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PHOTOCHEMISTRY OF THE NUCLEOSIDE MEMBRANE TRANSPORT INHIBITOR 6-[(4-NITROBENZYL)THIO]-9-(β-D-RIBOFURANOSYL)PURINE

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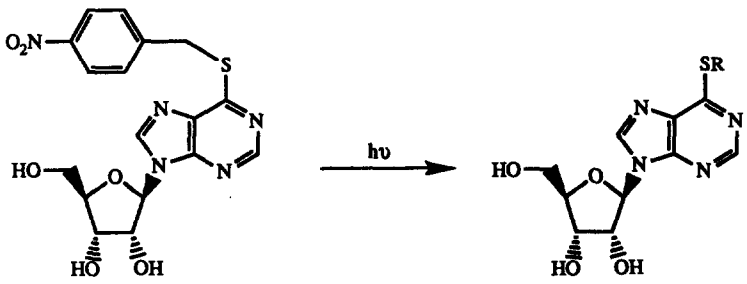
Summary: The photoactive site of 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine, an inhibitor of nucleoside transport across cellular membranes, was studied by radical trapping. Homolytic cleavage of the sulfur-benzyl bond occurred and thiol coupling was observed.

Photoaffinity labeling is a powerful tool for analysis of molecular binding to proteins. Unfortunately, functional groups that have limited stability frequently must be introduced into the molecule to be studied. For example, azido,[1] diazoketone,[2] or diazirine[3] groups are commonly used to facilitate light-induced reactivity for labeling purposes. These functionalities are sometimes difficult to introduce into biomolecules and can have other disadvantages.[1] The nucleoside transport inhibitor 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine (NBMPR, **1**)[4] has been found to undergo photoaffinity labeling of the band 4.5 polypeptide of the erythrocyte membrane.[5] We now report model studies and a plausible mechanism of photolabeling with this easily synthesized benzyl-substituted thioether.

In the initial study,[5] no attempt was made to determine the mode of labeling of the transporter protein by the title compound. NBMPR has several potential sites of photoreactivity: (1) the nitro group is known to undergo photoreduction in the presence of alcohols bearing abstractable hydrogens,[6] (2) addition of radical centers at the 8-position of adenosine upon irradiation has been reported,[7] and (3) sulfur-carbon bonds are susceptible to cleavage upon photolysis.[8] Since NBMPR bears no group with overt photoreactivity, but was found to label the transporter protein with comparable efficiency to that of a *p*-azidobenzyl analogue,[5] we have investigated its photochemical behavior.

NBMPR (**1**) was recovered and minor amounts of 6-thioinosine [6-mercaptopurine riboside, 9-(β-D-ribofuranosyl)purin-6(1*H*)-thione, **2**] were detected when **1** was irradiated in 2-propanol/water with no attempt to exclude oxygen.[9] An identical photolysis (see Table I, entry 1) after deoxygenation of the solution with purified nitrogen resulted in >90% cleavage of the benzyl group from the thioether. The cleaved photoproduct, 6-thioinosine (**2**), was confirmed by TLC,[10] spectral data, and HPLC comparison with a commercially available sample.[11] In the presence of propanethiol, an efficient radical trapping agent, photolysis (entry 2) gave **2**, **3**, and **4**, each of which had undergone benzyl cleavage. A photoproduct apparently resulting from thiol trapping of an intermediate with a reduced nitro group was also observed.[12] Compound **3** was identified as the disulfide resulting from thiol coupling with the cleaved sulfur radical by its mass

and NMR spectra (^{1}H, COSY).[13] Confirmation of its structure was provided by the synthesis of **3**, via the procedure of Mukaiyama and Takahashi,[14] and comigration (TLC, HPLC) of the two samples. The suspected secondary photochemistry of **3** was verified by photolysis of the synthetic sample. Two major processes occurred: (1) sulfur-sulfur bond cleavage to give thione **2**, and (2) sulfur extrusion[15] to yield **4**. Compound **4** was identified by HPLC and ^{1}H NMR comparison with a synthetic sample.[16] ^{1}H and ^{13}C NMR spectra of the photo-reduced product were consistent with a propanethiol-coupled aminobenzylthioinosine derivative.[12] Addition of propanamine to the aqueous photolysis mixture (entry 3) resulted in moderate conversion to **2**, only.



1 R = $CH_2C_6H_4NO_2(p)$
2 = H (thione tautomer)
3 = $SCH_2CH_2CH_3$
4 = $CH_2CH_2CH_3$

Table I. Photolyses of 6-[(4-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)purine[a]

Entry No.	Solvent[b]	Additions[c]	1(%)	2(%)	(3 + 4)(%)	Red. Nitro(%)
1	A	none	6.0	93.9	—	0.1
2	A	PrSH	6.0	45.5	29.0	19.5
3	A	$PrNH_2$	52.4	47.5	—	<0.1
4	B	none	77.5	17.7	—	4.8
5	B	2-PrOH	71.5	22.1	—	6.4
6	B	$PrNH_2$	69.3	25.0	—	5.7
7	B	PrSH	1.3	76.1	14.3	8.3

[a]Irradiations were performed with a 100 W medium-pressure Hg lamp through a pyrex filter for 2 h. Deoxygenated solutions (7.0 mL) were 3.4 x 10^{-3} M of NBMPR (**1**) in the solvents noted. [b]A = 2-propanol/water (5:1, v:v); B = methanol/chloroform (1:1, v:v). [c]0.20 mL of the indicated compound was added to the 7.0-mL solution of **1**.

When **1** was irradiated in deoxygenated methanol/chloroform (1:1) (entry 4) benzyl cleavage and nitro group reduction occurred. Addition of 2-propanol (entry 5) or propanamine (entry 6) had essentially no effect. Both photolyses gave **2** and small amounts of unidentified nitro-reduced compounds. Addition of propanethiol to the methanol/chloroform mixture and photolysis (entry 7) resulted in formation of **2**, **3**, and **4**. The major cleavage product was 6-thioinosine (**2**) (TLC, HPLC, spectral data, and isolation). The reduced-nitro photocoupling product found in entry 2[12] was again produced. Monitoring of the photocleavage of **1** also

revealed the formation of *p*-nitrotoluene (GC comparison) and *p*-nitrobenzylthiopropane. The identity of the latter thioether was confirmed by independent synthesis,[17] and isolation by HPLC. Further studies on related benzylic cleavage processes are underway.

In conclusion, it has been found that the primary photoreactive site on NBMPR (**1**) (in two solvent systems) is the sulfur-benzyl bond. Coupling of the putative purinethiol radical with propanethiol has been demonstrated in both model systems. The aqueous 2-propanol and methanolic chloroform solvent systems employed in this study were chosen to approximate the aqueous-protein boundary and interior hydrophobic protein regions, respectively, of the nucleoside transporter. Analogous photoinduced coupling of the purinethiol radical, or a radical species generated from nitro group reduction, with the thiol function of a cysteine residue in the NBMPR-binding domain of the transporter protein is our working hypothesis for continuing studies. The present results have provided a foundation for the future design of other benzylic photoaffinity-labeling compounds. The demonstration of protein binding with an aromatic substituent would invite the examination of *p*-nitrobenzylthio-modified substrates.

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9. All solvents were checked for purity and control experiments were run on each solution.

10. TLC plates were developed with iodine-azide/ethanol as described in Touchstone, J. C.; Dobbins, M. F. *Practice of Thin Layer Chromatography*, 2nd. Ed., Wiley: New York, **1983**, p. 212. This reagent is specific for thiols and disulfides; thioethers do not stain.

11. 6-Mercaptopurine riboside (99+%) was obtained from Aldrich Chemical Co.

12. ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 9.77 (bs, 1, NH), 8.78, 8.71 (s, s; 1, 1; H2,8), 7.41 (d, J = 8.5 Hz, 2, ArH_A), 7.13 (d, J = 8.5 Hz, 2, ArH_B), 5.99 (d, J = 5.5 Hz, 1, H1'), 5.55 (d, J = 5.9 Hz, 1, OH2'), 5.26 (d, J = 5.1 Hz, OH3'), 5.12 (t, J = 5.5 Hz, OH5'), 4.61 (s, 2, CH_2Ar), 4.59 (bq, J = 5.2 Hz, 1, H2'), 4.16 (bq, J = 5.1 Hz, 1, H3'), 3.96 (q, J = 3.7 Hz, 1, H4'), 3.62 (m, 2, H5',5"), 3.03 (t, J = 7.6 Hz, 2, SCH_2), 1.65 (sextet, J = 7.6 Hz, 2, CH_2), 0.91 (t, J = 7.6 Hz, 3, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6) 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).

13. Colorless crystalline 6-(propanethio)thio-9-(β-D-ribofuranosyl)purine (3) had mp 73-74 °C, R_f 0.6 (SiO_2, upper phase of EtOAc/PrOH/H_2O, 4:1:2); MS, 20 eV, m/z 358, (M^+), 284 (M-SC_3H_7); IR (KBr) 3318, 2926, 1567, 1436, 1336, 1207, 1120, 1085, 640 cm^{-1}; UV (MeOH/H_2O, 1:1) 283 nm (ε 18,500); ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 8.86, 8.80 (s, s; 1, 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) D_2O exchange and COSY experiments confirmed these assignments. *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.

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15. Fujihara, H.; Chiu, J-J.; Furukawa, N. *Tetrahedron Lett.* **1989**, *30*, 7441.

16. This compound was synthesized by the procedure for NBMPR.[4] Colorless crystalline 6-propylthio-9-(β-D-ribofuranosyl)purine (4) had mp 118-121 °C, R_f 0.7 (SiO_2, upper phase of EtOAc/PrOH/H_2O, 4:1:2); MS, 20 eV, m/z 326, (M^+); IR (KBr) 3318, 2926, 1569, 1416, 1335, 1207, 1120, 1081, 634 cm^{-1}; UV (MeOH/H_2O,1:1) 293 nm (ε 30,100); ^{1}H NMR (200 MHz, Me_2SO-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); *Anal.* Calcd. for $C_{13}H_{18}N_4O_4S$: C, 47.85; H, 5.56; N, 17.17. Found: C, 47.75; H, 5.55; N, 17.03.

17. The standard thioether synthesis of *p*-nitrobenzylthiopropane utilized PrSH/*p*-nitrobenzyl bromide/KOH/THF.

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