 126.84 (C_{DIBO}), 127.1 (C_{DIBO}), 128.1 ($C_{Quinoline}$), 128.2 (C_{DIBO}), 129.27 (C_{DIBO}), 129.34 ($C_{Quinoline}$), 131.1 (C_{DIBO}), 131.2 (C_{DIBO}), 135.0 (C_q), 140.1 (C_{13}), 143.2 (C_q), 145.8 (C_q), 149.8 (C_q), 151.8 ($C_{Quinoline}$), 152.4 (C_q), 153.3 (C_q), 156.8 (C_q), 166.3 (C_q), 168.2 (C_q), 168.5 (C_q). Two C_q are not resolved.

^{1}H NMR spectrum of **DIBO-PEG$_3$-FAPi**



^{13}C NMR spectrum of **DIBO-PEG$_3$-FAPi**

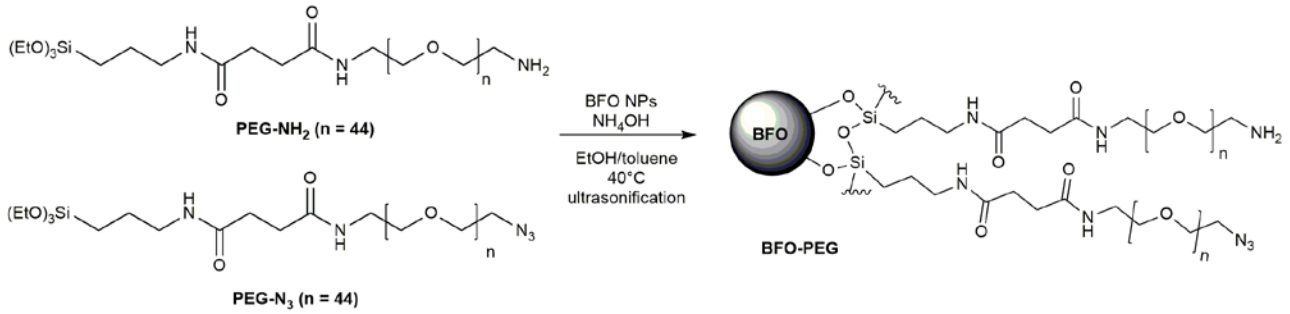


Coating of BFO NPs with heterobifunctional PEG derivatives

Adapted from protocols described in:

D. Staedler, S. Passemard, T. Magouroux, A. Rogov, C. M. Maguire, B. M. Mohamed, S. Schwung, D. Rytz, T. Juestel, S. Hwu, Y. Mugnier, R. Le Dantec, Y. Volkov, S. Gerber-Lemaire, A. Prina-Mello, L. Bonacina and J.-P. Wolf, *Nanomedicine: NBM*, 2015, **11**, 815-824.

Scheme S5. Surface coating of BFO NPs with heterobifunctional PEG derivatives



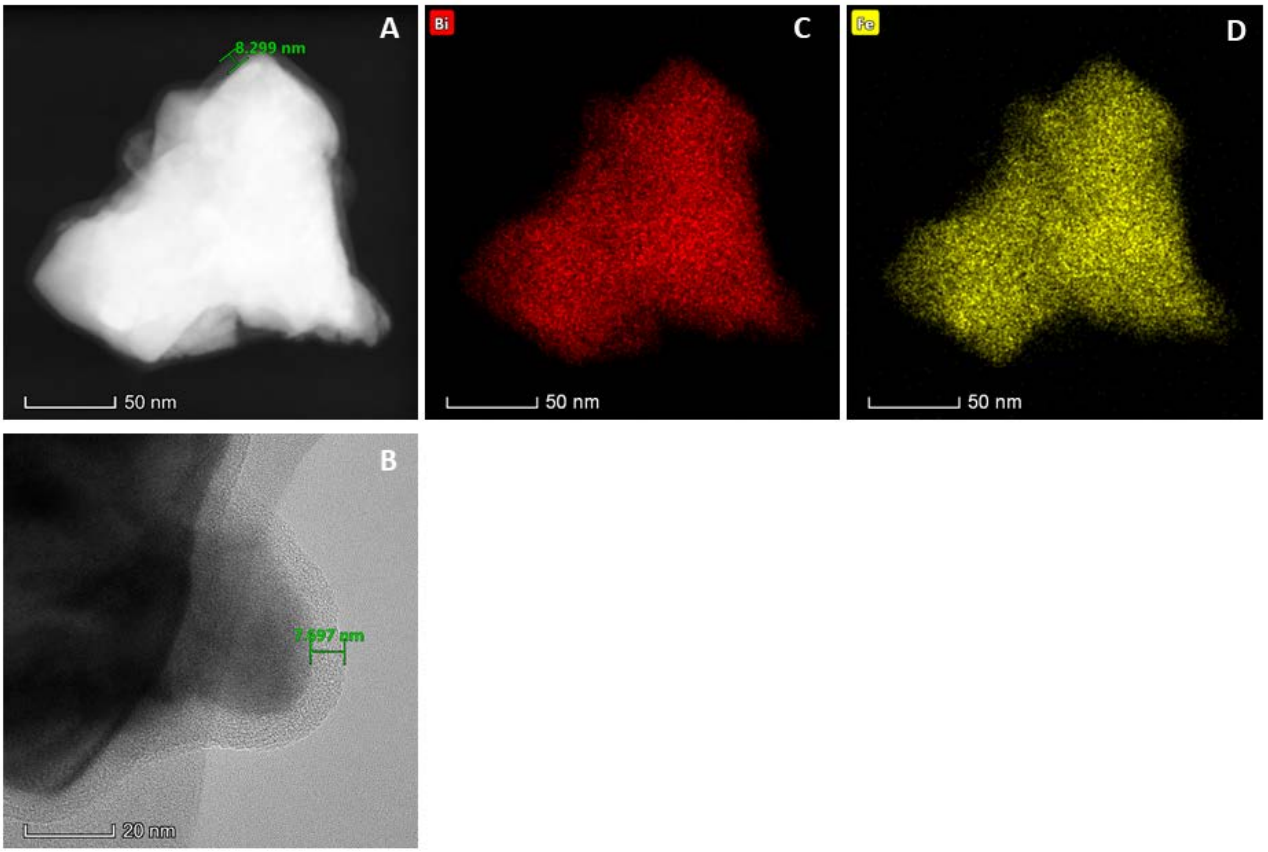
To a suspension of BFO NPs (2 mg) in EtOH (2 mL) was added a mixture of toluene (1 mL) and aqueous NH_3 25 % (320 µL). The suspension was ultra-sonicated for 30 min. **PEG-NH_2** and **PEG-N_3** (1:1, 21 µmol, 50 mg) were added and the suspension was ultrasonicated for 16 h at 40 °C. The suspension was divided into eppendorfs and centrifuged (10 min, 13 000 rpm). The supernatant was discarded and the NPs were resuspended in EtOH/H_2O 1:1 (1 mL). The eppendorfs were shaken until emulsification and centrifuged (10 min, 13 000 rpm). The procedure was repeated 4 times. **BFO-PEG** NPs were stored in EtOH at a concentration of 1 mg/mL.

A sample (10 µL) was diluted with distilled water (1 mL) and ultra-sonicated for 30 minutes. Mean hydrodynamic diameter and zeta potential were measured on a Malvern NanoZ instrument.

NPs	Mean hydrodynamic diameter [nm]	Zeta Potential [mV]
BFO	192 ± 28	- 28.1 ± 0.48
BFO-PEG	92 ± 11	-13.4 ± 1.1

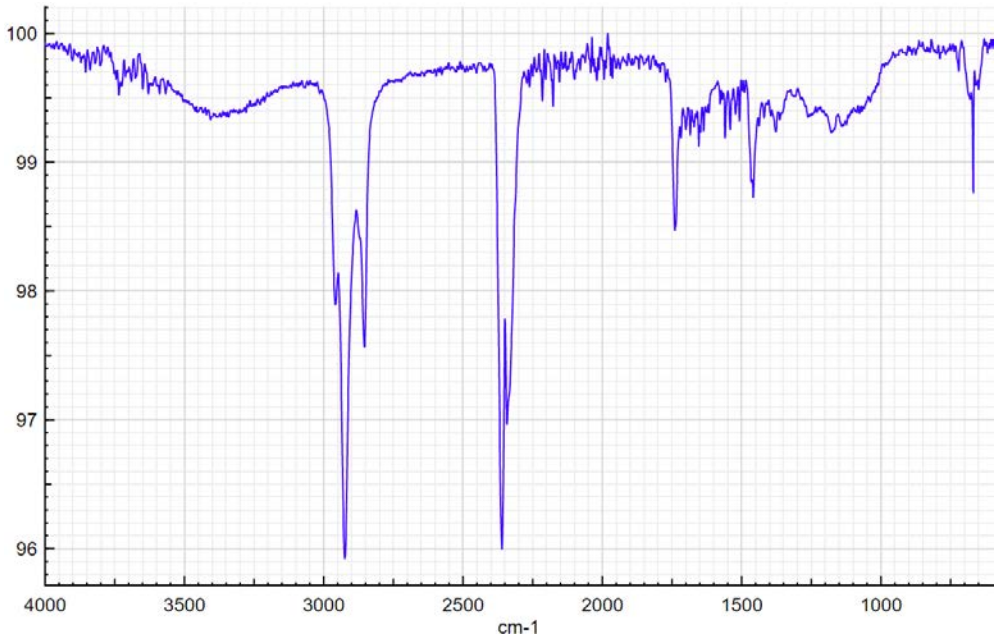
Scanning transmission electron microscopy (STEM) images of BFO-PEG-FAPi NPs

Figure S1. Representative STEM images of **BFO-PEG-FAPi**. (A) High-angle annular dark-field image; (B) Zoom on the functionalization layer; (C) Bi EDX map; (D) Fe EDX map.



FT-IR characterization of BFO-PEG-FAPi NPs

Figure S2. FT-IR spectrum of **BFO-PEG-FAPi** NPs



Qualitative analysis by ESI-HRMS

Mass spectrometry analyses were performed on a LTQ Orbitrap FTMS instrument (LTQ Orbitrap Elite FTMS, Thermo Scientific, Bremen, Germany) operated in the positive mode coupled with a robotic chip-based nano-ESI source (TriVersa Nanomate, Advion Biosciences, Ithaca, NY, U.S.A.). A standard data acquisition and instrument control system was utilized (Thermo Scientific) whereas the ion source was controlled by Chipsoft 8.3.1 software (Advion BioScience). Samples were loaded onto a 96-well plate (Eppendorf, Hamburg, Germany) with a volume of 5 µl. The ionization voltage was +1.4kV and the gas pressure was set at 0.30 psi. The temperature of the ion transfer capillary was set to 275 °C. FTMS spectra were obtained in the 80-1000 *m/z* range in the reduce profile mode with a resolution set to 120,000. In all spectra, one microscan was acquired with a maximum injection time value of 1000ms.

HRMS analyses of compounds **2**, **3**, **5**, **6**, **7**, **DIBO-PEG$_3$-FAPi** and **Biotin-PEG$_3$-FAPi** were conducted on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). The injection volume was 5 µL. Mass spectrometer detection was operated in positive ionization using the ZSpray™ dual-orthogonal multimode ESI/APCI/ESCi® source. The TOF mass spectra were acquired in the resolution mode over the range of *m/z* 50-1200. The instrument was calibrated using a solution of sodium formate (0.01 mg/L in isopropanol/H_2O 90:10). A mass accuracy below 5 ppm was achieved using a Leucine Enkephalin solution as lock-mass (200 pg/µL in MeCN/H_2O (50:50)) infused continuously using the LockSpray source. Source settings were as follows: cone, 25V; capillary, 3 kV, source temperature, 150°C; desolvation temperature, 500°C, cone gas, 10 L/h, desolvation gas, 500 L/h. Data were processed using MassLynx™ 4.1 software.

Multiphoton multispectral microscopy

Target-specific association of **BFO-PEG-FAPi** NPs to FAP was assessed by multiphoton microscopy. hrFAP (100 ng) was incubated for 2 h at 37°C with **BFO-PEG-FAPi** NPs (100 μg/mL) or **BFO-PEG** NPs (100 μg/mL) in the presence of Human Fibroblast Activation Protein alpha /FAP Alexa Fluor® 594-conjugated Antibody (dilution 1:5). Multiphoton imaging of the samples was performed on a Nikon multiphoton inverted microscope (A1R-MP) coupled with a Mai-Tai tunable Ti:Sapphire oscillator from Newport-Spectra-Physics (100 fs, 80 MHz, 700 – 1000 nm). A Plan APO 20 × WI N.A. 0.75 objective was used to focus the excitation 840 nm and to epi-collect second harmonic and fluorescence. The collected signals were processed by a Nikon A1 descanned grating-spectrometer equipped with an array of 32-photomultipliers. The detection range used here was 410 nm to 650 nm with 6 nm step-size (Figure S1). Four regions (each region: 635 x 635 μm) of both samples were analyzed to calculate the ratio of the area of structures emitting in both SH and fluorescence channels to the area of SH emitting structures. Mean values calculated for the four regions are presented in Figure 4 with standard deviation.

Figure S3. Multiphoton imaging of **BFO-PEG** NPs-hrFAP (A) and **BFO-PEG-FAPi**-hrFAP conjugates (B). hrFAP (100 ng) was incubated for 2 h at 37°C with **BFO-PEG-FAPi** NPs (100 μg/mL) or **BFO-PEG** NPs (100 μg/mL) in the presence of Human Fibroblast Activation Protein alpha /FAP Alexa Fluor® 594-conjugated Antibody (dilution 1:5). The samples were analyzed by multiphoton microscopy. Left column: SHG (420 nm) channel images; middle column: fluorescence emission (580-650 nm); right column: merging of all channels.