Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Electronic Supplementary Information

Protein-mediated dethreading of a biotin-functionalised pseudrotaxane

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General experimental.

Melting points are uncorrected. The NMR spectra were recorded as $CDCl_3$ solutions. ^1H and ^{13}C spectra were recorded at 400 MHz and 100 MHz, respectively, and were referenced to TMS or chloroform (δ 7.26, ^1H; δ 77.0, ^{13}C). 2D-NMR techniques were used to confirm the structures and to assign signals. When required reactions were performed under dry oxygen-free nitrogen and in oven-dried glassware. Dry solvents were obtained using the Pure-Solv™ Solvent Purification System (Innovative Technology). UV-vis spectroscopy measurements were performed using a Perkin Elmer Lambda 25 instrument. Fluorescence spectroscopy measurements were performed using a Shimadzu RF-5301 PC instrument. Avidin and neutravidin were used in their purified form and were purchased from Sigma Aldrich and Thermo Scientific, respectively.

1. ITC data

Isothermal titration calorimetry (ITC) was used to compare the apparent binding affinities of the biotin-functionalised compound **4** to avidin, neutravidin and **1** in water at 25 ºC. Because of poor solubilities of some of the compounds in standard buffers, these measurements were done in unbuffered water, giving rise to anomalous heat effects during the first 2-3 injections due to weak acid/base interactions and pH fluctuations (Figure S1). These initial transient effects were ignored in subsequent data analysis.

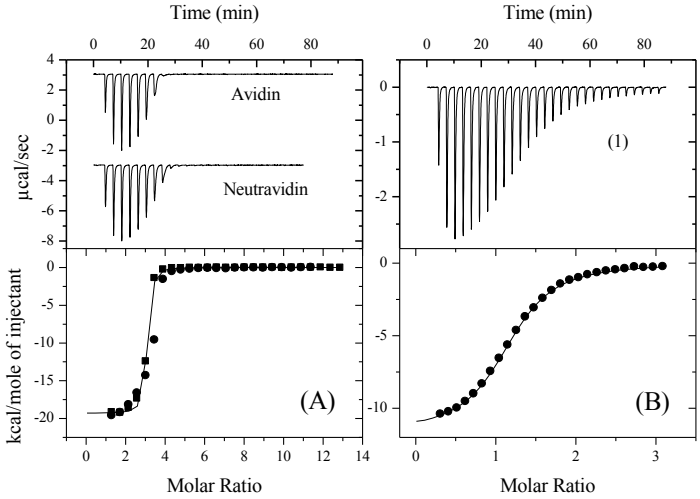

Figure S1: Isothermal titration calorimetry (ITC) data for the binding of **4** to avidin, neutravidin, or pseudorotaxane (**1**) in aqueous solution at 25 ºC. In each case, raw data in the upper panel shows the heat pulses per 10 µl injection of **4** (0.7 mM) into: (A) avidin (■) or neutravidin (•) (0.012 mM); (B) **1** (0.05 mM). The lower panels show integrated heat data (1 cal = 4.184 J), normalized per mole of injectant, and fit (solid curve) to a standard one-set-of-sites binding model, with parameters given in Table S1.

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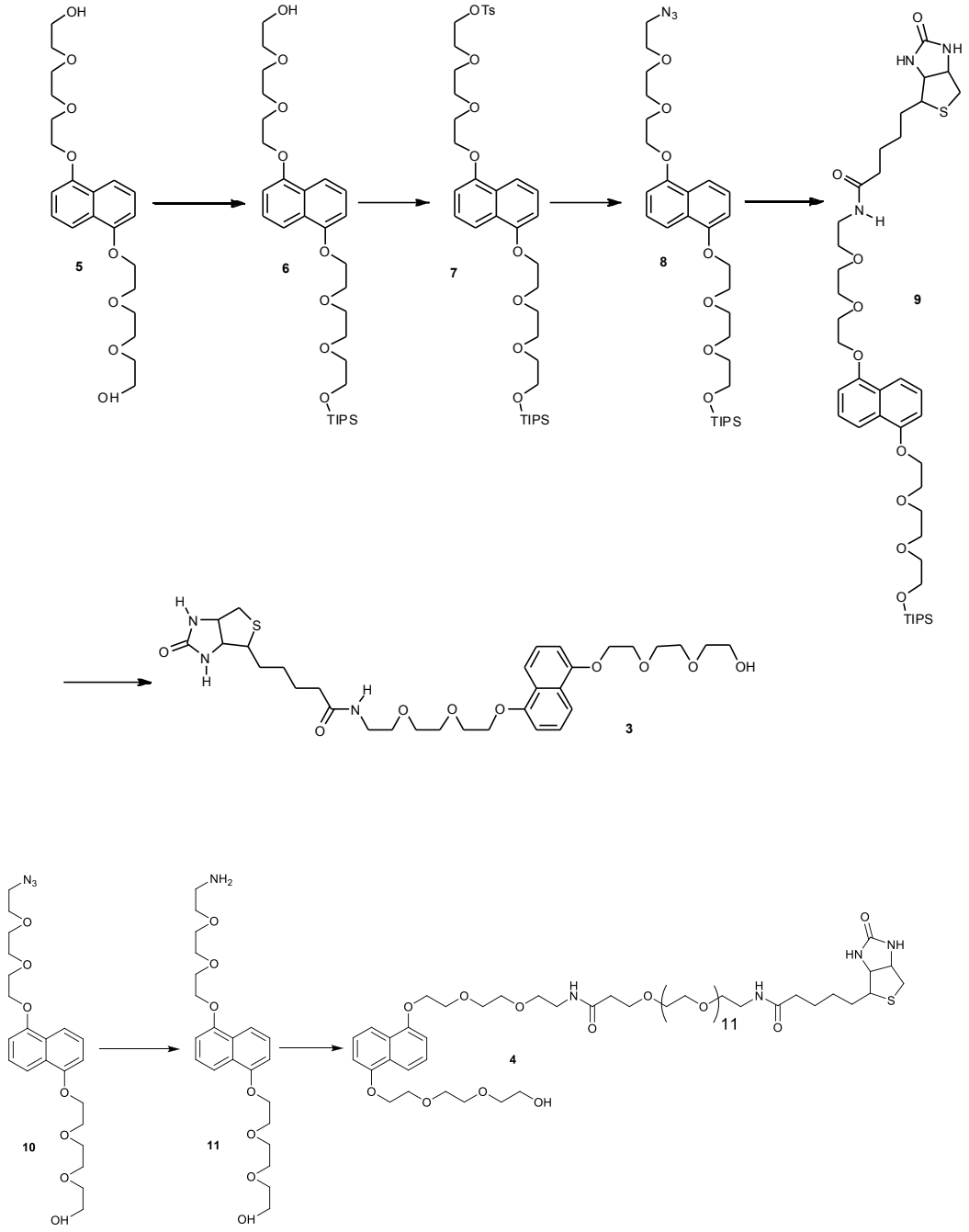
Table S1: ITC-derived thermodynamic binding parameters for binding of **4** to target compounds in water at 25 ºC.

	K_a / M^{-1}	ΔH / kJ mol^{-1}	N
Avidin	$2.0 (\pm 0.3) \times 10^7$	-81 (±2)	2.9 (± 0.1)
Neutravidin	$4.7 (\pm 1.1) \times 10^6$	-84 (±5)	3.2 (± 0.1)
1	$2.6 (\pm 0.1) \times 10^5$	-48.4 (±0.2)	1.18 (± 0.01)

2. Synthesis.



Scheme S1: Synthesis of compounds **3** and **4.**

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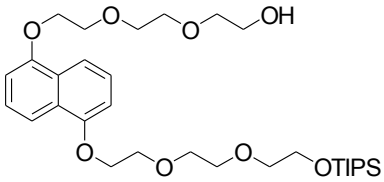
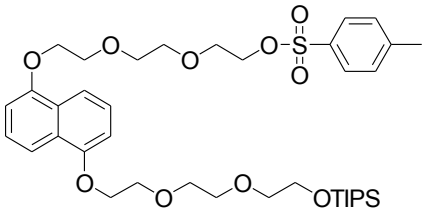
Compound **10** was prepared using previously described protocols.[1]

Synthesis of 6



Triisopropylsilyl trifluoromethanesulfonate (1.9 ml, 7.07 mmol) was added slowly to a solution of diol **5** (3.0 g, 7.07 mmol) and pyridine (1.14 ml, 14.14 mmol) in dry CH_2Cl_2 (40 ml). The solution was stirred at r.t. for 1h then concentrated under reduced pressure. The resulting residue was purified by column chromatography on SiO_2 eluting with EtOAc to give the alcohol as a pale brown oil. (1.47 g, 36%).
δ_H (400 MHz: $CDCl_3$): 1.04-1.08 (21H, m, 3 x ^{i}Pr), 2.61 (1H, broad s, OH), 3.58-3.62 (4H, m, 2 x OCH_2), 3.67-3.73 (6H, m, 3 x OCH_2), 3.77-3.79 (4H, m, 2 x OCH_2), 3.84 (2H, t, J 5.6 Hz, OCH_2), 3.96-3.99 (4H, m, 2 x OCH_2), 4.26-4.28 (4H, m, 2 x OCH_2), 6.82 (2H, d, J 7.7 Hz, H-2+H-6), 7.31-7.36 (2H, m, H-3+H-6), 7.85-7.88 (2H, m, H-4+H-8). δ_C (100 MHz: $CDCl_3$): 11.8 (CH), 17.9 (CH_3), 61.6 (CH_2), 62.9 (CH_2), 67.8 (CH_2), 67.8 (CH_2), 69.7 (CH_2), 70.3 (CH_2), 70.8 (CH_2), 70.9 (CH_2), 70.9 (CH_2), 72.4 (CH_2), 72.7 (CH_2), 105.5 (CH), 105.6 (CH), 114.4 (CH), 114.6 (CH), 124.9 (CH), 124.9 (CH), 126.6 (C), 126.7 (C), 154.1 (C), 154.3 (C). m/z (FAB): 581.2 ($(M+H)^+$, 60%), 187.6 (50), 158.0 (100). Found: 581.3515. $C_{31}H_{53}O_8Si$ requires $(M+H)^+$, 581.3510.

Synthesis of 7



Tosyl chloride (1.13 g, 5.95 mmol) was added to a solution of alcohol **6** (1.73 g, 2.98 mmol) and triethylamine (0.83 ml, 5.95 mmol, 2.0 equiv.) in dry CH_2Cl_2. The solution was stirred at r.t. overnight then concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica eluting with Et_2O:petroleum ether 1:2 then 100% Et_2O to give the tosylate as pale yellow oil. (1.63 g, 75%).
δ_H (400 MHz: $CDCl_3$): 1.03-1.06 (21H, m, 3 x ^{i}Pr), 2.31 (3H, s, CH_3), 3.53-3.66 (8H, m, 4 x OCH_2), 3.68-3.70 (2H, m, OCH_2), 3.74-3.76 (2H, m, OCH_2), 3.82 (2H, t, J 5.5 Hz, OCH_2), 3.86-3.88 (2H, m, OCH_2), 3.93-3.96 (2H, m, OCH_2), 4.07-4.10 (2H, m, OCH_2), 4.17-4.20 (2H, m, OCH_2), 4.21-4.24 (2H, m, OCH_2), 6.77-6.80 (2H, m, H-2+H-6), 7.21 (2H, d, J 8.2 Hz, Ar-H), 7.31 (2H, t, J 8.0 Hz, H-3+H-6), 7.72 (2H, d, J 8.2 Hz, Ar-H), 7.82 (1H, d, J 8.5 Hz, H-4 or H-8), 7.86 (1H, d, J 8.5 Hz, H-4 or H-8).

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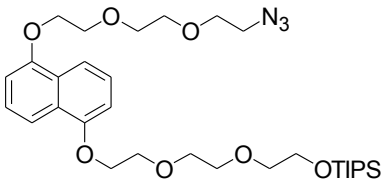
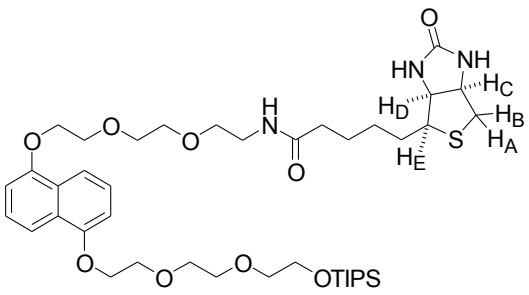
δ_C (100 MHz: $CDCl_3$): 11.6 (CH), 17.7 (CH_3), 21.2 (CH_3), 62.7 (CH_2), 67.5 (CH_2), 67.5 (CH_2), 68.3 (CH_2), 69.0 (CH_2), 69.4 (CH_2), 70.4 (CH_2), 70.5 (CH_2), 70.6 (CH_2), 70.7 (CH_2), 72.5 (CH_2), 105.3 (CH), 114.1 (CH), 114.4 (CH), 124.7 (CH), 124.8 (CH), 126.4 (CH), 126.4 (CH), 127.6 (CH), 129.3 (CH), 132.6 (C), 144.4 (C), 153.9 (C), 154.0 (C).

Synthesis of 8



Sodium azide (288 mg, 4.44 mmol) was added to a solution of tosylate **7** (1.63 g, 2.22 mmol) in DMF (30 ml). The solution was heated to 80°C overnight. After cooling the solution was extracted into Et_2O then washed with water (3 x 200 ml). The organic layer was dried over magnesium sulphate, filtered and then concentrated under reduced pressure to give the azide as an off-white viscous oil. (859 mg, 64%).
δ_H (400 MHz: $CDCl_3$): 1.04-1.07 (21H, m, 3 x ^{i}Pr), 3.29 (2H, t, J 5.0 Hz, CH_2N_3), 3.58-3.64 (6H, m, 3 x OCH_2), 3.68-3.71 (2H, m, OCH_2), 3.73-3.76 (4H, m, OCH_2), 3.83 (2H, t, J 5.5 Hz, OCH_2), 3.91-3.96 (4H, m, 2 x OCH_2), 4.21-4.24 (4H, m, 2 x OCH_2), 6.79 (2H, d, J 7.7 Hz, H-2+H-6), 7.30-7.35 (2H, m, H-3+H-6), 7.86 (1H, d, J 8.5 Hz, H-4 or H-8), 7.87 (1H, d, J 8.4 Hz, H-4 or H-8). δ_C (100 MHz: $CDCl_3$): 11.6 (CH), 17.7 (CH_3), 50.3 (CH_2), 62.7 (CH_2), 67.5 (CH_2), 67.5 (CH_2), 69.4 (CH_2), 69.5 (CH_2), 69.7 (CH_2), 70.4 (CH_2), 70.6 (CH_2), 70.7 (CH_2), 70.7 (CH_2), 72.5 (CH_2), 105.2 (CH), 114.2 (CH), 114.3 (CH), 124.8 (CH), 126.4 (C), 126.4 (C), 153.9 (C), 154.0 (C).m/z (FAB): 605.4 (M^+, 100%), 578.4 (65), 158.1 (85). Found: 605.3495. $C_{31}H_{51}O_7N_3Si$ requires M^+, 605.3496.

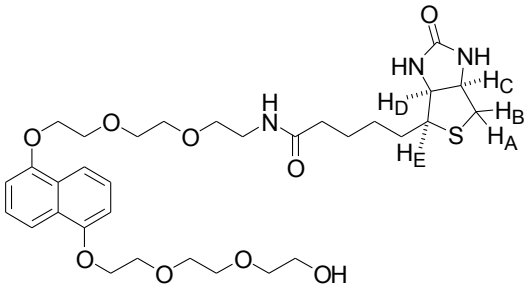
Synthesis of 9



Triphenyl phosphine (520 mg, 1.98 mmol) was added to a solution of azide **8** (801 mg, 1.32 mmol) in THF (20 ml). The solution was stirred at r.t. overnight then water (1 ml) was added and the solution was stirred for a further 3 h. The solvent was removed under vacuum and then residue purified by column chromatography on silica eluting with EtOAc then acetone to give the impure amine which was used without further purification. Biotin (291 mg, 1.19 mmol) was added to a solution of impure amine (628 mg, 1.08 mmol), EDCI (311 mg, 1.62 mmol) and DMAP (12 mg, 0.1

mmol) in dry DMF (10 ml). The solution was stirred overnight at room temperature, extracted in EtOAc then washed with water (3 x 100 ml). The organic layer was dried over magnesium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica eluting with CH_2Cl_2:MeOH 10:1 to give the amide as a off white solid. (391 mg, 45%).
δ_H (400 MHz: $CDCl_3$): 1.02-1.10 (21H, m, 3 x ^{i}Pr), 1.25-1.33 (2H, m, CH_2), 1.50-1.68 (4H, m, 2 x CH_2), 2.12 (2H, t, J 7.5 Hz, (C=O)CH_2), 2.64 (1H, d, J 12.7 Hz, CH_AH$_B$), 2.74 (1H, dd, J 4.8 and 12.7 Hz, CH$_A$$H_B$), 2.92 (1H, dt, J 4.5 and 7.2 Hz, CH$_E$), 3.56-3.61 (4H, m, OCH_2 + NCH_2), 3.64-3.62 (2H, m, OCH_2), 3.70-3.73 (2H, m, OCH_2), 3.76-3.79 (4H, m, 2 x OCH_2), 3.83 (2H, t, J 5.5 Hz, OCH_2), 3.95-4.00 (4H, m, 2 x OCH_2), 4.08-4.11 (1H, m, CH$_D$), 4.26-4.28 (4H, m, 2 x OCH_2), 4.34-4.37 (1H, m, CH$_C$), 5.79 (1H, s, NH), 6.71 (1H, s, NH), 6.82 (2H, d, J 7.3 Hz, H-2+H-6), 6.85 (1H, t, J 5.8 Hz, NH), 7.31-7.35 (2H, m, H-3+H-6), 7.82 (1H, d, J 8.5 Hz, H-4 or H-8), 7.85 (1H, d, J 8.5 Hz, H-4 or H-8). δ_C (100 MHz: $CDCl_3$): 11.9 (CH), 17.9 (CH_3), 25.5 (CH_2), 27.9 (CH_2), 28.1 (CH_2), 35.9 (CH_2), 39.1 (CH_2), 40.4 (CH_2), 55.5 (CH), 60.1 (CH), 61.5 (CH), 62.9 (CH_2), 67.9 (CH_2), 69.7 (CH_2), 69.7 (CH_2), 69.9 (CH_2), 70.1 (CH_2), 70.6 (CH_2), 70.9 (CH_2), 70.9 (CH_2), 72.7 (CH_2), 105.6 (CH), 114.3 (CH), 114.8 (CH), 125.0 (CH), 125.2 (CH), 126.6 (C), 126.7 (C), 154.1 (C), 154.3 (C), 164.2 (C), 173.3 (C).m/z (FAB): 806.3 ($(M+H)^+$, 100%), 762.3 (30), 270.2 (30). Found: 806.4448. $C_{41}H_{68}O_9N_3SiS$ requires $(M+H)^+$, 806.4446.

Synthesis of 3



Tetrabutylammonium fluoride (1.0 M in THF) (0.35 ml, 0.35 mmol) was added to a solution of amide **9** (237 mg, 0.29 mmol) in dry THF (3 ml). The solution was stirred at r.t. for 1 h then extracted into CH_2Cl_2 then washed with water (3 x 50 ml). The organic layer was dried over magnesium sulphate, filtered and concentrated under reduced pressure to give the alcohol as an off white solid. (86 mg, 45%).

δ_H (400 MHz: $CDCl_3$): 1.20-1.27 (2H, m, CH_2), 1.45-1.62 (4H, m, 2 x CH_2), 2.07 (2H, t, J 7.5 Hz, (C=O)CH_2), 2.60 (1H, d, J 12.7 Hz, CH_AH$_B$), 2.70 (1H, dd, J 4.9 and 12.7 Hz, CH$_A$$H_B$), 2.85 (1H, dt, J 4.6 and 7.2 Hz, CH$_E$), 3.01 (1H, broad s, OH), 3.36-3.41 (2H, m, NCH_2), 3.53-3.66 (2H, m, OCH_2), 3.57-3.59 (2H, m, OCH_2), 3.62-3.64 (2H, m, OCH_2), 3.66-3.69 (4H, m, 2 x OCH_2), 3.73-3.78 (4H, m, 2 x OCH_2), 3.93-3.97 (4H, m, 2 x OCH_2), 3.99-4.02 (1H, m, CH$_D$), 4.25-4.27 (4H, m, 2 x OCH_2), 4.28-4.31 (1H, m, CH$_C$), 5.81 (1H, s, NH), 6.53 (1H, s, NH), 6.81-6.83 (3H, m, H-2+H-6+NH), 7.30-7.35 (2H, m, H-3+H-6), 7.83 (2H, t, J 9.2 Hz, H-4+H-8).
δ_C (100 MHz: $CDCl_3$): 25.4 (CH_2), 27.9 (CH_2), 28.1 (CH_2), 35.8 (CH_2), 39.1 (CH_2), 40.4 (CH_2), 55.5 (CH), 60.0 (CH), 61.6 (CH+CH_2), 67.7 (CH_2), 67.9 (CH_2), 69.7

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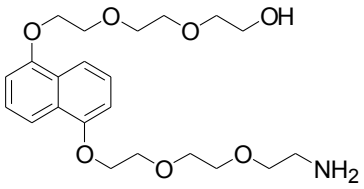
(CH_2), 69.7 (CH_2), 69.9 (CH_2), 70.1 (CH_2), 70.4 (CH_2), 70.6 (CH_2), 70.9 (CH_2), 72.5 (CH_2), 105.7 (CH), 114.4 (CH), 114.7 (CH), 125.1 (CH), 125.2 (CH), 126.6 (C), 126.7 (C), 154.1 (C), 154.2 (C), 164.1 (C), 173.3 (C). m/z (FAB): 650.0 ($(M+H)^+$, 100%), 270.1 (65), 84.9 (55). Found: 650.3118. $C_{32}H_{48}O_9N_3SS$ requires $(M+H)^+$, 650.3111.

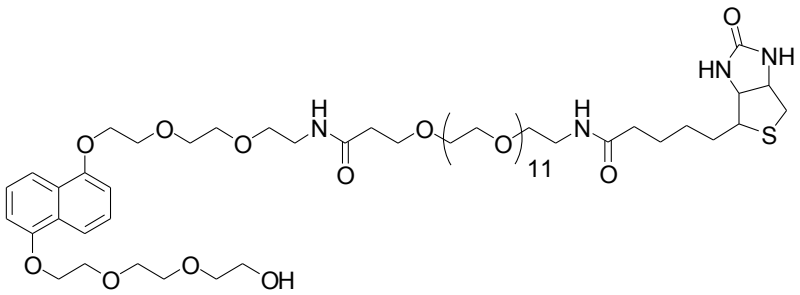
Synthesis of 11



Triphenylphospine (598 mg, 2.28 mmol) was added to a solution of azide **10** (682 mg, 1.52 mmol) in dry THF (10 ml). The solution was stirred at r.t. overnight then water (1 ml) was added. The solution was stirred for a further 3 hours then concentrated under reduced pressure. The residue was purified by column chromatography on SiO_2 eluting chloroform:MeOH:triethylamine (89:10:1) to give the amine as a pale yellow oil. (253 mg, 39%).

δ_H (400 MHz: $CDCl_3$): 2.22 (3H, broad s, OH + NH_2), 2.80 (2H, broad s, CH_2NH_2), 3.46 (2H, t, J 5.0 Hz, OCH_2), 3.55-3.58 (2H, m, OCH_2), 3.61-3.63 (2H, m, OCH_2), 3.65-3.68 (2H, m, OCH_2), 3.75-3.78 (4H, m, 2 x OCH_2), 3.95 (4H, t, J 4.9 Hz, OCH_2), 4.24-4.27 (4H, m, 2 x OCH_2), 6.81 (2H, d, J 7.5 Hz, H-2+H-6), 7.30-7.34 (2H, m, H-3+H-6), 7.48 (2H, d, J 8.3 Hz, H-4+H-8).
δ_C (100 MHz: $CDCl_3$): 41.5 (CH_2), 61.4 (CH_2), 67.7 (CH_2), 67.8 (CH_2), 69.6 (CH_2), 70.2 (CH_2), 70.3 (CH_2), 70.8 (CH_2), 70.8 (CH_2), 72.5 (CH_2), 73.1 (CH_2), 105.5 (CH), 114.4 (CH), 114.5 (CH), 124.9 (CH), 124.9 (CH), 126.6 (C), 126.6 (C), 154.1 (C), 154.1 (C). m/z (FAB): 424.2 ($(M+H)^+$, 100%), 219.5 (25). Found: 424.2331. $C_{22}H_{34}O_7N$ requires $(M+H)^+$, 424.2335.

Synthesis of 4



O-[2-(Biotinylamino)ethyl]-O′-(2-carboxyethyl)undecaethylene glycol (100 mg, 0.118 mmol) was added to a solution of amine **11** (65 mg, 0.154 mmol), EDCI (34 mg, 0.177 mmol) and DMAP (2 mg, 0.012 mmol) in dry dichloromethane (2 ml). The

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resulting solution was stirred overnight at r.t. under an atmosphere of nitrogen. The solution was diluted with dichloromethane then was washed with water (2 x 50 ml). The organic layer was dried over magnesium sulphate, filtered and concentrated under reduced pressure to give the amide as a light brown oil. (135 mg, 91%).

δ_H (400 MHz: $CDCl_3$): 1.33-1.40 (2H, m, CH_2), 1.54-1.73 (4H, m, 2 x CH_2), 2.17 (2H, t, J 7.2 Hz, $(C=O)CH_2$), 2.37 (2H, t, J 5.8 Hz, $(C=O)CH_2$), 2.67 (1H, d, J 12.7 Hz, CH_AH_B), 2.80 (1H, dd, J 4.4 and 12.7 Hz, CH_AH_B), 2.86 (1H, broad s, OH), 3.03 (1H, m, CH_E), 3.36-3.40 (4H, m, 2 x NCH_2), 3.49-3.67 (58 H, m, OCH_2), 3.72-3.77 (4H, m, 2 x OCH_2), 3.94 (4H, broad s, 2 x OCH_2), 4.19-4.26 (5H, m, 2 x OCH_2 + CH_D), 4.39-4.41 (1H, m, CH_C), 5.74 (1H, s, NH), 6.49 (1H, s, NH), 6.71 (1H, broad s, NH), 6.79 (2H, d, J 7.5 Hz, H-2+H-6), 6.92 (1H, broad s, NH), 7.30 (2H, t, 8.0 Hz, H-3+H-6), 7.80 (1H, d, J 8.0 Hz, H-4 or H-8), 7.81 (1H, d, J 8.0 Hz, H-4 or H-8).
δ_C (100 MHz: $CDCl_3$): 25.4 (CH_2), 27.9 (CH_2), 28.1 (CH_2), 35.7 (CH_2), 36.7 (CH_2), 38.9 (CH_2), 40.3 (CH_2), 55.5 (CH), 60.0 (CH), 61.5 (CH_2), 61.5 (CH), 67.0 (CH_2), 67.7 (CH_2), 67.7 (CH_2), 69.6 (CH_2), 69.7 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.2 (CH_2), 70.2 (CH_2), 70.2 (CH_2), 70.3 (CH_2), 70.7 (CH_2), 70.8 (CH_2), 72.4 (CH_2), 105.5 (CH), 114.4 (CH), 114.4 (CH), 124.9 (CH), 126.5 (C), 126.6 (C), 154.1 (C), 163.9 (C), 171.3 (C), 173.3 (C). m/z (FAB): 1249.4 ($(M+H)^+$, 100%), 579.5 (65), 270.3 (90).

1. O.S. Miljanic, W. R. Dichtel, S. I. Khan, S. Mortezaei, J. R. Heath, J. F. Stoddart. J. Am. Chem. Soc. 2007, **129**, 8236.

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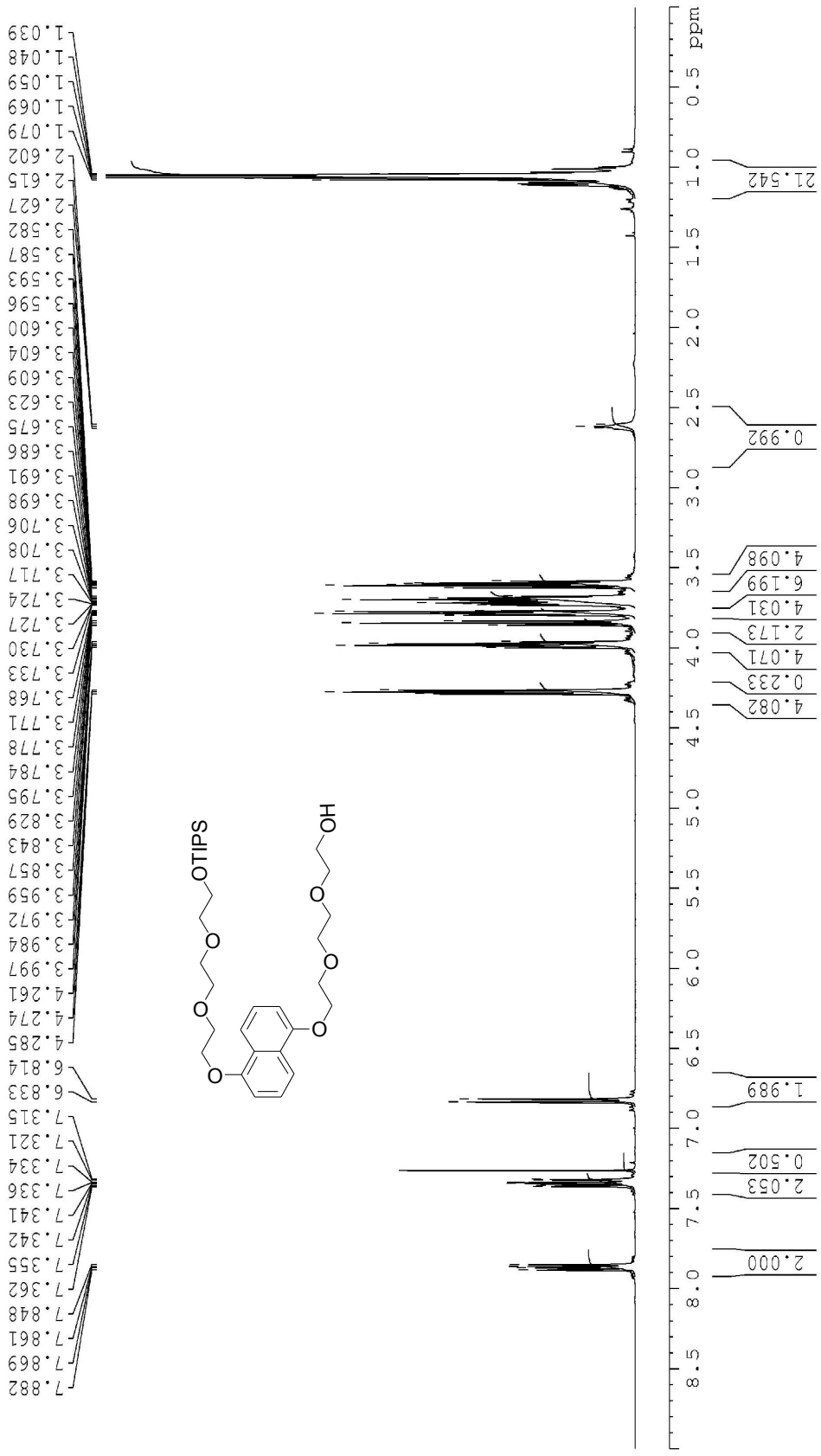

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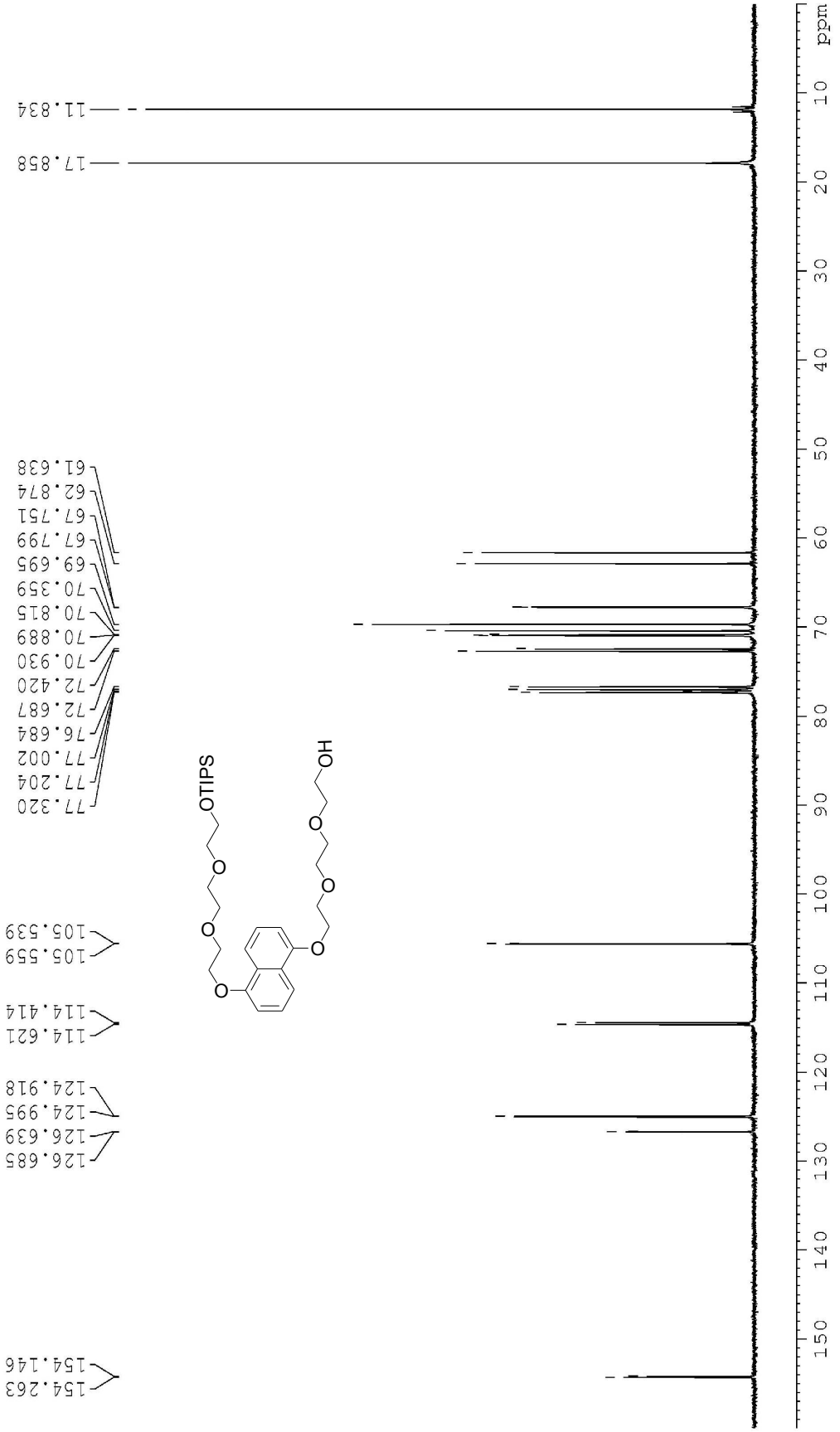

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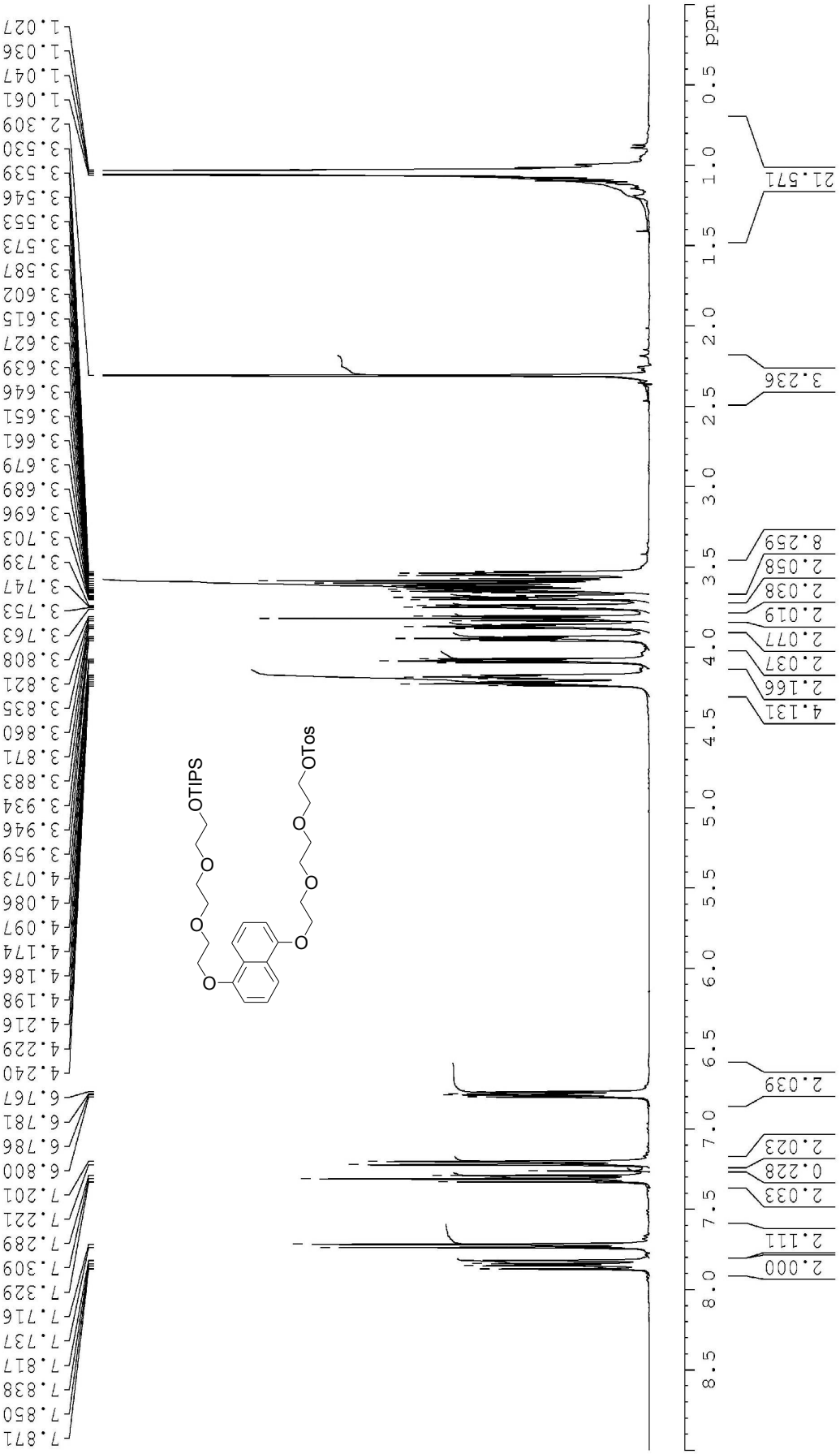

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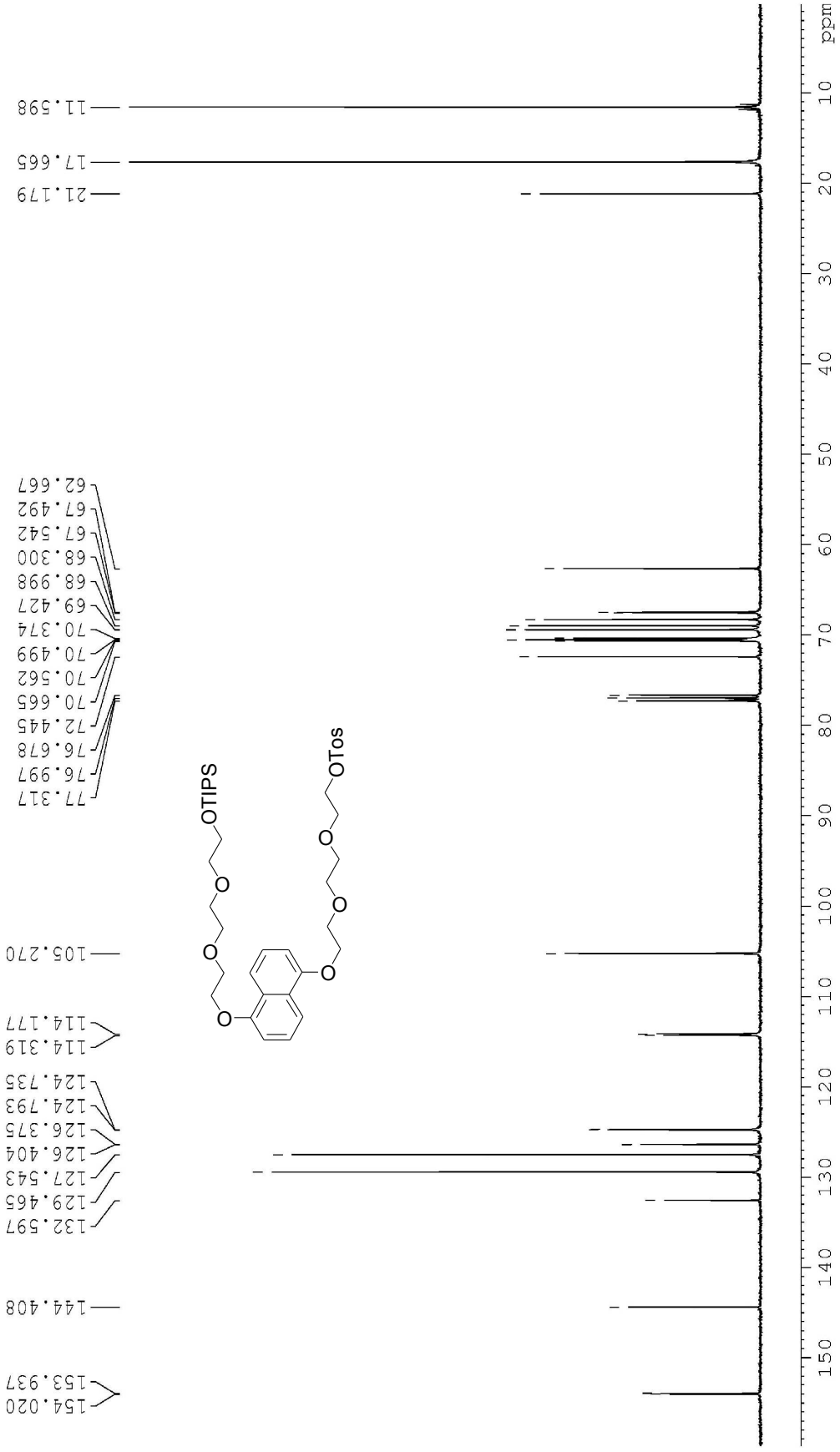

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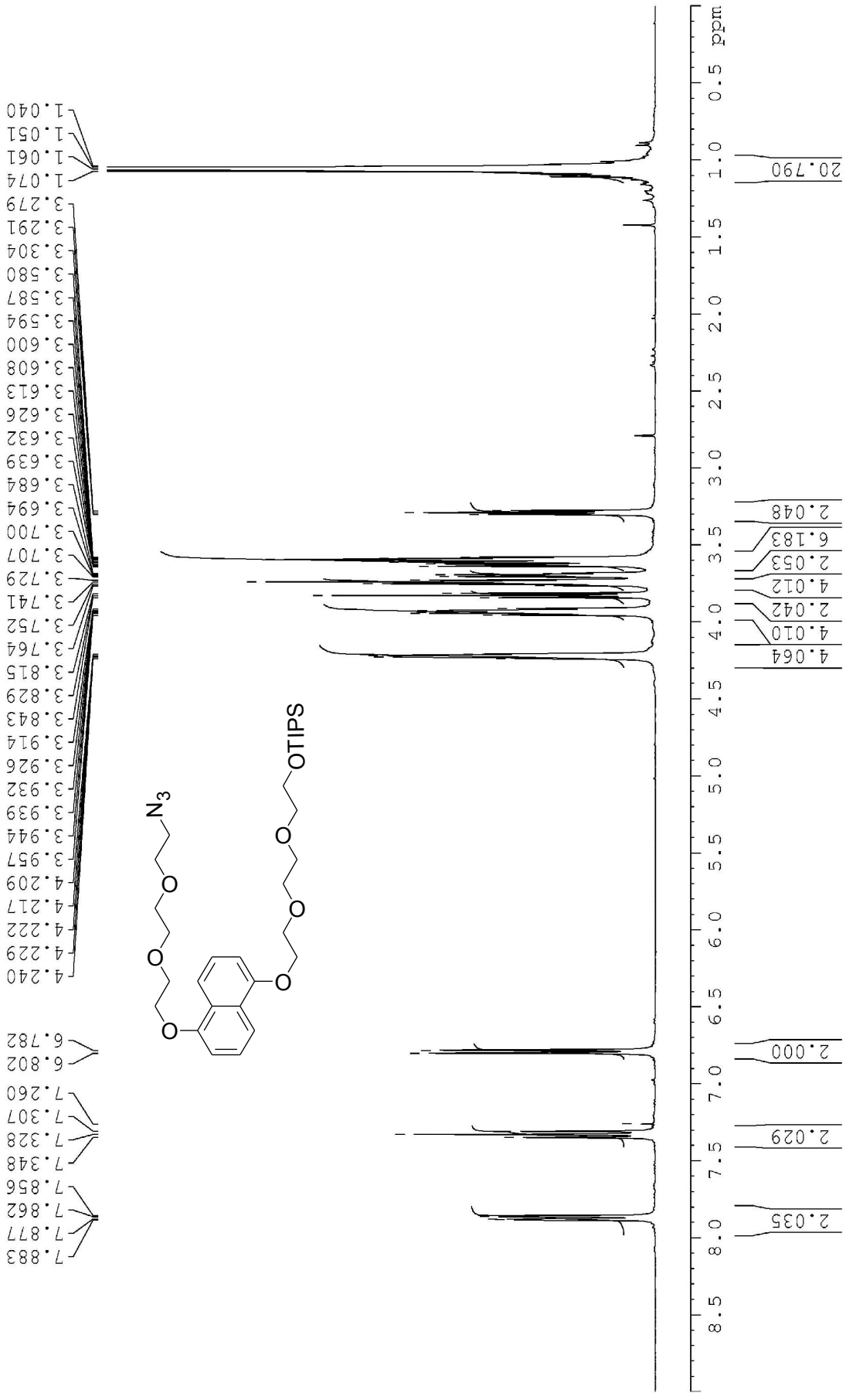

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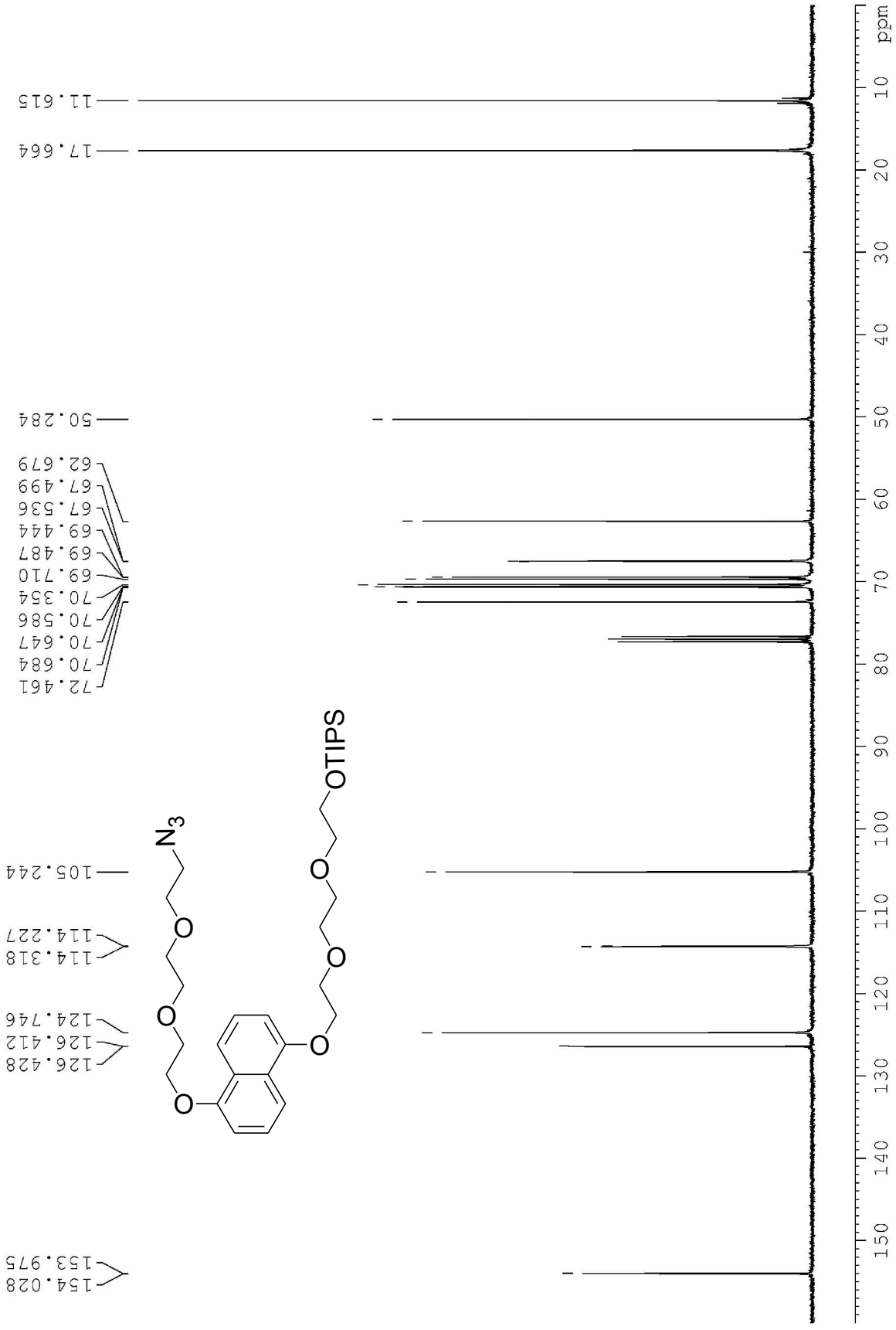

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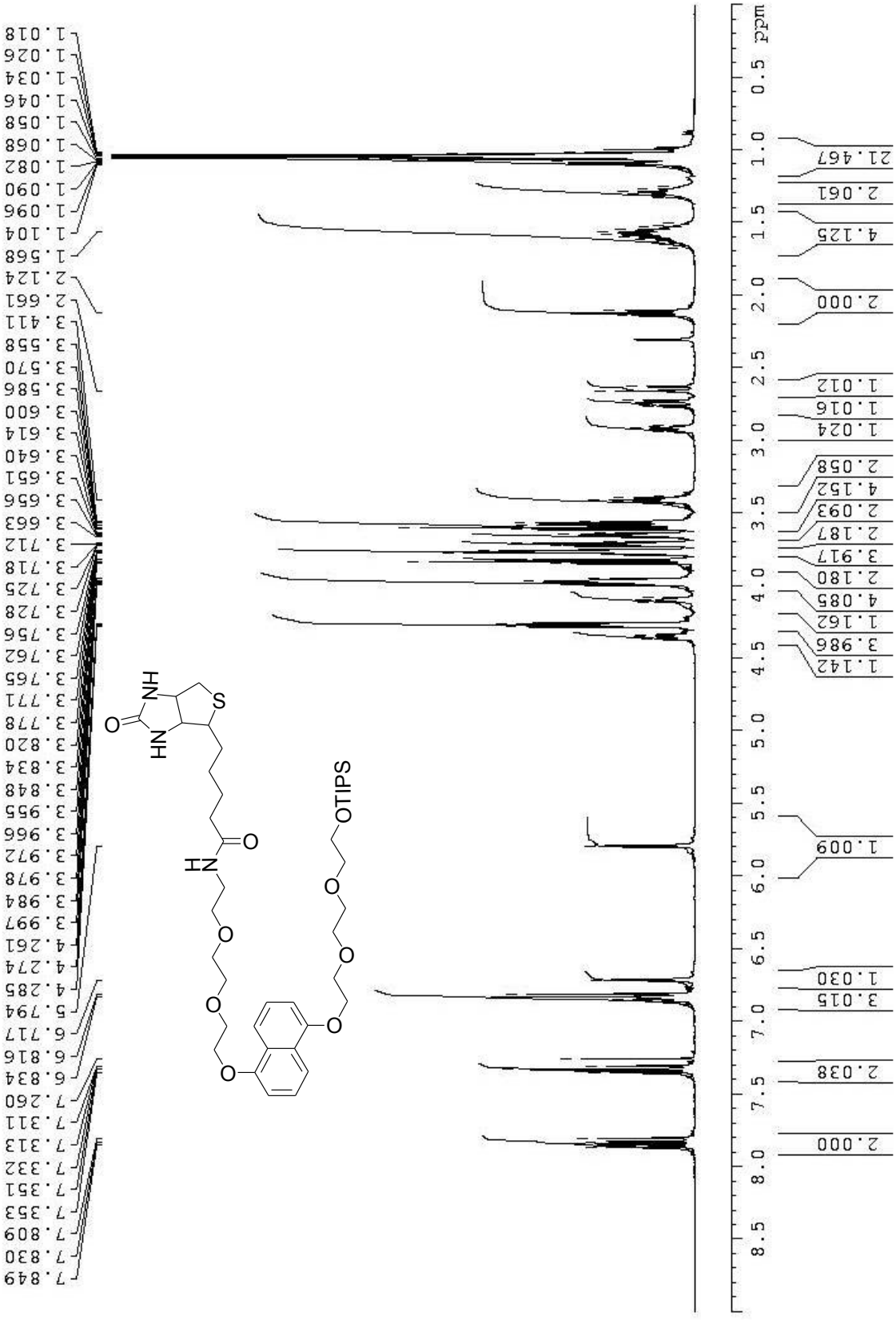

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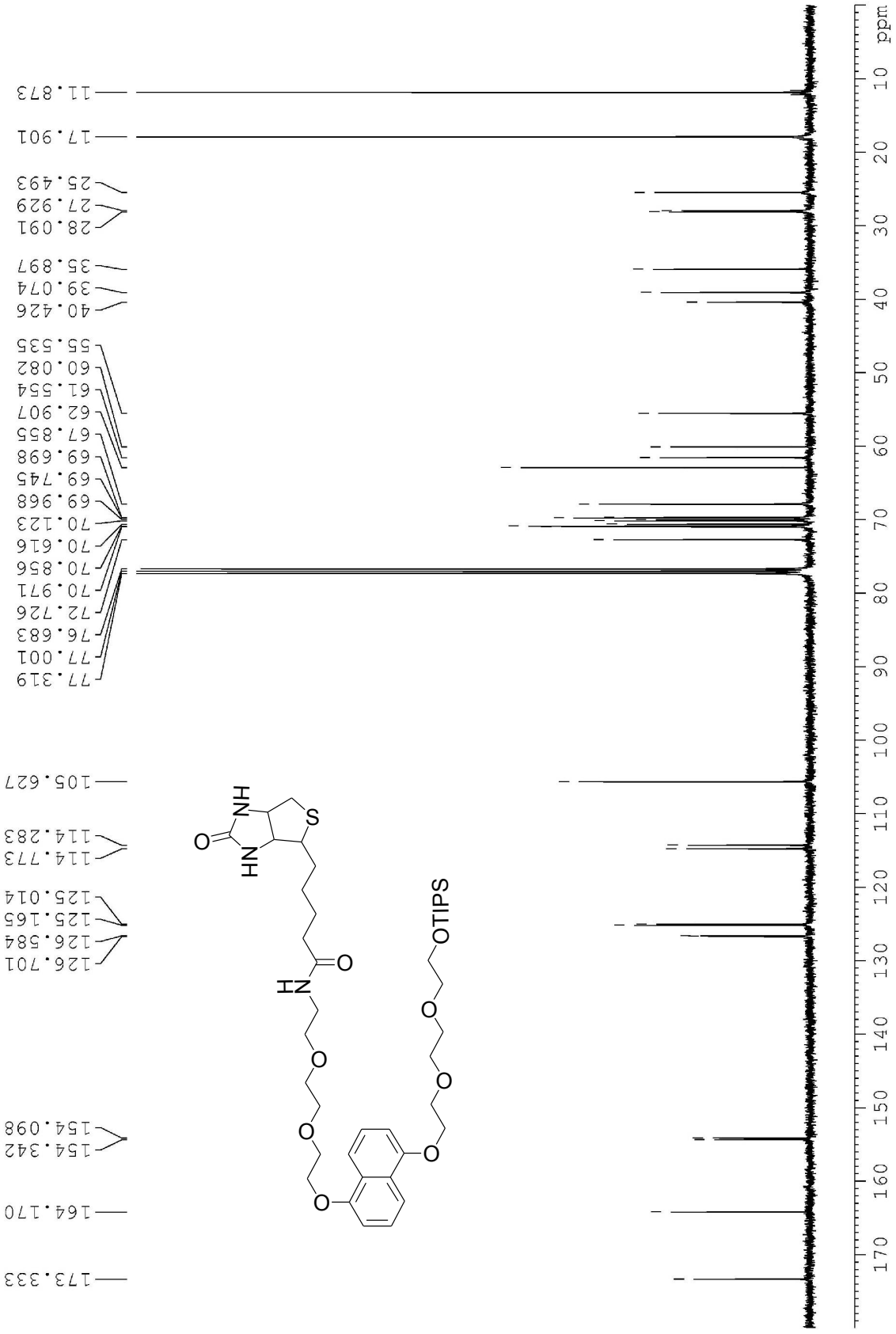

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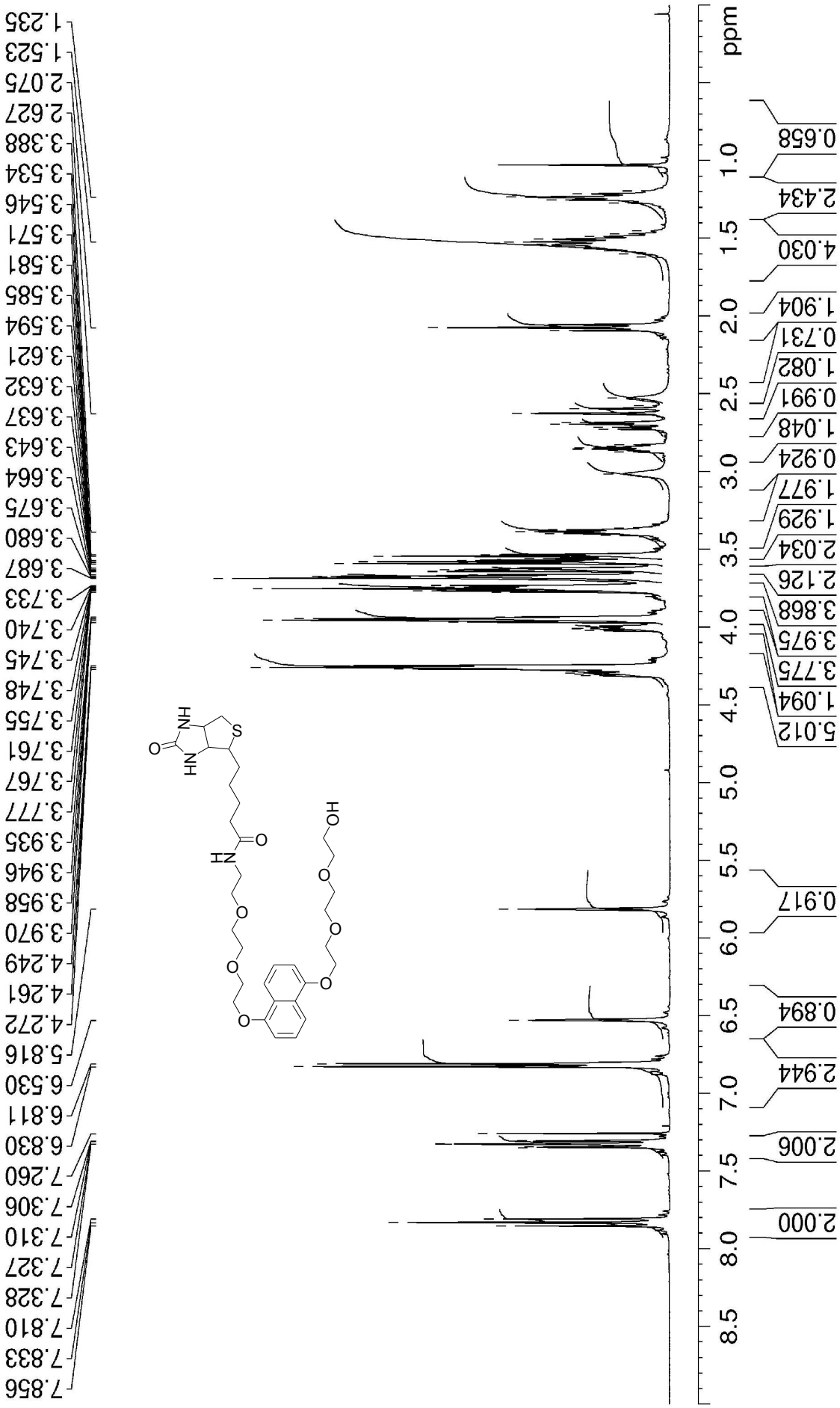

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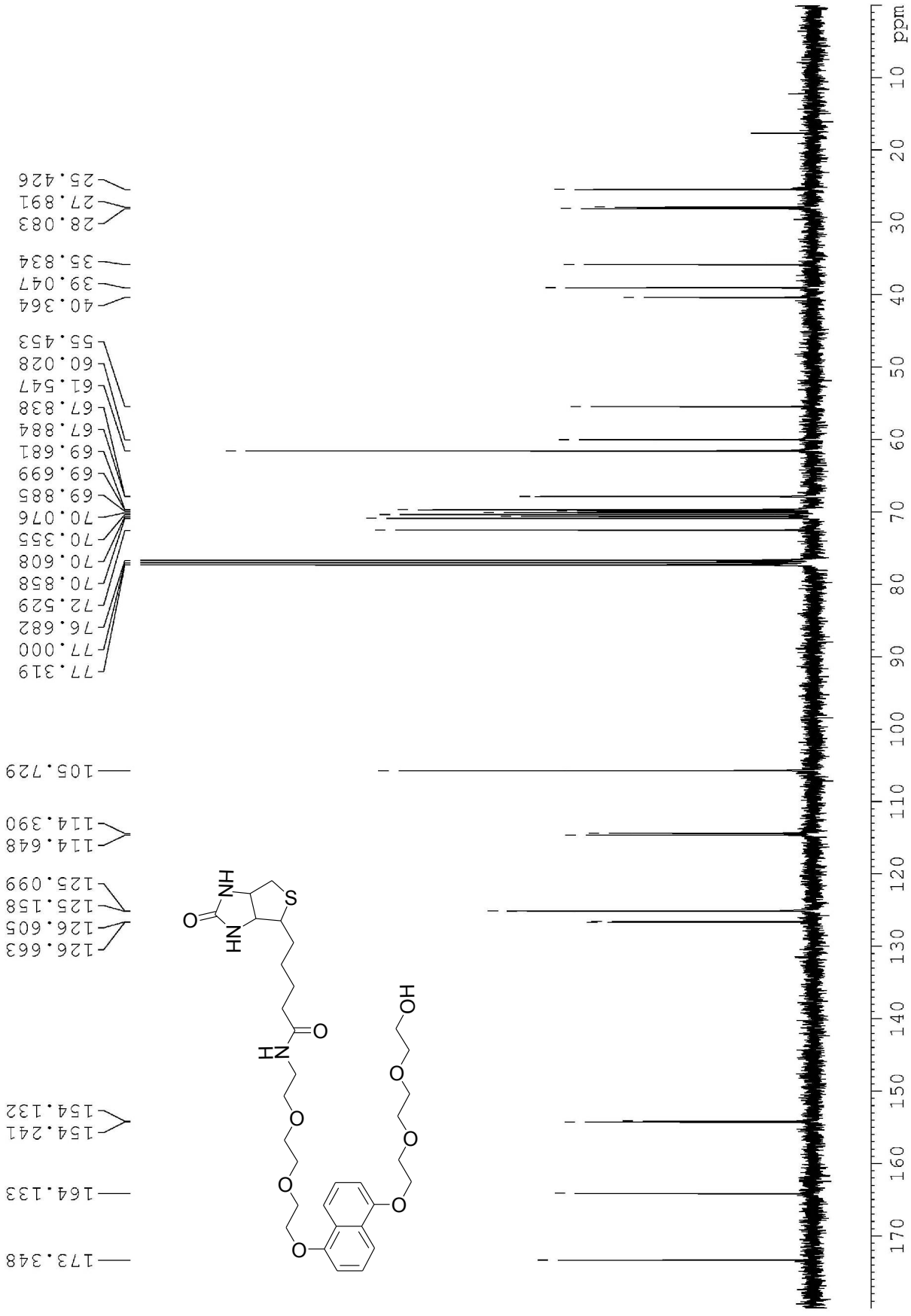

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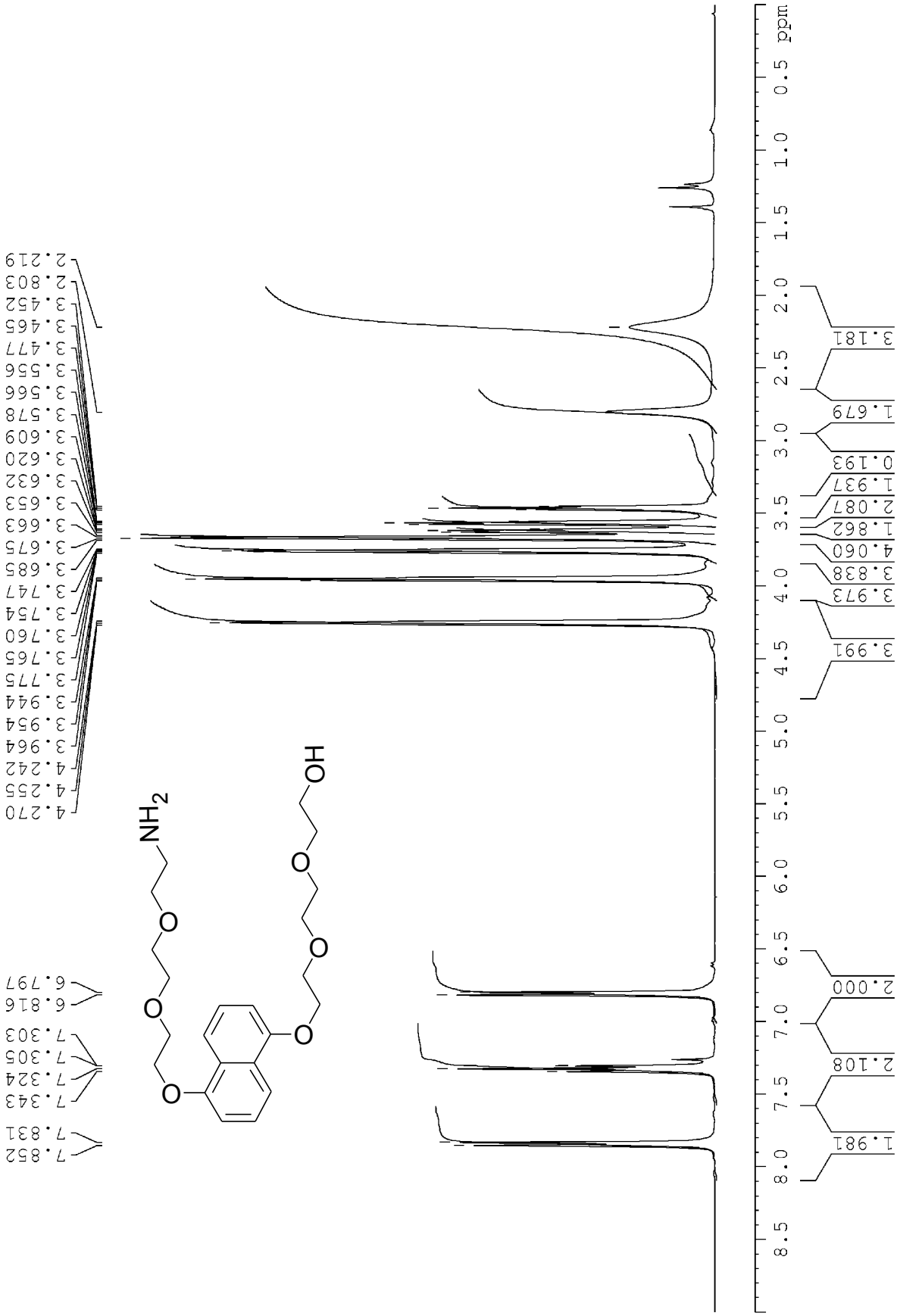

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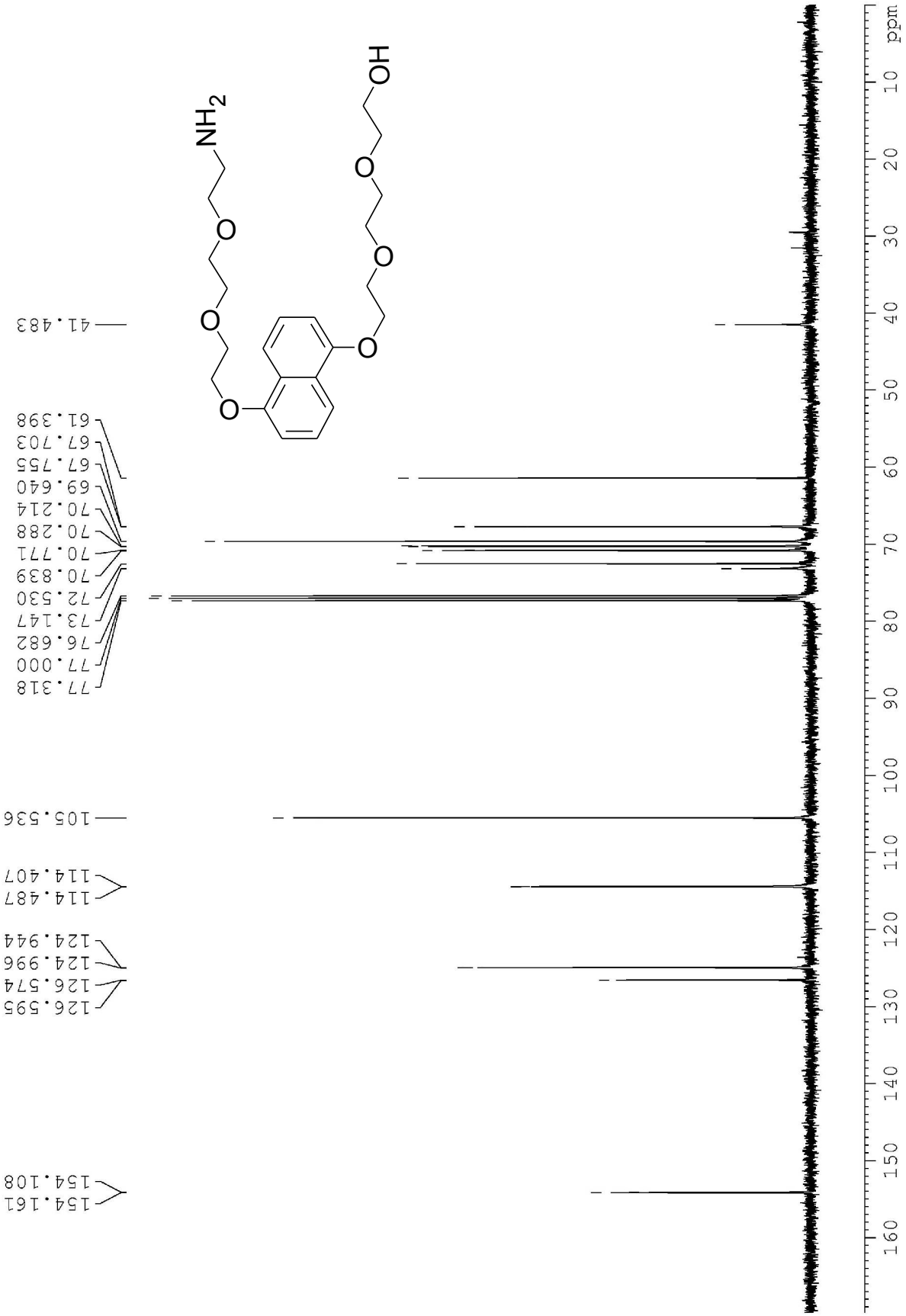

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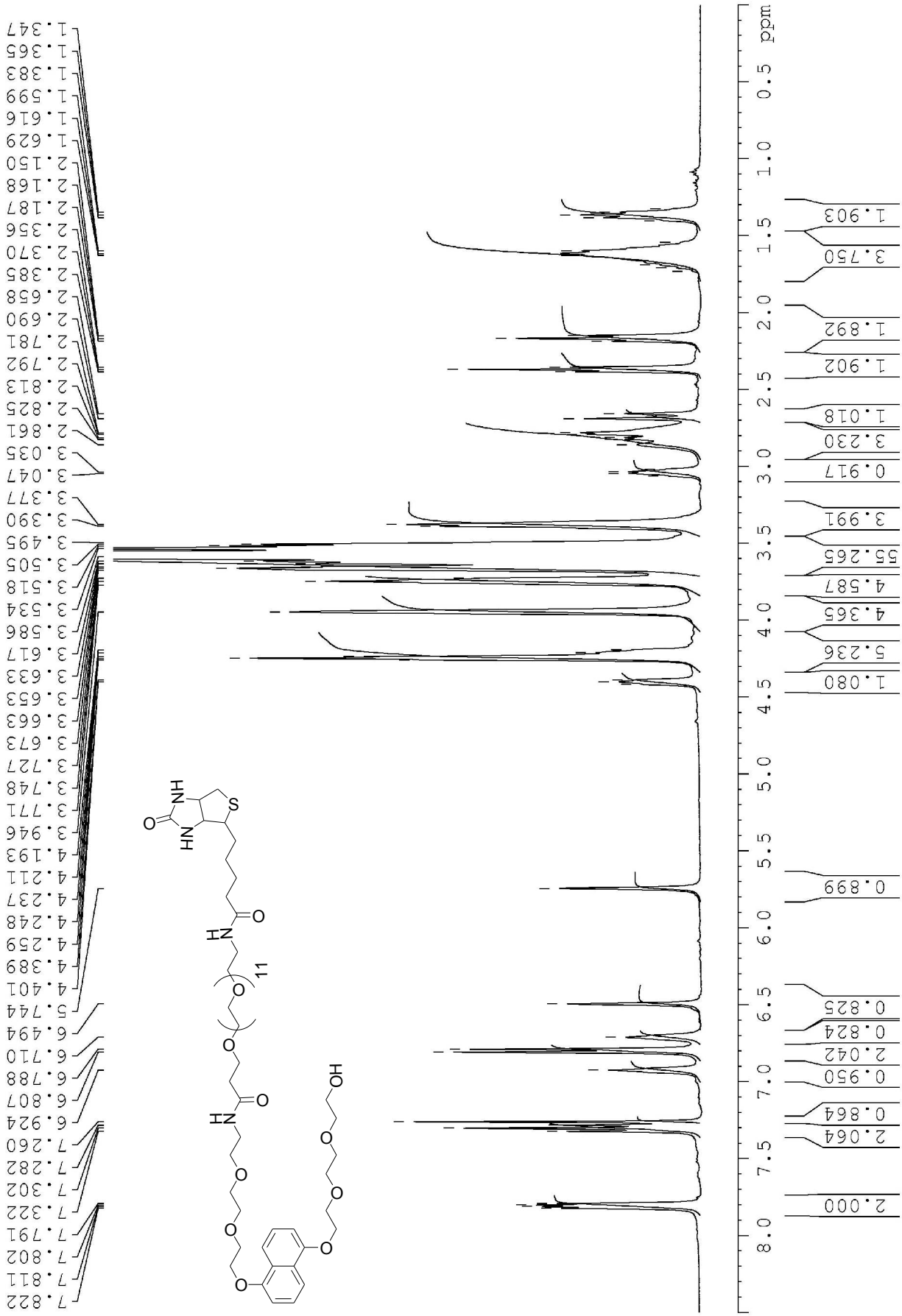

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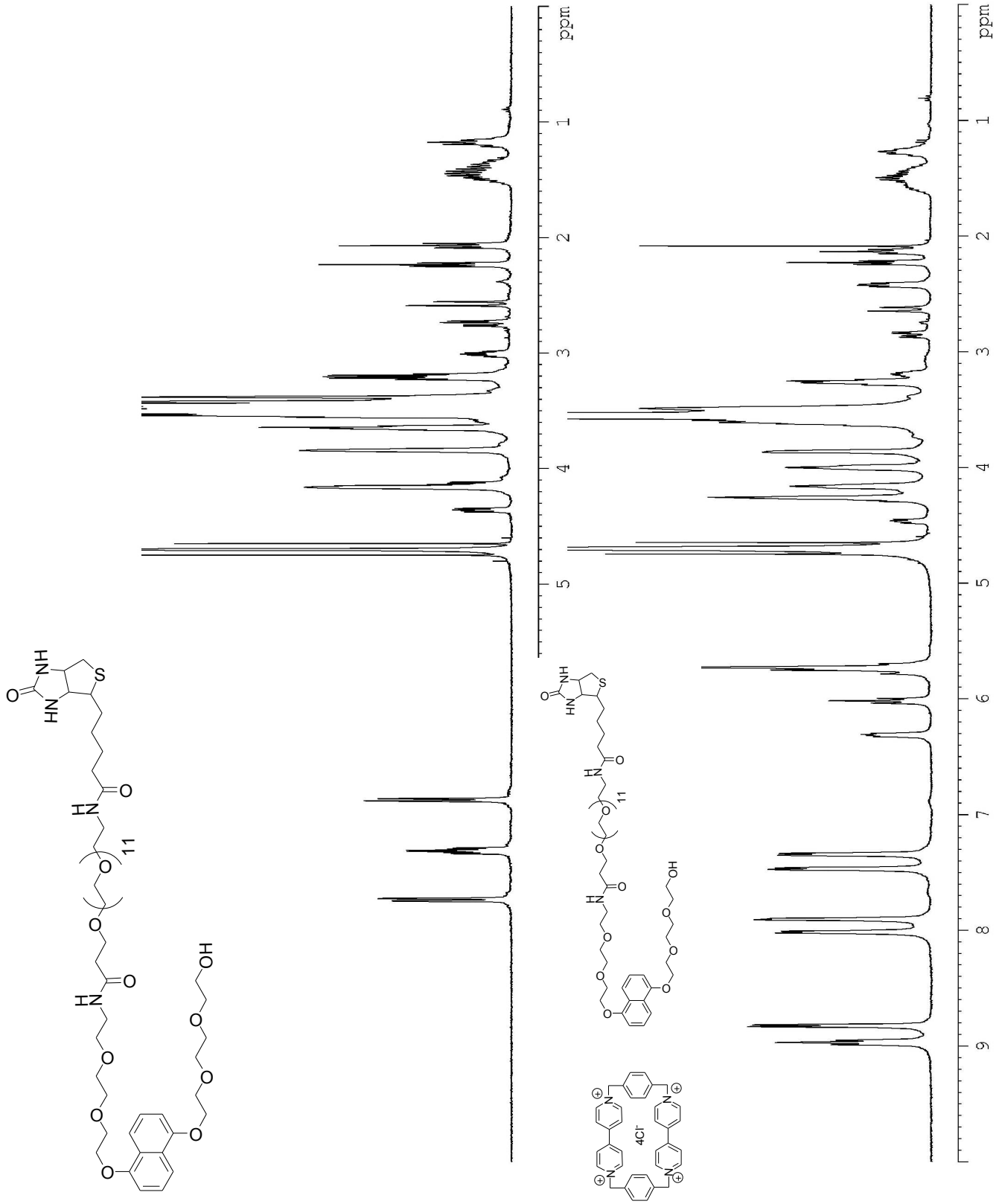
3. NMR Investigation of the complexation between 1 and 4