Supplementary Information for:

Light-Responsive Bicyclic Peptides

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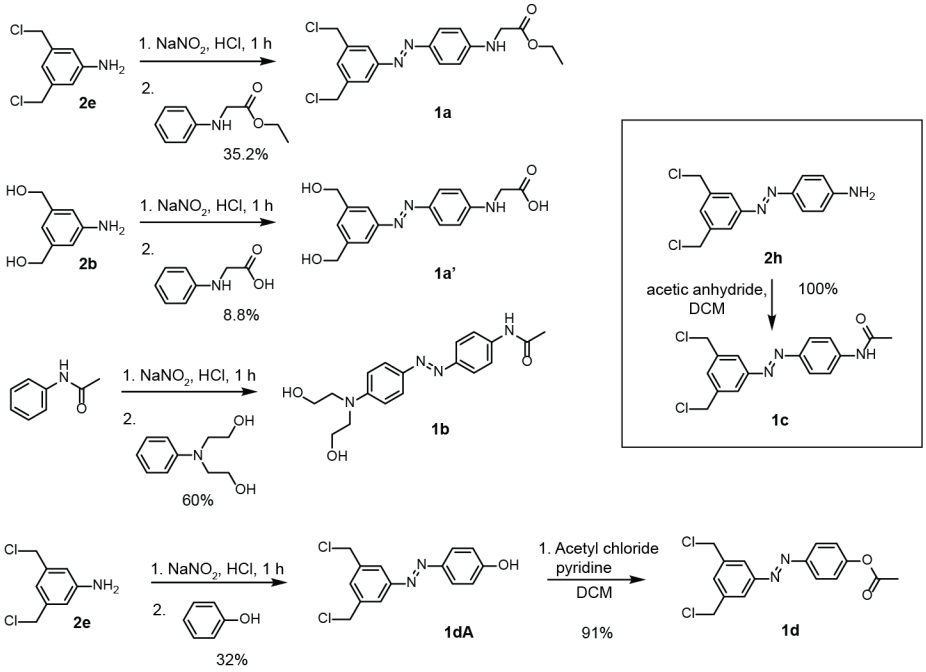
Abbreviations

1D	one-dimensional
AM resin	aminomethyl resin
APT	attached proton test
AU	arbitrary unit
Azb	azobenzene
Boc	*tert*-butyloxycarbonyl
calc.	calculated
Da	dalton(s)
DCM	dichloromethane
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	dimethylformamide
EDC.HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDT	1,2-ethanedithiol
eq.	equivalent(s)
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	fluorenylmethoxycarbonyl chloride
h	hour(s)
HBTU	*N*,*N*,*N'*,*N'*,-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uranium hexafluorophosphate
HADCAz	hydroxyl amine and di-chlorobenzene containing azobenzene
HRMS	high-resolution mass spectrometry
LCMS	liquid chromatography-mass spectrometry
MALDI-MS	matrix-assisted laser desorption ionization-mass spectrometry
MeCN	acetonitrile
MHz	megahertz
MeOH	methanol
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
mL	milliliter(s)
min	minute(s)
mmol	millimoles
nm	nanometer(s)
ppm	parts per million
ref.	reference
ROESY	rotating frame overhauser effect spectroscopy
r.t.	room temperature
TCEP	tris(2-carboxyethyl) phosphine
temp.	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TOCSY	total correlation spectroscopy
UV	ultraviolet
v/v	volume/volume
w/v	weight/volume

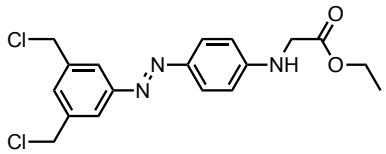
Materials and methods



Scheme S1. Syntheses of azobenzene derivatives **1a**, **1a'**, **1b**, **1c**, **1d**.

Synthesis of 1a-1d, HADCAz and intermediates

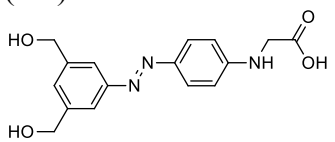
Synthesis of **1a**



To an ice cold solution of 450 mg (2.4 mmol) of compound **2e** (see the following) in 10 mL EtOH, we added 2 mL of 2 M HCl and stirred the solution on ice for 30 min. To this mixture we added a solution of 165 mg (2.4 mmol) of $NaNO_2$ in water drop wise with vigorous stirring for 1 h to form the diazo salt. Then we added the diazo salt solution drop wise to an ice cold solution of 537 mg (3 mmol) of *N*-phenyl-ethylglycine in 50 mL of EtOH/2 M aqueous glycine (0.2 M, pH 5) = 1/1. After stirring for 15 min at this temperature, the solution was warmed up to r.t. and stirred for an additional 2 h. Then we evaporated the solvents on a rotary evaporator and dissolved the solids in a 100 mL mixture of DCM/2 M HCl = 1/1. The organic phase was separated and the aqueous phase was extracted twice with 50 mL of DCM. The organic phases were combined and the solvent was removed on a rotary evaporator. The resulting solid was purified on silica by column chromatography (eluted in hexane/EtOAc = 85/15) to yield the azobenzene **1c** as orange crystals (620 mg, 32.5%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 2 H), 7.86 (s, 2 H), 7.47 (s, 1 H), 6.71 (d, *J* = 8.4 Hz, 2 H), 4.67 (s, 4 H), 4.29 (q, *J* = 7.2 Hz, 2 H), 4.02 (s, 2 H), 1.33 (t, *J* = 7.2 Hz, 3 H) ppm. **^{13}C-NMR** (100.5 MHz, $CDCl_3$) δ 170.1, 152.7, 150.6, 144.6, 139.0, 129.4, 126.2, 122.2, 112.8, 61.7, 45.26, 45.2, 14.2

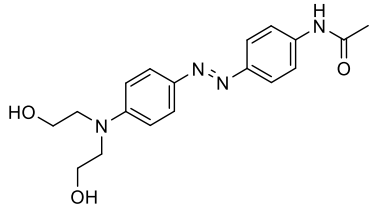
ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{18}H_{19}Cl_2N_3O_2$: 380.0927, observed: 380.0918; 2.23 ppm.

Synthesis of [(4-{(E)-[3,5-bis(hydroxymethyl)phenyl]diazenyl}phenyl)amino]acetic acid (**1a'**)



To an ice cold solution of 462 mg (3 mmol) of compound **2b** (see the following) in 4 mL methanol, we added, 2 mL of 2 M HCl and the solution was stirred on ice for 30 min. To this mixture we added a solution of 104.3 mg (3 mmol) of $NaNO_2$ in water drop wise with vigorous stirring for 1 h to form the diazo salt. Then we added the diazo salt solution drop wise to an ice cold solution of 453 mg (3 mmol) of phenyl glycine in 50 mL of THF/NaHCO3 = 5/1. After stirring for 1 h at this temperature, the solution was warmed up to r.t. and further stirred overnight. Then we evaporated the solvents and dissolved the solids in a 100 mL mixture of DCM/brine = 1/1. The organic phase was separated and the aqueous phase was extracted twice with 50 mL of DCM. The organic phase was combined and the solvent was removed by rotary evaporator. The resulting solid was purified on silica by column chromatography to yield the azobenzene **1c** as orange crystals (8.8%). **^{1}H-NMR** (399.8 MHz, DMSO) δ 7.70 (d, *J* = 8.8 Hz, 2 H), 7.57 (s, 2H), 7.29 (s, 1 H), 6.64 (d, *J* =8.8 Hz, 2 H), 4.52 (s, 4 H), 3.92 (s, 2 H) ppm. **^{13}C-NMR** (125.7 MHz, DMSO) δ171.8, 152.0, 151.7, 143.5, 143.1, 125.5, 125.0, 118.0, 112.0, 62.6, 44.3 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$calc. for$C_{16}H_{18}N_3O_4$: 316.1292, observed: 316.1295; -0.88 ppm.

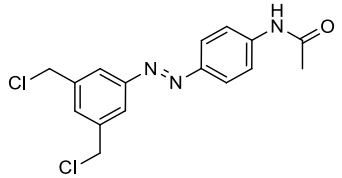
Synthesis of N-{4-[(E)-{4-[bis(2-hydroxyethyl)amino]phenyl}diazenyl]phenyl} acetamide (**1b**)



To an ice cold solution of 4-aminoacetanilide (182 mg, 1.2 mmol) in 4 mL of 1 M HCl 1M, we added 1.2 mL of a 1 M solution of $NaNO_2$ drop wise. After stirring the reaction mixture on ice for 1 h, we slowly added the mixture to an ice cold solution of *N*-phenyldiethanolamin (253 mg 1.4 mmol) in EtOH/H_2O = 1/1 with vigorous stirring. We stirred the solution for 3 h, then removed the solvent on a rotary evaporator and purified the products over silica by column chromatography. The product was eluted by DCM/MeOH = 4/1 to yield the compound **1b** as an orange solid (288 mg, 60%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 10.2 (s, 1 H), 7.72 – 7.68 (m, 6 H), 6.80 (d, *J* = 8.8 Hz, 2 H), 3.76 (t, *J* = 5.6 Hz, 4 H), 4.82 (t, *J* = 5.2, 2 H), 3.60 – 3.51 (m, 4 H), 2.10 (s, 3 H) ppm. **^{13}C-NMR** (100.5 MHz, DMSO) δ 169.0, 151.0, 148.4, 142.8, 141.1, 125.0, 123.0,

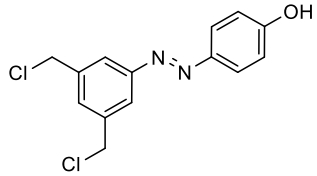
119.6, 111.7, 58.6, 53.7, 24.6 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{18}H_{22}N_4O_3$: 343.1765, observed: 343.1763; 0.47 ppm.

Synthesis of N-(4-{(E)-[3,5-bis(chloromethyl)phenyl]diazenyl}phenyl)acetamide (**1c**)



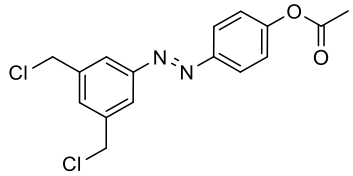
To 100 mg of a solution of compound **2h** in dichloromethane, we added 0.2 mL of acetic anhydride and stirred the solution for 1 h at r.t. The excess acetic anhydride was then removed by rotary evaporator and the compound **1c** was obtained as an orange solid (100 mg, 100%). **^{1}H-NMR** (400.0 MHz, CDOD3/$CDCl_3$: 3/1) 7.88 (d, J = 8.8 Hz, 2 H), 7.79 (s, 2 H), 7.74 (d, J = 8.8 Hz, 2 H), 7.48 (s, 1 H), 4.71 (s, 4 H), 2.17 (s, 3 H) ppm. **^{13}C-NMR** (100.6 MHz, CDOD3/$CDCl_3$: 3/1) δ 171.7, 154.2, 149.9, 144.1, 142.7, 128.4, 124.6, 120.9, 120.8, 64.7, 24.0 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for: $C_{16}H_{17}Cl_2N_4O_2$: 367.0723, observed: 367.0723; -0.22 ppm.

Synthesis of **1dA**



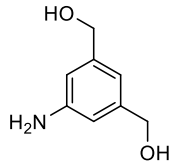
To an ice cold solution of **2e** (190 mg, 1 mmol) in THF, we added 2 mL of 2 M HCl. Then we added 1 mL of a 1 M solution of $NaNO_2$ in water dropwise. After stirring on ice for 1 h we slowly added this mixture to an ice cold solution of phenol (113 mg, 1 mmol) in saturated $NaHCO_3$. The reaction mixture was stirred at this temperature for 30 min and then was warmed up to r.t., then stirred at r.t. for another 2 h. The solvent was removed on a rotary evaporator and the resulting mixture was purified over silica by column chromatography (eluted in DCM/MeOH = 9/1) to yield **1dA** as orange crystals (94 mg, 32%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 8.8 Hz, *J* = 2 Hz, 2 H), 7.87 (d, *J* = 0.8 Hz, 2 H), 7.52 (d, *J* = 0.8 Hz, 1 H), 6.96 (dd, *J* = 8.8 Hz, *J* = 2 Hz, 2 H), 4.69 (s, 4 H) ppm. **^{13}C-NMR** (399.8 MHz, $CDCl_3$) δ 158.6, 153.1, 147.0, 139.1, 130.1, 125.2, 122.6, 116.0, 45.4 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{14}H_{13}N_2Cl_2O$: 295.0399, observed: 295.0399; 0.46 ppm.

Synthesis of **1d**



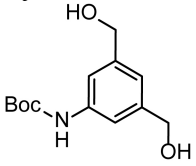
To a solution of **1dA** (60 mg, 0.2 mmol) in dichloromethane, we added acetyl chloride (212 µL, 0.3 mmol) followed 100 µL of pyridine. The reaction was then warmed to r.t. and stirred for 1 h at this temperature. After the completion of the reaction, the solvent was evaporated on a rotary evaporator and the resulting solid was purified on silica by column chromatography. The product eluted in DCM/MeOH = 9/1 and solvent was removed on a rotary evaporator to yield **1d** (62 mg, 91%) as orange crystals. **^{1}H-NMR** (499.8 MHz, $CDCl_3$) δ 7.98 (d, J = 8.8, 2 H), 7.92 (s, 2 H), 7.57 (s, 1 H), 7.29, (d, *J* = 8.8. Hz, 2 H), 4.70 (s, 4 H), 2.37 (s, 3H) ppm. **^{13}C-NMR** (125.7 MHz, $CDCl_3$) δ 169.0, 153.0, 153.0, 150.0, 139.2, 130.8, 124.3, 122.9, 122.3, 45.4, 21.2 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for: $C_{16}H_{15}Cl_2N_2O_2$: 337.0505, observed: 337.0502,-0.75 ppm.

Synthesis of 3,5-bis(hydroxymethyl) aniline (**2b**)



We synthesized 3,5-bis(hydroxymethyl) aniline through a previously published method.[1] To an ice cold solution of diethyl-5-(amino)isophtalate (**2a**) (5.23 g, 25 mmol, TCI Chemicals) in THF (300 mL) we slowly added an ice cold suspension of $LiAlH_4$ (2.77 g, 75 mmol) in THF (200 mL) with vigorous stirring. After stirring for 30 min at this temperature, the solution was warmed up to room temperature and was stirred for an additional 2 h. Upon the completion of the reaction, the excess $LiAlH_4$ was quenched by slowly adding 60 mL ethyl acetate and stirring for 1 h, followed by 20 mL of methanol. Then we added 50 mL of a solution of saturated NH_4Cl to hydrolyze the aluminum salt. The resulting mixture was filtered off and the filtrate was concentrated using a rotary evaporator. The resulting solids were further purified by silica gel column chromatography to give compound **2b** (69% yield). **^{1}H-NMR** (400.0 MHz, $CDOD_3$): δ 6.67 (s, 1H) 6.61 (s, 2H), 4.46 (s, 4 H) ppm. **^{13}C-NMR** (100.6 MHz, $CDOD_3$): δ 147.3, 142.3, 115.2, 113.0, 63.5 ppm. **HRMS-ESI** (*m/z*): calc. for $C_8H_{12}NO_2$ $(M+H)^+$: 154.0868; found: 154.0861; 0.89 ppm.

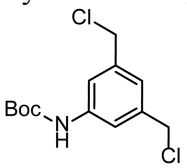
Synthesis of 3,5-bis(hydroxymethyl) aniline-*tert*-butyl carbamate (**2c**)



To a solution of 3,5-bis(hydroxymethyl) aniline (compound **2b**) (7 g, 45.5 mmol) in 200 mL DMF, we added di-tert-butyl dicarbonate (10.9 g, 50 mmol) with vigorous stirring.

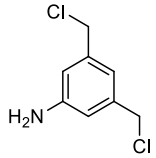
After stirring for 2 h, the solvent was evaporated and the crude solid was purified over silica by column chromatography to give the white solid compound **2c** (94%). **^{1}H-NMR** (399.7 MHz, $CDOD_3$): δ 7.31 (s, 2 H), 6.99 (s, 1 H), 4.54 (s, 4 H), 1.50 (s, 9 H). **^{13}C-NMR** (100.5 MHz, $CDOD_3$): δ 153.9, 142.1, 139.2, 119.3, 115.9, 79.4, 63.7, 27.3 **HRMS-ESI** (*m/z*): calc. for $C_{13}H_{19}NO_4$ $(M+H)^+$:271.1652; found: 241.1649; 0.33 ppm.

Synthesis of 3,5-bis(chloromethyl) aniline-*tert*-butyl carbamate (**2d**)



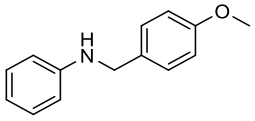
To an ice cold suspension of compound **2c** (7 g, 27.6 mmol) in 200 mL THF, we slowly added methanesulfonyl chloride (5.3 mL, 69 mmol), followed by triethylamine (7.6 mL, 55 mmol). The mixture was stirred at 0 ºC for 30 min followed by stirring at room temperature for an hour. To the resulting solution, we slowly added a solution of LiCl (2.8 g, 69 mmol) in 100 mL DMF and the reaction mixture was stirred for 2 h at r.t. After completion of the reaction, the solvent was evaporated and the resulting mixture was dissolved in 200 mL of a 1:1 mixture of dichloromethane (DCM) and 0.1 M aqueous solution of $NaHCO_3$ in water. The organic phase was separated and the aqueous phase was extracted twice by 100 mL of DCM. The organic phases were combined and the solvent was evaporated. The resulting crude mixture was purified over silica by column chromatography to yield **2d** as a fluffy powder (7.6 g, 82%). **^{1}H-NMR** (399.7 MHz, $CDCl_3$): δ 7.39 (d, J=1.2 Hz, 2 H), 7.39 (d, J=1.2 Hz, 1 H), 6.65 (broad, 1 H), 4.53 (s, 4 H), 1.53 (s, 9 H). **^{13}C-NMR** (100.5 MHz, CD_3OD): δ 152.6, 139.2, 138.9, 123.0, 118.35, 81.1 ,45.7 , 28.3 **HRMS-ESI** (*m/z*): ESI (m/z): calc. for $C_{13}H_{17}Cl_2NO_2$ Na (M+Na)+:312.0529; found: 312.0527; 0.65 ppm

Synthesis of 3,5-bis(chloromethyl) aniline (**2e**)



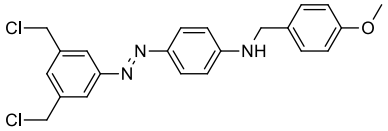
To 1.17 g of compound **2d** we added 6 mL of trifluoroacetic acid (TFA) at room temperature, with vigorous stirring. After 30 min, the TFA was evaporated and the resulting solid was used with no further purification (0.71 g, quantitative). **^{1}H-NMR** (399.8 MHz, $CDCl_3$): δ 6.79 (s, 1 H), 6.69 (s, 1H), 4.50 (s, 4H) 3.69 (br, 2H) **^{13}C-NMR** (100.5 MHz, $CDCl_3$): δ 147.2, 139.1, 118.7, 115.0, 46.0 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_8H_{10}C_{l2}N$: 190.0184, observed: 190.0184; 0.3ppm

Synthesis of 4-methoxy-N-phenyl-benzylamine **(2f)**



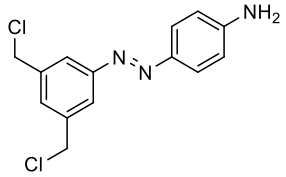
To a solution of 5.1 mL (55 mmol) of aniline in 100 mL methanol, we added 6.1 mL (55 mmol) of 4-methoxy benzaldehyde with vigorous stirring. After refluxing overnight, the reaction was cooled down to 0 ºC and $NaBH_4$ (1.9 g, 500 mmol) was added in small portions along with vigorous stirring. We stirred the reaction for 30 min on ice and an additional 1 h at room temperature. We evaporated the solvent by rotary evaporator and re-dissolved the solid in DCM/0.1 M HCl = 1:1. The organic phase was washed three times with HCl solution, pH 3. The combined organic phases were evaporated on rotary evaporator. Crude product was purified by column chromatography over silica. The product eluted in hexane/EtOAc = 85.15 to yield compound **2f** as a white solid (10.4 g, 88%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$): δ 7.34 (dd, *J*=6.4 Hz, *J*=2 Hz, 2 H), 7.23 (dd, *J* = 8.4 Hz, *J* = 7.6 Hz, 2 H) 6.93 (dd, *J*=6.4 Hz, *J*=2 Hz, 2 H), 6.77 (dd, *J* = 8.4 Hz, J = 2 Hz, 1 H), 6.68 (dd, *J*=7.6 Hz, *J*= 2 Hz, 2 H) 4.30 (s, 2 H), 3.99 (br, 1 H), 3.85 (s, 3H) **^{13}C-NMR** (100.5 MHz, $CDCl_3$): δ 158.9, 148.3, 138.5, 129.3, 128.9, 117.5, 114.0, 112.9, 55.3, 47.8. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{14}H_{15}NO$: 214.1226, observed: 214.1223; 1.67 ppm.

Synthesis of **2g**



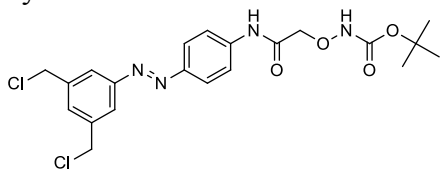
To an ice cold solution of 760 mg (4 mmol) of compound **2e** in 4 mL methanol, we added, 4 mL of 2 M HCl and the solution was stirred on ice for 30 min. To this mixture we added a solution of 276 mg of $NaNO_2$ (4 mmol) in water drop wise with vigorous stirring for 1 h to form the diazo salt. Then we added the diazo salt solution drop wise to an ice cold solution of **2f** in 50 mL of THF and the pH was adjusted to 5 with a saturated solution of $NaHCO_3$. After stirring for 1 h at this temperature, the solution was warmed up to r.t. and further stirred overnight. Then we evaporated the solvents on a rotary evaporator and dissolved the solids in a 100 mL mixture of DCM/brine = 1/1. The organic phase was separated and the aqueous layer was extracted twice with 50 mL of DCM. The organic layers were combined and dried on a rotary evaporator. The resulting solid was purified on silica by column chromatography; the product was eluted in hexane/EtOAc = 3/1 to yield the **2g** as orange crystals (1.03 g, 62%). **^{1}H NMR** (399.9 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.8 Hz, 2 H), 7.83 (d, *J* = 2 Hz, 2 H), 7.45 (t, *J* = 2 Hz, 1 H), 7.29 (dd, *J* = 6.8 Hz, *J* = 2.4 Hz, 2 H), 6.91 (dd, *J* = 6.8 Hz, *J* = 2.4 Hz, 2 H), 6.69 (d, *J* = 8.8 Hz, 2 H), 4.65 (s, 4 H), 4.35 (s, 2H), 3.81 (s, 3 H) ppm. **^{13}C-NMR (**100.6 MHz, $CDCl_3$) δ 159.1, 153.5, 151.2, 144.6, 138.9, 130.2, a29.2, 128.8, 125.6, 122.3, 114.2, 122.4, 55.3, 47.3, 45.6 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{22}H_{20}Cl_2N_3O$:412.0978, observed: 412.0982; 0.89 ppm.

Synthesis of **2h**



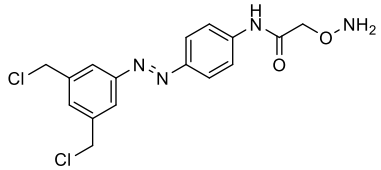
To 100 mg of compound **2g**, we added 500 μL of anisole, followed by 5 mL of TFA and the resulting mixture was stirred in dark for at least 2 h at r.t. After completion of the reaction, the solvent was removed by rotary evaporator and the resulting solid was re-dissolved in MeOH and neutralized by saturated $NaHCO_3$ solution. The resulting mixture was removed by rotary evaporator and further purified by column chromatography, the product was eluted in hexane/EtOAc = 65/75 to give compound **2h** as an orange solid (600 mg, 85%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 7.843 (s, 2 H), 7.83 (d, *J* = 8.5 Hz, 2H) 7.48 (s, 1 H), 6.757 (d, *J* = 8.5 Hz, 2H), 4.68 (s, 2 H) **^{13}C-NMR** (100.5 MHz, $CDCl_3$) δ 153.5, 150.1, 145.4, 138.9, 129.5, 125.4, 122.4, 114.6, 45.5 **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{14}H_{13}Cl_2N_3$:294.0559, observed: 294.0557; 0.87 ppm.

Synthesis of **2i**



To a solution of 70 mg (0.24 mmol) of compound **2h** in 25 mL of THF, we added (BOC-aminooxy)acetic acid (92 mg, 0.48 mmol) followed by EDC.HCl (115 mg, 0.6 mmol) and N,N-diisopropylethylamine (81 μL, 0.48 mmol) with vigorous stirring. The reaction was allowed to stir for 2 h and then was washed with 20 mL of water followed by 20 mL of brine. The resulting solid was purified over silica by column chromatography; the product was eluted in hexane/EtOAc = 3/1 to yield compound **2i** as an orange solid (80 mg, 72%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 10.6 (br, 1 H)7.97-7.88 (m, 7 H), 7.5 (s, 1 H) 4.68 (s, 4H), 4.49 (s, 2 H), 1.53 (s, 9 H) **^{13}C-NMR** (100.5 MHz, $CDCl_3$) δ 167.5, 158.5, 153.16, 148.82, 141.0, 139.0, 130.4, 124.1, 122.7, 129.9, 83.9, 77.1, 45.4, 28.1 **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{21}H_{24}Cl_2N_4O_4$: 467.1247, observed: 467.1244; 0.5 ppm.

Synthesis of **2j (HADCAz)**



To 150 mg of the compound **2i**, we added 2 mL of TFA and stirred the mixture for 15 min. The TFA was then evaporated by rotary evaporator, and the resulting compound was purified on silica by column chromatography with no further workup. The product was eluted in hexane/EtOAc = 7/3 to yield compound **2j** as orange solid (108 mg, 92%). **^{1}H-NMR** (399.8 MHz, $CDCl_3$) δ 7.94 (dt, *J* = 8.8 Hz, *J* = 2 Hz, 2 H), 7.90 (d, J = 1.6 Hz,

2 H), 7.83 (dt, *J* = 8.8 Hz, *J* = 2 Hz, 2 H), 7.61 (t, J = 1.6 Hz, 1 H), 4.74 (s, 4 H), 4.68 (s, 2 H) ppm. **^{13}C-NMR** (100.6 MHz, $CDOD_3$) δ168.6, 154.4, 150.3, 142.2, 141.2, 132.0, 125.0, 123.6, 121.3 73.1, 46.0 ppm. **HRMS-ESI** (*m/z*): $(M+H)^+$ calc. for $C_{16}H_{17}Cl_2N_4O_2$: 367.0723 Da, observed: 367.0723, -0.22 ppm.

General Procedure for Synthesis of Peptides Using FMOC Chemistry

We synthesized the peptides using Rink Amide AM resin (Chempep) standard solid phase amide coupling. Briefly, we transferred 250 mg (0.24 mmol, 0.96 mmol/g) into a PolyPrep® chromatography column. We set up the column on a manifold vacuum and equipped it with a three-way stopcock that allows draining of the solvent by vacuum filtration. Agitation of the resin was done by purging nitrogen gas as described by Verdine and co-workers.[2] We added 3 mL of DCM to the dry resin and let it swell for 15 min. We drained the DCM and re-suspended the resin in 3 mL of DMF for 15 min. Then, we drained the DMF and deprotected the amine with 20% (v/v) piperidine in DMF (4 mL) for 1 min. The deprotection was repeated for another 9 min using fresh 20% (v/v) piperidine in DMF (4 mL). We washed the resin with DMF (4 x 4 mL), and dissolved Fmoc-protected amino acid (0.96 mmol, 4 eq.) in 3 mL of DMF (4 mL). HBTU was added (0.96 mmol, 4 eq) to the solution until dissolution occured. We added this mixture to the resin, followed by 0.3 mL of DIPEA (1.92 mmol, 8 eq). After 30 minutes of mixing, we removed the reagents by vacuum filtration and washed the resin with DMF (4 x 4 mL). The Fmoc-deprotection, amide coupling, and washing steps were subsequently repeated to elongate the sequence up to the N-terminal residue. After Fmoc deprotection, we washed the resin with DMF (5 x 4 mL), followed by DCM (5 x 4 mL) and left the resin on the manifold for 10 min to dry under vacuum. We added the cleavage cocktail (4 mL, TFA/H_2O/TIPS/Phenol/EDT, 90/2.5/5/5/2.5 (v/v/v/w/v)), to the dried resin and rocked the column for 2 h to cleave the peptide. Next, we collected the flow through from the column and washed the resin with TFA (1 mL), and then added this combined cleavage mixture drop-wise to 40 mL of ice cold diethyl ether in a 50 mL polypropylene centrifuge tube (Falcon, Thermo Fisher). The mixture was incubated on ice for 30 min, and the precipitates separated by centrifugation (5 min, 3000 rpm). Then, we decanted the supernatant and washed the precipitates with cold diethyl ether (2 × 40 mL). After air drying the peptides were dissolved in 40 mL of 50% aqueous MeCN solution with pH adjusted to 8 by aqueous ammonia. We then added 500 µL of DMSO to this solution, and stirred the mixture for 24 h at room temperature in the presence of air to form the disulfide bonds. The solution was then concentrated to 5 mL by speedvac (Thermo Scientific) and was purified by HPLC. Addition of 10% acetic acid was often necessary to dissolve the peptide, before purification.

For HPLC purification, we injected the peptide solution into a semi preparative RP-HPLC system equipped with C18 column (Waters Symmetryprep 19 × 50 mm). A gradient of solvent A (MQ water, 0.1% (v/v) TFA) and solvent B (MeCN, 0.1% (v/v) TFA) was run at a flow rate of 8 mL/min (0-2 min: 2% A; 2-18 min: 2→50% B). We used same method for all the purifications with HPLC, unless otherwise noted. The fractions corresponding to the main peak were collected. We removed the acetonitrile by evaporation under reduced pressure. Lyophilizing the aqueous fraction yielded the

peptide as white powder. Identity and purity of the peptides was confirmed by LCMS (Fig. S7).

General Procedure for Periodate Oxidation of Serine in Peptide Sequence SX_nCRRRRC

To an ice cold solution of 10 mg peptide in 10 mL of 100 mM phosphate buffer (pH 7.4) we added a solution of $NaIO_4$ (2 eq) in one portion. The reaction mixture was incubated on ice for 10 min and at r.t. for an additional 10 min and then was purified with HPLC on C18 silica. In some cases, we observed precipitation. In this case, the solution was acidified to pH~ 5 with 10% AcOH to dissolve the peptide and then was immediately injected to HPLC. Identity and purity of the peptides was confirmed by LCMS (Fig. S8).

General Procedure for Synthesis of Bicyclic Peptide

To a solution of 5 mg of oxidized peptide in 500 µL DMF, we added 100 µL of a solution of **HADCAz** (1.2 eq) in DMF. We incubated the reaction for 2 h on a 55 °C bath and then diluted it to 2.5 mL with DMF. We further diluted the mixture by adding 2.5 mL of H_2O and then added 1.2 equivalent of TCEP solution (25 mM, pH 8.0) in two portions in 15 min intervals. The pH of the mixture was carefully adjusted to 8 and the reaction mixture was incubated at r.t. for 45 min. We then acidified the reaction to pH 5 with 10% AcOH solution and purified the product by HPLC. The identity of the peptides was confirmed by HRMS and/or NMR (Figs. S2, S9-S13).

Kinetics and Switching Efficiency of HADCAz and Bicyclic Peptides.

To quantify switching efficiency of the azobenzene, we injected 5 µL of a 100 µM solution of the Azb containing compound to HPLC. We repeated the injections 0,11, 22, 60, 120, 240 and 720 min after irradiation by 365 nm light. The absorption of the peaks was measured at 420 nm (isosbestic point) (Figs. S1, S5 and S6).

Simulation Methods

Molecular dynamics (MD) simulations were performed for four cyclic peptides (CP3a, CP3b, CP3c, and CP3d). For each peptide, two different initial structures were built using the Maestro 10.2 software of Schrödinger.[1] The topology file for each peptide was generated using the Schrödinger utility ffld_server and converted to the GROMACS format using the ffconv.py script.[2] All MD simulations in this study were performed using the GROMACS 4.6.7 suite[3] with OPLS 2005 force field and TIP4P water model.[4] The initial structure was first energy minimized for 1000 steps and then solvated in a cubic box of water molecules. The box size was chosen such at the minimum distance between the peptide and the box wall is 1.5 nm. Explicit counter ions were also added to neutralize the net charge of the system. With all heavy atoms of the peptide restrained, the solvated system was further energy minimized for 5000 steps.

Each initial structure was subjected to 100 independent runs starting from different initial geometries. With all the heavy atoms of the peptide remain restrained to their initial coordinates; a 50 ps NVT (isochoric-isothermal) equilibration followed by a 50 ps NPT (isobaric-isothermal) equilibration at 300K/1atm was first implemented for each run to adjust the solvent density. The well-equilibrated system then underwent a simulated annealing process in the NVT ensemble, during which the system temperature was first increased to 600 K within 500 ps and maintained at 600 K for an additional 500 ps. The temperature was then decreased gradually to 300 K within 1 ns. During the simulated annealing, the position restraints for the peptide were removed; the azobenzene motif and the oxime bond were harmonically restrained to the *cis* configuration with a force constant of 1000 kcal/mol/rad^2. In all the simulations, the temperature was regulated using the v-rescale thermostat[5] with a coupling time constant of 0.1 ps. To avoid the "hot solvent/cold solute" artifact,[6,7] two separated thermostats were applied to the solvent (water and ions) and the peptide. The pressure was maintained using the isotropic Parrinello-Rahman barostat[8] with a coupling time of 2.0 ps and a compressibility of 4.5 $\times 10^{-5}$ bar^{-1}. All bonds were constrained with the LINCS algorithm[9] allowing a 2 fs time step to be used with the leapfrog integrator.[10] The non-bonded interactions (Lennard-Jones and electrostatic) were truncated at 1.0 nm. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) summation method.[11] A long-range analytic dispersion correction was applied to both the energy and pressure to account for the truncation of Lennard-Jones interactions.[12]

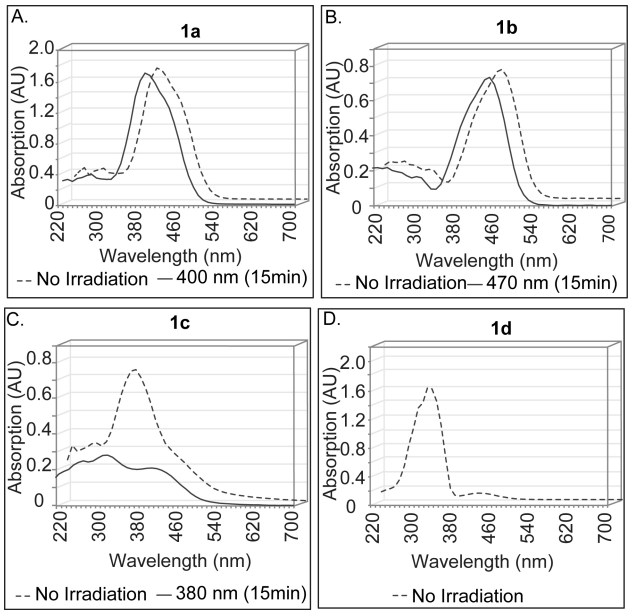


Fig. S1 (A)-(D) Absorption spectra of compounds 1a-1d before and after irradiation.

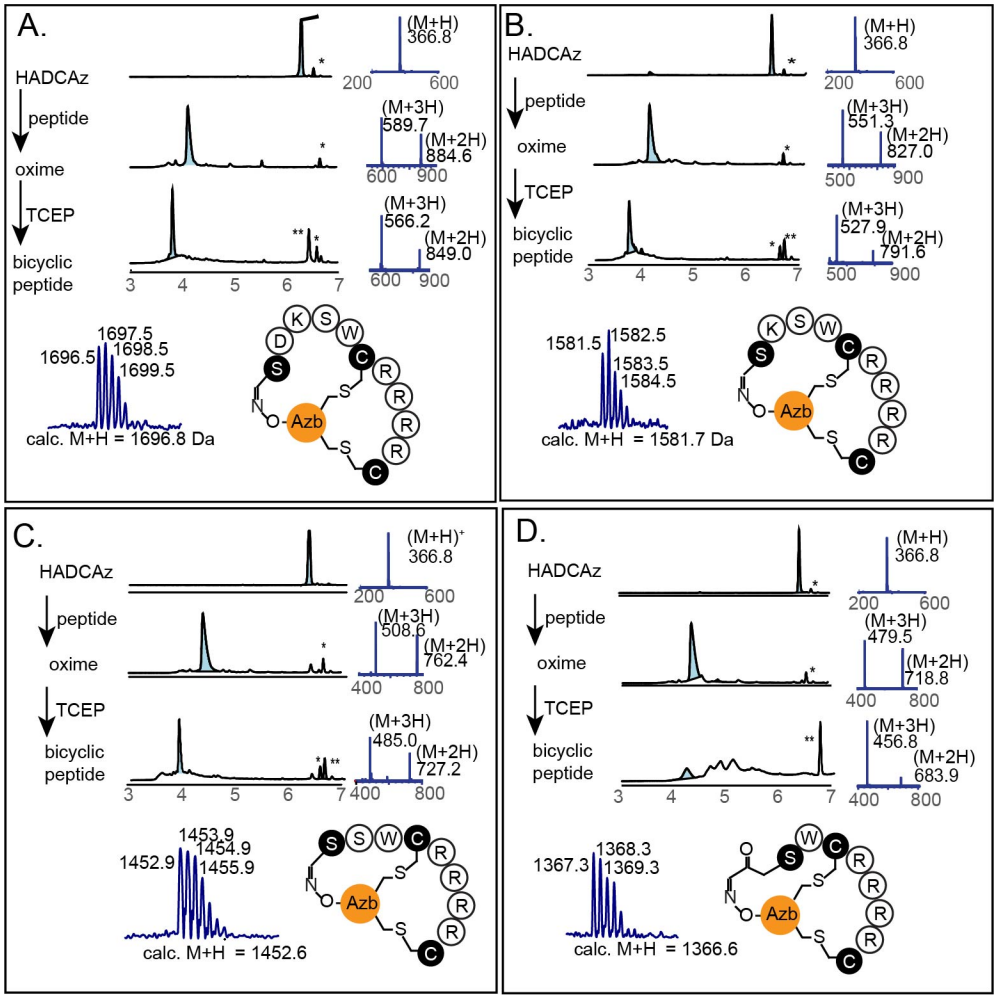


Fig. S2 (A)-(D) LCMS traces confirm the synthesis of bicyclic peptides 3a-3d. MALDI-MS shows the isotopic pattern for the product (for high resolution MS see Fig. S9). * indicates **HADCAz** adduct of reaction with formaldehyde (DMF impurity). ** indicates **HADCAz** byproducts with unidentified structure.

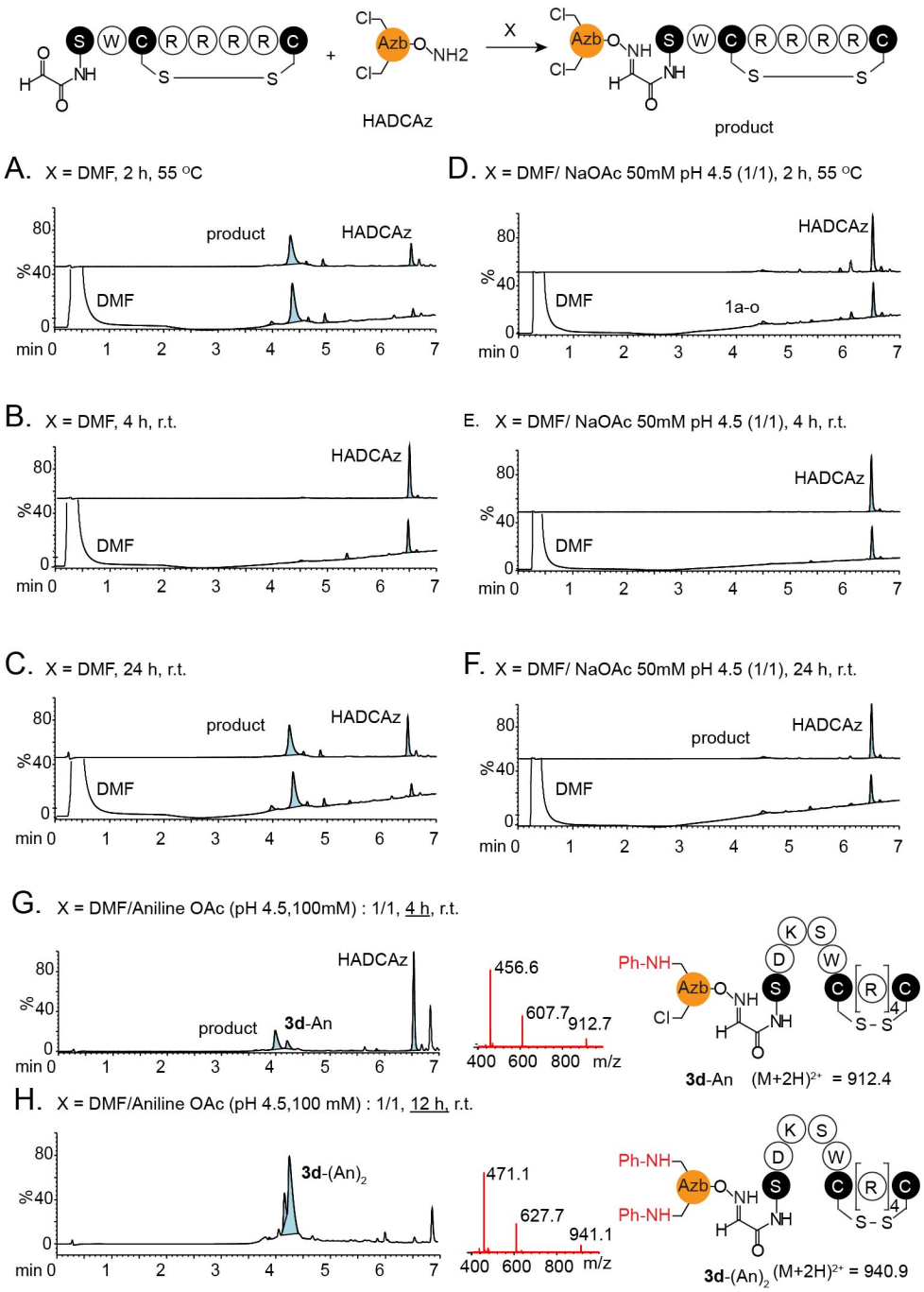


Fig. S3 Optimization of the formation of oxime bond with and without aniline catalyst (SDKSWRRRRC peptide sequence was used in the latter case). (A)-(F) Formation of products is undetectable in solvents containing water, but occurs efficiently in pure DMF. (G)-(H) Addition of aniline resulted in undesired substitution of one or both chlorides in the linker.

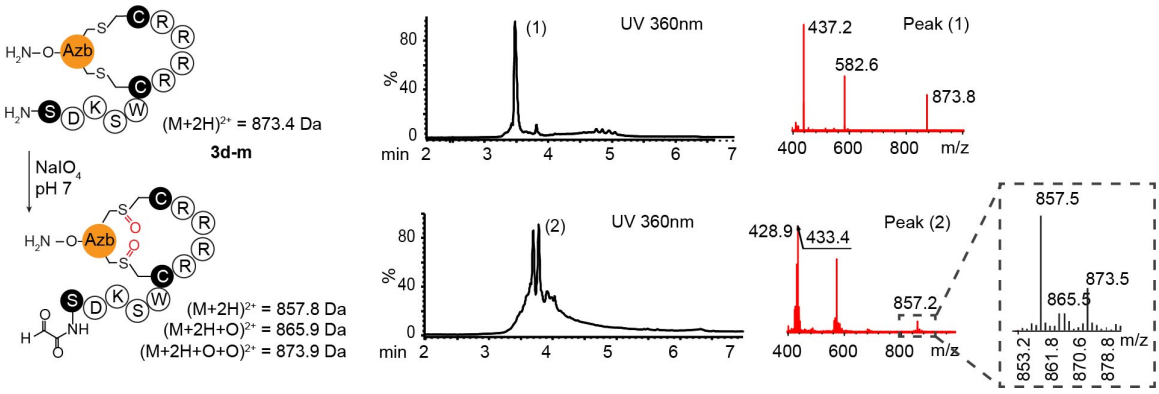


Fig. S4 Oxidation of 4 results in a mixture of byproducts, including oxidized sulfides.

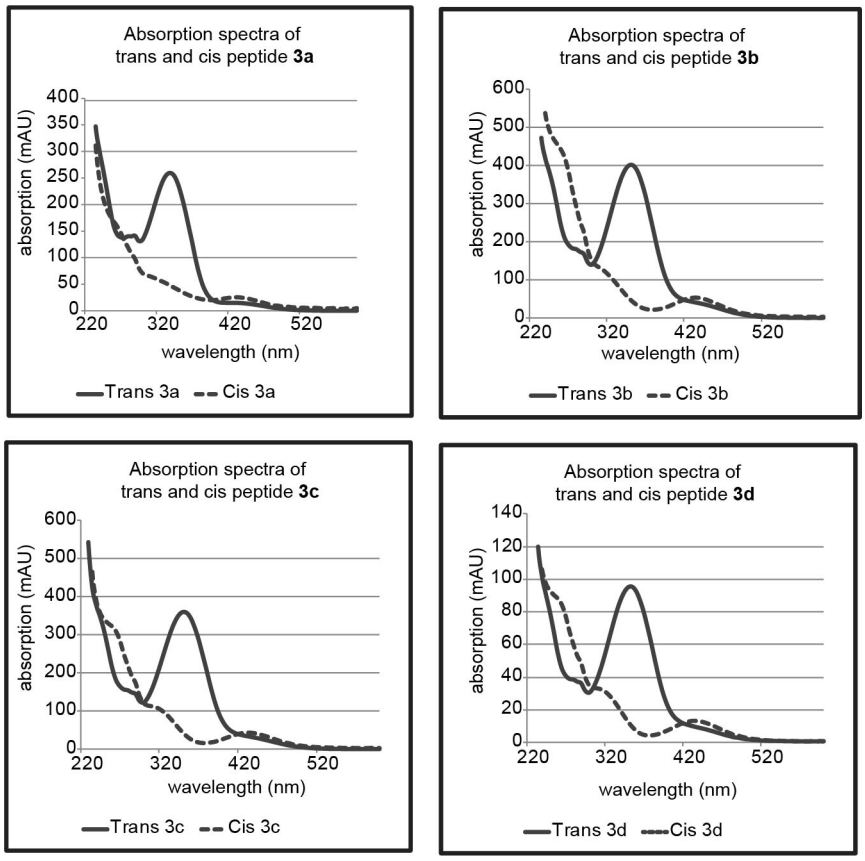


Fig. S5 Absorption spectra of 3a-3d before and after irradiation with 365 nm light.

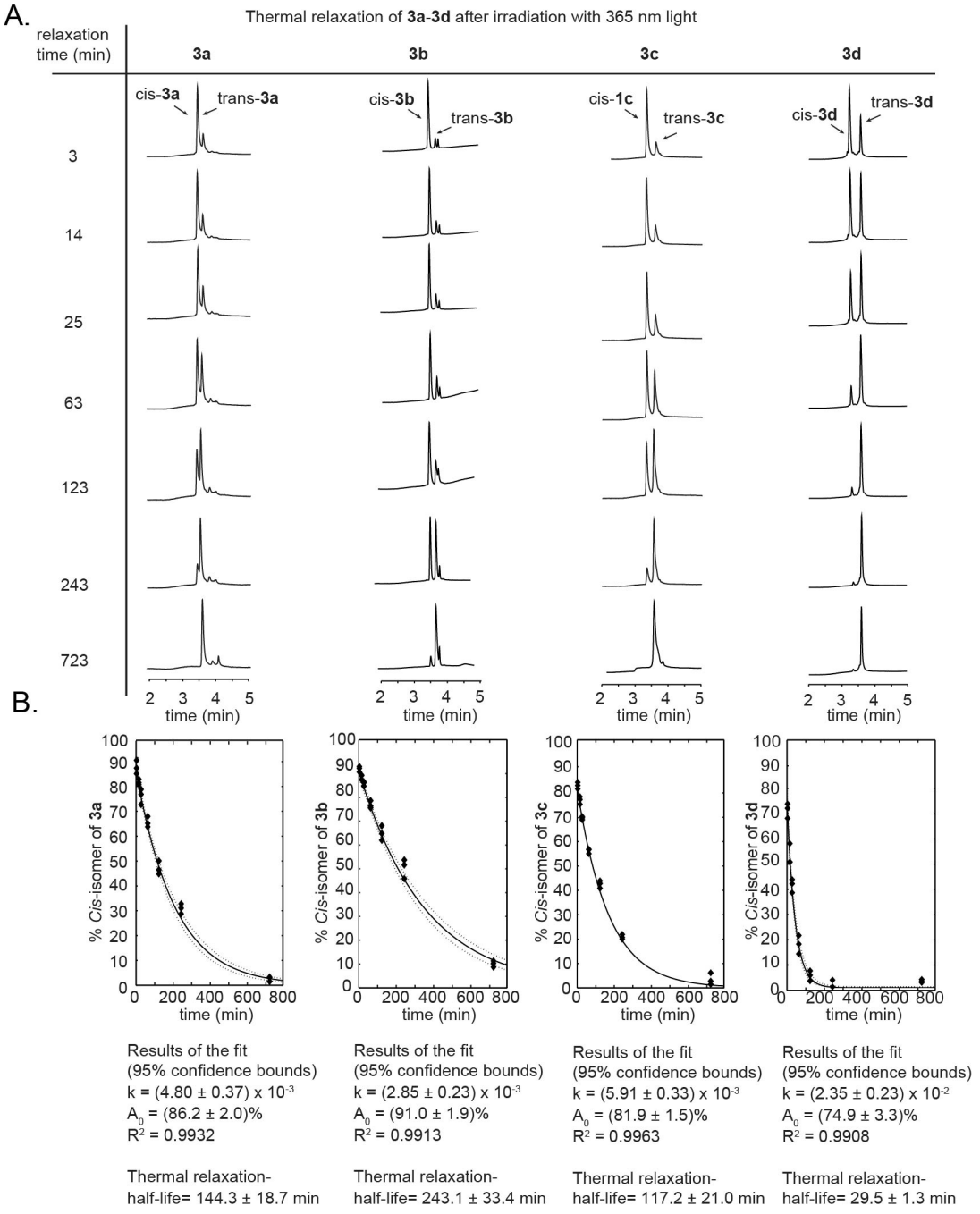


Fig. S6 Kinetics of thermal relaxation for 3a-3d. (A) LCMS traces for thermal relaxation of **3a-3d** after irradiation with 365 nm light. (B) Fitting the kinetic data in a first order kinetic model determined the relaxation rate, *cis*-ratio at photostationary state (A_0) and relaxation half-life.

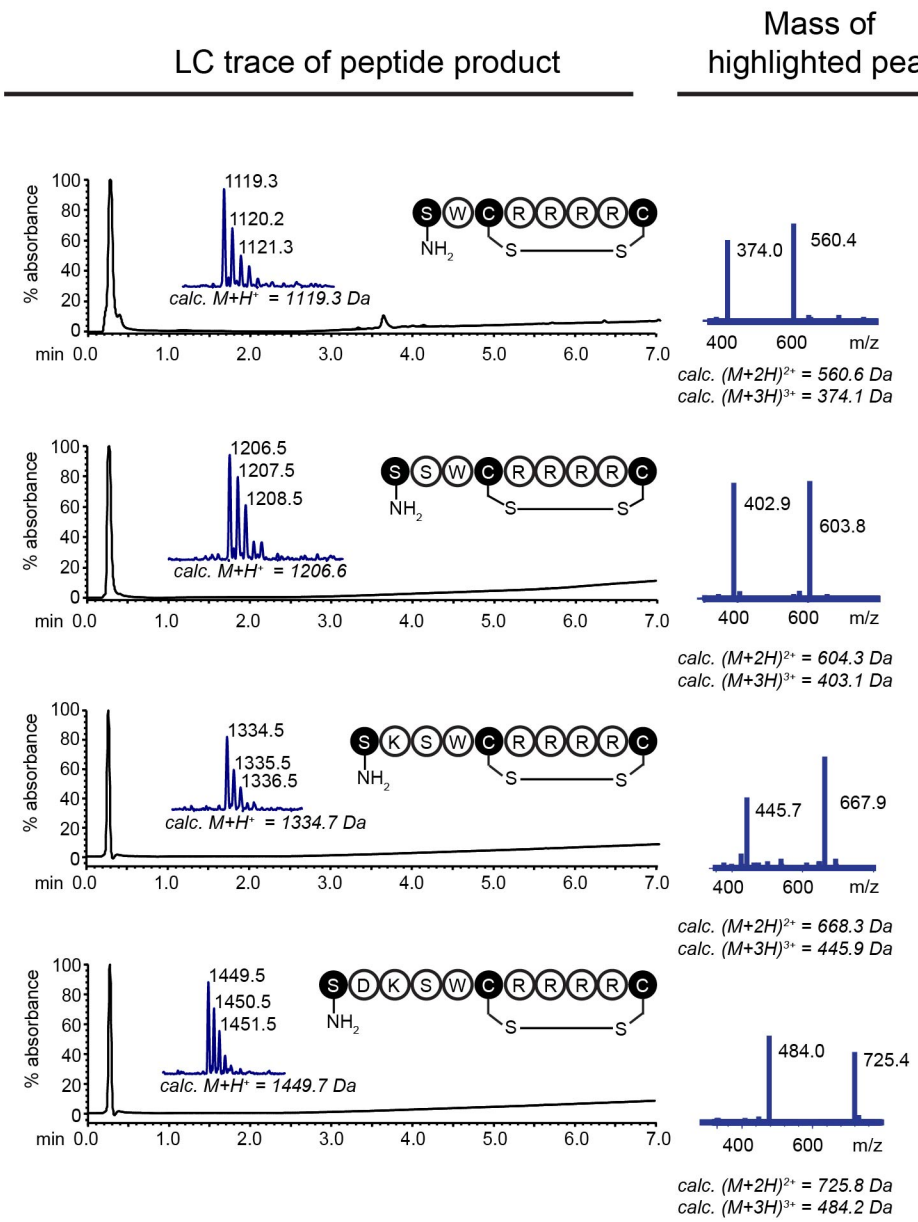


Fig. S7 LCMS traces of disulfide peptides. MALDI–MS results show the isotope pattern.

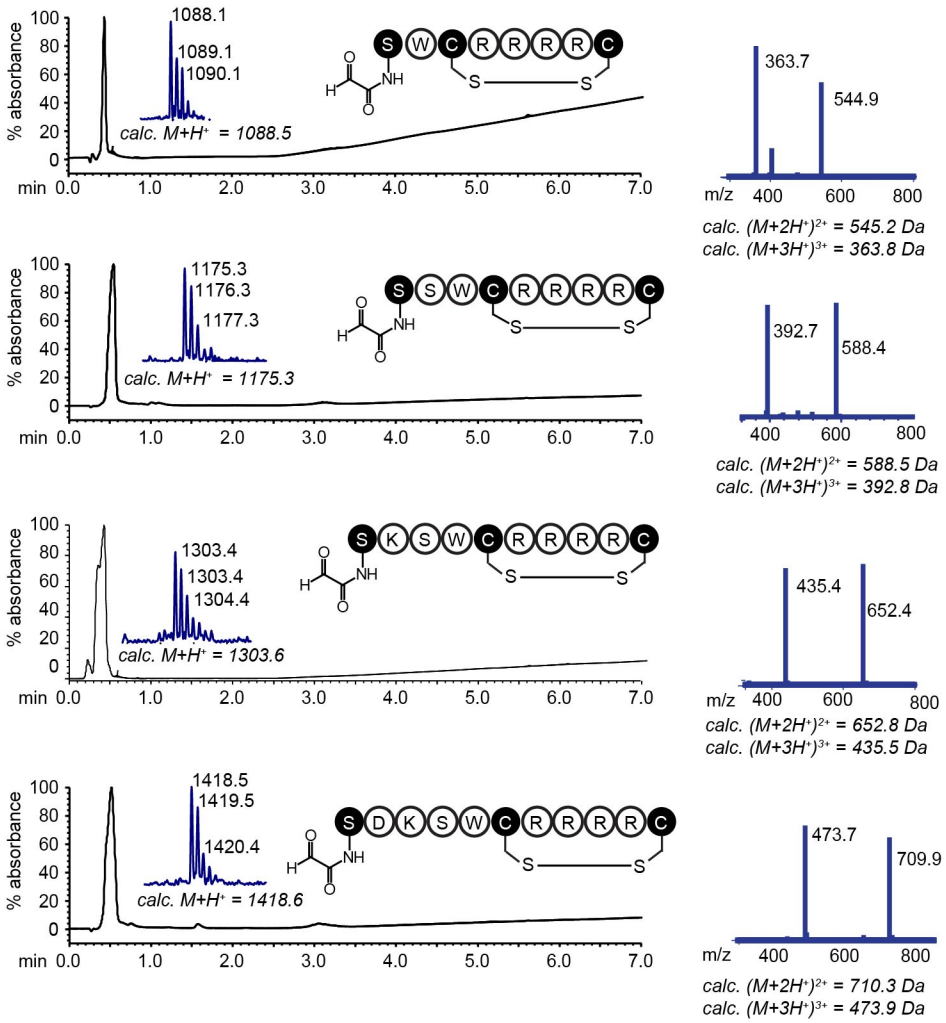


Fig. S8 LCMS traces of the peptides after oxidation of the N-terminal serine with sodium periodate. MALDI–MS results shows the isotope pattern.

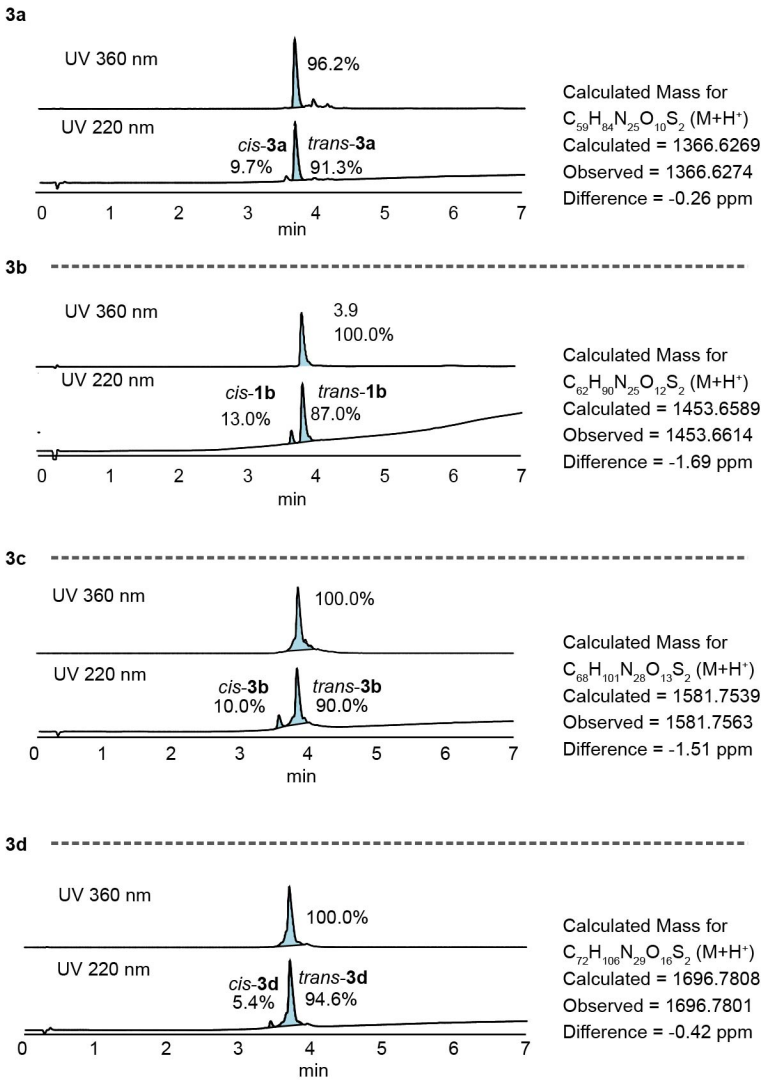


Fig. S9 LCMS traces of purified peptide **3a-3d** and HRMS characterization. The *cis*-isomer is visible when measured at 220 nm, but is not detectable at 360 nm chromatogram, because of low absorption of **Azb** at 360 nm for *cis* form.

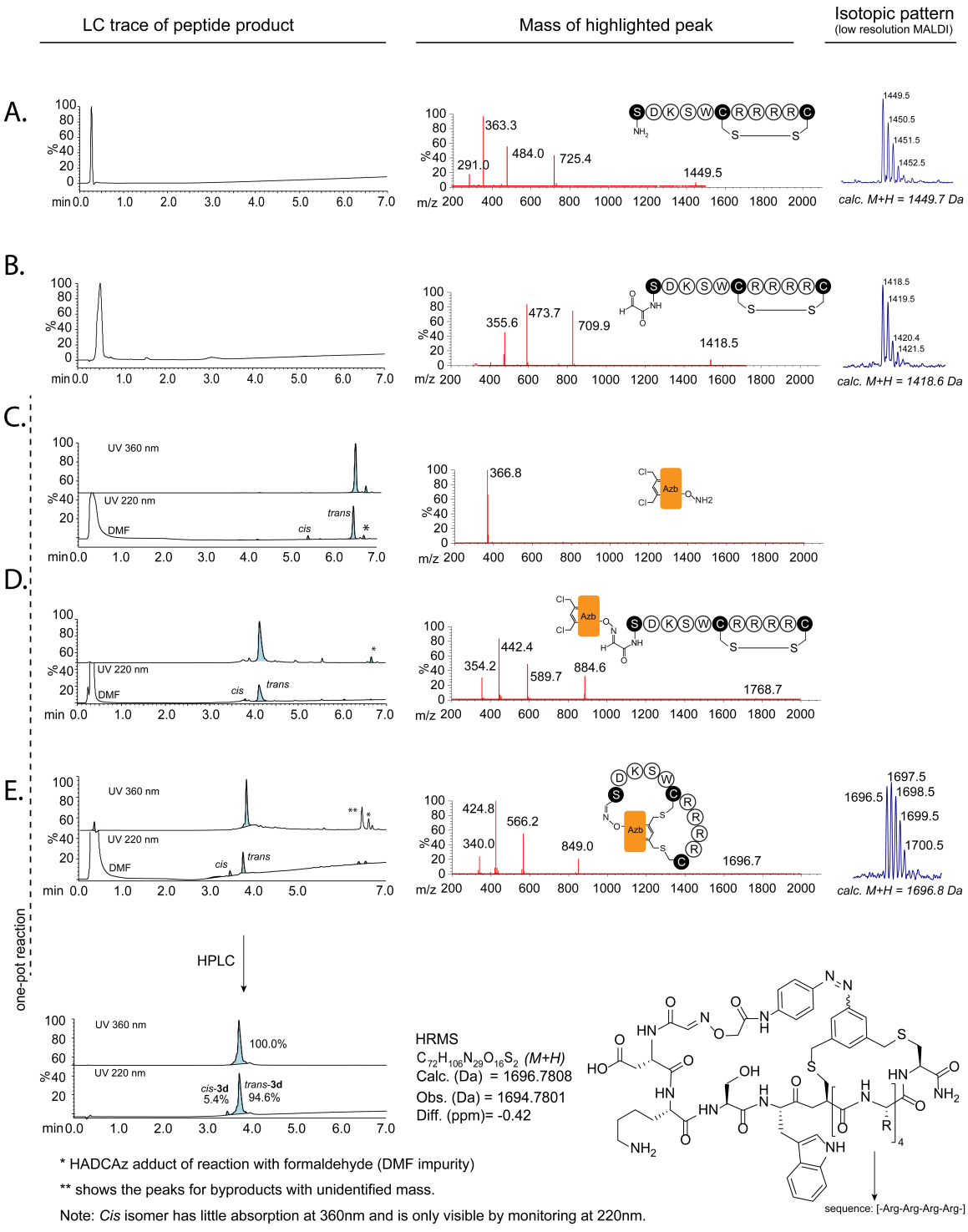


Fig. S10 LCMS traces of the reaction between the peptide SDKSWCRRRRC and **HADCAz**. (A) LCMS trace of the purified peptide SDKSWCRRRRC. (B) SDKSWCRRRRC after oxidation of the N-terminal serine with sodium periodate. The periodate oxidation does not break the disulfide bond. (C) LCMS trace of **HADCAz** prior to reaction. (D) An oxime bond is formed after reaction of **HADCAz** with the oxidized peptide. (E) LCMS trace of the bicyclized peptide after reduction with TCEP.

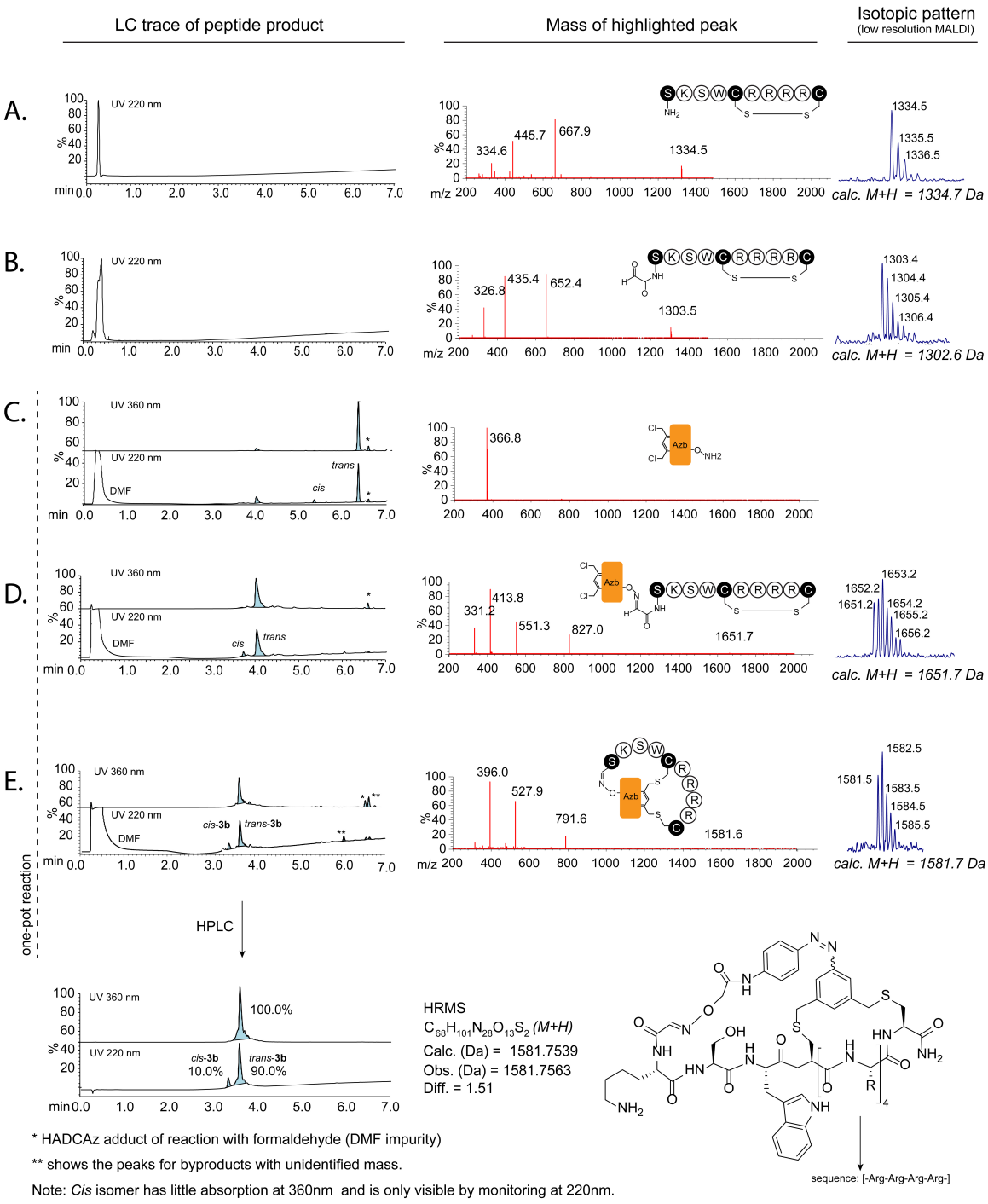


Fig. S11 LCMS traces of the reaction between the peptide SKSWCRRRRC and **HADCAz**. (A) LCMS trace of the purified peptide SKSWCRRRRC. (B) SKSWCRRRRC after oxidation of the N-terminal serine with sodium periodate. The periodate oxidation does not break the disulfide bond. (C) LCMS trace of **HADCAz** prior to reaction. (D) An oxime bond is formed after reaction of **HADCAz** with the oxidized peptide. (E) LCMS trace of the bicyclized peptide after reduction with TCEP.

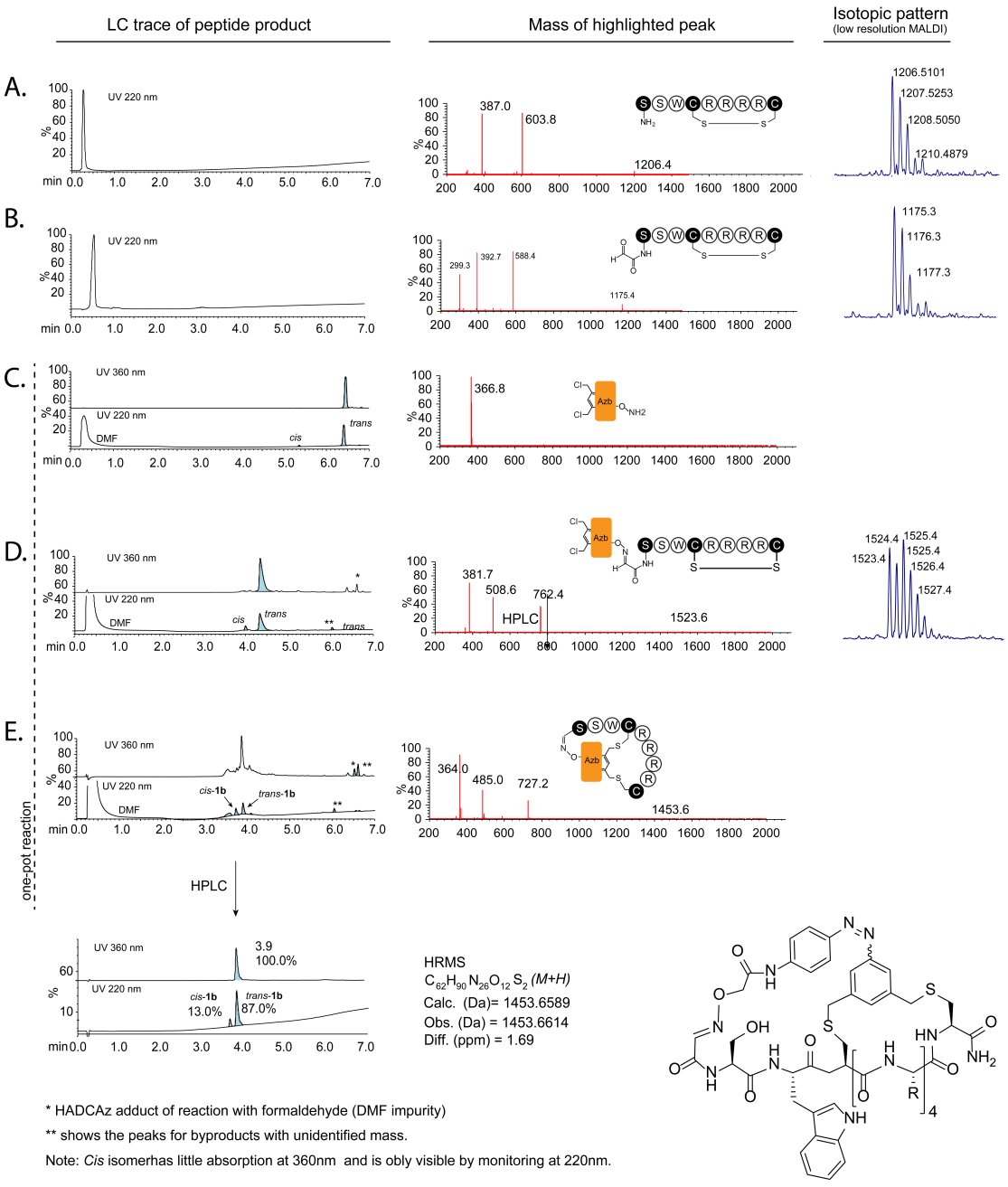
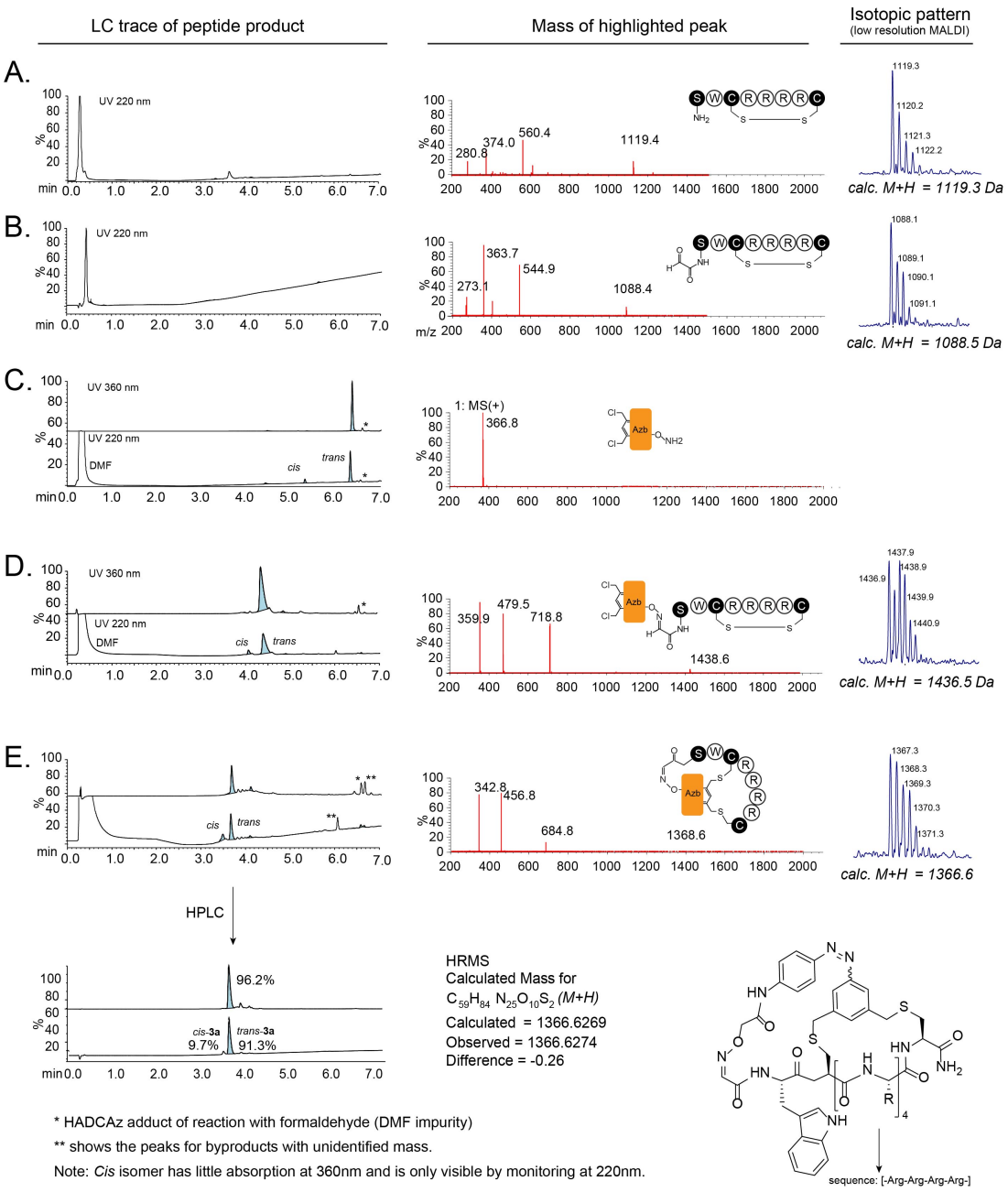


Fig. S12 LCMS traces of the reaction between the peptide SSWCRRRRC and **HADCAz**. (A) LCMS trace of the purified peptide SSWCRRRRC. (B) SSWCRRRRC after oxidation of the N-terminal serine with sodium periodate. The periodate oxidation does not break the disulfide bond. (C) LCMS trace of **HADCAz** prior to reaction. (D) An oxime bond is formed after reaction of **HADCAz** with the oxidized peptide. (E) LCMS trace of the bicyclized peptide after reduction with TCEP.



* HADCAz adduct of reaction with formaldehyde (DMF impurity)

** shows the peaks for byproducts with unidentified mass.

Note: *Cis* isomer has little absorption at 360nm and is only visible by monitoring at 220nm.

Fig. S13 LCMS traces of the reaction between the peptide SWCRRRRC and **HADCAz.** (A) LCMS trace of the purified peptide SWCRRRRC. (B) SWCRRRRC after oxidation of the N-terminal serine with sodium periodate. The periodate oxidation does not break the disulfide bond. (C) LCMS trace of **HADCAz** prior to reaction. (D) An oxime bond is formed after reaction of **HADCAz** with the oxidized peptide. (E) LCMS trace of the bicyclized peptide after reduction with TCEP.

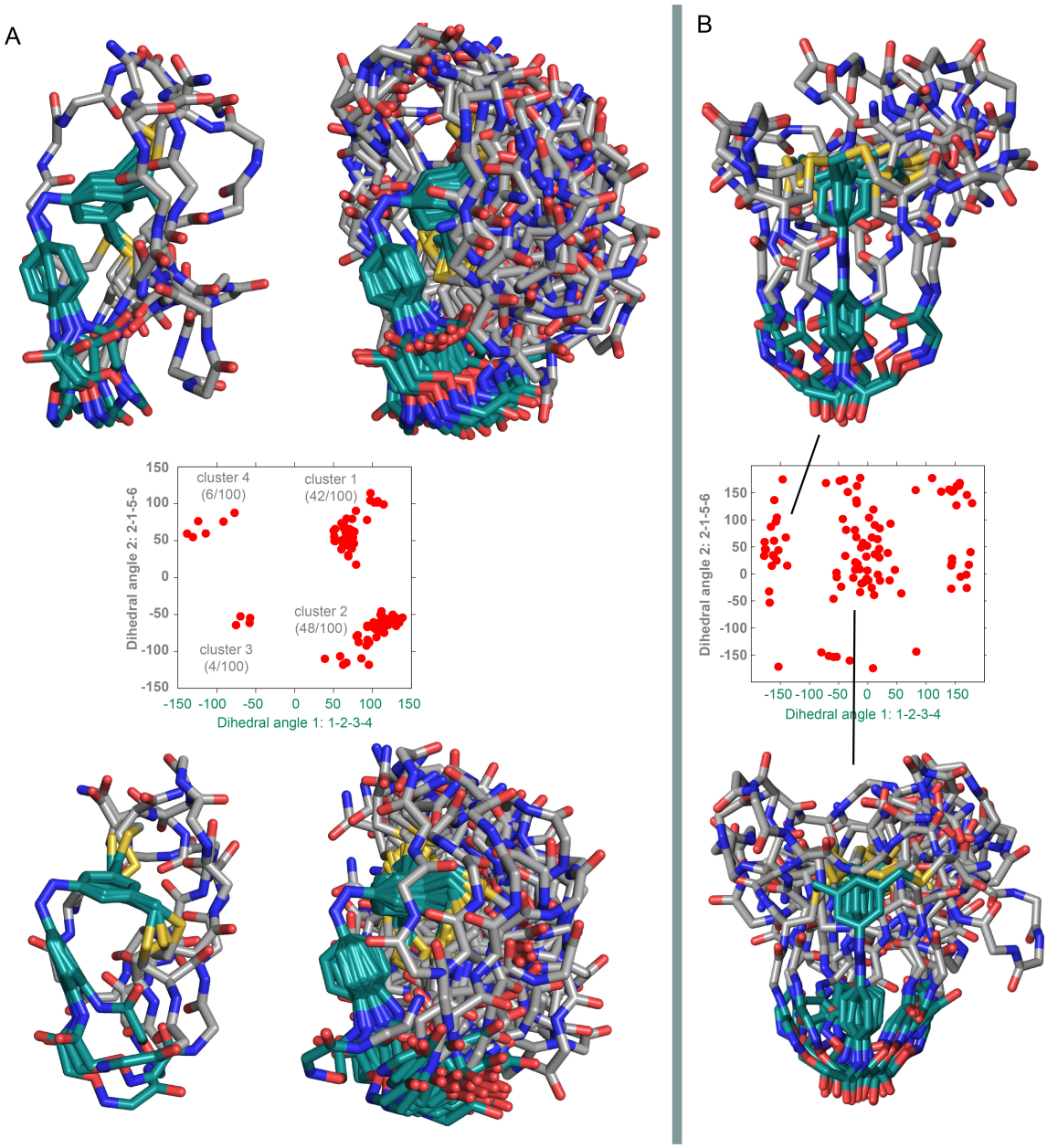


Fig. S14 The structures of both **HADCAz** and peptide backbone in (A) light **3a** and (B) dark **3a** diverged in 100 simulations (see Movies S1 and PBD files in *Derda_PDB.zip* in Supporting Information). In (A) the images represent all structures from each cluster; (B) shows 10 representative structures from each "cluster". In all cases, the structures are grouped in space to overlay N=N bond and maximize the overlay of the azobenzene core.

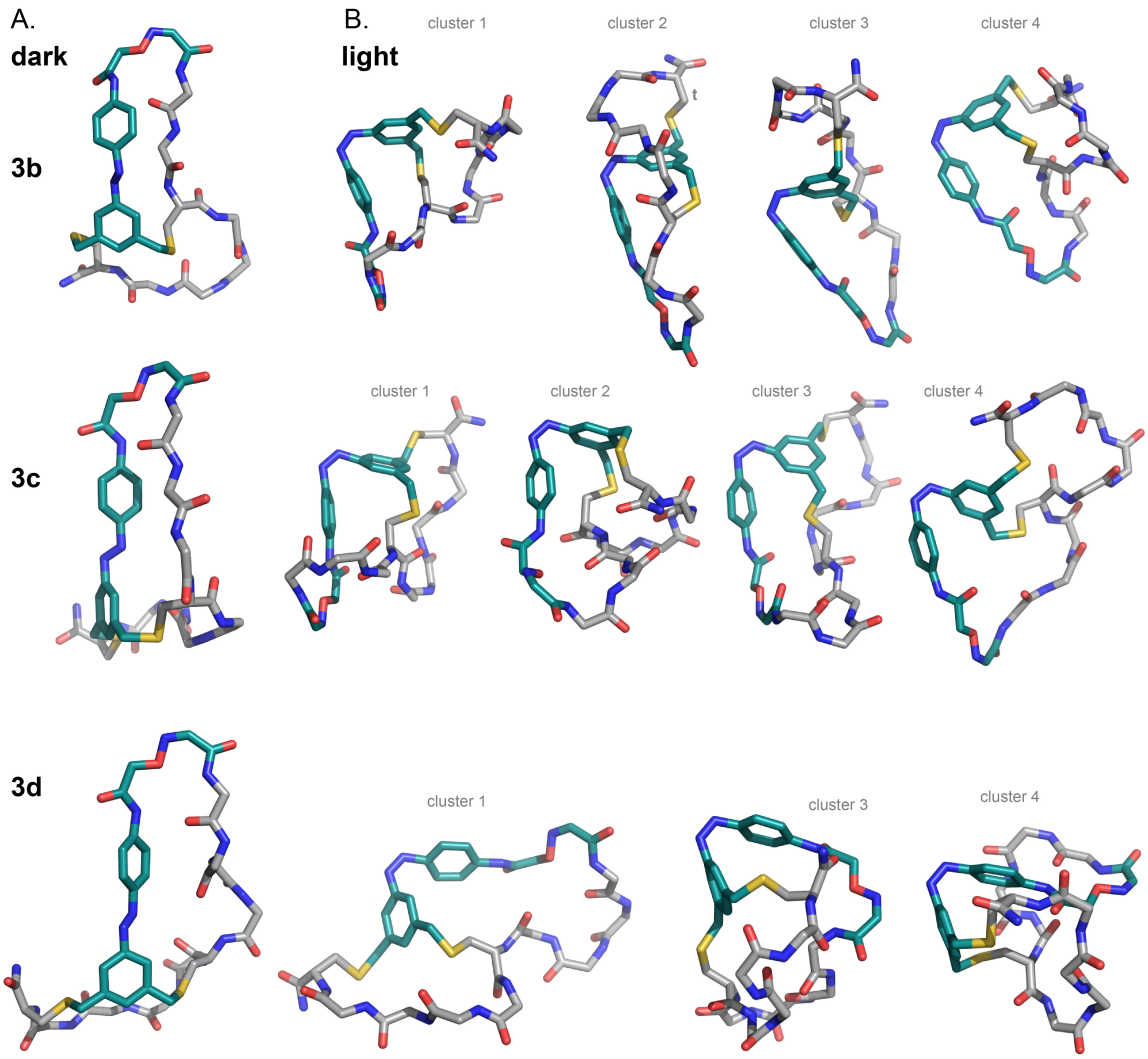


Fig. S15 (A) Representative structure of dark **3b**, **3c**, **3d** and (B) four representative structures from four cluster of light **3b**, **3c**, **3d** obtained after 100 simulations (see PBD files in *Derda_PDB.zip* in Supporting Information).

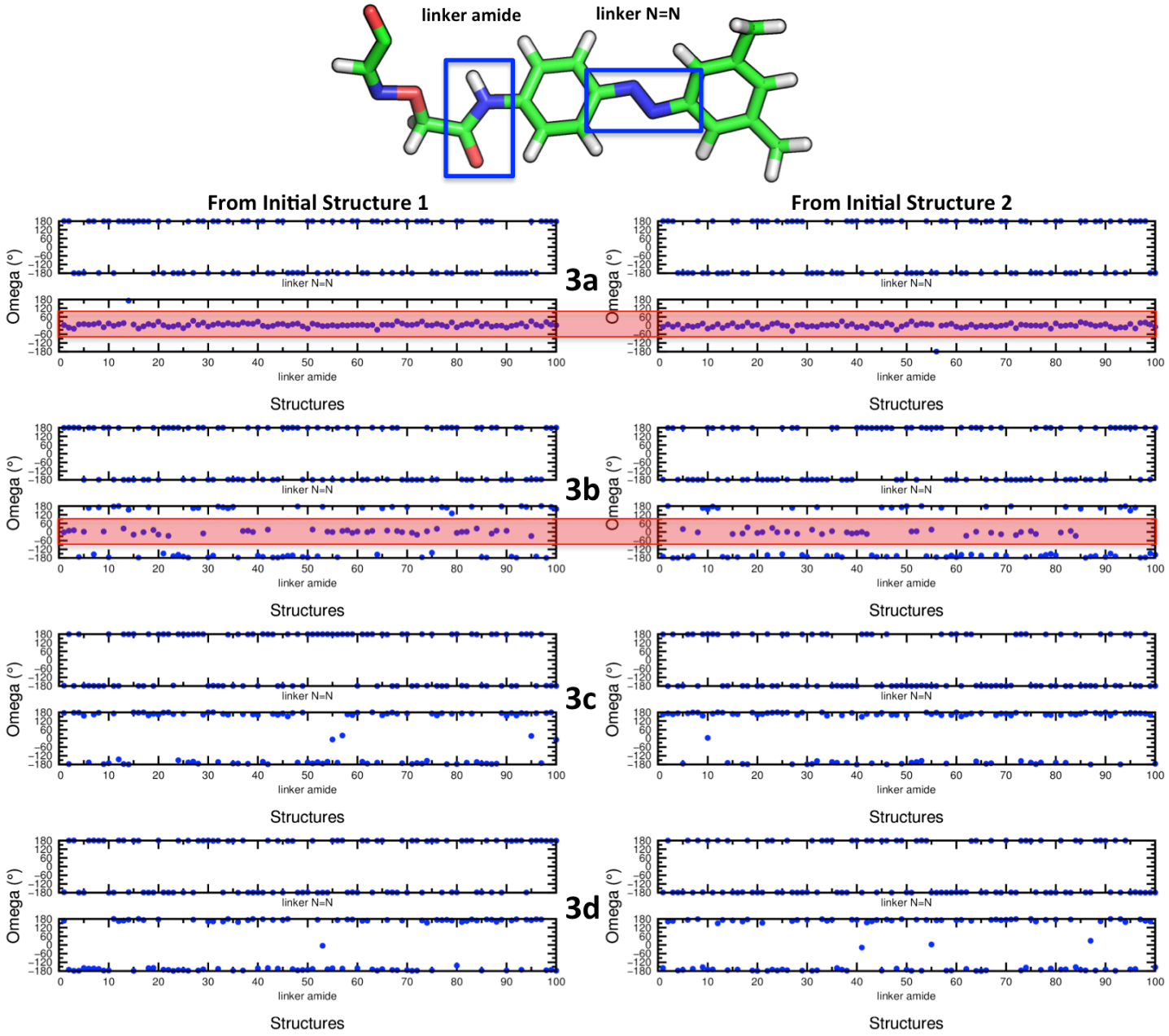


Fig. S16 Omega dihedral plot of the azobenzene N=N bond (top plot in each group) and the linker amide bond (bottom plot in each group) for dark **3a**, **3b**, **3c**, and **3d**. The dihedral angle of 0° in **3a** indicates *cis* conformation of the amide bond. Each dot represent the result of a separate MD simulation.

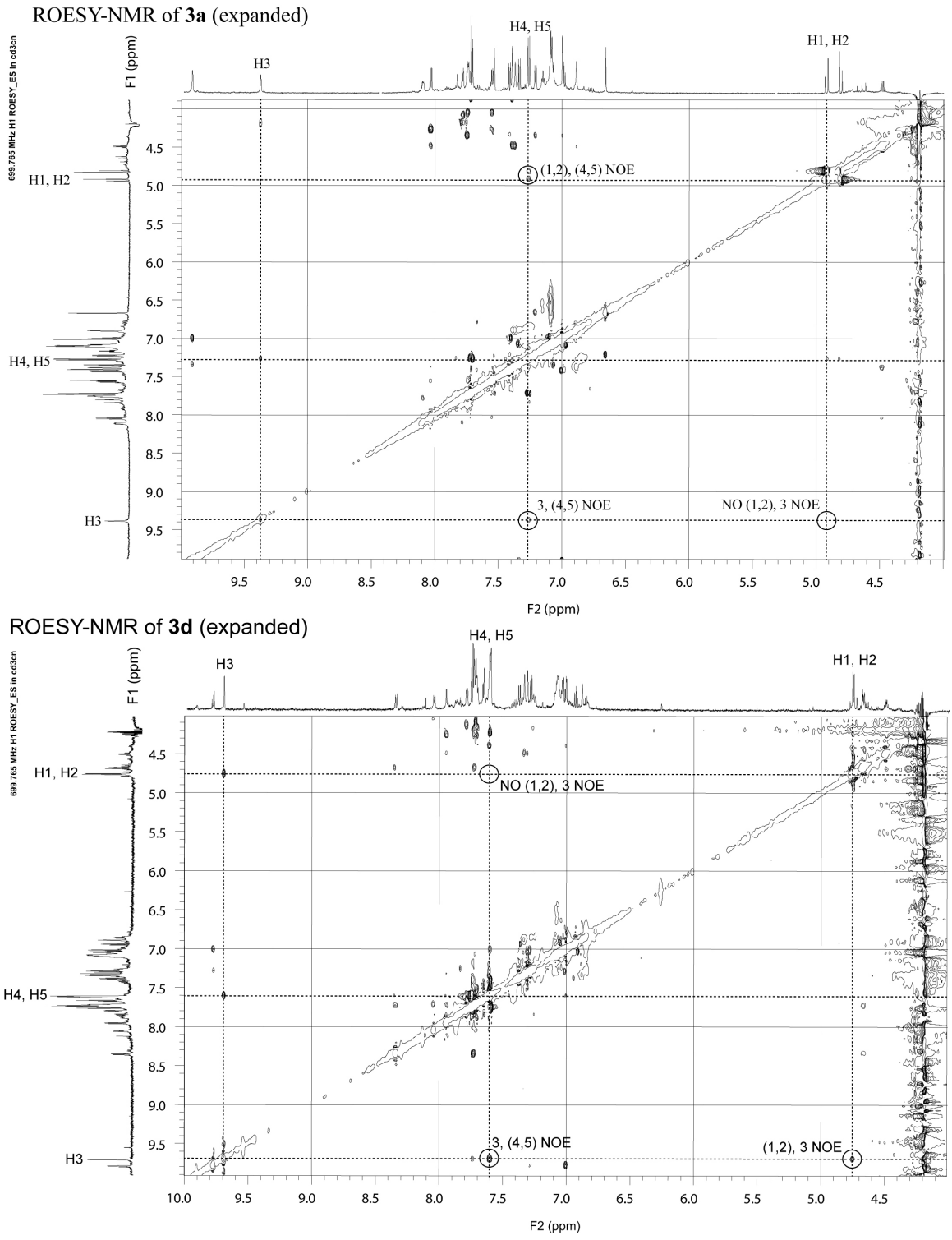
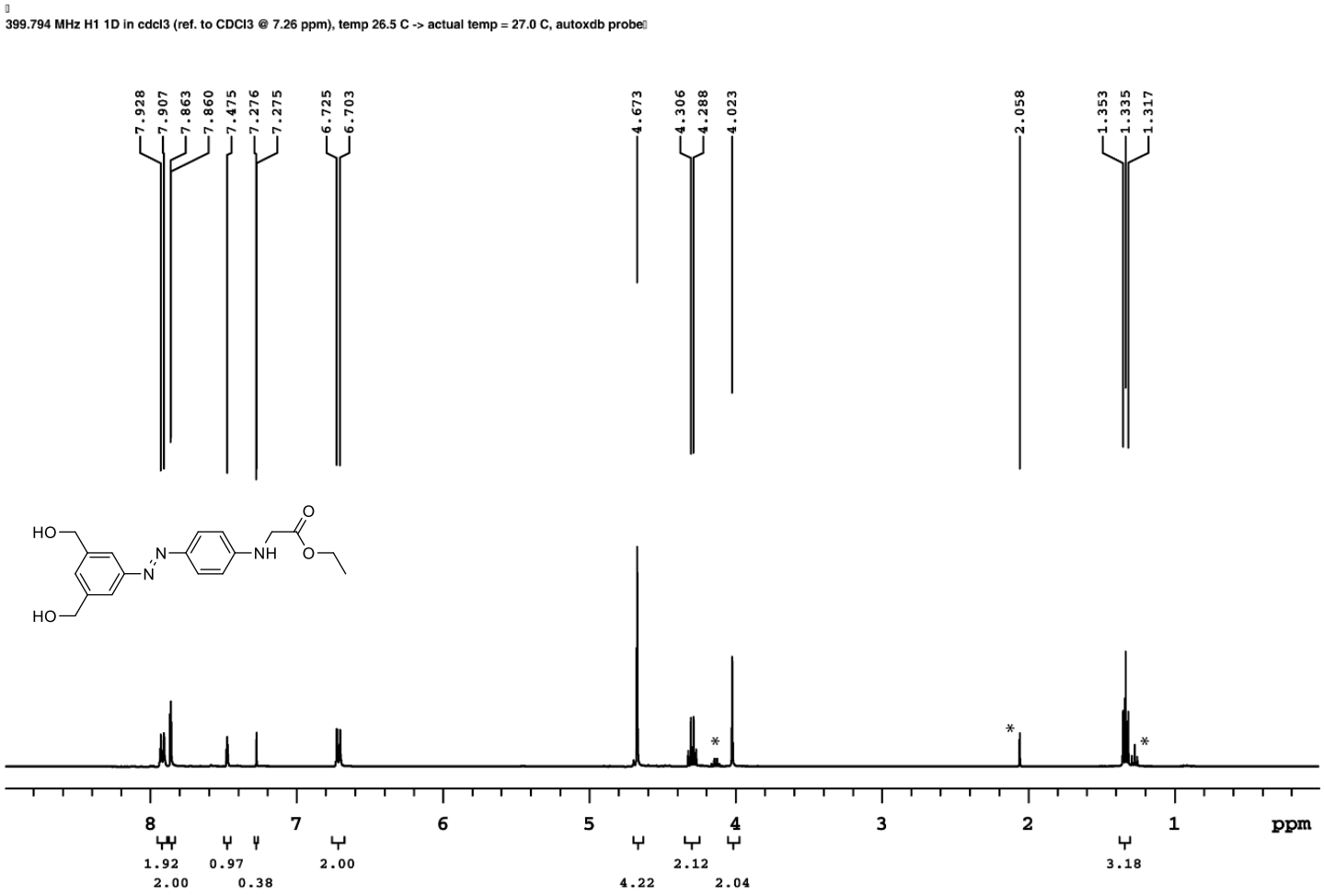


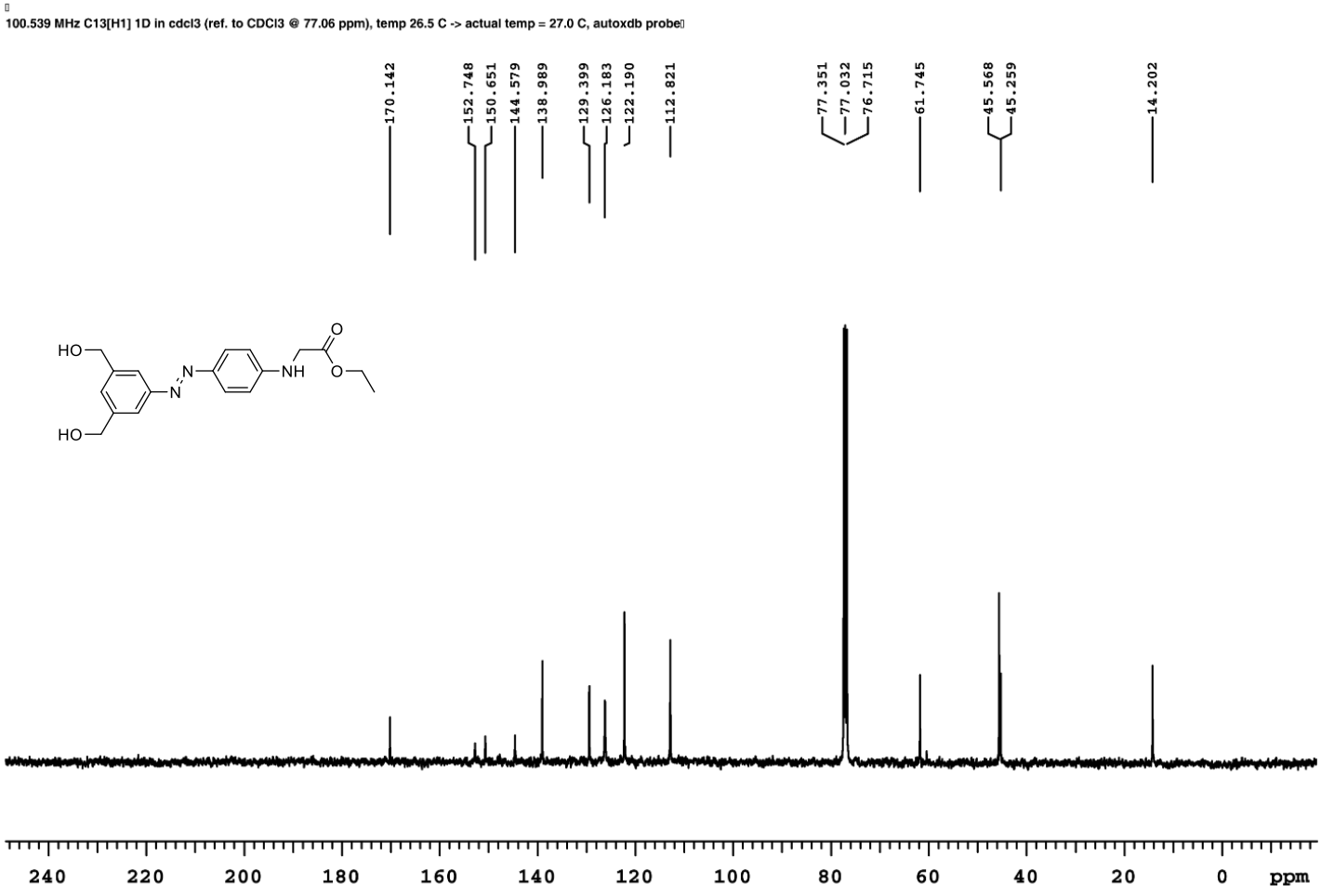
Fig. S17 Comparison of the NOE spectra of compounds **3a** and **3d** indicating presence of *cis* and *trans* aromatic amide bond respectively.

NMR spectra of synthesized compounds

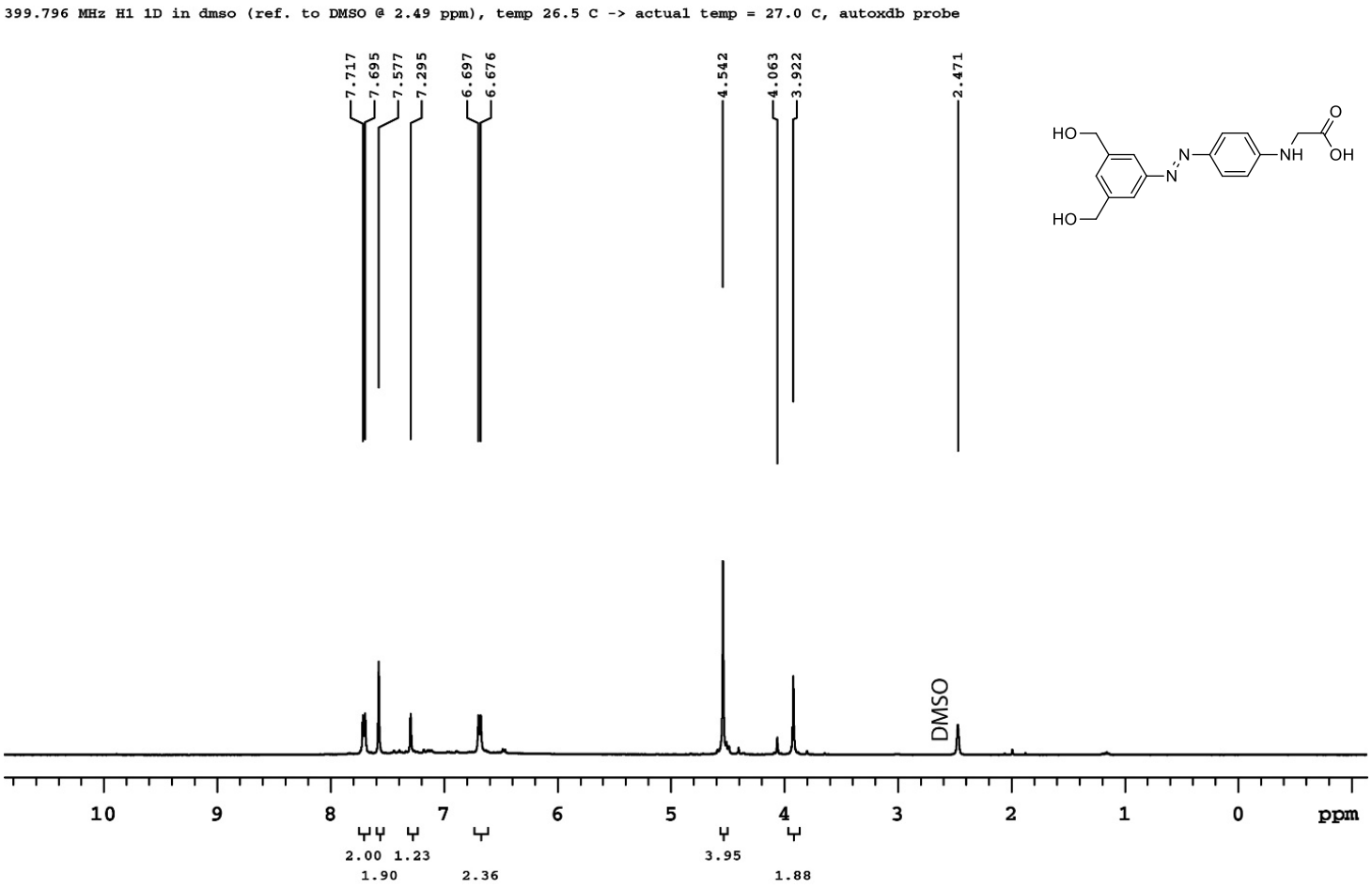
^{1}H-NMR of **1a** (asterisk shows residual EtOAc)



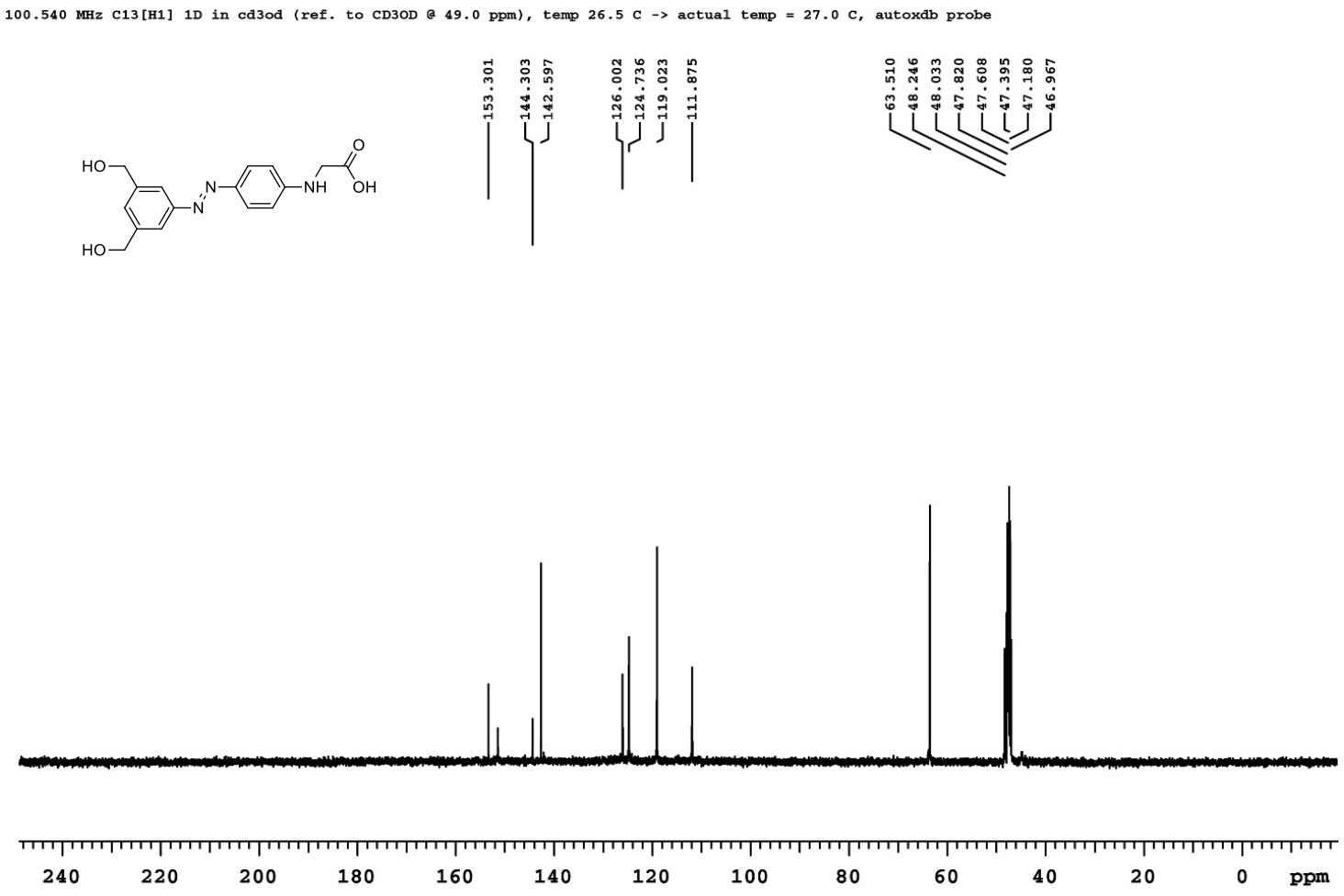
^{13}C-NMR of **1a**


100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
170.142
152.748
150.651
144.579
138.989
129.399
126.183
122.190
112.821
77.351
77.032
76.715
61.745
45.568
45.259
14.202
HO
HO
N
N
NH
O
O
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

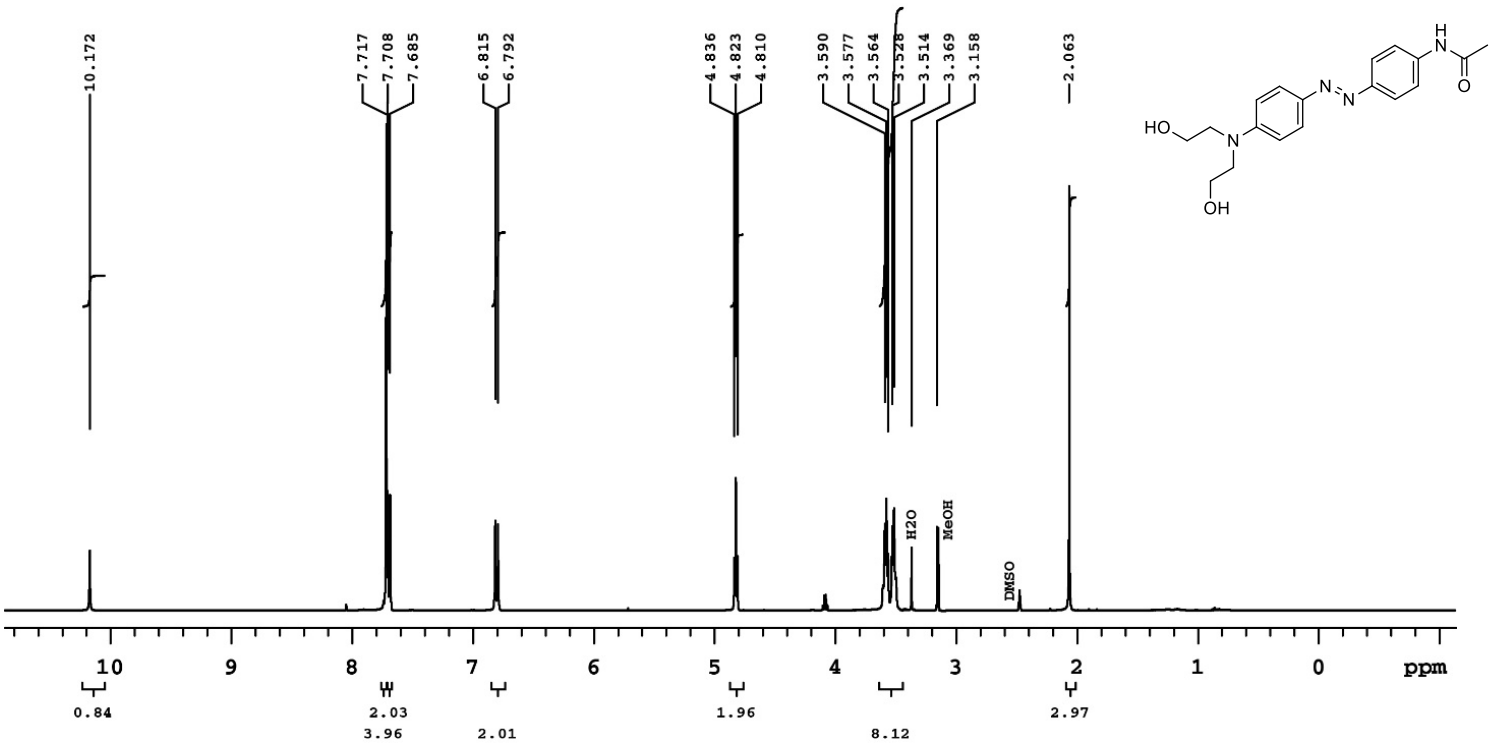
^{1}H-NMR of **1a'**


399.796 MHz H1 1D in dmso (ref. to DMSO @ 2.49 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.717
7.695
7.577
7.295
6.697
6.676
4.542
4.063
3.922
2.471
HO
HO
N
N
NH
O
OH
DMSO
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.23
1.90
2.36
3.95
1.88

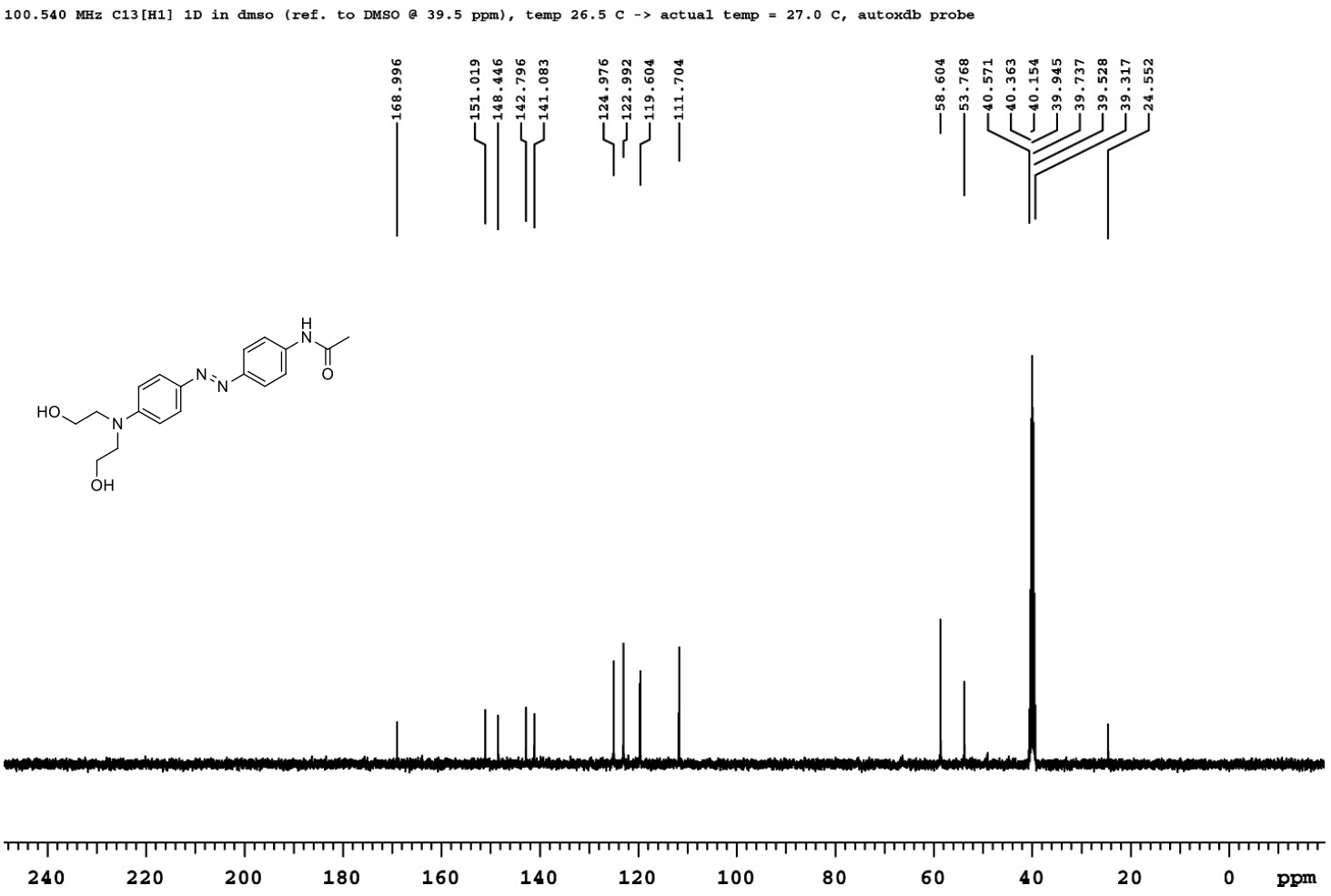
^{13}C-NMR of **1a'**


100.540 MHz C13[H1] 1D in cd3od (ref. to CD3OD @ 49.0 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
153.301
144.303
142.597
126.002
124.736
119.023
111.875
63.510
48.246
48.033
47.820
47.608
47.395
47.180
46.967
HO
HO
N
N
NH
O
OH
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

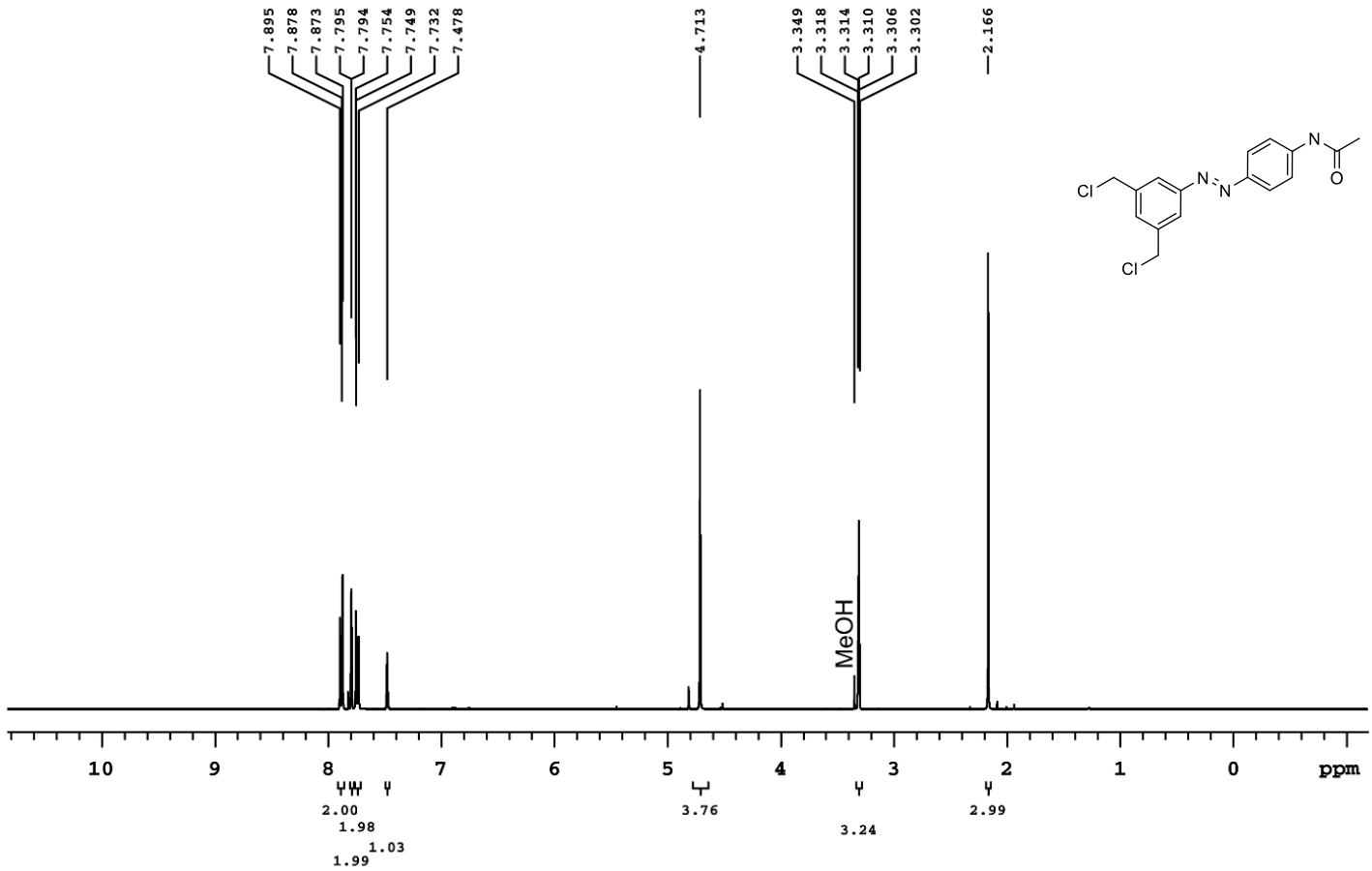
^{1}H-NMR of **1b**


399.796 MHz H1 1D in dmso (ref. to DMSO @ 2.49 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
10.172
7.717
7.708
7.685
6.815
6.792
4.836
4.823
4.810
3.590
3.577
3.564
3.528
3.514
3.369
3.158
2.063
H2O
MeOH
DMSO
HO
N
OH
N
N
H
N
O
10
9
8
7
6
5
4
3
2
1
0
ppm
0.84
2.03
3.96
2.01
1.96
8.12
2.97

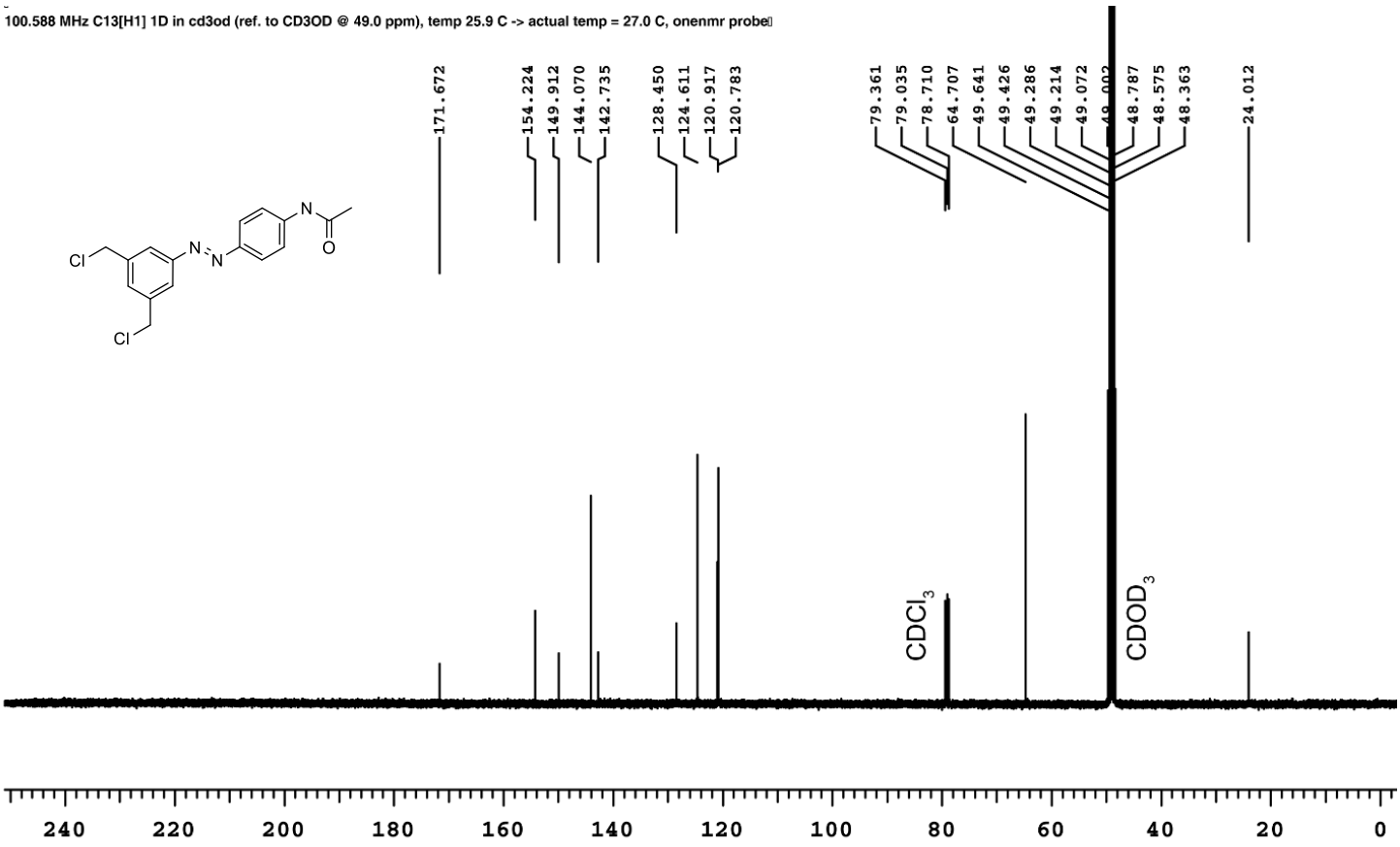
^{13}C-NMR of **1b**


100.540 MHz C13[H1] 1D in dmso (ref. to DMSO @ 39.5 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
168.996
151.019
148.446
142.796
141.083
124.976
122.992
119.604
111.704
58.604
53.768
40.571
40.363
40.154
39.945
39.737
39.528
39.317
24.552
HO
N
OH
N
N
H
N
O
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

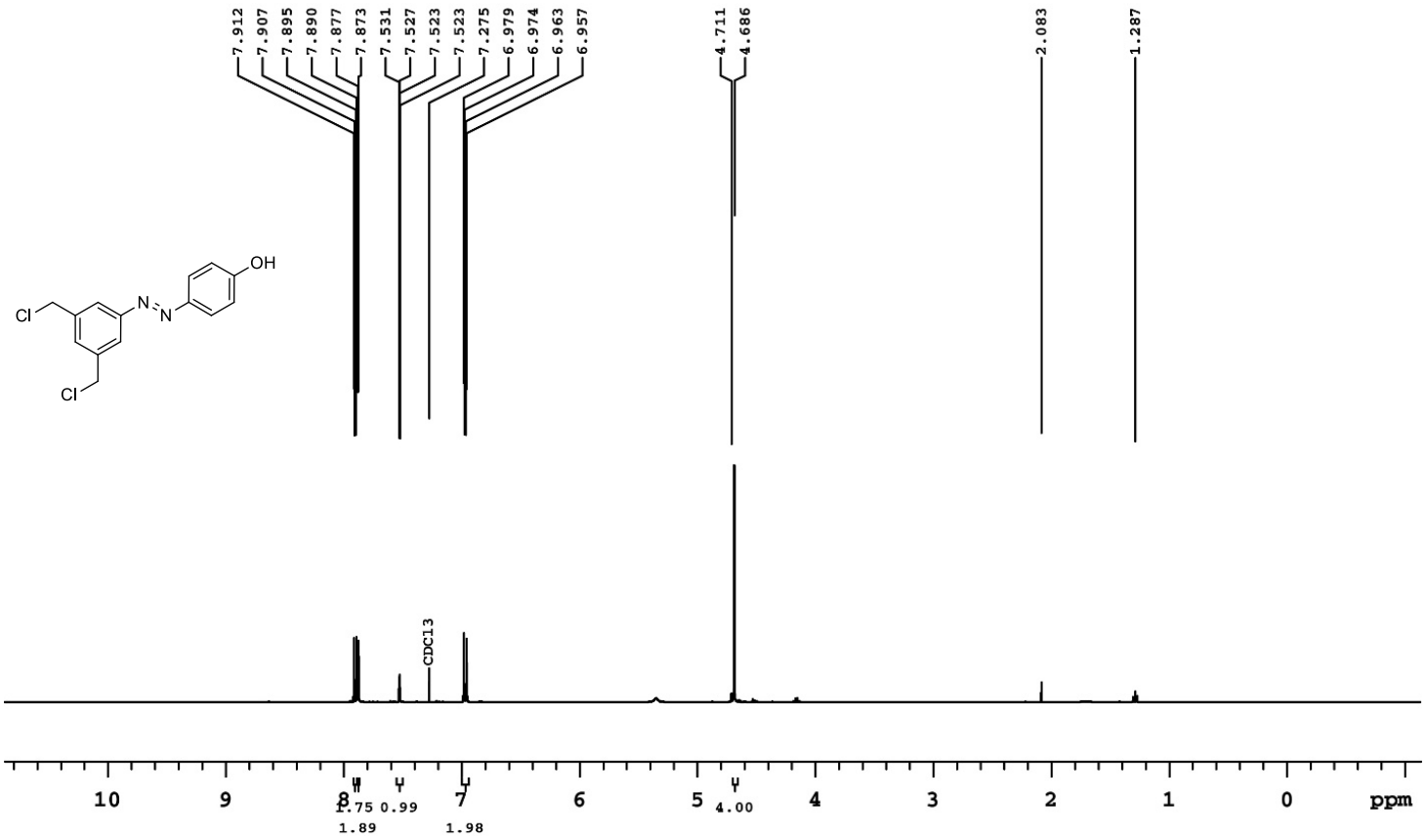
^{1}H-NMR of **1c**


399.986 MHz H1 1D in cd3od (ref. to CD3OD @ 3.30 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
7.895
7.878
7.873
7.795
7.794
7.754
7.749
7.732
7.478
4.713
3.349
3.318
3.314
3.310
3.306
3.302
2.166
MeOH
Cl
Cl
N
N
N
O
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.98
1.03
1.99
3.76
3.24
2.99

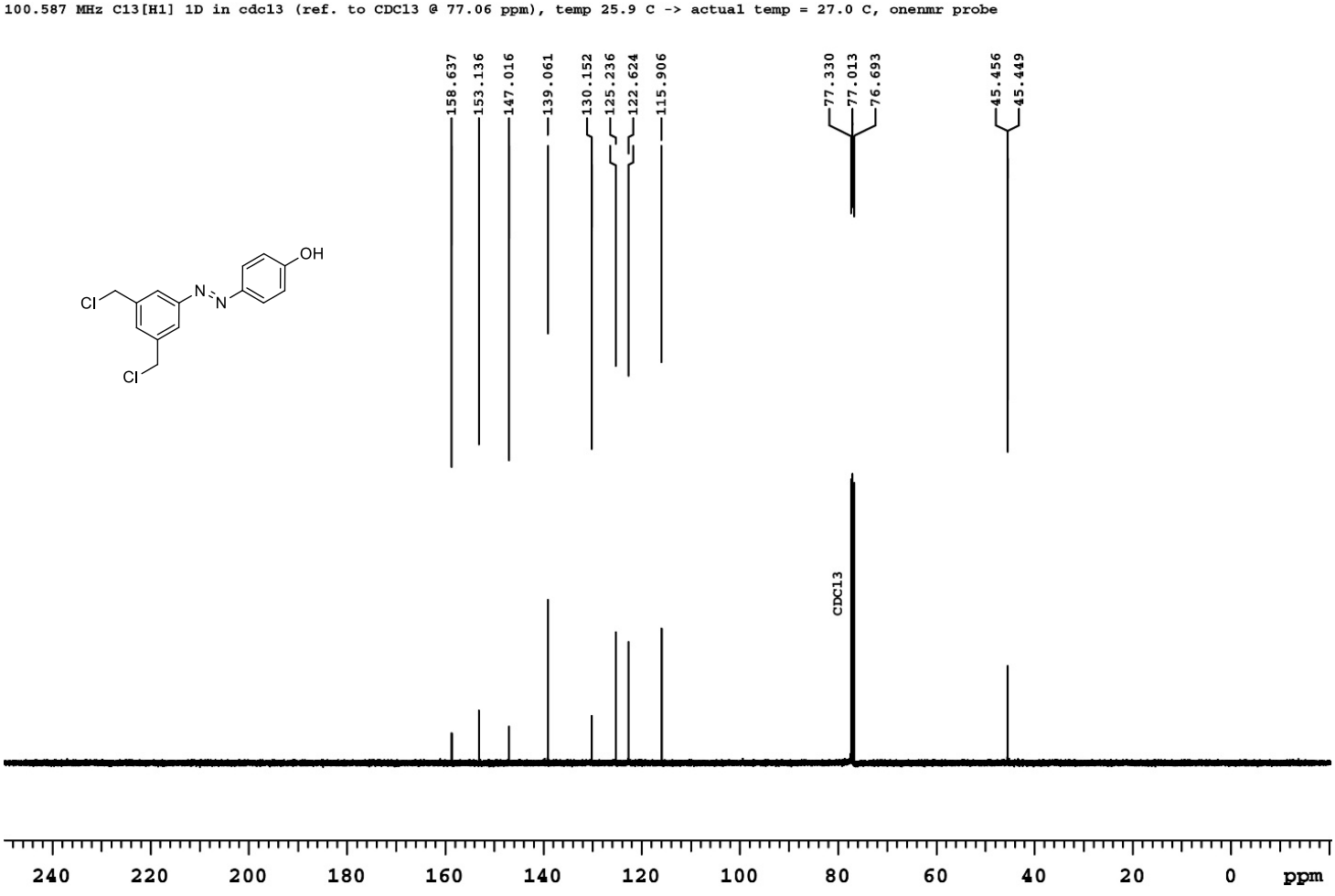
^{13}C-NMR of **1c**


100.588 MHz C13[H1] 1D in cd3od (ref. to CD3OD @ 49.0 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
171.672
154.224
149.912
144.070
142.735
128.450
124.611
120.917
120.783
79.361
79.035
78.710
64.707
49.641
49.426
49.286
49.214
49.072
49.002
48.787
48.575
48.363
24.012
CDCl3
CDOD3
Cl
Cl
N
N
N
O
240
220
200
180
160
140
120
100
80
60
40
20
0

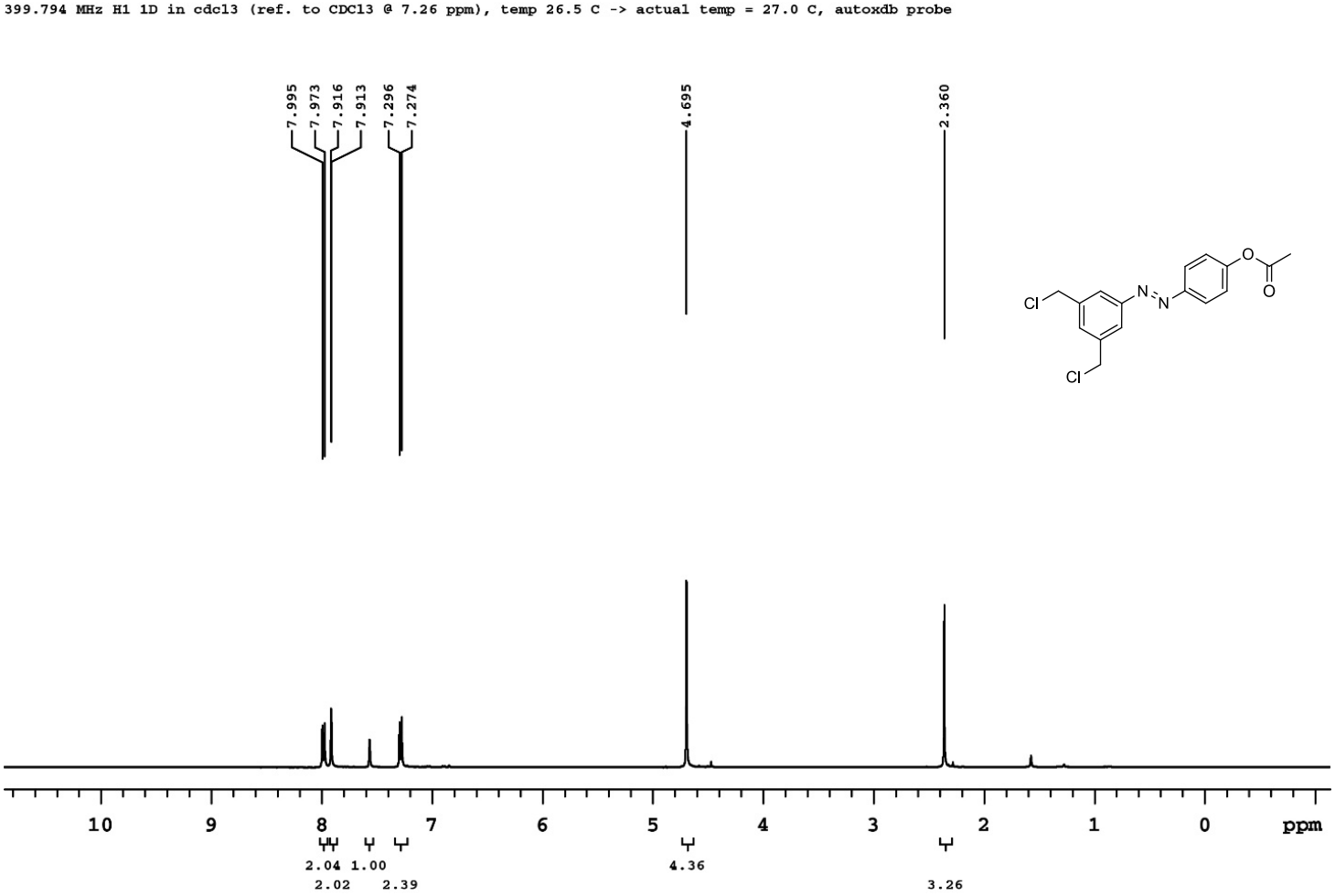
^{1}H-NMR of **1dA**


399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.912
7.907
7.895
7.890
7.877
7.873
7.531
7.527
7.523
7.523
7.275
6.979
6.974
6.963
6.957
4.711
4.686
2.083
1.287
OH
Cl
N
N
Cl
CDCl3
10
9
8
7
6
5
4
3
2
1
0
ppm
1.75
0.99
1.89
1.98
4.00

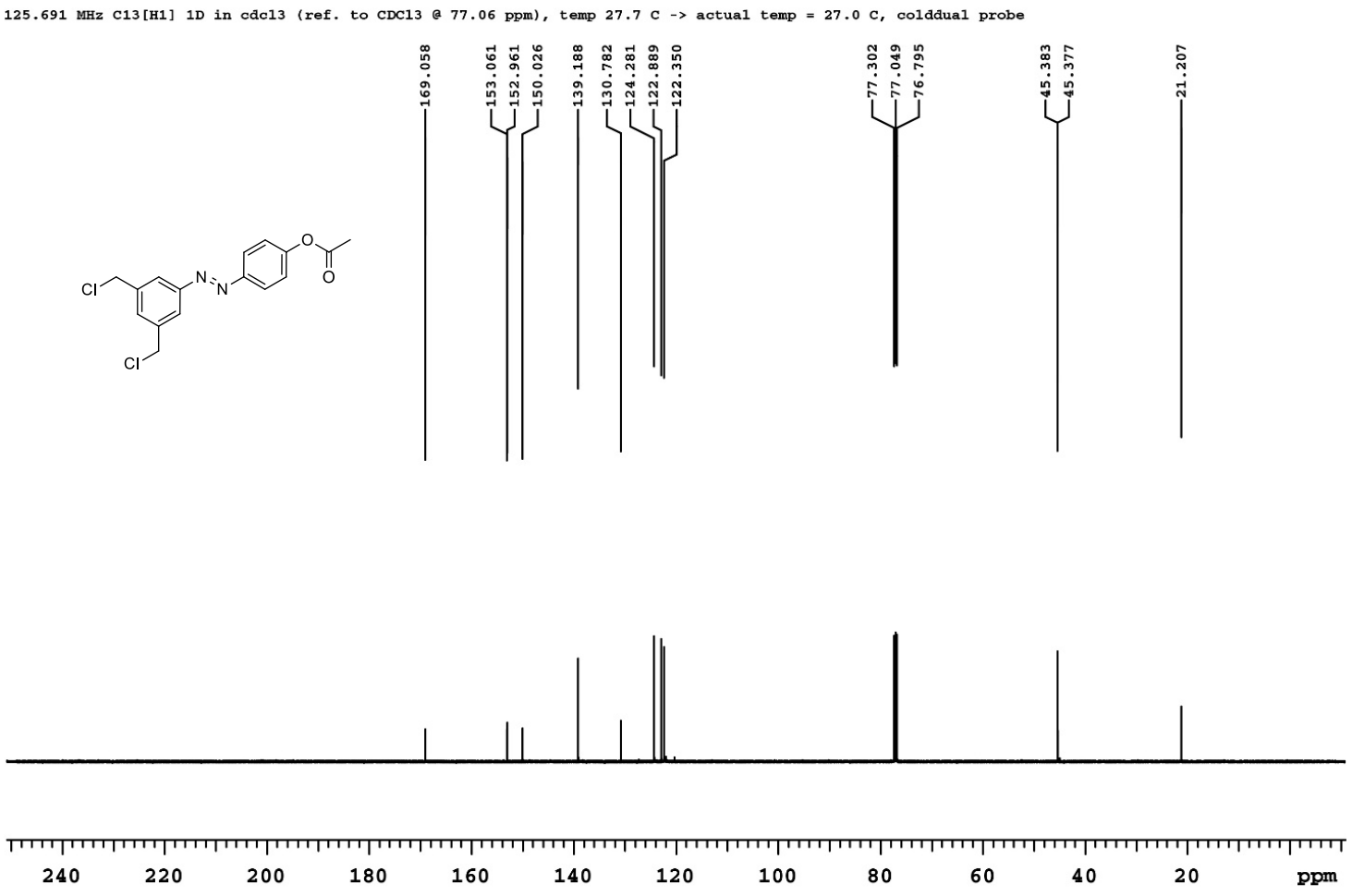
^{13}C-NMR of **1dA**



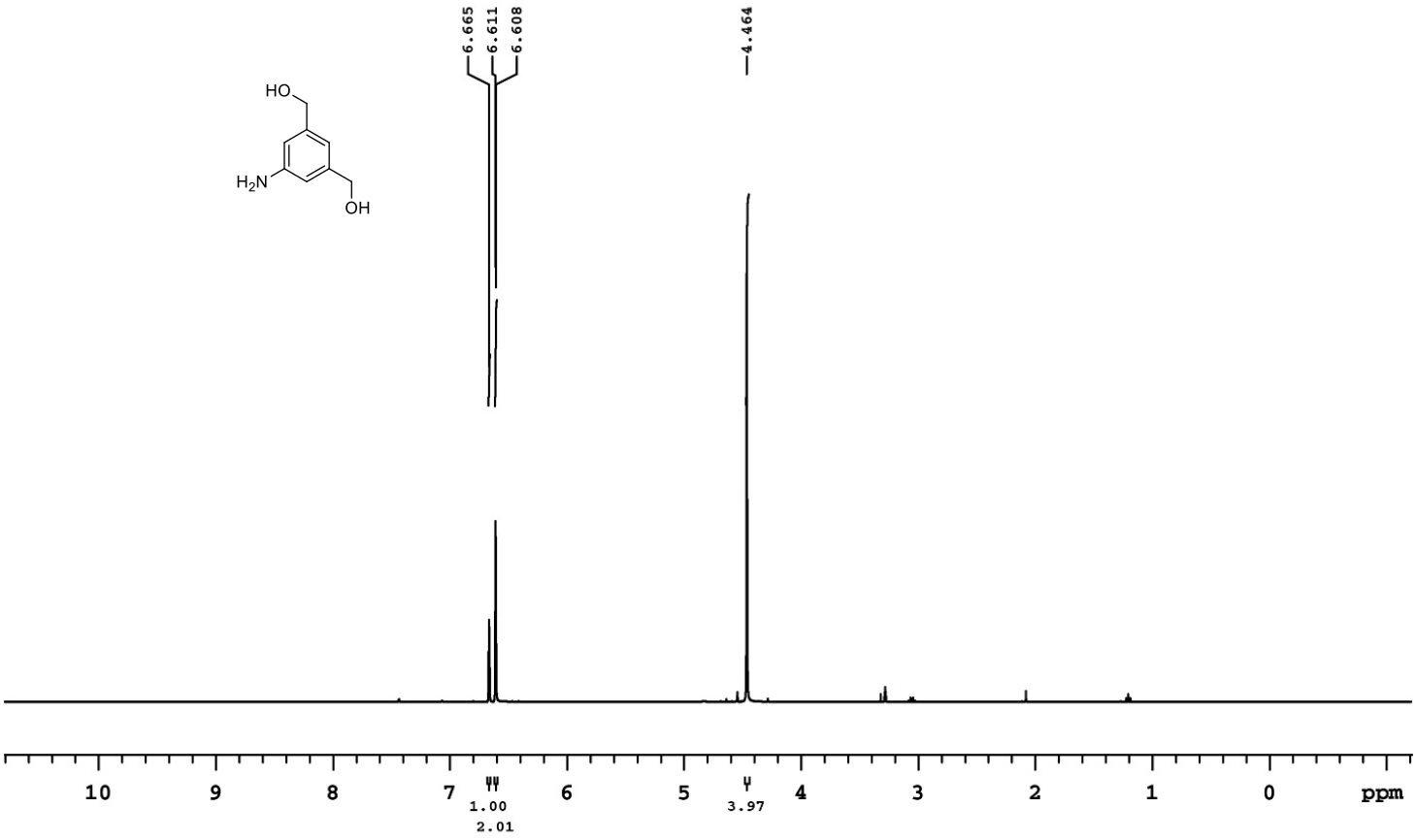
[1]H-NMR of **1d**


399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.995
7.973
7.916
7.913
7.296
7.274
4.695
2.360
Cl
Cl
N
N
O
O
10
9
8
7
6
5
4
3
2
1
0
ppm
2.04
1.00
2.02
2.39
4.36
3.26

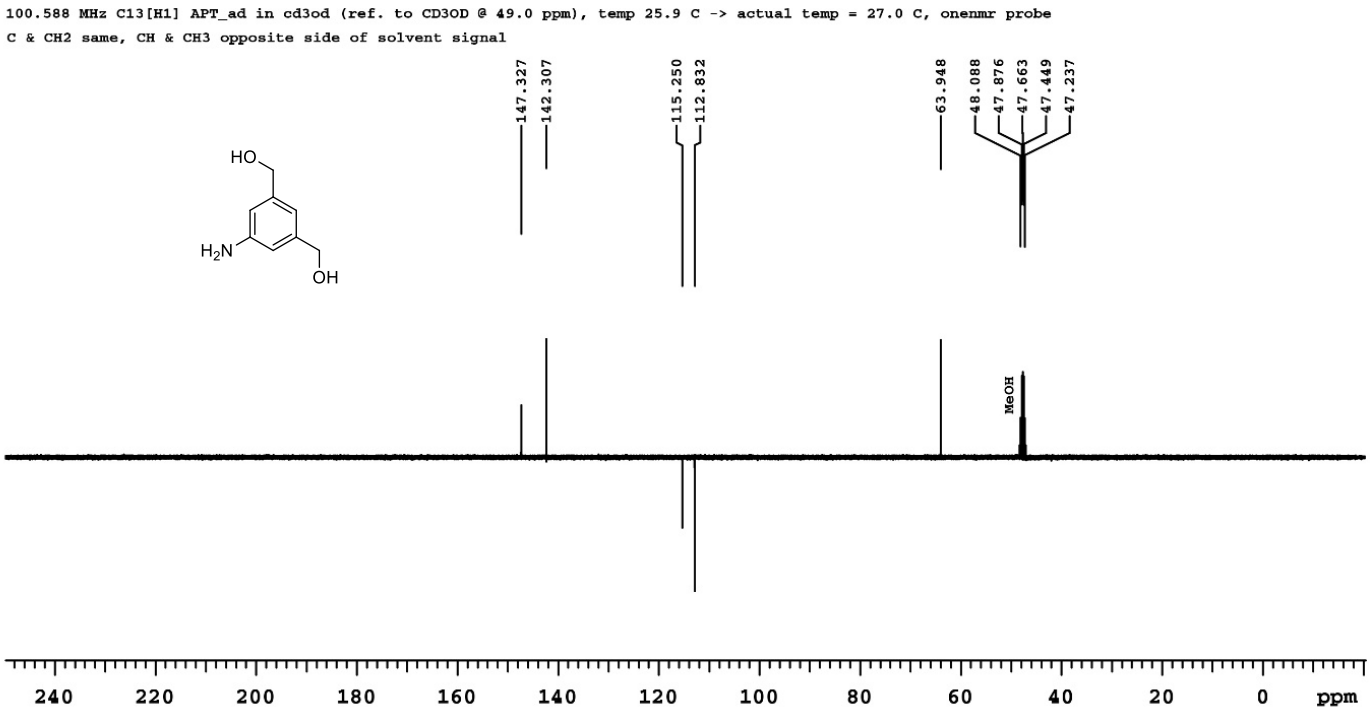
^{13}C-NMR of **1d**


125.691 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 27.7 C -> actual temp = 27.0 C, colddual probe
169.058
153.061
152.961
150.026
139.188
130.782
124.281
122.889
122.350
77.302
77.049
76.795
45.383
45.377
21.207
Cl
Cl
N
N
O
O
240
220
200
180
160
140
120
100
80
60
40
20
ppm

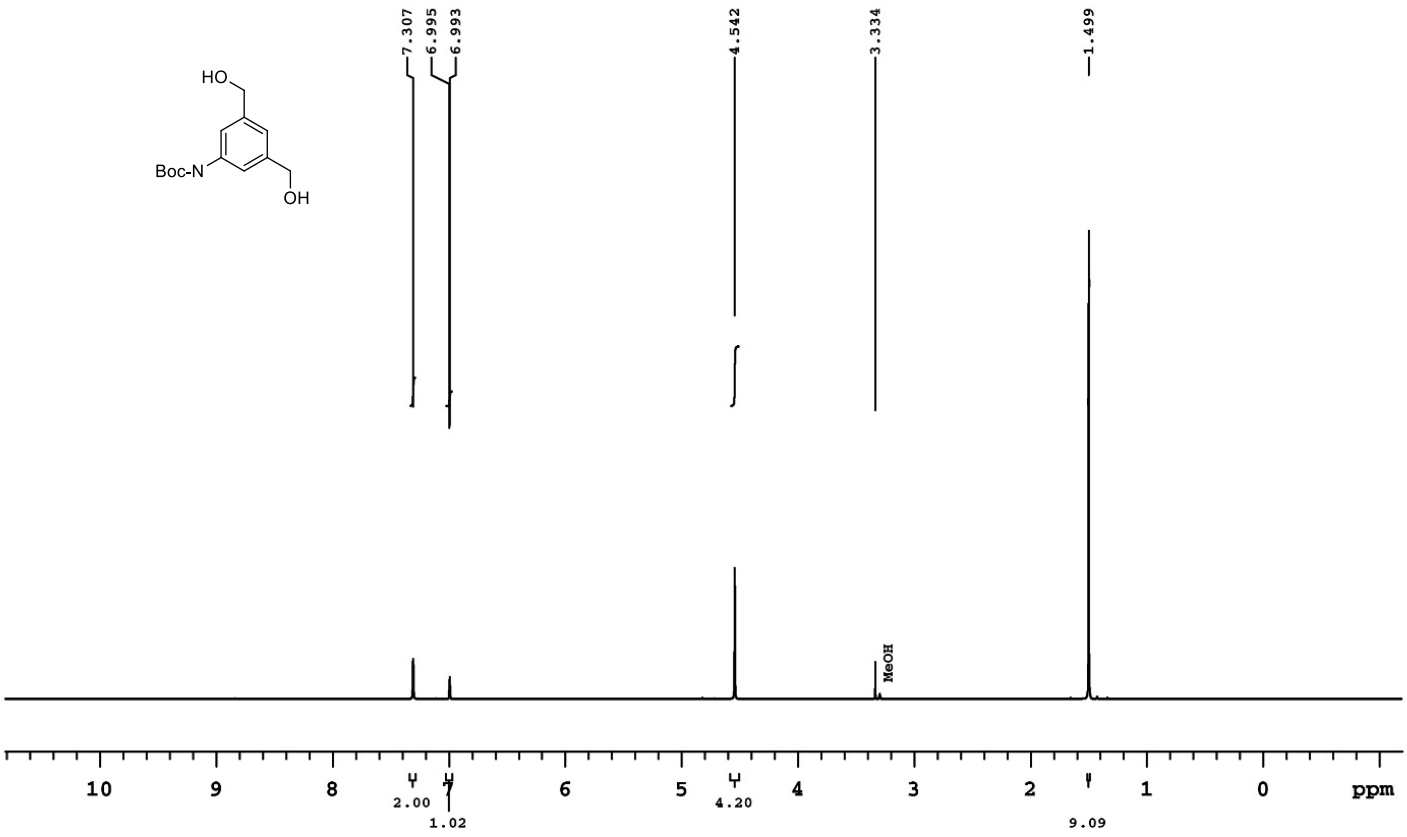
^{1}H-NMR of **2b**


399.986 MHz H1 1D in cd3od (ref. to CD3OD @ 3.30 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
6.665
6.611
6.608
4.464
HO
H2N
OH
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.01
3.97

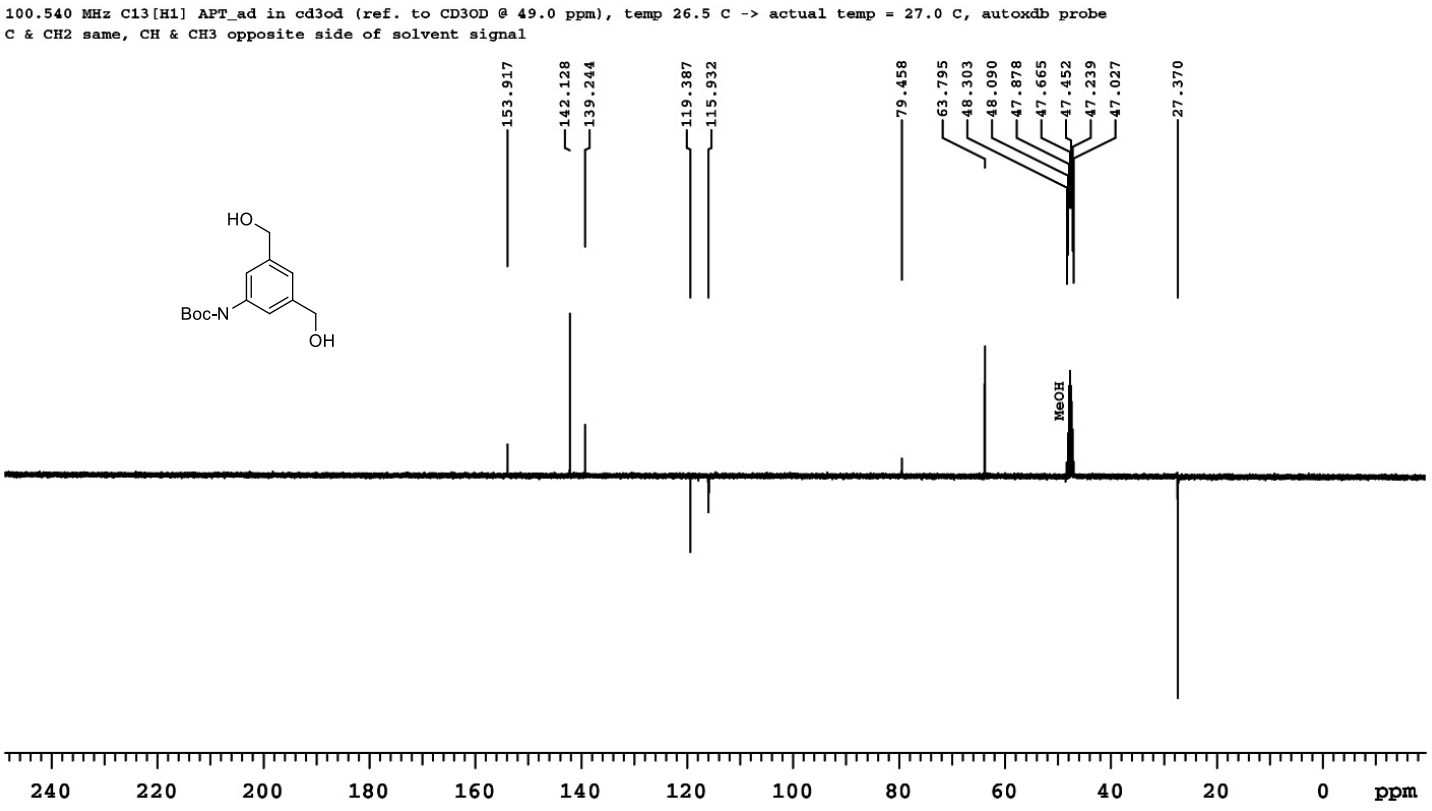
^{13}C-NMR of **2b**


100.588 MHz C13[H1] APT_ad in cd3od (ref. to CD3OD @ 49.0 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
C & CH2 same, CH & CH3 opposite side of solvent signal
147.327
142.307
115.250
112.832
63.948
48.088
47.876
47.663
47.449
47.237
MeOH
HO
H2N
OH
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

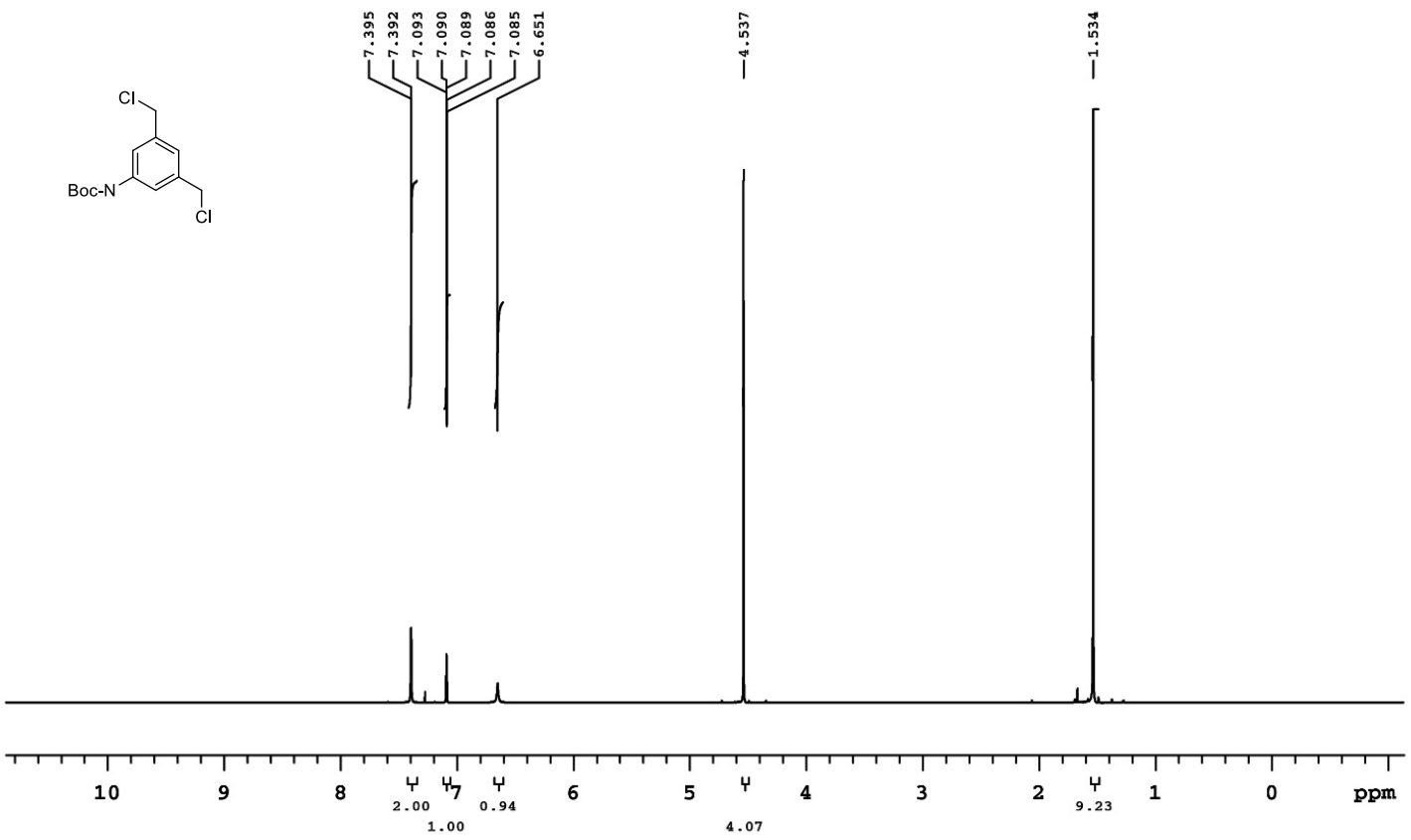
^{1}H-NMR of **2c**


399.796 MHz H1 1D in cd3od (ref. to CD3OD @ 3.30 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
HO
Boc-N
OH
7.307
6.995
6.993
4.542
3.334
1.499
MeOH
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.02
4.20
9.09

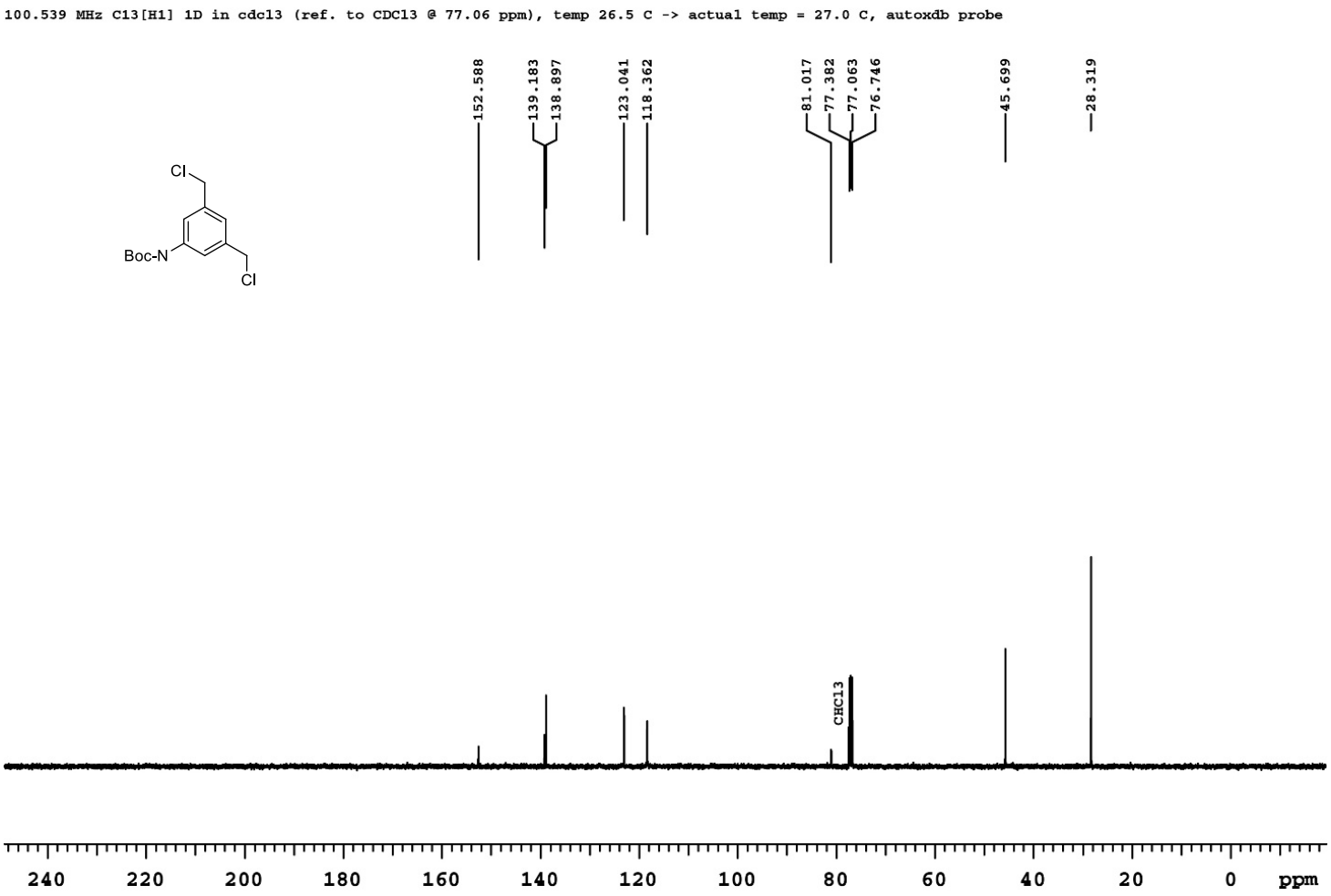
^{13}C-NMR of **2c**


100.540 MHz C13[H1] APT_ad in cd3od (ref. to CD3OD @ 49.0 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal
153.917
142.128
139.244
119.387
115.932
79.458
63.795
48.303
48.090
47.878
47.665
47.452
47.239
47.027
27.370
MeOH
HO
Boc-N
OH
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

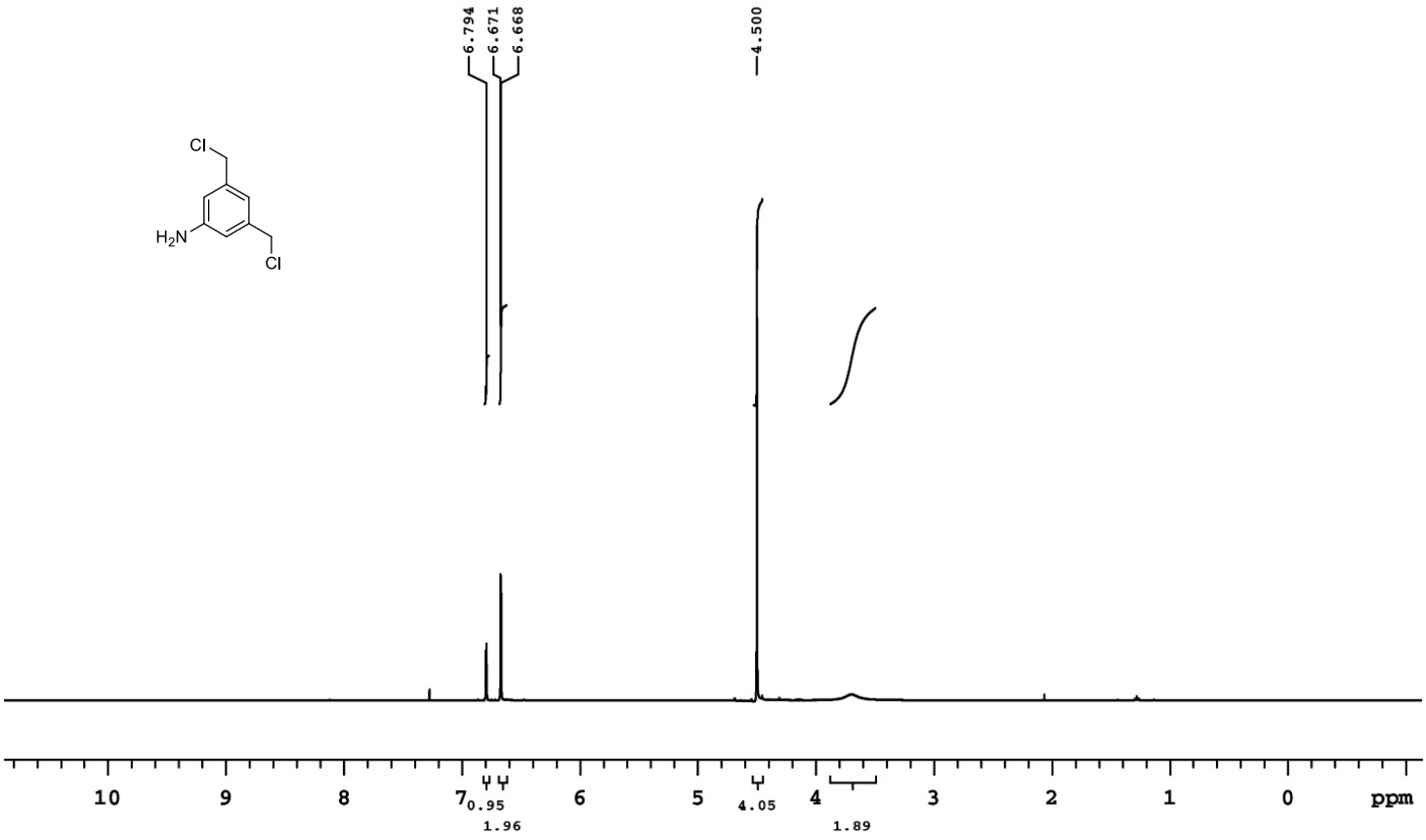
^{1}H-NMR of **2d**


399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.395
7.392
7.093
7.090
7.089
7.086
7.085
6.651
4.537
1.534
Cl
Boc-N
Cl
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.00
0.94
4.07
9.23

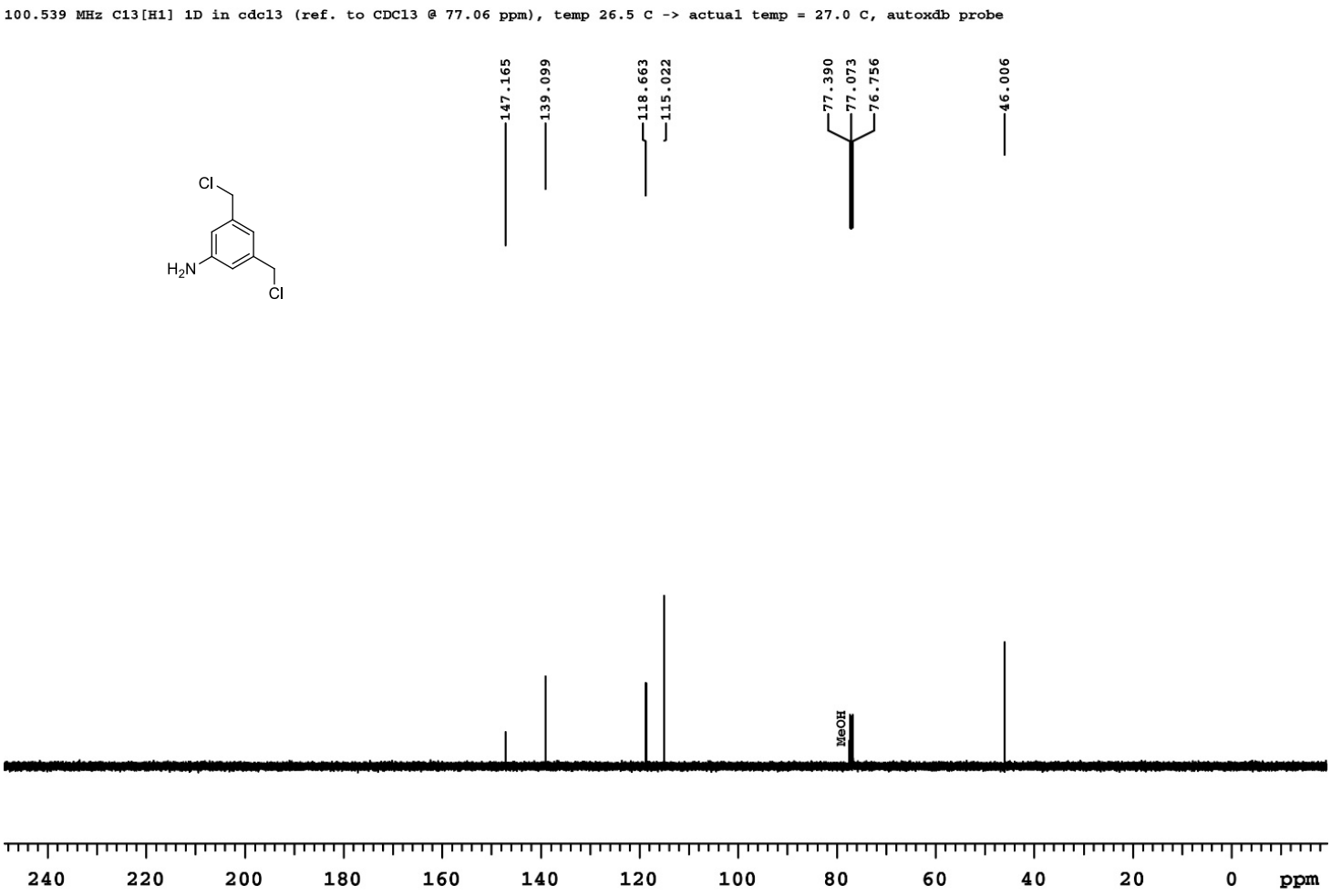
^{13}C-NMR of **2d**


100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
152.588
139.183
138.897
123.041
118.362
81.017
77.382
77.063
76.746
45.699
28.319
Cl
Boc-N
Cl
CHCl3
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

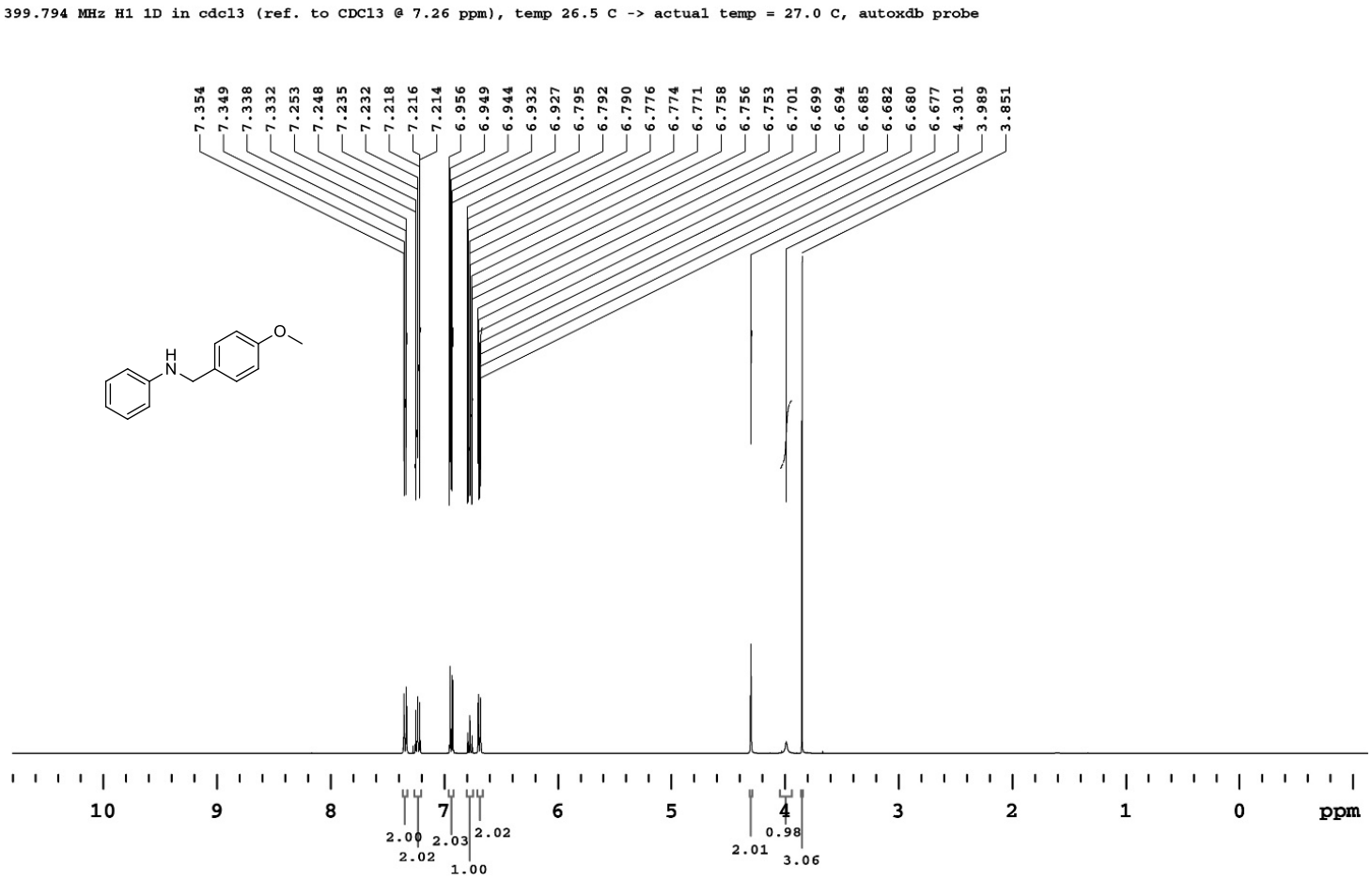
^{1}H-NMR of **2e**


399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
6.794
6.671
6.668
4.500
Cl
H2N
Cl
10
9
8
7
6
5
4
3
2
1
0
ppm
0.95
1.96
4.05
1.89

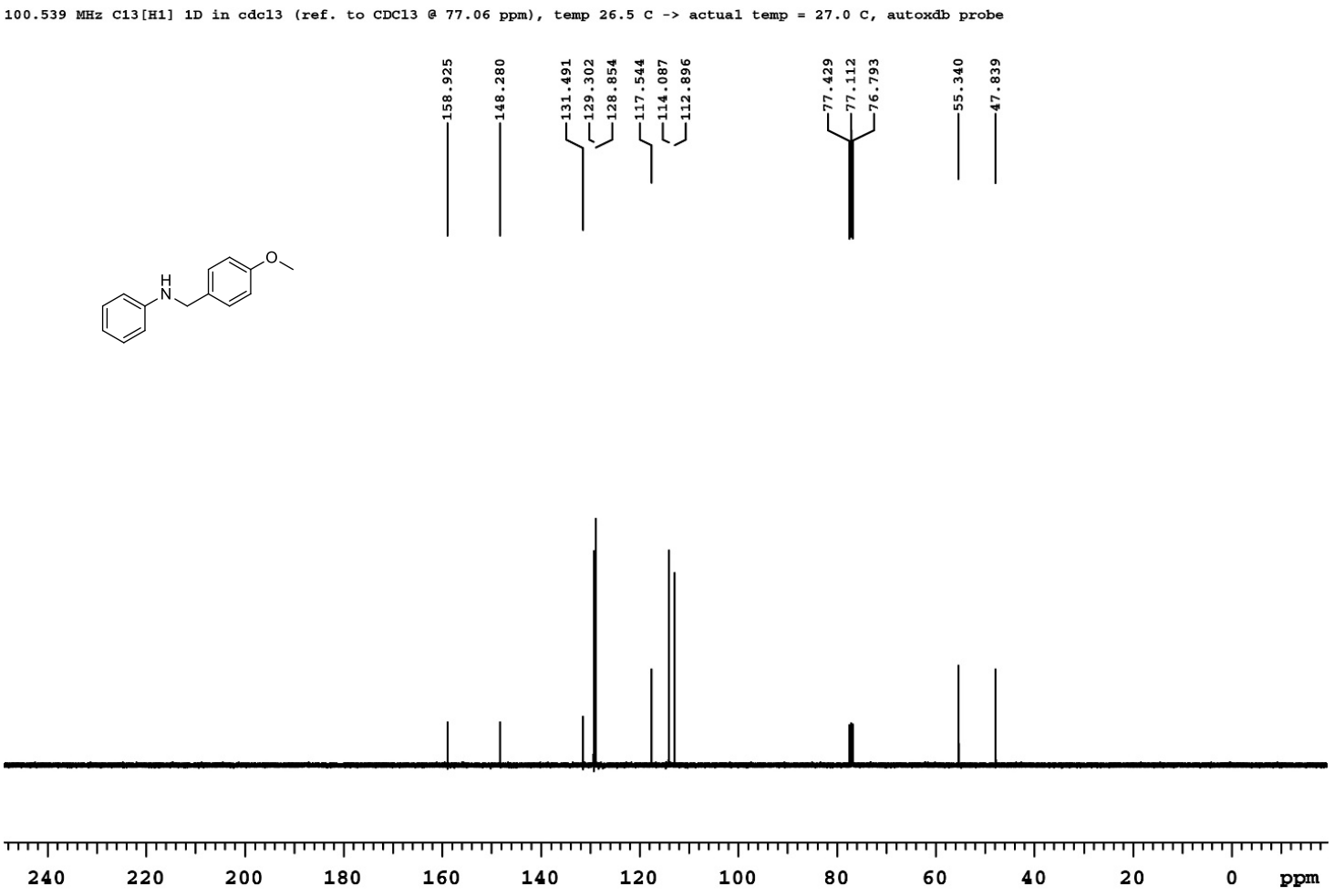
^{13}C-NMR of **2e**


100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
147.165
139.099
118.663
115.022
77.390
77.073
76.756
46.006
Cl
H2N
Cl
MeOH
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

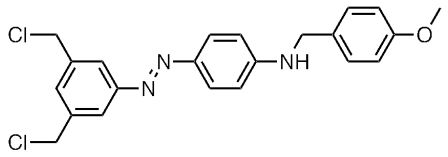
^{1}H-NMR of **2f**

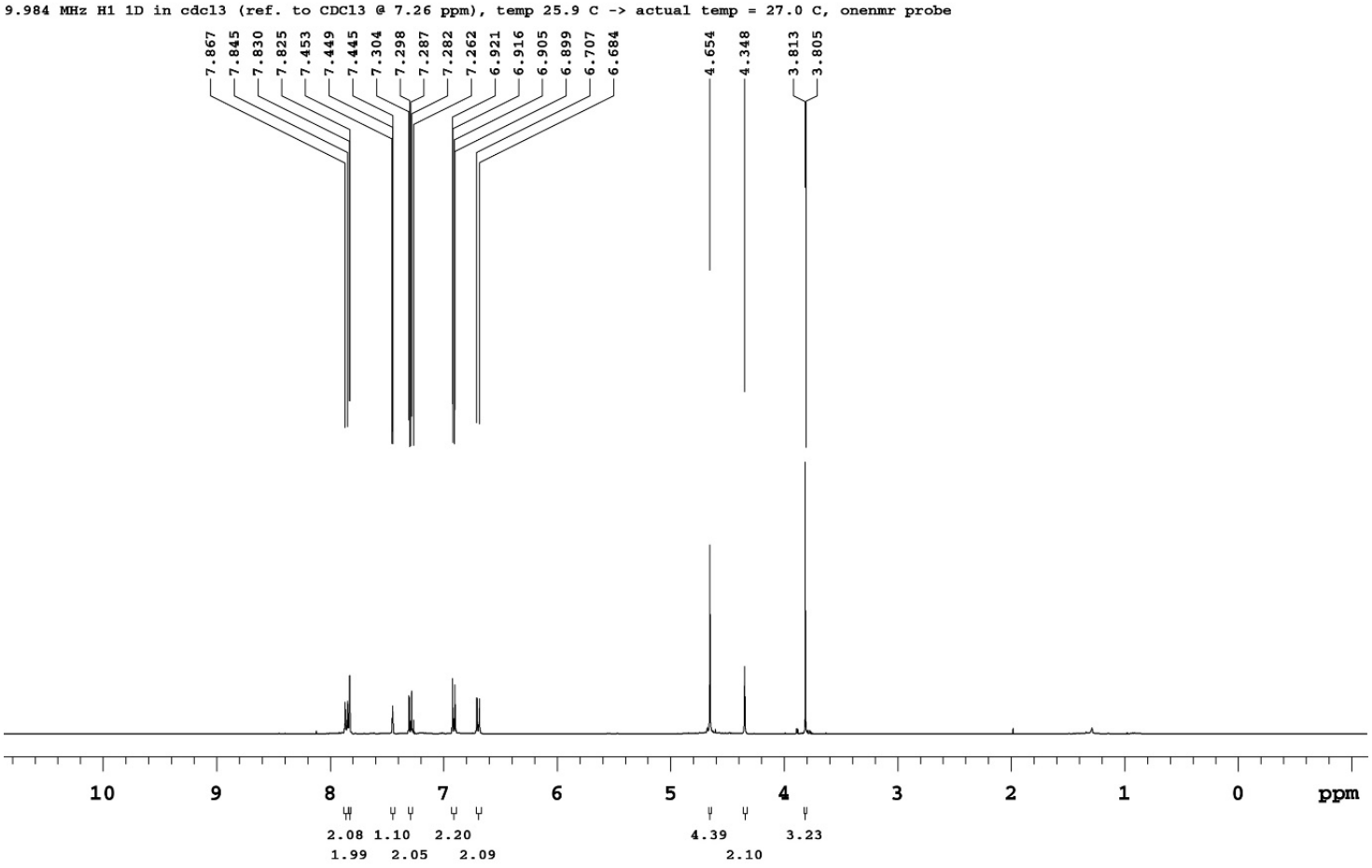

399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.354
7.349
7.338
7.332
7.253
7.248
7.235
7.232
7.218
7.216
7.214
6.956
6.949
6.944
6.932
6.927
6.795
6.792
6.790
6.776
6.774
6.771
6.758
6.756
6.753
6.701
6.699
6.694
6.685
6.682
6.680
6.677
4.301
3.989
3.851
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
2.02
2.03
1.00
2.02
2.01
0.98
3.06

^{13}C-NMR of **2f**

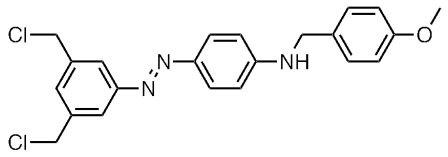

100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
158.925
148.280
131.491
129.302
128.854
117.544
114.087
112.896
77.429
77.112
76.793
55.340
47.839
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

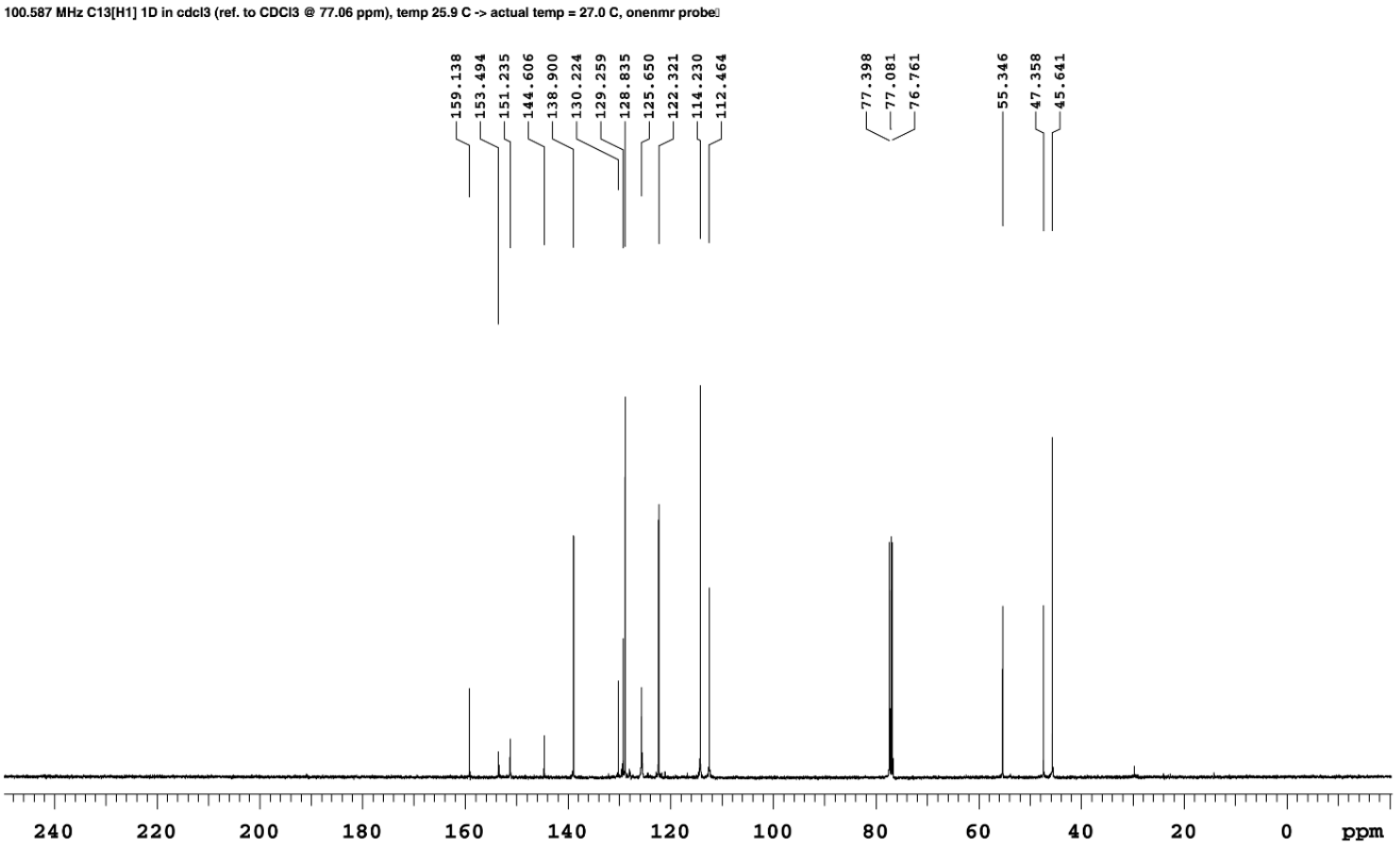
^{1}H-NMR of **2g**


Cl
Cl
N
N
NH
O

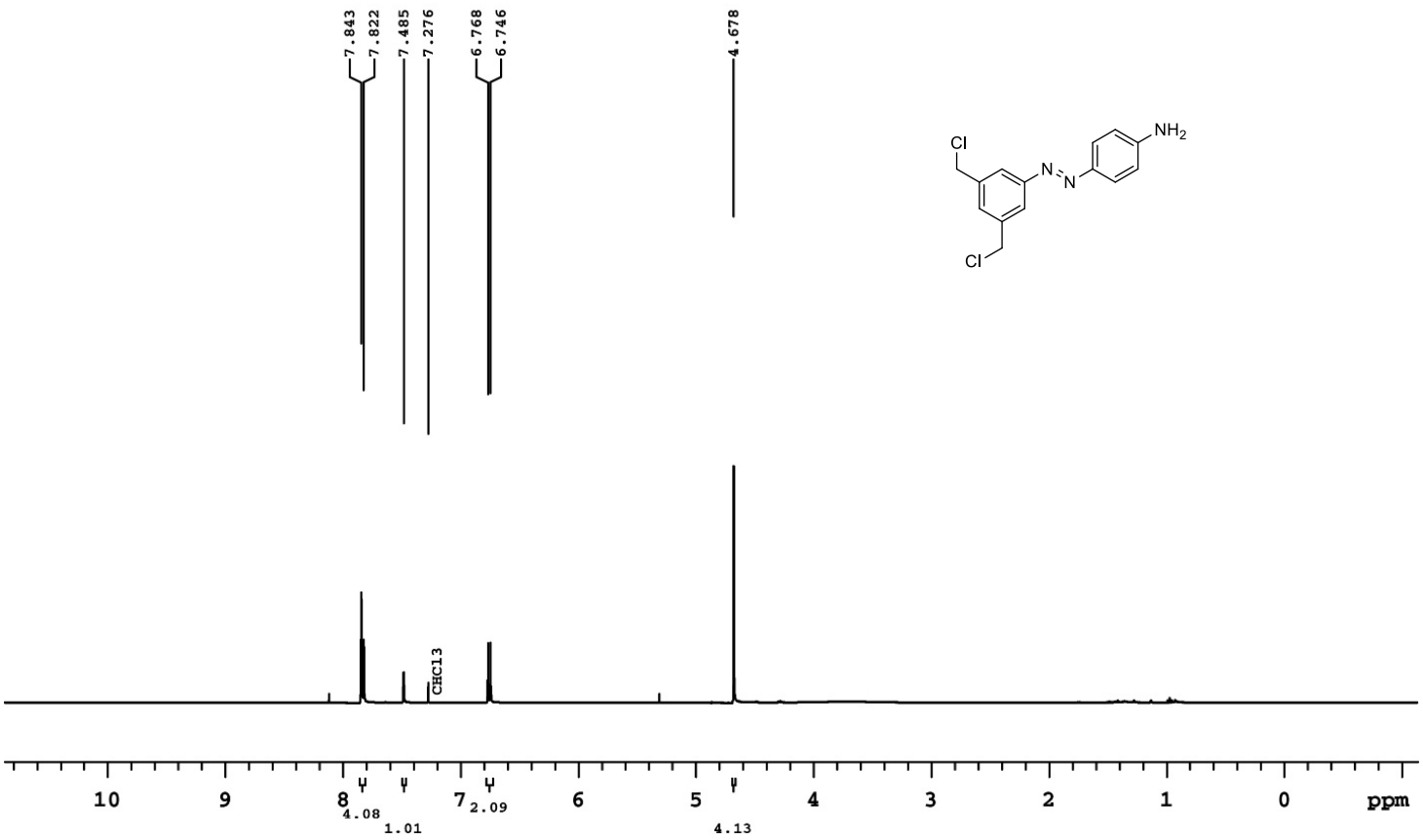

9.984 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
7.867
7.845
7.830
7.825
7.453
7.449
7.445
7.304
7.298
7.287
7.282
7.262
6.921
6.916
6.905
6.899
6.707
6.684
4.654
4.348
3.813
3.805
10
9
8
7
6
5
4
3
2
1
0
ppm
2.08
1.99
1.10
2.05
2.20
2.09
4.39
2.10
3.23

^{13}C-NMR of **2g**

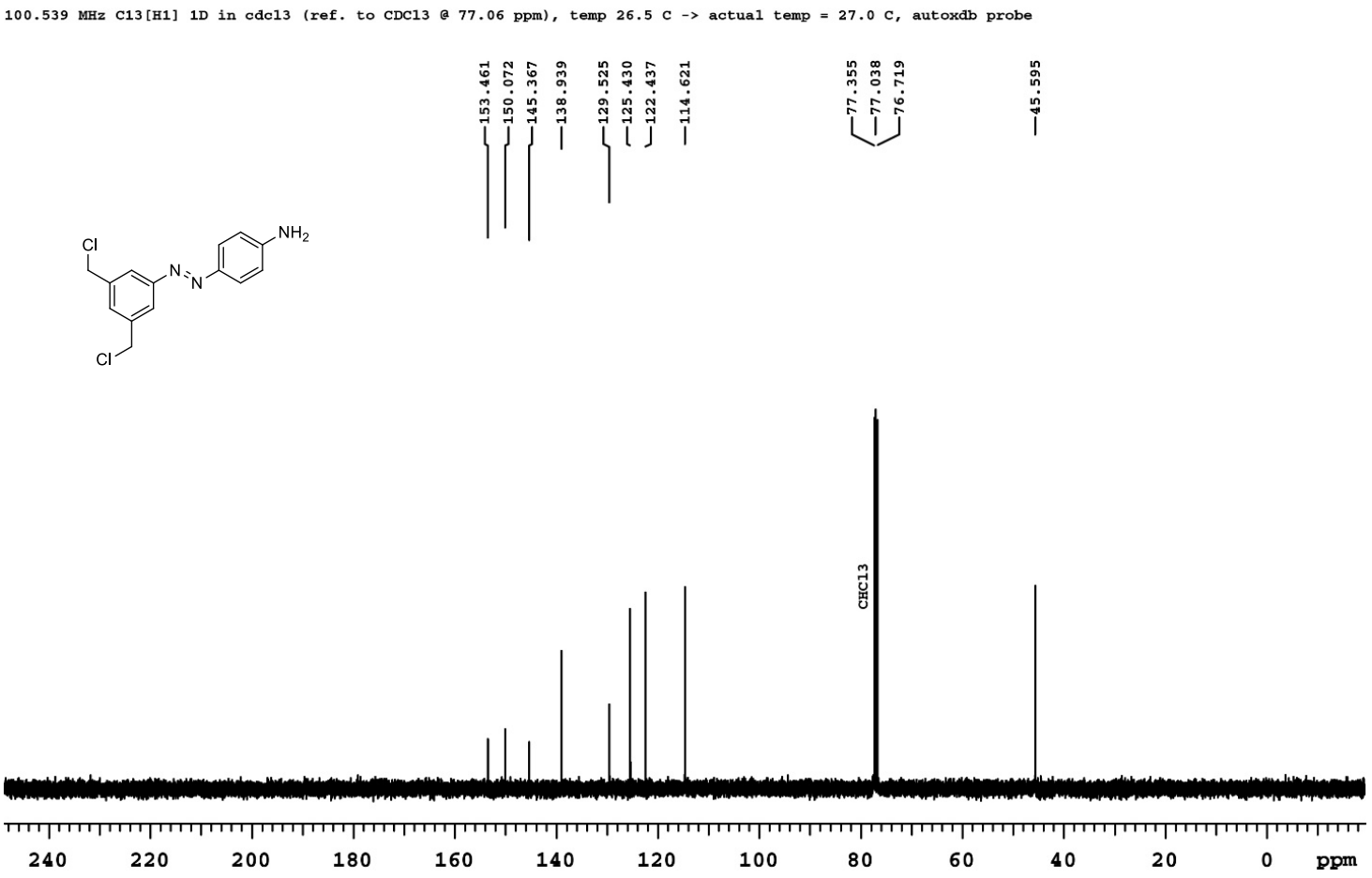

Cl
Cl
N
N
NH
O


100.587 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
159.138
153.494
151.235
144.606
138.900
130.224
129.259
128.835
125.650
122.321
114.230
112.464
77.398
77.081
76.761
55.346
47.358
45.641
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

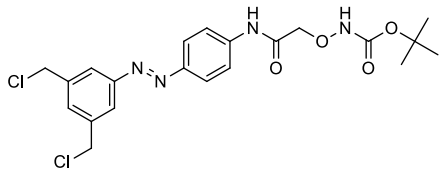
^{1}H-NMR of **2h**

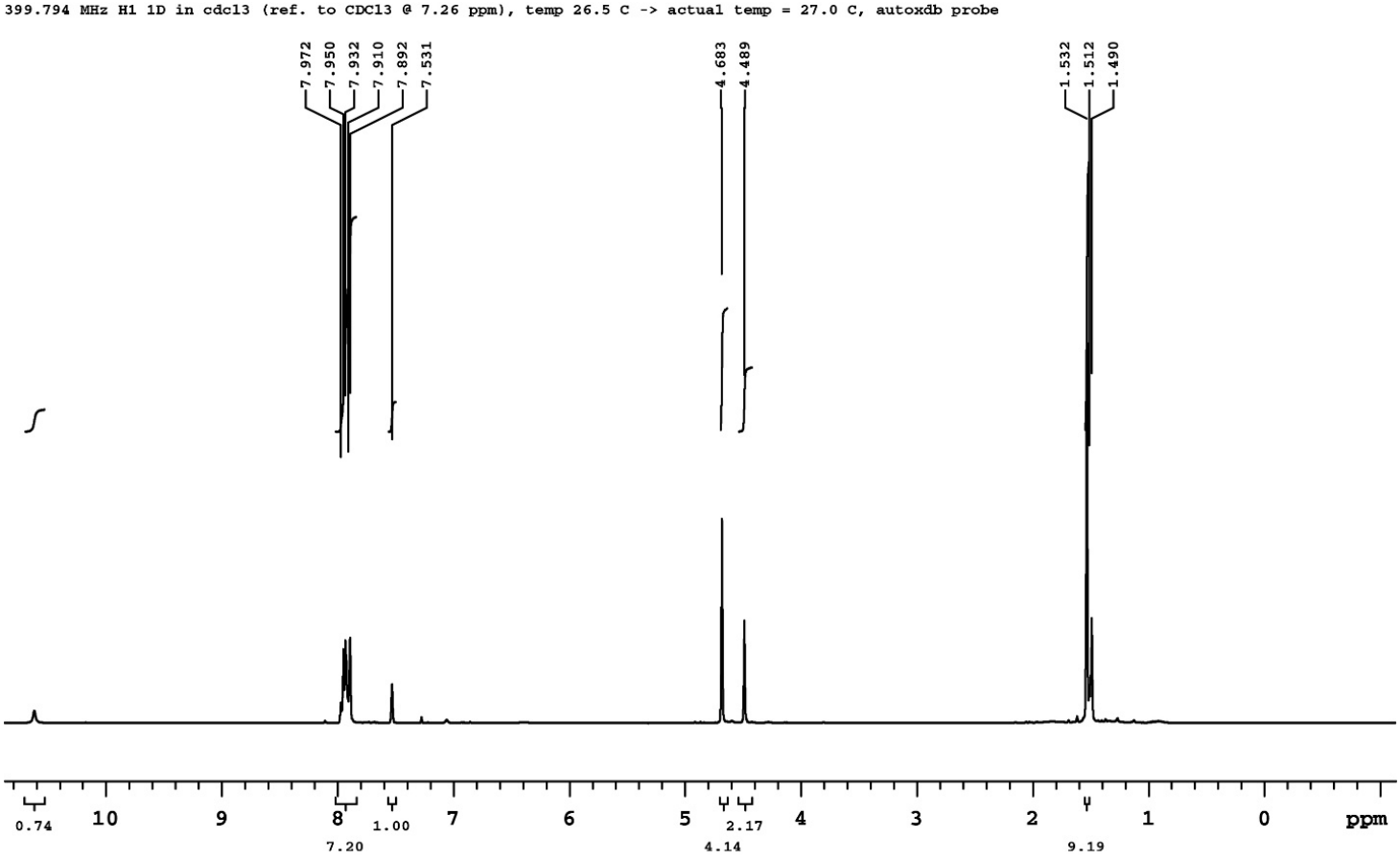

399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.843
7.822
7.485
7.276
6.768
6.746
4.678
CHCl3
Cl
Cl
N
N
NH2
10
9
8
7
6
5
4
3
2
1
0
ppm
4.08
1.01
2.09
4.13

^{13}C-NMR of **2h**

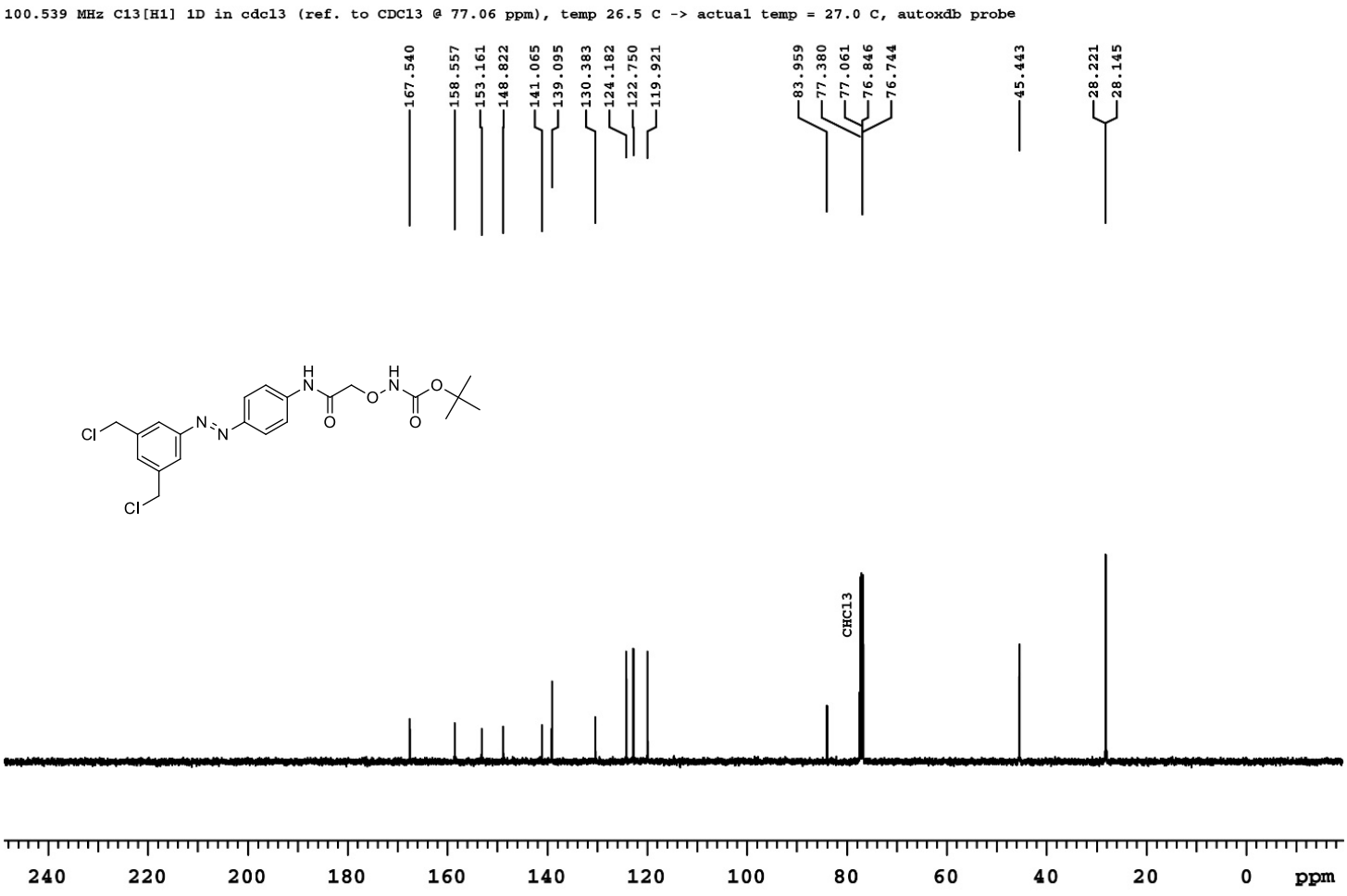

100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
153.461
150.072
145.367
138.939
129.525
125.430
122.437
114.621
77.355
77.038
76.719
45.595
CHC13
Cl
N
N
NH2
Cl
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

^{1}H-NMR of **2i**

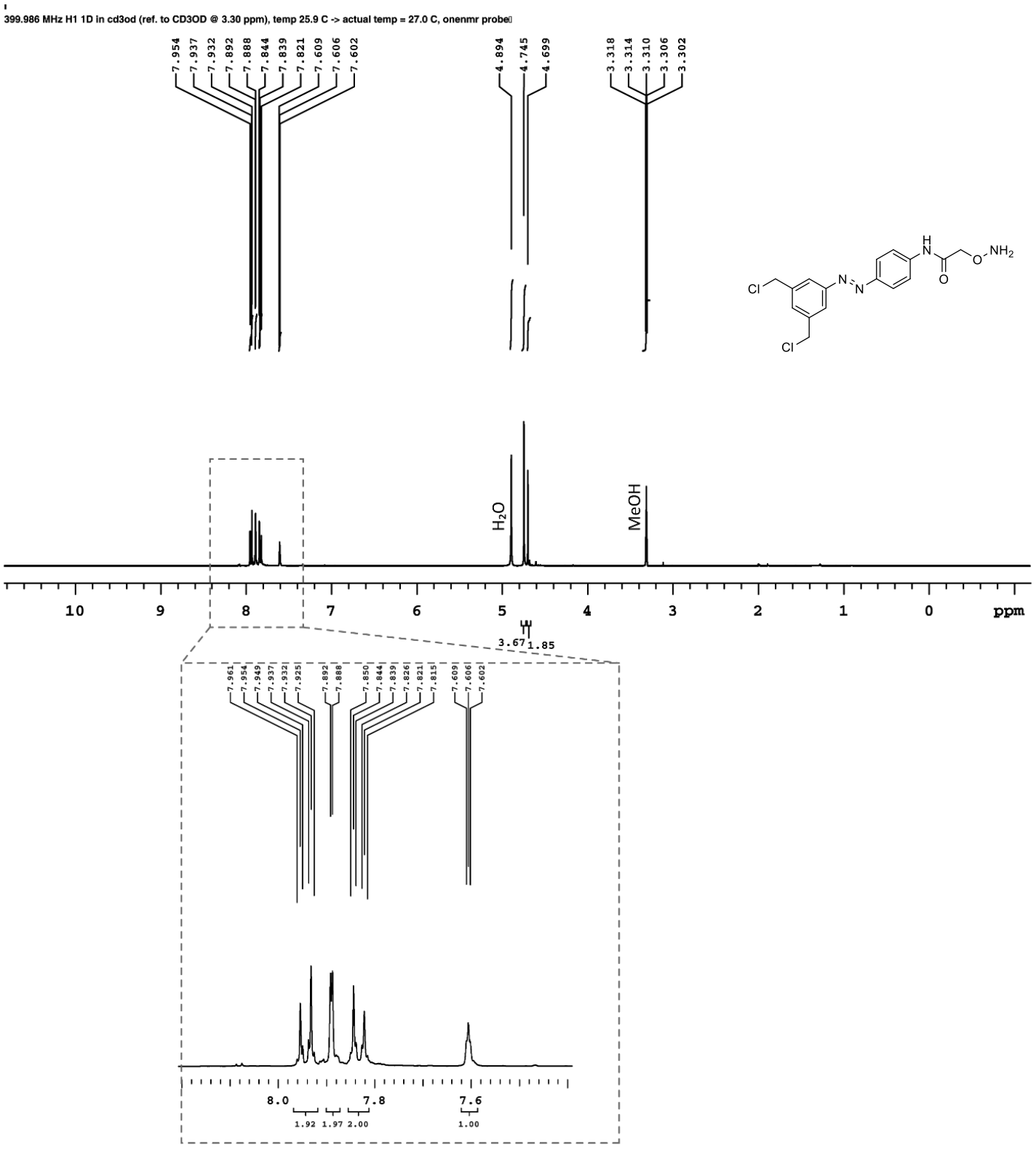



399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
7.972
7.950
7.932
7.910
7.892
7.531
4.683
4.489
1.532
1.512
1.490
0.74
1.00
2.17
7.20
4.14
9.19
ppm

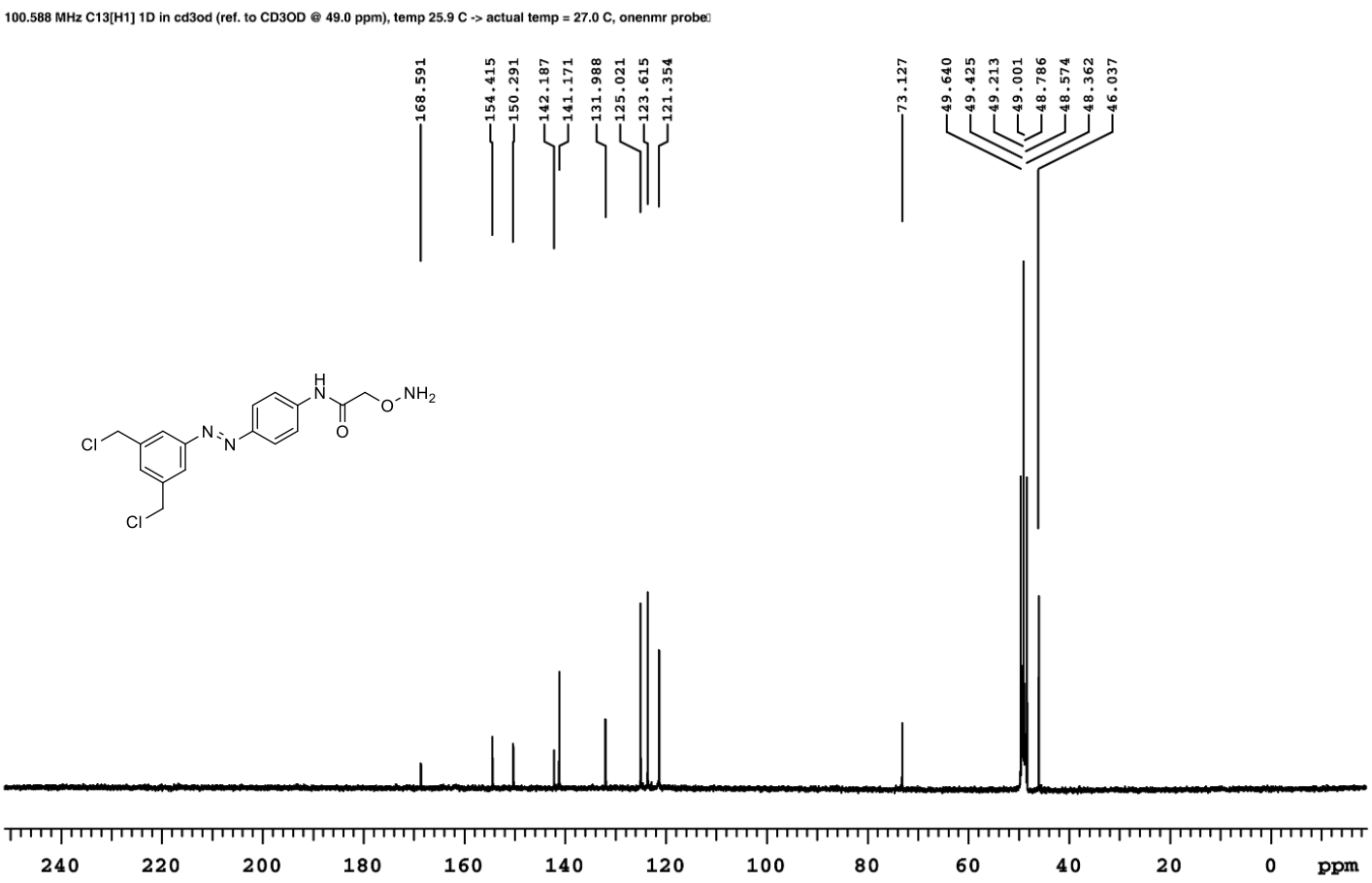
^{13}C-NMR of **2i**


100.539 MHz C13[H1] 1D in cdcl3 (ref. to CDC13 @ 77.06 ppm), temp 26.5 C -> actual temp = 27.0 C, autoxdb probe
167.540
158.557
153.161
148.822
141.065
139.095
130.383
124.182
122.750
119.921
83.959
77.380
77.061
76.846
76.744
45.443
28.221
28.145
CHC13
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

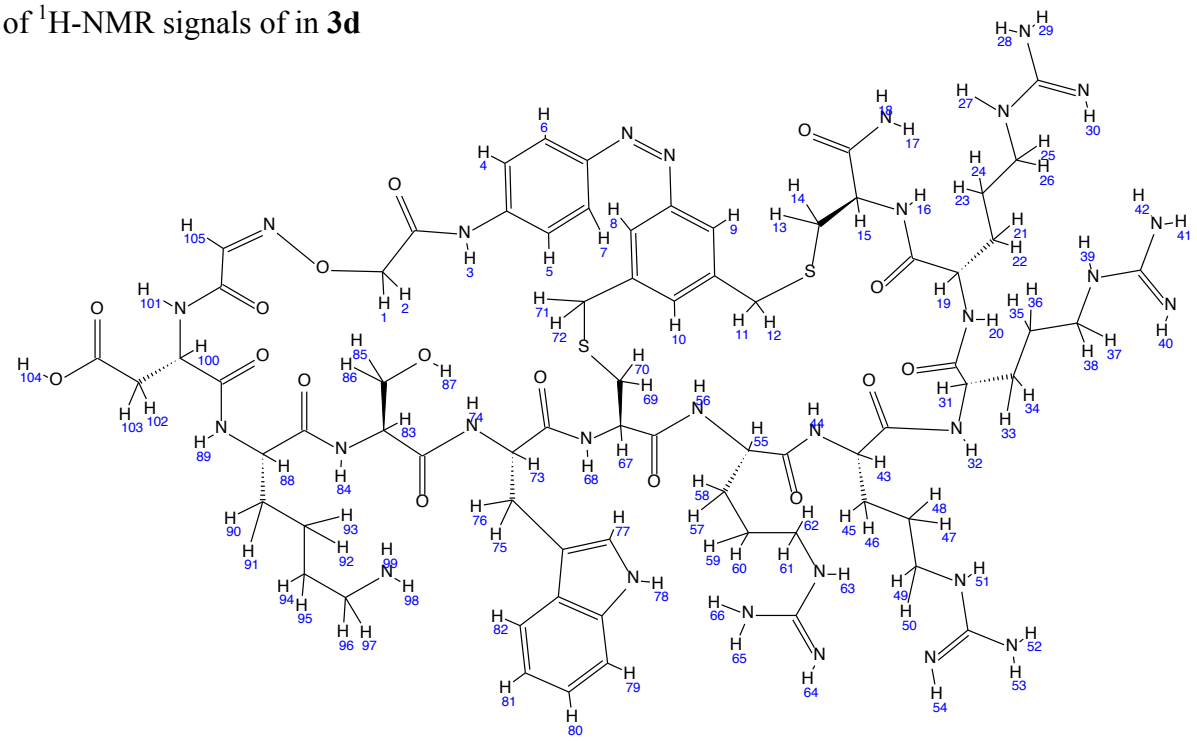
^{1}H-NMR of **2j**


399.986 MHz H1 1D in cd3od (ref. to CD3OD @ 3.30 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
7.954
7.937
7.932
7.892
7.888
7.844
7.839
7.821
7.609
7.606
7.602
4.894
4.745
4.699
3.318
3.314
3.310
3.306
3.302
H2O
MeOH
10
9
8
7
6
5
4
3
2
1
0
ppm
3.67
1.85
7.961
7.954
7.949
7.937
7.932
7.925
7.892
7.888
7.850
7.844
7.839
7.826
7.821
7.815
7.609
7.606
7.602
8.0
7.8
7.6
1.92
1.97
2.00
1.00
Cl
Cl
N
N
H
N
O
O
NH2

^{13}C-NMR of **2j**

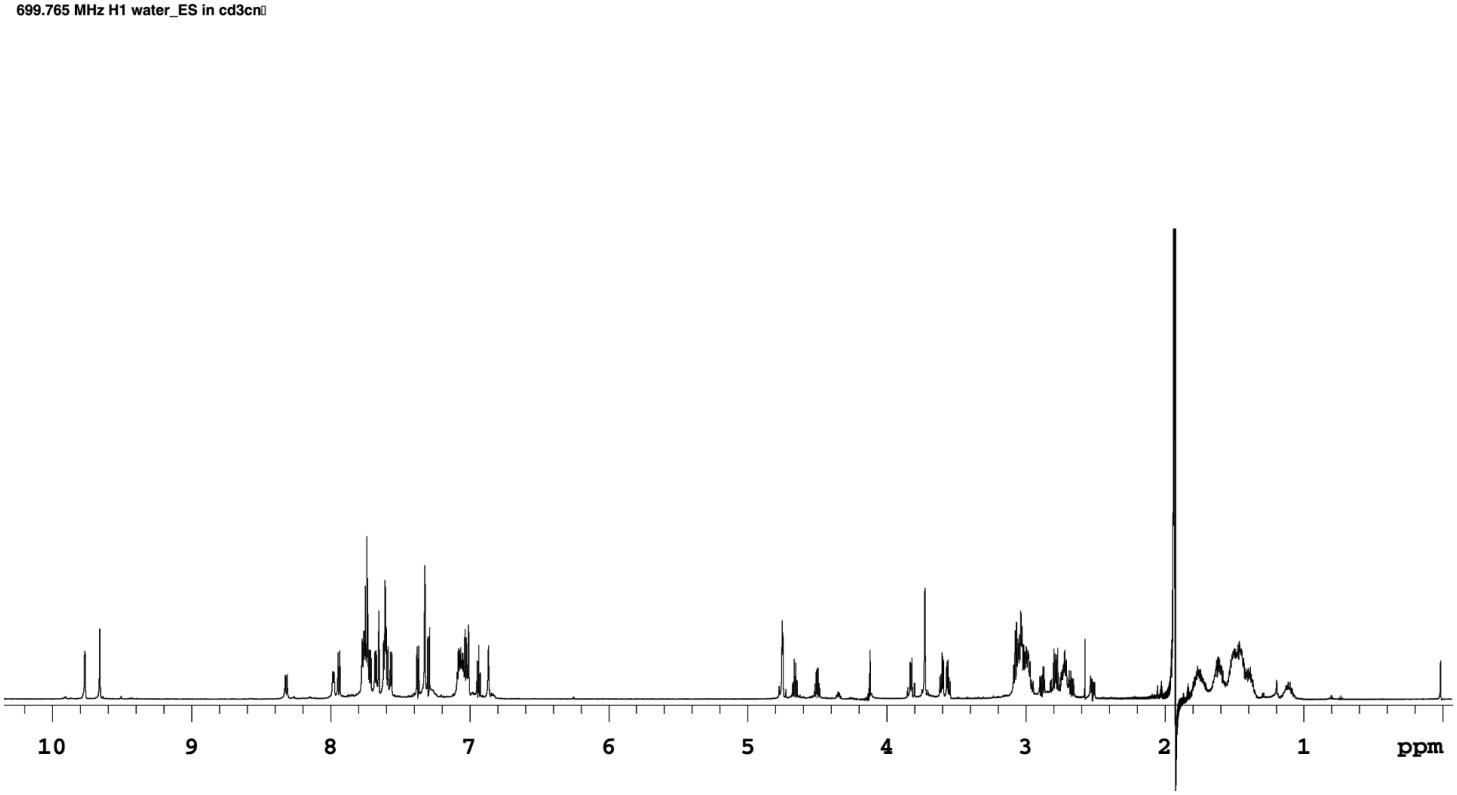

100.588 MHz C13[H1] 1D in cd3od (ref. to CD3OD @ 49.0 ppm), temp 25.9 C -> actual temp = 27.0 C, onenmr probe
168.591
154.415
150.291
142.187
141.171
131.988
125.021
123.615
121.354
73.127
49.640
49.425
49.213
49.001
48.786
48.574
48.362
46.037
Cl
Cl
N
N
H
N
O
O
NH2
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

List of ^{1}H-NMR signals of in **3d**

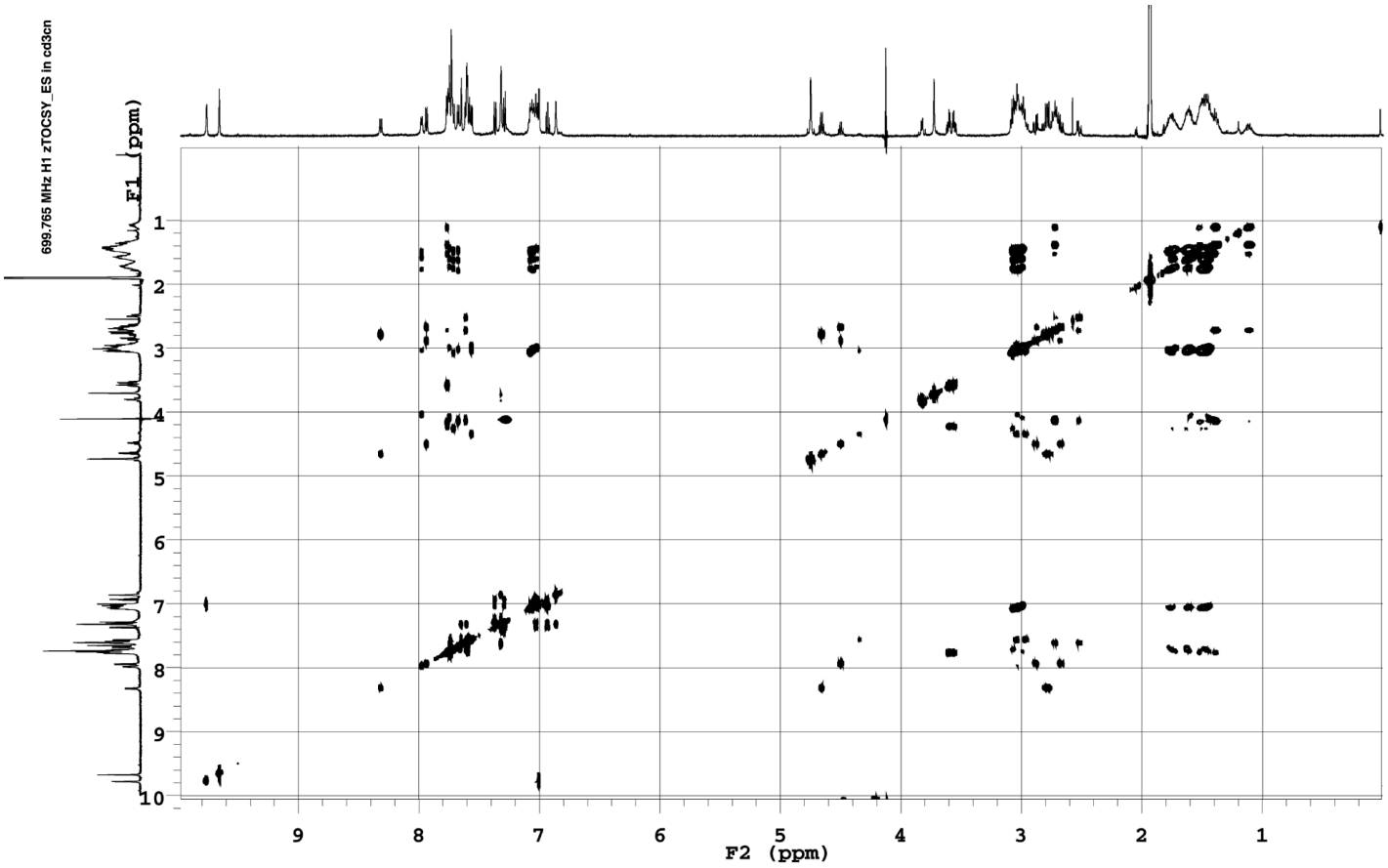


Residue	NH	ppm	H_α	ppm	H_β	ppm	H_γ	ppm	H_δ	ppm	H_ε	ppm
Asp	101	8.31	100	4.66	102,103	2.81	---	---	---	---	---	---
Lys	89	7.65	88	4.15	90,91	1.53, 1.38	92, 93	1.38	94, 95	2.71	96, 07	3.01
Ser	84	7.76	83	4.2	85,86	3.58	---	---	---	---	---	---
Trp	74	7.56	73	4.34	75,76	3.05, 2.96	---	---	---	---	---	---
Cys	68	7.93	67	4.5	69,70	2.87, 2.66	---	---	---	---	---	---
Arg	56	7.74	55	4.26	57,58	1.7, 1.63	59, 60	1.47	61, 62	3.06	63-66	7.02
Arg	44	7.77	43	4.08	45,46	1.74, 1.61	47, 48	1.46	49, 50	3.01	51-54	7.06
Arg	32	7.68	31	4.12	33,34	1.78, 1.63	35, 36	1.48	37, 38	3.03	39-42	7.05
Arg	20	7.98	19	4.05	21,22	1.78, 1.57	23, 24	1.48	25, 26	3.03	27-30	7.07
Cys	16	7.62	15	4.15	13,14	2.72, 2.53	---	---	---	---	---	---
Trp (Ar)	77	9.79	78	7.01	79	7.32	80	7.03	81	6.93	82	7.38
HADCAz	1,2	4.76	71, 72	3.83	11,12	3.71	---	---	---	---	---	---
	3	9.67	---	---	---	---	---	---	---	---	---	---
	4,5	7.59	6,7	7.73	---	---	---	---	---	---	---	---
	8	7.65	9	7.61	10	7.32	---	---	---	---	---	---
	105	6.86	---	---	---	---	---	---	---	---	---	---

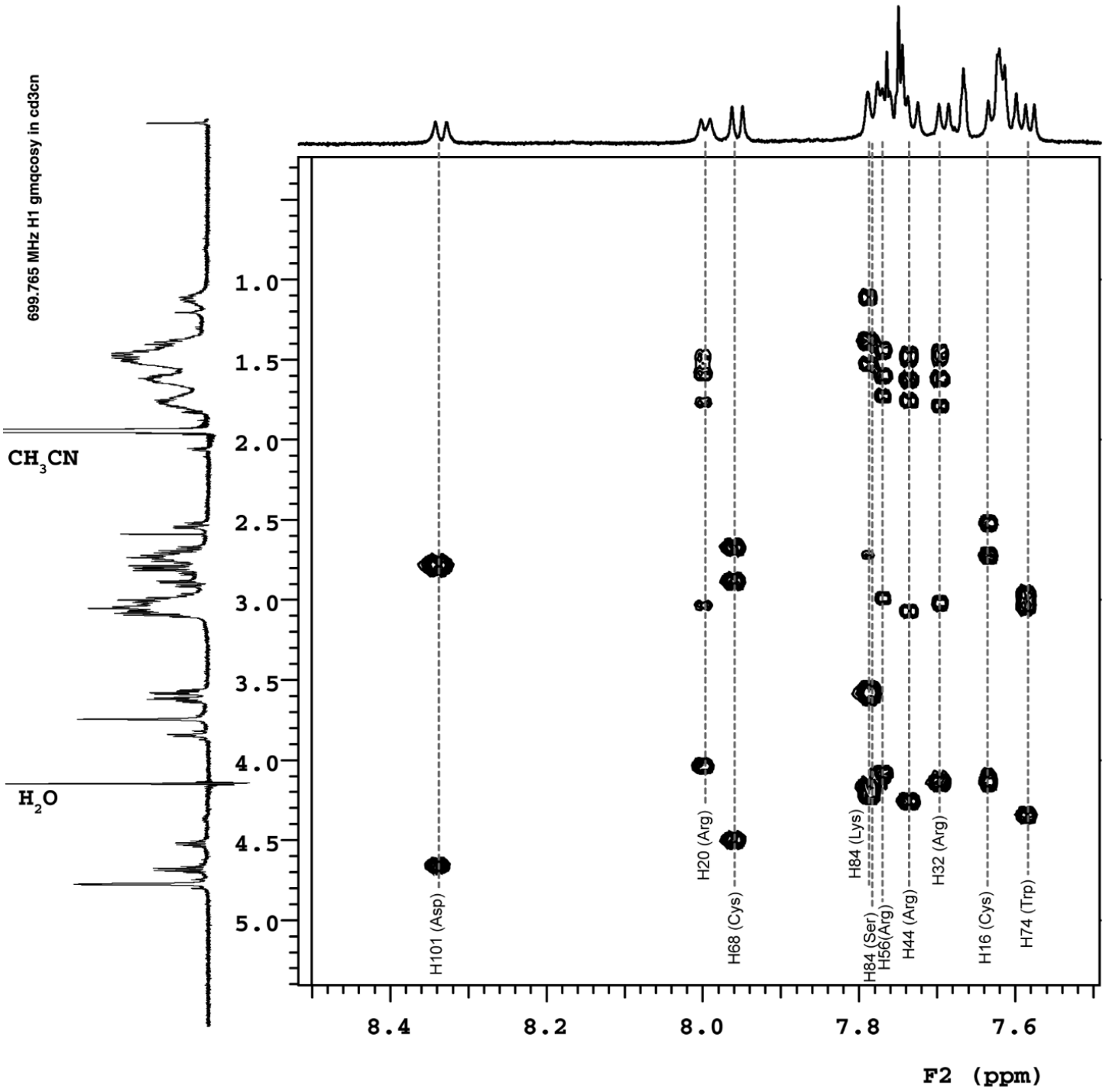
^{1}H-NMR of **3d**



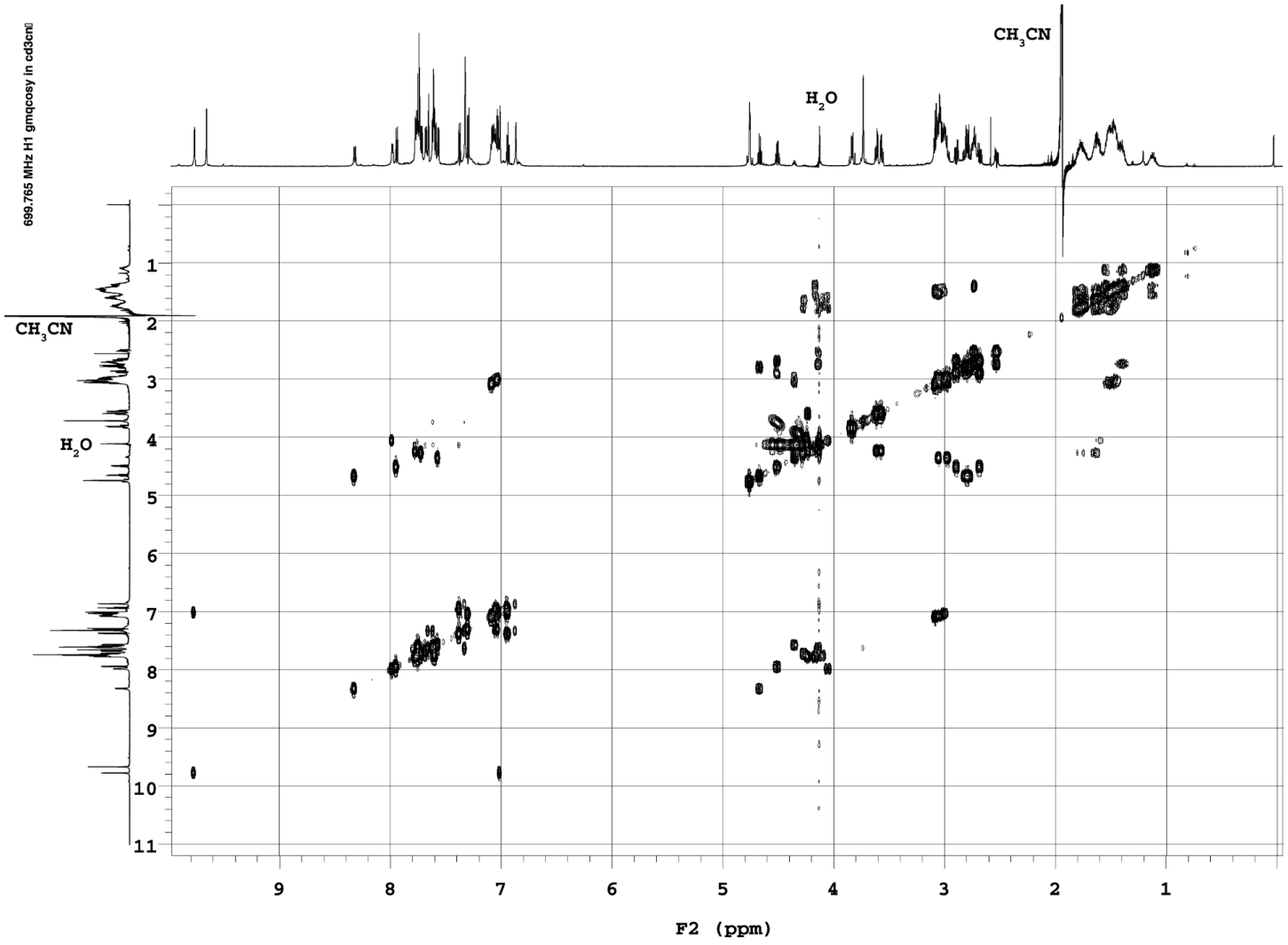
TOCSY-NMR of **3d**



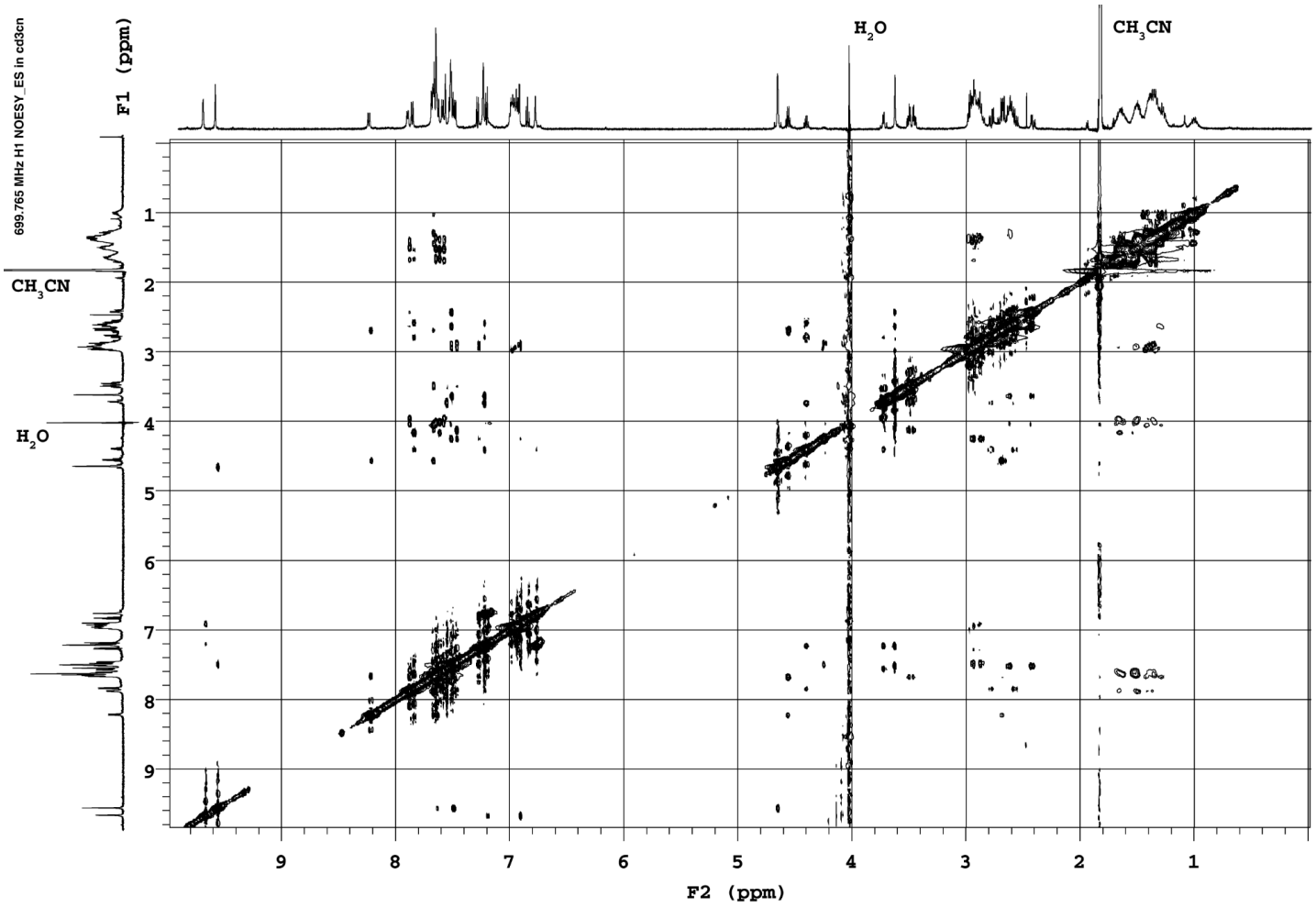
TOCSY-NMR of **3d** (expanded)



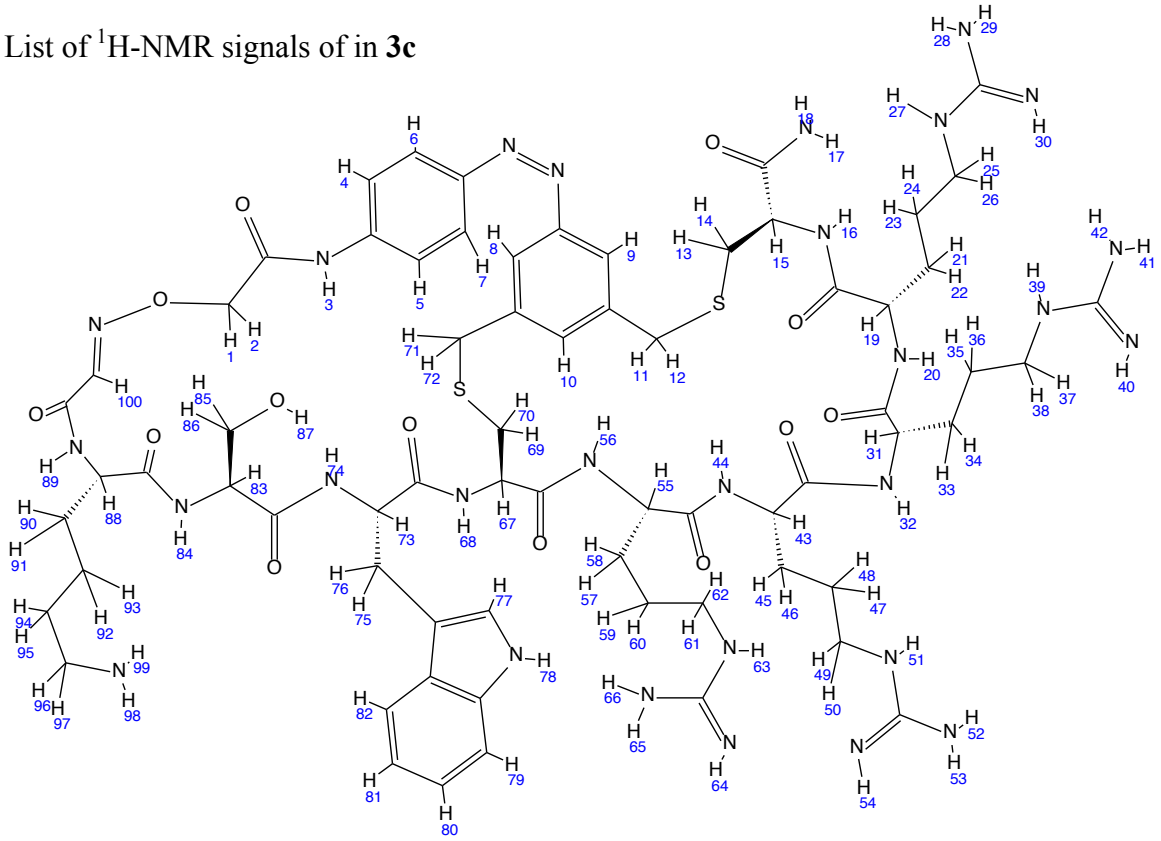
gmCOSY-NMR of **3d**



NOESY-NMR of **3d**

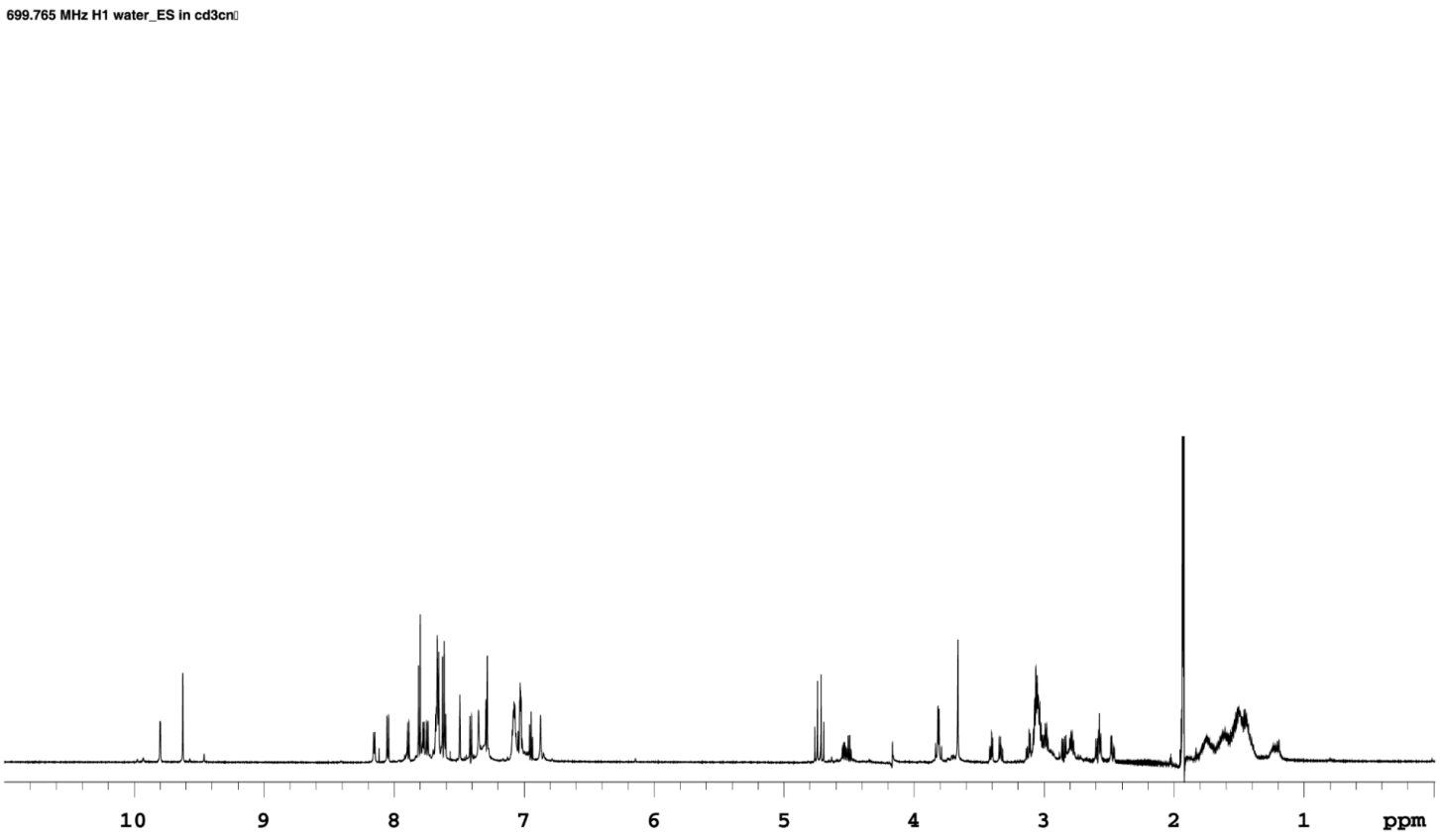


List of ^{1}H-NMR signals of in **3c**

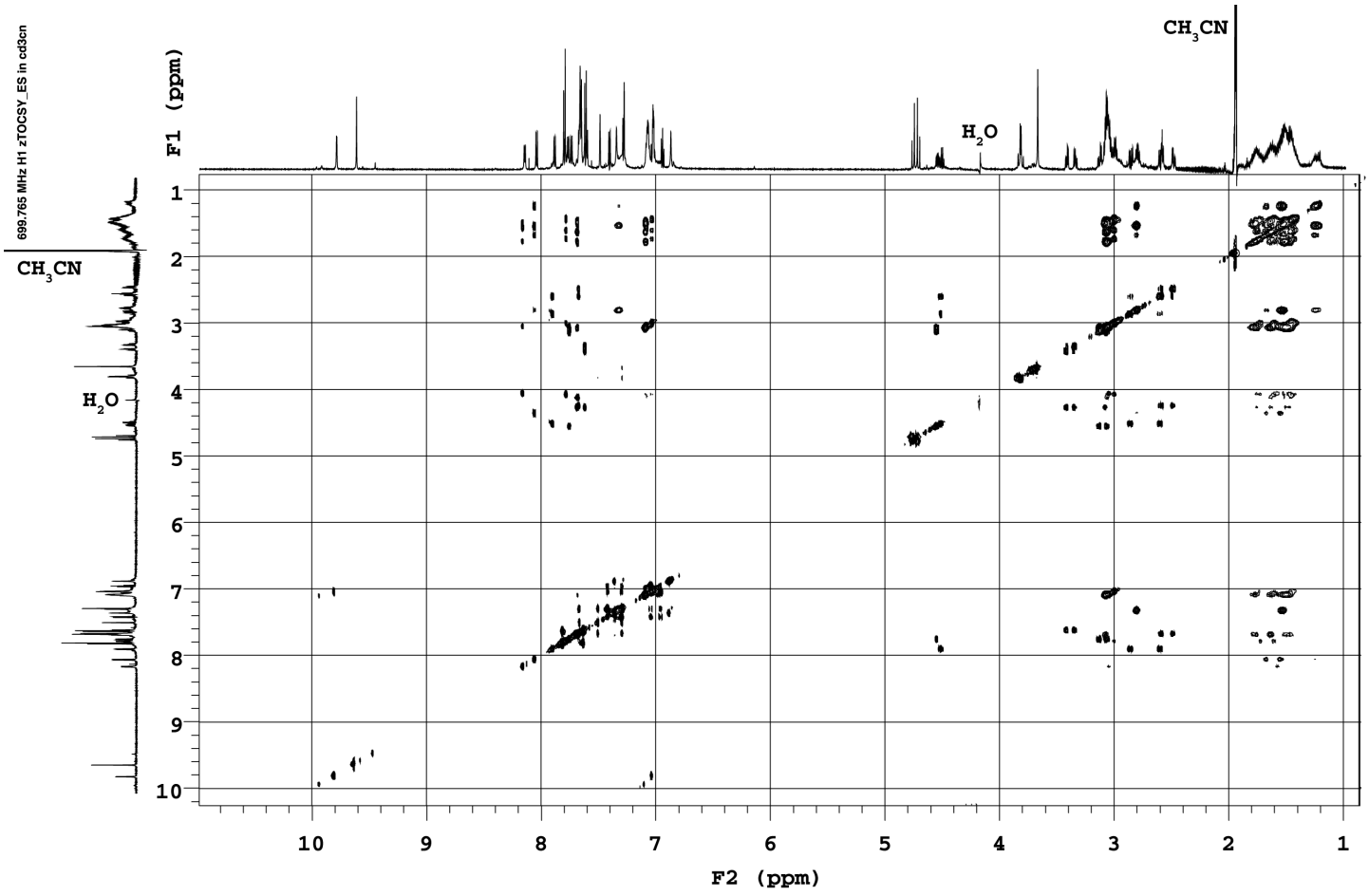


Residue	NH	ppm	H_{α}	ppm	H_{β}	ppm	H_{γ}	ppm	H_{δ}	ppm	H_{ε}	ppm
Lys	89	8.05	88	4.35	90, 91	1.67, 1.54	92, 93	1.24	94, 95	1.54	96, 97	2.82
Ser	84	7.62	83	4.27	85, 86	3.34, 3.43	---	---	---	---	---	---
Trp	74	7.75	73	4.56	75, 76	3.13, 3.08	---	---	---	---	---	---
Cys	68	7.90	67	4.52	69, 70	2.88, 2.60	---	---	---	---	---	
Arg	56	7.78	55	4.08	57, 58	1.74, 1.62	59, 60	1.43	61, 62	3.01	63-66	7.09
Arg	44	7.66	43	4.12	45, 46	1.80, 1.63	47, 48	1.49	49, 50	3.07	51-54	7.04
Arg	32	7.66	31	4.25	33, 34	1.80, 1.63	35, 36	1.49	37, 38	3.07	39-42	7.08
Arg	20	7.98	19	4.06	21, 22	1.77, 1.57	23, 24	1.50	25, 26	3.07	27-30	7.09
Cys	16	8.13	15	4.24	13, 14	2.59, 2.49	---	---	---	---	---	---
Trp (Ar)	77	9.81	78	7.05	79	7.3	80	7.04	81	6.96	82	7.43
HADCAz	1, 2	4.72	71, 72	3.68	11, 12	3.83	---	---	---	---	---	---
	3	9.63	---	---	---	---	---	---	---	---	---	---
	4,5	7.62	6, 7	7.80	---	---	---	---	---	---	---	
	8	7.50	9	7.66	10	7.28	---	---	---	---	---	---
	100	6.88	---	---	---	---	---	---	---	---	---	---

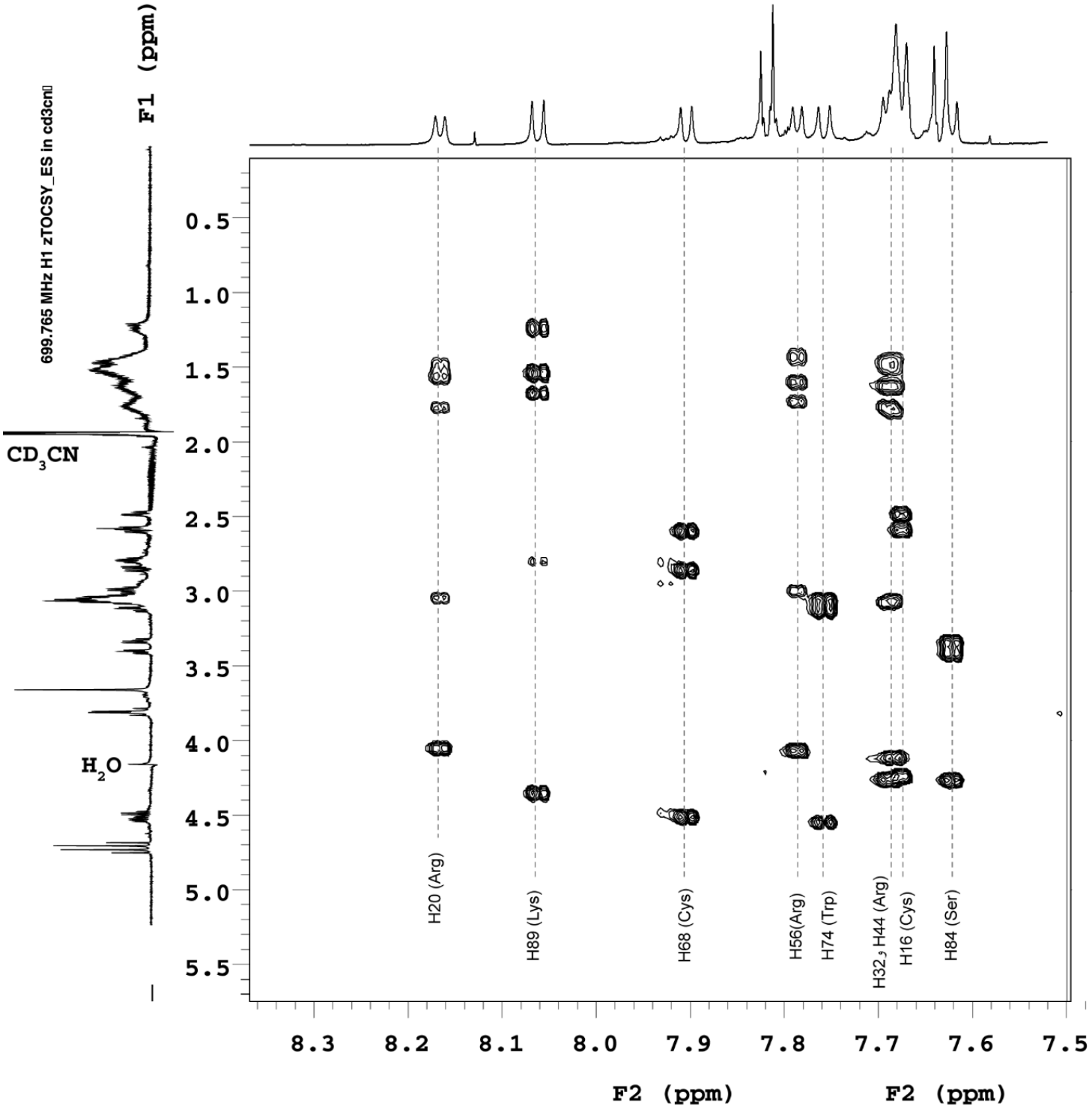
^{1}H-NMR of **3c**



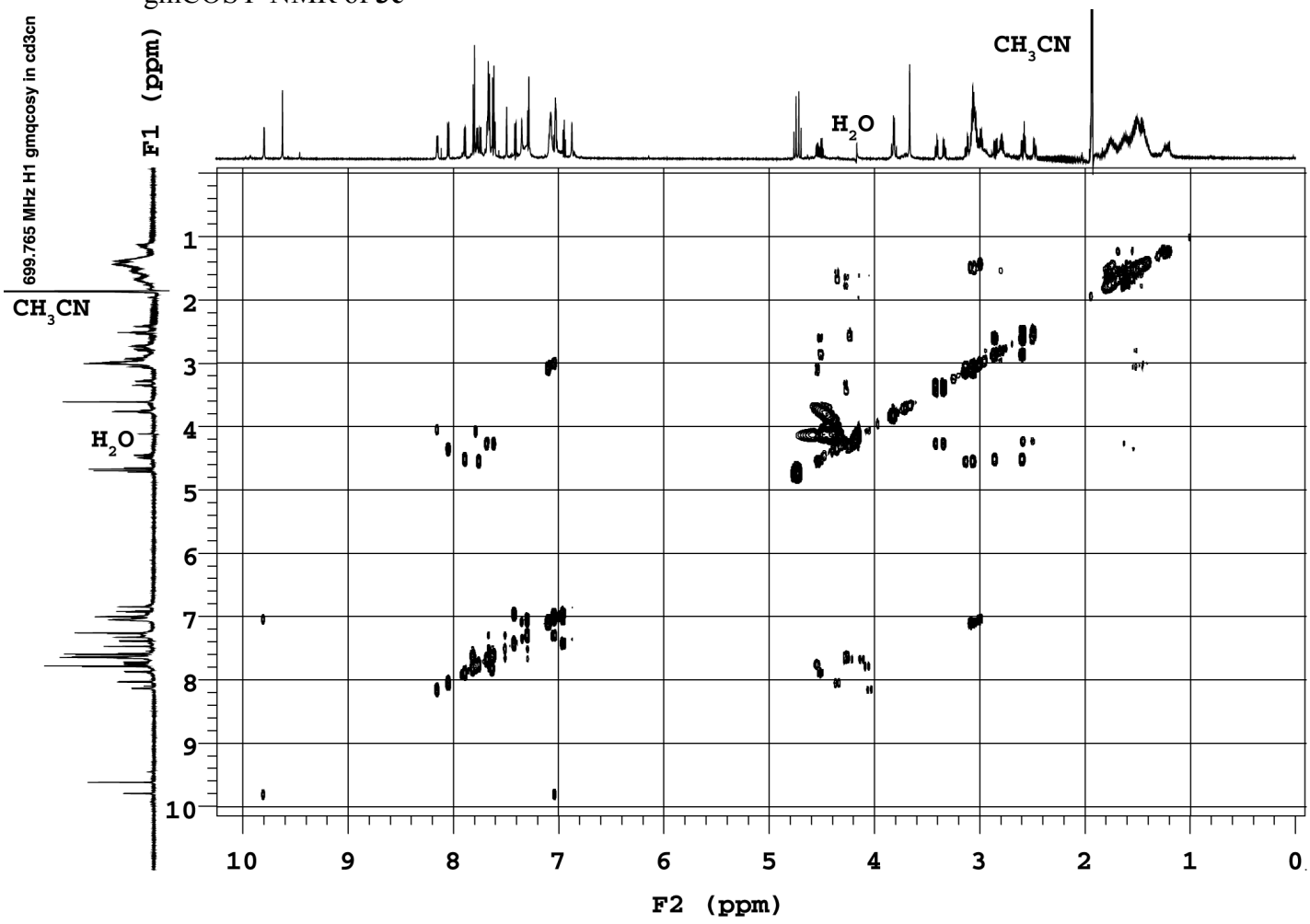
TOCSY-NMR of **3c**



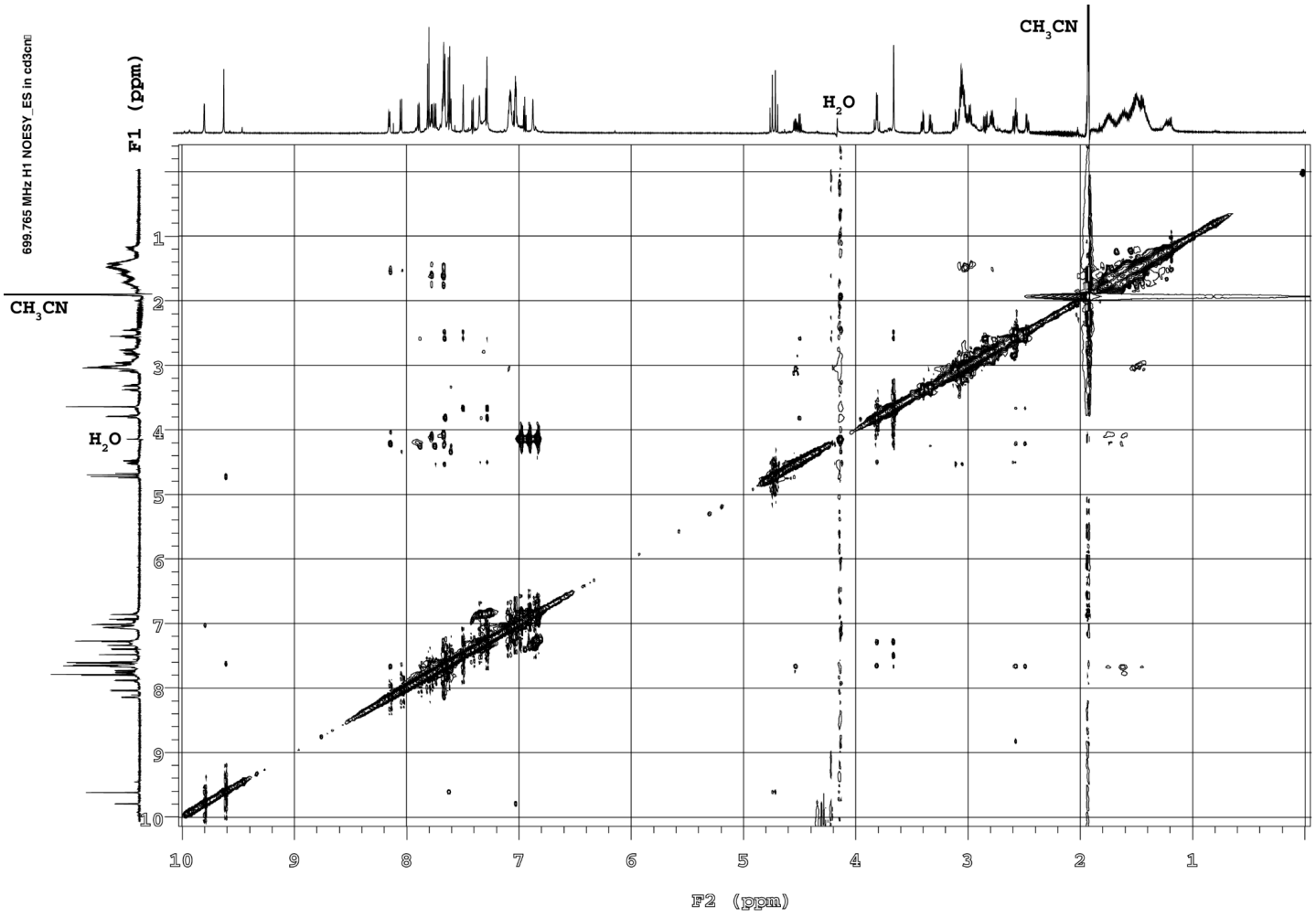
TOCSY-NMR of **3c** (expanded)



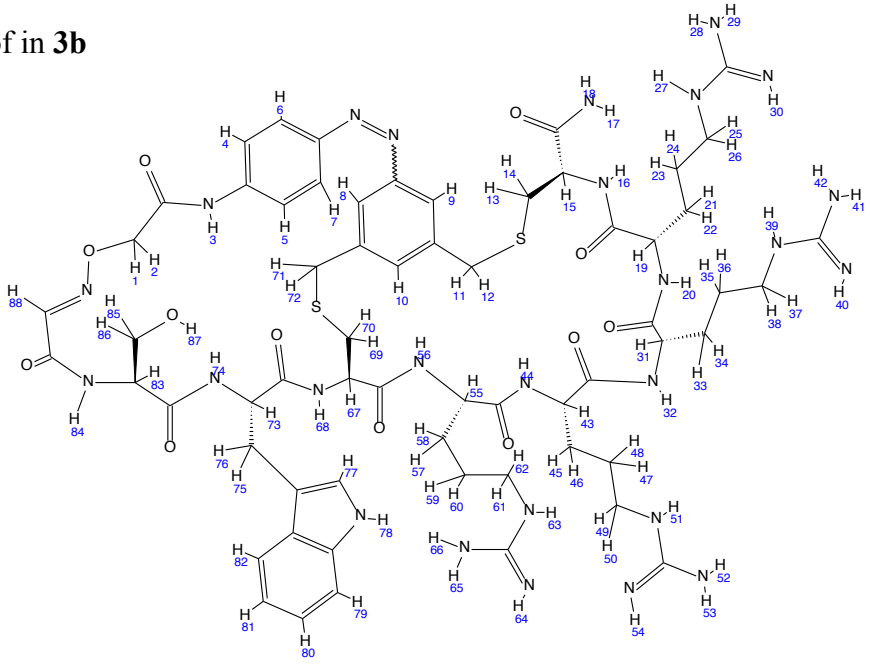
gmCOSY-NMR of **3c**



NOESY-NMR of **3c**

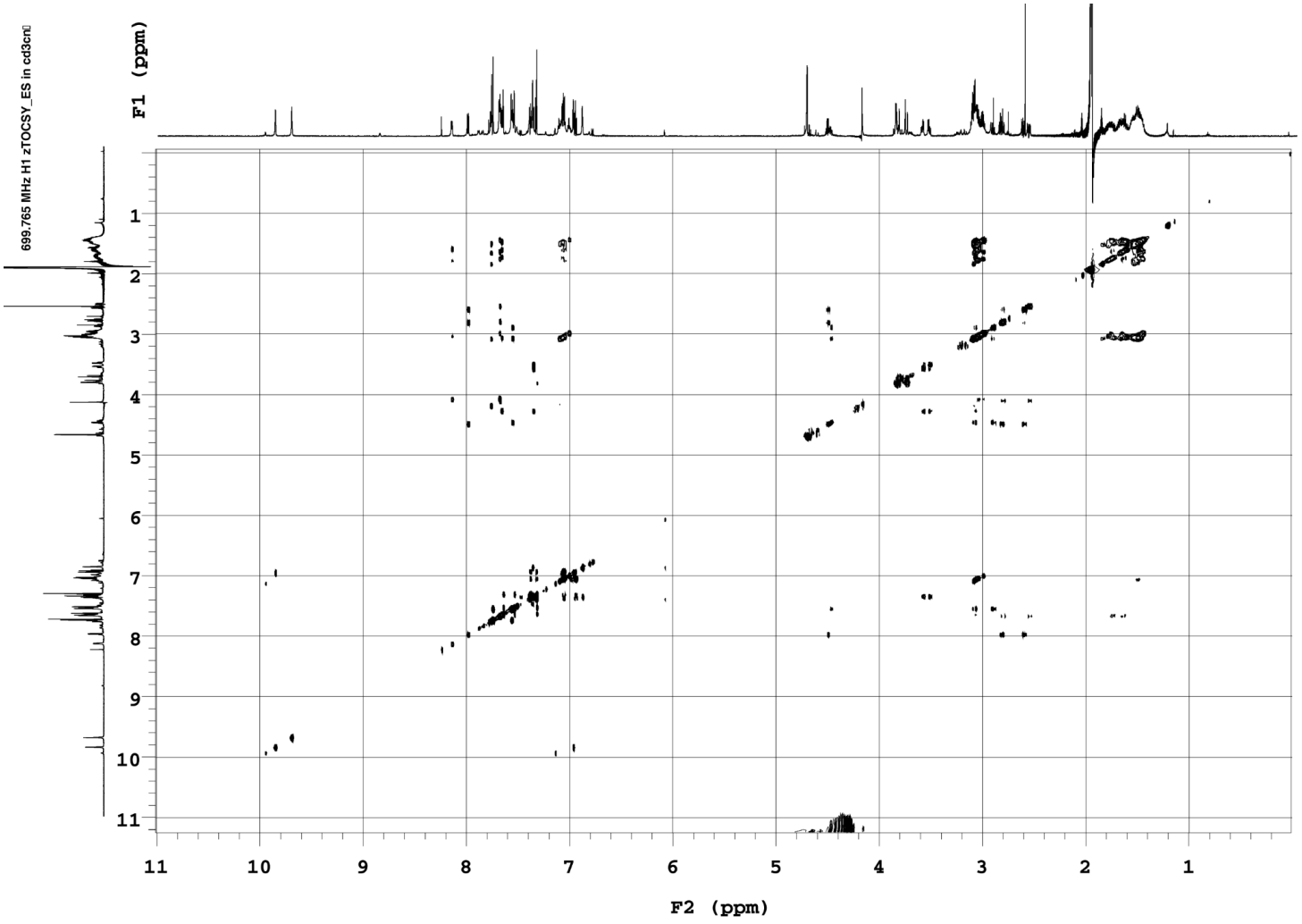


List of ^{1}H-NMR signals of in **3b**

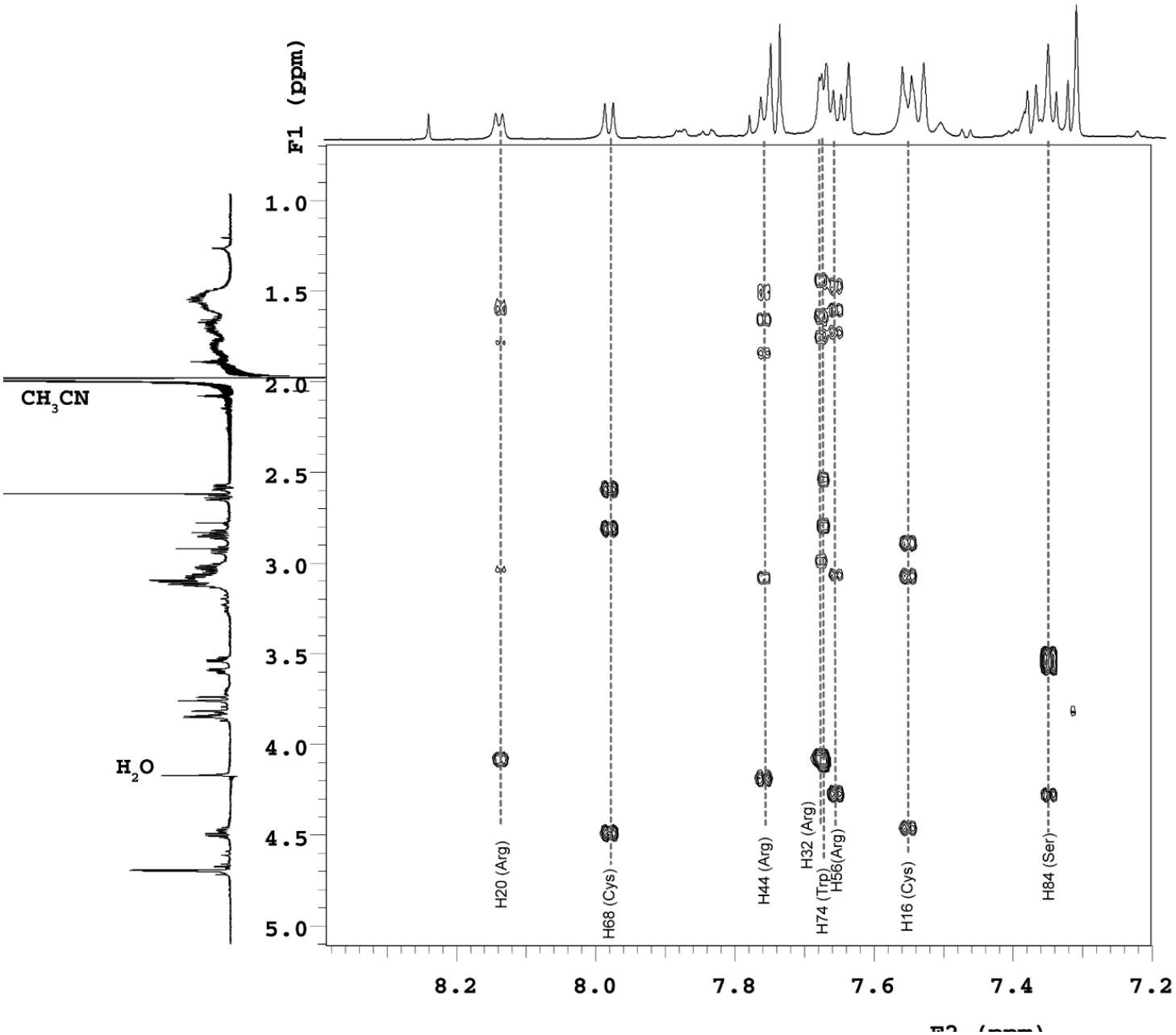


Residue	**NH**	ppm	**H_α**	ppm	**H_β**	ppm	**H_γ**	ppm	**H_δ**	ppm	**H_ε**	ppm
Ser	84	7.34	83	4.03	85, 86	3.22	---	---	---	---	---	---
Trp	74	7.55	73	4.67	75, 76	2.87, 3.06	---	---	---	---	---	---
Cys	68	7.98	67	4.50	69, 70	2.82, 2.58	---	---	---	---	---	---
Arg	56	7.65	55	4.26	57, 58	1.75, 1.60	59, 60	1.48	61, 62	3.06	63-66	7.10
Arg	44	7.75	43	4.19	45, 46	2.05, 1.67	47, 48	1.51, 1.52	49, 50	3.08	51-54	7.07
Arg	32	7.67	31	4.10	33, 34	1.76, 1.64	35, 36	1.45	37, 38	2.97	39-42	7.00
Arg	20	8.14	19	4.08	21, 22	1.78, 1.59	23, 24	1.59, 1.50	25, 26	3.05	27-30	7.06
Cys	16	7.68	15	4.09	13, 14	3.09, 2.89	---	---	---	---	---	---
Trp (Ar)	77	9.85	78	6.97	79	7.32	80	7.07	81	6.93	82	7.38
HADCAz	1, 2	4.17	71, 72	3.82	11, 12	3.74	---	---	---	---	---	---
	3	9.69	---	---	---	---	---	---	---	---	---	---
	4,5	7.65	6,7	7.75	---	---	---	---	---	---	---	---
	8	7.64	9	7.53	10	7.32	---	---	---	---	---	---
	88	6.87	---	---	---	---	---	---	---	---	---	---

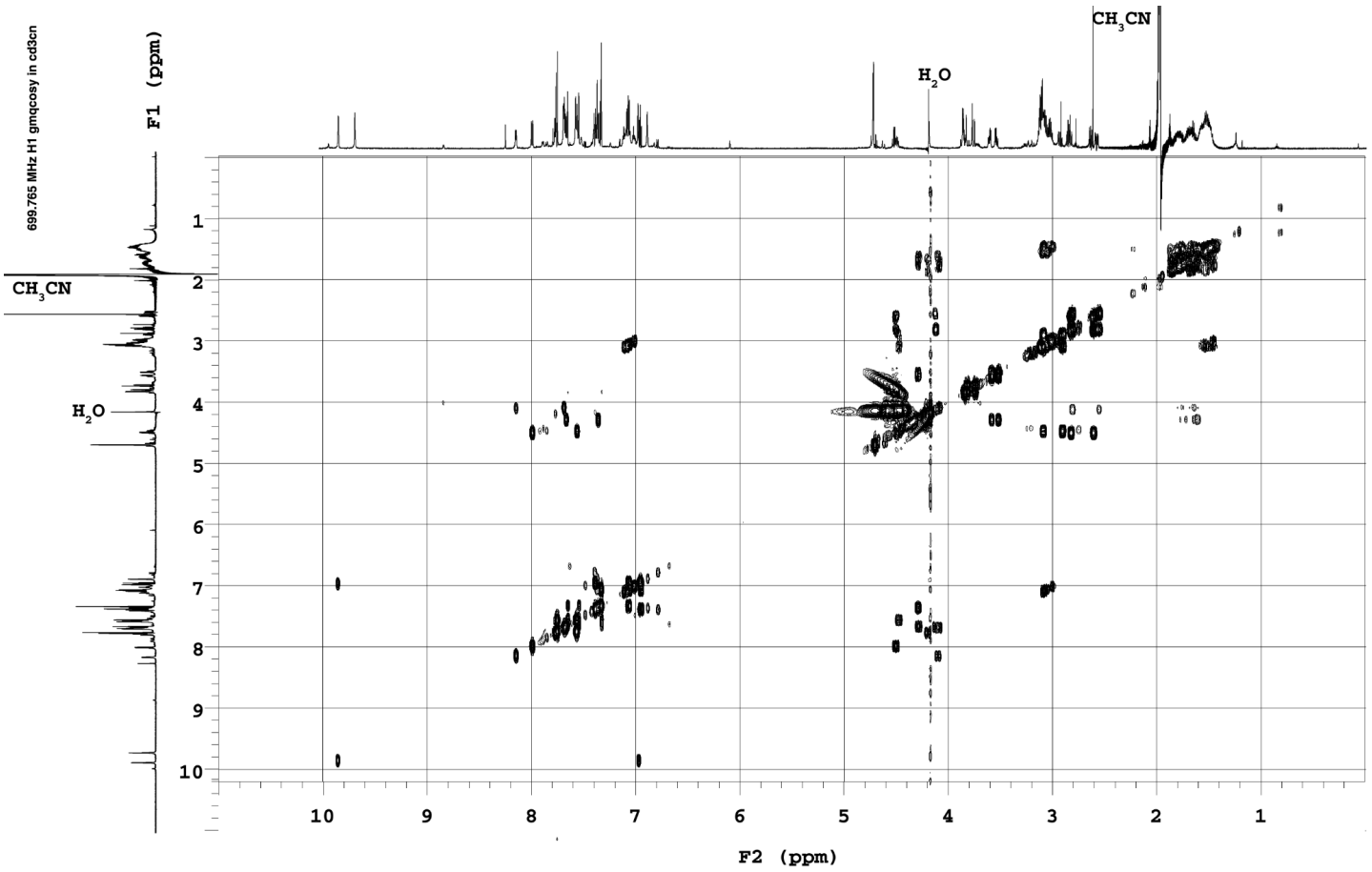
TOCSY-NMR of **3b**



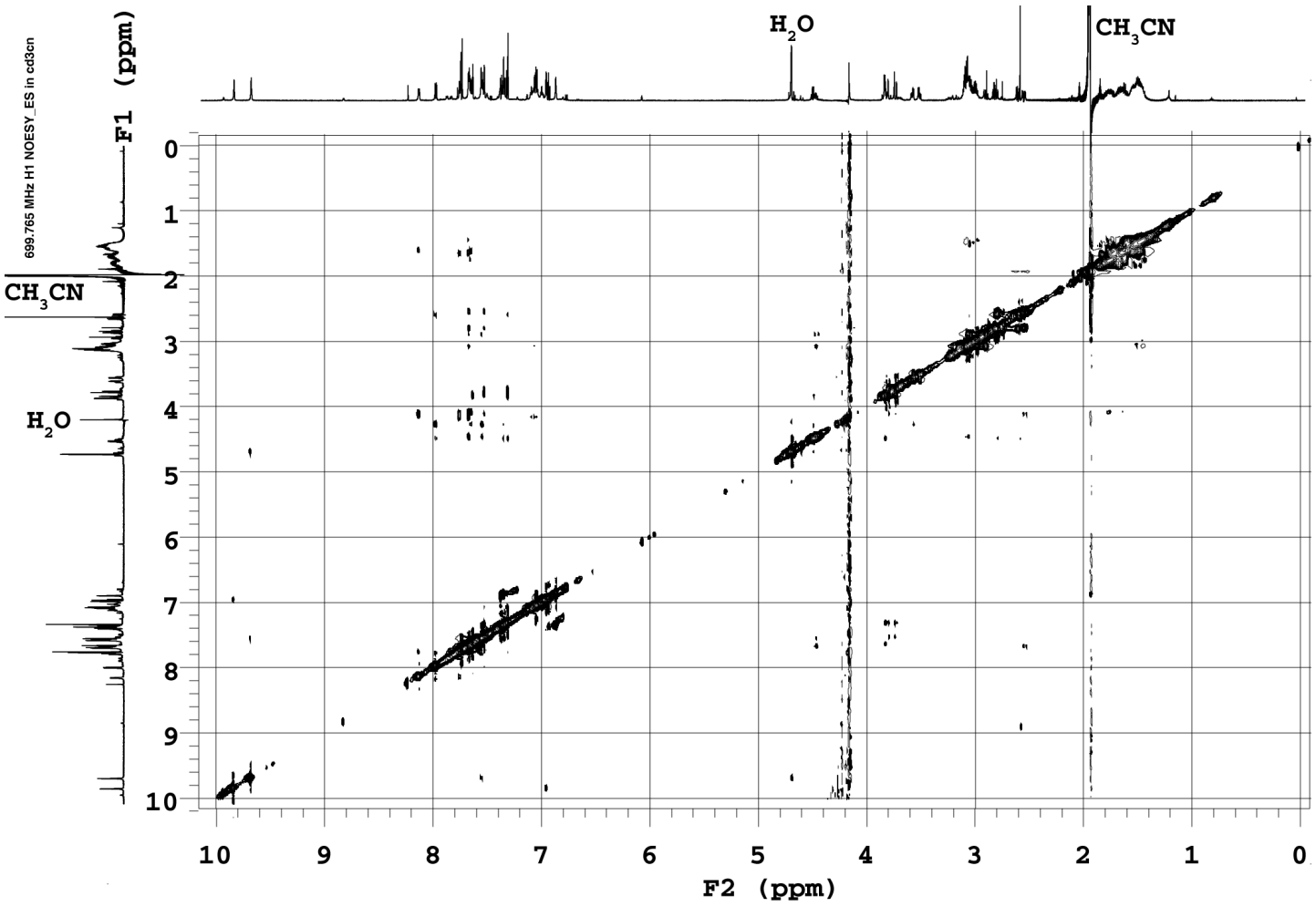
TOCSY-NMR of **3b** (expanded)



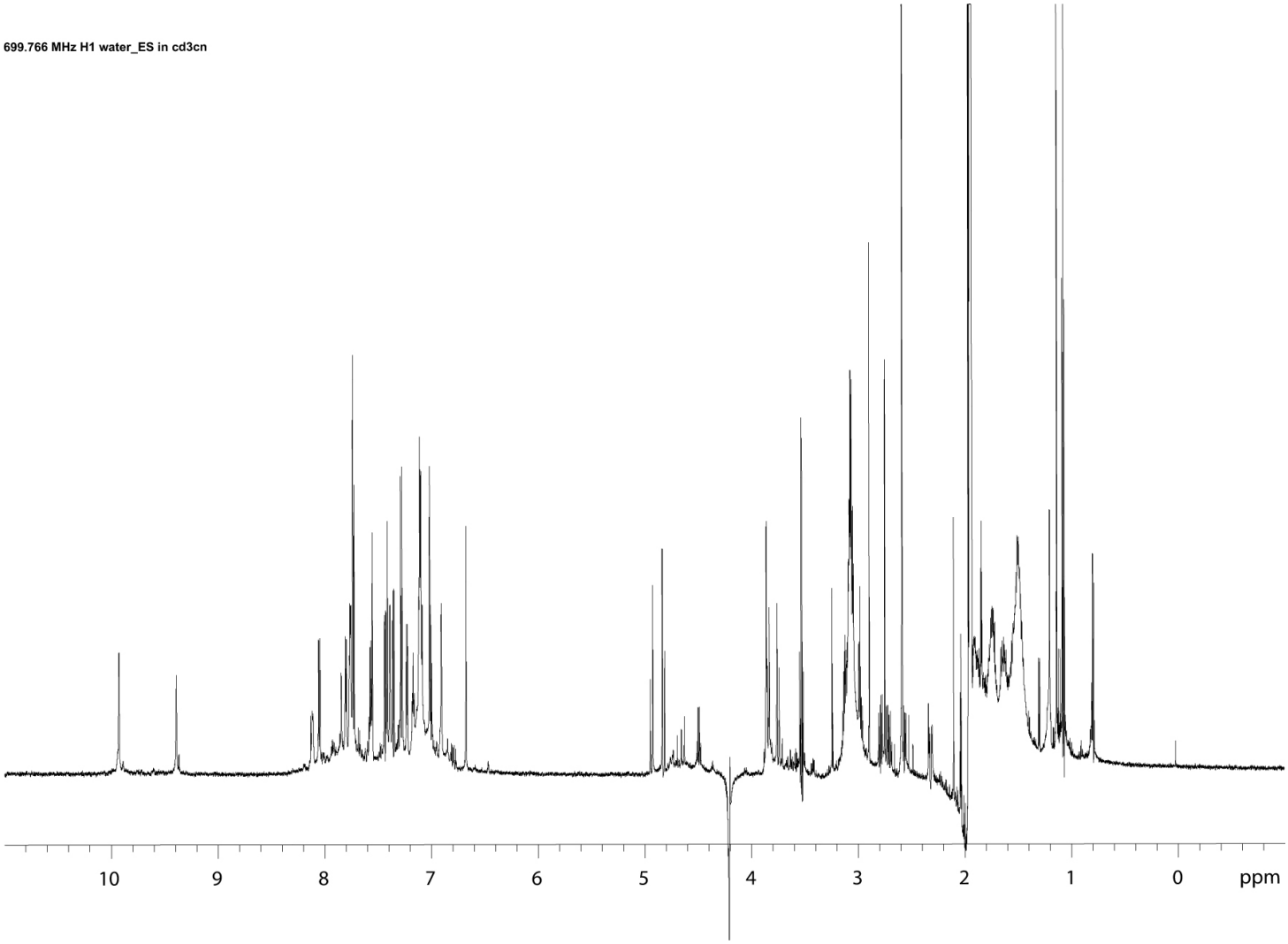
COSY-NMR of **3b**



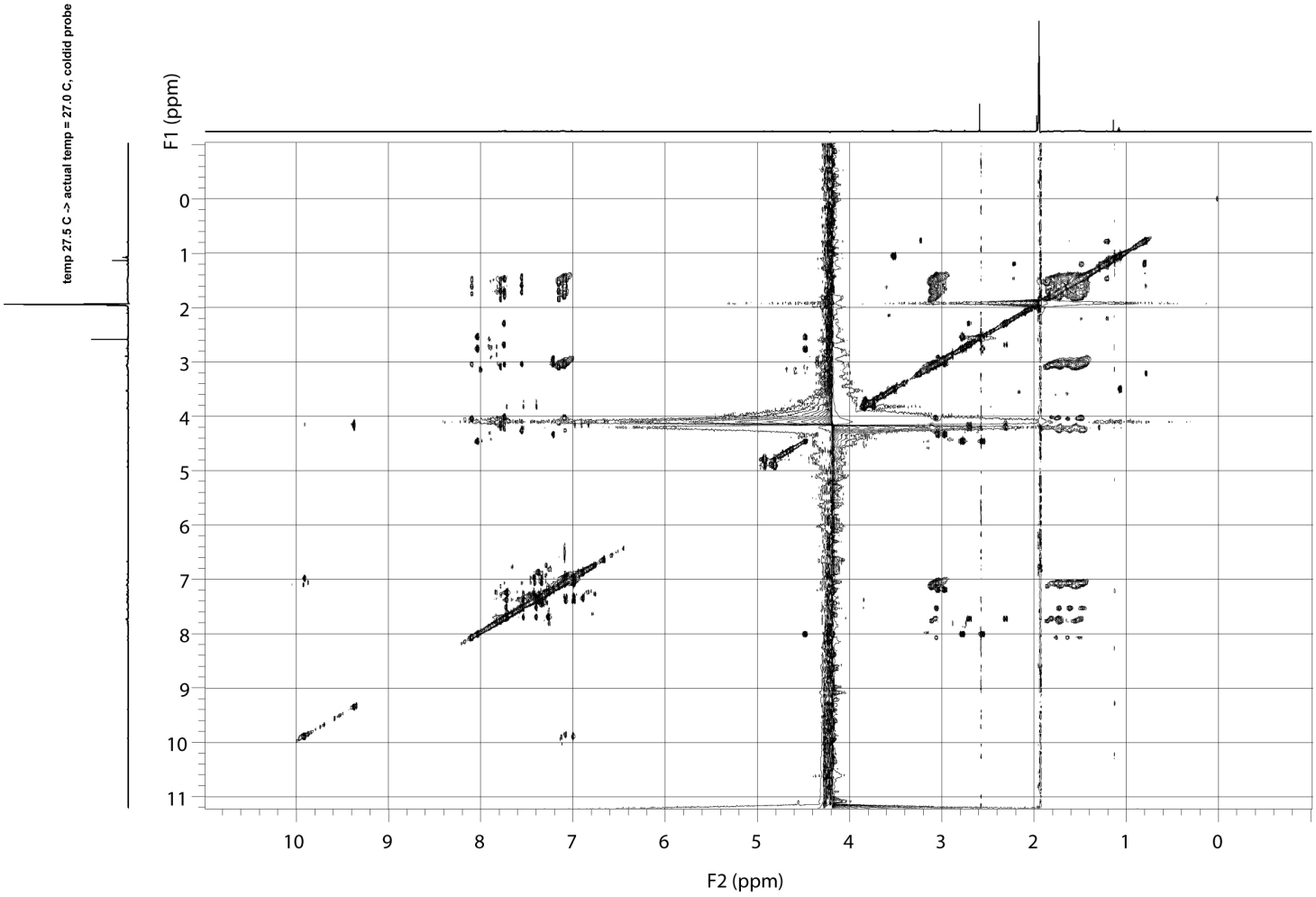
NOESY-NMR of **3b**



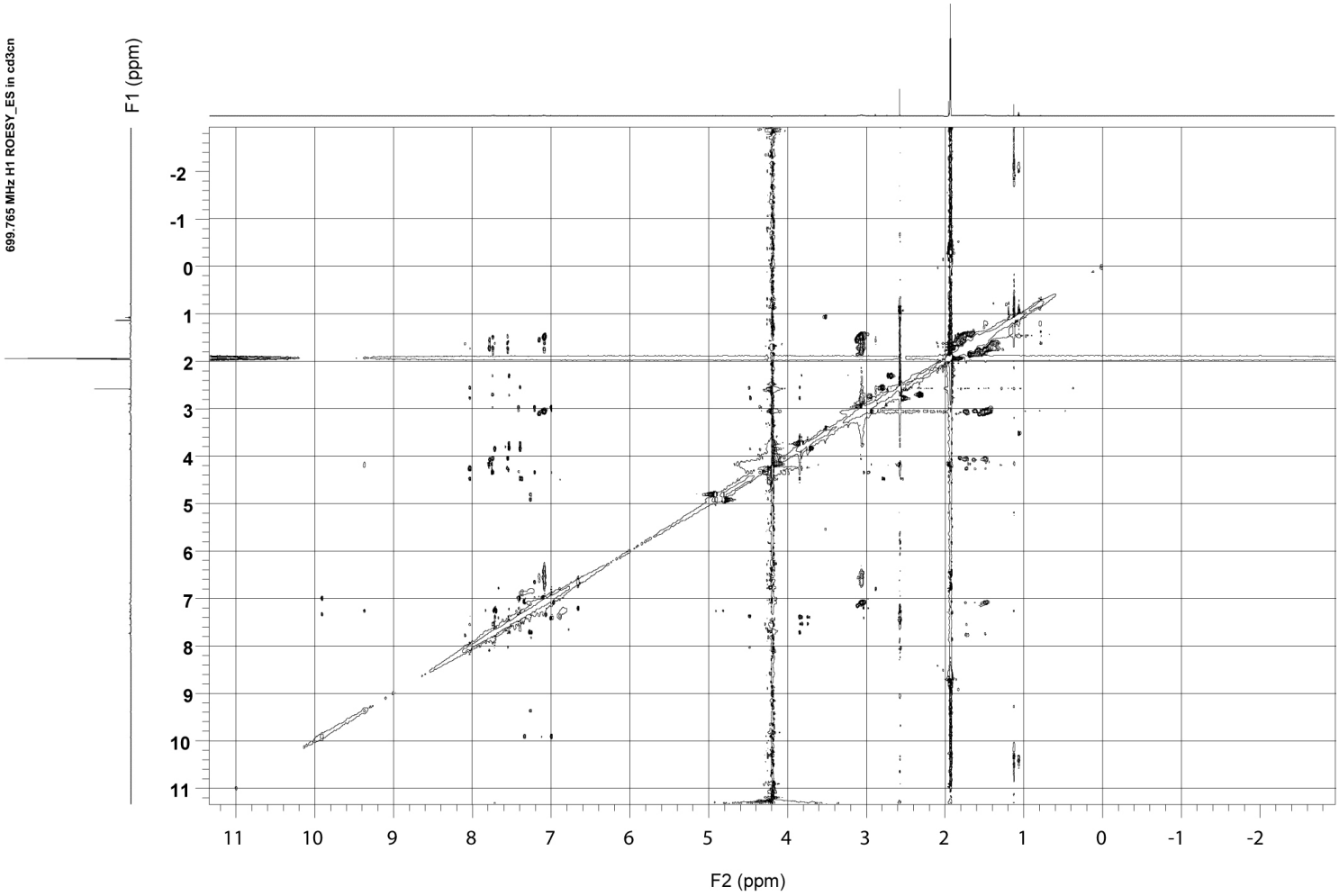
^{1}H-NMR of **3a**

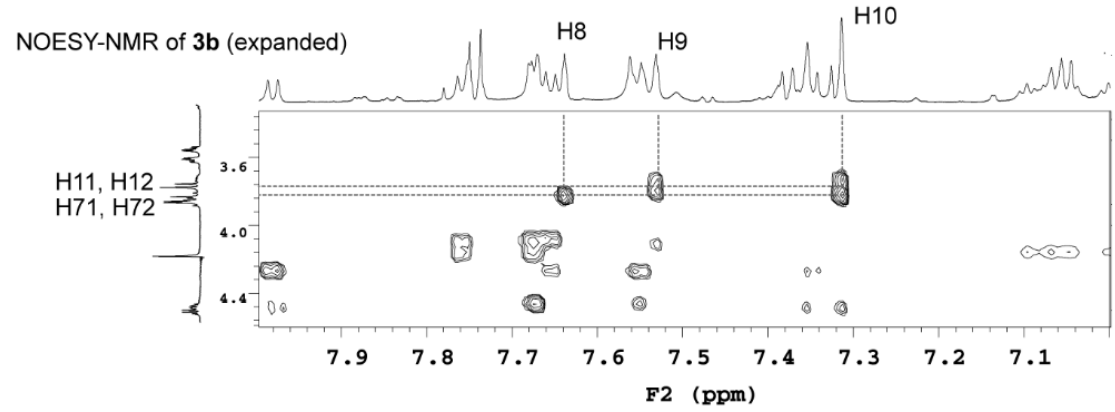


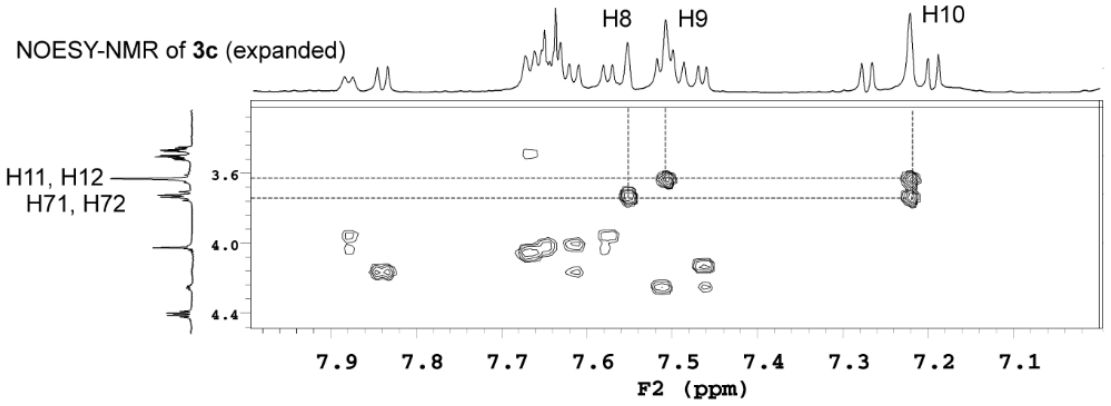
TOCSY-NMR of **3a**

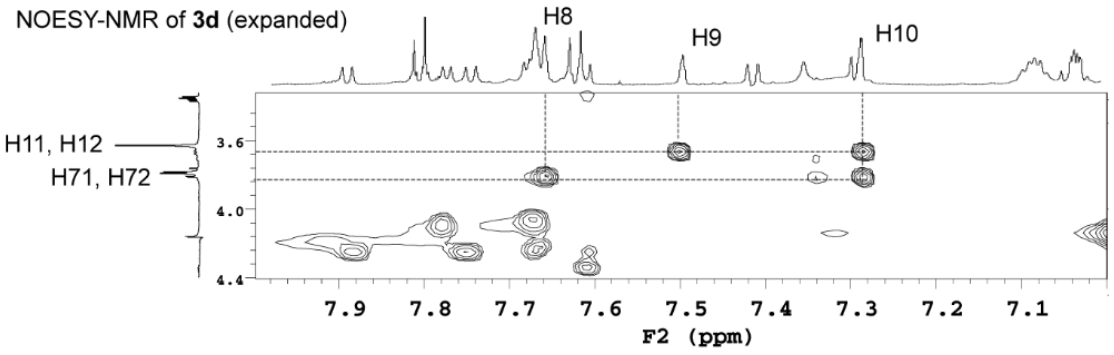


ROESY-NMR of **3a**

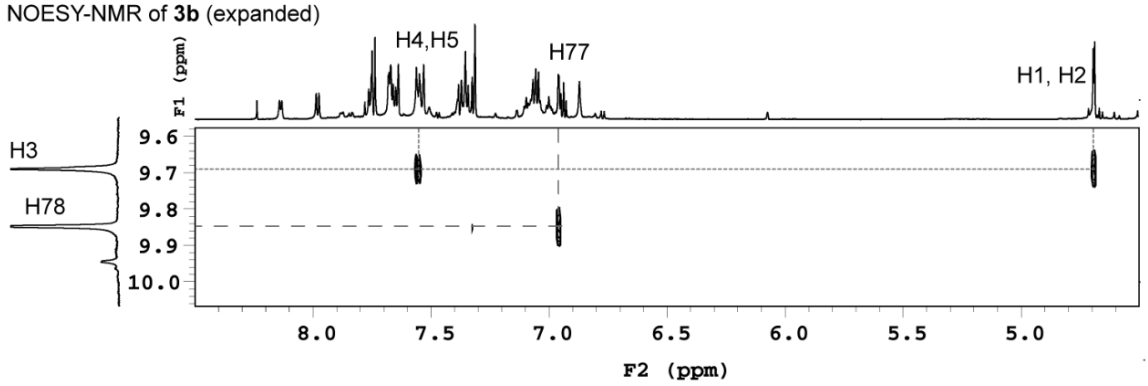



NOESY-NMR of **3b** (expanded)
H8
H9
H10
H11, H12
H71, H72
3.6
4.0
4.4
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
F2 (ppm)

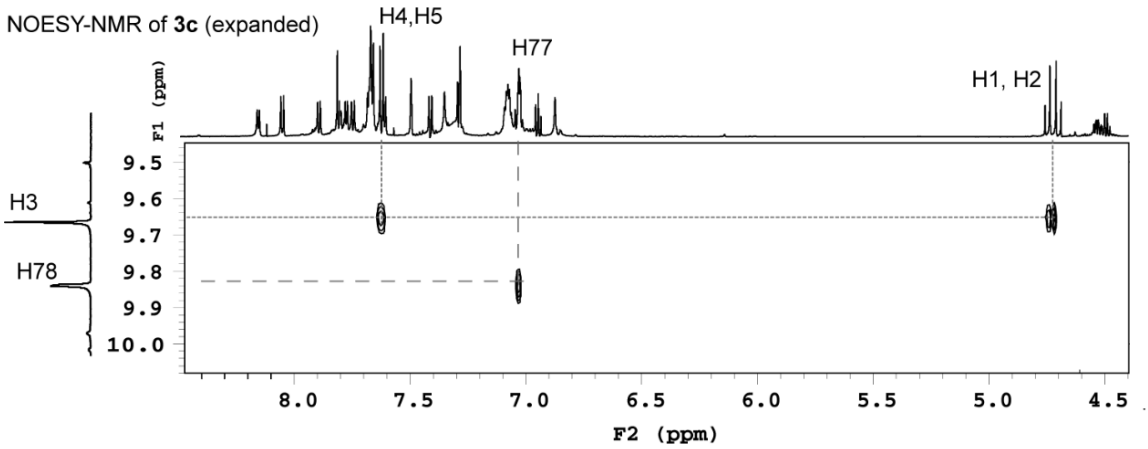

NOESY-NMR of **3c** (expanded)
H8
H9
H10
H11, H12
H71, H72
3.6
4.0
4.4
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
F2 (ppm)


NOESY-NMR of **3d** (expanded)
H8
H9
H10
H11, H12
H71, H72
3.6
4.0
4.4
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
F2 (ppm)

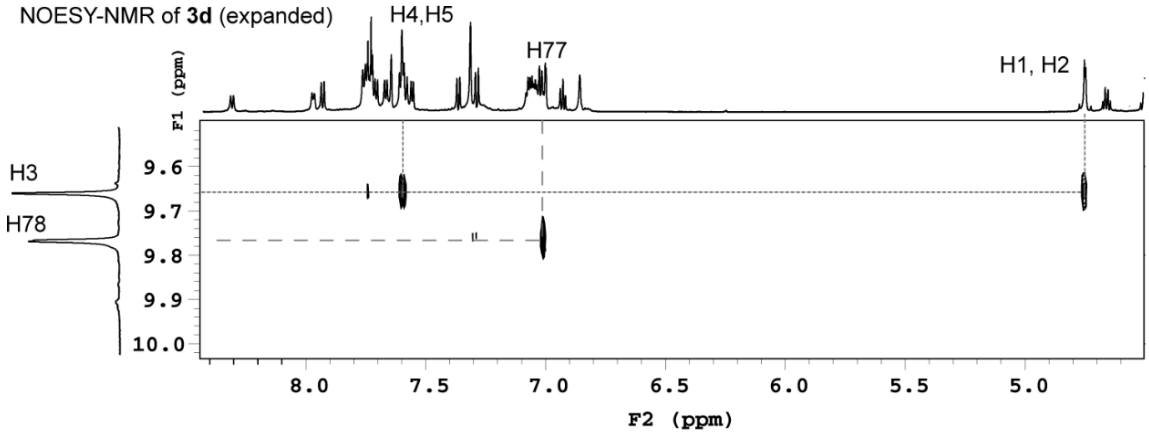
NOESY-NMR of **3b** (expanded)



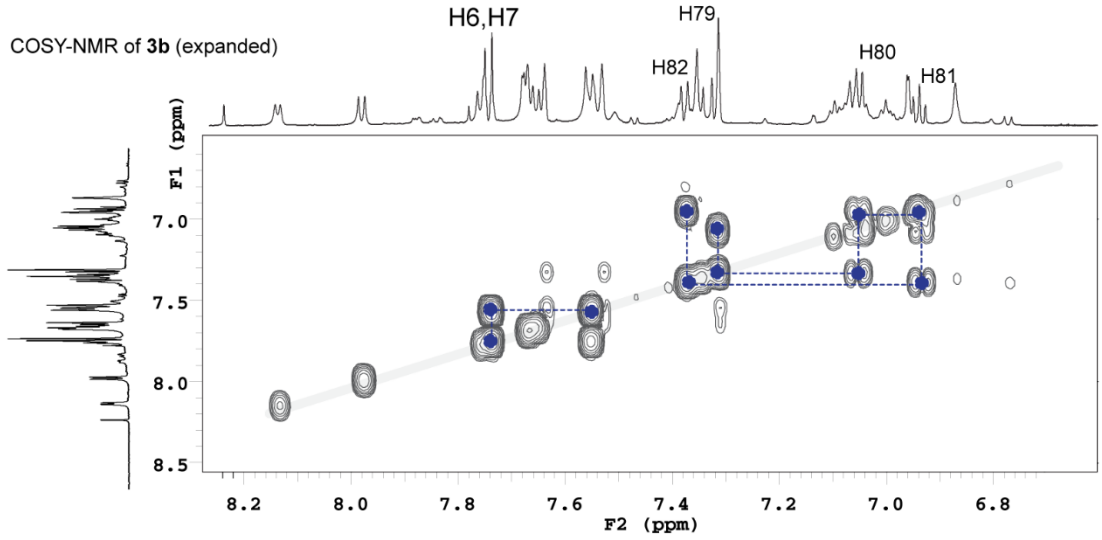
NOESY-NMR of **3c** (expanded)



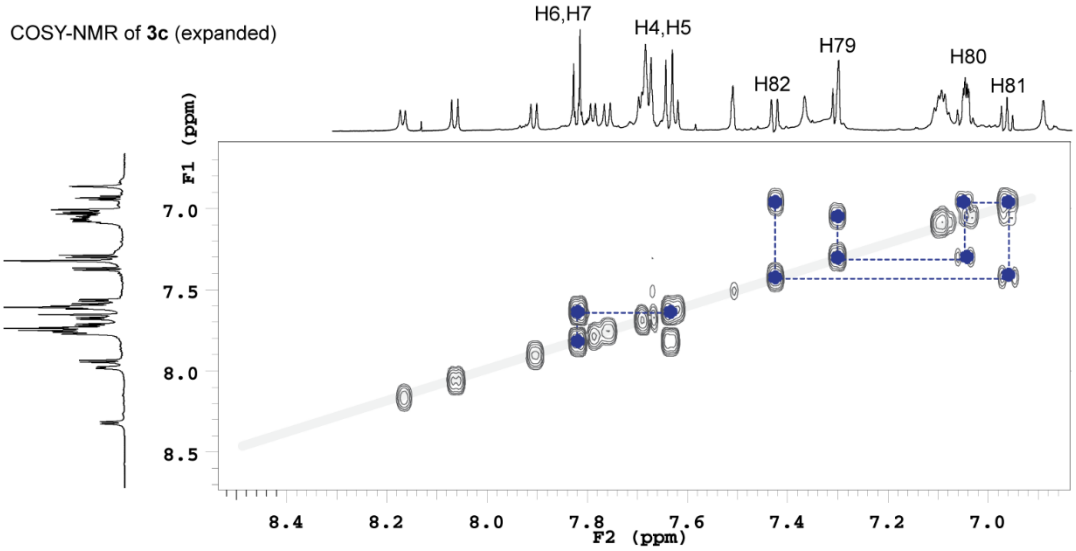
NOESY-NMR of **3d** (expanded)



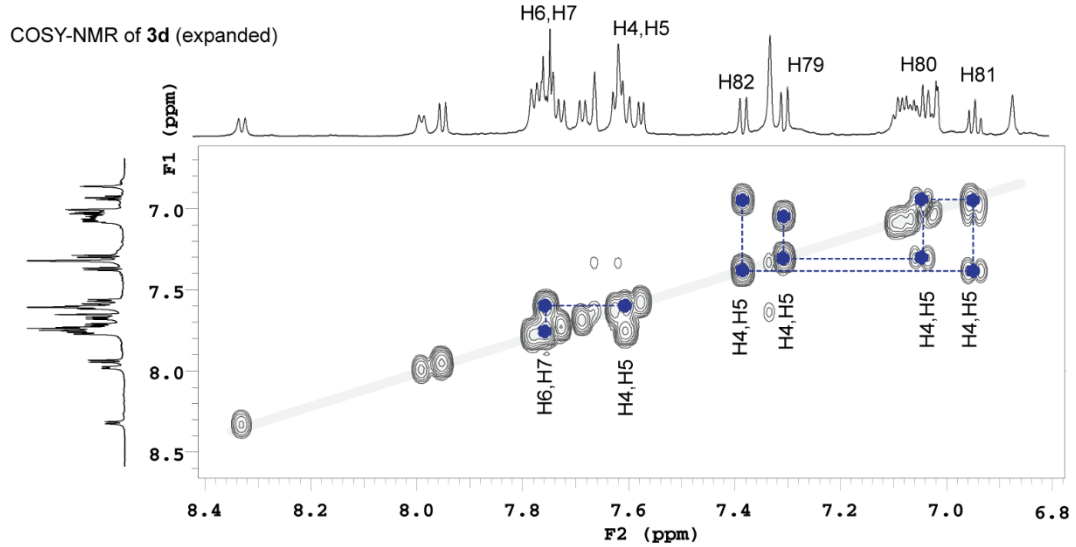
COSY-NMR of **3b** (expanded)



COSY-NMR of **3c** (expanded)



COSY-NMR of **3d** (expanded)



Supporting Information References

1. Zheng, J.; Lin, S.; Jiang, B. W.; Marder, T. B.; Yang, Z. *Can. J Chem.* **2012**, 90, 138.

2. Kim, Y.-W.; Grossmann, T.N.; Verdine, G.L. *Nat. Protocols* **2011**, 6, 761.