

First synthesis of *N*-[(aziridin-2-yl)methyl]benzimidazolequinone and analysis of toxicity towards normal and Fanconi anemia cells

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Experimental

General

All materials were obtained from Sigma-Aldrich and solvents were purified and dried prior to use according to conventional methods. Monitoring of reactions by thin layer chromatography (TLC) was performed using aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F₂₅₄). Column chromatography using silica gel was carried out using Merck Kieselgel 60 H silica. All reactions were conducted under a nitrogen atmosphere apart from those involving aqueous solutions.

Melting points were measured on a Stuart Scientific melting point apparatus SMP3. Optical rotations were measured on a Perkin Elmer 241 polarimeter using a 1 dm path length. IR spectra were acquired using a Perkin-Elmer Spec 1 with ATR attached. All ¹H and ¹³C NMR spectra were recorded using a Joel GXFT 400 MHz instrument equipped with a DEC AXP 300 computer workstation. Data are expressed in parts per million downfield from SiMe₄ as internal standard or relative to CHCl₃. NMR assignments were supported by ¹H-¹³C NMR 2D spectra for compounds **4** and DEPT for **2** and **4-8** and **10**. High resolution mass spectra for compounds **2**, **6-8** and **10** were carried out by University College London, Mass Spectroscopy Facility using a Thermo Finnigan MAT900xp operating in chemical ionization (CI, CI⁺) mode with methane as the carrier gas and accurate masses were calculated against reference 'heptacosyl'. The EPSRC National Mass Spectrometry Service (University of Swansea) carried out high resolution mass spectrometry using electrospray ionization (ESI, ES⁺) on the Finnigan MAT 900 XLT for compound **5** by manual peak matching. The precision of all accurate mass measurements is better than 3 ppm. Elemental analysis was carried out on a Perkin-Elmer 2400 Series II analyzer for compound **4**. UV absorbance of **2** was measured on a Cary 50 UV-VIS Spectrophotometer. HPLC analysis of **2** was carried out using an Agilent Technologies 1200 series instrument with a UV detector at the specified wavelength. Absorbance was measured in the MTT assay using a Wallac Victor2 1420 multi-label Counter (plate reader).

[(2*S*)-1-Tritylaziridin-2-yl]methyl methanesulfonate **4**

Triethylamine (0.20 mL, 1.40 mmol) was added dropwise to a solution of methanesulfonyl chloride (0.11 mL, 1.40 mmol), and (2*S*)-1-tritylaziridine-2-methanol **3** (0.300 g, 0.95 mmol) in dichloromethane (5 mL) at -78 °C. The solution was allowed to return to room temperature and was stirred for a further 18 h. Water was added (10 mL), the mixture extracted with dichloromethane (3 x 5 mL) and the combined organic extracts dried (MgSO₄). The organic extracts were evaporated to dryness to give a yellow solid, which was recrystallised from hexane to yield the **title compound 4**, as white needles (0.289 g, 77%), mp 90-91 °C; *R*_f = 0.40 (4:1 hexane/diethyl ether); [α]_D²⁴ -17.2° (*c* 1.00 in CH₂Cl₂); (Found: C, 70.7; H, 5.9; N,

3.4%. C₂₃H₂₃NO₃S requires C, 70.2; H, 5.9; N, 3.6%); $\nu_{\max}/\text{cm}^{-1}$ 3029, 1488, 1352 (S=O), 1173 (S=O), 1068; δ_{H} (400 MHz; CDCl₃) 1.25 (1 H, d, $J = 5.9$ Hz, NCHH), 1.65-1.67 (1 H, m, CH), 1.84 (1 H, d, $J = 2.8$ Hz, NCHH), 2.97 (3 H, s, CH₃), 4.30-4.34 (1 H, dd, $J^2 = 10.2$ Hz, $J^3 = 5.5$ Hz, CHHOMs), 4.39-4.43 (1 H, dd, $J^2 = 10.2$ Hz, $J^3 = 6.2$ Hz, CHHOMs), 7.20-7.31 (9 H, m, Ph-H), 7.47-7.49 (6 H, m, Ph-ortho-H); δ_{C} (100 MHz; CDCl₃) 25.6 (CH₂), 30.9 (CH), 37.8 (CH₃), 71.8 (CH₂O), 73.9 (CPh₃), 127.0, 127.7, 129.5 (all Ph-CH), 144.1 (Ph-*ipso*-C).

4,7-Dimethoxy-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole **5**

4,7-Dimethoxy-1*H*-benzimidazole (40 mg, 0.22 mmol) and sodium hydride (8 mg, 0.33 mmol) in *N,N*-dimethylformamide (5 mL) were heated at 80 °C for 0.5 h. Mesylate **4** (65 mg, 0.17 mmol) was added and heating continued for an additional 4 h. The reaction was cooled, filtered through Celite and evaporated to dryness to yield a brown residue, which was purified using column chromatography with silica gel as absorbent and ethyl acetate/hexane as eluent. Evaporation of the fractions containing the second component gave the **title compound 5**, as a brown solid (65 mg, 80%), mp 85-88 °C; $R_{\text{f}} = 0.60$ (3:1 ethyl acetate/hexane) [α]_D²⁴ +13.2° (*c.* 0.50 in CH₂Cl₂); $\nu_{\max}/\text{cm}^{-1}$ 2934, 1522, 1492, 1448; δ_{H} (400 MHz; CDCl₃) 1.09 (1 H, d, $J = 6.2$ Hz, aziridinyl-CHHN), 1.70 (1 H, d, $J = 3.0$ Hz, aziridinyl-CHHN), 1.79-1.86 (1 H, m, aziridinyl-CHN), 3.69 (3 H, s, CH₃), 3.96 (3 H, s, CH₃), 4.21-4.26 (1 H, dd, $J^2 = 14.1$ Hz, $J^3 = 6.6$ Hz, NCHH), 4.95-4.99 (1 H, dd, $J^2 = 14.1$ Hz, $J^3 = 4.1$ Hz, NCHH), 6.50 (2 H, s, Ar-5,6-H), 7.09-7.26 (9 H, m, Ph-H), 7.45 (6 H, d, $J = 7.3$ Hz, Ph-ortho-H), 7.74 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl₃) 26.5 (aziridinyl-CH₂), 33.2 (CHN), 49.5 (CH₂), 55.5 (CH₃), 56.1 (CH₃), 74.1 (CPh₃), 101.8 (ArCH), 103.1 (ArCH), 124.9 (C), 126.9, 127.6, 129.4 (all Ph-CH), 136.0 (C), 141.6 (C), 142.4 (Im-2-CH), 144.3 (C), 146.3 (C); (HRMS (ESI): found MH⁺, 476.2335. C₃₁H₃₀N₃O₂ requires, 476.2333); m/z (ESI) 476 ([M+H]⁺, 100%), 951 ([M₂+H]⁺, 12).

N-[(1-Tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione **2**

4,7-Dimethoxybenzimidazole **5** (0.100 g, 0.21 mmol), *N*-bromosuccinimide (NBS, 41 mg, 0.23 mmol), concentrated H₂SO₄ (12 μ L) and water (1.1 mL) in tetrahydrofuran (THF, 3.2 mL) were stirred for 40 min at 0 °C. The mixture was basified with saturated sodium hydrogen carbonate (10.0 mL), extracted with ethyl acetate (3 x 5 mL), dried (MgSO₄) and evaporated to dryness to give a solid residue. This was purified using column chromatography with silica gel as absorbent using ethyl acetate/hexane as eluent to give in order of elution; **6-bromo-1-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione 7** as a yellow solid (16 mg, 14%), mp 121-124 (dec) °C; $R_{\text{f}} = 0.72$ (1:1 ethyl acetate/hexane); [α]_D²⁴ -25.2° (*c.* 0.23 in CH₂Cl₂); $\nu_{\max}/\text{cm}^{-1}$ 3056, 1669 (C=O), 1579 (C=O), 1490, 1143; δ_{H} (400 MHz; CDCl₃) 1.20 (1 H, d, $J = 6.0$ Hz, aziridinyl-CHHN), 1.74 (1 H, d, $J = 3.0$ Hz, aziridinyl-CHHN), 1.84-1.87 (1 H, m, aziridinyl-CHN), 4.39-4.44 (1 H, dd, $J^2 = 14.1$ Hz, $J^3 = 5.4$ Hz, NCHH), 4.64-4.69 (1 H, dd, $J^2 = 14.1$ Hz, $J^3 = 5.6$ Hz, NCHH), 7.16-7.26 (10 H, m, Ph-H and Ar-5-H), 7.38 (6 H, d, $J = 7.1$ Hz, Ph-ortho-H), 7.82 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl₃) 26.5 (aziridinyl-CH₂), 31.9 (CHN), 49.8 (CH₂), 74.2 (CPh₃), 127.1, 127.8, 129.3 (all Ph-CH), 137.2 (C), 137.7 (Ar-5-CH), 143.3 (C), 143.8 (Im-2-CH), 144.2 (C), 145.2 (C), 170.7 (C=O), 178.2 (C=O); (HRMS (CI): found MH⁺, 524.0976. C₂₉H₂₃N₃O₂⁷⁹Br requires, 524.0974); m/z (CI) 526 (C₂₉H₂₃N₃O₂⁸¹Br, 67%), 524 (C₂₉H₂₃N₃O₂⁷⁹Br, 64), 446 (31), 419 (39), 224 (23), 243 (100); **5-bromo-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione 6**, as a yellow solid (trace), mp 109-111 (dec) °C; $R_{\text{f}} = 0.62$ (1:1 ethyl acetate/hexane);

$[\alpha]_{\text{D}}^{24}$ -40.8° (c. 0.23 in CH_2Cl_2); $\nu_{\text{max}}/\text{cm}^{-1}$ 3016, 1689 (C=O), 1661 (C=O), 1489, 1346, 1211; δ_{H} (400 MHz; CDCl_3) 1.19 (1 H, d, J = 6.0 Hz, aziridinyl-CHHN), 1.73 (1 H, d, J = 3.2 Hz, aziridinyl-CHHN), 1.86-1.91 (1 H, m, aziridinyl-CHN), 4.39-4.44 (1 H, dd, J^2 = 14.0 Hz, J^3 = 5.2 Hz, NCHH), 4.59-4.64 (1 H, dd, J^2 = 14.0, J^3 = 5.7 Hz NCHH), 7.10 (1 H, s, Ar-6-H), 7.20-7.27 (9 H, m, Ph-H), 7.38 (6 H, d, J = 6.9 Hz, Ph-ortho-H), 7.81 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl_3) 26.5 (aziridinyl-CH₂), 31.8 (CHN), 49.7 (CH₂), 76.8 (CPh₃), 127.1, 127.8, 129.3 (all Ph-CH), 130.5 (C), 137.1 (Ar-6-CH), 138.8 (C), 141.6 (C), 143.8 (Im-2-CH), 172.9 (C=O), 175.7 (C=O); (HRMS (CI): found MH^+ , 524.0970. $\text{C}_{29}\text{H}_{23}\text{N}_3\text{O}_2$ ^{79}Br requires, 524.0974); m/z 526 ($\text{C}_{29}\text{H}_{23}\text{N}_3\text{O}_2$ ^{81}Br , 15%), 524 ($\text{C}_{29}\text{H}_{23}\text{N}_3\text{O}_2$ ^{79}Br , 14), 244 (18), 243 (100), and the **title compound 2**, as a yellow solid (52 mg, 56%), mp 95-98 (dec) °C; R_{f} = 0.50 (1:1 ethyl acetate/hexane); $[\alpha]_{\text{D}}^{24}$ +8.1° (c. 0.32 in CH_2Cl_2); $\nu_{\text{max}}/\text{cm}^{-1}$ 2891, 1684 (C=O), 1664, (C=O), 1459, 1355; δ_{H} (400 MHz; CDCl_3) 1.18 (1 H, d, J = 5.9 Hz, aziridinyl-CHHN), 1.73 (1 H, d, J = 3.2 Hz, aziridinyl-CHHN), 1.84-1.87 (1 H, m, aziridinyl-CHN), 4.39-4.44 (1 H, dd, J^2 = 14.1 Hz, J^3 = 5.5 Hz, NCHH), 4.65-4.70 (1 H, dd, J^2 = 14.1 Hz, J^3 = 5.7 Hz, NCHH), 6.57 (1 H, AB-q, J = 10.3 Hz, Ar-(5)6-H), 6.65 (1 H, AB-q, J = 10.3 Hz, Ar-5(6)-H), 7.12-7.26 (9 H, m, Ph-H), 7.39-7.41 (6 H, m, Ph-ortho-H), 7.82 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl_3) 26.3 (aziridinyl-CH₂), 31.8 (CHN), 49.3 (CH₂), 76.7 (CPh₃), 126.9, 127.6, 129.2 (all Ph-CH), 130.1, (C), 136.0 (Ar-CH), 136.6 (Ar-CH), 142.4 (C), 143.3 (C), 143.7 (Im-2-CH), 178.3 (C=O), 180.7 (C=O); (HRMS (CI): found MH^+ , 446.1865, $\text{C}_{29}\text{H}_{24}\text{N}_3\text{O}_2$ requires. 446.1869); m/z 447 (4%), 446 ($[\text{M}+\text{H}]^+$, 17), 244 (21), 243 (100); λ_{max} (in acetonitrile) = 256 nm, ϵ = 1636 $\text{Lmol}^{-1}\text{cm}^{-1}$. HPLC chromatogram of **2** is included on page 20.

Reaction of 4,7-dimethoxybenzimidazole **5** with NBS under acid-free conditions

4,7-Dimethoxybenzimidazole **5** (50 mg, 0.11 mmol), NBS (93 mg, 0.53 mmol) and water (0.5 mL) in THF (1.6 mL) were stirred for 40 min at room temperature. Water was added (10 mL) and the mixture extracted with ethyl acetate (3 x 10 mL), dried (MgSO_4) and evaporated to dryness to give a solid residue. This was purified using column chromatography with silica gel as absorbent using ethyl acetate/hexane as eluent to give in order of elution; **5,6-dibromo-4,7-dimethoxy-N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 10**, as a yellow solid (21 mg, 30%), mp 115-118 °C, R_{f} = 0.80 (1:1 ethyl acetate/hexane); $\nu_{\text{max}}/\text{cm}^{-1}$ 3013, 2867, 1509, 1396, 1367, 1197; δ_{H} (400 MHz; CDCl_3) 1.15 (1 H, d, J = 6.0 Hz, aziridinyl-CHHN), 1.74 (1 H, d, J = 2.8 Hz, aziridinyl-CHHN), 1.84-1.88 (1 H, m, aziridinyl-CHN), 3.74 (3 H, s, CH₃), 4.23-4.27 (4 H, m and s, NCHH and CH₃, respectively), 4.81-4.86 (1 H, dd, J^2 = 14.7 Hz, J^3 = 4.6 Hz, NCHH), 7.18-7.26 (9 H, m, Ph-H), 7.43 (6 H, d, J = 8.2 Hz, Ph-ortho-H), 7.81 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl_3) 26.6 (aziridinyl-CH₂), 32.6 (CHN), 49.0 (CH₂), 61.6 (CH₃), 62.0 (CH₃) 74.2 (CPh₃), 110.3 (C-Br), 114.4 (C-Br), 127.0, 127.6, 129.3 (all Ph-CH), 137.2 (C), 139.7 (C), 143.5 (C), 144.0 (Im-2-CH), 146.4 (C); (HRMS (CI): found MH^+ , 631.0471. $\text{C}_{31}\text{H}_{28}\text{N}_3\text{O}_2$ $^{79}\text{Br}^{79}\text{Br}$ requires, 631.0470, m/z 635 ($\text{C}_{31}\text{H}_{28}\text{N}_3\text{O}_2$ $^{81}\text{Br}^{81}\text{Br}$, 52%), 633 ($\text{C}_{31}\text{H}_{28}\text{N}_3\text{O}_2$ $^{81}\text{Br}^{79}\text{Br}$, 95), 631 ($\text{C}_{31}\text{H}_{28}\text{N}_3\text{O}_2$ $^{79}\text{Br}^{79}\text{Br}$, 51), 556 (21), 554 (20), 244 (20), 243 (100); **6-bromo-4,7-dimethoxy-N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 9**, as a yellow solid (trace), R_{f} = 0.70 (1:1 ethyl acetate/hexane); δ_{H} (400 MHz; CDCl_3) 1.17 (1 H, d, J = 6.2 Hz, aziridine-CHHN), 1.71-1.73 (1 H, m, aziridine-CHHN), 1.85-1.89 (1 H, m, aziridinyl-CHN), 3.69-3.71 (3 H, m, CH₃), 4.20-4.27 (3 H, m, CH₃), 4.38-4.43 (1 H, dd, J^2 = 14.0 Hz, J^3 = 5.4 Hz, NCHH), 4.58-4.63 (1 H, dd, J^2 = 14.0 Hz, J^3 = 5.5 Hz, NCHH), 7.10 (1 H, s, Ar-5-H), 7.17-7.28 (9 H, m, Ph-H), 7.37 (6 H, d, J = 6.9 Hz,

Ph-*ortho*-H), 7.80 (1 H, s, Im-2-H), and **5-bromo-4,7-dimethoxy-N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole 8**, as a yellow solid (37 mg, 60%), mp 132-136 °C, R_f = 0.65 (ethyl acetate/hexane); $\nu_{\max}/\text{cm}^{-1}$ 3057, 2937, 1489, 1446, 1278, 1241; δ_{H} (400 MHz; CDCl_3) 1.13 (1 H, d, J = 6.0 Hz, aziridiny-CHHN), 1.74 (1 H, d, J = 2.8 Hz, aziridiny-CHHN), 1.81-1.89 (1 H, m, aziridiny-CHN), 3.70 (3 H, s, CH_3), 4.17 (3 H, s, CH_3), 4.22-4.27 (1 H, dd, J^2 = 14.1 Hz, J^3 = 6.6 Hz, NCHH), 4.84-4.89 (1 H, dd, J^2 = 14.1 Hz, J^3 = 4.6 Hz, NCHH), 6.72 (1 H, s, Ar-6-H), 7.17-7.25 (9 H, m, Ph-H), 7.42 (6 H, d, J = 7.3 Hz, Ph-*ortho*-H), 7.74 (1 H, s, Im-2-H); δ_{C} (100 MHz; CDCl_3) 26.6 (aziridiny- CH_2), 33.2 (CHN), 49.9 (CH_2), 55.8 (CH_3), 61.4 (CH_3) 74.1 (CPh₃), 106.1 (C-Br), 107.7 (Ar-6-CH), 124.7 (C), 126.9, 127.6, 129.3 (all Ph-CH), 138.4 (C), 142.8 (C), 142.9 (C), 143.0 (C), 144.2 (Im-2-CH); (HRMS (CI): found MH^+ , 554.1439. $\text{C}_{31}\text{H}_{29}\text{N}_3\text{O}_2^{79}\text{Br}$ requires 554.1443), m/z (CI) 556 ($\text{C}_{31}\text{H}_{29}\text{N}_3\text{O}_2^{81}\text{Br}$, 30%), 554 ($\text{C}_{31}\text{H}_{29}\text{N}_3\text{O}_2^{79}\text{Br}$, 29), 350 (22), 348 (17), 244 (27), 243 (100).

Synthesis of 5-bromo-N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole-4,7-dione 6

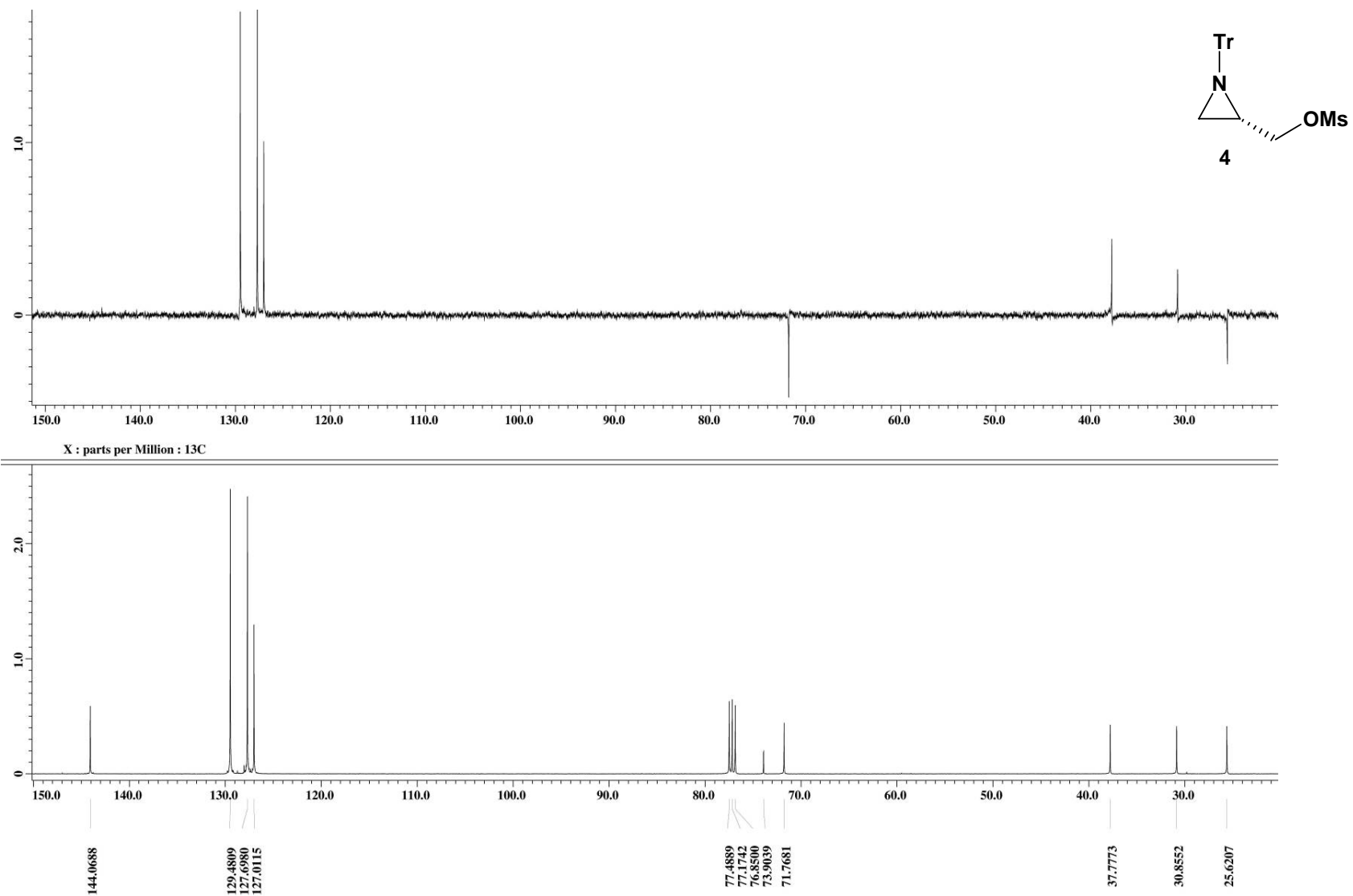
5-Bromo-4,7-dimethoxy-N-[(1-tritylaziridin-(2S)-yl)methyl]-1H-benzimidazole **8** (0.100 g, 0.18 mmol), NBS (35 mg, 0.20 mmol), concentrated H_2SO_4 (10 μL) and water (1.0 mL) in THF (3.0 mL) were stirred for 40 min at 0 °C. The mixture was basified with saturated sodium hydrogen carbonate (10.0 mL), extracted with ethyl acetate (3 x 5 mL), dried (MgSO_4) and evaporated to dryness to give a solid residue. This was purified using column chromatography with silica gel as absorbent using ethyl acetate/hexane as eluent to give the **title compound 6**, as a yellow solid (65 mg, 69%). Physical and spectroscopic data was consistent with previous experiments.

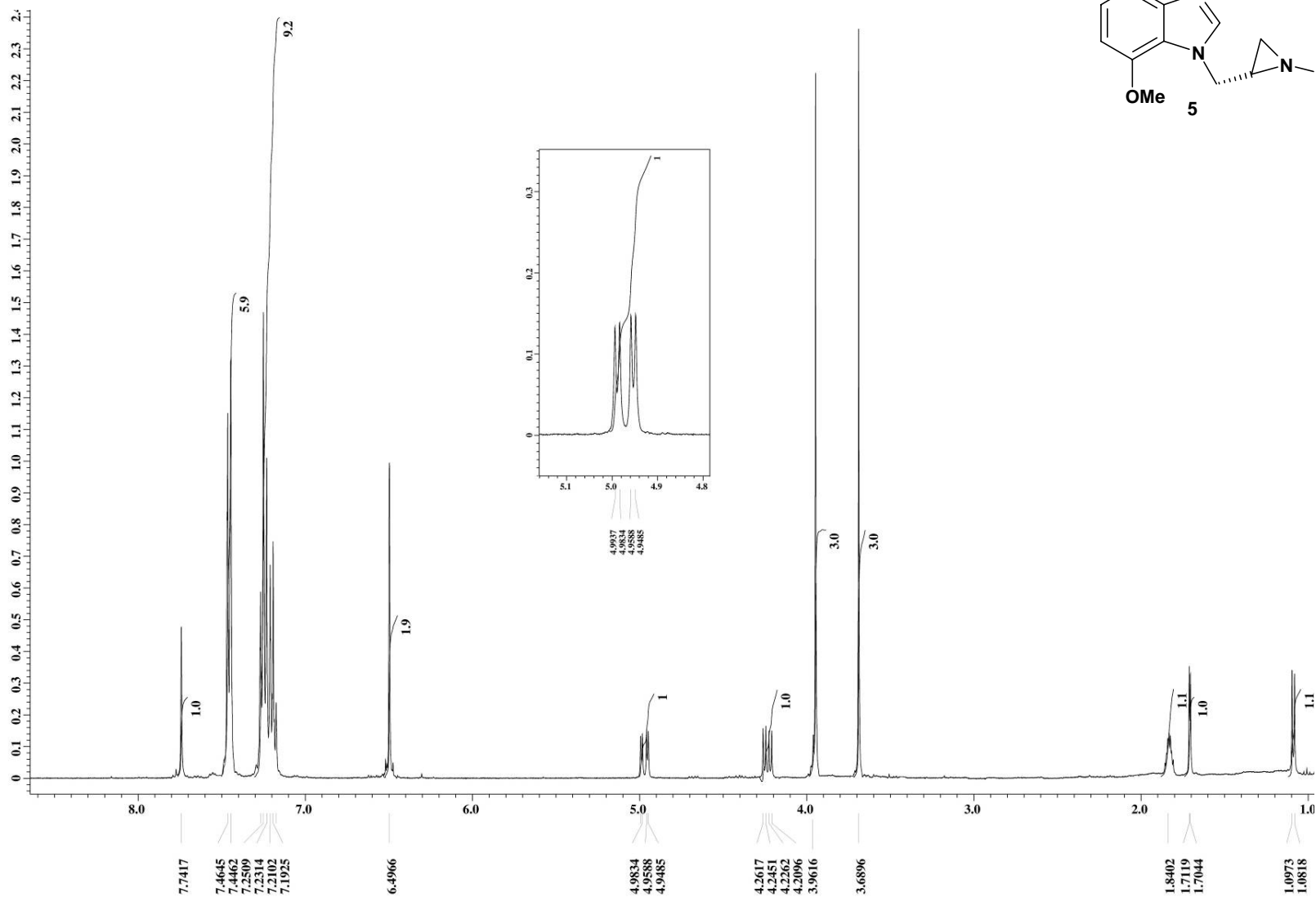
Cell culture: A SV40-transformed normal human skin fibroblast cell line (repository number GM00637) was obtained from the National Institute for General Medical Sciences (NIGMS) Human Genetic Cell Repository (Coriell Institute for Medical Research, New Jersey, USA). The PD20i and PD20:RV human fibroblast cell lines were obtained from the Oregon Health and Science University (OHSU) Fanconi Anemia Cell Repository (Portland, OR, USA). The SV40-transformed normal human skin fibroblast cell line (GM00637) was grown in minimum essential media (MEM) Eagle-Earle BSS supplemented with 15% uninactivated fetal bovine serum, (FBS), penicillin- streptomycin, 2 x essential and non-essential amino acids and vitamins, and with 2 mM L-glutamine. PD20i and PD20:RV human fibroblast cell lines were grown in minimal essential media (alpha modification) supplemented with 15% uninactivated fetal bovine serum, penicillin-streptomycin and 2 mM L-glutamine. All cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 .

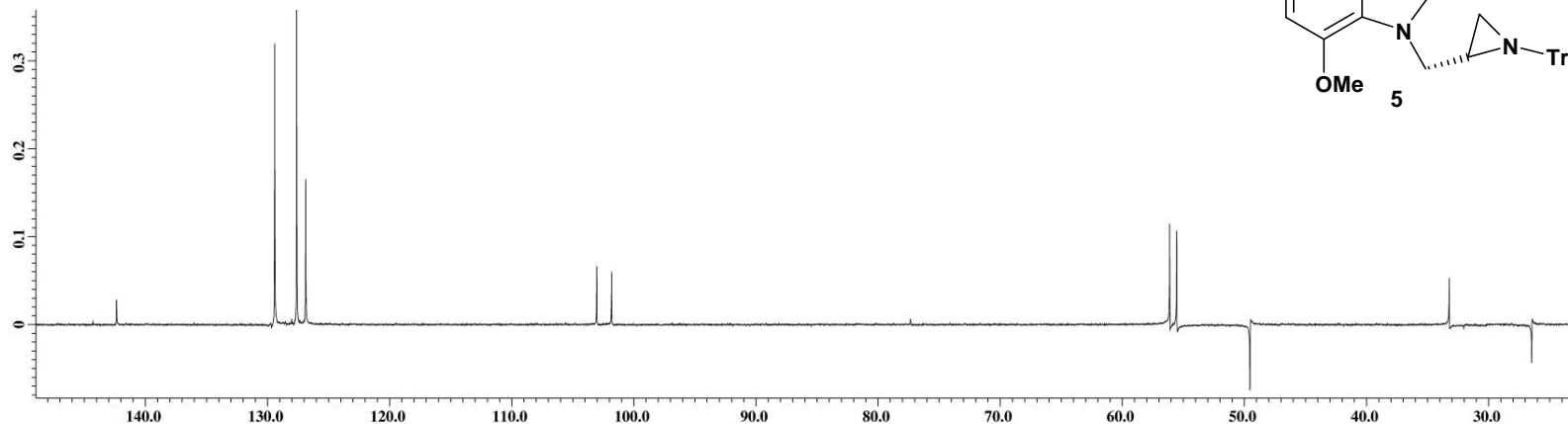
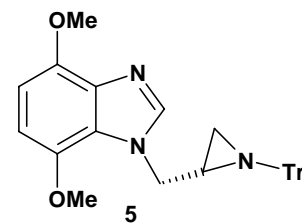
Cytotoxicity measurement: Growth inhibition (cell viability) was determined using the MTT colorimetric assay. Cells were plated into 96-well plates at a density of 10,000 cells per well (PD20i and PD20:RV cells were plated in parallel in the same plate) and allowed to adhere over a period of 48 hours. Benzimidazolequinone and mitomycin C (Sigma) solutions (5 μL) were applied in DMSO (2.5% final concentration in well), and the plates were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 hours.

Control cells were exposed to an equivalent concentration of DMSO alone. MTT (100 μg) was added and the cells were incubated for another 3 hours. The supernatant was then removed carefully by pipetting. The resultant MTT formazan

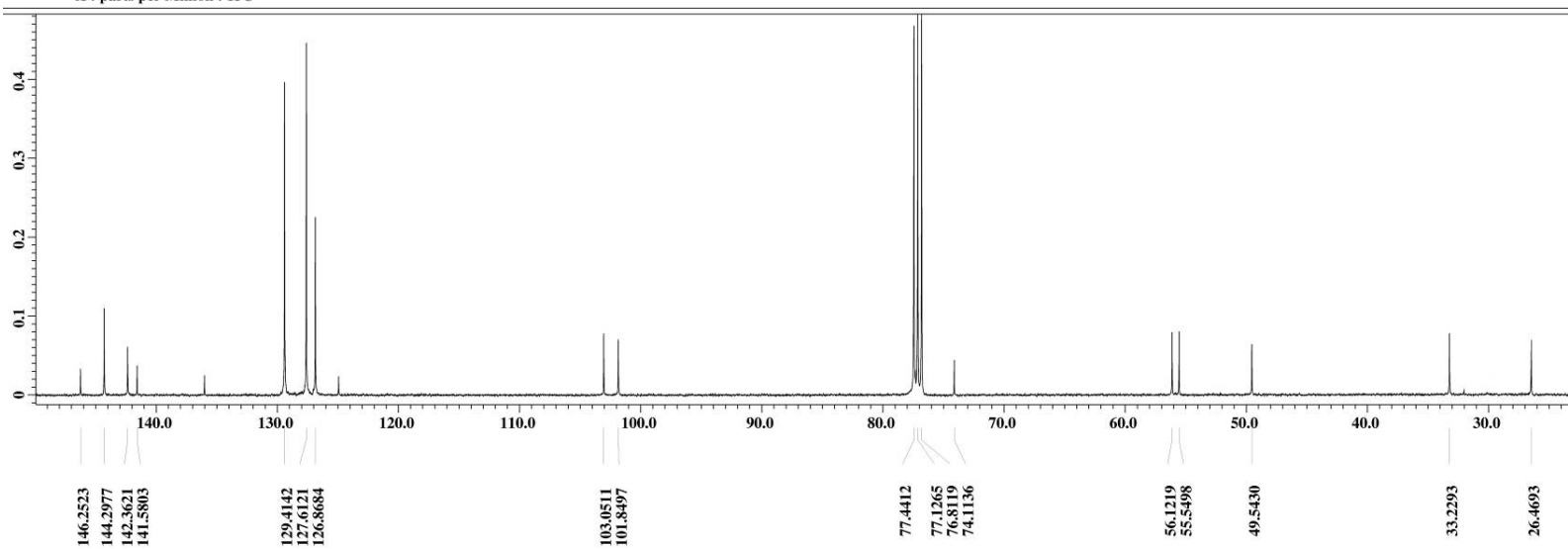
crystals were dissolved in 100 μL of DMSO and absorbance was determined on a plate reader at 550 nm with a reference at 690 nm. Cell viability is expressed as a percentage of the DMSO-only treated control value.

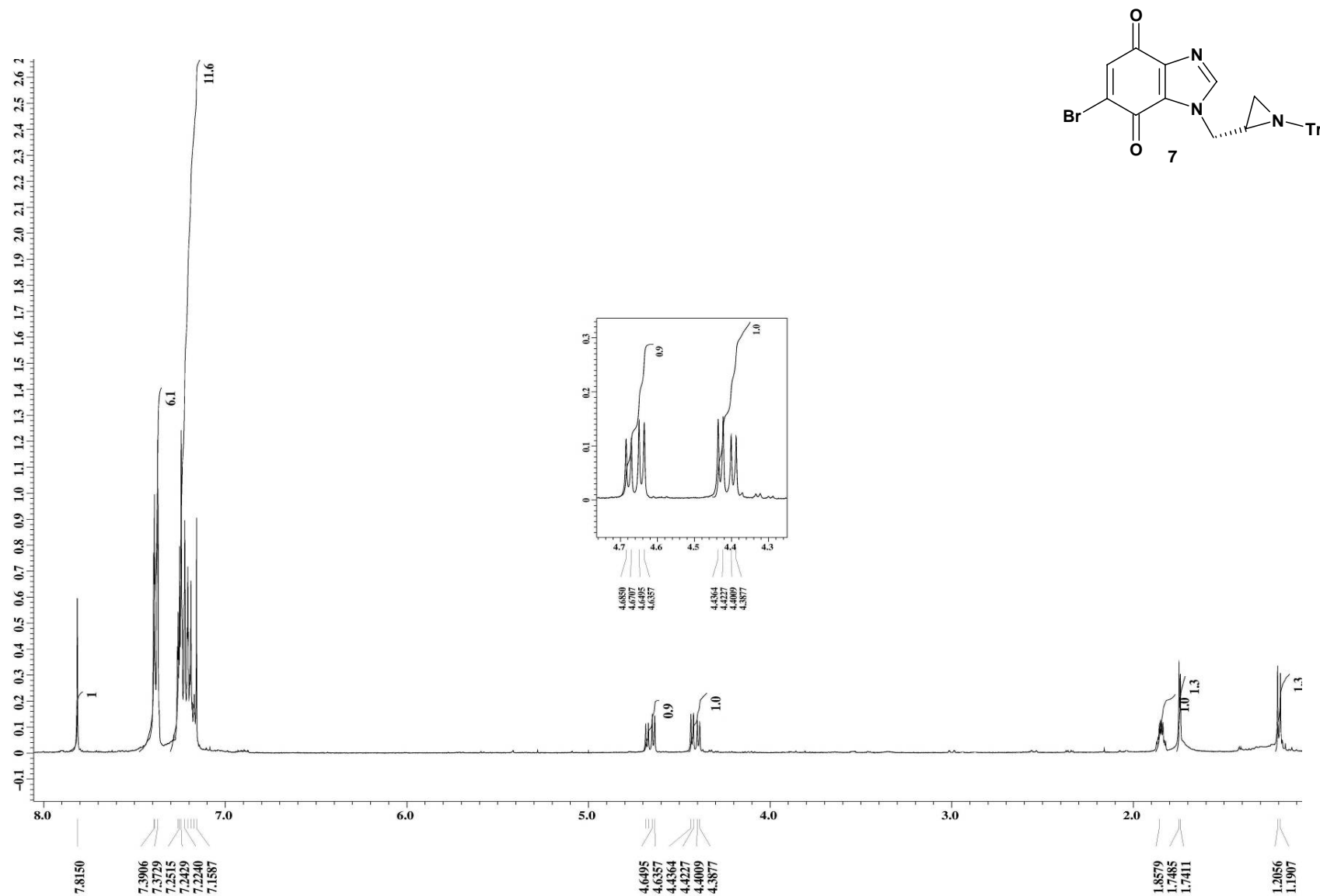


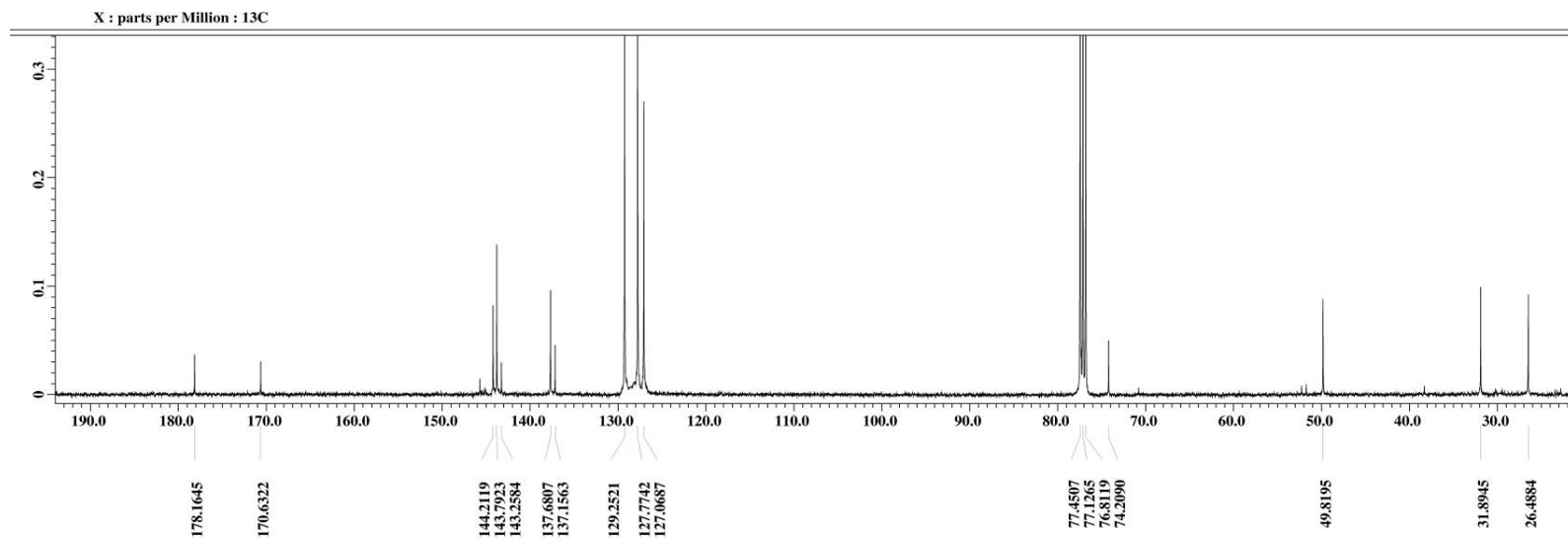
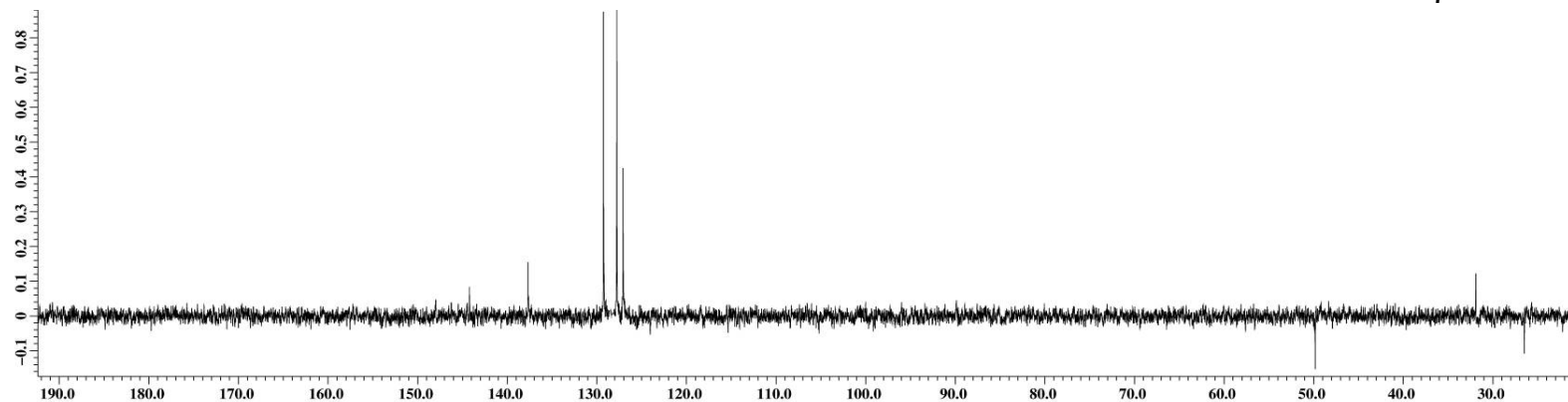
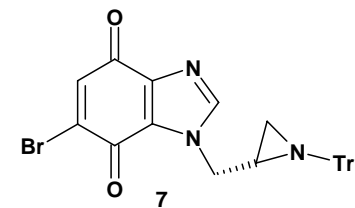


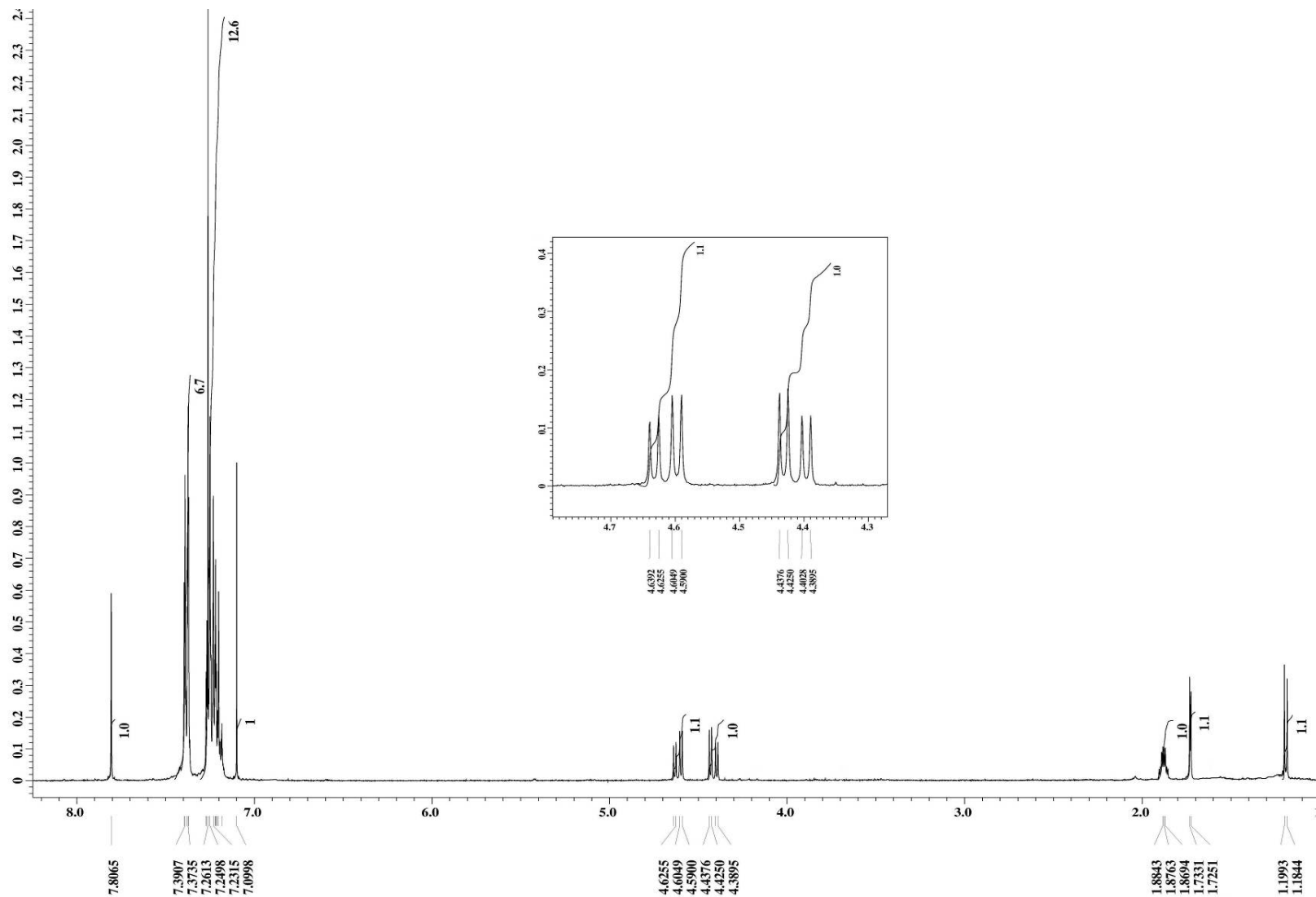
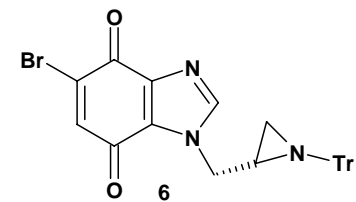


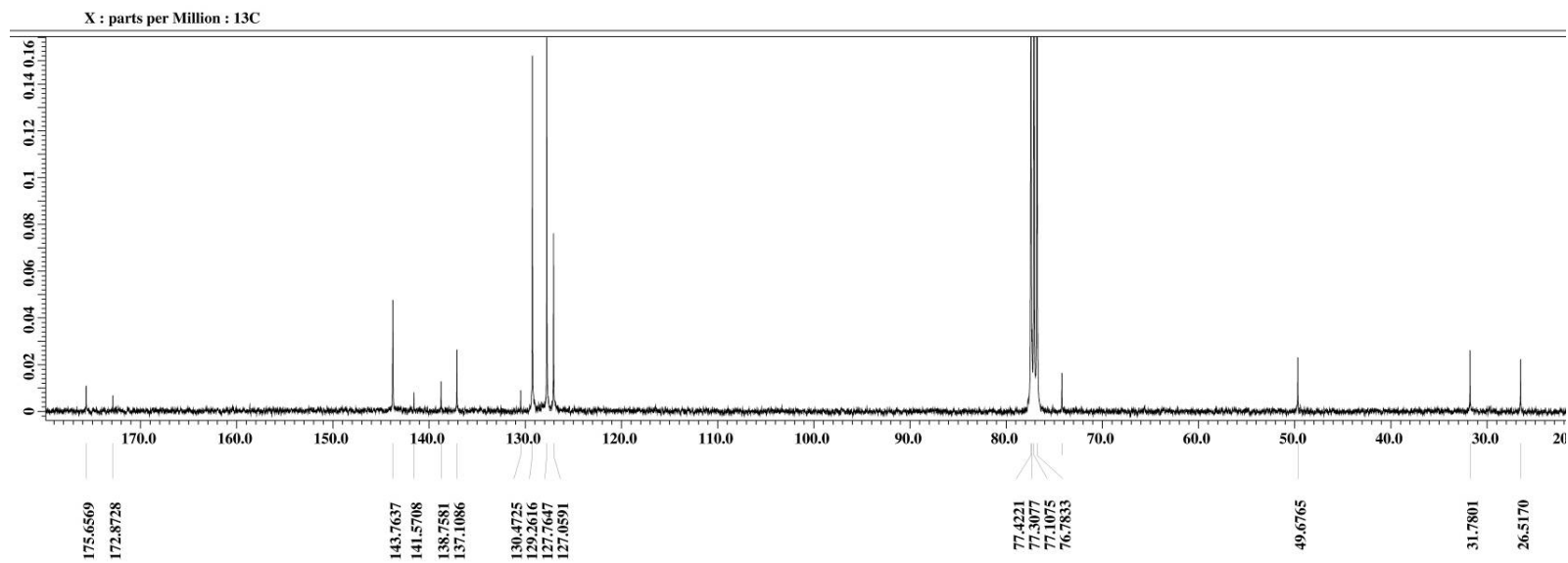
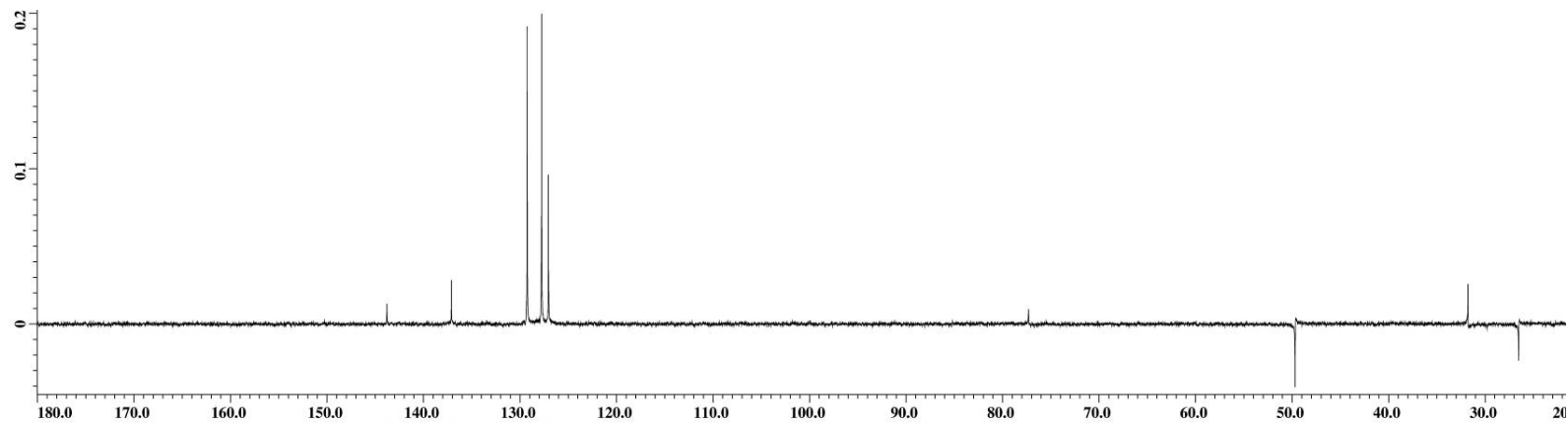
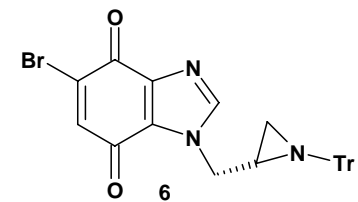
X : parts per Million : ^{13}C

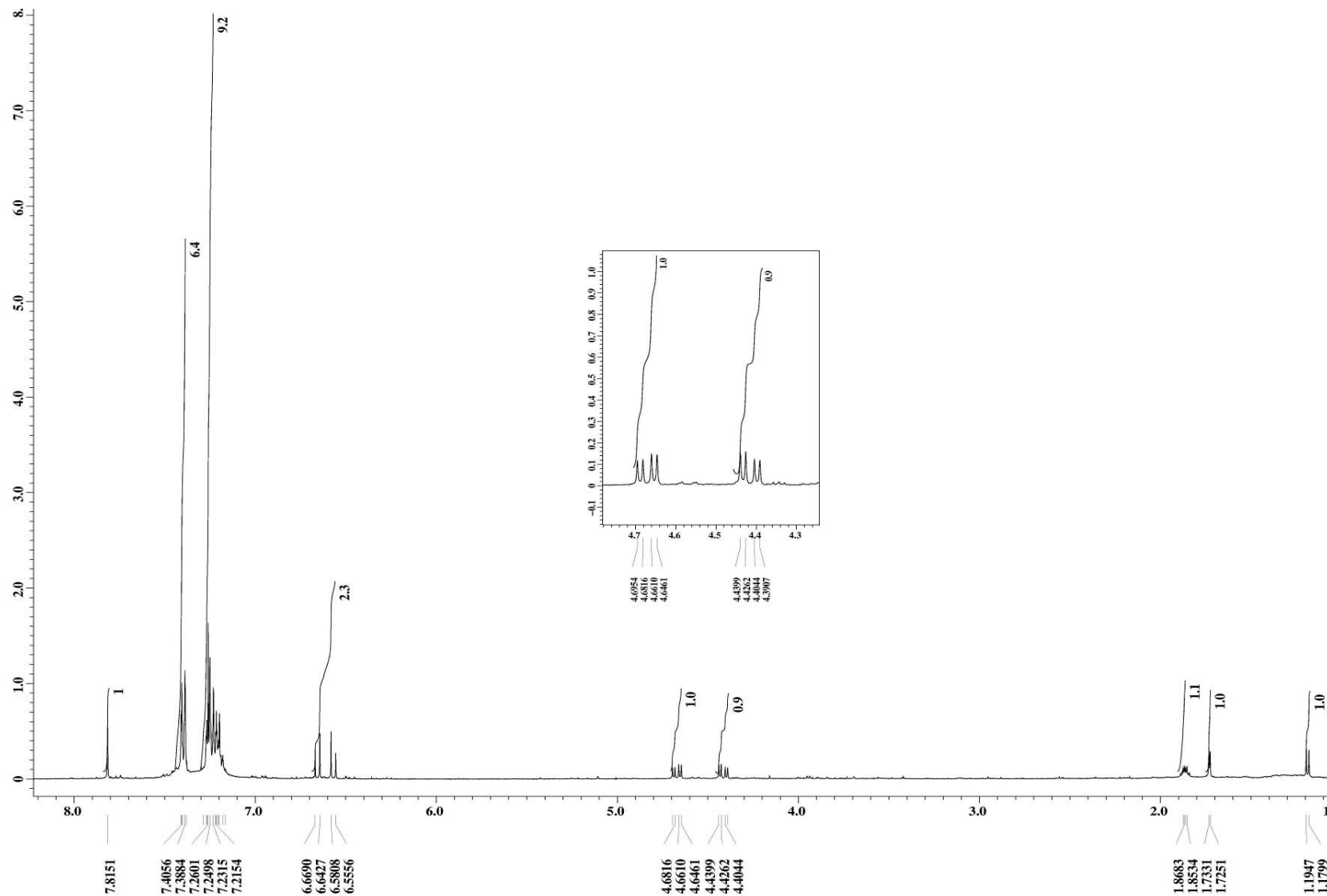
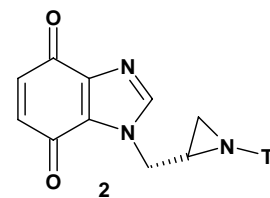


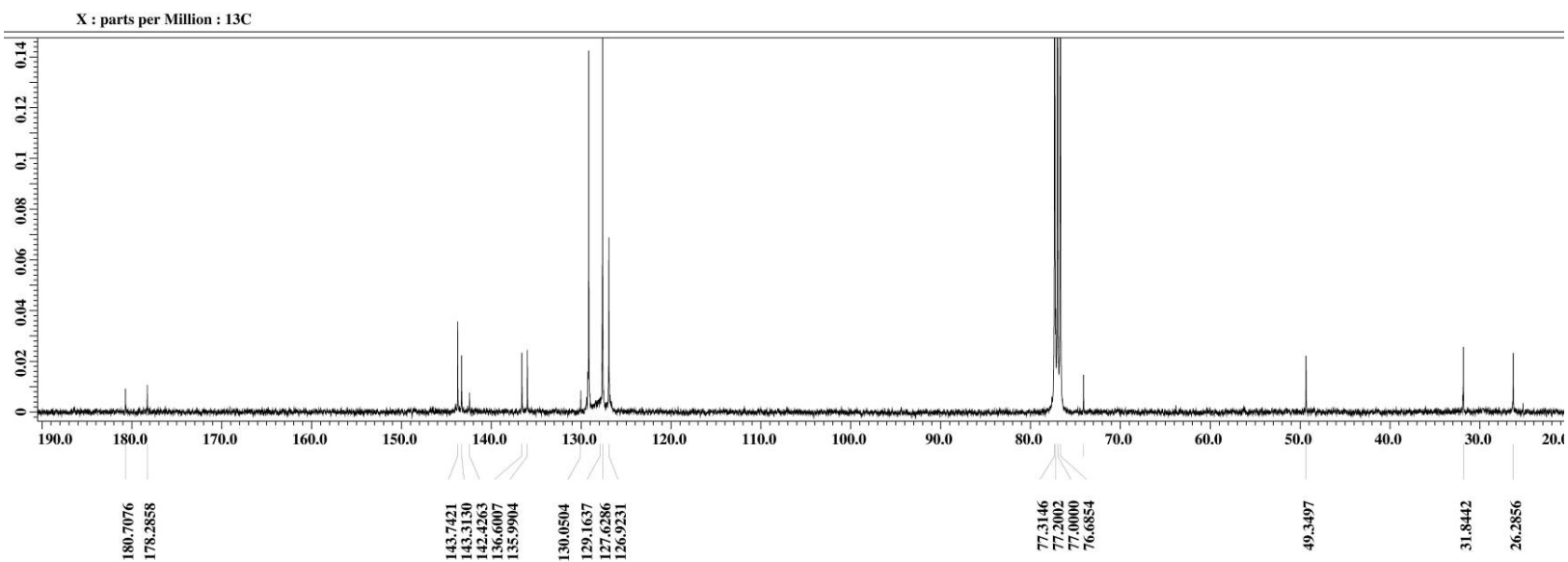
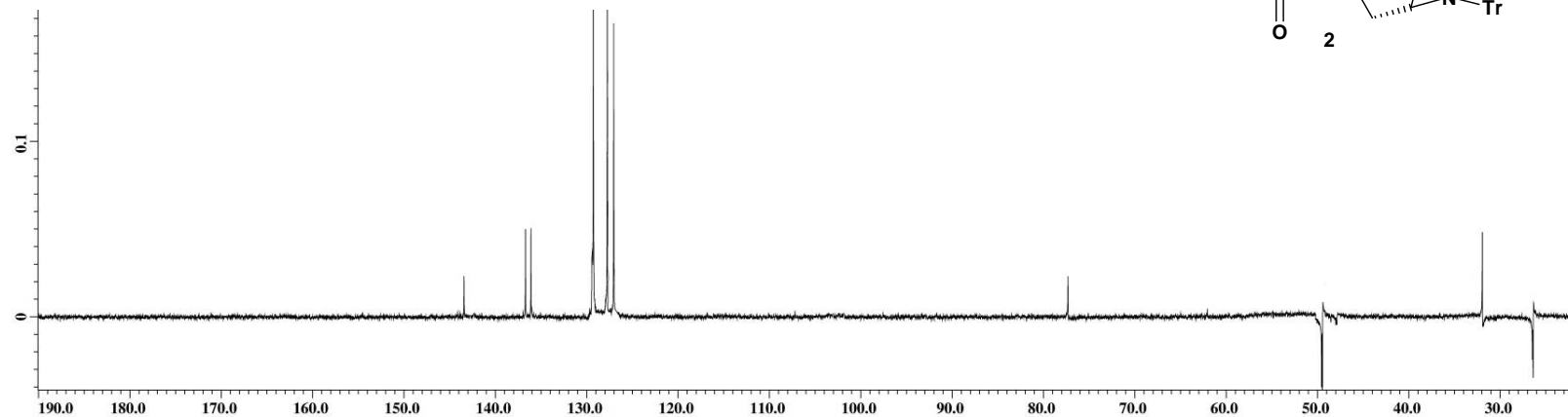
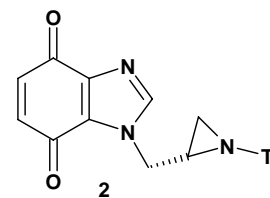


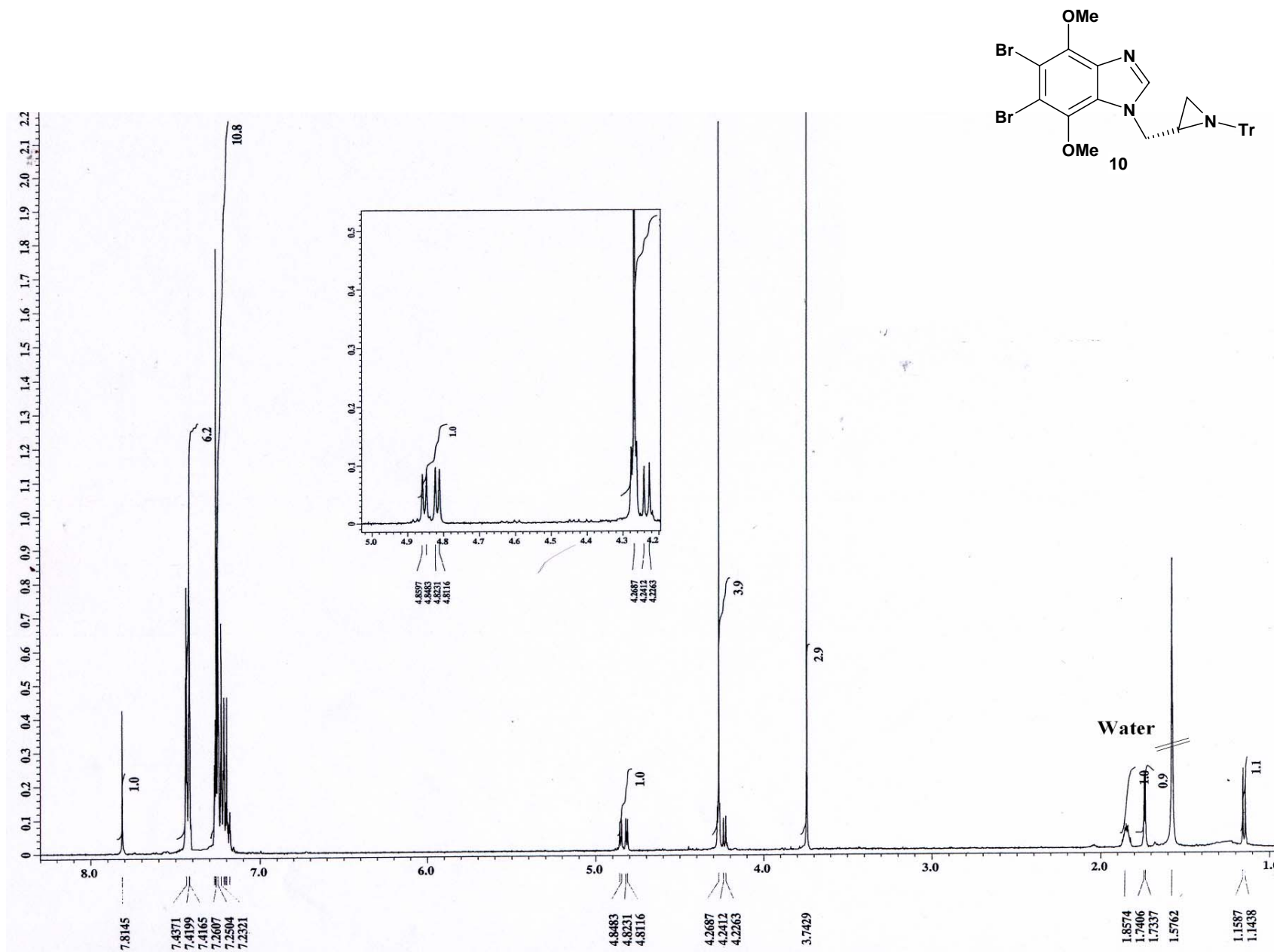


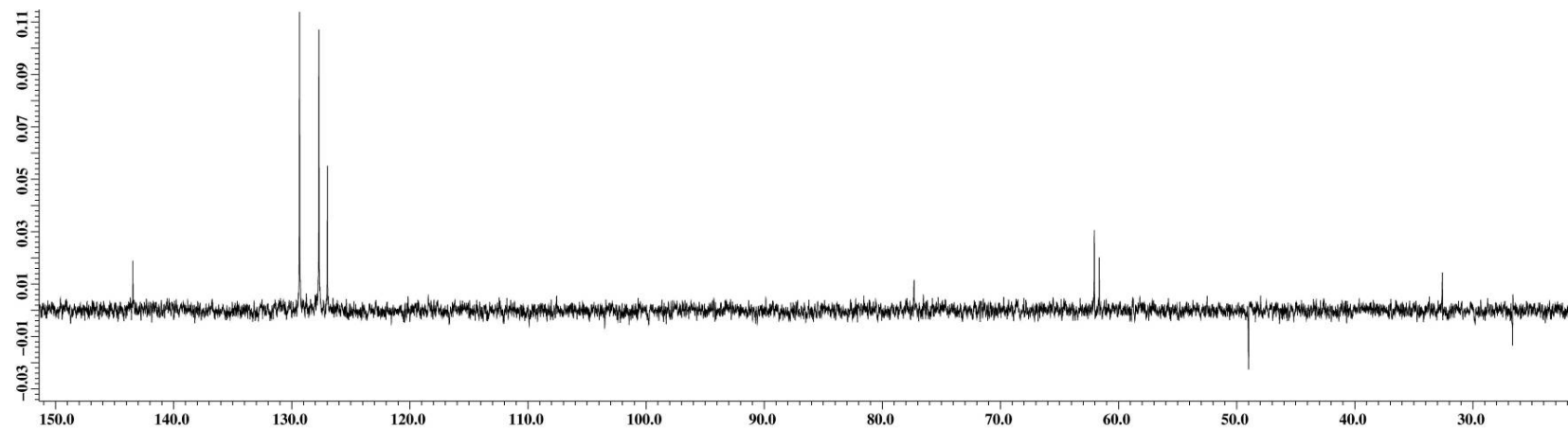
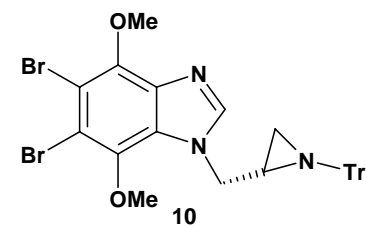




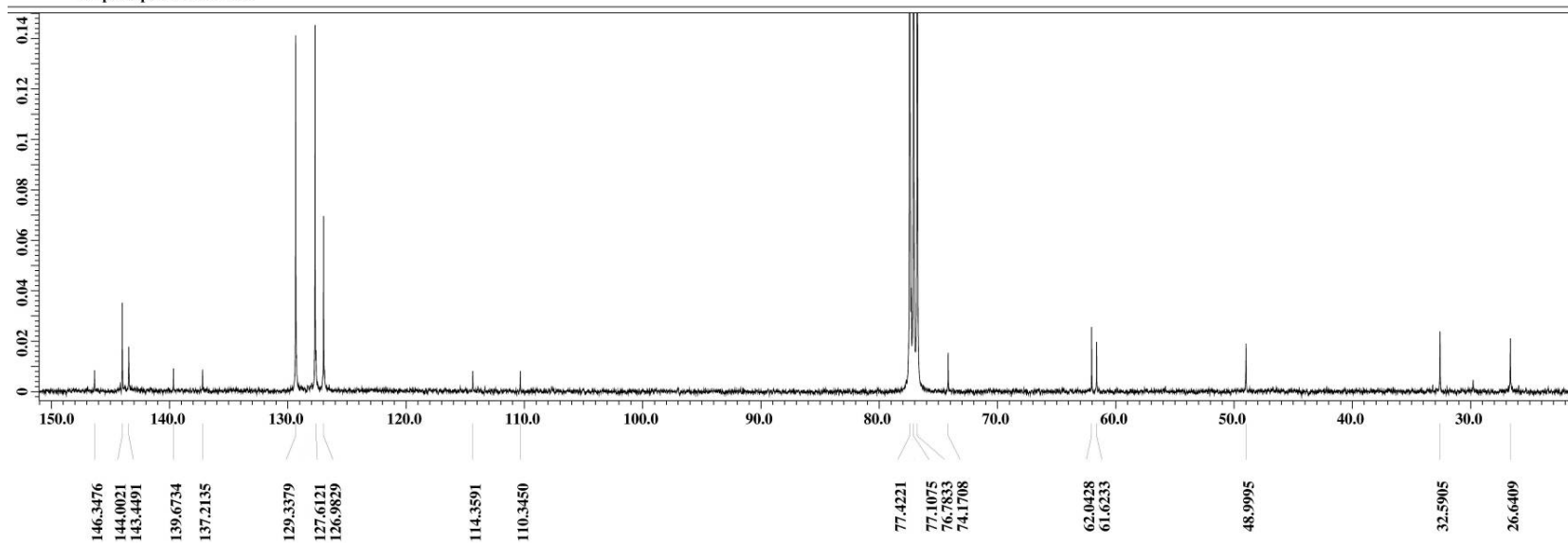


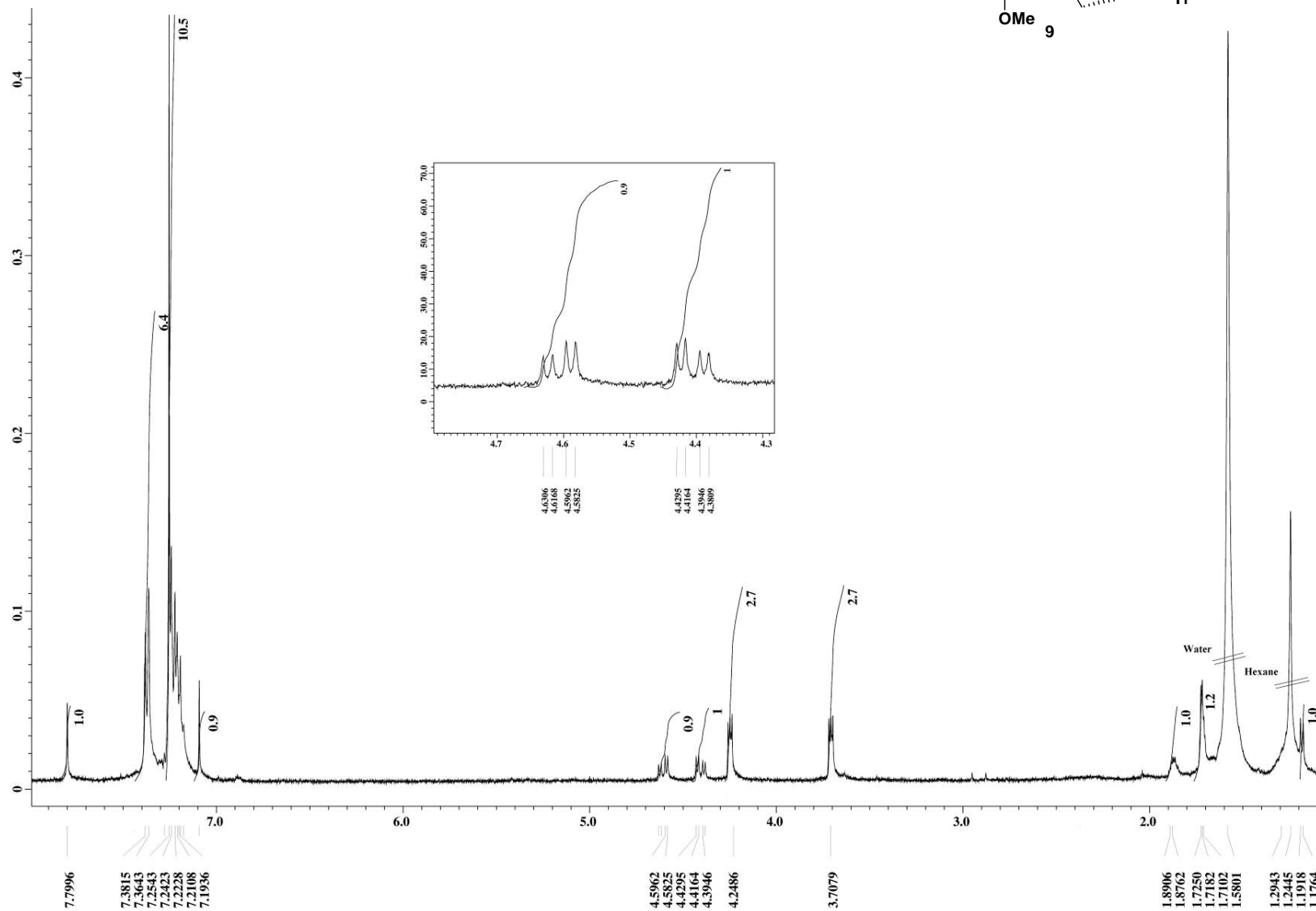
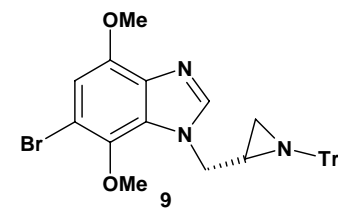


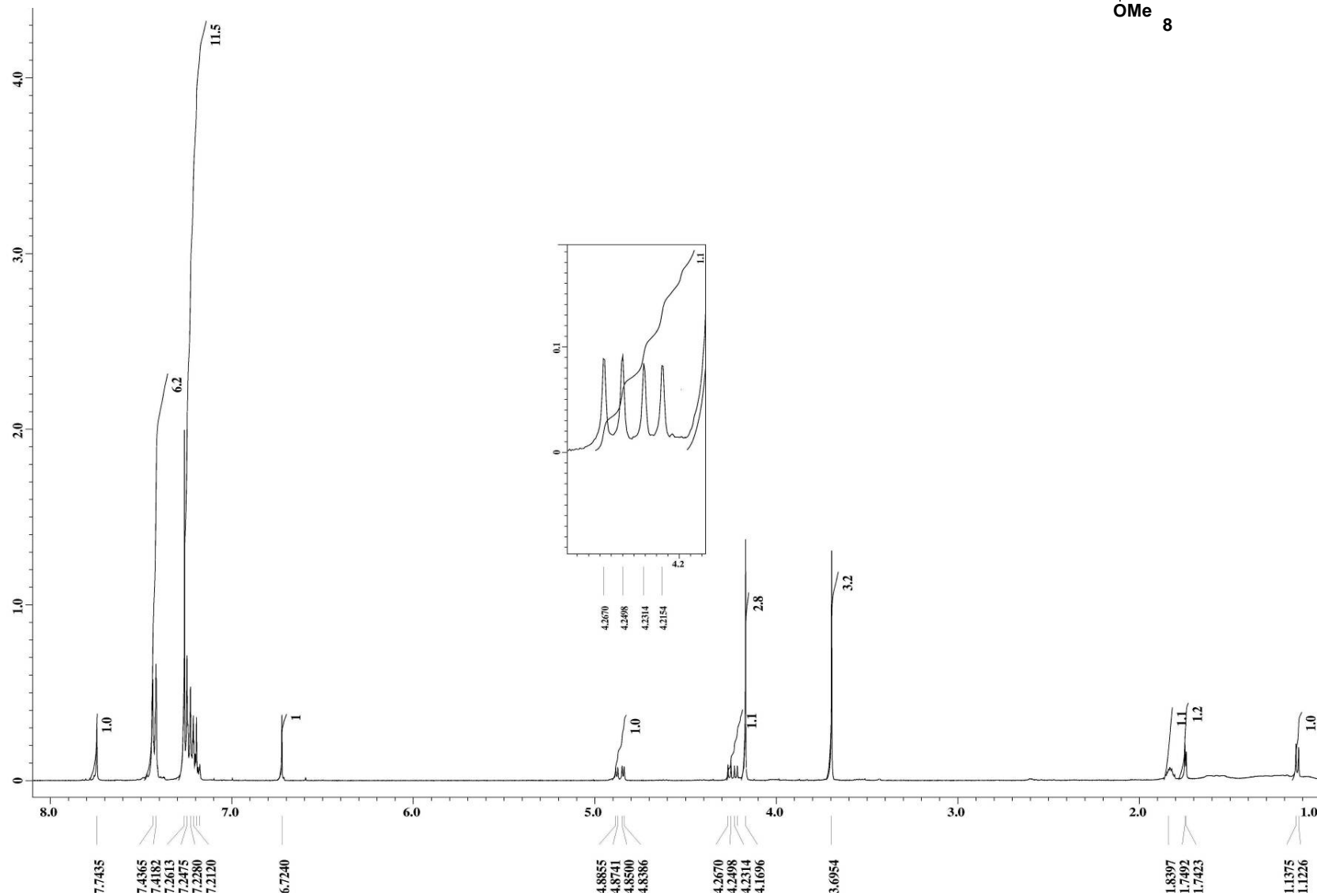
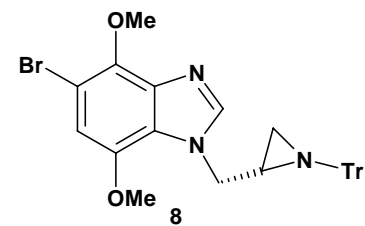


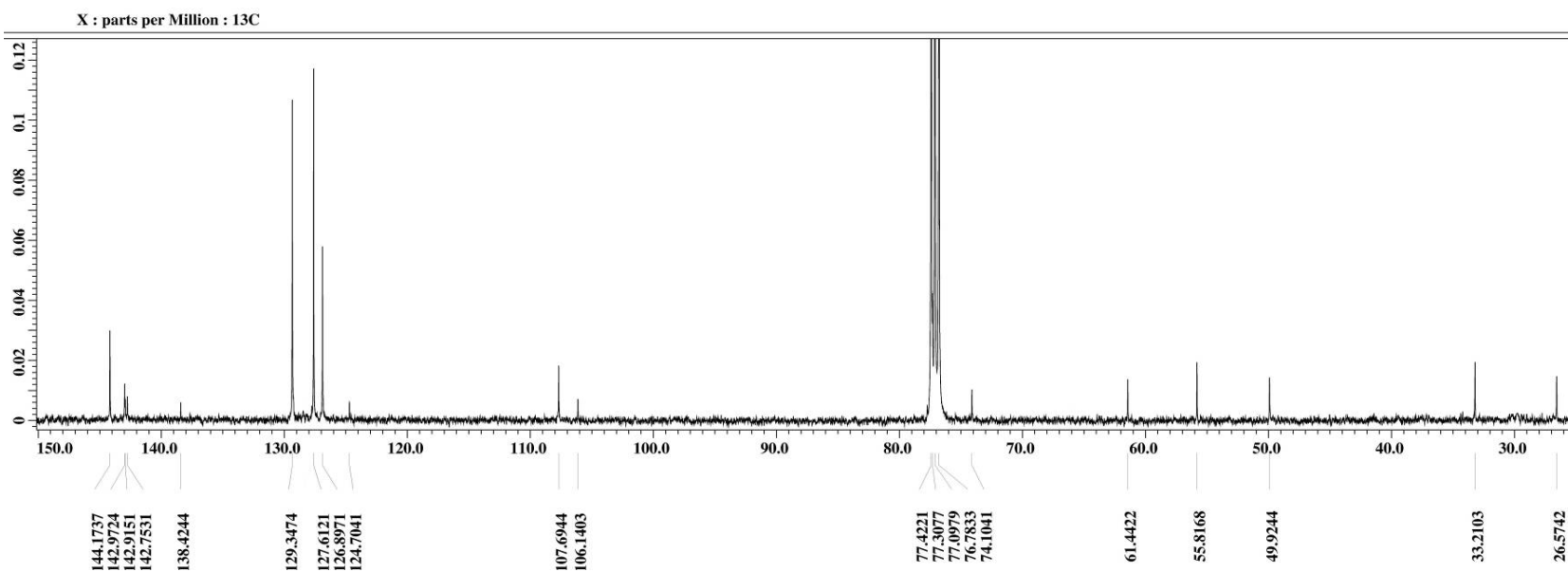
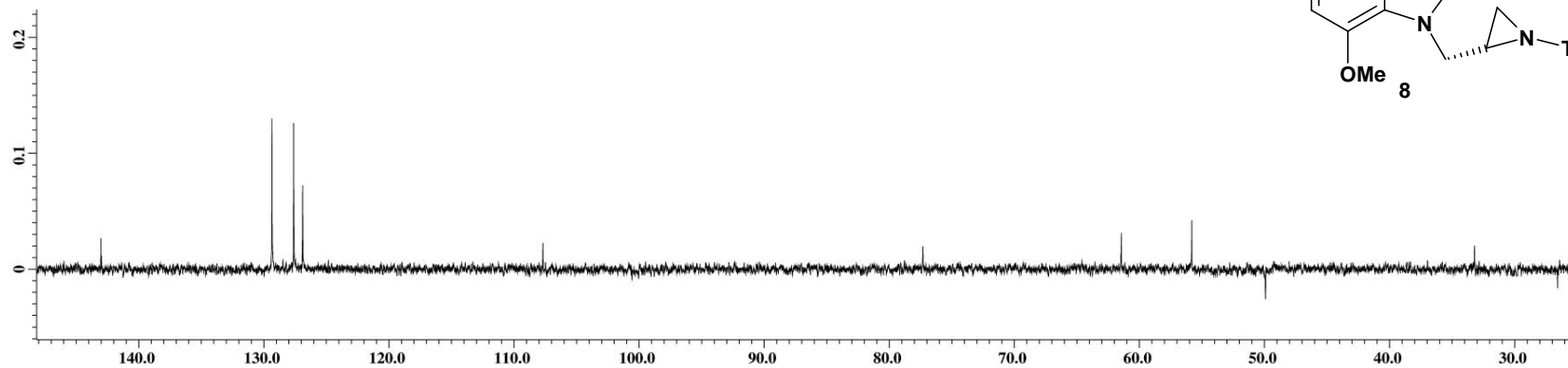
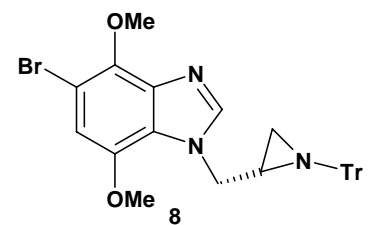


X : parts per Million : ^{13}C

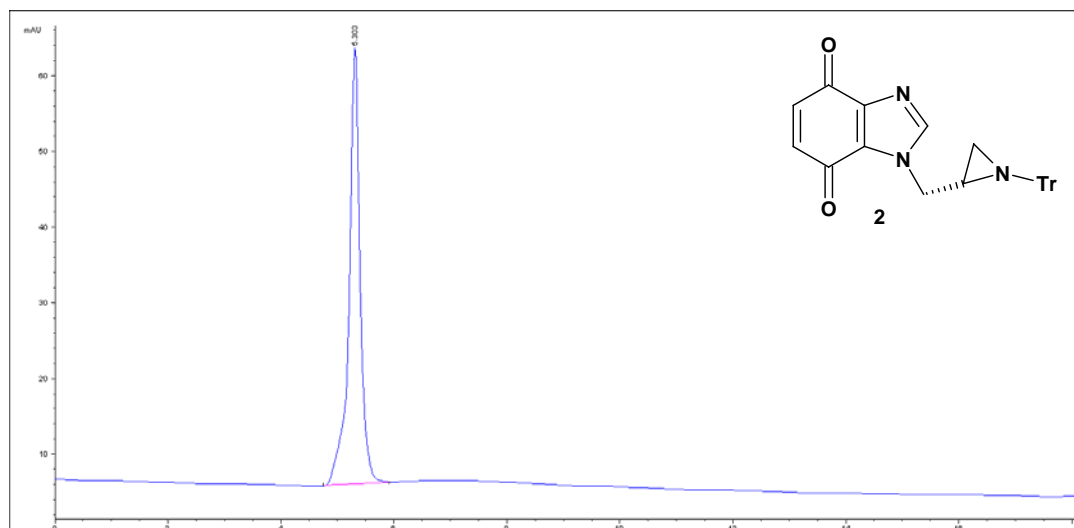








Column: Hyperclone 5u ODS (C18) 120A, 250 x 4.6 mm, eluent: diisopropyl ether/methanol (50:50); flow rate: 1 mLmin⁻¹; wavelength: 254 nm.



Index	Time (Min)	Quantity (% Area)	Height (mAU)	Area (mAU.s)	Area % (%)
1	5.303	100	57.72	784.45	100
Total		100	57.72	784.45	100