Supporting information for:

Efficient discovery of fluorescent chemosensors based on a biarylpyridine scaffold.

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Contents

1. General notes and procedures

^{1}H NMR spectra were obtained on Bruker AV-300 (300 MHz) or AV2-400 (400 MHz) spectrometers. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm). Multiplicities are given as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet). Coupling constants are reported in Hz.

^{1}H-decoupled ^{13}C NMR spectra were obtained on AV-300 (75 MHz) or AV2-400 (100 MHz) spectrometers. ^{13}C NMR chemical shifts are reported relative to tetramethylsilane (0.00 ppm). IR frequencies are given in cm^{-1}; spectra were obtained on a Perkin Elmer Spectrum One (FT-IR) spectrometer in KBr pellets.

Fluorescence measurements were carried out in spectroscopic grade DMSO or DMSO/aqueous MOPS on Edinburg FLS920, using 450W Xenon lamp excitation and 1 nm excitation and 5 nm emission slit widths. All samples were flushed with nitrogen to remove oxygen. Reported emission spectra were recorded at the global absorption maximum for each compound.

Quantum yields were determined by standard methods,[1,2] using pyrene (ϕ = 0.32) as the standard.[3] The samples were diluted to optical transparency (Abs = 0.04–0.05) and the integrated emission intensity was compared to an iso-absorptive solution of pyrene in degassed dichloromethane.

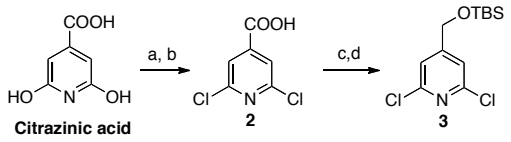
UV-Vis measurements were carried out on a Perkin-Elmer Lambda 19 UV-Vis spectrophotometer.

For extinction coefficient determination four independent solutions with different concentration were prepared with absorption between 0.04–0.10 AU. The value of ε was calculated by linear least-squares fitting of plots of A vs. concentration. All fits gave R^2 values of >0.98.

Flash chromatographic purification was performed by with Merck silica gel 60 (particle size 0.040–0.063 mm) packed in glass columns; eluting solvent for each purification was determined by thin layer chromatography (TLC). Analytical TLC was performed with Macherey–Nagel POLYGRAM SIL NHR/UV254 or ALOX N/UV254. Preparative TLC purification was carried out using PCS-plates silica gel 60 F254, 2 mm.

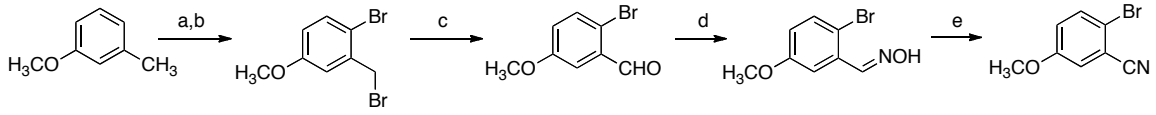
Synthetic procedures were carried out under an inert atmosphere, in dry solvent, using standard Schlenk techniques, unless otherwise noted. All reagents and solvents were reagent grade and were used without further purification unless otherwise specified.

2. Synthetic schemes.



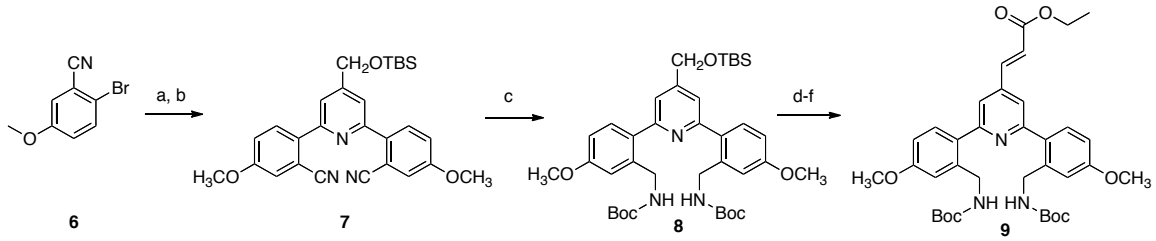
Citrazinic acid **2** **3**

Reagents and conditions: a. $POCl_3$, Me_4NCl, Δ then CH_3OH, 86%; b. LiOH, THF/H_2O, ~100%; c. BH_3*$S(CH_3)_2$, THF, Δ, 70%; d. TBSCl, imidazole, DMAP, CH_2Cl_2, 85%



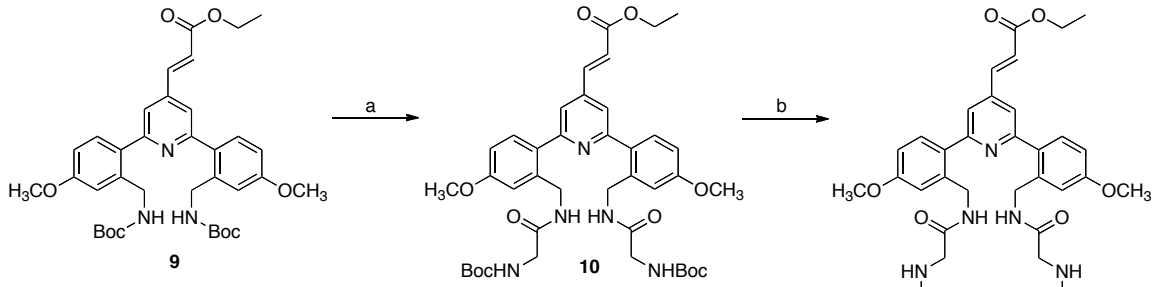
4 **5** **5a** **6**

Reagents and conditions: a. NBS, acetonitrile, reflux, 89%; b. NBS, AIBN, PhH, reflux, 70%; c. hexamethylenetetramine, AcOH (50% solut.), Δ, 90%; d. NH_2OH*H_2SO_4, NaOH, MeOH, 84%; e. $(CF_3CO)_2O$, pyridine, dioxane, 93%



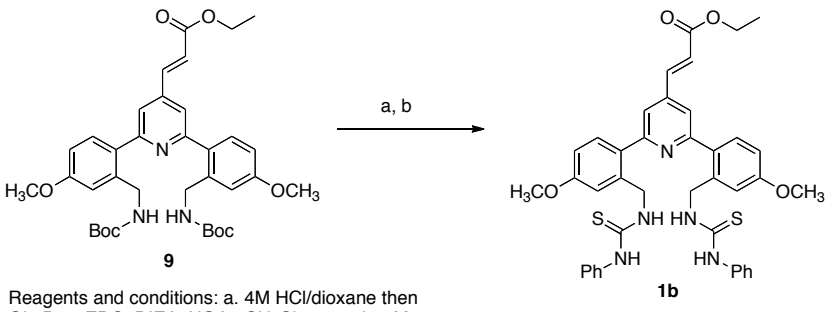
6 **7** **8** **9**

Reagents and conditions: a. BuLi, -100°C, THF; b. $ZnCl_2$, THF then **3**, 69% over two steps c. H_2, 50 psi, Raney-Ni, $(Boc)_2O$, EtOH, 57-67%; d. Bu_4NF, THF, 0°C, 95%; e. $(COCl)_2$, DMSO, -78°C, then Et_3N, 77%; f. $(EtO)_2P(O)$-CH_2COOEt, KO^tBu, THF, 0°C, 74%;

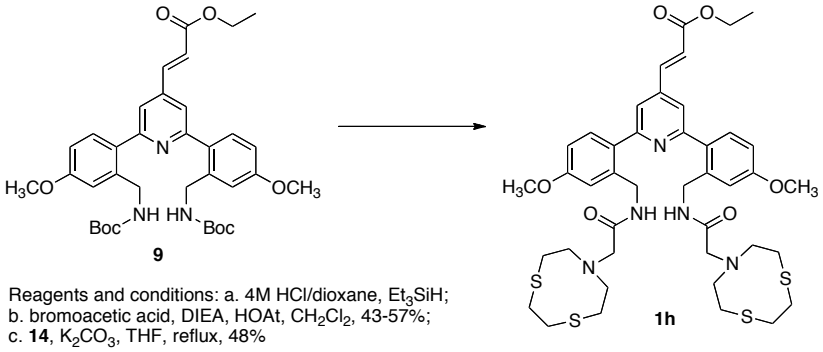


9 **10** **1a**

Reagents and conditions: a. 4M HCl/dioxane then Gly-Boc, EDC, DIEA, HOAt, CH_2Cl_2, 85%; b. 4M HCl/dioxane then Et_3N, PhNCS, 54%



Reagents and conditions: a. 4M HCl/dioxane then Gly-Boc, EDC, DIEA, HOAt, CH_2Cl_2, 85%; b. 4M HCl/dioxane then Et_3N, PhNCS, 54%

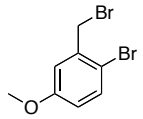


Reagents and conditions: a. 4M HCl/dioxane, Et_3SiH; b. bromoacetic acid, DIEA, HOAt, CH_2Cl_2, 43-57%; c. **14**, K_2CO_3, THF, reflux, 48%

Abbreviations:

NBS = N-Bromosuccinimide
AIBN = 2,2'-Azobis(2-methylpropionitrile)
EDC = *N*-Ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide
DIEA = *N,N*-Diisopropylethylamine
TBSCl = *tert*-Butyldimethylsilyl chloride
HOAt = 1-Hydroxy-7-azabenzotriazole

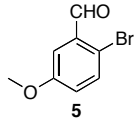
3. Synthetic details and tabulated spectroscopic data.



4

The compound was synthesized as described.[4] The ^{1}H NMR of this material was consisted with previously reported data.

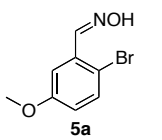
^{1}H-NMR (300 MHz, $CDCl_3$): 7.45 (*d*, 1H, *J*=8.8 Hz), 6.99 (*d*, 1H, *J*=3.2 Hz), 6.74 (*dd*, 1H, *J*=8.8, 2.8 Hz), 4.55 (*s*, 2H), 3.80 (*s*, 3H).



5

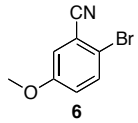
The solution of 4-bromo-3-bromomethylanisole **4** (0.70 g, 2.50 mmol, 1.00 eq) and hexamethylenetetramine (0.75 g, 5.40 mmol, 2.20 eq) 50% acetic acid (3.00 mL) was refluxed for 2 h.[5] After cooling to room temperature the mixture was diluted with 10 mL water and extracted with CH_2Cl_2. The organic fraction was washed with H_2O, then $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated. The purification on silica gel (hexane/Et_2O 10/1) provided 0.45 g (90%) of the product.
The ^{1}H NMR of this material was consisted with previously reported data.[4]

^{1}H-NMR (300 MHz, $CDCl_3$): 10.32 (*s*, 1H), 7.53 (*d*, 1H, *J*=8.8 Hz), 7.41 (*d*, 1H, *J*=2.8 Hz), 7.03 (*dd*, 1H, *J*=8.8, 2.8 Hz), 3.85 (*s*, 3H).



5a

The oxime **5a** was synthesized as described.[4] The ^{1}H NMR of this material was consisted with previously reported data.

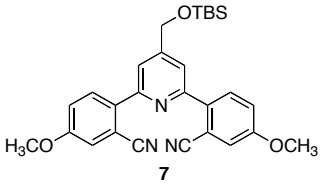


6

The solution of oxime **5a** (1.00 g, 4.00 mmol, 1 eq) and pyridine (1.40 g, 18.00 mmol, 4.40 eq) in dry dioxane (5 mL) was cooled in an ice bath. Trifluoroacetic acid anhydride (0.90 g, 4.40 mmol, 1.10 eq) was added dropwise.[6] The resulting mixture was stirred at 0°C for 1 h and 10 h at r.t. Then 50 mL HCl (~10%) was added. The solution was transferred to a separation funnel and extracted with CH_2Cl_2. The organic phase was washed with water, dried over Na_2SO_4 and concentrated. The residue was passed through a plug of silica gel (hexane/EtOAc 20/1) to remove baseline impurities and the eluent concentrated to yield 0.79 g (93%) of the product.
The ^{1}H NMR of this material was consisted with previously reported data.[4]

^{1}H-NMR (300 MHz, $CDCl_3$): 7.54 (*d*, 1H, *J*=8.8 Hz), 7.15 (*d*, 1H, *J*=3.2 Hz), 7.00 (*dd*, 1H, *J*=9.2, 3.2 Hz), 3.83 (*s*, 3H).

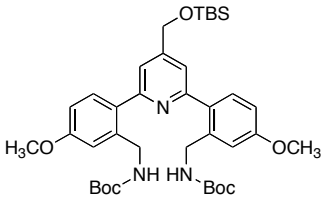
Dichloropyridines **2-3** were prepared as described.[4] The ^{1}H NMR and MS of all compounds were consisted with previously reported data.



7

Aryl bromide **6** (0.43 g, 2.00 mmol, 2.00 eq) was dissolved in THF (10 mL) and cooled to -100°C, BuLi (1.26 mL, 1.60 M in hexane) was added dropwise and the resulting solution was stirred for 5 min at -100°C. The solution of $ZnCl_2$ (0.27 g, 2.00 mmol, 2.00 eq) in THF (5 mL) was added via syringe and the flask warmed to 23°C. The resulting solution was transferred to the second flask containing dichloropyridine derivative **3** (0.28 g, 1.00 mmol, 1.00 eq), $Pd(PPh)_4$ (0.055 g, 5%) in THF (5 mL) and this was heated for 14 h at 80°C. The solvent was removed with the rotary evaporator and the residue was purified by flash column chromatography (CH_2Cl_2/acetone 50/1) to yield 0.34 g (69%) of the product.
The ^{1}H NMR of this material was consisted with previously reported data.[4]

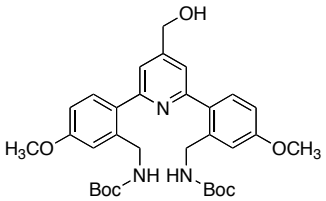
^{1}H-NMR (300 MHz, $CDCl_3$): 8.01 (*d*, 2H, *J*=8.8 Hz), 7.72 (*s*, 2H), 7.25 (*m*, 4H), 4.93 (*s*, 2H), 3.88 (*s*, 6H), 0.99 (*s*, 9H), 0.18 (*s*, 6H).



8

The dinitrile **7** (0.60 g, 1.24 mmol, 1.00 eq) was submitted to catalytic hydrogenation (50 psi) over Raney nickel (ca. 5 g) as a catalyst in ethanol (20.00 mL) in the presence of Boc-anhydride (0.75 g, 3.40 mmol, 2.70 eq) in a standard Parr instrument. After 30 h the reaction mixture was filtered through a plug of celite and concentrated. Purification by flash column chromatography (CH_2Cl_2/acetone 50/1) provided 1.50 g (57%) of the product.
The ^{1}H NMR of this material was consisted with previously reported data.[4]

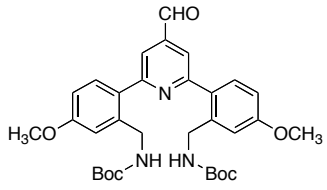
^{1}H-NMR (300 MHz, $CDCl_3$): 7.41 (*d*, 2H, *J*=8.4 Hz), 7.33 (*s*, 2H), 7.04 (*d*, 2H, *J*=2.0 Hz), 6.91 (*dd*, 2H, *J*=8.8, 2.4 Hz), 5.73 (*b*, 2H), 4.85 (*s*, 2H), 4.26 (*d*, 4H, *J*=5.6 Hz), 3.86 (*s*, 6H), 1.38 (*s*, 18H), 0.99 (*s*, 9H), 0.17 (*s*, 6H).



8a

The compound **8a** was synthesized as described.[4] The ^{1}H NMR of this material was consisted with previously reported data.

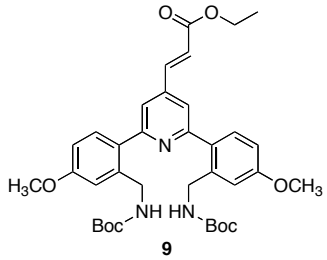
^{1}H-NMR (300 MHz, $CDCl_3$): 7.47 (*d*, 2H, *J*=8.4), 7.39 (*s*, 2H), 7.00 (*d*, 2H, *J*=2.4), 6.91 (*dd*, 2H, *J*=8.4, 2.4), 5.67 (*b*, 2H), 4.81 (*d*, 2H, *J*=3.6), 4.30 (*d*, 4H, *J*=5.2), 3.86 (*s*, 6H), 2.76 (*b*, 1H), 1.37 (*s*, 18H).



8b

The compound **8b** was synthesized as described.[4] The ^{1}H NMR of this material was consisted with previously reported data.

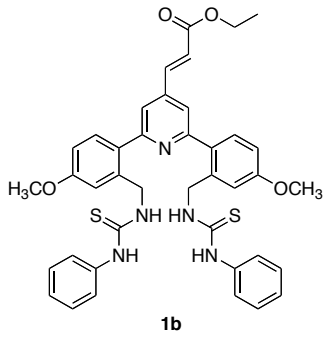
^{1}H-NMR (300 MHz, $CDCl_3$): 10.15 (*s*, 1H), 7.79 (*s*, 2H), 7.47 (*d*, 2H, *J*=8.4 Hz), 7.06 (*d*, 2H, *J*=2.8), 6.94 (*dd*, 2H, *J*=8.4, 2.8 Hz), 5.61 (*b*, 2H), 4.30 (*s*, 4H), 3.88 (*s*, 6H), 1.38 (*s*, 18H).



9

The compound **9** was synthesized as described.[4] The ^{1}H NMR of this material was consisted with previously reported data.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.68 (*d*, 1H, *J*=16 Hz), 7.46 (*s*, 2H), 7.42 (*d*, 2H, *J*=8.8), 7.04 (*d*, 2H, *J*=2.4), 6.91 (*dd*, 2H, *J*=8.4, 2.4 Hz), 6.67 (*d*, 1H, *J*=16 Hz), 5.65 (*b*, 2H), 4.28 (*m*, 6H), 3.85 (*s*, 6H), 1.36 (*m*, 21H).



1b

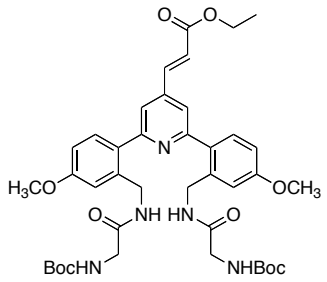
The Boc diamine **9** (30.00 mg, 0.046 mmol, 1.00 eq) was dissolved in 4 M HCl/dioxane ^{i}Pr$_3$SiH (19:1, 5.00 mL) and stirred for 1 h at r.t. The volatile compounds were removed in vacuum and the residue redissolved in 10 mL CH_2Cl_2 and Et_3N (19.00 mg, 0.184 mmol, 4.00 eq). Phenylisothiocyanate (16.00 mg, 0.115 mmol, 2.50 eq) was added and the reaction stirred for 16 h at r.t. Purification by flash chromatography (SiO_2, hexane/acetone 2/1) provided 20.00 mg (60%) of the product **1b**.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.64 (*d*, 1H, *J*=16.1 Hz), 7.38 (*s*, 1H), 7.28-7.22 (*m*, 3H), 7.09-6.98 (*m*, 6H), 6.96-6.83 (*m*, 4H), 6.65 (*d*, 1H, *J*=16.1 Hz), 4.83 (*d*, 4H, *J*=4.98 Hz), 4.28 (*q*, 2H, *J*=7.12 Hz), 3.83 (*s*, 6H), 1.34 (*t*, 3H, *J*=7.14 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$): 179.98, 165.92, 160.23, 158.92, 143.40, 141.42, 136.84, 131.52, 131.22, 129.54, 126.54, 124.40, 123.64, 120.21, 115.45, 113.79, 61.04, 55.44, 47.82, 14.26.

IR (KBr), cm^{-1}: 3276 (s), 2957 (m), 2836 (m), 1710 (vs), 1644 (m), 1607 (vs), 1449 (vs), 1310 (vs), 1182 /vs), 1036 (s).

HRMS-ESI: Calculated for $C_{40}H_{40}N_5O_4S_2$ $[(M+H)]^+$ 718.2522, found 718.2531.



10

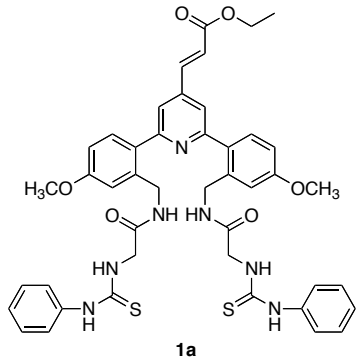
The Boc diamine **9** (8.00 mg, 0.0124 mmol, 1.00 eq) was dissolved in 4 M HCl/dioxane : $^{i}Pr_3SiH$ (19/1, 1 mL) and stirred for 1 h at r.t. The volatile compounds were removed in vacuum and the residue redissolved in 5 mL CH_2Cl_2, Boc-Gly (4.30 mg, 0.0248 mmol, 2.00 eq), HOAt (6.60 mg, 0.0496 mmol, 4.00 eq) was added at 0°C, followed by EDC (7.10 mg, 0.0372 mmol, 3 eq) and the resulting solution stirred for 2 h at 0°C and 14 h at r.t. The reaction mixture was filtered, concentrated and purified by preparative TLC (CH_2Cl_2/acetone 10/1, 5/1) provided 6.10 mg (65%) of the product.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.68 (*d*, 1H, *J*=16.0 Hz), 7.45 (*b*, 4H), 7.06-7.01 (*m*, 2H), 6.93 (*dd*, 2H, *J*=2.4, 8.4 Hz), 6.67 (*d*, 1H, *J*=16.0 Hz), 4.42 (*d*, 4H, *J*= 4.6 Hz), 4.30 (*q*, 2H, *J*=7.1 Hz), 3.39 (*s*, 6H), 1.46 (*b*, 18H), 1.38 (*t*, 3H, *J*=7.1 Hz).

^{13}C-NMR (100 MHz, d_6-acetone): 170.99, 167.67, 162.20, 161.09, 145.21, 143.88, 140.91, 134.10, 133.42, 125.23, 122.09, 114.51, 80.34, 62.29, 56.78, 45.69, 42.96, 29.64, 15.67, 6.96.

IR (KBr), cm^{-1}: 3426 (s), 2978 (w), 1712 (vs), 1669 (vs), 1608 (s), 1546 (s), 1506 (s), 1286 (s), 1169 (vs), 1038 (m).

HRMS-ESI: Calculated for $C_{40}H_{51}N_5NaO_{10}$ $[(M+Na)]^+$ 784.3534, found 784.3533.



1a

The Boc-Gly compound **10** (37.00 mg, 0.049 mmol, 1.00 eq) was dissolved in 4 M HCl/dioxane $^{i}Pr_3SiH$ (19:1, 5.00 mL) and stirred for 1 h at r.t. The volatile compounds were removed in vac. and the residue redissolved in 10 mL CH_2Cl_2 and Et_3N (50.00 mg, 0.49 mmol, 10.00 eq). Phenylisothiocyanate (80.00 mg, 0.123 mmol, 2.50 eq) was added and the reaction stirred for 16 h at r.t. Purification by flash chromatography (SiO_2, CH_2Cl_2/acetone 10/1) provided 22.00 mg (54%) of the product **1a**.

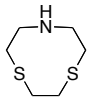
^{1}H-NMR (300 MHz, $CDCl_3$): 8.40 (*b*, 2H), 7.68 (*d*, 1H, *J*=16.04), 7.54 (*t*-like, *b*, 2H), 7.47 (*s*, 2H), 7.41 (*d*, 2H, *J*=8.49), 7.36-7.16 (*m*, 11H), 7.12 (*t-like*, 2H), 7.15 (*d*, 2H, *J*=2.64), 6.92 (*dd*, 2H, *J*=8.52, 2.64), 6.69 (*d*, 1H, *J*=16.04), 4.42 (*d*, 4H, *J*=5.84), 4.25 (*q*, 2H, *J*=7.13), 3.79 (*s*, 6H), 1.32 (*t*, 3H, *J*=7.12).

^{13}C-NMR (100 MHz, $CDCl_3$): 181.22, 168.67, 166.51, 160.92, 159.54, 144.19, 142.04, 138.28, 132.53, 131.96, 127.06, 126.95, 125.18, 123.97, 120.73, 115.87, 113.73, 61.55, 56.06, 48.69, 42.37, 14.61.

IR (KBr), cm^{-1}: 3337 (s), 1661 (vs), 1608 (vs), 1498 (s), 1311 (m), 1241 (m).

HRMS-ESI: Calculated for $C_{44}H_{46}N_7O_6S_2$ [$(M+H)^+$] 832.2951, found 832.2951.

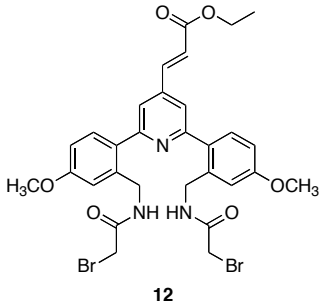
7-Aza-1,4-dithiacyclononane (**11)**



11

7-Aza-1,4-dithiacyclononane was prepared according to the literature.[7] After deprotection, **11** was isolated in 11% overall yield.

^{1}H-NMR (300 MHz, $CDCl_3$): 3.04-2.99 (*m*, 6H), 2.83-2.79 (*m*, 6H).



12

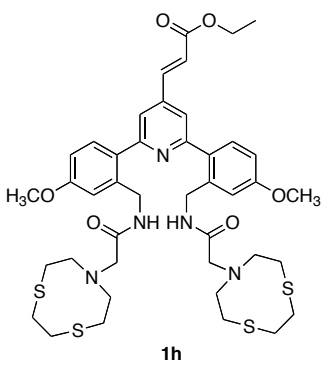
Compound **9** (8.00 mg, 0.0124 mmol, 1.00 eq) was dissolved in 4 M HCl/dioxane ^{i}Pr$_3$SiH (19:1, 1.00 mL) and stirred for 1 h at r.t. The volatile compounds were removed in vacuum and the residue redissolved in 3 mL CH_2Cl_2 and DIEA (4.80 mg, 0.0372 mmol, 3.00 eq). α-Bromoacetic acid (3.40 mg, 0.0248 mmol, 2.00 eq), HOAt (6.60 mg, 0.0496 mmol, 4.00 eq), EDC (7.10 mg, 0.0372 mmol, 3.00 eq) was added at 0°C and the resulting solution stirred for 2 h at 0°C and for 4h at r.t. The reaction mixture was filtered, concentrated and purified by preparative TLC (CH_2Cl_2/acetone 10/1) provided 4.50 mg (53%) of **12**. The yield in this reaction varies from 43% to 57%.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.70 (*d*, 1H, *J*=16.1 Hz), 7.56-7.37 (*m*, 6 H), 7.08-7.01 (*m*, 2H), 6.99-6.91 (*m*, 2H), 6.66 (*d*, 1H, *J*=16.06 Hz), 4.30 (*q*, 2H, *J*=7.15 Hz), 3.86 (*s*, 6H), 3.80 (*d*, 4H, *J*=2.19 Hz), 3.62 (*d*, 4H, *J*=1.89), 1.35 (*t*, 3H, *J*=7.15 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$): 165.34, 160.43, 160.39, 159.61, 141.36, 137.41, 131.14, 131.07, 120.24, 115.79, 115.76, 113.79, 61.08, 55.57, 42.41, 42.27, 14.26.

IR (KBr), cm^{-1}: 3377 (m), 3265 (m), 3063 (w), 2957 (m), 2837 (w), 1721 (vs), 1667 (vs), 1609 (vs), 1311 (vs), 1039 (vs).

HRMS-ESI: Calculated for $C_{30}H_{32}Br_2N_3O_6$ [$(M+H)^+$] 688.0658, found 688.0652.



1h

To the solution of **12** (28.00 mg, 0.41 mmol, 1eq) and 7-aza-1,4-dithiacyclononane **16** (20.00 mg, 0.123 mmol, 3 eq) in 5 mL THF K_2CO_3 (170.00 mg, 0.123 mmol, 3 eq) was added. The reaction mixture was stirred and refluxed for 20 h. Then water (20 mL) was added. After extraction with CH_2Cl_2 (x 2) the organic phase was dried over $MgSO_4$ and concentrated. Purification by flash column chromatography yielded 16.00 mg (48%) of the product **1h**.

^{1}H-NMR (400 MHz, $CDCl_3$): 8.52 (*t-like*, 2H), 7.62 (*d*, 1H, *J*=16.04), 7.42 (*s*, 2H), 7.38 (*d*, 2H, *J*=8.49), 6.85 (*dd*, 2H, *J*=2.64, 8.48), 6.59 (*d*, 1H, *J*=16.01), 4.49 (*d*, 4H, *J*=5.68), 4.22 (*q*, 2H, *J*=7.12), 3.78 (*s*, 6H), 3.06 (*s*, 4H), 2.73-2.65 (*m*, 14H), 2.57-2.52 (*m*, 24H), 1.28 (*t*, 3H, *J*=7.12).

^{13}C-NMR (100 MHz, $CDCl_3$): 171.09166.52, 160.60, 159.67, 143.28, 142.39, 138.52, 133.03, 131.94, 123.68, 120.68, 115.76, 113.76, 61.44, 60.87, 56.69, 56.00, 41.89, 34.68, 33.26, 14.78.

IR (KBr), cm^{-1}: 3259 (s), 2908 (s), 2832 (m), 1713 (s), 1666 (vs), 1607 (vs), 1183 (s), 1038 (s).

HRMS-ESI: Calculated for $C_{42}H_{55}N_5NaO_6S_4$ $[(M+Na+H)^+]$ 876.2933, found 876.2932.

4. Absorption spectra.

Figure 1. Absorption spectra of **1a** (5×10^{-5} M, DMSO/$MOPS_{aq}$).

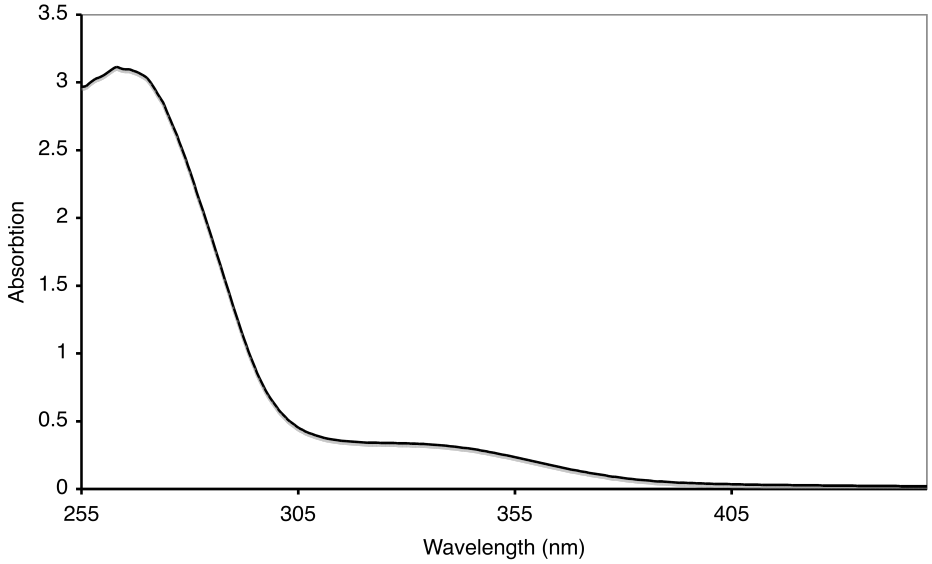


Figure 2. Absorption spectra of **1b** (5×10^{-5} M, DMSO/$MOPS_{aq}$).

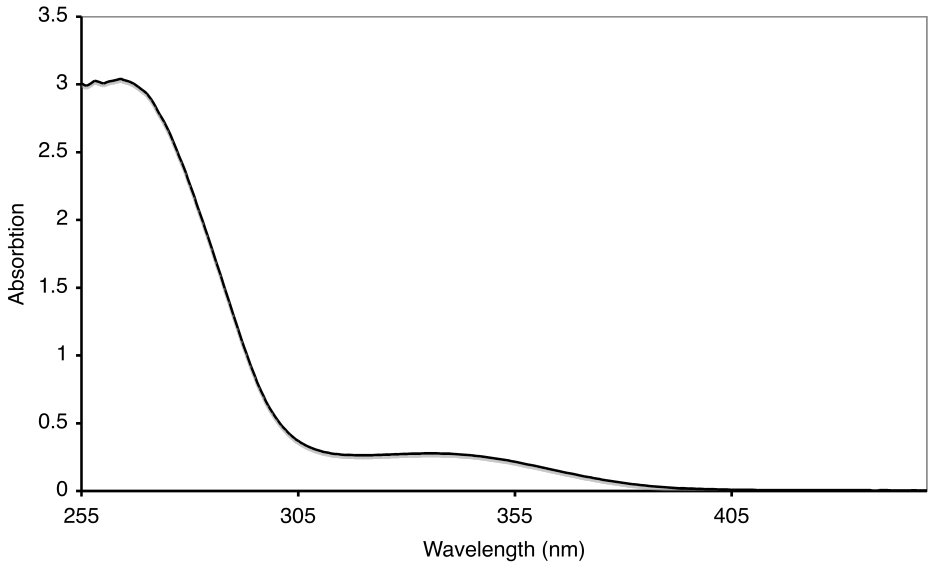
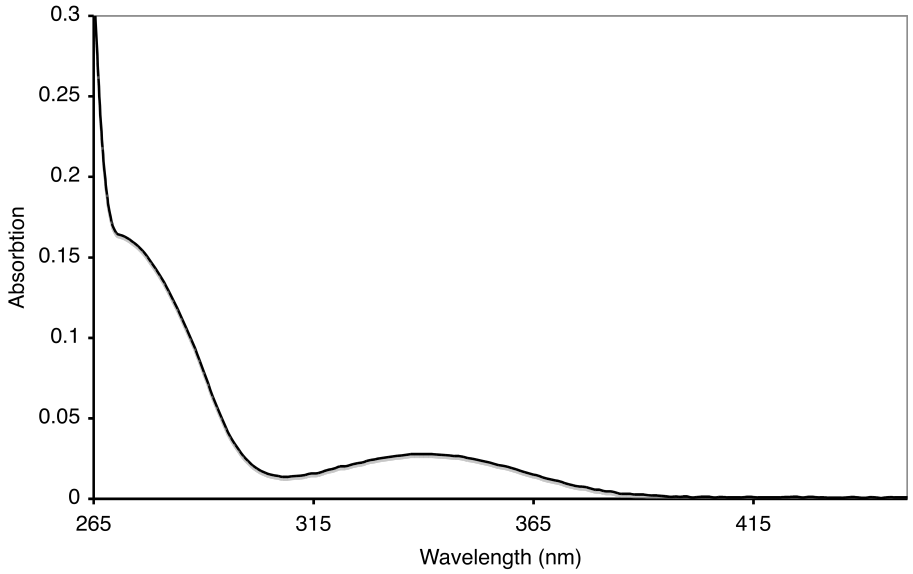


Figure 3. Absorption spectra of **1h** (5×10^{-6} M, DMSO).



5. Emission spectra and metal titrations

All metal titrations were carried out using 5×10^{-5} M solutions in 1:1 DMSO/pH 7.4 aqueous MOPS unless otherwise noted. Mercury (II) was added as a 10^{-3} M solution of $HgCl_2$ in 1:1 DMSO/aqueous MOPS via micropipette to 2.500 mL of fluorophore solution in a quartz cuvette. The solutions were equilibrated by stirring prior to acquiring fluorescence spectra.

Figure 4. Emission spectra of Hg^{2+} titration of **1a**.

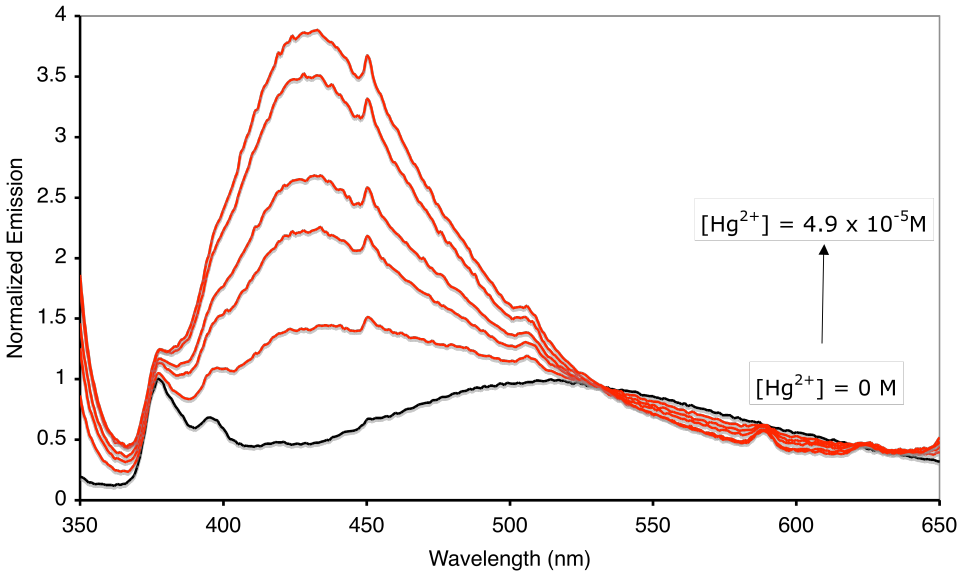


Figure 5. Change of I_{430}/I_{550} ration upon titration with Hg^{2+}.

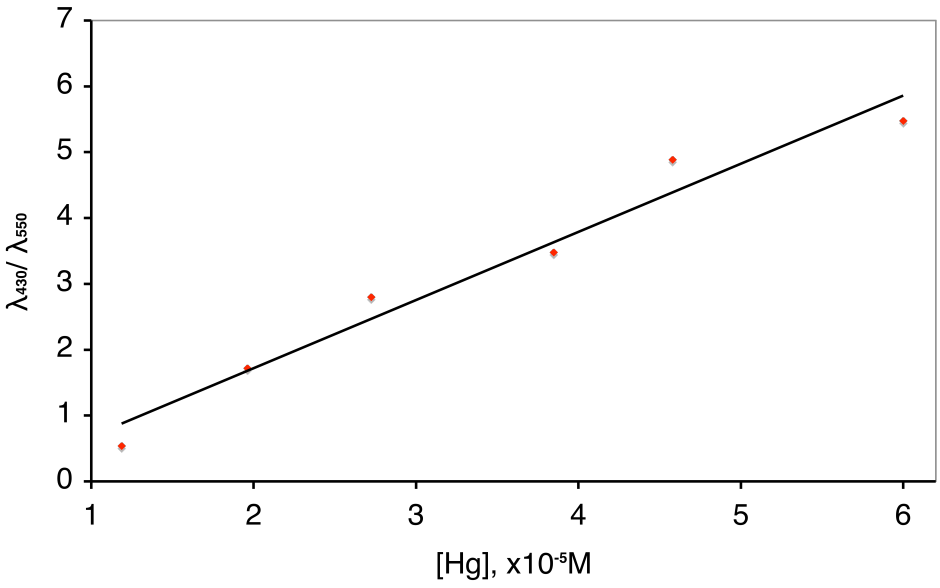


Figure 6. Emission spectra of Ag^{+} titration of **1a** (1×10^{-4}M).

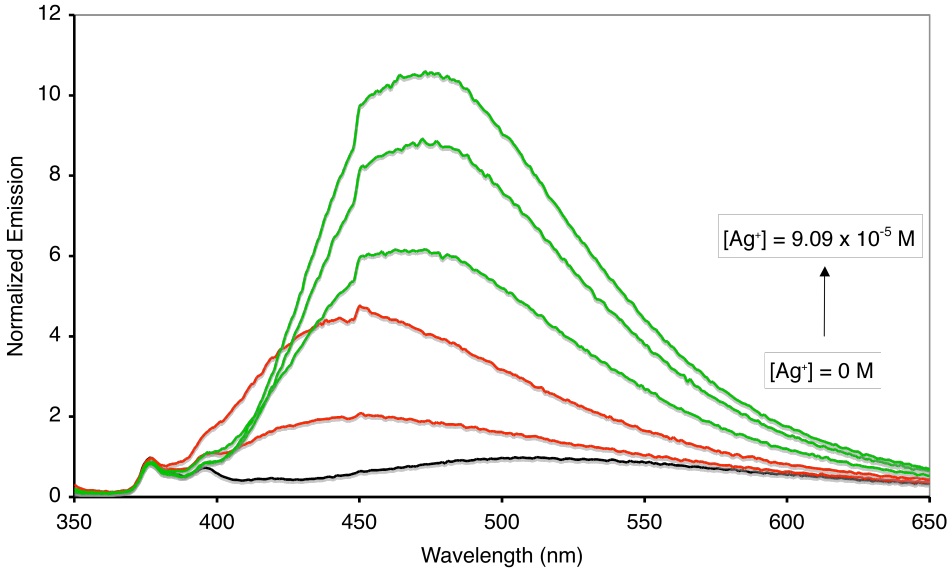


Figure 7. Emission spectra of Hg^{2+} titration of **1b**.

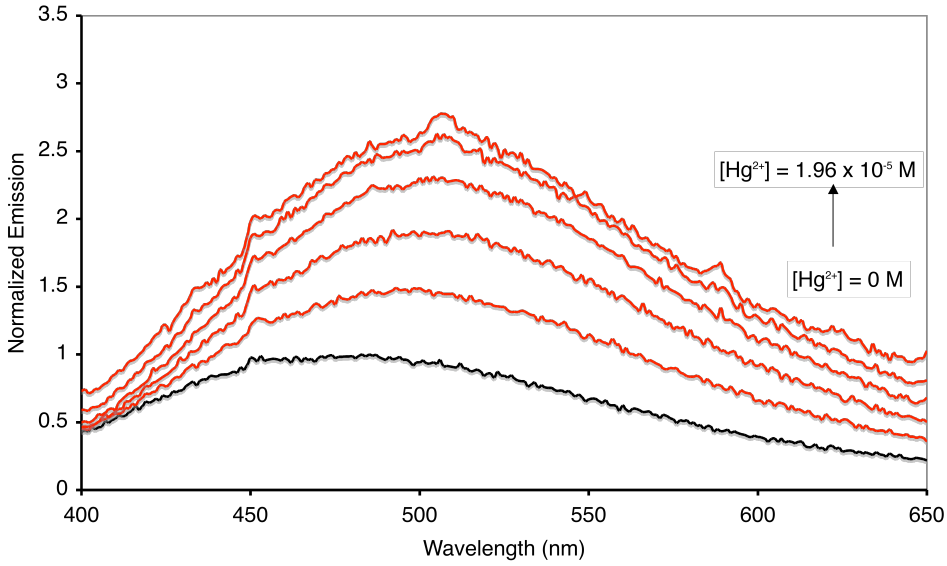


Figure 8. Emission spectra of Ag^{+} titration of **1b**.

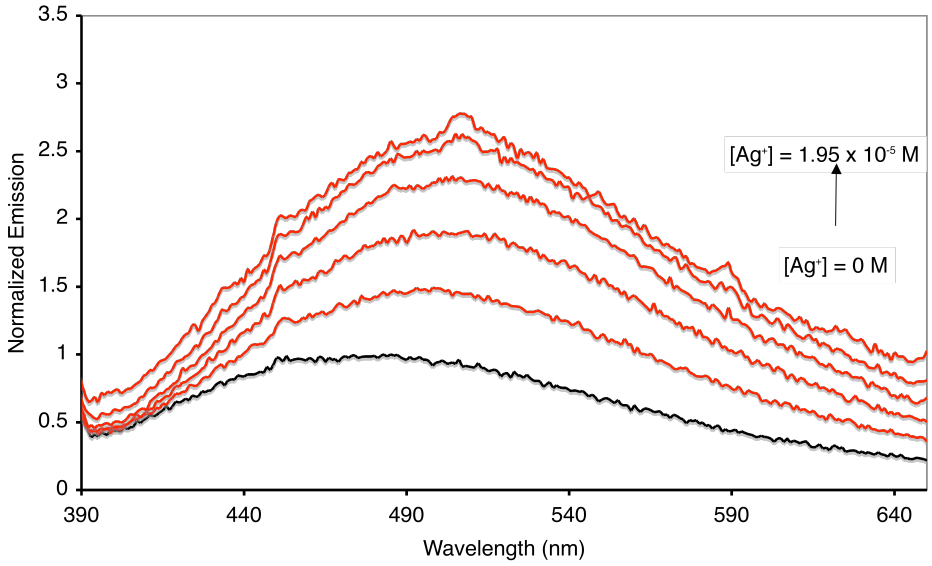


Figure 9. Emission spectra of Hg^{2+} titration of **1h** (1×10^{-4}M).

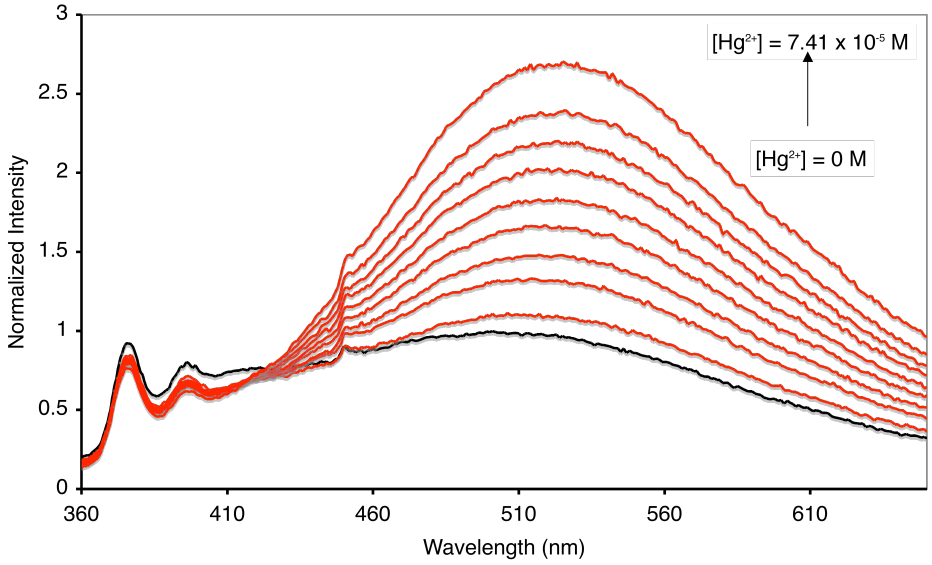
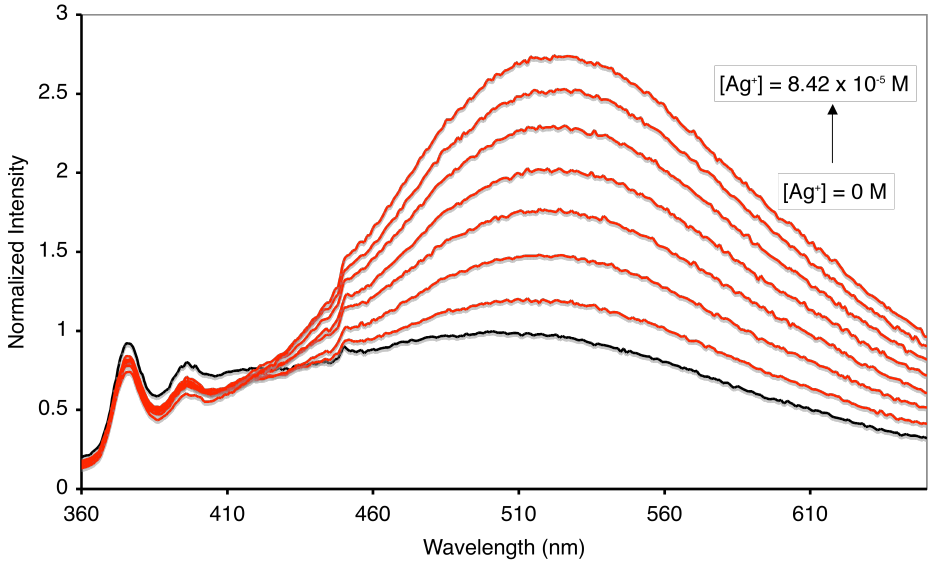


Figure 10. Emission spectra of Ag^+ titration of **1h** (1×10^{-4}M).



K_{assoc} Determination

The association constants K_{assoc} for the interaction of **1a, b, h** with Hg^{2+} and Ag^+ ions were estimated by the nonlinear curve-fitting of plots of fluorescence intensity vs. log[M] using *Prism3* (Graphpad, Inc., San Diego, CA). The titrations were carried out in a fluorescence cuvette by adding aliquots of $HgCl_2$ or AgOTs solutions via micropipette to a fluorophore solution of known concentration. DMSO/aqueous MOPS (5μM) solvent mixture was used in all cases. The solutions were equilibrated by stirring prior to acquiring the fluorescence spectra.

Determination of Stoichiometry

To determine binding stoichiometry the method of continuous variation (Job's method) was used, where titrations were performed holding the total concentration of Hg(II) or Ag(I) and ligand constant while varying the mole fraction of both.[8] To correct data for dilution UV spectra of all solutions were taken and the intensity at the emission maximum (I_{max}) obtained from fluorescent spectra was divided by absorption at the excitation wavelength. The absorbance varies linearly with concentration in all cases.

Figure 11. Job's plot for **1a** +AgOTs (3×10^{-5} M, DMSO/MOPS)

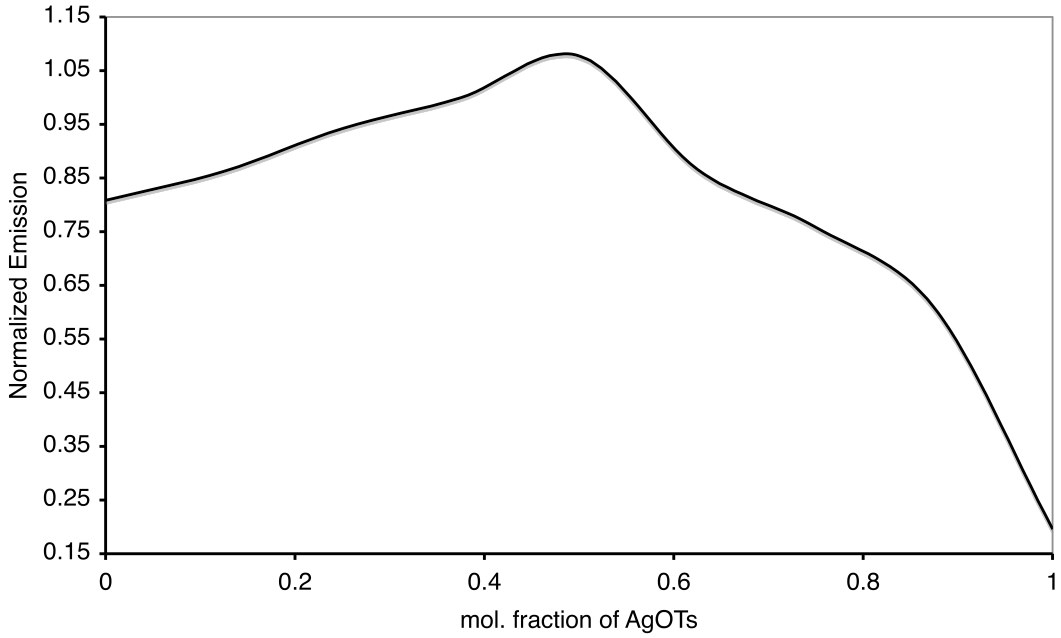


Figure 12. Job's plot for **1b** +AgOTs (3×10^{-5} M, DMSO/MOPS)

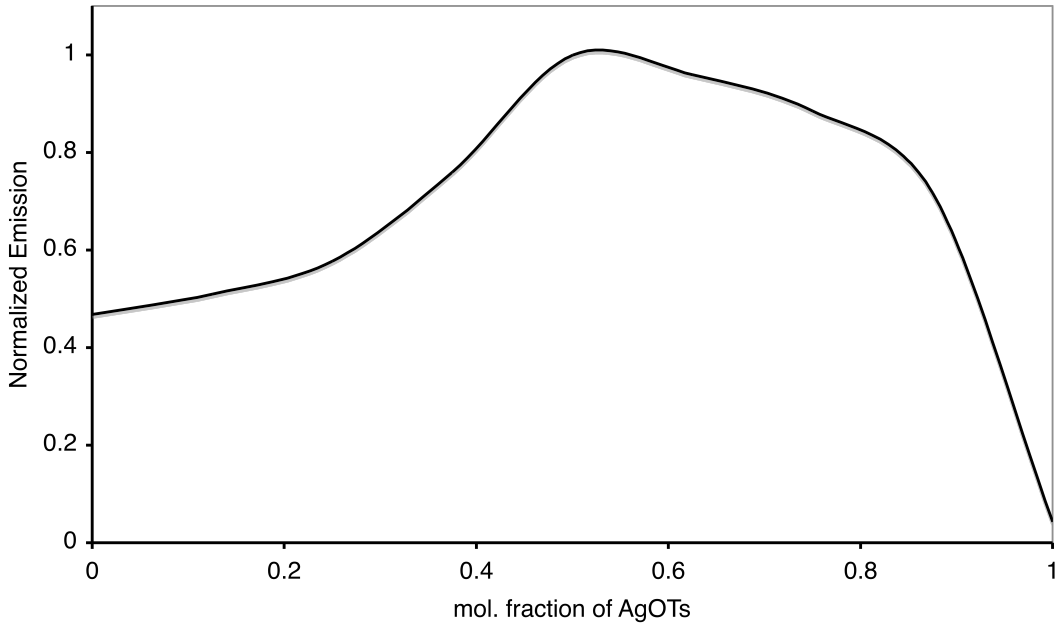
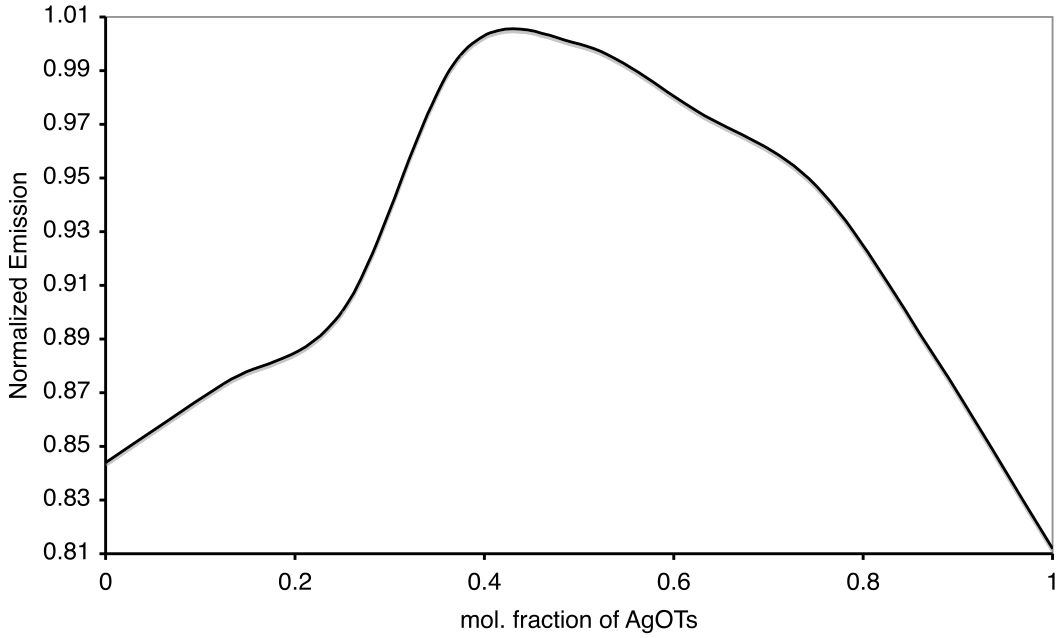


Figure 13. Job's plot for **1c** +AgOTs (3×10^{-5} M, DMSO/MOPS)



6. Calculated structures and relative energies

See ref. 20 (manuscript) for details of computational methodology.

Minimum-energy structures for **1a**•$HgCl_2$.

B98/DZ(2d,p) optimized

Structure	E_{rel} / kcal/mol
1a•$HgCl_2$ O/O	8.3
1a•$HgCl_2$ S/S	3.4
1a•$HgCl_2$ S/O	0.0

Three minimum energy structures found, two similar in energy, one significantly higher in energy.

1a•$HgCl_2$ O/O – $HgCl_2$ coordinated to two carbonyl oxygen atoms.
Relative energy + 8.5 kcal/mol. (Figures 14, 15.)

1a•$HgCl_2$ S/S – $HgCl_2$ coordinated to two thiocarbonyl sulfur atoms.
Relative energy + 3.4 kcal/mol. (Figures 16, 17.)

1a•$HgCl_2$ S/O – $HgCl_2$ coordinated to one carbonyl oxygen and one thiocarbonyl sulfur atom. Relative energy 0 kcal/mol. (Figures 15, 16.)

Minimum-energy structures for **1b**•$HgCl_2$.

B98/DZ(2d,p) optimized

Structure	E_{rel} / kcal/mol
1b•$HgCl_2$ A	1.5
1b•$HgCl_2$ B	0.0

Two minimum energy structures found, differing only slightly in energy (Figures 20-23).

Figure 14. Ball-and-stick view of **1a**•$HgCl_2$ O/O.

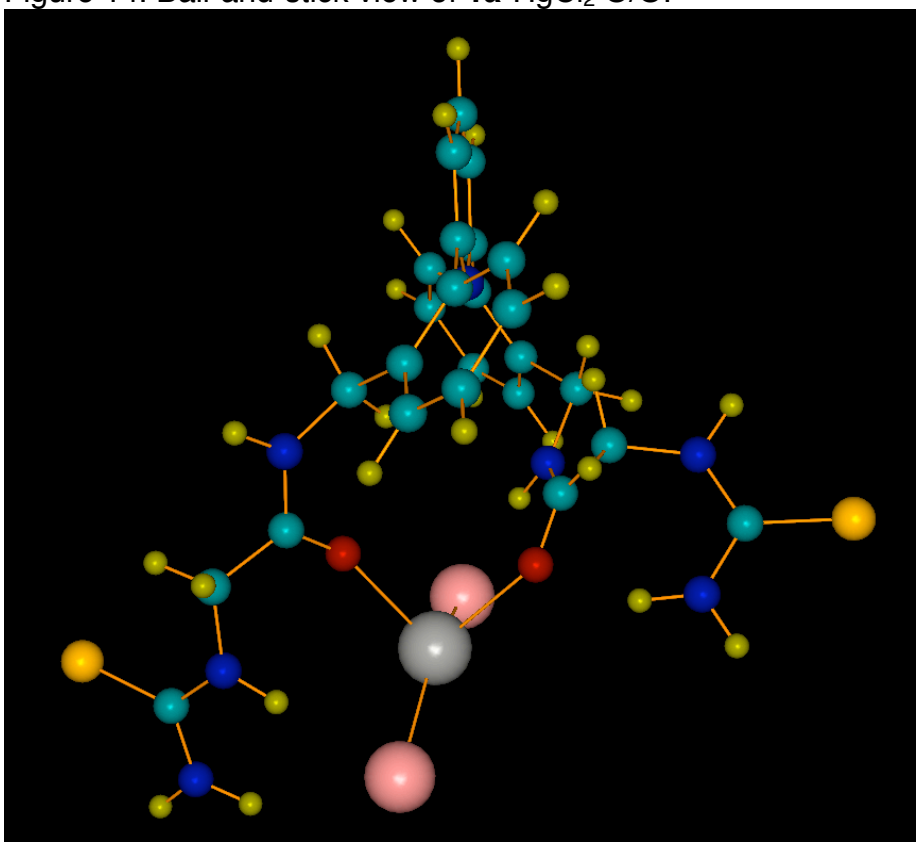


Figure 15. Space-filling view of **1a**•$HgCl_2$ O/O, with and without H atoms.

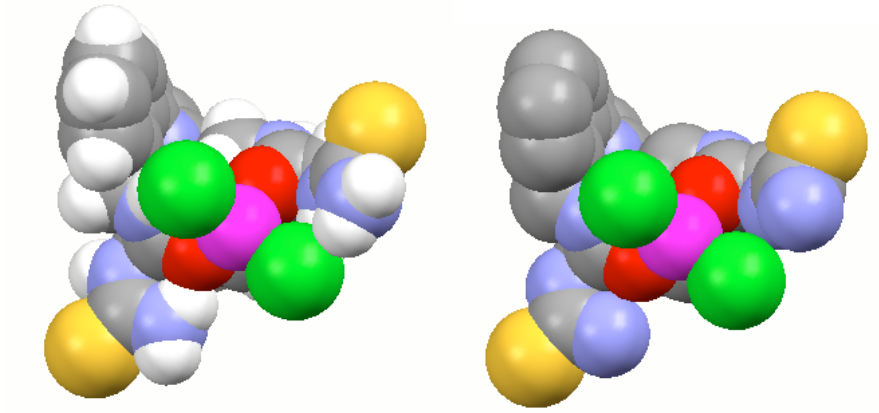


Figure 16. Ball-and-stick view of **1a**•$HgCl_2$ S/S.

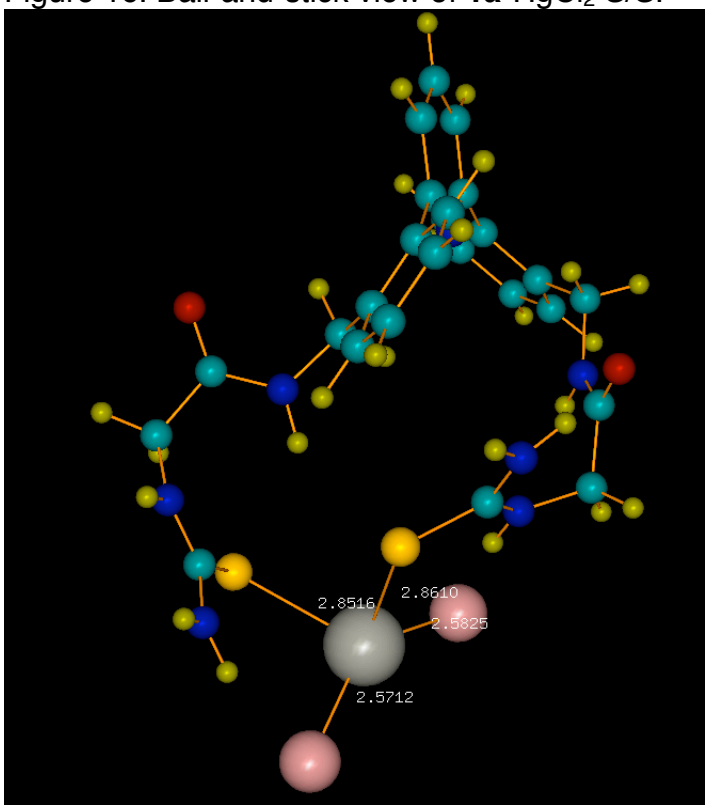


Figure 17. Space-filling view of **1a**•$HgCl_2$ S/S, with and without H atoms.

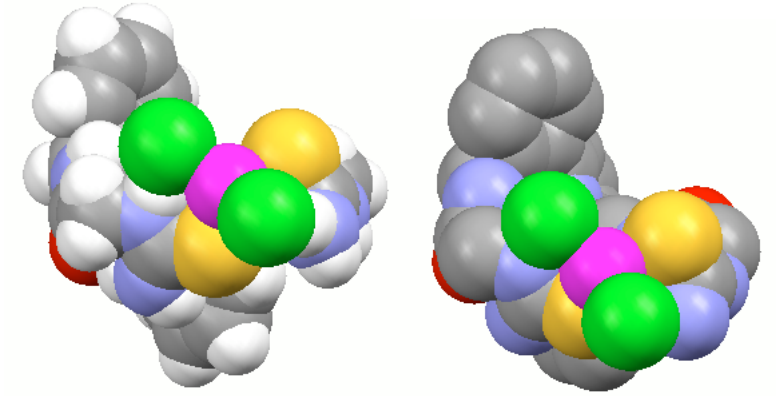


Figure 18. Ball-and-stick view of **1a**•$HgCl_2$ S/O.

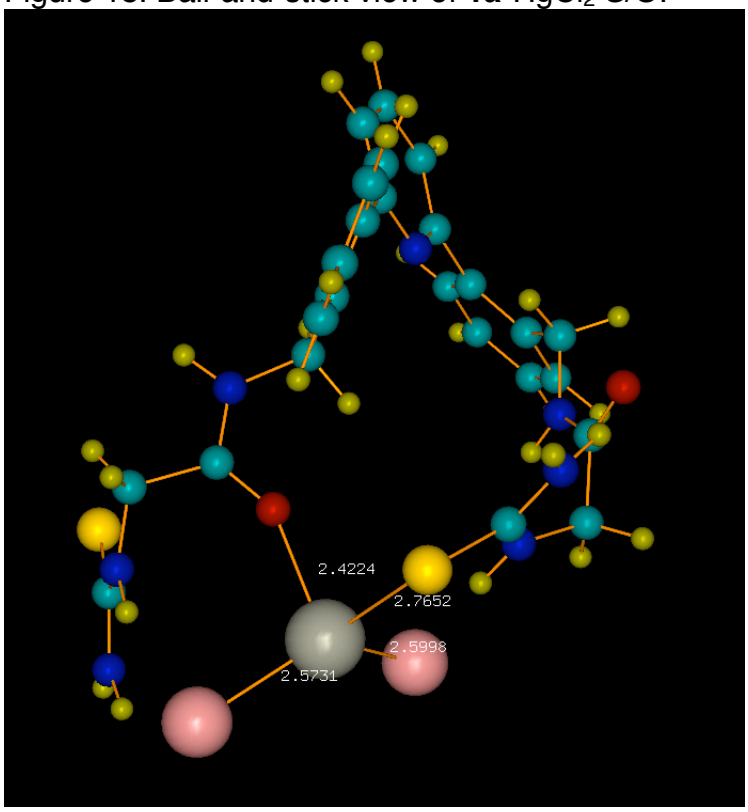


Figure 19. Space-filling view of **1a**•$HgCl_2$ S/O, with and without H atoms.

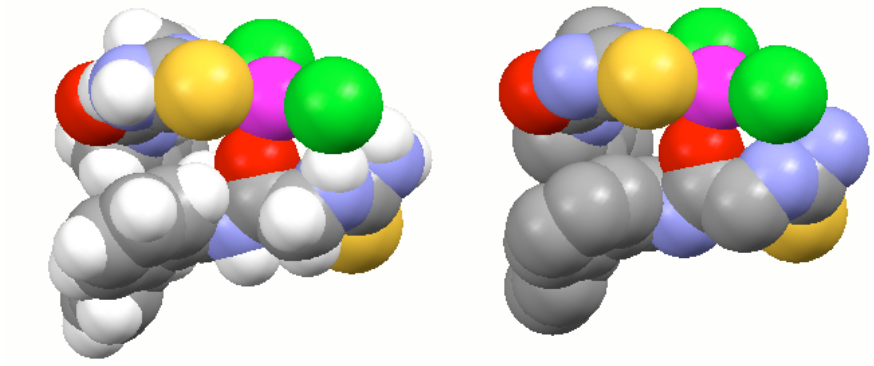


Figure 20. Ball-and-stick view of **1b**•$HgCl_2$ A.

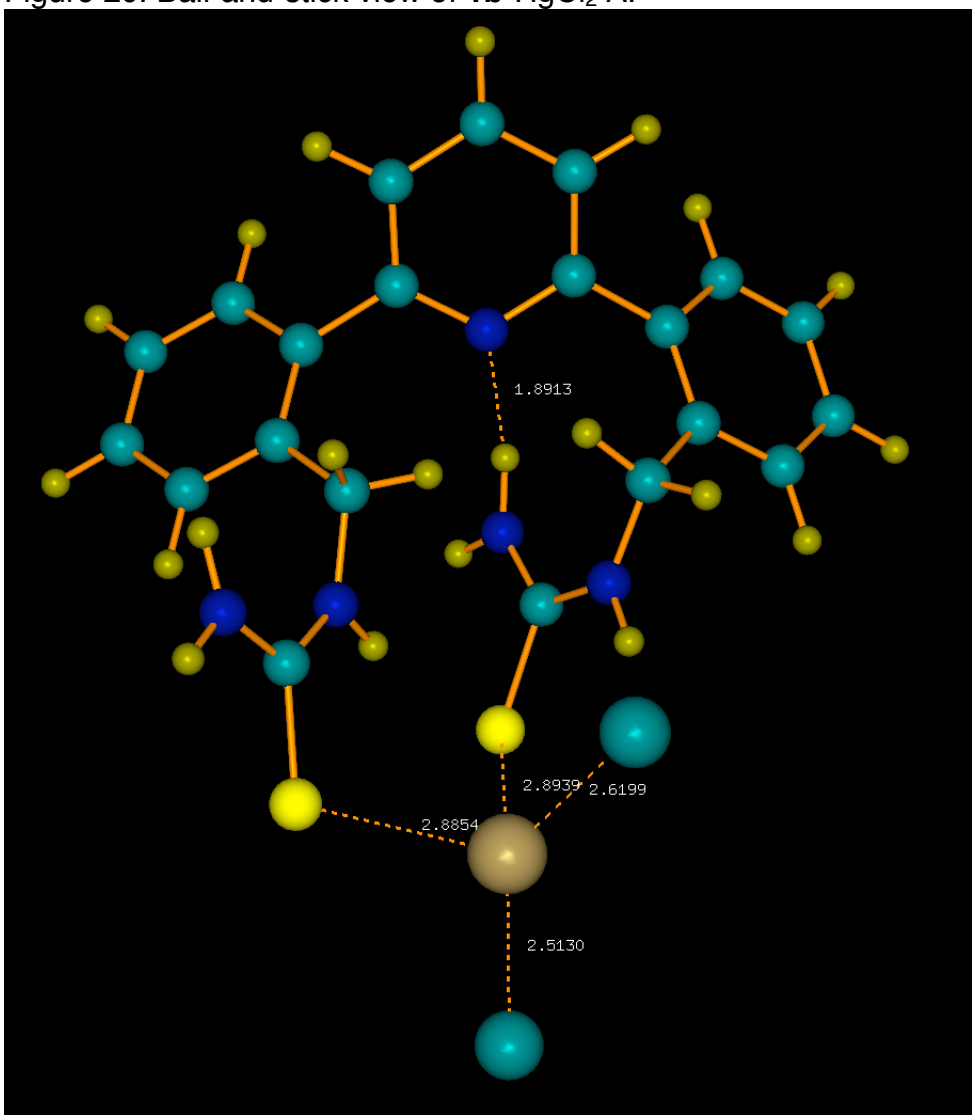


Figure 21. Space-filling view of **1b**•$HgCl_2$ A, with and without H atoms.

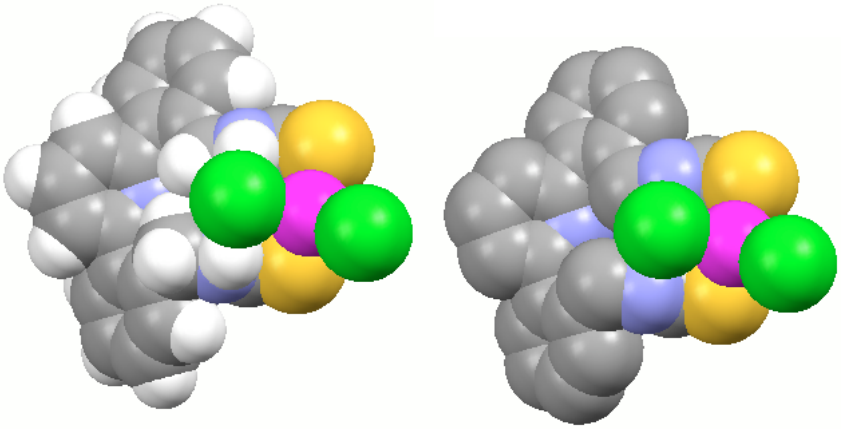


Figure 22. Ball-and-stick view of **1b**•$HgCl_2$ B.

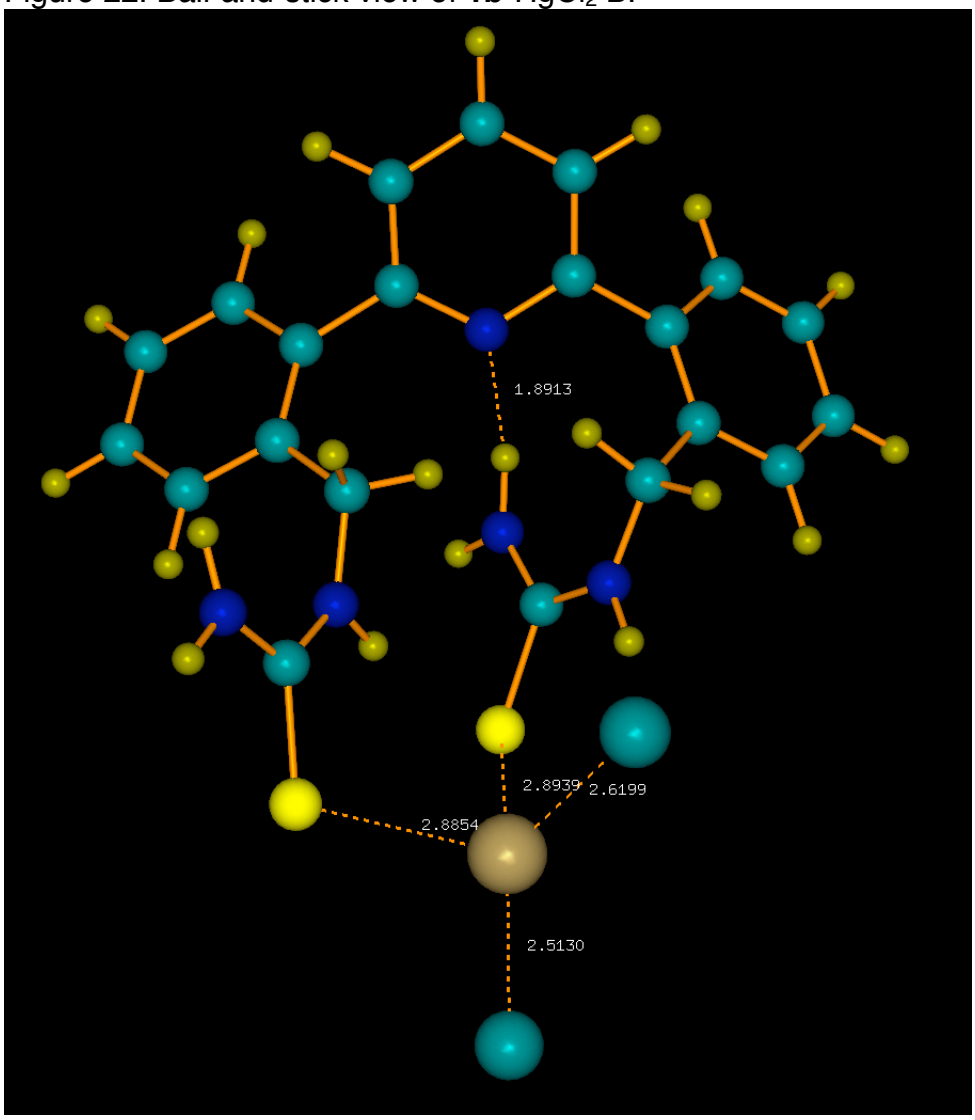
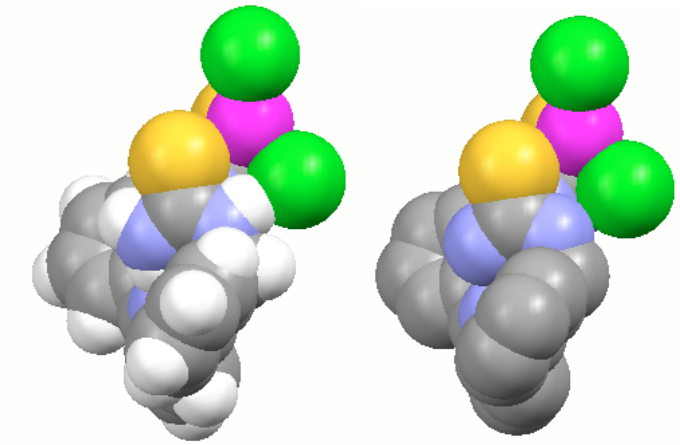


Figure 23. Space-filling view of **1b**•$HgCl_2$ B, with and without H atoms.



7. References

(1) Lakowicz, J.R. *Principles of Fluorescent Spectroscopy, 2nd ed.* **1999**, New York, Kluwer Akademic.
(2) Demas, J.M.; Grosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.
(3) Berlman, I.B. *Handbook of Fluorescent Spectra* **1965**, New York, Academic Press.
(4) Mello, J.V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124.
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(6) Carotti, A.; Campagna, F. *Synthesis*, **1979**, 56.
(7) van de Water, L.G.A.; ten Hoonte, F.; Driessen, W. L.; Reedijk, J.; Sherrington, D. C. *Inorg. Chim. Acta* **2000**, *303*, 77.
(8) Connors, K.A. *Binding Constants: The Measurement of Molecular Complex Stability* **1987**, New York, John Wiley and Sons.

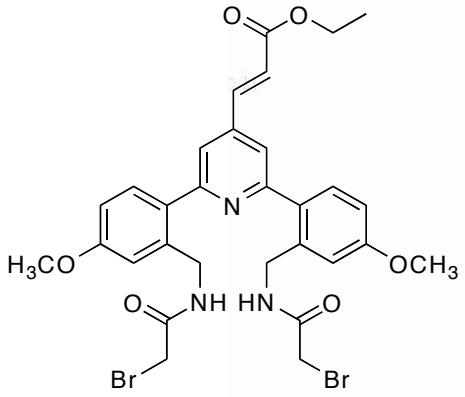
8. ^{1}H and ^{13}C NMR Spectra

We have noted that all biarylpyridine compounds bearing a vinylogous amide at C4 of the pyridine ring undergo gradual photochemical isomerization under fluorescent light. The NMR spectra thus show the presence of small amounts of the *(Z)*-isomer in addition to the dominant *(E)*-isomer. We have confirmed the identity of the *(Z)*-isomer by deliberate photochemical isomerization to prepare samples with sufficient amounts of the minor isomer to allow full peak assignment in the case of **1a**.

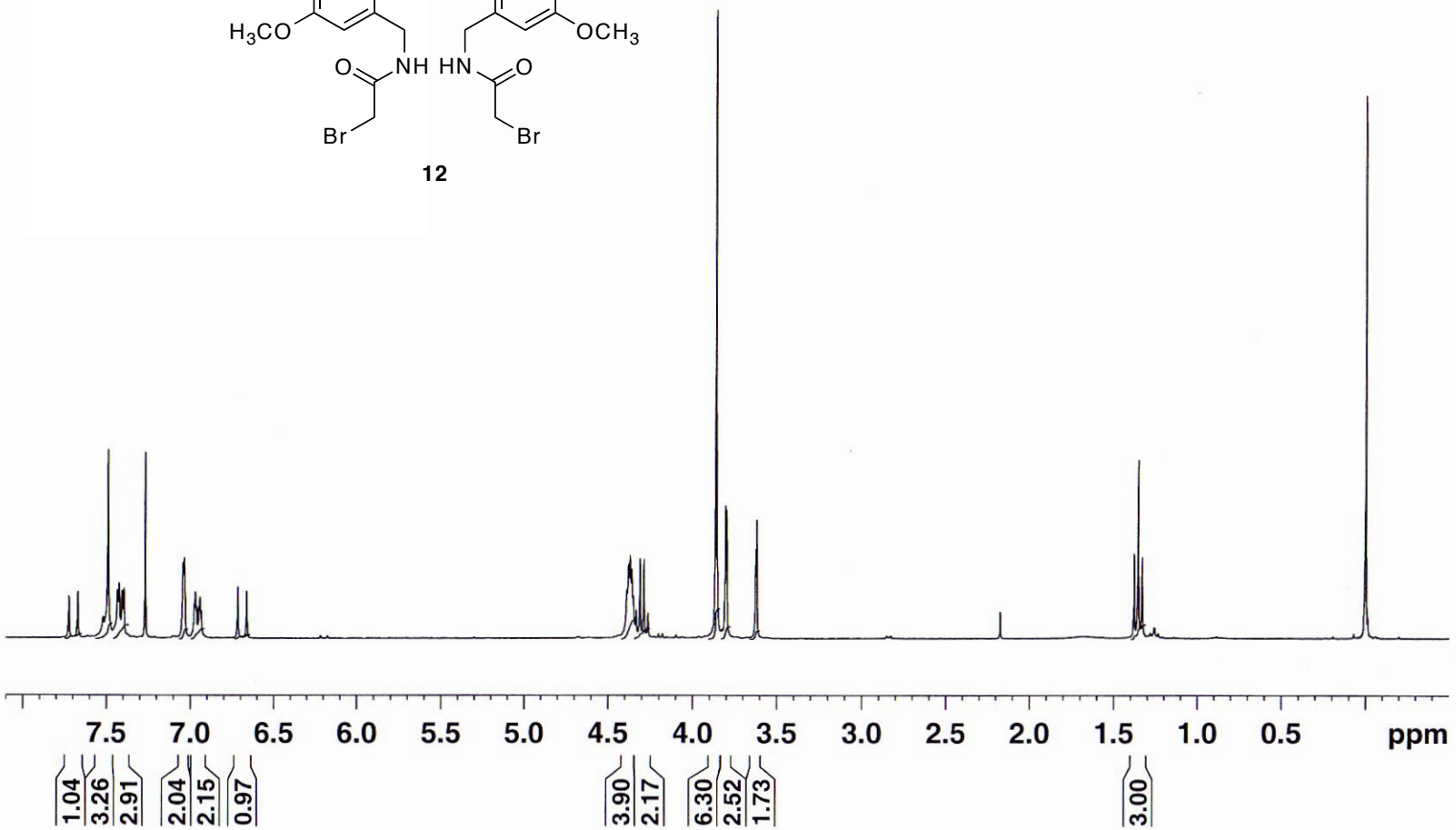
DUAL_1H_no_peak CDCl3 v sienmr 7

7.67 7.52 7.49 7.43 7.42 7.40 7.39 7.04 6.97 6.96 6.95 6.94 6.93 6.66

4.39 4.38 4.37 4.36 4.35 4.34 4.31 4.29 4.26 3.81 3.80 3.63 3.62



12



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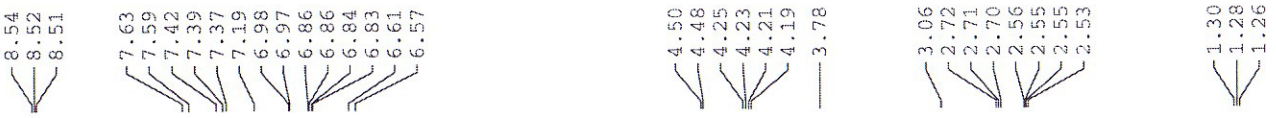

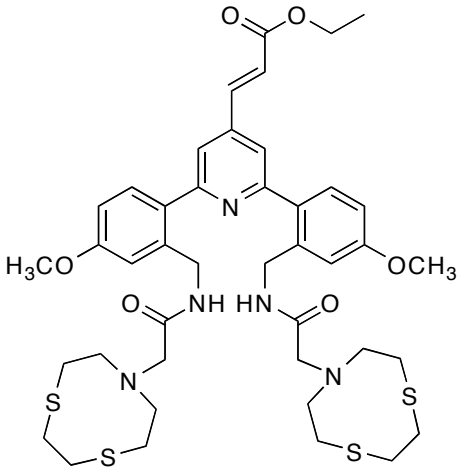

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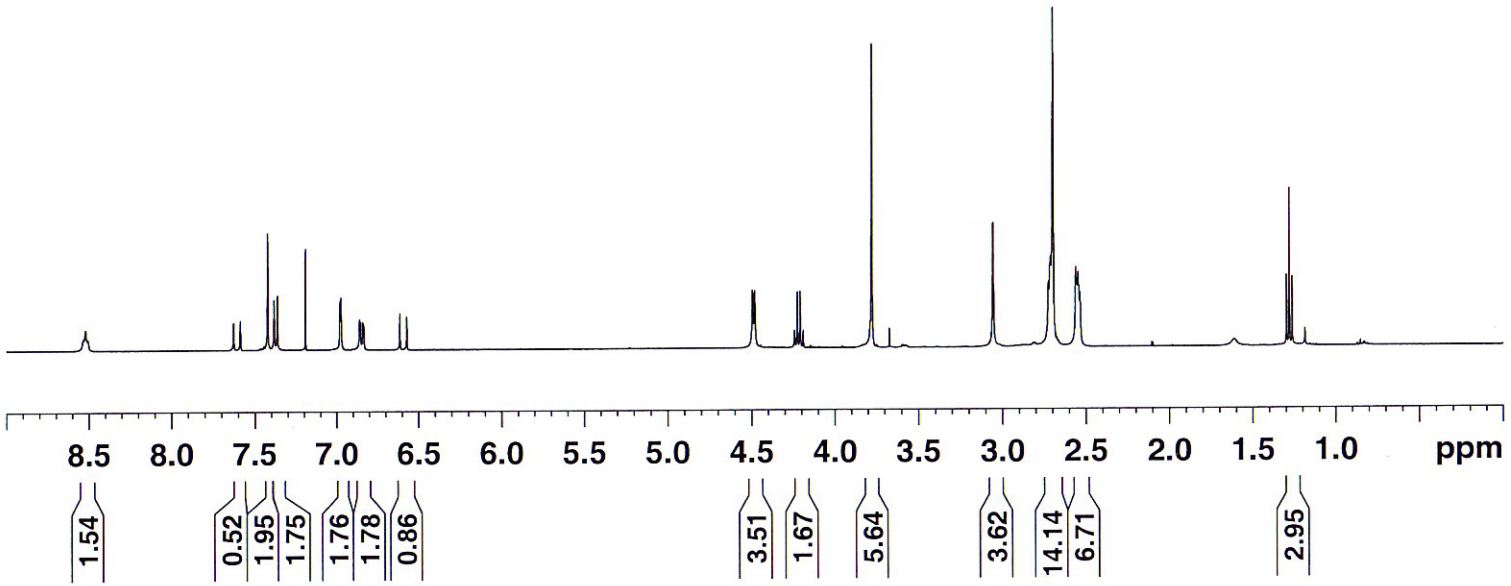

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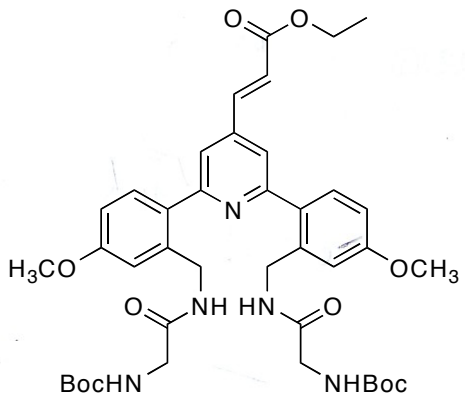

10

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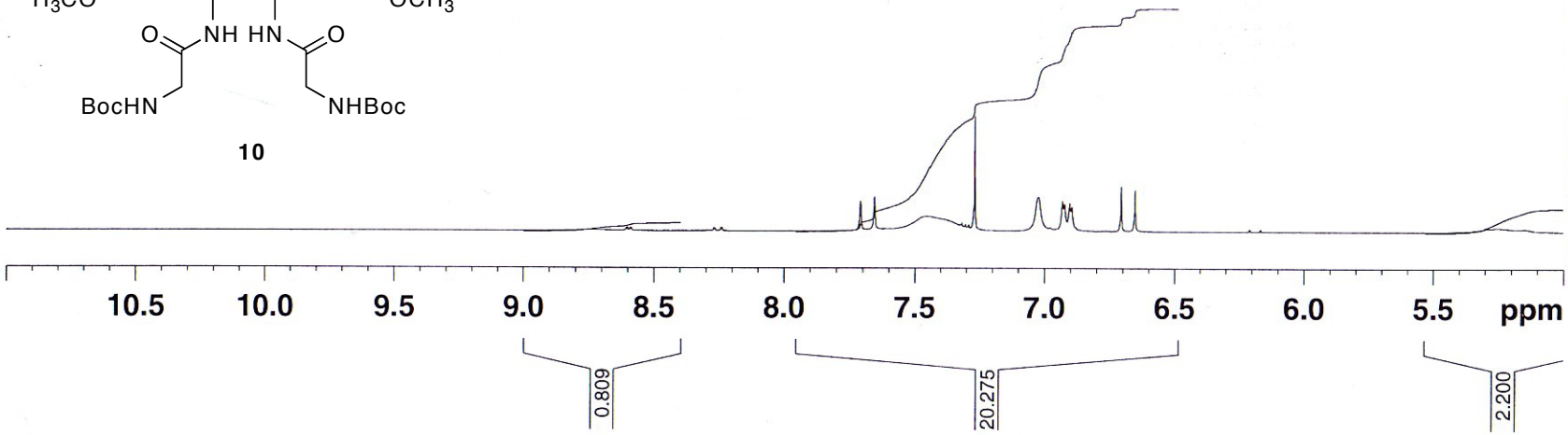

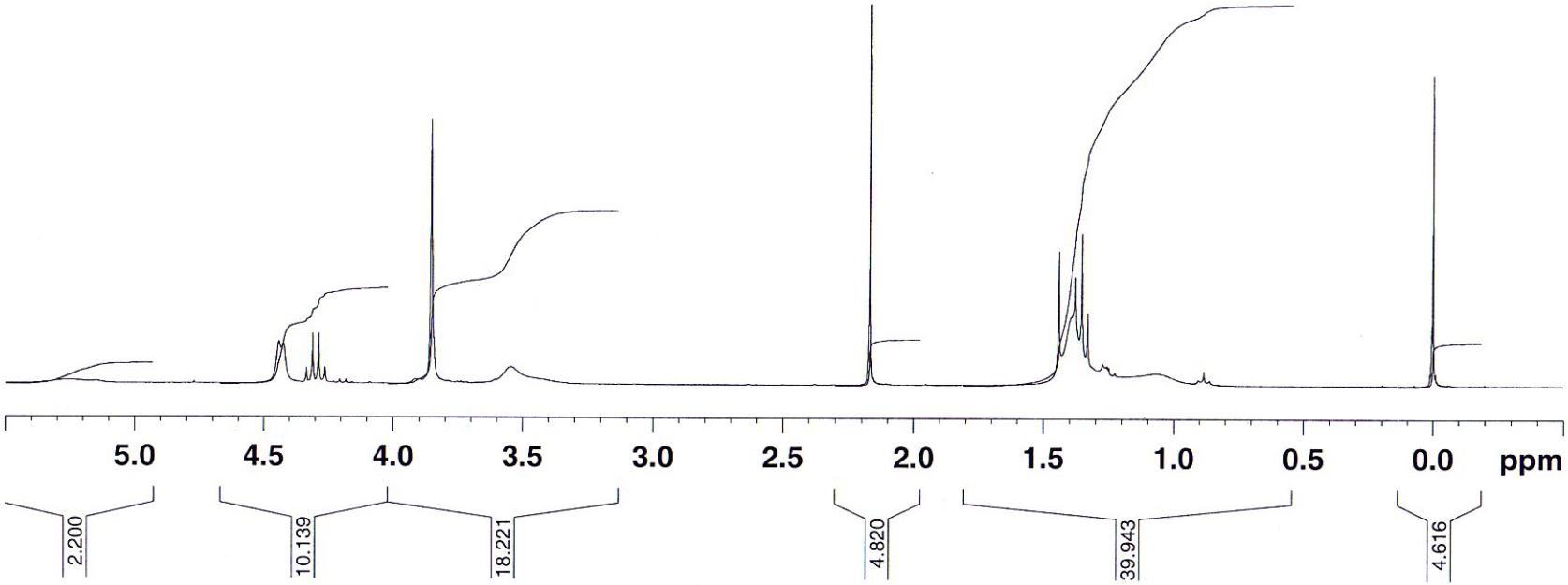

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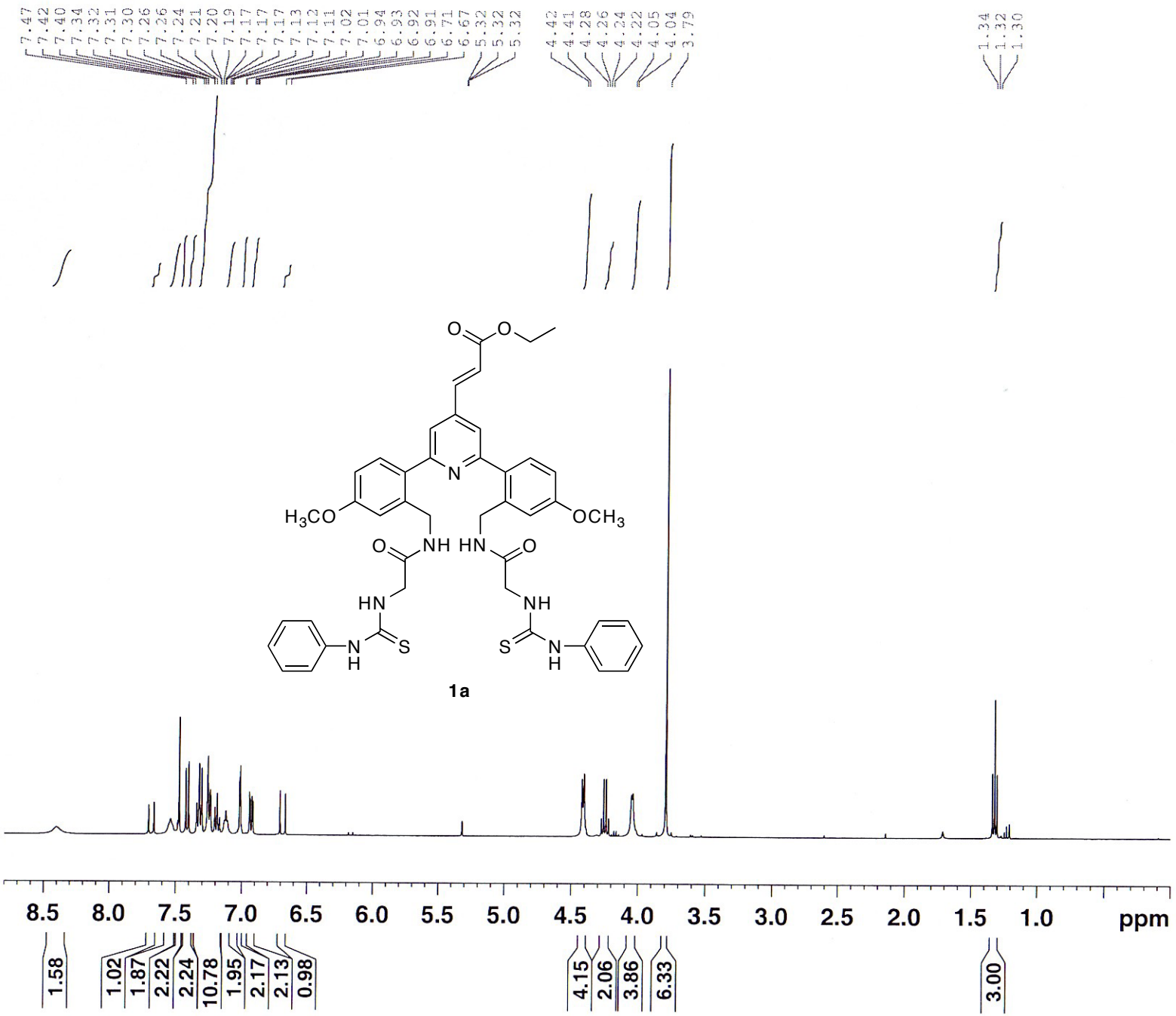

BRUKER

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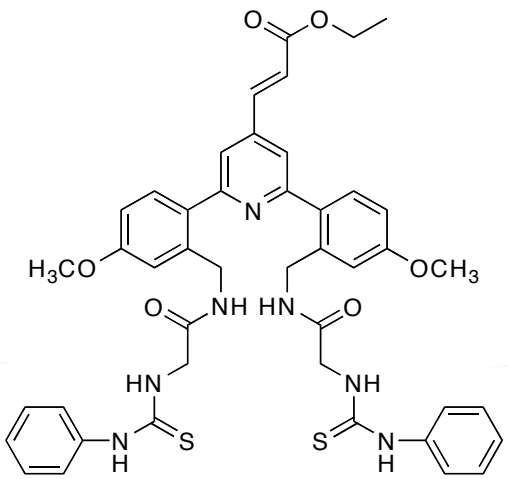
DW                    40.533 usec
DE                     6.00 usec
TE                     300.0 K
D1                 3.00000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                       1H
P1                     12.00 usec
PL1                    -3.00 dB
SFO1           400.1324008 MHz

F2 - Processing parameters
SI                     65536
SF             400.1300153 MHz
WDW                       no
SSB                        0
LB                      0.00 Hz
GB                         0
PC                      1.00
```



1a

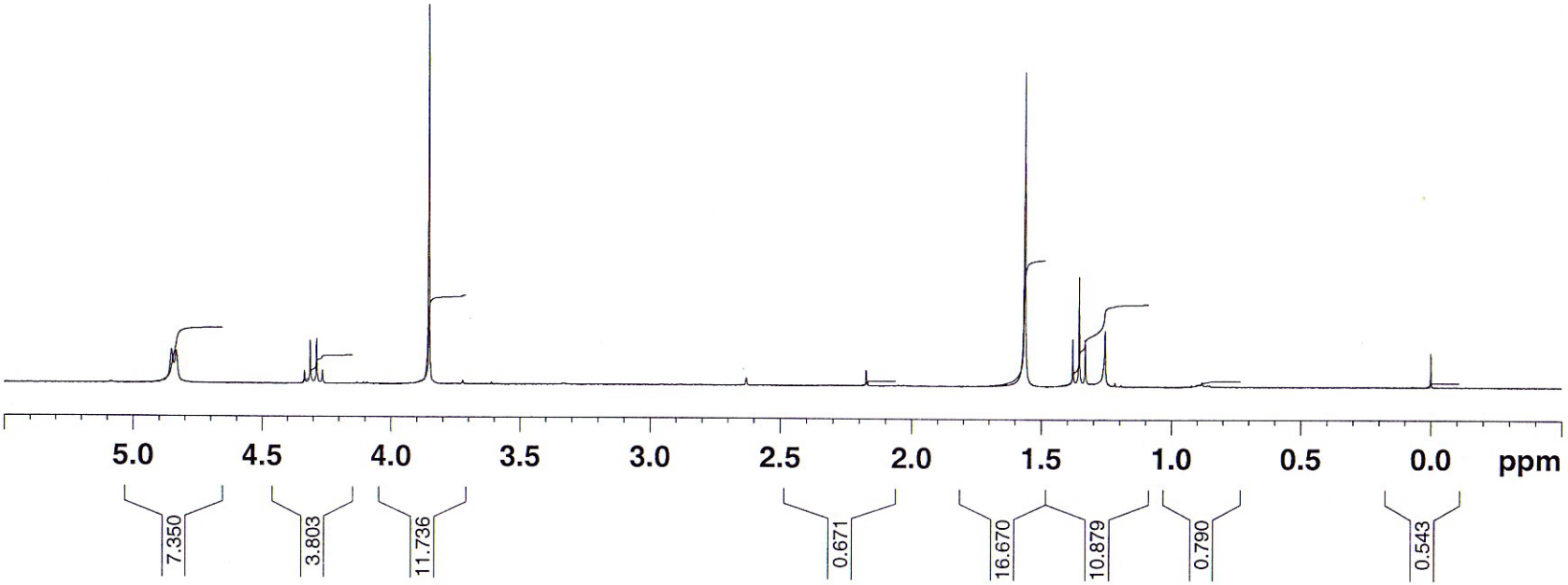
DUAL_1H_no_peak CDCl3 v sienmr 56


```
Current Data Parameters
NAME                  SM81.3
EXPNO                      1
PROCNO                     1

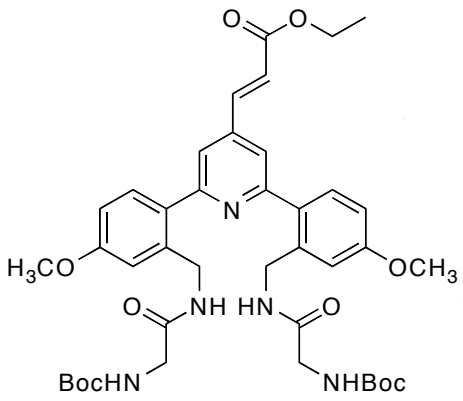
F2 - Acquisition Parameters
Date_              20061201
Time                 10.14
INSTRUM              arx300
PULPROG              zg30
TD                   32768
NS                      16
DS                       2
SWH               6250.000 Hz
FIDRES            0.190735 Hz
D1             1.00000000 sec
P1                 7.50 usec

F2 - Processing parameters
SI                   32768
SF           300.1300063 MHz
WDW                     EM
SSB                      0
LB                0.20 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
PPMCM             0.30263 ppm/cm
HZCM             90.82882 Hz/cm
```



DUAL_13C.1H Aceton v sienmr 28



10

Current Data Parameters

NAME	SM061.1
EXPNO	2
PROCNO	1

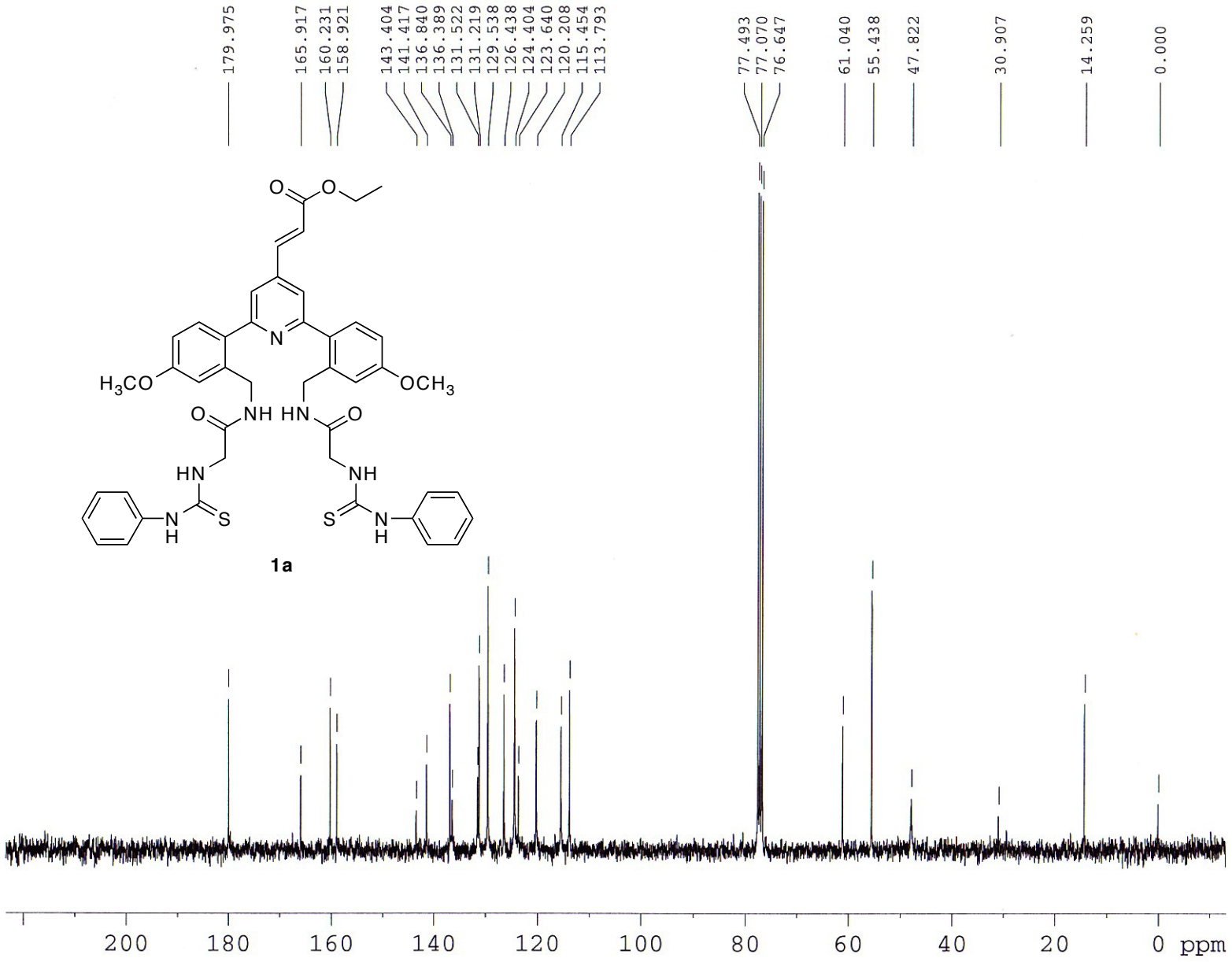
F2 - Acquisition Parameters

Date_	20080129
Time	19.06
INSTRUM	arx300
PULPROG	zgpg30
TD	32768
NS	512
DS	4
SWH	17857.143 Hz
FIDRES	0.544957 Hz
D1	1.00000000 sec
P1	6.50 usec
D12	0.0000200 sec
D11	0.0300000 sec

F2 - Processing parameters

SI	32768
SF	75.4675982 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40

DUAL_13C.1H CDCl3 v sienmr 6



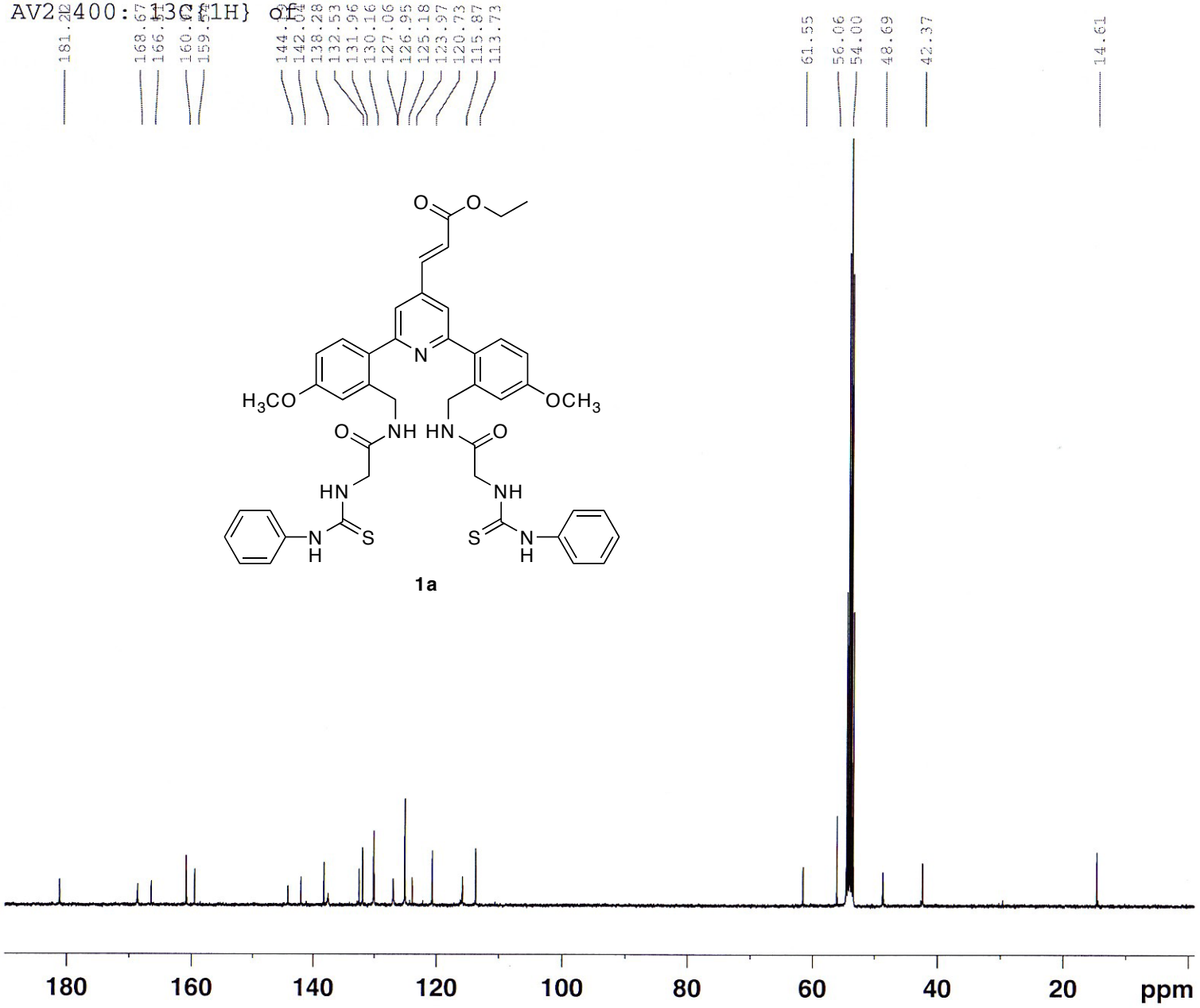
1a

```
Current Data Parameters
NAME                I19SM81.2
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_                20060508
Time                    14.12
INSTRUM                arx300
PULPROG                zgpg30
TD                      32768
NS                        512
DS                          4
SWH                 17857.143 Hz
FIDRES              0.544957 Hz
D1                  1.00000000 sec
P1                      6.50 usec
D12                 0.0000200 sec
D11                 0.0300000 sec

F2 - Processing parameters
SI                      32768
SF                 75.4677469 MHz
WDW                        EM
SSB                         0
LB                       2.00 Hz
GB                          0
PC                       1.40
```

AV2 400: 13C{1H} of



1a

BRUKER

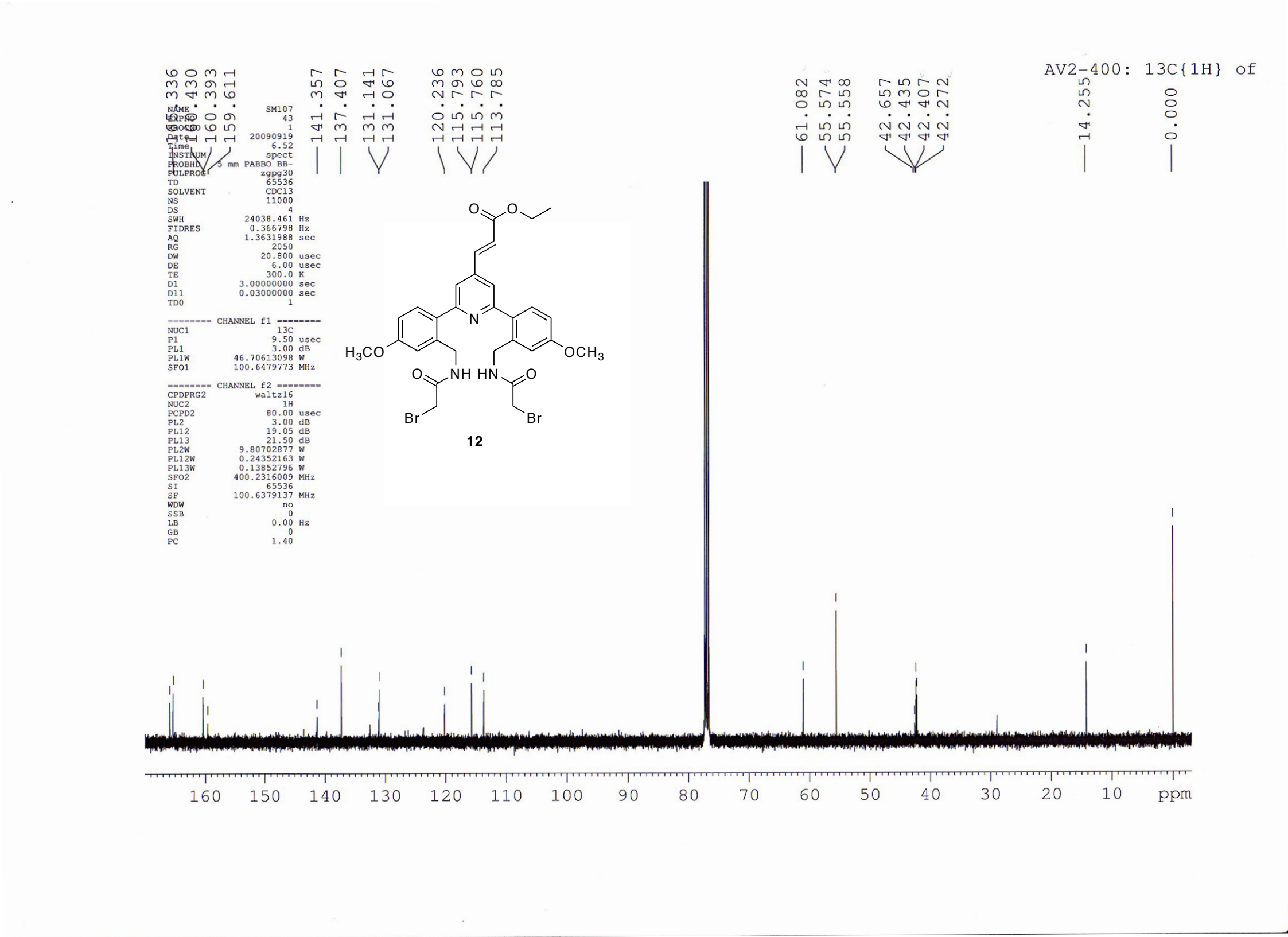
```
Current Data Parameters
NAME                 SM073.2
EXPNO                     42
PROCNO                     1

F2 - Acquisition Parameters
Date_              20080216
Time                   5.57
INSTRUM               spect
PROBHD    5 mm PAQNP Swi
PULPROG              zgpg30
TD                    65536
SOLVENT              CD2Cl2
NS                    10240
DS                        4
SWH               39062.500 Hz
FIDRES             0.596046 Hz
AQ                0.8389108 sec
RG                     2050
DW                   12.800 usec
DE                    6.00 usec
TE                   300.0 K
D1              3.00000000 sec
d11             0.03000000 sec
DELTA           2.90000010 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                     13C
P1                    8.40 usec
PL1                  -2.00 dB
SFO1          100.6238364 MHz

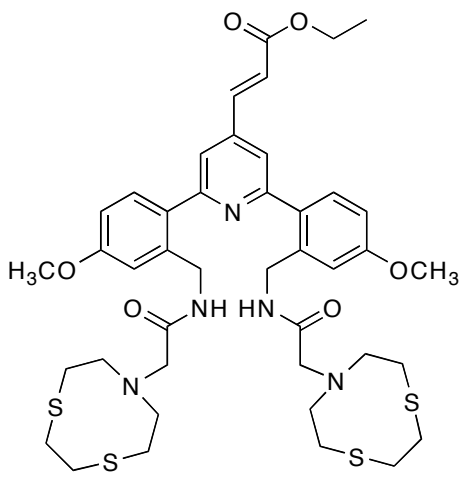
======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                     1H
PCPD2                80.00 usec
PL12                 16.00 dB
PL13                 18.00 dB
PL2                  -3.00 dB
SFO2          400.1316005 MHz

F2 - Processing parameters
SI                    65536
SF            100.6127100 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.40
```


AV2-400: 13C{1H} of
NAME SM107
EXPNO 43
PROCNO 1
Date_ 20090919
Time 6.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 11000
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 2050
DW 20.800 usec
DE 6.00 usec
TE 300.0 K
D1 3.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 3.00 dB
PL1W 46.70613098 W
SFO1 100.6479773 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 19.05 dB
PL13 21.50 dB
PL2W 9.80702877 W
PL12W 0.24352163 W
PL13W 0.13852796 W
SFO2 400.2316009 MHz
SI 65536
SF 100.6379137 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
161.336
160.430
160.393
159.611
141.357
137.407
131.141
131.067
120.236
115.793
115.760
113.785
61.082
55.574
55.558
42.657
42.435
42.407
42.272
14.255
0.000
O
O
N
H3CO
OCH3
O
NH HN
O
Br
Br
12
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

AV2-400: 13C{1H} of

171.09 166.52 160.60 159.67 143.28 142.39 138.52 133.03 131.94 123.68 120.68 115.76 113.76 61.44 60.87 56.69 56.00 41.89 34.68 33.26 14.78



1h


```
Current Data Parameters
NAME                  SM162
EXPNO                     3
PROCNO                    3

F2 - Acquisition Parameters
Date_              20081212
Time                  11.37
INSTRUM               spect
PROBHD   5 mm PAQNP Swi
PULPROG             zgpg30
TD                   65536
SOLVENT              CDC13
NS                    1536
DS                       4
SWH              39062.500 Hz
FIDRES           0.596046 Hz
AQ               0.8389108 sec
RG                    2050
DW                12.800 usec
DE                20.00 usec
TE                   300.0 K
D1            3.00000000 sec
d11           0.03000000 sec
DELTA         2.90000010 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                  8.40 usec
PL1                -2.00 dB
SFO1         100.6238364 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2              80.00 usec
PL12               16.00 dB
PL13               18.00 dB
PL2                -3.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                   65536
SF           100.6127182 MHz
WDW                     no
SSB                      0
LB                0.00 Hz
GB                       0
PC                    1.40
```