SERS Enhancement of silver nanoparticles prepared by a template-directed triazole ligand strategy

Heba A. Kashmery,[1] David G. Thompson,[2] Ruggero Dondi,[3] Duncan Graham,[1,2] Alasdair W. Clark,[4] Glenn A. Burley[1*]*

[1] *Department* of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK.

[2] Centre for Molecular Nanometrology, Department of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK.

[3] University of Bath, Department of Pharmacy and Pharmacology, Claverton Down, Bath, BA2 7AY, UK.

[4] Biomedical Engineering Research Division, School of Engineering, Rankine Building, Oakfield Avenue, University of Glasgow, Glasgow, UK.

* Corresponding author. E-mail: duncan.graham@strath.ac.uk; alasdair.clark@gla.ac.uk; glenn.burley@strath.ac.uk

Table of Contents

1.0 Abbreviations

^{1}H NMR: Proton nuclear magnetic resonance

^{13}C NMR: ^{13}C nuclear magnetic resonance

HMBC: Heteronuclear Multiple-Bond Correlation

HRMS: High resolution mass spectrometry

HSQC: Heteronuclear Single Quantum Coherence

NOESY: Nuclear Overhauser Effect Spectroscopy

ROESY: Rotating-frame Overhauser Effect Spectroscopy

TEM: Transmission electron microscope

UV-Vis: Ultraviolet-visible

2.0 Experimental Section

2.1 General.

Silver nitrate (99.9999% and NH_3 (28%) were purchased from Sigma Aldrich. Compounds (**5**)[1] and (**6**)[2] were prepared as reported previously. Cyclooctyne-EG4 (**7**) was prepared from literature procedures.[3] UV-Vis measurements were acquired using a Thermo-Scientific Nanodrop 1000. Time-course kinetics experiments were acquired using a Varian CaryWin 300Bio UV-Visible spectrometer. Electron microscopy images were taken using an FEI Tecnai T20 TEM. SERS analysis was performed using an Avalon Instruments Plate reader (532 nm) using a 96 well plate. High resolution mass spectrometry was performed on a Waters Acquity XEVO Q ToF machine. Nuclear magnetic resonance (NMR) (^{1}H, ^{13}C, HSQC, HMBC, ROSY and NOSY) spectra were recorded using a Bruker

400, 500 and 600 MHz spectrometer. Analytical and semi-preparative RP-HPLC was performed at room temperature on an ULTIMAT 3000 Instrument (DIONEX). UV absorbance was measured using a photodiode array detector at 210 and 260 nm. An ACE C18 column (4.6 X 250 mm, 5 µm, 300 Å) was used for analytical RP-HPLC. A solvent gradient of increasing amount of MeCN was used for HPLC of compounds (**3, 4a** and **4b**). A typical gradient started with 90 % H_2O (solvent A) and 10% MeCN (solvent B). This was held at 2 min. then increased to 90% solvent B over 20 min. For semi-preparative HPLC, an ACE C18 column (21.2 X 250 mm, 5 µm, 300 Å) was used.

2.2 Synthesis of compound (S1)

Error! No topic specified. To a solution of (**5**) (0.10 g, 0.12 mmol) and (**6**) (0.17 g, 0.73 mmol) in THF:H_2O:DMSO (3:1:2, 1.4 mL) was added a solution of 0.5 M $CuSO_4$ in H_2O (0.28 mL) followed by solid sodium ascorbate (0.05 g, 0.25 mmol). The reaction mixture was stirred overnight at room temperature. The suspension was diluted with H_2O (2 mL), cooled to 0°C and treated with conc. NH_4OH (0.17 mL) for 10 min. The reaction mixture was diluted with DCM (100 mL) and the organic layer washed with brine (2 × 20 mL), followed by H_2O (2 × 20 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (SiO_2) eluting with 10 % of acetone in DCM afforded (**S1**) (0.09, 70%) as a white solid.

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{48}H_{70}N_9O_{17}$ 1044.4890; Found 1044.4935.

MP. 65-66°C.

^1H NMR ($CDCl_3$, 500 MHz): δ 1.29 (s, 6H, CH_3), 1.36 (s, 6H, CH_3), 1.38 (s, 6H, CH_3), 1.50 (s, 6H, CH_3), 3.58-3.71 (m, 16H, CH_2-EG), 4.19-4.21 (m, 4H, CH-sugar), 4.33 (dd, 2H, *J* =

2.5, 4.9 Hz, H_2), 4.47 (dd, 2H, *J* = 8.5, 14.3 Hz, $H_{6'}$), 4.62-4.65 (m, 4H, CH-sugar, H_6), 4.68 (s, 2H, CH_2 [19]), 5.14 (s, 4H, CH_2O [9]), 5.44 (s, 2H, CH_2 [16]), 5.51 (d, 2H, *J* = 4.9 Hz, H_1), 6.52 (d, 2H, *J* = 2.1 Hz, *o*-Ar-H_{13}/H_{15}), 6.62 (t, 1H, *J* = 2.1 Hz, *p*-Ar-H_{11}), 7.57 (s, 1H, NCH=C [17]), 7.80 (s, 2H, NCH=C [7]).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.6 (CH_3, 2C), 25.1 (CH_3, 2C), 26.1 (CH_3, 2C), 26.2 (CH_3, 2C), 41.2 (CH_2, 1C [19]), 50.8 (CH_6/$H_{6'}$, 2C), 54.2 (CH_2, 1C [16]), 61.8 (CH_2-EG), 62.3 (OCH_2, 2C [9]), 64.9 (CH_2-EG), 67.4 (CH-sugar, 2C), 69.9 (CH_2-EG), 70.4 (CH_2-EG), 70.5 (CH-sugar, 2C), 70.65 (CH_2-EG), 70.7 (CH_2-EG), 70.74 (CH_2-EG), 71.0 (CH-sugar, 2C), 71.4 (CH-sugar, 2C), 72.8 (CH_2-EG), 96.4 (CH_1, 2C), 102.1 (*p*-Ar-CH_{11}, 1C), 107.7 (*o*-Ar-CH_{13}/CH_{15}, 2C), 109.3 (Cq, 2C), 110.1 (Cq, 2C), 123.0 (N**C**H=C, 1C [17]), 124.4 (N**C**H=C, 2C [7]), 137.1 (*p*-Ar-C_{14}, 1C), 143.4 (C_8, 2C), 145.7 (C_{18}, 1C), 160.1 (*m*-Ar-C_{10}/C_{12}, 2C).

2.3 Synthesis of compound (3)

Error! No topic specified. To a mixture of TFA:H_2O (1:1, 8 mL) was added (**S1**) (0.08 g, 0.08 mmol) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 4 h. The mixture was then cooled to room temperature followed by concentrated *in vacuo*. The crude residue was diluted with H_2O (20 mL) and concentrated *in vacuo* again to remove excess TFA. The product was diluted with MeOH and precipitated using Et_2O. The crude residue was diluted in H_2O and purified by semi-preparative HPLC using H_2O and MeCN. The gradient was started at 5% MeCN (solvent B), held at 5 min. then increased to 90% solvent B over 20 min. The product was freeze-dried to afford (**3**) (0.028g, 40%) as a white powder. This compound was isolated as a mixture of diastereomers.

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{36}H_{54}N_9O_{17}$ 884.3638; Found 884.3612.

MP. 93-95°C.

^{1}H NMR (D_2O, 600 MHz): δ 3.55-3.68 (m), 3.70-3.72 (m), 3.86-3.91 (m), 3.95 (d, *J* = 3.3 Hz), 4.02 (d, *J* = 2.5 Hz), 4.06-4.08 (m), 4.46 (dd, *J* = 4.1, 8.9 Hz), 4.52 (d, *J* = 7.9 Hz), 4.60-4.73 (m), 5.15 (s), 5.24 (d, *J* = 3.5 Hz), 5.54 (s), 6.61 (d, *J* = 1.9 Hz), 6.64 (t, J = 1.9 Hz), 8.07 (s), 8.09 (s).

^{13}C NMR (D_2O, 150 MHz): δ 50.8, 51.0, 53.5, 60.3, 61.3, 63.1, 68.2, 68.8, 68.9, 68.91, 69.0, 69.4, 69.43, 69.46, 69.5, 69.52, 69.6, 71.66, 71.7, 72.6, 73.2, 92.4, 96.5, 102.5, 108.0, 125.2, 125.7, 125.9, 137.7, 142.9, 144.3, 159.1.

2.4 Synthesis of compound (S2a/S2b)

Error! No topic specified. To a solution of **(5**) (0.30 g, 0.37 mmol) in DMSO (2 mL) was added (**7**) (0.20 g, 0.67 mmol) under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (50 mL) and the organic layer washed with H_2O (3 × 25 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* followed by purification by column chromatography (SiO_2) eluting with 5% MeOH in EtOAc afforded (**S2a**) (0.15 g, 37%) and (**S2b**) (0.107, 26%) as white crystals. Identification of both regioisomers was achieved using 2D NMR NOESY, HSQC, HMBC and ROESY.

2.4.1 Characterisation of compound (S2a)

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{53}H_{78}N_9O_{17}$ 1112.5516; Found 1112.5554.

MP. 75-77°C.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.14-1.23 (m, 1H, OCT), 1.29 (s, 6H, CH_3), 1.37 (s, 6H, CH_3), 1.39 (s, 6H, CH_3), 1.34-1.48 (m, 2H, CH_2, OCT), 1.50 (s, 6H, CH_3), 1.55-1.62 (m,

1H, OCT), 1.64-1.75 (m, 2H, CH_2, OCT), 1.79-1.88 (m, 1H, OCT), 1.90-1.98 (m, 1H, OCT), 2.78-2.86 (m, 1H, OCT), 3.07-3.13 (m, 1H, OCT), 3.46-3.70 (m, 16H, CH_2-EG), 4.19-4.21(m, 4H, CH-sugar), 4.33 (dd, 2H, *J* = 2.5, 4.9 Hz, H_2), 4.44-4.51 (m, 3H, H_{19} + $H_{6'}$), 4.61-4.66 (m, 4H, CH-sugar + H_6), 5.10 (s, 4H, CH_2O [9]), 5.52 (d, 2H, *J* = 4.9 Hz, H_1), 5.70 (s, 2H, CH_2 [16]), 6.48 (s, 2H, *o*-Ar-H_{13}/H_{15}), 6.54 (s, 1H, *p*-Ar-H_{11}), 7.79 (s, 2H, NCH=C [7]).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.1 (CH_2-OCT, 1C), 24.5 (CH_2-OCT, 1C), 24.6 (CH_3, 2C), 24.9 (CH_2-OCT, 1C), 25.1 (CH_3, 2C), 26.1 (CH_3, 2C), 26.2 (CH_3, 2C), 28.2 (CH_2-OCT, 1C), 30.3 (CH_2-OCT, 1C), 50.8 (CH_6/$H_{6'}$, 2C), 52.4 (CH_2, 1C [16]), 61.8 (CH_2-EG), 62.2 (OCH_2, 2C [9]), 67.4 (CH-sugar, 2C), 68.2 (CH_2-EG), 70.5 (CH-sugar, 2C), 70.7 (CH_2-EG), 70.8 (CH_2-EG), 70.84 (CH_2-EG), 71.0 (CH-sugar, 2C), 71.3 (CH-sugar, 2C), 72.2 (CH_{19}, 1C), 72.8 (CH_2-EG), 96.4 (CH_1, 2C), 101.7 (*p*-Ar-CH_{11}, 1C), 106.8 (*o*-Ar-CH_{13}/CH_{15}, 2C), 109.3 (Cq, 2C), 110.1 (Cq, 2C), 124.38 (N**C**H=C, 1C), 124.4 (N**C**H=C, 1C), 133.3 (C_{17}, 1C), 138.8 (*p*-Ar-C_{14}, 1C), 143.5 (C_8, 2C), 145.2 (C_{18}, 1C), 159.9 (*m*-Ar-C_{10}/C_{12}, 2C).

2.4.2 Characterisation of compound (S2b)

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{53}H_{78}N_9O_{17}$ 1112.5516; Found 1112.5559.

MP. 65-67°C.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.92-0.99 (m, 1H, OCT), 1.29 (s, 6H, CH_3), 1.37 (s, 6H, CH_3), 1.39 (s, 6H, CH_3), 1.50 (s, 6H, CH_3), 1.34-1.66 (m, 5H, OCT), 1.79-1.86 (m, 1H, OCT), 2.13-2.20 (m, 1H, OCT), 2.52-2.58 (m, 1H, OCT), 2.99-3.06 (m, 1H, OCT), 3.49-3.73 (m, 16H, CH_2-EG), 4.18-4.21 (m, 4H, CH-sugar), 4.33 (dd, 2H, *J* = 2.6, 4.9 Hz, H_2), 4.46 (dd, 2H, *J* = 8.4, 14.3 Hz, $H_{6'}$), 4.61-4.66 (m, 4H, CH-sugar + H_6), 4.87 (dd, 1H, *J* =

3.8, 5.7 Hz, H_{19}), 5.10 (s, 4H, CH_2O [9]), 5.33 (dd, 1H, *J* = 2.6, 15.7 Hz, $H_{16'}$), 5.45 (dd, 1H, *J* = 2.5, 15.7 Hz, H_{16}), 5.52 (d, 2H, *J* = 4.9 Hz, H_1), 6.40 (d, 2H, *J* = 2.2 Hz, *o*-Ar-H_{13}/H_{15}), 6.57 (t, 1H, *J* = 2.2 Hz, *p*-Ar-H_{11}), 7.79 (s, 2H, NCH=C [7]).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4 (CH_2-OCT, 1C), 21.1 (CH_2-OCT, 1C), 24.6 (CH_3, 2C), 25.1 (CH_3, 2C), 25.7 (CH_2-OCT, 1C), 26.1 (CH_3, 2C), 26.2 (CH_3, 2C), 26.6 (CH_2-OCT, 1C), 35.5 (CH_2-OCT, 1C), 50.8 (CH_6/$H_{6'}$, 2C), 51.8 (CH_{16}/$H_{16'}$, 2C), 61.9 (CH_2-EG), 62.2 (OCH_2, 2C [9]), 67.4 (CH-sugar, 2C), 68.1 (CH_2-EG), 70.5 (CH_2-EG), 70.52 (CH-sugar, 2C), 70.6 (CH_2-EG), 70.7 (CH_2-EG), 70.8 (CH_2-EG), 71.0 (CH, 1H-sugar, 2C), 71.4 (CH-sugar, 2C), 72.8 (CH_2-EG), 74.8 (CH_{19}, 1C), 96.4 (CH_1, 2C), 101.8 (*p*-Ar-CH_{11}, 1C), 106.6 (*o*-Ar-CH_{13}/CH_{15}, 2C), 109.3 (Cq, 2C), 110.1 (Cq, 2C) , 124.4 (N**C**H=C, 2C [7]), 134.5 (C_{18}, 1C), 138.0 (*p*-Ar-C_{14}, 1C), 143.4 (C_8, 2C), 145.8 (C_{17}, 1C), 160.1 (*m*-Ar-C_{10}/C_{12}, 2C).

2.5 Synthesis of compound (4a)

Error! No topic specified. To a mixture of TFA:H_2O (1:1, 8 mL) was added (**S2a**) (0.13 g, 0.12 mmol) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 3 h. The mixture was then cooled to room temperature followed by concentrated *in vacuo*. The crude residue was diluted with H_2O (20 mL) and concentrated *in vacuo* again to remove excess TFA. The product was diluted with MeOH and precipitated using Et_2O. The crude residue was diluted in H_2O and purified by semi-preparative HPLC using H_2O and MeCN. The gradient was started at 5% MeCN (solvent B), held at 5 min. then increased to 90% solvent B over 20 min. The product was freeze-dried to afford **(4a)** (0.088 g, 77%) as a white powder. This compound was isolated as a mixture of diastereomers.

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{41}H_{62}N_9O_{17}$ 952.4264; Found 952.4309.

MP. 93-95°C.

^{1}H NMR (D_2O, 600 MHz): δ 1.01-1.86 (m), 2.80-2.83 (m), 3.00-3.05 (m), 3.39-3.65 (m), 3.66-3.68 (m), 3.83-3.88 (m), 3.92-3.93 (m), 3.99-4.00 (m), 4.05-4.07 (m), 4.42-4.44 (m), 4.50 (dd, *J* = 0.5, 7.9 Hz), 4.58-4.71 (m), 5.16 (s), 5.21 (d, *J* = 3.6 Hz), 5.60 (s), 6.43 (d, *J* = 1.7 Hz), 6.66 (s), 8.05 (s), 8.07 (s).

^{13}C NMR (D_2O, 150 MHz): δ 22.0, 23.4, 23.9, 27.9, 30.6, 50.9, 51.0, 52.1, 60.4, 61.4, 67.4, 67.44, 68.2, 68.8, 68.9, 69.1, 69.5, 69.6, 69.65, 69.7, 71.7, 71.73, 72.6, 73.2, 92.4, 96.5, 102.6, 107.3, 125.7, 125.8, 134.3, 138.5, 143.1, 146.3, 159.1.

2.6 Synthesis of compound (4b)

To a mixture of TFA:H_2O (1:1, 8 mL) was added (**S2b**) (0.09 g, 0.08 mmol) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 3 h. The mixture was then cooled to room temperature followed by concentrated *in vacuo*. The crude residue was diluted with H_2O (20 mL) and concentrated *in vacuo* again to remove excess TFA. The product was diluted with MeOH and precipitated using Et_2O. The crude residue was diluted in H_2O and purified by semi-preparative HPLC using H_2O and MeCN. The gradient was started at 5% MeCN (solvent B), held at 5 min. then increased to 90% solvent B over 20 min. The product was freeze-dried to afford **(4b)** (0.059 g, 77%) as a white powder. This compound was isolated as a mixture of diastereomers.

HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{41}H_{62}N_9O_{17}$ 952.4264; Found 952.4293.

MP. 96-98°C.

^{1}H NMR (D_2O, 600 MHz): δ 0.81-1.48 (m), 1.87-2.00 (m), 2.62-2.67 (m), 2.88-2.93 (m), 3.52-3.68 (m), 3.70-3.72 (m), 3.82-3.88 (m), 3.92 (d, *J* = 3.5 Hz), 3.99 (d, *J* = 2.6 Hz),

4.04-4.06 (m), 4.42 (dd, *J* = 4.1, 9.0 Hz), 4.49 (d, *J* = 7.9 Hz), 4.57-4.70 (m), 5.14 (s), 5.21 (d, *J* = 3.5 Hz), 5.47 (d, *J* = 4.3 Hz), 6.45 (s), 6.63 (s), 8.06 (s), 8.07 (s).

^{13}C NMR (D_2O, 150 MHz): δ 20.0, 21.0, 24.7, 25.9, 34.3, 50.9, 51.0, 51.2, 60.4, 61.4, 67.5, 68.2, 68.8, 68.9, 69.1, 69.5, 69.6, 69.7, 69.8, 71.7, 71.8, 72.6, 73.2, 74.4, 92.4, 96.5, 102.7, 107.4, 125.7, 125.9, 136.0, 138.2, 143.0, 145.3, 159.1

3.0 Silver nanoparticle (AgNP) formation

Preparation of sugar stock solutions: The corresponding sugar triazole (**3**, **4a** and **4b**) was dissolved in ultrapure water and diluted to a standard concentration of 50 mM. This stock solution was then used to optimise conditions for AgNP formation (Tables S1-S3).

Preparation of Tollens' reagent stock solutions: Stock solutions of Tollens' reagent were prepared in three different concentrations (100, 20 and 3 mM) and diluted as required with ultrapure water for the preparation of the nanoparticle arrays.

100 mM Tollens: To 1.8 ml H_2O was added $AgNO_3$ (0.5 M, 500 µL), followed by NaOH (3 M, 100 µL) and finally NH_4OH (28 %, 110 µL)

20 mM Tollens: To 4.100 µL H_2O was added $AgNO_3$ (0.5 M, 279 uL), followed by NaOH (3 M, 56 uL) and finally NH_4OH (28%, 61 µL)

3 mM Tollens: To 9.9 ml H_2O was added $AgNO_3$ (0.5 M, 60 µL), followed by NaOH (3 M, 12 µL) and finally NH_4OH (28 %, 13 µL)

AgNPs were formed by the addition of 25 µL of Tollens' reagent to 25 µL of a solution of an appropriate sugar ligand in a plastic tube. The solution was vortexed and left in the dark overnight. The mixture was centrifuged for 30 seconds to afford a suspension of colloidal of AgNPs.

3.1 Preparation of AgNP@(3) series

Table S1. *AgNP@(**3**) screening array prepared using (**3**) and the Tollens' reagent. White boxes represent no AgNP formation, yellow boxes represent AgNP formation and grey boxes represent the formation of silver mirrors.*

[(3)]

[Tolle	25 mM	10 mM	1 mM	100 µM	10 µM	1 µM
10 µM	#1	#2	#3	#4	#5	#6
100 µM	#7	#8	#9	#10	#11	#12
1 mM	#13	#14	#15	#16	#17	#18
10 mM	#19 15 ± 4 nm	#20 15 ± 4 nm	#21	#22	#23	#24
20 mM	#25	#26 16 ± 2 nm	#27	#28	#29	#30
50 mM	#31	#32	#33	#34	#35	#36

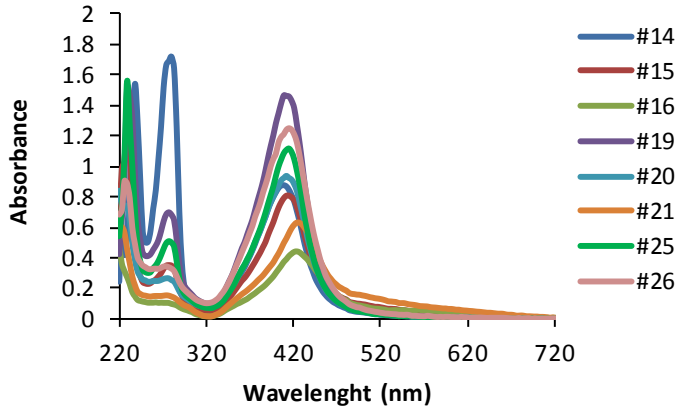


Figure S1. *UV-vis spectra of reactions #14-16, 19-21 and 25-26 which formed AgNP@(**3**) as observed by a SPR peak. Samples #19, 21, 25 were diluted 1:10 and #20, 26 were diluted 1:20 prior to each measurement.*

3.2 Preparation of AgNP@(4a) series

Table S2. *AgNP@(**4a**) screening array prepared using (**4a**) and the Tollens' reagent. White boxes represent no AgNP formation, yellow boxes represent AgNP formation and grey box represents the formation of silver mirror.*

[(4a)]

[Tolle

	25 mM	10 mM	1 mM	100 µM	10 µM	1 µM
10 µM	#1	#2	#3	#4	#5	#6
100 µM	#7	#8	#9	#10	#11	#12
1 mM	#13	#14	#15	#16	#17	#18
10 mM	#19 19 ± 10 nm	#20 18 ± 7 nm	#21	#22	#23	#24
20 mM	#25	#26 15 ± 6 nm	#27	#28	#29	#30
50 mM	#31	#32	#33	#34	#35	#36

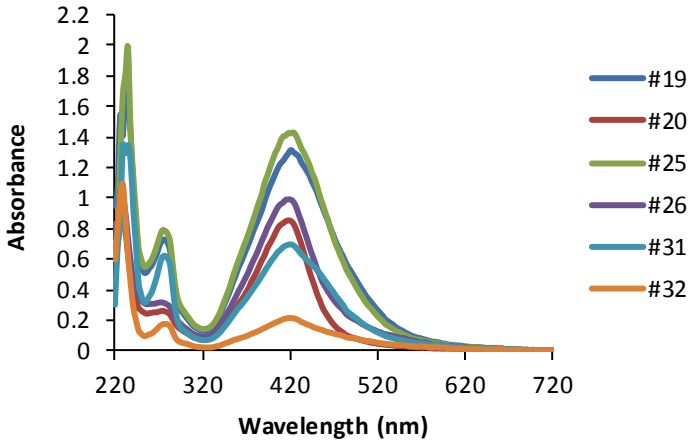


Figure S2. *UV-vis spectra of reactions #19-20, 25-26 and 31-32 which formed AgNP@(**4a**) as observed by a SPR peak at 420 nm. Samples #19, 25, 31-32 were diluted 1:10 and #20, 26 were diluted 1:20 prior to each measurement.*

3.3 Preparation of AgNP@(4b) series

Table S3. *AgNP@(**4b**) screening array prepared using (**4b**) and the Tollens' reagent. White boxes represent no AgNP formation, yellow boxes represent AgNP formation and grey boxes represent the formation of silver mirrors.*

[(4b)]

[Tolle

	25 mM	10 mM	1 mM	100 µM	10 µM	1 µM
10 µM	#1	#2	#3	#4	#5	#6
100 µM	#7	#8	#9	#10	#11	#12
1 mM	#13	#14	#15	#16	#17	#18
10 mM	#19 38 ± 7 nm	#20 17 ± 5 nm	#21	#22	#23	#24
20 mM	#25	#26 25 ± 5 nm	#27	#28	#29	#30
50 mM	#31	#32	#33	#34	#35	#36

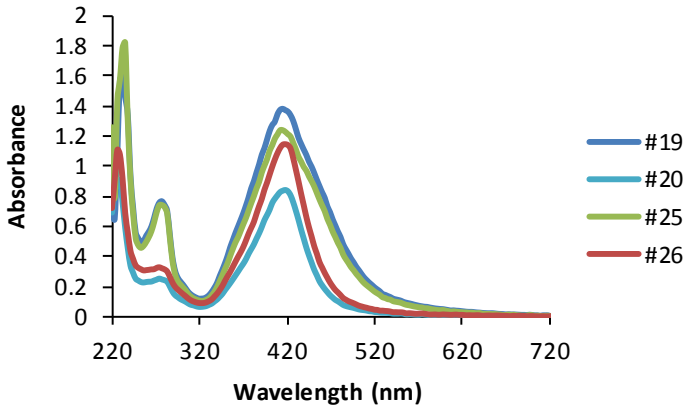
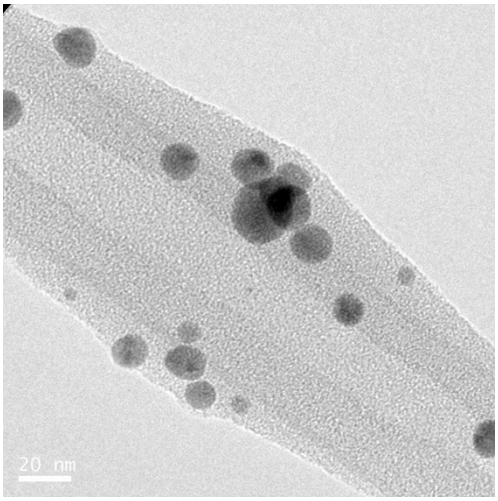


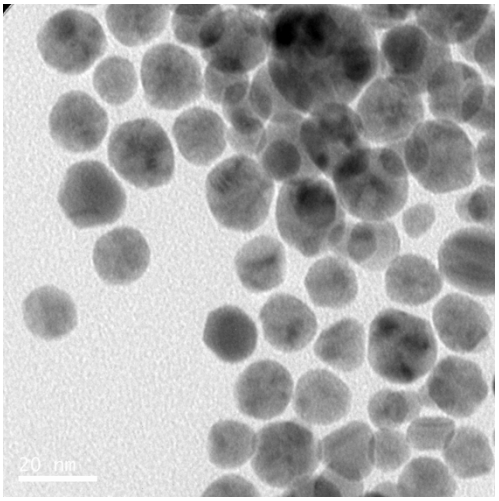
Figure S3. *UV-vis spectra of reactions #19-20 and 25-26 which formed AgNP@(**4b**) as observed by a SPR peak. Samples #19, 25 were diluted 1:10 and #20, 26 were diluted 1:20 prior to each measurement.*

4.0 TEM images of AgNPs

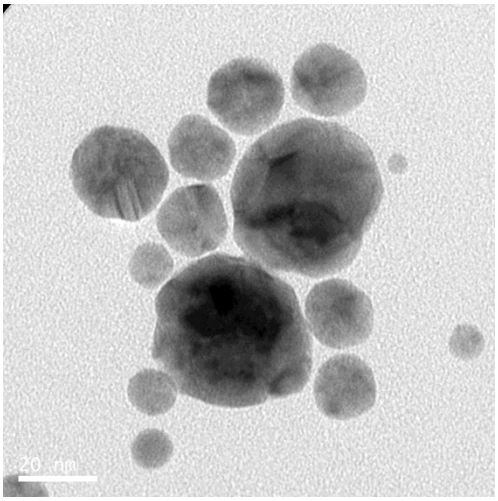
(a)



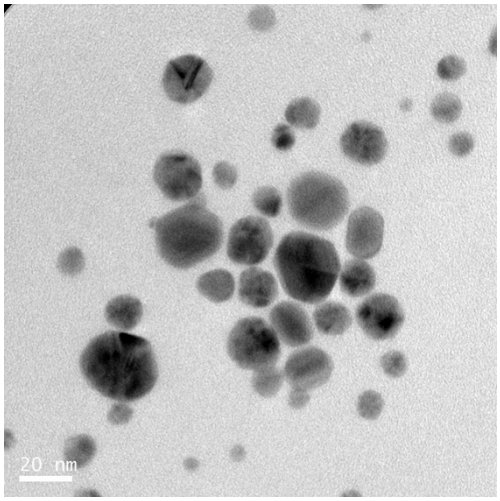
(b)



(c)



(d)



(e)

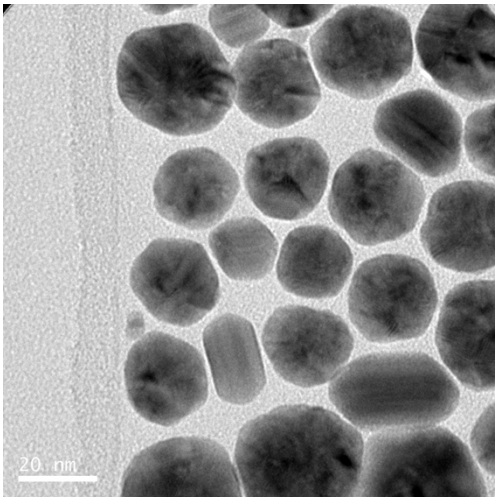


Figure S4. *Exemplar TEM images of AgNP prepared using* ***(a)*** *10 mM Tollens' and 25 mM (**3**), Ø = 15 ± 4 nm,* ***(b)*** *20 mM Tollens' and 10 mM (**3**), Ø = 16 ± 2 nm,* ***(c)*** *10 mM Tollens' and 25 mM (**4a**), Ø = 19 ± 10 nm,* ***(d)*** *20 mM Tollens' and 10 mM (**4a**), Ø = 15 ± 6 nm and* ***(e)*** *20 mM Tollens' and 10 mM (**4b**), Ø = 25 ± 5 nm.*

5.0 Reaction kinetics of AgNP formation

Time course: 150 µL of sugar solutions (**3, 4a** or **4b**) at 20 µM and 150 µL of Tollens' solution (20 mM) were mixed in a low-volume quartz cuvette, UV-Vis measurements were taken at 400 nm every 5 seconds using a UV-Vis spectrophotometer.

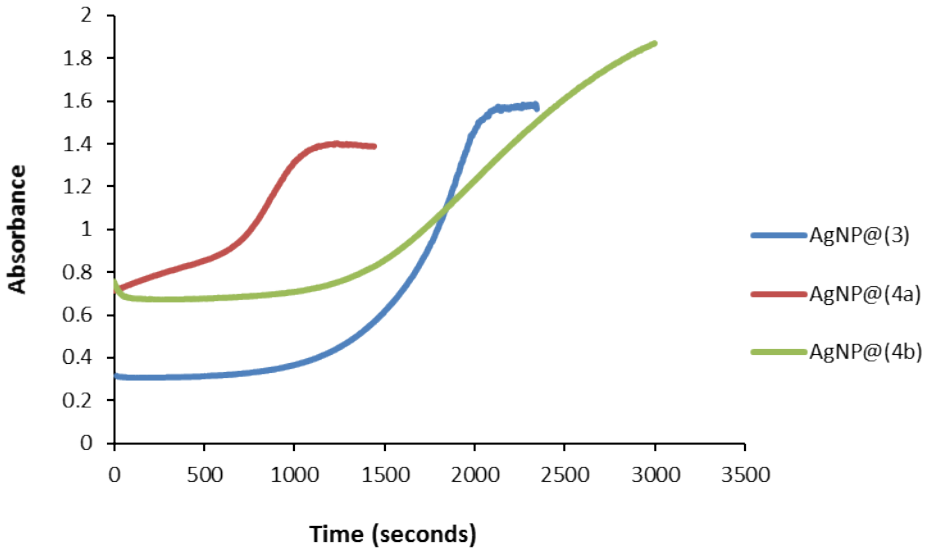


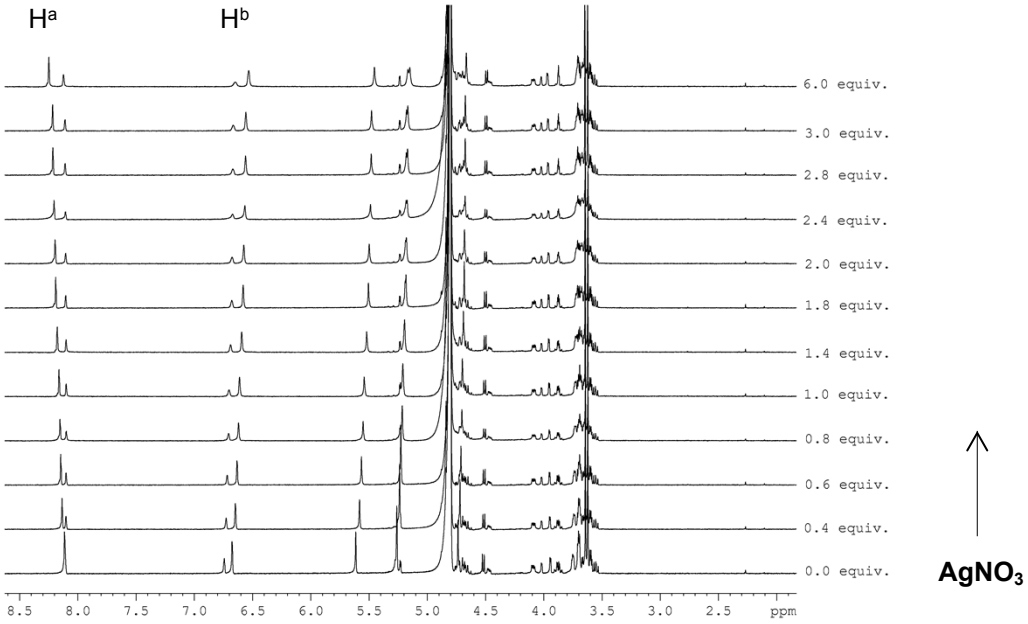
Figure S5. *Kinetics of formation of AgNP using (**3**, blue), (**4a**, red) and (**4b**, green) as monitored by the formation of the SPR peak at 400nm.*

6.0 ^{1}H NMR titration studies using ligands (3, 4a and 4b) with $AgNO_3$

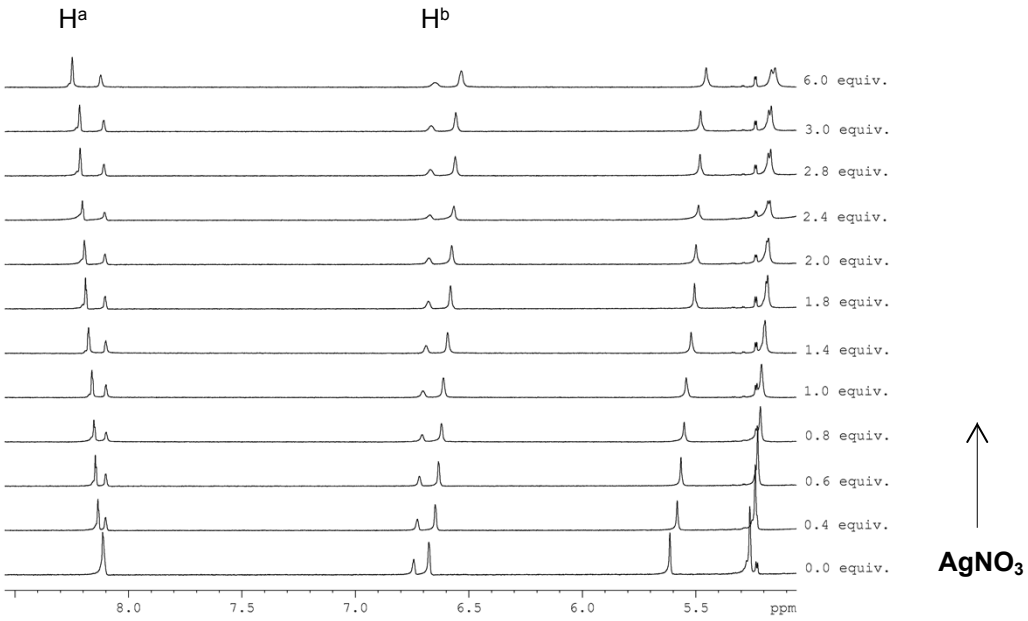
Stock solutions of triazole ligands (**3**, **4a** or **4b**) at 2 mM and $AgNO_3$ (12 mM) were prepared in D_2O. 300 µL of aliquots of the ligands were mixed with increasing amounts of $AgNO_3$ and diluted with D_2O up to 600 µL. The recorded spectra are shown in Figures S6-8 and ordered at different concentrations of $AgNO_3$ from 0 to 6 mM.

6.1 ^{1}H NMR titration studies of Ag(I)-binding using ligand (3)

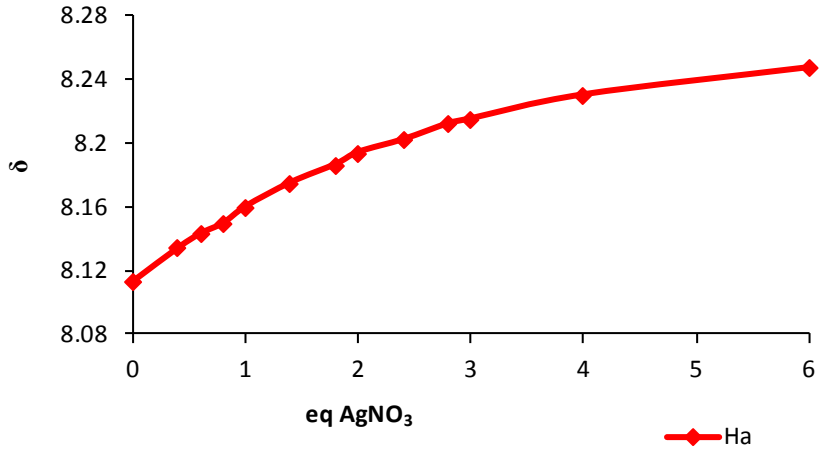
(a)



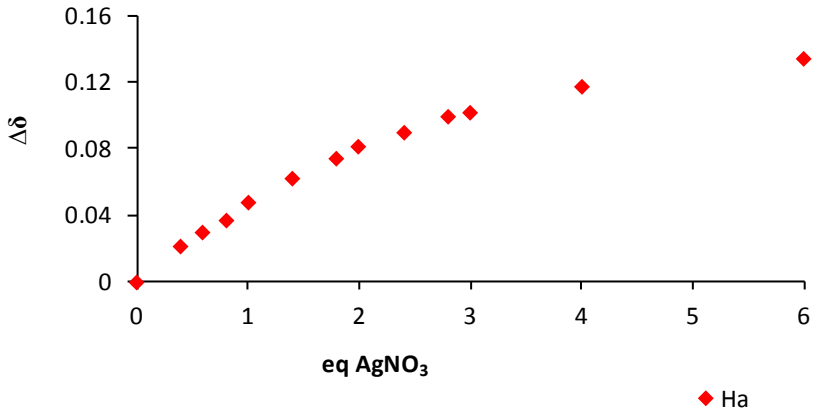
(b)


H^a
H^b
6.0 equiv.
3.0 equiv.
2.8 equiv.
2.4 equiv.
2.0 equiv.
1.8 equiv.
1.4 equiv.
1.0 equiv.
0.8 equiv.
0.6 equiv.
0.4 equiv.
0.0 equiv.
8.0
7.5
7.0
6.5
6.0
5.5
ppm
AgNO3

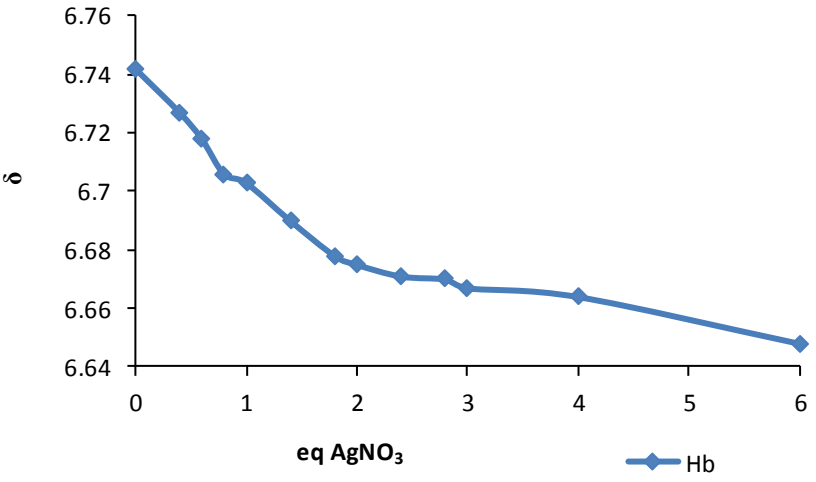
(c)


8.28
8.24
8.2
8.16
8.12
8.08
δ
0
1
2
3
4
5
6
eq AgNO3
Ha

(d)


0.16
0.12
0.08
0.04
0
Δδ
0
1
2
3
4
5
6
eq AgNO3
Ha

(e)


6.76
6.74
6.72
6.7
6.68
6.66
6.64
δ
0
1
2
3
4
5
6
eq AgNO3
Hb

(f)

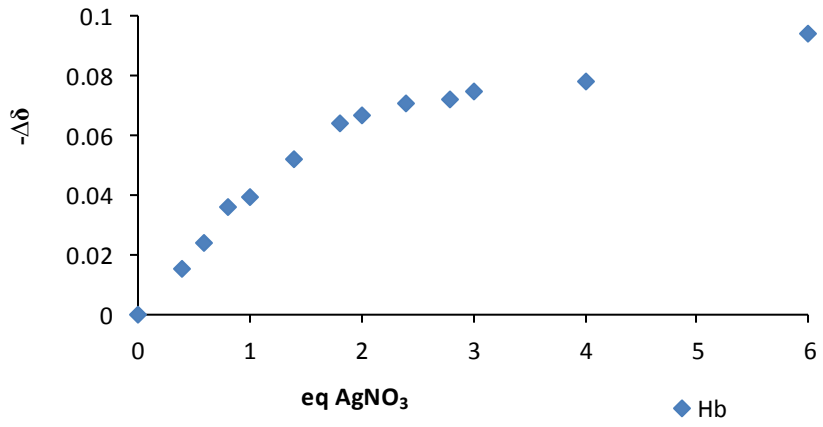
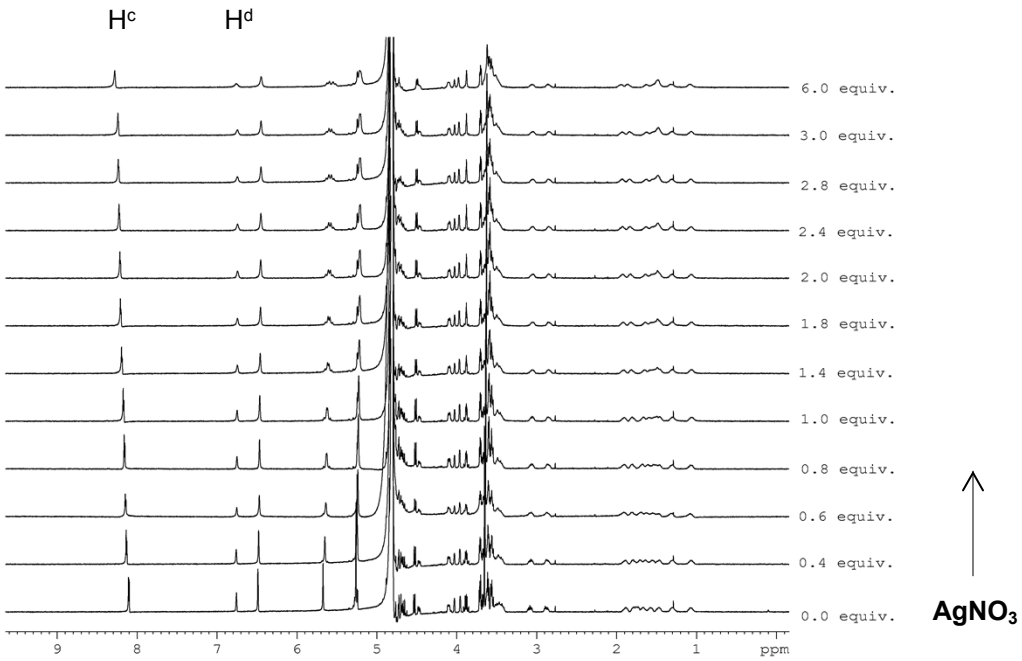


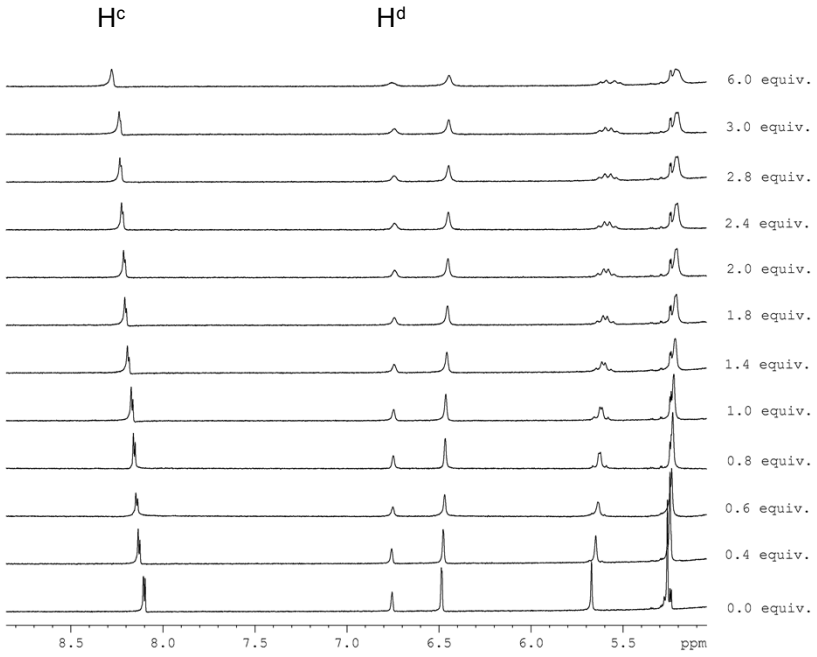
Figure S6. ***(a)*** *Stack plot of ^{1}H-NMR (500 MHz, D_2O) of (**3**) (stock concentration 2 mM) with an increasing amount of $AgNO_3$.* ***(b)*** *Stack plot of selected areas ^{1}H-NMR (500 MHz, D_2O) of (**3**) (2 mM) with an increasing amount of $AgNO_3$.* ***(c)*** *Plot of the ^{1}H-NMR titration of H^a with $AgNO_3$ in D_2O.* ***(d)*** *Change in chemical shift of H^a as a function of $AgNO_3$.* ***(e)*** *Plot of the ^{1}H-NMR titration of H^b with $AgNO_3$ in D_2O.* ***(f)*** *Change in chemical shift of H^b as a function of $AgNO_3$.*

6.2 ^{1}H NMR titration studies of Ag(I)-binding using ligand (4a)

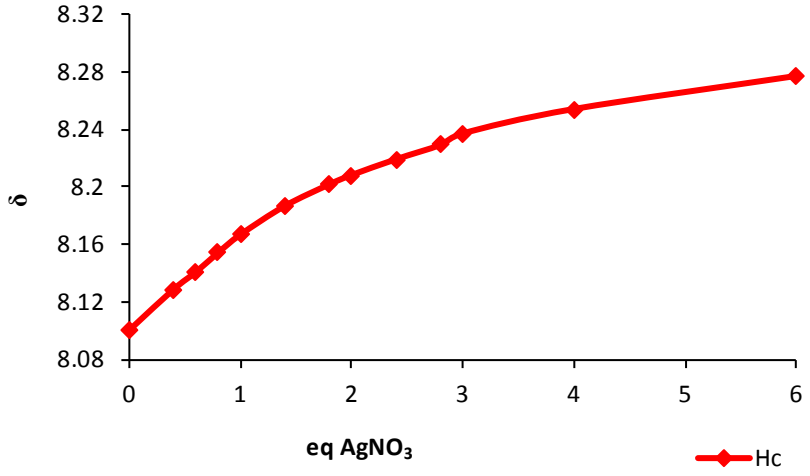
(a)



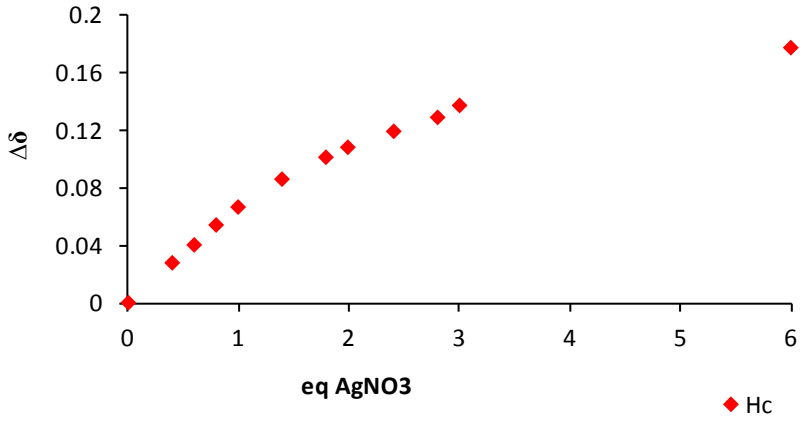
(b)


Hc
Hd
6.0 equiv.
3.0 equiv.
2.8 equiv.
2.4 equiv.
2.0 equiv.
1.8 equiv.
1.4 equiv.
1.0 equiv.
0.8 equiv.
0.6 equiv.
0.4 equiv.
0.0 equiv.
8.5
8.0
7.5
7.0
6.5
6.0
5.5
ppm
AgNO3

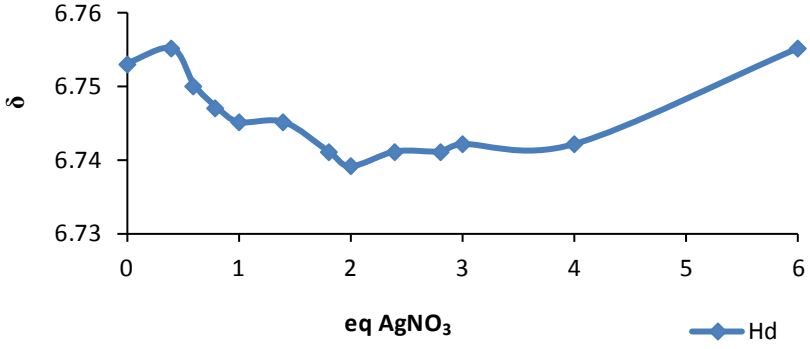
(c)


8.32
8.28
8.24
8.2
8.16
8.12
8.08
δ
0
1
2
3
4
5
6
eq AgNO3
Hc

(d)


0.2
0.16
0.12
0.08
0.04
0
Δδ
0
1
2
3
4
5
6
eq AgNO3
Hc

(e)


6.76
6.75
6.74
6.73
δ
0
1
2
3
4
5
6
eq AgNO3
Hd

(f)

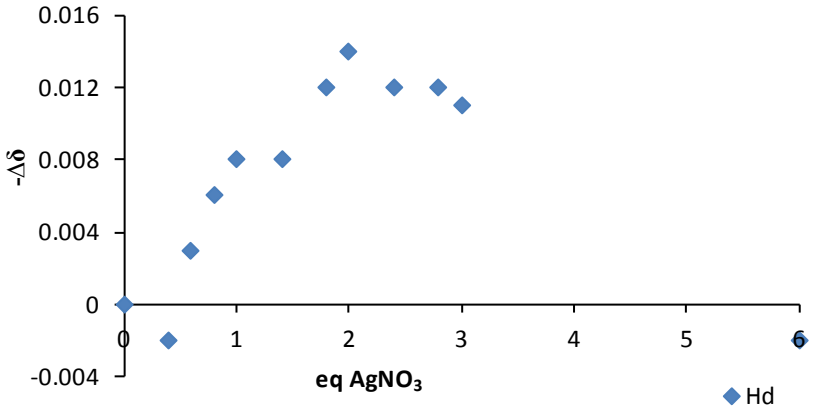
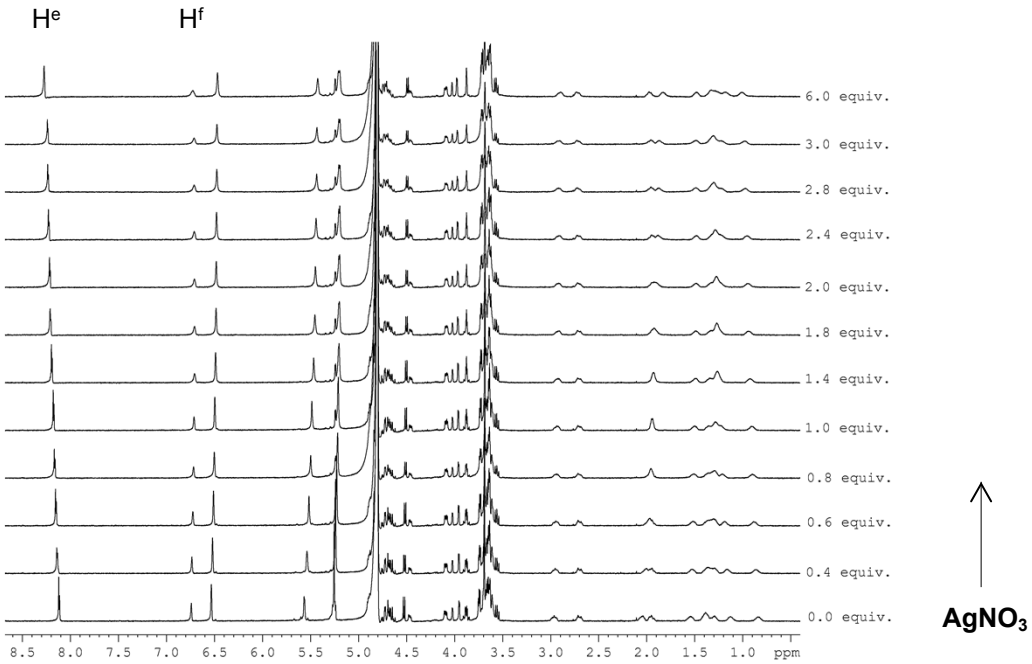


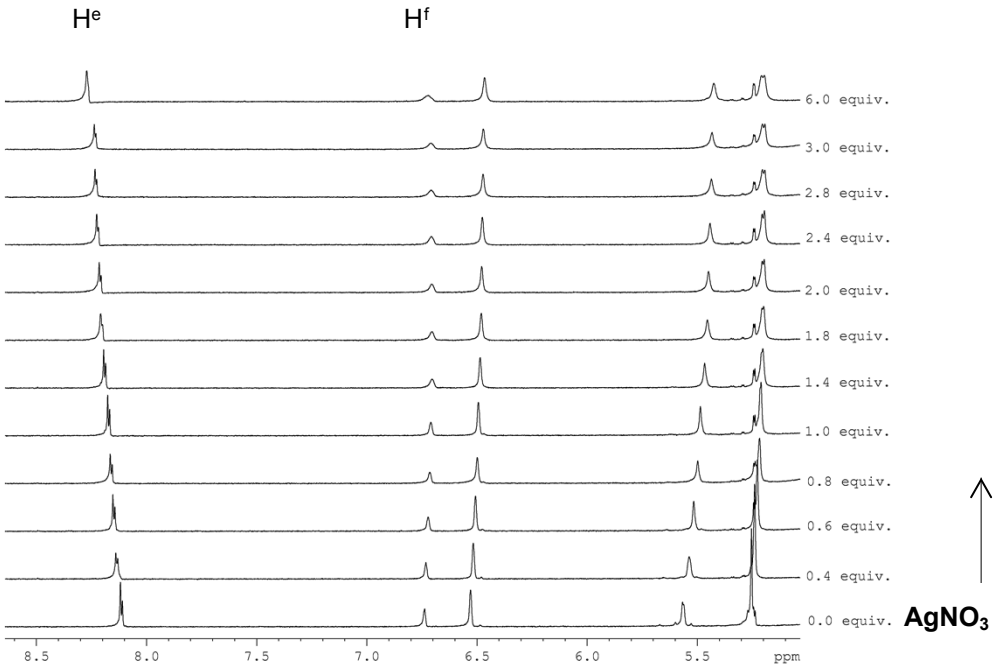
Figure S7. *(**a**) Stack plot of ^{1}H-NMR (500 MHz, D_2O) of (**4a**) (stock concentration 2 mM) with an increasing amount of $AgNO_3$. **(b)** Stack plot of selected areas ^{1}H-NMR (500 MHz, D_2O) of (**4a**) (2 mM) with an increasing amount of $AgNO_3$. **(c)** Plot of the ^{1}H-NMR titration of H^c with $AgNO_3$ in D_2O. **(d)** Change in chemical shift of H^c as a function of $AgNO_3$. **(e)** Plot of the ^{1}H-NMR titration of H^d with $AgNO_3$ in D_2O. **(f)** Change in chemical shift of H^d as a function of $AgNO_3$.*

6.3 ^{1}H NMR titration studies of Ag(I)-binding using ligand (4b)

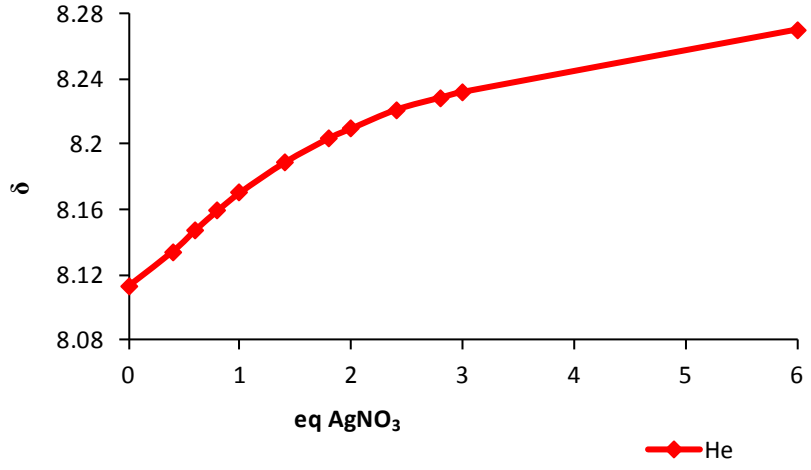
(a)



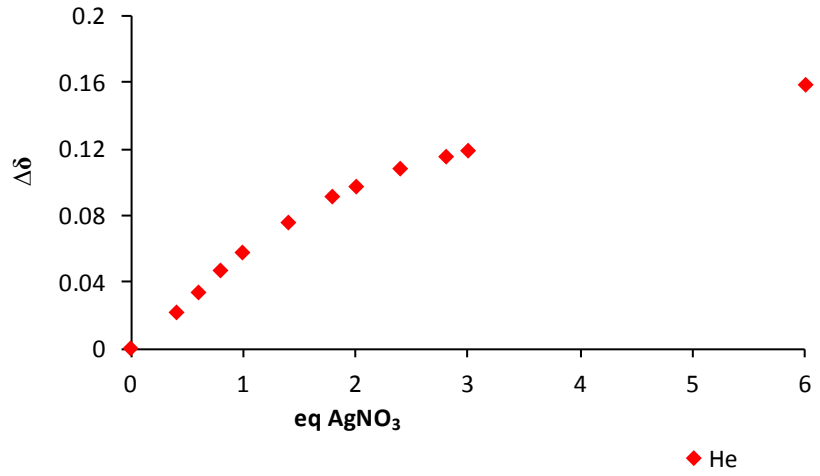
(b)


He
Hf
6.0 equiv.
3.0 equiv.
2.8 equiv.
2.4 equiv.
2.0 equiv.
1.8 equiv.
1.4 equiv.
1.0 equiv.
0.8 equiv.
0.6 equiv.
0.4 equiv.
0.0 equiv.
AgNO3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
ppm

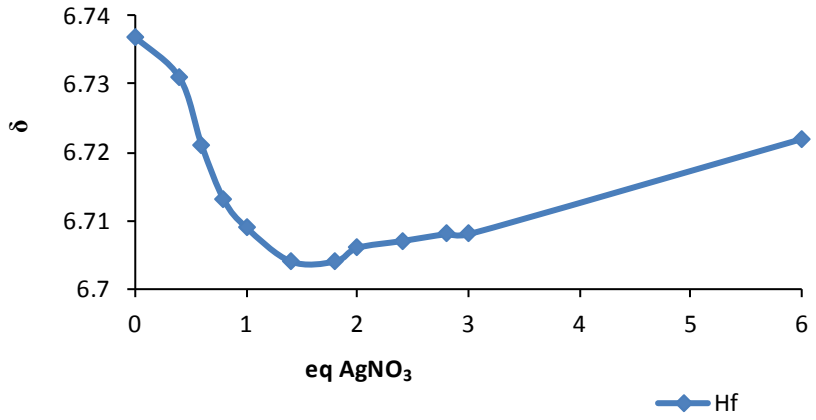
(c)


8.28
8.24
8.2
8.16
8.12
8.08
δ
0
1
2
3
4
5
6
eq AgNO3
He

(d)



(e)



(f)

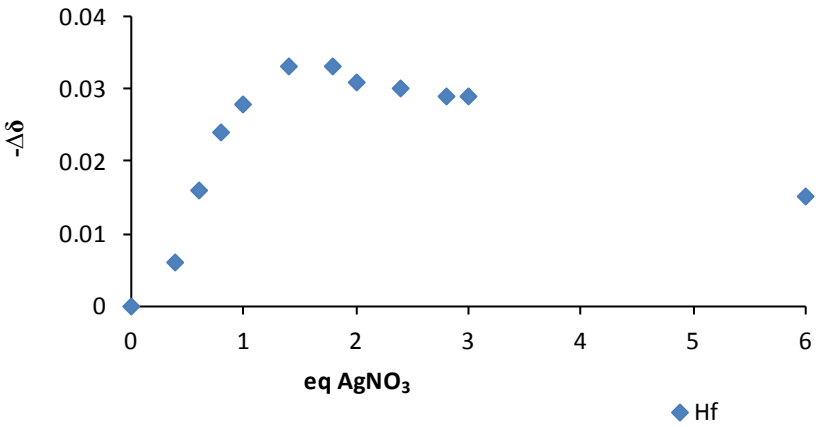


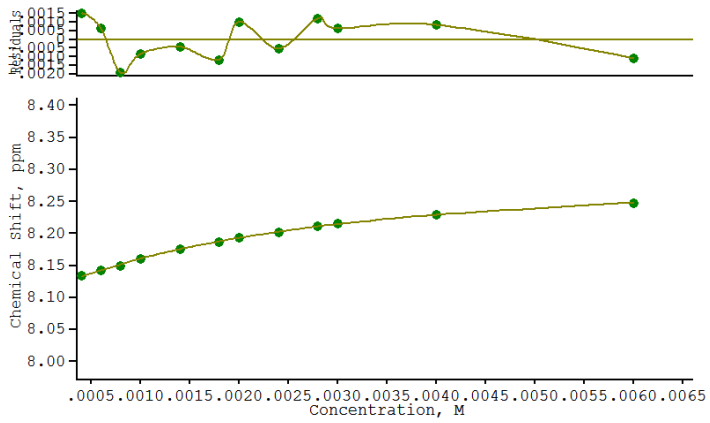
Figure S8. *(**a**) Stack plot of ^{1}H-NMR (500 MHz, D_2O) of (**4b**) (stock concentration 2 mM) with an increasing amount of $AgNO_3$. **(b)** Stack plot of selected areas ^{1}H-NMR (500 MHz, D_2O) of (**4b**) (2 mM) with an*

*increasing amount of $AgNO_3$. **(c)** Plot of the ^{1}H-NMR titration of H^e with $AgNO_3$ in D_2O. **(d)** Change in chemical shift of H^e as a function of $AgNO_3$. **(e)** Plot of the ^{1}H-NMR titration of H^f with $AgNO_3$ in D_2O. **(f)** Change in chemical shift of H^f as a function of $AgNO_3$.*

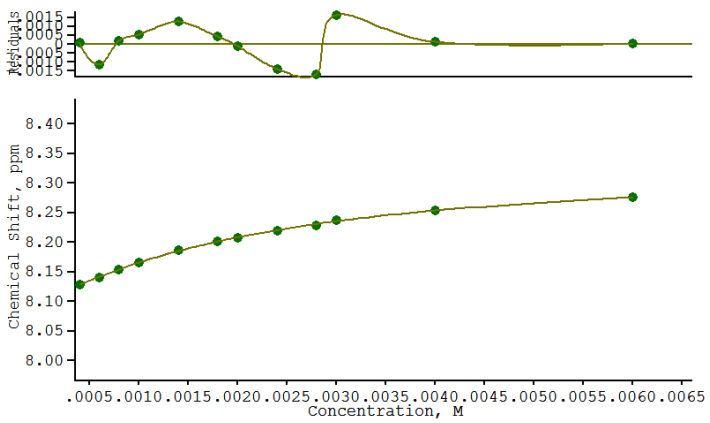
7.0 Calculation of the Ag(I) binding constant

The binding affinity of Ag(I) for ligands (**3, 4a** and **4b**) was calculated by non-linear least squares fitting. The acquired ^{1}H NMR data of the downfield shift observed for the triazole protons and the concentration of the Ag(I) was used to calculate the Ag(I) binding constants using WinEQNMR2 software.[4]

(a)



(b)



(c)

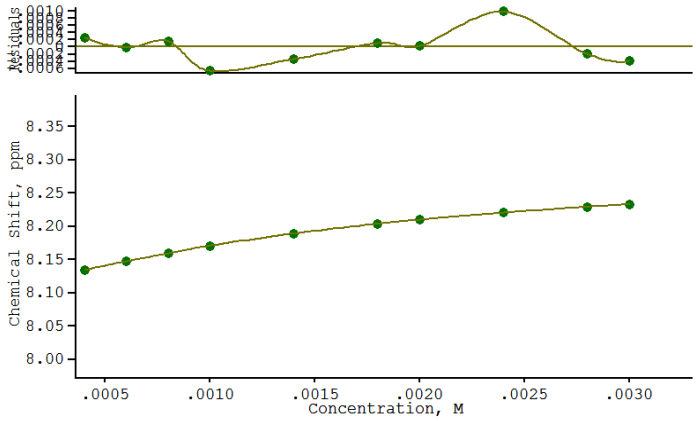
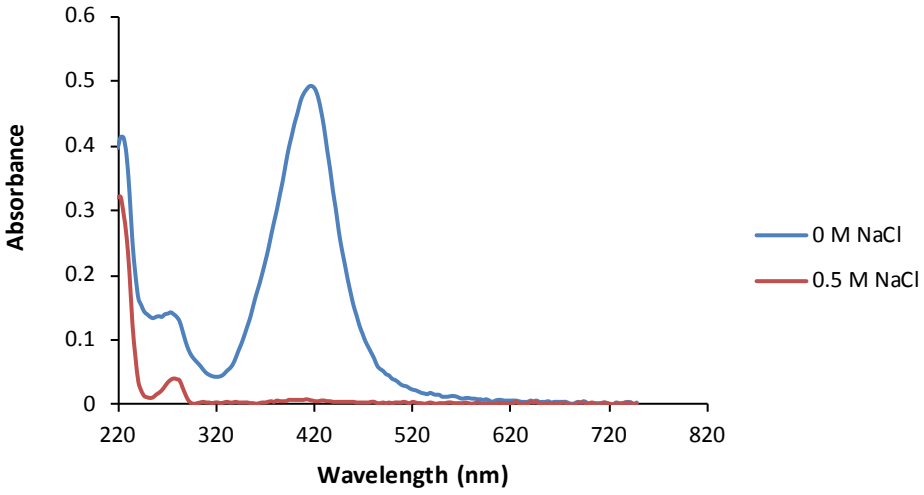


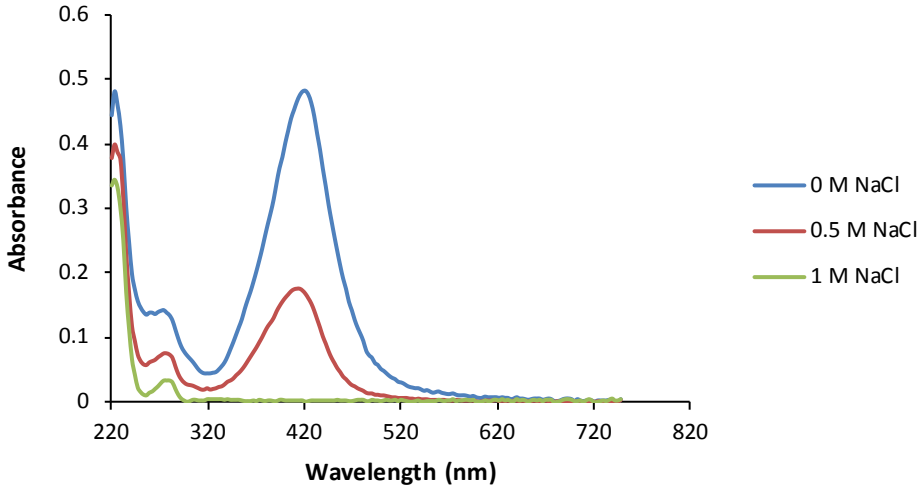
Figure S9. *Plots of the experimental points and the calculated best fit line against concentration of titrant Ag using* ***(a) (3)****,* ***(b) (4a)*** *and* ***(c) (4b)****.*

8.0 Stability of AgNP@(3), AgNP@(4a) and AgNP@(4b) in salt buffer

(a)



(b)



(c)

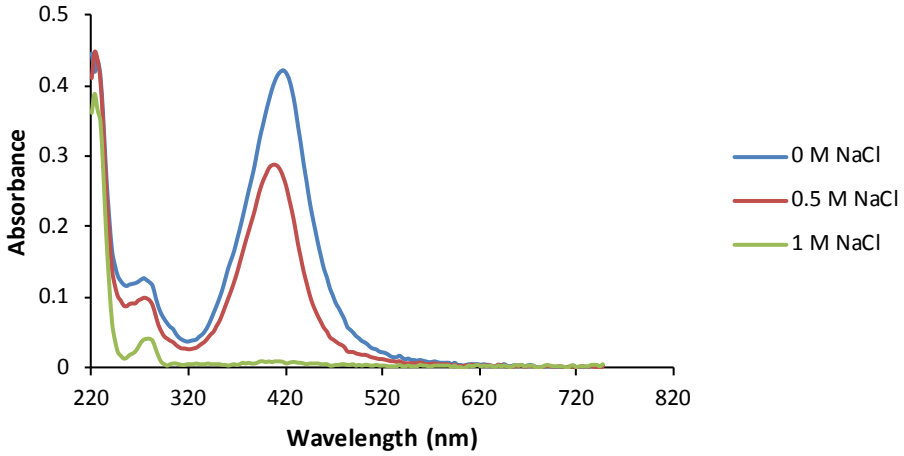


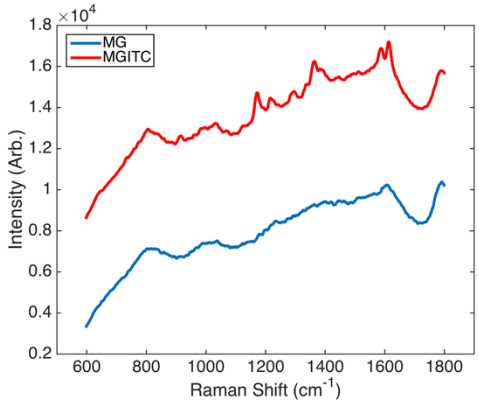
Figure S 10. *Stability of* ***(a)*** *AgNP@(**3**),* ***(b)*** *AgNP@(**4a**) and* ***(c)*** *AgNP@(**4b**) to increasing concentrations of an aqueous solution of NaCl.*

9.0 Surface Enhanced Raman scattering

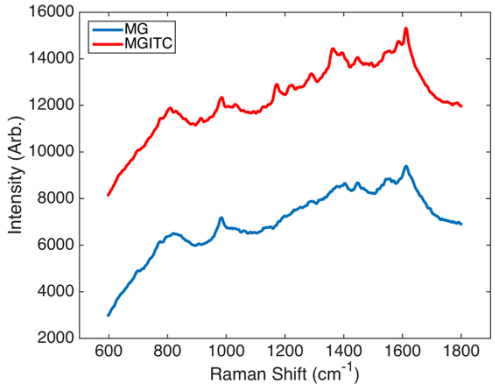
Below outlines the procedure used for detection of both malachite green (MG) and malachite green isothiocyanate (MGITC) at concentrations of 100 nM. The same experimental was also used in the generation of concentration plots relating to MGITC.

The solution of prepared nanoparticle was diluted 1:200 with double distilled deionised H_2O. 15 µL of MG or MGITC was added to a well followed by 25 µL of double distilled deionised H_2O and 100 µL of the diluted nanoparticles. This solution was thoroughly aspirated and 10 µL of 0.1 M spermine hydrochloride was added and the nanoparticles allowed to aggregate for 1 minute before immediate SERS analysis. Analysis was carried out using an Avalon Ramanstation spectrometer (PerkinElmer, Waltham, MA). The system is equipped with a 100 mW 532 nm diode laser. All measurements were collected for 10 s using a resolution of 2 cm^{-1} over a range of 200-2500 wavenumbers.

(a)



(b)



(c)

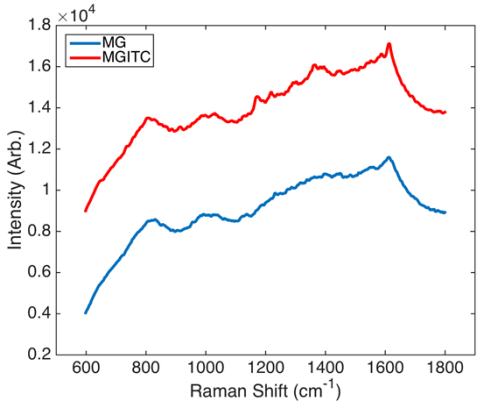


Figure S11. *Stacked plots comparing the SERRS signal of MG and MGITC (both 100 nM) exhibited using* ***(a)*** *AgNP@(**3**),* ***(b)*** *AgNP@(**4a**),* ***(c)*** *AgNP@(**4b**).*

10.0 HRMS, HPLC, ^{1}H and ^{13}C NMR spectra

Figure S12. *HRMS (ESI) spectra of compound (**S1**).*

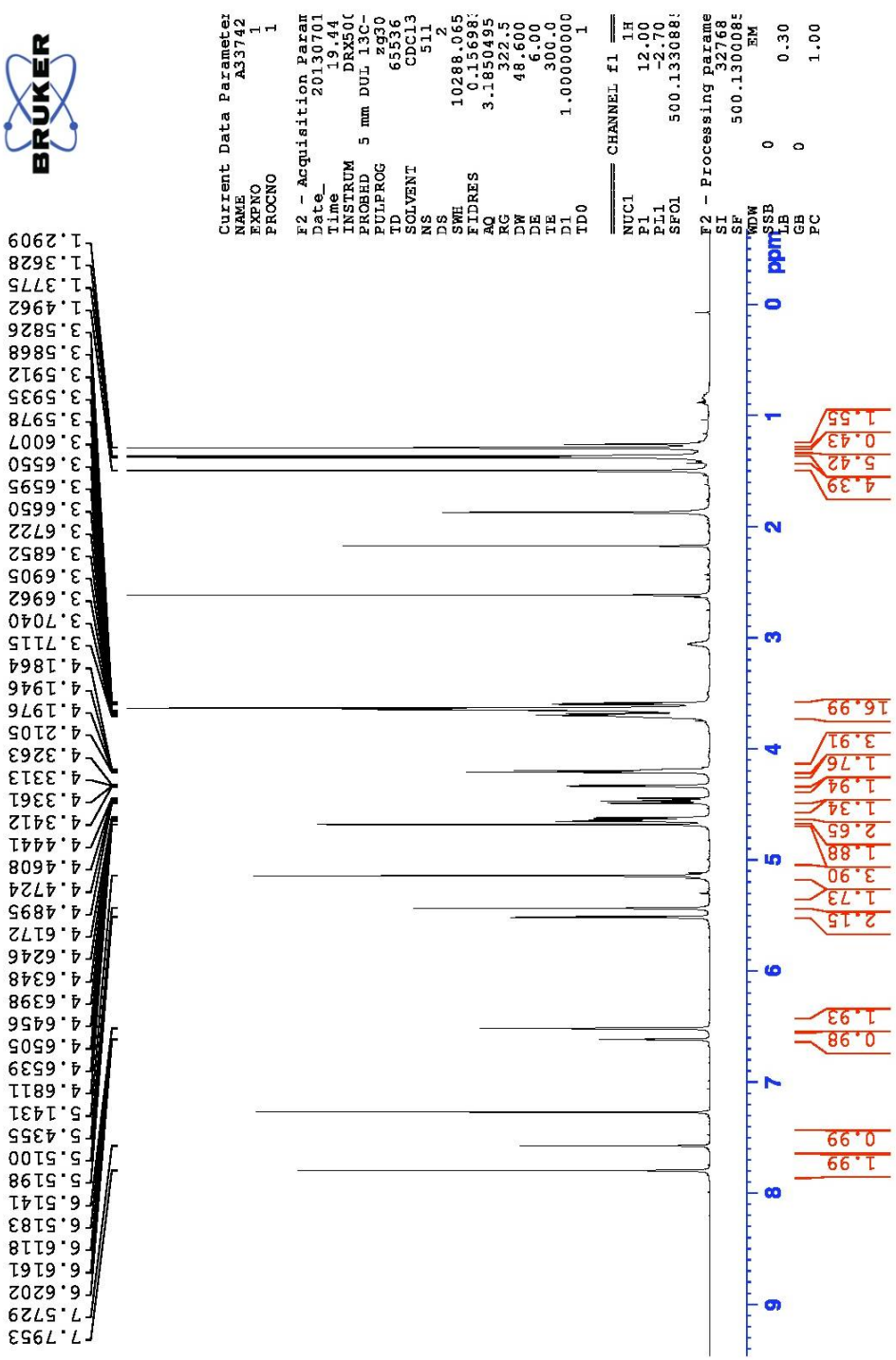


Figure S13. *[1]H NMR spectrum of compound (**S1**).*

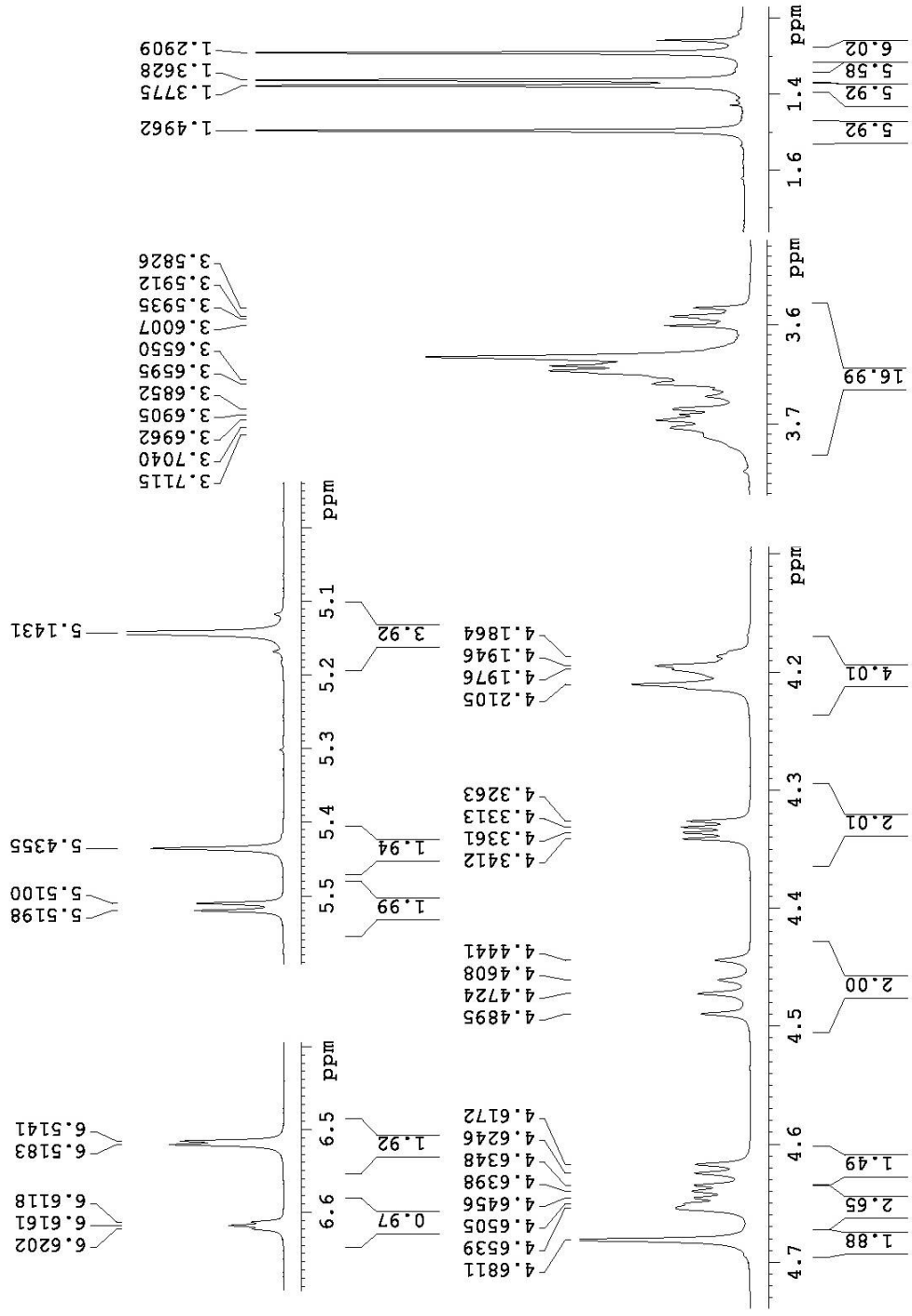


Figure S14. *Selected areas [1]H NMR of compound (**S1**).*

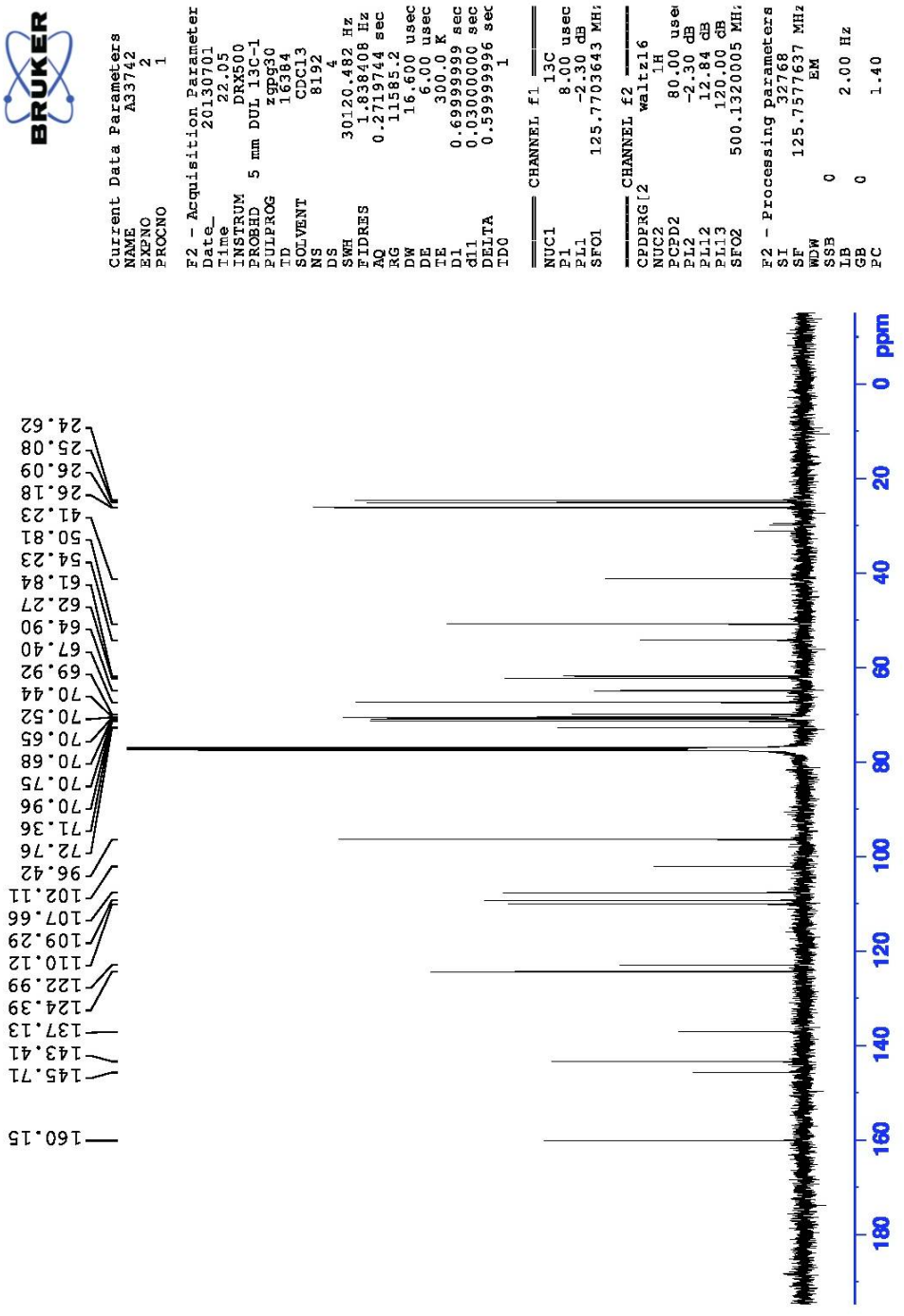


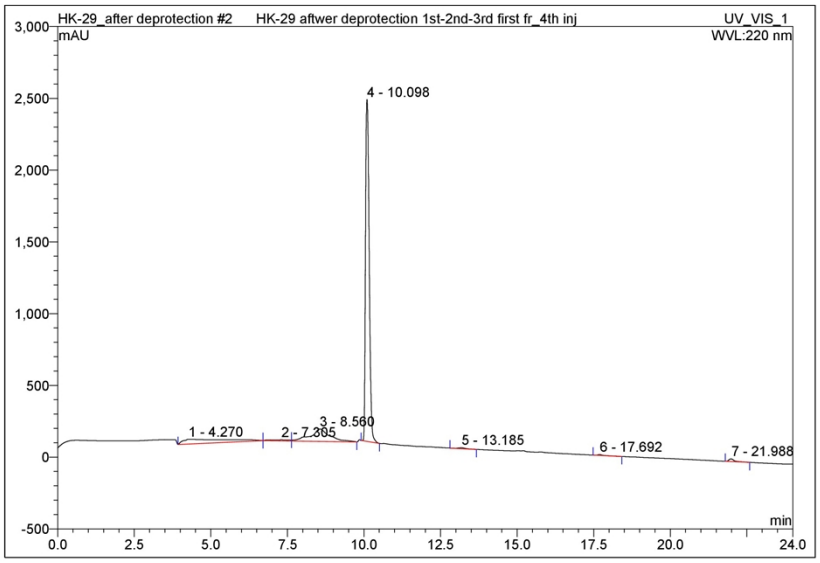
Figure S15. *^{13}C NMR spectrum of compound (**S1**).*

Operator:Administrator Timebase:analyticalhplc Sequence:HK-29_after deprotection

Page 1-1
21/2/2015 5:48 PM

2 HK-29 aftwer deprotection 1st-2nd-3rd first fr_4th inj

Sample Name:	**HK-29 aftwer deprotection 1st-2nd-3rd first fr_4th inj**	*Injection Volume:*	**20.0**
Vial Number:	**RA2**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**220**
Control Program:	**poly-p4 28min +230nm**	*Bandwidth:*	**10**
Quantif. Method:	**dna method**	*Dilution Factor:*	**1.0000**
Recording Time:	**3/7/2013 17:32**	*Sample Weight:*	**1.0000**
Run Time (min):	**24.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4.27	n.a.	32.958	49.937	10.92	n.a.	BMB
2	7.31	n.a.	6.678	5.158	1.13	n.a.	bM
3	8.56	n.a.	85.808	63.372	13.86	n.a.	MB
4	10.10	n.a.	2382.633	332.268	72.66	n.a.	BMB
5	13.19	n.a.	6.096	1.637	0.36	n.a.	BMB
6	17.69	n.a.	6.034	1.129	0.25	n.a.	BMB
7	21.99	n.a.	18.242	3.809	0.83	n.a.	BMB
Total:			2538.449	457.310	100.00	0.000	

default/Integration

Chromeleon (c) Dionex 1996-2006
Version 6.80 SP4 Build 2361 (130805)

Figure S16. *HPL chromatogram of compound (**3**).*

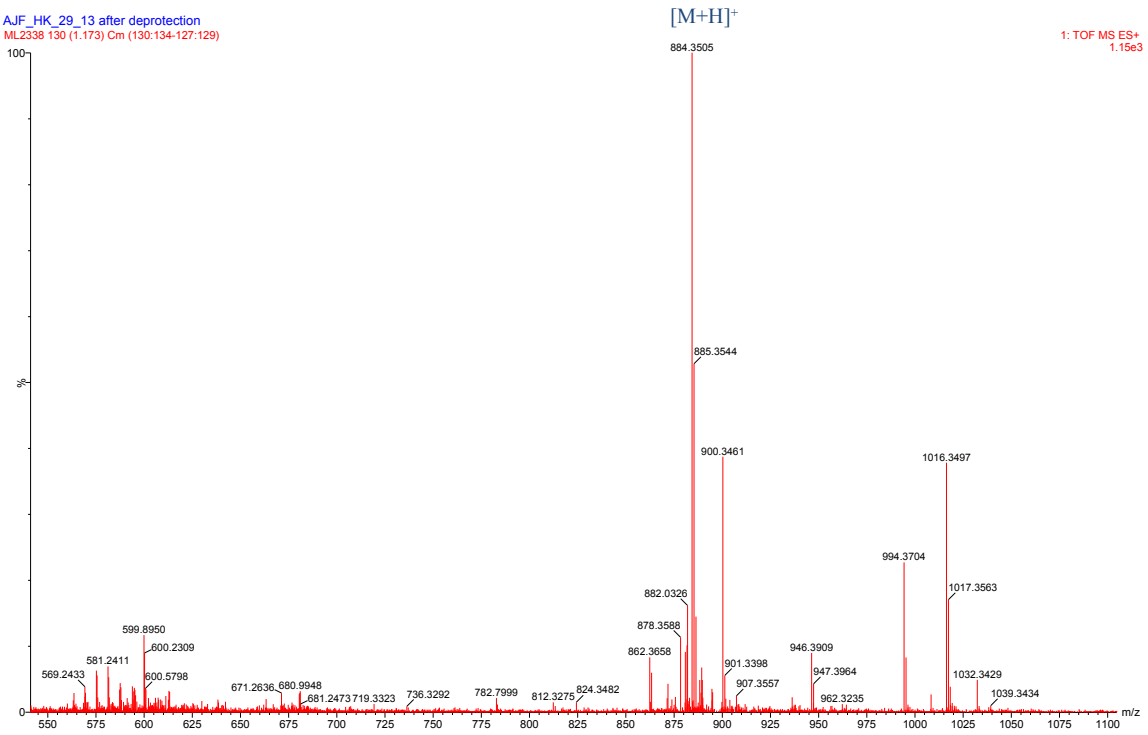


Figure S17. *HRMS (ESI) spectra of compound (**3**).*

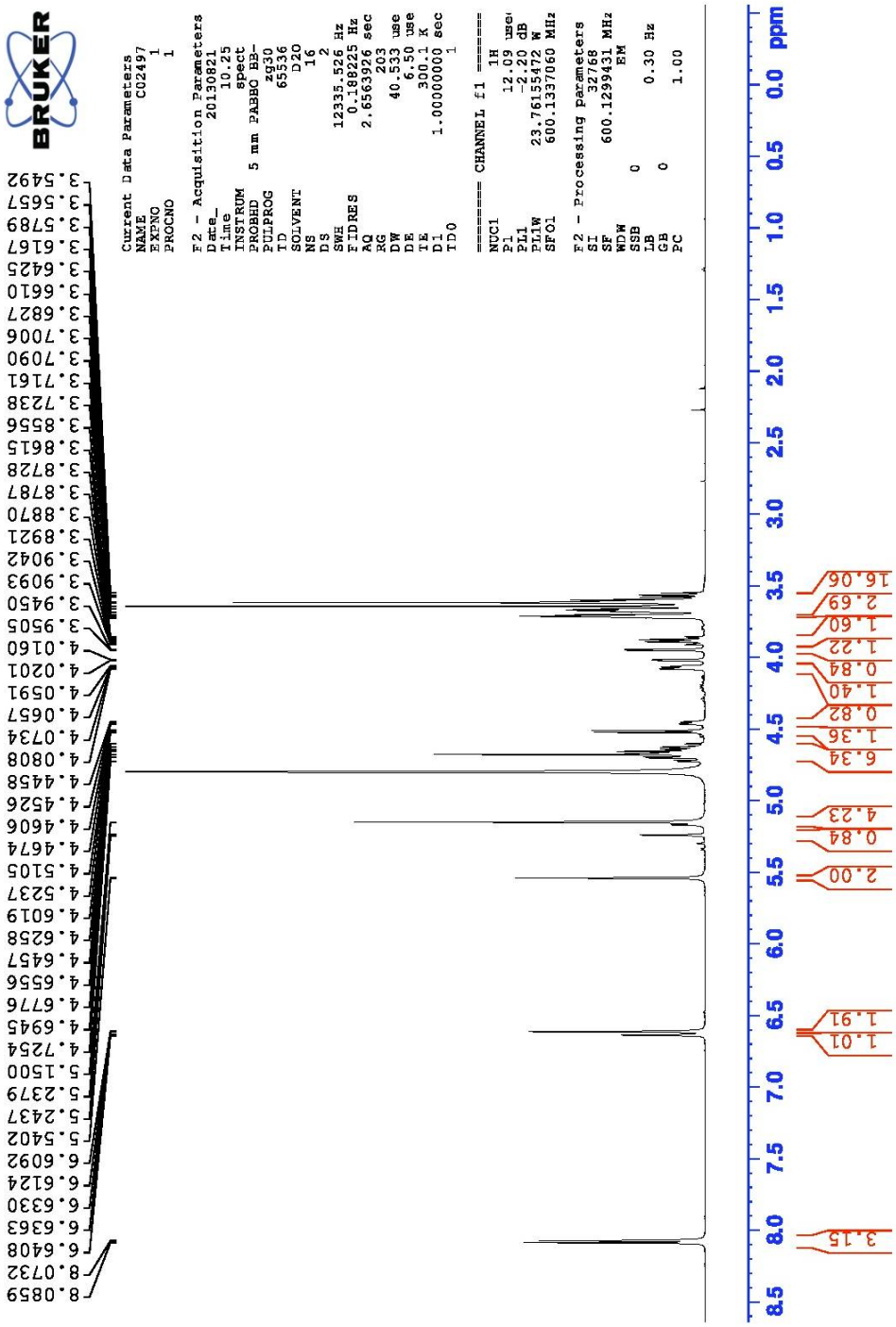


Figure S18. *[1]H NMR spectrum of compound (**3**).*

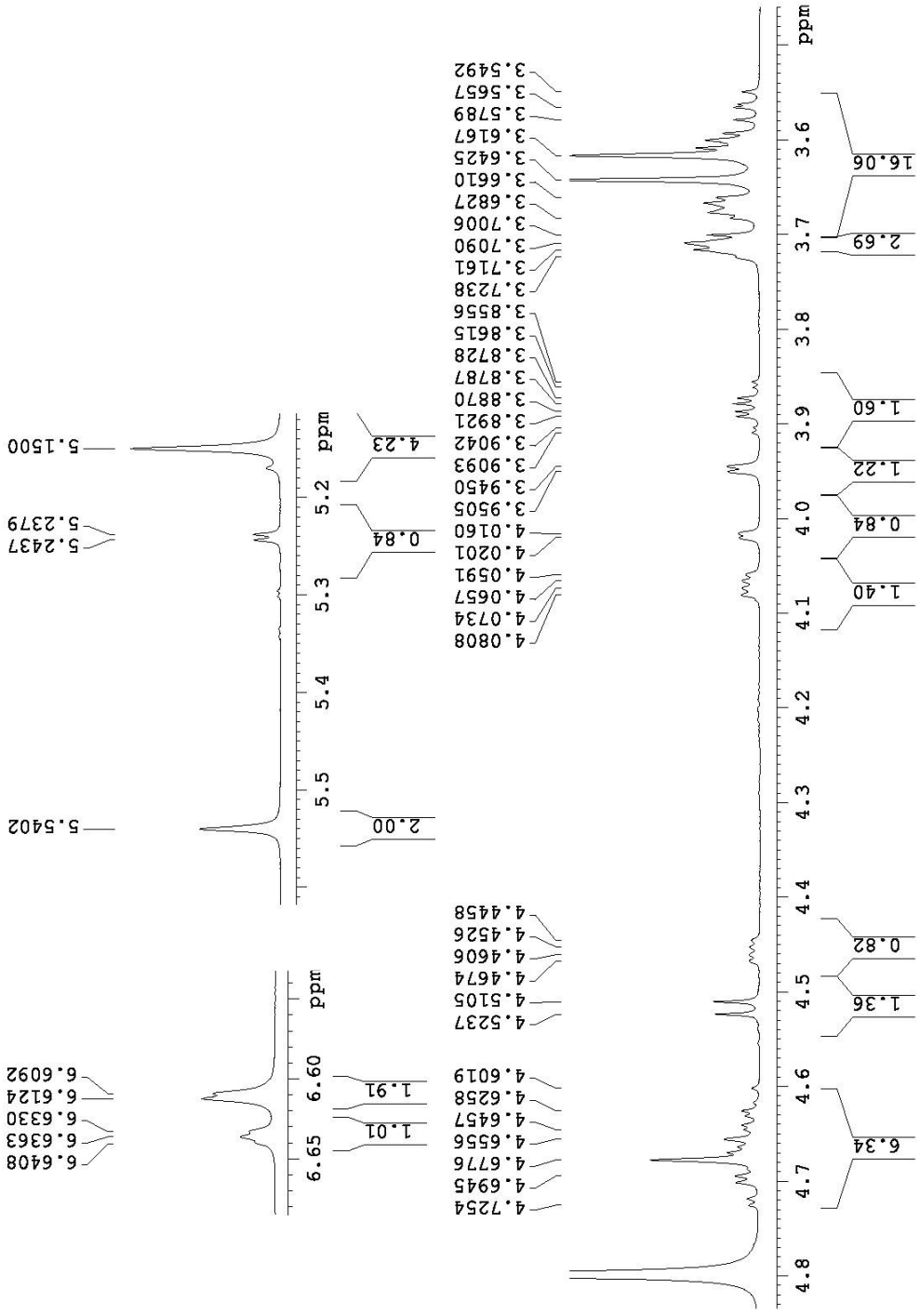


Figure S19. *Selected areas [1]H NMR of compound (**3**).*

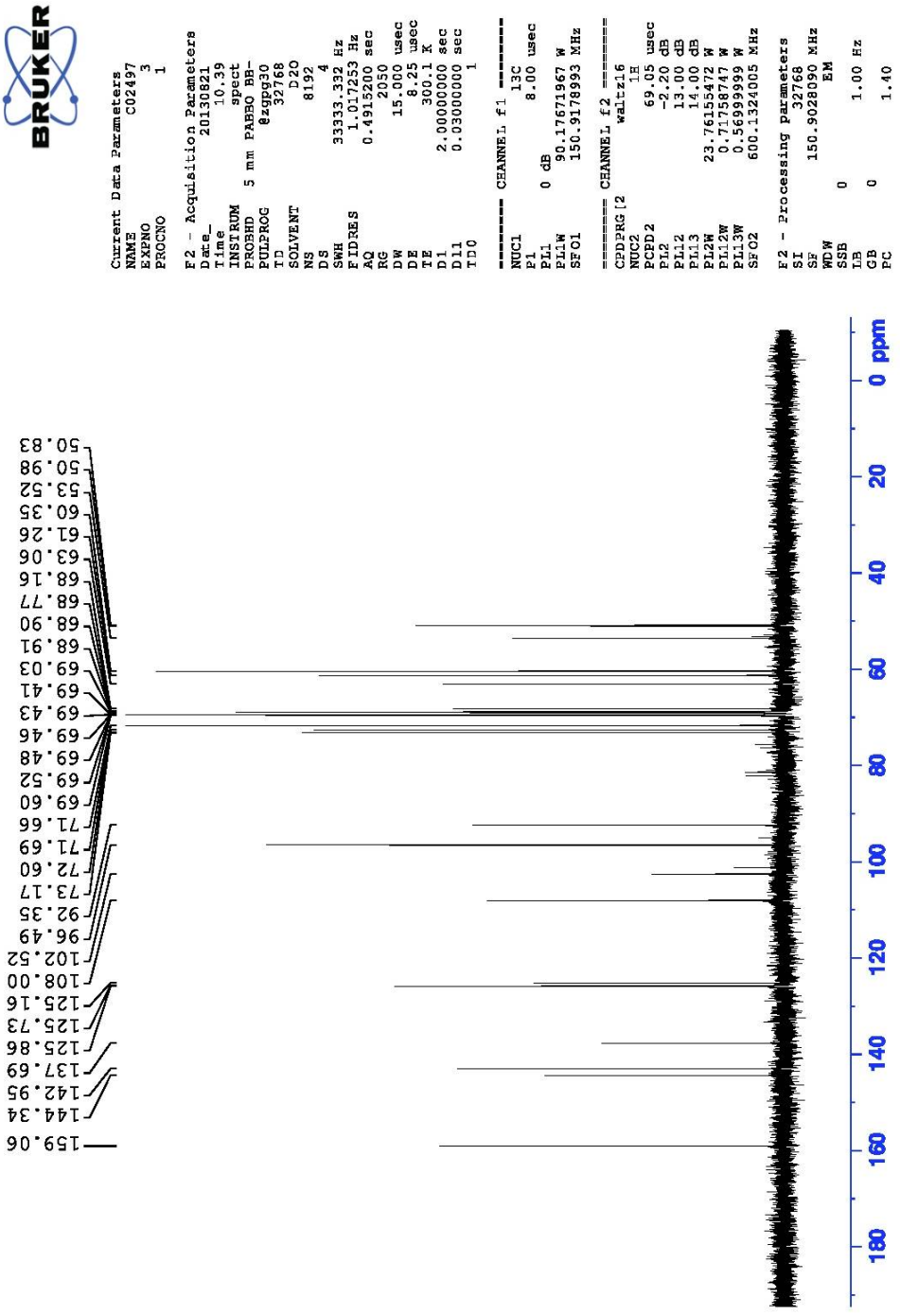


Figure S20. *^{13}C NMR spectrum of compound (**3**).*

Figure S21. *HRMS (ESI) spectra of compound (**S2a**).*

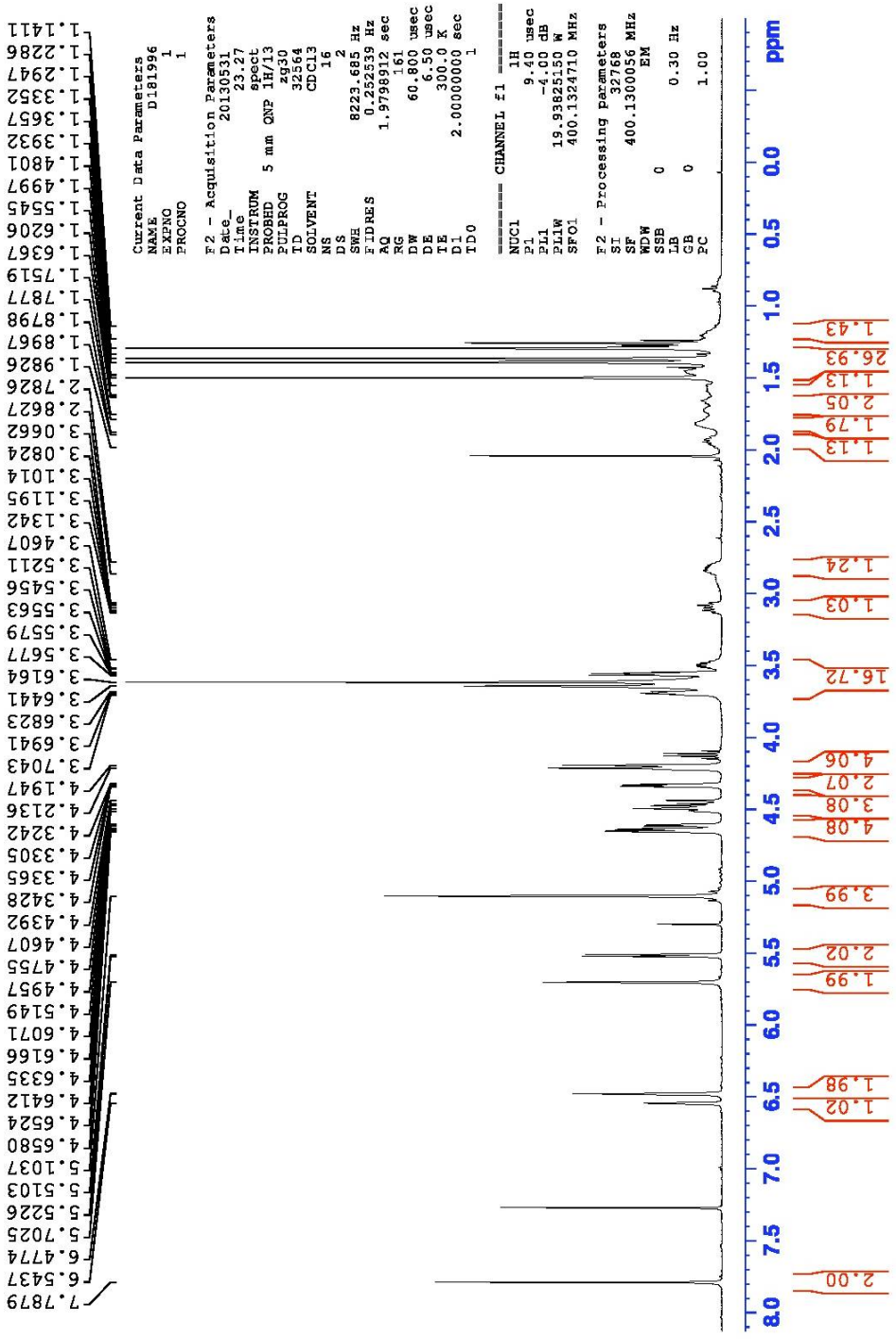


Figure S22. *[1]H NMR spectrum of compound (**S2a**).*

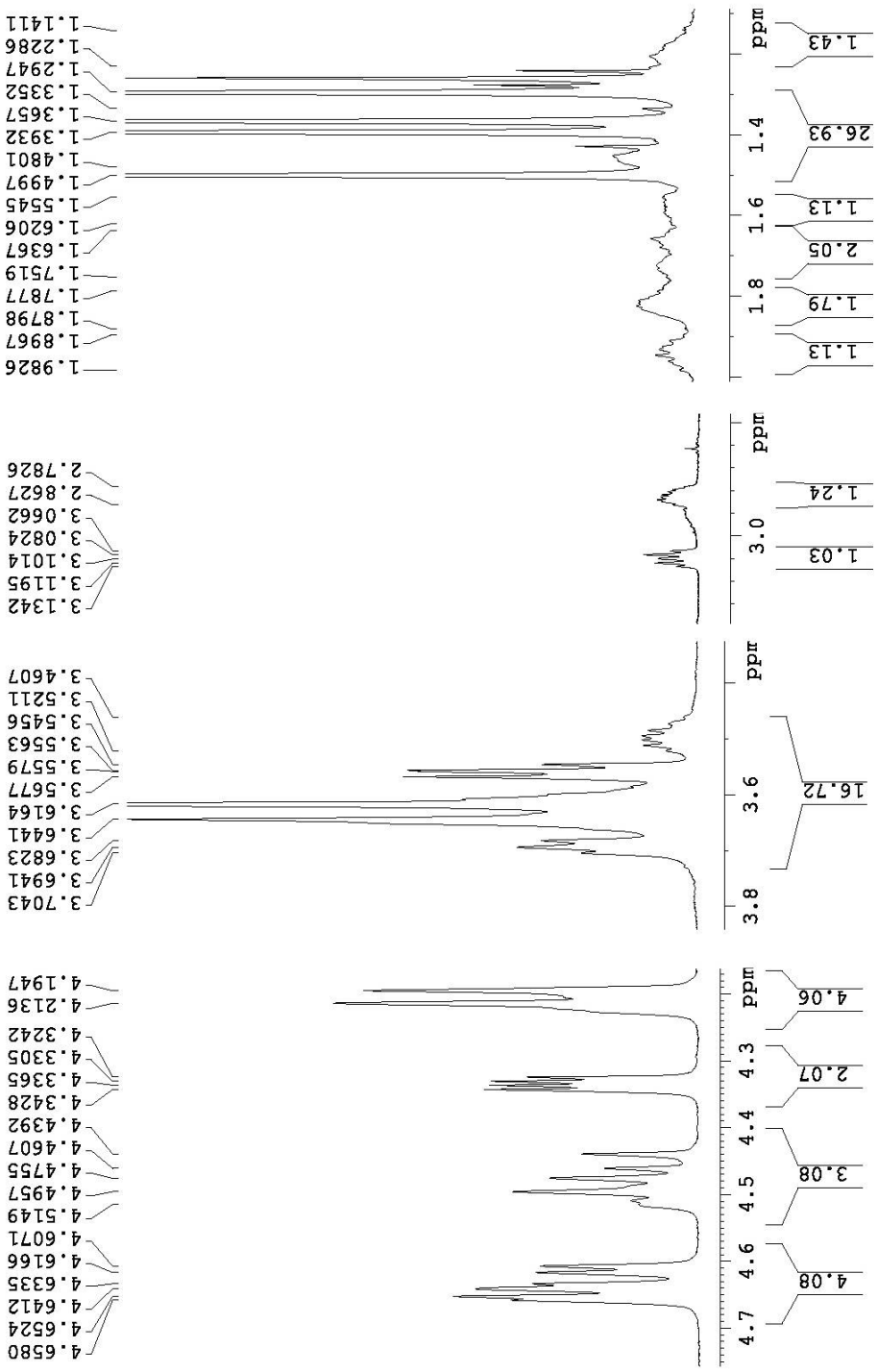


Figure S23. *Selected areas [1]H NMR of compound (**S2a**).*

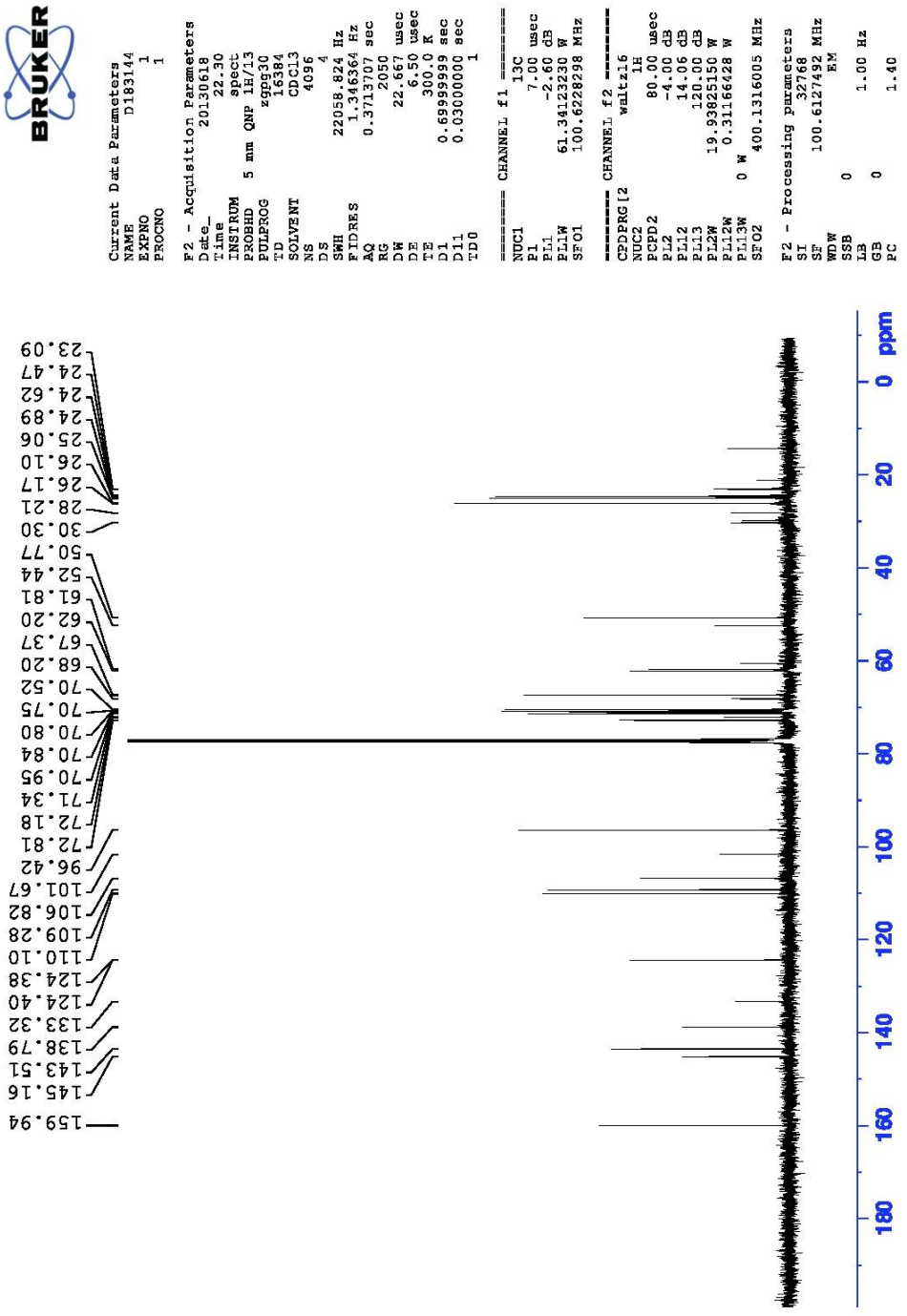


Figure S24. *^{13}C NMR spectrum of compound (**S2a**).*

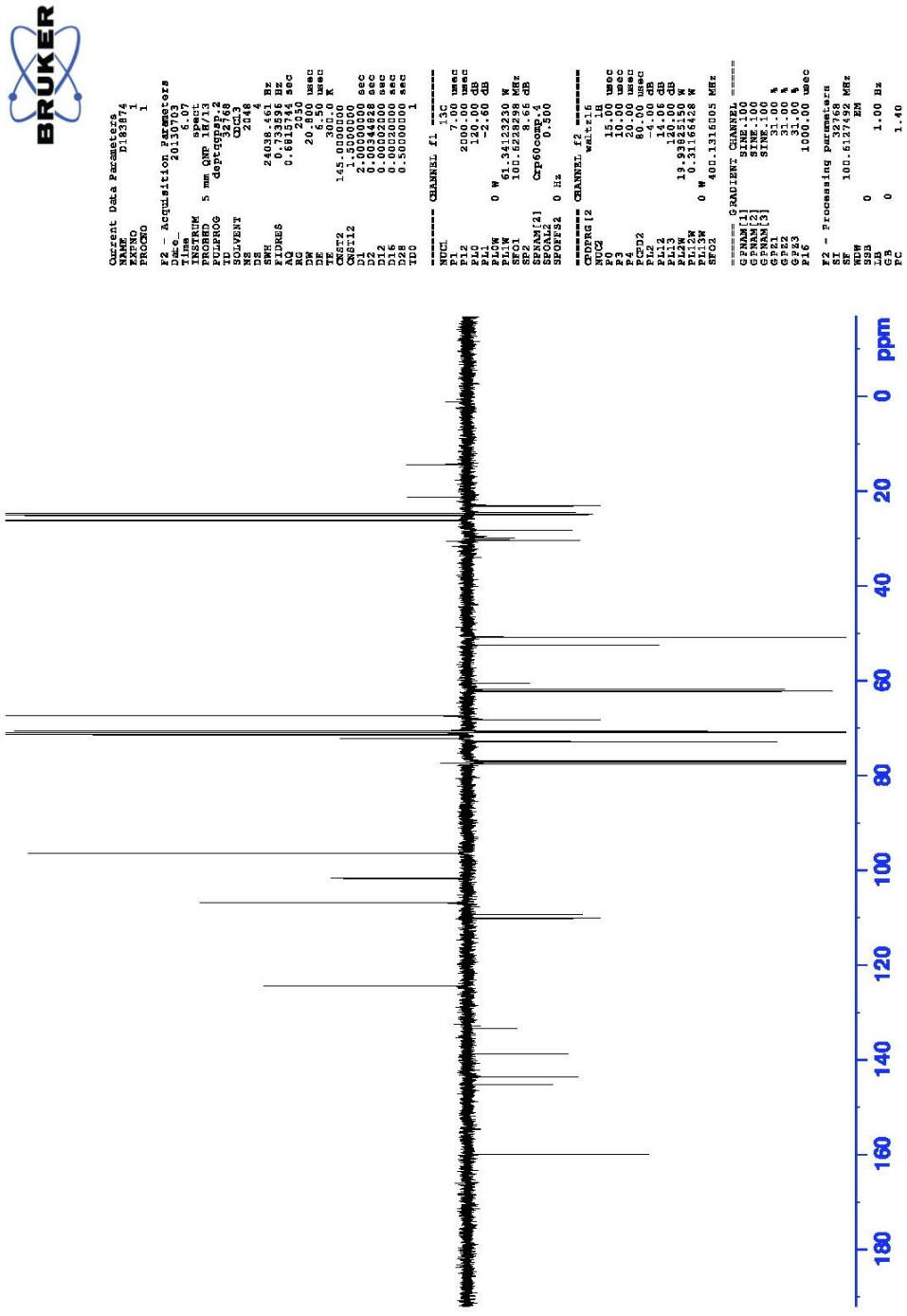


Figure S25. *DEPT C- NMR spectrum of compound (**S2a**).*

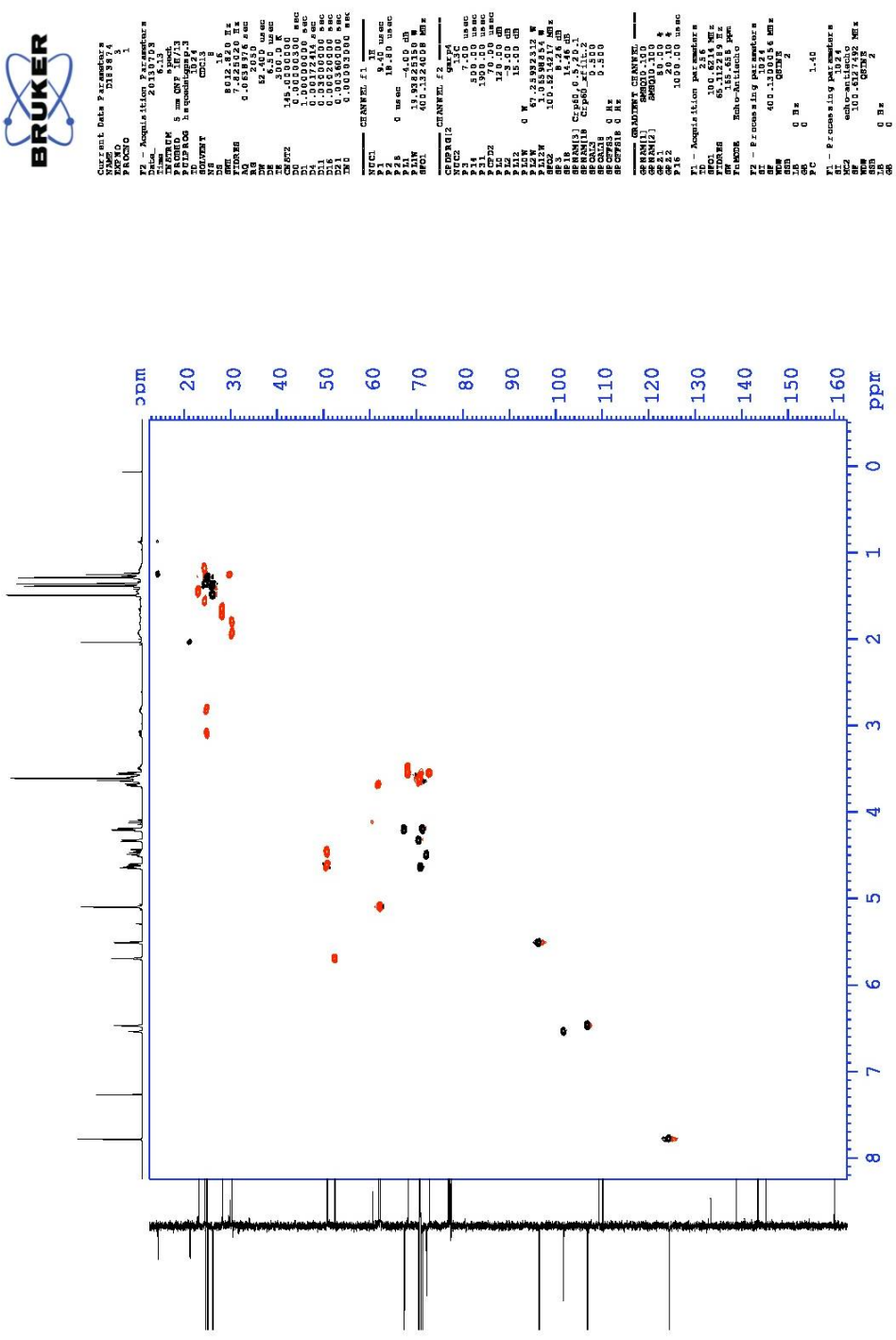


Figure S26. *HSQC NMR spectrum of compound (**S2a**).*

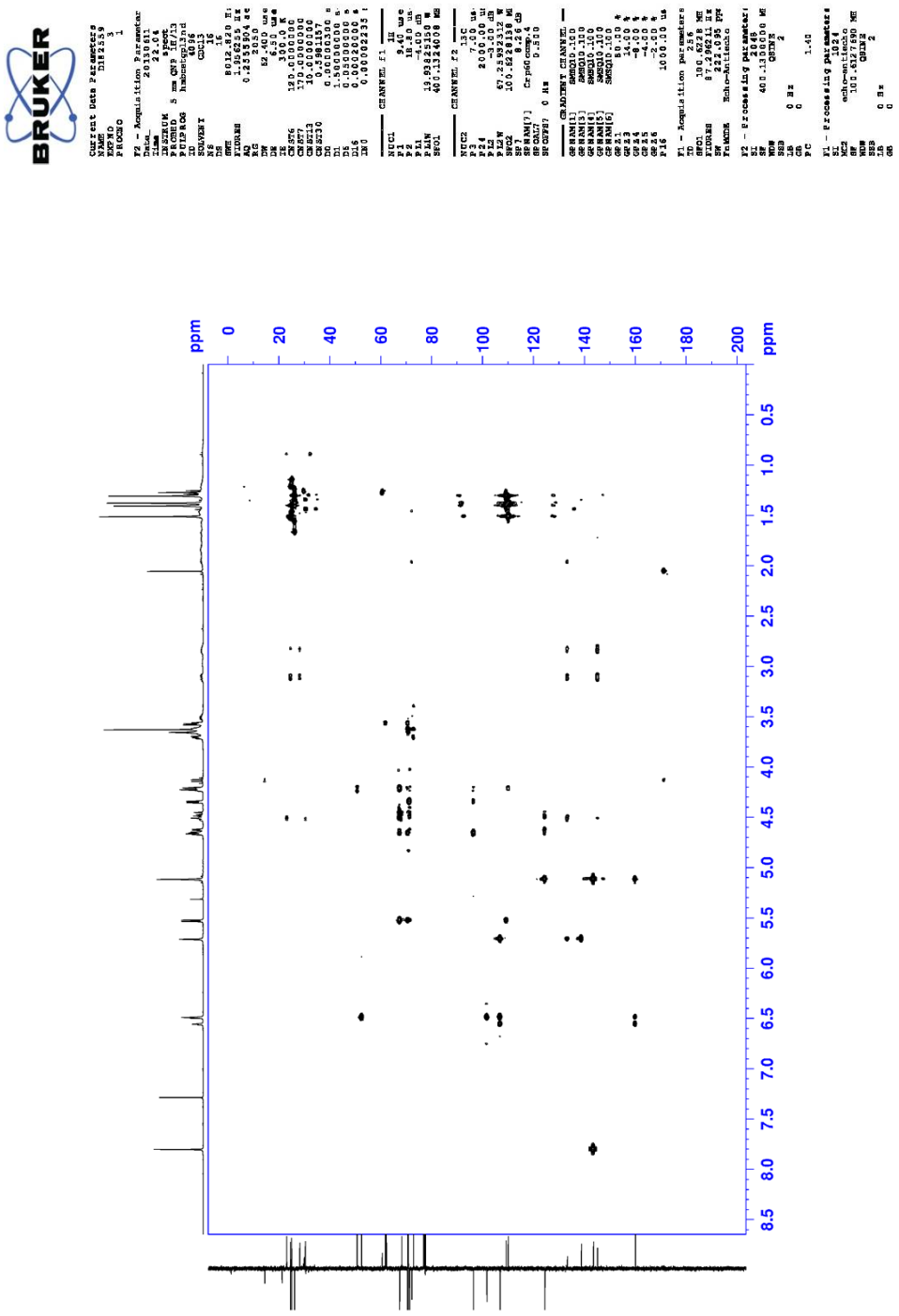


Figure S27. *HMBC NMR spectrum of compound (**S2a**).*

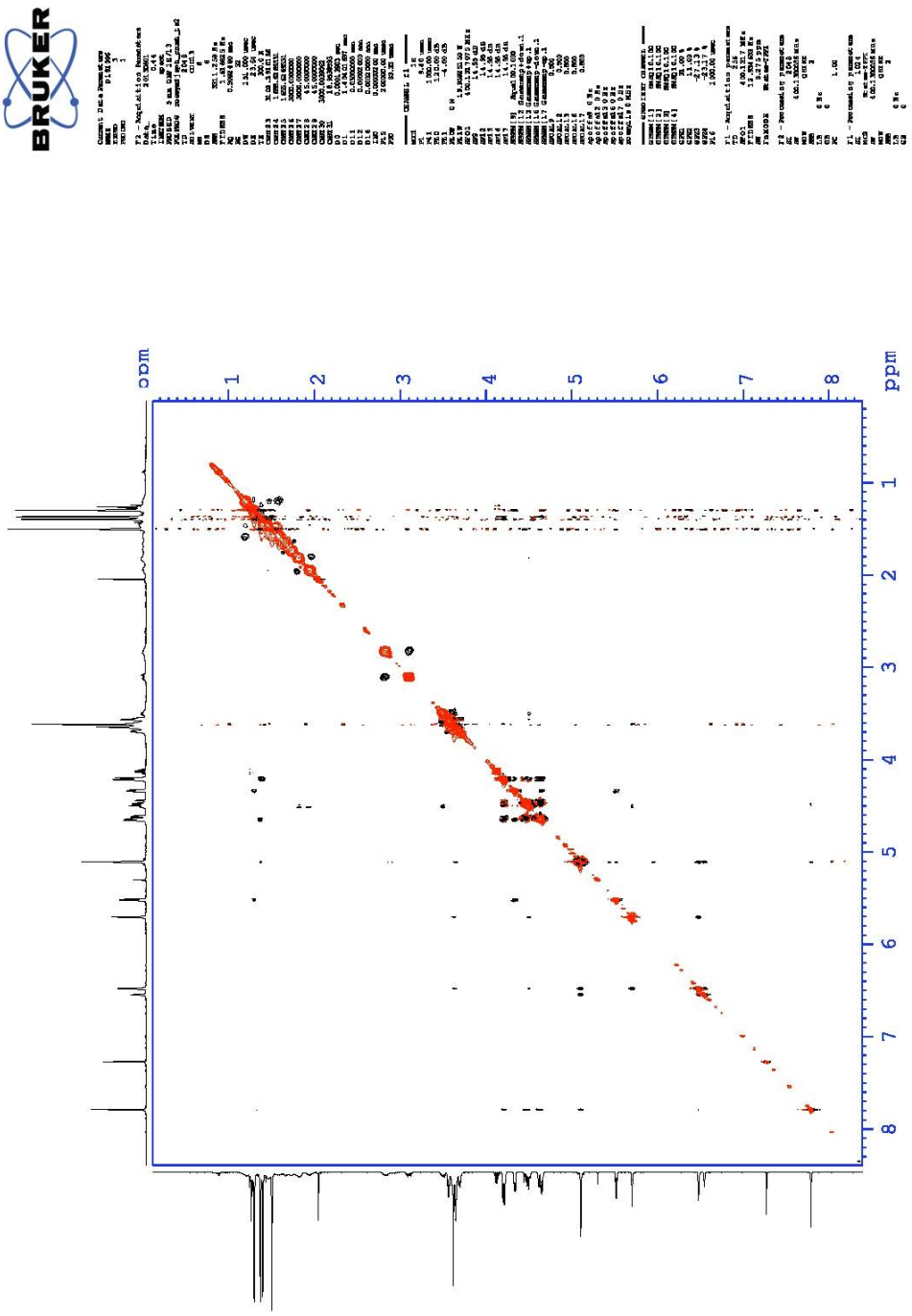


Figure S28. *ROSY NMR spectrum of compound (**S2a**).*

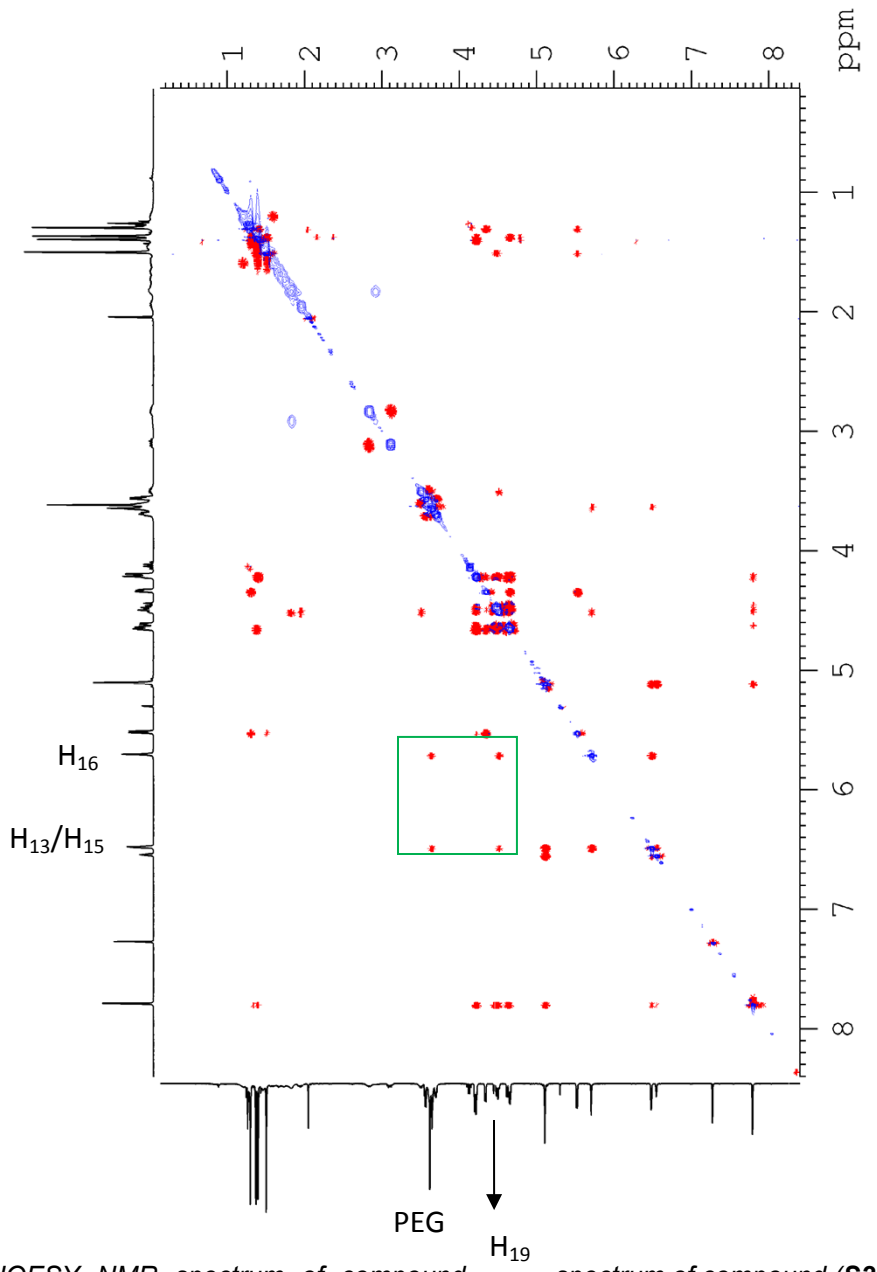


Figure S 29. *NOESY NMR spectrum of compound spectrum of compound (**S2a**).*

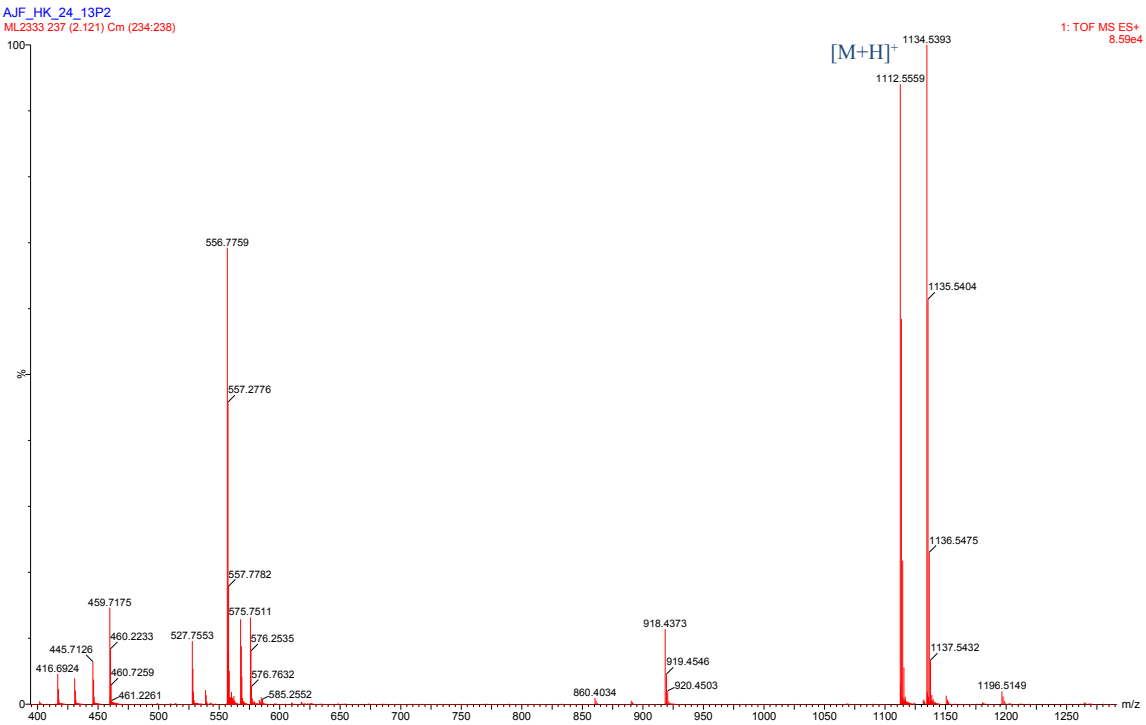


Figure S30. *HRMS (ESI) spectra of compound (**S2b**).*

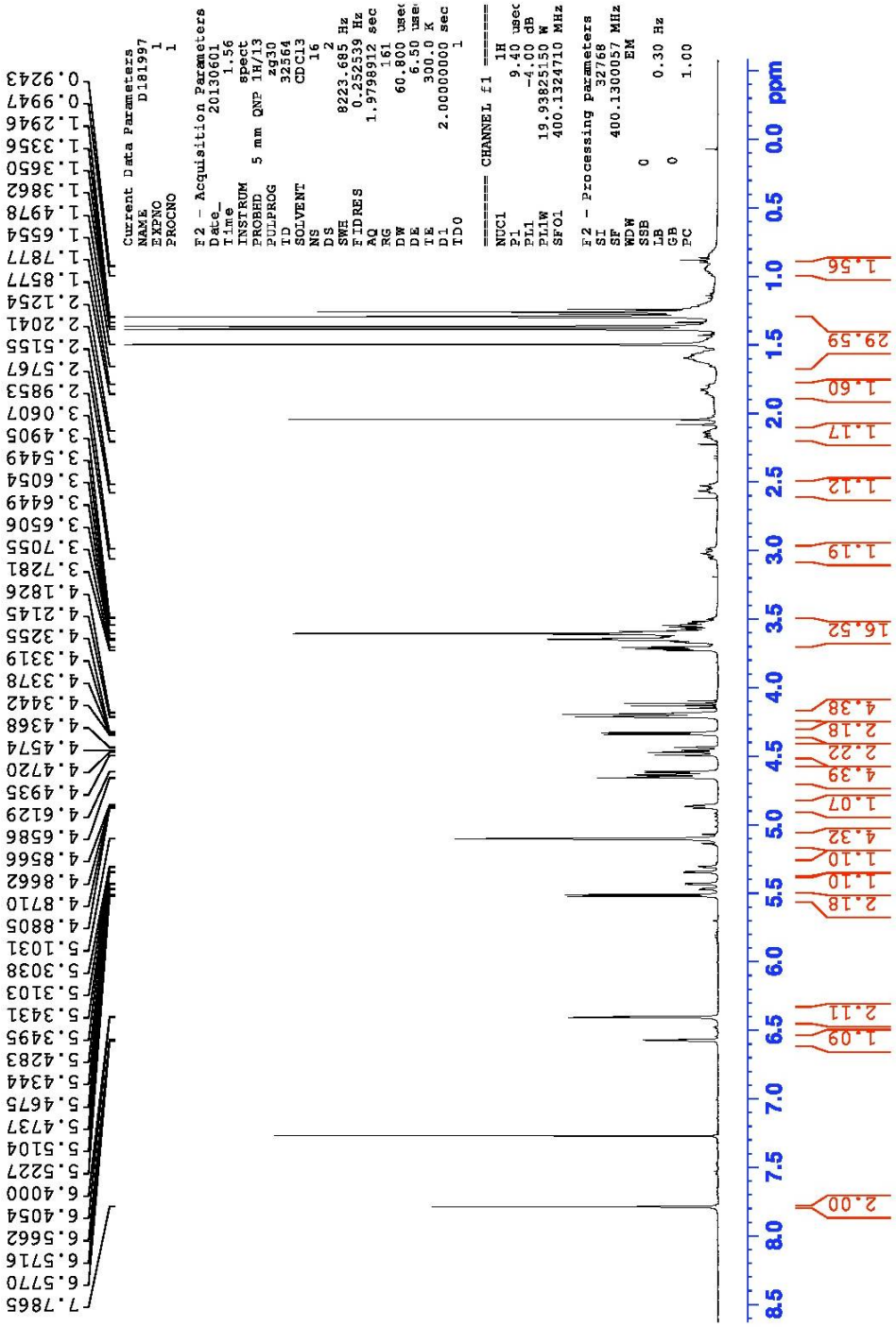


Figure S31. *^{1}H NMR spectrum of compound (**S2b**).*

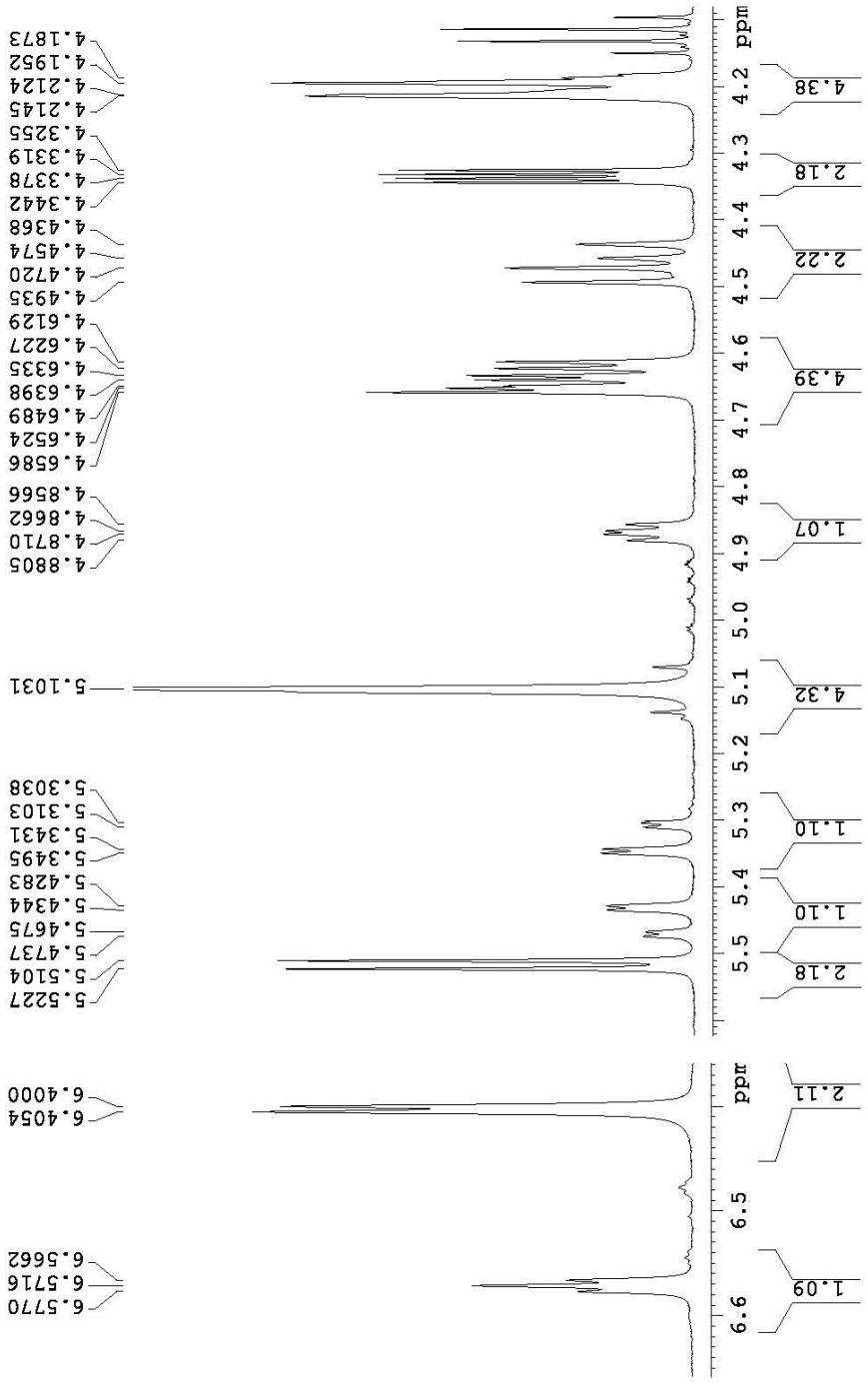


Figure S32. *Selected areas [1]H NMR of compound (**S2b**).*

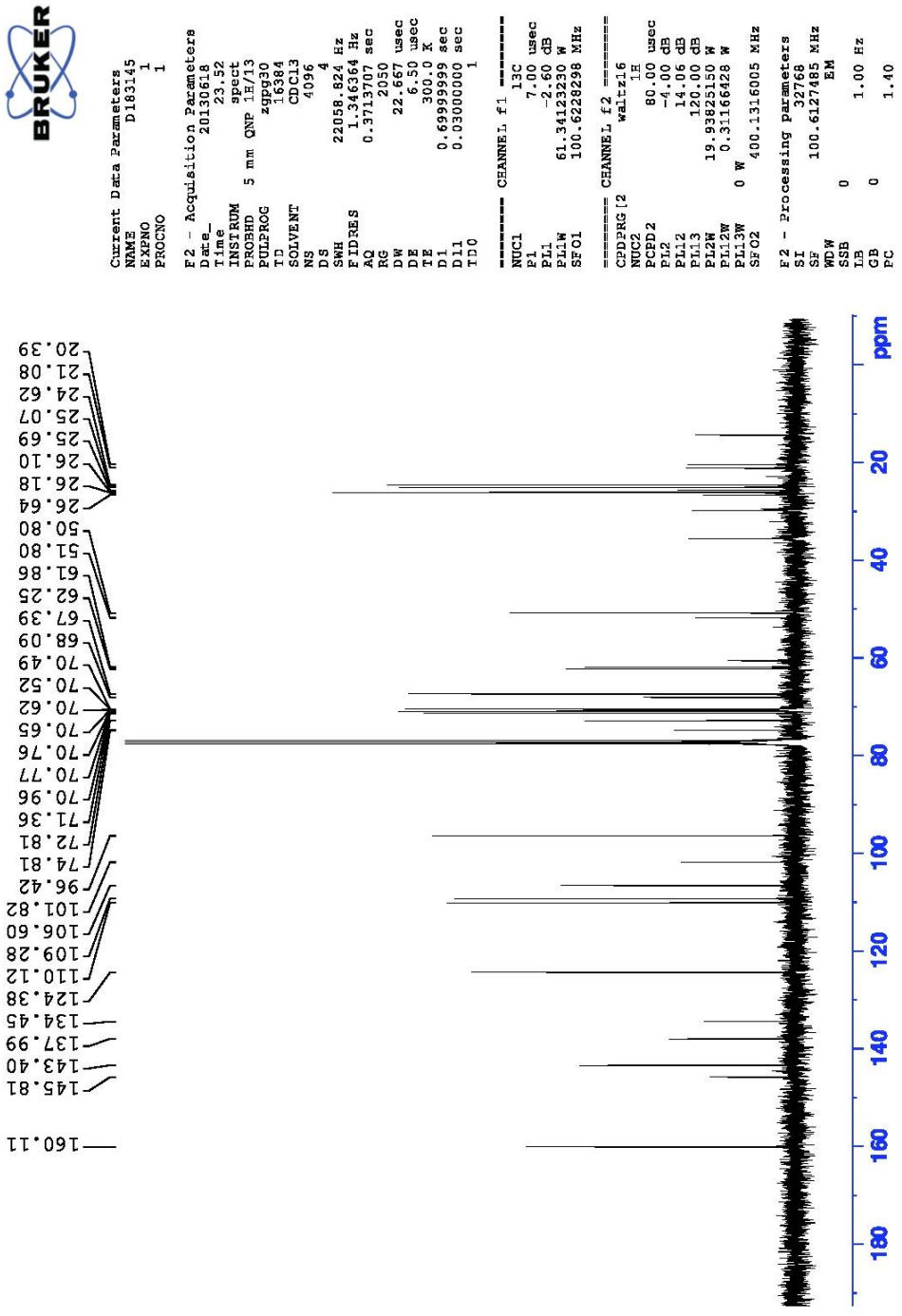


Figure S33. *^{13}C NMR spectrum of compound (**S2b**).*

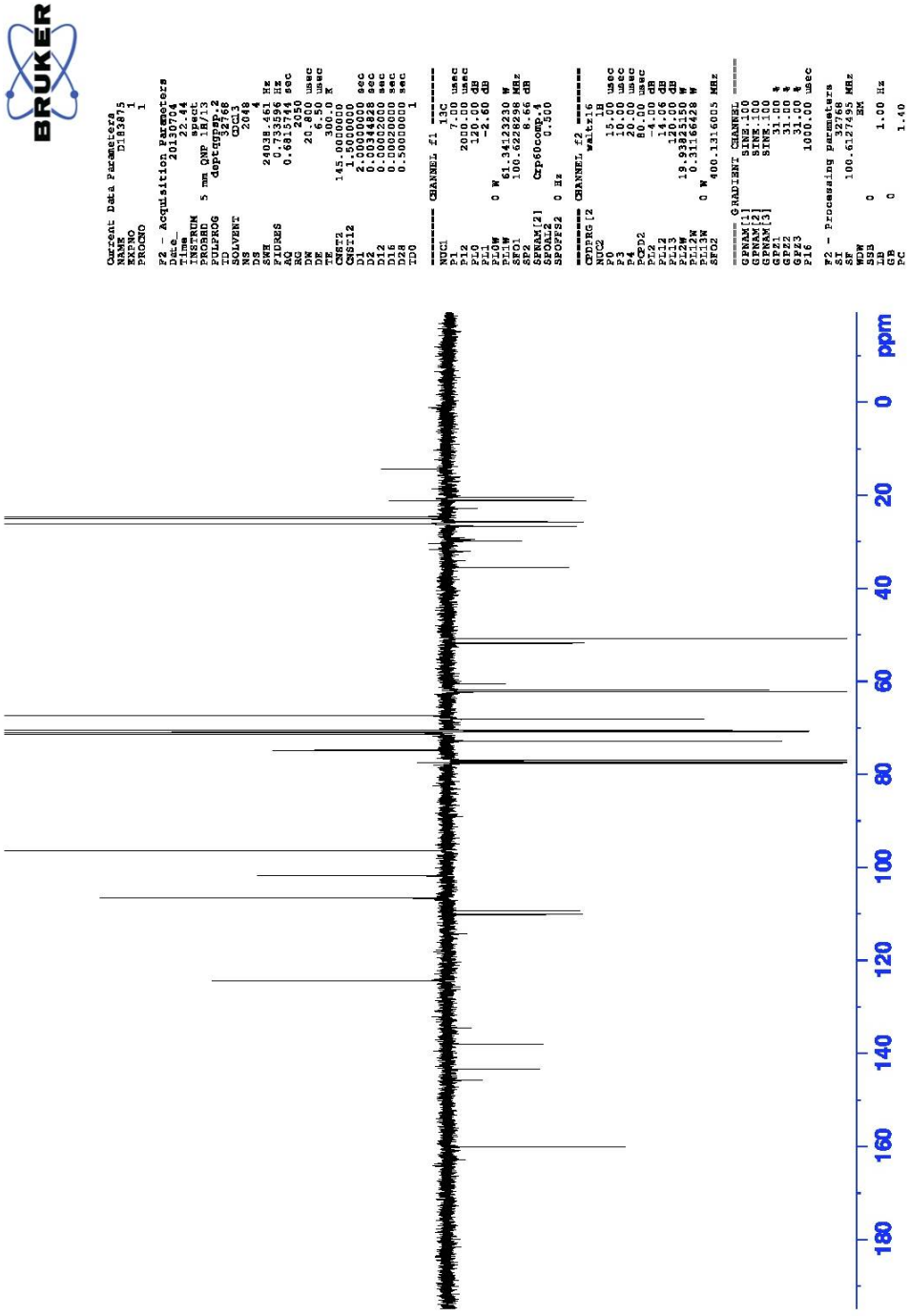


Figure S34. *DEPT C- NMR spectrum of compound (**S2b**).*

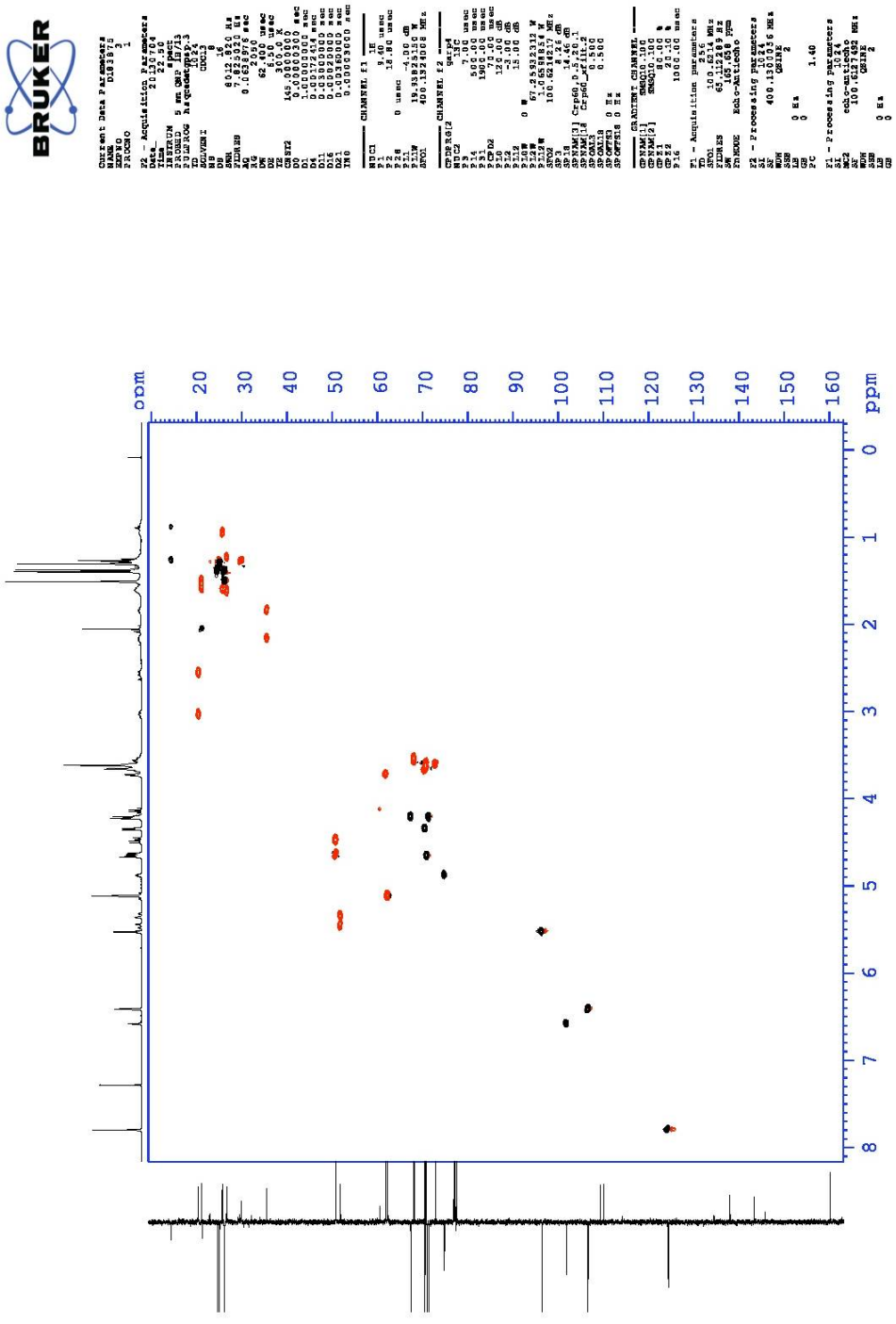


Figure S35. *HSQC NMR spectrum of compound (**S2b**).*

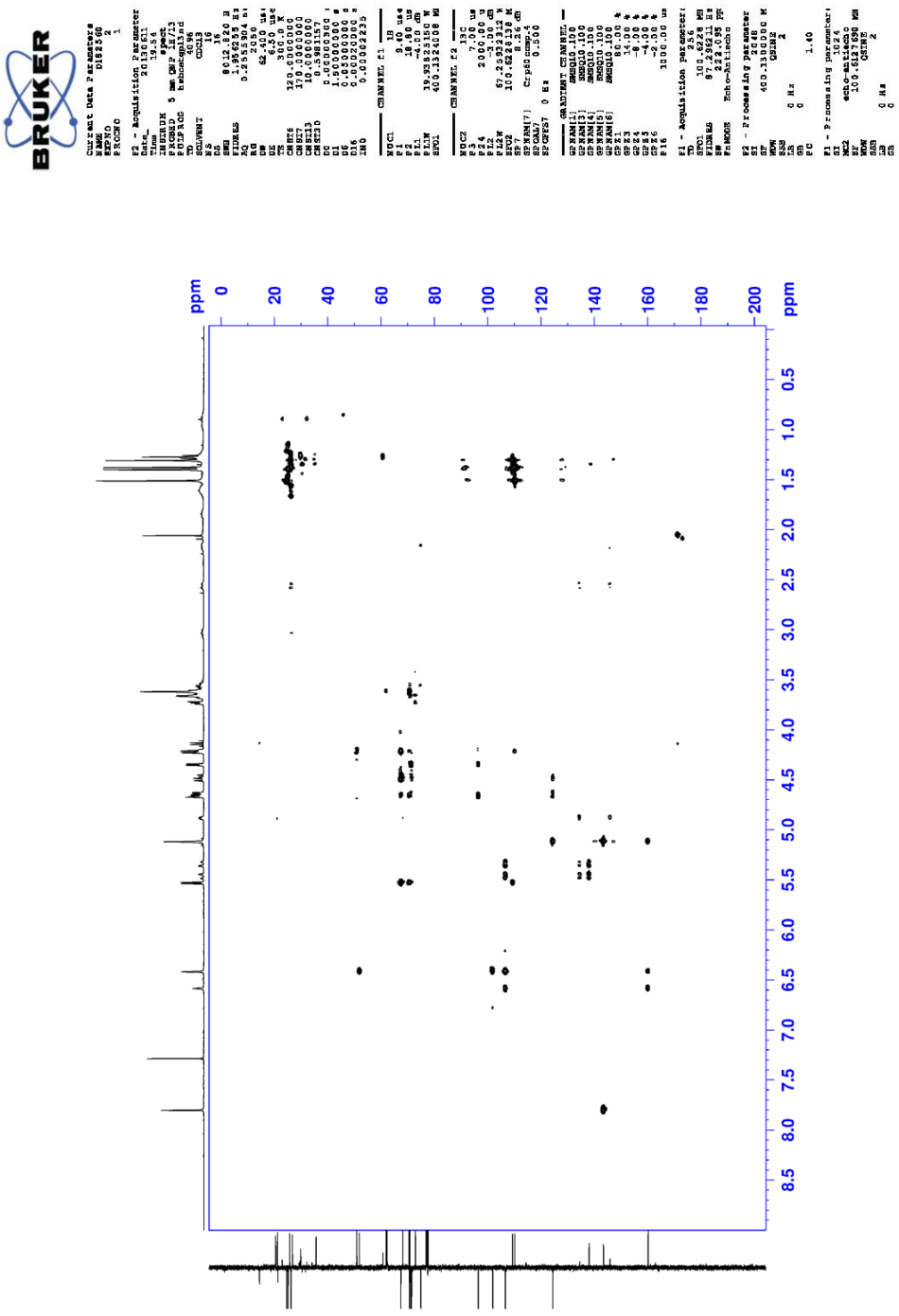


Figure S36. *HMBC NMR spectrum of compound (**S2b**).*

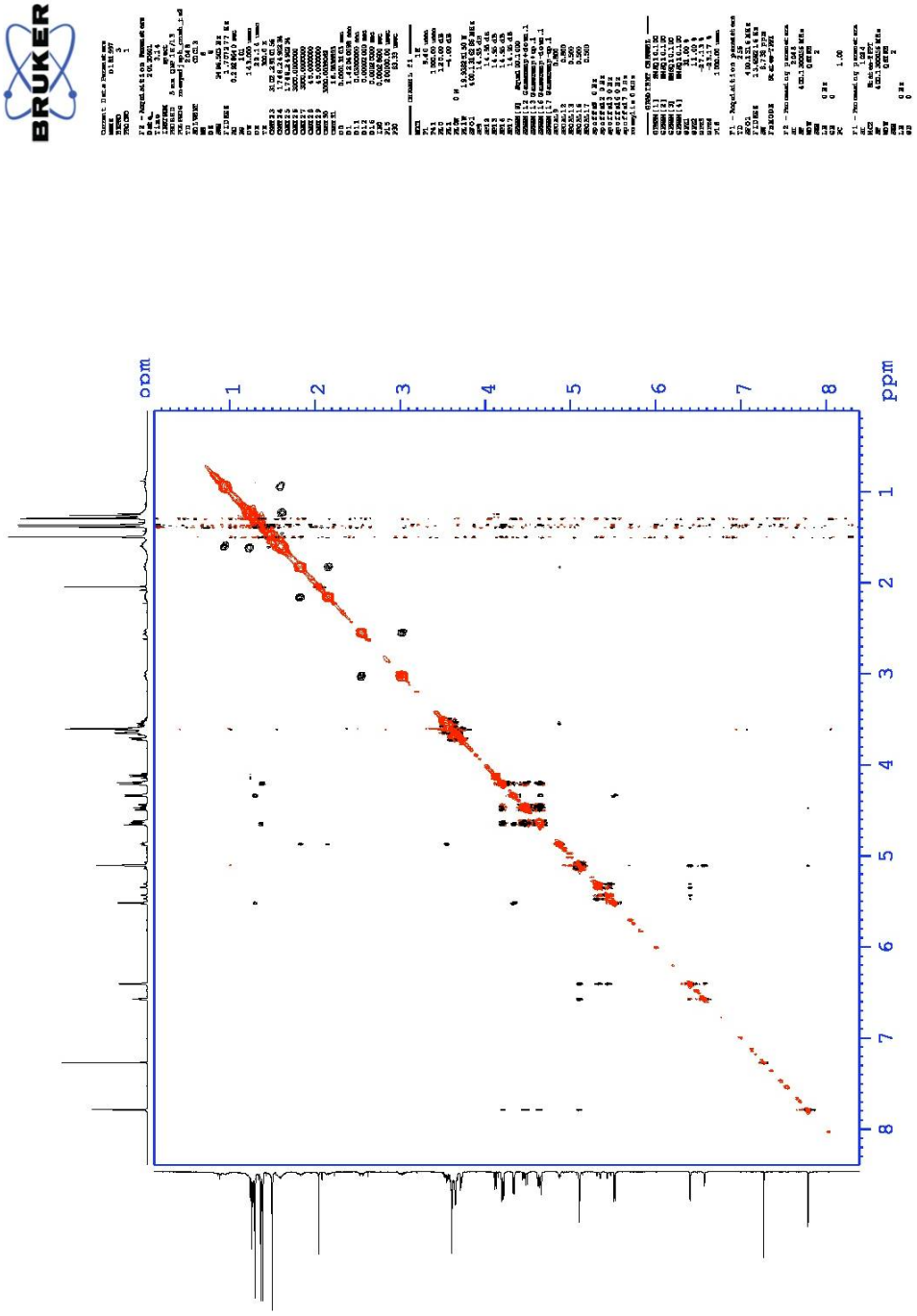


Figure S37. *ROSY NMR spectrum of compound (**S2b**).*

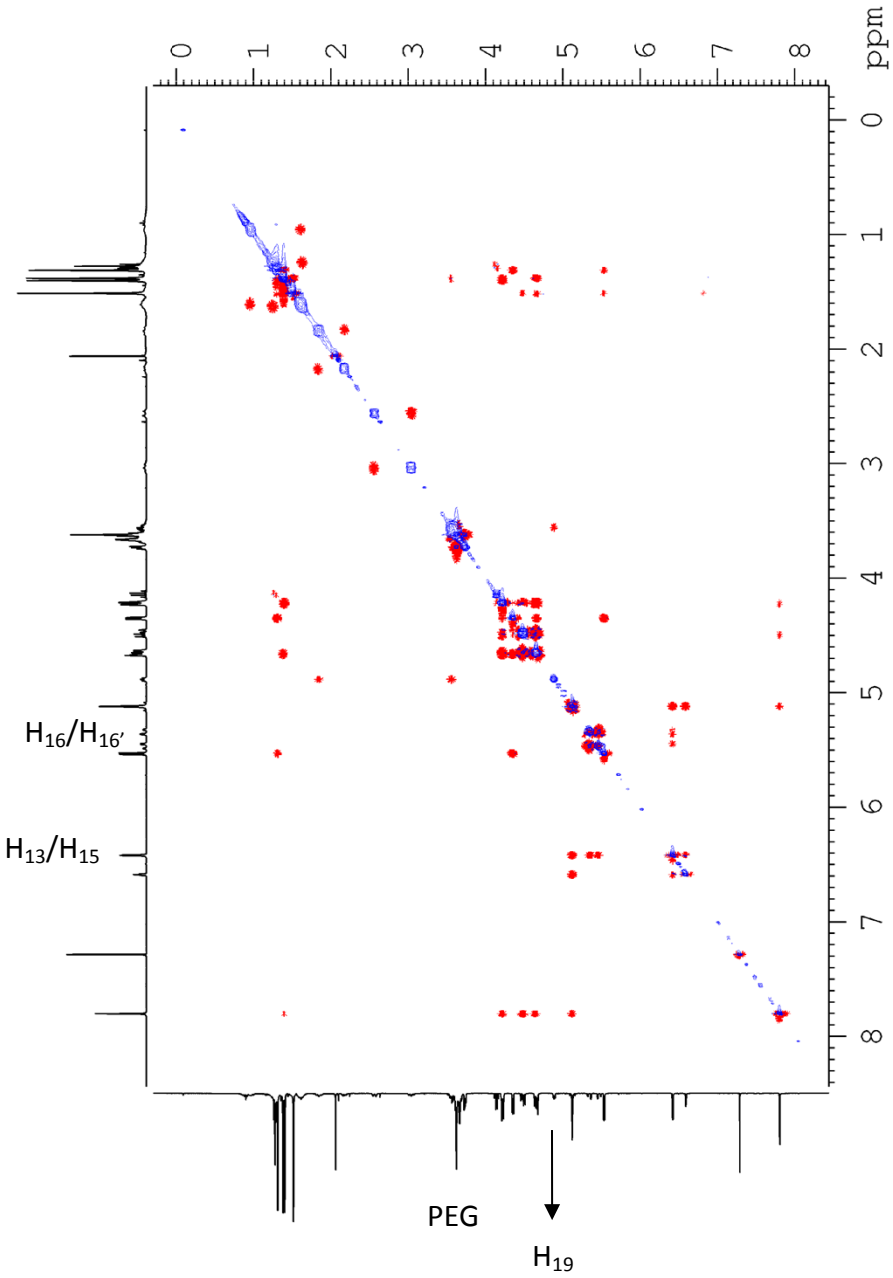


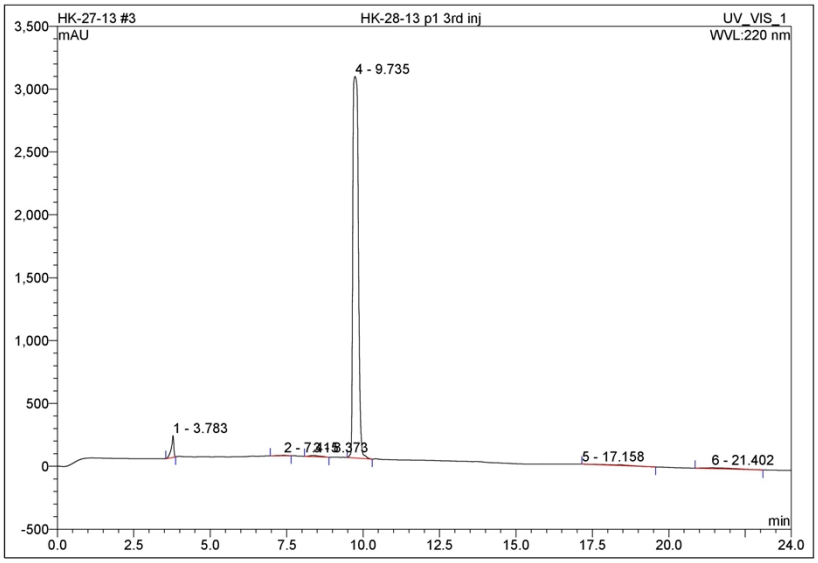
Figure S38. *NOESY NMR spectrum of compound (**S2b**).*

Operator:Administrator Timebase:analyticalhplc Sequence:HK-27-13

Page 1-1
21/2/2015 5:46 PM

3 HK-28-13 p1 3rd inj

Sample Name:	**HK-28-13 p1 3rd inj**	*Injection Volume:*	**20.0**
Vial Number:	**RA3**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**220**
Control Program:	**poly-p4 28min +230nm**	*Bandwidth:*	**10**
Quantif. Method:	**dna method**	*Dilution Factor:*	**1.0000**
Recording Time:	**24/6/2013 17:49**	*Sample Weight:*	**1.0000**
Run Time (min):	**24.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.78	n.a.	173.268	16.223	2.42	n.a.	BMB
2	7.42	n.a.	3.950	1.622	0.24	n.a.	BMB
3	8.37	n.a.	10.140	4.226	0.63	n.a.	BMB
4	9.74	n.a.	3033.827	633.805	94.59	n.a.	BMB
5	17.16	n.a.	0.000	5.923	0.88	n.a.	BMB
6	21.40	n.a.	6.434	8.236	1.23	n.a.	BMB
Total:			3227.620	670.035	100.00	0.000	

default/Integration

Chromeleon (c) Dionex 1996-2006
Version 6.80 SP4 Build 2361 (130805)

Figure S39. *HPL chromatogram of compound (**4a**).*

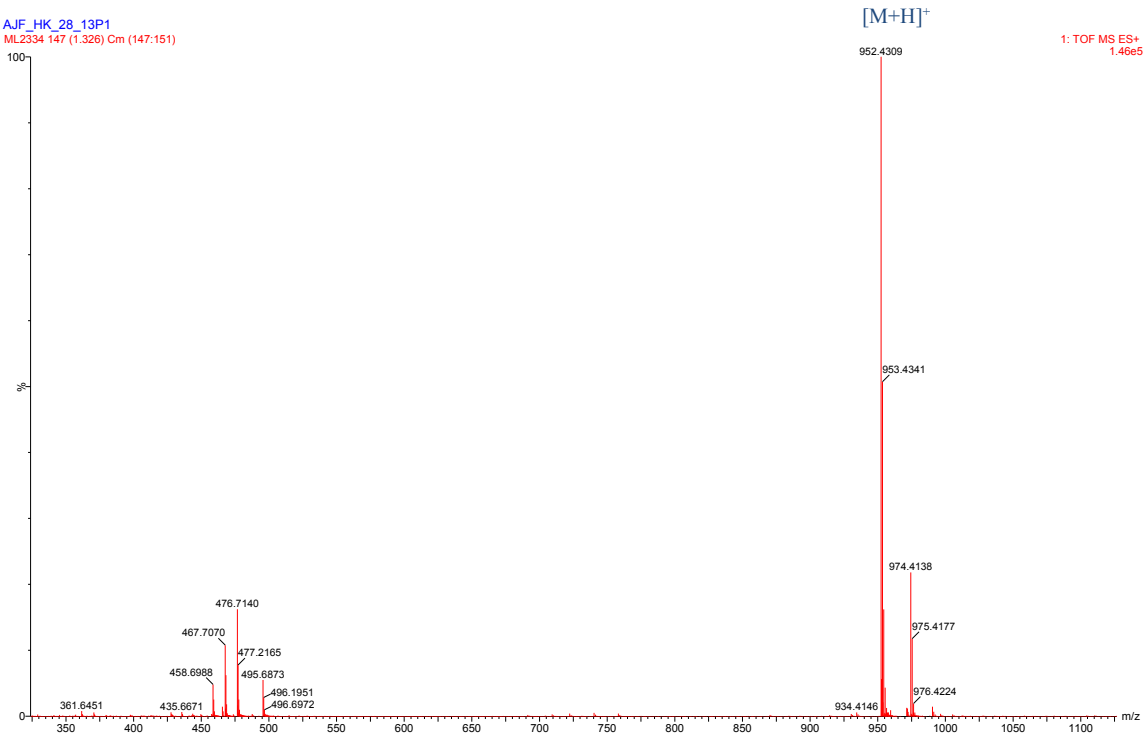


Figure S40. *HRMS (ESI) spectra of compound (***4a***).*

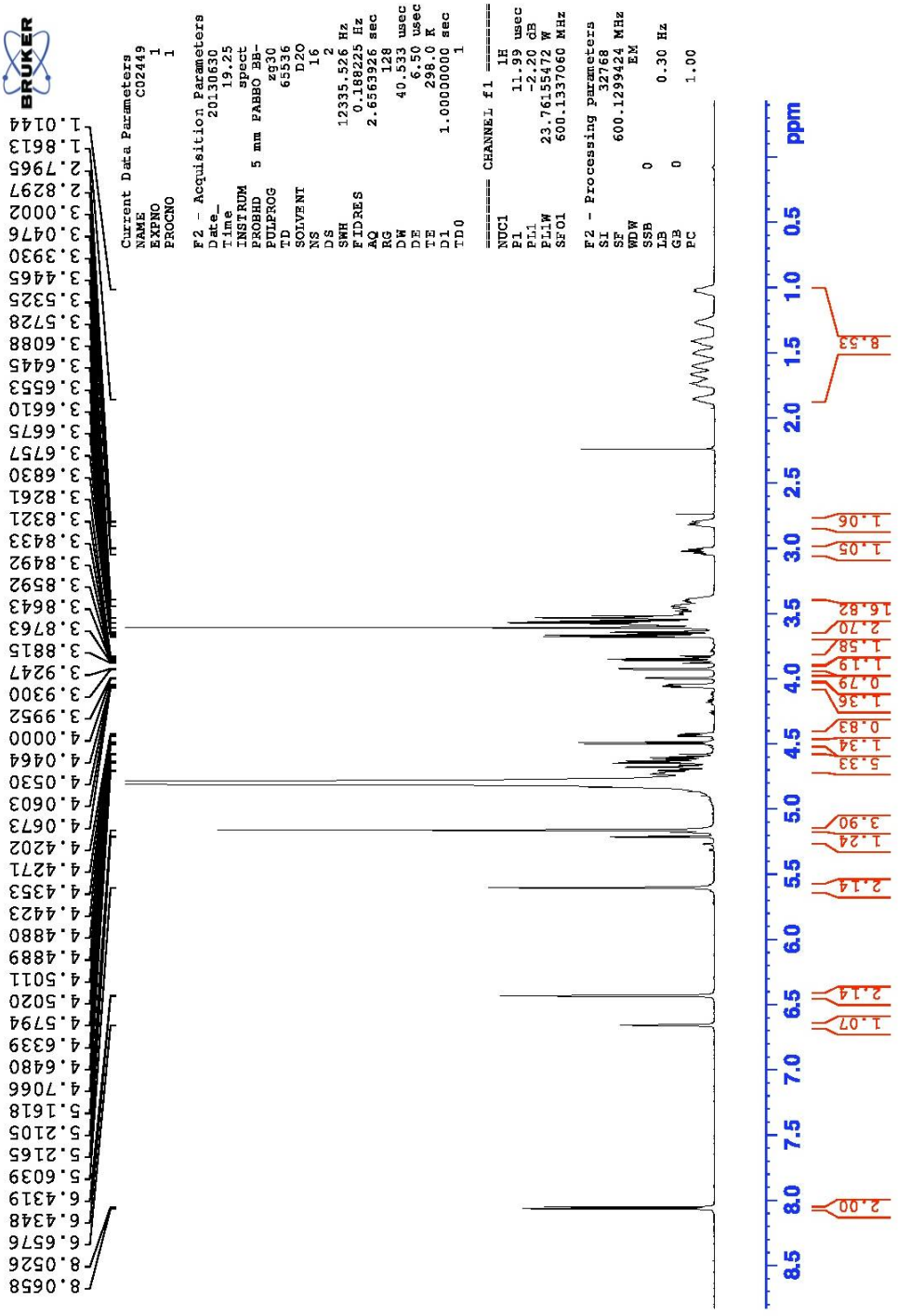


Figure S41. *^{1}H NMR spectrum of compound (**4a**).*

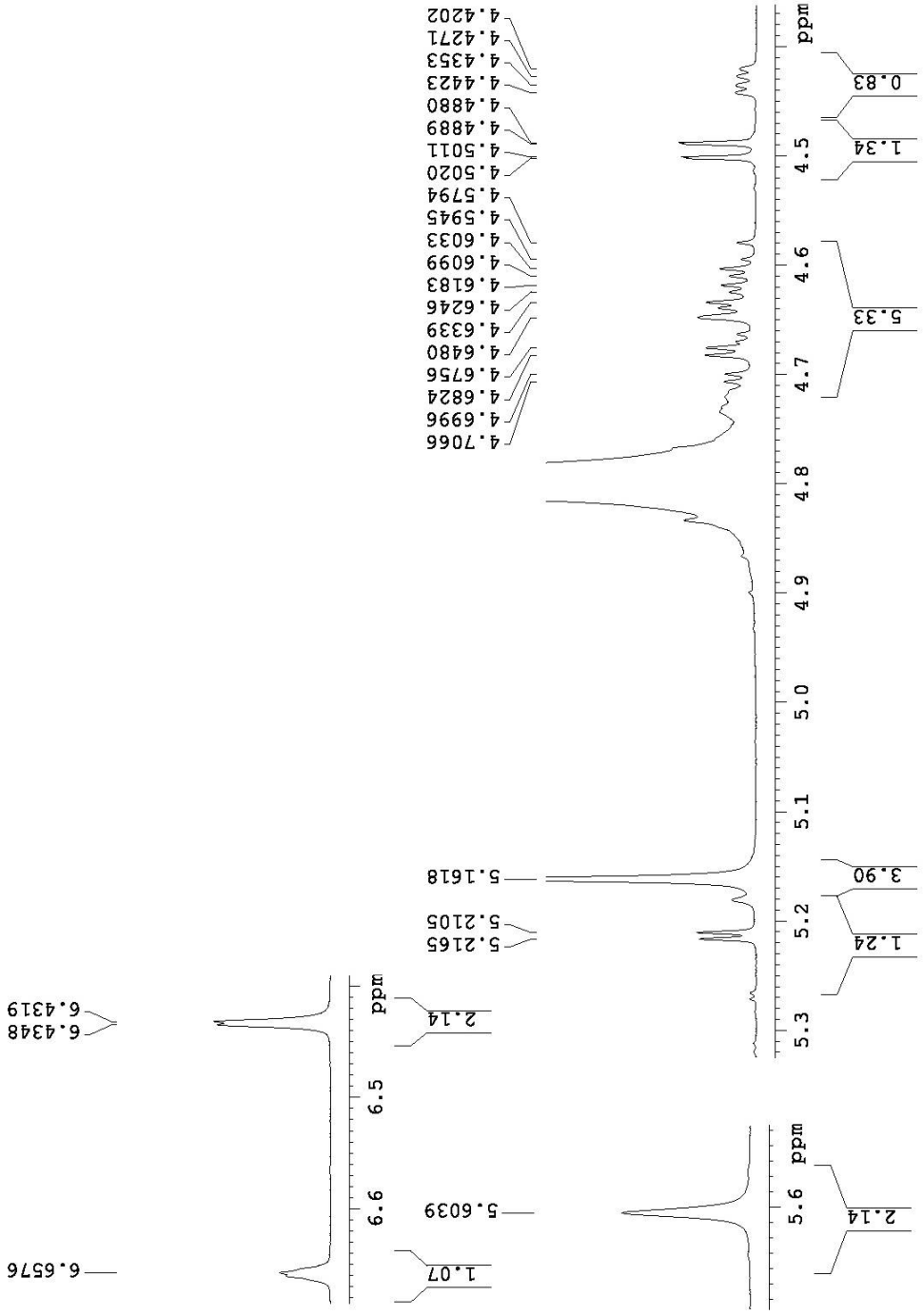


Figure S42. *Selected areas [1]H NMR of compound (**4a**).*

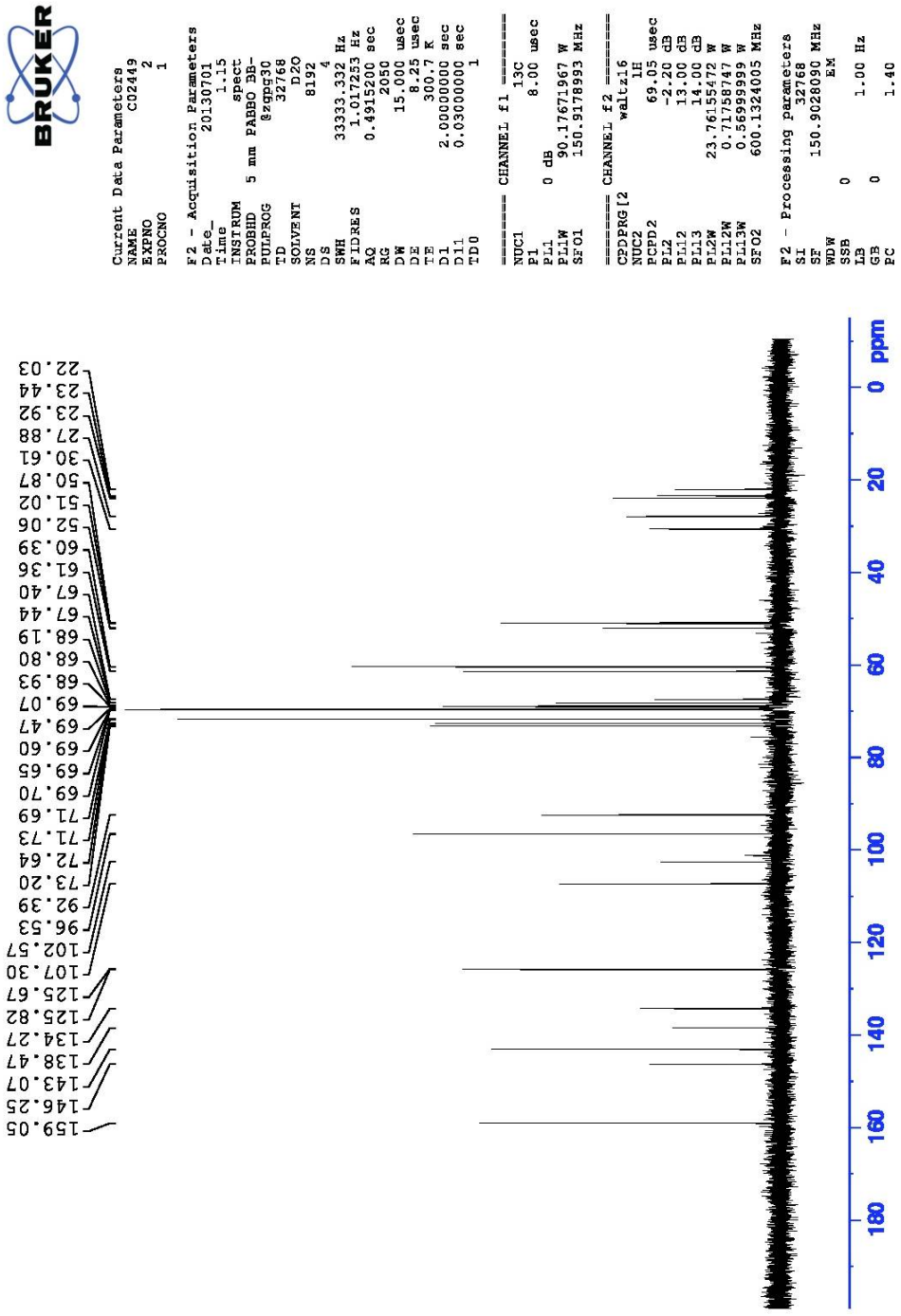


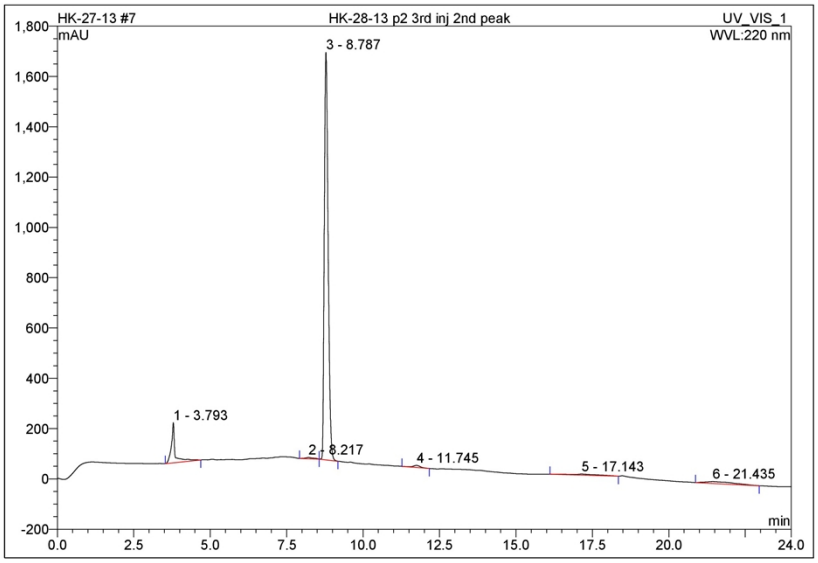
Figure S43. *^{13}C NMR spectrum of compound (**4a**).*

Operator:Administrator Timebase:analyticalhplc Sequence:HK-27-13

Page 1-1
21/2/2015 5:47 PM

7 HK-28-13 p2 3rd inj 2nd peak

Sample Name:	**HK-28-13 p2 3rd inj 2nd peak**	*Injection Volume:*	**20.0**
Vial Number:	**RA7**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**220**
Control Program:	**poly-p4 28min +230nm**	*Bandwidth:*	**10**
Quantif. Method:	**dna method**	*Dilution Factor:*	**1.0000**
Recording Time:	**24/6/2013 19:44**	*Sample Weight:*	**1.0000**
Run Time (min):	**24.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.79	n.a.	159.996	23.094	8.37	n.a.	BMB
2	8.22	n.a.	5.056	1.612	0.58	n.a.	BMb
3	8.79	n.a.	1620.621	235.657	85.42	n.a.	bMB
4	11.75	n.a.	8.153	2.139	0.78	n.a.	BMB
5	17.14	n.a.	4.423	4.775	1.73	n.a.	BMB
6	21.44	n.a.	7.267	8.617	3.12	n.a.	BMB
Total:			1805.516	275.894	100.00	0.000	

default/Integration

Chromeleon (c) Dionex 1996-2006
Version 6.80 SP4 Build 2361 (130805)

Figure S44. *HPL chromatogram of compound (**4b**).*

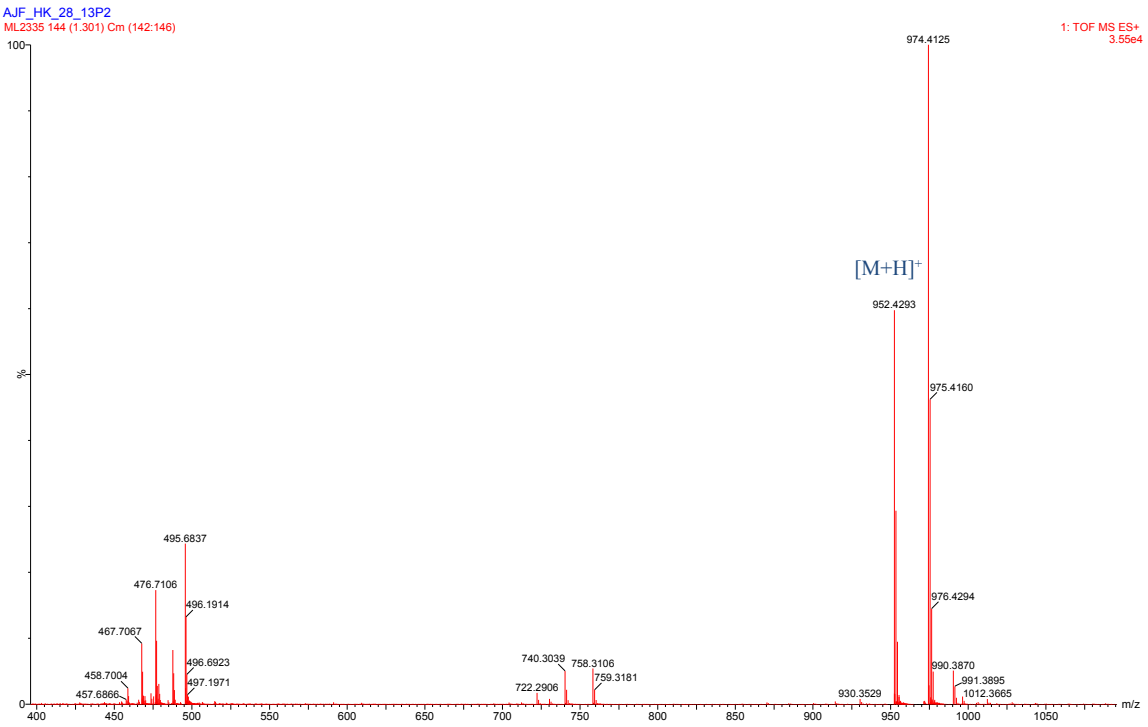


Figure S45. *HRMS (ESI) spectra of compound (**4b**).*

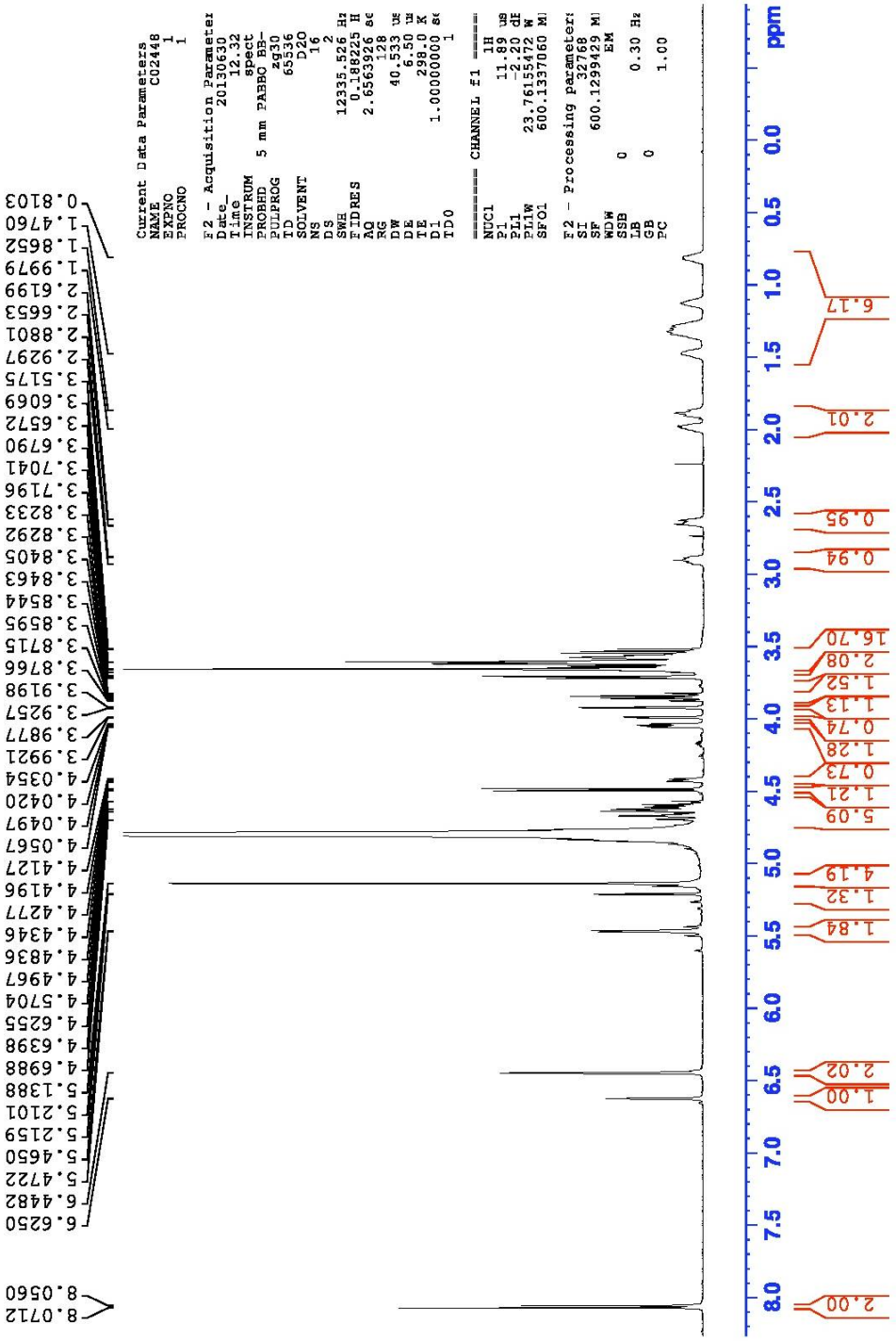


Figure S46. *^{1}H NMR spectrum of compound (**4b**).*

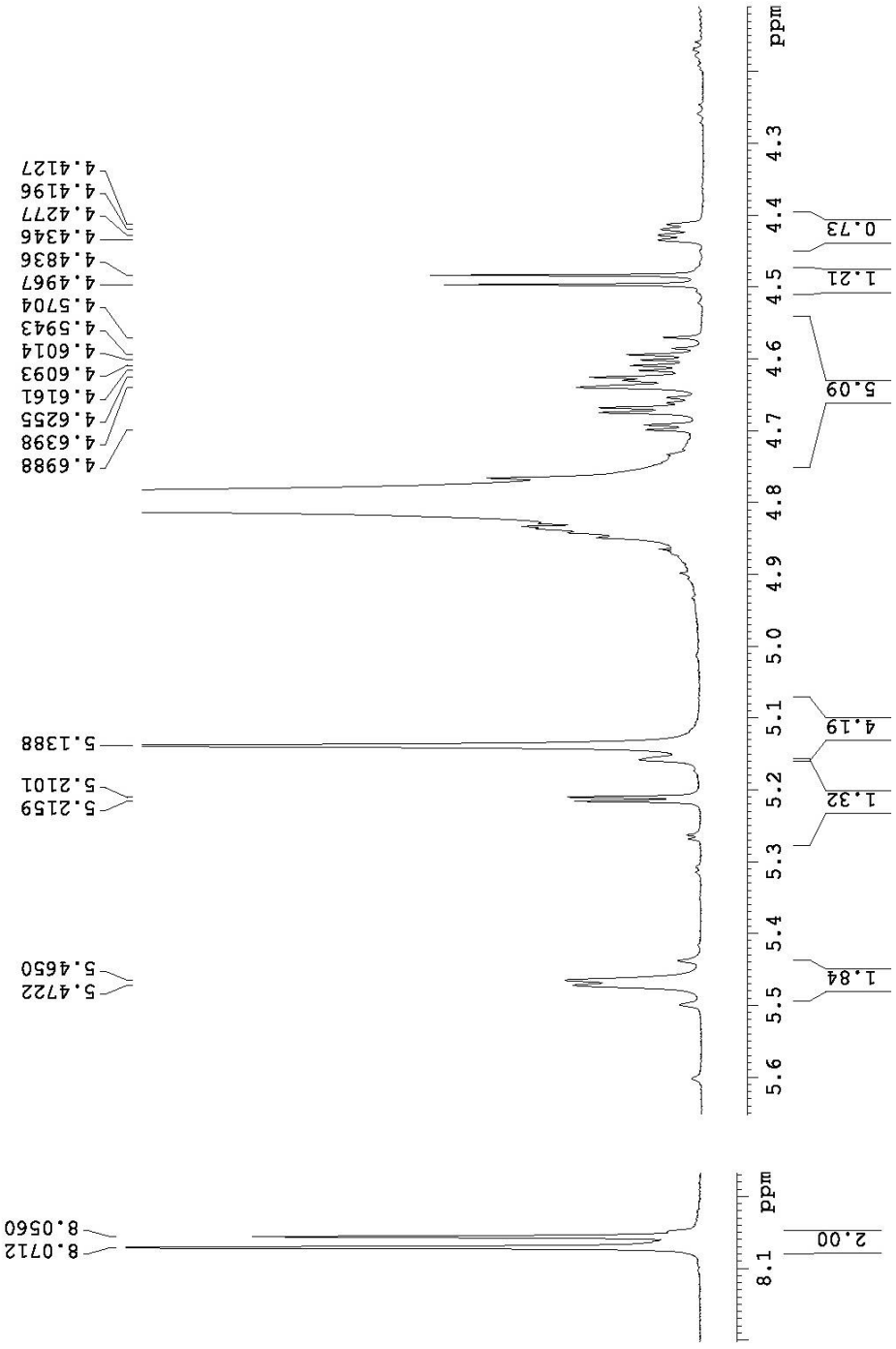


Figure S47. *Selected areas [1]H NMR of compound (**4b**).*

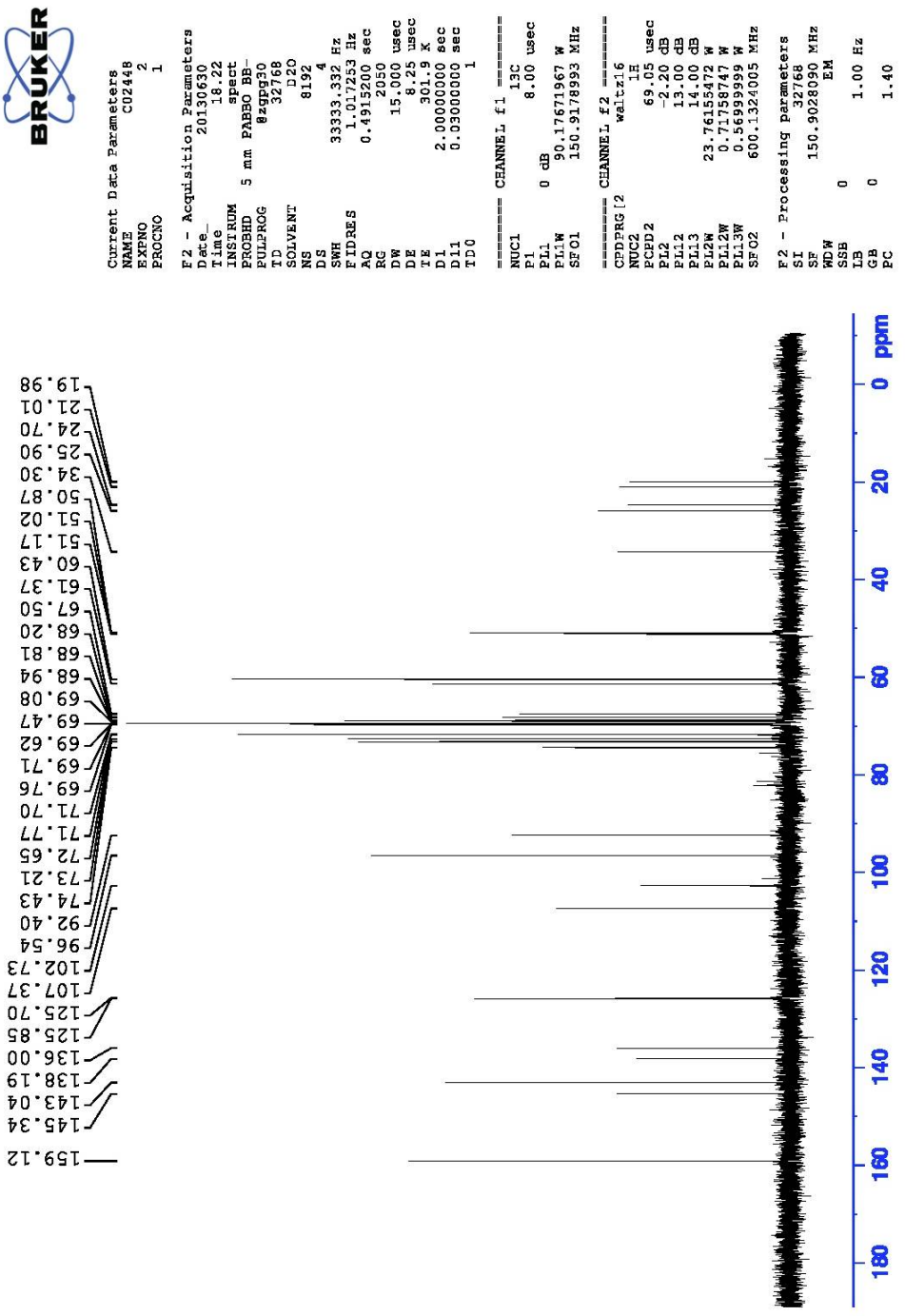


Figure S48. ^{13}C NMR spectrum of compound (4b).

11.0 References

[1] Burley, G. A.; Gierlich, J.; Mofid, M. R.; Nir, H.; Tal, S.; Eichen, Y.; Carell, T., Directed DNA Metallization. *Journal of the American Chemical Society* **2006,** *128* (5), 1398-1399.

[2] Pinter, G.; Bereczki, I.; Roth, E.; Sipos, A.; Varghese, R.; Udo, E. E.; Ostorhazi, E.; Rozgonyi, F.; Phillips, O. A.; Herczegh, P., The Effect of Systematic Structural Modifications on the Antibacterial Activity of Novel Oxazolidinones. *Medicinal Chemistry* **2011,** *7* (1), 45-55.

[3] Lallana, E.; Fernandez-Megia, E.; Riguera, R., Surpassing the Use of Copper in the Click Functionalization of Polymeric Nanostructures: A Strain-Promoted Approach. *Journal of the American Chemical Society* **2009,** *131* (16), 5748-5750.

[4] Hynes, M. J., EQNMR: a computer program for the calculation of stability constants from nuclear magnetic resonance chemical shift data. *Journal of the Chemical Society, Dalton Transactions* **1993,** (2), 311-312.