Supporting Information

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

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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 $\delta$ values (ppm) using tetramethylsilane (TMS) ($\delta^1$H 0, $\delta^1$C 0) or solvents (DMSO: $\delta^1$H 2.49, $\delta^1$C 39.5; D$_2$O: $\delta^1$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

![Diagram of Analog 1]
2.1.1. 5'-Acetylthio-5'-deoxy-2',3'-O-isopropylideneadenosine  (11).\cite{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 (CHCl₃–MeOH, 100:0, 95:5, 90:10) to give 11 (1.14 g, 96%) as a yellowish oil. \(^1\)H NMR (CDCl₃, 400 MHz) \(\delta\) 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\(_2\)O, 400 MHz) \(\delta\) 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\(_2\)CO\(_3\) (1.29 g, 12.1 mmol) were dissolved in CH\(_2\)Cl\(_2\)–H\(_2\)O (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\(_2\)SO\(_4\), and evaporated to afford 13 (1.16 g, 95%) as a white solid. \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 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 CHCl3–MeOH (100 ml, 95:5), washed with water, and dried over Na2SO4. The organic layer was evaporated and subsequently purified by silica gel column chromatography (CHCl3–MeOH, 95:5) to give 14 (428 mg, 49%) as a yellowish oil. 1H NMR (CDCl3, 400 MHz) δ 0.87 (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). 13C NMR (CDCl3, 100 MHz) δ 14.1, 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–H2O (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 (CHCl3–MeOH, 97:3) to give 15 (282 mg, 72%) as a yellowish gum. 1H NMR (DMSO-d6, 400 MHz) δ 0.84 (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). 13C NMR (DMSO-d6, 100 MHz) δ 14.0, 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\(_6\)N\(_6\)NaS, 547.2315).

### 2.1.6. 5’-Deoxy-5’-[3-(carboxy)-3-(octanamido)propythio]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\(_4\)OH (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) \( \delta \) 0.83 (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) \( \delta \) 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\(_6\)N\(_6\)KS, 549.1900).

### 2.2. Analog 4

![Chemical Structure]

**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) $\delta$ 0.90 (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) $\delta$ 0.87 (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) $\delta$ 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) $\delta$ 0.83 (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) $\delta$ 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$_4$N$_6$NaS, 489.2256).
2.3. Analog 20

2.3.1. \textit{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 \(\mu\)l, 1.84 mmol) were used. 18 was obtained as a colorless oil (88 mg, 36%). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 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. \textit{5’-Deoxy-2’\textasciitilde3’-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) \(\delta\) 1.41 (3H, s), 1.62 (3H, s), 1.71 (2H, m), 1.96 (3H, s), 2.53 (2H, \(J = 7.4\) 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) \(\delta\) 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. \textit{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) \(\delta\) 1.59 (2H, quint, \(J = 6.8\) Hz), 1.76 (3H, s), 2.79 (1H, dd, \(J = 7.1, 13.9\) Hz).
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). 13C NMR (DMSO-\( d_6 \), 100 MHz) \( \delta \) 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\(_4\)N\(_6\)NaS, 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 \( \mu \)l, 3.70 mmol) were used. 21 was obtained as a colorless oil (686 mg, quant.). 1H NMR (CDCl\(_3\), 400 MHz) \( \delta \) 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)propythio]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%). 1H NMR (CDCl\(_3\), 400 MHz) \( \delta \) 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) $\delta$ 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) $\delta$ 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) $\delta$ 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$_4$N$_6$NaS, 461.1947).

2.5. Analog 26

![Diagram](attachment://image.png)

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 \(\text{CH}_2\text{Cl}_2\) (15 ml). After stirring at rt overnight, the urea that formed was removed by filtration. The filtrate was evaporated, dissolved in \(\text{EtOAc}\) (20 ml), and washed with 2 M \(\text{aq HCl}\) and \(\text{brine}\). The organic layer was dried over \(\text{Na}_2\text{SO}_4\) 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 \(\text{Et}_3\text{N}\) (361 \(\mu\)l, 2.60 mmol) were dissolved in dry \(\text{MeCN}\) (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 \(\text{EtOAc}\) (40 ml), and washed with sat. \(\text{NaHCO}_3\) and \(\text{brine}\). The organic layer was dried over \(\text{Na}_2\text{SO}_4\) and purified by silica gel column chromatography (hexane–\(\text{EtOAc}\), 80:20) to afford 24 (195 mg, 35%) as a yellow solid. \(^1\text{H} \text{NMR (CDCl}_3, 400 \text{MHz)} \delta 0.89 \text{ (3H, t, } J = 6.9 \text{ Hz)}, 1.24–1.33 \text{ (4H)}, 2.09 \text{ (2H, quint, } J = 6.6 \text{ Hz)}, 2.52 \text{ (2H, quint, } J = 7.4 \text{ Hz)}, 3.41 \text{ (2H, s)}, 3.42–3.46 \text{ (4H)}, 7.21 \text{ (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: (\(\text{CHCl}_3–\text{MeOH}, 97.5:2.5\)). 25 was obtained as a colorless paste (231 mg, 50%). \(^1\text{H} \text{NMR (CDCl}_3, 400 \text{ MHz)} \delta 0.89 \text{ (3H, t, } J = 7.1 \text{ Hz)}, 1.22–1.35 \text{ (4H)}, 1.41 \text{ (3H, s)}, 1.58 \text{ (2H, m)}, 1.62 \text{ (3H, s)}, 1.71 \text{ (2H, m)}, 2.49–2.55 \text{ (4H)}, 2.76 \text{ (1H, dd, } J = 7.0, 13.5 \text{ Hz)}, 2.84 \text{ (1H, dd, } J = 7.0, 13.5 \text{ Hz)}, 3.29 \text{ (2H, s)}, 3.29 \text{ (2H, m)}, 4.40 \text{ (1H, m)}, 5.06 \text{ (1H, dd, } J = 3.0, 6.5 \text{ Hz)}, 5.54 \text{ (1H, dd, } J = 2.2, 6.5 \text{ Hz)}, 5.77 \text{ (2H, br)}, 6.09 \text{ (1H, d, } J = 2.2 \text{ Hz)}, 7.14 \text{ (1H, br)}, 7.97 \text{ (1H, s)}, 8.36 \text{ (1H, s)}. \(^{13}\text{C} \text{NMR (CDCl}_3, 100 \text{ MHz)} \delta 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: (\(\text{CHCl}_3–\text{MeOH}, 95:5, 90:10\)). 26 was obtained as a yellowish solid (143 mg, 67%). \(^1\text{H} \text{NMR}
(DMSO-$d_6$, 400 MHz) $\delta$ 0.83 (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) $\delta$ 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$_5$N$_6$NaS, 503.2053).

2.6. Analog 29

![Diagram](image.png)

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) $\delta$ 2.02 (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) $\delta$ 1.40 (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) \( \delta \) 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) \( \delta \) 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) \( \delta \) 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\(_4\)N\(_6\)NaS, 481.1634).

2.7. Analog 31

![Diagram of analog 31](image)

2.7.1. 5'-Deoxy-2',3'-O-isopropylidene-5'-(dodecanthio)adenosine (30). The reaction procedure described in Section 2.1.4. was followed. 11 (260 mg, 0.711 mmol) and 1-bromododecan (256 \( \mu \)l, 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) \( \delta \) 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). 13C NMR (CDCl3, 100 MHz) \( \delta \) 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′-(dodecanthio)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%). 1H NMR (DMSO-\( d_6 \), 400 MHz) \( \delta \) 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). 13C NMR (DMSO-\( d_6 \), 100 MHz) \( \delta \) 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 \( \mu l \), 1.64 mmol) were used. Chromatography: (CHCl₃–MeOH, 95:5). 32 was obtained as a colorless solid (314 mg, 67%). 1H NMR (CDCl₃, 400 MHz) \( \delta \) 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). 13C 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. 5ʹ-Deoxy-5ʹ-(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%). 1H 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). 13C 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 C18H22O3N5S, 388.1443).

2.9. Analog 35

2.9.1. 5ʹ-Deoxy-2ʹ,3ʹ-O-isopropylidene-5ʹ-(2-indolyethylthio)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%). 1H 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) \(\delta\) 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-indolyethylthio)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) \(\delta\) 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), 7.42 (2H, s), 7.63 (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) \(\delta\) 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\(_3\)N\(_6\)S, 427.1552).

2.10. Analog 36

![Diagram](image)

5′-Deoxy-5′-[3-(octanamido)propylsulfanyl]adenosine (36).\(^{[82]}\) Thirty percent aq H\(_2\)O\(_2\) (232 \(\mu\)l, 2.07 mmol) was added to a solution of 4 (100 mg, 0.228 mmol) in MeOH−H\(_2\)O (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) $\delta$ 0.83 (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) $\delta$ 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$_5$N$_6$NaS, 505.2209).

2.11. Analog 40

![Diagram](image)

2.11.1. o-Nitro-N-methylbenzenesulfonamide (37). Et$_3$N (691 $\mu$L, 4.96 mmol) and 40% aq MeNH$_2$ (587 $\mu$L, 6.77 mmol) were added with stirring to a solution of o-nitrobenzenesulfonyl chloride (1.00 g, 4.51 mmol) in dry CH$_2$Cl$_2$ (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$_2$SO$_4$. 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) $\delta$ 2.80 (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-nitrobenzenesulfonlamino) adenosine (38). The reaction procedure described in Section 2.1.1. was followed. 2′,3′-O-Isopropylidenedeadenosine (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%).
1H NMR (DMSO-d₆, 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). Thiophenol (354 µl, 3.46 mmol) and Cs₂CO₃ (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 CHCl₃ (50 ml ×3) and dried over Na₂SO₄. The solvents were removed by evaporation and purified by silica gel column chromatography (CHCl₃–MeOH, 9:1) to give 39 (383 mg, 69%) as a light brown solid. 1H NMR (DMSO-d₆, 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 (CHCl₃–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: (CHCl₃–MeOH, 9:1). 40 (150 mg, 36%, 2 steps) was obtained as a light brown paste. 1H NMR (DMSO-d₆, 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). 13C NMR (DMSO-d₆, 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_{4}N_{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 (CHCl₃–MeOH, 9:1) to give 41 (5.40 g, 85%) as a colorless solid. 

\[
\begin{align*}
\delta &\text{0.04 (6H, s), 0.86 (9H, s), 3.74 (1H, dd, } J = 4.0, 11.3 \text{ Hz), 3.87 (1H, dd, } J = 4.0, 11.3 \text{ Hz), 3.94 (1H, m), 4.17 (1H, m), 4.53 (1H, m), 5.17 (1H, d, } J = 5.4 \text{ Hz), 5.51 (1H, d, } J = 5.6 \text{ Hz), 5.90 (1H, d, } J = 5.1 \text{ Hz), 7.26 (2H, br), 8.13 (1H, s), 8.27 (1H, s).}
\end{align*}
\]

2.12.2. 2',3'-O-Thiocarbonylene-5'-O-tert-butyldimethylsilyladenosine (42).[^54] 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 (CHCl₃–MeOH, 97:3) to give 42 (6.00 g, quant.) as a colorless solid. 

\[
\begin{align*}
\delta &\text{−0.10 (6H, s), 0.77 (9H, s), 3.69 (1H, dd, } J = 6.1, 11.0 \text{ Hz), 3.74 (1H, dd, } J = 6.1, 11.0 \text{ Hz), 4.52 (1H, dt, } J = 2.6, 6.0 \text{ Hz), 5.81 (1H, d, } J
\end{align*}
\]
= 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. 2ʹ,3ʹ-Didehydro-2ʹ,3ʹ-dideoxy-5ʹ-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) \( \delta -0.10 \) (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. 2ʹ,3ʹ-Didehydro-5ʹ-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) \( \delta 0.10 \) (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. 2ʹ,3ʹ-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) \( \delta 2.00–2.10 \) (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. 5ʹ-Acethylthio-2ʹ,3ʹ,5ʹ-trideoxyadenosine \( (46) \)

The reaction procedure described in Section 2.1.1. was followed. \( 45 \) (678 mg, 2.88 mmol) was used. Chromatography:
20

(CHCl₃–MeOH, 95:5). 46 was obtained as a yellowish solid (704 mg, 83%). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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. 2ʹ,3ʹ,5ʹ-Trimethoxy-5ʹ-[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: (CHCl₃–MeOH, 9:1). 47 was obtained as a yellowish solid (256 mg, 52%). ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₂₁H₃₄O₂N₆K, 457.2362).

2.13. Analog 50

2.13.1. 2ʹ,3ʹ-Didehydro-2ʹ,3ʹ-dideoxyadenosine (48). The reaction procedure described in Section 2.12.5. was followed. 43 (1.00 g, 2.87 mmol) was used. Chromatography: (CHCl₃–MeOH, 94:6). 48 was obtained as a white powder (722 mg, quant.). ¹H NMR (DMSO-d₆, 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
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: (CHCl₃–MeOH, 97:3). 49 was obtained as a yellowish solid (700 mg, 78%). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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: (CHCl₃–MeOH, 9:1). 50 was obtained as a yellowish solid (146 mg, 45%). ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₂₁H₃₃O₂N₆S, 433.2386).

![Chemical Diagram]

2.14.1. (2-Acetoxyethoxy)methyl bromide (51).\textsuperscript{[55]} 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. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta 2.10 (3H, s), 3.87 (2H, t, J = 4.5 Hz), 4.28 (2H, m), 5.70 (2H, s)\).

2.14.2. 9-\{(2-Acetoxyethoxy)methyl\}adenine (52).\textsuperscript{[55]} 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 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\textsubscript{3} at 0°C. After removing the solvents, the residue was purified by silica gel column chromatography (CHCl\textsubscript{3}–MeOH, 95:5) to give 52 (1.30 g, 70%) as a white solid. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta 1.92 (3H, s), 3.70 (2H, t, J = 4.7 Hz), 4.06 (2H, t, J = 4.7 Hz), 5.56 (2H, s), 7.23 (2H, br), 8.16 (1H, s), 8.26 (1H, s)\).

2.14.3. 9-\{(2-Hydroxyethoxy)methyl\}adenine (53).\textsuperscript{[55]} 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\textsubscript{3}–MeOH, 95:5, 90:10) to give 53 (232 mg, 87%) as a white solid. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta 2.08 (3H, s), 3.70 (2H, t, J = 4.7 Hz), 4.06 (2H, m), 5.56 (2H, s), 7.23 (2H, br), 8.16 (1H, s), 8.26 (1H, s)\).
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: (CHCl3–MeOH, 97:3). 54 was obtained as a yellowish solid (231 mg, 58%). 1H NMR (CDCl3, 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). 13C NMR (CDCl3, 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: (CHCl3–MeOH, 98:2). 55 was obtained as a yellowish solid (76.9 mg, 39%). 1H NMR (DMSO-d6, 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). 13C NMR (DMSO-d6, 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 C19H32O2N6NaS, 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: (CHCl₃–MeOH, 95:5). 56 was obtained as a white powder (163 mg, 52%). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 1.53 (2H, dt, \(J_1 = 6.2, 15.9\) 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: (CHCl₃–MeOH, 95:5, 90:10). 57 was obtained as a white powder (123 mg, 90%). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 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: (CHCl₃–MeOH, 95:5). 58 was obtained as a yellowish solid (163 mg, 52%). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 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: (CHCl₃–MeOH, 95:5). 59 was obtained as a yellowish solid (491 mg, 43%). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 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) \(\delta\) 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\(_{34}\)O\(_6\)N\(_6\)NaS, 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$_2$O, 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) $\delta$ 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) $\delta$ 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) $\delta$ 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$_4$N$_6$NaS, 503.2416).

### 2.17. Analog 6

![Diagram of the analog](image)

#### 2.17.1. 5-Bromopentan-1-amine hydrobromide (63).

The reaction procedure described in Section 2.16.1. was followed. 5-Aminopentan-1-ol (700 µl, 6.44 mmol) was used. 63 was obtained as an ocher solid (2.33 g, quant.). $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 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) $\delta$ 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) $\delta$ 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) $\delta$ 14.1, 22.6, 25.3, 25.8, 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) $\delta$ 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) $\delta$ 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$_4$N$_6$NaS, 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$_2$O, 400 MHz) $\delta$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) $\delta$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) $\delta$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) $\delta$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: (CHCl₃–MeOH, 95:5, 90:10). **7** was obtained as a white solid (157 mg, 52%). \(^1^H\) NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 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). \(^1^C\) NMR (DMSO-\(d_6\), 100 MHz) \(\delta\) 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\(_4\)N\(_6\)NaS, 531.2729).

2.19. Analog 8

2.19.1. 7-Aminoheptan-1-ol (69).\(^{[66]}\) 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 0°C 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 CHCl₃ (20 ml ×3). The CHCl₃ layer was washed with water and dried over Na₂SO₄. Evaporation of the solvents afforded 69 (colorless liquid) with some impurities. ¹H NMR (CDCl₃, 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. ¹H NMR (D₂O, 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). ¹H NMR (CDCl₃, 400 MHz) δ 0.88 (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 = 6.1, 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). ¹³C NMR (CDCl₃, 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.

2.19.4. 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: (CHCl₃–MeOH, 97.5:2.5). 72 was obtained as a white powder (405 mg, 88%). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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) $\delta$0.84 (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) $\delta$13.9, 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$_4$N$_6$NaS, 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) $\delta$0.83 (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) $\delta$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, 71.0, 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) $\delta$0.84 (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). 13C NMR (DMSO-d6, 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-1H-1,2,3-triazol-4-yl)methyl]amine, 125 μM CuSO4, 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–H2O (4:1:4, three times) and MeOH–H2O (1:1, three times), suspended in MeOH–H2O (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.[8] Apo-ACP was prepared from E. coli K-12 cells grown in TB medium.[8] The chemical acylation of apo-ACP by N-octanoylimidazole was carried out according to the method of Cronan et al.[9] 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 MgSO4, and 0.1 mM FeSO4.[10] 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 Na2SO4 and subjected to GC–MS.[11,12] 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 (Ki 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 C₈-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 C₈-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 C₈-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 C₈-ACP. Each point represents the average of triplicate runs.
3.2.5. Analog 7

![Dixon plot for TofI with varying concentrations of analog 7 and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C8-ACP. Each point represents the average of triplicate runs.]

Figure S4. Dixon plot for TofI with varying concentrations of analog 7 and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C8-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 C8-ACP. Each point represents the average of triplicate runs.]

Dixon plot for TofI with varying concentrations of analog 8 and 10 μM (●), 15 μM (▲), and 20 μM (■) SAM and C8-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 \(\mu\)M. 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 ToFIL-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 C8-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.*[^14] 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 MgCl₂, 0.2 mM CaCl₂, 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 1 Chromatogram](image)

Analog 4

![Analog 4 Chromatogram](image)
8. $^1$H and $^{13}$C NMR spectra of acyl-SAM analogs

Analog 1
Analog 23

[Graph of a spectral analysis with various peaks and values标注]

[Table of values related to the spectral analysis]
Analog 33
Analog 56
References