Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

A Mechanism-Based Fluorescent Probe for Labeling O^6-Methylguanine-DNA Methyltransferase in Live Cells

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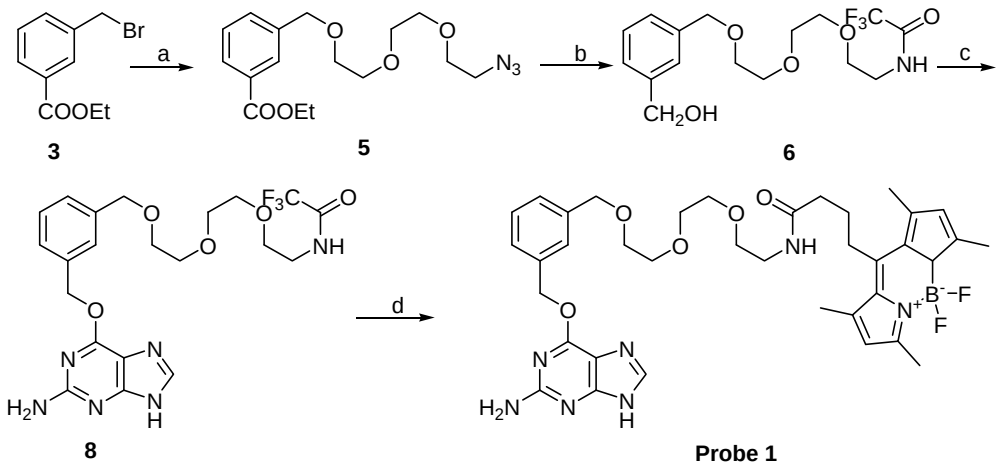
Synthesis and spectroscopic data

General: Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Anhydrous THF was distilled from Na prior to use. Anhydrous DMF was distilled from CaH_2 under reduced pressure. The intermediates **4**, **7**, **9** were prepared as reported. Reactions were monitored by thin layer chromatography using TLC Silica gel 60 F254 supplied by Qingdao Puke Seperation Meterial Corporation, Qingdao, P. R. Chin. Silica gel for column chromatography was 200-300 mesh and was supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. Characterization of intermediates and final compounds was done using NMR spectroscopy and mass spectrometry. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 or Bruker DPX-500 Fourier transform spectrometer or with *d*-$CHCl_3$ or d_6-DMSO as solvents and tetramethylsilane (TMS) as the internal standard. All spectra were recorded at 25^{o}C and chemical shifts were given in ppm and coupling constants (*J*) in Hz. Low-resolution mass data were obtained on a Finnigan LCQ DECA XP plus LCMS spectrometer. High-resolution mass data were obtained on a Waters GCT Premier^{TM} Micromass instrument. FT-IR spectra were taken on a Bruker Vector 22 spectrophotometer as KBr pellets.

Synthesis

Scheme 2. Synthesis of the mechanism-based probe **1** [a]



[a] Reagents and conditions: (a) **4**, NaH, dry THF, 0°C-r.t., 3h, 58%; (b) (i) $LiAlH_4$, dry THF, reflux, 2h; (ii) CF_3COOEt, Et_3N, anhydrous EtOH, r.t., 5h, 87% in two steps; (c) ^{t}BuOK, **7**, DMF, 0°C-r.t., 3h, 58%; (d) (i) K_2CO_3, MeOH, 60°C, 3h; (ii) Et_3N, **9**, anhydrous MeOH, r.t., 10h, 91% in two steps.

Ethyl 3-((2-(2-(2-azidoethoxy)ethoxy)ethoxy)methyl)benzoate (5) To a solution of 2-(2-(2-azidoethoxy)ethoxy)ethanol (**4**) (1.260 g, 7.2 mmol) in ice-cold dry THF (35 mL) was added NaH (60% in oil, 576 mg, 14.4 mmol). After 5 minutes of stirring, ethyl 3-(bromomethyl)benzoate (**3**) (1.750 g, 7.2 mmol) dissolved in 10 mL dry THF, was added through syringe and the system was stirred at r.t. for 3 h. H_2O (15 mL) was then added to quench the reaction and the solution was transferred to a separating funnel and diluted with EtOAc (50 mL). The organic phase was washed with H_2O (1 × 20 ml) and brine (1 × 20 ml), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product which was purified by silica gel chromatography eluted with PE/EtOAc (3:1) to afford **5** as a colorless oil (1.400 g, 4.1 mmol, 58%).

δ_H (500 MHz, $CDCl_3$) 8.01 (1 H, s), 7.96 (1 H, d, J =7.7), 7.56 (1 H, d, J =7.7), 7.42 (1 H, t, J =7.7), 4.61 (2 H, s), 4.38 (2 H, q, J= 7.1), 3.72 - 3.65 (10 H, m), 3.38 (2 H, t, J =5.1), 1.40 (3 H, t, J =7.1).

(3-((2-(2-(2-(2,2,2-Trifluoroacetamido)ethoxy)ethoxy)ethoxy)methyl)) benzyl alcohol (6) $LiAlH_4$ (385 mg, 10.1 mmol) was added to a solution of **5** (1.690 g, 5.0 mmol) in dry THF (50 ml) at 0°C. The solution was then heated to reflux and stirred for 2h. The reactant was then cooled in an ice-cold water bath and $Na_2SO_4 \cdot nH_2O$ was added to quench the reaction. The solution turned slowly from light gray to white. The solid was then removed by filtration and the filtrate was concentrated under reduced pressure to give a pale yellow oil (1.350 g), which was then dissolved in anhydrous EtOH (40 mL) and treated with CF_3COOEt (710 µl, 6.0 mmol) and Et_3N (832 µl, 6.0 mmol). After 5 h of stirring at r.t., H_2O (10 ml) was added to the reaction and the solution was extracted with EtOAc (3 × 20 mL). The organic extracts were combined, washed with brine (2 × 20 mL), dried over Na_2SO_4, filtered and concentrated under

reduced pressure to give the crude product, which was purified by silica gel chromatography eluted with CH_2Cl_2/MeOH (100:1) to afford **6** as a colorless oil (1.610 g, 4.4 mmol, 87 % for two steps).

δ_H (500 MHz, $CDCl_3$) 7.75 (1 H, m), 7.34 - 7.22 (4 H, m), 4.64 (2 H, s), 4.54 (2 H, s), 3.66 - 3.58 (10 H, m), 3.47 (2 H, q, J =5.2).

δ_C (101 MHz, $CDCl_3$) 157.70 (q, J =37), 141.39, 138.21, 128.58, 126.96, 126.40, 126.35, 117.43 (q, J =288), 73.20, 70.52,70.50, 70.21, 69.42, 68.90, 64.84, 39.79.

***O*^{6}-((3-((2-(2-(2-(2,2,2-Trifluoroacetamido)ethoxy)ethoxy)ethoxy)methyl)) benzyl) guanine (8)** Compound **6** (520 mg, 1.4 mmol) was dissolved in dry DMF (7 mL) and ^{t}BuOK (320 mg , 2.8 mmol) was added at 0^{o}C. After 5 minutes of stirring, 180 mg (0.7 mmol) of 1-(2-amino-9*H*-purin-6-yl)-1-methylpyrrolidinium chloride (**7**) were added and the solution was stirred for 3 h at r.t.. After removal of the solvent *in vacuo*, the crude product was purified by silica gel chromatography eluted with CH_2Cl_2/methanol in a gradient of 50/1 to 20/1 yielded the product in 58% as a colorless gum (205 mg, 0.4 mmol).

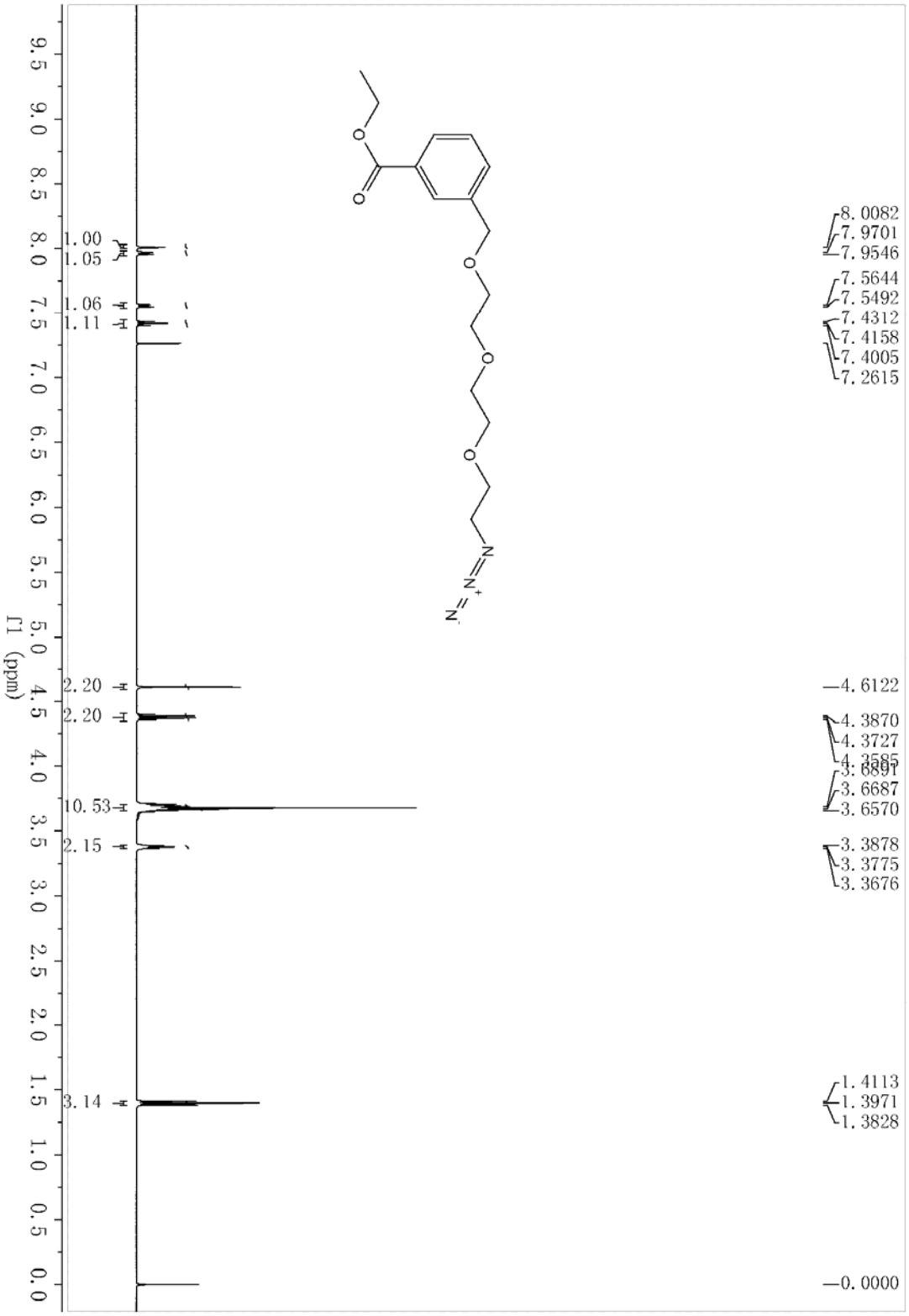
δ_H (500 MHz, DMSO) 12.45 (1 H, brs), 9.51 (1 H, brs), 7.81 (1 H, s), 7.45 (1 H, s), 7.42 (1 H, d, J =7.5), 7.38 (1 H, t, J =7.5), 7.30 (1 H, d, J =7.5), 6.33 (2 H, brs), 5.48 (2 H, s), 4.51 (2 H, s), 3.56 (4 H, s), 3.52 – 3.48 (6 H, m), 3.32 (2 H, t, J =5.7).

Probe 1 K_2CO_3 (134 mg, 1.0 mmol) was added to a solution of compound **8** (96 mg, 0.2 mmol) in MeOH (20 mL) and H_2O (0.5 mL). The reaction was stirred at 60^{o}C for 3 h till the disappearance of the starting material as shown by TLC, and then evaporated to dryness *in vacuo*. The crude product was dissolved in MeOH (10 mL) while the insoluble solid (inorganic salt) was abandoned. Concentration under reduced pressure yielded the deprotected product as a colorless gum, which was used in the next step without further purification. The gum was then dissolved in anhydrous MeOH (15 mL) and treated with *N*-hydroxysuccinimidyl-4-(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-8-yl)-butyric acid (**9**) (82 mg, 0.2 mmol) and EtN_3 (25 μL, 0.2 mmol). After 10 h of stirring at r.t., the reaction was concentrated

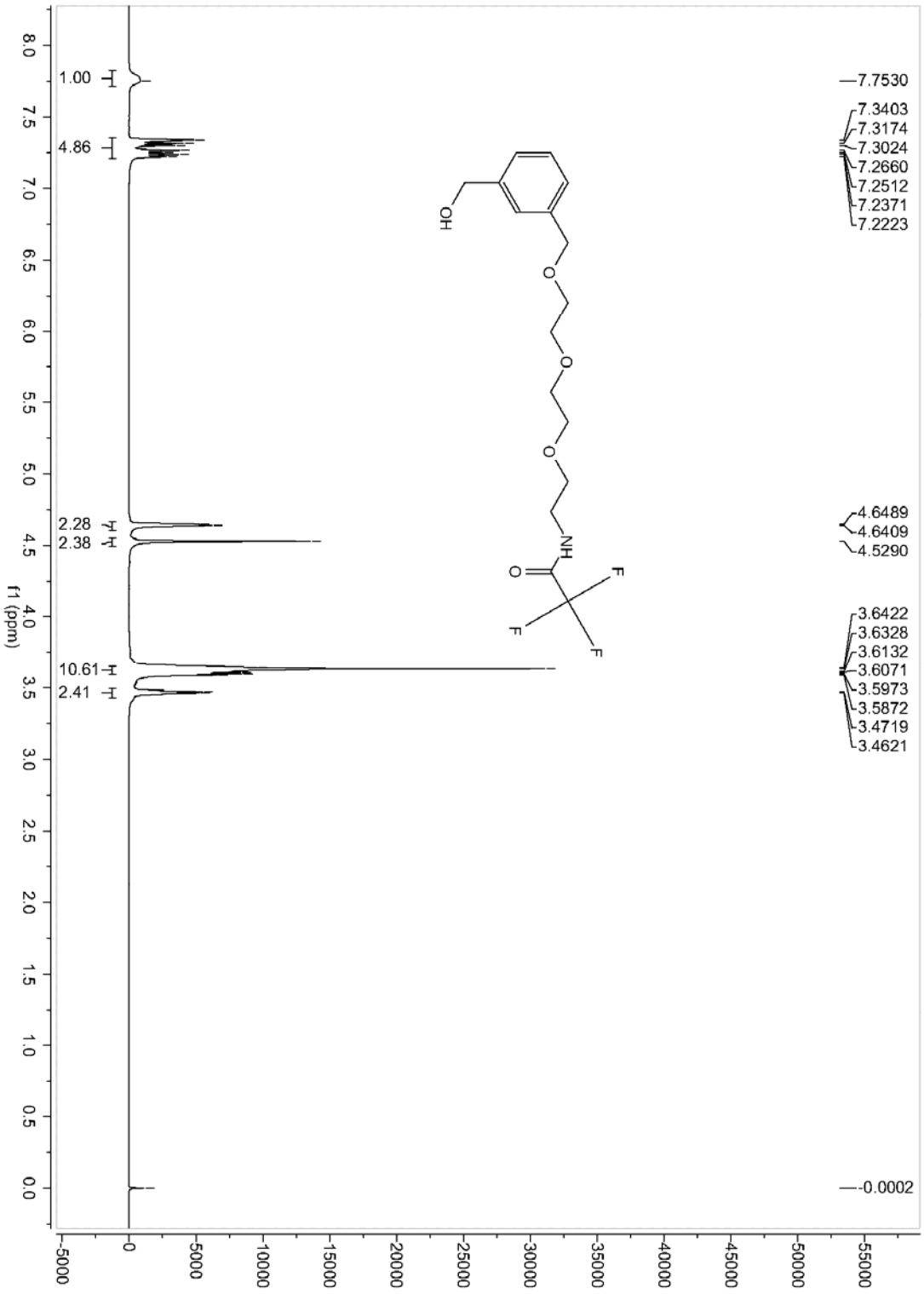
under reduced pressure and purified by silica gel chromatography eluted with CH_2Cl_2/MeOH in a gradient of 20:1 to 5:1 to afford the probe as a brick red gum (140 mg, 0.2mmol, 91%).

δ_H (400 MHz, DMSO) 12.45 (1 H, brs), 8.06 (1 H, m), 7.81 (1 H, s), 7.45 – 7.33 (3 H, m), 7.28 (1 H, d, J =6.9), 6.32 (2 H, s), 6.22 (2 H, s), 5.47 (2 H, s), 4.47 (2 H, s), 3.53 (4 H, s), 3.50 (4 H, s), 3.41 (2 H, t, J =5.2), 3.21 (2 H, q, J =5.2), 2.92 (2 H, m), 2.50 (6 H, s), 2.39 (6 H, s), 2.28 (2 H, t, J =6), 1.76 (2 H, m); δ_C (101 MHz, DMSO) 171.39, 159.86, 159.69, 155.23, 153.15, 146.32, 141.01, 138.71, 137.85, 136.79, 130.74, 128.36, 127.53, 127.45, 127.14, 121.72, 113.48, 71.88, 69.79, 69.75, 69.64, 69.22, 69.10, 66.65, 45.70, 35.40, 27.65, 27.14, 15.82, 14.11; ESI-MS *m/z* 719.20 $[M+H]^+$; HRMS (TOF-EI) *m/z* Calculated for $C_{36}H_{45}BN_8O_5F_2$ 718.3574, found 718.3588, Δ = 1.9 ppm; IR (KBr) ν_{max} = 3415, 2931, 2870, 2363, 1630, 1548, 1506, 1467, 1403, 1198, 1081, 980 cm^{-1}.

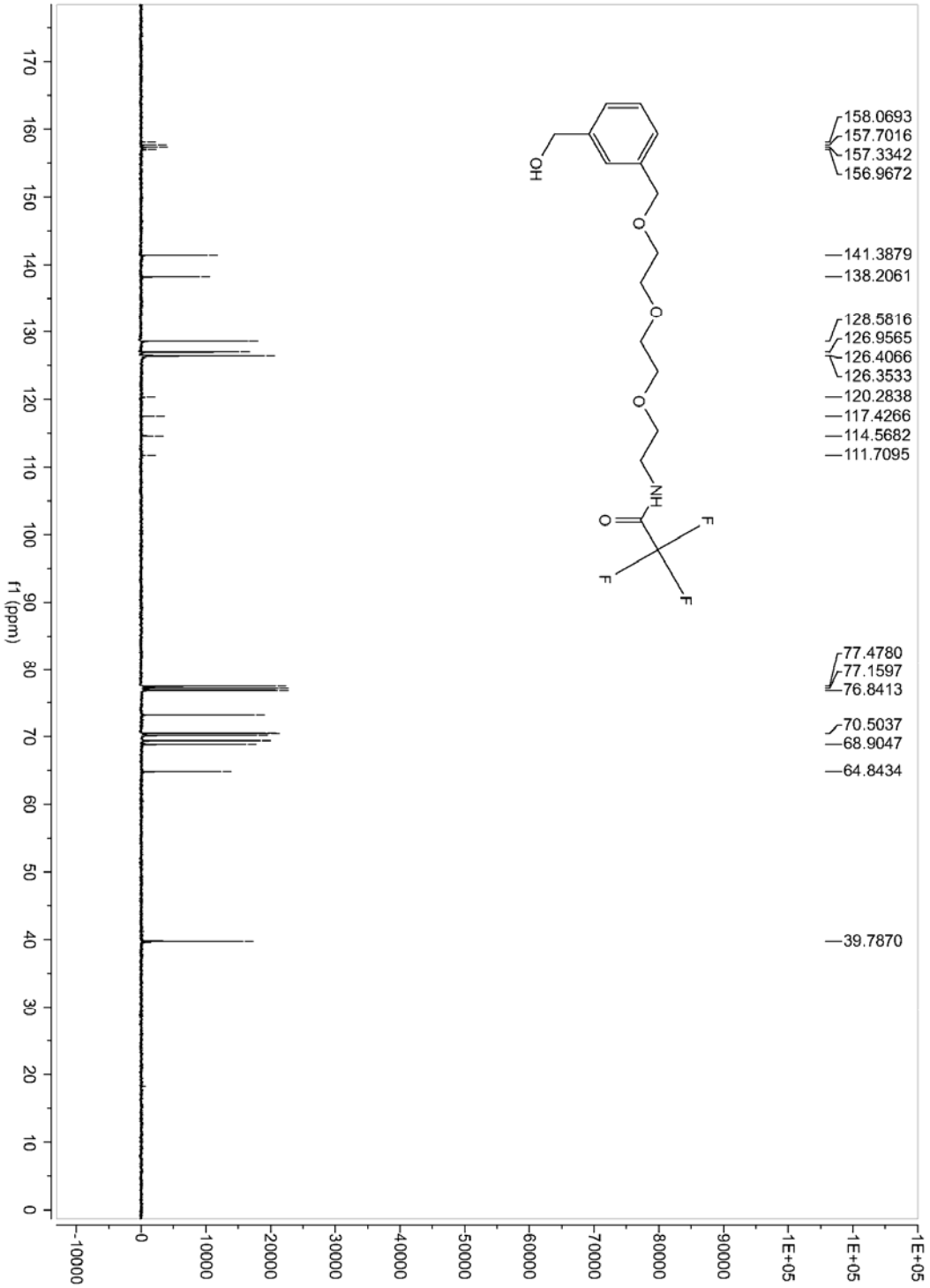
NMR traces



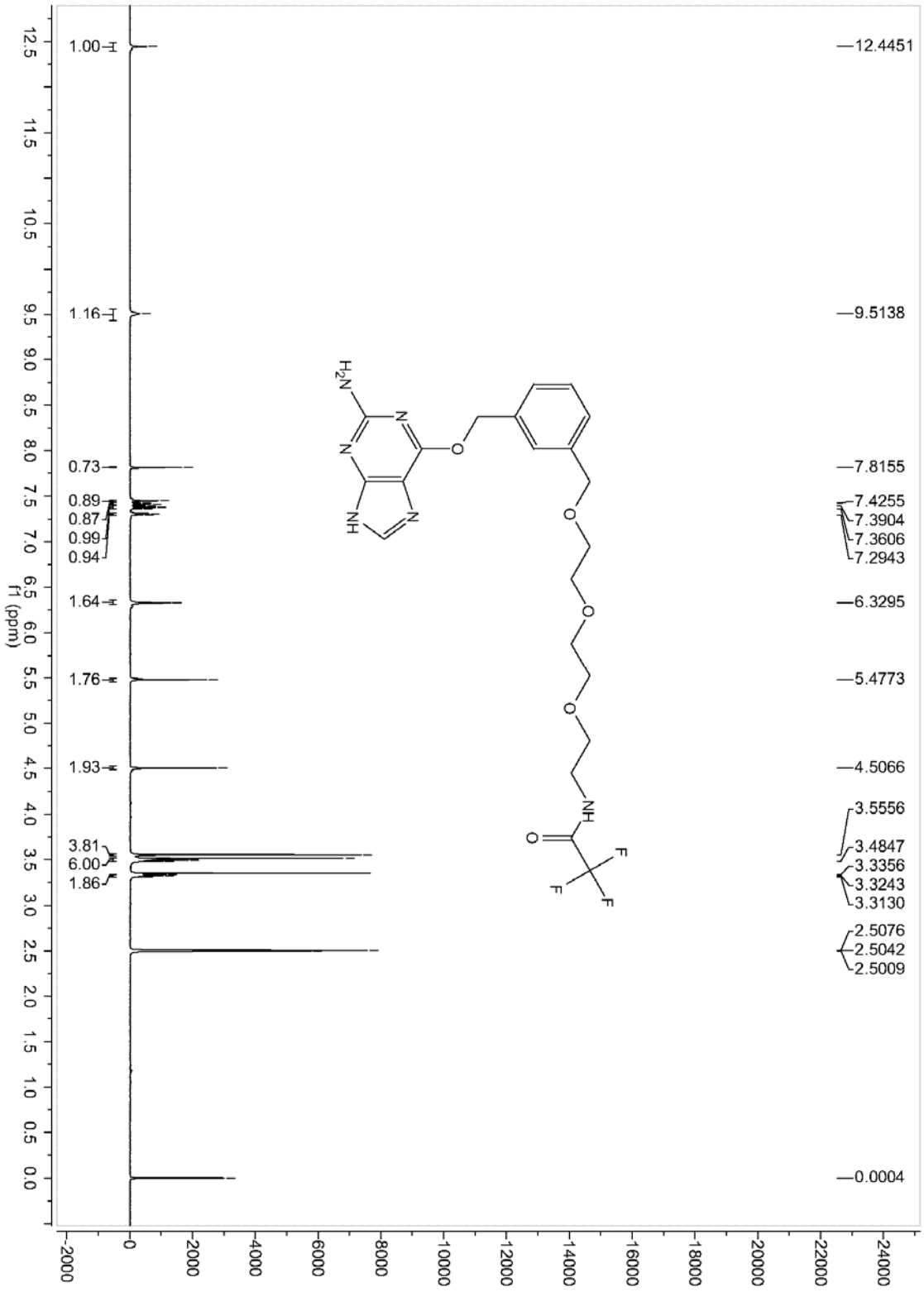
^{1}H NMR of compound 5



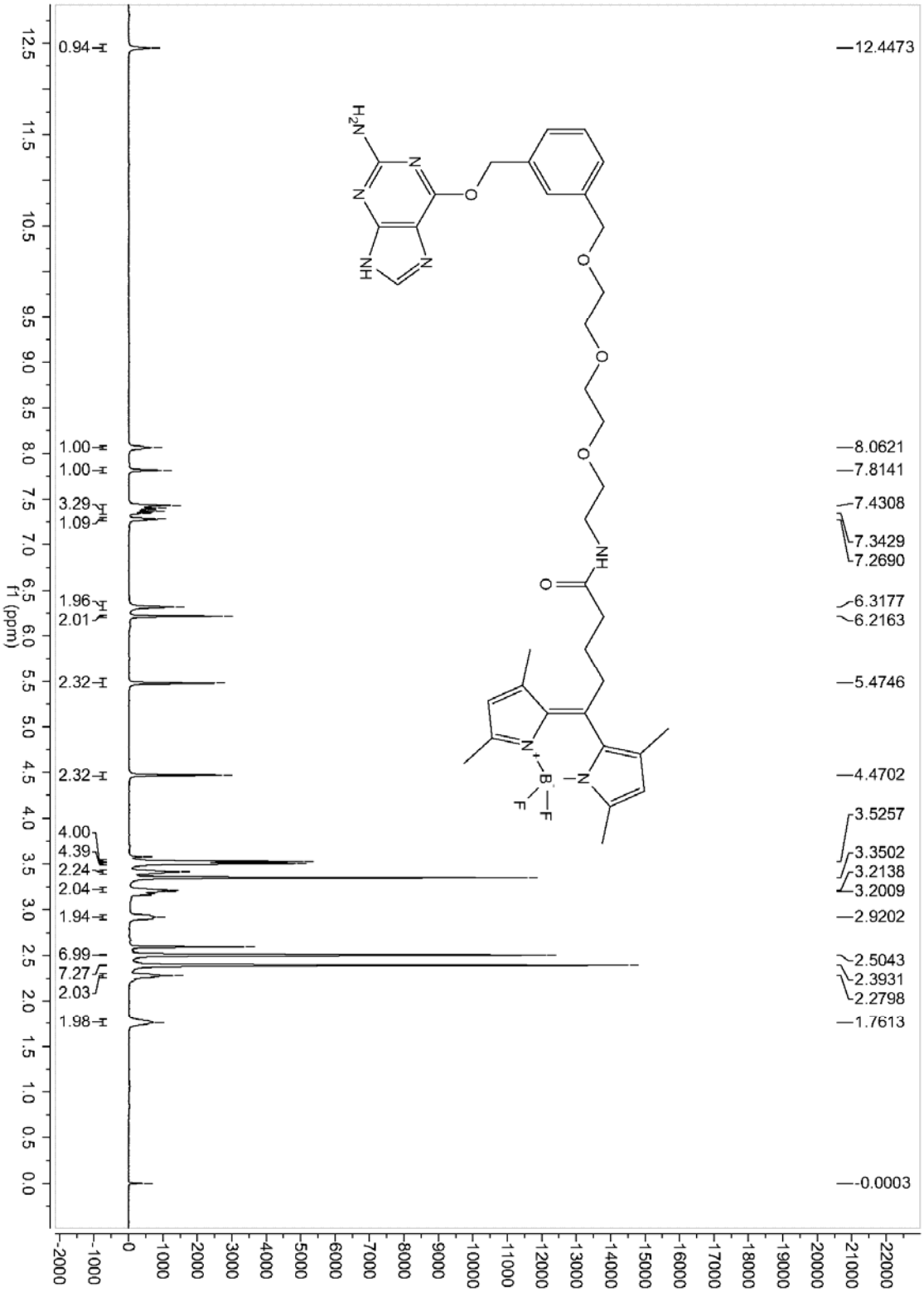
^{1}H NMR of compound 6



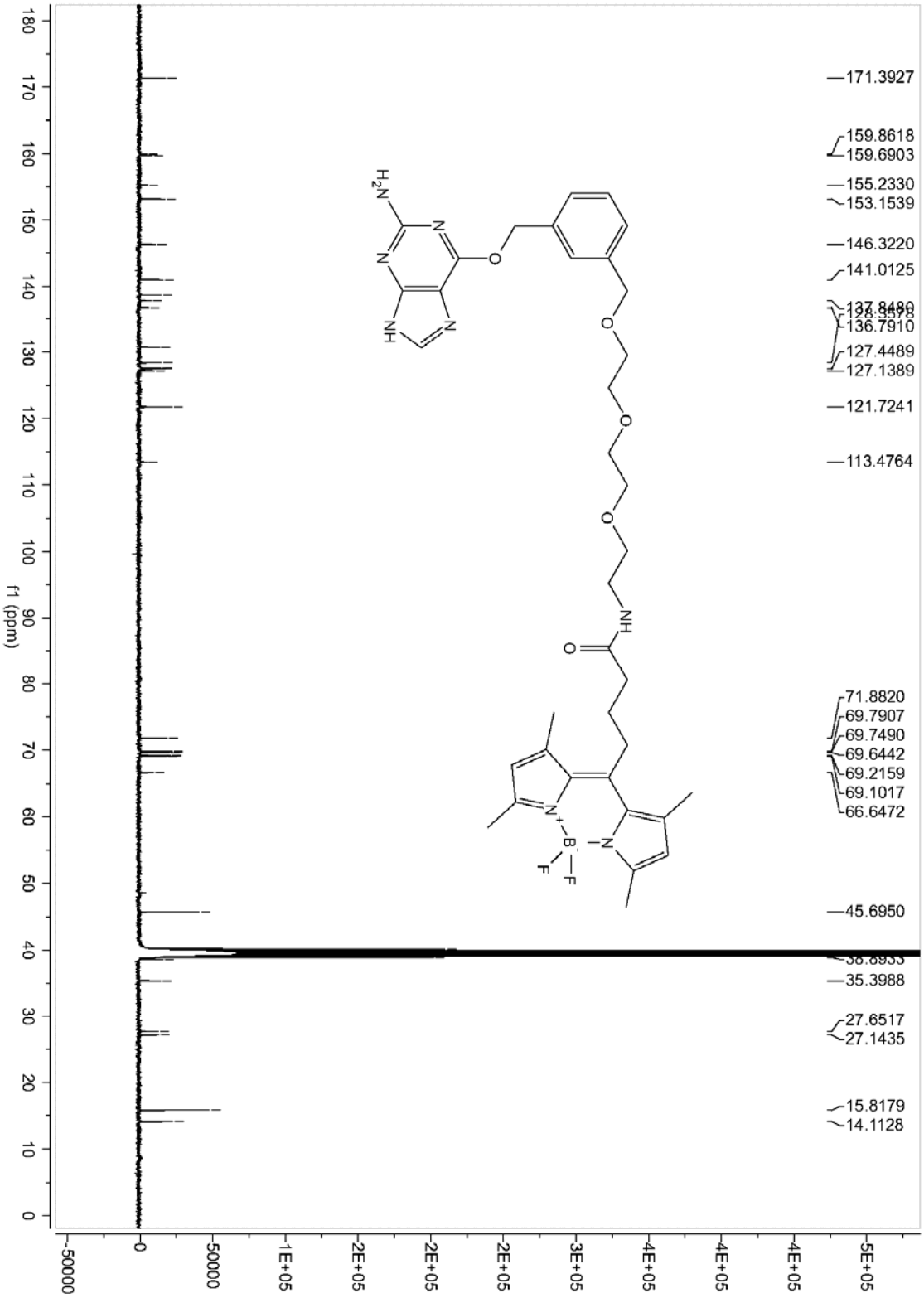
^{13}C NMR of compound 6



^{1}H NMR of compound 8



^{1}H NMR of probe 1



^{13}C NMR of probe 1

Elemental Composition Report **Page 1**

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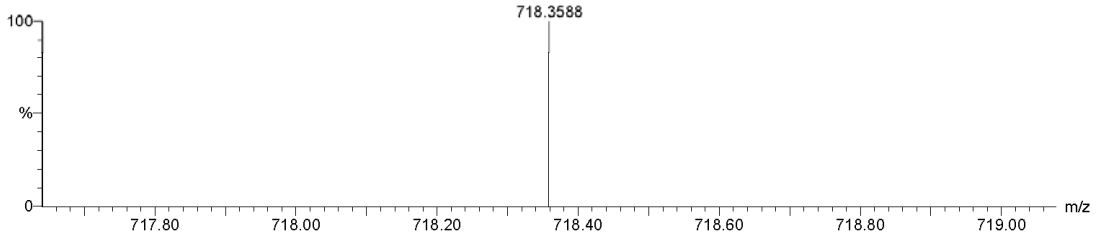
Element prediction: Off

Monoisotopic Mass, Odd and Even Electron Ions
107 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-300 B: 0-1 N: 8-8 O: 0-6 F: 2-2
13-Jul-2011GCT Premier ZJU
Ix 405 (1.485) Cm (403:409)

TOF MS EI+
2.38e+002



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HR-MS of probe 1