Inhibition of Nucleoside Transport Proteins by C^8-Alkylamine-Substituted Purines

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4-Nitrobenzylthioinosine (NBTI, **1**) is a well-known inhibitor for the nucleoside transport protein ENT1. Here we report on the synthesis and the biological evaluation of compounds that are less polar than NBTI. Compound screening in our laboratory indicated that introduction of an alkylamine substituent at the C^8-position of N^6-cyclopentyladenosine (CPA, **2**) led to an increment in affinity for the transport protein. It was investigated whether this would also apply for NBTI derivatives. Two series of C^8-alkylamine-substituted compounds were prepared, one in which the nitro group was absent (**46**−**58**) and another in which the ribose moiety was replaced by a benzyl group (**72**−**75**). Comparison of the biological data of these compounds with 6-benzylthioinosine (**4**, K_i = 53 nM) and 9-benzyl-6-(4-nitrobenzylsulfanyl)purine (**59**, K_i = 135 nM) confirmed the hypothesis. The K_i values improved upon elongation of the alkylamine chain from methylamine to *n*-hexylamine with an optimum for *n*-pentylamine (**50**, K_i = 2.3 nM). Substitution with 2-methylbutylamine (**52**), cyclopropylamine (**53**), cyclopentylamine (**54**, **72**), and cyclohexylamine (**55**, **73**) revealed that the presence of a bulky group enhanced the affinity. The presence of tertiary amines obtained by substitution with pyrrolidine, piperidine, and morpholine gave only poor results. For both series substitution with cyclopentylamine was most effective. Compound **54** (LUF5942) proved the most active, showing a comparable affinity (K_i = 0.64 nM) to NBTI but a significantly lower polar surface area.

Introduction

Nucleosides, such as adenosine, rely on active transport via specialized proteins to cross the cell membrane as a consequence of their hydrophilic nature.[1] Two classes of these nucleoside transport proteins exist. The first, the concentrative nucleoside proteins, or CNTs, depends on sodium ions and drives the nucleoside flow against its concentration. Subtypes of this family are CNT1, CNT2, and CNT3.[2] The second family, the equilibrative nucleoside transport proteins, or ENTs, drives the nucleoside flow following its concentration gradient and includes the subtypes ENT1 and ENT2 and the more recently discovered ENT3 and ENT4.[3] Differences in the subtypes is expressed in different substrate specificity.

Both nucleoside transport protein classes have been recognized as a target for the treatment of several diseases. They may be able to modulate the uptake of nucleoside analogues that act as antiviral or anticancer agents into target tissues.[2,3] Inhibition of the nucleoside transport protein ENT1 is an approach to combat ischemic heart diseases and stroke and has also been found to protect host tissue in chemotherapy.[4−6] Here, the basic principle is that blockage of the transport protein leads to an increased extracellular concentration of adenosine. This results in a more profound occupancy of the adenosine A_1 receptor through which adenosine exerts its physiological effects, e.g. counteracting pain.[7,8] A highly potent inhibitor is 4-nitrobenzylthioinosine [NBTI (**1**), Figure 1]. However, its highly polar nature hinders its oral bioavailability and passage through the blood−brain barrier.

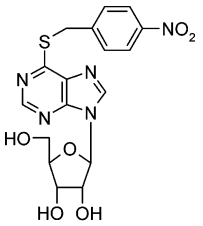

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Figure 1. 4-Nitrobenzylthioinoisine (NBTI).

In our present research we are exploring new compounds that can act as ENT1 inhibitors but are less polar than NBTI itself. Both the nitro group and the ribose moiety of NBTI (**1**) add greatly to its affinity, and thus removal of these highly polar fragments would lower a compound's polarity but concomitantly also decrease its affinity. However, screening of compounds prepared earlier in our laboratory revealed that, when N^6-cyclopentyladenosine (CPA, **2**), a highly potent reference agonist for adenosine A_1 receptors, was substituted at the 8-position of the purine ring with *n*-propylamine (**3**), the compound's potency for ENT1 was increased by a factor of 11 (Figure 2). This improvement of affinity by C^8-substitution with alkylamines could compensate for this loss in potency when omitting either the ribose moiety or the nitro group, resulting in compounds that are less polar but still show affinity in the low nanomolar range.

Here, we describe the synthesis and biological evaluation of C^8-alkylamine-substituted analogues of 6-benzylthioinosine (**4**).[9] We also prepared a number of C^8-alkylamine-substituted analogues of 9-benzyl-6-(4-nitrobenzylsulfanyl)purine[10] (**59**), since substitution of the ribose group for a benzyl moiety also reduces the

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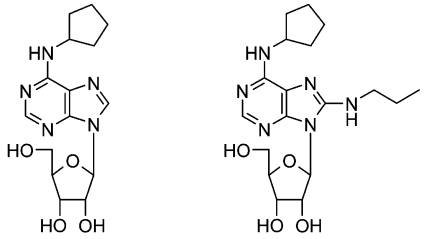
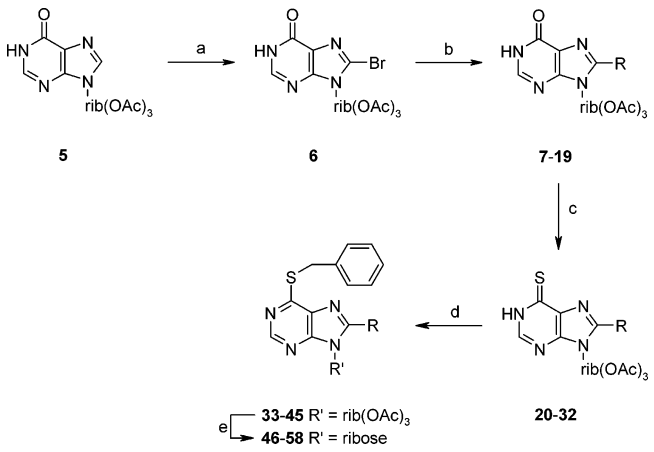


Figure 2. C^8-Substitution of N^6-cyclopentyladenosine (CPA) with *n*-propylamine improved its affinity for the ENT1 nucleoside transport protein.

Scheme 1[a]



R = NHMe, NHEt, NH(*n*-Pr), NH(*n*-Bu), NH(*n*-Pent), NH(*n*-Hex), NH(2-Me-Butyl), NH(*c*-Pr), NH(*c*-Pent), NH(*c*-Hex), pyrrolidine, piperidine, morpholine

[a] Reagents: (a) Br_2, Na_2HPO_4, H_2O, dioxane, rt, 7 d; (b) alkylamine, dioxane (/H_2O), 80 °C, 3 d; (c) 1) phosphorus pentasulfide, pyridine, reflux, 7 h; (2) H_2O, 50 °C, 1 h; (d) benzylbromide, K_2CO_3, DMF, rt, overnight; (e) NH_3, MeOH, 0 °C to room temperature, overnight.

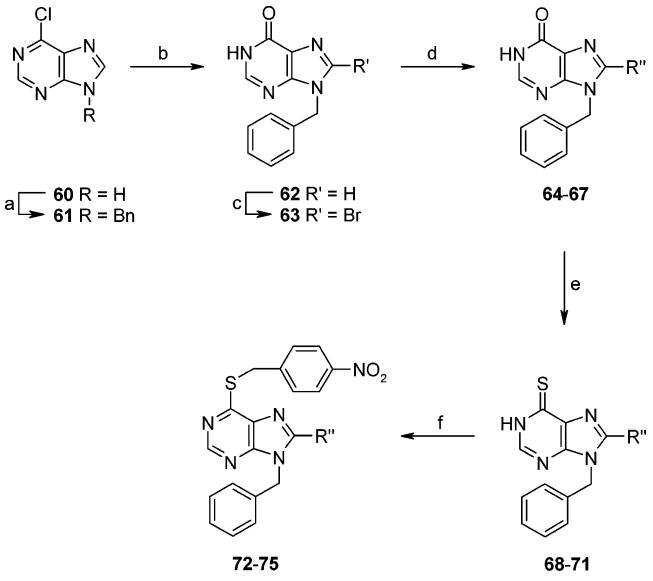
number of both hydrogen-bond donors and acceptors, important in terms of cell permeability and uptake into the central nervous system.

Chemistry. The synthetic route toward the C^8-alkylamino-substituted benzylthioinosines (**46**−**58**) was based on a route designed for the synthesis of amine-substituted inosines.[11] The synthesis of reference compound 6-benzylthioinosine (**4**) has been described before.[9]

First, acetylated inosine (**5**) was brominated to provide **6** as described by Roelen et al. (Scheme 1).[11] Next, the alkylamine groups were introduced by stirring the respective amine in dioxane for 3 days at elevated temperatures in the presence of **6**, giving **7**−**19**. The thiol function was introduced by refluxing the purines with phosphorus pentasulfide in pyridine, yielding thioinosines **20**−**32**.[12] Benzylation[13] (**33**−**45**) and subsequent deacetylation[14] provided compounds **46**−**58**.

The C^8-substituted analogues of 9-benzyl-6-(4-nitrobenzylsulfanyl)purine **59**[10] were prepared by following the same route as the one described above. N^9-Benzylhypoxanthine (**62**) was prepared from commercially available 6-chloropurine (**60**) in two steps (Scheme 2).[15,16] After bromination, which afforded **63**, the introduction of the alkylamines was performed under microwave conditions. Attempts to introduce primary amines under the conditions used for the synthesis of **7**−**19** failed. Therefore, mixtures of bromide **63** and either cyclopentylamine or cyclohexylamine in dioxane/water were heated for 5 h at 175 °C to provide **64** and **65**. Pyrrolidine and morpholine containing **66** and **67** could be obtained under both regular and microwave conditions (2.5 h at 150 °C). Thiols **68**−**71** were synthesized by heating **64**−**67** in pyridine in the presence of phosphorus pentasulfide.[12] Benzylation[13] of the sulfur using 4-nitrobenzyl bromide provided **72**−**75**.

Scheme 2[a]



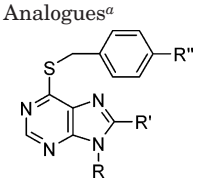
R" = NH(*c*-Pent), NH(*c*-Hex), pyrrolidino, morpholino

[a] Reagents: (a) benzyl bromide, NaH, DMF, 0 °C to room temperature, overnight; (b) 1 M HCl, reflux, 2 h; (c) Br_2, Na_2HPO_4, H_2O, dioxane, rt, 7 d; (d) alkylamine, dioxane, H_2O, microwave, 175 °C, 5 h; (e) (1) phosphorus pentasulfide, pyridine, reflux, 7 h; (2) H_2O, 50 °C, 1 h; (f) 4-nitrobenzyl bromide, K_2CO_3, DMF, rt, overnight.

Polar Surface Area. The polar surface area (PSA) was calculated as a measure to quantify the polarity of the compounds described in this study. The PSA is defined as the van der Waals surface of a molecule occupied by nitrogen and oxygen and the hydrogen atoms that are attached to these atoms. Literature values for the upper limit for good intestinal absorption range from 120 to 140 Å^2.[17−19] To allow for passage through the blood−brain barrier, a compound's PSA value should be lower than 60−90 Å^2.[18,20] Variations in the given limits result from differences in the calculation methods.

The polar surface area of the molecules presented in this report were calculated using Spartan 5.0 for SGI,[21] in combination with an in-house developed application called PolSurf 1.0. The PSA value of NBTI (**1**) was determined to be 154 Å^2 based on the conformation (syn) of the crystal structure.[22] The value for the anti-conformation was calculated to be 163 Å^2. The difference results from the overlap of the N^3 of the purine ring with the ribose moiety in the case of the syn-conformation leading to a lower van der Waals surface compared to that of the anti-conformation in which both the 5′-OH and the N^3 are exposed. The same holds for parent compound **4**, giving PSA values of 108 and 118 Å^2 for

Table 1. Affinities and PSA Values for the Novel NBTI Analogues[a]



compd	R	R′	R″	PSA (Å^2)[b]	K_i (nM)
4	ribose	H	H	108 (syn)/118 (anti)	53 (±3)
46	ribose	NHMe	H	125	28 (±11)
47	ribose	NHEt	H	124	9.5 (±0.7)
48	ribose	NH(n-Pr)	H	124	5.3 (±1.4)
49	ribose	NH(n-Bu)	H	124	5.4 (±1.9)
50	ribose	NH(n-Pent)	H	122	2.3 (±0.1)
51	ribose	NH(n-Hex)	H	122	3.7 (±0.9)
52	ribose	NH(2-Me-Butyl)	H	122	3.6 (±1.0)
53	ribose	NH(c-Pr)	H	124	3.0 (±0.8)
54	ribose	NH(c-Pent)	H	123	0.64 (±0.31)
55	ribose	NH(c-Hex)	H	120	0.94 (±0.28)
56	ribose	pyrrolidino	H	121	410 (±262)
57	ribose	piperidino	H	120	744 (±43)
58	ribose	morpholino	H	132	33%c
59	benzyl	H	NO_2	86	135 (±30)[d]
72	benzyl	NH(c-Pent)	NO_2	100	29 (±8)
73	benzyl	NH(c-Hex)	NO_2	99	55 (±15)
74	benzyl	pyrrolidino	NO_2	97	1519 (±523)
75	benzyl	morpholino	NO_2	109	51 (±15)

[a] Binding study: [^{3}H]NBTI (K_D = 0.59 nM) as radioligand and human erythrocyte membranes (n = 3). K_i values are shown with SEM in parentheses. [b] See the Experimental Section for details on the calculation of the polar surface area (PSA). The given PSA values for **46**−**58** concern the syn-conformer. PSA values for the anti-conformer were up to 3 Å^2 higher. [c] Percentage of displacement at a concentration of 10 μM. [d] Taken from the literature.[10]

its syn- and anti-conformation, respectively (Table 1). The PSA values for compounds **46**−**58** and **72**−**75** are depicted in Table 1 together with their biological data. For ribose-containing **46**−**58**, the PSA values represent those obtained for the syn-conformer. Values for the anti-conformation were determined to be only slightly higher (up to 3 Å^2) compared to those of the syn-conformation. The low variation between the PSA values for the syn- or the anti-conformation in case of the C^8-amino-substituted compounds results from the fact that either the exocyclic alkylamine at C^8 is exposed while the N^3 overlaps with the ribose moiety or visa versa.

Biological Studies. Compounds were tested in a radioligand binding assay using human erythrocyte membranes as the source of the nucleoside transport protein and [^{3}H]NBTI as the radioligand (K_D value 0.59 ± 0.07 nM). Compounds that inhibited radioligand binding for 50% or more at a single concentration of 10 μM were further analyzed over a range of concentrations. Their IC_{50} values were converted to K_i values, which are represented in Table 1.

The most active compounds of both series (**54** and **72**) were also tested for their affinity at the adenosine A_1 receptor. CHO cells expressing the human adenosine A_1 receptor were used with [^{3}H]DPCPX as the radioligand. Radioligand displacement was determined at a single concentration of 1 μM.

Results and Discussion

Parent compound benzylthioinosine (**4**) showed a K_i value of 53 nM (Table 1). Substitution at the C^8-position of the purine ring with methylamine (**46**) led to an almost 2-fold improvement in affinity (K_i = 28 nM), while the K_i value of ethylamine-substituted **47** (9.5 nM) was four times lower than that of parent compound **4**. The presence of n-propyl- (**48**) and n-butylamine (**49**) resulted in affinities of 5.4 and 5.3 nM, repectively. n-Pentylamine (**50**) yielded a K_i value of 2.3 nM, only slightly higher than NBTI (**1**, K_D = 0.59 nM), but compound **50** is considerably less polar. Further elongation of the alkyl chain did not lead to further improvement in affinity as shown by n-hexylamine substituted **51** (K_i = 3.7 nM).

Introduction of a bulkier alkylamine proved to give a higher affinity as was shown by 2-methylbutylamine-substituted **52** (K_i = 3.7 nM), which proved to be more potent than n-butylamine **49**. This was confirmed by compounds **53**−**55**, carrying cycloalkylamines. In comparison with ethylamine-substituted **47**, the presence of cyclopropylamine at the C^8-position (**53**) gave rise to a 3-fold increase in affinity (K_i = 3.0 nM). Larger alkyl rings further improved the potency. Introduction of cyclopentylamine (**54**) showed a similar affinity as NBTI (**1**) with a K_i value of 0.64 nM, while its polarity is considerably lower, with a PSA value of 123 Å^2 (versus 154 Å^2 for NBTI), well within the limits for intestinal absorption as suggested by Clark.[19] Cyclohexylamine-substituted **55** proved almost equipotent with a K_i value of 0.94 nM.

C^8-Substitution with pyrrolidine (**56**) and piperidine (**57**) indicated that the presence of tertiary amines at the C^8-position greatly decreased the affinity for ENT1, showing K_i values of 410 and 744 nM, respectively. The presence of an oxygen in the morpholine function present in **58** even further decreased the affinity in comparison with **57**, showing only 33% displacement at 10 μM. A possible explanation for these large differences in affinity may be the steric hindrance between the C^8-substituents of **56**−**58** and the ribose moiety. While the actual binding conformation to ENT1 is not clear, the

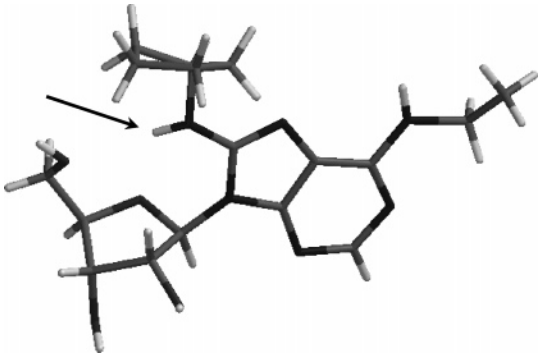


Figure 3. The crystal structure[11] of 8-(cyclopentylamino)-N^6-ethyladenosine, the C^6-ethylamine substituted derivative of **54**. The amine hydrogen of the cyclopentylamine substituent at C^8 (see arrow) is situated above the ribose ring.

crystal structure[11] of a derivative of **54** that carried an *n*-ethylamine substituent at C^6 instead of a benzyl thioether was found to have the anti-conformation (Figure 3). Here the hydrogen attached to the exocyclic amine at C^8 is positioned directly above the ribose moiety close to both the ring oxygen and the 5′-OH. The steric hindrance that occurs when the hydrogen is replaced by an alkyl group as in **56**−**58** will not only lead to a different orientation of the C^8-substituent but also have an effect on the orientation of the purine ring with respect to the ribose moiety.

In the case of the N^9-benzylthiohypoxanthine derivatives **72**−**75**, the presence of cyclopentylamine (**72**) or cyclohexylamine (**73**) also improved the affinity compared to parent compound **54**, although to a lesser extent than found for the ribose-containing compounds. Where for **59** an improvement in affinity of over 80-fold with respect to unsubstituted **4** was found, compound **72** (K_i = 29 nM) was just over 4.5 times more potent than parent compound **59**. As for the ribose compounds, cyclohexylamine-substituted **73** (K_i = 55 nM) was less active than its cyclopentylamine analogue **72**. Substitution with pyrrolidine (**74**) again resulted in a lower affinity compared to the parent compound (**59**), showing a K_i value of 1519 nM. In contrast to ribose-containing **58**, the presence of a morpholino function in combination with a benzyl group at the N^9-position (**75**) increased the affinity with respect to unsubstituted **59**, giving a K_i value of 51 nM.

To study their selectivity, compounds **54** and **72** were tested for their affinity toward the human adenosine A_1 receptor. Both compounds showed only poor displacement of the radioligand in the binding assay, i.e., 12% at 1 μM for each of them, indicating good selectivity for the ENT1 nucleoside transport protein over the human adenosine A_1 receptor.

Conclusions

In this report the synthesis and biological evaluation of new NBTI analogues were addressed. In our search for compounds that are less polar than NBTI, the nitro functionality was omitted. To compensate for the loss in affinity, the C^8-position of the purine ring was substituted with alkylamines. Compounds with affinities at the low nanomolar level and PSA values close to the limits given for blood−brain barrier passage were obtained. Substitution with primary amines resulted in improved affinities in comparison with the unsubstituted parent compound (**4**). In general, a bulkier alkylamine resulted in a more potent compound. However, C^8-substitution with secondary amines, resulting in tertiary amines, greatly decreased a ligand's affinity for ENT1. This may result from steric hindrance between the amine substituent and the ribose moiety. Cyclopentylamine-substituted **54** gave the best affinity, with a K_i value of 0.64 nM, an 80-fold increase compared to unsubstituted **4**. In addition, a high selectivity toward the nucleoside transport protein was observed over the human A_1 receptor. With a K_i value comparable to that of NBTI, but considerably less polar, compound **54** may have more favorable characteristics in aspects of absorption and distribution.

Experimental Section

Column chromatography was performed on Baker silica gel (0.063−0.200 mm). For TLC analysis, Schleicher and Schuell F1500/LS 254 silica plates were used. Spots were visualized with ultraviolet light. Microwave reactions were performed in an Emrys Optimizer. ^{1}H NMR and ^{13}C NMR were recorded with a Bruker AC 200 spectrometer at room temperature unless indicated otherwise. Tetramethylsilane was used as internal standard; δ values are reported in ppm and *J* in Hz. Melting points were determined with a Büchi melting point apparatus and are uncorrected. High-resolution mass spectroscopy was performed on a PE-Sciex API Qstar instrument. Elemental analysis was accomplished on a PE-2400 series II CHNS/O analyzer.

Analytical HPLC for compound **72** was performed on a RP-18 column, 5.0 μm, 125 × 4 mm. Mobile phase: system A = 10 mM AcOH in MeOH/H_2O (75/25 v/v), flow rate 0.6 mL/min; system B = gradient between (A) MeCN/H_2O (1/9 v/v), 10 mM AcOH, and 5 mM SDS and (B) MeCN/H_2O (9/1 v/v), 10 mM AcOH, and 5 mM SDS; gradient elution t = 0 min (40% B), t = 15 min (100% B), t = 18 min (100% B), flow rate 0.6 mL/min.

The polar surface areas of the molecules were calculated using Spartan 5.0 for SGI,[21]21 in combination with an in-house developed application called PolSurf 1.0

(A copy of PolSurf and its C source code can be obtained from the corresponding author). First Spartan was used to build the molecule and to optimize its 3D-structure by molecular mechanics (Merck force field) followed by semiempirical AM1 single point energy calculation. The electrostatic potentials were calculated over the entire accessible surface of the molecules (roughly equal to the van der Waals contact surface). Subsequently PolSurf was applied to convert the raw data from the Spartan "input" and "proparc" files into the polar surface area of the molecule.

The crystal structure[11] (CSD code TASWEP) as depicted in Figure 3 was retrieved from the Cambridge Structural Database[23] using ConQuest[24] and visualized with PC Spartan Pro.[25]

General Procedure A.[11] To a solution of 2′,3′,5′-tri-*O*-acetyl-8-bromoinosine (**6**) in dioxane (10 mL per mmol) was added the appropiate alkylamine (14 equiv). After stirring for 3 days at 80 °C the reaction mixture was evaporated to dryness. The residue was dissolved in dry pyridine and evaporated to dryness (two times). To a solution of the resulting residue in pyridine (3.5 mL per mmol) were added acetic acid anhydride (0.85 mL per mmol) and a catalytic amount of DMAP. The mixture was stirred for 4 h before methanol was added (1.5 mL per mmol). After evaporation of the solvents, the residue was dissolved in dichloromethane (20 mL per mmol) and the organic layer was washed with 10% $NaHCO_3$ (10 mL per mmol) and water (10 mL per mmol). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. The remaining pyridine was removed by coevaporation with toluene and dichloromethane. The product was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 100/0→95/5, v/v).

General Procedure B.[12] Per mmol of purine (3× coevaporated with dry pyridine) in 25 mL of dry pyridine was added

1.75 g of phosphorus pentasulfide, and the resulting mixture was refluxed for 7 h. After evaporation of the solvent, the residual solvent was removed by coevaporation with MeOH (2×). Water was added and the resulting mixture was stirred for 1 h at 50 °C. After extraction with EtOAc (3 × 15 mL), the combined organic layers were dried (Na_2SO_4) and filtered, and the solvent was evaporated. The product was purified by column chromatography (eluent: EtOAc/MeOH, 97/3→95/5 v/v).

General Procedure C.[13] Per mmol of substituted purine in 7.5 mL of dry DMF was added 1 equiv of K_2CO_3 and 1.2 equiv of (4-nitro-) benzyl bromide. After stirring overnight at room temperature, 10 mL of water was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and filtered, and the solvent was evaporated. The product was purified by column chromatography (eluent: EtOAc/PE 40−60, 1/1→6/4 v/v).

General Procedure D.[14] Per mmol of purine was added 25 mL of MeOH saturated with NH_3 at 0 °C. After stirring overnight the solvent was evaporated and the product purified by column chromatography (eluent: EtOAc/MeOH, 95/5→90/10 v/ v). The appropriate fractions were collected and evaporated to dryness. Residual EtOAc was removed by dissolving the product in CH_2Cl_2 followed by evaporation (two times).

General Procedure E. Per mmol of purine **63** were added 6 mL of dioxane and 2.4 mL of water followed by 7.5 equiv of the required amine. The mixture was heated in an Emrys Optimizer microwave for 5 h at 175 °C (**64** and **65**) or 2.5 h at 150 °C (**66** and **67**). After removal of the solvents by evaporation, dioxane was added and the mixture evaporated to dryness. The product was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 99/1→90/10 v/v).

6-Benzylthioinosine (4). This compound was prepared according to a literature procedure.[9]

2′,3′,5′-Tri-*O*-acetylinosine (5). This compound was prepared according to a literature procedure.[11]

2′,3′,5′-Tri-*O*-acetyl-8-bromoinosine (6). This compound was prepared according to a literature procedure.[11]

2′,3′,5′-Tri-*O*-acetyl-8-(methylamino)inosine (7). This compound was prepared according to general procedure A with methylamine (40% in H_2O, 15 mL per mmol): yield 65%; white solid; ^{1}H NMR ($CDCl_3$) δ 2.04, 2.15, 2.16 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.11 (d, 3H, J = 4.9 C*H*$_3$N), 4.26−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.54−4.68 (m, 1H, 1 × *H-5′*), 5.09 (q, 1H, J = 4.9, CH$_3$N*H*), 5.46 (dd, 1H, J = 3.8, J = 6.2, *H-3′*), 5.71 (t, 1H, J = 6.6, *H-2′*), 6.18 (d, 1H, J = 6.8, *H-1′*), 7.97 (s, 1H, *H-2*), 11.55 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(ethylamino)inosine (8). This compound was prepared according to a literature procedure.[11]

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-propylamino)inosine (9). This compound was prepared according to general procedure A with *n*-propylamine (11.5 mL per mmol) and H_2O (5 mL per mmol): yield 58%; yellowish foam; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.3, C*H*$_3$CH$_2$), 1.70 (sextet, 2H, J = 7.3, CH$_3$*CH*$_2$), 2.05, 2.13, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.35−3.67 (m, 2H, C*H*$_2$N), 4.27−4.42 (m, 2H, *H-4′*, 1 × *H-5′*), 4.51−4.63 (m, 1H, 1 × *H-5′*), 5.06 (t, 1H, J = 5.9, CH$_2$N*H*), 5.48 (dd, 1H, J = 4.0, J = 5.8, *H-3′*), 5.79 (t, 1H, J = 6.2, *H-2′*), 6.12 (d, 1H, J = 6.6, *H-1′*), 7.92 (s, 1H, *H-2*), 11.55 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-butylamino)inosine (10). This compound was prepared according to general procedure A with *n*-butylamine: yield 47%; yellowish foam; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.3, C*H*$_3$CH$_2$), 1.29−1.69 (m, 2H, CH$_3$C*H*$_2$), 1.57−1.75 (m, 2H, C*H*$_2$CH$_2$N), 2.05, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.37−3.68 (m, 2H, C*H*$_2$N), 4.27−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.48−4.63 (m, 1H, 1 × *H-5′*), 5.00 (t, 1H, J = 5.9, CH$_2$N*H*), 5.49 (dd, 1H, J = 4.4, J = 5.9, *H-3′*), 5.79 (t, 1H, J = 6.2, *H-2′*), 6.11 (d, 1H, J = 6.6, *H-1′*), 7.91 (s, 1H, *H-2*), 11.40 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-pentylamino)inosine (11). This compound was prepared according to general procedure A with *n*-pentylamine: yield 81%; yellow foam; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, J = 6.6, C*H*$_3$CH$_2$), 1.21−1.47 (m, 4H, 2 × C*H*$_2$), 1.56−1.79 (m, 2H, C*H*$_2$CH$_2$N), 2.05, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.35−3.68 (m, 2H, C*H*$_2$N), 4.87−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.49−4.63 (m, 1H, 1 × *H-5′*), 5.03 (t, 1H, J = 5.9, CH$_2$N*H*), 5.49 (dd, 1H, J = 4.4, J = 6.2, *H-3′*), 5.80 (t, 1H, J = 6.2, *H-2′*), 6.12 (d, 1H, J = 6.2, *H-1′*), 7.97 (s, 1H, *H-2*), 11.24 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-hexylamino)inosine (12). This compound was prepared according to general procedure A with *n*-hexylamine: yield 43%; yellowish oil; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.6, C*H*$_3$CH$_2$), 1.21−1.48 (m, 6H, 3 × C*H*$_2$), 1.60−1.74 (m, 2H, C*H*$_2$CH$_2$N), 2.05, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.37−3.67 (m, 2H, C*H*$_2$N), 4.27−4.43 (m, 2H, *H-4′*, 1 × *H-5′*), 4.49−4.63 (m, 1H, 1 × *H-5′*), 5.03 (t, 1H, J = 5.8, CH$_2$N*H*), 5.51 (dd, 1H, J = 4.2, J = 6.0, *H-3′*), 5.80 (t, 1H, J = 6.2, *H-2′*), 6.13 (d, 1H, J = 6.2, *H-1′*), 7.98 (s, 1H, *H-2*), 11.12 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-[(2-methylbutyl)amino]inosine (13). This compound was prepared according to general procedure A applying 2-methylbutylamine: yield 27%; colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.87−0.99 (m, 6H, 2 × C*H*$_3$), 1.05−1.28 (m, 1H, CH$_3$CH*H*), 1.39−1.62 (m, 1H, CH$_3$CH*H*), 1.69−1.90 (m, 1H, CH$_3$C*H*), 2.06, 2.11, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.17−3.34 (m, 0.5H, CH*H*NH), 3.36−3.46 (m, 1H, CH*H*NH), 3.47−3.66 (m, 0.5H, CH*H*NH), 4.28−4.61 (m, 3H, *H-4′*, *H-5′*), 5.07 (t, 1H, J = 5.9, N*H*), 5.46−5.55(m, 1H, *H-3′*), 5.79−5.88 (m, 1H, *H-2′*), 6.07−6.15 (m, 1H, *H-1′*), 7.99 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-8-(cyclopropylamino)inosine (14). This compound was prepared according to general procedure A with cyclopropylamine (1.7 mL per mmol) and H_2O (3.5 mL per mmol): yield 57%; yellowish solid; ^{1}H NMR ($CDCl_3$) δ 0.51−0.68 (m, 2H, C*H*$_2$CHN), 0.80−0.98 (m, 2H, C*H*$_2$CHN), 2.05, 2.14, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 2.88−3.05 (m, 1H, C*H*N), 4.29−4.40 (m, 2H, *H-4′*, 1 × *H-5′*), 4.50 (ABX, 1H, J = 4.8, J = 12.8, 1 × *H-5′*), 5.41 (br s, 1H, CH$_2$CHN*H*), 5.50 (dd, 1H, J = 4.8, J = 5.9, *H-3′*), 5.75 (t, 1H, J = 6.2, *H-2′*), 6.10 (d, 1H, J = 6.2, *H-1′*), 8.05 (s, 1H, *H-2*), 11.21 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(cyclopentylamino)inosine (15). This compound was prepared according to a literature procedure.[11]

2′,3′,5′-Tri-*O*-acetyl-8-(cyclohexylamino)inosine (16). This compound was prepared according to general procedure A with cyclohexylamine: yield 28%; colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.07−1.84 (m, 8H, 2 × NHCHC*H*H, 3 × C*H*$_2$), 2.06, 2.09, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 2.07−2.25 (m, 2H, 2 × NHCHC*H*H), 3.87−4.08 (m, 1H, CH$_2$C*H*NH), 4.29−4.41 (m, 3H, *H-4′*, *H-5′*), 4.50 (ABX, 1H, J = 4.7, J = 12.8, 1 × *H-5′*), 4.73 (d, 1H, J = 7.5, CH$_2$CHN*H*), 5.53 (dd, 1H, J = 4.6, J = 5.9, *H-3′*), 5.88 (t, 1H, J = 5.9, *H-2′*), 6.03 (d, 1H, J = 5.9, *H-1′*), 7.91 (s, 1H, *H-2*), 11.31 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-pyrrolidinoinosine (17). This compound was prepared according to general procedure A with pyrrolidine: yield 65%; white foam; ^{1}H NMR ($CDCl_3$) δ 1.72−2.09 (m, 4H, 2 × C*H*$_2$CH$_2$N), 2.06, 2.08, 2.12 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.52−3.75 (m, 4H, 2 × C*H*$_2$N), 4.29−4.37 (m, 2H, *H-4′*, 1 × *H-5′*), 4.48 (ABX, 1H, J = 6.0, J = 13.7, 1 × *H-5′*), 5.86 (dd, 1H, J = 5.1, J = 5.9, *H-3′*), 5.98 (d, 1H, J = 4.9, *H-1′*), 6.40 (dd, 1H, J = 4.9, J = 5.9, *H-2′*), 7.97 (s, 1H, *H-2*), 11.44 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-piperidinoinosine (18). This compound was prepared according to general procedure A with piperidine: yield 60%; white solid; ^{1}H NMR ($CDCl_3$) δ 1.51−1.82 (m, 6H, (C*H*$_2$)$_3$CH$_2$N), 2.07, 2.09, 2.13 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.14−3.38 (m, 4H, 2 × C*H*$_2$N), 4.28−4.38 (m, 2H, *H-4′*, 1 × *H-5′*), 4.51 (ABX, 1H, J = 6.2, J = 13.9, 1 × *H-5′*), 5.84−5.96 (m, 2H, *H-1′*, *H-3′*), 6.25 (dd, 1H, J = 4.8, J = 6.2, *H-2′*), 8.07 (s, 1H, *H-2*), 11.50 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-*N*-morpholinoinosine (19). This compound was prepared according to general procedure A with morpholine: yield 40%; white solid; ^{1}H NMR ($CDCl_3$) δ 2.06, 2.10, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.21−3.49 (m, 4H, 2 × C*H*$_2$N), 3.77−3.95 (m, 4H, 2 × C*H*$_2$O), 4.26−4.55 (m, 3H, *H-4′*, *H-5′*), 5.85−5.94 (m, 2H, *H-1′*, *H-3′*), 6.29 (dd, 1H, J = 4.6, J = 6.0, *H-2′*), 8.10 (s, 1H, *H-2*), 11.52 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(methylamino)thioinosine (20). This compound was prepared according to general procedure B: yield 63%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.03, 2.14, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.15−3.78 (d, 3H, *J* = 4.8, C*H*$_3$N), 4.28−4.44 (m, 2H, *H-4′*, 1 × *H-5′*), 4.53−4.66 (m, 1H, 1 × *H-5′*), 5.45 (dd, 1H, *J* = 4.0, *J* = 6.2, *H-3′*), 5.59 (q, 1H, *J* = 4.8, N*H*), 5.66−5.76 (m, 1H, *H-2′*), 6.17 (d, 1H, *J* = 6.6, *H-1′*), 8.11 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-8-(ethylamino)thioinosine (21). This compound was prepared according to general procedure B: yield 67%; yellow solid; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 2.03, 2.13, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.56−3.78 (m, 2H, C*H*$_2$N), 4.28−4.42 (m, 2H, *H-4′*, 1 × *H-5′*), 4.59 (dd, 1H, *J* = 4.0, *J* = 12.1, 1 × *H-5′*), 5.29 (t, 1H, *J* = 4.4, N*H*), 5.45 (dd, 1H, *J* = 4.0, *J* = 6.6, *H-3′*), 5.72 (t, 1H, *J* = 6.6, *H-2′*), 6.12 (d, 1H, *J* = 6.6, *H-1′*), 8.01 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-propylamino)thioinosine (22). This compound was prepared according to general procedure B: yield 60%; brown oil; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 1.59−1.81 (m, 2H, CH$_3$C*H*$_2$), 2.03, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.42−3.75 (m, 2H, C*H*$_2$N), 4.26−4.45 (m, 2H, *H-4′*, 1 × *H-5′*), 4.58 (dd, 1H, *J* = 4.0, *J* = 12.1, 1 × *H-5′*), 5.36 (t, 1H, *J* = 5.5, N*H*), 5.46 (dd, 1H, *J* = 4.4, *J* = 6.6, *H-3′*), 5.76 (t, 1H, *J* = 6.6, *H-2′*), 6.14 (d, 1H, *J* = 6.6, *H-1′*), 8.05 (s, 1H, *H-2*), 11.67 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-butylamino)thioinosine (23). This compound was prepared according to general procedure B: yield 61%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 1.30−1.50 (m, 2H, CH$_3$C*H*$_2$), 1.57−1.75 (m, 2H, C*H*$_2$CH$_2$NH), 2.04, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.46−3.77 (m, 2H, C*H*$_2$N), 4.26−4.44 (m, 2H, *H-4′*, 1 × *H-5′*), 4.58 (dd, 1H, *J* = 4.0, *J* = 12.1, 1 × *H-5′*), 5.30 (t, 1H, *J* = 5.5, N*H*), 5.45 (dd, 1H, *J* = 4.0, *J* = 6.2, *H-3′*), 5.76 (dd, 1H, *J* = 6.2, *J* = 6.6, *H-2′*), 6.13 (d, 1H, *J* = 6.6, *H-1′*), 8.04 (s, 1H, *H-2*), 11.63 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-pentylamino)thioinosine (24). This compound was prepared according to general procedure B: yield 45%; yellow-brown foam; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.6, C*H*$_3$CH$_2$), 1.23−1.43 (m, 4H, 2 × C*H*$_2$), 1.56−1.75 (m, 2H, C*H*$_2$CH$_2$N), 2.03, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.42−3.76 (m, 2H, C*H*$_2$N), 4.22−4.44 (m, 2H, *H-4′*, 1 × *H-5′*), 4.57 (ABX, 1H, *J* = 4.4, *J* = 11.7, 1 × *H-5′*), 5.34 (t, 1H, *J* = 5.1, CH$_2$N*H*), 5.46 (dd, 1H, *J* = 3.7, *J* = 5.9, *H-3′*), 5.74 (dd, 1H, *J* = 5.9, *J* = 6.6, *H-2′*), 6.13 (d, 1H, *J* = 6.6, *H-1′*), 8.07 (s, 1H, *H-2*), 11.47 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(*n*-hexylamino)thioinosine (25). This compound was prepared according to general procedure B: yield 58%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.6, 1 × C*H*$_3$CH$_2$), 1.25−1.44 (m, 6H, 3 × C*H*$_2$), 1.55−1.77 (m, 2H, C*H*$_2$CH$_2$NH), 2.04, 2.12, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$ CO), 3.44−3.77 (m, 2H, C*H*$_2$N), 4.26−4.44 (m, 2H, *H-4′*, 1 × *H-5′*), 4.58 (dd, 1H, *J* = 4.4, *J* = 11.7, 1 × *H-5′*), 5.29 (t, 1H, *J* = 5.1, N*H*), 5.45 (dd, 1H, *J* = 4.4, *J* = 6.6, *H-3′*), 5.73 (t, 1H, *J* = 6.6, *H-2′*), 6.13 (d, 1H, *J* = 6.6, *H-1′*), 8.02 (s, 1H, *H-2*), 11.68 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-[(2-methylbutyl)amino]thioinosine (26). This compound was prepared according to general procedure B: yield 44%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.87−1.00 (m, 6H, 2 × C*H*$_3$), 1.07−1.31 (m, 1H, CH$_3$CH*H*), 1.37−1.60 (m, 1H, CH$_3$CH*H*), 1.64−1.92 (m, 1H, CH$_3$C*H*), 2.04, 2.10, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.27−3.70 (m, 2H, C*H*$_2$N), 4.26−4.45 (m, 2H, *H-4′*, 1 × *H-5′*), 4.55 (dd, 1H, *J* = 3.7, *J* = 12.1, 1 × *H-5′*), 5.30 (t, 1H, *J* = 5.5, N*H*), 5.46 (dd, 1H, *J* = 4.4, *J* = 6.2, *H-3′*), 5.77 (t, 1H, *J* = 6.2, *H-2′*), 6.11 (d, 1H, *J* = 6.2, *H-1′*), 8.01 (s, 1H, *H-2*), 11.76 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(cyclopropylamino)thioinosine (27). This compound was prepared according to general procedure B: yield 49%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.58−0.70 (m, 2H, 2 × CH*H*), 0.81−0.94 (m, 2H, C*H*$_2$CH$_2$NH), 2.05, 2.14, 2.16 (3 × s, 3 × 3H, 3 × C*H*$_3$ CO), 2.98−3.13 (m, 1H, C*H*N), 4.26−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.46−4.60 (m, 1H, 1 × *H-5′*), 5.46 (dd, 1H, *J* = 4.4, *J* = 5.9, *H-3′*), 5.65−5.77 (m, 2H, *H-2′*, CHN*H*), 6.13 (d, 1H, *J* = 5.8, *H-1′*), 8.17 (s, 1H, *H-2*), 11.75 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(cyclopentylamino)thioinosine (28). This compound was prepared according to general procedure B: yield 22%; brownish oil; ^{1}H NMR ($CDCl_3$) δ 1.41−1.70 (m, 8H, 4 × C*H*$_2$), 2.05, 2.06, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$ CO), 4.29−4.63 (m, 4H, *H-4′*, *H-5′*, C*H*NH), 5.15 (d, 1H *J* = 5.6, CHN*H*), 5.49 (t, 1H, *J* = 5.9, *H-3′*), 5.81 (t, 1H, *J* = 5.9, *H-2′*), 6.04 (d, 1H, *J* = 5.9, *H-1′*), 8.01 (s, 1H, *H-2*), 11.77 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-(cyclohexylamino)thioinosine (29). This compound was prepared according to general procedure B: yield 41%; yellow solid; ^{1}H NMR ($CDCl_3$) δ 1.05−1.85 (m, 8H, 2 × NHCHC*H*H, 3 × C*H*$_2$), 2.05, 2.11, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 1.98−2.24 (m, 2H, 2 × NHCHC*H*H), 4.02−4.23 (m, 1H, CH$_2$C*H*NH), 4.28−4.58 (m, 3H, *H-4′*, *H-5′*), 5.07 (d, 1H, *J* = 8.0, CH$_2$CHN*H*), 5.48 (dd, 1H, *J* = 4.4, *J* = 5.9, *H-3′*), 5.81 (dd, 1H, *J* = 5.9, *J* = 6.6, *H*-2′), 6.05 (d, 1H, *J* = 6.6, *H-1′*), 7.99 (s, 1H, *H-2*), 11.79 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-pyrrolidinothioinosine (30). This compound was prepared according to general procedure B: yield 55%; brownish oil; ^{1}H NMR ($CDCl_3$) δ 1.78−2.09 (m, 4H, 2 × C*H*$_2$CH$_2$N), 2.03, 2.05, 2.11 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.60−3.82 (m, 4H, 2 × C*H*$_2$N), 4.21−4.53 (m, 3H, *H-4′*, *H-5′*), 5.80 (t, 1H, *J* = 5.1, *H-3′*), 5.98 (d, 1H, *J* = 5.1, *H-1′*), 6.40 (t, 1H, *J* = 5.1, *H-2′*), 8.05 (s, 1H, *H-2*), 11.74 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-piperidinothioinosine (31). This compound was prepared according to general procedure B: yield 82%; brown oil; ^{1}H NMR ($CDCl_3$) δ 1.56−1.83 (m, 6H, (C*H*$_2$)$_3$CH$_2$N), 2.05, 2.08, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.22−3.49 (m, 4H, 2 × C*H*$_2$N), 4.26−4.55 (m, 3H, *H-4′*, *H-5′*), 5.79−5.88 (m, 2H, *H-1′*, *H-3′*), 6.25 (t, 1H, *J* = 5.5, *H-2′*), 8.10 (s, 1H, *H-2*), 12.05 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-8-*N*-morpholinothioinosine (32). This compound was prepared according to general procedure B: yield 84%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.07, 2.09, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.27−3.42 (m, 2H, 2 × CH*H*N), 3.44−3.59 (m, 2H, 2 × CH*H*N), 3.78−3.99 (m, 4H, 2 × C*H*$_2$N), 4.25−4.51 (m, 3H, *H-4′*, *H-5′*), 5.81−5.90 (m, 2H, *H-1′*, *H-3′*), 6.30 (dd, 1H, *J* = 4.8, *J* = 5.9, *H-2′*), 8.10 (s, 1H, *H-2*), 12.15 (br s, 1H, N*H*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(methylamino)thioinosine (33). This compound was prepared according to general procedure C: yield 61%; white solid; ^{1}H NMR ($CDCl_3$) δ 2.01, 2.13, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.13 (d, 2H, *J* = 5.1, C*H*$_3$N), 4.61 (m, 2H, *H-4′*, 1 × *H-5′*), 4.61 (ABX, 1H, *J* = 3.7, *J* = 11.7, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.36 (t, 1H, *J* = 5.1, N*H*), 5.47 (dd, 1H, *J* = 3.4, *J* = 6.2, *H-3′*), 5.73 (dd, 1H, *J* = 6.2, *J* = 6.9, *H-2′*), 6.24 (d, 1H, *J* = 6.9, *H-1′*), 7.21−7.34 (m, 3H, C*H* arom), 7.43−7.49 (m, 2H, C*H* arom), 8.52 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(ethylamino)thioinosine (34). This compound was prepared according to general procedure C: yield 52%; white solid; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 2.03, 2.09, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.46−3.69 (m, 2H, C*H*$_2$N), 4.24−4.58 (m, 3H, *H-4′*, *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.24 (t, 1H, *J* = 5.5, N*H*), 5.51 (dd, 1H, *J* = 3.7, *J* = 5.9, *H-3′*), 5.84 (t, 1H, *J* = 6.2, *H-2′*), 6.17 (d, 1H, *J* = 6.6, *H-1′*), 7.22−7.36 (m, 3H, C*H* arom), 7.38−7.47 (m, 2H, C*H* arom), 8.50 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(*n*-propylamino)thioinosine (35). This compound was prepared according to general procedure C: yield 59%; yellowish solid; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 2.03, 2.07, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.36−3.66 (m, 2H, C*H*$_2$N), 4.25−4.42 (m, 1H, *H-4′*, 1 × *H-5′*), 4.56 (ABX, 1H, *J* = 3.7, *J* = 11.7, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.30 (t, 1H, *J* = 5.9, N*H*), 5.52 (dd, 1H, *J* = 4.0, *J* = 5.9, *H-3′*), 5.87 (t, 1H, *J* = 6.2, *H-2′*), 6.16 (d, 1H, *J* = 6.2, *H-1′*), 7.17−7.34 (m, 3H, C*H* arom), 7.38−7.49 (m, 2H, C*H* arom), 8.50 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(*n*-butylamino)thioinosine (36). This compound was prepared according to general procedure C: yield 66%; off-white solid; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 1.30−1.50 (m, 2H, C*H*$_2$CH$_3$), 1.55−1.75 (m, 2H, C*H*$_2$CH$_2$N), 2.03, 2.07, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.39−3.69 (m, 2H, C*H*$_2$N), 4.27−4.43 (m, 2H, *H-4′*, 1 × *H-5′*), 4.56 (ABX, 1H, *J* = 3.7, *J* = 11.7, 1 × *H-5′*),

4.62 (s, 2H, C*H*$_2$S), 5.25 (t, 1H, *J* = 5.4, N*H*), 5.53 (dd, 1H, *J* = 4.4, *J* = 5.9, *H-3′*), 5.87 (t, 1H, *J* = 5.9, *H-2′*), 6.15 (d, 1H, *J* = 5.9, *H-1′*), 7.17−7.34 (m, 3H, C*H* arom), 7.41−7.49 (m, 2H, C*H* arom), 8.50 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(*n*-pentylamino)thioinosine (37). This compound was prepared according to general procedure C: yield 95%; yellowish oil; ^{1}H NMR (CDCl$_3$) *δ* 0.85−0.94 (m, 3H, C*H*$_3$CH$_2$), 1.23−1.45 (m, 4H, 2 × C*H*$_2$), 1.60−1.73 (m, 2H, C*H*$_2$CH$_2$N), 2.05, 2.07, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.38−3.67 (m, 2H, C*H*$_2$N), 4.27−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.58 (dd, 1H, *J* = 4.4, *J* = 11.7, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.03 (t, 1H, *J* = 5.1, CH$_2$N*H*), 5.53 (dd, 1H, *J* = 3.7, *J* = 5.9, *H-3′*), 5.87 (t, 1H, *J* = 5.9, *H-2′*), 6.16 (d, 1H, *J* = 5.9, *H-1′*), 7.18−7.33 (m, 3H, C*H* arom), 7.45 (dd, 2H, *J* = 1.5, *J* = 8.0, C*H* arom), 8.50 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(*n*-hexylamino)thioinosine (38). This compound was prepared according to general procedure C: yield 86%; white solid; ^{1}H NMR (CDCl$_3$) *δ* 0.88 (t, 3H, *J* = 6.6, C*H*$_3$CH$_2$), 1.25−1.45 (m, 4H, C*H*$_2$CH$_3$), 1.53−1.77 (m, 4H, C*H*$_2$CH$_2$N), 2.03, 2.07, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.37−3.69 (m, 2H, C*H*$_2$N), 4.27−4.42 (m, 2H, *H-4′*, 1 × *H-5′*), 4.56 (ABX, 2H, *J* = 3.7, *J* = 11.7, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.25 (t, 1H, *J* = 5.4, N*H*), 5.53 (dd, 1H, *J* = 3.7, *J* = 5.9, *H-3′*), 5.85 (t, 1H, *J* = 5.9, *H-2′*), 6.15 (d, 1H, *J* = 5.9, *H-1′*), 7.17−7.37 (m, 3H, C*H* arom), 7.45 (dd, 2H, *J* = 1.5, *J* = 8.0 C*H* arom), 8.50 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-[(2-methylbutyl)amino]-thioinosine (39). This compound was prepared according to general procedure C: yield 64%; yellow oil; ^{1}H NMR (CDCl$_3$) *δ* 0.87−0.99 (m, 6H, 2 × C*H*$_3$), 1.07−1.31 (m, 1H, CH$_3$CH*H*), 1.36−1.58 (m, 1H, CH$_3$CH*H*), 1.64−1.86 (m, 1H, CH$_3$C*H*), 2.04, 2.05, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.18−3.33 (m, 0.5H, CH*H*NH), 3.36−3.46 (m, 1H, CH*H*NH), 3.49−3.65 (m, 0.5H, CH*H*NH), 4.25−4.43 (m, 2H, *H-4′*, 1 × *H-5′*), 4.48−4.59 (m, 1H, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.28 (m, 1H, N*H*), 5.49−5.58 (m, 1H, *H-3′*), 5.86−5.95 (m, 1H, *H-2′*), 6.12−6.18 (m, 1H, *H-1′*), 7.16−7.34 (m, 3H, C*H* arom), 7.41−7.49 (m, 2H, C*H* arom), 8.49 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(cyclopropylamino)thioinosine (40). This compound was prepared according to general procedure C: yield 64%; white solid; ^{1}H NMR (CDCl$_3$) *δ* 0.59−0.69 (m, 2H, C*H*$_2$), 0.82−0.93 (m, 2H, C*H*$_2$), 2.02, 2.12, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 2.88−3.01 (m, 2H, C*H*$_2$N), 4.27−4.39 (m, 2H, *H-4′*, 1 × *H-5′*), 4.46−4.58 (m, 1H, 1 × *H-5′*), 4.64 (s, 2H, C*H*$_2$S), 5.47−5.56 (m, 1H, *H-3′*), 5.57 (d, 1H, *J* = 1.5, N*H*), 5.78 (t, 1H, *J* = 6.2, *H-2′*), 6.14 (d, 1H, *J* = 6.2, *H-1′*), 7.17−7.35 (m, 3H, C*H* arom), 7.41−7.50 (m, 2H, C*H* arom), 8.51 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(cyclopentylamino)thioinosine (41). This compound was prepared according to general procedure C: yield 63%; colorless oil; ^{1}H NMR (CDCl$_3$) *δ* 1.41−1.80 (m, 6H, NHCHCH*H*, 2 × C*H*$_2$), 2.03, 2.05, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 2.08−2.12 (m, 2H, NHCHC*H*H), 4.27−4.56 (m, 4H, *H-4′*, *H-5′*, C*H*NH), 4.62 (s, 2H, C*H*$_2$S), 5.08 (d, *J* = 6.8, 1H, N*H*), 5.57 (dd, 1H, *J* = 4.8, *J* = 5.7, *H-3′*), 5.97 (t, 1H, *J* = 5.7, *H-2′*), 6.05 (d, 1H, *J* = 5.7, *H-1′*), 7.21−7.34 (m, 3H, C*H* arom), 7.45 (dd, 2H, *J* = 1.8, *J* = 8.1, C*H* arom), 8.49 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-(cyclohexylamino)thioinosine (42). This compound was prepared according to general procedure C: yield 59%; colorless oil; ^{1}H NMR (CDCl$_3$) *δ* 1.12−1.84 (m, 8H, 2 × NHCHC*H*H, 3 × C*H*$_2$), 2.04, 2.05, 2.15 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 2.01−2.12 (m, 2H, 2 × NHCHC*H*H), 3.81−4.03 (m, 1H, CH$_2$C*H*NH), 4.29−4.57 (m, 3H, *H-4′*, *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.01 (d, *J* = 7.7, 1H, N*H*), 5.57 (dd, 1H, *J* = 4.8, *J* = 5.5, *H-3′*), 5.97 (t, 1H, *J* = 5.5, *H-2′*), 6.07 (d, 1H, *J* = 5.5, *H-1′*), 7.18−7.33 (m, 3H, C*H* arom), 7.45 (dd, 2H, *J* = 1.8, *J* = 8.0, C*H* arom), 8.48 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-pyrrolidinothioinosine (43). This compound was prepared according to general procedure C: yield 42%; colorless oil; ^{1}H NMR (CDCl$_3$) *δ* 1.90−2.09 (m, 4H, 2 × C*H*$_2$N), 2.05, 2.06, 2.13 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.62−3.78 (m, 4H, 2 × C*H*$_2$N), 4.26−4.37 (m, 1H, *H-4′*, 1 × *H-5′*), 4.45−4.57 (m, 1H, 1 × *H-5′*), 4.61 (s, 2H, C*H*$_2$S), 5.95−6.03 (m, 2H, *H-1′*, *H-3′*), 6.59 (dd, 1H, *J* = 4.8, *J* = 5.8, *H-2′*), 7.20−7.33 (m, 3H, C*H* arom), 7.40−7.48 (m, 2H, C*H* arom), 8.51 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-piperidinothioinosine (44). This compound was prepared according to general procedure C: yield 48%; colorless oil; ^{1}H NMR (CDCl$_3$) *δ* 1.57−1.84 (m, 6H, 3 × C*H*$_2$), 2.06, 2.07, 2.13 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.19−3.45 (m, 4H, 2 × C*H*$_2$N), 4.29−4.41 (m, 2H, *H-4′*, 1 × *H-5′*), 4.52 (ABX, 1H, *J* = 6.2, *J* = 13.9, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.84 (d, 1H, *J* = 4.8, *H-1′*), 6.00 (t, 1H, *J* = 5.9, *H-3′*), 6.38 (dd, 1H, *J* = 4.8, *J* = 5.9, *H-2′*), 7.17−7.34 (m, 3H, C*H* arom), 7.44 (dd, 2H, *J* = 1.8, *J* = 8.0, C*H* arom), 8.58 (s, 1H, *H-2*).

2′,3′,5′-Tri-*O*-acetyl-6-benzyl-8-morpholinothioinosine (45). This compound was prepared according to general procedure C: yield 66%; colorless oil; ^{1}H NMR (CDCl$_3$) *δ* 2.06, 2.07, 2.14 (3 × s, 3 × 3H, 3 × C*H*$_3$CO), 3.24−3.29 (m, 2H, 2 × CH*H*N), 3.42−3.57 (m, 2H, 2 × CH*H*N), 3.77−3.97 (m, 4H, 2 × C*H*$_2$O), 4.27−4.39 (m, 2H, *H-4′*, 1 × *H-5′*), 4.43−5.56 (m, 1H, 1 × *H-5′*), 4.62 (s, 2H, C*H*$_2$S), 5.86 (d, 1H, *J* = 4.6, *H-1′*), 6.01 (t, 1H, *J* = 5.7, *H-3′*), 6.44 (dd, 1H, *J* = 4.6, *J* = 5.7, *H-2′*), 7.17−7.34 (m, 3H, C*H* arom), 7.45 (dd, 2H, *J* = 1.8, *J* = 8.0, C*H* arom), 8.61 (s, 1H, *H-2*).

6-Benzyl-8-(methylamino)thioinosine (46). This compound was prepared according to general procedure D: yield 83%; white solid; ^{1}H NMR (MeOD) *δ* 3.02 (s, 3H, CH$_3$), 3.76−3.89 (m, 2H, *H-5′*), 4.15 (dd, 1H, *J* = 1.8, *J* = 4.0, *H-4′*), 4.27 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.70 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.61 (d, 1H, *J* = 7.7, *H-1′*), 7.17−7.33 (m, 3H, C*H* arom), 7.42 (dd, 2H, *J* = 2.2, *J* = 8.0, C*H* arom), 8.39 (s, 1H, *H-2*). Anal. (C$_{18}$H$_{21}$N$_5$O$_4$S·0.15CH$_2$Cl$_2$) C, H, N, S.

6-Benzyl-8-(ethylamino)thioinosine (47). This compound was prepared according to general procedure D: yield 96%; white solid; ^{1}H NMR (CDCl$_3$/MeOD 1/1, v/v) *δ* 1.21 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 3.34−3.56 (m, 2H, C*H*$_2$N), 3.77 (ABX, 1H, *J* = 1.1, *J* = 12.4, 1 × *H-5′*), 3.92 (ABX, 1H, *J* = 2.0, *J* = 12.4, 1 × *H-5′*), 4.18−4.24 (m, 1H, *H-4′*), 4.30 (dd, 1H, *J* = 1.6, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.70 (dd, 1H, *J* = 5.5, *J* = 7.1, *H-2′*), 5.90 (d, 1H, *J* = 7.1, *H-1′*), 7.18−7.34 (m, 3H, C*H* arom), 7.40−7.48 (m, 2H, C*H* arom), 8.39 (s, 1H, *H-2*). Anal. (C$_{19}$H$_{23}$N$_5$O$_4$S·0.05CH$_2$Cl$_2$) C, H, N, S.

6-Benzyl-8-(*n*-propylamino)thioinosine (48). This compound was prepared according to general procedure D: yield 78%; white solid; ^{1}H NMR (MeOD) *δ* 0.97 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 1.56−1.77 (m, 2H, CH$_3$C*H*$_2$), 3.34−3.50 (m, 2H, C*H*$_2$N), 3.74−3.86 (m, 2H, *H-5′*), 4.10−4.17 (m, 1H, *H-4′*), 4.21−4.29 (m, 1H, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.69 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.13 (d, 1H, *J* = 7.7, *H-1′*), 7.13−7.31 (m, 3H, C*H* arom), 7.36−7.45 (m, 2H, C*H* arom), 8.37 (s, 1H, *H-2*). Anal. (C$_{20}$H$_{25}$N$_5$O$_4$S) C, H, N, S.

6-Benzyl-8-(*n*-butylamino)thioinosine (49). This compound was prepared according to general procedure D: yield 87%; white solid; ^{1}H NMR (MeOD) *δ* 0.95 (t, 3H, *J* = 7.3, C*H*$_3$CH$_2$), 1.31−1.51 (m, 2H, CH$_3$C*H*$_2$), 1.55−1.73 (m, 2H, C*H*$_2$CH$_2$N), 3.34−3.58 (m, 2H, C*H*$_2$N), 3.77−3.83 (m, 2H, *H-5′*), 4.11−4.16 (m, 1H, *H-4′*), 4.26 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.68 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.14 (d, 1H, *J* = 7.7, *H-1′*), 7.15−7.31 (m, 3H, C*H* arom), 7.37−7.46 (m, 2H, C*H* arom), 8.37 (s, 1H, *H-2*). Anal. (C$_{21}$H$_{27}$N$_5$O$_4$S) C, H, N, S.

6-Benzyl-8-(*n*-pentylamino)thioinosine (50). This compound was prepared according to general procedure D: yield 83%; white foam; ^{1}H NMR (MeOD) *δ* 0.89 (t, 3H, *J* = 6.9, C*H*$_3$CH$_2$), 1.28−1.43 (m, 4H, 2 × C*H*$_2$), 1.61−1.76 (m, 2H, C*H*$_2$CH$_2$N), 3.32−3.60 (m, 2H, C*H*$_2$N), 3.74−3.87 (m, 2H, *H-5′*), 4.12−4.18 (m, 1H, *H-4′*), 5.51 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.60 (s, 2H, C*H*$_2$S), 4.69, (dd, 1H, *J* = 5.5, *J* = 7.3, *H-2′*), 6.14 (d, 1H, *J* = 7.3, *H-1′*), 7.12−7.31 (m, 3H, C*H* arom), 7.40 (dd, 2H, *J* = 1.5, *J* = 8.0, C*H* arom), 8.36 (s, 1H, *H-2*). Anal. (C$_{22}$H$_{29}$N$_5$O$_4$S·0.1CH$_2$Cl$_2$) C, H, N, S.

6-Benzyl-8-(*n*-hexylamino)thioinosine (51). This compound was prepared according to general procedure D: yield 61%; colorless glass; ^{1}H NMR (MeOD) *δ* 0.88 (m, 3H, C*H*$_3$CH$_2$),

1.21−1.48 (m, 6H, 3 × C*H*$_2$), 1.57−1.74 (m, 2H, C*H*$_2$CH$_2$N), 3.33−3.56 (m, 2H, C*H*$_2$N), 3.74−3.87 (m, 2H, *H-5′*), 4.14 (d, 1H, *J* = 1.8, *J* = 3.7, *H-4′*), 4.27 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.60 (s, 2H, C*H*$_2$S), 4.68 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.14 (d, 1H, *J* = 7.7, *H-1′*), 7.13−7.30 (m, 3H, C*H* arom), 7.36−7.44 (m, 2H, C*H* arom), 8.36 (s, 1H, *H-2*). Anal. ($C_{23}H_{31}N_5O_4S$·0.25CH_2Cl_2) C, H, N, S.

6-Benzyl-8-[(2-methylbutyl)amino]thioinosine (52). This compound was prepared according to general procedure D: yield 87%; white solid; ^{1}H NMR (MeOD) δ 0.86−0.97 (m, 6H, 2 × C*H*$_3$), 1.03−1.29 (m, 1H, CH$_3$CH*H*), 1.39−1.63 (m, 1H, CH$_3$CH*H*), 1.69−1.89 (m, 1H, CH$_3$C*H*), 3.13−3.50 (m, 2H, C*H*$_2$N), 3.73−3.82 (m, 2H, *H-5′*), 4.11−4.17 (m, 1H, *H-4′*), 4.25 (dd, 1H, *J* = 1.5, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.67 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.15 (d, 1H, *J* = 7.7, *H-1′*), 7.13−7.30 (m, 3H, C*H* arom), 7.40 (dd, 2H, *J* = 1.8, *J* = 7.7, C*H* arom), 8.36 (s, 1H, *H-2*). Anal. ($C_{22}H_{29}N_5O_4S$·0.05CH_2Cl_2) C, H, N, S.

6-Benzyl-8-(cyclopropylamino)thioinosine (53). This compound was prepared according to general procedure D: yield 58%; white solid; ^{1}H NMR (MeOD) δ 0.56−0.66 (m, 2H, 2 × CH*H*), 0.76−0.85 (m, 2H, 2 × CH*H*), 2.78−2.90 (m, 2H, C*H*$_2$N), 3.72−3.88 (m, 2H, *H-5′*), 4.11 (dd, 1H, *J* = 1.8, *J* = 3.7, *H-4′*), 4.23 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.61 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 4.62 (s, 2H, C*H*$_2$S), 6.14 (d, 1H, *J* = 7.7, *H-1′*), 7.15−7.32 (m, 3H, C*H* arom), 7.41−7.47 (m, 2H, C*H* arom), 8.41 (s, 1H, *H-2*). Anal. ($C_{20}H_{23}N_5O_4S$·0.2CH_2Cl_2) C, H, N, S.

6-Benzyl-8-(cyclopentylamino)thioinosine (54). This compound was prepared according to general procedure D: yield 80%; white solid; ^{1}H NMR (MeOD) δ 1.50−1.84 (m, 6H, 2 × C*H*$_2$, 2 × CH*H*CH), 1.94−2.13 (m, 2H, 2 × CH*H*CH), 3.76−3.85 (m, 2H, *H-5′*), 4.12 (dd, 1H, *J* = 1.8, *J* = 3.7, *H-4′*), 4.25 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.29−4.44 (m, 1H, C*H*NH), 4.62 (s, 2H, C*H*$_2$S), 4.65 (dd, 1H, *J* = 5.5, *J* = 7.7, *H-2′*), 6.15 (d, 1H, *J* = 7.7, *H-1′*), 7.15−7.32 (m, 3H, C*H* arom), 7.42 (dd, 2H, *J* = 1.8, *J* = 8.0, C*H* arom), 8.37 (s, 1H, *H-2*). Anal. ($C_{22}H_{27}N_5O_4S$·0.05CH_2Cl_2) C, H, N, S.

6-Benzyl-8-(cyclohexylamino)thioinosine (55). This compound was prepared according to general procedure D: yield 93%; white solid; ^{1}H NMR (MeOD) δ 1.08−1.87 (m, 8H, 2 × NHCHC*H*H, 3 × C*H*$_2$), 1.93−2.09 (m, 2H, 2 × NHCHC*H*H), 3.73−3.97 (m, 3H, *H-5′*, CH$_2$C*H*NH), 4.11−4.17 (m, 1H, *H-4′*), 4.26 (dd, 1H, *J* = 1.8, *J* = 5.8, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 4.67 (dd, 1H, *J* = 5.8, *J* = 7.7, *H-2′*), 6.14 (d, 1H, *J* = 7.7, *H-1′*), 7.14−7.32 (m, 3H, C*H* arom), 7.38−7.44 (m, 2H, C*H* arom), 8.36 (s, 1H, *H-2*). Anal. ($C_{22}H_{27}N_5O_4S$·0.25CH_2Cl_2) C, H, N, S.

6-Benzyl-8-pyrrolidinothioinosine (56). This compound was prepared according to general procedure D: yield 90%; white solid; ^{1}H NMR (CDCl$_3$/MeOD 1/9, v/v) δ 1.95−2.09 (m, 4H, 2 × C*H*$_2$CH$_2$N), 3.66−3.80 (m, 5H, 2 × C*H*$_2$N, 1 × *H-5′*), 3.88 (ABX, 1H, *J* = 2.6, *J* = 12.4, 1 × *H-5′*), 4.14 (dd, 1H, *J* = 2.6, *J* = 4.4, *H-4′*), 4.38 (dd, 1H, *J* = 1.8, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 5.19 (dd, 1H, *J* = 5.1, *J* = 7.3, *H-2′*), 6.05 (d, 1H, *J* = 7.3, *H-1′*), 7.21−7.32 (m, 3H, C*H* arom), 7.42 (dd, 2H, *J* = 1.5, *J* = 8.0, C*H* arom), 8.40 (s, 1H, *H-2*). Anal. ($C_{21}H_{25}N_5O_4S$) C, H, N, S.

6-Benzyl-8-piperidinothioinosine (57). This compound was prepared according to general procedure D: yield 86%; colorless glass; ^{1}H NMR (CDCl$_3$/MeOD 1/9, v/v) δ 1.57−1.84 (m, 6H, 3 × C*H*$_2$), 3.24−3.50 (m, 4H, 2 × C*H*$_2$N), 3.80 (ABX, 1H, *J* = 2.9, *J* = 12.4, *H-5′*), 4.08−4.15 (m, 1H, *H-4′*), 4.40 (dd, 1H, *J* = 2.2, *J* = 5.5, *H-3′*), 4.61 (s, 2H, C*H*$_2$S), 5.19 (dd, 1H, *J* = 5.5, *J* = 7.3, *H-2′*), 5.81 (d, 1H, *J* = 7.3, *H-1′*), 7.14−7.32 (m, 3H, C*H* arom), 7.37−7.46 (m, 2H, C*H* arom), 8.48 (s, 1H, *H-2*). Anal. ($C_{22}H_{27}N_5O_4S$·0.2CH_2Cl_2) C, H, N, S.

6-Benzyl-8-morpholinothioinosine (58). This compound was prepared according to general procedure D: yield 94%; colorless glass; ^{1}H NMR (MeOD) δ 3.27−3.40 (m, 2H, C*H*$_2$N), 3.45−3.58 (m, 2H, C*H*$_2$N), 3.73 (ABX, 1H, *J* = 2.9, *J* = 12.4, 1 × *H-5′*), 3.81−3.94 (m, 5H, 2 × C*H*$_2$O, 1 × *H-5′*), 4.13 (dd, 1H, *J* = 2.9, *J* = 4.5, *H-4′*), 4.38 (dd, 1H, *J* = 2.2, *J* = 5.1, *H-3′*), 4.62 (s, 2H, C*H*$_2$S), 5.17 (dd, 1H, *J* = 5.1, *J* = 7.3, *H-2′*), 5.87 (d, 1H, *J* = 7.3, *H-1′*), 7.14−7.33 (m, 3H, C*H* arom), 7.42 (dd, 2H, *J* = 1.5, *J* = 8.0, C*H* arom), 8.52 (s, 1H, *H-2*). Anal. ($C_{21}H_{25}N_5O_4S$·0.2CH_2Cl_2) C, H, N, S.

9-Benzyl-6-(4-nitrobenzylsulfanyl)purine (59). This compound was prepared according to a literature procedure.[10]

9-Benzyl-6-chloropurine (61). This compound was prepared according to a literature procedure.[15]

9-Benzyl-hypoxanthine (62). This compound was prepared according to a literature procedure.[16]

9-Benzyl-8-bromohypoxanthine (63). This compound was prepared according to a literature procedure.[11] After workup the product was washed with CH_2Cl_2 and dried in vacuo to provide **62** as an off-white solid: yield 83%; white solid; ^{1}H NMR (DMSO-*d*$_6$) δ 5.37 (s, 2H, C*H*$_2$N), 7.20−7.42 (m, 5H, C*H* arom), 8.11 (s, 1H, *H-2*), 12.53 (br s, 1H, N*H*).

9-Benzyl-8-(cyclopentylamine)hypoxanthine (64). This compound was prepared according to general procedure E: yield 67%; white solid; ^{1}H NMR (DMSO-*d*$_6$) δ 1.43−1.96 (m, 8H, 4 × C*H*$_2$), 4.06−4.19 (m, 1H, C*H*N), 5.22 (s, 2H, PhC*H*$_2$N), 6.46 (d, 1H, *J* = 6.6, CHN*H*), 7.12−7.22 (m, 2H, C*H* arom), 7.24−7.38 (m, 3H, C*H* arom), 8.71 (s, 1H, *H-2*), 12.01 (br s, 1H, N*H*).

9-Benzyl-8-(cyclohexylamine)hypoxanthine (65). This compound was prepared according to general procedure E: yield 67%; off-white solid; ^{1}H NMR (CDCl$_3$) δ 0.96−2.03 (m, 10H, 5 × C*H*$_2$), 3.79−4.02 (m, 2H, CHN*H*, NHC*H*), 5.18 (s, 2H, C*H*$_2$N), 7.18−7.43 (m, 5H, C*H* arom), 8.02 (s, 1H, *H-2*), 11.54 (br s, 1H, N*H*).

9-Benzyl-8-pyrrolidinohypoxanthine (66). This compound was prepared according to general procedure A with omittance of the acetylation step (yield 74%) or general procedure E (yield: 56%): white solid; ^{1}H NMR (CDCl$_3$) δ 1.84−1.95 (m, 4H, 2 × C*H*$_2$CH$_2$N), 3.50−3.62 (m, 4H, 2 × C*H*$_2$N), 5.41 (s, 2H, C*H*$_2$N), 7.07−7.15 (m, 2H, C*H* arom), 7.25−7.39 (m, 3H, C*H* arom), 7.92 (s, 1H, *H-2*), 11.79 (br s, 1H, N*H*).

9-Benzyl-8-morpholinohypoxanthine (67). This compound was prepared according to general procedure A with omittance of the acetylation step (yield 55%) or general procedure E (yield 88%): white solid; ^{1}H NMR (CDCl$_3$) δ 3.20 (t, 4H, *J* = 4.7, 2 × C*H*$_2$N), 3.76 (t, 4H, *J* = 4.7, 2 × C*H*$_2$O), 5.29 (s, 2H, C*H*$_2$N), 7.15−7.41 (m, 5H, C*H* arom), 8.01 (s, 1H, *H-2*), 11.72 (br s, 1H, N*H*).

9-Benzyl-8-(cyclopentylamine)-6-mercaptopurine (68). This compound was prepared according to general procedure B: yield 8%; white solid; ^{1}H NMR (CDCl$_3$/MeOD 3/1, v/v) δ 1.29−1.69 (m, 6H, 2 × CH*H*CHNH, 2 × C*H*$_2$), 4.32−4.52 (m, 1H, C*H*NH), 5.24 (s, 2H, C*H*$_2$N), 7.13−7.42 (m, 5H, C*H* arom), 7.98 (br s, 1H, *H-2*).

9-Benzyl-8-(cyclohexylamine)-6-mercaptopurine (69). This compound was prepared according to general procedure B: yield 44%; pink solid; ^{1}H NMR (CDCl$_3$/MeOD 1/1, v/v) δ 1.03−2.04 (m, 10H, 5 × C*H*$_2$), 3.83−4.05 (m, 1H, NHC*H*), 5.27 (s, 2H, C*H*$_2$N), 7.12−7.39 (m, 5H, C*H* arom), 8.02 (s, 1H, *H-2*), 11.54 (br s, 1H, N*H*).

9-Benzyl-8-pyrrolidino-6-mercaptopurine (70). This compound was prepared according to general procedure B: yield 53%; white solid; ^{1}H NMR (CDCl$_3$/ MeOD 5/1, v/v) δ 1.81−2.02 (m, 4H, 2 × C*H*$_2$CH$_2$N), 3.55−3.72 (m, 4H, 2 × C*H*$_2$N), 5.46 (s, 2H, C*H*$_2$N), 7.02−7.14 (m, 2H, C*H* arom), 7.27−7.40 (m, 3H, C*H* arom), 8.00 (s, 1H, *H-2*).

9-Benzyl-8-morpholino-6-mercaptopurine (71). This compound was prepared according to general procedure B: yield 54%; yellowish solid; ^{1}H NMR (CDCl$_3$/ MeOD 3/2, v/v) δ 3.23−3.31 (m, 4H, 2 × C*H*$_2$N), 3.71−3.79 (m, 4H, 2 × C*H*$_2$O), 5.32 (s, 2H, C*H*$_2$N), 7.14−7.22 (m, 2H, C*H* arom), 7.29−7.39 (m, 3H, C*H* arom), 8.02 (s, 1H, *H-2*).

9-Benzyl-8-(cyclopentylamine)-6-(4-nitrobenzyl)-6-mercaptopurine (72). This compound was prepared according to general procedure C: yield 21%; yellow solid; ^{1}H NMR (CDCl$_3$) δ 1.18−1.64 (m, 6H, 2 × C*H*$_2$, 2 × CH*H*CHNH), 1.89−2.08 (m, 2H, 2 × CH*H*CHNH), 4.12 (d, 1H, *J* = 7.3, CHN*H*), 4.25−4.42 (m, 1H, C*H*NH), 4.70 (s, 2H, C*H*$_2$S), 5.19 (s, 2H, C*H*$_2$N), 7.17 (dd, 2H, *J* = 1.8, *J* = 7.3, C*H* arom), 7.29−7.40 (m, 3H, C*H* arom), 7.64 (d, 2H, *J* = 8.8, C*H* arom), 8.12 (d, 2H, *J* =

8.8, C*H* arom), 8.51 (s, 1H, *H-2*); HPLC (system A) t_R = 8.00 min, (system B) t_R = 11.23 min.

9-Benzyl-8-(cyclohexylamine)-6-(4-nitrobenzyl)-6-mercaptopurine (73). This compound was prepared according to general procedure C: yield 19%; yellow solid; ^{1}H NMR ($CDCl_3$) δ 0.96−1.64 (m, 8H, 3 × CH_2, 2 × CH*H*CHNH), 1.83−1.97 (m, 2H, 2 × CH*H*CHNH), 3.82−4.01 (m, 1H, NHC*H*), 4.08 (d, 1H, J = 8.0, CHN*H*), 4.70 (s, 2H, CH_2S), 5.20 (s, 2H, CH_2N), 7.12−7.24 (m, 2H, C*H* arom), 7.28−7.42 (m, 3H, C*H* arom), 7.64 (d, 2H, J = 8.8, C*H* arom), 8.13 (d, 2H, J = 8.8, C*H* arom), 8.51 (s, 1H, *H-2*). Anal. ($C_{25}H_{26}N_6O_2S\cdot0.3CH_2Cl_2$) C, H, N, S.

9-Benzyl-6-(4-nitrobenzyl)-8-pyrrolidino-6-mercaptopurine (74). This compound was prepared according to general procedure C: yield 83%; yellow solid; ^{1}H NMR ($CDCl_3$) δ 1.85−1.93 (m, 4H, 2 × CH_2CH$_2$N), 3.56−3.63 (m, 4H, 2 × CH$_2$CH_2N), 4.71 (s, 2H, CH_2S), 5.46 (s, 2H, CH_2N), 7.02−7.09 (m, 2H, C*H* arom), 7.24−7.37 (m, 3H, C*H* arom), 7.65 (d, 2H, J = 8.8, C*H* arom), 8.13 (d, 2H, J = 8.8, C*H* arom), 8.50 (s, 1H, *H-2*). Anal. ($C_{23}H_{22}N_6O_2S\cdot0.2CH_2Cl_2$) C, H, N, S.

9-Benzyl-8-morpholino-6-(4-nitrobenzyl)-6-mercaptopurine (75). This compound was prepared according to general procedure C: yield 78%; white solid; ^{1}H NMR (Bruker DMX 600 MHz, DMSO-d_6, 60 °C) δ 3.28 (t, 4H, J = 4.8, 2 × CH_2N), 3.63 (t, 4H, J = 4.8, 2 × CH_2O), 4.77 (s, 2H, CH_2S), 5.38 (s, 2H, CH_2N), 7.17−7.19 (m, 2H, C*H* arom), 7.26−7.34 (m, 3H, C*H* arom), 7.74 (d, 2H, J = 8.8, C*H* arom), 8.15 (d, 2H, J = 8.8, C*H* arom), 8.57 (s, 1H, *H-2*). Anal. ($C_{23}H_{22}N_6O_3S\cdot0.1CH_2Cl_2$) C, H, N, S.

Erythrocytes and Membrane Preparation. Whole human blood (Blood Bank, Leiden University Medical Center) was stirred in lysis buffer (1/2 v/v, 10 mM $MgCl_2$ in 10 mM Tris-HCl, pH 8.0 at 25 °C) for 1 h. After homogenization it was centrifuged for 50 min at 19 000 rpm. The supernatant was removed and the pellet was dissolved in ice-cold water and centrifuged again for 50 min. This procedure was repeated two more times. After removal of the last supernatant, 25 mL of buffer (50 mM Tris-HCl, pH 7.4 at 25 °C) was added to the final pink pellet. This suspension was homogenized and the ghosts were collected. Aliquots were stored at −80 °C until further use.

[^{3}H]NBTI Binding Assay. Saturation and displacement equilibrium NBTI binding to membranes prepared from human erythrocytes (ghosts) was determined at 25 °C based on a method previously described.[26]

Adenosine A_1 Receptor. The affinity for the A_1 receptor was determined on CHO cells expressing the human receptors, using [^{3}H]DPCPX as the radioligand. Membranes containing 10 μg of protein were incubated in a total volume of 400 μL of 50 mM Tris/HCl (pH 7.4) and [^{3}H]DPCPX (final concentration 1.6 nM) for 1 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 10 μM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h radioactivity was counted in an LKB rack β scintillation counter.

Data Analysis. K_i values were calculated using a nonlinear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA). The K_D value of the radioligand, [^{3}H]DPCPX, was 1.6 nM.

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Supporting Information Available: ^{13}C NMR spectra for **7**, **9**−**14**, **16**−**58**, **63**−**67**, and **69**−**75**; melting points for **46**−**58** and **72**−**75**; (HR)MS data of **20**, **21**, **46**−**58**, and **72**−**75**; and details on elemental analyses for **46**−**58** and **73**−**75**. This material is available free of charge via the Internet at http://pubs.acs.org.

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