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Synthesis and cytotoxicity of 9-(2-deoxy-2-alkyldithio-β-D-arabino-furanosyl)purine nucleosides which are stable precursors to potential mechanistic probes of ribonucleotide reductases

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A series of 2′-thionucleosides, as potential inhibitors of ribonucleotide reductases, has been synthesized. Treatment of the 3′,5′-*O*-TPDS-2′-*O*-(trifluoromethanesulfonyl)adenosine with potassium thioacetate gave the arabino epimer of 2′-*S*-acetyl-2′-thioadenosine which was deacetylated to give 9-(3,5-*O*-TPDS-2-thio-β-D-arabinofuranosyl)adenine in high yield. Treatment of the latter with diethyl azodicarboxylate–C_3H_7SH–THF gave 2′-propyl disulfide which was desilylated to give 9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine. Subsequent tosylation (O5′) and displacement of the tosylate with pyrophosphate afforded the 5′-*O*-diphosphate in a stable form as propyl mixed-disulfide, which upon treatment with dithiothreitol releases 9-(2-thio-β-D-arabinofuranosyl)adenine 5′-diphosphate. The arabino 2′-mercapto group might interact with the crucial thiyl radical at cysteine 439 leading to the inhibition of ribonucleotide reductases *via* formation of a Cys439–2′-mercapto disulfide bridge. The 2,6-diamino-, 2-amino-6-chloro- and 2-amino-6-methoxypurine ribosides were also converted to the corresponding 2′-deoxy-2′-propyldithio-β-D-arabinofuranosyl nucleosides, which might serve as convenient precursors to the arabino epimer of 2′-thioguanosine. Analogously, 2′-deoxy-2′-propyldithioadenosine was prepared from 9-(β-D-arabinofuranosyl)-adenine. The nucleoside disulfides show modest cytotoxicity in a panel of human tumor cell lines.

Introduction

Ribonucleotide reductases (RNRs) are ubiquitous enzymes that execute conversion of 5′-(di- or tri)phosphate esters of ribonucleosides into the requisite 2′-deoxy building blocks.[1] Inhibition of RNRs disrupts the primary source of DNA components and is an appealing target for the rational drug design.[2] The ribonucleoside diphosphate reductase (RDPR) from *Escherichia coli* consists of two subunits (R1 and R2) whose X-ray structure have been determined.[3] The R1 subunit contains allosteric control sites and cysteine residues that participate in catalytic turnover and/or as redox dithiol/disulfide pairs. The R2 subunit contains an essential tyrosine (Tyr122) free radical that is responsible for generation of a proximate thiyl radical (Cys439) on R1. A thiyl radical initiates nucleotide reduction by abstraction of H3′ from the substrate ribonucleotide and water (O2′) is then lost from C2′ of the resulting hydrogen-bonded C3′ radical.[1a,4]

In spite of numerous attempts using modified substrates as radical traps for both enzyme and substrate radicals, direct observation of nucleotide radical intermediates has remained elusive until very recently.[5–7] Elegant EPR studies with (*E*)-2′-deoxy-2′-(fluoromethylene)cytidine 5′-diphosphates and its [^{2}H] and [^{13}C] isotopomers has allowed detection and characterization of a substrate-derived allyl radical during inactivation of RDPR.[2,5] Spectroscopic evidence has been provided for new radical intermediates in the reduction of cytidine 5′-diphosphate (CDP) by Glu441Gln R1 mutant of RDPR.[6,7] Furthermore, radicals at C3′ of adenosine,[8] and 2′-substituted homonucleosides[9a] and homoribofuranose[9b] have been selectively generated to simulate the initiation–elimination cascades that occur during enzymatic 2′-deoxygenation of ribonucleotides.

Fontecave and Decout and their coworkers demonstrated that 2′-thiouridine 5′-diphosphate **A** is a potent inactivator of RDPR (Fig. 1).[10] They postulated that the mode of inactivation involved substrate-protein disulfide **B** which after generation of a radical at C3′ **C** underwent fragmentation to the protein-based perthiyl radical R1–SS·**D**.[10a] The perthiyl radical **D** can then add to the proximal 2′-deoxy-3′-ketouridine intermediate generated at the active site. Such an addition may produce new radicals which would resemble structures that were proposed to be formed during inactivation of RDPR by 2′-azido-2′-deoxyuridine 5′-diphosphate.[11]

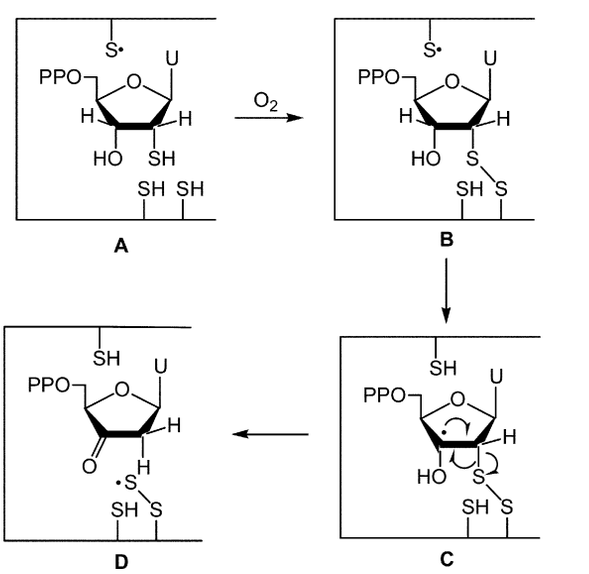

Fig. 1 The proposed mechanism[10a] for the inhibition of RDPR by 2′-deoxy-2′-thiouridine 5′-diphosphate A.

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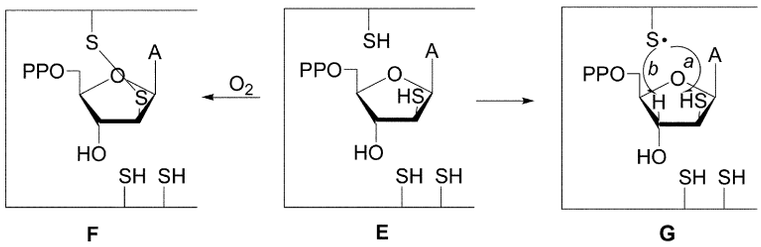
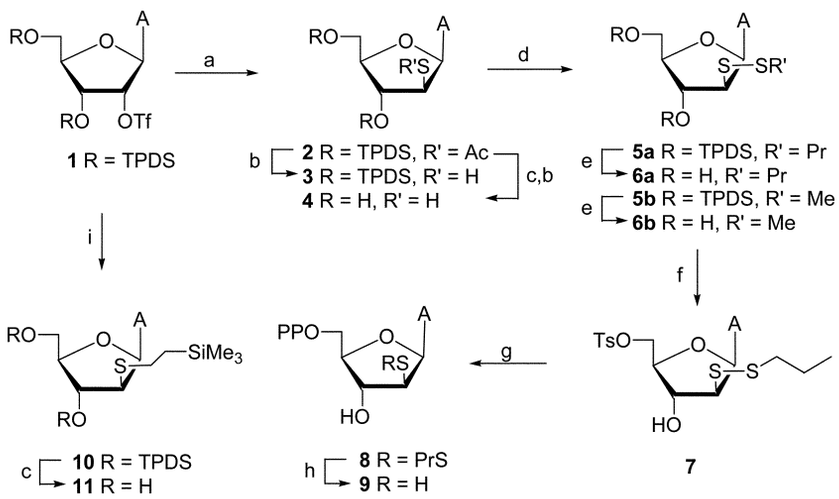


Fig. 2 Possible interaction of RDPR with 9-(2-thio-β-D-arabinofuranosyl)adenine 5′-diphosphate.



Scheme 1 *Reagents and conditions*: (a) CH_3COSK–DMF; (b) NH_3–MeOH; (c) TBAF–THF; (d) DEAD–PrSH or MeSH–THF; (e) NH_4F–MeOH; (f) TsCl–pyridine; (g) $(Bu_4N)_3{\cdot}HP_2O_7$–CH_3CN; (h) DTT–H_2O; (i) $Me_3SiCH_2CH_2SH$–K_2CO_3–DMF.

We now report the syntheses of stable mixed-disulfide precursors to 9-(2-thio-β-D-arabinofuranosyl)adenine 5′-diphosphate (**E**, Fig. 2). The arabino 2′-mercapto group in **E** may interact with Cys439 leading to the inhibition of RDPR *via* formation of R1 Cys439–2′-SH disulfide bridge **F**. Alternatively, competitive abstraction of hydrogen from the 2′-thiol group (path *a*) rather than the H3′ (path *b*) by Cys439 radical **G** may also be considered as a possible interaction pathway. The homolytic bond dissociation energies of 381 kJ mol^{-1} for S–H and 393 kJ mol^{-1} for H–C–O make hydrogen atom abstraction from the 2′-mercapto group (path *a*) thermodynamically more feasible.[4] It is noteworthy that arabinonucleosides have some preferences[12] for C2′ *endo* conformation which is conducive for the formation of disulfide bridge **F** or abstraction of a proton from the 2′-thiol (**G**, path *a*).

Results and discussion

From the available arabino thionucleosides,[13] we chose an adenosine 2′-mercapto analog (*e.g.*, **4**[14]) because attempts to synthesize 1-(2-thio-β-D-arabinofuranosyl)uracil failed due to the rapid Michael addition of the arabino 2′-mercapto function across the double bond of the uracil moiety to give 2′-deoxy-2′,6-epithio-5,6-dihydro derivative.[15] The synthesis of arabino 2′-thio analogs was attempted using two different approaches. In our first approach, treatment of the 3′,5′-*O*-[1,1,3,3-tetraisopropyldisiloxane(TPDS)-1,3-diyl]-2′-*O*-(trifluoromethanesulfonyl)adenosine[16] (**1**) with potassium thioacetate gave the arabino epimer of the protected 2′-*S*-acetyl-2′-thioadenosine (**2**, Scheme 1). Sequential removal of the 3′,5′-*O*-TPDS [tetrabutylammonium fluoride (TBAF)] and 2′-*S*-acetyl (NH_3–MeOH) groups afforded 9-(2-thio-β-D-arabinofuranosyl)-adenine[14] (**4**; 41% from **1**) after purification on Dowex 1 × 2 (OH^-) column. Compound **4** had spectroscopic data as reported[14b] but slow formation of the corresponding disulfide was apparent since treatment of the mixture with dithiothreitol (DTT) led to a sharper spot on TLC.

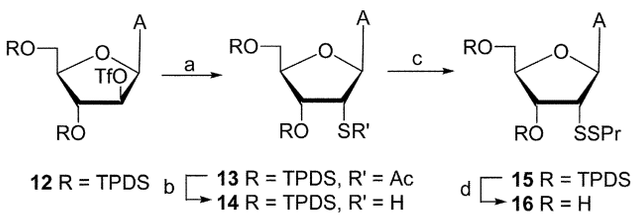
The thiols protected as mixed-disulfides were prepared using diethyl azodicarboxylate (DEAD) as an oxidizing agent.[10b,17] Thus, treatment of **4** with DEAD and 1-propanethiol in THF gave disulfide **6a** (18%) plus unchanged **4** (25%). In order to overcome the limited solubility of deprotected thiol **4** in THF, 9-(3,5-*O*-TBDS-2-thio-β-D-arabinofuranosyl)adenine **3** was prepared by deacetylation of **2** with NH_3–MeOH (97%). Treatment of **3** with DEAD–C_3H_7SH–THF gave protected disulfide **5a** (45%) but separation from the diethyl hydrazinedicarboxylate byproduct was tedious. Desilylation (NH_4F–MeOH[18]) gave **6a** (60%) which was readily purified. Diagnostic peaks for the propyl group were observed in the ^1H and ^{13}C NMR spectra of **5a** and **6a**.

In the second approach, chemistry utilizing 2-(trimethylsilyl)ethyl sulfides[19] was employed for the attempted preparation of mixed-methyl disulfide **6b**. Thus, displacement of triflate from **1** with 2-(trimethylsilyl)ethanethiolate in DMF proceeded smoothly at 60 °C to give 2′-*S*-[(2-trimethylsilyl)-ethyl]-2′-thionucleoside **10** in good yield. Deprotection of **10** with TBAF gave **11**, which also demonstrated stability of the 2-(trimethylsilyl)ethyl group towards fluoride.[20] However, reaction of **10** or **11** with dimethyl(methylthio)sulfonium tetrafluoroborate[19,20] in the presence of large excess of dimethyl sulfide failed to produce methyl mixed-disulfides **5b** or **6b**. Instead unchanged **10** or **11** and fluorescent-type byproduct(s) were isolated from the reaction mixtures indicating instability of the purine ring (as opposed to pyrimidine[19]) under the reaction conditions. However, treatment of thiol **3** with MeSH–DEAD–THF gave **5b** that was deprotected to yield less sterically hindered **6b**.

From the methods available for the 5′-phosphorylation of nucleosides,[21] we chose the methodology of Poulter and coworkers[22] that is based on the displacement of the 5′-*O*-tosylate ester with the corresponding pyrophosphate ion. Thus, disulfide **6a** was converted to the 5′-*O*-tosylate **7** using standard chemistry with the bulky propyl group allowing selective 5′-*O*-tosylation. Treatment of **7** with tris(tetrabutylammonium) hydrogen

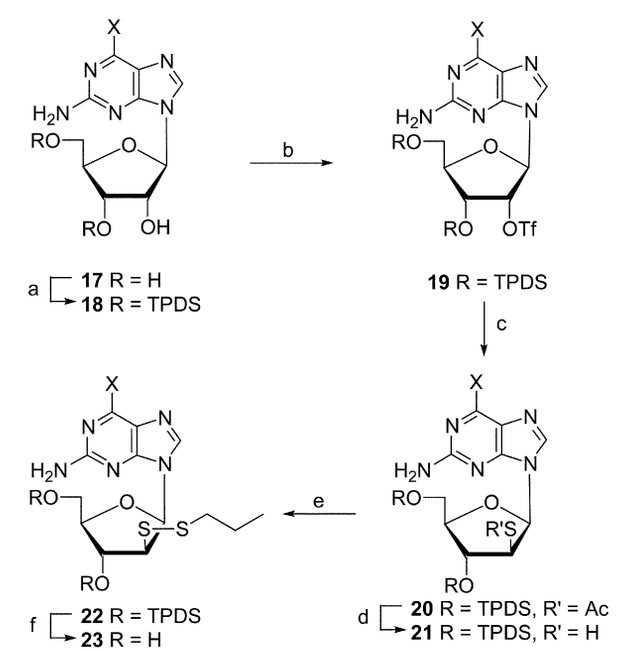
pyrophosphate[22] effected displacement to give 5′-diphosphate **8**. The nucleotide **8** was purified by ion-exchange chromatography on a DEAE Sephadex A-25 (HCO_3^-) column using triethyl ammonium bicarbonate (TEAB, 0.05 ⟶ 0.50 M) as an elutant. The ^{31}P NMR spectrum showed two doublets (J = 23.5 Hz) at δ −9.75 and −5.26 and the ^{1}H NMR spectrum confirmed the structure. This material was converted to its sodium salt by passage through a Dowex 50 (Na^+) column. Reduction of diphosphate **8** with a slight excess of DTT immediately generated[10a] the mercaptonucleotide **9**. Dithiothreitol was chosen as a reducing agent since it is one of the electron sources used in the assays for ribonucleotide reductase activity *in vitro*.

In addition to the propyl **6a** and methyl **6b** mixed-disulfides, the ribo analogue **16** was synthesized in order to test differences in the biological activities between ribo and arabino epimers. Subjection of 9-(3,5-*O*-TPDS-2-*O*-triflate-β-D-arabinofuranosyl)adenine[16] **12** to the similar dithiolation sequence (**12** ⟶ **16**, Scheme 2) gave ribo disulfide **16** in 11% overall yield.



Scheme 2 *Reagents and conditions*: (a) CH_3COSK–DMF; (b) NH_3–MeOH; (c) DEAD–PrSH–THF; (d) NH_4–MeOH.

The 2-amino-6-chloro-, 2-amino-6-methoxy-, and 2,6-diaminopurine ribosides are known to be substrates of adenosine deaminase.[23] The deaminase-mediated hydrolysis at C6 of 2-amino-6-(substituted) mixed-disulfides **23a**–**c** (Scheme 3) would generate the arabino epimer of 2′-thioguanosine. Subjection of 2-amino-6-chloro- (**17a**), 2-amino-6-methoxy- (**17b**) and 2,6-diaminopurine riboside (**17c**) to our triflation–dithiolation sequence (**17** ⟶ **23**), produced arabino mixed-disufides **23a** (14%), **23b** (10%), and **23c** (24%; overall yield).



Scheme 3 *Reagents and conditions*: (a) TPDS-Cl_2–pyridine; (b) Tf_2NPh–DMAP–$CHCl_2$; (c) CH_3COSK–DMF; (d) NH_3–MeOH; (e) DEAD–PrSH–THF; (f) NH_4F–MeOH.

Cytotoxic activities

The disulfide nucleosides **6a**, **6b**, **16**, and **23a**–**c** were evaluated for their cytotoxicities in a panel of human tumor cell lines. This panel was comprised of SNB-7 (CNS), DLD-1 (colon), CCRF-CEM (leukemia), NCI-H23 (lung), ZR-75-1 (mammary), LOX IMVI (melanoma), PC-3 (prostate), and CAKI-1 (renal) cells. The two mixed *arabino* disulfides with adenine as the base, **6a** and **6b**, were not cytotoxic up to the highest doses evaluated (>150 μM) in any of the eight cell lines. The mixed *ribo* disulfide **16** had a weak IC_{50} of 110 μM in CCRF-CEM cells, and exhibited no cytotoxicity up to the highest doses in any other cell lines. The three mixed *arabino* disulfides containing 2,6-disubstituted purines were similarly non-toxic to most of the cell lines. In CCRF-CEM cells, which are known to be sensitive to many nucleosides, **23a** had an IC_{50} of 14 μM and **23b** had an IC_{50} of 19 μM. The only other cytotoxicities seen were for **23a** (100 μM, NCI-H23), **23b** (160 μM, DLD-1, and 140 μM, NCI-H23), and **23c** (130 μM, CCRF-CEM). The lack of or very modest cytotoxicity seen with these analogs may be the result of their inability to be activated by the appropriate enzymes to the monophosphate level and then up to the di- and/or triphosphate levels.

We have synthesized 9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine and 2-amino-6-substituted(amino/chloro/methoxy)purine analogues which are stable precursors of arabino 2′-deoxy-2′-mercaptonucleotides. The mixed propyldisulfides were chosen as in general they could be deprotected under mild reductive conditions compatible with biological assays to form the corresponding mercaptonucleosides. These mercaptonucleosides can serve as valuable probes for understanding the role of the Cys439 radical during enzymatic deoxygenation reaction catalyzed by ribonucleotide reductases. Studies on inhibition of RDPR with **9** are in progress.[24]

Experimental

^{1}H (Me_4Si) NMR spectra were determined with solution in $CDCl_3$ at 400 MHz, ^{13}C (Me_4Si) at 100.6 MHz and ^{31}P (H_3PO_4) at 161.9 MHz unless otherwise noted. Mass spectra (MS) were obtained by atmospheric pressure chemical ionization (APCI) technique. Reagent grade chemicals were used and solvents were dried by reflux over and distillation from CaH_2 under an argon atmosphere except THF (K–benzophenone). TLC was performed on Merck kieselgel 60-F_{254} with MeOH–$CHCl_3$ (1 : 19) and EtOAc–hexane (4 : 1) as developing systems, and products were detected with 254 nm light. Merck kieselgel 60 (230–400 mesh) was used for column chromatography. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. The 2,6-(disubstituted)purine ribosides **17a**–**c** were purchased from Berry & Associates, Inc, Ann Arbor, MI.

9-[2-*S*-Acetyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]adenine (2)

Procedure A. CH_3COSK (434 mg, 3.8 mmol) was added to a solution of **1**[16] (1.53 g, 2.38 mmol) in dried DMF (10 mL) and the resulting mixture was stirred overnight at ambient temperature. Volatiles were evaporated and the residue was partitioned (EtOAc–$NaHCO_3$–H_2O). The organic layer was washed (NaCl–H_2O), dried ($MgSO_4$), evaporated and the residue was column chromatographed (EtOAc–hexanes; 70 : 30) to give **2**[25] (1.01 g, 75%): mp 68–76 °C; UV max 260 nm (ε 15 100), min 233 nm (ε 6200); ^{1}H NMR δ 0.8–1.3 (m, 28, 4 × *i*-Pr), 2.19 (s, 3, Ac), 3.99–4.08 (m, 2, H4′,5′), 4.28 (dd, J = 4.5, 12.5 Hz, 1, H5″), 4.59 (dd, J = 7.3, 10.3 Hz, 1, H2′), 5.06 (dd, J = 8.2, 10.0 Hz, 1, H3′), 6.45 (d, J = 7.3 Hz, 1, H1′), 6.30 (br s, 2, NH_2), 7.95 (s, 1, H2), 8.29 (s, 1, H8); MS *m*/*z* 568 (100, MH^+). Anal. calcd for $C_{24}H_{41}N_5O_5Si_2S$ (567.86): C, 50.76; H, 7.28; N, 12.33. Found: C, 50.39; H, 7.49; N, 11.94%.

9-[3,5-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]adenine (3)

Procedure B. A solution of **2** (241 mg, 0.43 mmol) in saturated (0 °C) NH_3–MeOH (50 mL) was stirred for 30 min at ~0 °C. Volatiles were evaporated, and the residue was column chromatographed ($CHCl_3$–MeOH; 95 : 5) to give **3** [254 mg, 97%; contaminated (^{1}H NMR) with 0.5 equiv. of CH_3CONH_2]: mp 78–82 °C; UV max 261 nm (ε 14 400), min 230 nm (ε 3100); ^{1}H NMR δ 1.02–1.19 (m, 28, 4 × *i*-Pr), 1.47 (d, *J* = 8.1 Hz, 1, SH), 3.87 (dd, *J* = 7.6, 9.7 Hz, 1, H2′), 3.91 (dt, *J* = 2.8, 8.2 Hz, 1, H4′), 4.07 (dd, *J* = 2.7, 12.9 Hz, 1, H5′), 4.24 (dd, *J* = 2.8, 12.9 Hz, 1, H5″), 4.62 (t, *J* = 8.9 Hz, 1, H3′), 6.12 (br s, 2, NH_2), 6.42 (d, *J* = 7.1 Hz, 1, H1′), 8.14 (s, 1, H2), 8.35 (s, 1, H8); ^{13}C NMR δ 12.8–17.9 (12, 4 × *i*-Pr), 48.7 (C2′), 61.4 (C5′), 75.7 (C3′), 84.2 (C4′), 85.3 (C1′), 120.1 (C5), 139.7 (C8), 150.2 (C4), 153.2 (C2), 155.9 (C6); MS *m*/*z* 526 (100, MH^+). For elemental analysis, this material was repurified [column chromatography ($CHCl_3$–MeOH; 97 : 3)]. Anal. calcd for $C_{22}H_{39}N_5O_4Si_2S$ (525.54): C, 50.28; H, 7.43; N, 13.33. Found: C, 49.91; H, 7.68; N, 12.91%.

9-(2-Thio-β-D-arabinofuranosyl)adenine (4)

Treatment of **2** (283 mg, 0.50 mmol) with TBAF (1 M solution in THF; 1 mL) in THF (15 mL) at 0 °C for 30 min followed by NH_3–MeOH (10 mL; 2 h, ambient temperature) gave crude **4**. This material was partitioned (EtOAc–H_2O) and the water layer was chromatographed [Dowex 1 × 2 (OH^-); H_2O ⟶ 25% MeOH–H_2O) to give **4**[14b] [89 mg, 55%; contaminated (^{1}H NMR) by the corresponding dimer-disulfide].

9-[3,5-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-propyldithio-β-D-arabinofuranosyl]adenine (5a)

Procedure C. A solution of **3** (370 mg, 0.70 mmol) and DEAD (113 μL, 0.72 mmol) in THF (7 mL) was stirred overnight at ambient temperature and then propane-1-thiol (3.68 mL, 3.09 g, 41 mmol) was added. Reaction mixture was refluxed for 8 h and volatiles were evaporated. The residue was partitionated (Na_2CO_3–H_2O–EtOAc) and the organic layer was washed (NaCl), dried ($MgSO_4$), evaporated and column chromatographed (EtOAc–hexane; 1 : 1) to give **5a** (190 mg, 45%): mp 110–118 °C (soften); UV max 261 nm (ε 7600), min 233 nm (ε 1400); ^{1}H NMR δ 0.94 (t, *J* = 7.4 Hz, 3, CH_3), 1.07–1.17 (m, 28, 4 × *i*-Pr), 1.62 (sextet, *J* = 7.4 Hz, 2, CH_2), 2.61 (t, *J* = 7.4 Hz, 2, SCH_2), 3.91–3.97 (m, 2, H2′,4′), 4.05–4.10 (m, 2, H5′,5″), 4.60 (t, *J* = 8.5 Hz, 1, H3′), 6.54 (d, *J* = 6.8 Hz, 1, H1′), 8.15 (s, 1, H2), 8.33 (s, 1, H8); MS *m*/*z* 600 (100, MH^+).

9-[3,5-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-methyldithio-β-D-arabinofuranosyl]adenine (5b)

A solution of **3** (100 mg, 0.19 mmol) and DEAD (29 μL, 0.19 mmol) in THF (5 mL) was stirred overnight at ambient temperature and then methanethiol (183 mg, 3.8 mmol) in THF (3 mL) was added. Stirring was continued at ambient temperature for 96 h, and then volatiles were evaporated and the residue was column chromatographed (EtOAc–hexane; 1 : 1) to give **5b** (41 mg, 38%): ^{1}H NMR δ 1.04–1.14 (m, 28, 4 × *i*-Pr), 2.32 (s, 3, SCH_3), 3.92–3.99 (m, 2, H2′,4′), 4.05–4.21 (m, 2, H5′,5″), 4.76 (t, *J* = 7.8 Hz, 1, H3′), 6.07 (br s, 2, NH_2), 6.53 (d, *J* = 7.1 Hz, 1, H1′), 8.01 (s, 1, H2), 8.33 (s, 1, H8); MS *m*/*z* 572 (100, MH^+).

9-(2-Deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine (6a)

Procedure D. NH_4F (222 mg, 6.0 mmol) was added to a stirred solution of **5a** (180 mg, 0.3 mmol) in MeOH (25 ml). After 18 h, volatiles were removed *in vacuo* and the residue was chromatographed ($CHCl_3$–MeOH; 95 : 5) to give **6a** (64 mg, 60%): mp 120–124 °C (dec.); UV max 261 nm (ε 14 800), min 231 nm (ε 5100); ^{1}H NMR (MeOH-d_4) δ 0.87 (t, *J* = 7.3 Hz, 3, CH_3), 1.53 (sextet, *J* = 7.3 Hz, 2, CH_2), 2.57 (t, *J* = 7.2 Hz, 2, CH_2S), 3.86–3.95 (m, 4, H2′,4′,5′,5″), 4.53 (t, *J* = 7.6 Hz, 1, H3′), 6.58 (d, *J* = 7.1 Hz, 1, H1′), 8.21 (s, 1, H2), 8.44 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 12.13 (CH_3), 22.03 (CH_2), 40.96 (SCH_2), 60.22 (C2′), 62.54 (C5′), 71.94 (C3′), 84.92 & 85.32 (C4′ & C1′), 119.0 (C5), 140.79 (C8), 149.50 (C4), 152.68 (C2), 156.30 (C6); MS *m*/*z* 358 (100, MH^+). Anal. calcd. for $C_{13}H_{19}N_5O_3S_2 \cdot H_2O$ (375.47): C, 41.59; H, 5.64; N, 18.65. Found: C, 41.95; H, 5.65; N, 18.27%.

Treatment of **4** (159 mg, 0.56 mmol) with DEAD (98 mg, 0.57 mmol) and propane-1-thiol (2.9 mL, 2.4 g, 32 mmol) in THF (15 mL), as described for **5a**, also gave **6a** (36 mg, 18%).

9-(2-Deoxy-2-methyldithio-β-D-arabinofuranosyl)adenine (6b)

Treatment of **5b** (19 mg, 0.033 mmol) by procedure D (chromatography: EtOAc ⟶ 4% MeOH–EtOAc) gave **6b** (7.3 mg, 67%) as a white powder: UV max 261 nm (ε 12 700), min 230 nm (ε 2800); ^{1}H NMR (MeOH-d_4) δ 2.33 (s, 3, CH_3S), 3.87–4.00 (m, 4, H2′,4′,5′,5″), 4.57 (t, *J* = 7.7 Hz, 1, H3′), 6.57 (d, *J* = 7.0 Hz, 1, H1′), 8.20 (s, 1, H2), 8.39 (s, 1, H8); MS *m*/*z* 330 (100, MH^+). Anal. calcd. for $C_{11}H_{15}N_5O_3S_2 \cdot 0.5\ C_4H_8O_2$ (373.45; ^{1}H NMR integration of residual EtOAc): C, 41.81; H, 5.13; N, 18.75. Found: C, 41.47; H, 5.38; N, 18.39%.

9-[2-Deoxy-2-propyldithio-5-*O*-(*p*-tolylsulfonyl)-β-D-arabinofuranosyl]adenine (7)

p-Tolylsulfonyl chloride (106 mg, 0.55 mmol) was added to a solution of **6a** (40 mg, 0.11 mmol) in anhydrous pyridine (2 mL) and the reaction mixture was stirred for 3 h at 0 °C. Volatiles were evaporated, and the residue was partitioned ($NaHCO_3$–H_2O–EtOAc). The organic layer was washed (AcOH–H_2O, $NaHCO_3$–H_2O), dried ($MgSO_4$), evaporated and column chromatographed (EtOAc ⟶ 2.5% MeOH–EtOAc) to give **7** (28 mg, 52%): ^{1}H NMR δ 0.90 (t, *J* = 7.2 Hz, 3, CH_3), 1.56 (sextet, *J* = 7.2 Hz, 2, CH_2), 2.42 (s, 3, $PhCH_3$), 2.59 (m, 2, CH_2S), 3.90 (t, *J* = 8.2 Hz, 1, H2′), 4.16 (m, 1, H4′), 4.42 (dd, *J* = 3.1, 11.2 Hz, 1, H5′), 4.45 (dd, *J* = 4.5, 11.3 Hz, 1, H5″), 4.81 (t, *J* = 8.2 Hz, 1, H3′), 6.48 (d, *J* = 7.2 Hz, 1, H1′), 7.27 (d, *J* = 8.0 Hz, 2, H_{Ar}), 7.87 (d, *J* = 8.1 Hz, 2, H_{Ar}), 7.99 (s, 1, H2), 8.29 (s, 1, H8); MS *m*/*z* 512 (100, MH^+).

9-(2-Deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine 5′-diphosphate (8)

Tris(tetra-*n*-butylammonium) hydrogenpyrophosphate[22] (31 mg, 0.035 mmol) was added to a stirred solution of **7** (12 mg, 0.023 mmol) in CH_3CN (0.5 mL) and stirring was continued at ambient temperature for 72 h. Volatiles were removed *in vacuo* and the residue was dissolved in water and was purified on an ion exchange resin [Sephadex-DEAE A-25, 40–125 μm; gradient elution with triethylammonium bicarbonate (0.05 ⟶ 0.5 M)]. Appropriated fractions were evaporated, and the residue was dissolved in H_2O and was converted to a sodium salt by passing the solution through a Dowex (Na^+) column to yield **8** (4 mg, 29%) as a trisodium salt: ^{1}H NMR (MeOH-d_4) δ 0.64 (t, *J* = 6.3 Hz, 3, CH_3), 1.27 (sextet, *J* = 6.5 Hz, 2, CH_2), 2.32 (t, *J* = 6.7 Hz, 2, CH_2S), 3.88 (dd, *J* = 7.4, 8.8 Hz, 1, H2′), 4.03–4.07 (m, 1, H4′), 4.19–4.23 (m, 2, H5′,5″), 4.49 (t, *J* = 8.3 Hz, 1, H3′), 6.84 (d, *J* = 7.1 Hz, 1, H1′), 8.11 (s, 1, H2), 8.33 (s, 1, H8); ^{31}P NMR δ −9.75 (d, *J* = 23.5 Hz, 1, P_α), −5.26 (d, *J* = 23.5 Hz, 1, P_β).

Treatment of **8** (1 mg) with dithiothreitol (1 mg) in H_2O (1 mL) produced **9** as judged by the appearance of a new spot on TLC and immediate releasing of the PrSH odor.

9-[2-*S*-(2-Trimethylsilylethyl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]adenine (10)

2-(Trimethylsilyl)ethanethiol (0.1 mL, 90 mg, 0.7 mmol) was added to a stirred suspension of **1** (371 mg, 0.60 mmol) and

K_2CO_3 (276 mg, 2.0 mmol) in DMF (3 mL) at ambient temperature, and the reaction mixture was heated at 60 °C overnight. Volatiles were evaporated and the residue was partitioned [$CHCl_3$–$NaHCO_3$–H_2O (ice-cold)]. The organic layer was washed (NaCl–H_2O), dried ($MgSO_4$), evaporated and column chromatographed (EtOAc–hexanes; 1 : 1) to give **2** (108 mg, 30%): ^{1}H NMR δ −0.07 (s, 9, $SiMe_3$), 0.57 (dt, J = 5.2, 14.0 Hz, 1, CH_2Si), 0.61 (dt, J = 5.2, 14.0 Hz, 1, CH_2Si), 0.80–1.31 (m, 28, 4 × i-Pr), 2.49 (dt, J = 5.6, 12.7 Hz, 1, SCH_2), 2.53 (dt, J = 5.6, 12.7 Hz, 1, SCH_2), 3.72 (dd, J = 7.0, 9.7 Hz, 1, H2′), 3.89 (dt, J = 3.0, 7.9 Hz, 1, H4′), 4.09 (dd, J = 2.8, 12.9 Hz, 1, H5′), 4.16 (dd, J = 3.2, 12.9 Hz, 1 H5″), 4.55 (dd, J = 8.3, 9.3 Hz, 1, H3′), 6.50 (d, J = 7.0 Hz, 1, H1′), 8.08 (s, 1, H2), 8.32 (s, 1, H8); MS m/z 626 (100, MH^+). Anal. calcd for $C_{27}H_{51}N_5O_4SSi_3 \cdot H_2O$ (644.07): C, 50.35; H, 8.29; N, 10.87. Found: C, 50.39; H, 8.11; N, 10.49%.

Treatment (ambient temperature to ~45 °C, 2 h to overnight) of **10** (97 mg, 0.16 mmol) [or **11** (27 mg, 0.07 mmol)] with dimethyl(methylthio)sulfonium tetrafluoroborate (219 mg, 1.12 mmol) and dimethyl sulfide (0.72 mL, 496 mg, 8 mmol) in anhydrous THF (5 mL) gave recovered **10** or **11** (~20% to 80% depends on reaction conditions) plus other byproduct(s).

9-[2-*S*-(2-Trimethylsilylethyl)-2-thio-β-D-arabinofuranosyl]-adenine (11)

TBAF–THF (1M; 0.3 mL, 0.3 mmol) was added to a solution of **10** (50 mg, 0.08 mmol) in THF (2 mL) and was stirred for 2h at ambient temperature. Volatiles were evaporated and the residue was column chromatographed (EtOAc ⟶ 8% MeOH–EtOAc) to give **11** (27 mg, 90%): ^{1}H NMR δ −0.08 (s, 9, $SiMe_3$), 0.59–0.63 (m, 2, $SiCH_2$), 2.40–2.45 (m, 2, SCH_2), 3.74 (dd, J = 6.9, 8.8 Hz 1, H2′), 3.80–4.01 (m, 3, H4′,5′,5″), 4.32–4.38 (m, 1, H3′), 6.57 (d, J = 6.9 Hz, 1, H1′), 8.20 (s, 1, H2), 8.41 (s, 1, H8); MS m/z 384 (100, MH^+).

2′-*S*-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2′-thioadenosine (13)

Treatment of **12**[16] (290 mg, 0.45 mmol) by procedure A gave **13**[25] (200 mg, 78%): ^{1}H NMR δ 1.05–1.12 (m, 28, 4 × i-Pr), 2.31 (s, 3, Ac), 4.02–4.09 (m, 3, H4′,5′,5″), 4.70 (dd, J = 4.8, 7.4 Hz, 1, H2′), 5.26 (dd, J = 5.4, 7.2 Hz, 1, H3′), 6.07 (d, J = 4.8 Hz, 1, H1′), 7.97 (s, 1, H2), 8.29 (s, 1, H8); MS m/z 568 (100, MH^+).

3′,5′-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2′-thioadenosine (14)

Treatment of **13** (180 mg, 0.31 mmol) by procedure B gave **14** (160 mg, 96%): ^{1}H NMR δ 1.03–1.15 (m, 28, 4 × i-Pr), 2.19 (d, J = 6.4 Hz, 1, SH), 4.08 (d, J = 4.7 Hz, 1, H2′), 4.17–4.25 (m, 3, H4′,5′,5″), 4.88 (t, J = 6.4 Hz, 1, H3′), 5.98 (d, J = 7.1 Hz, 1, H1′), 6.22 (br s, 2, NH_2), 8.01 (s, 1, H2), 8.34 (s, 1, H8); MS m/z 526 (100, MH^+).

3′,5′-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2′-deoxy-2′-propyldithioadenosine (15)

Treatment of **14** (140 mg, 0.27 mmol) by procedure C gave **15** (67 mg, 42%): ^{1}H NMR δ 0.89 (t, J = 7.3 Hz, 3, CH_3), 1.07–1.12 (m, 28, 4 × i-Pr), 1.59 (sextet, J = 7.2 Hz, 2, CH_2), 2.56 (t, J = 7.4 Hz, 2, SCH_2), 4.05–4.07 (m, 2, H2′,4′), 4.19–4.26 (m, 2, H5′,5″), 5.20 (t, J = 7.3 Hz, 1, H3′), 6.07 (br s, 2, NH_2), 6.34 (d, J = 3.5 Hz, 1, H1′), 8.03 (s, 1, H2), 8.29 (s, 1, H8); MS m/z 600 (100, MH^+).

2′-Deoxy-2′-propyldithioadenosine (16)

Treatment of **15** (60 mg, 0.1 mmol) by procedure D gave **16** (12.4 mg, 35%): mp 145–147 °C (dec; EtOAc–MeOH); UV max 260 nm (ε 15 400), min 229 nm (ε 4100); ^{1}H NMR (MeOH-d_4) δ 0.74 (t, J = 7.3 Hz, 3, CH_3), 1.36 (sextet, J = 7.1 Hz, 2, CH_2), 2.18–2.24 (m, 2, CH_2S), 3.78 (dd, J = 2.4, 12.6 Hz, 1, H5′), 3.92 (dd, J = 2.6, 12.6 Hz, 1, H5″), 4.19–4.25 (m, 1, H4′), 4.30 (dd, J = 5.0, 9.2 Hz, 1, H2′), 4.57 (d, J = 5.0 Hz, 1, H3′), 6.20 (d, J = 9.2 Hz, 1, H1′), 8.22 (s, 1, H2), 8.35 (s, 1, H8); MS m/z 358 (100, MH^+). Anal. calcd. for $C_{13}H_{19}N_5O_3S_2 \cdot 0.5\ C_4H_8O_2$ (401.50; ^{1}H NMR integration of residual EtOAc): C, 44.87; H, 5.77; N, 17.44. Found: C, 44.48; H, 5.71; N, 17.21%.

2-Amino-6-methoxy-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-ribofuranosyl]purine (18b)

TPDS-Cl_2 (317 mg, 0.32 mL, 1.01 mmol) was added to a stirred solution of 2-amino-6-methoxy-(β-D-ribofuranosyl)-purine (**17b**; 300 mg, 1.01 mmol) in dry pyridine (8 mL), and the mixture was stirred overnight at ambient temperature. Volatiles were evaporated and the residue was partitioned (EtOAc–H_2O). The organic phase was washed [cold 0.1 M HCl–H_2O (2 × 30 mL), H_2O, saturated $NaHCO_3$–H_2O, and brine], dried (Na_2SO_4), and evaporated. Column chromatography ($CHCl_3$ ⟶ 4% MeOH–$CHCl_3$) gave **18b** (375 mg, 69%): ^{1}H NMR δ 0.93–1.12 (m, 28, 4 × i-Pr), 4.03–4.15 (m, 6, H4′,5′,5″, OCH_3), 4.49 (d, J = 4.7 Hz, 1, H2′), 4.82 (m, 1, H3′), 5.32 (br s, 2, NH_2), 6.04 (s, 1, H1′), 7.82 (s, 1, H8); MS m/z 540 (100, MH^+). Anal. calcd for $C_{23}H_{41}N_5O_6Si_2$ (539.77): C, 51.18; H, 7.66; N, 12.97. Found: C, 51.38; H, 7.88; N, 12.88%.

2-Amino-6-chloro-9-[2-*O*-trifluoromethanesulfonyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-ribofuranosyl]purine (19a)

Procedure E. *N*-Phenyltrifluoromethanesulfonimide (237 mg, 0.66 mmol) was added to a cold (0 °C) stirred solution of **18a**[26] (300 mg, 0.55 mmol) and DMAP (201 mg, 1.65 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was stirred for 2 h and partitioned between cold 0.1 M AcOH–H_2O (50 mL) and CH_2Cl_2 (2 × 50 mL). The combined organic phase was washed with cold $NaHCO_3$–H_2O (50 mL), brine (50 mL) and dried $MgSO_4$, filtered and evaporated. The residue was column chromatographed (EtOAc–hexane; 30 : 70) to give **19a** (310 mg, 83%): ^{1}H NMR δ 1.04–1.12 (m, 28, 4 × i-Pr), 4.07 (dd, J = 2.7, 13.5 Hz, 1, H5′), 4.13 (dt, J = 2.4, 7.0 Hz, 1, H4′), 4.50 (d, J = 13.5 Hz, 1, H5″), 4.86 (dd, J = 4.5, 9.3 Hz, 1, H3′), 5.64 (d, J = 4.5 Hz, 1, H2′), 6.10 (s, 1, H1′), 8.02 (s, 1, H8); MS m/z 676 (100, MH^+[^{35}Cl]), 678 (30, MH^+[^{37}Cl]).

2-Amino-6-methoxy-9-[2-*O*-trifluoromethanesulfonyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-ribofuranosyl]purine (19b)

Treatment of **18b** (200 mg, 0.37 mmol) by procedure E gave **19b** (156 mg, 64%): ^{1}H NMR δ 1.04–1.12 (m, 28, 4 × i-Pr), 4.03–4.23 (m, 6, H4′,5′,5″,OCH_3), 4.85 (br s, 2, NH_2), 4.94 (dd, J = 4.7, 9.0 Hz, 1, H3′), 5.70 (d, J = 4.7 Hz, 1, H2′), 6.04 (s, 1, H1′), 7.82 (s, 1, H8); MS m/z 672 (100, MH^+).

2,6-Diamino-9-[2-*O*-trifluoromethanesulfonyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-β-D-ribofuranosyl]purine (19c)

Treatment of **18c**[27] (370 mg, 0.706 mmol) by procedure E (column chromatography: EtOAc–hexane, 75 : 25) gave amorphous **19c** (360 mg, 78%), which failed to crystallize (MeOH): ^{1}H NMR δ 1.01–1.12 (m, 28, 4 × i-Pr), 4.04–4.20 (m, 3, H4′,5′,5″), 4.66 (br s, 2, NH_2), 4.99 (dd, J = 4.5, 8.9 Hz, 1, H3′), 5.41 (br s, 2, NH_2), 5.81 (d, J = 4.5 Hz, 1, H2′), 6.05 (s, 1, H1′), 7.71 (s, 1, H8); ^{13}C NMR δ 13.2–17.4 (12, 4 × i-Pr), 60.1 (C2′), 68.6 (C5′), 81.6 (C3′), 86.9 (C4′), 88.4 (C1′), 115.0 (C5), 136.7 (C8), 151.7 (C4), 155.8 (C2), 159.7 (C6); MS m/z 657 (100, MH^+). Anal. calcd for $C_{23}H_{39}F_3N_6O_7SSi_2 \cdot CH_4O$ (688.86, ^{1}H NMR integration of MeOH): C, 41.84; H, 6.29; N, 12.20. Found: C, 41.56; H, 5.99; N, 12.25%.

2-Amino-6-chloro-9-[2-*S*-acetyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (20a)

Treatment of **19a** (250 mg, 0.37 mmol) by procedure A (column chromatography: EtOAc–hexanes, 20 : 80) gave **20a** (150 mg, 67%): mp 78–86 °C; ^{1}H NMR δ 0.8–1.3 (m, 28, 4 × *i*-Pr), 2.20 (s, 3, Ac), 3.94–3.97 (m, 1, H4′), 4.09 (dd, J = 3.2, 12.0 Hz, 1, H5′), 4.16 (dd, J = 3.2, 12.0 Hz, 1, H5″), 4.56 (dd, J = 7.0, 10.5 Hz, 1, H2′), 4.68 (t, J = 10.5 Hz, 1, H3′), 5.24 (br s, 2, NH_2), 6.39 (d, J = 7.0 Hz, 1, H1′), 7.96 (s, 1, H8); ^{13}C NMR δ 13.3–17.6 (12, 4 × *i*-Pr), 30.7 (CH_3) 52.2 (C2′), 61.4 (C5′), 71.66 (C3′), 83.7 (C4′), 83.9 (C1′), 125.7 (C5), 141.3 (C8), 151.5 (C4), 153.8 (C2), 159.1 (C6), 194.0 (CO); MS *m*/*z* 602 (100, MH$^+$[^{35}Cl]), 604 (40, MH$^+$[^{37}Cl]). Anal. calcd for $C_{24}H_{40}ClN_5O_5SSi_2$ (602.30): C, 47.86; H, 6.69; N, 11.63. Found: C, 47.87; H, 6.67; N, 11.24.

2-Amino-6-methoxy-9-[2-*S*-acetyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (20b)

Treatment of **19b** (100 mg, 0.15 mmol) by procedure A (column chromatography: EtOAc–hexanes, 20 : 80) gave **20b** (65 mg, 73%): ^{1}H NMR δ 1.04–1.20 (m, 28, 4 × *i*-Pr), 2.19 (s, 3, Ac), 3.93–3.96 (m, 1, H4′), 4.05–4.09 (m, 4, H5′, OCH_3), 4.16 (dd, J = 3.8, 12.8 Hz, 1, H5″), 4.55 (dd, J = 7.2, 10.4 Hz, 1, H2′), 4.77 (dd, J = 8.2, 10.4 Hz, 1, H3′), 4.87 (br s, 2, NH_2), 6.35 (d, J = 7.2 Hz, 1, H1′), 7.79 (s, 1, H8); MS *m*/*z* 598 (100, MH$^+$).

2,6-Diamino-9-[2-*S*-acetyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (20c)

Treatment of **19c** (340 mg, 0.52 mmol) by procedure A (column chromatography: EtOAc–hexanes, 20 : 80) gave **20c** (220 mg, 73%): ^{1}H NMR δ 1.04–1.20 (m, 28, 4 × *i*-Pr), 2.22 (s, 3, Ac), 3.95 (dt, J = 3.2, 7.9 Hz, 1, H4′), 4.06 (dd, J = 3.1, 12.8 Hz, 1, H5′), 4.09 (dd, J = 4.2, 12.8 Hz, 1, H5″), 4.57 (dd, J = 7.2, 10.4 Hz, 1, H2′), 4.65 (br s, 2, NH_2), 4.84 (t, J = 10.2 Hz, 1, H3′), 5.35 (br s, 2, NH_2), 6.30 (d, J = 7.2 Hz, 1, H1′), 7.72 (s, 1, H8); ^{13}C NMR δ 12.8–17.8 (12, 4 × *i*-Pr), 30.6 (CH_3), 52.4 (C2′), 61.9 (C5′), 72.6 (C3′), 83.4 (C4′), 83.8 (C1′), 114.9 (C5), 137.3 (C8), 152.2 (C4), 156.0 (C2), 159.9 (C6), 194.0 (CO); MS *m*/*z* 583 (100, MH$^+$).

2-Amino-6-chloro-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (21a)

Treatment of **20a** (110 mg, 0.18 mmol) by procedure B (column chromatography: EtOAc–hexanes, 35 : 65) gave **21a** (95 mg, 93%): mp 270 °C (dec.); ^{1}H NMR δ 1.02–1.19 (m, 28, 4 × *i*-Pr), 1.42 (d, J = 8.0 Hz, 1, SH), 3.83–3.89 (m, 2, H2′,4′), 4.12 (dd, J = 2.8, 13.2 Hz, 1, H5′), 4.15 (dd, J = 2.8, 13.2 Hz, 1, H5″), 4.44 (t, J = 8.5 Hz, 1, H3′), 5.25 (br s, 2, NH_2), 6.32 (d, J = 7.0 Hz, 1, H1′), 8.10 (s, 1, H8); MS *m*/*z* 560 (100, MH$^+$[^{35}Cl]), 562 (40, MH$^+$[^{37}Cl]).

2-Amino-6-methoxy-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (21b)

Treatment of **20b** (120 mg, 0.2 mmol) by procedure B (column chromatography: EtOAc–hexane, 35 : 65) gave **21b** (102 mg, 91%): ^{1}H NMR δ 1.03–1.18 (m, 28, 4 × *i*-Pr), 1.39 (d, J = 8.2 Hz, 1, SH), 3.80–3.86 (m, 2, H2′,4′), 4.04–4.07 (m, 4, H5′,OCH_3), 4.15 (dd, J = 2.6, 13.0 Hz, 1, H5″), 4.46 (t, J = 8.5 Hz, 1, H3′), 5.02 (br s, 2, NH_2), 6.31 (d, J = 7.1 Hz, 1, H1′), 7.95 (s, 1, H8); MS *m*/*z* 556 (100, MH$^+$).

2,6-Diamino-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-thio-β-D-arabinofuranosyl]purine (21c)

Treatment of **20c** (210 mg, 0.36 mmol) by procedure B (column chromatography: EtOAc–hexane, 75 : 15) gave amorphous **21c** (160 mg, 82%), which failed to crystallize (MeOH): ^{1}H NMR δ 1.03–1.19 (m, 28, 4 × *i*-Pr), 1.51 (d, J = 8.0 Hz, 1, SH), 3.81 (dd, J = 7.5, 13.2 Hz, 1, H2′), 3.87–3.89 (m, 1, H,4′), 4.07 (dd, J = 2.6, 13.0 Hz, 1, H5′), 4.19 (dd, J = 2.6, 13.0 Hz, 1, H5″), 4.51 (t, J = 8.7, Hz, 1, H3′), 4.75 (br s, 2, NH_2), 5.46 (br s, 2, NH_2), 6.27 (d, J = 7.1 Hz, 1, H1′), 7.84 (s, 1, H8); ^{13}C NMR δ 12.8–17.9 (12, 4 × *i*-Pr), 48.5 (C2′), 61.2 (C5′), 75.7 (C3′), 83.9 (C4′), 84.4 (C1′), 114.5 (C5), 137.0 (C8), 152.3 (C4), 155.8 (C2), 159.6 (C6); MS *m*/*z* 541 (100, MH$^+$). Anal. calcd for $C_{22}H_{40}N_6O_4SSi_2 \cdot CH_4O$ (572.87; ^{1}H NMR integration of MeOH): C, 48.22; H, 7.74; N, 14.67. Found: C, 48.65; H, 7.56; N, 14.92%.

2-Amino-6-chloro-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-propyldithio-β-D-arabinofuranosyl]purine (22a)

Treatment of **21a** (122 mg, 0.212 mmol) by procedure C (after thiol was added, the reaction mixture was stirred overnight at ambient temperature) and column chromatography (EtOAc–hexane, 30 : 70) gave **22a** (66 mg, 48%): ^{1}H NMR δ 0.92 (t, J = 7.2 Hz, 3, CH_3), 1.05–1.27 (m, 28, 4 × *i*-Pr), 1.60 (sextet, J = 7.2 Hz, 2, CH_2), 2.56 (t, J = 7.2 Hz, 2, SCH_2), 3.86–3.89 (m, 2, H2′,4′), 4.08 (dd, J = 3.0, 12.7 Hz, 1, H5′), 4.10 (dd, J = 3.0, 12.7 Hz, 1, H5″), 4.51 (dd, J = 8.0, 9.6 Hz, 1, H3′), 5.20 (br s, 2, NH_2), 6.42 (d, J = 7.0 Hz, 1, H1′), 7.98 (s, 1, H8); MS *m*/*z* 634 (100, MH$^+$[^{35}Cl]), 636 (55, MH$^+$[^{37}Cl]).

2-Amino-6-methoxy-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-propyldithio-β-D-arabinofuranosyl]purine (22b)

Treatment of **21b** (110 mg, 0.2 mmol) by procedure C (after thiol was added, the reaction mixture was stirred overnight at ambient temperature) and column chromatography (EtOAc–hexane, 30 : 70) gave **22b** (52 mg, 42%): ^{1}H NMR δ 0.90 (t, J = 7.2 Hz, 3, CH_3), 1.05–1.27 (m, 28, 4 × *i*-Pr), 1.58 (sextet, J = 7.2 Hz, 2, CH_2), 2.56 (t, J = 7.2 Hz, 2, SCH_2), 3.85–3.90 (m, 2, H2′,4′), 4.07–4.09 (m, 5, H5′,5″,OCH_3), 4.59 (dd, J = 8.4, 9.5 Hz, 1, H3′), 4.82 (br s, 2, NH_2), 6.41 (d, J = 7.0 Hz, 1, H1′), 7.80 (s, 1, H8); MS *m*/*z* 630 (100, MH$^+$).

2,6-Diamino-9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-propyldithio-β-D-arabinofuranosyl]purine (22c)

Treatment of **21c** (145 mg, 0.268 mmol) by procedure C (after thiol was added, the reaction mixture was stirred overnight at ambient temperature) and column chromatography (EtOAc–hexane, 90 : 10) gave **22c** (110 mg, 67%): ^{1}H NMR δ 0.87 (t, J = 7.2 Hz, 3, CH_3), 1.04–1.27 (m, 28, 4 × *i*-Pr), 1.55 (sextet, J = 7.2 Hz, 2, CH_2), 2.53 (t, J = 7.2 Hz, 2, SCH_2), 3.84–3.87 (m, 2, H2′,4′), 4.06–4.12 (m, 2, H5′,5″), 4.56 (t, J = 8.2 Hz, 1, H3′), 4.92 (br s, 2, NH_2), 5.86 (br s, 2, NH_2), 6.39 (d, J = 7.0 Hz, 1, H1′), 7.70 (s, 1, H8); MS *m*/*z* 615 (100, MH$^+$).

2-Amino-6-chloro-9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)purine (23a)

Treatment of **22a** (30 mg, 0.047 mmol) by procedure D (column chromatography: $CHCl_3$–MeOH, 96 : 4) gave **23a** (17 mg, 91%): mp 190–192 °C (dec.); UV max 309 nm (ε 8800), max 248 nm (ε 8200), min 272 nm (ε 1900), min 236 nm (ε 6800); ^{1}H NMR (MeOH-d_4) δ 0.89 (t, J = 7.2 Hz, 3, CH_3), 1.53 (sextet, J = 7.2 Hz, 2, CH_2), 2.58 (t, J = 7.2 Hz, 2, CH_2S), 3.84–3.92 (m, 4, H2′,4′,5′,5″), 4.49 (t, J = 8.0 Hz, 1, H3′), 6.52 (d, J = 7.0 Hz, 1, H1′), 8.35 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 12.1 (CH_3), 22.0 (CH_2), 41.0 (SCH_2), 60.3 (C2′), 62.7 (C5′), 72.7 (C3′), 84.8 & 84.9 (C4′ & C1′), 123.7 (C5), 142.4 (C8), 150.6 (C4), 154.0 (C2), 160.6 (C6); MS *m*/*z* 392 (100, MH$^+$[^{35}Cl]), 394 (55, MH$^+$[^{37}Cl]). Anal. calcd. for $C_{13}H_{18}ClN_5O_3S_2$ (391.90): C, 39.84; H, 4.63; N, 17.87. Found: C, 39.63; H, 4.79; N, 17.49%.

2-Amino-6-methoxy-9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)purine (23b)

Treatment of **22b** (40 mg, 0.064 mmol) by procedure D (column chromatography: $CHCl_3$–MeOH, 96 : 4) gave **23b** (20 mg,

81%): mp 81–85 °C; UV max 282 nm (ε 9700), max 249 nm (ε 9600), min 262 nm (ε 5300), min 227 nm (ε 4600); ^{1}H NMR (MeOH-d_4) δ 0.89 (t, J = 7.3 Hz, 3, CH_3), 1.53 (sextet, J = 7.3 Hz, 2, CH_2), 2.58 (t, J = 7.3 Hz, 2, CH_2S), 3.82–3.96 (m, 4, H2′,4′,5′,5″), 4.07 (s, 3, OCH_3), 4.52 (t, J = 8.2 Hz, 1, H3′), 6.47 (d, J = 7.1 Hz, 1, H1′), 8.08 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 12.1 (CH_3), 22.0 (CH_2), 41.0 (SCH_2), 53.3 (OCH_3), 60.4 (C2′), 63.1 (C5′), 72.4 (C3′), 84.7 & 84.9 (C4′ & C1′), 114.1 (C5), 139.1 (C8), 153.7 (C4), 160.7 (C2), 161.7 (C6); MS *m*/*z* 388 (100, MH^+). Anal. calcd. for $C_{14}H_{21}N_5O_4S_2 \cdot CH_4O$ (419.52; ^{1}H NMR integration of MeOH): C, 42.94; H, 6.01; N, 16.69. Found: C, 43.01; H, 5.76; N, 16.66%.

2,6-Diamino-9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)-purine (23c)

Treatment of **22c** (110 mg, 0.18 mmol) by procedure D (column chromatography: $CHCl_3$–MeOH, 96 : 4) gave **23c** (57 mg, 85%): mp 82–90 °C (soften), 110 °C; UV max 283 nm (ε 11 200), max 257 nm (ε 9600), min 267 nm (ε 7900), min 238 nm (ε 6300); ^{1}H NMR (MeOH-d_4) δ 0.89 (t, J = 7.2 Hz, 3, CH_3), 1.53 (sextet, J = 7.2 Hz, 2, CH_2), 2.58 (t, J = 7.2 Hz, 2, CH_2S), 3.81–3.96 (m, 4, H2′,4′,5′,5″), 4.51 (t, J = 8.8 Hz, 1, H3′), 6.41 (d, J = 7.2 Hz, 1, H1′), 7.98 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 12.1 (CH_3), 22.0 (CH_2), 41.0 (SCH_2), 60.4 (C2′), 63.2 (C5′), 72.4 (C3′), 84.7 & 84.9 (C4′ & C1′), 113.1 (C5), 137.8 (C8), 151.6 (C4), 156.6 (C2), 160.7 (C6); MS *m*/*z* 373 (100, MH^+). Anal. calcd. for $C_{13}H_{20}N_6O_3S_2 \cdot H_2O$ (390.49): C, 39.99; H, 5.68; N, 21.52. Found: C, 40.35; H, 5.78; N, 21.33%.

Cell culture

The eight human cell lines used were obtained from the Developmental Therapeutics Program Tumor Repository, National Cancer Institute (Frederick, MD). The cell lines were grown in RPMI 1640 medium containing 9% fetal bovine serum, 1% iron-supplemented calf serum, and 2 mmol L-glutamine. For the *in vitro* evaluation of the sensitivity of the human cell lines to the compounds, cells were plated in 96-well microtiter plates (5.0×10^3 cells per well) and then were exposed continuously to various concentrations of the compounds for 72 h at 37 °C. Cell viability was based on the reduction of MTS (in conjunction with phenazine ethosulfate) to a water soluble formazan product, which was measured with a microplate reader at 490 nm. The background absorbance mean was subtracted from the data followed by conversion to percent of control. The drug concentrations producing survival just above and below the 50% level were used in a linear regression analysis to calculate the IC_{50}.

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