Supporting Information

Polybrominated diphenyl ethers: structure determination and trends in antibacterial activity

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Contents:

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Table S1. ^{1}H and ^{13}C NMR data of **3a** and **5a** in $CDCl_3$ at 298 K

	3a		**5a**	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		150.9, C		150.1, C
2		145.7, C		147.9, C
3		119.3, C		119.5, C
4	7.40 d (2.2)	129.7, CH	7.53 d (2.1)	131.6, CH
5		116.8, C		117.0, C
6	6.48 d (2.2)	117.1, CH	6.96 d (2.1)	122.3, CH
1’		150.5, C		148.8, C
2’		146.5, C		149.1, C
3’		120.8, C	7.15 s	122.9, CH
4’		120.9, C		120.1, C
5’	7.82 s	133.9, CH		123.4, C
6’		117.9, C		120.1, C
2-OCH_3	3.81 s, 3H	61.7, CH_3	3.89 s, 3H	61.4, CH3
1’-OCH_3	4.00 s, 3H	61.4, CH3	3.87 s, 3H	61.4, CH3

Figure S1. ^{1}H NMR spectrum of **8** in $CDCl_3$

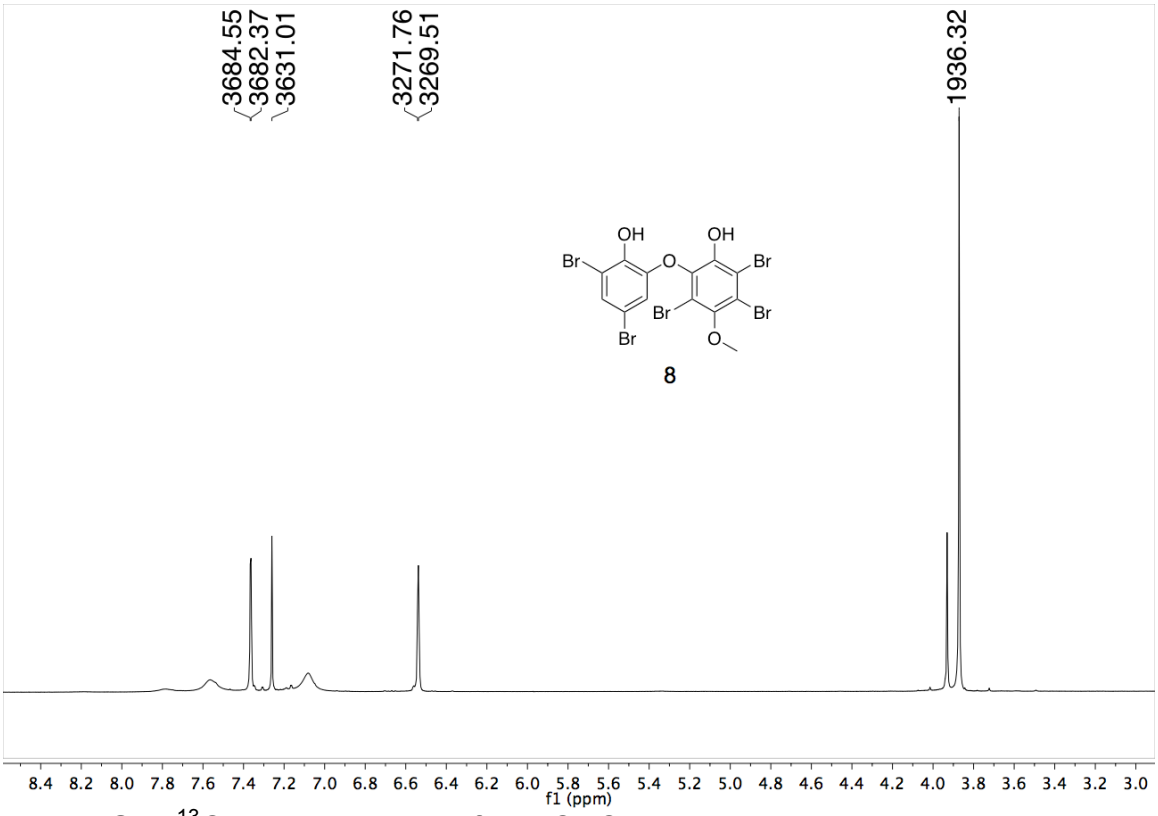


Figure S2. ^{13}C NMR spectrum of **8** in $CDCl_3$

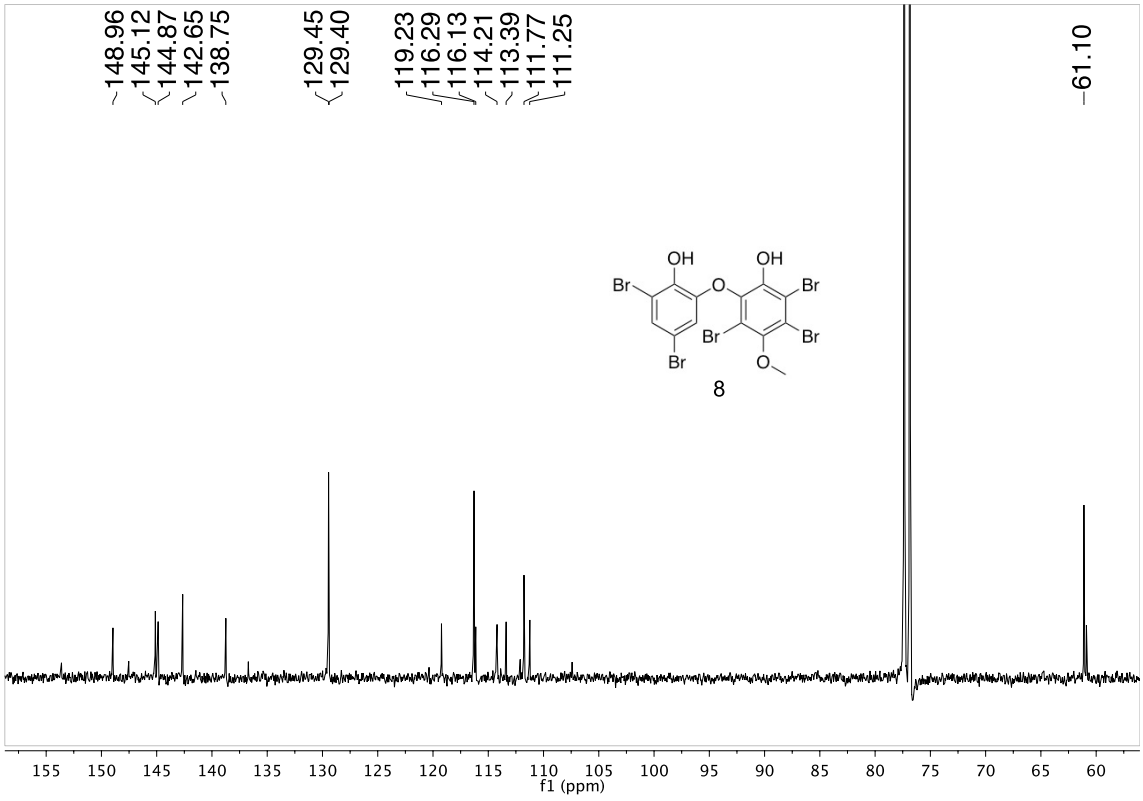


Figure S3. HSQC spectrum of **8** in $CDCl_3$

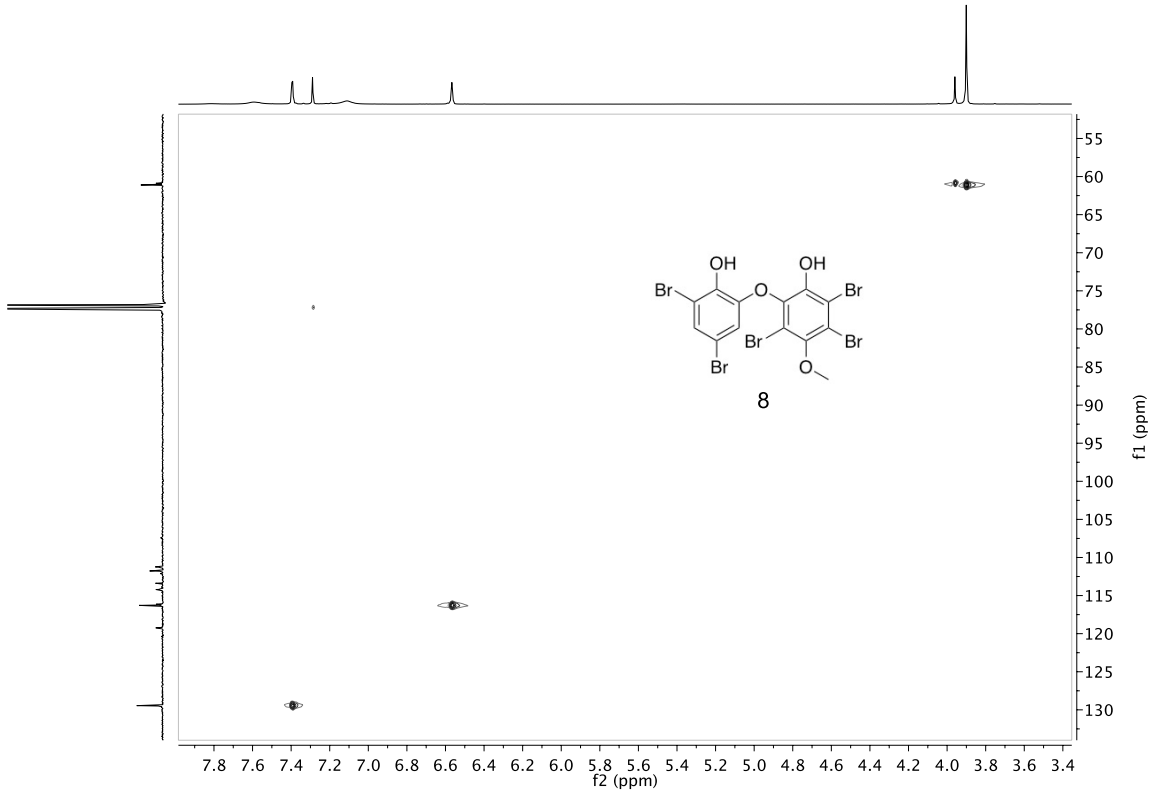


Figure S4. HMBC spectrum of **8** in $CDCl_3$

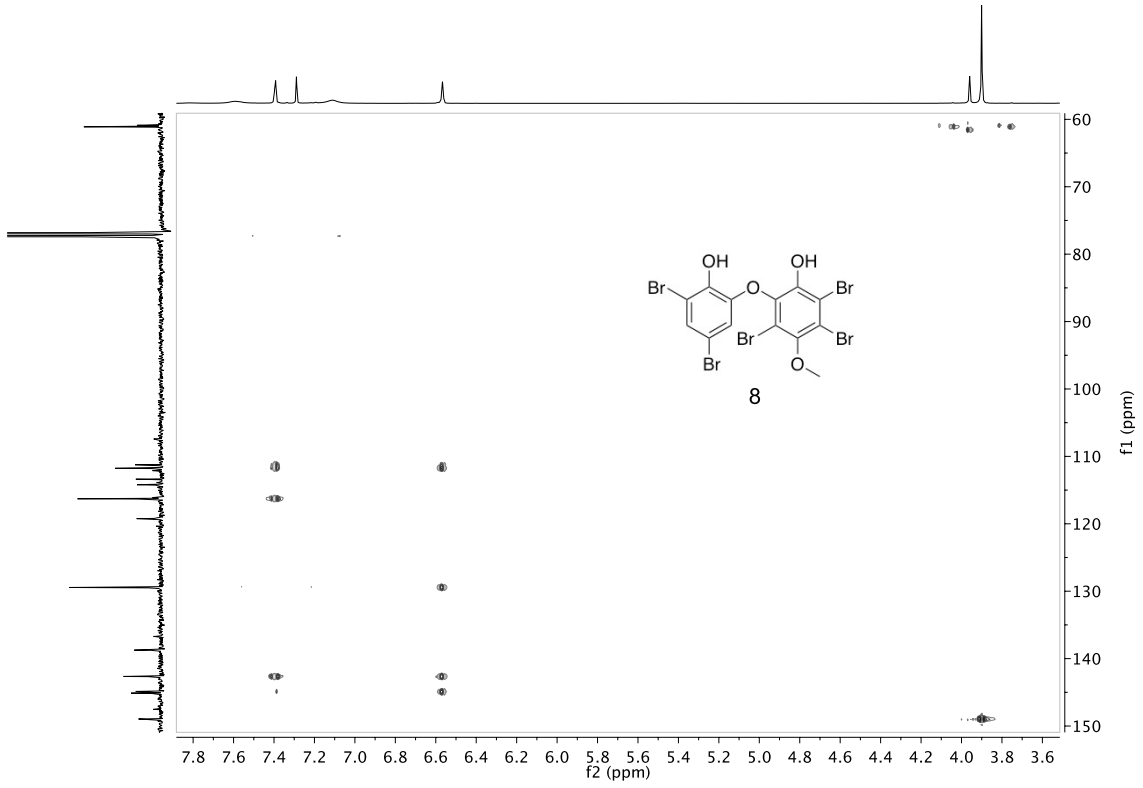


Figure S5. IR spectrum of **8**

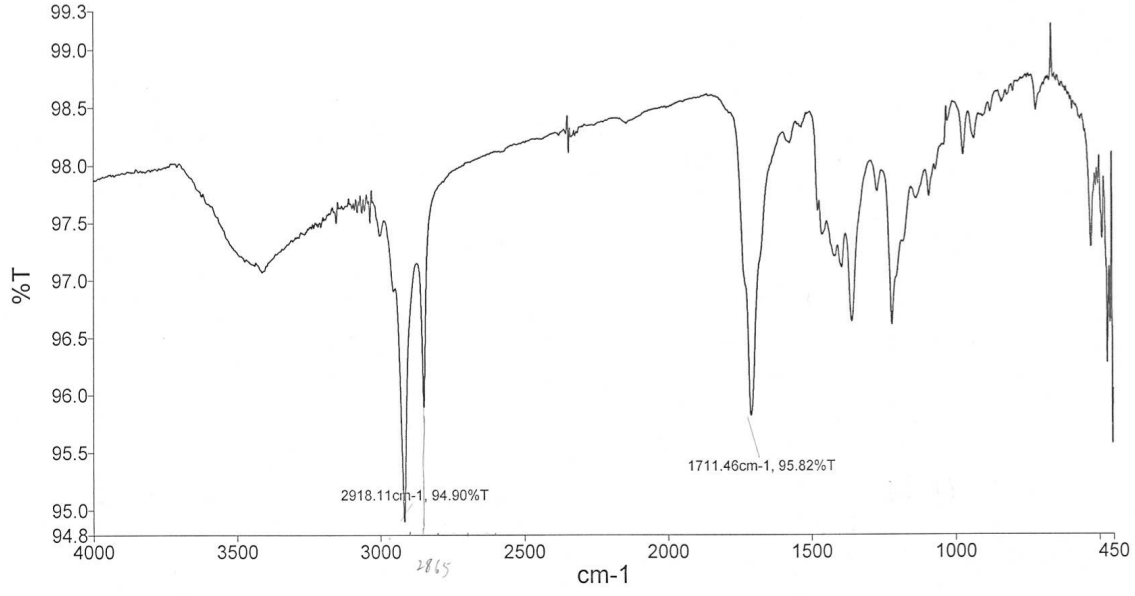


Figure S6. HRESIMS spectrum of **8**

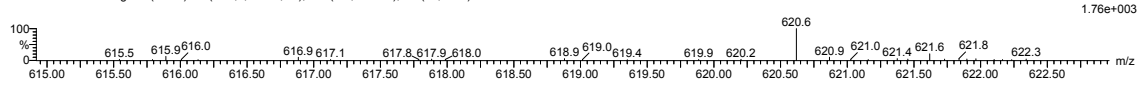
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
29 formula(e) evaluated with 2 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 O: 0-50 79Br: 5-5

11-Feb-2015
hbl-11feb15-25-25-neg 79 (1.461) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES-
1.76e+003



Minimum:				-2.0		
Maximum:		10.0	10.0	500.0		
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**Formula**
620.6187	620.6183	0.4	0.6	8.5	n/a	C13 H6 O4 79Br5
	620.6242	-5.5	-8.9	-0.5	n/a	C6 H10 O9 79Br5

Figure S9. ^{1}H NMR spectrum of **8a** in $CDCl_3$

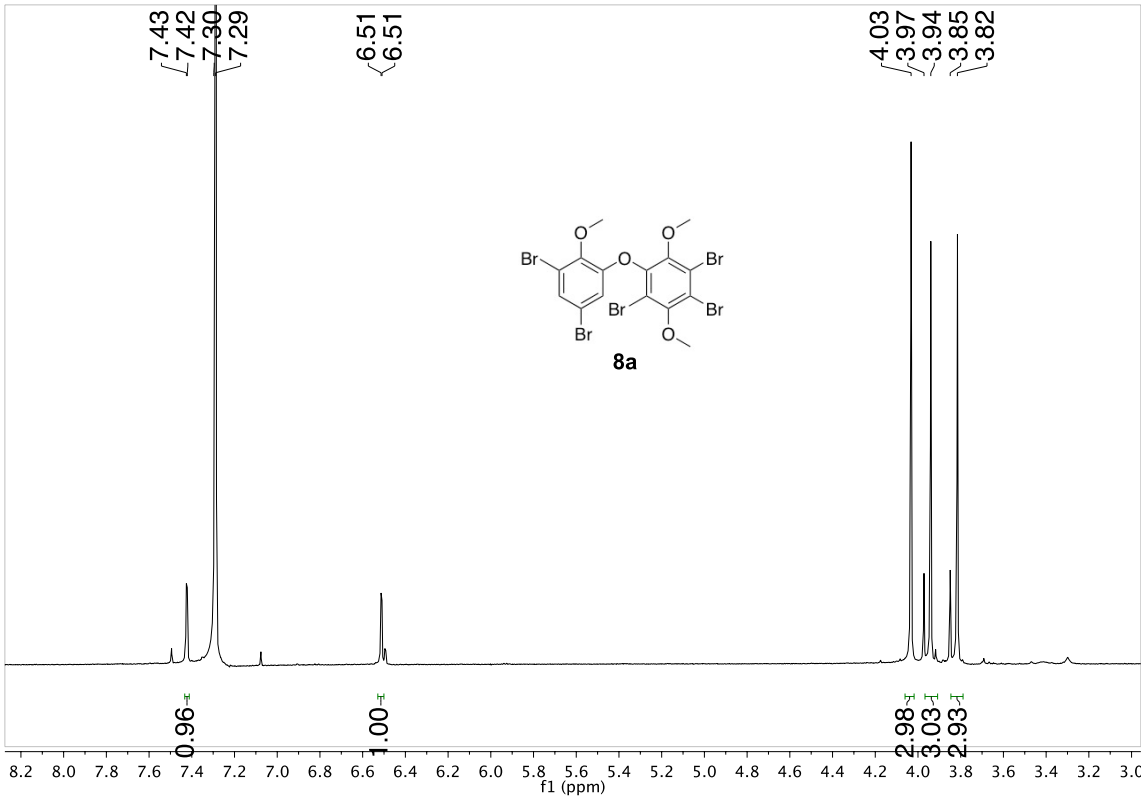


Figure S10. ^{13}C NMR spectrum of **8a** in $CDCl_3$

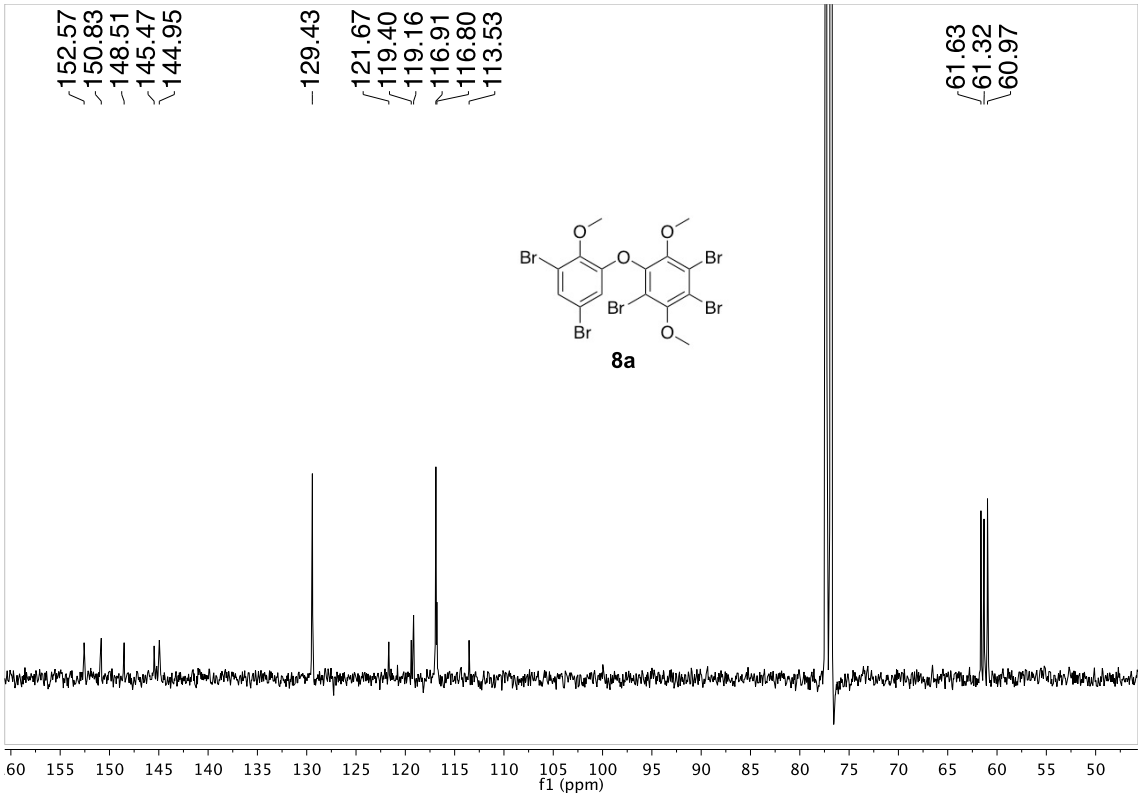


Figure S11. HSQC spectrum of **8a** in $CDCl_3$

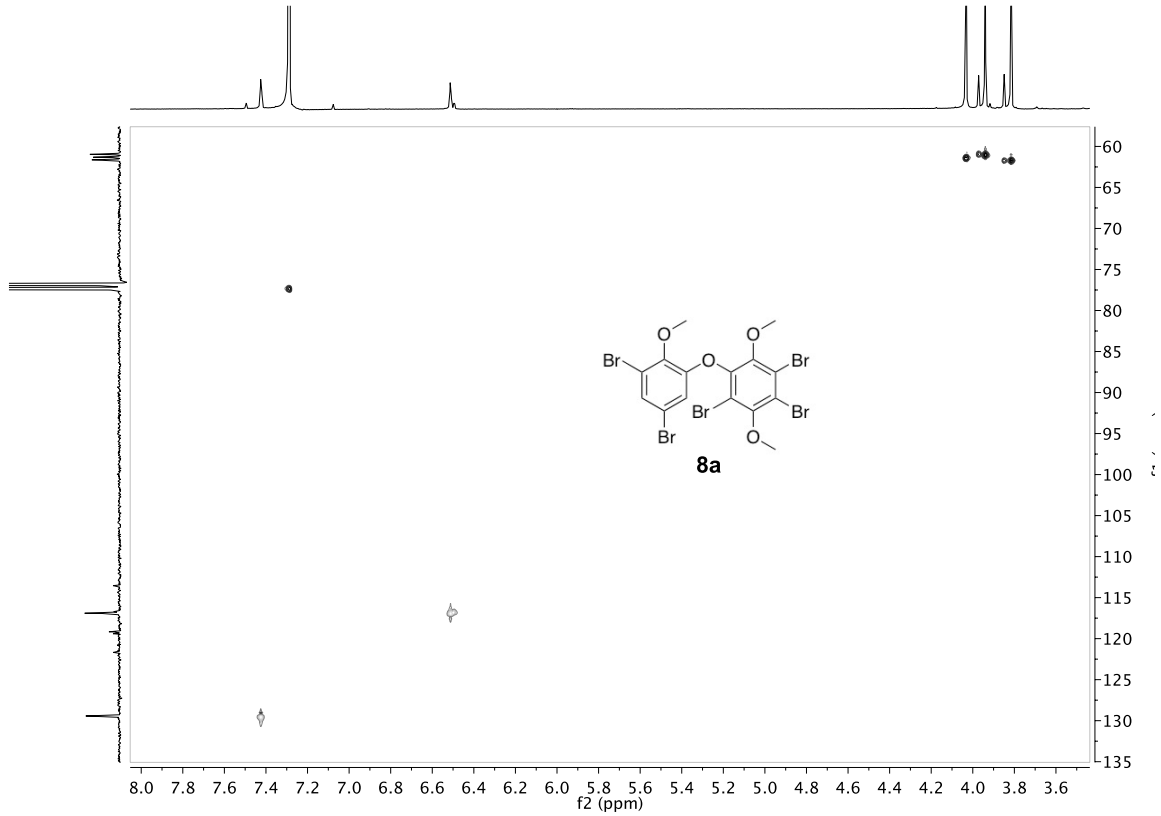


Figure S12. HMBC spectrum of **8a** in $CDCl_3$

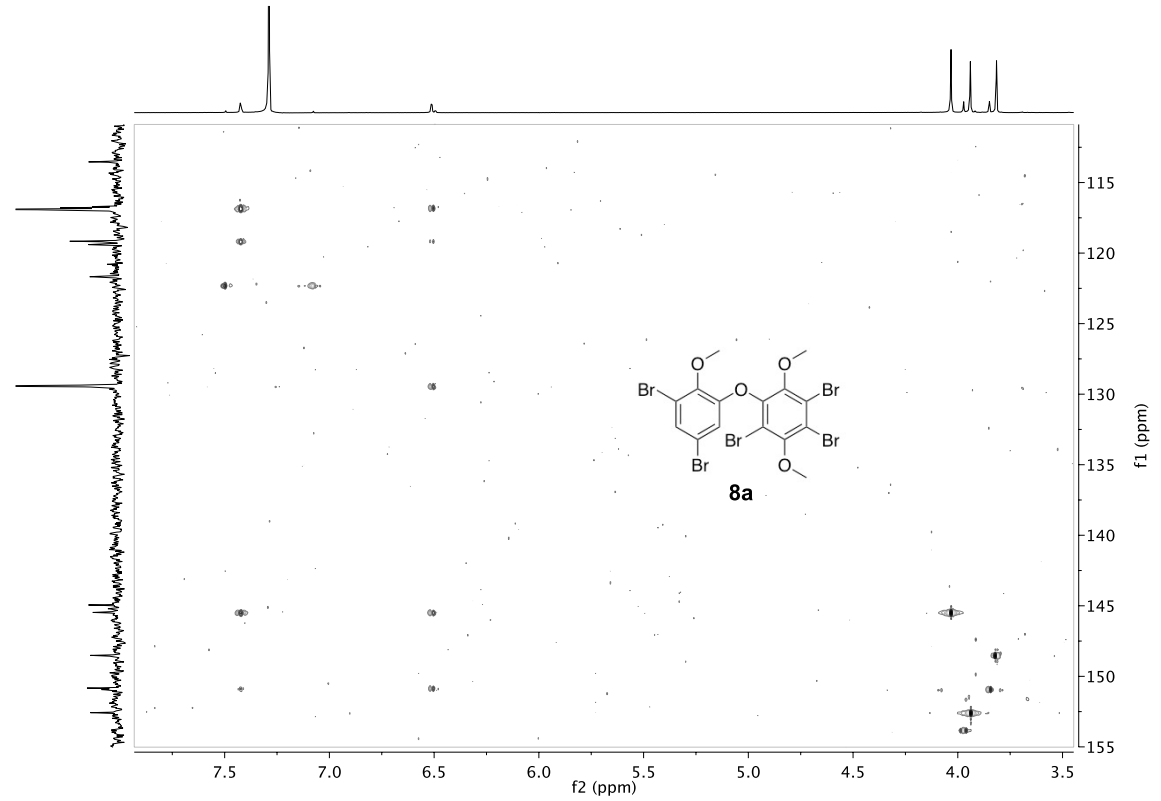


Figure S13. Selective 1-D NOE spectra of **8** and **8a**.

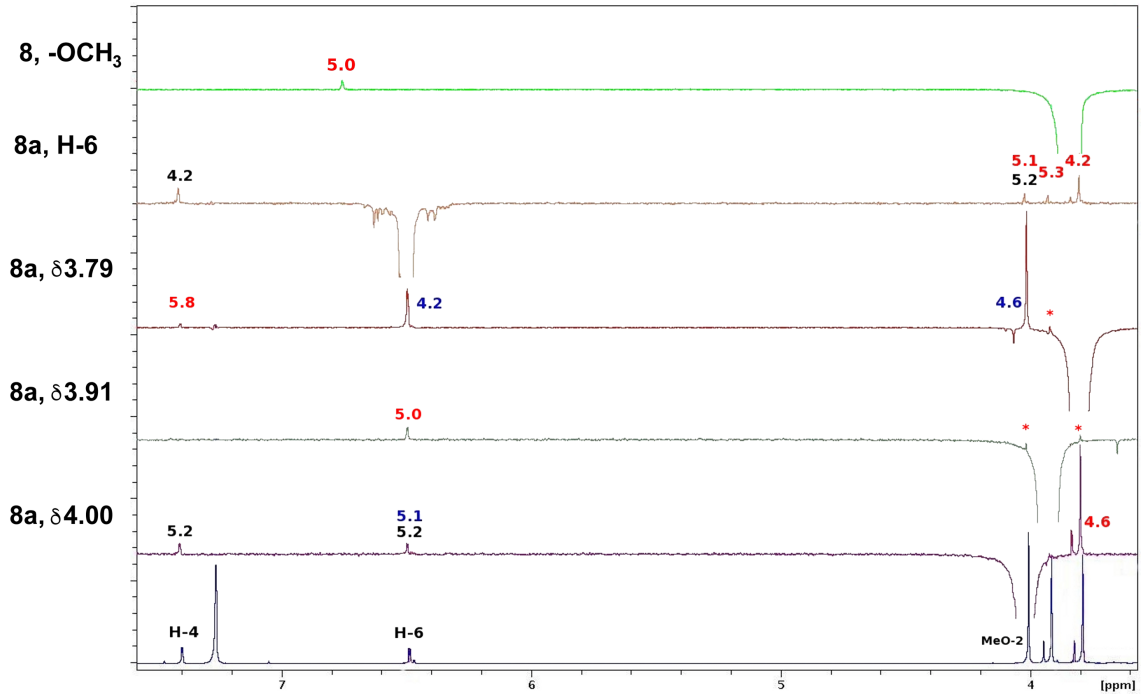


Figure S7. 1D-DPFGSE-NOE spectra of **8** (top only) and **8a**. The bottom spectrum serves as a reference to which the NOE selected resonances have been similarly scaled. For clarity the spectra have been expanded to include selected resonances. Numbers in black are distances (Å) known from molecular geometry (methyl 5.2 Å = $<1/r^6>$); numbers in blue correspond to the complimentary NOE experiment; and those in red are calculated distances. The asterisks designate very weak NOE responses that correspond to >7 Å. If comparing heights, the H-4 and H-6 responses should be approximately doubled to account for line width. The difference between known 5.2 and calculated 5.1 Å for the ring A methoxy to H-6 distance (second to top spectrum) is suggestive of the error in the calculations (less than 2%).

1D NOE analysis for ring B. To investigate the substitution pattern of hydroxy, the naturally occurring methoxy and bromine atoms in ring B, we recorded transient 1D-NOE spectra for **8** and **8a** wherein each proton was irradiated in individual spectra. NOE peak intensities were integrated and distances calculated as a function of $<1/r^6>$ by comparing to experimental intensities between atoms with known distances. Along with the molecular connectivity described in the paper the NOEs led to some specific conclusions. First, the NOE from the two B ring methoxy groups (Me^B) suggested that they were separated by >6 Å, which ruled out an ortho relationship to one another and, with the exception of the 1'/5' configuration, conformers with the Me^Bs on the same side of the plane of the aromatic ring. Further analysis indicated that the methoxy at δ_H 3.79 was close to both the C-2 $-OCH_3$

and H-6 (4.6 and 4.2 Å, respectively), and the other methoxy at δ_H 3.91 was ~6 Å from C-2 –OCH_3 and 5 Å from H-6. This eliminated methoxy substitution at C-1'/C-3' (where both methoxy groups are always close to the methoxy and H-6 in ring A) and C-4'/C-6' (in which no conformer is close to both) but could not unambiguously distinguish between the C-1'/C-4' and C-1'/C-5' positions. This left either the 1'/4' or 1'/5' arrangement with the 1'-Me^B positioned close to both Me^A and H-6. For both of these, the ether bond rotational barrier (MM2 level) is ~12 kcal/mol, thus rotating on the NMR timescale, and ~2 kcal/mol advantage (>95% of population) for the lowest energy conformer which has H-6 positioned over ring-B as opposed to C-2 –OCH_3. This major conformer is consistent with the shielded shift of H-6, comparatively deshielded shift of Me^A, with one Me^B located close and one distant to Me^A, and the H-6 contact with both Me^Bs. Considering this conformation, a 5'-Me^B would be over 8 Å from Me^A whereas a 4'-Me^B would be roughly equidistant (6-7 Å) from Me^A and 1'-Me^B, which is consistent with the NOE data and the 4' shift change, vide infra. The similar NOE response from the methoxy at δ_H 3.91 in **8** compared to **8a** suggested that this was the original Me^B. Finally, a shorter ^{13}C-T_1 relaxation time for the resonance at δ_C 119, the chemical shift itself, and the changes in chemical shift were consistent with the Br resonance assignments.

Figure S14. IR spectrum of **8a**

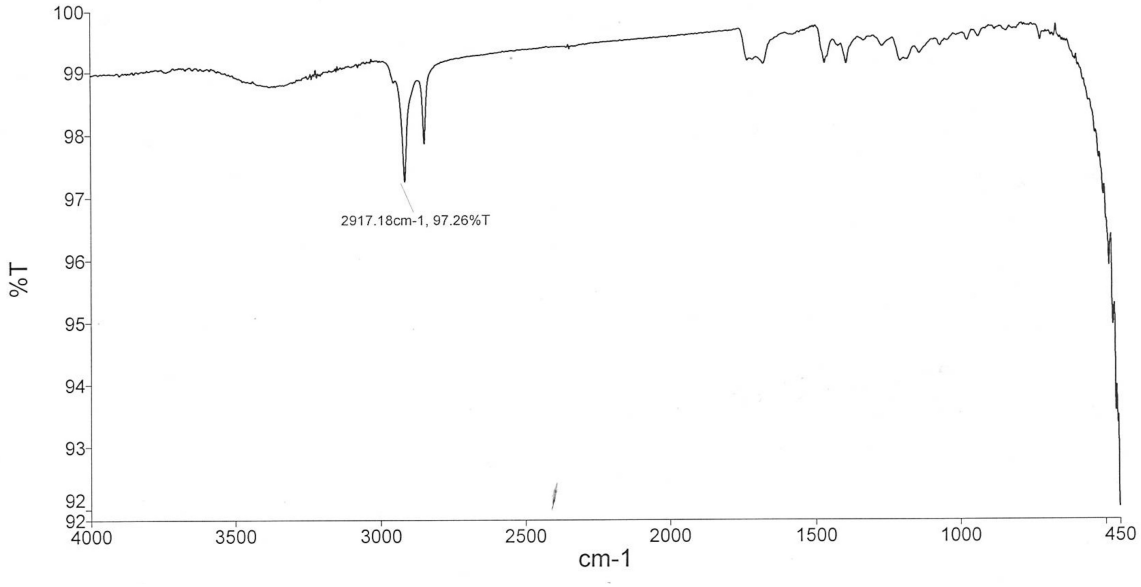


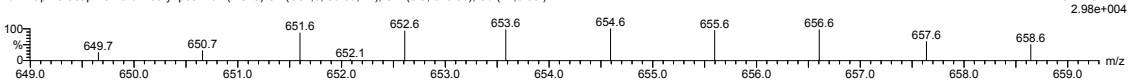
Figure S15. HRESIMS spectrum of **8a**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -50.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions
131 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 O: 0-30 79Br: 5-5

14-Apr-2015
hbl-14apr15-asap-25-25-dimethyl-pos 164 (2.813) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)
TOF MS AP+
2.98e+004



Minimum:				-50.0		
Maximum:		10.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
649.6567	649.6574	-0.7	-1.1	8.0	16675.3	C15 H11 O4 79Br5
	649.6633	-6.6	-10.2	-1.0	17340.8	C8 H15 O9 79Br5
	649.6480	8.7	13.4	-5.0	17327.2	C4 H15 O12 79Br5

Figure S16. ^{1}H NMR spectrum of **3a** in $CDCl_3$

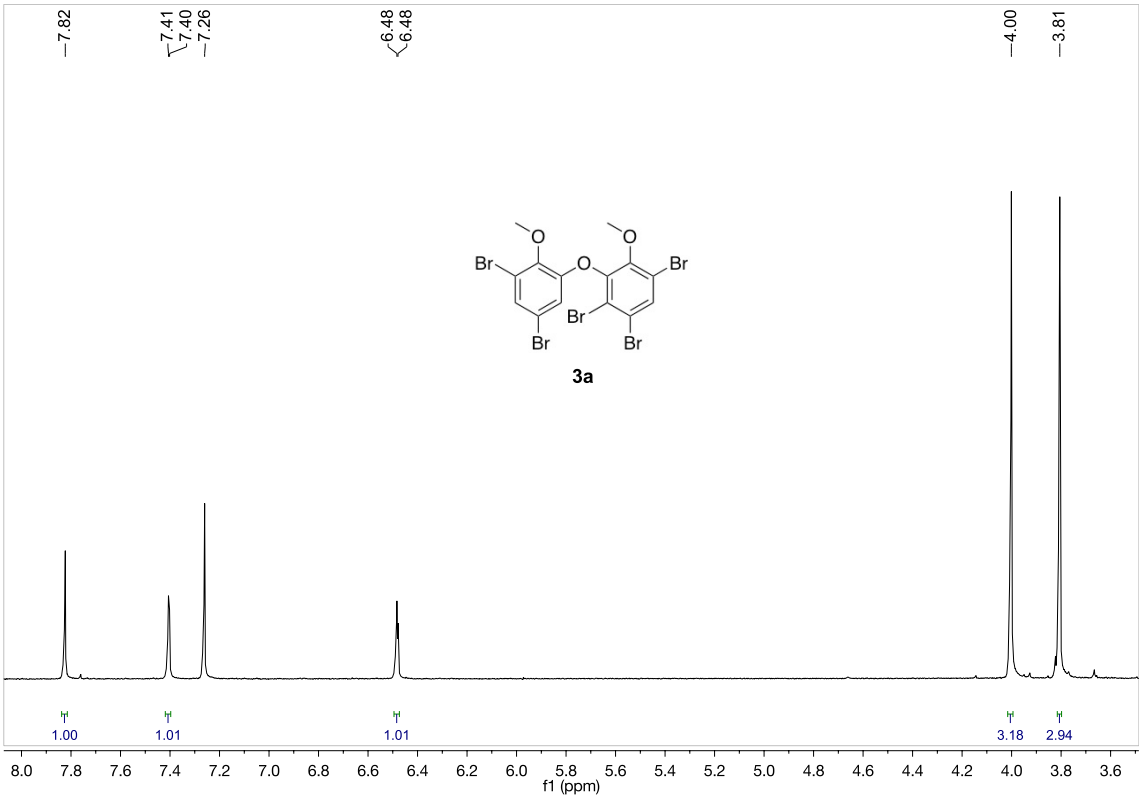


Figure S17. ^{13}C NMR spectrum of **3a** in $CDCl_3$

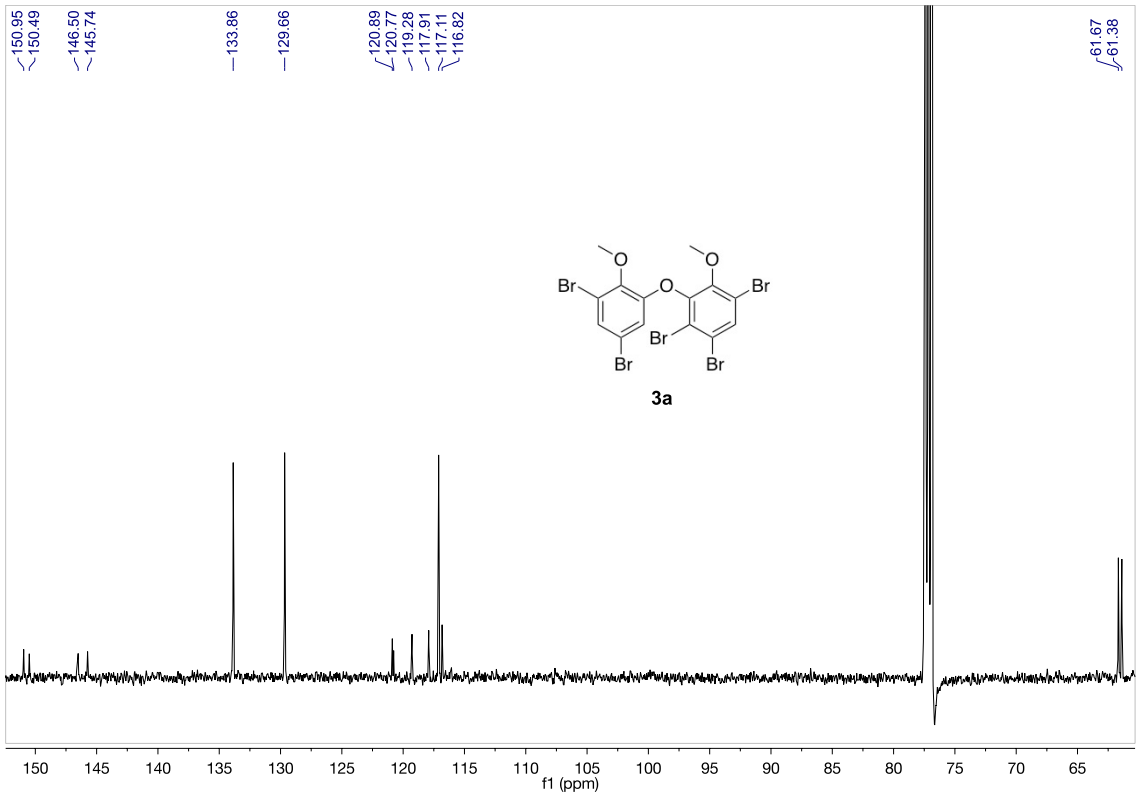


Figure S18. HSQC spectrum of **3a** in $CDCl_3$

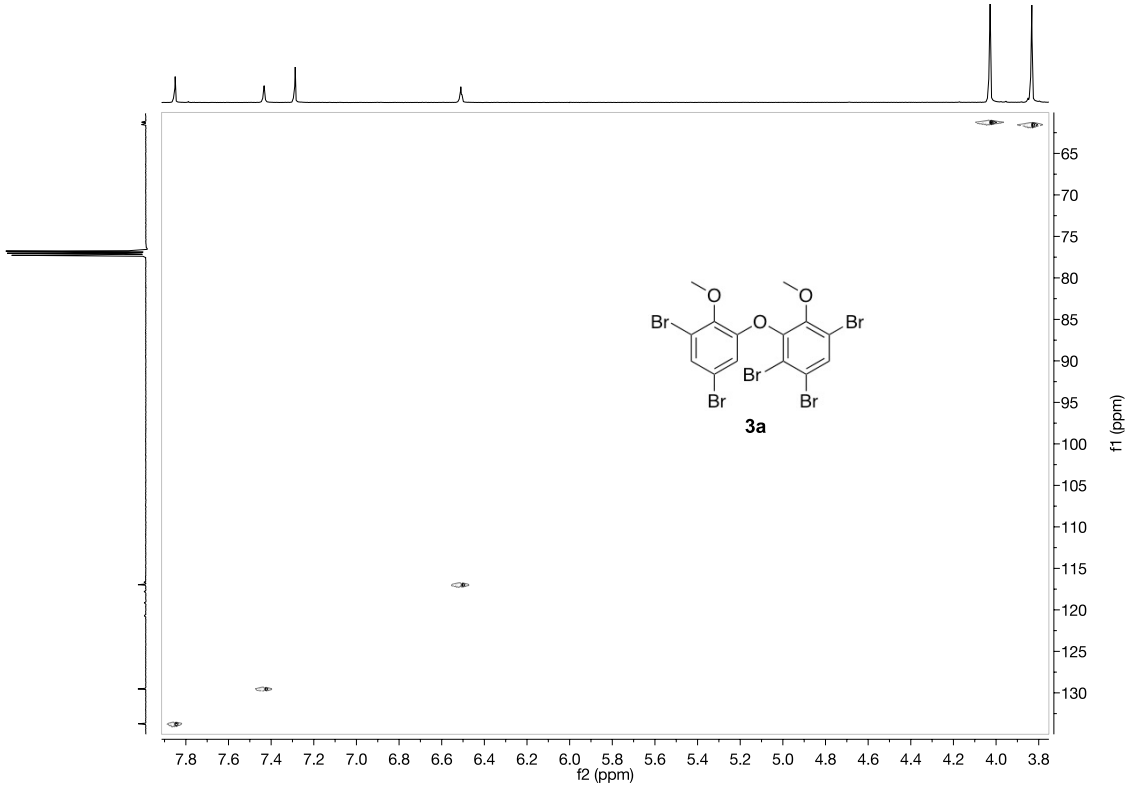


Figure S19. HMBC spectrum of **3a** in $CDCl_3$

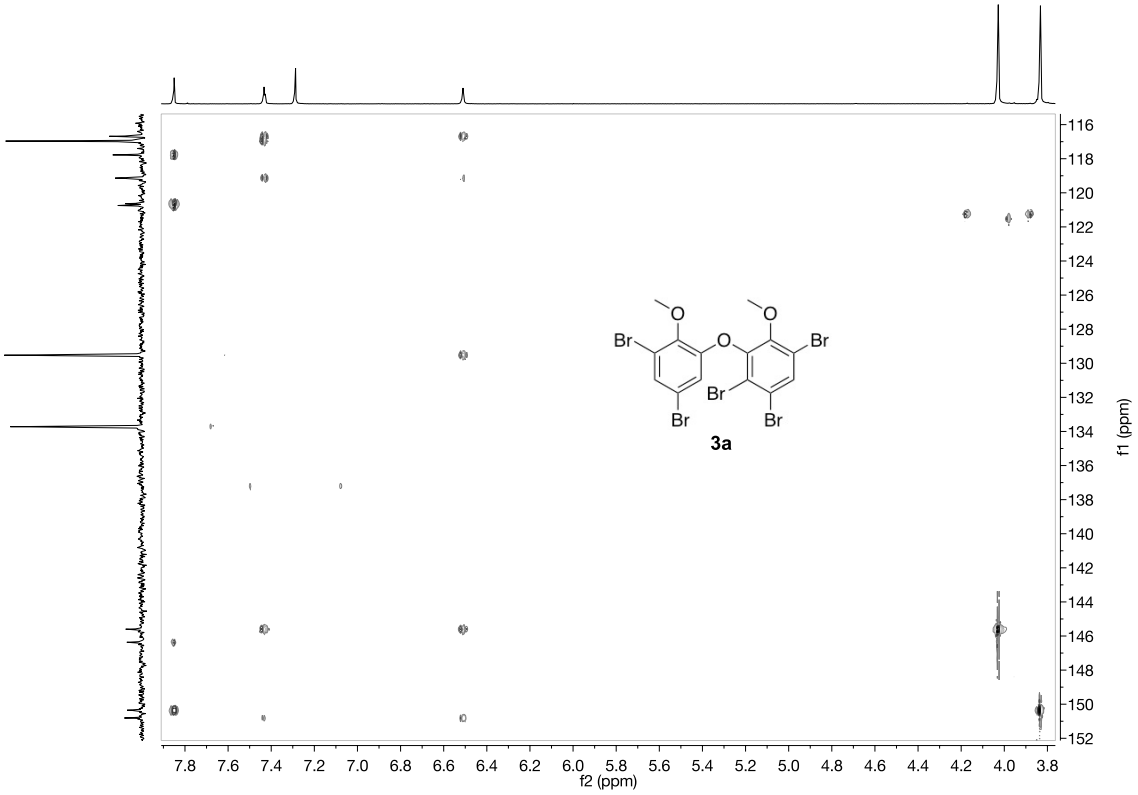


Figure S20. IR spectrum of **3a**

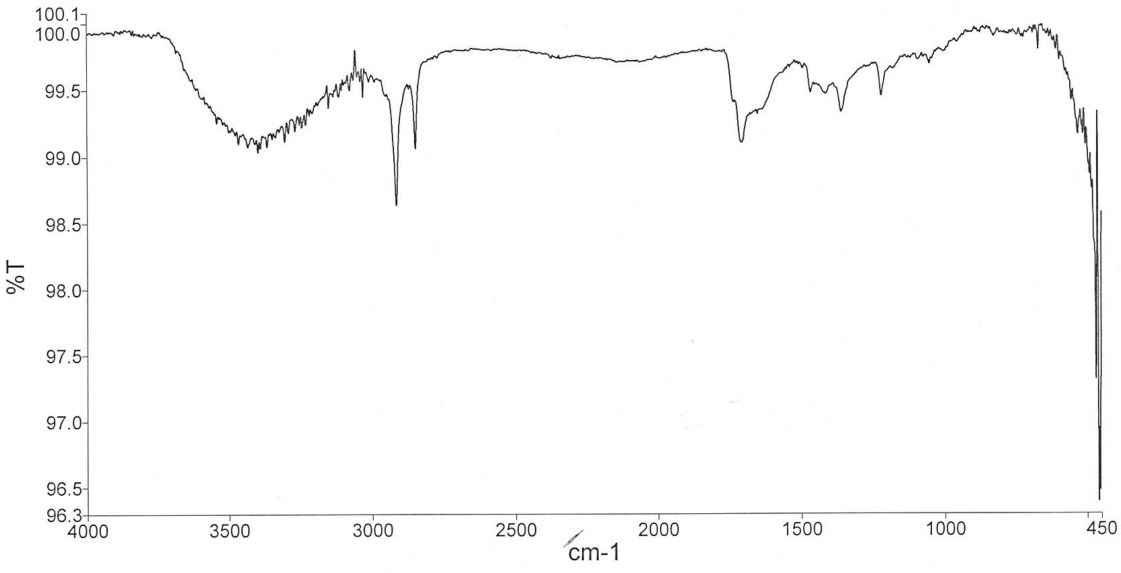


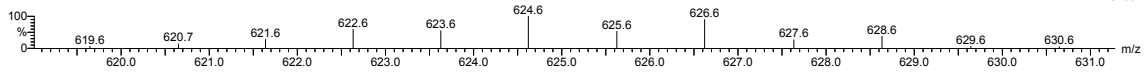
Figure S21. HRESIMS spectrum of **3a**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 25.0 mDa / DBE: min = -50.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions
109 formula(e) evaluated with 8 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 O: 0-30 79Br: 5-5

15-Apr-2015
hbl-15apr15-asap-25-27-methylated 75 (1.287) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)
TOF MS AP+
1.17e+004



Minimum:				-50.0		
Maximum:		25.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
619.6458	619.6468	-1.0	-1.6	8.0	2282.2	C14 H9 O3 79Br5
	619.6527	-6.9	-11.1	-1.0	2341.6	C7 H13 O8 79Br5
	619.6375	8.3	13.4	-5.0	2363.4	C3 H13 O11 79Br5
	619.6586	-12.8	-20.7	-10.0	2399.2	H17 O13 79Br5
	619.6316	14.2	22.9	4.0	2313.4	C10 H9 O6 79Br5
	619.6621	-16.3	-26.3	12.0	2269.1	C18 H9 79Br5
	619.6257	20.1	32.4	13.0	2260.3	C17 H5 O 79Br5
	619.6680	-22.2	-35.8	3.0	2337.5	C11 H13 O5 79Br5

Figure S22. ^{1}H NMR spectrum of **5a** in $CDCl_3$

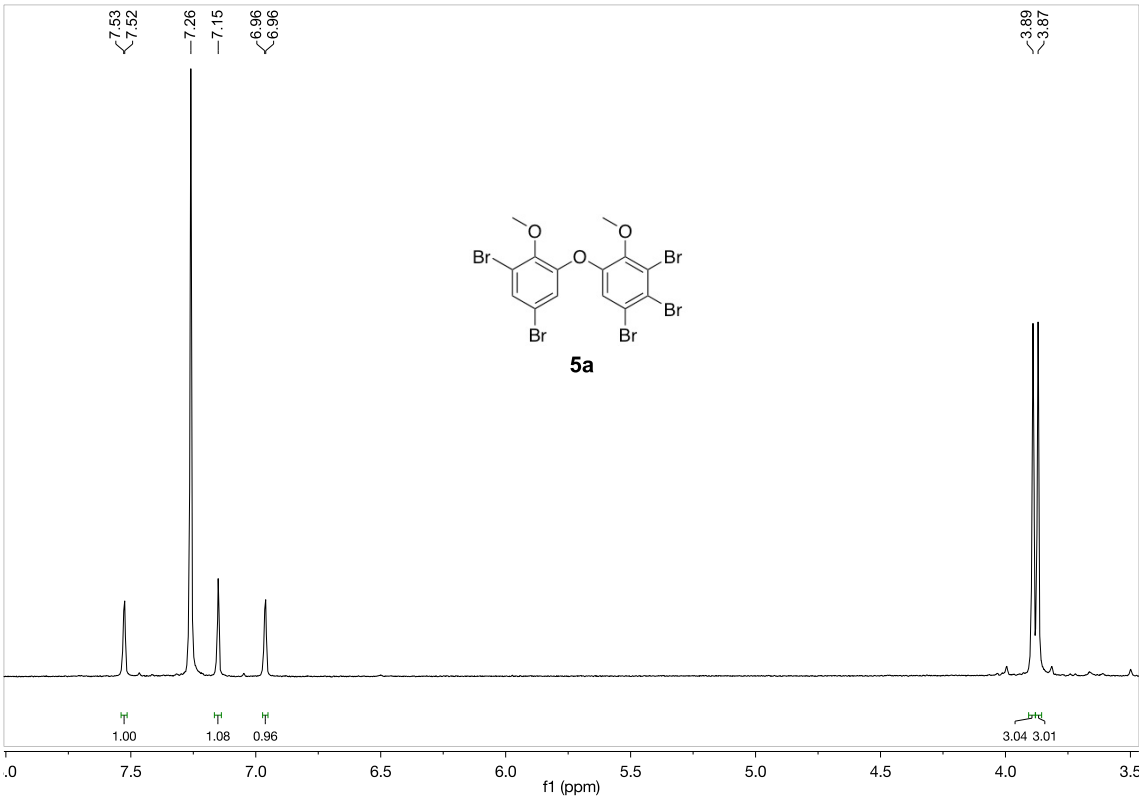


Figure S23. ^{13}C NMR spectrum of **5a** in $CDCl_3$

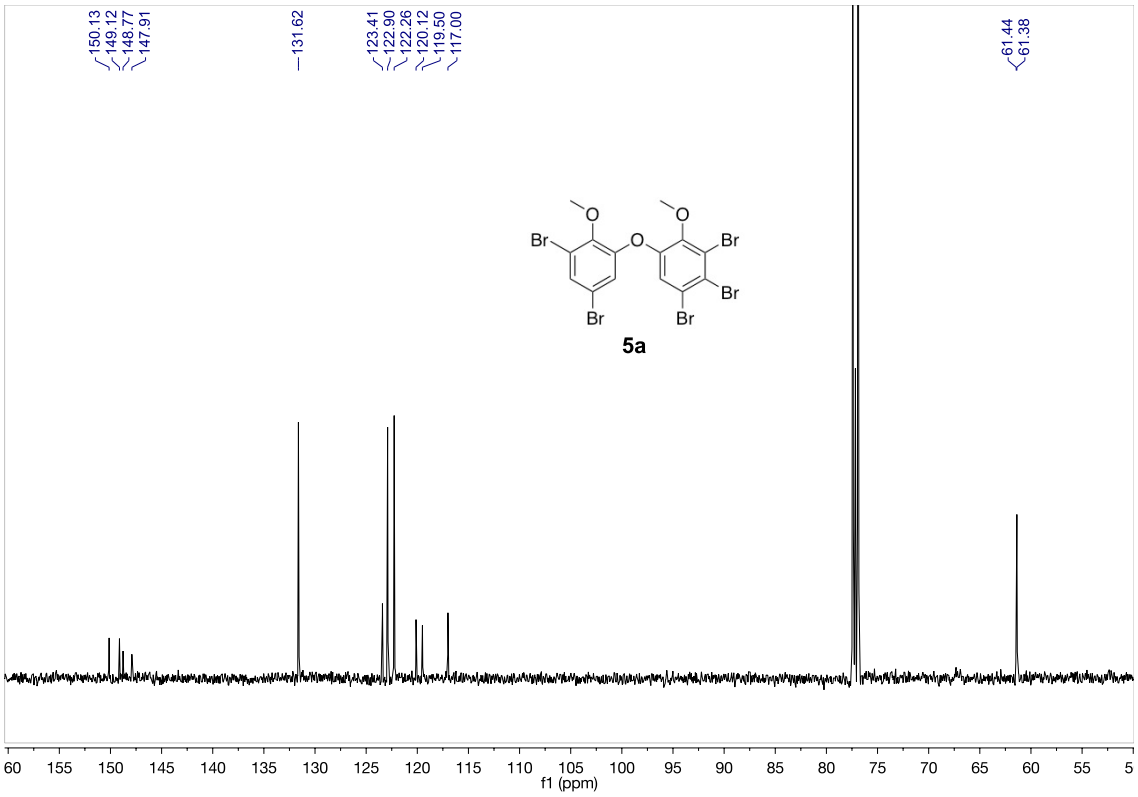


Figure S24. HSQC spectrum of **5a** in $CDCl_3$

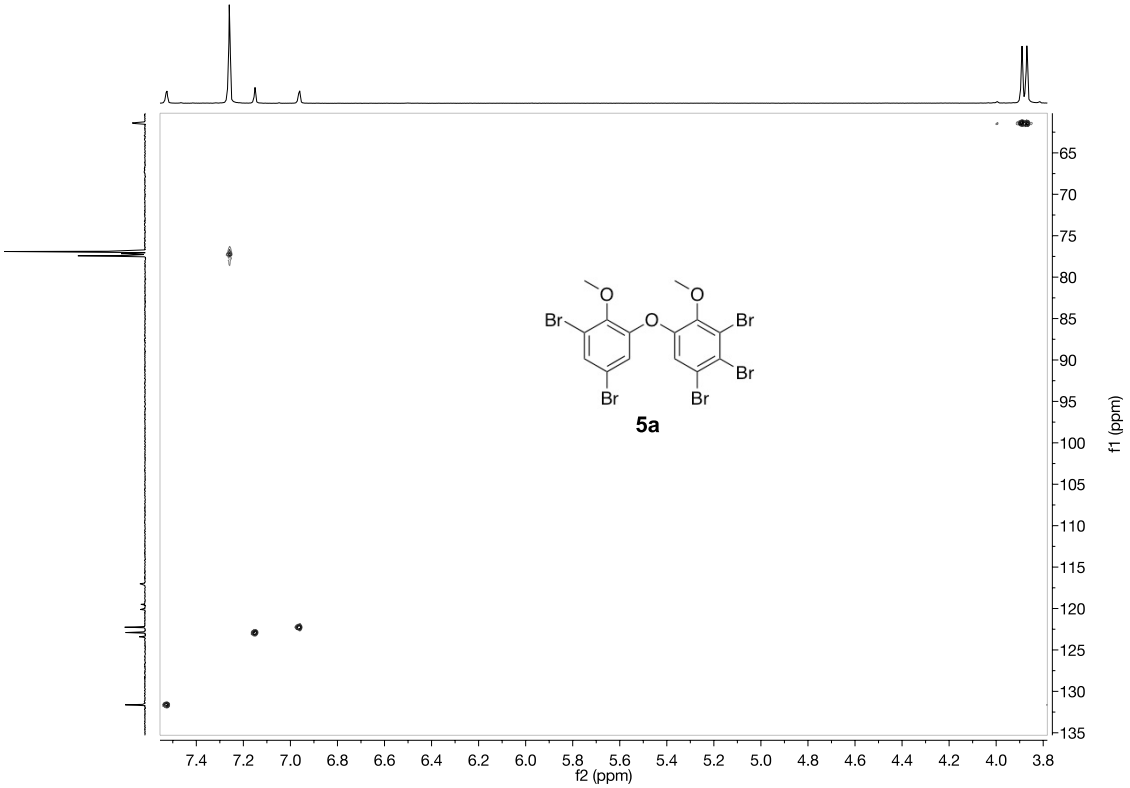


Figure S25. HMBC spectrum of **5a** in $CDCl_3$

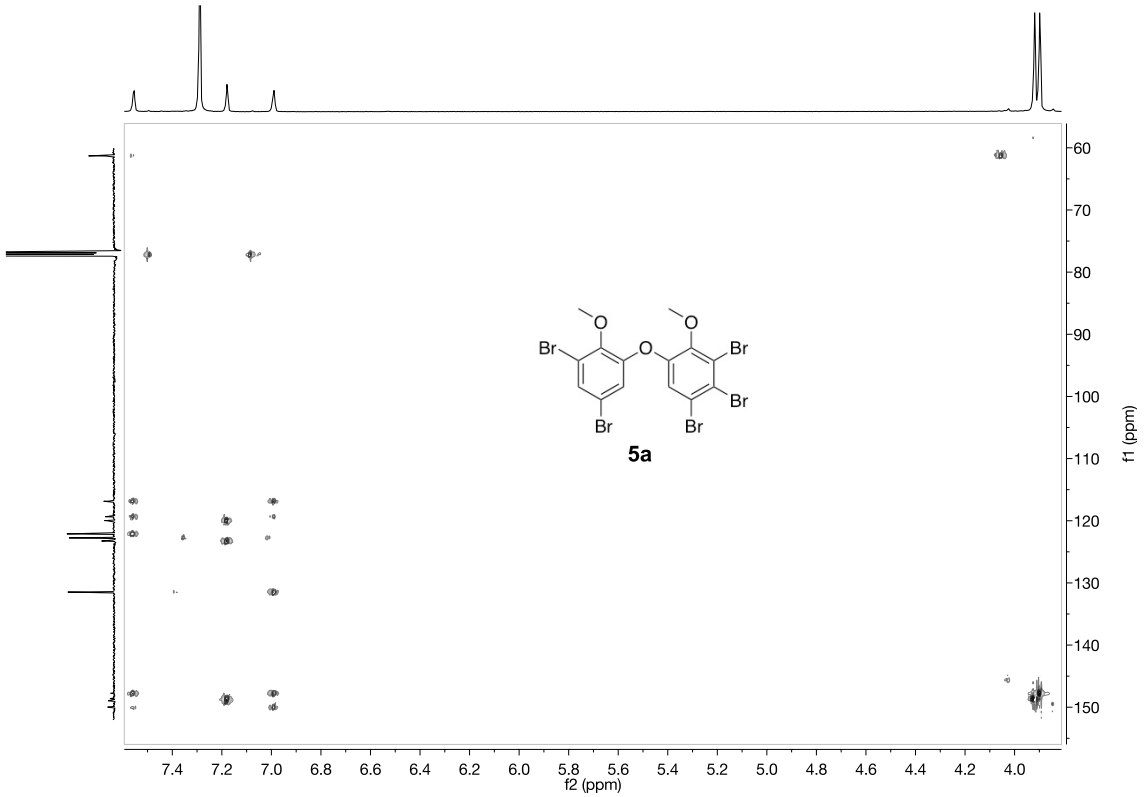


Figure S26. IR spectrum of **5a**

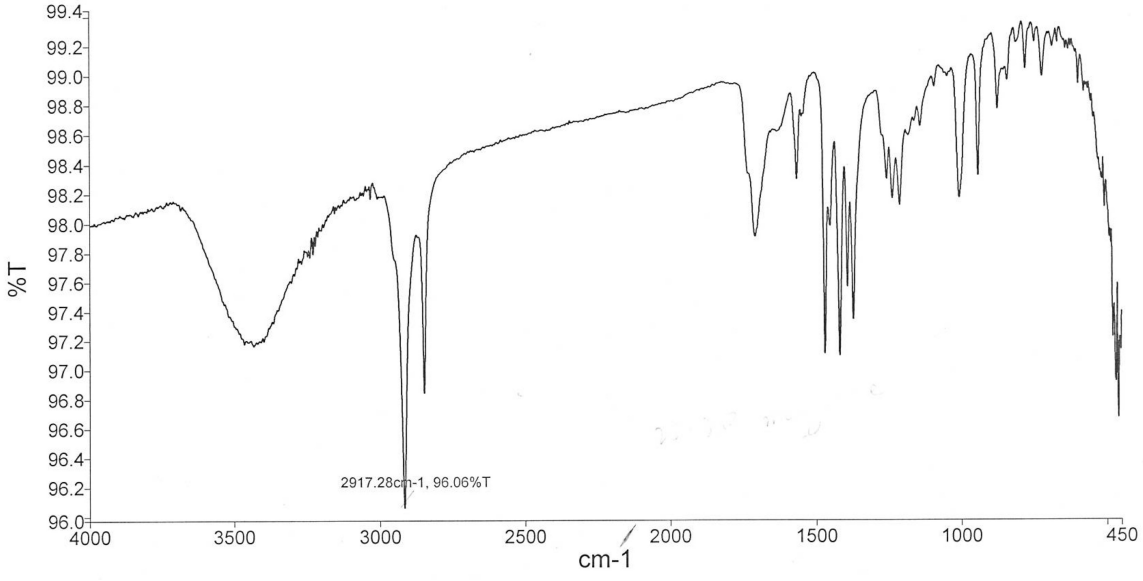


Figure S27. HRESIMS spectrum of **5a**

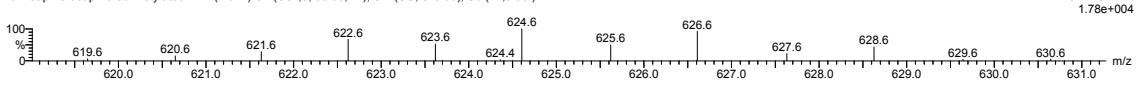
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 25.0 mDa / DBE: min = -50.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions
109 formula(e) evaluated with 8 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 O: 0-30 79Br: 5-5

15-Apr-2015
hbl-15apr15-asap-25-30-metylated 112 (1.921) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS AP+
1.78e+004



Minimum:				-50.0		
Maximum:		25.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
619.6473	619.6468	0.5	0.8	8.0	3661.1	C14 H9 O3 79Br5
	619.6527	-5.4	-8.7	-1.0	3748.4	C7 H13 O8 79Br5
	619.6375	9.8	15.8	-5.0	3767.9	C3 H13 O11 79Br5
	619.6586	-11.3	-18.2	-10.0	3833.7	H17 O13 79Br5
	619.6621	-14.8	-23.9	12.0	3655.6	C18 H9 79Br5
	619.6316	15.7	25.3	4.0	3694.9	C10 H9 O6 79Br5
	619.6680	-20.7	-33.4	3.0	3756.7	C11 H13 O5 79Br5
	619.6257	21.6	34.9	13.0	3617.6	C17 H5 O 79Br5

Bacterial strains and Minimal Inhibitory Concentration determination

Compounds were tested in duplicate in at least two independent experiments for their antimicrobial activity against laboratory strains listed in Table 3 and as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines. Laboratory strains were obtained from the American Type Culture Collection (ATCC).

Cytotoxicity assays

The effects of compounds **1** and **2** on HCT-116 (human colorectal cancer cell line) and BSC-1 (monkey kidney cell line) cells were determined using an MTT cell proliferation assay kit (American Type Culture Collection, Manassas, VA). HCT-116 (1×10^4 cells/well) or BSC-1 (2×10^4 cells/well) cells were seeded in 96-well tissue culture plates and allowed to adhere for 18 h. Cells were then exposed for 24 hr to two-fold dilutions of compounds where concentrations ranged from 0.4 to 100 µg/ml, followed by addition of fresh growth media. After 48 h of additional incubation, the media was replaced with 10% MTT and plates were incubated for 4 hr. Cells were lysed with detergent (100 µl) and plates were stored in the dark at room temperature for 2 hr. A_{570} values were read using a multi-mode microplate reader (Synergy HT, Biotek Instruments, Inc.).