Online-ISSN 2411-2933, Print-ISSN 2411-3123 *December 2016*
DOI: https://doi.org/10.31686/ijier.vol4.iss12.50

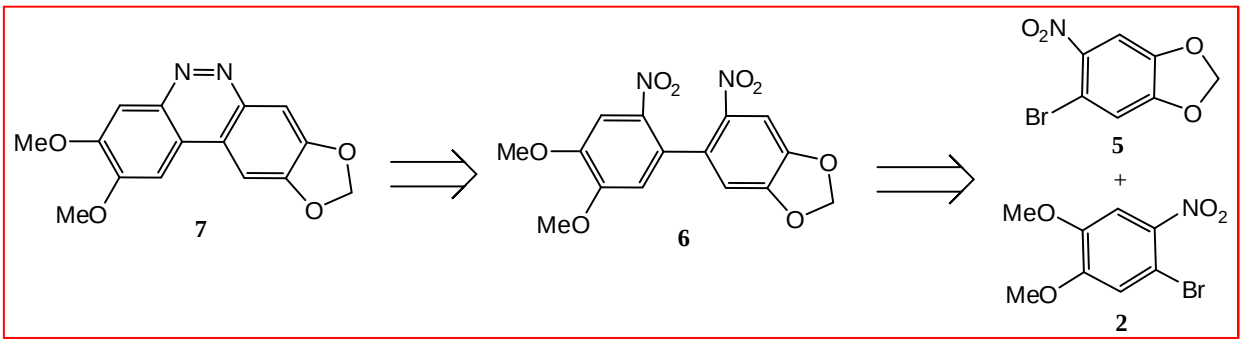
Synthesis of 2,3-dimethoxy-8,10-methylenedioxy benzo[c]cinnoline 7 As Potential Topoisomerase I Inhibitor

Ghislain R Mandouma, Tahera Nembhard, Brittney Bender

Abstract

*A novel and green synthesis of 2,3-dimethoxy-8,10-methylenedioxy benzo[c]cinnoline **7** is herein described. This compound is structurally related to benzo[i]phenanthridine, a class of potent topoisomerase I inhibitors such as nitidine. While nitidine was found to be toxic, the benzo[c]cinnoline derivative **7** may circumvent that by not being a Schiff base. A conveniently short synthetic plan was implemented involving a novel solvent- and catalyst-free cross-coupling biarylation of halogenated nitroarenes **2** and **5** using a high speed ball milling (HSBM) procedure. Indeed, using a copper vial as reaction vessel, and a copper ball as a collider in a shaker, we observed clean cross-coupling reactions of **2**, and **5** leading up to substituted biphenyl **6** in quantitative yield. Reductive diazotization of **6** with lithium aluminum hydride produced 2,3-dimethoxy-8,10-methylenedioxybenzo[c]cinnoline **7** in good yield.*

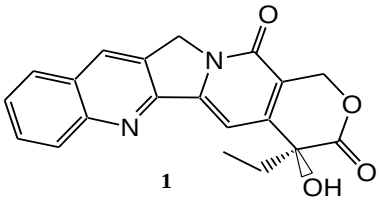
Graphical Abstract



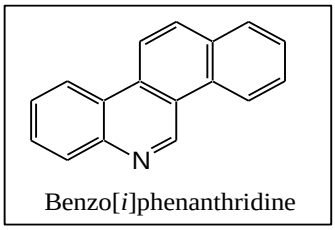
Introduction

Cancer is the second cause of death for people under the age of 85 in the United States. Camptothecin is a broad spectrum drug that was used to treat cancer. Its targets are topoisomerase I (TOP I) and topoisomerase II (TOP II). The onset of resistance to camptothecin has led the scientific community to seek new TOP I and TOP II inhibitors. In 1998 Nitidine was discovered as a replacement to camptothecin. Nitidine is within the class benzo[*i*]phenanthridine. Several reports have indicated that certain derivatives of polyaromatic hydrocarbons (PAHs), including pyrene, chrysene, and benzo[*c*]cinnoline derivatives, reduced the viability of transformed cell lines by induction of apoptosis. [1-6] The structures of benzo[*c*]cinnoline derivatives have been the subject of much interest.[7-10] While most PAHs are hydrocarbons, the benzo[*c*]cinnoline ring incorporates a diaza (N=N) moiety when making a fusion of two

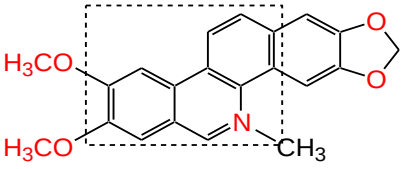
benzene rings. The ring is structurally similar to phenanthrene, except for the diaza junction. It has been shown more recently that several derivatives of benzo[*c*]cinnoline and dibenzo[*c,h*]cinnoline exhibited potent topoisomerase I-targeting activity while being capable of overcoming multi-drug resistance.[11-14] DNA topoisomerases (TOPI and TOPII) are enzymes that regulate the topological state of DNA through breaking and rejoining DNA strands. These enzymes are also involved during RNA transcription by controlling DNA template supercoiling.[15-16] Topoisomerase I (TOPI) is involved in the formation of a single-strand DNA break while topoisomerase II (TOPII) creates a double-strand DNA break. Because the enzyme-DNA complex is cleavable, its stabilization by drugs has been shown to offer antitumor activity.[17-20]



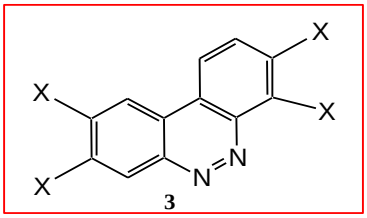
1
Camptothecin



Benzo[*i*]phenanthridine



2
Nitidine



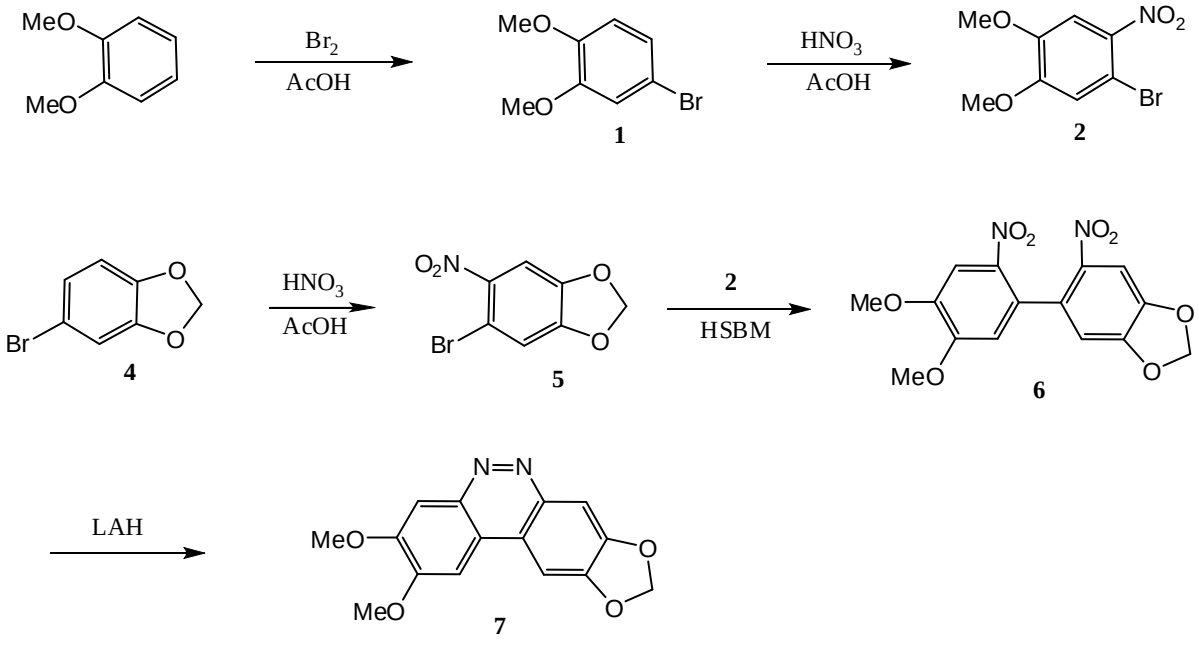
3
Halogenated Benzo[*c*]cinnoline

Materials and Methods

Biaryl carbon-carbon bond formation has been successfully attained through, mostly, organometallic chemistry. Various catalysts have been successfully utilized in both the homo- and the cross coupling biaryl syntheses. These include but are not limited to Palladium (Stille, Suzuki, Sonogashira), Zinc (Negishi), and Copper (Ullmann). Notwithstanding the cost of catalysts, several of the mostly used techniques have limitations. The traditional Ullmann's coupling reaction is often substrate specific. It is also limited by the high temperature requirement, which precludes sensitive function groups. Our approach to the Ullmann's coupling reaction, which does not require a catalyst, or solvent is based on High Speed Ball Milling (HSBM). We have recently adapted the use of HSBM to conduct copper-, and solvent-free Ullmann's homo- and cross- coupling of nitro-substituted aryl halides to achieve quantitative yields on Ullmann type reactions. While copper-catalyzed Ullmann's coupling reaction has been achieved in wet (using a solvent) and/or dry conditions, copper bronze, copper oxide, copper-zinc, or some combination of catalysts were always part of the reaction. Since in HSBM method, pyrex glass is replaced by a metal (or teflon) vial, the choice of "copper vial" provided us with a built-in catalyst, and waste production was minimized by not having unreacted copper to dispose.

The synthetic method employed for the preparation of the benzo[*c*]cinnolines and dibenzo[*c*,*h*]cinnolines compounds 4-7 is outlined in the scheme below featuring our innovative high speed ball milling (HSBM) Copper-Mediated biaryl couplings that are accomplished in solvent-free conditions, with no added catalysts excepted in the copper vials used as vessels for these Ullmann-like coupling reactions. The key HSBM Ullmann cross coupling of the nitroaryl bromides 1 and 2 led to the biaryl compound 3 (Scheme 1). Likewise the cross coupling of 2 and 5 afforded the biaryl compound **6**. These reaction were, gratifyingly high yielding as self-coupling of either nitroarylbromide proved unfavorable under the conditions of Ullmann coupling which is promoted by an adjacent electron-withdrawing group (such as a nitro group) to the leaving halo group. Both dinitrobiaryl compounds **3** and **6** were, subsequently subjected to intramolecular diazotization by lithium aluminum hydride (LAH) to afford benzo[c]cinnoline **7** (Scheme 1). Nitroaryl halides were valuable precursors to the HSBM Ullmann cross coupling reactions above. These were synthesized by classical methods described below. Commercially available 1,2-dimethoxybenzene was brominated with elemental bromine in glacial acetic acid. After 5 min of adding bromine, a thick solid precipitated out of the solution corresponding to 1,2-dimethoxy-4-bromobenzene. The latter was nitrated with a mixture of nitric acid in concentrated sulfuric acid to afford 1-bromo-3,4-dimethoxynitrobenzene 1 in quantitative yield (Scheme 4).

Synthetic Scheme:

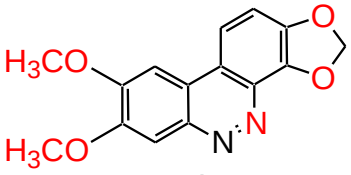


Discussion

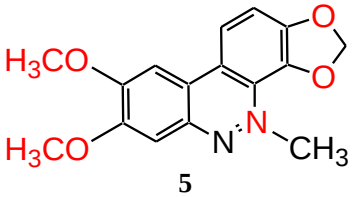
Resistance to camptothecin, and the need for drugs with better toxicity profiles have led the research community to the use of organic synthesis as the ultimate tool. In the last few years, new methodologies have been invented to prepare gram amounts of drug candidates for screening. Structure-activity relationship studies have established the benzo[i]phenanthridine ring as one of the most potent for TOPI and TOPII-targeting activity, with nitidine 2 having the best cytotoxic profile.[21] Significant in that

determination has been the structural features of nitidine.[22-23] The positions of the 2,3-methoxy groups and the 8,9-methylenedioxy as well as the nitrogen of the cinnoline are all key features associated with enhanced topoisomerase targeting activity and cytotoxicity. The present study explores the structure activity relationships of substituted benzo[c]cinnolines, which have incorporated into their structure two adjacent heteroatoms, are structurally similar to benzo[i]phenanthridines. The influence of substituents in the rings (in red) of benzo[c]cinnolines on both TOP1- targeting activity and cytotoxicity will be investigated. Among the compounds selected for synthesis and toxicological evaluation were 2,3-dimethoxy-7,8-methylenedioxybenzo[c]cinnoline, **7** and 2,3-dimethoxy-8,9-methylenedioxydibenzo[c,h]cinnoline in future studies. To further evaluate the structure–activity relationships within this class of compounds will be synthesized and their pharmacological activities evaluated through toxicity studies involving the zebrafish embryo model.[24-26]

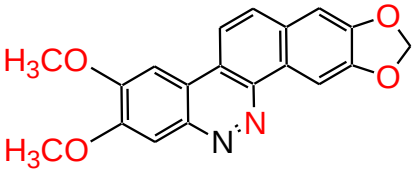
Analogs of Nitidine to be Synthesized



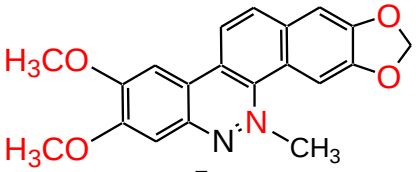
4



5



6



7

Toxicology Studies on Zebrafish Embryo

Owing to the numerous advantages conferred by this transparent and inexpensive species to maintain, we have been looking into the use of the zebrafish embryo to profile the therapeutic nature of the synthetic drugs 4-7 above. For, unlike rodents, embryological development in zebrafish can be monitored in live individual embryos. In addition, the survival ability of zebrafish embryos that are malformed, missing organs, or displaying organ dysfunction has been demonstrated as they can usually survive substantially past the time in which those organs start to function in healthy individuals. The rapid maturation of zebrafish is an important experimental factor when conducting mutagenesis screening as it establishes the required transgenerational endpoints, transgenic lines, and in assessing the teratogenicity of different therapeutic agents being tested. Gene expression can be visualized using light microscopy throughout the growth of this optically clear species from the larvae stage until the maturation of its tissues. One goal is the co-localization of different drugs receptor and the nuclear translocator mRNAs in the zebrafish cardiovascular system. Fluorescence labeling and confocal microscopy may also be used to enhance gene expression imagery, and assessment of larvae. Therefore, using little magnification, adverse effects of

chemical exposure on development of the brain, notochord, heart, and jaw, trunk segmentation, and measurements of size can be assessed quantitatively. Once a specific gene has been identified either as a marker for specific tissues or as an essential part of a developmental pathway, these genes can be assessed for disruption after chemical exposure. Second, when a chemical has disrupted gene expression or morphology, recovery of normal gene expression can be assessed after application of therapeutic agents. Instead of performing lengthy staining methods over several days to identify the spatial and temporal expression pattern of the gene, a line of zebrafish can be created to express a transgene with a fluorescent reporter such as green fluorescent protein (GFP), thereby enabling assessments at any stage of early development easily with fluorescence microscopy. Although in the present study, there is no provision to use zebrafish mutants, their importance in research is undeniable. As well as being useful to identify genes affected by the exposure to various toxicants, mutant models are useful for studying human diseases. Phenotypic comparisons between zebrafish manifesting chemical-specific endpoints of toxicity and zebrafish mutants may provide insight into the specific genes affected by the drug being tested. Likewise, zebrafish carrying a certain mutation may be resistant to a particular toxicant and hence may help demonstrate the necessity for a specific developmental or detoxification pathway in mediating the toxic response.

Experimental Methods

All NMR spectra were recorded on a Varian Mercury 400MHz spectrometer. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories, Inc., Andover MA, and used without further purification. All products were confirmed by 1H, 13C NMR, pending GCMS results. Starting materials were purchased from Sigma-Aldrich and used without further purification. Ball milling experiments was carried out in a 2500 Parr Hydrogenator/Shaker. Ball bearings were purchased from Small Parts incorporated. Custom made vials were gifts from Dr. Lon Knight who manufactured them from parts in the machine shop at Furman University, Greenville, SC.

1.1. Synthesis of 3,4-dimethoxy-bromobenzene 1:

15.2g of 1,2-dimethoxybenzene were dissolved in 50ml of glacial acetic acid at room temperature. Pyridinium bromide (0.055 mol or 17.6g), was added in portion and the mixture was shaken at room temperature. The reaction was followed by TLC, then it was quenched by pouring the reaction mixture in a beaker of ice. The precipitate was collected in a Buchner funnel with suction until dry. The crystals were then recrystalized with methanol to give 18g (97%) of 1,2-dimethoxy-4-bromobenzene. M.p. 165 °C
^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 3.8(s, 6H), 6.6(d, 1H), 6.95(s, 1H), 7.00(d, 1H)

1.2. Synthesis of 3,4-methylenedioxy-bromobenzene 4:

1,2-methylenedioxybenzene (15.2g) were dissolved in 50ml of glacial acetic acid at room temperature. 0.055 mol or 17.6g of Pyridinium bromide was added in portion and the mixture was allowed to react at room temperature. The reaction was followed by TLC, then it was quenched by pouring the reaction mixture in a beaker of ice. The precipitate was collected in a Buchner funnel with suction until dry. The

crystals were then recrystalized with methanol to give 18g of 3,4-methylenedioxy-bromobenzene **4**. (M.p. 185-7 °C)

^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 5.95(s, 2H), 6.67(d, 1H), 6.93(d, 1H), 6.94(s, 1H)

^{13}C NMR (400 MHz, CDCl3, TMS): δ (ppm) 101, 109, 112, 113, 124, 146.5, 148.2

1.3. Synthesis of 1,2-dimethoxy-4-bromo-5-nitrobenzene 2:

In a clean and dry 125-ml Erlenmeyer flask, equipped with a magnetic bar, were added 25 ml of sulfuric acid and 1.15g of potassium nitrate. The mixture was then heated to 135 °C and 1, 2-dimethoxy-4-bromobenzene (2.0g) was added in portion. The mixture was then heated for 1 hour before being cooled to room temperature and poured into a beaker containing ice and water (400g/1000ml beaker). The precipitated crystals were collected in a Buchner funnel using two filter papers. The crystals were recrystallized in hot methanol to afford 1,2g of 1,2-dimethoxy-4-bromo-5-nitrobenzene **2**. M.p. 213 °C.

^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 3.95(S, 3H), 3.97(S, 3H), 6.60(S, 1H), 7.15(S, 1H)

1.4. Synthesis of 1,2-methylenedioxy-4-bromo-5-nitrobenzene 5

In a clean and dry 125-ml Erlenmeyer flask, equipped with a magnetic bar, were added 25 ml of sulfuric acid and 1.15g of potassium nitrate. The mixture was then heated to 135 °C and 1, 2-methylenedioxy-4-bromobenzene (2.0g) was added in portion. The mixture was then heated for 1 hour before being cooled to room temperature and poured into a beaker containing ice and water (400g/1000ml beaker). The precipitated crystals were collected in a Buchner funnel using two filter papers. The crystals were recrystallized in hot methanol to afford 1,2g of 1,2-methylenedioxy-4-bromo-5-nitrobenzene **5**. (M.p. 220-2 °C).

^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 6.18(s, 1H), 7.10(s, 1H), 7.41(s, 1H)

^{13}C NMR (400 MHz, CDCl3, TMS): δ (ppm) 103.4, 106, 108.2, 114, 143.5, 147.5, 151.6

1.5 Synthesis of 2,2'-dinitro-4,5-dimethoxy-4',5'-methylenedioxy biphenyl 6

In a 50ml oven-dried round-bottom flask equipped with a stirring bar and an oven-dried condenser topped with KOH guard, were mixed 1,2-methylenedioxy-4-bromo-5-nitrobenzene **5** (.500g - .002 mol) and 1,2-dimethoxy-4-bromo-5-nitrobenzene **2** (.500g - .002mol) in 40 ml of dry DMF. Copper powder was added and the mixture was refluxed for about 3 hrs. Then, the mixture was cooled and poured into a beaker containing ice and water. Extraction with ethyl acetate (2X200 mL) and washing with diluted acid (HCl 50%) provided organic layers that were combined and dried over Sodium sulfate anhydrous. Filtration of the drying agent and evaporation of the solvent under reduced pressure to afford an oil that was chromatographed in a gradient of 10% ethyl acetate in petroleum ether.

^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 3.95(s, 3H), 4.0(s, 3H), 6.20(s, 2H), 6.60(s, 1H), 6.61(s, 1H), 7.70(s, 1H), 7.80(s, 1H)

1.6. Synthesis of 2,3-dimethoxy-8,10-methylenedioxy benzo[c]cinnoline 7:

In a clean and oven-dried three-necked 250-ml round-bottomed flask (rbf), equipped with a magnetic bar, an addition funnel and a condenser fitted with a $CaCl_2$ drying guard and an nitrogen/argon gas inlet. In the addition funnel 2.20g of the biphenyl **6** were suspended in 60 ml of benzene. 100 mL of diethyl ether were added into the rbf and lithium aluminum hydride LAH (1.65g) in one portion. The rbf was cooled in an ice-water bath, before a slow, dropwise addition of the content of the funnel to the rbf under vigorous stirring. The mixture was then allowed to stir at room temperature for 1 hour before being cooled to ice-water temperature. Under an inert atmosphere, water was carefully added to the mixture to quench any excess of LAH. Solid NaOH was added to further dissolve the inorganic salts formed under these conditions. The solution was extracted with ethyl acetate (2X200mL) and washed with sodium bisulfite first and then with 20% aqueous hydrochloric acid, before being dried (anhydrous magnesium sulfate) and concentrated under reduced pressure to afford red-brick crystals of the benzo[c]cinnoline **7**. The crystals were recrystallized in hot methanol to afford 0.65g of 2,3-dimethoxy-8,10-methylenedioxy benzo[c]cinnoline **7**. M.p. > 220 C.

^{1}H NMR (400 MHz, CDCl3, TMS): δ (ppm) 3.95(s, 3H), 4.0(s, 3H), 6.20(s, 2H), 6.60(s, 1H), 6.61(s, 1H), 7.70(s, 1H), 7.80(s, 1H)

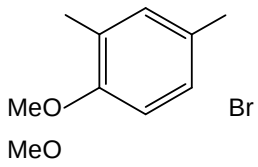
References

1. Banik BK and Becker FF, Curr. Med. Chem. 8; 1513-1533, 2001;
2. Banik BK and Becker FF, Eur J Med Chem. 45, 4687-4691, 2010;
3. Vichai V and Kirtikara K, Nat Protoc. 1, 1112-1116, 2006;
4. Bandyopadhyay B, Granados JC, Short JD and Banik BK, Oncology Letters 3, 45-49, 2012;
5. Leary JA, Lafleur AL, Liber HL and Blemann K, Anal. Chem. 55, 758-761, 1983;
6. Ashby J, styles JA and Paton D, Carcinogenesis 1, 1-7, 1980;
7. Hokelec T, acta Cryst. E57, o645-o647, 2001 and Refs cited therein;
8. Secüken, N.; Aksu, M. L.; Solak, A. O.; Kilicü, E. Turk. J. Chem. 2002, 26, 617.
9. (a) Hill, J. E.; Fanwick, P. E.; Rothwell, I. P. Inorg. Chem. 1991, 30, 1143.
10. Aubart, M. A.; Bergman, R. G. Organometallics 1999, 18, 811.
11. Lentz, M. R.; Fanwick, P. E.; Rothwell, I. P. Chem. Commun. 2002, 2482.
12. Mercando, L. A.; Handwerker, B. M.; MacMillan, H. J.; Geoffroy, G. L. Organometallics 1993, 12, 1559.
13. Emslie, D. J. H.; Piers, W. E.; Parvez, M. Angew. Chem., Int. Ed. 2003, 42, 1252.
14. Komorsky-Lovric´, Sÿ. Electroanalysis 2002, 14, 888.
15. Choi, B. K.; Yamamoto, T. Electrochem. Commun. 2003, 5, 566.
16. Mugnier, Y.; Laviron, E. J. Org. Chem. 1988, 53, 5781.
17. Durmusü, Z.; Solak, A. O.; Durmusü, S.; Kilicü, E. Talanta 2001, 55, 357.
18. Ho¨kelek, T.; Kilicü, E.; Dincüer, S. Acta Crystallogr. 2001, E57, O645.
19. Wang, J. C. Annu. Rev. Biochem. 1985, 54, 665.
20. Liu, L. Annu. Rev. Biochem. 1989, 58, 351.
21. Chen, A. Y.; Liu, L. F. Annu. Rev. Pharmacol. Toxicol. 1994, 34, 191.

22. Li, T.-K.; Liu, L. F. Annu. Rev. Pharmacol. Toxicol. 2001, 41, 53.
23. Liu, L. F.; Wang, J. C. Proc. Natl. Acad. Sci. U.S.A. 1987, 84, 7024.
24. Tsao, Y.-P.; Wu, H.-Y.; Liu, L. F. Cell 1989, 56, 111.
25. Li,D.; Makhey,D. B.; Zhao, B.; Sim, S.-P.; Liu, A.; Li, L. F.; LaVoie, E. J. Proc. Amer. Assoc. Res. 2001, 42, 720.
26. Makhey, D.; Li, D.; Zhao, B.; Sim, S.-P.; Lin, T. K.; Liu, A.; Liu, A. F.; LaVoie, E. J. Bioorg. Med. Chem., In press.

Supplemental Information – Spectral Data

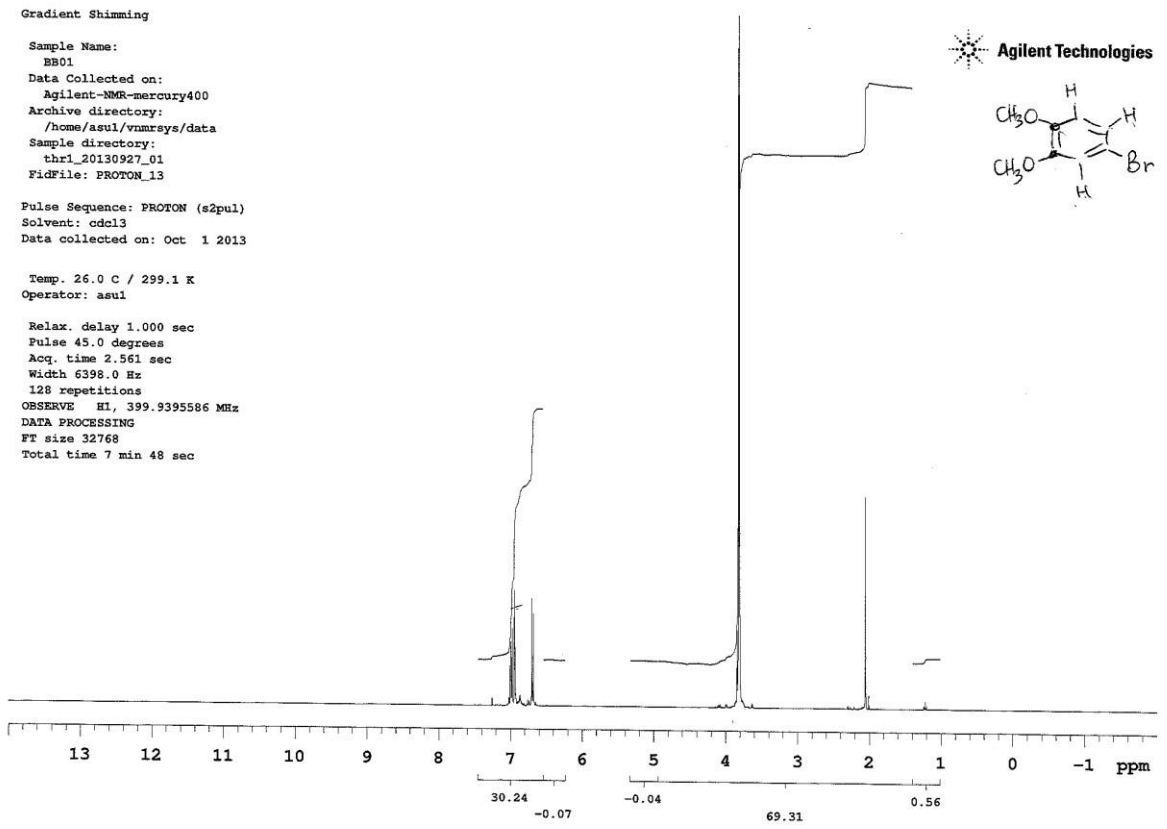
1. 3,4-Dimethoxy-bromobenzene 1



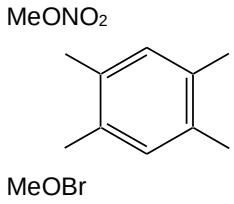
Chemical Formula: $C_8H_9BrO_2$ Exact Mass: 215.98

Molecular Weight: 217.06

Elemental Analysis: C, 44.27; H, 4.18; Br, 36.81; O, 14.74



2. 1,2-Dimethoxy-4-bromo-5-nitrobenzene **3**



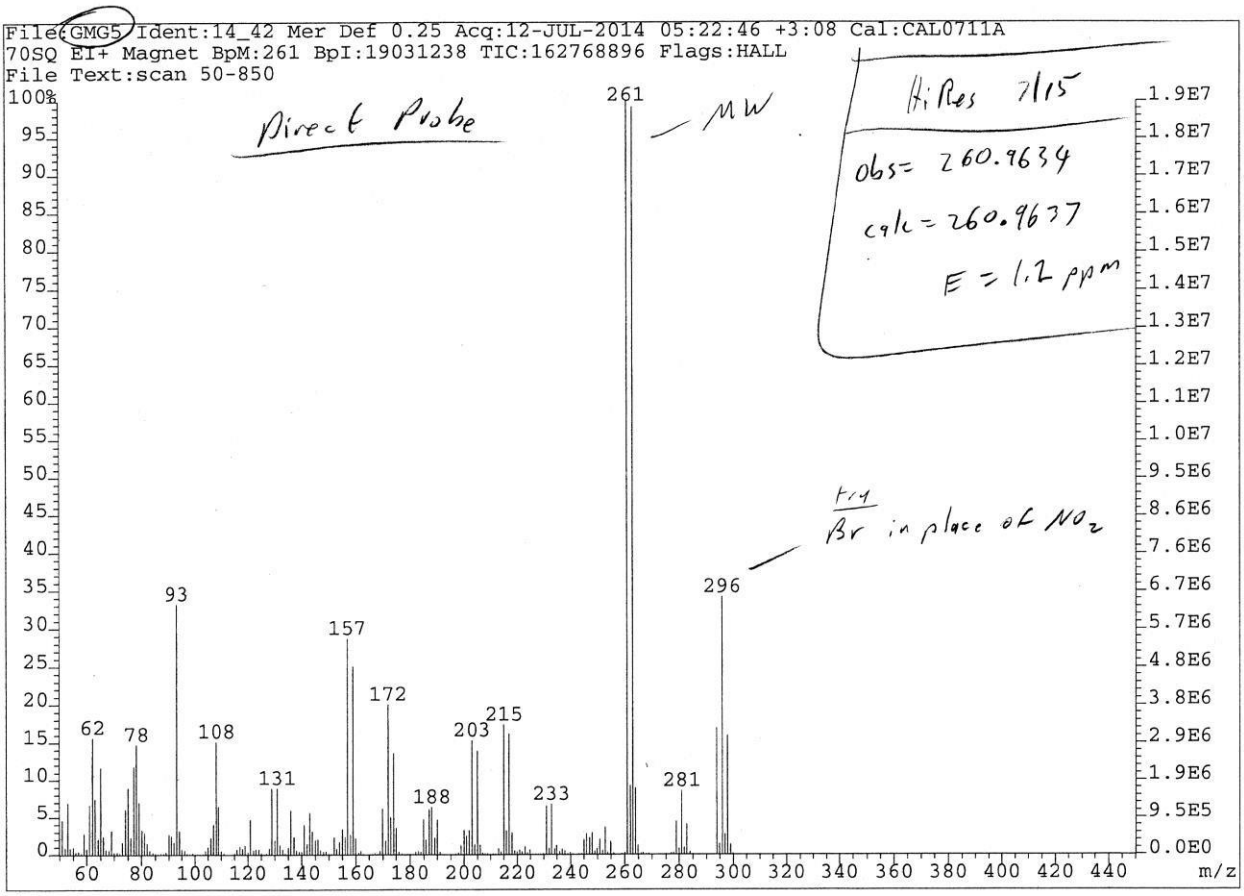
3

$C_8H_8BrNO_4$

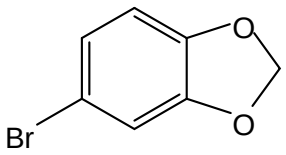
Exact Mass: 260.96

Mol. Wt.: **262.06**

C, 36.67; H, 3.08; Br, 30.49; N, 5.34; O, 24.42



3. 3,4-Methylenedioxy-bromobenzene 4



4

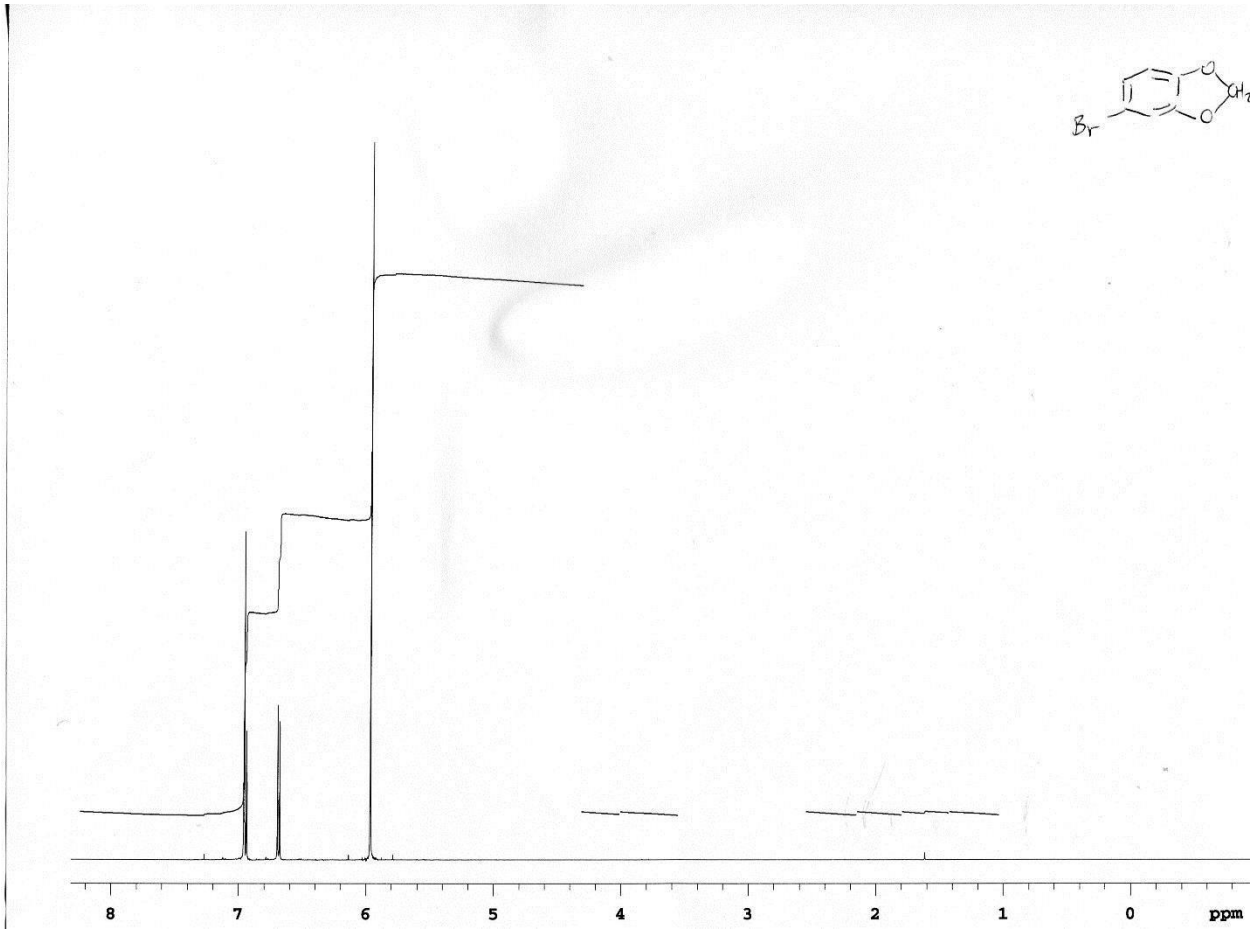
Chemical Formula: $C_7H_5BrO_2$

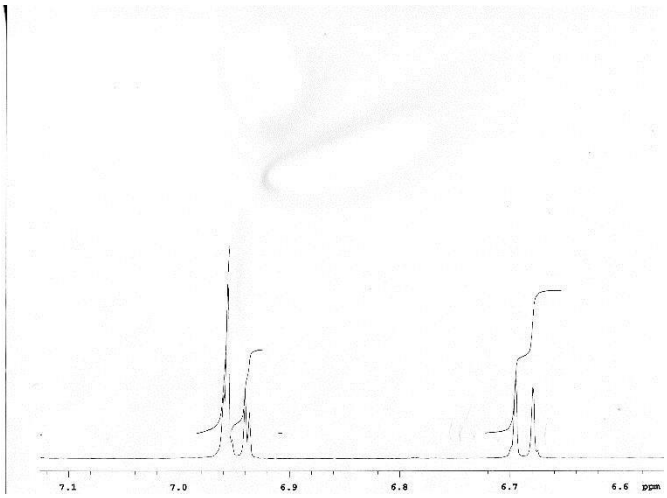
Exact Mass: 199.95

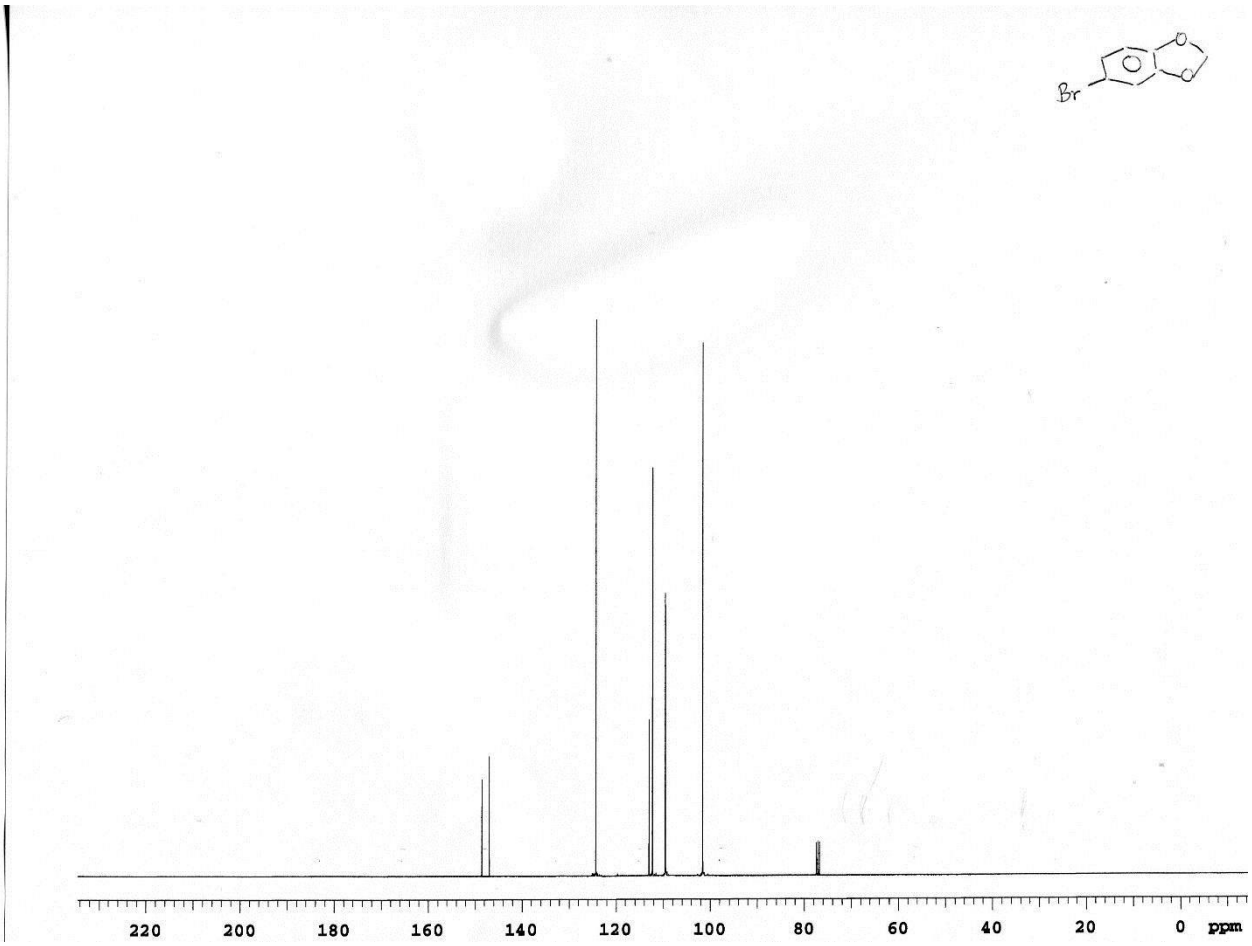
Molecular Weight: 201.02

ElementalAnalysis: C, 41.82; H, 2.51; Br, 39.75;

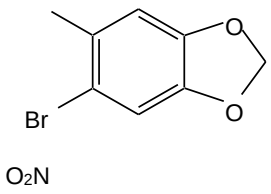
O, 15.92


Br
O
O
CH2
8
7
6
5
4
3
2
1
0
ppm


7.1
7.0
6.9
6.8
6.7
6.6
ppm



4. 1,2-methylenedioxy-4-bromo-5-nitrobenzene 5



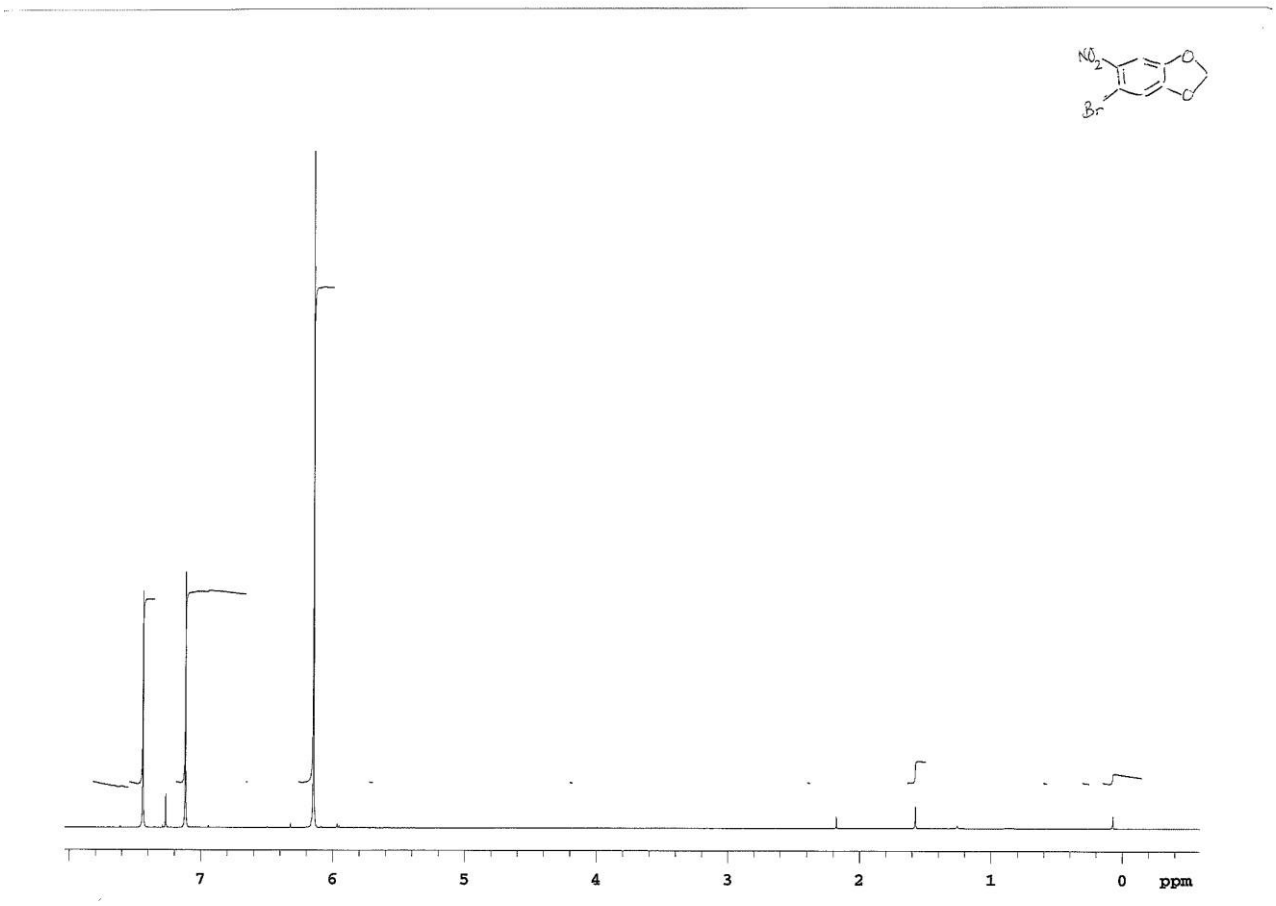
5

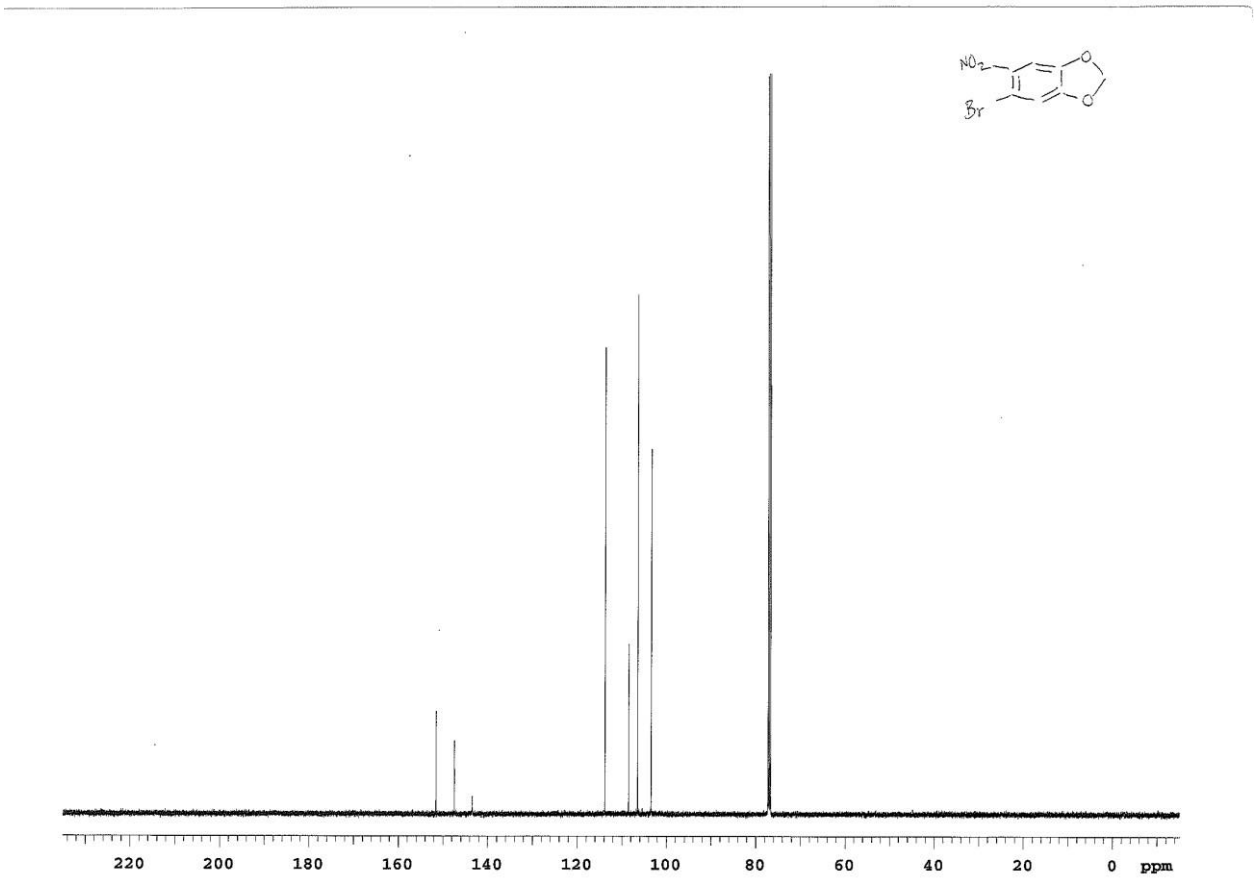
$C_7H_4BrNO_4$

Exact Mass: 244.93

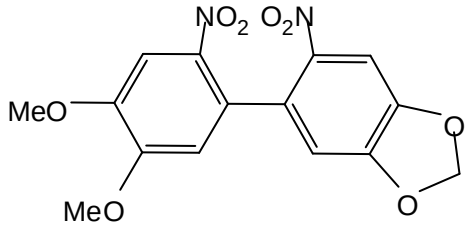
Mol. Wt.: **246.02**

C, 34.17; H, 1.64; Br, 32.48; N, 5.69; O, 26.01


NO2
O
O
Br
7
6
5
4
3
2
1
0
ppm


NO2
Br
220
200
180
160
140
120
100
80
60
40
20
0
ppm

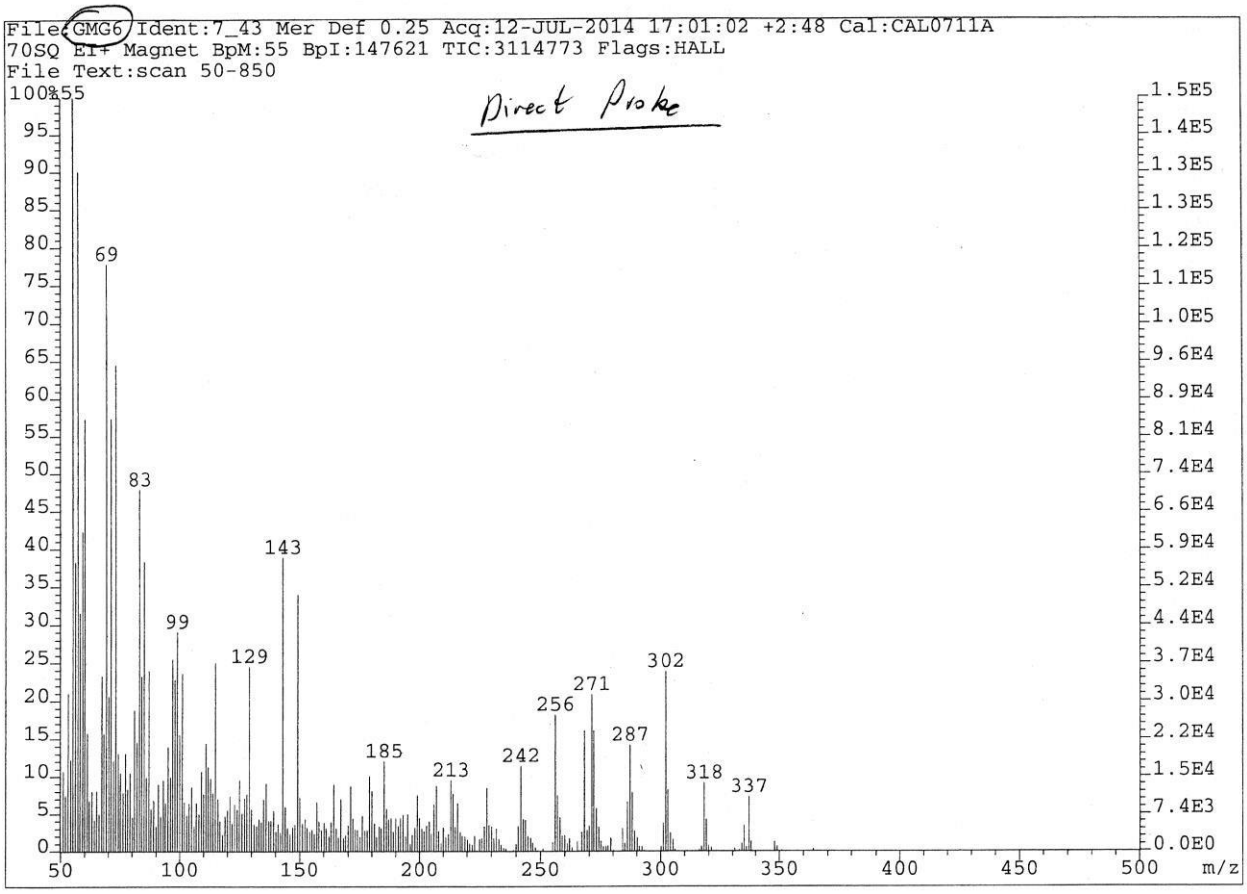
5. **2,2'-dinitro-4,5-dimethoxy-4',5'-methylenedioxy biphenyl 6**

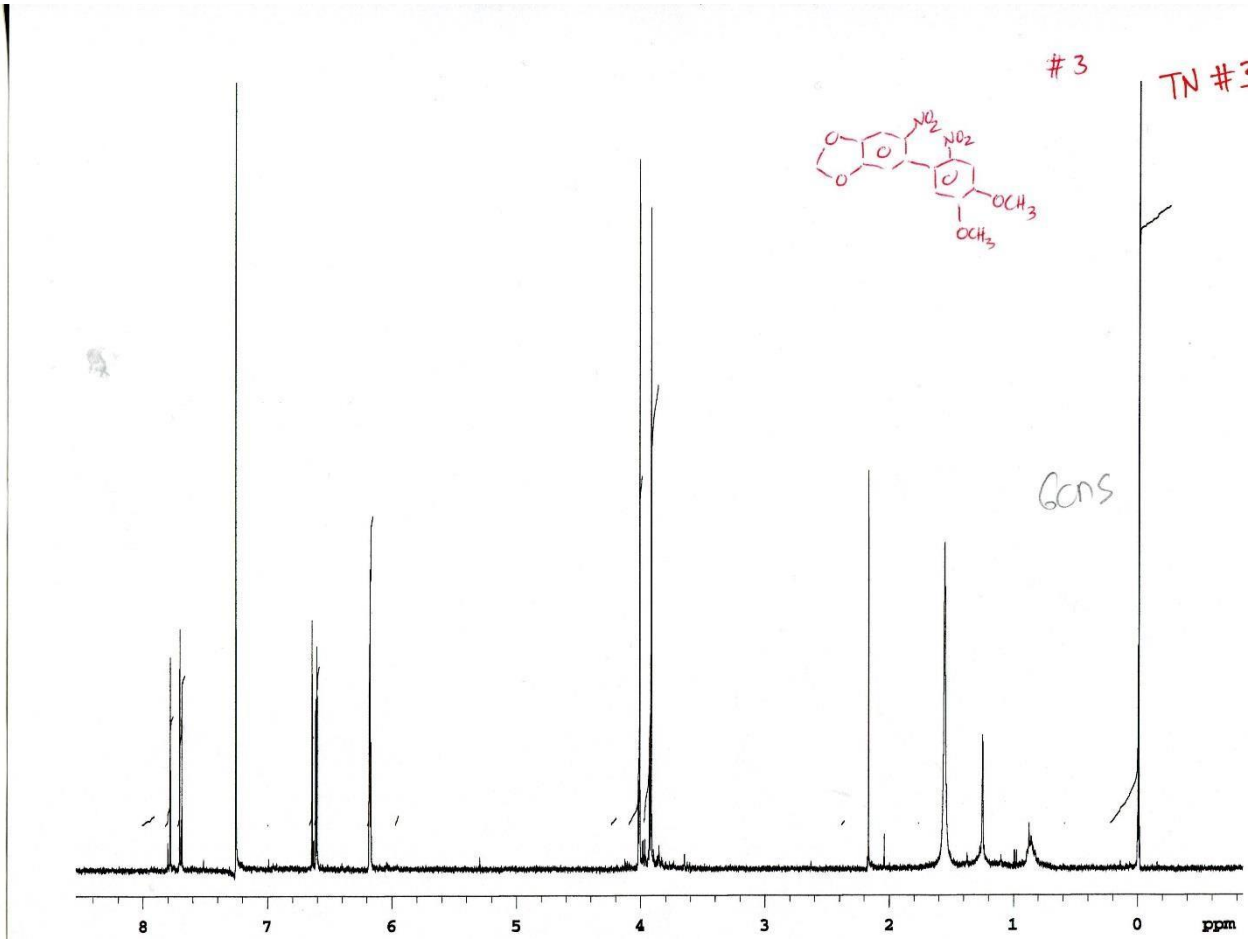


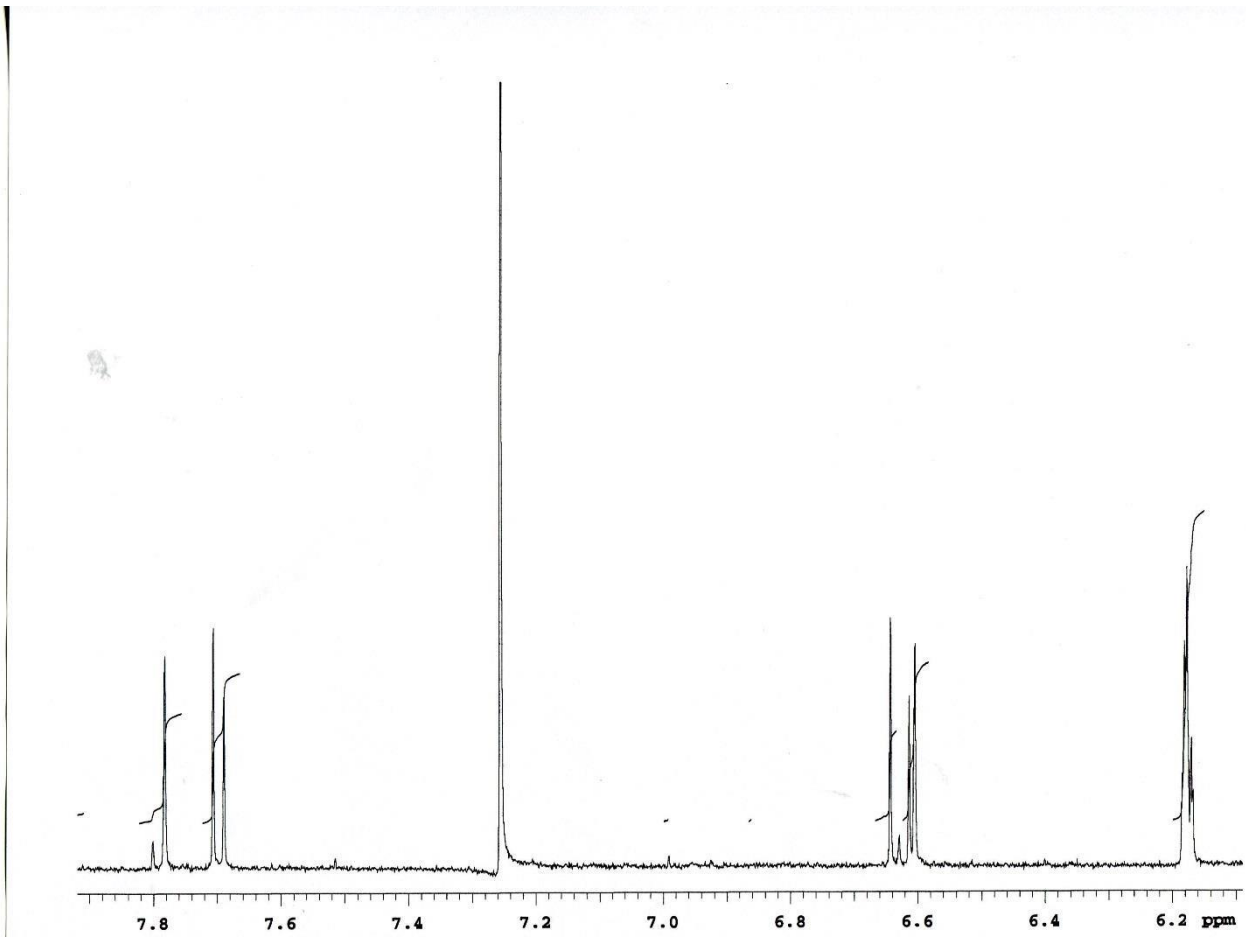
Chemical Formula: $C_{15}H_{12}N_2O_8$ Exact Mass: 348.06

Molecular Weight: 348.26

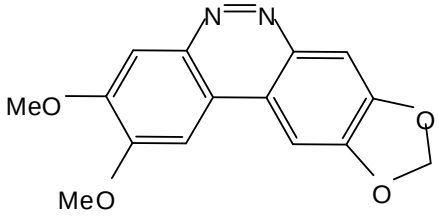
Elemental Analysis: C, 51.73; H, 3.47; N, 8.04; O, 36.75




#3
TN #3
6cns
8
7
6
5
4
3
2
1
0
ppm



6. **2,3-dimethoxy-8,10-methylenedioxy benzo[c]cinnoline 7:**

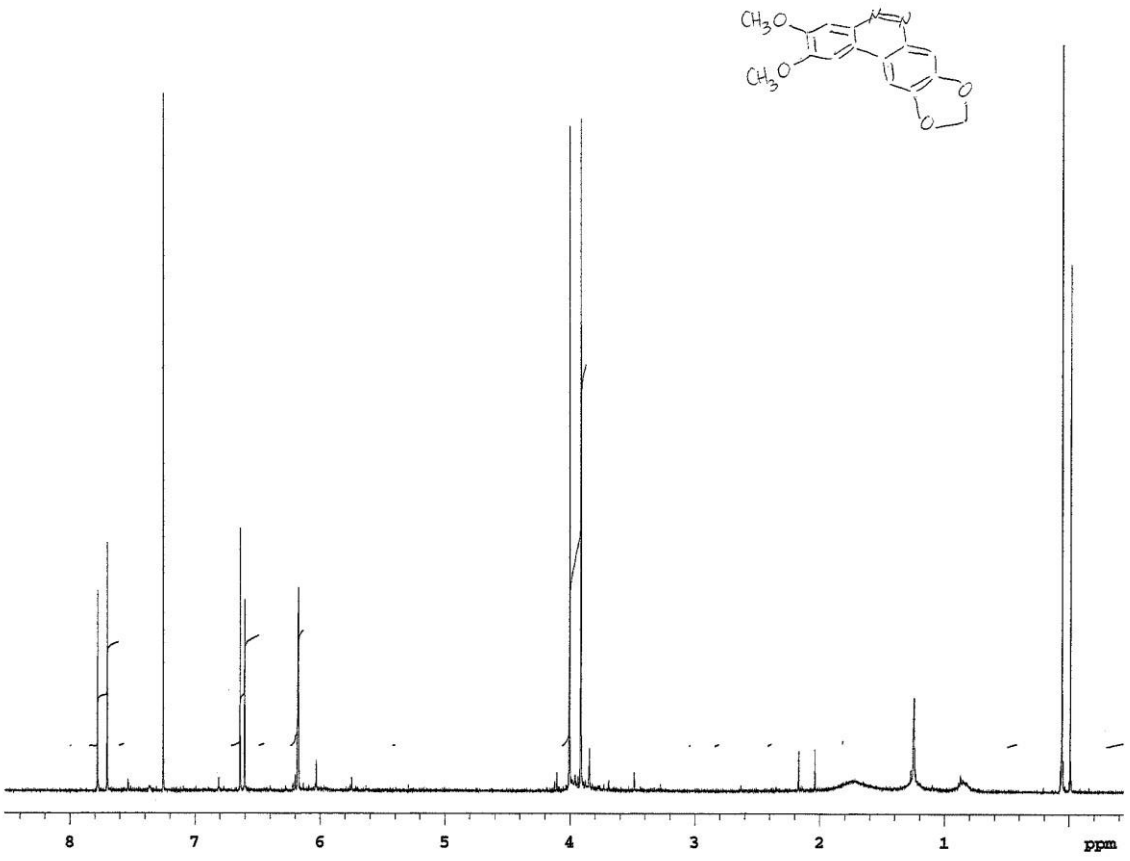


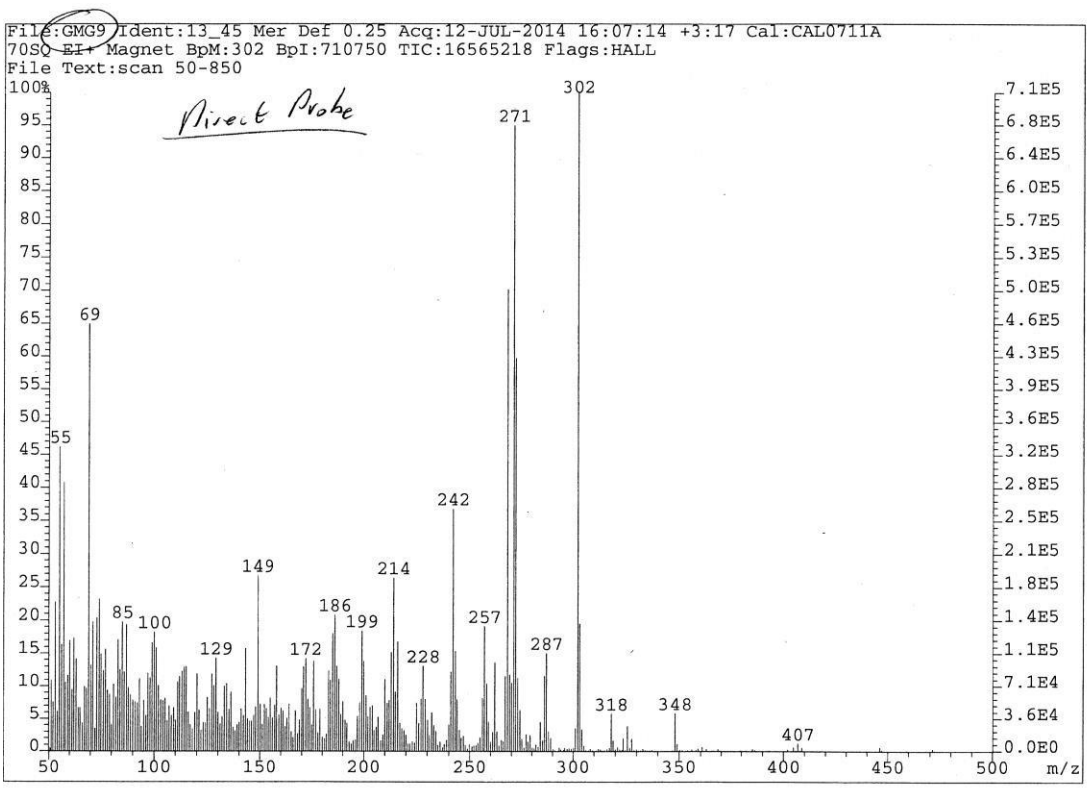
Chemical Formula: $C_{15}H_{12}N_2O_4$

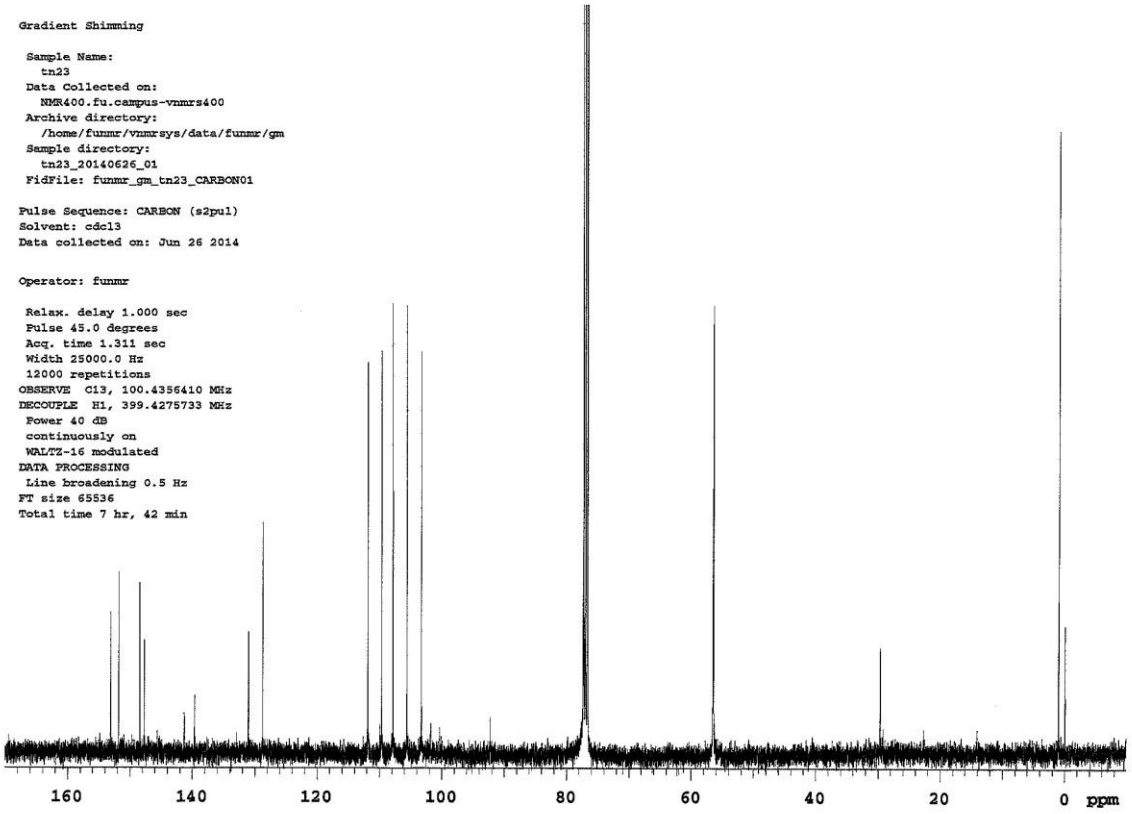
Exact Mass: 284.08

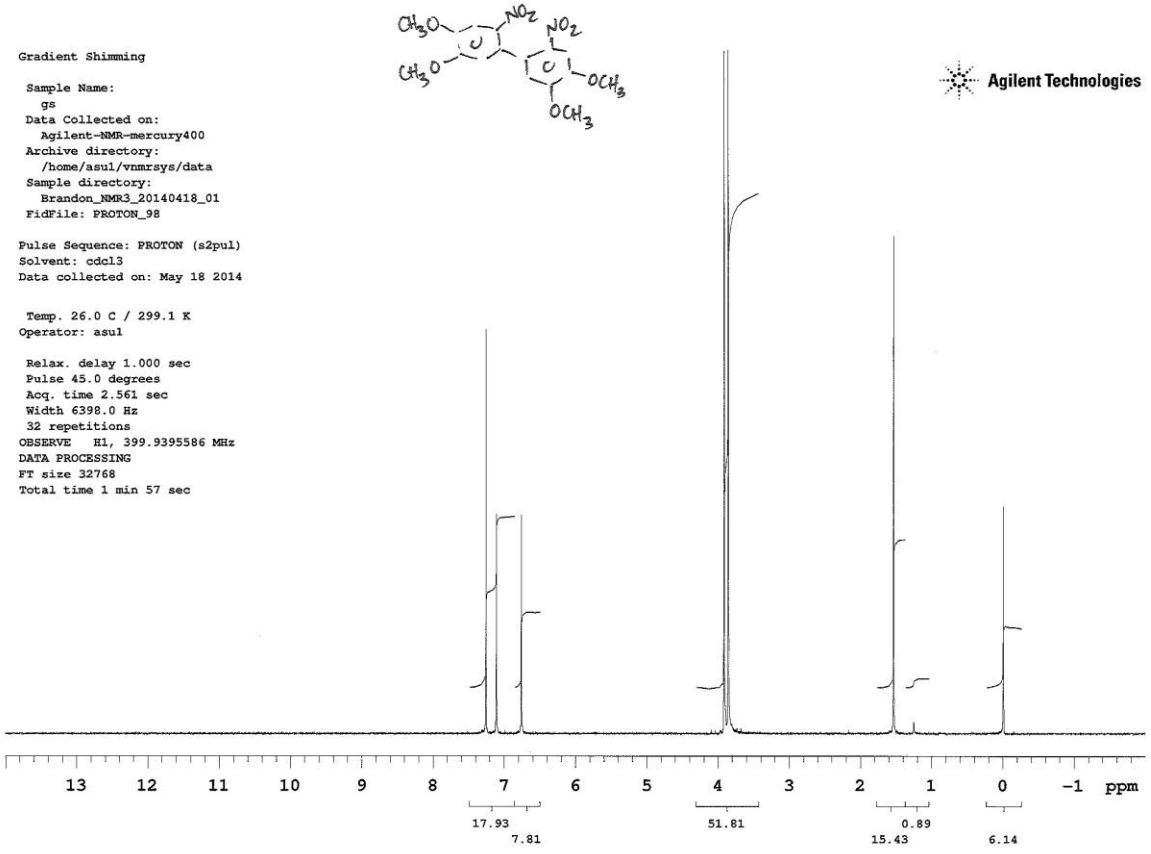
Molecular Weight: 284.27

Elemental Analysis: C, 63.38; H, 4.25; N, 9.85; O, 22.51



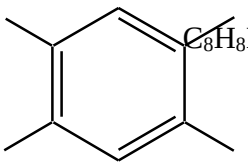

File:GMG9 Ident:13_45 Mer Def 0.25 Acq:12-JUL-2014 16:07:14 +3:17 Cal:CAL0711A
70SQ EI+ Magnet BpM:302 BpI:710750 TIC:16565218 Flags:HALL
File Text:scan 50-850
Direct Probe
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
7.1E5
6.8E5
6.4E5
6.0E5
5.7E5
5.3E5
5.0E5
4.6E5
4.3E5
3.9E5
3.6E5
3.2E5
2.8E5
2.5E5
2.1E5
1.8E5
1.4E5
1.1E5
7.1E4
3.6E4
0.0E0
302
271
69
55
242
149
214
85
100
186
199
257
129
172
228
287
318
348
407
50
100
150
200
250
300
350
400
450
500
m/z


Gradient Shimming
Sample Name:
tn23
Data Collected on:
NMR400.fu.campus-vnmrs400
Archive directory:
/home/funmr/vnmrsys/data/funmr/gm
Sample directory:
tn23_20140626_01
FidFile: funmr_gm_tn23_CARBON01
Pulse Sequence: CARBON (s2pul)
Solvent: cdcl3
Data collected on: Jun 26 2014
Operator: funmr
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.311 sec
Width 25000.0 Hz
12000 repetitions
OBSERVE C13, 100.4356410 MHz
DECOUPLE H1, 399.4275733 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 7 hr, 42 min
160
140
120
100
80
60
40
20
0
ppm



H3COBr

Exact Mass: **260.96**

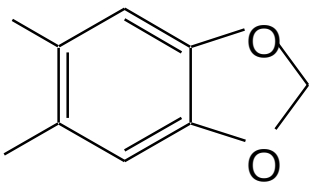


$C_8H_8BrNO_4$

H3CO **G5** NO2

Mol. Wt.: 262.06

C, 36.67; H, 3.08; Br, 30.49; N, 5.34; O, 24.42



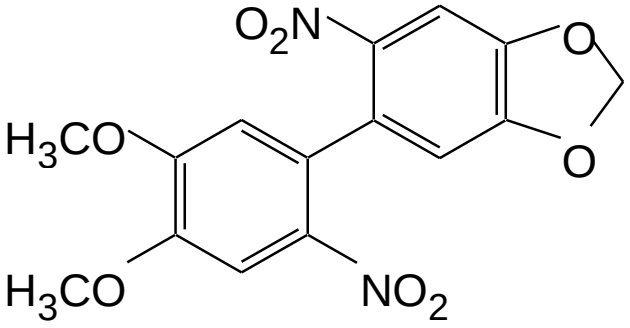
O2N$C_7H_4BrNO_4$

Exact Mass: **244.93**

Mol. Wt.: 246.02

BrC, 34.17; H, 1.64; Br, 32.48; N, 5.69; O, 26.01

G4

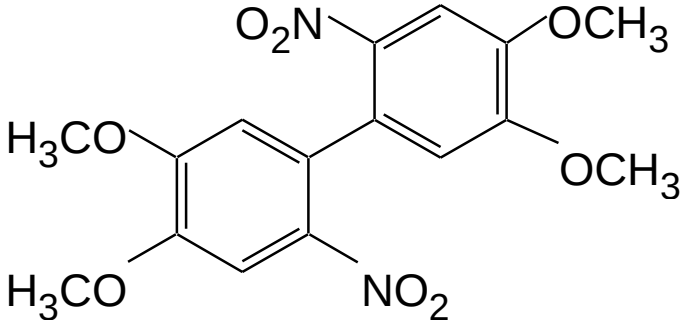


$C_{15}H_{12}N_2O_8$

Exact Mass: **348.06**

Mol. Wt.: 348.26

C, 51.73; H, 3.47; N, 8.04; O, 36.75



G6

$C_{16}H_{16}N_2O_8$

Exact Mass: **364.09**

Mol. Wt.: 364.31

C, 52.75; H, 4.43; N, 7.69; O, 35.13

G3