

Supporting information

Regioselective Triclocarban-Catalyzed Synthesis of Spiroindeno- and Spirooxindolo-Pyrrolizidines with Apoptosis Induction in MCF-7 Cells

V. Rukyanaik,^a Samata Pradhan,^b Chandraiah Godugu,^b Srinu Tothadi,^c Kadiyam Rama Krishna,^d Ravinder pawar,^d Srinivas Basavoju,^{a,*}

^{a,d}*Department of Chemistry, National Institute of Technology Warangal, Hanamkonda-506 004, Telangana, India.*

^b*Department of Regulatory Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, Balanagar, 500037, Telangana, India.*

^c*CSIR-Central Salt and Marine Chemicals Research Institute, Gijub Badheka Marg, Bhavnagar-364002, Gujarat, India.*

*Corresponding author

E-mail: basavojusrinivas@nitw.ac.in

Mobile: (+91) 9703351571.

S. No.	Contents	Page number
1	General information	S1
2	Experimental section	S1-S2
3	Gram-scale reaction	S2
4	Plausible reaction mechanism	S2-S3
5	Spectral data of the compounds 4a-s and 6a-r	S3-S17
6	NMR, Mass, FTIR spectral images of the Compounds 4a-s and 6a-r	S17-S93
7	Salient feature of the crystallographic data of the compounds 4m and 4n	S93-S94
8	Computational studies	S95-S99
9	Computational methodology	S99-S100
10	Cell culture and growth conditions	S100-S102
11	References	S102-S104

1. General information

All reagents and solvents were obtained from commercial suppliers and used as received unless otherwise stated. Isatin (98% purity) was obtained from Spectrochem Pvt. Ltd., Mumbai, while L-proline (99% purity) was sourced from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai. The starting materials 3-(2-nitrovinyl)-1*H*-indoles and 11*H*-indeno[1,2-*b*]quinoxalin-11-ones were synthesized according to reported literature procedures (Cited in main paper). Triclocarban (TCC) (99% purity) was procured from Sigma-Aldrich. Analytical-grade solvents were purchased from Merck Chemicals, Mumbai, and borosilicate glassware was used throughout all experimental work. The course of the reactions was routinely monitored by thin-layer chromatography (TLC) using E. Merck silica gel plates. Melting points were measured in open capillaries with a Stuart SMP30 instrument (Bibby Scientific Ltd., UK). Infrared spectra (IR) were collected in the solid state using KBr pellets on a Bruker Alpha-II spectrophotometer, spanning the 400–4000 cm⁻¹ region. Nuclear magnetic resonance (NMR) spectra, including ¹H, ¹³C, DEPT-135 and ¹⁹F were recorded on a Bruker Avance-III 400 MHz spectrometer (Bruker Corporation, Germany) with DMSO-*d*₆ and CDCl₃ as solvents; tetramethylsilane (TMS) served as an internal reference and chemical shifts are reported in ppm. HRMS spectra were recorded by using the ESI-TOF technique with acetonitrile as the solvent. For crystallographic analysis, single-crystal X-ray diffraction data of compounds **4m** and **4n** were measured on a Bruker Kappa APEX-II CCD diffractometer and the structures were solved using the ShelXT program. Additionally, molecular docking studies were performed using AutoDock Tools (version 1.5.6) and the docking results were analyzed and visualized with Discovery Studio software.

2. Experimental section

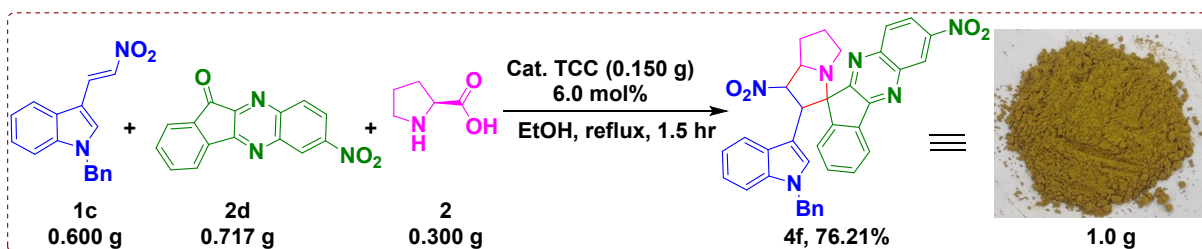
General procedure for the synthesis of target compounds **4a–s** and **6a–r**.

To a dry 50 mL round-bottom flask equipped with a magnetic stir bar and a reflux condenser, add ethanol (5.0 mL), indole-nitroalkene **1a–g** (1.0 equiv.), 11*H*-indeno[1,2-*b*]quinoxalin-11-one **2a–e** or isatin **5a–c** (1.2 equiv.), L-proline **3** (1.2 equiv.) and TCC catalyst (6 mol%). Stir the reaction mixture vigorously under reflux for 60 minutes, monitoring progress by thin-layer chromatography (TLC). Upon completion, cool the reaction mixture to room temperature, add cold water and stir for 5 minutes at room temperature to induce precipitation. Collect the crude product by vacuum filtration, wash with cold water and dry until constant weight is achieved. Purify the product by

silica gel column chromatography using ethyl acetate/n-hexane (3:7, v/v) as the eluent to yield the desired products **4a–s**. The same procedure was followed for the synthesis of compounds **6a–r**.

3. Gram-scale reaction

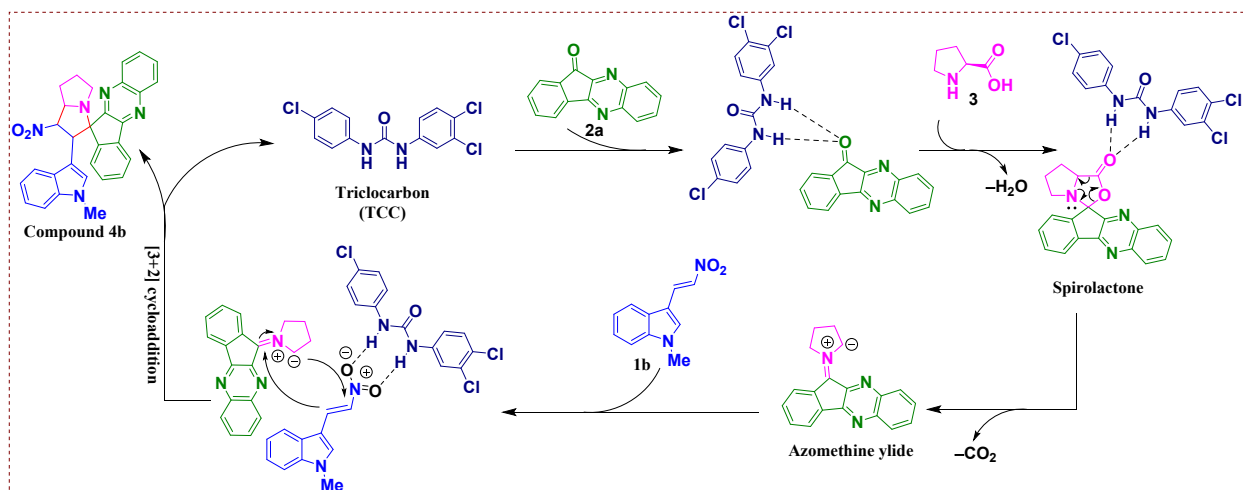
Furthermore, a gram-scale reaction was carried out using same procedure with substrate **1c** (0.600 g, 2.16 mmol, 1 eq), **2d** (0.717 g, 2.59 mmol, 1.2 eq), and L-proline **3** (0.300 g, 2.59 mmol, 1.2 eq) in the presence of TCC catalyst (0.150 g, 0.474 mmol, 6 mol%, 0.22 eq). The reaction proceeded smoothly and afforded **4f** in 76.21% yield (1.0 g), as shown in Scheme 1.



Scheme 1. Gram scale reaction of the compound **4f**.

4. Plausible reaction mechanism

A common reaction mechanism for the synthesized spiroindeno-pyrrolizidine and spirooxindolo-pyrrolizidine derivatives was outlined in Scheme 2.¹⁻⁵ Initially, trilocarban (TCC) activates the carbonyl group of 11*H*-indeno[1,2-*b*]quinoxalin-11-one (**2a**) through hydrogen-bond interactions, thereby enhancing π -bond polarization. This activation facilitates condensation with L-proline (**3**), leading to the loss of H₂O and the formation of an unstable spirolactone intermediate. Subsequently, spirolactone undergoes decarboxylation to form highly reactive azomethine ylide. Further, the *in situ*-generated azomethine ylide undergoes a [3+2] cycloaddition with indole-nitroalkene (**1b**), wherein the negative charge of the ylide attacks at the α -carbon of the nitroalkene with respect to the nitro group, leading to the formation of regioselective compound **4b**. Consistently, only a single regioisomer was observed in all the synthesized compounds. Furthermore, the formation of regioisomer was confirmed by single-crystal X-ray diffraction (SCXRD) analysis.



Scheme 2. Plausible reaction mechanism of the compounds **4a-s** and **6a-r**.

5. Spectral data for the compounds **4a-s** and **6a-r**

1-(3-(1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizin]-2'-yl)-1*H*-indol-1-yl)ethan-1-one (4a). Yellow **crystalline** solid. mp: 182 – 183 °C. IR (KBr, cm^{-1}): 1701, 1614, 1543, 1338. ^1H NMR (400 MHz, CDCl_3) δ 8.21 – 8.15 (m, 1H), 8.10 (d, $J = 7.8$ Hz, 1H), 8.06 – 8.01 (m, 1H), 7.98 – 7.93 (m, 1H), 7.76 – 7.69 (m, 3H), 7.58 (td, $J = 7.6, 1.3$ Hz, 1H), 7.47 – 7.39 (m, 2H), 7.03 – 6.86 (m, 3H), 6.54 (t, $J = 9.6$ Hz, 1H), 6.38 (d, $J = 2.7$ Hz, 1H), 5.41 (d, $J = 9.9$ Hz, 1H), 5.27 – 5.18 (m, 1H), 3.56 (ddd, $J = 10.8, 8.3, 5.6$ Hz, 1H), 2.88 (t, $J = 6.7$ Hz, 1H), 2.24 (dt, $J = 14.2, 7.3$ Hz, 1H), 2.15 – 2.05 (m, 1H), 1.95 – 1.70 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.20, 153.34, 143.77, 142.45, 141.11, 138.73, 135.14, 131.29, 130.17, 129.75, 129.62, 129.14, 129.10, 127.16, 126.39, 122.63, 122.05, 119.54, 118.50, 110.81, 108.89, 95.77, 74.98, 64.97, 51.05, 46.26, 27.52, 26.03. HRMS (ESI, m/z): $[\text{M}+\text{NH}_4]^+$ calcd. For $\text{C}_{29}\text{H}_{27}\text{N}_6\text{O}_2$: 491.2195; Found: 492.2247.

2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4b). Yellow **crystalline** solid. mp: 223 – 224 °C. IR (KBr, cm^{-1}): 1612, 1536, 1333. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.48 (d, $J = 7.8$ Hz, 1H), 8.29 (dd, $J = 8.0, 2.0$ Hz, 1H), 8.04 (dd, $J = 7.8, 2.0$ Hz, 1H), 7.89 – 7.80 (m, 3H), 7.66 (t, $J = 7.0$ Hz, 1H), 7.55 – 7.47 (m, 2H), 7.05 (d, $J = 8.1$ Hz, 1H), 6.90 (t, $J = 7.3$ Hz, 1H), 6.79 (t, $J = 7.5$ Hz, 1H), 6.68 (t, $J = 10.0$ Hz, 1H), 6.60 (s, 1H), 5.33 (d, $J = 10.6$ Hz, 1H), 4.91 (q, $J = 7.8$ Hz, 1H), 3.61 – 3.53 (m, 1H), 3.27 (s, 3H), 2.59 (t, $J = 7.2$ Hz, 1H), 2.15 – 1.95 (m, 2H), 1.71 – 1.49 (m, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 163.44, 153.05, 144.00, 142.05, 140.84, 138.23, 136.18, 132.13, 130.81,

130.63, 130.11, 130.02, 129.20, 128.20, 128.13, 127.59, 122.31, 121.63, 119.13, 119.08, 110.01, 106.78, 94.85, 74.89, 64.29, 51.08, 45.41, 32.68, 27.87, 26.04. HRMS (ESI, m/z): [M+H]⁺ calcd. For C₃₀H₂₅N₅O₂: 488.2087; Found: 488.2068.

7-methyl-2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4c). Off-white **crystalline** solid. mp: 210 – 211 °C. IR (KBr, cm⁻¹): 1620, 1547, 1373. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.46 (d, *J* = 7.8 Hz, 1H), 8.22 – 8.08 (m, 1H), 7.93 – 7.81 (m, 2H), 7.69 – 7.61 (m, 2H), 7.54 (dd, *J* = 8.0, 4.2 Hz, 1H), 7.51 – 7.46 (m, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.94 – 6.88 (m, 1H), 6.84 – 6.78 (m, 1H), 6.66 (td, *J* = 10.0, 1.4 Hz, 1H), 6.57 (s, 1H), 5.32 (d, *J* = 10.6 Hz, 1H), 4.91 (q, *J* = 8.4 Hz, 1H), 3.61 – 3.55 (m, 1H), 3.26 (s, 3H), 2.60 (t, *J* = 4.8 Hz, 1H 1H), 2.54 (s, 3H), 2.14 – 1.95 (m, 2H), 1.70 – 1.50 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.56, 152.92, 143.94, 142.11, 140.73, 139.25, 138.37, 136.16, 132.08, 131.93, 130.72, 129.55, 128.20, 128.13, 128.07, 127.62, 122.19, 121.63, 119.13, 119.08, 110.00, 106.84, 94.96, 74.83, 64.26, 51.13, 45.34, 32.68, 27.85, 26.04, 21.68. HRMS (ESI, m/z): [M+H]⁺ calcd. For C₃₁H₂₈N₅O₂: 502.2243; Found: 502.2229.

2'-(1-benzyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4d). Yellow **crystalline** solid. mp: 172 – 174 °C. IR (KBr, cm⁻¹): 1614, 1539, 1334. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.50 (d, *J* = 7.8 Hz, 1H), 8.31 – 8.22 (m, 1H), 8.11 – 8.03 (m, 1H), 7.88 – 7.80 (m, 3H), 7.67 (td, *J* = 7.6, 1.3 Hz, 1H), 7.51 (t, *J* = 7.2 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.08 (d, *J* = 8.3 Hz, 1H), 7.01 – 6.96 (m, 1H), 6.92 (s, 1H), 6.86 – 6.77 (m, 3H), 6.74 – 6.65 (m, 2H), 6.34 (dd, *J* = 8.1, 1.3 Hz, 2H), 5.35 (d, *J* = 10.4 Hz, 1H), 5.01 – 4.84 (m, 3H), 3.61 – 3.55 (m, 1H), 2.63 (t, *J* = 6.8 Hz, 1H), 2.18 – 1.97 (m, 2H), 1.74 – 1.52 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 163.46, 152.93, 144.10, 142.05, 140.91, 138.23, 137.64, 135.37, 132.09, 130.79, 130.53, 130.06, 130.01, 129.27, 128.48, 128.34, 128.08, 127.76, 127.50, 126.84, 122.26, 121.67, 119.21, 119.13, 110.31, 107.32, 94.51, 75.00, 64.62, 50.95, 49.07, 45.94, 27.90, 26.07. HRMS (ESI, m/z): [M+H]⁺ calcd. For C₃₆H₃₀N₅O₂: 564.2400; Found: 564.2379.

2'-(1-benzyl-1*H*-indol-3-yl)-7,8-dimethyl-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4e). White **crystalline** solid. mp: 203 – 204 °C. IR (KBr, cm⁻¹): 1615, 1539, 1331. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (d, *J* = 7.8 Hz, 1H), 8.00 (s, 1H), 7.83 (s, 1H), 7.78 (d, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 6.87 –

6.78 (m, 4H), 6.73 (t, $J = 7.5$ Hz, 1H), 6.66 (t, $J = 9.9$ Hz, 1H), 6.36 (d, $J = 7.6$ Hz, 2H), 5.32 (d, $J = 10.5$ Hz, 1H), 4.99 – 4.85 (m, 3H), 3.59 (dt, $J = 9.8, 4.6$ Hz, 1H), 2.62 (t, $J = 7.0$ Hz, 1H), 2.47 (s, 6H), 2.16 – 1.97 (m, 2H), 1.73 – 1.51 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.46, 151.99, 143.76, 140.86, 140.55, 140.08, 139.72, 138.61, 137.59, 135.41, 131.56, 130.63, 129.08, 128.45, 128.18, 127.84, 127.77, 127.47, 126.91, 121.90, 121.67, 119.21, 119.11, 110.27, 107.47, 94.69, 74.94, 64.55, 51.01, 49.04, 45.75, 27.84, 26.07, 20.24. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{38}\text{H}_{34}\text{N}_5\text{O}_2$: 592.2713; Found: 592.2671.

2'-(1-benzyl-1*H*-indol-3-yl)-1',7-dinitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4f). White crystalline solid. mp: 202 – 203 °C. IR (KBr, cm^{-1}): 1583, 1542, 1340. ^1H NMR (400 MHz, DMSO- d_6) δ 9.108 (d, $J = 2.8$ Hz, 1H), 8.613 (d, $J = 7.8$ Hz, 1H), 8.527 (dd, $J = 9.0, 2.7$ Hz, 1H), 8.227 (d, $J = 9.2$ Hz, 1H), 7.882 (d, $J = 7.7$ Hz, 1H), 7.773 (t, $J = 7.7$ Hz, 1H), 7.550 (t, $J = 7.5$ Hz, 1H), 7.386 (d, $J = 8.1$ Hz, 1H), 7.119 (d, $J = 8.2$ Hz, 1H), 7.060 – 6.931 (m, 2H), 6.926 – 6.654 (m, 5H), 6.400 (d, $J = 7.6$ Hz, 2H), 5.402 (d, $J = 10.5$ Hz, 1H), 5.062 – 4.941 (m, 2H), 4.891 – 4.852 (m, 1H), 3.692 – 3.586 (m, 1H), 2.667 (t, $J = 7.2$ Hz, 1H), 2.231 – 1.978 (m, 2H), 1.824 – 1.551 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.65, 155.72, 147.17, 145.12, 144.93, 139.73, 137.74, 137.33, 135.48, 133.45, 131.14, 130.84, 128.65, 128.37, 128.14, 127.73, 127.44, 126.87, 126.23, 123.92, 123.11, 121.73, 119.25, 119.07, 110.30, 107.20, 94.32, 74.97, 64.63, 50.93, 48.97, 45.81, 28.03, 26.10. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{36}\text{H}_{29}\text{N}_6\text{O}_4$: 609.2250; Found: 609.2256

1'-nitro-2'-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4g). Light yellow crystalline solid. mp: 201 – 202 °C. IR (KBr, cm^{-1}): 1614, 1537, 1337. ^1H NMR (400 MHz, DMSO- d_6) δ 8.48 (d, $J = 7.8$ Hz, 1H), 8.29 (dd, $J = 7.8, 2.0$ Hz, 1H), 8.03 (dd, $J = 7.6, 2.0$ Hz, 1H), 7.89 – 7.79 (m, 3H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.52 (q, $J = 7.6$ Hz, 2H), 7.15 (d, $J = 8.2$ Hz, 1H), 6.94 (t, $J = 7.2$ Hz, 1H), 6.83 (t, $J = 7.6$ Hz, 1H), 6.70 (s, 1H), 6.65 (t, $J = 10.0$ Hz, 1H), 5.34 (d, $J = 10.4$ Hz, 1H), 4.93 (q, $J = 7.8$ Hz, 1H), 4.60 (t, $J = 2.8$ Hz, 2H), 3.62 – 3.56 (m, 1H), 2.83 (t, $J = 2.4$ Hz, 1H), 2.60 (t, $J = 7.0$ Hz, 1H), 2.17 – 1.95 (m, 2H), 1.74 – 1.46 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 163.32, 152.97, 143.90, 142.12, 140.91, 138.24, 135.30, 132.11, 130.83, 130.61, 130.06, 129.17, 128.18, 127.95, 126.60, 122.32, 121.95, 119.64, 119.23, 110.27, 107.91, 95.01, 78.69, 75.71, 74.90, 64.41, 51.06, 45.34, 35.23, 27.87, 26.04. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{32}\text{H}_{26}\text{N}_5\text{O}_2$: 512.2087; Found: 512.2072.

7-methyl-1'-nitro-2'-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1',2',5',6',7',7a'-

hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4h).

Light brown **crystalline** solid. mp: 198 – 199 °C. IR (KBr, cm⁻¹): 1612, 1542, 1335. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.46 (d, *J* = 7.8 Hz, 1H), 8.21 – 8.08 (m, 1H), 7.93 – 7.80 (m, 2H), 7.72 – 7.59 (m, 2H), 7.57 – 7.46 (m, 2H), 7.15 (d, *J* = 8.2 Hz, 1H), 6.98 – 6.90 (m, 1H), 6.84 (td, *J* = 7.4, 3.2 Hz, 1H), 6.70 – 6.59 (m, 2H), 5.32 (d, *J* = 10.4 Hz, 1H), 4.92 (q, *J* = 7.6 Hz, 1H), 4.66 – 4.55 (m, 2H), 3.61 – 3.55 (m, 1H), 2.87 (t, *J* = 2.6 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 1H), 2.54 (s, 3H), 2.16 – 1.96 (m, 2H), 1.71 – 1.49 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.43, 152.85, 143.84, 142.19, 140.71, 139.31, 138.40, 135.29, 132.04, 131.91, 130.75, 129.58, 128.12, 127.99, 126.58, 122.20, 121.94, 119.65, 119.24, 110.28, 107.95, 95.11, 78.72, 75.80, 74.85, 64.36, 51.11, 45.26, 35.26, 27.85, 26.04, 21.70. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₃H₂₈N₅O₂: 526.2243; Found: 526.2243.

7,8-dimethyl-1'-nitro-2'-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1',2',5',6',7',7a'-

hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4i). White **crystalline** solid. mp: 218 – 219 °C. IR (KBr, cm⁻¹): 1616, 1536, 1333. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (d, *J* = 7.8 Hz, 1H), 8.06 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.63 – 7.55 (m, 2H), 7.48 (t, *J* = 7.1 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.95 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.88 – 6.82 (m, 1H), 6.66 – 6.59 (m, 2H), 5.32 (d, *J* = 10.6 Hz, 1H), 4.95 – 4.87 (m, 1H), 4.62 (d, *J* = 2.6 Hz, 2H), 3.62 – 3.56 (m, 1H), 2.87 (t, *J* = 2.5 Hz, 1H), 2.60 (t, *J* = 7.0 Hz, 1H), 2.51 (s, 3H), 2.45 (s, 3H), 2.15 – 1.96 (m, 2H), 1.71 – 1.50 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.34, 152.05, 143.54, 140.94, 140.67, 140.19, 139.73, 138.62, 135.29, 131.57, 130.68, 129.16, 128.33, 128.07, 126.46, 121.95, 119.65, 119.25, 110.28, 108.02, 95.21, 78.81, 75.77, 74.83, 64.27, 51.16, 45.04, 35.27, 27.84, 26.05, 20.25. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₄H₃₀N₅O₂: 540.2400; Found: 540.2383.

1'-nitro-2'-(1-tosyl-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-

***b*]quinoxaline-11,3'-pyrrolizine] (4j).** White **crystalline** solid. mp: 200 – 201 °C. IR (KBr, cm⁻¹): 1590, 1545, 1368, 1286. ¹H NMR (400 MHz, CDCl₃) δ 8.31 – 8.26 (m, 1H), 8.13 – 8.05 (m, 2H), 7.93 (d, *J* = 6.7 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.64 – 7.56 (m, 2H), 7.46 (td, *J* = 7.5, 1.0 Hz, 1H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.07 (ddd, *J* = 8.4, 7.3, 1.3 Hz, 1H), 7.01 – 6.95 (m, 2H), 6.92 – 6.79 (m, 4H), 6.54 (t, *J* = 9.4 Hz, 1H), 5.30 (d, *J* = 9.7 Hz, 1H), 5.25 – 5.17 (m, 1H), 3.46 (ddd, *J* = 10.5, 8.3, 5.8 Hz, 1H), 2.82 (td, *J* = 5.9, 2.7 Hz, 1H), 2.26 (s, 3H), 2.23 (dd, *J* = 8.5, 1.5 Hz, 1H), 2.11 – 2.03 (m, 1H), 1.90 – 1.79 (m, 1H), 1.75 – 1.65 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 162.22,

153.00, 144.64, 143.22, 142.43, 140.97, 138.64, 134.30, 134.17, 131.28, 130.36, 130.11, 129.97, 129.76, 129.64, 129.08, 126.29, 126.00, 125.12, 124.69, 122.98, 122.63, 119.16, 115.87, 113.27, 95.17, 74.62, 65.17, 50.69, 45.92, 27.44, 25.94, 21.55. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{36}H_{30}N_5O_4S$: 628.2019; Found: 628.1998.

7,8-dimethyl-1'-nitro-2'-(1-tosyl-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4k). Off-white **crystalline** solid. mp: 238 – 239 °C. IR (KBr, cm^{-1}): 1597, 1532, 1367, 1280. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.99 (m, 2H), 7.89 (dt, $J = 7.6, 1.0$ Hz, 1H), 7.87 – 7.83 (m, 1H), 7.62 (dt, $J = 8.2, 1.0$ Hz, 1H), 7.52 (td, $J = 7.6, 1.2$ Hz, 1H), 7.42 (td, $J = 7.4, 1.0$ Hz, 1H), 7.30 – 7.26 (m, 1H), 7.07 (ddd, $J = 8.4, 7.2, 1.2$ Hz, 1H), 6.99 (ddd, $J = 8.2, 7.2, 1.0$ Hz, 1H), 6.93 (s, 1H), 6.87 – 6.84 (m, 4H), 6.53 (t, $J = 9.4$ Hz, 1H), 5.28 (d, $J = 9.8$ Hz, 1H), 5.22 (td, $J = 8.6, 7.0$ Hz, 1H), 3.46 (ddd, $J = 10.6, 8.2, 5.6$ Hz, 1H), 2.82 (td, $J = 7.0, 3.6$ Hz, 1H), 2.57 (s, 3H), 2.53 (s, 3H), 2.26 (s, 3H), 2.25 – 2.05 (m, 2H), 1.86 – 1.79 (m, 1H), 1.72 – 1.65 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.22, 152.02, 144.57, 142.84, 141.21, 140.52, 140.05, 139.91, 138.99, 134.24, 134.17, 130.76, 129.60, 129.06, 128.34, 126.18, 126.03, 125.02, 124.67, 123.00, 122.26, 119.15, 116.15, 113.28, 95.25, 74.60, 65.07, 50.82, 45.63, 29.71, 27.43, 25.94, 21.54, 20.40. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{38}H_{34}N_5O_4S$: 656.2332; Found: 656.2280.

1',7-dinitro-2'-(1-tosyl-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4l). Light yellow **crystalline** solid. mp: 200 – 201 °C. IR (KBr, cm^{-1}): 1587, 1542, 1373, 1340. ^1H NMR (400 MHz, $DMSO-d_6$) δ 9.35 (d, $J = 2.6$ Hz, 1H), 8.62 (dd, $J = 9.0, 2.4$ Hz, 2H), 8.35 (d, $J = 9.0$ Hz, 1H), 7.91 (d, $J = 7.8$ Hz, 1H), 7.72 (t, $J = 7.6$ Hz, 1H), 7.64 (d, $J = 8.0$ Hz, 1H), 7.56 (q, $J = 8.0$ Hz, 2H), 7.26 (s, 1H), 7.14 – 7.01 (m, 5H), 6.94 (d, $J = 8.0$ Hz, 2H), 5.37 (d, $J = 10.4$ Hz, 1H), 4.92 (q, $J = 8.4$ Hz, 1H), 3.68 – 3.58 (m, 1H), 2.61 (t, $J = 7.2$ Hz, 1H), 2.27 (s, 3H), 2.21 – 2.00 (m, 2H), 1.71 – 1.55 (m, 2H). ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 164.95, 155.59, 147.44, 145.75, 145.13, 144.12, 139.73, 137.15, 133.93, 133.77, 133.46, 131.39, 130.86, 130.26, 128.82, 126.27, 126.07, 125.73, 125.39, 124.37, 123.53, 123.13, 120.43, 116.78, 113.26, 93.23, 74.63, 64.51, 50.95, 44.12, 28.46, 26.05, 21.46. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{36}H_{29}N_6O_6S$: 673.1869; Found: 673.1860.

2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4m). Yellow crystalline solid. mp: 227 – 228 °C. IR (KBr, cm⁻¹): 1620, 1538, 1338. ¹H NMR (400 MHz, CDCl₃) δ 8.22 – 8.16 (m, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.07 – 8.02 (m, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.73 (qd, *J* = 7.0, 3.4 Hz, 2H), 7.60 (td, *J* = 7.6, 1.4 Hz, 1H), 7.48 (td, *J* = 7.4, 1.0 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 2.4 Hz, 1H), 6.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (t, *J* = 9.6 Hz, 1H), 6.26 (s, 1H), 5.33 (d, *J* = 10.0 Hz, 1H), 5.20 (q, *J* = 8.0 Hz, 1H), 3.73 (s, 3H), 3.55 (ddd, *J* = 10.6, 8.2, 5.6 Hz, 1H), 3.24 (s, 3H), 2.86 (t, *J* = 7.4 Hz, 1H), 2.27 – 2.20 (m, 1H), 2.15 – 2.05 (m, 1H), 1.92 – 1.80 (m, 1H), 1.73 – 1.65 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 163.24, 153.78, 153.29, 144.06, 142.44, 141.13, 138.94, 131.56, 131.15, 130.16, 129.70, 129.60, 129.16, 129.07, 127.93, 127.79, 126.35, 122.69, 112.07, 109.72, 106.52, 100.54, 95.44, 74.81, 64.88, 55.96, 50.94, 46.75, 32.73, 27.46, 26.02. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₁H₂₈N₅O₃: 518.2192; Found: 518.2153.

2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-7-methyl-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4n). Light green crystalline solid. mp: 218 – 219 °C. IR (KBr, cm⁻¹): 1622, 1539, 1337. ¹H NMR (400 MHz, CDCl₃) δ 8.11 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.97 – 7.93 (m, 1H), 7.82 (s, 1H), 7.62 – 7.53 (m, 2H), 7.46 (t, *J* = 7.4 Hz, 1H), 6.84 – 6.74 (m, 2H), 6.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (td, *J* = 9.6, 2.4 Hz, 1H), 6.24 (d, *J* = 4.0 Hz, 1H), 5.32 (d, *J* = 10.0 Hz, 1H), 5.20 (q, *J* = 8.0 Hz, 1H), 3.73 (s, 3H), 3.55 (ddd, *J* = 11.4, 8.2, 5.6 Hz, 1H), 3.22 (s, 3H), 2.87 (t, *J* = 7.2 Hz, 1H), 2.58 (s, 3H), 2.29 – 2.17 (m, 1H), 2.14 – 2.04 (m, 1H), 1.96 – 1.69 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 162.33, 153.77, 140.18, 139.56, 139.10, 131.84, 131.54, 131.22, 130.95, 130.08, 129.10, 128.78, 128.65, 128.27, 127.98, 127.76, 126.30, 122.58, 112.04, 109.69, 106.58, 100.52, 95.52, 74.76, 64.85, 55.96, 51.00, 46.66, 32.72, 27.44, 26.02, 21.79. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₂H₃₀N₅O₃: 532.2349; Found: 532.2328.

2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-7,8-dimethyl-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4o). Yellow crystalline solid. mp: 224 – 225 °C. IR (KBr, cm⁻¹): 1622, 1543, 1375. ¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, *J* = 7.8 Hz, 1H), 7.98 – 7.92 (m, 2H), 7.79 (d, *J* = 1.2 Hz, 1H), 7.55 (td, *J* = 7.6, 1.4 Hz, 1H), 7.45 (td, *J* = 7.4, 1.0 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.50 (t, *J* = 9.6 Hz, 1H), 6.21 (s, 1H), 5.32 (d, *J* = 10.0 Hz, 1H), 5.20 (td, *J* = 8.6, 7.0 Hz, 1H),

3.74 (s, 3H), 3.55 (ddd, $J = 10.6, 8.4, 5.6$ Hz, 1H), 3.24 (s, 3H), 2.87 (t, $J = 6.8$ Hz, 1H), 2.53 (s, 3H), 2.49 (s, 3H), 2.28 – 2.18 (m, 1H), 2.14 – 2.04 (m, 1H), 1.94 – 1.67 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 162.23, 153.74, 152.36, 143.71, 141.21, 140.00, 139.41, 139.29, 131.53, 130.65, 130.02, 128.94, 128.43, 128.02, 127.74, 126.23, 122.36, 112.01, 109.67, 106.65, 100.56, 95.63, 74.77, 64.81, 55.94, 51.04, 46.54, 32.73, 27.42, 26.02, 20.32. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{33}\text{H}_{32}\text{N}_5\text{O}_3$: 546.2505; Found: 546.2491.

2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4p). Light yellow **crystalline** solid. mp: 224 – 225 °C. IR (KBr, cm^{-1}): 1613, 1536, 1336, 1289. ^1H NMR (400 MHz, CDCl_3) δ 8.31 – 8.23 (m, 1H), 8.13 – 8.06 (m, 2H), 7.96 (d, $J = 7.4$ Hz, 1H), 7.81 (qd, $J = 7.0, 3.4$ Hz, 2H), 7.59 (td, $J = 7.6, 1.2$ Hz, 1H), 7.52 – 7.45 (m, 2H), 7.01 (s, 1H), 6.87 (d, $J = 8.2$ Hz, 2H), 6.81 (d, $J = 8.2$ Hz, 2H), 6.68 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.59 (d, $J = 2.4$ Hz, 1H), 6.50 (t, $J = 9.2$ Hz, 1H), 5.25 (d, $J = 9.4$ Hz, 1H), 5.19 (q, $J = 8.0$ Hz, 1H), 3.66 (s, 3H), 3.50 – 3.38 (m, 1H), 2.82 – 2.74 (m, 1H), 2.25 (s, 3H), 2.24 – 2.02 (m, 2H), 1.92 – 1.68 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 162.14, 156.20, 152.92, 144.55, 143.49, 142.41, 141.01, 138.75, 134.23, 131.21, 130.95, 130.39, 130.09, 129.79, 129.59, 129.12, 128.91, 126.19, 125.89, 122.78, 115.92, 114.13, 114.03, 101.58, 94.93, 74.36, 65.20, 55.63, 50.42, 46.33, 27.32, 25.91, 21.53. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{37}\text{H}_{32}\text{N}_5\text{O}_5\text{S}$: 658.2124; Found: 658.2073.

7,8-dichloro-2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4q). Light brown **crystalline** solid. mp: 206 – 207 °C. IR (KBr, cm^{-1}): 1590, 1545, 1368, 1286. ^1H NMR (400 MHz, CDCl_3) δ 8.36 (s, 1H), 8.14 (s, 1H), 8.11 (d, $J = 7.8$ Hz, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 7.67 – 7.63 (m, 1H), 7.56 – 7.50 (m, 2H), 7.07 – 7.01 (m, 2H), 6.95 (s, 1H), 6.90 (d, $J = 8.1$ Hz, 2H), 6.69 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.53 (d, $J = 2.4$ Hz, 1H), 6.39 (t, $J = 9.0$ Hz, 1H), 5.23 (d, $J = 9.2$ Hz, 1H), 5.11 (q, $J = 8.0$ Hz, 1H), 3.65 (s, 3H), 3.43 – 3.35 (m, 1H), 2.77 – 2.70 (m, 1H), 2.30 (s, 3H), 2.25 – 2.05 (m, 2H), 1.91 – 1.67 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.31, 156.20, 153.89, 144.79, 143.92, 141.14, 139.56, 138.07, 134.43, 133.76, 131.95, 130.62, 130.16, 129.71, 129.60, 129.08, 126.26, 125.94, 125.53, 123.11, 115.68, 114.19, 114.09, 101.74, 94.18, 74.23, 65.42, 55.63, 50.03, 46.98, 27.21, 25.90, 21.55. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{37}\text{H}_{30}\text{Cl}_2\text{N}_5\text{O}_5\text{S}$: 726.1345; Found: 726.1284.

2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-7-methyl-1'-nitro-1',2',5',6',7',7a'-

hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4r). Off-white **crystalline** solid. mp: 204 – 205 °C. IR (KBr, cm⁻¹): 1597, 1542, 1367, 1274. ¹H NMR (400 MHz, CDCl₃) δ 8.19 – 8.05 (m, 2H), 8.01 – 7.86 (m, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.56 (q, *J* = 8.0 Hz, 1H), 7.52 – 7.43 (m, 2H), 7.00 – 6.95 (m, 1H), 6.90 – 6.79 (m, 4H), 6.68 (d, *J* = 9.0 Hz, 1H), 6.60 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.49 (td, *J* = 9.4, 2.4 Hz, 1H), 5.27 – 5.14 (m, 2H), 3.66 (s, 3H), 3.44 (q, *J* = 8.4 Hz, 1H), 2.79 (t, *J* = 7.6 Hz, 1H), 2.63 (s, 3H), 2.26 (s, 3H), 2.24 – 2.03 (m, 2H), 1.91 – 1.70 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 162.05, 156.20, 152.77, 144.48, 142.46, 140.62, 138.94, 134.22, 132.27, 131.75, 131.01, 130.86, 130.31, 129.57, 129.30, 128.91, 128.61, 128.22, 126.13, 125.95, 125.85, 116.03, 114.13, 114.01, 101.56, 94.97, 74.33, 65.18, 55.63, 50.48, 46.26, 27.31, 25.91, 21.85, 21.54. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₈H₃₄N₅O₅S: 672.2281; Found: 672.2266.

2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-7,8-dimethyl-1'-nitro-1',2',5',6',7',7a'-

hexahydrospiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] (4s). Light yellow **crystalline** solid. mp: 230 – 231 °C. IR (KBr, cm⁻¹): 1603, 1538, 1375, 1334, 1283. ¹H NMR (400 MHz, CDCl₃) δ 8.10 – 7.99 (m, 2H), 7.92 (d, *J* = 7.4 Hz, 1H), 7.85 (s, 1H), 7.57 – 7.43 (m, 3H), 6.95 (s, 1H), 6.85 (q, *J* = 8.4 Hz, 4H), 6.67 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 6.49 (t, *J* = 9.4 Hz, 1H), 5.26 – 5.15 (m, 2H), 3.67 (s, 3H), 3.44 (ddd, *J* = 10.0, 8.2, 5.8 Hz, 1H), 2.80 (t, *J* = 7.4 Hz, 1H), 2.57 (s, 3H), 2.54 (s, 3H), 2.26 (s, 3H), 2.25 – 2.00 (m, 2H), 1.91 – 1.66 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 161.14, 156.21, 151.96, 144.46, 143.13, 141.21, 140.48, 140.04, 139.94, 139.11, 134.17, 131.11, 130.69, 130.23, 129.55, 129.08, 128.91, 128.38, 126.07, 125.96, 125.81, 122.40, 116.19, 114.16, 114.00, 101.60, 95.00, 74.35, 65.12, 55.62, 50.53, 46.11, 27.30, 25.91, 21.53, 20.38. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₉H₃₆N₅O₅S: 686.2437; Found: 686.2381.

2'-(1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one

(6a). White **crystalline** solid. mp: 236 – 237 °C. IR (KBr, cm⁻¹): 3055, 1613, 1590, 1536, 1336. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.98 (s, 1H), 10.25 (s, 1H), 7.87 (d, *J* = 7.4 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.20 (dd, *J* = 5.4, 2.8 Hz, 2H), 7.13 – 7.06 (m, 1H), 6.98 – 6.91 (m, 2H), 6.85 – 6.78 (m, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 6.22 (dd, *J* = 10.6, 9.2 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.61 (q, *J* = 8.2 Hz, 1H), 3.42 – 3.33 (m, 1H), 2.60 (t, *J* = 7.2 Hz, 1H), 2.04 – 1.89 (m, 2H), 1.71 – 1.60 (m, 1H), 1.40 (tt, *J* = 11.6, 7.6 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.69, 143.53, 135.94,

130.13, 127.55, 127.19, 125.99, 123.72, 121.86, 121.61, 119.08, 118.69, 111.82, 110.05, 107.54, 94.15, 74.67, 63.58, 51.08, 43.93, 27.89, 25.60. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{22}H_{21}N_4O_3$: 389.1614; Found: 389.1605.

2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6b). White **crystalline** solid. mp: 223 – 224 °C. IR (KBr, cm^{-1}): 3179, 1701, 1533, 1338. ^1H NMR (400 MHz, DMSO- d_6) δ 7.94 (s, 1H), 7.66 (d, $J = 7.6$ Hz, 1H), 7.32 (d, $J = 8.0$ Hz, 1H), 7.15 (td, $J = 7.8, 1.4$ Hz, 1H), 7.10 – 7.04 (m, 3H), 6.98 – 6.89 (m, 2H), 6.62 (d, $J = 7.6$ Hz, 1H), 6.13 (t, $J = 9.6$ Hz, 1H), 4.98 (d, $J = 10.0$ Hz, 1H), 4.85 (q, $J = 8.0$ Hz, 1H), 3.39 – 3.31 (m, 1H), 3.28 (s, 3H), 2.88 (t, $J = 7.6$ Hz, 1H), 2.19 – 2.10 (m, 1H), 2.06 – 1.98 (m, 1H), 1.90 – 1.79 (m, 1H), 1.59 – 1.51 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.00, 141.98, 136.30, 129.95, 127.78, 127.17, 126.15, 125.94, 122.44, 121.61, 119.19, 118.48, 110.18, 109.22, 106.65, 94.62, 74.99, 64.13, 50.97, 45.05, 32.36, 27.67, 25.66. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{23}H_{23}N_4O_3$: 403.1770; Found: 403.1773.

2'-(1-benzyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6c). White **crystalline** solid. mp: 214 – 215 °C. IR (KBr, cm^{-1}): 3192, 1699, 1533, 1336. ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.65 (dd, $J = 7.4, 1.4$ Hz, 1H), 7.39 (dt, $J = 8.0, 1.2$ Hz, 1H), 7.20 – 7.15 (m, 3H), 7.11 – 6.99 (m, 5H), 6.97 – 6.92 (m, 1H), 6.78 – 6.71 (m, 2H), 6.59 (dd, $J = 7.4, 1.4$ Hz, 1H), 6.16 (dd, $J = 10.0, 9.2$ Hz, 1H), 5.00 (d, $J = 10.0$ Hz, 1H), 4.87 (dt, $J = 9.2, 7.6$ Hz, 1H), 4.81 (s, 2H), 3.35 (ddd, $J = 10.6, 8.4, 5.4$ Hz, 1H), 2.90 (dd, $J = 8.2, 6.4$ Hz, 1H), 2.19 – 2.10 (m, 1H), 2.08 – 1.98 (m, 1H), 1.90 – 1.78 (m, 1H), 1.59 – 1.51 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.04, 141.95, 137.18, 135.95, 129.97, 128.63, 127.99, 127.47, 126.90, 126.33, 126.14, 125.88, 122.47, 121.90, 119.51, 118.71, 110.04, 109.81, 107.35, 94.27, 75.06, 64.33, 50.94, 49.68, 45.37, 27.69, 25.69. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{29}H_{27}N_4O_3$: 479.2083; Found: 479.2069.

1'-nitro-2'-(1-tosyl-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6d). White **crystalline** solid. mp: 227 – 228 °C. IR (KBr, cm^{-1}): 3208, 1706, 1607, 1536, 1362, 1287. ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, $J = 8.4$ Hz, 1H), 7.65 – 7.54 (m, 5H), 7.28 (s, 1H), 7.21 – 7.12 (m, 4H), 7.04 (q, $J = 7.4$ Hz, 2H), 6.59 (d, $J = 7.6$ Hz, 1H), 6.16 (t, $J = 9.6$ Hz, 1H), 4.89 (q, $J = 8.0$ Hz, 1H), 4.84 (d, $J = 10.0$ Hz, 1H), 3.29 (ddd, $J = 10.6, 8.4, 5.6$ Hz, 1H), 2.89 (t, $J = 7.4$ Hz, 1H), 2.32 (s, 3H), 2.16 (dd, $J = 13.6, 7.4$ Hz, 1H), 2.06 – 1.97 (m, 1H), 1.90 – 1.76

(m, 1H), 1.51 (ddt, $J = 13.2, 10.6, 7.6$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 177.81, 145.01, 141.76, 134.85, 134.63, 130.19, 130.04, 129.87, 126.75, 125.94, 125.10, 124.89, 124.83, 123.15, 122.50, 119.19, 115.51, 113.45, 110.27, 93.52, 74.51, 64.46, 50.78, 44.83, 27.67, 25.63, 21.58. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{29}\text{H}_{27}\text{N}_4\text{O}_5\text{S}$: 543.1702; Found: 543.1658.

5-iodo-2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6e). Light green crystalline solid. mp: 233 – 234 °C. IR (KBr, cm^{-1}): 3385, 1712, 1536, 1333. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.35 (s, 1H), 8.40 (s, 1H), 7.54 – 7.42 (m, 2H), 7.25 (s, 2H), 7.04 (t, $J = 7.6$ Hz, 1H), 6.90 (t, $J = 7.6$ Hz, 1H), 6.39 (d, $J = 8.2$ Hz, 1H), 6.24 (t, $J = 10.0$ Hz, 1H), 4.95 (d, $J = 11.0$ Hz, 1H), 4.57 (q, $J = 8.0$ Hz, 1H), 3.65 (s, 3H), 3.48 (s, 1H), 2.61 (t, $J = 7.6$ Hz, 1H), 1.97 (t, $J = 14.3$ Hz, 2H), 1.71 – 1.60 (m, 1H), 1.49 – 1.41 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 178.00, 143.34, 138.60, 136.46, 135.84, 128.51, 127.89, 121.72, 119.24, 119.00, 112.33, 110.08, 106.65, 93.11, 84.63, 74.60, 63.63, 50.92, 43.64, 32.95, 27.98, 25.69. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{23}\text{H}_{22}\text{IN}_4\text{O}_3$: 529.0737; Found: 529.0743.

5-methoxy-2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6f). White crystalline solid. mp: 208 – 209 °C. IR (KBr, cm^{-1}): 3168, 1699, 1538, 1447. ^1H NMR (400 MHz, CDCl_3) δ 7.88 (s, 1H), 7.69 (d, $J = 7.4$ Hz, 1H), 7.16 (td, $J = 7.6, 1.4$ Hz, 1H), 7.07 (td, $J = 7.6, 1.2$ Hz, 1H), 6.99 – 6.93 (m, 2H), 6.73 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.66 (d, $J = 2.4$ Hz, 1H), 6.63 – 6.58 (m, 1H), 6.12 (t, $J = 9.6$ Hz, 1H), 4.92 (d, $J = 10.0$ Hz, 1H), 4.84 (q, $J = 8.0$ Hz, 1H), 3.73 (s, 3H), 3.37 (s, 3H), 3.36 – 3.30 (m, 1H), 2.88 (td, $J = 7.0, 3.6$ Hz, 1H), 2.17 – 2.00 (m, 2H), 1.90 – 1.81 (m, 1H), 1.61 – 1.51 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 178.81, 153.86, 142.10, 131.79, 130.00, 127.99, 127.70, 126.14, 126.02, 122.31, 112.18, 110.32, 109.95, 106.07, 100.24, 94.17, 74.88, 64.21, 55.89, 50.83, 45.65, 32.68, 27.61, 25.65. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}_4$: 433.1876; Found: 433.1867.

5-methoxy-1'-nitro-2'-(1-tosyl-1*H*-indol-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6g). White crystalline solid. mp: 210 – 211 °C. IR (KBr, cm^{-1}): 3184, 1704, 1601, 1543, 1371, 1302. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.38 (s, 1H), 7.84 (d, $J = 8.0$ Hz, 1H), 7.78 – 7.58 (m, 5H), 7.31 (d, $J = 8.0$ Hz, 2H), 7.21 (t, $J = 7.8$ Hz, 1H), 7.15 (t, $J = 7.4$ Hz, 1H), 6.63 (d, $J = 8.4$ Hz, 1H), 6.50 (d, $J = 8.4$ Hz, 1H), 6.43 (t, $J = 10.0$ Hz, 1H), 4.93 (d, $J = 10.8$ Hz, 1H), 4.63 (q, $J = 8.4$ Hz, 1H), 3.70 (s, 3H), 3.53 – 3.43 (m, 1H), 2.60 (d, $J = 7.4$ Hz, 1H), 2.29 (s, 3H), 2.05 – 1.92 (m, 2H), 1.73 – 1.59 (m, 1H), 1.49 – 1.35 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-$

d_6) δ 178.41, 154.99, 145.80, 136.52, 134.08, 133.96, 131.05, 130.63, 126.90, 126.33, 125.40, 125.36, 123.61, 120.58, 117.09, 115.55, 114.37, 113.33, 110.31, 92.91, 79.65, 75.02, 63.78, 56.18, 50.76, 42.02, 28.41, 25.64, 21.47. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{30}H_{29}N_4O_6S$: 573.1808; Found: 573.1795.

2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-5-(trifluoromethoxy)-1',2',5',6',7',7a'-

hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6h). White **crystalline** solid. mp: 232 – 233 °C. IR (KBr, cm^{-1}): 3380, 1710, 1548, 1336. ^1H NMR (400 MHz, DMSO- d_6) δ 10.42 (s, 1H), 8.22 (d, $J = 2.4$ Hz, 1H), 7.52 (d, $J = 8.0$ Hz, 1H), 7.29 (s, 1H), 7.24 (d, $J = 8.2$ Hz, 1H), 7.15 – 7.10 (m, 1H), 7.02 (t, $J = 7.6$ Hz, 1H), 6.83 (t, $J = 7.4$ Hz, 1H), 6.62 (d, $J = 8.4$ Hz, 1H), 6.32 – 6.24 (m, 1H), 5.01 (d, $J = 10.8$ Hz, 1H), 4.59 (q, $J = 8.2$ Hz, 1H), 3.66 (s, 3H), 3.54 – 3.47 (m, 1H), 2.61 (t, $J = 7.2$ Hz, 1H), 2.03 – 1.92 (m, 2H), 1.69 – 1.61 (m, 1H), 1.50 – 1.40 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 178.50, 143.28, 142.88, 136.42, 128.00, 127.89, 127.59, 123.70, 122.14, 122.01, 121.69, 119.47, 119.15, 118.92, 110.68, 110.02, 106.54, 93.21, 74.84, 63.54, 50.82, 43.54, 32.94, 28.02, 25.66. ^{19}F NMR (376 MHz, DMSO- d_6) δ -57.29. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{24}H_{22}F_3N_4O_4$: 487.1593; Found: 487.1598.

1'-nitro-2'-(1-tosyl-1*H*-indol-3-yl)-5-(trifluoromethoxy)-1',2',5',6',7',7a'-

hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6i). White **crystalline** solid. mp: 240 – 241 °C. IR (KBr, cm^{-1}): 3409, 1722, 1545, 1365. ^1H NMR (400 MHz, DMSO- d_6) δ 10.67 (s, 1H), 8.20 (d, $J = 2.4$ Hz, 1H), 7.77 (s, 1H), 7.74 – 7.64 (m, 4H), 7.32 (d, $J = 8.0$ Hz, 2H), 7.19 (t, $J = 7.8$ Hz, 1H), 7.13 – 7.03 (m, 2H), 6.64 (d, $J = 8.4$ Hz, 1H), 6.56 – 6.46 (m, 1H), 5.01 (d, $J = 10.8$ Hz, 1H), 4.61 (q, $J = 8.4$ Hz, 1H), 3.52 – 3.45 (m, 1H), 2.59 (t, $J = 7.2$ Hz, 1H), 2.29 (s, 3H), 2.05 – 1.91 (m, 2H), 1.70 – 1.62 (m, 1H), 1.48 – 1.38 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 178.35, 145.88, 143.26, 142.60, 134.06, 134.02, 130.70, 130.61, 126.95, 126.82, 125.62, 125.34, 123.90, 123.39, 122.19, 121.93, 120.51, 119.39, 116.52, 113.29, 110.76, 92.00, 74.77, 63.81, 50.70, 42.22, 28.51, 25.63, 21.46. ^{19}F NMR (376 MHz, DMSO- d_6) δ -57.33. HRMS (ESI, m/z): $[M+H]^+$ calcd. For $C_{30}H_{26}F_3N_4O_6S$: 627.1525; Found: 627.1521.

5-methyl-2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6j). White **crystalline** solid. mp: 240 – 241 °C. IR (KBr, cm^{-1}): 3377, 1705, 1542, 1337. ^1H NMR (400 MHz, DMSO- d_6) δ 10.15 (s, 1H), 7.79 (s, 1H), 7.49 (d, $J = 8.0$ Hz, 1H), 7.24 (t, $J = 4.0$ Hz, 2H), 7.02 (t, $J = 7.6$ Hz, 1H), 6.93 – 6.84 (m, 2H), 6.45 (d, $J = 7.8$ Hz, 1H),

6.27 – 6.19 (m, 1H), 4.89 (d, J = 10.6 Hz, 1H), 4.62 (q, J = 8.2 Hz, 1H), 3.65 (s, 3H), 3.44 – 3.39 (m, 1H), 2.64 (t, J = 7.2 Hz, 1H), 2.28 (s, 3H), 2.05 – 1.91 (m, 2H), 1.67 (tt, J = 11.6, 4.8 Hz, 1H), 1.46 – 1.36 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 178.49, 141.10, 136.44, 130.69, 130.21, 127.99, 127.94, 126.00, 121.65, 119.06, 110.04, 109.66, 106.90, 94.09, 74.65, 63.58, 51.03, 43.92, 32.92, 27.93, 25.64, 21.16. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}_3$: 417.1927; Found: 417.1932.

5,7-dimethyl-2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6k). Light yellow crystalline solid. mp: 203 – 204 °C. IR (KBr, cm^{-1}): 3139, 1706, 1624, 1538, 1337. ^1H NMR (400 MHz, DMSO- d_6) δ 10.23 (s, 1H), 7.59 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.25 (t, J = 4.0 Hz, 2H), 7.03 (t, J = 7.2 Hz, 1H), 6.90 (t, J = 7.4 Hz, 1H), 6.72 (s, 1H), 6.26 – 6.20 (m, 1H), 4.88 (d, J = 10.8 Hz, 1H), 4.62 (q, J = 8.2 Hz, 1H), 3.64 (s, 3H), 3.49 – 3.42 (m, 1H), 2.59 (t, J = 7.4 Hz, 1H), 2.23 (s, 3H), 2.04 – 1.94 (m, 2H), 1.95 (s, 3H), 1.71 – 1.60 (m, 1H), 1.44 – 1.35 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 178.97, 139.74, 136.42, 131.80, 130.55, 128.03, 125.63, 125.19, 121.68, 119.12, 119.08, 118.73, 110.06, 107.05, 94.46, 74.71, 63.43, 56.51, 50.99, 43.65, 32.93, 27.89, 25.60, 21.06, 19.03, 16.61. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{25}\text{H}_{27}\text{N}_4\text{O}_3$: 431.2083; Found: 431.2084.

2'-(1-methyl-1*H*-indol-3-yl)-1'-nitro-1-phenyl-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6l). White crystalline solid. mp: 190 – 191 °C. IR (KBr, cm^{-1}): 1715, 1541, 1369. ^1H NMR (400 MHz, DMSO- d_6) δ 8.15 – 8.09 (m, 1H), 7.50 (t, J = 7.6 Hz, 2H), 7.46 – 7.40 (m, 2H), 7.32 (s, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.16 – 7.09 (m, 2H), 7.04 – 7.00 (m, 3H), 6.87 (t, J = 7.4 Hz, 1H), 6.39 (dd, J = 6.6, 1.8 Hz, 1H), 6.26 (t, J = 10.0 Hz, 1H), 5.03 (d, J = 10.6 Hz, 1H), 4.68 (q, J = 8.2 Hz, 1H), 3.69 (s, 3H), 3.56 – 3.51 (m, 1H), 2.76 (t, J = 7.4 Hz, 1H), 2.09 – 1.97 (m, 2H), 1.77 – 1.68 (m, 1H), 1.53 – 1.44 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 176.28, 144.67, 136.45, 133.97, 130.32, 130.15, 128.71, 127.96, 127.92, 127.59, 126.88, 125.18, 123.20, 121.75, 119.15, 118.92, 110.07, 109.37, 106.53, 93.62, 74.60, 64.07, 50.95, 44.88, 32.90, 28.03, 25.75. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{29}\text{H}_{27}\text{N}_4\text{O}_3$: 479.2083; Found: 479.2080.

2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6m). White crystalline solid. mp: 179–180 °C. IR (KBr, cm^{-1}): 3259, 1714, 1543, 1339. ^1H NMR (400 MHz, CDCl_3) δ 7.88 (s, 1H), 7.69 (d, J = 7.4 Hz, 1H), 7.16 (td, J = 7.6, 1.4 Hz, 1H), 7.07 (td, J = 7.6, 1.2 Hz, 1H), 6.99 – 6.93 (m, 2H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H),

6.66 (d, $J = 2.4$ Hz, 1H), 6.63 – 6.58 (m, 1H), 6.12 (t, $J = 9.6$ Hz, 1H), 4.92 (d, $J = 10.0$ Hz, 1H), 4.84 (q, $J = 8.0$ Hz, 1H), 3.73 (s, 3H), 3.37 (s, 3H), 3.36 – 3.30 (m, 1H), 2.88 (td, $J = 7.0, 3.6$ Hz, 1H), 2.17 – 2.00 (m, 2H), 1.90 – 1.81 (m, 1H), 1.61 – 1.51 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 178.81, 153.86, 142.10, 131.79, 130.00, 127.99, 127.70, 126.14, 126.02, 122.31, 112.18, 110.32, 109.95, 106.07, 100.24, 94.17, 74.88, 64.21, 55.89, 50.83, 45.65, 32.68, 27.61, 25.65. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}_4$: 433.1876; Found: 433.1867.

2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6n). Off-white **crystalline** solid. mp: 220 – 221 °C. IR (KBr, cm^{-1}): 3138, 1719, 1621, 1544, 1369, 1281. ^1H NMR (400 MHz, CDCl_3) δ 7.70 (d, $J = 9.0$ Hz, 1H), 7.62 – 7.55 (m, 3H), 7.53 (s, 1H), 7.37 (s, 1H), 7.21 – 7.14 (m, 3H), 7.05 (t, $J = 7.6$ Hz, 1H), 6.78 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.65 – 6.56 (m, 2H), 6.12 (t, $J = 9.4$ Hz, 1H), 4.88 (q, $J = 8.0$ Hz, 1H), 4.78 (d, $J = 9.8$ Hz, 1H), 3.70 (s, 3H), 3.28 (ddd, $J = 10.4, 8.2, 5.6$ Hz, 1H), 2.88 (t, $J = 7.4$ Hz, 1H), 2.33 (s, 3H), 2.20 – 2.10 (m, 1H), 2.06 – 1.98 (m, 1H), 1.89 – 1.80 (m, 1H), 1.57 – 1.47 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 177.55, 156.30, 144.89, 141.80, 134.80, 131.02, 130.24, 129.81, 129.38, 126.69, 125.83, 125.64, 125.34, 122.45, 115.64, 114.38, 114.27, 110.34, 101.48, 93.34, 74.31, 64.54, 55.62, 50.57, 45.35, 27.56, 25.62, 21.57. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{30}\text{H}_{29}\text{N}_4\text{O}_6\text{S}$: 573.1808; Found: 573.1788.

5-chloro-2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6o). White **crystalline** solid. mp: 232 – 233 °C. IR (KBr, cm^{-1}): 3189, 1717, 1616, 1547, 1358, 1282. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.60 (s, 1H), 8.27 (d, $J = 2.2$ Hz, 1H), 7.71 (s, 1H), 7.63 (d, $J = 8.0$ Hz, 2H), 7.57 (d, $J = 9.0$ Hz, 1H), 7.31 (d, $J = 8.0$ Hz, 2H), 7.24 (d, $J = 2.4$ Hz, 1H), 7.14 (dd, $J = 8.2, 2.0$ Hz, 1H), 6.78 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.58 (d, $J = 8.4$ Hz, 1H), 6.51 – 6.43 (m, 1H), 4.95 (d, $J = 10.8$ Hz, 1H), 4.60 (q, $J = 8.4$ Hz, 1H), 3.78 (s, 3H), 3.51 – 3.44 (m, 1H), 2.61 (t, $J = 7.2$ Hz, 1H), 2.29 (s, 3H), 2.06 – 1.93 (m, 2H), 1.73 – 1.62 (m, 1H), 1.49 – 1.38 (m, 1H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 178.02, 156.58, 145.68, 142.27, 133.92, 132.03, 130.54, 130.27, 128.65, 128.08, 127.25, 126.83, 126.32, 125.95, 117.05, 115.01, 114.27, 111.33, 102.41, 92.02, 79.65, 74.75, 63.77, 56.07, 50.79, 42.14, 28.50, 25.61, 21.47. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{30}\text{H}_{28}\text{ClN}_4\text{O}_6\text{S}$: 607.1418; Found: 607.1411.

5-methoxy-2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-

hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6p). Light **crystalline** brown solid. mp: 178 – 179 °C. IR (KBr, cm⁻¹): 3253, 1711, 1543, 1486. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.01 (s, 1H), 7.75 (d, *J* = 2.6 Hz, 1H), 7.22 (s, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.69 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.46 (d, *J* = 8.4 Hz, 1H), 6.26 (dd, *J* = 10.8, 9.4 Hz, 1H), 4.92 (d, *J* = 10.8 Hz, 1H), 4.60 (q, *J* = 8.2 Hz, 1H), 3.76 (s, 3H), 3.70 (s, 3H), 3.62 (s, 3H), 3.51 (ddd, *J* = 11.0, 8.4, 5.2 Hz, 1H), 2.65 (t, *J* = 7.4 Hz, 1H), 2.04 – 1.93 (m, 2H), 1.73 – 1.62 (m, 1H), 1.50 – 1.40 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.34, 154.99, 153.74, 136.86, 131.78, 128.35, 128.32, 127.34, 114.98, 114.65, 112.17, 110.67, 110.23, 106.57, 100.94, 93.50, 74.99, 63.50, 56.07, 55.72, 50.80, 43.88, 33.06, 27.98, 25.67. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₂₅H₂₇N₄O₅: 463.1981; Found: 463.1957.

5-methoxy-2'-(5-methoxy-1-tosyl-1*H*-indol-3-yl)-1'-nitro-1',2',5',6',7',7a'-

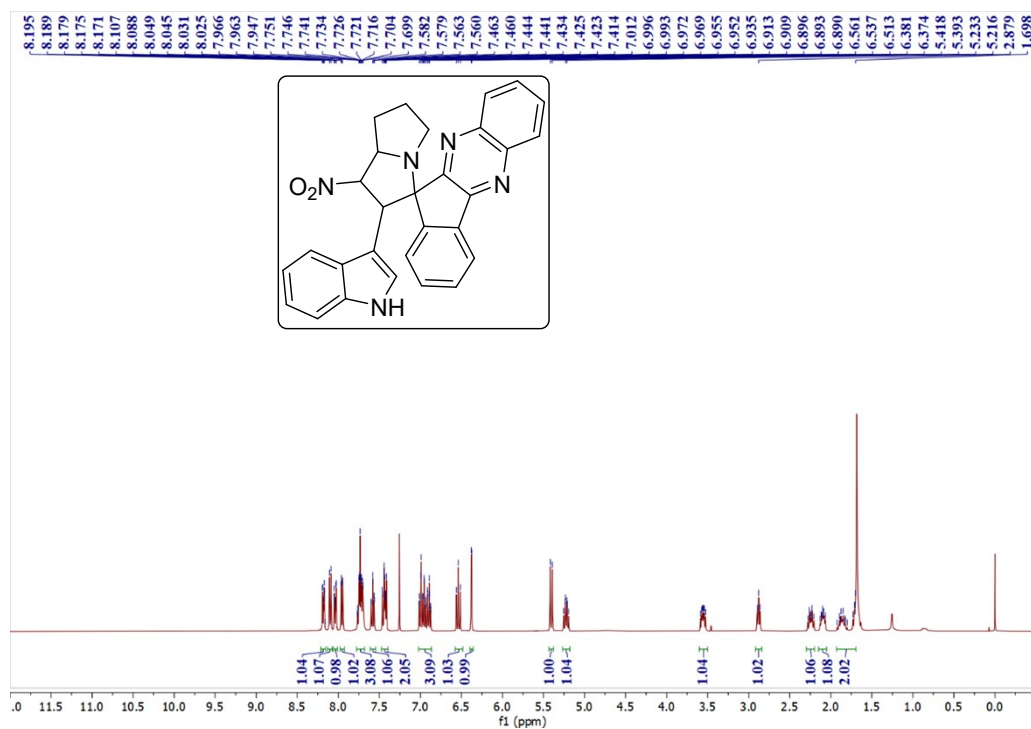
hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6q). White **crystalline** solid. mp: 221 – 222 °C. IR (KBr, cm⁻¹): 3375, 1715, 1545, 1366, 1291. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.31 (s, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.69 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 6.44 (t, *J* = 10.0 Hz, 1H), 4.91 (d, *J* = 10.6 Hz, 1H), 4.62 (q, *J* = 8.4 Hz, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.49 (q, *J* = 6.2 Hz, 1H), 2.62 (t, *J* = 7.4 Hz, 1H), 2.29 (s, 3H), 2.06 – 1.92 (m, 2H), 1.72 – 1.62 (m, 1H), 1.47 – 1.38 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.23, 156.49, 154.94, 145.63, 136.51, 133.96, 132.17, 130.55, 128.71, 126.82, 126.47, 126.26, 117.42, 114.70, 114.54, 114.16, 110.28, 103.04, 92.65, 79.65, 74.99, 63.74, 56.02, 55.94, 50.69, 42.27, 28.43, 25.63, 21.47. HRMS (ESI, *m/z*): [M+H]⁺ calcd. For C₃₁H₃₀N₄O₇S: 603.1913; Found: 603.1892.

2'-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1'-nitro-5-(trifluoromethoxy)-1',2',5',6',7',7a'-

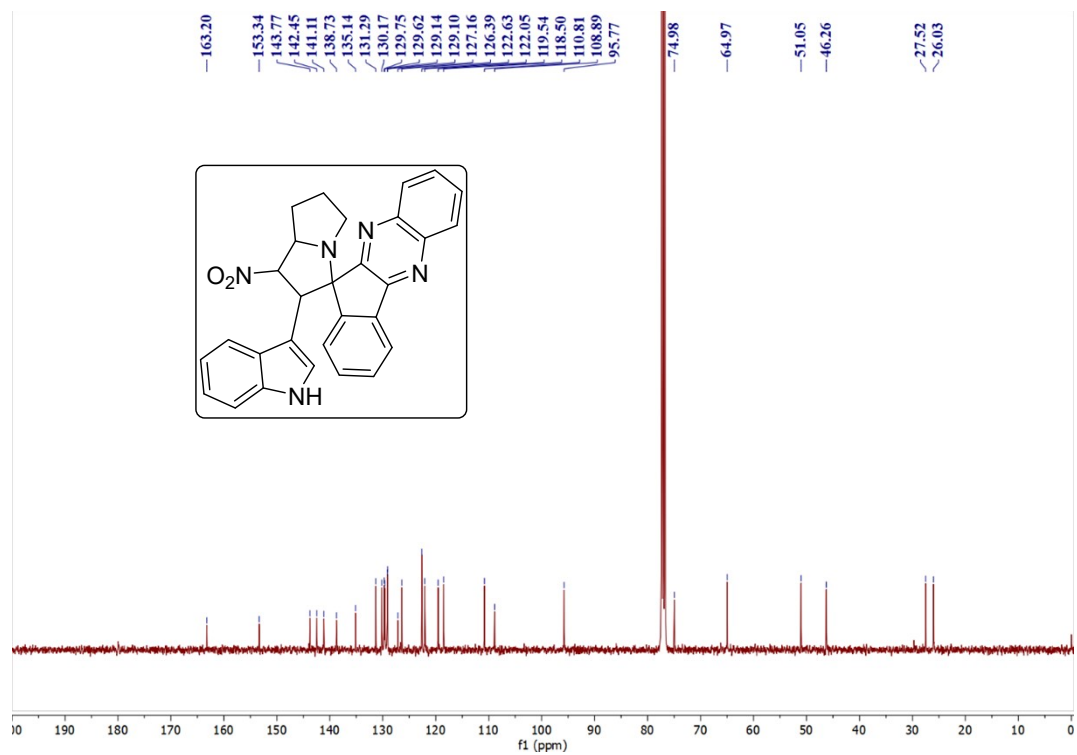
hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (6r). White **crystalline** solid. mp: 222 – 223 °C. IR (KBr, cm⁻¹): 3377, 1708, 1626, 1548, 1338. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.44 (s, 1H), 8.28 (d, *J* = 2.4 Hz, 1H), 7.21 (s, 1H), 7.14 – 7.11 (m, 3H), 6.68 – 6.61 (m, 2H), 6.28 – 6.20 (m, 1H), 5.00 (d, *J* = 10.8 Hz, 1H), 4.58 (q, *J* = 8.4 Hz, 1H), 3.72 (s, 3H), 3.61 (s, 3H), 3.54 – 3.48 (m, 1H), 2.60 (t, *J* = 7.2 Hz, 1H), 2.00 – 1.93 (m, 2H), 1.71 – 1.62 (m, 1H), 1.50 – 1.40 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.60, 153.91, 143.31, 142.84, 131.70, 128.39, 128.32, 127.73,

123.34, 122.08, 121.96, 119.42, 112.21, 110.74, 110.64, 106.16, 100.84, 93.34, 74.83, 63.47, 55.73, 50.71, 43.39, 33.07, 27.97, 25.62. ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ -57.30. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{25}\text{H}_{24}\text{F}_3\text{N}_4\text{O}_5$: 517.1699; Found: 517.1710.

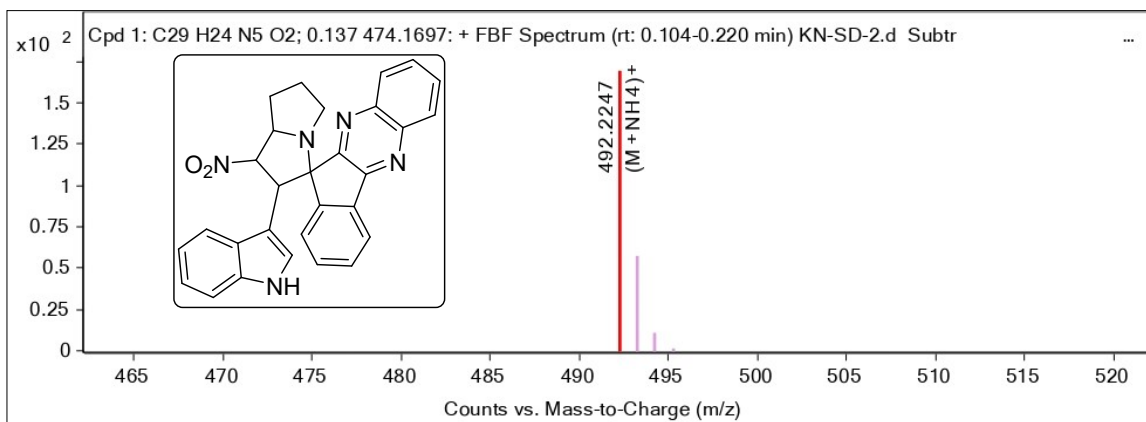
6. NMR, Mass, FTIR spectral images of the Compounds 4a-s and 6a-r



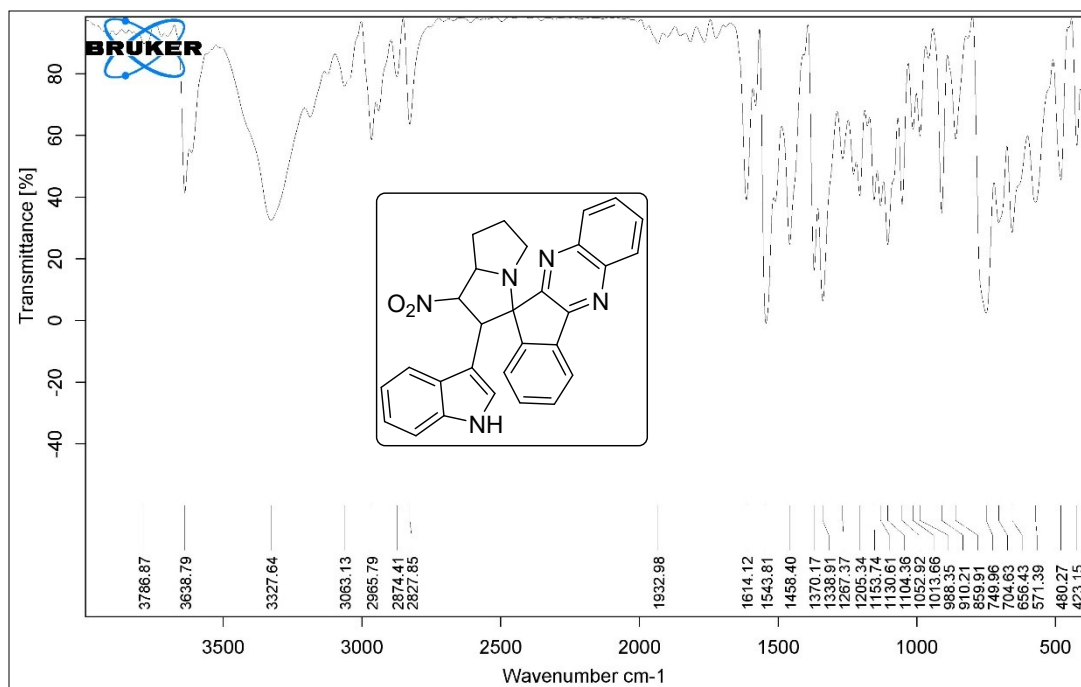
^1H NMR spectrum of the compound **4a** (400 MHz, CDCl_3)



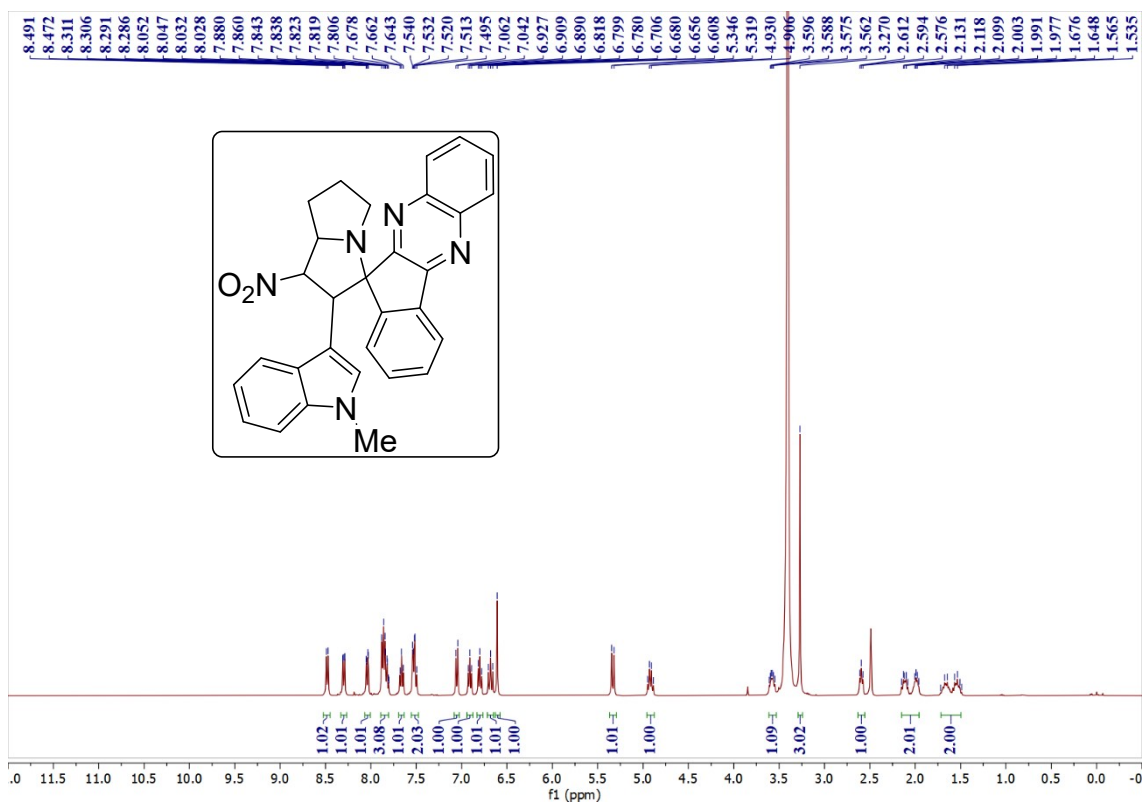
^{13}C NMR spectrum of the compound **4a** (100 MHz, CDCl_3)



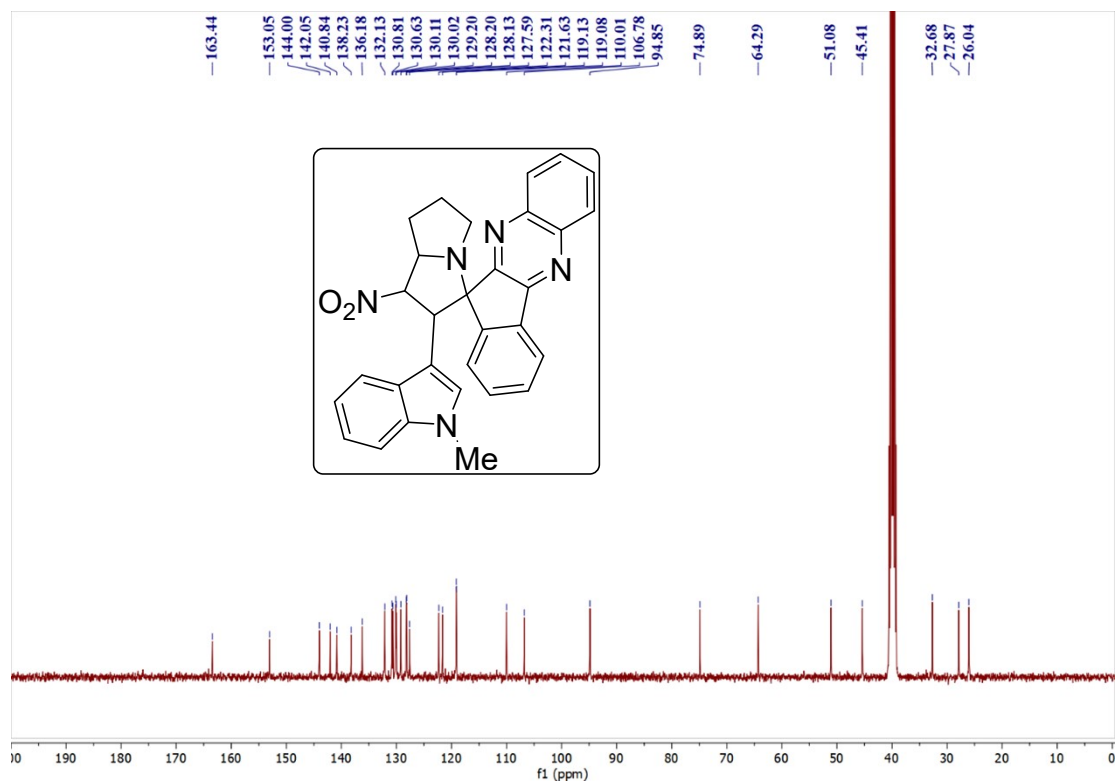
Mass spectrum of the compound **4a**



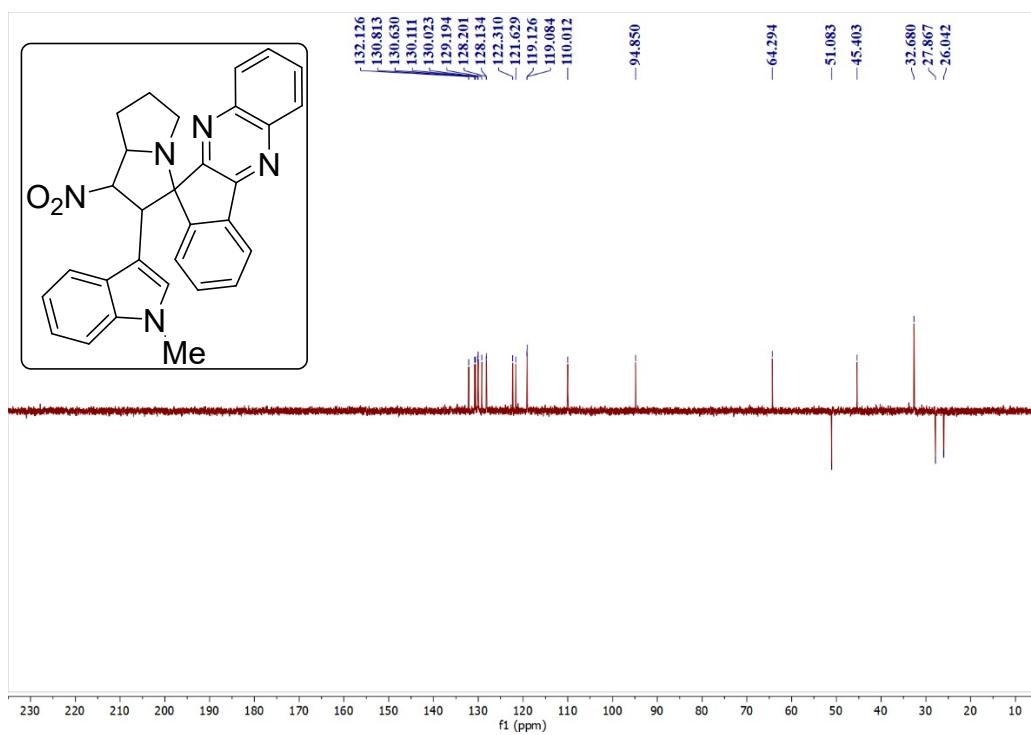
IR spectrum of the compound **4a**



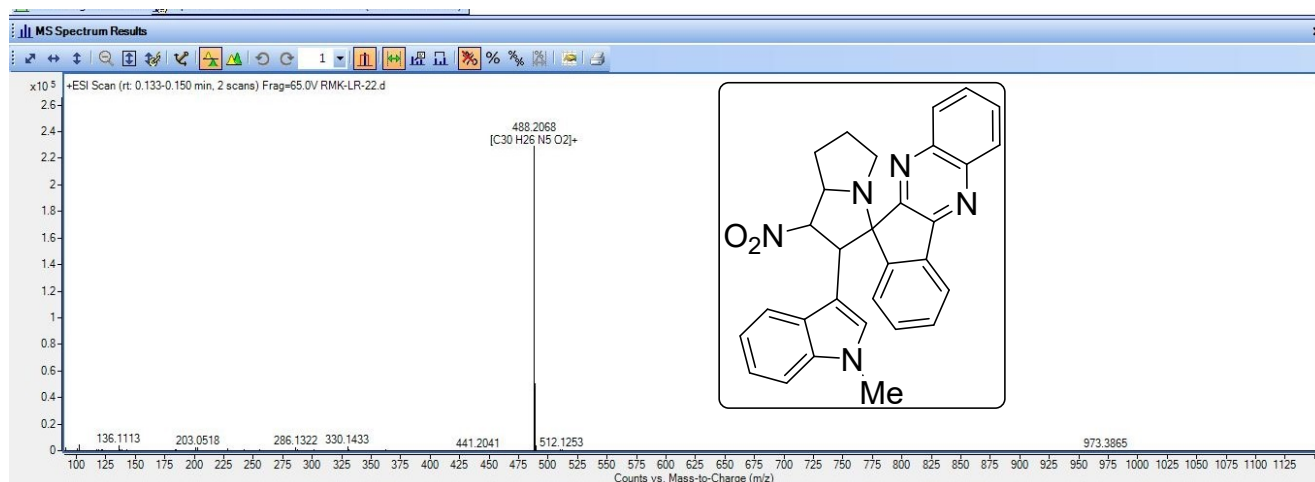
¹H NMR spectrum of the compound **4b** (400 MHz, DMSO-*d*₆)



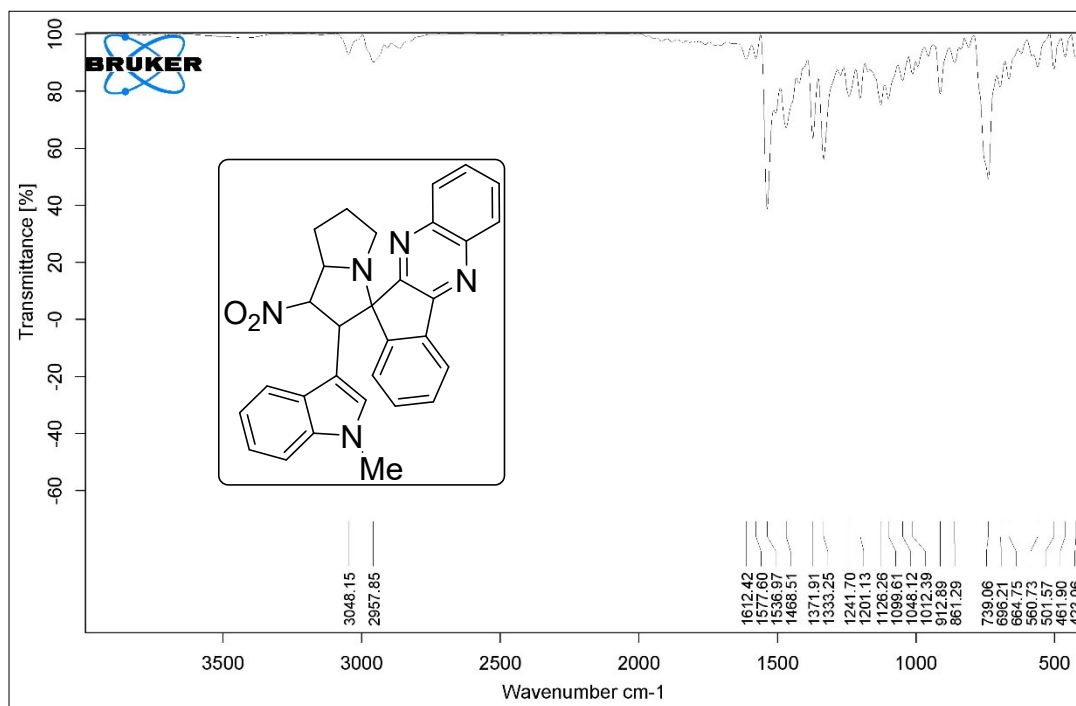
¹³C NMR spectrum of the compound **4b** (100 MHz, DMSO-*d*₆)



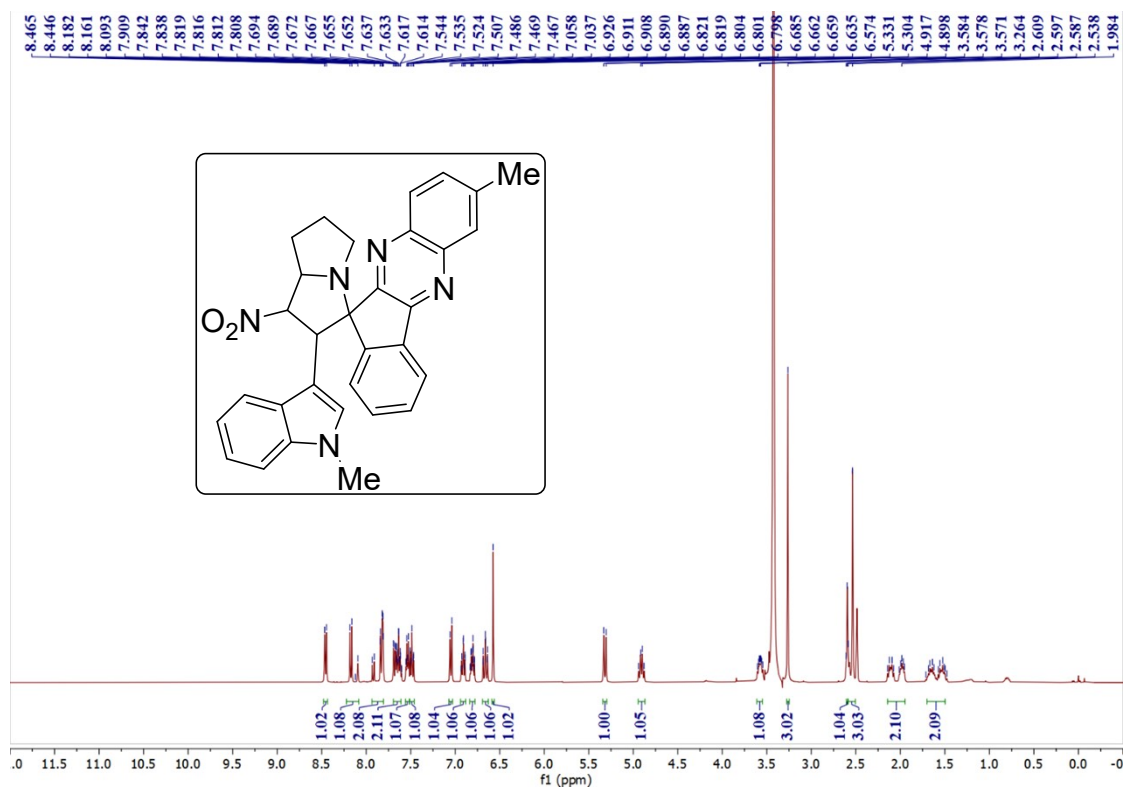
DEPT-135 NMR spectrum of the compound **4b** (100 MHz, DMSO-*d*₆)



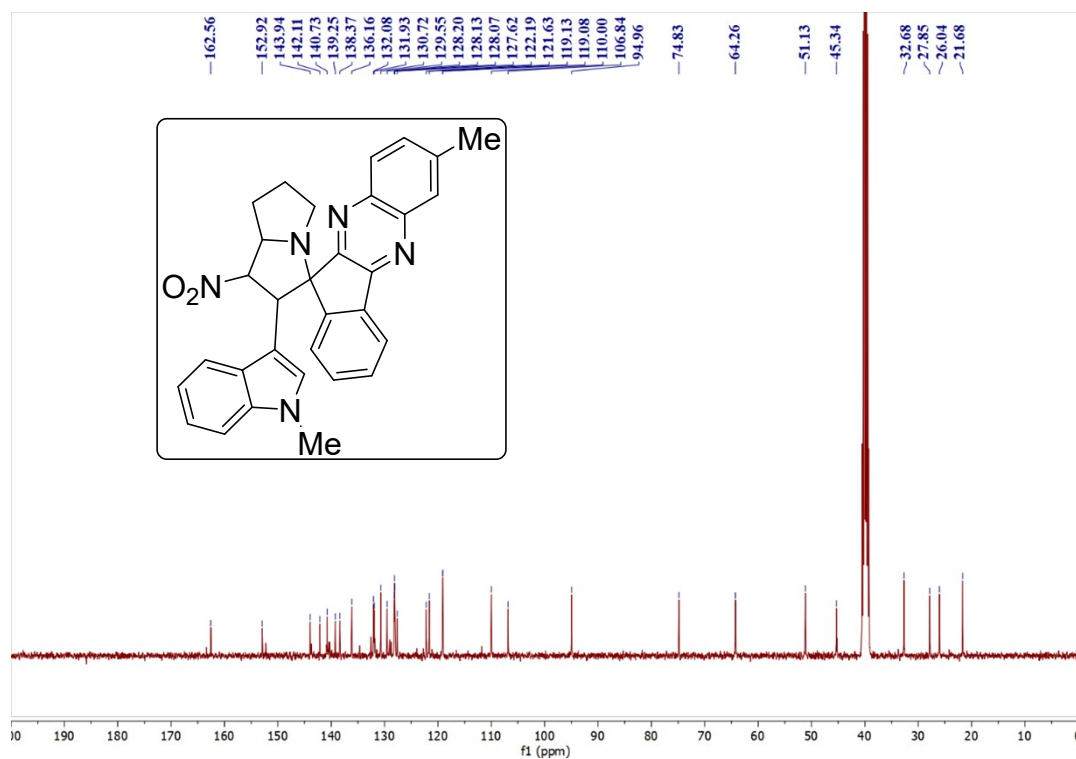
Mass spectrum of the compound **4b**



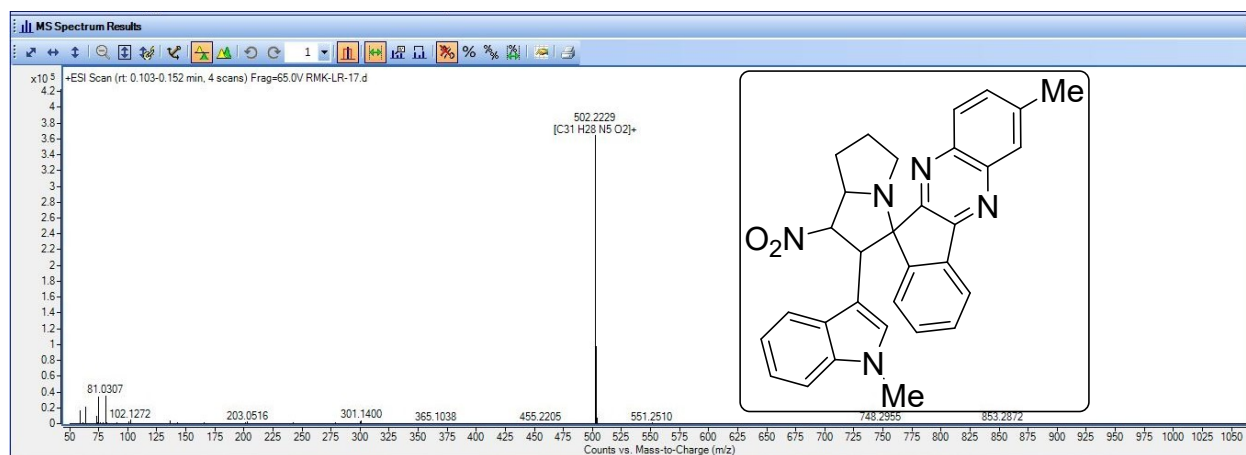
IR spectrum of the compound **4b**



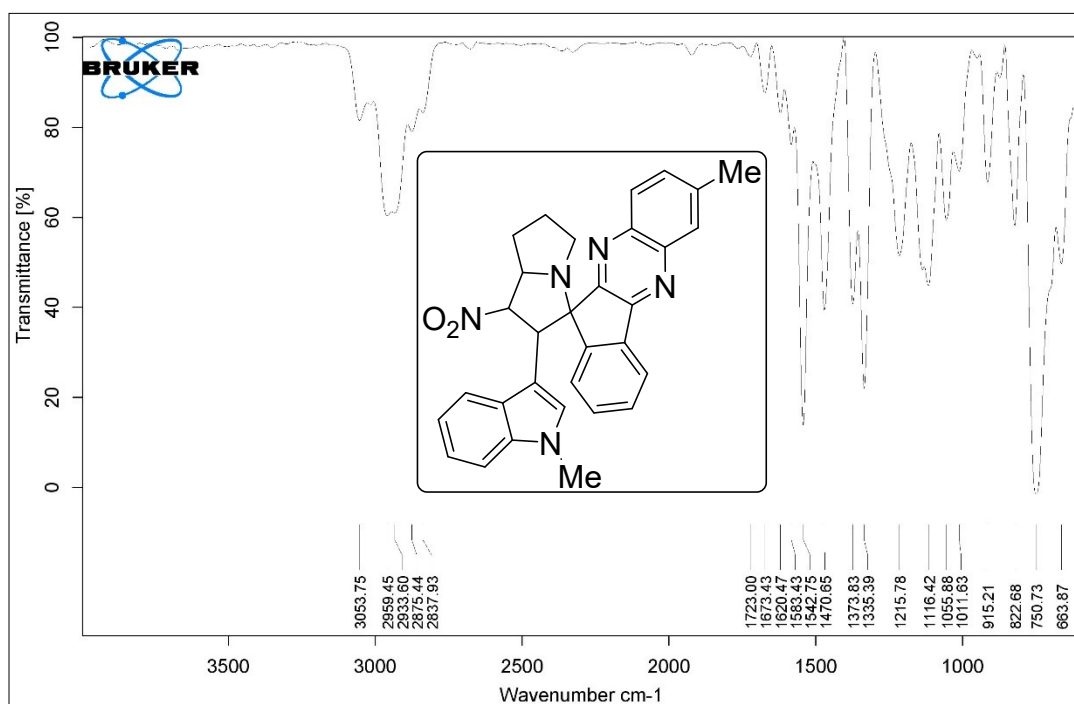
¹H NMR spectrum of the compound **4c** (400 MHz, DMSO-*d*₆)



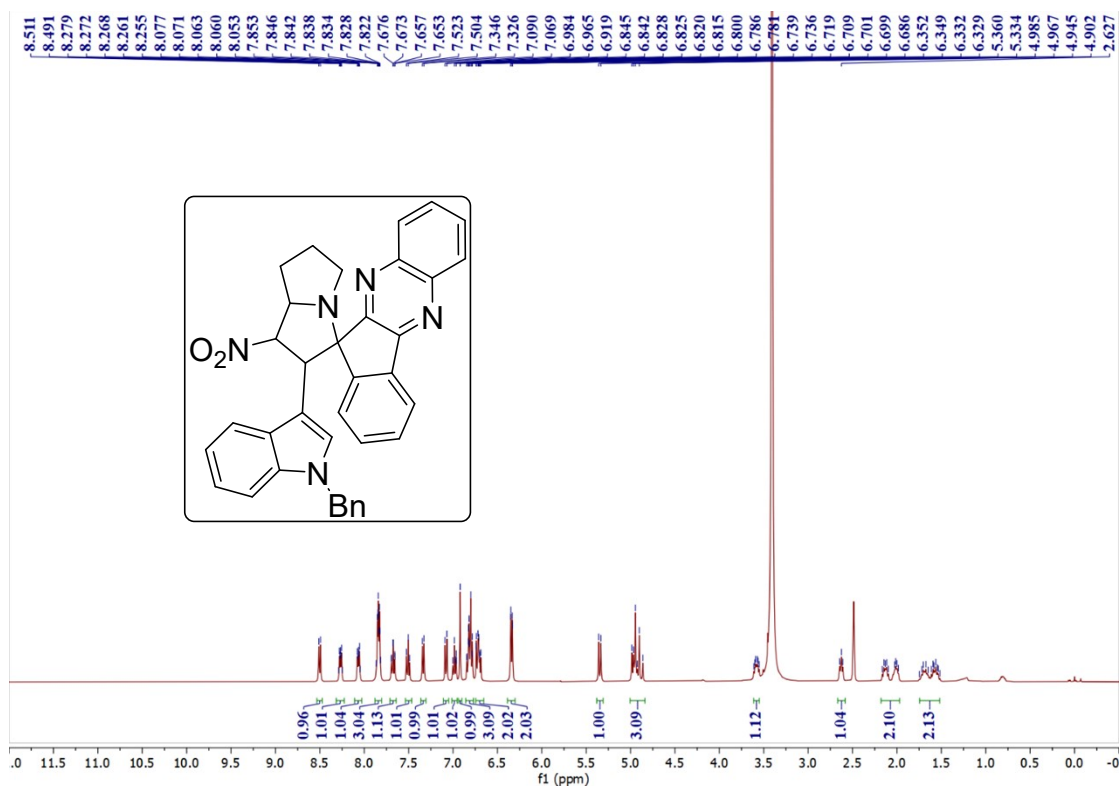
¹³C NMR spectrum of the compound **4c** (100 MHz, DMSO-*d*₆)



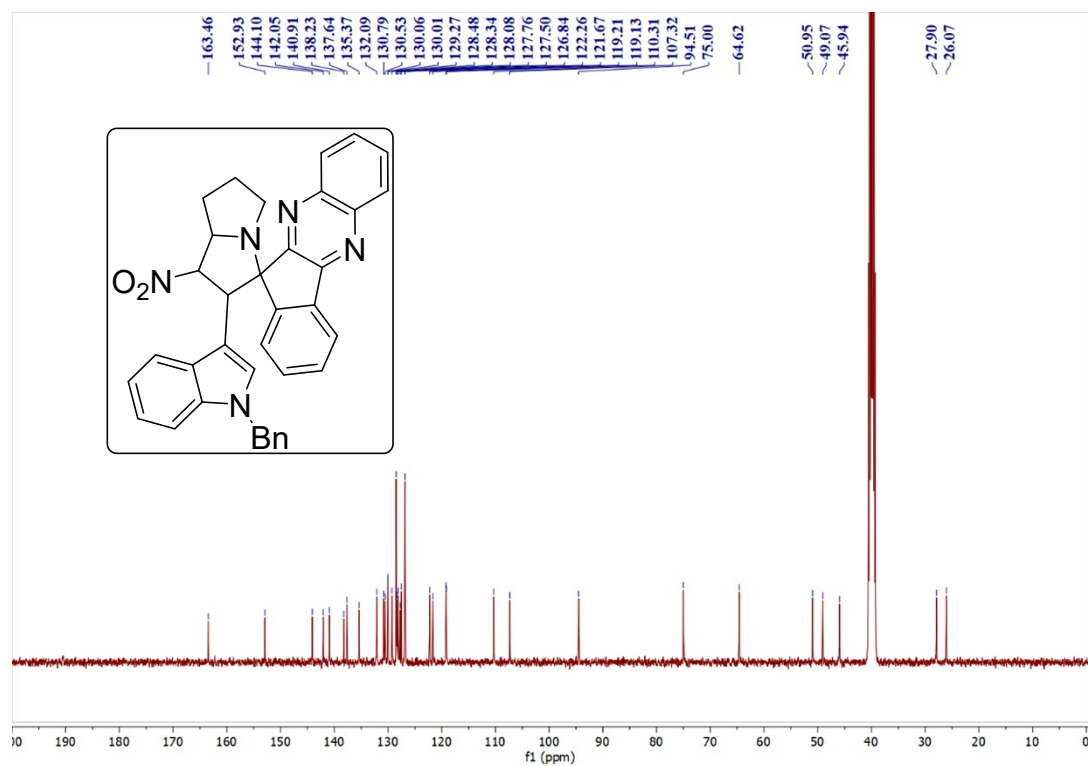
Mass spectrum of the compound **4c**



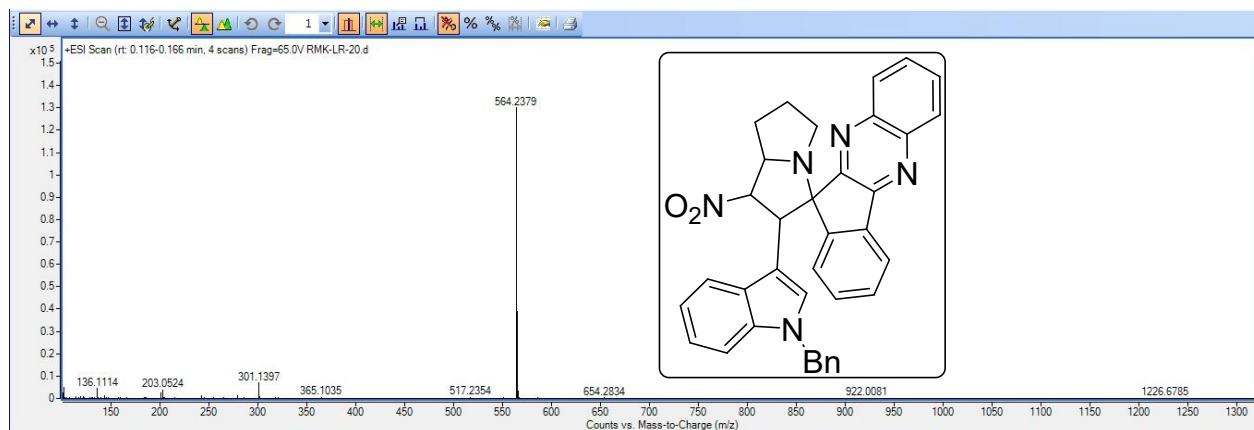
IR spectrum of the compound **4c**



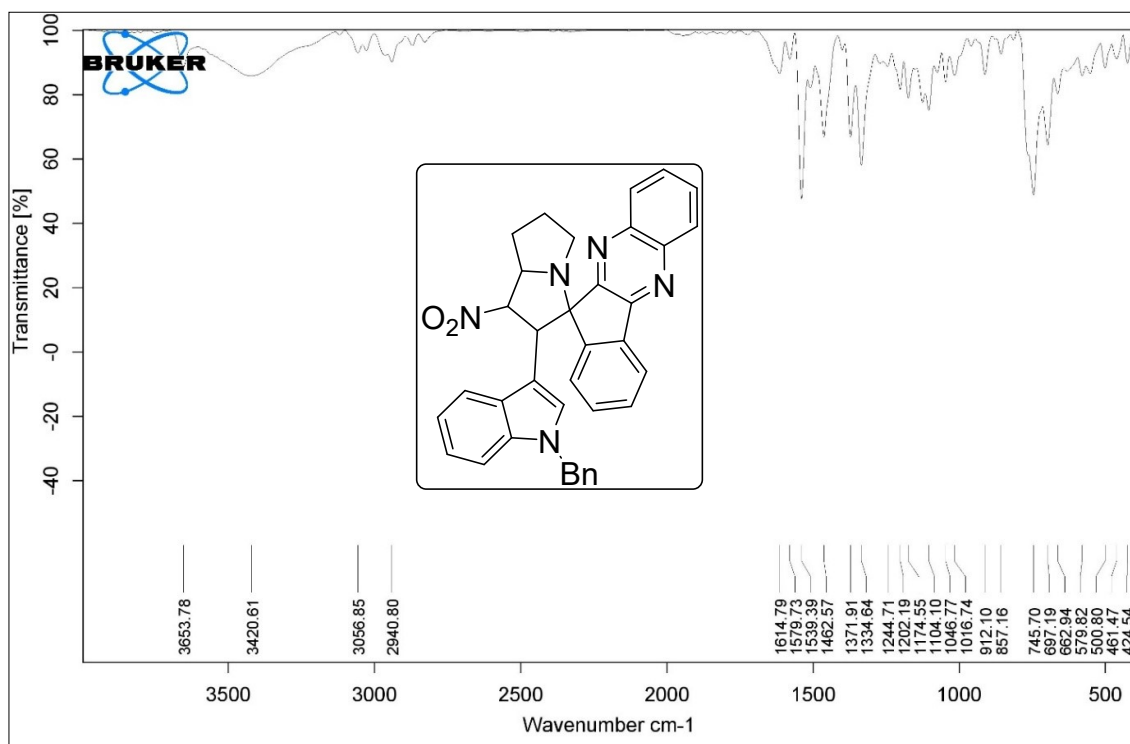
¹H NMR spectrum of the compound **4d** (400 MHz, DMSO-*d*₆)



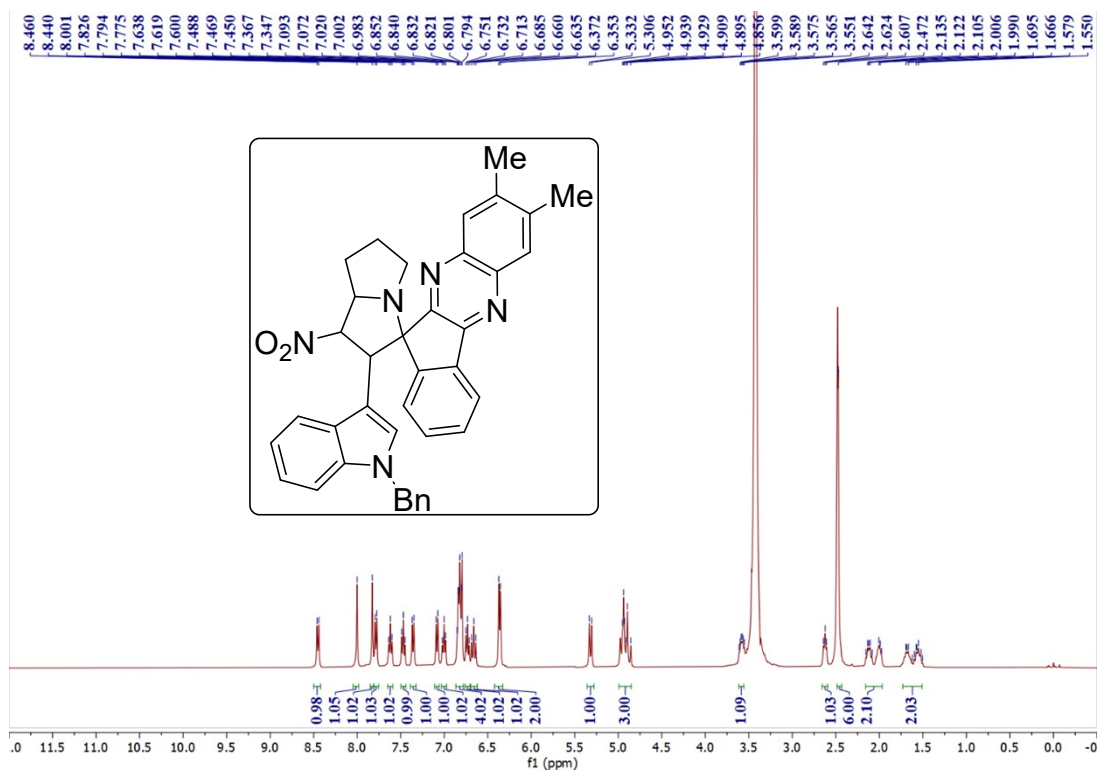
¹³C NMR spectrum of the compound **4d** (100 MHz, DMSO-*d*₆)



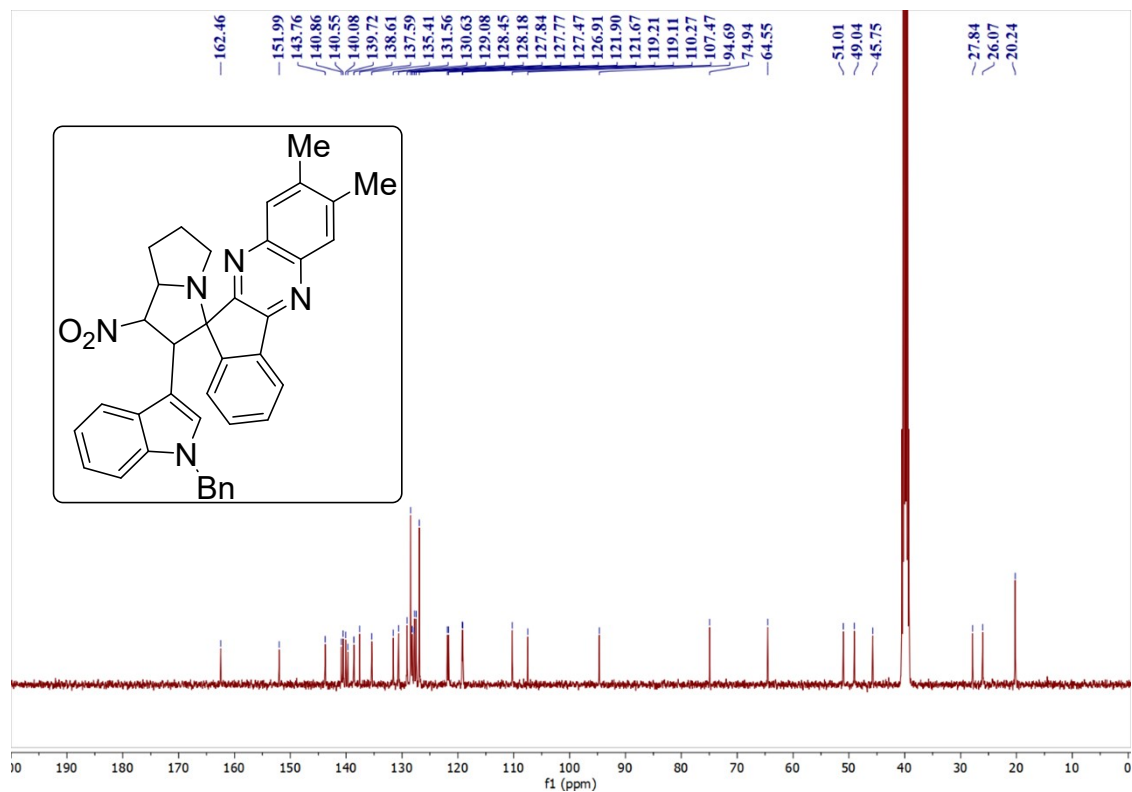
Mass spectrum of the compound **4d**



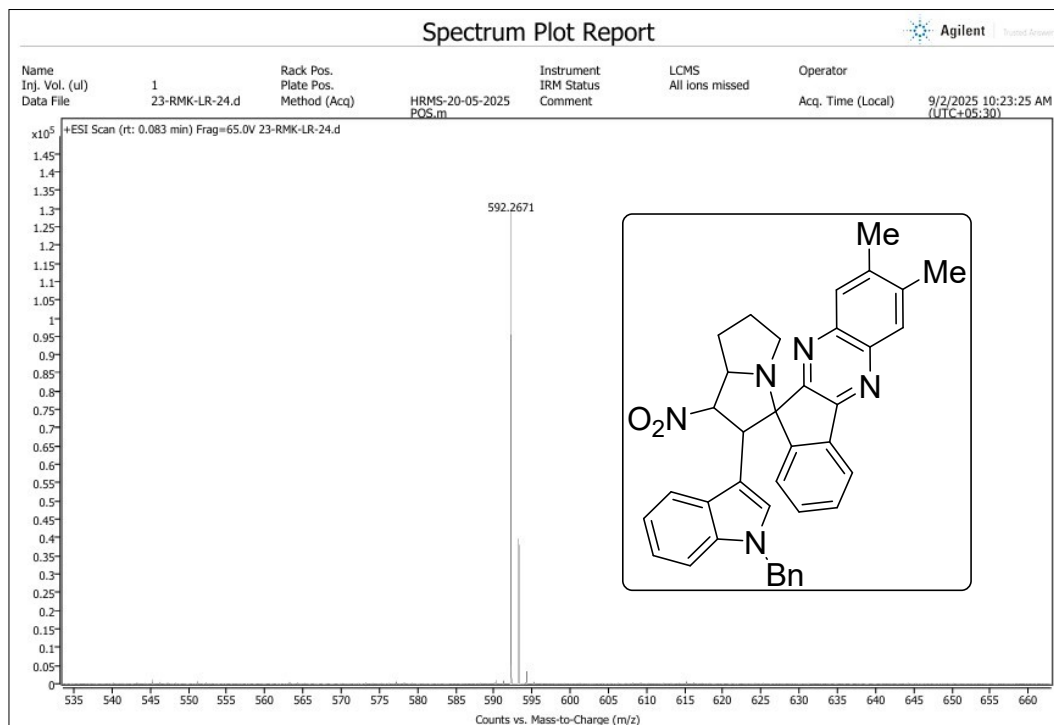
IR spectrum of the compound **4d**



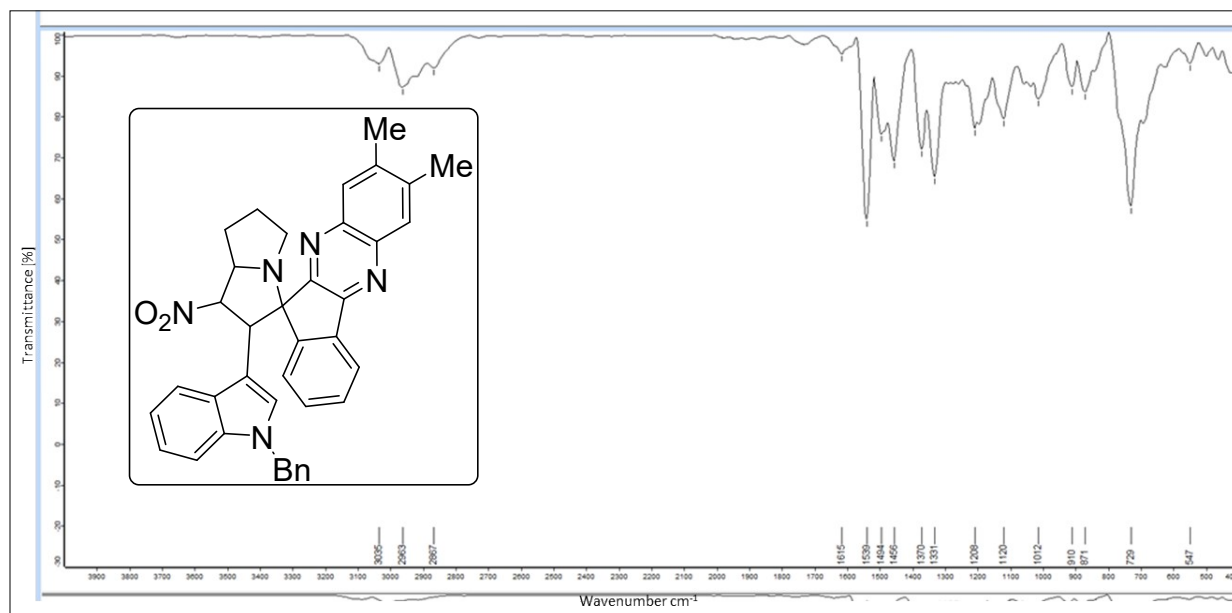
¹H NMR spectrum of the compound **4e** (400 MHz, DMSO-*d*₆)



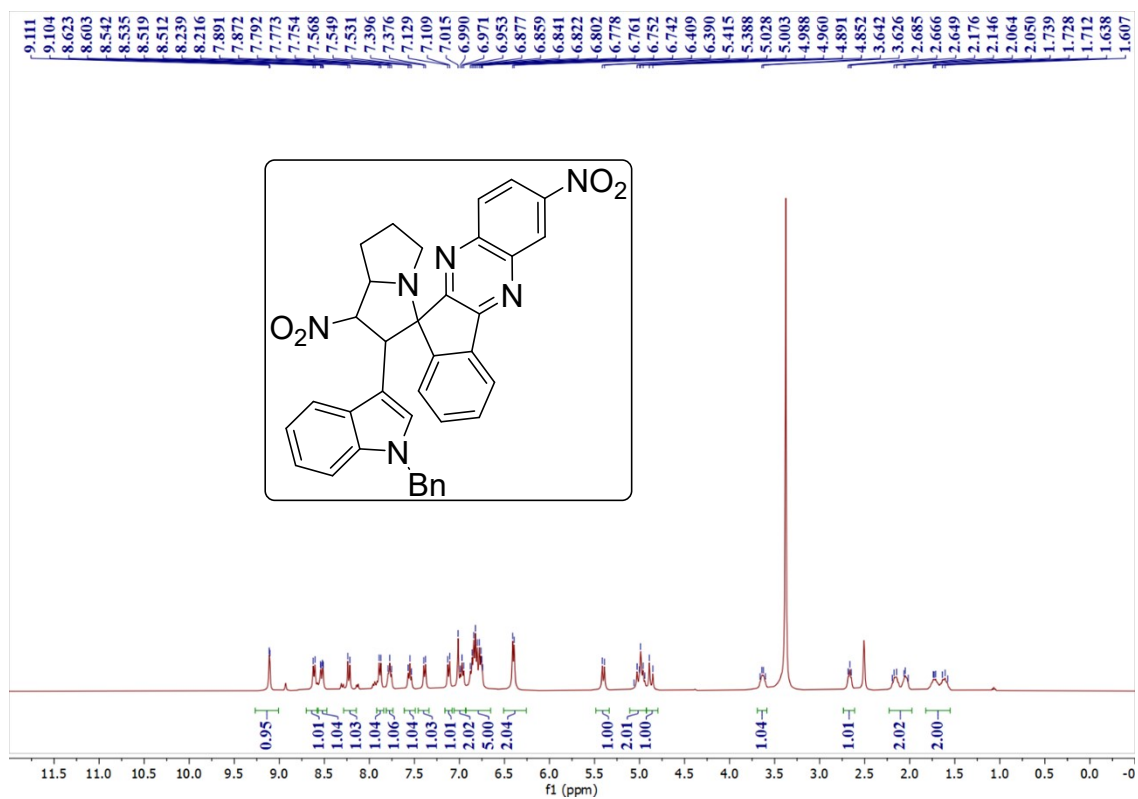
^{13}C NMR spectrum of the compound **4e** (100 MHz, $\text{DMSO}-d_6$)



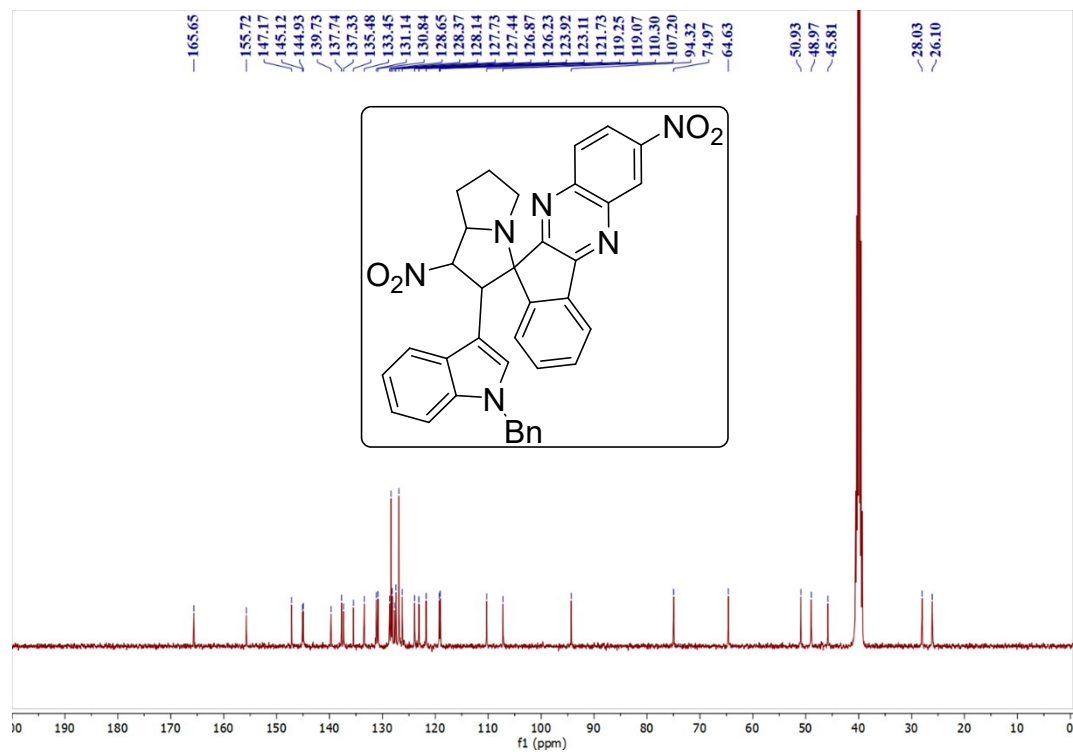
Mass spectrum of the compound **4e**



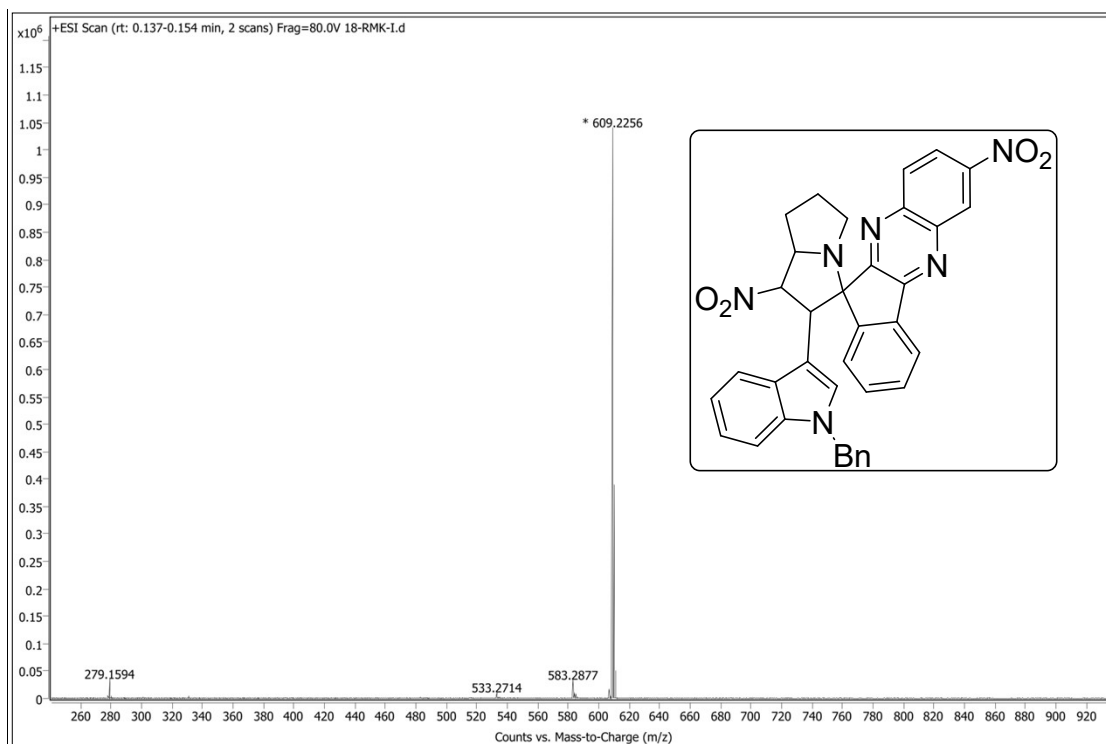
IR spectrum of the compound **4e**



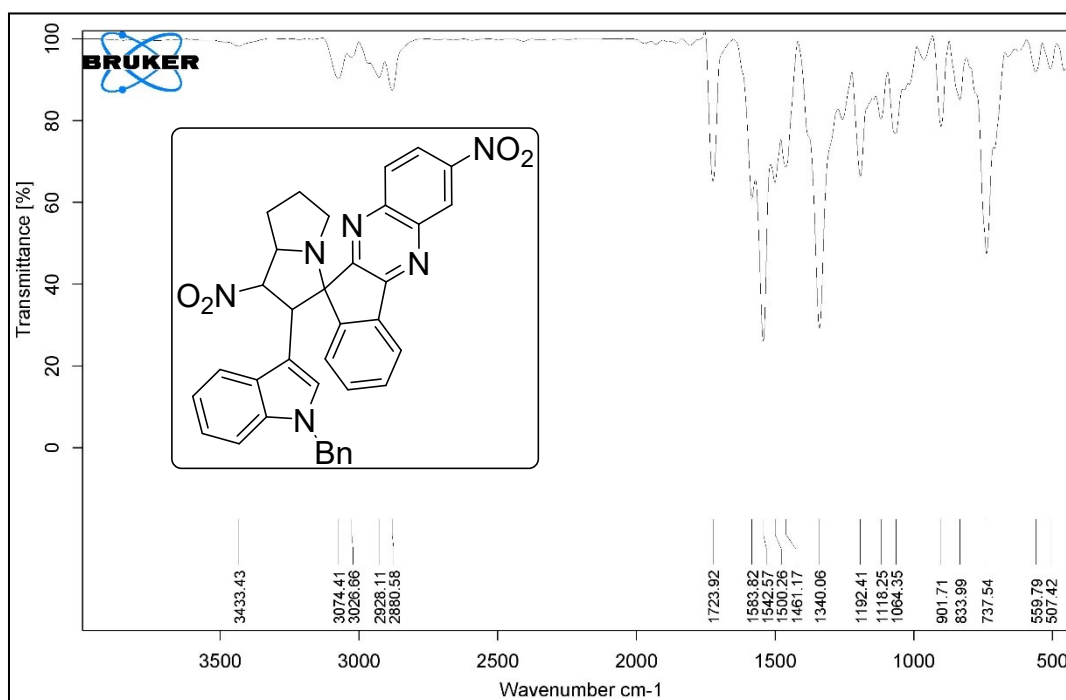
¹H NMR spectrum of the compound **4f** (400 MHz, DMSO-*d*₆)



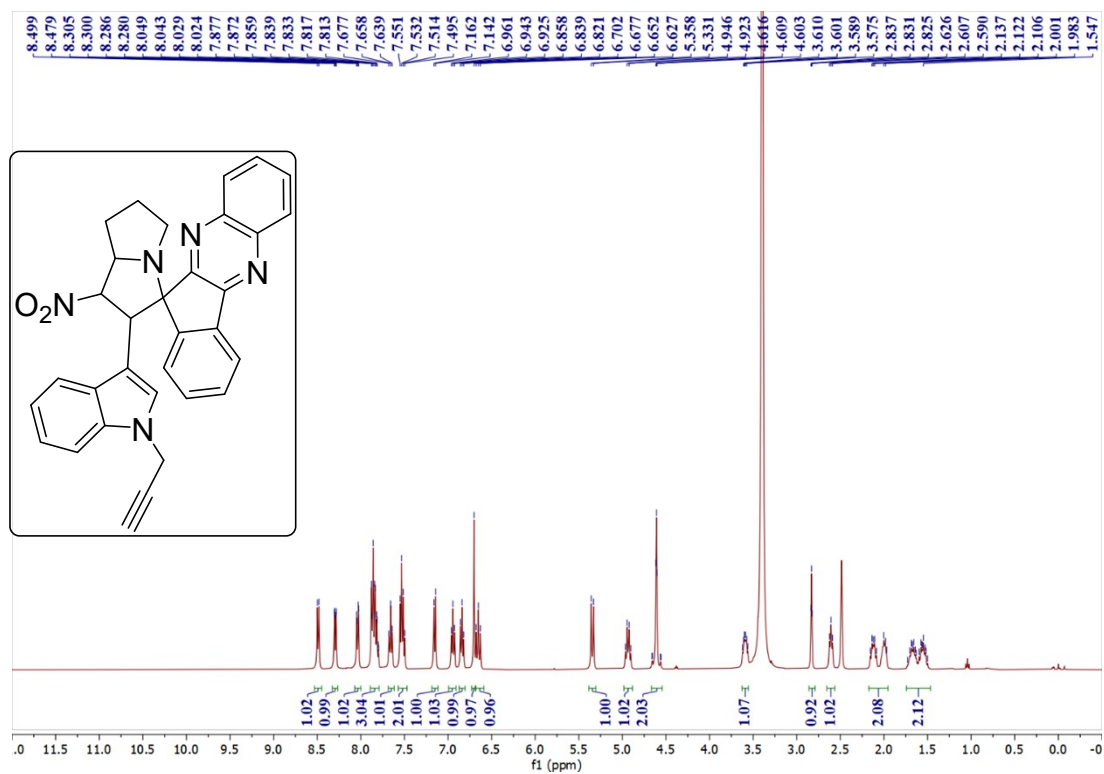
¹³C NMR spectrum of the compound **4f** (100 MHz, DMSO-*d*₆)



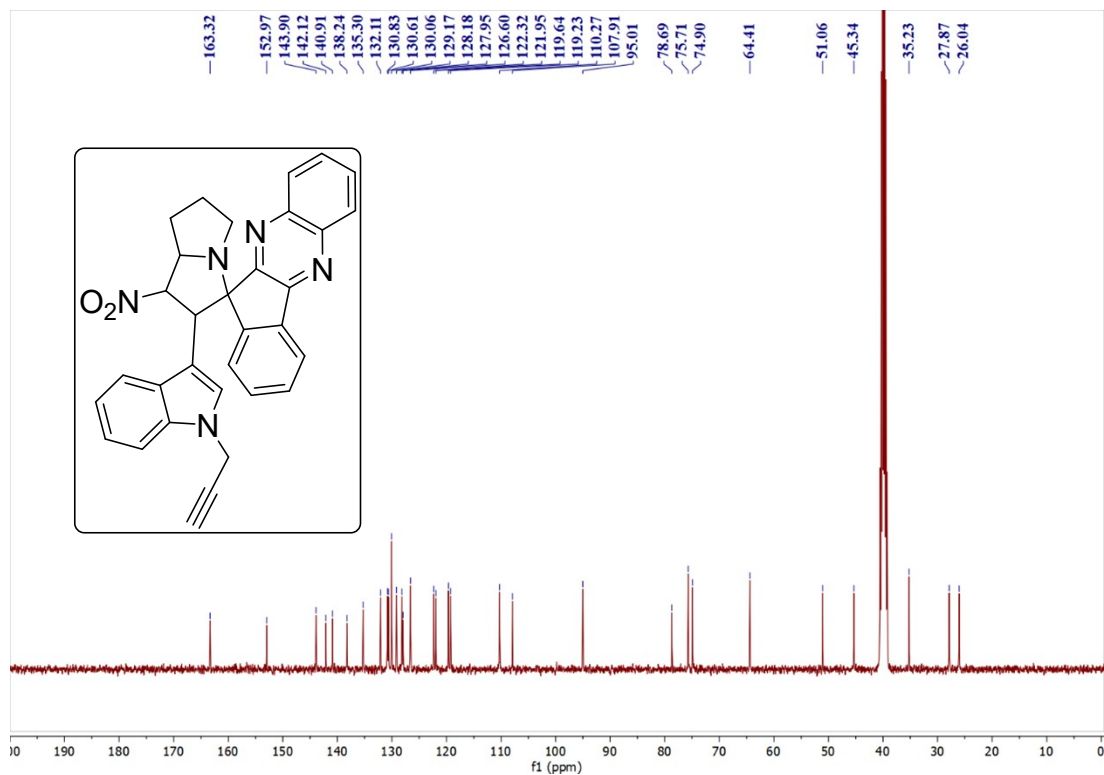
Mass spectrum of the compound 4f



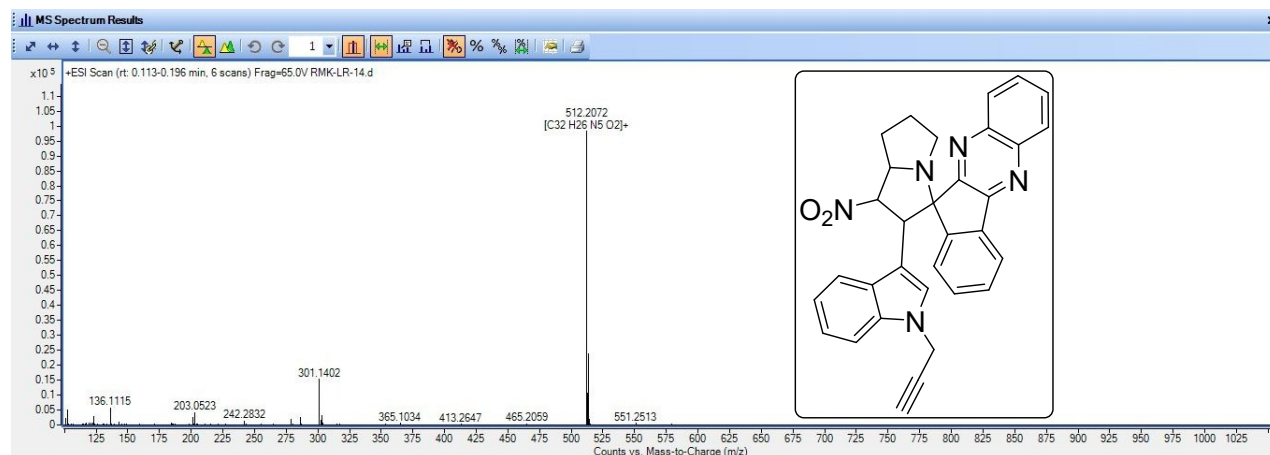
IR spectrum of the compound 4f



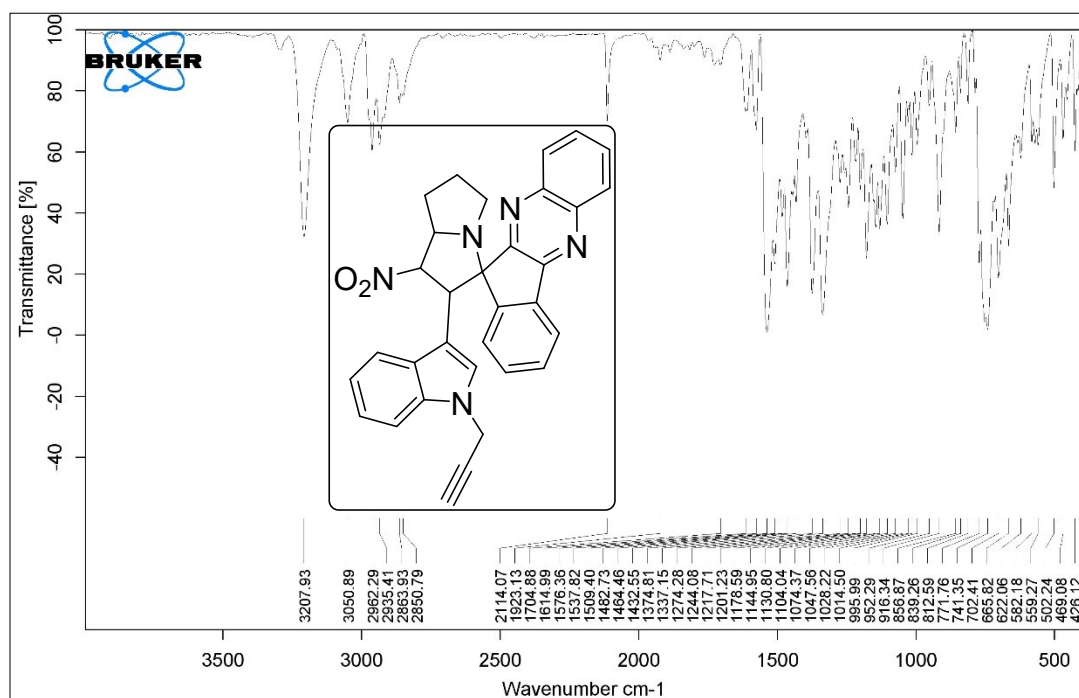
¹H NMR spectrum of the compound **4g** (400 MHz, DMSO-*d*₆)



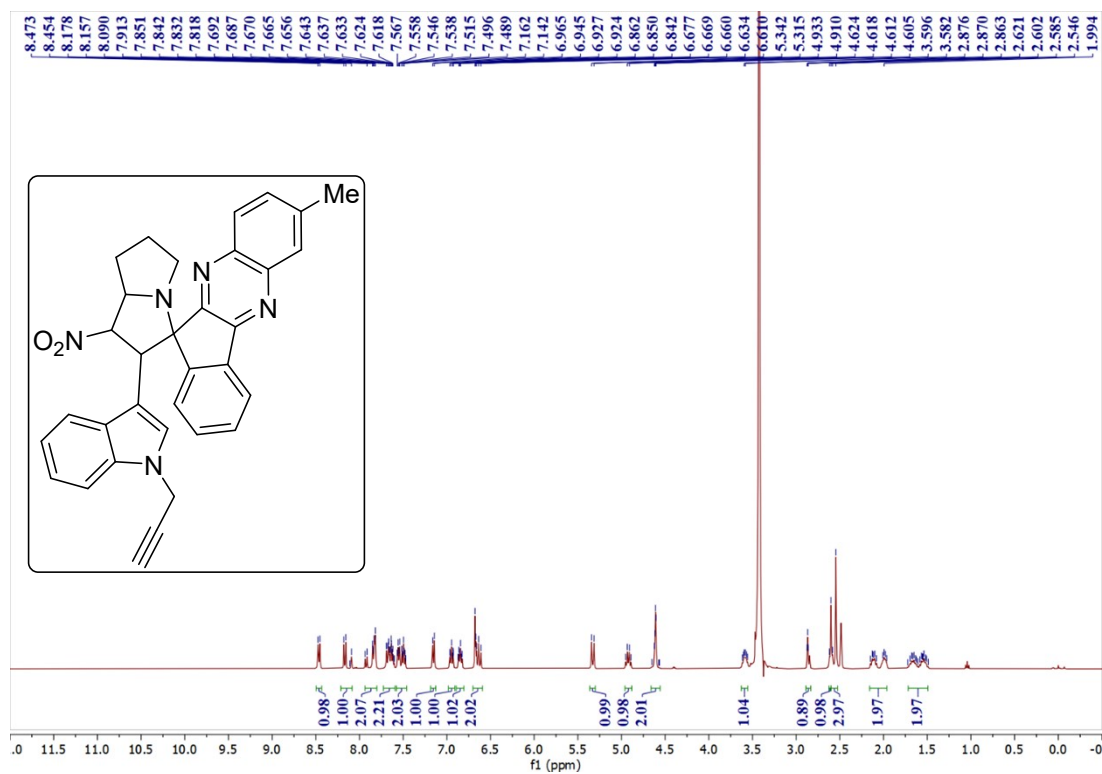
¹³C NMR spectrum of the compound **4g** (100 MHz, DMSO-*d*₆)



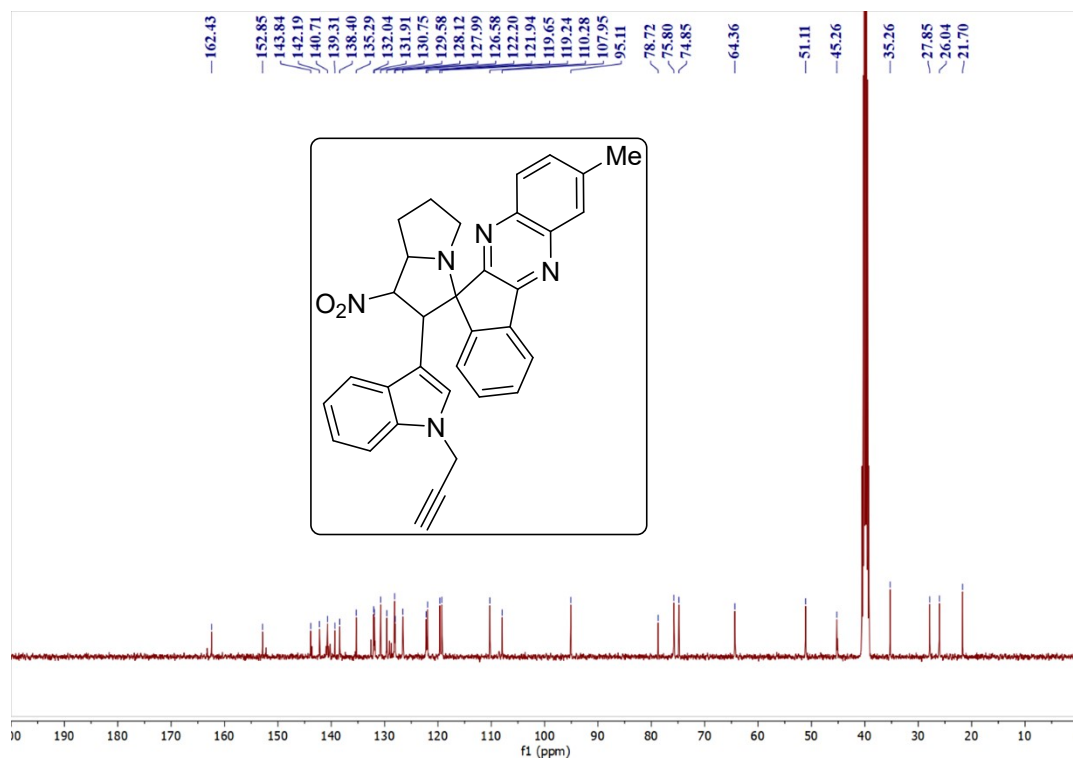
Mass spectrum of the compound **4g**



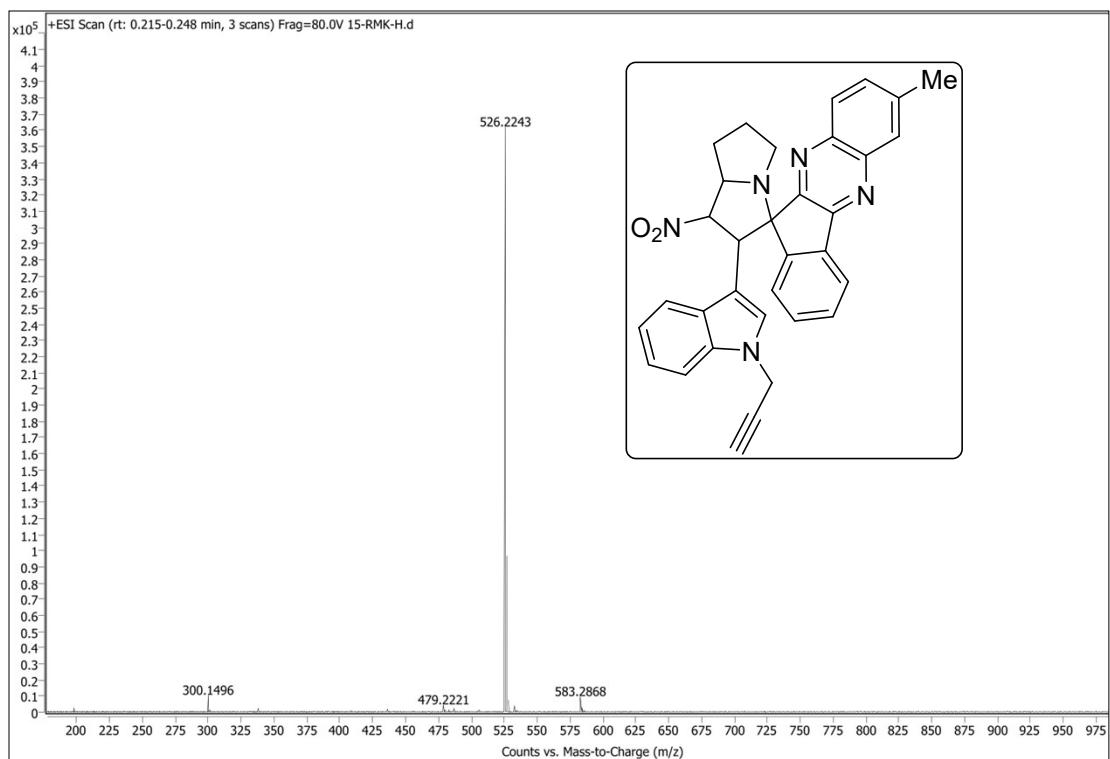
IR spectrum of the compound **4g**



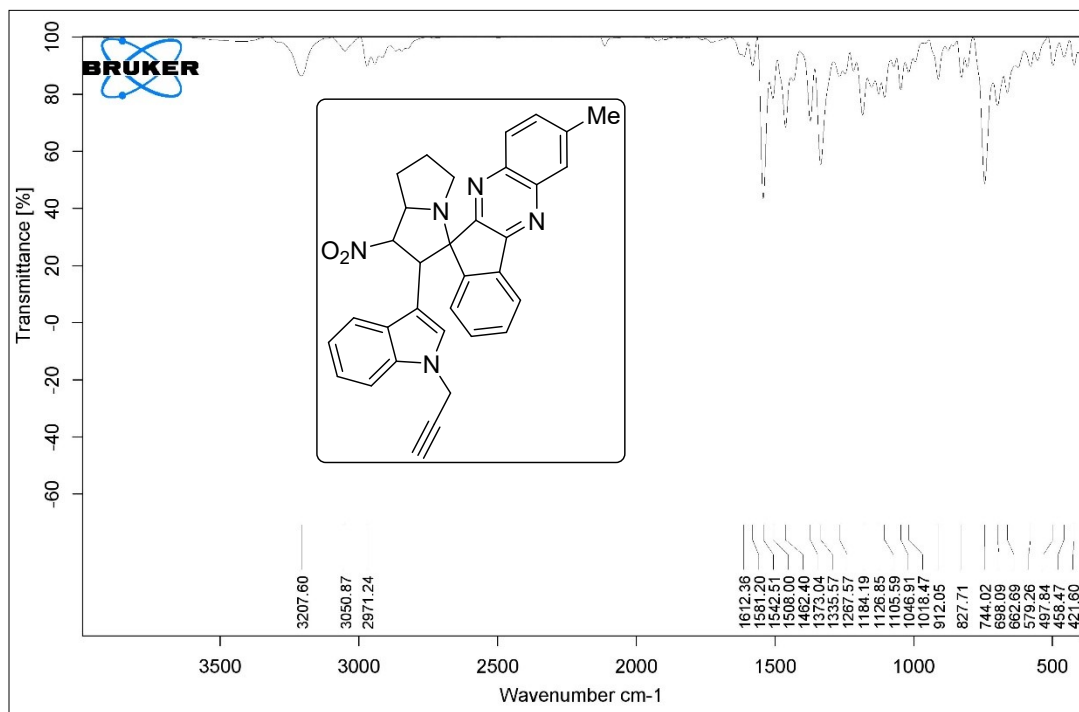
¹H NMR spectrum of the compound **4h** (400 MHz, DMSO-*d*₆)



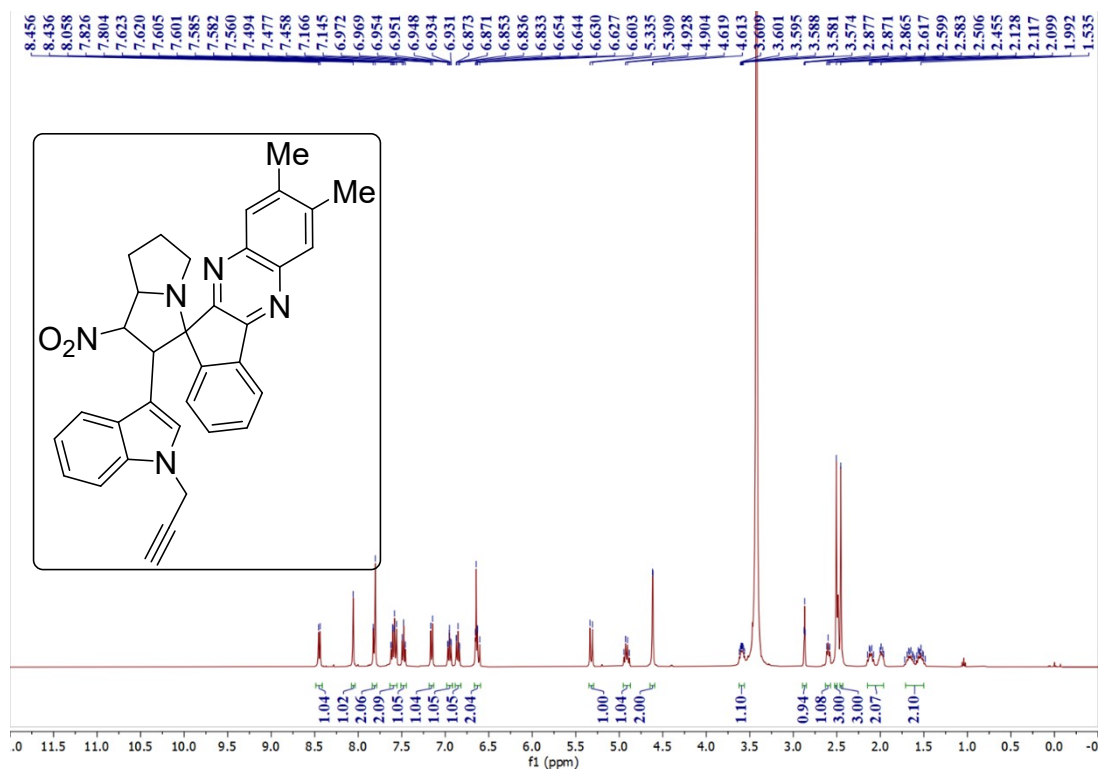
¹³C NMR spectrum of the compound **4h** (100 MHz, DMSO-*d*₆)



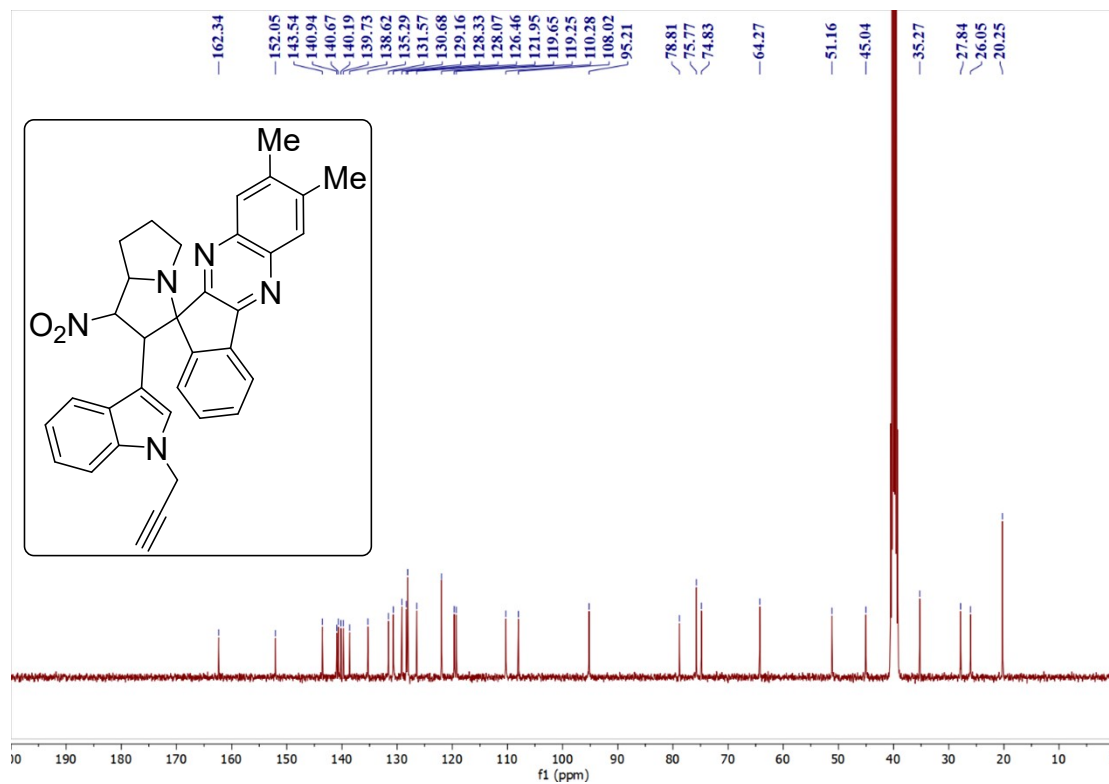
Mass spectrum of the compound **4h**



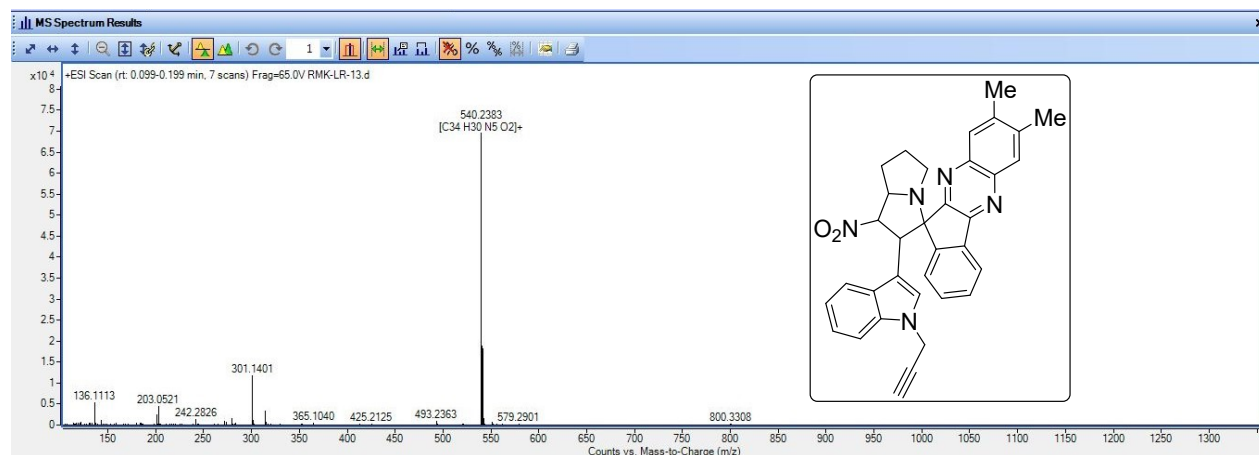
IR spectrum of the compound **4h**



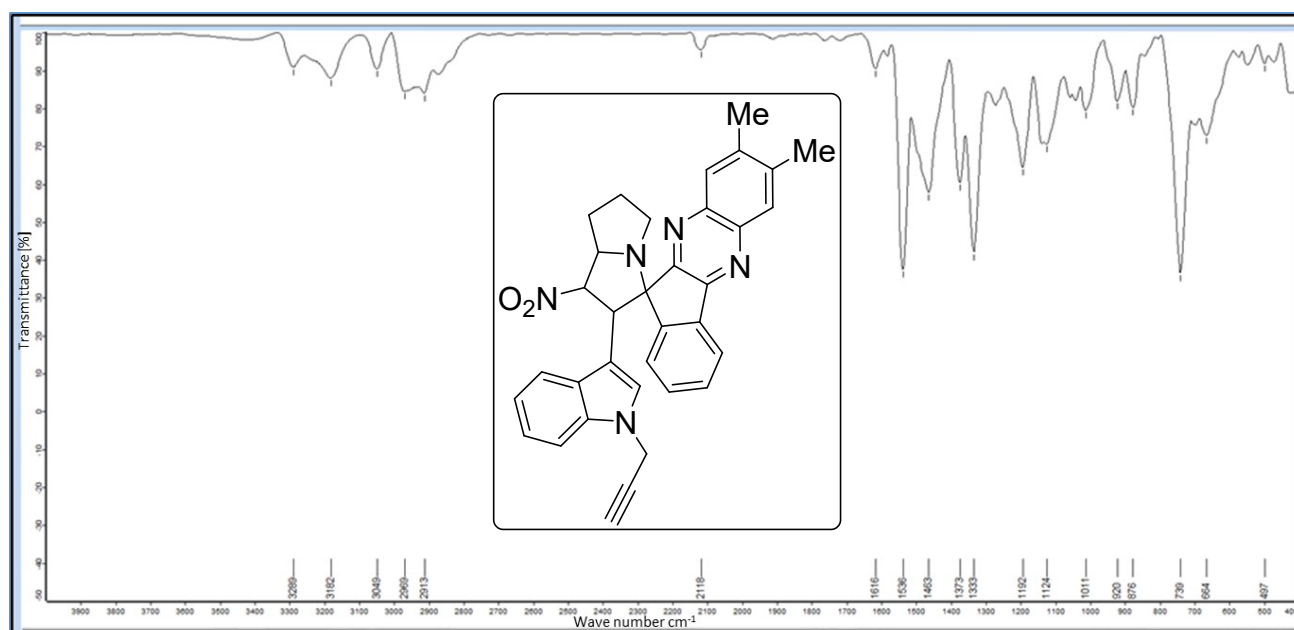
¹H NMR spectrum of the compound **4i** (400 MHz, DMSO-*d*₆)



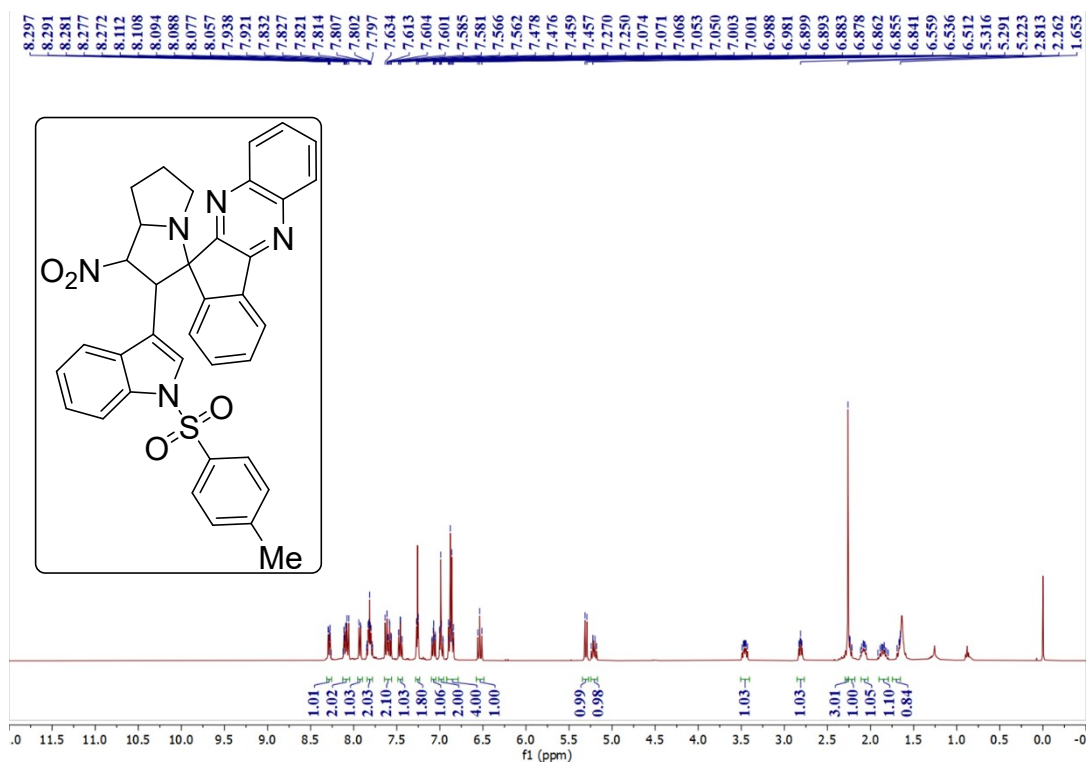
¹³C NMR spectrum of the compound **4i** (100 MHz, DMSO-*d*₆)



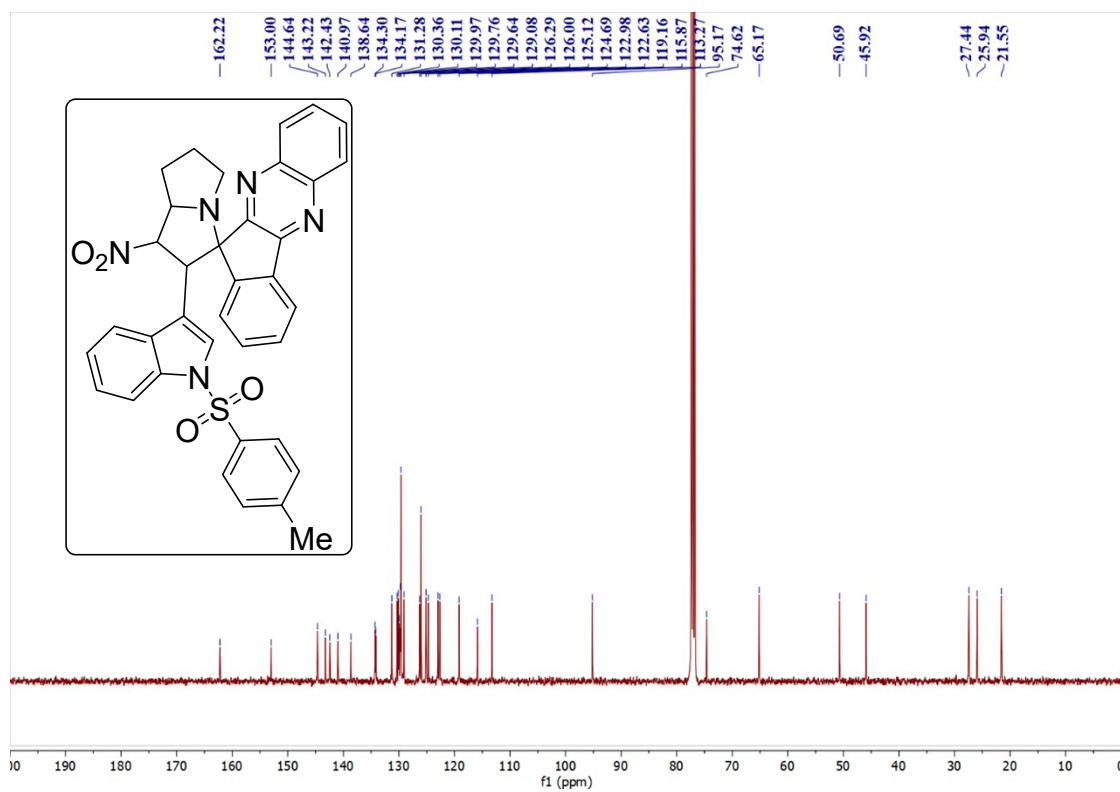
Mass spectrum of the compound **4i**



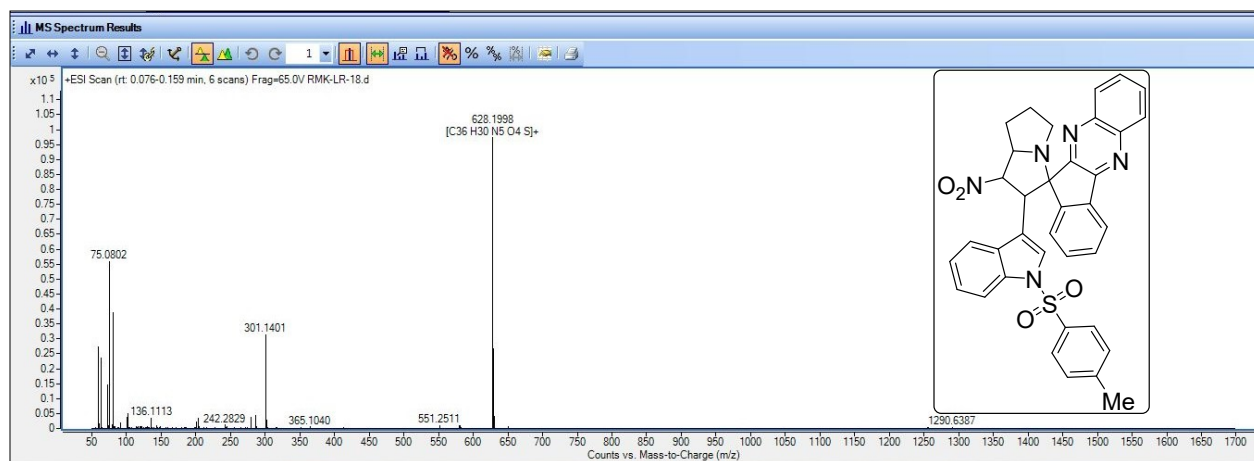
IR spectrum of the compound **4i**



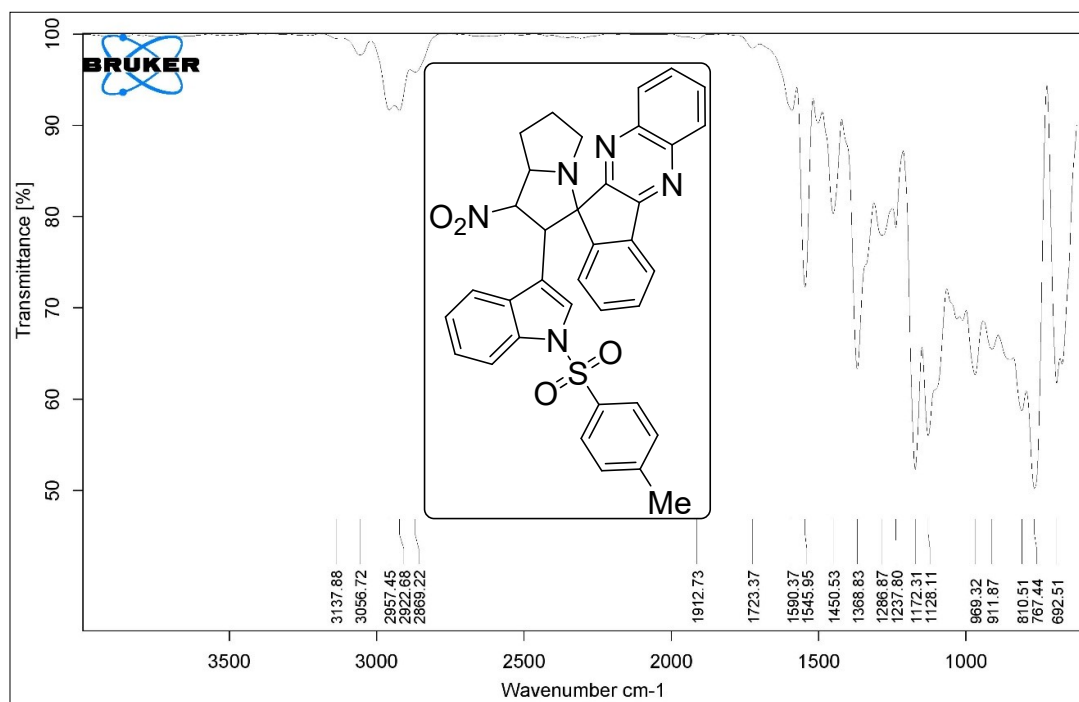
¹H NMR spectrum of the compound **4j** (400 MHz, CDCl₃)



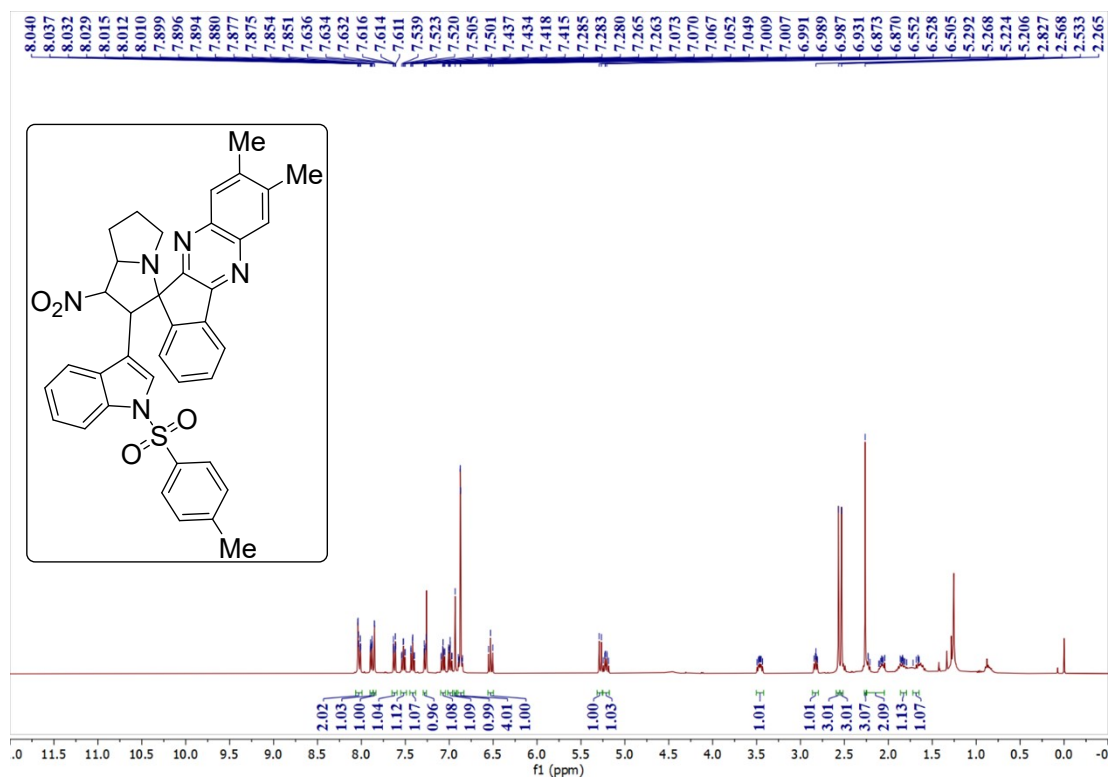
¹³C NMR spectrum of the compound **4j** (100 MHz, CDCl₃)

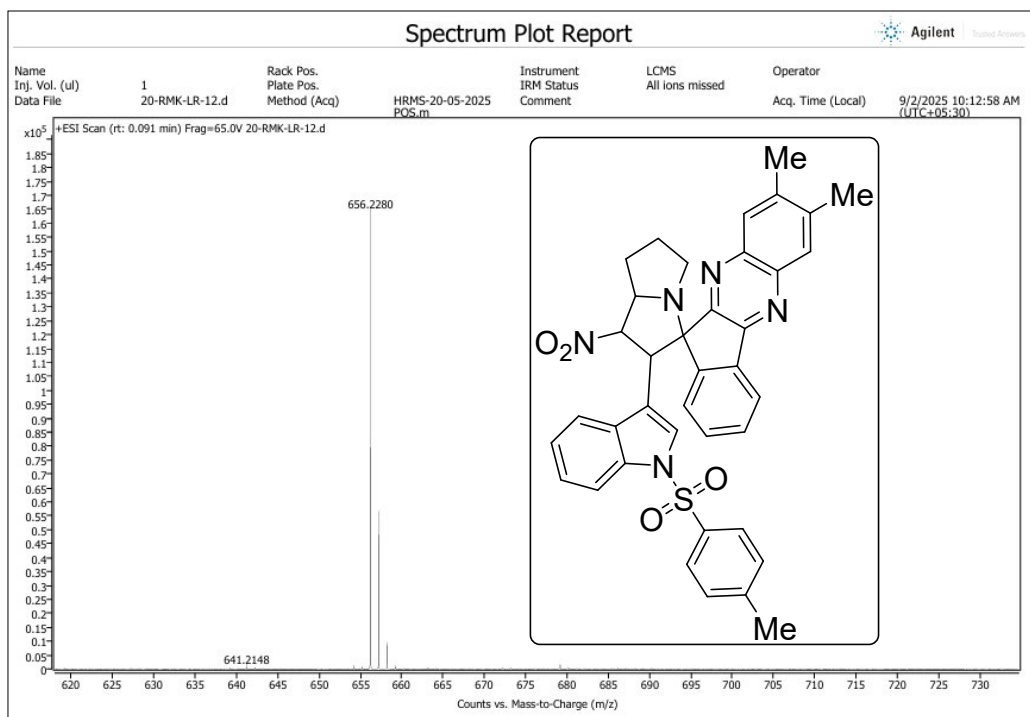


Mass spectrum of the compound **4j**

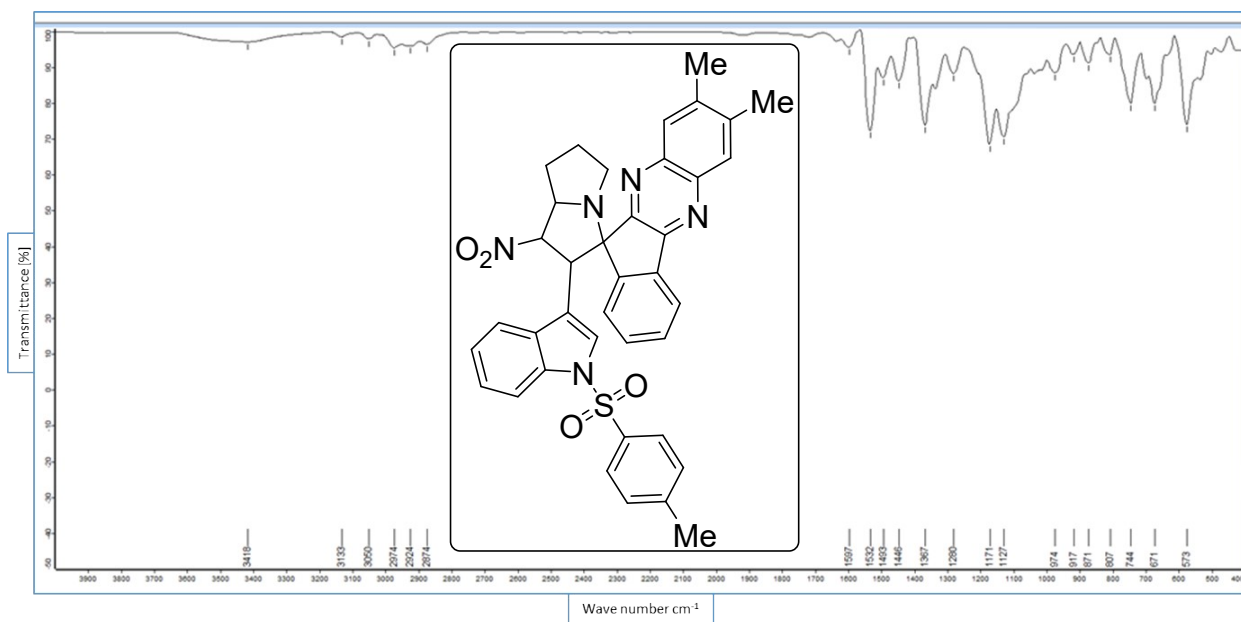


IR spectrum of the compound **4j**

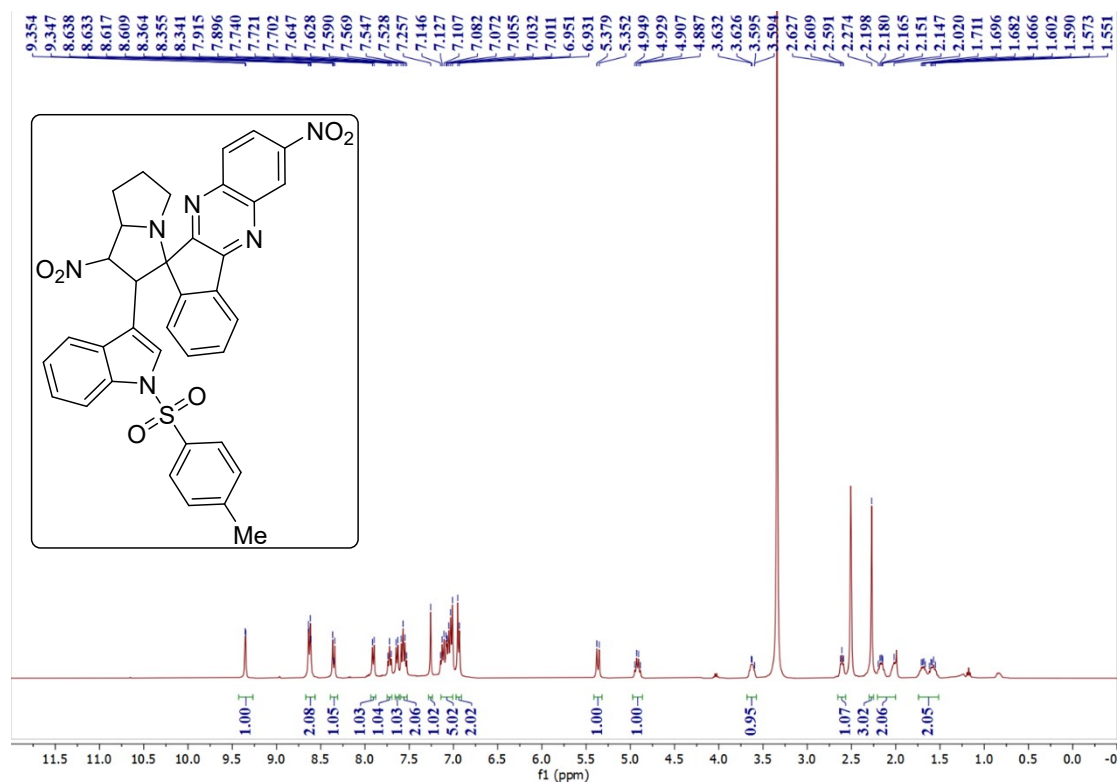




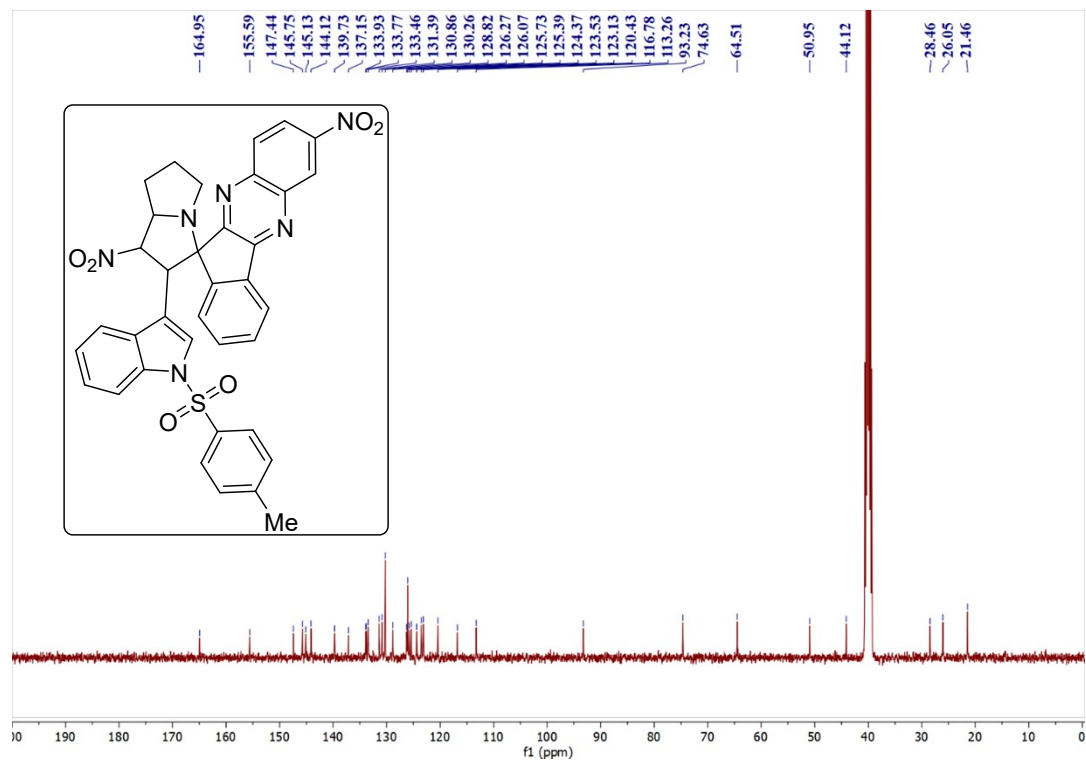
Mass spectrum of the compound **4k**



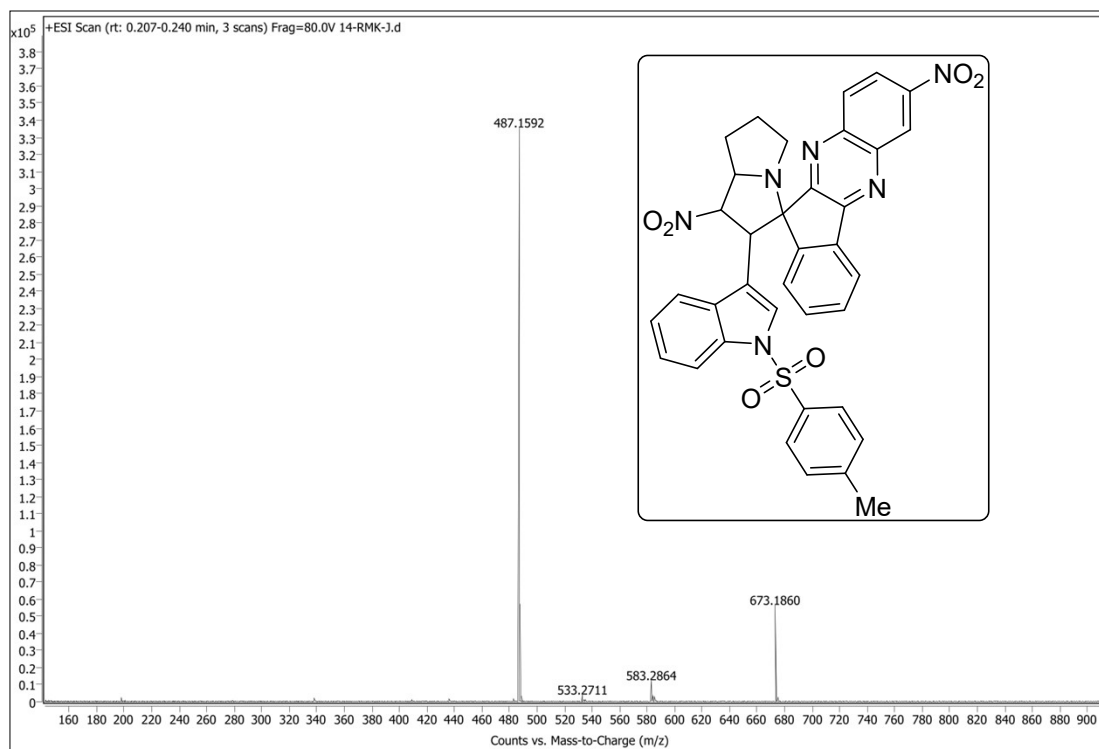
IR spectrum of the compound **4K**



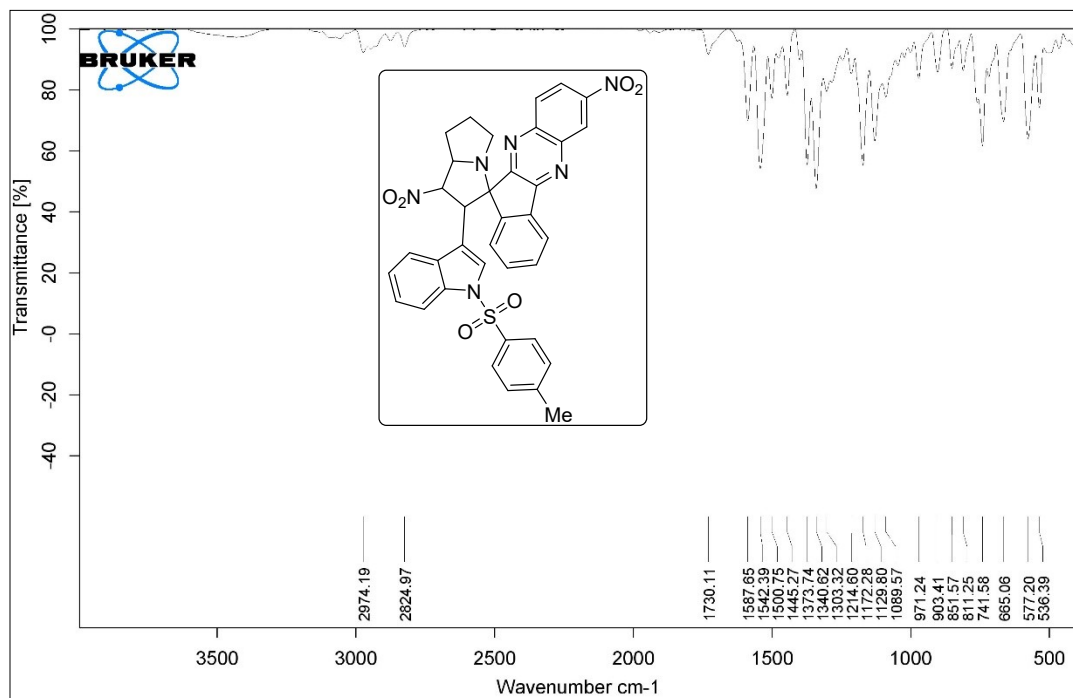
¹H NMR spectrum of the compound **4l** (400 MHz, DMSO-*d*₆)



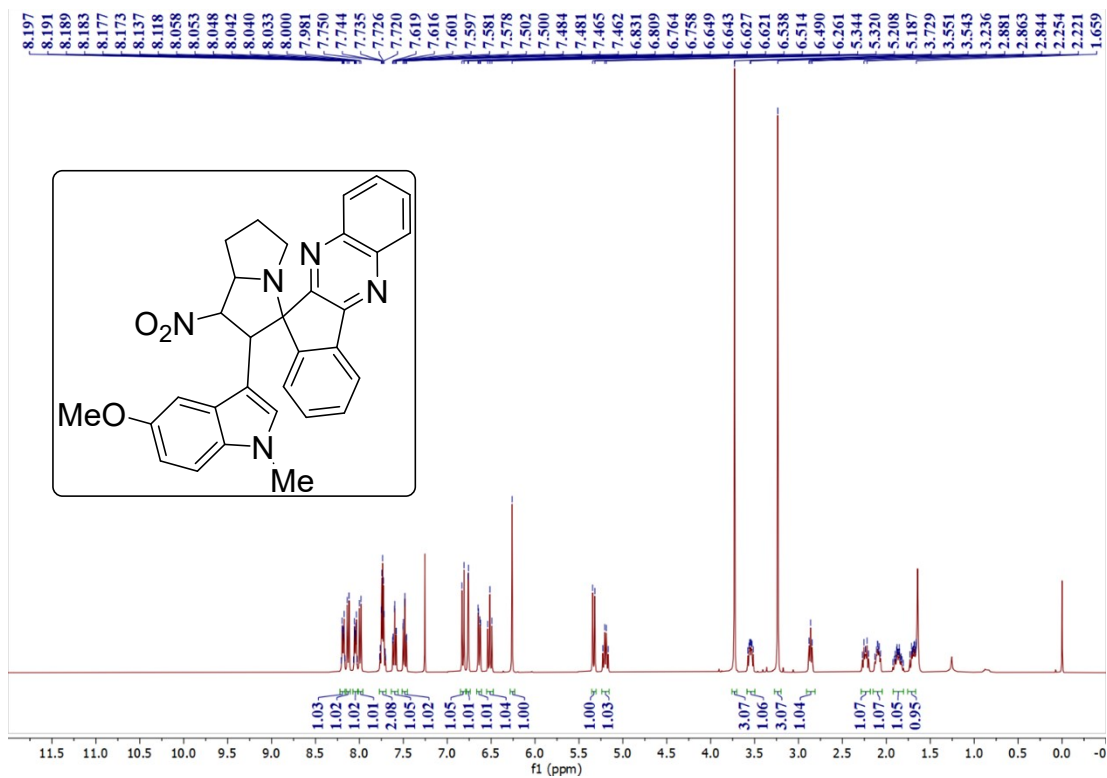
¹³C NMR spectrum of the compound **4l** (100 MHz, DMSO-*d*₆)



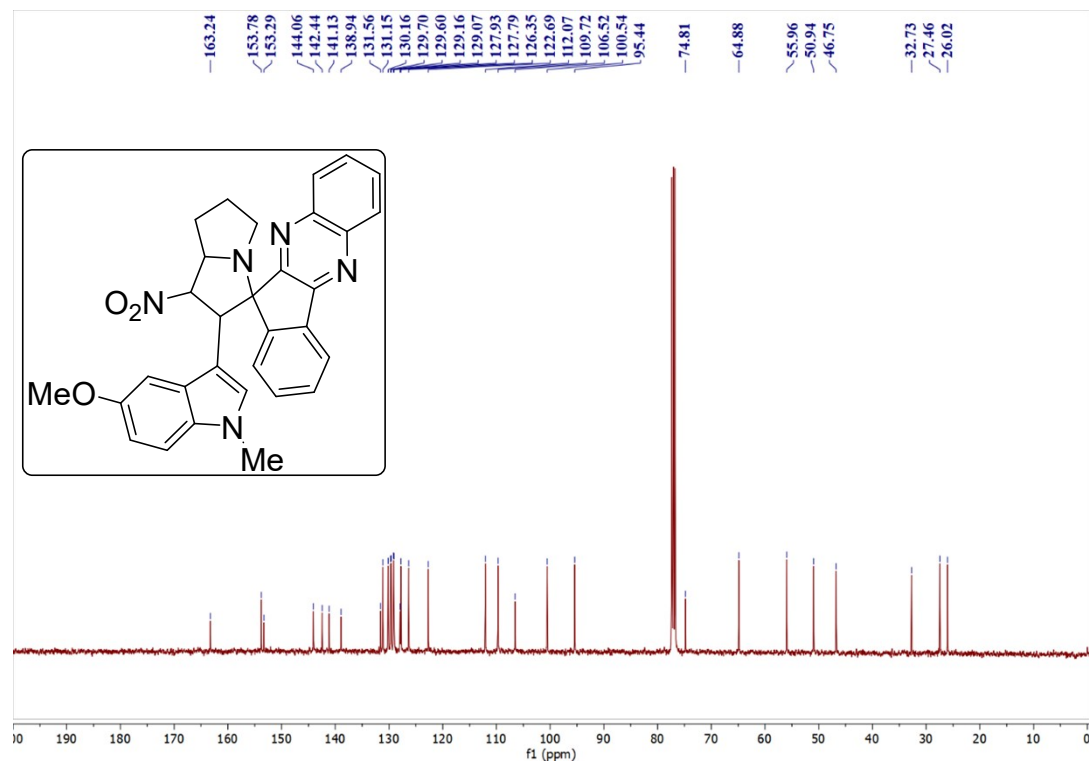
Mass spectrum of the compound **41**



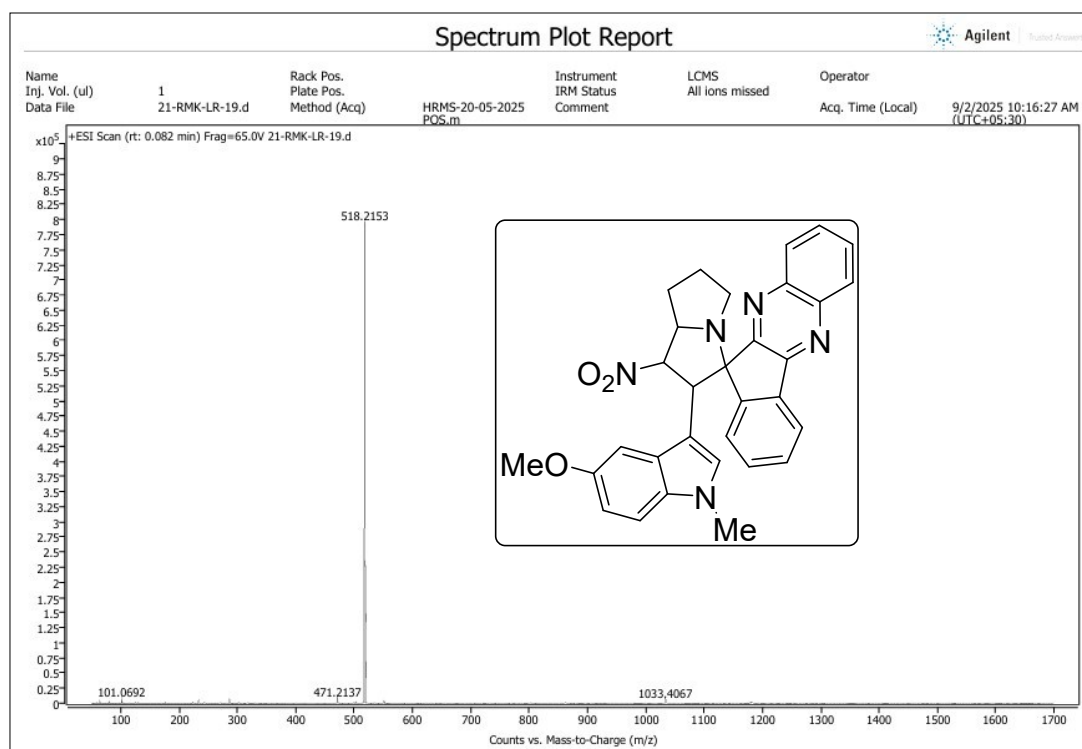
IR spectrum of the compound **41**



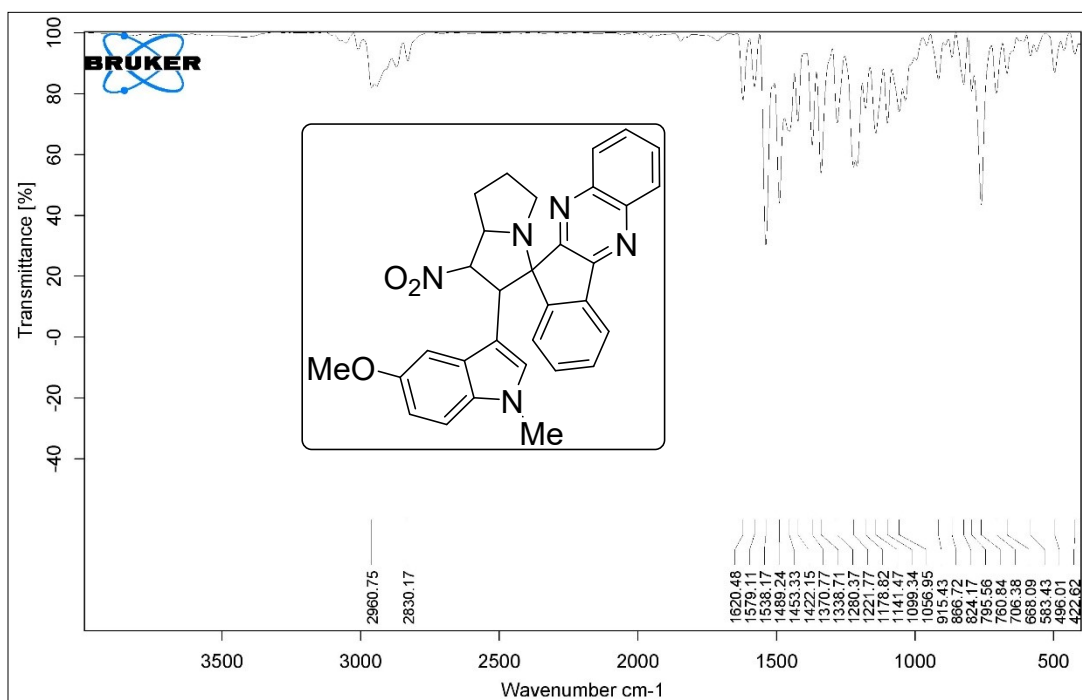
¹H NMR spectrum of the compound **4m** (400 MHz, CDCl₃)



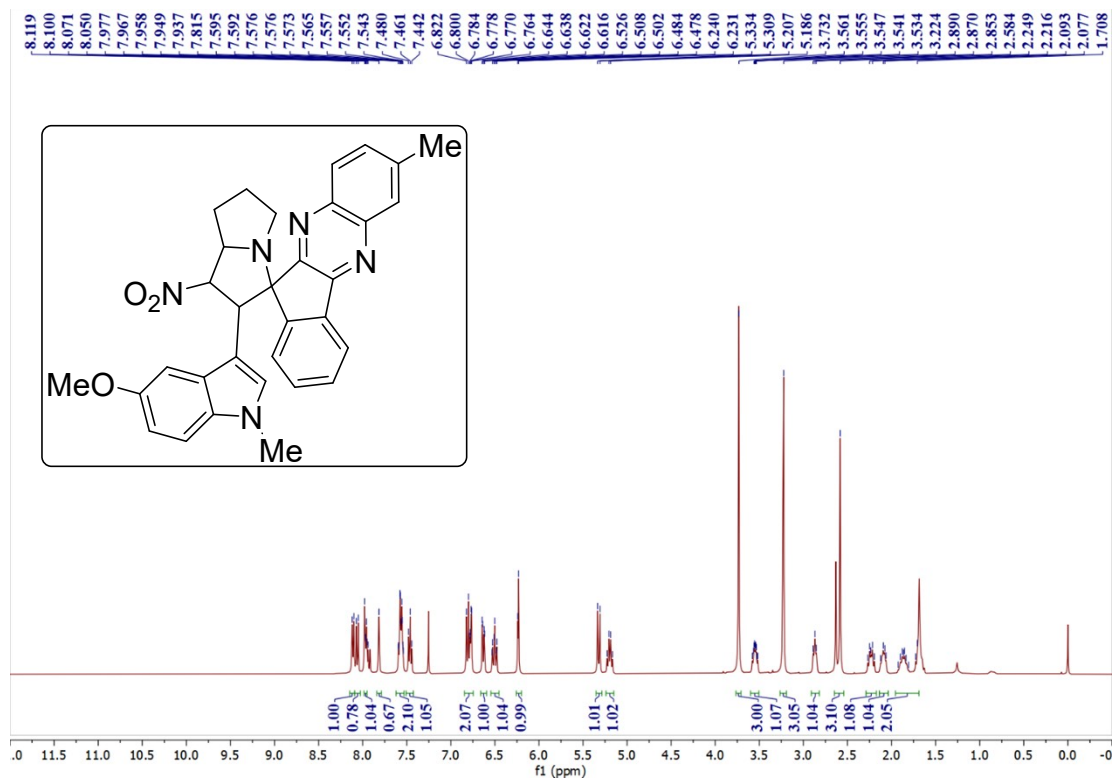
¹³C NMR spectrum of the compound **4m** (100 MHz, CDCl₃)



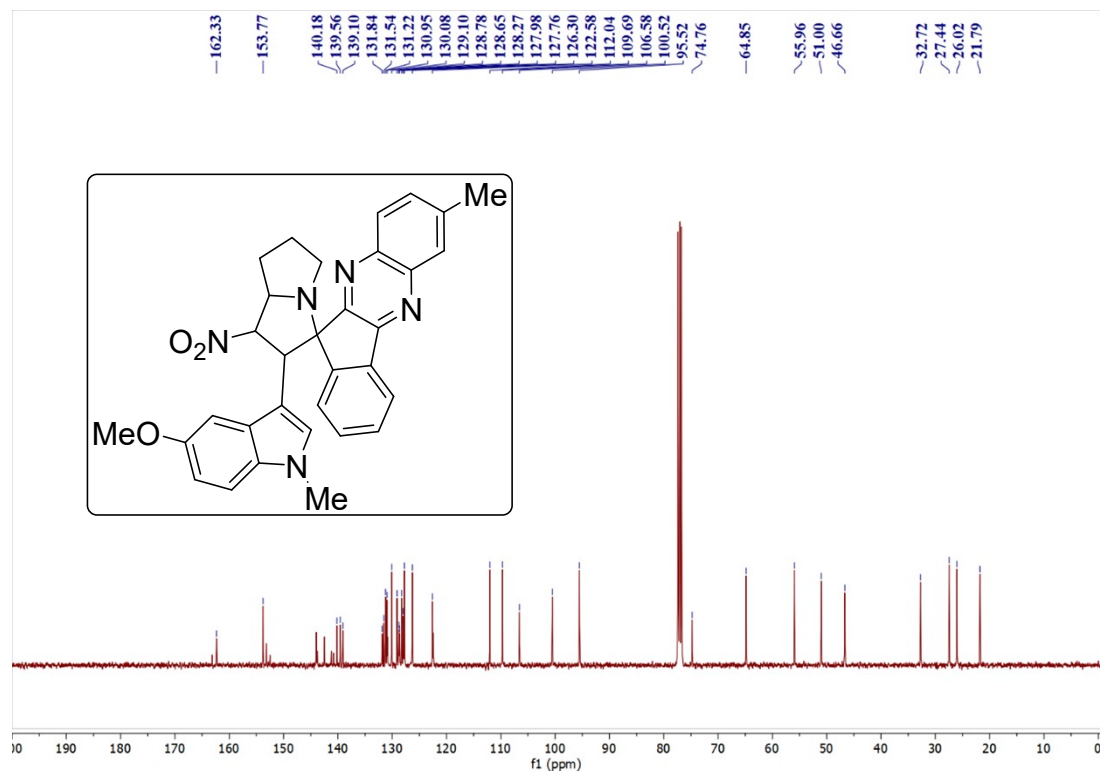
Mass spectrum of the compound **4m**



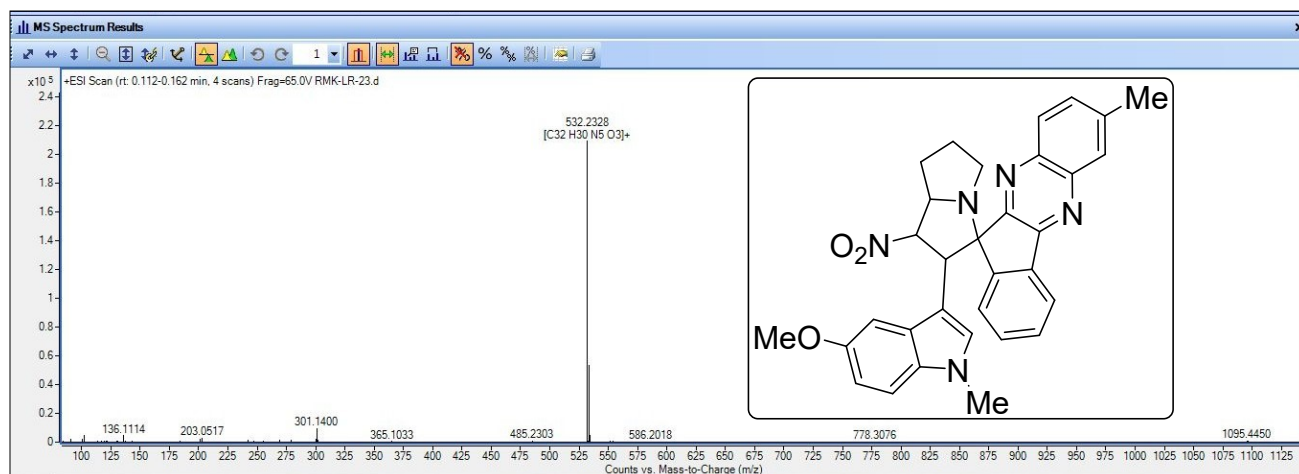
IR spectrum of the compound **4m**



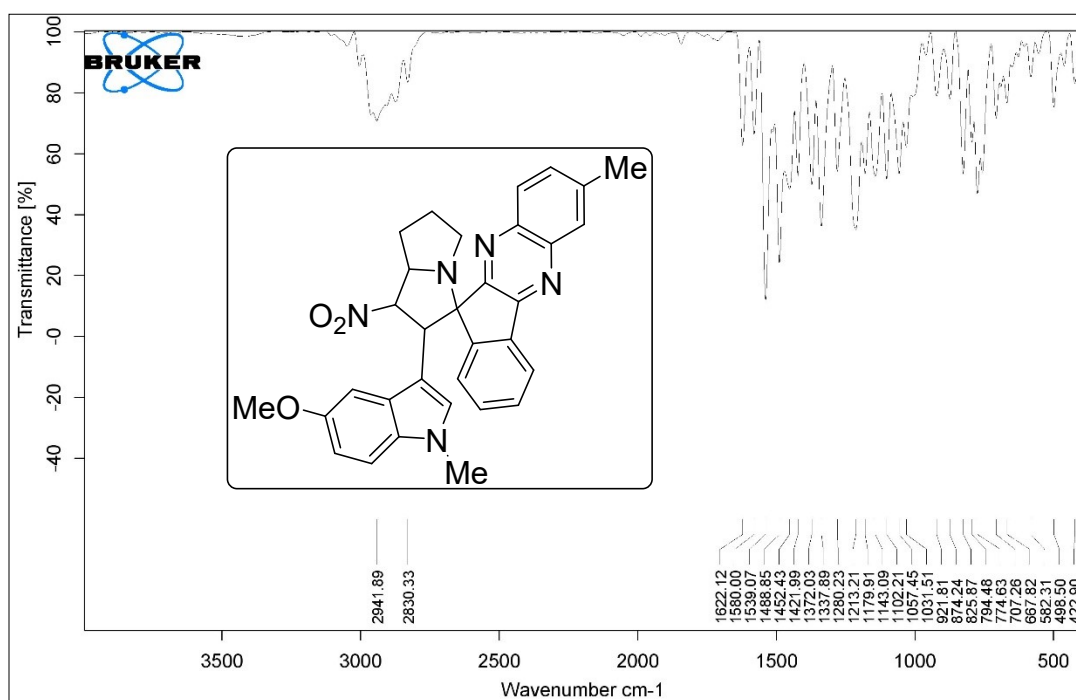
^1H NMR spectrum of the compound **4n** (400 MHz, CDCl_3)



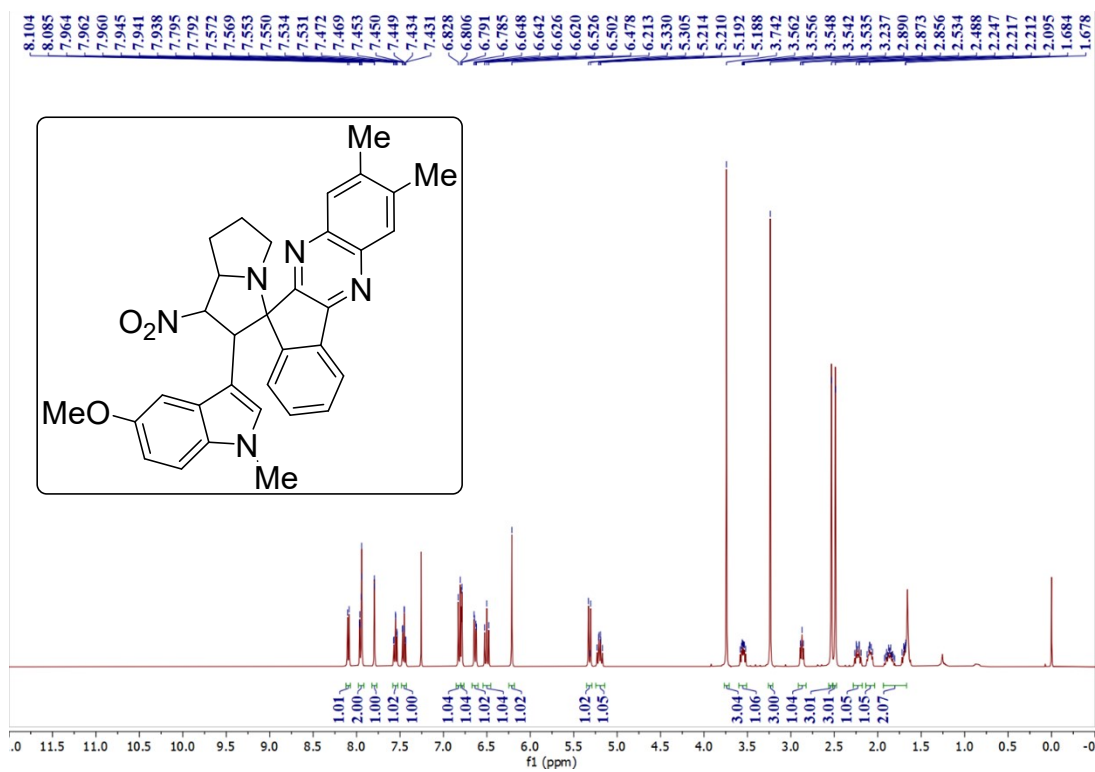
^{13}C NMR spectrum of the compound **4n** (100 MHz, CDCl_3)



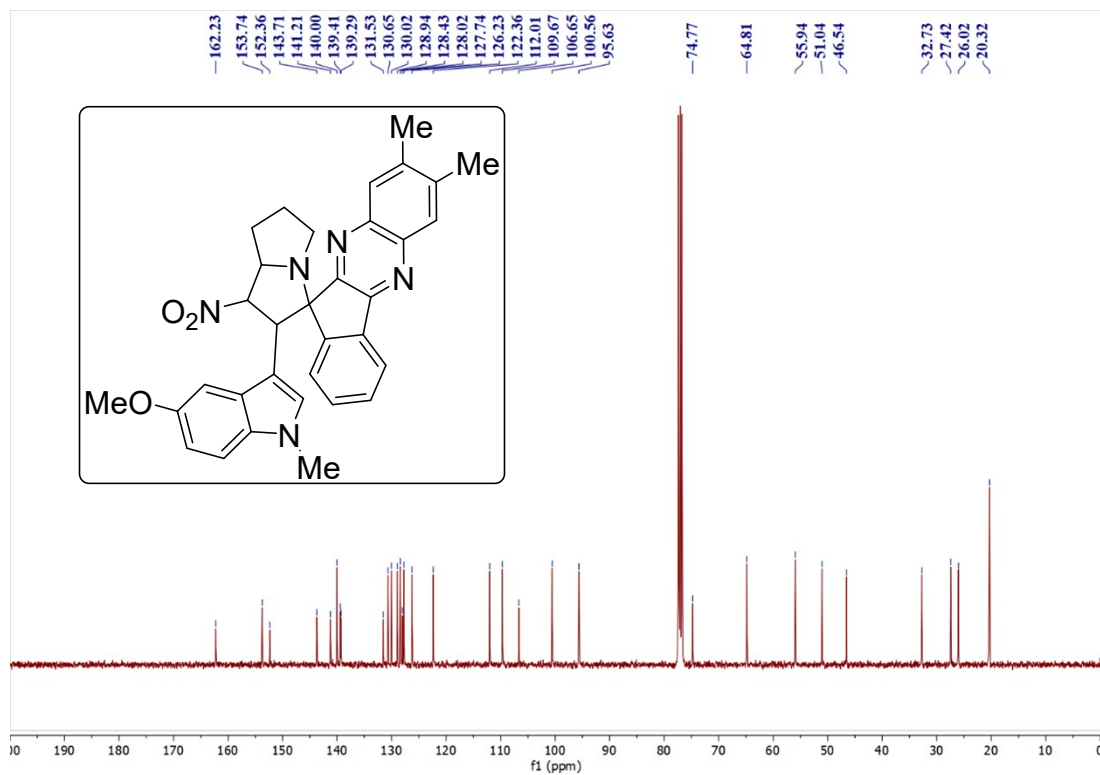
Mass spectrum of the compound **4n**



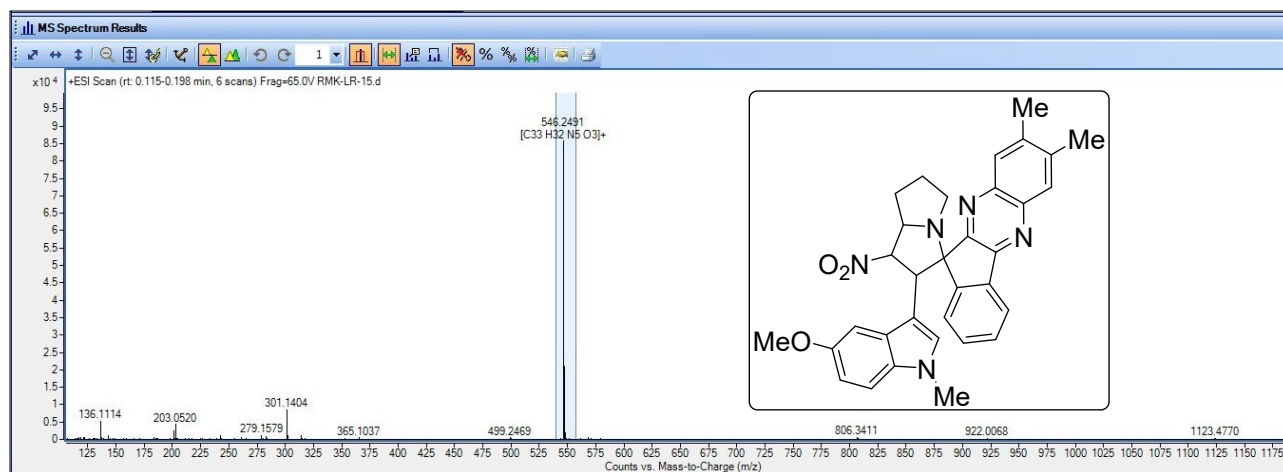
IR spectrum of the compound **4n**



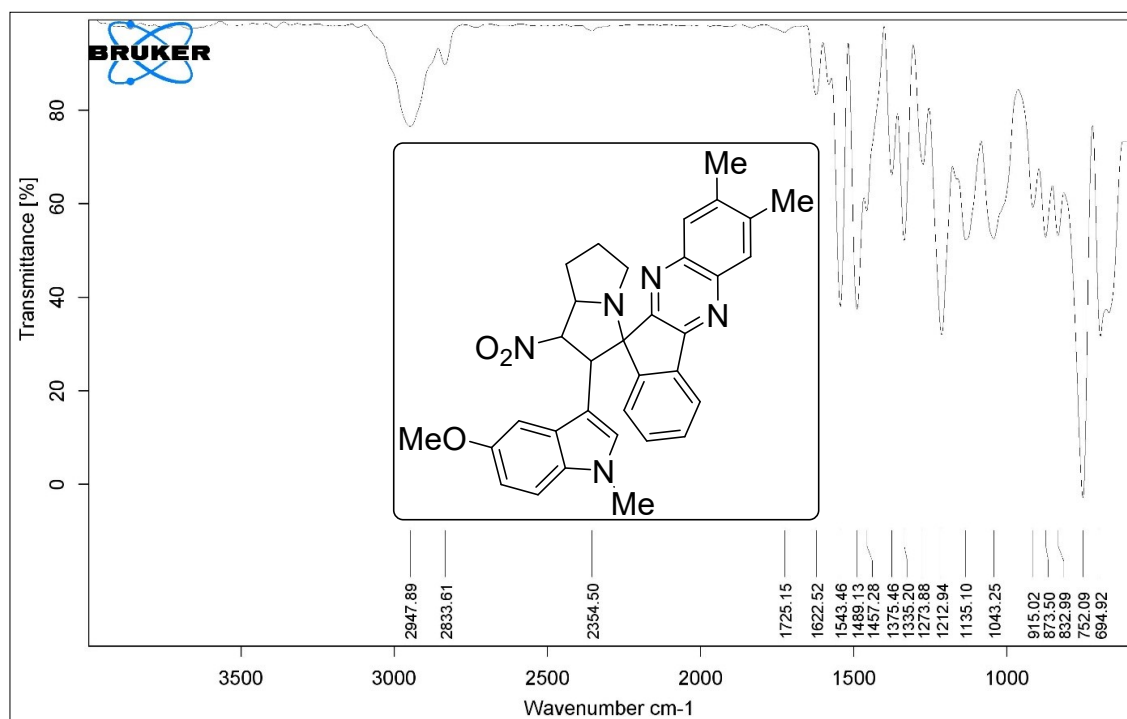
¹H NMR spectrum of the compound **4o** (400 MHz, CDCl₃)



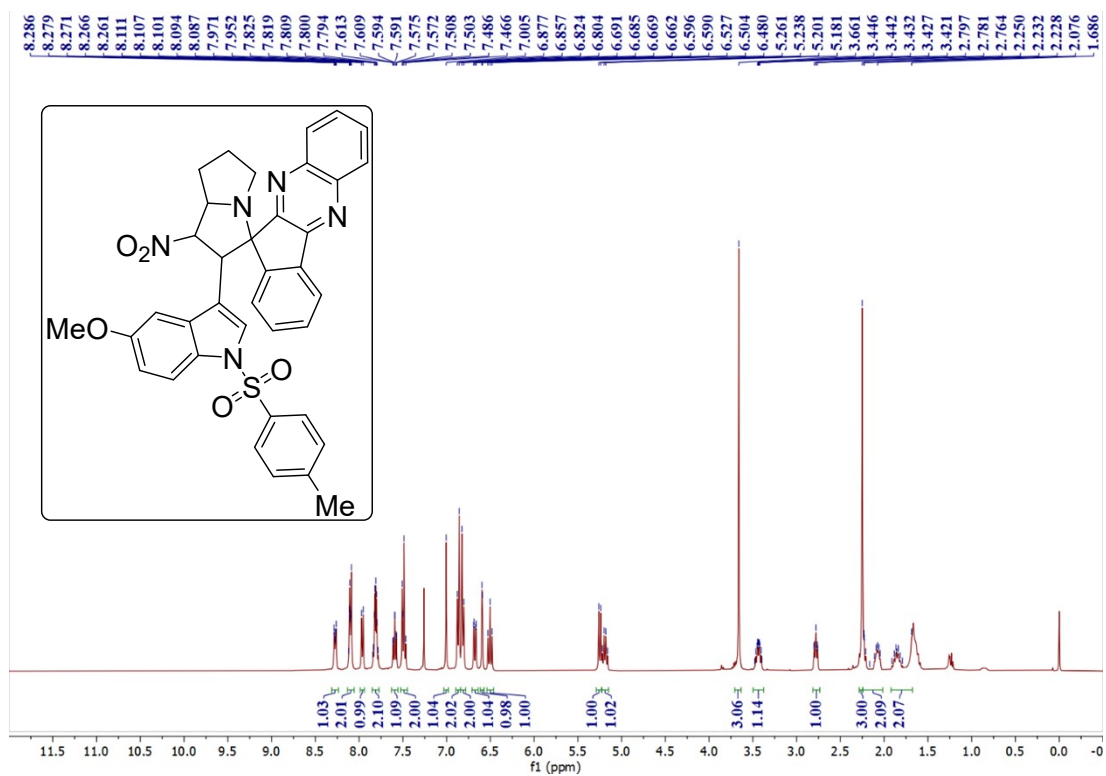
¹³C NMR spectrum of the compound **4o** (100 MHz, CDCl₃)



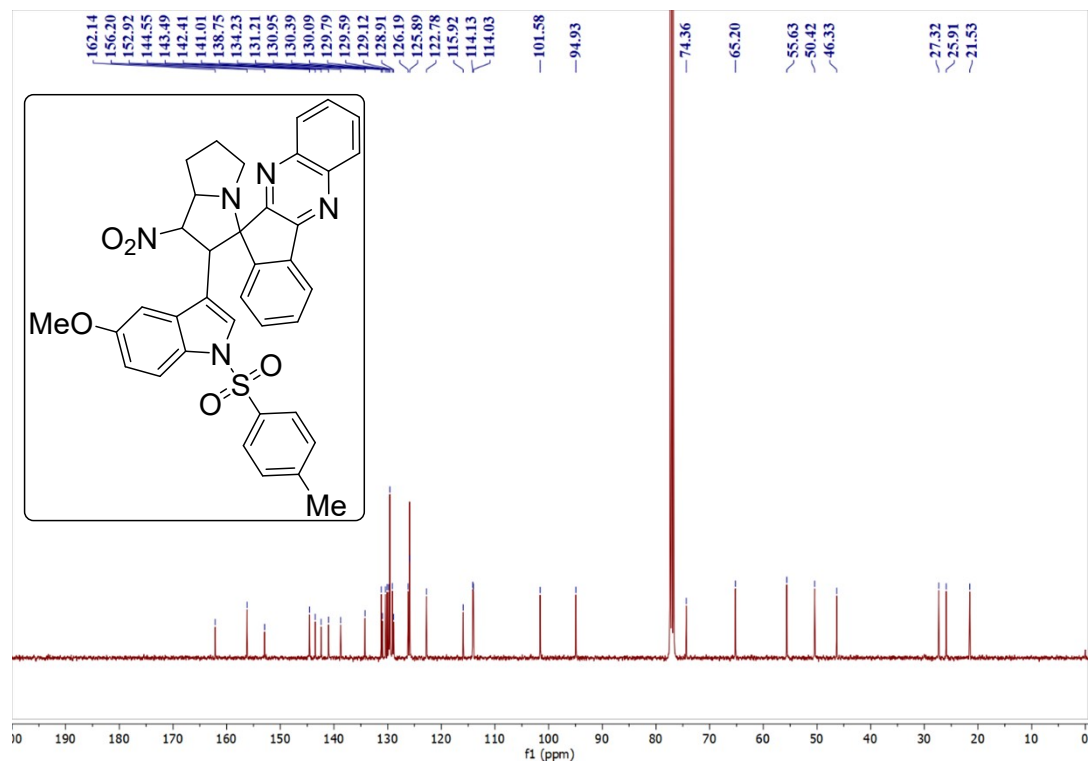
Mass spectrum of the compound **4o**



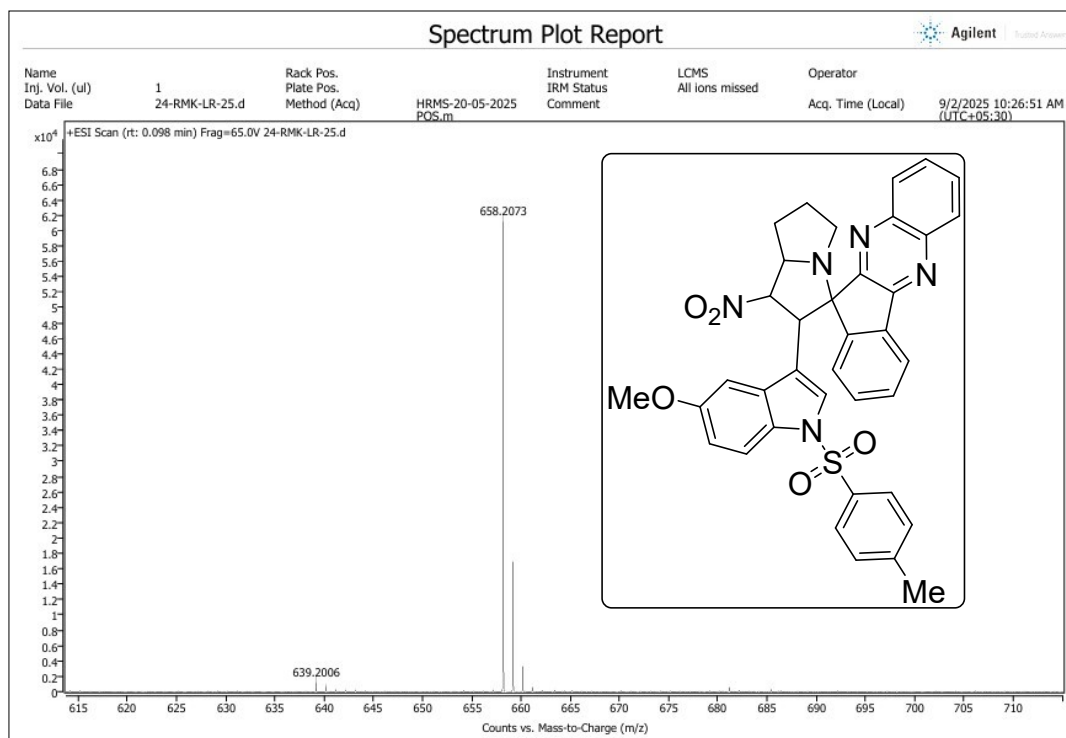
IR spectrum of the compound **4o**



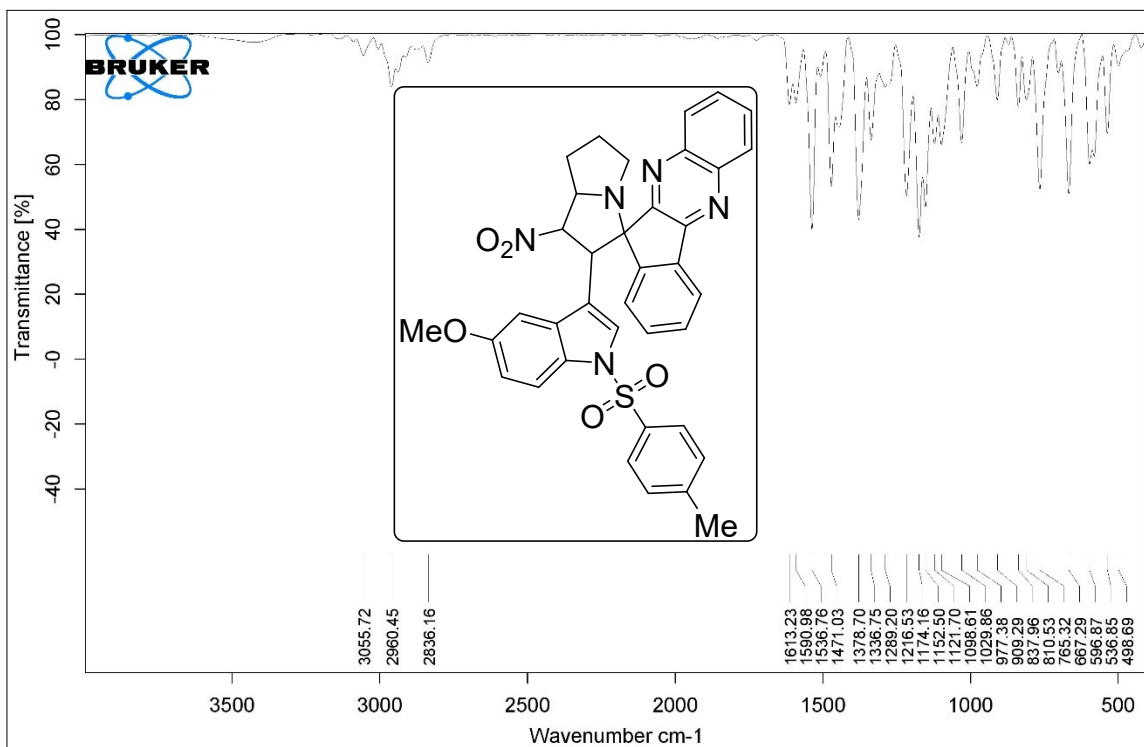
¹H NMR spectrum of the compound **4p** (400 MHz, CDCl₃)



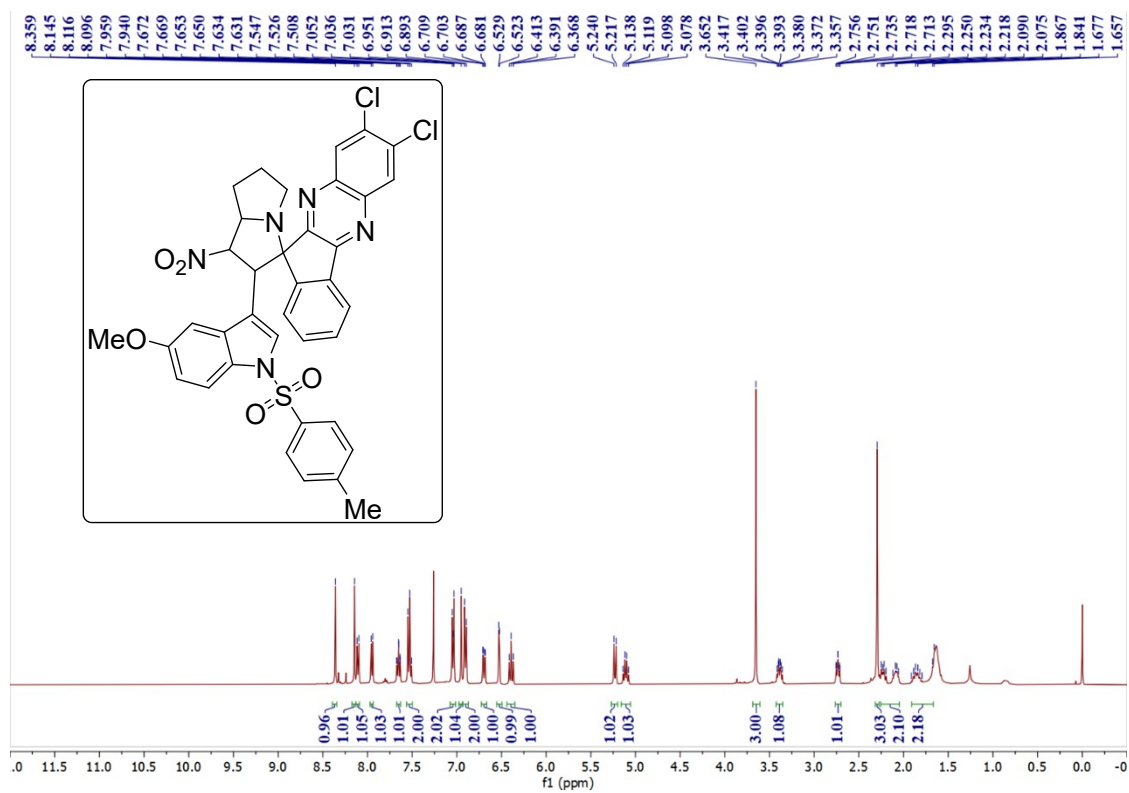
¹³C NMR spectrum of the compound **4p** (100 MHz, CDCl₃)



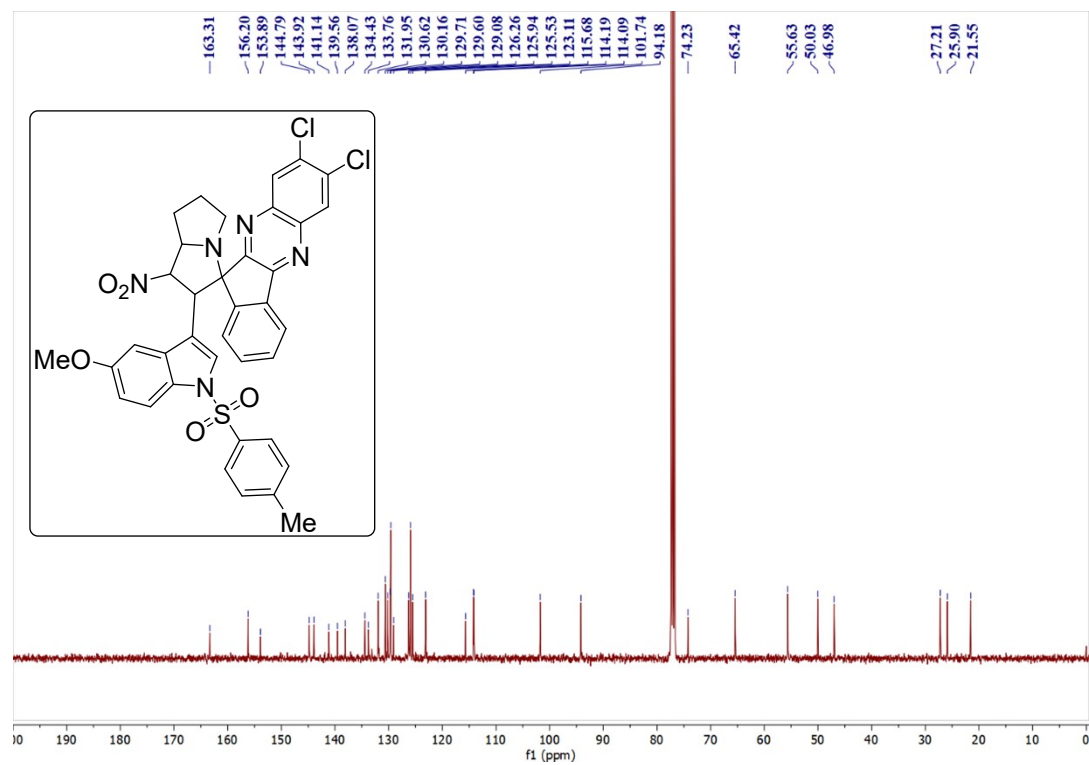
Mass spectrum of the compound **4p**



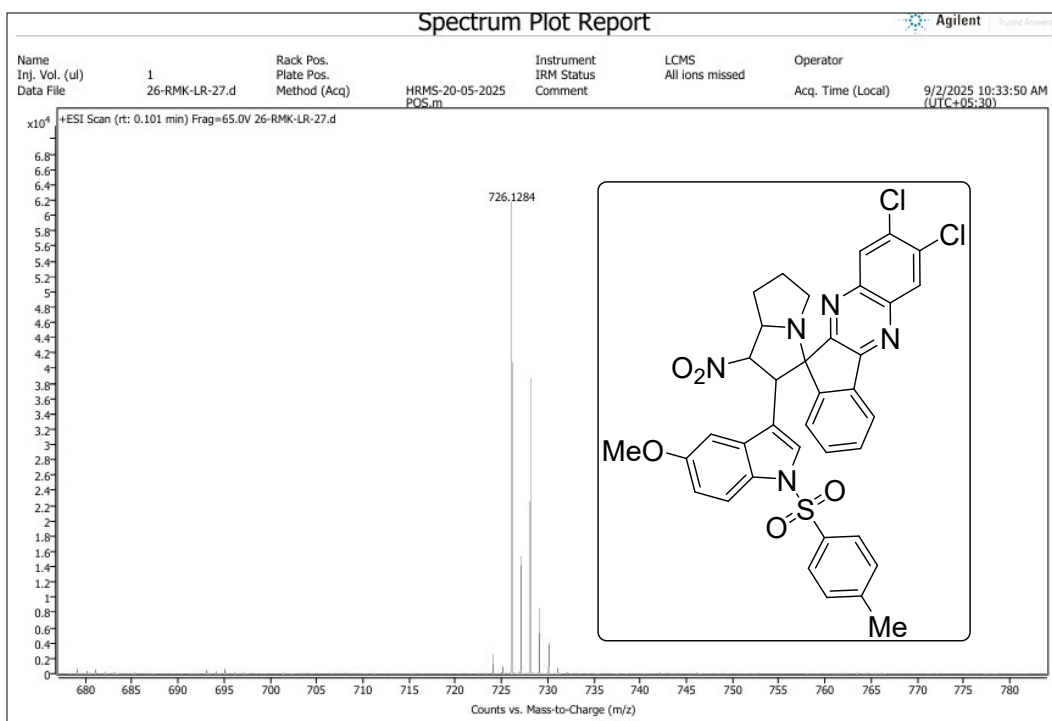
IR spectrum of the compound **4p**



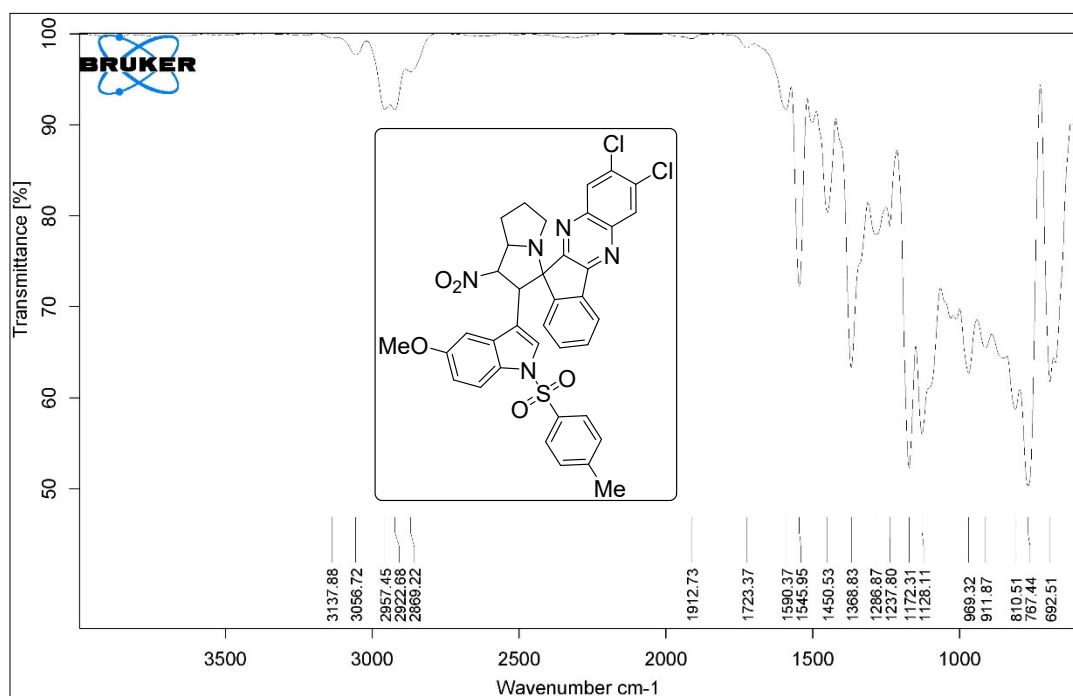
¹H NMR spectrum of the compound **4q** (400 MHz, CDCl₃)



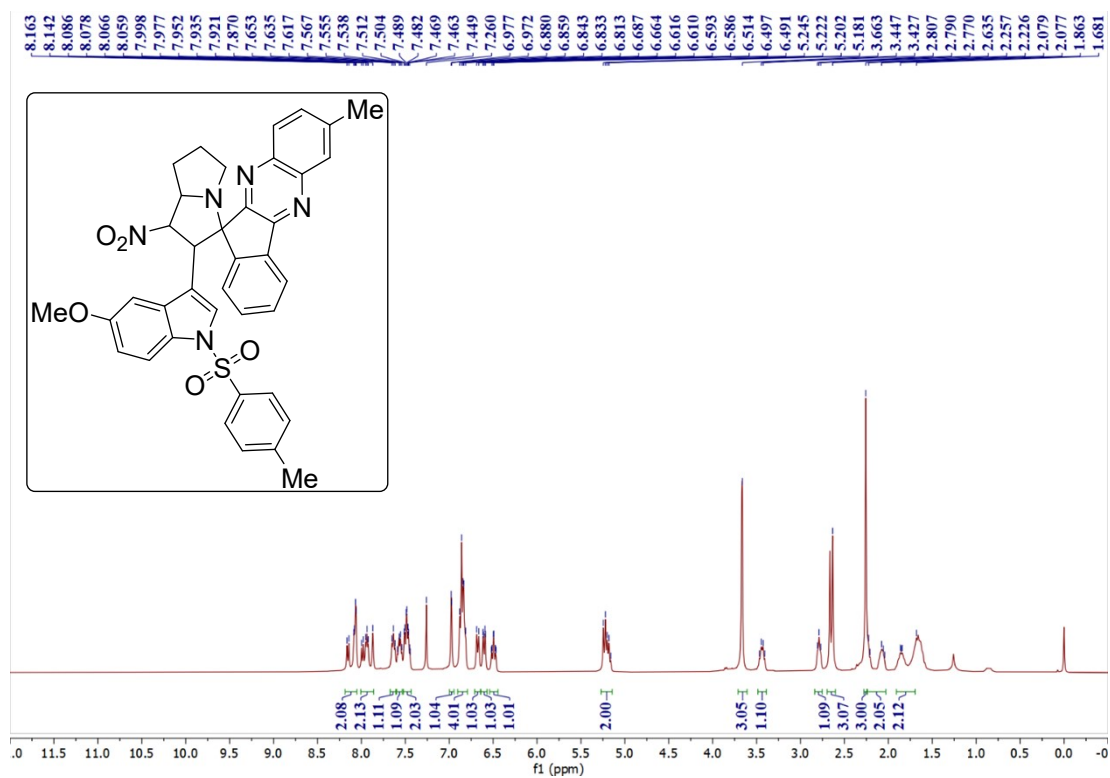
¹³C NMR spectrum of the compound **4q** (100 MHz, CDCl₃)



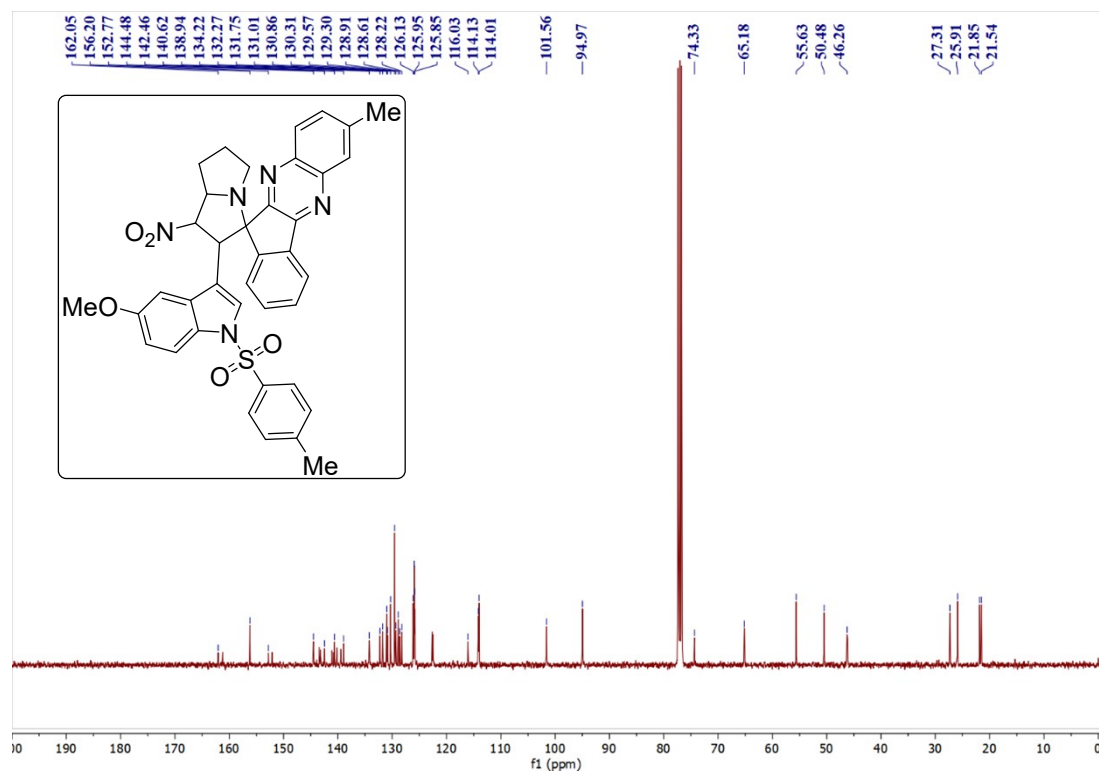
Mass spectrum of the compound **4q**



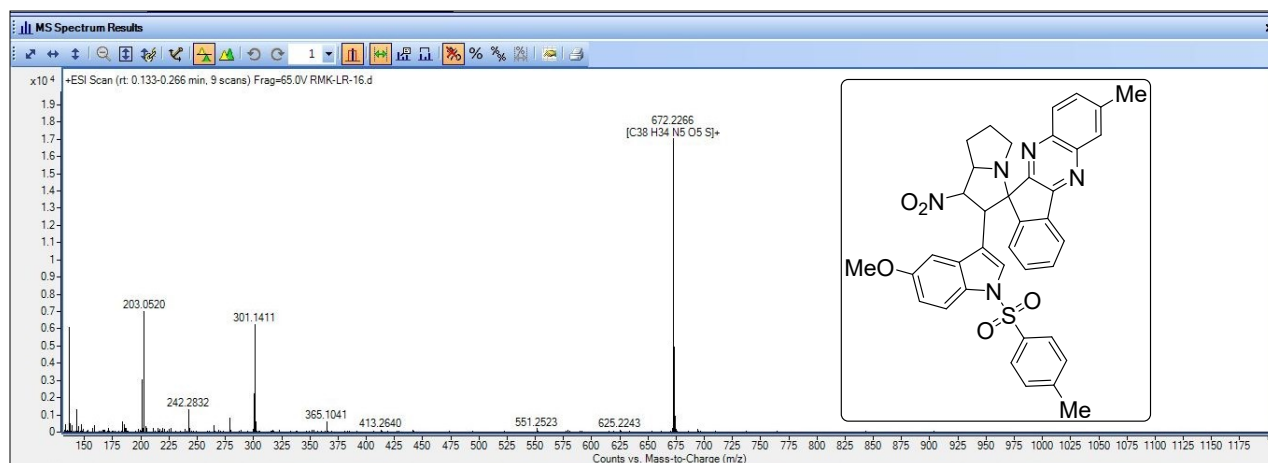
IR spectrum of the compound **4q**



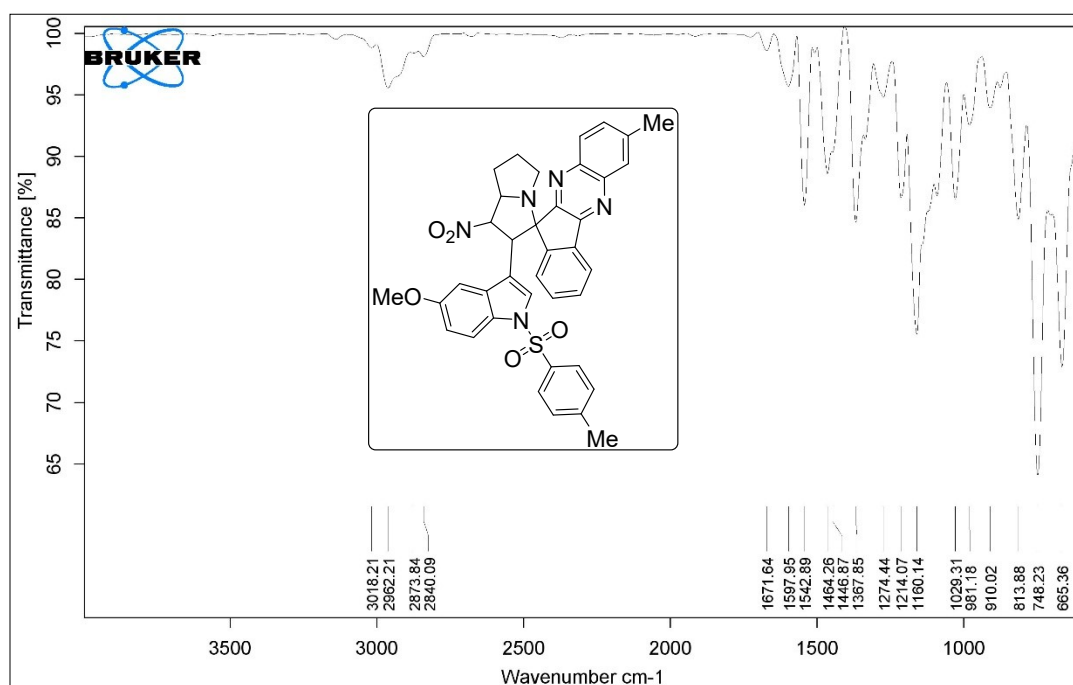
¹H NMR spectrum of the compound **4r** (400 MHz, CDCl₃)



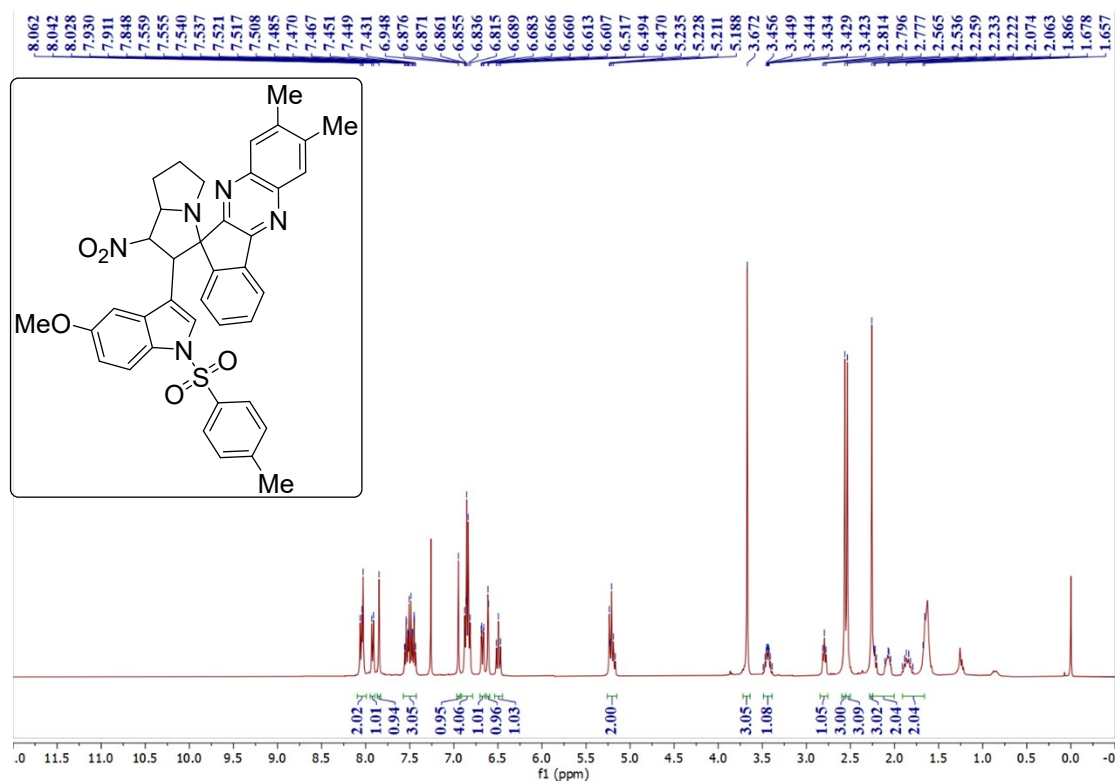
¹³C NMR spectrum of the compound **4r** (100 MHz, CDCl₃)



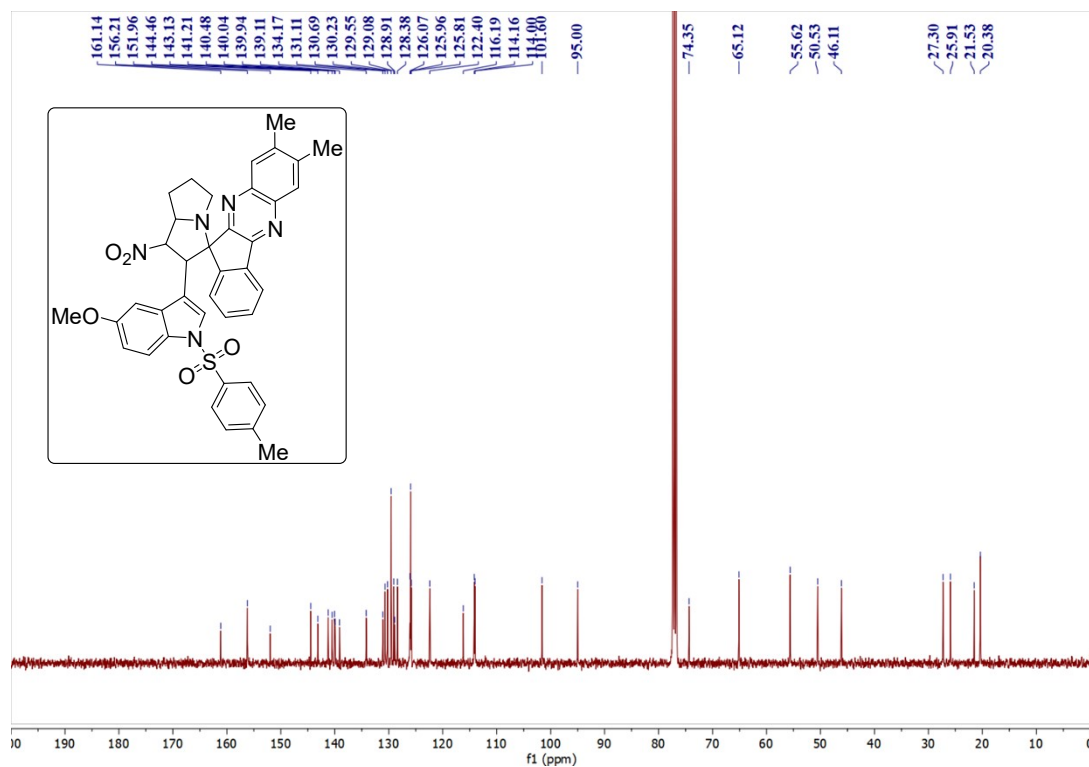
Mass spectrum of the compound **4r**



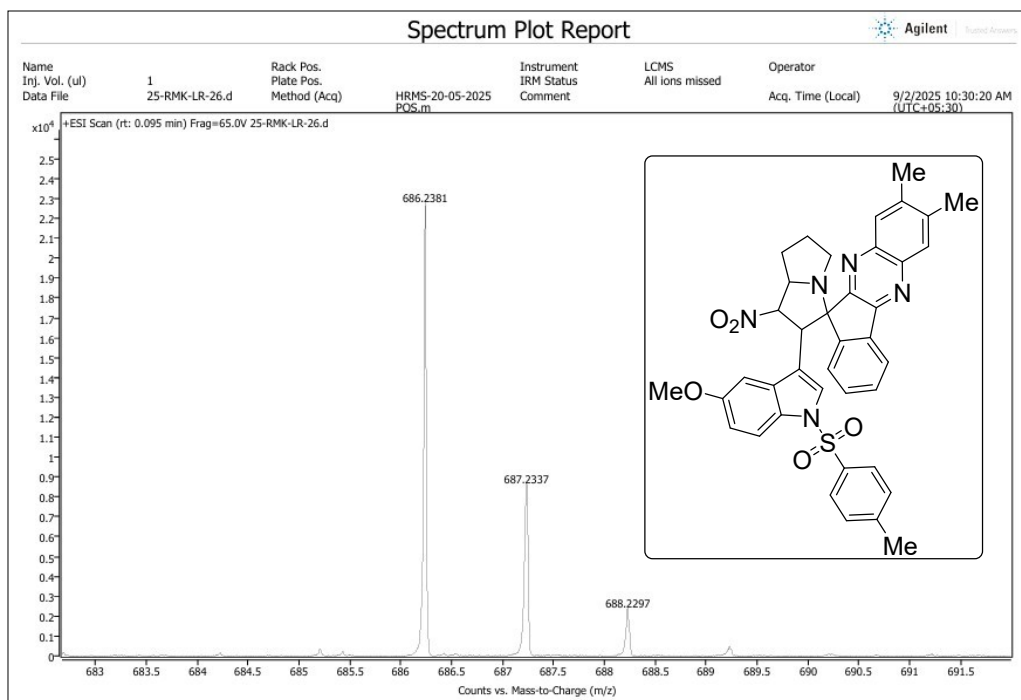
IR spectrum of the compound **4r**



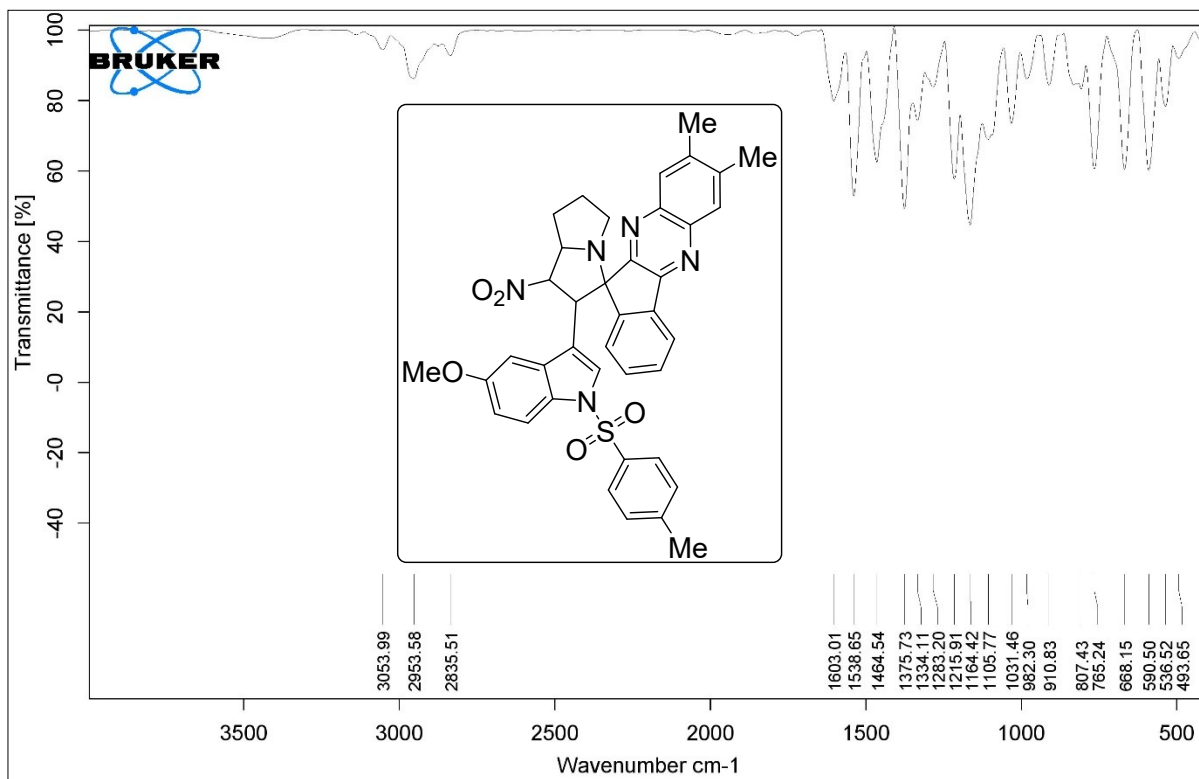
¹H NMR spectrum of the compound 4s (400 MHz, CDCl₃)



¹³C NMR spectrum of the compound 4s (100 MHz, CDCl₃)

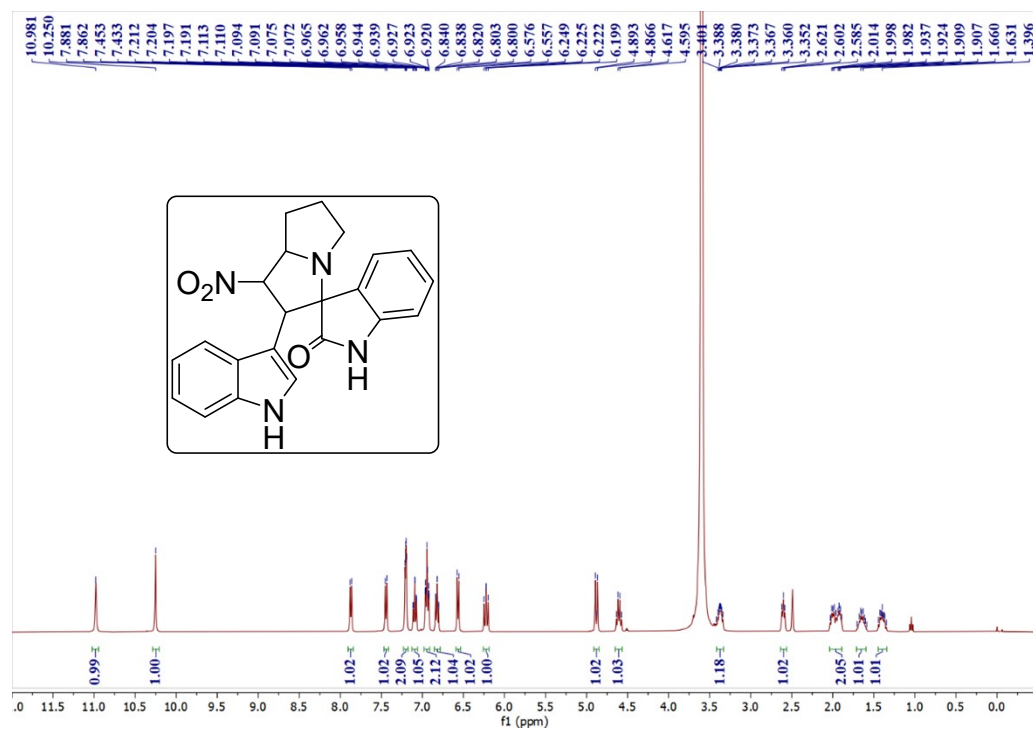


Mass spectrum of the compound **4s**

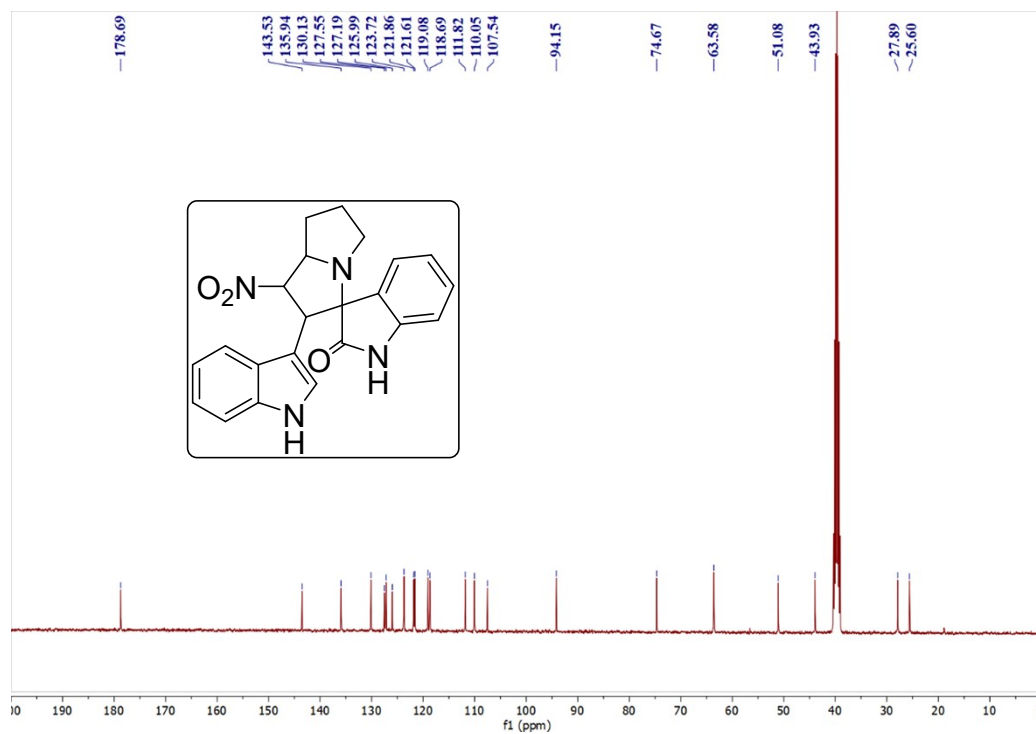


IR spectrum of the compound **4s**

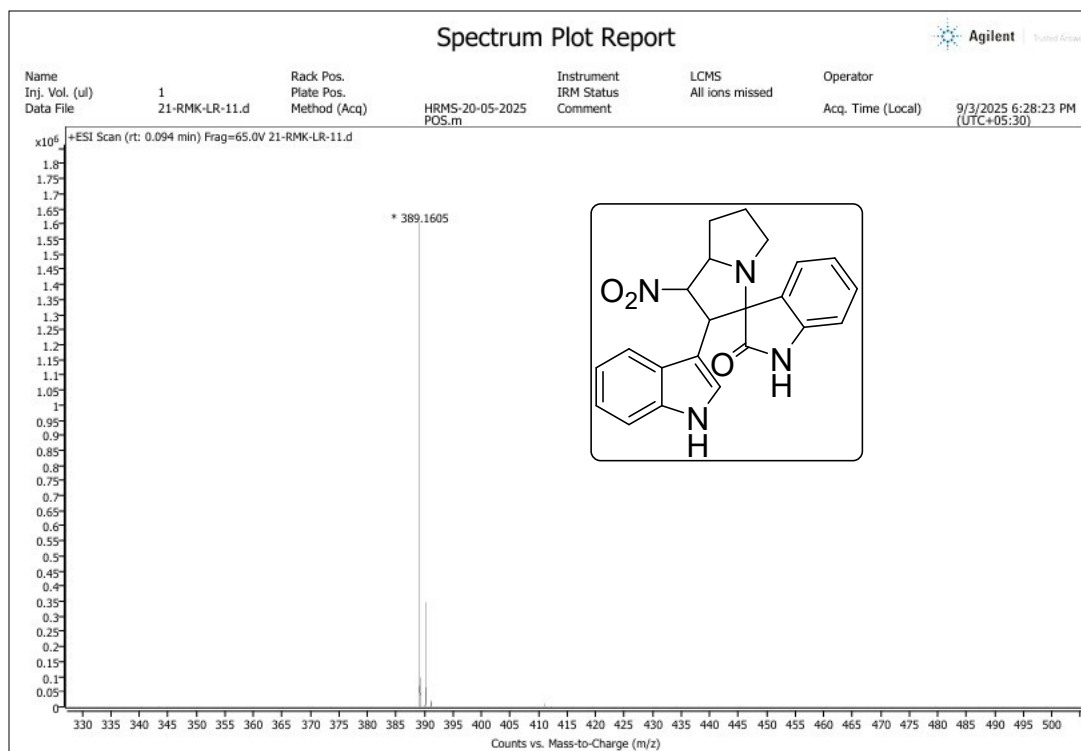
NMR, Mass, FTIR spectral images of the compounds **6a-r**.



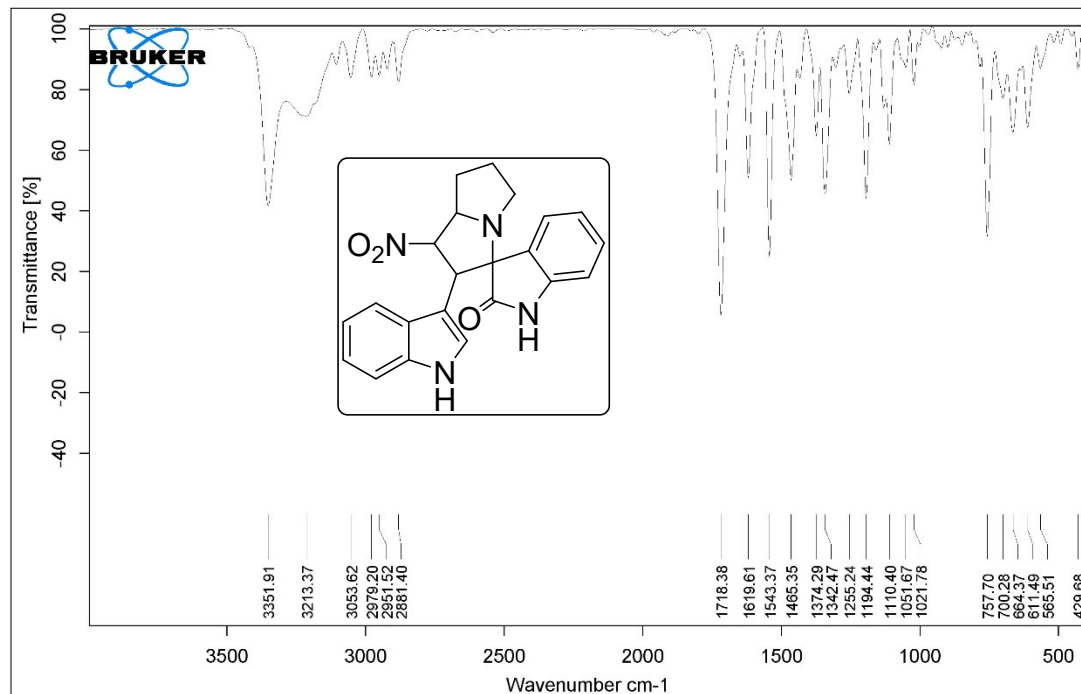
¹H NMR spectrum of the compound **6a** (400 MHz, DMSO-*d*₆)



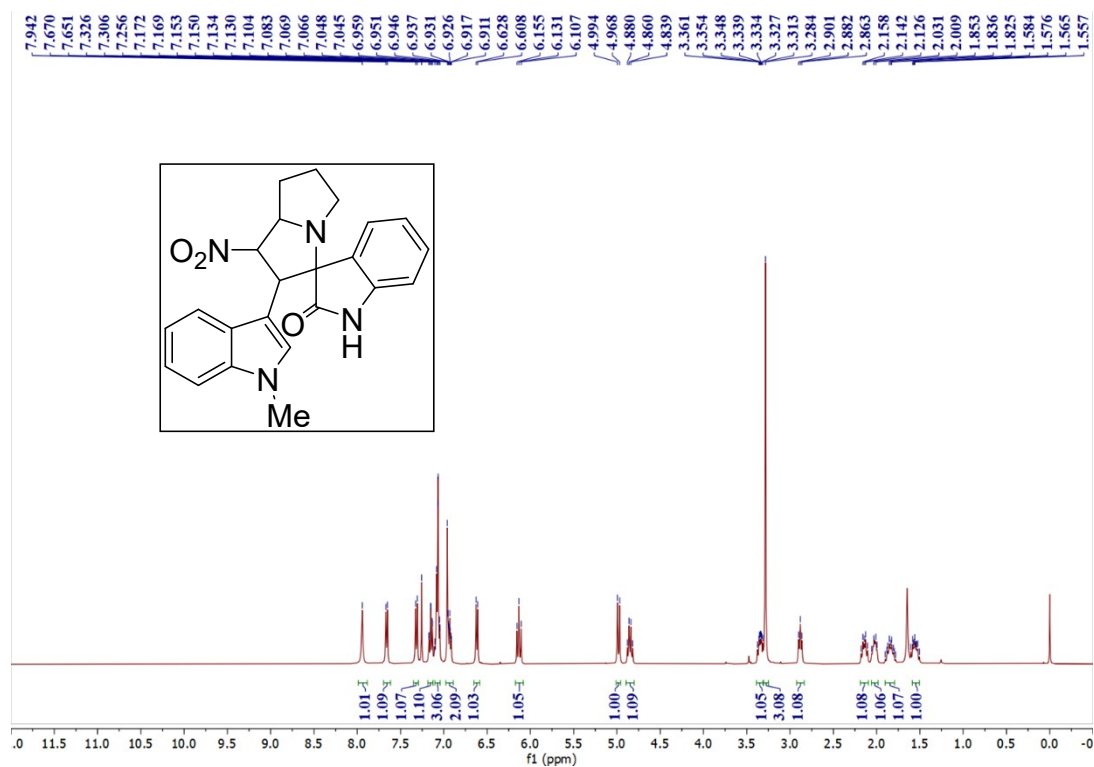
¹³C NMR spectrum of the compound **6a** (100 MHz, DMSO-*d*₆)



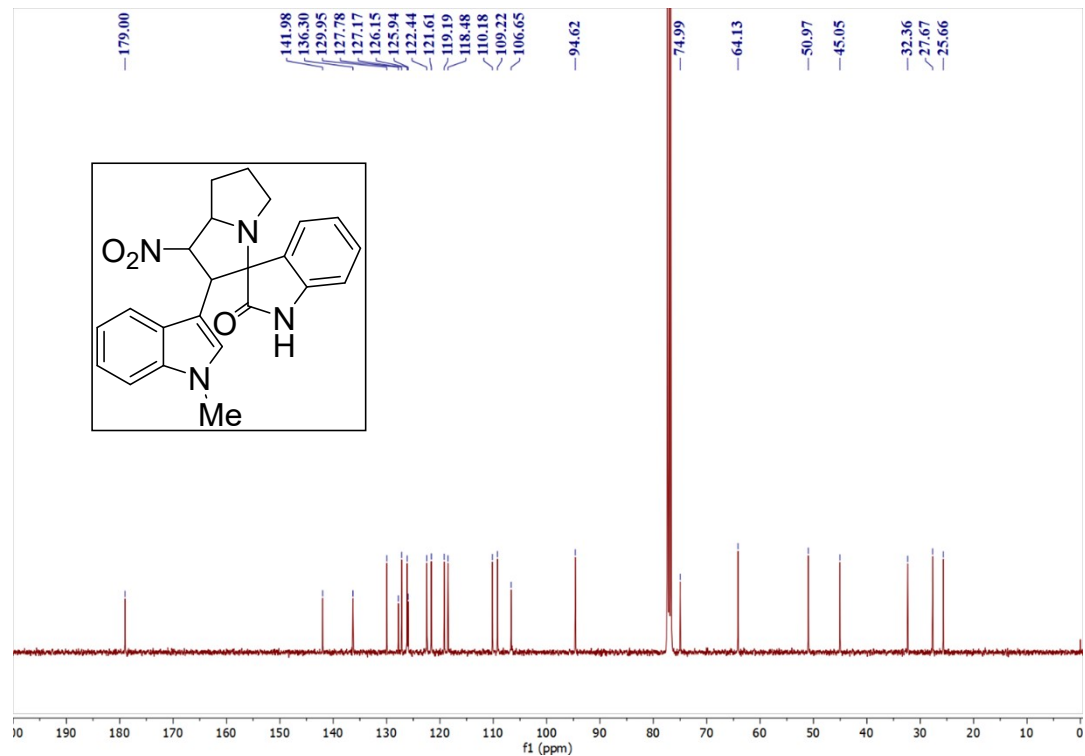
Mass spectrum of the compound **6a**



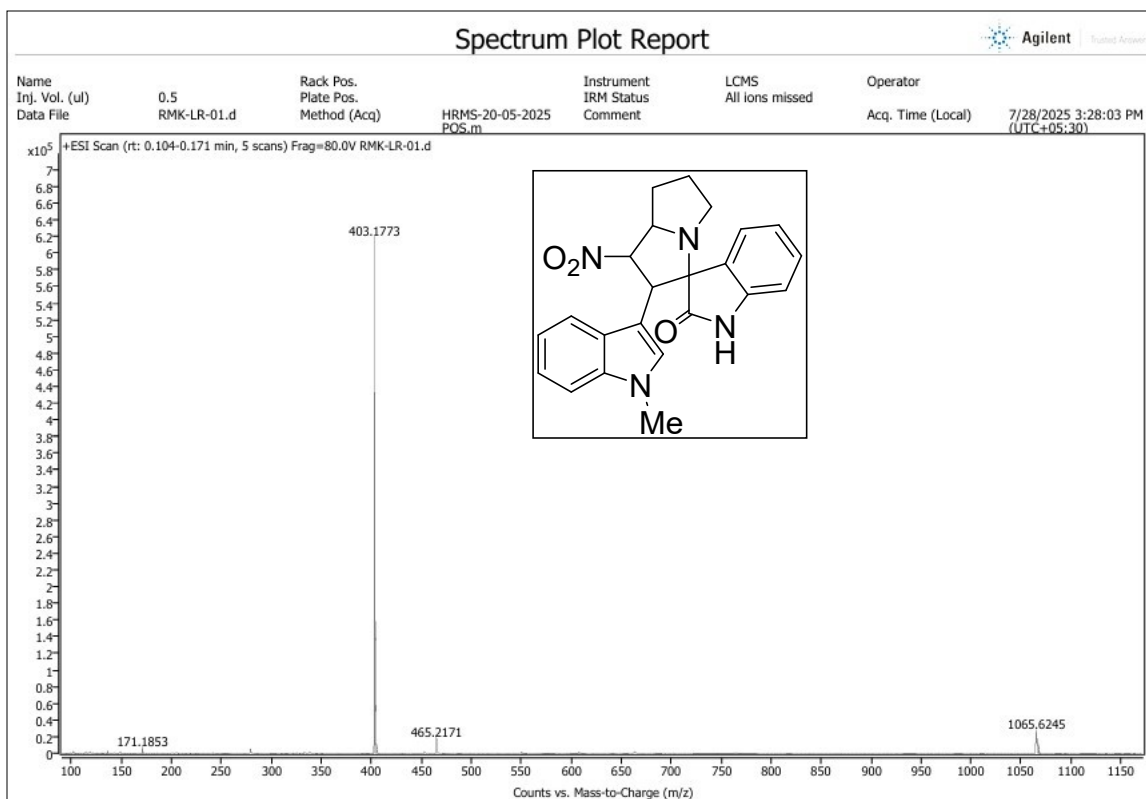
IR spectrum of the compound **6a**



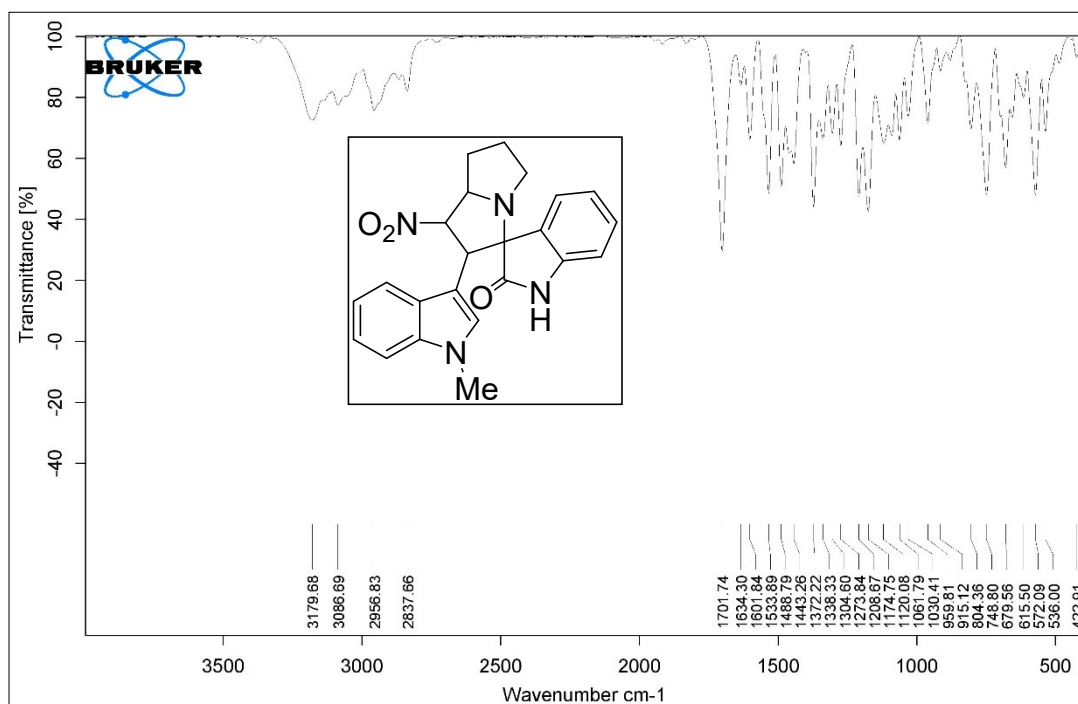
¹H NMR spectrum of the compound **6b** (400 MHz, CDCl₃)



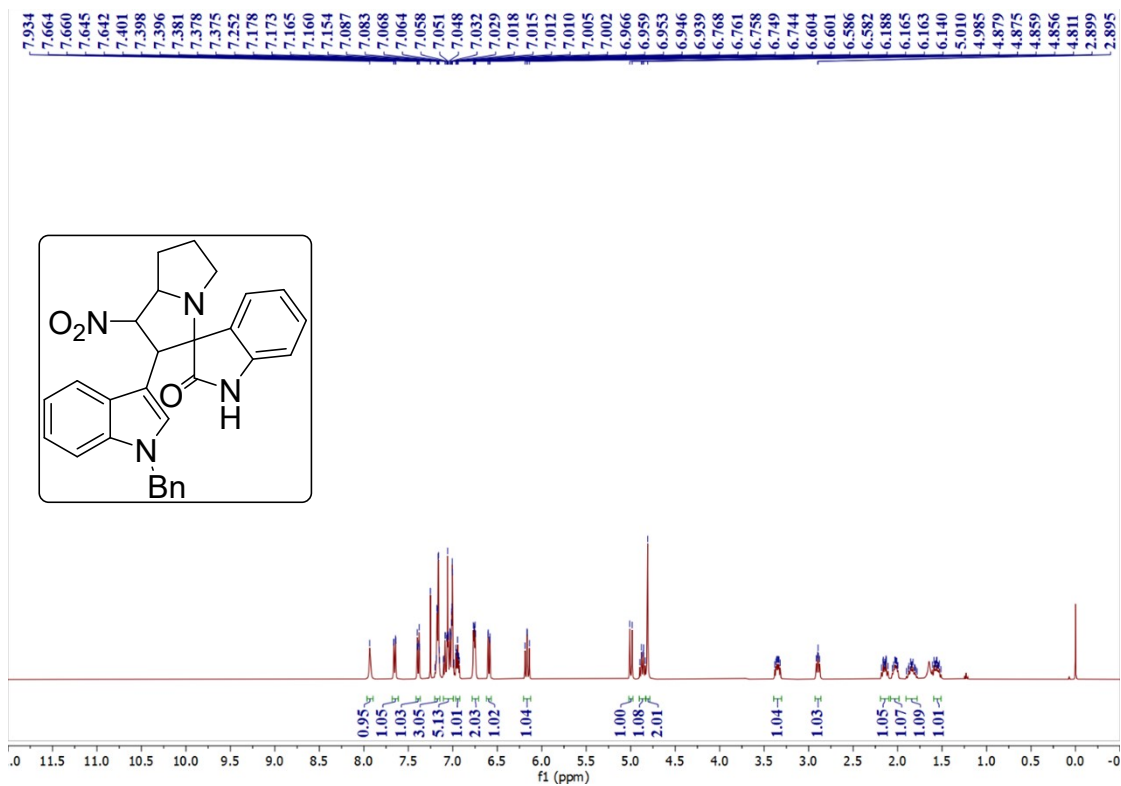
¹³C NMR spectrum of the compound **6b** (100 MHz, CDCl₃)



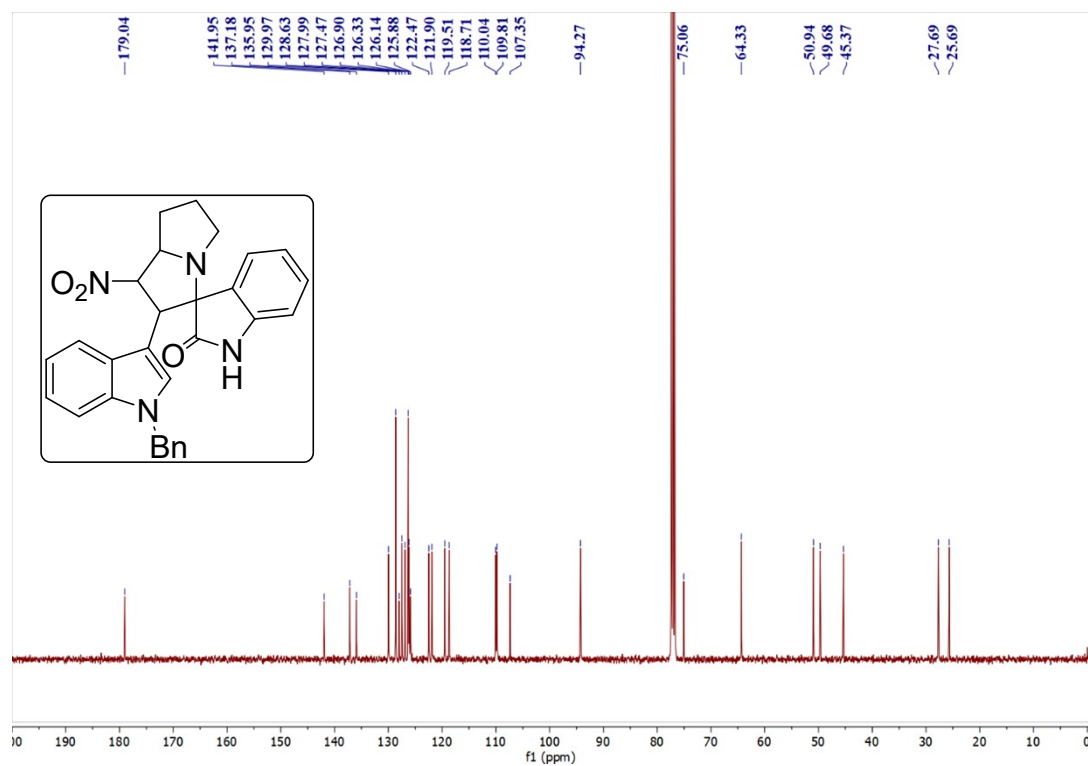
Mass spectrum of the compound **6b**



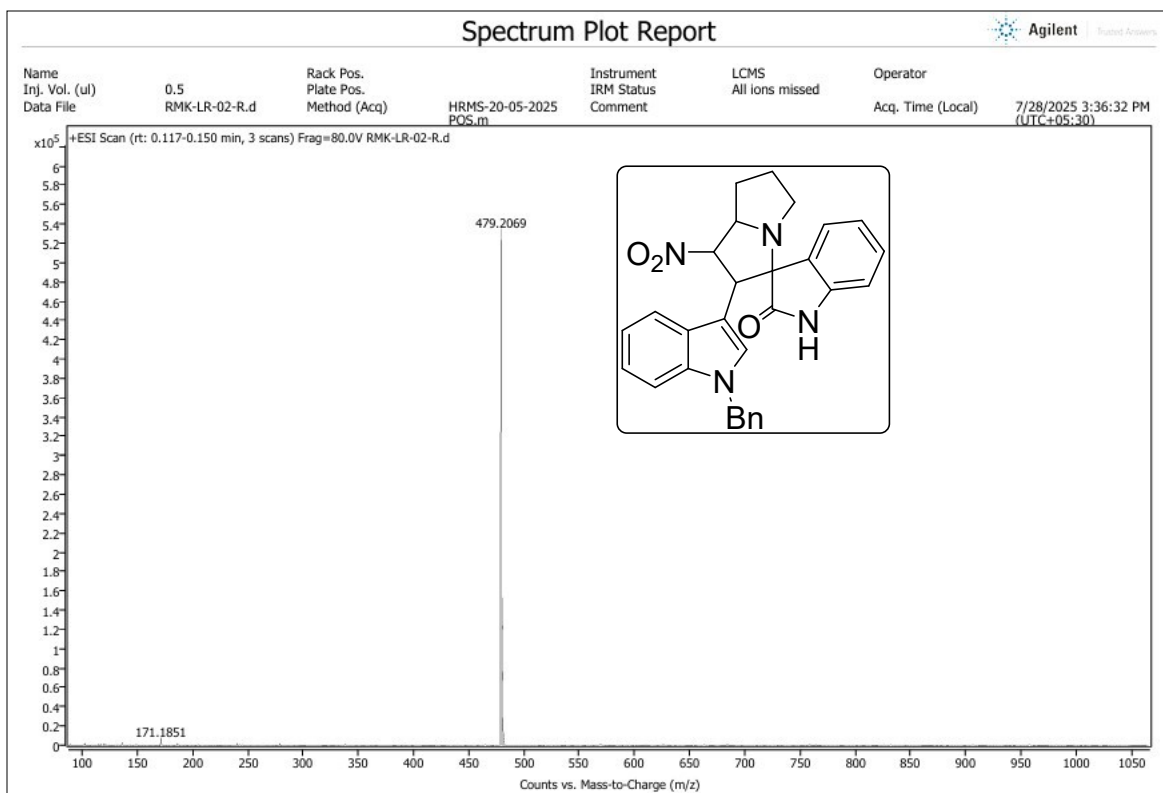
IR spectrum of the compound **6b**



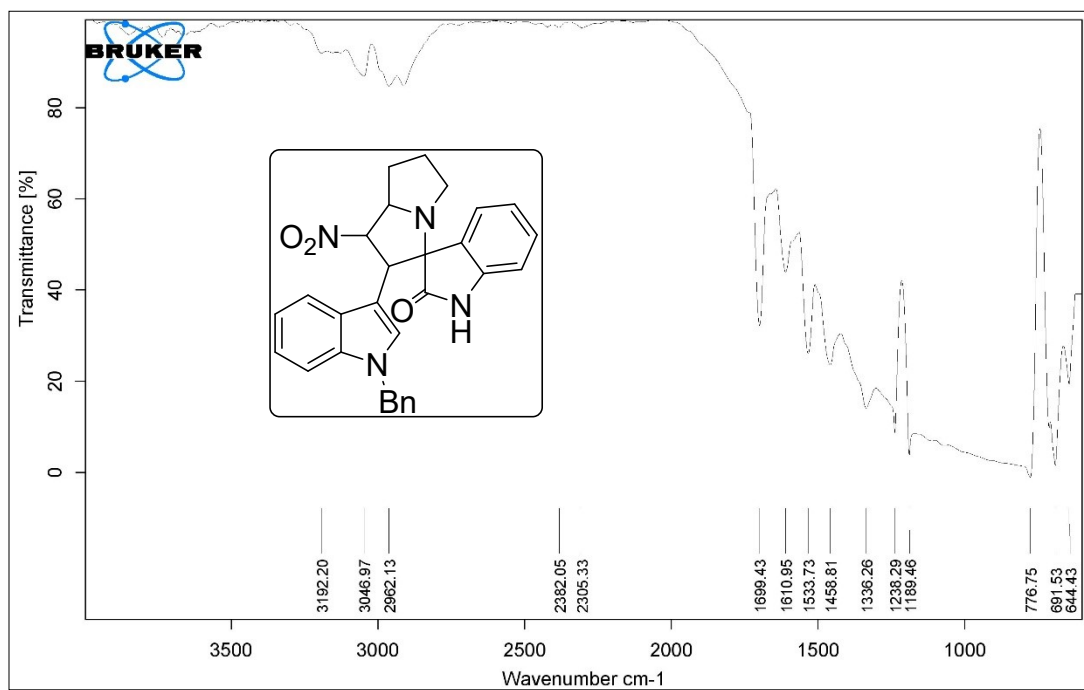
¹H NMR spectrum of the compound **6c** (400 MHz, CDCl₃)



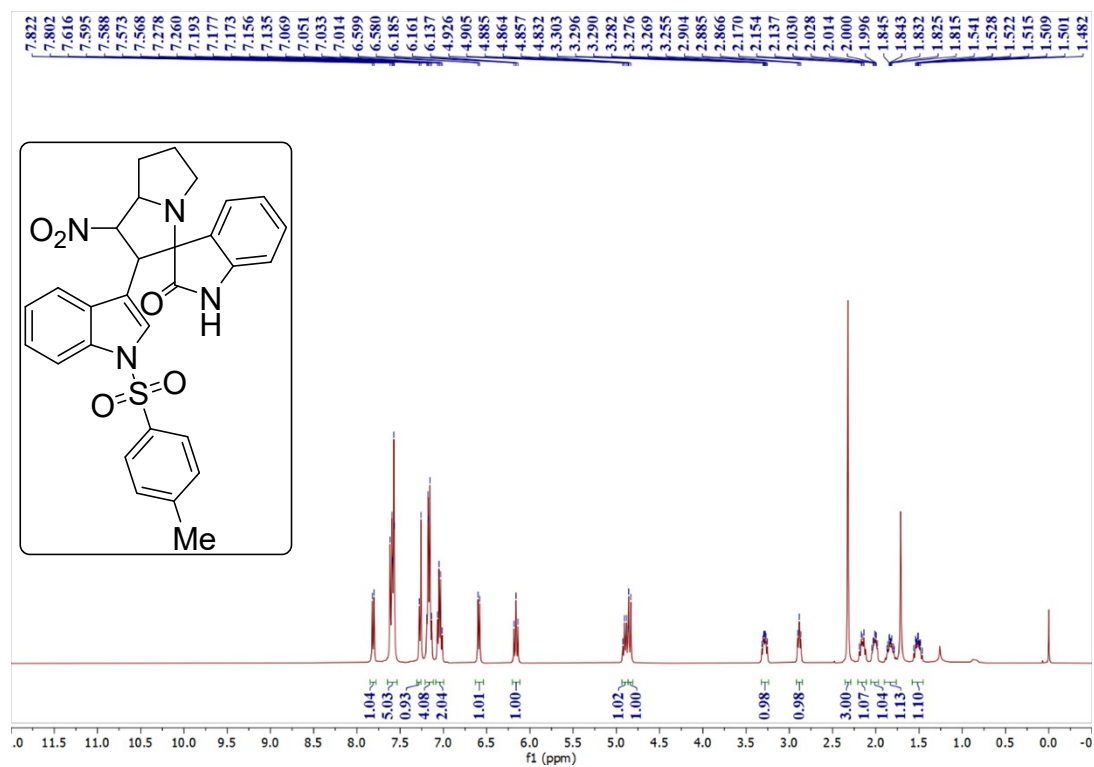
¹³C NMR spectrum of the compound **6c** (100 MHz, CDCl₃)



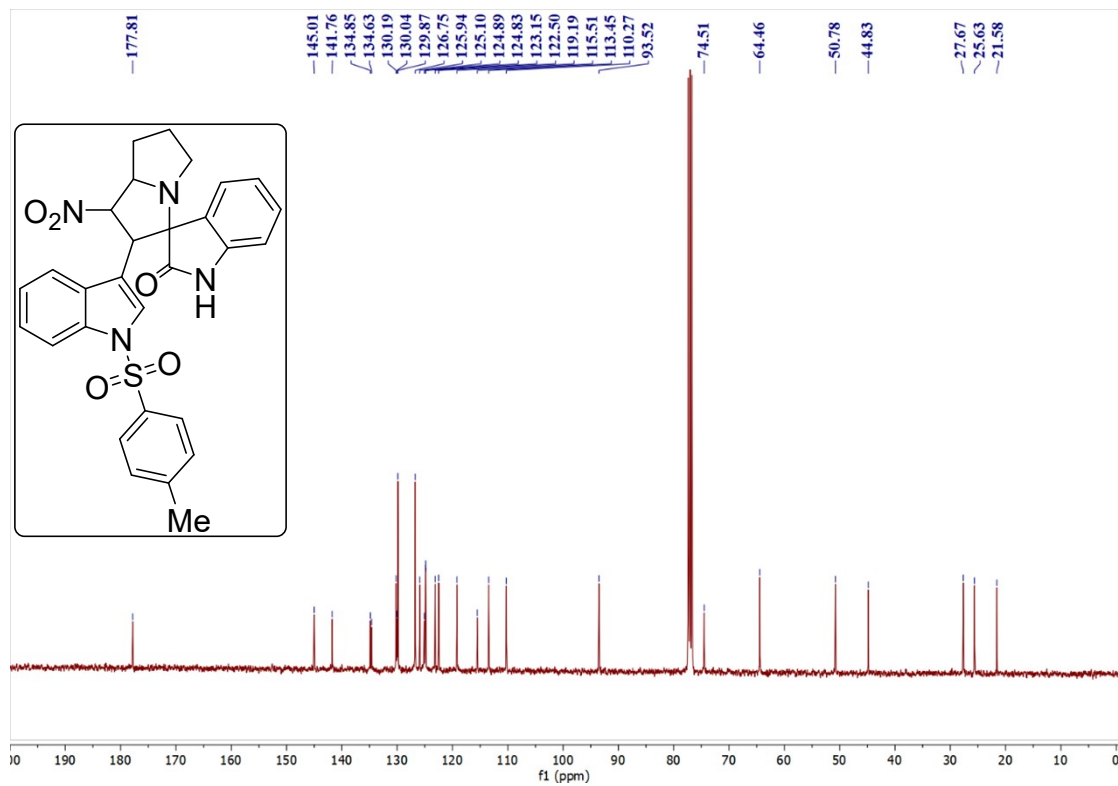
Mass spectrum of the compound **6c**



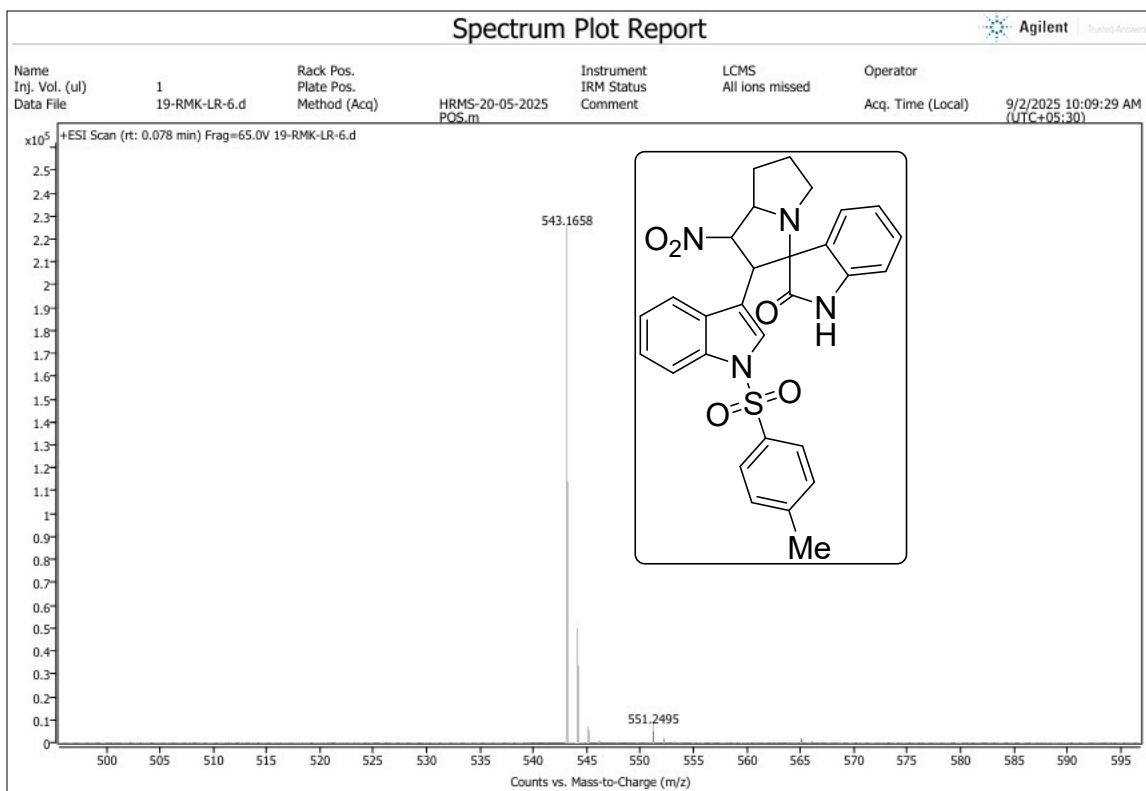
IR spectrum of the compound **6c**



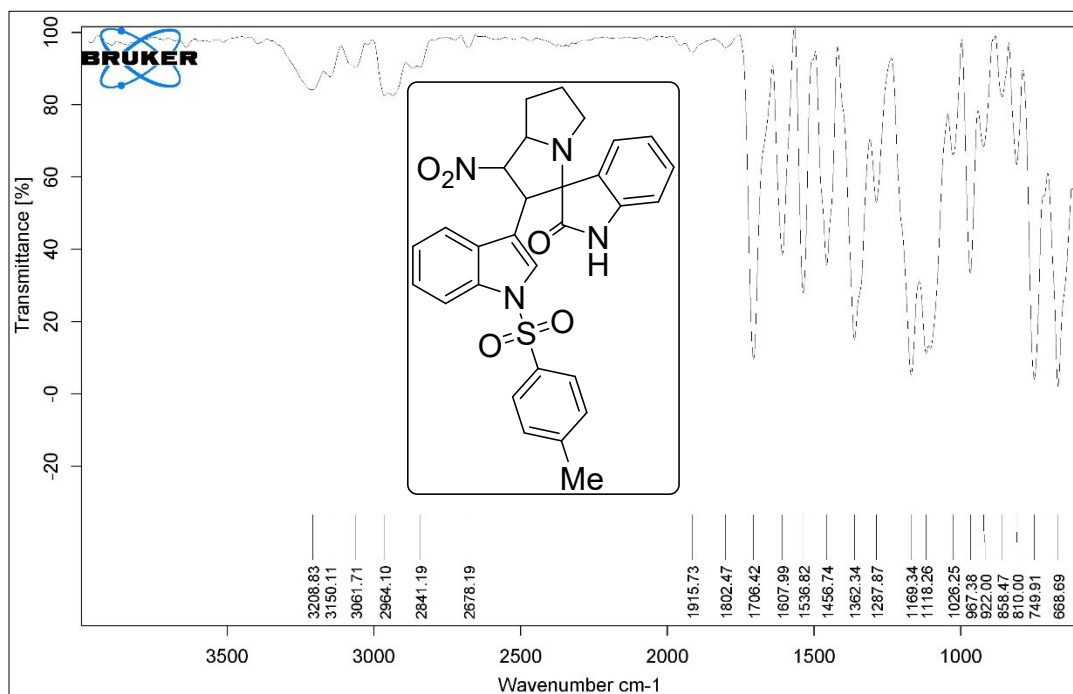
¹H NMR spectrum of the compound **6d** (400 MHz, CDCl₃)



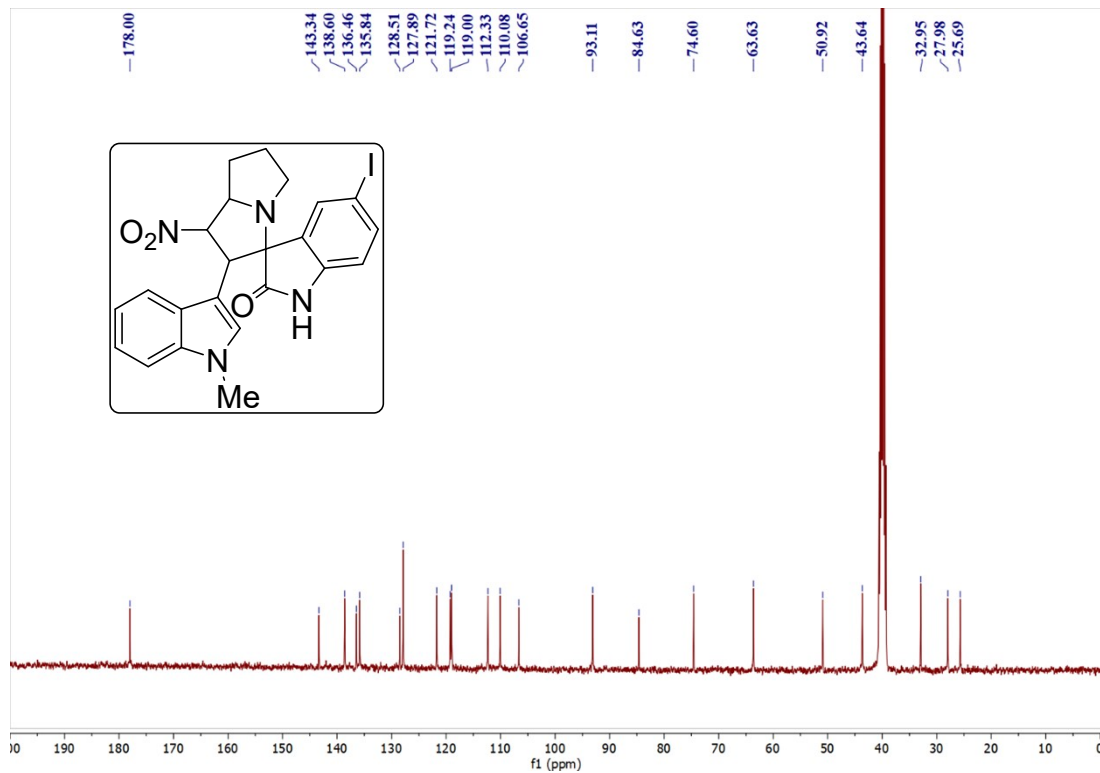
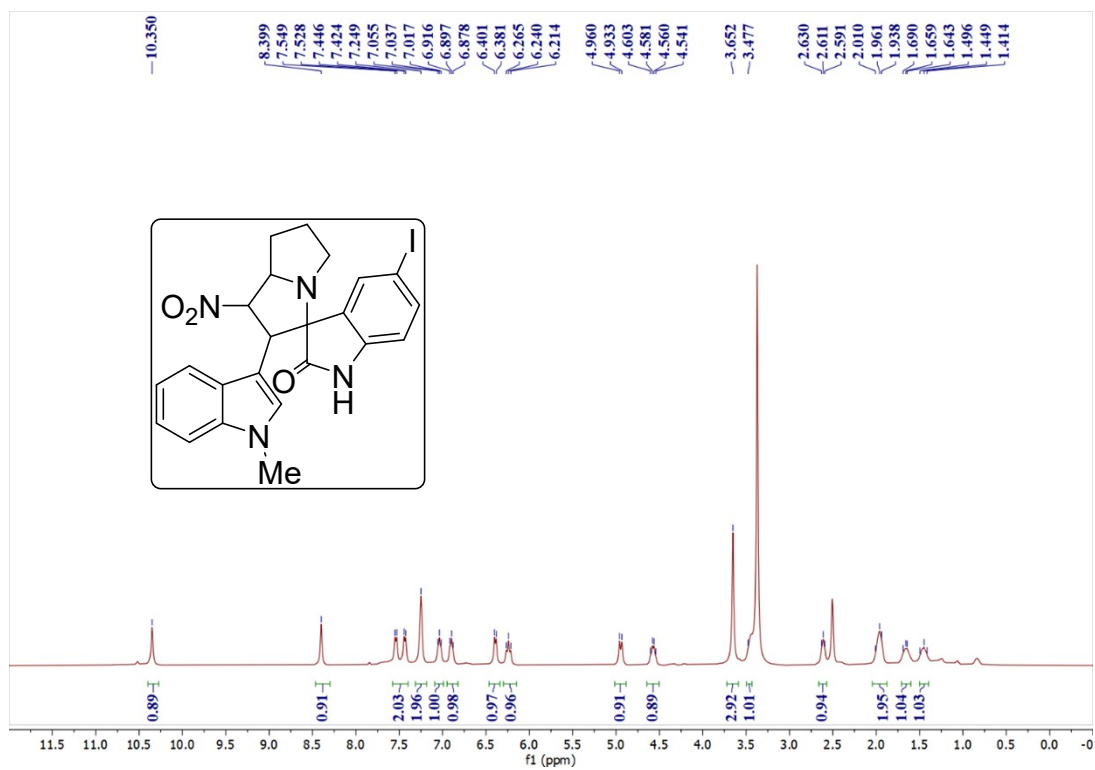
¹³C NMR spectrum of the compound **6d** (100 MHz, CDCl₃)

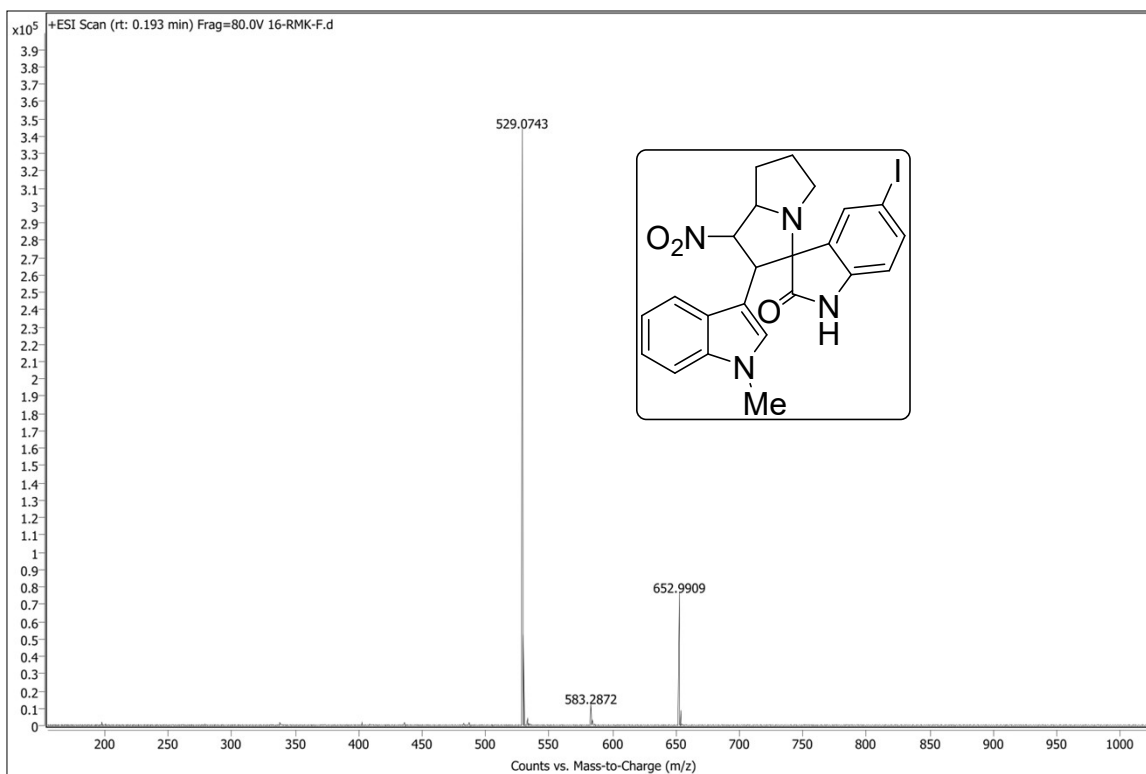


Mass spectrum of the compound **6d**

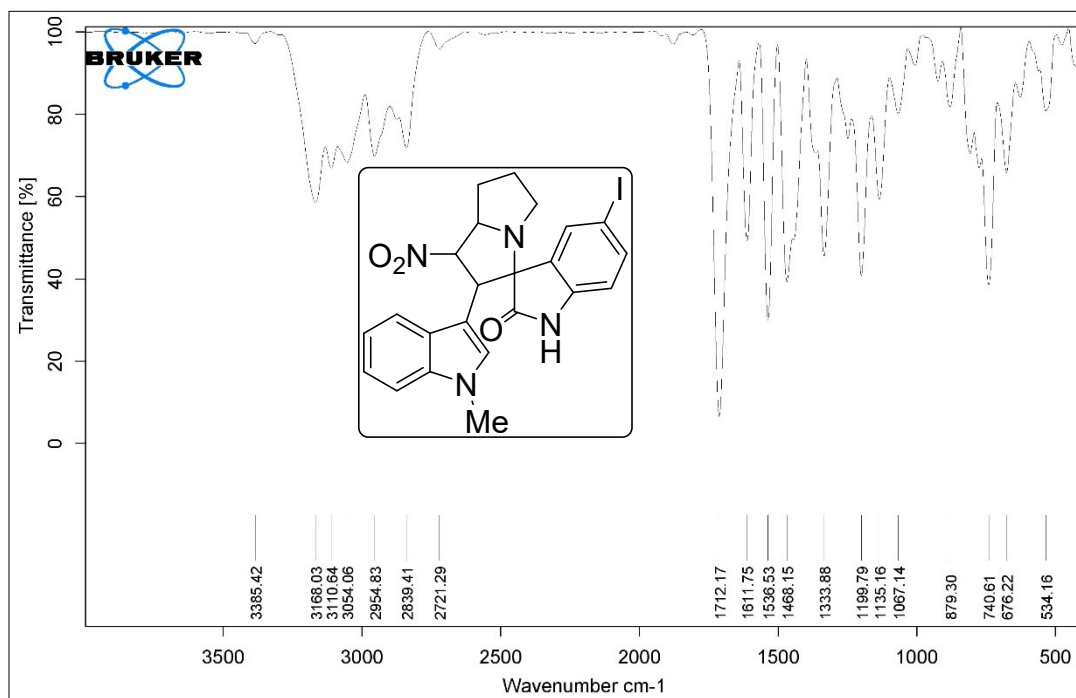


IR spectrum of the compound **6d**

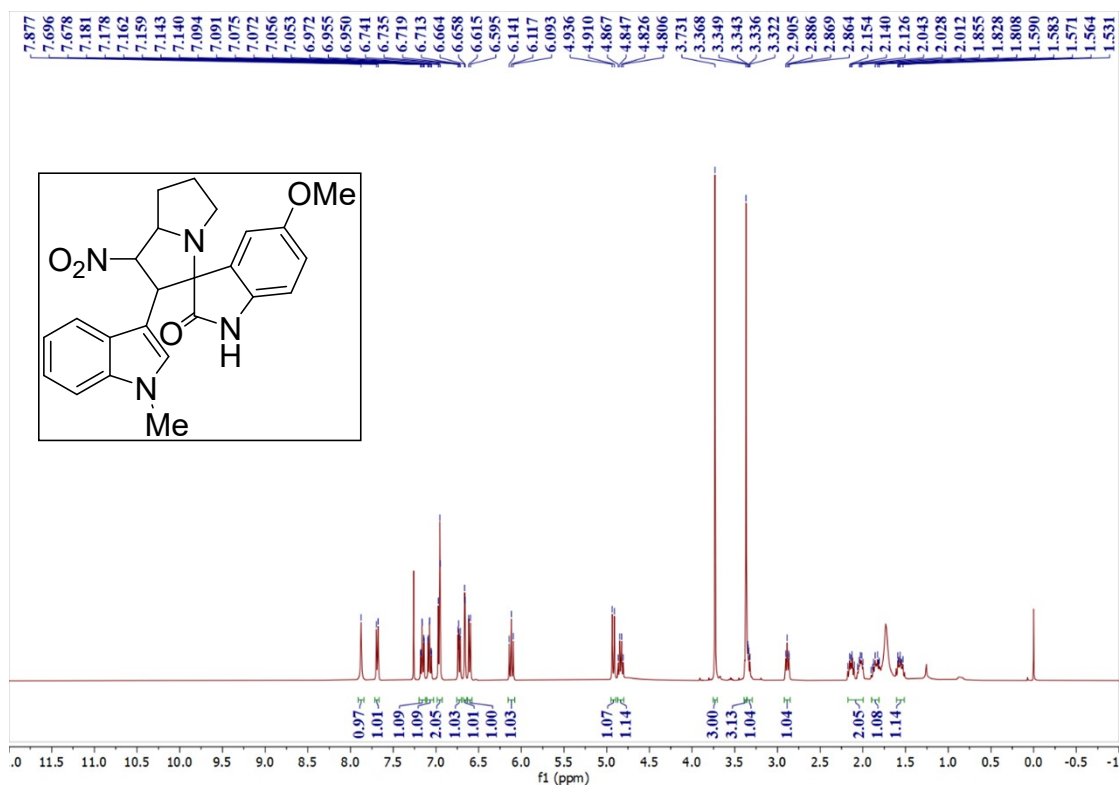




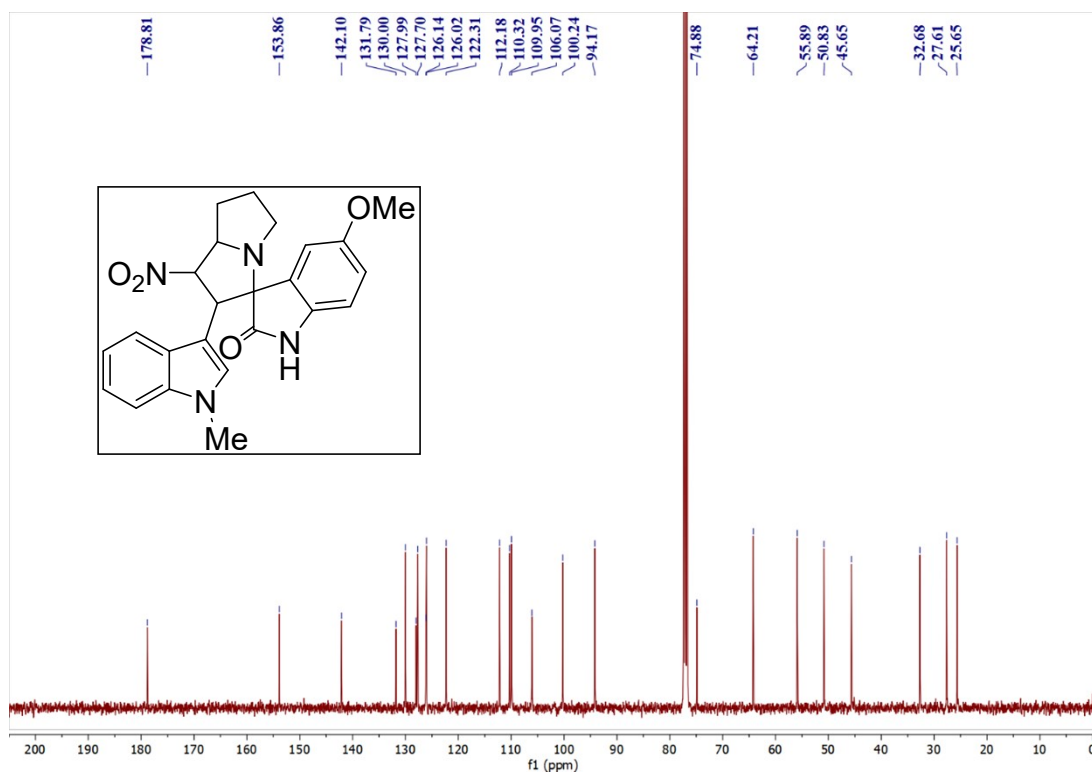
Mass spectrum of the compound **6e**



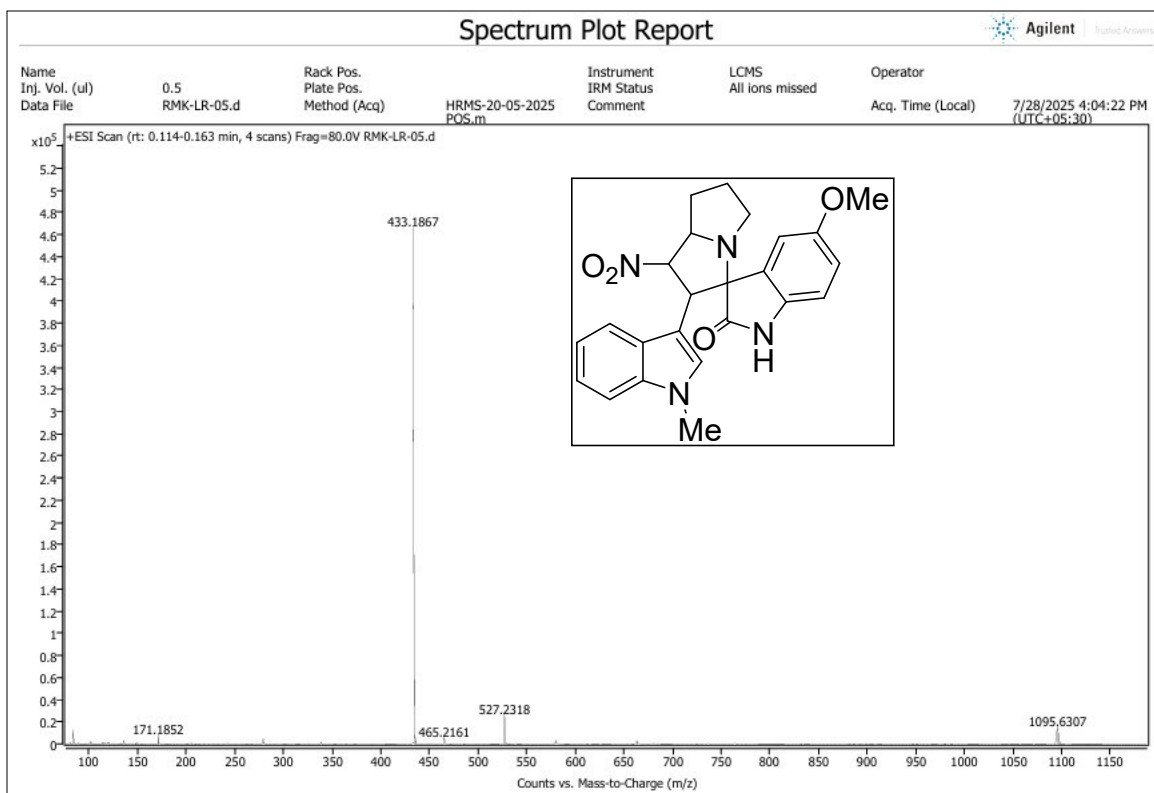
IR spectrum of the compound **6e**



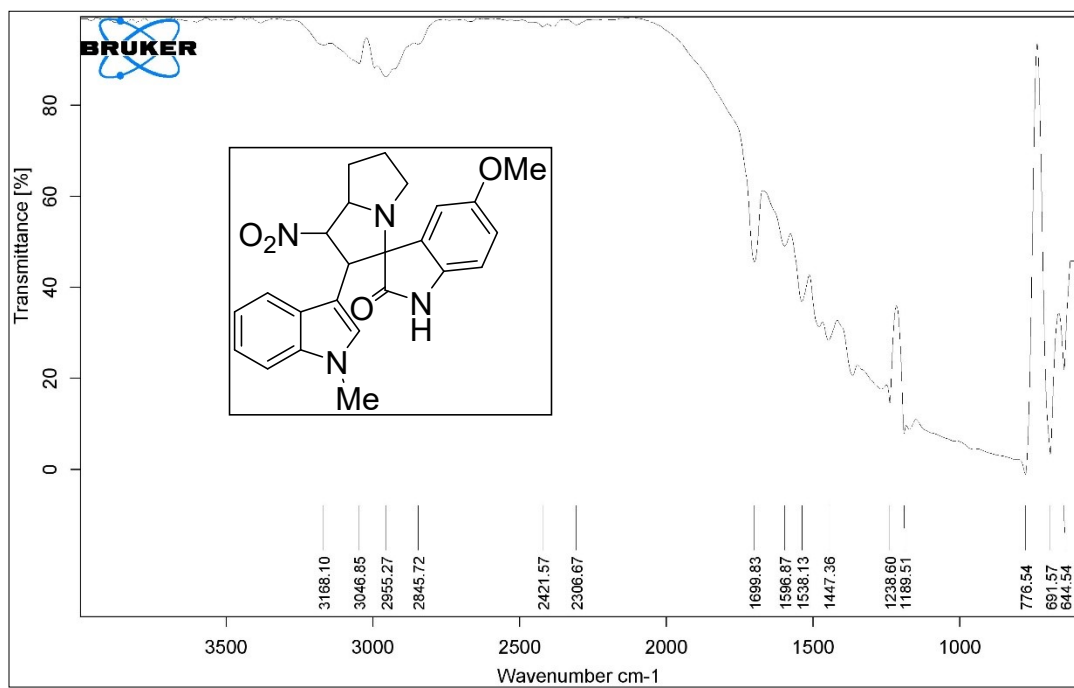
¹H NMR spectrum of the compound **6f** (400 MHz, CDCl₃)



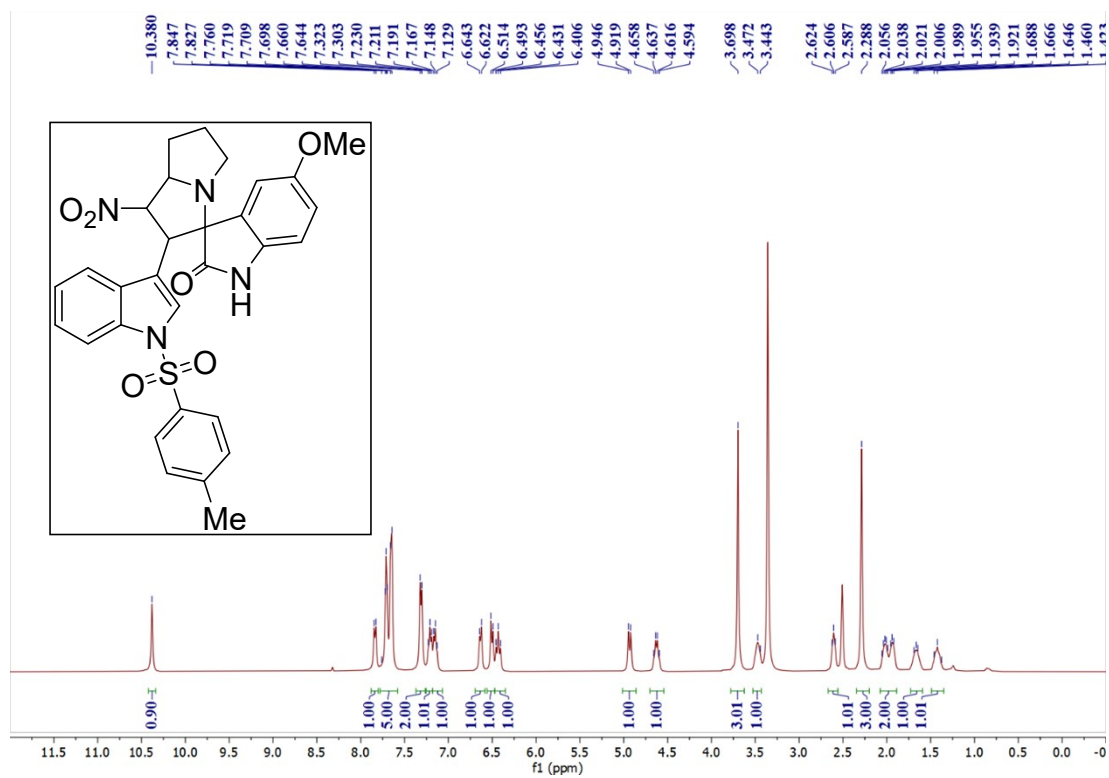
¹³C NMR spectrum of the compound **6f** (100 MHz, CDCl₃)



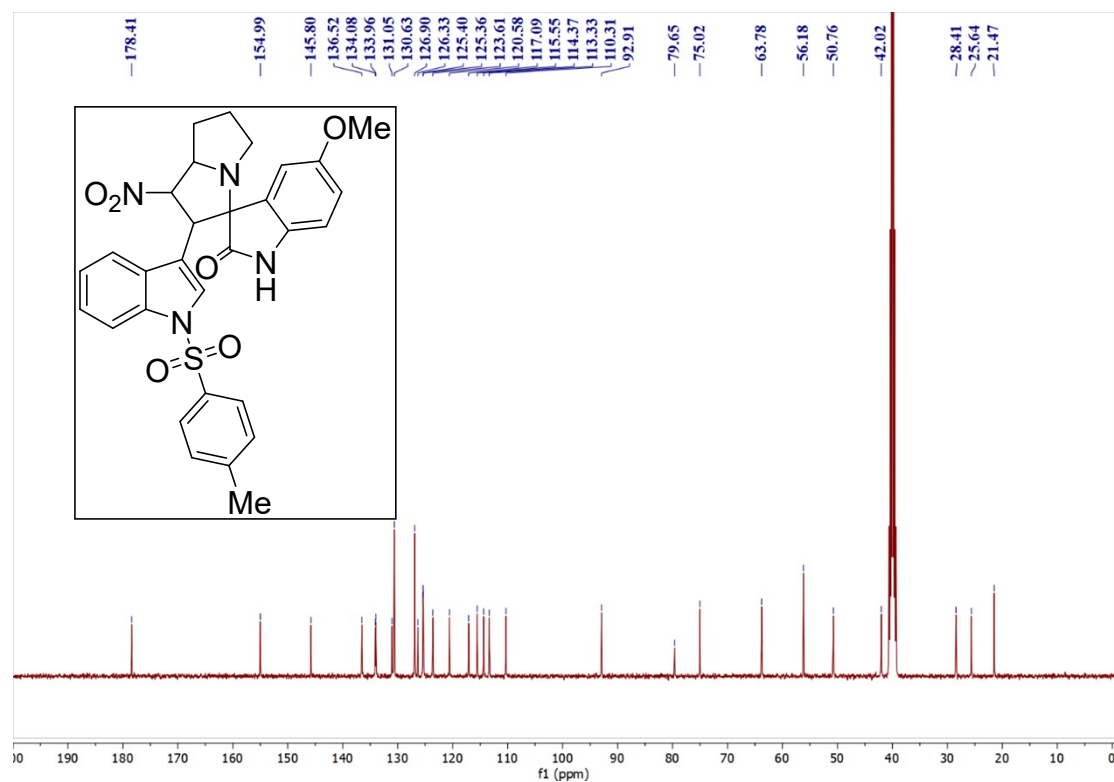
Mass spectrum of the compound **6f**



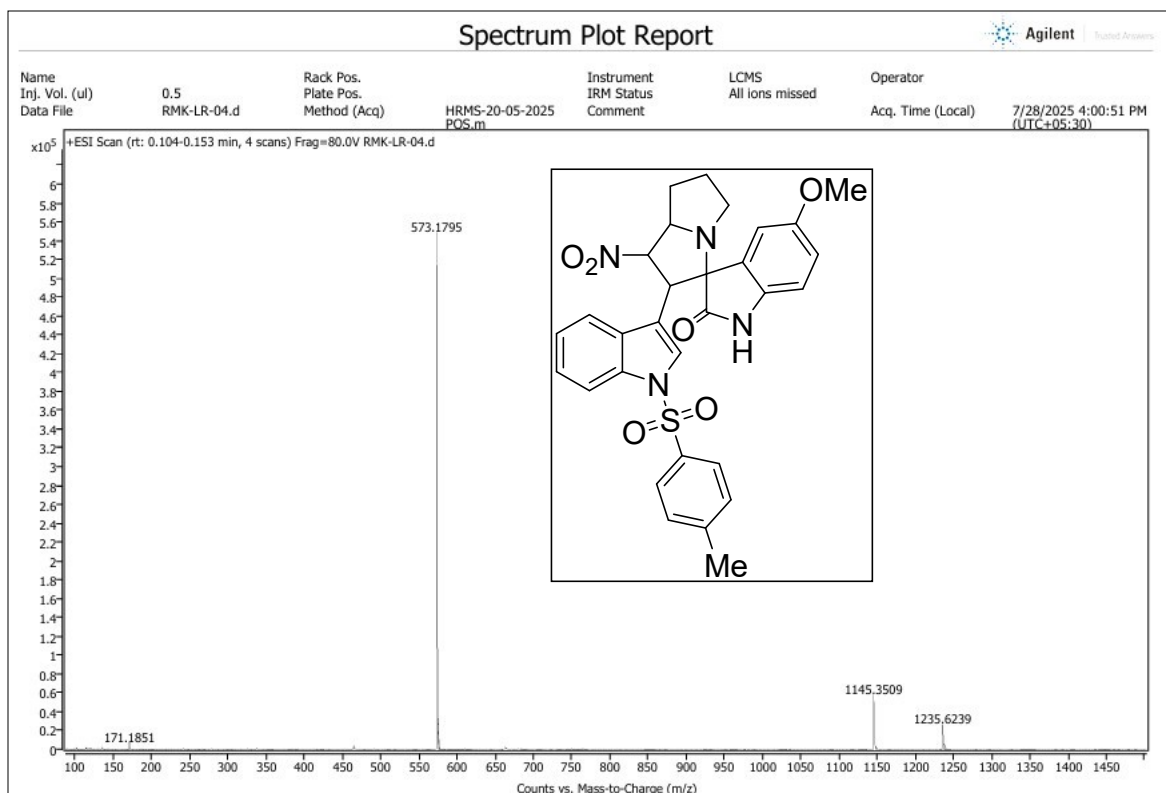
IR spectrum of the compound **6f**



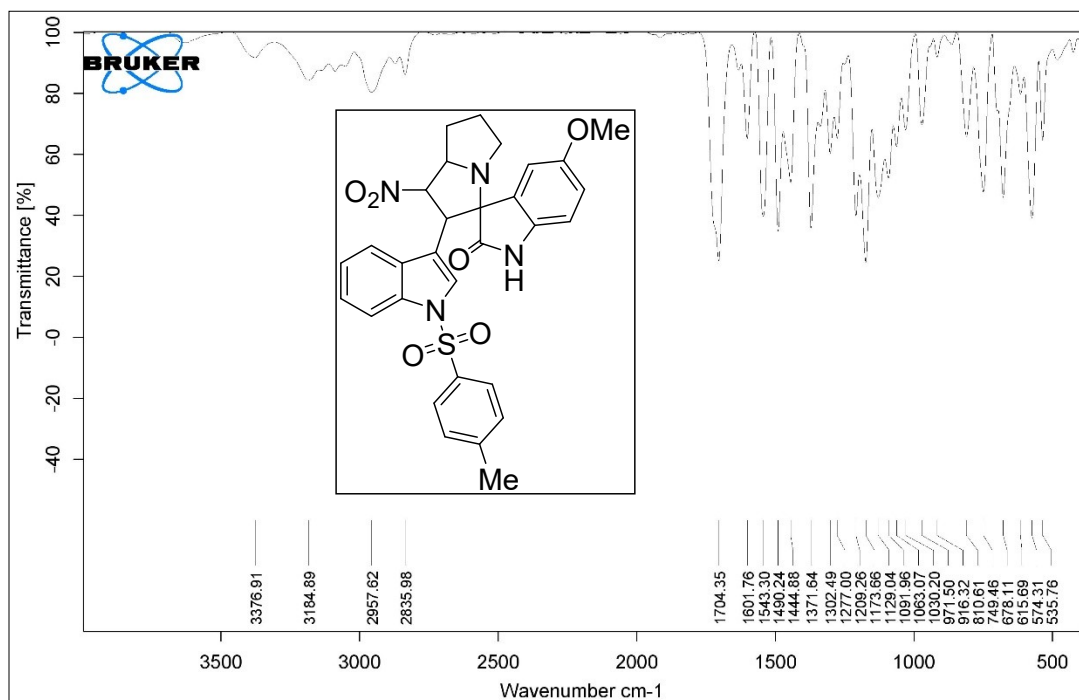
¹H NMR spectrum of the compound **6g** (400 MHz, DMSO-*d*₆)



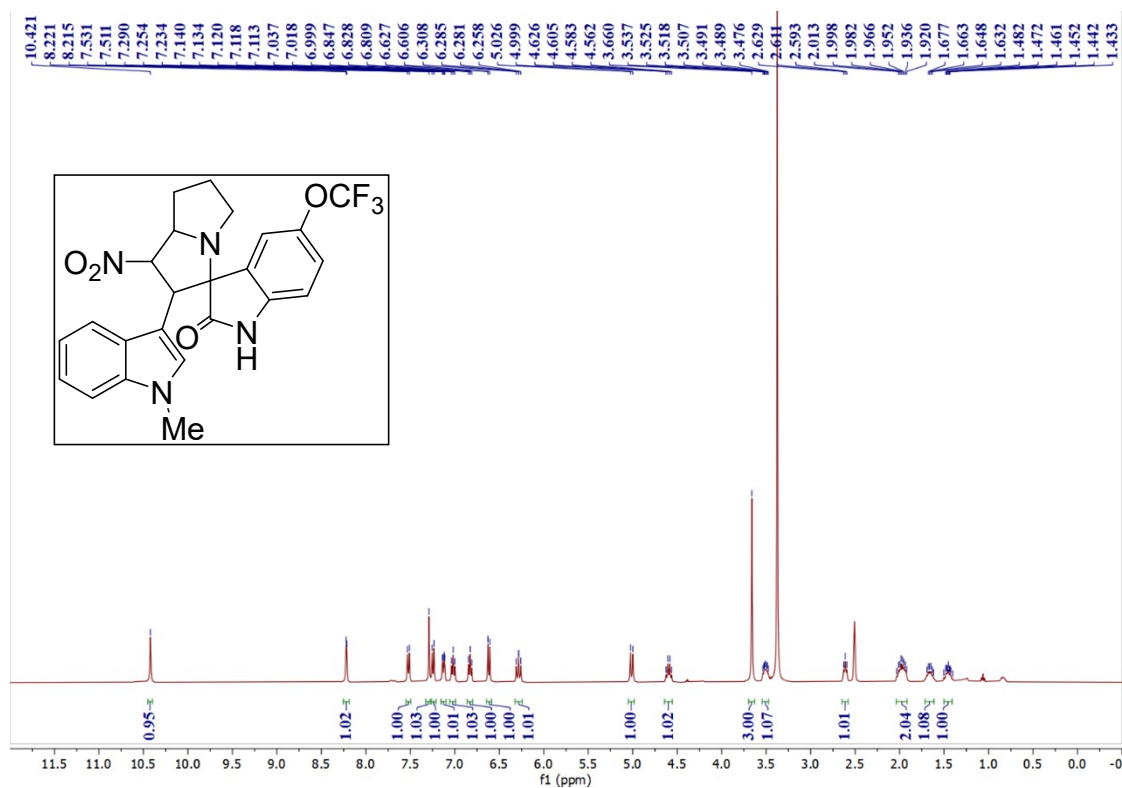
¹³C NMR spectrum of the compound **6g** (100 MHz, DMSO-*d*₆)



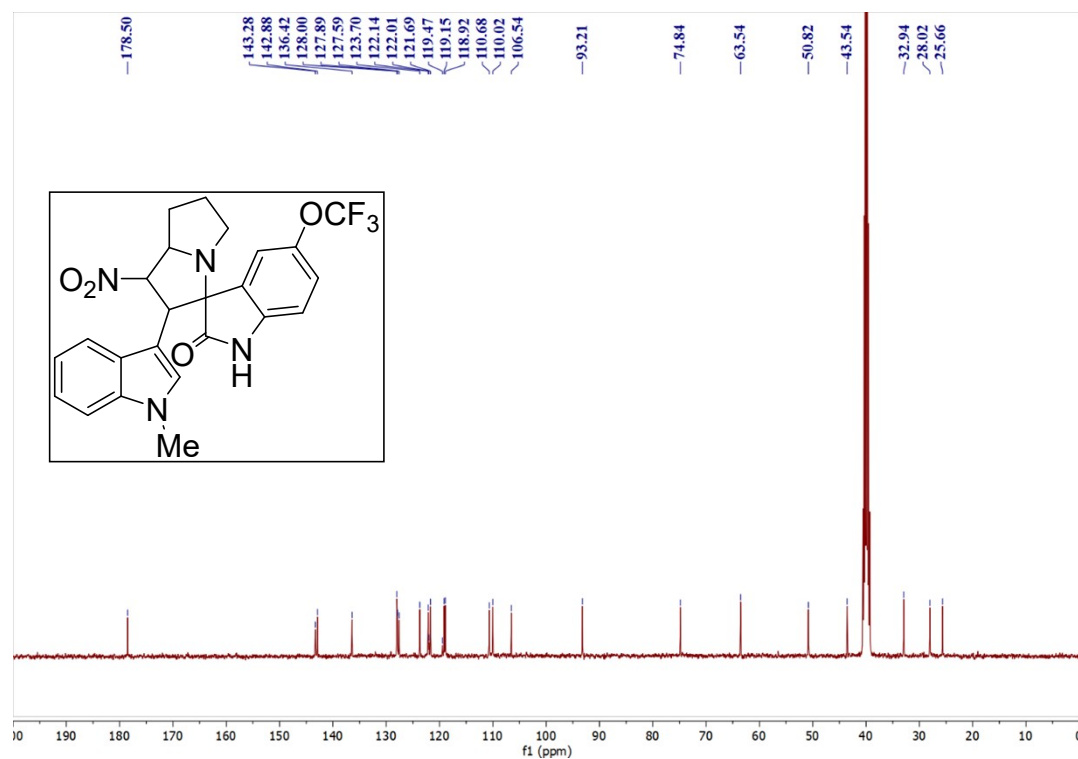
Mass spectrum of the compound **6g**



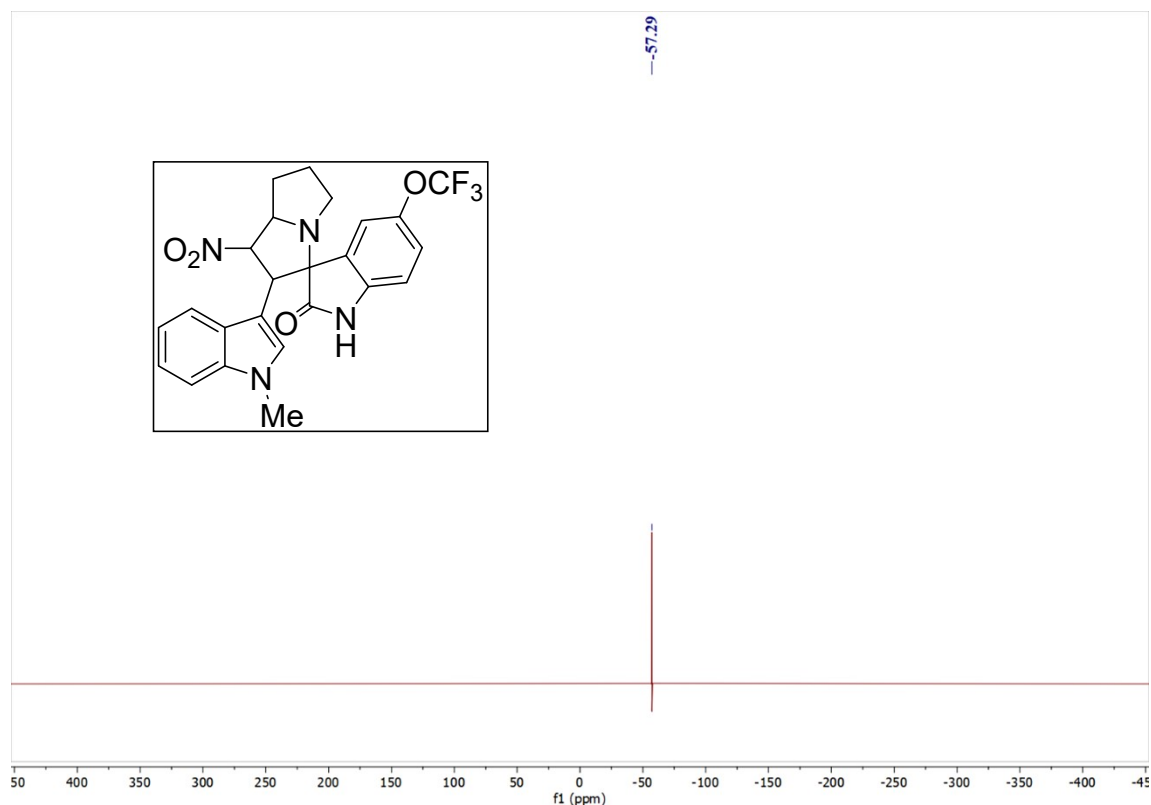
IR spectrum of the compound **6g**



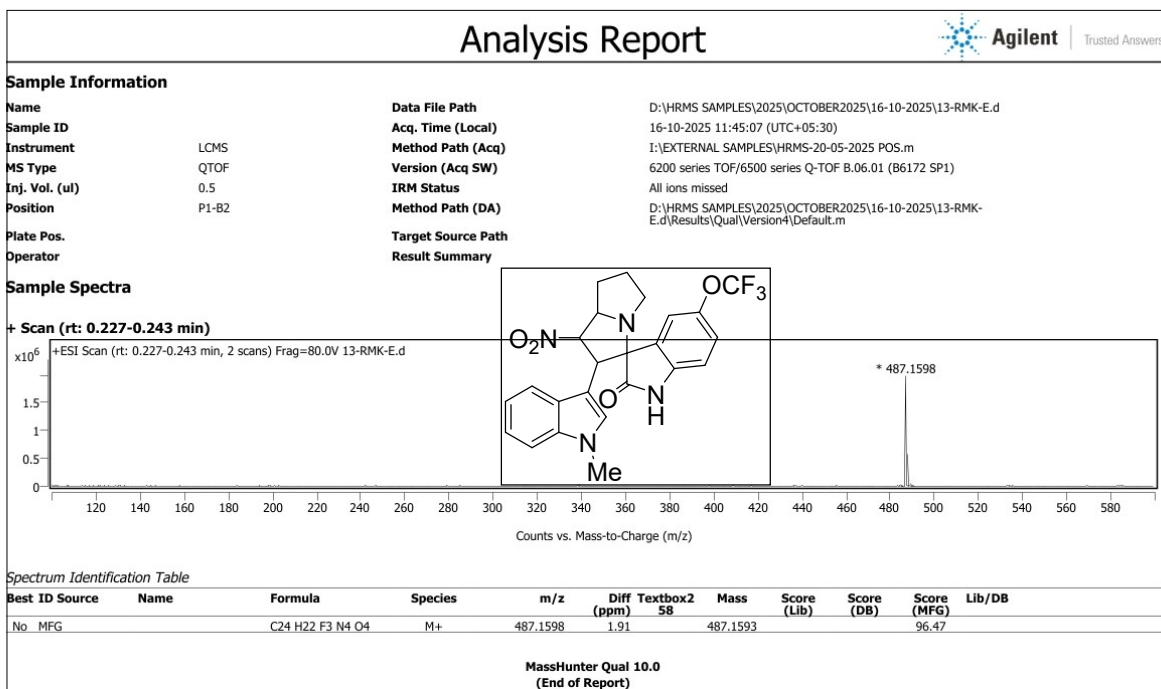
¹H NMR spectrum of the compound **6h** (400 MHz, DMSO-*d*₆)



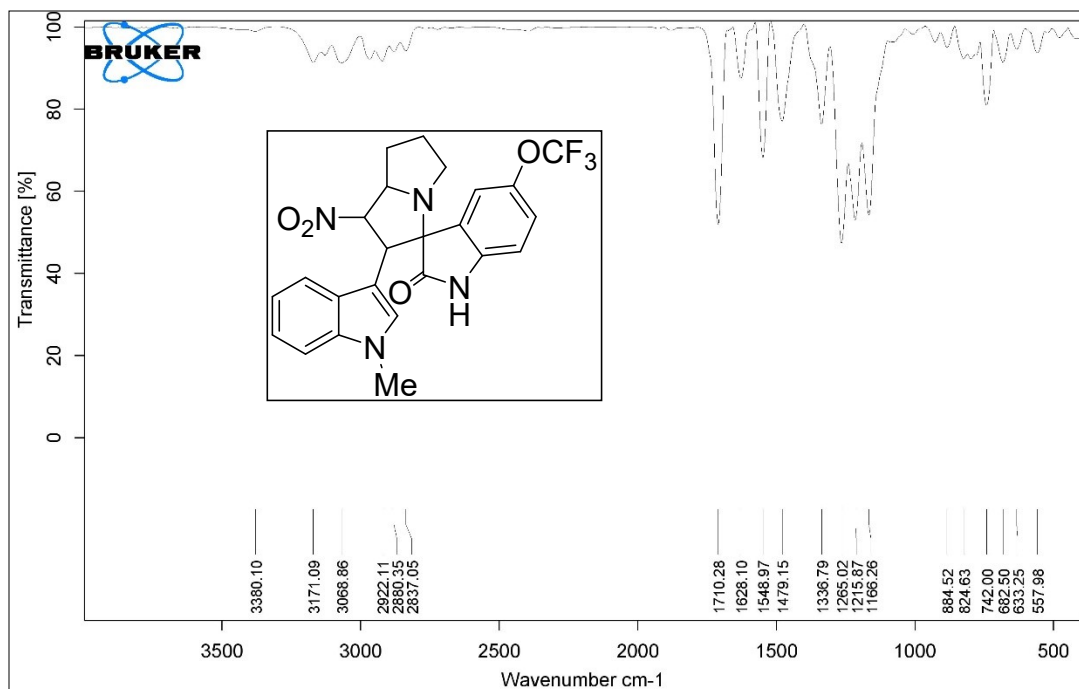
¹³C NMR spectrum of the compound **6h** (100 MHz, DMSO-*d*₆)



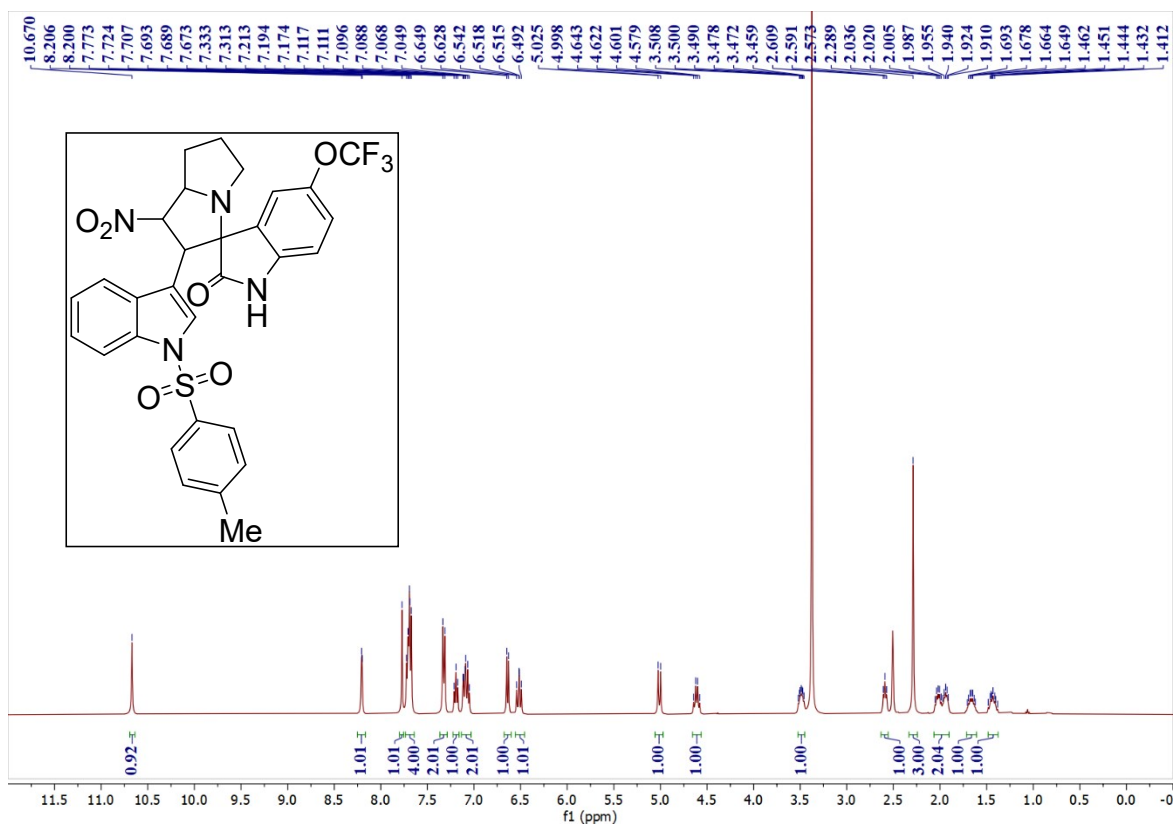
^{19}F NMR spectrum of the compound **6h** (376 MHz, DMSO- d_6)



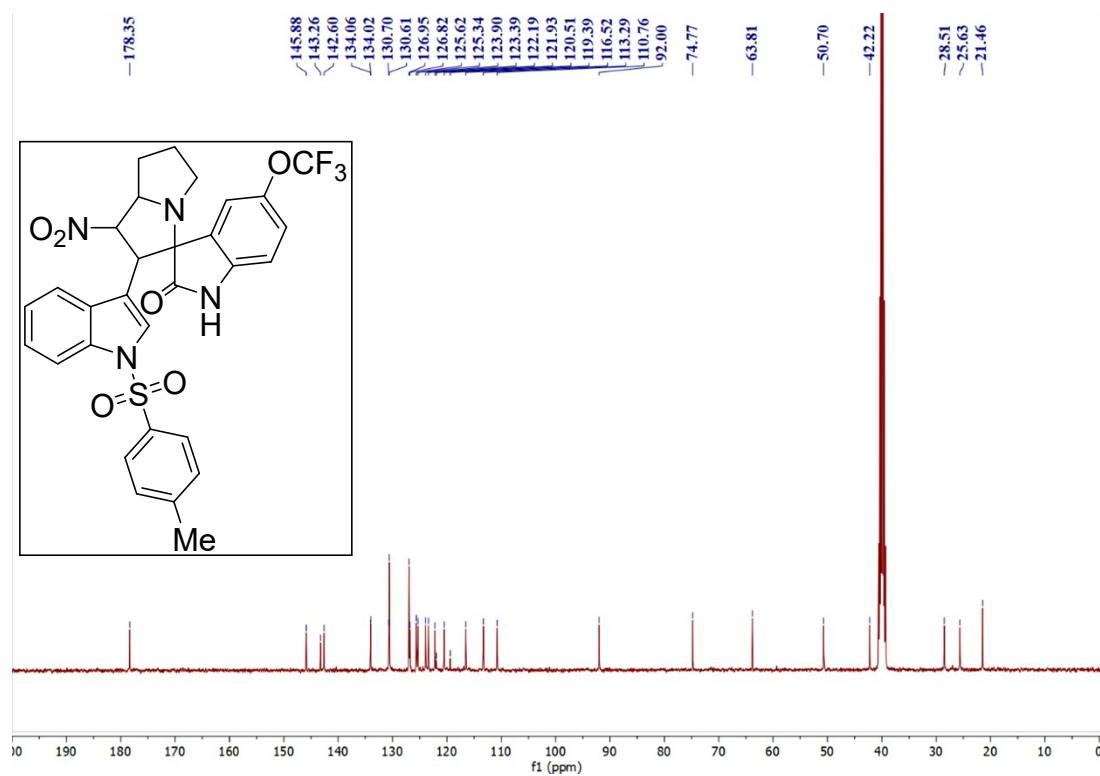
Mass spectrum of the compound **6h**



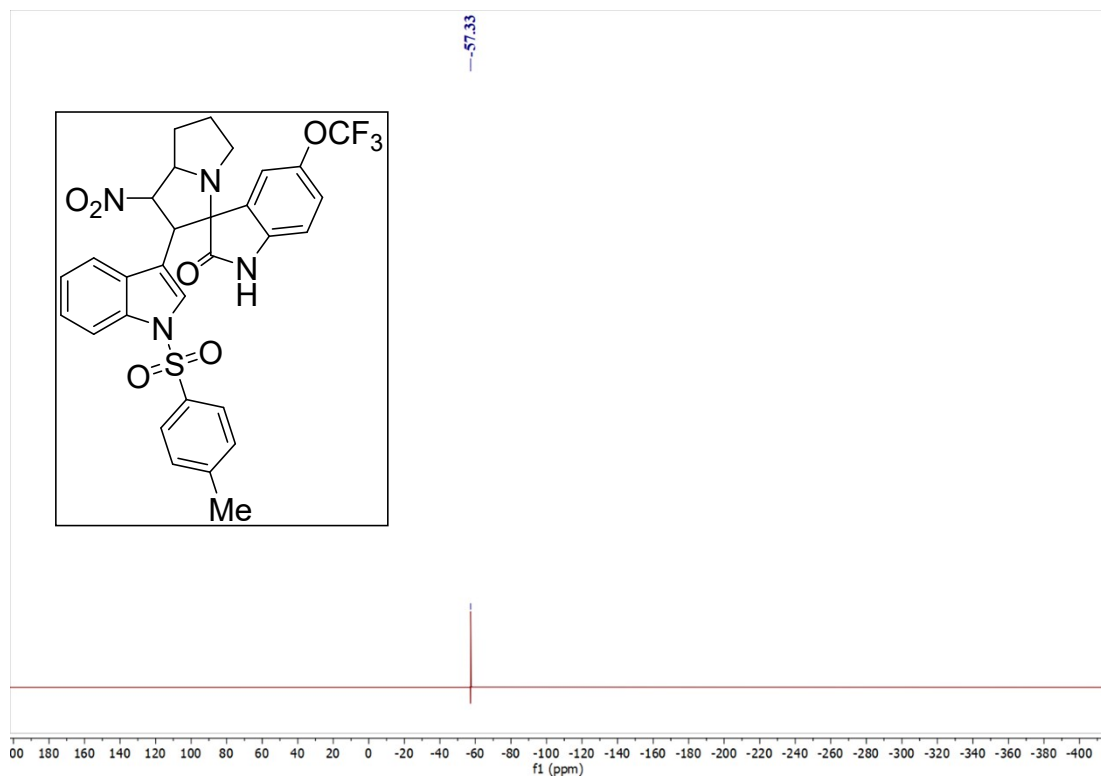
IR spectrum of the compound **6h**



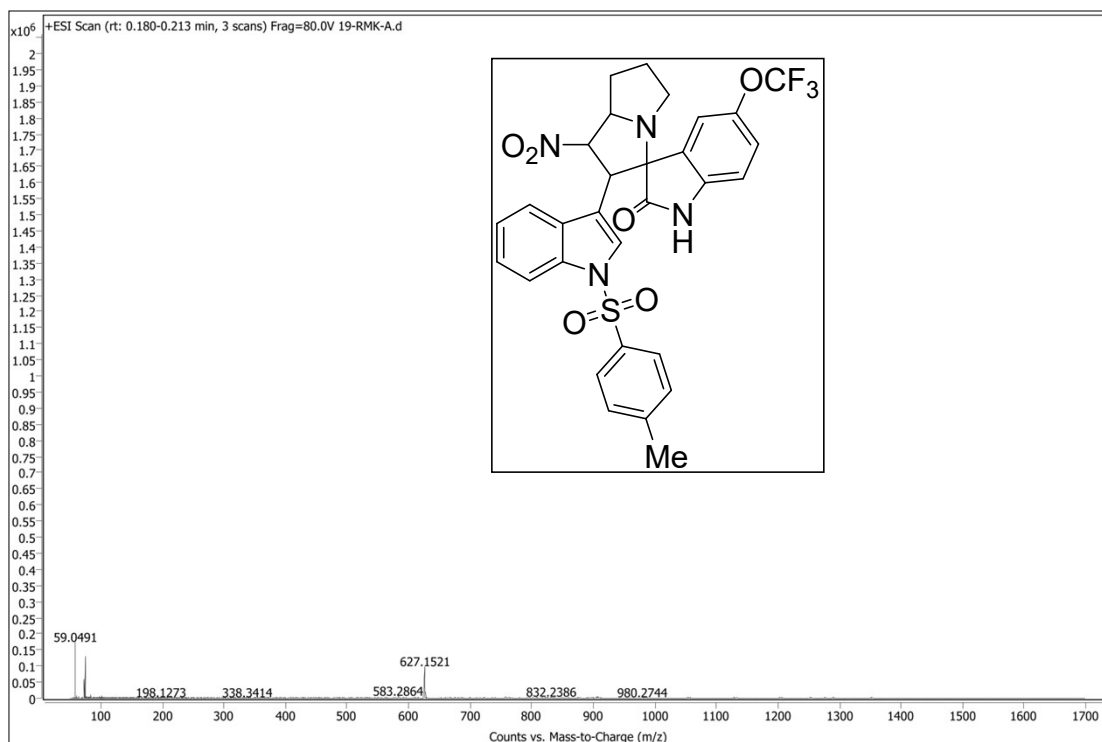
¹H NMR spectrum of the compound **6i** (400 MHz, DMSO-*d*₆)



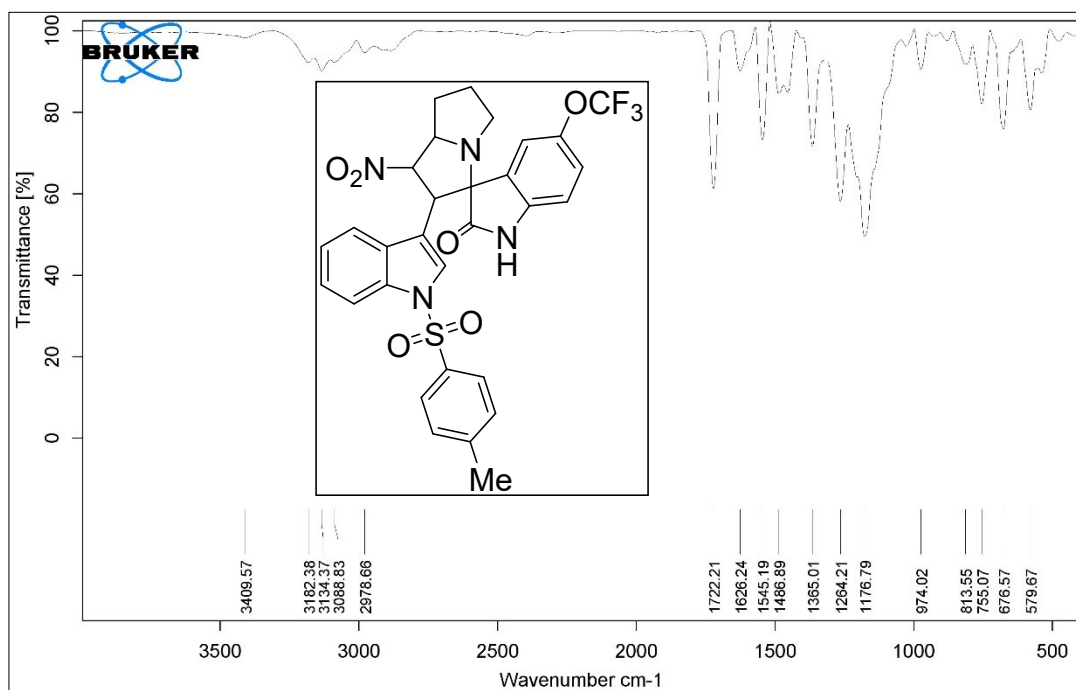
^{13}C NMR spectrum of the compound **6i** (100 MHz, $\text{DMSO}-d_6$)



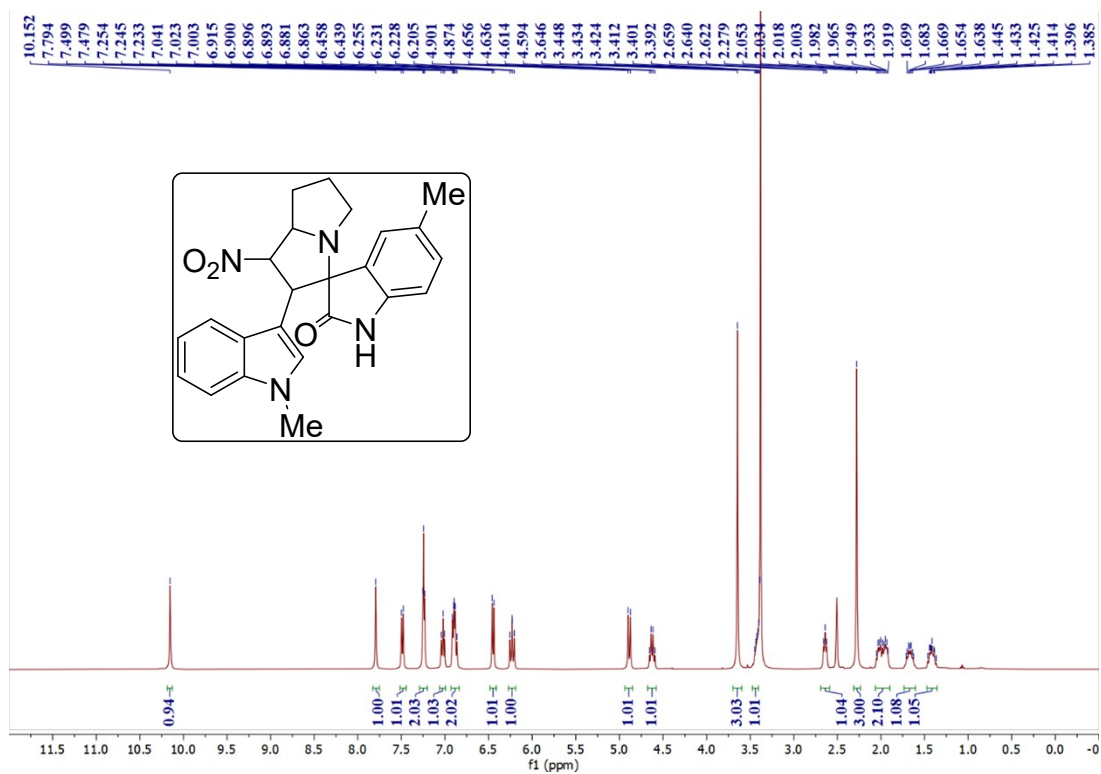
^{19}F NMR spectrum of the compound **6i** (376 MHz, $\text{DMSO}-d_6$)



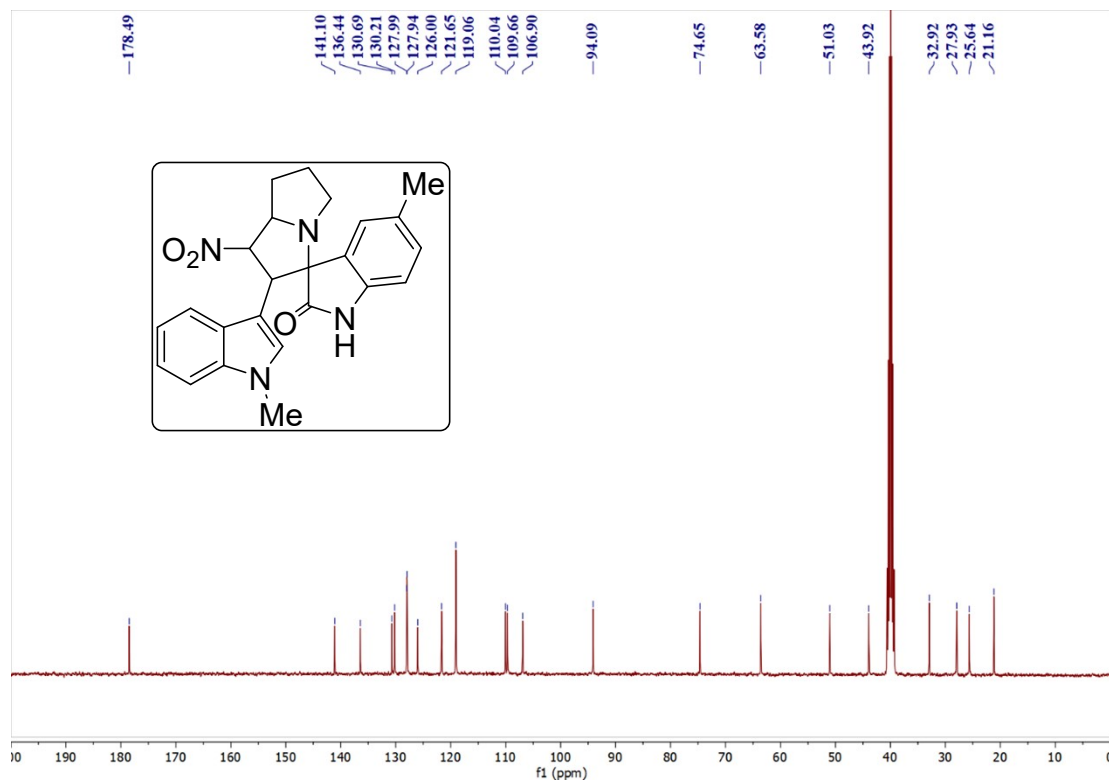
Mass spectrum of the compound **6i**



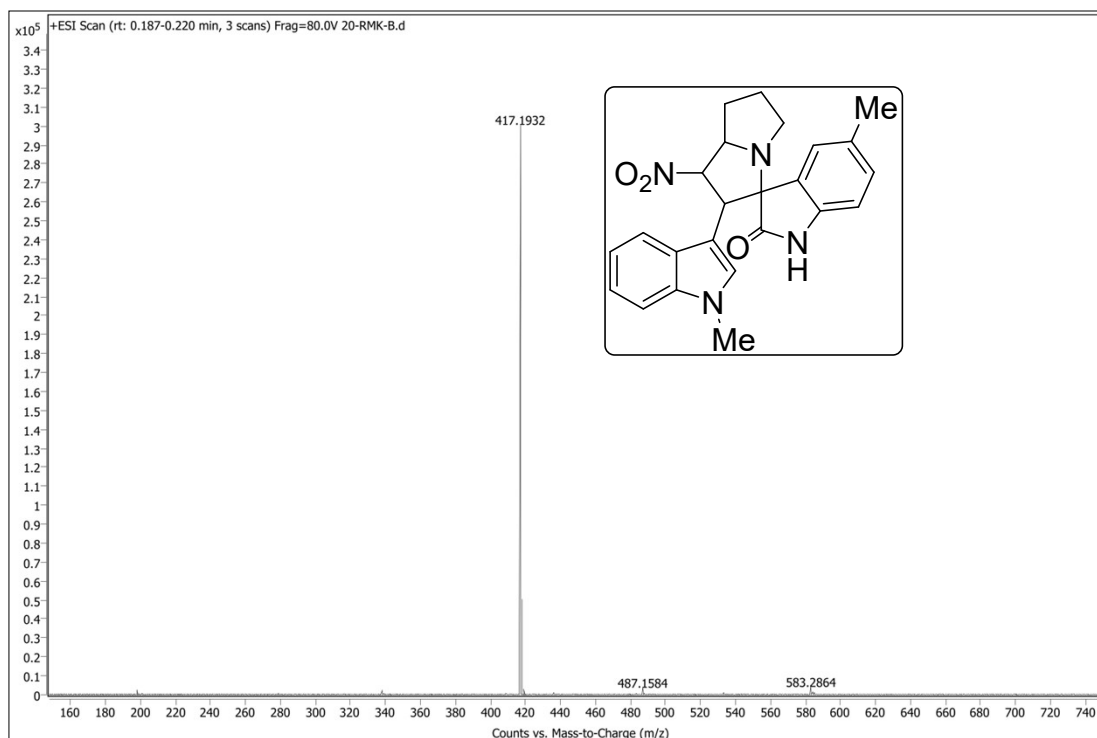
IR spectrum of the compound **6i**



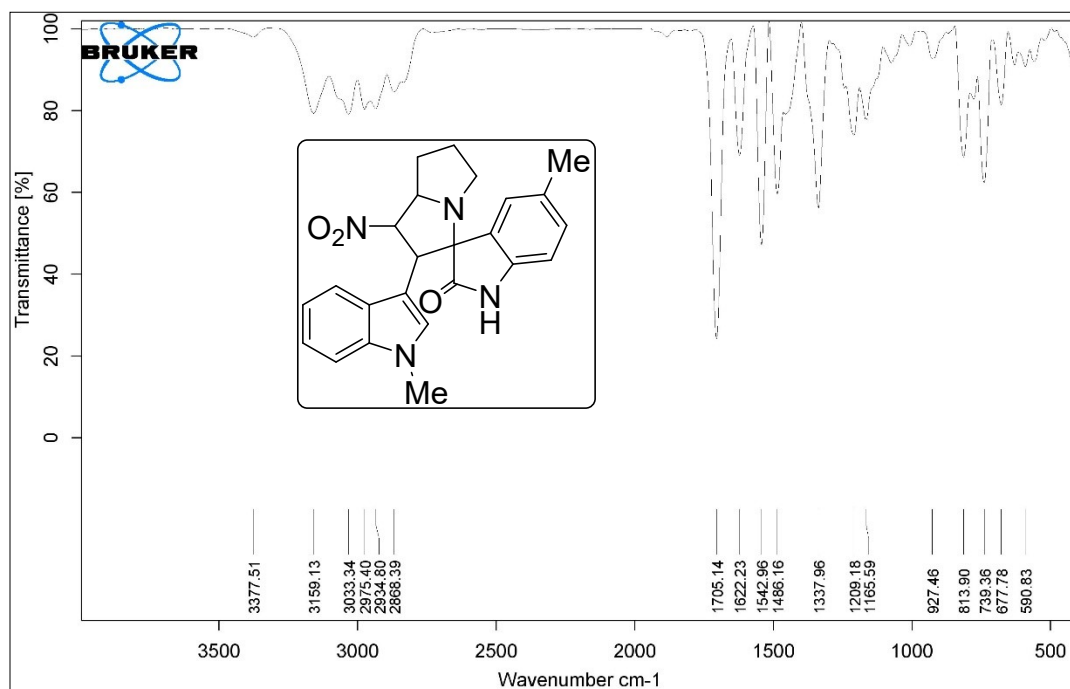
¹H NMR spectrum of the compound **6j** (400 MHz, DMSO-*d*₆)



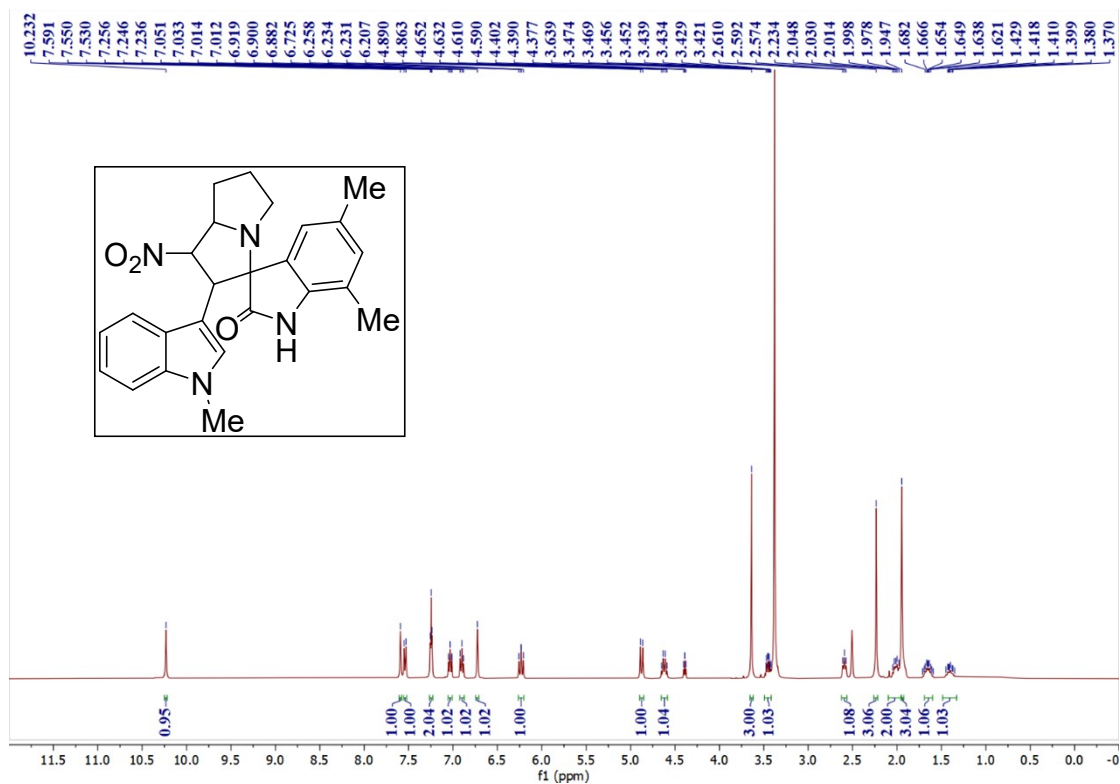
¹³C NMR spectrum of the compound **6j** (100 MHz, DMSO-*d*₆)



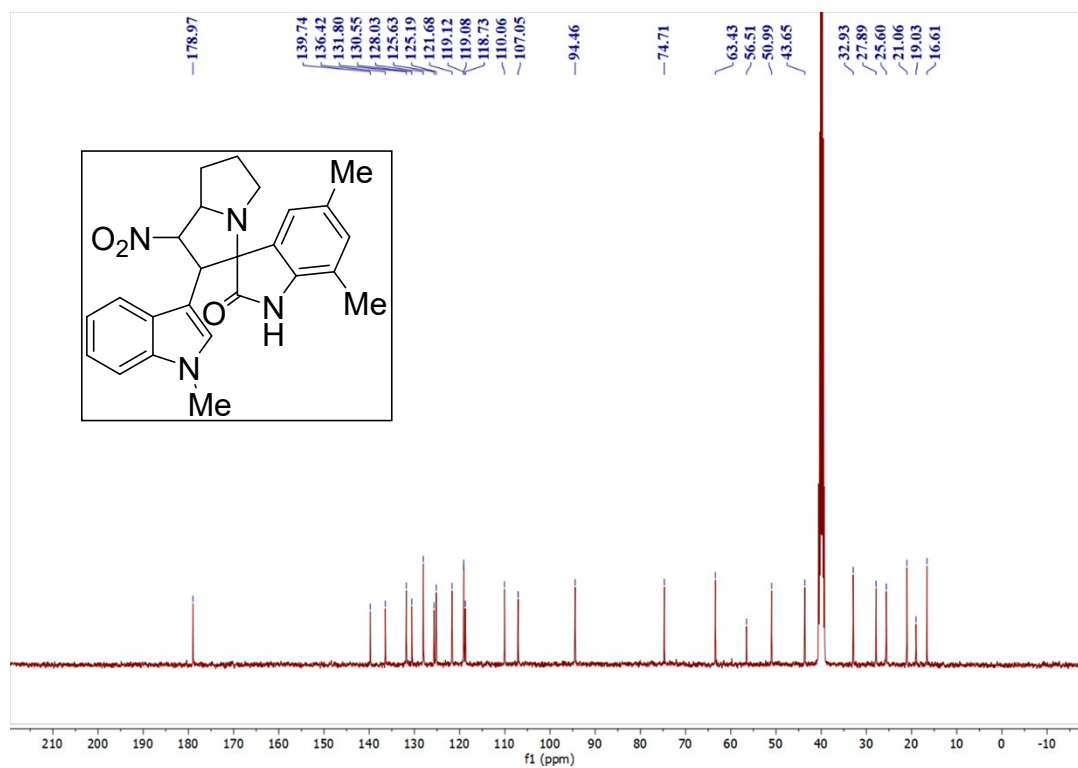
Mass spectrum of the compound **6j**



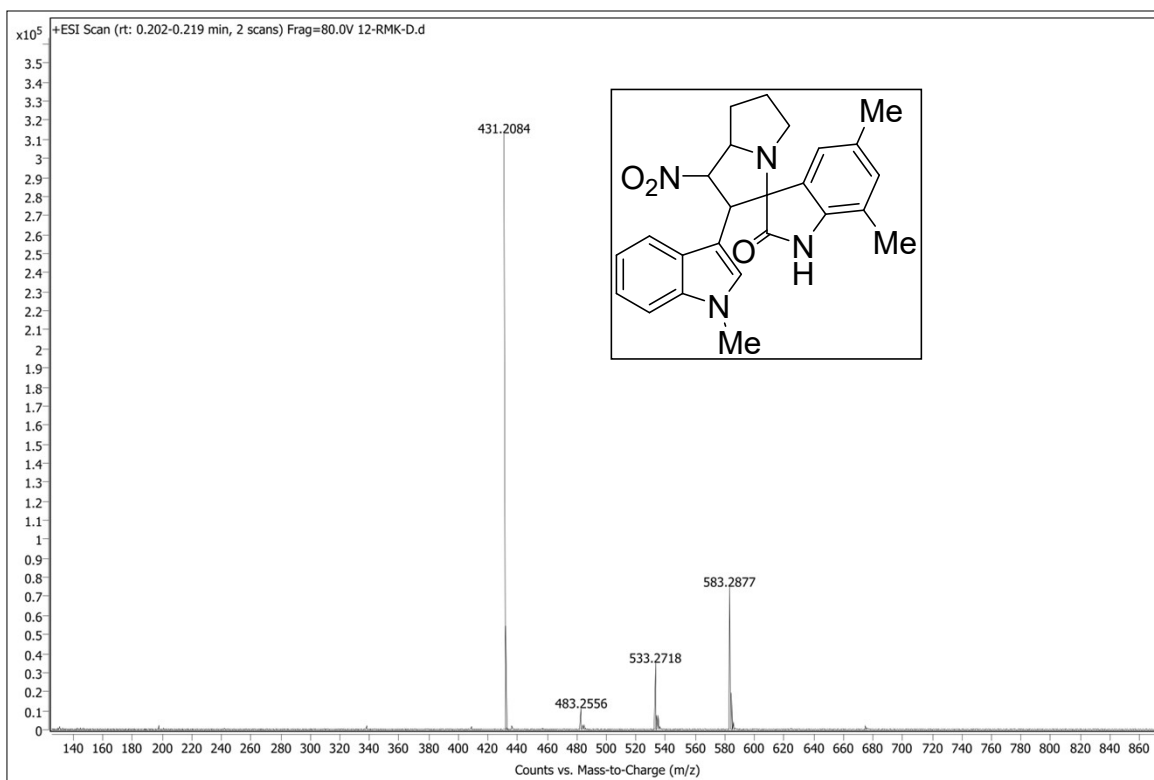
IR spectrum of the compound **6j**



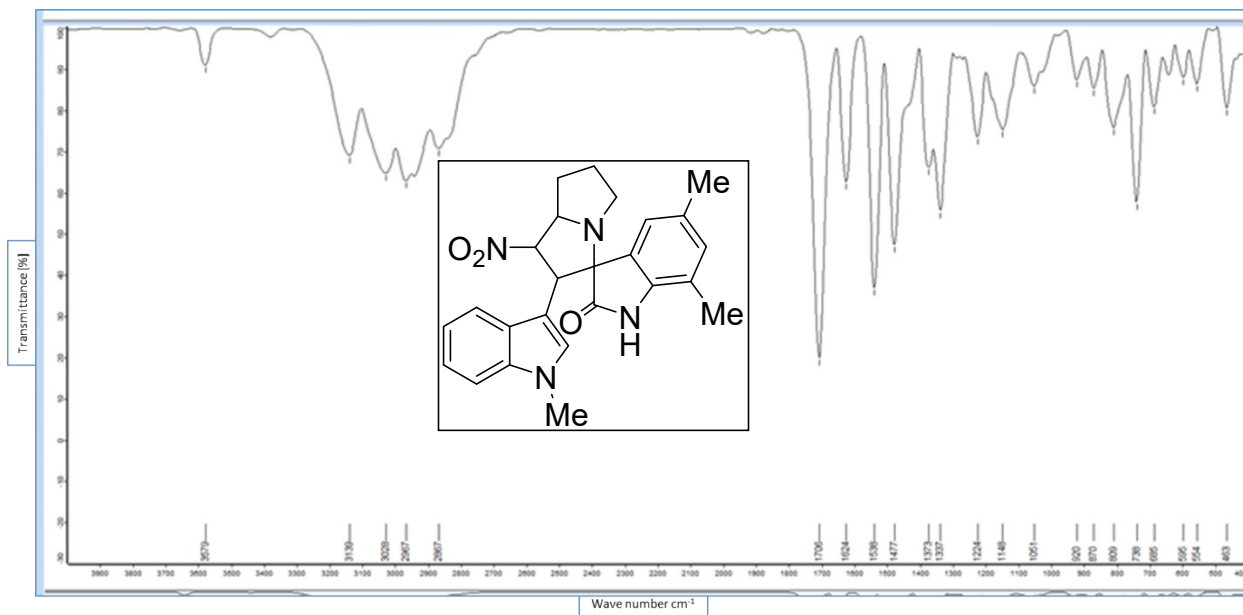
¹H NMR spectrum of the compound **6k** (400 MHz, DMSO-*d*₆)



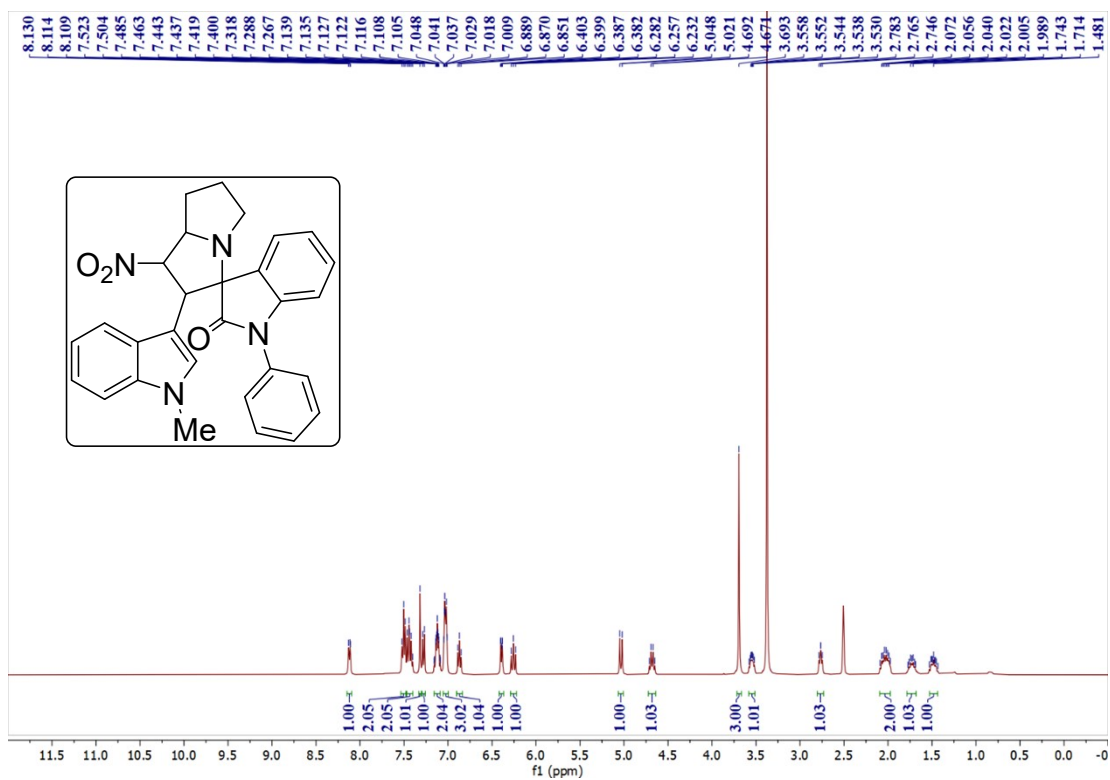
¹³C NMR spectrum of the compound **6k** (100 MHz, DMSO-*d*₆)



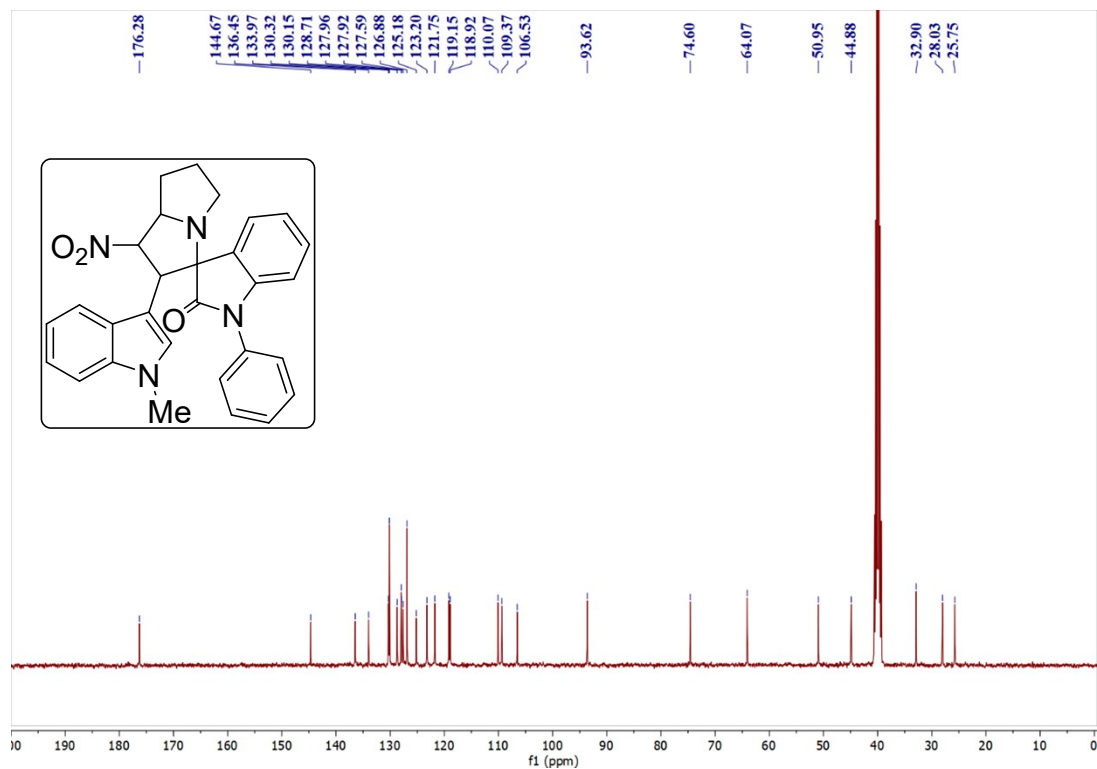
Mass spectrum of the compound **6k**



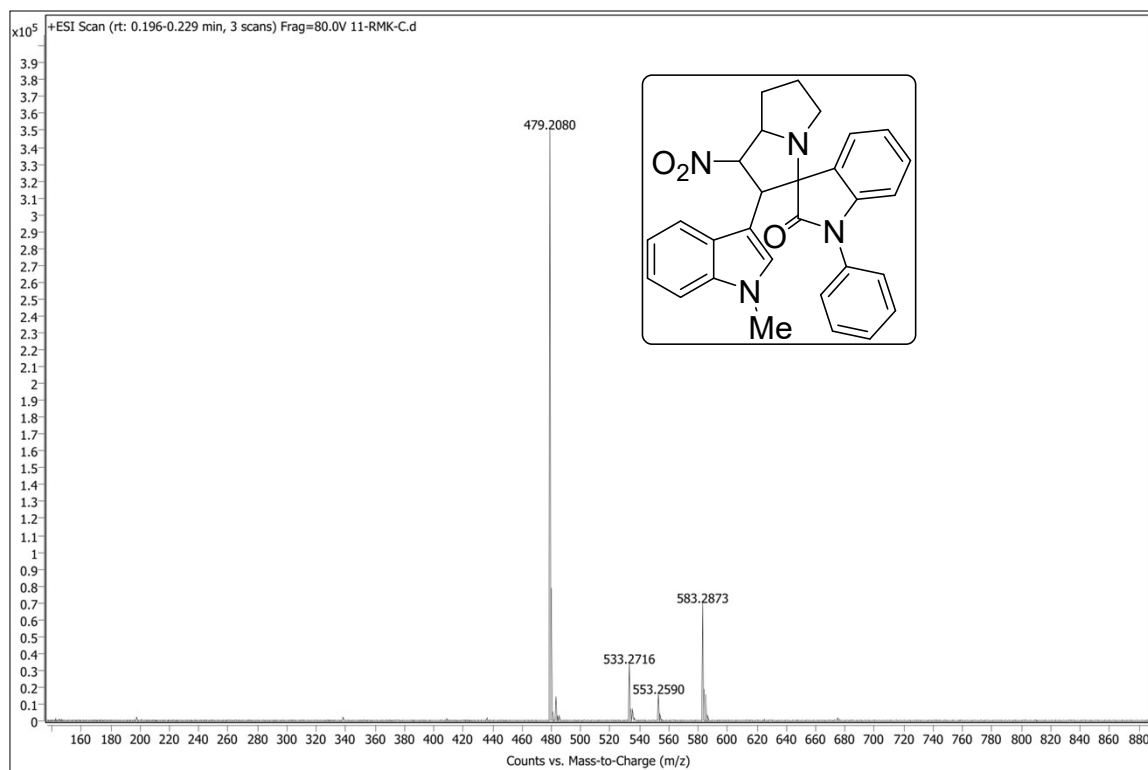
IR spectrum of the compound **6k**



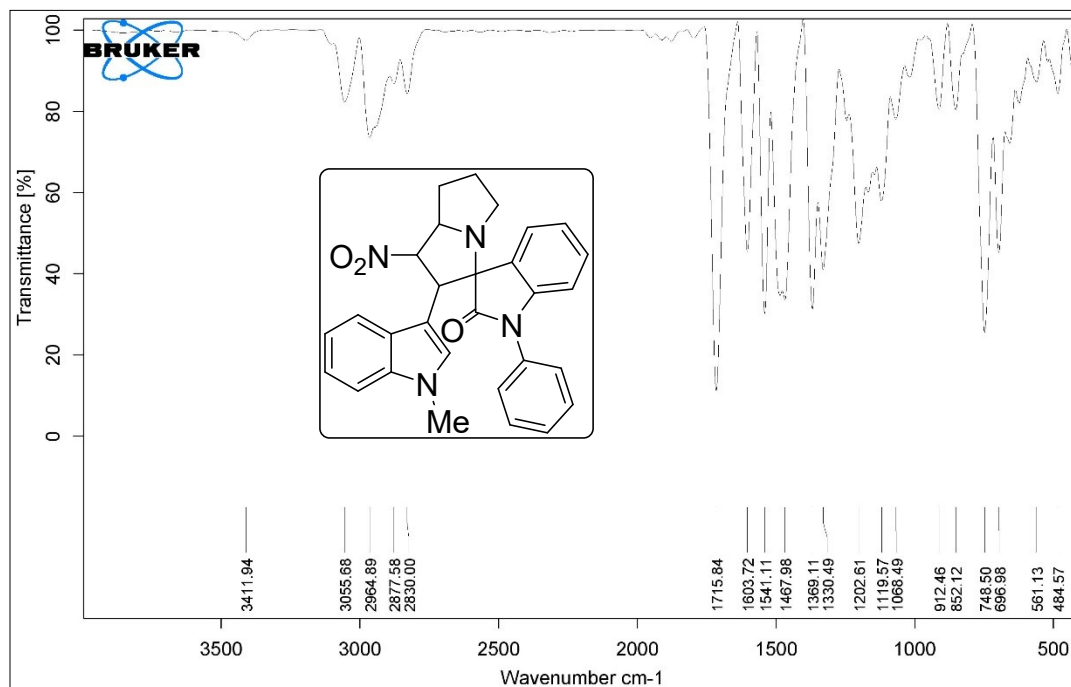
¹H NMR spectrum of the compound **6l** (400 MHz, DMSO-*d*₆)



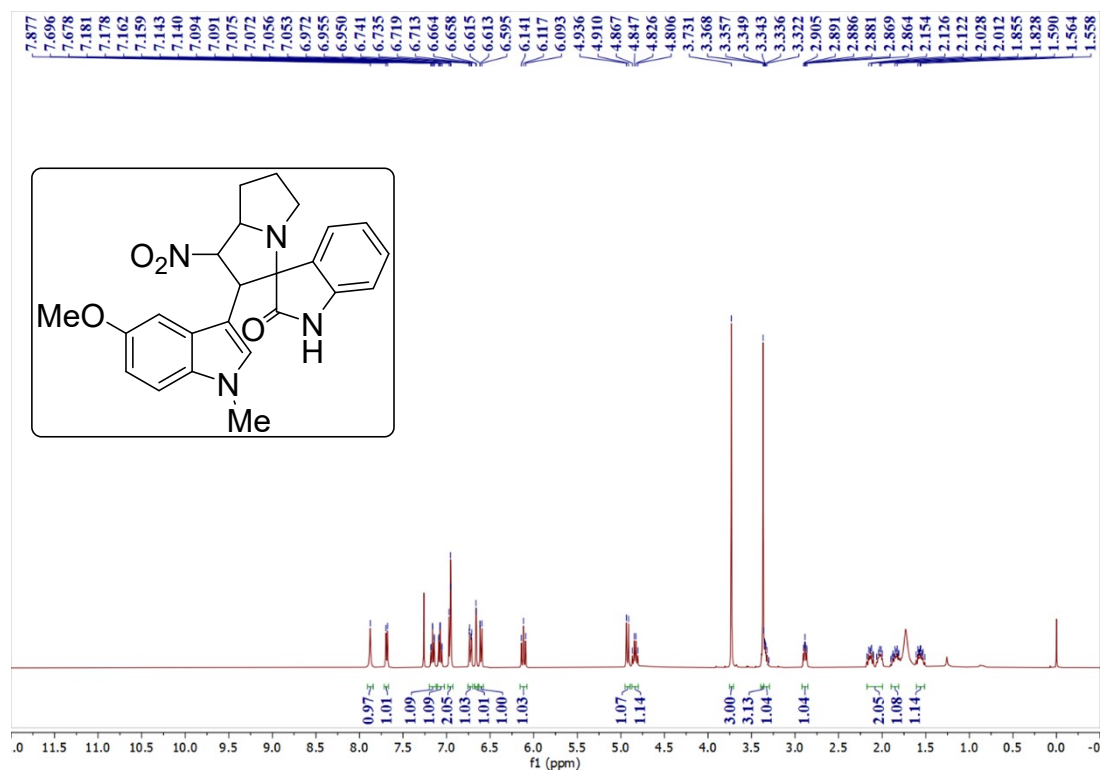
¹³C NMR spectrum of the compound **6l** (100 MHz, DMSO-*d*₆)



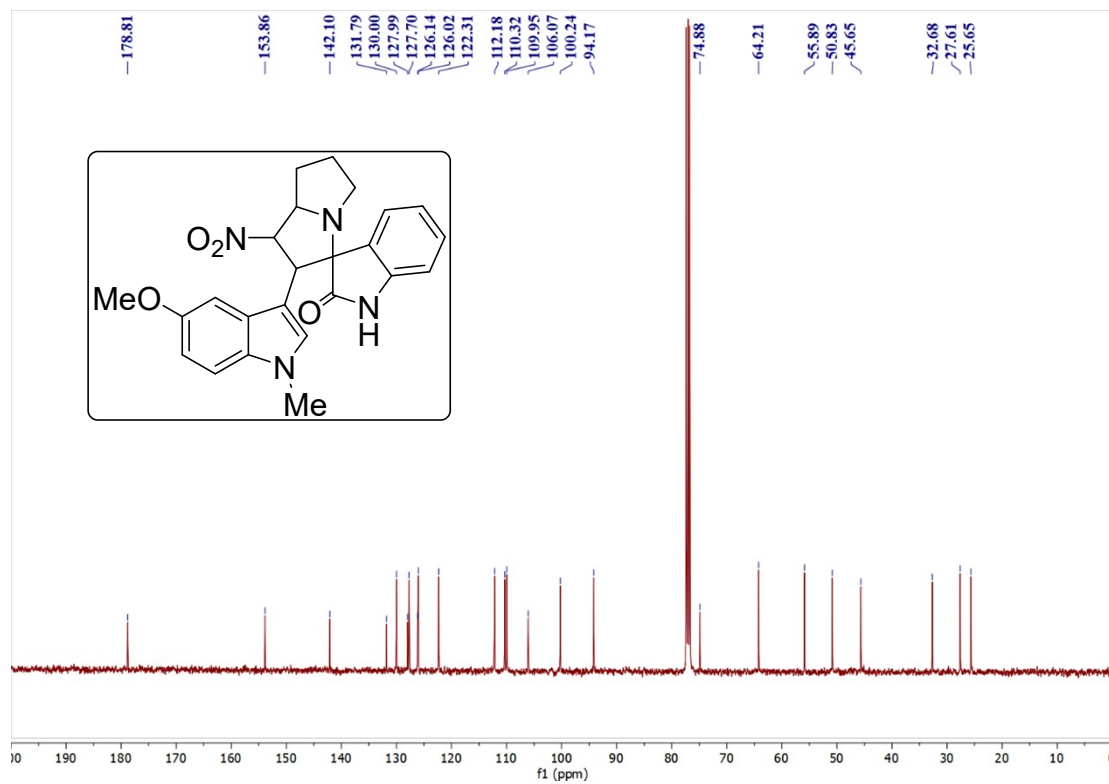
Mass spectrum of the compound **6l**



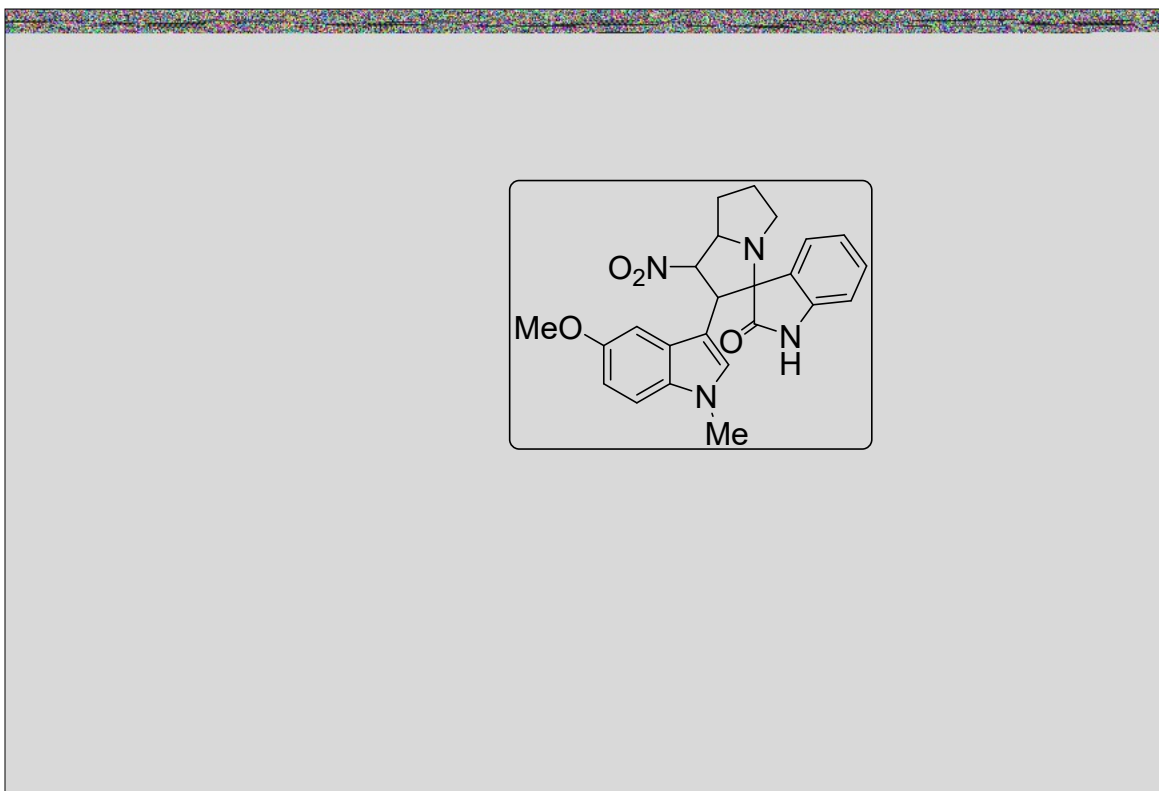
IR spectrum of the compound **6l**



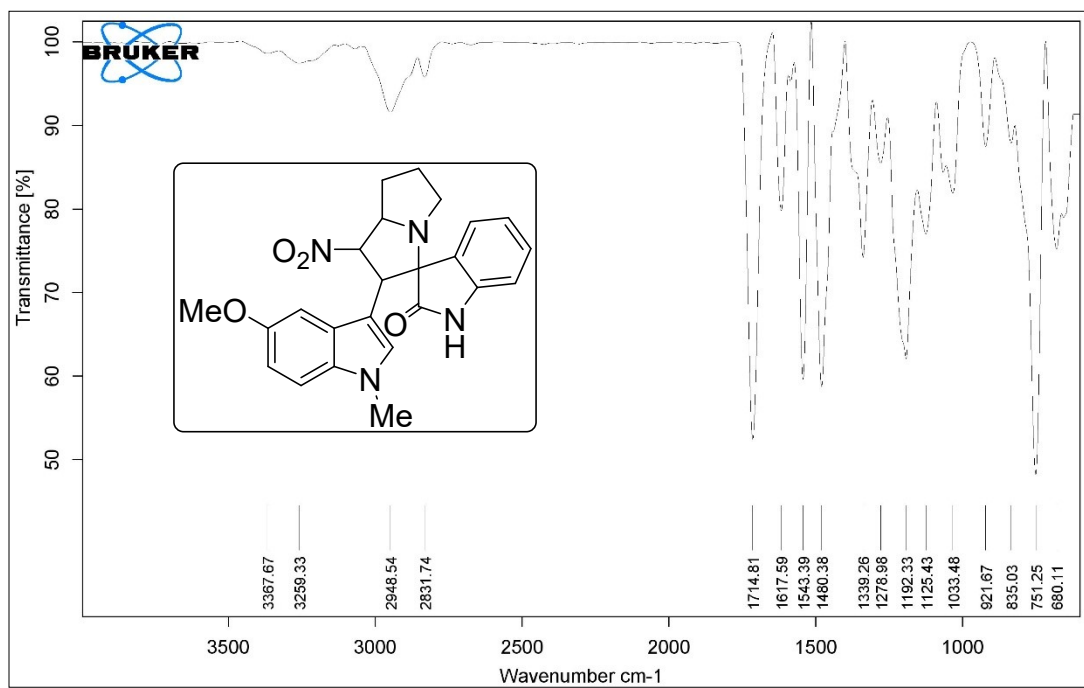
^1H NMR spectrum of the compound **6m** (400 MHz, CDCl_3)



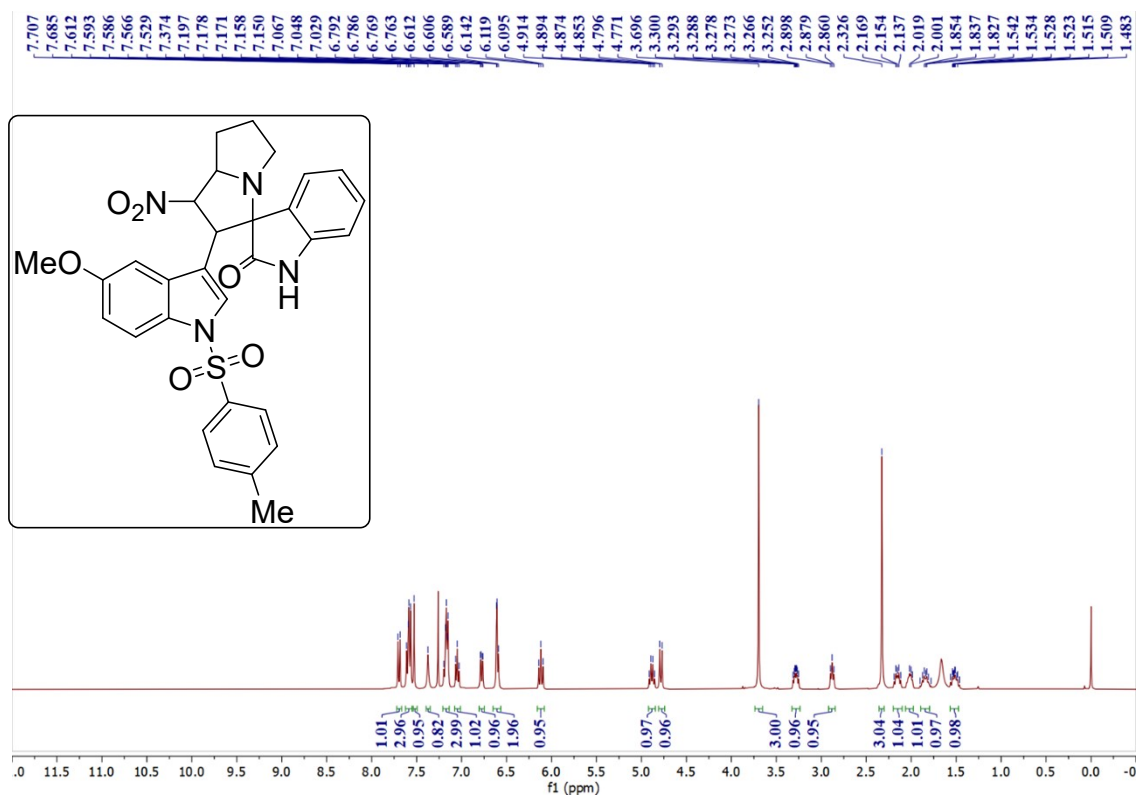
^{13}C NMR spectrum of the compound **6m** (100 MHz, CDCl_3)



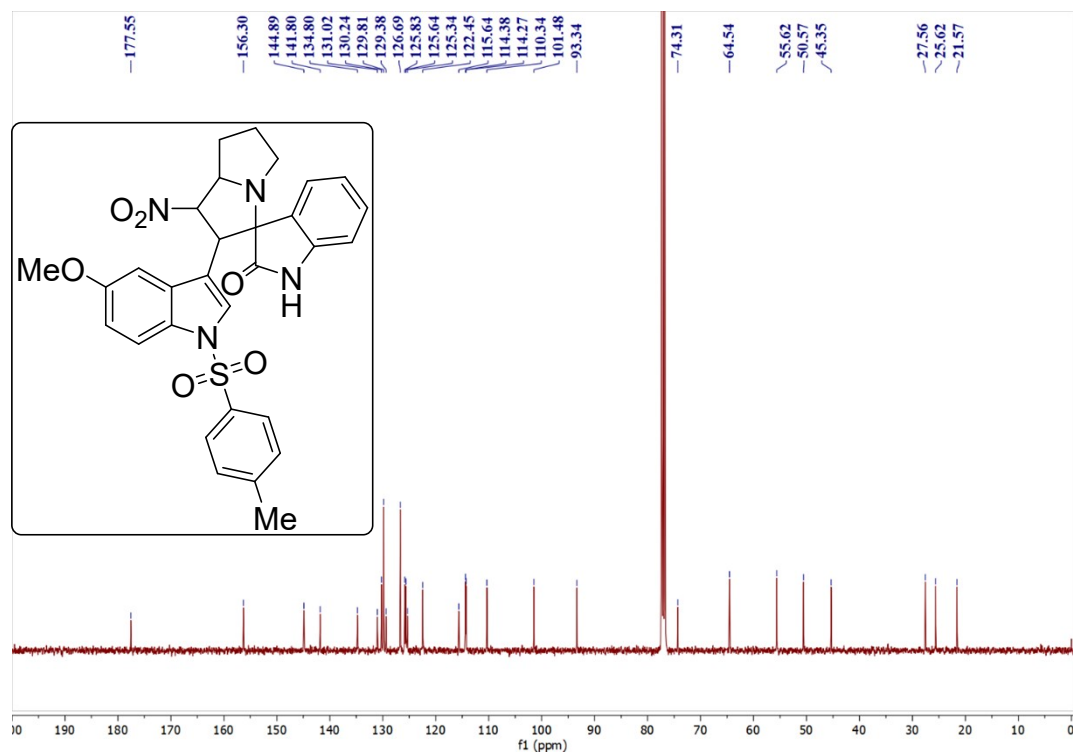
Mass spectrum of the compound **6m**



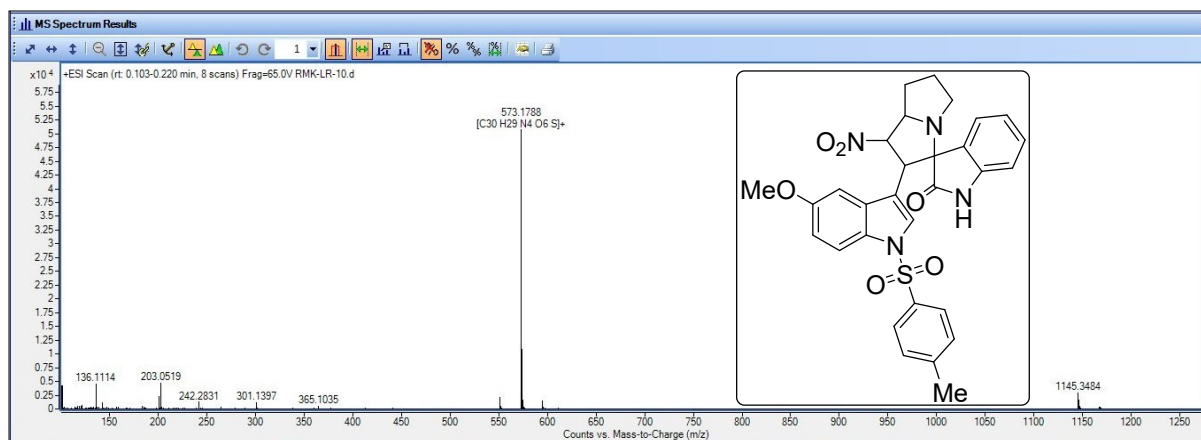
IR spectrum of the compound **6m**



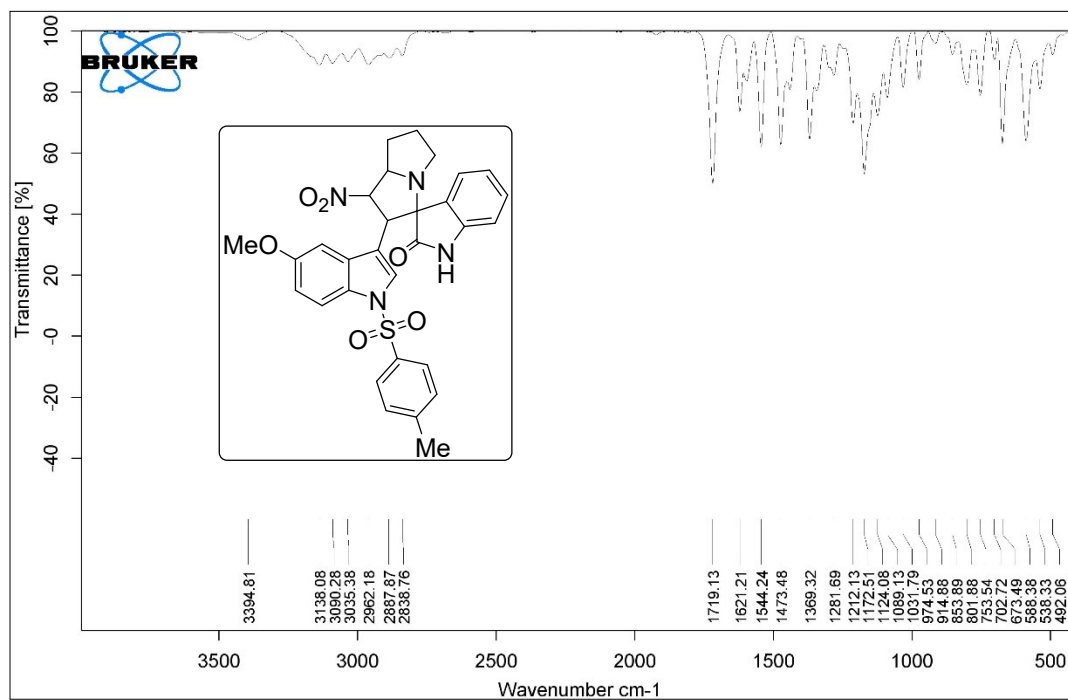
¹H NMR spectrum of the compound **6n** (400 MHz, CDCl₃)



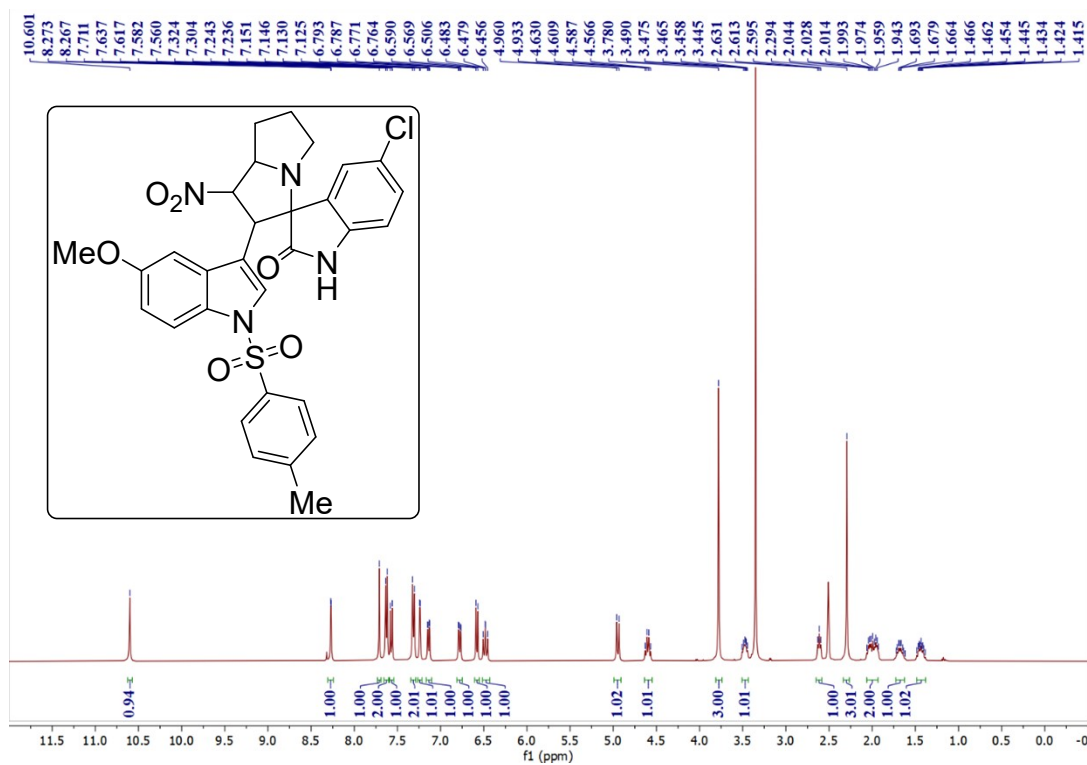
¹³C NMR spectrum of the compound **6n** (100 MHz, CDCl₃)



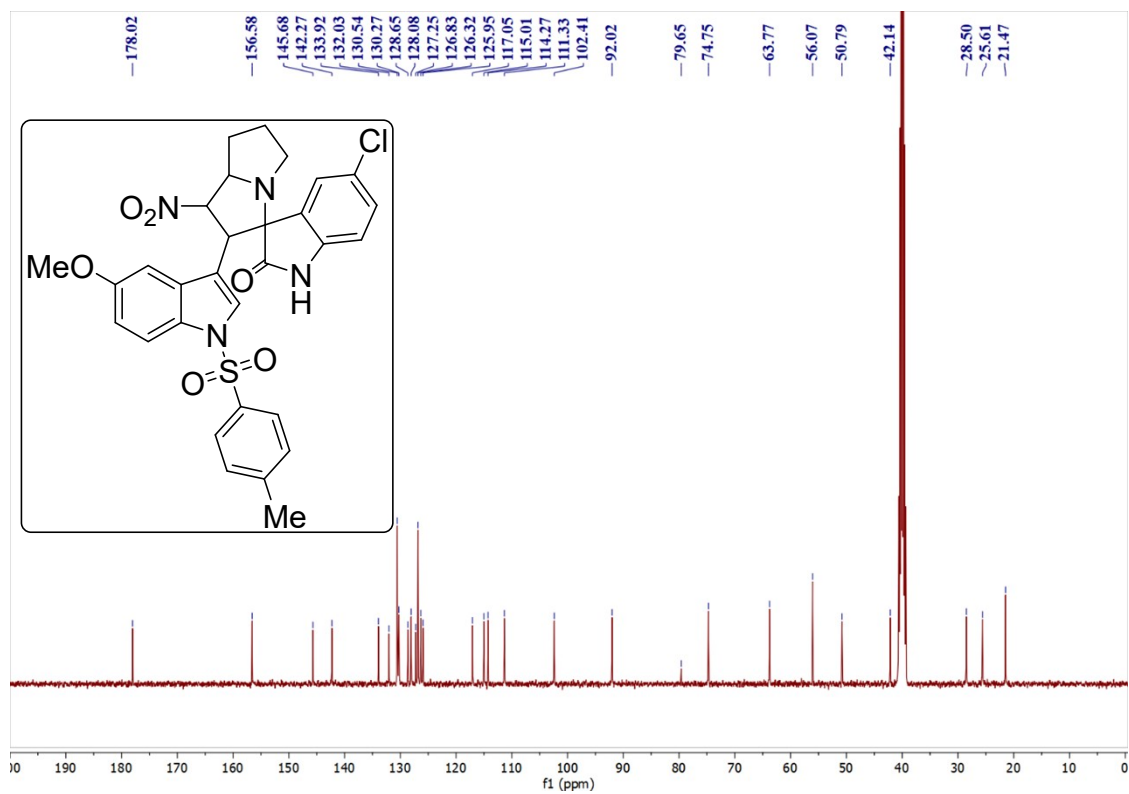
Mass spectrum of the compound **6n**



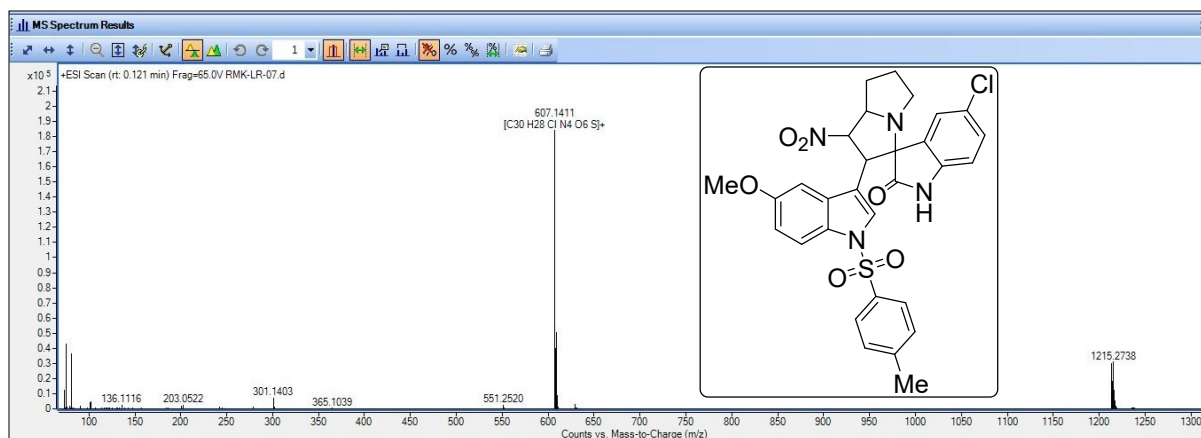
IR spectrum of the compound **6n**



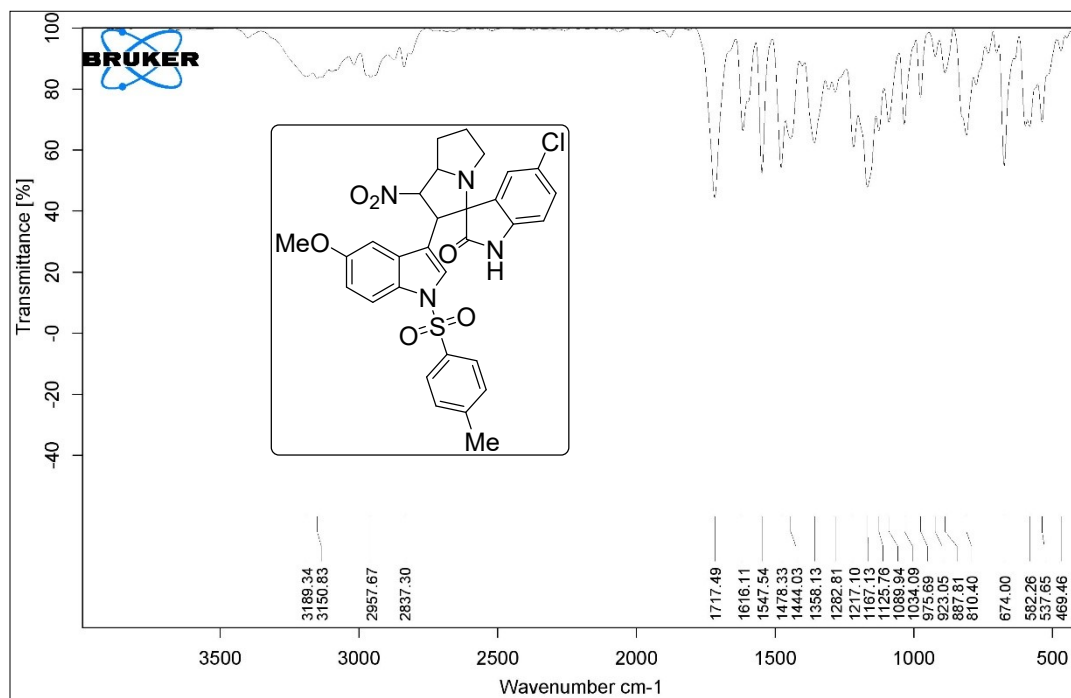
¹H NMR spectrum of the compound **6o** (400 MHz, DMSO-*d*₆)



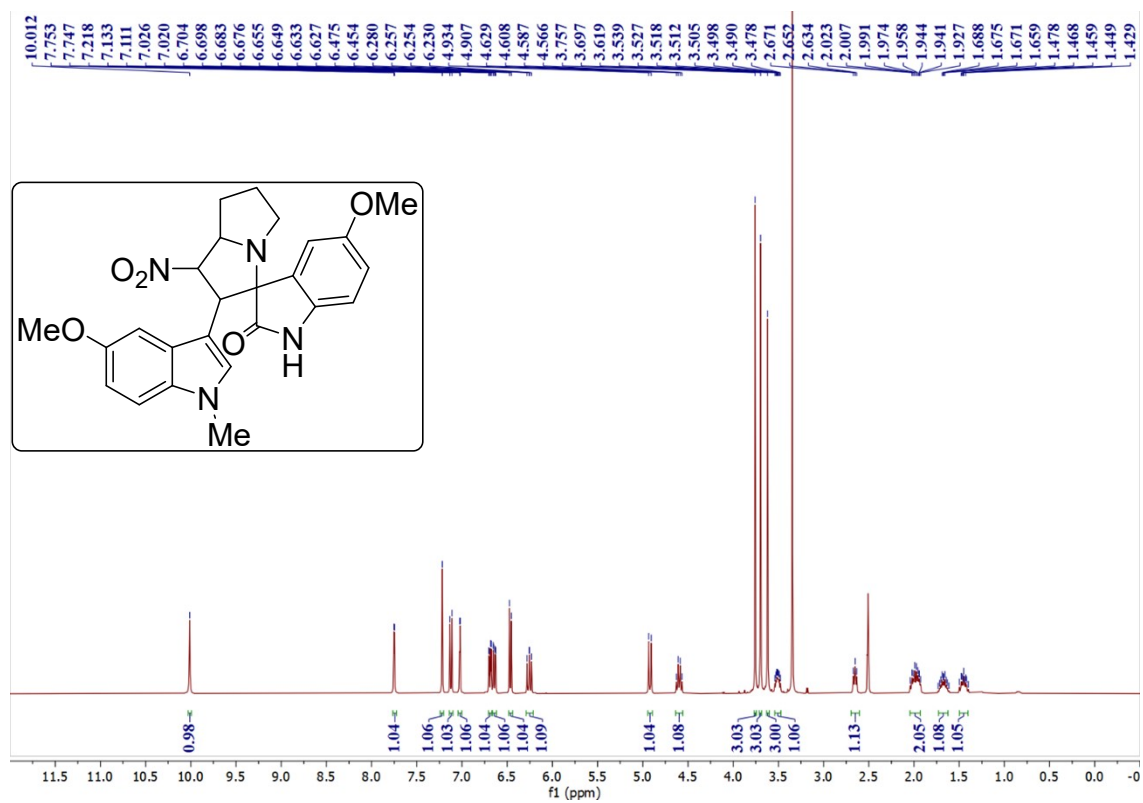
¹³C NMR spectrum of the compound **6o** (100 MHz, DMSO-*d*₆)



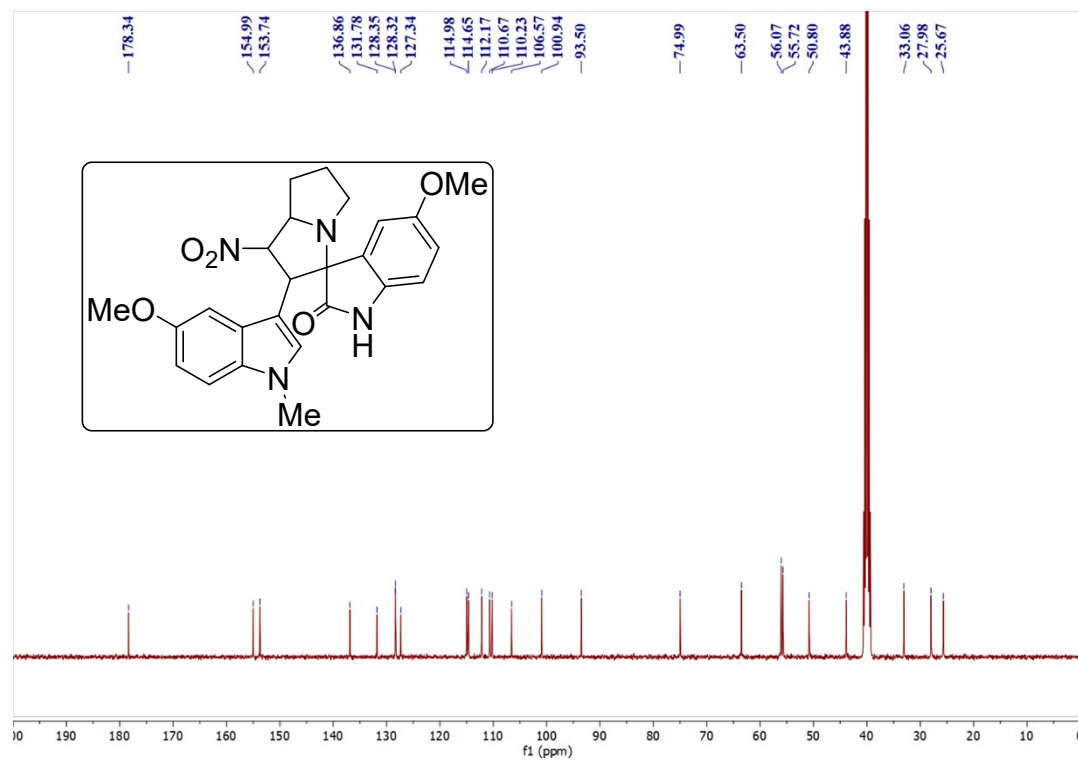
Mass spectrum of the compound **60**



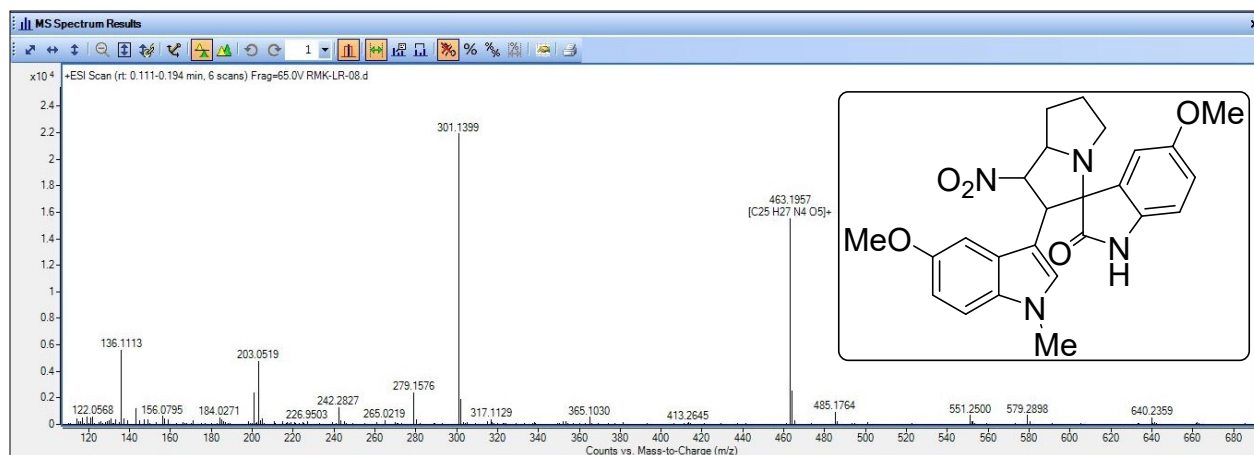
IR spectrum of the compound **60**



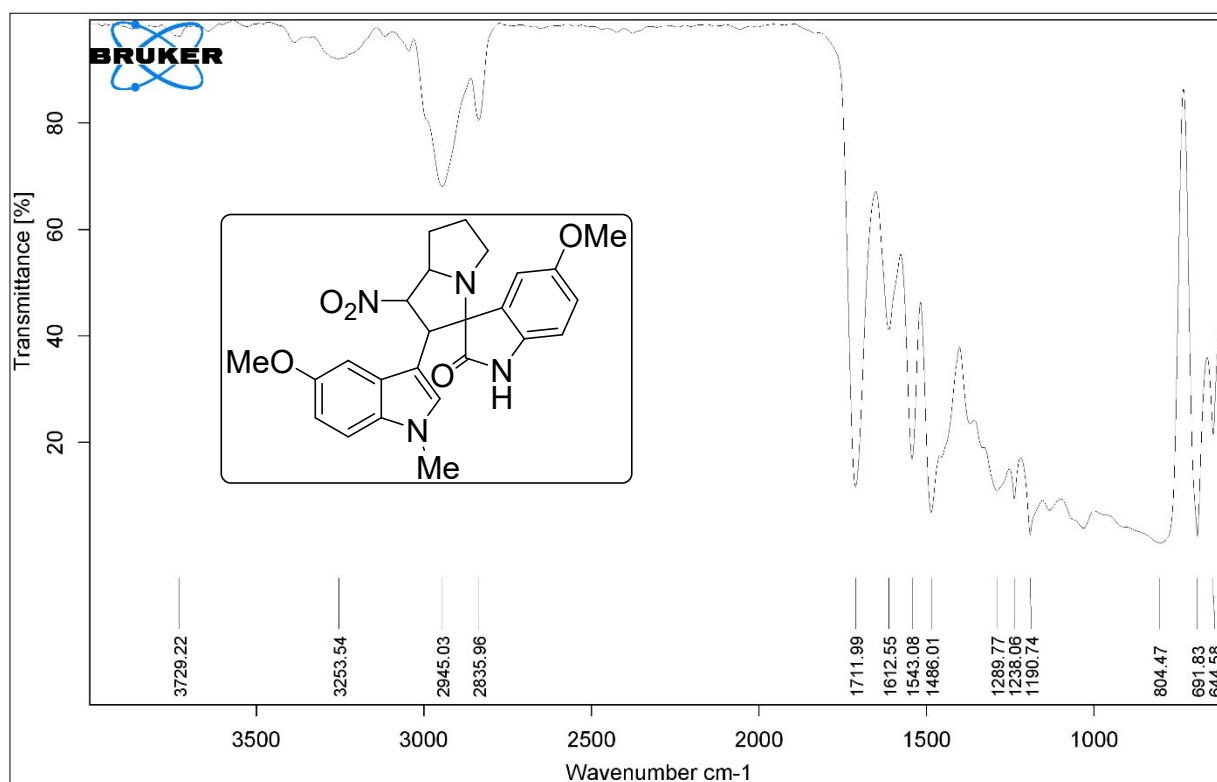
¹H NMR spectrum of the compound **6p** (400 MHz, DMSO-*d*₆)



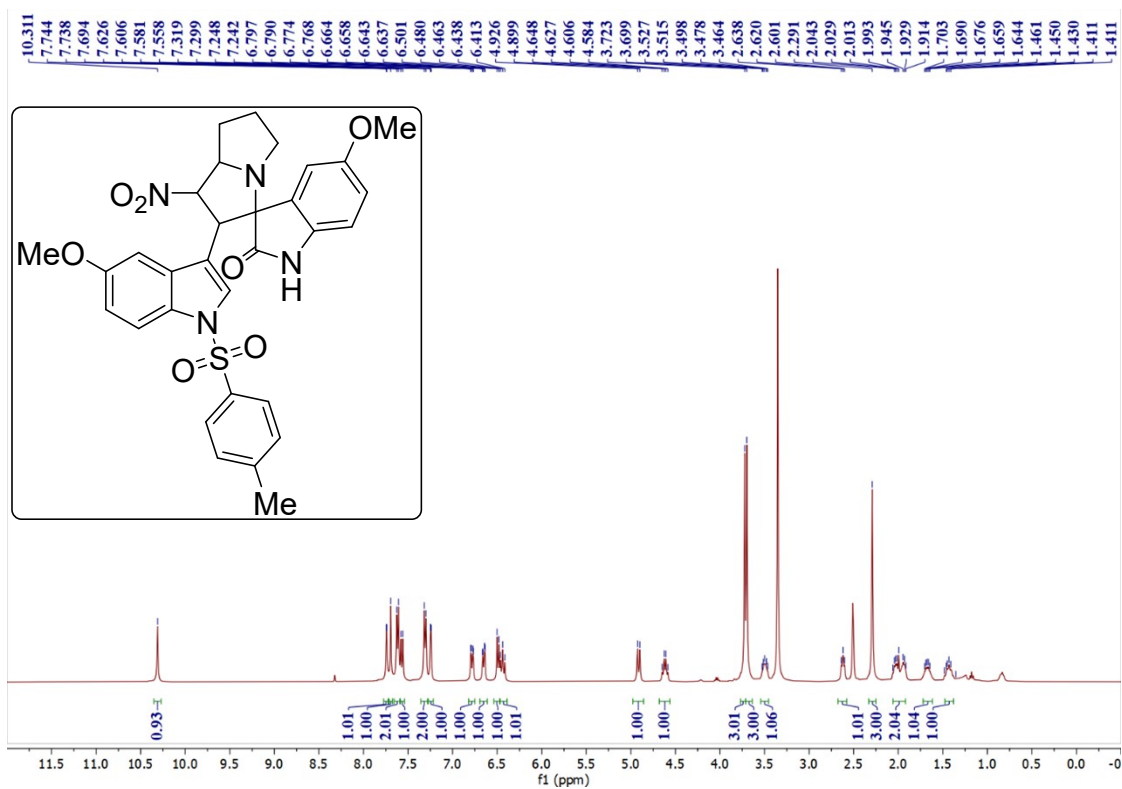
¹³C NMR spectrum of the compound **6p** (100 MHz, DMSO-*d*₆)



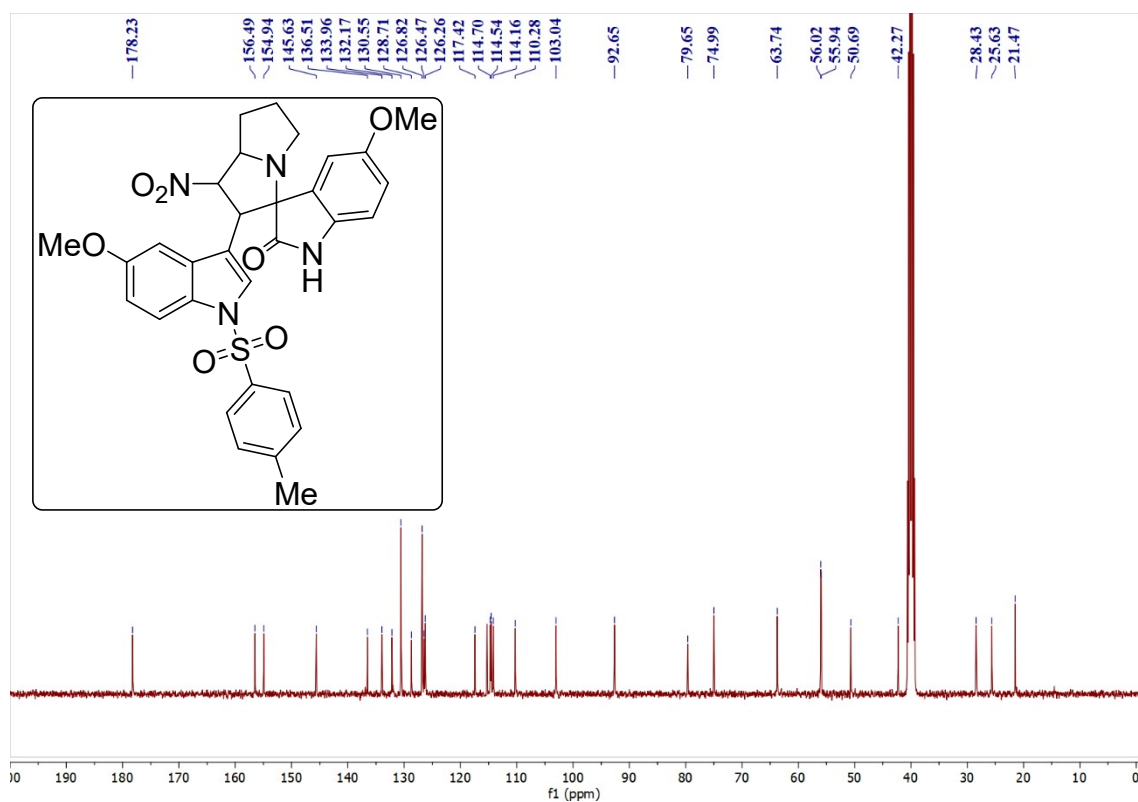
Mass spectrum of the compound 6p



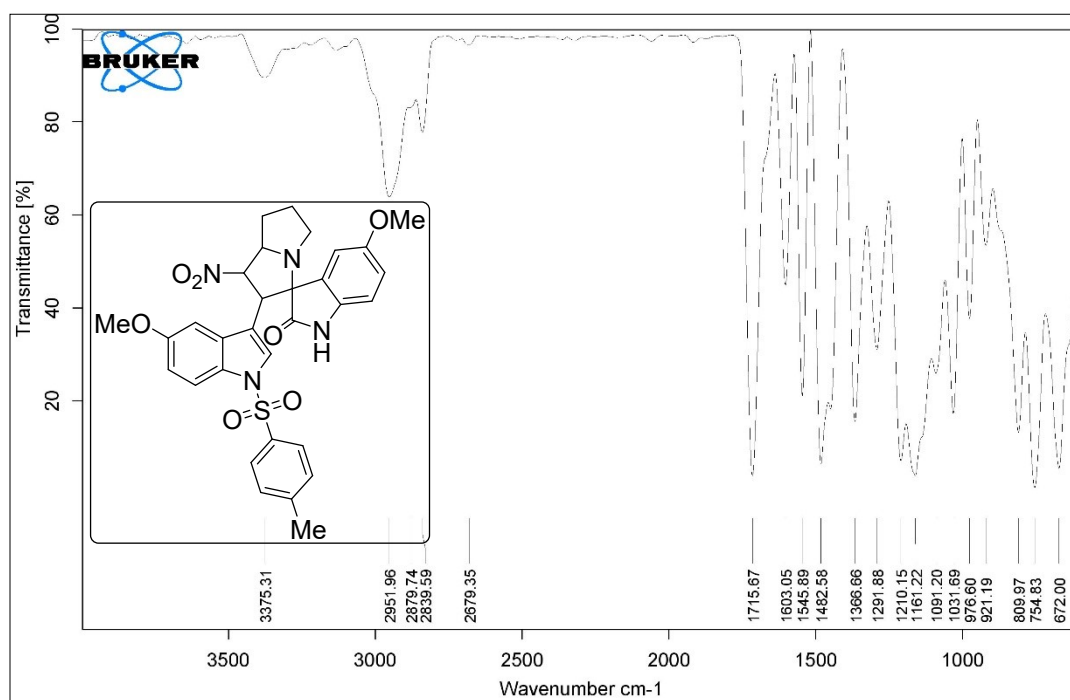
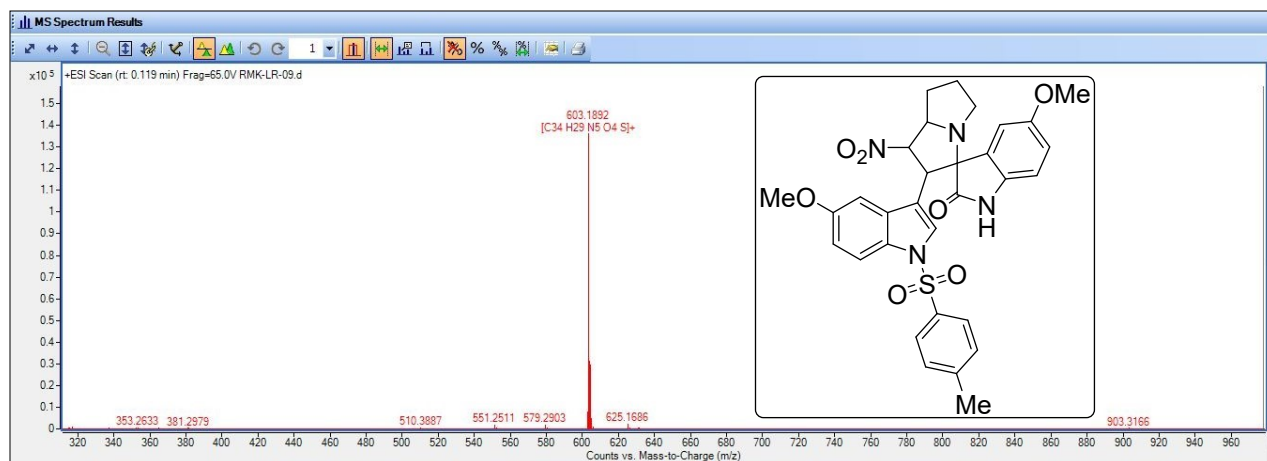
IR spectrum of the compound 6p

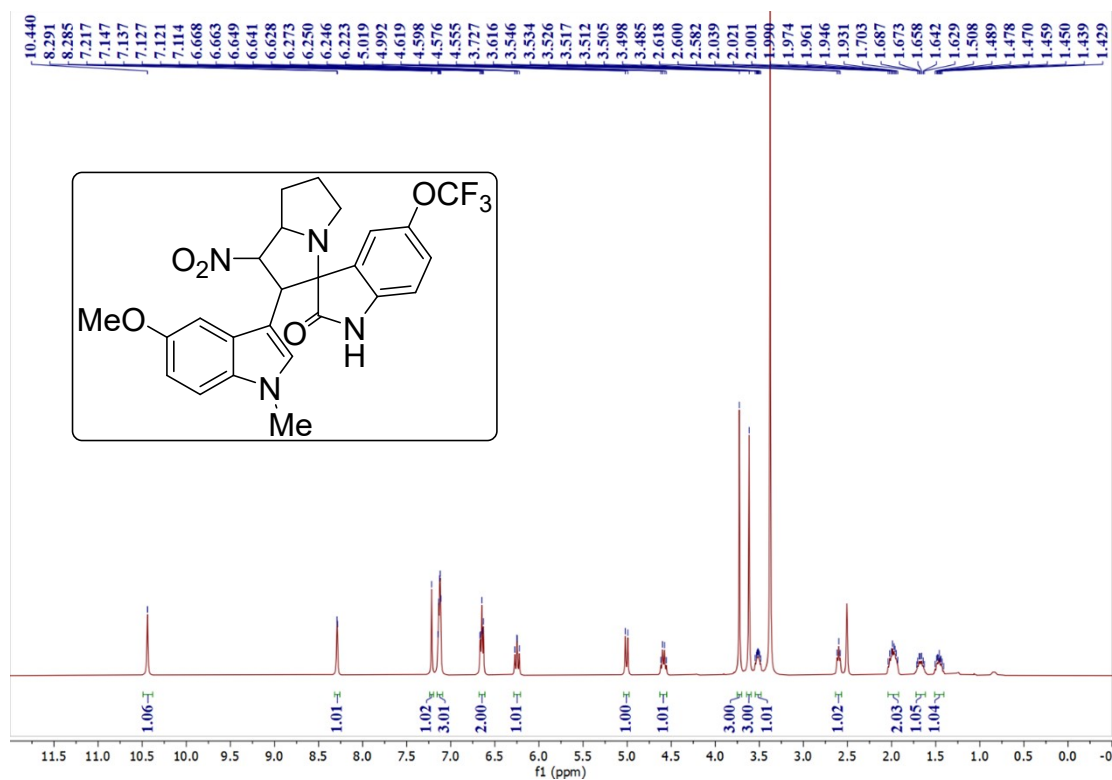


¹H NMR spectrum of the compound **6q** (400 MHz, DMSO-*d*₆)

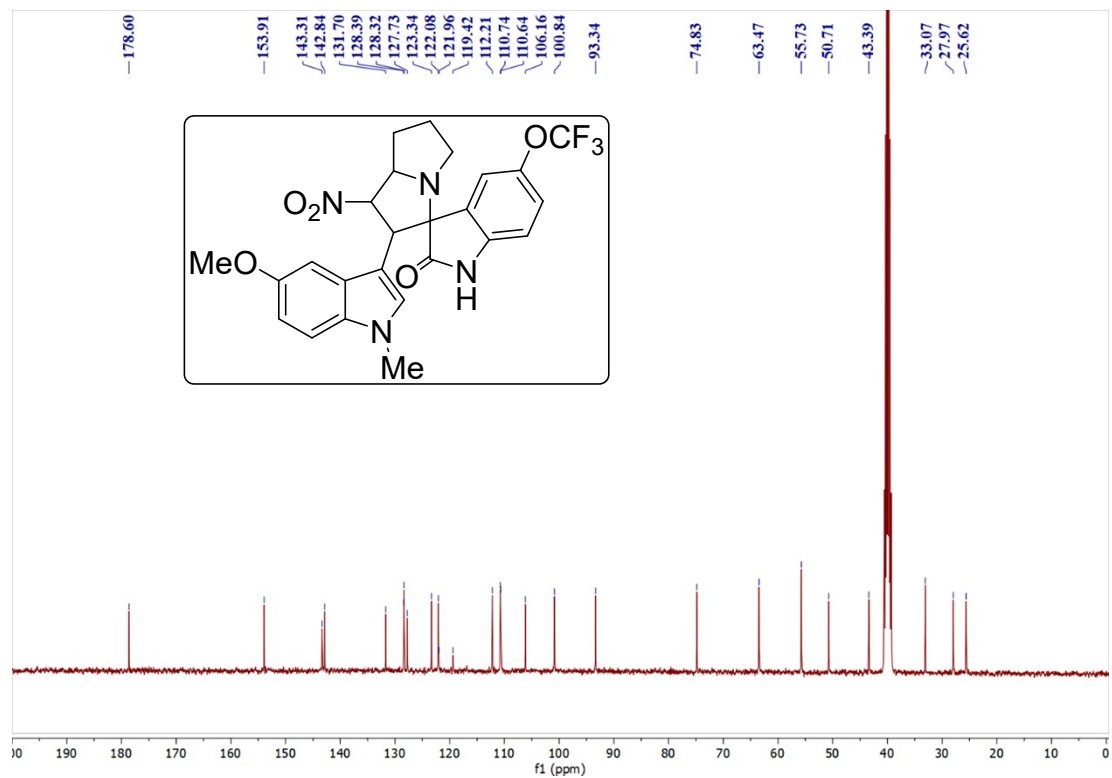


¹³C NMR spectrum of the compound **6q** (100 MHz, DMSO-*d*₆)

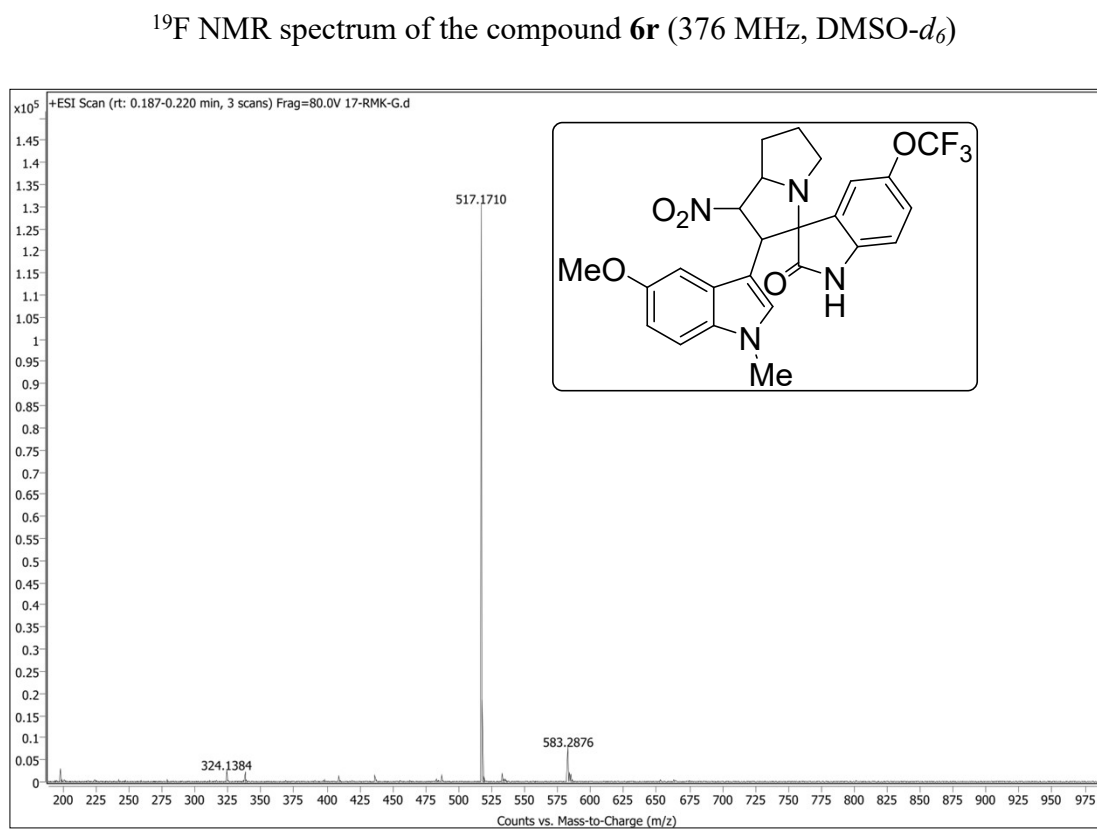
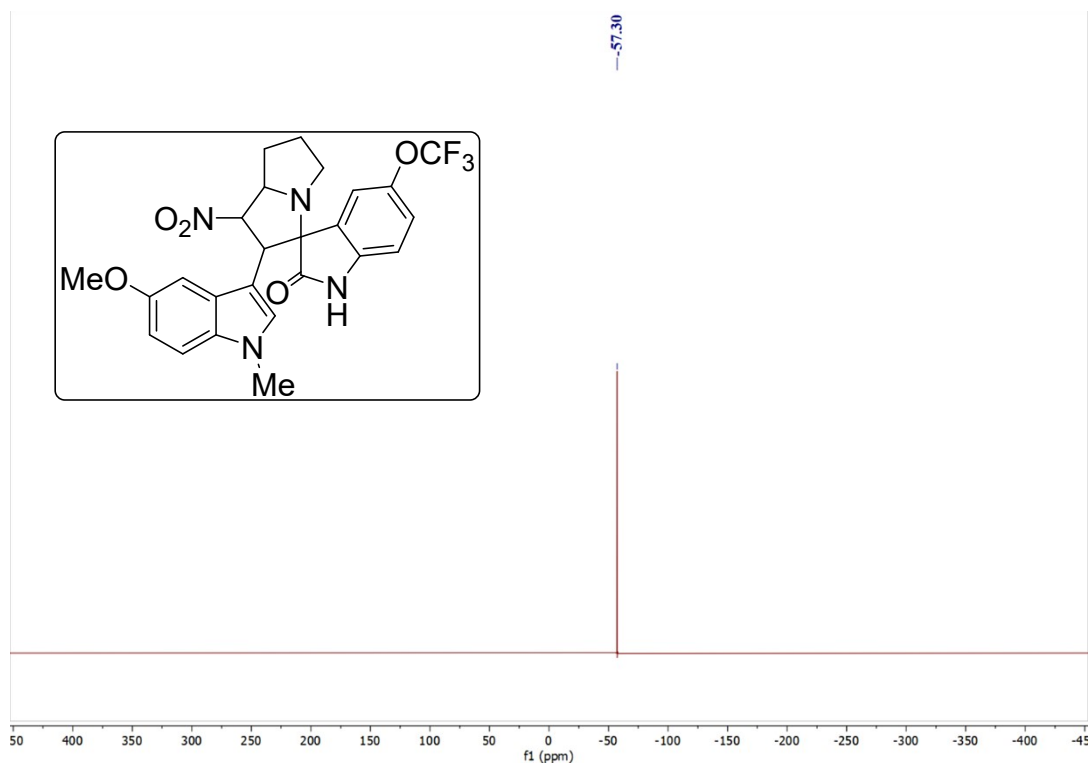


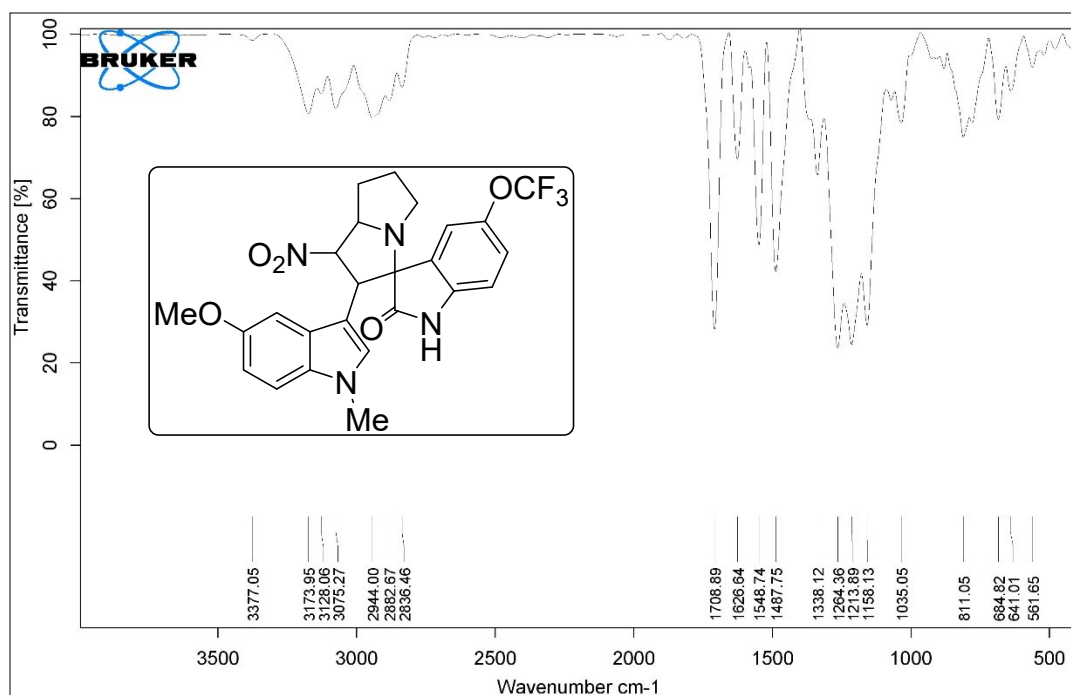


¹H NMR spectrum of the compound **6r** (400 MHz, DMSO-*d*₆)



¹³C NMR spectrum of the compound **6r** (100 MHz, DMSO-*d*₆)





IR spectrum of the compound **6r**

7. Crystallographic data and structure refinement parameters of the compounds **4m** and **4n**.

CCDC numbers for compounds **4m** and **4n** have been deposited with the Cambridge Crystallographic Data Centre.

Table S1. Crystallographic data

Identification code	4m	Identification code	4n
Empirical formula	C ₃₁ H ₂₈ N ₅ O ₃	Empirical formula	C ₃₂ H ₂₈ N ₅ O ₃
Formula weight	518.58	Formula weight	530.59
Crystal system	Monoclinic	Crystal system	Monoclinic
Space group	P2 ₁ /c	Space group	P2 ₁ /c
<i>T</i> /K	298 K	<i>T</i> /K	298 K
<i>a</i> /Å	15.6361 (12)	<i>a</i> /Å	16.0530(15)
<i>b</i> /Å	16.2505 (11)	<i>b</i> /Å	16.2367(15)
<i>c</i> /Å	10.9406 (9)	<i>c</i> /Å	11.1482(10)
α /°	90	α /°	90
β /°	108.354 (3)	β /°	107.943(4)

$\gamma/^\circ$	90	$\gamma/^\circ$	90
Z	4	Z	4
$V/\text{\AA}^3$	2638.5 (3)	$V/\text{\AA}^3$	2764.4(4)
$D_{\text{calc}}/\text{g/cm}^3$	1.306	$D_{\text{calc}}/\text{g/cm}^3$	1.275
$F(000)$	1092.0	$F(000)$	1116.0
μ/mm^{-1}	0.086	μ/mm^{-1}	0.084
$\theta/^\circ$	25.250 (max)	$\theta/^\circ$	24.998 (max)
Index ranges	$-18 \leq h \leq 18$ $-19 \leq k \leq 19$ $-13 \leq l \leq 13$	Index ranges	$-19 \leq h \leq 19$ $-19 \leq k \leq 19$ $-13 \leq l \leq 13$
N-total	4785	N-total	4874
N-parameters	354	N-parameters	364
$R_I (I > 2\sigma(I))$	0.0620 (2981)	$R_I (I > 2\sigma(I))$	0.0906 (3089)
wR_2 (all data)	0.2101 (4784)	wR_2 (all data)	0.3278 (4873)
GOF	1.131	GOF	1.118
CCDC	2525543	CCDC	2525544

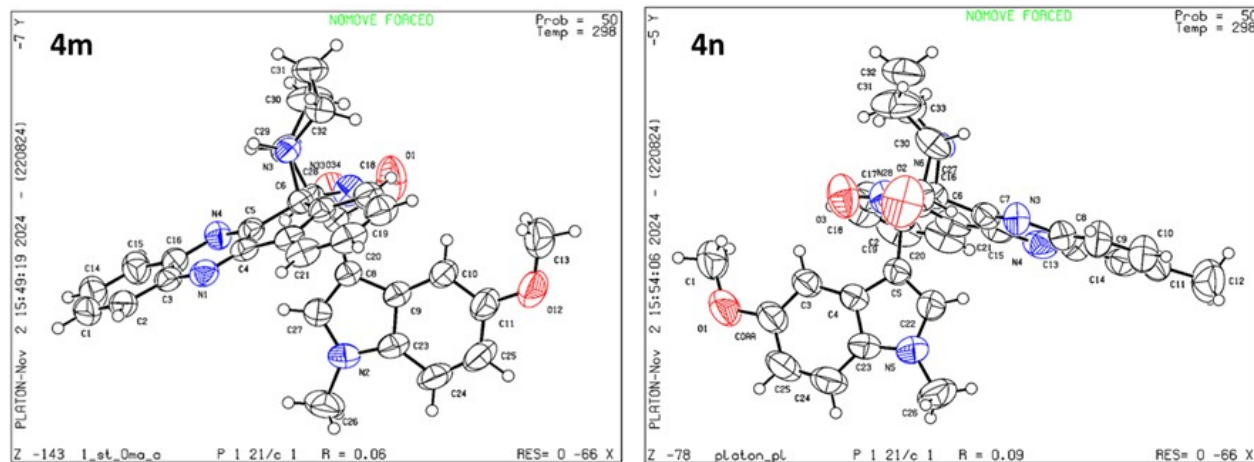


Figure S1. Crystal images of the compounds **4m** and **4n**.

8. Computational studies

Molecular docking studies

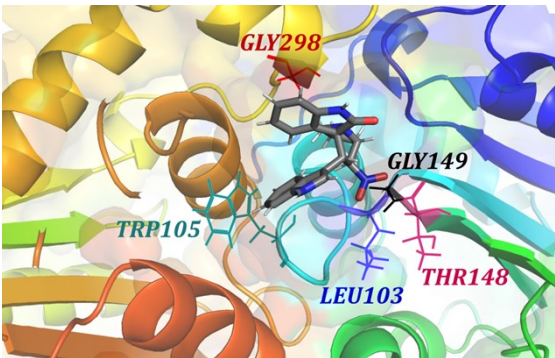
Molecular docking studies were carried out to investigate the binding affinity and interaction profiles of the most potent compound **6b** against a panel of breast cancer–associated proteins, namely human NAD(P)H: quinone oxidoreductase 2 (NQO2; PDB ID: 4ZVM), along with few breast cancer-associated proteins (PDB ID: 4DRH, 4Y18, 3ERT and 1UU7). The protein structures and compound **6b** were prepared for docking using AutoDock Tools 1.5.6. software.^{6,7}

Compound **6b** exhibited favorable binding energies toward all five selected proteins, with the least binding energy values of −11.0, −10.5, −9.2, −9.1 and −9.0 kcal/mol against 4ZVM, 4DRH, 4Y18, 3ERT and 1UU7, respectively (Table S2). Notably, the strongest binding affinity was observed for NQO2 (4ZVM), where compound **6b** exhibited a well-defined binding orientation within the NQO2 active site, stabilized by hydrogen bond interactions with GLY149, THR148 and LEU103 (2.8–3.0 Å). Additional stabilization was provided by multiple noncovalent interactions, including π – π stacking with aromatic residues, amide– π stacking, and extensive hydrophobic interactions, involving residues such as GLY298, TRP105, TYR104, PHE356, PHE408, ILE358 and PRO102. These interactions collectively stabilized compound **6b** within the binding pocket and support its favorable complementarity with the enzyme active site (Figure S2). Overall, the docking results highlight a favorable and specific interaction profile of compound **6b** with NQO2, providing a structural rationale for its observed anticancer activity and justifying further validation through molecular dynamics simulations.

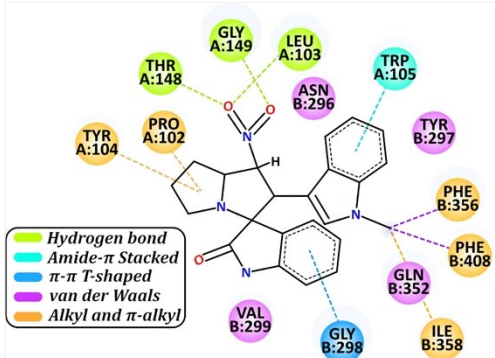
Table S2. Docking results of the potent compound **6b** against five breast cancer proteins 4ZVM, 4DRH, 4Y18, 3ERT and 1UU7.

Compounds	Binding energy (kcal/mol)	No. of hydrogen bonds	Residues involved in the hydrogen bonding	Hydrogen bond length (Å)
PDB ID: 4ZVM				
6b	-11.0	3	GLY149, LEU103, THR148	3.0, 2.9, 2.8
Doxorubicin	-11.3	3	ASN66, ASP117, TRP105	2.85, 2.75, 2.38
PDB ID: 4DRH				

6b	-10.5	1	ARG175	2.0
Doxorubicin	-10.5	4	GLY84, SER2035, TYR113	2.72, 2.68, 2.40, 2.04
PDB ID: 4Y18				
6b	-9.2	1	ARG1753	2.78
Doxorubicin	-10.1	7	ARG175, ASP1813, ASP1840, GLU1754, GLU1836, HIS732	2.84, 2.61, 2.38, 2.35, 2.33, 2.21, 2.15
PDB ID: 3ERT				
6b	-9.1	0	-	-
Doxorubicin	-8.0	3	CYS530	3.05, 2.47, 1.82
PDB ID: 1UU7				
6b	-9.0	1	ASP223	2.62
Doxorubicin	-9.5	2	GLU130, LYS111	2.72, 2.00



(a)



(b)

Figure S2. (a) 3D binding mode of compound **6b** within the NQO2 active site, highlighting key interacting residues and (b) 2D interaction diagram of the complex (PDB ID: 4ZVM).

Physicochemical properties and drug-likeness parameters of the compound **6b**

The compound **6b** was further evaluated using Lipinski's rule to determine its potential for oral bioavailability, as summarized in Table S3. The compound **6b** met all of Lipinski's rule criteria, suggesting it is suitable for oral administration. The bioavailability radar plot for compound **6b** and doxorubicin (DOX) shown in Figure S3, illustrates six physicochemical parameters: lipophilicity, size, polarity, solubility, flexibility and saturation. The pink area on the plot indicates the optimal range for each parameter. Compound **6b** fell within this optimal range for all criteria

and was compared to the standard drug doxorubicin. Compound **6b** exhibited a bioavailability score of 0.55, reflecting a topological polar surface area (TPSA) of 83.09 Å², which suggests enhanced permeability compared to standard drugs. Additionally, moderate solubility was observed for this compound a key factor in drug absorption.

Table S3. Physicochemical properties of the compound **6b** and standard drug doxorubicin.

En try	Mol. Form & Mol. Wt	HBA	HBD	RBN	TPSA	Log <i>P</i>	Log <i>S</i>	Bioav ailabi lity	Lipins ki
6b	C ₂₃ H ₂₂ N ₄ O ₃ 402.45	4	1	2	83.09	1.95	-4.31	0.55	Yes: 0
D O X	C ₂₇ H ₂₉ NO ₁₁ 543.52	12	6	5	206.07	0.50	-3.91	0.17	No: 3

Lipinski (RO5) criteria ranges are: **Mol. Form & Mol. Wt** = Molecular formula and molecular weight (≤ 500); **HBA** = Hydrogen bond acceptor (≤ 10); **HBD** = Hydrogen bond donor (≤ 5); **RBN** = Number of rotatable bonds (≤ 10); **TPSA** = topological polar surface area (Å²); **Log Po/w** = Lipophilicity (≤ 5); **Log S (ESOL)** = Water solubility (≤ 0.5); **DOX** = Doxorubicin standard drug.

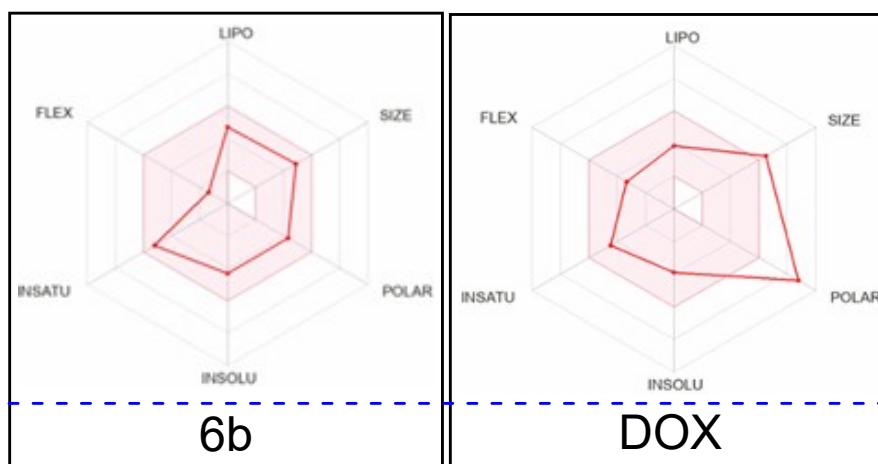


Figure S3. Bioavailability radar plot of the predicted compounds: The pink region indicates the ideal range for each property-lipophilicity (XLOGP3: -0.7 to $+5.0$), molecular size (MW: 150–500 g/mol), polarity (TPSA: 20–130 Å²), solubility ($\log S \leq 6$), saturation (sp^3 hybridized carbon fraction ≥ 0.25), and flexibility (≤ 9 rotatable bonds).

***In silico* pharmacokinetic properties and toxicity prediction of the compound 6b**

The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) prediction results for compound **6b** and the standard drug DOX are presented in Table S4. Doxorubicin has a log

BB value of -1.562 , indicating low brain permeation. Furthermore, it shows no activity as a substrate or inhibitor for CYP2D6, CYP3A4, CYP1A2, CYP2C9 or CYP2C19, thereby minimizing the risk of drug–drug interactions. However, compound **6b** exhibits a log BB value of 0.509 , suggesting good brain permeation. It acts as a substrate for CYP3A4, CYP1A2, CYP2C19, and CYP2D6, which indicates a potential increase in drug–drug interactions. Its water solubility (-3.27), Caco-2 permeability (0.982), intestinal absorption (ABS), skin permeability (log Kp: -2.742) and P-gp substrate status (yes) are comparable to those of DOX. The volume of distribution (0.997) and clearance (0.558) are also noted for compound **6b**, which additionally shows a positive renal OCT2 substrate status. However, compound **6b** and DOX is predicted to be AMES toxic and exhibit acute oral toxicity (2.5) as well as rat oral toxicity (1.363). Notably, the predicted AMES toxicity of compound **6b** is comparable to that of the clinically used anticancer drug doxorubicin; however, such *in silico* predictions require experimental validation and do not preclude further lead optimization. Importantly, compound **6b** demonstrates no risk of skin sensitization, indicating no allergic skin reactions and is predicted to be non-carcinogenic.

To evaluate their potential as drug candidates, the compounds were analyzed for physicochemical properties and drug-likeness parameters. These characteristics are crucial, as they determine pharmacokinetics, pharmacodynamics and overall safety. The Lipinski rule of five was applied as the primary guideline for assessing drug-likeness and prioritizing candidates. Detailed physicochemical properties and bioavailability were assessed using the SwissADME web tool.⁸ In addition, pharmacokinetic behaviour and toxicity were evaluated using pkCSM,⁹ admetSAR¹⁰ and Deep-PK.¹¹

Table S4. *In silico* pharmacokinetic properties and toxicity predictions of the compound **6b**.

Model Name	6b	Doxorubicin
Absorption		
Water solubility (log mol/L)	-3.27	-3.14
Caco2 permeability log Papp in 10^{-6} cm/s	0.928	0.6
Intestinal absorption	ABS	ABS
Skin Permeability (log Kp)	-2.742	-2.735
P-glycoprotein substrate and inhibitor	Yes	Yes
Distribution		

VDss (human; (log L/kg)	0.997	1.144
BBB permeability (log BB)	0.509	-1.562
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	No
CYP1A2, 2C19, 2D6 inhibitor	Yes	No
CYP3A4, 2C9 inhibitor	No	
Excretion		
Total Clearance (log ml/min/kg)	0.558	1.035
Renal OCT2 substrate	Yes	No
Toxicity		
AMES toxicity	Yes	Yes
Acute Oral Toxicity	2.5	2.443
Oral Rat Acute Toxicity (LD50; mol/kg)	1.363	3.354
Skin Sensitization	No	No
Carcinogenesis	Safe	Safe

9. Computational methodology

Molecular Docking Simulations: The X-Ray crystallographic structure of quinone reductase 2 in oxidized form was retrieved from the RCSB Protein Data Bank (PDB ID: 4ZVM). Prior to docking, the protein structure was prepared by removing the co-crystallized doxorubicin, FAD cofactor, crystallographic water molecules, and associated ions to ensure unobstructed ligand binding within the active site. Polar hydrogen atoms were subsequently added, and appropriate partial charges were assigned to the enzyme to enable accurate interaction calculations. Molecular docking of the synthesized spiro compounds was carried out to identify favourable binding conformations and estimate binding affinities within the NQO2 active site. A three-dimensional grid box with dimensions of $40 \times 40 \times 40 \text{ \AA}^3$ along the x, y, and z axes was employed, with a grid spacing of $0.375 \text{ \AA}$. The grid center was positioned at coordinates (50.146, 4.999, 12.823) to encompass the catalytic pocket. Docking calculations were performed using AutoDock Vina, and the resulting binding poses were visualized using AutoDockTools and the Protein–Ligand Interaction Profiler.¹²⁻¹⁴

6.2. Molecular Dynamics (MD) Simulations: The NQO2 enzyme was parameterized using the AMBER99SB-ILDN force field, while the spiro compound parameters were generated using the GAFF2 force field. Ligand topology and parameters were derived *via* the Antechamber module implemented in AmberTools, ensuring compatibility with the AMBER force field.^{15,16} The protein–ligand complex was placed in a dodecahedral simulation box and solvated with extended simple point charge (SPC/E) water molecules to replicate physiological aqueous conditions.¹⁷ System electroneutrality was achieved by substituting water molecules with eight Na⁺ counterions. Energy minimization was followed by a two-step equilibration protocol comprising an NVT ensemble at 300 K and a subsequent NPT ensemble equilibration at 1 bar to stabilize temperature and pressure. The equilibrated system was subjected to a 150 ns production MD run using a time step of 2 fs. All MD simulations were carried out using the GROMACS simulation package, and trajectory analyses were performed using Visual Molecular Dynamics (VMD) software.^{18,19}

The binding free energies of the compound within the enzyme binding site was computed using the gmx_MMPBSA module interfaced with GROMACS. Frames extracted from the final 40 ns of the production MD trajectory were uniformly sampled at 1 ns interval for molecular mechanics/generalized Born surface area (MM/GBSA) calculations.²⁰ Principal component analysis (PCA) was performed on the mass-weighted C α backbone fluctuations from the last 10 ns of the MD trajectory to identify dominant collective motions.²¹ The projections onto the first two principal components was used to assess conformational stability and free energy distribution.

10. Materials and methods for biological evaluation

Cell Culture: Normal human embryonic kidney cells (HEK-293) and cancer cell lines, including lung (A549), breast (MCF-7), colon (HCT-116) and liver (HepG2), were cultured in their respective growth media supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution (Sigma-Aldrich). Cells were maintained at 37 °C in a humidified incubator with 5% CO₂. When cultures reached 80–90% confluency, they were sub-cultured using 0.25% trypsin/1 mM EDTA. Stock solutions (10 mM) of the test compound and reference drugs were prepared in DMSO and working concentrations were obtained by dilution with the appropriate culture medium.

***In vitro* cytotoxicity assay:** To assess cytotoxic effects, normal and cancer cell lines were seeded in 96-well plates at densities suitable for their respective doubling times and allowed to adhere

overnight. Cells were then treated with compound **6b** at concentrations ranging from 2.11, 4.23 and 8.46 μM for 48 h. Following treatment, the medium was replaced with 100 μL of fresh medium containing MTT (0.5 mg/mL) and incubated for an additional 3–4 h at 37 °C in the dark. After carefully discarding the supernatant, formazan crystals were dissolved in 100 μL DMSO. Absorbance was measured at 570 nm using a microplate reader and IC_{50} values were calculated. All assays were performed in triplicate.

Cell morphology assessment: MCF-7 cells were seeded into 6-well culture plates at a density of 1×10^5 cells/mL and allowed to adhere overnight. Cells were then treated with compound **6b** at concentrations of 2.11, 4.23, and 8.46 μM for 48 hours. Morphological changes were examined under 100x magnification using a bright field microscope and representative images were recorded. All experiments were performed in triplicate.

Cell migration (Wound-healing) assay: MCF-7 cells were seeded in 12-well plates at a density of 3.5×10^5 cells/well in DMEM, high glucose medium and allowed to grow to a confluent monolayer. A uniform scratch was created in each well using a sterile 200 μL pipette tip. Cells were then treated with various concentrations (2.11, 4.23 and 8.46 μM) of compound **6b** and doxorubicin (0.34 μM) and wound closure was monitored by capturing images at 0 h and 48 h under a 100x magnification microscope.

DCFDA reactive Oxygen Species (ROS) detection assay: To measure intracellular reactive oxygen species (ROS), MCF-7 cells were plated in 12-well plates at 1×10^6 cells/well and treated with varying concentrations (2.11, 4.23 and 8.46 μM) of compound **6b** and doxorubicin (0.34 μM) for 48 h. After treatment, cells were incubated with 10 μM DCFDA for 30 minutes at 37 °C, washed with PBS to remove excess dye and imaged using a fluorescence microscope (Nikon, Japan) at 200 $\times$ magnification. Green fluorescence intensity indicated ROS generation due to DCFDA oxidation into dichlorofluorescein (DCF).

Mitochondrial membrane potential analysis: MCF-7 cells were seeded in 12-well plates at a density of 1×10^6 cells/mL and treated for 48 h with compound **6b** (2.11, 4.23 and 8.46 μM) or doxorubicin (0.34 μM). After treatment, the medium was replaced with 500 μL of fresh medium containing JC-1 dye (8.46 $\mu\text{g/mL}$) and cells were incubated for 20 min at 37 °C. Excess dye was removed by washing three times with PBS. Fluorescence images were then captured at 200 $\times$ magnification in both red and green channels using a fluorescence microscope.

Acridine orange/ethidium bromide (AO/EB) staining: MCF-7 cells were seeded at 60,000 cells/well and exposed to different concentrations (2.11, 4.23 and 8.46 μM) of compound **6b** and doxorubicin (0.34 μM) for 24 h in a 5% CO_2 incubator at 37 $^\circ\text{C}$. Following treatment, a mixture of acridine orange and ethidium bromide, each at 10 μM , was added to the wells and incubated for 10 minutes. Cells were examined using a fluorescence microscope (Nikon, Japan) at 200 $\times$ magnification, with excitation at 488 nm and emission at 550 nm, to distinguish viable, apoptotic and necrotic cells based on fluorescence colour.

DAPI (4',6-diamidino-2-phenylindole) staining: To visualize nuclear changes, MCF-7 cells were treated with compound **6b** (2.11, 4.23 and 8.46 μM) or doxorubicin (0.34 μM) for 48 h, then washed with PBS. Cells were permeabilized with 0.1% Triton X-100 for 10 minutes and stained with 10 $\mu\text{g/mL}$ DAPI. Fluorescence images were acquired at 200 $\times$ magnification using a Nikon microscope equipped with a DAPI filter (excitation 359 nm, emission 461 nm). Both vehicle-treated controls and drug-treated samples were analyzed for chromatin condensation and nuclear fragmentation.

Colony formation assay: Exponentially growing MCF-7 cells were seeded in 6-well plates. After 24 h, the culture medium was replaced with fresh medium containing compound **6b** (2.11, 4.23 and 8.46 μM) and doxorubicin (0.34 μM). Cells were maintained for 7 days, with drug-containing medium refreshed every 3 days. Treatments were performed in triplicate. At the end of incubation, cells were rinsed twice with PBS, fixed with 4% paraformaldehyde for 20 min and stained with crystal violet for 15 min. The number of colonies was analyzed using ImageJ software (NIH, USA).

11. REFERENCES

1. A. Y. Barkov, N. S. Zimnitskiy, I. B. Kutyashev, V. Y. Korotaev, V. Y. Sosnovskikh, Regio- and stereoselective 1,3-dipolar cycloaddition reactions between arylideneacetones and stabilized azomethine ylides obtained from ninhydrin and indenoquinoxalinones. *Chem. Heterocycl. Compd.*, 2017, **53**, 1315–1323.
2. A. Y. Barkov, N. S. Zimnitskiy, I. B. Kutyashev, V. Y. Korotaev, V. Y. Sosnovskikh, Unexpected regiochemistry in [3+2] cycloaddition reaction of azomethine ylides of indenoquinoxalinone series to arylidene malononitriles. *Chem. Heterocycl. Compd.* **2018**, *54*, 43–50.

3. I. B. Kutyashev, A. V. Barkov, V. Y. Korotaev, V. Y. Sosnovskikh, 3-Nitro-2*H*-chromenes in [3+2] cycloaddition reaction with azomethine ylides derived from *N*-unsubstituted α -amino acids and isatins: regio- and stereoselective synthesis of spirochromeno[3,4-*c*]pyrrolidines. *Chem. Heterocycl. Compd.*, 2019, **55**, 529–540.
4. C. E. Puerto Galvis, V. V. Kouznetsov, Regio- and stereoselective synthesis of spirooxindole 1'-nitro pyrrolizidines with five concurrent stereocenters under aqueous medium and their bioprospection using the zebrafish (*Danio rerio*) embryo model. *Org. Biomol. Chem.*, 2013, **11**, 7372–7386.
5. V. Rukyanai, R. K. Gamidi, J. Kumari, D. Sriram, S. Basavoju, A green one-pot three component synthesis of thiazolidine-2,4-dione based bispirooxindolo-pyrrolidines with [Bmim]BF₄: Their *in vitro* and *in silico* anti-TB studies. *Mol. Divers.*, 2024, **29**, 303–317.
6. F. Thaler, L. Moretti, R. Amici, A. Abate, A. Colombo, G. Carenzi, M. C. Fulco, R. Boggio, G. Dondio, S. Gagliardi, S. Minucci, L. Sartori, M. Varasi, C. Mercurio, Synthesis, biological characterization and molecular modeling insights of spirochromanes as potent HDAC inhibitors. *Eur. J. Med. Chem.*, 2016, **108**, 53–67.
7. A. Allouche, Software news and updates gabedit—A graphical user interface for computational chemistry softwares. *J. Comput. Chem.*, 2012, **32**, 174–182.
8. A. Daina, O. Michielin, V. Zoete, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, 2017, **7**, 1–13.
9. D. E. V. Pires, T. L. Blundell, D. B. Ascher, PkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J. Med. Chem.*, 2015, **58**, 4066–4072.
10. H. Yang, C. Lou, L. Sun, J. Li, Y. Cai, Z. Wang, W. Li, G. Liu, Y. Tang, AdmetSAR 2.0: Web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics.*, 2019, **35**, 1067–1069.
11. Y. Myung, A. G. C. De Sá, D. B. Ascher, Deep-PK: deep learning for small molecule pharmacokinetic and toxicity prediction. *Nucleic Acids Res.*, 2024, **52**, W469–W475.
12. O. Trott, A. J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem.*, 2010, **31**, 455–461.

13. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*., 2009, **30**, 2785–2791.
14. S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, Schroeder, M. PLIP: Fully automated protein-ligand interaction profiler. *Nucleic Acids Res.*, 2015, 43, W443-447.
15. D. A. Case, H. M. Aktulga, K. Belfon, D. S. Cerutti, G. A. Cisneros, V. W. D. Cruzeiro, N. Forouzes, T. J. Giese, A. W. Götz, H. Gohlke, S. Izadi, K. Kasavajhala, M. C. Kaymak, E. King, T. Kurtzman, T. S. Lee, P. Li, J. Liu, T. Luchko, R. Luo, M. Manathunga, M. R. Machado, H. M. Nguyen, K. A. O’Hearn, A. V. Onufriev, F. Pan, S. Pantano, R. Qi, A. Rahnamoun, A. Rishch, S. Schott-Verdugo, A. Shajan, J. Swails, J. Wang, H. Wei, X. Wu, Y. Wu, S. Zhang, S. Zhao, Q. Zhu, T. E. I. Cheatham, D. R. Roe, A. Roitberg, C. Simmerling, D. M. York, M. C. Nagan, K. M. Merz, Jr. AmberTools. *J. Chem. Inf. Model.*, 2023, **63**, 6183–6191.
16. A. W. Sousa da Silva, W. F. Vranken, ACPYPE - AnteChamber PYthon Parser interface. *BMC Research Notes*., 2012, **5**, 367.
17. H. J. C. Berendsen, J. R. Grigera, T. P. Straatsma, The missing term in effective pair potentials. *J. Phys. Chem.*, 1987, **91**, 6269–6271.
18. D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark, H. J. C. Berendsen, GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*., 2005, **26**, 1701–1718.
19. W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics. *Journal of Molecular Graphics*., 1996, **14**, 33–38.
20. M. S. Valdés-Tresanco, M. E. Valdés-Tresanco, P. A. Valiente, E. Moreno, gmx_MMPBSA: A new tool to perform end-state free energy calculations with GROMACS. *J. Chem. Theory Comput.*, 2021, **17**, 6281–6291.
21. A. Altis, M. Otten, P. H. Nguyen, R. Hegger, G. Stock, construction of the free energy landscape of biomolecules *via* dihedral angle principal component analysis. *The Journal of Chemical Physics*., 2008, **128**, 245102.