Supporting Information

An acyl-SAM analog as an affinity ligand for identifying quorum sensing signal synthases

Kenji Kai,* Hiroki Fujii, Rui Ikenaka, Mitsugu Akagawa, and Hideo Hayashi

Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

E-mail: kai@biochem.osakafu-u.ac.jp

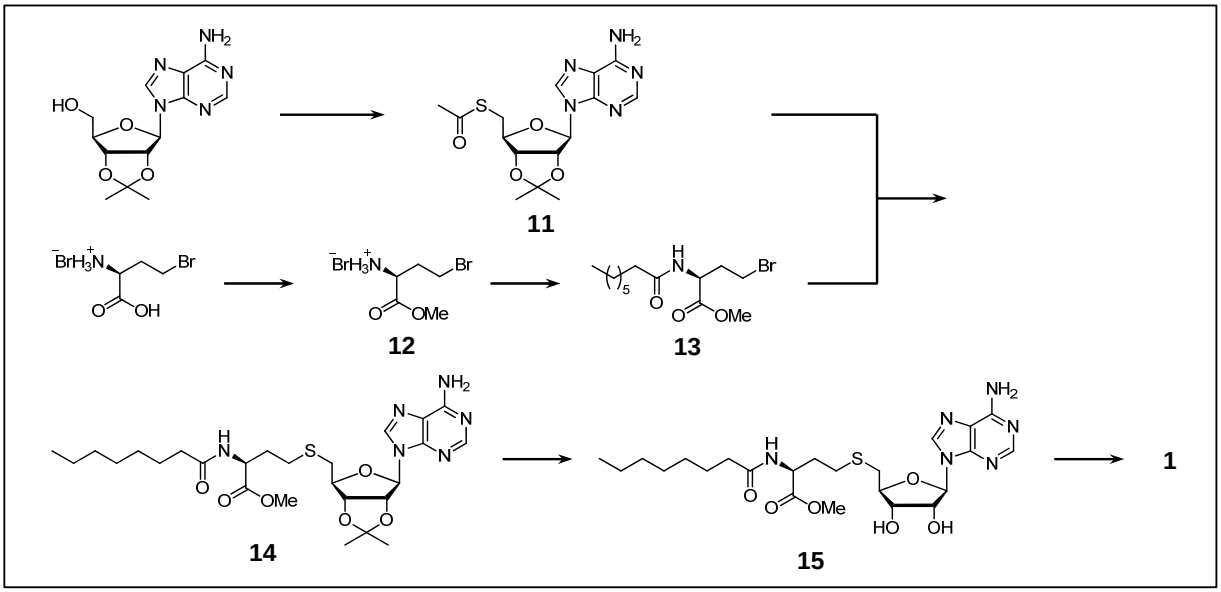
Contents

1. General information

Unless otherwise noted, all materials were purchased from suppliers (Aldrich, Wako Pure Chemical, Kanto Chemical, and TCI) and were used without further purification. All reactions were conducted under a nitrogen atmosphere unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-AL400 spectrometer (JEOL). Chemical shifts were reported in δ values (ppm) using tetramethylsilane (TMS) (δ_H 0, δ_C 0) or solvents (DMSO: δ_H 2.49, δ_C 39.5; D_2O: δ_H 4.65) as an internal reference. High-resolution mass spectra (HRMS) were obtained with a JMS-T100LP AccuTOF mass spectrometer (JEOL) and LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific). Silica gel column chromatography was performed on Wakogel C-200 (Wako Pure Chemical). HPLC experiments were performed with a LaChrom Elite HPLC system (Hitachi) and Prominence HPLC system (Shimadzu). Solvents for HPLC were purchased from Kanto Chemical. GC/MS data were obtained with a GCMS-QP2010 Plus (Shimadzu).

2. Synthesis of acyl-SAM analogs and preparation of ligand-fixed beads

2.1. Analog 1



2.1.1. 5′-Acetylthio-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (11).[S1] Diethyl azodicarboxylate (1.01 ml, 6.50 mmol) was added dropwise over 5 min to an ice-cold solution of triphenylphosphane (1.70 g, 6.50 mmol) in dry THF (10 ml). After stirring for 30 min, 2′,3′-*O*-isopropylideneadenosine (1.00 g, 3.25 mmol) was added, and stirring was continued for 10 min at 0°C. A solution of thioacetic acid (462 µl, 6.50 mmol) in dry THF (2 ml) was added dropwise to the resulting yellow suspension and stirring was continued for another 1 h at 0°C. The solvent was removed under reduced pressure, and the resulting yellowish residue was purified by chromatography on silica gel ($CHCl_3$−MeOH, 100:0, 95:5, 90:10) to give **11** (1.14 g, 96%) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, s), 1.60 (3H, s), 2.35 (3H, s), 3.19 (1H, dd, J = 7.1, 13.7 Hz), 3.29 (1H, dd, J = 7.1, 13.7 Hz), 4.35 (1H, dt, J = 3.1, 7.0 Hz), 4.98 (1H, dd, J = 3.1, 6.3 Hz), 5.51 (1H, dd, J = 2.2, 6.3 Hz), 5.69 (2H, br), 6.07 (1H, d, J = 2.2 Hz), 7.90 (1H, s), 8.37 (1H, s).

2.1.2. (*S*)-Methyl 2-amino-4-bromobutanoate hydrobromide (12). $SOCl_2$ (1.50 ml, 20.9 mmol) was added dropwise to ice-cold dry MeOH (10 ml). (*S*)-2-Amino-4-bromobutanoic acid hydrobromide (1.00 g, 3.80 mmol) was then added and refluxed for 3 h with stirring. After cooling to rt, the solution was evaporated to give **12** as a yellow amorphous solid, which contained some impurities. ^{1}H NMR (D_2O, 400 MHz) δ 2.23−2.55 (2H), 3.52−3.73 (2H), 3.78 (3H, s), 4.27 (1H, m).

2.1.3. (*S*)-Methyl 4-bromo-2-octanamidobutanoate (13). Compound **12** (3.80 mmol) and Na_2CO_3 (1.29 g, 12.1 mmol) were dissolved in CH_2Cl_2−H_2O (20 ml, 1:1) and the mixture was cooled to 0°C. After stirring for 5 min, octanoyl chloride (930 µl, 5.45 mmol) was added dropwise to the mixture at 0°C. The mixture was stirred for 4 h at rt and subsequently diluted with EtOAc (30 ml). The solution was then washed with brine, dried over Na_2SO_4, and evaporated to afford **13** (1.16 g, 95%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.22−1.35 (8H), 1.64 (2H, quint, J = 7.3 Hz), 2.24 (2H, t, J = 7.7 Hz), 2.15−2.53

(2H), 3.35−3.60 (2H), 6.09 (1H, br), 4.71−4.77 (2H, m).

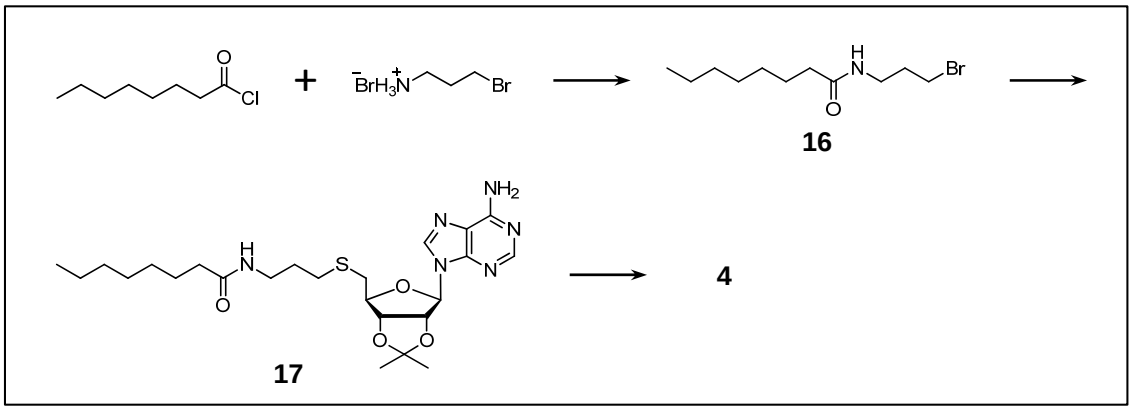
2.1.4. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(methoxycarbonyl)-3-(octanamido) propylthio]adenosine (14).[S1] Compounds **11** (748 mg, 1.55 mmol) and **13** (568 mg, 1.55 mmol) were dissolved in dry MeOH (20 ml). After cooling to −20°C, MeONa (186 mg, 3.41 mmol) was added to the solution. The solution was warmed slowly to rt and stirred for 10 h. The mixture was evaporated to remove the solvents. The residue was dissolved in $CHCl_3$−MeOH (100 ml, 95:5), washed with water, and dried over Na_2SO_4. The organic layer was evaporated and subsequently purified by silica gel column chromatography ($CHCl_3$−MeOH, 95:5) to give **14** (428 mg, 49%) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ0.87 (3H, t, J = 6.8 Hz), 1.23−1.32 (8H), 1.40 (3H, s), 1.62 (3H, s), 1.58−1.64 (2H), 1.85−2.13 (2H), 2.20 (2H, t, J = 7.3 Hz), 2.50−2.55 (2H), 2.77 (1H, dd, J = 6.3, 13.7 Hz), 2.86 (1H, dd, J = 7.3, 13.7 Hz), 3.73 (3H, s), 4.37 (1H, dt, J = 3.1, 6.8 Hz), 4.66−4.71 (1H), 5.06 (1H, dd, J = 3.1, 6.3 Hz), 5.50 (1H, dd, J = 2.2, 6.3 Hz), 5.63 (2H, br), 6.06 (1H, br), 6.08 (1H, d, J = 2.2 Hz), 7.93 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ14.1, 22.6, 25.3, 25.5, 27.1, 28.5, 29.0, 29.2, 31.7, 32.3, 34.3, 36.6, 51.3, 52.6, 83.7, 84.0, 86.7, 90.8, 114.6, 120.3, 140.1, 149.3, 153.2, 155.5, 172.5, 173.0.

2.1.5. 5′-Deoxy-5′-[3-(methoxycarbonyl)-3-(octanamido)propylthio]adenosine (15). Compound **14** (424 mg, 0.750 mmol) was dissolved in ice-cold HCOOH−H_2O (10 ml, 1:1) and warmed to rt with stirring. After stirring for 20 h at rt, the solvents were removed by evaporation and the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 97:3) to give **15** (282 mg, 72%) as a yellowish gum. ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.84 (3H, t, J = 6.9 Hz), 1.16−1.27 (8H), 1.42−1.49 (2H), 1.76−1.82 (2H), 2.07 (2H, t, J = 7.1 Hz), 2.44−2.58 (2H), 2.84 (1H, m), 2.90 (1H, m), 3.58 (3H, s), 3.98 (1H, dt, J = 3.7, 6.3 Hz), 4.12 (1H, m), 4.30 (1H, m), 4.73 (1H, m), 5.27 (1H, d, J = 5.1 Hz), 5.46 (1H, d, J = 6.1 Hz), 5.87 (1H, d, J = 5.6 Hz), 7.24 (2H, s), 8.11 (1H, s), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ14.0, 22.2, 25.3, 28.3, 28.5, 28.6, 31.3 (2), 34.0, 35.1, 50.9, 51.9, 72.7, 72.8, 84.1, 87.6, 119.3, 140.0, 149.6,

152.8, 156.2, 172.5, 172.7. HRMS (ESI, positive) *m/z* 547.2307 $[M+Na]^+$ (calcd for $C_{23}H_{36}O_6N_6NaS$, 547.2315).

2.1.6. 5′-Deoxy-5′-[3-(carboxy)-3-(octanamido)propylthio]adenosine (1). Compound **15** (267 mg, 0.509 mmol) was dissolved in MeOH (11 ml) and the mixture was cooled to 0°C. Ice-cold 25% NH_4OH (2 ml) was added to the solution and the mixture was stirred for 30 h at rt. The mixture was evaporated and white crystals then formed. These crystals were collected by filtration, washed with water, and dried to give **1** (127 mg, 49%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.83 (3H, t, J = 6.9 Hz), 1.16−1.27 (8H) 1.42−1.49 (2H), 1.75 (1H, m), 1.89 (1H, m), 2.09 (2H, t, J = 7.1 Hz), 2.41−2.55 (2H), 2.79 (1H, dd, J = 6.3, 13.7 Hz), 2.90 (1H, dd, J = 6.3, 13.7 Hz), 3.98 (1H, dt, J = 3.8, 6.2 Hz), 4.12 (1H, m), 4.23 (1H, m), 4.73 (1H, m), 5.28 (1H, d, J = 4.9 Hz), 5.46 (1H, d, J = 5.9 Hz), 5.87 (1H, d, J = 5.9 Hz), 6.96, (1H, s), 7.24 (2H, s), 7.81 (1H, d, J = 8.0 Hz), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.1, 22.2, 25.3, 28.5, 28.6, 28.7, 31.3, 32.5, 34.1, 35.3, 51.6, 72.8 (2), 83.8, 87.5, 119.3, 140.0, 149.6, 152.8, 156.2, 172.4, 173.5. HRMS (ESI, positive) *m/z* 549.1900 $[M+K]^+$ (calcd for $C_{22}H_{34}O_6N_6KS$, 549.1900).

2.2. Analog 4



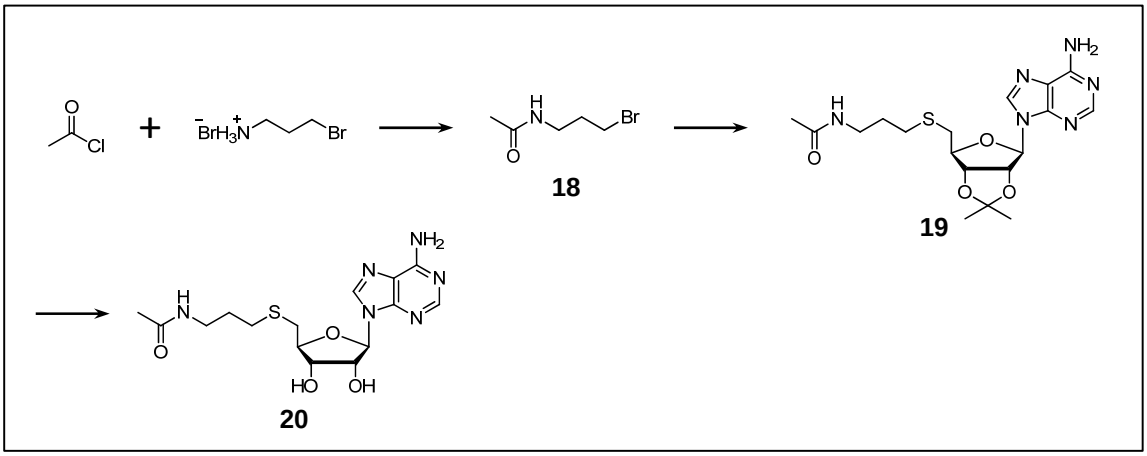
2.2.1. *N*-(3-Bromopropyl)octanamide (16). The reaction procedure described in Section 2.1.3. was followed. 3-Bromo-1-propylamine hydrobromide (300 mg, 1.37 mmol) and octanoyl

chloride (316 μl, 1.84 mmol) were used. **16** was obtained as a yellow oil (374 mg, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ0.90 (3H, t , J = 6.8 Hz), 1.20−1.40 (8H), 1.61−1.66 (2H), 2.09 (2H, quint, J = 6.5 Hz), 2.18 (2H, t, J = 7.1 Hz), 3.41 (2H, dt, J = 6.5, 13.2 Hz), 3.44 (2H, t, J = 6.5 Hz), 5.65 (1H, br).

2.2.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(octanamido)propylthio]adenosine (17). The reaction procedure described in Section 2.1.4. was followed. **11** (501 mg, 1.37 mmol) and **16** (374 mg, 1.37 mmol) were used. **17** was obtained as an orange oil (259 mg, 37%). Chromatography: ($CHCl_3$−MeOH, 95:5). ^{1}H NMR ($CDCl_3$, 400 MHz) δ0.87 (3H, t, J = 6.9 Hz), 1.23−1.32 (8H), 1.41 (3H, s), 1.57−1.64 (2H), 1.62 (3H, s), 1.72 (2H, quint, J = 7.1 Hz), 2.14 (2H, t, J = 7.6 Hz), 2.53 (2H, t, J = 7.1 Hz), 2.78 (1H, dd, J = 7.1, 13.7 Hz), 2.85 (1H, dd, J = 7.1, 13.7 Hz), 3.29 (2H, q, J = 6.5 Hz), 4.40 (1H, dt, J = 3.1, 6.7 Hz), 5.05 (1H, dd, J = 3.1, 6.3 Hz), 5.49 (1H, dd, J = 2.2, 6.3 Hz), 5.64 (1H, br), 6.09 (1H, d, J = 2.2 Hz), 6.13 (2H, br), 7.99 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 25.3, 25.7, 27.1, 29.0, 29.1, 29.2, 30.0, 31.7, 34.4, 36.8, 38.3, 83.7, 84.1, 86.7, 90.9, 114.6, 120.1, 140.4, 149.1, 151.9, 154.9, 173.2.

2.2.3. 5′-Deoxy-5′-[3-(octanamido)propylthio]adenosine (4). The reaction procedure described in Section 2.1.5. was followed. **17** (258 mg, 0.509 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5). **4** was obtained as a yellow oil (168 mg, 70%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.83 (3H, t, J = 6.8 Hz), 1.16−1.27 (8H), 1.41−1.48 (2H), 1.59 (2H, quint, J = 7.0 Hz), 2.00 (2H, t, J = 7.4 Hz), 2.49 (2H, m), 2.78 (1H, dd, J = 6.0, 13.8 Hz), 2.89 (1H, dd, J = 6.0, 13.8 Hz), 3.05 (2H, m), 3.99 (1H, dt, J = 3.7, 6.3 Hz), 4.13 (1H, m) , 4.74 (1H, m), 5.27 (1H, d, J = 4.9 Hz), 5.46 (1H, d, J = 6.1 Hz), 5.88 (1H, d, J = 5.8 Hz), 7.25 (2H, br), 7.71 (1H, t, J = 5.5 Hz), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.1, 22.2, 25.4, 28.5, 28.7, 29.4, 29.5, 31.3, 34.1, 35.5, 37.6, 72.7 (2), 84.0, 87.5, 119.3, 140.0, 149.6, 152.8, 156.2, 172.2. HRMS (ESI, positive) *m/z* 489.2257 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_4N_6NaS$, 489.2256).

2.3. Analog 20



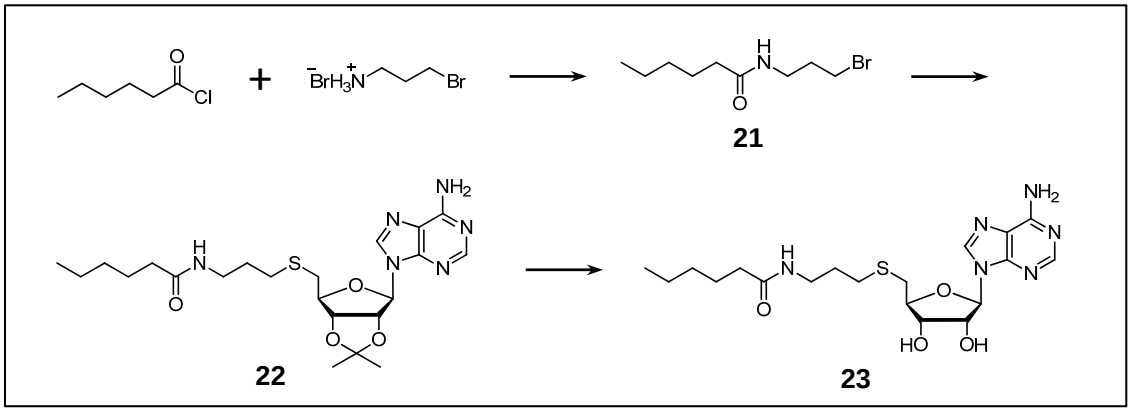
2.3.1. *N*-(3-Bromopropyl)acetamide (18). The reaction procedure described in Section 2.1.3. was followed. 3-Bromo-1-propylamine hydrobromide (300 mg, 1.37 mmol) and acetyl chloride (131 µl, 1.84 mmol) were used. **18** was obtained as a colorless oil (88 mg, 36%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (3H, s), 2.09 (2H, quint, J = 6.5 Hz), 3.38−3.49 (4H, m), 5.83 (1H, br).

2.3.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(acetamido)propylthio]adenosine (19). The reaction procedure described in Section 2.1.4. was followed. **11** (388 mg, 1.06 mmol) and **18** (287 mg, 1.59 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 97.5:2.5, 95:5). **19** was obtained as a yellowish oil (207 mg, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (3H, s), 1.62 (3H, s), 1.71 (2H, m), 1.96 (3H, s), 2.53 (2H, t, J = 7.4 Hz), 2.78 (1H, dd, J = 6.6, 13.3 Hz), 2.85 (1H, dd, J = 7.3, 13.3 Hz), 3.27 (2H, m), 4.39 (1H, m), 5.07 (1H, dd, J = 2.0, 6.4 Hz), 5.52 (1H, dd, J = 2.1, 6.4 Hz), 5.85 (2H, br), 6.06 (1H, br), 6.09 (1H, d, J = 2.1 Hz), 7.95 (1H, s), 8.34 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2, 25.3, 27.0, 29.0, 29.9, 34.3, 38.4, 83.8, 84.0, 86.8, 90.8, 114.5, 120.0, 140.0, 149.1, 153.2, 155.6, 170.8.

2.3.3. 5′-Deoxy-5′-[3-(acetamido)propylthio]adenosine (20). The reaction procedure described in Section 2.1.5. was followed. **19** (196 mg, 0.463 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **20** was obtained as a yellowish solid (147 mg, 83%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.59 (2H, quint, J = 6.8 Hz), 1.76 (3H, s), 2.79 (1H, dd, J = 7.1, 13.9

Hz), 2.89 (1H, dd, J = 5.9, 13.9 Hz), 3.04 (2H, m), 3.79 (1H, m), 4.13 (1H, br), 4.74 (1H, m), 5.25 (1H, br), 5.46 (1H, br), 5.88 (1H, d, J = 5.6 Hz), 7.25 (2H, s), 7.77 (1H, br), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.5, 29.2, 29.3, 33.9, 37.5, 72.5 (2), 83.8, 87.3, 119.1, 139.8, 149.4, 152.6, 156.0, 169.0. HRMS (ESI, positive) m/z 405.1313 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_4N_6NaS$, 405.1321).

2.4. Analog 23



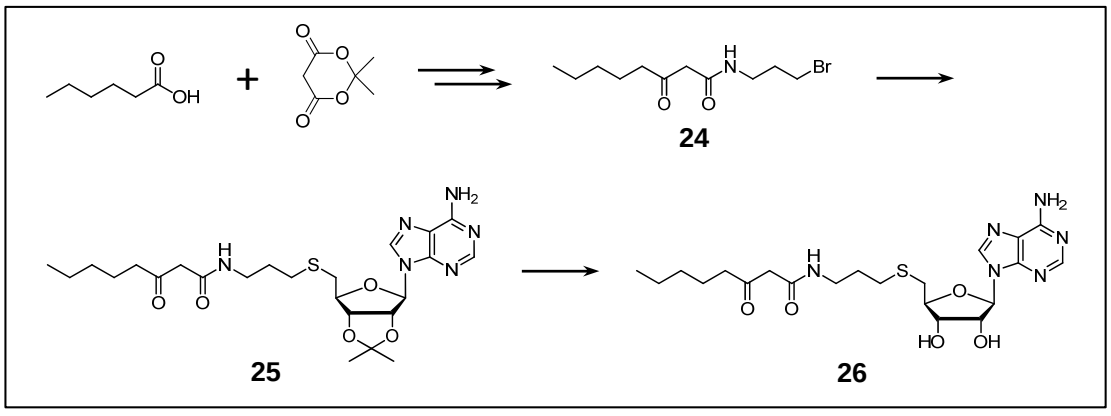
2.4.1. *N*-(3-Bromopropyl)hexanamide (21). The reaction procedure described in Section 2.1.3. was followed. 3-Bromo-1-propylamine hydrobromide (600 mg, 2.74 mmol) and hexanoyl chloride (517 µl, 3.70 mmol) were used. **21** was obtained as a colorless oil (686 mg, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (3H, t, J = 7.0 Hz), 1.24−1.35 (4H), 1.62 (2H, m), 2.09 (2H, quint, J = 6.5 Hz), 2.17 (2H, t, J = 7.7 Hz), 3.41 (2H, m), 3.44 (2H, t, J = 6.4 Hz), 5.69 (1H, br).

2.4.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(hexanamido)propylthio]adenosine (22). The reaction procedure described in Section 2.1.4. was followed. **11** (590 mg, 1.62 mmol) and **21** (644 mg, 2.74 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 95:5). **22** was obtained as a yellowish oil (764 mg, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, J = 6.9 Hz), 1.41 (3H, s), 1.62 (3H, s), 1.24−1.35 (4H), 1.57−1.65 (2H), 1.72 (2H, quint, J = 6.9 Hz), 2.14 (2H, t, J = 7.6 Hz), 2.53 (2H, t, J = 7.1 Hz), 2.85 (1H, dd, J = 7.3, 13.7 Hz), 2.77 (1H, dd, J = 7.3, 13.7 Hz),

3.28 (2H, q, J = 6.5 Hz), 4.39 (1H, dt, J = 3.1, 6.9 Hz), 5.07 (1H, dd, J = 3.1, 6.3 Hz), 5.53 (1H, dd, J = 2.2, 6.3 Hz), 5.61 (1H, br), 5.71 (2H, br), 6.08 (1H, d, J = 2.2 Hz), 7.94 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 22.4, 25.3, 25.4, 27.1, 29.1, 30.0, 31.5, 34.3, 36.8, 38.3, 83.8, 84.0, 86.8, 90.9, 114.5, 120.3, 140.1, 144.7, 153.2, 155.5, 173.2.

2.4.3. 5′-Deoxy-5′-[3-(hexanamido)propylthio]adenosine (23). The reaction procedure described in Section 2.1.5. was followed. **22** (723 mg, 1.51 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 9:1). **23** was obtained as a white solid (537 mg, 81%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 7.0 Hz), 1.13−1.29 (4H), 1.45 (2H, quint, J = 7.4 Hz), 1.59 (2H, quint, J = 7.1 Hz), 2.00 (2H, t, J = 7.4 Hz), 2.79 (1H, dd, J = 6.6, 13.5 Hz), 2.89 (1H, dd, J = 5.9, 13.5 Hz), 3.04 (2H, m), 3.99 (1H, m), 4.12 (1H, m), 4.74 (1H, m), 5.28 (1H, d, J = 4.9 Hz), 5.46 (1H, d, J = 5.9 Hz), 5.87 (1H, d, J = 5.9 Hz), 7.24 (2H, s), 7.71 (1H, br), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.8, 21.8, 24.9, 29.2, 30.8 (2), 33.9, 35.3, 37.4, 72.5 (2), 83.8, 87.3, 119.1, 139.8, 149.4, 152.6, 156.0, 172.0. HRMS (ESI, positive) m/z 461.1950 $[M+Na]^+$ (calcd for $C_{19}H_{30}O_4N_6NaS$, 461.1947).

2.5. Analog 26



2.5.1. *N*-(3-Bromopropyl)-3-oxooctanamide (24). Hexanoic acid (251 μl, 2.00 mmol) was added to a solution of 4-dimethylaminopyridine (268 mg, 2.20 mmol),

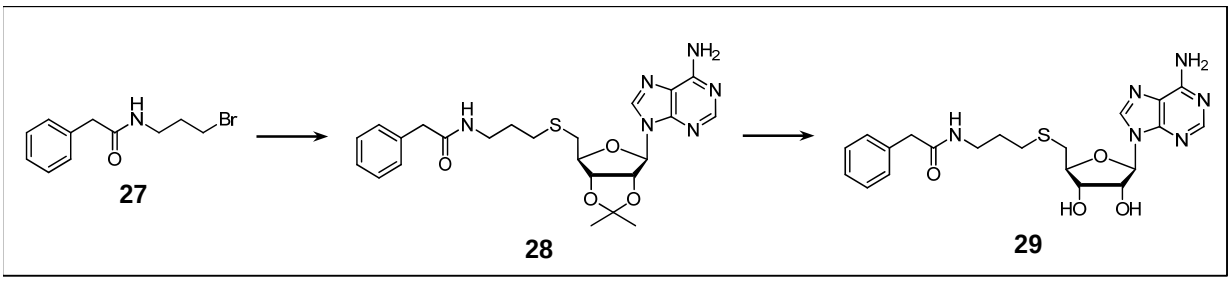
N,*N*′-dicyclohexylcarbodiimide (453 mg, 2.20 mmol), and Meldrum's acid (288 mg, 2.00 mmol) in dry CH_2Cl_2 (15 ml). After stirring at rt overnight, the urea that formed was removed by filtration. The filtrate was evaporated, dissolved in EtOAc (20 ml), and washed with 2 M aq HCl and brine. The organic layer was dried over Na_2SO_4 and evaporated dry to quantitatively yield hexanoyl Meldrum's acid. Hexanoyl Meldrum's acid, 3-bromo-1-propylamine hydrobromide (437 mg, 2.00 mmol), and Et_3N (361 μl, 2.60 mmol) were dissolved in dry MeCN (15 ml) and stirred for 2 h at rt and for another 3 h under reflux. After cooling to rt, the reaction mixture was evaporated, dissolved in EtOAc (40 ml), and washed with sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and purified by silica gel column chromatography (hexane−EtOAc, 80:20) to afford **24** (195 mg, 35%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, J = 6.9 Hz), 1.24−1.33 (4H), 2.09 (2H, quint, J = 6.6 Hz), 2.52 (2H, t, J = 7.4 Hz), 3.41 (2H, s), 3.42−3.46 (4H), 7.21 (1H, br).

2.5.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(3-oxooctanamido)propylthio] adenosine (25). The reaction procedure described in Section 2.1.4. was followed. **11** (326 mg, 0.892 mmol) and **24** (372 mg, 1.33 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 97.5:2.5). **25** was obtained as a colorless paste (231 mg, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, J = 7.1 Hz), 1.22−1.35 (4H), 1.41 (3H, s), 1.58 (2H, m), 1.62 (3H, s), 1.71 (2H, m), 2.49−2.55 (4H), 2.76 (1H, dd, J = 7.0, 13.5 Hz), 2.84 (1H, dd, J = 7.0, 13.5 Hz), 3.29 (2H, s), 3.29 (2H, m), 4.40 (1H, m), 5.06 (1H, dd, J = 3.0, 6.5 Hz), 5.54 (1H, dd, J = 2.2, 6.5 Hz), 5.77 (2H, br), 6.09 (1H, d, J = 2.2 Hz), 7.14 (1H, br), 7.97 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9, 22.4, 23.0, 25.3, 27.1, 29.0, 29.9, 31.1, 34.4, 38.3, 44.0, 48.8, 83.8, 84.3, 86.9, 91.0, 114.4, 120.3, 140.1, 149.3, 153.2, 155.5, 165.6, 207.1.

2.5.3. 5′-Deoxy-5′-[3-(3-oxooctanamido)propylthio]adenosine (26). The reaction procedure described in Section 2.1.5. was followed. **25** (231 mg, 0.443 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **26** was obtained as a yellowish solid (143 mg, 67%). ^{1}H NMR

(DMSO-d_6, 400 MHz) δ0.83 (3H, t, J = 7.1 Hz), 1.13−1.28 (4H), 1.43 (2H, m), 1.52 (2H, m), 2.45 (2H, t, J = 7.3 Hz), 2.51 (2H, m), 2.79 (1H, dd, J = 7.0, 13.8 Hz), 2.90 (1H, dd, J = 6.0, 13.8 Hz), 3.07 (2H, m), 3.24 (2H, s), 3.99 (1H, m), 4.13 (1H, m), 4.74 (1H, m), 5.27 (1H, d, J = 4.8 Hz), 5.46 (1H, d, J = 6.3 Hz), 5.88 (1H, d, J = 5.9 Hz), 7.24 (2H, s), 7.98 (1H, t, J = 5.6 Hz), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.7, 21.8, 22.5, 29.0, 29.2, 30.6, 30.6, 33.9, 37.6, 41.9, 72.5 (2), 83.8, 87.3, 119.1, 139.8, 149.4, 152.6, 156.0, 165.9, 205.0. HRMS (ESI, positive) *m/z* 503.2050 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_5N_6NaS$, 503.2053).

2.6. Analog 29



27 28 29

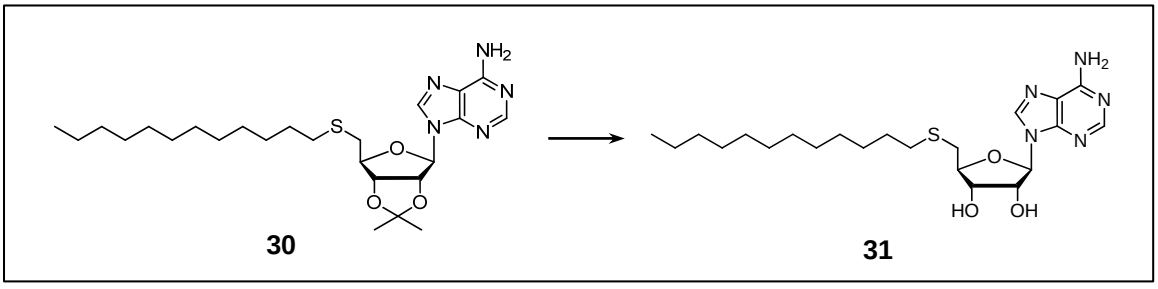
2.6.1. *N*-(3-Bromopropyl)-2-phenylacetamide (27). The reaction procedure described in Section 2.1.3. was followed. 3-Bromo-1-propylamine hydrobromide (300 mg, 1.37 mmol) and phenylacetyl chloride (316 µl, 1.84 mmol) were used. **27** was obtained as a colorless oil (383 mg, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ2.02 (2H, quint, J = 6.5 Hz), 3.34 (2H, t, J = 6.5 Hz), 3.36 (2H, dt, J = 6.5, 12.8 Hz), 3.58 (2H, s), 5.56 (1H, br), 7.26−7.39 (5H).

2.6.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(2-phenylacetamido)propylthio] adenosine (28). The reaction procedure described in Section 2.1.4. was followed. **11** (453 mg, 1.24 mmol) and **27** (383 mg, 1.37 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 95:5). **28** was obtained as a yellowish oil (520 mg, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ1.40 (3H, s), 1.61 (3H, s), 1.64 (2H, quint, J = 7.1 Hz), 2.43 (2H, t, J = 7.2 Hz), 2.70 (1H, dd, J = 7.3, 13.7 Hz), 2.78 (1H, dd, J = 7.3, 13.7 Hz), 3.23 (2H, dt, J = 6.4, 6.8 Hz), 3.56 (2H, s), 4.34 (1H, dt, J = 3.1,

6.9 Hz), 5.04 (1H, dd, J = 3.1, 6.3 Hz), 5.51 (1H, dd, J = 2.2, 6.3 Hz), 5.56 (1H, br), 5.72 (2H, br), 6.07 (1H, d, J = 2.2 Hz), 7.23−7.69 (5H), 7.91 (1H, s), 8.34 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.3, 27.1, 28.9, 29.9, 34.3, 38.5, 43.8, 83.8, 84.0, 86.8, 90.9, 114.5, 127.4, 120.3, 128.4, 129.4, 132.0, 132.1, 134.9, 140.1, 149.2, 153.2, 155.5, 166.2, 171.1.

2.6.3. 5′-Deoxy-5′-[3-(2-phenylacetamido)propylthio]adenosine (29). The reaction procedure described in Section 2.1.5. was followed. **28** (516 mg, 1.03 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **29** was obtained as a yellowish solid (279 mg, 59%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.61 (2H, quint, J = 7.3 Hz), 2.46−2.50 (2H, m), 2.78 (1H, dd, J = 5.8, 13.9 Hz), 2.88 (1H, dd, J = 5.8, 13.9 Hz), 3.04−3.09 (2H), 3.16 (2H, s), 4.00 (1H, dt, J = 3.7, 6.5 Hz), 4.13 (1H, m), 4.74 (1H, m), 5.28 (1H, d, J = 4.8 Hz), 5.46 (1H, d, J = 6.0 Hz), 5.89 (1H, d, J = 5.9 Hz), 7.16−7.29 (5H, m), 8.00 (1H, t, J = 5.4 Hz), 8.15 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 29.1, 29.2, 34.0, 37.6, 42.4, 72.6 (2), 83.8, 87.4, 119.1, 126.2, 128.1 (2), 128.9 (2), 136.5, 139.8, 149.4, 152.6, 156.0, 170.0. HRMS (ESI, positive) m/z 481.1625 $[M+Na]^+$ (calcd for $C_{21}H_{26}O_4N_6NaS$, 481.1634).

2.7. Analog 31



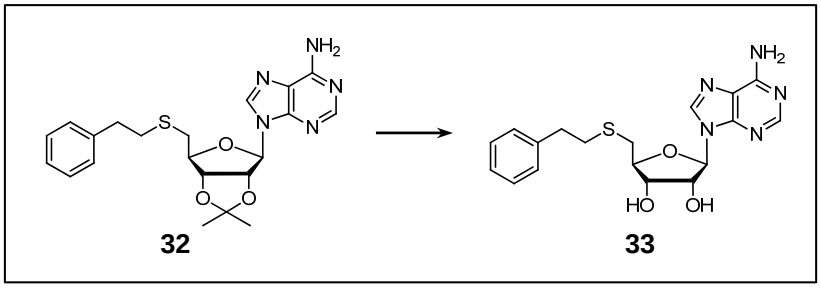
30 **31**

2.7.1. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-(dodecanylthio)adenosine (30). The reaction procedure described in Section 2.1.4. was followed. **11** (260 mg, 0.711 mmol) and 1-bromododecane (256 µl, 1.06 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 95:5). **30** was obtained as a colorless paste (162 mg, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J =

6.9 Hz), 1.18−1.35 (18H), 1.41 (3H, s), 1.52 (2H, quint, J = 7.5 Hz), 1.62 (3H, s), 2.50 (2H, t, J = 7.3 Hz), 2.77 (1H, dd, J = 7.8, 13.7 Hz), 2.84 (1H, dd, J = 7.8, 13.7 Hz), 4.39 (1H, m), 5.06 (1H, dd, J = 3.1, 6.3 Hz), 5.51 (1H, dd, J = 2.2, 6.3 Hz), 5.72 (2H, br), 6.08 (1H, d, J = 2.2 Hz), 7.94 (1H, s), 8.34 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 25.3, 27.1, 28.8, 29.2, 29.4, 29.5 (3), 29.6, 29.7, 31.9, 32.7, 34.3, 83.8, 84.1, 86.8, 90.9, 114.4, 120.3, 140.0, 149.3, 153.2, 155.5.

2.7.2. 5′-Deoxy-5′-(dodecanylthio)adenosine (31). The reaction procedure described in Section 2.1.5. was followed. **30** (152 mg, 0.309 mmol) was used. Crystallization from EtOH. **31** was obtained as a white crystal (93 mg, 66%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.84 (3H, t, J = 6.8 Hz), 1.13−1.30 (8H), 1.45 (2H, m), 2.46 (2H, m), 2.79 (1H, dd, J = 6.7, 13.8 Hz), 2.88 (1H, dd, J = 5.7, 13.8 Hz), 3.99 (1H, m), 4.13 (1H, m), 4.74 (1H, m), 5.25 (1H, br), 5.44 (1H, br), 5.87 (1H, d, J = 5.6 Hz), 7.23 (2H, s), 8.13 (1H, s), 8.32 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 28.1, 28.5, 28.6, 28.9 (3), 29.1, 31.2, 31.8, 33.9, 72.6 (2), 84.1, 87.4, 119.1, 139.8, 149.4, 152.5, 156.0, 163.0. HRMS (ESI, positive) *m/z* 474.2506 $[M+Na]^+$ (calcd for $C_{22}H_{37}O_3N_5NaS$, 474.2515).

2.8. Analog 33

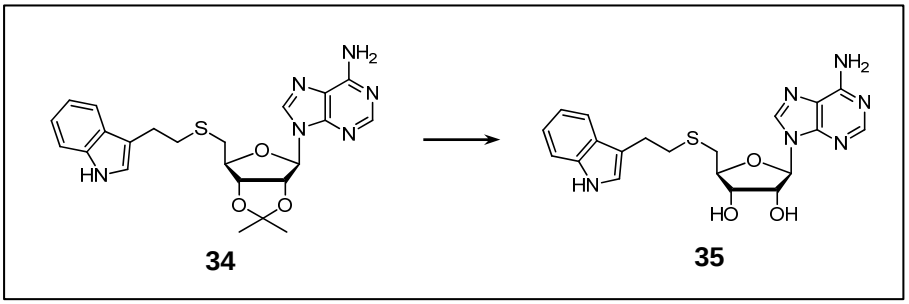


2.8.1. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-(2-phenylethylthio)adenosine (32). The reaction procedure described in Section 2.1.4. was followed. **11** (400 mg, 1.09 mmol) and (2-bromoethyl)benzene (222 µl, 1.64 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 95:5). **32** was obtained as a colorless solid (314 mg, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (3H, s),

1.62 (3H, s), 2.74−2.90 (6H), 4.37 (1H, dt, J = 3.2, 6.4 Hz), 5.06 (1H, dd, J = 3.2, 6.3 Hz), 5.50 (1H, dd, J = 2.1, 6.3 Hz), 5.76 (2H, br), 6.08 (1H, d, J = 2.1 Hz), 7.11 (2H, d, J = 7.4 Hz), 7.13 (2H, t, J = 7.0 Hz), 7.26 (2H, t, J = 8.0 Hz), 7.91 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.3, 27.1, 34.1, 34.5, 36.2, 83.8, 84.0, 86.7, 90.9, 114.5, 120.3, 126.4, 128.4 (2), 128.5 (2), 140.0, 140.1, 149.3, 153.2, 155.5.

2.8.2. 5′-Deoxy-5′-(2-phenylethylthio)adenosine (33). The reaction procedure described in Section 2.1.5. was followed. **32** (304 mg, 0.711 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5). **33** was obtained as a white powder (196 mg, 71%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.69−2.78 (4H), 2.90 (2H, m), 4.08 (1H, m), 4.17 (1H, m), 4.75 (1H, m), 5.28 (1H, d, J = 5.1 Hz), 5.47 (1H, d, J = 5.9 Hz), 5.89 (1H, d, J = 5.6 Hz), 7.06−7.23 (5H), 7.25 (2H, br), 8.14 (1H, s), 8.34 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 33.3, 33.8, 35.5, 72.5, 72.6, 84.4, 87.5, 119.2, 126.0, 128.1 (2), 128.3 (2), 139.8, 140.4, 149.4, 152.6, 156.1. HRMS (ESI, positive) m/z 388.1437 $[M+H]^+$ (calcd for $C_{18}H_{22}O_3N_5S$, 388.1443).

2.9. Analog 35

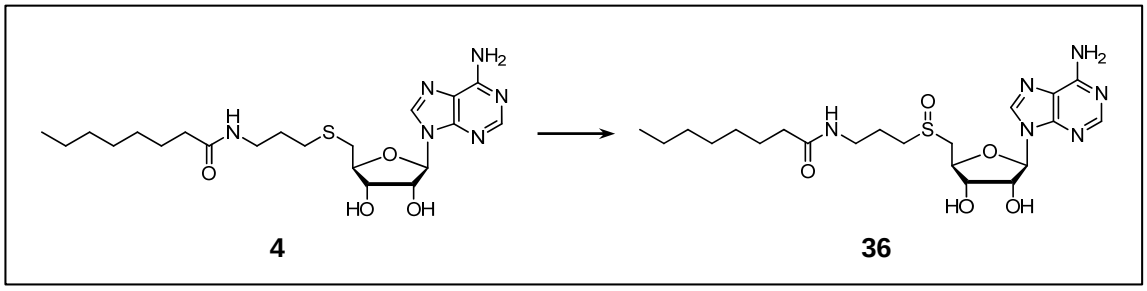


2.9.1. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-(2-indolylethylthio)adenosine (34). The reaction procedure described in Section 2.1.4. was followed. **11** (329 mg, 0.900 mmol) and 3-(2-bromoethyl)indole (302 mg, 1.35 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 100:0, 95:5). **34** was obtained as a yellowish solid (276 mg, 66%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, s), 1.61 (3H, s), 2.79−3.01 (6H), 4.41 (1H, dt, J = 3.2, 6.6 Hz), 5.04 (1H, dd, J = 3.2,

6.5 Hz), 5.45 (1H, dd, J = 2.3, 6.5 Hz), 5.72 (2H, s), 6.07 (1H, d, J = 2.3 Hz), 6.96−7.88 (5H), 7.90 (1H, s), 8.14 (1H, br), 8.34 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4, 25.3, 25.9, 27.1, 33.3, 34.6, 62.3, 83.8, 86.9, 90.9, 111.2, 114.5, 114.6, 118.6, 119.3, 120.2, 121.7, 122.1, 127.1, 128.5, 128.6, 132.1, 132.2, 136.2, 140.0, 149.3, 153.2, 155.5.

2.9.2. 5′-Deoxy-5′-(2-indolylethylthio)adenosine (35). The reaction procedure described in Section 2.1.5. was followed. **34** (270 mg, 0.578 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5). **35** was obtained as a brown paste (139 mg, 56%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.90 (1H, dd, J = 7.3, 13.8 Hz), 2.98 (1H, dd, J = 5.7, 13.8 Hz), 4.00−4.11 (4H), 4.18 (1H, m), 4.74 (1H, m), 5.29 (1H, d, J = 4.9 Hz), 5.47 (1H, d, J = 6.1 Hz), 5.90 (1H, d, J = 5.6 Hz), 6.89 (1H, m), 7.02 (1H, m), 7.09 (1H, d, J = 2.4 Hz), 7.24 (2H, s), 7.30 (1H, d, J = 8.3 Hz), 7.40 (1H, d, J = 7.8 Hz), 8.15 (1H, s), 8.35 (1H, s), 10.8 (1H, br). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.6, 32.8, 34.0, 72.5, 72.6, 84.1, 87.4, 111.3, 113.0, 118.1, 118.2, 119.1, 120.8, 122.6, 126.8, 136.1, 139.8, 149.4, 152.6, 156.0. HRMS (ESI, positive) *m/z* 427.1550 $[M+H]^+$ (calcd for $C_{20}H_{23}O_3N_6S$, 427.1552).

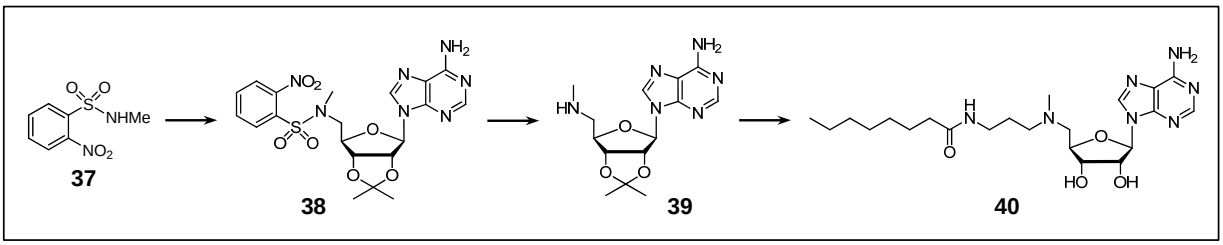
2.10. Analog 36



5′-Deoxy-5′-[3-(octanamido)propylsulfinyl]adenosine (36).[S2] Thirty percent aq H_2O_2 (232 µl, 2.07 mmol) was added to a solution of **4** (100 mg, 0.228 mmol) in MeOH−H_2O (1.2 ml, 2:1), and the mixture was stirred for 1 h at rt. After quenching the reaction with sodium thiosulfate, the solvents were removed by evaporation. The residue was purified by silica gel column

chromatography to afford **36** (102 mg, 99%, diastereomixture of sulfoxide) as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.83 (3H, m), 1.12−1.30 (8H), 1.39−1.50 (2H), 1.66−1.77 (2H), 2.01 (2H), 2.62−2.79 (2H), 3.01−3.18 (2H), 4.22−4.32 (2H), 4.75 (1H, m), 5.43 (1H, m), 5.53 (1H, m), 5.91 (1H, m), 7.26 (2H, s), 7.80 (1H, m), 8.13 (1H, m), 8.33 (1H, m). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.35, 21.48, 21.87, 22.07, 24.67, 27.84, 28.06, 30.59, 34.87, 36.83, 36.93, 48.09, 48.96, 53.00, 54.81, 72.03, 72.10, 72.56, 72.69, 77.43, 77.61, 87.32, 87.69, 118.83, 139.36, 139.74, 148.65, 148.79, 152.04, 152.09, 155.57, 171.59, 171.61. HRMS (ESI, positive) *m/z* 505.2210 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5N_6NaS$, 505.2209).

2.11. Analog 40



2.11.1. *o*-Nitro-*N*-methylbenzenesulfonamide (37).[S3] Et_3N (691 µl, 4.96 mmol) and 40% aq $MeNH_2$ (587 µl, 6.77 mmol) were added with stirring to a solution of *o*-nitrobenzenesulfonyl chloride (1.00 g, 4.51 mmol) in dry CH_2Cl_2 (7 ml). After stirring at rt for 20 h, the resulting mixture was diluted with EtOAc (50 ml) and washed with 2 M aq HCl, sat. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4. The solvents were removed by evaporation and purified by crystallization from $CHCl_3$−hexane to give **37** (803 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ2.80 (3H, d, J = 5.4 Hz), 5.23 (1H, br), 7.76 (2H, m), 7.88 (1H, m), 8.15 (1H, m).

2.11.2. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-(*N*-methyl-*o*-nitrobenzenesulfonylamino) adenosine (38).[S3] The reaction procedure described in Section 2.1.1. was followed. 2′,3′-*O*-Isopropylideneadenosine (885 mg, 2.88 mmol) and **37** (1.25 g, 5.76 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 97:3). **38** was obtained as a light brown solid (812 mg, 61%).

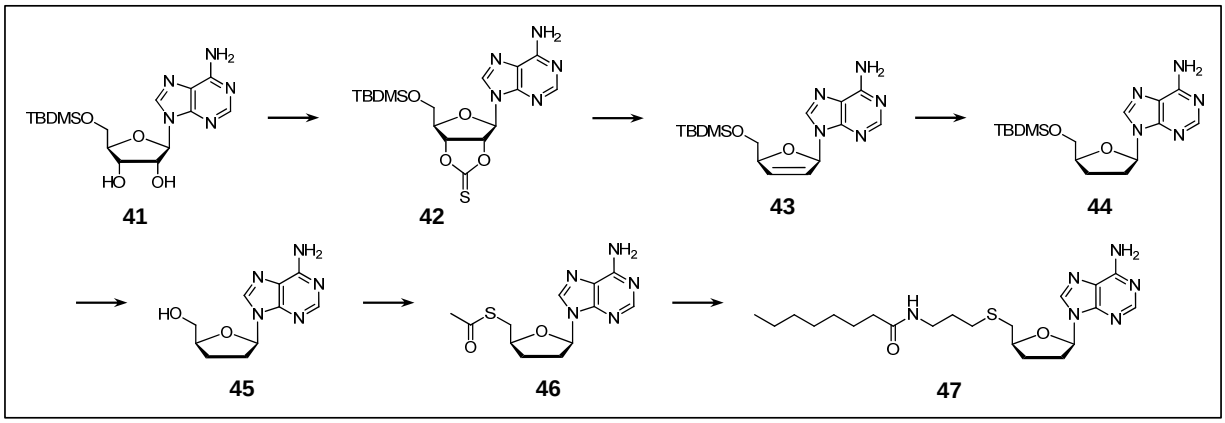
^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.32 (3H, s), 1.53 (3H, s), 2.76 (3H, s), 3.43 (1H, dd, J = 7.7, 14.4 Hz), 3.62 (1H, dd, J = 5.1, 14.4 Hz), 4.31 (1H, m), 5.06 (1H, dd, J = 3.4, 6.3 Hz), 5.44 (1H, dd, J = 2.3, 6.3 Hz), 6.19 (1H, d, J = 2.3 Hz), 7.29 (2H, br), 7.72 (1H, m), 7.82 (2H, m), 7.92 (1H, m), 8.15 (1H, s), 8.29 (1H, s).

2.11.3. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-(*N*-methylamino)adenosine (39).[S3] Thiophenol (354 μl, 3.46 mmol) and Cs_2CO_3 (1.12 g, 3.46 mmol) were added to a solution of **38** (805 mg, 1.73 mmol) in dry MeCN (30 ml). After stirring at rt for 20 h, the reaction was quenched by 1 M aq KOH (50 ml). The resulting mixture was extracted by $CHCl_3$ (50 ml ×3) and dried over Na_2SO_4. The solvents were removed by evaporation and purified by silica gel column chromatography ($CHCl_3$−MeOH, 9:1) to give **39** (383 mg, 69%) as a light brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.31 (3H, s), 1.53 (3H, s), 2.24 (3H, s), 2.62 (1H, dd, J = 6.3, 12.3 Hz), 2.68 (1H, dd, J = 5.9, 12.3 Hz), 4.20 (1H, dt, J = 2.9, 6.1 Hz), 4.94 (1H, dd, J = 2.9, 6.3 Hz), 5.44 (1H, dd, J = 3.2, 6.3 Hz), 6.08 (1H, d, J = 3.2 Hz), 7.27 (2H, br), 8.15 (1H, s), 8.33 (1H, s).

2.11.4. 5′-Deoxy-5′-[*N*-methyl-3-(octanamido)propylamino]adenosine (40). *N,N*-Diisopropylethylamine (236 μl, 1.37 mmol) was added to a solution of **16** (385 mg, 1.46 mmol) and **39** (300 mg, 0.936 mmol) in dry MeCN (10 ml), and the mixture was stirred for 24 h at 60°C. The mixture was evaporated and the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 9:1) to give protected **40** (637 mg) as a light brown solid. The deprotection of protected **40** was conducted in accordance with the procedure described in Section 2.1.5. Chromatography: ($CHCl_3$−MeOH, 9:1). **40** (150 mg, 36%, 2 steps) was obtained as a light brown paste. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 6.9 Hz), 1.14−1.28 (8H), 1.44 (2H, m), 1.53 (2H, m), 1.98 (2H, t, J = 7.3 Hz), 2.23 (3H, s), 2.42 (2H, m), 2.57−2.85 (2H, m), 3.02 (2H, m), 4.02 (1H, m), 4.10 (1H, m), 4.65 (1H, m), 5.18 (1H, m), 5.40 (1H, m), 5.87 (1H, d, J = 5.4 Hz), 7.21 (2H, s), 7.68 (1H, br) , 8.14 (1H, s), 8.31 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 25.2, 28.4 (2), 28.5 (2), 31.1 (2), 35.4, 36.6, 48.5, 71.9, 72.5, 88.0 (2), 119.2,

139.8, 149.3, 152.6, 156.0, 171.9. HRMS (ESI, positive) *m/z* 464.2983 $[M+H]^+$ (calcd for $C_{22}H_{38}O_4N_7$, 464.2985).

2.12. Analog 47



2.12.1. 5′-*O*-*tert*-Butyldimethylsilyladenosine (41). *tert*-Butyldimethylsilyl chloride (3.06 g, 19.9 mmol) was added with stirring to a solution of adenosine (4.46 g, 16.5 mmol) and imidazole (2.42 g, 35.0 mmol) in dry DMF (100 ml) at 0°C. The mixture was warmed to rt and subsequently stirred for 14 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 9:1) to give **41** (5.40 g, 85%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.04 (6H, s), 0.86 (9H, s), 3.74 (1H, dd, *J* = 4.0, 11.3 Hz), 3.87 (1H, dd, *J* = 4.0, 11.3 Hz), 3.94 (1H, m), 4.17 (1H, m), 4.53 (1H, m), 5.17 (1H, d, *J* = 5.4 Hz), 5.51 (1H, d, *J* = 5.6 Hz), 5.90 (1H, d, *J* = 5.1 Hz), 7.26 (2H, br), 8.13 (1H, s), 8.27 (1H, s).

2.12.2. 2′,3′-*O*-Thiocarbonylene-5′-*O*-*tert*-butyldimethylsilyladenosine (42).[S4] A mixture of **41** (5.40 g, 14.2 mmol) and 1,1′-thiocarbonyl diimidazole (5.20 g, 28.3 mmol) in dry 1,2-dichloroethane (100 ml) was refluxed for 3 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 97:3) to give **42** (6.00 g, quant.) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.10 (6H, s), 0.77 (9H, s), 3.69 (1H, dd, *J* = 6.1, 11.0 Hz), 3.74 (1H, dd, *J* = 6.1, 11.0 Hz), 4.52 (1H, dt, *J* = 2.6, 6.0 Hz), 5.81 (1H, d, *J*

= 2.6, 7.3 Hz), 6.37 (1H, dd, *J* = 1.7, 7.3 Hz), 6.56 (1H, d, *J* = 1.7 Hz), 7.38 (2H, br), 8.14 (1H, s), 8.25 (1H, s).

2.12.3. 2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-*tert*-butyldimethylsilyladenosine (43).[S4] A solution of **42** (6.02 g, 14.2 mmol) in triethyl phosphite (100 ml) was refluxed for 3 h. Excess triethyl phosphite was removed by evaporation following completion of the reaction. The residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 98:2) to give **43** (3.40 g, 69%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.10 (6H, s), 0.82 (9H, s), 3.73−3.81 (2H), 4.89−4.93 (1H), 6.16−6.18 (1H), 6.44−6.47 (1H), 6.44−6.94 (1H), 7.24 (2H, br), 8.09 (1H, s), 8.15 (1H, s).

2.12.4. 2′,3′-Didehydro-5′-*O*-*tert*-butyldimethylsilyladenosine (44) A mixture of **43** (1.70 g, 4.89 mmol) and 10% Pd/C (170 mg) in 0.5% triethylamine−MeOH (30 ml) was stirred under a hydrogen atmosphere for 4.5 h. The mixture was filtrated and purified by silica gel column chromatography ($CHCl_3$−MeOH, 98:2) to give **44** (1.56 g, 91%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.10 (3H, s), 0.11 (3H, s), 0.92 (9H, s), 1.99−2.21 (2H), 2.38−2.57 (2H, m), 3.78 (1H, dd, *J* = 3.2, 8.5 Hz), 4.01 (1H, dd, *J* = 3.2, 11.2 Hz), 4.22−4.28 (1H, m), 5.63 (2H, br), 6.34 (1H, dd, *J* = 2.9, 6.6 Hz), 8.32 (1H, s), 8.34 (1H, s).

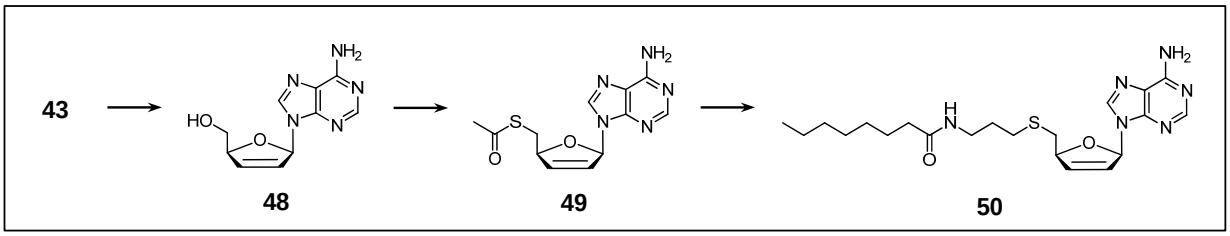
2.12.5. 2′,3′-Dideoxyadenosine (45). TBAF (1.0 M solution in THF, 8.92 ml, 8.92 mmol) was added with stirring to a solution of **44** (1.56 g, 4.46 mmol) in dry THF (50 ml) at 0°C. The mixture was warmed to rt and stirred for 1 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 95:5) to give **45** (940 mg, 90%) as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.00−2.10 (2H), 2.38−2.43 (2H), 3.47−3.64 (2H), 4.09−4.12 (1H), 5.00 (1H, t, *J* = 5.2 Hz), 6.20 (1H, t, *J* = 5.4 Hz), 7.18 (2H, br), 8.12 (1H, s), 8.32 (1H, s).

2.12.6. 5′-Acetylthio-2′,3′,5′-trideoxyadenosine (46). The reaction procedure described in Section 2.1.1. was followed. **45** (678 mg, 2.88 mmol) was used. Chromatography:

($CHCl_3$−MeOH, 95:5). **46** was obtained as a yellowish solid (704 mg, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.93−2.27 (2H), 2.37 (3H, s), 2.49−2.64 (2H), 3.27 (1H, dd, J = 6.1, 13.9 Hz), 3.31 (1H, dd, J = 5.4, 13.9 Hz), 4.29−4.36 (1H), 5.71 (2H, br), 6.26 (1H, dd, J = 3.4, 6.8 Hz), 8.06 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.0, 30.6, 32.5, 33.0, 80.1, 85.4, 125.6, 138.8, 149.3, 152.9, 155.3, 195.4.

2.12.7. 2′,3′,5′-Trideoxy-5′-[3-(octanamido)propylthio]adenosine (47). The reaction procedure described in Section 2.1.4. was followed. **16** (498 mg, 1.88 mmol) and **46** (335 mg, 1.14 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 9:1). **47** was obtained as a yellowish solid (256 mg, 52%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.87 (3H, t, J = 7.1 Hz), 1.23−1.33 (8H), 1.56−1.70 (2H), 1.77 (2H, quint, J = 7.1 Hz), 2.14 (2H, t, J = 7.7 Hz), 2.03−2.30 (2H), 2.58 (2H, t, J = 7.1 Hz), 2.54−2.60 (2H), 2.84 (1H, dd, J = 5.9, 13.7 Hz), 2.90 (1H, dd, J = 5.6, 13.7 Hz), 3.30 (2H, m), 4.36 (1H, m), 5.62 (3H, br), 6.30 (1H, dd, J = 3.7, 6.3 Hz), 8.11 (1H, s), 8.36 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.1, 22.6, 25.7, 29.0, 29.2, 29.3 (2), 30.6, 31.7, 32.5, 36.6, 36.8, 38.4, 81.1, 85.3, 120.1, 138.8, 149.3, 153.0, 155.3, 173.2. HRMS (ESI, positive) *m/z* 457.2368 $[M+K]^+$ (calcd for $C_{21}H_{34}O_2N_6K$, 457.2362).

2.13. Analog 50



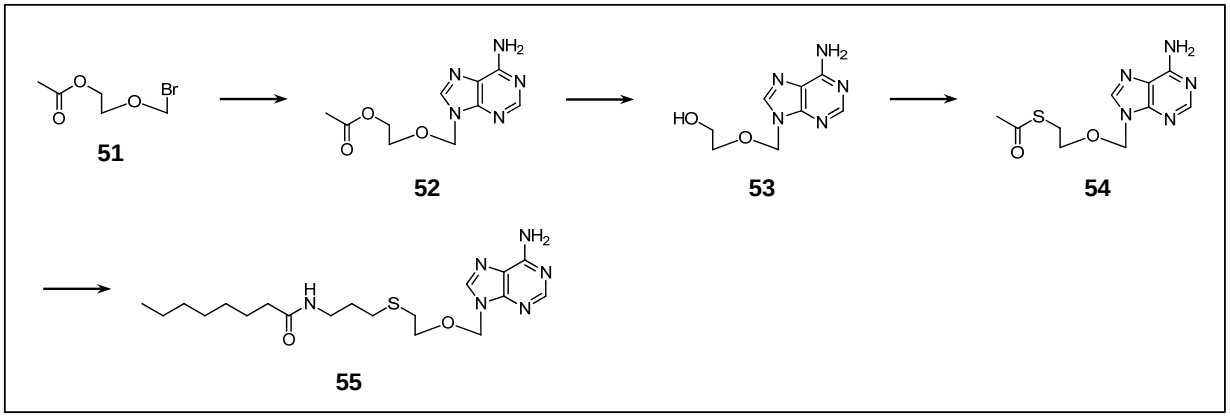
2.13.1. 2′,3′-Didehydro-2′,3′-dideoxyadenosine (48). The reaction procedure described in Section 2.12.5. was followed. **43** (1.00 g, 2.87 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 94:6). **48** was obtained as a white powder (722 mg, quant.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.58 (2H, m), 4.88 (1H, m), 5.02 (H, t, J = 5.5 Hz), 6.13 (1H, m), 6.46 (1H, m), 6.93

(1H, m), 7.24 (2H, br), 8.14 (1H, s), 8.16 (1H, s).

2.13.2. 5′-Acetylthio-2′,3′-didehydro-2′,3′,5′-trideoxyadenosine (49). The reaction procedure described in Section 2.1.1. was followed. **48** (722 mg, 3.09 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 97:3). **49** was obtained as a yellowish solid (700 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.17 (1H, dd, J = 5.6, 14.2 Hz), 3.31 (1H, dd, J = 4.9, 14.2 Hz), 5.65 (1H, m), 5.70 (1H, br), 6.11 (1H, m), 6.36 (1H, m), 7.05 (1H, m), 8.02 (1H, s), 8.39 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.6, 33.1, 85.4, 88.1, 119.7, 125.6, 135.4, 138.8, 149.9, 150.0, 153.4, 155.4.

2.13.3. 2′,3′-Didehydro-2′,3′,5′-trideoxy-5′-[3-(octanamido)propylthio]adenosine (50). The reaction procedure described in Section 2.1.4. was followed. **16** (416 mg, 1.57 mmol) and **49** (308 mg, 1.05 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 9:1). **50** was obtained as a yellowish solid (146 mg, 45%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.86 (3H, t, J = 7.0 Hz), 1.22−1.30 (8H), 1.60 (2H, m), 1.67 (2H, quint, J = 7.1 Hz), 2.11 (2H, t, J = 7.7 Hz), 2.44 (2H, m), 2.77 (1H, dd, J = 6.1, 14.0 Hz), 2.88 (1H, dd, J = 4.9, 14.0 Hz), 3.22 (2H, m), 5.16 (1H, br), 5.56 (1H, br), 5.71 (2H, br), 6.12 (1H, m), 6.46 (1H, m), 7.08 (1H, m), 8.06 (1H, s), 8.39 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.1, 22.6, 25.7, 29.0, 29.2, 29.3, 30.7, 31.7, 36.6, 36.8, 38.3, 86.5, 88.3, 119.7, 125.2, 136.0, 139.0, 150.0, 153.4, 155.4, 173.2. HRMS (ESI, positive) *m/z* 433.2386 $[M+H]^+$ (calcd for $C_{21}H_{33}O_2N_6S$, 433.2386).

2.14. Analog 55



2.14.1. (2-Acetoxyethoxy)methyl bromide (51).[S5] Acetyl bromide (4.00 ml, 54.0 mmol) was stirred at 0°C while 1,3-dioxolane (3.40 ml, 49.0 mmol) was added dropwise over 5 min. Vacuum distillation of the mixture gave **51** (6.60 g, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.10 (3H, s), 3.87 (2H, t, J = 4.5 Hz), 4.28 (2H, m), 5.70 (2H, s).

2.14.2. 9-[(2-Acetoxyethoxy)methyl]adenine (52).[S5] NaH (60% in oil, 325 mg, 8.20 mmol) was added with stirring to a solution of adenine (1.00 g, 7.40 mmol) in dry DMF (25 ml) at 0°C. The mixture was warmed to rt and subsequently stirred for 30 min. The mixture was then cooled to −60°C and **51** (1.31 g, 6.70 mmol) in dry DMF (10 ml) was slowly added dropwise. The mixture was warmed slowly to rt and stirred for 6 h. The reaction was quenched by 1 M aq $NaHCO_3$ at 0°C. After removing the solvents, the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 95:5) to give **52** (1.30 g, 70%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.92 (3H, s), 3.70 (2H, t, J = 4.7 Hz), 4.06 (2H, t, J = 4.7 Hz), 5.56 (2H, s), 7.23 (2H, br), 8.16 (1H, s), 8.26 (1H, s).

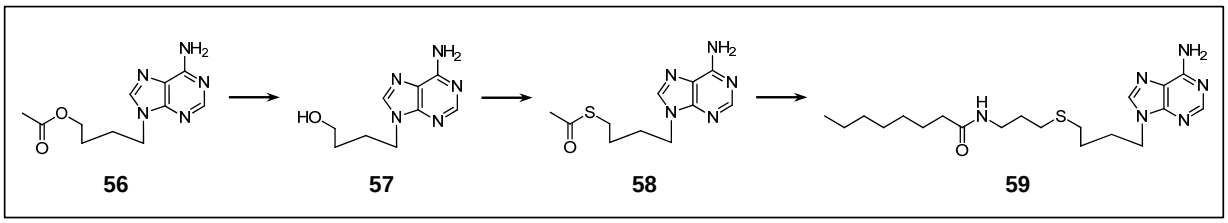
2.14.3. 9-[(2-Hydroxyethoxy)methyl]adenine (53).[S5] Compound **52** (323 mg, 1.29 mmol) was dissolved in 1 M aq MeONa−MeOH (11 ml, 1:10) and stirred for 2 h at rt. The solvents were removed by evaporation and the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 95:5, 90:10) to give **53** (232 mg, 87%) as a white solid. ^{1}H NMR ($CDCl_3$, 400

MHz) δ 3.43−3.52 (4H), 4.62 (1H, t, J = 5.4 Hz), 5.55 (2H, s), 7.21 (2H, br), 8.16 (1H, s), 8.25 (1H, s).

2.14.4. 9-[(2-Acetylthioethoxy)methyl]adenine (54). The reaction procedure described in Section 2.1.1. was followed. **53** (309 mg, 1.48 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 97:3). **54** was obtained as a yellowish solid (231 mg, 58%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.26 (3H, s), 2.97 (2H, t, J = 6.2 Hz), 3.61 (2H, t, J = 6.2 Hz), 5.53 (2H, s), 7.22 (2H, br), 8.16 (1H, s), 8.24 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.0, 30.3, 67.3, 71.7, 118.4, 141.0, 149.6, 152.8, 155.9, 194.7.

2.14.5. 9-{[2-(3-Octanamidopropylthio)ethoxy]methyl}adenine (55). The reaction procedure described in Section 2.1.4. was followed. **16** (127 mg, 0.481 mmol) and **54** (128 mg, 0.480 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 98:2). **55** was obtained as a yellowish solid (76.9 mg, 39%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 6.8 Hz), 1.13−1.31 (8H), 1.46 (2H, m), 1.56 (2H, m), 2.01 (2H, t, J = 7.4 Hz), 2.44 (2H, t, J = 7.3 Hz), 2.59 (2H, t, J = 6.7 Hz), 3.05 (2H, q, J = 6.7 Hz), 3.64 (2H, t, J = 6.7 Hz), 5.54 (2H, s), 7.25 (2H, br), 7.71 (1H, br), 8.16 (1H, s), 8.26 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.8, 21.9, 25.2, 28.3, 28.5, 28.8, 29.3, 30.3, 31.1, 35.3, 37.4, 68.5, 71.8, 118.5, 141.1, 149.7, 152.9, 156.0, 172.0. HRMS (ESI, positive) m/z 431.2201 $[M+Na]^+$ (calcd for $C_{19}H_{32}O_2N_6NaS$, 431.2205).

2.15. Analog 59



2.15.1. 9-(4-Acetoxybutyl)adenine (56). The reaction procedure described in Section 2.14.2. was followed. Adenine (162 mg, 1.20 mmol) and 4-bromobutyl acetate (174 µl, 1.20 mmol) were

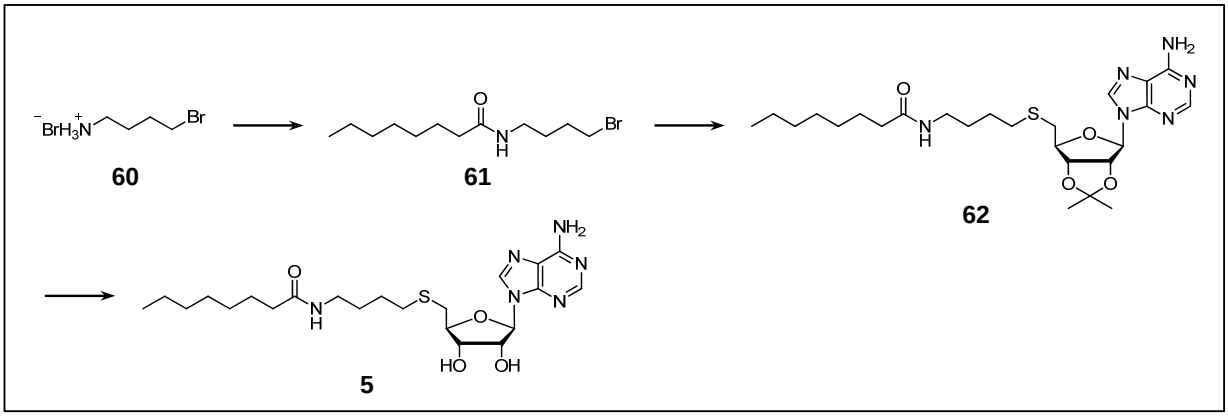
used. Chromatography: ($CHCl_3$−MeOH, 95:5). **56** was obtained as a white powder (163 mg, 52%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.53 (2H, dt, J = 6.2, 15.9 Hz), 1.84 (2H, dt, J = 7.4, 14.9 Hz), 1.96 (3H, s), 3.99 (2H, t, J = 6.6 Hz), 4.14 (2H, t, J = 7.1 Hz), 7.11 (2H, br), 8.12 (2H, s).

2.15.2. 9-(4-Hydroxybutyl)adenine (57). The reaction procedure described in Section 2.14.3. was followed. **56** (163 mg, 0.653 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **57** was obtained as a white powder (123 mg, 90%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.37 (2H, m), 1.82 (2H, m), 3.39 (2H, dd, J = 6.3, 11.5 Hz), 4.13 (2H, t, J = 7.1 Hz), 4.38 (1H, t, J = 5.0 Hz), 7.11 (2H, br), 8.11 (1H, s), 8.12 (1H, s).

2.15.3. 9-(4-Acetylthiobutyl)adenine (58). The reaction procedure described in Section 2.1.1. was followed. **57** (120 mg, 0.579 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5). **58** was obtained as a yellowish solid (163 mg, 52%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.47 (2H, m), 1.83 (2H, m), 2.29 (3H, s), 2.85 (2H, t, J = 7.2 Hz), 4.13 (2H, t, J = 7.0 Hz), 7.11 (2H, br), 8.11 (1H, s), 8.12 (1H, s).

2.15.4. 9-[(4-Octanamidepropylthio)butyl]adenine (59). The reaction procedure described in Section 2.1.4. was followed. **16** (880 mg, 3.33 mmol) and **58** (740 mg, 2.79 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 95:5). **59** was obtained as a yellowish solid (491 mg, 43%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 6.6 Hz), 1.13−1.29 (8H, m), 1.42−1.50 (4H), 1.59 (2H, quint, J = 7.2 Hz), 1.88 (2H, quint, J = 7.4 Hz), 2.02 (2H, t, J = 7.4 Hz), 2.42 (2H, t, J = 7.3 Hz), 2.48 (2H, t, J = 7.3 Hz), 3.06 (2H, dd, J = 6.7, 12.6 Hz), 4.15 (2H, t, J = 7.1 Hz), 7.15 (2H, br), 7.74 (1H, t, J = 5.5 Hz), 8.128 (1H, s), 8.131 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 25.3, 26.1, 28.4, 28.5, 28.6 (2), 29.3, 30.4, 31.2, 35.4, 37.5, 42.4, 118.7, 140.8, 149.5, 152.3, 155.9, 172.1. HRMS (ESI, positive) *m/z* 429.2418 $[M+Na]^+$ (calcd for $C_{20}H_{34}ON_6NaS$, 429.2413).

2.16. Analog 5



2.16.1. 4-Bromobutan-1-amine hydrobromide (60). The mixture of 4-bromobutan-1-ol (517 µl, 5.58 mmol) and 48% aq HBr (5 ml) was refluxed for 3 h with stirring. Evaporation of the mixture gave **60** (1.15 g, 88%) as an ocher solid. ^{1}H NMR (D_2O, 400 MHz) δ 1.63−1.83 (4H), 2.88 (2H, t, J = 7.4 Hz), 3.37 (2H, t, J = 6.3 Hz).

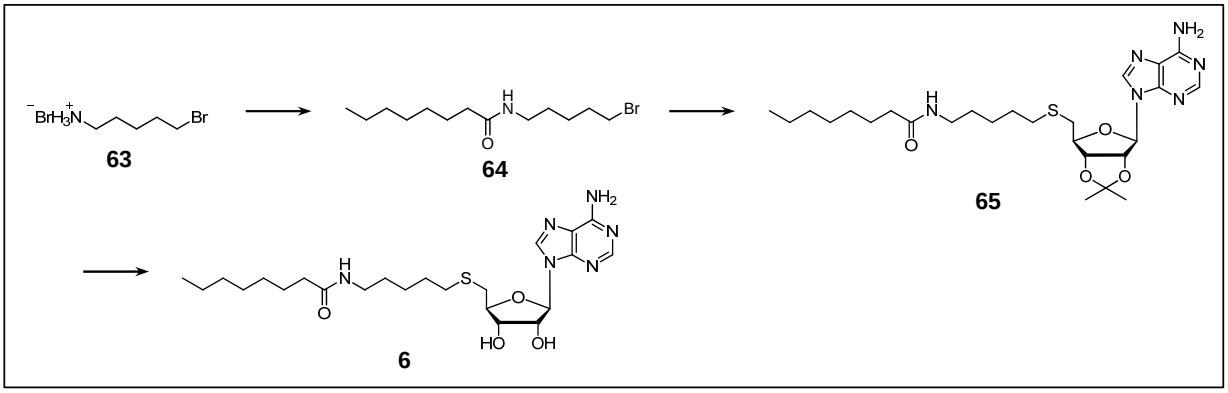
2.16.2. *N*-(4-Bromobutyl)octanamide (61). The reaction procedure described in Section 2.1.3. was followed. **60** (253 mg, 1.09 mmol) and octanoyl chloride (517 µl, 3.70 mmol) were used. **61** was obtained as a colorless paste (383 mg, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.23−1.38 (8H), 1.65 (2H, quint, J = 7.6 Hz), 1.81−1.98 (4H), 2.25 (2H, t, J = 7.7 Hz), 3.41 (2H, t, J = 6.7 Hz), 3.46 (2H, t, J = 6.8 Hz), 5.46 (1H, br).

2.16.3. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[4-(octanamido)butylthio]adenosine (62). The reaction procedure described in Section 2.1.4. was followed. **11** (208 mg, 0.569 mmol) and **61** (237 mg, 0.851 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 100:0, 95:5). **62** was obtained as a yellowish solid (139 mg, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.9 Hz), 1.23−1.31 (8H), 1.40 (3H, s), 1.52 (2H, m), 1.59−1.64 (4H), 1.62 (3H, s), 2.15 (2H, t, J = 7.6 Hz), 2.48−2.52 (2H), 2.78 (1H, dd, J = 6.9, 13.7 Hz), 2.83 (1H, dd, J = 6.9, 13.7 Hz), 3.18−3.23 (2H), 4.39 (1H, dt, J = 3.0, 6.8 Hz), 5.06 (1H, dd, J = 3.0, 6.3 Hz), 5.46 (1H, br), 5.53 (1H, dd, J = 2.2, 6.3 Hz), 5.62 (2H, br), 6.09 (1H, d, J = 2.2 Hz), 7.94 (1H, s), 8.36 (1H, s). ^{13}C

NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 25.4, 25.8, 26.8, 27.1, 28.8, 29.0, 29.2, 31.7, 32.3, 34.4, 36.9, 38.9, 83.9, 84.0, 87.0, 90.9, 114.5, 120.3, 140.1, 149.7, 153.2, 155.5, 173.2.

2.16.4. 5′-Deoxy-5′-[4-(octanamido)butylthio]adenosine (5). The reaction procedure described in Section 2.1.5. was followed. **62** (135 mg, 0.259 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **5** was obtained as a white solid (69.2 mg, 66%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 7.0 Hz), 1.18−1.26 (8H), 1.35−1.50 (6H), 2.01 (2H, t, J = 7.4 Hz), 2.78 (1H, dd, J = 6.8, 13.8 Hz), 2.89 (1H, dd, J = 6.8, 13.8 Hz), 2.98 (2H, m), 3.99 (1H, dt, J = 3.8, 6.4 Hz), 4.13 (1H, m), 4.74 (1H, m), 5.27 (1H, d, J = 5.1 Hz), 5.45 (1H, d, J = 6.1 Hz), 5.87 (1H, d, J = 5.9 Hz), 7.24 (2H, br), 7.68 (1H, t, J = 5.6 Hz), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 25.3, 26.5, 28.2, 28.3, 28.5, 31.1, 31.5, 33.9, 35.4, 37.8, 72.5 (2), 83.9, 87.3, 119.1, 139.8, 149.4, 152.6, 156.0, 171.8. HRMS (ESI, positive) *m/z* 503.2420 $[M+Na]^+$ (calcd for $C_{22}H_{36}O_4N_6NaS$, 503.2416).

2.17. Analog 6



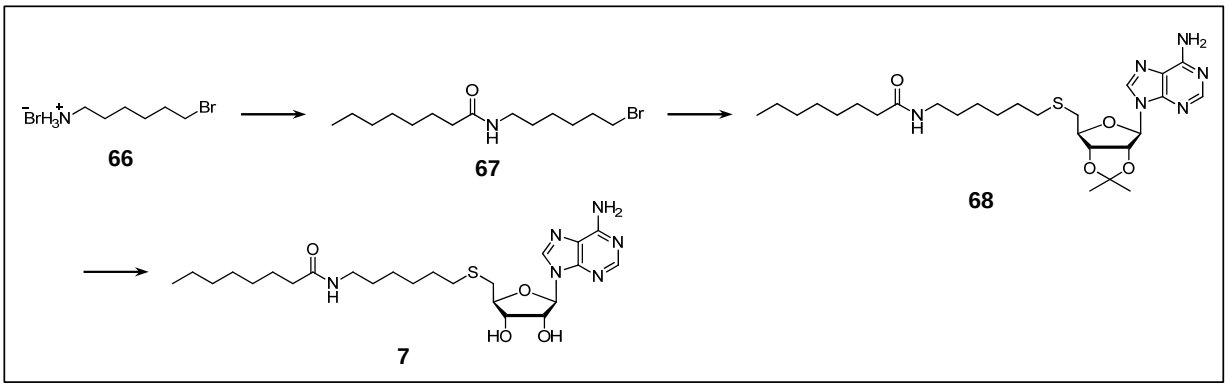
2.17.1. 5-Bromopentan-1-amine hydrobromide (63). The reaction procedure described in Section 2.16.1. was followed. 5-Aminopetan-1-ol (700 µl, 6.44 mmol) was used. **63** was obtained as an ocher solid (2.33 g, quant.). ^{1}H NMR (D_2O, 400 MHz) δ 1.48 (2H, m), 1.65 (2H, quint, J = 7.3 Hz), 1.85 (2H, quint, J = 7.1 Hz), 2.96 (2H, t, J = 7.3 Hz), 3.41 (2H, t, J = 6.6 Hz).

2.17.2. *N*-(5-Bromopentyl)octanamide (64). The reaction procedure described in Section 2.1.3. was followed. **63** (350 mg, 1.42 mmol) and octanoyl chloride (230 µl, 1.42 mmol) were used. **64** was obtained as a colorless paste (383 mg, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 7.1 Hz), 1.22−1.36 (10H), 1.43−1.69 (4H), 1.88 (2H, m), 2.16 (2H, t, J = 7.7 Hz), 3.27 (2H, q, J = 6.5 Hz), 3.42 (2H, t, J = 6.7 Hz), 5.43 (1H, br).

2.17.3. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[5-(octanamido)pentylthio]adenosine (65). The reaction procedure described in Section 2.1.4. was followed. **11** (306 mg, 0.837 mmol) and **64** (369 mg, 1.26 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 100:0, 97.5:2.5). **65** was obtained as a yellowish solid (341 mg, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.8 Hz), 1.19−1.35 (12H), 1.36−1.55 (4H), 1.40 (3H, s), 1.55−1.60 (2H), 1.61 (3H, s), 2.15 (2H, t, J = 7.6 Hz), 2.46 (2H, t, J = 7.3 Hz), 2.79 (2H, t, J = 6.8 Hz), 3.20 (2H, m), 4.40 (1H, dt, J = 3.0, 6.8 Hz), 5.06 (1H, dd, J = 3.0, 6.5 Hz), 5.51 (1H, br), 5.54 (1H, dd, J = 2.0, 6.5 Hz), 5.62 (2H, br), 6.08 (1H, d, J = 2.0 Hz), 7.94 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 25.3, 25.8, 25.9, 27.1, 29.0, 29.1, 29.2, 29.3, 31.7, 32.5, 34.3, 36.9, 39.2, 83.9, 84.0, 87.1, 90.9, 114.4, 120.3, 140.1, 149.2, 153.2, 155.4, 173.1.

2.17.4. 5′-Deoxy-5′-[5-(octanamido)pentylthio]adenosine (6). The reaction procedure described in Section 2.1.5. was followed. **65** (340 mg, 0.636 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **6** was obtained as a white solid (154 mg, 49%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83 (3H, t, J = 6.8 Hz), 1.13−1.37 (12H), 1.39−1.50 (4H), 2.00 (2H, t, J = 7.3 Hz), 2.47 (2H, m), 2.78 (1H, dd, J = 7.1, 13.8 Hz), 2.88 (1H, dd, J = 5.9, 13.8 Hz), 2.97 (2H, q, J = 6.3 Hz), 3.99 (1H, m), 4.13 (1H, m), 4.75 (1H, m), 5.27 (1H, d, J = 5.1 Hz), 5.46 (1H, d, J = 6.0 Hz), 5.87 (1H, d, J = 6.0 Hz), 7.26 (2H, br), 7.67 (1H, br), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 25.3, 25.5, 28.4, 28.5, 28.7, 28.8, 31.1, 31.8, 33.9, 35.4, 38.1, 72.5, 72.6, 84.0, 87.4, 119.1, 139.8, 149.4, 152.7, 156.0, 171.8. HRMS (ESI, positive) *m/z* 517.2574 $[M+Na]^+$ (calcd for $C_{23}H_{38}O_4N_6NaS$, 517.2573).

2.18. Analog 7



2.18.1. 5-Bromohexan-1-amine hydrobromide (66). The reaction procedure described in Section 2.16.1. was followed. 6-Aminohexan-1-ol (545 µl, 4.65 mmol) was used. **66** was obtained as an ocher solid (1.22 g, quant.). ^{1}H NMR (D_2O, 400 MHz) δ 1.25−1.42 (4H), 1.58 (2H, quint, J = 7.4 Hz), 1.77 (2H, quint, J = 7.3 Hz), 2.90 (2H, t, J = 7.6 Hz), 3.42 (2H, t, J = 6.6 Hz).

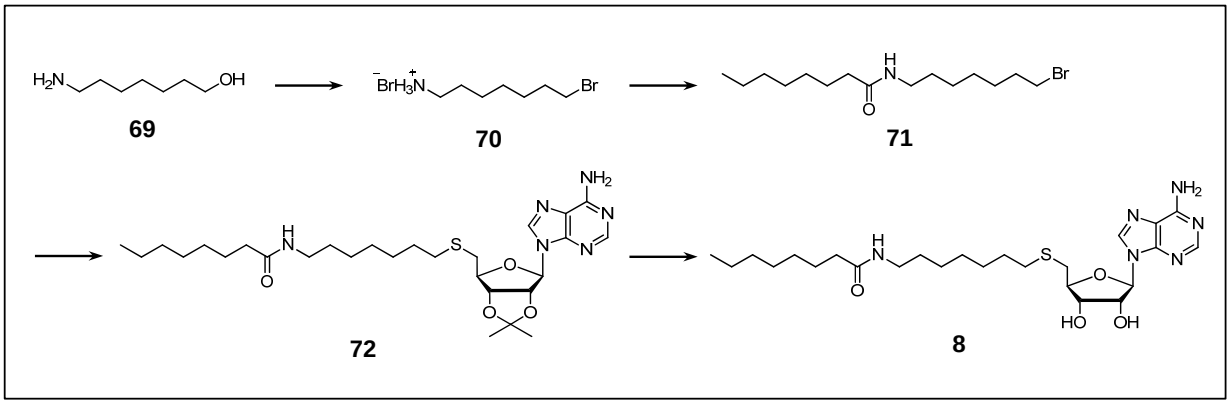
2.18.2. *N*-(5-Bromohexyl)octanamide (67). The reaction procedure described in Section 2.1.3. was followed. **66** (350 mg, 1.34 mmol) and octanoyl chloride (308 µl, 1.81 mmol) were used. **67** was obtained as a colorless paste (410 mg, quant). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.21−1.39 (12H), 1.42−1.71 (4H), 1.86 (2H, m), 2.15 (2H, t, J = 7.6 Hz), 3.25 (2H, q, J = 7.1 Hz), 3.41 (2H, t, J = 6.7 Hz), 5.47 (1H, br).

2.18.3. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[6-(octanamido)hexylthio]adenosine (68). The reaction procedure described in Section 2.1.4. was followed. **11** (300 mg, 0.821 mmol) and **67** (448 mg, 1.46 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 97.5:2.5). **68** was obtained as a yellowish solid (327 mg, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 7.0 Hz), 1.19−1.35 (12H), 1.41 (3H, s), 1.45 (2H, m), 1.58−1.68 (2H), 1.62 (3H, s), 2.16 (2H, m), 2.45 (2H, t, J = 7.4 Hz), 2.81 (2H, dd, J = 2.7, 6.8 Hz), 3.23 (2H, m), 4.40 (1H, dt, J = 3.0, 6.8 Hz), 5.07 (1H, dd, J = 3.0, 6.5 Hz), 5.54 (2H, dd, J = 2.2, 6.5 Hz), 5.67 (2H, br), 6.09 (1H, d, J = 2.2 Hz), 7.93 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 25.3, 25.8, 26.5, 27.1,

28.4, 29.0, 29.3, 29.4, 29.6, 31.7, 32.6, 34.3, 36.9, 39.4, 83.9, 84.0, 87.2, 91.0, 114.4, 120.4, 140.2, 149.2, 153.2, 155.5, 173.2.

2.18.4. 5′-Deoxy-5′-[6-(octanamido)hexylthio]adenosine (7). The reaction procedure described in Section 2.1.5. was followed. **68** (340 mg, 0.596 mmol) was used. Chromatography: ($CHCl_3$−MeOH, 95:5, 90:10). **7** was obtained as a white solid (157 mg, 52%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.84 (3H, t, J = 7.0 Hz), 1.12−1.36 (14H), 1.39−1.51 (4H), 2.01 (2H, t, J = 7.3 Hz), 2.47 (2H, m), 2.79 (1H, dd, J = 7.1, 13.9 Hz), 2.88 (1H, dd, J = 5.8, 13.9 Hz), 2.97 (2H, dd, J = 5.8, 6.8 Hz), 3.99 (1H, m), 4.14 (1H, m), 4.76 (1H, m), 5.27 (1H, d, J = 4.9 Hz), 5.45 (1H, d, J = 5.9 Hz), 5.87 (1H, d, J = 5.9 Hz), 7.25 (2H, br), 7.65 (1H, t, J = 5.6 Hz), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.9, 22.0, 25.3, 25.9, 27.9, 28.4, 28.5, 29.0, 29.1, 31.1, 31.8, 33.9, 35.4, 38.2, 72.5, 72.6, 84.1, 87.4, 119.1, 139.8, 149.4, 152.6, 156.0, 171.8. HRMS (ESI, positive) *m/z* 531.2735 $[M+Na]^+$ (calcd for $C_{24}H_{40}O_4N_6NaS$, 531.2729).

2.19. Analog 8



2.19.1. 7-Aminoheptan-1-ol (69).[S6] $NaBH_4$ (6.25 mg, 16.5 mmol) and 7-aminoheptanoic acid (1.00 g, 6.89 mmol) were dissolved in dry THF (10 ml) and the solution was cooled to 0ºC in an ice bath. I_2 (1.74 g, 6.89 mmol, in 5 ml of dry THF) was added dropwise to the solution over 30 min with stirring. After the addition of I_2 was complete and gas evolution had ceased, the solution

was heated to reflux for 18 h and then cooled to rt. MeOH was added until the mixture became clear. After stirring for 30 min, the solvents were removed by evaporation and the residue was dissolved in 20% aq KOH (20 ml). The solution was stirred for 4 h at rt and extracted with $CHCl_3$ (20 ml ×3). The $CHCl_3$ layer was washed with water and dried over Na_2SO_4. Evaporation of the solvents afforded **69** (colorless liquid) with some impurities. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30−1.70 (10H), 2.66−2.88 (2H), 3.65 (2H, m).

2.19.2. 7-Bromoheptan-1-amine hydrobromide (70). The reaction procedure described in Section 2.16.1. was followed. **69** (16.5 mmol) was used. **70** was quantitatively obtained as an ocher solid. ^{1}H NMR (D_2O, 400 MHz) δ 1.22−1.41 (6H), 1.58 (2H, m), 1.78 (2H, quint, J = 7.2 Hz), 2.91 (2H, t, J = 7.4 Hz), 3.43 (2H, t, J = 6.7 Hz).

2.19.3. *N*-(7-Bromoheptyl)octanamide (71). The reaction procedure described in Section 2.1.3. was followed. **70** (6.89 mmol) and octanoyl chloride (1.17 ml, 6.89 mmol) were used. **71** was obtained as a colorless oil (2.07 g, 94% for three steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 6.8 Hz), 1.18−1.70 (18H), 1.85 (2H, quint, J = 7.4 Hz), 2.15 (2H, t, J = 7.7 Hz), 3.24 (2H, dt, J = 6.1, 7.0 Hz), 3.41 (2H, t, J = 6.7 Hz), 5.40 (1H, br).

2.19.4. 5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[7-(octanamido)heptylthio]adenosine (72). The reaction procedure described in Section 2.1.4. was followed. **11** (300 mg, 0.821 mmol) and **71** (394 mg, 1.23 mmol) were used. Chromatography: ($CHCl_3$−MeOH, 97.5:2.5). **72** was obtained as a white powder (405 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.8 Hz), 1.18−1.37 (16H), 1.40 (3H, s), 1.42−1.52 (4H), 1.62 (3H, s), 2.16 (2H, t, J = 7.6 Hz), 2.47 (2H, t, J = 7.4 Hz), 2.80 (2H, t, J = 6.1 Hz), 3.23 (2H, dt, J = 6.6, 6.8 Hz), 4.40 (1H, m), 5.06 (1H, m), 5.53 (2H, m), 5.77 (2H, br), 6.09 (1H, s), 7.93 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 25.3, 25.8, 26.7, 27.1, 28.6, 28.8, 29.0, 29.3, 29.4, 29.6, 31.7, 32.6, 34.3, 36.9, 39.4, 83.9, 84.0, 87.1, 90.9, 114.4, 120.3, 140.0, 149.2, 153.2, 155.5, 173.1.

2.19.5. 5′-Deoxy-5′-[7-(octanamido)heptylthio]adenosine (8). The reaction procedure

described in Section 2.1.5. was followed. **72** (400 mg, 0.711 mmol) was used. **8** was purified by crystallization from MeOH (233 mg, 63%, white powder). ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.84 (3H, t, J = 6.7 Hz), 1.12−1.28 (14H), 1.32 (2H, m), 1.33−1.50 (4H), 2.01 (2H, t, J = 7.3 Hz), 2.47 (2H, m), 2.79 (1H, dd, J = 7.1, 13.8 Hz), 2.87 (1H, dd, J = 5.6, 13.8 Hz), 2.98 (2H, dd, J = 6.1, 6.8 Hz), 3.99 (1H, m), 4.14 (1H, m), 4.75 (1H, m), 5.26 (1H, d, J = 4.9 Hz), 5.45 (1H, d, J = 5.9 Hz), 5.87 (1H, d, J = 5.6 Hz), 7.24 (2H, br), 7.66 (1H, t, J = 5.6 Hz), 8.14 (1H, s), 8.32 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ13.9, 22.0, 25.3, 26.3, 28.1, 28.2, 28.4, 28.5, 29.0 (2), 31.1, 31.8, 33.9, 35.4, 38.3, 72.5, 72.6, 84.1, 87.4, 119.1, 139.8, 149.4, 152.6, 156.0, 171.8. HRMS (ESI, positive) *m/z* 545.2889 $[M+Na]^+$ (calcd for $C_{25}H_{42}O_4N_6NaS$, 545.2886).

2.20. 9- and 10-fixed beads

2.20.1. Ligands 9 and 10. The mixture of **6** (500 mg, 1.01 mmol), NaH (60% in oil, 48.3 mg, 1.21 mmol), tetrabutylammonium iodide (74.6 mg, 0.202 mmol), and propargyl bromide (117 µl, 1.01 mmol) in dry DMF (10 ml) was stirred for 2.5 h at 55°C. After evaporating the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 98.5:1.5) and HPLC (Inertsil ODS-3 10 × 250 mm, 45% aq MeCN, 4 ml/min) to give **9** (110 mg, 21%, white powder) and **10** (43 mg, 8%, white powder). Spectroscopic data for **9**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.83 (3H, t, J = 6.8 Hz), 1.15−1.37 (12H), 1.40−1.50 (4H), 2.01 (2H, t, J = 7.4 Hz), 2.47 (2H, m), 2.80 (1H, dd, J = 7.0, 13.9 Hz), 2.90 (1H, dd, J = 5.9, 13.9 Hz), 2.97 (2H, q, J = 6.4 Hz), 3.31 (1H, t, J = 2.4 Hz), 4.01 (1H, m), 4.21 (1H, dd, J = 2.4, 15.9 Hz), 4.29 (1H, dd, J = 2.4, 15.9 Hz), 4.37 (1H, m), 4.82 (1H, t, J = 5.2 Hz), 5.42 (1H, br), 6.00 (1H, d, J = 5.6 Hz), 7.26 (2H, br), 7.63 (1H, br), 8.15 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ13.8, 21.9, 25.2, 25.5, 28.3, 28.5, 28.6, 28.8, 31.1, 31.8, 33.8, 35.4, 38.1, 57.1, 71.0, 77.5, 78.8, 79.7, 84.5, 85.7, 119.2, 139.8, 149.2, 152.6, 156.0, 171.8. MS (ESI, positive) *m/z* 534 $[M+H]^+$. Spectroscopic data for **10**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ0.84 (3H, t, J = 6.5 Hz), 1.14−1.37 (12H), 1.40−1.51 (4H), 2.01

(2H, t, J = 7.3 Hz), 2.49 (2H, m), 2.82 (1H, dd, J = 6.3, 13.9 Hz), 2.89 (1H, dd, J = 6.1, 13.9 Hz), 2.98 (2H, q, J = 6.3 Hz), 3.45 (1H, br), 4.10−4.18 (2H), 4.32 (1H, dd, J = 2.4, 16.1 Hz), 4.40 (1H, dd, J = 2.4, 16.1 Hz), 4.97 (1H, t, J = 5.6 Hz), 5.63 (1H, br), 5.88 (1H, d, J = 6.3 Hz), 7.23 (2H, br), 7.64 (1H, br), 8.14 (1H, s), 8.33 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.8, 21.9, 25.2, 25.5, 28.3, 28.5, 28.6, 28.8, 31.1, 31.8, 33.8, 35.4, 38.1, 57.1, 71.6, 77.2, 79.1, 80.2, 81.9, 87.3, 119.1, 139.7, 149.4, 152.6, 156.0, 171.8. MS (ESI, positive) *m/z* 534 $[M+H]^+$.

2.20.2. 9- and 10-fixed beads. Magnetic FG beads with azide linkers were purchased from Tamagawa Seiki (Nagano, Japan). Beads (2.5 mg) were incubated with 125 μM **9** or **10**, 125 μM Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine, 125 μM $CuSO_4$, and 125 μM (+)-sodium L-ascorbate in *t*-BuOH−DMSO (4:1, 500 μl) for 24 h at rt. The beads were then washed with *t*-BuOH−DMSO−H_2O (4:1:4, three times) and MeOH−H_2O (1:1, three times), suspended in MeOH−H_2O (1:1, 100 μl), and stored at 4°C.

3. Enzyme assay

3.1. Assay methods. The TofI enzyme (His-tag) was expressed in *E. coli* BL21(DE3)pLysS cells (Novagen) and purified using the HisTrap HP column (1 ml, GE Healthcare) in accordance with the method of Chung *et al*.[S7] Apo-ACP was prepared from *E. coli* K-12 cells grown in TB medium.[S8] The chemical acylation of apo-ACP by *N*-octanoylimidazole was carried out according to the method of Cronan *et al.*[S9] Enzyme reactions for the TofI inhibition assay were conducted for 20 min at 37°C using 1 μM TofI, 10 μM octanoyl-ACP, 10 μM SAM, and the analog's DMSO solutions in a buffer containing 10 mM Tris-HCl (pH 7.4), 330 mM NaCl, 15% glycerol, 0.7 mM dithioerythritol, 2 mM EDTA, 25 mM $MgSO_4$, and 0.1 mM $FeSO_4$.[S10] The reaction was stopped by the addition of two volumes of EtOAc followed by vortexing. The EtOAc layers were dried over a small amount of Na_2SO_4 and subjected to GC−MS.[S11,S12] The GC conditions were as follows: column, InertCap 5MS/NP capillary column (25 m × 0.25 mm

i.d., 0.25 mm film, GL Sciences); injection, 1 μl, splitless, 60 s valve time; injector temperature, 230°C; carrier gas, He at 0.8 ml/min; transfer line temperature, 280°C; electron energy, 70 eV. The temperature of the column oven was programmed as follows: 150°C for 3 min, followed by an increase to 275°C at 8.33°C/min, with the temperature being maintained at 275°C for 5 min. Inhibition constants (K_i values) were estimated graphically using Dixon plots.[S13]

3.2. Dixon plots.

3.2.1. Analog 1

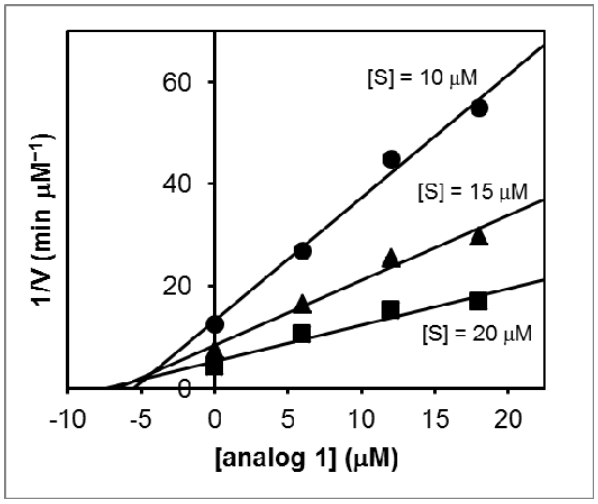


Figure S1. Dixon plot for TofI with varying concentrations of analog **1** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

3.2.2. Analog 4

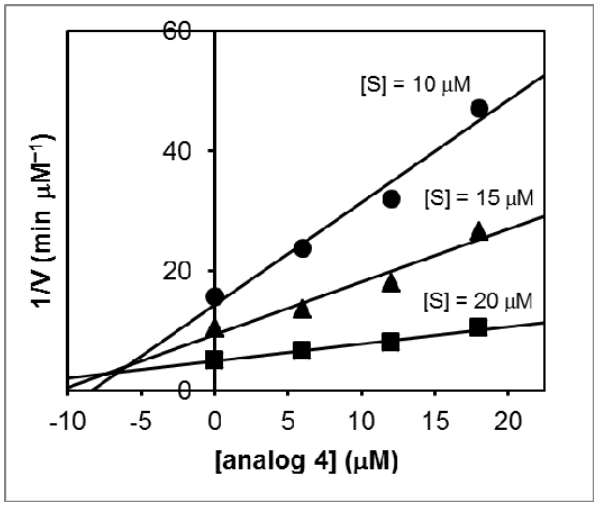
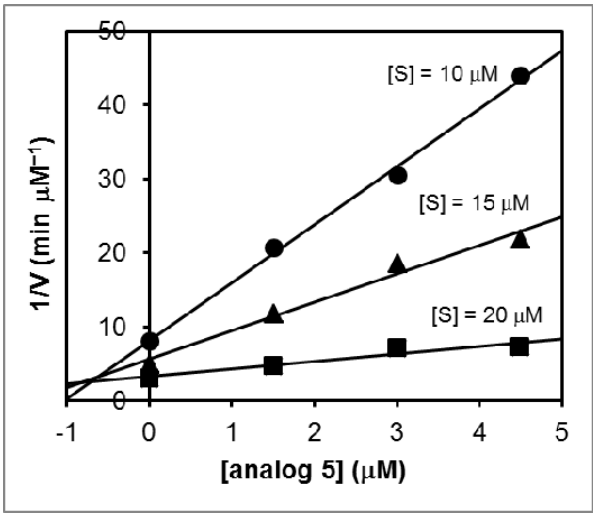


Figure S2. Dixon plot for TofI with varying concentrations of analog **4** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

3.2.3. Analog 5



Dixon plot for TofI with varying concentrations of analog **5** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

3.2.4. Analog 6

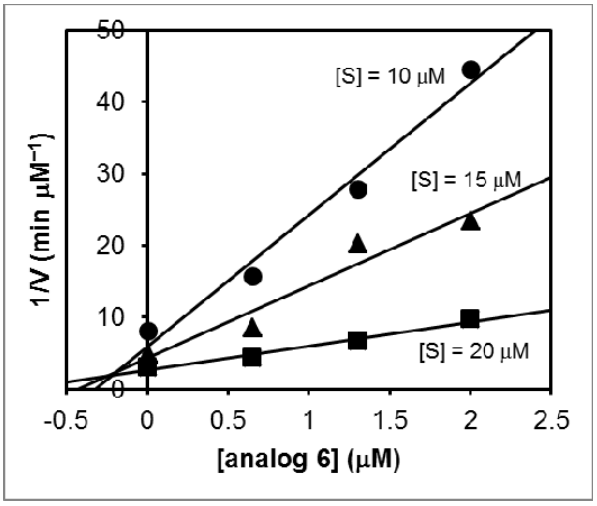


Figure S3. Dixon plot for TofI with varying concentrations of analog **6** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

3.2.5. Analog 7

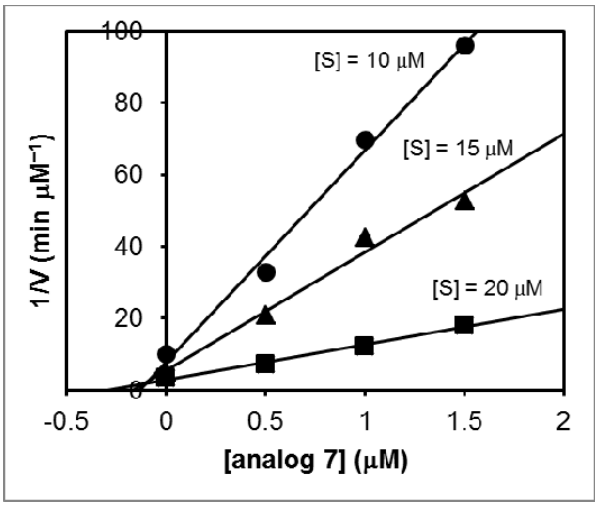
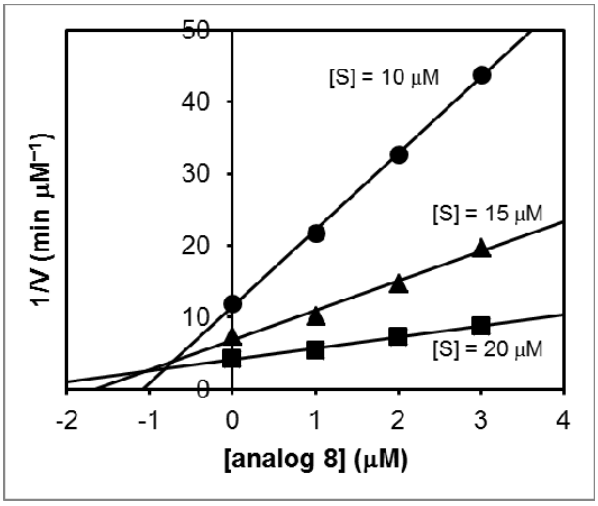


Figure S4. Dixon plot for TofI with varying concentrations of analog **7** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

3.2.6. Analog 8



Dixon plot for TofI with varying concentrations of analog **8** and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C_8-ACP. Each point represents the average of triplicate runs.

4. Structure-activity relationships of analog 4

We selected **4** as a promising lead compound for a strong TofI inhibitor, and attempted to identify the potential structural motifs within **4** that were responsible for the observed TofI inhibition. We dissected **4** into three substructures: a side chain, sulfide, and ribose, and designed thirteen compounds in which these substructures were changed. The side chain-changed analogs, *N*-acylaminopropane-type **20**, **23**, **26**, and **29** having acetyl and acyl chains mimicking those of natural AHLs and synthetic antagonists, alky-type **31** having the same chain length as that of octanoyl-SAM, and arylethyl-type **33** and **35** having different side chains from **4** (Figure S5a), were synthesized based on the methods of **4**. To mimic the 5′-methylsulfur motif of octanoyl-SAM, sulfoxide-type **36** and aza-desthio (N-Me)-type **40** (Figure S5b) were synthesized. Ribose-modified analogs **47**, **50**, **56**, and **59** (Figure S5c) were also synthesized. The analogs obtained were tested for TofI inhibition at concentrations of 100 and 10 μM. Analog **26** inhibited the TofI reaction at a slightly lower level than that of **4**. However, analogs **20**, **23**, **29**, **31**, **33**, and **35** exhibited weak or no inhibition. Thus, the inhibition of TofI was highly dependent on the length of the acyl chain and presence of an amide moiety at the appropriate position on the inhibitors. The inhibitory activities of **36** and **40** were lower than that of **4**, which indicated that mimicking the methylsulfur moiety of acyl-SAM was not needed for TofI inhibition. The inhibitory activities of ribose-modified analogs **47**, **50**, **56**, and **59** against TofI were slightly lower than that of **4**. Thus, the ribose unit in acyl-SAM analogs was suggested to be of some importance for inhibitory activity.

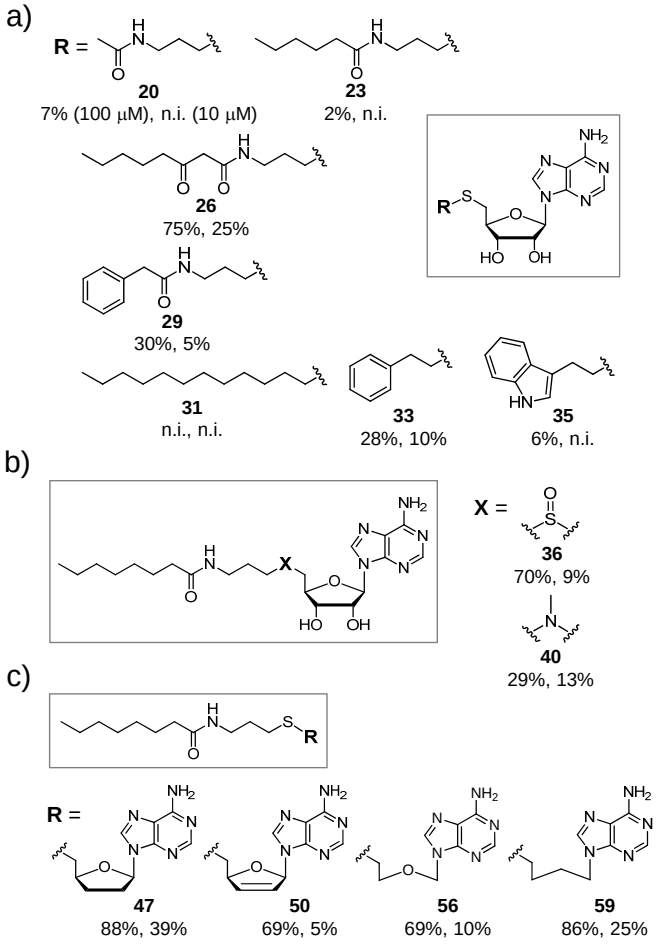


Figure S5. Structures and TofI-inhibition activities of decarboxy-type acyl-SAM analogs. a) Side chain-changed analogs. b) 5′-Methylsulfur motif-changed analogs. c) Ribose-modified analogs. The percentage inhibition was calculated from the ratio of C_8-HSL production with and without an inhibitor; n.i. = no inhibition (*i.e.*, <5% inhibition). Data represent the average of triplicate determinations.

5. Docking simulation

The docking simulation of acyl-SAM analogs to TofI was conducted using AutoDock VINA software (ver. 1.1.2). The crystal structure of the J8-C8/MTA/TofI ternary complex (3P2H) was downloaded from the PDB website. The ligands, J8-C8 and MTA, were removed from their binding site in the crystal and docking of the acyl-SAM analogs to the binding site was performed. Data were visualized using Discovery Studio Visualizer software (ver. 3.5).

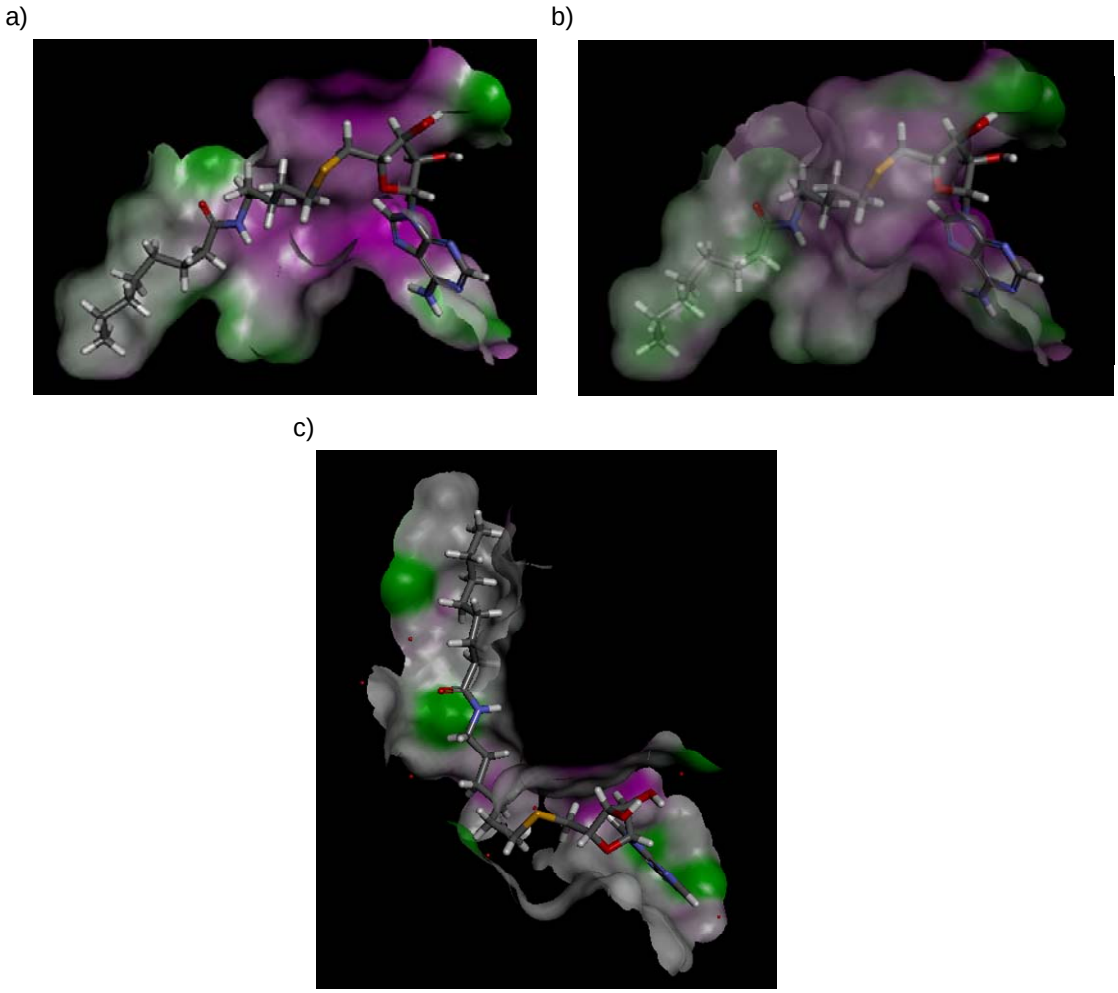


Figure S6. Docking models of analogs **6** and **7** to the TofI enzyme. a) Analog **6**. b) Analog **6**, different view. c) Analog **7**. The binding site of acyl-SAM analogs in TofI is shown as a surface model.

6. Pull-down assays

Whole cell protein extracts of *E. coli* were prepared using BugBuster HT Protein Extraction Reagent (Merck Millipore) as recommended by the supplier. BmaI1 (His-tag) and YspI (MBP-tag) were prepared as described by Christensen *et al*.[S14] Control, **9**-fixed, and **10**-fixed beads (0.5 mg) were equilibrated with binding buffer (20 mM HEPES−NaOH, pH 7.9, 100 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM EDTA, 10% glycerol, 0.1% NP-40, 1 mM DTT, and 0.2 mM PMSF) and incubated with 200 μl of TofI (0.5 mg/ml) or whole cell protein extracts (*E. coli* expressing TofI, 1.0 mg/ml; *E. coli* expressing BmaI1, 1.2 mg/ml; *E. coli* expressing YspI, 1.0 mg/ml) at 4°C for 4 h (purified enzyme) or 12 h (whole cell protein extracts) with gentle rotation. After washing with binding buffer three times, the beads were incubated with 30 μl of **6** solution (250 μM, in binding buffer) for 5 min. After magnetic separation, the eluates were transferred to new tubes (ligand elution samples). SDS sample buffer was added to the remaining beads, and then the suspensions were heated at 98°C for 5 min (boiling elution samples). Both ligand elution and boiling elution samples were subjected to SDS-PAGE and then silver stained. For the competitive binding assay, ligand-fixed beads (0.25 mg) were incubated with the whole cell protein extracts of *E. coli* (1.0 mg/ml, 200 μl) containing 0.2, 0.5, or 1.0 mM SAM.

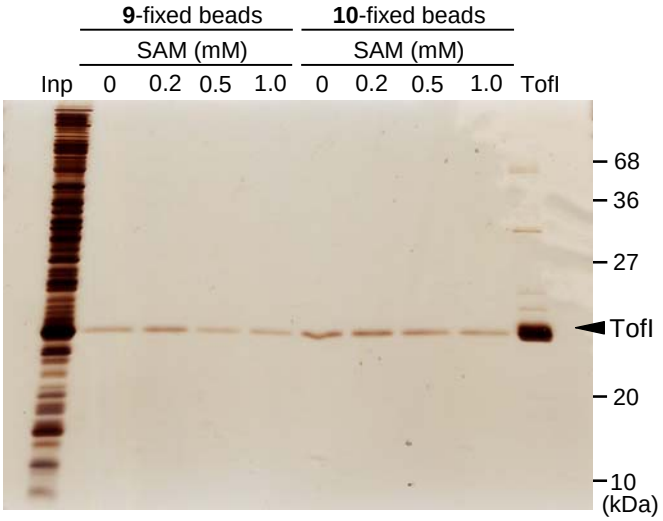


Figure S7. Inhibition of the binding of TofI to ligand-fixed beads by SAM. **9**- and **10**-fixed beads were incubated with the whole cell protein extracts containing 0 (control), 0.2, 0.5, or 1.0 mM SAM. Proteins eluted with buffer containing **6** (ligand elution) were analyzed by SDS-PAGE (15% acrylamide) followed by silver staining. Inp: whole cell protein extracts of *E. coli*.

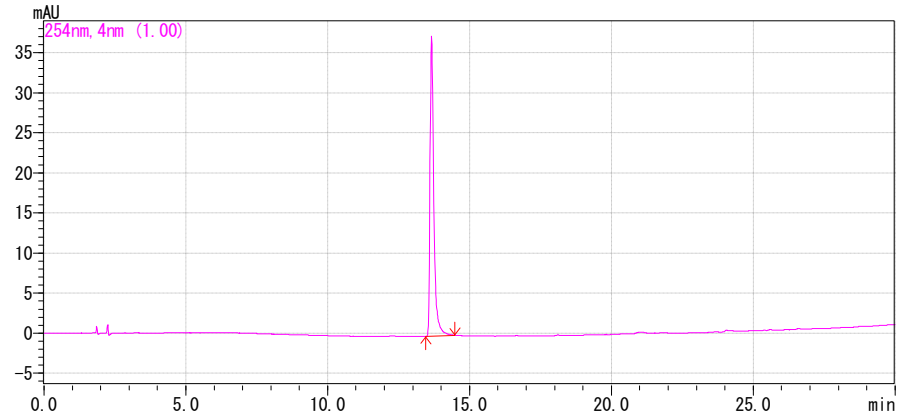
7. HPLC chromatograms of acyl-SAM analogs

7.1. HPLC conditions

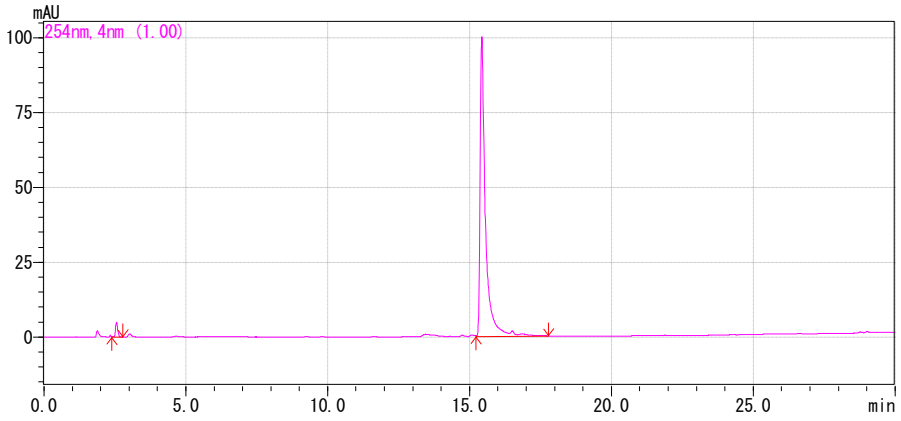
Purity checks of acyl-SAM analogs were performed using the following parameters: column, Inertsil ODS-3 column (150 mm × 4.6 mm i.d., 5 μm, GL Sciences); flow rate, 1 ml/min; eluent system, 10−90% acetonitrile in water (linear gradient in 30 min); chromatogram, Abs 254 nm.

7.2. HPLC chromatograms

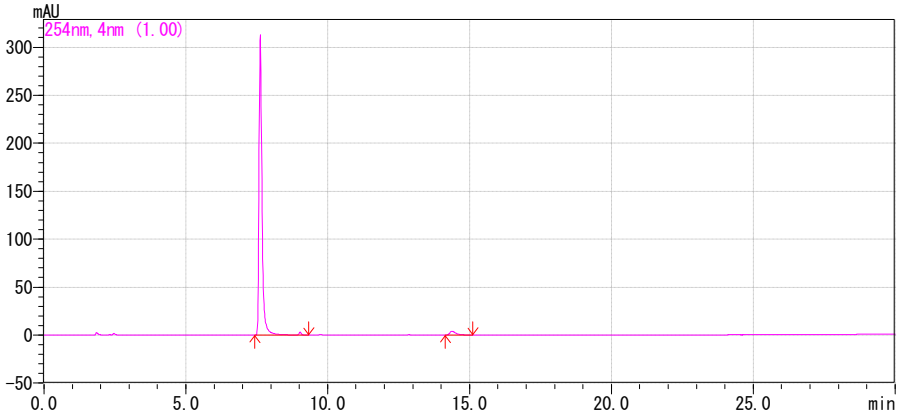
Analog 1



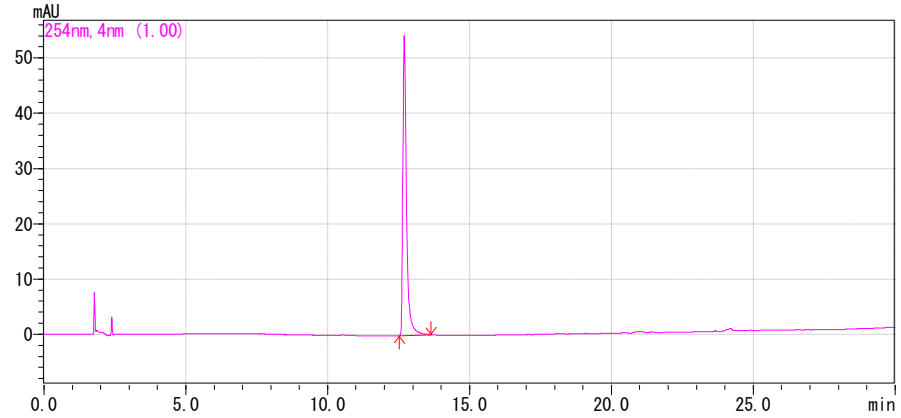
Analog 4



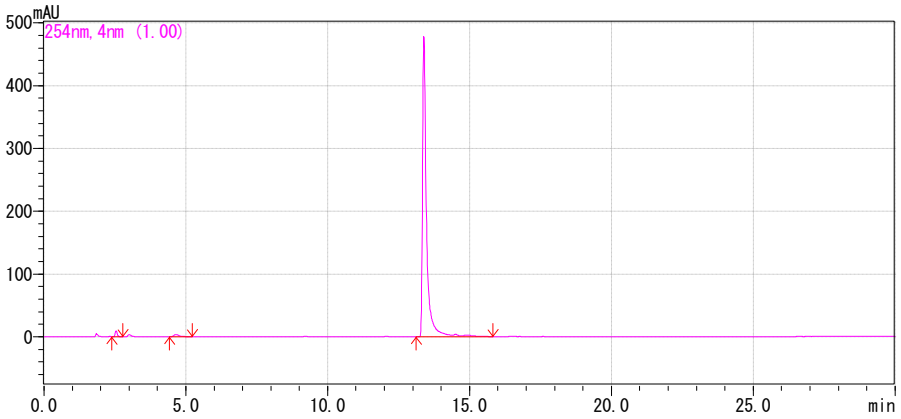
Analog 20



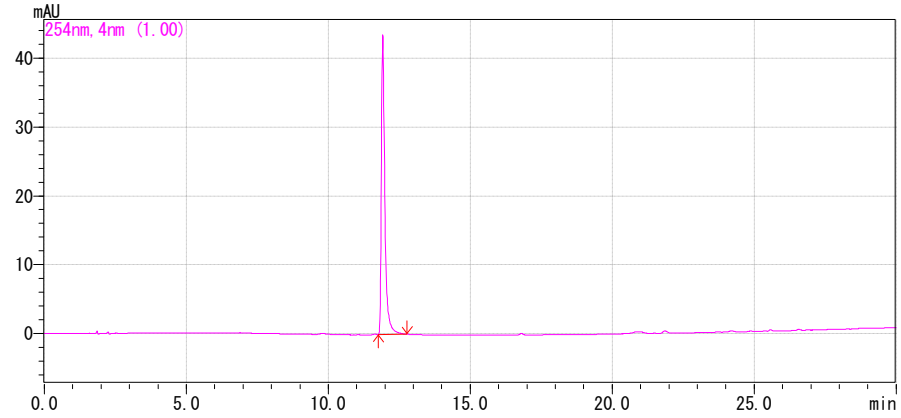
Analog 23



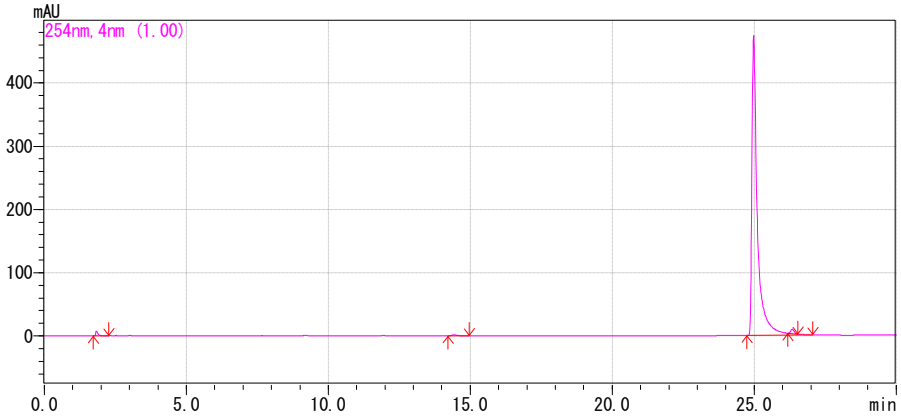
Analog 26



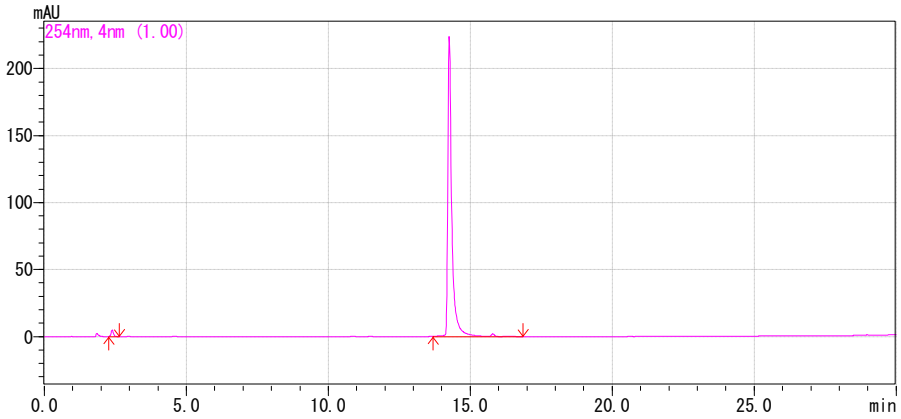
Analog 29



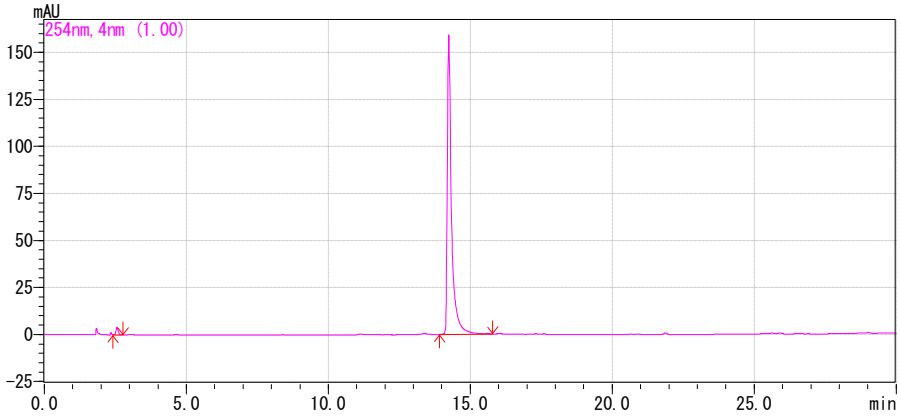
Analog 31



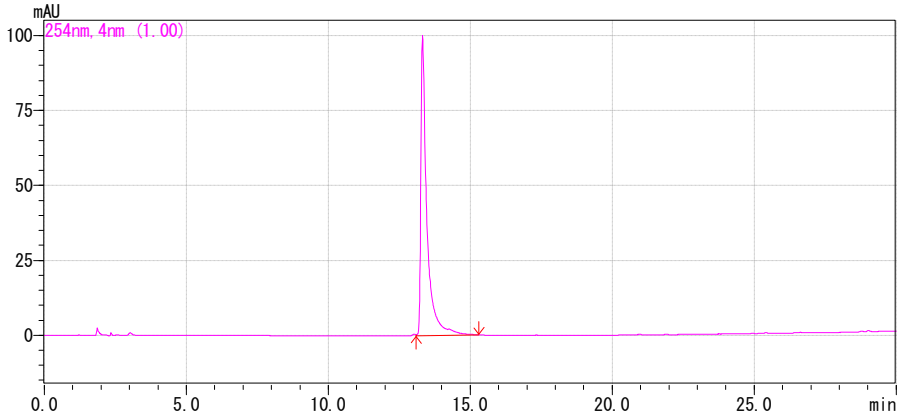
Analog 33



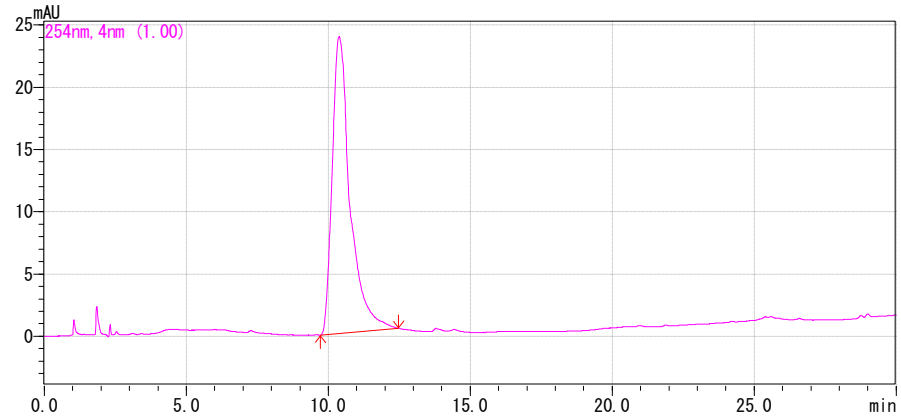
Analog 35



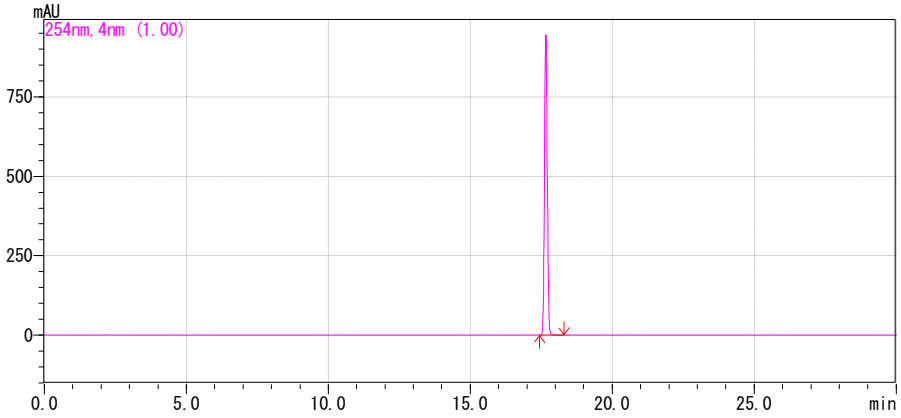
Analog 36



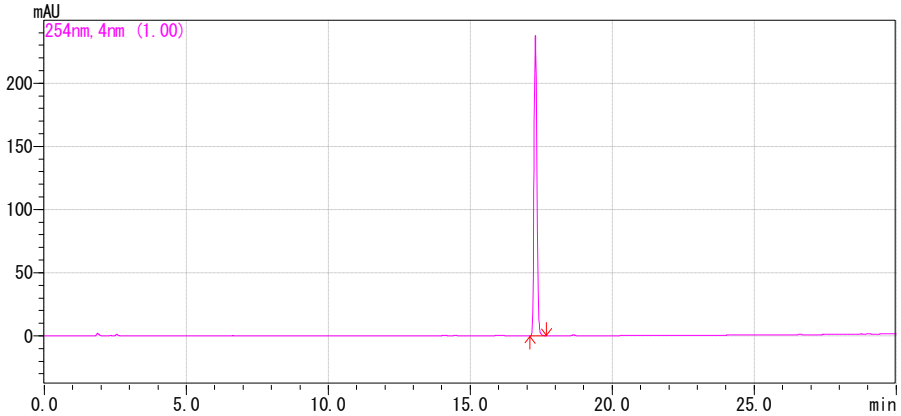
Analog 40



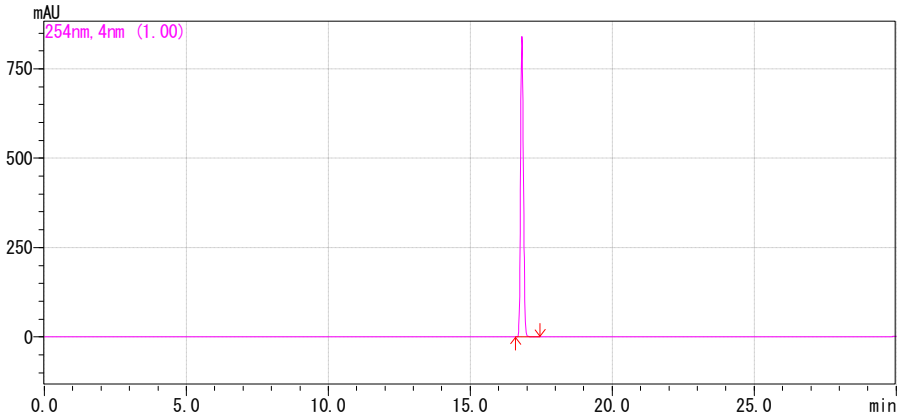
Analog 47



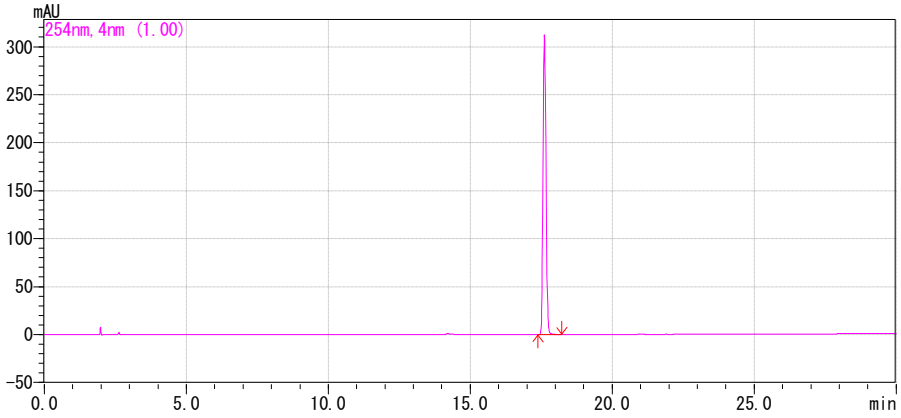
Analog 50



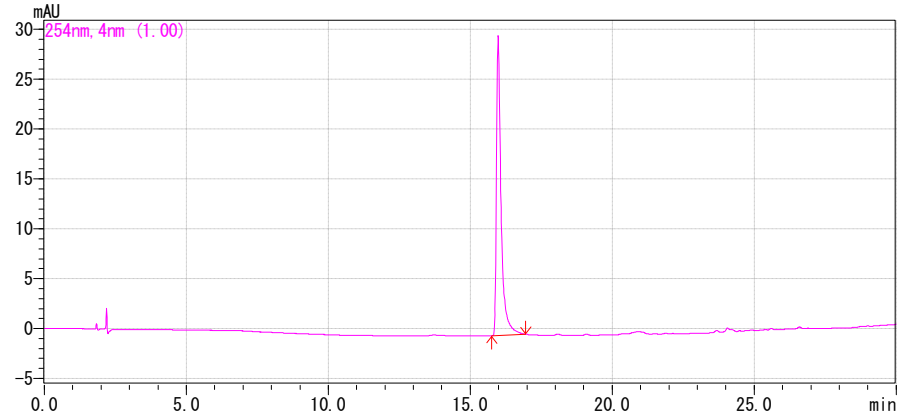
Analog 56



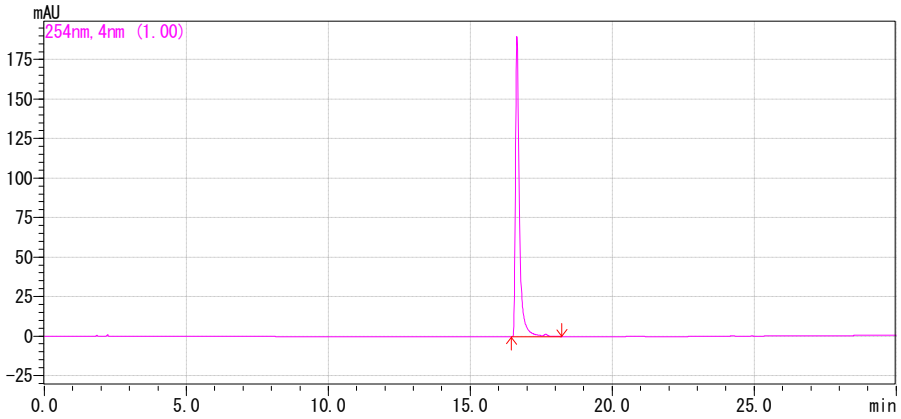
Analog 59



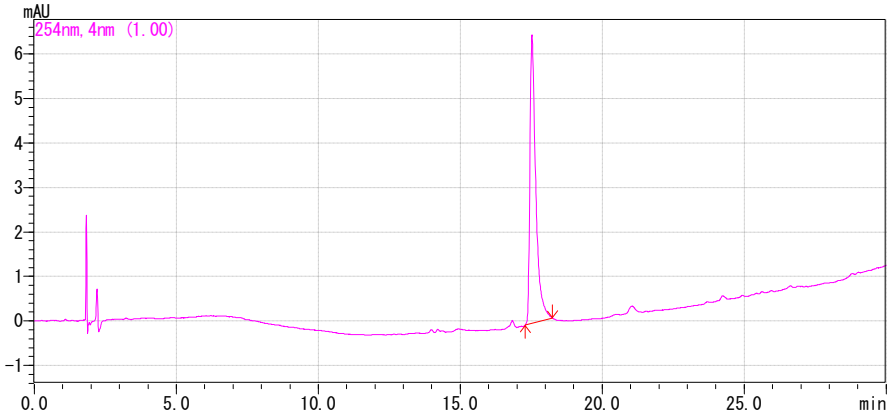
Analog 5



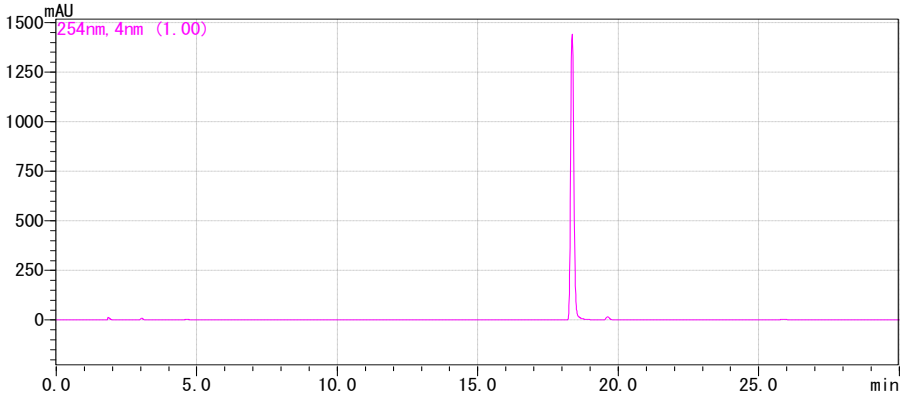
Analog 6



Analog 7

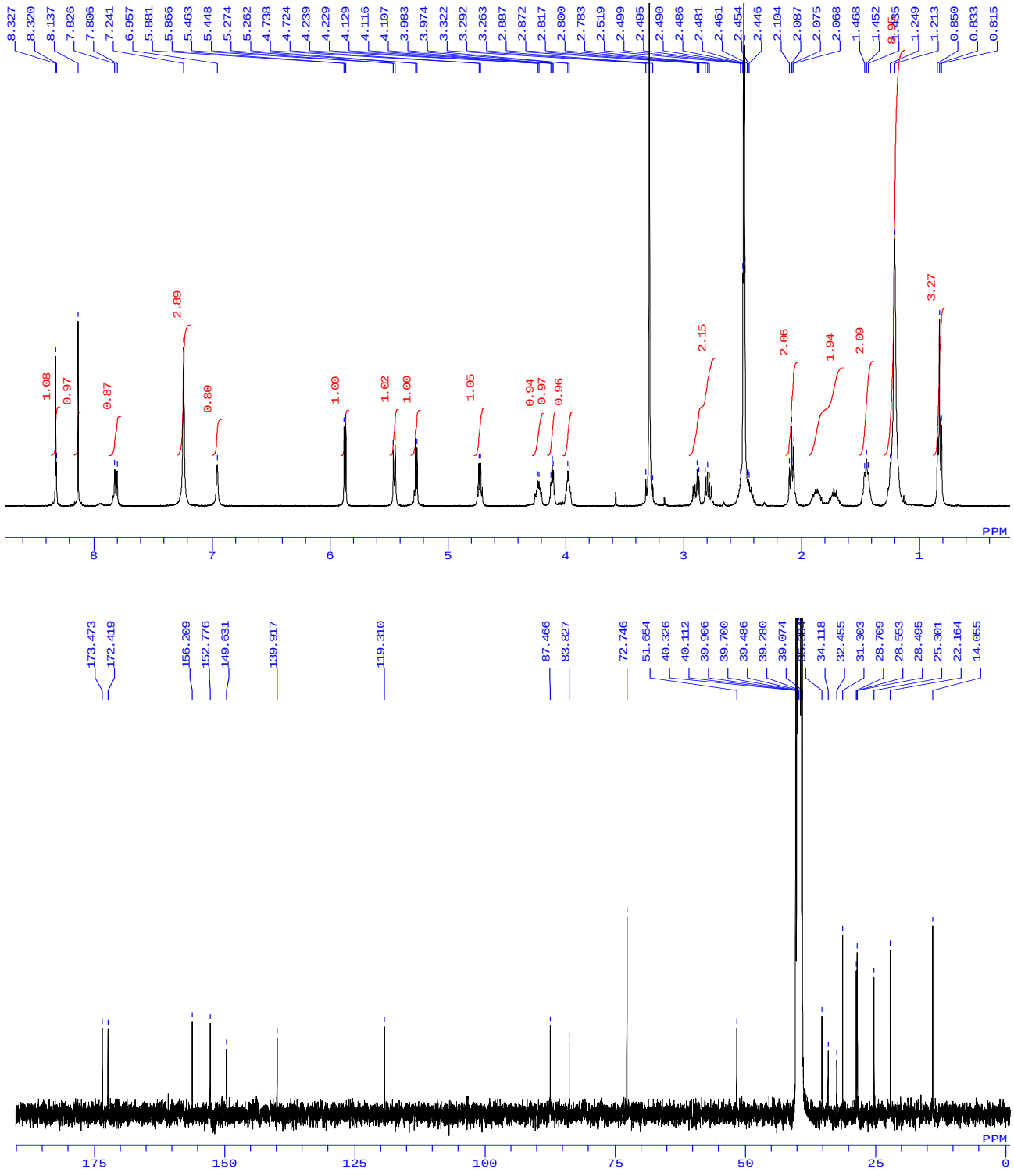


Analog 8

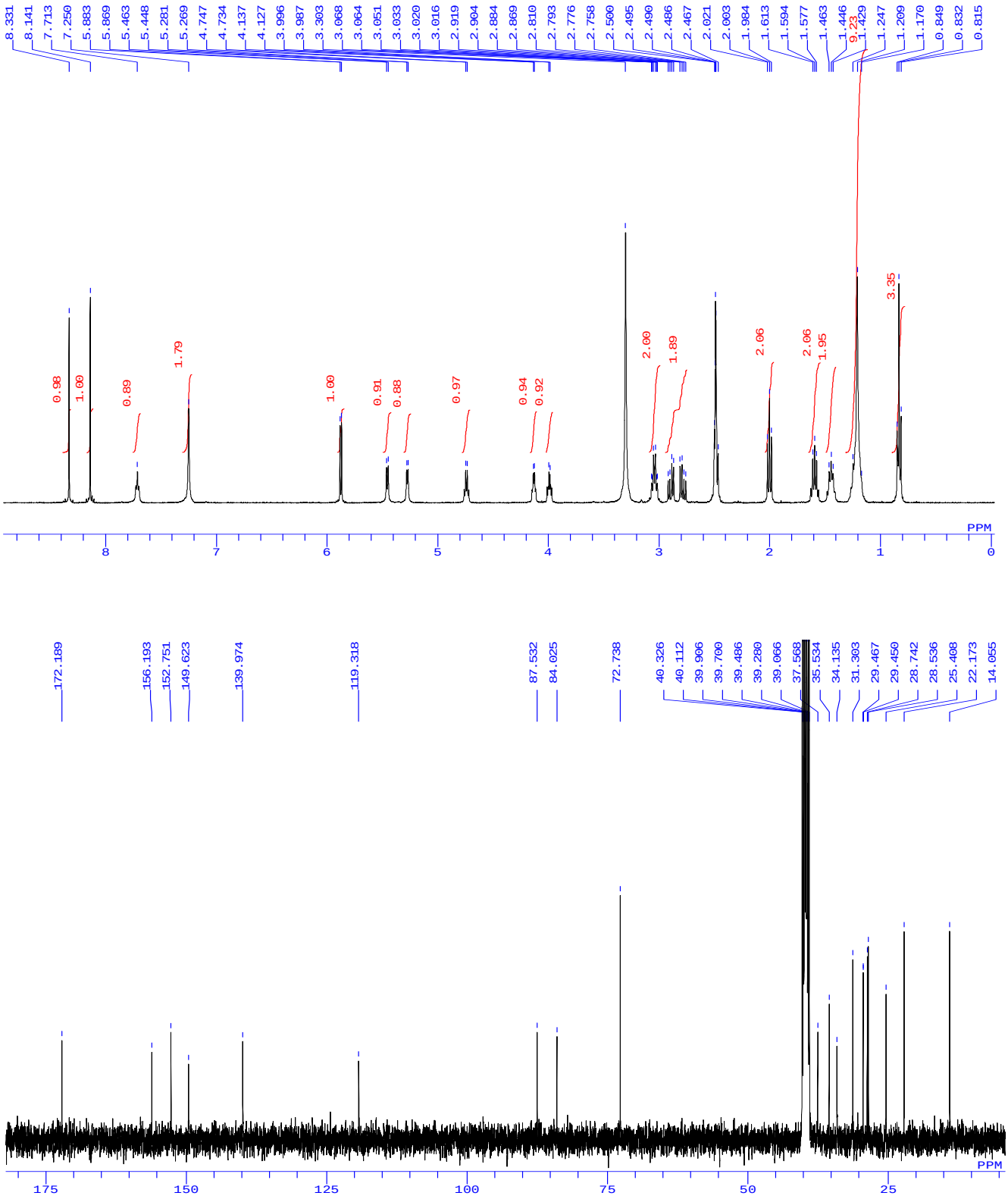


8. ^{1}H and ^{13}C NMR spectra of acyl-SAM analogs

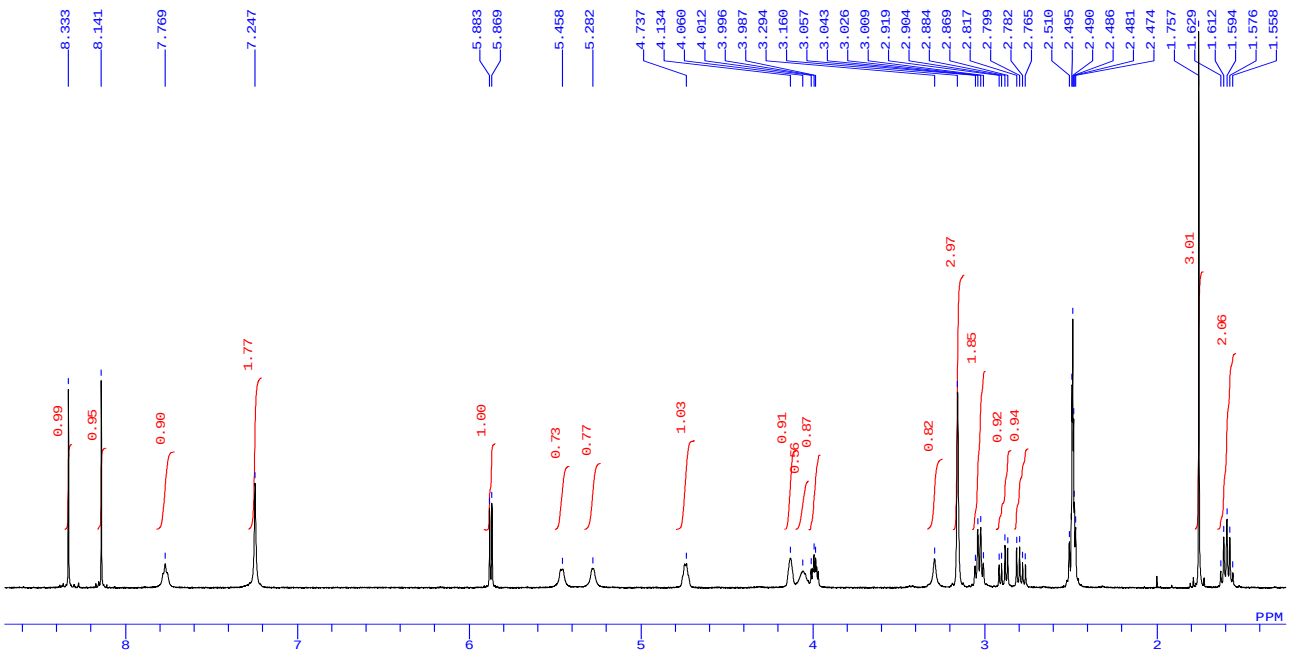
Analog 1

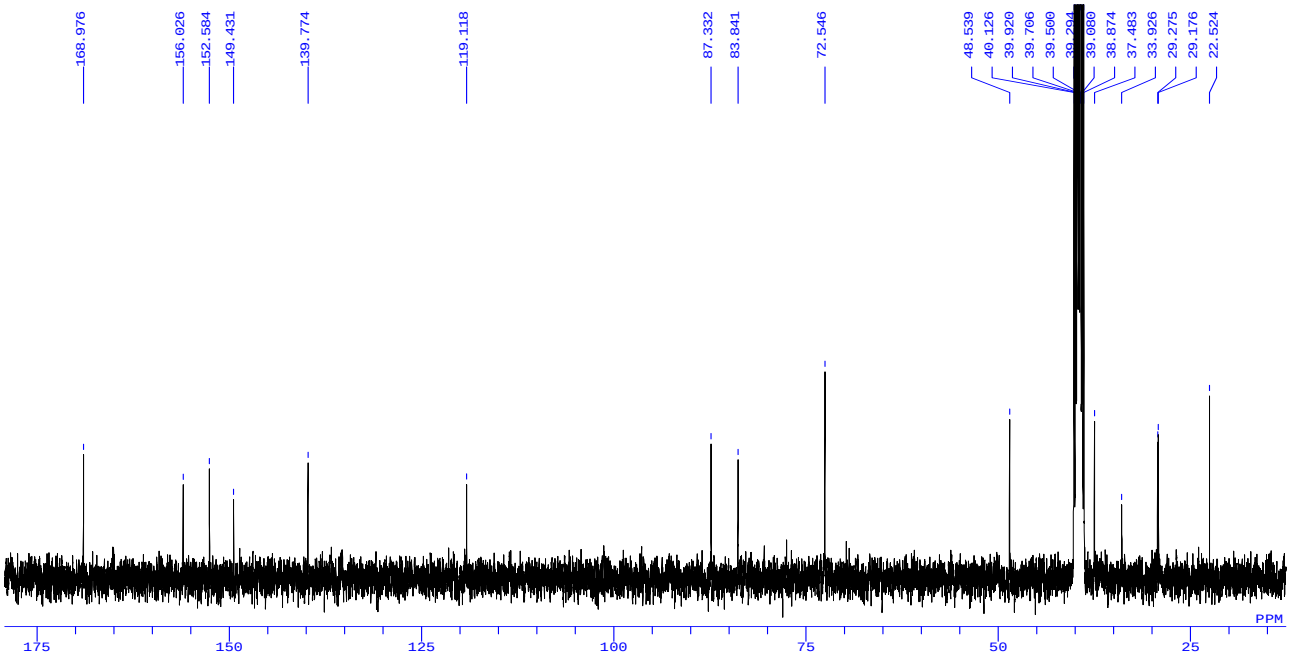


Analog 4

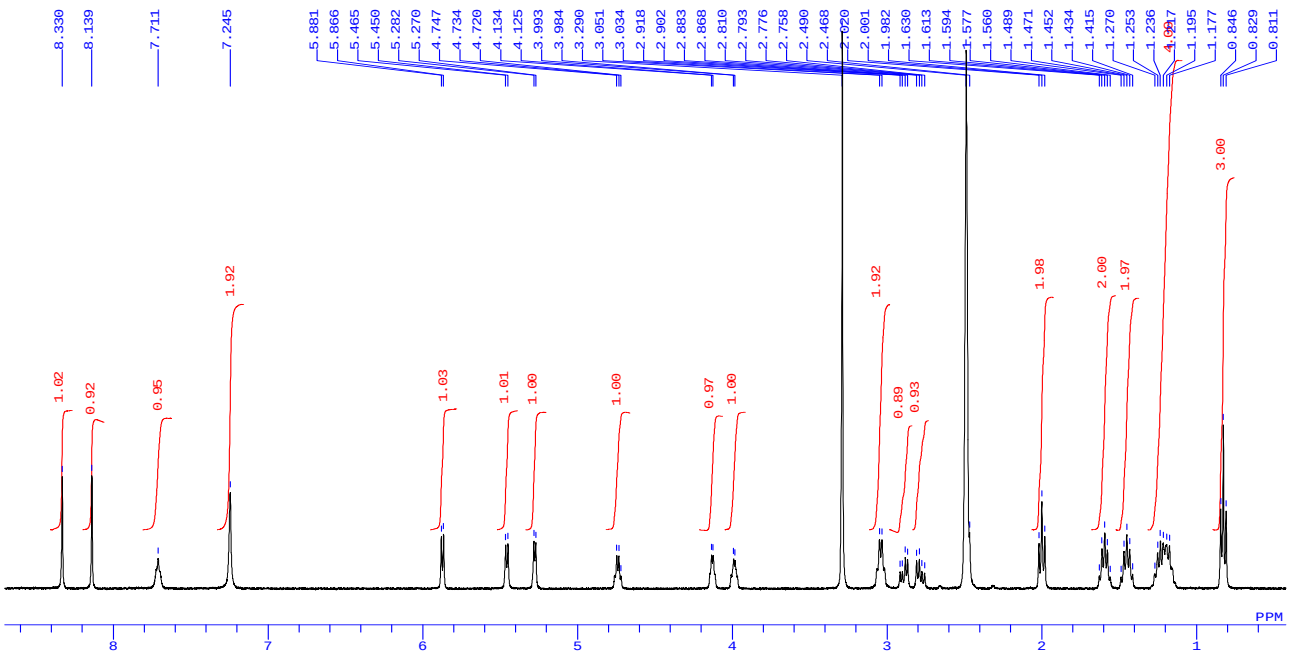

8.331
8.141
7.713
7.250
5.883
5.869
5.463
5.448
5.281
5.269
4.747
4.734
4.137
4.127
3.996
3.987
3.303
3.068
3.064
3.051
3.033
3.020
3.016
2.919
2.904
2.884
2.869
2.810
2.793
2.776
2.758
2.500
2.495
2.490
2.486
2.467
2.021
2.003
1.984
1.613
1.594
1.577
1.463
1.446
9.23
1.429
1.247
1.209
1.170
0.849
0.832
0.815
0.98
1.00
0.89
1.79
1.00
0.91
0.88
0.97
0.94
0.92
2.00
1.89
2.06
2.06
1.95
3.35
PPM
8
7
6
5
4
3
2
1
0
172.189
156.193
152.751
149.623
139.974
119.318
87.532
84.025
72.738
40.326
40.112
39.906
39.700
39.486
39.280
39.066
37.568
35.534
34.135
31.303
29.467
29.450
28.742
28.536
25.408
22.173
14.055
PPM
175
150
125
100
75
50
25

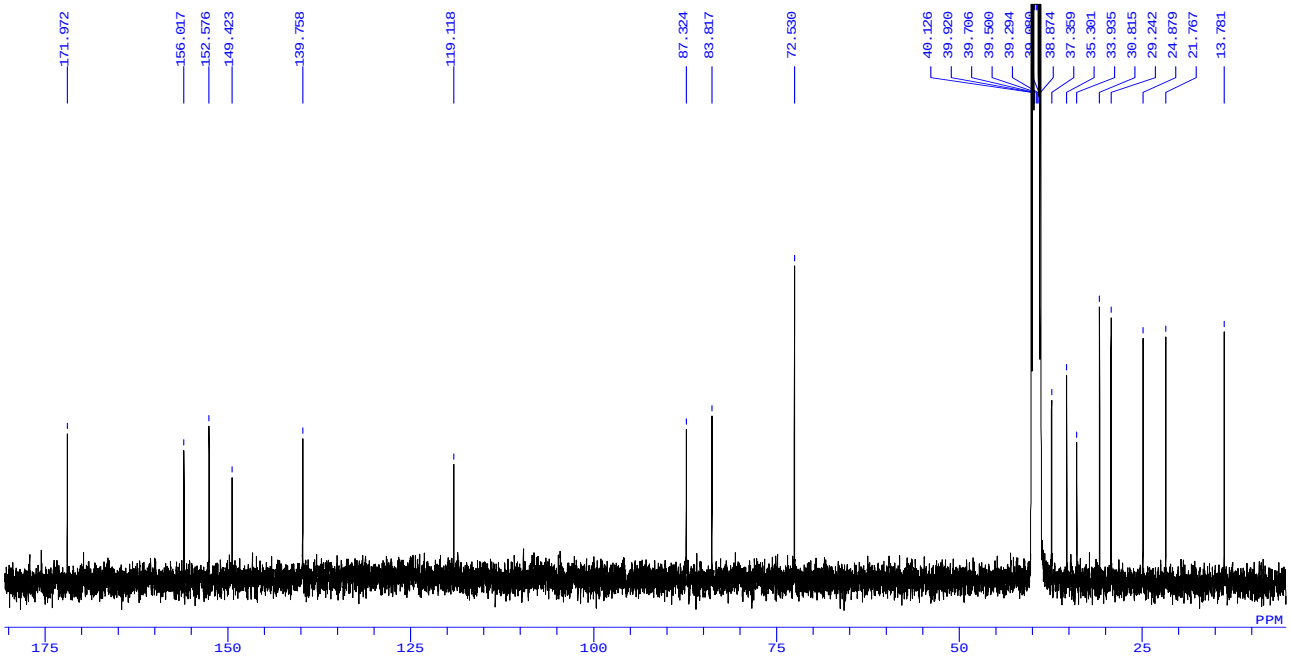
Analog 20


8.333
8.141
7.769
7.247
5.883
5.869
5.458
5.282
4.737
4.134
4.060
4.012
3.996
3.987
3.294
3.160
3.057
3.043
3.026
3.009
2.919
2.904
2.884
2.869
2.817
2.799
2.782
2.765
2.510
2.495
2.490
2.486
2.481
2.474
1.757
1.629
1.612
1.594
1.576
1.558
0.99
0.95
0.90
1.77
1.00
0.73
0.77
1.03
0.91
0.86
0.87
0.82
2.97
1.85
0.92
0.94
3.01
2.06
PPM
8
7
6
5
4
3
2

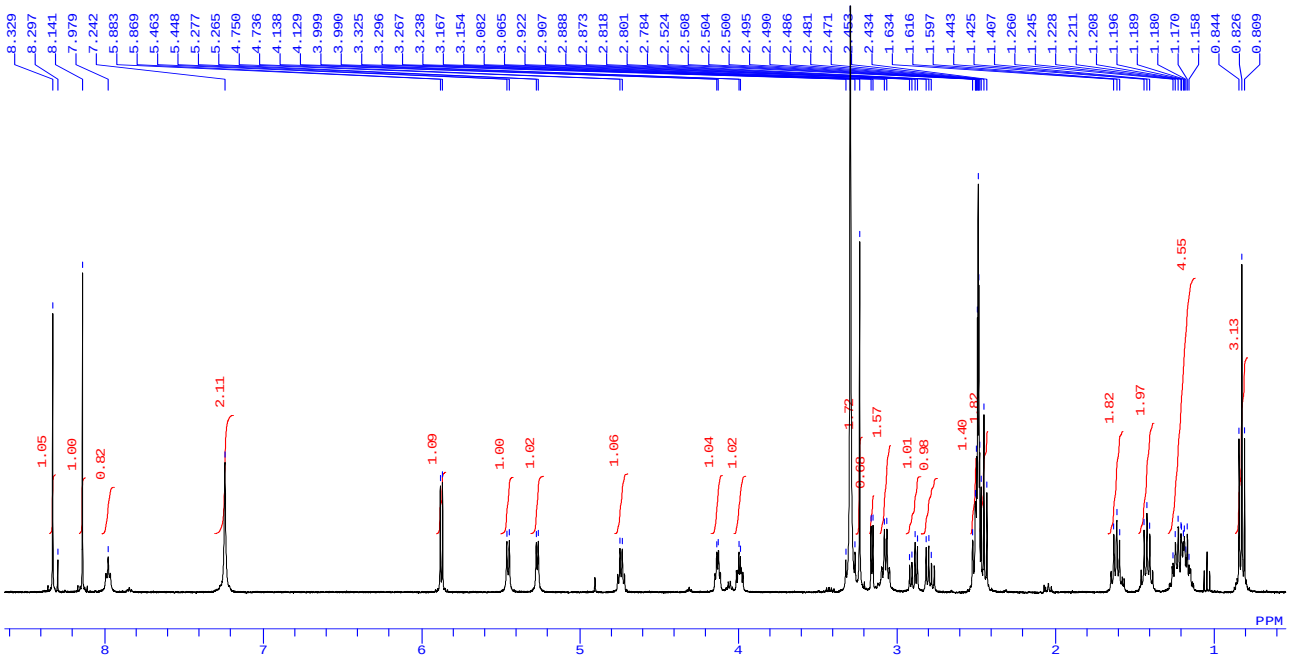

168.976
156.026
152.584
149.431
139.774
119.118
87.332
83.841
72.546
48.539
40.126
39.920
39.706
39.500
39.294
39.080
38.874
37.483
33.926
29.275
29.176
22.524
PPM
175
150
125
100
75
50
25

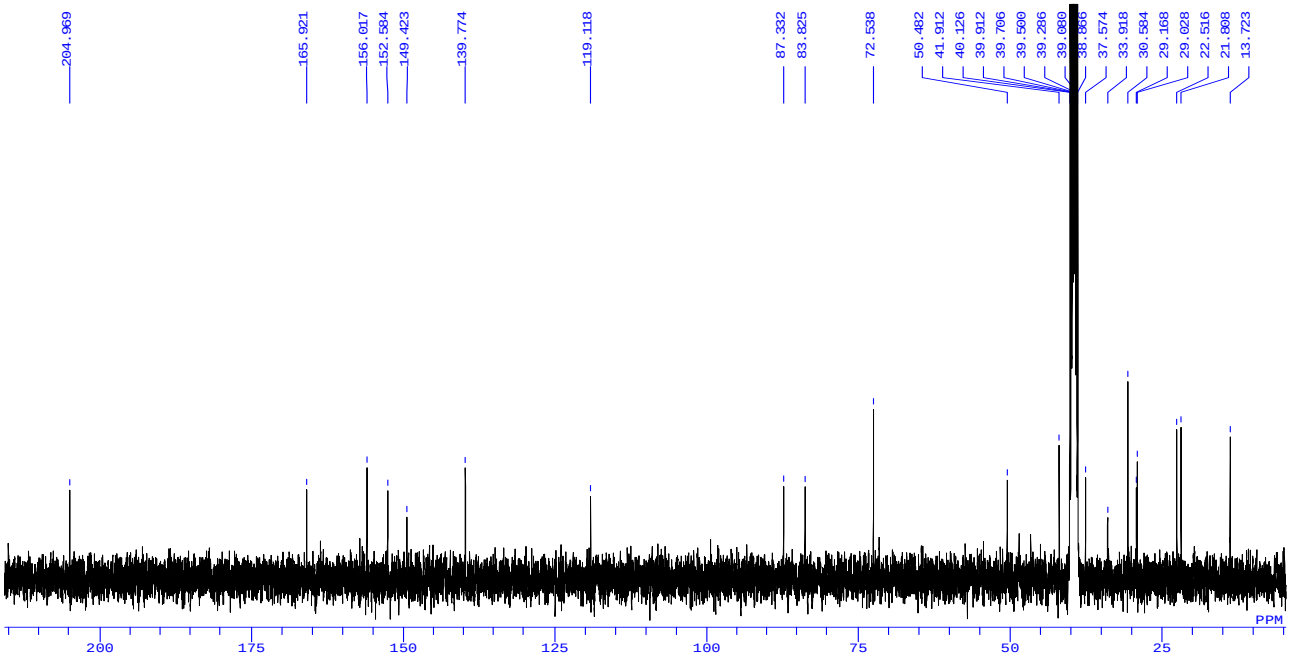
Analog 23


8.330
8.139
7.711
7.245
5.881
5.866
5.465
5.450
5.282
5.270
4.747
4.734
4.720
4.134
4.125
3.993
3.984
3.290
3.051
3.034
2.918
2.902
2.883
2.868
2.810
2.793
2.776
2.758
2.490
2.468
2.020
2.001
1.982
1.630
1.613
1.594
1.577
1.560
1.489
1.471
1.452
1.434
1.415
1.270
1.253
1.236
4.000
1.195
1.177
0.846
0.829
0.811
1.02
0.92
0.95
1.92
1.03
1.01
1.00
1.00
0.97
1.00
1.92
0.89
0.93
1.98
2.00
1.97
3.00
PPM
8
7
6
5
4
3
2
1

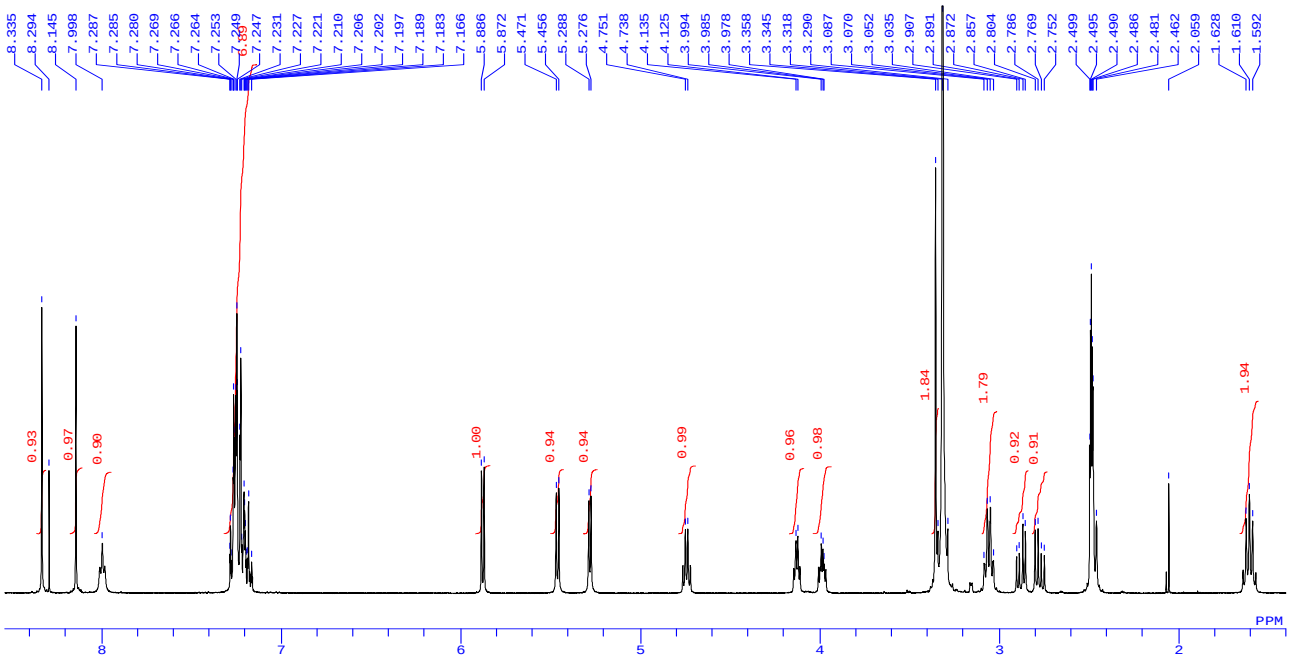

171.972
156.017
152.576
149.423
139.758
119.118
87.324
83.817
72.530
40.126
39.920
39.706
39.500
39.294
38.874
37.359
35.301
33.935
30.815
29.242
24.879
21.767
13.781
PPM
175
150
125
100
75
50
25

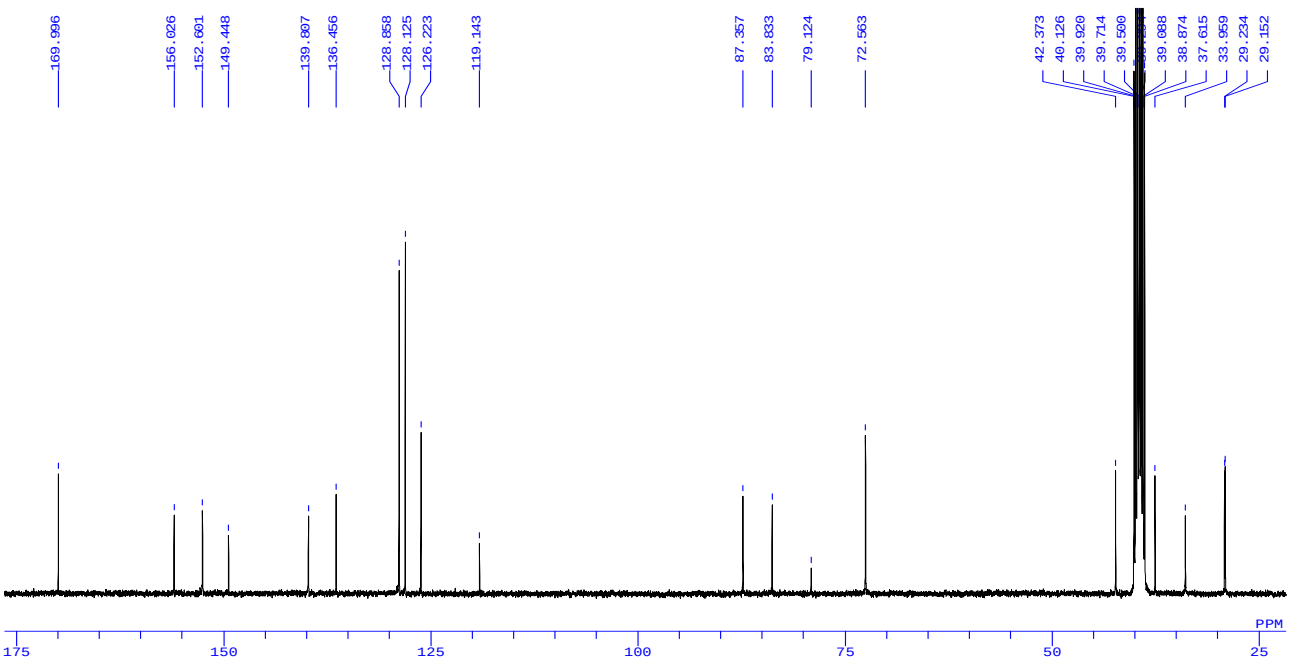
Analog 26


8.329
8.297
8.141
7.979
7.242
5.883
5.869
5.463
5.448
5.277
5.265
4.750
4.736
4.138
4.129
3.999
3.990
3.325
3.296
3.267
3.238
3.167
3.154
3.082
3.065
2.922
2.907
2.888
2.873
2.818
2.801
2.784
2.524
2.508
2.504
2.500
2.495
2.490
2.486
2.481
2.471
2.453
2.434
1.634
1.616
1.597
1.443
1.425
1.407
1.260
1.245
1.228
1.211
1.208
1.196
1.189
1.180
1.170
1.158
0.844
0.826
0.809
1.05
1.00
0.82
2.11
1.09
1.00
1.02
1.06
1.04
1.02
1.72
0.66
1.57
1.01
0.98
1.40
1.82
1.82
1.97
4.55
3.13
PPM
8
7
6
5
4
3
2
1

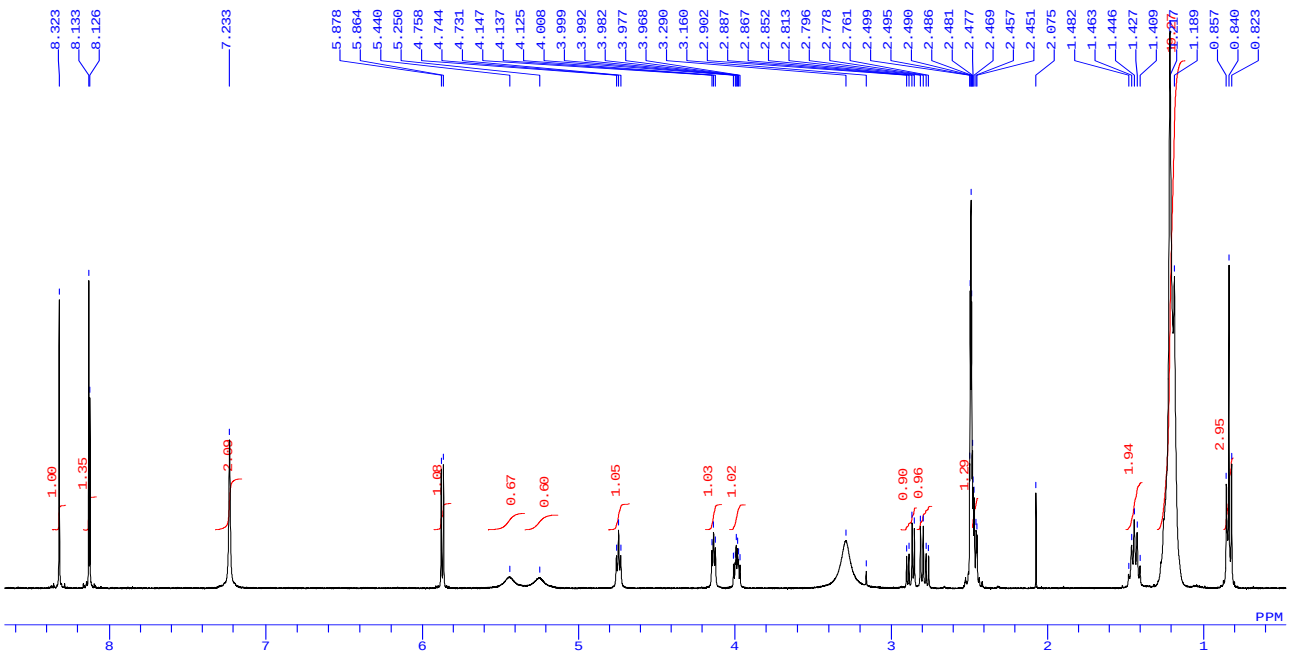

204.969
165.921
156.017
152.584
149.423
139.774
119.118
87.332
83.825
72.538
50.482
41.912
40.126
39.912
39.796
39.560
39.296
39.080
38.866
37.574
33.918
30.584
29.168
29.028
22.516
21.808
13.723
PPM
200
175
150
125
100
75
50
25

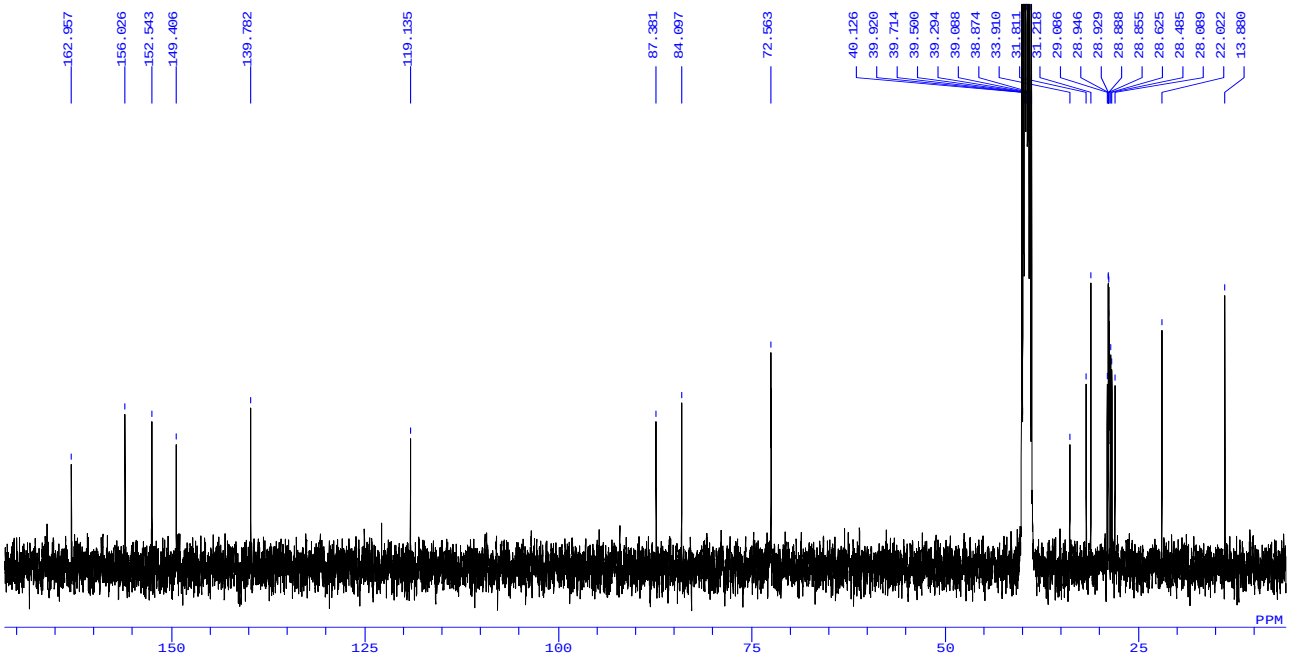
Analog 29


8.335
8.294
8.145
7.998
7.287
7.285
7.280
7.269
7.266
7.264
7.253
7.249
7.247
7.231
7.227
7.221
7.210
7.206
7.202
7.197
7.189
7.183
7.166
5.886
5.872
5.471
5.456
5.288
5.276
4.751
4.738
4.135
4.125
3.994
3.985
3.978
3.358
3.345
3.318
3.290
3.087
3.070
3.052
3.035
2.907
2.891
2.872
2.857
2.804
2.786
2.769
2.752
2.499
2.495
2.490
2.486
2.481
2.462
2.059
1.628
1.610
1.592
0.93
0.97
0.90
1.00
0.94
0.94
0.99
0.96
0.98
1.84
1.79
0.92
0.91
1.94
PPM
8
7
6
5
4
3
2

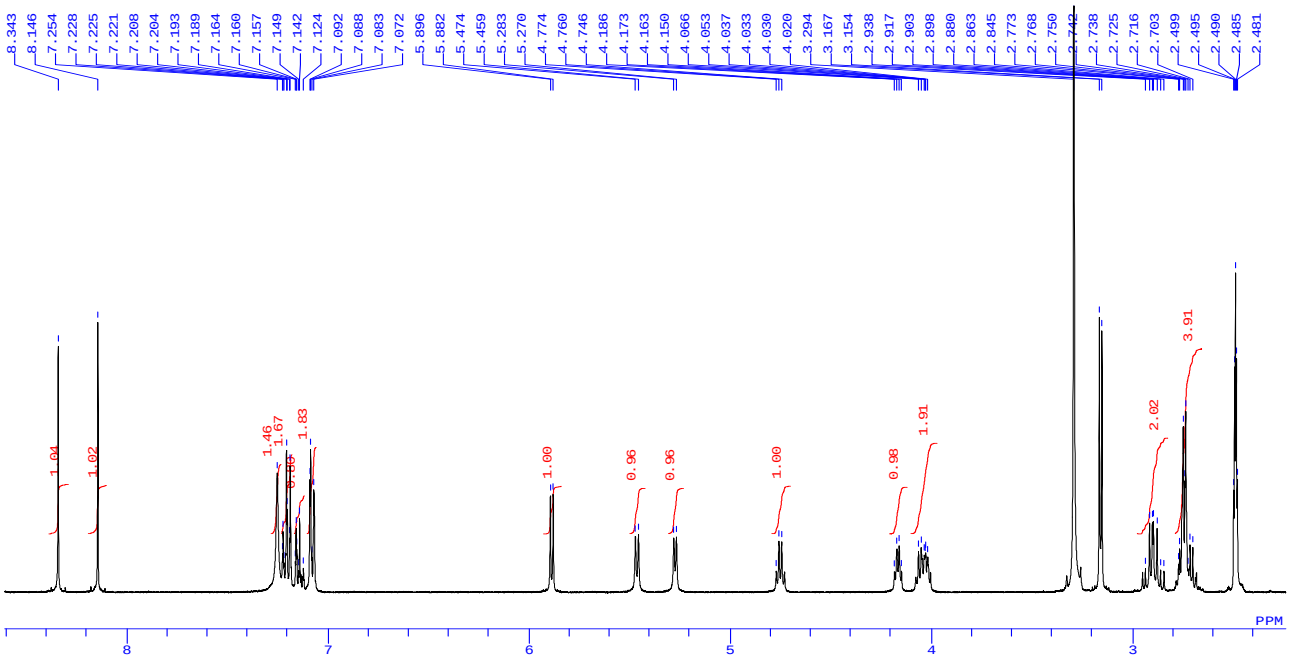

169.996
156.026
152.601
149.448
139.807
136.456
128.858
128.125
126.223
119.143
87.357
83.833
79.124
72.563
42.373
40.126
39.920
39.714
39.500
39.068
38.874
37.615
33.959
29.234
29.152
PPM
175
150
125
100
75
50
25

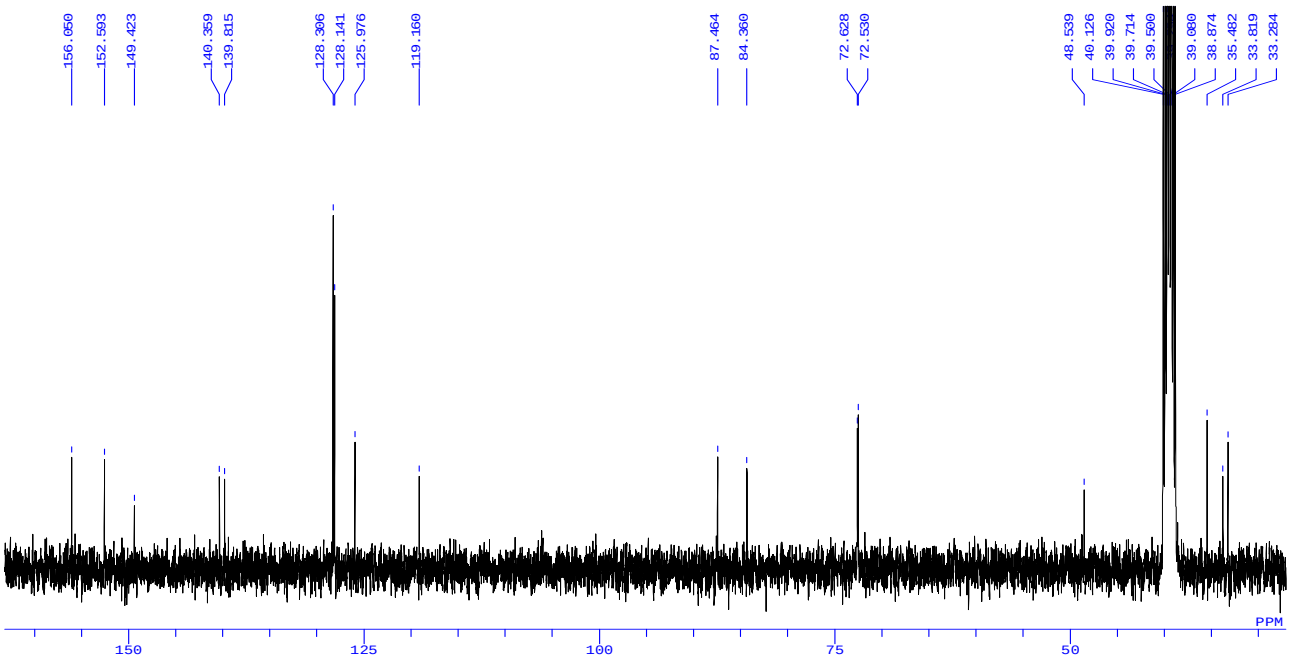
Analog 31


8.323
8.133
8.126
7.233
5.878
5.864
5.440
5.250
4.758
4.744
4.731
4.147
4.137
4.125
4.008
3.999
3.992
3.982
3.977
3.968
3.290
3.160
2.902
2.887
2.867
2.852
2.813
2.796
2.778
2.761
2.499
2.495
2.490
2.486
2.481
2.477
2.469
2.457
2.451
2.075
1.482
1.463
1.446
1.427
1.409
1.189
0.857
0.840
0.823
1.00
1.35
2.00
1.08
0.67
0.60
1.05
1.03
1.02
0.90
0.96
1.29
1.94
2.95
PPM
8
7
6
5
4
3
2
1

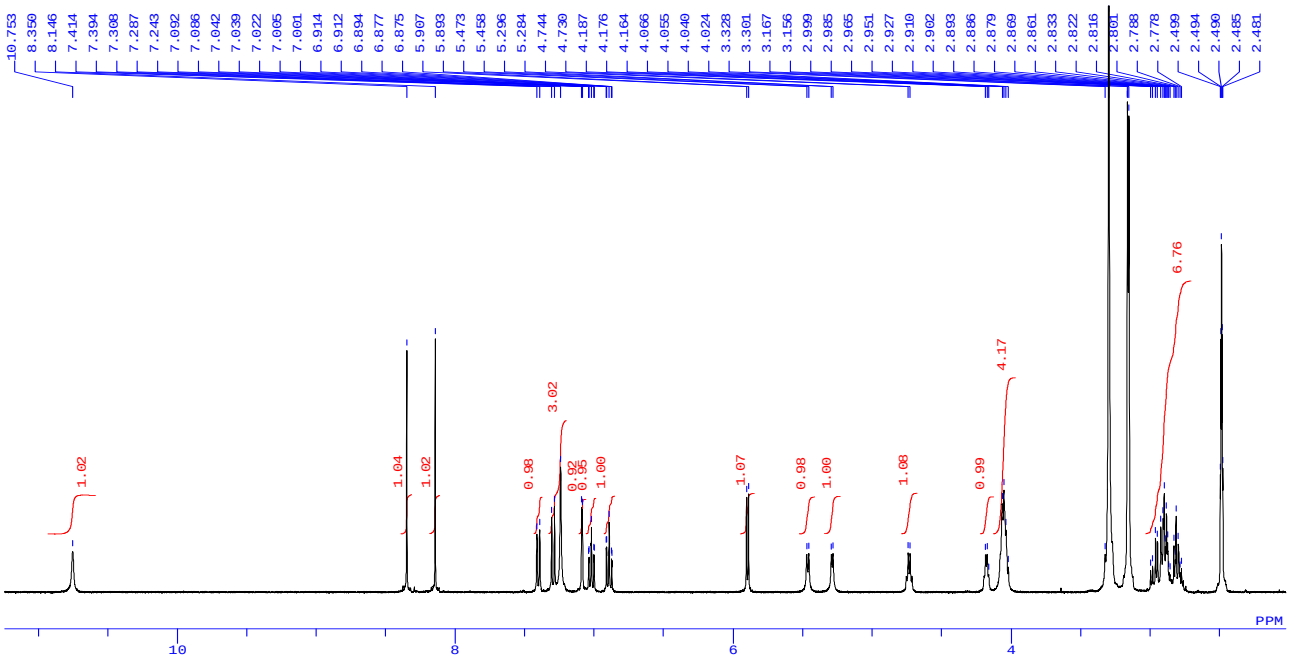

162.957
156.026
152.543
149.406
139.782
119.135
87.381
84.097
72.563
40.126
39.920
39.714
39.500
39.294
39.088
38.874
33.910
31.811
31.218
29.086
28.946
28.929
28.888
28.855
28.625
28.495
28.089
22.022
13.880
PPM
150
125
100
75
50
25

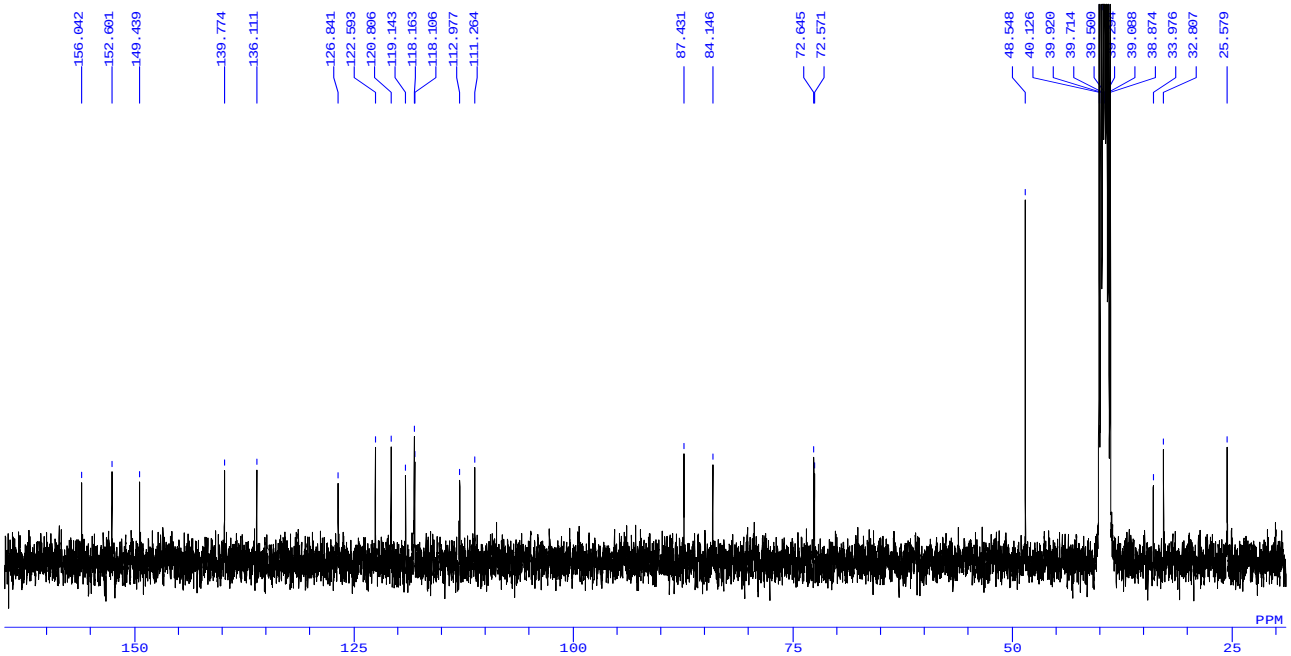
Analog 33


8.343
8.146
7.254
7.228
7.225
7.221
7.208
7.204
7.193
7.189
7.164
7.160
7.157
7.149
7.142
7.124
7.092
7.088
7.083
7.072
5.896
5.882
5.474
5.459
5.283
5.270
4.774
4.760
4.746
4.186
4.173
4.163
4.150
4.066
4.053
4.037
4.033
4.030
4.020
3.294
3.167
3.154
2.938
2.917
2.903
2.898
2.880
2.863
2.845
2.773
2.768
2.750
2.742
2.738
2.725
2.716
2.703
2.499
2.495
2.490
2.485
2.481
1.04
1.02
1.46
1.67
1.83
1.00
0.96
0.96
1.00
0.98
1.91
2.02
3.91
PPM
8
7
6
5
4
3

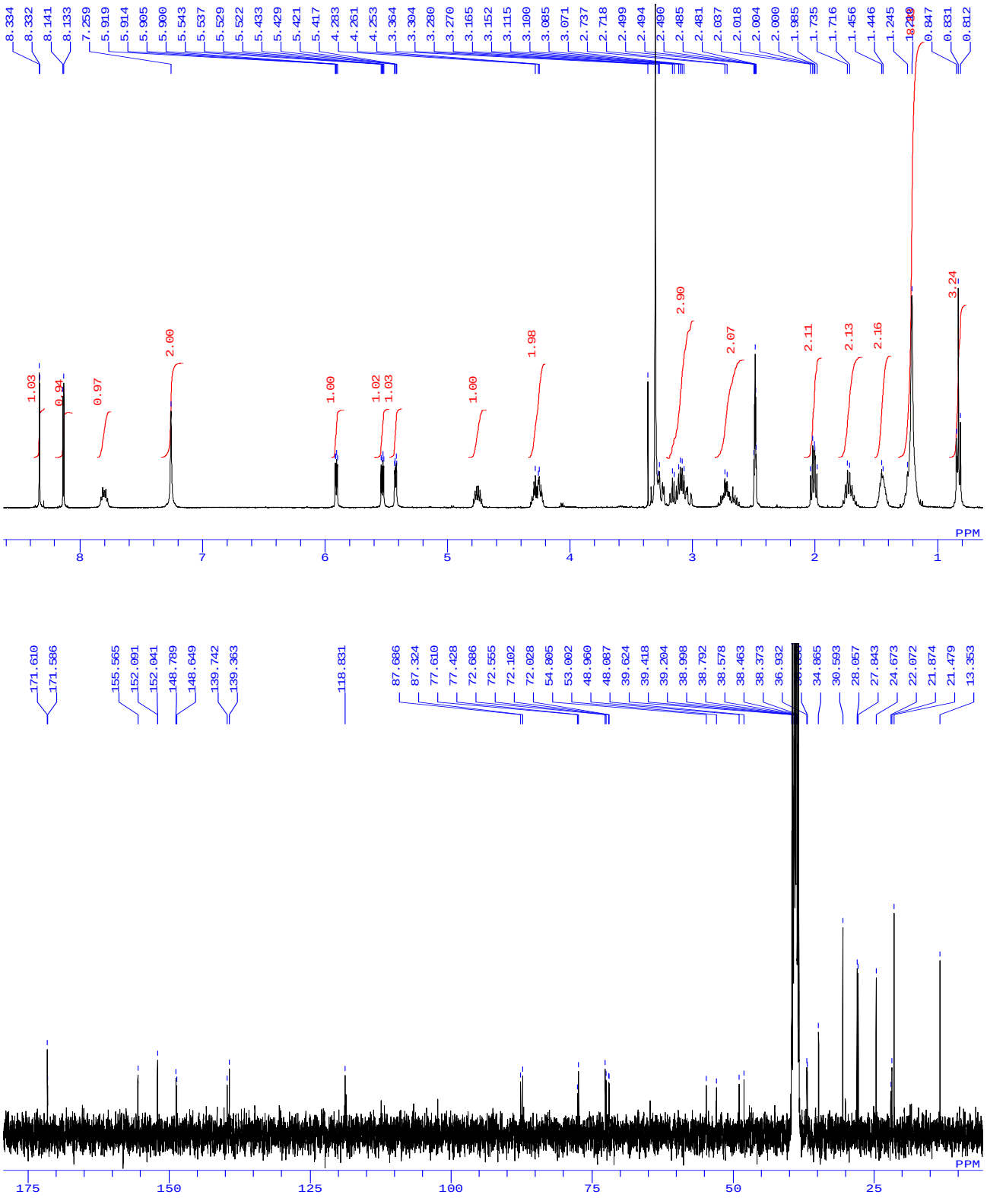

156.650
152.593
149.423
140.359
139.815
128.306
128.141
125.976
119.160
87.464
84.360
72.628
72.530
48.539
40.126
39.920
39.714
39.500
39.080
38.874
35.482
33.819
33.284
PPM
150
125
100
75
50

Analog 35

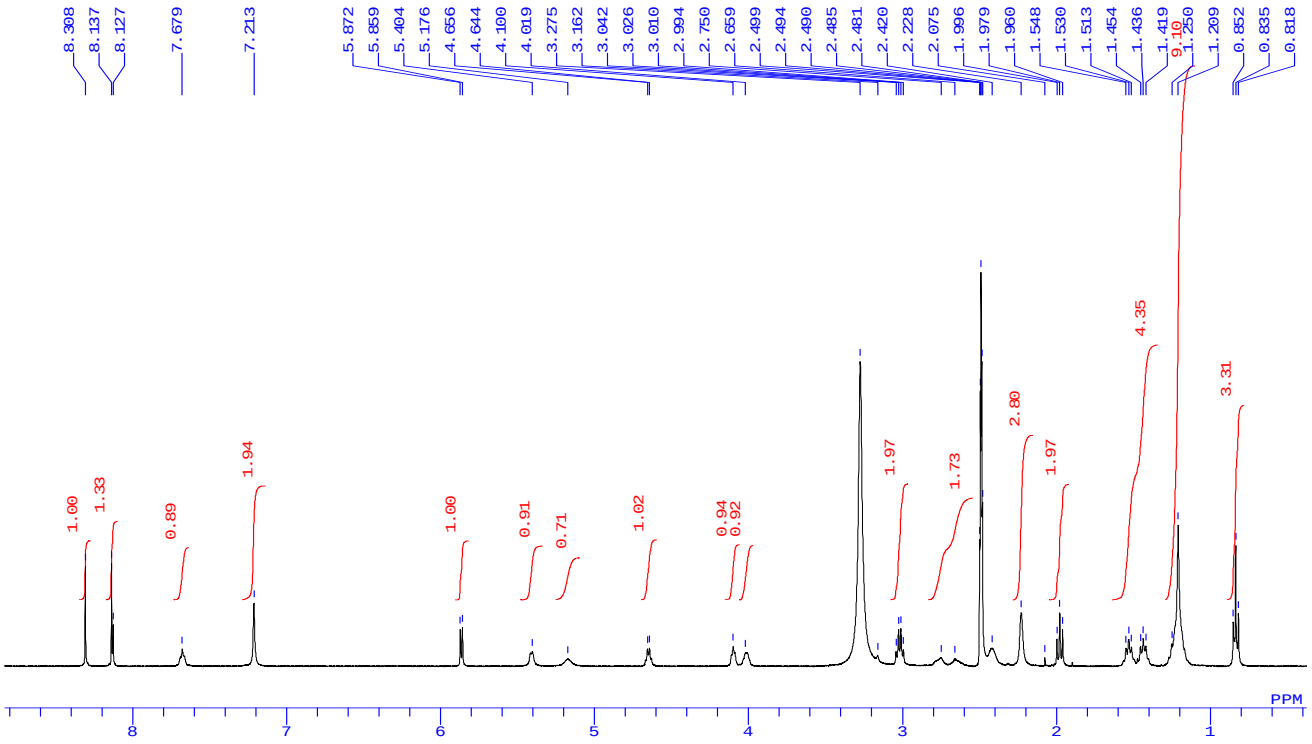

10.753
8.350
8.146
7.414
7.394
7.308
7.287
7.243
7.092
7.086
7.042
7.039
7.022
7.005
7.001
6.914
6.912
6.894
6.877
6.875
5.907
5.893
5.473
5.458
5.296
5.284
4.744
4.730
4.187
4.176
4.164
4.066
4.055
4.040
4.024
3.328
3.301
3.167
3.156
2.999
2.985
2.965
2.951
2.927
2.910
2.902
2.893
2.886
2.879
2.869
2.861
2.833
2.822
2.816
2.801
2.788
2.778
2.499
2.494
2.490
2.485
2.481
1.02
1.04
1.02
0.98
3.02
0.92
0.85
1.00
1.07
0.98
1.00
1.08
0.99
4.17
6.76
PPM
10
8
6
4

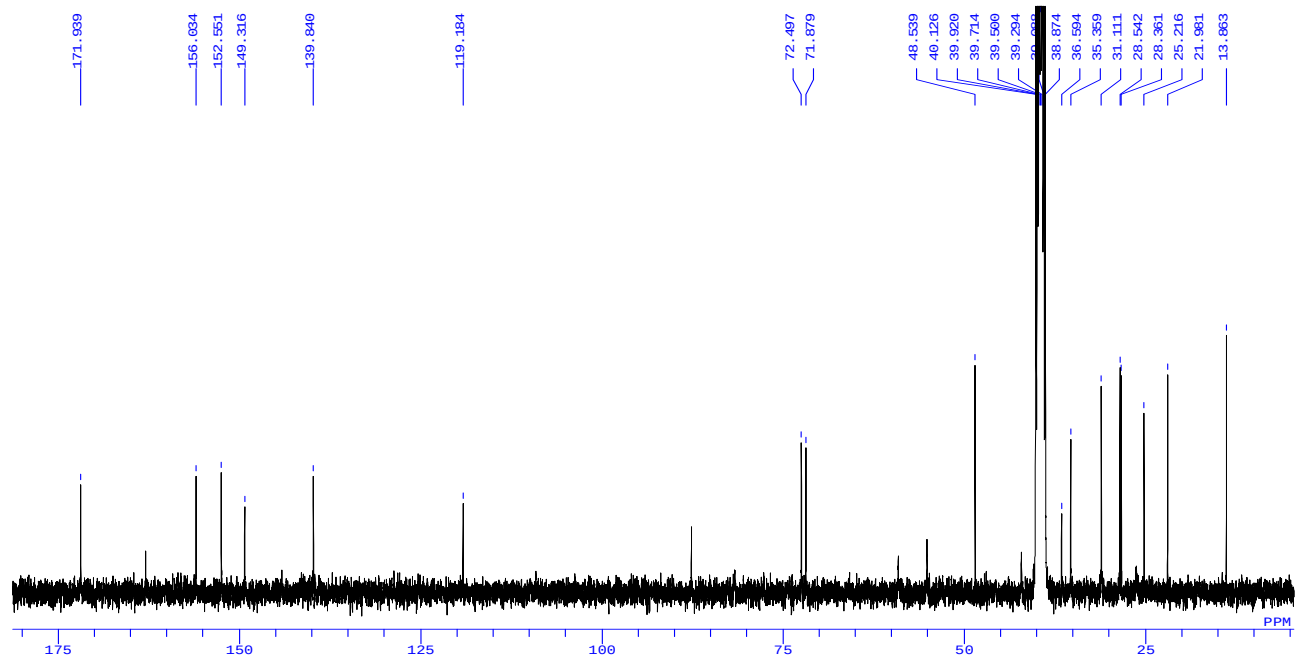

156.042
152.601
149.439
139.774
136.111
126.841
122.593
120.806
119.143
118.163
118.106
112.977
111.264
87.431
84.146
72.645
72.571
48.548
40.126
39.920
39.714
39.500
39.294
39.088
38.874
33.976
32.807
25.579
PPM
150
125
100
75
50
25

Analog 36

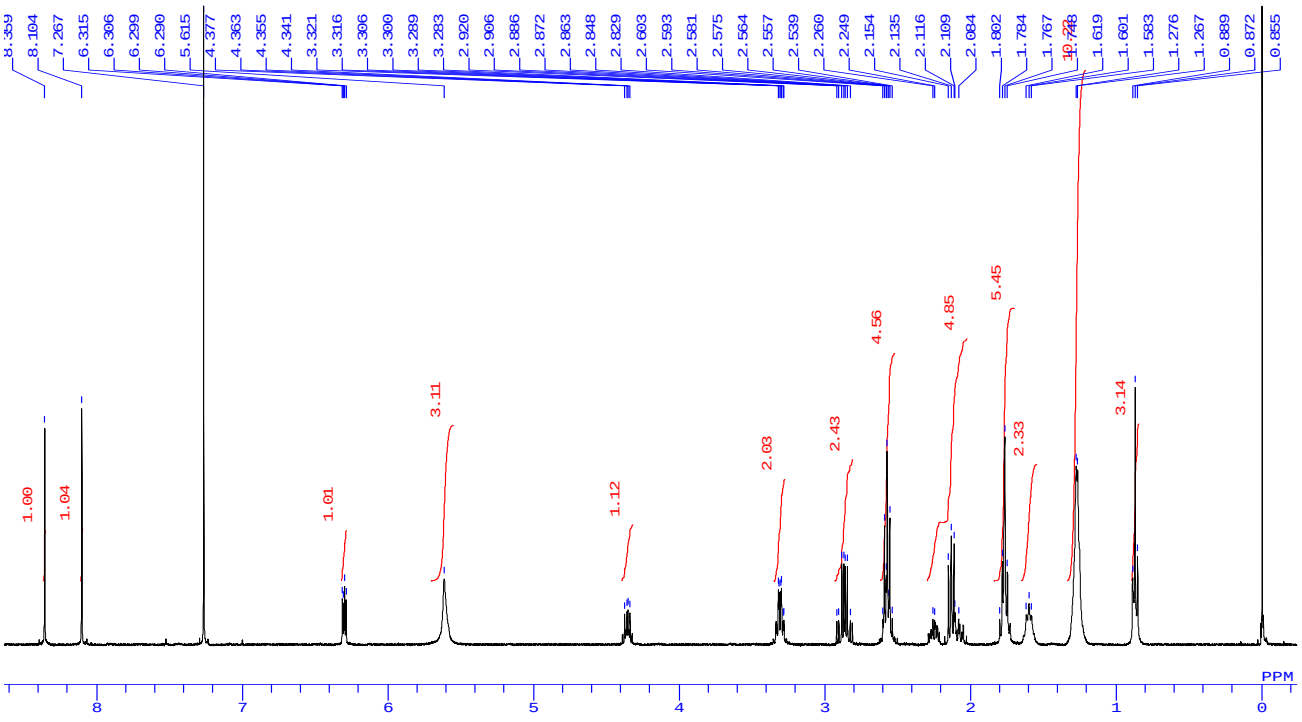

8.334
8.332
8.141
8.133
7.259
5.919
5.914
5.905
5.900
5.543
5.537
5.529
5.522
5.433
5.429
5.421
5.417
4.283
4.261
4.253
3.364
3.304
3.280
3.270
3.165
3.152
3.115
3.100
3.085
3.071
2.737
2.718
2.499
2.494
2.490
2.485
2.481
2.037
2.018
2.004
2.000
1.985
1.735
1.716
1.456
1.446
1.245
1.210
0.847
0.831
0.812
1.03
0.94
0.97
2.00
1.00
1.02
1.03
1.00
1.98
2.90
2.07
2.11
2.13
2.16
3.24
PPM
8
7
6
5
4
3
2
1
171.610
171.586
155.565
152.091
152.041
148.789
148.649
139.742
139.363
118.831
87.686
87.324
77.610
77.428
72.686
72.555
72.102
72.028
54.805
53.002
48.960
48.087
39.624
39.418
39.204
38.998
38.792
38.578
38.463
38.373
36.932
34.865
30.593
28.057
27.843
24.673
22.072
21.874
21.479
13.353
PPM
175
150
125
100
75
50
25

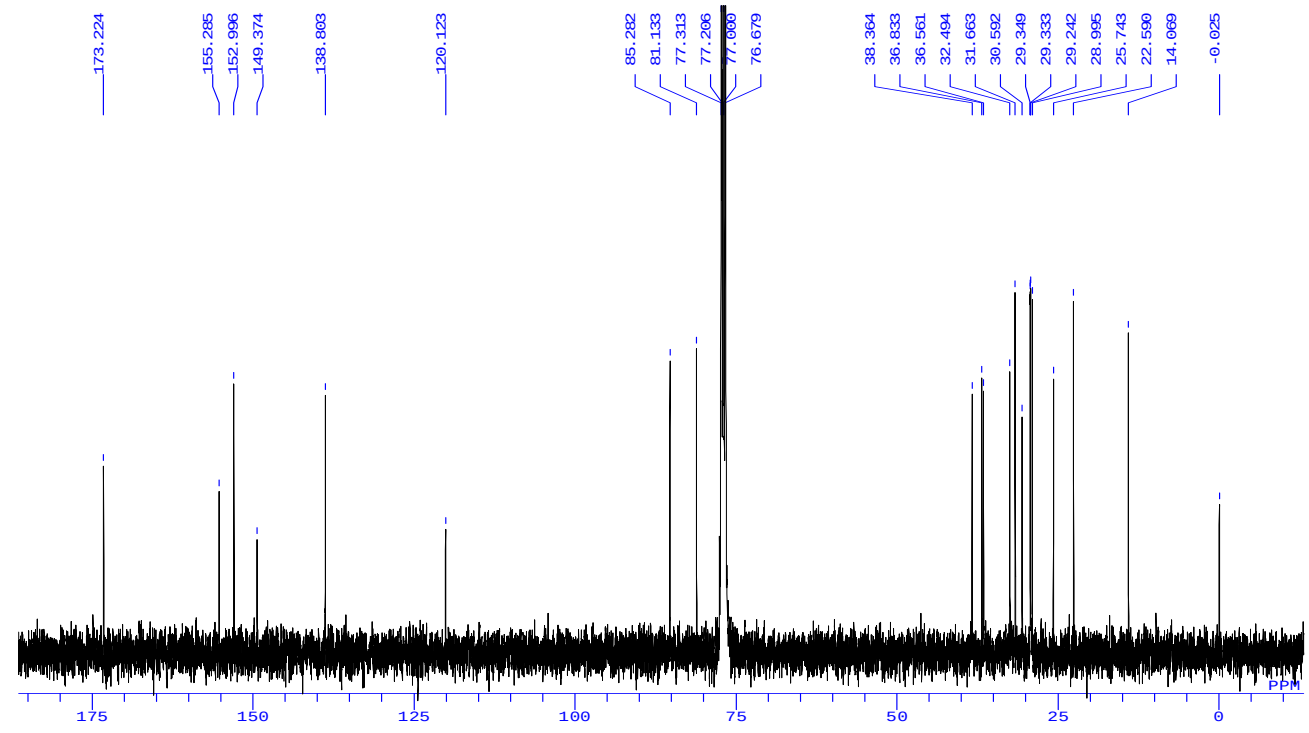
Analog 40


8.308
8.137
8.127
7.679
7.213
5.872
5.859
5.404
5.176
4.656
4.644
4.100
4.019
3.275
3.162
3.042
3.026
3.010
2.994
2.750
2.659
2.499
2.494
2.490
2.485
2.481
2.420
2.228
2.075
1.996
1.979
1.960
1.548
1.530
1.513
1.454
1.436
1.419
1.250
1.209
0.852
0.835
0.818
1.00
1.33
0.89
1.94
1.00
0.91
0.71
1.02
0.94
0.92
1.97
1.73
2.80
1.97
4.35
3.31
PPM
8
7
6
5
4
3
2
1

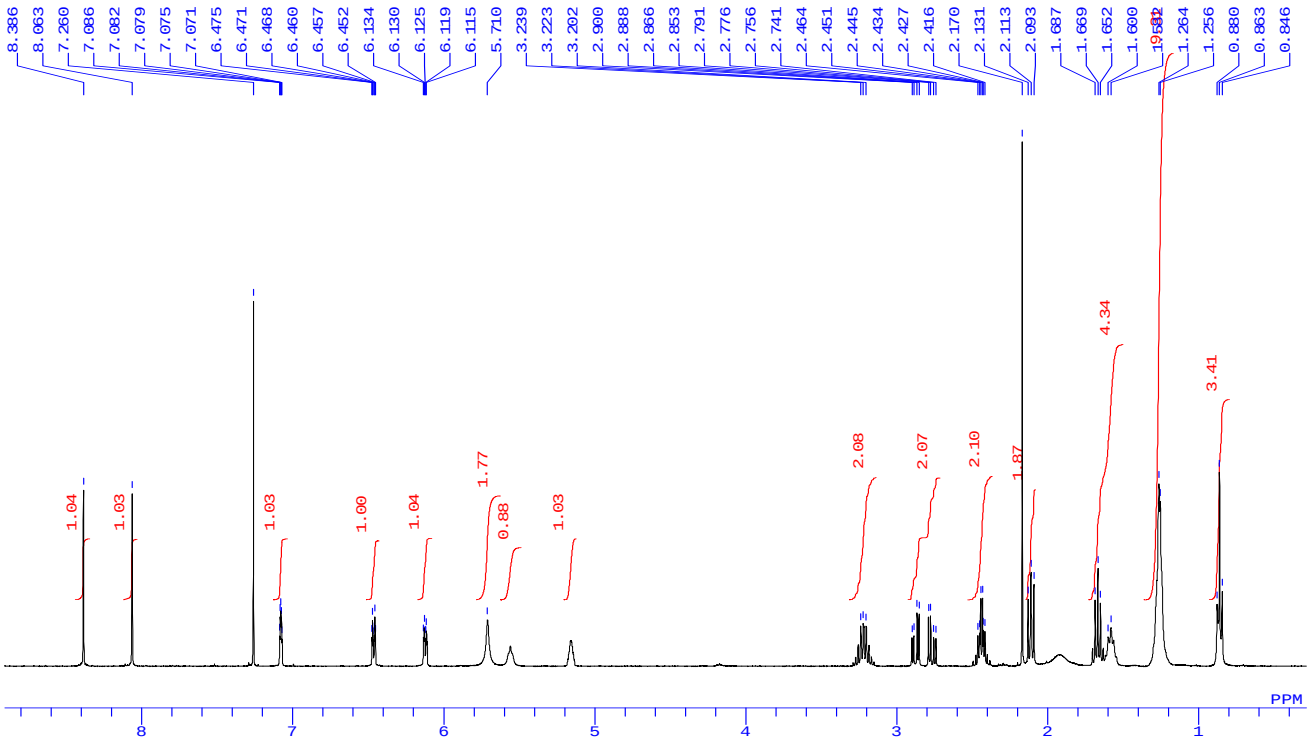

171.939
156.034
152.551
149.316
139.840
119.184
72.497
71.879
48.539
40.126
39.920
39.714
39.500
39.294
38.874
36.594
35.359
31.111
28.542
28.361
25.216
21.981
13.863
PPM
175
150
125
100
75
50
25

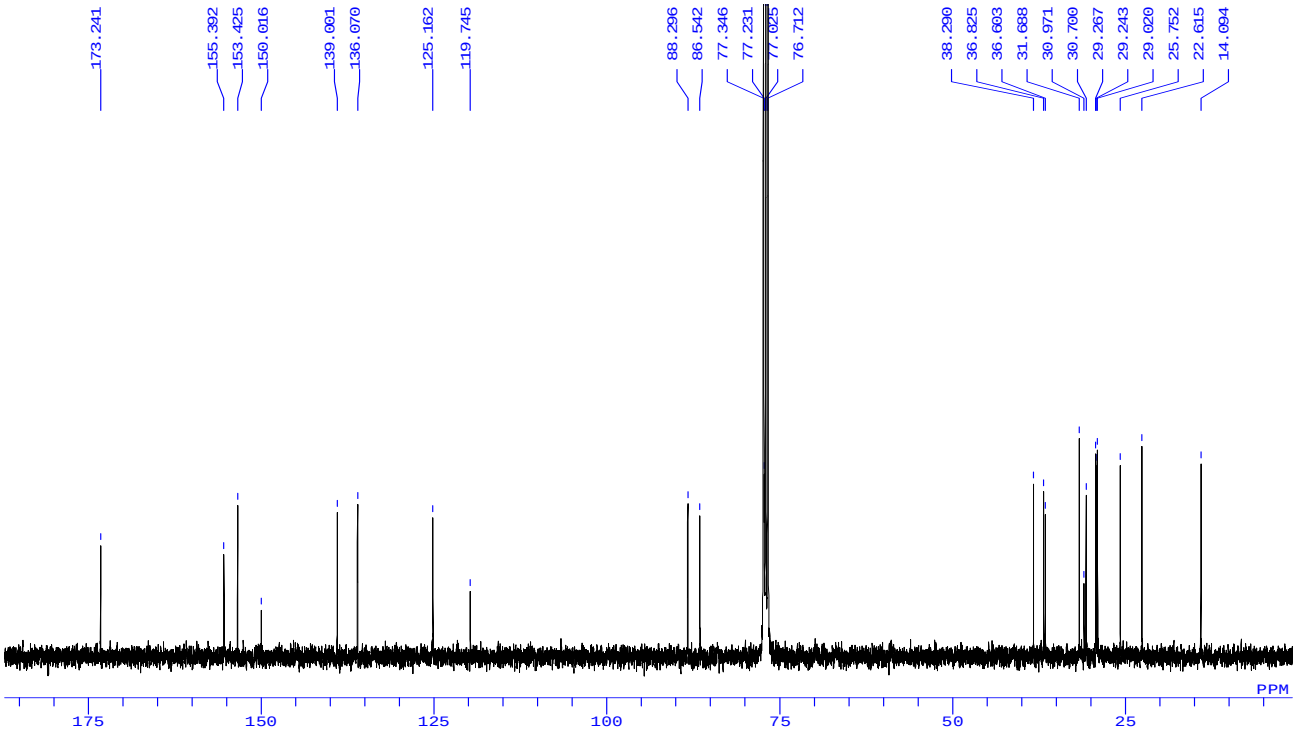
Analog 47


8.104
7.267
6.315
6.306
6.299
6.290
5.615
4.377
4.363
4.355
4.341
3.321
3.316
3.306
3.300
3.289
3.283
2.920
2.906
2.886
2.872
2.863
2.848
2.829
2.603
2.593
2.581
2.575
2.564
2.557
2.539
2.260
2.249
2.154
2.135
2.116
2.109
2.084
1.802
1.784
1.767
1.748
1.619
1.601
1.583
1.276
1.267
0.889
0.872
0.855
1.00
1.04
1.01
3.11
1.12
2.03
2.43
4.56
4.85
5.45
2.33
10.27
3.14
PPM
8
7
6
5
4
3
2
1
0

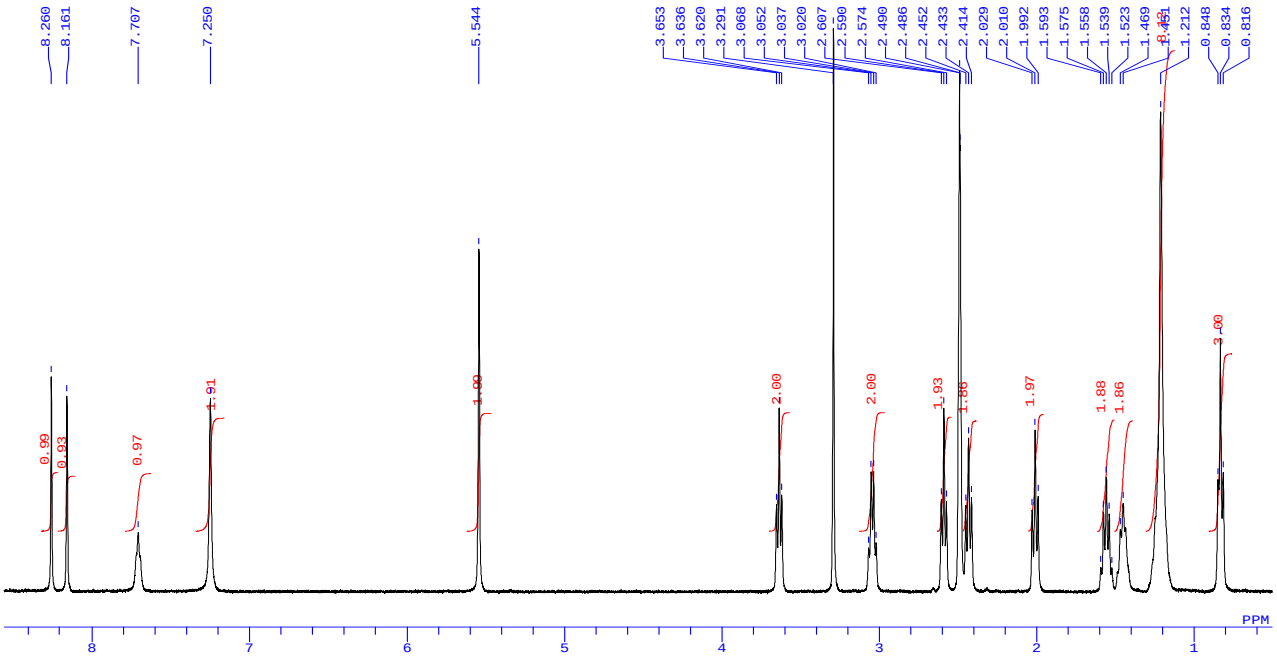

173.224
155.285
152.996
149.374
138.803
120.123
85.282
81.133
77.313
77.206
77.000
76.679
38.364
36.833
36.561
32.494
31.663
30.592
29.349
29.333
29.242
28.995
25.743
22.590
14.069
-0.025
PPM
175
150
125
100
75
50
25
0

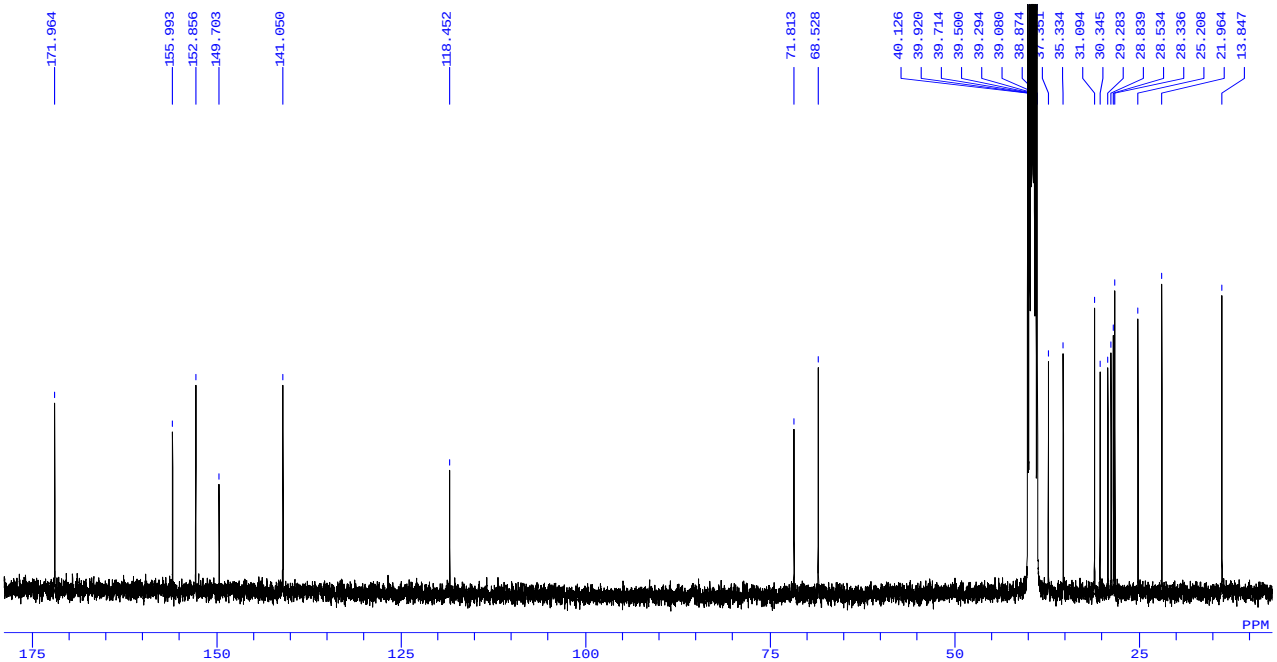
Analog 50


8.386
8.063
7.260
7.086
7.082
7.079
7.075
7.071
6.475
6.471
6.468
6.460
6.457
6.452
6.134
6.130
6.125
6.119
6.115
5.710
3.239
3.223
3.202
2.900
2.888
2.866
2.853
2.791
2.776
2.756
2.741
2.464
2.451
2.445
2.434
2.427
2.416
2.170
2.131
2.113
2.093
1.687
1.669
1.652
1.600
1.264
1.256
0.880
0.863
0.846
1.04
1.03
1.03
1.00
1.04
1.77
0.88
1.03
2.08
2.07
2.10
1.87
4.34
3.41
PPM
8
7
6
5
4
3
2
1

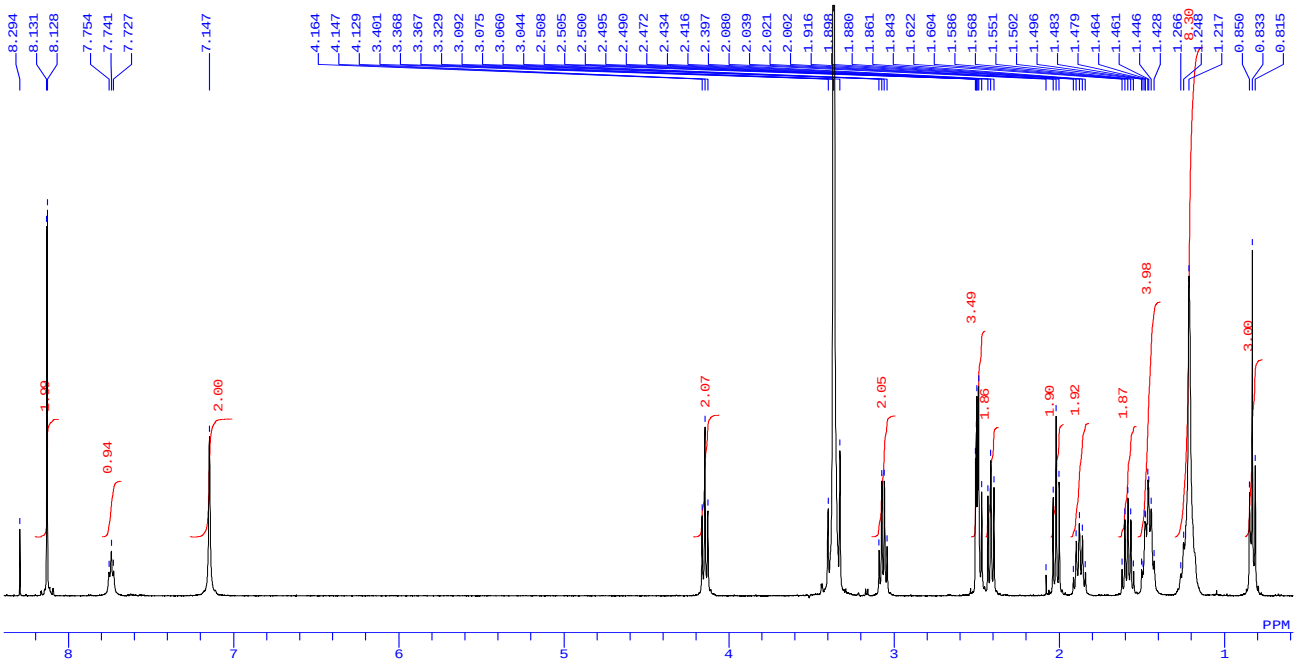

173.241
155.392
153.425
150.016
139.001
136.070
125.162
119.745
88.296
86.542
77.346
77.231
77.025
76.712
38.290
36.825
36.603
31.688
30.971
30.700
29.267
29.243
29.020
25.752
22.615
14.094
PPM
175
150
125
100
75
50
25

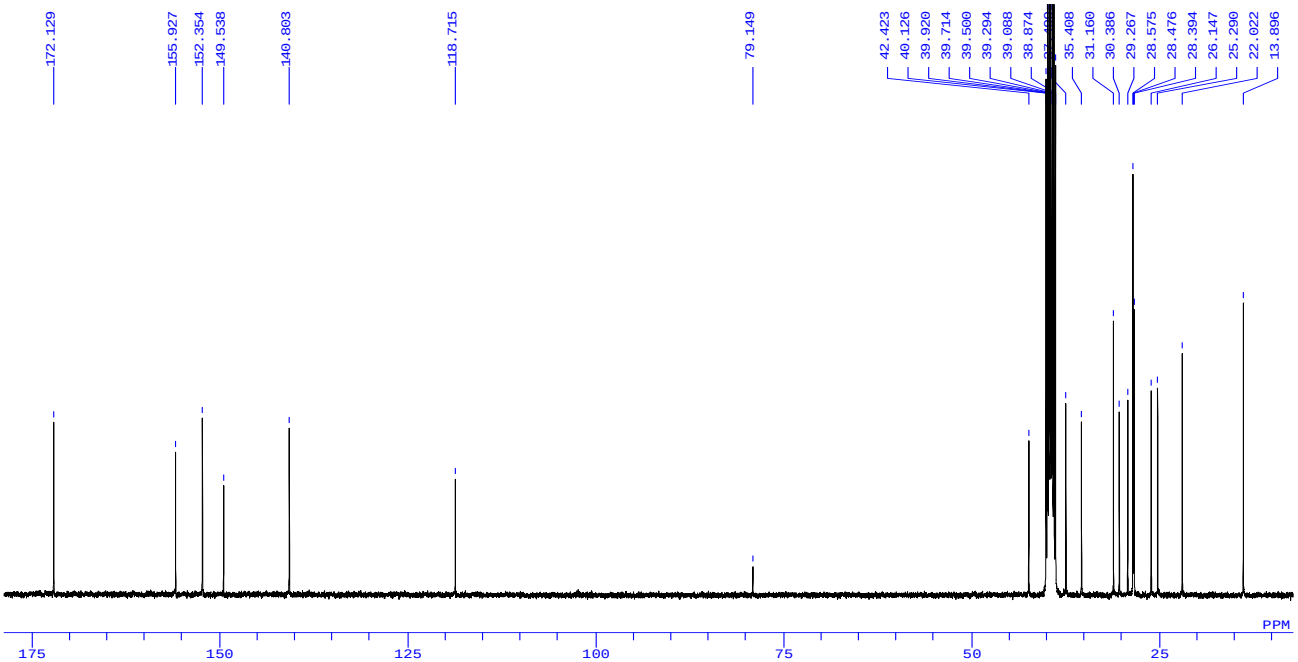
Analog 56


8.260
8.161
7.707
7.250
5.544
3.653
3.636
3.620
3.291
3.068
3.052
3.037
3.020
2.607
2.590
2.574
2.490
2.486
2.452
2.433
2.414
2.029
2.010
1.992
1.593
1.575
1.558
1.539
1.523
1.469
1.212
0.848
0.834
0.816
0.99
0.93
0.97
1.91
1.00
2.00
2.00
1.93
1.86
1.97
1.88
1.86
3.00
PPM
8
7
6
5
4
3
2
1

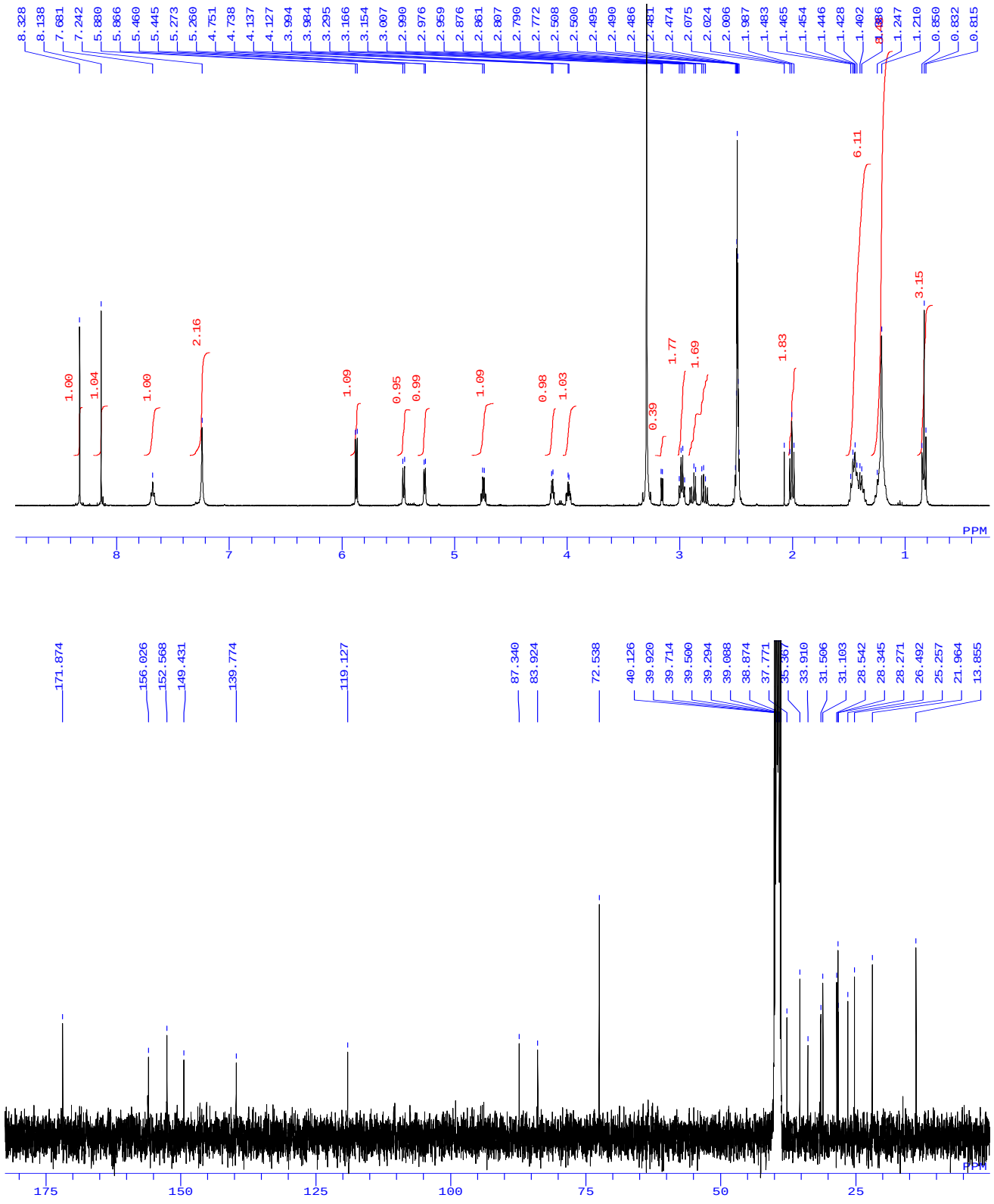

171.964
155.993
152.856
149.703
141.050
118.452
71.813
68.528
40.126
39.920
39.714
39.500
39.294
39.080
38.874
35.334
31.094
30.345
29.283
28.839
28.534
28.336
25.208
21.964
13.847
PPM
175
150
125
100
75
50
25

Analog 59

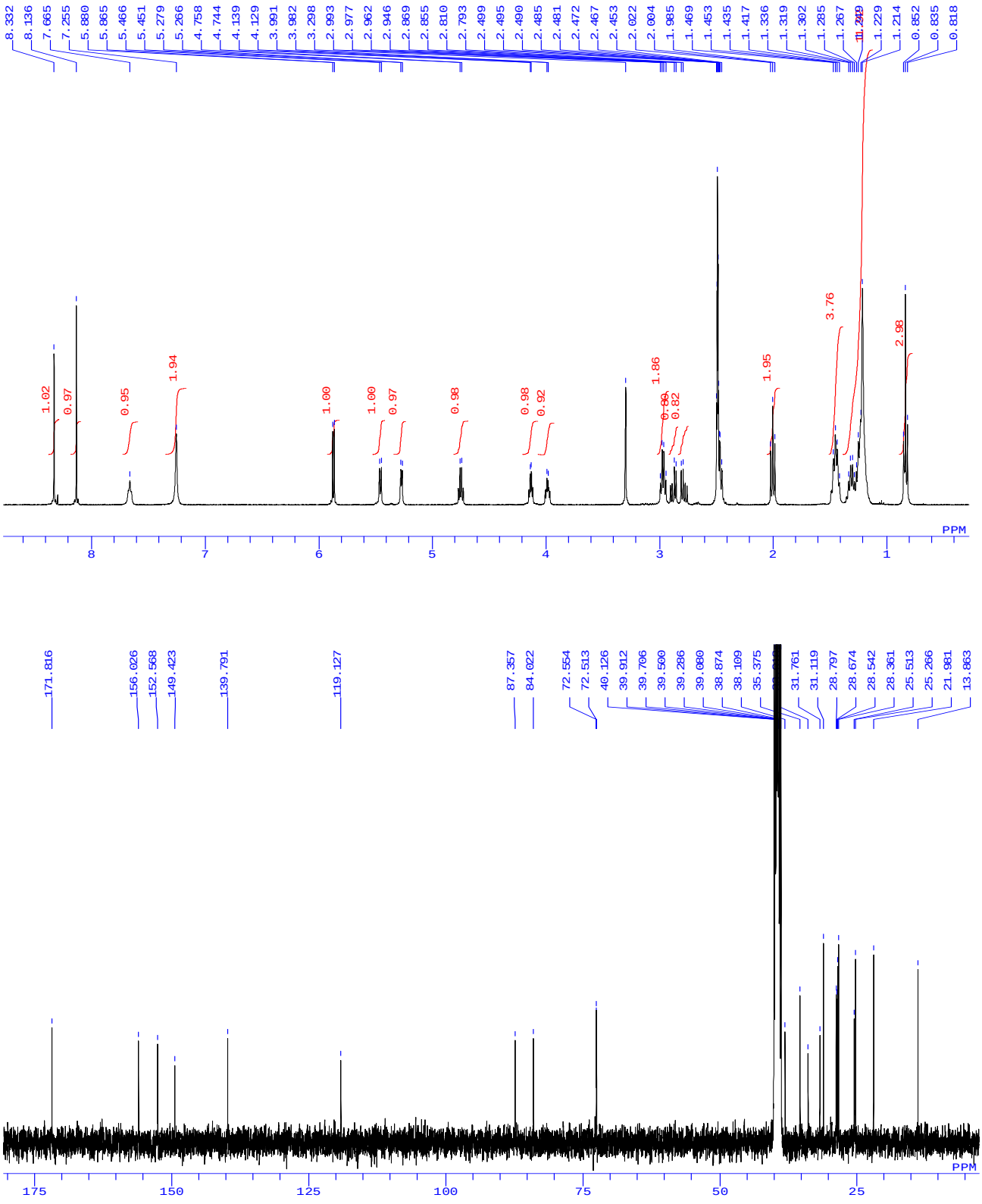

8.294
8.131
8.128
7.754
7.741
7.727
7.147
4.164
4.147
4.129
3.401
3.368
3.367
3.329
3.092
3.075
3.060
3.044
2.508
2.505
2.500
2.495
2.490
2.472
2.434
2.416
2.397
2.080
2.039
2.021
2.002
1.916
1.898
1.880
1.861
1.843
1.622
1.604
1.586
1.568
1.551
1.502
1.496
1.483
1.479
1.464
1.461
1.446
1.428
1.266
8.30
1.248
1.217
0.850
0.833
0.815
1.00
0.94
2.00
2.07
2.05
3.49
1.86
1.90
1.92
1.87
3.98
3.00
PPM
8
7
6
5
4
3
2
1


172.129
155.927
152.354
149.538
140.803
118.715
79.149
42.423
40.126
39.920
39.714
39.500
39.294
39.088
38.874
35.408
31.160
30.386
29.267
28.575
28.476
28.394
26.147
25.290
22.022
13.896
PPM
175
150
125
100
75
50
25

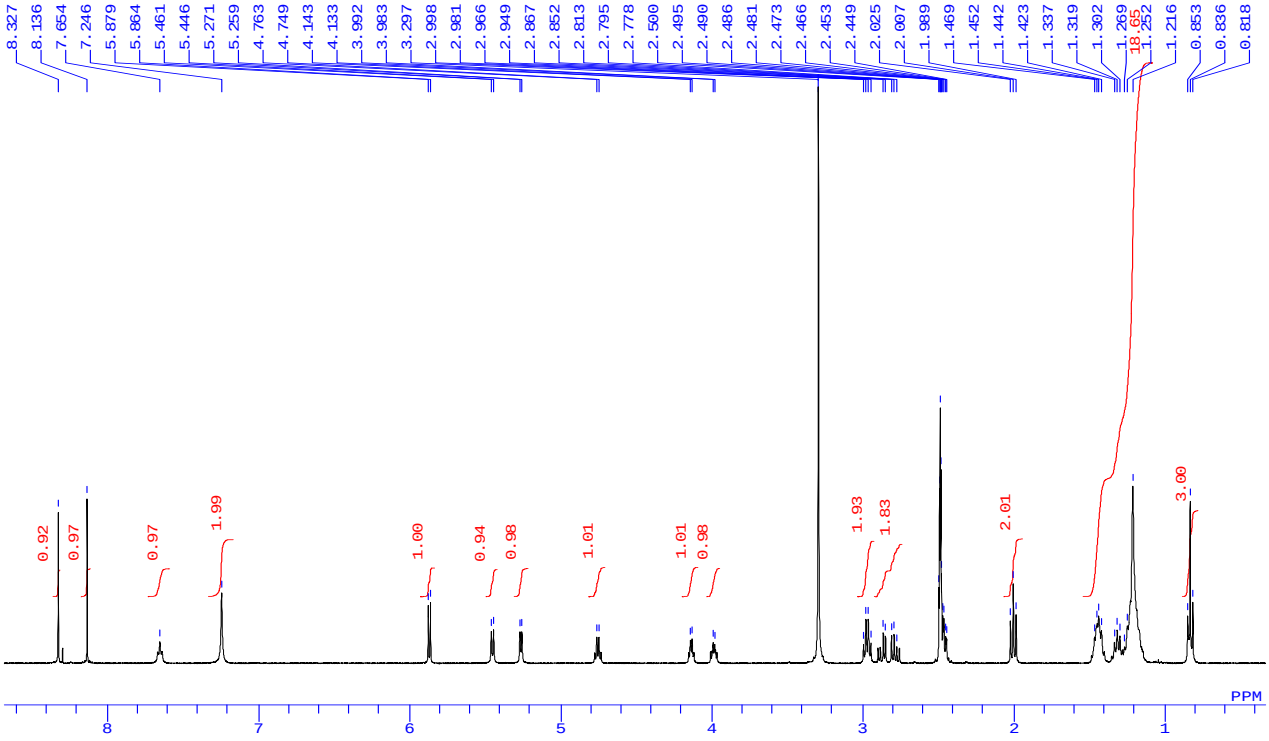
Analog 5

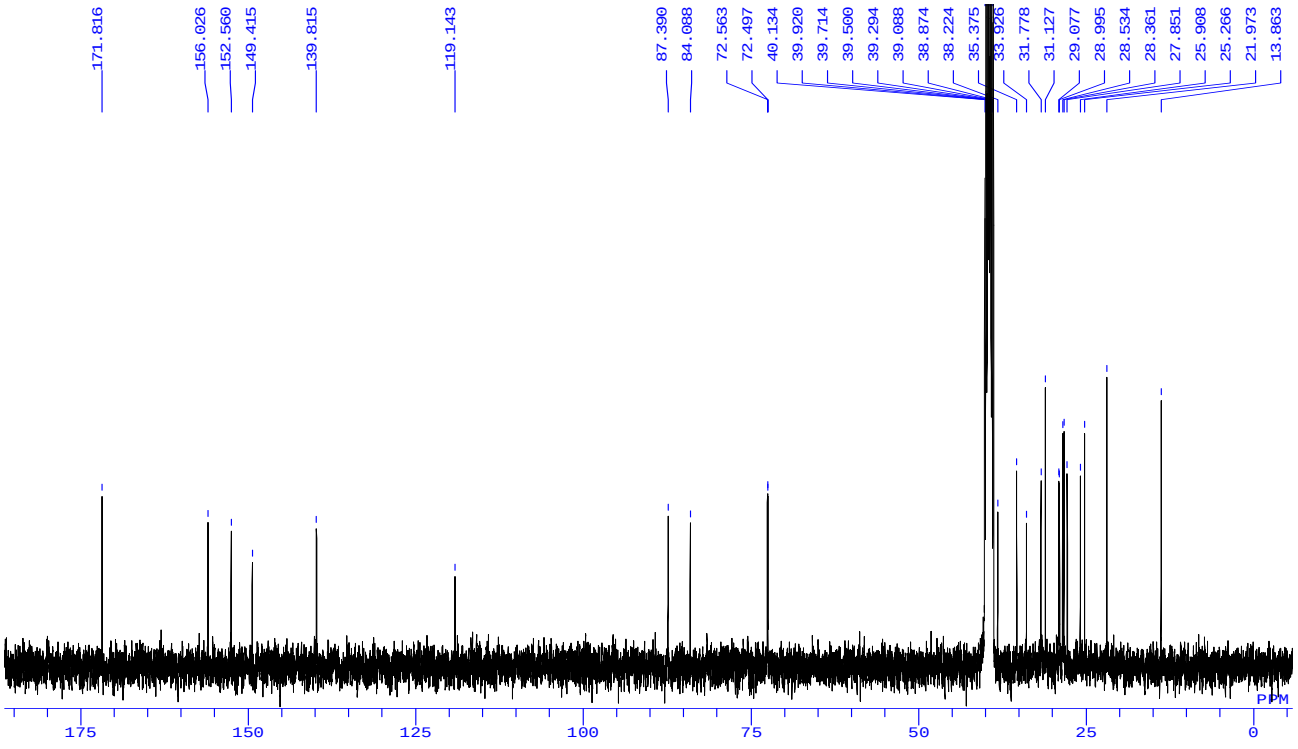

8.328
8.138
7.681
7.242
5.880
5.866
5.460
5.445
5.273
5.260
4.751
4.738
4.137
4.127
3.994
3.984
3.295
3.166
3.154
3.007
2.990
2.976
2.959
2.876
2.861
2.807
2.790
2.772
2.508
2.500
2.495
2.490
2.486
2.481
2.474
2.075
2.024
2.006
1.987
1.483
1.465
1.454
1.446
1.428
1.402
1.247
1.210
0.850
0.832
0.815
1.00
1.04
1.00
2.16
1.09
0.95
0.99
1.09
0.98
1.03
0.39
1.77
1.69
1.83
6.11
3.15
PPM
8
7
6
5
4
3
2
1
171.874
156.026
152.568
149.431
139.774
119.127
87.340
83.924
72.538
40.126
39.920
39.714
39.500
39.294
39.088
38.874
37.771
35.367
33.910
31.506
31.103
28.542
28.345
28.271
26.492
25.257
21.964
13.855
PPM
175
150
125
100
75
50
25

Analog 6

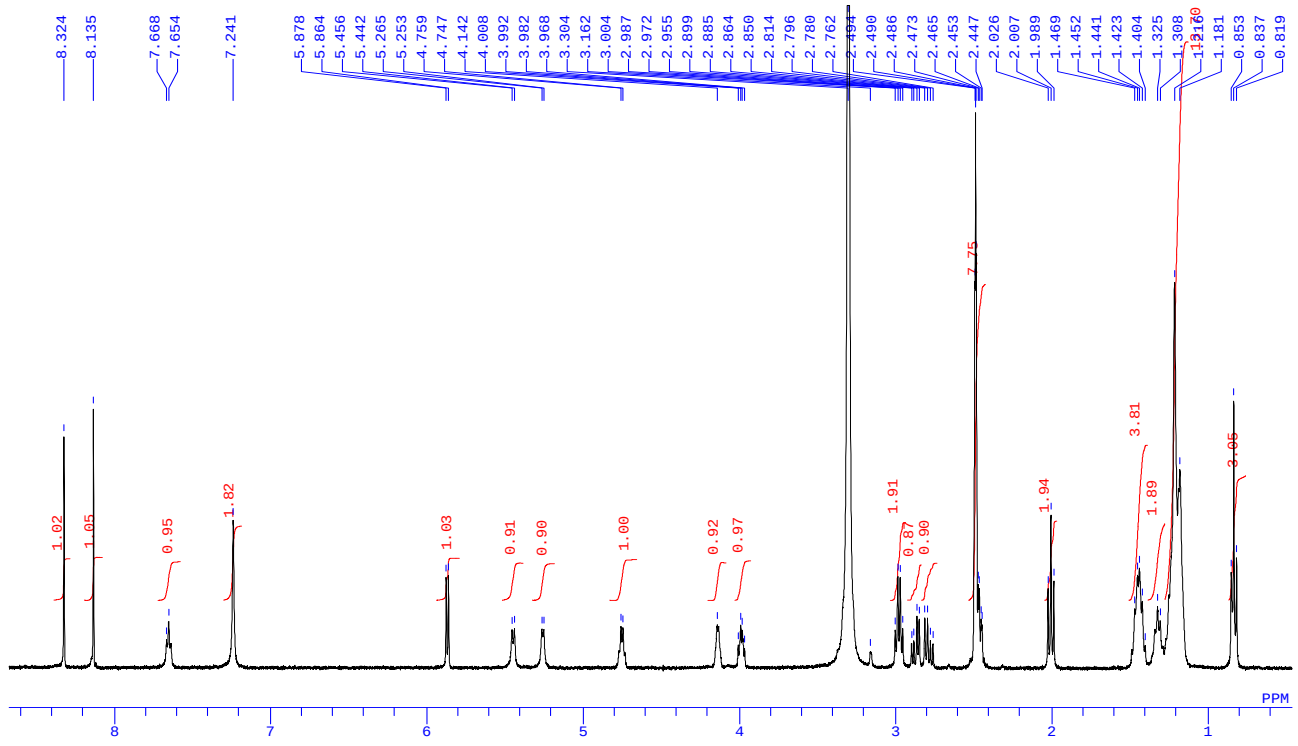

8.332
8.136
7.665
7.255
5.880
5.865
5.466
5.451
5.279
5.266
4.758
4.744
4.139
4.129
3.991
3.982
3.298
2.993
2.977
2.962
2.946
2.869
2.855
2.810
2.793
2.499
2.495
2.490
2.485
2.481
2.472
2.467
2.453
2.022
2.004
1.985
1.469
1.453
1.435
1.417
1.336
1.319
1.302
1.285
1.267
1.229
1.214
0.852
0.835
0.818
1.02
0.97
0.95
1.94
1.00
1.00
0.97
0.98
0.98
0.92
1.86
0.88
0.82
1.95
3.76
2.98
PPM
8
7
6
5
4
3
2
1
171.816
156.026
152.568
149.423
139.791
119.127
87.357
84.022
72.554
72.513
40.126
39.912
39.706
39.500
39.286
39.080
38.874
38.109
35.375
31.761
31.119
28.797
28.674
28.542
28.361
25.513
25.266
21.981
13.863
PPM
175
150
125
100
75
50
25

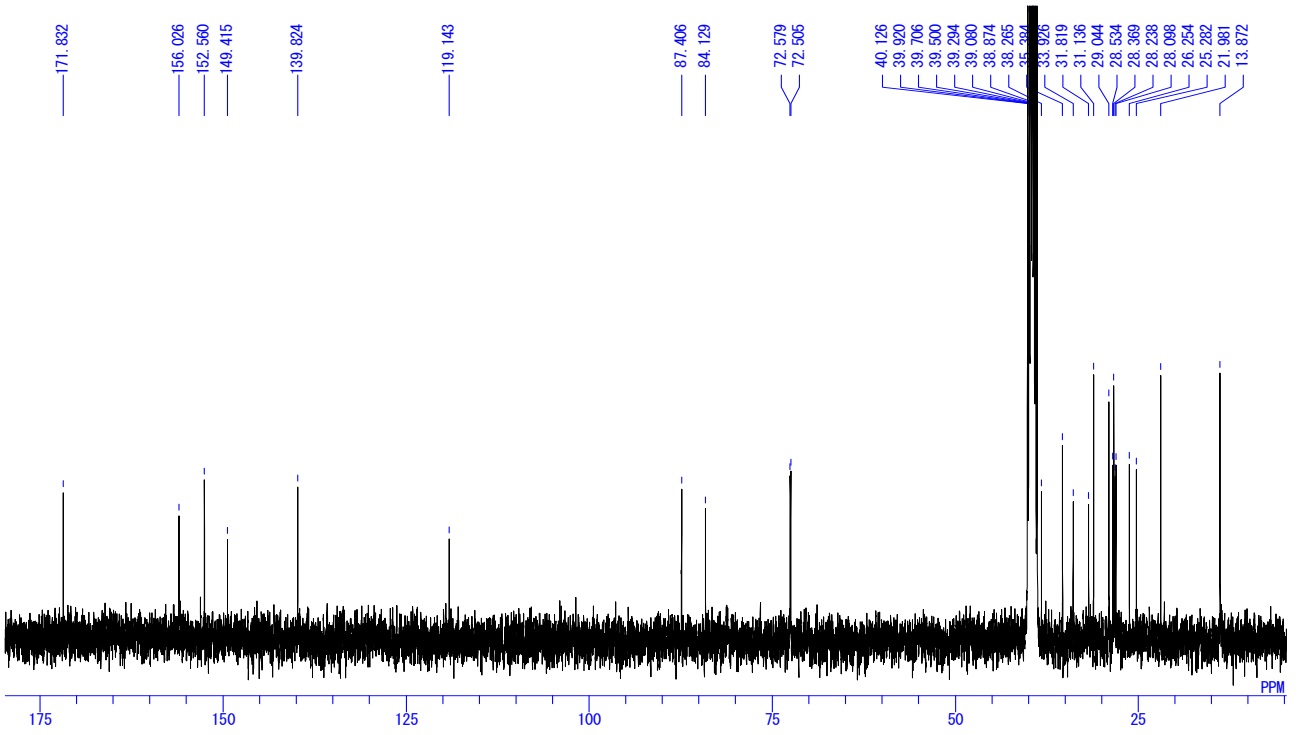
Analog 7


8.327
8.136
7.654
7.246
5.879
5.864
5.461
5.446
5.271
5.259
4.763
4.749
4.143
4.133
3.992
3.983
3.297
2.998
2.981
2.966
2.949
2.867
2.852
2.813
2.795
2.778
2.500
2.495
2.490
2.486
2.481
2.473
2.466
2.453
2.449
2.025
2.007
1.989
1.469
1.452
1.442
1.423
1.337
1.319
1.302
1.269
18.65
1.252
1.216
0.853
0.836
0.818
0.92
0.97
0.97
1.99
1.00
0.94
0.98
1.01
1.01
0.98
1.93
1.83
2.01
3.00
PPM
8
7
6
5
4
3
2
1

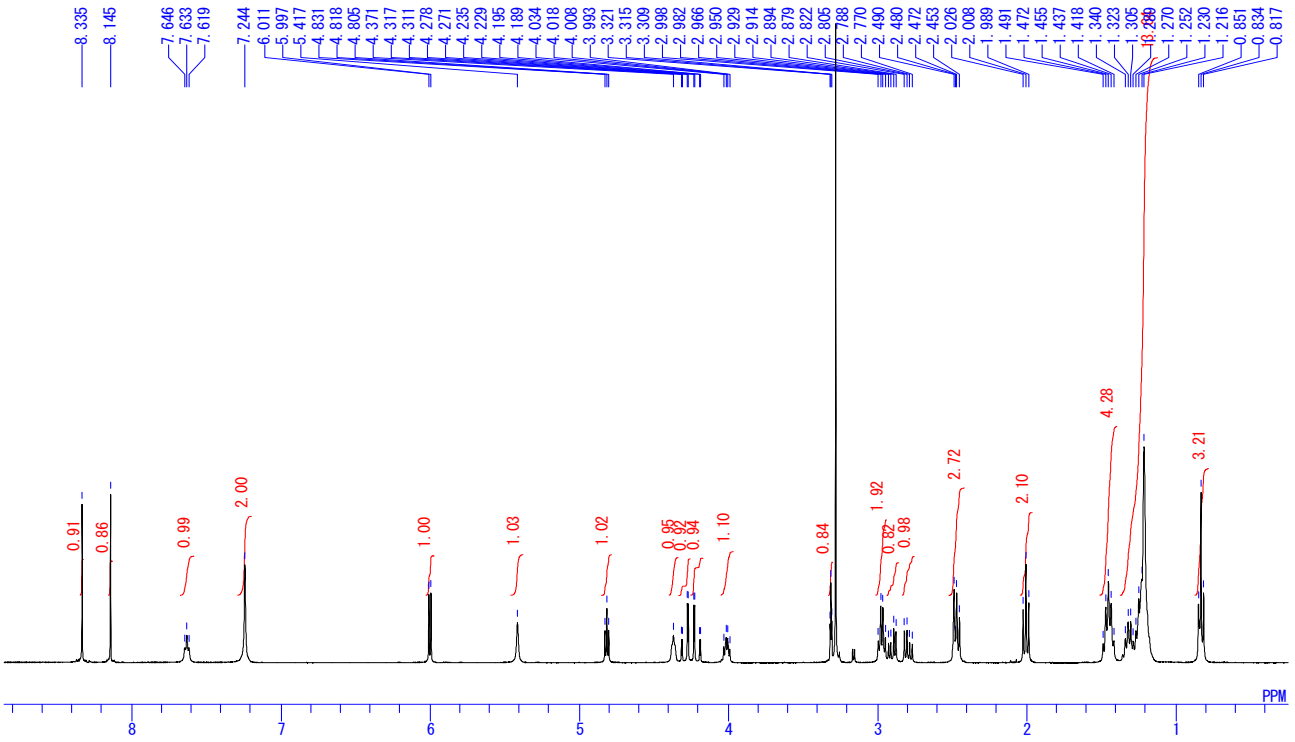

171.816
156.026
152.560
149.415
139.815
119.143
87.390
84.088
72.563
72.497
40.134
39.920
39.714
39.500
39.294
39.088
38.874
38.224
35.375
33.926
31.778
31.127
29.077
28.995
28.534
28.361
27.851
25.908
25.266
21.973
13.863
PPM
175
150
125
100
75
50
25
0

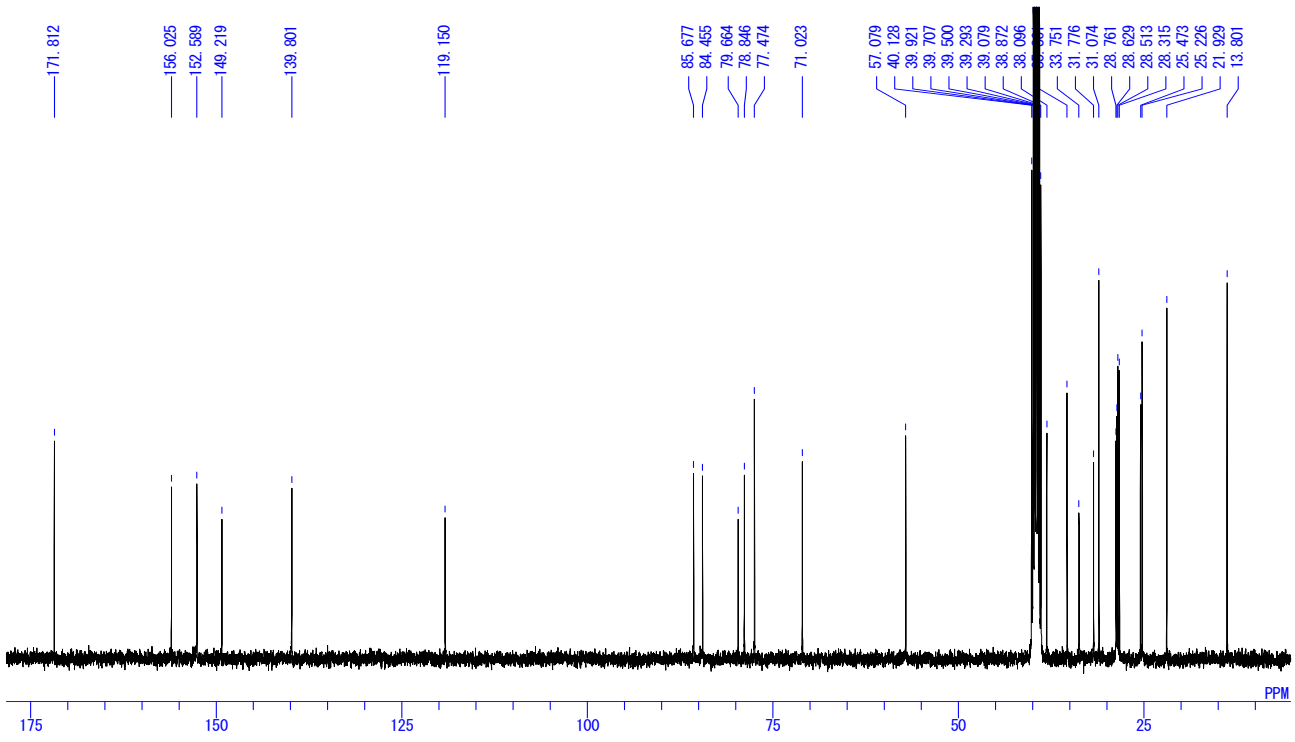
Analog 8


8.324
8.135
7.668
7.654
7.241
5.878
5.864
5.456
5.442
5.265
5.253
4.759
4.747
4.142
4.008
3.992
3.982
3.968
3.304
3.162
3.004
2.987
2.972
2.955
2.899
2.885
2.864
2.850
2.814
2.796
2.780
2.762
2.494
2.490
2.486
2.473
2.465
2.453
2.447
2.026
2.007
1.989
1.469
1.452
1.441
1.423
1.404
1.325
1.308
1.181
0.853
0.837
0.819
1.02
1.05
0.95
1.82
1.03
0.91
0.90
1.00
0.92
0.97
1.91
0.87
0.90
7.75
1.94
3.81
1.89
3.05
PPM
8
7
6
5
4
3
2
1

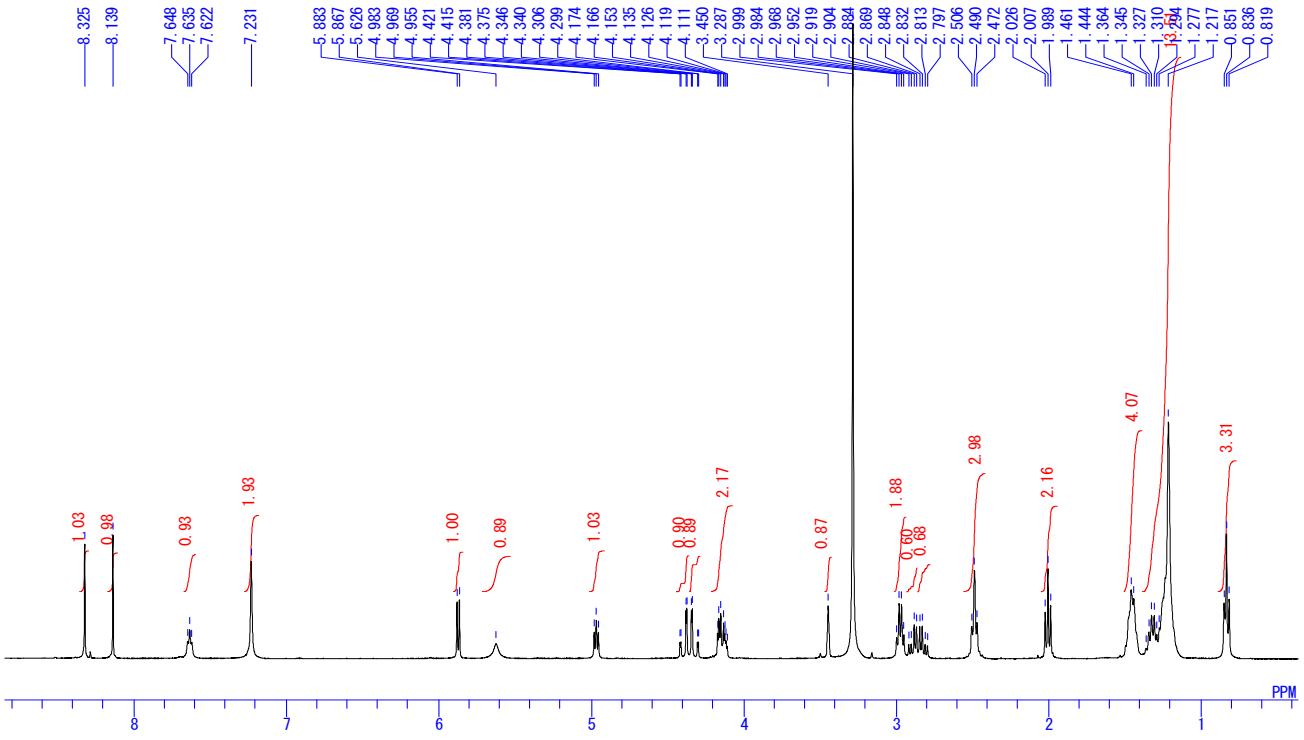

171.832
156.026
152.560
149.415
139.824
119.143
87.406
84.129
72.579
72.505
40.126
39.920
39.706
39.500
39.294
39.080
38.874
38.265
33.926
31.819
31.136
29.044
28.534
28.369
28.238
28.098
26.254
25.282
21.981
13.872
PPM
175
150
125
100
75
50
25

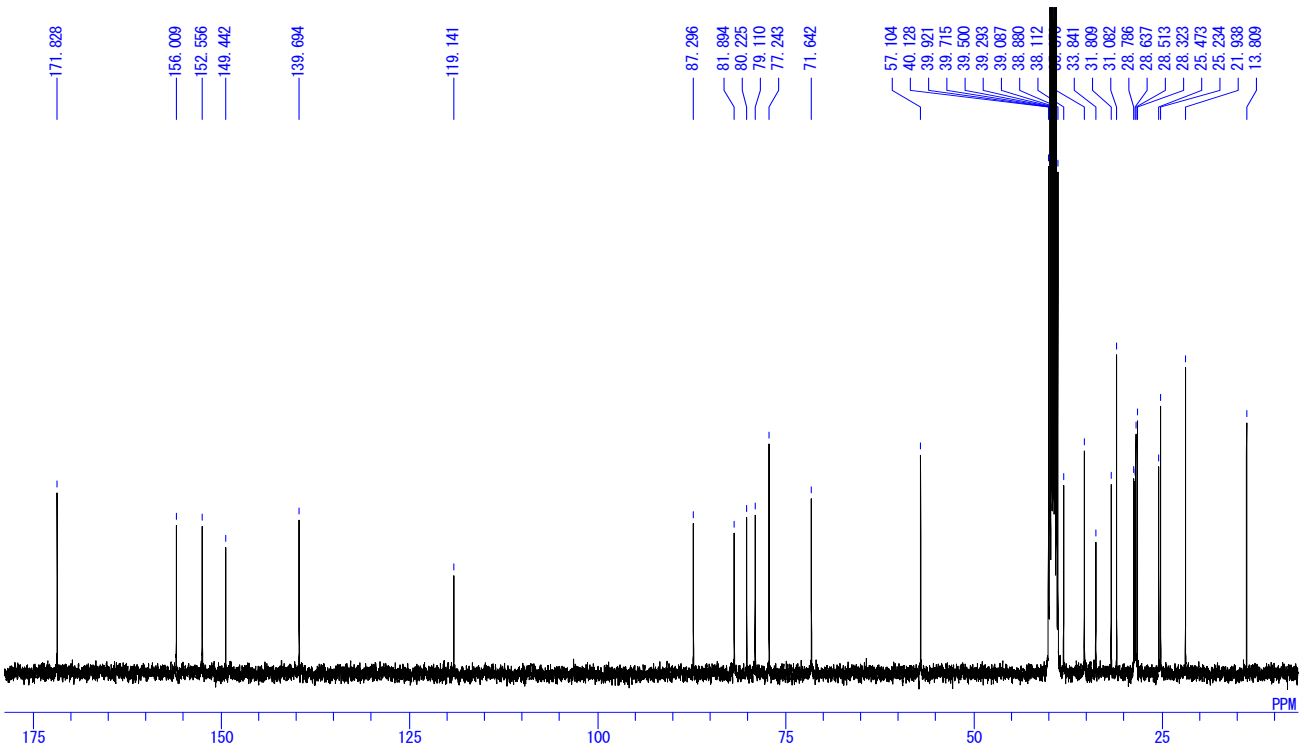
Ligand 9


8.335
8.145
7.646
7.633
7.619
7.244
6.011
5.997
5.417
4.831
4.818
4.805
4.371
4.317
4.311
4.278
4.271
4.235
4.229
4.195
4.189
4.034
4.018
4.008
3.993
3.321
3.315
3.309
2.998
2.982
2.966
2.950
2.929
2.914
2.894
2.879
2.822
2.805
2.788
2.770
2.490
2.480
2.472
2.453
2.026
2.008
1.989
1.491
1.472
1.455
1.437
1.418
1.340
1.323
1.305
1.270
1.252
1.230
1.216
0.851
0.834
0.817
0.91
0.86
0.99
2.00
1.00
1.03
1.02
0.95
0.94
1.10
0.84
1.92
0.98
2.72
2.10
4.28
3.21
PPM
8
7
6
5
4
3
2
1


171.812
156.025
152.589
149.219
139.801
119.150
85.677
84.455
79.664
78.846
77.474
71.023
57.079
40.128
39.921
39.707
39.500
39.293
39.079
38.872
38.096
33.751
31.776
31.074
28.761
28.629
28.513
28.315
25.473
25.226
21.929
13.801
PPM
175
150
125
100
75
50
25

Ligand 10


8.325
8.139
7.648
7.635
7.622
7.231
5.883
5.867
5.626
4.983
4.969
4.955
4.421
4.415
4.381
4.375
4.346
4.340
4.306
4.299
4.174
4.166
4.153
4.135
4.126
4.119
4.111
3.450
3.287
2.999
2.984
2.968
2.952
2.919
2.904
2.884
2.869
2.848
2.832
2.813
2.797
2.506
2.490
2.472
2.026
2.007
1.989
1.461
1.444
1.364
1.345
1.327
1.310
1.294
1.277
1.217
0.851
0.836
0.819
1.03
0.98
0.93
1.93
1.00
0.89
1.03
0.90
0.89
2.17
0.87
1.88
0.60
0.68
2.98
2.16
4.07
3.31
PPM
8
7
6
5
4
3
2
1


171.828
156.009
152.556
149.442
139.694
119.141
87.296
81.894
80.225
79.110
77.243
71.642
57.104
40.128
39.921
39.715
39.500
39.293
39.087
38.880
38.112
33.841
31.809
31.082
28.786
28.637
28.513
28.323
25.473
25.234
21.938
13.809
PPM
175
150
125
100
75
50
25

References

[S1] M. Pignot, G. Pljevaljcic, E. Weinhold, *Eur. J. Org. Chem.*, **2000**, 549−555.

[S2] C. Guérard, M. Bréard, F. Courtois, T. Drujon, O. Ploux, *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1661−1664.

[S3] C. Joce, J. Caryl, P. G. Stockley, S. Warriner, A. Nelson, *Org. Biomol. Chem.*, **2009**, *7*, 635−638.

[S4] Y. Saito, T. A. Zevaco, L. A. Agrofoglio, *Tetrahedron*, **2002**, *58*, 9593−9603.

[S5] M. J. Robins, P. W. Hatfield, *Can. J. Chem.*, **1982**, *60*, 547−553.

[S6] M. J. McKennon, A. I. Meyers, *J. Org. Chem.*, **1993**, *58*, 3568−3571.

[S7] J. Chung, E. Goo, S. Yu, O. Choi, J. Lee, J. Kim, H. Kim, J. Igarashi, H. Suga, J. S. Moon, I. Hwang, S. Rhee, *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, *108*, 12089−12094.

[S8] C. O. Rock, J. E. Cronan, *Methods Enzymol.*, **1981**, *71*, 341−351.

[S9] J. E. Cronan, A. L. Klages, *Proc. Natl. Acad. Sci. U.S.A.*, **1981**, *78*, 5440−5444.

[S10] M. I. Moré, L. D. Finger, J. L. Stryker, C. Fuqua, A. Eberhard, S. C. Winans, *Science*, **1996**, *272*, 1655−1658.

[S11] K. Kai, A. Tani, H. Hayashi, H. *Bioorg. Med. Chem.*, **2010**, *18*, 3776−3782.

[S12] K. Kai, K. Furuyabu, A. Tani, H. Hayashi, *ChemBioChem*, **2012**, *13*, 1776−1754.

[S13] M. Dixon, *Biochem. J.*, **1953**, *55*, 170−171.

[S14] C. H. Christensen, T. L. Grove, S. J. Booker, E. P. Greenberg, *Proc. Natl. Acad. Sci. U.S.A.*, **2013**, *110*, 13815−13820.