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Graphical Abstract

Total Synthesis of Calothrixins A and B via Oxidative Radical Reaction of Cyclohexenone with Aminophenanthridinedione

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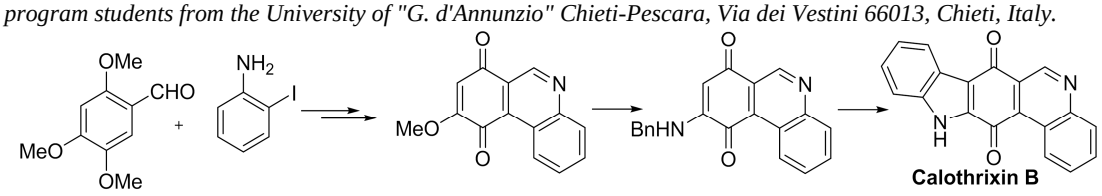

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ABSTRACT

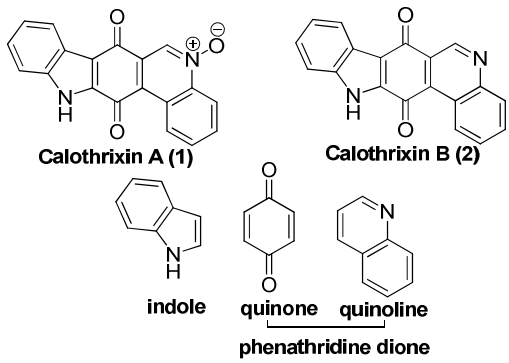
Bioactive indolo[3,2-*j*]phenanthridine alkaloids, calothrixin B and its N-oxide derivative calothrixin A have been synthesized via an oxidative free radical reaction. calothrixin B is generated from the commercially available 2,4,5-trimethoxybenzaldehyde in only 7 steps. The key step in this synthesis is the $Mn(OAc)_3$ mediated oxidative free radical reaction of 9-(benzylamino)phenanthridine -7,10-dione with cyclohexenone to form 12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione.

Introduction

Natural products have traditionally played a major role in drug discovery by serving as the source for many of the earliest medicines.[1] In spite of the scientific advancements and the promise of alternative drug discovery strategies in the recent decades , there is still a shortage of drug leads progressing into clinical trials especially in the areas of oncology, immunosuppression, and metabolic diseases.[2] Natural products continue to play a major role in providing leads particularly in these areas as shown by a recent review published by Newman and Cragg which summarizes natural product derived drugs over past 30 years (1981-2010).[3] Even though many natural products exhibit potent biological activities, their systematic biological evaluation is precluded because they are often isolated in very minute quantities. Further, natural products are known to have unique chemical structures which provide challenging synthetic targets for organic chemists. For these reasons, development of new synthetic methods for natural products remains as a very important research area.

Calothrixin B and its N-oxide derivative, calothrixin A (Fig. 1) are two bioactive metabolites isolated from the cyanobacteria *Calothrix* in 1999.[4] They are also known as indolophenanthridines because they contain the unusual pentacyclic indolo[3,2-*j*]phenanthridine ring system. This ring system consists of indole, quinone, and quinoline moieties and is unique amongst natural products (Fig. 1).

Figure 1. Calothrixin A and B and the three moieties present in them



Calothrixins possess a wide array of biological activities that are of interest to medicinal chemists. They display nanomolar antiproliferative activity against certain human cancer cell lines such as human cervical cancer cell, HeLa[4-5], CEM leukemia cells,[6] and human Jurkat cancer cells.[7] Furthermore, calothrixins A and B act as a new class of human DNA topoisomerase I poisons. They stabilize the topoisomerase I-DNA binary complex

to prevent DNA religation and cause DNA damage which results in apoptosis.[8] In addition, calothrixin A binds to DNA quadruplex to inhibit DNA replication and DNA directed RNA synthesis resulting in impaired protein synthesis and cell death.[9] They also exhibit *in vitro* antiparasitic activity against chloroquine resistant strains of *Plasmodium falciparum*, which is the causative organism for malaria.[4] These biological activities make calothrixins the potential lead compounds for anticancer and antiparasitic drug discovery. Due to their unique structural features and potent bioactivity, calothrixins are notable synthetic targets.[4-9] The first total synthesis of calothrixins was reported by Kelly, using an *o*-lithiation strategy. [10] Several other syntheses of calothrixins have also been reported exploring different synthetic strategies such as metallations,[11] hetero Diels-Alder,[12] Friedel-Crafts acylation reaction,[11a, 13] etc. A few other syntheses of calothrixins have also been reported. [14]

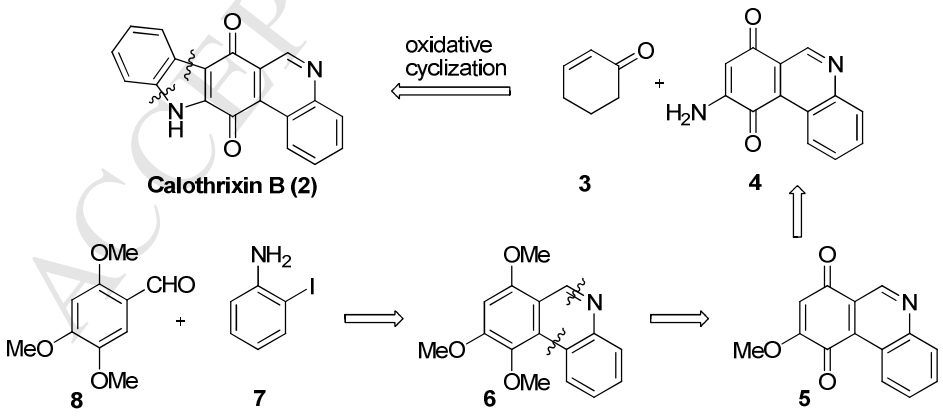
As a part of our interest in deriving lead drug molecules from natural products,[15] we have been particularly interested in developing a shorter and a better yielding synthesis of calothrixins. Majority of the reported calothrixin syntheses are focused on the formation of the middle benzoquinone ring between the indole and quinoline units as the last step. Our synthetic approach relies on the construction of the indole ring on to a phenanthridine dione via a novel oxidative free radical reaction mediated by $Mn(OAc)_3$. The method of $Mn(OAc)_3$ mediated oxidative reaction of 2-cyclohexenone with quinones was originally developed by Chuang *et al*.[16] None of the existing reports of calothrixin synthesis utilizes the late stage indole construction strategy and thus our synthetic approach is unique and different.

Research literature published in the recent three decades highlights the importance of oxidative free radical reactions mediated by transition metals. These reactions result in intermolecular / intramolecular formation of carbon-carbon bonds through transient electrophilic carbon radicals[17] and are most commonly promoted by transition metal compounds. Manganese triacetate ($Mn(OAc)_3$) and ceric (IV) ammonium nitrate (CAN) are the most commonly used and efficient catalysts for the oxidative reaction between 1,3-diketones and aminoquinones. The mechanism for this type of oxidative free radical reactions has been reported.[16, 17b, 17e] . $Mn(OAc)_3$ and CAN promoted oxidative free radical cyclizations have also been used extensively in the synthesis of naphthaquinones, which is an important skeleton of natural products. [18] The synthesis of several interesting compounds in our laboratory takes advantage of oxidative free radical reactions. Bispyrroloquinone, and bispyrroloiminoquinone ring systems were synthesized via CAN mediated oxidative free radical reaction of 1,3-dicarbonyl compounds with aminoquinones[19] while Zyzzyanones were synthesized using $Mn(OAc)_3$ mediated oxidative free radical reactions.[20] Apart from being a powerful tool to construct polycyclic ring systems, these reactions are generally high yielding and are relatively easy to perform. We report herein the synthesis of calothrixins taking advantage of an oxidative radical reaction between cyclohexenone and an aminophenanthridinedione derivative.

Results and Discussion

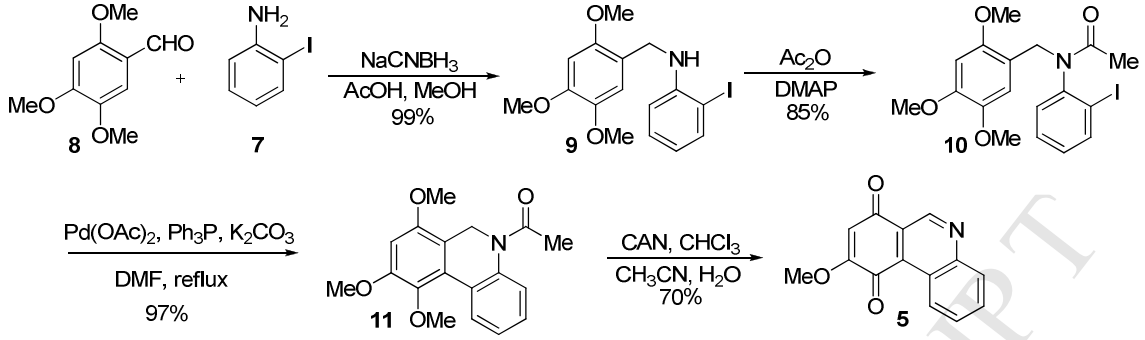
Our retrosynthetic analysis for calothrixin B utilizes an oxidative free radical reaction strategy as outlined in Scheme 1. We envisaged that the intermediate compound **6** for calothrixin synthesis could be prepared by the amination of 2,4,5-trimethoxybenzaldehyde (**8**) with *ortho*-iodoaniline (**7**) followed by a palladium catalyzed coupling. Oxidation of compound **6** with CAN can afford the corresponding methoxy quinone **5**, which could be aminated to form the aminoquinone **4**. Oxidative free radical reaction of the aminoquinone **4** with cyclohexenone (**3**) in the presence of $Mn(OAc)_3$ could give calothrixin B, which could be further oxidized with *m*-CPBA to form calothrixin A. Construction of the indole ring to the phenanthridine dione using cyclohexenone and $Mn(OAc)_3$ is the novel aspect of our calothrixin synthesis.

Scheme 1. Retrosynthetic analysis of calothrixin B



Our investigation started with the synthesis of the key intermediate compound **5** as outlined in Scheme 2. Reductive amination of commercially available 2,4,5-trimethoxybenzaldehyde (**8**) with *ortho*-iodoaniline (**7**) in the presence of $NaCNBH_3$ in a mixture of MeOH and acetic acid gave the compound **9** in 99% yield. In order to avoid potential complications, the NH group present in compound **9** was protected as an acetyl amide. This was achieved by the treatment of compound **9** with Ac_2O in the presence of catalytic amount of DMAP at room temperature for 15 hours to form compound **10** in 85% yield. Then the acetyl protected compound **10** was treated with $Pd(OAc)_2$, PPh_3, and K_2CO_3 in DMF at reflux condition for 7 hours to generate the cyclized product **11** in 97% yield.

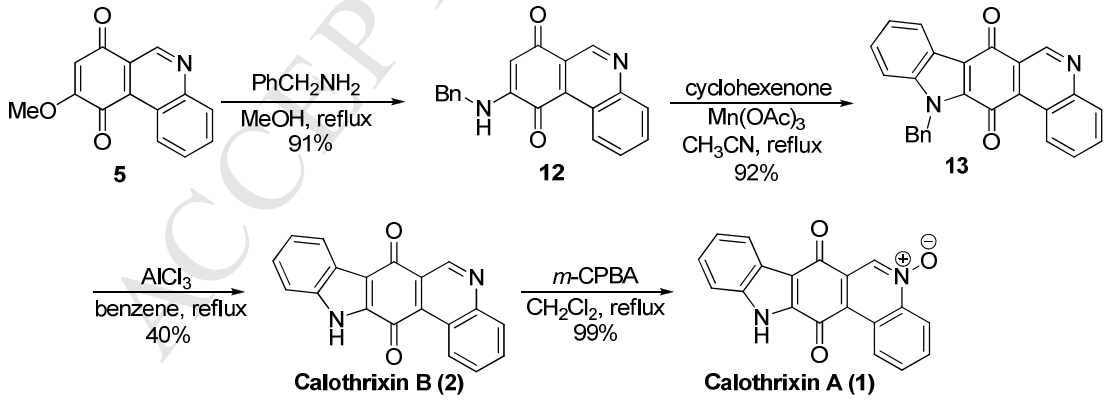
Scheme 2. Synthesis of intermediate quinone **5**



Complications occurred with the assignment of CH_2 proton signal within the ^{1}H-NMR spectra of compound **11** as seen by the broad multiplet ranging from 4.2 to 5.5 ppm. These protons were almost invisible in the spectra. This might be due to the fact that the molecule exists as a pair of amide rotamers that are in dynamic equilibrium. In order to confirm the structure of compound **11** unambiguously, we recorded a ^{1}H-NMR in DMSO-d_6 at 90 ºC. The CH_2 proton signal was clearly visible as a sharp singlet in the high temperature NMR, which confirms the structure of compound **11**. This might be due to the faster equilibration of the amide rotamers at higher temperature. Compound **11** was then oxidized using CAN in a mixture of CH_3CN, chloroform and water to afford the quinone **5** in 70% yield. Surprisingly, the deacetylation and aromatization of the N containing ring also occurred under the same reaction conditions.

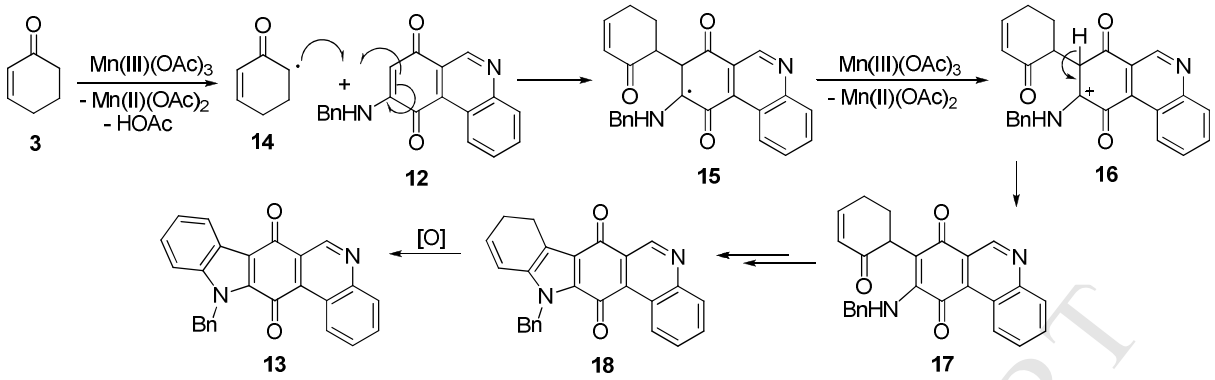
Conversion of quinone **5** to calothrixins A and B is outlined in Scheme 3. In order to construct the indole ring on to the quinone **5** by oxidative free radical reaction we needed to substitute the methoxy group in **5** with an amino functionality. We chose a benzyl amino group in this case as the benzyl group could serve as a protecting group and could be removed at the end of the synthesis. Reaction of the quinone **5** with benzyl amine in anhydrous MeOH under reflux conditions resulted in the formation of benzylamino phenanthridine dione **12** in 91% yield. Then the oxidative free radical reaction was performed by refluxing the mixture of benzylamino phenanthridine dione **12** and cyclohexenone in the presence of $Mn(OAc)_3$ in anhydrous CH_3CN for three days to afford compound **13** in 92% yield. Finally, the debenzylation of compound **13** was carried out by following a previously reported literature procedure using $AlCl_3$ and anhydrous benzene to furnish calothrixin B (**2**) in 40 % yield.[12b] In order to improve the yield of this step, we attempted other debenzylation reaction conditions such as H_2 in the presence of Pd/C or Pd black/$HCOONH_4$. But, these reactions resulted in lower yields than what we obtained with $AlCl_3$. Calothrixin B (**2**) was oxidized to calothrixin A (**1**) in 99 % yield using *m*-CPBA in DCM following the literature procedure.[10]

Scheme 3. Conversion of quinone **5** to calothrixin A and B



Based on the previous literature reports on the mechanism of oxidative free radical reactions,[16, 17e, 21] a plausible mechanism for the conversion of compound **12** to **13** is outlined in Scheme 4. Initiation of the reaction occurs with the interaction of $Mn(OAc)_3$ with 2-cyclohexenone to generate the radical **14** by a one-electron oxidation. Intermolecular addition of radical **14** to the quinone **12** generates another radical **15**. Oxidation of **15** by another molecule of $Mn(OAc)_3$ produces the corresponding cation **16**, which is deprotonated to form the intermediate **17**. Intramolecular condensation of **17** forms the compound **18** which up on aromatization yields the product **13**.

Scheme 4. Mechanism of oxidative radical reaction of 2-cyclohexenone with aminophenanthridinedione.



Conclusions

In summary, we have described a new and efficient synthesis of calothrixin A and B with good overall yields. Synthesis of calothrixin B was achieved from 2,4,5-trimethoxybenzaldehyde in only 7 steps. This novel synthetic strategy involves the construction of an indole ring on to a phenanthridine-7,10-dione to form the calothrixin core. The key step in this synthesis is $Mn(OAc)_3$ mediated oxidative free radical reaction of 9-(benzylamino)phenanthridine -7,10-dione to form 12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione in excellent yield.

Experimental Section

General Methods for Synthesis: Solvent evaporations were carried out *in vacuo* using a rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Dynamic Adsorbents, Inc., Aluminum backed TLC, 20 X 20 cm F-254, 200 μm). Spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using silica gel (32-63 μm) from Dynamic Absorbent in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. The NMR spectra were recorded on Bruker DPX 300 spectrometer. Chemical shifts are reported in ppm relative to TMS or $CDCl_3$ as internal standard. The values of chemical shifts (δ) and coupling constants *J* were given in parts per million and in Hz, respectively. Mass spectra were recorded on a MicroMass Platform LCC instrument. HRMS were obtained on a Waters AutoSpec-UltimaTM NT mass spectrometer with an EI source. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich chemical company. Other reagents were purchased from Aldrich, Lancaster or Fisher chemical companies and used as received.

***N*-(2,4,5-trimethoxybenzyl)-2-iodobenzenamine (9):** To a stirred solution of *o*-iodoaniline **7** (3.85 g, 17.5 mmol) in MeOH (50 mL), 2,4,5-trimethoxybenzaldehyde **8** (3.45 g, 17.5 mmol) and a solution of acetic acid (1.58 g, 26.3 mmol) in MeOH (10 mL) were added and the reaction mixture was stirred at rt under N_2 atm for 30 min. The reaction mixture was cooled to 0 ^oC using an ice bath and $NaCNBH_3$ (1.44 g, 22.8 mmol) was added in 4 portions over a period of 15 min and the solution was stirred for another 20 min at 0 ^oC. The reaction mixture was then stirred at rt for 1 h. TLC examination (30% EtOAc in hexanes) revealed that the reaction was complete. Reaction mixture was then quenched with sat. $NaHCO_3$ (100 mL) and the solvent was removed to obtain a brown residue. The residue was diluted with water (30 mL) and filtrated to afford the pure *N*-(2,4,5-trimethoxybenzyl)-2-iodobenzenamine **9** (6.95 g, 99%); Mp: 84-86 ^oC; ^1H NMR ($CDCl_3$) δ 3.80 (s, 3H), 3.87 (s, 3H), 3.90 (s, 3H), 4.32 (d, J = 5.5 Hz, 2H), 4.63 (t, J = 5.5 Hz, 1H), 6.43 (t, J = 7.6 Hz, 1H), 6.56 (s, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.86 (s, 1H), 7.17 (t, J = 7.6 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 43.6, 56.5 (2C), 57.0, 85.8, 97.9, 111.5, 113.5, 118.3, 119.0, 129.6, 139.2, 143.3, 147.7, 149.2, 151.9; MS (ES+) *m/z* 400 (M + H) and HRMS calcd for $C_{16}H_{18}INO_3$ 399.0331, found 399.0343.

***N*-(2,4,5-trimethoxybenzyl)-*N*-(2-iodophenyl)acetamide (10):** To a stirred solution of *N*-(2,4,5-trimethoxybenzyl)-2-iodobenzenamine **9** (6.42 g, 16.1 mmol) in acetic anhydride (35 mL), a cat amount of DMAP (196 mg, 1.6 mmol) was added and the reaction mixture was stirred at rt under N_2 atm for 15 h. TLC examination (50% EtOAc in hexanes) revealed that the reaction was complete. The solvent was removed and the residue was dissolved in EtOAc (400 mL), which was washed with water (3 × 100 mL) and brine (1 × 100 mL) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated on a rotary evaporator to afford the pure *N*-(2,4,5-trimethoxybenzyl)-*N*-(2-iodophenyl)acetamide **10** (6.04 g, 85%); Mp: 126-127 ^oC; ^1H NMR ($CDCl_3$) δ 1.79 (s, 3H), 3.41 (s, 3H), 3.79 (s, 3H), 3.83 (s, 3H), 4.36 (d, J = 13.8 Hz, 1H), 5.28 (d, J = 13.8 Hz, 1H), 6.33 (s, 1H), 6.73 (d, J = 7.8 Hz, 1H), 6.91 (s, 1H), 6.98 (t, J = 7.2 Hz, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 23.1, 44.9, 56.2 (2C), 56.7, 97.2, 100.6, 115.3, 116.6, 129.0, 129.5, 130.6, 139.8, 143.1, 144.8, 149.3, 152.4, 170.1; MS (ES^+) *m/z* 442 (M + H) and HRMS calcd for $C_{18}H_{20}INO_4$ 441.0437, found 441.0432.

1-(7,9,10-trimethoxyphenanthridin-5(6*H*)-yl)ethanone (11): To a stirred solution of *N*-(2,4,5-trimethoxybenzyl)-*N*-(2-iodophenyl)acetamide **10** (300 mg, 0.68 mmol) in anhyd DMF (5 mL), PPh_3 (52 mg, 0.20 mmol), anhyd K_2CO_3 (136 mg, 0.99 mmol) and $Pd(OAc)_2$ (15 mg, 0.06 mmol) were added and the reaction mixture was refluxed under N_2 atm for 7 h. TLC examination (50% EtOAc in hexanes) revealed that the reaction was complete. The reaction mixture was cooled to rt and filtered through celite and washed with EtOAc (3 × 50 mL). The combined filtrates were washed with water (3 × 50 mL) and brine (1 × 50 mL) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated to afford the crude product, which was purified by column chromatography over Si gel (20 × 2 cm) using EtOAc / hexanes (1:3) as eluent to afford the pure 1-(7,9,10-trimethoxyphenanthridin-5(6*H*)-yl)ethanone

11 (210 mg, 97%); Mp: 173-175 °C; ^{1}H NMR ($CDCl_3$) δ 2.15-2.39 (m, 3H), 3.68 (s, 3H), 3.84 (s, 3H), 3.90 (s, 3H) 4.14-5.52 (brs, 2H), 6.50 (s, 1H), 7.12-7.34 (m, 3H), 8.38-8.50 (m, 1H); ^{1}H NMR (90 °C, DMSO-d_6) δ 2.15 (s, 3H), 3.71 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 4.72 (s, 2H), 6.83 (s, 1H), 7.31-7.43 (m, 2H), 7.53 (d, J = 7.7 Hz, 1H), 8.35 (d, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 22.2, 39.1, 56.1, 56.3, 60.5, 97.0, 117.9, 124.5, 125.7, 126.0, 127.7, 128.3, 128.7, 138.7, 140.8, 151.4, 152.9, 169.0; MS (ES+) *m/z* 314 (M + H) and HRMS calcd for $C_{18}H_{19}NO_4$ 313.1314, found 313.1315.

9-Methoxyphenanthridine-7,10-dione (5): To a stirred solution of 1-(7,9,10-trimethoxyphenanthridin-5(6H)-yl)ethanone **11** (400 mg, 1.3 mmol) in a mixture of CH_3CN (100 mL) and $CHCl_3$ (5mL), a solution of CAN (2.79 g, 5.1 mmol) in water (100 mL) was added. The reaction mixture was stirred at rt for 7 h. TLC examination (50% EtOAc in hexanes) revealed that the reaction was complete. CH_3CN was removed under reduced pressure and the residue obtained was extracted with $CHCl_3$ (4 × 100 mL). The combined organic layers were washed with water (3 × 100 mL), brine (2 × 100 mL) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated to obtain the crude product, which was purified by column chromatography over Si gel (20 × 2 cm) using $CHCl_3$ as eluent to afford the pure 9-methoxyphenanthridine-7,10-dione **5** (214 mg, 70%); Mp: 243-245 °C; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H), 6.20 (s, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.89 (t, J = 7.2 Hz, 1H), 8.21 (d, J = 8.7 Hz, 1H), 9.41 (d, J = 8.4 Hz, 1H), 9.66 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.0, 107.9, 122.3, 122.9, 127.5, 130.7, 131.0, 131.1, 132.1, 147.8, 152.1, 160.7, 183.0, 185.1; MS (ES+) *m/z* 240 (M + H) and HRMS calcd for $C_{14}H_9NO_3$ 239.0582, found 239.0592.

9-(Benzylamino)phenanthridine-7,10-dione (12): To a stirred solution of 9-methoxyphenanthridine-7,10-dione **5** (200 mg, 0.83 mmol) in MeOH (50 mL), a solution of benzyl amine (358 mg, 3.3 mmol) in MeOH (25 mL) was added in 2 potions over 5 h and the reaction mixture was refluxed under N_2 atm for another 6 h. TLC analysis (50% EtOAc in hexanes) revealed that the reaction was complete. The solvent was removed by a rotary evaporator to afford the crude product, which was purified by column chromatography over Si gel (20 × 2 cm) using EtOAc / hexanes (1:1) as eluent to afford the pure 9-(benzylamino)phenanthridine-7,10-dione **12** (239 mg, 91%); Mp: 183-185 °C; ^{1}H NMR ($CDCl_3$) δ 4.43(d, J = 6.0 Hz, 2H), 5.82 (s, 1H), 6.30 (brs, 1H), 7.28-7.48 (m, 5H), 7.73-7.90 (m, 2H), 8.20 (d, J = 8.1 Hz, 1H), 9.37 (d, J = 8.4 Hz, 1H), 9.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 47.3, 100.2, 122.3, 124.1, 127.0, 127.9, 128.6, 129.4, 130.2, 130.8 (2C), 131.5, 135.9, 148.2, 148.52, 151.6, 183.1, 185.0; MS (ES^+) *m/z* 315 (M + H) and HRMS calcd for $C_{20}H_{14}N_2O_2$ 314.1055, found 314.1067.

12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione (13): To a stirred solution of 9-(benzylamino)phenanthridine-7,10-dione **12** (12 mg, 0.04 mmol) in CH_3CN (16 mL), $Mn(OAc)_3 \cdot 2H_2O$ (142 mg, 0.53 mmol) and a solution of 2-cyclohexen-1-one **3** (15 mg, 0.15 mmol) in CH_3CN (4 mL) were added. The reaction mixture was refluxed under N_2 atm for 3 days. TLC examination (30% EtOAc in hexanes) revealed that the reaction was complete. The reaction mixture was then allowed to attain rt and the solvent was removed under reduced pressure. The residue obtained was then dissolved in EtOAc (50 mL) and washed with saturated $NaHSO_3$ (3 × 30 mL), water (3 × 30 mL) and brine (2 × 25 mL) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated to afford the crude product, which was purified by column chromatography over Si gel using EtOAc / hexanes (1:19) as eluent to afford the pure 12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione **13** (14 mg, 92 %); Mp: 255-257 °C; ^{1}H NMR ($CDCl_3$) δ 6.02 (s, 2H), 7.17-7.37 (m, 5H), 7.38-7.51 (m, 3H), 7.74 (t, J = 8.4 Hz, 1H), 7.83(t, J = 6.9 Hz, 1H), 8.18 (d, J = 7.5 Hz, 1H), 8.43-8.50 (m, 1H), 9.53 (d, J = 8.4 Hz, 1H), 9.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 48.8, 111.8, 118.0, 123.4, 123.6, 124.2, 124.8, 125.4, 126.8, 127.9, 128.1, 128.2, 129.1, 130.3, 130.6, 131.6, 133.6, 135.4, 136.5, 140.4, 148.2, 152.5, 181.3, 182.3; MS (ES^+) *m/z* 389 (M + H) and HRMS calcd for $C_{26}H_{16}N_2O_2$ 388.1212, found 388.1218.

Calothrixin B (1): To a solution of 12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione **13** (10 mg, 0.03 mmol) in anhyd benzene (10 mL), $AlCl_3$ (17 mg, 0.13 mmol) was added and the reaction mixture was refluxed for 5 h. TLC examination (20 % EtOAc in hexanes) revealed that the reaction was complete. The reaction mixture was then allowed to attain rt, quenched with water (10 mL), and extracted by DCM (2 × 30 mL). The combined organic layers were dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated on a rotary evaporator to afford the crude product, which was purified by column chromatography over Si gel (20 × 2 cm) using EtOAc/hexanes (1:9) as eluent to afford the pure calothrixin B (3 mg, 40 %); Mp: 298-300 °C; ^{1}H NMR (DMSO-d_6) δ 7.42 (t, J = 8.1 Hz, 1H), 7.50 (t, J = 8.4 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 6.9 Hz, 1H), 7.97 (t, J = 6.9 Hz,1H), 8.19 (m, 2H), 9.59 (d, J = 8.7 Hz, 1H), 9.63(s, 1H), 13.18 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 113.9, 115.5, 122.3, 122.6, 123.3, 124.3, 124.9, 127.2, 129.8, 130.2, 131.6 (2C), 132.6, 138.0, 138.4, 147.5, 151.2, 180.4, 180.8; MS (ES+) *m/z* 299 (M + H) and HRMS calcd for $C_{19}H_{10}N_2O_2$ 298.0742, found 298.0745.

Calothrixin A (2): To a suspension of calothrixin B (5 mg, 0.02 mmol) in DCM (10 mL), under nitrogen, *m*-CPBA (77 %, 13 mg, 0.08 mmol) in DCM (10 mL) was added and the reaction mixture was refluxed overnight. TLC (50 % EtOAc in hexanes) examination revealed that the reaction was complete. The reaction mixture was diluted with DCM (50 mL) to afford a red orange solution, which was washed with saturated K_2CO_3 (3 × 15 mL), water (3 × 15 mL), brine (15 mL) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated on a rotary evaporator to afford the crude product, which was purified by column chromatography over Si gel (20 × 2 cm) using EtOAc / hexanes (3:17) as eluent to afford the pure calothrixin A (5 mg, 99 %); Mp: 283-285 °C; ^{1}H NMR (DMSO-d_6) δ 7.38 (t, J = 7.2 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.99-7.97 (m, 2H), 8.13 (d, J = 7.5 Hz, 1H), 8.61 (d, J = 9.9 Hz, 1H), 8.89 (s, 1H), 9.68 (d, J = 9.3 Hz, 1H), 13.22 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 114.0, 115.1, 119.1, 121.9, 122.0, 123.4, 124.5, 126.8, 127.0, 128.1, 129.9, 131.8, 131.9, 132.0, 138.1, 138.7, 143.1, 177.8, 178.3; MS (ES+) *m/z* 315 (M + H) and HRMS calcd for $C_{19}H_{10}N_2O_3$ 314.0691, found 314.0701.

Acknowledgements

Authors also wish to acknowledge the financial support by the Collaborative Programmatic Development Grant from the University of Alabama at Birmingham (UAB) Comprehensive Cancer Center. Beginning Grant-in-Aid (AHA0865323E) from American Heart Association Greater Southeast Affiliate is also acknowledged. Authors would like to thank Ms. Bhavitavya Nijampatnam for the proof reading of this manuscript.

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Supplementary material

Copies of ^{1}H NMR, ^{13}C NMR spectra of all compounds are available as Supplementary Material.

Supporting Information

Total Synthesis of Calothrixins A and B via Oxidative Radical Reaction of Cyclohexenone with Aminophenanthridinedione

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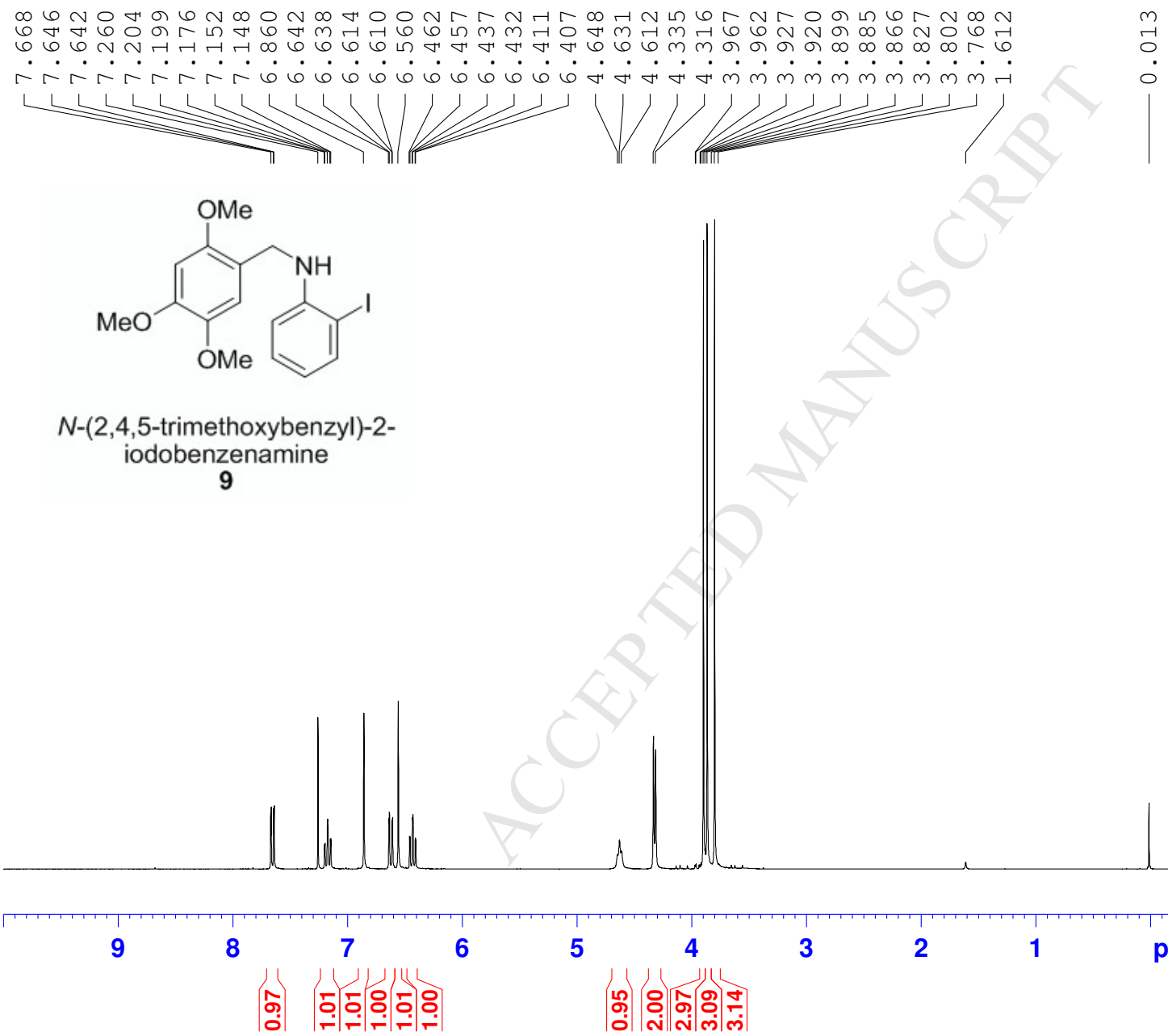
Table of Contents

SX-I-248-Pure 1HNMR CDCl3



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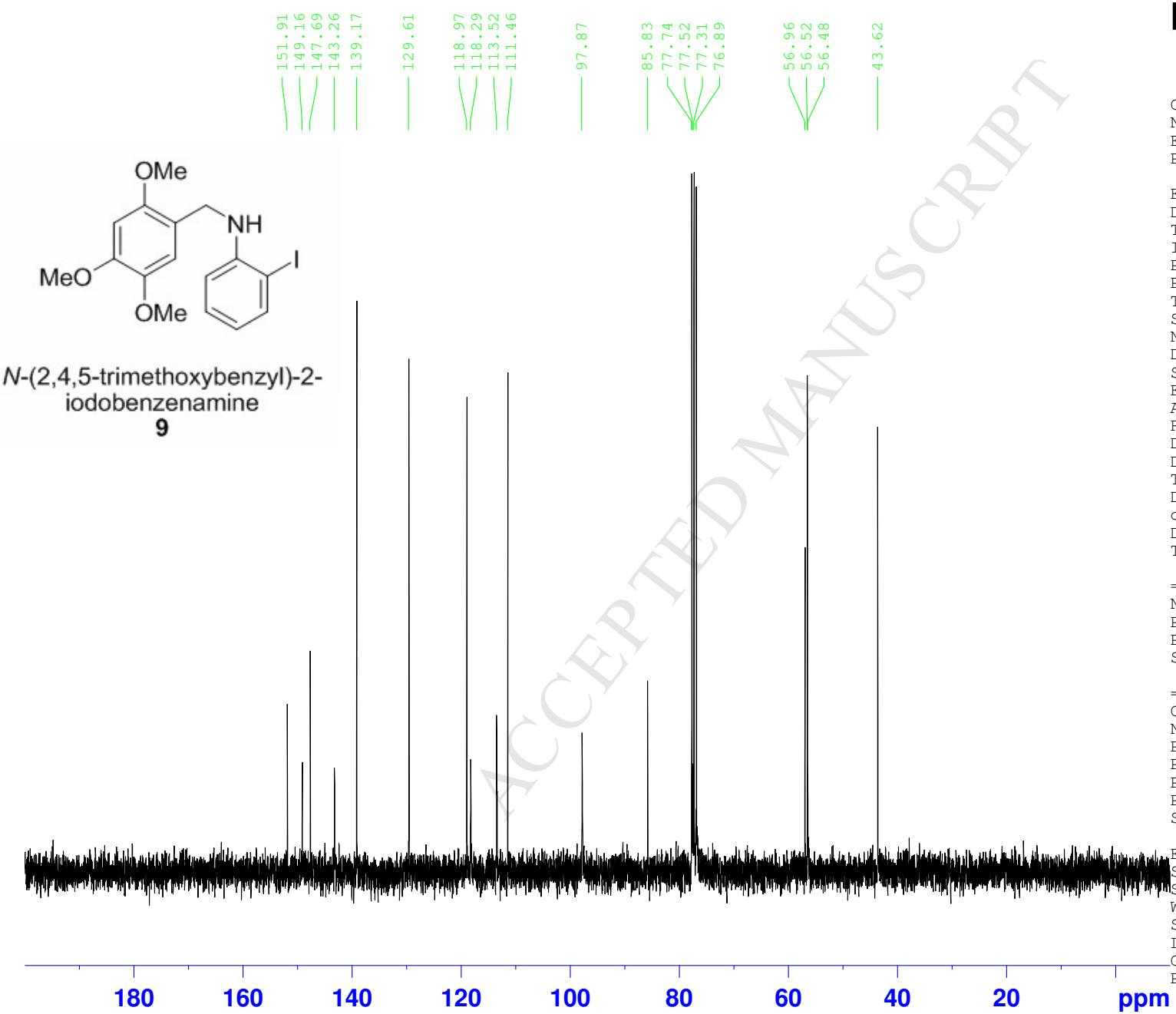

N-(2,4,5-trimethoxybenzyl)-2-iodobenzenamine
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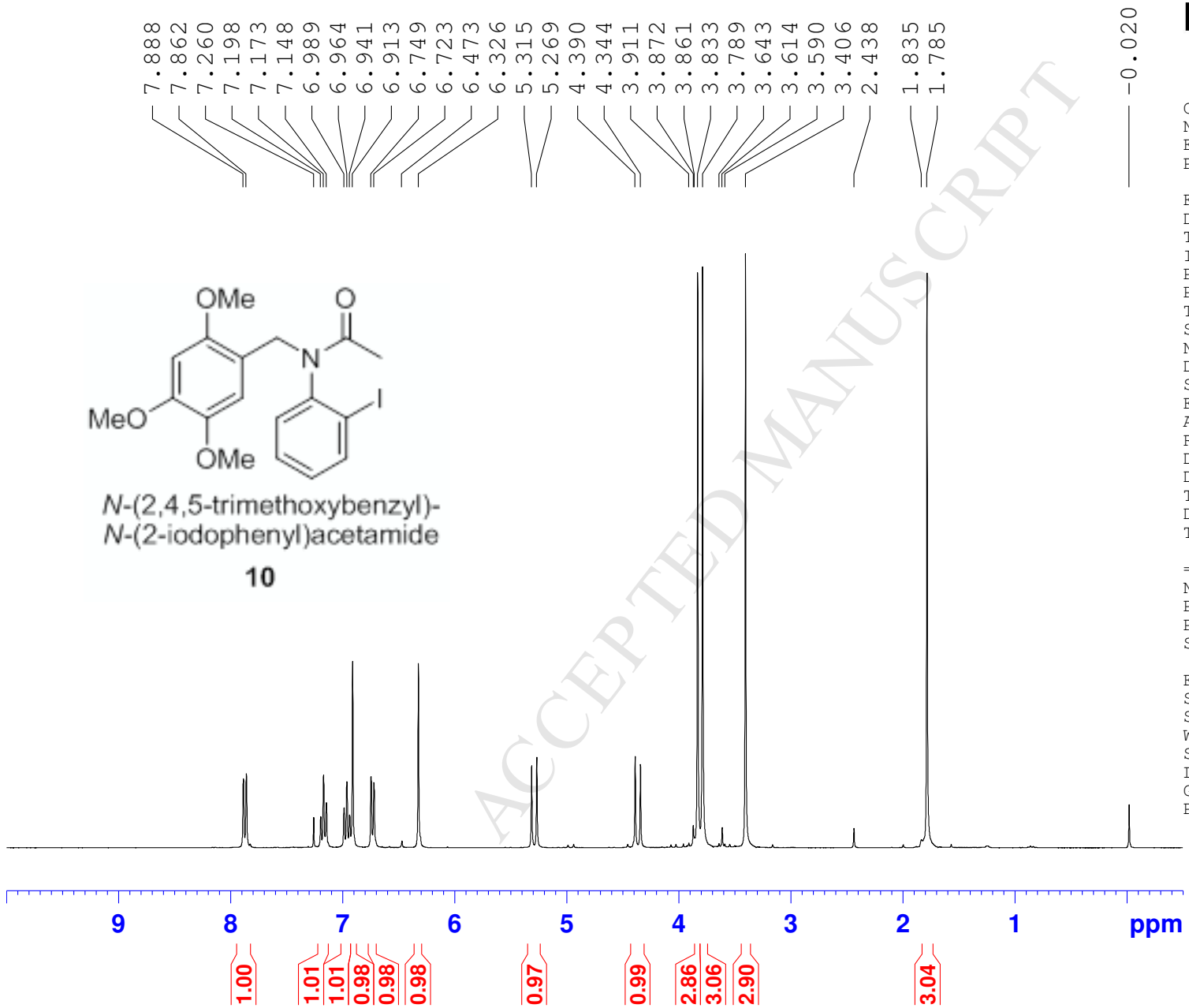

N-(2,4,5-trimethoxybenzyl)-*N*-(2-iodophenyl)acetamide

10

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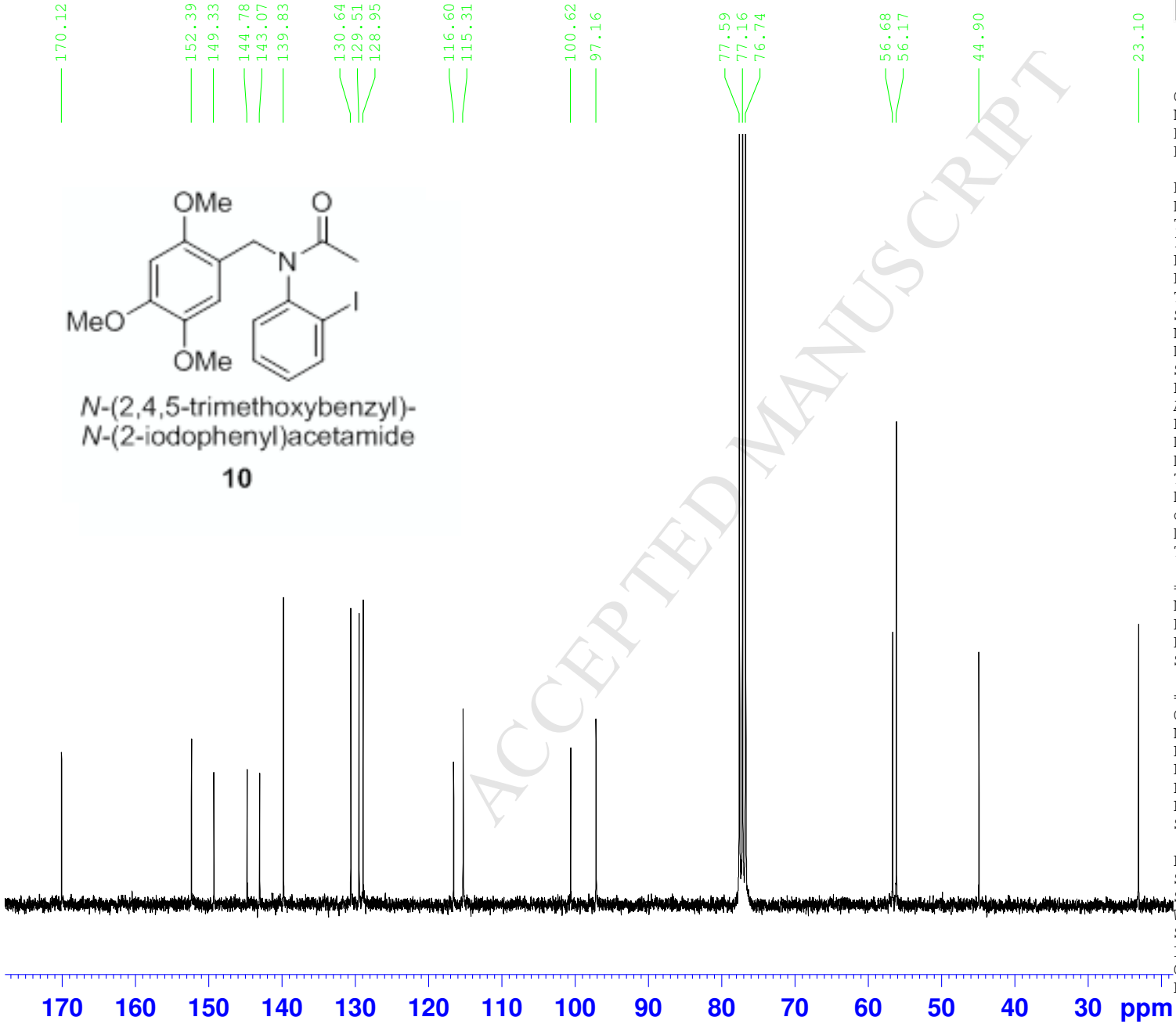

N-(2,4,5-trimethoxybenzyl)-
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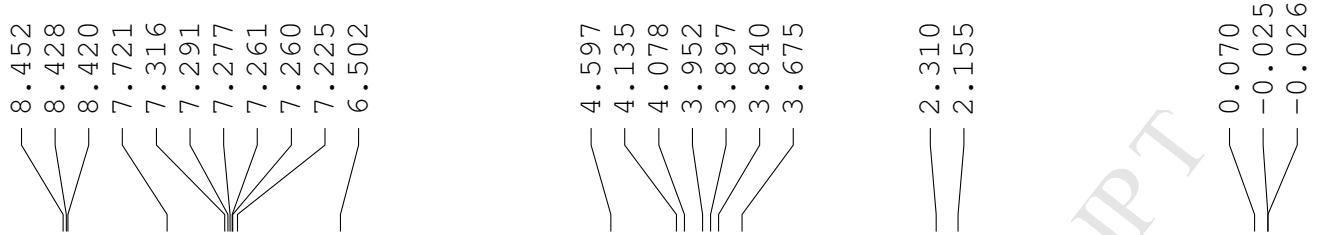
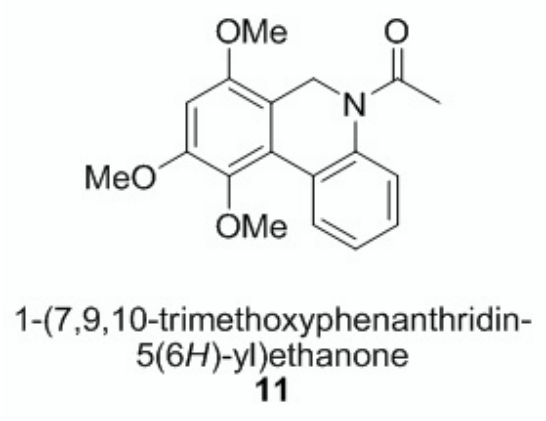
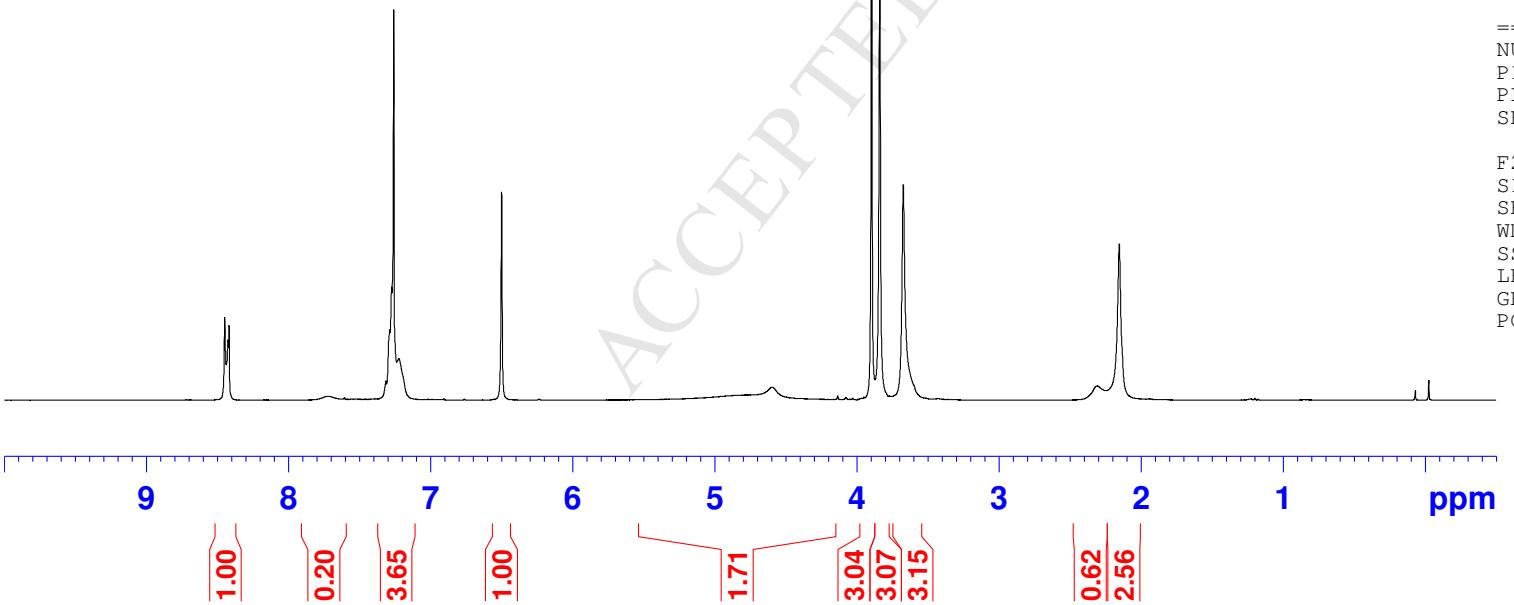




1-(7,9,10-trimethoxyphenanthridin-5(6*H*)-yl)ethanone
11

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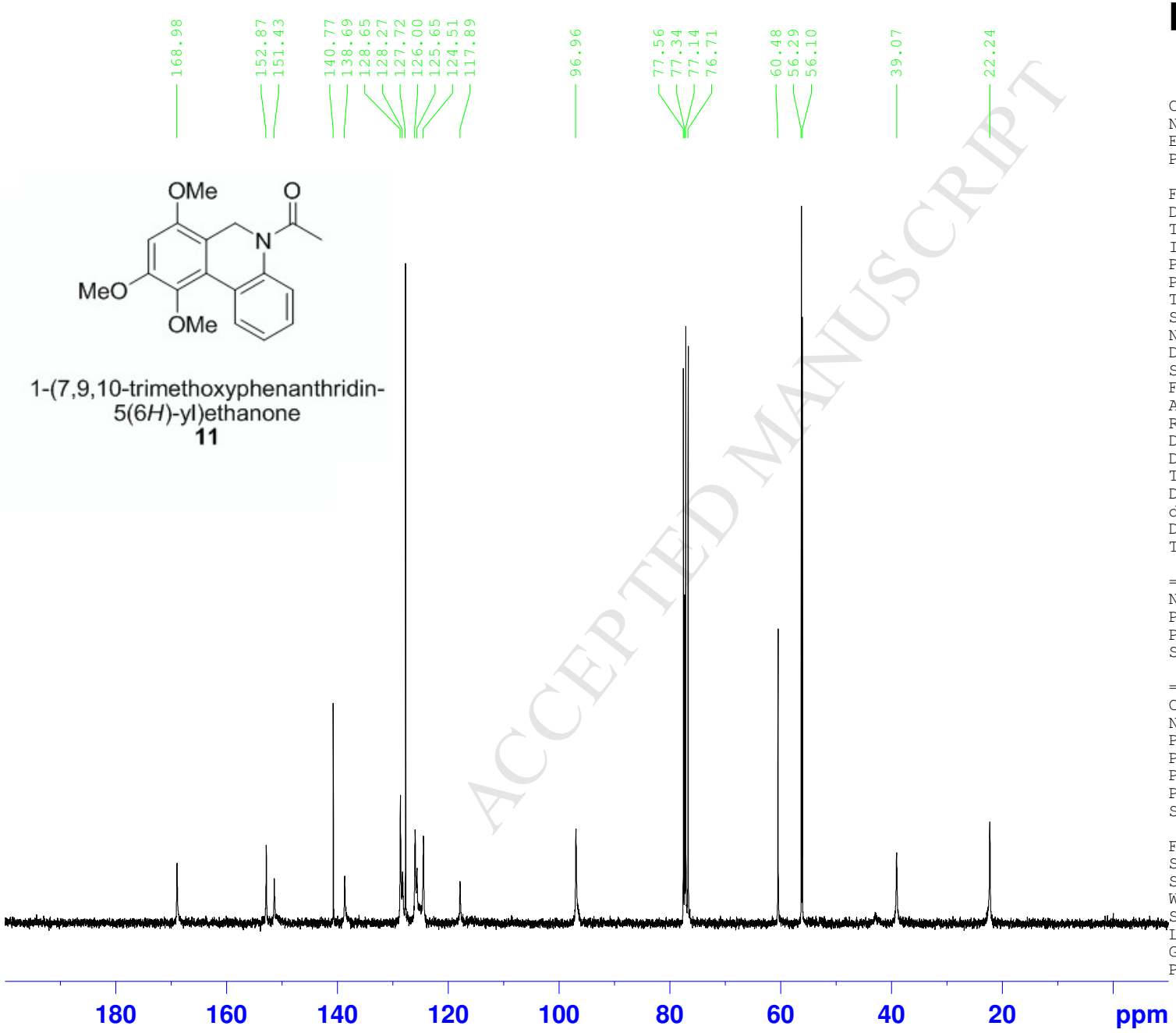
SX-I-254B-WhiteSolid 13CNMR CDCl3



1-(7,9,10-trimethoxyphenanthridin-5(6*H*)-yl)ethanone
11

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PROCNO                       1

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TE                  300.0 K
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d11             0.03000000 sec
DELTA           1.89999998 sec
TD0                      1

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PL1                  -1.80 dB
SFO1           75.4124265 MHz

======== CHANNEL f2 ========
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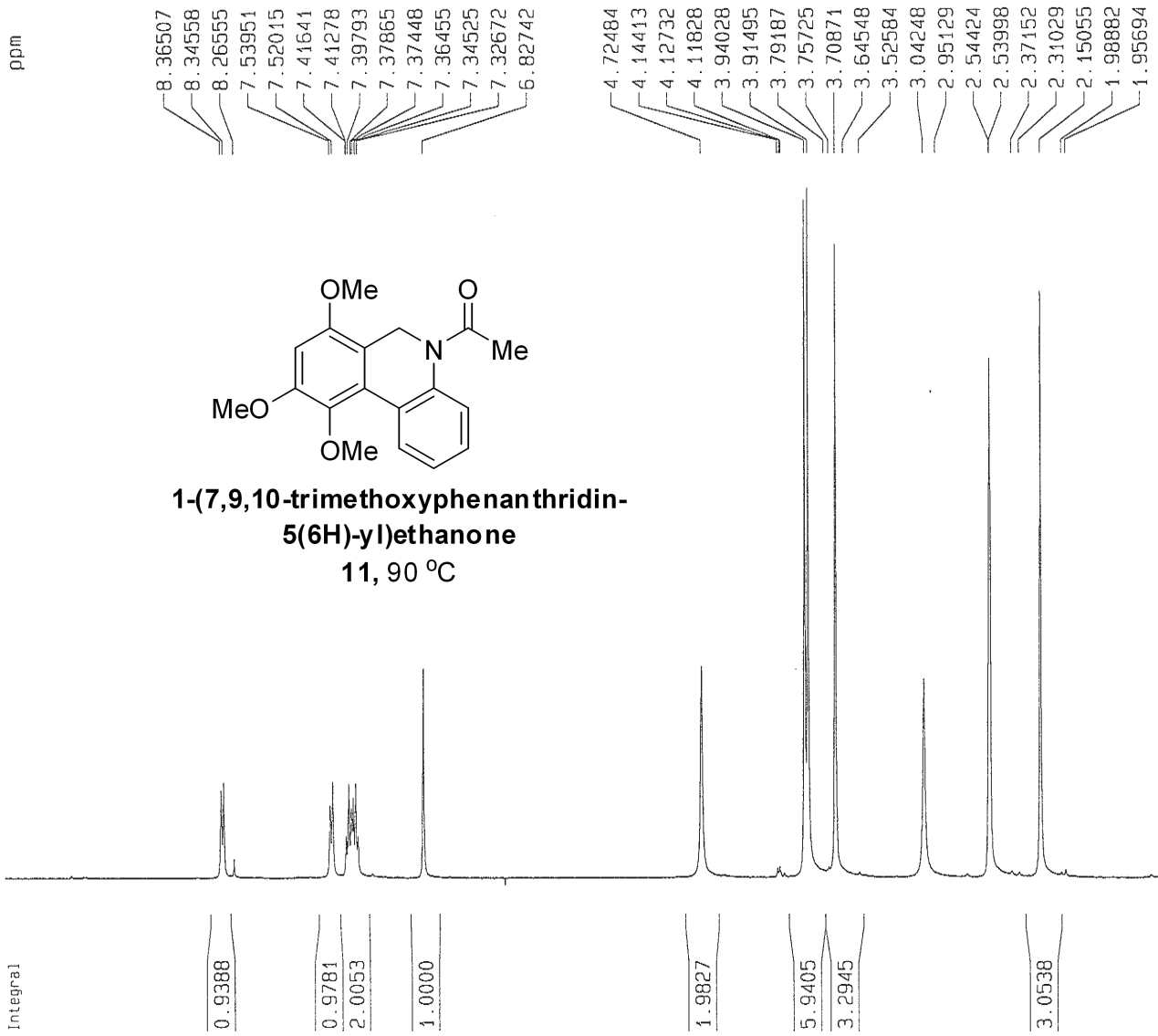
SX-I-254-90oC 1HNMR DMSO-d6



1-(7,9,10-trimethoxyphenanthridin-5(6H)-yl)ethanone

11, 90 °C

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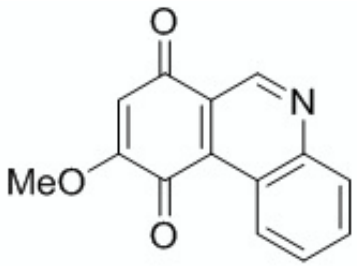
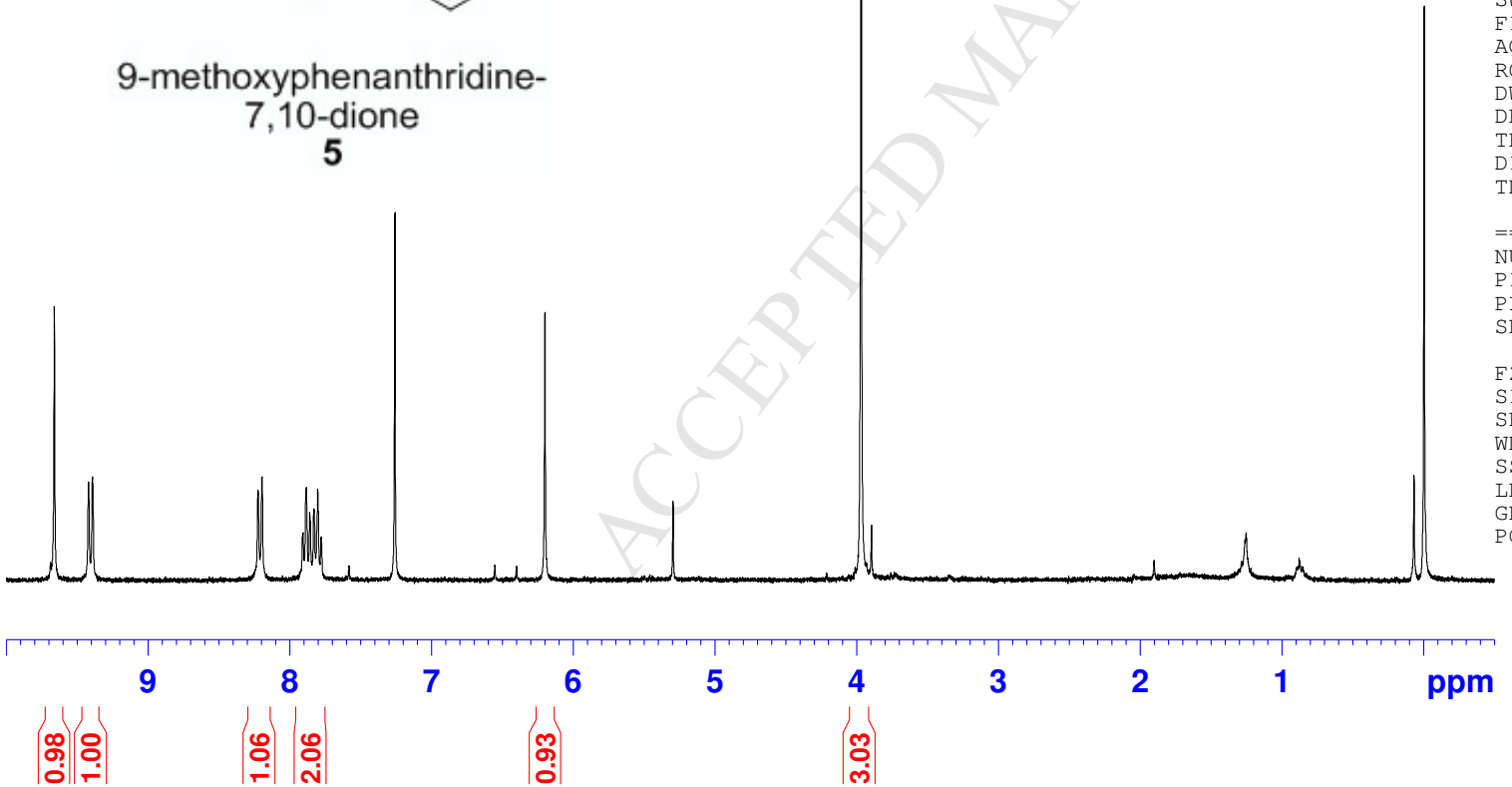
F2P               -0.500 ppm
F2              -200.06 Hz
PPMCM          0.52500 ppm/cm
HZCM         210.06824 Hz/cm
```

SX-I-246-Column-F1 1HNMR CDCl3

9.687 9.660 9.419 9.391 8.227 8.198 7.909 7.885 7.861 7.833 7.805 7.781 7.585 7.260 6.556 6.401 6.201 5.297 4.213 4.011 3.969 3.934 3.896 3.871 3.760 3.736 2.044 1.902 1.814 1.717 1.653 1.619 1.605 1.564 1.345 1.335 1.285 1.252 1.218 0.878 0.855 0.828 0.067 -0.005



9-methoxyphenanthridine-7,10-dione
5



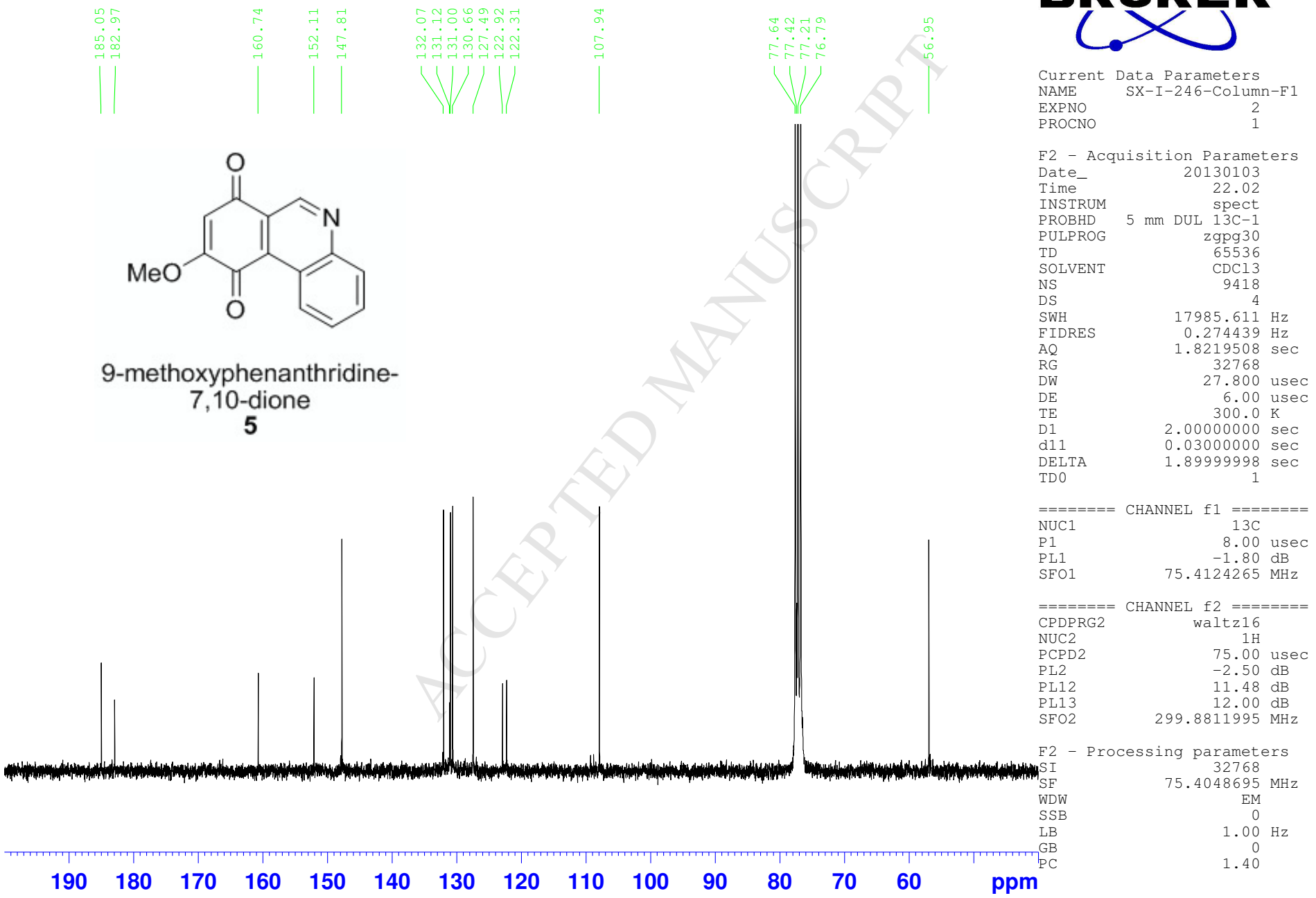
Current Data Parameters
NAME SX-I-246-Column-F1
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20130103
Time 21.54
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 812.7
DW 80.800 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 -2.50 dB
SFO1 299.8818519 MHz

F2 - Processing parameters
SI 32768
SF 299.8800036 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

SX-I-246-Column-F1 13CNMR CDCl3




```
Current Data Parameters
NAME      SX-I-246-Column-F1
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_           20130103
Time               22.02
INSTRUM            spect
PROBHD    5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 9418
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                32768
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

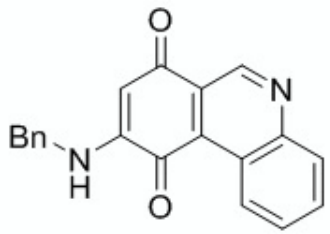
======== CHANNEL f1 ========
NUC1                 13C
P1                8.00 usec
PL1               -1.80 dB
SFO1         75.4124265 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            75.00 usec
PL2               -2.50 dB
PL12              11.48 dB
PL13              12.00 dB
SFO2        299.8811995 MHz

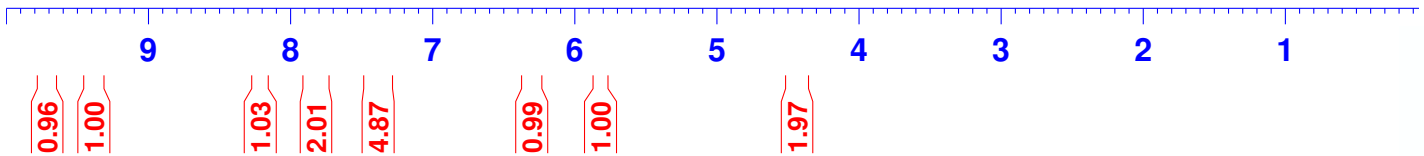
F2 - Processing parameters
SI                32768
SF          75.4048695 MHz
WDW                  EM
SSB                   0
LB                1.00 Hz
GB                    0
PC                1.40
```

SX-I-252-Column 1HNMR CDCl3

9.710 9.395 9.393 9.390 9.367 9.364 9.362 8.216 8.189 7.878 7.873 7.855 7.850 7.827 7.822 7.803 7.798 7.774 7.769 7.751 7.441 7.435 7.421 7.407 7.405 7.389 7.374 7.364 7.340 7.297 7.261 7.259 6.301 5.819 4.441 4.421 1.568 1.253 0.070 0.010 0.008 -0.001 -0.003 -0.011



9-(benzylamino)phenanthridine-7,10-dione
12




```
Current Data Parameters
NAME      SX-I-252-Column
EXPNO                   1
PROCNO                  1

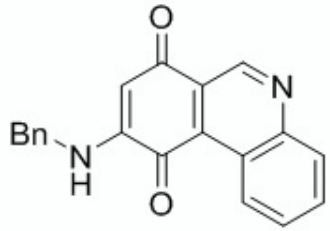
F2 - Acquisition Parameters
Date_           20130110
Time               17.20
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6188.119 Hz
FIDRES          0.094423 Hz
AQ             5.2953587 sec
RG                 912.3
DW                80.800 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                15.00 usec
PL1               -2.50 dB
SFO1        299.8818519 MHz

F2 - Processing parameters
SI                 32768
SF          299.8800036 MHz
WDW                   no
SSB                    0
LB                0.00 Hz
GB                     0
PC                1.00
```

SX-I-252-Column 13CNMR CDCl3

185.04
183.06
151.58
148.52
148.23
135.87
131.46
130.80
130.76
130.21
129.37
128.55
127.91
127.03
124.13
122.29
100.21
77.66
77.23
76.81
47.27



9-(benzylamino)phenanthridine-7,10-dione
12


```
Current Data Parameters
NAME      SX-I-252-Column
EXPNO                  2
PROCNO                 1

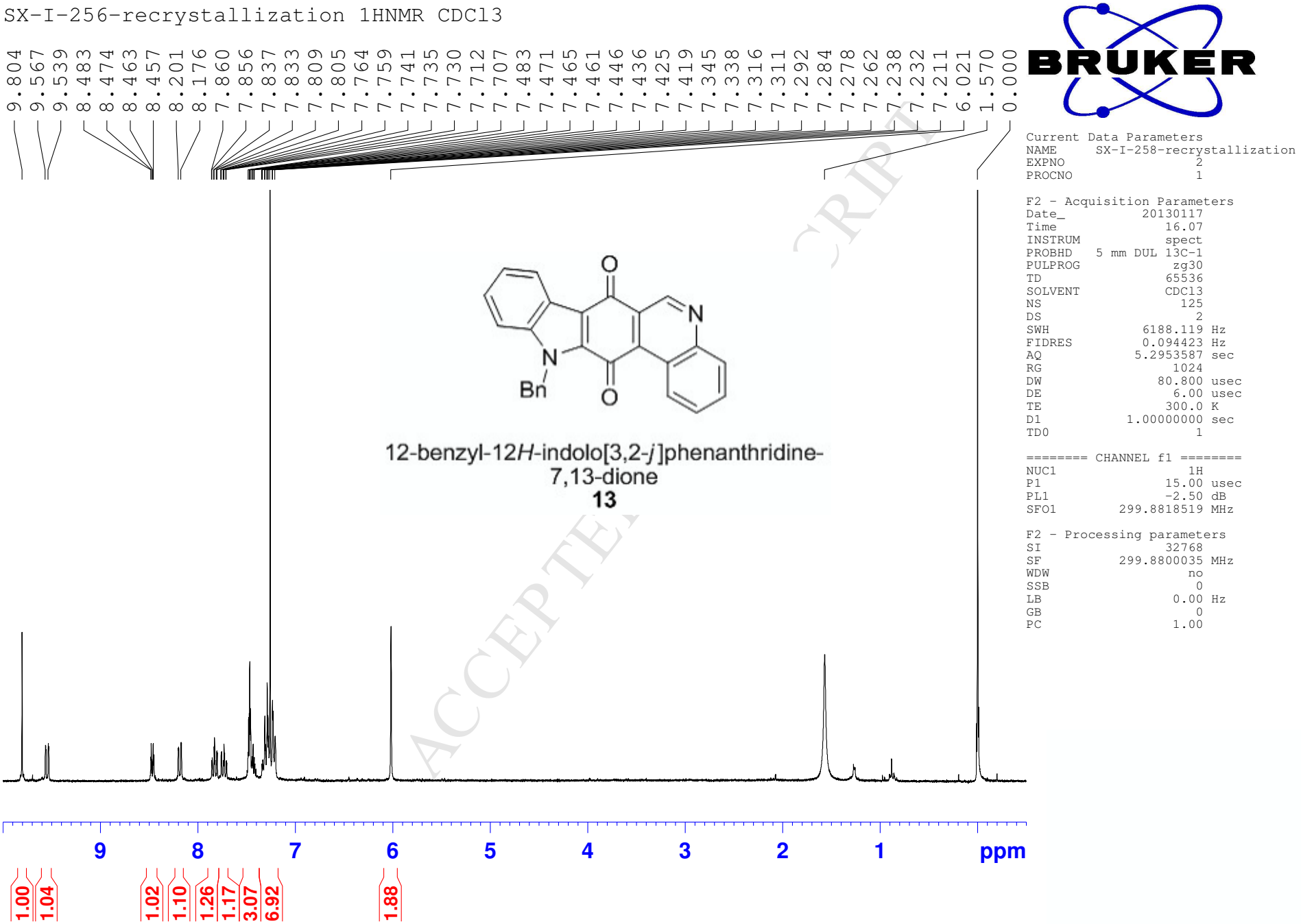
F2 - Acquisition Parameters
Date_           20130110
Time               17.28
INSTRUM            spect
PROBHD    5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                13843
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                32768
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                8.00 usec
PL1               -1.80 dB
SFO1         75.4124265 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            75.00 usec
PL2               -2.50 dB
PL12              11.48 dB
PL13              12.00 dB
SFO2        299.8811995 MHz

F2 - Processing parameters
SI                32768
SF           75.4048677 MHz
WDW                  EM
SSB                   0
LB                1.00 Hz
GB                    0
PC                1.40
```

SX-I-256-recrystallization 1HNMR CDCl3



12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione

13

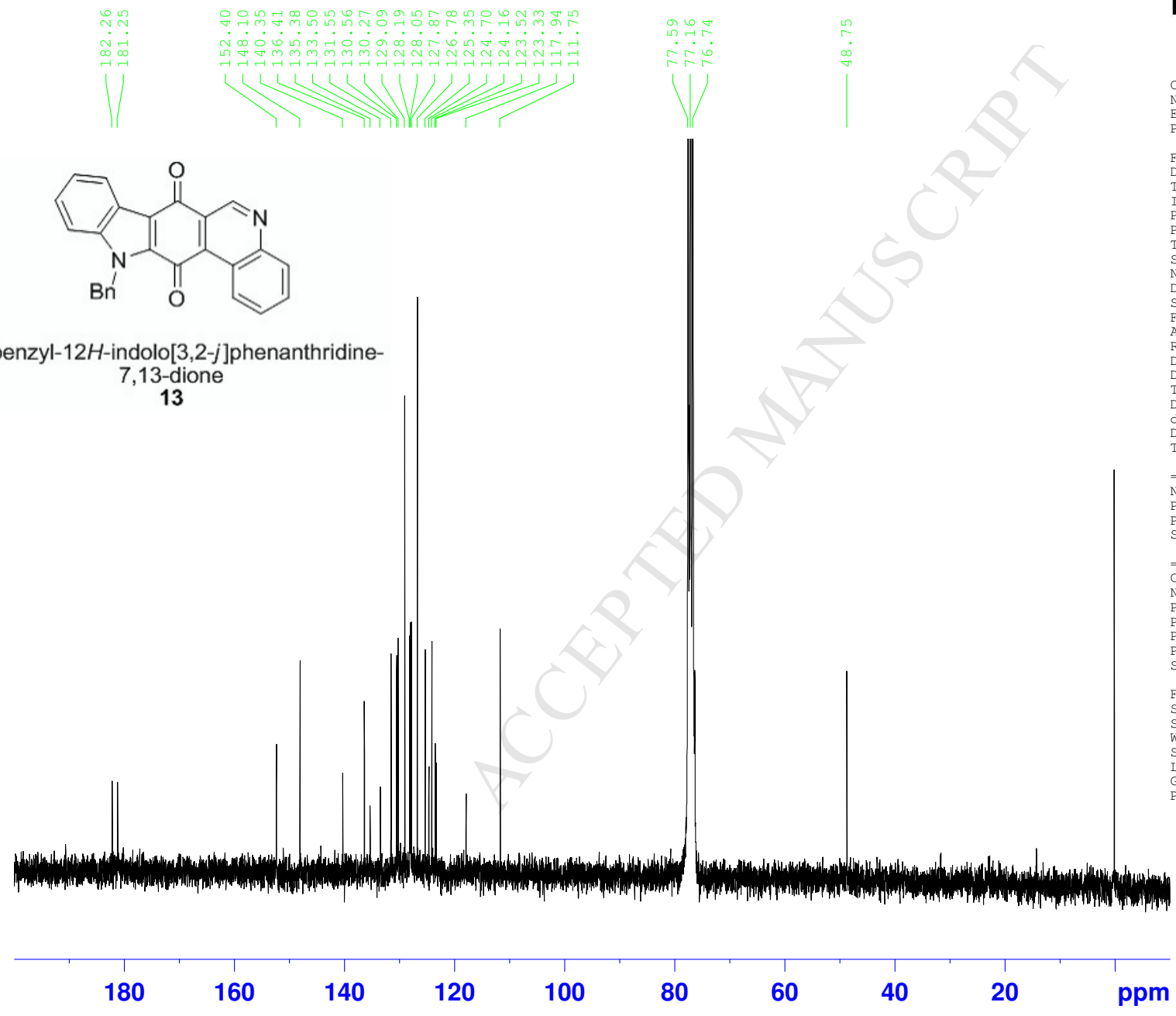
```
Current Data Parameters
NAME      SX-I-258-recrystallization
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130117
Time               16.07
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                   125
DS                     2
SWH             6188.119 Hz
FIDRES          0.094423 Hz
AQ              5.2953587 sec
RG                  1024
DW                80.800 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 15.00 usec
PL1                -2.50 dB
SFO1         299.8818519 MHz

F2 - Processing parameters
SI                 32768
SF          299.8800035 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
```

SX-I-256-recrystallization 13CNMR CDCl3



12-benzyl-12*H*-indolo[3,2-*j*]phenanthridine-7,13-dione

13

```
Current Data Parameters
NAME      SX-I-258-recrystallization
EXPNO                  5
PROCNO                 1

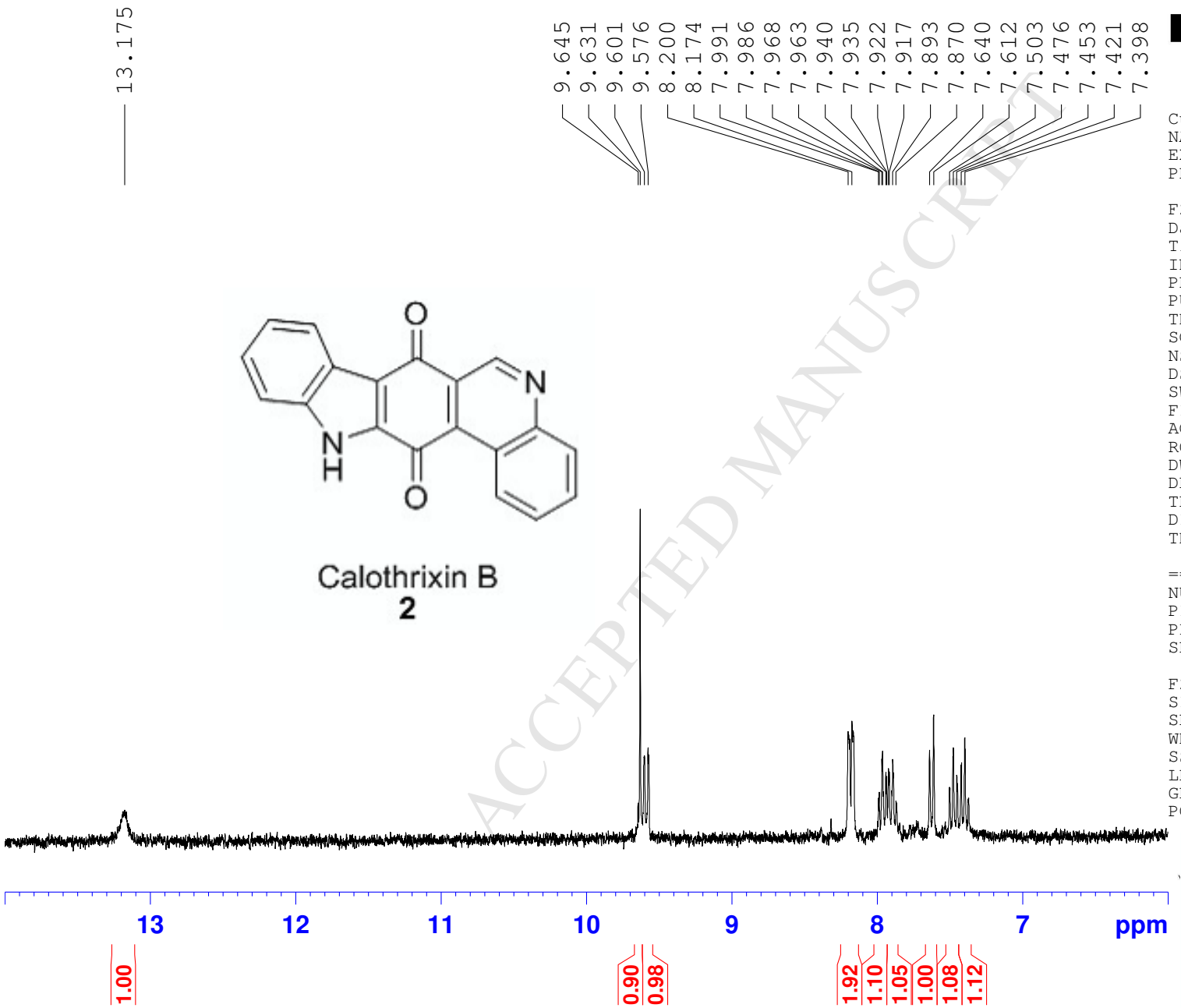
F2 - Acquisition Parameters
Date_           20130119
Time               15.48
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                37109
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                32768
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                8.00 usec
PL1               -1.80 dB
SFO1         75.4124265 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2               -2.50 dB
PL12              11.48 dB
PL13              12.00 dB
SFO2        299.8811995 MHz

F2 - Processing parameters
SI                32768
SF           75.4048735 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

SX-II-8-Solid 1HNMR DMSO



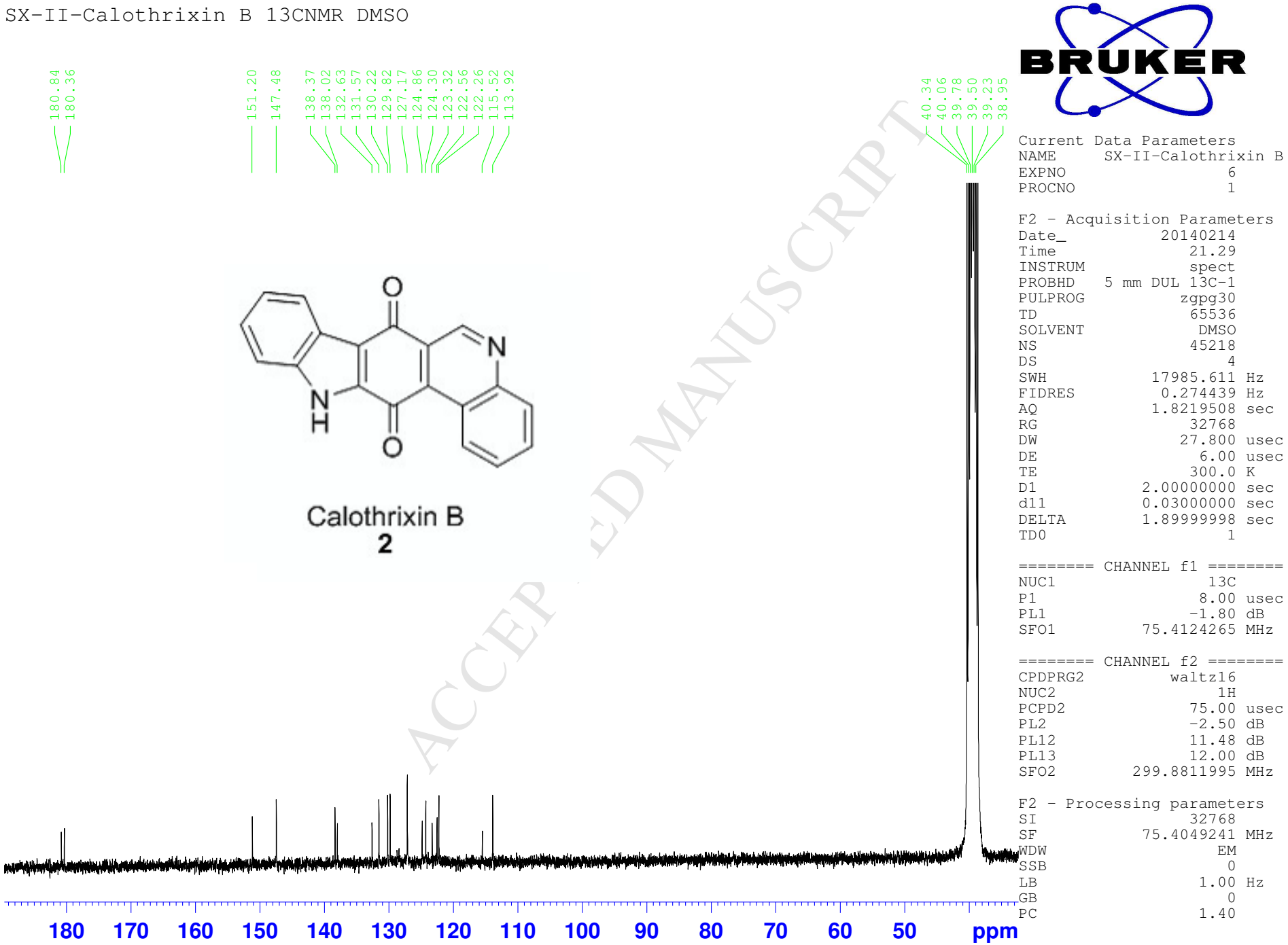
Current Data Parameters
NAME SX-II-8-Solid
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20131011
Time 23.02
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 40
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 812.7
DW 80.800 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 -2.50 dB
SFO1 299.8818519 MHz

F2 - Processing parameters
SI 32768
SF 299.8799971 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

SX-II-Calothrixin B 13CNMR DMSO




```
Current Data Parameters
NAME      SX-II-Calothrixin B
EXPNO                       6
PROCNO                      1

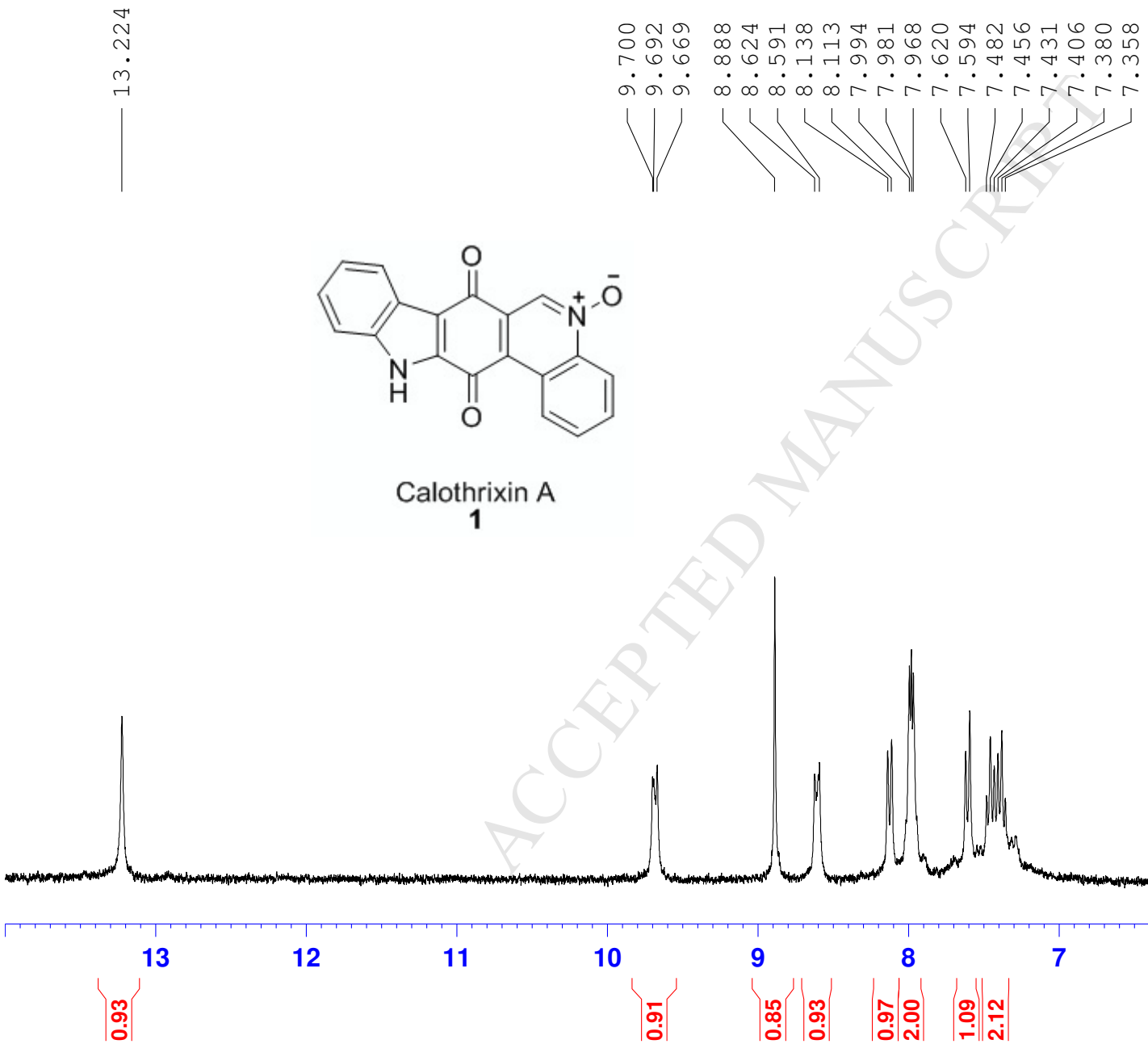
F2 - Acquisition Parameters
Date_              20140214
Time                 21.29
INSTRUM              spect
PROBHD    5 mm DUL 13C-1
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                   45218
DS                       4
SWH             17985.611 Hz
FIDRES          0.274439 Hz
AQ              1.8219508 sec
RG                   32768
DW                 27.800 usec
DE                  6.00 usec
TE                 300.0 K
D1              2.00000000 sec
d11             0.03000000 sec
DELTA           1.89999998 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                  8.00 usec
PL1                -1.80 dB
SFO1          75.4124265 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2               75.00 usec
PL2                -2.50 dB
PL12               11.48 dB
PL13               12.00 dB
SFO2         299.8811995 MHz

F2 - Processing parameters
SI                   32768
SF            75.4049241 MHz
WDW                     EM
SSB                      0
LB                  1.00 Hz
GB                       0
PC                   1.40
```

SX-II-41 1HNMR DMSO-d6



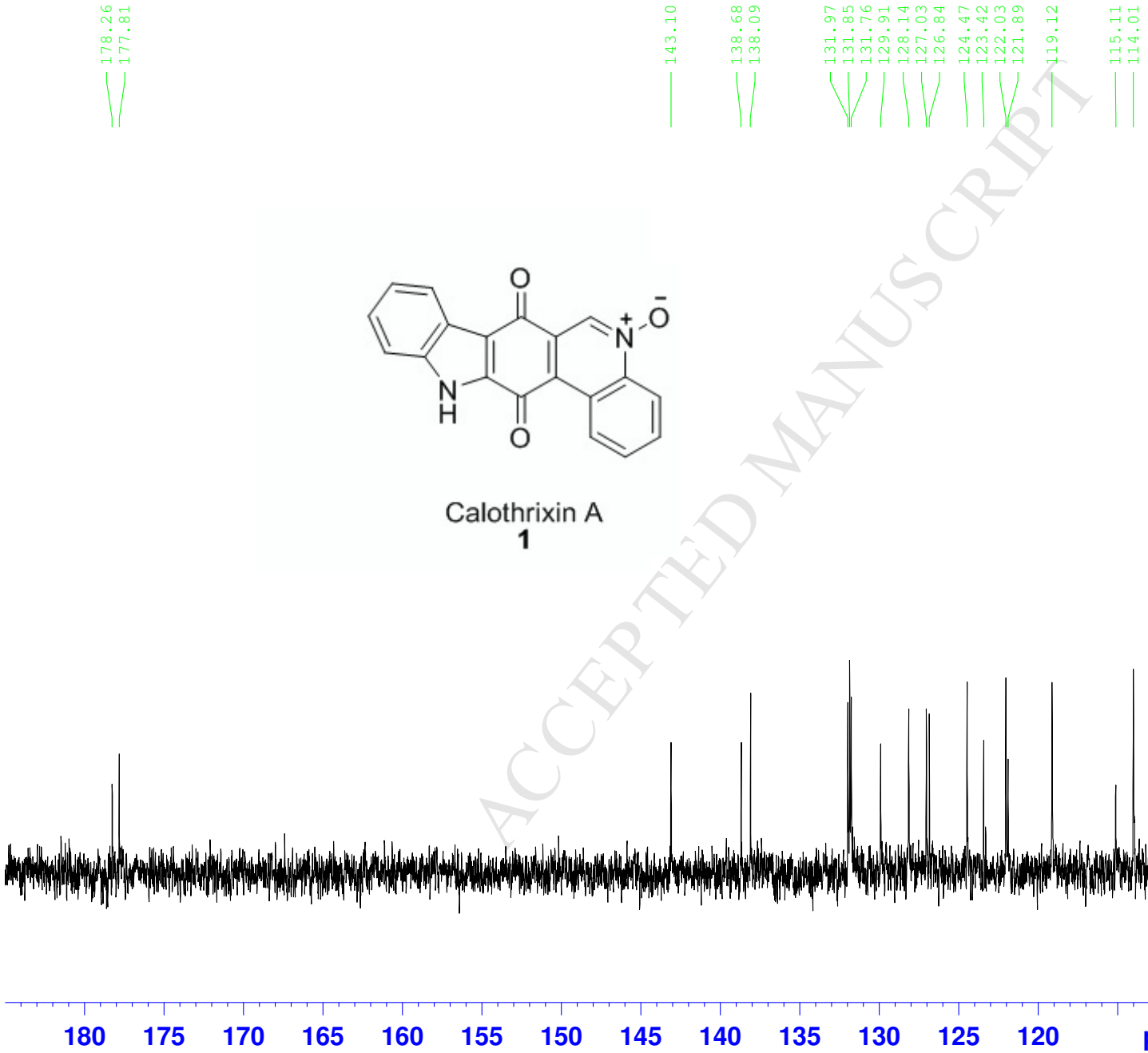
Current Data Parameters
NAME SX-II-41
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20140228
Time 9.39
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 64
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 1024
DW 80.800 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 -2.50 dB
SFO1 299.8818519 MHz

F2 - Processing parameters
SI 32768
SF 299.8799979 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

SX-II-41 13CNMR DMSO-d6




```
Current Data Parameters
NAME            SX-II-41
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140301
Time                8.31
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                 44265
DS                     4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                 32768
DW               27.800 usec
DE                6.00 usec
TE                300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                8.00 usec
PL1               -1.80 dB
SFO1         75.4124265 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            75.00 usec
PL2               -2.50 dB
PL12              11.48 dB
PL13              12.00 dB
SFO2        299.8811995 MHz

F2 - Processing parameters
SI                 32768
SF          75.4049227 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                1.40
```