Supporting Information for

New dual CK2/HDAC1 inhibitors with nanomolar inhibitory activity against both enzymes

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KEYWORDS. Multi-target drugs, CK2, HDAC1, Dual inhibitors.

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PART A. Synthesis and characterization of compounds

1. General experimental

All non-aqueous reactions were performed using oven-dried glassware and were magnetically stirred unless otherwise stated. Yields refer to chromatographically purified and spectroscopically pure compounds, unless otherwise stated.

All **reagents** bought from commercial sources were used as sold. Organic **solvents** were evaporated under reduced pressure using a Büchi rotary evaporator.

Dimethylformamide and dioxane was dried and stored over 4Å MS.

Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F254 plates and visualised by fluorescence quenching under UV light. In addition, TLC plates were stained with a dipping solution of potassium permanganate (1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL water) and ninhydrin (1.5 g. ninhydrin in 100 mL of ethanol and 3 mL of acetic acid).

Chromatographic purification was performed on VWR 60 silica gel (230-400 mesh) and Merck neutral alumina using synthesis grade solvents that were used as supplied.

Final compounds **15s**, **15b**, **15c**, and **15d** were purified by preparative HPLC using the ACE 5 C18 column 250 x 10 mm; acidic conditions:

Time (minutes)	Gradient	Flow
0	10 (acetonitrile): 90 (H_20 TFA 0.1 %)	3 mL/min
12	40 (acetonitrile): 60 (H_20 TFA 0.1 %)	3 mL/min
13	95 (acetonitrile): 5 (H_20 TFA 0.1 %)	3 mL/min
15	95 (acetonitrile): 5 (H_20 TFA 0.1 %)	3 mL/min

Melting points were obtained on a Stuart Scientific SMP3 melting point apparatus and microscope and are uncorrected.

Mass spectra (MS) were recorded on a Bruker, Esquire 3000 model (coupled to HPLC 1100 Agilent technologies with ESI, APCI interfaces).

Infrared spectra were recorded on a Perkin-Elmer 1330 spectrometer. Only selected maximum absorbances are reported.

NMR spectra were recorded at 400 MHz (^{1}H) y a 101 MHz (^{13}C) using a Bruker 400-AC spectrometer, chemical shifts (δ) are quoted in parts per million referenced to the residual solvent peak. The multiplicity of each signal is designated using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet. Coupling constants (*J*) are reported in Hertz (*Hz*). NMR were performed at room temperature using DMSO-d6, $CDCl_3$, en MeOD or D_2O as solvents.

Analytical purity (considered as a value ≥ 95 %) were determined using analytical high-performance liquid chromatography (HPLC) performed on an Agilent Technologies 1260 Infinity II Series systems HPLC; conditions:

Time (minutes)	Gradient	Flow
0	5 (acetonitrile): 95 (H_20 TFA 0.1 %)	1 mL/min
20	95 (acetonitrile): 5 (H_20 TFA 0.1 %)	1 mL/min
25	95 (acetonitrile): 5 (H_20 TFA 0.1 %)	1 mL/min

(column and solvent conditions are specified for each compound).

2. Practical experimental.

2.1 Synthesis of starting materials 3, 8, 10a-10d.

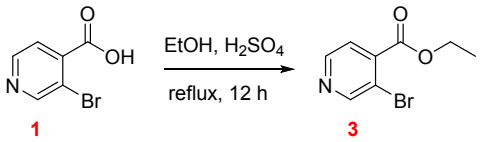
The azides of the carboxylic acids **10a**, **10b**, **10c** and **10d** were synthesized according to the literatures,[1]H and [13]C are described to confirm the data reported in the literature. [1]

The pyridine ester **3** was synthesized according to the procedure described in the literature, [1]H and [13]C are described to confirm the data reported in the literature..[2]

Tricyclic **8** was synthesized adapting the procedure described in the literature, [1]H and [13]C are described to confirm the data reported in the literature.[3]

2.1.1 Synthesis of methyl 5-chlorobenzo[c][2,6]naphthyridine-8-carboxylate 8

2.1.1.1 Synthesis of ethyl 3-bromoisonicotinate 3

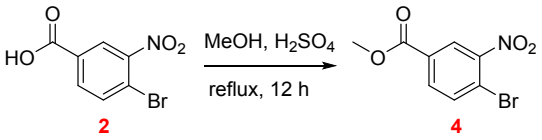


1 3

To a solution of **3-bromoisonicotinic acid 1** (5.0 g, 24.8 mmol) in ethanol (160 mL) was added sulfuric acid (8.3 mL) and the reaction mixture was left to reflux for 12 hours. Then, a saturated aqueous solution of $NaHCO_3$ (40 mL) was carefully added. Compound was extracted with DCM (2 x 70 mL) and the combined organic layers were washed with water (50 mL), saturated sodium chloride solution (30 mL), dried over anhydrous Sodium sulphate, filtered and concentrated under reduced pressure. The crude compound was purified by silica gel flash column chromatography using DCM:EtOAc 95:5 as eluent mixture to yield **ethyl 3-bromoisonicotinate 3 as a pale yellow oil** (5 g, 88%).

IR ν: 2983, 1741 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.82 (s, 1H, C**H**Ar), 8.58 (d, J=4.9 Hz, 1H, C**H**), 7.58 (dd, J= 4.9, 0.5 Hz, 1H, C**H**), 4.40 (q, J=7.1 Hz, 2**H**, C$\mathbf{H}_2$), 1.38 (t, J= 7.1 Hz, 3H, C$\mathbf{H}_3$) **^{13}C NMR (101 MHz, $CDCl_3$) δ** 164.6 (**C**=O), 153.7 (**C**H), 148.5 (**C**H), 139.4 (**C**), 124.3 (**C**H), 118.8 (**C**-Br), 62.5 (O-**C**H_2), 14.2 (**C**H_3).

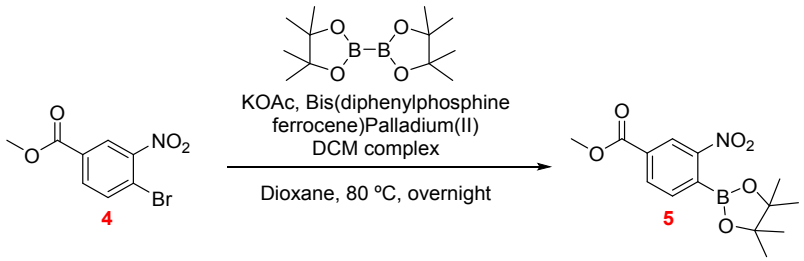
2.1.1.2 Synthesis of methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 6



2 4

To a solution of **4-bromo-3-nitrobenzoic acid 2** (10.0 g, 40.6 mmol) in methanol (100 mL) was added sulfuric acid (2 mL) and the reaction mixture was left to reflux for 12 hours. Then, a saturated aqueous solution of $NaHCO_3$ (20 mL) was carefully added. Compound was extracted with DCM (2 x 70 mL) and the combined organic layers were washed with water (50 mL), saturated sodium chloride solution (30 mL), dried over anhydrous Sodium sulphate, filtered and concentrated under reduced pressure. The crude compound was purified by silica gel flash column chromatography using DCM as eluent to yield **methyl 4-bromo-3-nitrobenzoate 4 as a white solid** (9.9 g, 93%).

MP: 105 ºC; **IR** v = 3095, 2964, 1719 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.46 (d, J=1.7 Hz, 1H, C**H**Ar), 8.06 (dd, J=8.3, 1.7 Hz, 1H, C**H**Ar), 7.84 (d, J=8.3 Hz , 1H, C**H**Ar), 3.97 (s, 3H, OC**H**$_3$); **^{13}C NMR (101 MHz, $CDCl_3$) δ** 164.5 (**C**=O), 150.0 (**C**-NO_2) 135.6 (**C**H), 133.6 (**C**H), 130.8 (**C**-COO), 126.6 (**C**H), 119.7 (**C**(Br)), 53.1 (O**C**H$_3$).

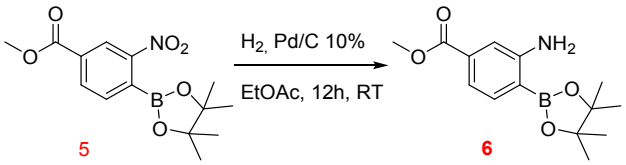


4 5

A solution of **methyl 4-bromo-3-nitrobenzoate 4** (2.0 g, 7.7 mmol) in dioxane (40 mL) was placed in a pressure vessel. After bubbling solution with argon for 5 minutes, bis(pinacolato)diboron (2.9 g, 11.5 mmol), bis(diphenylphosphineferrocene)Palladium(II) DCM complex (626 mg, 0.77 mmol), potassium acetate (2.3 g, 23.0 mmol) were sequentially. Once the vessel was sealed, reaction mixture was heated at 80 ºC overnight. Then, water (50 mL) was added and compound was extracted with EtOAc (2 x 70 mL). The combined organic layers were washed with water (50 mL), saturated sodium chloride solution (30 mL), dried over anhydrous Sodium sulphate, filtered and concentrated under reduced pressure. The crude compound was purified by silica gel flash column chromatography using hexane:EtOAc as eluent mixture to yield **methyl 3-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 5 as a yellow oil** (1.3 g, 57%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, J = 1.3 Hz, 1H, C**H**Ar), 8.31 (dd, J = 7.6, 1.4 Hz, 1H, C**H**Ar), 7.65 (d, J = 7.6 Hz, 1H, C**H**Ar), 3.98 (s, 3H, OC**H**$_3$), 1.44 (s, 12H, C**H**$_3$ x 4); **^{13}C NMR (101 MHz,**

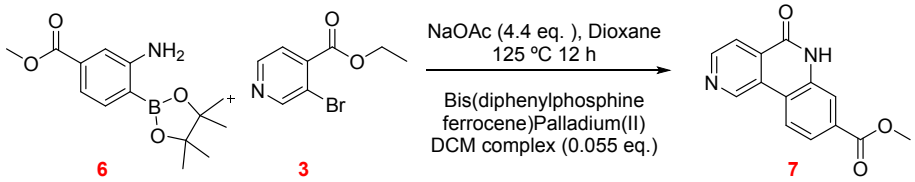
$CDCl_3$) δ 165.2 (**C**=O), 151.3 (**C**-NO_2) , 134.3 (**C**HAr), 133.4 (**C**HAr), 132.5 (**C**Ar), 124.1 (**C**HAr), 85.2 (**C**-O), 52.9 (O**C**H_3), 24.9 (**C**H_3 x 4), **C**-B does not appear.



To a solution of **methyl 3-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 5** (1.3 g, 4.4 mmoles) was dissolved in EtOAc (100 mL) and was placed in a round bottom flask followed by the addition of 10% w/w palladium on carbon 10 wt. % loading (135 mg). The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 12 h. Palladium on carbon was removed by filtration through celite and then the solvent was evaporated under reduced pressure to yield **methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 6 as a yellow oil** (1.2 g, 99 %). The resulting amine was freshly used in the next step due to its instability.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.7 Hz, 1H, C**H**Ar), 7.23 (d, J = 1.3 Hz, 1H, C**H**Ar), 7.04 (dd, J = 7.7, 1.5 Hz, 1H, C**H**Ar), 5.76 (s, 2H, N**H**$_2$), 3.81 (s, 3H, OC**H**$_3$), 1.30 (s, 12H, C**H**$_3$ x 4); **^{13}C NMR (101 MHz, $CDCl_3$) δ** 166.7 (**C**=O), 154.7 (**C**-NH_2), 136.7 (**C**HAr), 133.3 (**C**Ar), 115.3 (**C**HAr), 114.9 (**C**HAr), 83.8 (**C**-O), 52.2 (O**C**H_3), 24.8 (**C**H_3 x 4), **C**-B does not appear.

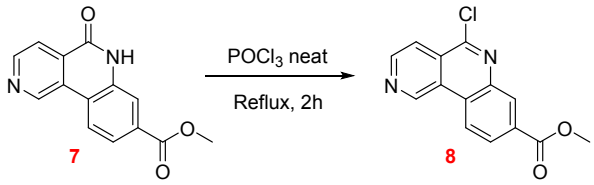
2.1.1.3 Synthesis of methyl 5-chlorobenzo[c][2,6]naphthyridine-8-carboxylate 8



In a pressure vessel **methyl 3-amino-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 6** (860 mg, 3.1 mmol), **ethyl 3-bromoisonicotinate 3** (785 mg, 3.4 mmol), bis(diphenylphosphineferrocene)Palladium(II) DCM complex (139 mg, 0.17 mmol) and sodium acetate (1.1 g, 13.6 mmol) were dissolved in DMF (9 mL)and the resulting solution was purged with argon. After sealing the vessel, the reaction mixture was heated at 125 ºC for 12 hours. Then, the reaction mixture was cooled down to room temperature and 88 mL of water were added to obtain a precipitate which was filtered and washed with water. **Methyl 5-chlorobenzo[c][2,6]naphthyridine-8-carboxylate 7** (562 mg, 71%) was obtained as a **brown solid** and was used without further purification in the next step.

MP: 307 ºC; **IR ν:** 2951, 1713, 1680, 1550 cm^{-1}**; ^{1}H NMR (400 MHz, DMSO) δ** 12.18 (s, 1H, N**H**), 9.95 (s, 1H, C**H**Ar), 8.89 (d, J=5.1 Hz , 1H, C**H**Ar), 8.72 (d, J=8.5 Hz ,1H, C**H** Ar), 8.15 (d, J= 5.1 Hz, 1H, C**H**Ar), 8.02 (d, 1H, J = 1.6 Hz, C**H**Ar), 7.83 (dd, 1H, J = 8.5, 1.6 Hz, C**H**Ar), 3.91 (s, 3H, OC**H**$_3$)**; ^{13}C NMR (101 MHz, DMSO) δ** 165.6 (**C**=O), 159.7 (**C**ON), 149.0 (**C**HAr), 147.1 (**C**HAr),

136.9 (**C**Ar), 131.7 (**C**Ar), 130.7 (**C**Ar), 127.6 (**C**Ar), 123.7 (**C**HAr), 122.7 (**C**HAr), 119.8 (**C**HAr), 119.6 (**C**Ar), 117.3 (**C**HAr), 52.5 (O**C**H_3).

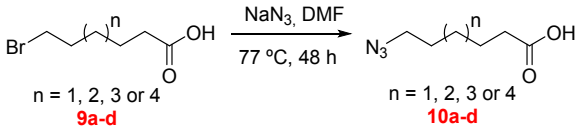


In a round bottom flask containing **methyl 5-oxo-5,6-dihydrobenzo[c][2,6]naphthyridine-8-carboxylate 7** (392 mg, 1.5 mmol) was added $POCl_3$ (2 mL)**.** After 2 hours under reflux the volatiles were removed under reduced pressure and the residue was dissolved in DCM (100 mL), the organic layer was washed with a saturated aqueous $NaHCO_3$ solution (2 x 30 mL), saturated sodium chloride solution (25 mL), dried over anhydrous Sodium sulphate, filtered and concentrated under reduced pressure. The crude compound was purified by neutral alumina flash column chromatography using DCM:EtOAc 1:1 as eluent mixture to yield **methyl 5-chlorobenzo[c][2,6]naphthyridine-8-carboxylate as a pale yellow solid 8** (200 mg, 48%).

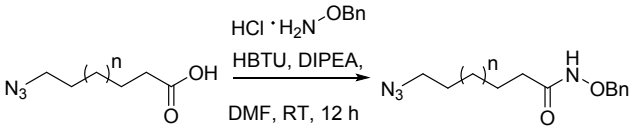
MP: 198 ºC; **IR ν:** 2934, 1725, 1569 cm^{-1}**; ^{1}H NMR (400 MHz, $CDCl_3$) δ** 10.08 (s, 1H, C**H**Ar), 9.03 (d, J = 5.6 Hz, 1H, C**H**Ar), 8.82 (d, J = 1.7 Hz, 1H, C**H**Ar), 8.73 (d, J = 8.6 Hz, 1H, C**H**Ar), 8.40 (dd, J = 8.6, 1.7 Hz, 1H, C**H**Ar), 8.26 (d, J = 5.6 Hz, 1H, C**H**Ar), 4.03 (s, 3H, OC**H**$_3$).; **^{13}C NMR (101 MHz, $CDCl_3$) δ** 166.2 (**C**=O), 151.2 (**C**-Cl), 148.3 (**C**HAr), 147.3 (**C**HAr), 143.6 (**C**Ar), 132.0 (**C**Ar), 131.7 (**C**HAr), 129.6 (**C**Ar), 128.9 (**C**HAr), 127.7 (**C**Ar), 125.5 (**C** Ar), 122.1 (**C**HAr), 119.5 (**C**HAr), 52.8 (O**C**H_3).

2.1.2 Synthesis of hydroxamic linkers 12a-12d

General procedure A for the synthesis of hydroxamic linkers:



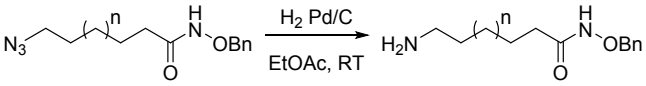
To a solution of the corresponding **carboxylic acid 9a-d** (1.0 equivalent) in dimethylformamide (1.6 mL/mmol), placed in a round bottom flask, was added sodium azide (5.0 equivalents). Reaction was heated for 48 hours at 77 ºC. After cooling to room temperature, ethyl acetate (5 mL/mmol) was added. The organic layer was washed with a saturated solution sodium bicarbonate solution (2 mL/mmol), with water (2 mL/mmol) and saturated sodium chloride solution (2 mL/mmol), was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to yield the crude **azide 10a-d** which was purified by silica gel flash column chromatography in the way described for each compound.



n = 1, 2, 3 or 4
10a-d

n = 1, 2, 3 or 4
11a-d

Once the corresponding **azide 10a-d** was purified it was placed in a round bottom flask and dissolved in DMF (2.5 mL/mmol). After cooling the solution to 0 ºC, DIPEA (2.2 equivalents), HBTU (1.2 equivalents) and *O*-Benzylhydroxylamine hydrochloride (1.2 equivalents) were added sequentially. Reaction was stirred at room temperature for 12 hours. Then, DCM (5 mL/mmol) and the organic layer was washed with water (2 x 3 mL/mmol) and saturated sodium chloride solution (1 mL/mmol), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to yield the crude azido-***N*-(benzyloxy)amide 11a-d** which was purified by silica gel flash column chromatography in the way described for each compound.

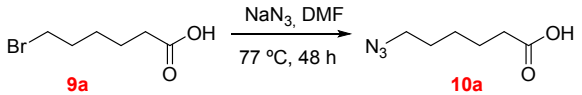


n = 1, 2, 3 or 4
11a-d

n = 1, 2, 3 or 4
12a-d

The corresponding **azido-*N*-(benzyloxy)amide 11a-d** (1.0 equivalent) was dissolved in ethyl acetate (8.5 mL/mmol) and was placed in a round bottom flask followed by the addition of 10%w/w palladium on carbon 10 wt. % loading. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 2 hours. Palladium on carbon was removed by filtration through celite and then the solvent was evaporated under reduced pressure to yield the corresponding **amino-*N*-(benzyloxy)amide 12a-d**. The resulting amine was freshly used in the next step due to its instability.

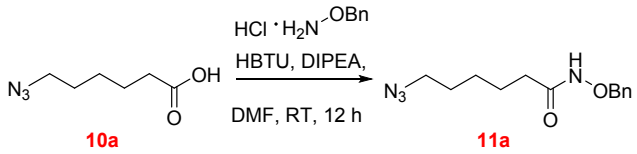
2.1.2.1 Synthesis of 6-amino-*N*-(benzyloxy)hexanamide 12a



9a **10a**

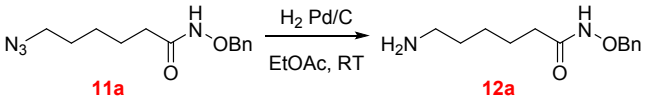
According to general procedure A, **6-azidohexanoic acid 10a** was obtained using **6-bromohexanoic acid 9a** (6.3 g, 32.3 mmol), NaN_3 (10.4 g, 161.5 mmol), and DMF as solvent (40 mL). Crude was purified using hexane:EtOAc 7:3 as eluent mixture to yield **6-azidohexanoic acid 10a as a pale yellow oil** (4.3 g, 84 %).

IR: 3200, 2942, 2096, 1709 cm^{-1}**; ^{1}H NMR (400 MHz, $CDCl_3$) δ** 3.28 (t, J = 6.8 Hz, 1H, C**H_2**), 2.38 (t, J = 7.4 Hz, 1H, C**H_2**), 1.74 – 1.54 (m, 4H, C**H_2**), 1.49 – 1.36 (m, 1H, C**H_2**); **^{13}C NMR (101 MHz, $CDCl_3$) δ** 180.0 (**C**=O), 51.3 (**C**H_2), 33.9 (**C**H_2), 28.7 (**C**H_2), 26.3 (**C**H_2), 24.3 (**C**H_2).



According to general procedure A, **6-azido-*N*-(benzyloxy)hexanamide 11a** was obtained using **6-azidohexanoic acid 10a** (2.6 g, 16.7 mmol), DIPEA (6.4 mL, 36.8 mmol), HBTU (7.6 g, 20.1 mmol) and *O*-Benzylhydroxylamine hydrochloride (3.2 g, 20.1 mmol) and DMF as solvent (25 mL). Crude was purified using hexane:EtOAc 6:4 as eluent mixture to yield **6-azido-*N*-(benzyloxy)hexanamide 11a as a pale yellow oil** (2.7 g, 60 %).

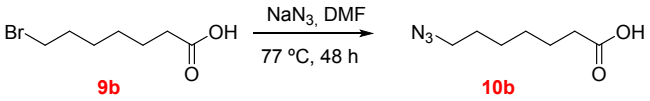
HRMS (ESI) calculated for $C_{13}H_{17}N_4O_2^-$ [M-H]$^-$ 261.1347; found m/z 261.1000**; IR:** 3188, 2942, 2860, 2098, 1655 cm^{-1}**; ^{1}H NMR (400 MHz, DMSO) δ** 10.96 (s, 1H, N**H**), 7.92 – 6.45 (m, 5H, **H**Ar), 4.78 (s, 2H, OC**H**$_2$), 3.30 (t, *J* = 6.8 Hz, 2H, C**H**$_2$), 1.95 (t, *J* = 7.2 Hz, 2H, C**H**$_2$), 1.60 – 1.39 (m, 4H, C**H**$_2$), 1.41 – 1.18 (m, 2H, C**H**$_2$); **^{13}C NMR (101 MHz, DMSO) δ** 169.2 (**C**=O), 136.1 (**C**Ar), 128.8 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 76.8 (O**C**H$_2$), 50.5 (**C**H$_2$), 32.1 (**C**H$_2$), 28.0 (**C**H$_2$), 25.6 (**C**H$_2$), 24.5 (**C**H$_2$).



According to general procedure A, **6-amino-*N*-(benzyloxy)hexanamide 12a** was obtained using **6-azido-*N*-(benzyloxy)hexanamide 11a** (1.3 g, 4.9 mmol), Pd/C 10 wt. % loading (130 mg) and EtOAc as solvent (40 mL). Yielding the crude **6-amino-*N*-(benzyloxy)hexanamide 12a as a colourless oil** (1.0 g, 89 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 5H, **H**Ar), 4.91 (s, 2H, OC**H**$_2$), 2.66 (t, J = 6.5 Hz, 2H, C**H**$_2$), 2.05 (brs, 2H, C**H**$_2$), 1.66 – 1.58 (m, 2H, C**H**$_2$), 1.51 – 1.37 (m, 2H, C**H**$_2$), 1.37 – 1.27 (m, 2H, C**H**$_2$).

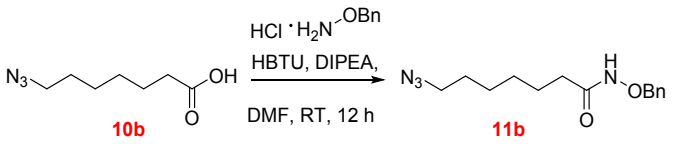
2.1.2.2. Synthesis of 7-amino-*N*-(benzyloxy)heptanamide 12b



According to general procedure A, **7-azidoheptanoic acid 10b** was obtained using **7-bromoheptanoic acid 9b** (5.0 g, 23.9 mmol), NaN_3 (7.7 g, 119.8 mmol), and DMF as solvent (25

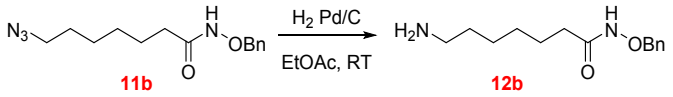
mL). Crude was purified using hexane:EtOAc 7:3 as eluent mixture to yield **7-azidoheptanoic acid 10b as a pale yellow oil** (3.6 g, 87 %).

IR ν: 2938, 2098, 1713 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 11.99 (s, 1H, O**H**), 3.30 (t, J = 6.9 Hz, 2H, C**H$_2$**), 2.19 (t, J = 7.3 Hz, 2H, C**H$_2$**), 1.64–1.41 (m, 4H, C**H$_2$**), 1.35-1.26 (m, 4H, C**H$_2$**); **^{13}C NMR (101 MHz, DMSO) δ** 174.5 (**C**=O), 50.6 (**C**H_2), 33.6 (**C**H_2), 28.1 (**C**H_2), 25.9 (**C**H_2), 24.4 (**C**H_2).



According to general procedure A, **7-azido-*N*-(benzyloxy)heptanamide 11b** was obtained using **7-azidoheptanoic acid 10b** (2 g, 11.7 mmol), DIPEA (4.5 mL, 25.7 mmol), HBTU (5.3 g, 14.0 mmol) and *O*-Benzylhydroxylamine hydrochloride (2.2 g, 14.0 mmol) and DMF as solvent (25 mL). Crude was purified using hexane:EtOAc 6:4 as eluent mixture to yield **7-azido-*N*-(benzyloxy)heptanamide 11b as a pale yellow oil** (2.4 g, 73 %).

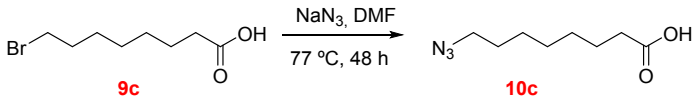
HRMS (ESI) calculated for $C_{14}H_{19}N_4O_2^-$ [M-H]$^-$ 275.1513; found m/z 27561.1900**; IR ν:** 3188, 2930, 2860, 2094, 1655 cm^{-1}**; ^{1}H NMR (400 MHz, DMSO) δ** 10.95 (s, 1H, N**H**), 7.45 – 7.31 (m, 5H, **H**Ar), 4.77 (s, 2H, OC**H$_2$**), 3.30 (t, J = 6.8 Hz, 2H, C**H$_2$**), 1.94 (t, J = 7.3 Hz, 2H, C**H$_2$**), 1.54-1,44 (m, 4H, C**H$_2$**), 1.30 – 1.18 (m, 4H, C**H$_2$**) ; **^{13}C NMR (101 MHz, DMSO) δ** 169.3 (**C**=O), 136.1 (**C**Ar), 128.8 (**CH**Ar), 128.3 (**CH**Ar), 128.2 (**CH**Ar), 76.7 (O-**C**H_2-Ph), 50.6 (**C**H_2), 32.1 (**C**H_2), 28.1 (**C**H_2), 28,0 (**C**H_2), 25.8 (**C**H_2), 24.8 (**C**H_2).



According to general procedure A, **7-amino-*N*-(benzyloxy)heptanamide 12b** was obtained using **7-azido-*N*-(benzyloxy)heptanamide 11b** (1.3 g, 4.7 mmol), Pd/C 10 wt. % loading (130 mg) and EtOAc as solvent (40 mL). Yielding the crude **7-amino-*N*-(benzyloxy)heptanamide 12b as a pale yellow oil** (613 mg, 52 %).

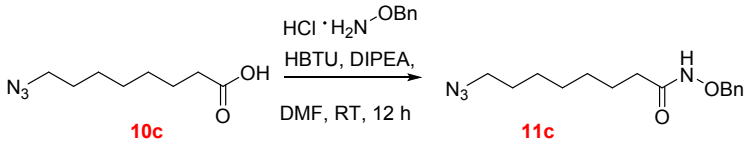
^{1}H NMR (400 MHz, DMSO) δ 7.38 (s, 1H, **H**Ar), 4.77 (s, 2H, OC**H$_2$**), 2.50-2.44 (m, 2H, C**H$_2$**), 1.94 (t, *J* = 7.2 Hz, 1H, C**H$_2$**), 1.54 – 1.39 (m, 2H, C**H$_2$**), 1.35-1.26 (m, 2H, C**H$_2$**), 1.26 – 1.13 (m, 4H, C**H$_2$**) ; **^{13}C NMR (101 MHz, DMSO) δ** 169.5 (**C**=O), 136.4 (**C**Ar), 129.0 (**CH**Ar), 128.5 (**CH**Ar), 128.4 (**CH**Ar), 76.9 (O**C**H_2), 41.9 (**C**H_2), 33.5 (**C**H_2), 32.4 (**C**H_2), 28.6 (**C**H_2), 26.3 (**C**H_2), 25.2 (**C**H_2).

2.1.2.3 Synthesis of 8-amino-*N*-(benzyloxy)octanamide 12c



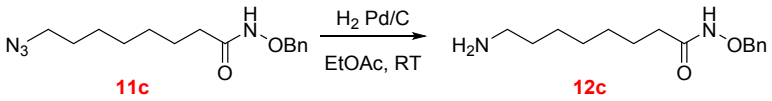
According to general procedure A, **8-azidooctanoic acid 10c** was obtained using **8-bromooctanoic acid 9c** (6 g, 26.9 mmol), NaN_3 (8.7 g, 134.5 mmol), and DMF as solvent (30 mL). Crude was purified using hexane:EtOAc 8:2 as eluent mixture to yield **8-azidooctanoic acid 10c as a pale yellow oil** (4.2 g, 84 %).

IR (film) v = 2934, 2098, 1708 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 11.94 (s, 1H, O**H**), 3.29 (t, J = 6.9 Hz, 2H, C**H$_2$**), 2.18 (t, J = 7.4 Hz, 2H, C**H$_2$**), 1.61 – 1.42 (m, 4H, C**H$_2$**), 1.28 (brs, J = 8.6 Hz, 6H, C**H$_2$**); **^{13}C NMR (101 MHz, DMSO) δ** 174.6 (**C**=O), 50.8 (**C**H$_2$), 33.7 (**C**H$_2$), 28.6 (**C**H$_2$), 28.4 (**C**H$_2$), 28.4 (**C**H$_2$), 26.2 (**C**H$_2$), 24.5 (**C**H$_2$).



According to general procedure A, **8-azido-*N*-(benzyloxy)octanamide 11c** was obtained using **8-azidooctanoic acid 10c** (2.2 g, 11.7 mmol), DIPEA (4.5 mL, 25.7 mmol), HBTU (5.3 g, 14.0 mmol) and *O*-Benzylhydroxylamine hydrochloride (2.2 g, 14.0 mmol) and DMF as solvent (25 mL). Crude was purified using hexane:EtOAc 1:1 as eluent mixture to yield **8-azido-*N*-(benzyloxy)octanamide 11c as a pale yellow oil** (2.8 g, 83 %).

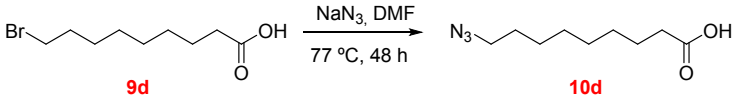
HRMS (ESI) calculated for $C_{15}H_{21}N_4O_2^-$ [M-H]$^-$ 289.1670; found m/z 289.1000**; IR** (film) v = 3176, 2930, 2864, 2094, 1659 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 10.93 (s, 1H, N**H**), 7.75 – 7.00 (m, 5H, C**H**Ar), 4.78 (s, 2H, OC**H$_2$**), 3.30 (t, J = 6.9 Hz, 2H, C**H$_2$**), 1.94 (t, J = 7.3 Hz, 2H, C**H$_2$**), 1.59 – 1.37 (m, 4H, C**H$_2$**), 1.37 – 1.16 (m, 6H, C**H$_2$**); **^{13}C NMR (101 MHz, DMSO) δ** 169.3 (**C**=O), 136.1 (**C**Ar), 128.7 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 76.7 (O **C**H$_2$), 50.6 (**C**H$_2$), 32.2 (**C**H$_2$), 28.3 (**C**H$_2$), 28.2 (**C**H$_2$), 26.0 (**C**H$_2$), 24.8 (**C**H$_2$).



According to general procedure A, **8-amino-*N*-(benzyloxy)octanamide 12c** was obtained using **8-azido-*N*-(benzyloxy)octanamide 11c** (1.4 g, 4.8 mmol), Pd/C 10 wt. % loading (139 mg) and EtOAc as solvent (40 mL). Yielding the crude **8-amino-*N*-(benzyloxy)octanamide 12c as a colourless oil** (706 mg, 56 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (brs, 5H, C**H**Ar), 4.91 (s, 2H, OC**H_2**), 2.66 (t, J = 6.9 Hz, 2H, C**H_2**), 2.04 (brs, 2H, C**H_2**), 1.66 – 1.56 (m, 2H, C**H_2**), 1.48 – 1.36 (m, 2H, C**H_2**), 1.29 (brs, 6H, C**H_2**).

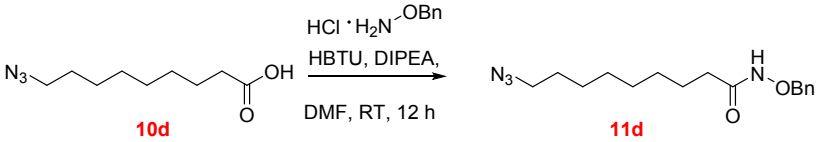
2.1.2.4 Synthesis of 9-amino-*N*-(benzyloxy)nonanamide 12d



9d 10d

According to general procedure A, **9-azidononanoic acid 10d** was obtained using **9-bromononanoic acid 9d** (4.0 g, 16.9 mmol), NaN_3 (5.5 g, 84.3 mmol), and DMF as solvent (25 mL). Crude was purified using hexane:EtOAc 1:1 as eluent mixture to yield **9-azidononanoic acid 10d as a colourless oil** (3.1 g, 92 %).

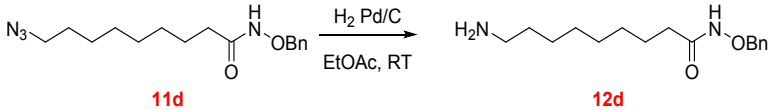
IR (film) v = 2930, 2860, 2098, 1713 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 3.25 (t, J = 6.9 Hz, 2H, C**H_2**), 2.35 (t, J = 7.4 Hz, 2H, C**H_2**), 1.71 – 1.47 (m, 4H, C**H_2**x 2), 1.33 (s, 8H, C**H_2**x4); **^{13}C NMR (101 MHz, $CDCl_3$) δ** 180.2 (**C**=O), 51.6 (**C**H_2), 34.1 (**C**H_2), 29.2 (**C**H_2), 29.1 (**C**H_2), 28.9 (**C**H_2), 26.8 (**C**H_2), 24.7 (**C**H_2).



10d 11d

According to general procedure A, **9-azido-*N*-(benzyloxy)nonanamide 11d** was obtained using **9-azidononanoic acid 10d** (1.8 g, 8.9 mmol), DIPEA (3.4 mL, 19.7 mmol), HBTU (4.1 g, 10.7 mmol) and *O*-Benzylhydroxylamine hydrochloride (1.7 g, 10.7 mmol) and DMF as solvent (20 mL). Crude was purified using hexane:EtOAc 6:4 as eluent mixture to yield **9-azido-*N*-(benzyloxy)nonanamide 11d as a colourless oil** (2.6 g, 97 %).

HRMS (ESI) calculated for $C_{16}H_{23}N_4O_2^-$ [M-H]$^-$ 303.1826; found m/z 303.1800**; IR** (film) v = 3197, 2934, 2860, 2098, 1655 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 10.94 (s, 1H, N**H**), 7.46 – 7.27 (m, 5H, C**H**Ar), 4.78 (s, 2H, O C**H_2**), 3.30 (t, J = 6.8 Hz, 2H, C**H_2**), 1.94 (t, J = 7.1 Hz, 2H, C**H_2**), 1.58 -1.40 (m, 4H, C**H_2**), 1.35 – 1.15 (s, 8H, C**H_2**); **^{13}C NMR (101 MHz, DMSO) δ** 169.4 (**C**=O), 136.1 (**C**Ar), 128.7 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 76.7 (O**C**H_2), 50.6 (**C**H_2), 32.2 (**C**H_2), 28.6 (**C**H_2), 28.4 (**C**H_2), 28.4 (**C**H_2), 28.2 (**C**H_2), 26.1 (**C**H_2), 24.9 (**C**H_2).



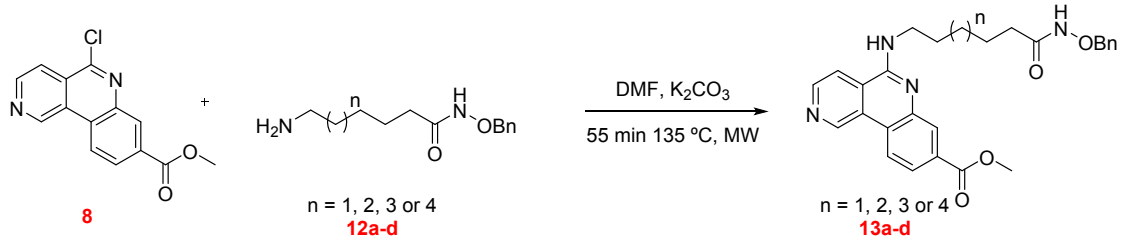
11d 12d

According to general procedure A, **9-amino-*N*-(benzyloxy)nonanamide 12d** was obtained using **9-azido-*N*-(benzyloxy)nonanamide 11d** (1.5 g, 4.8 mmol), Pd/C 10 wt. % loading (145 mg) and EtOAc as solvent (45 mL). Yielding the crude **9-amino-*N*-(benzyloxy)nonanamide 12d as a colourless oil** (1.2 g, 94 %).

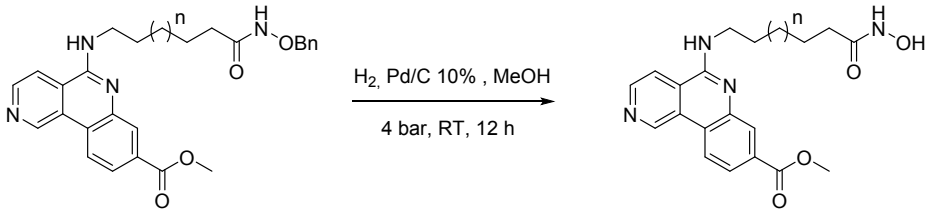
^{1}H NMR (400 MHz, DMSO) δ 7.38 (brs, 5H, C**H**Ar), 4.77 (s, 2H, O C**H$_2$**), 2.63 – 2.36 (m, 2H, C**H$_2$**), 1.93 (t, J = 6.8 Hz, 2H, C**H$_2$**), 1.47 (brs, 2H, C**H$_2$**), 1.32 (brs, 2H, C**H$_2$**), 1.22 (brs, 8H, C**H$_2$**).

2.1.3 Synthesis of CK2-HDAC1 dual inhibitors with hydroxamic acid as ZBG 15a-15d

General procedure B for the synthesis of CK2-HDAC1 dual inhibitors with hydroxamic acid as ZBG:

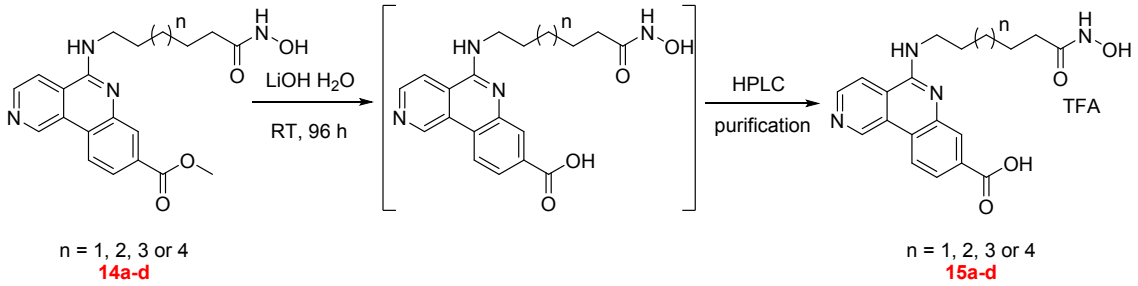


To a solution of **5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate 8** (1.0 equivalent) and the corresponding **amine 12a-d** (3.5 equivalents) in dimethylformamide (4 mL/mmol), placed in a pressure vessel, was added potassium carbonate (1.0 equivalent). Once the vessel was sealed, reaction was heated in the Microwave for 55 minutes at 135 ºC. After cooling to room temperature, water was added (20 mL/mmol) and compound was extracted with dichloromethane (3 x 25 mL/mmol). The organic layer was washed with water (15 mL/mmol) and saturated sodium chloride solution (10 mL/mmol) and finally was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to yield the corresponding ***N*-(benzyloxy)acetamide 13a-d** derivative which was purified by silica gel flash column chromatography in the way described for each one.



The corresponding ***N*-(benzyloxy)acetamide 13a-d** derivative (1.0 equivalent) was dissolved in methanol (95 mL/mmol) and was placed in a pressure vessel followed by the addition of 40%w/w palladium on carbon 10 wt. % loading. The reaction mixture was stirred under 4 psi of hydrogen at room temperature for 12 h. Palladium on carbon was removed by filtration through celite and then the solvent was evaporated under reduced pressure to yield the corresponding ***N*-**

hydroxyacetamide 14a-d derivative which was purified by silica gel flash column chromatography in the way described for each one.

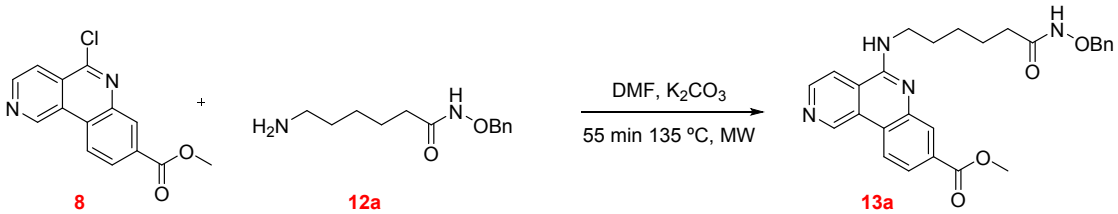


n = 1, 2, 3 or 4
14a-d

n = 1, 2, 3 or 4
15a-d

Once the corresponding ***N*-hydroxyacetamide 14a-d** derivative was purified it was placed in a round bottom flask and dissolved in MeOH:H_2O:THF 2:2:1 (60 mL/mmol). After addition of LiOH H_2O (1.2 equivalents), reaction was stirred at room temperature for 96 hours. After evaporation of solvents, the crude was directly purified by preparative HPLC using the ACE 5 C18 column 250 x 10 mm under acidic conditions (general experimental part 1.2) to yield the **final compound as TFA salt 15a-d**.

2.1.3.1 Synthesis of 8-carboxy-5-((6-(hydroxyamino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15a

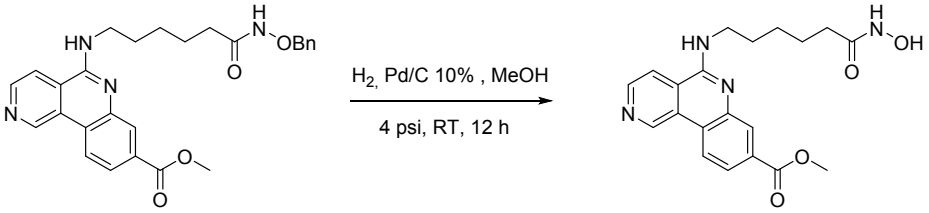


8 **12a** **13a**

According to general procedure B, **methyl 5-((6-((benzyloxy)amino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13a** was obtained using **5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate 8** (300 mg, 1.1 mmol), **6-amino-*N*-(benzyloxy)hexanamide 12a** (1.0 g, 4.4 mmol), K_2CO_3 (167 mg, 1.2 mmol) and DMF as solvent (4 mL). Crude was purified using DCM:MeOH 97:3 as eluent mixture to yield **methyl 5-((6-((benzyloxy)amino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate as a pale yellow solid 13a** (223 mg, 43 %).

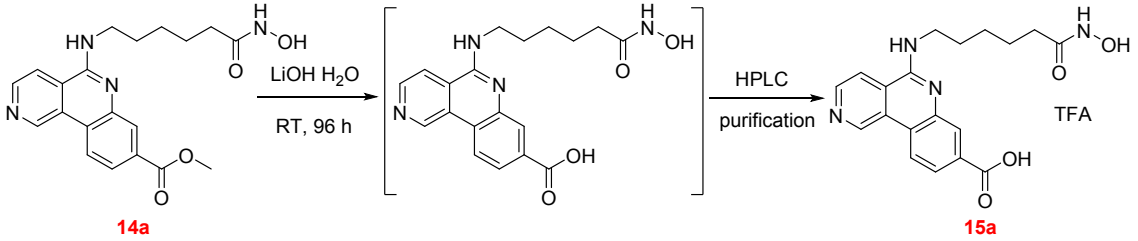
HRMS (ESI) calculated for $C_{27}H_{29}N_4O_4^+$ $[M+H]^+$ 473.2183; found m/z 473.1800**; MP:** 104-105 ºC; **IR** (film) v = 3442, 3233, 2934, 1712, 1651 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 10.94 (s, 1H, N**H**-OBn), 10.05 (s, 1H, C**H**Ar), 8.88 (d, J = 5.5 Hz, 1H, C**H**Ar), 8.74 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.29 (d, J = 5.6 Hz, 1H, C**H**Ar), 8.15 (d, J = 1.2 Hz, 1H, C**H**Ar), 7.99 (t, J = 4.9 Hz, 1H, N**H**), 7.81 (dd, J = 8.4, 1.3 Hz, 1H, C**H**Ar), 7.48 – 7.10 (m, 5H, C**H**Ar), 4.74 (s, 2H, OC**H$_2$**), 3.90 (s, 3H, OC**H$_3$**), 3.65 – 3.55 (m, 2H, C**H$_2$**), 1.98 (t, J = 7.2 Hz, 2H, C**H$_2$**), 1.79 – 1.63 (m, 2H, C**H$_2$**), 1.63 - 1.52 (m, 2H, C**H$_2$**), 1.43 – 1.32 (m, 2H, C**H$_2$**); **^{13}C NMR (101 MHz, DMSO)** δ 169.4 (**C**ON), 166.3

(**C**=O), 152.9 (**C**=N), 147.6 (**C**HAr), 147.6 (**C**HAr), 145.0 (**C**Ar), 136.1 (**C**Ar), 130.2 (**C**Ar), 128.8 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 127.5 (**C**HAr), 126.7 (**C**Ar), 124.0 (**C**Ar), 122.7 (**C**HAr), 121.8 (**CH**Ar), 121.6 (**C**Ar), 116.2 (**C**HAr), 76.8 (O**C**H_2), 52.3 (O**C**H_3), 40.7 (**C**H_2), 32.3 (**C**H_2), 28.3 (**C**H_2), 26.2 (**C**H_2), 24.9 (**C**H_2).



According to general procedure B, **methyl 5-((6-(hydroxyamino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14a** was obtained using **methyl 5-((6-((benzyloxy)amino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13a** (223 mg, 0.47 mmol), Pd/C 10 wt. % loading (89 mg) and MeOH as solvent (45 mL). Crude was purified using DCM:MeOH 9:1 as eluent mixture to yield **methyl 5-((6-(hydroxyamino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14a as a yellow solid** (93 mg, 52 %).

HRMS (ESI) calculated for $C_{20}H_{23}N_4O_4^+$ $[M+H]^+$ 383.1714; found m/z 383.1000**; MP:** 190-192 ºC; **IR** (film) v = 3545, 3385, 3213, 2926, 1704, 1639 cm^{-1}; **^{1}H NMR (400 MHz, DMSO)** δ 10.34 (s, 1H, N**H**-OH), 10.06 (s, 1H, C**H**Ar), 8.89 (d, J = 5.5 Hz, 1H, C**H**Ar), 8.75 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.67 (s, 1H, NH-O**H**), 8.29 (d, J = 5.5 Hz, 1H, C**H**Ar), 8.14 (s, 1H, C**H**Ar), 8.00 (t, J = 5.1 Hz, 1H, N**H**), 7.81 (d, J = 8.3 Hz, 1H, C**H**Ar), 3.91 (s, 3H, OC**H**$_3$), 3.63 – 3.55 (m, 2H, C**H**$_2$), 1.98 (t, J = 7.3 Hz, 2H, C**H**$_2$), 1.76 – 1.66 (m, 2H, C**H**$_2$), 1.62 – 1.52 (m, 2H, C**H**$_2$), 1.43 – 1.33 (m, 2H, C**H**$_2$); **^{13}C NMR (101 MHz, DMSO)** δ 169.1 (**C**ON), 166.3 (**C**=O), 152.8 (**C**=N), 147.6 (**C**HAr), 147.6 (**C**HAr), 145.0 (**C**Ar), 130.2 (**C**Ar), 127.5 (**C**HAr), 126.7 (**C**Ar), 124.1 (**C**Ar), 122.7 (**C**HAr), 121.8 (**C**HAr), 121.6 (**C**Ar), 116.2 (**C**HAr), 52.3 (O**C**H_3), 40.8 (**C**H_2), 32.3 (**C**H_2), 28.3 (**C**H_2), 26.4 (**C**H_2), 25.1 (**C**H_2).

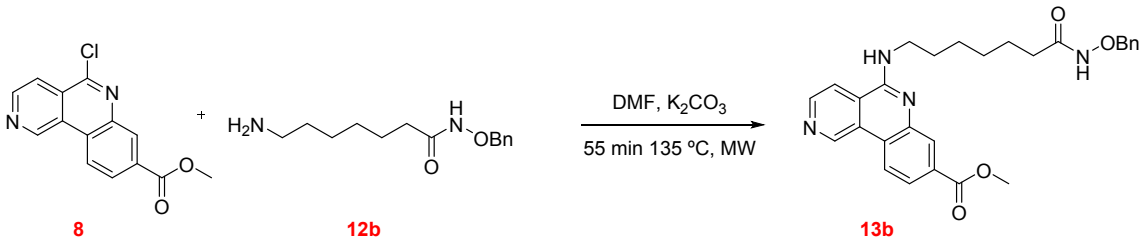


According to general procedure B, **8-carboxy-5-((6-(hydroxyamino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15a** was obtained using **methyl 5-((6-(hydroxyamino)-6-oxohexyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14a** (88 mg, 0.23 mmol), LiOH H_2O (12 mg, 0.28 mmol) and THF:MeOH:H_2O 2:2:1

as solvent (6 mL). After HPLC purification in acidic conditions, **compound 15a** was obtained as a **pale yellow solid** (41 mg, 37 %). Purity was determined according to general experimental 1.7 using Kromasil C8 5μ, 250 x 4.6 mm as column.

LRMS (ESI) **calcd for** $C_{19}H_{21}N_4O_4^+$ ([M+H]$^+$ 369.1, found 369.0 m/z (rel. intensity 100 %); **Purity:** 97%; **MP:** 155-156 ºC; **IR** (film) v = 3434, 3213, 2948, 1684, 1626 cm^{-1}; **^{1}H NMR (400 MHz, DMSO)** δ 10.38 (brs, 1H, N**H**-OH), 10.12 (brs, 1H, C**H**Ar), 8.99 (brs, 1H, C**H**Ar), 8.81 (brs, 1H, C**H**Ar), 8.44 (brs, 2H, C**H**Ar), 7.93 (brs, 1H, C**H**Ar), 3.67 (brs, 2H, C**H$_2$**), 1.99 (brs, 2H, C**H$_2$**), 1.76 (brs, 2H, C**H$_2$**), 1.59 (brs, 2H, C**H$_2$**), 1.42 (brs, 2H, C**H$_2$**); **^{13}C NMR (101 MHz, DMSO)** δ 169.1 (**C**ON), 166.8 (**C**=O), 158.1 (q, J = 33.4 Hz, **C**O-CF$_3$), 152.0 (**C**=N), 148.2 (**C**HAr), 147.8 (**C**HAr), 132.1 (**C**Ar), 126.3 (**C**Ar), 124.6 (**C**Ar), 123.8 (**C**HAr), 123.1 (**C**HAr), 121.0 (**C**Ar), 118.0 (**C**HAr), 117.0 (**C**Ar), 116.9 (**C**HAr), 41.8 (**C**H$_2$), 32.2 (**C**H$_2$), 27.7 (**C**H$_2$), 26.2 (**C**H$_2$), 24.9 (**C**H$_2$).

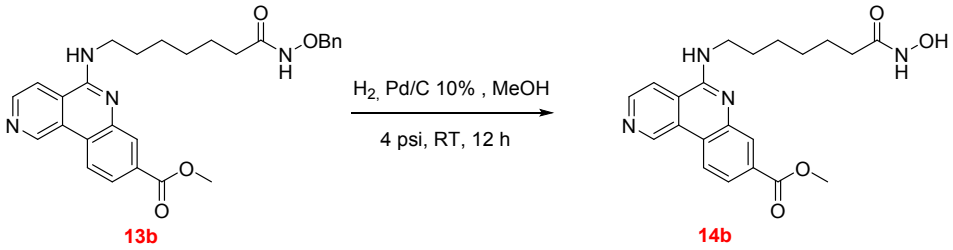
2.1.3.2 Synthesis of 8-carboxy-5-((7-(hydroxyamino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15b



8 12b 13b

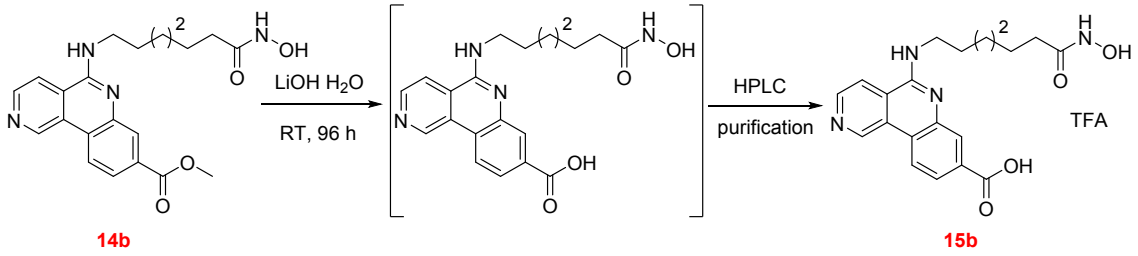
According to general procedure B, **methyl 5-((7-((benzyloxy)amino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13b** was obtained using **5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate 8** (233 mg, 0.9 mmol), **7-amino-*N*-(benzyloxy)heptanamide 12b** (749 mg, 3.0 mmol), K_2CO_3 (117 mg, 0.9 mmol) and DMF as solvent (3.5 mL). Crude was purified using DCM:MeOH 97:3 as eluent mixture to yield **methyl 5-((7-((benzyloxy)amino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13b as a pale yellow solid** (253 mg, 61 %).

HRMS (ESI) calculated for $C_{28}H_{31}N_4O_4^+$ [M+H]$^+$ 487.2340; found m/z 487.0600**; MP:** 124-125 ºC; **IR ν:** 3352, 3197, 2942, 2856, 1721 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 10.94 (s, 1H, N**H**-OBn), 10.06 (s, 1H, C**H**Ar), 8.89 (d, J = 5.5 Hz, 1H, C**H**Ar), 8.74 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.29 (d, J = 5.6 Hz, 1H, C**H**Ar), 8.14 (s, 1H, C**H**Ar), 7.98 (s, 1H, N**H**), 7.81 (d, J = 8.4 Hz, 1H, C**H**Ar), 7.47 – 7.21 (m, 5H, **H**Ar), 4.76 (s, 2H, OC**H$_2$**), 3.90 (s, 3H, OC**H$_3$**), 3.62-3.57 (m, 2H, C**H$_2$**), 1.96 (t, J = 7.2 Hz, 2H, C**H$_2$**), 1.80 – 1.61 (m, 2H, C**H$_2$**), 1.56 – 1.51 (m, 2H, C**H$_2$**), 1.41 – 1.36 (m, 2H, C**H$_2$**), 1.35 – 1.24 (m, 2H, C**H$_2$**).; **^{13}C NMR (101 MHz, DMSO) δ** 169.4 (**C**ON), 166.3 (**C**=O), 152.8 (**C**=N), 147.6 (**C**HAr), 147.6 (**C**HAr), 145.0 (**C**Ar), 136.1 (**C**Ar), 130.2 (**C**Ar), 128.8 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 127.5 (**C**HAr), 126.6 (**C**Ar), 124.1 (**C**Ar), 122.7 (**C**HAr), 121.8 (**C**HAr), 121.6 (**C**Ar), 116.3 (**C**HAr), 76.7 (O**C**H$_2$), 52.3 (O**C**H$_3$), 40.9 (**C**H$_2$), 32.2 (**C**H$_2$), 28.4 (**C**H$_2$), 26.5 (**C**H$_2$), 25.0 (**C**H$_2$).



According to general procedure C, **methyl 5-((7-(hydroxyamino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14b** was obtained using **methyl 5-((7-((benzyloxy)amino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13b** (186 mg, 0.4 mmol), Pd/C 10 wt. % loading (75 mg) and MeOH as solvent (27 mL). Crude was purified using DCM:MeOH 9:1 as eluent mixture to yield **methyl 5-((7-(hydroxyamino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14b as a yellow solid** (57 mg, 38 %).

LRMS (ESI) **calcd for** $C_{21}H_{25}N_4O_4^+$ ([M+H]$^+$ 397.1, found 397.0 m/z (rel. intensity 100 %); **MP:** 174-176 ºC; **IR**: 3291, 3184, 2930, 1717, 1659 cm^{-1}**; ^{1}H NMR (400 MHz, MeOD) δ** 9.89 (s, 1H, C**H**Ar), 8.76 (d, J = 5.6 Hz, 1H, C**H**Ar), 8.56 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.28 (s, 1H, C**H**Ar), 8.14 (d, J = 5.6 Hz, 1H, C**H**Ar), 7.89 (d, J = 8.4 Hz, 1H, C**H**Ar), 3.97 (s, 3H, OC**H**$_3$), 3.68 (t, J = 7.2 Hz, 2H, C**H**$_2$), 2.11 (t, J = 7.4 Hz, 2H, C**H**$_2$), 1.85 – 1.75 (m, 2H, C**H**$_2$), 1.72 – 1.61 (m, 2H, C**H**$_2$), 1.52 – 1.42 (m, 4H, C**H**$_2$); **^{13}C NMR (101 MHz, MeOD) δ** 173.0 (**C**ON), 168.5 (**C**=O), 154.4 (**C**=N), 148.1 (**C**HAr), 147.7 (**C**HAr), 146.6 (**C**Ar), 132.2 (**C**Ar), 129.2 (**C**HAr), 129.0 (**C**Ar), 126.3 (**C**Ar), 123.7 (**C**HAr), 123.1 (**C**HAr), 123.0 (**C**Ar), 117.8 (**C**HAr), 52.8 (O**C**H$_3$), 42.4 (**C**H$_2$), 33.8 (**C**H$_2$), 30.0 (**C**H$_2$), 28.0 (**C**H$_2$), 26.8 (**C**H$_2$).

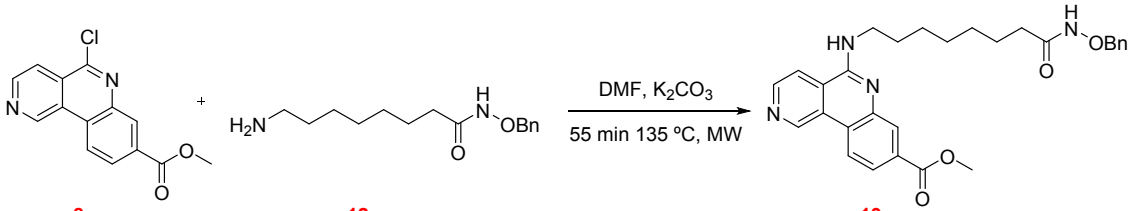


According to general procedure C, **8-carboxy-5-((7-(hydroxyamino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15b** was obtained using **methyl 5-((7-(hydroxyamino)-7-oxoheptyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14b** (54 mg, 0.14 mmol), LiOH H_2O (7mg, 0.16 mmol) and THF:MeOH:H_2O 2:2:1 as solvent (7.5 mL). After HPLC purification in acidic conditions, **compound 15b** was obtained as a **pale yellow solid** (40 mg, 59 %). Purity was determined according to general experimental 1.7 using Kromaphase C18 5μ, 250 x 4.6 mm as column.

LRMS (ESI) **calcd for** $C_{20}H_{23}N_4O_4^+$ ([M+H]$^+$ 383.1, found 382.9 m/z (rel. intensity 100 %); **MS (ESI) m/z** 383.0 [M + H]; **Purity:** 99%; **MP:** 138-139 ºC; **IR**: 3221, 3090, 2949, 1676, 1631 cm^{-1}**;**

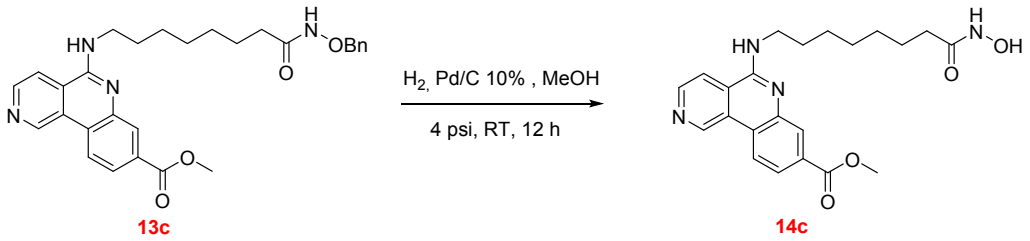
^{1}H NMR (400 MHz, D_2O) δ 9.47 (s, 1H, C**H**Ar), 8.81 (brs, 1H, C**H**Ar), 8.10 (s, 2H, C**H**Ar), 7.87 (s, 1H, C**H**Ar), 7.69 (s, 1H, C**H**Ar), 3.56 (brs, 2H, C$\mathbf{H}_2$), 2.23 (t, J = 6.6 Hz, 2H, C$\mathbf{H}_2$), 1.83 (brs, 2H, C$\mathbf{H}_2$), 1.68 (brs, 2H, C$\mathbf{H}_2$), 1.51 (brs, 2H, C$\mathbf{H}_2$), 1.43 (brs, 2H, C$\mathbf{H}_2$); **^{13}C NMR (101 MHz, D_2O) δ** 173.4 (**C**ON), 168.3 (**C**OOH), 163.1 (**C**O-CF_3), 150.6 (**C**=N), 148.1 (**C**HAr), 146.4 (**C**HAr), 133.1 (**C**Ar), 132.9 (**C**Ar), 125.8 (**C**HAr), 125.6 (**C**Ar), 124.4 (**C**Ar), 122.5 (**C**HAr), 119.7 (**C**H(Ar), 119.4 (**C**Ar), 117.7 (**C**F_3), 114.8 (**C**HAr), 43.2 (**C**H_2), 32.2 (**C**H_2), 27.7 (**C**H_2), 26.8 (**C**H_2), 25.7 (**C**H_2), 24.7 (**C**H_2).

2.1.3.3 Synthesis of 8-carboxy-5-((8-(hydroxyamino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15c



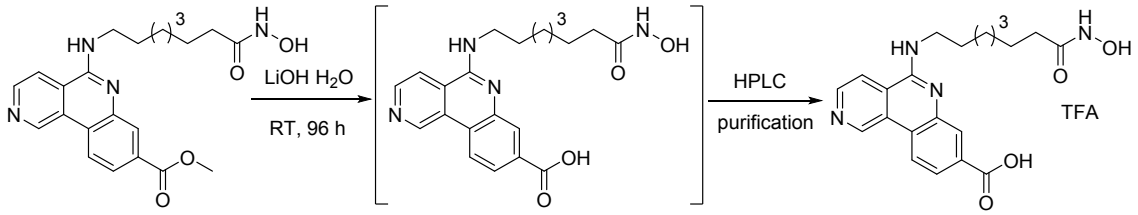
According to general procedure C, **methyl 5-((8-((benzyloxy)amino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13c** was obtained using **5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate 8** (219 mg, 0.8 mmol), **8-amino-*N*-(benzyloxy)octanamide 12c** (706 mg, 2.8 mmol), K_2CO_3 (121 mg, 0.9 mmol) and DMF as solvent (4 mL). Crude was purified using DCM:MeOH 97:3 as eluent mixture to yield **methyl 5-((8-((benzyloxy)amino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13c as a pale yellow solid** (272 mg, 68 %).

HRMS (ESI) calculated for $C_{29}H_{33}N_4O_4^+$ $[M+H]^+$ 501.2496; found m/z 501.1500**; MP:** 135-136 ºC; **IR** (film) v = 3397, 3303, 2926, 1704, 1676 cm^{-1}; **^{1}H NMR (400 MHz, DMSO) δ** 10.91 (s, 1H, N**H**-OBn), 10.06 (s, 1H, C**H**Ar), 8.89 (d, J = 5.5 Hz, 1H, C**H**Ar), 8.74 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.29 (d, J = 5.6 Hz, 1H, C**H**Ar), 8.15 (brs, 1H, C**H**Ar), 7.98 (brs, 1H, N**H**), 7.81 (d, J = 8. Hz, 1H, C**H**Ar), 7.54 – 7.20 (m, 5H, C**H**Ar), 4.76 (s, 2H, OC$\mathbf{H}_2$), 3.91 (s, 3H, OC$\mathbf{H}_3$), 3.63 – 3.56 (m, 2H, C$\mathbf{H}_2$), 1.94 (t, J = 7.2 Hz, 2H, C$\mathbf{H}_2$), 1.81 – 1.62 (m, 2H, C$\mathbf{H}_2$), 1.56 – 1.44 (m, 2H, C$\mathbf{H}_2$), 1.44 – 1.31 (m, 4H, C$\mathbf{H}_2$), 1.31 – 1.19 (m, 2H, C$\mathbf{H}_2$); **^{13}C NMR (101 MHz, DMSO) δ** 169.4 (**C**ON), 166.3 (**C**=O), 152.8 (**C**=N), 147.6 (**C**HAr), 147.6 (**C**HAr), 136.1 (**C**Ar), 130.2 (**C**Ar), 128.8 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 127.5 (**C**HAr), 126.6 (**C**Ar), 124.1 (**C**Ar), 122.7 (**C**HAr), 121.8 (**C**HAr), 121.6 (**C**Ar), 116.2 (**C**HAr), 76.7 (O**C**H_2), 52.3 (O**C**H_3), 40.8 (**C**H_2), 32.2 (**C**H_2), 28.6 (**C**H_2), 28.5 (**C**H_2), 28.5 (**C**H_2), 26.6 (**C**H_2), 25.0 (**C**H_2)



According to general procedure C, **methyl 5-((8-(hydroxyamino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14c** was obtained using **methyl 5-((8-((benzyloxy)amino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13c** (272 mg, 0.54 mmol), Pd/C 10 wt. % loading (109 mg) and MeOH as solvent (40 mL). Crude was purified using DCM:MeOH 9:1 as eluent mixture to yield **methyl 5-((8-(hydroxyamino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14c as a yellow solid** (96 mg, 43 %).

HRMS (ESI) calculated for $C_{22}H_{27}N_4O_4^+$ $[M+H]^+$ 411.2027; found m/z 411.1000**; MP:** 93-95 ºC; **IR** (film) v = 3426, 3233, 2930, 1717, 1647 cm^{-1}; **^{1}H NMR (400 MHz, MeOD)** δ 9.86 (s, 1H, C**H**Ar), 8.75 (d, J = 5.7 Hz, 1H, C**H**Ar), 8.52 (d, J = 8.4 Hz, 1H, C**H**Ar), 8.25 (s, 1H, C**H**Ar), 8.11 (d, J = 5.7 Hz, 1H, C**H**Ar), 7.87 (d, J = 8.4 Hz, 1H, C**H**Ar), 3.97 (s, 3H, OC**H**$_3$), 3.67 (t, J = 7.2 Hz, 2H, OC**H**$_2$), 2.10 (t, J = 7.4 Hz, 2H, C**H**$_2$), 1.86 – 1.74 (m, 2H, C**H**$_2$), 1.70 – 1.58 (m, 2H, C**H**$_2$), 1.53 – 1.42 (m, 4H, C**H**$_2$), 1.42 – 1.31 (m, 4H, C**H**$_2$); **^{13}C NMR (101 MHz, MeOD)** δ 173.0 (**C**ON), 168.5 (**C**=O), 154.3 (**C**=N), 148.1 (**C**HAr), 147.7 (**C**HAr), 146.6 (**C**Ar), 132.1 (**C**Ar), 129.2 (**C**HAr), 129.0 (**C**Ar), 126.2 (**C**Ar), 123.7 (**C**HAr), 123.1 (**C**HAr), 122.9 (**C**Ar), 117.7 (**C**HAr), 52.8 (O**C**H$_3$), 42.4 (**C**H$_2$), 33.8 (**C**H$_2$), 30.2 (**C**H$_2$), 30.1 (**C**H$_2$), 30.0 (**C**H$_2$), 28.1 (**C**H$_2$), 26.7 (**C**H$_2$).



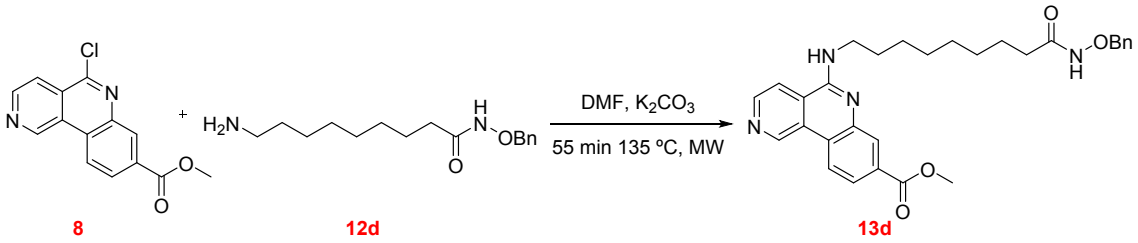
14c **15c**

According to general procedure C, **8-carboxy-5-((8-(hydroxyamino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridin-2-ium-2,2,2-trifluoroacetate 15c** was obtained using **methyl 5-((8-((benzyloxy)amino)-8-oxooctyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14c** (96 mg, 0.23 mmol), LiOH H_2O (12 mg, 0.3 mmol) and THF:MeOH:H_2O 2:2:1 as solvent (10 mL). After HPLC purification in acidic conditions, **compound 15c** was obtained as a **yellow solid** (25 mg, 22 %). Purity was determined according to general experimental 1.7 using Kromasil C8 5μ, 250 x 4.6 mm as column.

LRMS (ESI) **calcd for** $C_{21}H_{25}N_4O_4^+$ ($[M+H]^+$ 397.1, found 397.0 m/z (rel. intensity 100 %); **(ESI) m/z** 397.0 [M + H]; **Purity:** 99%; **MP:** 147-148 ºC; **IR** (film) v = 3434, 3221, 2938, 2865, 1677, 1659 cm^{-1}; **^{1}H NMR (400 MHz, DMSO)** δ 10.33 (s, 1H, N**H**-OH), 10.10 (s, 1H, C**H**Ar), 8.95 (s, 1H, C**H**Ar), 8.79 (d, J = 8.1 Hz, 1H, C**H**Ar), 8.39 (s, 1H, C**H**Ar), 8.31 (s, 1H, C**H**Ar), 7.89 (d, J = 7.8 Hz, 1H, C**H**Ar), 3.64 (brs, 2H, C**H**$_2$), 1.94 (t, J = 7.3 Hz, 2H, C**H**$_2$), 1.79 – 1.68 (m, 2H, C**H**$_2$), 1.58

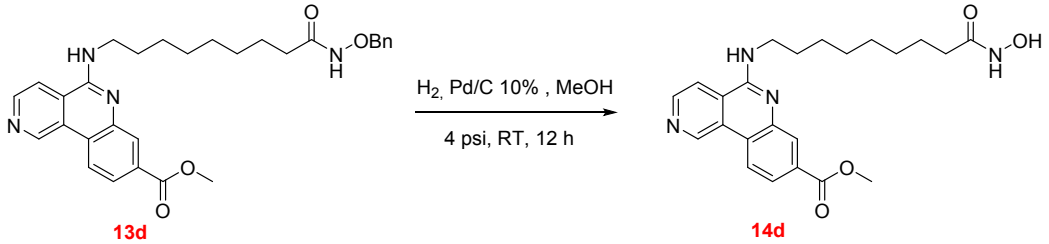
– 1.42 (m, 2H, C**H**$_2$), 1.43 – 1.04 (m, 6H, C**H**$_2$); **^{13}C NMR (101 MHz, DMSO)** δ 169.1 (**C**ON), 167.0 (**C**=O), 158.5 – 157.5 (m, **C**O-CF$_3$), 152.2 (**C**=N), 148.0 (**C**HAr), 147.7 (**C**HAr), 131.9 (**C**Ar), 126.4 (**C**Ar), 124.4 (**C**Ar), 123.6 (**C**HAr), 123.0 (**C**HAr), 121.1 (**C**Ar), 116.9 (**C**HAr), 116.8 (**C**F$_3$), 41.7 (**C**H$_2$), 32.3 (**C**H$_2$), 28.6 (**C**H$_2$), 28.1 (**C**H$_2$), 26.6 (**C**H$_2$), 25.1 (**C**H$_2$).

2.1.3.4 Synthesis of 8-carboxy-5-((9-(hydroxyamino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridin-2-ium -2,2,2-trifluoroacetate 15d



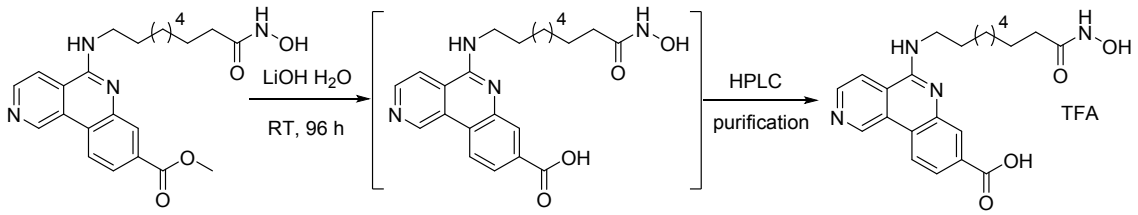
According to general procedure C, **methyl 5-((9-((benzyloxy)amino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13d** was obtained using **5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate 8** (347 mg, 1.3 mmol), **9-amino-*N*-(benzyloxy)nonanamide 12d** (1.2 g, 4.5 mmol), K_2CO_3 (175 mg, 1.3 mmol) and DMF as solvent (4.5 mL). Crude was purified using DCM:MeOH 99:1 as eluent mixture to yield **methyl 5-((9-((benzyloxy)amino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13d as a white solid** (302 mg, 46 %).

HRMS (ESI) calculated for $C_{30}H_{35}N_4O_4^+$ $[M+H]^+$ 515.2653; found m/z 515.1500**; MP:** 150-151 ºC; **IR** (film) v = 3459, 3229, 2934, 1729, 1655 cm^{-1}; **^{1}H NMR (400 MHz, DMSO)** δ 10.92 (s, 1H, N**H**-OBn), 10.04 (s, 1H, C**H**Ar), 8.88 (d, J = 5.0 Hz, 1H, C**H**Ar), 8.72 (d, J = 8.2 Hz, 1H, C**H**Ar), 8.28 (d, J = 4.8 Hz, 1H, C**H**Ar), 8.12 (s, 1H, C**H**Ar), 7.97 (brs, 1H, N**H**), 7.79 (d, J = 8.0 Hz, 1H, C**H**Ar), 7.36 (s, 5H, C**H**Ar), 4.76 (s, 2H, OC**H**$_2$), 3.90 (s, 3H, OC**H**$_3$), 3.64 - 3.53 (m, 2H, C**H**$_2$), 1.93 (brs, 2H, C**H**$_2$), 1.71 (brs, 2H, C**H**$_2$), 1.48 (brs, 2H, C**H**$_2$), 1.35 (brs, 4H, C**H**$_2$), 1.24 (brs, 4H, C**H**$_2$); **^{13}C NMR (101 MHz, DMSO)** δ 169.4 (**C**ON), 166.3 (**C**=O), 152.8 (**C**=N), 147.6 (**C**HAr), 147.5 (**C**HAr), 145.0 (**C**Ar), 136.1 (**C**Ar), 130.1 (**C**Ar), 128.8 (**C**HAr), 128.3 (**C**HAr), 128.2 (**C**HAr), 127.5 (**C**HAr), 126.6 (**C**Ar), 124.0 (**C**Ar), 122.7 (**C**HAr), 121.8 (C**H**Ar), 121.6 (**C**Ar), 116.2 (**C**HAr), 76.7 (O**C**H$_2$), 52.3 (O**C**H$_3$), 40.8 (**C**H$_2$), 32.2 (**C**H$_2$), 28.8 (**C**H$_2$), 28.7 (**C**H$_2$), 28.5 (**C**H$_2$), 26.7 (**C**H$_2$), 25.0 (**C**H$_2$).



According to general procedure C, **methyl 5-((9-(hydroxyamino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14d** was obtained using **methyl 5-((9-((benzyloxy)amino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 13d** (189 mg, 0.36 mmol), Pd/C 10 wt. % loading (75 mg) and methanol:tetrahydrofurane 1:1 as solvent (54 mL). Crude was purified using DCM:MeOH 9:1 as eluent mixture to yield **methyl 5-((9-(hydroxyamino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14d as a pale yellow solid** (70 mg, 46 %).

HRMS (ESI) calculated for $C_{23}H_{29}N_4O_4^+$ $[M+H]^+$ 425.2183; found m/z 425.1300**; MP:** 101-103 ºC; **IR** (film) v = 3393, 3213, 2930, 2852, 1713, 1647 cm^{-1}.



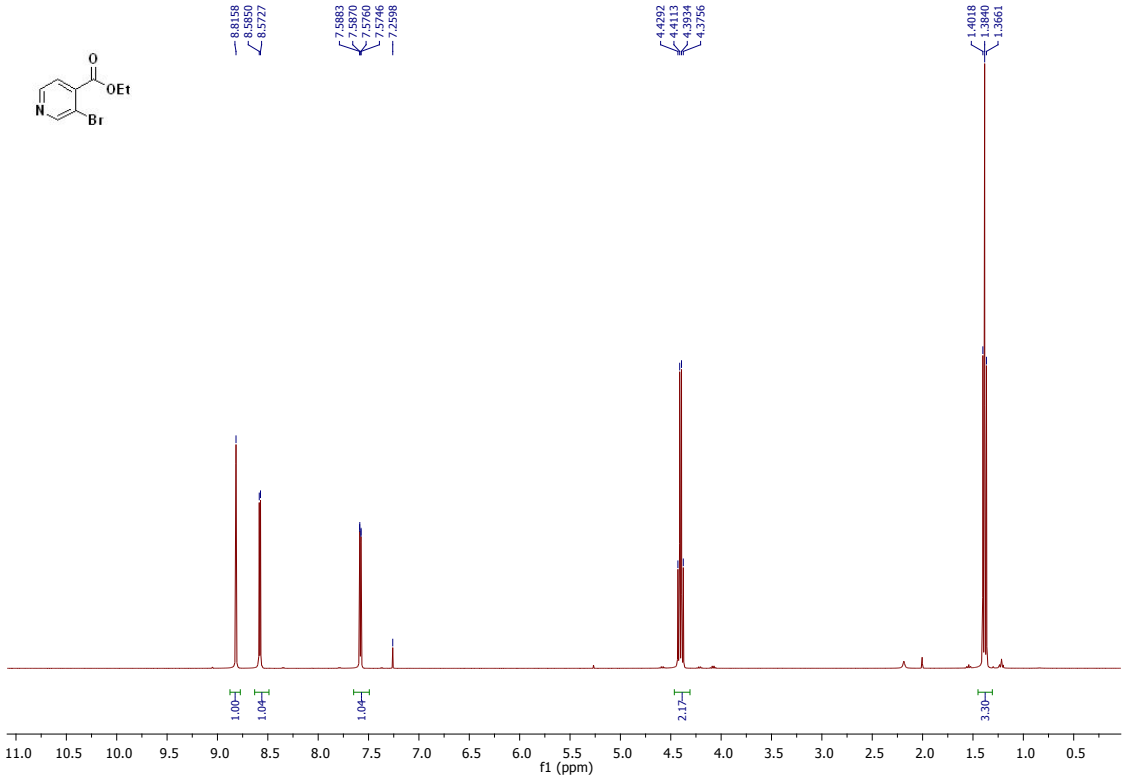
14d **15d**

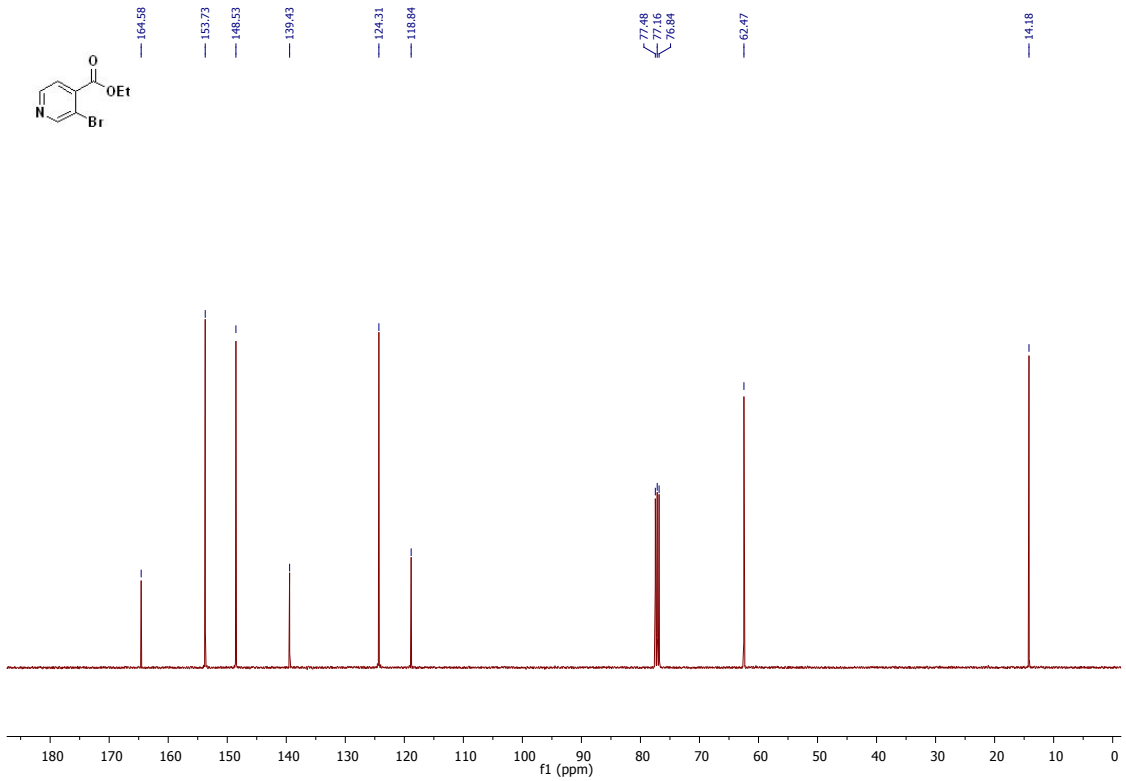
According to general procedure C, **8-carboxy-5-((9-(hydroxyamino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridin-2-ium -2,2,2-trifluoroacetate 15d** was obtained using **methyl 5-((9-(hydroxyamino)-9-oxononyl)amino)benzo[*c*][2,6]naphthyridine-8-carboxylate 14d** (20 mg, 0.047 mmol), LiOH H_2O (4 mg, 0.094 mmol) and THF:MeOH:H_2O 2:2:1 as solvent (2.5 mL). After HPLC purification in acidic conditions, **compound 15d** was obtained as a **pale yellow solid** (10 mg, 40 %). Purity was determined according to general experimental 1.7 using Kromaphase C18 5μ, 250 x 4.6 mm as column.

LRMS (ESI) **calcd for** $C_{22}H_{27}N_4O_4^+$ ($[M+H]^+$ 411.2, found 410.9 m/z (rel. intensity 100 %); **MS (ESI) m/z** 410.9 [M + H]; **Purity:** 98%; **MP:** 141-143 ºC; **IR** (film) v = 3217, 2930, 2865, 1672, 1622 cm^{-1}; **^{1}H NMR (400 MHz, DMSO)** δ 10.33 (s, 1H, N**H**-OH), 10.11 (s, 1H, C**H**Ar), 8.98 (s, 1H, C**H**Ar), 8.80 (d, J = 8.5 Hz, 1H, C**H**Ar), 8.42 (s, 1H, C**H**Ar), 8.36 (s, 1H, C**H**Ar), 7.91 (d, J = 6.9 Hz, 1H, C**H**Ar), 3.65 (brs, 2H, C$\mathbf{H}_2$), 1.92 (t, J = 7.2 Hz, 2H, C$\mathbf{H}_2$), 1.80 - 1.70 (m, 2H, C$\mathbf{H}_2$), 1.59 – 1.16 (m, 10H, C$\mathbf{H}_2$); **^{13}C NMR (101 MHz, DMSO)** δ 169.1 (**C**ON), 166.9 (**C**=O), 158.4 – 157.4 (m, **C**O-CF_3), 152.0 (**C**=N), 148.1 (**C**HAr), 147.7 (**C**HAr), 132.0 (**C**Ar), 126.3 (**C**Ar), 124.5 (**C**Ar), 123.8 (**C**HAr), 123.0 (**C**HAr), 121.0 (**C**Ar), 116.9 (**C**HAr), 41.8 (**C**H_2), 32.3 (**C**H_2), 28.8 (**C**H_2), 28.0 (**C**H_2), 26.6 (**C**H_2), 25.1 (**C**H_2).

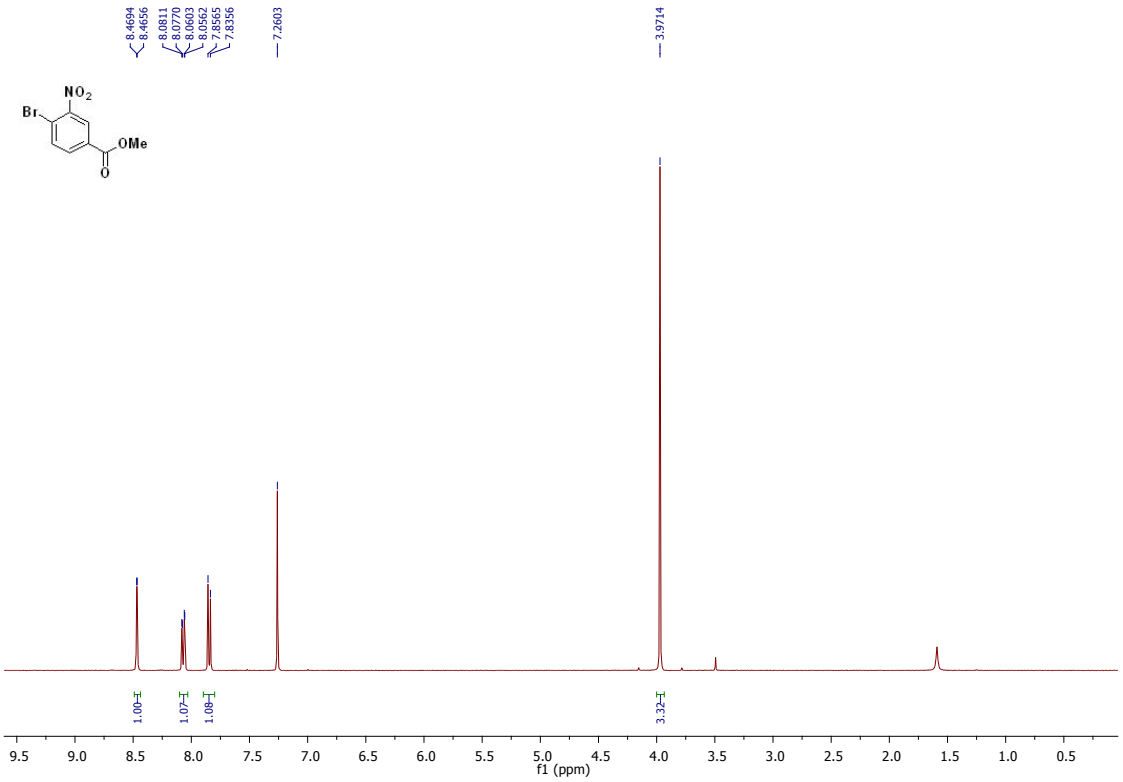
^{1}H and ^{13}C NMR spectra for the synthesis of compound **8**

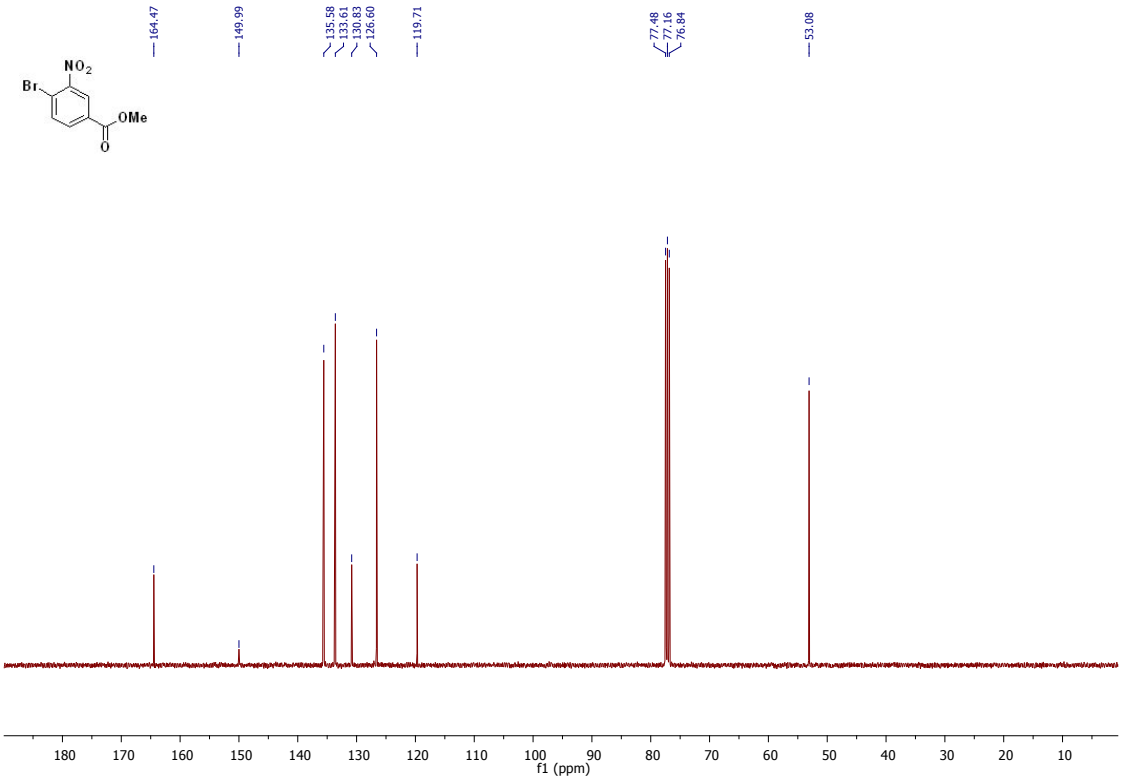
^{1}H and ^{13}C NMR spectra Compound 3 ($CDCl_3$)



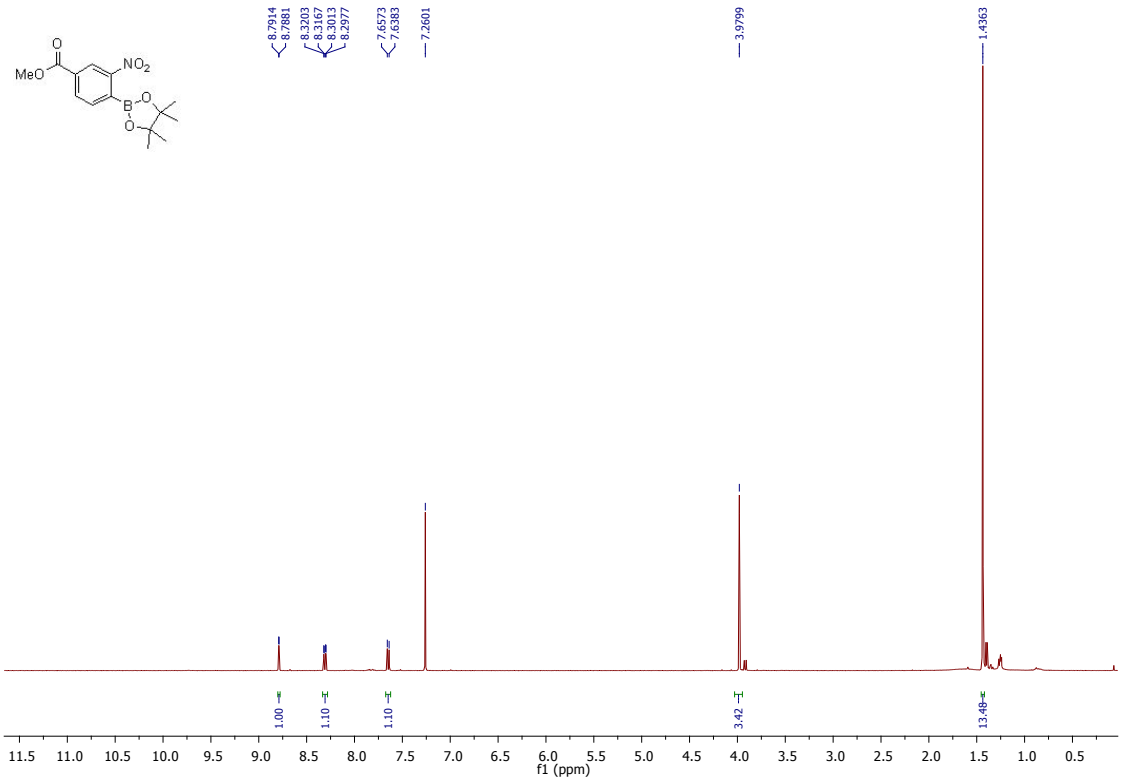


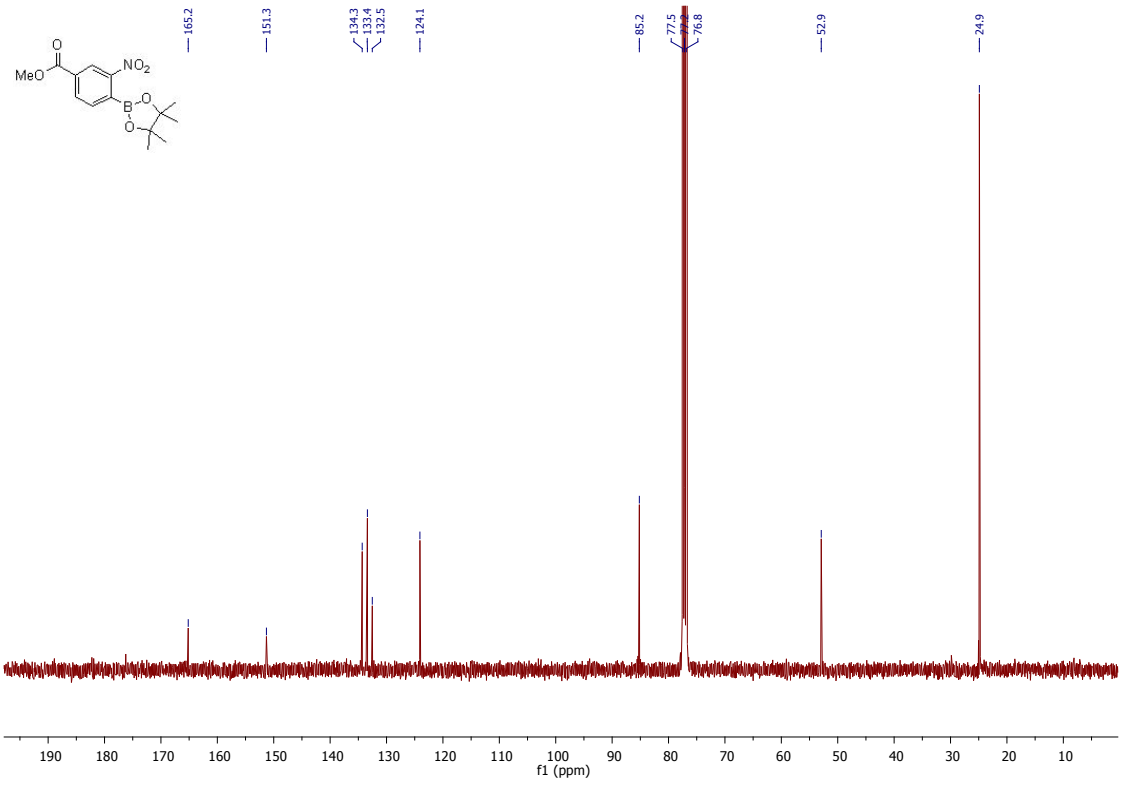
^{1}H and ^{13}C NMR spectra Compound 4 ($CDCl_3$)



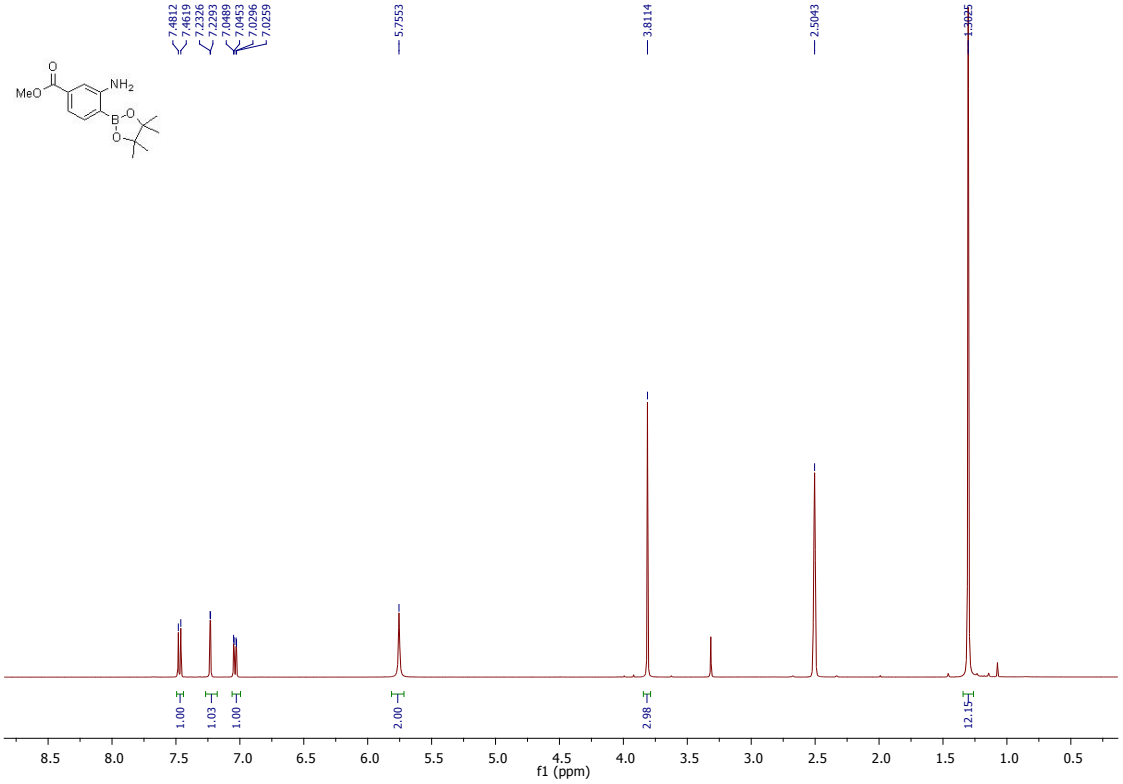


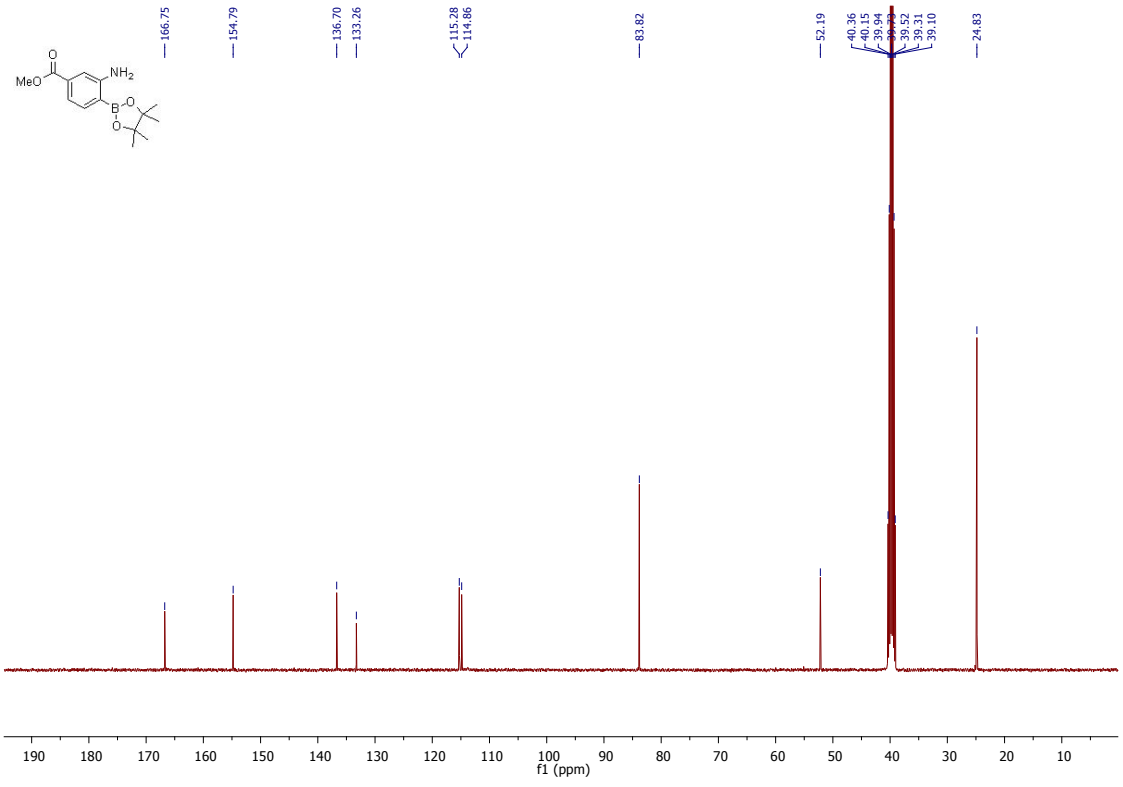
^{1}H and ^{13}C NMR spectra Compound 5 ($CDCl_3$)



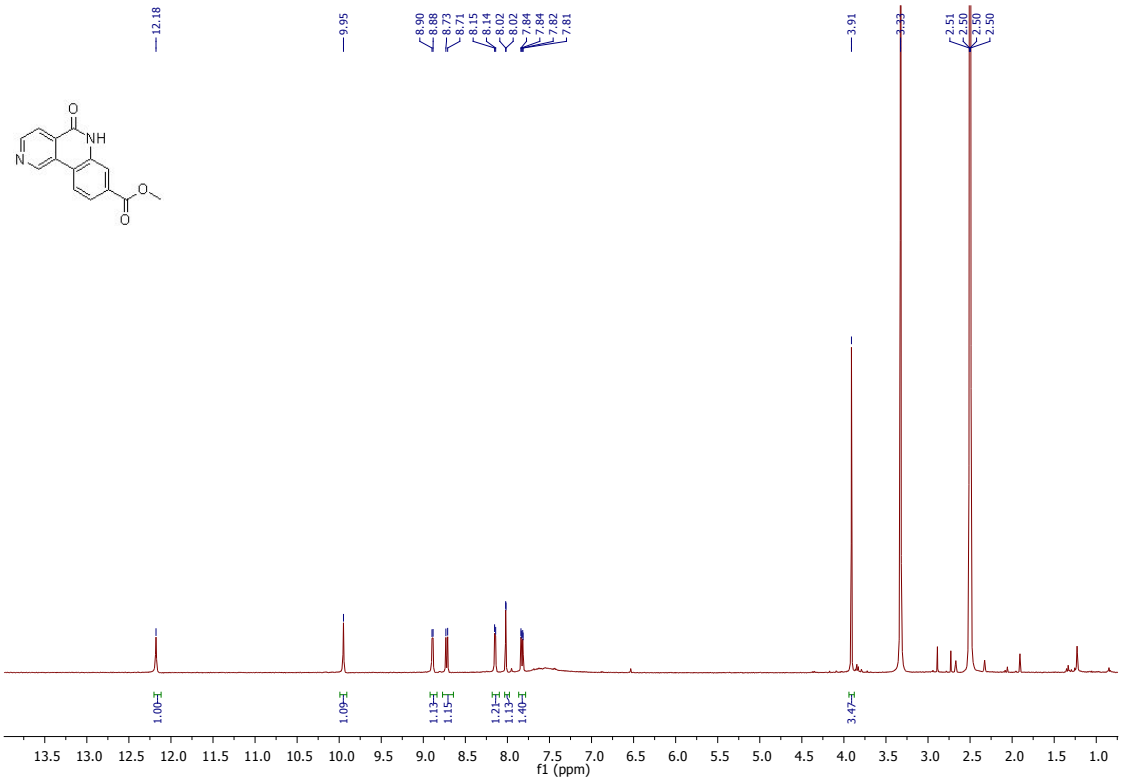


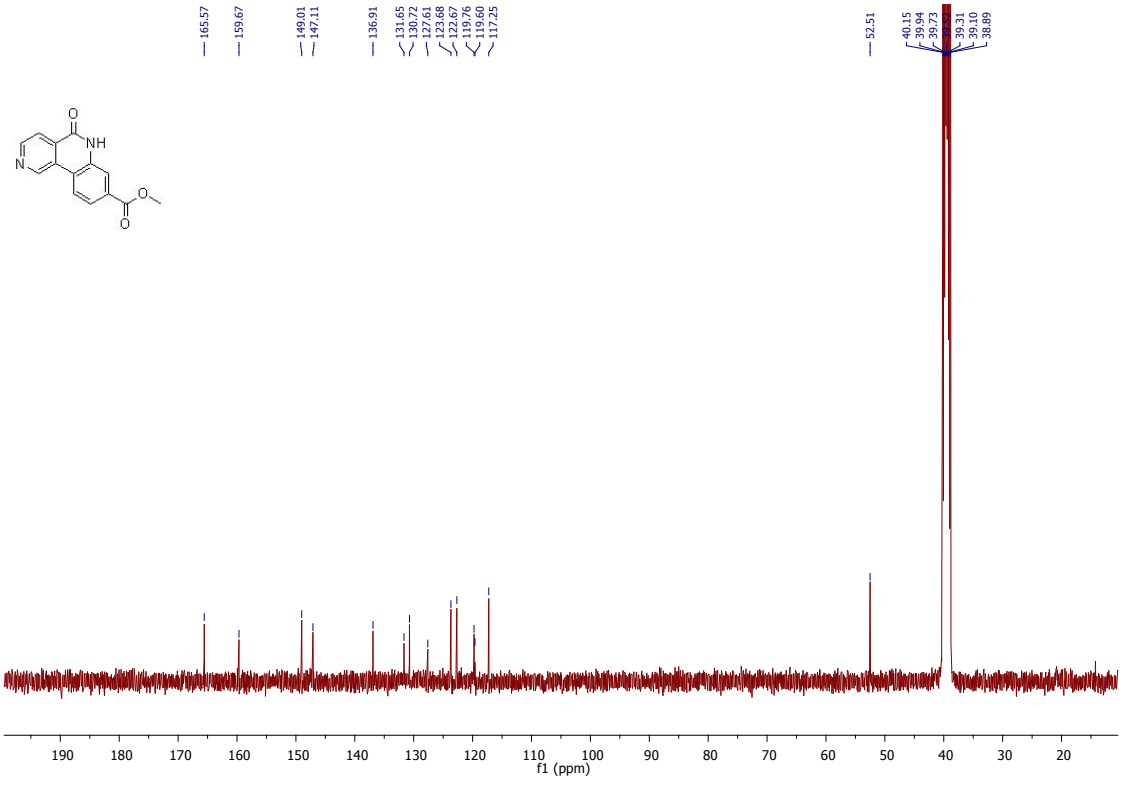
^{1}H and ^{13}C NMR spectra Compound 6 (DMSO-d_6)



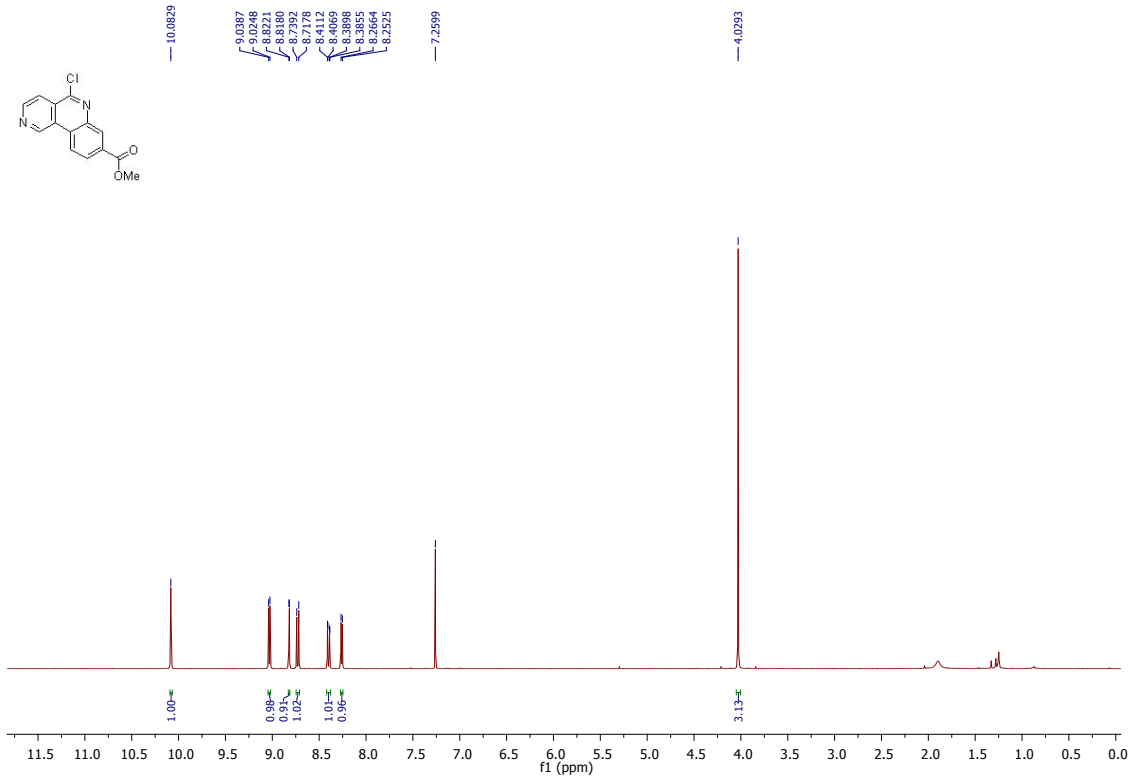


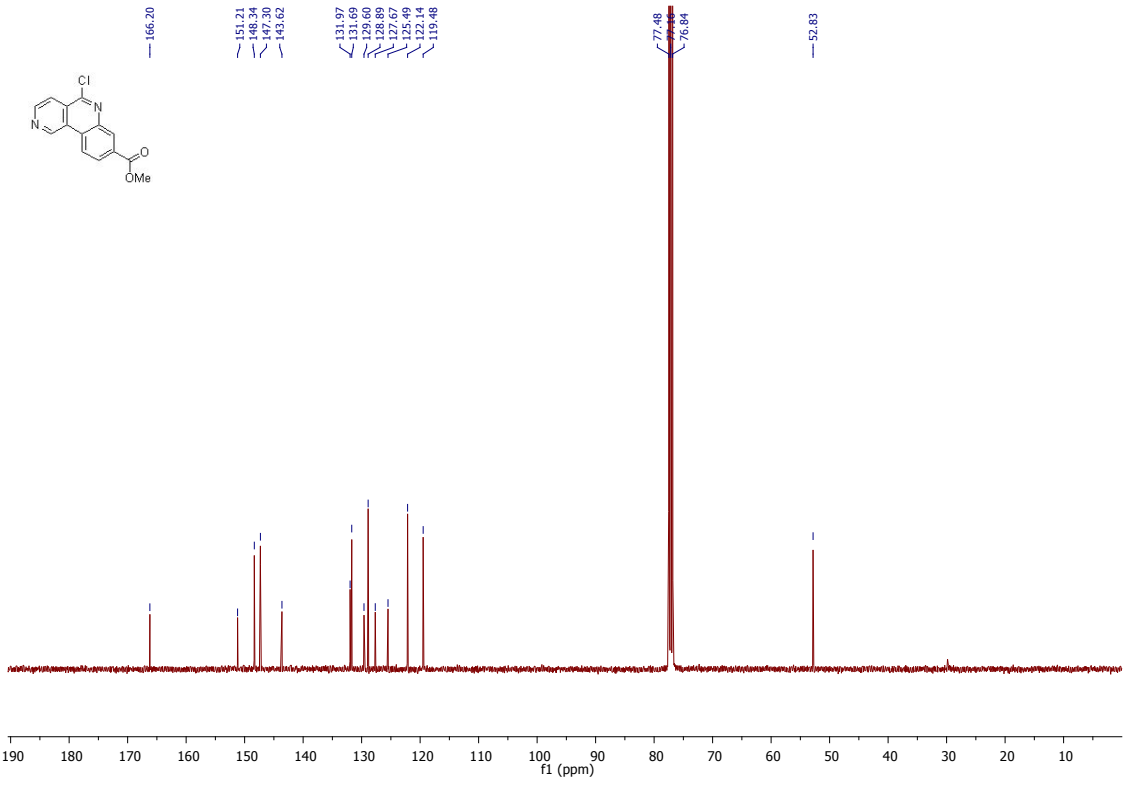
^{1}H and ^{13}C NMR spectra Compound 7 (DMSO-d_6)





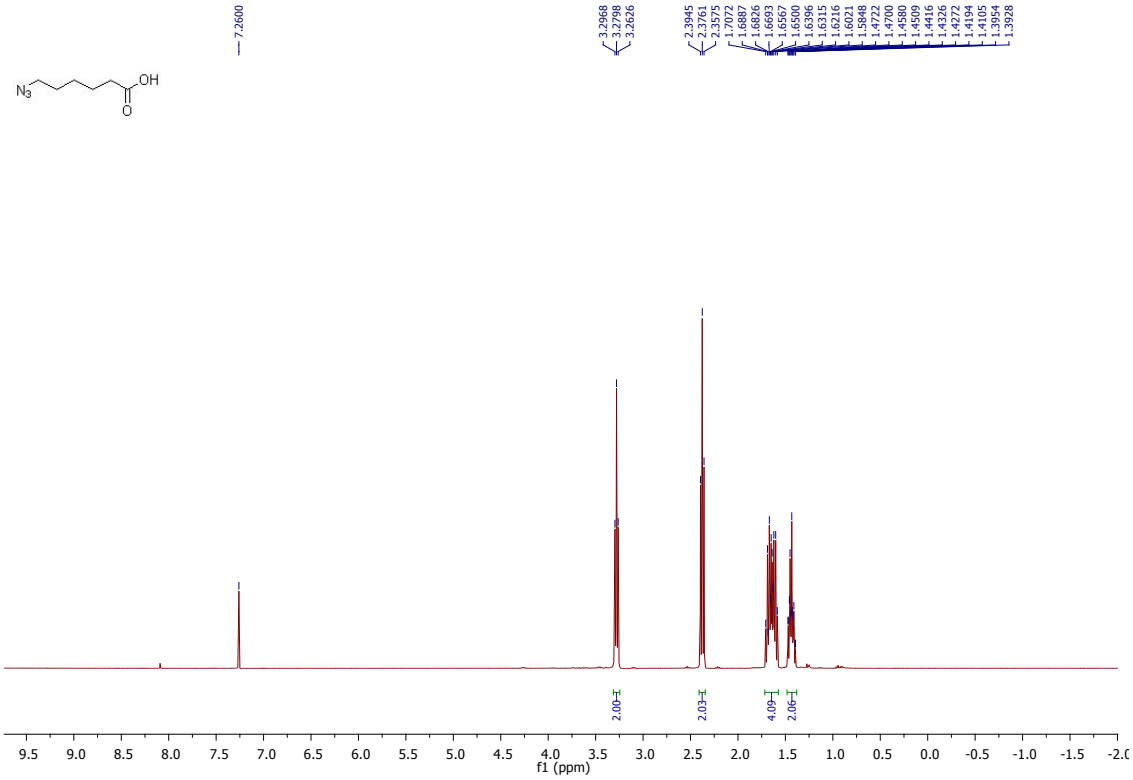
^{1}H and ^{13}C NMR spectra Compound 8 ($CDCl_3$)

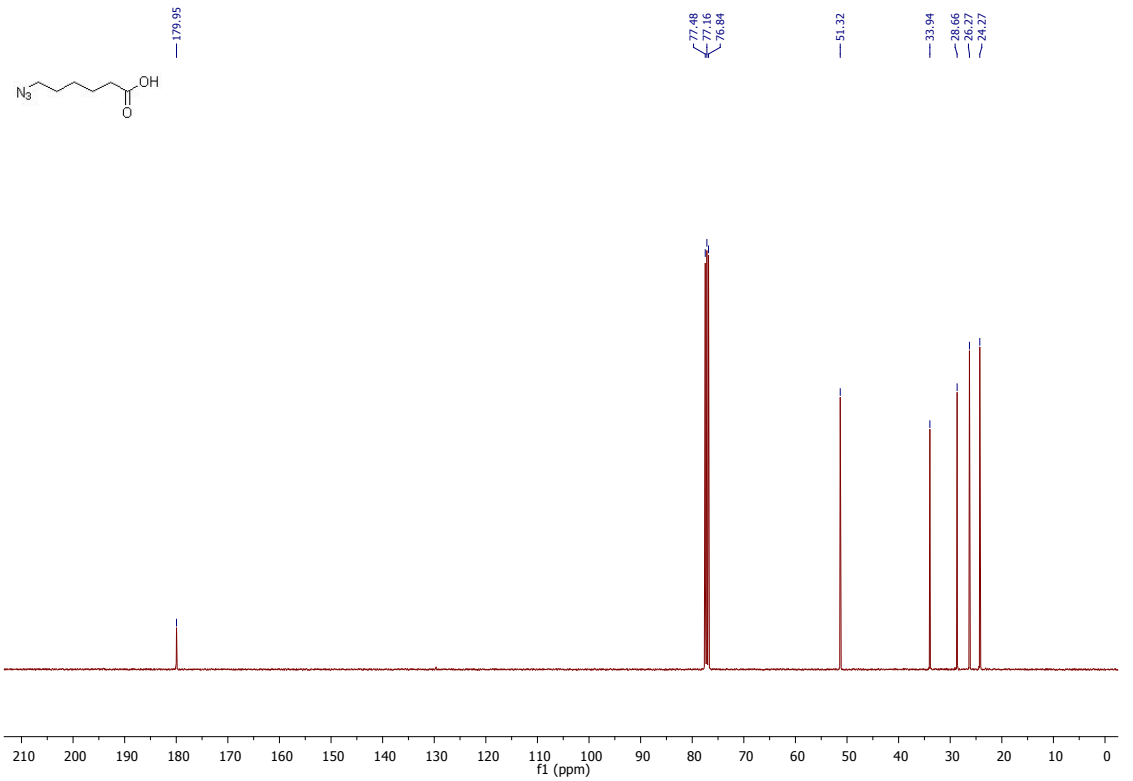




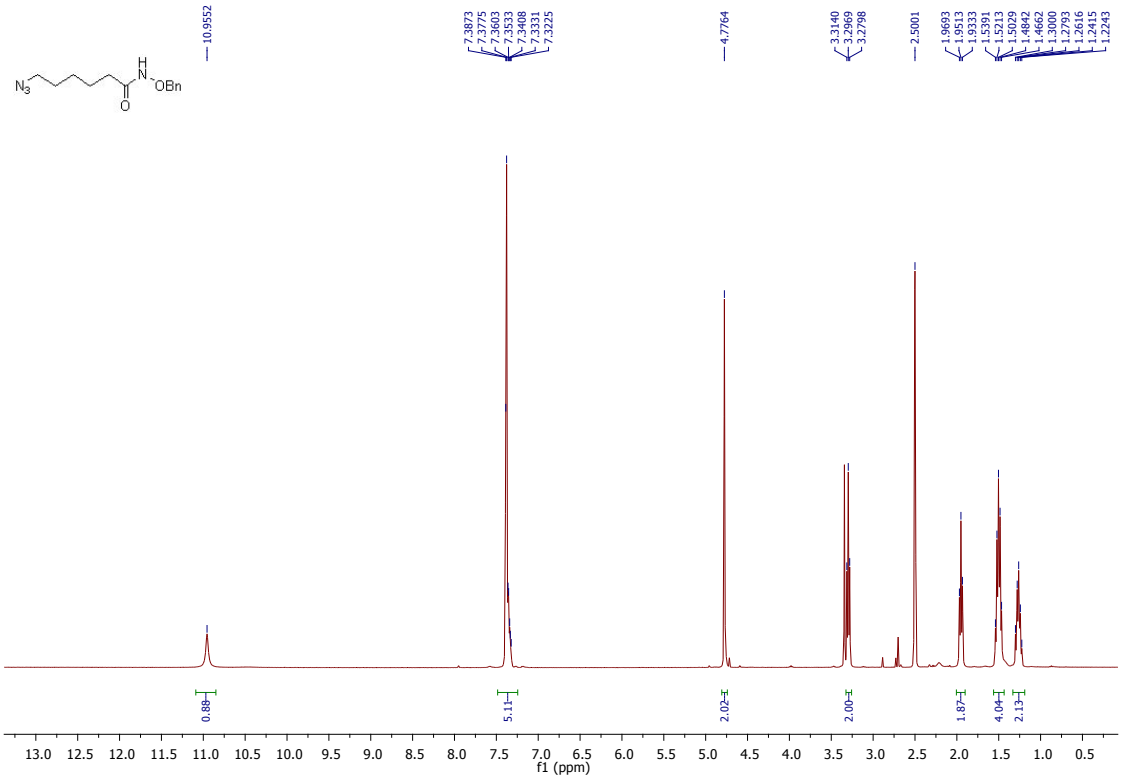
^{1}H and ^{13}C NMR spectra for the synthesis of Ω–amine (benzyloxy)amino compounds **12a-d**

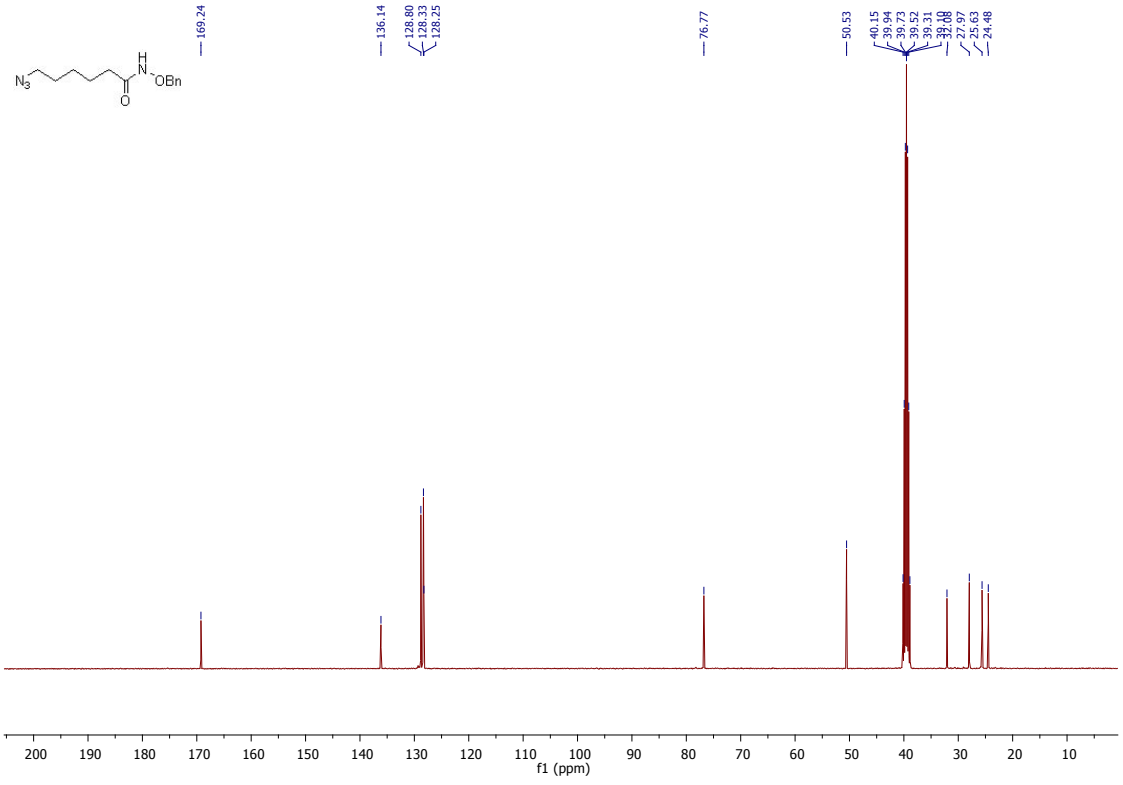
^{1}H and ^{13}C NMR spectra Compound 10a ($CDCl_3$)



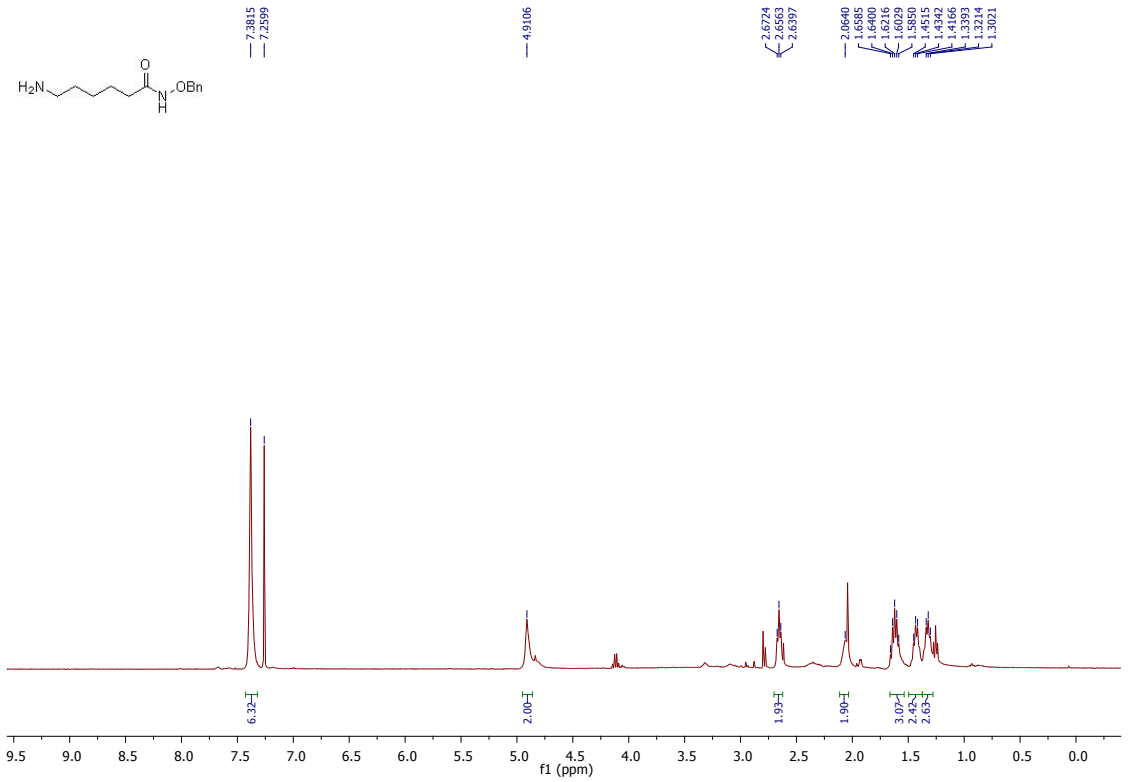


^{1}H and ^{13}C NMR spectra Compound 11a (DMSO-d_6)

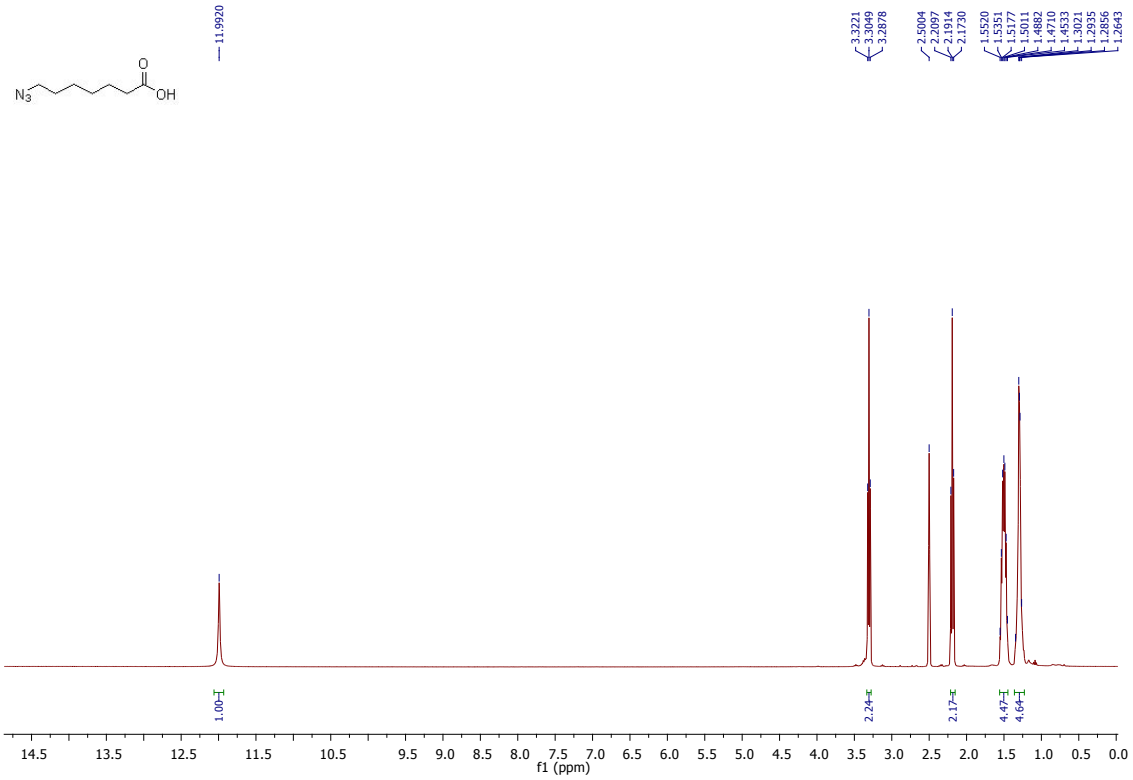


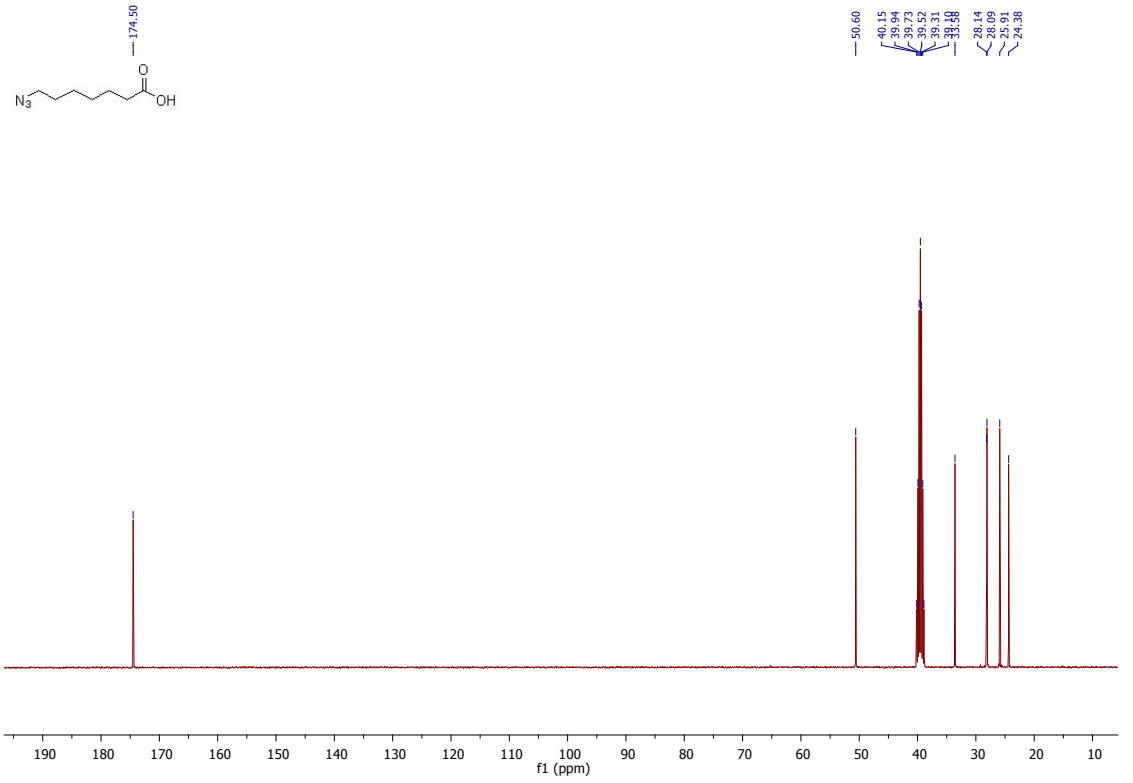


^{1}H NMR spectrum Compound 12a ($CDCl_3$)

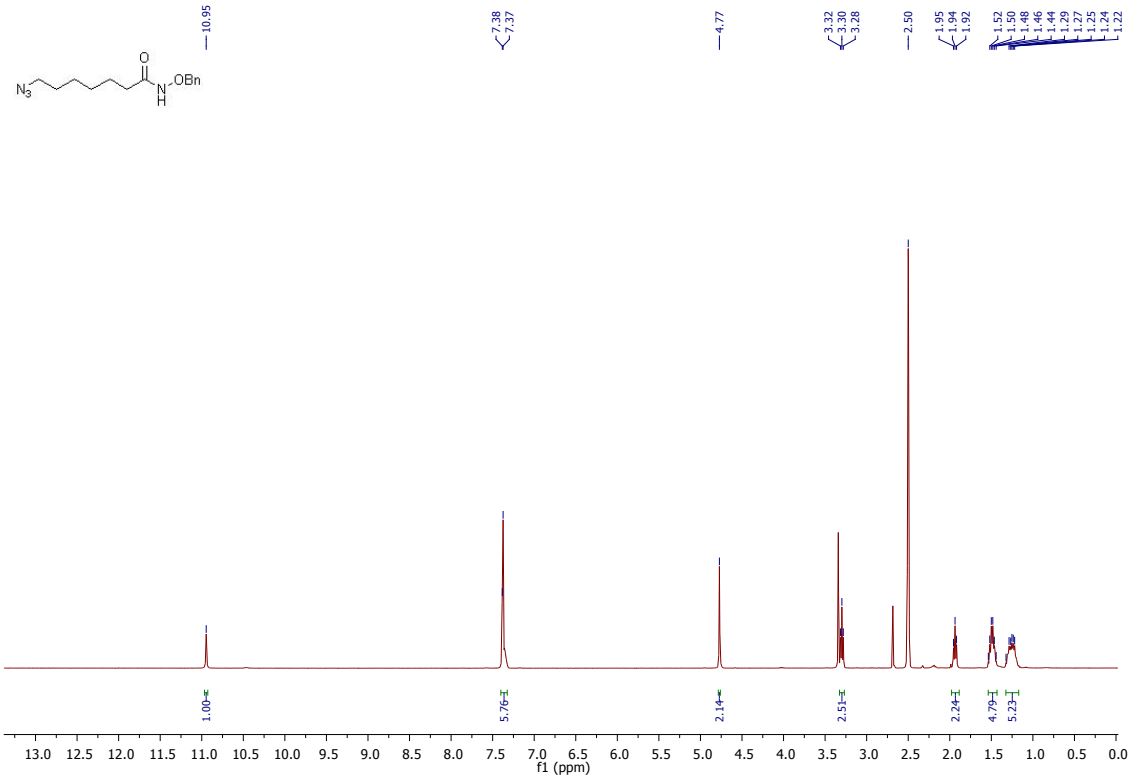


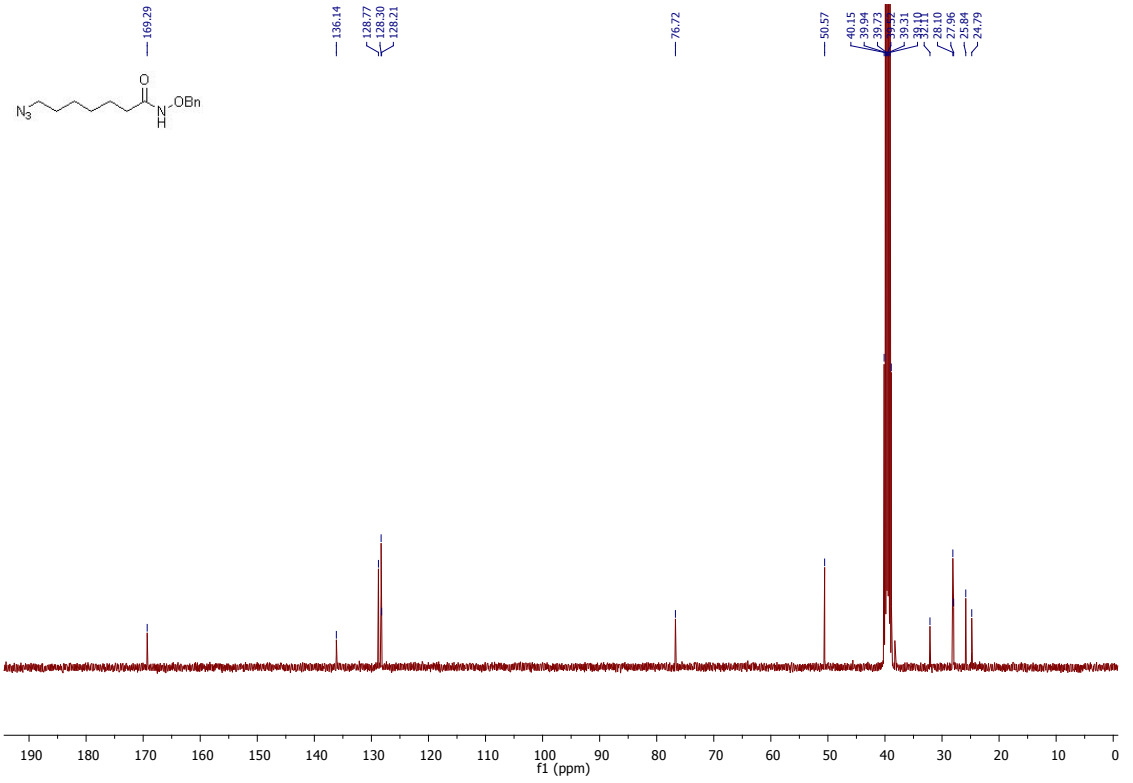
^{1}H and ^{13}C NMR spectra Compound 10b (DMSO-d_6)



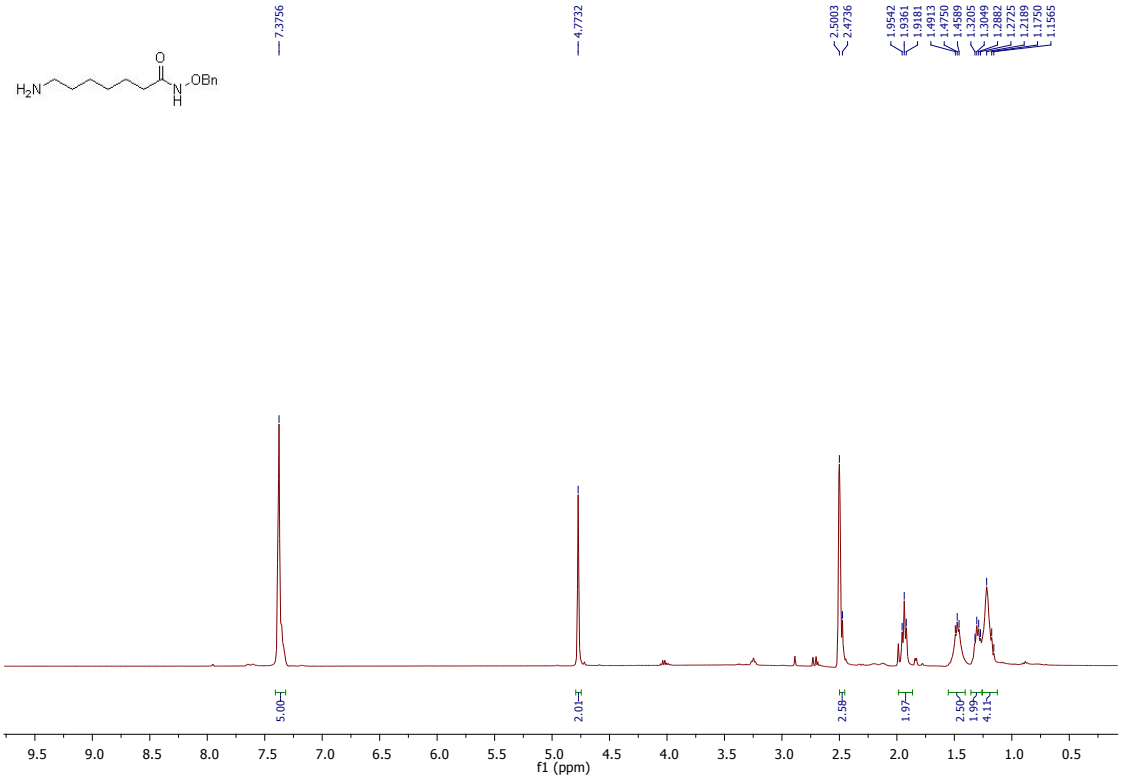


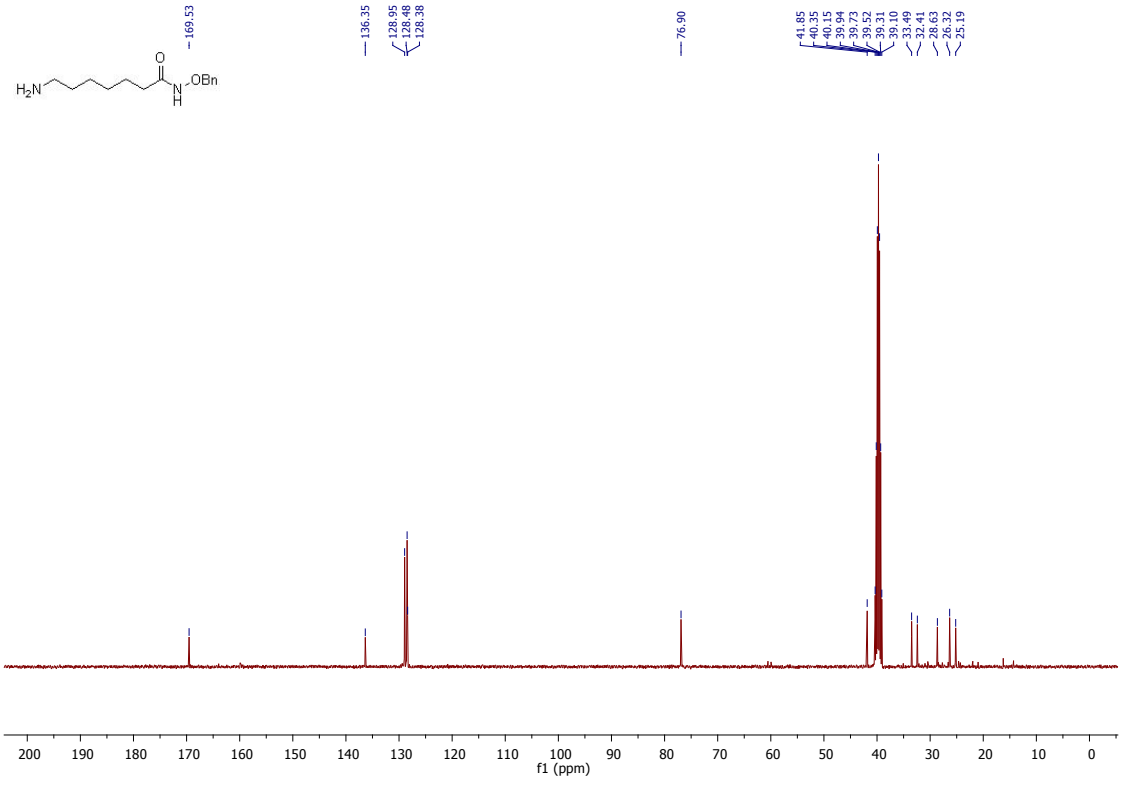
^{1}H and ^{13}C NMR spectra Compound 11b (DMSO-d_6)



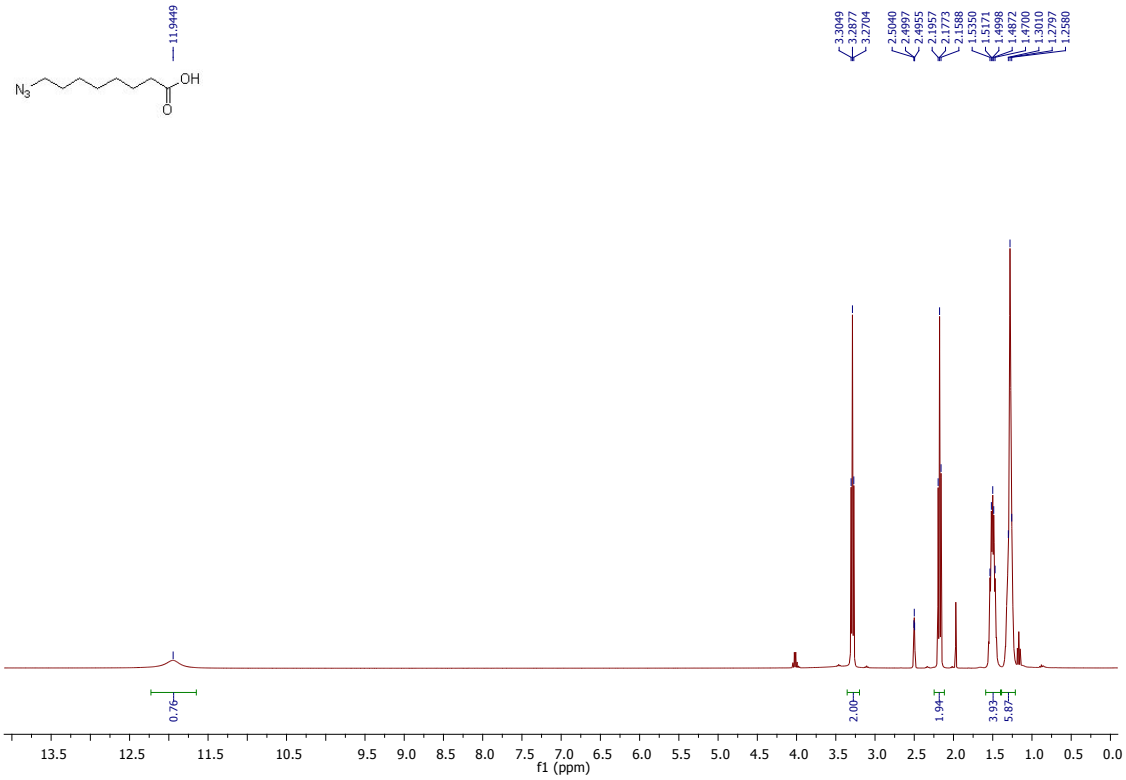


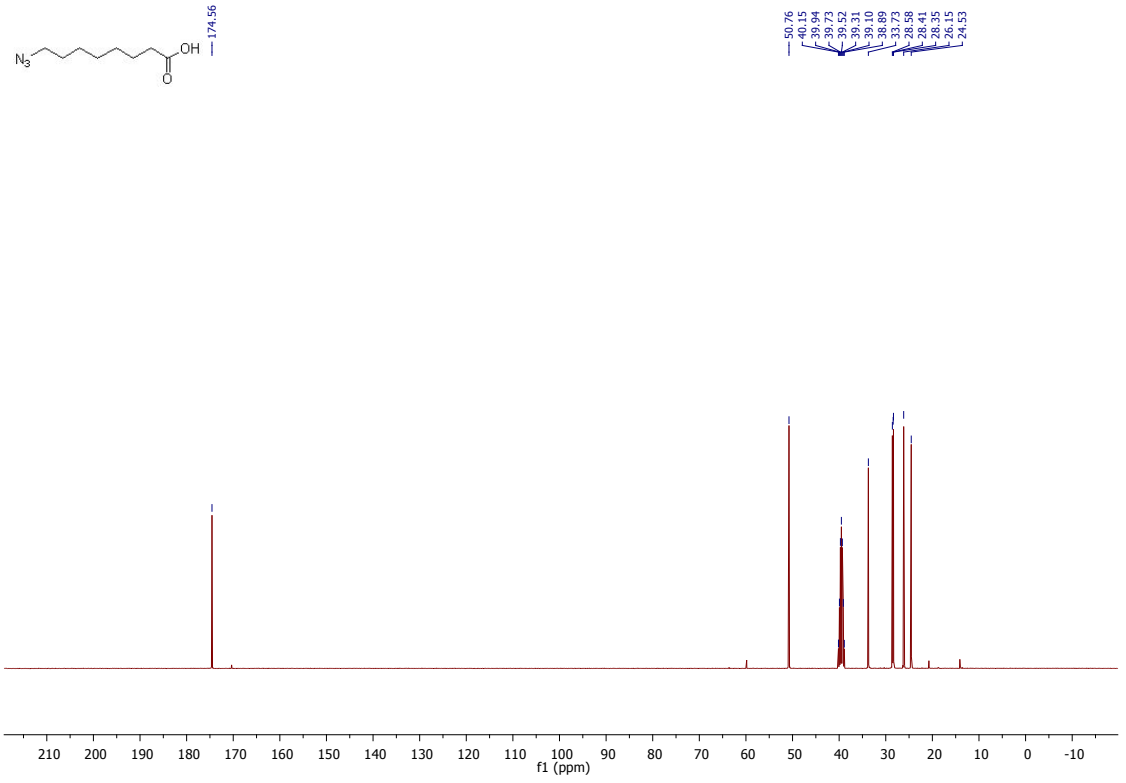
^{1}H and ^{13}C NMR spectra Compound 12b (DMSO-d_6)



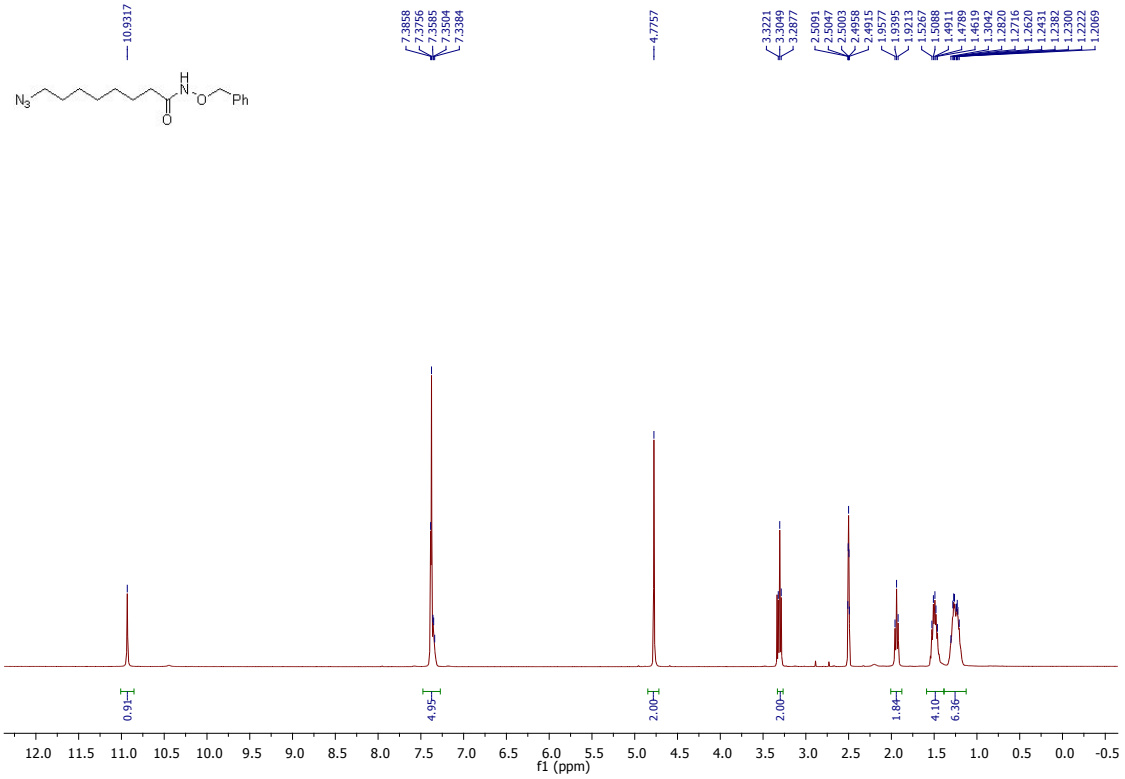


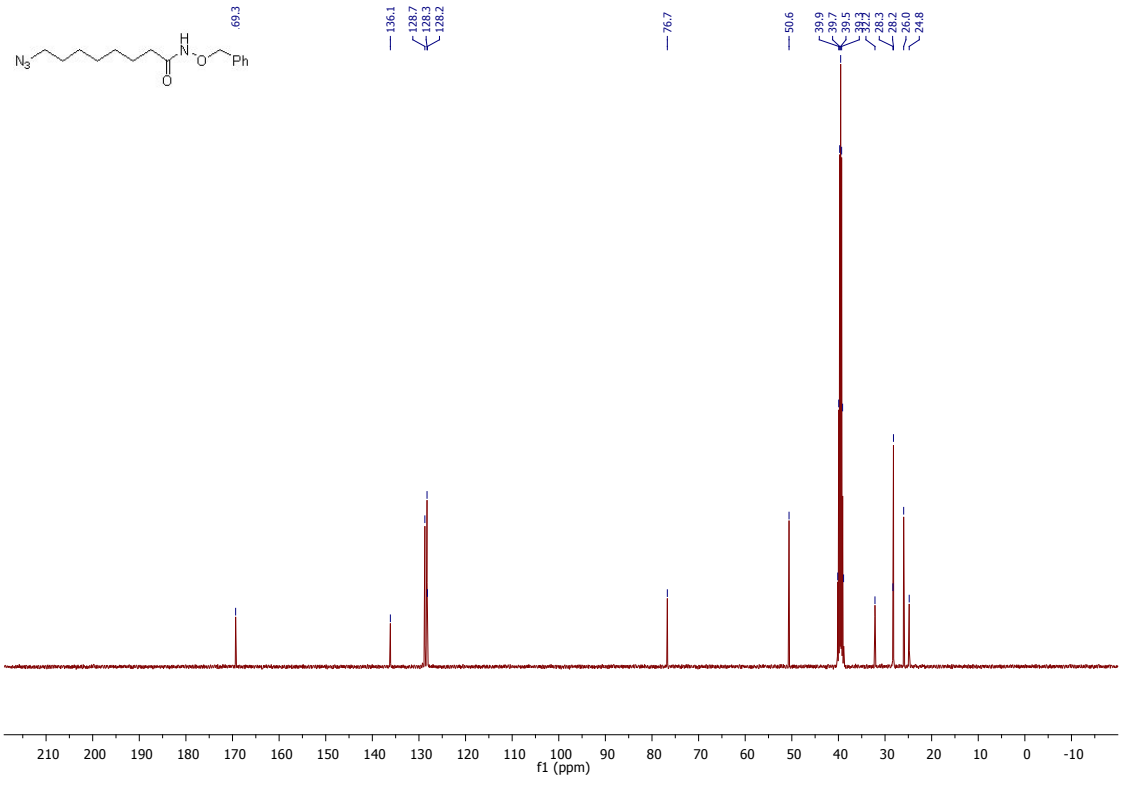
^{1}H and ^{13}C NMR spectra Compound 10c (DMSO-d_6)



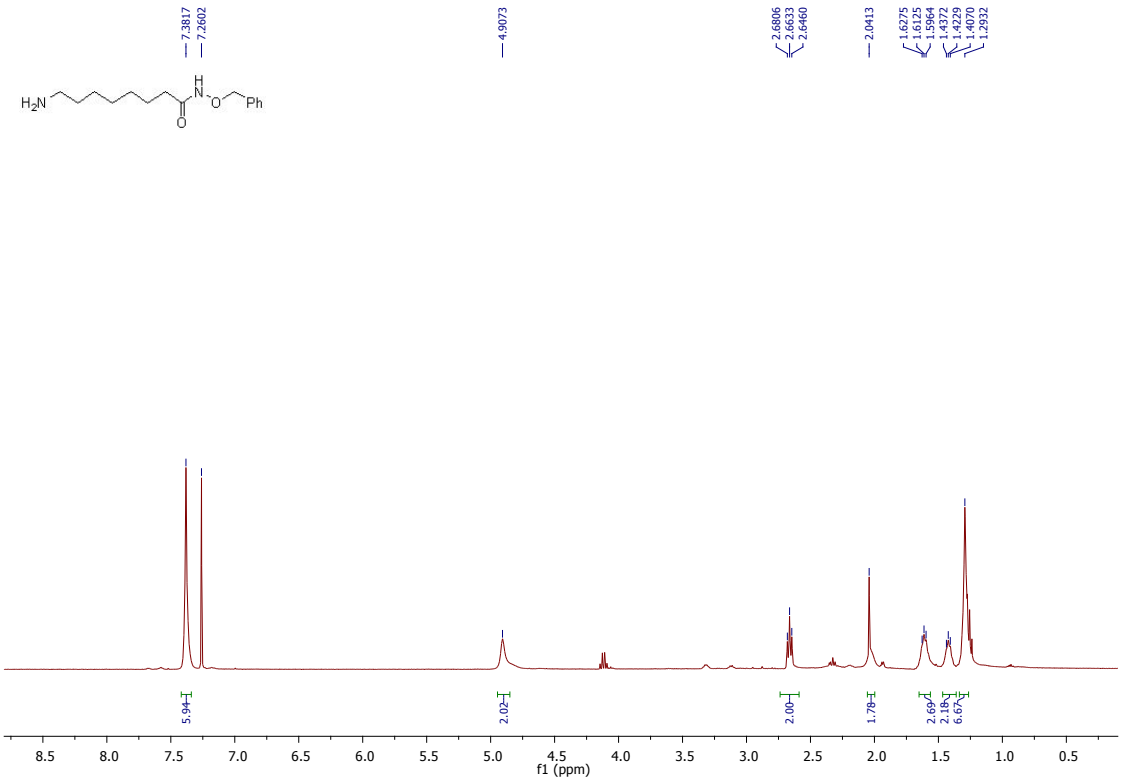


^{1}H and ^{13}C NMR spectra Compound 11c (DMSO-d_6)

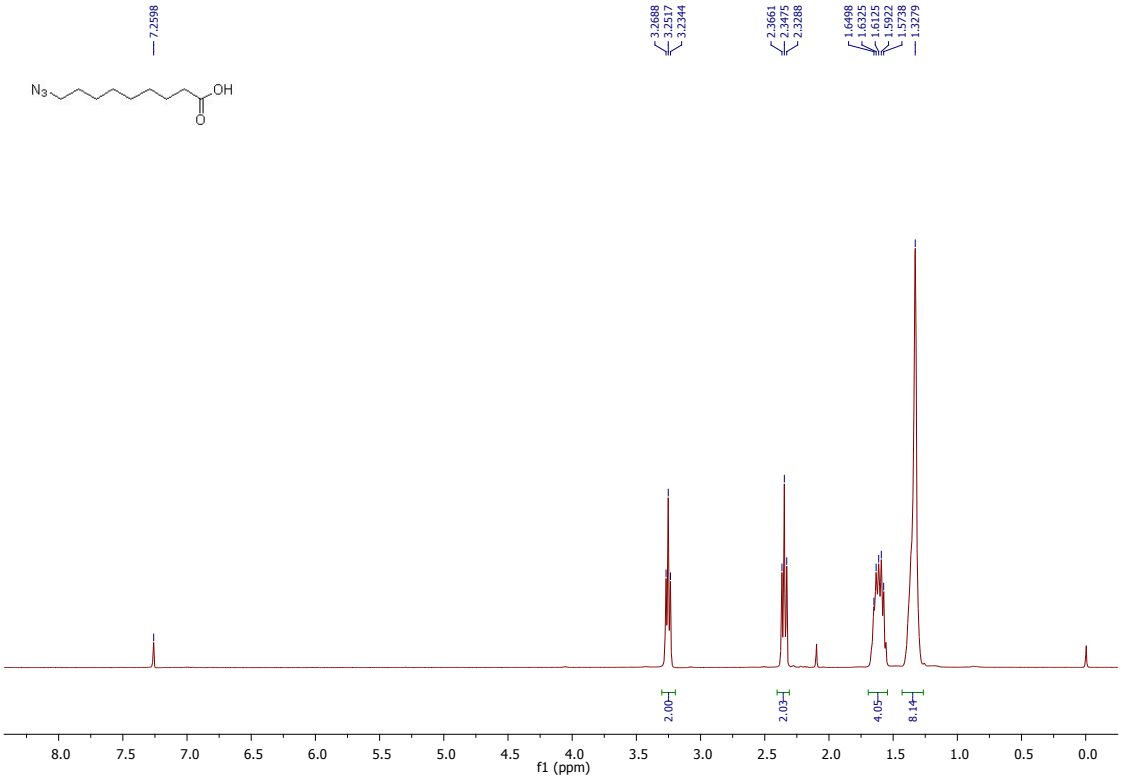


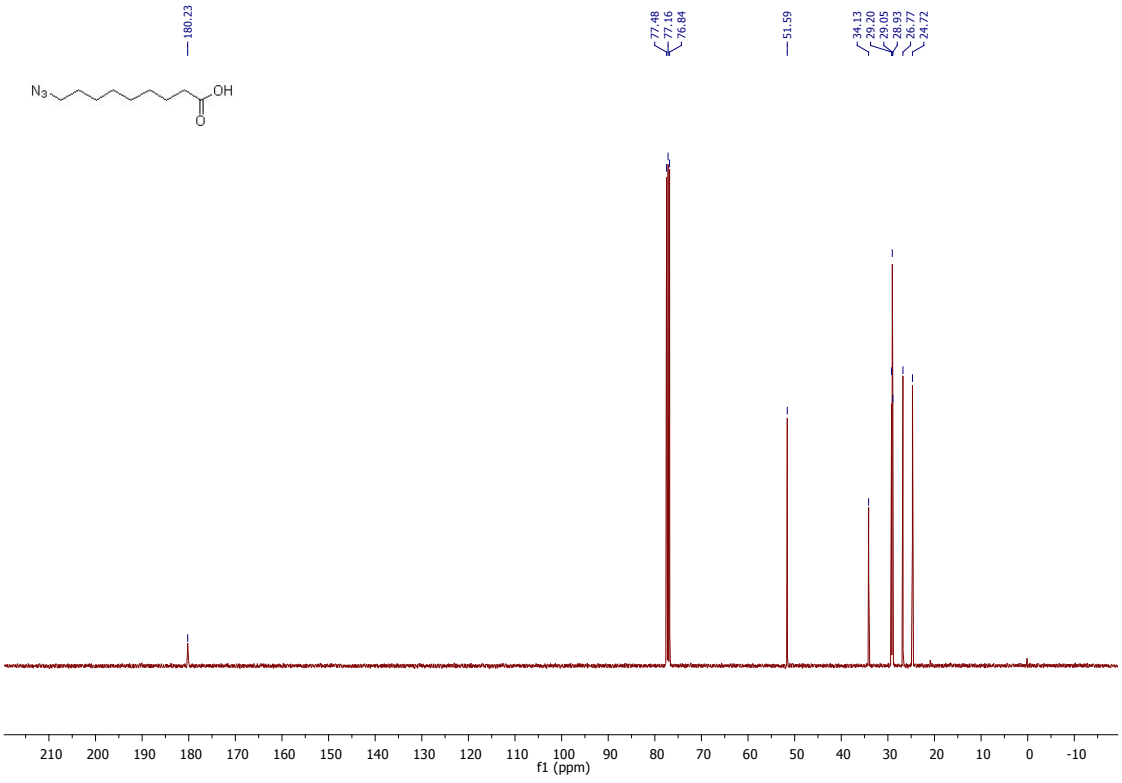


^{1}H and ^{13}C NMR spectra Compound 12c ($CDCl_3$)

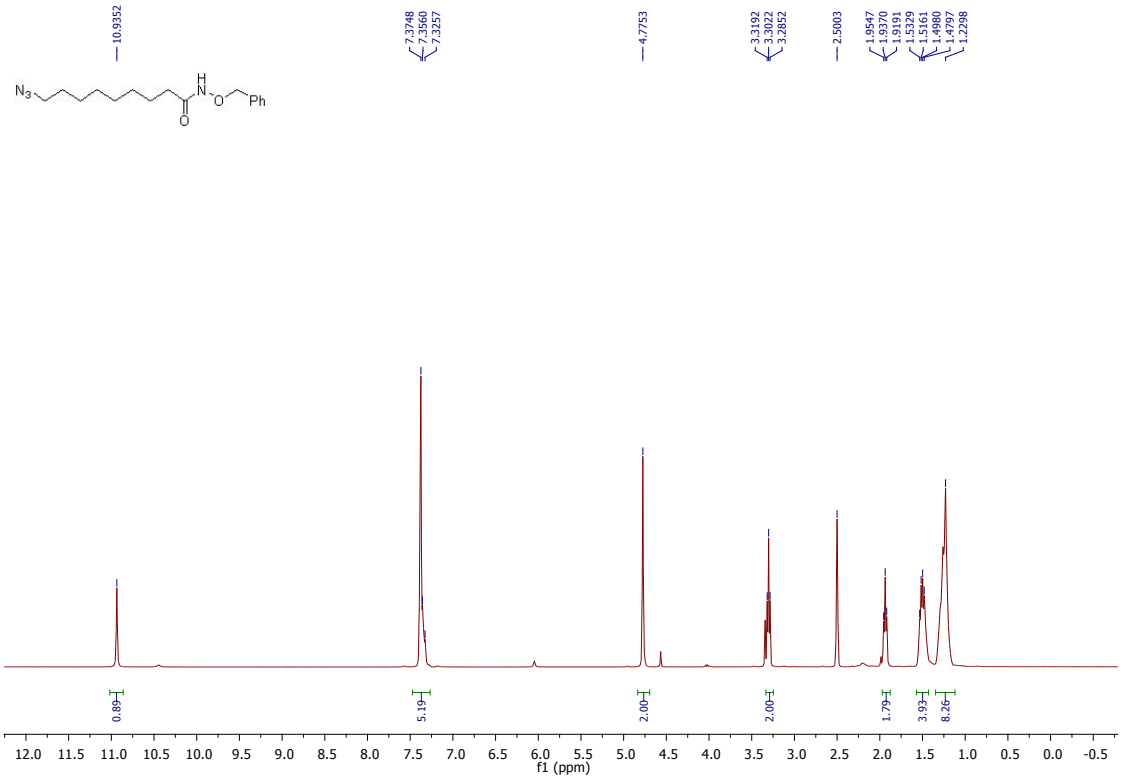


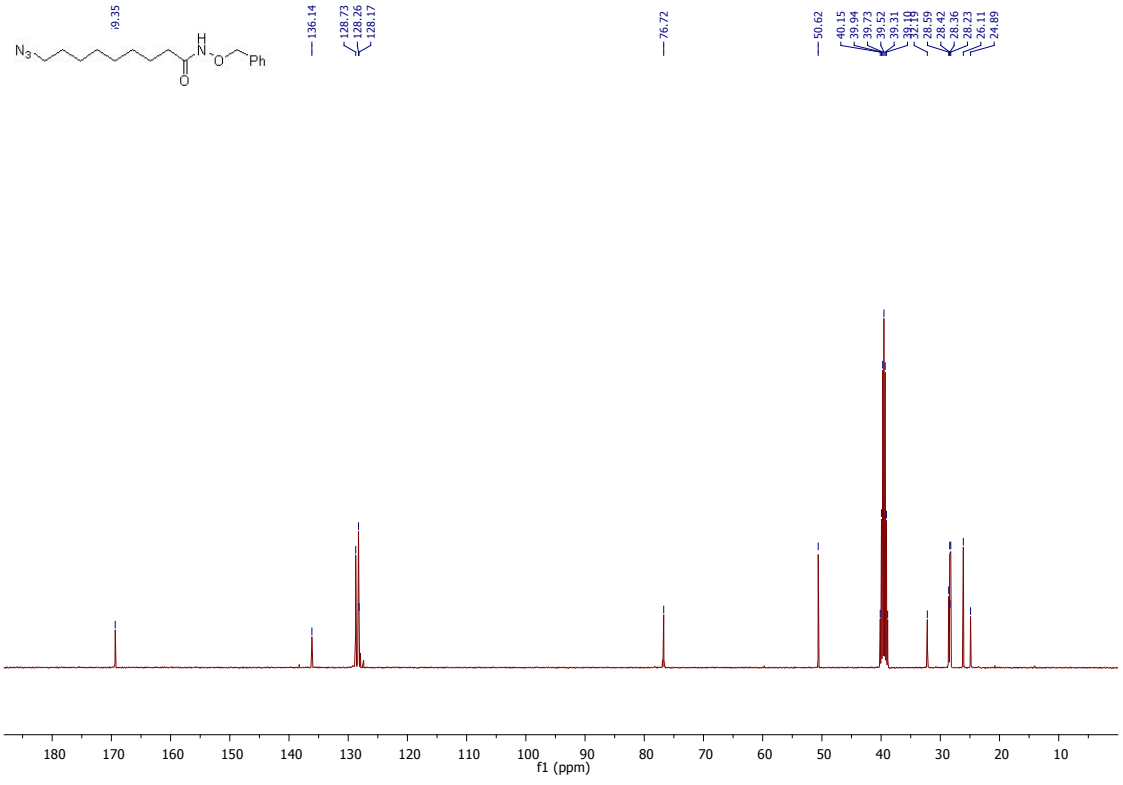
^{1}H and ^{13}C NMR spectra Compound 10d ($CDCl_3$)



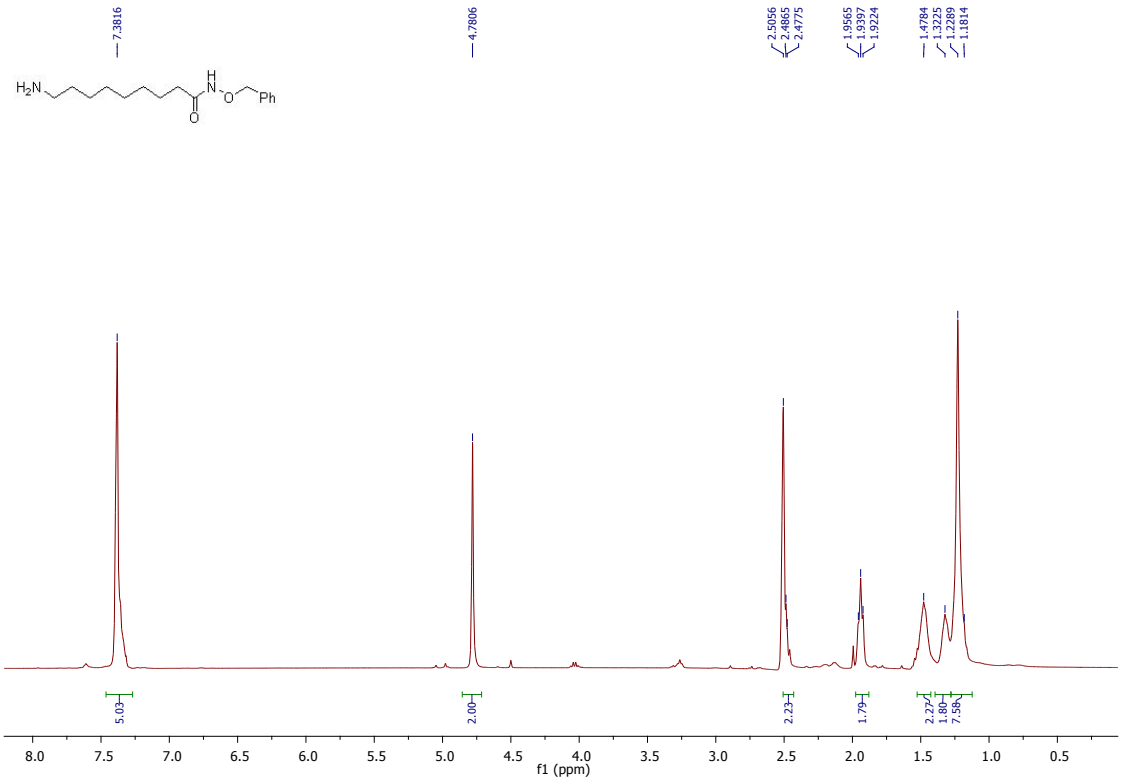


^{1}H and ^{13}C NMR spectra Compound 11d (DMSO-d_6)



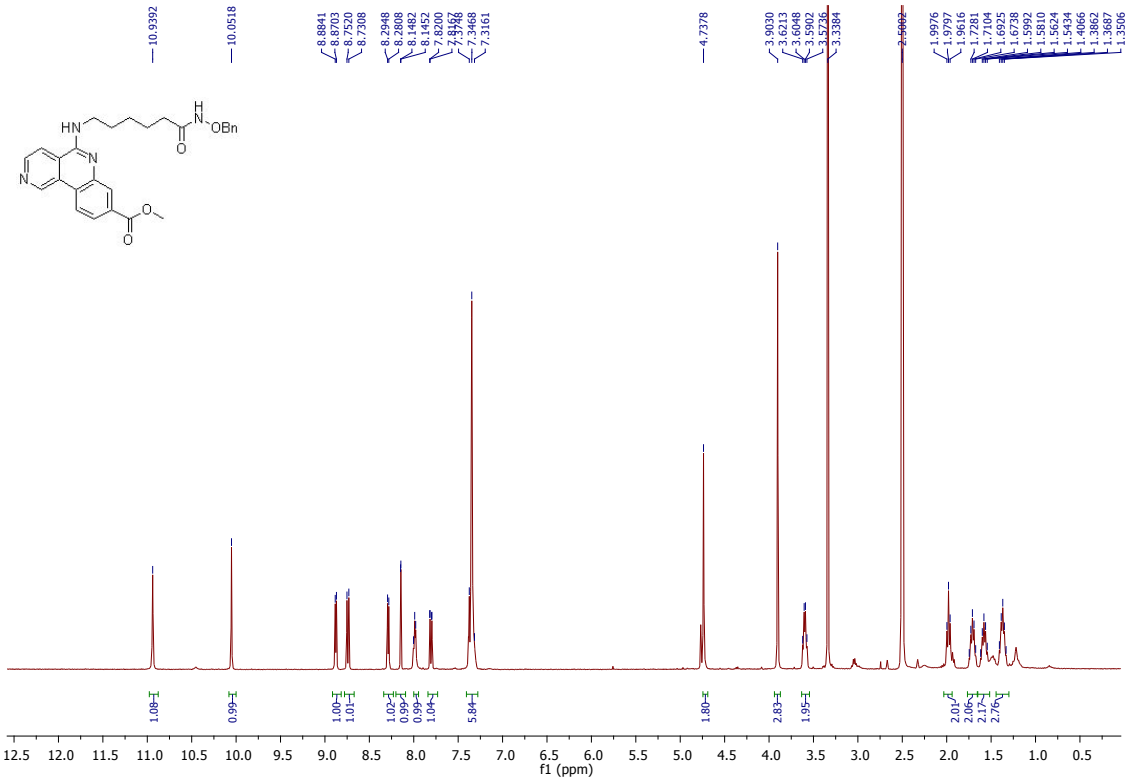


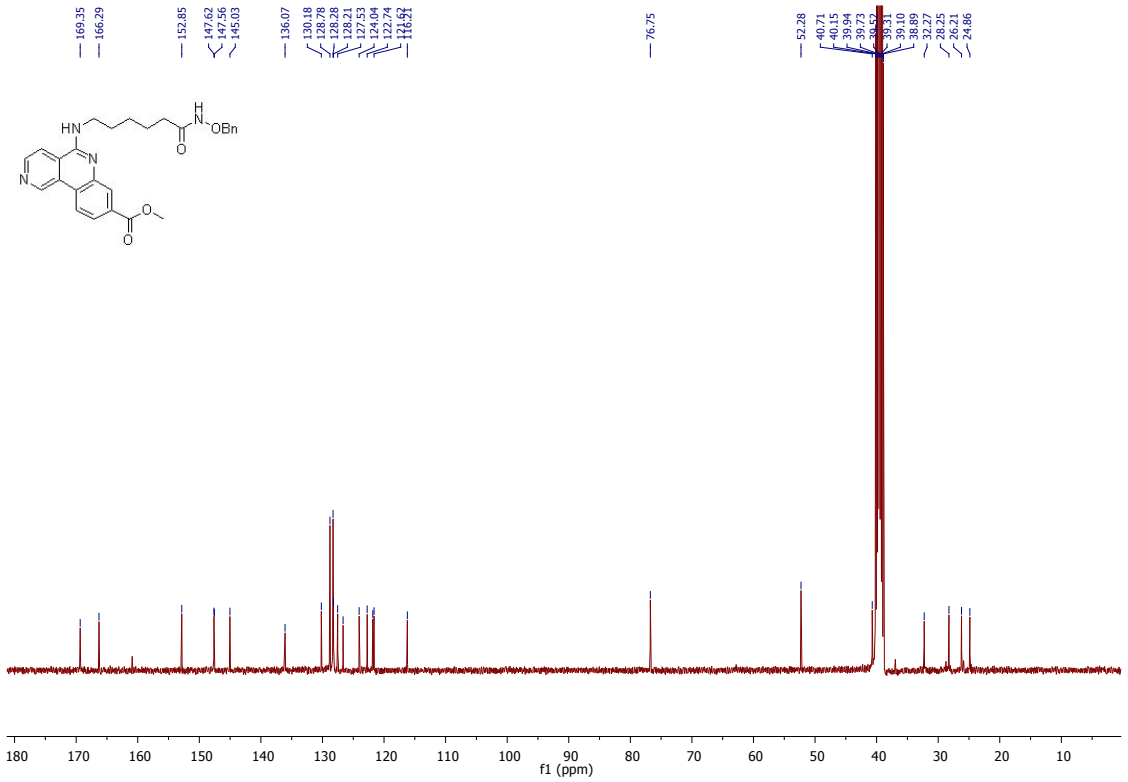
^{1}H NMR spectrum Compound 12d (DMSO-d_6)



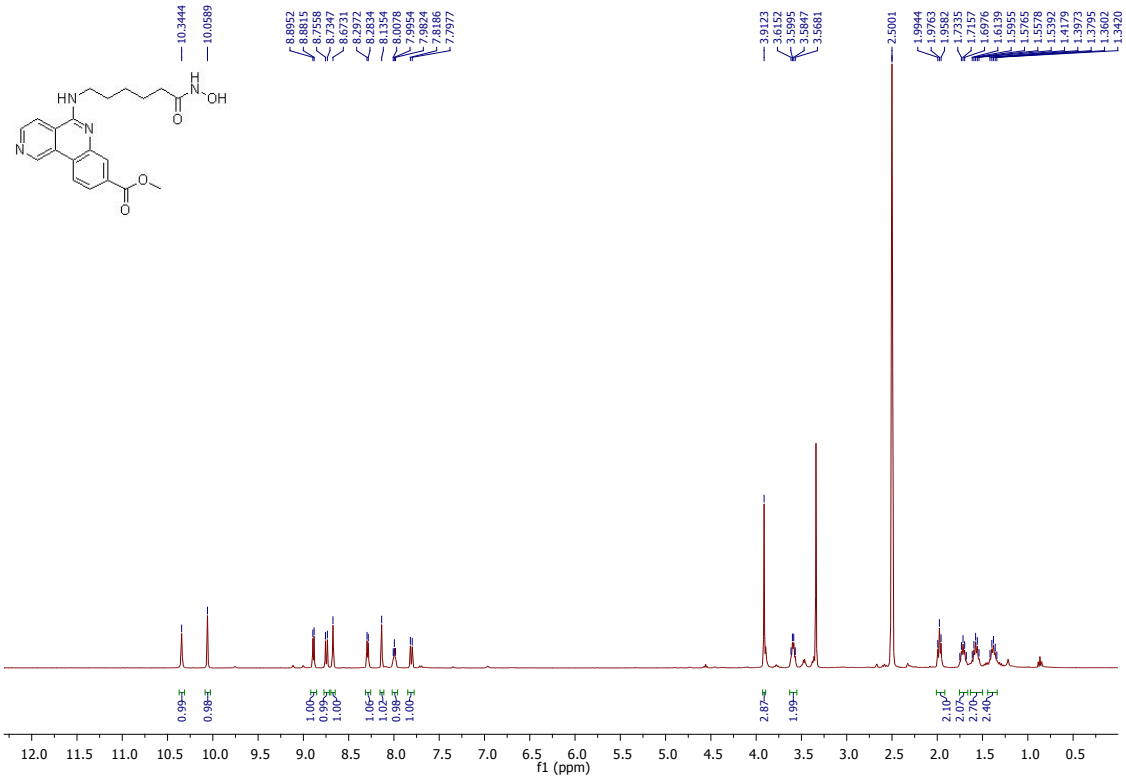
^{1}H, ^{13}C NMR spectra and HPLC traces for the synthesis of dual inhibitors **15a-d**

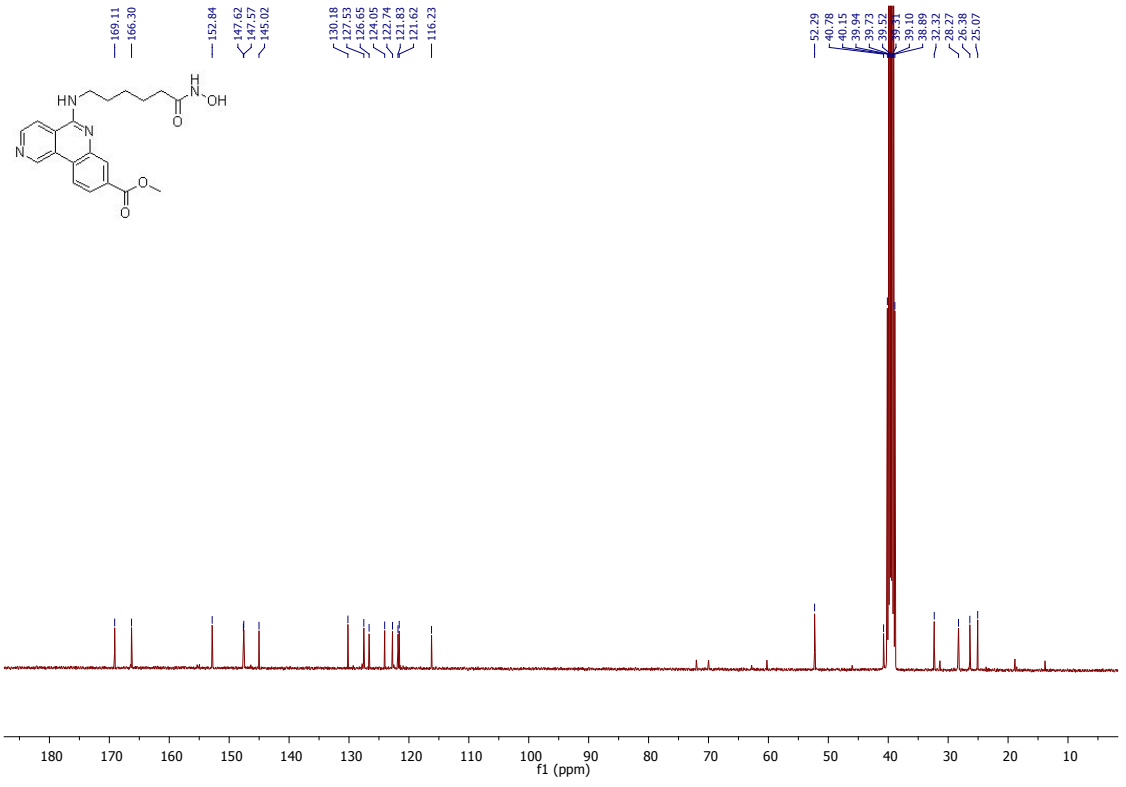
^{1}H and ^{13}C NMR spectra Compound 13a (DMSO-d_6)



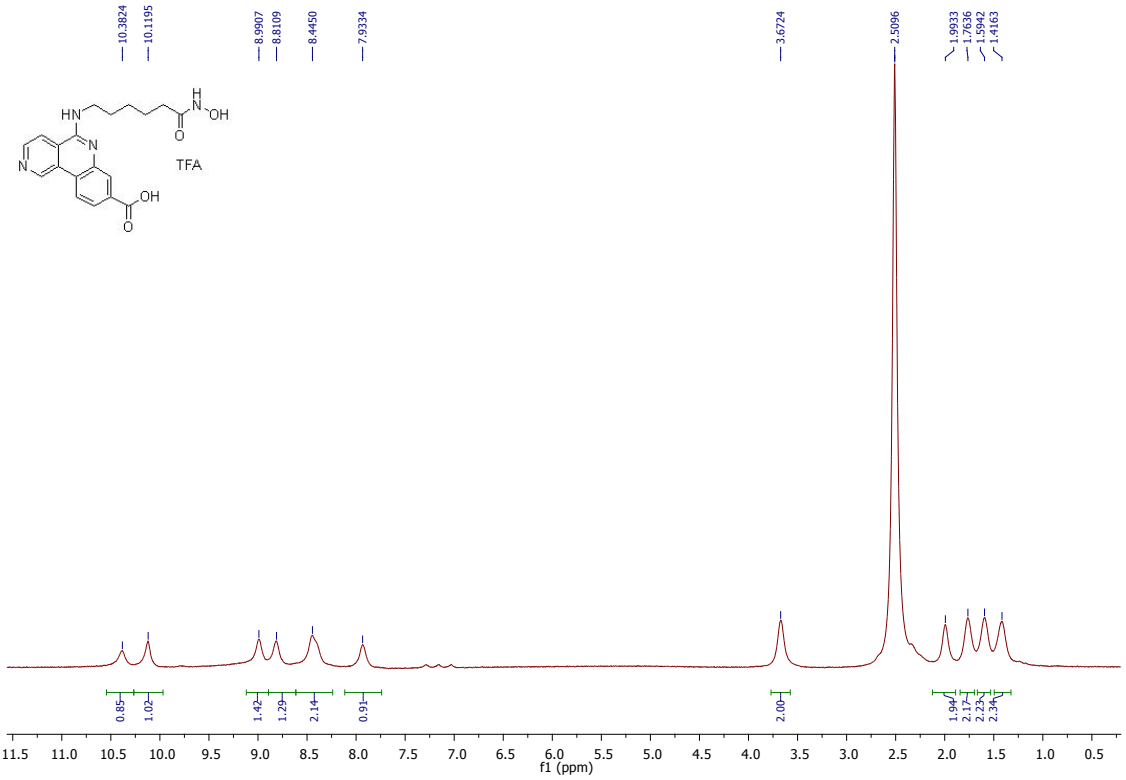


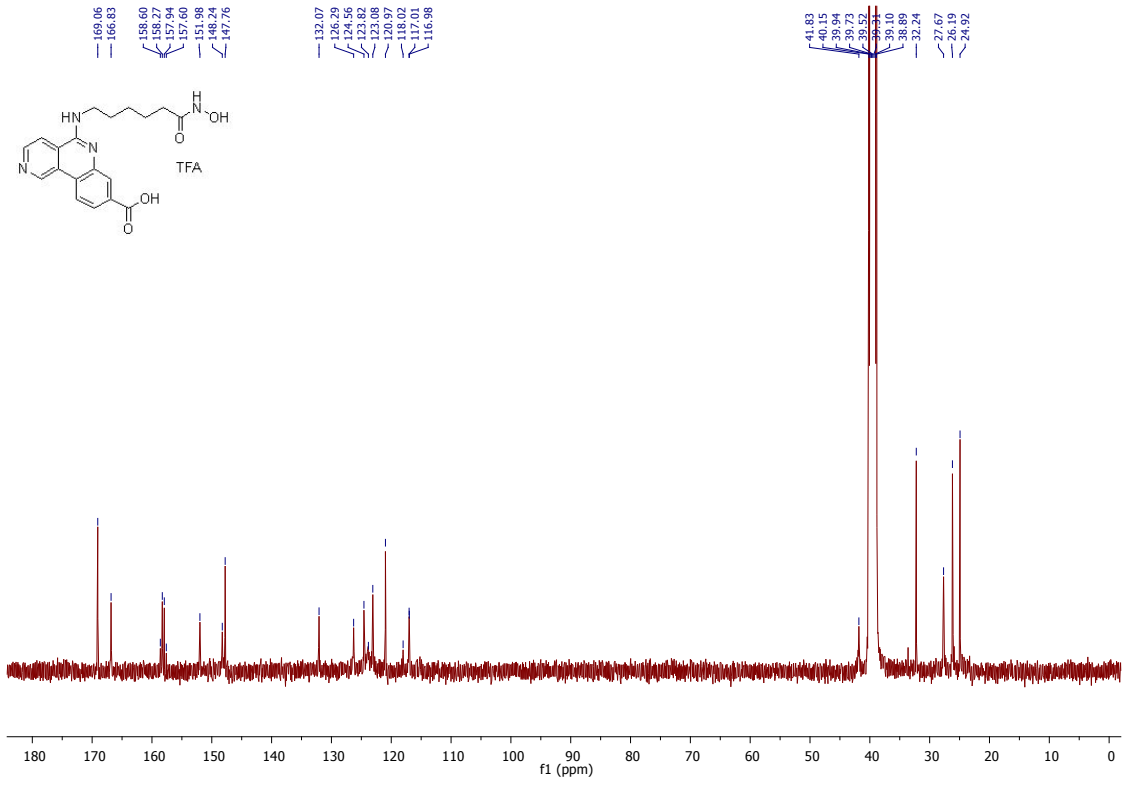
^{1}H and ^{13}C NMR spectra Compound 14a (DMSO-d_6)





^{1}H and ^{13}C NMR spectra Compound 15a (DMSO-d_6)





HPLC traces Compound 15a

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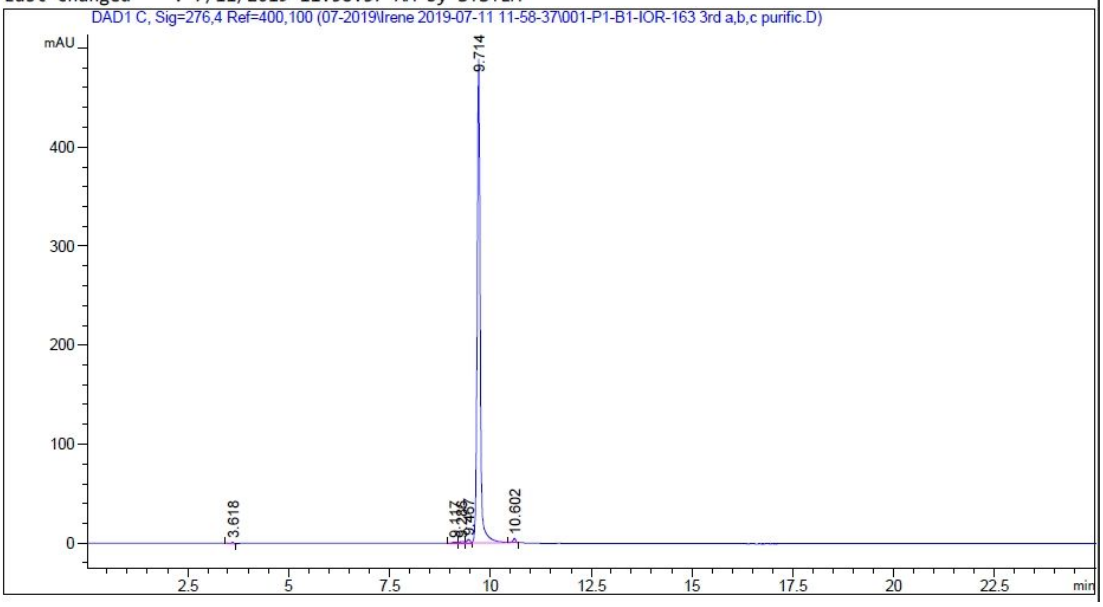


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                        Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=276,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.618 BB    0.0680    6.90536    1.43881   0.2620
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   3   9.286 VV E  0.1031   11.33095    1.51786   0.4299

   4   9.467 VV E  0.0843   20.83095    3.78182   0.7903
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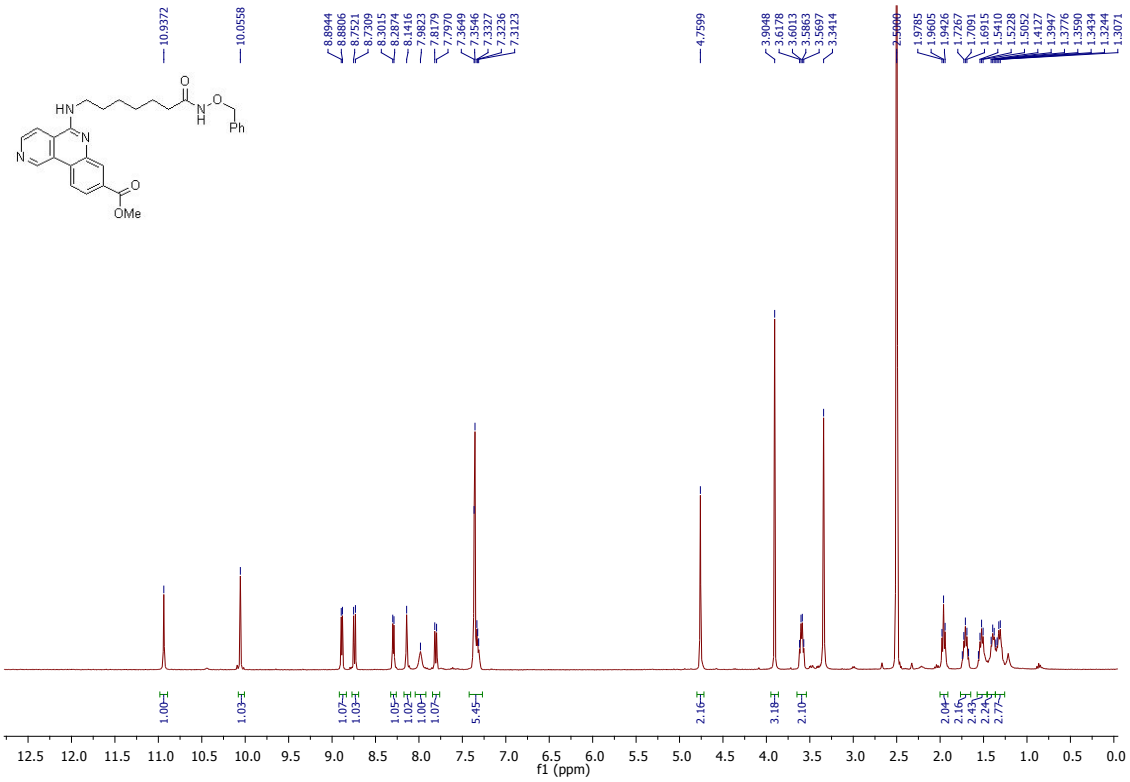
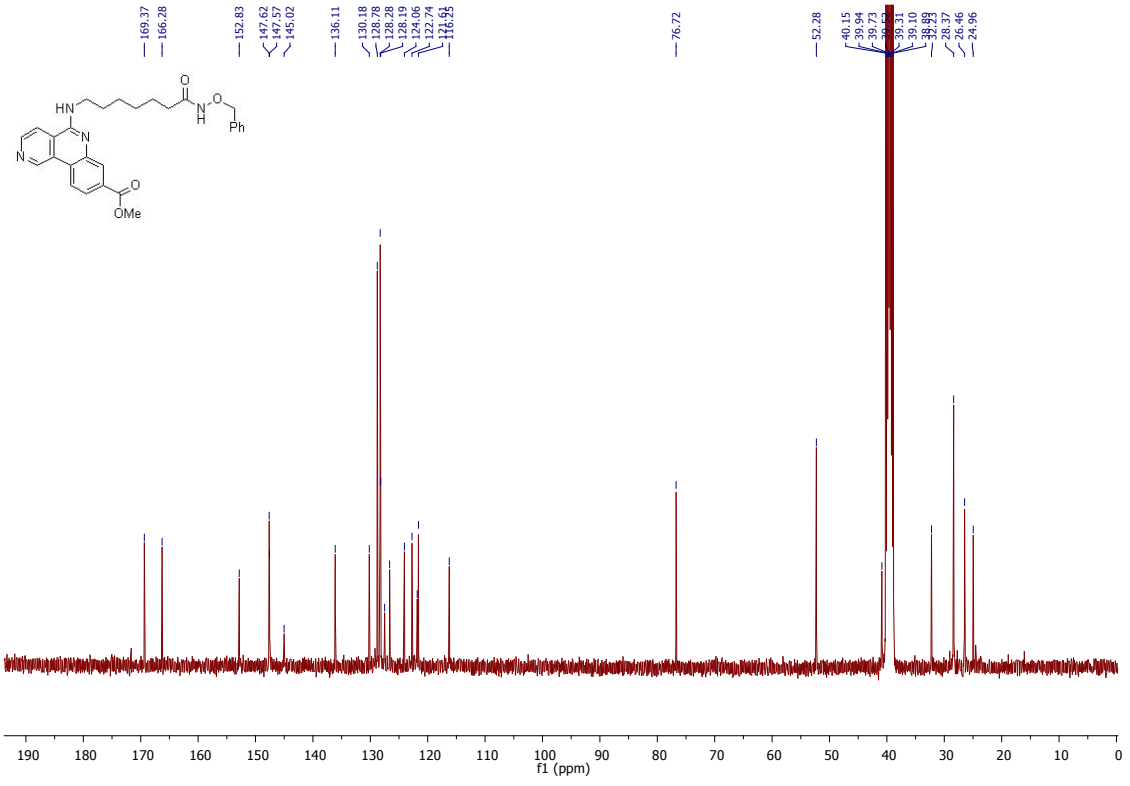
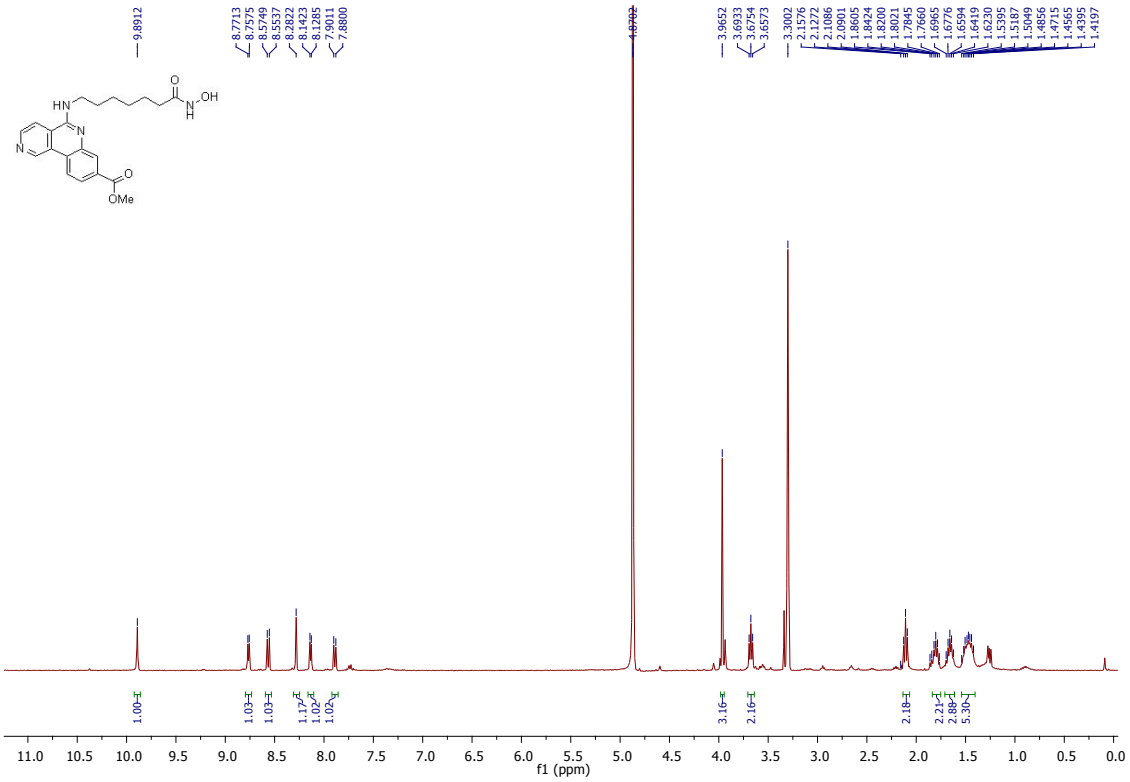
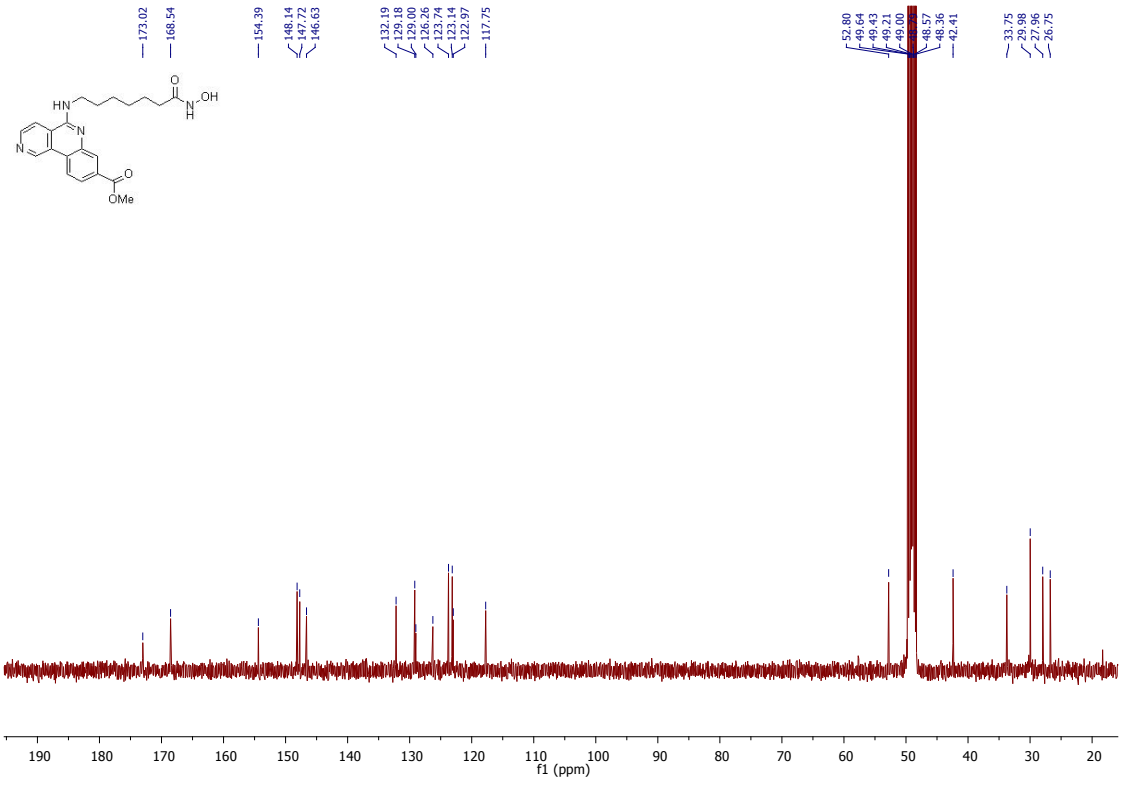
^{1}H and ^{13}C NMR spectra Compound 13b (DMSO-d_6)



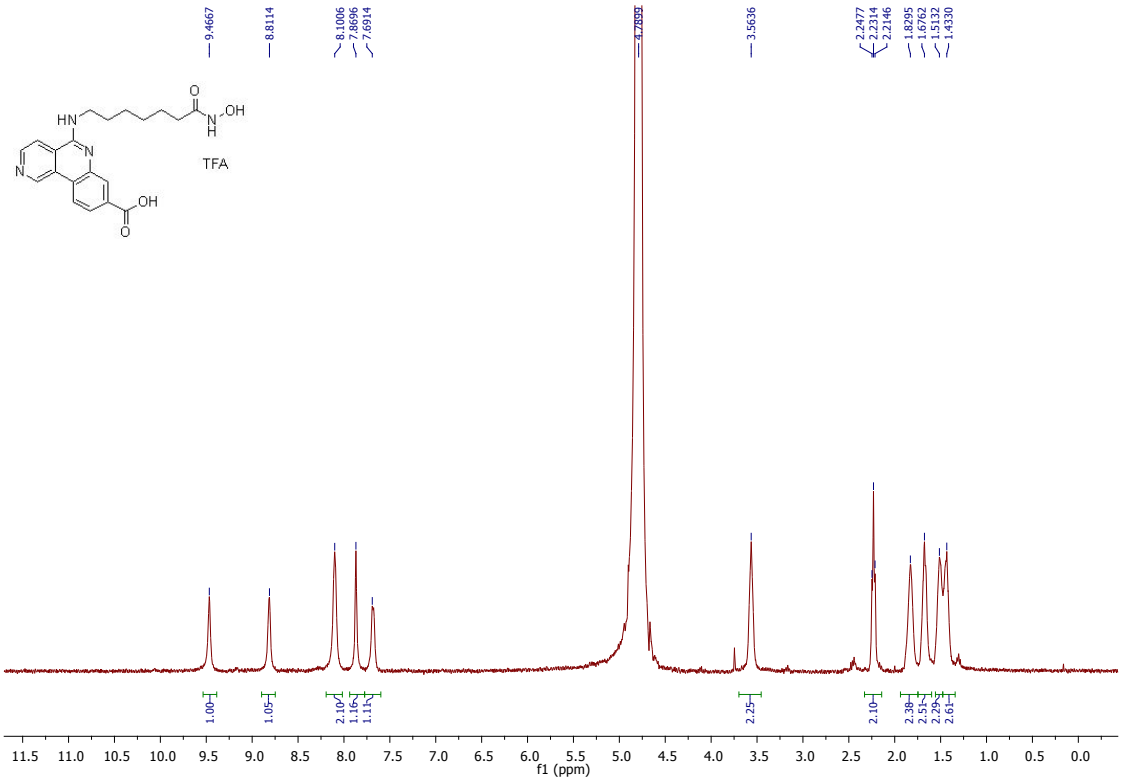


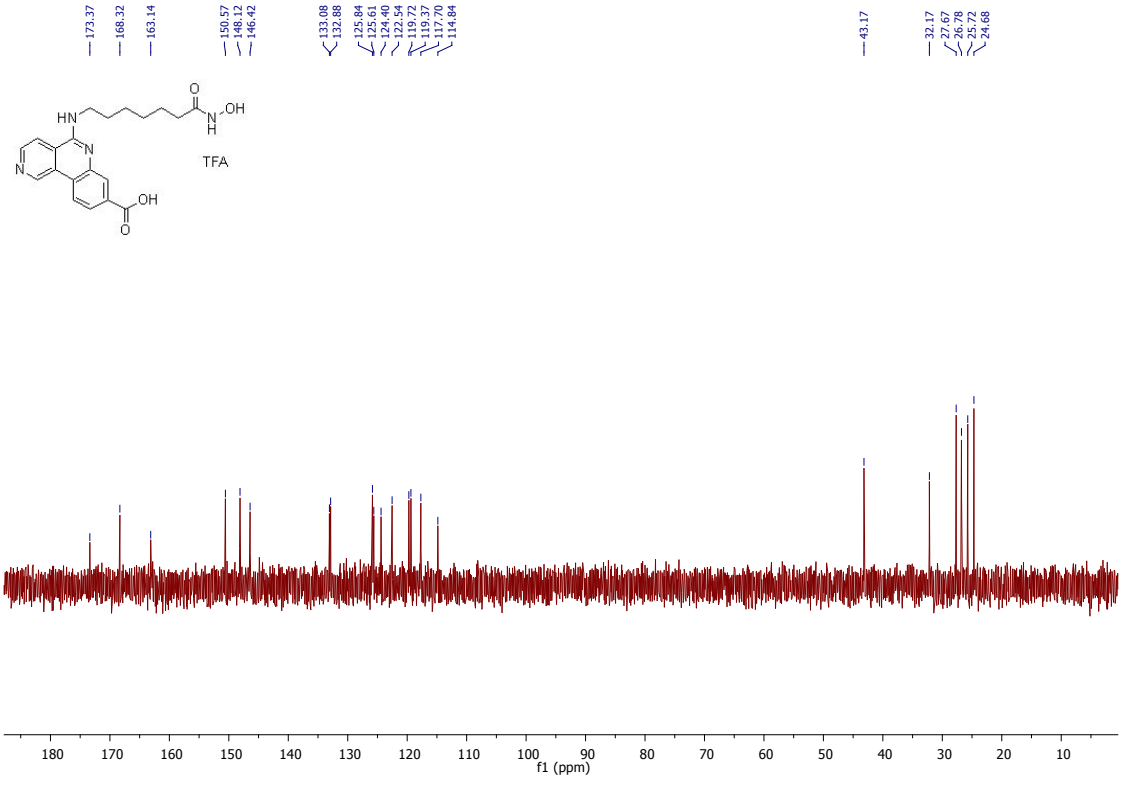
^{1}H and ^{13}C NMR spectra Compound 14b (MeOD)





^{1}H and ^{13}C NMR spectra Compound 15b (D_2O)





HPLC traces Compound 15b

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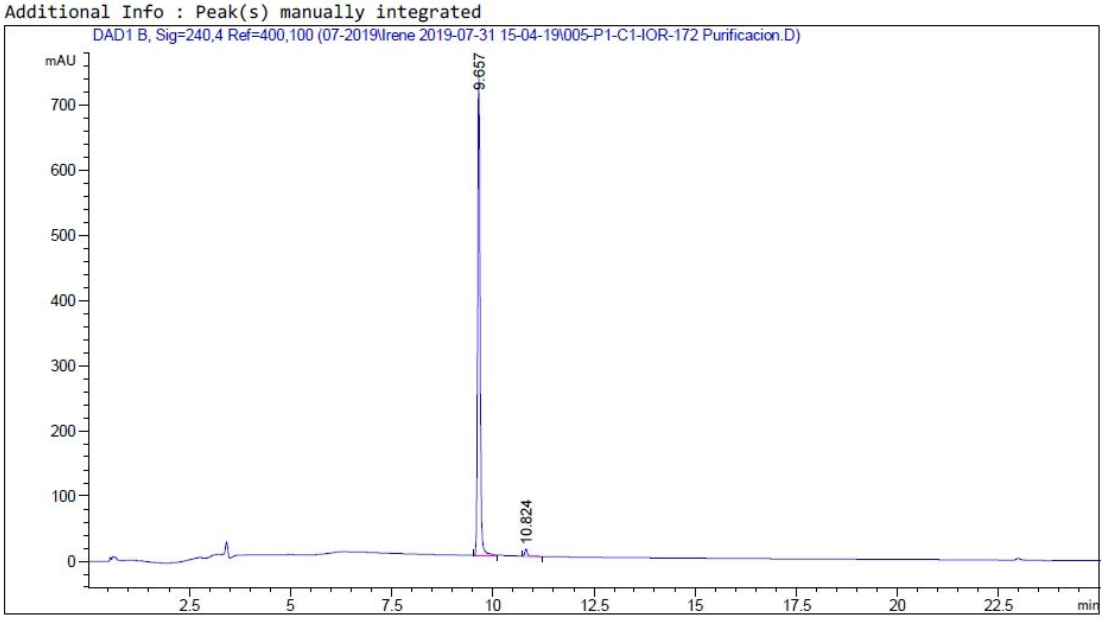


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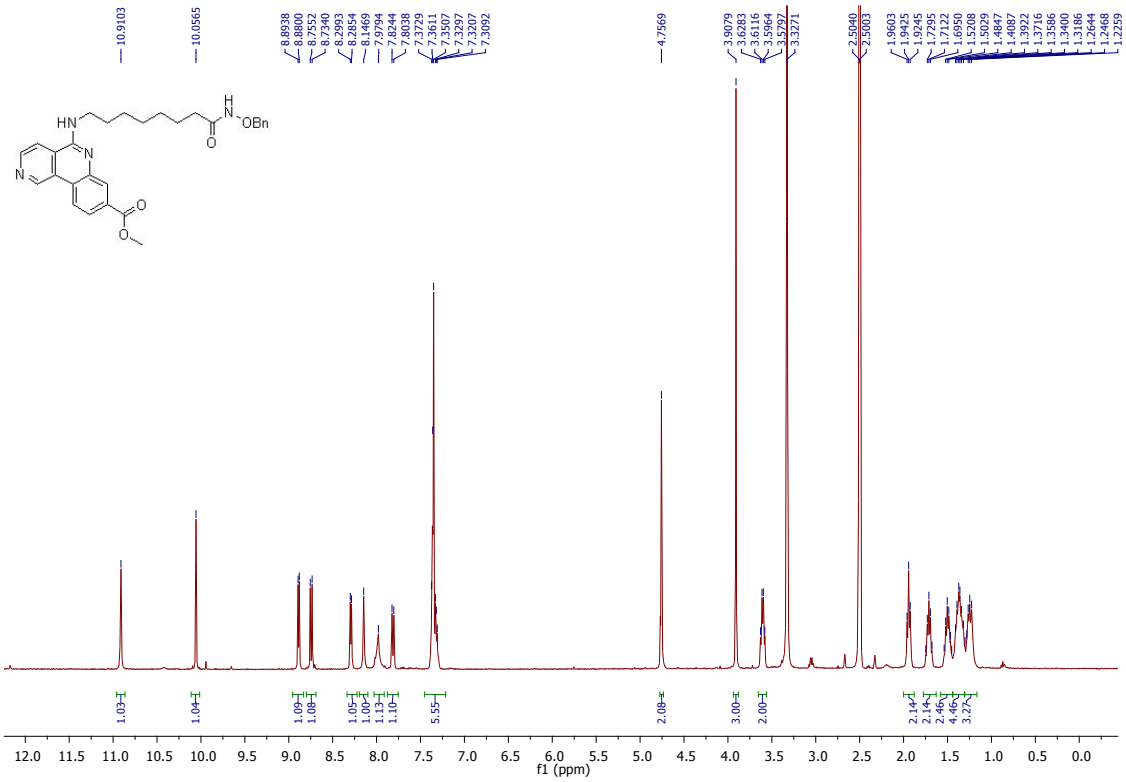

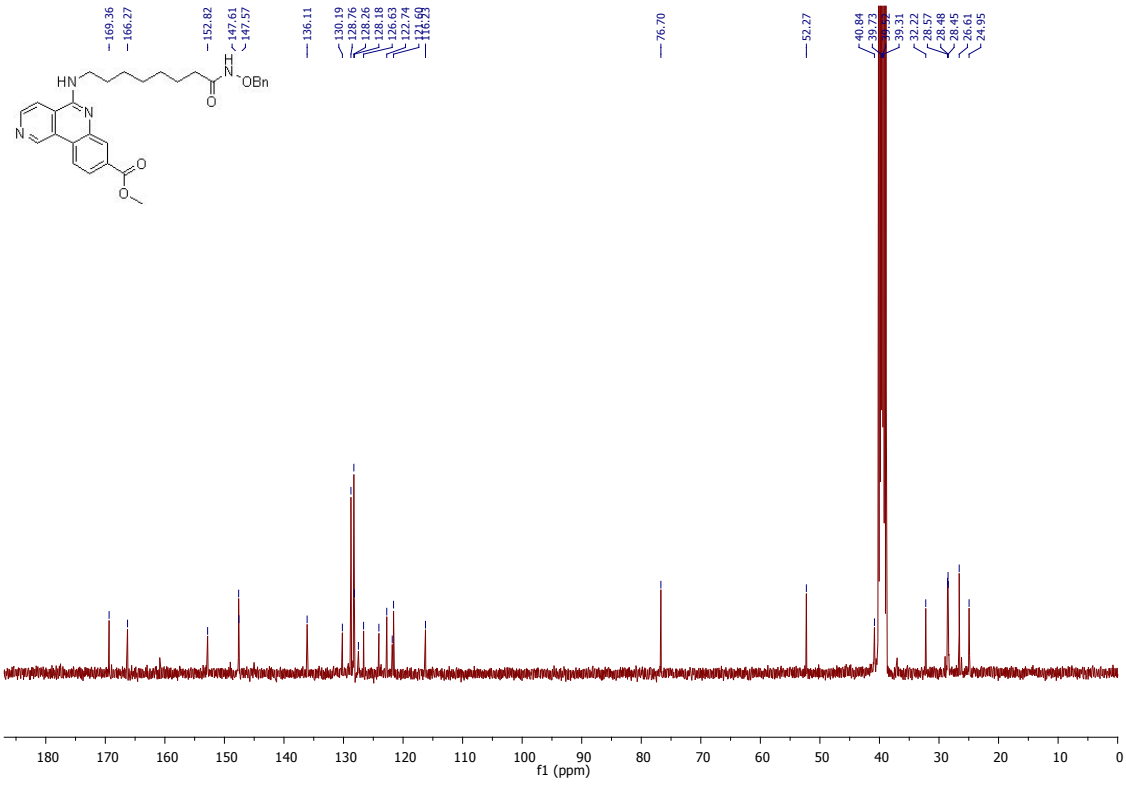

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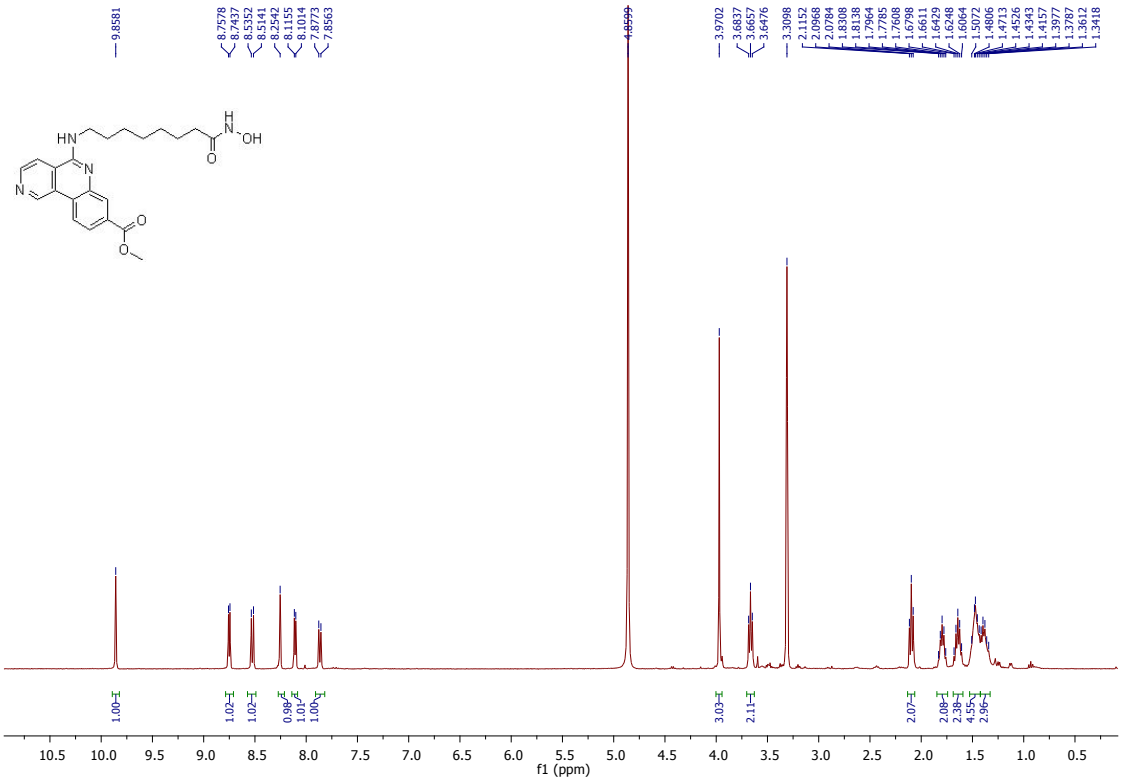

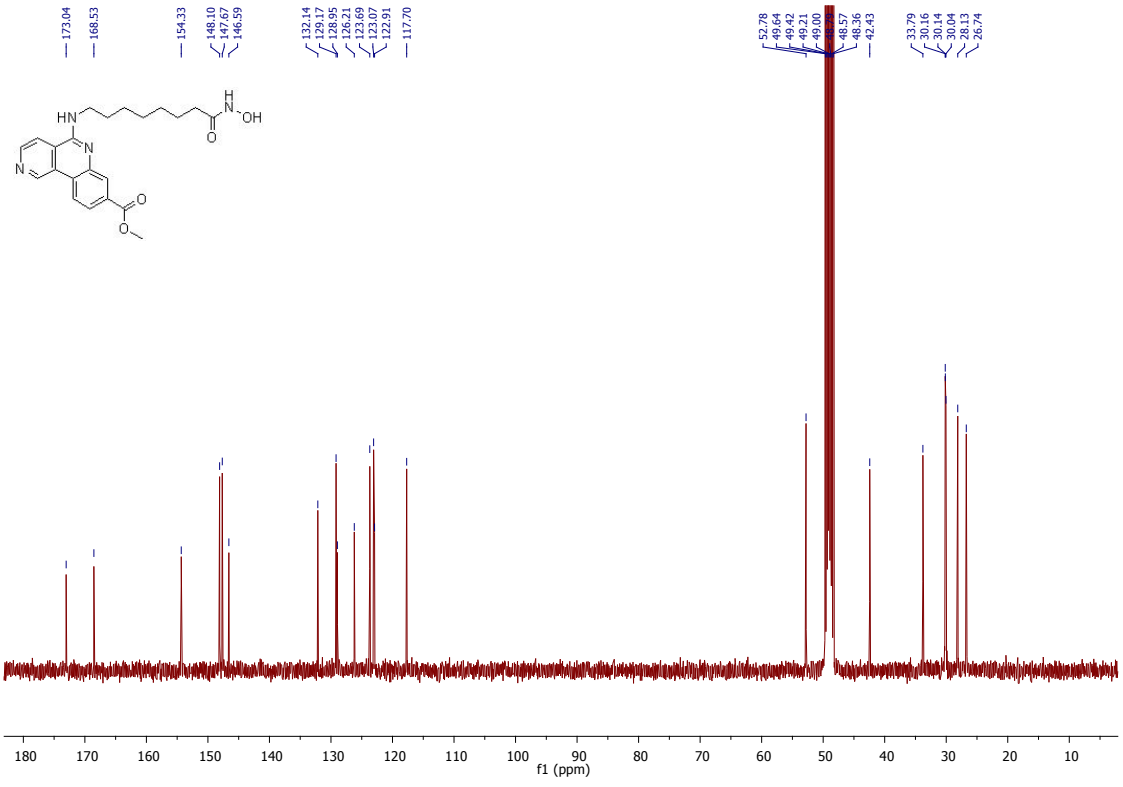

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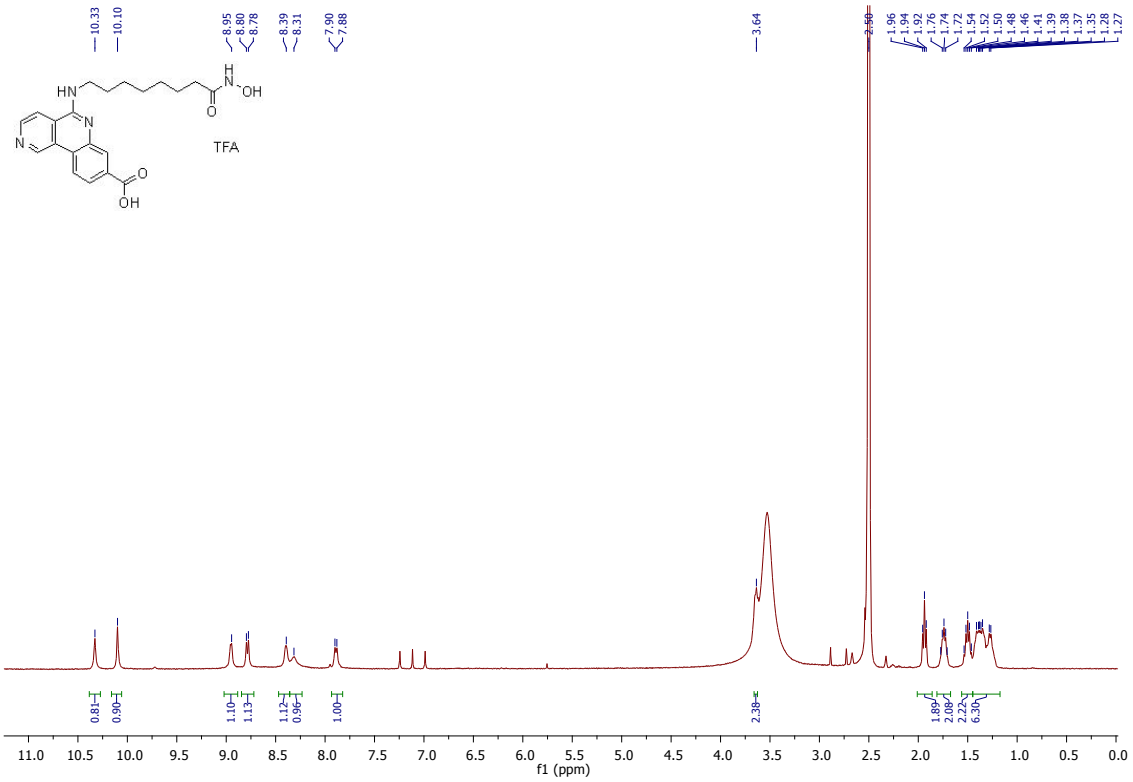

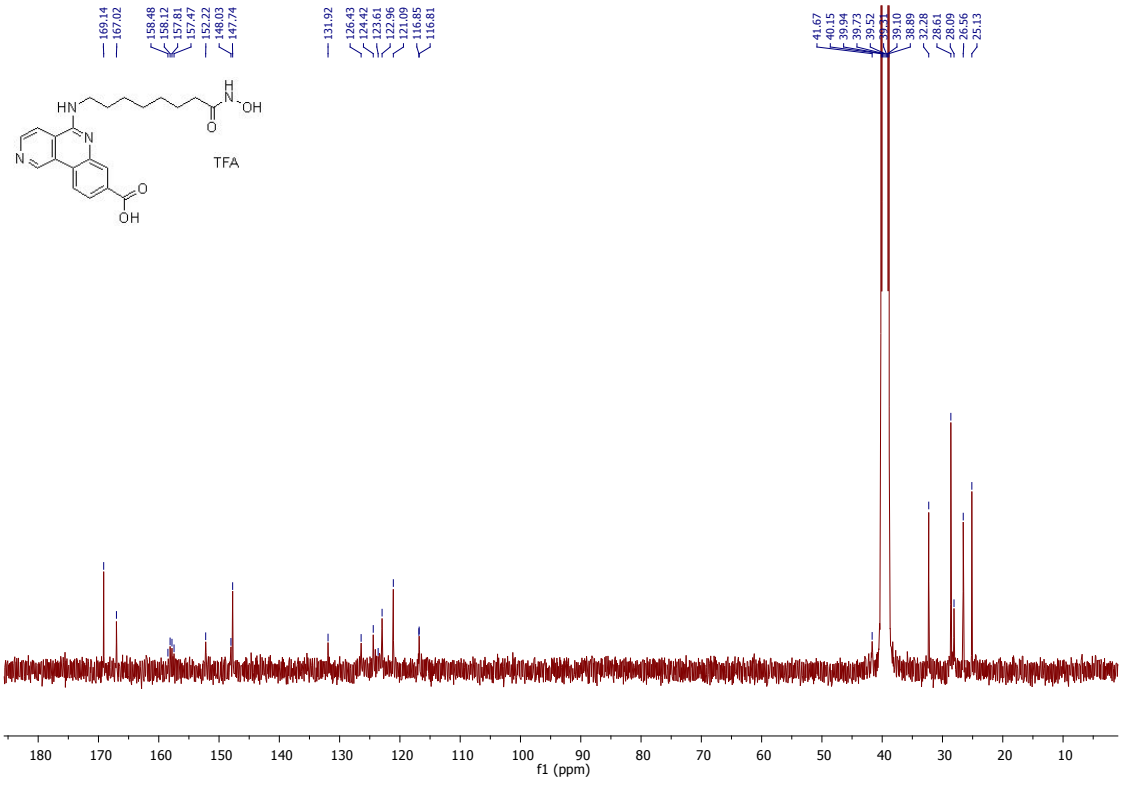

HPLC traces Compound 15c

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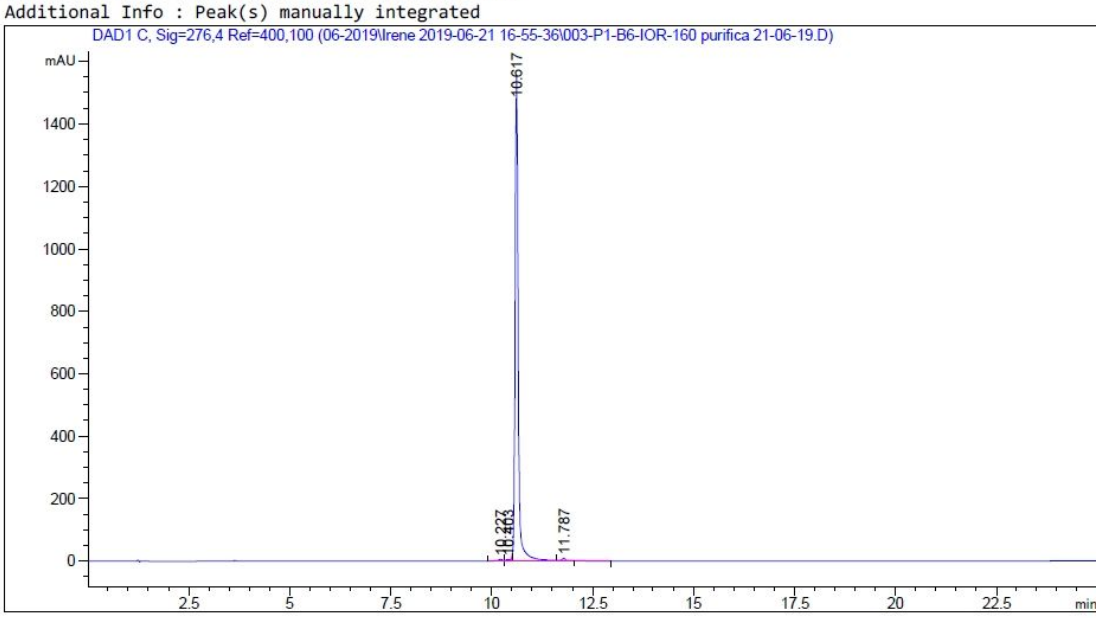


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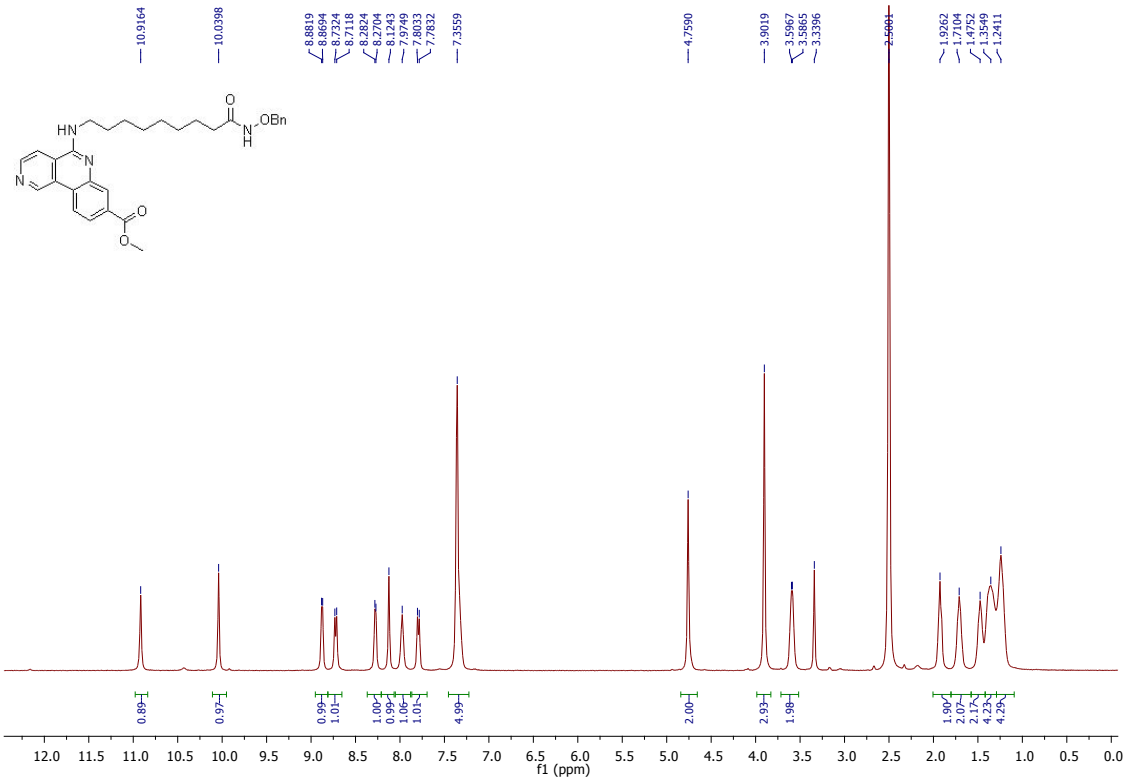

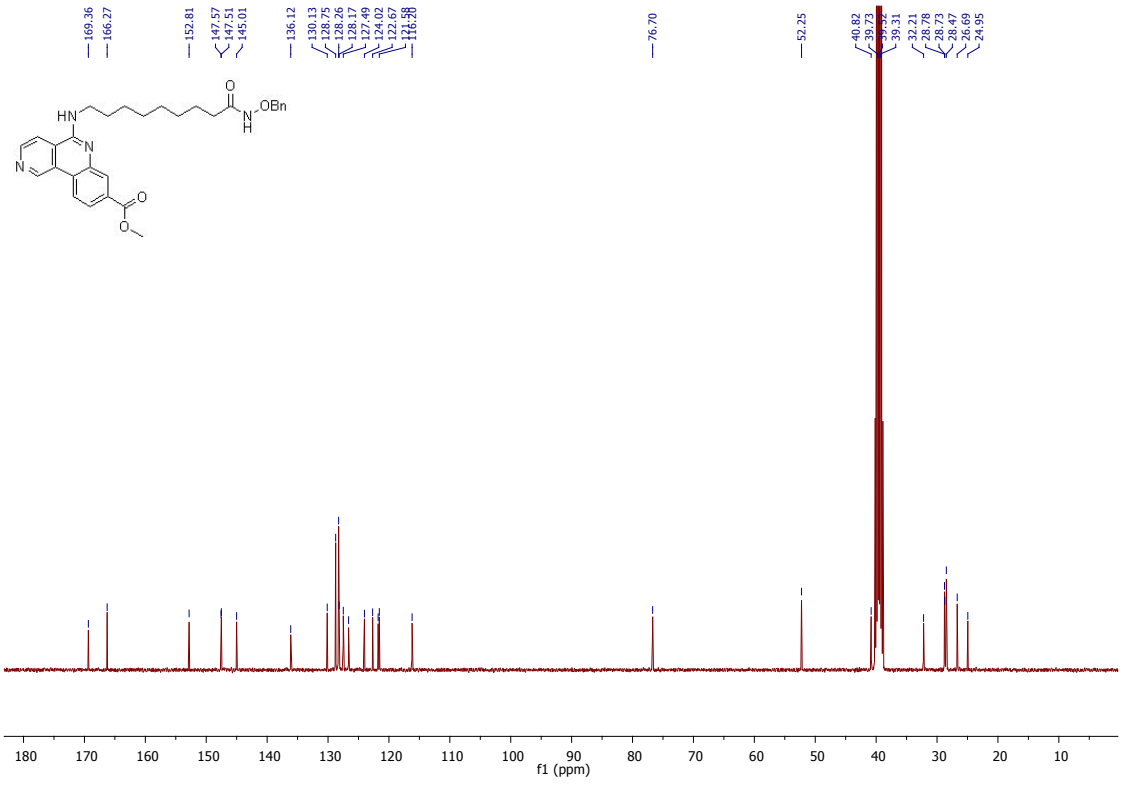

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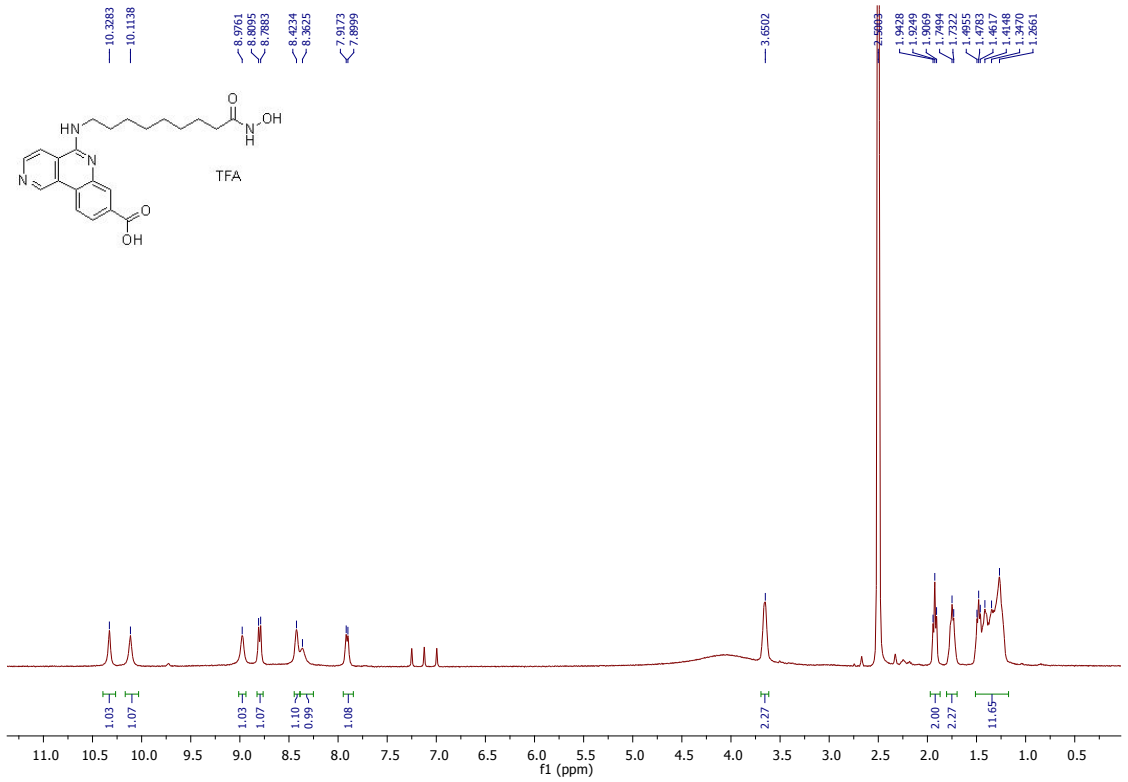

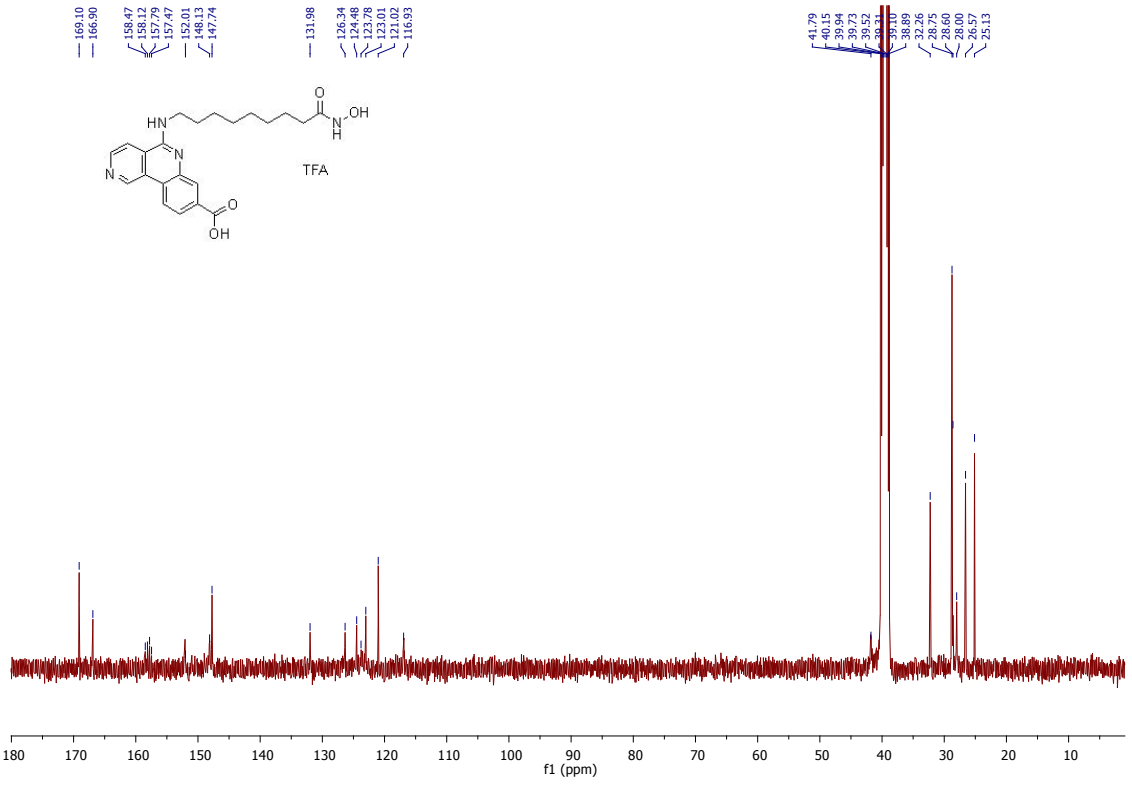

HPLC traces Compound 15d

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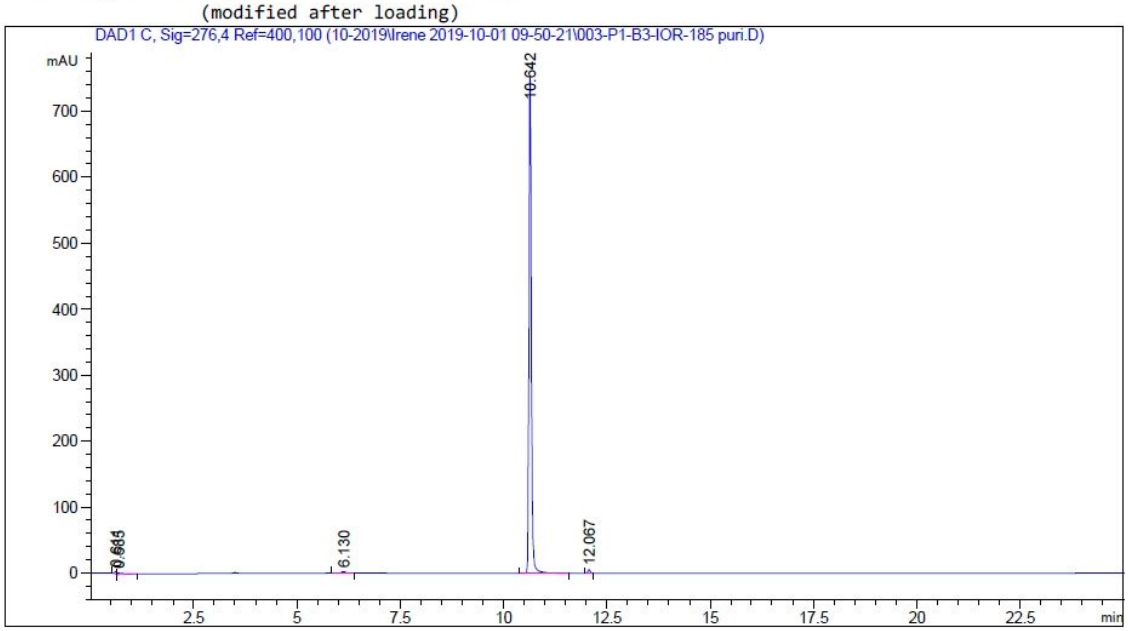


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PART B. Computational studies

Molecular Modeling

The **3PE1** CK2 structure bound to **CX4945** was used to build the CK2-bound complexes. On the other hand, the **5ICN** structure of HDAC1 bound to an inositol-6-phosphate unit and a modified peptide inhibitor with a hydroxamic acid as a ZBG was used to build the HDAC1-bound complexes. Previous studies have established that upon binding to the catalytic Zn^{2+}, the pKa of the hydroxamic acid decreases thus resulting in the deprotonation of the terminal hydroxyl group and transfer of the proton to the imidazole side chain if His140.[4] To prepare the proteins by adding the missing side chains and to calculating the protonation state of tritable groups we used the Protein Preparation Wizard of the Schrödinger suite (Protein Preparation Wizard, LLC, New York, NY, 2016.).[5] In the case of HDAC1 we set His140 to be protonated, His141 protonated at Nδ and His178 protonated at Nε.The ligands (compounds **15a**, **15a**, **15c** and **15d**) were built using Maestro LigPrep module (www. Schrodinger.com). Compounds were docked in CK2 in the hydroxamic acid form, whereas in HDAC1 compounds were docked in the hidroxamate form as the protein was prepared with a protonated His140. The Glide module[6-8] was used to perform the docking calculations. For the HDAC1 complexes the centre of the box was positioned on the catalytic Zinc ion present in the active site, while for CK2 it was located at the centre of the CX4945 structure. The box size was set up to enclose the ligand-binding domain to ensure a proper exploration of the binding poses. The docking procedure on CK2 was performed with the XP (extra precision) mode, and a van der Waals radii scale factor of 1.0/0.8 for receptor and ligand, respectively. For the HDAC1 complexes, the Induced Fit procedure was applied using a metal constraint on the Zn^{2+} and XP glide redocking.[9, 10] The best-obtained result for each ligand was considered for analysis of the ligand-receptor interactions and subsequent molecular modelling simulations.

For the MD simulations of the CK2-15c complex, the geometry optimization and charge distributions of all ligands were calculated quantum mechanically (RHF/6-31+G**) with aussian 09 (Gaussian, Inc., Wallingford, CT), Revision A.1. Available at www.gaussian.com/g_prod/g09.htm.). To assign the bonded and nonbonded parameters of the ligands the general AMBER force field 2 (GAFF2) was used (AMBER General Force Field for organic molecules (Version 2.1, April 2016). As for the MD simulations of the HDAC1-**15c** complex, both the ligands and the metal binding site were parametrized with the MCPB module embedded into the MTK++ software package of AMBER16.[11, 12] The optimized equilibrium values of bond lengths, angles and force constants of the atoms in the coordination spheres were obtained by geometry optimization in the gas phase using B3LYP/6-31G* with Gaussian09.

Classical molecular dynamics (MD) simulations using the classic ff14SB AMBER force field[13] along with the assigned active site and ligand parameters were performed on both complexes, with the AMBER16 program (http://ambermd.org/). After the complexes were minimized at vacuum, they were embedded in a TIP3P water octahedron of approximately 13000 to 19000

water molecules for the HDAC1 and CK2 complexes, respectively; and system neutrality was achieved on the CK2 complex by adding one chlorine ion. Water molecules and counter ions were minimized and then the systems were heated to 300 K for 25 ps at constant volume, keeping the protein restrained to initial positions using harmonic restraints with a constant force of 50 kcal mol-1Å-2. At all times the hydrogen bond lengths were kept at their equilibrium distance by means of the SHAKE algorithm,[14] the van der Waals atom pair distance cutoffs were applied at 10.0 Å, while long-range electrostatics were computed by means of Particle-Mesh Ewald (PME) method. Finally, MD simulations were performed for 20 ns generating snapshots each 20 ps for further analysis. The trajectories of all complexes were collected and analyzed by the cpptraj module of AMBER16[15] in order to obtain the root, mean square deviation (RMSD) value of the atomic positions of the ligands.

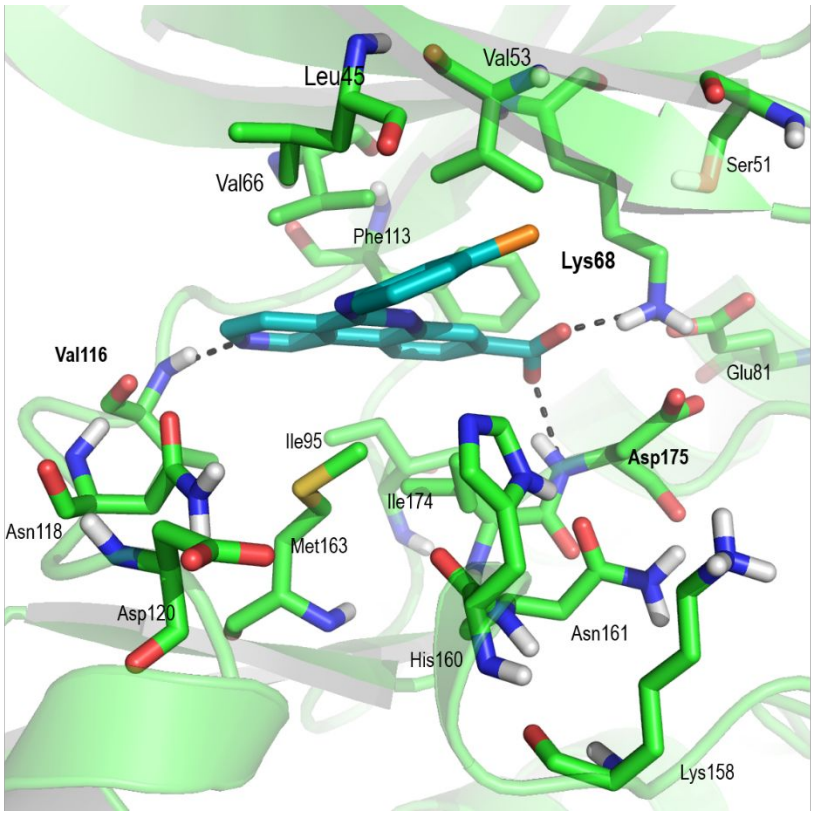


Figure S1: PyMOL stick and cartoon representation of the binding mode of **CX4945** to CK2 in PDB code **3PE1**. Hydrogen bonds have been highlighted with dashed lines.

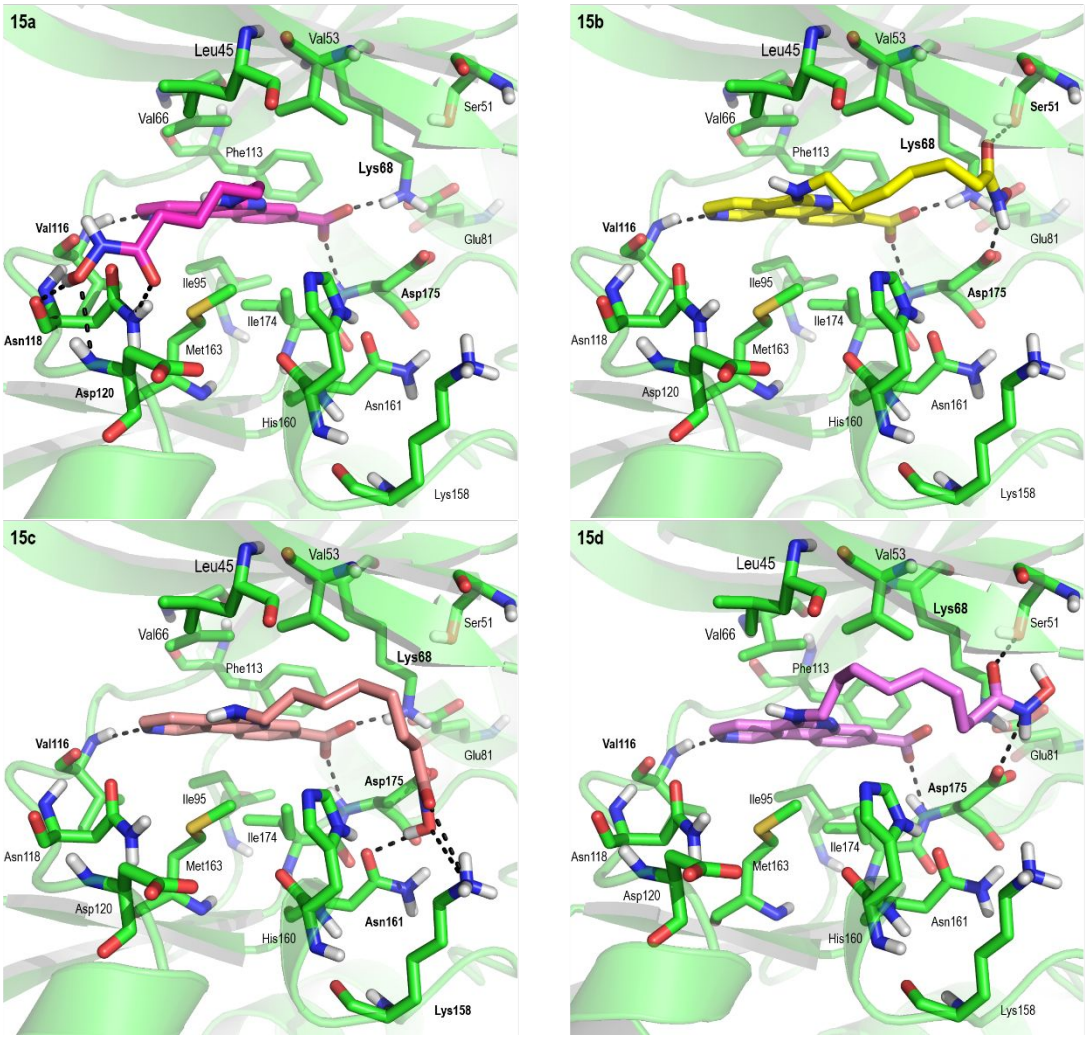


Figure S2: PyMOL stick and cartoon representation of the best docking poses of compounds **15a-d** to CK2. For the sake of clarity, only polar hydrogens are shown, and hydrogen bonds have been highlighted with dashed lines.

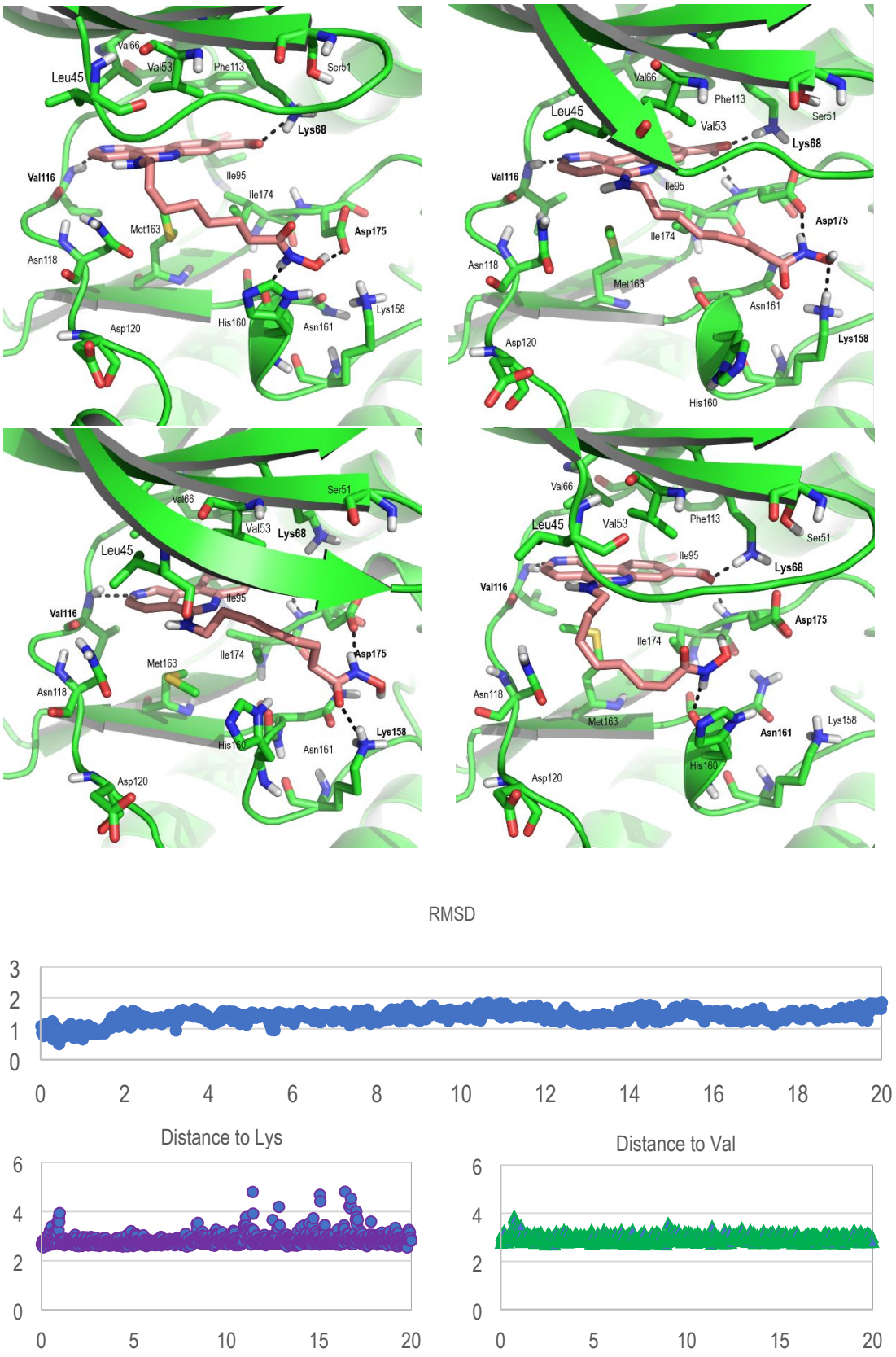
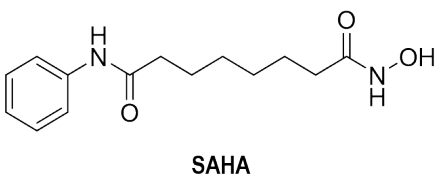
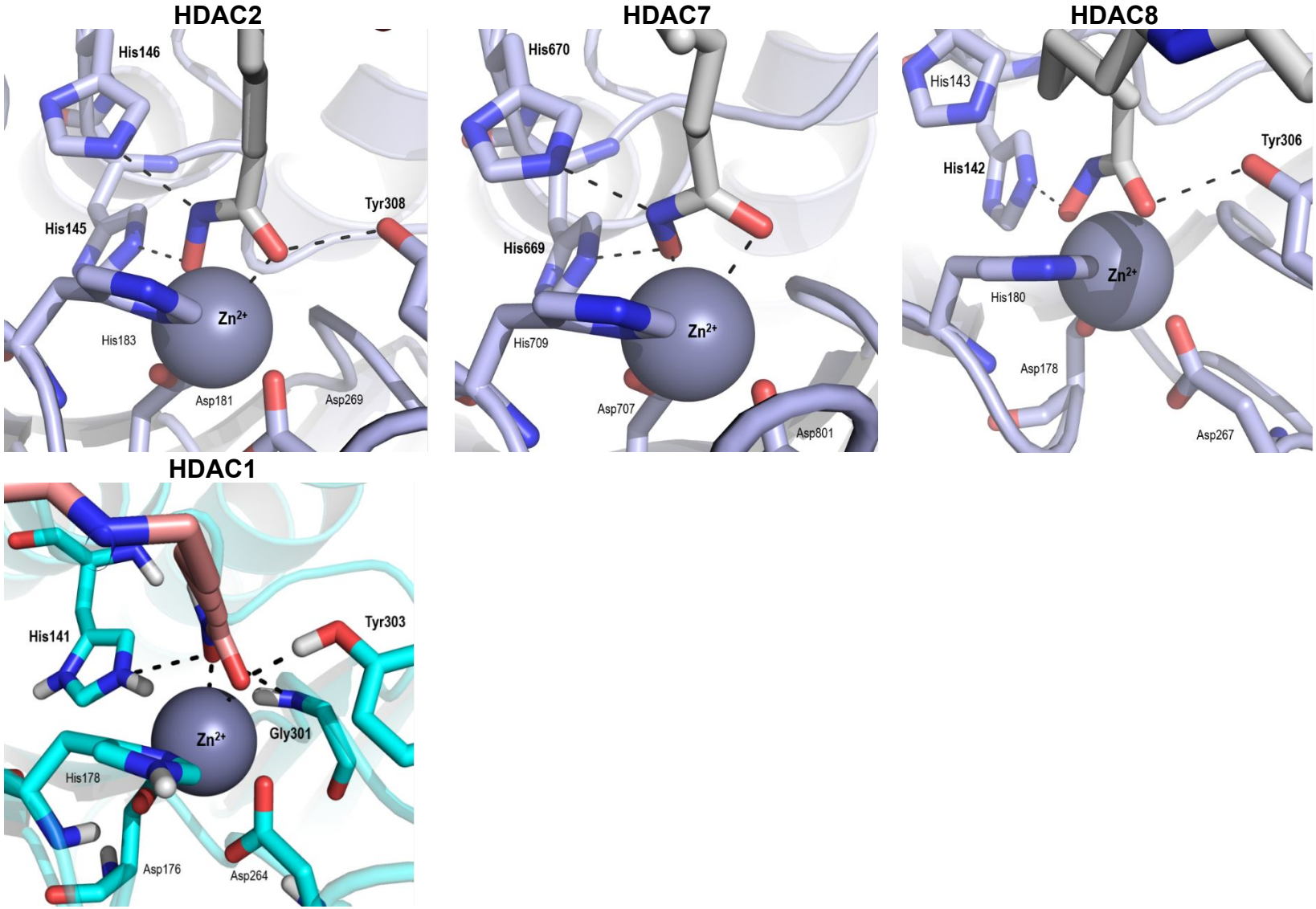


Figure S3: Top. PyMOL stick and cartoon representation of the most populated conformers of **15c** obtained in the MD dynamics. For the sake of clarity, only polar hydrogens are shown, and hydrogen bonds have been highlighted with dashed lines. **Bottom.** Graphical representation of the RMSD variation of **15c** during the simulation, and the variations of the hydrogen bond distances between the CX4945 moiety and the anchoring amino acids.





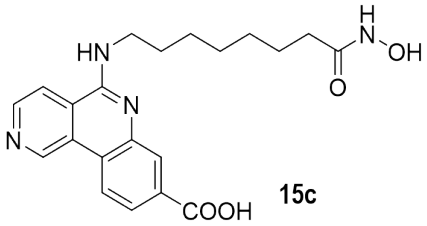


Figure S4: Structural comparison of the bidentate chelation of the catalytic Zn^{2+} by SAHA in HDAC2, 7 and 8 as found in PDB codes 4LXZ, 3C0Z and 1T69, respectively; and in HDAC1 by compound **15c** in the obtained docking result.

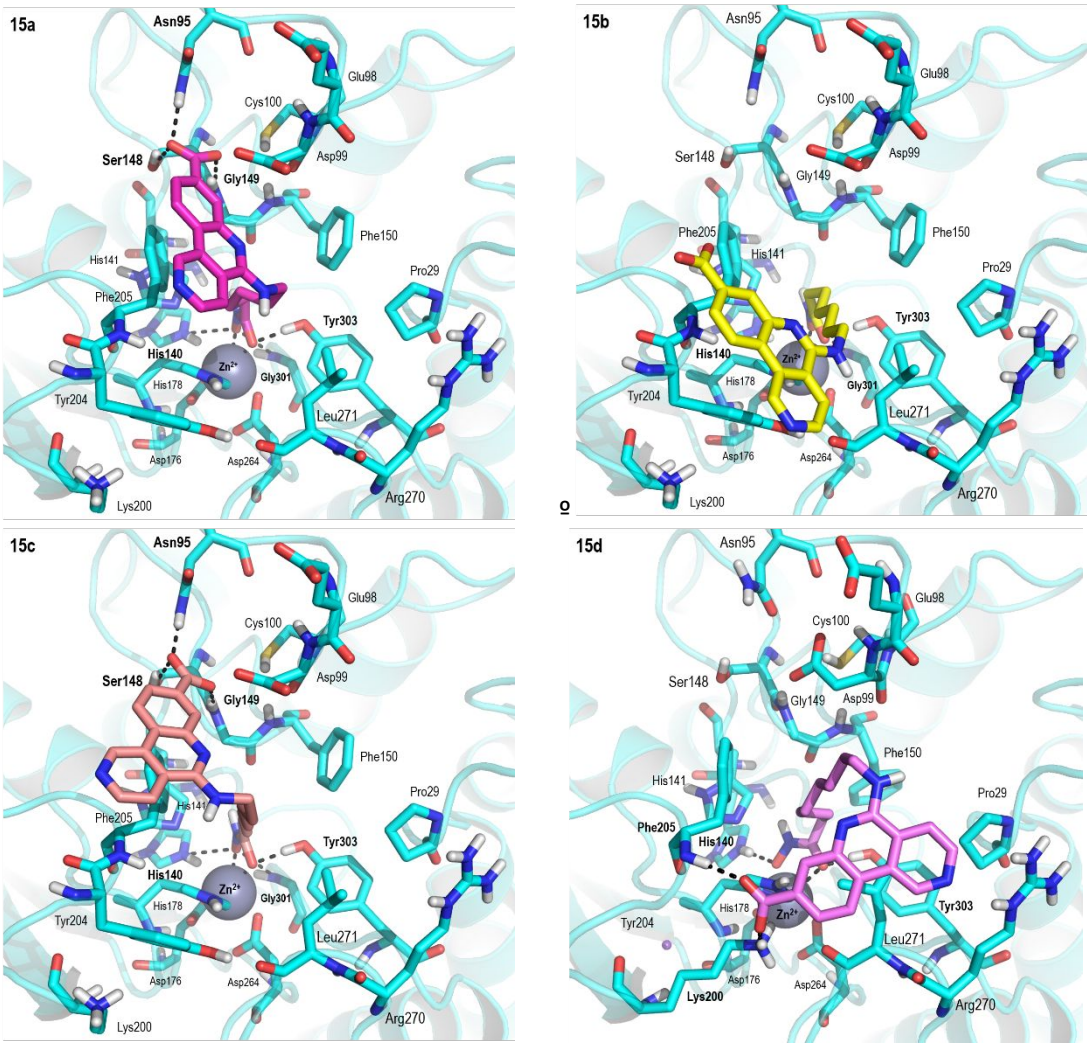
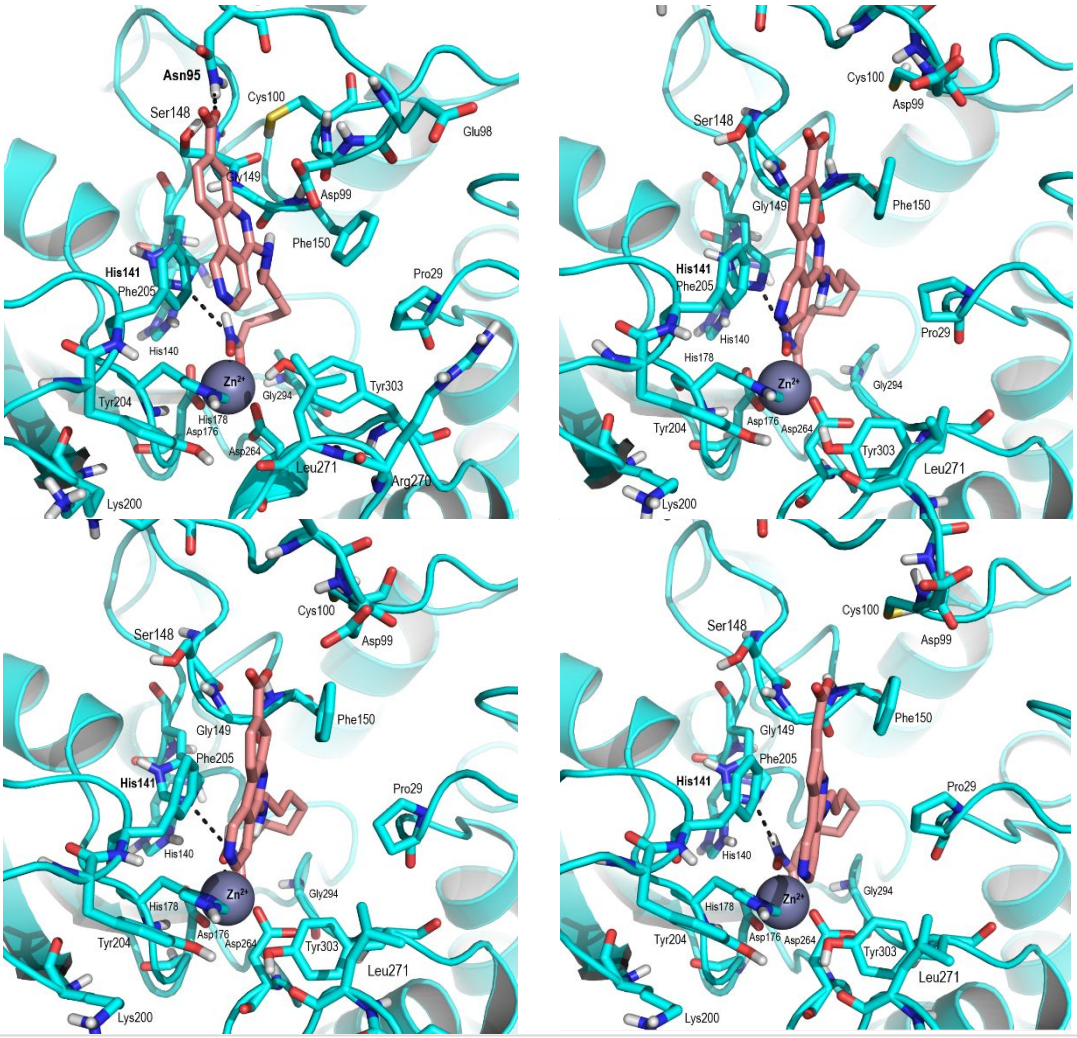


Figure S5: PyMOL stick and cartoon representation of the best induced fit docking poses of compounds **15a-d** to HDAC1. For the sake of clarity, only polar hydrogens are shown, and hydrogen bonds have been highlighted with dashed lines.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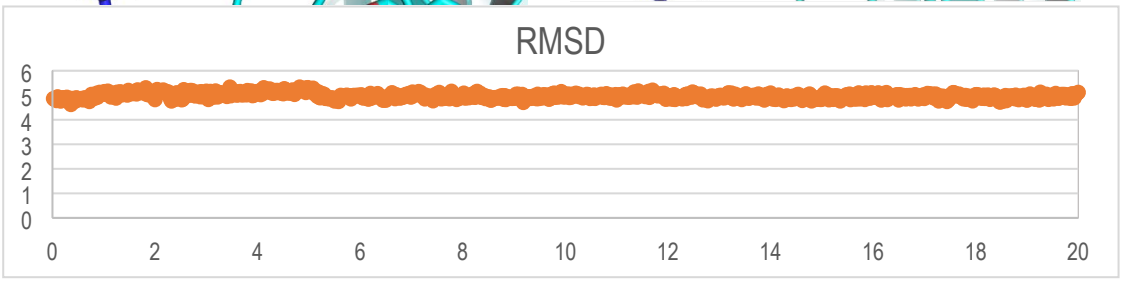
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10
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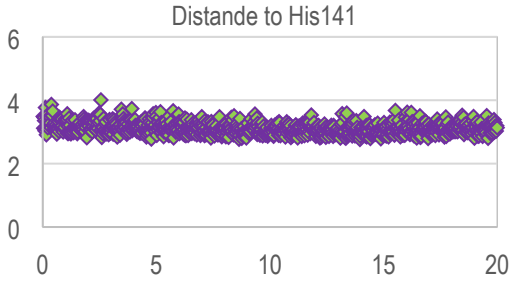
16
18
20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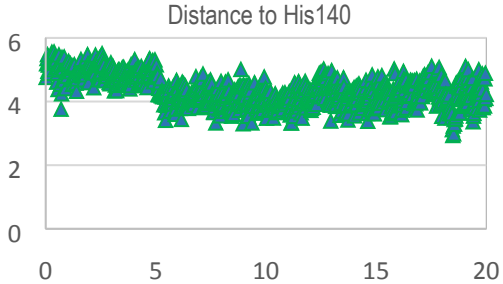
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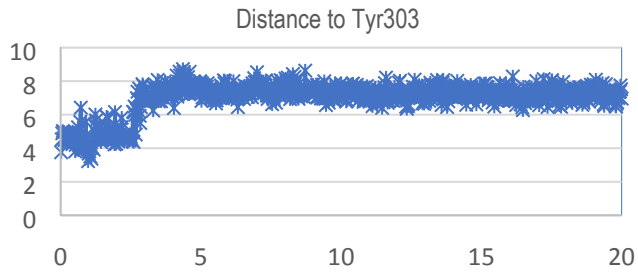

Figure S6: PyMOL stick and cartoon representation of the best induced fit docking poses of compounds **15a-d** to HDAC1. For the sake of clarity, only polar hydrogens are shown, and hydrogen bonds have been highlighted with dashed lines.

PART C. Enzymatic Biological Assays

HDAC1 and HDAC6 enzymatic assays

In vitro HDAC1 and HDAC6 inhibitions were measured using Fluorimetric Drug Discovery Assay Kits from Enzo Life Sciences, Inc. The reactions were prepared in HDAC1/HDAC6 assay buffers (50 mM Tris-HCl, pH=8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/mL BSA). 15 µL of HDAC1/HDAC6 (0.4 µg/well) was incubated at 37 °C for 30 min. with 10 µL of inhibitors at different concentrations (5% DMSO) and 25 µL of fluorogenic substrate (0.05 µL/well of 5 mM solution). Reactions were stopped with a mixture of developer and trichostatin A diluted in buffer without BSA. The plate was incubated at 30 °C for 45 min. followed by measuring the fluorescence (Ex. 360 nm, Em. 450 nm, Fluoroskan, Thermo Scientific)

CK2 enzymatic assays

CK2 assays were performed using ADP-Glo Kinase Assay (Promega).

500 pg/µL of CK2 (American Research Products, Inc.) in 10 µL of buffer (40 mM Tris, pH 7.5, 20 mM $MgCl_2$, and 0.1 mg/mL) were added to 10 µL of the tested compounds at different concentrations. After 30 min., 5 µL of a solution of 20 µM ATP (Promega) and substrate 140 µM (RRRDDDSDDD, Anaspec) were added to the wells followed by 60 min. incubation. Then, 25 µL of ADP-Glo Reagent was added to stop the kinase reaction and deplete the remaining ATP. After 40 min 50 µL of kinase detection solution converts ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction after 30 min. (Fluoroskan, Thermo Scientific). IC50 values were calculated using GraphPad Prism (version 4.0) software.

PART D. Cells Biological Assays

Cell cultures

Four human tumor cell lines (European Collection of Authenticated Cell Cultures number mentioned in brackets), Human caucasian prostate adenocarcinoma cells (PC3, 91072201) were maintained in Kaign's modified Ham's F12 + 45 mg/L ascorbic acid + 18 mg/L Inositol + 2 mM Glutamine + 10% Foetal Bovine Serum (FBS); Human caucasian lung carcinoma cells (A549,

86012804) were maintained in Ham's F12K or DMEM + 2 mM Glutamine + 10% FBS; Human caucasian breast adenocarcinoma (MCF-7, 86012803) were maintained in EMEM (EBSS) + 2 mM Glutamine + 1% Non-Essential Amino Acids (NEAA) + 10% (FBS) and Human caucasian prostate carcinoma (LNCaP clone FGC, 89110211) were maintained in RPMI 1640 + 2mM Glutamine + 1.0 mM sodium pyruvate + 10% FBS. All four cell lines were maintained as a monolayer culture in their respective nutrient medium supplemented with 100 U mL−1 penicillin and 100 mg mL−1 streptomycin. The cells were grown at 37 °C, in 5% CO2 and humidified air atmosphere.

Cytotoxicity studies

The cytotoxicity of the compounds toward lung (A549), breast (MCF-7), and prostate (PC3 and LNCaP) cancer cell lines was measured by a fluorometric cell viability assay using resazurin. Cells were plated in triplicates in 96-well plates at a density of 3 x 10^3 cells/well in 100 µL 24 h prior to treatment. Cells were then treated with increasing concentrations of compounds for 96 h. After 96 h in the incubator, the medium was replaced by 100 µL medium containing resazurin (0.2 mg/mL final concentration). After 4 h (for A549, MCF-7, LNCaP) or 24 (for PC3) of incubation at 37 °C, the fluorescence of the highly red fluorescent resorufin product was quantified at 590 nm emission with 540 nm excitation wavelength in a Varioskan microplate Reader.

Compound stocks were prepared in DMSO for cytotoxicity assay. DMSO concentration of <1% was maintained in each well. In general, each compound concentration was dosed in triplicate wells. Data Analysis: The reference fluorescence reading was subtracted from the resorufin fluorescence at 590 nm (background control well no compound added, 1% DMSO) and the data was plotted as a percentage of the vehicle (1% DMSO alone). Data analysis and curve fitting was performed using Graphpad Prism. For each cell line, there were n = 3 data points for each concentration. Each dose-response curve was performed at least thrice, providing n ≥ 9 for each data point.

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