Supporting information

Facile Synthesis of Novel Hybrid POSS Biomolecules via "Click" Reaction

Youssef El Aziz,[a] Nazia Mehrban,[b] Peter G. Taylor,[a] Martin A. Birchall[b] James Bowen,[a] Alan R. Bassindale,[a] Mateusz B. Pitak,[c] Simon J. Coles[c]*

a) The Open University, Faculty of Science, Technology, Engineering & Mathematics, Walton Hall, Milton Keynes, MK7 6AA, UK
b) University College London, Ear Institute, Brain Sciences, 332 Gray's Inn Rd, London WC1X 8EE, UK
c) UK National Crystallography Service, Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK

*To whom correspondence should be addressed. Fax: +441908 858 327; E-mail: youssef.elaziz@open.ac.uk

Keywords: Alkyne-Terminated-Silsesquioxane cage, Click reaction; Functionalized silsesquioxanes (POSS); Huisgen 1,3-dipolar cycloaddition; Biological scaffold, X-ray crystal structure.

Experimental Section

Materials

All the materials required in these reactions were commercial available. Octa(3-aminopropyl)silsesquioxanes hydrochloride (OctaAmmonium POSS-HCl) was purchased from hybrid plastic. 1-Hydroxy benzotriazole (HOBt), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI), N-Methylmorpholine (NMM), N-ethyldiisopropylamine (DIPEA), copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$), copper powder, sodium ascorbate, were supplied by Sigma-Aldrich, and used as received. Azido-N-Fmoc-norleucine was purchased from Iris Biotech GmbH. All the solvents used in the synthesis were analytical pure, dry and used without further purification. Silica column chromatography was carried out using silica gel (200-300 mesh) provide by Sigma-Aldrich. Thin layer chromatography was performed on commercially available Silica gel on TLC Al foils silica gel matrix, with fluorescent indicator 254 nm.

Measurements

Infrared spectra were performed using Thermo Nicolet Nexus 670 with Diamond ATR. NMR spectra were recorded as solutions in deuterochloroform with tetramethylsilane as internal standard on a JEOL Lamda 300 NMR spectrometer or a JEOL EX 400 NMR spectrometer (*J* values are given in Hz). MALDI TOF mass spectra were carried out by the University of Swansea using 2,5-dihydroxybenzoic acid as a matrix and ACN as the solvent.

T_8[N-propyl-hex-5-ynamide]$_8$ (2)

5-hexynoic acid (1.53 g, 13.63 mmol, 16 equiv), was dissolved in 20 mL DMF. NMM (2.75 g, 27.30 mmol, 32 equiv) was added, followed by the mixture of EDCI (2.614 g, 13.64 mmol, 16 equiv) and HOBt (2.01g, 14.92 mmol, 17.5 equiv) in an ice bath. After 5 min, octa-aminopropyl POSS-HCl (1 g, 0.85 mmol, 1 equiv) was added. The reaction mixture was allowed to warm to room temperature and stirred at room temperature for 24 h, and then 150 mL (0.5 M) citric acid aqueous solution was added. The precipitate was then treated with acetonitrile and saturated aqueous $NaHCO_3$ solution. The crude product was purified by silica gel column chromatography. The product was obtained as a white solid (1.54 g, 90%). ^{1}H NMR (300 MHz, DMSO)/ ppm: δ = 7.84 (s, 8H, NH), 3.35 (t, 16H, NHC**H**$_2$), 3.01 (m, 16H, O=CC**H**$_2$), 2.75 (s, 8H, C≡C**H**), 2.50 (t, 16H, C**H**$_2$C≡CH), 1.66 (m, 16H, C**H**$_2$), 1.45 (m, 16H, C**H**$_2$), 0.59 (t, 16H, SiC**H**$_2$). ^{13}CNMR (75.5 MHz, $CDCl_3$)/ ppm:δ = 176.54 (s, C=O), 89.17 (s, **C**≡CH), 76.53 (s, C≡**C**H), 39.32 (s, NH**C**H$_2$), 29.54 (C=O**C**H$_2$), 27.60 (CH$_2$), 22.55 (CH$_2$), 13.85 (s, **C**H$_2$C≡CH), 5.25 (s, SiCH$_2$). ^{29}Si NMR (79.3 MHz, $CDCl_3$)/ ppm: δ = -66.13. IR: ν/cm^{-1} 3289, 3086, 2936 (ν_{C-H}), 2871, 2150 ($\nu_{\equiv C-H}$), 1718, 1635 ($\nu_{C=O}$), 1556, 1455, 1435, 1369, 1195, 1094 ($\nu_{as(Si-O-Si)}$), 1024, 1003, 970, 799 ($\nu_{s(Si-O-Si)}$), 910, 680. Anal. Calcd for $C_{72}H_{112}N_8O_{20}Si_8$: C, 52.91; H,

6.91, Found C, 52.91; H, 6.92. MS (MALDI-TOF positive mode): m/z (%): 1633.00 [M^+], 1656.5 (100%) [M+Na]$^+$. The X-ray data of this compound are given in the supporting materials.

The preparation of T_8[6-(4-(3-(propylcarbamoyl)propyl)-1-*H*-1,2,3, triazol-4-yl)-Fmoc-norLeu-]$_8$ (3)

The click reaction was carried out in a 20 mL round-bottom flask with a small magnetic stirrer bar by adding the POSS-alkyne (200 mg, 122.36 μmol), copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$) (91.66 mg, 367.08 μmol, 3 equiv), copper powder (23.32 mg, 367.08 μmol, 3 equiv), sodium ascorbate (73 mg, 367.08 μmol, M=198.11, 3 equiv), and azido-N-Fmoc-norleucine (395.74 mg, 1003.35 μmol, 8.2 equiv) in DMF:water (2:1, 5 mL) followed by addition of N-ethyldiisopropylamine (DIPEA) (290 mg, 734 μmol, 6 equiv). The mixture was stirred at room temperature for 24-48 h. The completion of the reaction was confirmed by IR, ESI mass spectroscopy. The reaction mixture was filtered, and the solution was freeze dried to afford the crude product.The product was abstained as brown gel and was further purified by silica gel flash chromatography with (silica, CH_2Cl_2: MeOH, 94: 6) as eluent. The compound was obtained as a waxy white foam (0.5 g, 94%). ^{1}H NMR (300 MHz, DMSO)/ ppm: δ =7.96 (s, 8H, N**H**), 7.89-7.25 (m, 72H, Ar-**H** of Fmoc + C**H** of triazol), 4.29-4,23 (m, 32H, C**H**COOH + OC**H**$_2$Fluorene + C**H** of Fluorene), 3.93-3.80 (t, 16H, C**H**$_2$-N (triazol)), 3.02 (t, 16H, NHC**H**$_2$), 2.63 (t, 16H, C**H**$_2$-C=CH of triazol), 2.20-2.07 (m, 16H, -NHC=OC**H**$_2$), 1.93-1.60 (m, 48H, C**H**$_2$), 1.27-1.19 (m, 16 H, C**H**$_2$), 1.18-1.10 (m, 16H, C**H**$_2$), 0.60 (t, 16H, SiC**H**$_2$). ^{13}CNMR (75.5 MHz, $CDCl_3$)/ ppm:δ = 173.84 (**C**O_2H), 171.85 NH**C**=O), 162.35 (NH**C**O_2CH_2), 146.29, 143.73 (**C**q of Fmoc), 143.68 (HC=**Cq**, triazole), 140.60 (**C**q of Fmoc), 127.56 (**C**H of Fmoc), 127.00 (**C**H of Fmoc), 125.19 (=**C**H of triazol), 121.64 (**C**H of Fluorene), 120.00 (**C**H of Fluorene), 67.00 (O**C**H_2-Fluorene), 53.00 (NH**C**HCO$_2$H), 48.94 (**C**H_2-N of triazol), 46.56 (**C**H of Fluorene), 40.12 (NH**C**H_2), 34.81 (NHCO**C**H_2), 29.28 (**C**H_2-C=CH (triazol)), 25.16 (**C**H_2-CHCO$_2$H), 24.60 (CH_2), 22.57(**C**H_2), 8.57 (Si**C**H_2). ^{29}Si NMR (79.3 MHz, $CDCl_3$)/ ppm: δ = -65.68. IR: ν/cm^{-1} 3288, 2935 (ν_{C-H}), 1711, 1644 ($\nu_{C=O}$), 1537, 1448, 1372, 1243, 1094 ($\nu_{as(Si-O-Si)}$), 1044, 760 ($\nu_{s(Si-O-Si)}$), 739, 660. Anal. Calcd for $C_{240}H_{288}N_{40}O_{52}Si_8$: C, 60.18; H, 6.06, Found C, 60.37; H, 5.95. MS (MALDI-TOF positive mode): m/z (%) Calc. 4786 [M^+]. Found 4787 [M + H]$^+$.

T_8[4-(3-(propylcarbamoyl)propyl)-1-*H*-1,2,3, triazol-4-yl)-3'-thymidine)-]$_8$ (4)

The click reaction was carried out in a 20 mL round bottom flask with a small magnetic stirrer bar by adding the POSS-alkyne (18 mg, 11 μmol), copper(II) sulfate pentahydrate ($CuSO_4$.$5H_2O$) (92 mg, 33 μmol, 3 equiv), copper powder (2 mg, 33 μmol, 3 equiv), sodium ascorbate (7 mg, 33.02 μmol, 3 equiv), and 3′-azido-3′-deoxythymidine (25 mg, 94 μmol, 8.5 equiv) in DMF:water (2:1, 5 mL) followed by addition of N -ethyldiisopropylamine (DIPEA) (9 mg, 66 μmol, 6 equiv). The mixture was stirred at room temperature for 24 h. The completion of the reaction was confirmed by MALDI-TOF mass spectroscopy and infrared. The reaction mixture was filtered, and the solvent was freeze-dried to afford the crude product. The crude product was further purified by silica gel flash chromatography with ($CHCl_3$: MeOH, 0%→0.5%→1%→1.5%→2%) as eluent. The compound was obtained as a waxy white product (38 mg, 90%). NMR (300 MHz, DMSO)/ ppm: δ =11.33 (br s, 1H, NH, thymidine), 7.84 (br s, 8H, NH), 7.76 (s, 8H, triazole), 7.58 (br s, 8H, H-6, thymidine), 6.10 (t, 8H, J = 6.24 Hz, H-1' deoxyribose), 5.24 (m, 8H, J = 4.76 Hz, H-3' deoxyribose), 4.47-3.35 (m, 8H, H-4' deoxyribose), 3.87-3.56 (m, 16H, H-5' deoxyribose), 3.09-2.96 (t, 16H, NHC**H**$_2$), 2.54-2.48 (m, 16H, C**H**$_2$-triazole), 2.43-2.23 (m, 16H, H-2' deoxyribose), 2.22-2.12 (m, 16H, C=OC**H**$_2$), 1.81-1.76 (m, 40H, C**H**$_3$ of H-5 + C**H**$_2$,), 1.72-1.61 (m, 16H, C**H**$_2$), 1.54-1.36 (m, 16H, $SiCH_2$C**H**$_2$), 0.60 (t, 16H, J = 7.32 Hz, SiC**H**$_2$). ^{13}CNMR (75.5 MHz, $CDCl_3$)/ ppm:δ = ^{13}C NMR (100 MHz, DMSO) / ppm: δ = 8.62 (Si**C**H_2), 12.14 (**C**H_3), 22.37 (**C**H_2), 24.23 (**C**H_2), 34.75 (C=O**C**H_2), 36.74 (C-2'), 41.47 (NH**C**H_2), 60.71 (**C**-3') , 60.71 (**C**-5'), 83.30 (**C**-4'), 83.96 (**C**-1'), 109.44 (**C**-5), 123.27 (H**C**=C, triazole), 135.95 (C-6), 143.65 (HC=**Cq**, triazole), 150.32 (C-2), 163.65 (C-4), 171.37 (s, C=O). ^{29}Si NMR (79.3 MHz, $CDCl_3$)/ ppm: δ = -66.64 IR: ν/cm^{-1} 3413, 3280, 2900, 1660, 1550, 1052 ($\nu_{as(Si-O-Si)}$), 1025, 1006, 896, 822, 760 ($\nu_{s(Si-O-Si)}$). Anal. Calcd for $C_{152}H_{216}N_{48}O_{52}Si_8$: C, 48.40; H, 5.77. Found C, 48.52; H, 5.69. MS (MALDI-TOF positive mode): m/z (%) Calc. 3769.39 [M^+]. Found 3835.30 [M + Cu]$^+$ (base peak 100%).

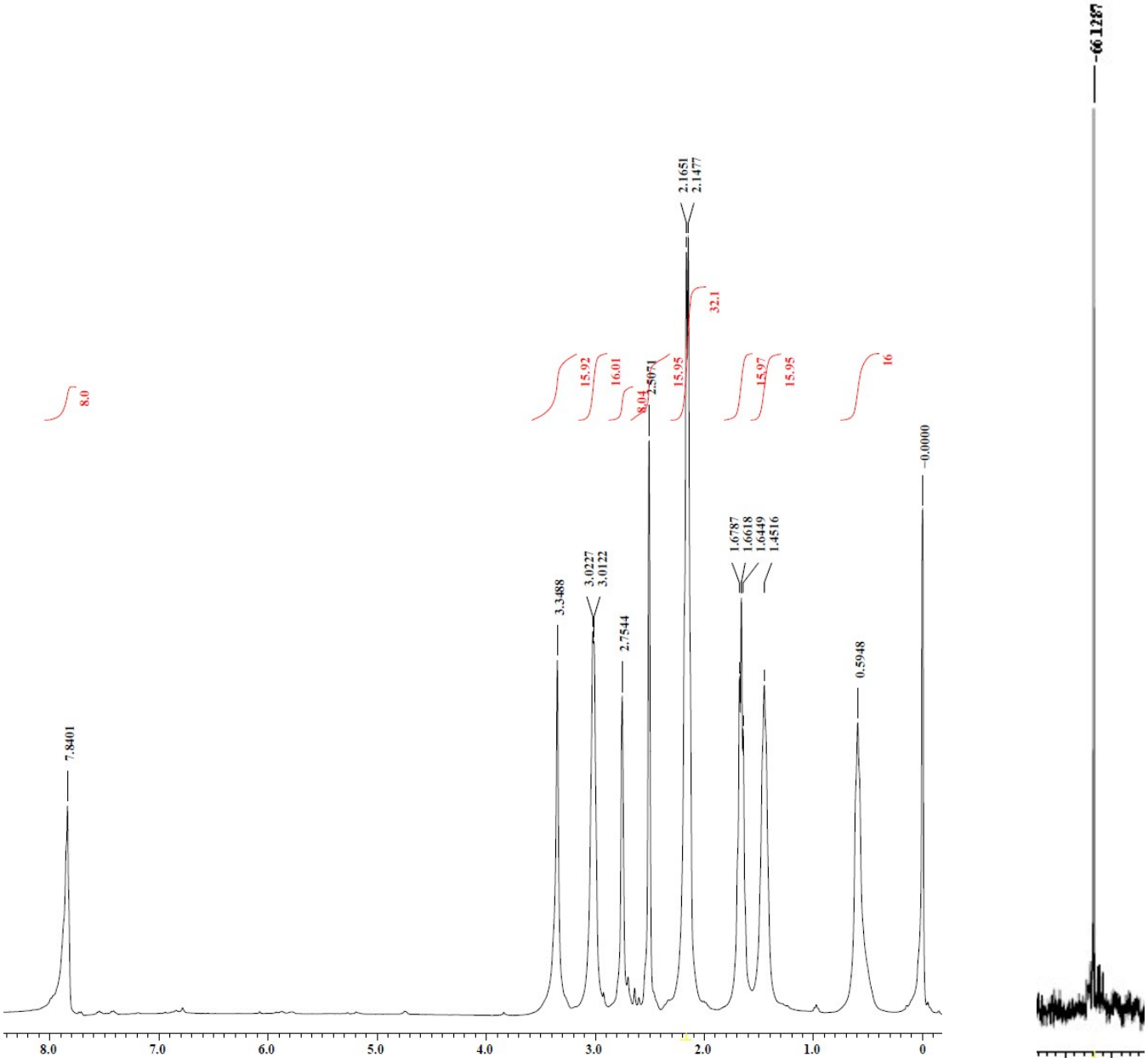

Figure S1. ^{1}H NMR and ^{29}Si NMR spectra of T_8[N-propyl-hex-5-ynamide]$_8$ (**2**)

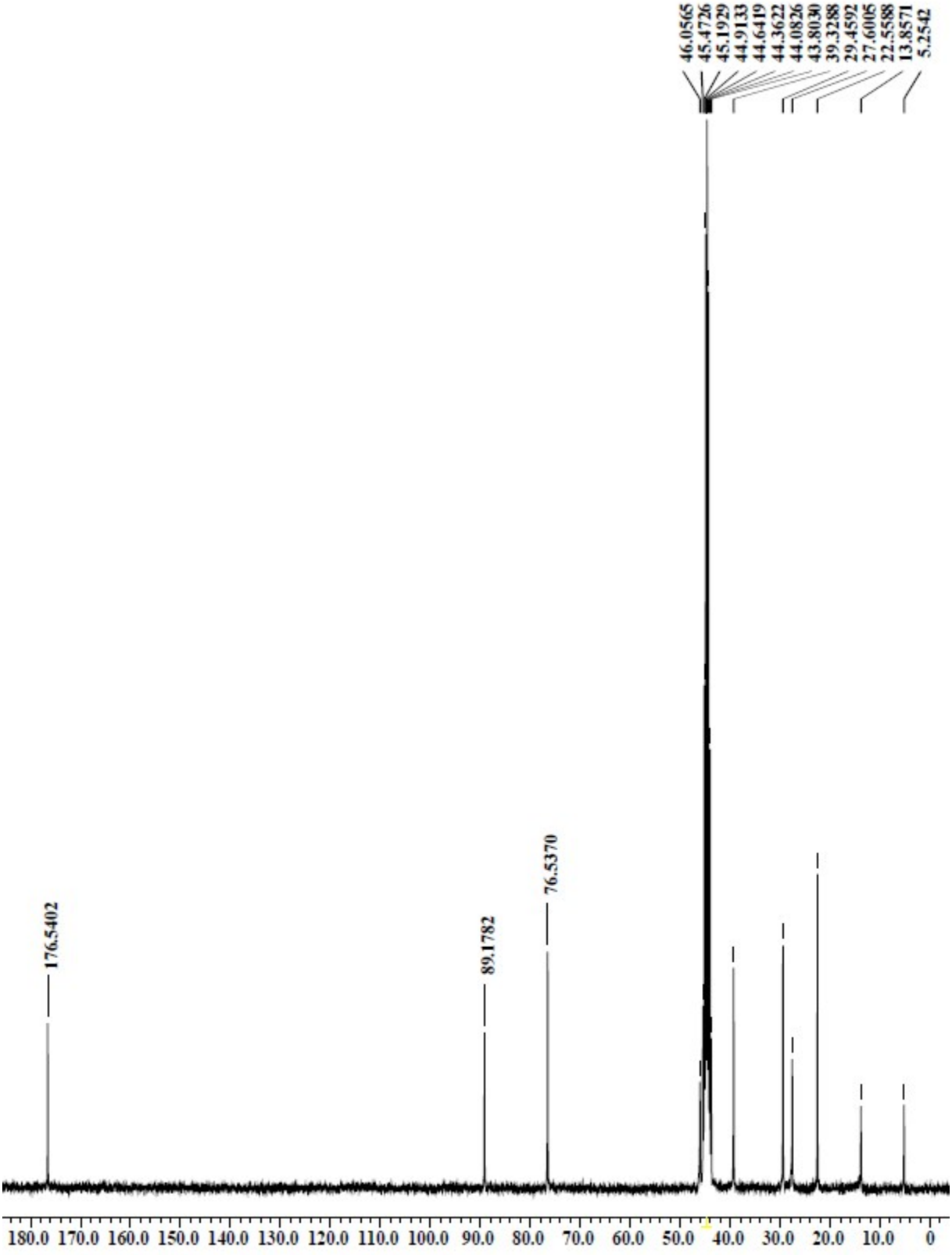

Figure S2. ^{13}C NMR spectrum of T_8[N-propyl-hex-5-ynamide]$_8$ (**2**)

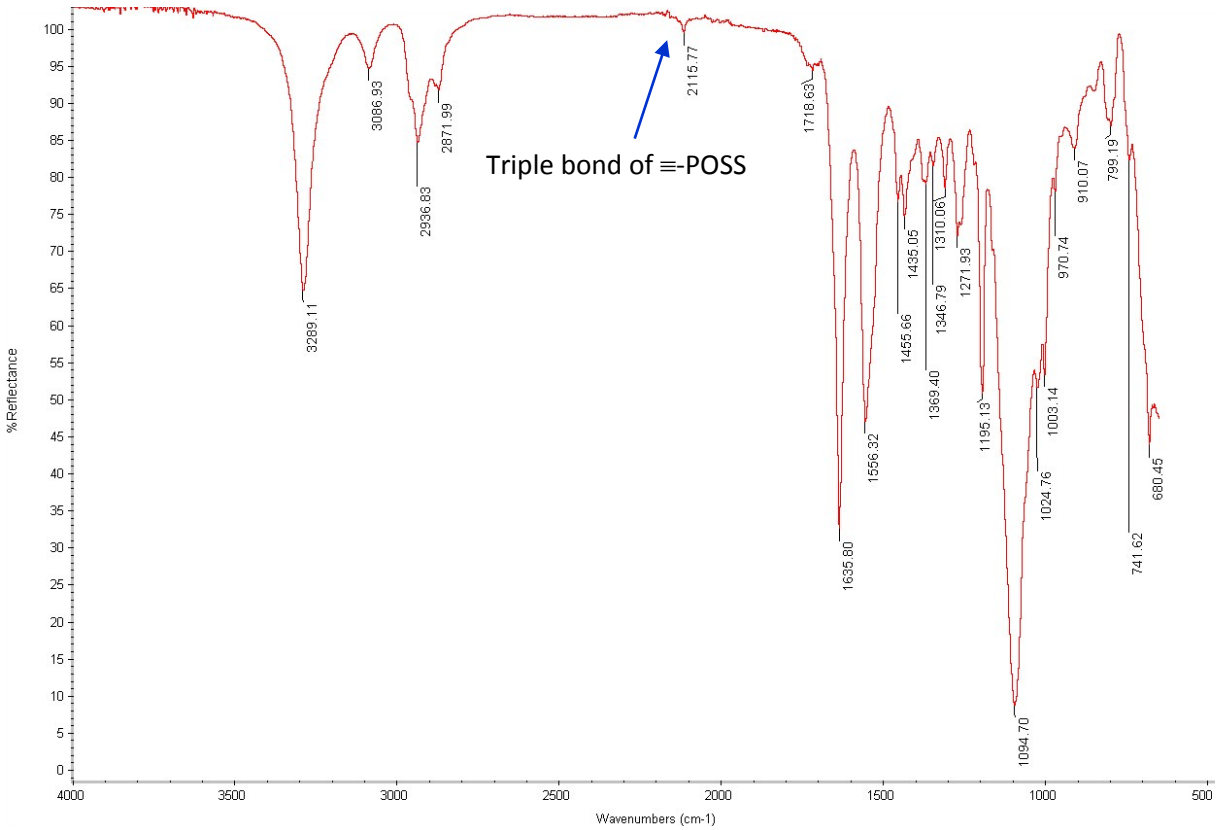

Figure S3. Infrared spectrum of **2**

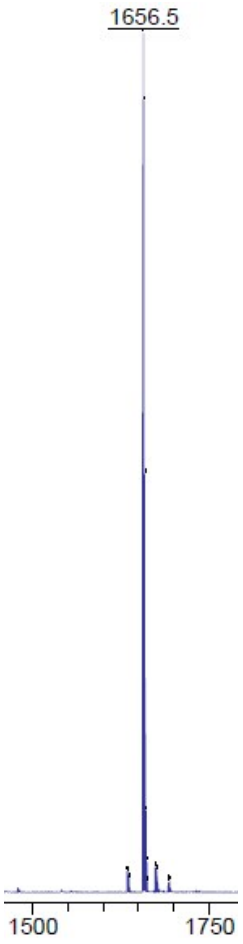

Figure S4. MALDI-TOF MS spectrum of **2**

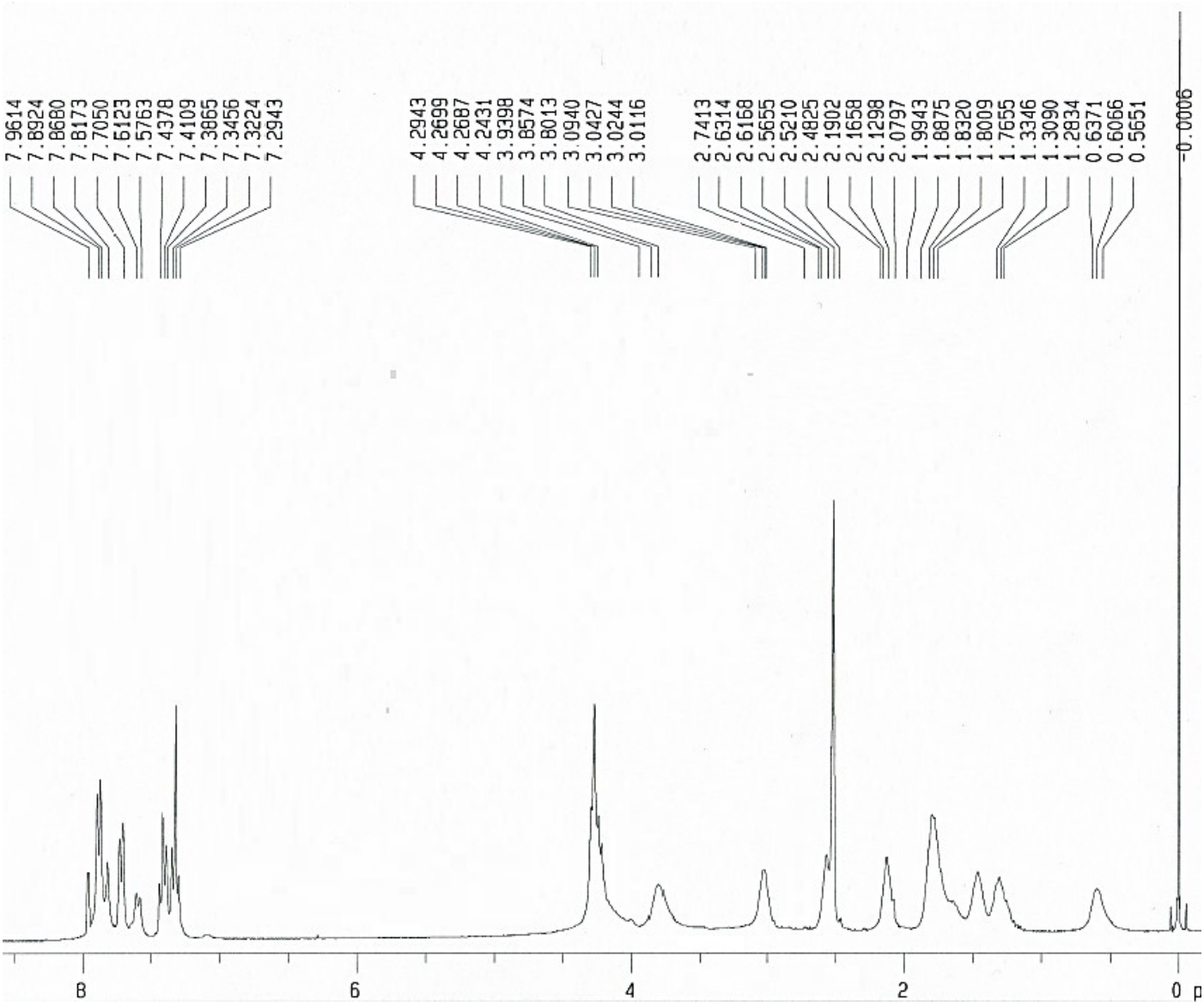

Figure S5. ^{1}-H NMR spectrum of **3**

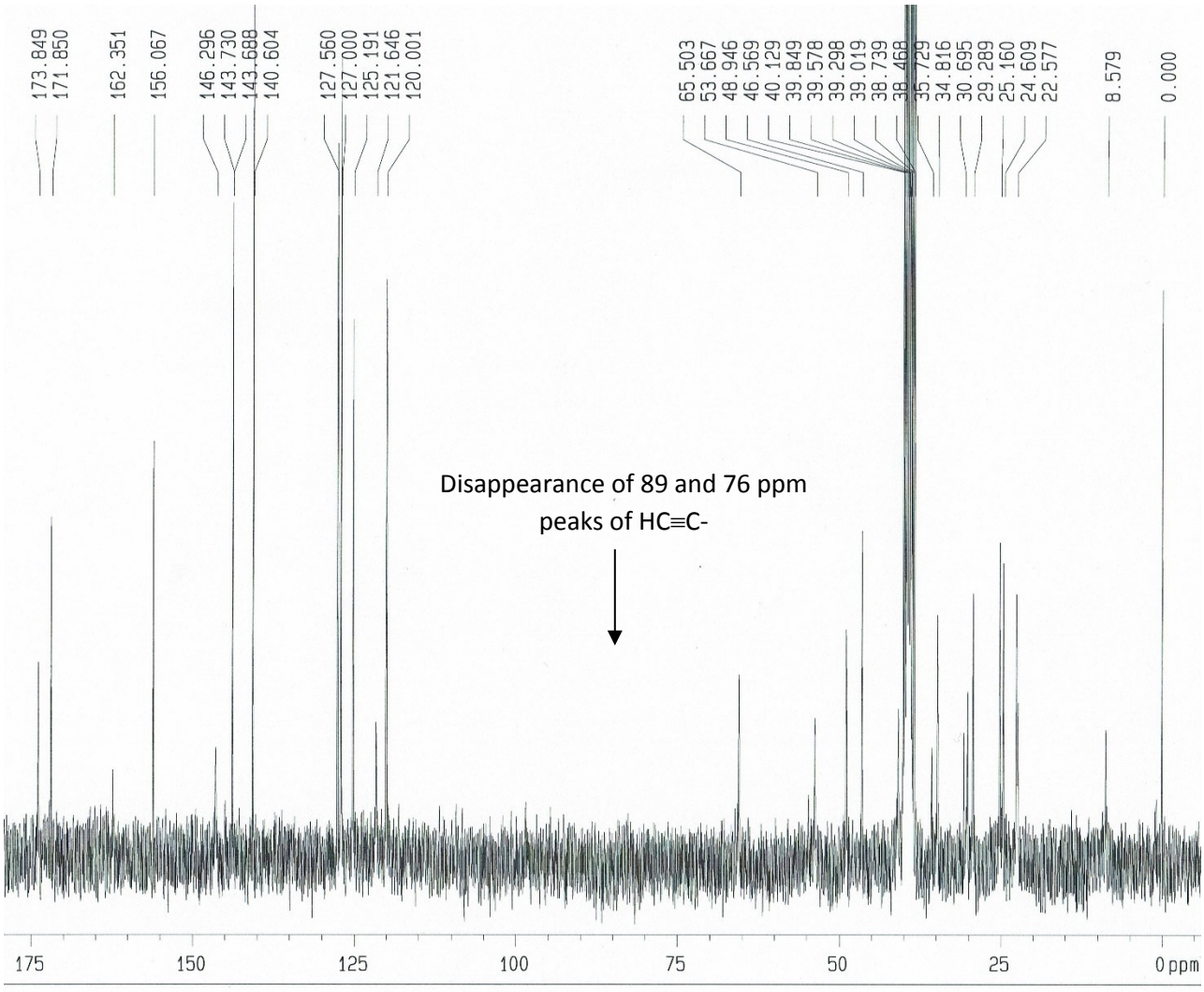

Figure S6. ^{13}C NMR spectrum of **3**

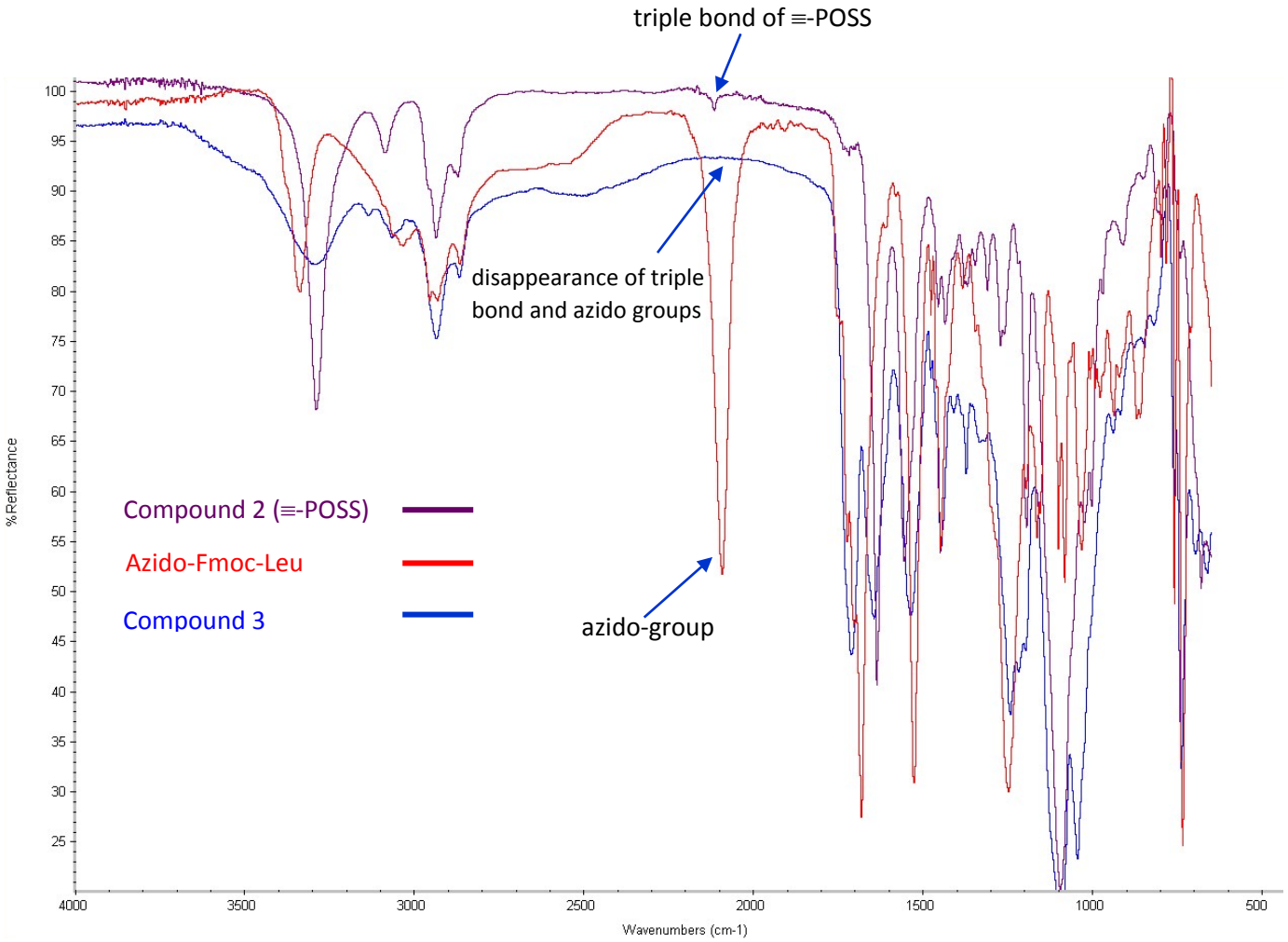

Figure S7. Infrared spectrum of **3**

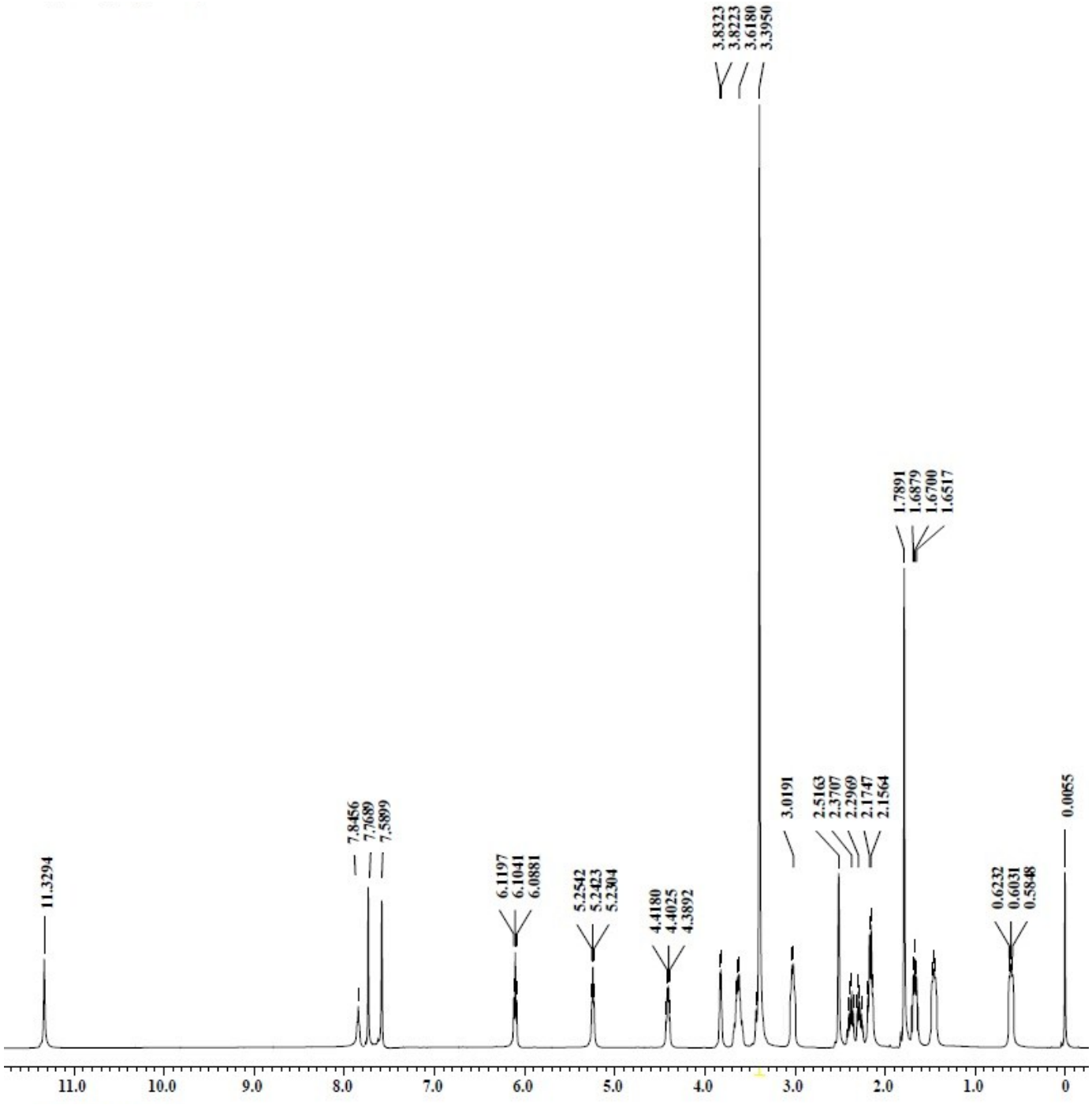

Figure S8. ^{1}H NMR spectrum of **4**

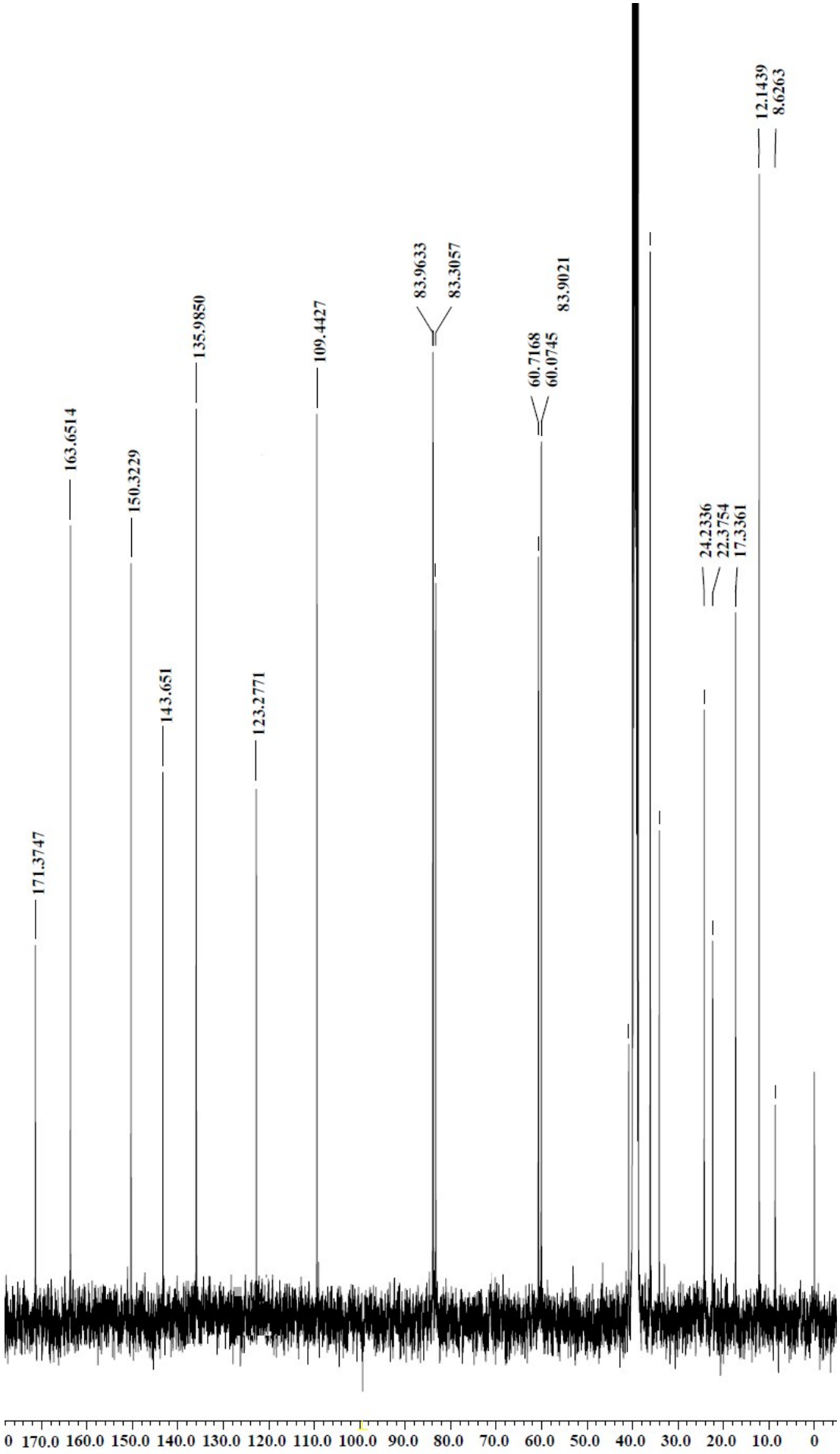

Figure S9. ^{13}C NMR spectrum of **4**

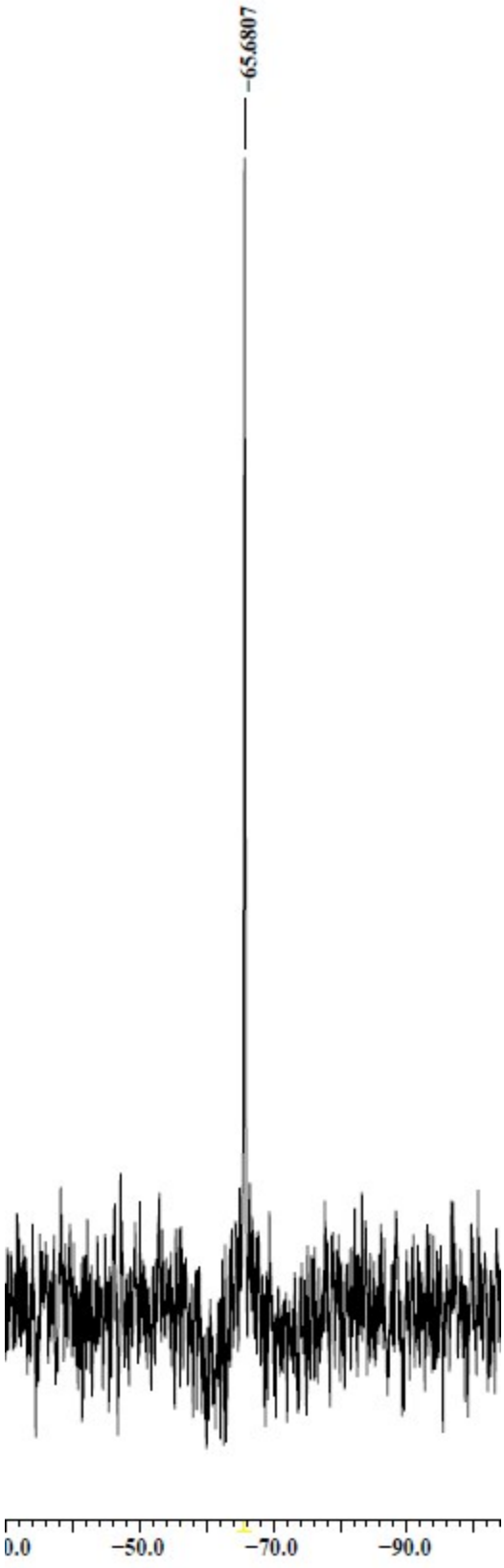

Figure S10. ^{29}Si-NMR spectrum **3**

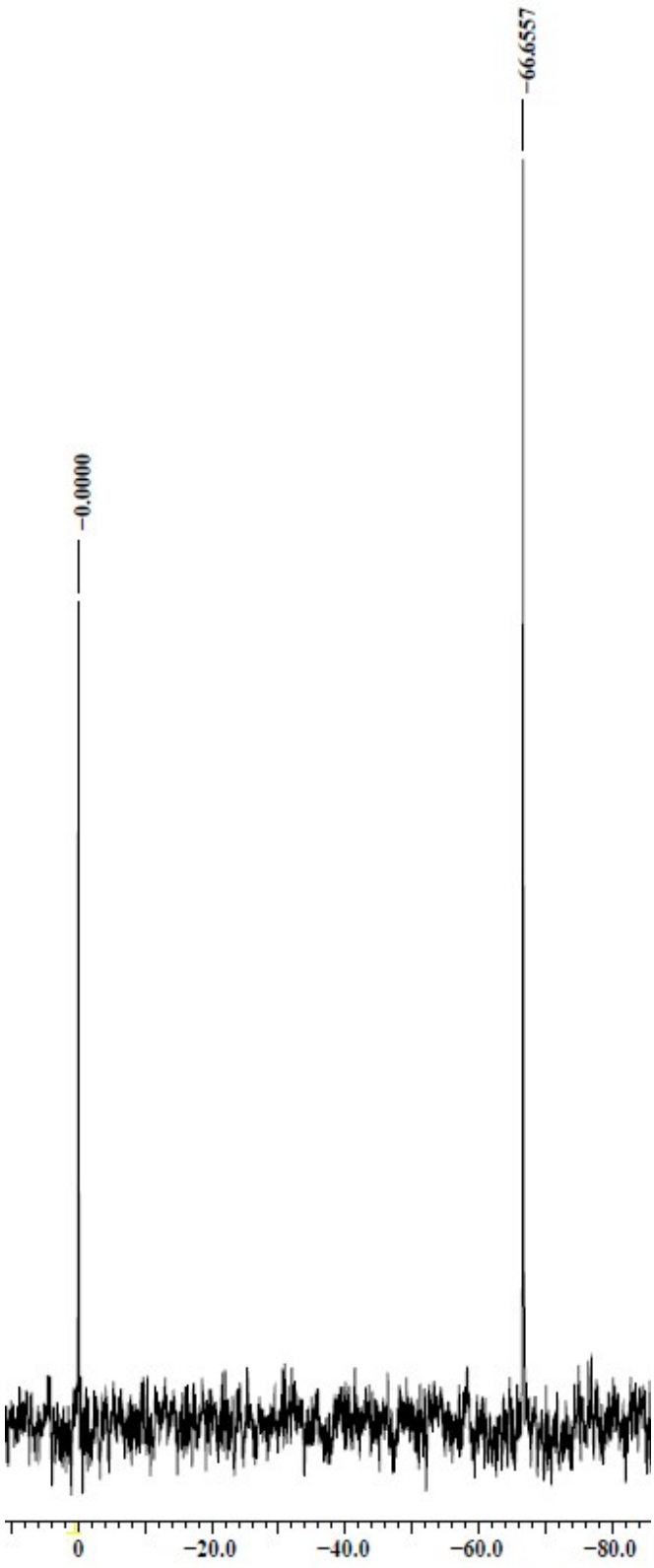

Figure S11. [29]-Si-NMR spectrum **4**

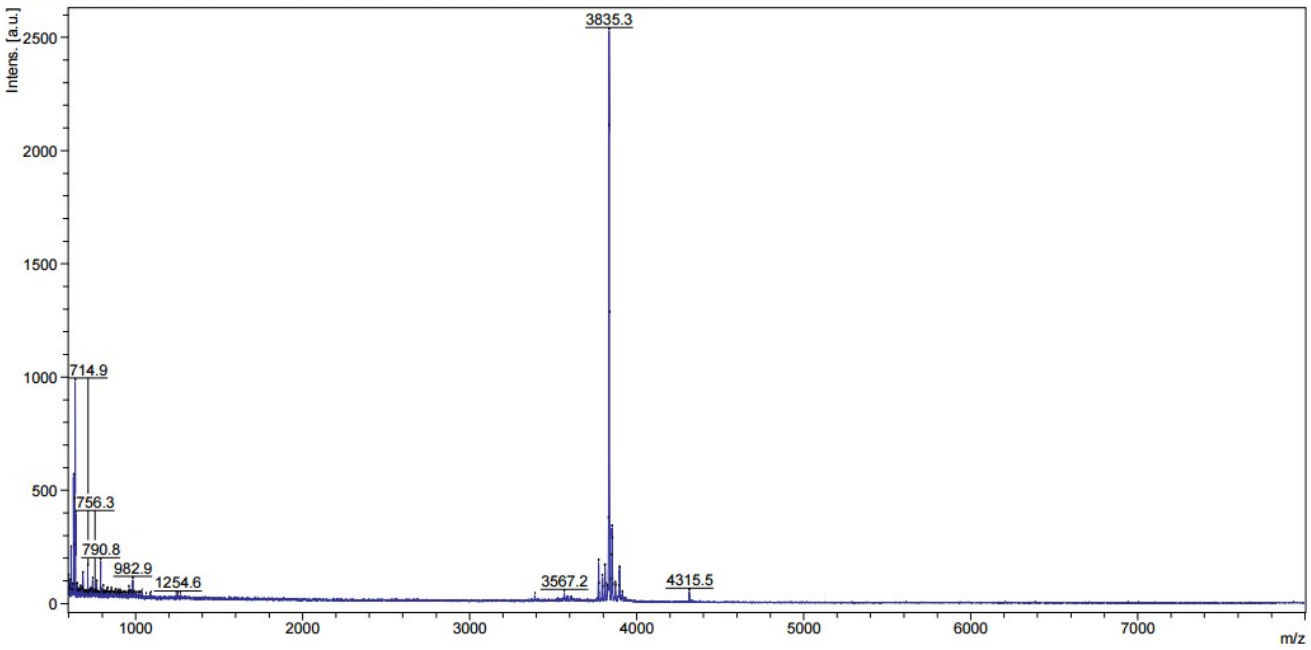

Figure S12. MALDI-TOF MS of **4**