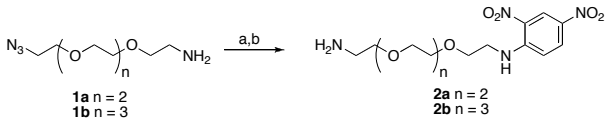
Supporting Information for "Trivalent Antigens for Degranulation of Basophils"

Materials for degranulation assays: Mouse monoclonal anti-DNP–IgE was obtained from hybridoma H1 26.8 and affinity purified. Final steps in the purification included ion exchange chromatography to remove bound DNP-glycine, then gel filtration to separate monomeric IgE from small amounts of IgE aggregates. RBL-2H3 cells were grown adherent in 75 cm^2 flasks, kept at 37 °C and 5% CO_2 and generally used 5 days after passage. Cell Media consisted of MEM 1× with Earle's salts, without glutamine (Gibco-BRL), 20% fetal bovine serum (Hyclone), 1% (v/v) -glutamine (Gibco-BRL) and 1% (v/v) penicillin and streptomycin (Gibco-BRL). Cells are harvested by first rinsing with trypsin–EDTA (Gibco-BRL) and then incubating with trypsin–EDTA for 5 min at 37° C. Cells in culture were incubated overnight at 37° C with 10 μg of anti-DNP FITC–IgE. After harvesting, the cells were washed and then resuspended in buffered salt solution (BSS: 135 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 1.8 m*M* $CaCl_2$, 5.6 m*M* glucose, 0.1% gelatin, 20 m*M* Hepes, pH 7.4).

Degranulation assays: The secretion response of RBL cells was monitored by measuring the activity of the granule-stored enzyme β-hexosaminidase secreted into the supernatant. RBL-2H3 cells (1 x 10^5 cells/100 mL/well), suspended in medium containing saturating concentrations of DNP-specific IgE were plated in 96-well plates. Cells were allowed to adhere for at least 2 h in the incubator followed by three washings with Tyrode's buffer and then were exposed to the indicated concentration of ligand. Twenty microliters of the supernatants was transferred to a new 96-well plate and reacted with 50 mL of substrate solution (*p*-nitrophenyl-*N*-acethyl-β-D-glucosamine, 1.3 mg/mL, in 0.1 *M* citrate, pH 4.5). The mixture was incubated at 37 °C for 1 h, and the reaction terminated and developed by addition of 150 mL 'stop' solution (0.2 *M* glycine, pH 10.7). The intensity of the yellow color formed at this pH, due to the nitrophenol produced by the enzymatic hydrolysis of the substrate, is proportional to the concentration of the secreted enzyme. The optical density of the color intensity was measured at 405 nm in an ELISA plate reader. All assays were repeated at least four times and their results are expressed as net percent of the cells' total enzyme activity contents ±5–10%.

Scheme 1. Preparation of dinitrophenyl-appended glycol linkers

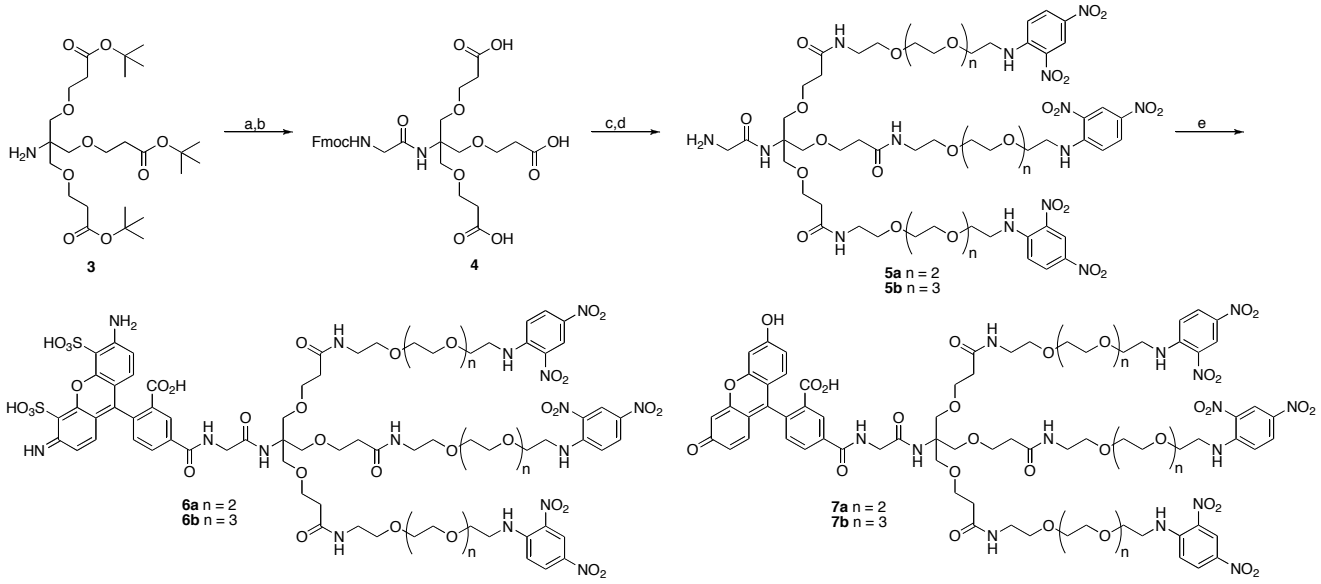


1a n = 2
1b n = 3

2a n = 2
2b n = 3

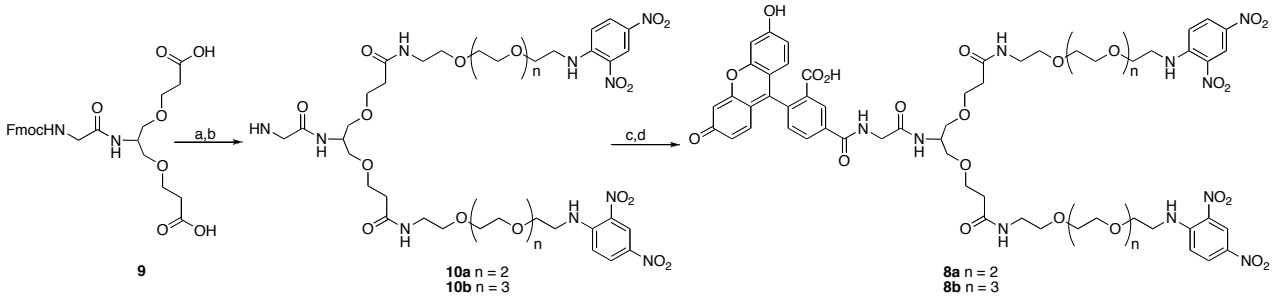
Reagents (yields in parentheses): a) 2,4-dinitrofluorobenzene, CH_2Cl_2 (85%); b) Ph_3P, EtOAc, Et_2O, H_2O (70%).

Scheme 2. Preparation of fluorophore-appended trivalent antigens.



Reagents (yields in parentheses): a) Fmoc-glycine, DCC, CH_2Cl_2 (82%); b) HCOOH (95%); c) **2a** or **2b**, HOBT, Et_3N, and EDCI, THF (60%); d) piperidine (90 %); e) Alexa-488 succinimidyl ester or 5,6-dicarboxyfluorescein succinimidyl ester, DMAP, DMF (50%).

Scheme S1. Preparation of fluorophore-appended divalent antigens **8a** and **8b**.



Reagents (yields in parentheses): a) **2a** or **2b**, HOBT, Et_3N, and EDCI, THF; b) piperidine (55-59% for two steps); b) 5,6-dicarboxyfluorescein succinimidyl ester, DMAP, DMF (50-52%).

Compound 2a: Compound **1a** (0.24 g, 1.1 mmol) and 2,4-dinitrofluorobenzene (0.32 g, 1.7 mmol) were dissolved in CH_2Cl_2 (20 mL) and stirred for 20 h at 40℃. The solvent was removed under vacuum and the residue was purified by column chromatography (SiO_2, 1:1 EtOAc/hexane) to yield the corresponding dinitrophenylamine (0.36 g, 85%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.05 (d, J = 2.5 Hz, 1 H), 8.78 (s, 1 H), 8.21 (dd, J = 2.0, 9.0, 1 H), 6.94 (d, J = 9.5 Hz, 1 H), 3.81 (t, J = 5.0 Hz, 2 H), 3.63-3.70 (m, 10 H), 3.59 (q, J = 5.5 Hz, 2 H), 3.35 (t, J = 5.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 148.3, 135.7, 130.1, 130.0, 123.9, 114.2, 70.5, 69.8, 68.4, 50.4, 43.1; FAB-MS: $[M+Na]^+$ 407.1293 (54.1%); cacld. 407.1291. The dinitrophenylamine (0.34 g, 0.9 mmol) was stirred in EtOAc (10 mL) and 1 *N* HCl (5 mL) while Ph_3P (0.25 g, 9.5 mmol) in Et_2O (10 mL) was added dropwise over 1 h. After stirring under N_2 for 24 h, the separated aqueous layer was washed with Et_2O (4x40 mL), and the pH was adjusted to 12 by addition of solid NaOH. The aqueous suspension was then extracted with CH_2Cl_2 (2 x 10 mL) and the combined extracts were dried over anhydrous Na_2SO_4. The solvent was removed under vacuum,

and the residue was purified by column chromatography (SiO_2, 10% CH_3OH in CH_2Cl_2) to afford **2a** (0.22 g, 70%). ^1H NMR ($CDCl_3$, 500 MHz) δ 9.12 (d, *J* = 4.5 Hz, 1 H), 8.82 (s, 1 H), 8.26 (dd, *J* = 4.5, 16, 1 H), 6.97 (d, *J* = 16 Hz, 1 H), 3.85 (t, *J* = 8.5 Hz, 2 H), 3.63-3.73 (m, 10 H), 3.51 (t, *J* = 8.5 Hz, 2 H), 2.86 (s, 2 H), 1.53 (s, 2 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 148.6, 136.1, 130.5, 124.4, 114.6, 70.7, 70.6, 70.3, 69.2, 68.7, 43.4, 40.5; FAB-MS: $[M+H]^+$ 359.1569 (100%); cacld. 359.1567.

Compound 2b: Same procedure as described above for preparation of the corresponding dinitrophenylamine (86% yield). ^1H NMR ($CDCl_3$, 500 MHz) δ 9.09 (d, *J* = 3.0 Hz,1 H), 8.79 (s, 1 H), 8.23 (dd, *J* = 2.5, 9.5 Hz, 1 H), 6.95 (d, *J* = 10.0 Hz, 1 H), 3.82 (t, *J* = 4.5 Hz, 2 H), 3.60-3.70 (m, 14 H), 3.59 (q, *J* = 4.5 Hz, 2 H), 3.36 (t, *J* = 4.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 148.5, 136.1, 130.6, 130.3, 124.4, 114.3, 70.9, 70.8, 70.7, 70.1, 68.7, 50.8, 43.4; FAB-MS: $[M+Na]^+$ 451.1507; cacld. 451.1548. Same procedure as described above for reducing the azide to give **2b** (75% yield). ^1H NMR ($CDCl_3$, 500 MHz) δ 9.17 (d, *J* = 6.5 Hz, 1 H), 8.83 (s, 1 H), 8.29 (dd, *J* = 4.5, 16 Hz, 1 H), 6.99 (d, *J* = 16 Hz, 1 H), 3.88 (t, *J* = 8.5 Hz, 2 H), 3.63-3.72 (m, 14 H), 3.53 (t, *J* = 8.5 Hz, 2 H), 2.89 (s, 2 H), 1.66 (s, 2 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 148.6, 136.3, 130.6, 124.5, 114.6, 77.5, 77.3, 77.0, 70.9, 70.5, 70.4, 70.2, 68.8, 68.1, 43.4, 40.3; FAB-MS: $[M+H]^+$ 403.1815 (100%); cacld. 403.1829.

Compound 4: Compound **3**[18] (2.4 g, 4.7 mmol) and Fmoc-glycine (1.4 g, 4.7 mmol) were dissolved in CH_2Cl_2 (200 mL) and cooled to 0° C. DCC (1.1 g, 5.3 mmol) in CH_2Cl_2 (20 mL) was added slowly over 1 h. The reaction mixture then was allowed to reach room temperature and left stirring for 24 h. The solvent was removed under vacuum, and the residue was purified by column chromatography (SiO_2, 1:2 EtOAc/hexane) to afford a colorless oil (3.0 g, 82%). The colorless oil was dissolved in HCOOH (10 mL), and stirred 24 h at 60℃. The HCOOH then was removed under vacuum and the residue was purified by column chromatography (SiO_2, 5% CH_3OH in CH_2Cl_2, with 0.5% v/v AcOH) to yield **5** (2.3 g, 95%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.68 (d, *J* = 7.0 Hz, 1 H), 7.57 (d, *J* = 7.0 Hz, 2 H), 7.36 (t, *J* = 7.5 Hz, 2 H), 7.28 (t, *J* = 7.5 Hz, 2 H), 6.57 (s, 1 H), 6.18 (s, 1 H), 4.33 (d, *J* = 7.5 Hz, 2 H), 4.17 (t, *J* = 6.5 Hz, 1 H), 3.88 (s, 2 H), 3.67 (m, 12 H), 2.53 (t, *J* = 6.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 175.8, 170.0, 157.5, 143.9, 141.4, 127.9, 127.3, 125.4, 120.2, 69.4, 67.7, 67.1, 60.4, 50.6, 47.2, 44.5, 35.1; FAB-MS: $[M+H]^+$ 617.2347 (100%); cacld. 617.2347.

Compound 5a: 1-Hydroxybenzotriazole (HOBT, 24.9 mg, 0.18 mmol), Et_3N (0.08 mL, 0.58 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 112.3 mg, 0.58 mmol) were added to **4** (120.2 mg, 0.19 mmol) in dry THF (10 mL). Compound **2a** (210.0 mg, 0.58 mmol) dissolved in dry THF (5.0 mL) was added, and the reaction mixture was stirred under N_2 for 24 h. After removal of the solvent at reduced pressure, the residue was purified by column chromatography (SiO_2, 1:1 EtOAc/hexane) to afford a yellow oil. The yellow oil (175 mg, 0.10 mmol) was dissolved in dry THF (5 mL), followed by the addition piperidine (1 mL). The reaction mixture was stirred for 10 min. The solvent was removed under vacuum, and the residue was purified by column chromatography (SiO_2, 5% CH_3OH in CH_2Cl_2 with 0.05% NH_4OH) to give **5a** (136 mg, 50% for two steps). ^1H NMR ($CDCl_3$, 500 MHz) δ 9.12 (t, *J* = 1.5 Hz, 3 H), 8.81(s, 3 H), 8.27 (dd, *J* = 2.5, 9.7 Hz, 3 H), 7.44(s, 1 H), 6.97 (d, *J* = 9.5 Hz, 3 H), 6.62 (t, *J* = 5.0 Hz, 3 H), 3.83 (t, *J* = 5.0 Hz, 6 H), 3.55-3.71 (m, 48 H), 3.44 (q, *J* = 5.5 Hz, 6 H), 3.28 (s, 2 H), 2.42 (t, *J* = 5.5 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 171.6, 148.6, 136.2, 130.6,

130.5, 124.5, 114.5, 70.9, 70.8, 70.7, 70.4, 70.1, 69.6, 68.7, 67.7, 59.9, 44.5, 43.427, 39.5, 36.9; FAB-MS: $[M+Na]^+$ 1437.5626 (100%); cacld. 1437.5634.

Compound 5b: The same procedure (above) for formation of **5a** was used. The overall yield for the two-step reaction sequence was 88%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.12 (d, *J* = 2.5 Hz, 3 H), 8.82(s, 1 H), 8.27 (dd, *J* = 2.5 Hz, 10.0, 3 H), 7.50(s, 1 H), 6.99 (d, *J* = 9.0 Hz, 3 H), 6.77(t, *J* = 6.0 Hz, 3 H), 3.84 (t, *J* = 5.0 Hz, 6 H), 3.61-3.73 (m, 60 H), 3.56(t, *J* = 5.5 Hz, 6 H), 3.43 (q, *J* = 5.5 Hz, 6 H), 3.28 (s, 2 H), 2.43 (t, *J* = 6.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 171.6, 148.6, 136.2, 130.6, 130.5, 124.4, 114.4, 70.9, 70.8, 70.7, 70.6, 70.3, 70.1, 69.6, 68.8, 67.6, 59.7, 44.9, 43.4, 39.4, 36.9; FAB-MS: $[M+Na]^+$ 1569.6423 (100%); cacld. 1569.6420.

Compound 6a: To the solution of **5a** (4.0 mg, 0.0028 mmol) in DMF (2 mL) was added Alexa Fluor 488 carboxylic acid, succinimidyl ester (Molecular Probes, 1 mg, 0.0015 mmol) and a catalytic amount of DMAP, and the mixture was stirred for 20 h under N_2. After removal of the solvent under vacuum, the residue was purified by chromatography (SiO_2, 25:65:4 MeOH/CH_2Cl_2/H_2O) to get **6a** (1.8 mg, 60%). ^{1}H NMR (d-DMSO, 500 MHz) δ 8.84 (d, *J* = 3.5 Hz, 3 H), 8.24 (q, *J* = 10.0, 2.5 Hz, 3 H), 8.14 (d, *J* = 8.0 Hz, 1 H), 8.02 (d, *J* = 8.5 Hz, 1 H), 7.89 (s, 1 H), 7.65 (s, 1 H), 7.26 (d, *J* = 10.0 Hz, 3 H), 6.43 (d, *J* = 9.0 Hz, 1 H), 6.32 (d, *J* = 8.5 Hz, 1 H), 5.76 (s, 1 H), 4.06 (s, 2 H), 3.63-3.67 (m, 6 H), 3.32-3.57 (m, 48 H), 3.14-3.26 (m, 6 H), 2.26 (t, *J* = 6.5 Hz, 6 H); FAB-MS: $[M+Na]^+$ 1953.5564 (100%); cacld. 1953.5567. **C13 NMR**

Compound 6b: The same procedure described above was used starting with **5b**. The yield was 55%. ^{1}H NMR (d-DMSO, 500 MHz) δ 8.83 (d, *J* = 3.0 Hz, 3 H), 8.23 (q, *J* = 10.0 Hz, 3 H), 8.13 (d, *J* = 7.5 Hz, 1 H), 8.01 (d, *J* = 8.0, 1 H), 7.89 (s, 1 H), 7.65 (s, 1 H), 7.26 (d, *J* = 9.5 Hz, 3 H), 6.43 (d, *J* = 9.0 Hz, 1 H), 6.32 (d, *J* = 8.5 Hz, 1 H), 5.40 (s, 1 H), 3.78(s, 2 H), 3.63-3.66 (m, 6 H), 3.31-3.56 (m, 60 H), 3.14-3.25 (m, 6 H), 2.26 (t, *J* = 6.5 Hz, 6 H); FAB-MS: $[M+Na]^+$ 2085.6343 (61.3%); cacld. 2085.6354. **C13 NMR**

Compound 7a: Compound **5a** (5 mg, 0.0035 mmol) and 5-(and-6)-carboxyfluorescein succinimidyl ester (Molecular Probes, 1.5 mg, 0.0031 mmol) were dissolved in dry THF (3 mL), and the mixture was stirred under N_2 for 24 h. After removal of the solvent under vacuum, the residue was purified by column chromatography (SiO_2, 10% CH_3OH in CH_2Cl_2) to afford **7a** (3 mg, 50%). ^{1}H NMR (d-DMSO, 500 MHz) δ 8.84 (t, *J* = 5.5 Hz, 3 H), 8.23 (dd, *J* = 2.5, 9.5 Hz, 3 H), 8.13 (d, *J* = 7.5 Hz, 1 H), 8.05 (d, *J* = 7.5 Hz, 1 H), 7.86 (t, *J* = 6.0 Hz, 3 H), 7.69 (s, 1 H), 7.24 (d, *J* = 9.5 Hz, 3 H), 6.65 (s, 1 H), 6.58 (d, *J* = 8.5 Hz, 1 H), 6.53 (s, 1 H), 3.80 (d, *J* = 5.5 Hz, 2 H), 3.61-3.67 (m, 12 H), 3.43-3.56 (m, 18 H), 3.29-3.35 (m, 24 H), 3.15 (q, *J* = 6.0 Hz, 6 H), 2.25 (t, *J* = 6.5 Hz, 6 H); ^{13}C NMR (d-DMSO, 500 MHz) δ 170.2, 148.3, 134.9, 129.9, 129.6, 123.5, 115.6, 69.8, 69.5, 69.1, 68.2, 67.3, 42.6, 38.5, 35.8; FAB-MS: $[M+H]^+$ 1773.6278 (100%); cacld. 1773.6292.

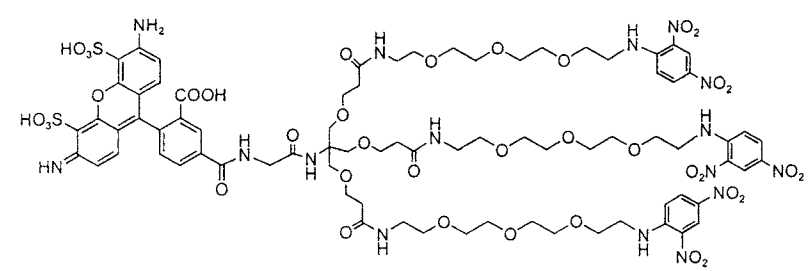
Compound 7b: The same procedure described above was used starting with **5b**. The yield was 52%. ^{1}H NMR (d-DMSO, 500 MHz) δ 8.85 (t, *J* = 5.5 Hz, 3 H), 8.50 (s, 1H), 8.24 (dd, *J* = 2.5, 9.5 Hz, 3 H), 8.14 (d, *J* = 7.0 Hz, 1 H), 7.95 (t, *J* = 5.5 Hz, 3 H), 7.32 (d, *J* = 8.0 Hz, 1 H), 7.26 (d, *J* = 9.5 Hz, 3 H), 6.59 (d, *J* = 9.0 Hz, 1 H), 6.51 (s, 1 H), 6.25 (s, 1 H), 3.92 (d, *J* = 5.5 Hz, 2 H), 3.63-3.69 (m, 12 H), 3.35-3.58 (m, 30 H), 3.34-3.39 (m, 24 H), 3.19 (q, *J* = 6.0 Hz, 6 H), 2.30 (t, *J* = 6.0 Hz, 6 H); ^{13}C NMR (d-DMSO, 500 MHz) δ 170.3, 148.4, 134.9, 129.9, 129.6, 123.5, 115.7, 69.8, 69.7, 69.5, 69.1, 68.2, 67.3, 42.6, 38.5, 35.9; FAB-MS: $[M+H]^+$ 1905.7061 (100%); cacld. 1905.7018.

Compound 10a: 1-Hydroxybenzotriazole (HOBT, 24.9 mg, 0.18 mmol), Et_3N (0.055 mL, 0.39 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCl, 75.5 mg, 0.39 mmol) were added to **9** (98.0 mg, 0.19 mmol) in dry THF (10 mL). Compound **2a** (141.0 mg, 0.39 mmol) dissolved in dry THF (5.0 mL) was added, and the reaction mixture was stirred under N_2 for 24 h. After removal of the solvent at reduced pressure, the residue was purified by column chromatography (SiO_2, 1:1 EtOAc/hexane) to afford a yellow oil (148 mg). The oil was dissolved in dry THF (5 mL), followed by the addition piperidine (1 mL). The reaction mixture was stirred for 10 min. The solvent was removed under vacuum, and the residue was purified by column chromatography (SiO_2, 5% CH_3OH in CH_2Cl_2 with 0.05% NH_4OH) to give **11a** (108 mg, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.81 (s, 2 H), 8.27 (dd, *J* = 2.5, 9.5 Hz, 2 H), 6.97 (d, *J* = 9.5 Hz, 2 H), 6.57 (t, *J* = 5.5 Hz, 2 H), 4.19 (m, 1 H), 3.84 (t, *J* = 5.0 Hz, 4 H), 3.55-3.76 (m, 28 H), 3.43-3.48 (m, 6 H), 3.36 (s, 2 H), 2.45 (m, 4 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 172.8, 171.4, 148.6, 136.3, 130.7, 130.5, 124.4, 114.4, 71.0, 70.9, 70.7, 70.4, 70.0, 69.5, 68.7, 67.5, 48.4, 45.1, 43.4, 39.5, 37.0; FAB-MS: $[M+Na]^+$ 995.39530 ; cacld. 995.39284.

Compound 10b: The same procedure described above was used starting with **2b**. The yield was 55%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.81 (s, 2 H), 8.27 (dd, *J* = 2.5, 9.5 Hz, 2 H), 6.98 (d, *J* = 9.5 Hz, 2 H), 6.66 (t, *J* = 4.5 Hz, 2 H), 4.19 (m, 1 H), 3.83 (t, *J* = 5.0 Hz, 4 H), 3.54-3.76 (m, 38 H), 3.42-3.48 (m, 6 H), 3.36 (s, 2 H), 2.45 (m, 4 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 172.9, 171.4, 148.6, 136.3, 130.7, 130.4, 124.4, 114.4, 71.0, 70.9, 70.8, 70.7, 70.6, 70.4, 70.0, 69.5, 68.8, 67.4, 48.4, 45.1, 43.4, 39.4, 37.0; FAB-MS: $[M+Na]^+$ 1083.44519; cacld. 1083.44527.

Compound 8a: Amine **10a** (3.4 mg, 0.0035 mmol) and 5-6-dicarboxyfluorescein succinimidyl ester (1.5 mg, 0.0031 mmol) were dissolved in dry THF (3 mL), and the mixture was stirred under N_2 for 24 h. After removal of the solvent under vacuum, the residue was purified by column chromatography (SiO_2, 10% CH_3OH in CH_2Cl_2) to afford **8a** (2.3 mg, 50%). ^{1}H NMR (d-DMSO, 500 MHz) δ 8.82-8.85 (m, 3 H), 8.16-8.25 (m, 2 H), 7.78-7.79 (m, 3 H), 7.26 (d, *J* = 9.5 Hz, 2 H), 6.58-6.62 (m, 3 H), 6.50 (d, *J* = 8.0 Hz, 2 H), 3.93 (d, *J* = 6.0 Hz, 2 H), 3.82 (d, *J* = 5.5 Hz, 1 H), 3.64-3.69 (m, 6 H), 3.45-3.60 (m, 20 H), 3.27-3.45 (m, 10 H), 3.14-3.20 (m, 4 H), 2.29 (m, 4 H); ^{13}C NMR (d-DMSO, 500 MHz) δ 170.1, 168.5, 168.4, 165.1, 164.9, 148.3, 135.6, 134.8, 129.8, 129.6, 129.3, 123.5, 115.6, 109.4, 102.3, 69.7, 69.5, 69.2, 68.2, 66.9, 48.6, 42.6, 42.3, 38.5, 35.8; FAB-MS: $[M+H]^+$ 1331.45979 ; cacld. 1331.45863.

Compound 8b: The same procedure described above was used starting with **10b**. The yield was 52%. ^{1}H NMR (d-DMSO, 500 MHz) δ 8.82-8.85 (m, 3 H), 8.23-8.26 (m, 2 H), 7.83-7.90 (m, 3 H), 7.26 (d, *J* = 9.5 Hz, 2 H), 6.68 (m, 2 H), 6.53-6.59 (m, 3 H), 3.93 (d, *J* = 5.5 Hz, 2 H), 3.81 (d, *J* = 5.5 Hz, 1 H), 3.63-3.69 (m, 6 H), 3.45-3.59 (m, 20 H), 3.28-3.40 (m, 18 H), 3.14-3.27 (m, 4 H), 2.30 (m, 4 H); ^{13}C NMR (d-DMSO, 500 MHz) δ 170.1, 168.4, 168.3, 164.9, 159.6, 148.3, 135.8, 134.8, 129.8, 129.6, 129.2, 126.4, 123.5, 115.6, 112.6, 109.1, 102.3, 69.7, 69.5, 69.2, 68.2, 66.9, 54.9, 48.6, 42.6, 42.3, 38.5, 35.8; FAB-MS: $[M+H]^+$ 1419.51502 ; cacld. 1419.51106.



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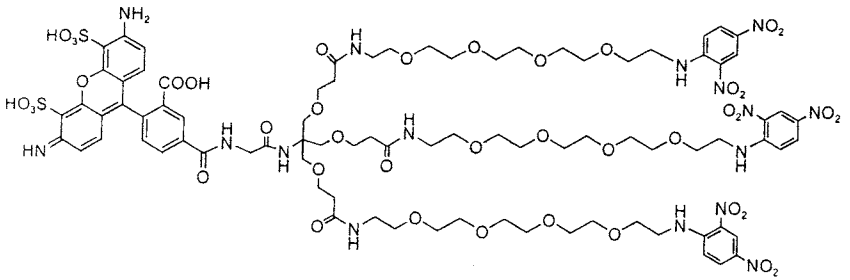
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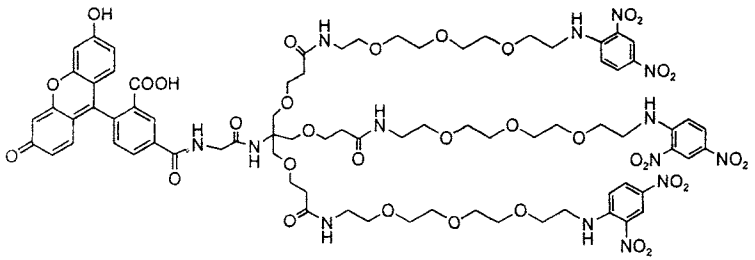
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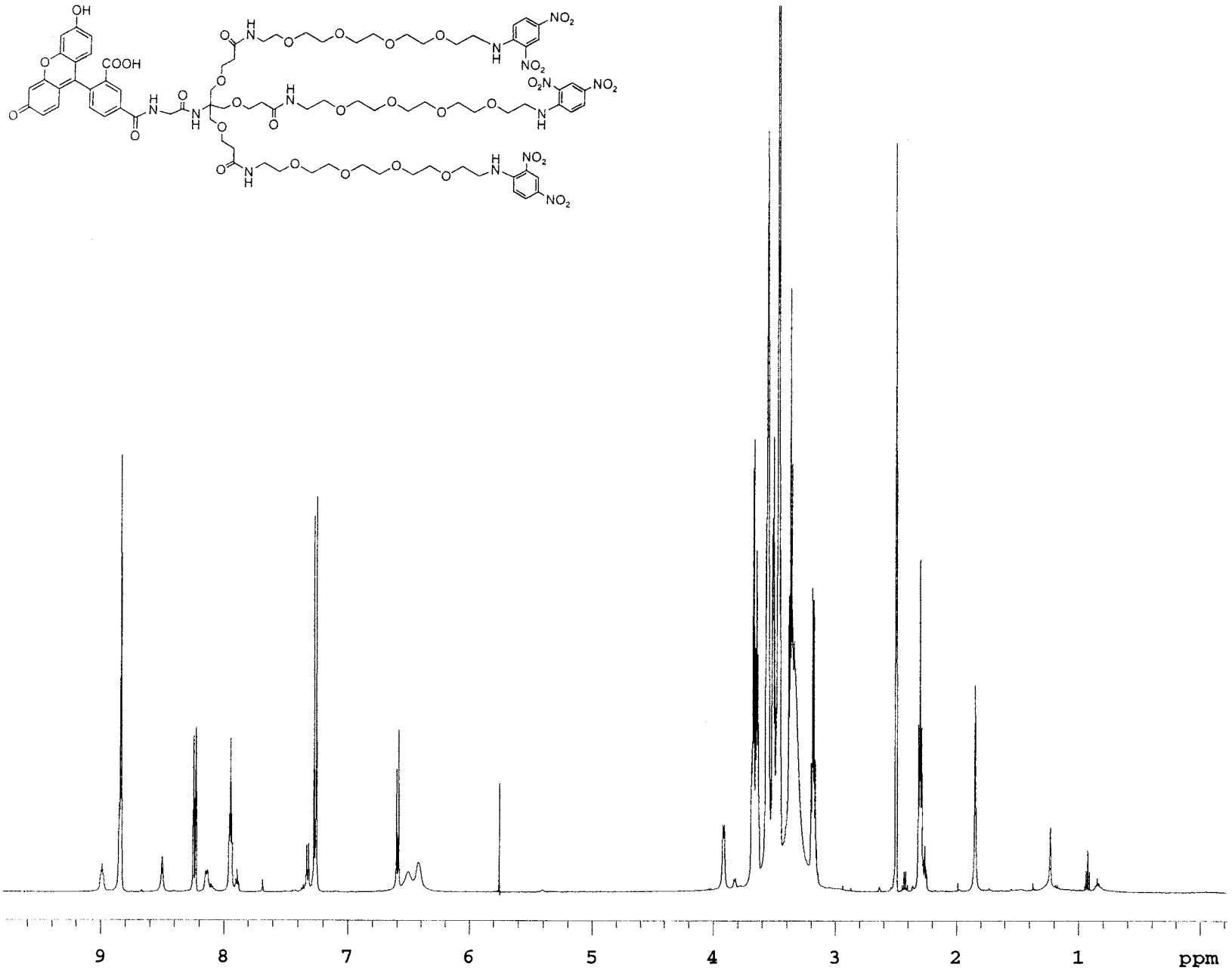
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3
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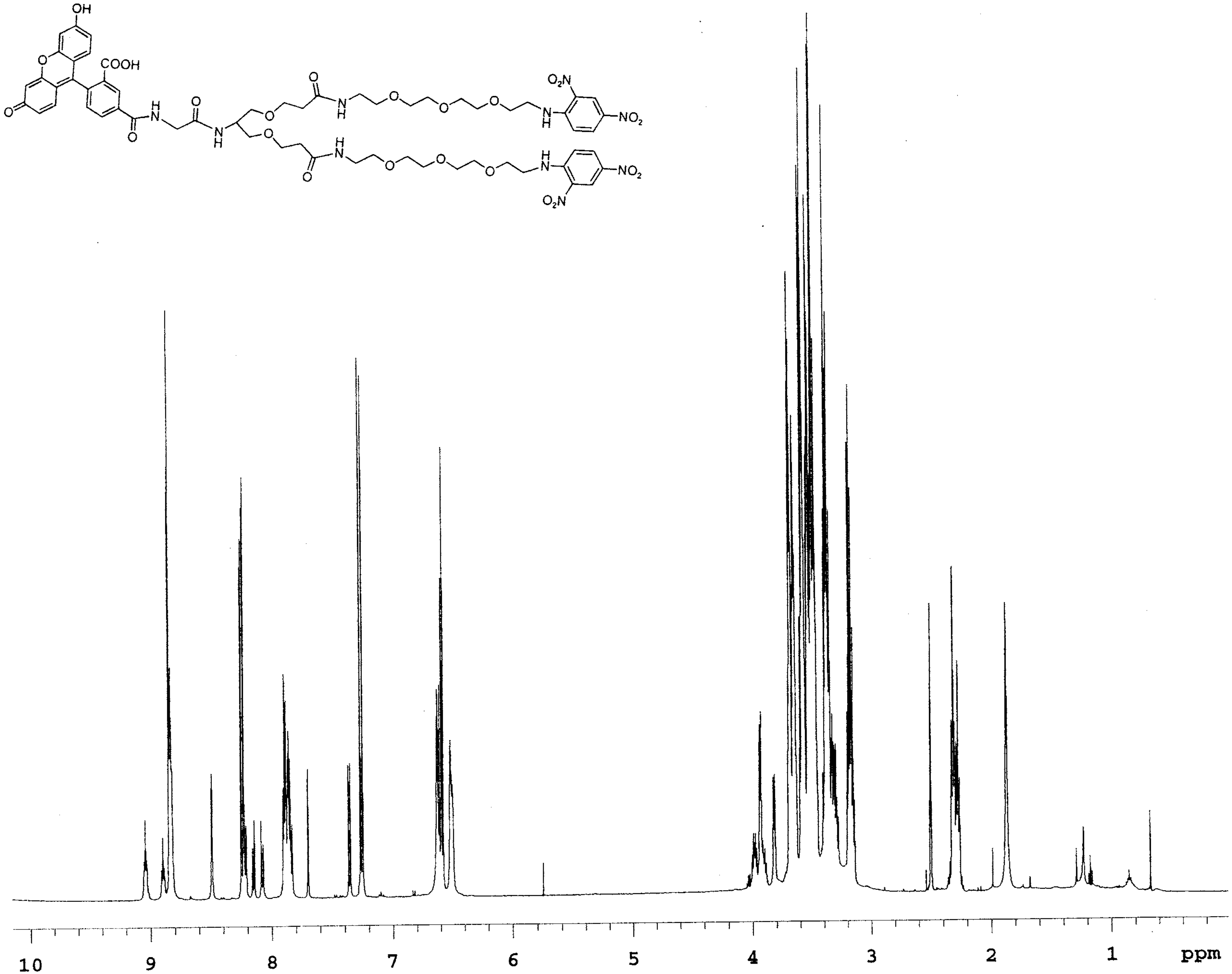
ppm



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O
H
N
NO2
O2N
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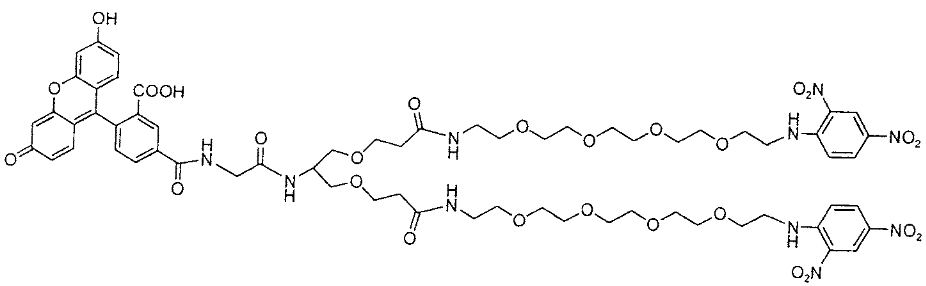
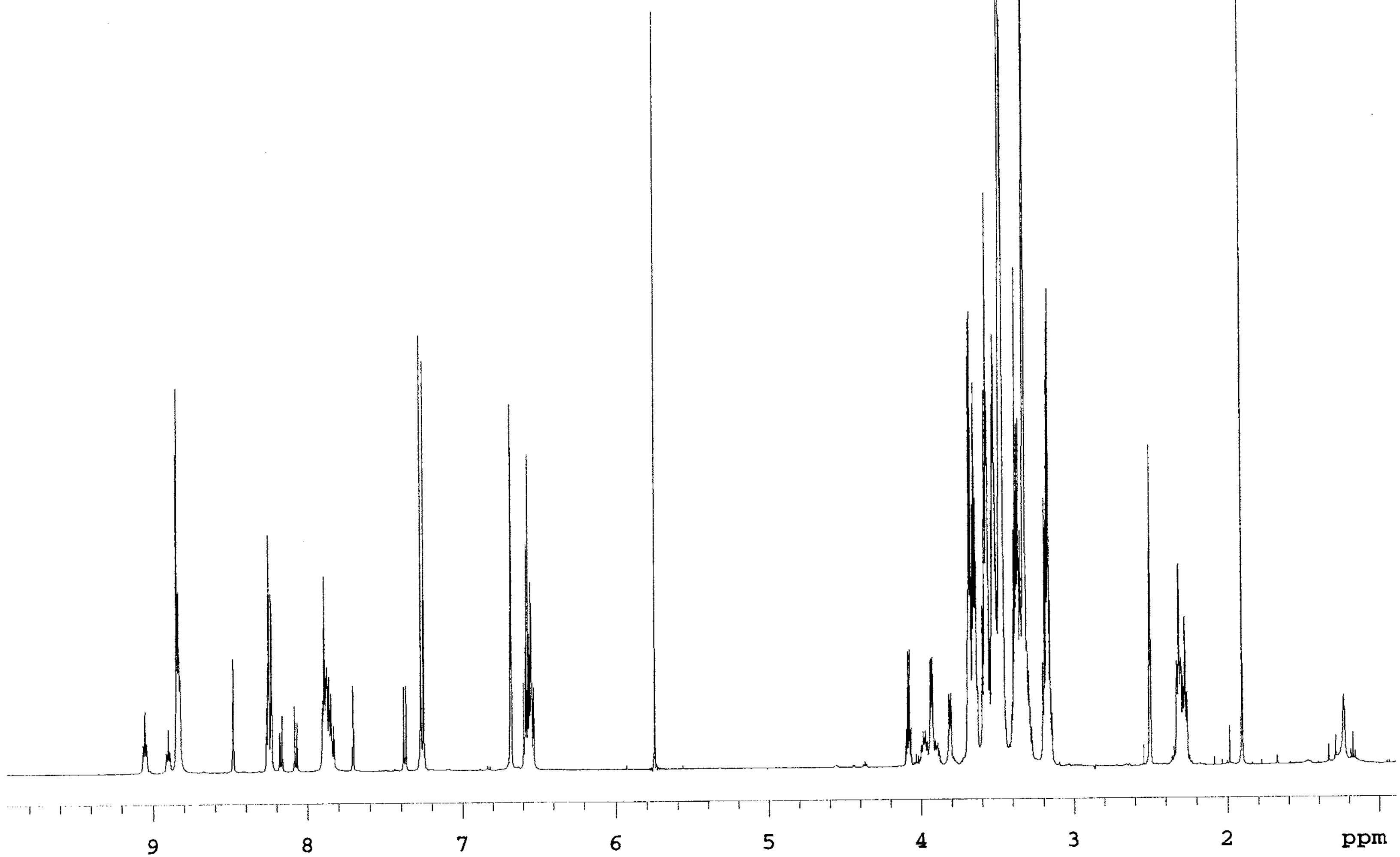
9
8
7
6
5
4
3
2
1
ppm


OH
COOH
O
H
N
NO2
O2N


9
8
7
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5
4
3
2
ppm