Photochemistry of Nucleoside Transport Inhibitor 6-S-Benzylated Thiopurine Ribonucleosides. Implications for a New Class of Photoaffinity Labels

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Photochemistry of the nucleoside transport inhibitor 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR, 1) yielded products from the initial homolytic cleavage of the benzyl–sulfur bond. Photoreduction of the nitro group also occurred to a minor extent. The quantum yield for the disappearance of 1 was ~0.04. Several photoproducts and secondary photoproducts were identified and confirmed by synthesis. Irradiation of 6-(benzylthio)-9-(β-D-ribofuranosyl)purine (BMPR, 3) also resulted in the formation of products from benzyl–sulfur cleavage with a quantum yield of ~0.01.

In the late 1970s and early 1980s, it was shown that 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR,[1] 1, Figure 1) competitively inhibited nucleoside transport in sheep erythrocytes.[2,3] It was found that 1 photolabeled a nucleoside transporter protein found in the erythrocyte membrane when the complex was exposed to UV light.[4] NBMPR was as efficient in labeling this protein as the analogous azido compound, 6-[(4-azidobenzyl)amino]-9-(β-D-ribofuranosyl)purine (ABA, Figure 1). The efficient photolabeling of this transport protein by 1 was unexpected. The azido group of ABA is frequently used in the design of reagents for photoaffinity labeling, whereas 1 has none of the groups normally employed. We now report studies on the photochemistry of NBMPR and related S- and N-benzylated analogues.

Photoaffinity Labeling

Photoaffinity labeling is a powerful tool for the analysis of host–guest relationships. Azide,[5] diazo ketone,[6] and diazirine[7] groups have been used to enhance the light-induced reactivity of photolabeling reagents. Benzophenone and acetophenone have also been used, although rarely, in photoaffinity labeling.[8] Unfortunately, the introduction of such functional groups into biomolecules is often difficult, and once introduced these groups sometimes are unstable. The phenylazido group is most commonly used, but it is somewhat bulky, is easily reduced by thiol groups, and can undergo other side reactions. The diazo ketone function is smaller than phenyl azide, but its introduction is more difficult. In addition it is unstable at low pH, can react with proteins in the absence of light, and can rearrange to a ketene upon irradiation. The diazirine group has high chemical and thermal stability but is more difficult to introduce and also can undergo side reactions.[8] Free radicals formed by homolytic bond cleavage or by excitation of a carbonyl group can couple with biomolecules. One terminus of the diradical formed from cleavage or excitation can abstract hydrogen from the host molecule, forming a new radical center. This host radical can couple with the remaining radical center on the guest molecule. This is the accepted mechanism for photolabeling by benzophenone and acetophenone.

In 1975 Paul, Chen, and Paterson reported[2] that the most potent inhibitors of nucleoside transport have a hydrophobic group (e.g. benzyl, cyclohexyl, 4-nitrobenzyl, or 4-methylbenzyl) attached to a sulfur, oxygen, or nitrogen atom at the 6-position of a purine nucleoside. The 6-S-substituted thiopurine ribonucleosides were the most effective, and 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)-purine (NBMPR, 1) was one of the best inhibitors with $IC_{50} = 1.5 \times 10^{-8}$ M. An important finding was that 1 bound with high affinity (apparent K_D 0.1–1.0 nM) to a nucleoside transport protein.[4] The inhibition of nucleoside transport by host cells has been used to counteract the toxicity of nucleoside analogs used in the treatment of diseases. The use of 1 in combination with a toxic nucleoside antibiotic, tubercidin, has been reported for the treatment of schistosomiasis[9] and malaria.[10] It also has been demonstrated that 1 reduced the toxicity of 1-(β-D-arabinofuranosyl)cytosine during treatment of leukemia in human cell lines.[11]

A nucleoside transport protein has been identified in the 4.5 band[12] (i.e. with an apparent molecular weight, M_r 45 000–65 000) with 1 as a photoaffinity labeling reagent. Changes in the UV spectra of 1 upon photolysis were reported in the initial investigation,[4] but no attempts have been made to determine the mode of photolabeling of the nucleoside transporter or to identify photoproducts. A nonspecifically tritiated sample of 1 was used, and it was not determined which part(s) of 1 bind(s) to the protein during photolysis. We have reported preliminary photochemical results with this compound[13] and now provide experimental details of our further investigation.

Potential sites of photoreactivity exist in 1. The nitro group is known to undergo photoreduction to hydroxylamines via a radical process in the presence of alcohols bearing abstractable hydrogens (Figure 2a).[14] Radical intermediates in this process might covalently bond to the protein. Sulfur–carbon bonds are susceptible to cleavage upon photolysis, especially benzyl- or allyl–sulfur bonds

(1) NBMPR is the acronym for "*p*-nitrobenzylmercaptopurine riboside".

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(4) Young, J. D.; Jarvis, S. M.; Robins, M. J.; Paterson, A. R. P. *J. Biol. Chem.* **1983**, *258*, 2202–2208.
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(14) Hashimoto, S.; Kano, K. *Tetrahedron Lett.* **1970**, 3509–3512.

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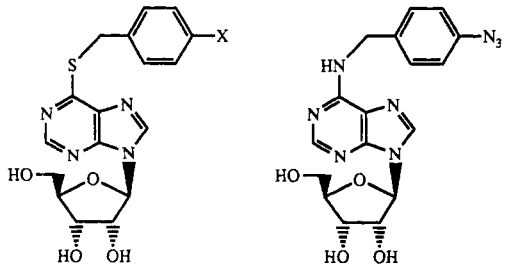

NBMPR (1), X = NO_2 ABA
BMPR (3), X = H

Figure 1. Structures of 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR, 1), 6-(benzylthio)-9-(β-D-ribofuranosyl)purine (BMPR, 3), and 6-[(4-azidobenzyl)amino]-9-(β-D-ribofuranosyl)purine (ABA).

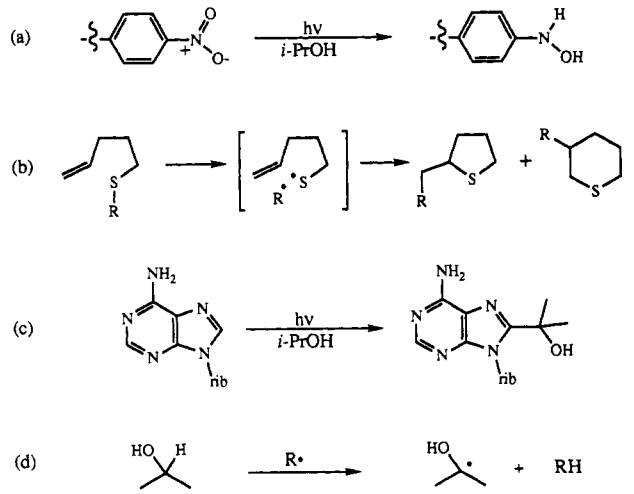

Figure 2. Examples of photochemistry possible with NBMPR (1).

(Figure 2b).[15] The resulting radicals might react like benzophenone through an abstraction–recombination process. The addition of radicals at the 8-position of adenosine upon irradiation has been reported,[16] and 1 might undergo a similar reaction (Figure 2c). If a host radical became bound to the purine, photolabeling would occur. Hydrogens on the sugar ring might be abstracted by a radical formed during photolysis (Figure 2d), and the resulting radical species might couple with a radical center on the host.

Results and Discussion

Synthesis of NBMPR (1) and BMPR (3). Nucleosides 1 and 3 were synthesized by benzylations of 6-thioinosine (2) as reported.[2] Formation of colored impurities was minimized by conducting the benzylations at <50 °C, and slightly higher yields were obtained by chilling the recrystallization solutions at −10 °C for extended periods.

Exploratory Photochemistry of 1. Initial photolyses were explored with 1 in *tert*-butyl alcohol containing 4% dimethyl sulfoxide. Analysis of the product mixture indicated reaction at the benzylic position. With this information we hypothesized that the sulfur–benzyl bond was the likely photoreactive site in 1. Combination reactions at this position or cleavage of the benzyl group from the nucleoside were viable possibilities.

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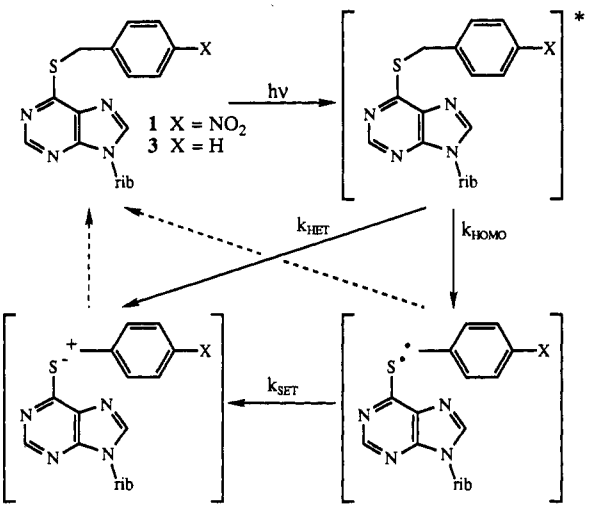
Scheme I. Initial Excited State and Cleavage Pathways



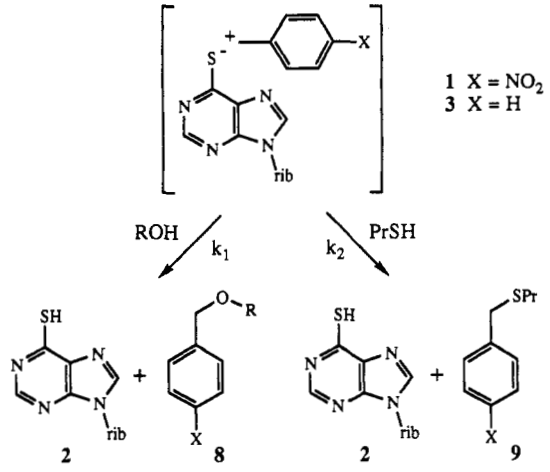
NBMPR (1) can be dissolved in methanol/chloroform (1:1, v:v) and 2-propanol/water (5:2, v:v), and these solvent systems were investigated as crude models of lipophilic "inner" and hydrated regions, respectively, of a protein. Irradiation of 1 in the first system and examination of photolysates by ^1H NMR spectroscopy indicated limited photoreactivity. However, in the 2-propanol/water mixtures essentially complete cleavage of the benzyl group from the nucleoside occurred. When oxygen was present, cleavage of the benzyl group was inhibited. Oxygen is known to quench triplet states, suggesting that the photochemical pathway involves a triplet.

Photochemistry of 1 with Additives. Compounds with substituents that might mimic protein functional groups at the binding site of the nucleoside transporter were added to photolyses mixtures. Propanethiol was chosen to imitate the sulfhydryl group of cysteine residues, dipropyl disulfide to mimic cysteine–cysteine disulfide linkages, propanamine to imitate lysine, and methanol or 2-propanol to imitate serine or threonine. Preliminary results from this study were reported earlier.[13] Addition of propanethiol to otherwise identical photolysis mixtures resulted in thiol trapping of benzyl-cleaved intermediates and a minor amount of a trapped nitro photoproduct. Addition of propanamine inhibited benzyl cleavage, possibly by making the solvent cage tighter and thereby favoring recombination reactions.

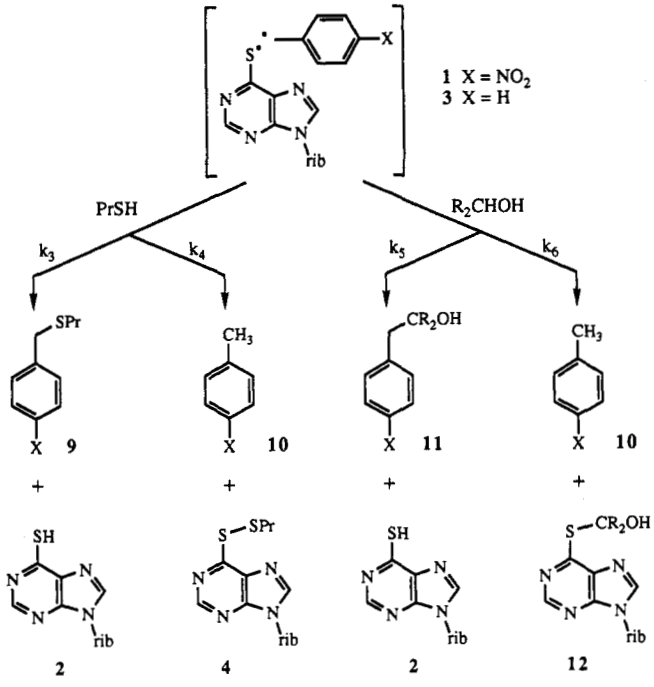
Pathways Involved in Photolysis of 1. Given that the primary site of photoreactivity in 1 is the benzyl–sulfur bond, it is possible to construct potential pathways that subsequent reactions might follow. The initial steps involved are shown in Scheme I. Upon photolysis either 1 or 3 would form an excited state that probably undergoes intersystem crossing to a triplet. This high-energy species could undergo cleavage at the benzyl-sulfur bond by one of two basic processes: heterolytic cleavage (pathway k_{HET}) to produce charged species or homolytic cleavage (pathway k_{HOMO}) to form radical intermediates. Single-electron transfer could occur between the radical intermediates to form charged species (pathway k_{SET}). Either set of intermediates could return to starting material if the solvent cage allowed recombination (dotted pathways) in preference to diffusion from the cage. The intermediates also could react with solvents or with additives in the photolysis mixtures.

Heterolytic Cleavage Products. If cleavage of the benzyl–sulfur bond were to occur by a heterolytic pathway or initial radical species were to undergo electron transfer (k_{SET}), the charged intermediates could be trapped by a

Scheme II. Products Formed by Heterolytic Cleavage of the Benzyl–Sulfur Bond



Scheme III. Products Formed by Homolytic Cleavage of the Benzyl–Sulfur Bond



solvent alcohol (pathway k_1) or added propanethiol. This is shown in Scheme II. The anion could be quenched by a proton from the alcohol to form 6-thioinosine (**2**), and the resulting alkoxide could attack the benzyl cation to form ether **8**. If the reaction mixture contained propanethiol (pathway k_2), the previously noted reactions would compete with a similar set involving propanethiol to form **2** and benzyl propyl sulfide (**9**). Alternatively, the sulfur cation and benzyl anion could be formed directly or from electron transfer. These intermediates would be expected to capture solvent to give substituted toluene and *S*-alkoxy-substituted thioinosine.

Homolytic Cleavage Products. Following homolytic cleavage, the radical intermediates could become involved in several alternative pathways to form different products as illustrated in Scheme III. In the presence of propanethiol, two possible pathways (k_3 and k_4) are noted. If the purinethiol radical were to abstract a hydrogen atom from propanethiol (k_3), 6-thioinosine (**2**) would be formed. The resulting propanethiol radical could couple with the benzylic radical to form sulfide **9**. These products are identical to those that would result from heterolytic cleavage

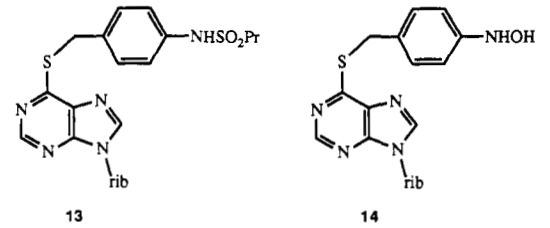
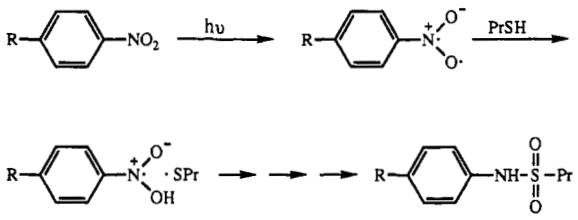


Figure 3. Products resulting from photoreduction of the nitro group.

Scheme IV. Pathway for Formation of 13



pathway k_2 (Scheme II). If the benzylic radical were to abstract a hydrogen atom from propanethiol first (pathway k_4), a toluene **10** would be formed and the resulting propanethiol radical could couple with the purinethiol radical to form disulfide **4**. Disulfide **4** and sulfide **9** also could be formed by individual trapping of both radical intermediates by propanethiol.

Alcohol present in the solvent mixture also could trap the radicals (pathways k_5 and k_6). If the purinethiol radical were to abstract a hydrogen from the alcohol (pathway k_5), **2** would be formed. The carbon radical could couple with the benzylic radical to form a phenethyl alcohol **11**. The benzylic radical also could abstract hydrogen from the alcohol solvent to form toluene or 4-nitrotoluene (**10**), and the resulting carbon radical could couple with the purinethiol radical to form the unstable purine hemithioacetal intermediate **12** (that would decompose to **2** and an aldehyde or ketone).

Isolation of Photoproducts. After photolysis (5 h) of **1** in methanol/chloroform with added propanethiol, photoproducts were separated by reversed-phase HPLC. Isolated products included 6-thioinosine (**2**), 4-nitrobenzyl propyl sulfide (**9**, X = NO_2), and a small amount of starting **1**. Three other fractions were isolated, two major and one minor. One of the major fractions contained the (propylthio)purine **7** (see below). The other major fraction was identified as 6-[[4-[*N*-(propylsulfonyl)amino]benzyl]thio]-9-(β-D-ribofuranosyl)purine (**13**) (Figure 3) by spectroscopic and chromatographic comparison with independently synthesized material.[17] This product presumably resulted from propanethiol trapping of a radical intermediate in the photoreduction of the nitro group followed by redox chemistry at the nitrogen and sulfur atoms as shown in Scheme IV.[18] The minor fraction was indicated to be 6-[[4-(hydroxyamino)benzyl]thio]-9-(β-D-ribofuranosyl)purine (**14**) by NMR and mass spectroscopy.

Distributions of the various purine-containing photoproducts were studied in different environments (Table I). It is noteworthy that photolyses in 2-propanol/water resulted in much more cleavage of the benzyl group than in methanol/chloroform, but the major product in both cases was 6-thioinosine (**2**).

Identification of Photoproducts. Comparisons of HPLC retention times allowed preliminary identification

(17) We thank the referees for suggesting the structure of compound **13**.

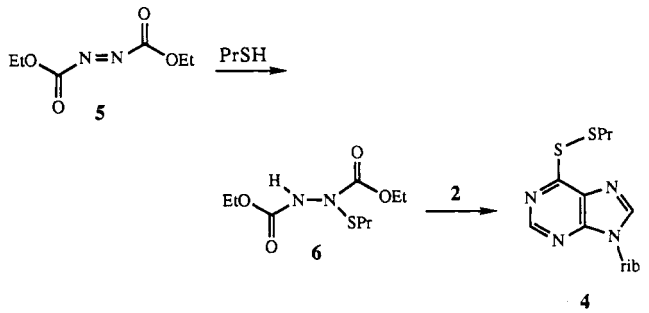
(18) Hart, H.; Link, J. W. *J. Org. Chem.* **1969**, *34*, 758–760.

Table I. Product Distribution for the Photolysis of NBMPR (1) with Additives[a]

run	solv[b]	additions[c]	1 (%)	2 (%)[d]	(4/7) (%)[d]	(13/14) (%)[d]
1	A	none	77.5	17.7	4.8	
2	A	2-PrOH	71.5	22.1	6.4	
3	A	$PrNH_2$	69.3	25.0	5.7	
4	A	Pr_2S_2	68.7	24.5	3.9	2.9
5	A	PrSH	1.3	76.1	14.3	8.3
6	B	none	6.0	93.9	0.1	
7	B	PrSH	6.0	45.5	29.0	19.5
8	B	$PrNH_2$	52.4	47.5	<0.1	

[a] Irradiations were performed with a 100-W medium-pressure Hg lamp through a Pyrex filter for 2 h. Deoxygenated solutions (7.0 mL) contained 3.4×10^{-3} M of NBMPR (1). [b] A = MeOH/$CHCl_3$ (1:1); B = *i*-PrOH/H_2O (5:2). [c] 0.20 mL of the compound was added to the 7.0-mL solution of 1. [d] 2 = 6-thioinosine; 4 = 6-[(propylthio)thio]-9-(β-D-ribofuranosyl)purine; 7 = 6-(propylthio)-9-(β-D-ribofuranosyl)purine; 13/14 = substituted-amine compounds.

Scheme V. Synthesis of 6-[(Propylthio)thio]-9-(β-D-ribofuranosyl)purine

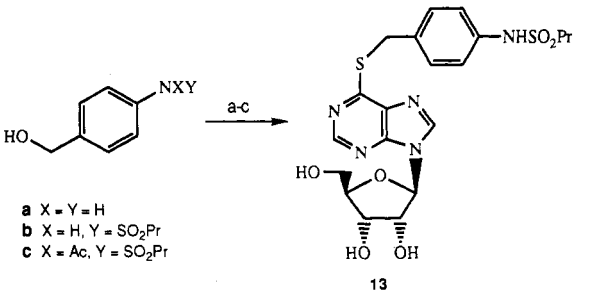


of the purine-containing products from photolyses of 1 as 6-thioinosine (2), disulfide 4, and sulfide 7 resulting from cleavage of the benzyl group, and comigrations upon coinjection with synthetic samples provided confirmatory evidence. Rapid migrations of the two nitro-reduced compounds 13 and 14 on reversed-phase HPLC were consistent with their polar structures. The benzyl cleavage fragment was trapped as the sulfide 9 (X = NO_2). No HPLC or NMR evidence was found for the presence of methyl 4-nitrobenzyl ether (8, X = NO_2). A minor amount of 4-nitrophenethyl alcohol (11, X = NO_2) was identified, and 4-nitrotoluene (10, X = NO_2) was detected by GC. This provides evidence compatible with three of the four homolytic cleavage pathways (k_3, k_4, and k_5 in Scheme III). No evidence was obtained for pathway k_1 (Scheme II) arising from heterolytic cleavage. Pathway k_2 cannot be ruled out since its products are identical to those from k_3.

Synthesis of Photoproducts. 6-[(Propylthio)thio]-9-(β-D-ribofuranosyl)purine (4) was synthesized by a modification of the procedure of Mukaiyama and Takahashi (Scheme V).[19] Diethyl azodicarboxylate (5) was treated with propanethiol to give adduct 6 which was isolated prior to its addition to a solution of 2. Spectral data of this resulting nucleoside were similar to those of a compound isolated from photolyses with added propanethiol. TLC comparisons of the synthetic disulfide 4 with the above crude photolysis mixture showed two compounds (4 and 7) with identical R_f values. An iodine–azide/ethanol solution that allows differentiation between sulfides and disulfides was used to develop the TLC plates.[20]

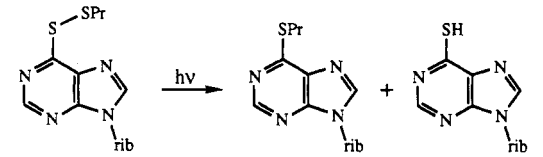
(19) Mukaiyama, T.; Takahashi, K. *Tetrahedron Lett.* **1968**, 5907–5908.
(20) Touchstone, J. C.; Dobbins, M. F. *Practice of Thin Layer Chromatography*, 2nd ed.; Wiley: New York, 1983; p 212.

Scheme VI. Synthesis of 6-[[4-[*N*-(Propylsulfonyl)amino]benzyl]thio]-9-(β-D-ribofuranosyl)purine[a]



[a] (a) $PrSO_2Cl$/pyridine/DMAP/MeCN; (b) (i) TMSCl/pyridine; (ii) AcCl; (iii) $NaHCO_3/H_2O$; (c) (i) 2′,3′,5′-tri-*O*-acetyl-6-thioinosine/Ph_3P/DEAD/THF; (ii) NH_3/MeOH.

Scheme VII. Secondary Photochemistry of Disulfide 4



Benzyl methyl ether (8, X = H), methyl 4-nitrobenzyl ether (8, X = NO_2), benzyl propyl sulfide (9, X = H), and 4-nitrobenzyl propyl sulfide (9, X = NO_2) were synthesized by standard Williamson ether synthesis methods. The potential photoproducts toluene (10, X = H), 4-nitrotoluene (10, X = NO_2), phenethyl alcohol (11, X = H, R = H), 4-nitrophenethyl alcohol (11, X = NO_2, R = H), and 6-thioinosine (2) were commercially available. The synthesis of 6-(propylthio)-9-(β-D-ribofuranosyl)purine (7) was accomplished analogously to those of 1 and 3. Comparisons of the mass spectra and NMR data for synthetic 7 with those of the isolated compound confirmed that sulfide 7 as well as disulfide 4 was a photoproduct with added propanethiol.

Treatment of 4-aminobenzyl alcohol with 1.5 equiv of propanesulfonyl chloride, DMAP, and pyridine in acetonitrile gave the *p*-(propanesulfonamido)benzyl alcohol (Scheme VI). Attempts to effect benzylic alkylation at S6 of 6-thioinosine derivatives with this compound by the Mitsunobu procedure[21] or by prior conversion to benzylic sulfonate or chloride intermediates resulted in formation of slowly migrating (TLC) decomposition products. Transient protection of the alcohol function with TMSCl[22] followed by treatment of the sulfonamide with acetyl chloride gave the *N*-acetyl-protected sulfonamidobenzyl alcohol. Mitsunobu benzylation of 2′,3′,5′-tri-*O*-acetyl-protected 6-thioinosine (2) with *p*-(*N*-acetylpropanesulfonamido)benzyl alcohol gave the desired benzylthio derivative plus a byproduct whose structure was not investigated. The major product was deacetylated (NH_3/MeOH) to give 6-[[4-[*N*-(propylsulfonyl)amino]benzyl]thio]-9-(β-D-ribofuranosyl)purine (13) with IR, ^1H, and ^{13}C NMR spectra and TLC and HPLC migration (comigration upon coinjection) identical to those of the major nitro-reduced photoproduct. MS and UV spectral data for the two samples were consistent, with minor intensity differences.

Secondary Photochemistry. It has been demonstrated that disulfides can extrude sulfur upon irradia-

(21) Mitsunobu, O. *Synthesis* **1981**, 1–28.
(22) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.

tion.[23] To determine whether this occurred in our systems, 1 was photolyzed for 18 h in a mixture of methanol/chloroform and propanethiol. The major compound was isolated and identified as 7 resulting from extrusion of a sulfur atom from disulfide 4. Following a 2-h photolysis of disulfide 4 in methanol/chloroform, the reaction mixture was examined by HPLC and NMR spectroscopy. Most of 4 (72%) remained, but sulfide 7 (9%) was produced and cleavage also occurred to form 2 (19%) (Scheme VII). The addition of propanethiol had a marked effect. In that case only 3% of starting 4 remained after photolysis, and 7 (31%) and 2 (66%) were produced.

Irregularities in the time study (see below) also indicated the possibility of secondary photochemistry of 2. This was verified by its photolysis in the presence of propanethiol. Irradiation of 2 for 2 h resulted in a mixture that contained 2 (78%), 7 (21%), and 4 (1%). It also was demonstrated that photolysis of sulfide 7 for 2 h caused cleavage to give 2 (~3%). With propanethiol added, that cleavage increased to 47%.

Photolysis of 3. Since 1 underwent homolytic benzyl–sulfur bond cleavage upon irradiation and the nitro group also suffered photoreduction, we examined the impact of the nitro group on the photocleavage reaction. Prolonged photolysis (19 h) of 3 in methanol/chloroform resulted in complete cleavage of the benzyl group from the nucleoside, as did photolysis of an identical sample with added propanethiol. Shorter photolyses (3 h) of 3 in methanol/chloroform with and without added propanethiol were examined by HPLC. A small amount of starting 3 remained in the sample without added propanethiol, and the only purine product was 2. Phenethyl alcohol (11, X = R = H) was identified by comparison of retention times with authentic material and by comigration upon coinjection. Neither benzyl methyl ether (8, X = H, R = CH_3) nor toluene (10, X = H) was detected. Photolysis of 3 with added propanethiol resulted in formation of 2, 7, and 4 as purine photoproducts. Phenethyl alcohol (11, X = R = H) and a small amount of toluene (10, X = H) were detected.

Photolysis of 6-*N*-(4-Nitrobenzyl)adenosine [6-[(4-Nitrobenzyl)amino]-9-(β-D-ribofuranosyl)purine]. The 6-amino analog of NBMPR is much less photoreactive. Preliminary studies showed that irradiation of this aminobenzylated adenosine derivative in methanol/chloroform resulted in minimal reaction unless propanethiol was added. With the thiol present, several new products were observed. A minor product was adenosine. Other products apparently resulted from nitro reduction. The benzyl–amine bond is not efficiently photocleaved.[24]

Summary of Observations. The major site of photoreactivity in NBMPR (1) is the sulfur–benzyl bond which is cleaved upon photolysis. The nitro group is not required for benzyl–sulfur bond cleavage.[25] The radical trapping agent, propanethiol, reacted with fragments generated by this cleavage to produce disulfide 4 and sulfide 7. In 2-propanol/water, cleavage occurred much more readily than in methanol/chloroform. Thus, it appears that the solvent cage is not as tight in 2-propanol/water solutions as in methanol/chloroform. In methanol/chloroform, the added propanethiol effects more efficient trapping of the radicals relative to their recombination to starting material. The other additives studied did not cause parallel effects. Since those additives can trap carbocation intermediates, significant involvement of the heterolytic cleavage pathways is precluded. No ether products that would result from heterolytic cleavage were observed.

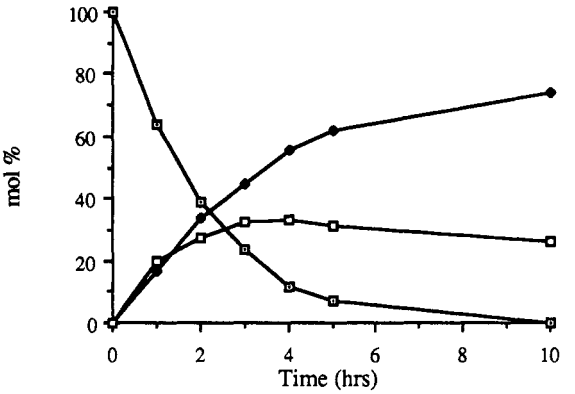


Figure 4. Time study of the photolysis of NBMPR (1) without added propanethiol: 1 (⊡), 2 (◆), reduced nitro products (□).

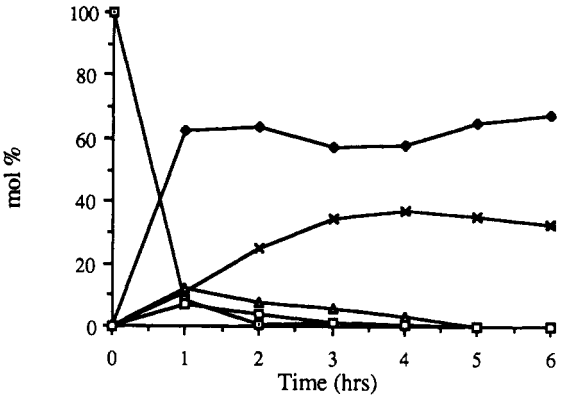


Figure 5. Time study of the photolysis of NBMPR (1) with added propanethiol: 1 (⊡), 2 (◆), 4 (△), 7 (×), 13/14 (□).

Time Studies of Photoproducts. Kinetics of the photoreactions were examined by monitoring photolyses every hour for product formation and ratios. When 1 was photolyzed in methanol/chloroform with no added propanethiol, a steady decrease in the amount of 1 and a steady increase in the formation of 2 was noted (Figure 4). A slower increase in the formation of compounds with reduced nitro groups also was seen, and within 4 h these also underwent significant cleavage to generate 2. Compound 1 was totally consumed within 10 h. Photolysis was continued for 22 h at which time 2 (89%) and the reduced nitro compounds (10.8%) remained.

An identical photolysis with added propanethiol showed a more complex distribution as expected (Figure 5). It is noteworthy that 1 was cleaved within 2 h and a much lower conversion to compounds with reduced nitro groups was observed. The major product again was 2. Disulfide 4 was formed, but was consumed within 5 h at which time the only remaining purine-containing products were 2 and sulfide 7. After 18 h, 2 (87%) and 7 (13%) remained.

BMPR (3) was photolyzed in methanol/chloroform without added propanethiol and examined every hour (Figure 6). Cleavage of 3 occurred within 4 h, whereas ~10 h was required for 1. After 4 h with 3, the only purine-containing product was 2. The time study of 3 with added propanethiol is informative (Figure 7). Disulfide 4 again was formed but was consumed within 6 h, similar to its lifetime in the photolysis of 1. Sulfide 7 was formed and steadily increased until most of the disulfide 4 was

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(24) For a recent example of benzyl–amine bond cleavage, see: Er-Rhaimini, A.; Mohsinaly, N.; Mornet, R. *Tetrahedron Lett.* **1990**, *31*, 5757–5760.

(25) The *p*-nitro group does serve to increase the efficiency of binding of 1 to the nucleoside transporter protein.[2]

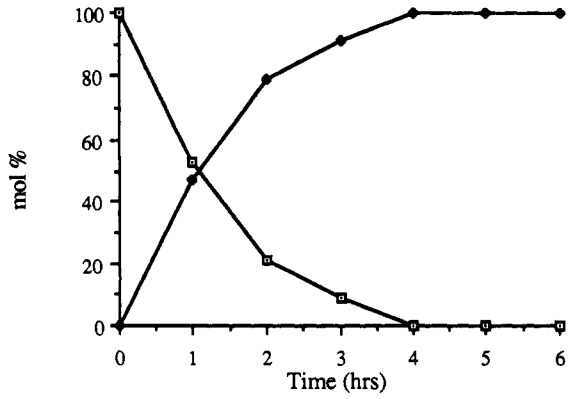

Figure 6. Time study of the photolysis of BMPR (3) without added propanethiol: 2 (◆), 3 (⊡).

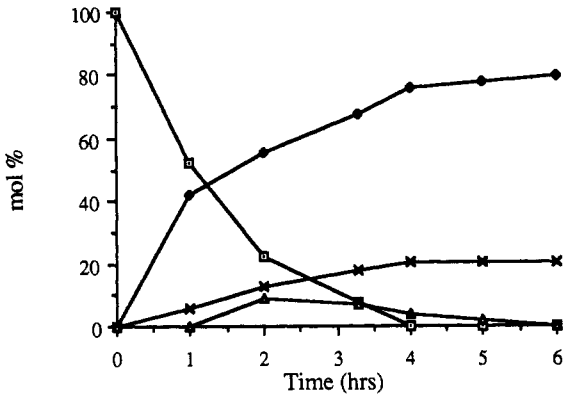

Figure 7. Time study of the photolysis of BMPR (3) with added propanethiol: 2 (◆), 3 (⊡), 4 (△), 7 (×).

Table II. Quantum Yields for Disappearance of 1 and 3[a]

	NBMPR (1)	BMPR (3)
without PrSH	0.04 ± 0.005	0.01 ± 0.005
with PrSH	0.02 ± 0.01	0.008 ± 0.002

[a] Errors based on scatter of experimental data. Light absorbed was determined on an optical bench equipped with a 200-W medium-pressure Hg–Xe lamp.[35] 1 and 3 were quantitated by calibrated HPLC.

consumed. This photolysis was not continued beyond 6 h. All products identified were consistent with those expected from initial homolytic cleavage of the benzyl–sulfur bond. Addition of propanethiol to photolysis mixtures with 3 did not accelerate its cleavage significantly, whereas this addition had a marked effect on the rate with 1.

Quantum Yield Determination. Quantum yields for the disappearance of 1 and 3 were determined in methanol/chloroform with and without added propanethiol (Table II).

Conclusions. We have shown that the nucleoside transport inhibitor 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR, 1) contains a photoreactive moiety, the benzyl–sulfur bond. This is cleaved homolytically upon photolysis, forming radical intermediates that are trapped by propanethiol. The nitro group is not required for this cleavage as evidenced by the parallel reactivity of the 6-(benzylthio)-9-(β-D-ribofuranosyl)purine (BMPR, 3) analog.

It is possible that a thiol group (i.e. a cysteine residue) present at the binding site of the nucleoside transport protein participates in photolabeling of this protein by 1. Proximity of a thiol group and 1 also might enhance photolabeling in the limited time during which the 1:protein complex is irradiated.[4] A difference noted between NBMPR-sensitive and -insensitive transporter proteins involves the exposure of a sulfhydryl group. The NBMPR-sensitive transporter protein is not inhibited by the *p*-(chloromercuri)phenyl sulfonate reagent that reacts with free thiol groups[26] whereas the NBMPR-insensitive protein is very susceptible to this organomercurial. However, the NBMPR-sensitive transporter is inhibited by this reagent if the cell membrane is inverted. The nucleoside transporter is presumed to consist of a protein dimer in the cell membrane.[27]

Previous studies[28] have demonstrated the increasing efficiency of photolabeling of the transporter protein by 1 with decreasing concentrations of dithiothreitol. We have confirmed that the disulfide bond of 4 is cleaved quickly by dithiothreitol to give 2. In a photolabeling experiment, added dithiothreitol might cause cleavage of a possible disulfide bond formed between the label and the bound residue.

The present studies indicate that if the binding site within a target biomolecule is large enought to accommodate a benzylthio moiety, an *S*-benzyl group should be considered as a candidate for photoaffinity labeling. Azidoaryl moieties used in many photoaffinity labeling studies might be replaced advantageously by benzylthio groups. A benzylthio unit usually is straightforward to introduce and stable under various experimental conditions, and benzyl thioethers normally can be stored indefinitely. Radicals formed upon photolysis of such thioethers are reactive intermediates that can bind readily to host molecules. Photolytic cleavage and binding may be enhanced in the proximity of a cysteine residue with a free thiol group. We are continuing to investigate the generality of this process and applications to photoaffinity labeling.

Experimental Section

General Procedures. Uncorrected melting points were determined on a hot-stage apparatus. ^{1}H NMR spectra were recorded at 200 mHz. Mass spectra (MS) were determined at 20 eV using direct probe sample introduction. Analytical HPLC at a flow rate of 1 mL/min, and preparative separations at 4.7 mL/min were performed with stainless steel columns packed with 8-μm, C_{18}-coated silica with a pore size of 60 Å. MeOH/H_2O (48:52, v:v) was used as eluant in all cases. Reactions were performed under an inert atmosphere of Ar or N_2. Anhydrous Na_2SO_4 was used as drying agent for extractions. EtOAc used for recrystallization was purified by distillation from P_4O_{10}. DMF and MeOH were dried and distilled from CaH_2. THF was purified by distillation from sodium benzophenone ketyl. Anhydrous Et_2O was used without purification. Benzyl ether and thioether comparison compounds were prepared by standard Williamson ether synthesis methods. 6-Thioinosine (6-mercaptopurine riboside) was purchased from Aldrich Chemical Co. All R_f values are from TLC (SiO_2; upper phase of EtOAc/PrOH/H_2O, 4:1:2).

6-[(4-Nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR, 1). Treatment of 6-thioinosine (2) as described[2] gave colorless crystalline 1 (75%): mp 194–195 °C (lit.[2] mp 198 °C); R_f ~0.6; IR (KBr) 3337, 3091, 2918, 1596, 1571, 1518, 1492, 1344, 940, 860, 638 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.78 and 8.73 (s and s, 1 and 1, H2,8), 8.16 (d, J = 8.8 Hz, 2, ArH_A), 7.74 (d, J = 8.8 Hz, 2, ArH_B), 5.99 (d, J = 5.5 Hz, 1 H1′), 5.52 (d, J = 5.9 Hz, 1, OH2′), 5.24 (d, J = 5.0 Hz, OH3′), 5.09 (t, J = 5.0 Hz, OH5′), 4.80 (s, 2, $ArCH_2$), 4.59 (bq, J = 5.9 Hz, 1, H2′), 4.16 (bq, J = 5.0 Hz,

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1, H3′), 3.96 (q, J = 4.0 Hz, 1, H4′), 3.62 (m, 2, H5′, 5″); MS m/z 419 (M^+).

Diethyl *N*-(Propylsulfenyl)hydrazodicarboxylate. A modification of the procedure of Mukaiyama and Takahashi[19] was used. Diethyl azodicarboxylate (1.00 mL, 6.35 mmol) and propanethiol (0.58 mL, 6.35 mmol) were added to anhydrous Et_2O (3 mL), and the solution was stirred for 3 h. White solid diethyl hydrazodicarboxylate was separated and the yellow liquid concentrated in vacuo. The concentrate was distilled with a Kugelrohr apparatus with collection bulbs cooled at 0 and −78 °C. At an oven temperature of 100 °C/0.05 Torr, dipropyl disulfide was collected in the bulb at −78 °C and unreacted diethyl azodicarboxylate at 0 °C. The light yellow title compound (1.19 g, 75%) was collected in a clean bulb at 150 °C/0.05 Torr: ^{1}H NMR ($CDCl_3$) δ 7.26 (bs, 1, NH), 4.23 (q, J = 6.0 Hz, 4, OCH_2), 2.96 (bt, J = 8.0 Hz, 2, SCH_2), 1.70 (sextet, J = 8.0 Hz, 2, CH_2), 1.28 (t, J = 6.0 Hz, 6, CH_3), 1.03 (t, J = 8.0 Hz, 3, CH_3).

6-[(Propylthio)thio]-9-(β-D-ribofuranosyl)purine (4). A solution of diethyl *N*-(propylsulfenyl)hydrazodicarboxylate (1.37 g, 5.47 mmol) in DMF (5 mL) was added to a solution of **2** (1.00 g, 3.52 mmol) in purified DMF (30 mL) and heated at 48–50 °C with stirring for 20 h. The reaction mixture was cooled, poured into cold H_2O (200 mL), and neutralized (10% HCl/H_2O) to pH 7. The clear solution was concentrated in vacuo, and the solid residue was washed with cold Me_2CO (15 mL × 3). The remaining solid was dissolved in warm EtOAc (150 mL), concentrated to 30 mL, and cooled at −25 °C. The crystalline material was filtered, washed with cold EtOAc, and dried (50 °C/0.025 Torr) to give colorless crystalline **4** (308 mg, 25%): mp 73–74 °C; R_f ~0.6; IR (KBr) 3318, 2926, 1567, 1436, 1336, 1207, 1120, 1085, 640 cm^{-1}; UV (1:1 H_2O/MeOH) max 283 nm (ϵ 18500); ^{1}H NMR (DMSO-d_6) δ 8.86 and 8.80 (s and s, 1 and 1, H2,8), 6.01 (d, J = 5.5 Hz, 1, H1′), 5.53 (d, J = 5.9 Hz, 1, OH2′), 5.24 (d, J = 5.1 Hz, OH3′), 5.09 (t, J = 5.5 Hz, OH5′), 4.59 (bq, J = 5.2 Hz, 1, H2′), 4.18 (bq, J = 5.1 Hz, 1, H3′), 3.97 (q, J = 3.7 Hz, 1, H4′), 3.63 (m, 2, H5′,5″), 2.91 (t, J = 7.2 Hz, 2, SCH_2), 1.65 (sextet, J = 7.1 Hz, 2, CH_2), 0.95 (t, J = 7.2 Hz, 3, CH_3); COSY experiments confirmed these assignments; MS m/z 358 (M^+), 284 (M − SPr). Anal. Calcd for $C_{13}H_{18}N_4O_4S_2$: C, 43.56; H, 5.06; N, 15.63. Found: C, 43.47; H, 5.01; N, 15.59.

6-(Benzylthio)-9-(β-D-ribofuranosyl)purine (BMPR, 3). The procedure to prepare **1** was used with **2** (200 mg, 0.70 mmol), K_2CO_3 (117 mg, 0.85 mmol), and benzyl bromide (1.45 mg, 0.85 mmol) in DMF (6 mL) and crystallization from EtOAc at −25 °C to give colorless **3** (2 crops; 102 mg, 40%). This product was dried (80 °C/0.025 Torr) for 15 h: mp softening at 72–75 °C (lit.[29] mp 156–158 °C); IR (KBr) 3321, 2926, 2360, 1568, 1493, 1120, 1082, 747, 701 cm^{-1}; UV (1:1 H_2O/MeOH) max 293 nm (ϵ 23400); ^{1}H NMR (DMSO-d_6) δ 8.79 and 8.72 (s and s, 1 and 1, H2,8), 7.46 (d, J = 7.2 Hz, 2, ArH_A), 7.30 (q, J = 7.2 Hz, 3, ArH_B), 5.99 (d, J = 5.5 Hz, 1, H1′), 5.53 (d, J = 5.9 Hz, 1, OH2′), 5.24 (d, J = 4.8 Hz, OH3′), 5.11 (t, J = 5.5 Hz, OH5′), 4.67 (s, 2, $ArCH_2$), 4.59 (bq, J = 5.2 Hz, 1, H2′), 4.17 (bq, J = 4.4 Hz, 1, H3′), 3.97 (q, J = 4.0 Hz, 1, H4′), 3.62 (m, 2, H5′, 5″); MS m/z 374 (M^+). Anal. Calcd for $C_{17}H_{18}N_4O_4S$: C, 54.68; H, 4.59; N, 15.00. Found: C, 54.43; H, 4.74; N, 14.81.

6-(Propylthio)-9-(β-D-ribofuranosyl)purine (7). The procedure to prepare **1** was used with **2** (200 mg, 0.70 mmol), bromopropane (121 mg, 0.98 mmol), and K_2CO_3 (117 mg, 0.85 mmol) in DMF (6 mL) at 50 °C for 20 h. The EtOAc solution was concentrated to ~10 mL and cooled at −25 °C to give colorless crystalline **7** (62 mg, 27%): mp 118–121 °C (lit. for monohydrate,[30] mp softening at 74 °C); R_f 0.7; IR (KBr) 3318, 2926, 1569, 1416, 1335, 1207, 1120, 1081, 634 cm^{-1}; UV (MeOH/H_2O, 1:1) max 293 nm (ϵ 30100); ^{1}H NMR (DMSO-d_6) δ 8.73, 8.70 (s, s; 1, 1; H2,8), 5.98 (d, J = 5.7 Hz, 1, H1′), 5.52 (d, J = 5.9 Hz, 1, OH2′), 5.23 (d, J = 5.0 Hz, OH3′), 5.11 (t, J = 5.6 Hz, OH5′), 4.59 (bq, J = 5.7 Hz, 1, H2′), 4.17 (bq, J = 5.1 Hz, 1, H3′), 3.96 (q, J = 3.7 Hz, 1, H4′), 3.62 (m, 2, H5′,5″), 3.33 (t, J = 7.2 Hz, 2, SCH_2), 1.73 (sextet, J = 7.1 Hz, 2, CH_2), 1.00 (t, J = 7.2 Hz, 3, CH_3); MS m/z 326 (M^+). Anal. Calcd for $C_{13}H_{18}N_4O_4S$: C, 47.84; H, 5.56; N, 17.17. Found: C, 47.75; H, 5.55; N, 17.03.

4-[*N*-(Propylsulfonyl)amino]benzyl Alcohol. To a solution of 4-aminobenzyl alcohol (0.37 g, 3.0 mmol) and pyridine (0.73 mL, 0.71 g, 9.0 mmol) in MeCN (15 mL) at 0 °C were added propanesulfonyl chloride (0.50 mL, 0.64 g, 4.5 mmol) and 4-(dimethylamino)pyridine (0.027 g, 0.22 mmol). After 1 h at 0 °C, the solvent was evaporated, and the residue was subjected to flash chromatography (hexanes/EtOAc, linear gradient 1:1 to 1:2) to give **2** (0.57 g, 84%) as a colorless oil that solidified on standing at ambient temperature: mp 40–42 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J = 8.4 Hz, 2, ArH_A), 7.19 (d, J = 8.4 Hz, 2, ArH_B), 6.79 (br s, 1, NH), 4.68 (s, 2, $ArCH_2$), 3.06 (t, J = 7.7 Hz, 2, SO_2CH_2), 1.83 ("sextet", J = 7.4 Hz, 2, CH_2), 1.80 (s, 1, OH), 1.01 (t, J = 7.4 Hz, 3, CH_3); MS m/z 229 (M^+).

4-[*N*-Acetyl-*N*-(propylsulfonyl)amino]benzyl Alcohol. To a stirred solution of 4-[*N*-(propylsulfonyl)amino]benzyl alcohol (0.25 g, 1.09 mmol) in pyridine (5.5 mL) was added TMSCl (0.35 mL, 0.29 g, 2.72 mmol). After 20 min at ambient temperature, the mixture was cooled to 0 °C, and acetyl chloride (0.19 mL, 0.21 g, 2.72 mmol) was slowly added. After 2 h at ambient temperature, the mixture was cooled to 0 °C, and saturated $NaHCO_3$/H_2O (10 mL) was added. After 4 h at ambient temperature, the mixture was extracted with EtOAc (2 × 30 mL), and the organic phase was washed with brine, dried, and evaporated. The residue was subjected to flash chromatography (hexanes/EtOAc, linear gradient 1:1 to 2:3) to give the title compound (0.25 g, 86%) as a pale yellow oil that solidified on standing at ambient temperature: mp 77–78 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, J = 8.2 Hz, 2, ArH_A), 7.37 (d, J = 8.2 Hz, 2, ArH_B), 4.73 (s, 2, $ArCH_2$), 3.64 (t, J = 7.8 Hz, 2, SO_2CH_2), 2.09 (br s, 1, OH), 1.94 (s, 3, $COCH_3$), 1.92 ("sextet", J = 7.6 Hz, 2, CH_2), 1.08 (t, J = 7.6 Hz, 3, CH_3); MS m/z 271 (M^+).

6-[[4-[*N*-(Propylsulfonyl)amino]benzyl]thio]-9-(β-D-ribofuranosyl)purine (13). To a suspension of 2′,3′,5′-tri-*O*-acetyl-6-thioinosine (0.30 g, 0.74 mmol), 4-[*N*-acetyl-*N*-(propylsulfonyl)amino]benzyl alcohol (0.20 g, 0.74 mmol), and triphenylphosphine (0.25 g, 0.95 mmol) in tetrahydrofuran (20 mL) under a nitrogen atmosphere was slowly added diethyl azodicarboxylate (DEAD; 150 μL, 166 mg, 0.95 mmol). After 18 h at ambient temperature, solvent was evaporated, and the residue was subjected to flash chromatography (hexanes/EtOAc, linear gradient 1:1 to 1:2) to provide crude 2′,3′,5′-tri-*O*-acetyl-**13** containing triphenylphosphine oxide. This material was stirred in NH_3/MeOH (20 mL) for 18 h at ambient temperature and evaporated. The residue was subjected to flash chromatography (CH_2Cl_2/MeOH, linear gradient 19:1 to 13:1) to give **13** (0.13 g, 36%) as a colorless foam with identical IR, ^{1}H, and ^{13}C NMR spectra to those of photoproduct **13**. Synthetic **13** had UV (MeOH) max 291, 227 nm (ϵ 22000, 20000), min 257, 214 nm (ϵ 5400, 15800) and MS peaks corresponding to those of photoproduct **13** except for intensity variations and minor peaks in the photoproduct spectrum. Anal. Calcd for $C_{20}H_{25}N_5O_6S_2 \cdot 0.5H_2O$: C, 47.61; H, 5.19; N, 13.88. Found: C, 47.90; H, 5.27; N, 13.72.

Spectral data for photoproduct **13**: IR (KBr) 3408, 2926, 1570, 1511, 1491, 1332, 1210, 1146, 982, 940, 929, 898, 840, 637 cm^{-1}; UV (1:1 H_2O/MeOH) max 292, 225 nm (ϵ 18200, 16700) min 254, 215 nm (ϵ 6000, 15500); ^{1}H NMR (DMSO-d_6) δ 9.77 (bs, 1, NH), 8.78, 8.71 (s, s; 1, 1; H2,8), 7.40 (d, J = 8.4 Hz, 2, ArH_A), 7.12 (d, J = 8.4 Hz, 2, ArH_B), 5.99 (d, J = 5.5 Hz, 1, H1′), 5.53 (d, J = 5.8 Hz, 1, OH2′), 5.25 (d, J = 5.0 Hz, OH3′), 5.11 (t, J = 5.6 Hz, OH5′), 4.61 (s, 2, CH_2Ar), 4.57 (t, J = 5.8 Hz, 1, H2′), 4.17 (bq, J = 3.7 Hz, 1, H3′), 3.96 (q, J = 3.6 Hz, 1, H4′), 3.62 (m, 2, H5′,5″), 3.02 (t, J = 7.5 Hz, 2, SO_2CH_2), 1.65 (sextet, J = 7.8 Hz, 2, CH_2), 0.90 (t, J = 7.4 Hz, 3, CH_3); ^{13}C NMR (50 MHz) δ 159.58 (s), 151.88 (d), 148.64 (s), 143.70 (d), 137.78 (s), 133.17 (s), 131.23 (s), 130.30 (d), 119.74 (d), 87.99 (d), 85.87 (d), 73.91 (d), 70.35 (d), 61.30 (t), 52.42 (t) 31.15 (t), 16.79 (t), 12.49 (q); MS (FAB) m/z 495 (M^+ $[C_{20}H_{25}N_5O_6S_2]$ = 495).

Methyl 4-Nitrobenzyl Ether. Kugelrohr distillation (collection bulb at 0 °C) at 180 °C/1.5 Torr gave light yellow material: mp 23–24 °C (lit.[31] mp 26–27 °C); IR (neat) 3111, 3079, 2930, 2823, 1606, 1522, 1347, 860 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.5 Hz, 2, ArH_A), 7.55 (d, J = 8.5 Hz, 2, ArH_B), 4.53 (s, 2, $ArCH_2$),

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3.34 (s, 3, OCH_3); MS m/z 167 (M^+).

Benzyl Propyl Sulfide. Distillation gave a clear liquid, bp 82 °C/4.5 Torr (lit.[32] bp 112 °C/14 Torr); IR (neat) 3027, 2960, 2930, 1602, 1584, 1494, 1376, 768, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.29 (s, 5, ArH), 3.70 (s, 2, $ArCH_2$), 2.35 (t, J = 7.0 Hz, 2, SCH_2), 1.50 (sextet, J = 7.0 Hz, 2, CH_2), 0.88 (t, J = 7.3 Hz, 3, CH_3); MS m/z 166 (M^+).

4-Nitrobenzyl Propyl Sulfide.[33] Distillation gave a light yellow oil: bp 86.5 °C/0.03 Torr; IR (neat) 3107, 3076, 2930, 2871, 1599, 1519, 1491, 1376, 1345, 858 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.18 (d, J = 8.6 Hz, 2, ArH_A), 7.59 (d, J = 8.6 Hz, 2, ArH_B), 3.84 (s, 2, $ArCH_2$), 2.37 (t, J = 7.3 Hz, 2, SCH_2), 1.50 (sextet, J = 7.1 Hz, 2, CH_2), 0.88 (t, J = 7.3 Hz, 3, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 147.22 (s), 130.12 (d), 124.21 (d), 35.93 (t), 33.83 (s), 22.62 (t), 13.56 (q); MS m/z 211 (M^+).

General Photolysis Procedures. Irradiations were performed with a Hanovia 100-W medium-pressure mercury lamp (except where noted) in a water-cooled quartz well. Small-scale photolyses were performed with 13- × 100-mm Pyrex test tubes held at a distance of 1.5 cm from the water-cooled well. All irradiation solutions were deoxygenated prior to photolysis by bubbling purified nitrogen[34] through the reaction mixture. Photograde Me_2CO, MeOH, and t-BuOH were dried by distillation from CaH_2. Photograde i-PrOH and $CHCl_3$ were purchased and used without purification. H_2O was distilled and used without further purification. All test tube runs contained 3.0×10^{-3} to 4.0×10^{-3} M of nucleoside in 7.0 mL of solvents. Solvent mixture ratios were $MeOH/CHCl_3$ (1:1, v:v) or i-$PrOH/H_2O$ (5:2, v:v) in all cases. Exploratory photolyses were performed with a Hanovia 450-W medium-pressure Hg lamp. Quantum yields were determined at 254 nm with a 200-W high-pressure Hg–Xe lamp, and light output was quantified with ferrioxalate actinometry.[35]

Exploratory Photolyses. With NBMPR (1). Run 1. NBMPR (1, 175 mg) was dissolved with heating in t-BuOH (175 mL)/DMSO (8 mL). The deoxygenated solution was photolyzed for 1.5 h, and the mixture was concentrated in vacuo. ^1H NMR analysis indicated reduction of the benzylic proton signal. TLC confirmed the presence of several new products.

Run 2. Photolysis of 1 in dry Me_2CO for 1.5 h with monitoring by TLC indicated loss of starting 1.

Run 3. Solutions of 1 (8 mg) in $MeOH/CHCl_3$ or i-$PrOH/H_2O$ in two test tubes were degassed, photolyzed for 3 h, and concentrated in vacuo. The $MeOH/CHCl_3$ solution showed little conversion of starting 1, whereas almost complete cleavage of the benzyl group had occurred in the i-$PrOH/H_2O$ solution.

Run 4. Duplicate pairs of test tubes with solutions of 1 (10 mg) in i-$PrOH/H_2O$ or $MeOH/CHCl_3$ were prepared, and one tube of each pair was degassed. Following photolysis for 3 h, the i-$PrOH/H_2O$ solution with oxygen present remained essentially unchanged whereas the degassed sample had undergone almost complete benzyl cleavage and had several new aromatic ^1H NMR signals. Benzyl cleavage had occurred ≤5% with both $MeOH/CHCl_3$ samples and starting 1 remained.

With BMPR (3). Run 1. Propanethiol (0.25 mL) was added to one of duplicate tubes with 3 (10 mg) in $MeOH/CHCl_3$. Both tubes were degassed and photolyzed for 19 h. ^1H NMR analysis showed complete cleavage of the benzyl group in both samples.

Run 2. Two tubes were prepared as in run 1 and photolyzed for 3 h. Minor benzyl cleavage to give 2 had occurred in the sample without propanethiol; 2, 4, and 7 were present in the sample with added propanethiol.

With 4. Run 1. A solution of synthetic 4 (10 mg) in $MeOH/CHCl_3$ was degassed and photolyzed for 2 h. ^1H NMR and HPLC showed starting 4 (72%), 7 (9%), and 2 (19%).

Run 2. A degassed solution as in run 1 with propanethiol (0.25 mL) added was photolyzed for 2.5 h. HPLC showed starting 4 (3%), 7 (31%), and 2 (66%).

With 7. Run 1. A solution of synthetic 7 (7 mg) in $MeOH/CHCl_3$ was degassed and photolyzed for 2 h. Starting 7 (97%) and a small amount of 2 (3%) were present.

Run 2. A degassed solution as in run 1 with propanethiol (0.25 mL) added was photolyzed for 2 h. HPLC showed starting 7 (53%) and 2 (47%).

With 2. A solution of 2 (7 mg) in $MeOH/CHCl_3$ containing propanethiol (0.20 mL) was degassed and photolyzed for 2 h. HPLC showed 2 (78%), 7 (21%), and 4 (1%).

Photolysis of 1 with Additives. Run 1. Solutions of 1 (10 mg) in $MeOH/CHCl_3$ were prepared in eight test tubes. To duplicate tubes was added 0.20 mL of propanethiol, dipropyl disulfide, propanamine, or 2-propanol. One tube of each pair was degassed prior to photolysis for 2 h, concentration, and analysis. No significant differences were seen between degassed and "oxygenated" sample pairs. In the tubes with added propanethiol, several new ^1H NMR peaks were seen corresponding to changes in both the aromatic region and cleavage of the benzyl group. TLC analysis followed by development of the plates with iodine–azide/ethanol solution[20] indicated the presence of disulfide 4. Additions of dipropyl disulfide, propanamine, or 2-propanol resulted in <5% reactions of starting 1.

Run 2. Solutions of 1 were prepared in five tubes as in run 1 and three tubes with 1 in i-$PrOH/H_2O$. One tube with each solvent was examined as a control. To each of the remaining four $MeOH/CHCl_3$ solutions was added 0.20 mL of propanethiol, propanamine, dipropyl disulfide, or 2-propanol. To the two remaining i-$PrOH/H_2O$ solutions was added 0.20 mL of propanethiol or propanamine. All tubes were degassed, photolyzed for 2 h, and subjected directly to HPLC analysis. The molar percentage of each purine compound was determined by integration after normalizing its UV activity at 254 nm in $MeOH/H_2O$ (1:1) with spectra of the compounds (synthetic, purchased, or isolated by HPLC) in the same solvent.

Photolysis of 1 and HPLC Separation. Propanethiol (8.5 mL) was added to a solution of 1 (300 mg) in $MeOH/CHCl_3$ (300 mL). This solution was degassed and irradiated. Every half hour a sample was withdrawn and analyzed, and after 5 h starting 1 was consumed. The reaction mixture was concentrated in vacuo and separated into eight fractions by HPLC. The first fraction contained 2 and propanethiol. Fraction 2 contained material eluting between major fractions and was not examined. Fraction 3 contained a mixture whose spectral data were too complex to interpret. Fraction 4 contained a mixture of a modified purine riboside with a benzyl group attached to the purine and a trace of 7. Spectral data for the modified compound suggested reduction of the nitro group to a hydroxylamine function to give 14. Fraction 5 contained 7. Fraction 6 contained another modified purine riboside identified as 6-[[4-[*N*-(propylsulfonyl)amino]benzyl]-thio]-9-(β-D-ribofuranosyl)purine (13) (see above). Fraction 7 contained a minor amount of starting 1. Fraction 8 contained 4-nitrobenzyl propyl sulfide.

Fraction 4 (14): ^1H NMR (DMSO-d_6) δ 8.76, 8.72 (s, s; 1, 1; H2,8), 7.61 (bs, 1, NOH), 7.45 (d, J = 8.4 Hz, 2, ArH_A), 6.81 (d, J = 8.4 Hz, 2, ArH_B), 6.04 (bs, 1, NH), 5.99 (d, J = 5.5 Hz, 1, H1′), 5.53 (d, J = 5.8 Hz, 1, OH2′), 5.25 (d, J = 5.0 Hz, OH3′), 5.11 (t, J = 5.6 Hz, OH5′), 4.61 (s, 2, CH_2Ar), 4.57 (t, J = 5.8 Hz, 1, H2′), 4.17 (bq, J = 3.7 Hz, 1, H3′), 3.96 (q, J = 3.6 Hz, 1, H4′), 3.62 (m, 2, H5′,5″); MS m/z 405 (M^+).

Time Course of the Photolysis of 1. Duplicate solutions of 1 (10 mg) in $MeOH/CHCl_3$ were prepared, and propanethiol (0.20 mL) was added to one tube. Both samples were degassed, photolyzed, and analyzed as described above. Data from the run without propanethiol are plotted in Figure 4 and from the run with propanethiol in Figure 5.

Time Course of the Photolysis of 3. Duplicate solutions of 3 were prepared and analyzed as described above for 1. Data are plotted in Figure 6 (without propanethiol) and Figure 7 (with propanethiol).

Quantum Yield with 1. A solution of 1 (120.4 mg) in $MeOH/CHCl_3$ (84 mL) was degassed for 45 min, irradiated for 4 h on the optical bench, and analyzed by HPLC. The solution absorbed 0.003 77 mE, and 0.139 mmol of 1 was reacted. The

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quantum yield of disappearance of 1 was 0.037.

Quantum Yield of 1 with Added Propanethiol. Propanethiol (2.4 mL) was added to an identical solution of 1 that was degassed for 30 min, irradiated for ~2h on the optical bench, and analyzed by HPLC. The solution absorbed 0.001 88 mE, and 0.0449 mmol of 1 was reacted. The quantum yield of disappearance of 1 was 0.024.

Quantum Yield with 3. A solution of 3 (65 mg) in $MeOH/CHCl_3$ (80 mL) was degassed for 45 min, irradiated on the optical bench, and analyzed by HPLC at approximately 1-h intervals.

Quantum Yield of 3 with Added Propanethiol. Propanethiol (2.4 mL) was added to an analogous solution of 3 (91.9 mg) in $MeOH/CHCl_3$ (78 mL). The solution was degassed for 30 min, irradiated on the optical bench, and aliquots were removed for HPLC analysis at ~30-min intervals.

Registry No. 1, 38048-32-7; 2, 574-25-4; 3, 6165-03-3; 4, 130948-35-5; 5, 1972-28-7; 6, 143330-50-1; 7, 143330-48-7; 14, 143330-49-8; propanethiol, 107-03-9; 4-[*N*-(propylsulfonyl)-amino]benzyl alcohol, 143330-51-2; 4-aminobenzyl alcohol, 623-04-1; propanesulfonyl chloride, 10147-36-1; 4-(*N*-acetyl-*N*-(propylsulfonyl)amino)benzyl alcohol, 143330-52-3; 2',3',5'-tri-*o*-acetyl-6-thioinosine, 3021-21-4.

Gas-Phase Ambident Reactivity of Monohydrated Enolate Anions

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The gas-phase reactions between a series of monohydrated enolate anions and unsaturated perfluorocarbon compounds have been studied using Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. The influence of selective solvation at the oxygen atom on the ambident reactivity of enolate anions in the gas phase is investigated through comparison of the observed product distributions with previously obtained data for the corresponding unsolvated anions. This analysis indicates that probably in order to make the nucleophilic addition reactions energetically accessible the water molecule is vaporized from the reaction complex prior to the addition reaction step. This evaporation of a water molecule from the reaction complex is considered to recover the intrinsic chemical reactivity of the enolate anions which is confirmed by the agreement between the observed ambident chemical behavior of the monohydrated and unsolvated enolate anions.

Introduction

Ambident ions can be characterized as ions in which reactive sites are connected through mesomerism.[1,2] The competition between the reactive sites is generally rationalized in terms of orbital versus charge control[3] or by the hard and soft acid/base concept.[4-6]

It is known from studies in the condensed phase that the reaction selectivity of ambident enolate anions is strongly influenced by temperature, counterion, and solvent. Variation of the solvent, for example, may drastically change the reaction selectivity.[1,7,8] From experimental studies it follows that the atom accommodating most of the charge[9-11] is selectively solvated in protic media.[12] It is generally believed that in protic polar media the solvent molecules preferentially associate with the hard oxygen atom (which carries the larger part of the charge) of enolate anions, thereby shielding the oxygen nucleophilic center. This promotes reaction via the carbon nucleophilic center of the ambident anion.[7,8,13,14]

Occasionally, however, the influence of the solvent is cancelled or even overruled by the counterion. In nonpolar aprotic solvents, for example, ion pairs of alkali enolates are strongly associated, which again promotes reaction via the carbon nucleophilic center.[1,12] The reaction via the oxygen nucleophilic center gains importance if the polarity and basicity of the solvent are increased. This effect can be accounted for by the enhanced solvation of the counterion. Clearly, the intrinsic ambident chemical behavior of enolate anions can be masked by solvent and counterion association effects.

In order to tackle this problem, the intrinsic behavior of ambident ions has also been studied in the gas phase, typically making use of mass spectrometric techniques. Among the substrates which have been used to probe the ambident reactivity of anions in the gas phase,[15-23] un-

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