Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Dearomative Intermolecular [2+2] Photocycloaddition for Construction of C(sp^3)-rich Heterospirocycles On-DNA

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1 General Considerations

1.1 General

All chemical transformations requiring inert atmospheric conditions were carried out using Schlenk line techniques with a 4- or 5-port dual-bank manifold. For blue light irradiation, one Kessil H150-Blue lamp (19 V DC 40 W Max) was placed 1.5 inches away from PCR tubes. NMR spectra (^{1}H, ^{13}C, and ^{19}F) were obtained at 298 K using 400, 500 or 600 MHz spectrometers. ^{1}H NMR spectra were referenced to residual $CHCl_3$ (δ 7.26 ppm) in $CDCl_3$. ^{13}C NMR spectra were referenced to $CDCl_3$ (δ 77.16 ppm). Reactions were monitored by LC/MS, GC/MS, ^{1}H NMR, and/or TLC on silica gel plates (60 Å porosity, 250 μm thickness). TLC analysis was performed using hexanes/EtOAc as the eluent and visualized with UV light. Flash chromatography was accomplished using an automated system (CombiFlash®, UV detector, λ = 254 nm and 280 nm) with RediSep® R_f silica gel disposable flash columns (60 Å porosity, 40–60 μm) or RediSep R_f Gold® silica gel disposable flash columns (60 Å porosity, 20–40 μm). Accurate mass measurement analyses were conducted using electron ionization (EI) or electrospray ionization (ESI). The signals were mass measured against an internal lock mass reference of perfluorotributylamine (PFTBA) for EI-GCMS, and leucine enkephalin for ESI-LC/MS. The utilized software calibrates the instruments and reports measurements by use of neutral atomic masses. The mass of the electron is not included. IR spectra were recorded on an FT-IR using either neat oil or solid products. Solvents were purified with drying cartridges through a solvent delivery system. Melting points (°C) are uncorrected. 10 W blue LED irradiation for preparation of BCP-I and off-DNA [2+2] reaction was accomplished *via* the LED reactor described in a previous report unless stated otherwise.[1] The set up for on-DNA reaction was described in a previous report.[2]

1.2 Chemicals

Deuterated NMR solvents were purchased and stored over 4Å molecular sieves. CH_2Cl_2, DMA, EtOAc, hexanes, MeCN, DMSO, DIPEA, Et_3N, and HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide) were purchased from commercial suppliers and used without further purification. The synthesis of all new carboxylic acids and Boc-protected heterocycles are outlined here. All other reagents were purchased commercially and used as received. Photoredox-catalyzed reactions were performed using PCR 8-strip tubes (Ref. Fisher 781320) with PCR strips of 8 caps (Ref. Fisher 781340). HyPure™ Molecular Biology Grade Water was purchased and used as received without further manipulation.

1.3 Analysis of on-DNA reactions

Analysis of on-DNA reactions was performed by LC/MS: After reaction completion, an aliquot of the reaction mixture was diluted with H_2O to approximately 0.05–0.13 mM. At this point, 4 - 8 μL aliquots of the LC/MS sample was injected onto a reverse-phase chromatography column (Clarity 2.6 μm Oligo-MS 100 Å 2.1x50 mm) and eluted (10-90% B over 4 min at 0.5 mL/min flow rate; Solvent A: 0.75% v/v/ HFIP / 0.038% Et_3N in H_2O; Solvent B: 0.75% HFIP, 0.038% Et_3N in 90/10 MeOH/deionized H_2O) with no UV monitoring. Effluent was analyzed on a Waters SQ Detector 2 ACQUITY UPLC System in Thermo Exactive Plus LC-esiMS with a Vanquish UHPLC. For the functionalized headpiece samples, % conversion was determined based on reported peak intensities following deconvolution (between 3,000-8,000 Da) of the DNA charge states using Intact Mass™ by Protein Metrics Inc. (version 3.7-32x64). For the photoredox

scope reactions, % conversion was determined using Intact Mass™ by Protein Metrics Inc. (version 3.7-32x64). Data was scanned between 1.0 - 2.4 min and deconvoluted between 3,000-8,000 Da, with a mass tolerance window of 2 Da, with 10% of base peak threshold set for reporting. Na, K, NH_4, Cu, Ni and HFIP adducts were included in the product percentage. Detailed parameters can be found later in the Supporting Information.

1.4 Materials for on-DNA synthesis

DNA headpiece HP (5'-/Phos/GAGTCA/iSp9-PEG/iAm C7_CO-PEG4-NH2/iSp9-PEG/TGACTCCC-3') was obtained from WuXi AppTec, Shanghai, China.

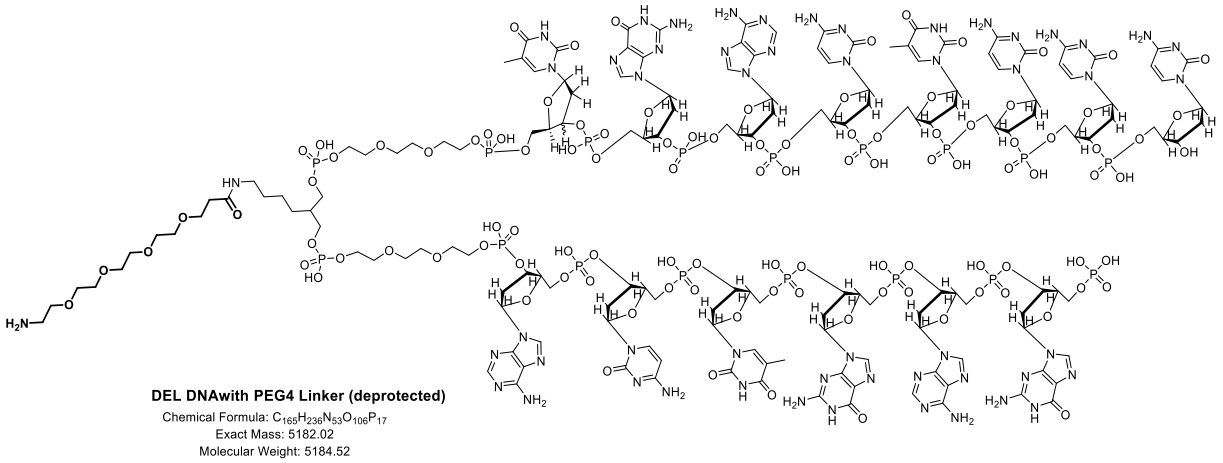
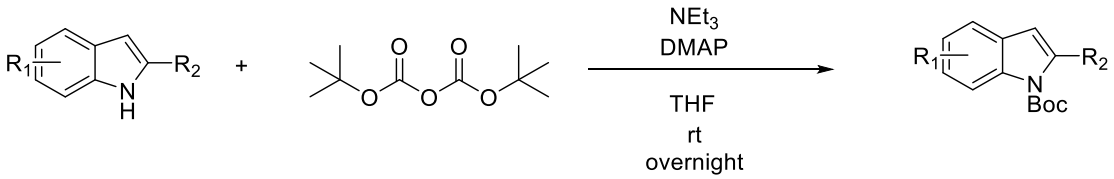


Figure S1. Sequence and structure of the DNA-headpiece (molecular weight = 5184.5220).

2 Synthesis of Indole/benzofuran substrates



Scheme S1

General procedure A: Boc-proteted indoles were prepared based on reported procedures[3]: *di*-tert-butyl dicarbonate (1.0-1.5 equiv), 4-dimethylaminopyridine (0.1-0.12 equiv), and Et_3N (1.0-1.2 equiv) were added to a solution indole (1.0 equiv) in THF (0.05 – 0.1 M). The reaction mixture was stirred at room temperature overnight. The reaction mixture was removed under reduced pressure. The product was filtered through silica gel or was purified by flash-column chromatography.

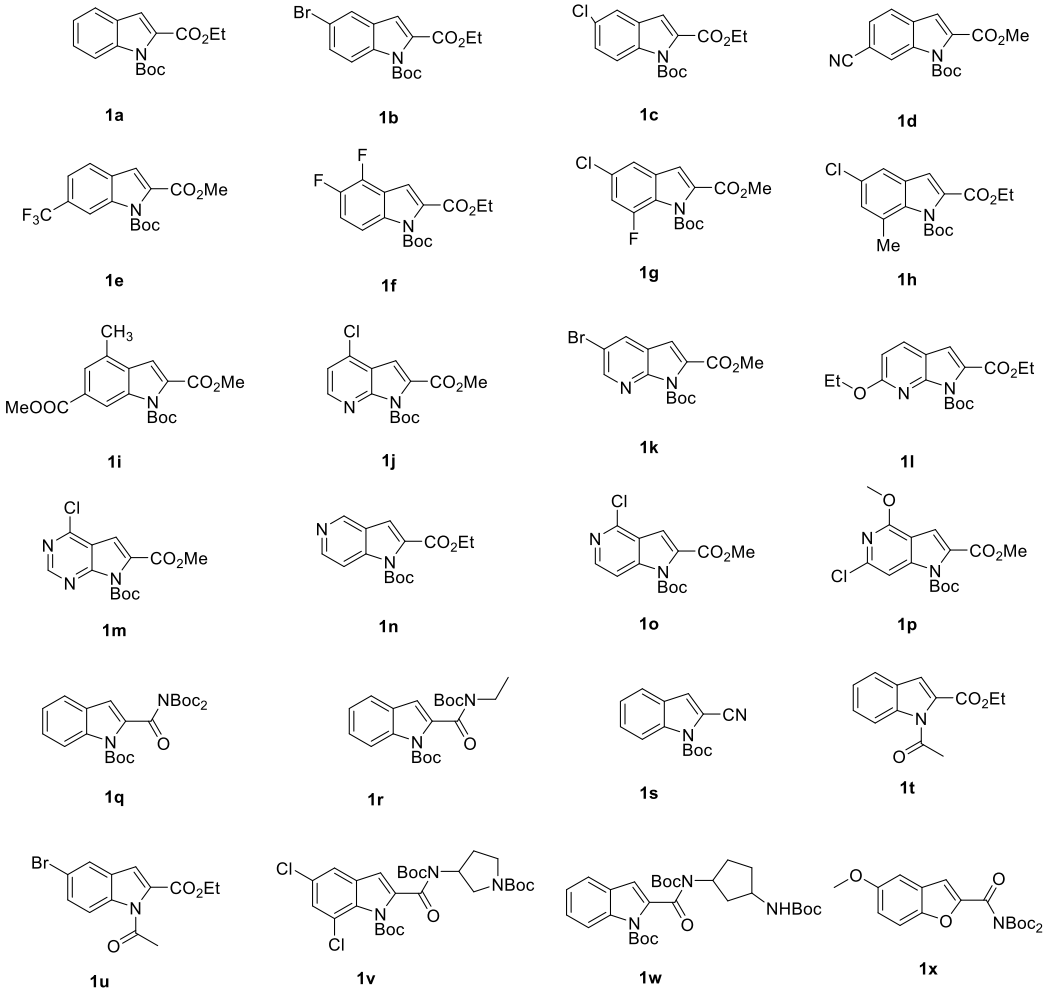
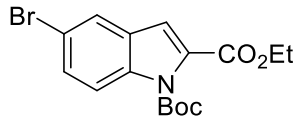


Figure S2. List of Boc-protected indoles and benzofurans used in this study.

1a[4], **1c**,[3] **1o**,[5] **1s**,[6] **1t**,[7] and **1u**[7] were synthesized following reported procedure and the characterization spectra are in accordance with the previous report.

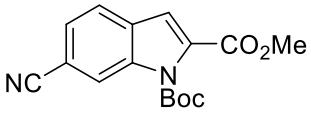
1-(*tert*-Butyl) 2-ethyl 5-bromo-1H-indole-1,2-dicarboxylate (1b)



Prepared following **General Procedure A**: ethyl 5-bromo-*1H*-indole-2-carboxylate (54 mg, 0.20 mmol, 1.0 equiv) and di-*tert*-butyl dicarbonate (65.5 mg, 69 µL, 0.3 mmol, 1.5 equiv), 4-dimethylaminopyridine (12.2 mg, 0.1 mmol, 0.5 equiv), and Et_3N (30.4 mg, 0.3 mmol, 1.5 equiv). After filtration on silica gel, the product **1b** was obtained as a yellow oil (73 mg, 0.20 mmol, >99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dt, *J* = 9.0, 0.8 Hz, 1H), 7.73 (d, *J* = 1.9 Hz, 1H), 7.49 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.00 (d, *J* = 0.9 Hz, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 1.62 (s, 9H), 1.40 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.5, 148.9, 136.4, 131.9, 129.5, 129.2, 124.6, 116.5, 116.4, 113.2, 85.1, 61.6, 27.8 (3*C), 14.2.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2986, 2844, 1728, 1443, 1393, 1370, 1338, 1317, 1275, 1227, 1187, 1156, 1137, 1017, 868.
HRMS (EI) calcd for $C_{14}H_{13}BrNO_4$ [M-Et]: 338.0028, found 338.0034.

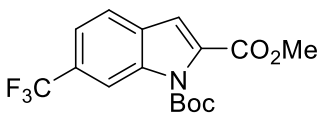
1-(*tert*-Butyl) 2-methyl 6-cyano-1*H*-indole-1,2-dicarboxylate (1d)



Prepared following **General Procedure A**: methyl 6-cyano-*1H*-indole-2-carboxylate (40 mg, 0.20 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (48 mg, 0.22 mmol, 1.1 equiv), 4-dimethylaminopyridine (2.9 mg, 0.024 mmol, 0.12 equiv), and Et_3N (22 mg, 0.22 mmol, 1.1 equiv). After filtration on silica gel, the product **1d** was obtained as a white solid (57 mg, 0.19 mmol, 95%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 – 8.44 (m, 1H), 7.69 (dd, *J* = 8.2, 0.7 Hz, 1H), 7.51 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.08 (d, *J* = 0.8 Hz, 1H), 3.95 (s, 3H), 1.64 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.9, 148.5, 136.5, 133.6, 130.9, 126.4, 123.1, 120.0, 119.6, 113.4, 109.7, 86.2, 52.9, 27.9 (3*C).
FT-IR (cm^{-1}, neat, ATR), ṽ = 2981, 2226, 1730, 1540, 1429, 1375, 1321, 1289, 1235, 1206, 1136, 1066, 1001, 938, 886, 840, 809, 767, 719, 625, 471.
HRMS (ESI) calcd for $C_{16}H_{17}N_2O_4$ [M+H]: 301.1188, found 301.1200.
Melting point (°C) 83.3 – 84.3.

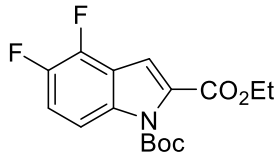
1-(*tert*-Butyl) 2-methyl 6-(trifluoromethyl)-*1H*-indole-1,2-dicarboxylate (1e)



Prepared following **General Procedure A**: methyl 6-(trifluoromethyl)-*1H*-indole-2-carboxylate (30 mg, 0.12 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (30 mg, 0.22 mmol, 1.1 equiv), 4-dimethylaminopyridine (1.8 mg, 0.015 mmol, 0.12 equiv), and Et_3N (14 mg, 0.14 mmol, 1.1 equiv). After filtration on silica gel, compound **1e** was obtained as a white solid (40 mg, 0.12 mmol, >99%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.46 – 8.39 (m, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.51 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.10 (d, *J* = 0.8 Hz, 1H), 3.95 (s, 3H), 1.64 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 162.1, 148.8, 136.8, 132.9, 130.1, 128.7 (d, *J* = 32.1 Hz), 124.6 (d, *J* = 282.0 Hz), 122.7, 120.2 (q, *J* = 3.6 Hz), 113.6, 112.9 (q, *J* = 4.3 Hz), 85.7, 52.8, 27.9 (3*C).
^{19}F NMR (376 MHz, $CDCl_3$) δ -61.45.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2991, 2956, 1747, 1733, 1432, 1370, 1316, 1293, 1231, 1202, 1155, 1131, 1117, 1078, 1051, 942, 898, 848, 818, 779, 763, 741.
HRMS (EI) calcd for $C_{16}H_{16}F_3NO_4$ [M]: 343.1031, found 343.1050.
Melting point (°C) 66.1 – 67.3

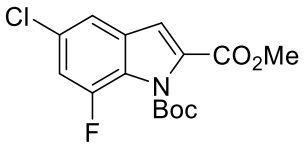
1-(*tert*-Butyl) 2-ethyl 4,5-difluoro-*1H*-indole-1,2-dicarboxylate (1f)



Prepared following **General Procedure A**: ethyl 4,5-difluoro-*1H*-indole-2-carboxylate (50 mg, 0.22 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (53 mg, 0.24 mmol, 1.1 equiv), 4-dimethylaminopyridine (3.3 mg, 0.027 mmol, 0.12 equiv), and Et_3N (25 mg, 0.24 mmol, 1.1 equiv). After filtration on silica gel, compound **1f** was obtained as a clear oil (58 mg, 0.18 mmol, 82%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 1H), 7.25 – 7.18 (m, 1H), 7.16 (d, *J* = 0.8 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 1.62 (s, 9H), 1.40 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.4, 149.0, 146.0 (d, *J* = 240.8, 10.4 Hz), 143.2 (d, *J* = 251.9, 14.2 Hz), 134.9 (d, *J* = 6.9 Hz), 132.3, 118.4 (d, *J* = 16.6 Hz), 116.3 (d, *J* = 20.9 Hz), 110.9 (dd, *J* = 7.1, 4.5 Hz), 109.5 (d, *J* = 5.0 Hz), 85.6, 61.9, 27.9 (3*C), 14.4.
^{19}F NMR (376 MHz, $CDCl_3$) δ -145.9, -145.9, -146.7, -146.7.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2983, 1732, 1506, 1437, 1381, 1370, 1328, 1280, 1260, 1239, 1206, 1179, 1144, 1097, 1044, 1036, 986, 847, 830, 800, 775.
HRMS (ESI) calcd for $C_{16}H_{18}F_2NO_4$ [M+H]: 326.1204, found 326.1214.

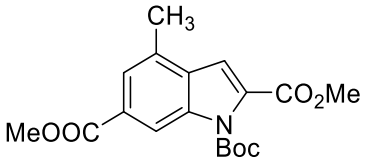
1-(*tert*-Butyl) 2-methyl 5-chloro-7-fluoro-*1H*-indole-1,2-dicarboxylate (1g)



Prepared following **General Procedure A**: methyl 5-chloro-7-fluoro-*1H*-indole-2-carboxylate (48 mg, 0.21 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (51 mg, 0.23 mmol, 1.1 equiv), 4-dimethylaminopyridine (3.1 mg, 0.025 mmol, 0.12 equiv) and Et_3N (23 mg, 0.23 mmol, 1.1 equiv). After filtration on silica gel, compound **1g** was obtained as a white solid (46 mg, 0.14 mmol, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 – 7.71 (m, 1H), 7.18 (d, *J* = 0.8 Hz, 1H), 7.08 (dd, *J* = 9.0, 2.2 Hz, 1H), 3.93 (s, 3H), 1.62 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.9 (d, *J* = 246.1 Hz), 161.7, 148.8, 138.3 (d, *J* = 14.2 Hz), 131.2 (d, *J* = 4.0 Hz), 128.0 (d, *J* = 12.9 Hz), 123.2 (d, *J* = 1.5 Hz), 112.7 (d, *J* = 9.1 Hz), 112.6 (d, *J* = 18.1 Hz), 101.1 (d, *J* = 28.5 Hz), 85.8, 52.7, 27.9 (3*C).
^{19}F NMR (376 MHz, $CDCl_3$) δ -111.7.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2979, 1733, 1481, 1437, 1412, 1394, 1371, 1327, 1295, 1253, 1212, 1146, 1129, 1072, 954, 907, 839, 794, 773, 759, 501.
HRMS (ESI) calcd for $C_{11}H_8ClFNO_4$ [M-*t*Bu+H]: 272.0126, found 272.0138.
Melting point (°C) 63.6 – 64.7.

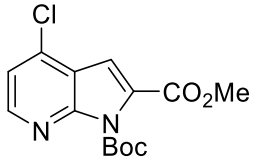
1-(*tert*-Butyl) 2,6-dimethyl 4-methyl-*1H*-indole-1,2,6-tricarboxylate (1i)



Prepared following **General Procedure A**: dimethyl 4-methyl-*1H*-indole-2,6-dicarboxylate (25 mg, 0.10 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (24 mg, 0.11 mmol, 1.1 equiv), 4-dimethylaminopyridine (1.5 mg, 0.012 mmol, 0.12 equiv) and Et_3N (11 mg, 0.11 mmol, 1.1 equiv). After filtration on silica gel, compound **1i** was obtained as a white solid (34 mg, 0.098 mmol, 98%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.67 – 8.62 (m, 1H), 7.80 – 7.72 (m, 1H), 7.14 (d, *J* = 0.9 Hz, 1H), 3.94 (s, 3H), 3.94 (s, 3H), 2.55 (s, 2H), 1.65 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 167.6, 162.3, 149.0, 137.1, 132.5, 131.8, 131.1, 128.5, 124.5, 114.7, 112.6, 85.4, 52.7, 52.3, 28.0 (3*C), 18.5.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2956, 1731, 1711, 1433, 1379, 1332, 1302, 1227, 1197, 1142, 1212, 1110, 1054, 999, 956, 897, 847, 835, 800, 782, 769, 739.
HRMS (ESI) calcd for $C_{18}H_{21}NO_6$ [M+H]: 370.1267, found 370.1266.
Melting point (°C) 85.6 – 86.3.

1-(*tert*-Butyl) 2-methyl 4-chloro-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (1j)

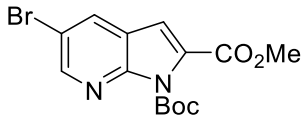


Prepared following **General Procedure A**: methyl 4-chloro-*1H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (105 mg, 0.50 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (115 mg, 0.525 mmol, 1.1 equiv), 4-dimethylaminopyridine (7.3 mg, 0.060 mmol, 0.12 equiv), and Et_3N (51 mg, 0.50 mmol, 1.0 equiv). After filtration on silica gel, compound **1j** was obtained as a white solid (132 mg, 0.42 mmol, 84%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (d, *J* = 5.2 Hz, 1H), 7.25 (d, *J* = 5.2 Hz, 1H), 7.21 (s, 1H), 3.95 (s, 3H), 1.64 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.3, 149.8, 148.3, 147.5, 138.3, 130.2, 119.4, 119.2, 109.1, 86.0, 52.7, 27.8 (3*C).
FT-IR (cm^{-1}, neat, ATR), ṽ = 2983, 1763, 1732, 1591, 1527, 1474, 1439, 1394, 1368, 1277, 1247, 1220, 1193, 1142, 1087, 937, 826, 802, 782, 746, 610.

HRMS (ESI) calcd for $C_{14}H_{16}ClN_2O_4$ [M+H]: 311.0799, found 311.0810.
Melting point (°C) 84.0 – 84.8.

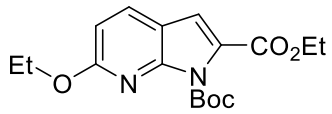
1-(*tert*-Butyl) 2-methyl 4-chloro-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (1k)



Prepared following **General Procedure A**: methyl 5-bromo-*1H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (51 mg, 0.20 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (46 mg, 0.21 mmol, 1.1 equiv), 4-dimethylaminopyridine (2.9 mg, 0.024 mmol, 0.12 equiv), and Et_3N (20 mg, 0.20 mmol, 1.0 equiv). The crude was purified by column chromatography (10% ethyl acetate/hexanes). The product **1k** was obtained as a white solid (33 mg, 0.093 mmol, 47%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.60 (d, *J* = 2.2 Hz, 1H), 8.09 (d, *J* = 2.2 Hz, 1H), 7.02 (s, 1H), 3.95 (s, 3H), 1.63 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.3, 148.6, 147.8, 147.5, 132.9, 131.2, 121.4, 115.0, 110.1, 85.9, 52.7, 27.8 (3*C).
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2976, 1755, 1723, 1524, 1442, 1408, 1393, 1367, 1306, 1256, 1225, 1208, 1193, 1148, 1132, 1074, 1035, 995, 885, 841, 739.
HRMS (ESI) calcd for $C_{14}H_{16}BrN_2O_4$ [M+H]: 355.0293, found 355.0298.
Melting point (°C) 107.8 – 108.9.

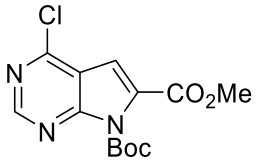
1-(*tert*-Butyl) 2-ethyl 6-ethoxy-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (1l)



Prepared following **General Procedure A**: ethyl 6-ethoxy-*1H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (60 mg, 0.26 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (61 mg, 0.28 mmol, 1.1 equiv), 4-dimethylaminopyridine (3.8 mg, 0.031 mmol, 0.12 equiv) and Et_3N (29 mg, 0.28 mmol, 1.1 equiv). After filtration on silica gel, compound **1l** was obtained as a white solid (82 mg, 0.25 mmol, 96%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (dd, *J* = 9.0, 0.8 Hz, 1H), 7.06 (d, *J* = 0.7 Hz, 1H), 6.76 (d, *J* = 9.0 Hz, 1H), 4.45 – 4.33 (m, 4H), 1.62 (s, 9H), 1.47 – 1.32 (m, 6H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.9, 161.9, 148.8, 142.6, 132.4, 127.3, 125.8, 114.2, 110.5, 85.3, 62.0, 61.7, 28.0 (3*C), 14.8, 14.4.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2981, 2931, 1740, 1722, 1528, 1415, 1380, 1352, 1329, 1302, 1212, 1178, 1147, 1120, 1090, 1070, 1043, 1012, 854, 824, 770, 749.
HRMS (ESI) calcd for $C_{17}H_{23}N_2O_5$ [M+H]: 335.1607, found 335.1612.
Melting point (°C) 52.9-54.1.

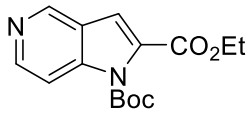
7-(*tert*-Butyl) 6-methyl 4-chloro-*7H*-pyrrolo[2,3-*d*]pyrimidine-6,7-dicarboxylate (1m)



Prepared following **General Procedure A**: methyl 4-chloro-*7H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (106 mg, 0.50 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (115 mg, 0.525 mmol, 1.1 equiv), 4-dimethylaminopyridine (7.3 mg, 0.060 mmol, 0.12 equiv) and Et_3N (51 mg, 0.50 mmol, 1.0 equiv). After filtration on silica gel, compound **1m** was obtained as a white solid (128 mg, 0.41 mmol, 82%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.22 (s, 1H), 3.97 (s, 3H), 1.66 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 160.5, 155.1, 154.9, 153.1, 146.6, 130.6, 117.2, 108.8, 87.2, 53.0, 27.7 (3*C).
FT-IR (cm^{-1}, neat, ATR), ṽ = 2971, 1771, 1732, 1441, 1427, 1396, 1292, 1250, 1233, 1209, 1189, 1154, 1129, 1084, 1015, 934, 835, 805, 788, 777, 747.
HRMS (ESI) calcd for $C_{13}H_{15}ClN_3O_4$ [M+H]: 312.0751, found 312.0759.
Melting point (°C) 105.1 – 105.9.

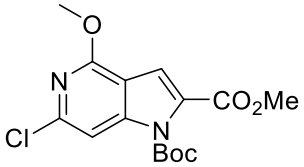
1-(*tert*-Butyl) 2-ethyl 1*H*-pyrrolo[3,2-*c*]pyridine-1,2-dicarboxylate (1n)



Prepared following **General Procedure A**: ethyl 1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (100 mg, 0.526 mmol, 1.0 equiv) and di-*tert*-butyl dicarbonate (138 mg, 150 µL, 0.6 mmol, 1.2 equiv), 4-dimethylaminopyridine (6.4 mg, 0.053 mmol, 0.1 equiv), and Et_3N (63.9 mg, 0.63 mmol, 1.2 equiv). After filtration on silica gel, the title compound **1n** was obtained as a colorless oil (110 mg, 0.379 mmol, 72%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.53 (d, *J* = 5.8 Hz, 1H), 7.92 (d, *J* = 5.8 Hz, 1H), 7.14 (s, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.63 (s, 9H), 1.39 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.4, 148.5, 145.9, 145.2, 141.9, 131.9, 124.3, 112.6, 109.8, 85.9, 61.9, 27.9, 14.3 (3*C).
FT-IR (cm^{-1}, neat, ATR), ṽ = 2982, 1732, 1600, 1477, 1455, 1439, 1394, 1371, 1355, 1337, 1321, 1300, 1234, 1186, 1152.
HRMS (EI) calcd for $C_{15}H_{18}N_2O_4$ [M]: 290.1267, found 291.1334.

1-(*tert*-Butyl) 2-methyl 6-chloro-4-methoxy-1*H*-pyrrolo[3,2-*c*]pyridine-1,2-dicarboxylate (1p)

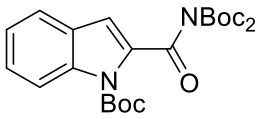


Prepared following **General Procedure A**: methyl 6-chloro-4-methoxy-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (100 mg, 0.416 mmol, 1.0 equiv) and di-*tert*-butyl dicarbonate (109 mg, 110 µL, 0.5 mmol, 1.2 equiv), 4-dimethylaminopyridine (5.1 mg, 0.042 mmol, 0.1 equiv) and Et_3N (50.5 mg, 0.50 mmol, 1.2

equiv). After filtration on silica gel, the title compound **1p** was obtained as a white solid (130 mg, 0.382 mmol, 92%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.14 (s, 1H), 4.07 (s, 3H), 3.91 (s, 3H), 1.62 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.5, 157.8, 148.4, 145.4, 145.4, 129.8, 112.2, 110.9, 103.9, 86.2, 54.4, 52.6, 27.8 (3*C).
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 1736, 1600, 1572, 1468, 1436, 1397, 1371, 1342, 1300, 1272, 1211, 1175, 1147, 1129, 912.
HRMS (EI) calcd for $C_{15}H_{17}ClN_2O_5$ [M]: 340.0826, found 341.0889.
Melting point (°C): 83.0 - 85.5.

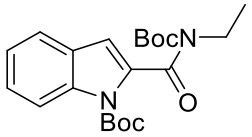
***tert*-Butyl 2-(bis(*tert*-butoxycarbonyl)carbamoyl)-1*H*-indole-1-carboxylate (1q)**



Prepared following **General Procedure A**: 1*H*-indole-2-carboxamide (100 mg, 0.624 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (400 mg, 400 µL, 2.0 mmol, 3.1 equiv), triethylamine (200 mg, 300 µL, 2.0 mmol, 3.0 equiv), and catalytic amount of DMAP. After filtration on silica gel, the title compound **1q** was obtained as a colorless oil (250 mg, 0.543 mmol, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.81 (s, 1H), 1.56 (s, 9H), 1.36 (s, 18H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.9, 149.7 (2*C), 149.2, 137.1, 133.1, 128.1, 126.8, 123.7, 122.2, 115.7, 111.7, 85.5, 85.3 (2*C), 28.2 (6*C), 27.9 (3*C).
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2980, 1783, 1738, 1704, 1476, 1451, 1370, 1330, 1299, 1247, 1153, 1116, 1086, 1037.
HRMS (EI) calcd for $C_{24}H_{32}N_2O_7Na$ [M+Na]: 483.2107, found 483.2092.

***tert*-Butyl 2-((*tert*-butoxycarbonyl)(ethyl)carbamoyl)-*1H*-indole-1-carboxylate (1r)**

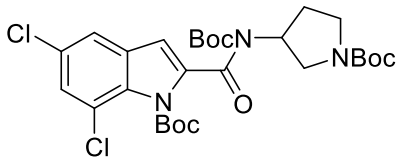


Prepared following **General Procedure A**: *N*-ethyl-*1H*-indole-2-carboxamide (110 mg, 0.584 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (281 mg, 1.29 mmol, 2.2 equiv), 4-dimethylaminopyridine (14.3 mg, 0.117 mmol, 0.2 equiv) and Et_3N (130 mg, 1.29 mmol, 2.2 equiv). After filtration on silica gel, compound **1r** was obtained as a white solid (126 mg, 0.324 mmol, 55%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.53 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.34 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.24 (ddd, *J* = 8.0, 7.2, 1.0 Hz, 1H), 6.54 (d, *J* = 0.8 Hz, 1H), 3.97 – 3.86 (m, 2H), 1.61 (s, 9H), 1.29 (t, *J* = 7.0 Hz, 3H), 1.14 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 164.8, 152.4, 149.2, 135.6, 135.5, 128.7, 125.5, 123.3, 121.4, 115.6, 107.3, 84.7, 83.3, 40.0, 28.2 (3*C), 27.7 (3*C), 13.7.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2979, 1731, 1674, 1440, 1392, 1368, 1330, 1314, 1238, 1216, 1140, 1119, 1088, 1063, 1036, 997, 851, 834, 823, 801, 755.

HRMS (ESI) calcd for $C_{21}H_{29}N_2O_5$ [M+H]: 389.2076, found 389.2089.
Melting point (°C) 63.0 – 65.8.

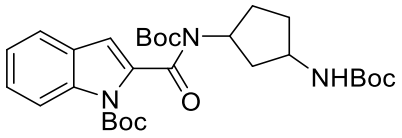
***ert*-Butyl 2-((*tert*-butoxycarbonyl)(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)carbamoyl)-5,7-dichloro-*1H*-indole-1-carboxylate (1v)**



Prepared following **General Procedure A**: 5,7-dichloro-*N*-(pyrrolidin-3-yl)-*1H*-indole-2-carboxamide (30 mg, 0.10 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (70 mg, 0.32 mmol, 3.2 equiv), 4-dimethylaminopyridine (2.5 mg, 0.020 mmol, 0.2 equiv) and Et_3N (33 mg, 0.32 mmol, 3.2 equiv). After filtration on silica gel, compound **1v** was obtained as a white solid (32 mg, 0.053 mmol, 53%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.29 (s, 1H), 6.64 (d, *J* = 9.6 Hz, 1H), 5.31 – 5.14 (m, 1H), 3.79 – 3.57 (m, 3H), 3.48 – 3.33 (m, 1H), 2.62 – 2.39 (m, 1H), 2.26 – 2.14 (m, 1H), 1.62 (d, *J* = 10.9 Hz, 9H), 1.47 (s, 9H), 1.16 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 164.1, 154.5, 152.0, 148.4, 136.4, 136.3, 131.8, 127.2, 125.9, 123.8, 114.6, 105.3, 105.1, 86.1, 84.5, 79.6, 53.8, 53.1, 47.3, 47.1, 45.0, 44.4, 28.7 (3*C), 28.0 (3*C), 28.0, 27.7 (3*C), 27.6.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2977, 1738, 1688, 1605, 1544, 1455, 1368, 1322, 1302, 1249, 1135, 1085, 852, 846, 811, 766, 589, 557.
HRMS (ESI) calcd for $C_{28}H_{38}Cl_2N_3O_7$ [M+H]: 598.2087, found 598.2073.
Melting point (°C) 60.6 – 63.6.

***tert*-Butyl 2-((*tert*-butoxycarbonyl)(3-((*tert*-butoxycarbonyl)amino)cyclopentyl)carbamoyl)-*1H*-indole-1-carboxylate (1w)**

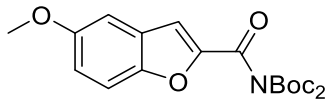


Prepared following **General Procedure A**: *N*-(3-aminocyclopentyl)-*1H*-indole-2-carboxamide (50 mg, 0.21 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (193 mg, 0.884 mmol, 4.3 equiv), 4-dimethylaminopyridine (10.0 mg, 0.082 mmol, 0.4 equiv) and Et_3N (89 mg, 0.88 mmol, 4.3 equiv). The crude was purified by column chromatography (60% ethyl acetate/hexanes). The product **1w** was obtained as a white solid (63 mg, 0.12 mmol, 57%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.57 – 7.48 (m, 1H), 7.41 – 7.32 (m, 1H), 7.26 – 7.22 (m, 1H), 6.54 (s, 1H), 5.57 (d, *J* = 8.5 Hz, 1H), 5.04 – 4.93 (m, 1H), 4.15 – 4.05 (m, 1H), 2.45 – 2.33 (m, 1H), 2.22 – 2.13 (m, 1H), 2.01 – 1.94 (m, 1H), 1.93 – 1.87 (m, 1H), 1.86 – 1.76 (m, 2H), 1.62 (s, 9H), 1.44 (s, 9H), 1.08 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 165.0, 155.6, 153.0, 149.0, 136.0, 135.9, 128.3, 125.9, 123.5, 121.6, 115.5, 107.6, 84.8, 83.8, 54.8, 51.2, 35.5, 33.0, 28.6 (3*C), 28.2 (3*C), 27.6 (3*C), 27.2.
FT-IR (cm^{-1}, neat, ATR), ṽ = 3408, 2976, 1732, 1677, 1501, 1450, 1367, 1333, 1305, 1240, 1141, 1084, 850, 772, 746, 588, 463.

HRMS (ESI) calcd for $C_{29}H_{42}N3O_7$ [M+H]: 544.3023, found 544.3043.
Melting point (°C) 56.1 – 57.6.

***tert*-Butyl (*tert*-butoxycarbonyl)(5-methoxybenzofuran-2-carbonyl)carbamate (1x)**

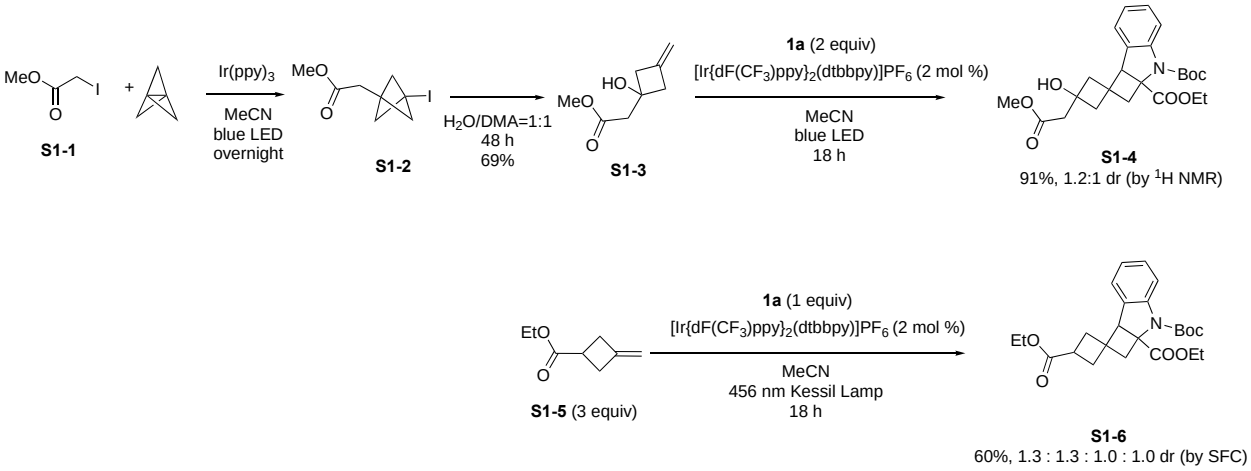


Prepared according to the *General Procedure* from methyl 5-methoxybenzofuran-2-carboxamide (50 mg, 0.3 mmol, 1.0 equiv) and di-*tert*-butyl dicarbonate (130 mg, 130 µL, 0.6 mmol, 2.2 equiv), 4-dimethylaminopyridine (3.2 mg, 0.026 mmol, 0.1 equiv) and Et_3N (58 mg, 0.58 mmol, 2.2 equiv). After filtration on silica gel, the title compound **1x** was obtained as a white solid (80 mg, 78%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.42 (d, *J* = 9.1 Hz, 1H), 7.11 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.09 (d, *J* = 2.5 Hz, 1H), 3.85 (s, 3H), 1.42 (s, 18H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 158.5, 155.9, 149.8, 148.3 (2*C), 147.9, 126.6, 118.1, 114.6, 112.2, 102.9, 83.6 (2*C), 55.0, 26.8 (6*C).
FT-IR (cm^{-1}, neat, ATR), ṽ =1797, 1738, 1704, 1558, 1476, 1457, 1370, 1351, 1251, 1210, 1148, 1100, 1029, 981, 900.
HRMS (EI) calcd for $C_{20}H_{25}NNaO_7$ [M+Na]: 414.1529, found 414.1541.
Melting point (°C) 93.2 - 95.1.

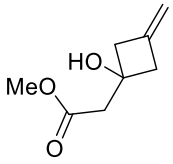
3 Small molecule model reaction

3.1 Off-DNA reaction for indoles



Scheme S2

Preparation of methyl 2-(1-hydroxy-3-methylenecyclobutyl)acetate (S1-3)

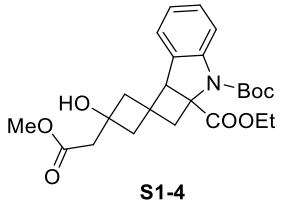


S1-3

To an 8 mL screwed-cap vial was added methyl 2-(3-Iodobicyclo[1.1.1]pentan-1-yl)acetate (**S1-2**) (250 mg, 0.94 mmol, **S1-2** was prepared following reported procedures[8,9]), 2.5 mL of DMA, and 2.5 mL of H_2O. The reaction was covered with alumina foil and stirred vigorously at rt. After 48 h, the reaction mixture was partitioned between Et_2O (20 mL) and H_2O (20 mL). The organic layer was washed two more times with brine (10 mL × 2). The combined organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by SiO_2 column chromatography (20% ethyl acetate/hexanes). The product **S1-3** was obtained as a colorless oil (101 mg, 0.647 mmol, 69%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.93 – 4.87 (m, 2H), 3.73 (s, 3H), 3.72 (s, 1H), 2.93 – 2.84 (m, 2H), 2.71 (s, 2H), 2.69 – 2.64 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.4, 140.0, 108.5, 69.3, 52.0, 45.6 (2*C), 42.9.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3455, 3076, 2955, 2918, 1720, 1682, 1438, 1405, 1370, 1321, 1204, 1166, 1100, 1075, 1000, 878, 766, 640, 585, 479.
HRMS (EI) calcd for $C_8H_{12}O_3$ [M]: 156.0786, found 156.0785.

Preparation of 3'-(*tert*-butyl) 2a'-ethyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)spiro[cyclobutane-1,1'-cyclobuta[b]indole]-2a',3'(2'H,7b'H)-dicarboxylate (S1-4)

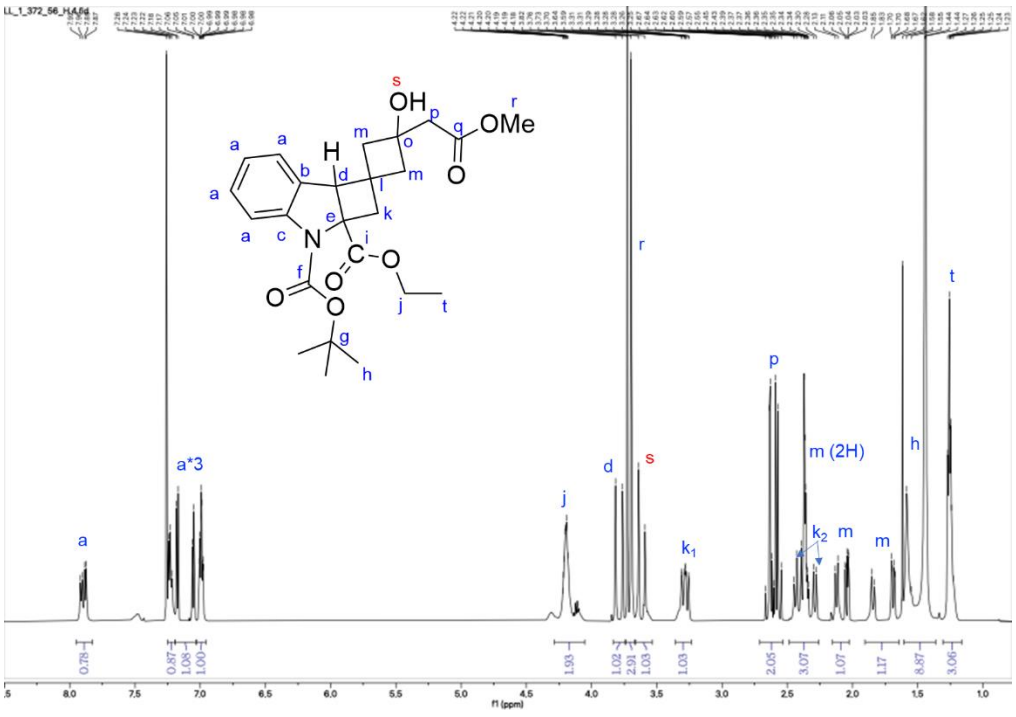


S1-4

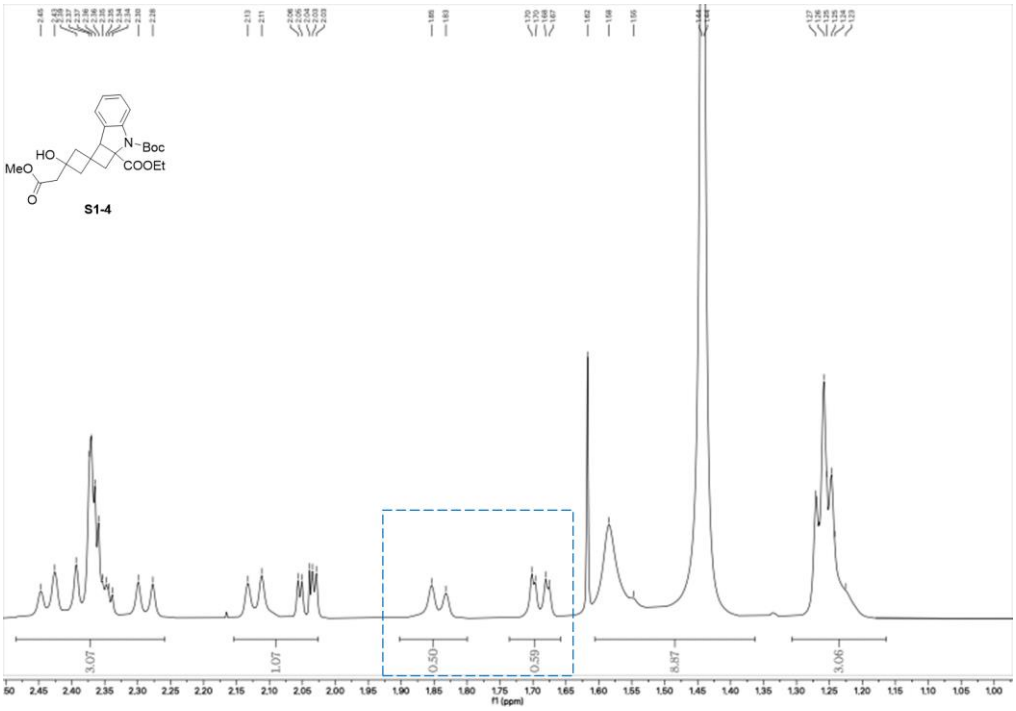
Mixture of diastereoisomers (dr = 1.2 : 1.0)

The procedure was adapted from reported literature:[10] To a 4 mL glass vial was added $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (4.5 mg, 0.0039 mmol, 1.8 mol%), indole substrate **1a** (130 mg, 0.45 mmol, 2.1 equiv), alkene **S1-3** (32.9 mg, 0.211 mmol, 1.0 equiv), and anhydrous CH_3CN (0.1 M, 2.0 mL). The reaction mixture was then degassed by sparging N_2 for 3 min. The vial was sealed. The stirred mixture was irradiated with ~10 W blue LED (strips, 470 nm) at a distance of 1 inch with a cooling fan to ensure reactions remained at or near room temperature for 18 h. The reaction mixture was concentrated, and the residue was purified by SiO_2 column chromatography (10% EtOAc/hexanes) to give the product (a mixture of diastereomers) as a clear oil (85.2 mg, 0.191 mmol, 91% yield).

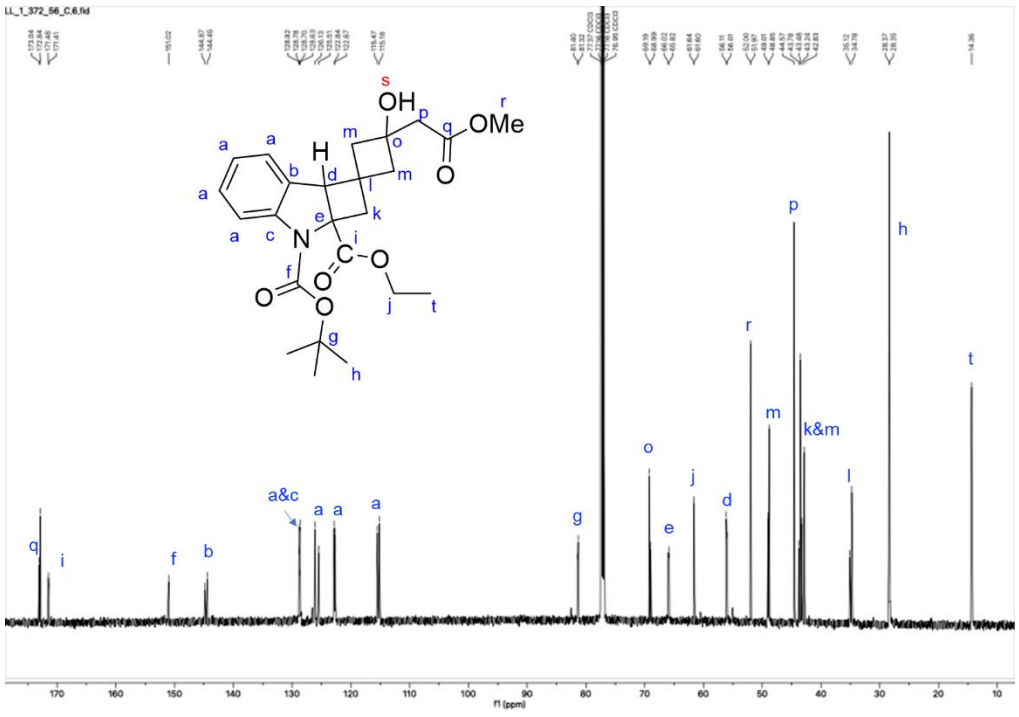
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 – 7.83 (m, 1H), 7.25 – 7.19 (m, 1H), 7.19 – 7.03 (m, 1H), 7.03 – 6.95 (m, 1H), 4.29 – 4.05 (m, 2H), 3.79 (d, *J* = 32.1 Hz, 1H), 3.71 (d, *J* = 17.1 Hz, 3H), 3.62 (d, *J* = 28.9 Hz, 1H), 3.36 – 3.24 (m, 1H), 2.71 – 2.53 (m, 2H), 2.48 – 2.26 (m, 3H), 2.15 – 2.03 (m, 1H), 1.90 – 1.64 (m, 1H), 1.61 – 1.36 (m, 9H), 1.31 – 1.16 (m, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0, 172.8, 171.5, 171.4, 151.0, 144.9, 144.5, 128.8, 128.8, 128.7, 128.6, 126.1, 125.5, 122.8, 122.7, 115.5, 115.2, 81.4, 81.3, 77.4, 77.2, 77.0, 69.2, 69.0, 66.0, 65.8, 61.6, 61.6, 56.1, 56.0, 52.0, 52.0, 49.0, 48.9, 44.6, 43.8, 43.5, 43.2, 42.8, 35.1, 34.8, 28.4 (3*C), 28.4 (3*C), 14.4.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3508, 2979, 2932, 1713, 1480, 1462, 1438, 1368, 1315, 1237, 1205, 1159, 1112, 1095, 1064, 1002, 911.
HRMS (ESI) calcd for $C_{24}H_{31}NO_7Na$ [M+Na]: 468.1998, found 468.2006.



^{1}H NMR (assigned), $CDCl_3$ of **S1-4**

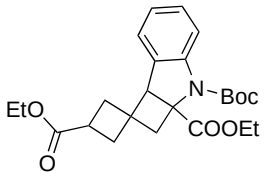


^{1}H NMR, $CDCl_3$ of **S1-4** for determination of diastereomeric ratio (peaks at 1.70ppm and 1.85ppm, dr = 1.2 : 1.0)



^{13}C NMR (assigned), $CDCl_3$ of **S1-4**

Preparation of 3'-(*tert*-butyl) 2a',3-diethyl spiro[cyclobutane-1,1'-cyclobuta[*b*]indole]-2a',3,3'(2'*H*,7b'*H*)-tricarboxylate (S1-6)



S1-6
60%, 1.3 : 1.3 : 1.0 : 1.0 dr (by SFC)

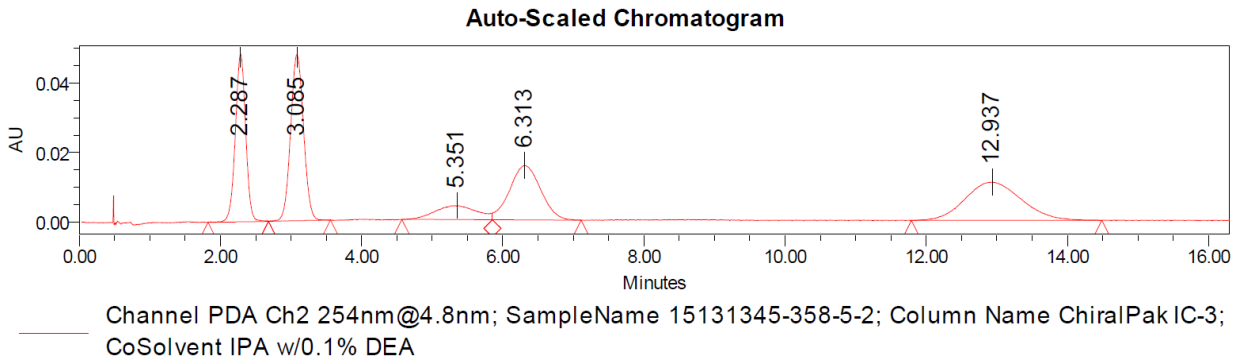
The procedure was adapted from reported literature:[10] To a 4 mL glass vial was added $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (2.24 mg, 0.002 mmol, 2 mol %), indole substrate (29 mg, 0.1 mmol, 1.0 equiv), alkene **S1-5** (42 mg, 0.3 mmol, 3.0 equiv, **S1-5** was prepared as reported[11]), and anhydrous CH_3CN (0.1 M, 1.0 mL). The reaction mixture was then degassed by sparging N_2 for 3 min. The vial was sealed. The stirred mixture was irradiated with 456 nm PR-160 Kessil Lamp at a distance of 1 inch with a cooling fan to ensure reactions remained at or near room temperature for 18 h. The reaction mixture was concentrated, and the residue was purified by SiO_2 column chromatography (10% EtOAc/hexanes) to give the product (a mixture of diastereomers) as a clear oil (Run 1: 41 mg, 0.095 mmol, 95% yield; Run 2: 30 mg, 0.07 mmol, 70% yield; Run 3 (2x scale): 52 mg, 0.12 mmol, 60% yield).

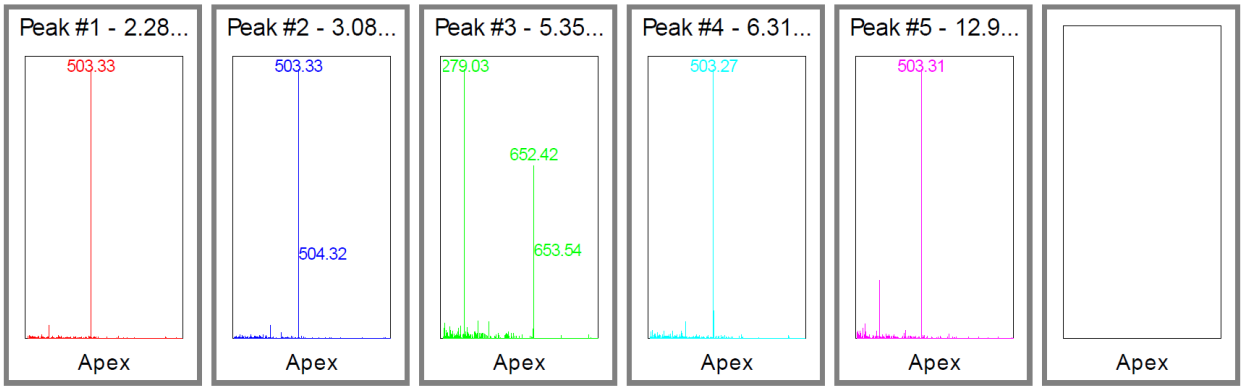
(*Note*: when indole/alkene = 2 : 1 was used, certain amount of indole dimer was formed. The dimer has similar polarity with the product which makes purification challenging.)

Analytical Chiral SFC: Ultra-High Performance SFC – Waters UPC^2

Analytical Ultra-High Performance SFC was performed on a Waters UPC^2 SFC system running under Empower software control. The SFC system included a 6-way column switcher, sample manager autosampler running with partial loop injections, CO_2 pump, binary modifier pump, column oven, convergence manager (ABPR), PDA detector and QDa mass detector.

Compound S1-6 analytical analysis took place on a 4.6x100mm ChiralPak IC-3 column held at 35 deg C and the backpressure regulator was set to maintain 2000 psi. The mobile phase comprised of CO_2 with a modifier mixture of 95:5 CO_2:isopropyl alcohol with 0.1% diethylamine held isocratically for 16 min at a flow rate of 3 mL/min.





RT	% Area	Column Name	
2.29	20.88	ChiralPak IC-3	Diastereomer 1
3.09	25.86	ChiralPak IC-3	Diastereomer 2
5.35	7.12	ChiralPak IC-3	Dimer Impurity
6.31	20.34	ChiralPak IC-3	Diastereomer 3
12.94	25.80	ChiralPak IC-3	Diastereomer 4

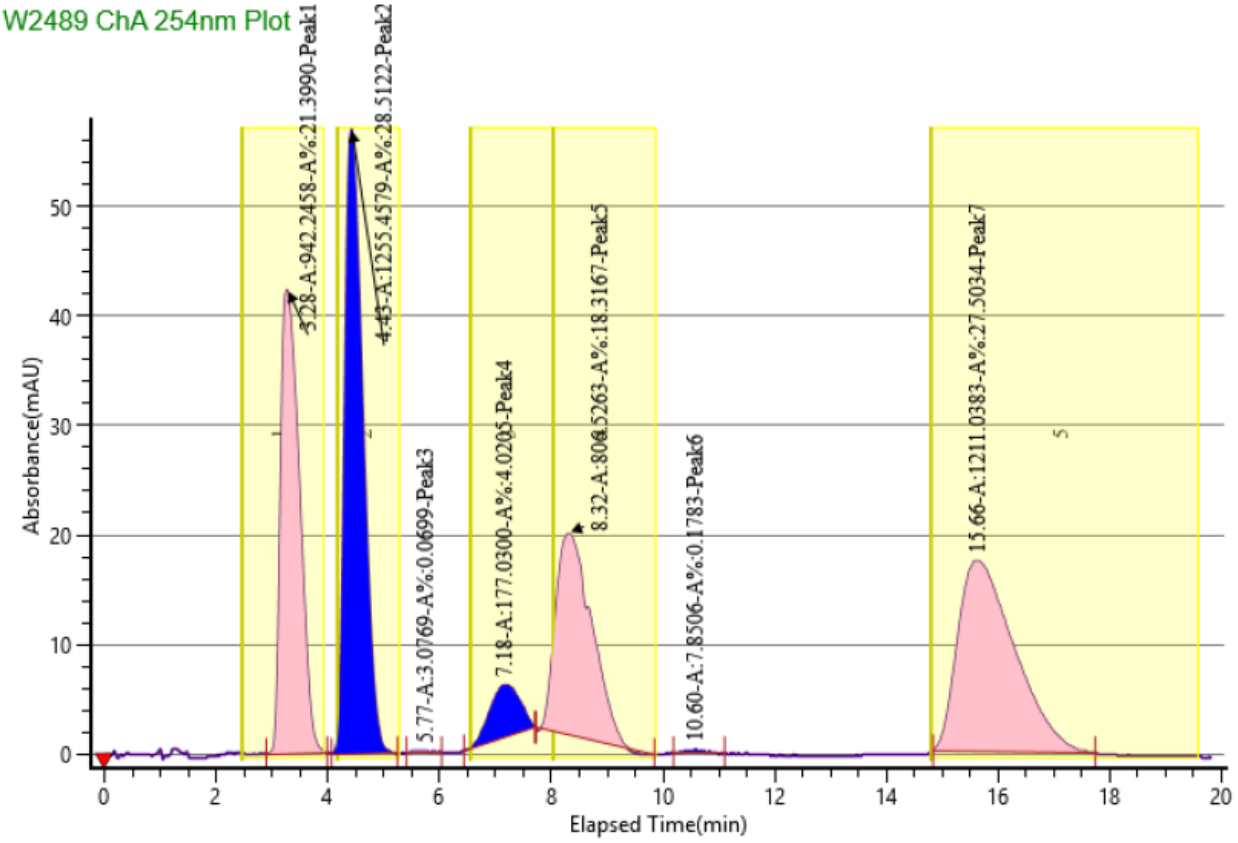
Therefore, dr = 1.0 : 1.3 : 1.0 : 1.3

Chiral Preparative SFC – Waters SFC80Q

Preparative SFC was performed on the Waters 150 Mgm system running under ChromScope software control. The preparative SFC system was equipped with a CO_2 pump, modifier pump with 4-port solvent selection valve, modifier stream injector, automated back pressure regulator (ABPR), UV detector, QDa

mass detector, gas liquid separator (GLS), make-up pump and 6-position fraction collector. Fraction collection was manual and/or time triggered.

Compound S1-6 was separated by chiral preparative supercritical fluid chromatography (SFC) on ChiralArt Cellulose-SC column (30 x 250 mm, 5 µm) at ambient temperature, using a mobile phase of 95:5 CO_2/*i*-PrOH with 0.1% DEA at a flow rate of 145 g/min, and backpressure of 120 bar. The sample was dissolved in 1:1 MeOH:EtOH at a concentration of 18 mg/mL, and the injection volume was 0.6 mL. Fractions were collected based on UV detection at 220 nm and 254 nm with main peaks detected at 3.28, 4.43, 8.32, 15.66 min.

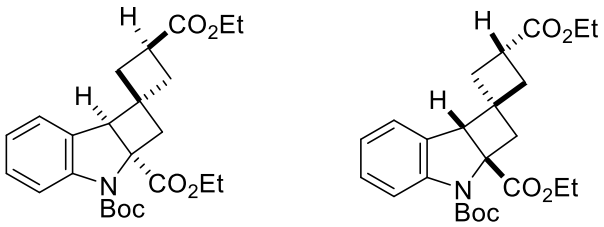


Peak Info[(W2489)2DChannel_1]

Peak #	Ret. Time	Peak Area	Area %	Height	Peak Width	SN Ratio	SelectivityPeak1	SelectivityPeak2
1	3.28 min	942.2458	21.3990	42.3635	1.1000 min	86.2132	1.0000	1.3538
2	4.43 min	1255.4579	28.5122	57.0428	1.1917 min	1065.7228	0.7387	1.0000
3	5.77 min	3.0769	0.0699	0.1883	0.6417 min	1.9550	0.5678	0.7687
4	7.18 min	177.0300	4.0205	4.7853	1.2750 min	49.6804	0.4564	0.6178
5	8.32 min	806.5263	18.3167	18.3757	2.1250 min	214.3906	0.3937	0.5330
6	10.60 min	7.8506	0.1783	0.3536	0.9250 min	1.9164	0.3089	0.4182
7	15.66 min	1211.0383	27.5034	17.4490	2.9083 min	76.7556	0.2091	0.2831

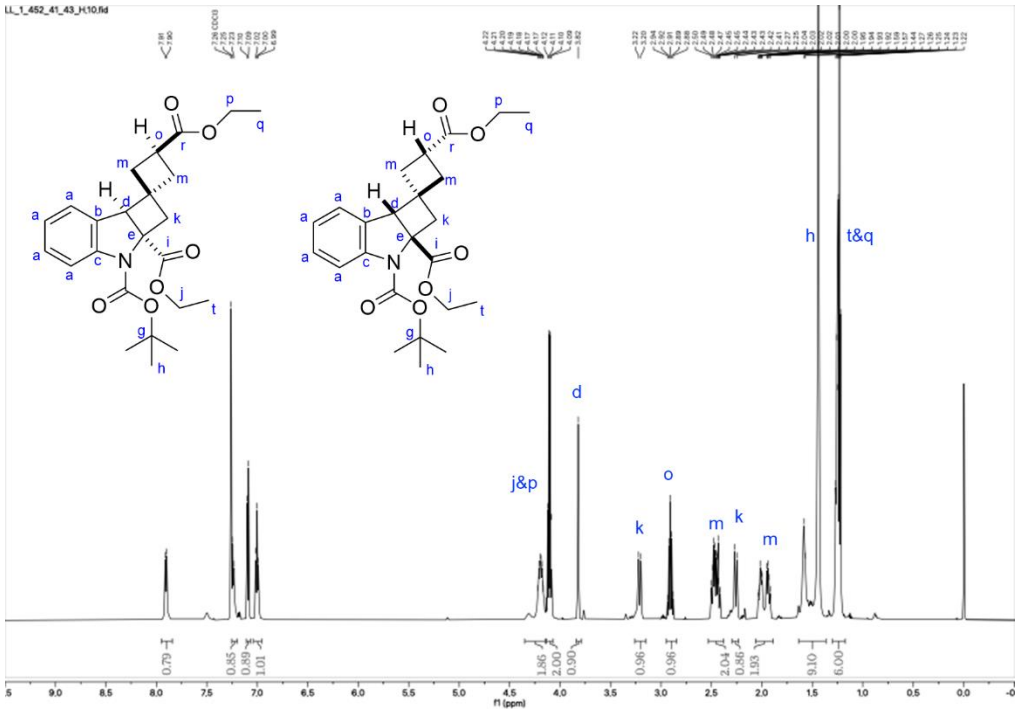
When SiO_2 column chromatography was used for separation, two diastereomers were isolated:

Characterization of Isomers A

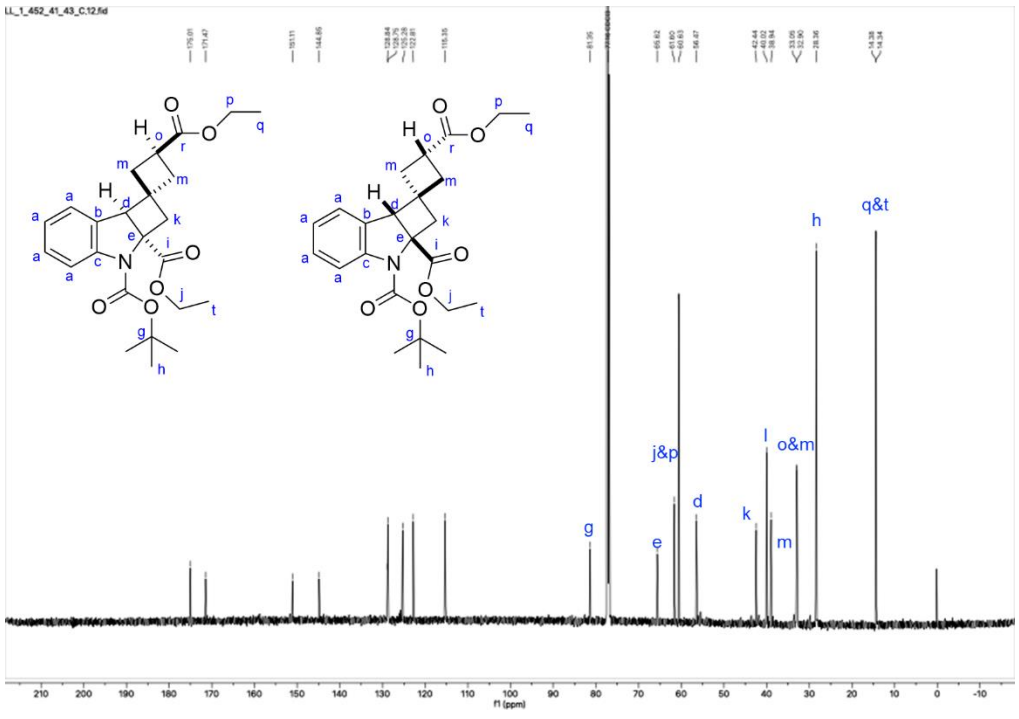


A mixture of enantiomers

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 7.3 Hz, 1H), 7.00 (t, *J* = 7.3 Hz, 1H), 4.19 (dq, *J* = 14.0, 6.7 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 1H), 3.21 (d, *J* = 13.9 Hz, 1H), 2.91 (p, *J* = 8.5 Hz, 1H), 2.53 – 2.38 (m, 2H), 2.26 (d, *J* = 13.7 Hz, 1H), 2.06 – 1.89 (m, 2H), 1.44 (s, 9H), 1.25 (dt, *J* = 16.2, 7.1 Hz, 6H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 175.0, 171.5, 151.1, 144.9, 128.8, 128.8, 125.3, 122.8, 115.4, 81.4, 65.6, 61.6, 60.6, 56.5, 42.4, 40.0, 38.9, 33.1, 32.9, 28.4 (3*C), 14.4, 14.3.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2977, 2932, 1714, 1602, 1480, 1463, 1367, 1238, 1182, 1161, 1107, 1091, 1066, 1033, 917.
HRMS (EI) calcd for $C_{24}H_{31}NO_6Na$ [M+Na]: 452.2049, found 452.2047.
Melting point (°C) 80.6 – 80.9.

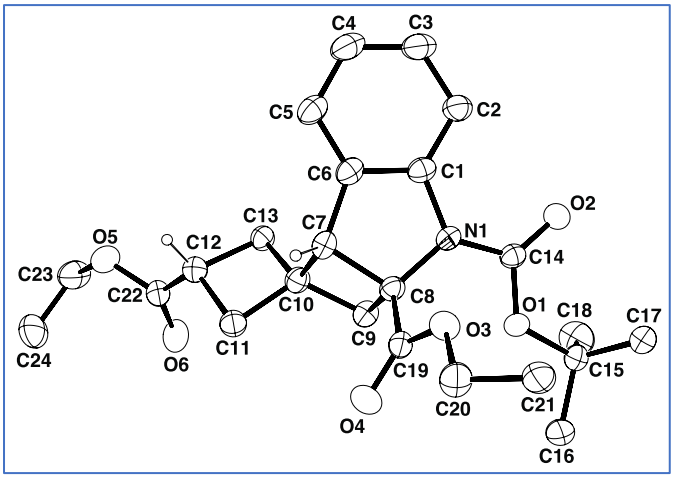


^{1}H NMR (assigned), $CDCl_3$ of **S1-6** (Isomer A)



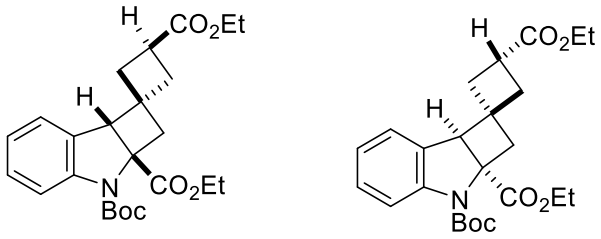
^{13}C NMR (assigned), $CDCl_3$ of **S1-6** (Isomer A)

The structure of **S1-6** isomers A was further confirmed by X-ray crystallography, see details in **Section 9**.



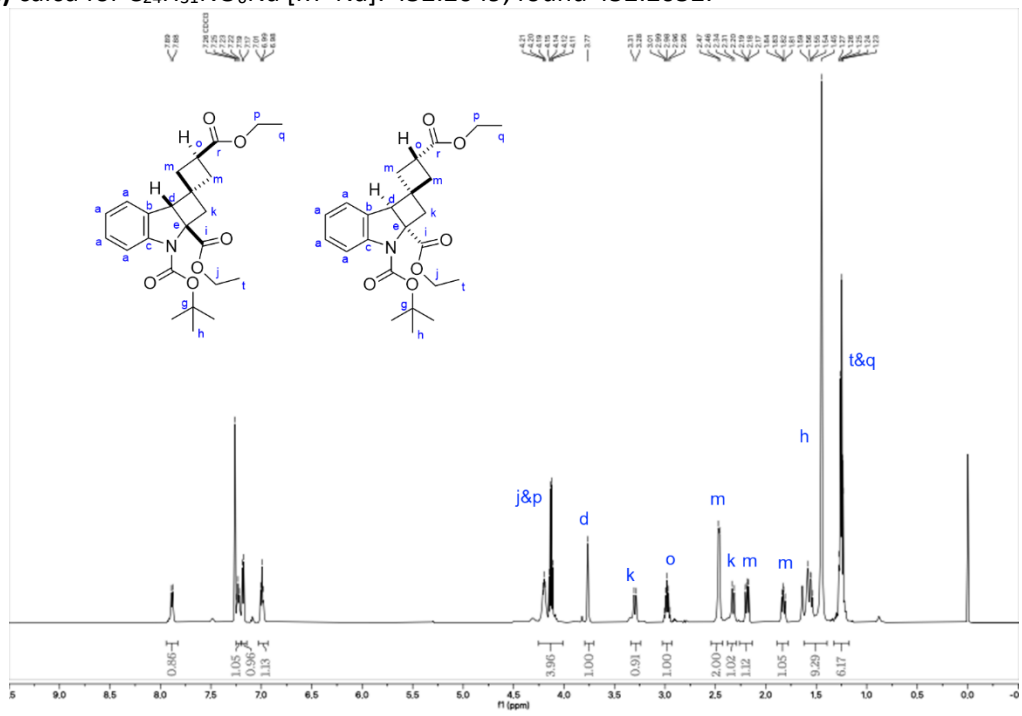
Note: Solvents used for recrystallization: hexanes/DCM.

Characterization of Isomers B

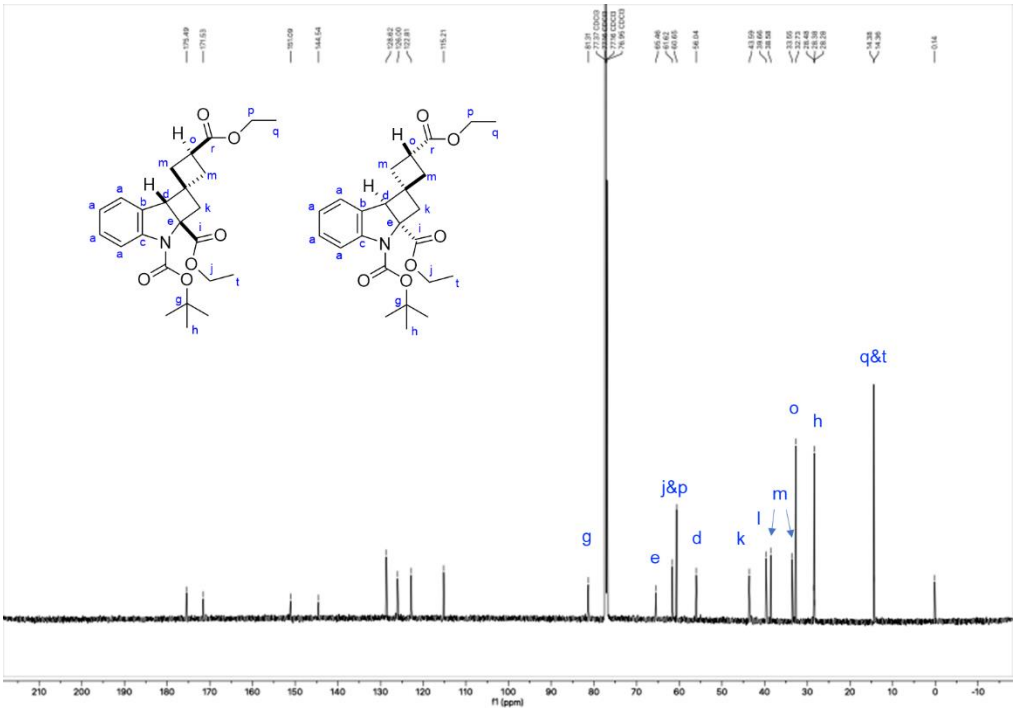


A mixture of enantiomers

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.2 Hz, 1H), 7.23 (t, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 6.99 (t, *J* = 7.3 Hz, 1H), 4.25 – 4.01 (m, 4H), 3.77 (s, 1H), 3.30 (d, *J* = 13.3 Hz, 1H), 2.98 (p, *J* = 8.2 Hz, 1H), 2.46 (d, *J* = 8.1 Hz, 2H), 2.32 (d, *J* = 13.2 Hz, 1H), 2.19 (dd, *J* = 12.2, 7.7 Hz, 1H), 1.83 (dd, *J* = 12.2, 8.7 Hz, 1H), 1.62 – 1.39 (m, 9H), 1.25 (t, *J* = 7.1 Hz, 6H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 175.5, 171.5, 151.1, 144.5, 128.6, 126.0, 122.8, 115.2, 81.3, 65.5, 61.6, 60.7 (2*C), 56.0, 43.6, 39.7, 38.6, 33.6, 32.7, 28.5, 28.4, 28.3, 14.4, 14.4.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2979, 2934, 1716, 1602, 1480, 1463, 1375, 1316, 1237, 1164, 1093, 1066, 852.
HRMS (EI) calcd for $C_{24}H_{31}NO_6Na$ [M+Na]: 452.2049, found 452.2052.



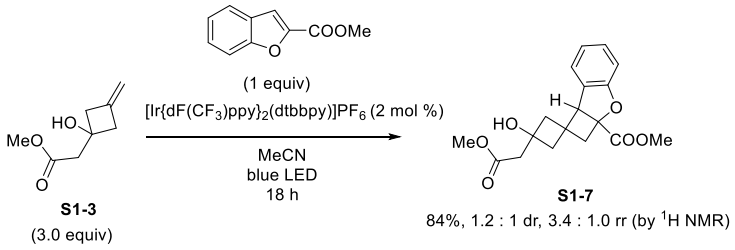
^{1}H NMR (assigned), $CDCl_3$ of **S1-6** (Isomers B)



^{13}C NMR (assigned), $CDCl_3$ of **S1-6** (Isomers B)

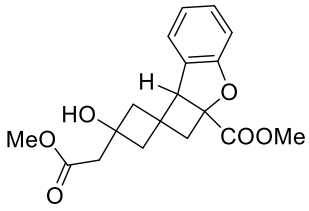
3.2 Off-DNA reaction for benzofuran

Preparation of methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-2'*H*-spiro[cyclobutane-1,1'-cyclobuta[*b*]benzofuran]-2a'(7b'*H*)-carboxylate (S1-7)



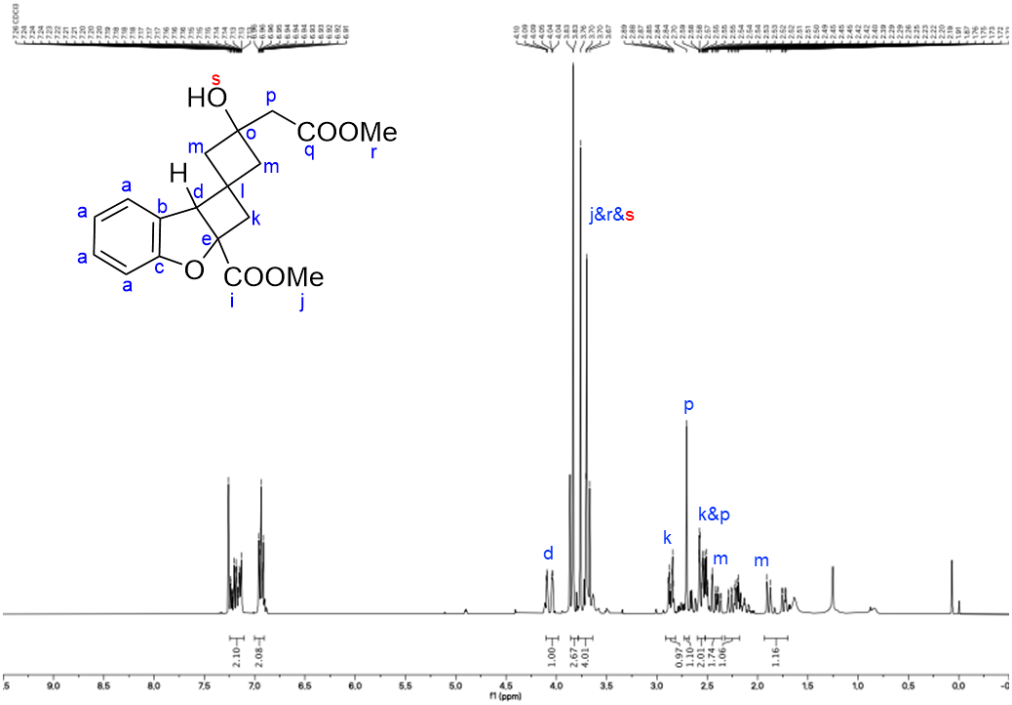
Mixture of isomers

The procedure was adapted from reported literature:[10] To a 4 mL glass vial was added [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$ (2.2 mg, 0.0019 mmol, 1.9 mol%), methyl benzofuran-2-carboxylate (17.6 mg, 0.10 mmol, 1.0 equiv), alkene **S1-3** (46.9 mg, 0.30 mmol, 3.0 equiv), and anhydrous CH_3CN (0.1 M, 1.0 mL). The reaction mixture was then degassed by sparging N_2 for 3 min. The vial was sealed. The stirred mixture was irradiated with ~10 W blue LED (strips, 470 nm) at a distance of 1 inch with a cooling fan to ensure reactions remained at or near room temperature for 18 h. The reaction mixture was concentrated, and the residue was purified by SiO_2 column chromatography (20% EtOAc/hexanes) to give the product (a mixture of diastereomers) as a clear oil (28.0 mg, 0.084 mmol, 84% yield).

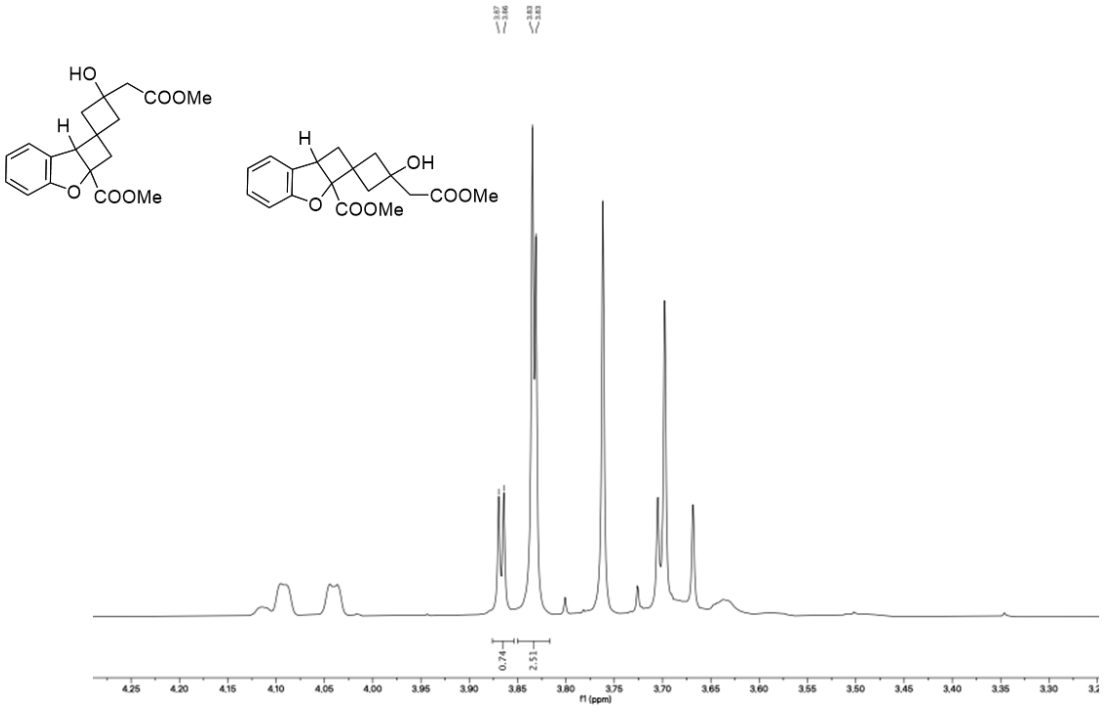


Mixture of diastereomers

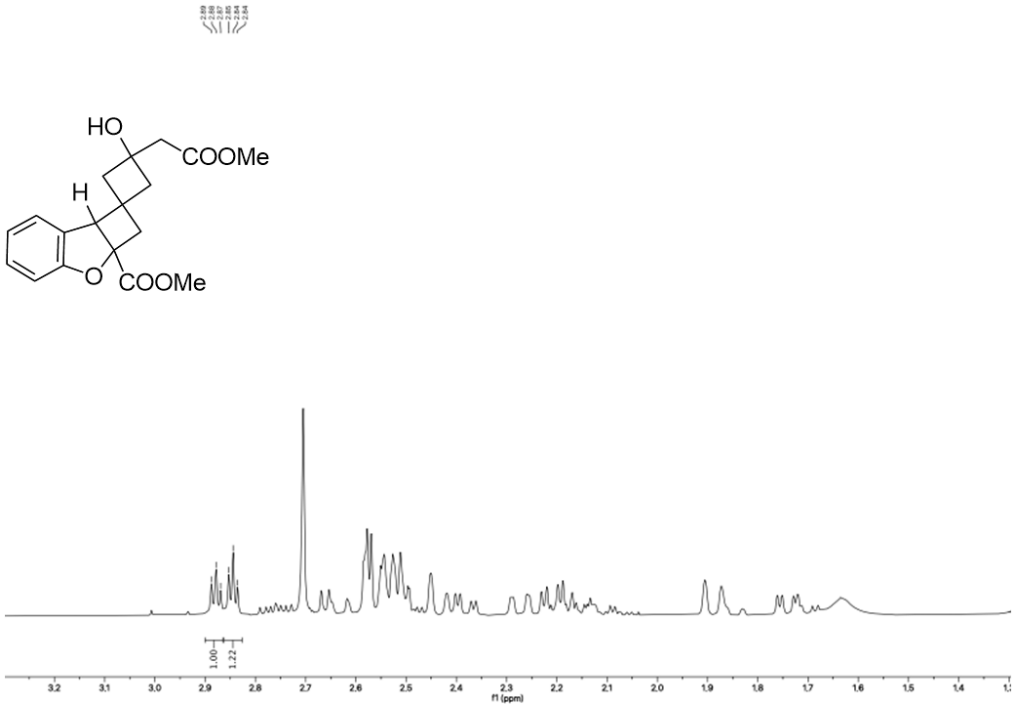
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.10 (m, 2H), 7.00 – 6.90 (m, 2H), 4.10 – 3.98 (m, 1H), 3.86 – 3.79 (m, 3H), 3.78 – 3.64 (m, 4H), 2.92 – 2.82 (m, 1H), 2.70 (s, 1H), 2.60 – 2.52 (m, 2H), 2.52 – 2.35 (m, 2H), 2.32 – 2.18 (m, 1H), 1.93 – 1.70 (m, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 172.9, 171.5, 171.4, 160.3, 160.2, 129.3, 129.1, 126.7, 126.6, 126.6, 126.2, 121.5, 121.5, 111.4, 111.0, 84.9, 84.7, 69.2, 68.8, 57.6, 57.2, 52.8 (2*C), 52.1, 52.0, 48.7, 48.5, 45.5, 45.3, 44.6, 44.5, 43.5, 42.7, 35.4, 35.0.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3511, 2954, 2927, 1732, 1476, 1460, 1437, 1329, 1284, 1231, 1203, 1171, 1122, 1090, 1059, 1017, 977.
HRMS (ESI) calcd for $C_{18}H_{20}O_6Na$ $[M+Na]^+$: 355.1158, found 355.1153.



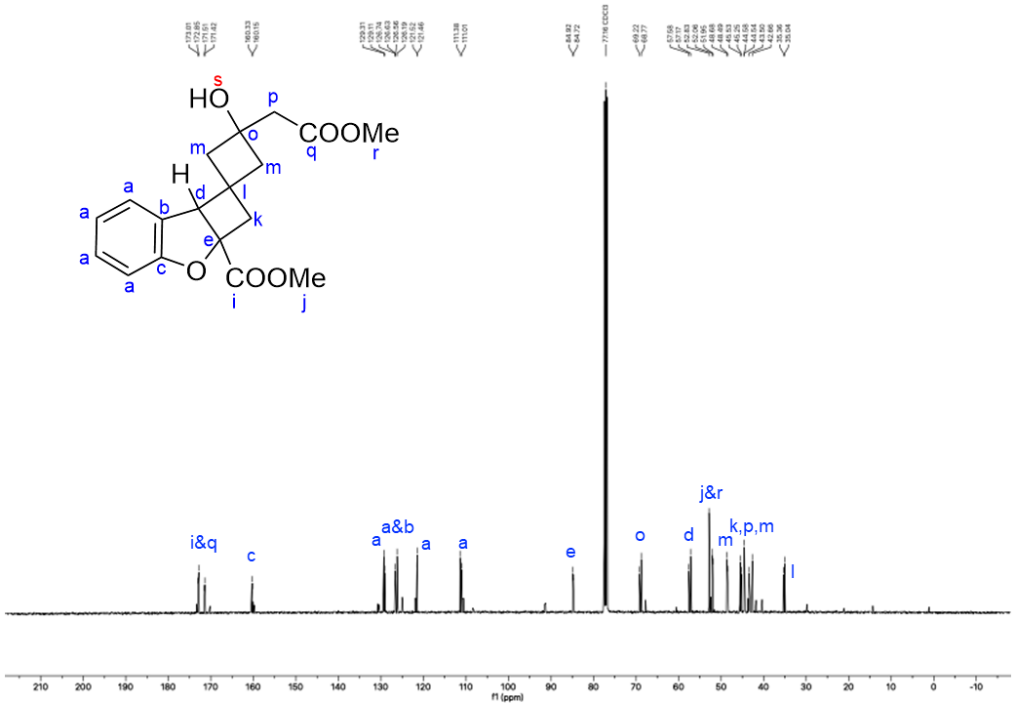
^{1}H NMR (assigned), $CDCl_3$ of **S1-7**



^{1}H NMR, $CDCl_3$ of **S1-7** for determination of regioisomeric ratio (peaks at 3.85ppm, rr = 3.4 : 1.0)



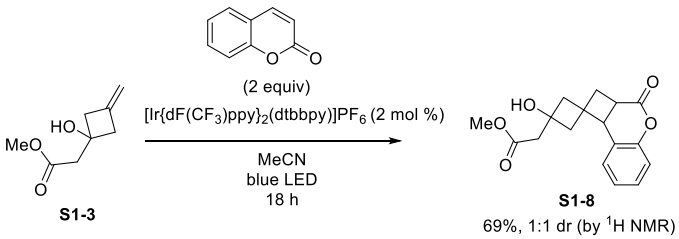
^{1}H NMR, $CDCl_3$ of **S1-7** for determination of diastereomeric ratio (peaks at 2.85 ppm, dr = 1.2 : 1.0)



^{13}C NMR (assigned), $CDCl_3$ of **S1-7**

3.3 Off-DNA reaction for coumarins

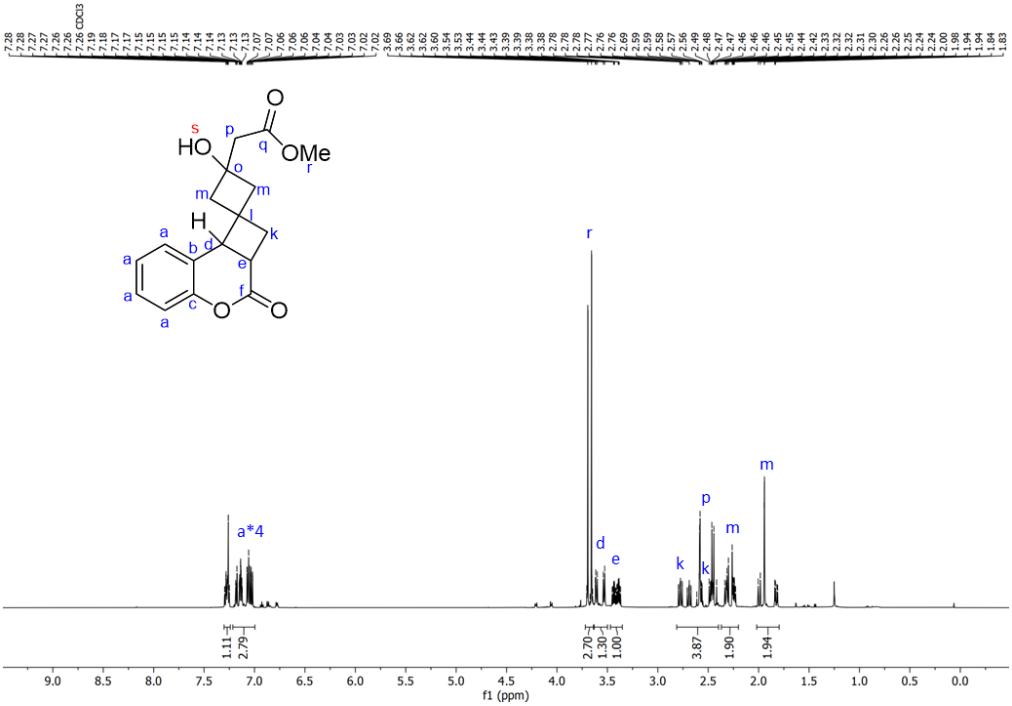
Preparation of methyl 2-((2a'*R*,8b'*R*)-3-hydroxy-3'-oxo-2a',8b'-dihydro-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromen]-3-yl)acetate (S1-8)



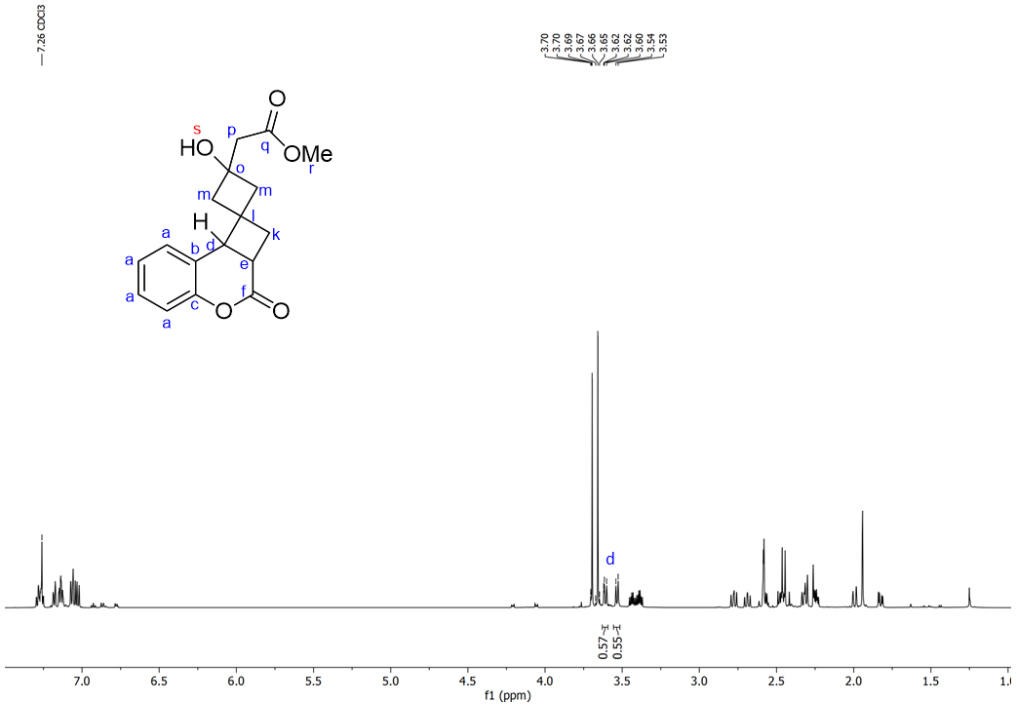
Mixture of diastereoisomers (dr = 1.0 : 1.0)

The procedure was adapted from reported literature:[10] To a 4 mL glass vial was added $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (3.6 mg, 0.0030 mmol, 1.9 mol %), 2*H*-chromen-2-one (47 mg, 0.32 mmol, 2.0 equiv), alkene **S1-3** (25 mg, 0.16 mmol, 1.0 equiv), and anhydrous CH_3CN (0.1 M, 1.6 mL). The reaction mixture was then degassed by sparging N_2 for 3 min. The vial was sealed. The stirred mixture was irradiated with ~10 W blue LED (strips, 470 nm) at a distance of 1 inch with a cooling fan to ensure reactions remained at or near room temperature for 18 h. The reaction mixture was concentrated, and the residue was purified by SiO_2 column chromatography (20% EtOAc/hexanes) to give the product **S1-8** (a mixture of diastereomers) as a yellow oil (34 mg, 0.11 mmol, 69% yield).

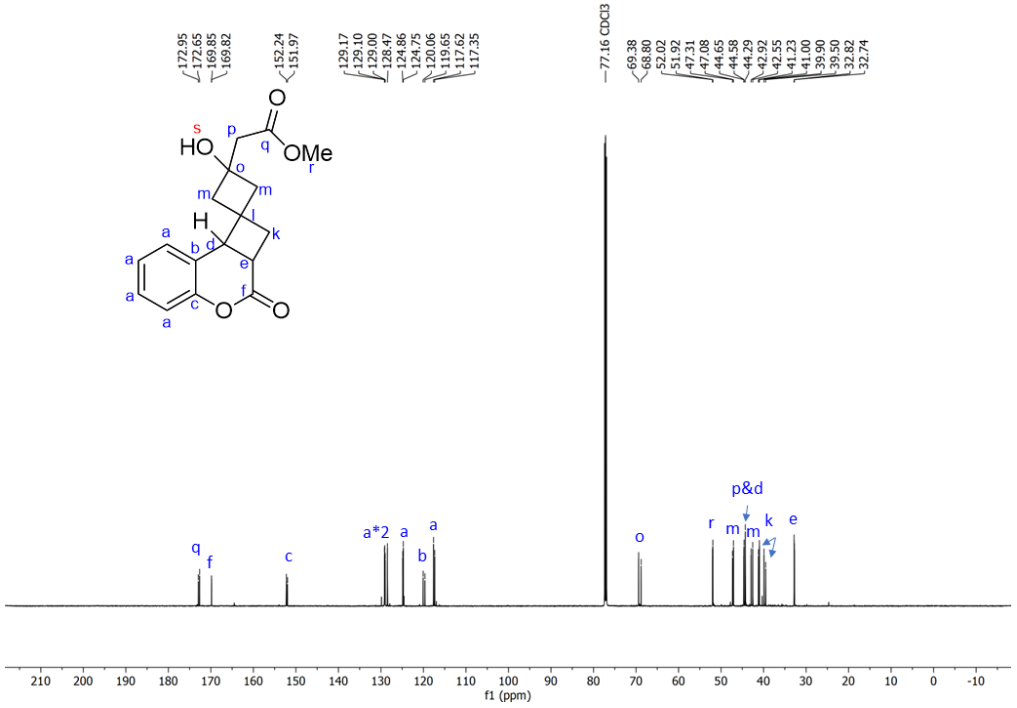
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 1H), 7.22 – 7.00 (m, 3H), 3.72 – 3.64 (m, 3H), 3.63 – 3.50 (m, 1H), 3.47 – 3.35 (m, 1H), 2.81 – 2.40 (m, 4H), 2.37 – 2.20 (m, 2H), 2.02 – 1.80 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0, 172.7, 169.9, 169.8, 152.2, 152.0, 129.2, 129.1, 129.0, 128.5, 124.9, 124.8, 120.1, 119.7, 117.6, 117.4, 69.4, 68.8, 52.0, 51.9, 47.3, 47.1, 44.7, 44.6, 44.3 (2*C), 42.9, 42.6, 41.2, 41.0, 39.9, 39.5, 32.8, 32.7.
FT-IR (cm^{-1}, neat, ATR), ṽ =3483, 2954, 2924, 1753, 1733, 1586, 1489, 1455, 1438, 1334, 1223, 1205, 1168, 1112, 959.
HRMS (ESI) calcd for $C_{17}H_{18}NaO_5$ [M+Na]: 325.1052, found 325.1025.



^{1}H NMR (assigned), $CDCl_3$ of **S1-8**

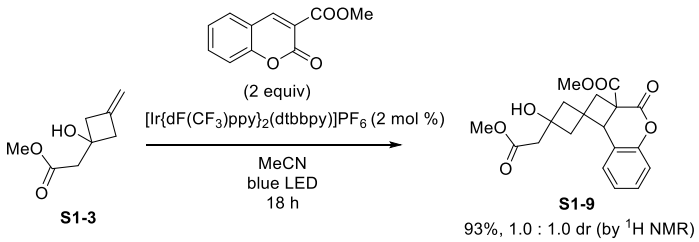


^{1}H NMR, $CDCl_3$ of **S1-8** for determination of diastereomeric ratio (peaks at 3.6 ppm, dr = 1.0 : 1.0)



^{13}C NMR (assigned), $CDCl_3$ of **S1-8**

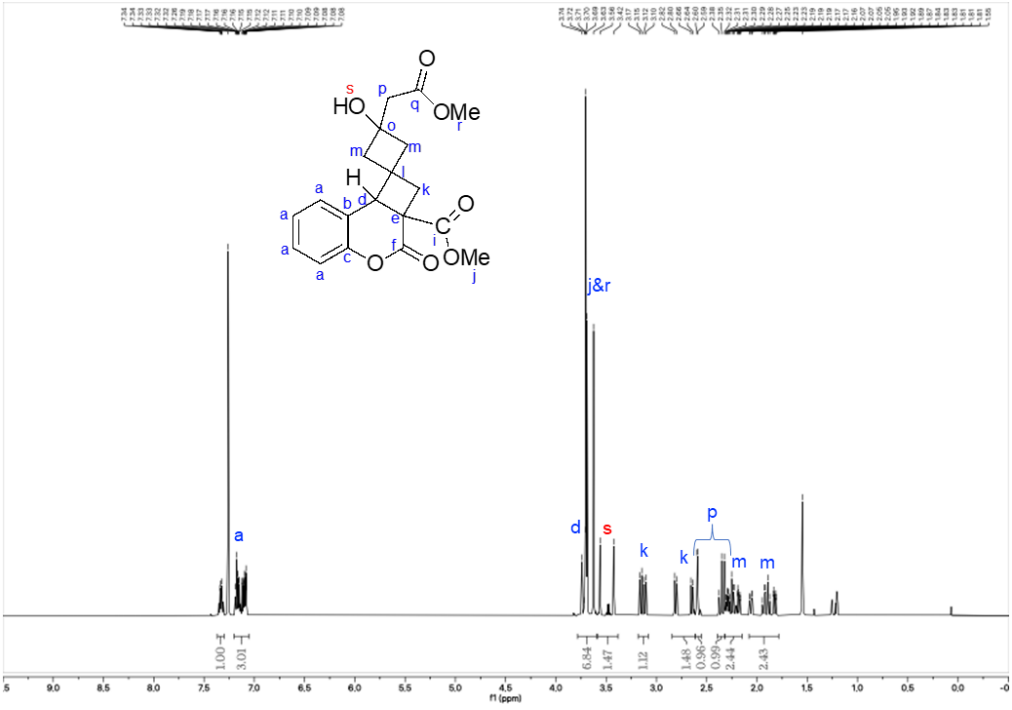
Preparation of methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-3'-oxo-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromene]-2a'(8b'*H*)-carboxylate (S1-9)



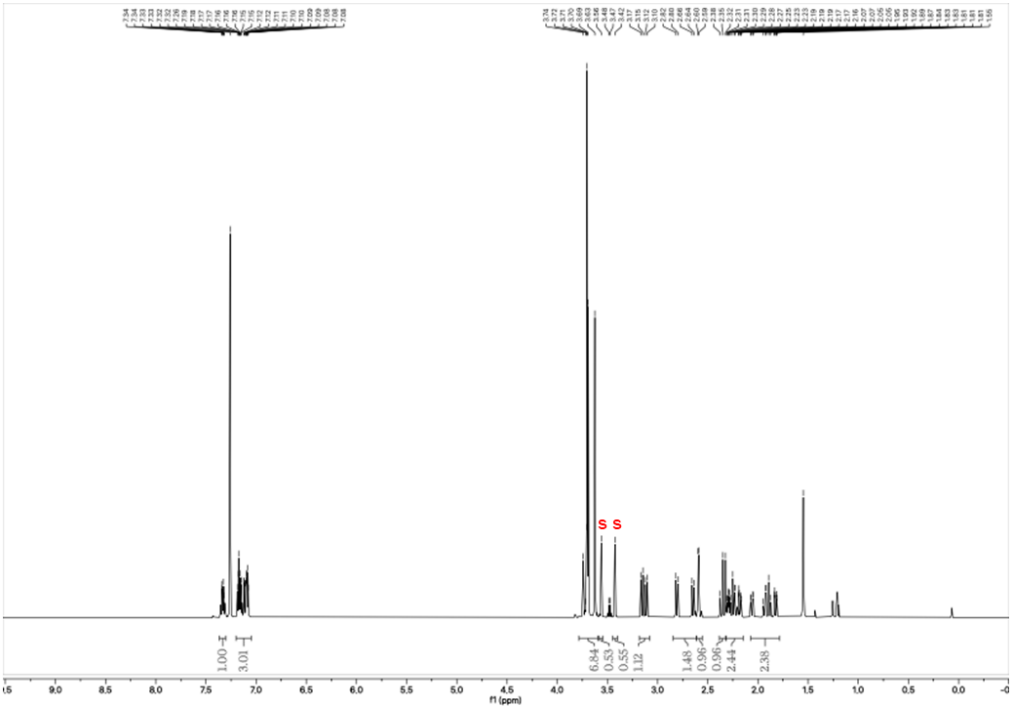
Mixture of diastereoisomers (dr = 1.0 : 1.0)

The procedure was adapted from reported literature:[10] To a 4 mL glass vial was addded $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (2.9 mg, 0.0025 mmol, 1.9 mol%), methyl 2-oxo-2H-chromene-3-carboxylate (52 mg, 0.26 mmol, 2.0 equiv), alkene **S1-3** (20 mg, 0.13 mmol, 1.0 equiv), and anhydrous CH_3CN (0.1 M, 1.3 mL). The reaction mixture was then degassed by sparging N_2 for 3 min. The vial was sealed. The stirred mixture was irradiated with ~10 W blue LED (strips, 470 nm) at a distance of 1 inch with a cooling fan to ensure reactions remained at or near room temperature for 18 h. The reaction mixture was concentrated, and the residue was purified by SiO_2 column chromatography (40% EtOAc/hexanes) to give the product **S1-9** (a mixture of diastereomers) as a clear oil (43 mg, 0.12 mmol, 93% yield).

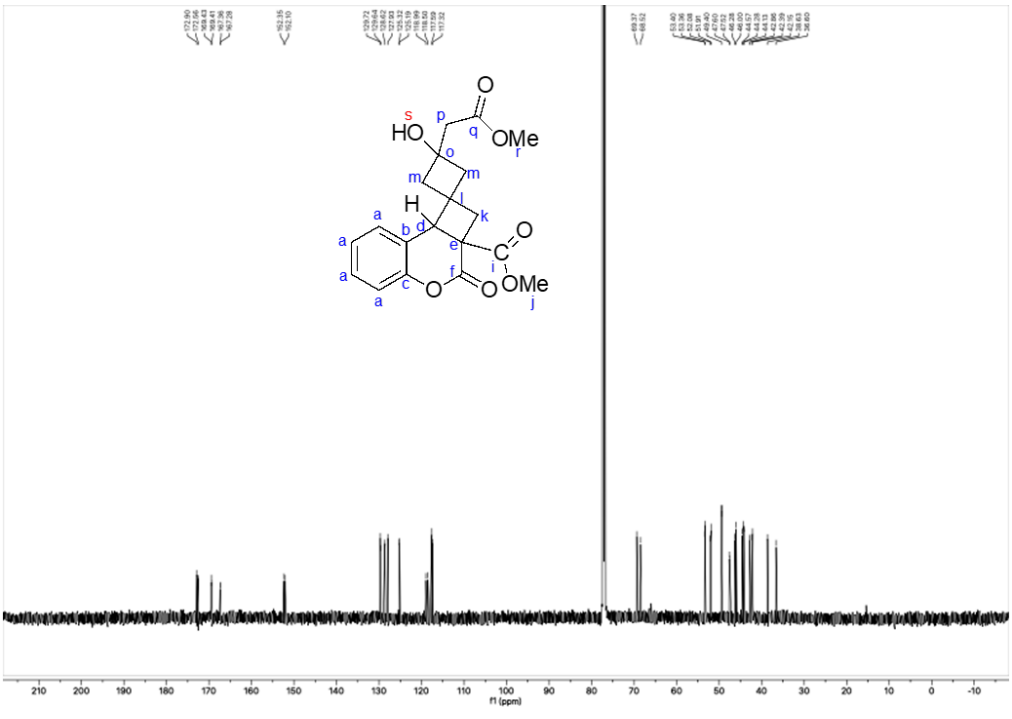
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 1H), 7.20 – 7.05 (m, 3H), 3.78 – 3.60 (m, 7H), 3.59 – 3.38 (m, 1H), 3.13 (dd, *J* = 25.6, 12.0 Hz, 1H), 2.84 – 2.61 (m, 1H), 2.59 (d, *J* = 3.6 Hz, 1H), 2.39 – 2.32 (m, 1H), 2.32 – 2.15 (m, 2H), 2.08 – 1.78 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 172.9, 172.6, 169.4, 169.4, 167.4, 167.3, 152.4, 152.1, 129.7, 129.6, 128.6, 127.9, 125.3, 125.2, 119.0, 118.5, 117.6, 117.3, 69.4, 68.5, 53.4, 53.4, 52.1, 51.9, 49.4 (2*C), 47.6, 47.5, 46.3, 46.0, 44.6, 44.3, 44.1, 42.9, 42.4, 42.2, 38.6, 36.6.
FT-IR (cm^{-1}, neat, ATR), ṽ = 3509, 2955, 1758, 1733, 1587, 1490, 1458, 1436, 1352, 1283, 1247, 1204, 1168, 1117, 1097, 1002, 967.
HRMS (ESI) calcd for $C_{19}H_{20}O_7Na$ [M+Na]: 383.1107, found 383.1114.



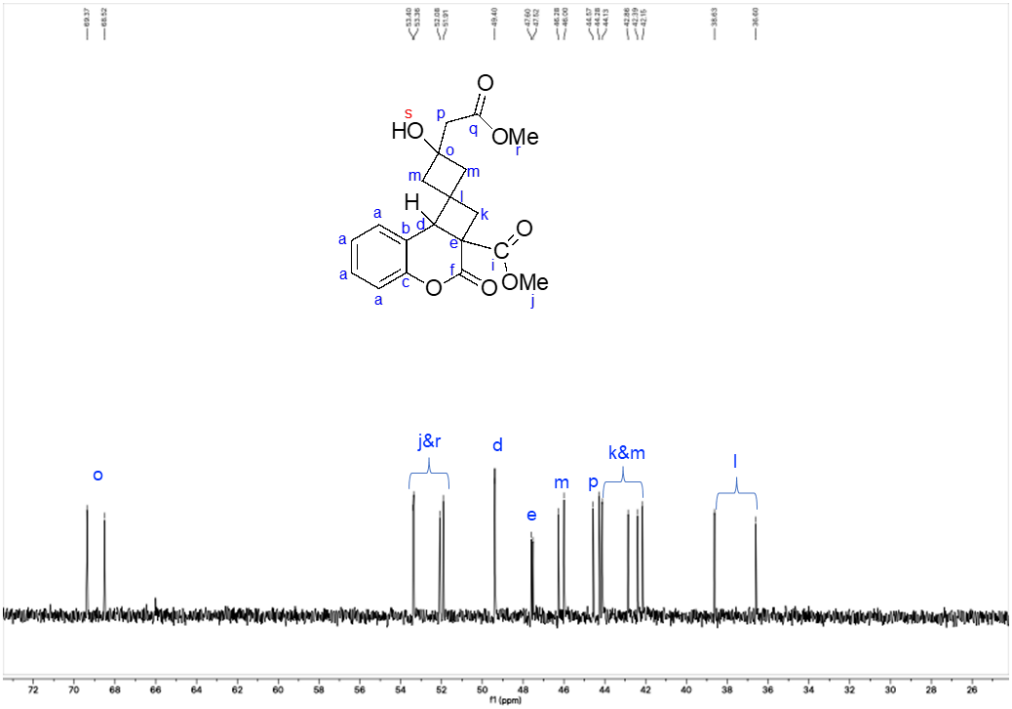
^{1}H NMR (assigned), $CDCl_3$ of **S1-9**



^{1}H NMR, $CDCl_3$ of **S1-9** for determination of diastereomeric ratio (peaks at 3.6 ppm, dr = 1.0 : 1.0)



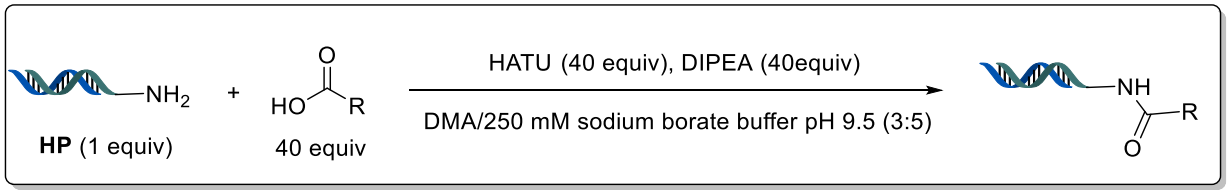
^{13}C NMR (full), $CDCl_3$ of **S1-9**



^{13}C NMR (25-73ppm, assigned), $CDCl_3$ of **S1-9**

4 Preparation of on-DNA Substrates

4.1 General Procedure B: Preparation of on-DNA Substrates



4.1.1 HATU premix protocol for acylation of DNA headpieces: The HATU (200 mM in DMA, 40.0 equiv), DIPEA (200 mM in DMA, 40.0 equiv), and the corresponding carboxylic acid (200 mM in DMA, 40.0 equiv) solutions were individually cooled at 4 °C for 5 min. Once chilled, the acid, DIPEA, and HATU solutions were mixed sequentially, vortexed briefly, and allowed to react at 4 °C for 20 min. The chilled mixture was then added to the DNA-NH_2 solution [1 mM in 250 mM pH 9.4 sodium borate buffer (diluted from commercially available sodium borate buffer 500 mM pH 9.5)], and the mixture was vortexed. The reaction was allowed to proceed at rt. After 20 min, the reaction was worked up following the EtOH precipitation protocol below.

4.1.2 EtOH precipitation protocol: A volume of 5 M aq NaCl equal to 1/10 of the reaction volume was then added, followed by cold (–20 °C) EtOH equal to 2.5 reaction volumes. The resulting mixture was vortexed and left to stand in a –20 °C freezer overnight. The chilled mixture was then centrifuged for 30 min at 4 °C at 4,000 rpm. The supernatant was then decanted, and the remaining sediment was allowed to dry under reduced pressure. The resulting pellet was re-dissolved in H_2O to give a theoretical concentration of 2 mM.

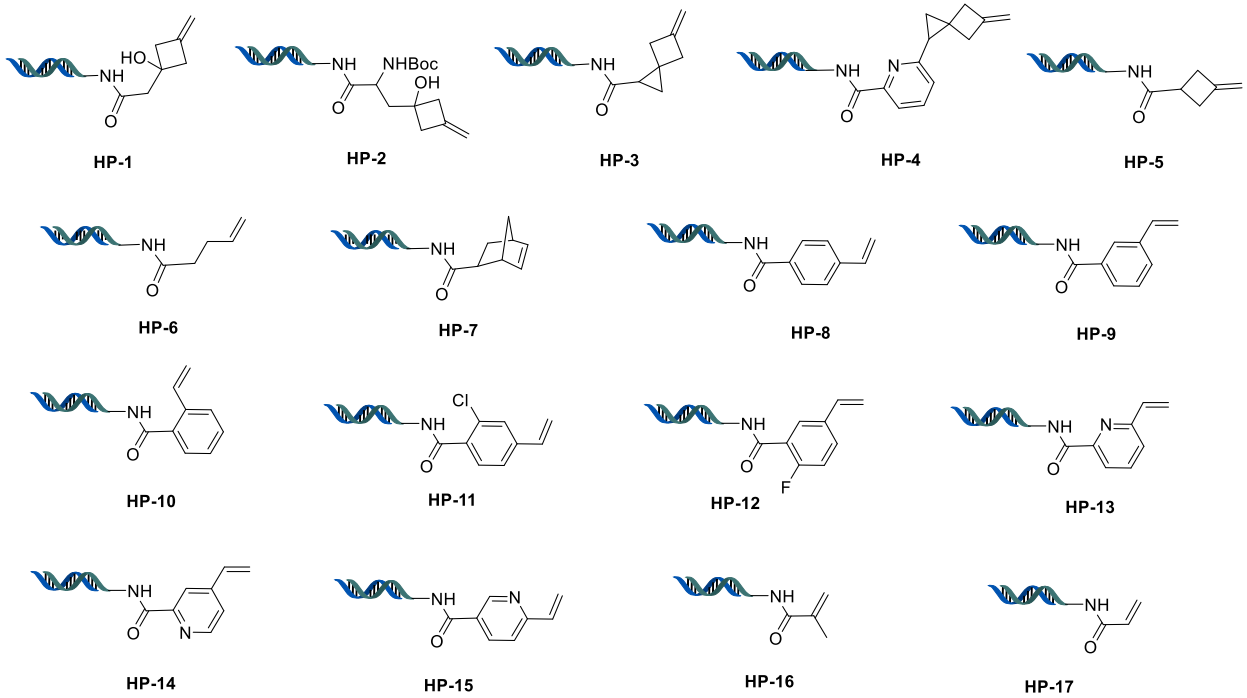


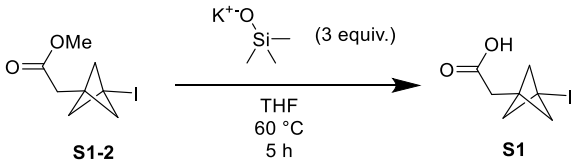
Figure S3. List of alkene-headpieces prepared in this study.

HP-1 to **HP-17** were prepared according to a described **general procedure B** starting from the corresponding carboxylic acid. The preparation and spectra of DNA-headpieces **HP-11, HP-13**, and **HP-14** were reported.[9]

4.2 Synthesis of small molecules carboxylic acid precursors for HP-1 – HP-4

4.2.1 Preparation of DNA headpiece HP-1

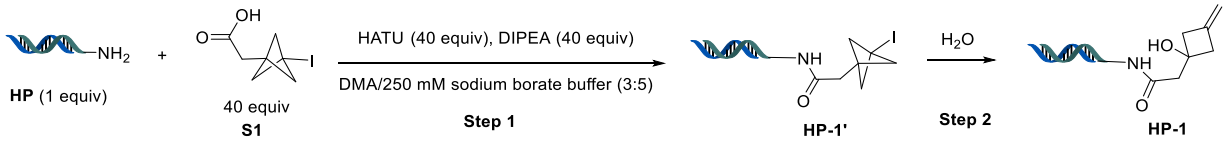
Preparation of 2-(3-Iodobicyclo[1.1.1]pentan-1-yl)acetic acid S1



Methyl 2-(3-Iodobicyclo[1.1.1]pentan-1-yl)acetate **S1** was prepared following reported procedures with modification:[12] To a solution of methyl 2-(3-Iodobicyclo[1.1.1]pentan-1-yl)acetate **S1-2** (130 mg, 0.49 mmol, **S1-2** was prepared following reported procedures[8,9]) in dry THF (6 mL) was added a solution of potassium trimethylsilanolate (188.5 mg, 1.47 mmol, 3.0 equiv) in 10 mL of dry THF while stirring at room temperature. The reaction was then heated to 60 °C under a N_2 atmosphere, avoiding ambiant light by covering the reaction using aluminum foil. The reaction was monitored by TLC. After 5 h, the reaction was cooled to room temperature. To the reaction mixture was added ~0.4 N HCl MeOH THF solution to a pH~6 (Preparation of the HCl methanol THF: commercially available HCl·MeOH 1.25 M solution was diluted with THF to ~0.4 N). The mixture was filtered to remove insoluble salts. The filtrate was concentrated *in vacuo*. Anhydrous Et_2O (20 mL) was added to the flask containing the residue. The heterogeneous solution was vigorously for 10 min. The resulting Et_2O solution was then filtered. The filtrate was concentrated and dried under vacuum to yield desired product **S1** as a pale-yellow solid (51.2 mg, 0.203 mmol, 41%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.60 (s, 1H), 2.35 (s, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 175.2, 61.0 (3*C), 43.3, 37.0, 5.9.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 2994, 2912, 2874, 1691, 1428, 1305, 1287, 1263, 1245, 1222, 1176, 1131, 1096, 1039, 973, 956, 911, 847, 801, 663.
HRMS (ESI) calcd for $C_7H_8IO_2$ [M-H]: 250.9569, found 250.9571.
Melting point (°C) 61.6 – 64.0.

Preparation of HP-1

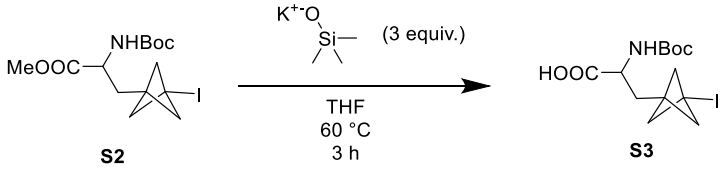


Step 1: Preparation of headpiece **HP-1'** followed **General Procedure B**.

Step 2: the water solution of the **HP-1'** was left in a refrigerator at ~8 °C to allow slow solvolysis. The completion was monitored by LCMS. It took about two days for the **HP-1'** to completely convert to the *exo*-methylene cyclobutanol **HP-1**.

4.2.2 Preparation of HP-2

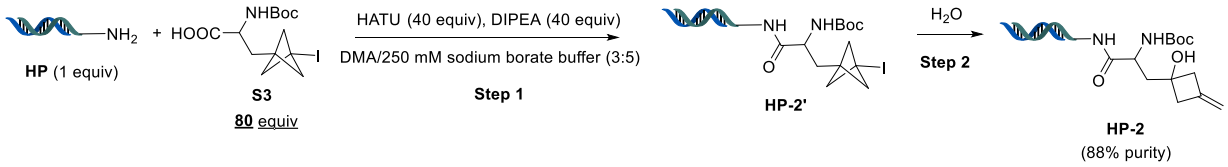
Preparation of 2-((*tert*-butoxycarbonyl)amino)-3-(3-iodobicyclo[1.1.1]pentan-1-yl)propanoic acid



Methyl 2-((*tert*-butoxycarbonyl)amino)-3-(3-iodobicyclo[1.1.1]pentan-1-yl)propanoate **S3** was prepared following reported procedure with modification:[12] To a solution of Methyl 2-((*tert*-butoxycarbonyl)amino)-3-(3-iodobicyclo[1.1.1]pentan-1-yl)propanoate (50 mg, 0.13 mmol, **S2** was prepared following reported procedure[8,9]) in dry THF (2 mL) was added a solution of potassium trimethylsilanolate (49 mg, 0.38 mmol, 3.0 equiv) in 2 mL of dry THF while stirring at room temperature. The reaction was then heated to 60 °C under a N_2 atmosphere, avoiding ambiant light by covering the reaction using aluminum foil. The reaction was monitored by TLC. After 3 h, the reaction was cooled to room temperature. To the reaction mixture was added ~0.4 N HCl MeOH THF solution to a pH~6 (Preparation of the HCl methanol THF: commercially available HCl·MeOH 1.25 M solution was diluted with THF to ~0.4 N). The mixture was filtered to remove insoluble salts. The filtrate was concentrated *in vacuo*, Anhydrous Et_2O (10 mL) was added to the flask containing the residue. The heterogeneous solution was vigorously for 10 min. The resulting Et_2O solution was then filtered. The filtrate was concentrated and dried under vacuum to yield desired product which was not pure as a pale-yellow oil (18 mg, 0.13 mmol, 37%, impure).

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.98 (d, *J* = 6.6 Hz, 1H), 4.34 – 4.17 (m, 1H), 2.27 (q, *J* = 9.8 Hz, 6H), 2.21 – 2.12 (m, 1H), 1.96 – 1.89 (m, 1H), 1.45 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 176.0, 155.5, 80.7, 61.1 (3*C), 52.1, 45.6, 34.2, 28.5 (3*C), 6.3.
FT-IR (cm^{-1}, neat, ATR), $\tilde{\nu}$ = 3375, 2929, 1738, 1722, 1660, 1519, 1436, 1403, 1364, 1250, 1201, 1180, 1160, 1101, 1064, 1032, 835, 777, 750, 579, 550.
HRMS (ESI) calcd for $C_{13}H_{19}INO_4$ [M-H]: 380.0359, found 380.0356.

Preparation of HP-2

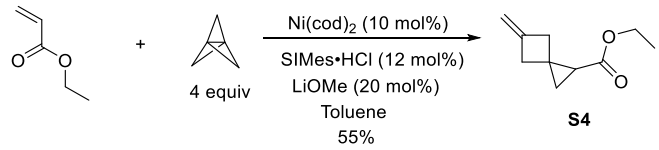


Step 1: Preparation of headpiece **HP-2'** followed **General Procedure B** with 80 equiv (400 mM in DMA) of acid instead of 40 equiv of acid because the acid used was not pure.

Step 2: The water solution of the **HP-2'** was left in a refrigerator at ~8 ^{o}C to allow slow solvolysis. The completion was monitored by LCMS. It took about 1 day for the **HP-2'** to completely convert to **HP-2** with 88% purity by LCMS.

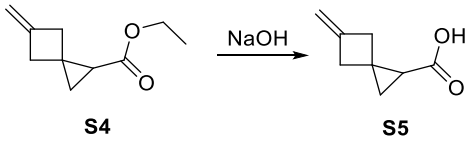
4.2.3 Preparation of HP-3

Preparation of 5-methylenespiro[2.3]hexane-1-carboxylic acid



Preparation of ethyl 5-methylenespiro[2.3]hexane-1-carboxylate **S4** was adapted from reported literature:[13] To a pressure tube was added $Ni(cod)_2$ (27.5 mg, 0.010 mmol, 10 mol%), SIMes·HCl (40.9 mg, 0.012 mmol, 12 mol%,) and LiOMe (7.6 mg, 0.020, 20 mol%,). The reaction vial was evacuated and back-filled with nitrogen three times. Toluene (5 mL) was added to the vial. The mixture was stirred for 15 min at RT. A solution of ethyl acrylate (100 mg, 1.00 mmol, 1.0 equiv) and [1.1.1]propellane (0.8 M in Et_2O, 4.0 mmol) in 10 mL of toluene was then added to the mixture. The reaction mixture was heated to 50 °C and stirred for 20 h. After cooling to rt, the reaction mixture was concentrated and purified by SiO_2 column chromatography (100% hexanes). The product **S4** was obtained as a clear oil (92 mg, 0.55 mmol, 55%).

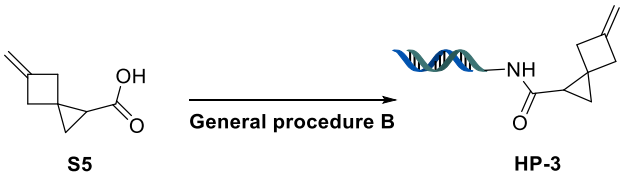
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.91 – 4.83 (m, 2H), 4.21 – 4.08 (m, 2H), 2.96 – 2.73 (m, 4H), 1.68 (dd, *J* = 8.5, 5.4 Hz, 1H), 1.31 – 1.22 (m, 4H), 1.10 (dd, *J* = 8.5, 4.7 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 172.9, 144.0, 106.8, 60.4, 39.8, 37.7, 26.1, 24.9, 20.4, 14.6.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2983, 2911, 1721, 1680, 1445, 1400, 1383, 1315, 1264, 1232, 1175, 1147, 1080, 1032, 967, 963, 877, 823, 767, 514.
HRMS (ESI) calcd for $C_{10}H_{15}O_2$ [M+H]: 167.1072, found 167.1098.



Preparation of **S5** was adapted from reported literature:[14] To a solution of **S4** (30 mg, 0.18 mmol, 1.0 equiv) in a mixture of THF (1 mL) and MeOH (1 mL) was added a solution of NaOH (0.14 g, 3.5 mmol, 20 equiv) in water (1 mL) dropwise. After 3 h, acidification with 2 M aq. HCl to pH= ~4.0. The reaction mixture was diluted with H_2O and extracted with DCM three times. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford product **S5** as white solid (24mg, 0.17 mmol, 96% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.95 – 4.76 (m, 2H), 3.06 – 2.73 (m, 4H), 1.71 (dd, *J* = 8.4, 5.3 Hz, 1H), 1.31 (t, *J* = 5.1 Hz, 1H), 1.19 (dd, *J* = 8.4, 4.8 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 178.5, 143.4, 107.0, 39.8, 37.7, 27.3, 24.6, 21.3.
FT-IR (cm^{-1}, neat, ATR), ṽ = 2947, 2912, 1675, 1455, 1425, 1403, 1313, 1243, 1218, 1191, 1167, 1151, 1033, 948, 914, 891, 857, 843, 823, 669.
HRMS (ESI) calcd for $C_8H_{11}O_2$ [M+H]: 139.0759, found 139.0772.
Melting point (°C) 62.6 – 63.7.

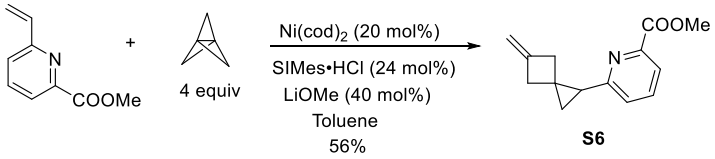
Preparation of HP-3



Preparation of **HP-3** followed **General Procedure B**.

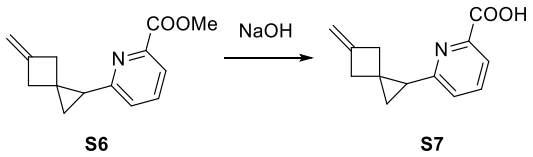
4.2.4 Preparation of HP-4

Preparation of methyl 6-(5-methylenespiro[2.3]hexan-1-yl)picolinate S6



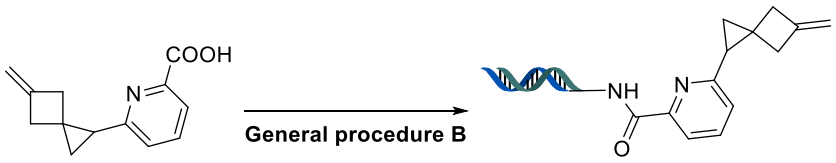
Preparation of **S6** was adapted from reported literature:[13] To a pressure tube was added $Ni(cod)_2$ (33 mg, 0.12 mmol, 20 mol%,), SIMes·HCl (49 mg, 0.14 mmol, 24 mol%) and LiOMe (9.1 mg, 0.24 mmol, 40 mol%). The reaction vial was evacuated and backfilled with nitrogen three times. Toluene (3 mL) was added to the vial. The mixture was stirred for 15 min at RT. A solution of methyl 6-vinylpicolinate (0.60 mmol) and [1.1.1]propellane (0.8 M in Et_2O, 3.4 mmol) in 6 mL of toluene was then added to the mixture. The reaction mixture was heated to 50 °C and stirred for 20 h. After cooling to RT, the reaction mixture was concentrated and purified by column chromatography (20% EtOAc/hexanes)to give product **S6** as a clear oil (77 mg, 56%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 6.96 (dd, *J* = 7.9, 1.0 Hz, 1H), 4.88 – 4.73 (m, 2H), 3.98 (s, 3H), 3.07 – 2.95 (m, 1H), 2.94 – 2.77 (m, 2H), 2.65 – 2.52 (m, 1H), 2.35 (dd, *J* = 8.4, 6.2 Hz, 1H), 1.36 – 1.20 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 166.3, 161.8, 147.5, 144.3, 136.7, 123.5, 122.0, 106.6, 52.9, 40.6, 36.6, 29.6, 26.6, 21.2.
FT-IR (cm^{-1}, neat, ATR), ṽ = 3072, 2950, 2905, 1742, 1720, 1678, 1589, 1459, 1436, 1318, 1291, 1246, 1204, 1192, 1163, 1136, 1092, 993, 876, 829, 780, 762, 741.
HRMS (EI) calcd for $C_{14}H_{16}NO_2$ [M+H]: 230.1181, found 230.1177.



Preparation of 6-(5-methylenespiro[2.3]hexan-1-yl)picolinic acid **S7** was adapted from reported literature:[14] To a solution of **S6** (40 mg, 0.17 mmol, 1.0 equiv) in a mixture of THF (1 mL) and MeOH (1 mL) was added a solution of NaOH (0.14 g, 3.5 mmol, 20 equiv) in water (1 mL) dropwise. After 5 h, acidification with 2 M aq. HCl to pH= ~4.0. The mixture was diluted with H_2O and extracted with DCM three time. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afforded **S7** as yellow solid (37 mg, 0.17 mmol, >99% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.39 (dd, *J* = 7.9, 1.0 Hz, 1H), 4.89 – 4.78 (m, 2H), 3.04 – 2.96 (m, 1H), 2.95 – 2.86 (m, 2H), 2.69 – 2.61 (m, 1H), 2.22 (dd, *J* = 8.1, 6.1 Hz, 1H), 1.42 – 1.35 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 164.28, 160.73, 145.17, 143.60, 138.26, 126.99, 120.14, 107.11, 40.61, 36.68, 28.50, 27.63, 21.99.
FT-IR (cm^{-1}, neat, ATR), ṽ = 3072, 2912, 2621, 1691, 1588, 1467, 1439, 1327, 1252, 1117, 1152, 1090, 990, 935, 873, 847, 815, 771, 755, 737, 661, 534.
HRMS (EI) calcd for $C_{13}H_{14}NO_2$ [M+H]: 216.1025, found 216.1026.
Melting point (°C) 88.2 – 89.5.
Preparation of HP-4

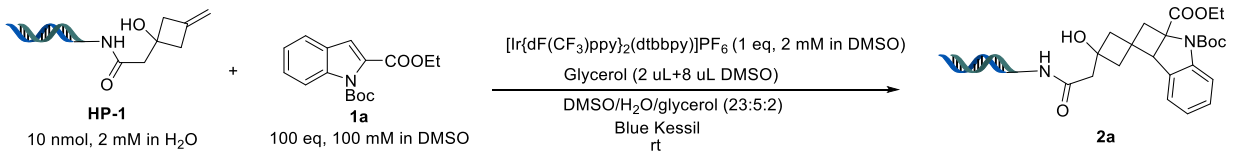


S7 **HP-4**

Preparation of **HP-4** followed **General Procedure B**.

5 Procedures for on-DNA 2+2 cycloaddition

5.1 Indole and benzofuran substrates (General Procedure C): 100 equiv



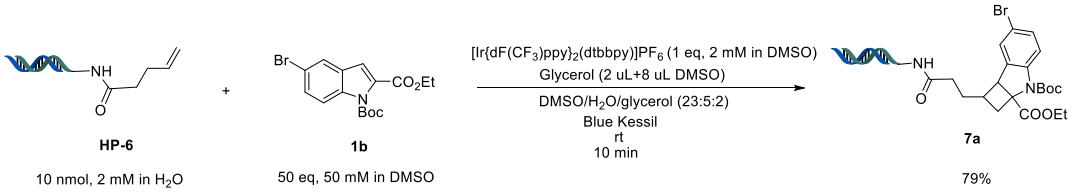
(**HP-1** and **1a** as example)

To a PCR Eppendorf tube was added {Ir[dF(CF_3)ppy]$_2$(dtbbpy)}PF_6 (5 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), indole (10 µL of a 100 nmol/µL soln in DMSO, 1000 nmol, 100 equiv), A glycerol/DMSO solution (10 µL, glycerol : DMSO = 2 : 8 v/v), and DNA-tethered alkene (5 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The mixture was mixed by pipetting back and forth. The PCR tube was then capped and irradiated by a Kessil H150-blue lamp at a distance of 1.5 inches for 10 min. The reaction was worked up following the EtOH precipitation protocol: to the reaction PCR Eppendorf tube was added 3 µL of cold (–20 °C) 5 M aq NaCl, followed by 75 µL of cold (–20 °C) EtOH. The resulting mixture was vortexed and left in a –20 °C freezer for 1 h. The chilled mixture was centrifuged for 15 min at 4 °C. The supernatant was decanted. The remaining solid was diluted with H_2O (100 µL) and analyzed by LC/MS.

Reaction workflow was similar to reported literature.[2]

100 nmol scale-up reaction (6b'): 10 × scale-up of **General Procedure C** with all the equiv ratios remaining the same**:** 0.6 mL PCR tube, {Ir[dF(CF_3)ppy]$_2$(dtbpy)}PF_6 (50 µL of a 2 nmol/µL soln in DMSO, 100 nmol, 1.0 equiv), indole (100 µL of a 100 nmol/µL soln in DMSO, 10000 nmol, 100 equiv), glycerol/DMSO solution (100 µL, glycerol : DMSO = 2 : 8 v/v), DNA-tethered alkene (50 µL of a 2 nmol/µL soln in H_2O, 100 nmol, 1.0 equiv), 10 min. Work up: 30 µL of cold (–20 °C) 5 M aq NaCl, followed by 750 µL of cold (–20 °C) EtOH.

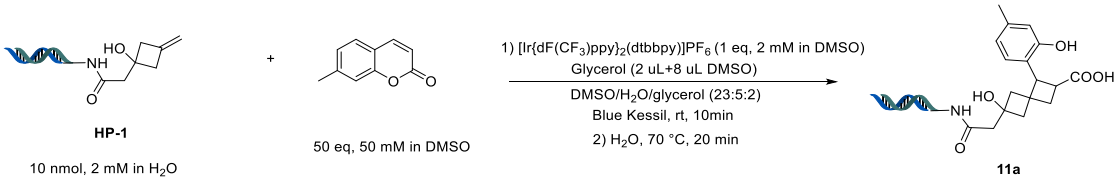
5.2 Indole and benzofuran substrates (General Procedure D): 50 equiv



(**HP-6** and **1b** as example)

To a PCR Eppendorf tube was added {Ir[dF(CF_3)ppy]$_2$(dtbbpy)}PF_6 (5 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), indole (10 µL of a 50 nmol/µL soln in DMSO, 500 nmol, 50 equiv), A glycerol/DMSO solution (10 µL, glycerol/DMSO = 2 : 8 v/v), and DNA-tethered alkene (5 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The mixture was mixed by pipetting back and forth. The PCR tube was then capped and irradiated by a Kessil H150-blue lamp at a distance of 1.5 inches for 10 min. The reaction was worked up following the EtOH precipitation protocol: to the reaction PCR Eppendorf tube was added 3 µL of cold (–20 °C) 5 M aq NaCl, followed by 75 µL of cold (–20 °C) EtOH. The resulting mixture was vortexed and left in a –20 °C freezer for 1 h. The chilled mixture was centrifuged for 15 min at 4 °C. The supernatant was decanted. The remaining solid was diluted with H_2O (100 µL) and analyzed by LC/MS.

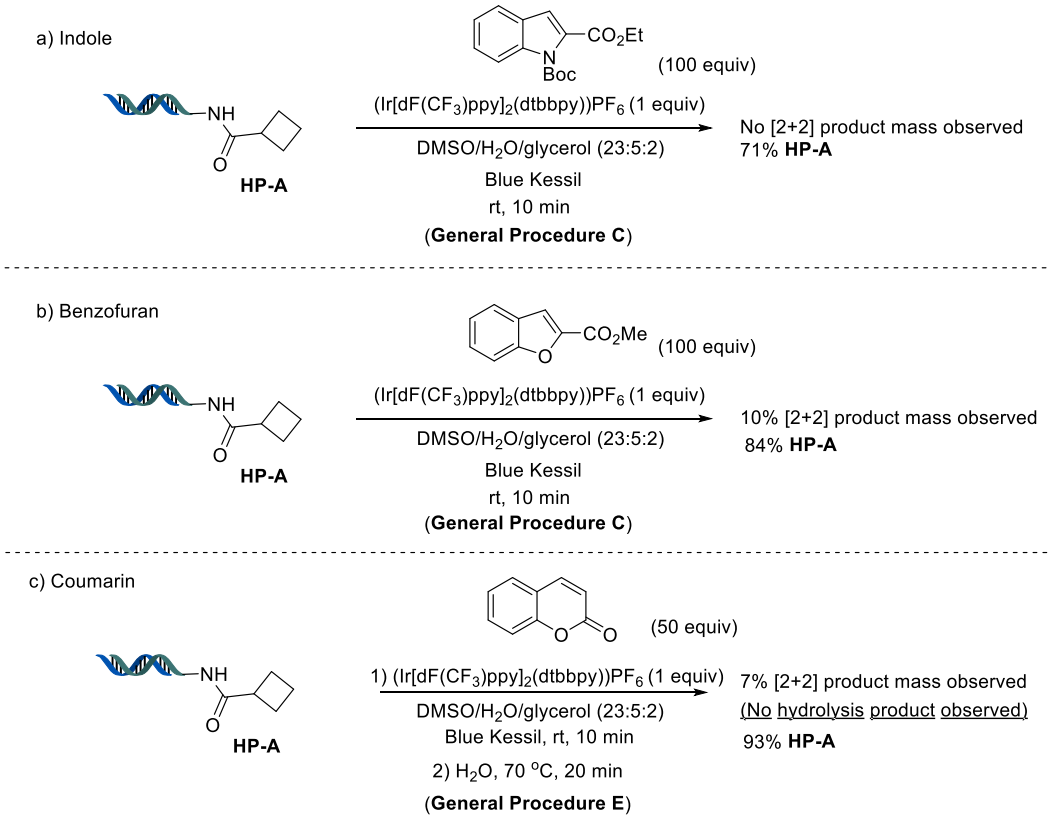
5.3 Coumarins (General Procedure E)



To a PCR Eppendorf tube was added {Ir[dF(CF_3)ppy]$_2$(dtbbpy)}PF_6 (5 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), coumarin (10 µL of a 50 nmol/µL soln in DMSO, 500 nmol, 50 equiv), A glycerol/DMSO solution (10 µL, glycerol/DMSO = 2 : 8 v/v), and DNA-tethered alkene (5 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The mixture was mixed by pipetting back and forth. The PCR tube was then capped and irradiated by a Kessil H150-blue lamp at a distance of 1.5 inches for 10 min. The reaction was worked up following the EtOH precipitation protocol: to the reaction PCR Eppendorf tube was added 3 µL of cold (–20 °C) 5 M aq NaCl, followed by 75 µL of cold (–20 °C) EtOH. The resulting mixture was vortexed and left in a –20 °C freezer for 1 h. The chilled mixture was centrifuged for 15 min at 4 °C. The supernatant was decanted. The hydrolysis of the product was performed by diluting the remaining solid with H_2O (150 µL). The PCR tube was then capped and heated at 70 °C for 20 min using an oil bath, and the final solution was analyzed by LC/MS.

6 Control Experiments

Control experiments of indole, benzofuran, and coumarin were conducted with headpiece **HP-A** under standard conditions (Scheme S3).



Scheme S3

7 Extra scope for on-DNA reactions

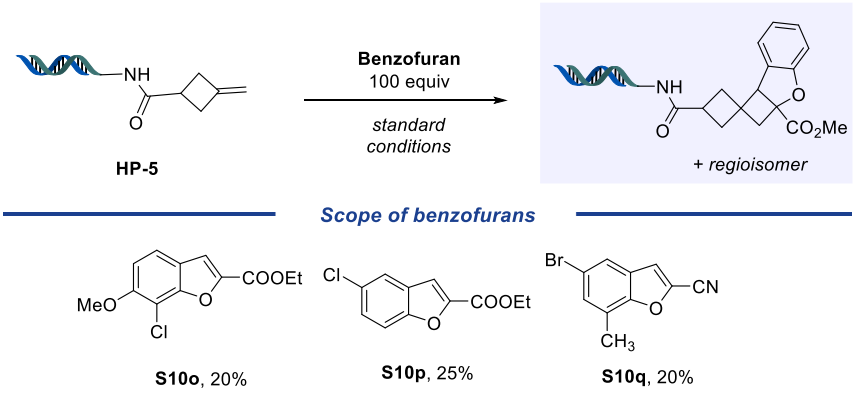
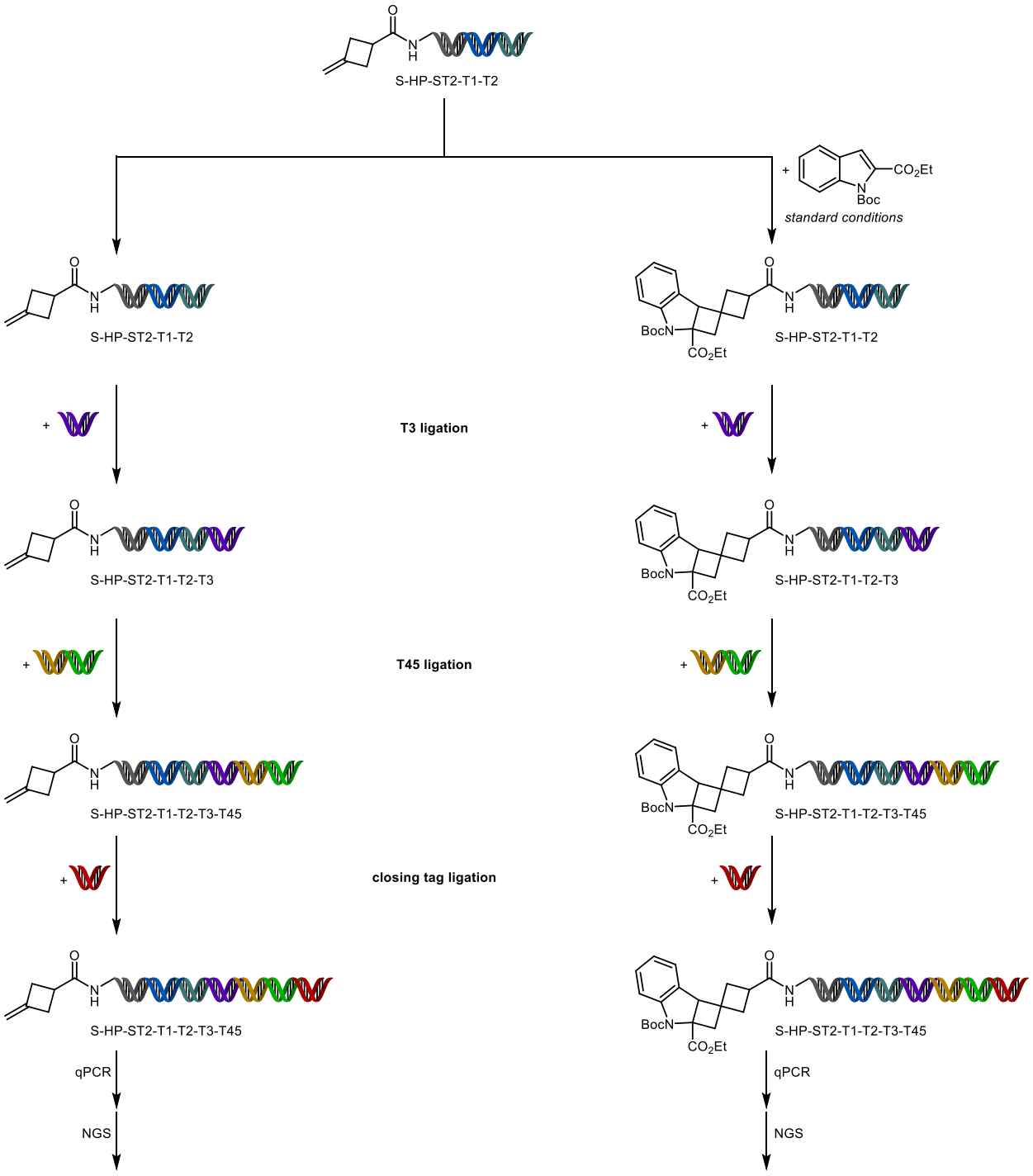


Figure S4 Scope of benzofuran for activated alkenes with low yields. DNA (2 mM in H_2O, 10 nmol, 1 equiv), **benzofurans** (100 mM in DMSO, 100 equiv), [Ir{dF(CF3)ppy}2(dtbbpy)]PF6 (2 mM in DMSO, 1 equiv), glycerol (2 µL in 8 µL of DMSO), rt, 10 min, blue Kessil. Work-up: Ethanol precipitation.

8 DNA Damage Assessment

Overview



Preparation of 3-*exo*-methylene cyclobutanamide capped cycle 2 DNA tag.

Cycle 2 tagged DNA material was defrosted and 40 µL (200 nmol, 5 mM), was transferred to a 0.2 mL PCR tube. To the 40 µL of DNA was added 160 µL of sodium borate buffer (pH 9.4). To the reaction solution was added 40 µL DMTMM (40 equiv, 8000 nmol, 200 mM in water) followed by 40 µL of methylene cyclobutane carboxylic acid (40 equiv, 8000 nmol, 200 mM in DMA). The reaction was equipped with a stir bar and allowed to stir at room temperature overnight. At the conclusion of the reaction, a 1 µL aliquot was diluted to 40 µL and used for LCMS analysis. The bulk solution was then subjected to an ethanol precipitation. The DNA pellet was then resuspended in 100 µL ddH_2O and lyophilized to provide a colorless solid.

Synthesis of cycle 2 DNA coupled [2+2] Indole Product.

To a PCR Eppendorf tube was added $[Ir(dF(CF_3)ppy)_2(dtbbpy)]PF_6$ (5 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), indole (10 µL of a 100 nmol/µL soln in DMSO, 1000 nmol, 100 equiv), Glycerol DMSO solution (2 µL of glycerol with 8 µL DMSO) (glycerol was measured by weight, converted to volume), and DNA-tethered alkene (5 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The mixture was mixed by pipetting back and forth. The PCR tube was then capped and irradiated for 10 min with 456 nm PR-160 Kessil lamps at a distance of 1.5 inches. Reactions were carried out on a 10 nmol scale, 3 reactions were run side-by-side, then combined to provide the 30 nmol sample that was used for DNA damage evaluation.

A - Chemistry – Air – Reaction samples A were run as outlined above with no alterations.

B - Chemistry - N_2 – Reaction samples B were sparged for 2 min with N_2, then capped and sealed with parafilm.

C - No light control – Reaction samples C were wrapped in aluminum foil to protect them from light then irradiated for 10 min.

D - No reagents control – Reaction samples D were run in the presence of blank water and DMSO.

E - No small molecule control – Reaction samples E were run in the absence of ethyl indole-2-carboxylate.

F - True blank – Reaction samples F were not subjected to reaction conditions.

The reaction was worked up following the EtOH precipitation protocol above: to the reaction PCR Eppendorf tube was added 3 µL of cold (-20 °C) 5 M aq NaCl, followed by 75 µL of cold (-20 °C) EtOH. The resulting mixture was vortexed and left in a -20 °C freezer for 1 h. The chilled mixture was centrifuged for 45 min at 4 °C. The supernatant was decanted. The DNA pellet was suspended in 30 µL of ddH_2O. A 1 µL aliquot was diluted with 40 µL of ddH_2O, filtered through a 13 mm PTFE (0.2 µm pore size) syringe filter, the resulting solution was used for LCMS analysis.

Following LCMS analysis, the reaction samples were frozen and lyophilized. The dried sample was then used for subsequent ligations, followed by qPCR and NGS analysis.

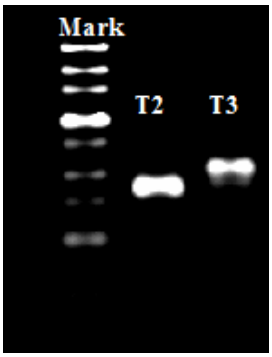
Ligations

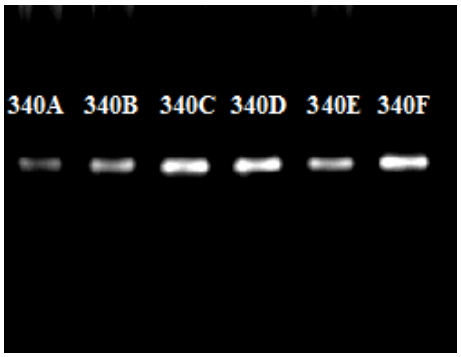
Cycle 3 (T3) ligations

General procedure: To each tube of 340A-F (10 nmol, 1 mM in ddH2O) was added 1.8 equiv T3 (18 nmol, 1.98 mM in ddH2O), 10X T4 DNA ligase buffer (4 µL), ddH2O (11.6 µL) and T4 DNA ligase (0.4 µL, 30 U/ µL). The tubes were vortexed, centrifuged and stood at 16°C for 16 h. After that the samples were subjected to ethanol precipitation and spin filtration with 10K membrane. The ligation efficiency was detected by gel electrophoresis and LC-MS analysis.

Ligation reactions with samples 340 A, 340 B, 340 C, 340 E, and 340 F were carried out on 10 nmol scale.

Ligation reaction with sample 340 D was carried out on 5 nmol scale.

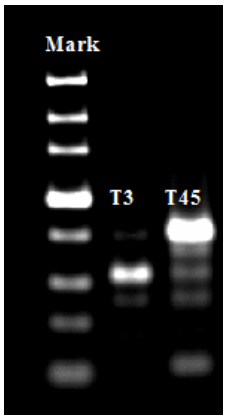


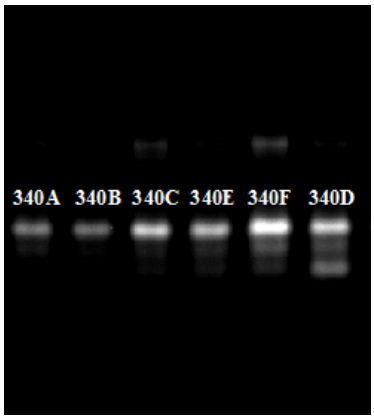


T3 Ligations	
Sample	**Yield (%)**
340 A	100%
340 B	100%
340 C	100%
340 D	100%
340 E	100%
340 F	100%

Cycle 4 and 5 (T45, library tag) ligation

To each tube of 340A-F (5 nmol, 1 mM in ddH2O) was added 1.3 equiv. T45 (6.5 nmol, 1 mM in ddH2O), 10X T4 DNA ligase buffer (2 µL) , ddH2O (5.8 µL) and T4 DNA ligase (0.2 µL, 30 U/ µL). The tubes were vortexed, centrifuged and stood at 16°C for 16 h. After that the samples were subjected to ethanol precipitation and spin filtration with 30K membrane. The ligation efficiency was detected by gel electrophoresis and LC-MS analysis.





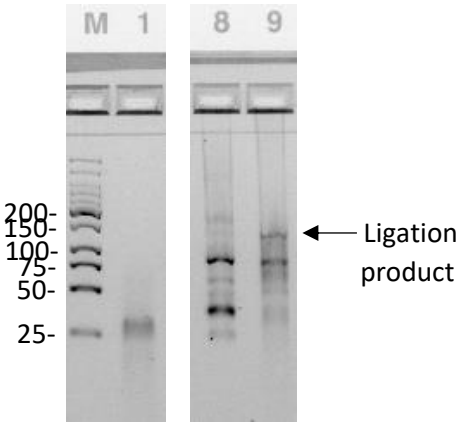
T45 Ligations	
Sample	**Yield (%)**
340 A	93%
340 B	100%
340 C	96%
340 D	96%
340 E	90%
340 F	80%

Closing tag ligation

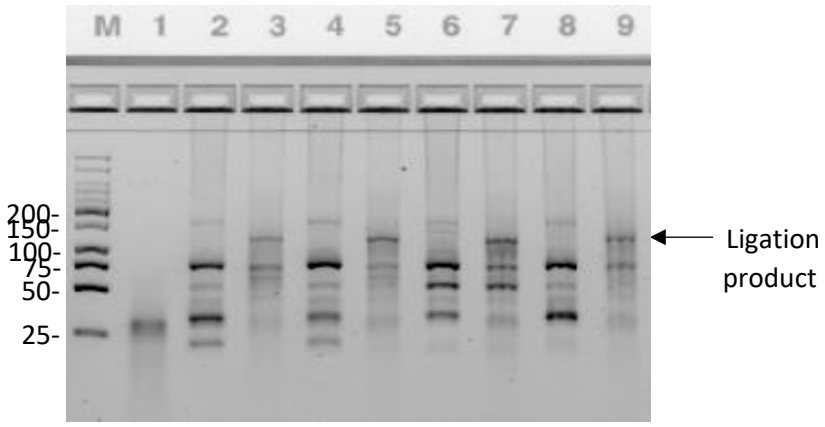
General Procedure: To each tube of 340A-F (1 nmol, 1 mM in ddH2O) was added 2 equiv closing tag (2 nmol, 1 mM in ddH2O), 10X T4 DNA ligase buffer (2 µL), ddH2O (15.6 µL) and T4 DNA ligase (0.4 µL, 30 U/ µL). The tubes were vortexed, centrifuged and stood at 16°C for 16 h. After that the samples were subjected to ethanol precipitation and gel electrophoresis.

Ligation reaction samples 340 A, 340 B, 340 C, 340 E and 340 F were carried out on 5 nmol scale.

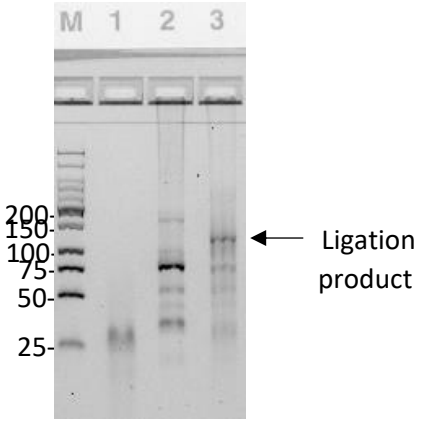
Ligation reaction sample 340 D was carried out on 2 nmol scale.



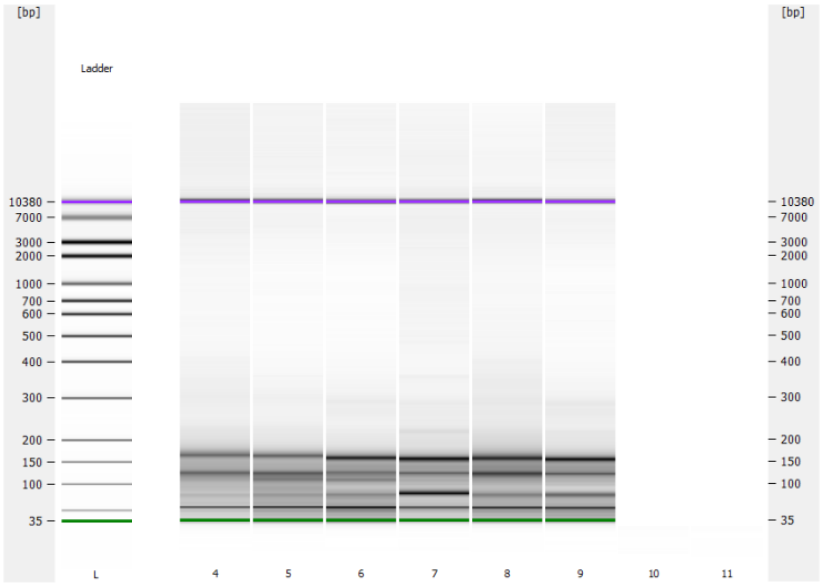
Lane No.	Sample
M	Marker
1	CT
8	LT - 340 A
9	340 A



Lane No.	Sample
M	Marker
1	CT
2	LT-340 B
3	340 B
4	LT-340 C
5	340 C
6	LT-340 D
7	340 D
8	LT-340 E
9	340 E



Lane No.	Sample
M	Marker
1	CT
2	LT-340 F
3	340 F



Lane No.	Sample
L	Ladder
4	340 A
5	340 B
6	340 C
7	340 D
8	340 E
9	340 F

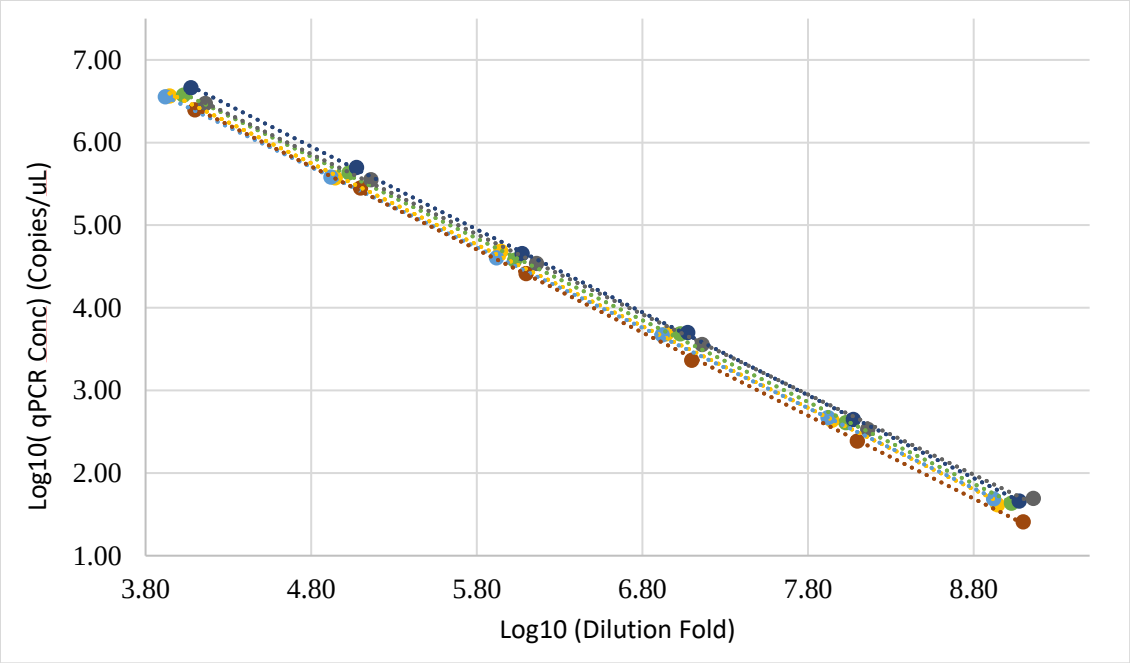
qPCR and Next Generation Sequencing (NGS)

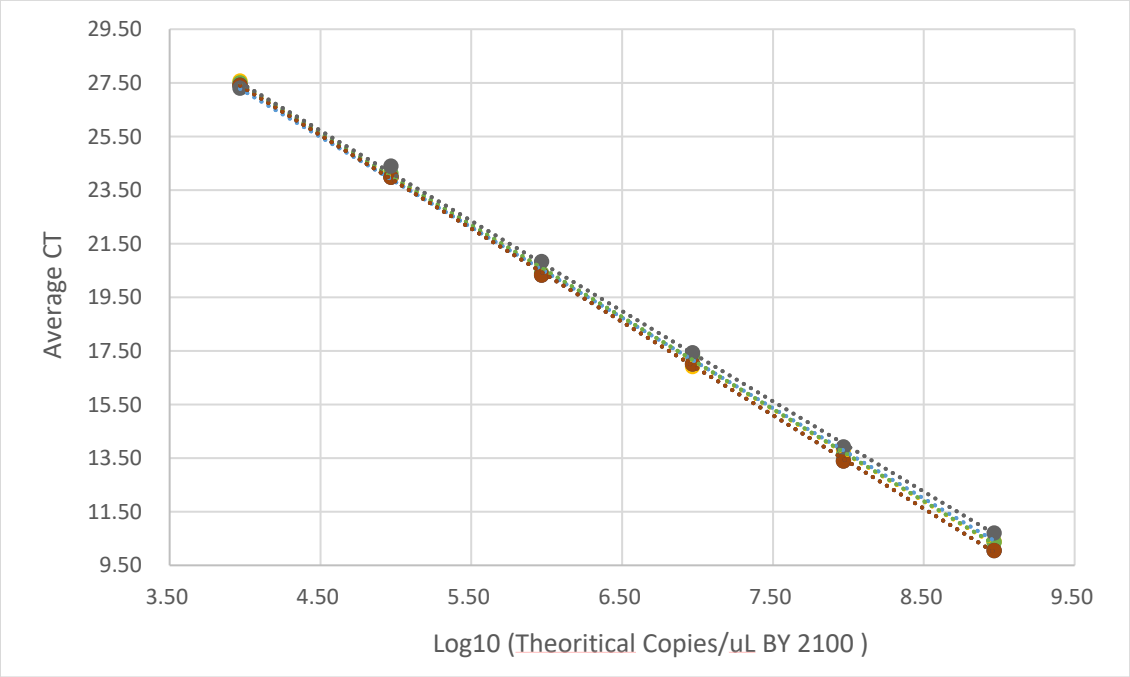
qPCR procedure

qPCR was performed with the SYBR Green Master Mix kit (Thermo) on a Real-Time PCR System (QuantStudio 7 Flex). All samples were subjected to PCR cycles as follows: 50°C incubation for 2 min, then 95°C heat activation for 5 min followed by 40 cycles of 95°C denaturation (10 seconds each), 55°C annealing (15 seconds each), and 72°C extension (30 seconds each).

To assess the amplification efficiency, the quantity of the full-length DNA templates was first normalized based on the Agilent 2100 Bioanalyzer result and qPCR with serial dilutions was performed. Linear fitting was then calculated respectively based on the CT values. The slope was then used to determine amplification efficacy, which was found to be comparable between the reaction sample and the controls (93-98%).

	Experiment Number					
	340 A	**340 B**	**340 C**	**340 D**	**340 E**	**340 F**
Slope	-3.42616	-3.37291	-3.43537	-3.4816	-3.48858	-3.36666
PCR Efficiency	96%	98%	95%	94%	93%	98%

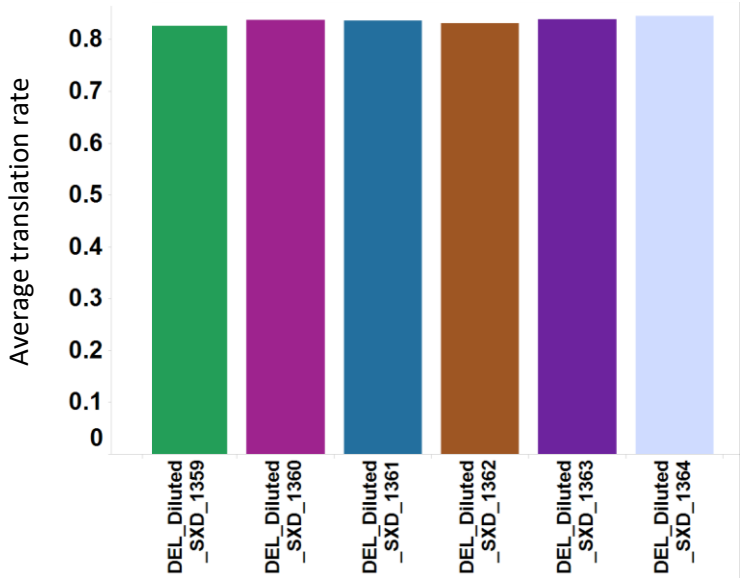


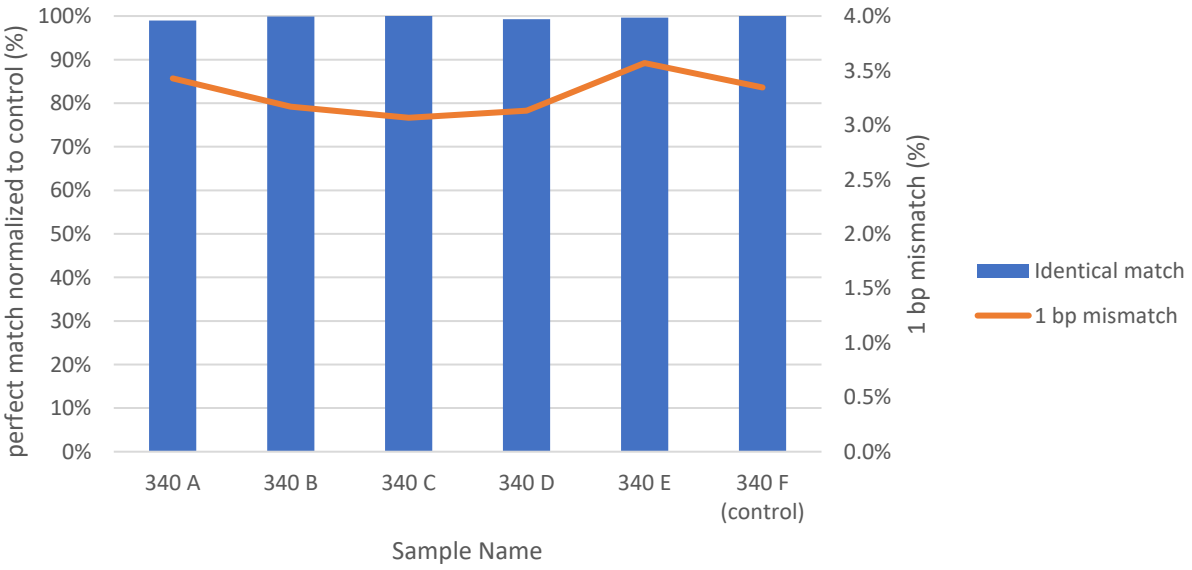


Next Generation Sequencing (NGS)

Samples were diluted to 1E+7 copies/35 µL as a template for PCR amplification. To a PCR tube was added diluted sample (35.0 µL), 10x high fidelity PCR buffer (5 µL), 50.0 mM MgSO4 (2 µL), 10 mM dNTP mix (1 µL), Platinum Taq DNA Polymerase (0.2 µL), 10 µM forward primer (2 µL), 10 µM reverse primer (2 µL), and nuclease-free water (2.8 µL). The PCR products were purified by the Agencourt AMPure XP Beads method. The purified samples underwent next-generation sequencing (Illumina NovaSeq). Bowtie2 was

used to map the sequenced reads to reference sequence (primer + coding region sequence) by local alignment. The detailed mapping identities were extracted from CIGAR string and XM flag in the SAM format. The translation rate of the six samples were found to be comparable. The percent of sequences that were a perfect match for each of the six samples was nearly identical and ranged from 77% for reaction sample 340 A to 78% for the control 340 F. When normalized to the control, reaction sample 340 A was a 99% perfect sequence match, indicating just 1% mutated sequences.

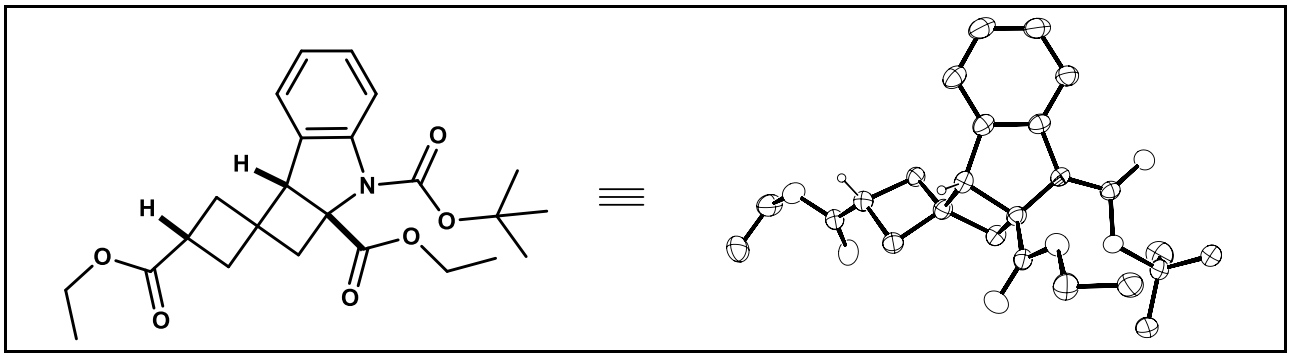




Note: For benzofuran and coumarin examples, we expect more DNA damage because of the potential for a small amount of addition to the DNA tag.

9 X-ray Structure Determination of Compound S1-6 (Isomers A)

X-ray Structure Determination of Compound 9259



Compound 9259, $C_{24}H_{31}NO_6$, crystallizes in the monoclinic space group Cc (systematic absences hkl: h+k=odd) with a=22.0386(15)Å, b=13.2446(3)Å, c=11.5475(8)Å, β=137.586(13)°, V=2273.4(4)Å^3, Z=4, and d_{calc}=1.255 g/cm^3. X-ray intensity data were collected on a Rigaku XtaLAB Synergy-S diffractometer [1] equipped with an HPC area detector (HyPix-6000HE) and employing confocal multilayer optic-monochromated Cu-Kα radiation (λ=1.54184 Å) at a temperature of 100K. Preliminary indexing was performed from a series of sixty 0.5° rotation frames with exposures of 0.625 seconds for θ = ±47.290° and 2.5 seconds for θ = 113.25°. A total of 4858 frames (46 runs) were collected employing ω scans with a crystal to detector distance of 34.0 mm, rotation widths of 0.5° and exposures of 2 seconds. Rotation frames were integrated using CrysAlisPro [2], producing a listing of unaveraged F^2 and σ(F^2) values. A total of 17402 reflections were measured over the ranges 8.942 ≤ 2θ ≤ 149.34°, -27 ≤ h ≤ 25, -16 ≤ k ≤ 16, -13 ≤ l ≤ 14 yielding 3997 unique reflections (R_{int} = 0.0430). The intensity data were corrected for Lorentz and polarization effects and for absorption using SCALE3 ABSPACK [3] (minimum and maximum transmission 0.4310, 1.0000). The structure was solved by dual space methods - SHELXT [4]. Refinement was by full-matrix least squares based on F^2 using SHELXL [5]. All reflections were used during refinement. The weighting scheme used was $w=1/[\sigma^2(F_o^2)+(0.0719P)^2+0.2868P]$ where $P = (F_o^2 + 2F_c^2)/3$. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using a riding model. Refinement converged to R1=0.0392 and wR2=0.1042 for 3771 observed reflections for which F > 4σ(F) and R1=0.0415 and wR2=0.1069 and GOF =1.079 for all 3997 unique, non-zero reflections and 285 variables. The maximum Δ/σ in the final cycle of least squares was 0.000 and the two most prominent peaks in the final difference Fourier were +0.23 and -0.21 e/Å^3.

Table S1. lists cell information, data collection parameters, and refinement data. Final positional and equivalent isotropic thermal parameters are given in **Tables S2**. and **S3**. Anisotropic thermal parameters are in **Table S4**., **Tables S5**., and **S6**. list bond distances and bond angles. **Figure S5.** is an ORTEP representation of the molecule with 50% probability thermal ellipsoids displayed.

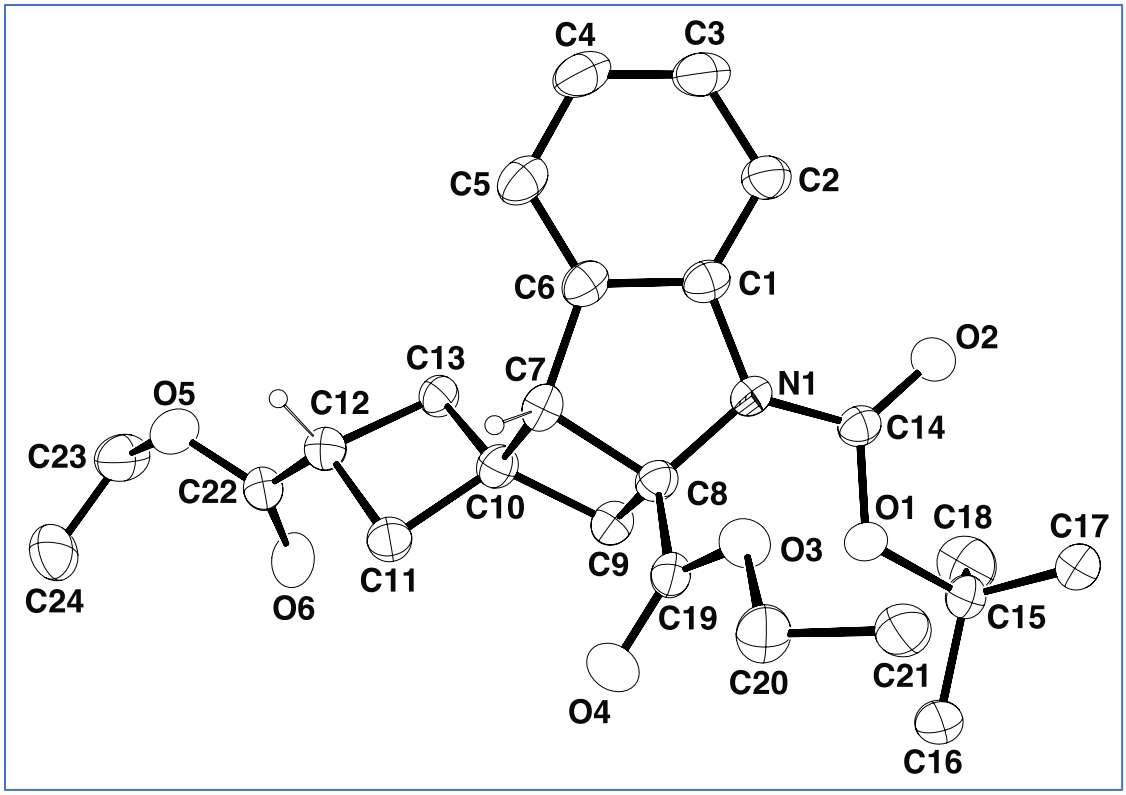


Figure S5. ORTEP drawing of the title compound with 50% thermal ellipsoids.

Table S1. Summary of Structure Determination of Compound 9259

Empirical formula	$C_{24}H_{31}NO_6$
Formula weight	429.50
Diffractometer	Rigaku XtaLAB Synergy-S (HyPix-6000HE)
Temperature/K	100
Crystal system	monoclinic
Space group	Cc
a	22.0386(15)Å
b	13.2446(3)Å
c	11.5475(8)Å
β	137.586(13)°
Volume	2273.4(4)Å^3
Z	4
d_{calc}	1.255 g/cm^3
μ	0.735 mm^{-1}
F(000)	920.0
Crystal size, mm	0.15 × 0.07 × 0.04
2θ range for data collection	8.942 - 149.34°
Index ranges	-27 ≤ h ≤ 25, -16 ≤ k ≤ 16, -13 ≤ l ≤ 14
Reflections collected	17402
Independent reflections	3997[R(int) = 0.0430]
Data/restraints/parameters	3997/2/285
Goodness-of-fit on F^2	1.079
Final R indexes [I>=2σ (I)]	R_1 = 0.0392, wR_2 = 0.1042
Final R indexes [all data]	R_1 = 0.0415, wR_2 = 0.1069
Largest diff. peak/hole	0.23/-0.21 eÅ^{-3}
Flack parameter	0.14(12)

Table S2 . Refined Positional Parameters for Compound 9259

Atom	*x*	*y*	*z*	U(eq)
O1	0.74839(12)	0.42427(13)	0.9950(2)	0.0272(4)
O2	0.79937(12)	0.58188(13)	1.1141(2)	0.0315(4)
O3	0.63281(13)	0.36617(13)	1.0321(2)	0.0302(4)
O4	0.54522(13)	0.28890(14)	0.7774(2)	0.0361(5)
O5	0.19442(14)	0.57522(16)	-0.0130(2)	0.0390(5)
O6	0.29552(14)	0.47419(18)	0.0449(3)	0.0416(5)
N1	0.65203(14)	0.53470(15)	0.9296(3)	0.0255(4)
C1	0.61452(17)	0.62887(18)	0.9105(3)	0.0270(5)
C2	0.6592(2)	0.7168(2)	1.0054(3)	0.0324(6)
C3	0.6059(2)	0.7989(2)	0.9647(4)	0.0390(7)
C4	0.5129(2)	0.7931(2)	0.8371(4)	0.0399(7)
C5	0.4696(2)	0.7042(2)	0.7425(3)	0.0335(6)
C6	0.52063(18)	0.62279(19)	0.7784(3)	0.0277(5)
C7	0.49004(16)	0.51913(19)	0.6986(3)	0.0265(5)
C8	0.58044(16)	0.46311(18)	0.7993(3)	0.0248(5)
C9	0.55425(18)	0.4588(2)	0.6332(3)	0.0278(5)
C10	0.46024(17)	0.50134(19)	0.5292(3)	0.0265(5)
C11	0.37596(18)	0.43548(19)	0.3909(3)	0.0302(5)
C12	0.32222(17)	0.53003(19)	0.2772(3)	0.0288(5)
C13	0.41340(17)	0.58754(18)	0.3953(3)	0.0261(5)
C14	0.74024(17)	0.51974(18)	1.0230(3)	0.0252(5)
C15	0.83400(17)	0.3888(2)	1.0658(3)	0.0312(6)
C16	0.8131(2)	0.2790(2)	1.0107(4)	0.0415(7)
C17	0.9093(2)	0.3962(2)	1.2599(4)	0.0438(8)
C18	0.8529(2)	0.4475(3)	0.9829(5)	0.0493(8)
C19	0.58626(16)	0.36248(19)	0.8691(3)	0.0260(5)
C20	0.6387(2)	0.2736(2)	1.1086(4)	0.0356(6)
C21	0.7309(2)	0.2672(2)	1.2882(4)	0.0376(6)
C22	0.27021(18)	0.5213(2)	0.0933(3)	0.0304(6)
C23	0.1449(2)	0.5795(2)	-0.1929(4)	0.0456(8)
C24	0.0901(2)	0.4868(3)	-0.2890(4)	0.0513(8)

Table S3 . Positional Parameters for Hydrogens in Compound 9259

Atom	*x*	*y*	*z*	U(eq)
H2	0.722954	0.721043	1.093915	0.039
H3	0.634436	0.860285	1.026387	0.047
H4	0.47864	0.849524	0.813816	0.048
H5	0.405875	0.699689	0.654634	0.04
H7	0.451459	0.484121	0.702684	0.032
H9a	0.590725	0.503993	0.634913	0.033
H9b	0.552877	0.389508	0.599258	0.033
H11a	0.352766	0.40372	0.429857	0.036
H11b	0.383322	0.384721	0.339056	0.036
H12	0.284308	0.55719	0.288175	0.035
H13a	0.435031	0.594896	0.345183	0.031
H13b	0.414095	0.653275	0.437135	0.031
H16a	0.793054	0.2455	1.053556	0.062
H16b	0.867323	0.245507	1.057873	0.062
H16c	0.765448	0.275121	0.885085	0.062
H17a	0.921891	0.467448	1.29401	0.066
H17b	0.963465	0.36391	1.305265	0.066
H17c	0.891134	0.362172	1.305978	0.066
H18a	0.902741	0.415646	1.011141	0.074
H18b	0.869281	0.517021	1.025895	0.074
H18c	0.799113	0.447829	0.858107	0.074
H20a	0.626872	0.214211	1.042054	0.043
H20b	0.593492	0.274543	1.107759	0.043
H21a	0.775189	0.265735	1.287668	0.056
H21b	0.736359	0.205578	1.34226	0.056
H21c	0.742018	0.326215	1.35305	0.056
H23a	0.188171	0.587242	-0.196531	0.055
H23b	0.10489	0.639244	-0.249215	0.055
H24a	0.055126	0.493973	-0.409771	0.077
H24b	0.049119	0.477233	-0.281204	0.077
H24c	0.130006	0.428162	-0.239319	0.077

Table S4 . Refined Thermal Parameters (U's) for Compound 9259

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0254(9)	0.0214(8)	0.0328(9)	-0.0008(7)	0.0209(8)	0.0007(7)
O2	0.0277(9)	0.0269(9)	0.0339(10)	-0.0034(8)	0.0209(8)	-0.0029(7)
O3	0.0338(9)	0.0257(8)	0.0284(9)	0.0029(7)	0.0221(8)	-0.0016(7)
O4	0.0376(10)	0.0283(9)	0.0319(9)	-0.0026(7)	0.0225(9)	-0.0058(8)
O5	0.0364(11)	0.0385(11)	0.028(1)	0.0019(8)	0.0194(9)	0.0100(8)
O6	0.0305(10)	0.0588(13)	0.032(1)	-0.0057(9)	0.0220(9)	0.0017(9)
N1	0.024(1)	0.0222(9)	0.0267(10)	0.0023(8)	0.0177(9)	0.0038(8)
C1	0.0324(13)	0.0249(12)	0.0275(12)	0.0039(9)	0.0233(11)	0.0043(10)
C2	0.0359(14)	0.0267(12)	0.0314(13)	0.0008(10)	0.0238(12)	0.0029(10)
C3	0.0495(17)	0.0296(13)	0.0368(14)	0.0014(11)	0.0315(14)	0.0077(12)
C4	0.0475(17)	0.0353(15)	0.0369(15)	0.0069(11)	0.0312(14)	0.0160(12)
C5	0.0353(14)	0.0366(14)	0.0295(13)	0.0072(11)	0.0242(12)	0.0115(12)
C6	0.0311(13)	0.0295(12)	0.0246(11)	0.0064(10)	0.0212(11)	0.0073(10)
C7	0.0233(12)	0.0300(12)	0.0263(12)	0.0043(10)	0.0183(11)	0.0038(10)
C8	0.0254(12)	0.0219(11)	0.0274(12)	0.0029(9)	0.0195(10)	0.0019(9)
C9	0.0275(12)	0.0298(12)	0.0270(12)	0.0025(9)	0.0204(11)	0.0033(10)
C10	0.0261(12)	0.0270(11)	0.0258(12)	0.0033(9)	0.0190(11)	0.0023(9)
C11	0.0295(12)	0.0269(11)	0.0308(12)	0.0026(10)	0.0212(11)	0.0004(10)
C12	0.0268(12)	0.0287(13)	0.0278(12)	0.000(1)	0.0192(11)	0.0002(10)
C13	0.0249(11)	0.0255(11)	0.0231(11)	0.0031(9)	0.0162(10)	0.0019(9)
C14	0.0272(12)	0.0227(11)	0.0257(12)	0.0030(9)	0.0195(10)	0.0025(9)
C15	0.0228(12)	0.0313(13)	0.0322(13)	-0.0036(10)	0.0181(11)	0.001(1)
C16	0.0326(14)	0.0343(14)	0.0451(16)	-0.0093(12)	0.0249(13)	0.0023(11)
C17	0.0318(14)	0.0338(14)	0.0353(15)	-0.0032(12)	0.0154(13)	0.0064(12)
C18	0.0490(18)	0.0567(19)	0.063(2)	-0.0091(17)	0.0477(18)	-0.0073(15)
C19	0.0207(11)	0.0264(12)	0.0262(11)	0.0012(9)	0.0158(10)	0.0011(9)
C20	0.0349(14)	0.0312(13)	0.0367(14)	0.0098(11)	0.0252(12)	-0.0002(11)
C21	0.0387(15)	0.0279(12)	0.0336(13)	0.0036(11)	0.0227(13)	0.0013(11)
C22	0.0254(12)	0.0301(12)	0.0301(13)	-0.0009(10)	0.0187(11)	-0.0012(10)
C23	0.0490(18)	0.0410(16)	0.0298(14)	0.0046(12)	0.0239(14)	0.0104(13)
C24	0.0369(16)	0.0541(19)	0.0342(16)	-0.0026(14)	0.0174(14)	-0.0027(14)

Table S5 . Bond Distances in Compound 9259, Å

O1-C14	1.349(3)	O1-C15	1.474(3)	O2-C14	1.208(3)
O3-C19	1.322(3)	O3-C20	1.460(3)	O4-C19	1.215(3)
O5-C22	1.334(3)	O5-C23	1.471(4)	O6-C22	1.214(3)
N1-C1	1.421(3)	N1-C8	1.465(3)	N1-C14	1.373(3)
C1-C2	1.390(4)	C1-C6	1.398(4)	C2-C3	1.402(4)
C3-C4	1.385(5)	C4-C5	1.397(4)	C5-C6	1.382(4)
C6-C7	1.507(4)	C7-C8	1.565(3)	C7-C10	1.554(3)
C8-C9	1.543(4)	C8-C19	1.513(3)	C9-C10	1.542(4)
C10-C11	1.543(4)	C10-C13	1.550(3)	C11-C12	1.549(3)
C12-C13	1.559(4)	C12-C22	1.497(4)	C15-C16	1.515(4)
C15-C17	1.515(4)	C15-C18	1.511(5)	C20-C21	1.488(4)
C23-C24	1.490(5)				

Table S6 . Bond Angles in Compound 9259, °

C14-O1-C15	120.6(2)	C19-O3-C20	117.3(2)	C22-O5-C23	116.1(2)
C1-N1-C8	110.3(2)	C14-N1-C1	124.9(2)	C14-N1-C8	122.7(2)
C2-C1-N1	128.4(2)	C2-C1-C6	121.6(2)	C6-C1-N1	110.0(2)
C1-C2-C3	117.1(3)	C4-C3-C2	121.9(3)	C3-C4-C5	119.9(3)
C6-C5-C4	119.2(3)	C1-C6-C7	110.7(2)	C5-C6-C1	120.2(3)
C5-C6-C7	129.0(3)	C6-C7-C8	103.2(2)	C6-C7-C10	119.4(2)
C10-C7-C8	89.25(19)	N1-C8-C7	105.73(19)	N1-C8-C9	115.6(2)
N1-C8-C19	114.79(19)	C9-C8-C7	89.48(18)	C19-C8-C7	112.0(2)
C19-C8-C9	115.9(2)	C10-C9-C8	90.49(19)	C9-C10-C7	89.96(19)
C9-C10-C11	119.9(2)	C9-C10-C13	124.6(2)	C11-C10-C7	117.1(2)
C11-C10-C13	88.71(18)	C13-C10-C7	119.7(2)	C10-C11-C12	90.18(19)
C11-C12-C13	88.18(19)	C22-C12-C11	116.4(2)	C22-C12-C13	114.9(2)
C10-C13-C12	89.54(18)	O1-C14-N1	108.0(2)	O2-C14-O1	126.8(2)
O2-C14-N1	125.2(2)	O1-C15-C16	101.9(2)	O1-C15-C17	110.8(2)
O1-C15-C18	108.9(2)	C16-C15-C17	110.2(2)	C18-C15-C16	111.6(3)
C18-C15-C17	113.0(3)	O3-C19-C8	113.4(2)	O4-C19-O3	124.5(2)
O4-C19-C8	121.8(2)	O3-C20-C21	108.1(2)	O5-C22-C12	113.0(2)
O6-C22-O5	122.7(2)	O6-C22-C12	124.2(2)	O5-C23-C24	111.6(3)

This report has been created with Olex2 [6], compiled on 2021.08.20 svn.r13c46975 for OlexSys.

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References for **Section 9**: X-ray Structure Determination (only)

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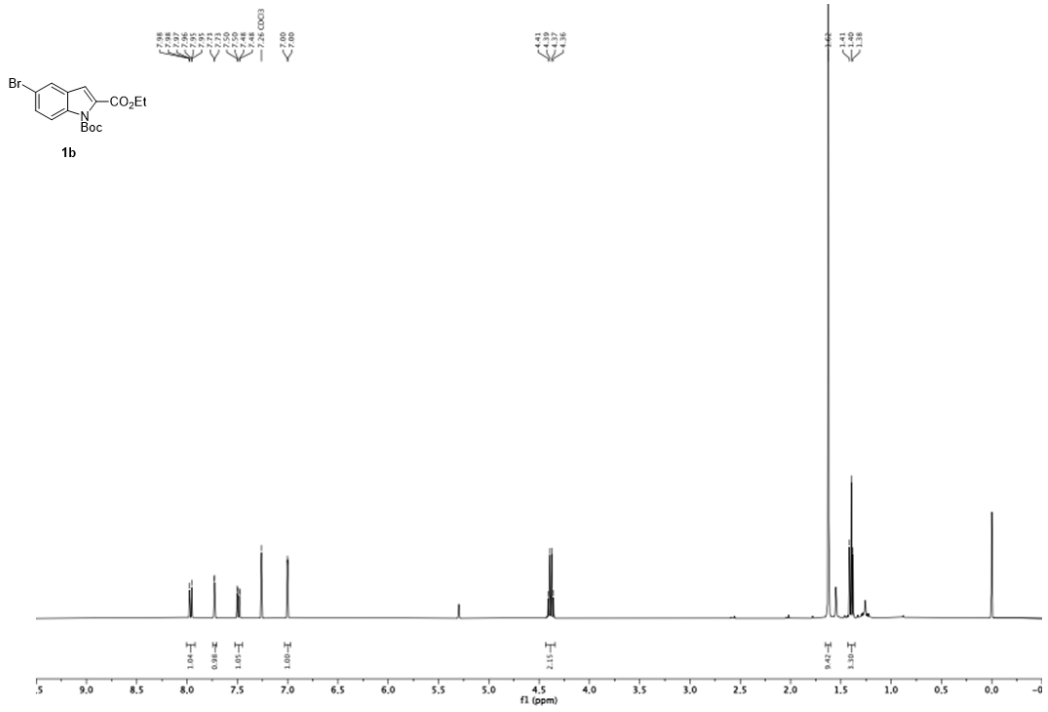
11 NMR spectra of small molecules

1-(*tert*-Butyl) 2-ethyl 5-bromo-1H-indole-1,2-dicarboxylate (**1b**)

^{1}H NMR, $CDCl_3$



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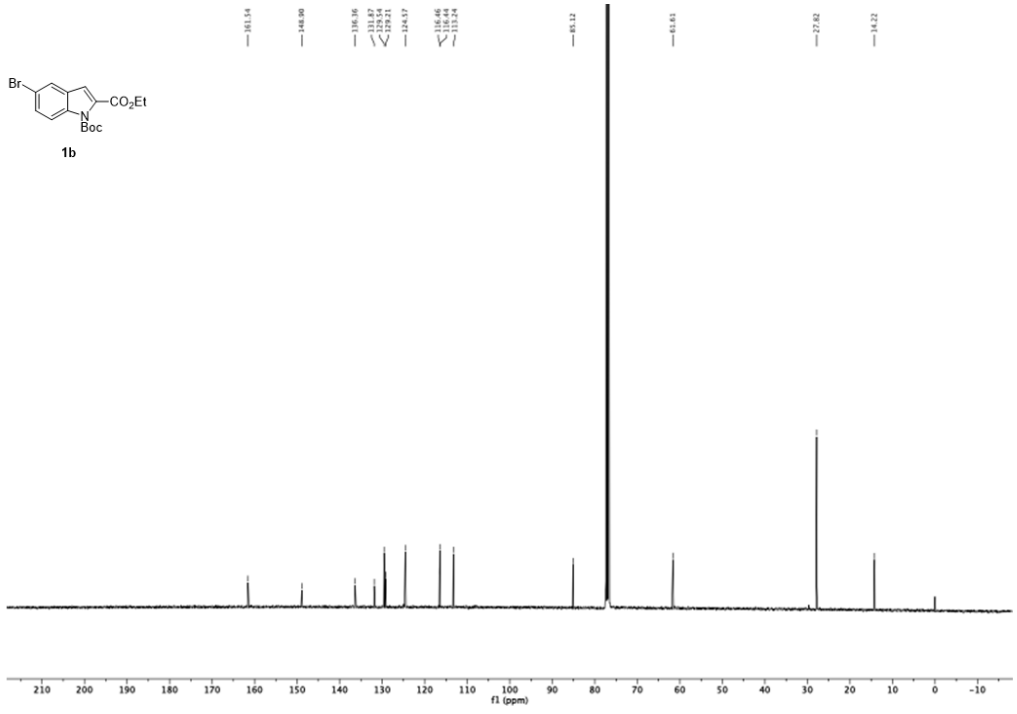

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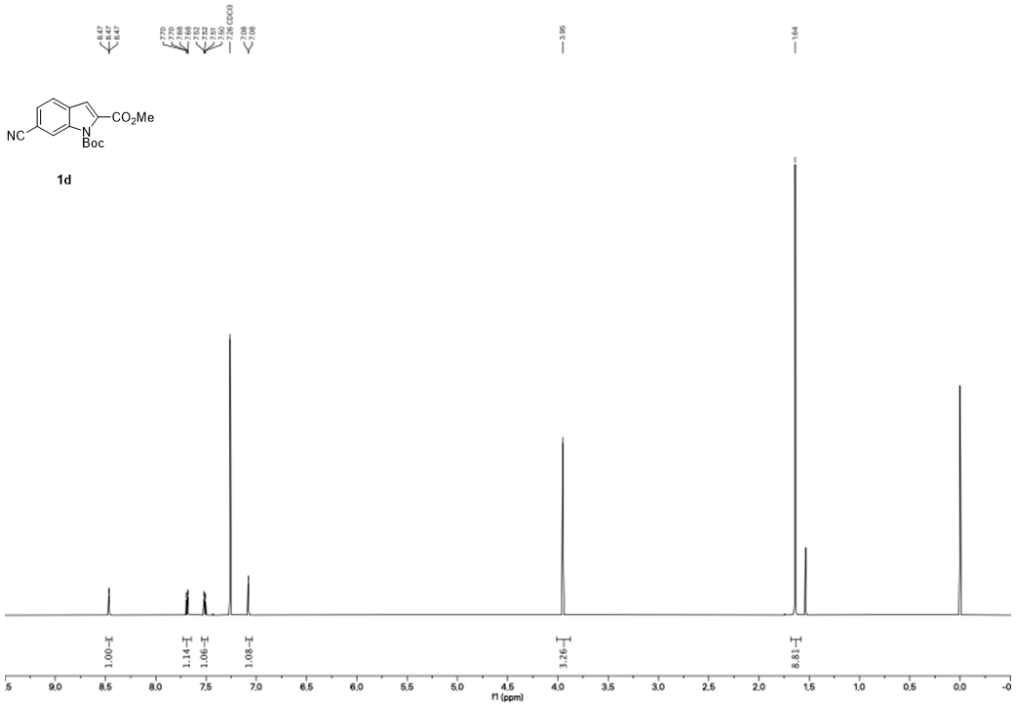

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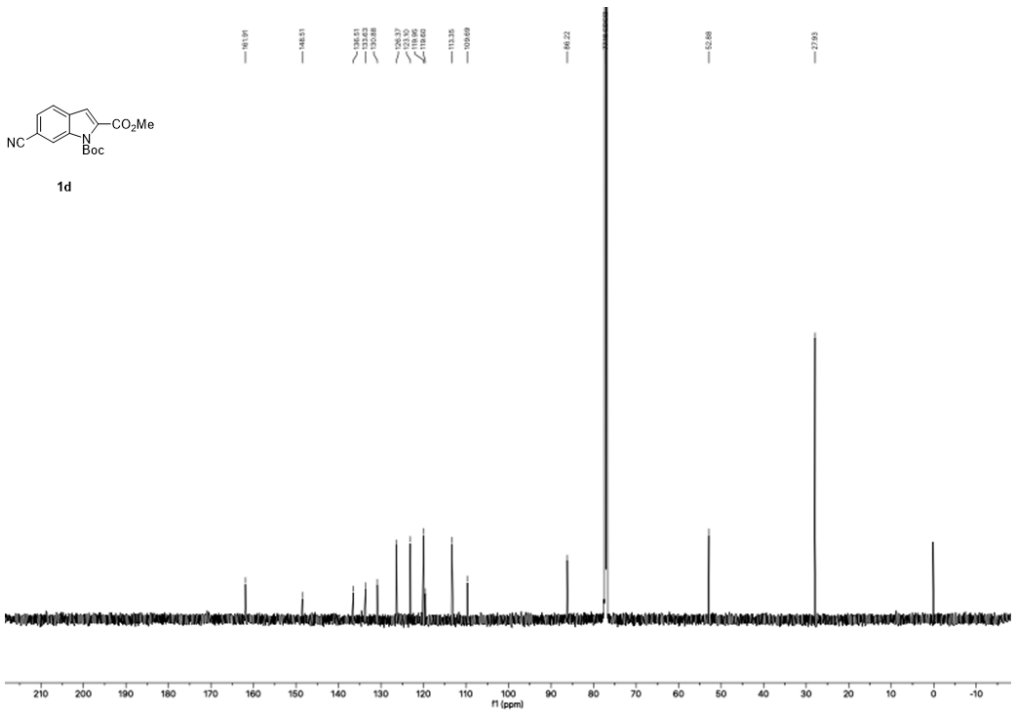

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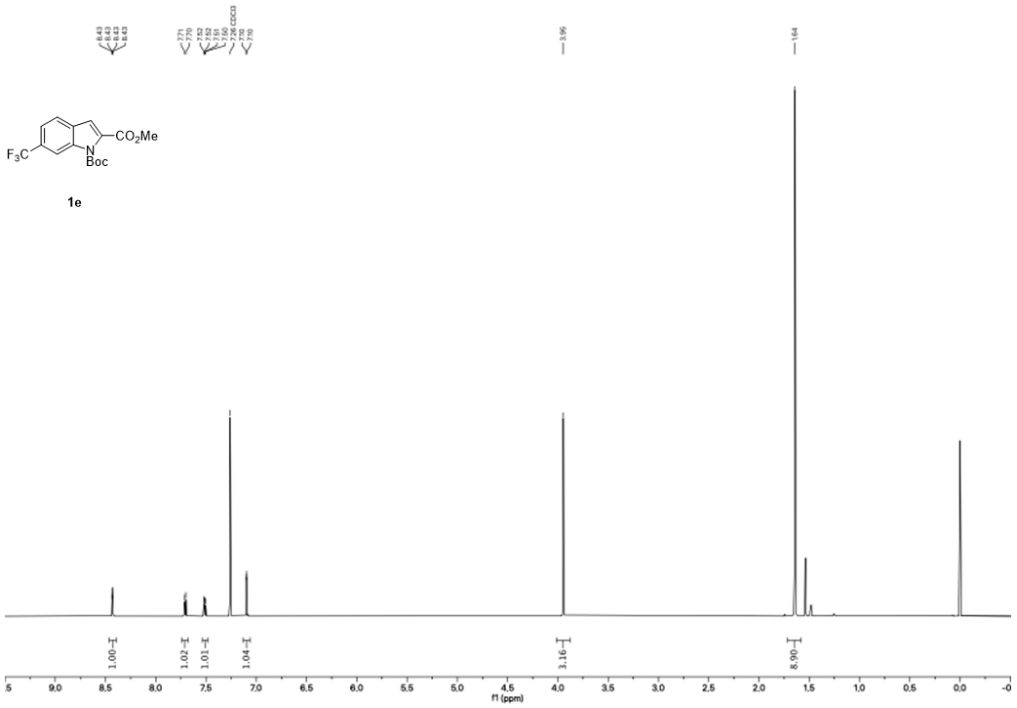

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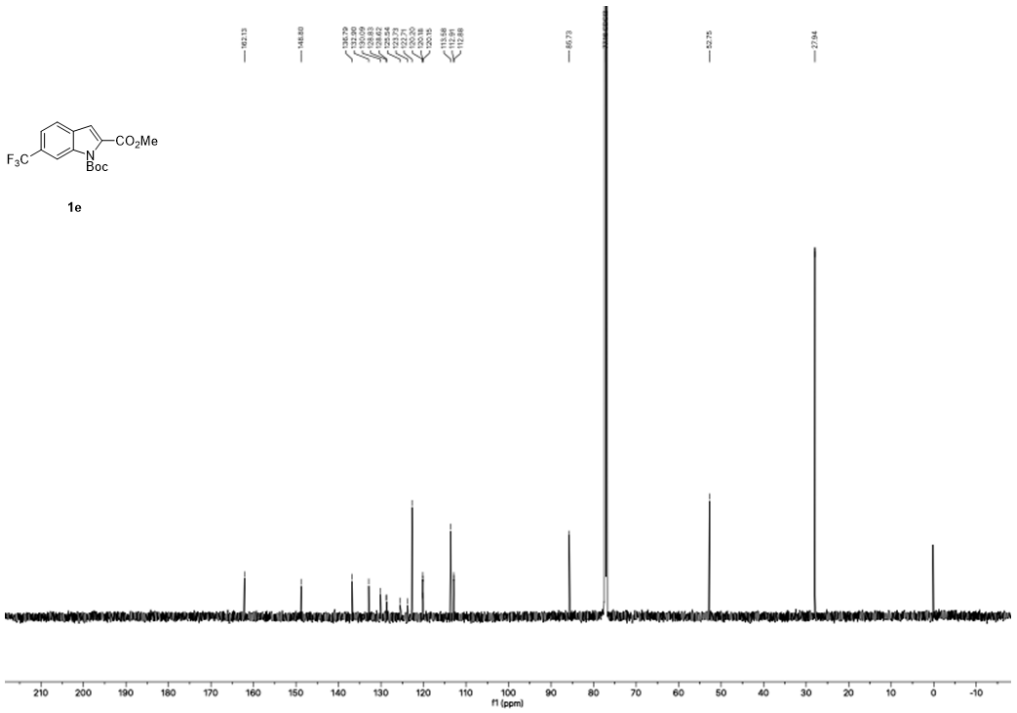

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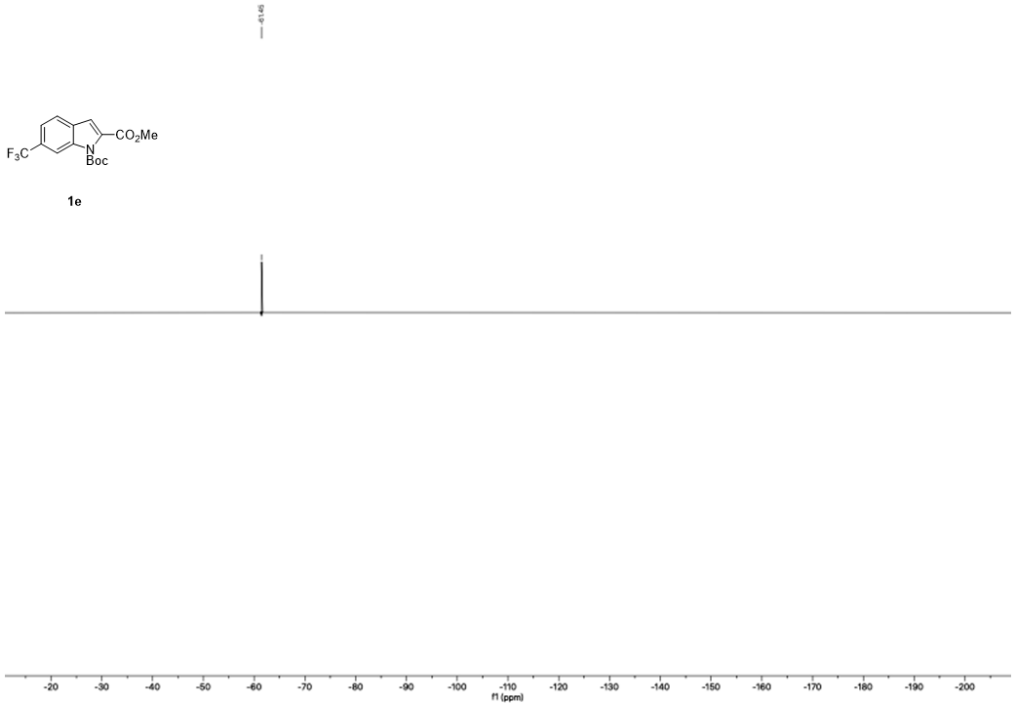
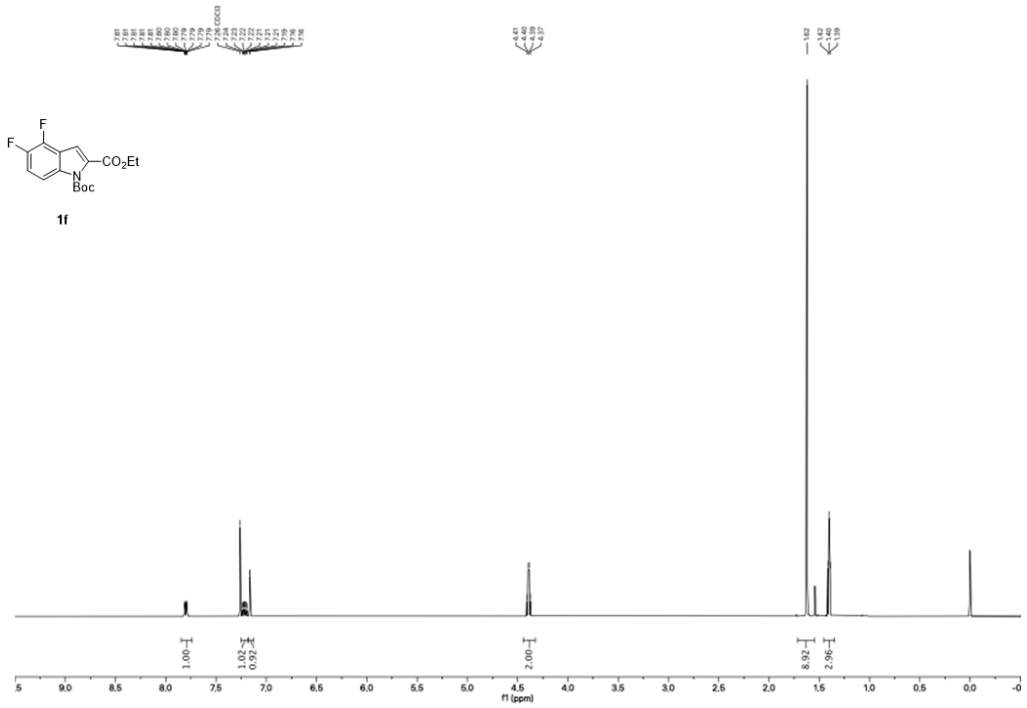


1-(*tert*-Butyl) 2-ethyl 4,5-difluoro-*1H*-indole-1,2-dicarboxylate (**1f**)

^{1}H NMR, $CDCl_3$



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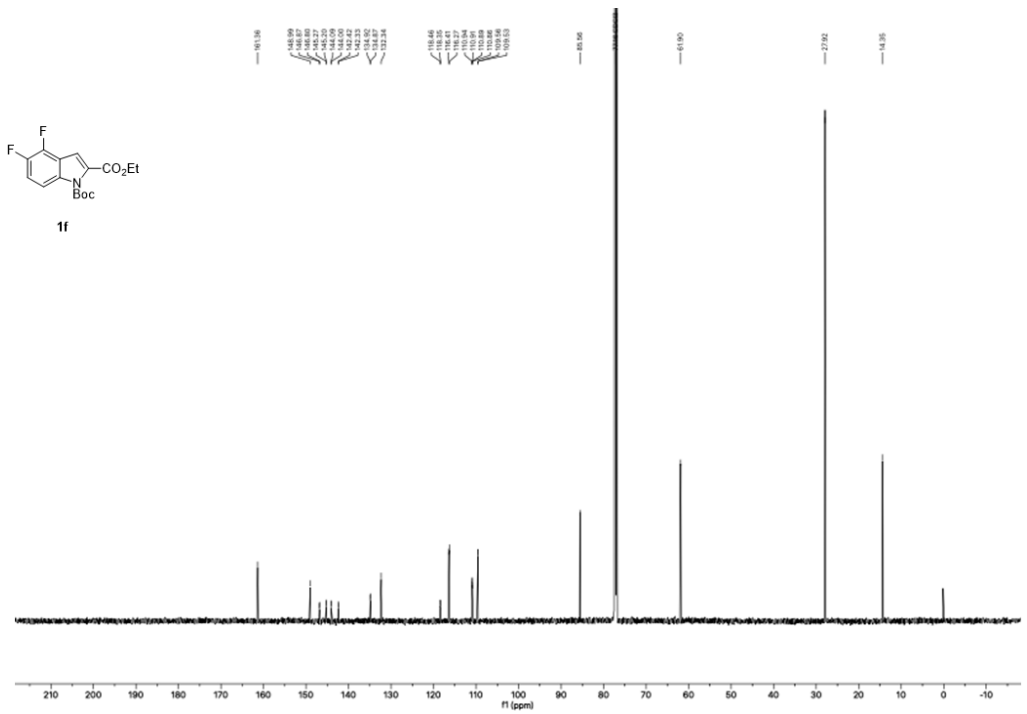

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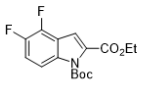
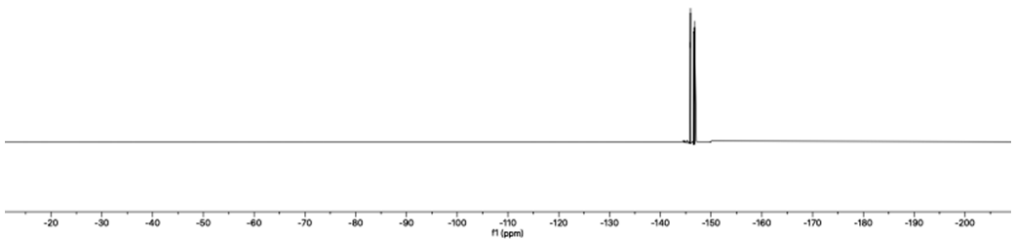
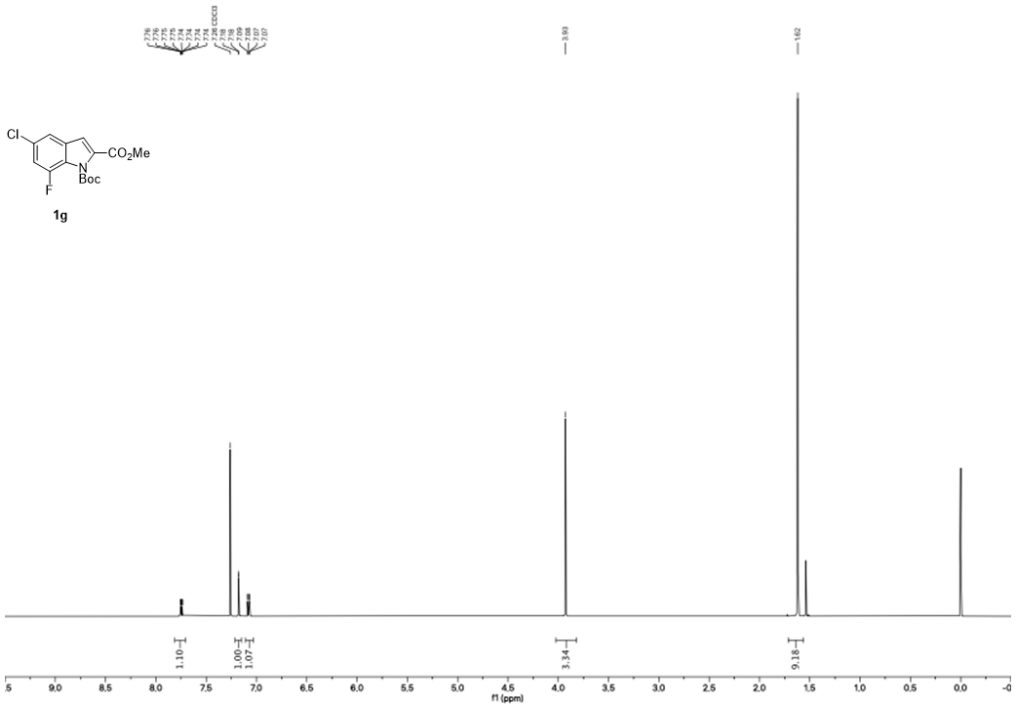


1f

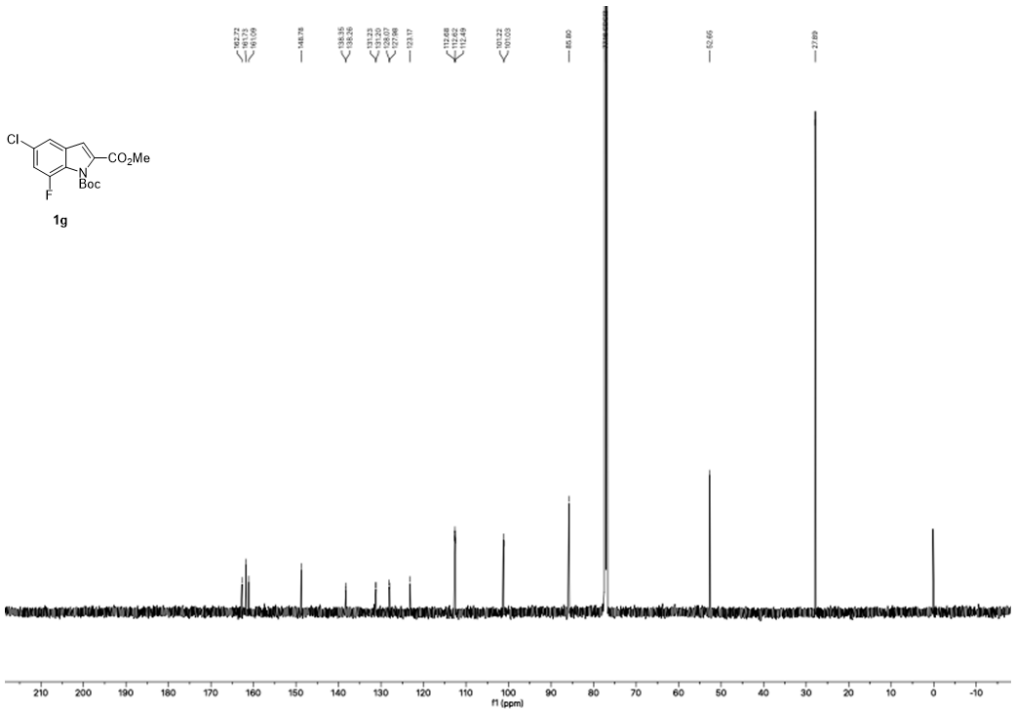


1-(*tert*-Butyl) 2-methyl 5-chloro-7-fluoro-*1H*-indole-1,2-dicarboxylate (**1g**)

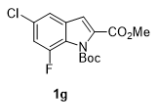
^{1}H NMR, $CDCl_3$



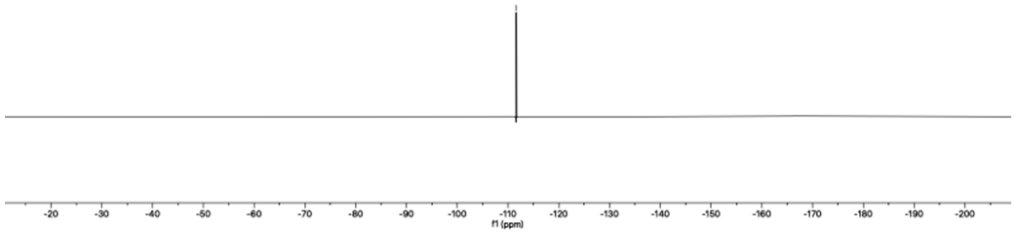
^{13}C NMR, $CDCl_3$



^{19}F NMR, $CDCl_3$

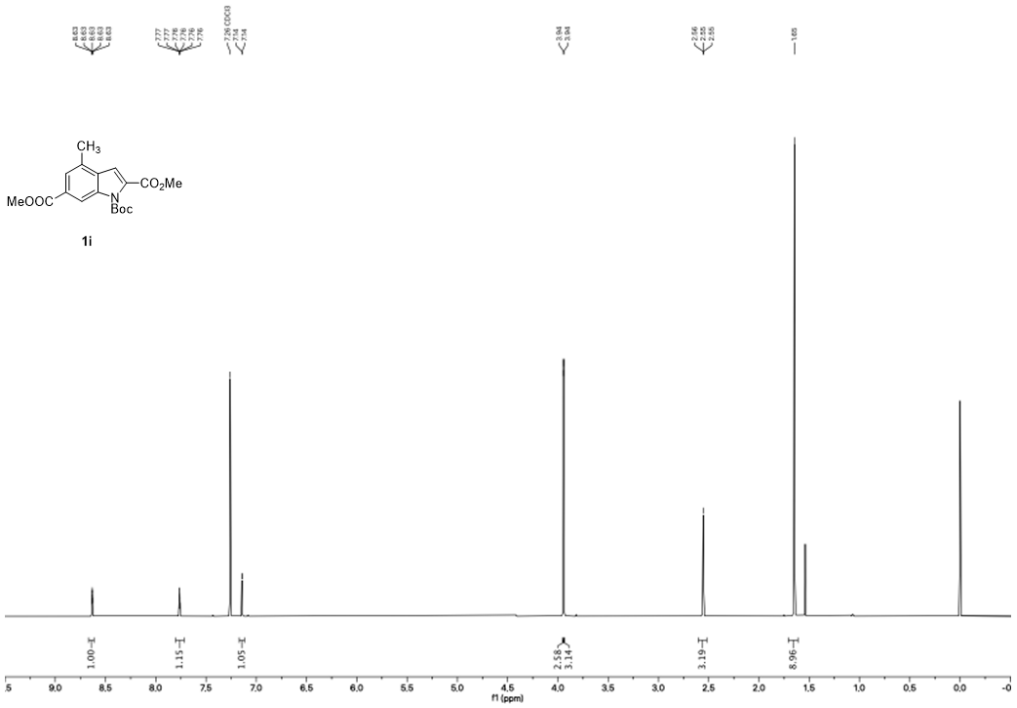


1g



1-(*tert*-Butyl) 2,6-dimethyl 4-methyl-*1H*-indole-1,2,6-tricarboxylate (**1i**)

^{1}H NMR, $CDCl_3$



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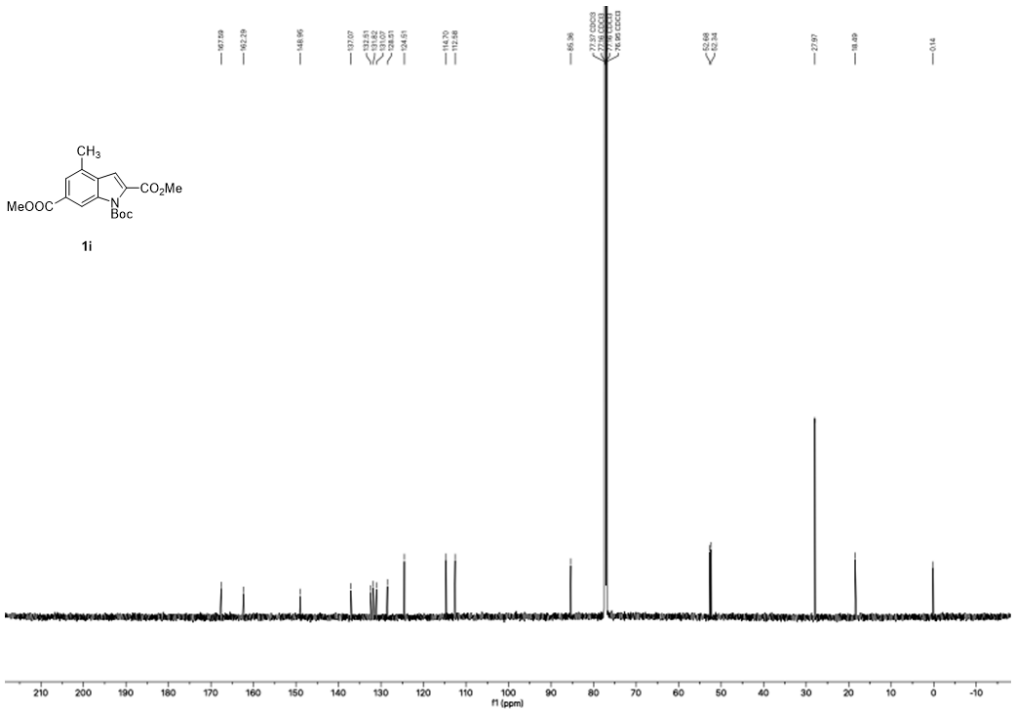
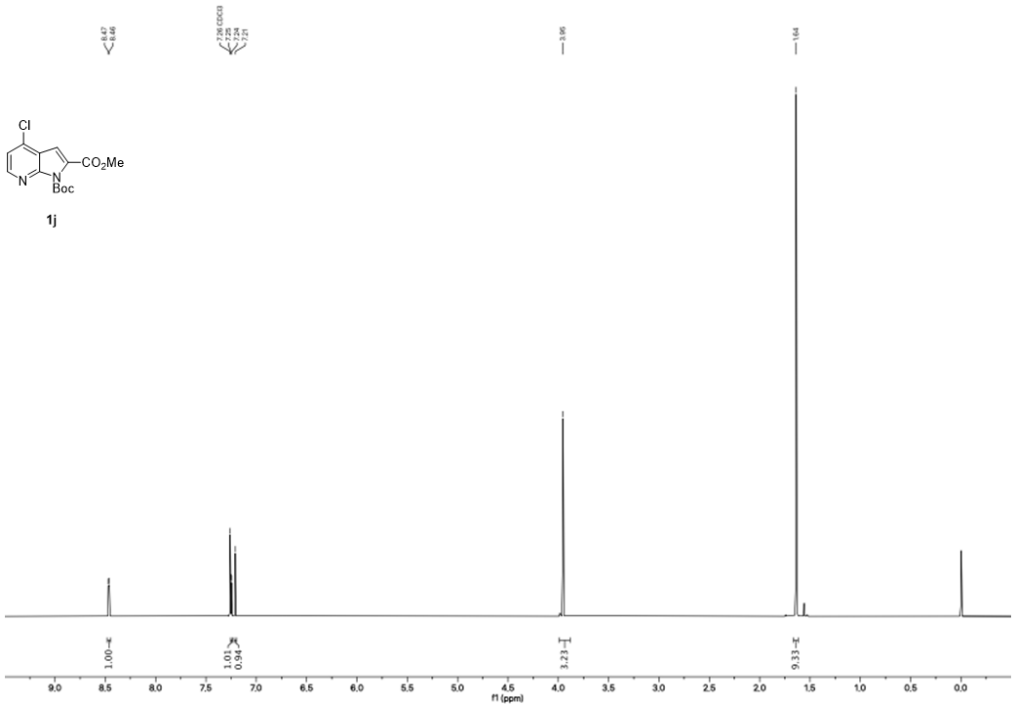


1-(*tert*-Butyl) 2-methyl 4-chloro-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (**1j**)

^{1}H NMR, $CDCl_3$



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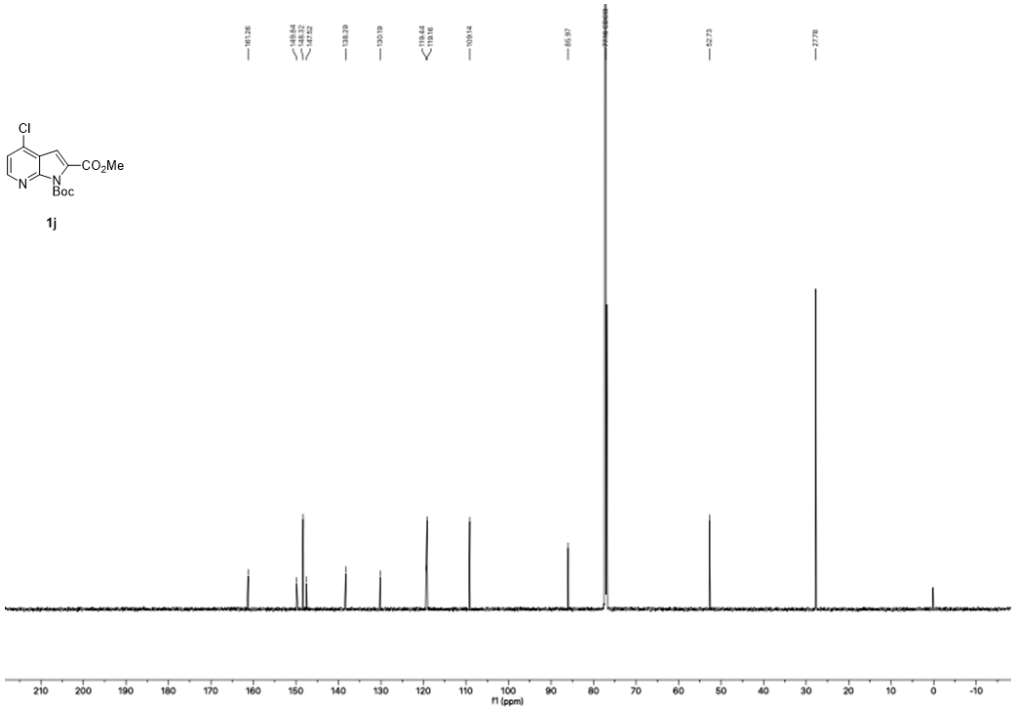
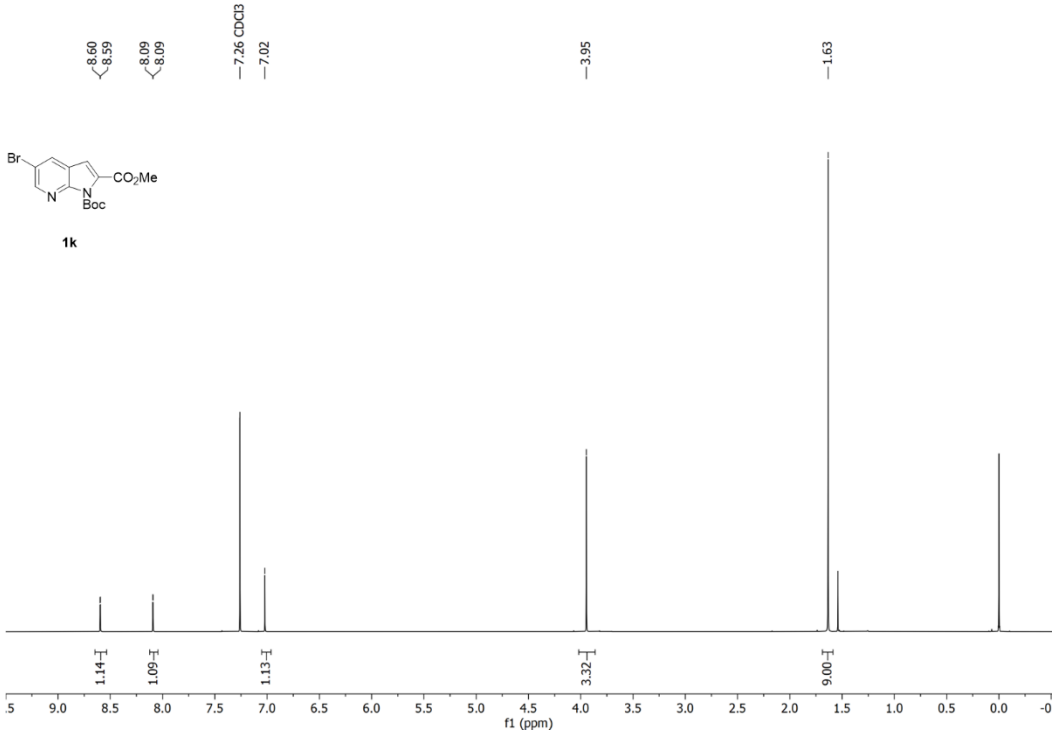


1-(*tert*-Butyl) 2-methyl 4-chloro-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (**1k**)

^{1}H NMR, $CDCl_3$



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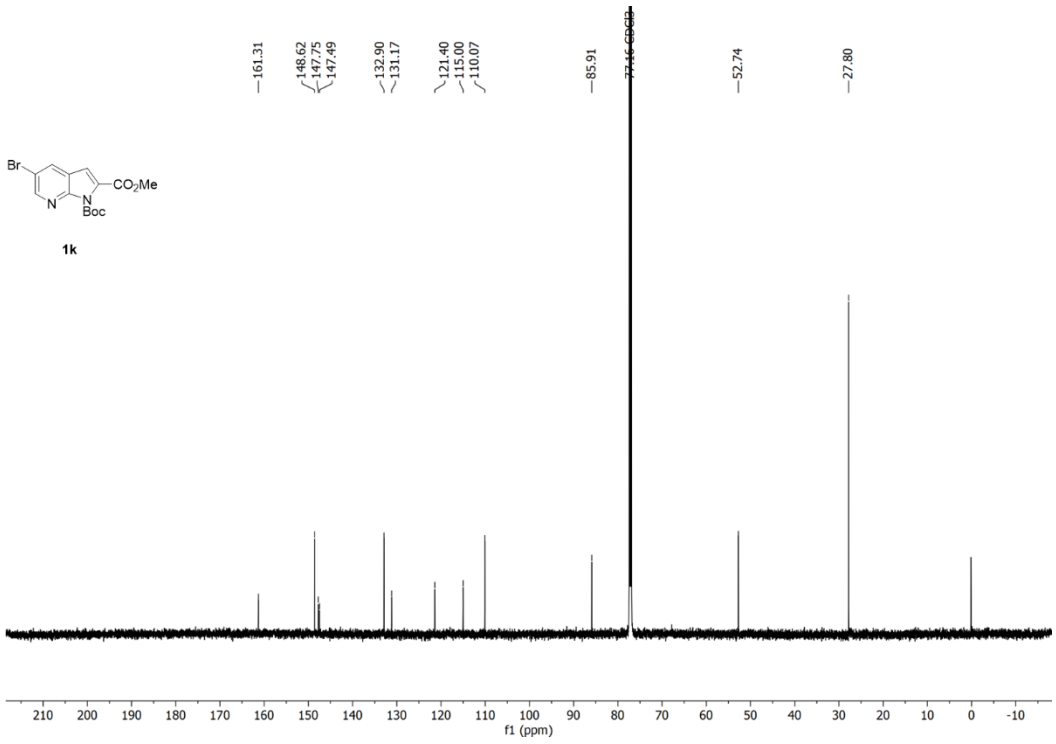
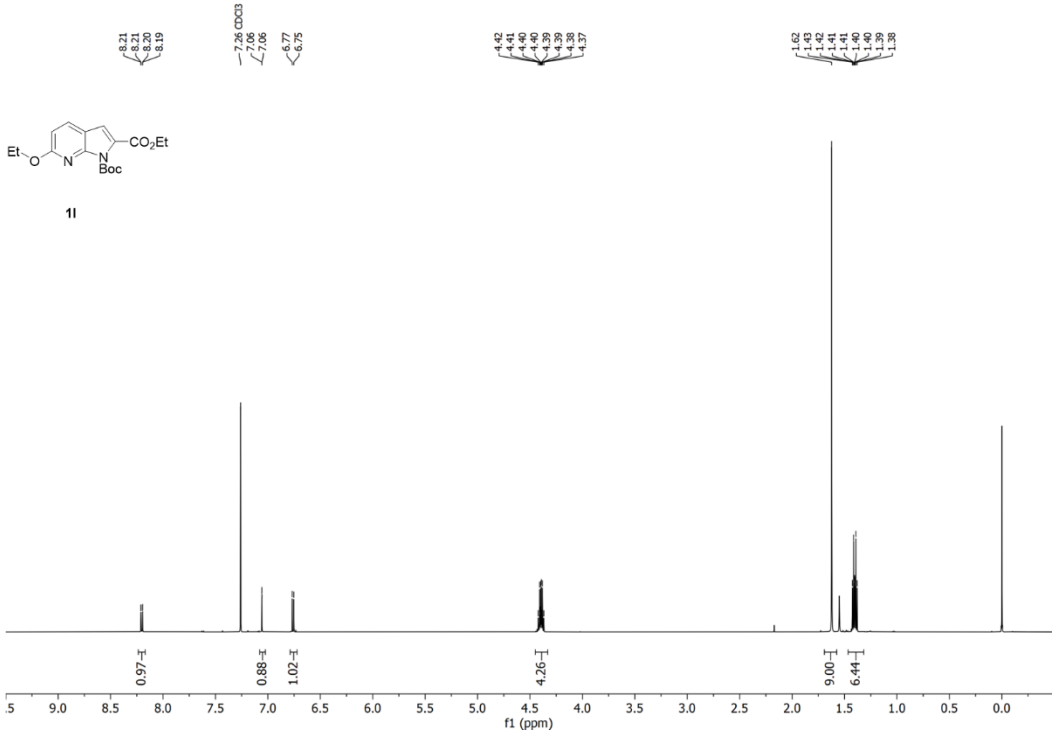
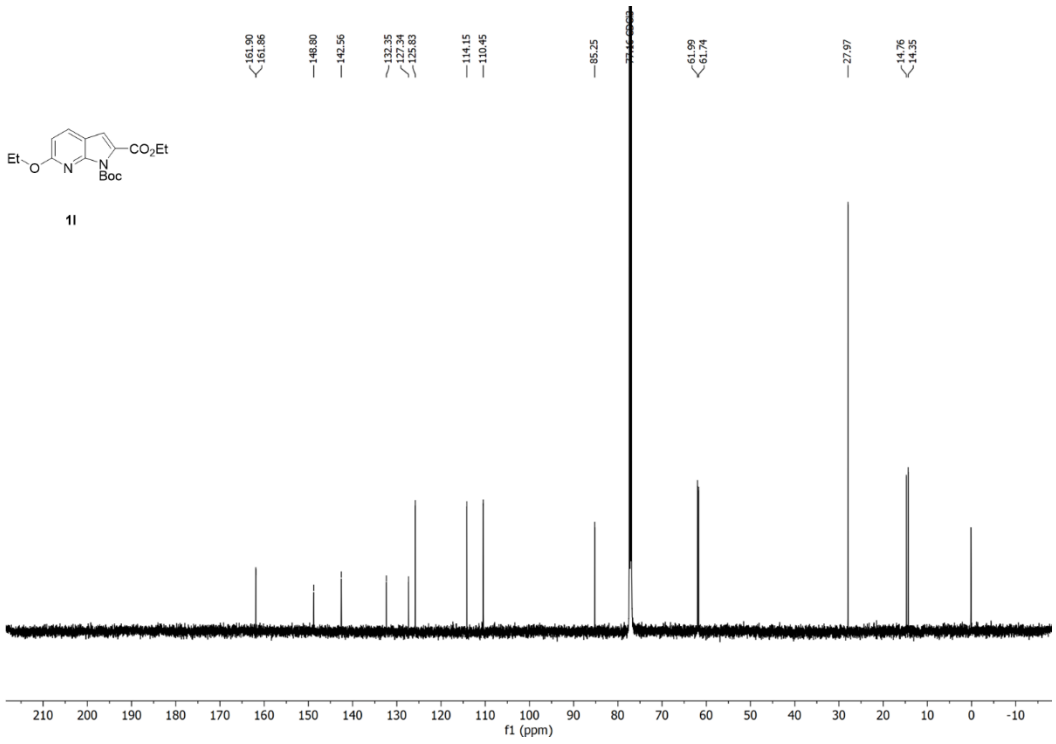


1-(*tert*-Butyl) 2-ethyl 6-ethoxy-*1H*-pyrrolo[2,3-*b*]pyridine-1,2-dicarboxylate (**1l**)

^{1}H NMR, $CDCl_3$



^{13}C NMR, $CDCl_3$



7-(*tert*-Butyl) 6-methyl 4-chloro-*7H*-pyrrolo[2,3-*d*]pyrimidine-6,7-dicarboxylate (**1m**)

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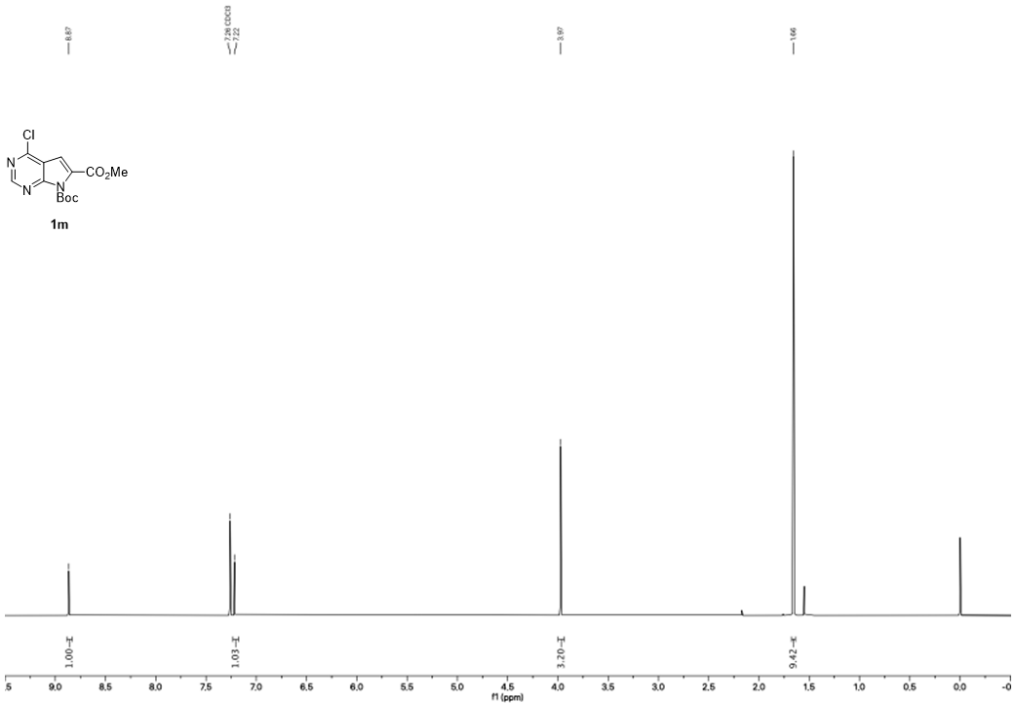
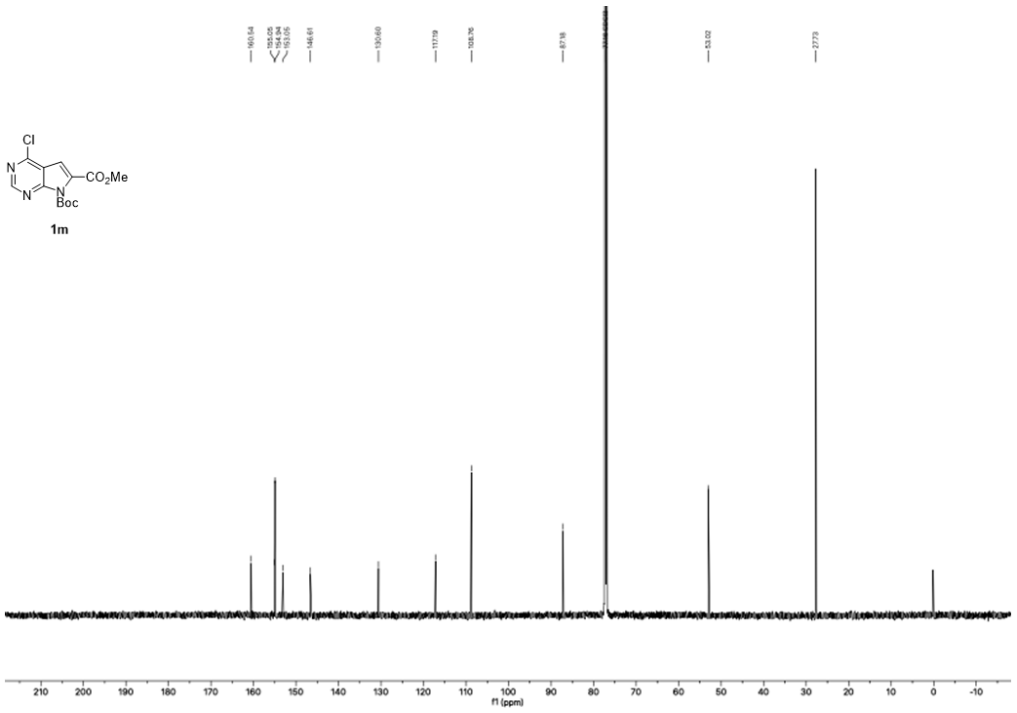
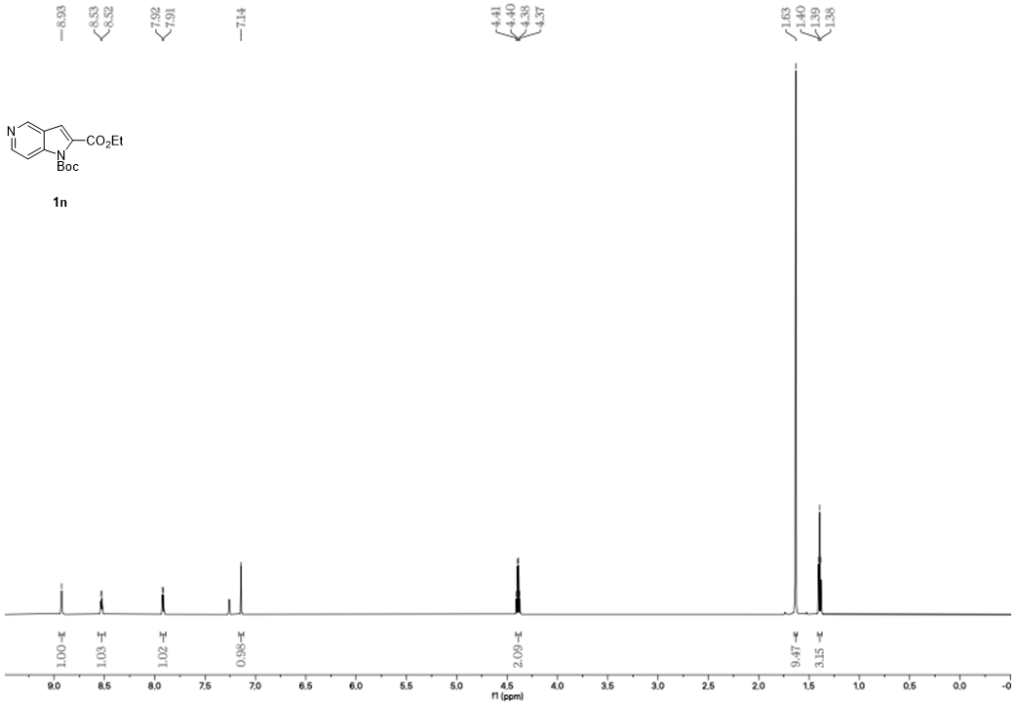


^{13}C NMR, $CDCl_3$

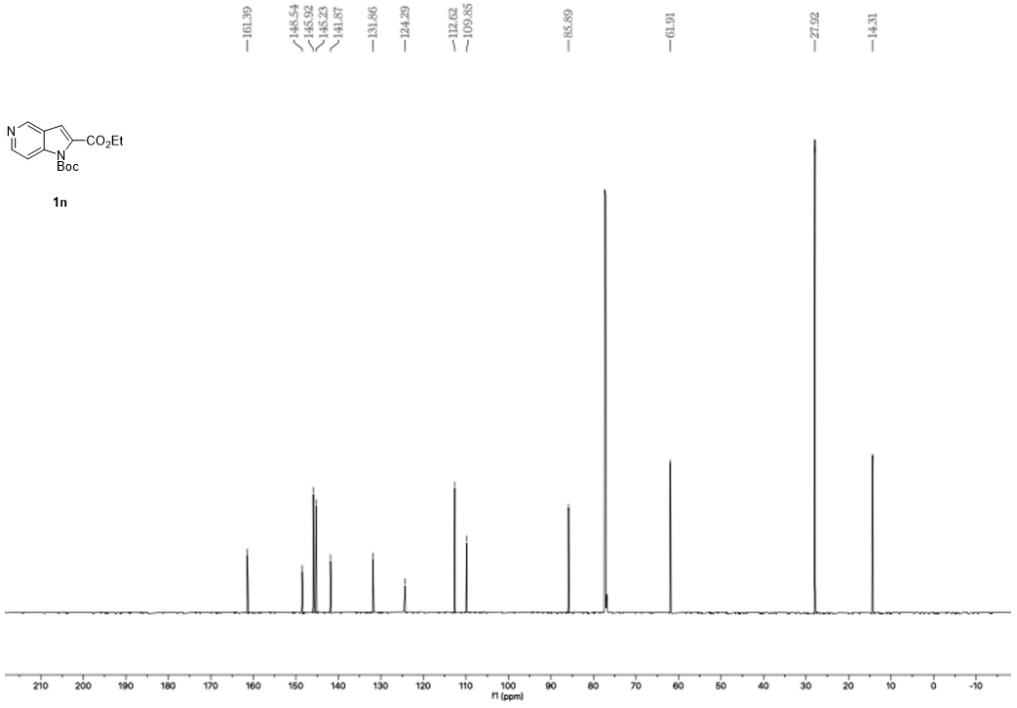


1-(*tert*-Butyl) 2-ethyl 1*H*-pyrrolo[3,2-*c*]pyridine-1,2-dicarboxylate (**1n**)

^{1}H NMR, $CDCl_3$

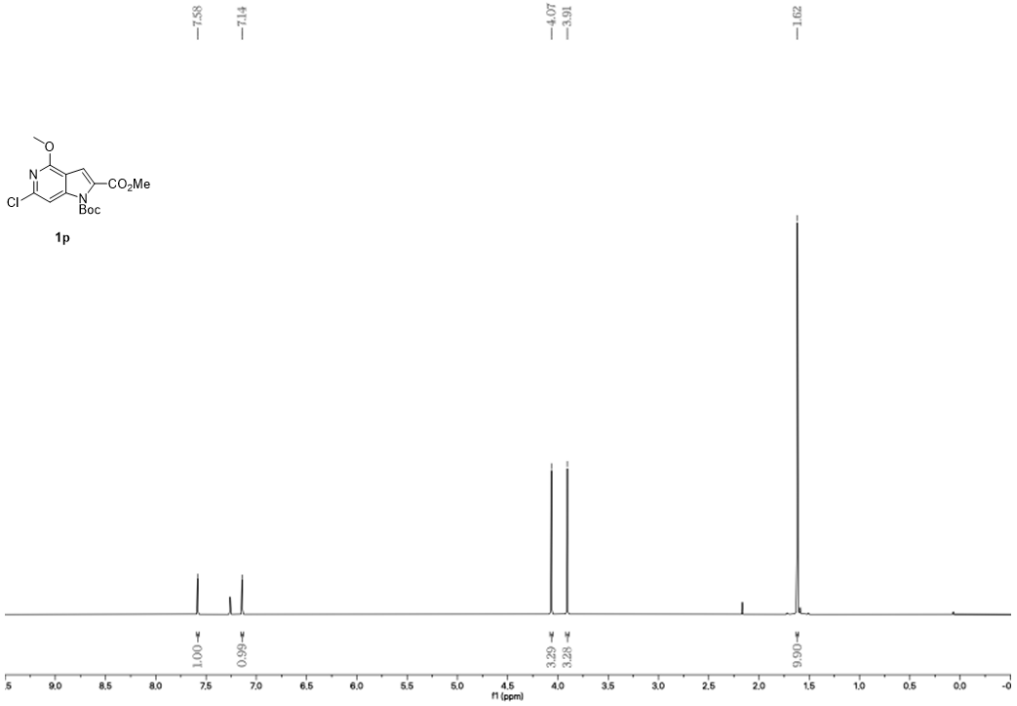


^{13}C NMR, $CDCl_3$

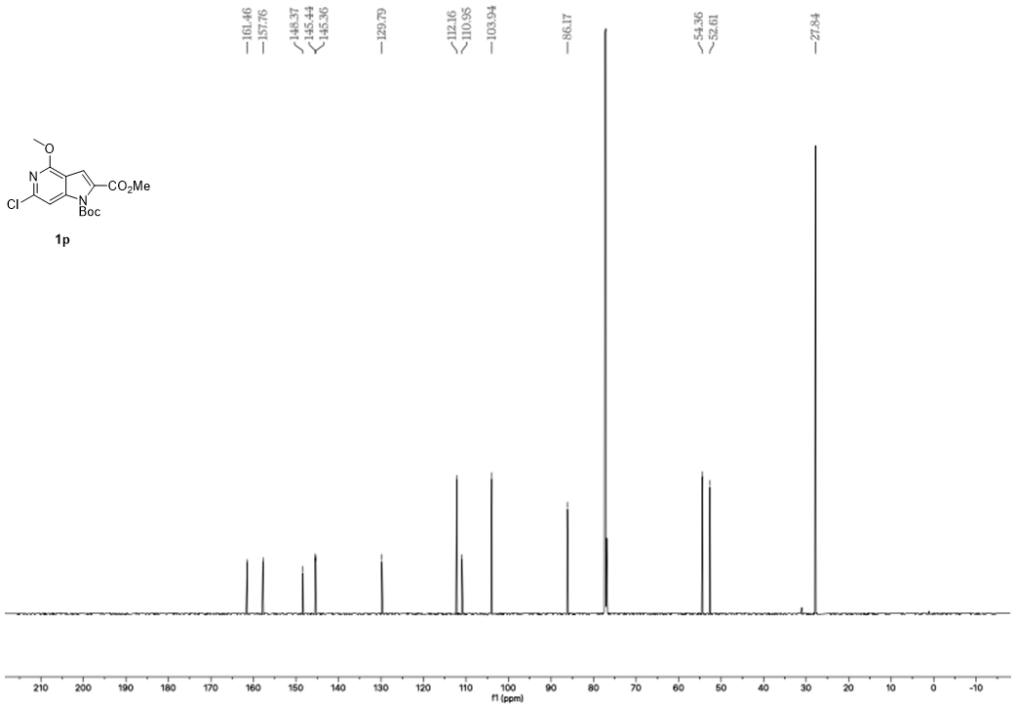


1-(*tert*-Butyl) 2-methyl 6-chloro-4-methoxy-1*H*-pyrrolo[3,2-*c*]pyridine-1,2-dicarboxylate (**1p**)

^{1}H NMR, $CDCl_3$

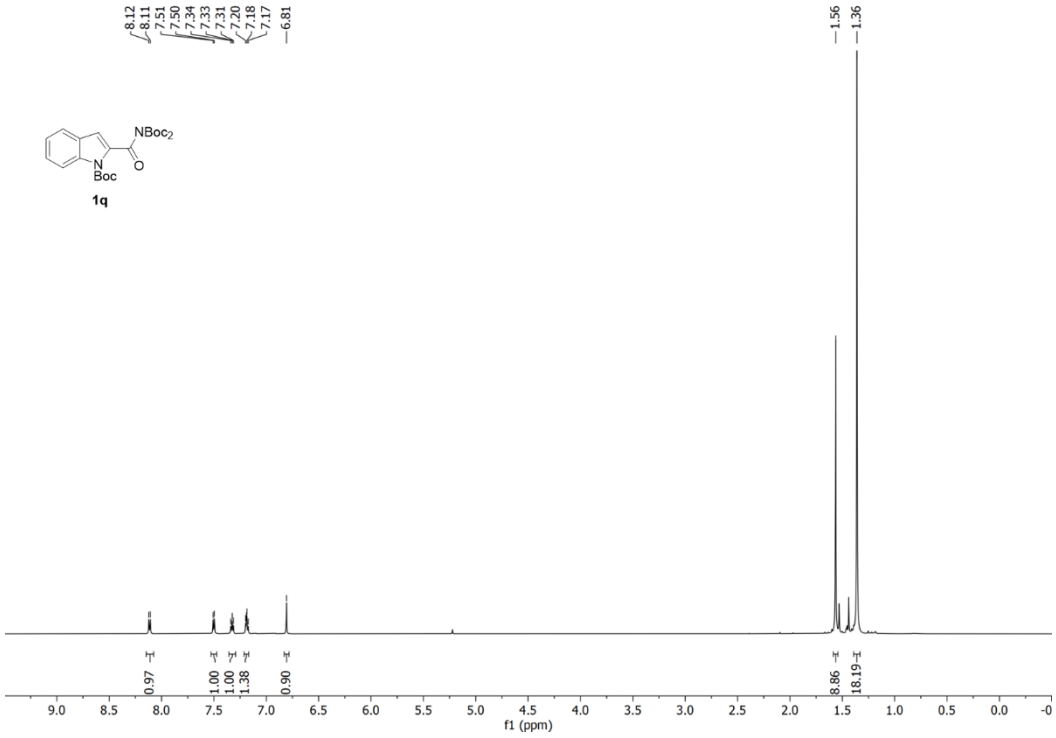


^{13}C NMR, $CDCl_3$

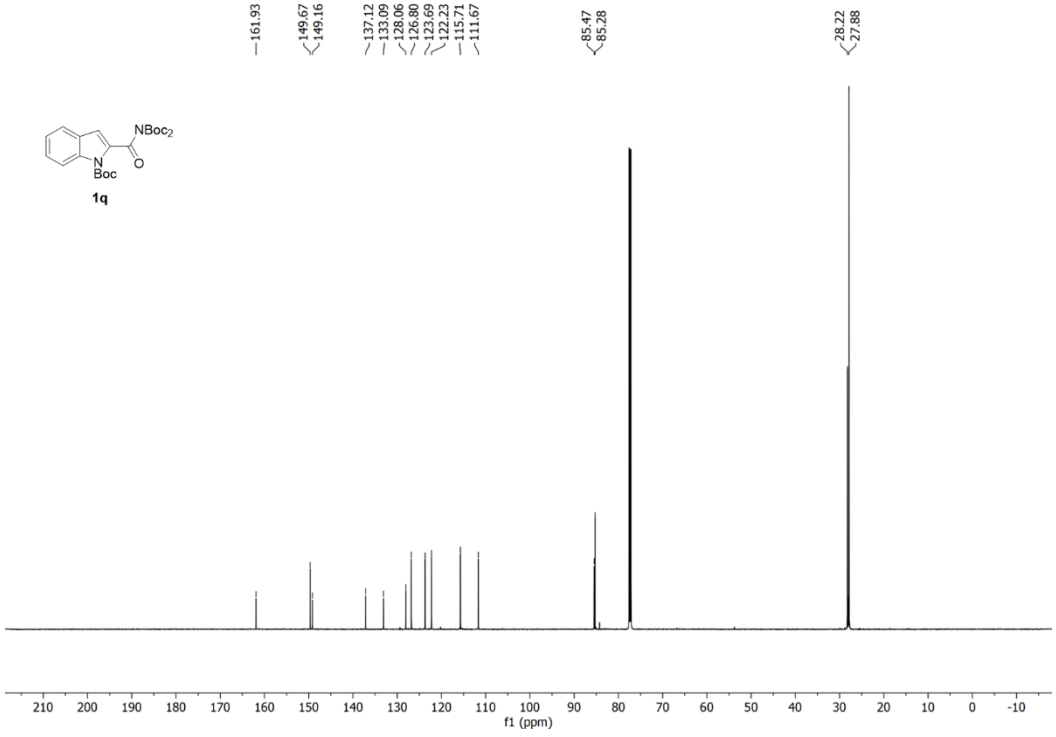


tert-Butyl 2-(bis(*tert*-butoxycarbonyl)carbamoyl)-1*H*-indole-1-carboxylate (**1q**)

^{1}H NMR, $CDCl_3$

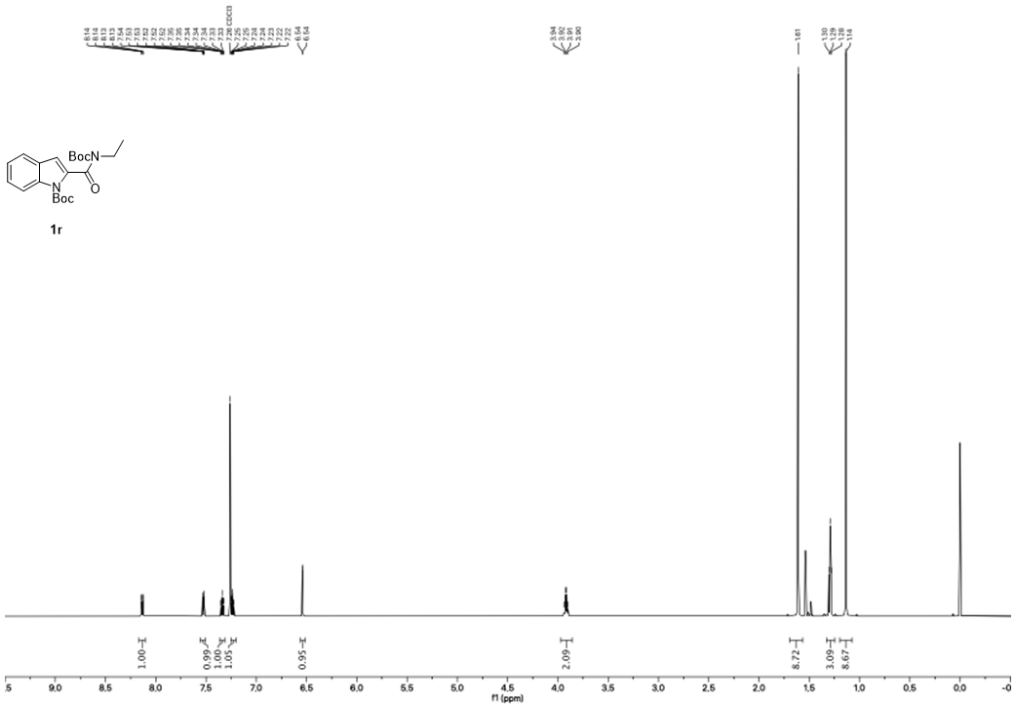


^{13}C NMR, $CDCl_3$

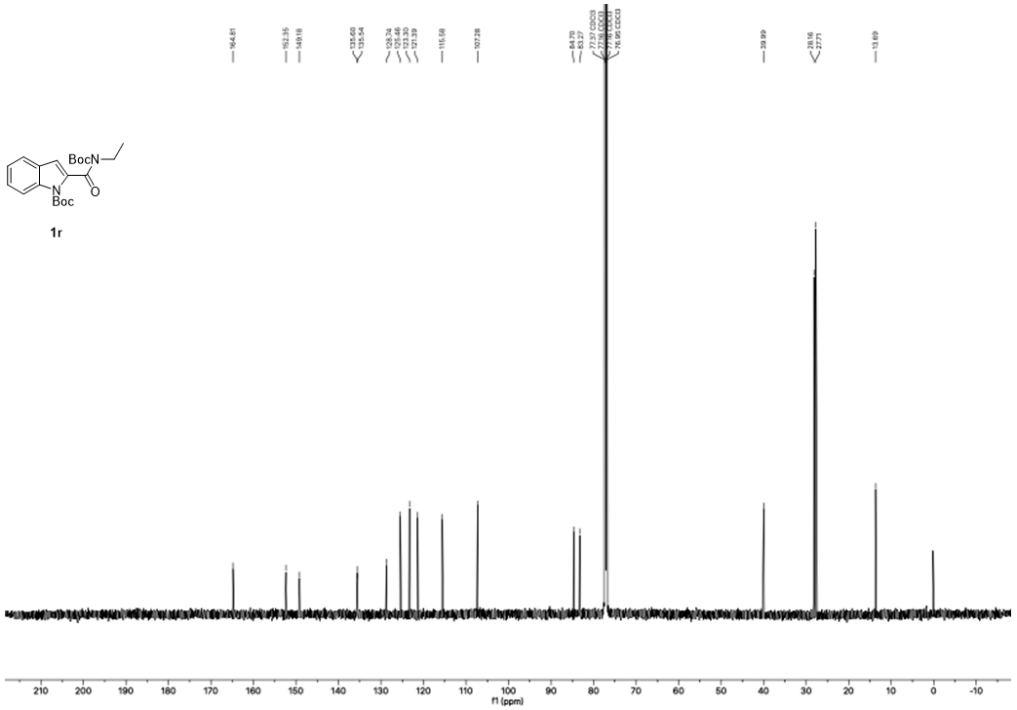


tert-Butyl 2-((*tert*-butoxycarbonyl)(ethyl)carbamoyl)-*1H*-indole-1-carboxylate (**1r**)

^{1}H NMR, $CDCl_3$

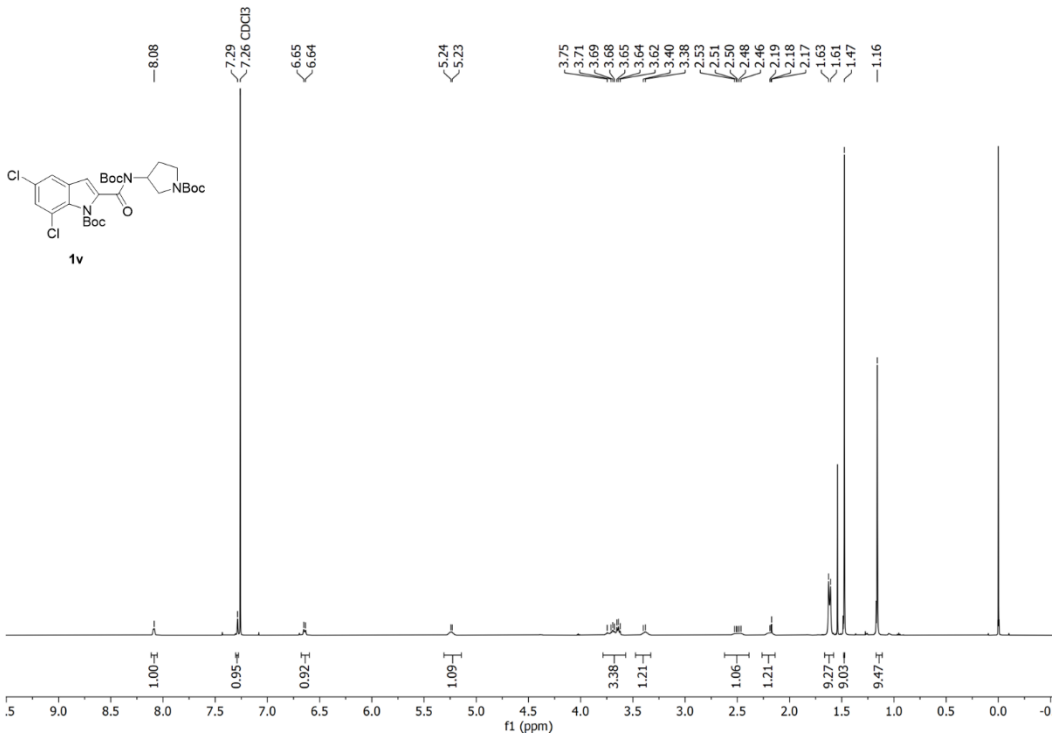


^{13}C NMR, $CDCl_3$

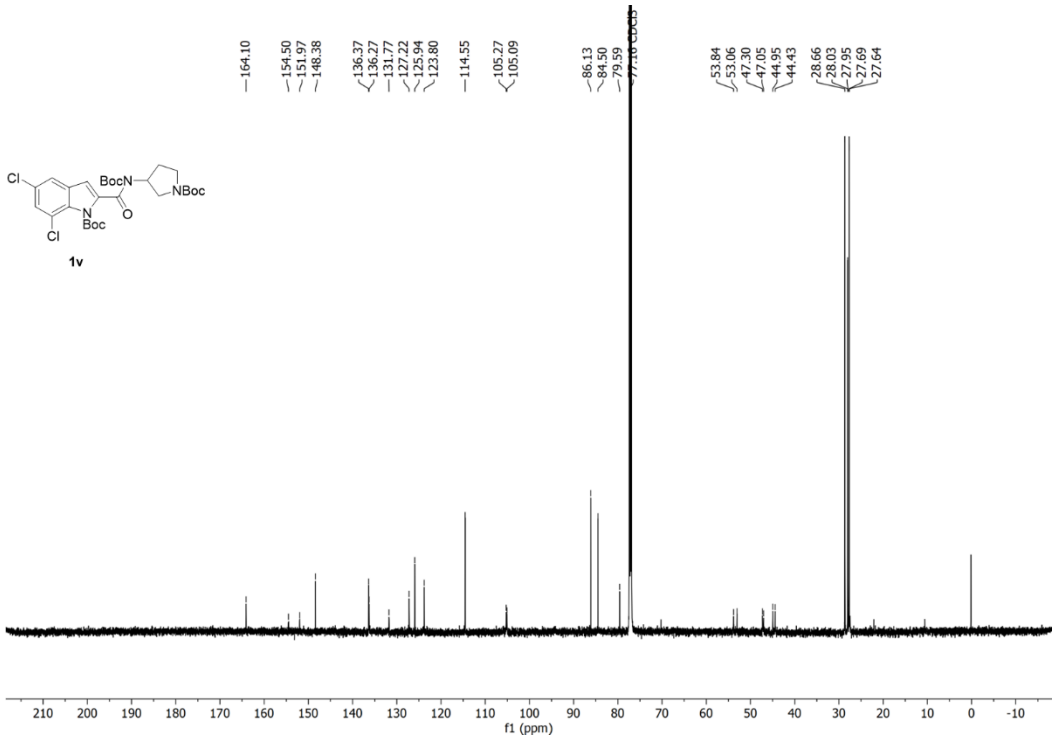


tert-Butyl 2-((*tert*-butoxycarbonyl)(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)carbamoyl)-5,7-dichloro-*1H*-indole-1-carboxylate (**1v**)

^{1}H NMR, $CDCl_3$

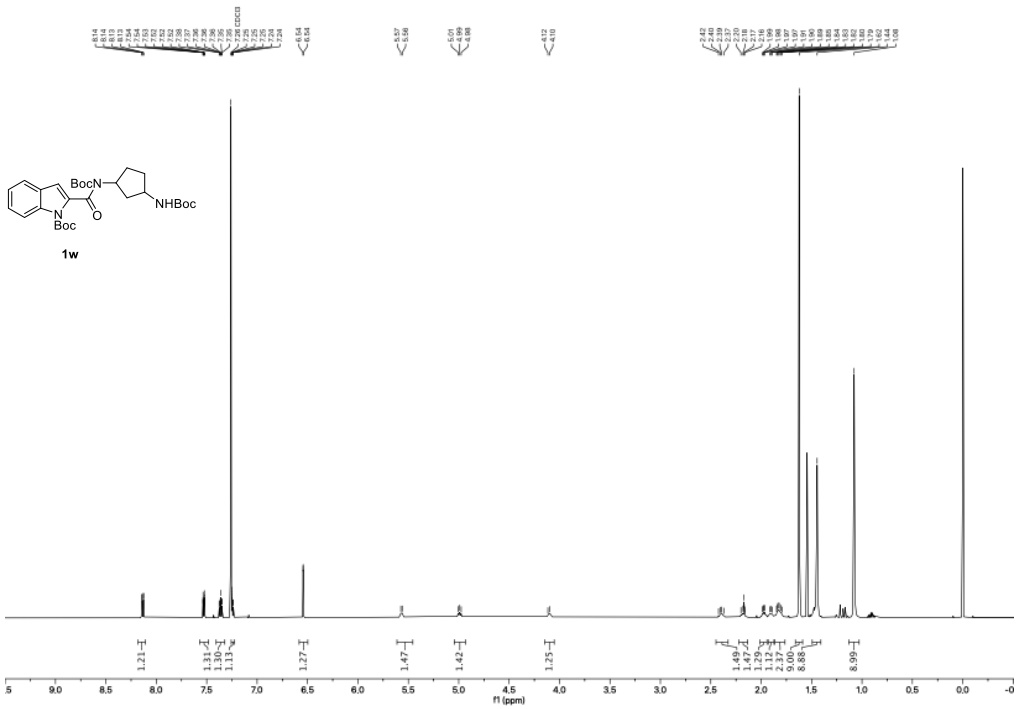


^{13}C NMR, $CDCl_3$

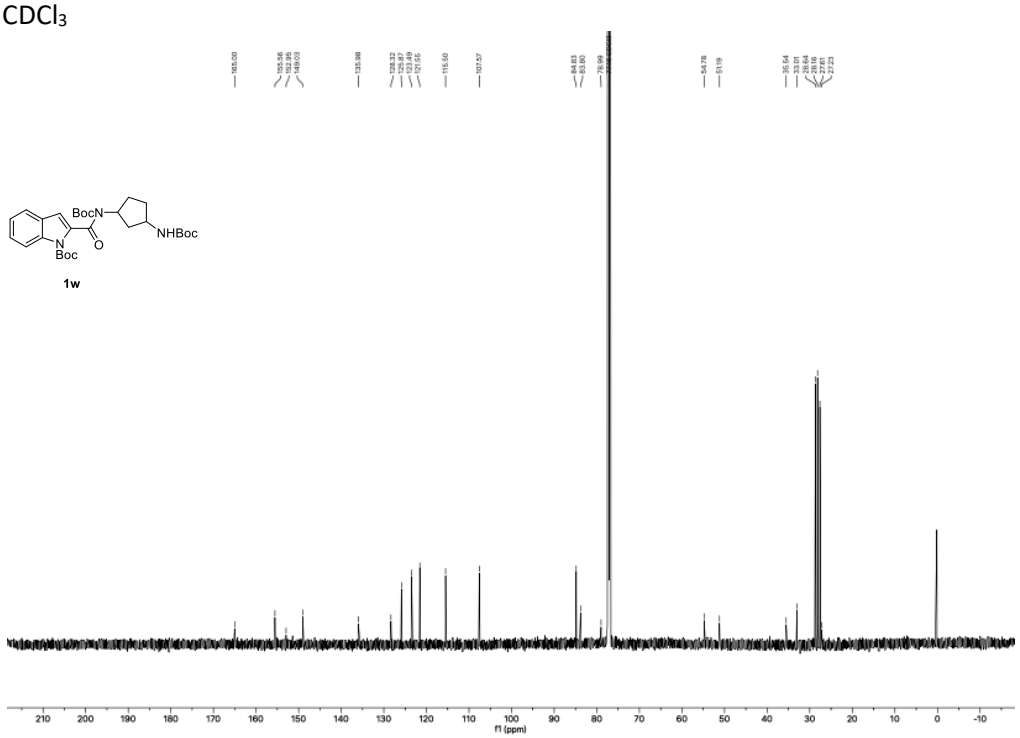


tert-Butyl 2-((*tert*-butoxycarbonyl)(3-((*tert*-butoxycarbonyl)amino)cyclopentyl)carbamoyl)-*1H*-indole-1-carboxylate (1w)

^{1}H NMR, $CDCl_3$

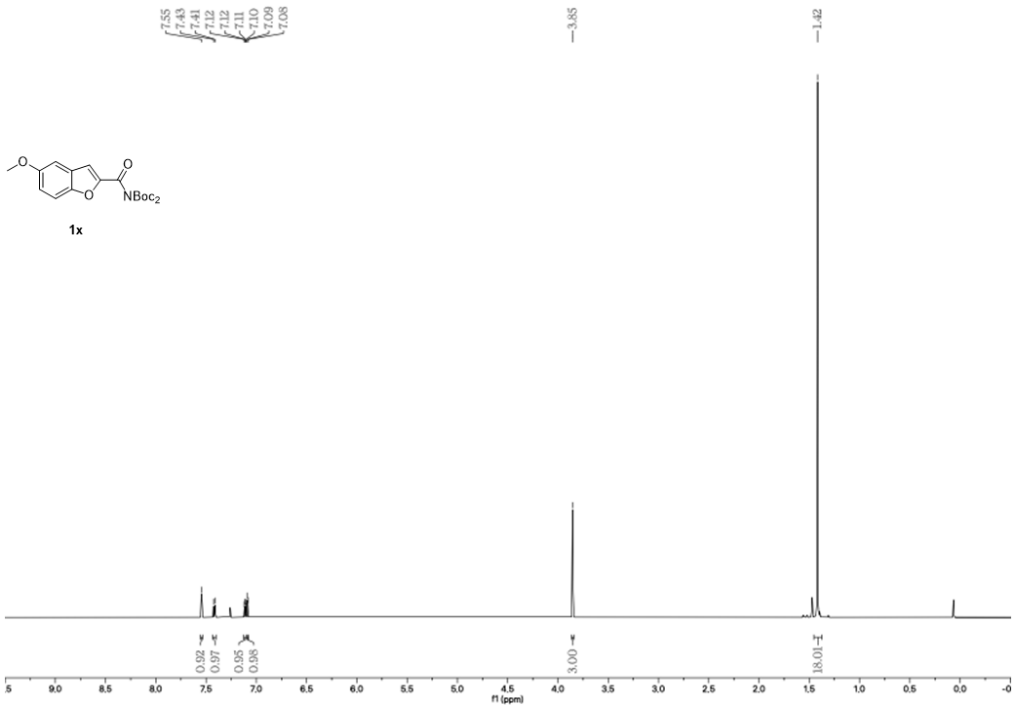


^{13}C NMR, $CDCl_3$

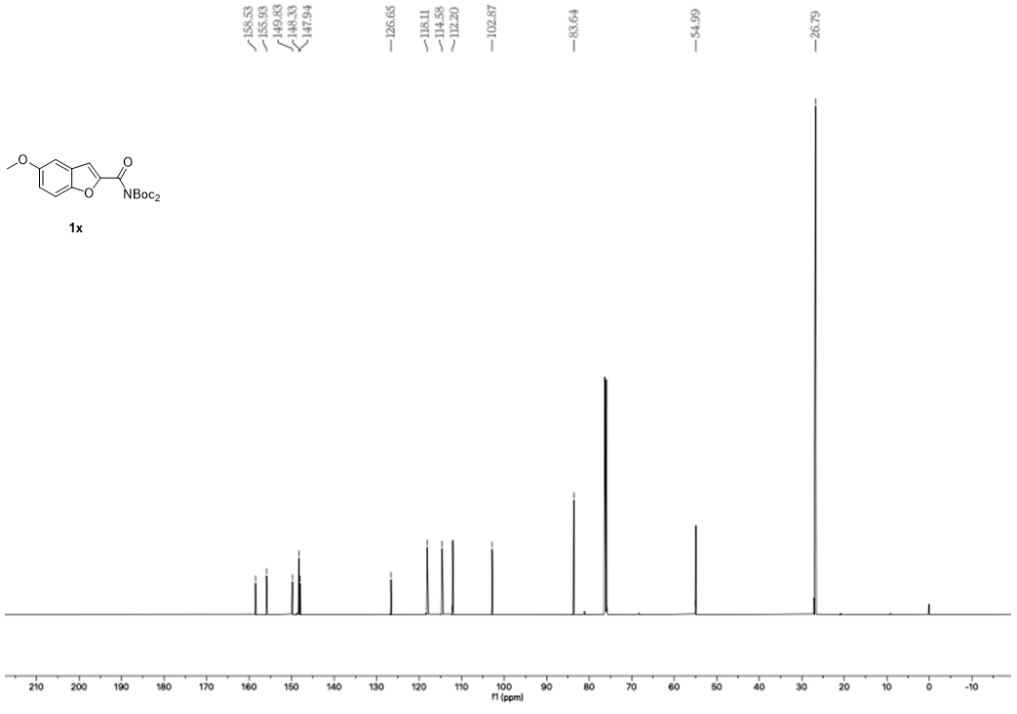


tert-Butyl 2-((*tert*-butoxycarbonyl)(3-((*tert*-butoxycarbonyl)amino)cyclopentyl)carbamoyl)-*1H*-indole-1-carboxylate (**1x**)

^{1}H NMR, $CDCl_3$

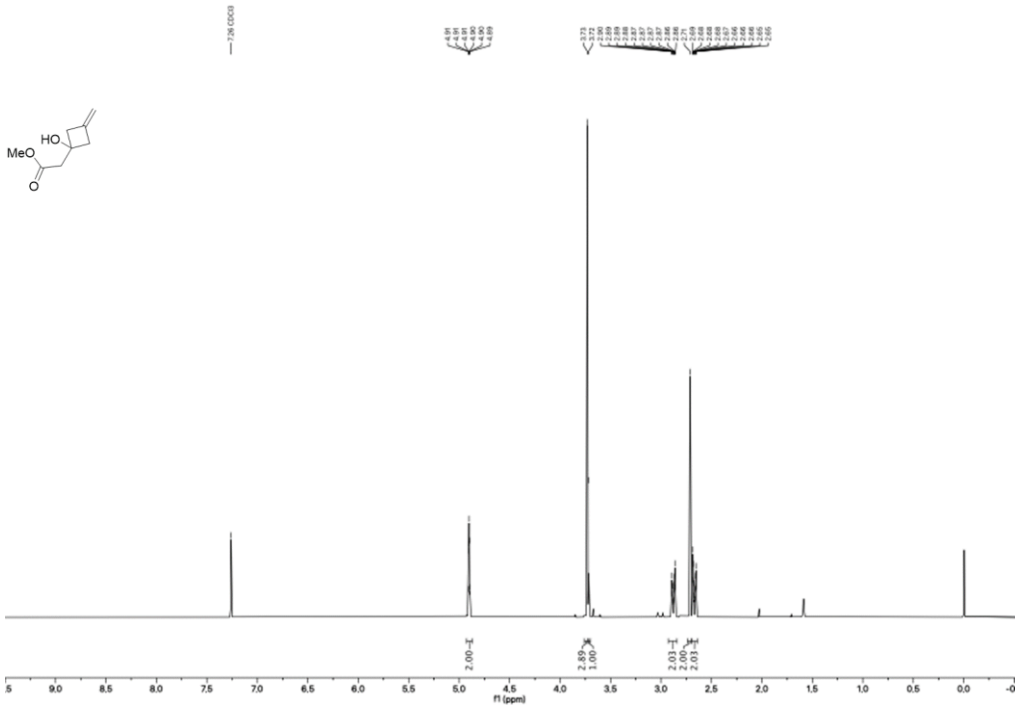


^{13}C NMR, $CDCl_3$

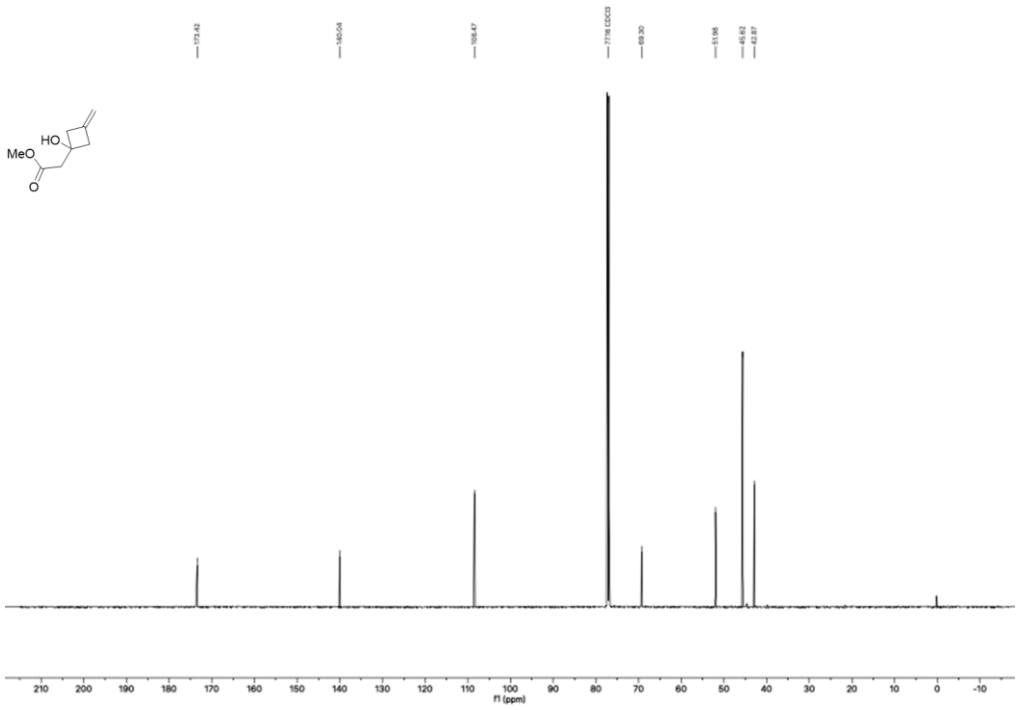


Preparation of methyl 2-(1-hydroxy-3-methylenecyclobutyl)acetate (**S1-3**)

^{1}H NMR, $CDCl_3$

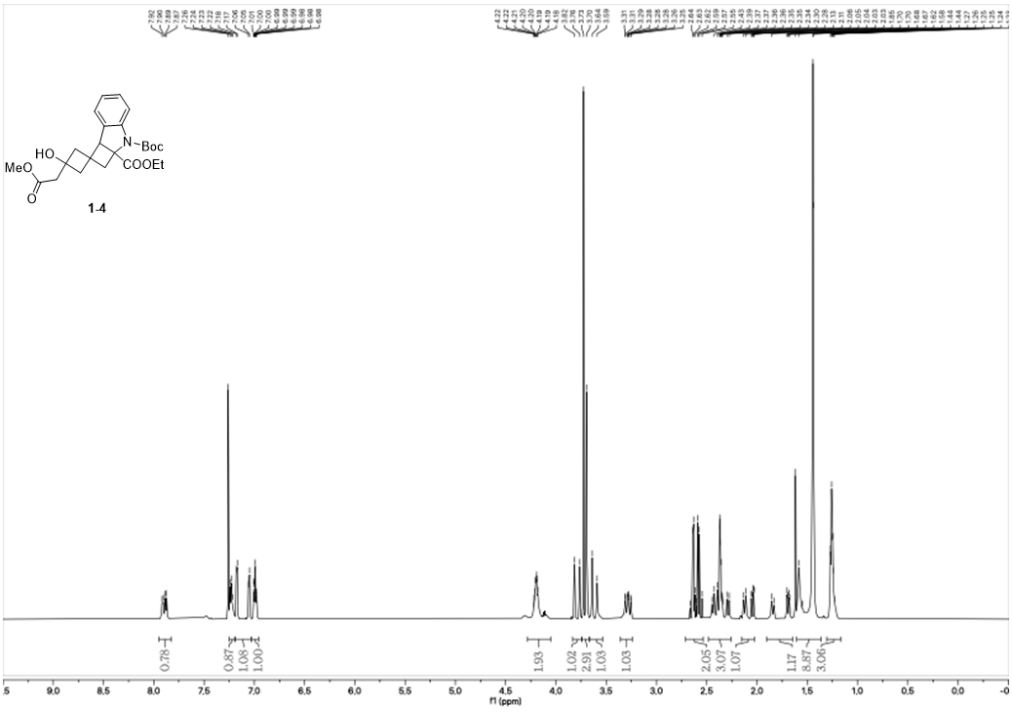


^{13}C NMR, $CDCl_3$

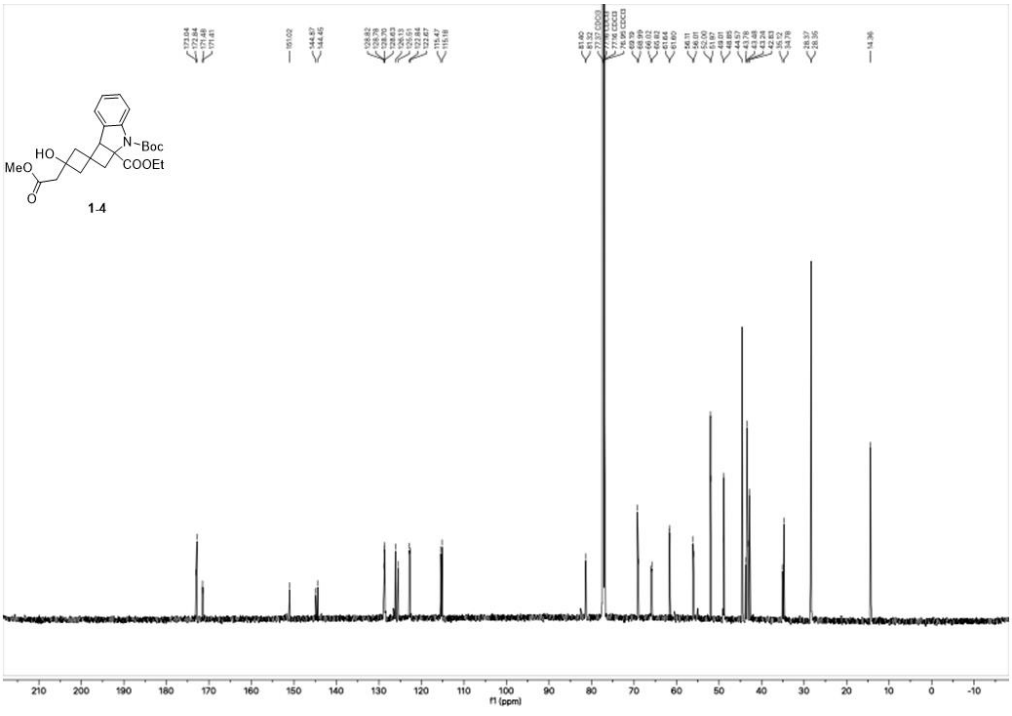


3'-(*tert*-Butyl) 2a'-ethyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)spiro[cyclobutane-1,1'-cyclobuta[b]indole]-2a',3'(2'H,7*b*'H)-dicarboxylate (**S1-4**)

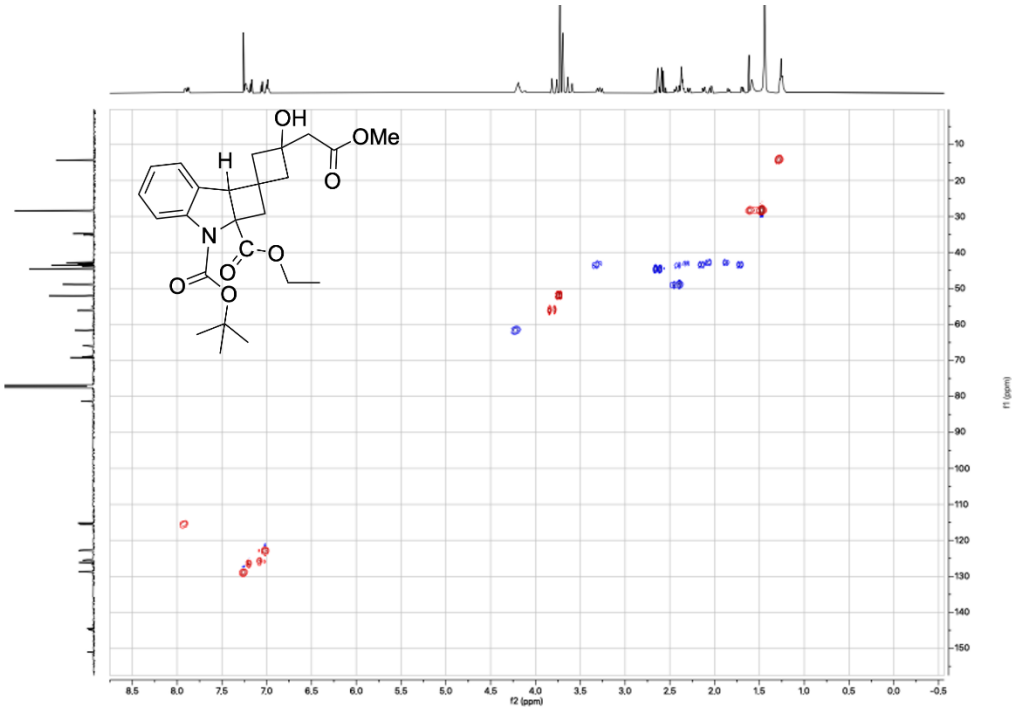
^{1}H NMR, $CDCl_3$



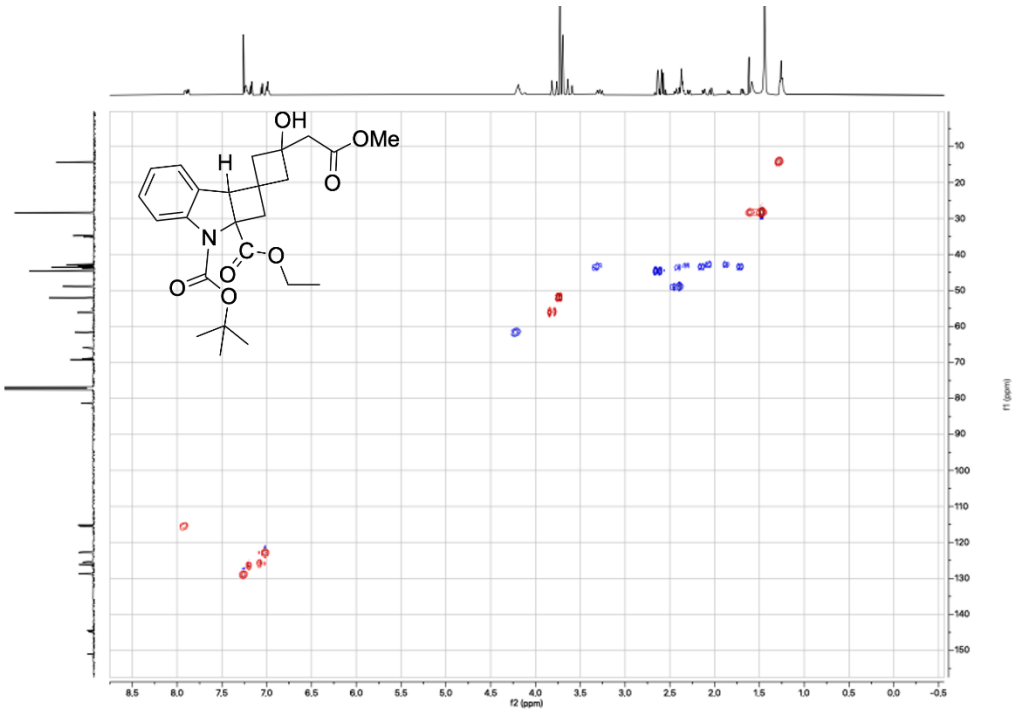
^{13}C NMR, $CDCl_3$



HSQC NMR, $CDCl_3$

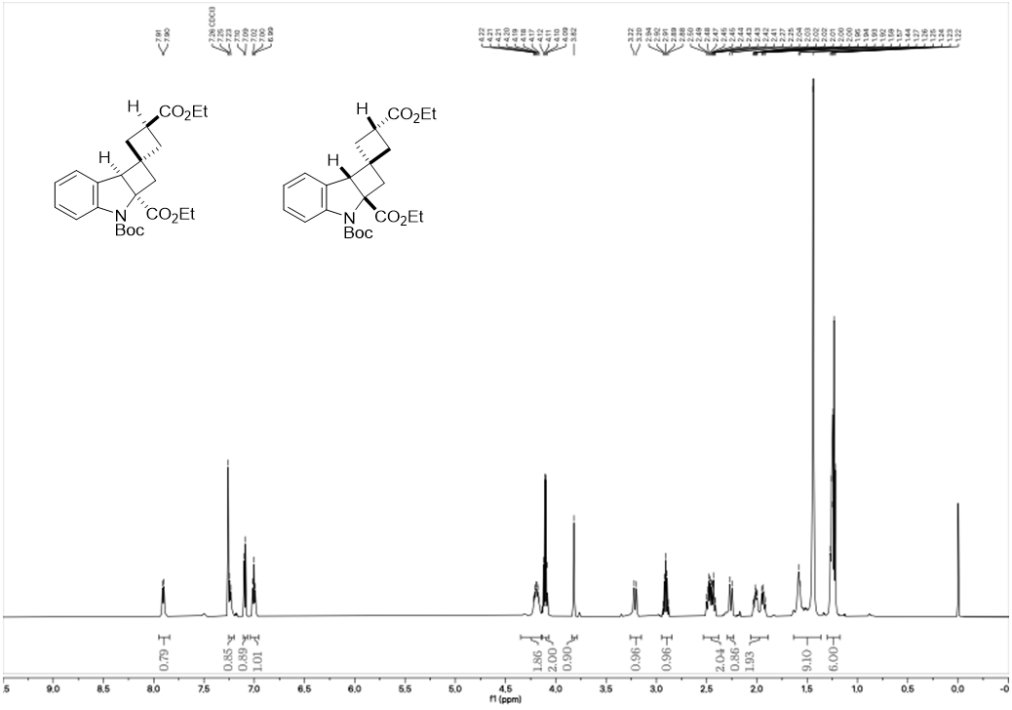


HMBC NMR, $CDCl_3$

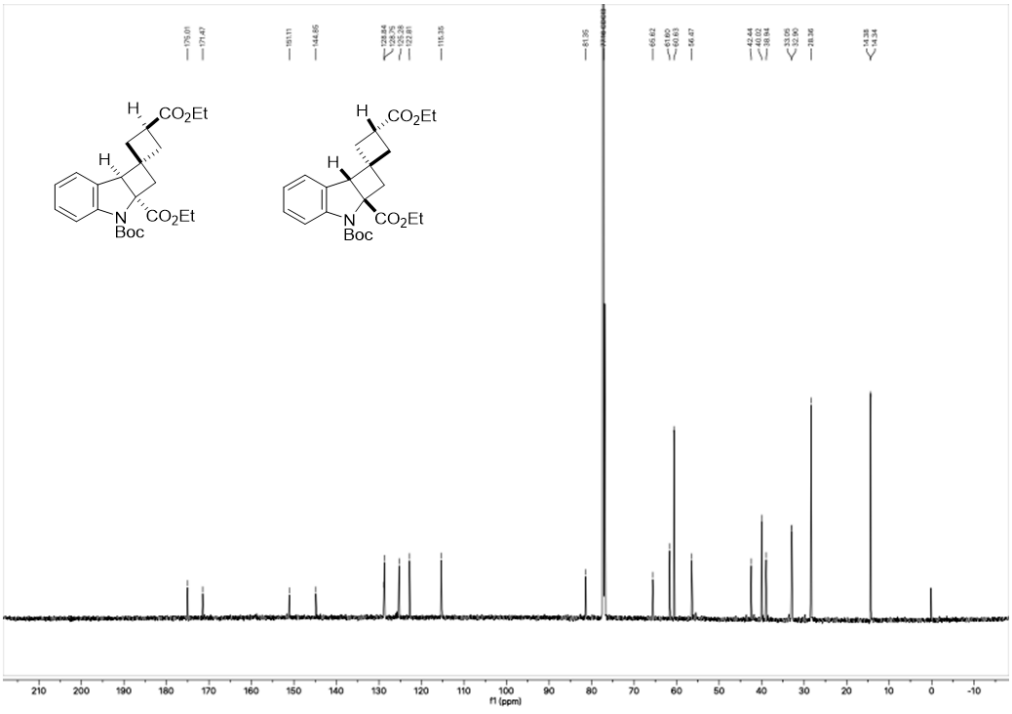


3'-(*tert*-Butyl) 2a',3-diethyl spiro[cyclobutane-1,1'-cyclobuta[*b*]indole]-2a',3,3'(2'*H*,7b'*H*)-tricarboxylate (**S1-6**)

^{1}H NMR, $CDCl_3$

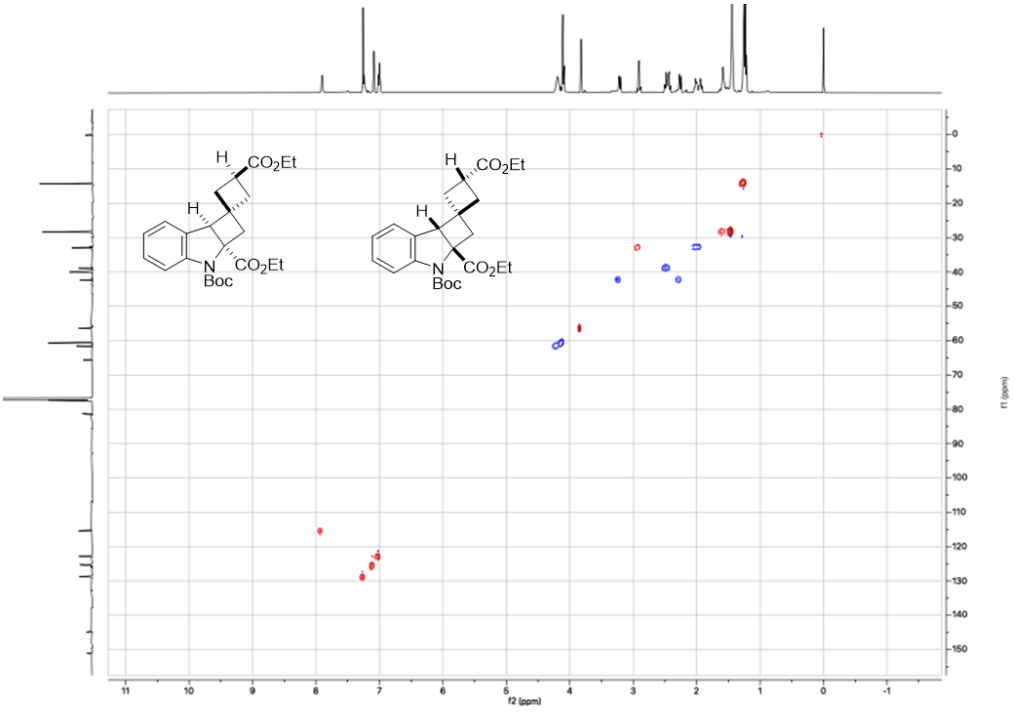


^{13}C NMR, $CDCl_3$

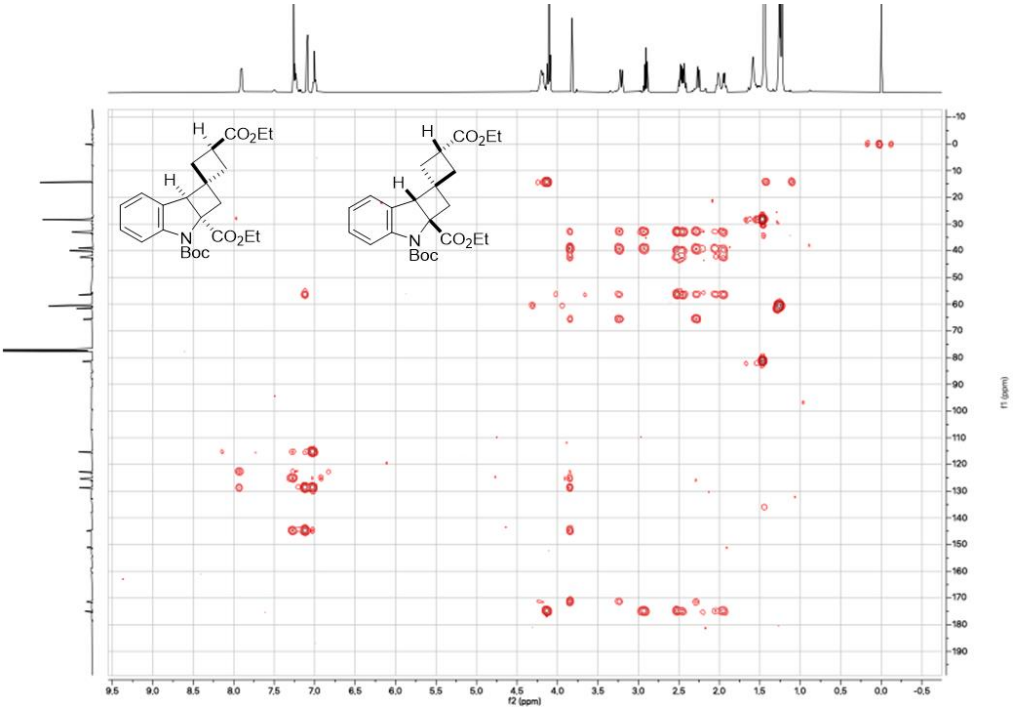


3'-(*tert*-Butyl) 2a',3-diethyl spiro[cyclobutane-1,1'-cyclobuta[*b*]indole]-2a',3,3'(2'*H*,7b'*H*)-tricarboxylate (**S1-6**)

HSQC NMR, $CDCl_3$

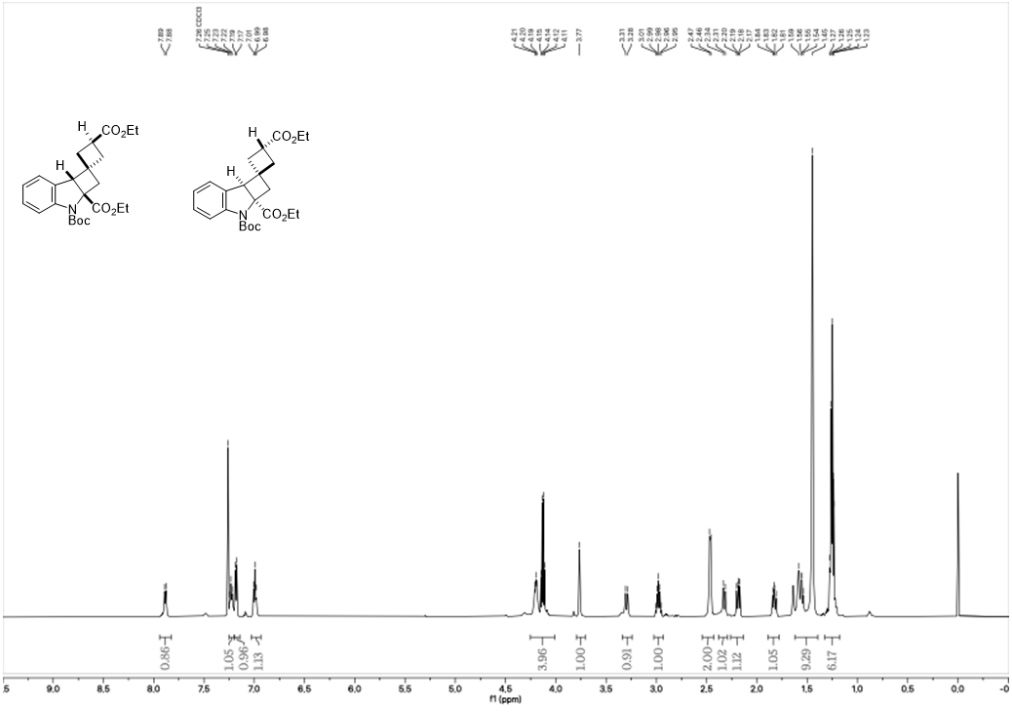


HMBC NMR, $CDCl_3$

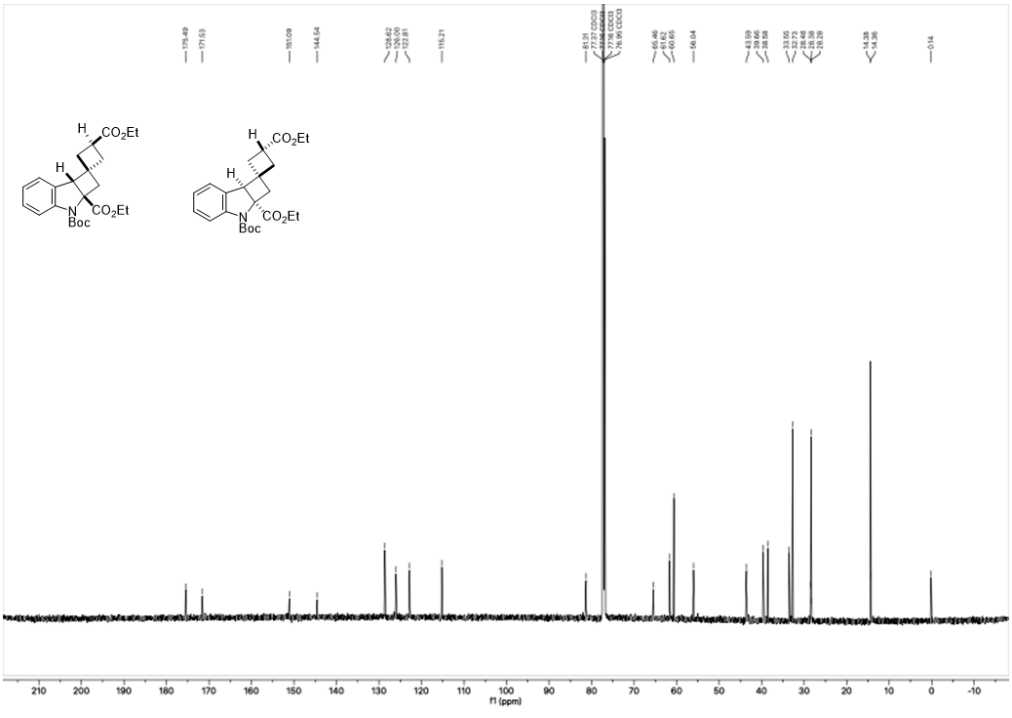


3'-(*tert*-Butyl) 2a',3-diethyl spiro[cyclobutane-1,1'-cyclobuta[*b*]indole]-2a',3,3'(2'*H*,7b'*H*)-tricarboxylate (**S1-6**)

^{1}H NMR, $CDCl_3$

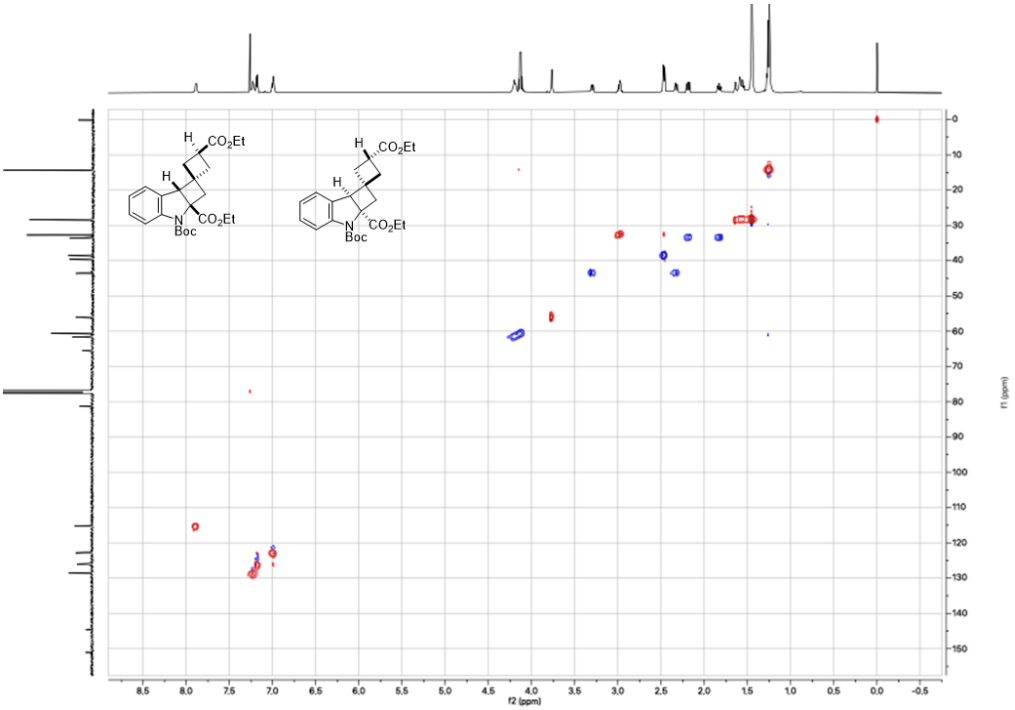


^{13}C NMR, $CDCl_3$

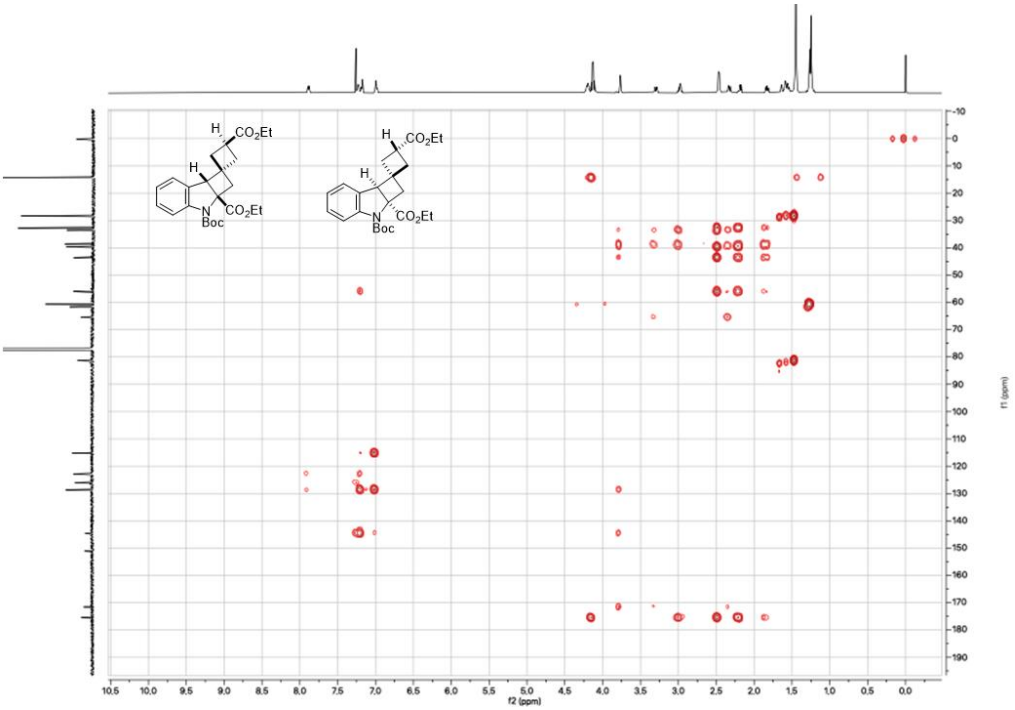


3'-(*tert*-Butyl) 2a',3-diethyl spiro[cyclobutane-1,1'-cyclobuta[*b*]indole]-2a',3,3'(2'*H*,7b'*H*)-tricarboxylate (**S1-6**)

HSQC NMR, $CDCl_3$

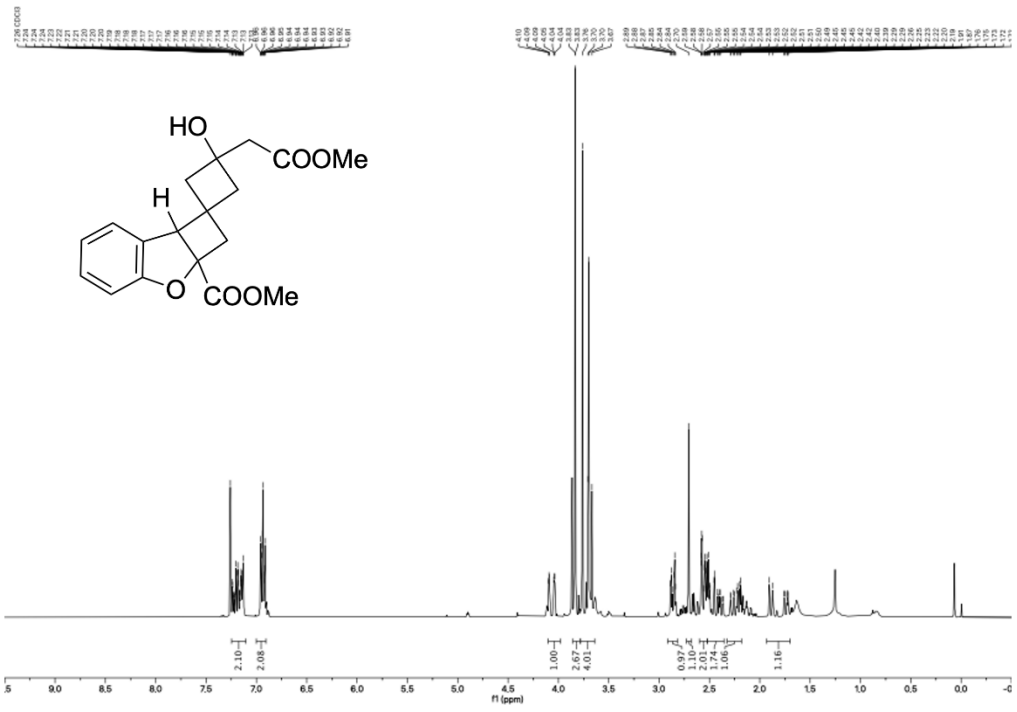


HMBC NMR, $CDCl_3$

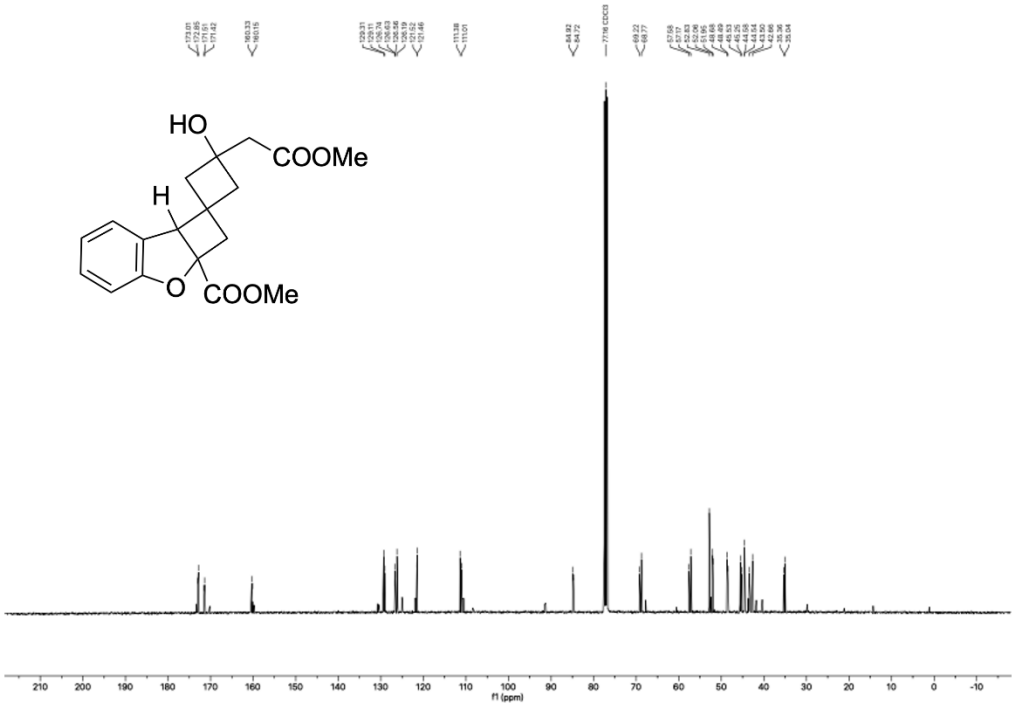


Methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-2'*H*-spiro[cyclobutane-1,1'-cyclobuta[*b*]benzofuran]-2a'(7b'*H*)-carboxylate (**S1-7**)

^{1}H NMR, $CDCl_3$

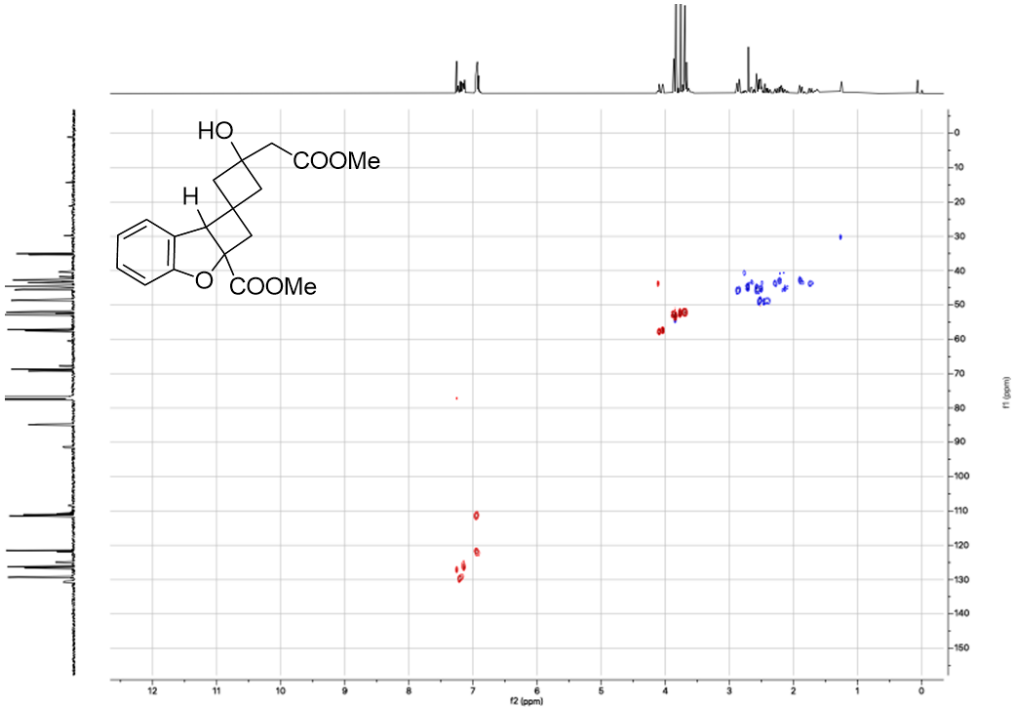


^{13}C NMR, $CDCl_3$

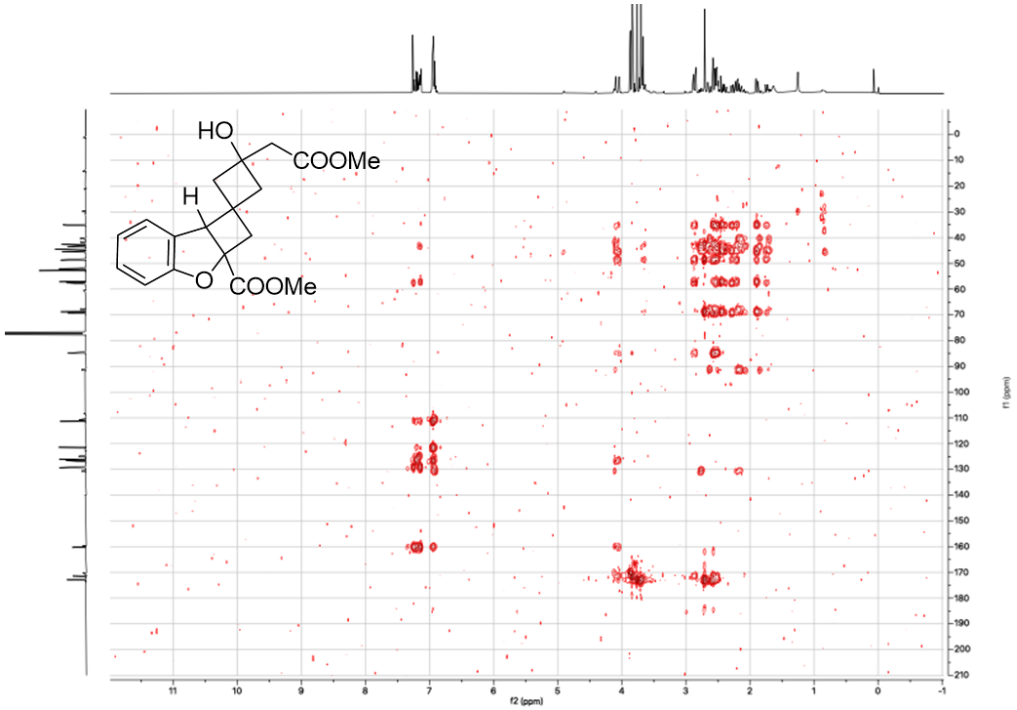


Methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-2'*H*-spiro[cyclobutane-1,1'-cyclobuta[*b*]benzofuran]-2a'(7b'*H*)-carboxylate (**S1-7**)

HSQC NMR, $CDCl_3$

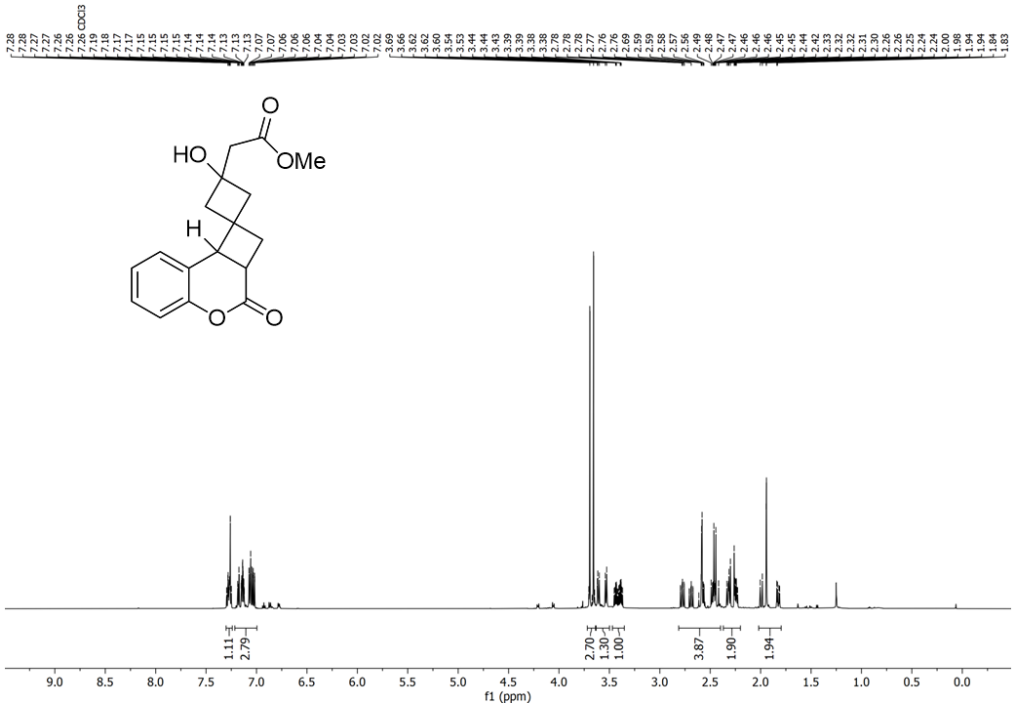


HMBC NMR, $CDCl_3$

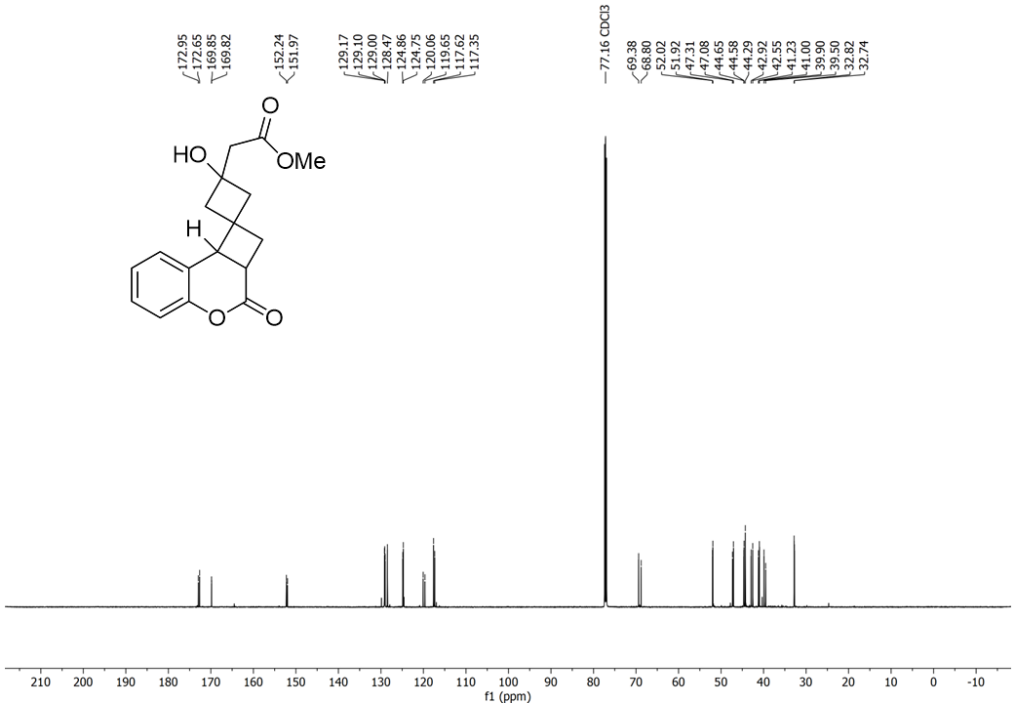


Methyl 2-((2a'*R*,8b'*R*)-3-hydroxy-3'-oxo-2a',8b'-dihydro-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromen]-3-yl)acetate (**S1-8**)

^{1}H NMR, $CDCl_3$

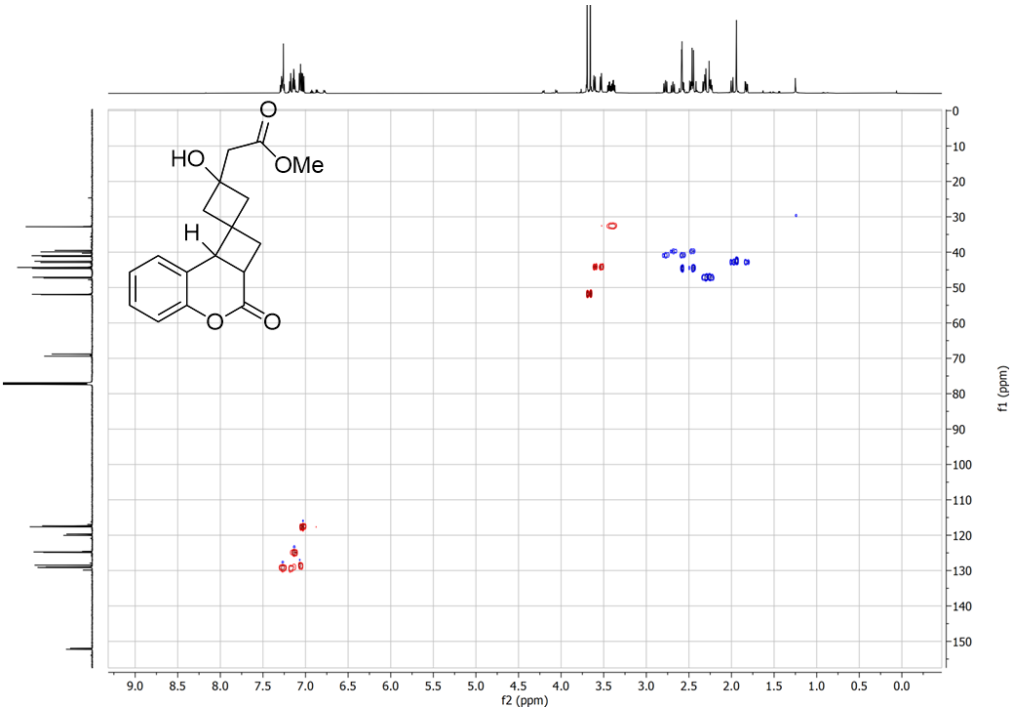


^{13}C NMR, $CDCl_3$

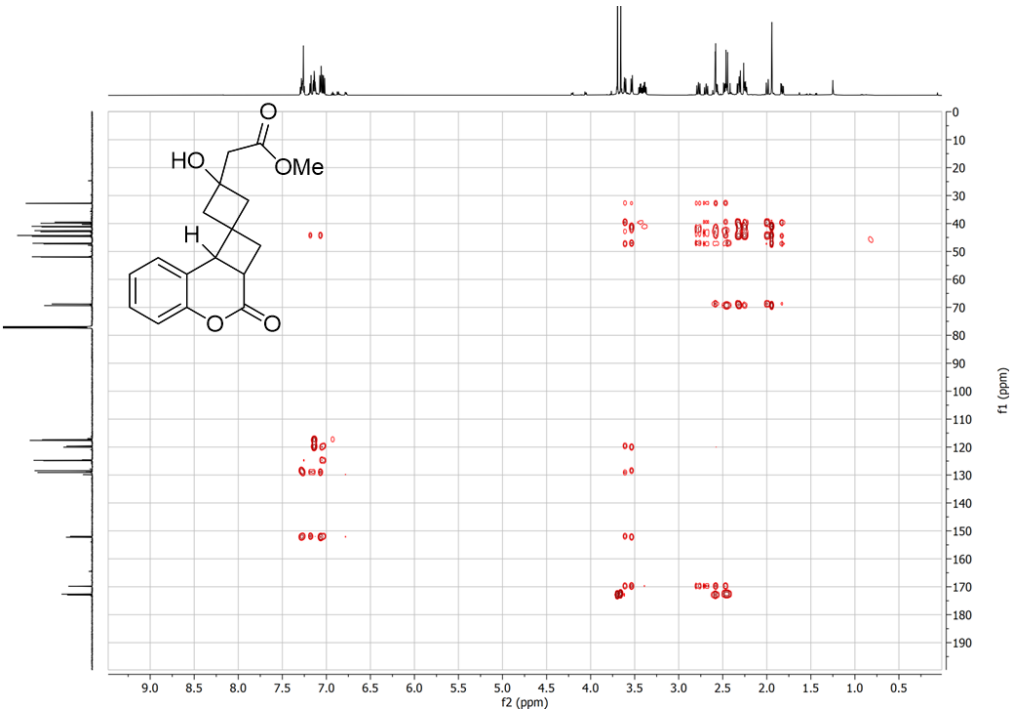


Methyl 2-((2a'*R*,8b'*R*)-3-hydroxy-3'-oxo-2a',8b'-dihydro-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromen]-3-yl)acetate (**S1-8**)

HSQC NMR, $CDCl_3$

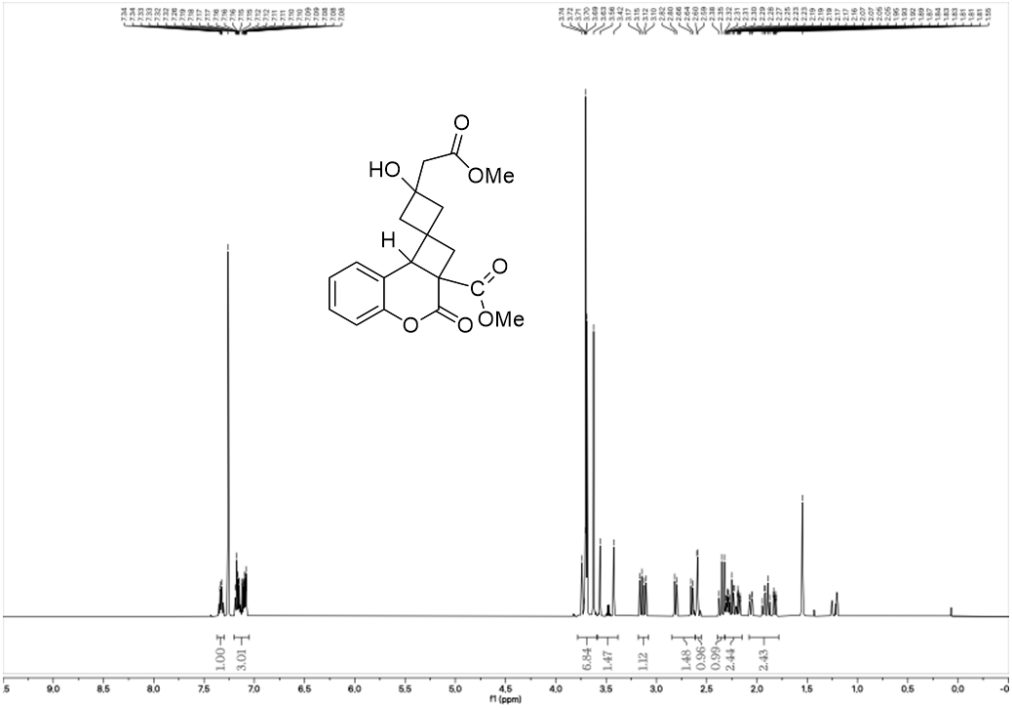


HMBC NMR, $CDCl_3$

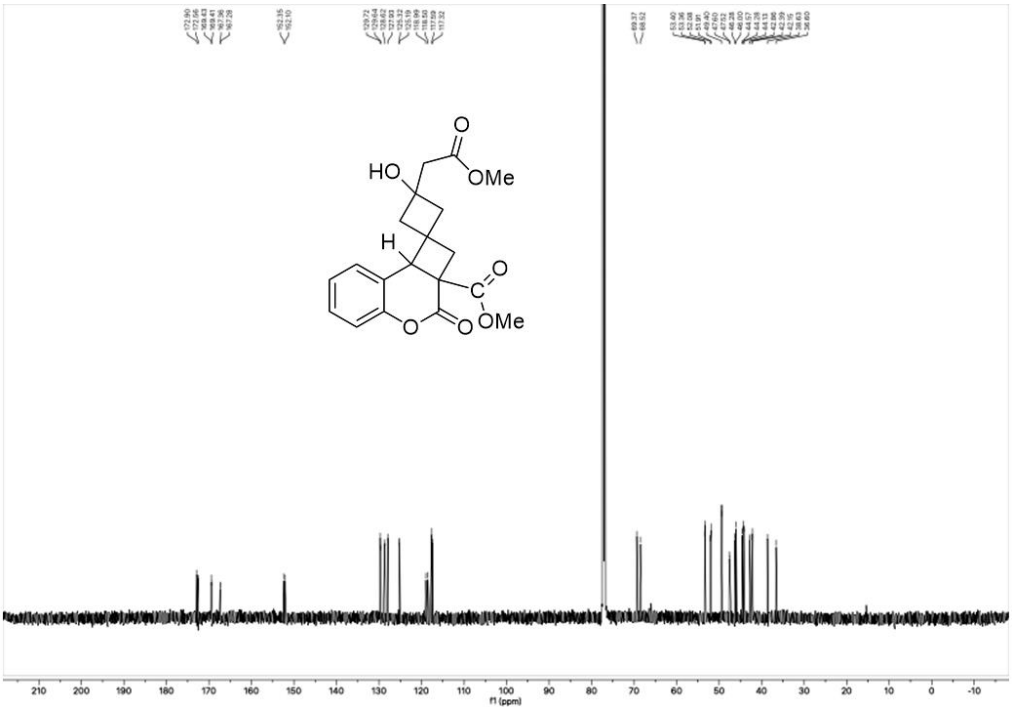


Methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-3'-oxo-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromene]-2a'(8b'*H*)-carboxylate (**S1-9**)

^{1}H NMR, $CDCl_3$

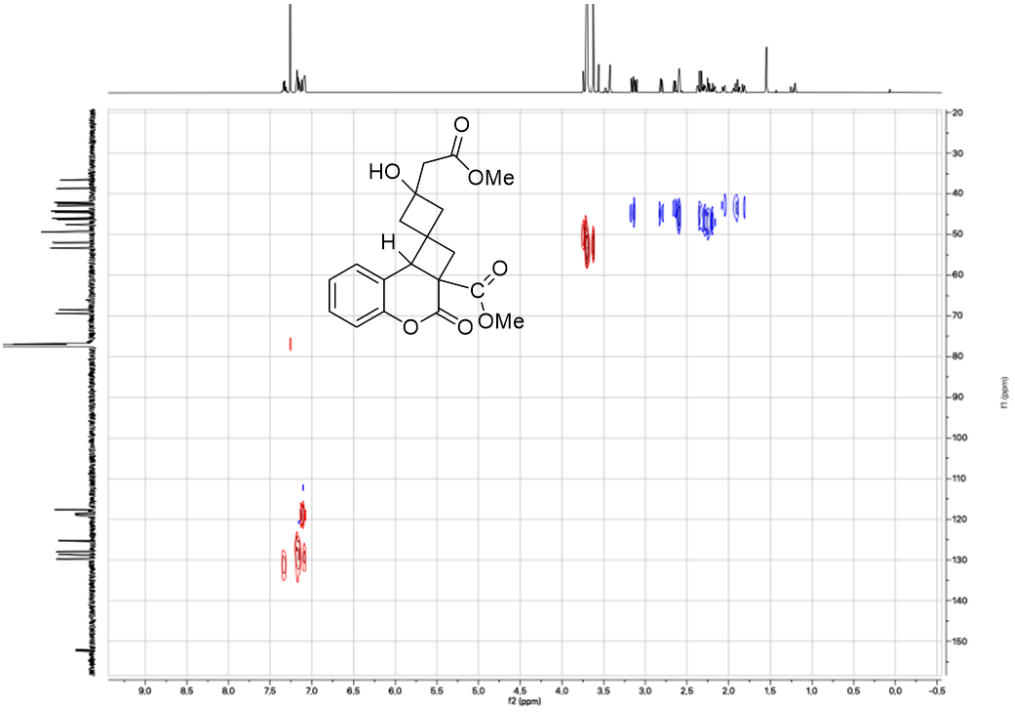


^{13}C NMR, $CDCl_3$

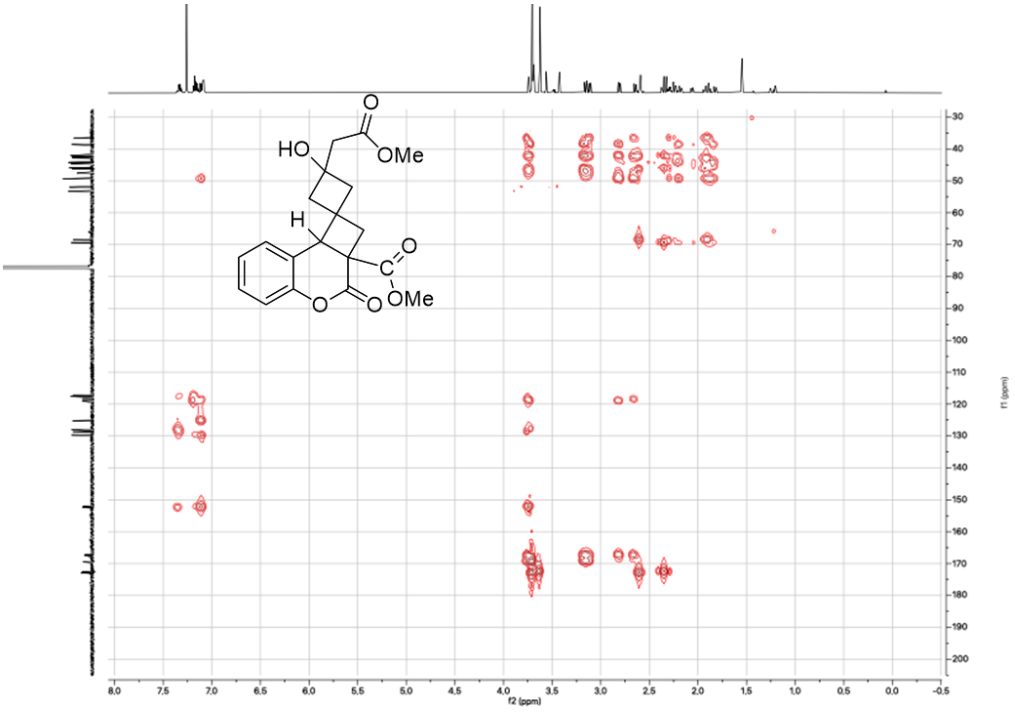


Methyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)-3'-oxo-2'*H*,3'*H*-spiro[cyclobutane-1,1'-cyclobuta[*c*]chromene]-2a'(8b'*H*)-carboxylate (**S1-9**)

HSQC NMR, $CDCl_3$

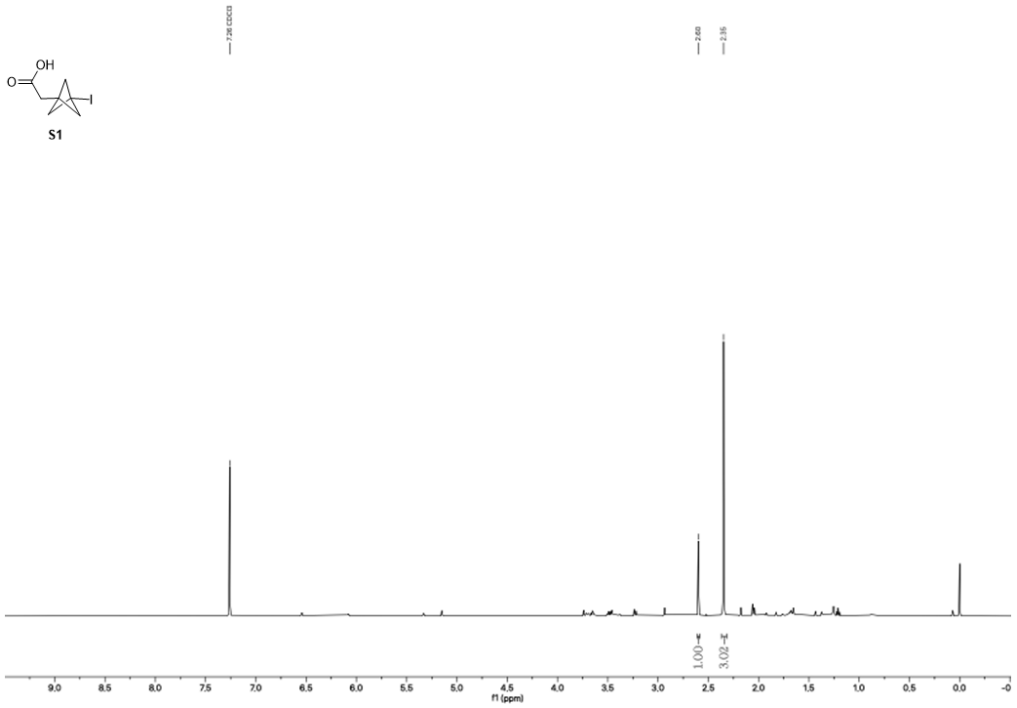


HMBC NMR, $CDCl_3$

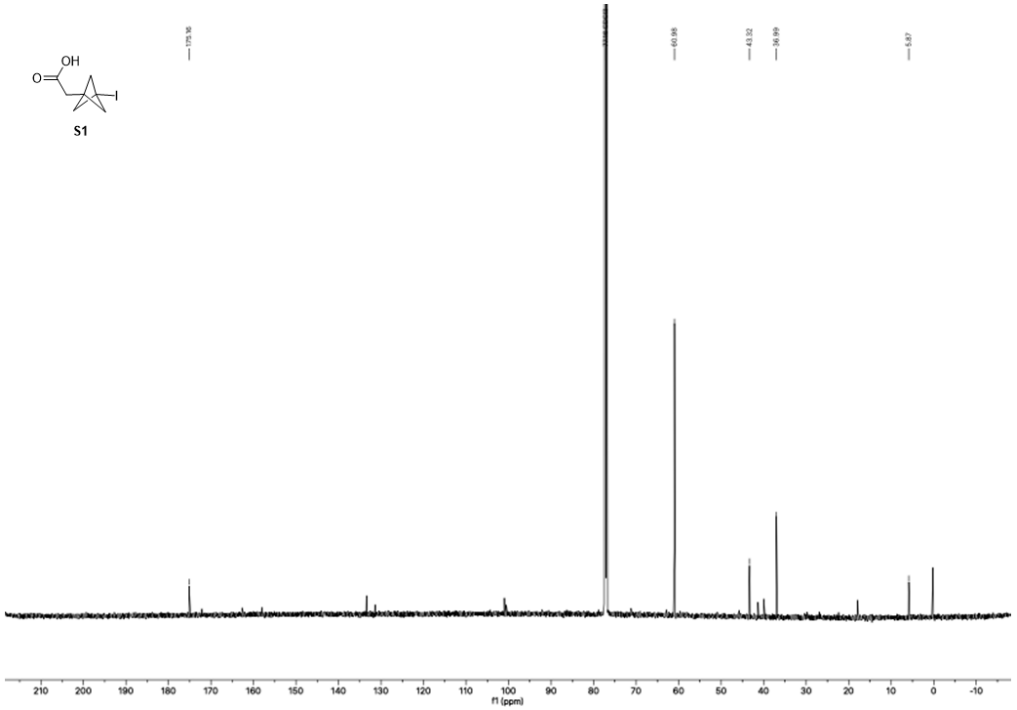


2-(3-Iodobicyclo[1.1.1]pentan-1-yl)acetic acid (**S1**)

^{1}H NMR, $CDCl_3$

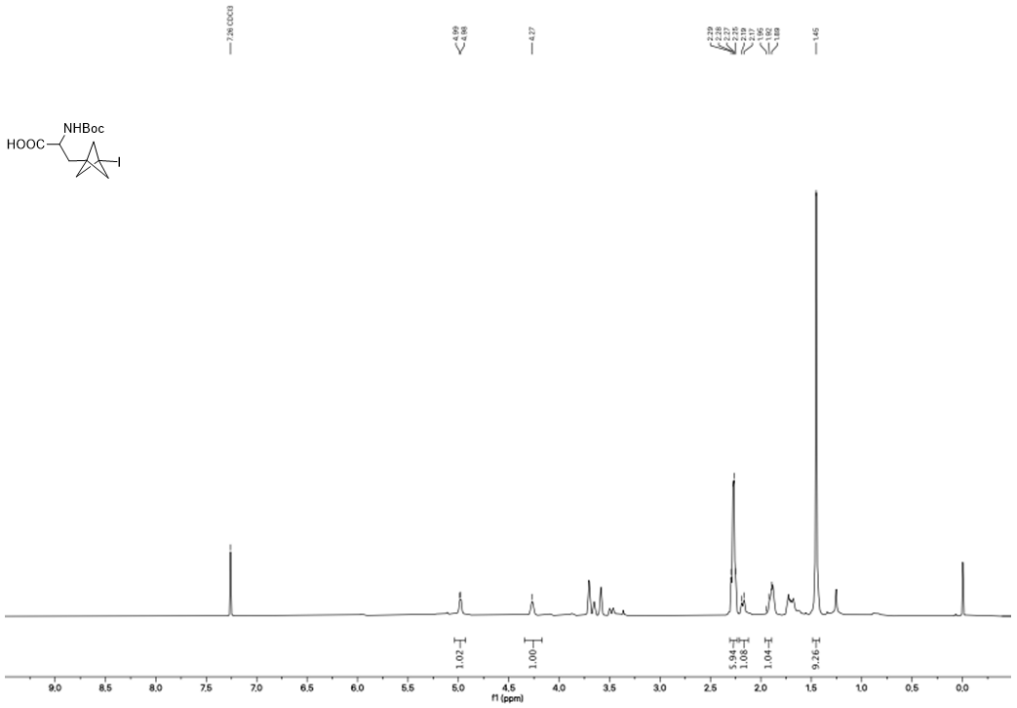


^{13}C NMR, $CDCl_3$

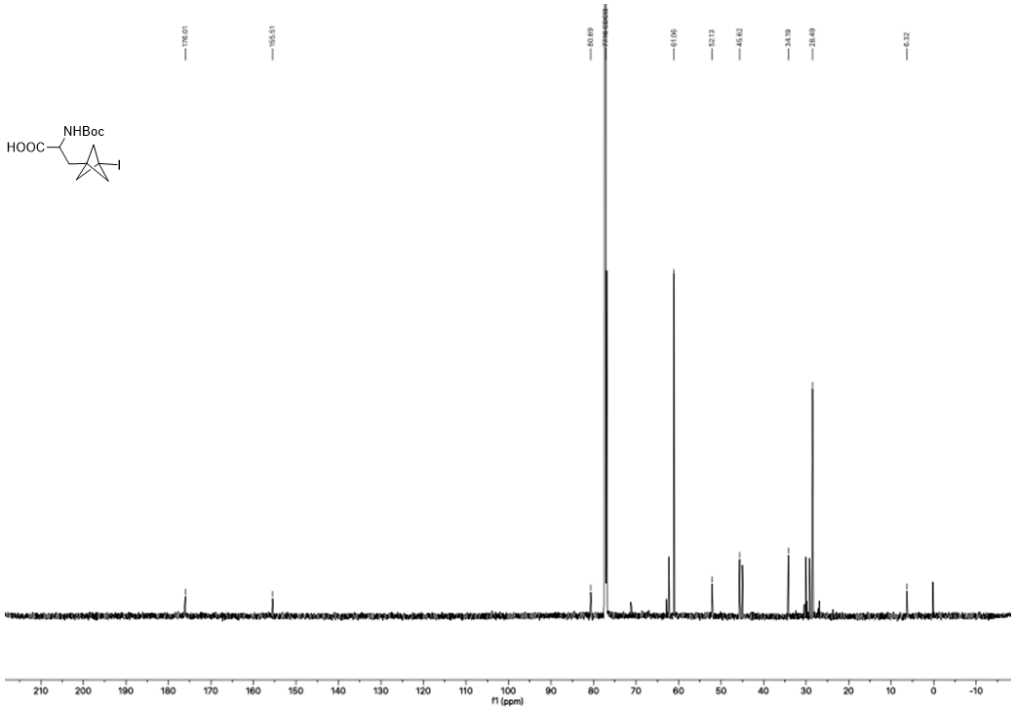


2-((*tert*-butoxycarbonyl)amino)-3-(3-iodobicyclo[1.1.1]pentan-1-yl)propanoic acid (**S3**)

^{1}H NMR, $CDCl_3$

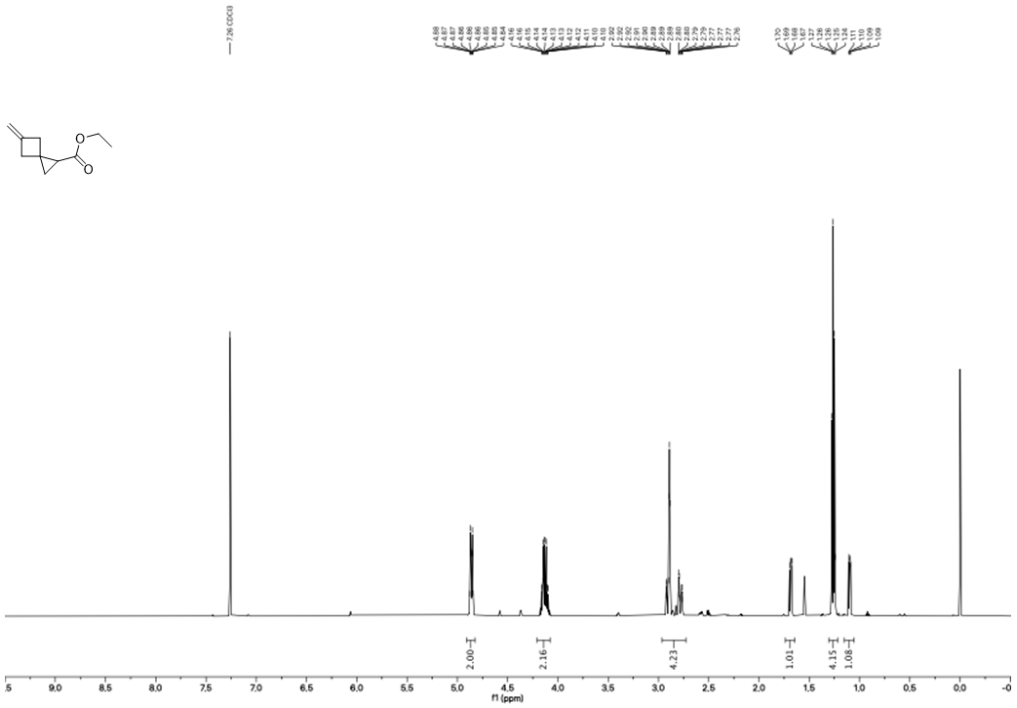


^{13}C NMR, $CDCl_3$

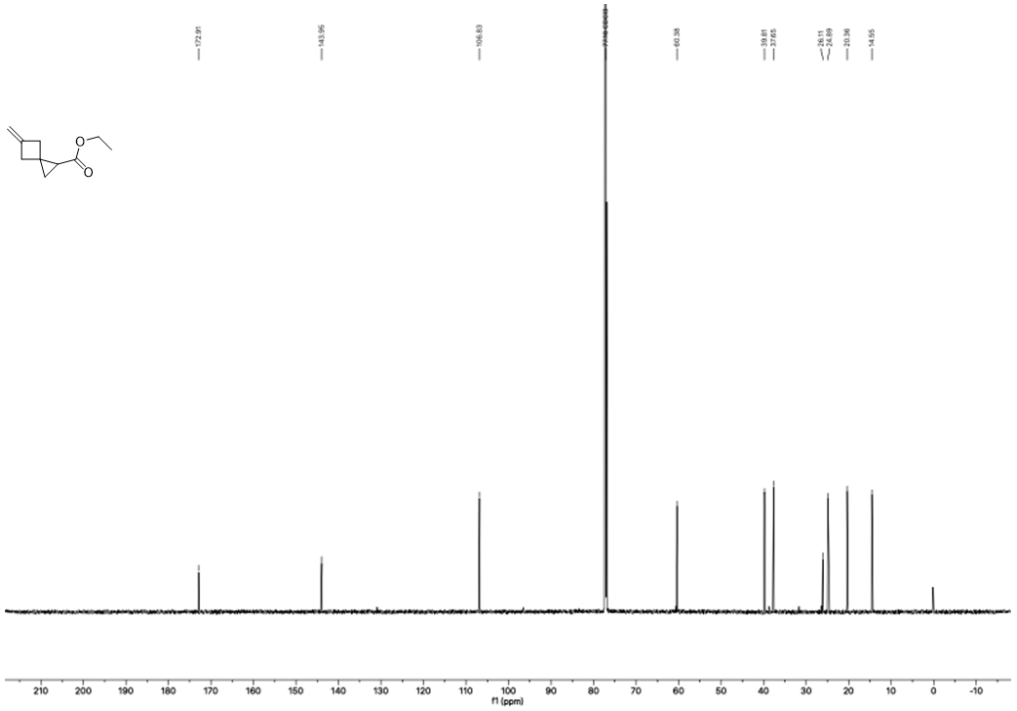


Ethyl 5-methylenespiro[2.3]hexane-1-carboxylate (**S4**)

^{1}H NMR, $CDCl_3$

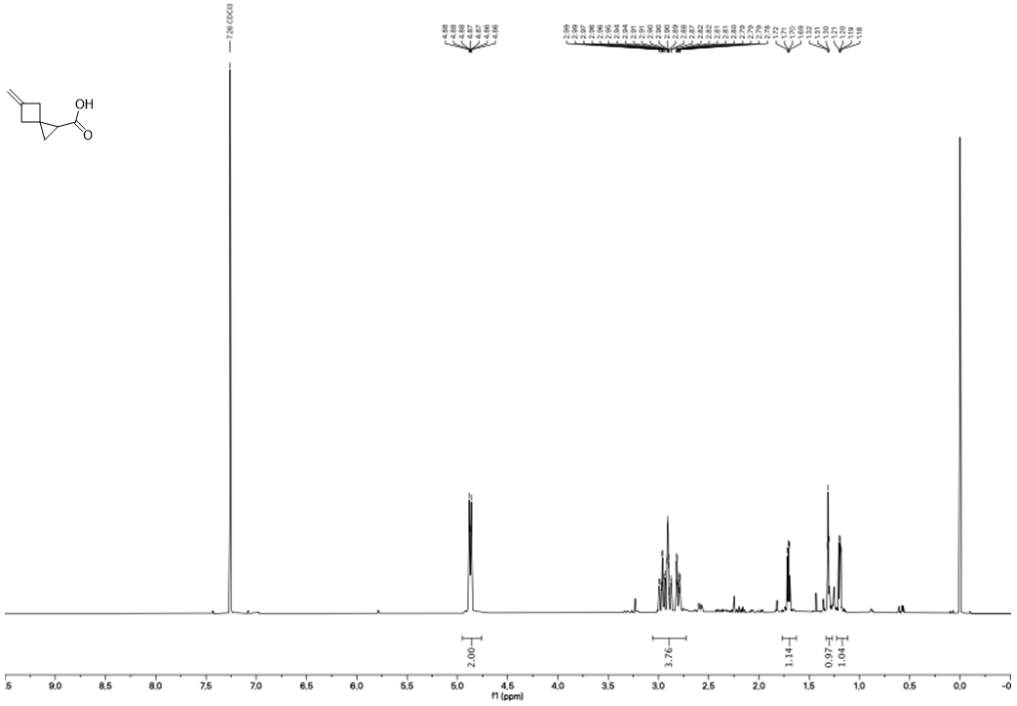


^{13}C NMR, $CDCl_3$

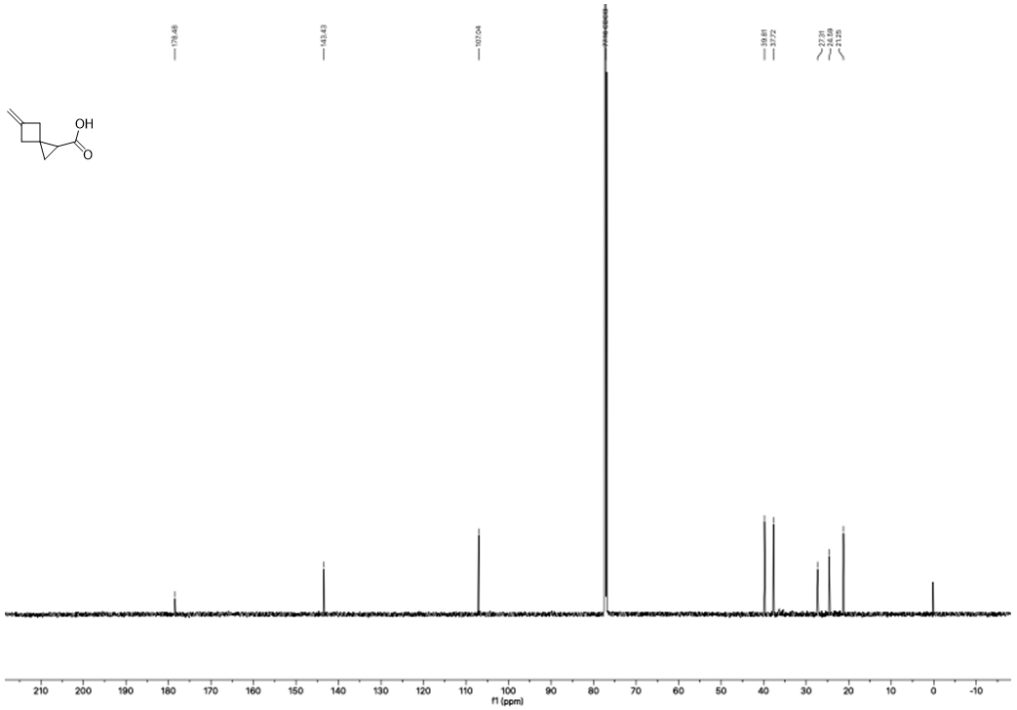


5-Methylenespiro[2.3]hexane-1-carboxylic acid (**S5**)

^{1}H NMR, $CDCl_3$

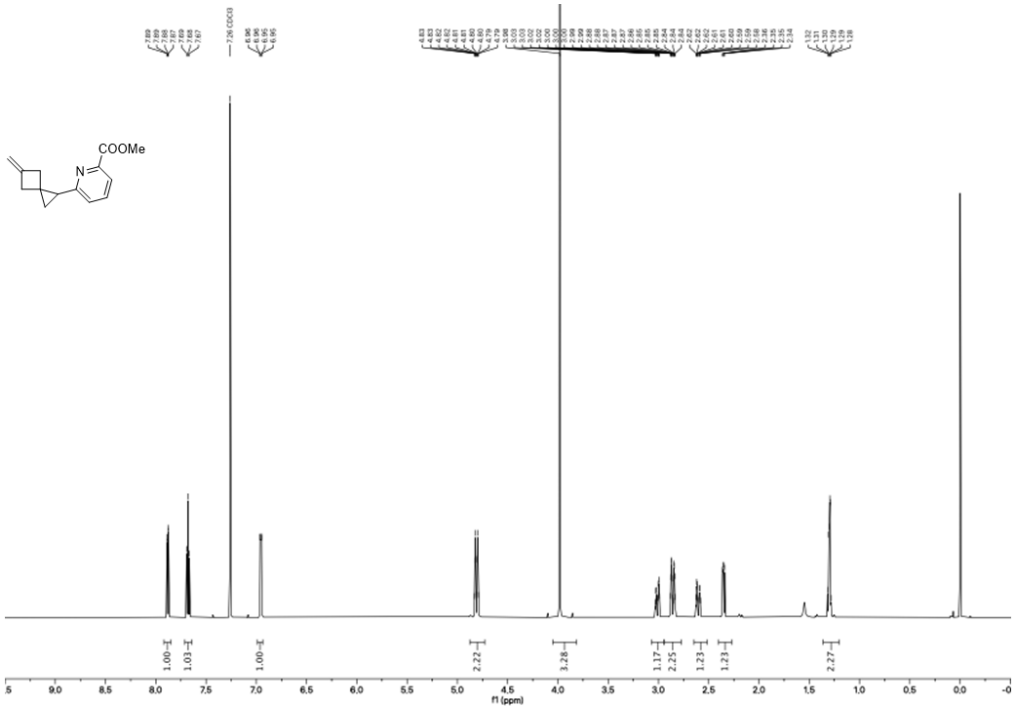


^{13}C NMR, $CDCl_3$

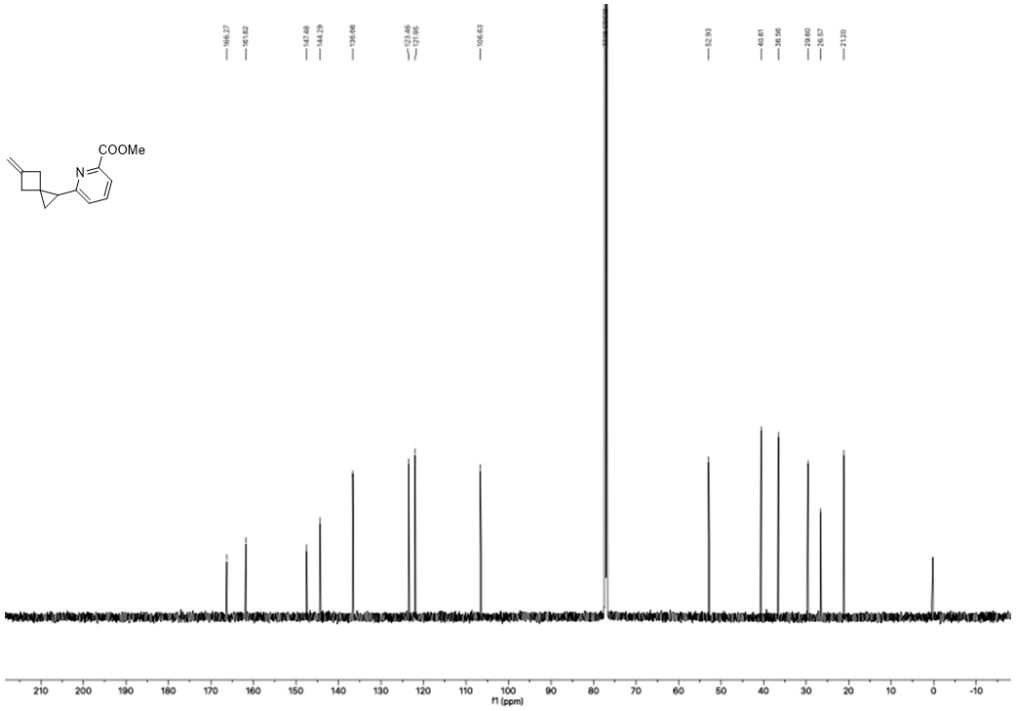


Methyl 6-(5-methylenespiro[2.3]hexan-1-yl)picolinate (**S6**)

^{1}H NMR, $CDCl_3$

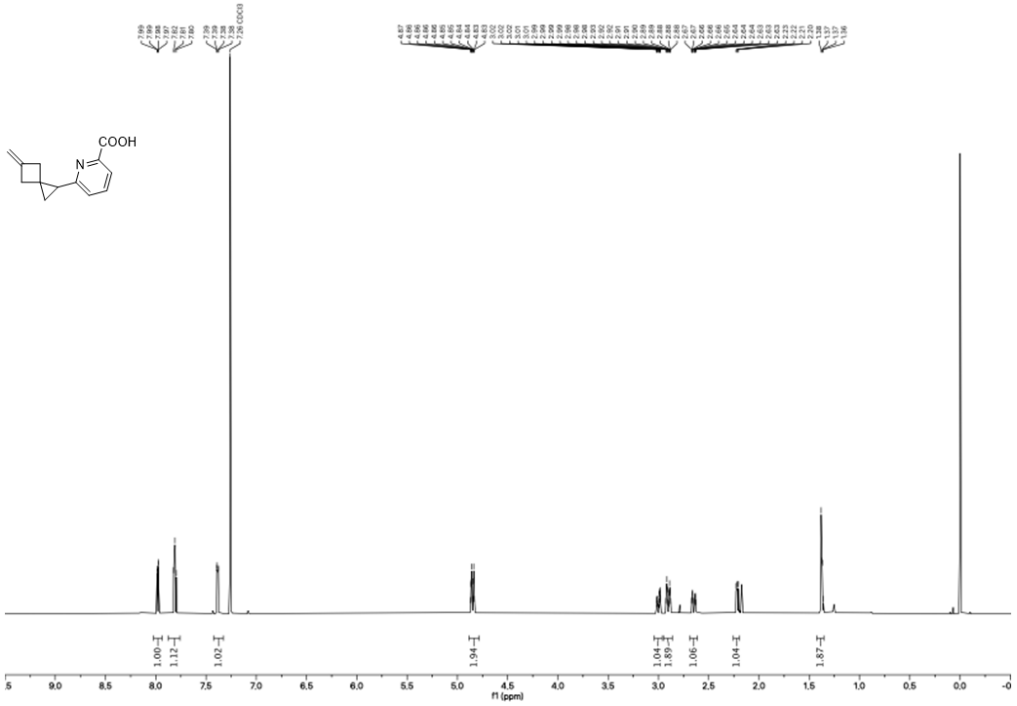


^{13}C NMR, $CDCl_3$

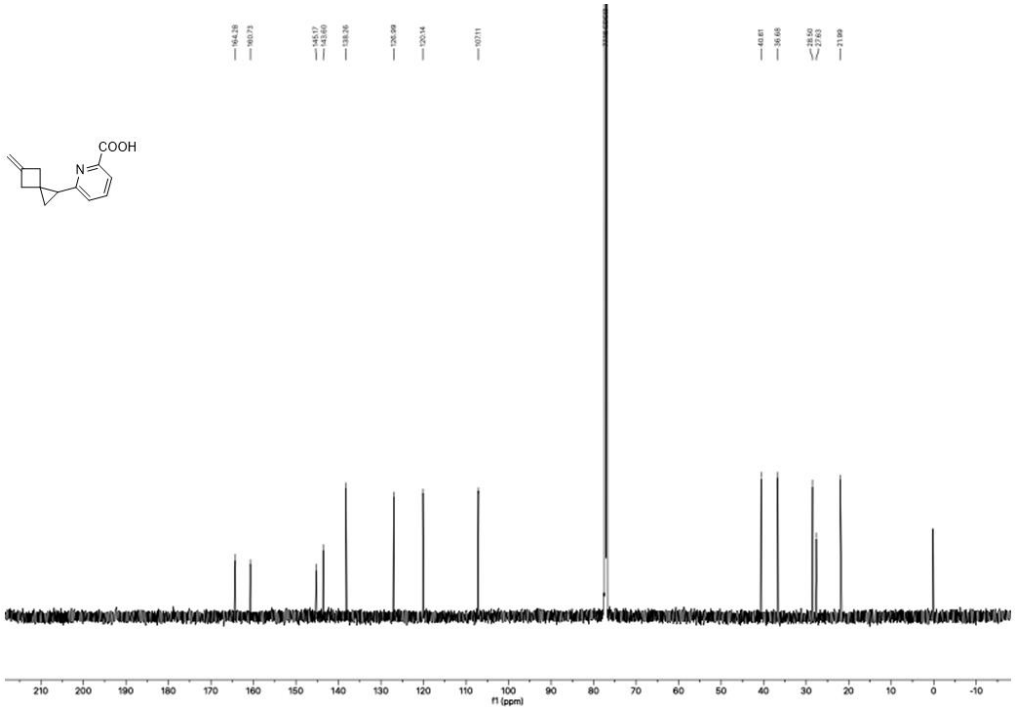


6-(5-Methylenespiro[2.3]hexan-1-yl)picolinic acid (S7)

^{1}H NMR, $CDCl_3$

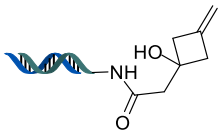


^{13}C NMR, $CDCl_3$

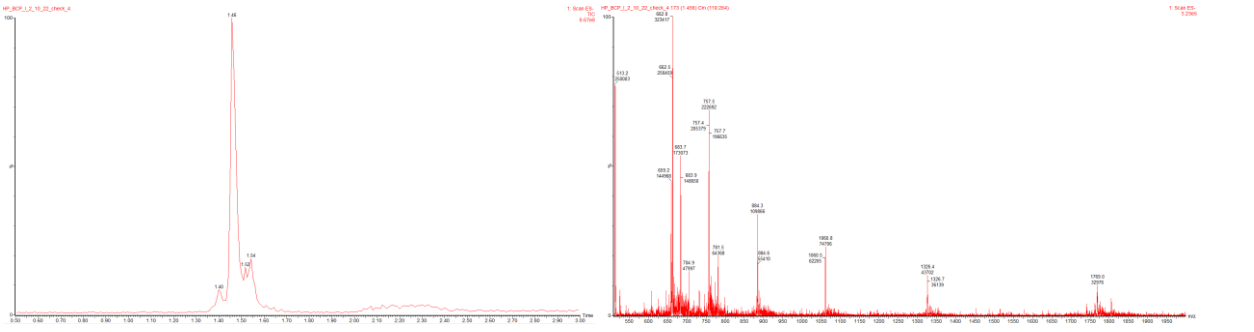


12 UPLC/MS Spectra of DNA headpieces

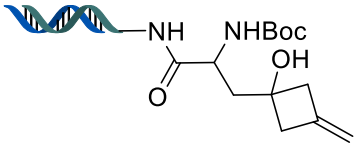
Mass spectra of HP-1



HP-1
5308.66 g/mol

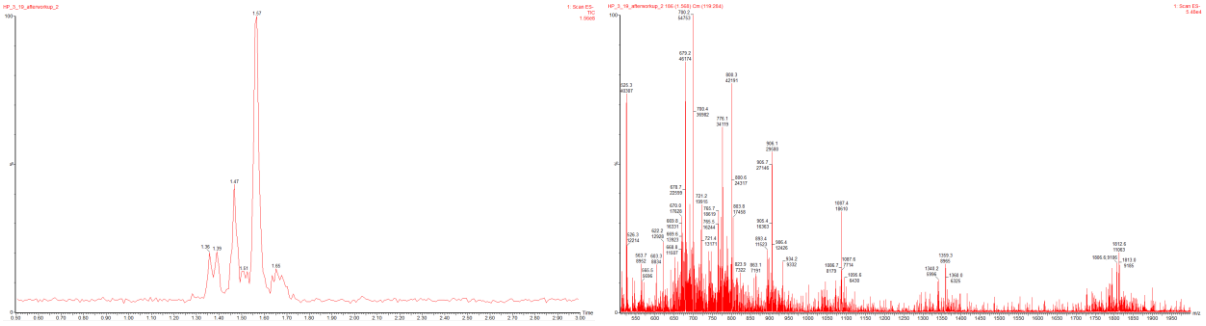


Mass spectra of HP-2 (Purity = 88%)

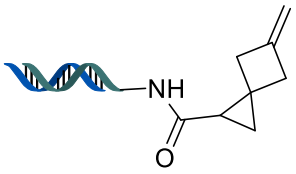


HP-2

5437.82 g/mol

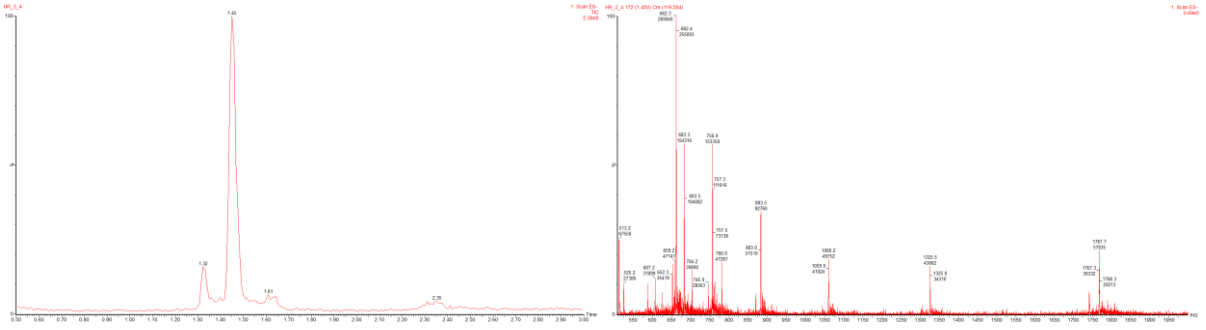


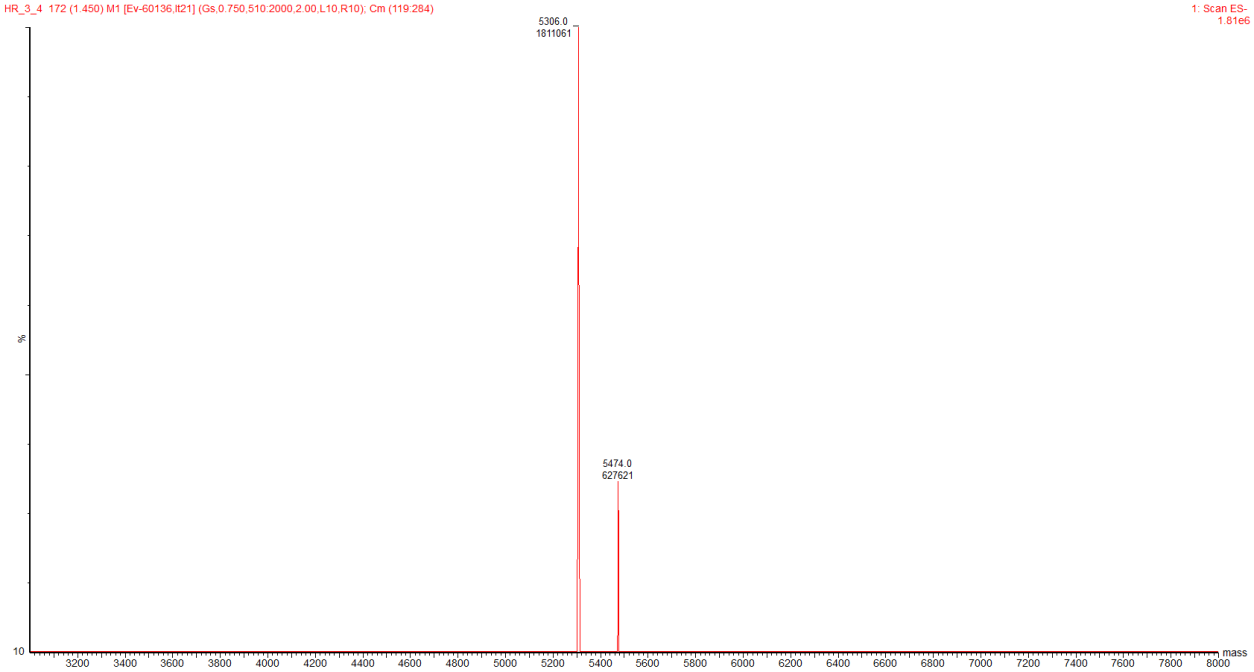
Mass spectra of HP-3



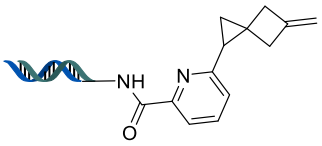
HP-3

5304.67 g/mol



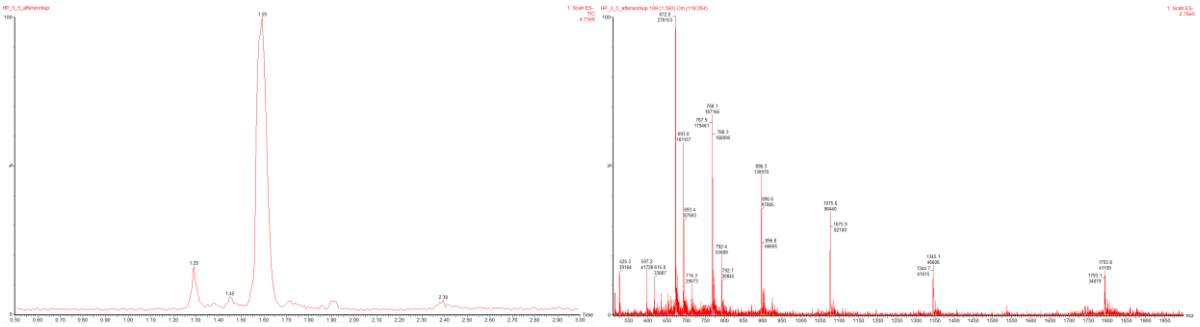


Mass spectra of HP-4

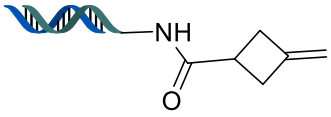


HP-4

5381.76 g/mol

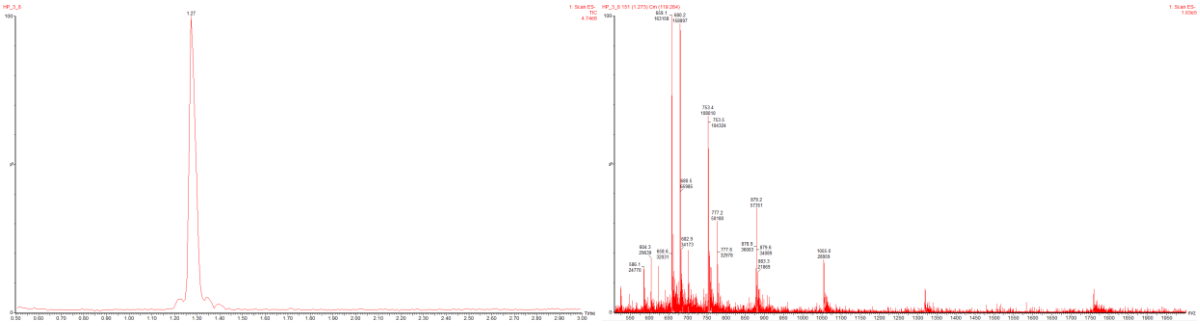


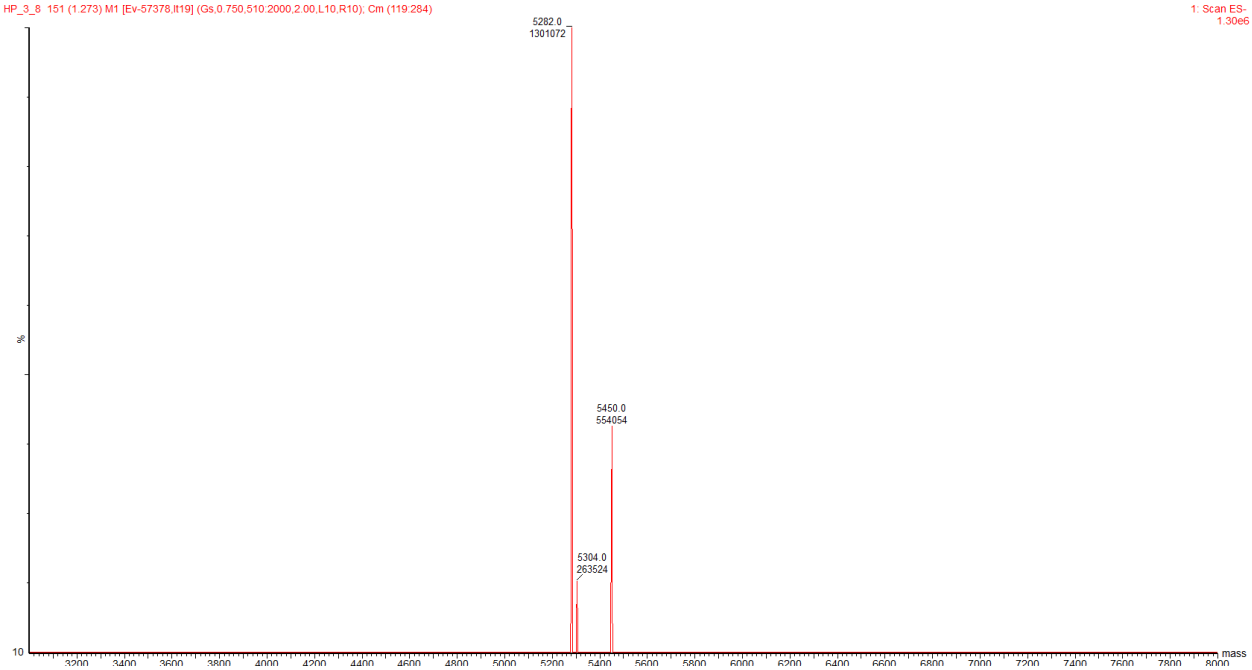
Mass spectra of HP-5



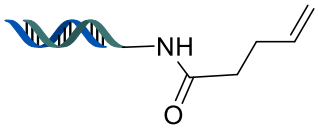
HP-5

5278.64 g/mol



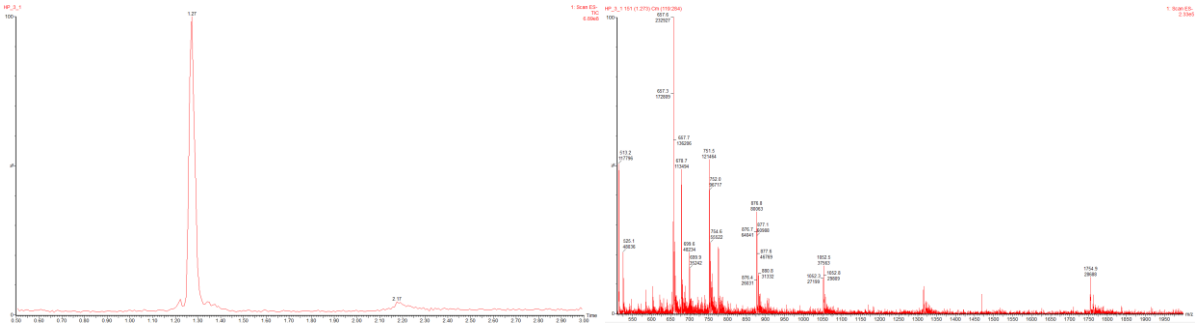


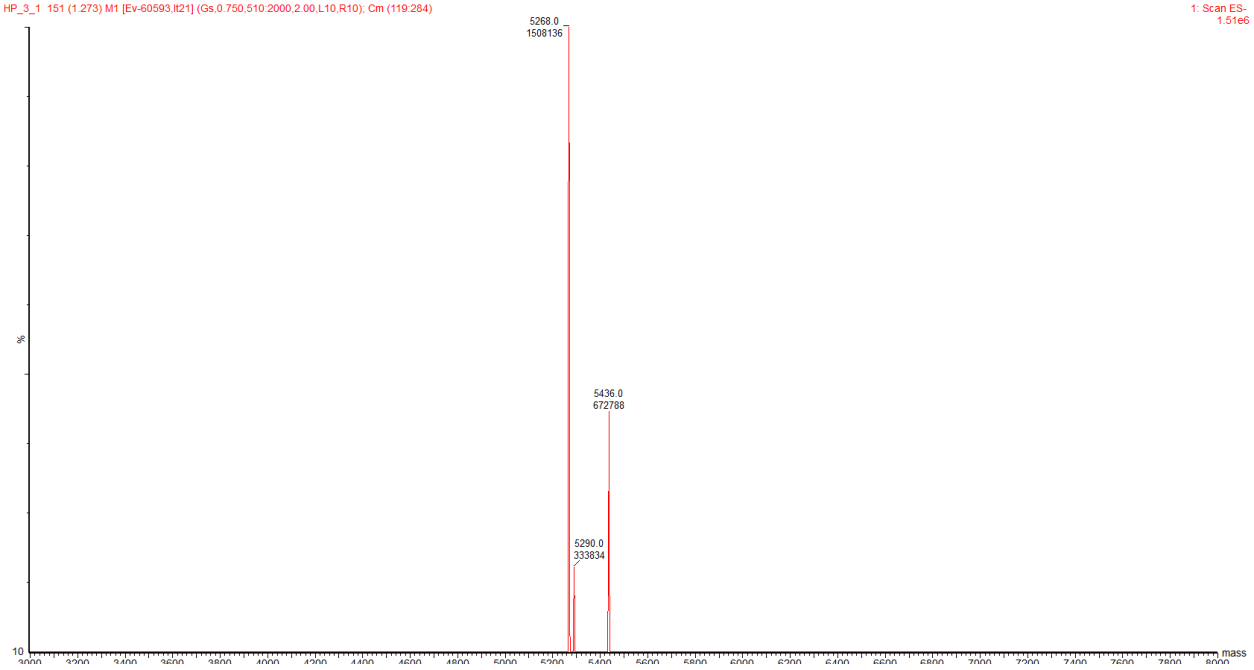
Mass spectra of HP-6



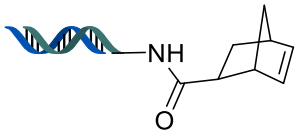
HP-6

5266.62 g/mol



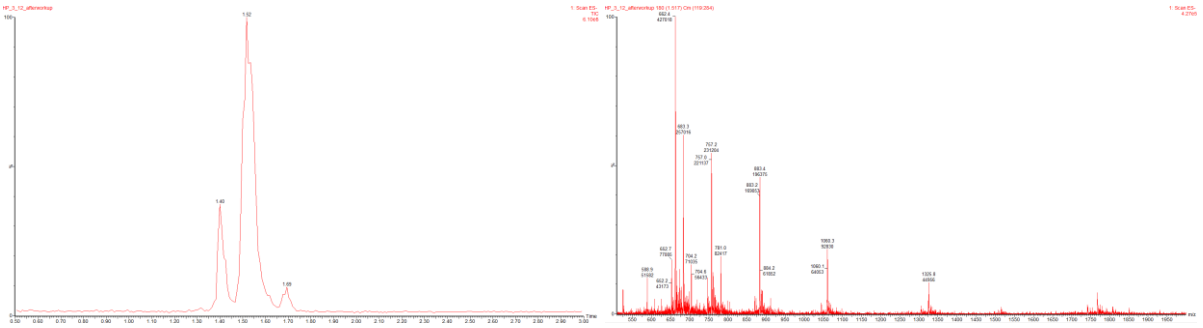


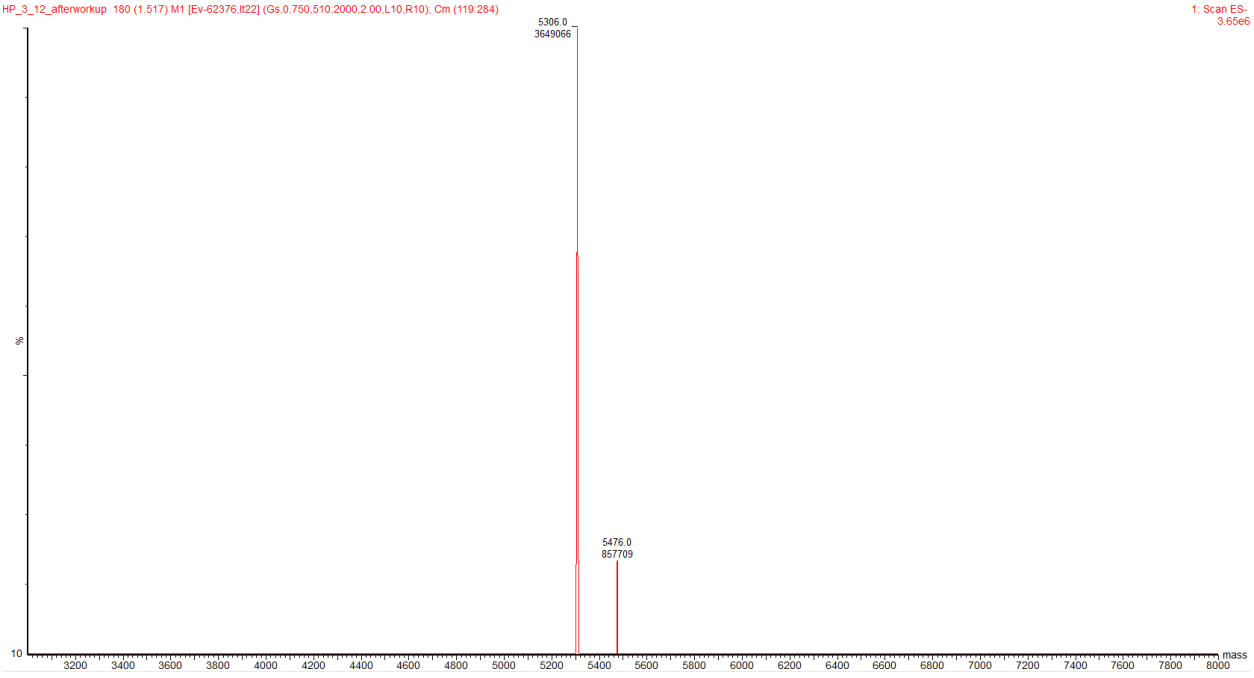
Mass spectra of HP-7



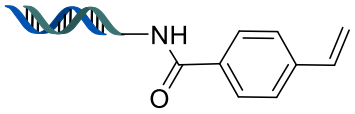
HP-7

5304.67 g/mol



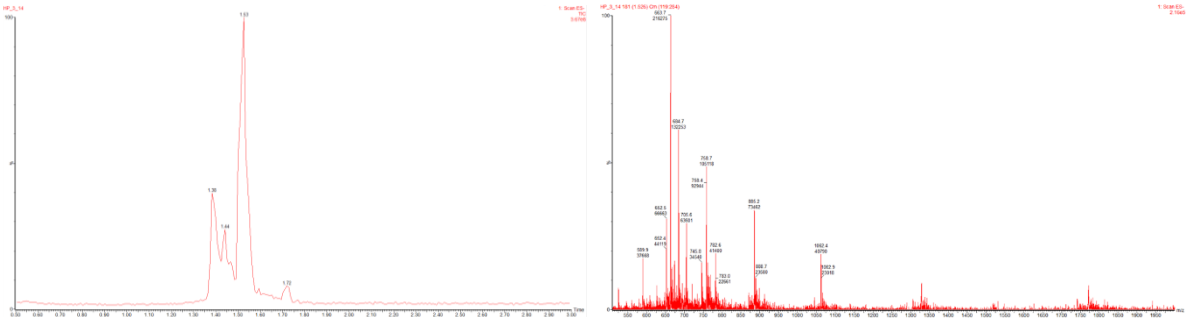


Mass spectra of HP-8

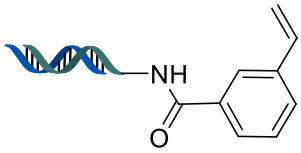


HP-8

5314.67 g/mol

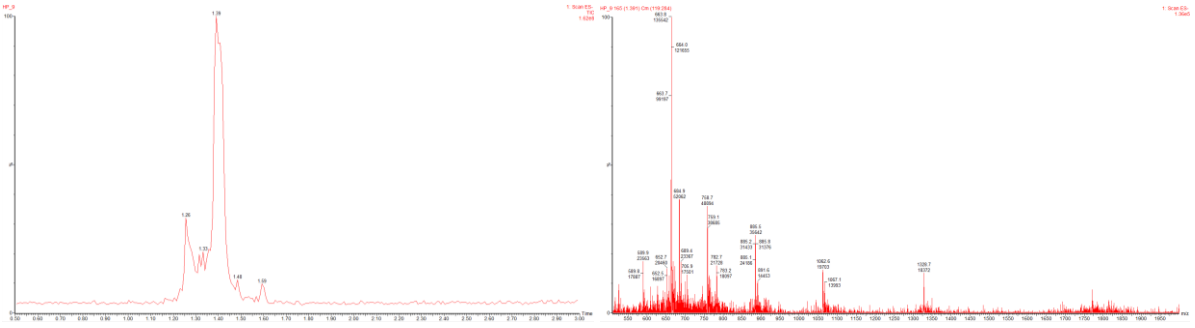


Mass spectra of HP-9

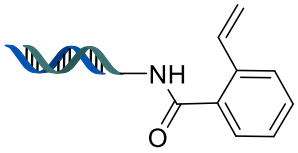


HP-9

5314.67 g/mol

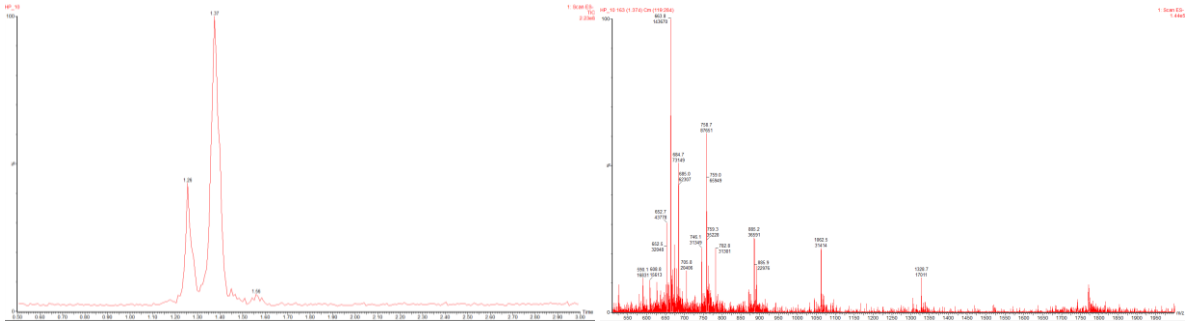


Mass spectra of HP-10

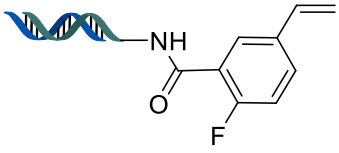


HP-10

5314.67 g/mol

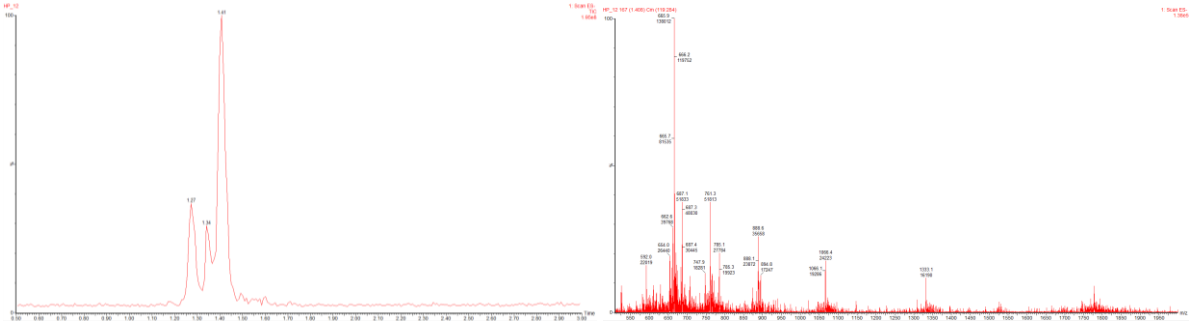


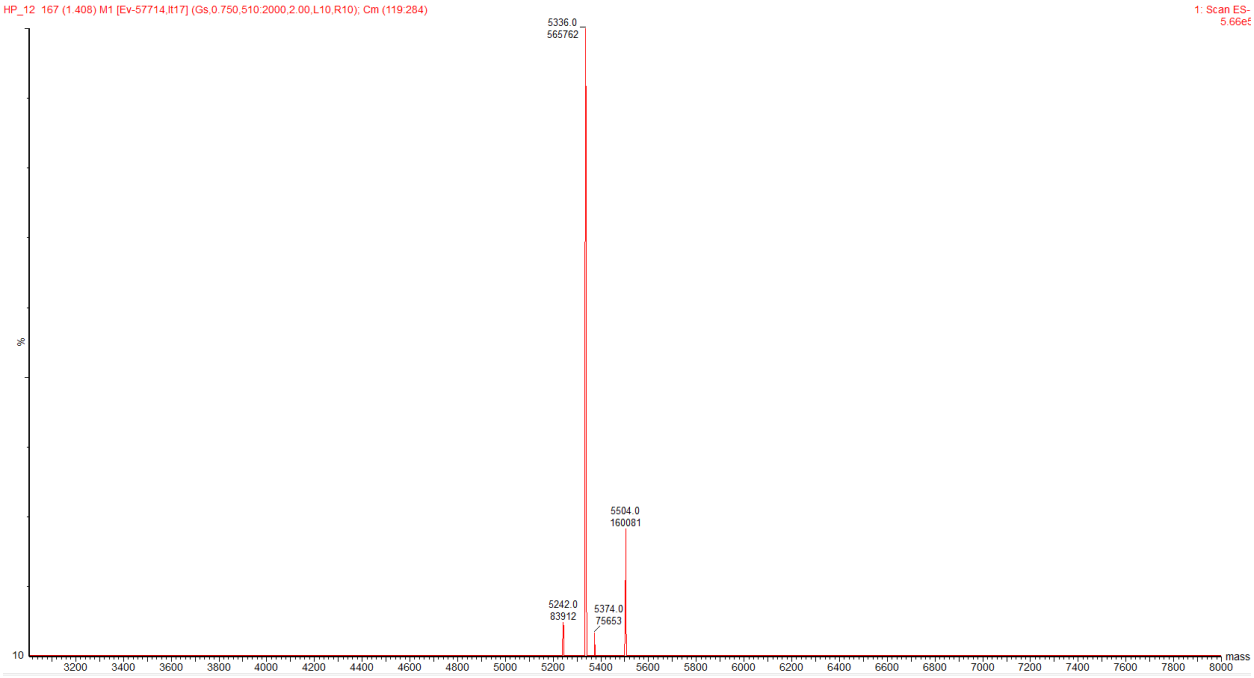
Mass spectra of HP-12



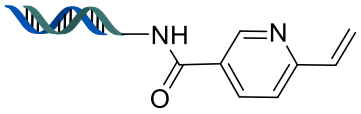
HP-12

5332.66 g/mol



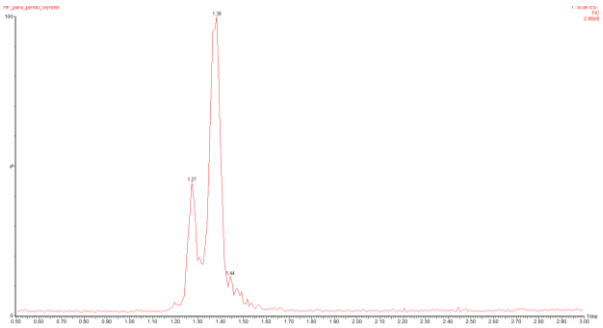


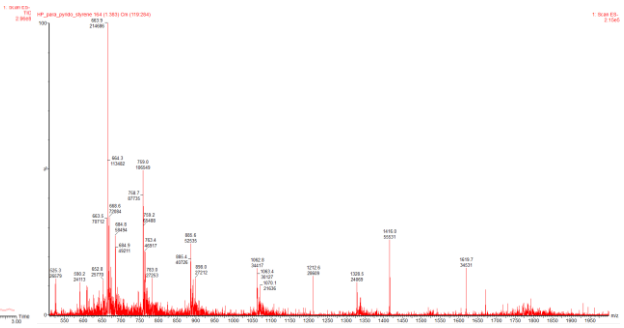
Mass spectra of HP-15

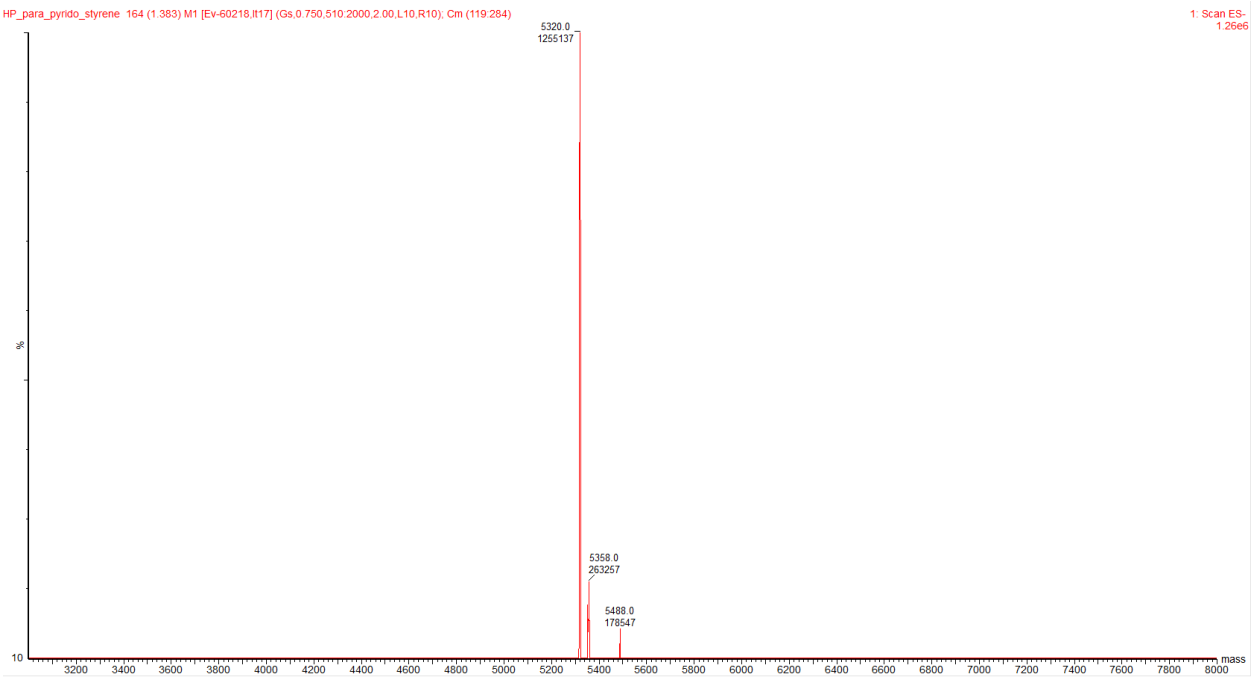


HP-15

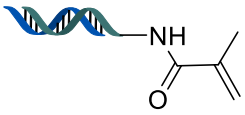
5314.67 g/mol





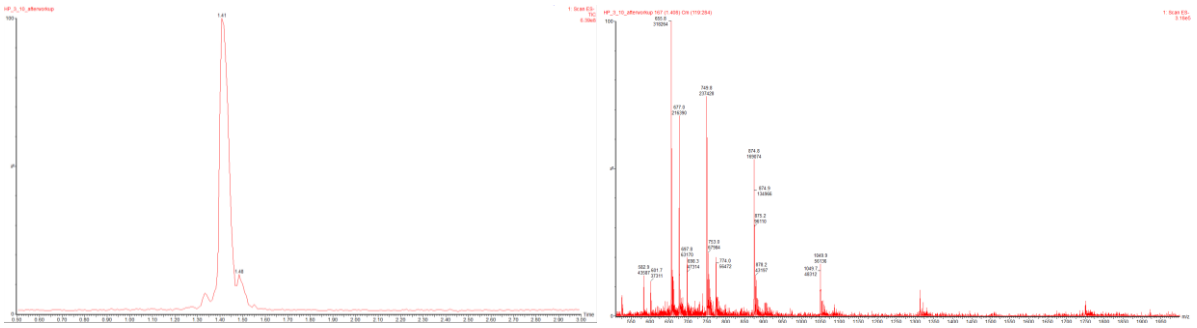


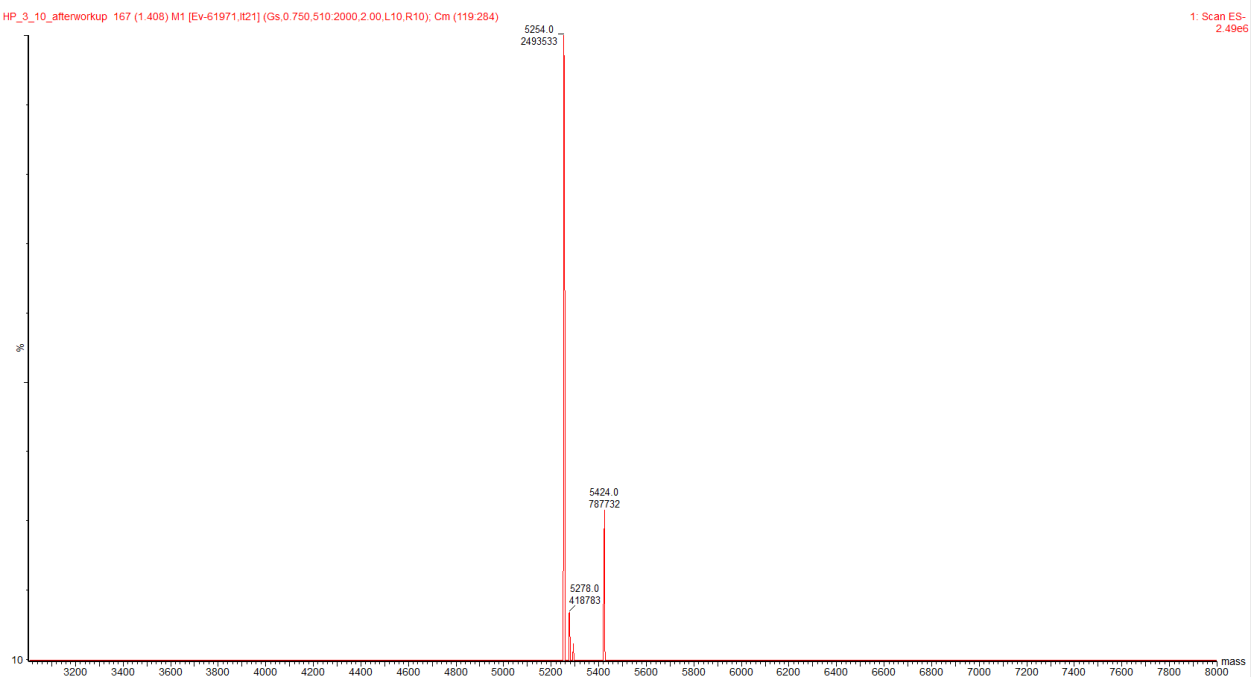
Mass spectra of HP-16



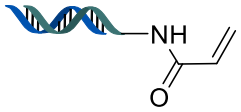
HP-16

5252.60 g/mol



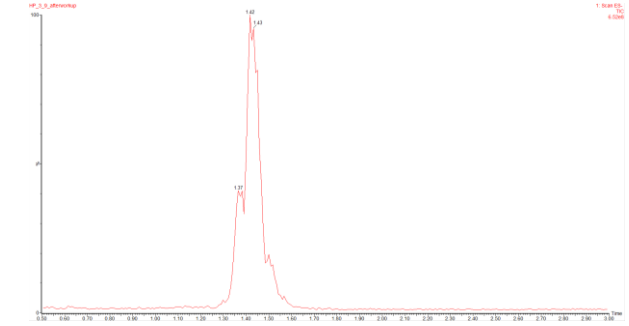


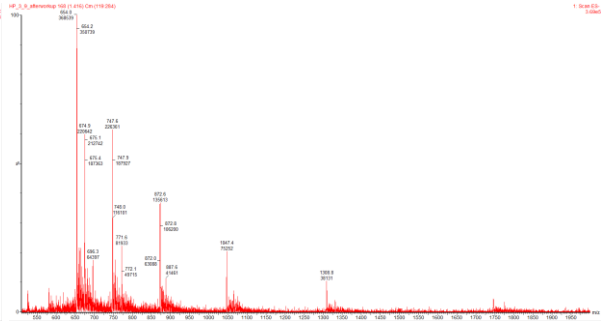
Mass spectra of HP-17



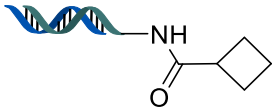
HP-17

5238.57 g/mol



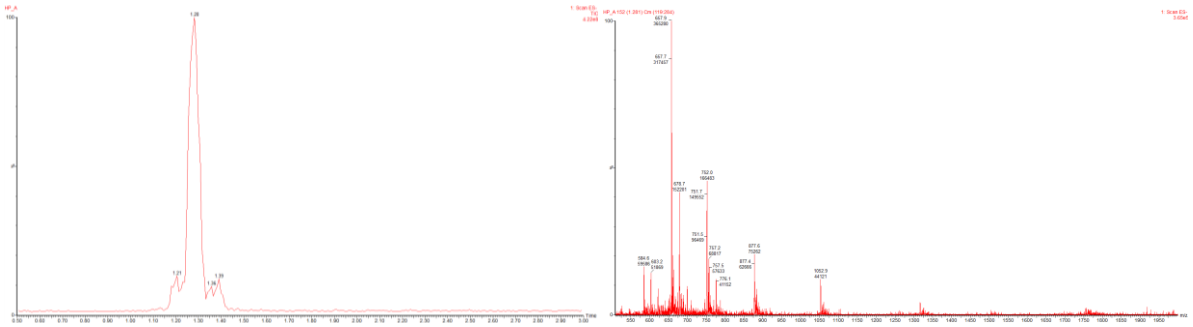


Mass spectra of HP-A



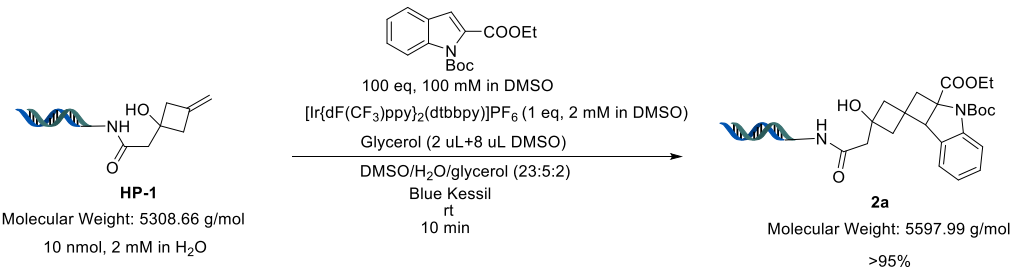
HP-A

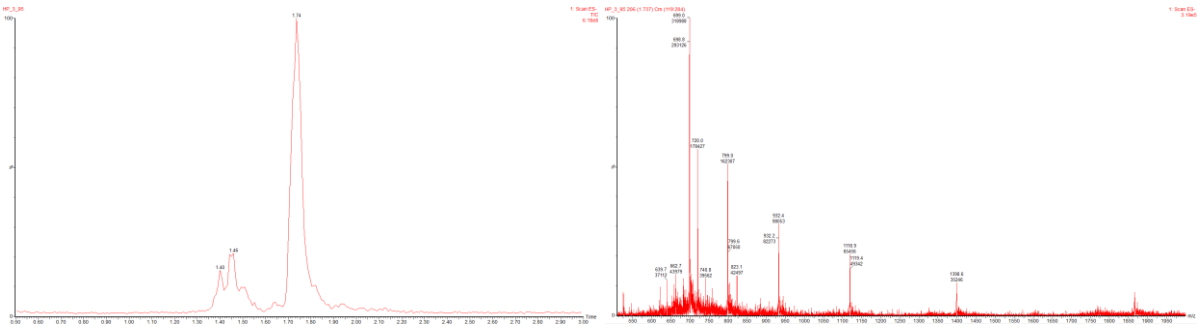
5266.62 g/mol

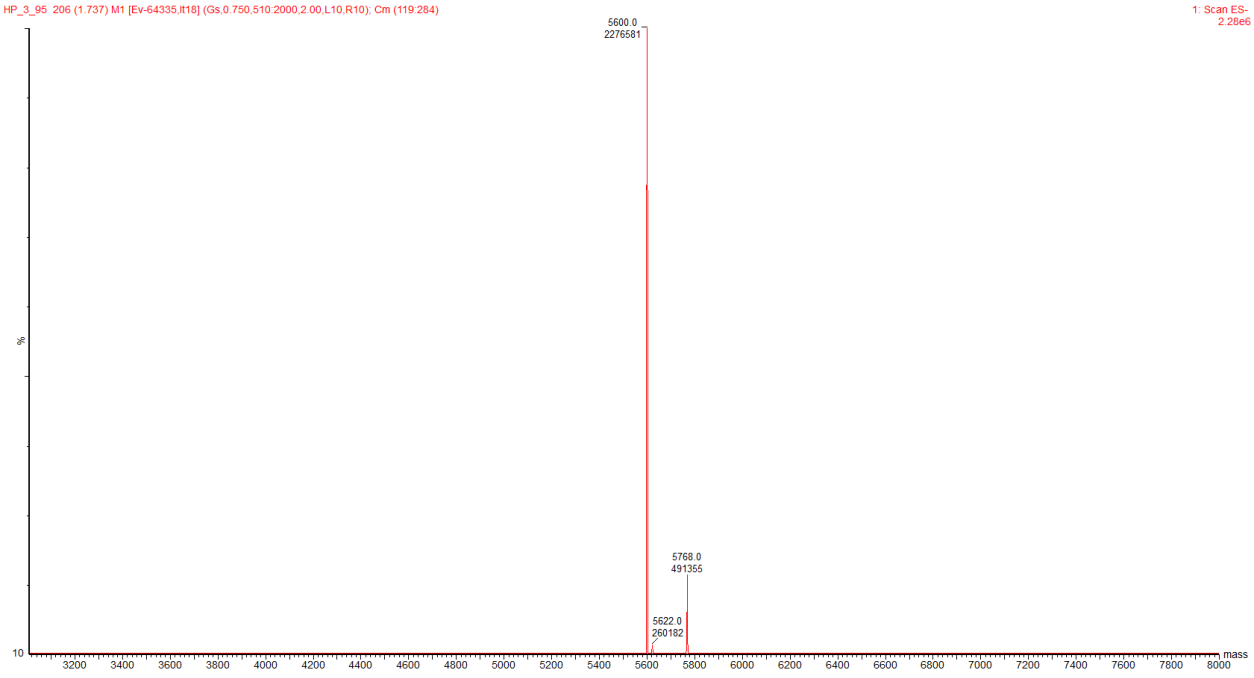


13 Control experiments for On-DNA reactions

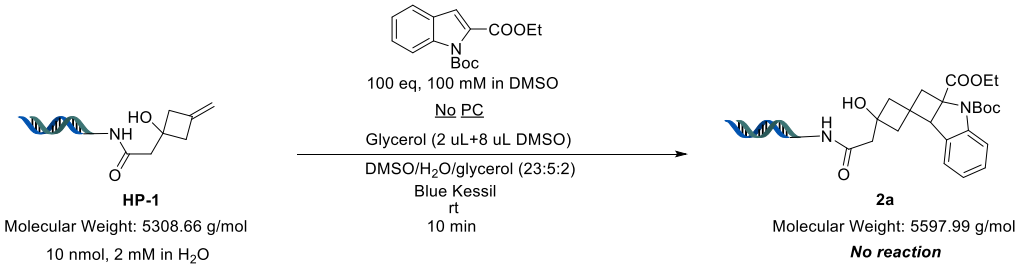
Standard conditions:

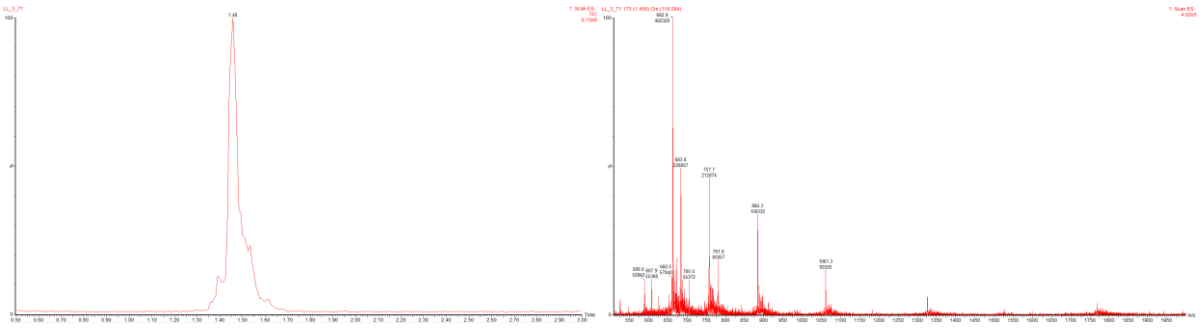


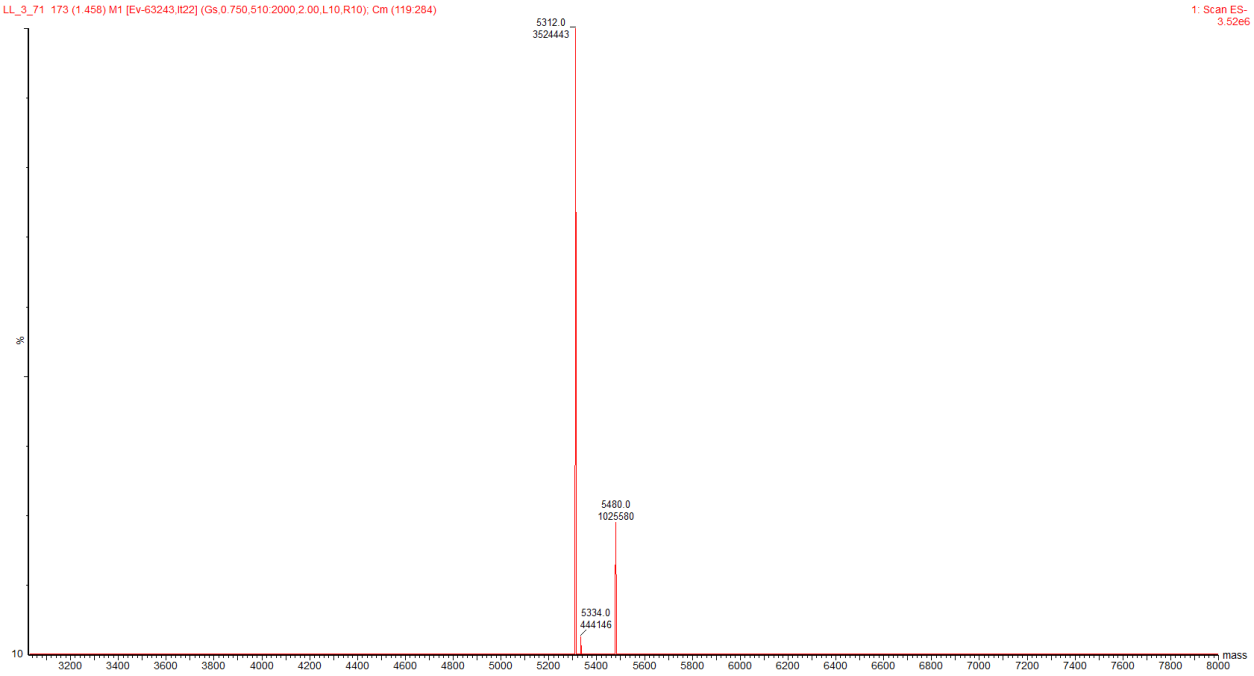




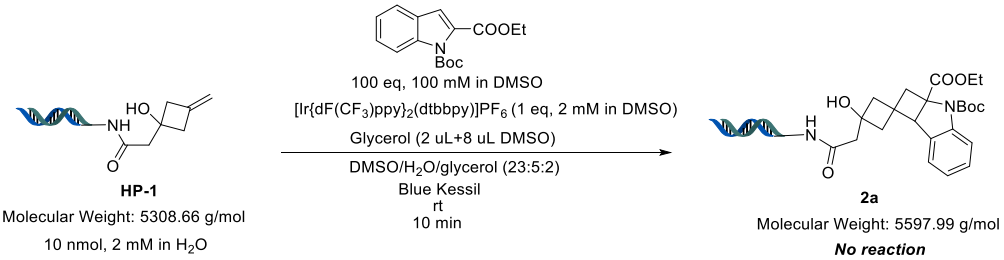
No photocatalyst:

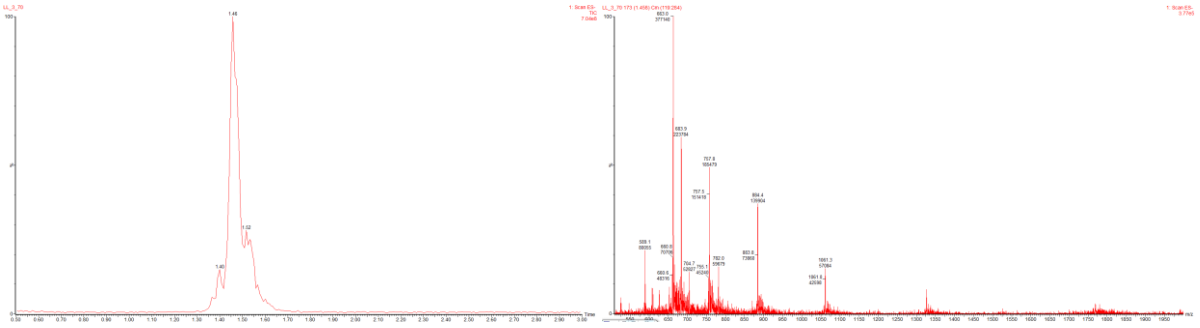


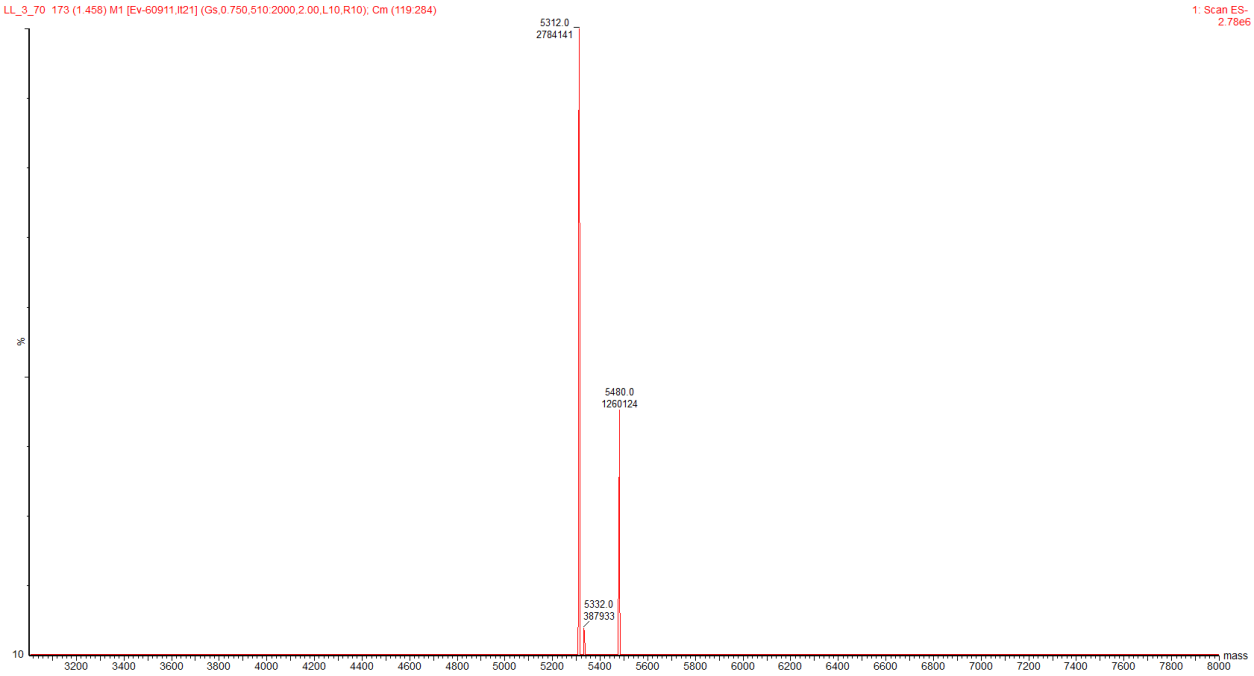




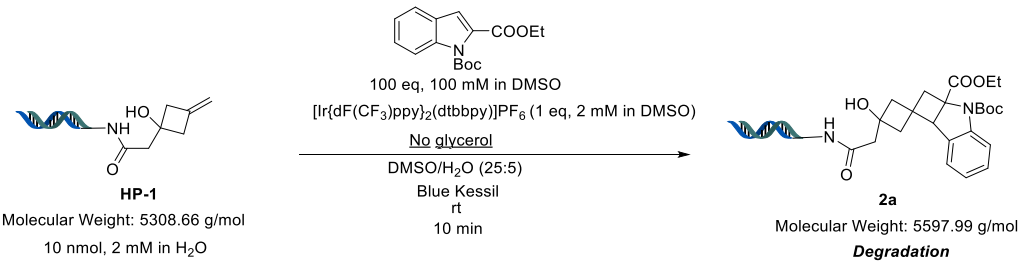
No light:


COOEt
N
Boc
100 eq, 100 mM in DMSO
[Ir{dF(CF3)ppy}2(dtbbpy)]PF6 (1 eq, 2 mM in DMSO)
Glycerol (2 uL+8 uL DMSO)
DMSO/H2O/glycerol (23:5:2)
Blue Kessil
rt
10 min
HO
NH
O
HP-1
Molecular Weight: 5308.66 g/mol
10 nmol, 2 mM in H2O
COOEt
HO
NBoc
H
N
O
2a
Molecular Weight: 5597.99 g/mol
No reaction




LL_3_70 173 (1.458) M1 [Ev-60911,It21] (Gs,0.750,510:2000,2.00,L10,R10); Cm (119:284)
1: Scan ES-
2.78e6
5312.0
2784141
5480.0
1260124
5332.0
387933
%
10
mass
3200
3400
3600
3800
4000
4200
4400
4600
4800
5000
5200
5400
5600
5800
6000
6200
6400
6600
6800
7000
7200
7400
7600
7800
8000

No glycerol:



HP-1

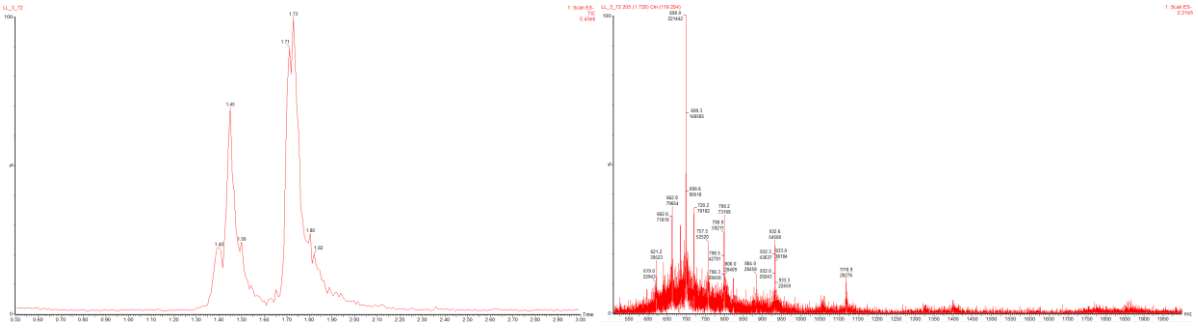
Molecular Weight: 5308.66 g/mol

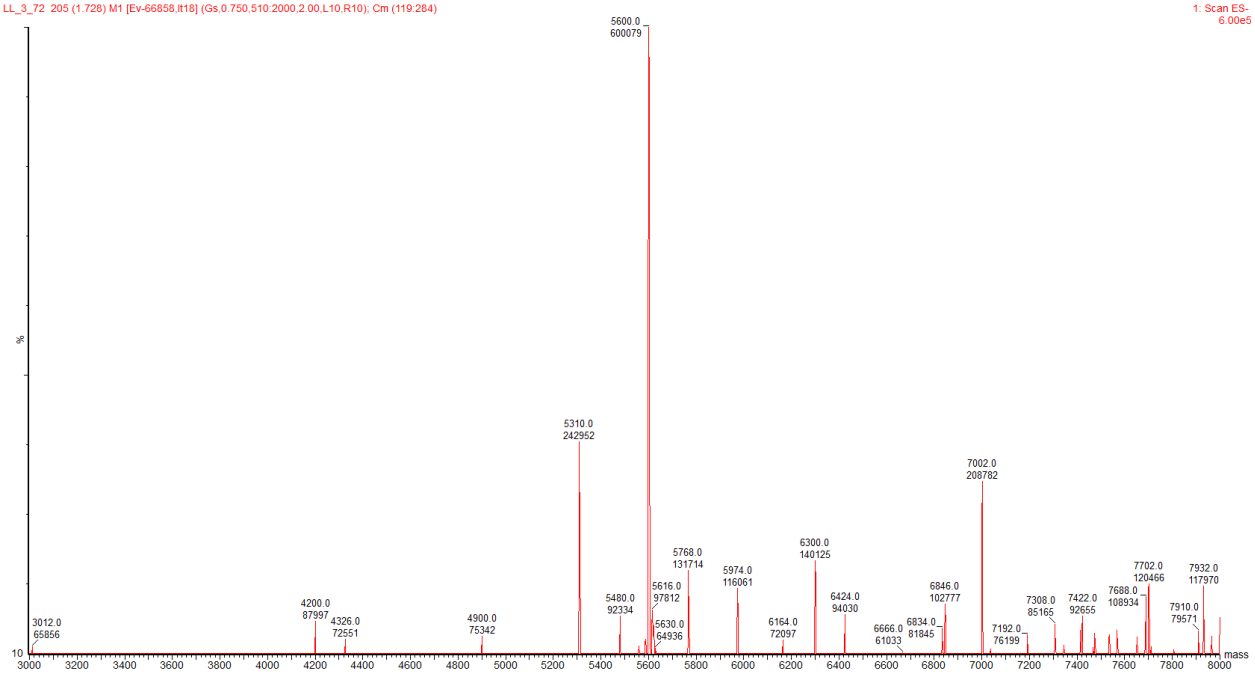
10 nmol, 2 mM in H_2O

2a

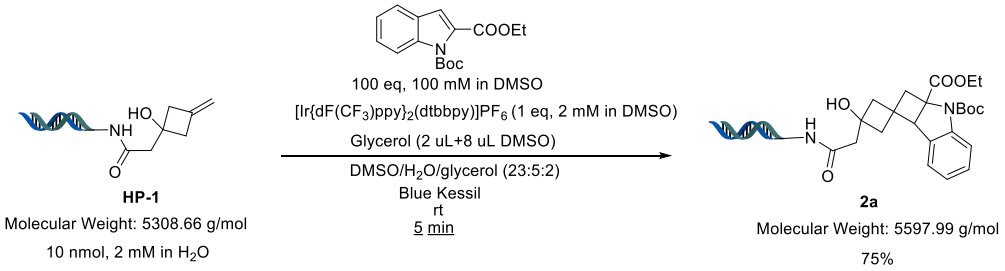
Molecular Weight: 5597.99 g/mol

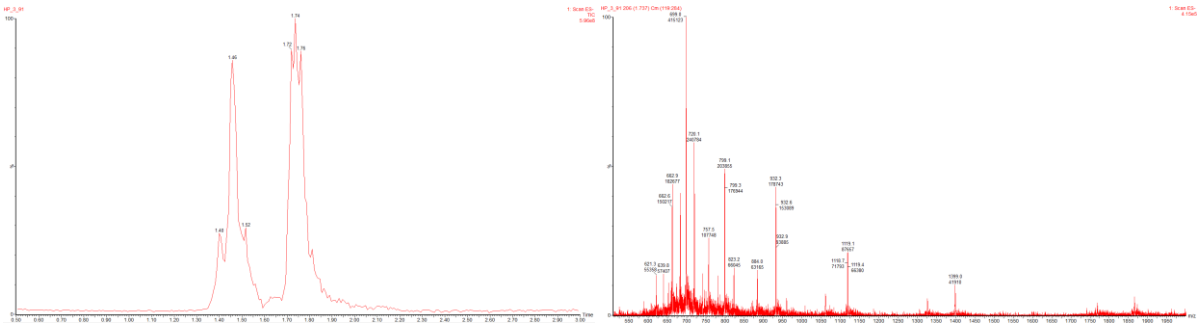
Degradation

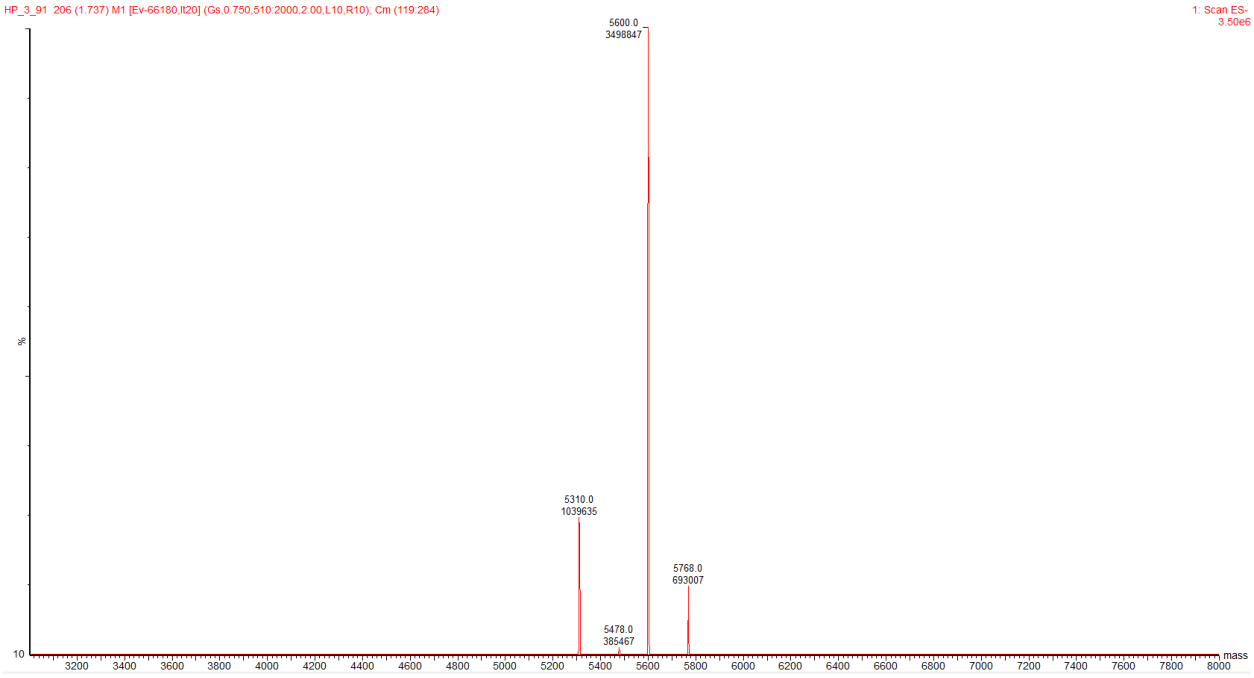




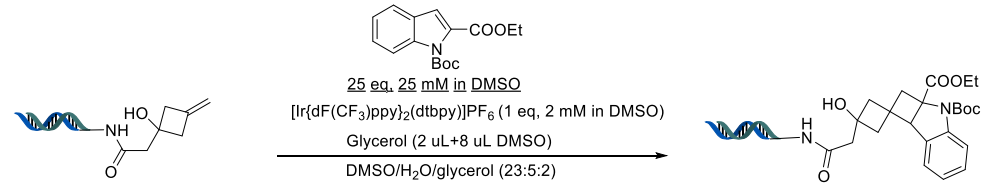
5 min instead of 10 min:







25 Equiv of indole:



HP-1

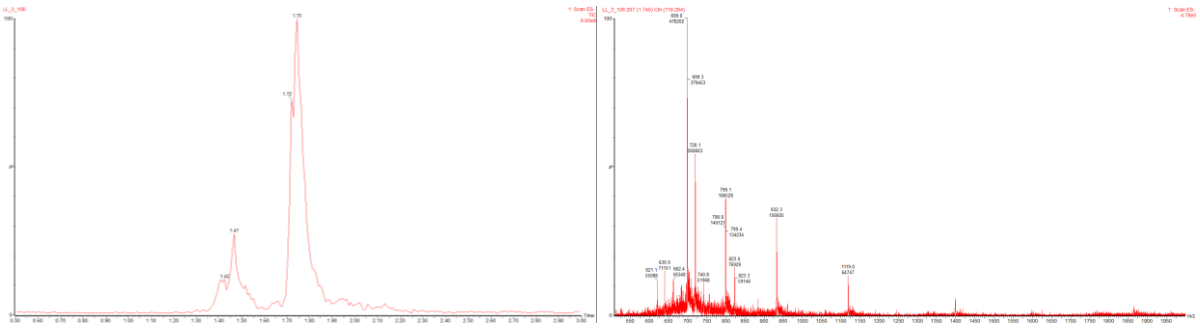
Molecular Weight: 5308.66 g/mol

10 nmol, 2 mM in H_2O

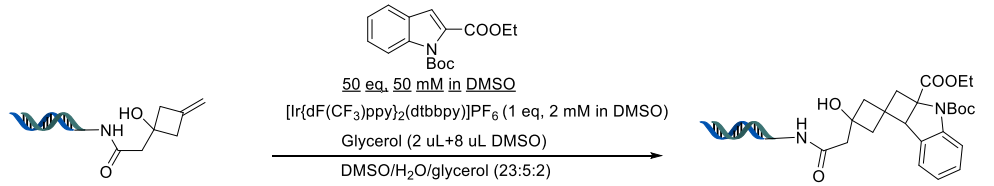
2a

Molecular Weight: 5597.99 g/mol

88%



50 Equiv of indole:



HP-1

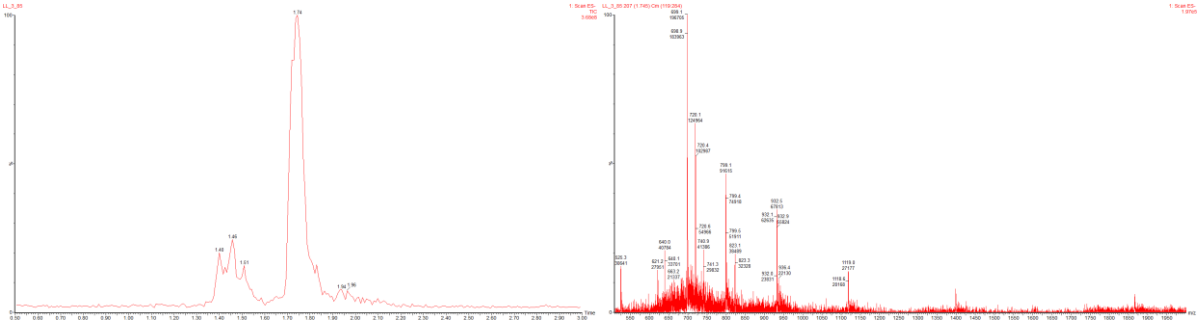
Molecular Weight: 5308.66 g/mol

10 nmol, 2 mM in H_2O

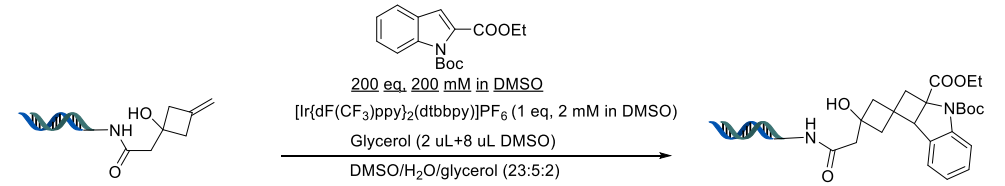
2a

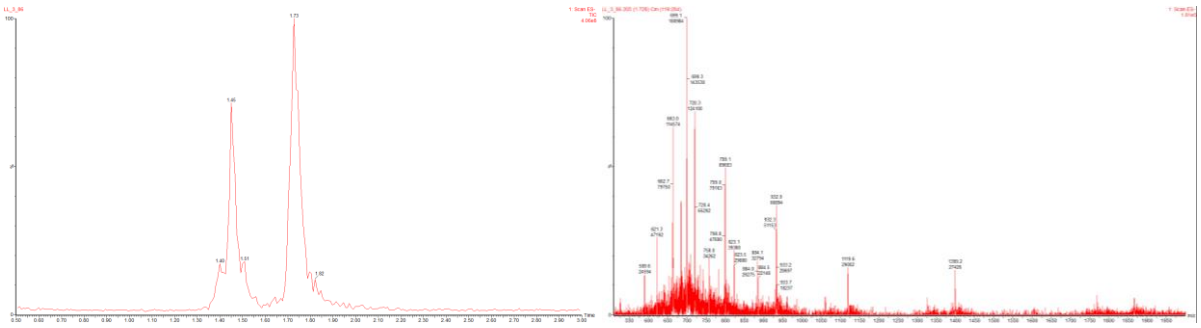
Molecular Weight: 5597.99 g/mol

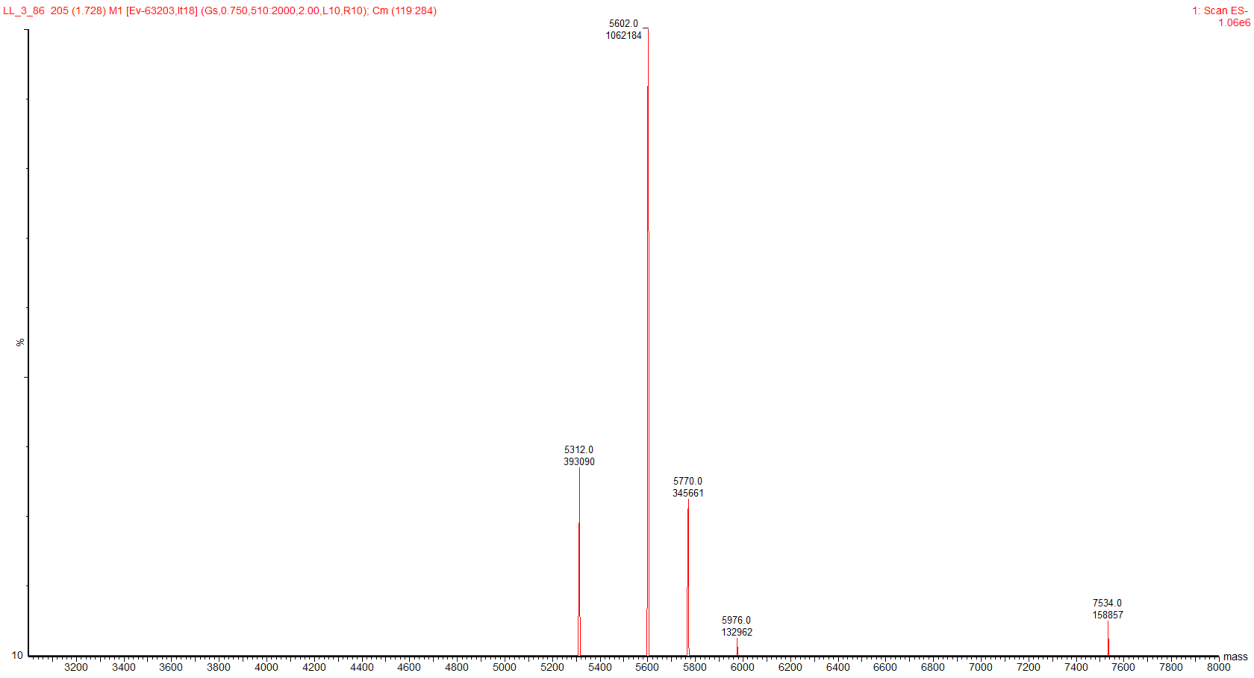
93%



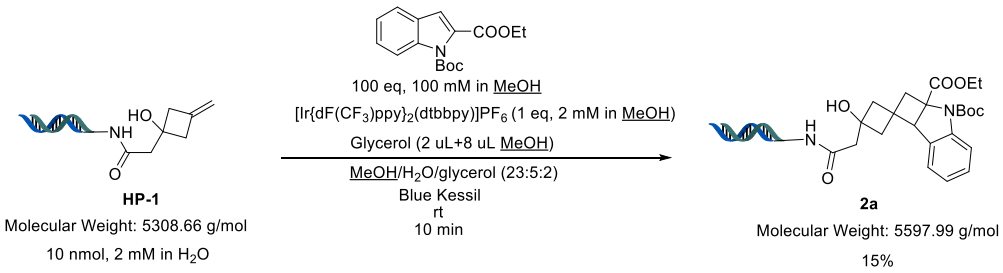
200 equiv of indole:

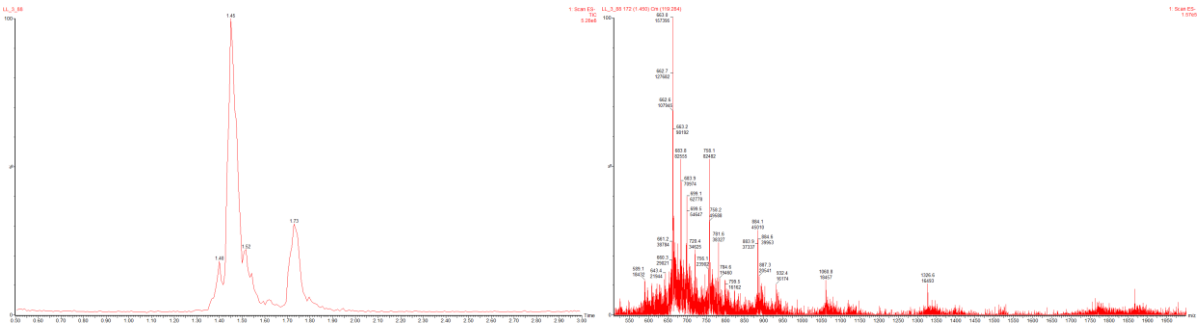


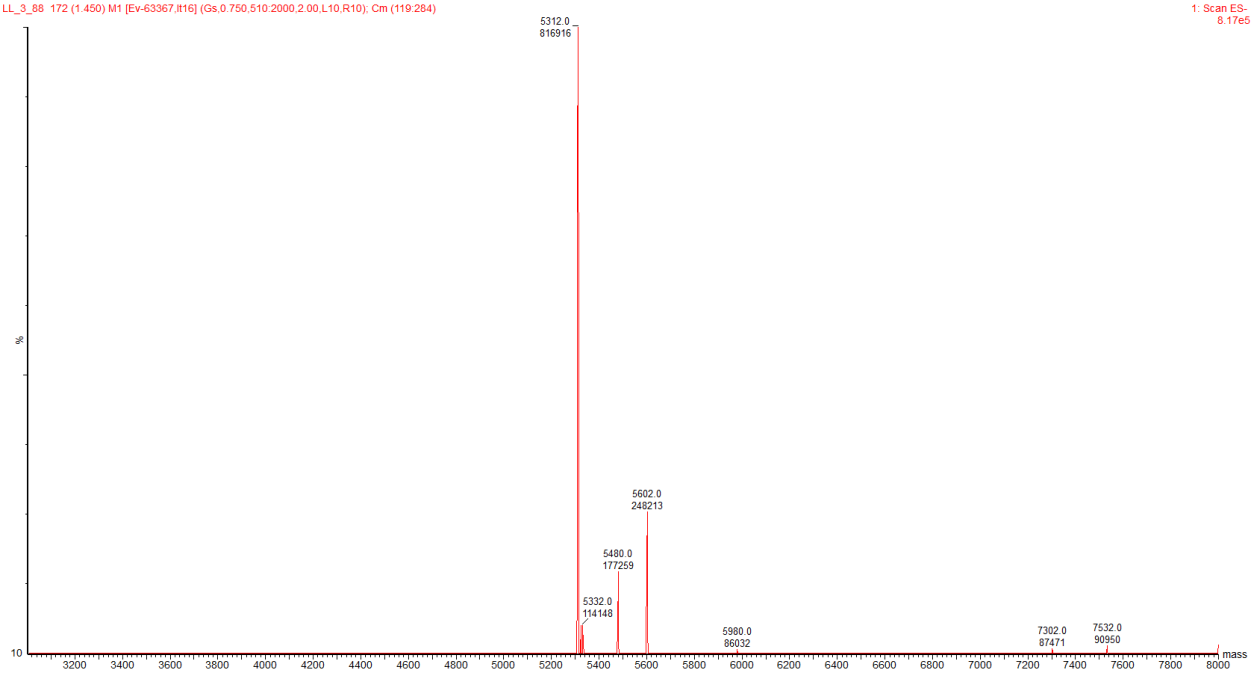




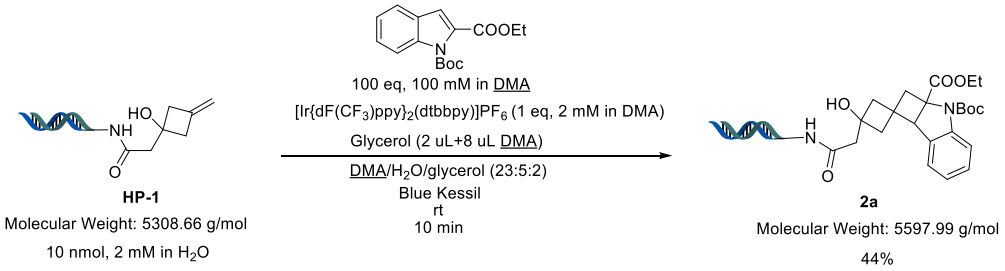
MeOH instead of DMSO:

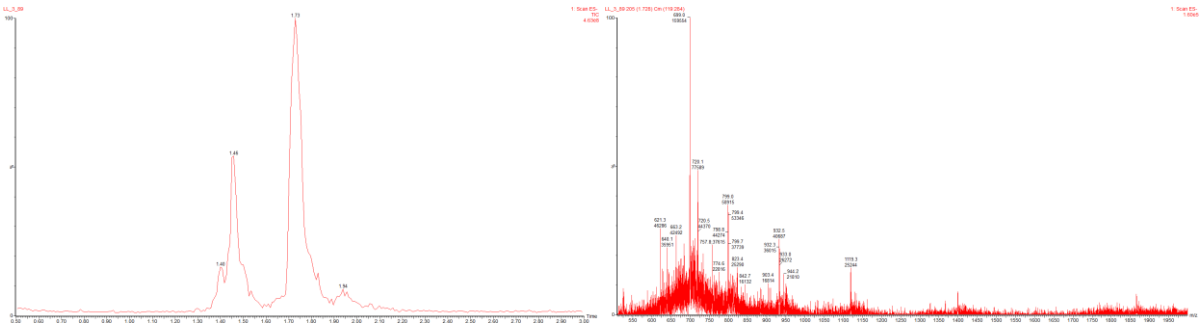


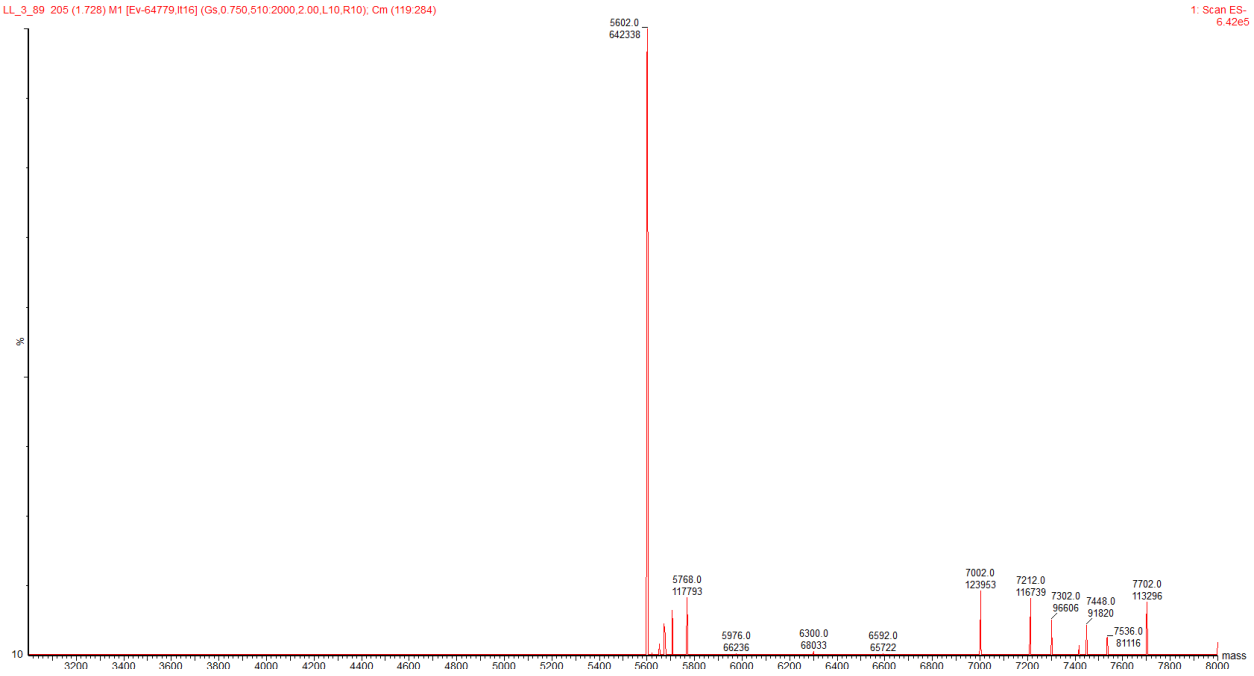




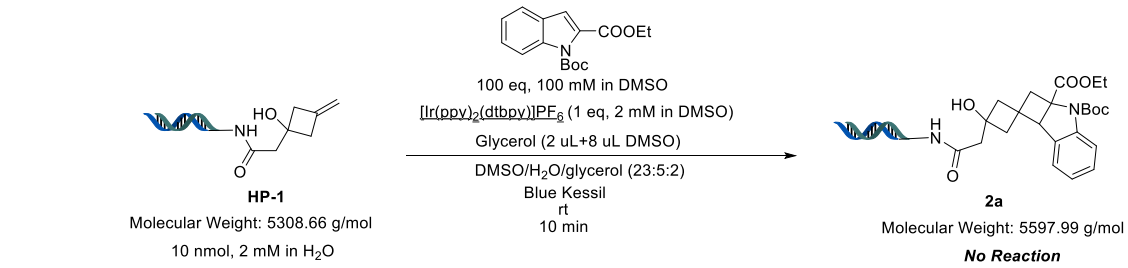
DMA instead of DMSO:

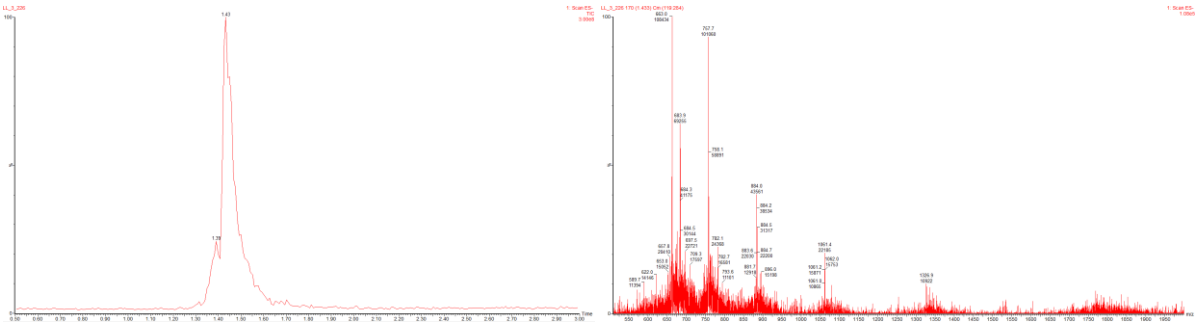




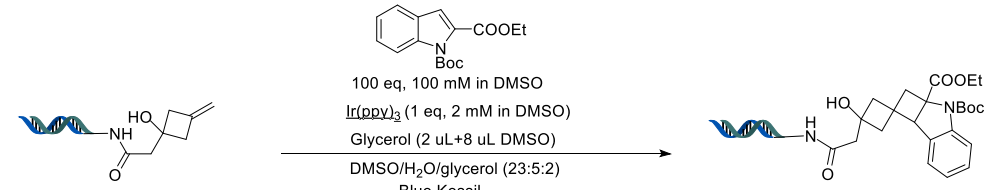


[Ir(ppy)$_2$(dtbpy)]PF$_6$ instead of [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$:





Ir(ppy)$_3$ instead of [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$:



HP-1

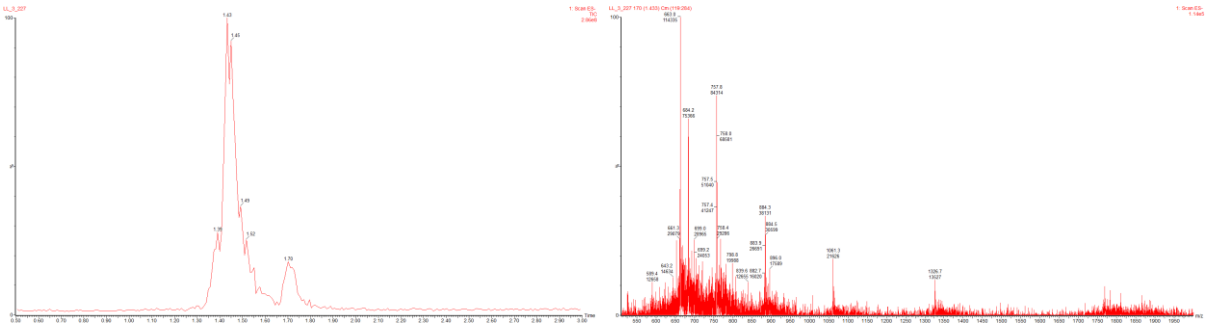
Molecular Weight: 5308.66 g/mol

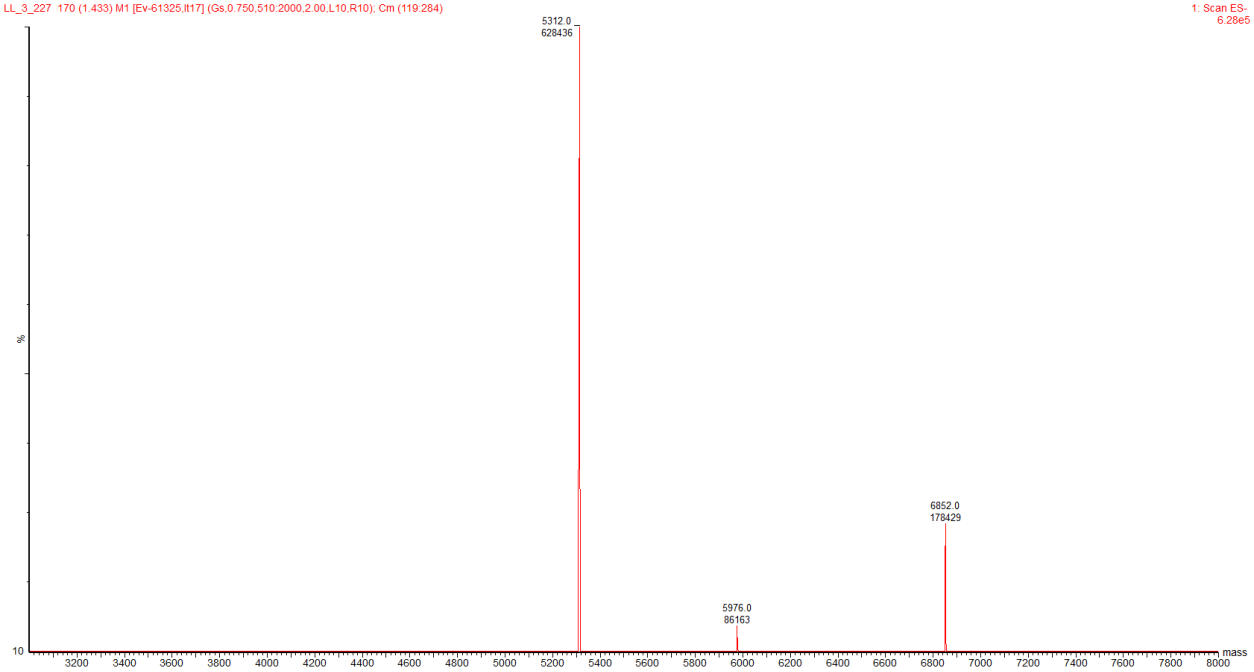
10 nmol, 2 mM in H_2O

2a

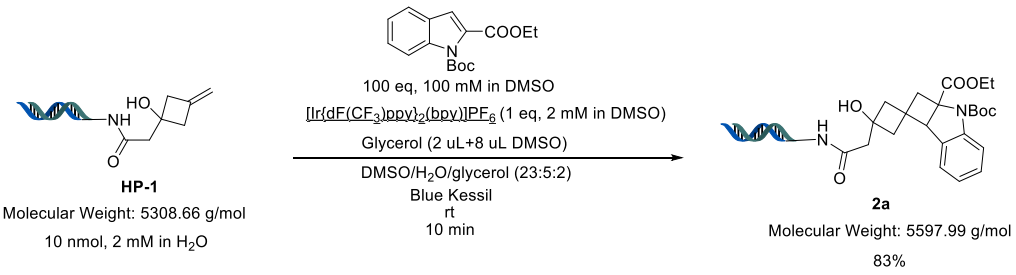
Molecular Weight: 5597.99 g/mol

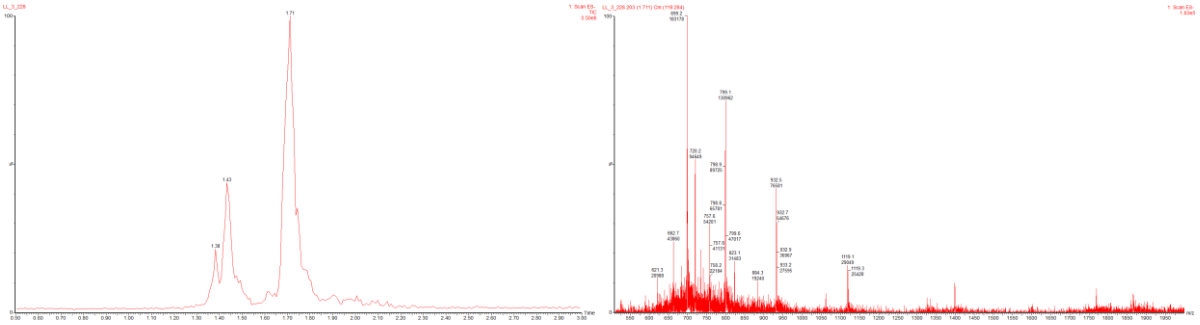
No Reaction



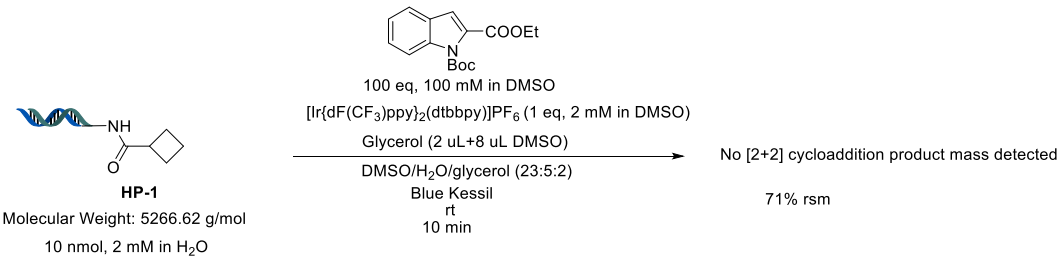


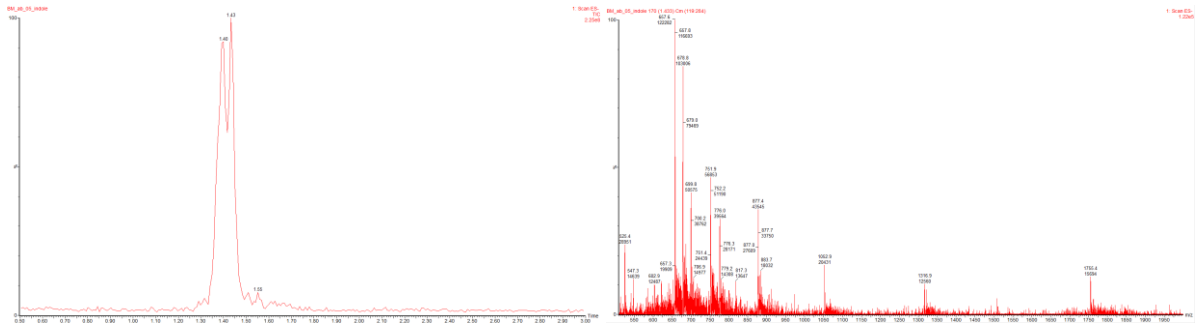
[Ir{dF(CF$_3$)ppy}$_2$(bpy)]PF$_6$ instead of [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$:

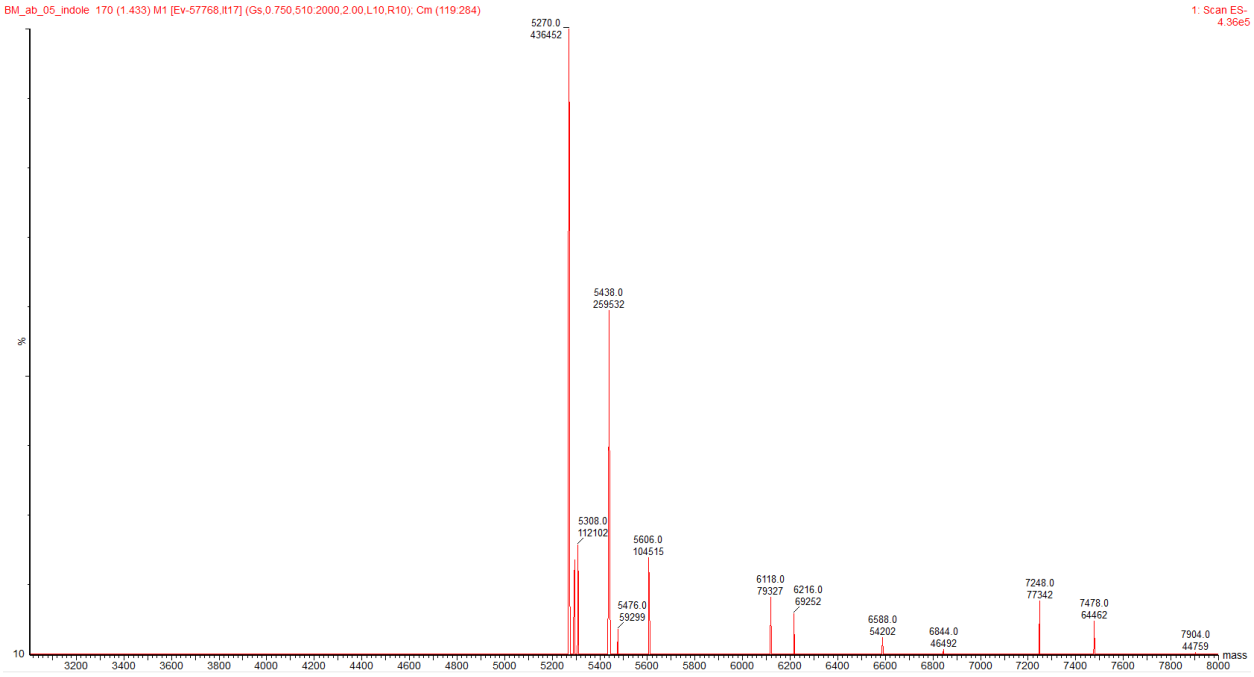




HP-A instead of **HP-1**

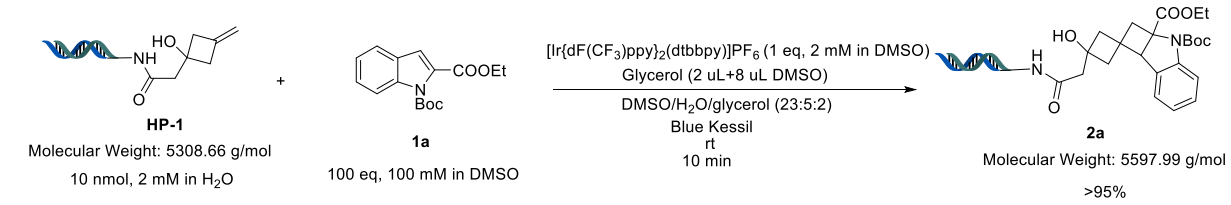


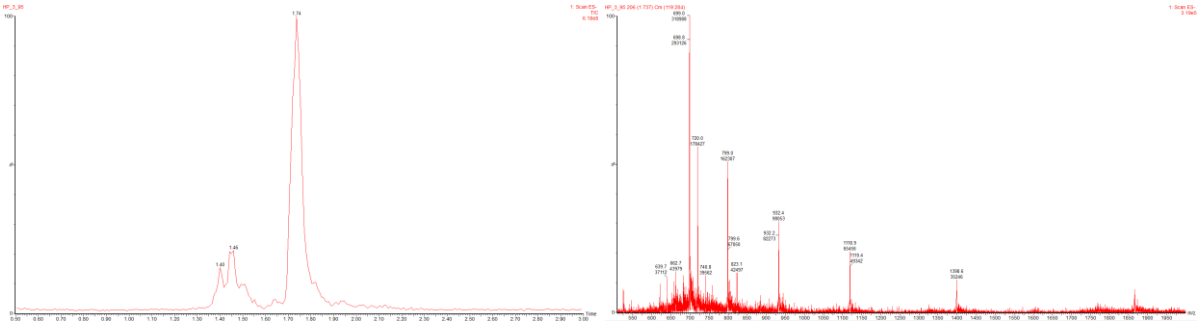




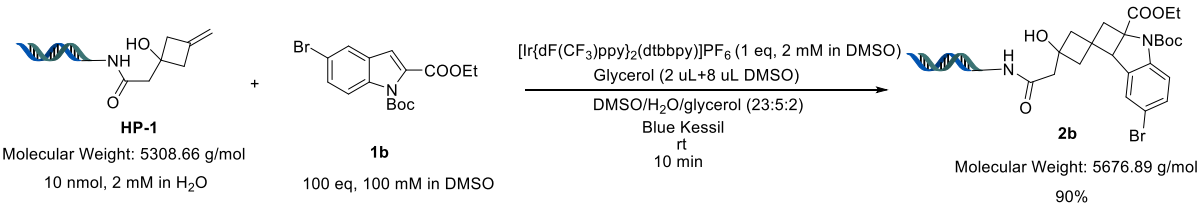
14 Determination of yields for On-DNA reactions

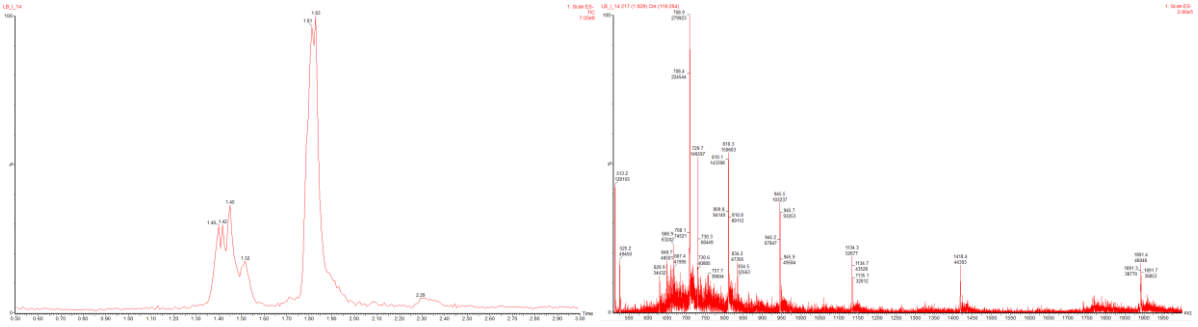
<u>General procedure C</u>: Compound **2a**, *>95% yield*



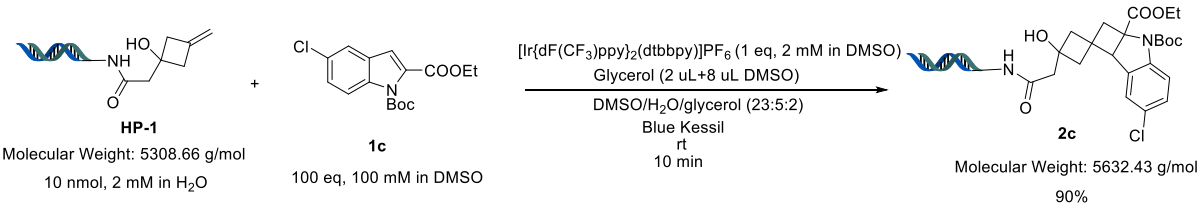


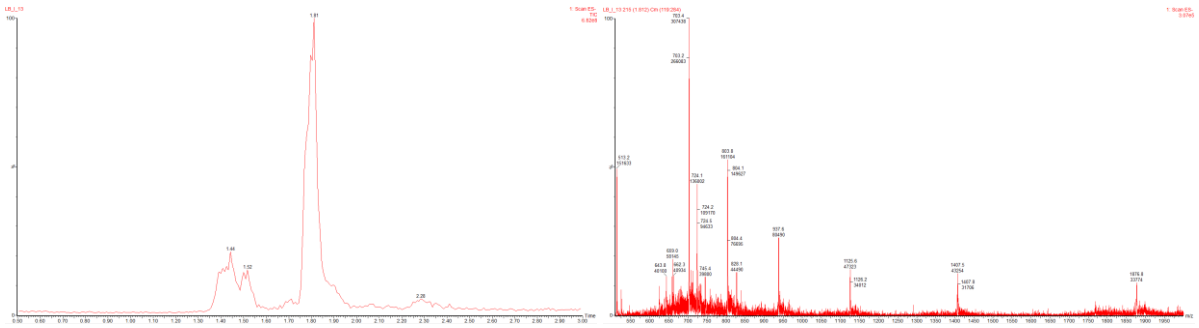
General procedure C: Compound **2b**, *90% yield*



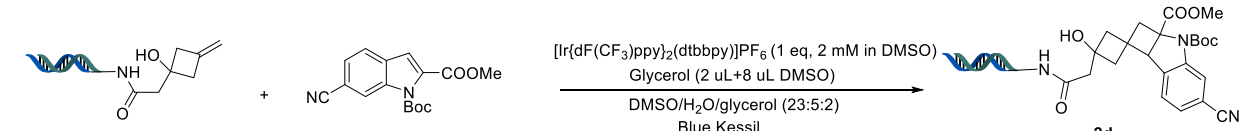


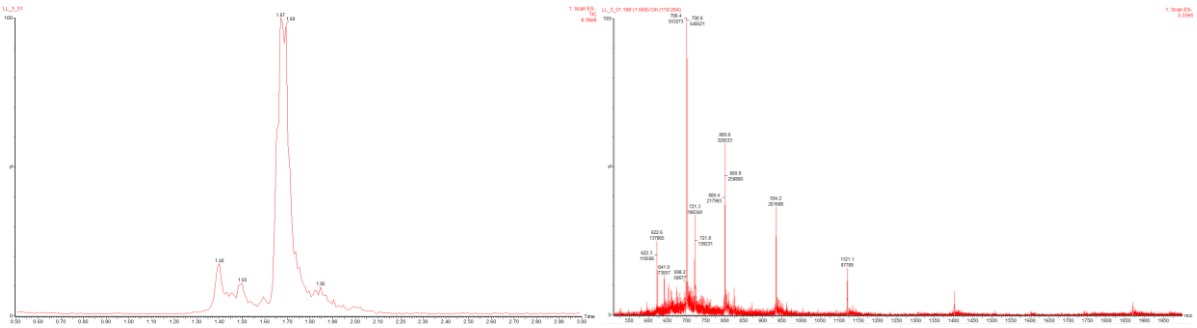
General procedure C: Compound **2c**, *90% yield*



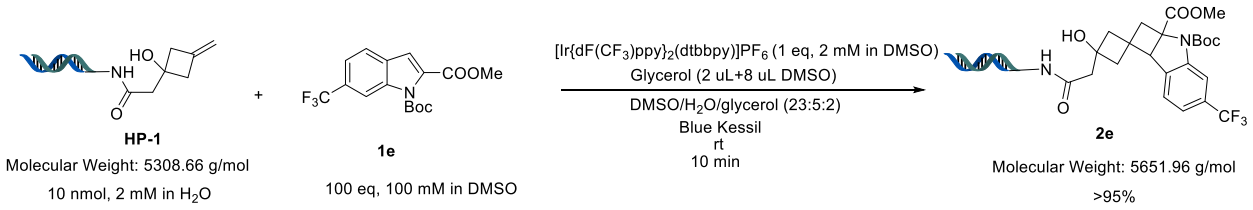


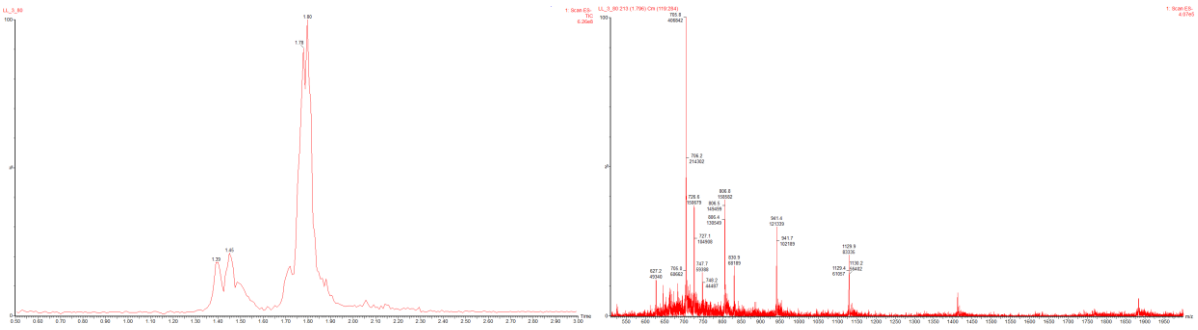
General procedure C: Compound **2d**, *>95% yield*

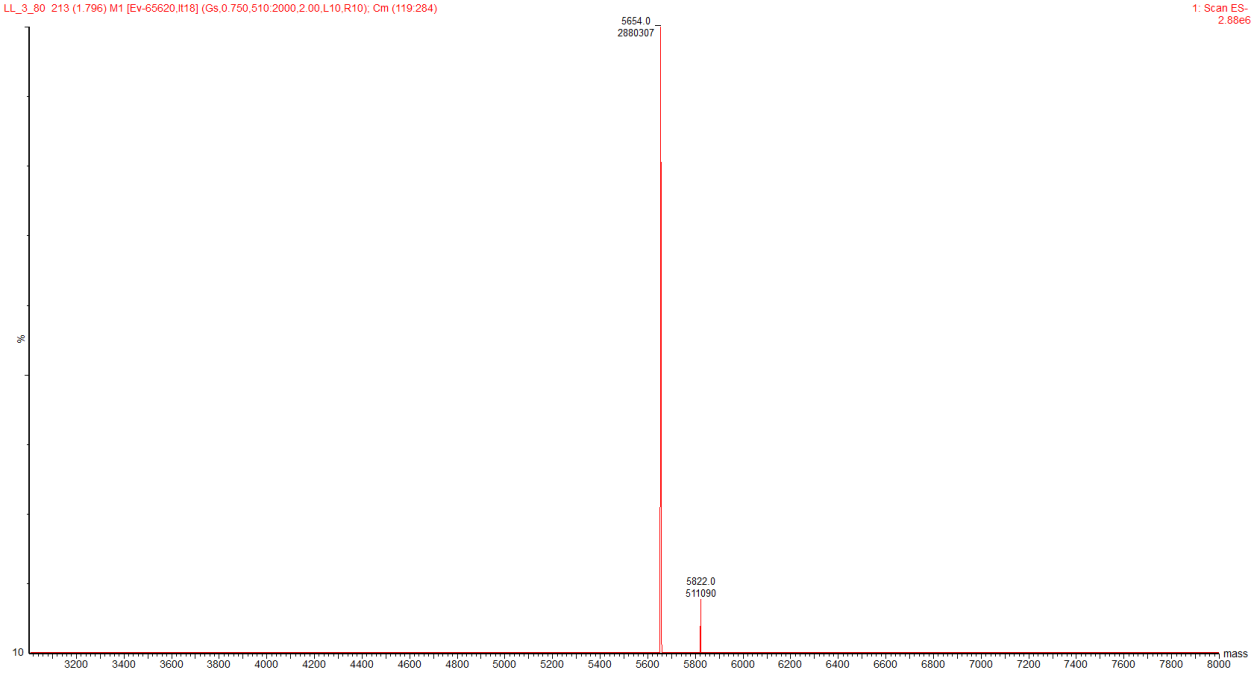




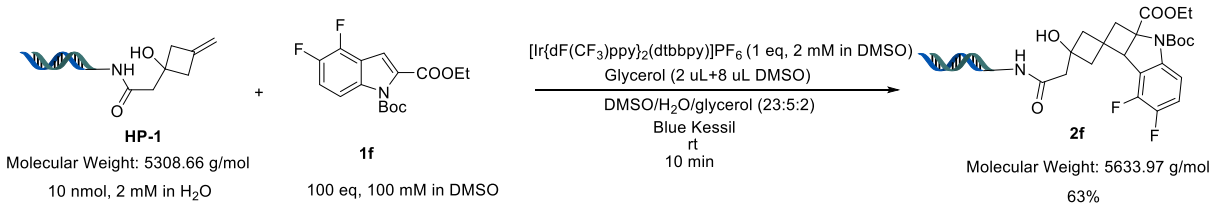
General procedure C: Compound **2e**, *>95% yield*

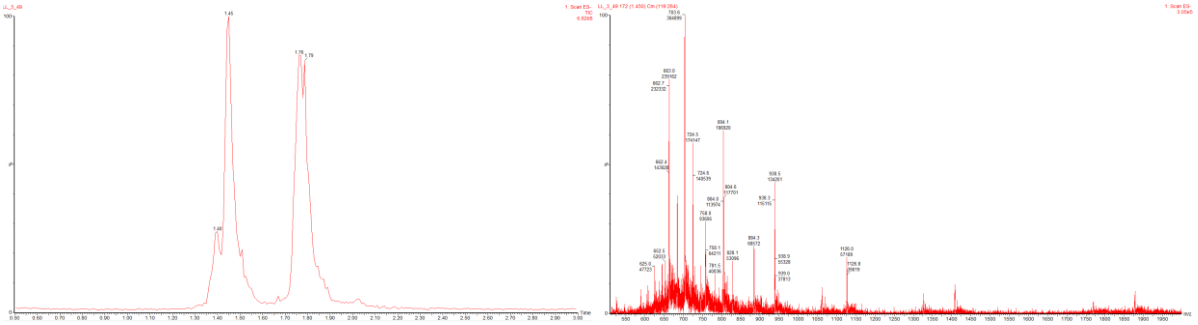




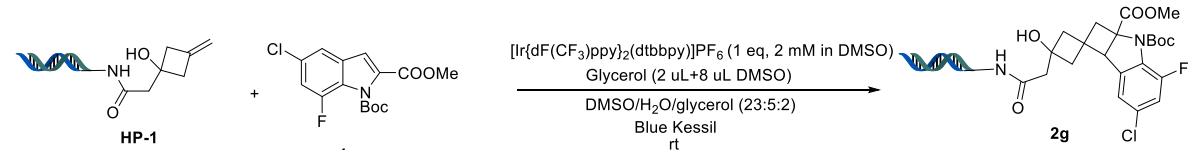


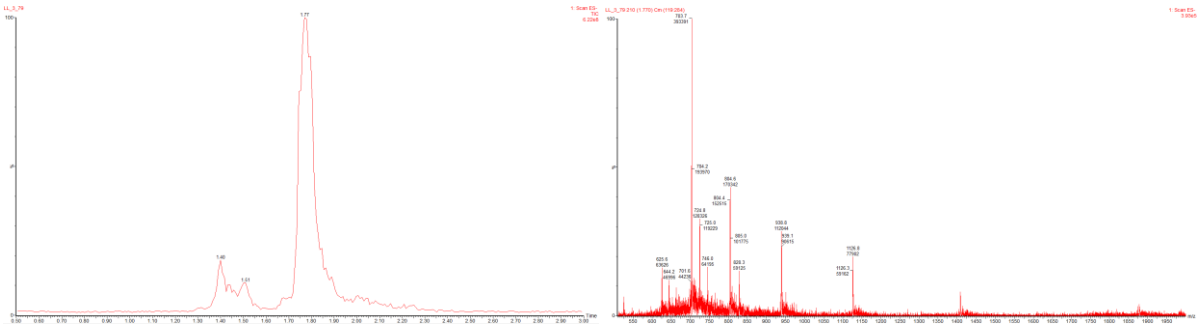
General procedure C: Compound **2f**, *63% yield*



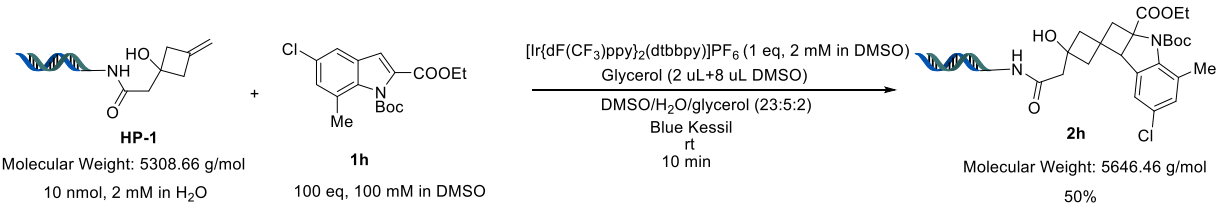


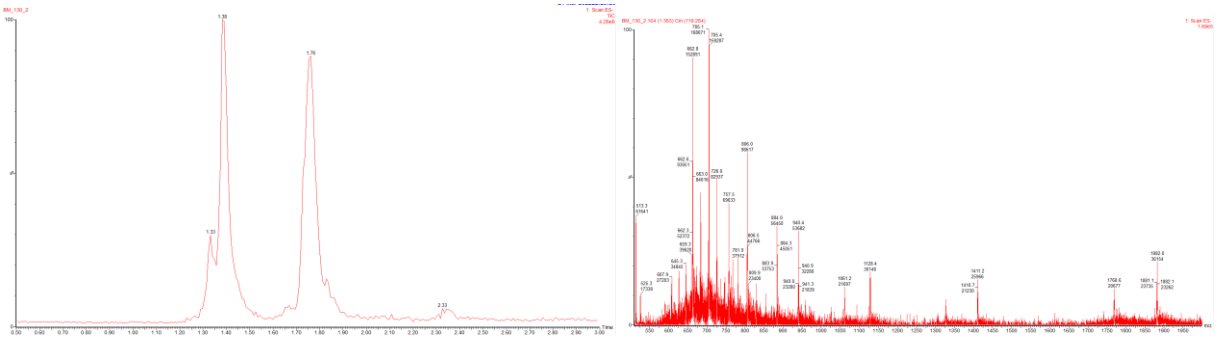
General procedure C: Compound **2g**, *70% yield*

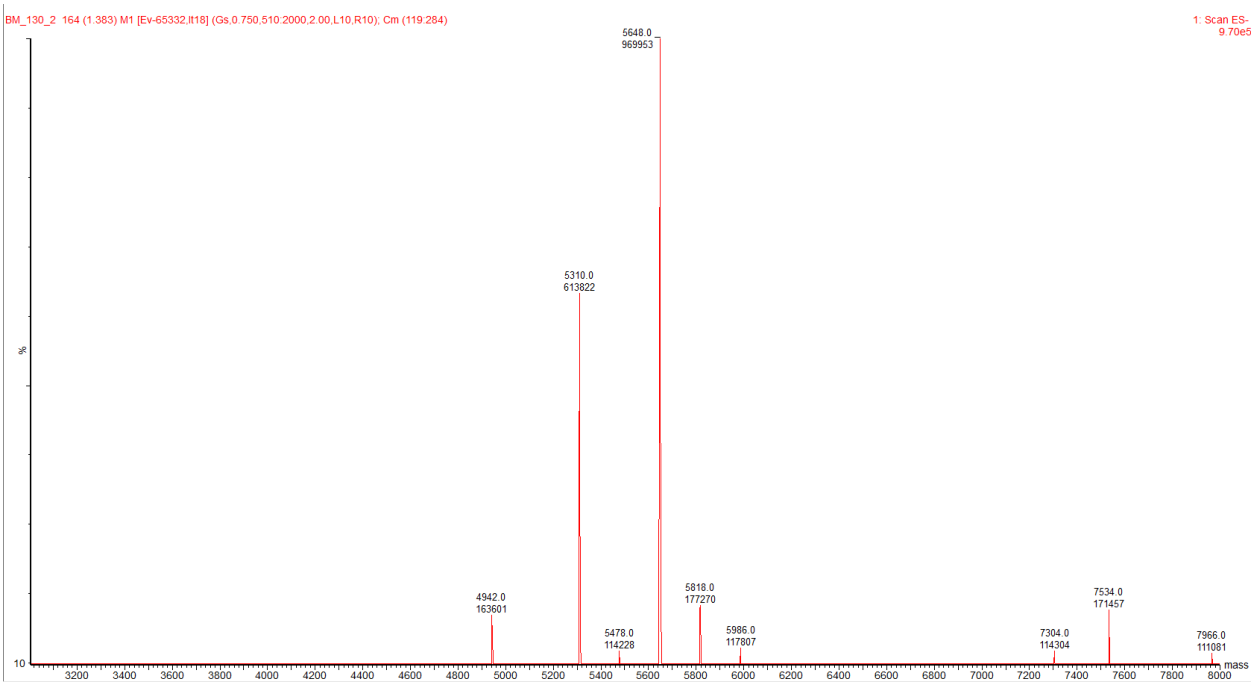




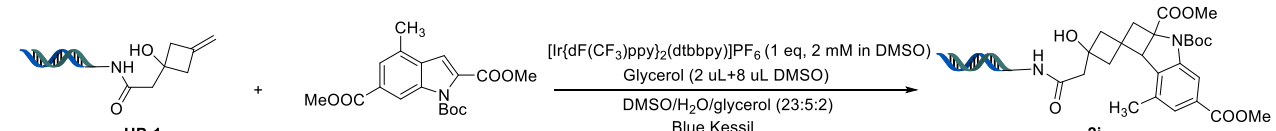
General procedure C: Compound **2h**, *50% yield*

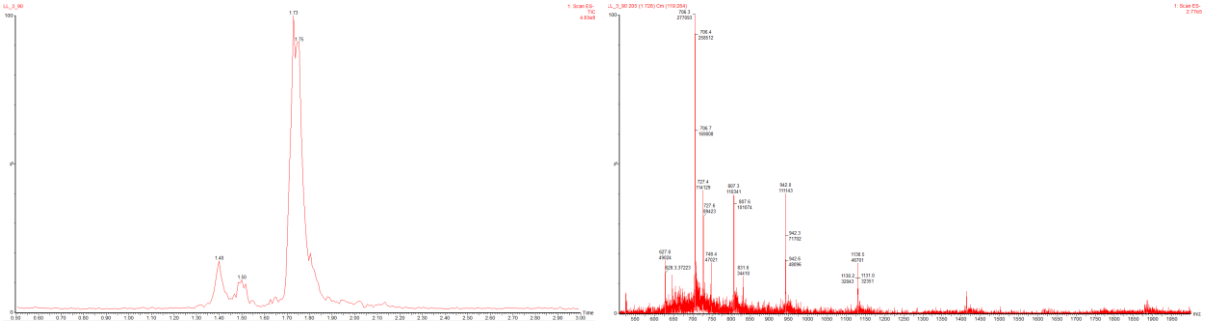


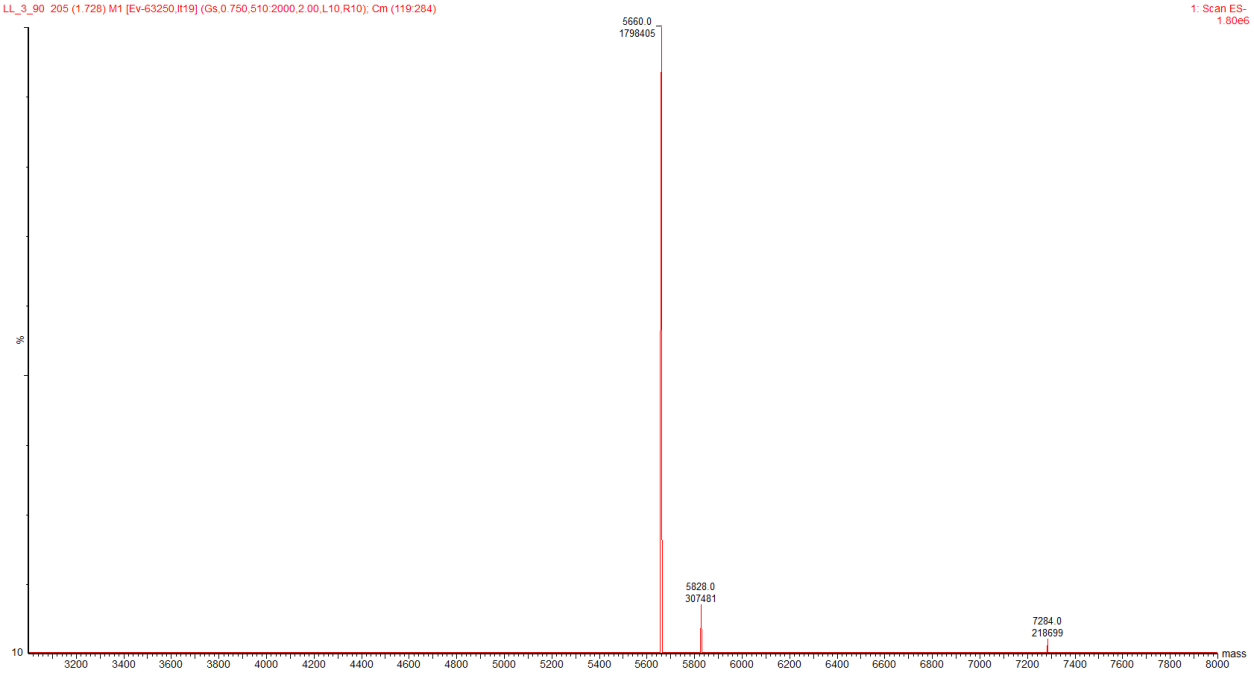




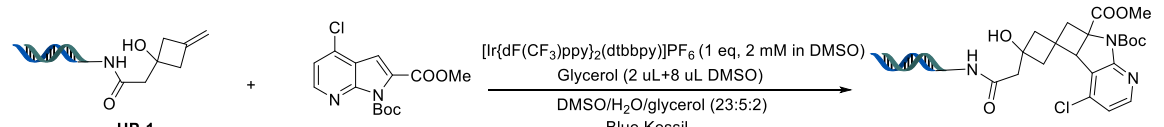
General procedure C: Compound **2i**, *91% yield*

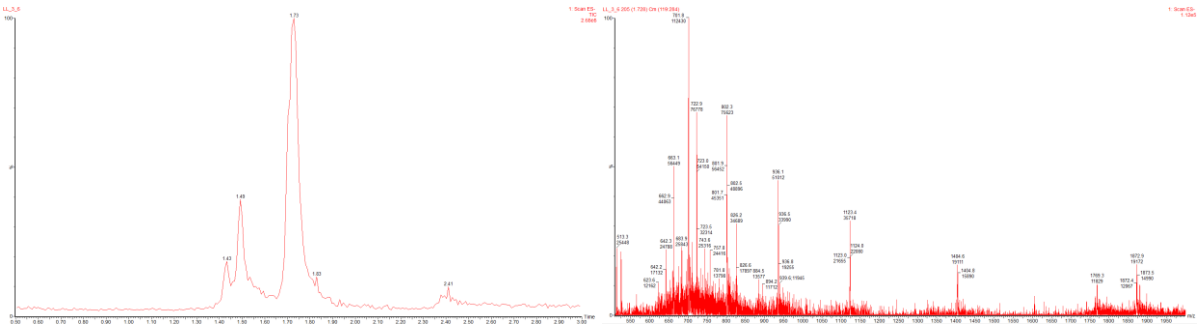




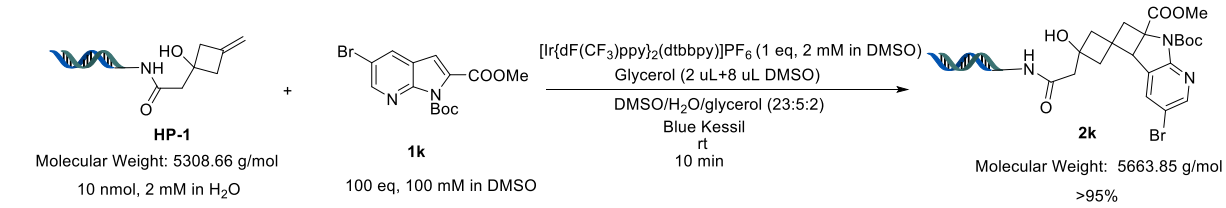


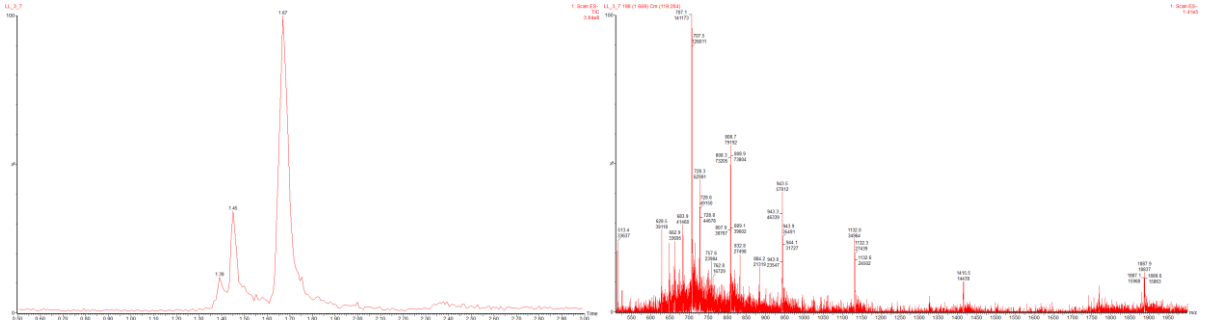
General procedure C: Compound **2j**, *>95% yield*

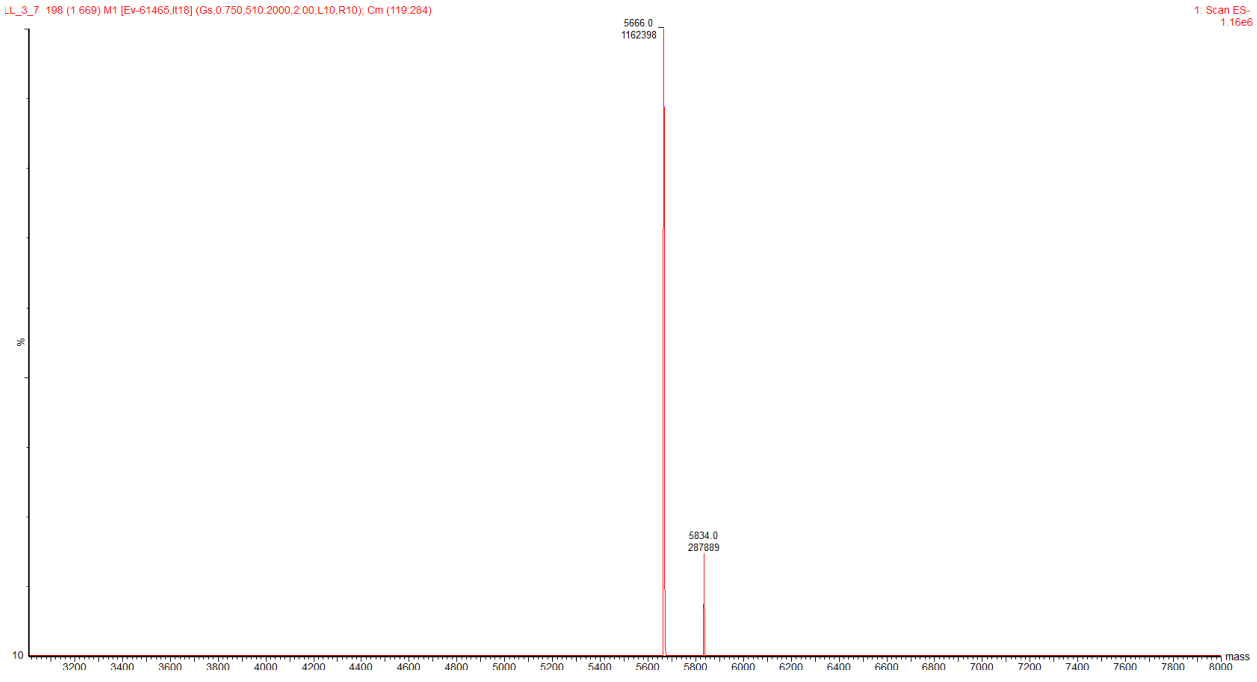




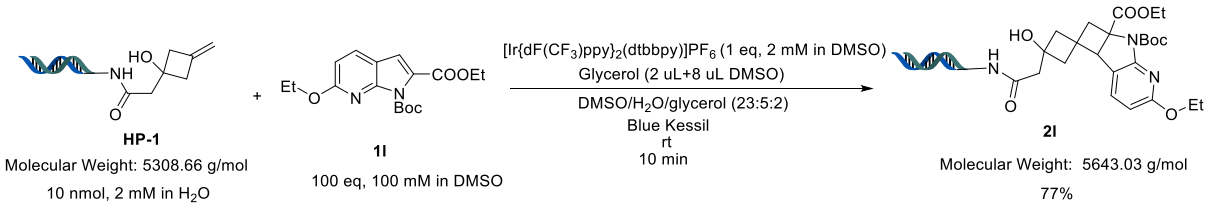
General procedure C: Compound **2k**, *>95% yield*

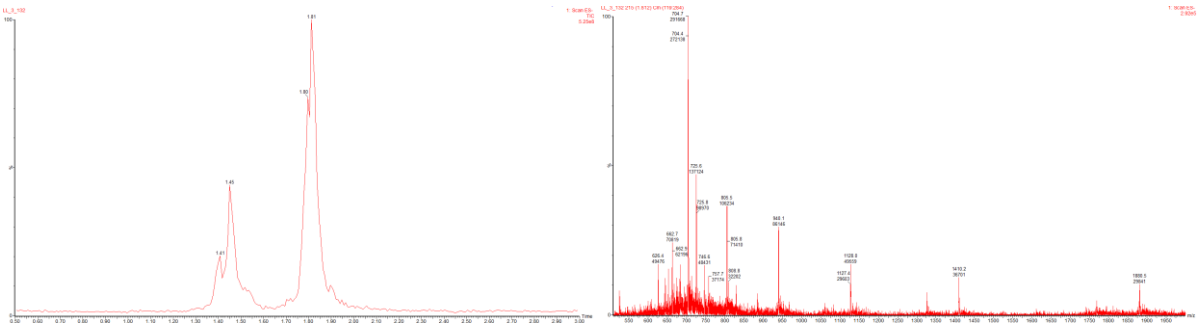




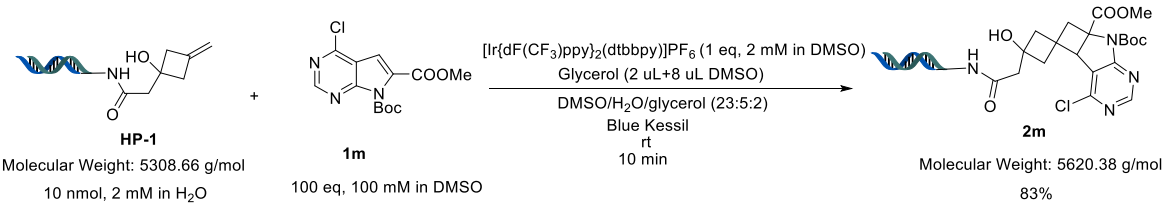


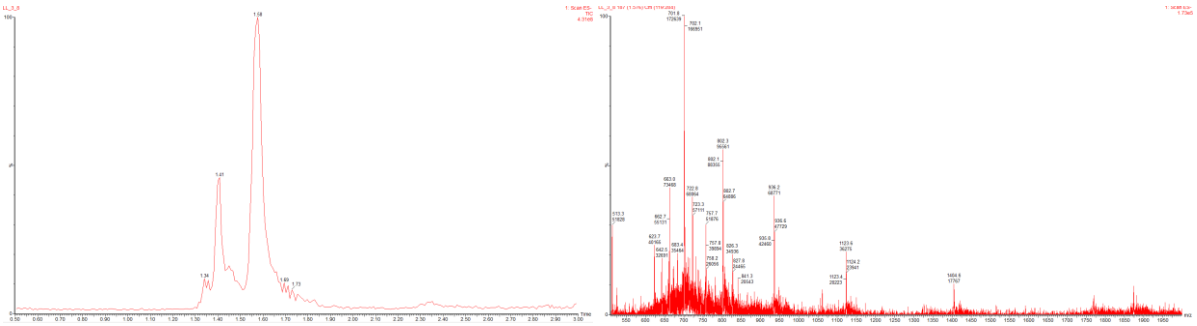
General procedure C: Compound **2l**, *77% yield*



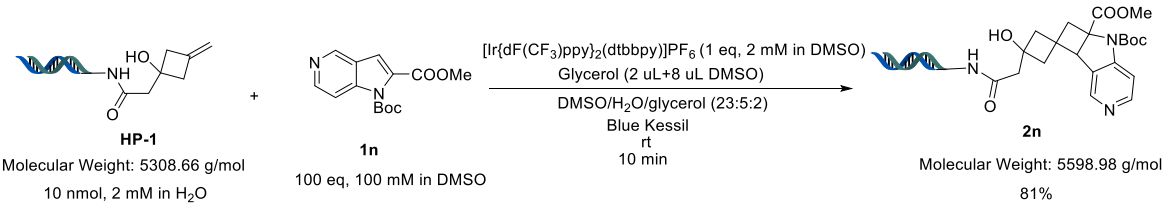


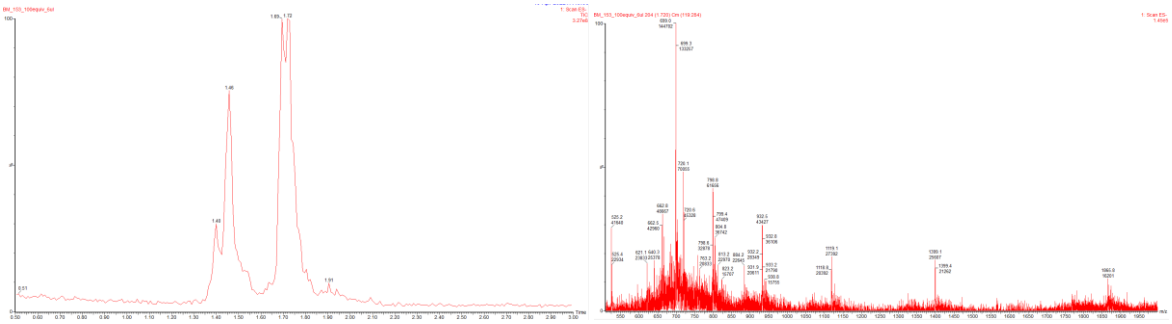
General procedure C: Compound **2m**, *83% yield*



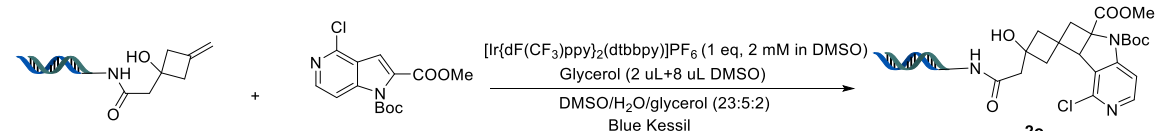


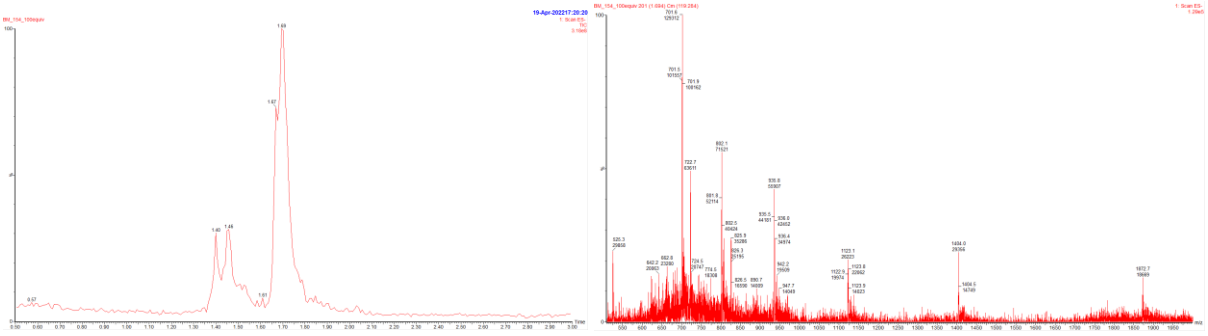
General procedure C: Compound **2n**, *81% yield*

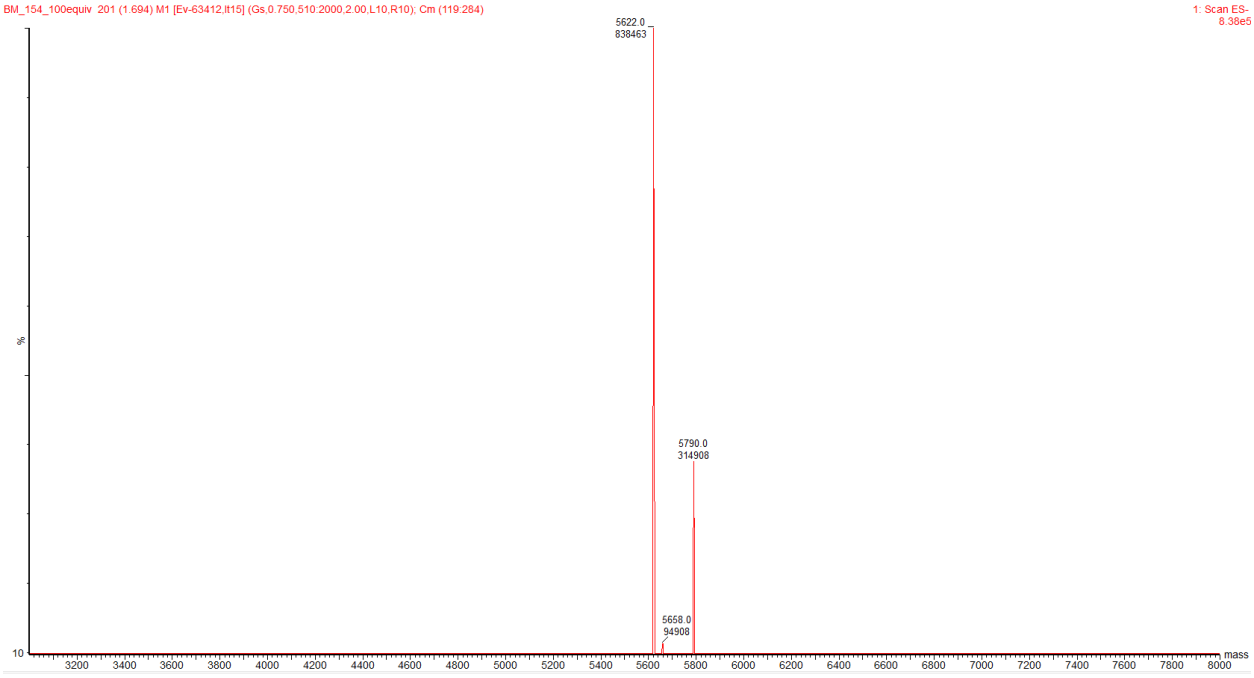




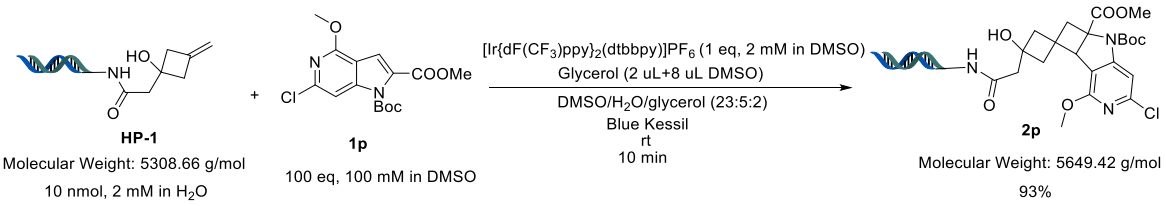
General procedure C: Compound **2o**, *>95% yield*

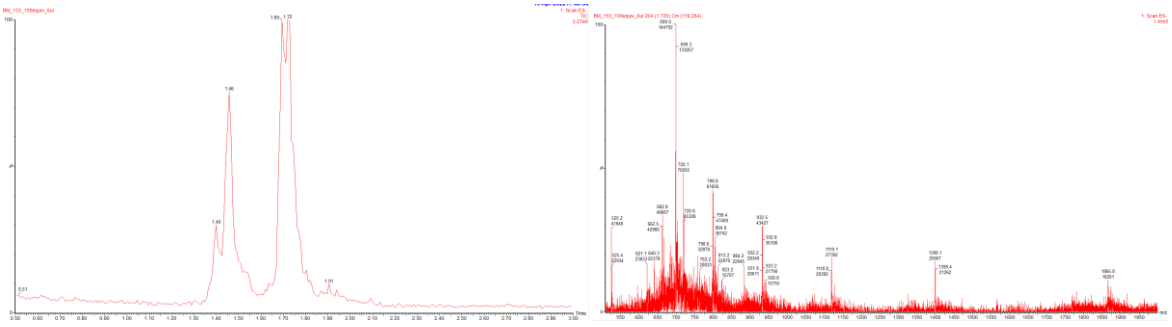




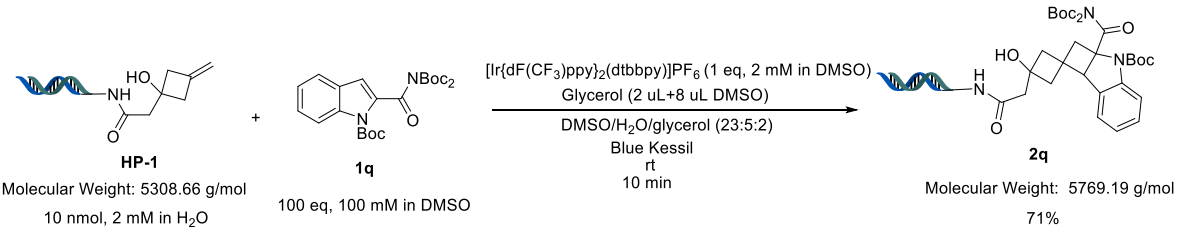


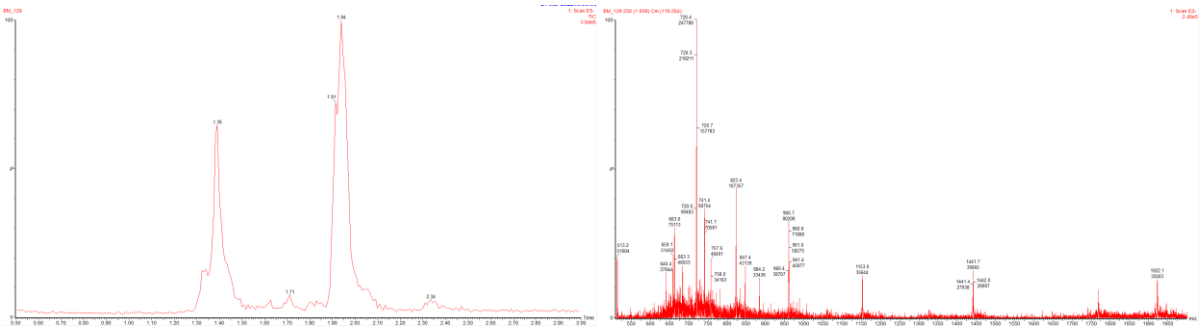
General procedure C: Compound **2p**, *93% yield*

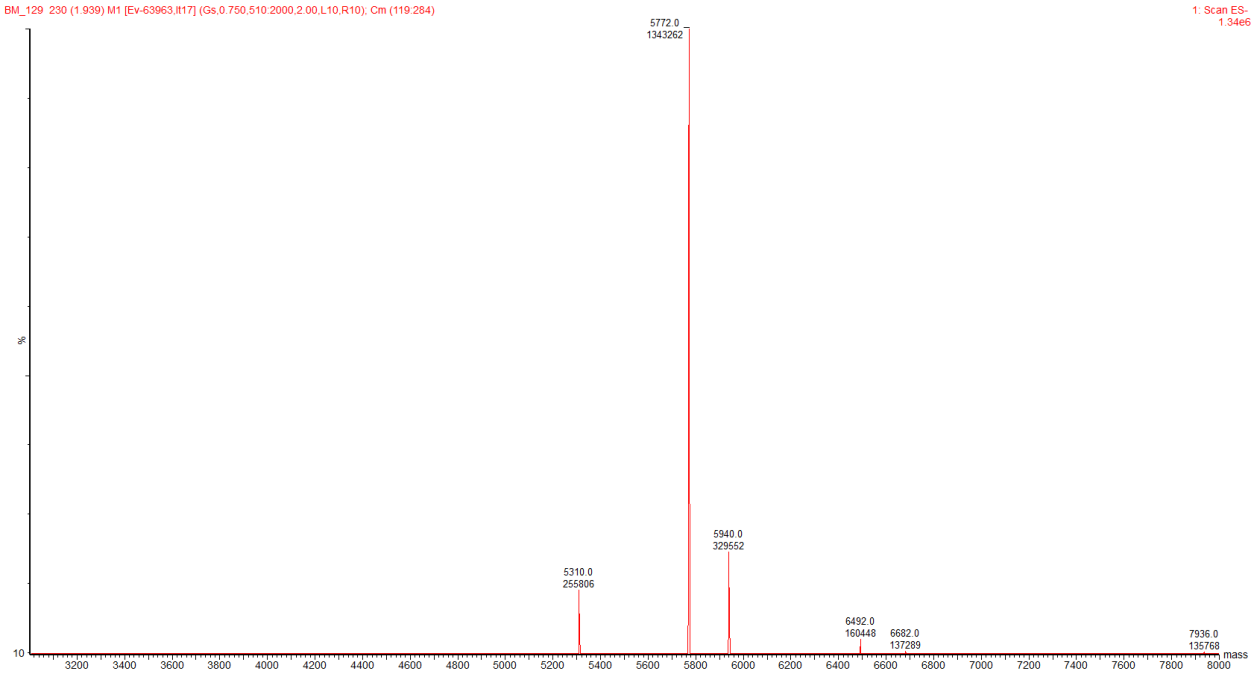




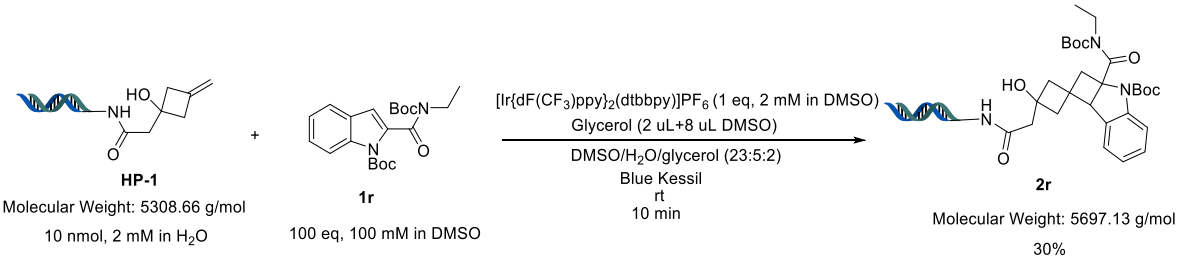
General procedure C: Compound **2q**, *71% yield*

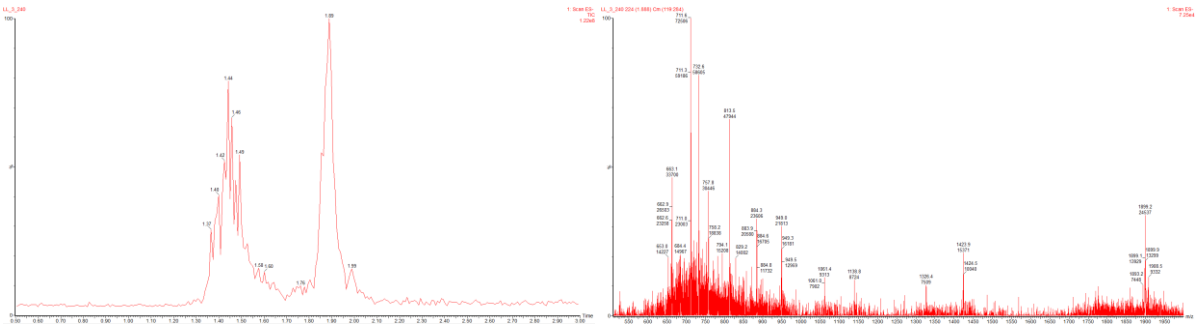


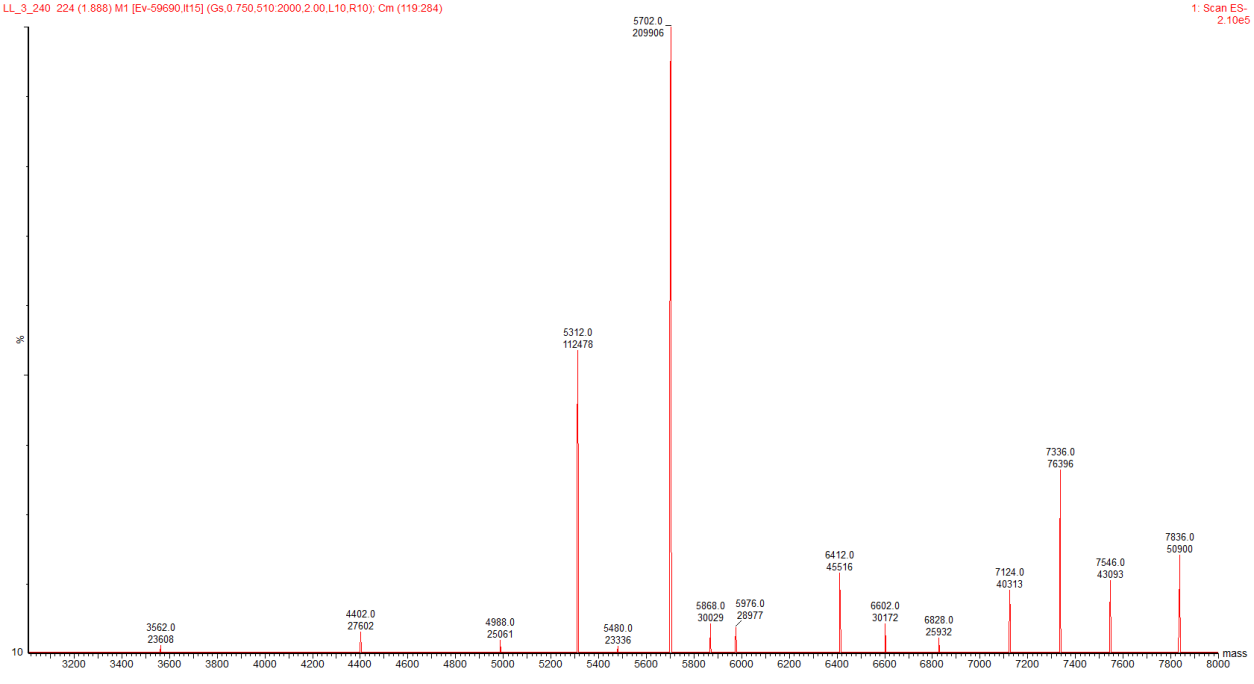




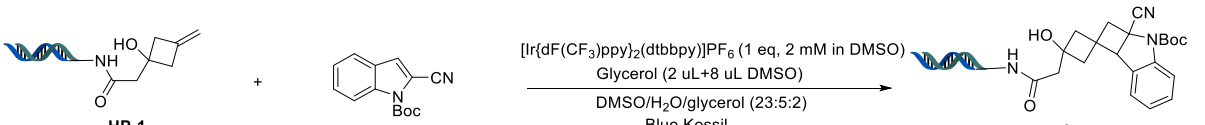
General procedure C: Compound **2r**, *30% yield*

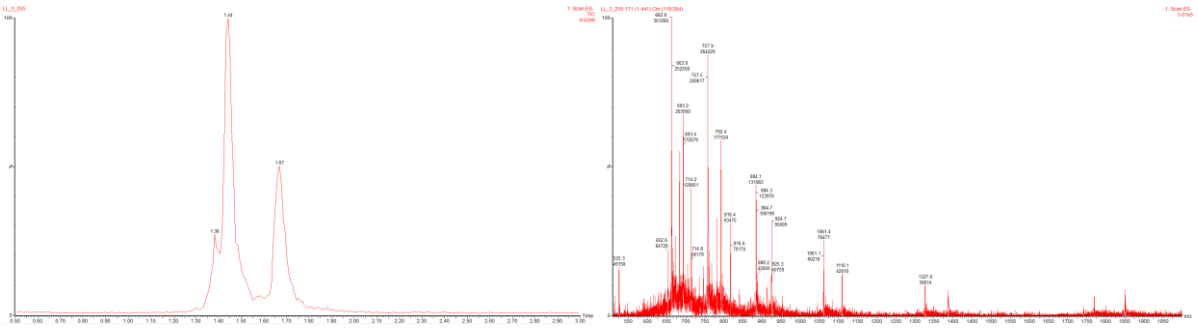


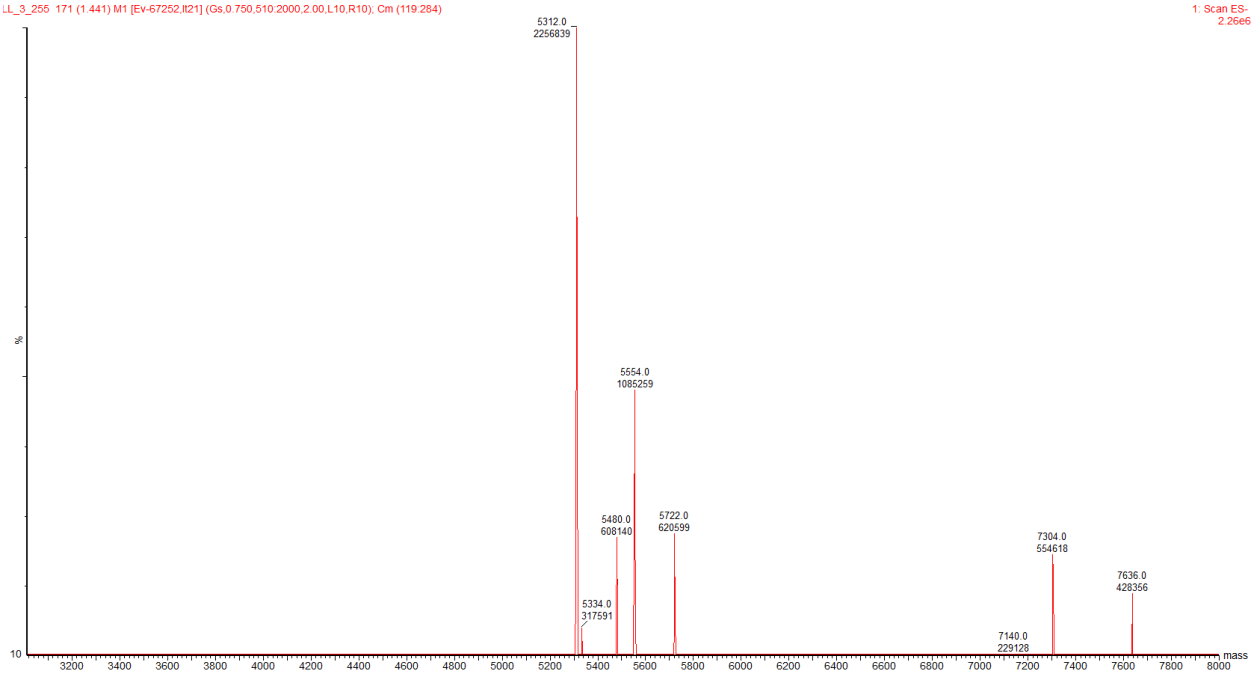




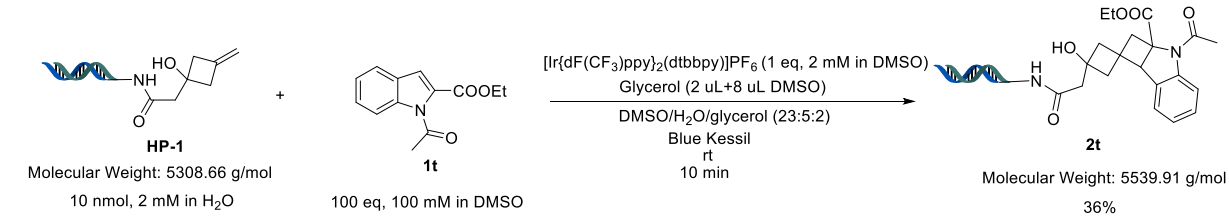
General procedure C: Compound **2s**, *28% yield*

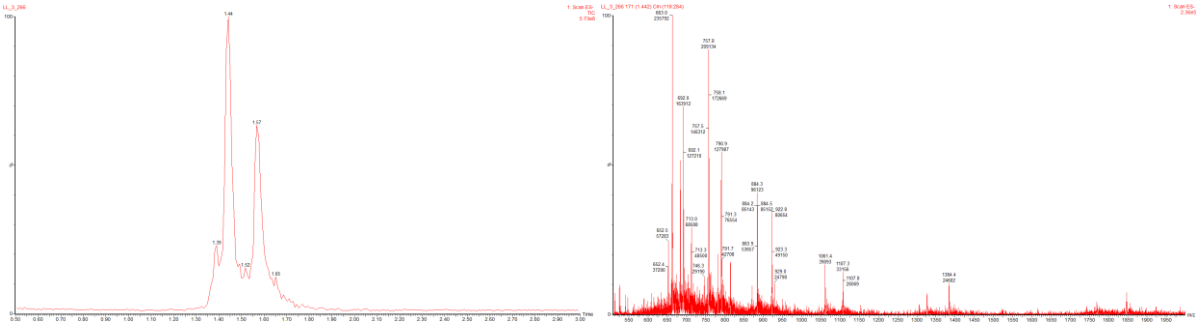




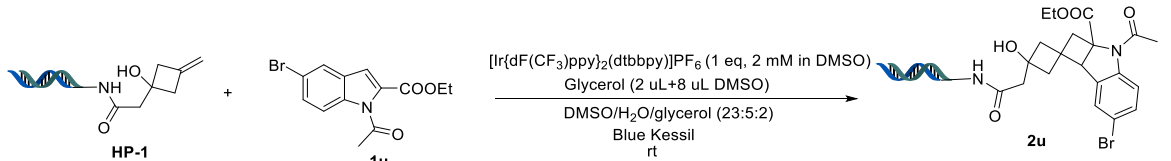


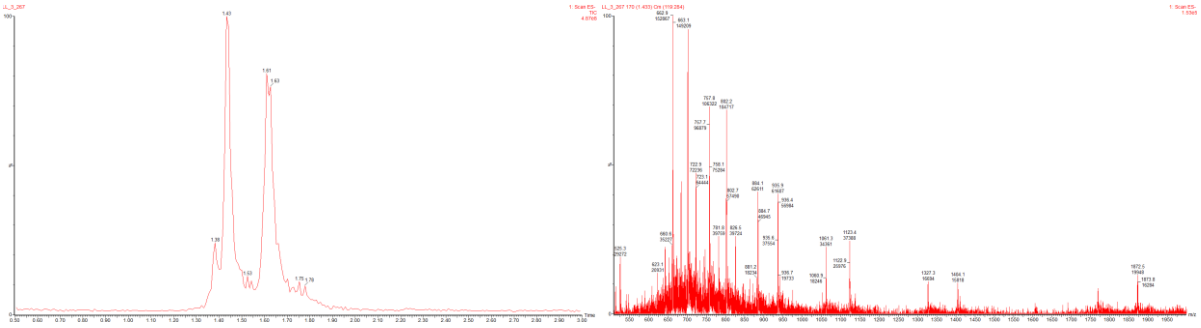
General procedure C: Compound **2t**, *36% yield*

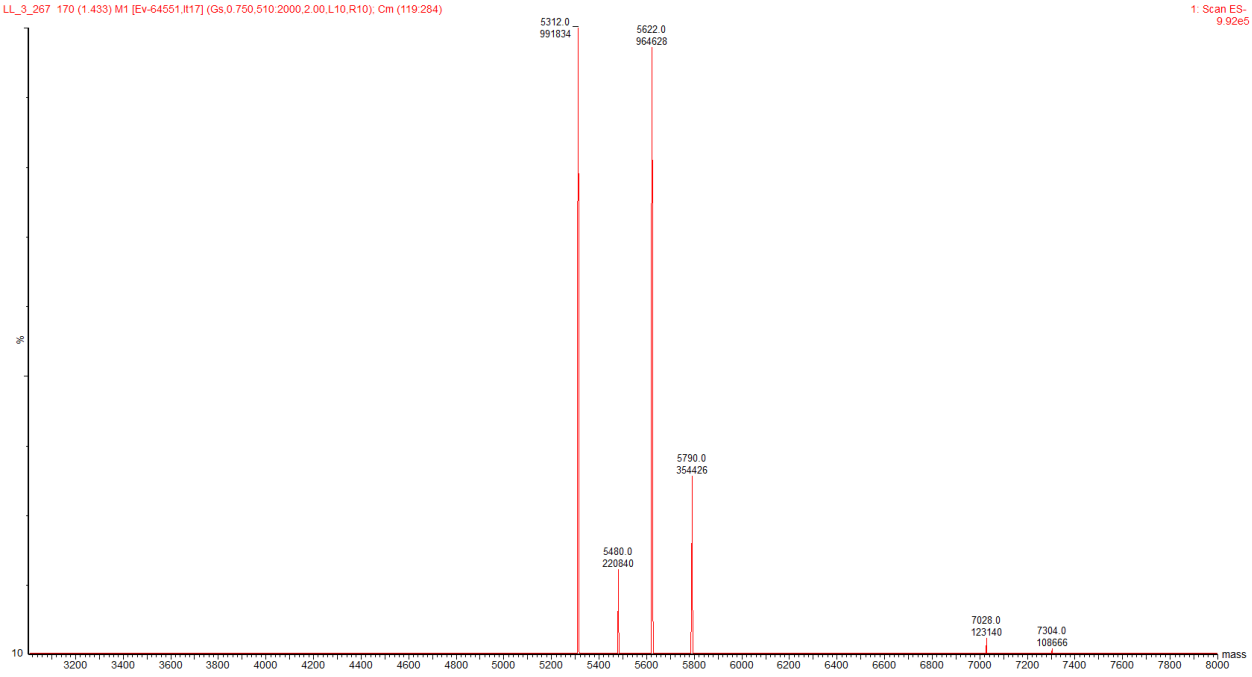




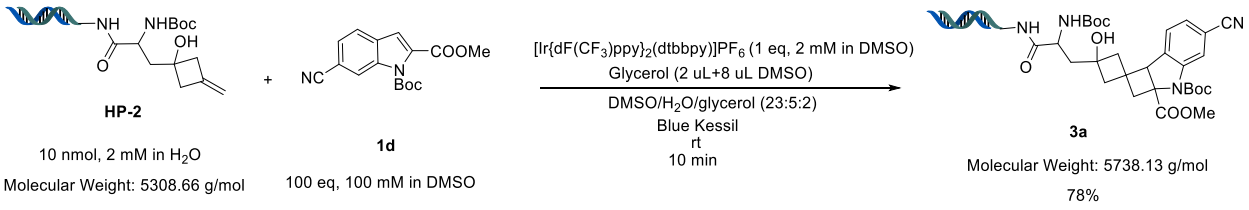
General procedure C: Compound **2u**, *48% yield*

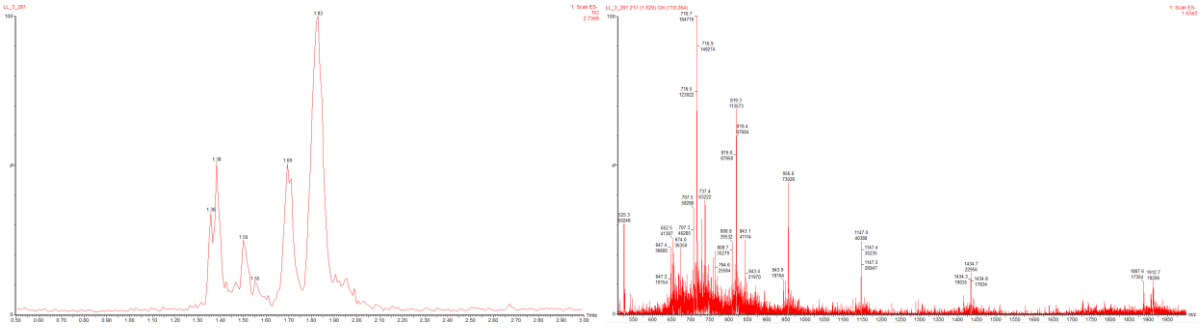




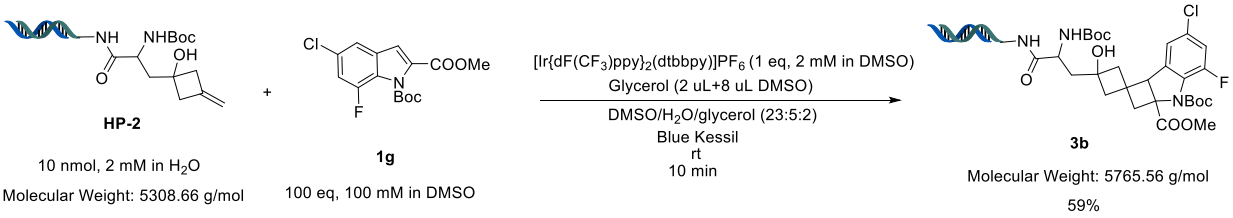


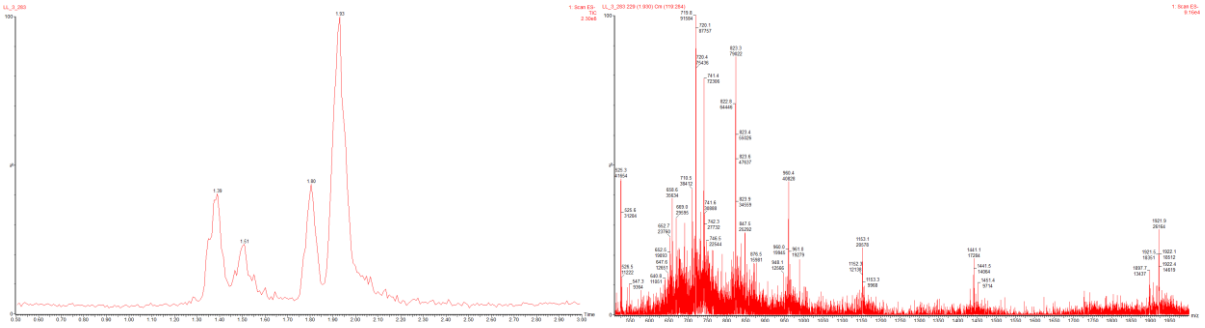
General procedure C: Compound **3a**, *78% yield*

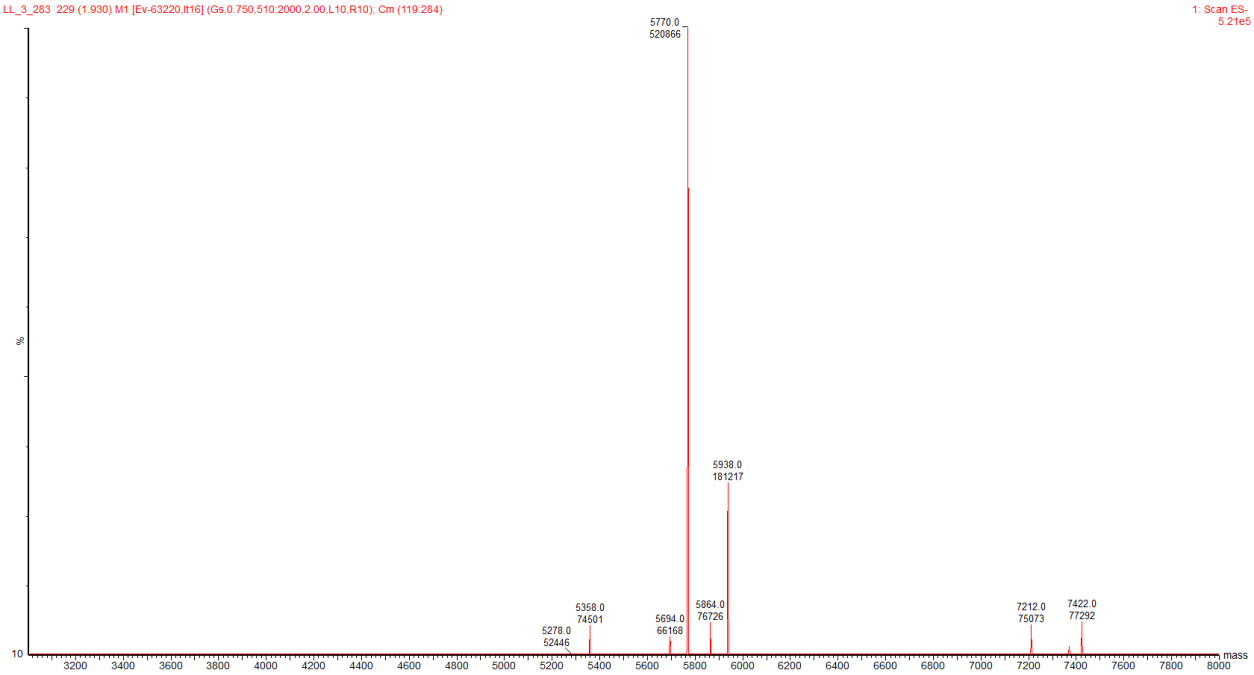




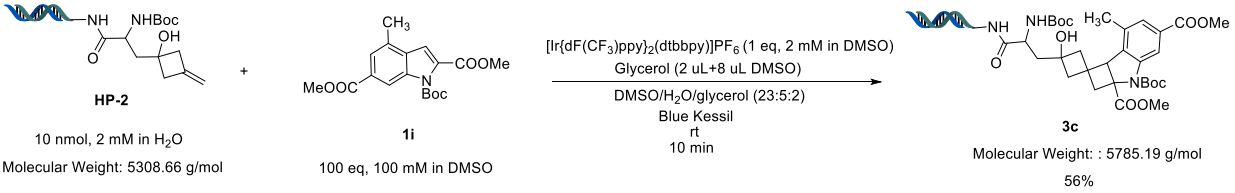
General procedure C: Compound **3b**, *59% yield*

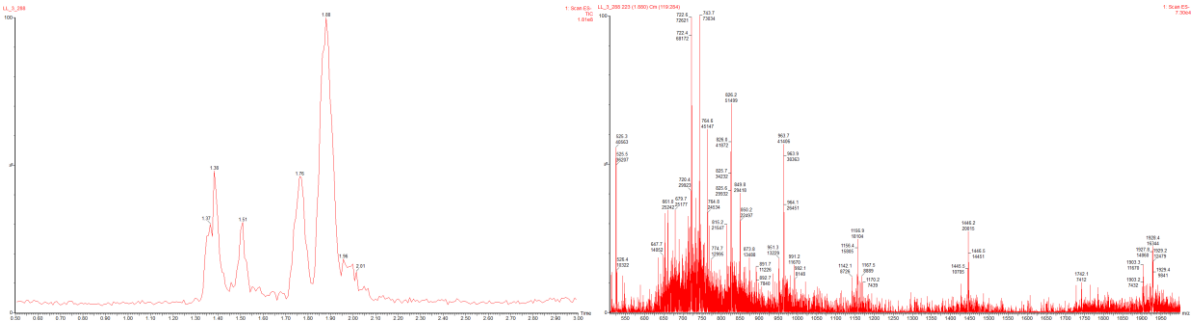


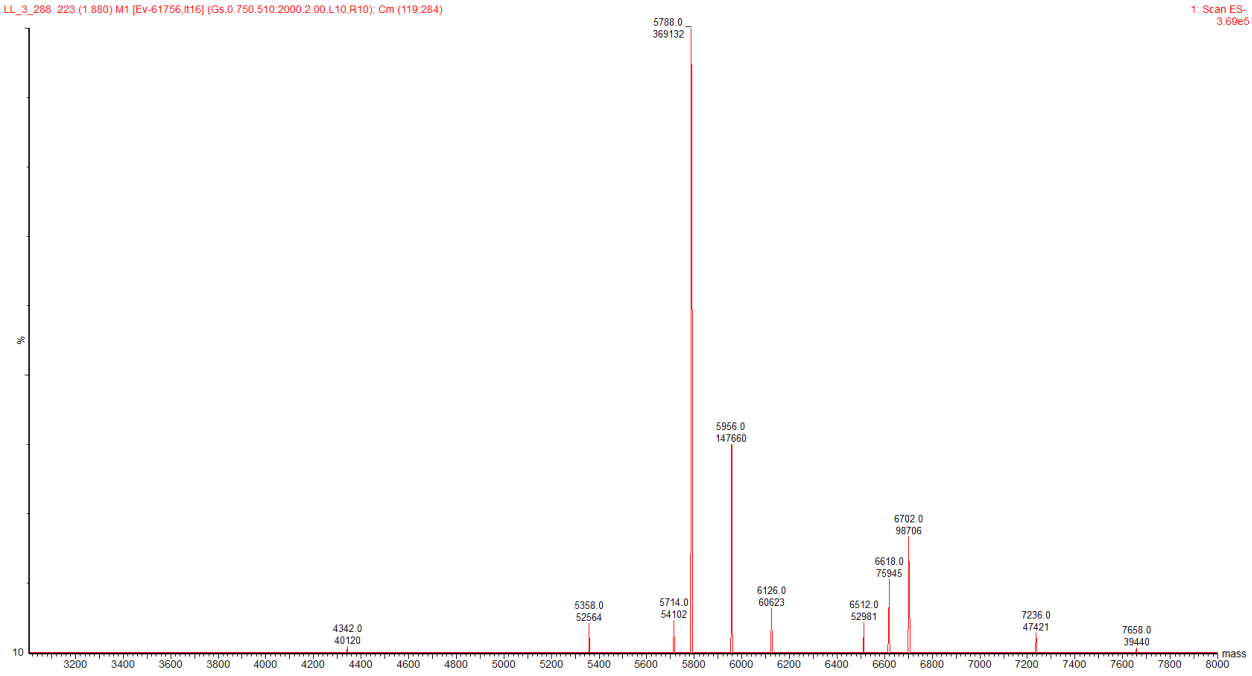




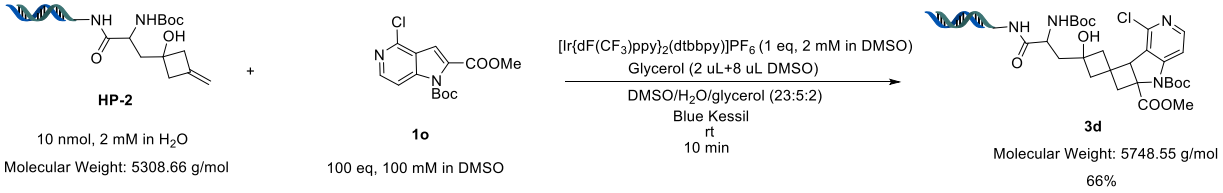
General procedure C: Compound **3c**, *56% yield*

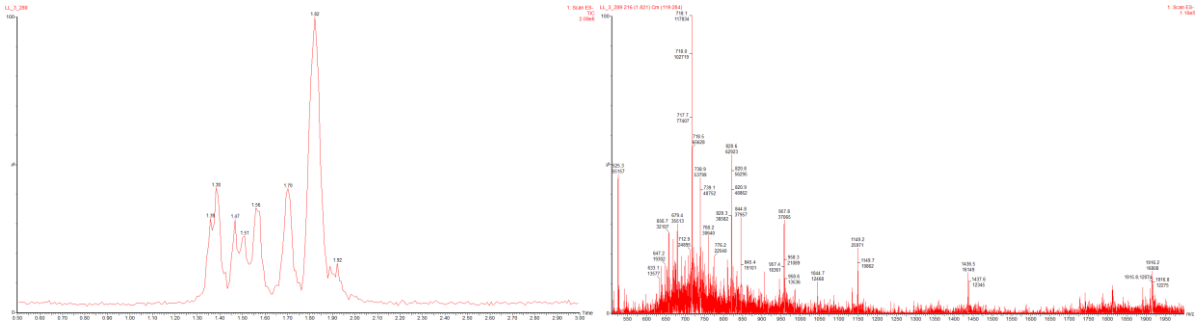




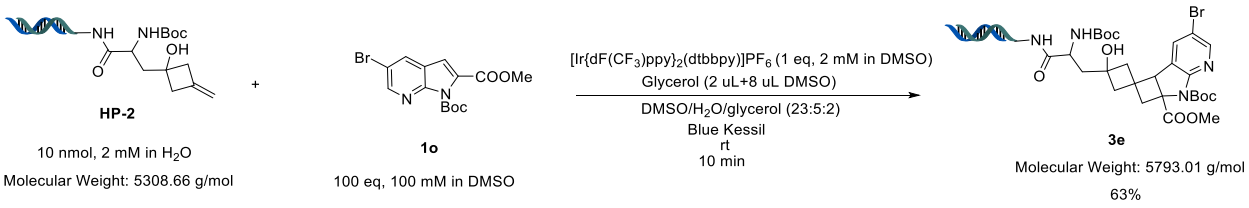


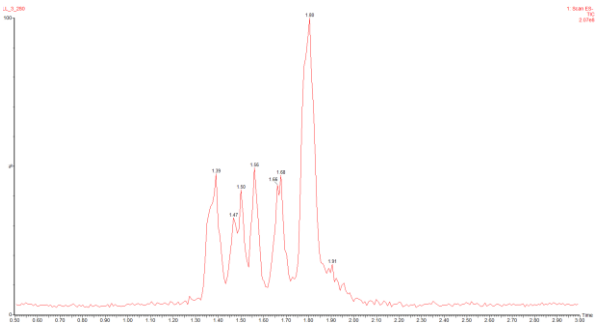
General procedure C: Compound **3d**, *66% yield*

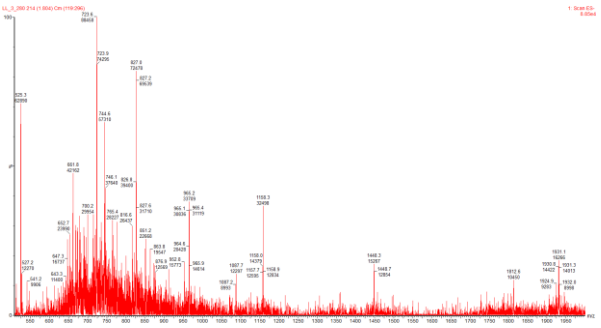




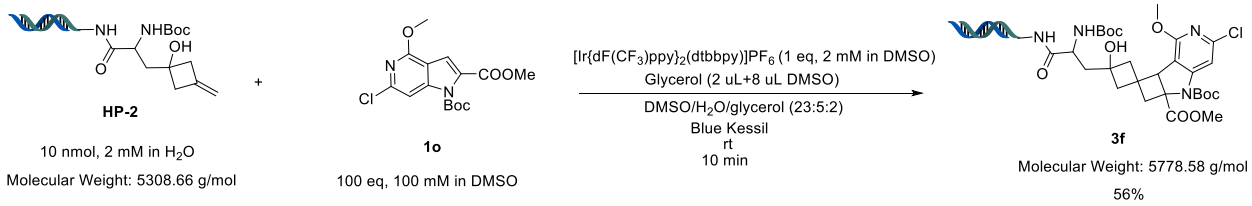
General procedure C: Compound **3e**, *63% yield*

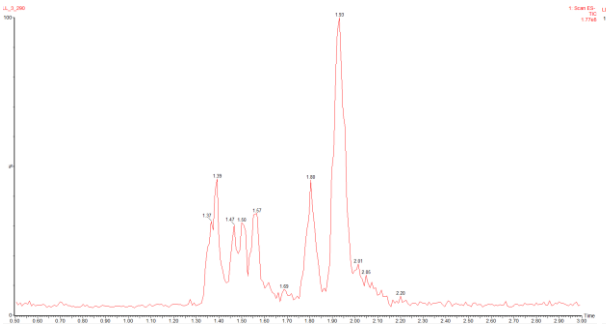


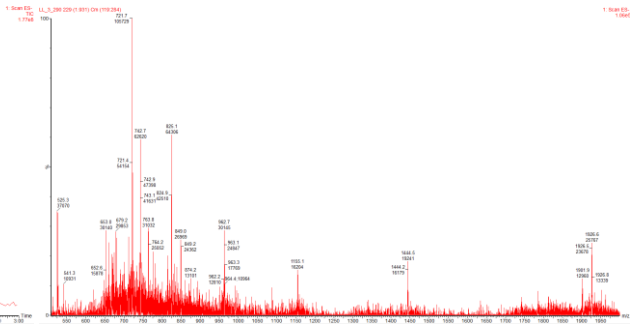


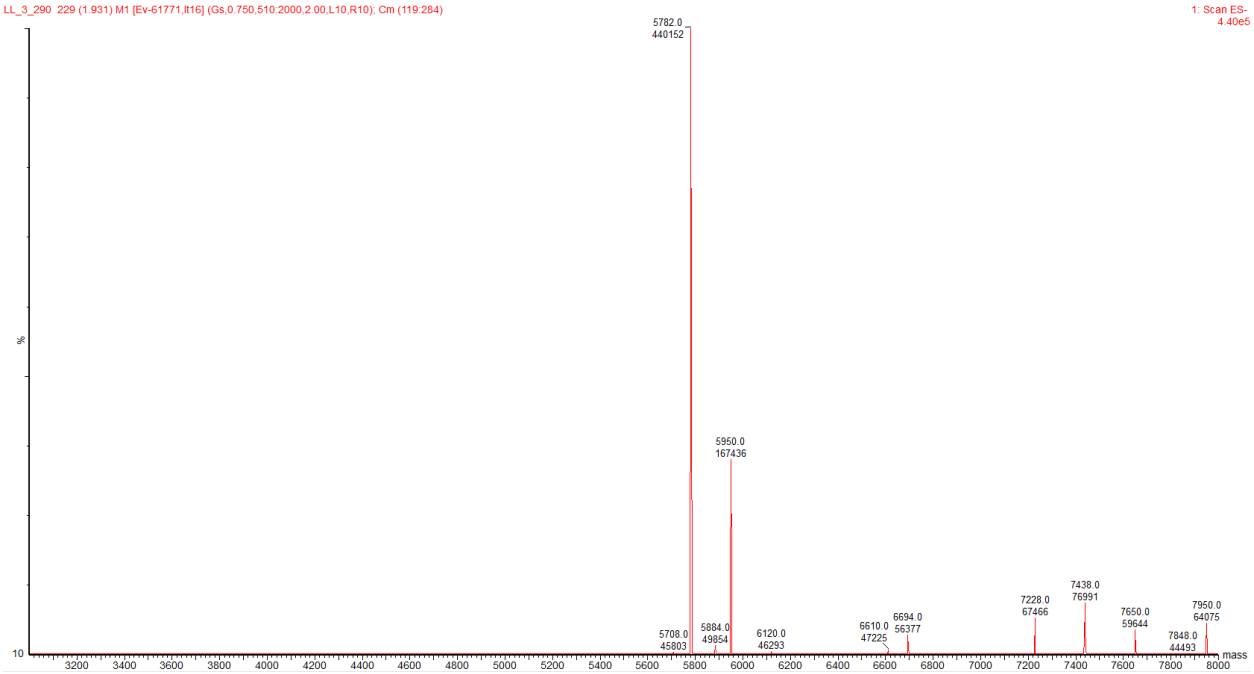


General procedure C: Compound **3f**, *56% yield*

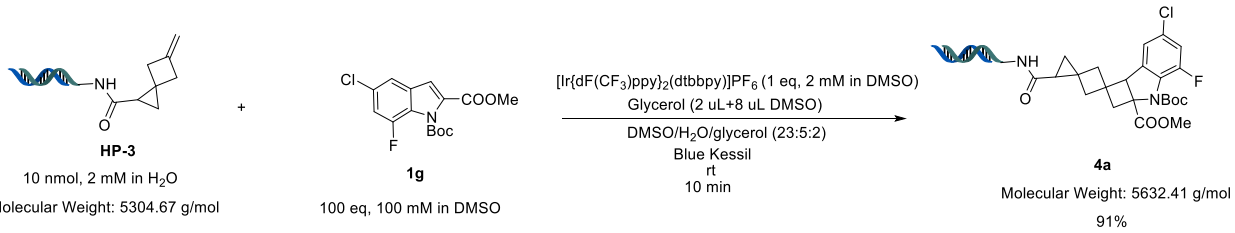


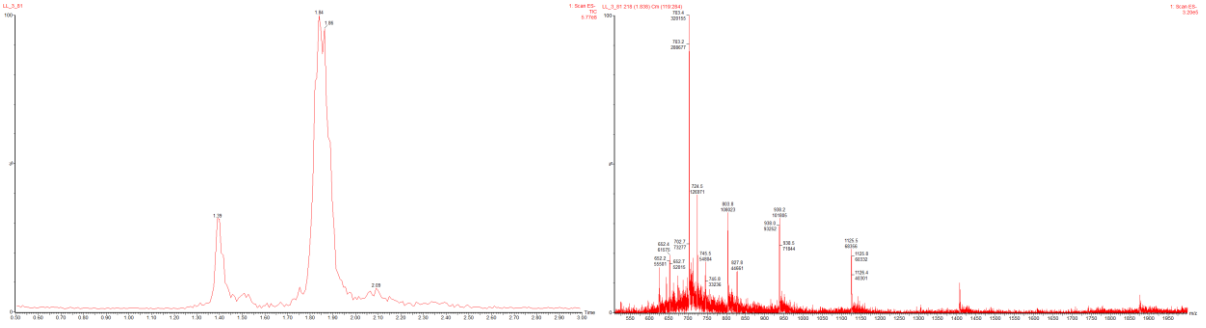




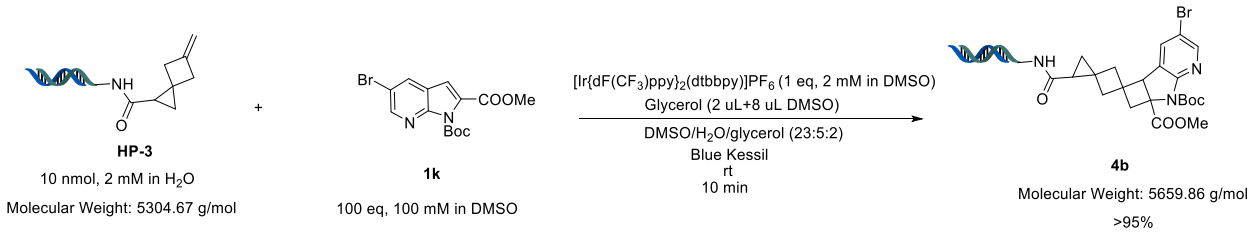


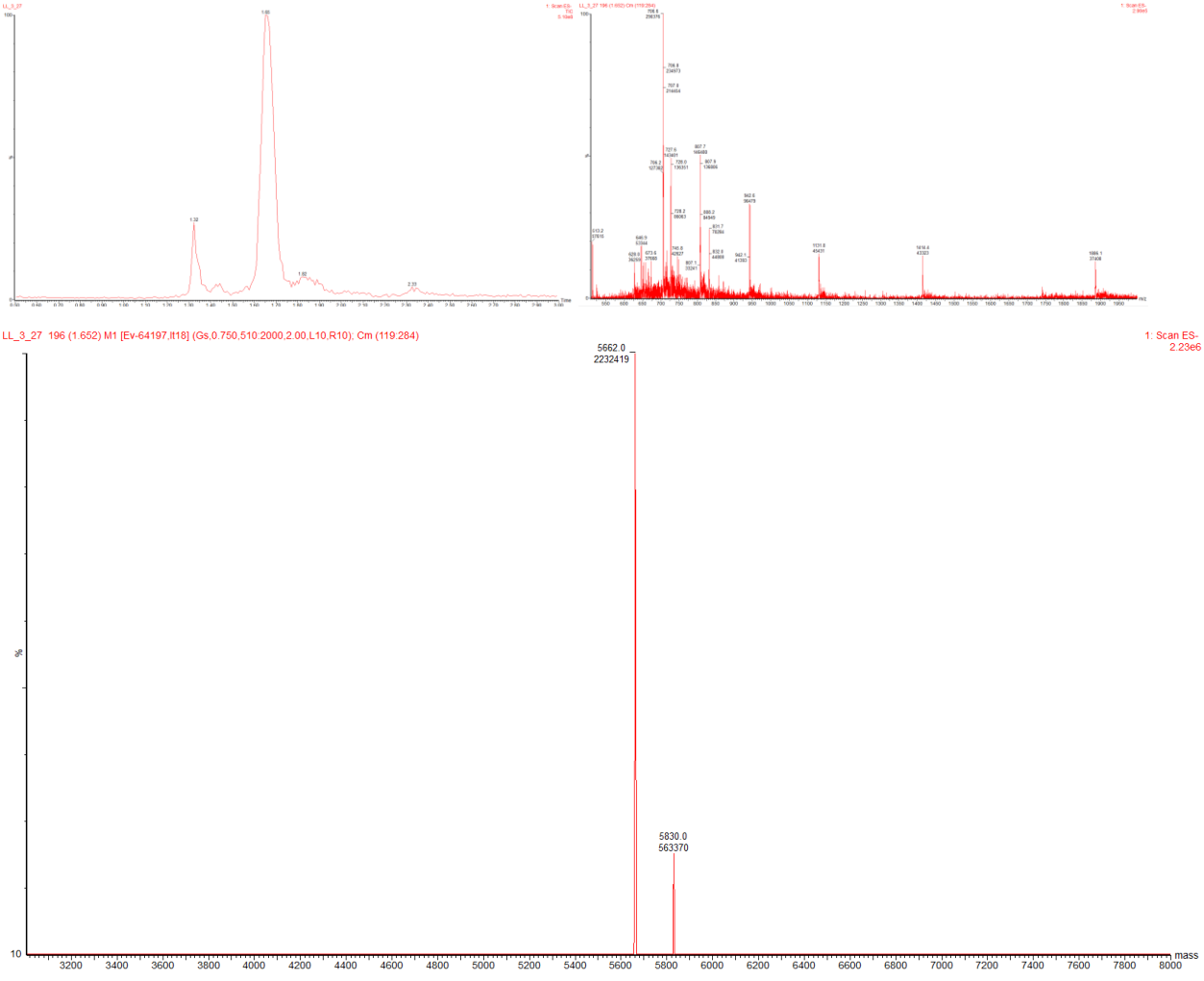
General procedure C: Compound **4a**, *91% yield*



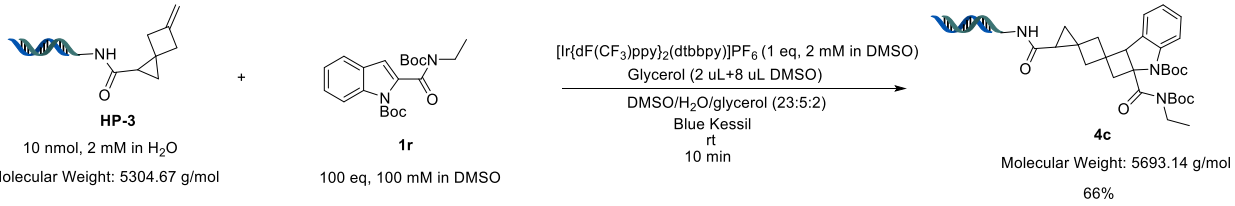


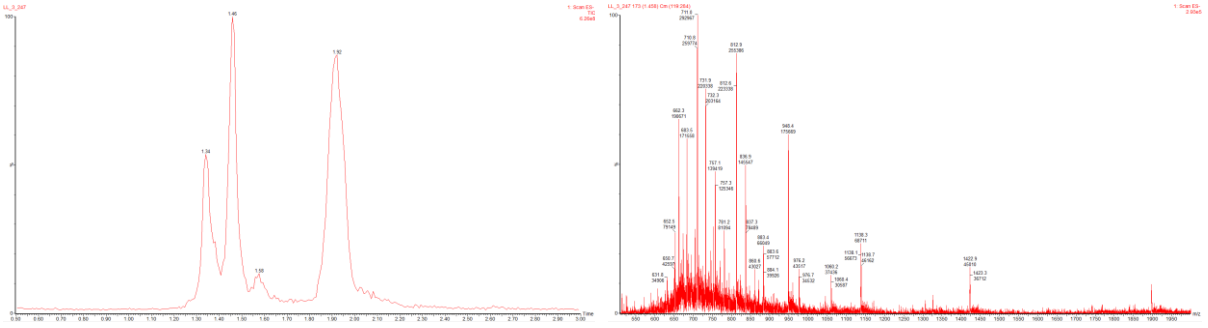
General procedure C: Compound **4b**, *>95% yield*

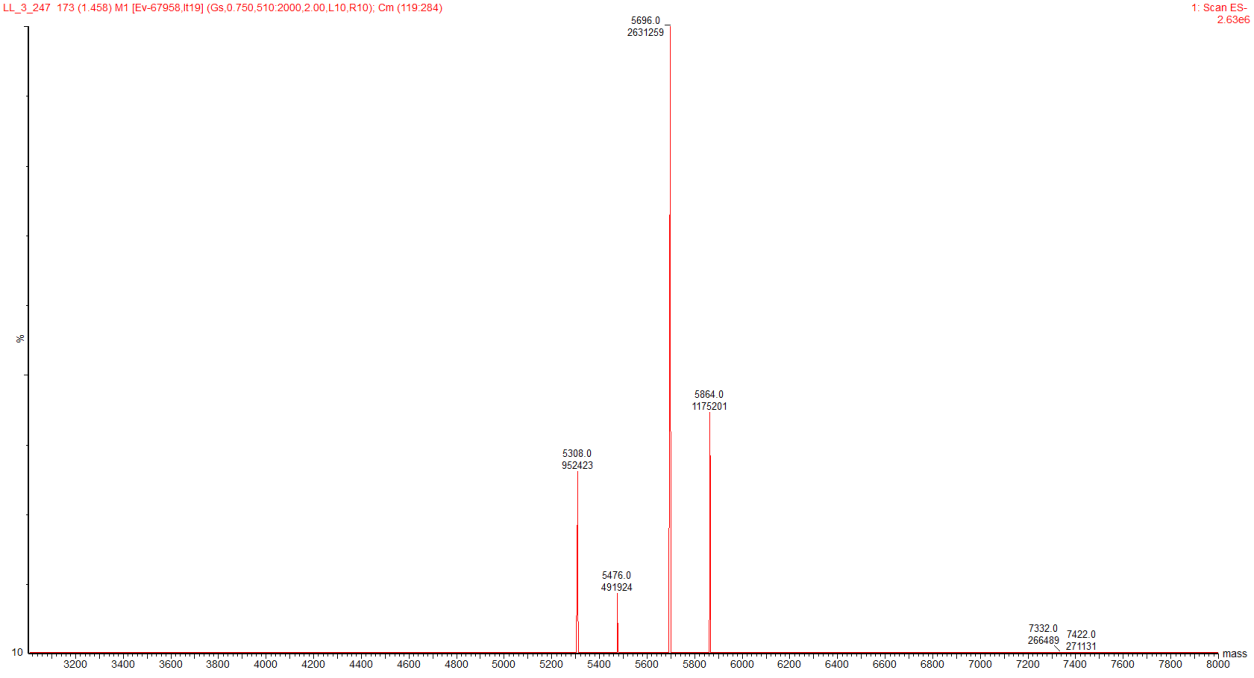




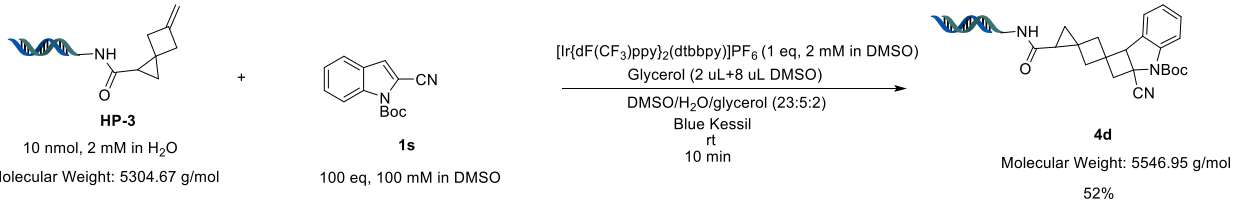
General procedure C: Compound **4c**, *66% yield*

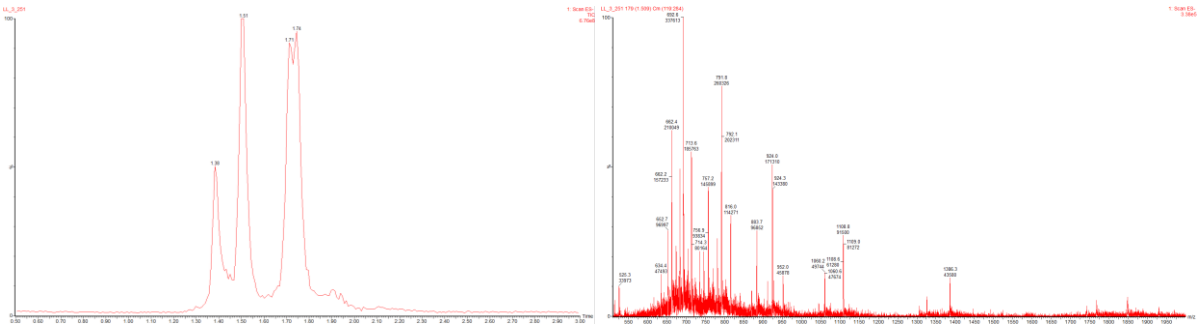




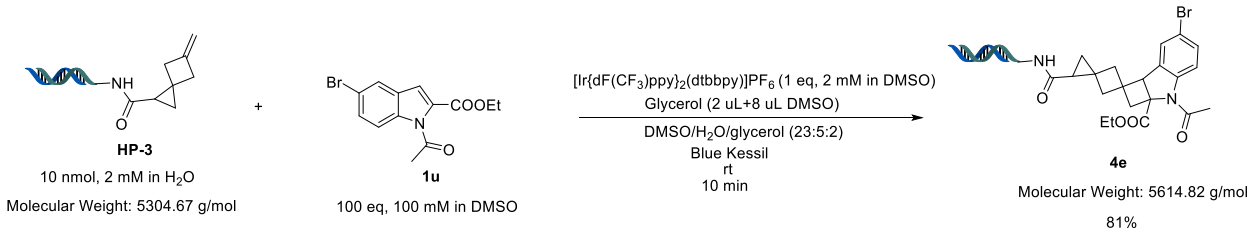


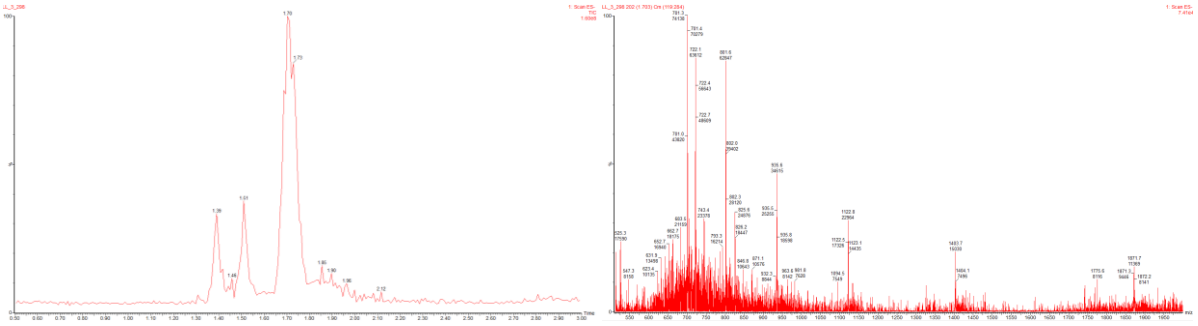
General procedure C: Compound **4d**, *52% yield*



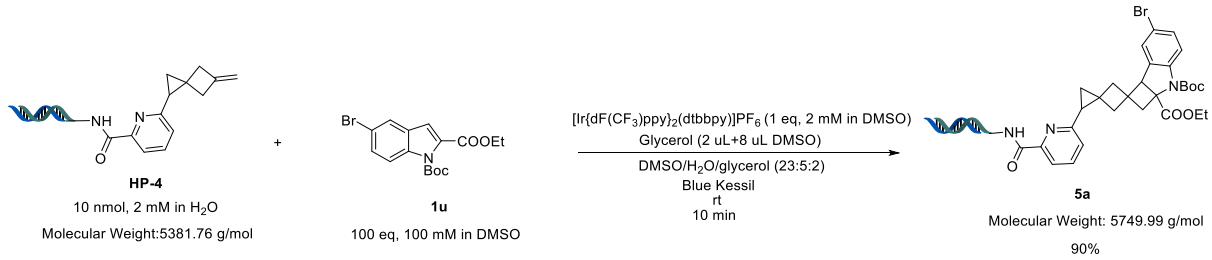


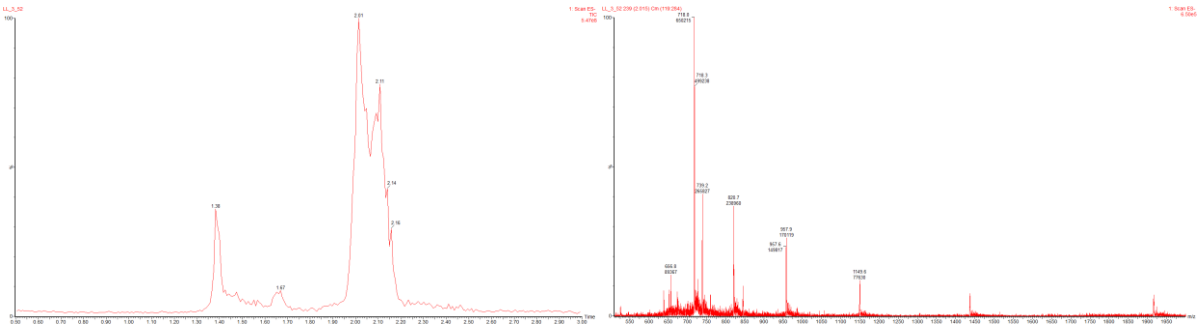
General procedure C: Compound **4e**, *81% yield*

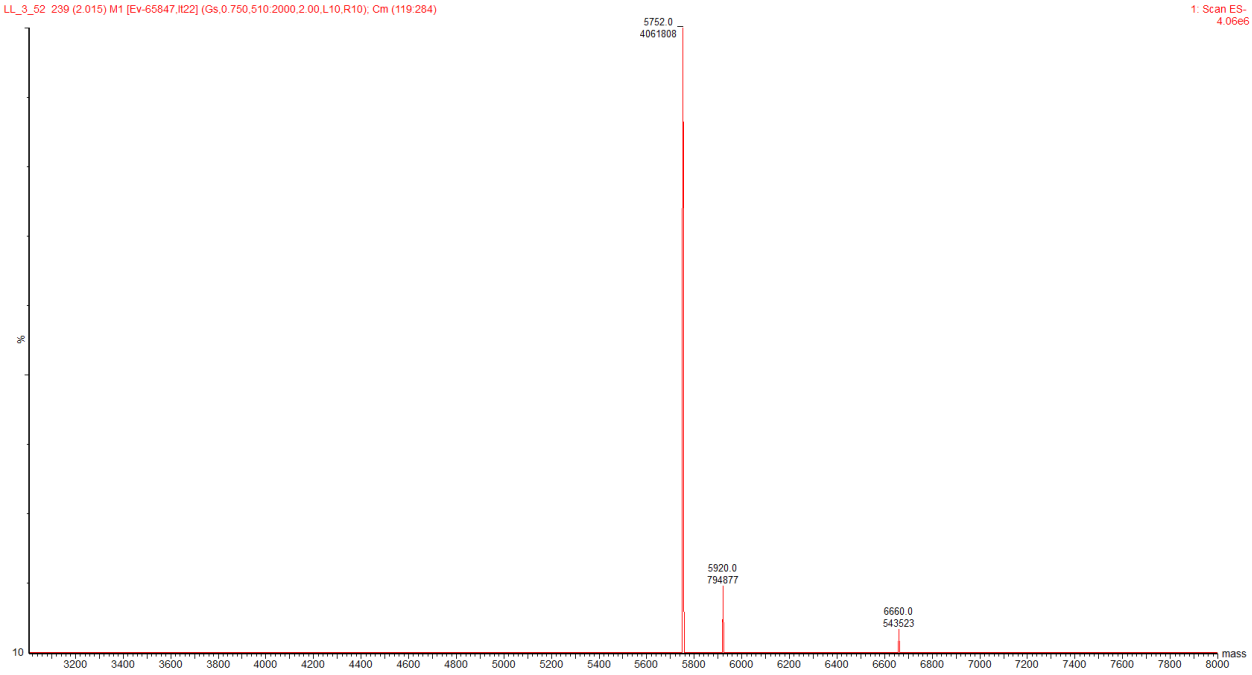




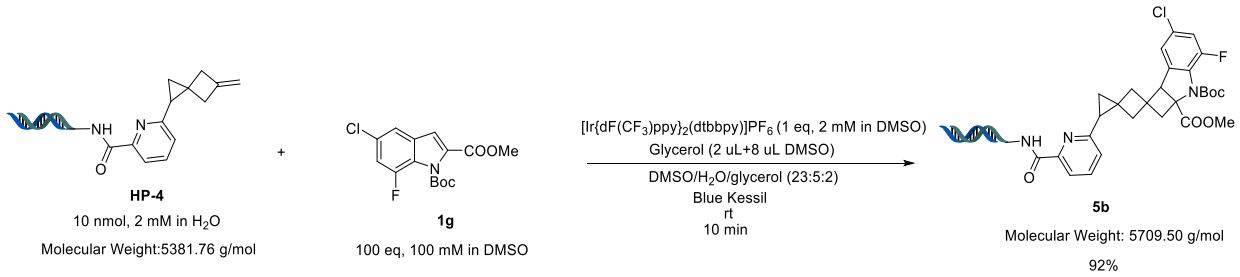
General procedure C: Compound **5a**, *90% yield*

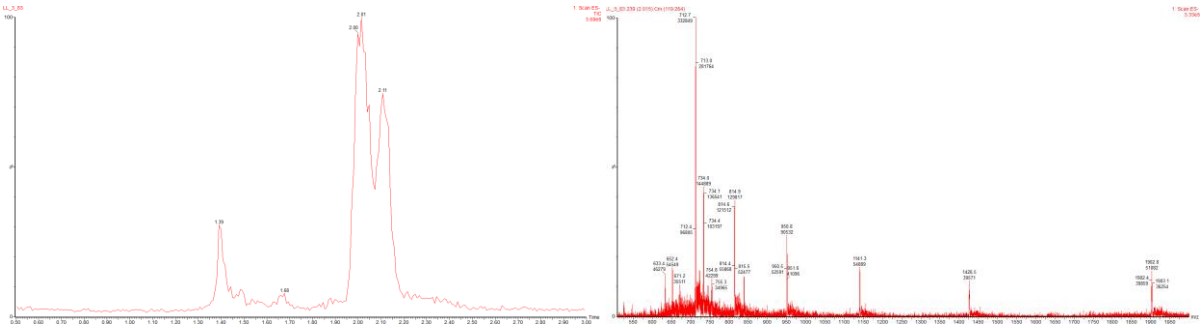


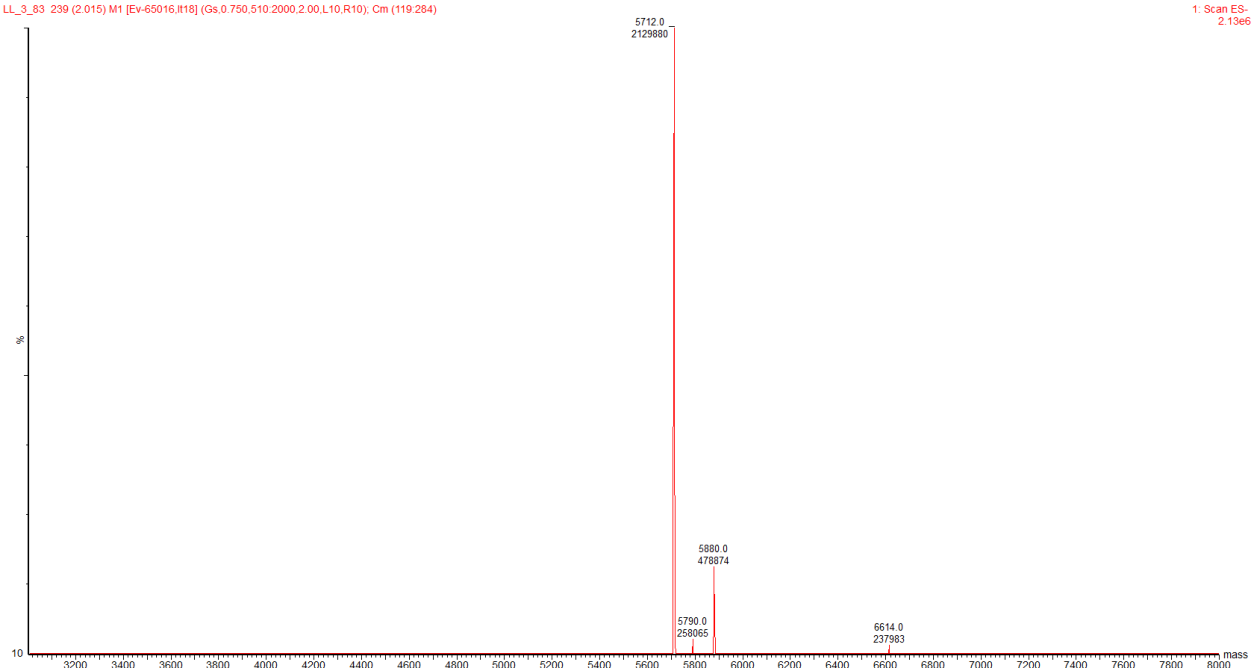




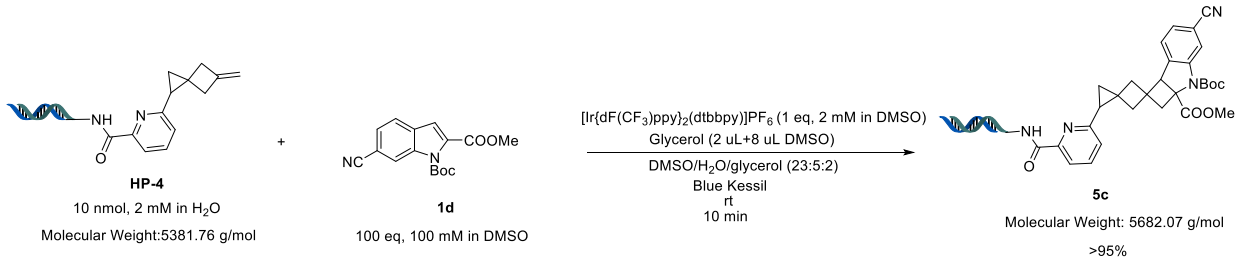
General procedure C: Compound **5b**, *92% yield*

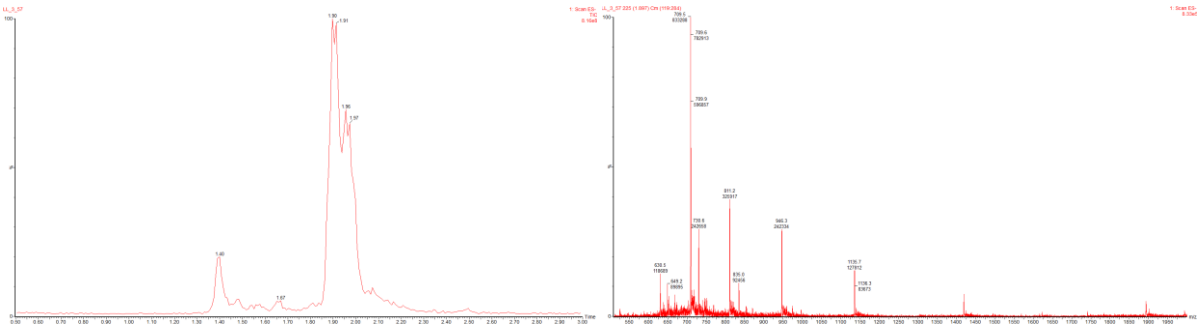




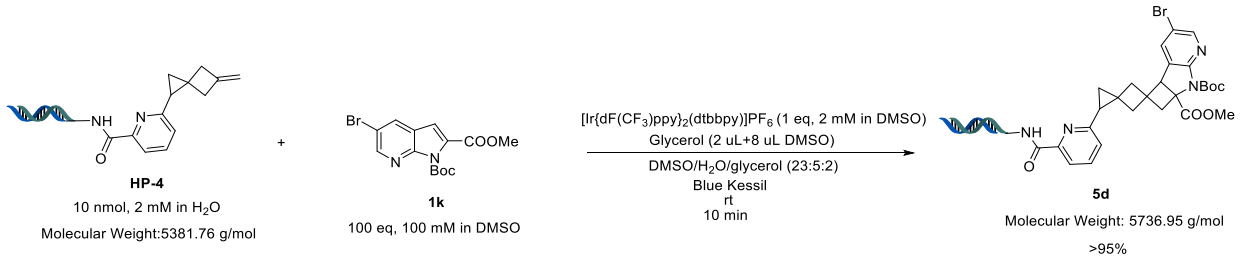


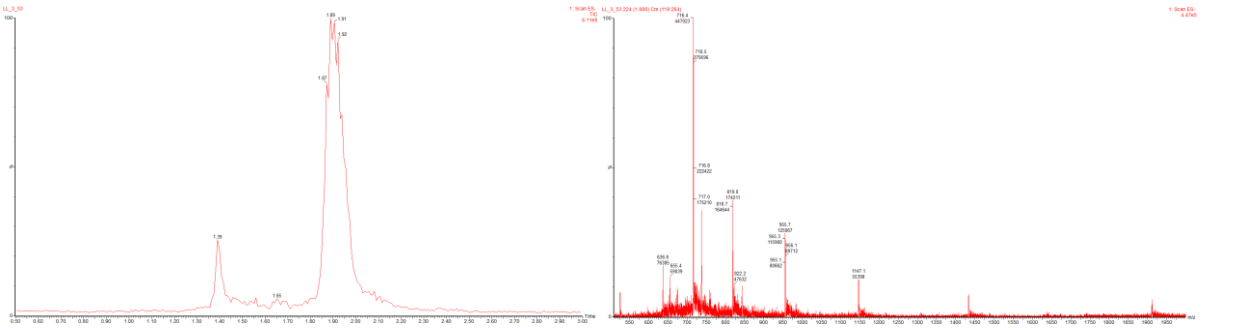
General procedure C: Compound **5c**, *>95% yield*

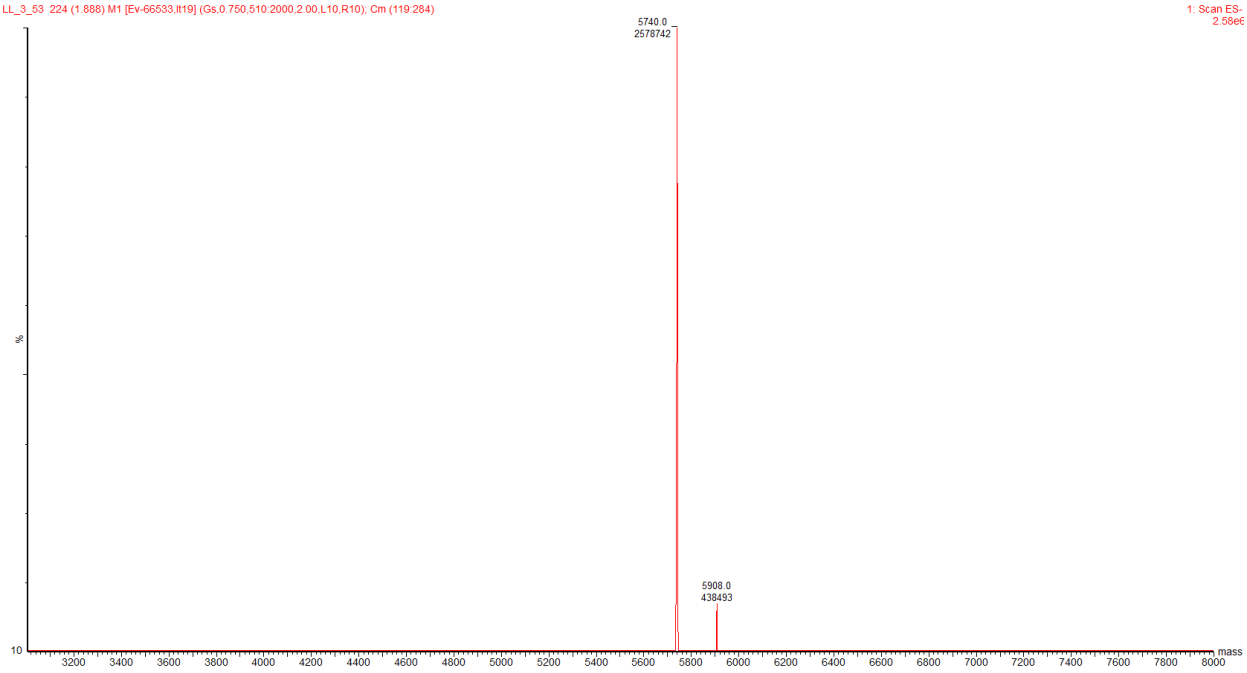




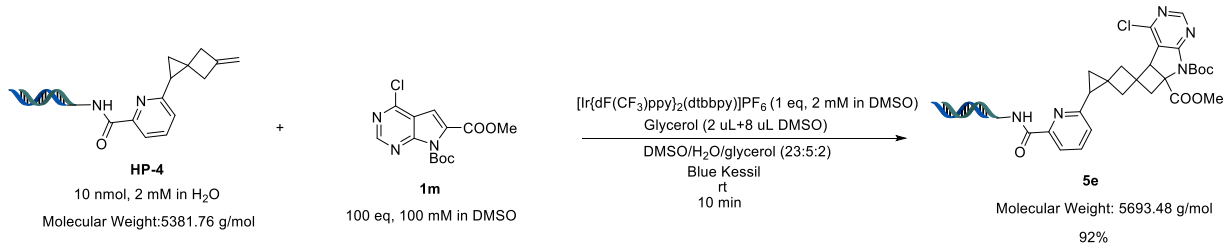
General procedure C: Compound **5d**, *>95% yield*

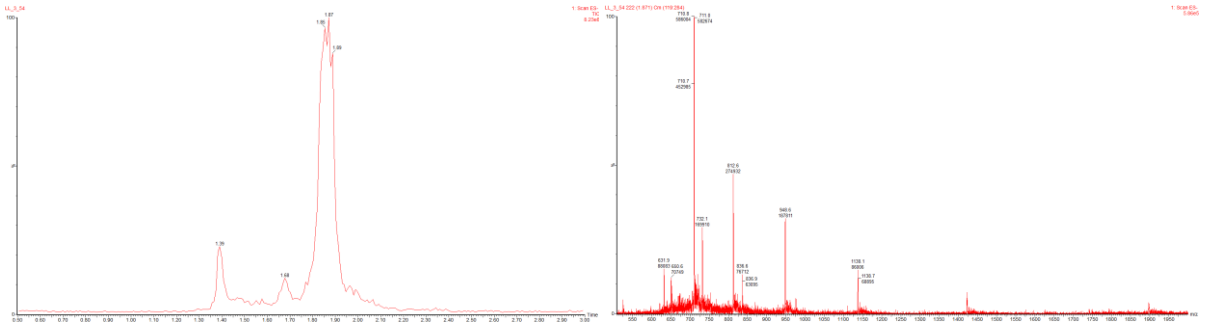




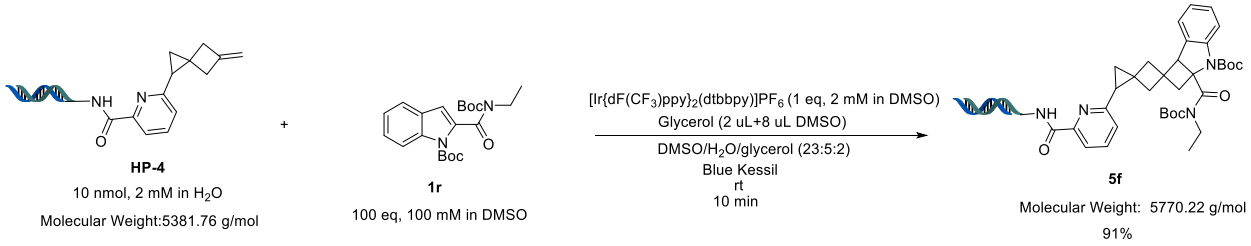


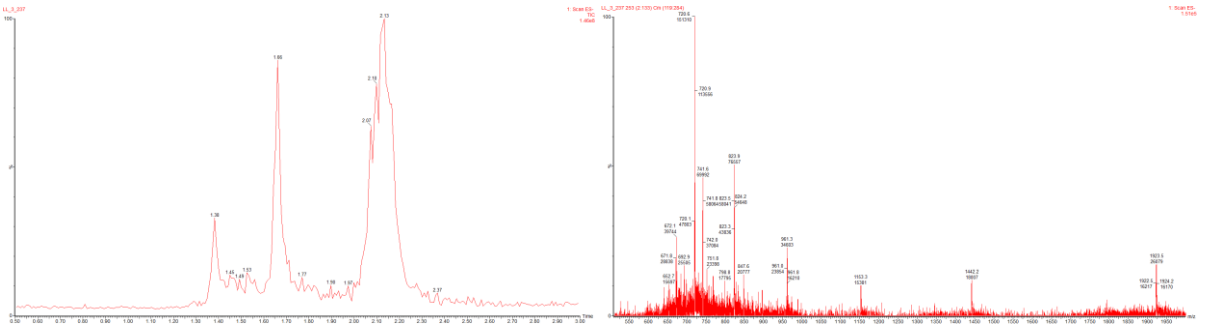
General procedure C: Compound **5e**, *92% yield*

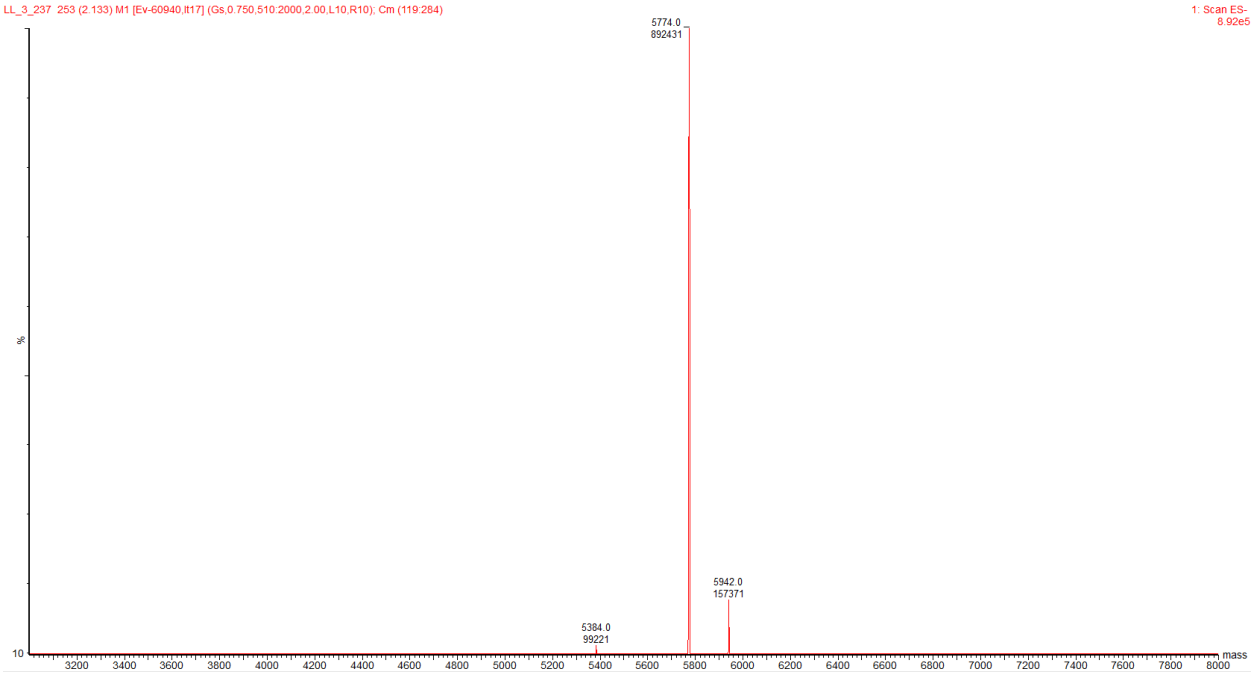




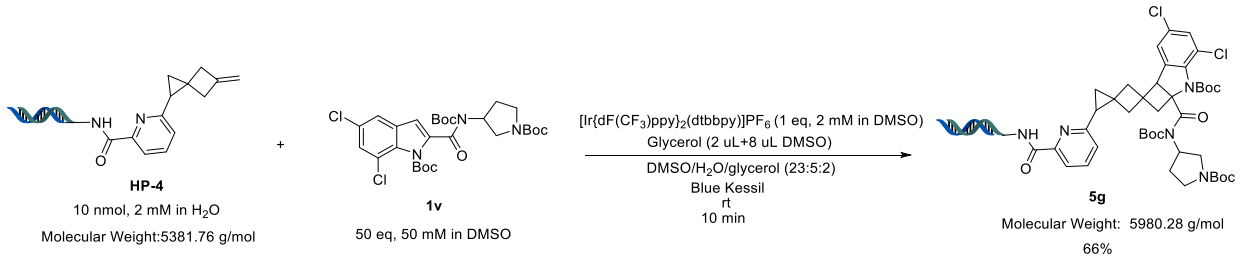
General procedure C: Compound **5f**, *91% yield*

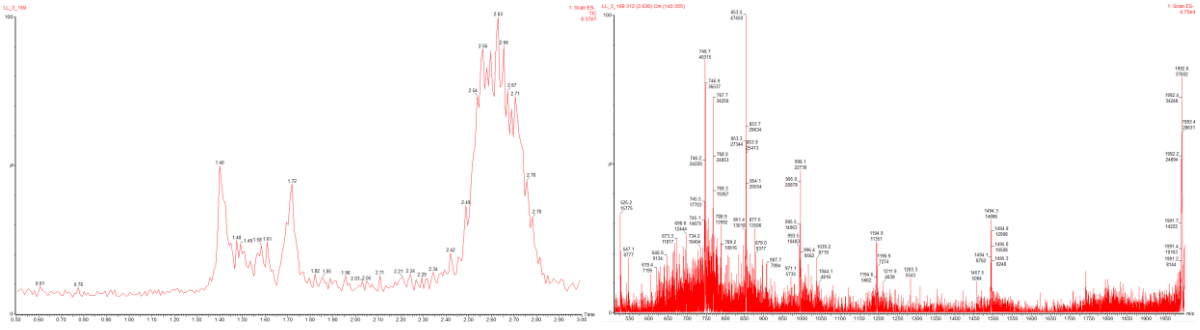


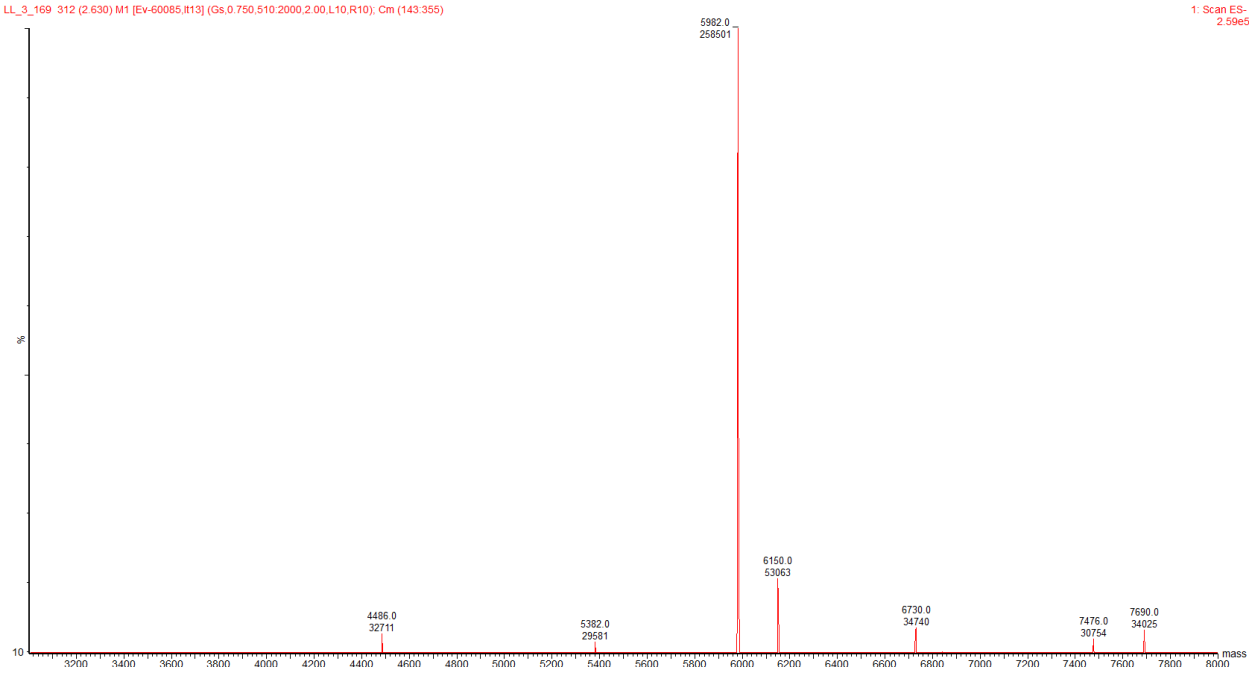




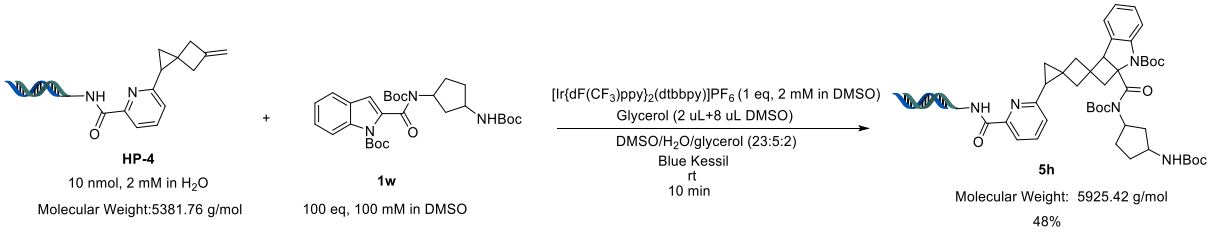
General procedure D: Compound **5g**, *66% yield*

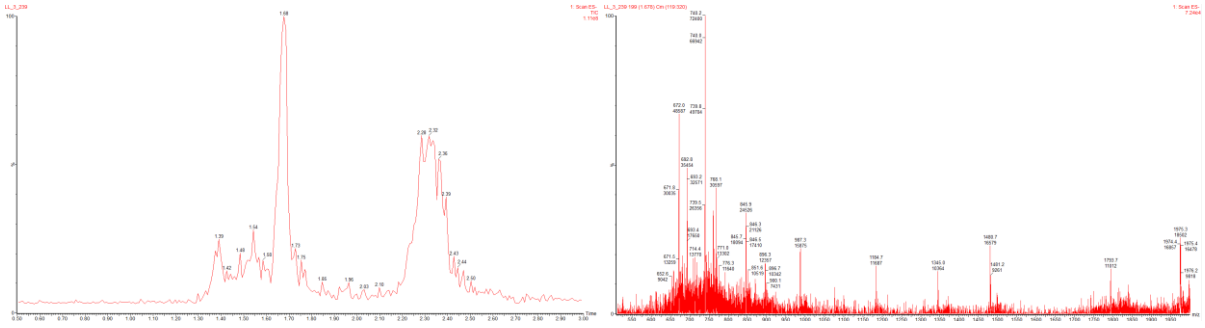




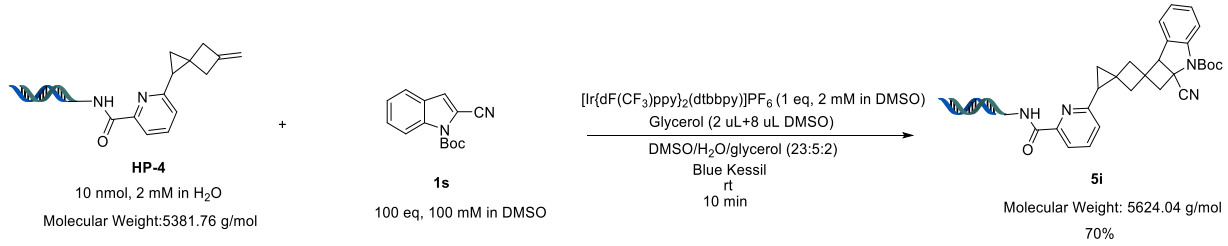


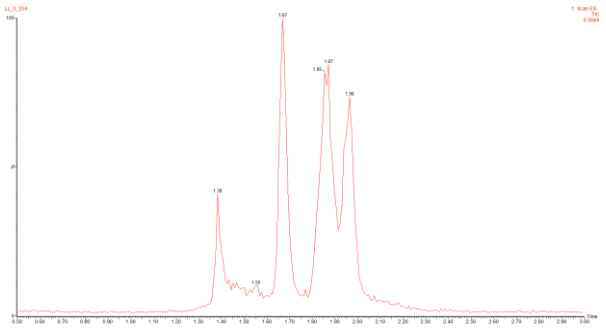
General procedure C: Compound **5h**, *48% yield*

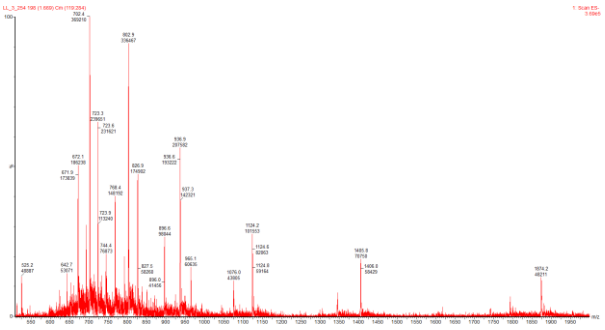




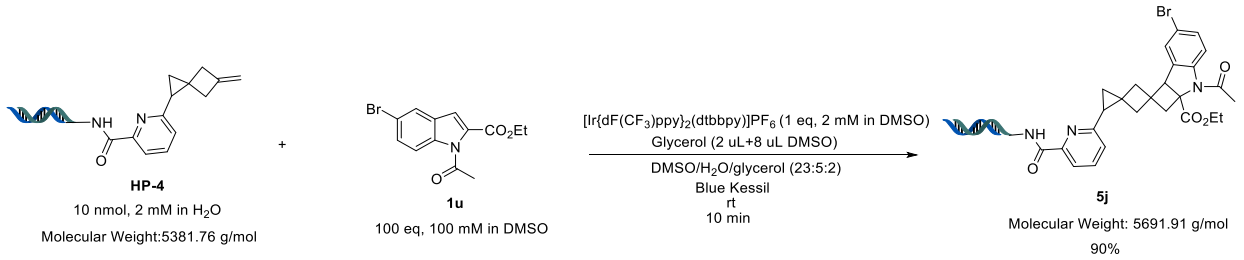
General procedure C: Compound **5i**, *70% yield*

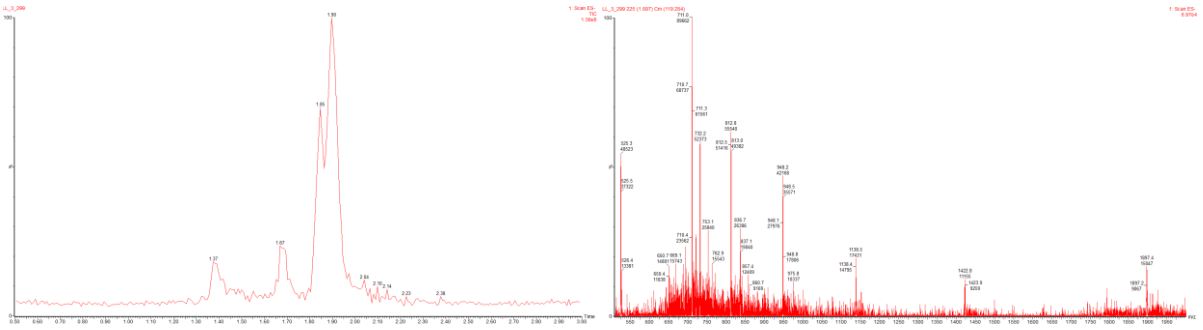




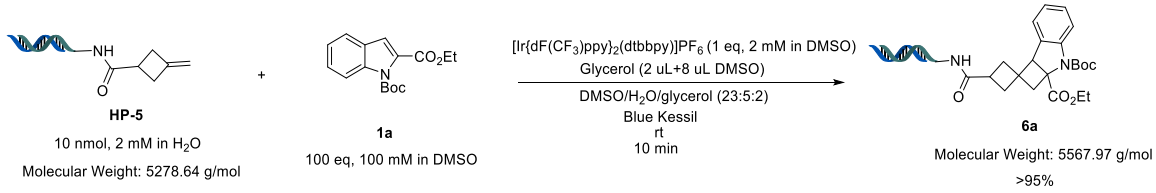


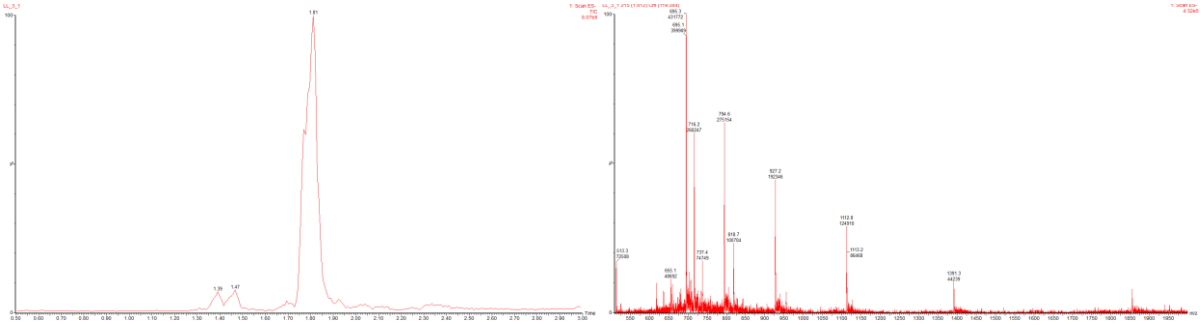
General procedure C: Compound **5j**, *90% yield*



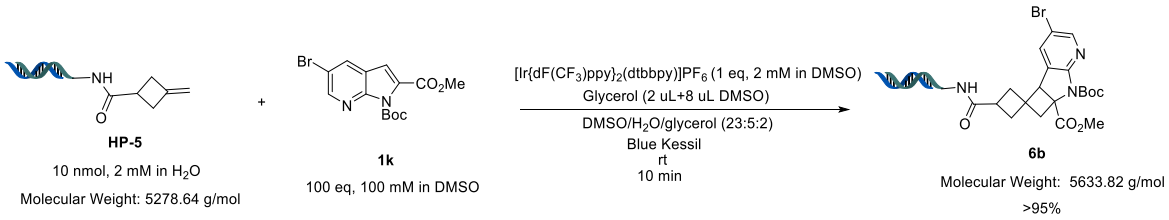


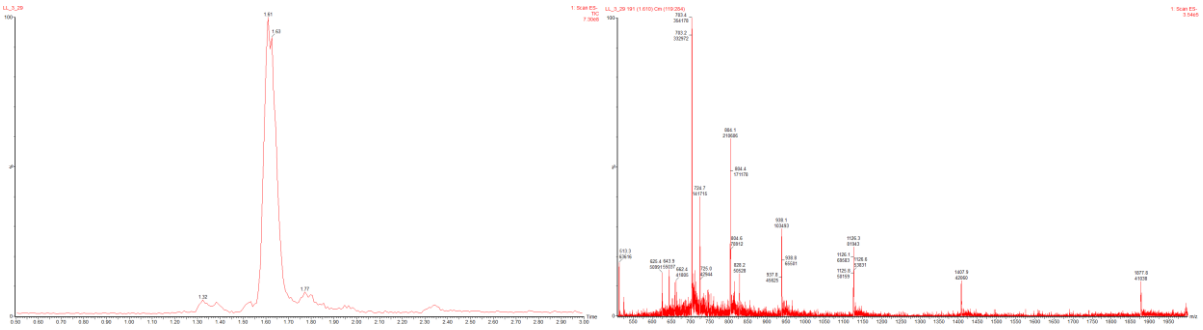
General procedure C: Compound **6a**, *>95% yield*



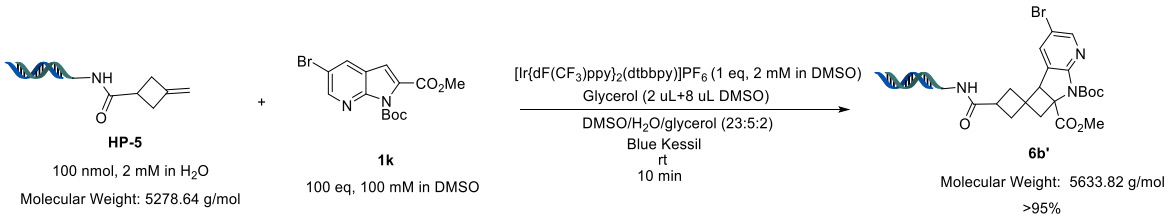


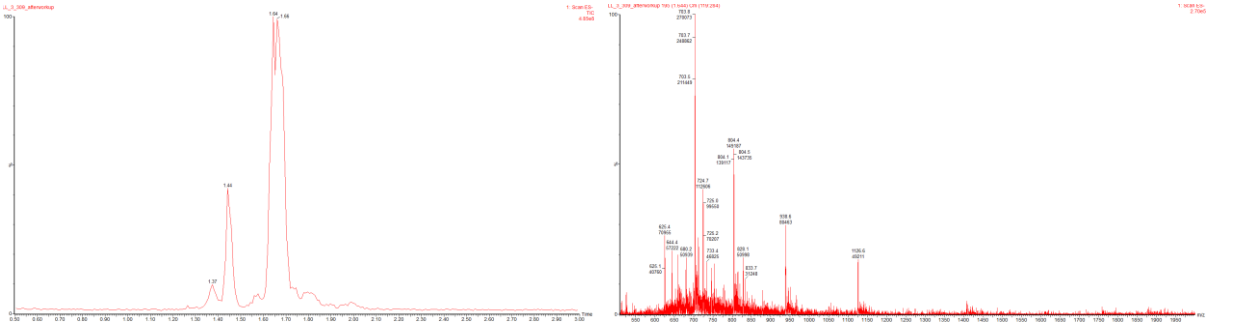
General procedure C: Compound **6b**, *>95% yield*

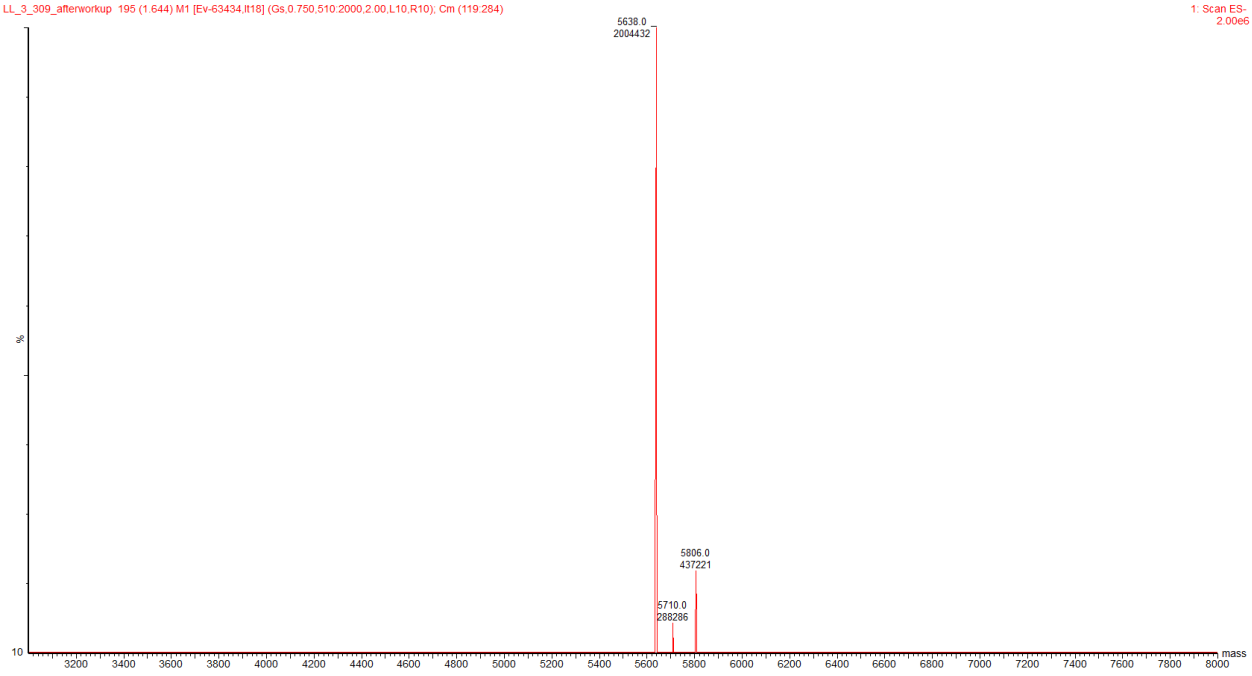




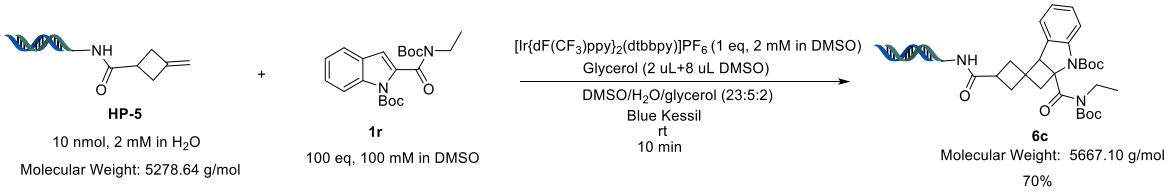
General procedure C: Compound **6b'** (100 nmol scale), *>95% yield*

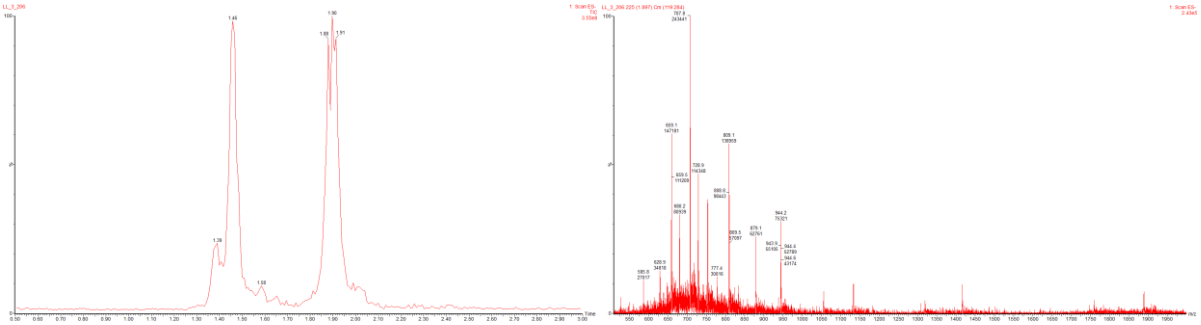




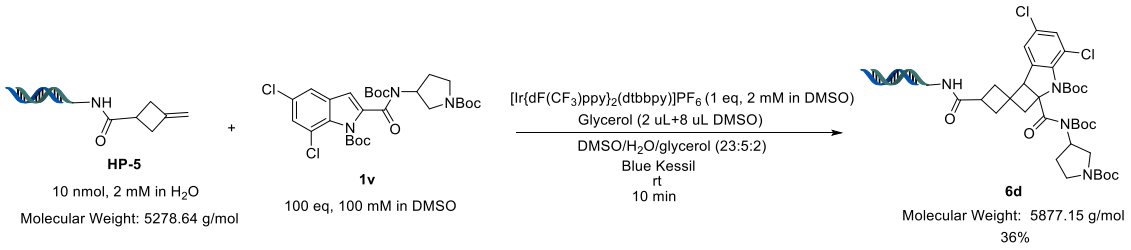


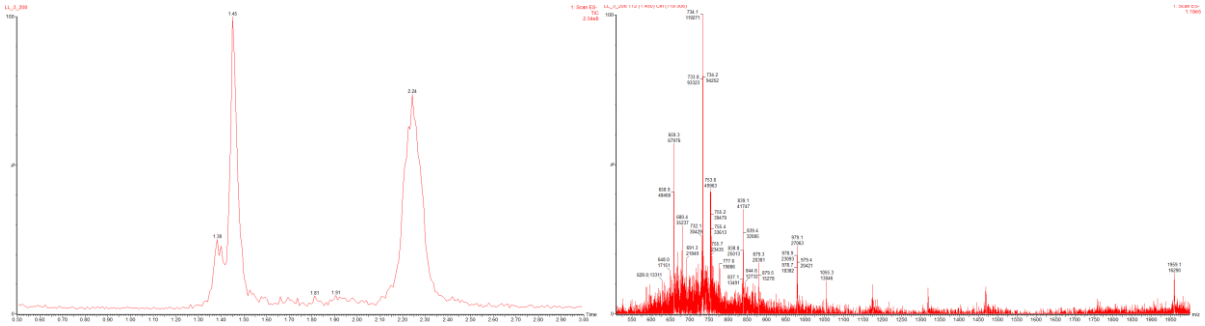
General procedure C: Compound **6c**, *70% yield*

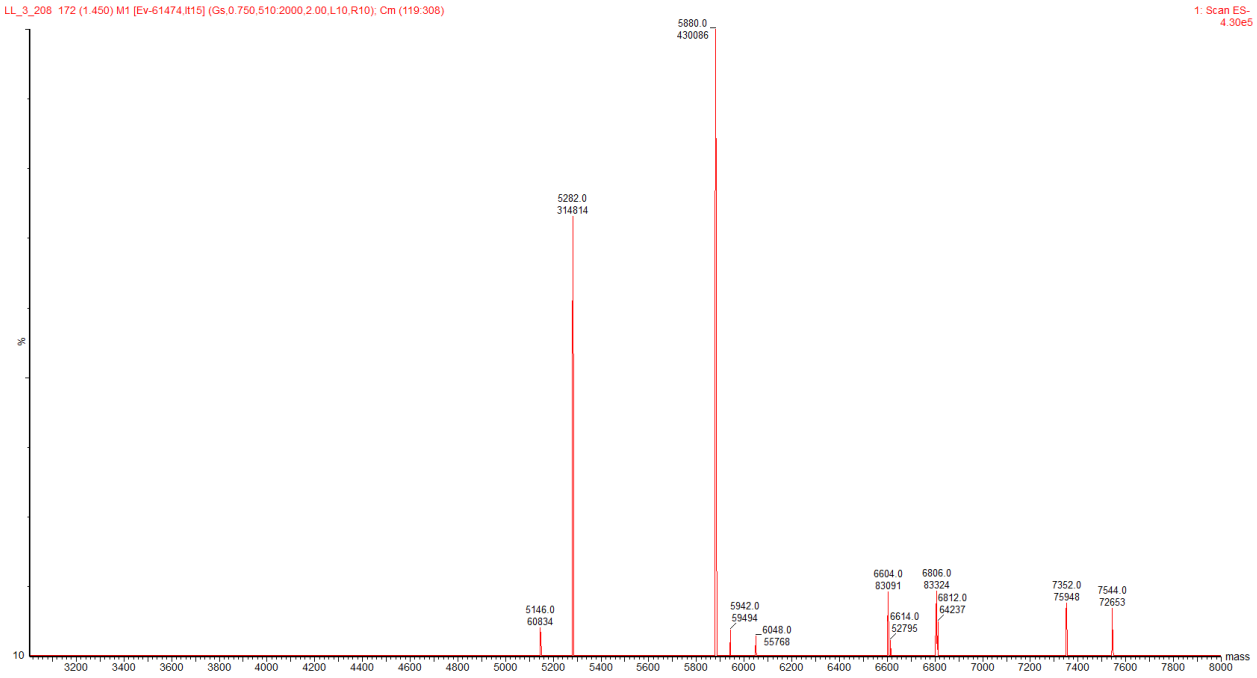




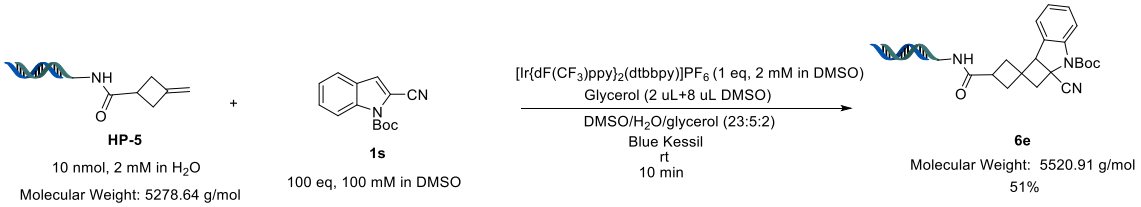
General procedure C: Compound **6d**, *36% yield*

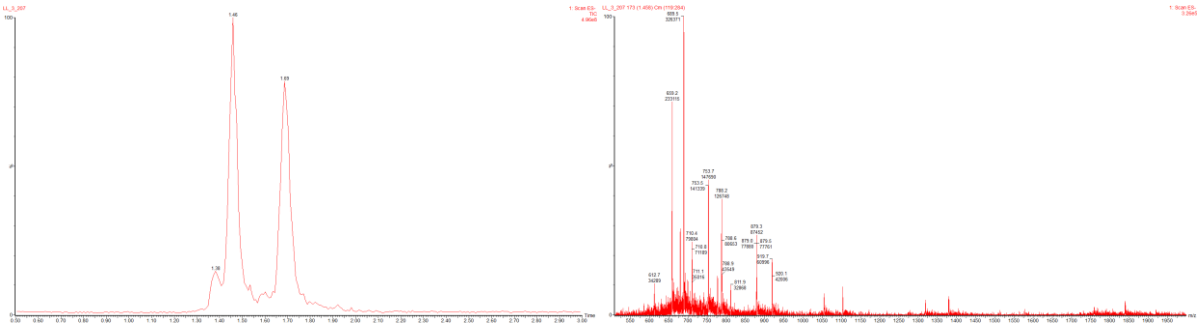




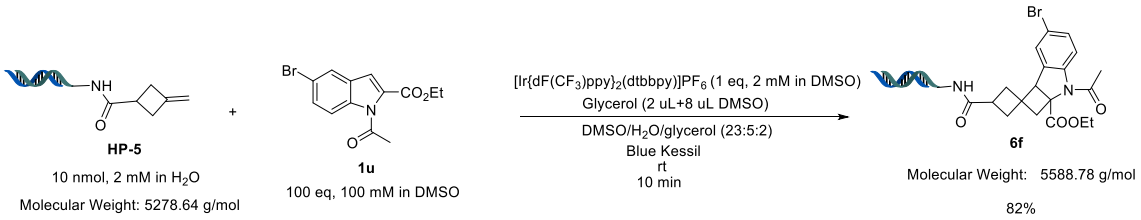


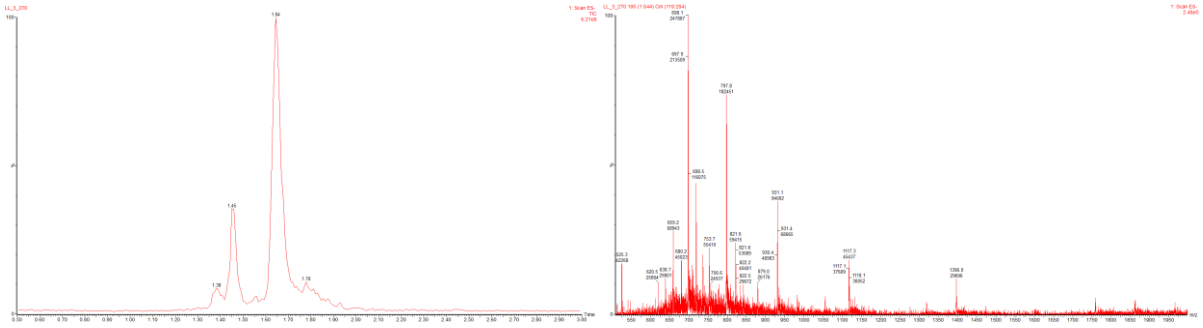
General procedure C: Compound **6e**, *51% yield*



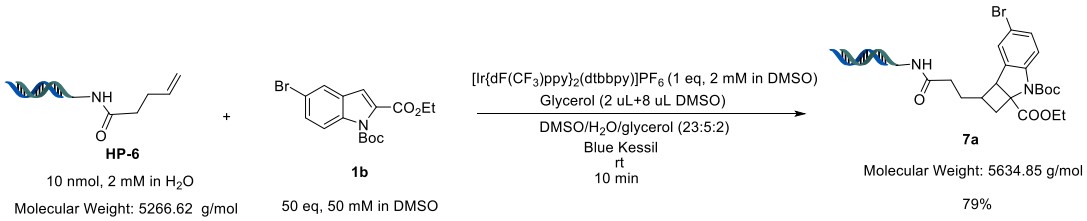


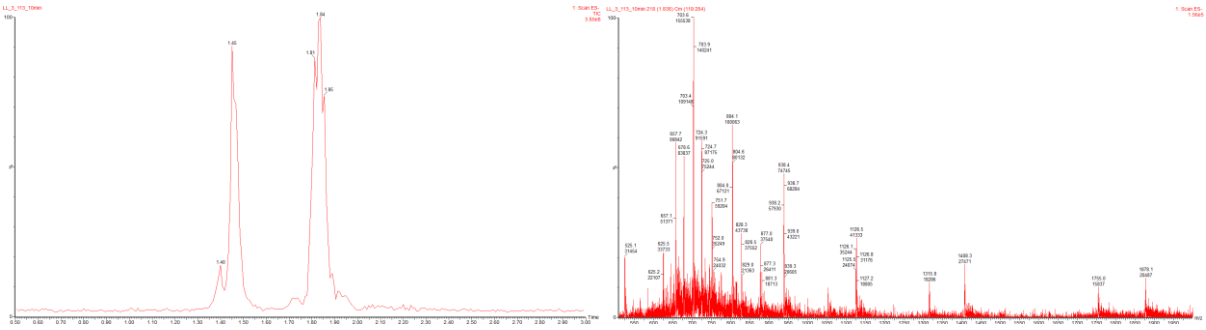
General procedure C: Compound **6f**, *82% yield*



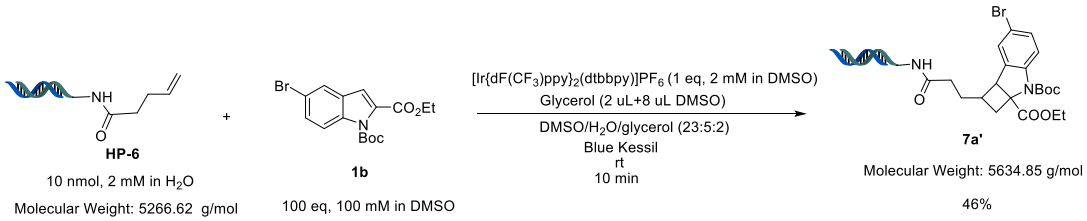


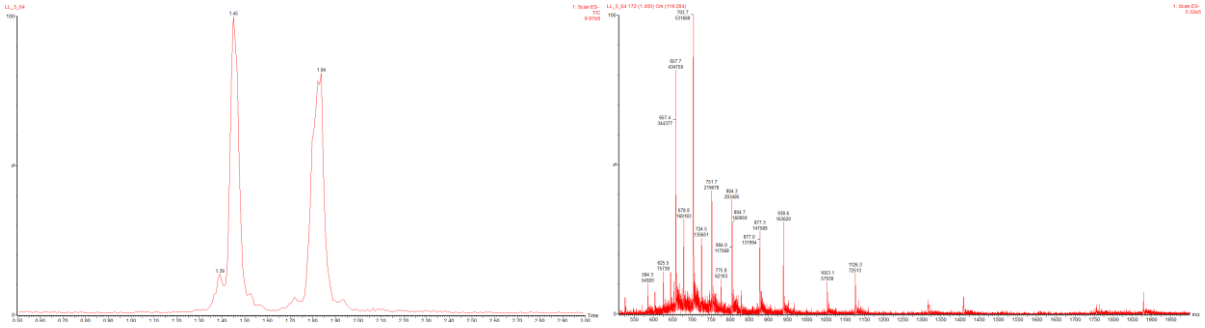
General procedure D: Compound **7a**, *79% yield*



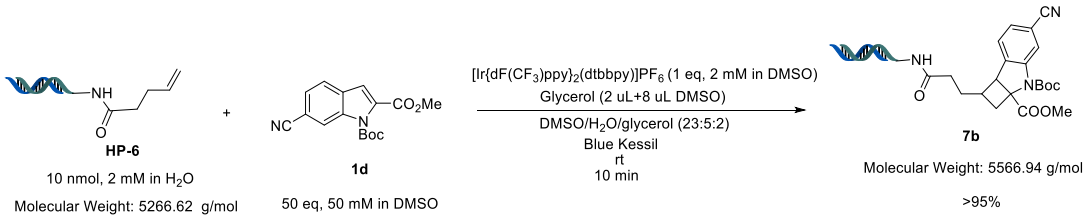


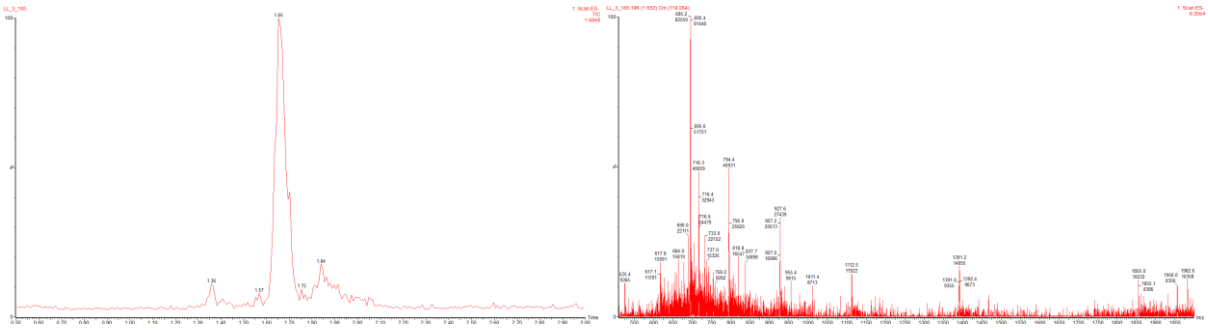
General procedure C: Compound **7a'**, *46% yield (rsm 39%)*



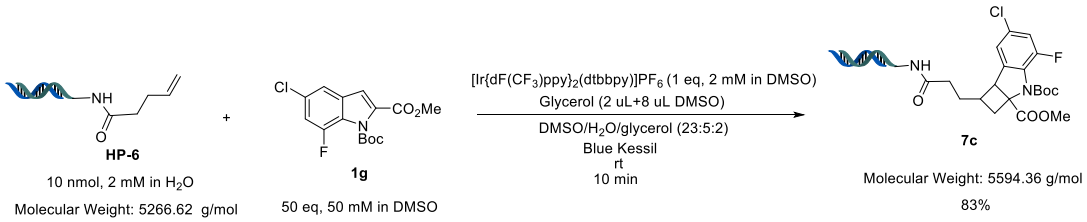


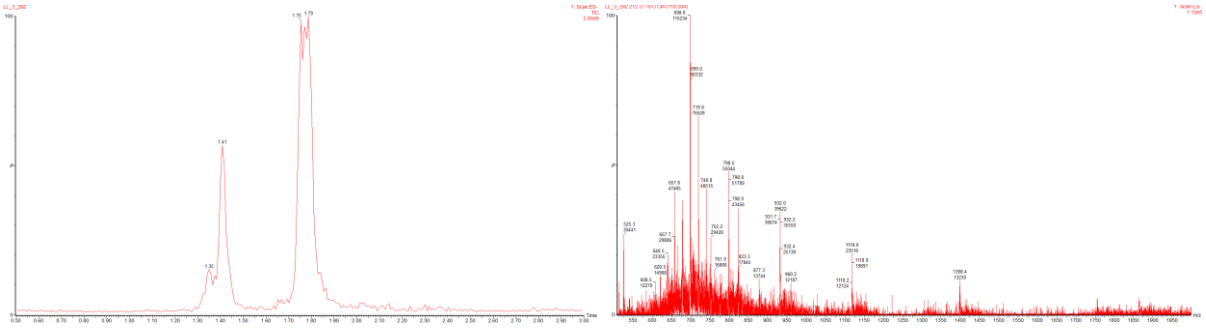
General procedure D: Compound **7b**, *>95% yield*

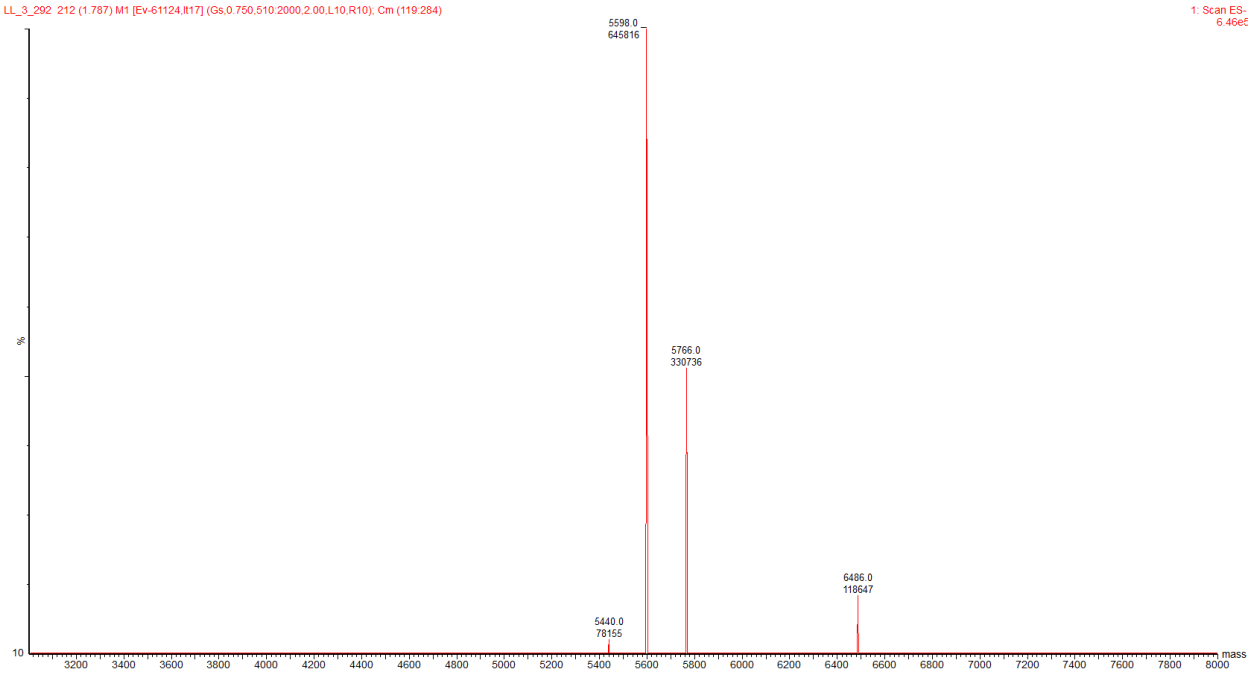




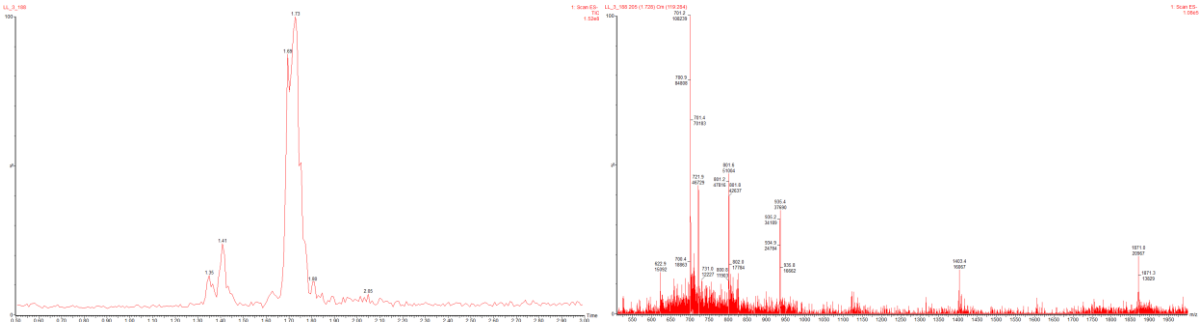
General procedure D: Compound **7c**, *83% yield*



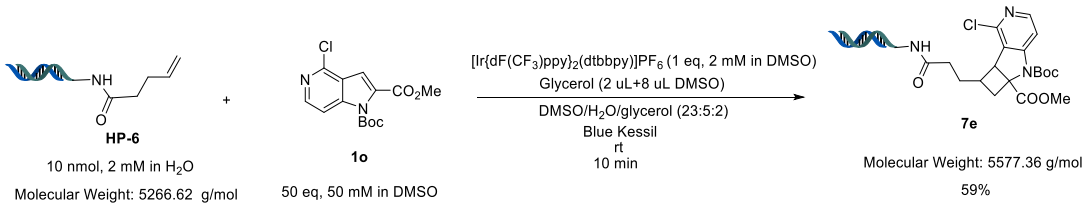


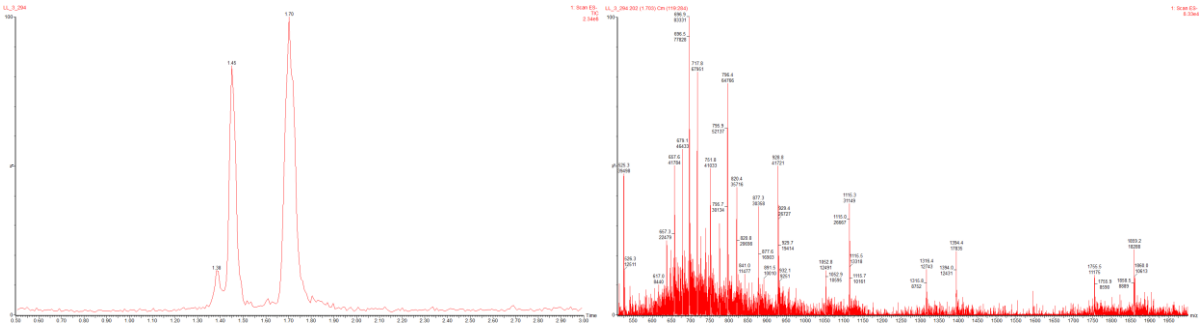


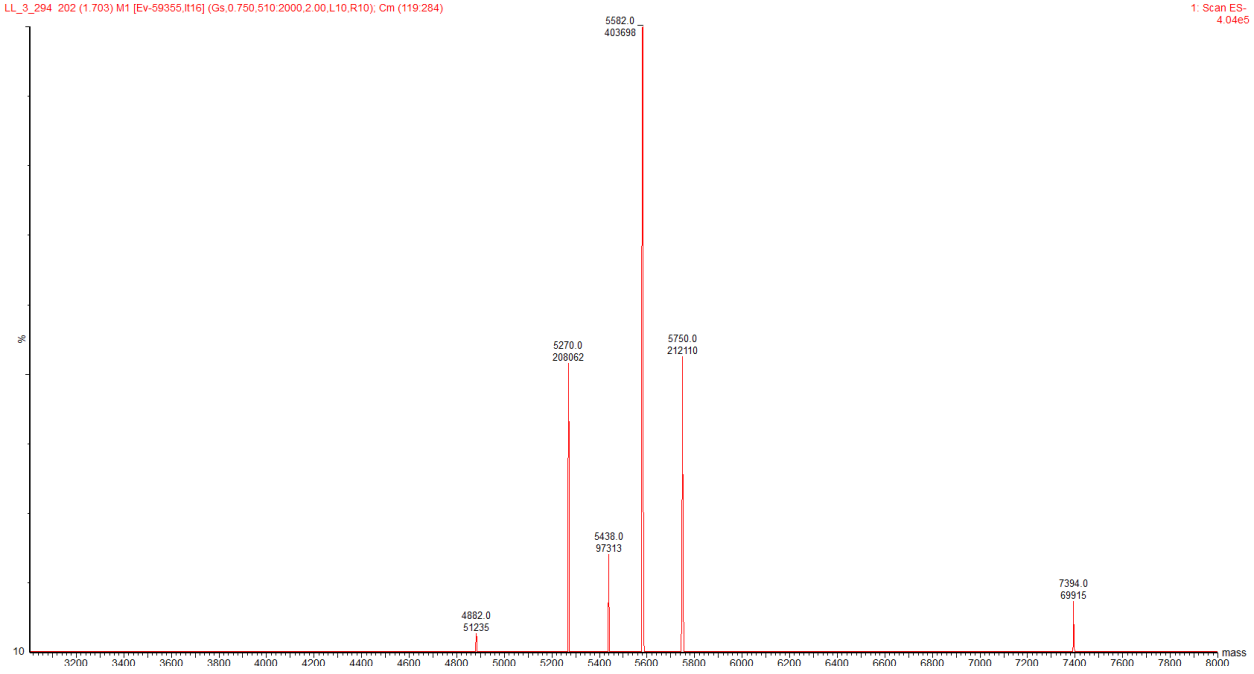
General procedure D: Compound **7d**, *>95% yield*



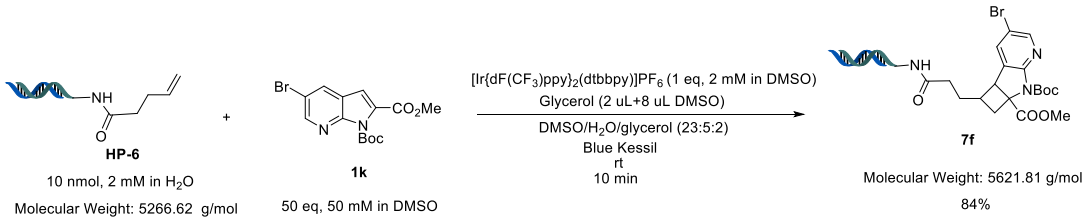
General procedure D: Compound **7e**, 59% *yield*

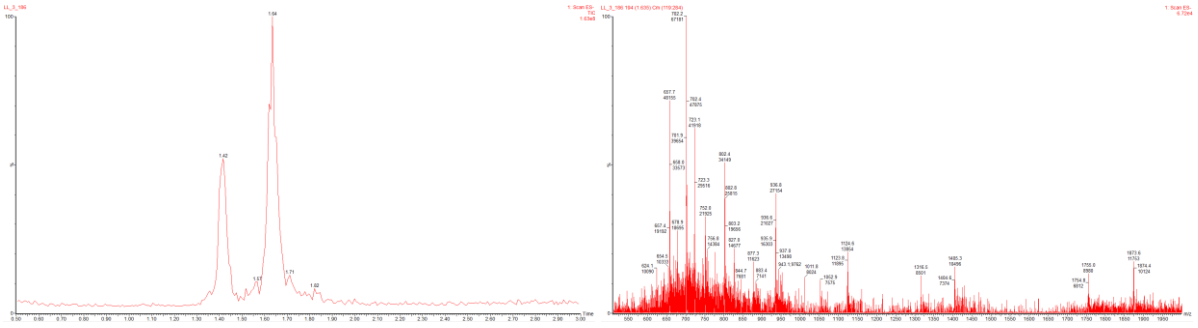




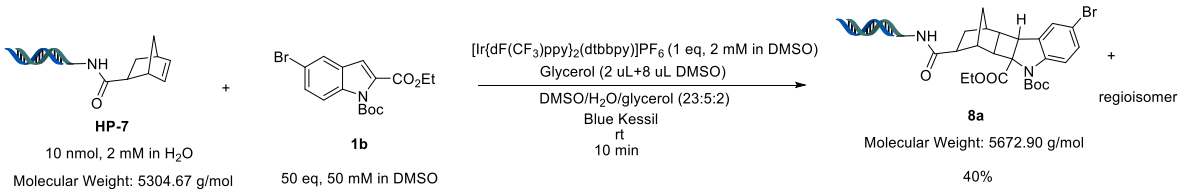


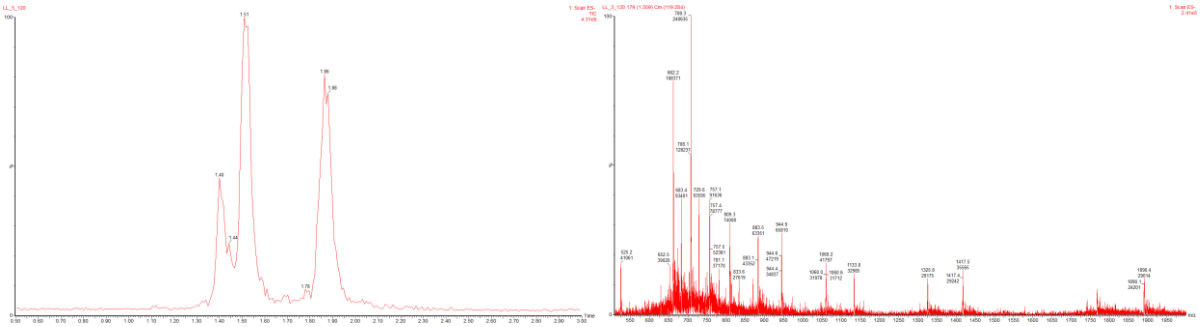
General procedure D: Compound **7f**, 84% *yield*



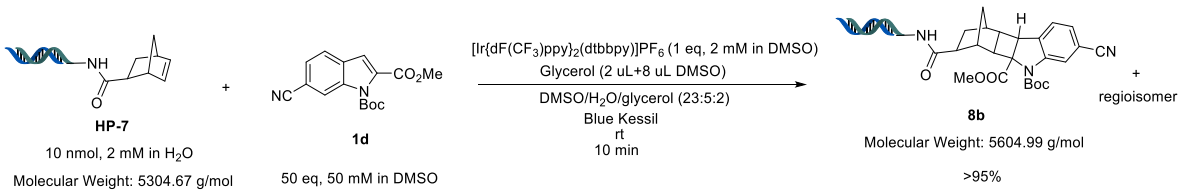


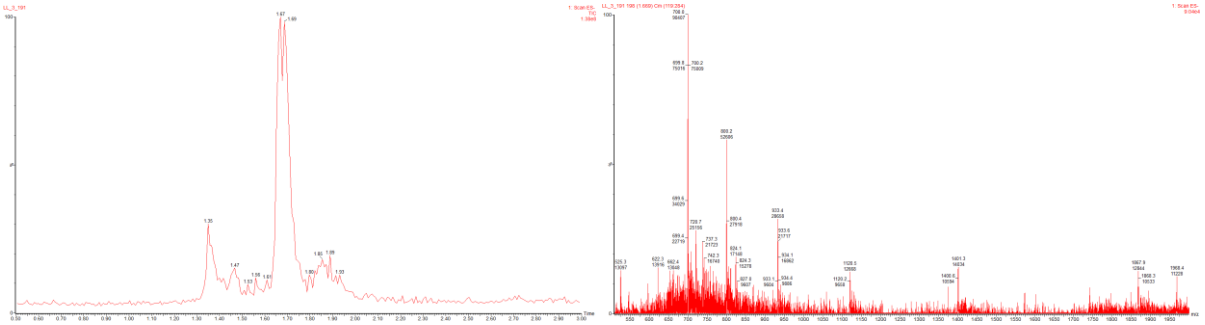
General procedure D: Compound **8a**, *40% yield*

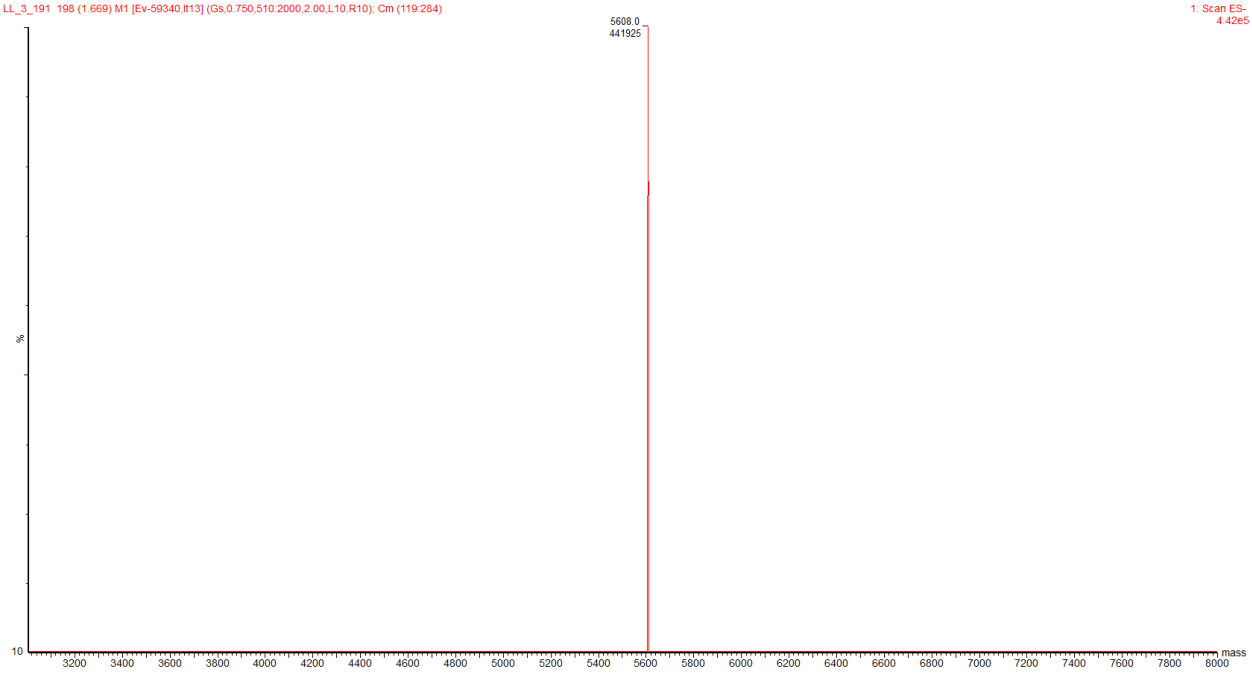




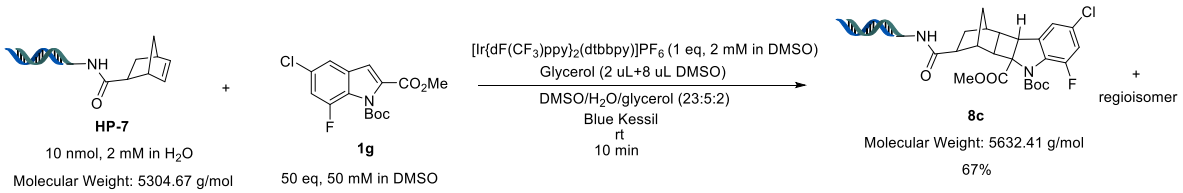
General procedure D: Compound **8b**, *>95% yield*

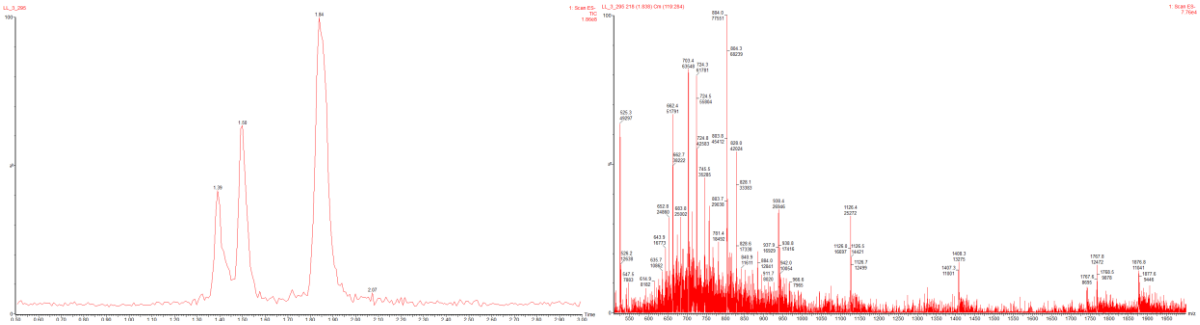




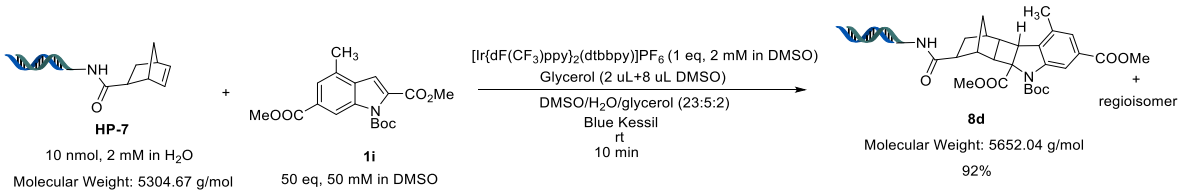


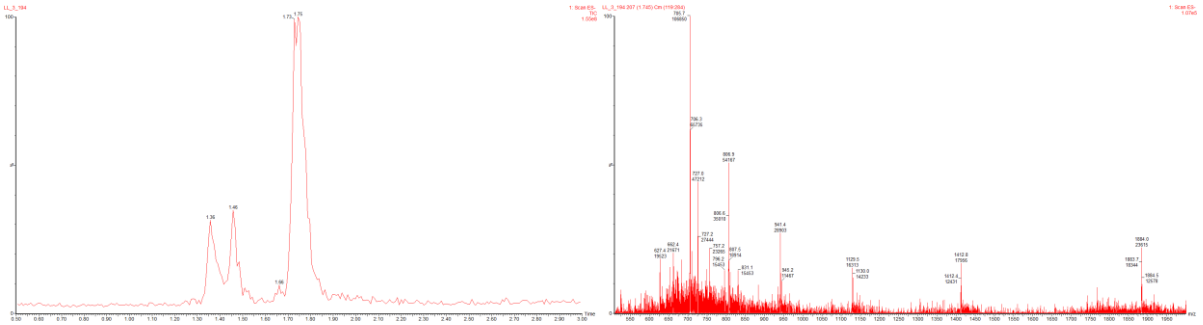
General procedure D: Compound **8c**, *67% yield*



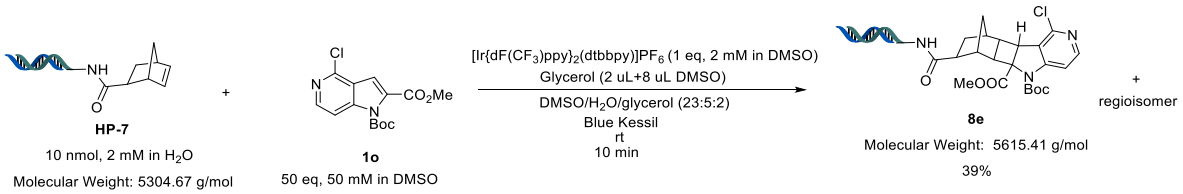


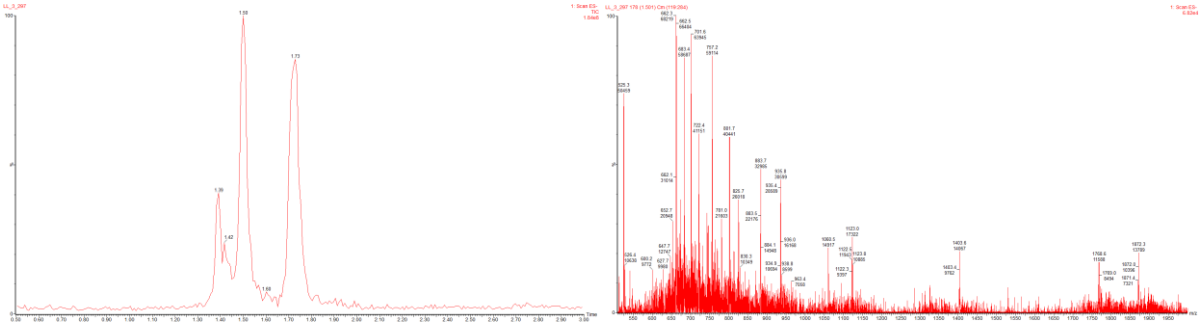
General procedure D: Compound **8d**, *92% yield*

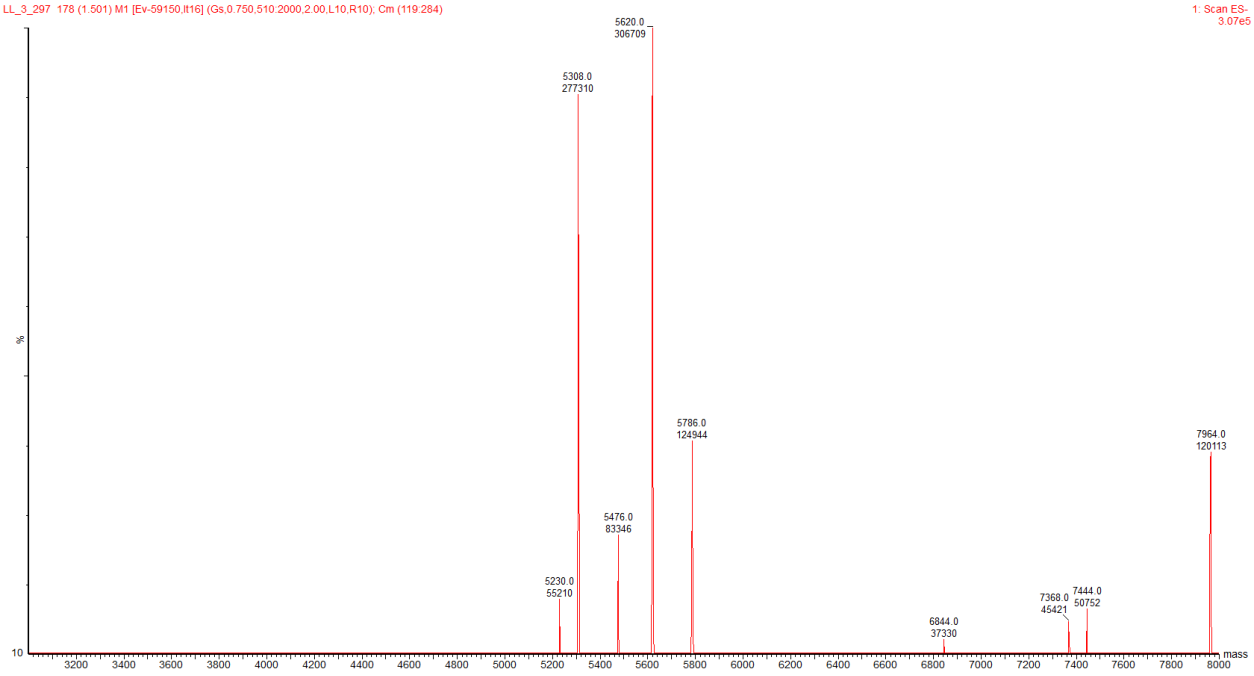




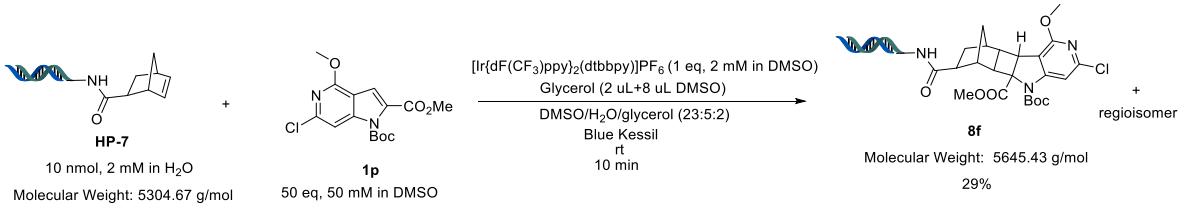
General procedure D: Compound **8e**, *39% yield*

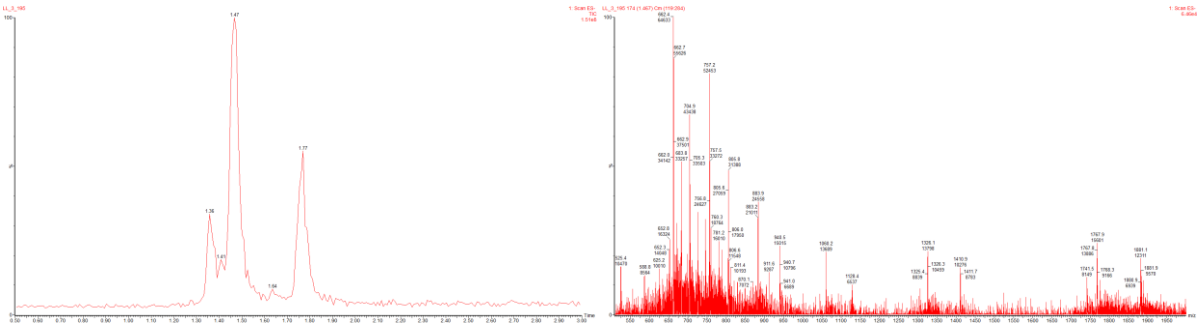




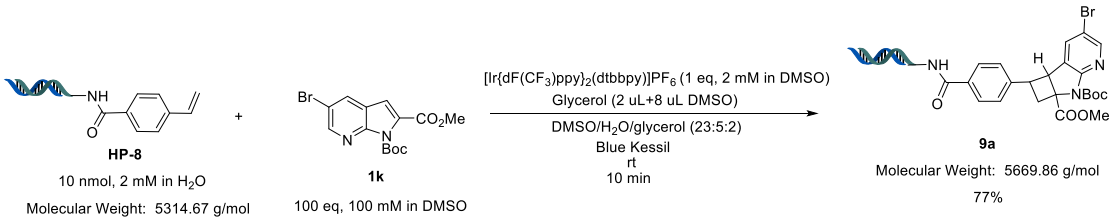


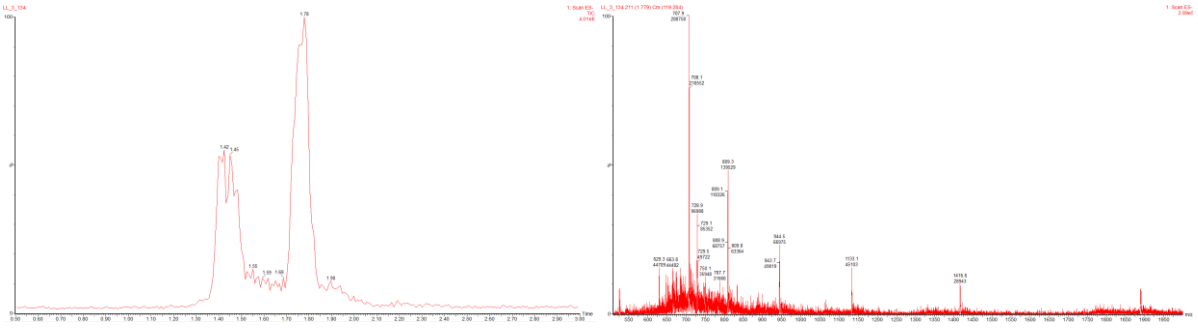
General procedure D: Compound **8f**, *29% yield (71% rsm)*

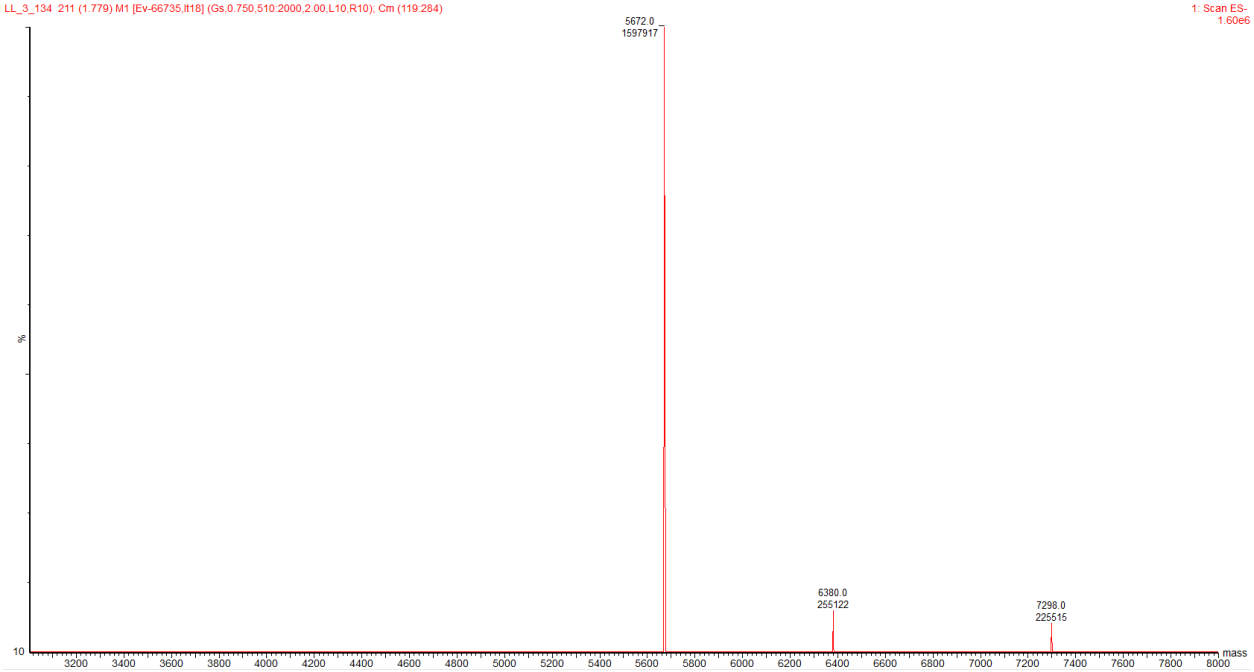




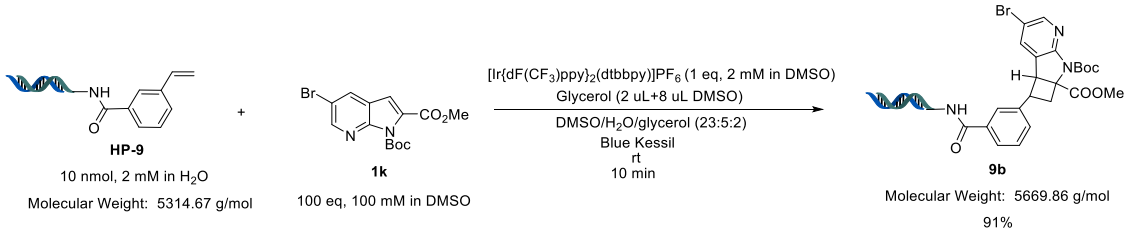
General procedure C: Compound **9a**, *77% yield*

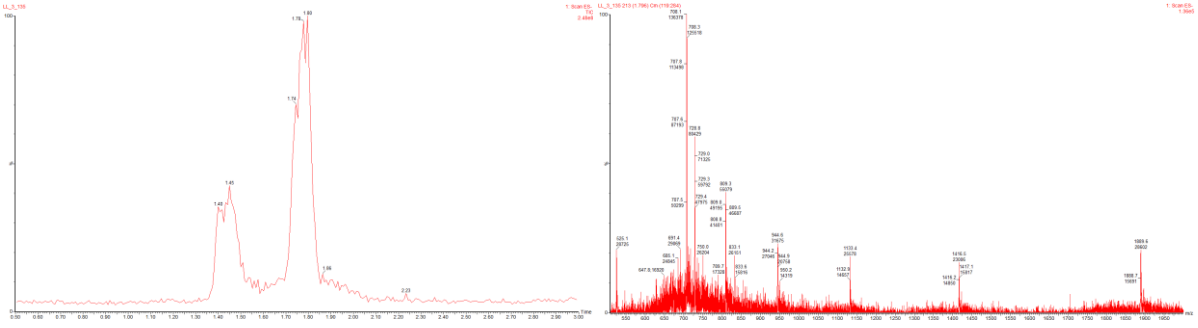


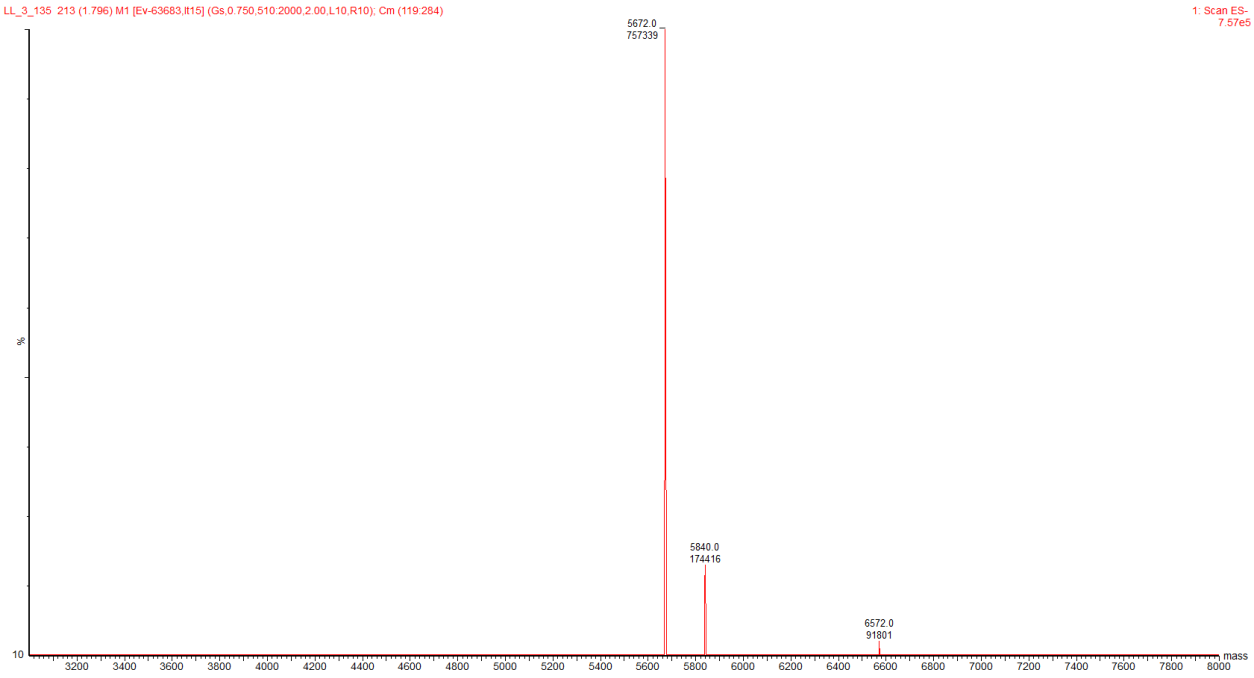




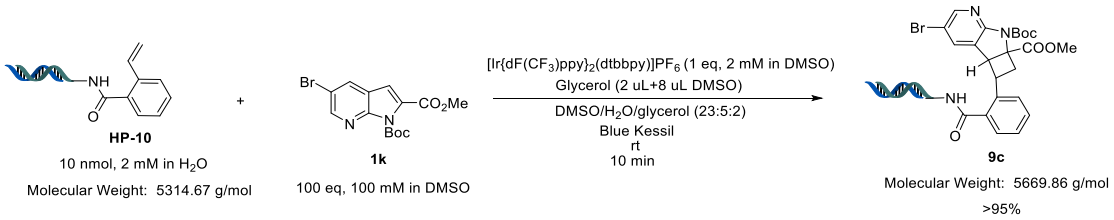
General procedure C: Compound **9b**, *91% yield*

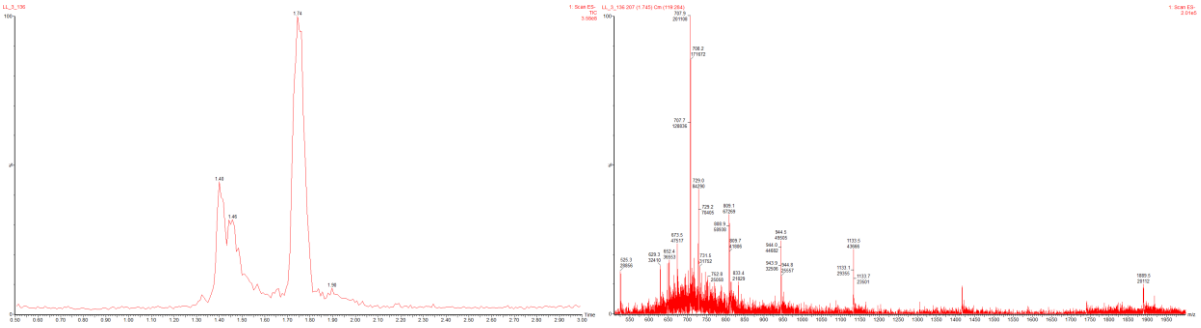




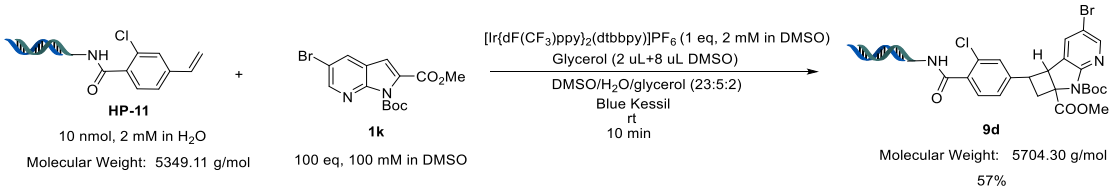


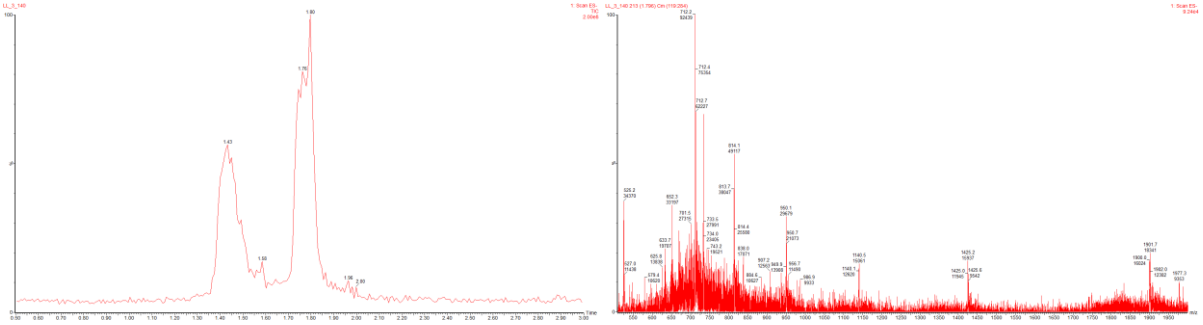
General procedure C: Compound **9c**, *>95% yield*

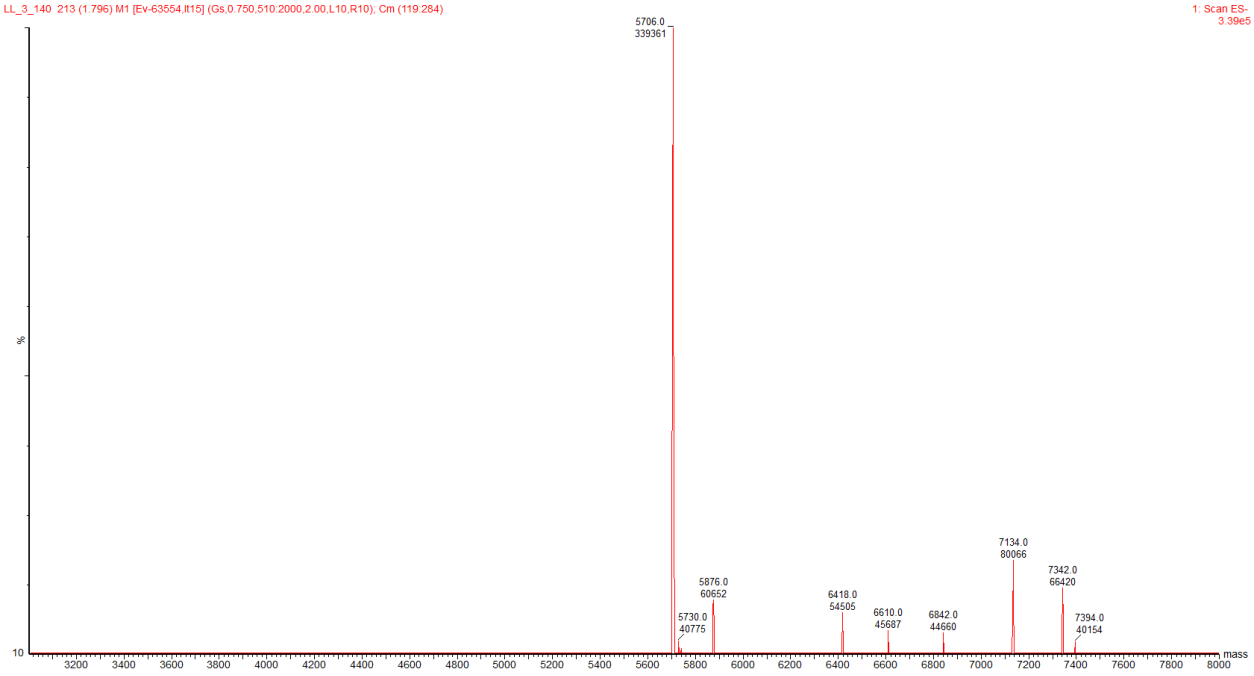




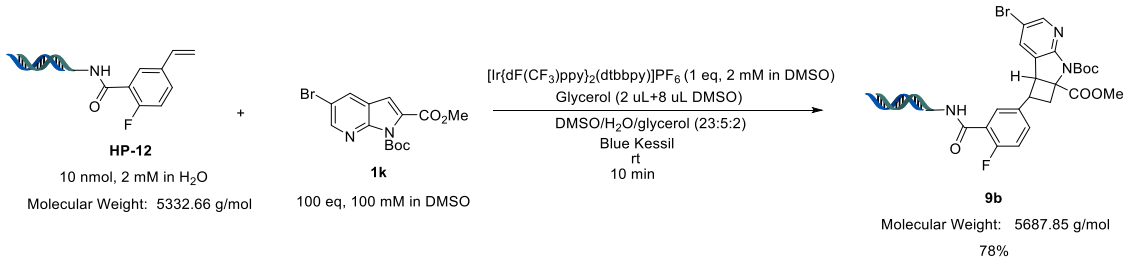
General procedure C: Compound **9d**, 57% *yield*

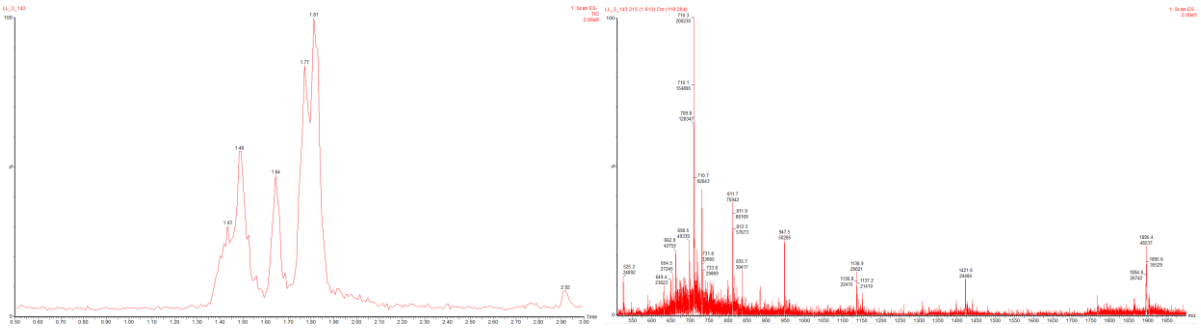


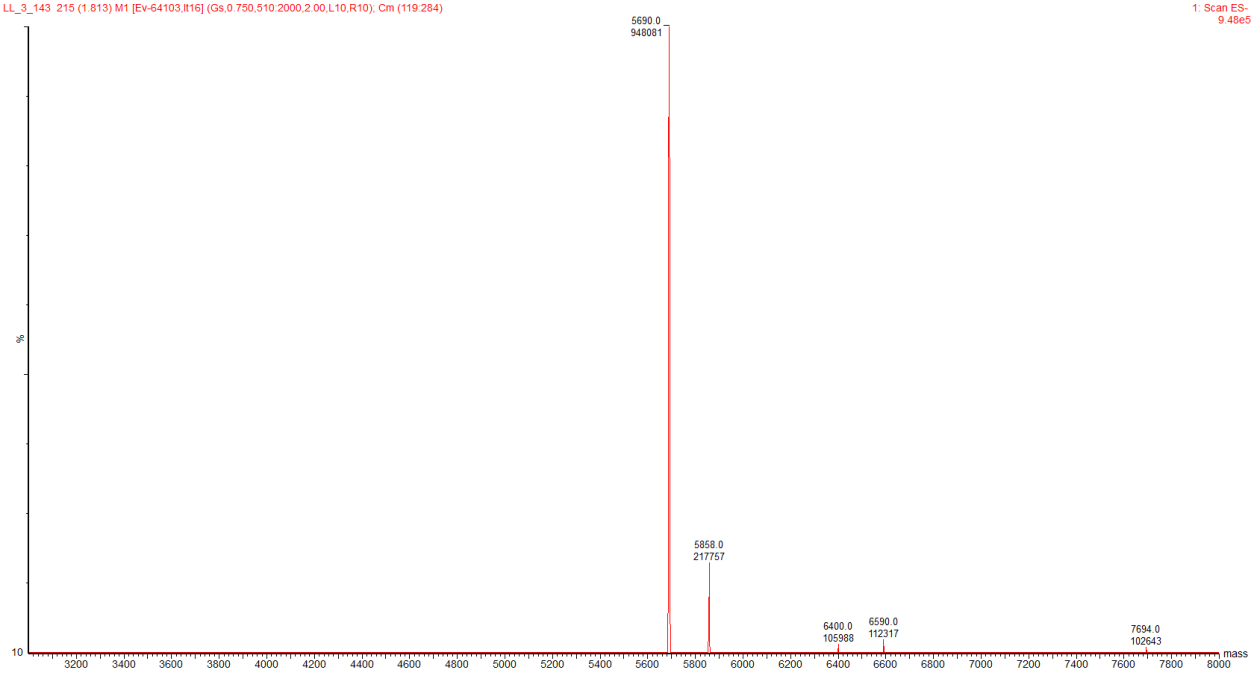




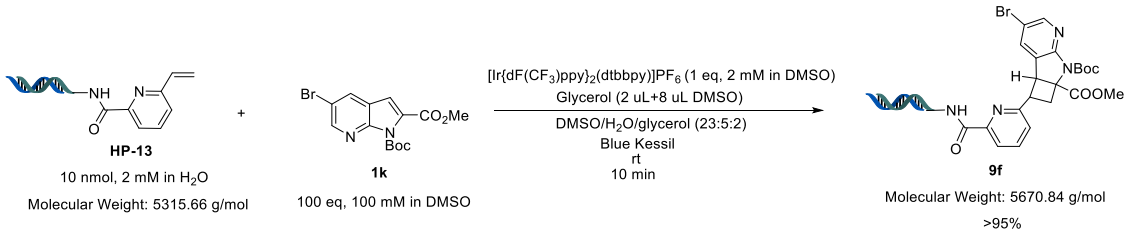
General procedure C: Compound **9e**, *78% yield*

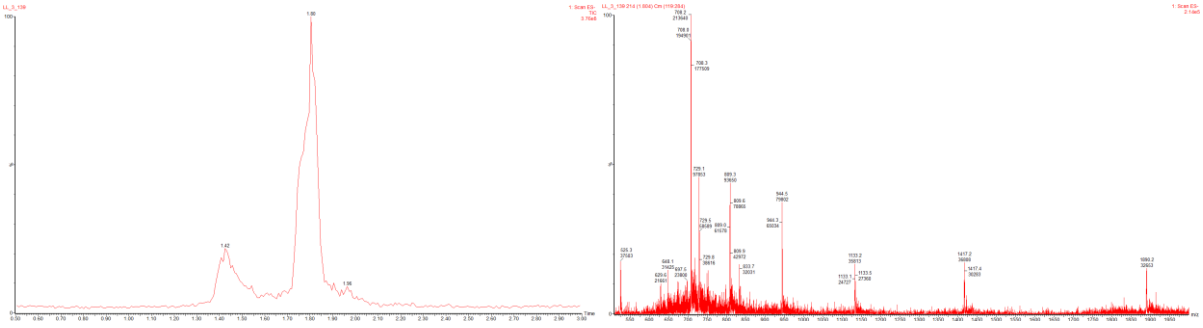




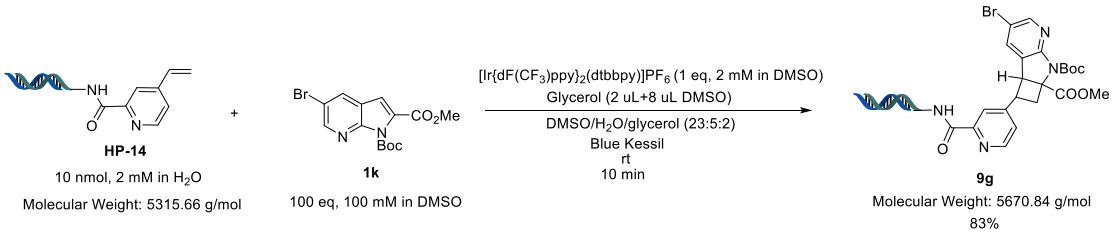


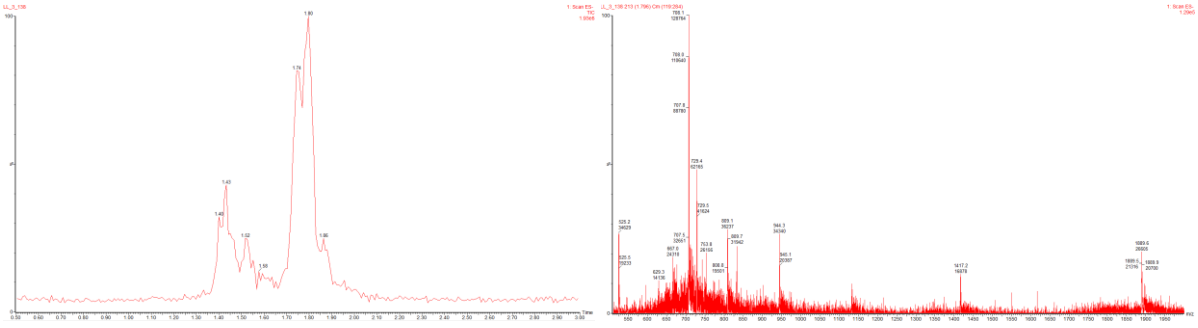
General procedure C: Compound **9f**, *>95% yield*

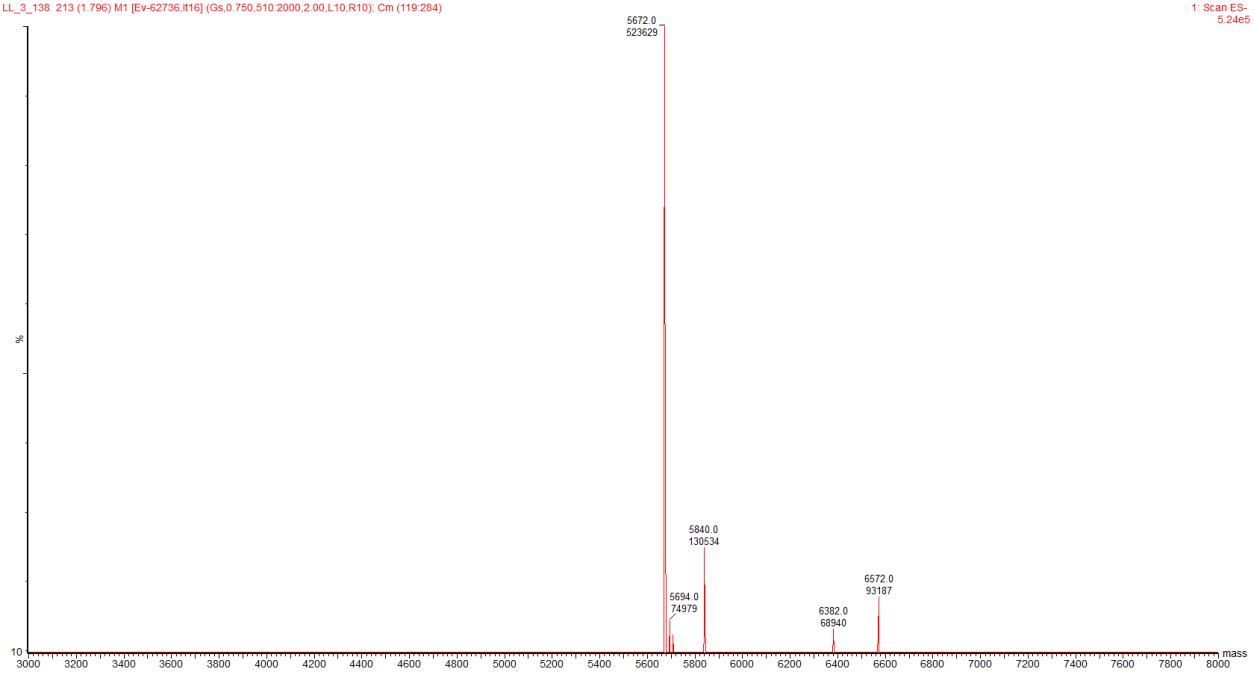




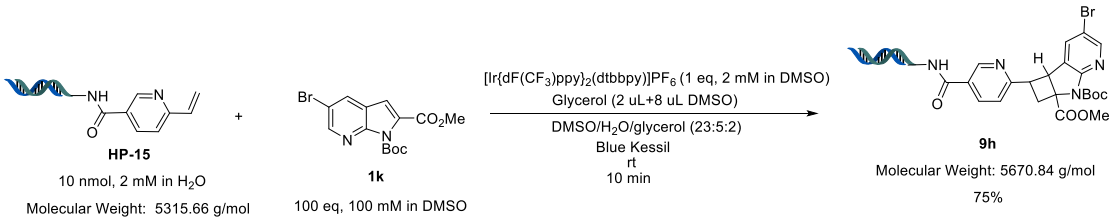
General procedure C: Compound **9g**, *83% yield*

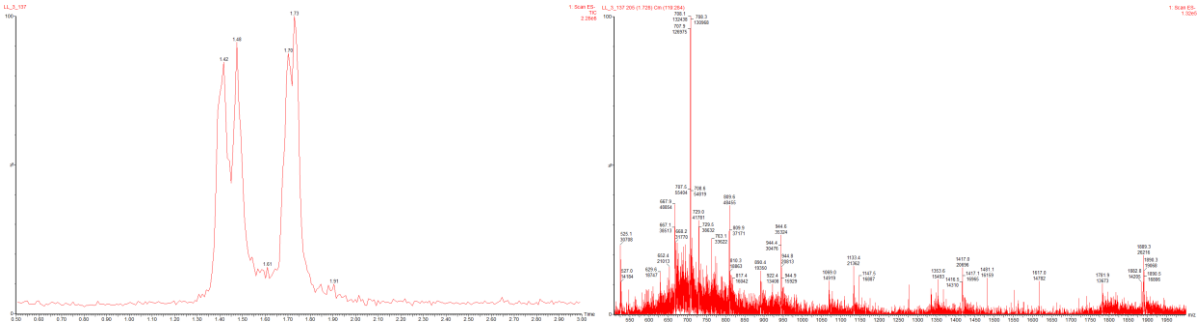


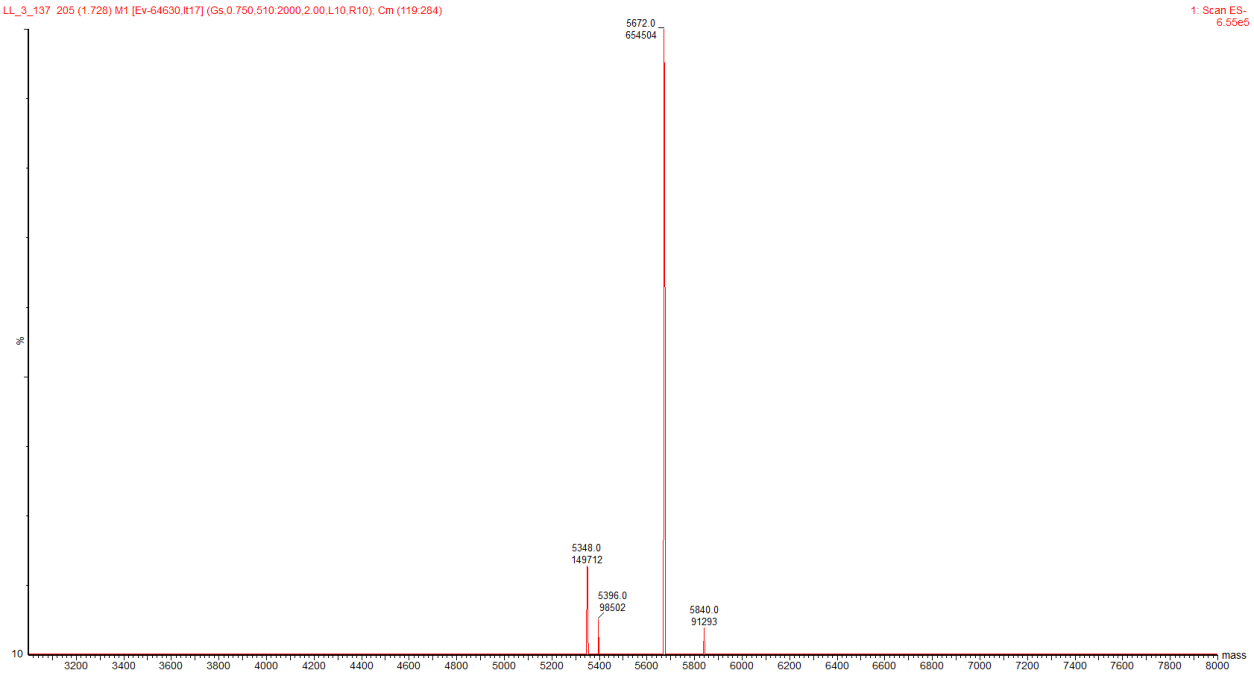




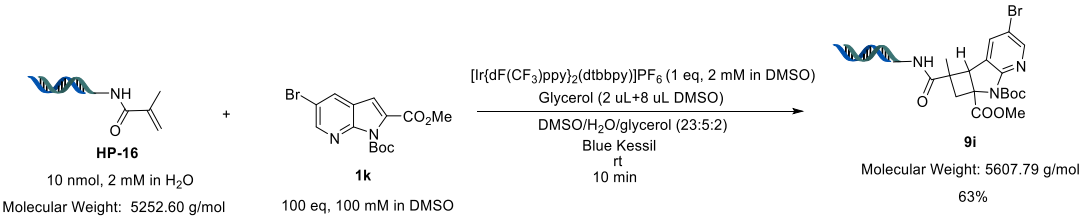
General procedure C: Compound **9h**, *75% yield*

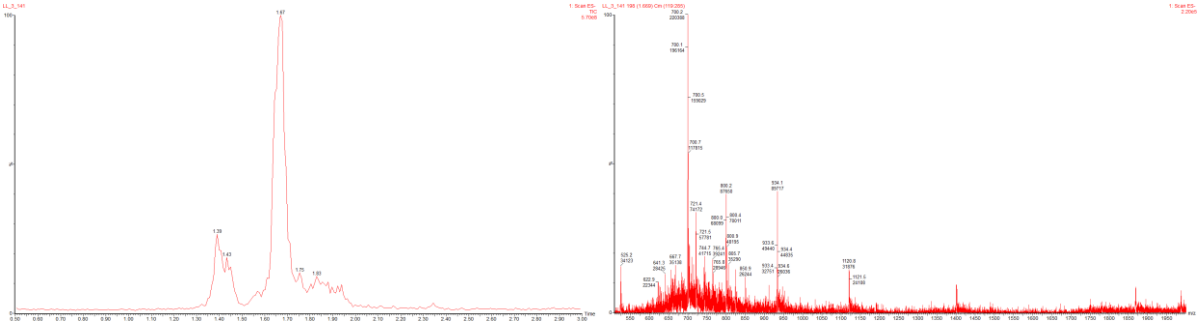




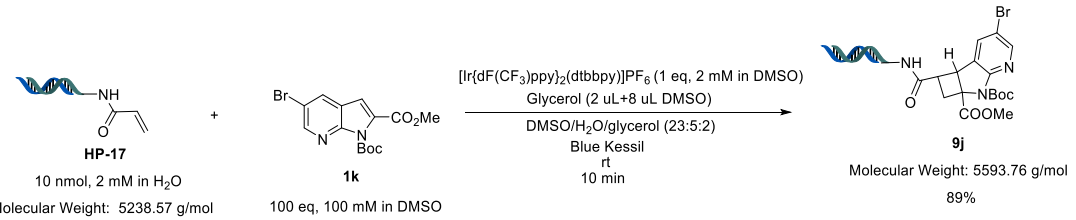


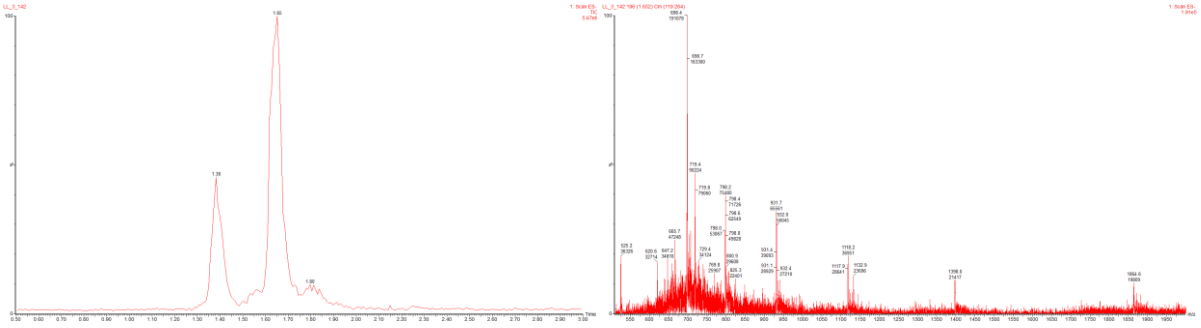
General procedure C: Compound **9i**, *63% yield*

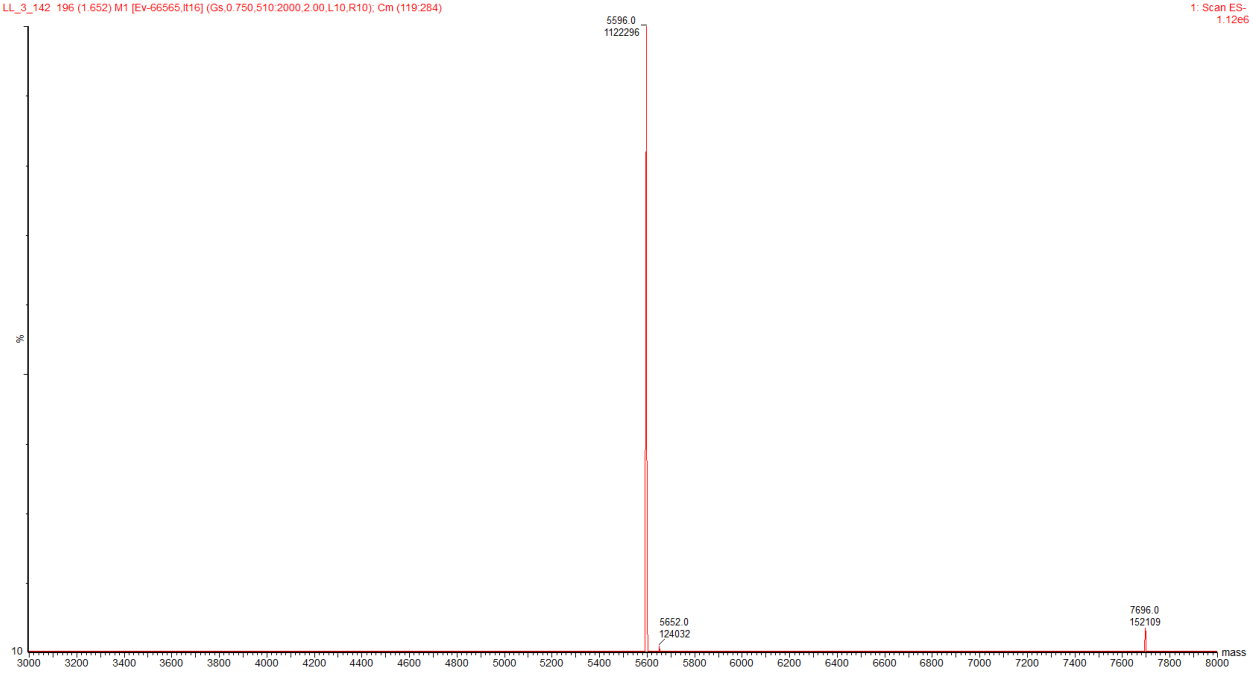




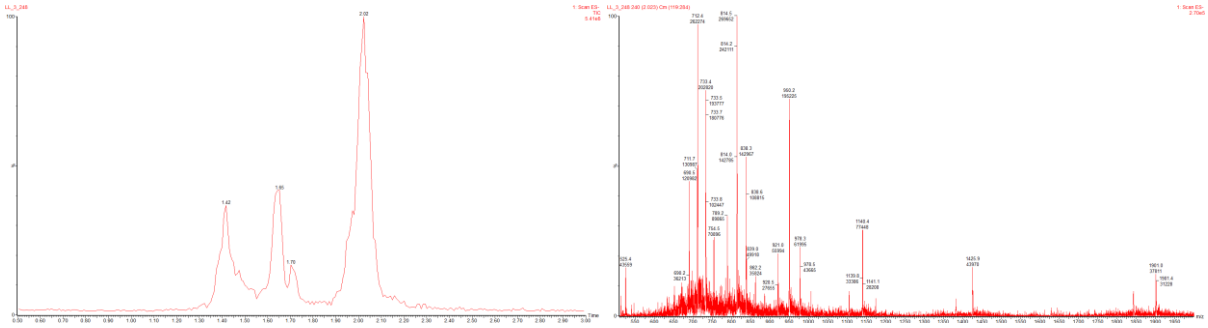
General procedure C: Compound **9j**, *89% yield*



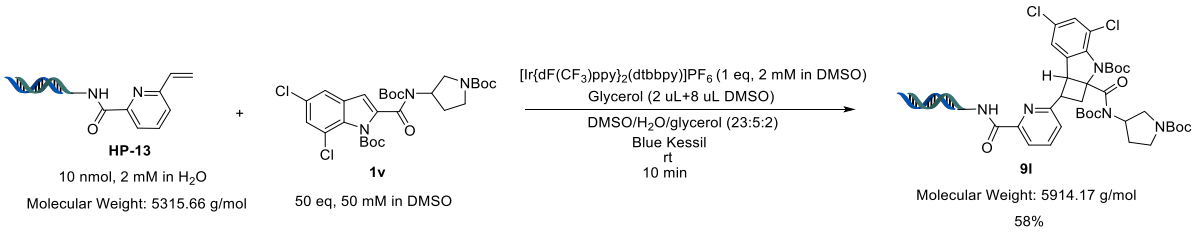


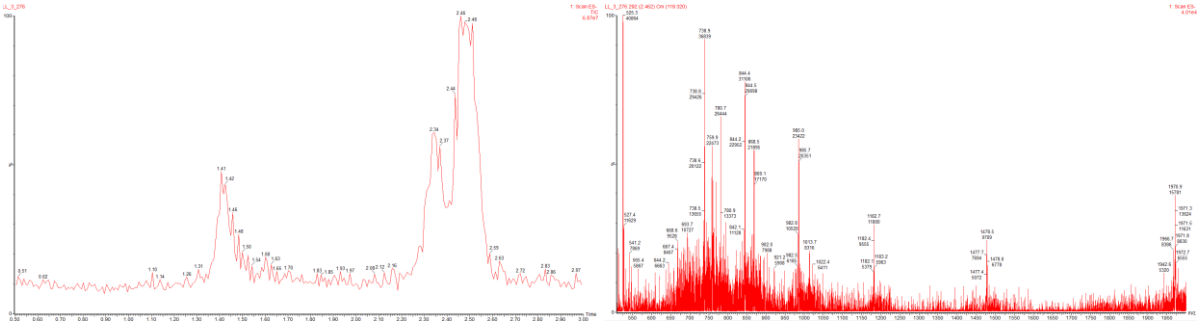


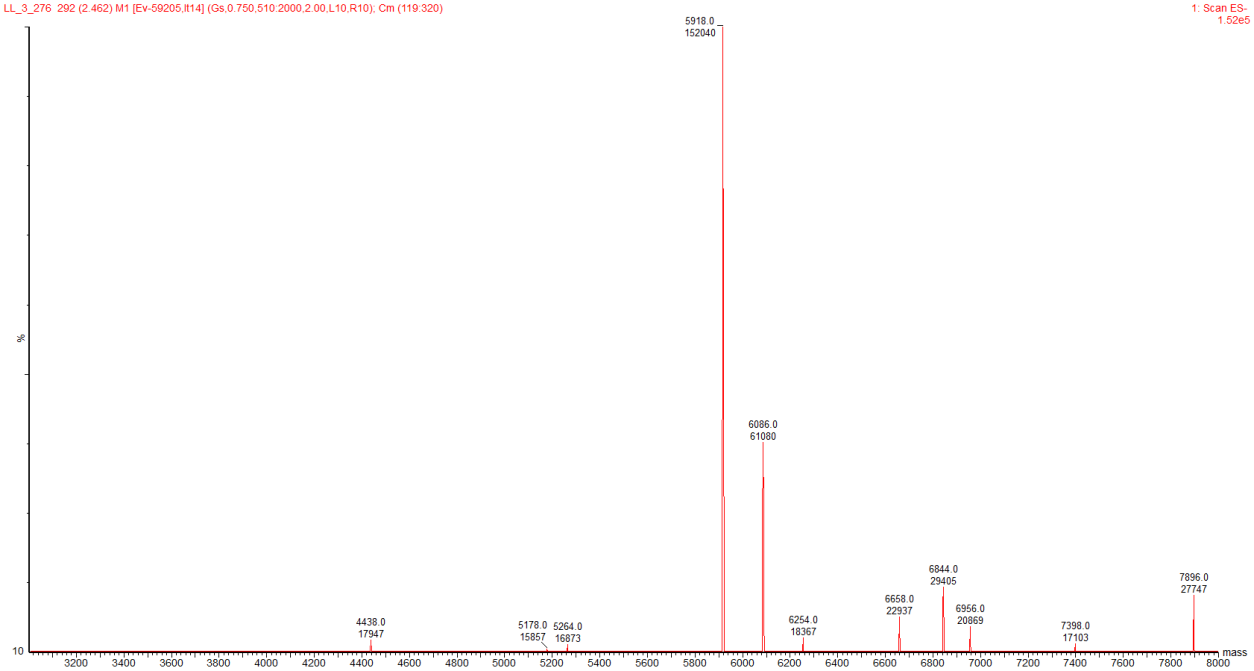
General procedure C: Compound **9k**, *91% yield*



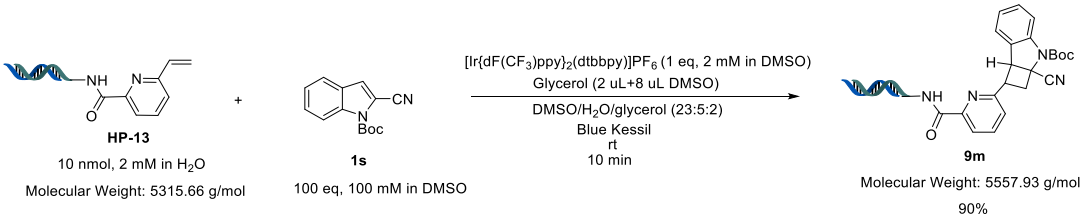
General procedure D: Compound **9l**, 58*% yield*

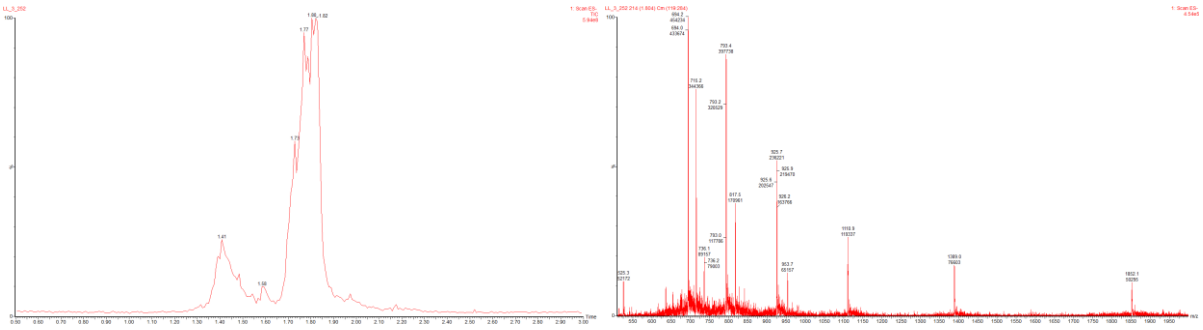




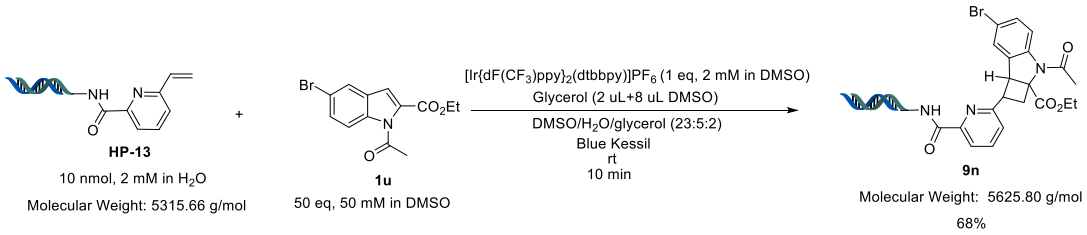


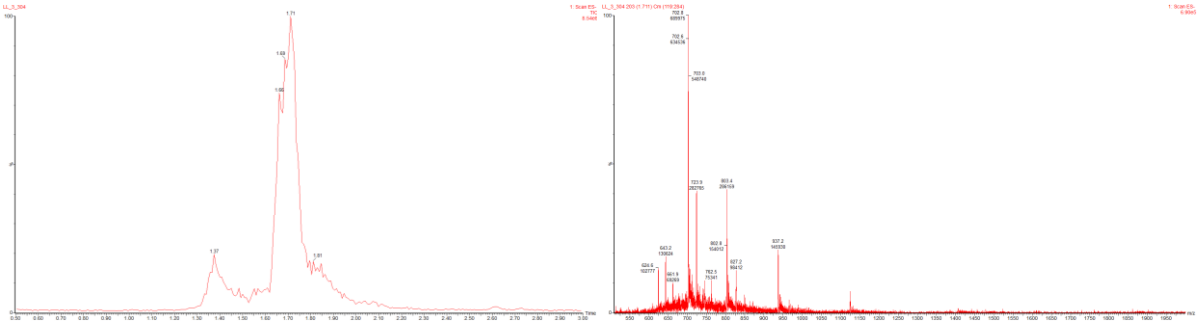
General procedure C: Compound **9m**, 90% *yield*



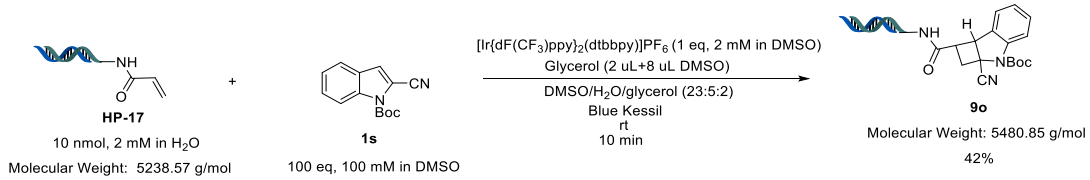


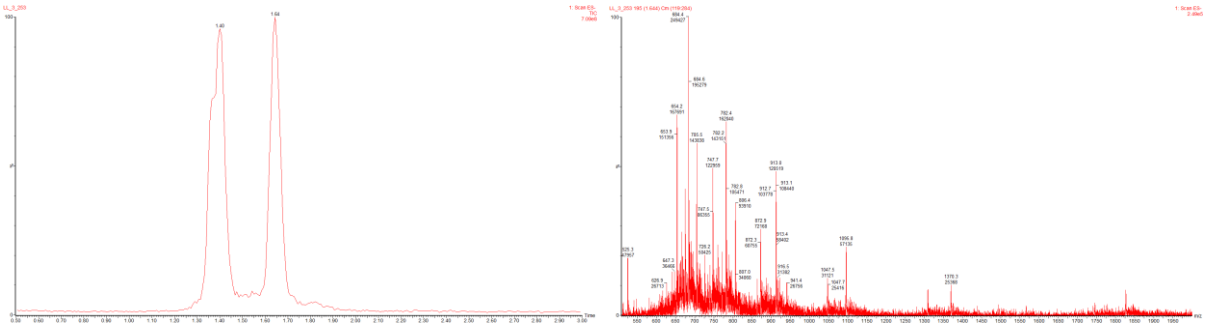
General procedure D: Compound **9n**, *68% yield*

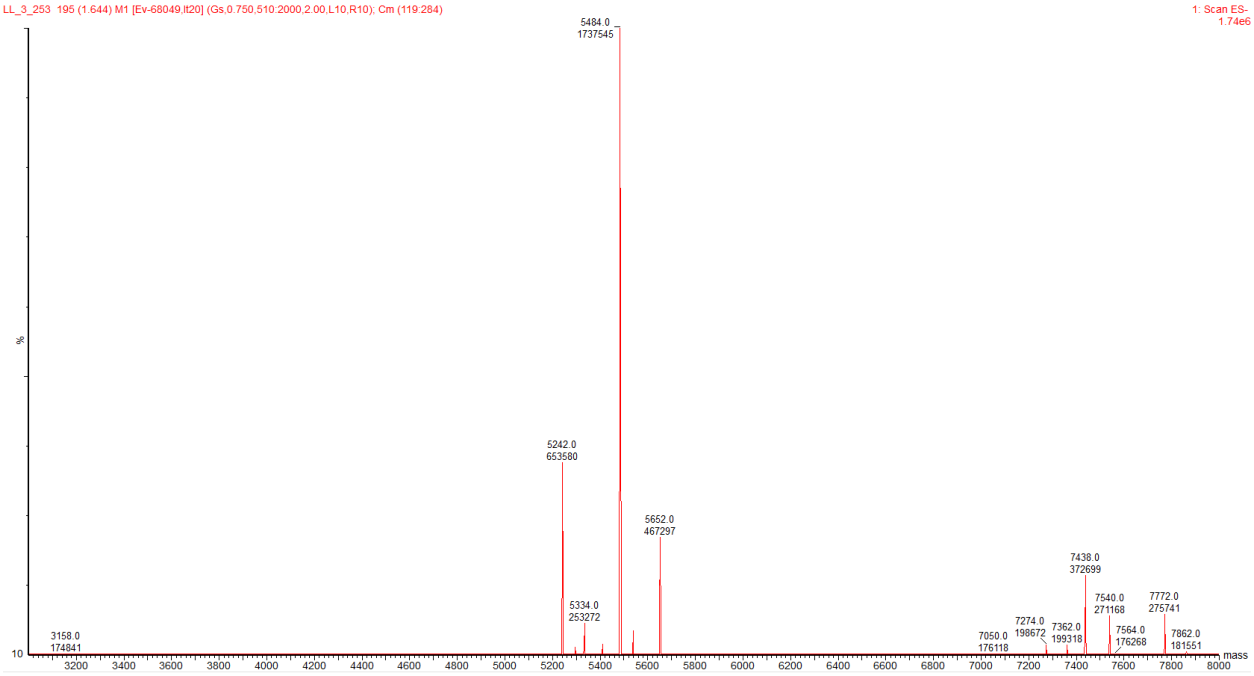




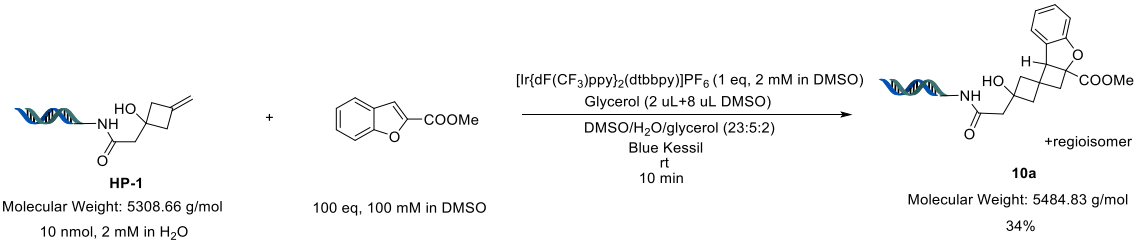
General procedure C: Compound **9o**, *42% yield*

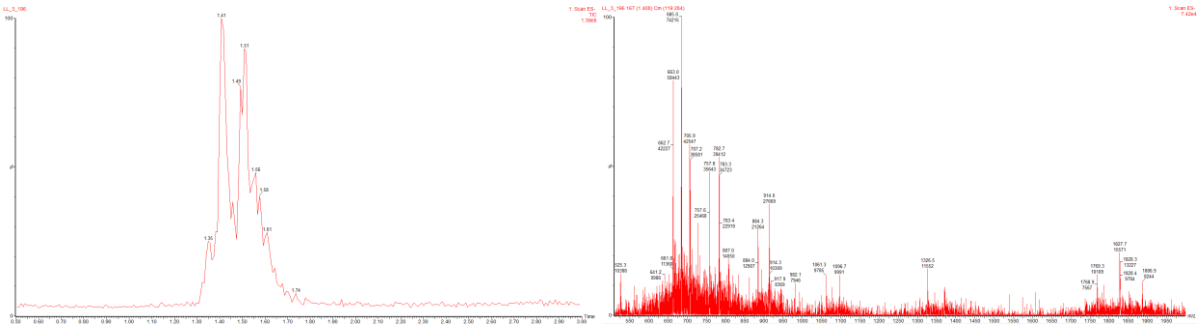




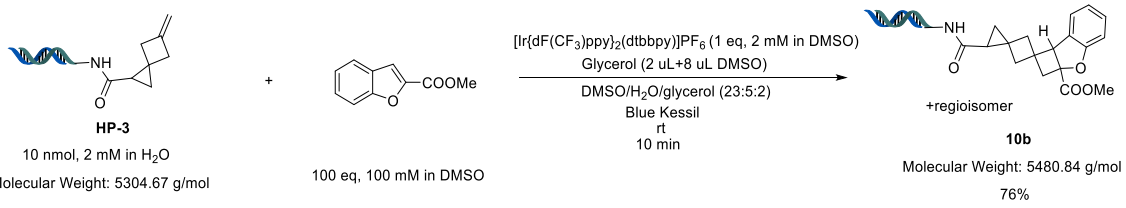


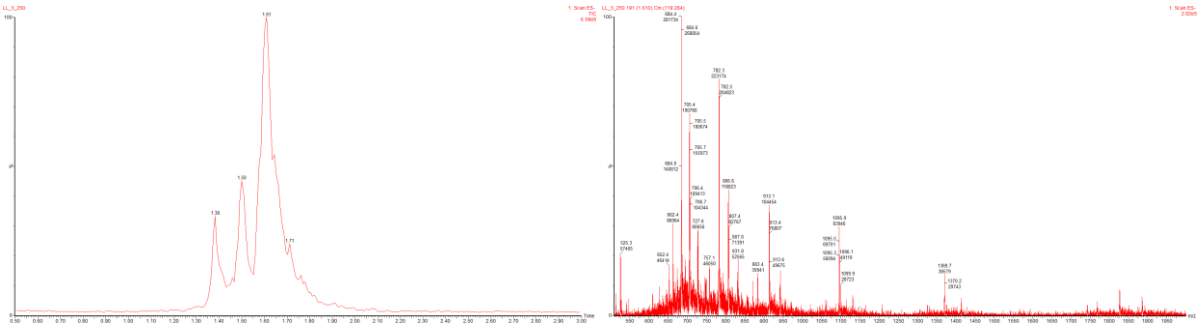
General procedure C: Compound **10a**, *34% yield*

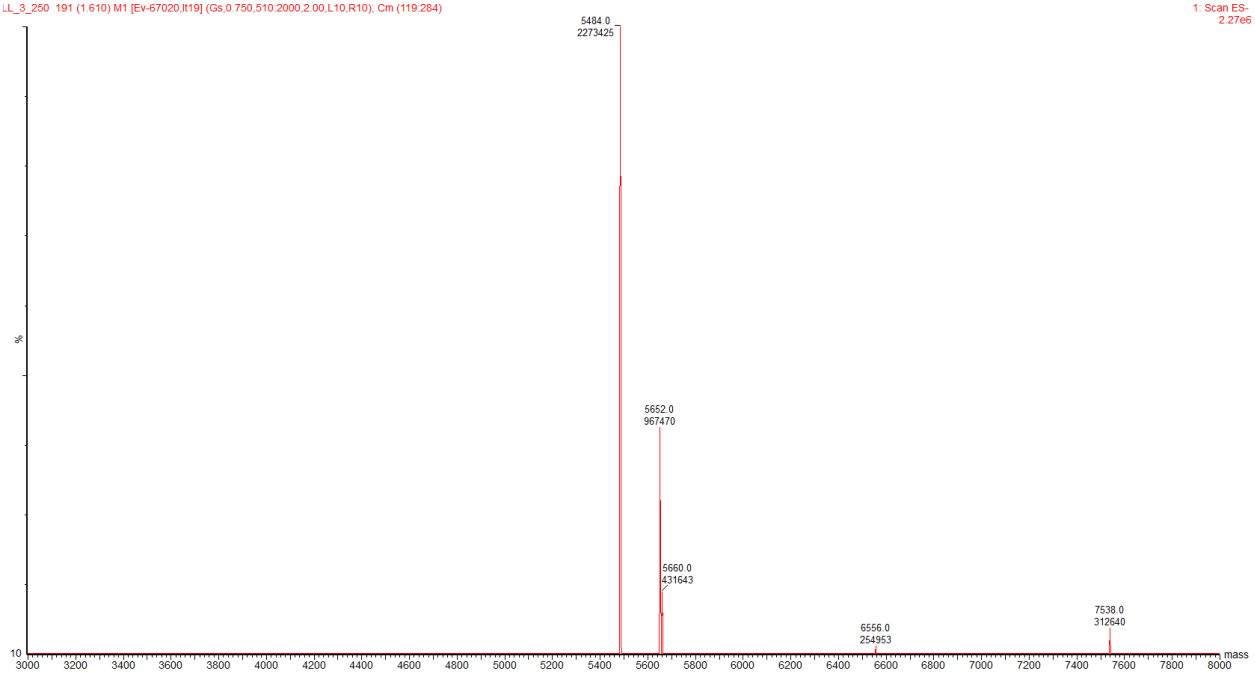




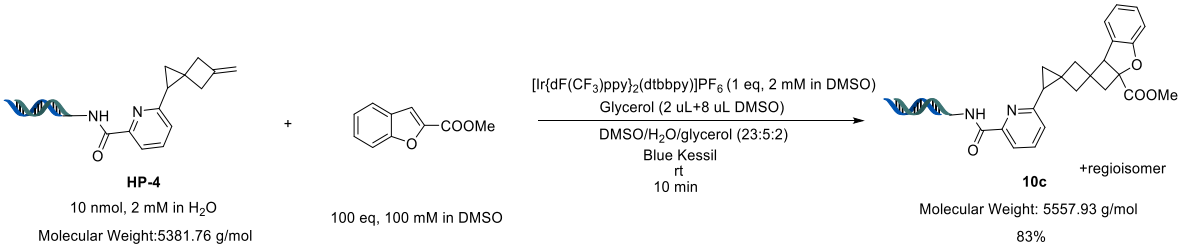
General procedure C: Compound **10b**, *76% yield*

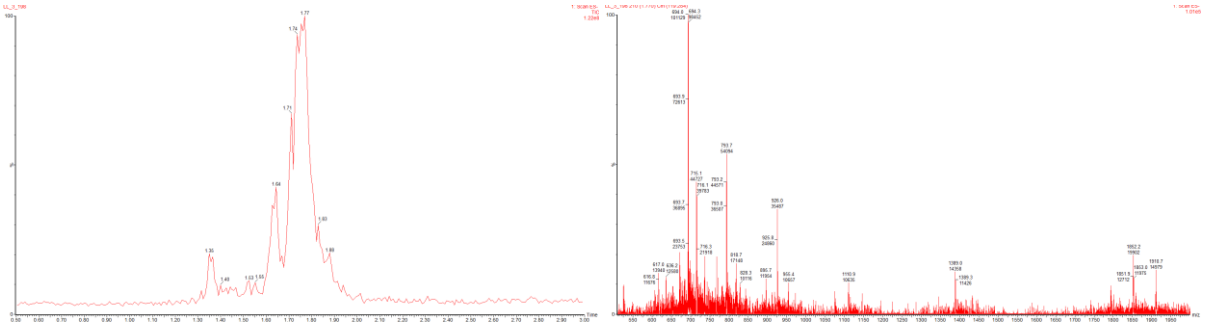




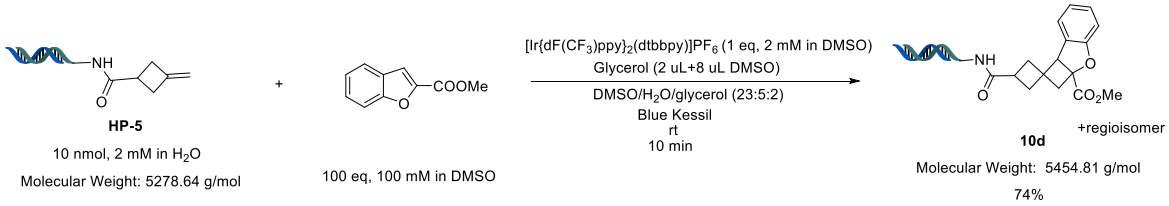


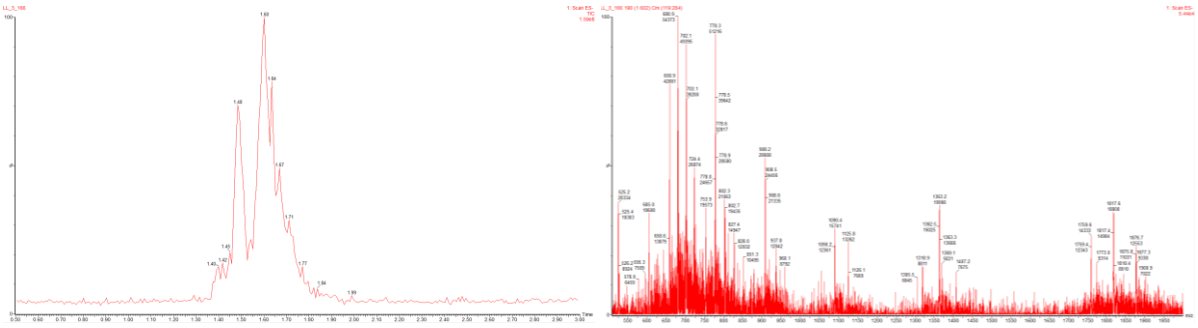
General procedure C: Compound **10c**, *83% yield*

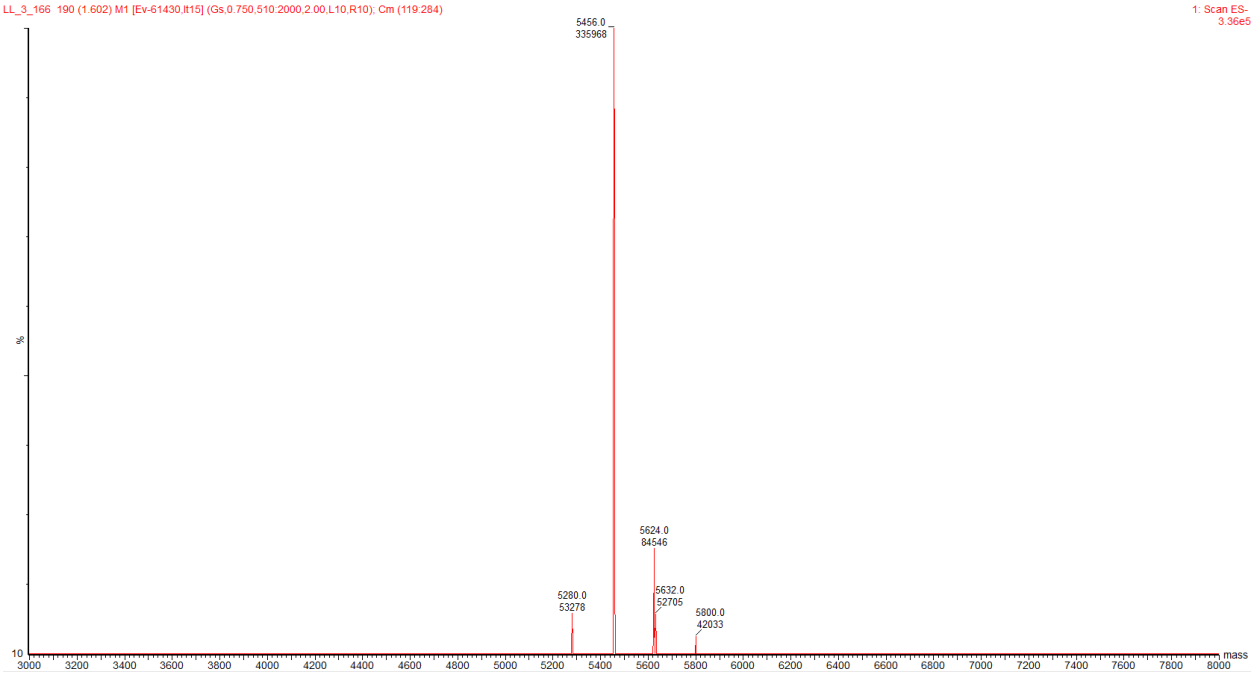




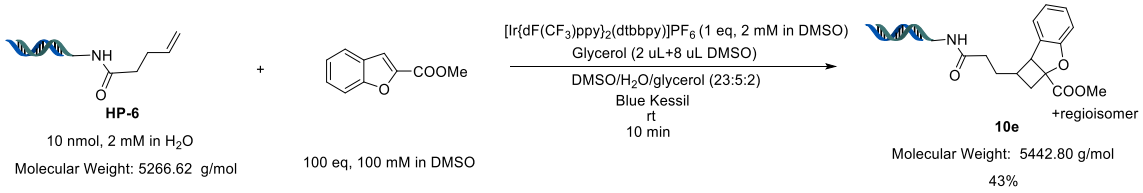
General procedure C: Compound **10d**, *74% yield*

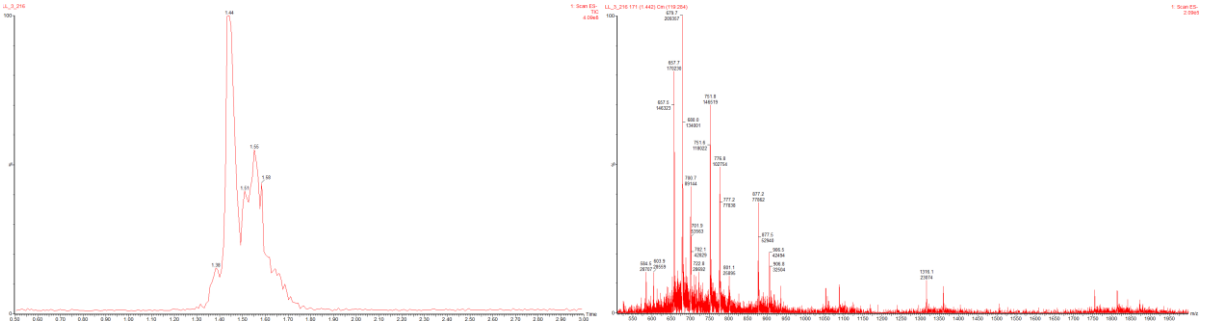


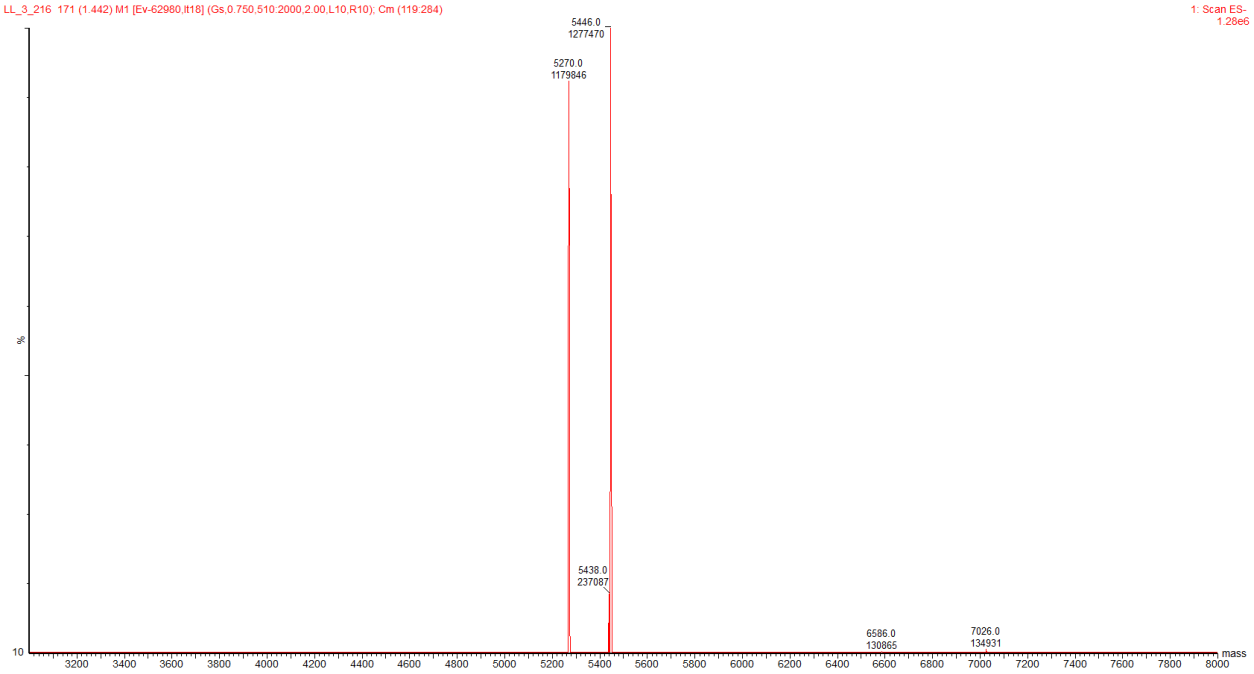




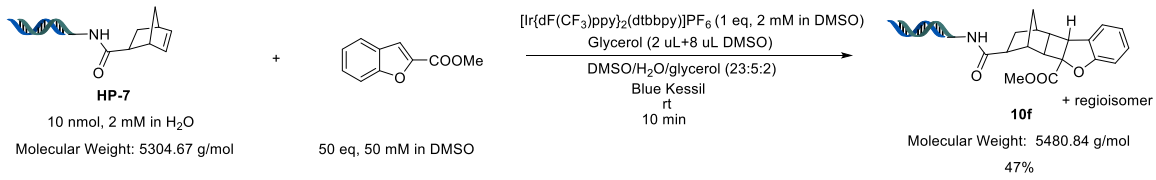
General procedure C: Compound **10e**, *43% yield*

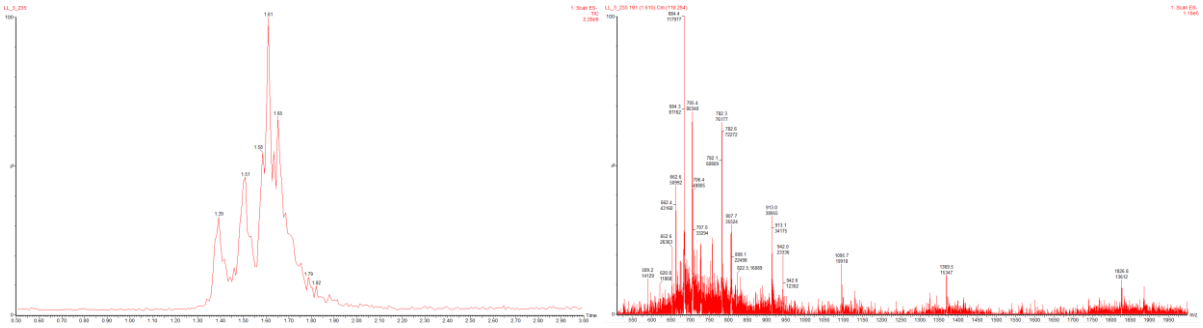




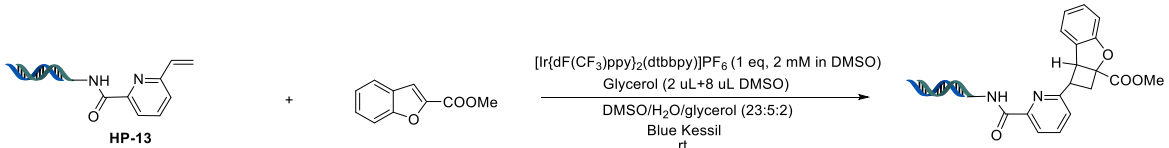


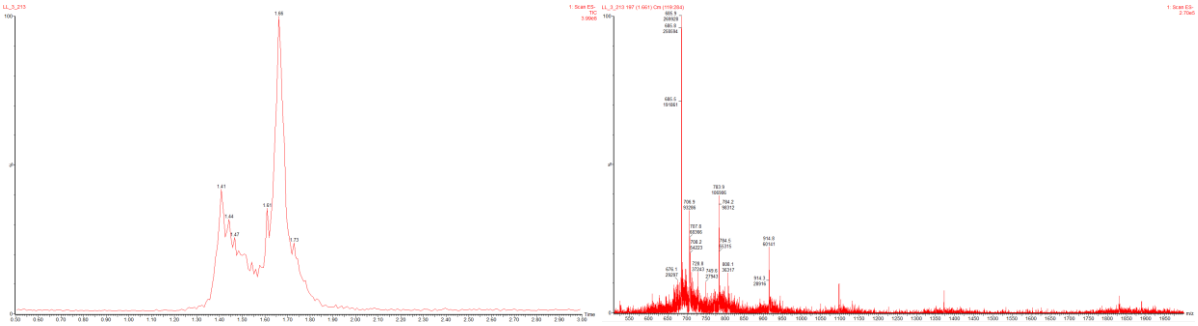
General procedure D: Compound **10f**, *47% yield*

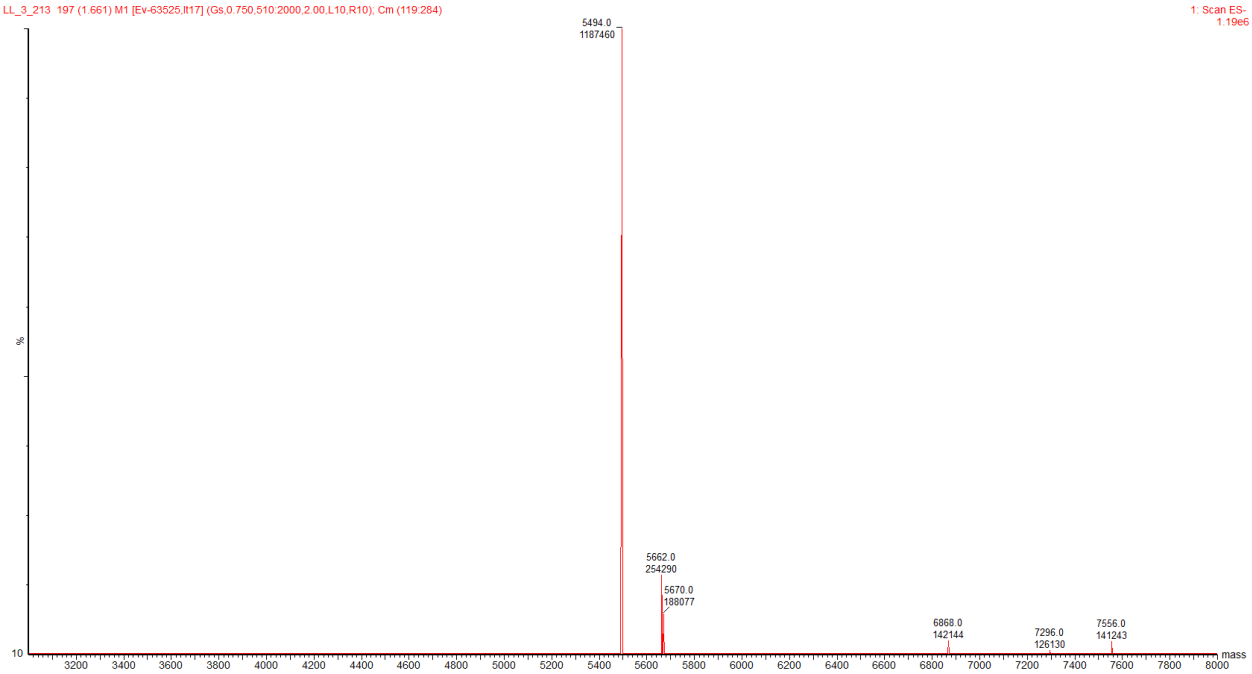




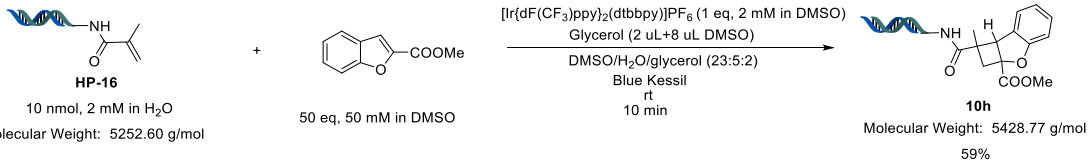
General procedure C: Compound **10g**, *71% yield*

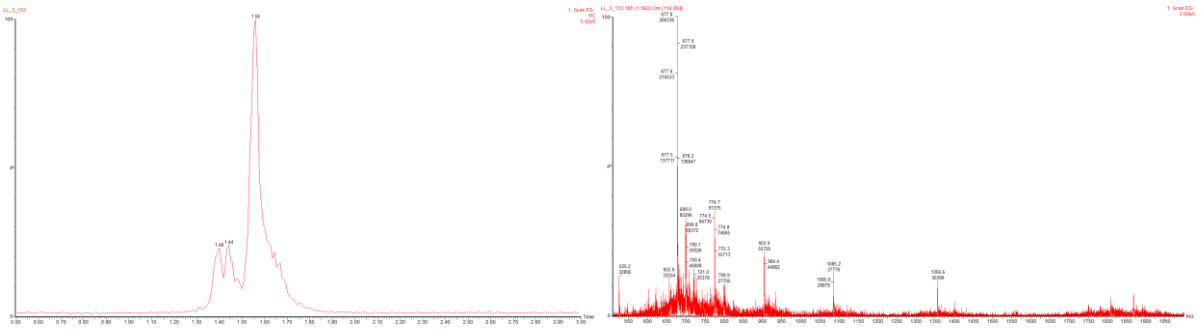




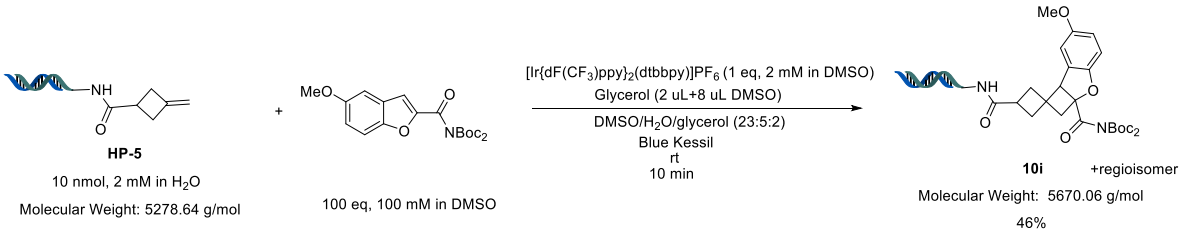


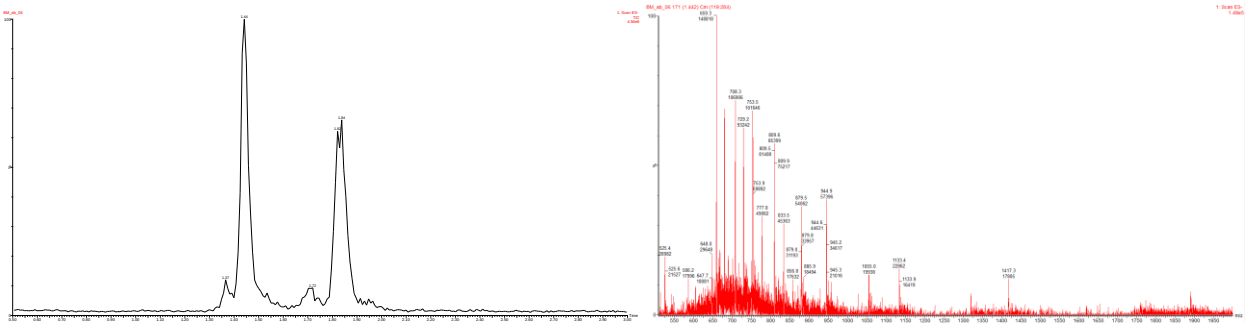
General procedure D: Compound **10h**, *59% yield*



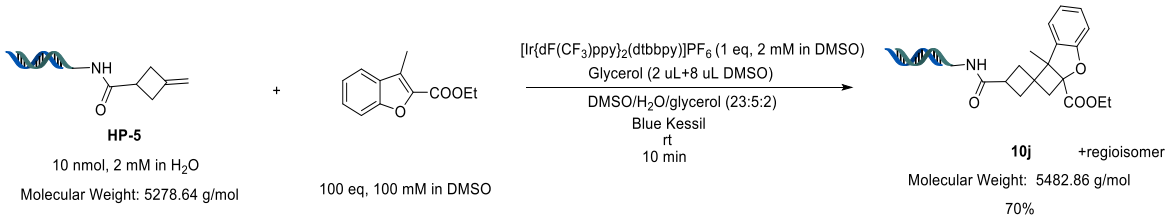


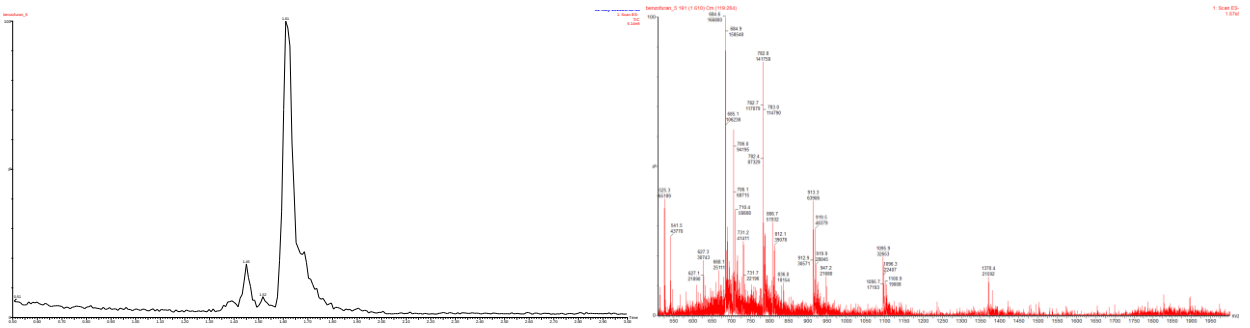
General procedure C: Compound **10i**, *46% yield*



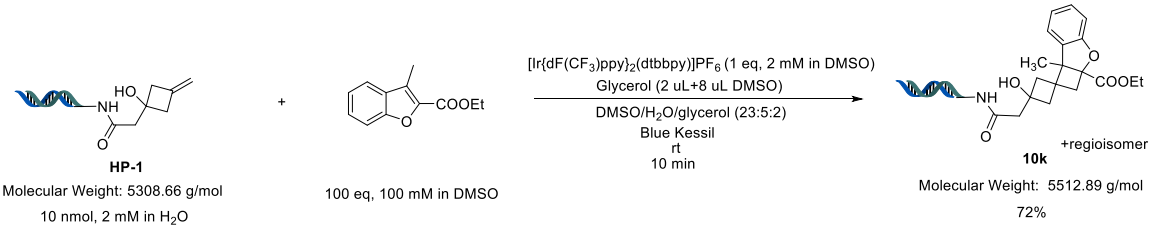


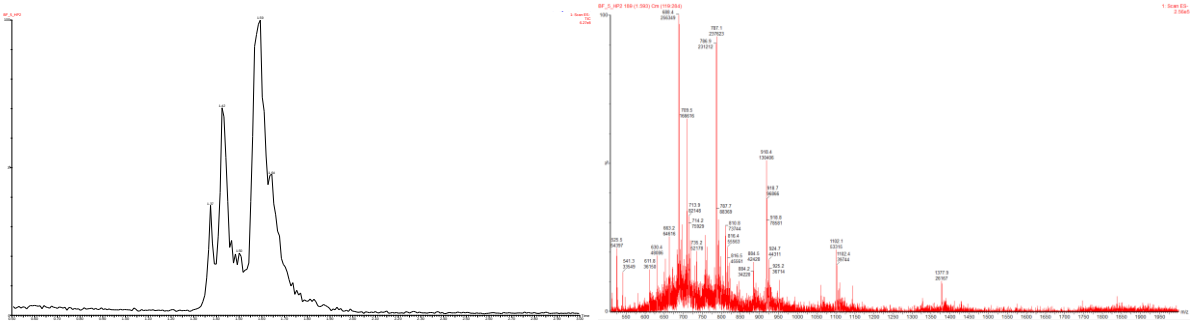
General procedure C: Compound **10j**, *70% yield*

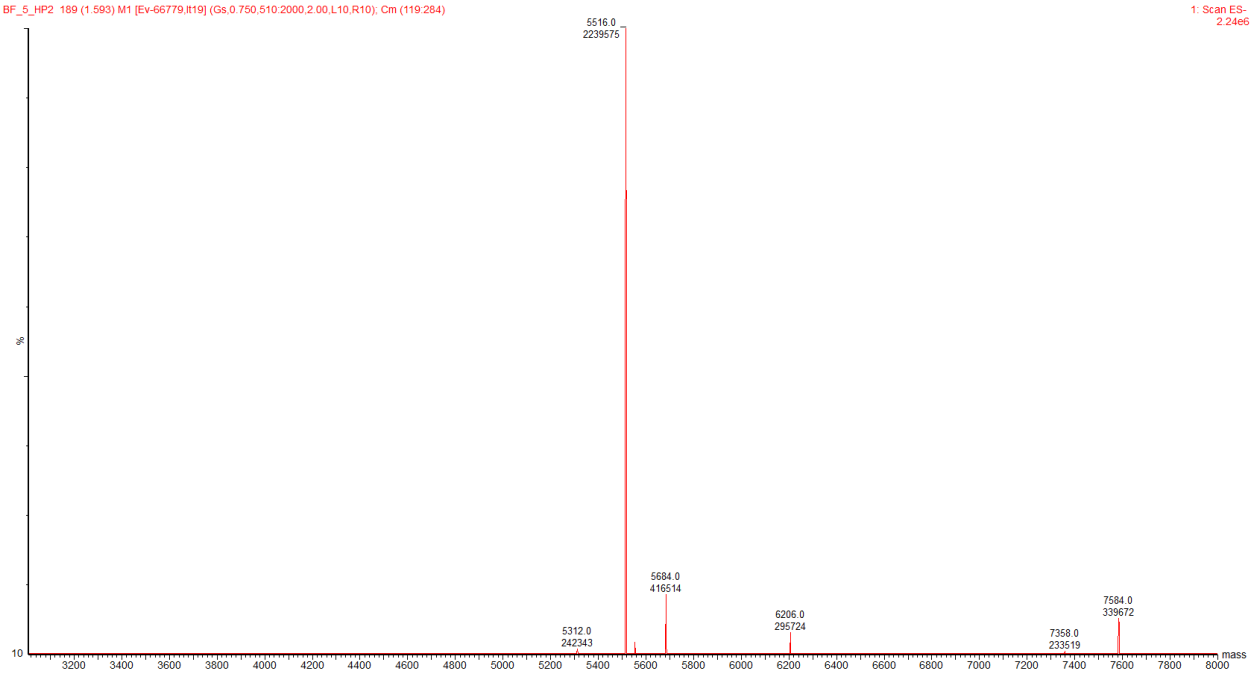




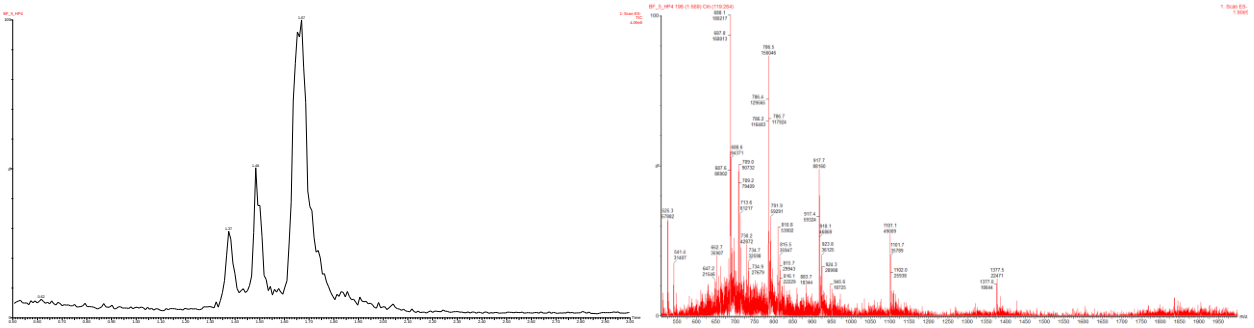
General procedure C: Compound **10k**, *72% yield*

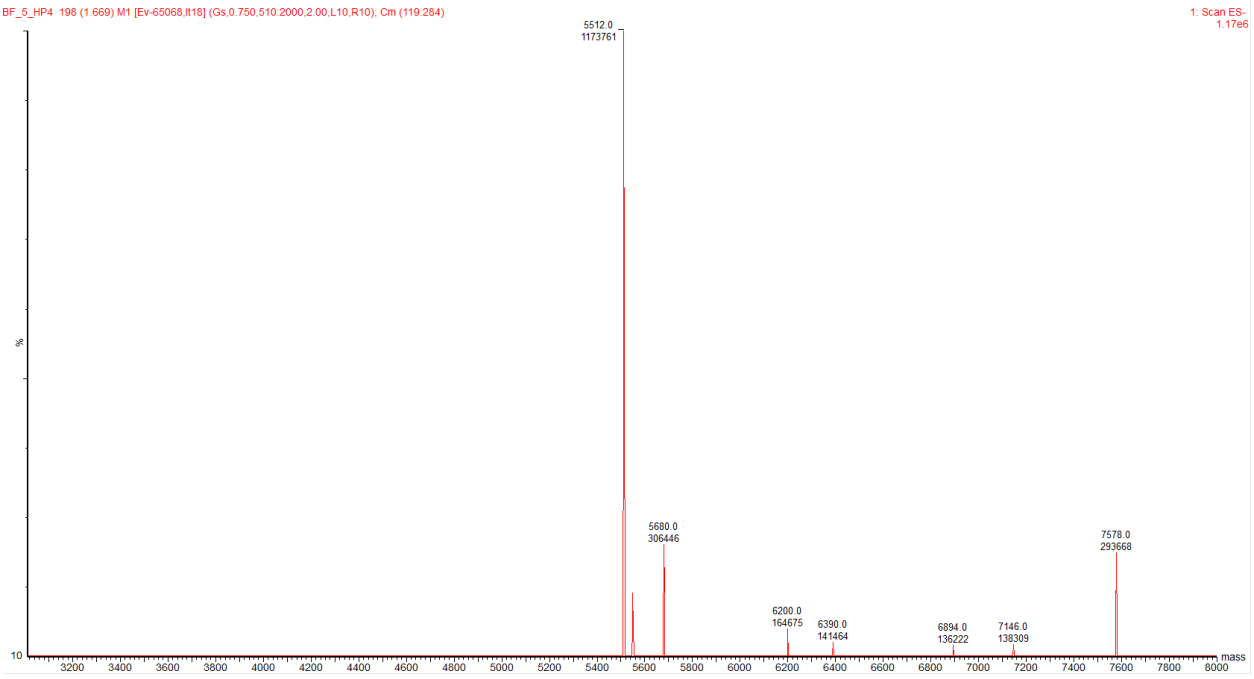




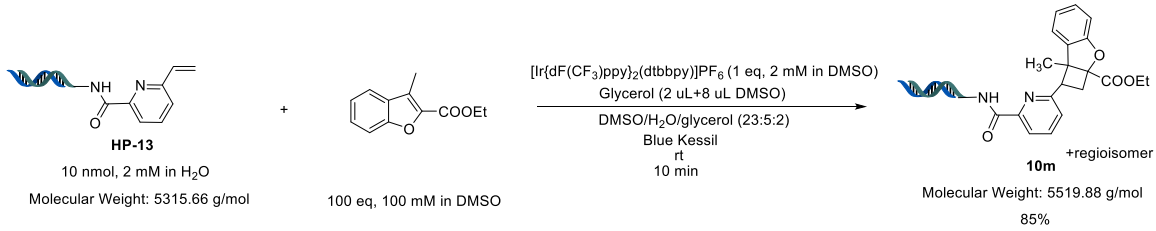


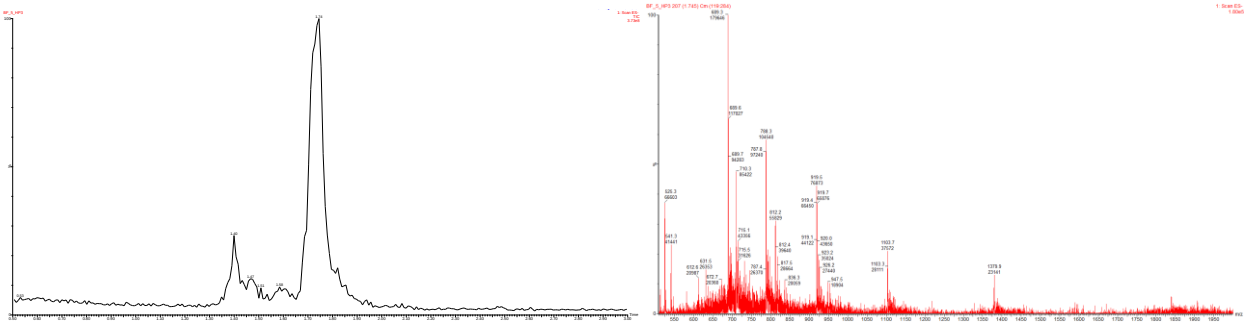
General procedure C: Compound **10l**, *66% yield*



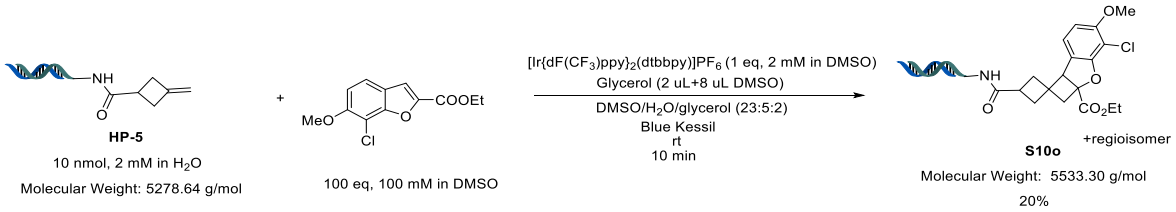


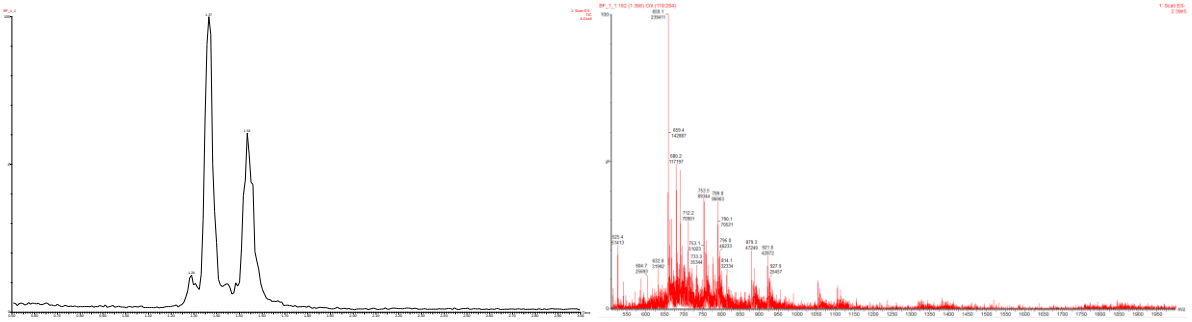
General procedure C: Compound **10m**, *85% yield*

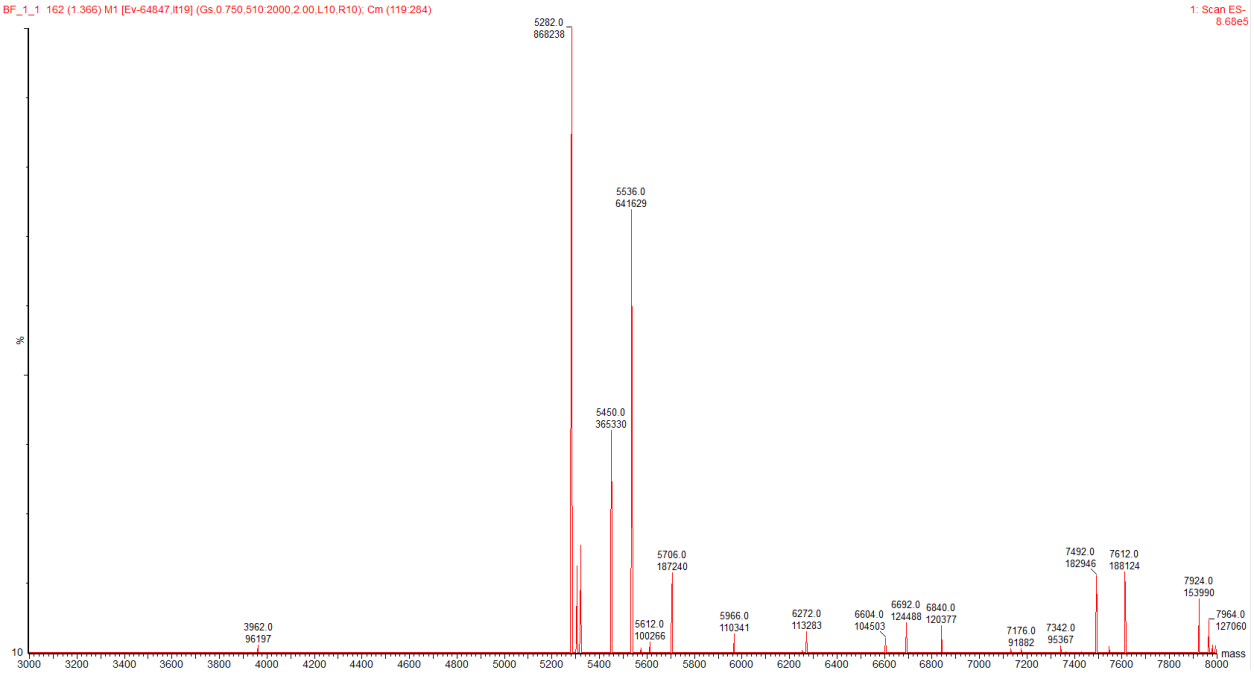




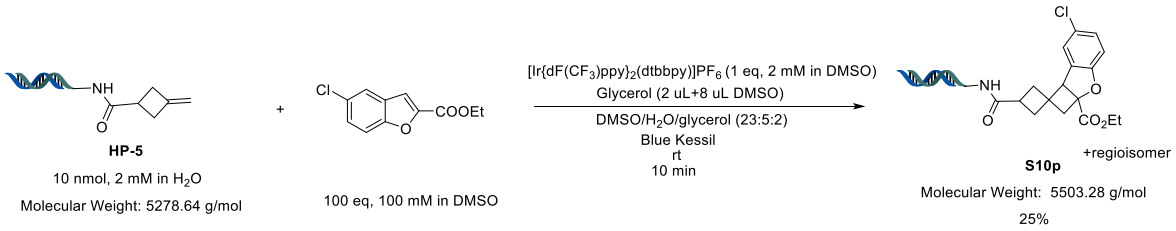
General procedure C: Compound **S10o**, *20% yield*

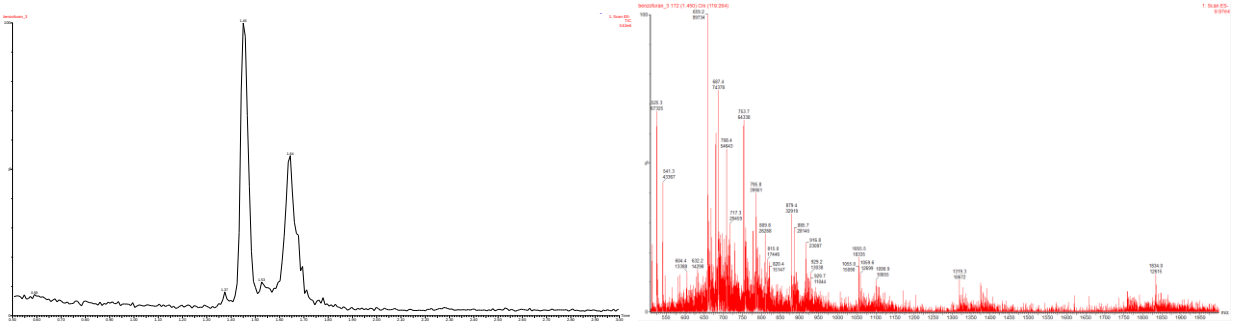


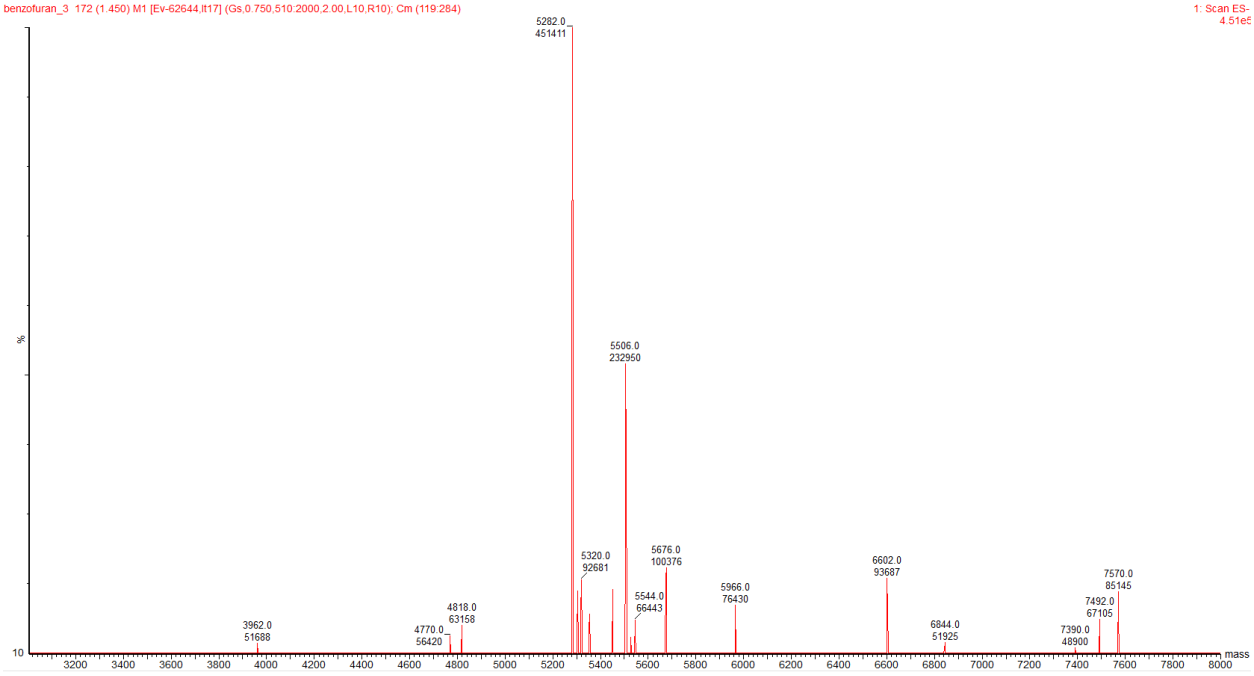




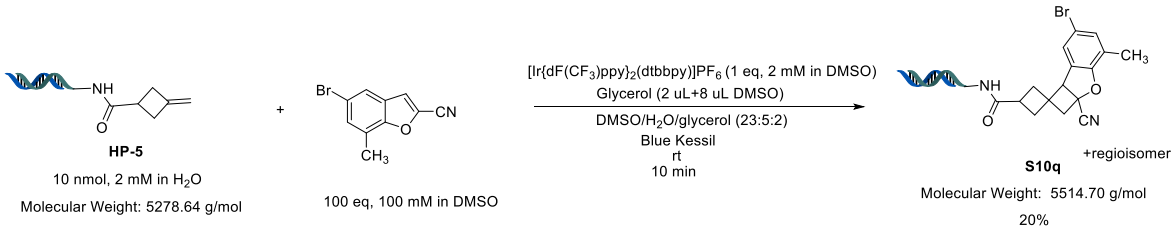
General procedure C: Compound **S10p**, *25% yield*

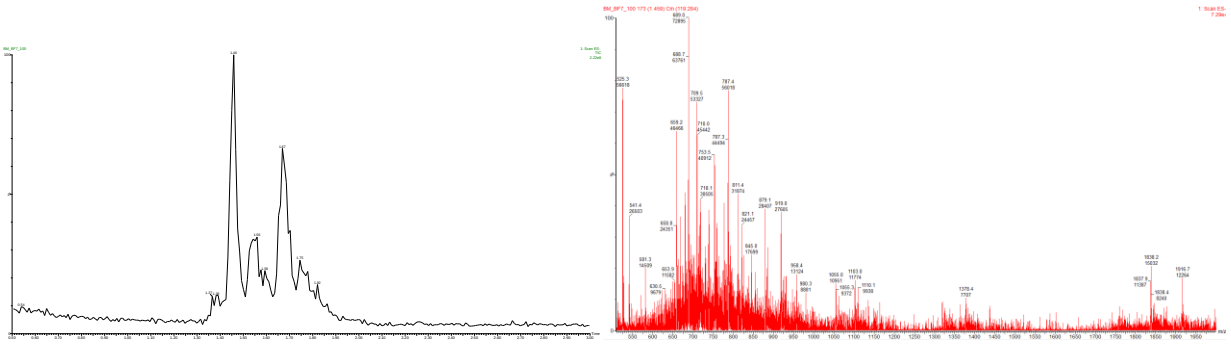


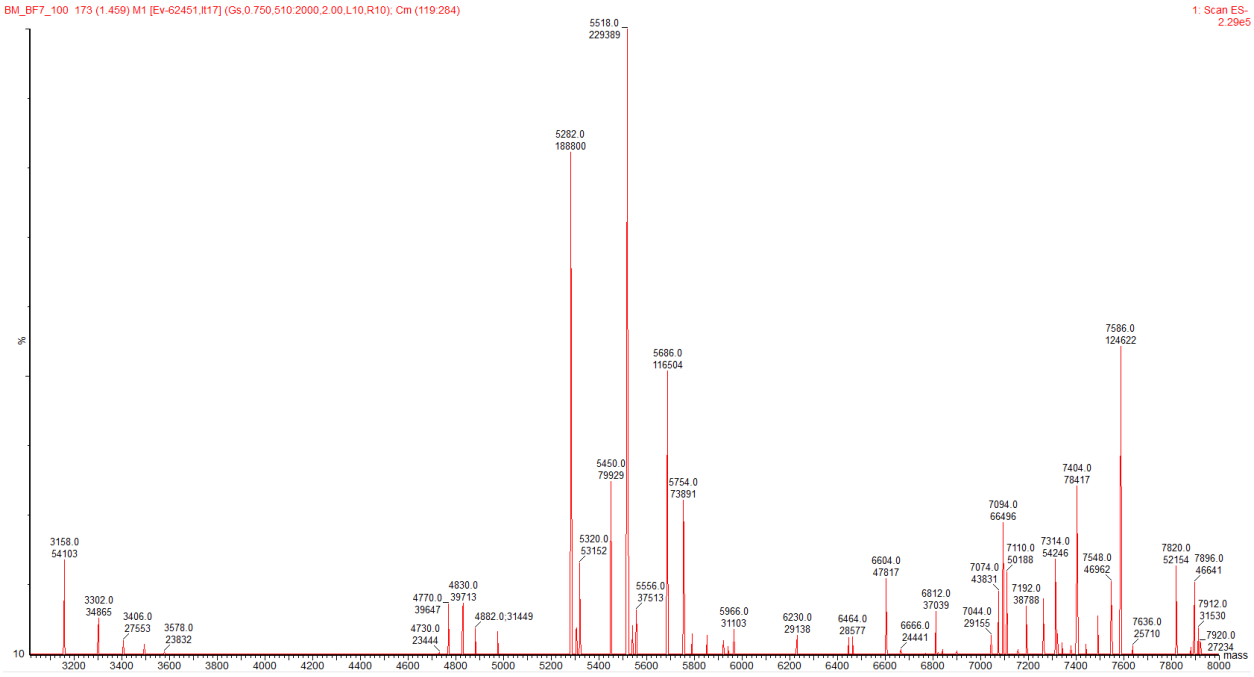




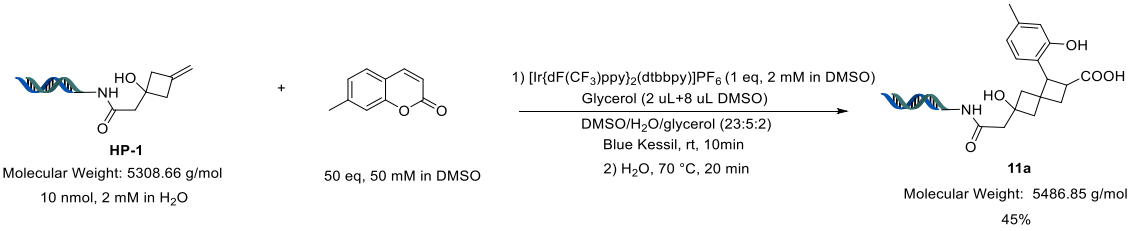
General procedure C: Compound **S10q**, *20% yield*

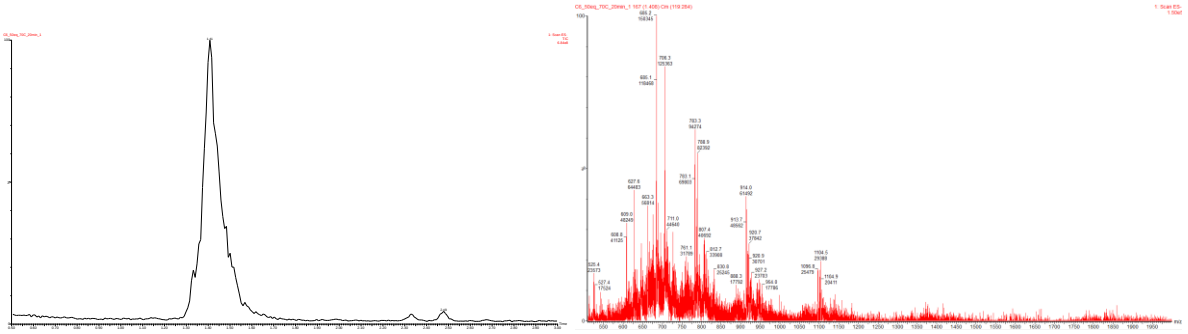


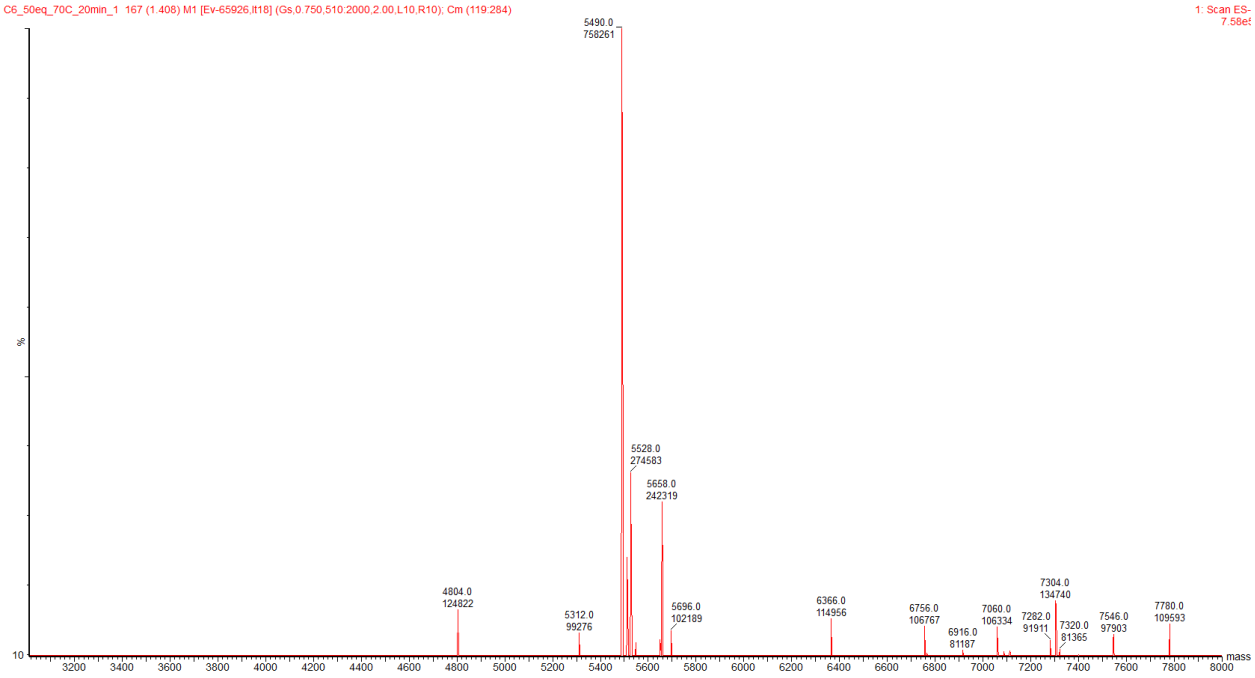




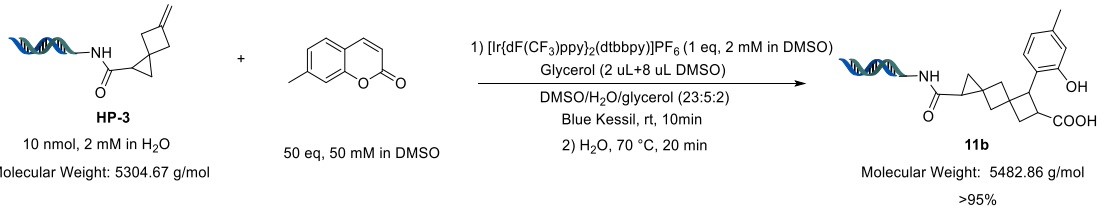
General procedure E: Compound **11a**, *45% yield*

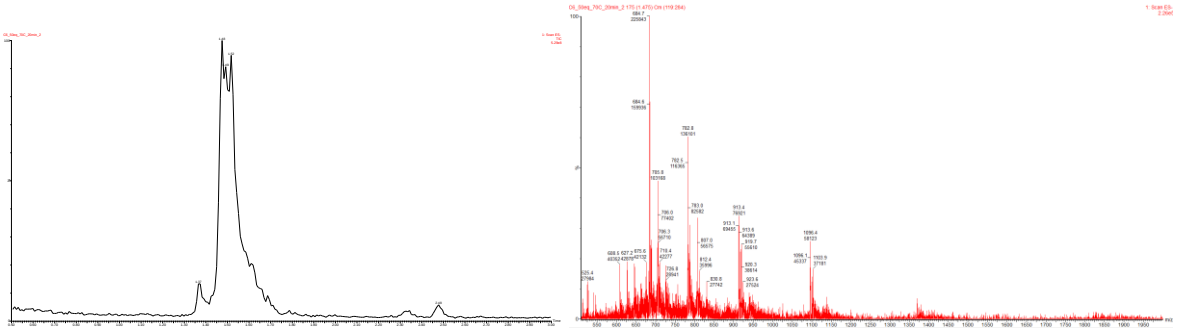




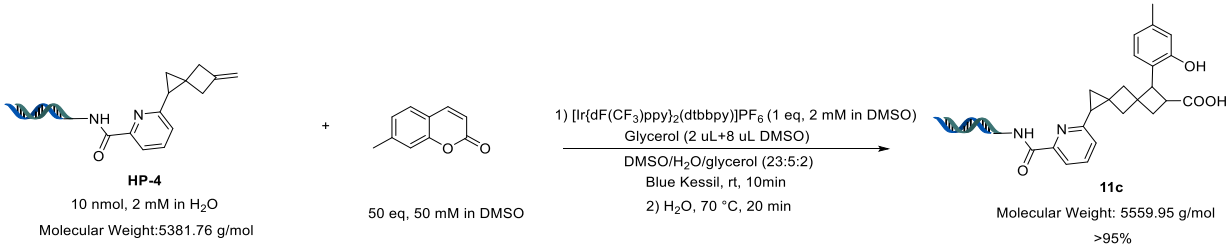


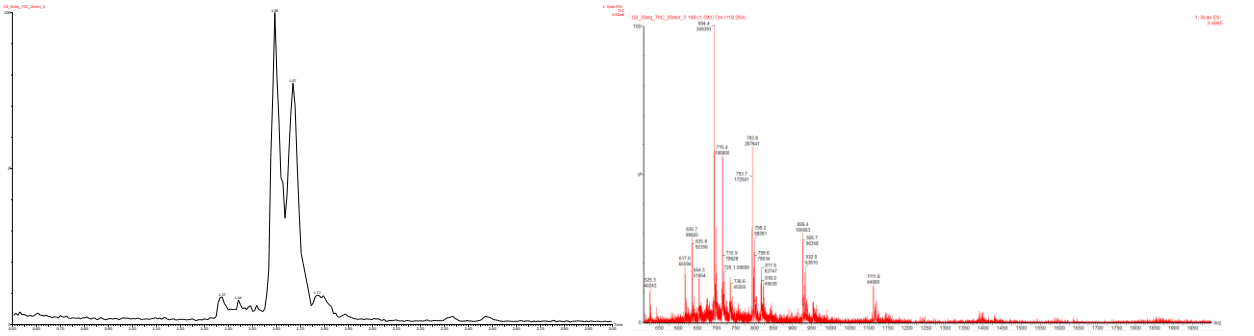
General procedure E: Compound **11b**, *>95% yield*



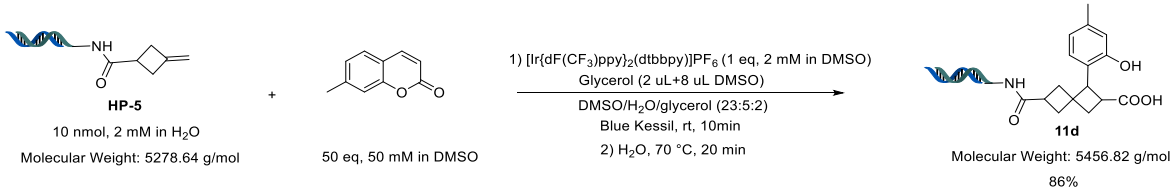


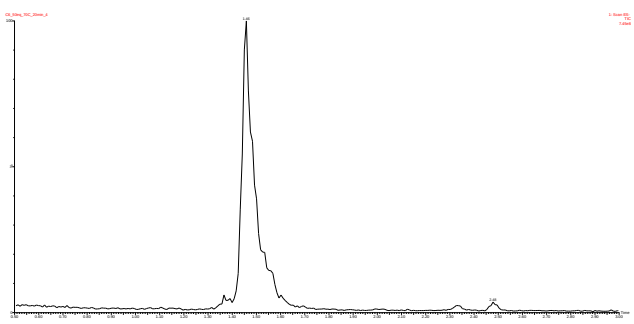
General procedure E: Compound **11c**, *>95% yield*

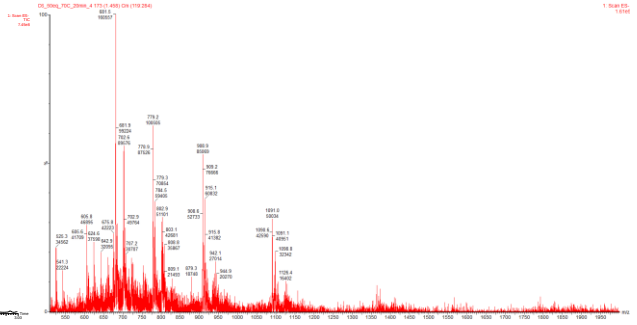


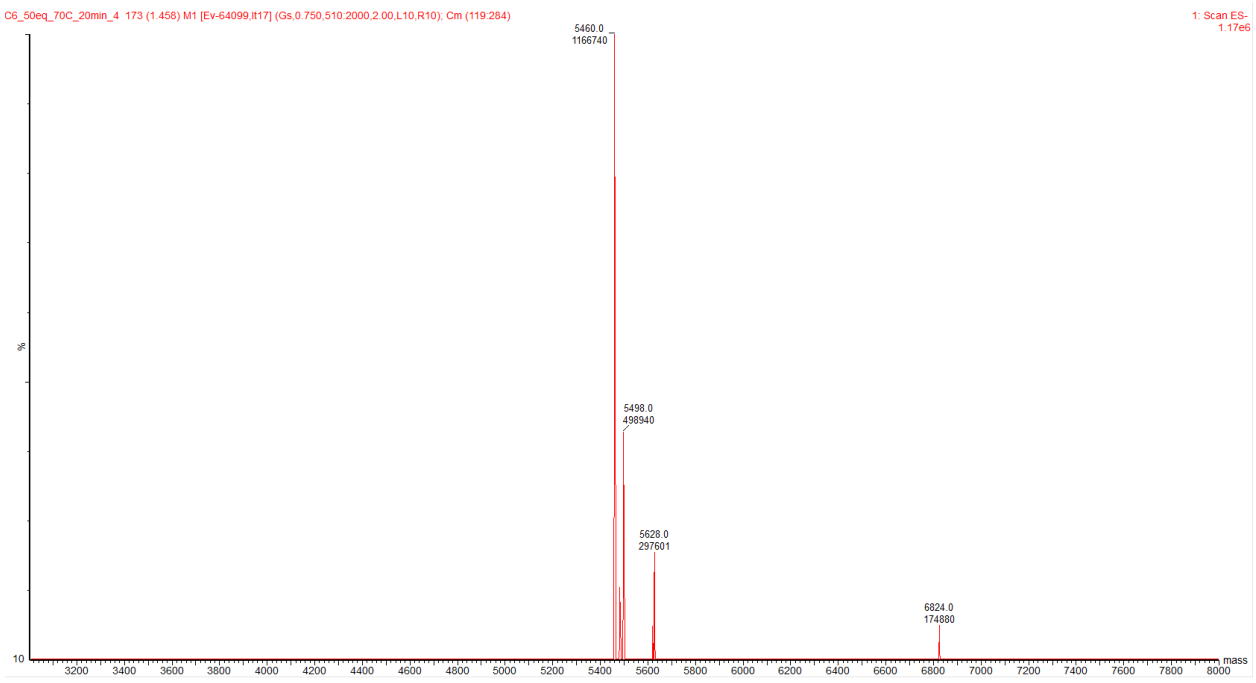


General procedure E: Compound **11d**, *86% yield*

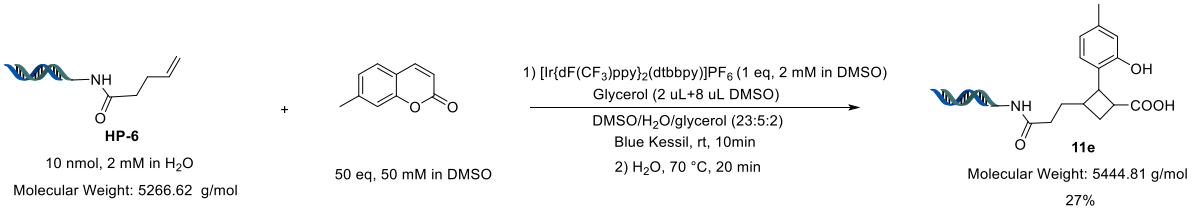


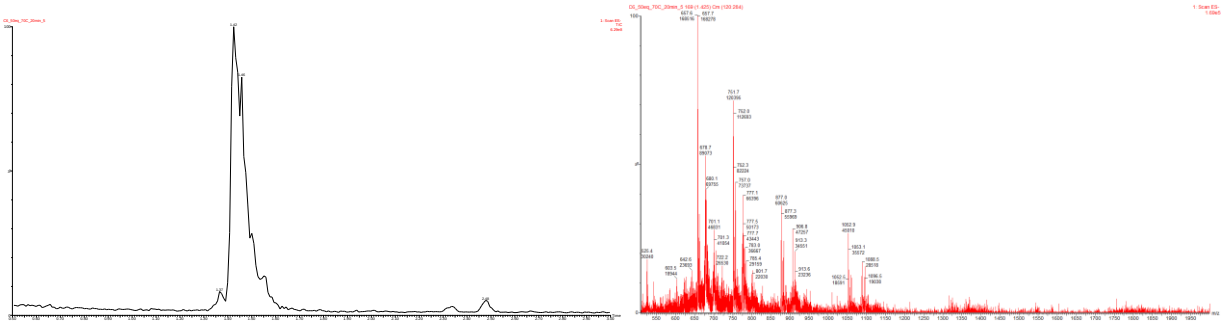


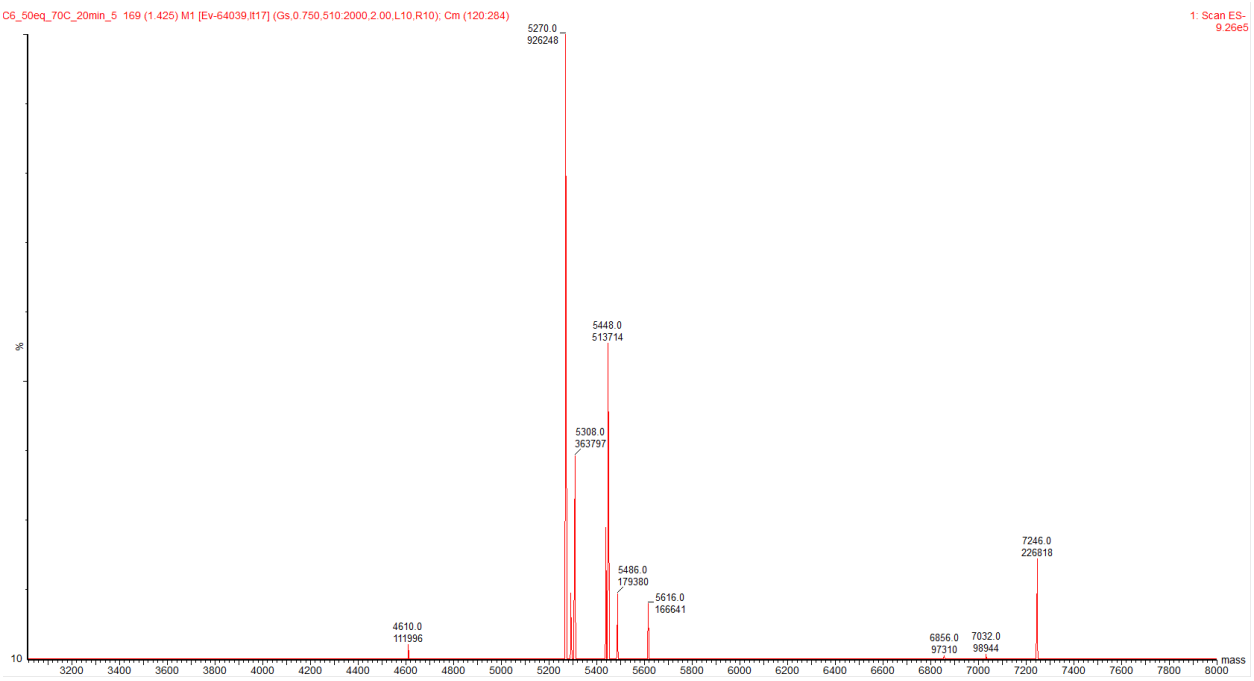




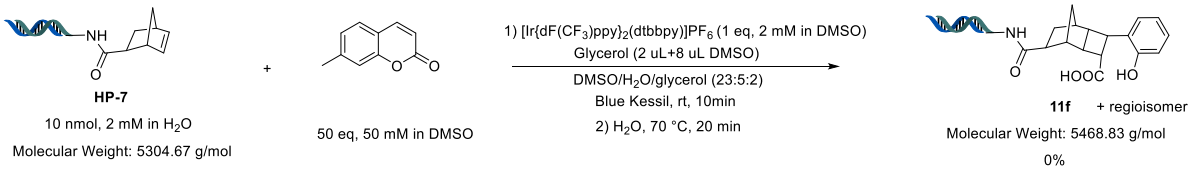
General procedure E: Compound **11e**, *27% yield*

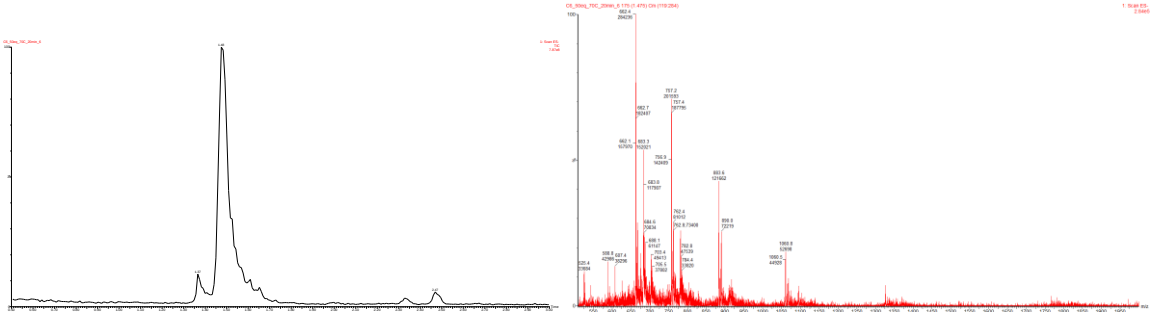




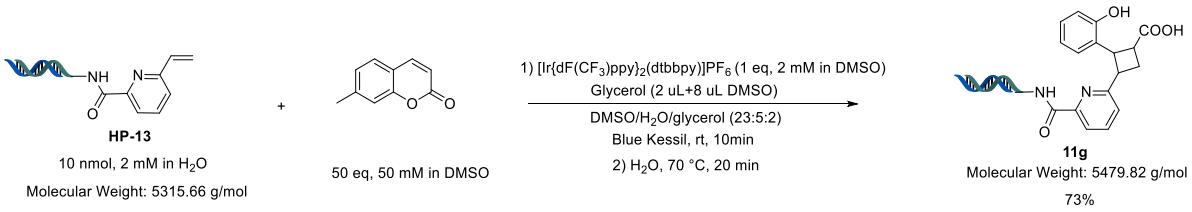


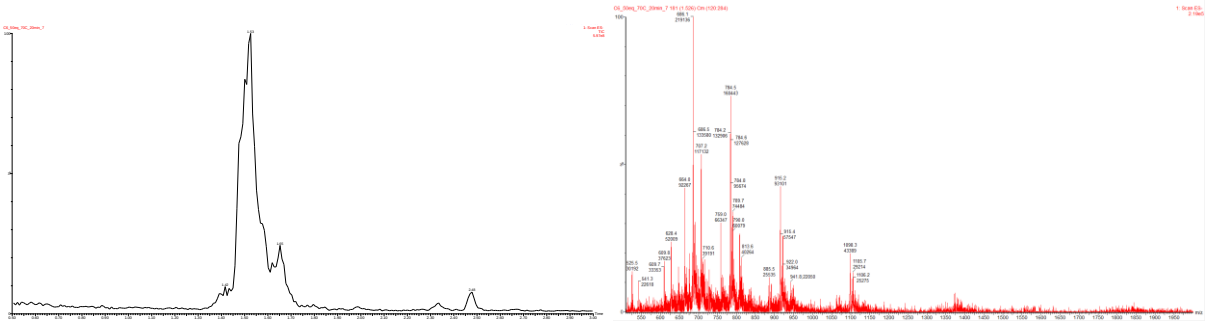
General procedure E: Compound **11f**, *0% yield*



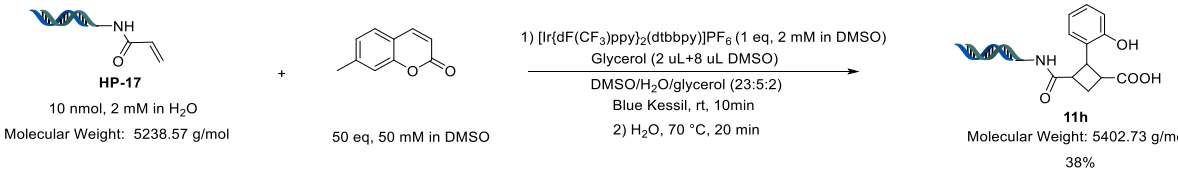


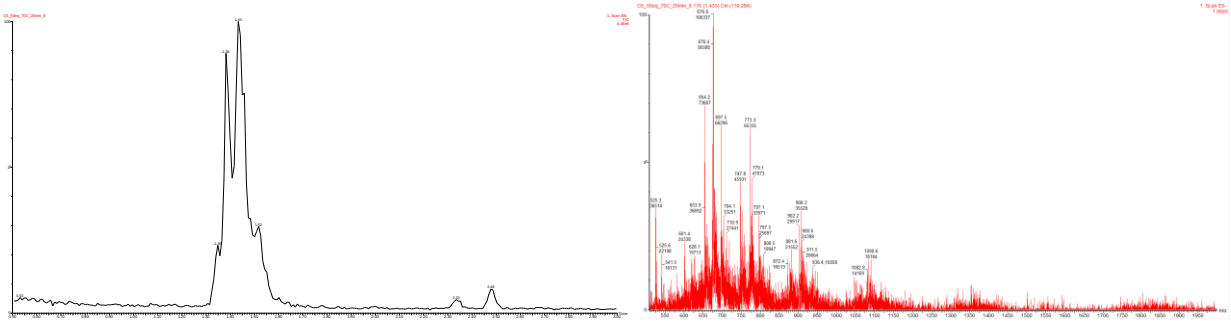
General procedure E: Compound **11g**, *73% yield*

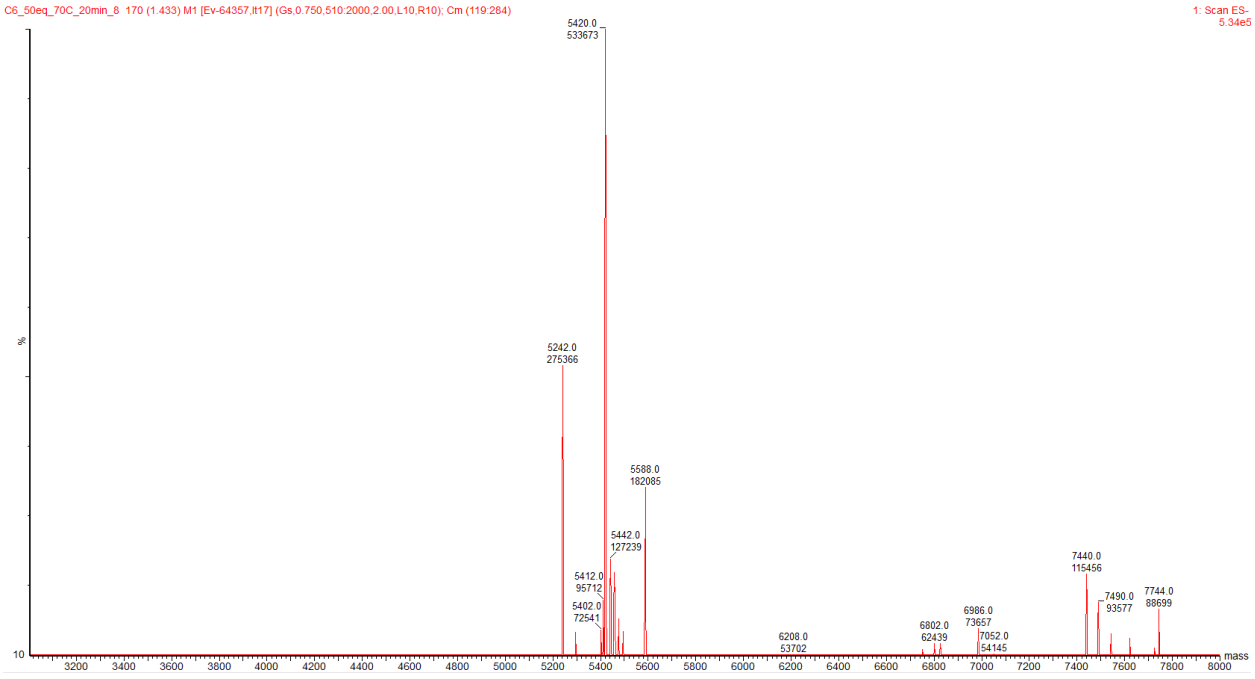




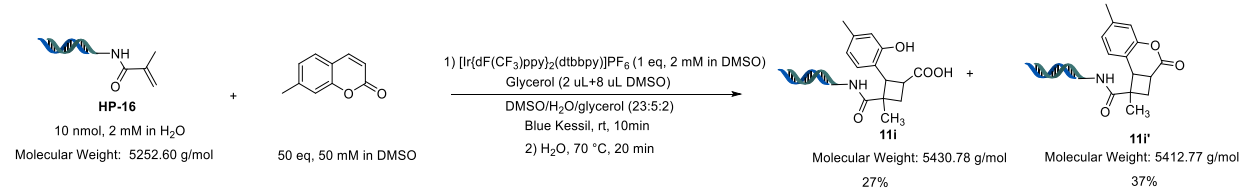
General procedure E: Compound **11h**, *38% yield*

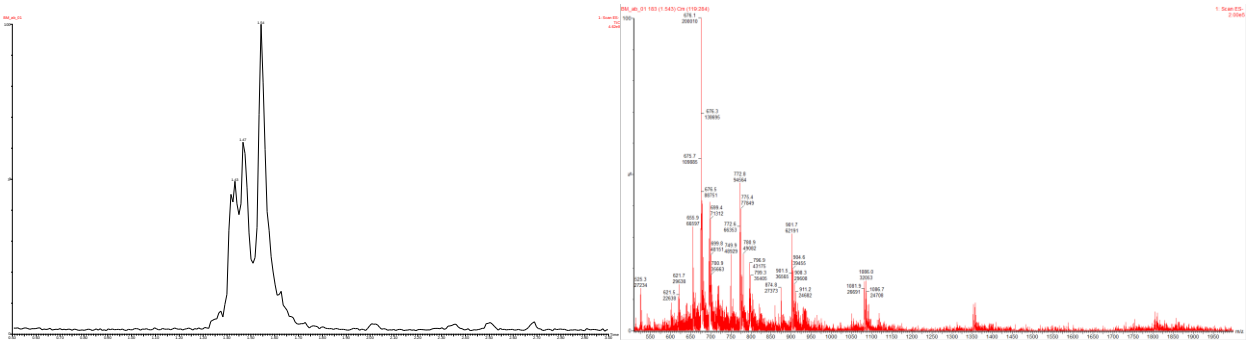


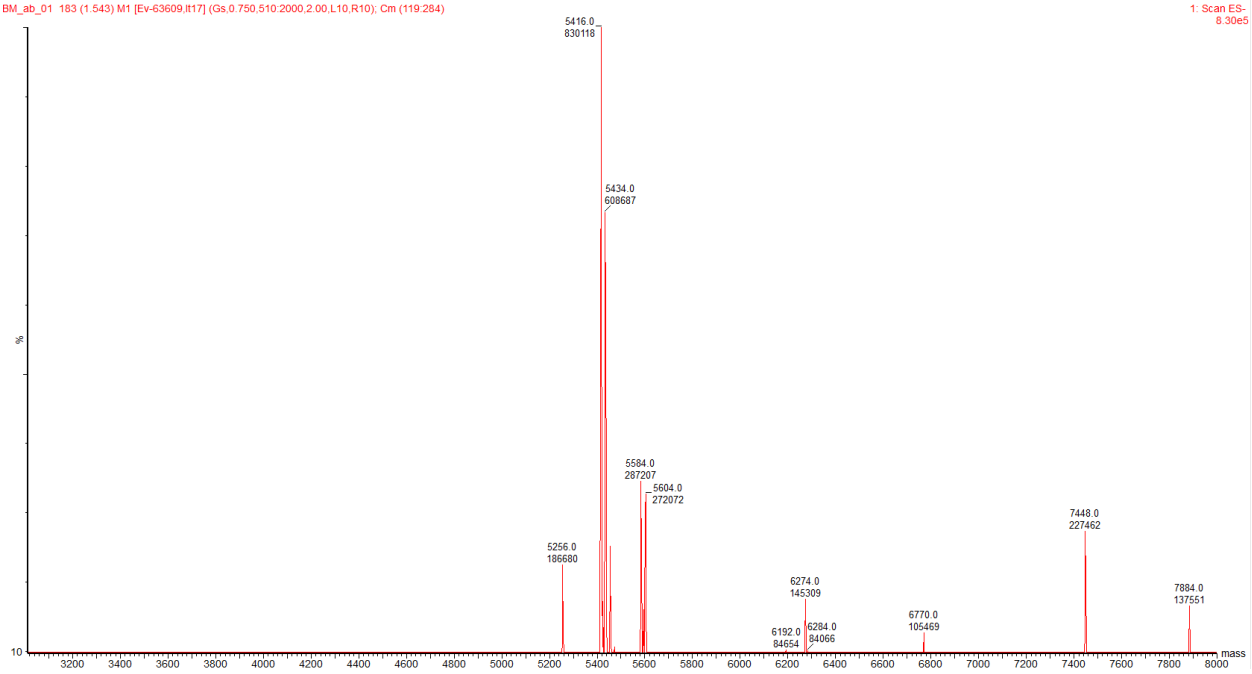




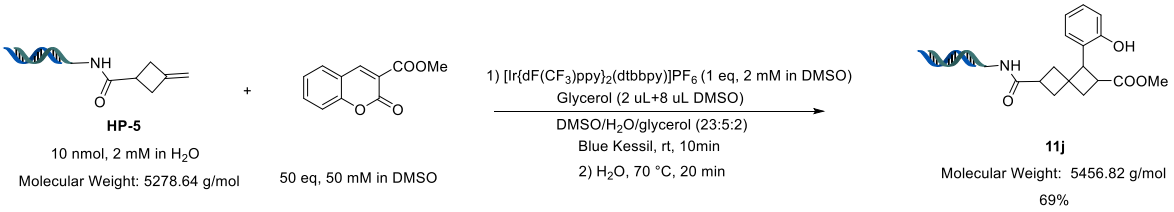
General procedure E: Compound **11i**, 27*% yield*

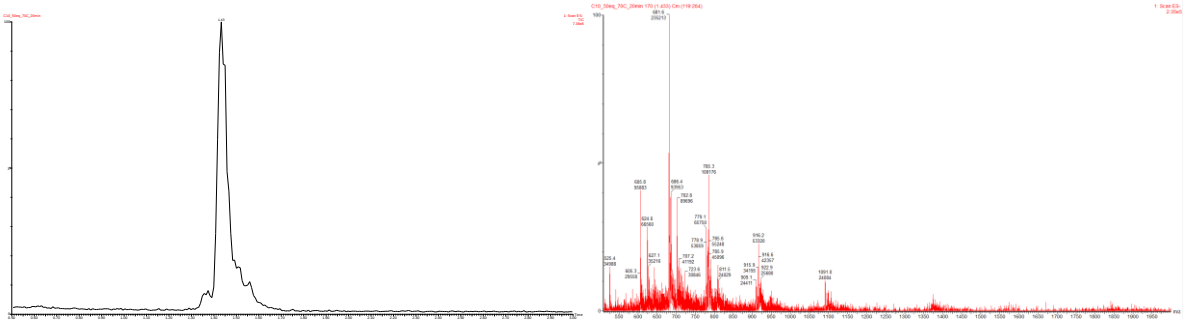


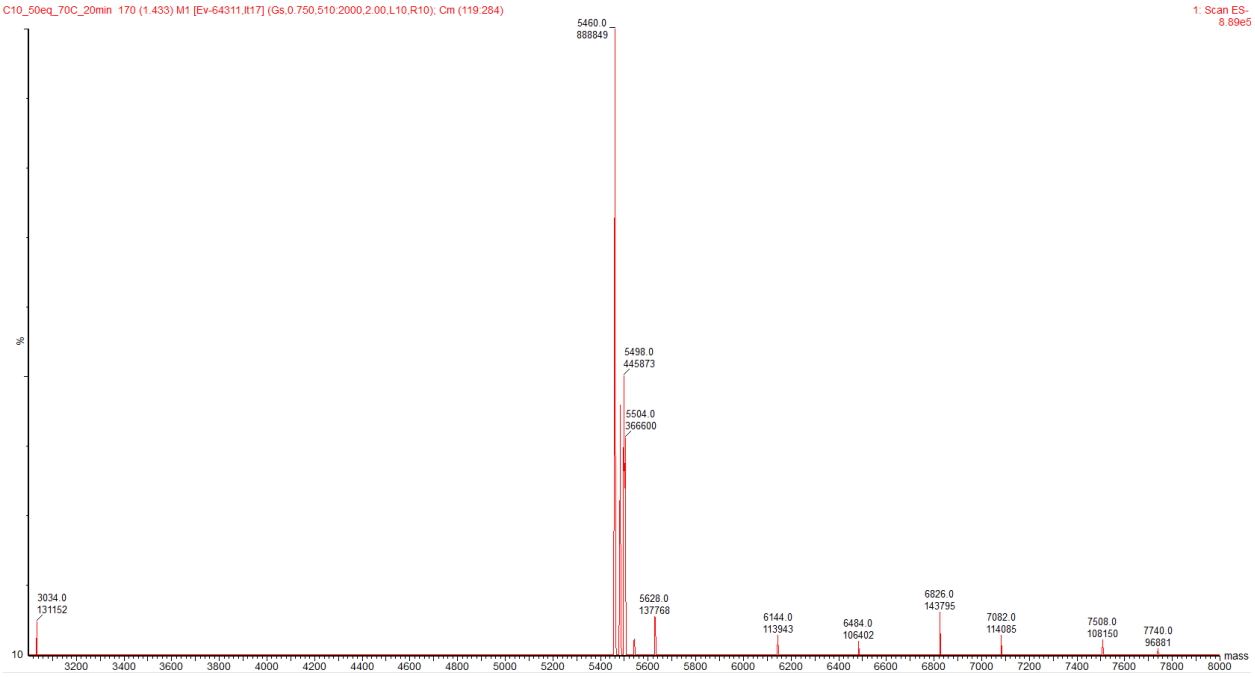




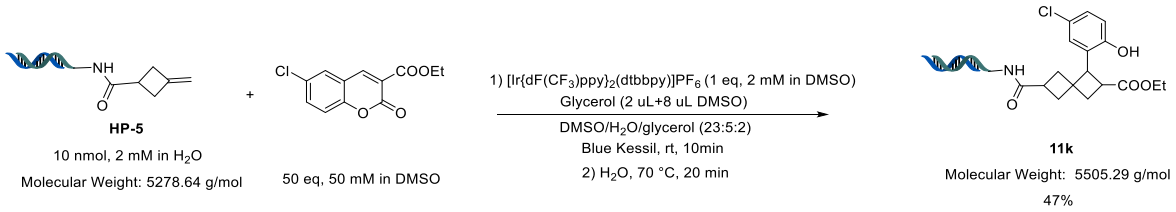
General procedure E: Compound **11j**, *69% yield*

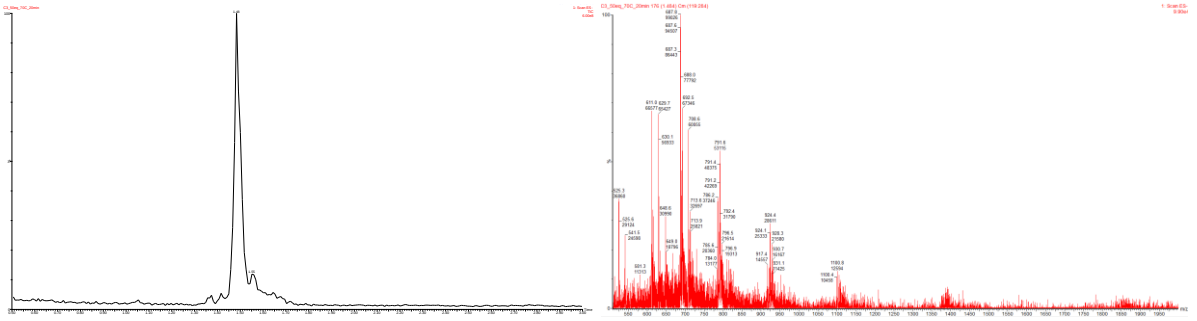


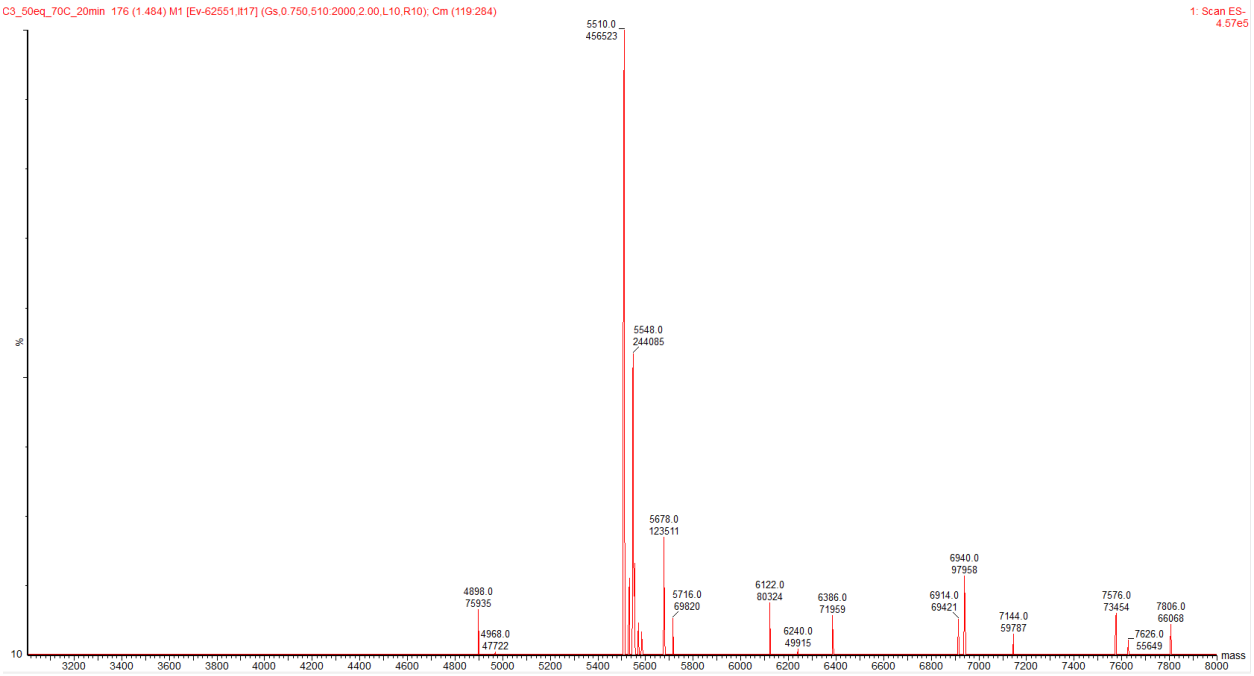




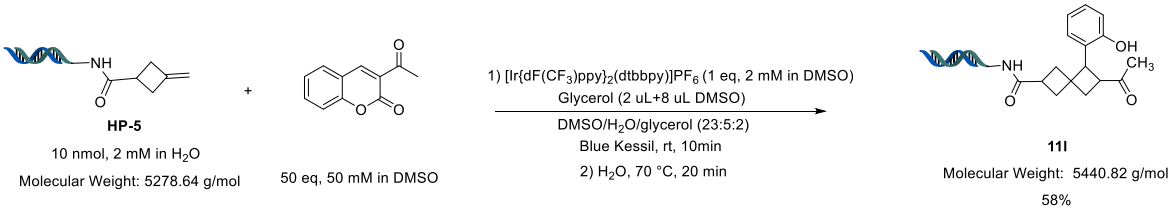
General procedure E: Compound **11k**, *47% yield*

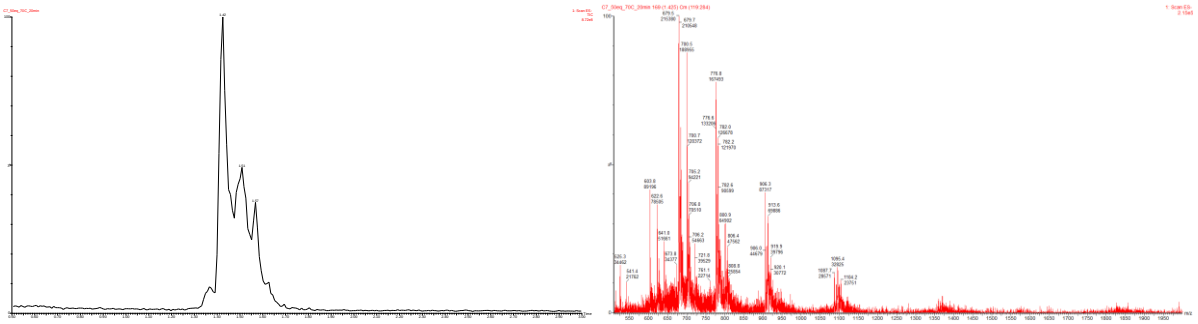


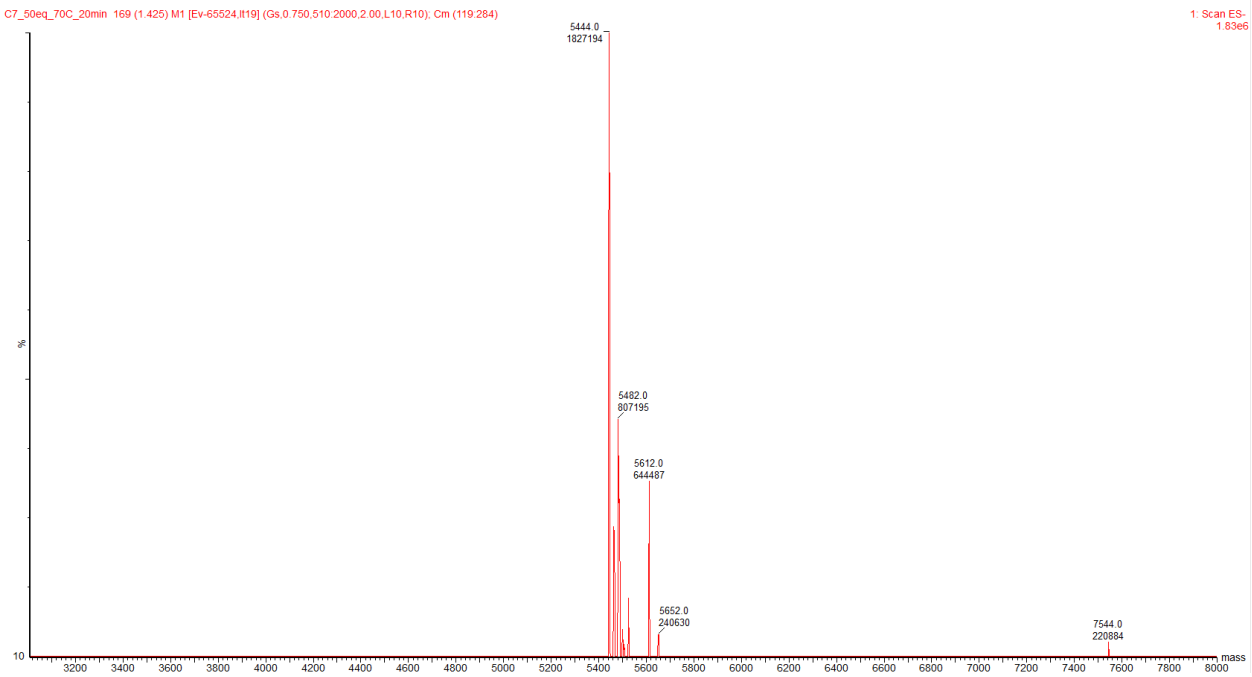




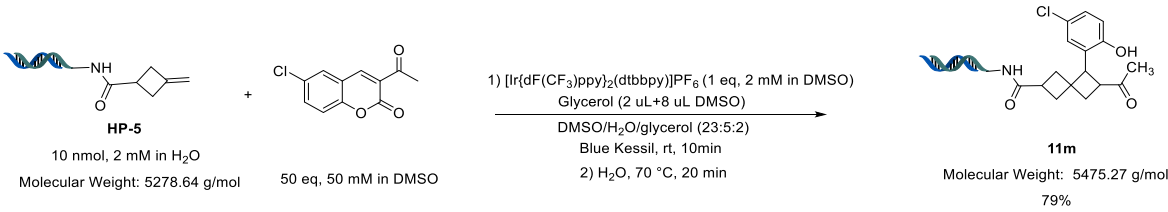
General procedure E: Compound **11l**, *58% yield*

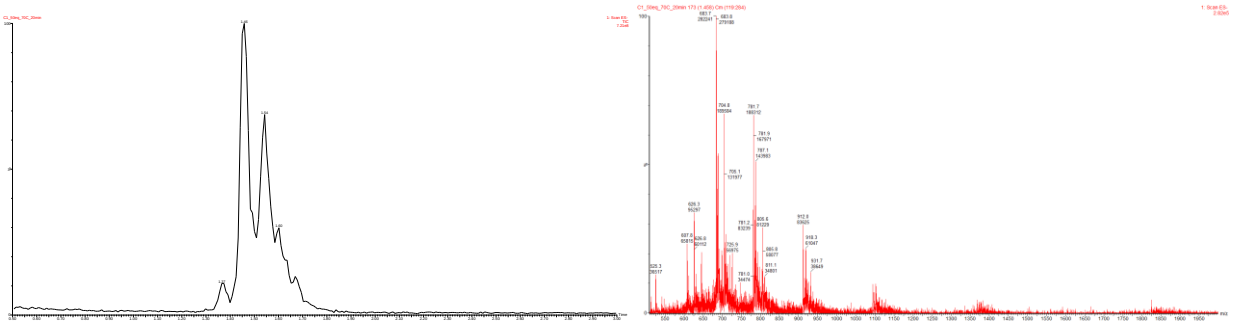


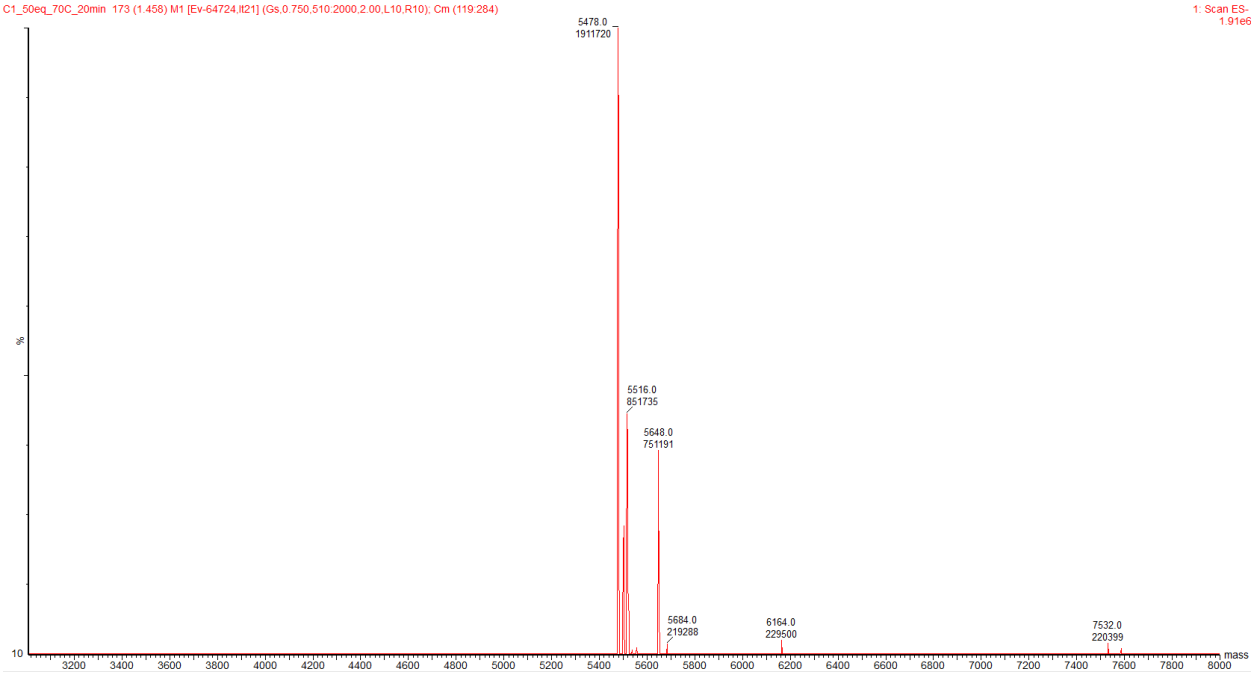




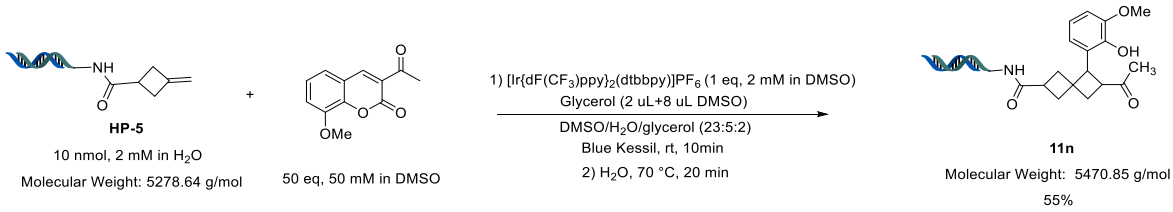
General procedure E: Compound **11m**, *79% yield*

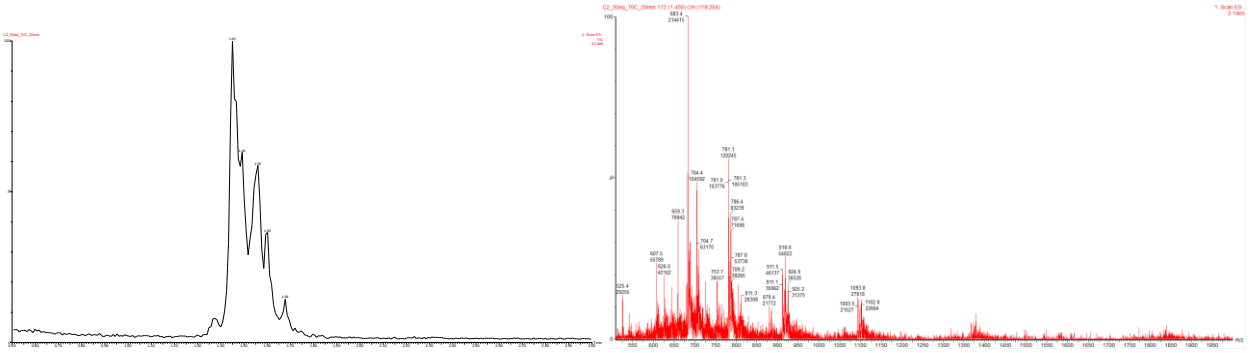


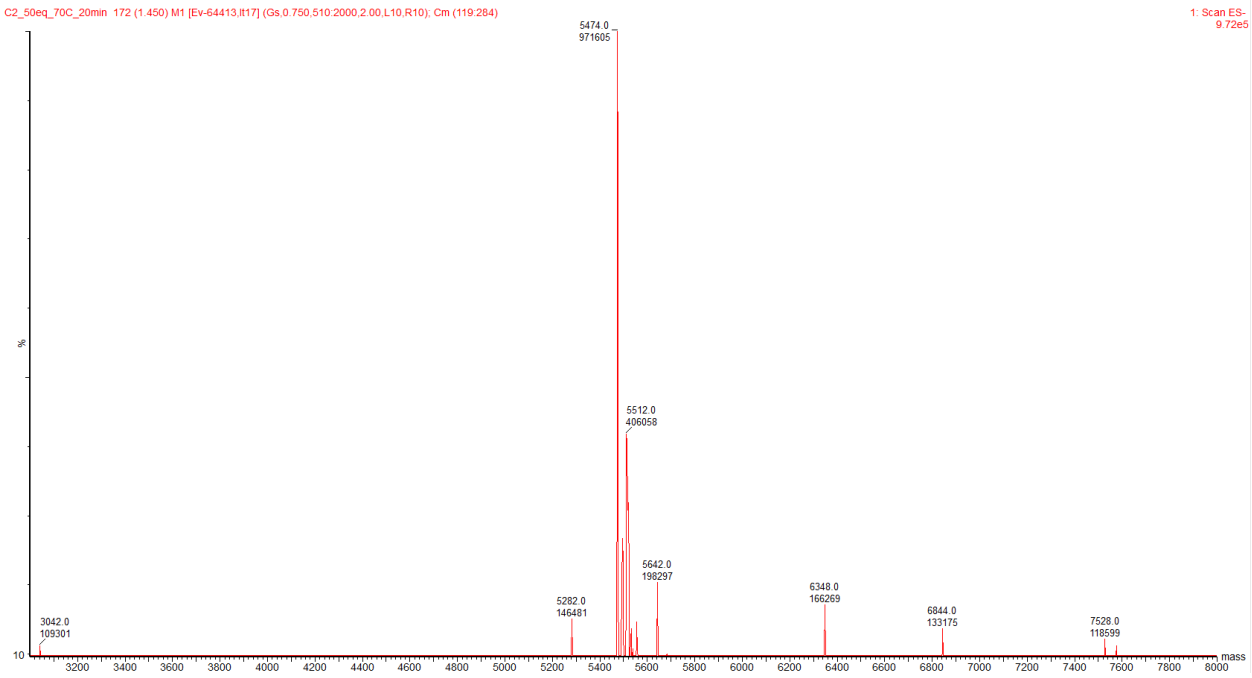




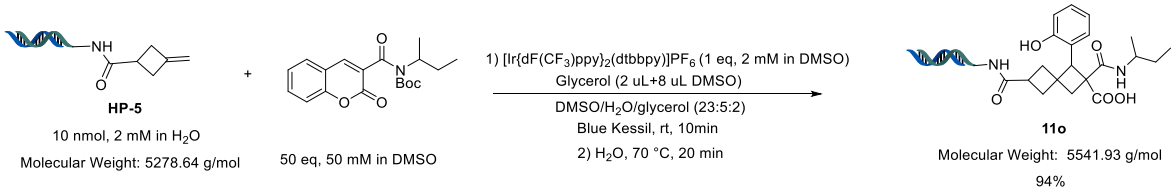
General procedure E: Compound **11n**, *55% yield*

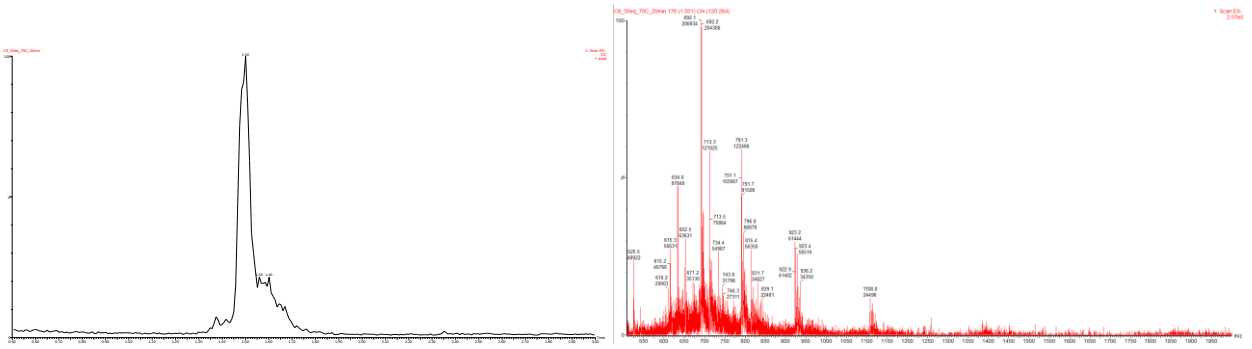


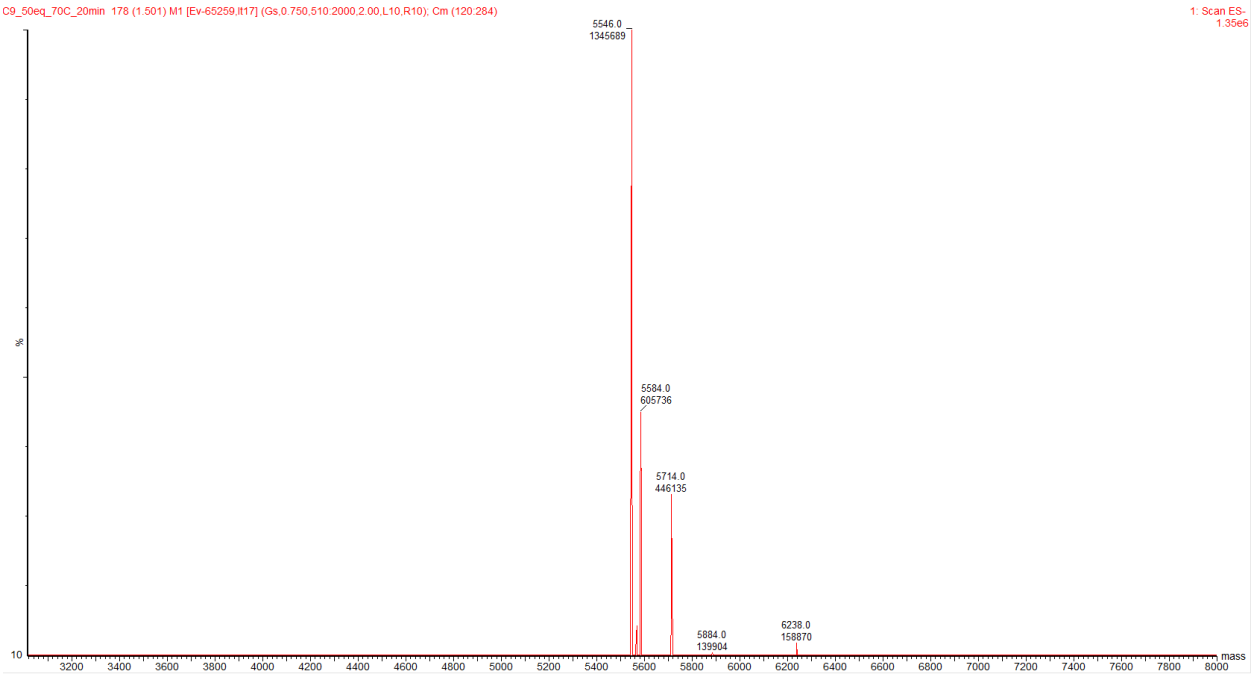




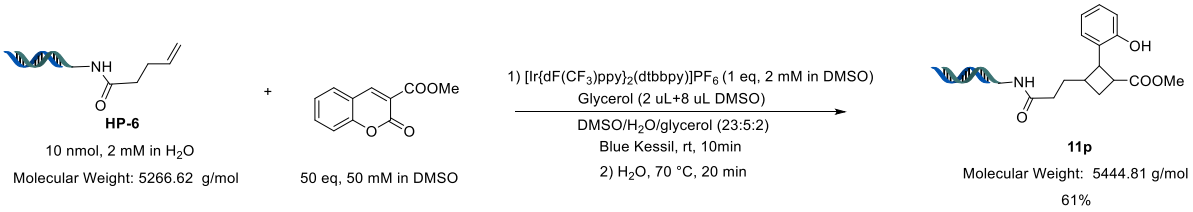
General procedure E: Compound **11o**, *94% yield*

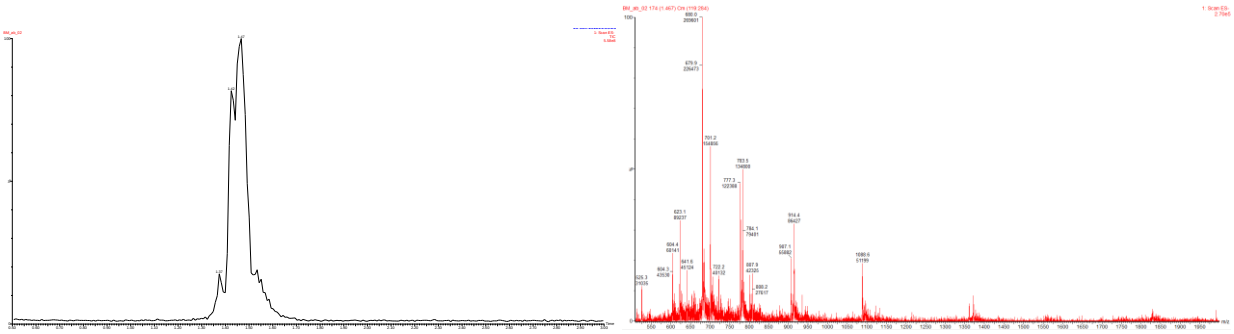


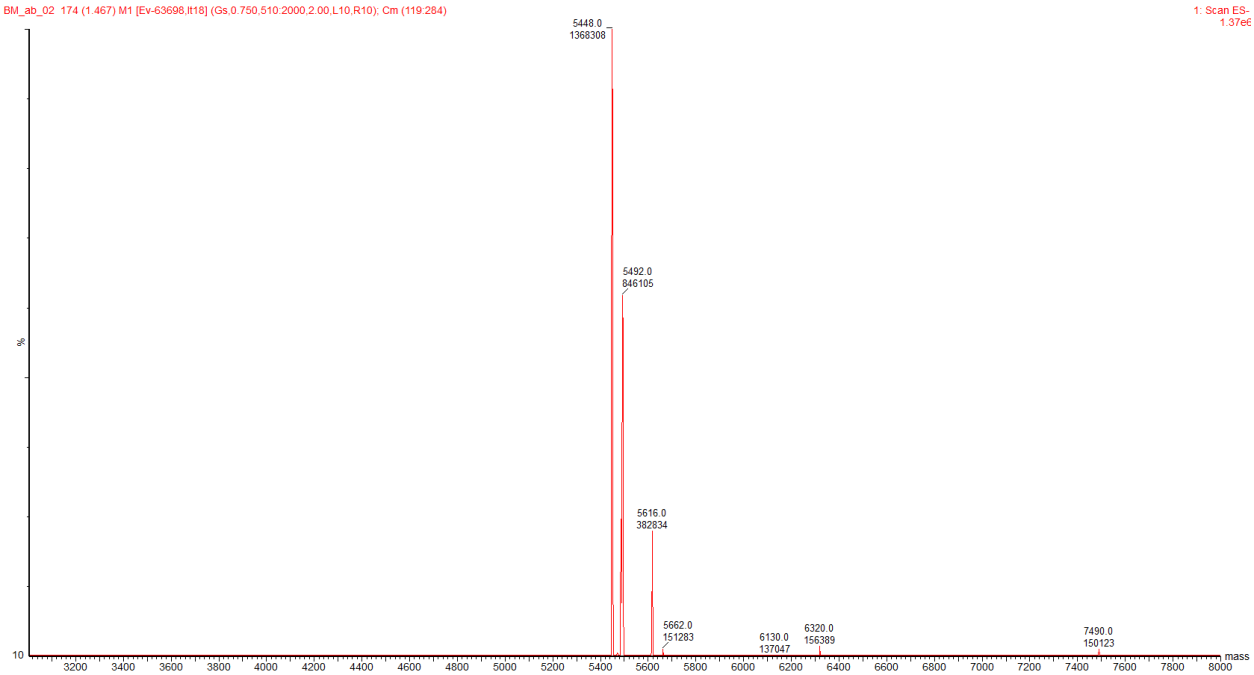




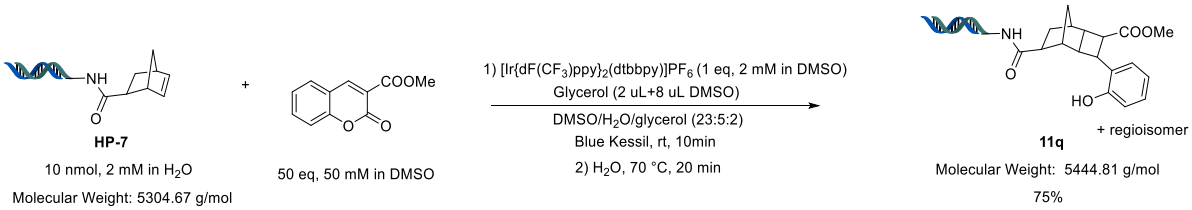
General procedure E: Compound **11p**, *61% yield*

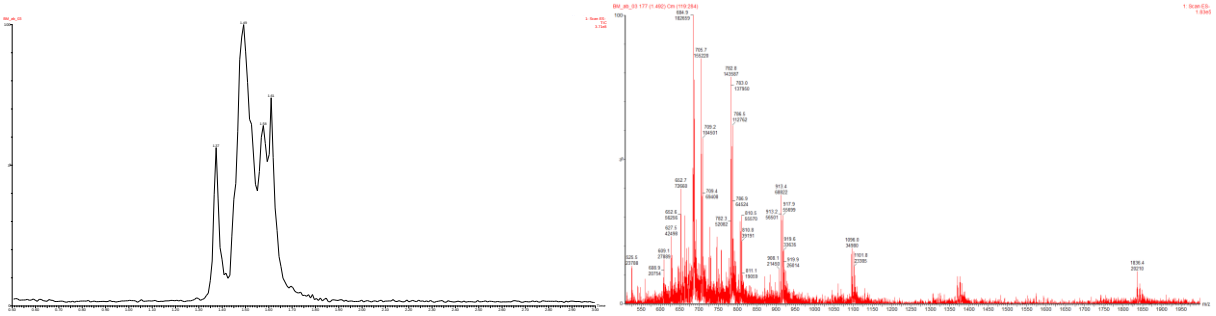






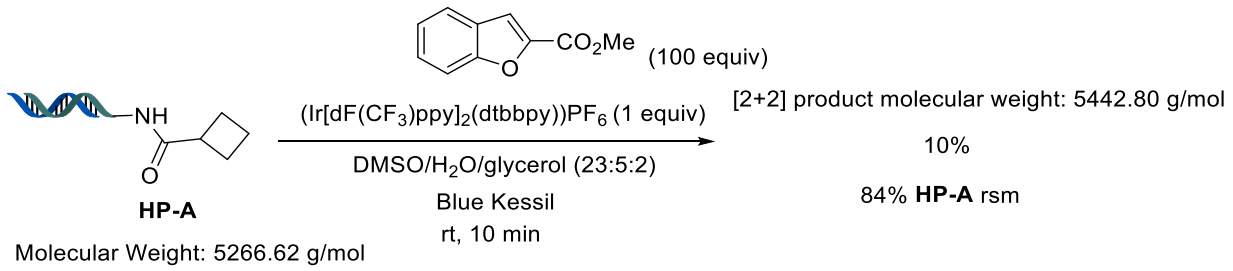
General procedure E: Compound **11q**, *75% yield*



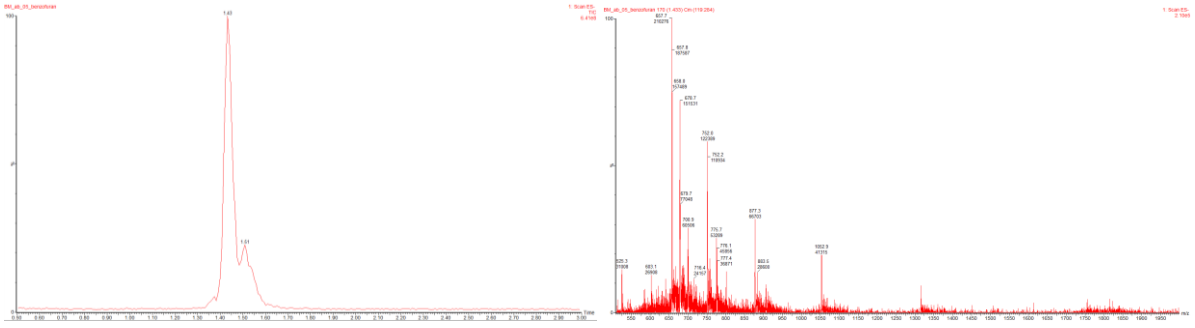


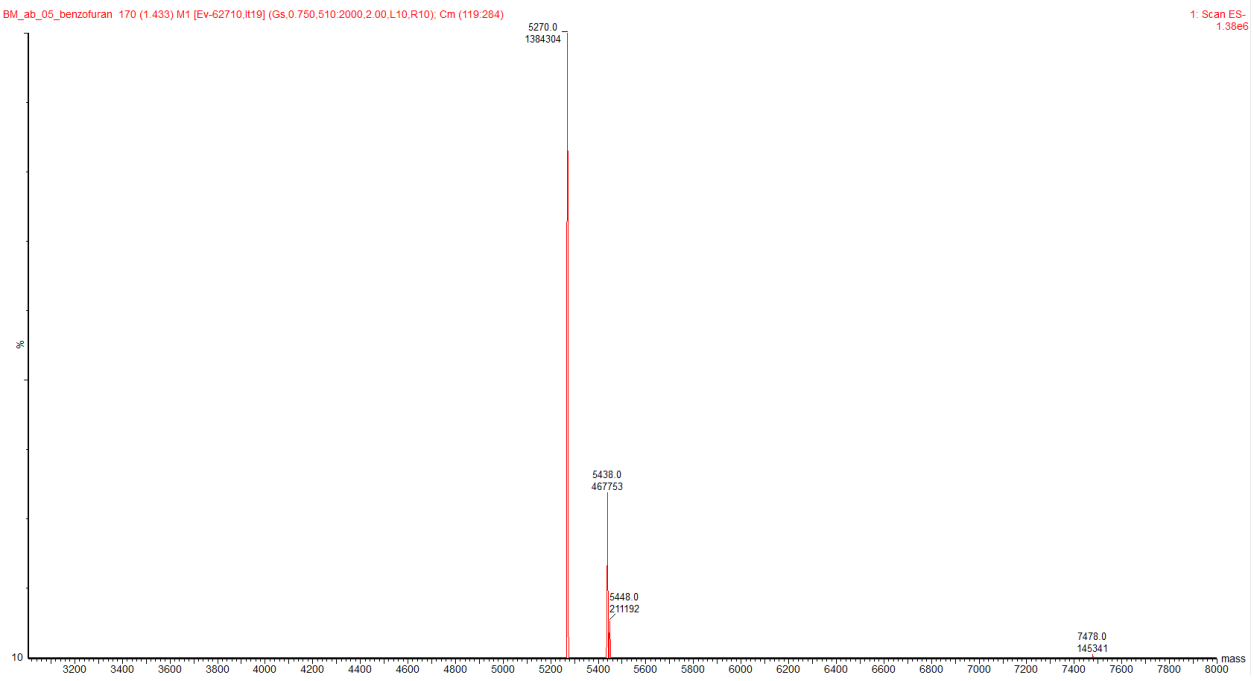
HP-A control experiment with indole see page 117

HP-A control experiment with benzofuran (**General Procedure C**)

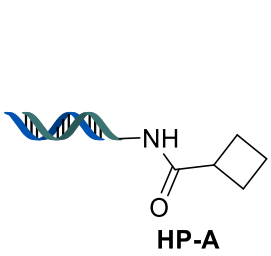


Molecular Weight: 5266.62 g/mol



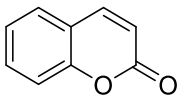


HP-A control experiment with coumarin (**General Procedure E**)



HP-A

Molecular Weight: 5266.62 g/mol



(50 equiv)

1) (Ir[dF(CF_3)ppy]$_2$(dtbbpy))PF_6 (1 equiv)

DMSO/H_2O/glycerol (23:5:2)

Blue Kessil, rt, 10 min

2) H_2O, 70 ^oC, 20 min

[2+2] product molecular weight: 5412.77 g/mol

7%

(No hydrolysis product observed)

93% **HP-A** rsm