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N-(*p*-Nitrophenoxy)carbonyl-3-morpholino-sydnonimine (NCMS) has been prepared from 3-morpholinosydnonimine hydrochloride. Using the Griess assay and the superoxide-mediated reduction of ferricytochrome c, the nitric oxide (NO•) and superoxide anion (O₂•⁻) - releasing properties in phosphate buffer pH 7.4 of this novel peroxynitrite donor was studied and compared with the known 3-morpholino-sydnonimine (SIN-1). From compound NCMS, a series of *N*-substituted sydnonimine derivatives were easily prepared that contain purine or melaminophenyl groups which specify a recognition by a trypanosomal purine transporter. The ability of these new sydnonimines to inhibit the uptake of [²³H]adenosine on *Trypanosoma equiperdum* was studied.

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Introduction.

Several types of cells, such as endothelial neutrophils or macrophages, produce significant amounts of both nitric oxide radical (NO•) and superoxide radical (O₂•⁻) which can recombine in a near diffusion-controlled reaction to give peroxynitrite (ONOO⁻) [1], a potent oxidant which decompose to generate highly reactive species capable of nitrating tyrosine residues in proteins and oxidizing DNA or lipids [2]. Decomposition of ONOO⁻ generates radicals such as carbonate, nitrogen dioxide or hydroxyl radicals that could account for its toxic properties [3], but the mechanism by which it decomposes has been a subject of controversy [4-6]. Sydnonimines are a class of compounds that generate simultaneously both nitric oxide radical and superoxide radical in neutral oxygenated aqueous media [7-8]. As a consequence, these compounds can be used as possible peroxynitrite donor and constitute an important class of compounds for understanding the contribution and the relevance of peroxynitrite in NO•-related biological processes. Sydnonimines exert different pharmacological actions [9], and also constitute an alternative to the organic nitrates in the treatment of cardiovascular diseases whose use is often limited by the development of nitrate tolerance [10]. They release nitric oxide either spontaneously or after enzymatic conversion and subsequent decarboxylation to SIN-1 (like molsidomine). In a preceding study [11], we reported the synthesis and the biological evaluation of a series of nitric oxide-releasing compounds (*S*-nitrosothiol and organic nitrate compounds) that contained melaminyl, adenine and adenosine moieties and exhibited a high affinity for a trypanosomal purine transporter (P2 transporter). We now extend this study to the preparation and the biological evaluation of a series of 3-morpholino-sydnonimines designed to deliver specifically peroxynitrite into the parasite *via* this transporter so that it accumulates and displays its cytotoxic effect. All these compounds were prepared starting from *N*-(*p*-nitrophenoxy)carbonyl-

3-morpholino-sydnonimine **1** (NCMS), a new sydnonimine that spontaneously decomposes in aqueous solution to yield both nitric oxide and superoxide anion radicals. The nitric oxide and superoxide releasing properties of this intermediate were investigated and compared with the known SIN-1.

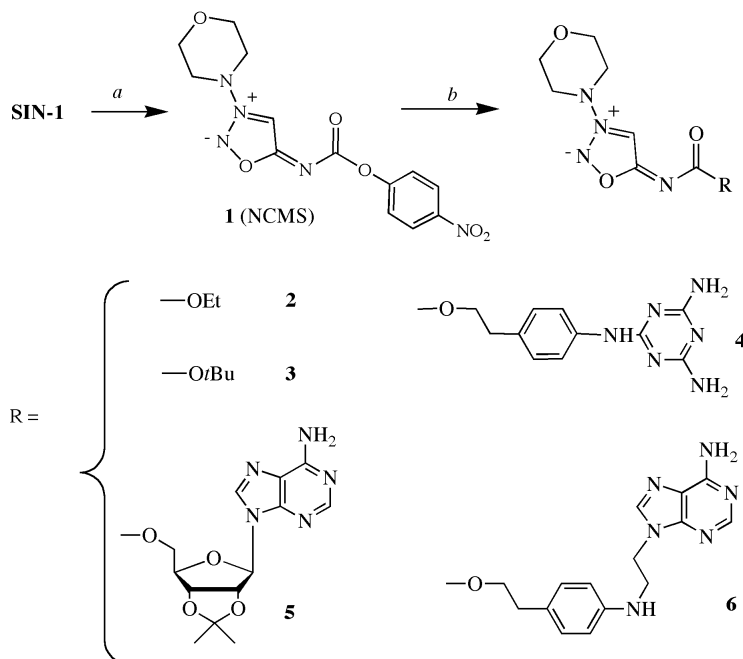
Results.

Synthesis of Sydnonimines.

SIN-1 was synthesized as previously described in a one-pot procedure from *N*-aminomorpholine and sodium formaldehyde bisulfite as starting material [12]. The imine intermediate was treated with potassium cyanide to yield a nitrile, which was then nitrosated to give the corresponding nitrosohydrazine. Cyclisation of this intermediate under acidic conditions gave SIN-1 with an overall yield of more than 50 %. The *p*-nitrophenyl sydnonimine compound, NCMS, was obtained by treatment of SIN-1 with 4-nitrophenyl chloroformate in pyridine. Reaction of NCMS in refluxing acetonitrile with various alcohols gave sydnonimines **4-6** (Scheme 1). For the synthesis of compound **4**, the alcohol precursor was obtained from 2-chloro-4,6-diamino-1,3,5-triazine and 4-aminophenethyl alcohol in refluxing aqueous sodium hydroxide. The alcohol used to obtain the adenine sydnonimine derivative **6** was synthesized in two steps from adenine, which was first alkylated with 1,2-dibromoethane, and the resulting intermediate was allowed to react with 2-(4-aminophenyl)-ethanol. The ethyl- (molsidomine, **2**) and *tert*-butyl- (**3**) derivatives of SIN-1, which lack a P2-recognition moiety were synthesized as reference compounds from NCMS in refluxing ethanol and *tert*-butanol, respectively.

Formation of Nitric Oxide and Superoxide.

The rate of formation of superoxide was measured with the commonly used spectrophotometric assay based on the superoxide-mediated reduction of ferricytochrome c



Scheme 1. Synthesis of Sydnonimine derivatives. (a) 4-Nitrophenyl chloroformate, pyridine 25 °C, 16 h (70 %). (b) 2: Refluxing ethanol, 3.5 h (83 %); 3: Refluxing *tert*-butanol, 18 h (70 %); 4: (2-4-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]phenyl)-1-ethanol, acetonitrile 82 °C, 4 h (26 %); 5: 2',3'-O-Isopropylidene-adenosine, acetonitrile 82 °C, 4 h (50 %); 6: 2-(4-[2-(6-Amino-9H-9-purinyl)ethyl]aminophenyl)-1-ethanol, acetonitrile 82 °C, 3 h (46 %).

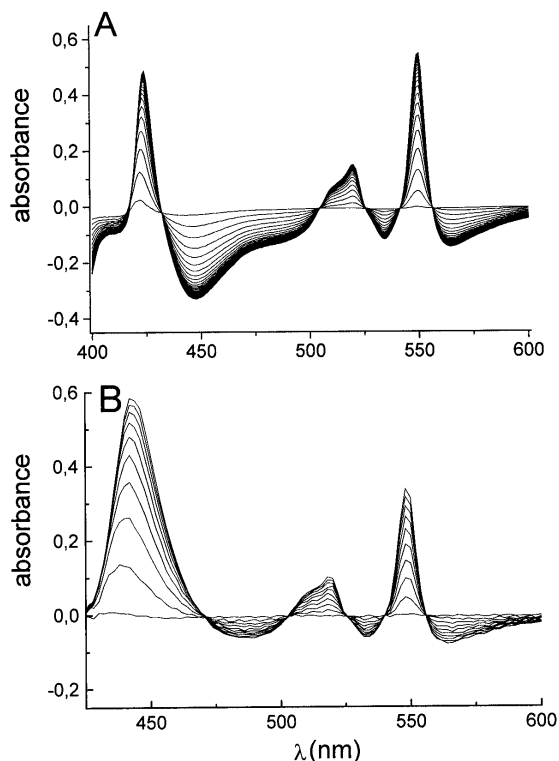


Figure 1. Difference spectral scans of reduction of ferricytochrome *c* (50 μ M) to ferrocytochrome *c* by $O_2^{\bullet-}$ generated from 500 μ M SIN-1 (A) and from 500 μ M NCMS (B). Repeated scans were performed at 2-min (A) or 10-min (B) intervals in 50 mM phosphate buffer (pH 7.4) containing 20 % DMSO and 1 mM EDTA

[13]. Figure 1A depicts the difference spectral scans of ferricytochrome *c* (5 μ M) to ferrocytochrome *c* during the decomposition of a solution of 3-morpholinosydnonimine (SIN-1, 500 μ M) in non-deoxygenated phosphate buffer (pH 7.4) at 25 °C, with a characteristic increase of the absorbance at 550 nm. The reaction of superoxide release followed a pseudo-first order kinetic with a half-life time value of around 30 minutes. The spontaneous hydrolysis of NCMS (50 μ M) was studied spectrophotometrically by monitoring the formation of the *p*-nitrophenoxide group at 420 nm. This reaction followed a typical first order kinetic with a half-life time value of around 40 minutes (not shown). As for SIN-1, the generation of $O_2^{\bullet-}$ from NCMS (500 μ M) was measured by the reduction of cytochrome *c*. The same characteristic increase in absorbance at 550 nm with a $t_{1/2}$ value of 55 minutes was observed, in addition of that at 420 nm due to the nitrophenoxide release (Figure 1B). The yields of nitric oxide from SIN-1 and NCMS were indirectly quantified using the Griess method [14] based on measurement of nitrite ions, products of the oxidative metabolism of $NO^{\bullet}$. The kinetics of $NO^{\bullet}$ release in a mixture of DMSO and phosphate buffer pH 7.4 followed a first order for the two compounds (17 mM) with $t_{1/2}$ values of about 6 h for SIN-1 (data not shown) and 14 h for NCMS (Figure 2). The difference of $NO^{\bullet}$ - liberation between the two compounds can be explained by the further hydrolytic step that is required for the formation of the $NO^{\bullet}$ - generating form NCMS.

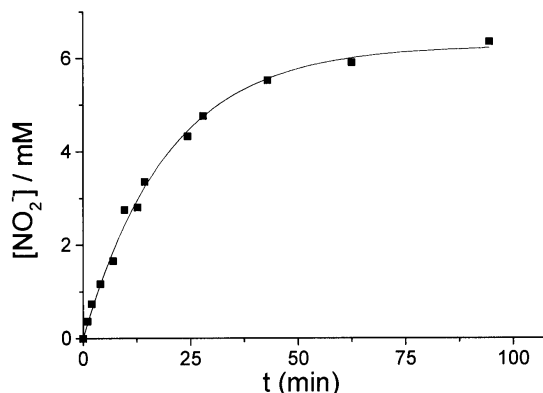


Figure 2. Nitrite ions formation from NCMS (17 mM) in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 90 % DMSO (25 °C). Concentrations of nitrite ions were determined using the assay based on Griess reaction [14].

Affinity for the P2 Purine Transporter.

Compounds **1-6** were tested on *Trypanosoma equiperdum* for their ability to inhibit the uptake of [2-³H]adenosine via the purine transporter P2 [15-16] (Table I). Because the natural substrate adenosine enters through both trypanosomal adenosine transporters P1 ($K_m = 0.6 \mu\text{M}$) and P2 ($K_m = 0.7 \mu\text{M}$), all experiments were carried out in the presence of a saturating concentration of inosine that is known to block transporter P1 [17]. Compared to the K_m value of the natural substrate adenosine, the values of inhibitory constants show that sydnonimines **4**, **5** and **6**, structurally related to melaminy, adenine and adenosine, respectively, efficiently inhibit adenosine transport with K_i ranging from 0.22 to 2.90 μM . In the same experimental conditions, the uptake of cymelarsan, an arsenical drug currently used in African trypanosomiasis treatment and known to enter through the P2-transporter [15], is less efficient. Compounds that lacked a P2 recognition group including SIN-1 and NCMS did not inhibit the adenosine transport with K_i values higher than 300 μM .

Table 1

Inhibition of P2 adenosine transporter in *Trypanosoma equiperdum* by Cymelarsan and Sydnonimine Compounds

Compounds	K_i (μM)
Adenosine	0.70 [a] $\pm$ 0.01
Cymelarsan	22.9 $\pm$ 0.2
SIN-1	> 300
NCMS (1)	> 300
2	> 300
3	> 300
4	0.22 $\pm$ 0.03
5	0.85 $\pm$ 0.10
6	2.90 $\pm$ 0.18

[a] K_m value of adenosine for transporter P2.

Discussion.

Early experiments have evidenced the mechanism of NO $\cdot$ - release from sydnonimines and the role of oxygen in initiating this reaction [7] (Figure 3): in a first step, SIN-1 decomposes spontaneously through ring opening under neutral conditions to give SIN-1A. Then, oxygen present in solution oxidises SIN-1A to give superoxide plus the corresponding cation radical (SIN-1B), which then spontaneously releases nitric oxide, H⁺ and SIN-1C. Therefore, sydnonimine derivatives may be considered as peroxynitrite donors as they release simultaneously stoichiometric amounts of NO $\cdot$ and O₂ $\cdot^-$ [18], thus mimicking the release of these radicals by macrophages, neutrophils, or endothelial cells, and the activity of iNOS [19]. For a therapeutic purpose, a strategy based on peroxynitrite releasing molecules could be exploited for the development of drugs with trypanocidal activity. Because of their general mode of

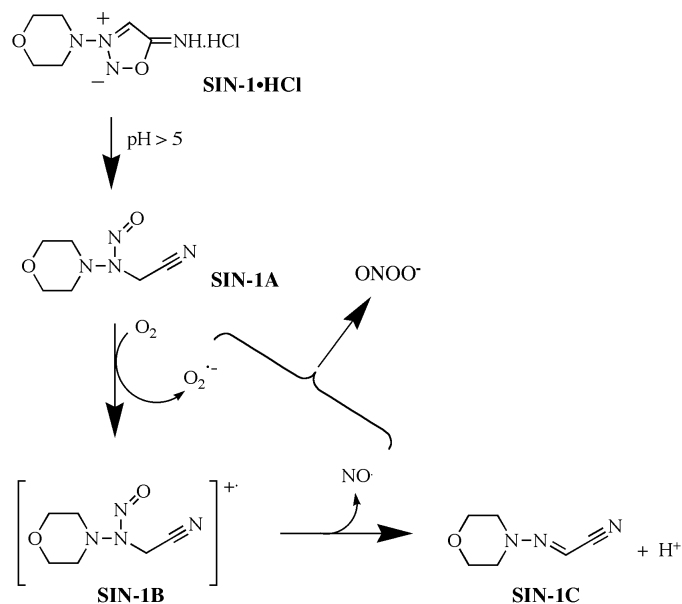


Figure 3. Mechanism of nitric oxide radical and superoxide anion radical release from sydnonimine SIN-1.

action, these compounds may overcome the drawbacks of current drugs such as resistance development. In the present work, three compounds (**4-6**) related to 3-morpholino-sydnonimine were designed in order to specifically target parasites that possess a P2-nucleoside transporter, so that they generate transient and high NO $\cdot$ and superoxide levels that might be responsible for the destruction of invading pathogens. These compounds that bear a P2-transporter recognition motif such as a melaminy, adenine or adenosine group, were found to efficiently inhibit the

uptake of adenosine with K_i values of the same order of magnitude of the K_m value of the natural substrate adenosine. The lethal dose (LD_{100}) of compounds **3** (*tert*-butyl ester) and **5** (adenosine derivative) in *Trypanosoma equiperdum* were determined to be 25 and 50 μ M within 48 h, respectively. In spite of a poor affinity for the transporter ($K_i > 300 \mu$ M), LD_{100} of NCMS was found to be 200 μ M. The other compounds were less toxic for the parasites with LD_{100} values superior to 200 μ M. In accordance with previous works, which showed that nitric oxide from NO•-donors inhibit the growth of several parasites including *Trypanosoma cruzi* or *Leishmania major* [20], we can hypothesized that the trypanocidal activity of the active compounds is due to the production of nitric oxide and superoxide with releasing rates compatible with the parasitic cellular replication cycle. Compounds that bear a P2-transporter recognition motif would probably require enzymatic activation, like molsidomine (**2**) that is known to be enzymatically converted in the liver to SIN-1 [21]. The new sydnonimine NCMS constitutes a key intermediate for the synthesis of these *N*-substituted compounds.

In contrast to most NO•-donor compounds, which require either enzymatic bioactivation or the presence of cofactors such as thiols (*i.e.* furoxans, organic nitrite and nitrate) or metal ions (*i.e.* *S*-nitrosothiols), NCMS decomposes spontaneously to yield the active NO•-releasing agent 3-morpholinosydnonimine, which then gives concomitantly nitric oxide and superoxide radicals. Despite the increasing discoveries relative to the biological role of peroxynitrite, compounds able to spontaneously release this potent oxidant, such as NCMS, are relatively limited. Moreover, this new sydnonimine provides a starting point for the synthesis of compounds with antimicrobial or other biological activities that could specifically deliver nitric oxide and/or peroxynitrite with controlled release rates, for example compatible with the rapid proliferation of trypanosomes.

EXPERIMENTAL

General.

Nuclear magnetic resonance spectra were recorded on a Bruker AC 250 at 250 MHz (^1H) or 60 MHz (^{13}C). Ultraviolet spectra were recorded with a CARY 1E spectrophotometer (VARIAN), and IR spectra on a Perkin-Elmer 1600 FTIR spectrophotometer. Silica gel 60 (70 – 230 mesh, Merck) was used for column chromatography and silica plates (60F254, Merck) were used for thin layer chromatography. SIN-1 hydrochloride was synthesized from *N*-aminomorpholine as previously described [12]. All chemicals were obtained from Aldrich, and used without further purification. Elemental analyses were performed by the *Ecole Nationale Supérieure de Chimie de Toulouse, France*.

Cytochrome *c* Assay.

Measurements of superoxide formation was carried out in 50 mM phosphate buffer, pH 7.4 (20 °C) containing cytochrome

c (50 μ M), superoxide-generating compound (SIN-1 or NCMS, 500 μ M), EDTA (1 mM) and DMSO (20 %). Spectra were recorded between 400 and 600 nm at 2- or 10-minutes intervals.

Griess Assay.

Nitrite ions were quantified using the Griess method: 10 μ L of a solution of SIN-1 or NCMS (16.7 mM) in a mixture of DMSO (90 %) and 50 mM phosphate buffer pH 7.4 (10 %) containing 1 mM EDTA were periodically taken and added to 890 μ L of water. 50 μ L of solution A and 50 μ L of solution B were then successively added, and the absorbance at 540 nm was recorded after 10 minutes (solution A: 1 g of sulfanilamide and 5 g of phosphoric acid in 100 ml water; solution B: 0.1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 100 ml water). A sodium nitrite solution (1 mM) in water was used as reference.

N-[(4-Nitrophenoxy)carbonyl]-3-morpholinosydnonimine (**1**, NCMS).

To a solution of 3-morpholinosydnonimine hydrochloride (2 g, 9.7 mmole) in dry pyridine (60 ml) was added 4-nitrophenyl chloroformate (2.9 g, 14.5 mmole). The reaction mixture was stirred at room temperature for 16 h. Pyridine was then removed under reduced pressure. The residue was triturated with hot dichloromethane and filtered to give **1** (2.26 g, 70 %) as a white powder. ir (KBr): 1691, 1614, 1510, 1261 cm^{-1} ; ^1H nmr (dimethyl sulfoxide- d_6): δ 3.62 (t, $J = 4.6$ Hz, 4H), 3.84 (t, $J = 4.6$ Hz, 4H), 7.41 (d, $J = 7$ Hz, 2H), 8.27 (d, $J = 7$ Hz, 2H), 8.42 (s, 1H); ^{13}C nmr (dimethyl sulfoxide- d_6): δ 53.3, 64.7, 100.2, 122.5, 124.9, 143.8, 157.3, 168.4, 173.7; ms (DCI, NH_3): m/z 336 [$\text{M}+\text{H}$] $^+$.

Anal. Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_5\text{O}_6$: C, 46.57; H, 3.91; N, 20.89. Found: C, 46.60; H, 3.91; N, 20.67.

N-(Ethoxycarbonyl)-3-morpholinosydnonimine (**2**).

A solution of **1** (0.3 g, 0.89 mmole) in ethanol (15 ml) was refluxed for 3.5 h. The solvent was evaporated and the residue was purified by column chromatography using ethyl acetate as eluent to yield **2** (0.18 g, 83 %) as a white powder; ir (KBr): 1653, 1562, 1266 cm^{-1} ; ^1H nmr (CD_3OD): δ 1.27 (t, $J = 7$ Hz, 3H), 3.60 (t, $J = 4.75$ Hz, 4H), 3.94 (t, $J = 4.75$ Hz, 4H), 4.12 (q, $J = 7$ Hz, 2H), 8.03 (s, 1H); ^{13}C nmr (CD_3OD): δ 14.9, 55.6, 62.3, 66.7, 100.8, 162.3, 175.4.

N-(*tert*-Butoxycarbonyl)-3-morpholinosydnonimine (**3**).

A solution of **1** (0.3 g, 0.89 mmole) in *tert*-butyl alcohol (15 ml) was refluxed for 18 h. The solvent was evaporated and the residue was purified by chromatography using a mixture of dichloromethane and ethyl acetate (8:2) as eluent to give **3** (0.17 g, 70 %) as a white powder; ir (KBr): 1662, 1614, 1295 cm^{-1} ; ^1H nmr (deuteriochloroform): δ 1.47 (s, 9H), 3.48 (t, $J = 4.7$ Hz, 4H), 3.93 (t, $J = 4.7$ Hz, 4H), 7.66 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 28.2, 54.8, 65.5, 78.9, 96.7, 160.6, 173.7.

Anal. Calcd. for $\text{C}_{11}\text{H}_{18}\text{N}_4\text{O}_4$: C, 48.88; H, 6.71; N, 20.73. Found: C, 49.24; H, 6.92; N, 19.89.

N-[(4-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]phenethyl)oxy]carbonyl]-3-morpholino-sydnonimine (**4**).

Alcohol precursor of **4** (2-4-[(4,6-diamino-1,3,5-triazin-2-yl)amino]phenyl-1-ethanol) was synthesized as follows: an aqueous 1 *M* sodium hydroxide solution (2 x 6.87 ml, 13.74 mmole) was slowly added during a period of 3.5 h to a refluxing solution of 2-chloro-4,6-diamino-1,3,5-triazin (2 g, 13.74 mmole) and 4-aminophenethyl alcohol (1.9 g, 13.7 mmole) in water (50 ml).

The mixture was then cooled (ice-bath), and the precipitate was collected by filtration and dried to yield the alcohol; ^1H nmr (dimethyl sulfoxide- d_6): δ 2.65 (t, J = 7 Hz, 2H), 3.56 (m, 2H), 4.60 (m, 1H), 6.29 (s, 2H), 7.05 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 8.73 (s, 1H); ^{13}C nmr (dimethyl sulfoxide- d_6): δ 38.36, 62.39, 119.63, 128.50, 132.09, 138.39, 164.72, 166.00. A solution of this alcohol (0.125 g, 0.5 mmole) and **1** (0.185, 0.5 mmole) in acetonitrile (10 ml) was refluxed for 4 h. The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was purified by column chromatography using a mixture of ethyl acetate and methanol (9:1) as eluent to yield **5** (0.056 g, 26 %) as a white powder; ir (KBr): 1664, 1597, 1264 cm^{-1} ; ^1H nmr (dimethyl sulfoxide- d_6): δ 2.84 (t, J = 6.5 Hz, 2H), 3.56 (t, J = 4.6 Hz, 4H), 3.84 (t, J = 4.6 Hz, 4H), 4.15 (t, J = 6.5 Hz, 2H), 6.25 (s, 4H), 7.09 (d, J = 8 Hz, 2H), 7.66 (d, J = 8 Hz, 2H), 8.15 (s, 1H), (s, 1H); ^{13}C nmr (dimethyl sulfoxide- d_6): δ 34.1, 53.4, 64.8, 65.2, 98.9, 119.6, 128.5, 130.8, 138.8, 160.1, 164.7, 167.0, 173.5; ms (DCI, NH_3): m/z 443 $[\text{M}+\text{H}]^+$.

Anal. Calcd. for $\text{C}_{18}\text{H}_{22}\text{N}_{10}\text{O}_4\cdot\text{H}_2\text{CO}_3$: C, 45.24; H, 4.80; N, 27.77. Found: C, 45.34; H, 4.85; N, 27.95.

N-(2',3'-Isopropylidene-5'-carbonyl-adenosine)-3-morpholinonydnonimine (**5**).

A solution of 2',3'-*O*-isopropylidene-adenosine (0.155 g, 0.5 mmole) and **1** (0.185, 0.5 mmole) in acetonitrile (10 ml) was heated to reflux for 4 h. The solvent was evaporated to dryness and the residue was purified by chromatography using a mixture of dichloromethane and methanol (95:5) as eluent to give **6** (0.125 g, 50 %) as a white powder; ir (KBr): 1652, 1594, 1263 cm^{-1} ; ^1H nmr (deuteriochloroform): δ 1.37 (s, 3H), 1.62 (s, 3H), 3.49 (t, J = 4.7 Hz, 4H), 3.92 (t, J = 4.7 Hz, 4H), 4.21 (dd, J = 12 and 4 Hz, 1H), 4.40 (dd, J = 12 and 4 Hz, 1H), 4.59 (q, J = 4 Hz, 1H), 5.06 (dd, J = 6 and 2 Hz, 1H), 5.40 (dd, J = 6 and 2 Hz, 1H), 6.19 (s, 2H), 6.22 (d, J = 2 Hz, 1H), 7.72 (s, 1H), 8.23 (s, 1H), 8.33 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 25.3, 27.2, 54.6, 65.2, 65.4, 81.9, 84.5, 84.6, 90.9, 99.4, 114.3, 119.7, 139.5, 149.7, 153.2, 155.6, 160.8, 174.4; ms (DCI, NH_3): m/z 504 $[\text{M}+\text{H}]^+$.

Anal. Calcd. for $\text{C}_{20}\text{H}_{25}\text{N}_9\text{O}_7\cdot 1.5\text{H}_2\text{CO}_3$: C, 43.29; H, 4.73; N, 21.13. Found: C, 43.84; H, 4.60; N, 20.88.

N-[(4-[2-(6-Amino-9H-9-puriny)ethyl]aminophenethyl)oxy]-carbonyl-3-morpholino sydnnonimine (**6**).

Alcohol precursor of **6** (2-(4-[2-(6-amino-9H-9-puriny)ethyl]-aminophenyl)-1-ethanol) was synthesized as follows: a solution of 9-(2-bromoethyl)-9H-6-purinamine (2 g, 8.26 mmole), 4-aminophenethyl alcohol (1.135 g, 8.26 mmole) and triethylamine (0.835 g, 8.26 mmole) in methanol (45 ml) was refluxed for 24 h. The solvent was evaporated to dryness and the residue was purified by chromatography using a mixture of ethyl acetate and methanol (9:1) as eluent to yield the alcohol (0.79 g, 32 %) as a yellow powder; ^1H nmr (dimethyl sulfoxide- d_6): δ 2.56 (t, J = 7 Hz), 3.42-3.53 (m, 4H), 4.29 (t, J = 6 Hz), 4.54 (s, 1H), 5.74 (s, 1H), 6.53 (d, J = 8 Hz, 2H), 6.92 (d, J = 8 Hz, 2H), 7.24 (s, 2H), 8.08 (s, 1H), 8.18 (s, 1H); ^{13}C nmr (dimethyl sulfoxide- d_6): δ 38.2, 42.4, 42.5, 62.7, 112.0, 118.7, 126.6, 129.3, 141.0, 146.3, 149.5, 152.3, 155.8. A solution of this alcohol (0.3 g, 1 mmole) and **1** (0.335 g, 1 mmole) in acetonitrile (15 ml) was refluxed for 3 h. The solvent was then removed under reduced pressure and

the residue was purified by chromatography using a mixture of ethyl acetate and methanol (7:3) as eluent to yield **6** (0.23 g, 46 %) as a white powder; ir (KBr): 1633, 1512, 1263 cm^{-1} ; ^1H nmr (dimethyl sulfoxide- d_6): δ 2.68 (t, J = 7 Hz, 2H), 3.53 (t, J = 6 Hz, 4H), 3.60 (t, J = 6 Hz, 2H), 3.80 (t, J = 4.7 Hz, 4H), 4.17 (m, 2H), 4.30 (t, J = 5 Hz, 1H), 4.65 (t, J = 4.7 Hz, 1H), 6.98 (d, J = 8 Hz, 2H), 7.08 (d, J = 8 Hz, 2H), 7.11 (s, 2H), 8.02 (s, 1H), 8.00 (s, 1H), 8.08 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 42.6, 48.0, 54.8, 53.5, 62.0, 64.9, 97.9, 118.6, 126.9, 128.6, 136.4, 140.7, 141.3, 149.6, 152.1, 155.7, 159.8, 171.6; ms (DCI, NH_3): m/z 495 $[\text{M}+\text{H}]^+$.

Anal. Calcd. for $\text{C}_{22}\text{H}_{26}\text{N}_{10}\text{O}_4\cdot 1.5\text{H}_2\text{CO}_3$: C, 49.64; H, 5.07; N, 25.17. Found: C, 49.51; H, 5.37; N, 25.22.

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