

## Sugar-Linked Dithiocarbamates as Modulators of Metabolic and Genotoxic Properties of *N*-Nitroso Compounds

Byung-Hoon Lee,<sup>†</sup> Barbara Bertram,<sup>\*‡</sup> Peter Schmezer,<sup>§</sup> Norbert Frank,<sup>‡</sup> and Manfred Wiessler<sup>‡</sup>

*Division of Molecular Toxicology and Division of Toxicology and Cancer Risk Factors, German Cancer Research Center, 69120 Heidelberg, Germany*

Received November 1, 1993<sup>\*</sup>

A series of putative anticarcinogenic and antimutagenic compounds was synthesized on the basis of tetraethylthiuram disulfide (disulfiram) and its metabolite, diethyldithiocarbamate (DDTC). Diallyldithiocarbamate was synthesized in order to combine the anticarcinogenic properties of diallyl sulfide, a known inhibitor of chemical carcinogenesis from *Allium* species, and those of DDTC. Several sugar-linked dithiocarbamates (SDTCs) were prepared using glucose, cellobiose, and lactose as glycosyl donors and DDTC and diallyldithiocarbamate as acceptors. All the S-glycoside bonds of SDTCs were very stable under physiological conditions in vitro. At low nitrosamine concentrations, glucose-DDTC inhibited microsomal nitrosamine dealkylases in vitro. In vivo these enzymes were also inhibited 4 h after ip administration of glucose-DDTC or lactose-DDTC to rats (1.7 mmol/kg); after 24 h, the values had returned to control levels. Glucose-DDTC induced the activity of glutathione-related enzymes. Concomitant treatment of rats with glucose-DDTC and *N*-nitrosodiethylamine (NDEA) led to a depression of the oxidative metabolism of [<sup>14</sup>C]NDEA to <sup>14</sup>CO<sub>2</sub> but increased the elimination of unchanged [<sup>14</sup>C]NDEA in the urine. Furthermore, glucose-DDTC totally inhibited the formation of DNA single-strand breaks induced by NDEA. All these effects may contribute to possible antimutagenic and anticarcinogenic actions of the dithiocarbamates investigated.

### Introduction

A number of compounds have been shown to inhibit or reduce the occurrence of neoplasia when administered in combination with carcinogens.<sup>1–3</sup> *N,N,N',N'*-tetraethylthiuram disulfide (disulfiram; DSF) and its main metabolite, *N,N*-diethyldithiocarbamate (**1**; DDTC), are potent anticarcinogenic substances containing a thionosulfur group in the molecule. Complete inhibition of carcinogenesis by DSF is described for some aromatic hydrocarbons and 1,2-dimethylhydrazine.<sup>4</sup> In the case of *N*-nitrosodiethylamine (NDEA) and *N*-nitrosodimethylamine (NDMA), DSF leads to a shift in the organotropy of tumor induction.<sup>5</sup> The mechanisms by which these compounds modulate toxic and carcinogenic properties of chemical carcinogens are well investigated with regard to some nitrosamines: (i) DSF and DDTC inhibit the mixed function oxidase system.<sup>6,7</sup> Treatment of rats exposed to nitrosamine with DSF or DDTC caused a diminished production of carcinogen-derived CO<sub>2</sub> and increased the elimination of non-metabolized nitrosamine in the urine.<sup>8</sup> The inhibition of metabolism results in a reduction of nitrosamine-induced alkylation<sup>8</sup> and strand breaks of rat liver DNA and RNA.<sup>9,10</sup> (ii) DSF increases glutathione contents and detoxifying phase II enzymes like glutathione *S*-transferase (GST)<sup>11,12</sup> and UDP-glucuronyl transferase.<sup>13</sup> (iii) DDTC serves as a nucleophile that acts as a scavenger for proximate or ultimate carcinogens and forms stable intermediates in vitro.<sup>14,15</sup>

Essential oils of garlic and onions possess anticarcinogenic activity.<sup>16</sup> The active principles of these antineoplastic plant oils have been found to be organosulfur compounds containing allyl groups. The most potent compounds out of this group are diallyl sulfide and diallyl disulfide.<sup>17,18</sup> Several studies suggest that the anticarcinogenic properties of these compounds are connected to the activation of glutathione related enzymes<sup>17,18</sup> and the inhibition of microsomal monooxygenase.<sup>19,20</sup>

As it seemed promising to combine their anticarcinogenic effects with those of dithiocarbamate, we have synthesized a dithiocarbamate analogue with the allyl function. Furthermore, we have attempted to design sugar derivatives of dithiocarbamates that may possess greater stability and better cell permeability. In recent years, monosaccharide conjugates of different types were synthesized to improve the therapeutic efficiency of the aglycons, e.g., azidothymidine (AZT) used in HIV therapy was conjugated with glucose and mannose for a better transport to the brain.<sup>21</sup> Glycosylated oligopeptides which function as renin inhibitors possess better pharmacokinetic parameters than the parent compound.<sup>22</sup> Glycosylation of antileishmanial drugs resulted in better internalization by macrophages.<sup>23</sup> Porphyrins,<sup>24,25</sup> which are used in photodynamic therapy, were much more water soluble after glycosylation.

In this paper, the synthesis of *N,N*-diallyldithiocarbamate (**2**; DATC) and sugar-linked dithiocarbamates (SDTCs), as well as their biological, biochemical, and pharmacokinetic properties, is reported.

### Chemistry

Diethyl- and diallyldithiocarbamate were used as glycosyl acceptors, and glucose, lactose, and cellobiose were chosen as sugar residues (Scheme 1). Dial-

<sup>\*</sup> Author to whom correspondence should be addressed.

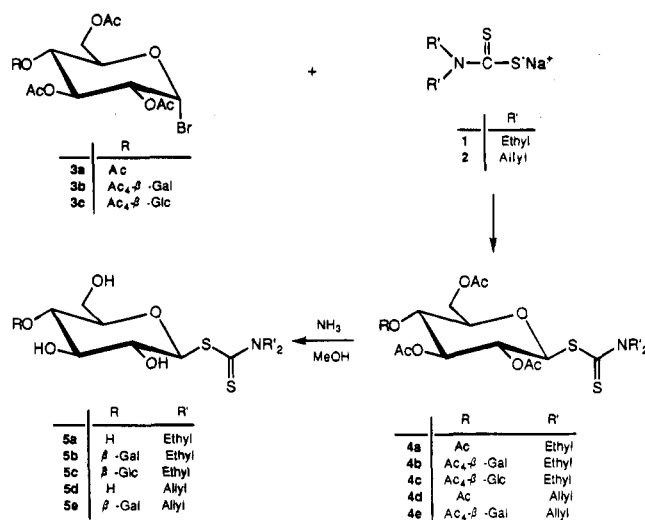
<sup>†</sup> Current address: Doping Control Center, Korea Institute of Science and Technology, P.O. Box 131 Cheongryang, Seoul 130-650, Korea.

<sup>‡</sup> Division of Molecular Toxicology.

<sup>§</sup> Division of Toxicology and Cancer Risk Factors.

<sup>\*</sup> Abstract published in *Advance ACS Abstracts*, August 15, 1994.

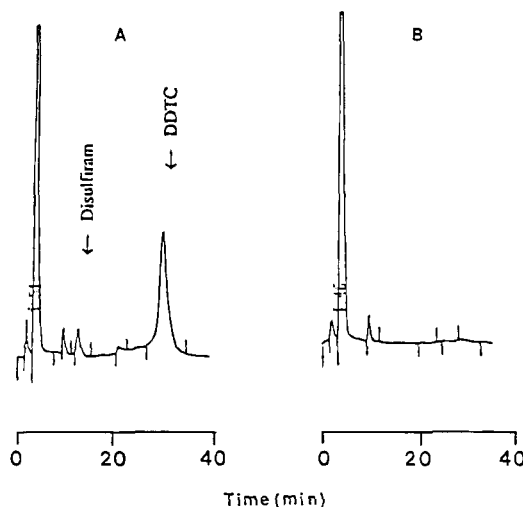
## Scheme 1



lyldithiocarbamate (2) was prepared by reacting CS<sub>2</sub> with diallylamine at basic pH and purified through recrystallization from Et<sub>2</sub>O. Because of its instability in the air, crystals were filtered and stored under N<sub>2</sub> gas. The SDTCs **5a** (Glc-DDTC), **5b** (Lac-DDTC), **5c** (Cel-DDTC), **5d** (Glc-DATC), and **5e** (Lac-DATC) were prepared according to Tejima and Ishiguro<sup>26</sup> with slight modifications. The acetylated glycosyl bromides **3a-c** were treated with dithiocarbamate 1 and 2 in acetonitrile or acetone to afford the corresponding acetylated thioglycosides **4a-e**. Hydrolysis of the protecting groups of **4a-e** with ammonia in methanol gave the free SDTCs **5a-e**. The compounds synthesized were characterized by <sup>1</sup>H NMR and elemental analysis.

## Biology

SDTCs were synthesized to act as prodrugs, which release their active moiety only after enzymatic cleavage. Therefore the possibility of enzymatic hydrolysis of the S-glycoside linkage was tested. Our primary biochemical assay measured the effects of these compounds on glutathione (GSH and GSSG) content, glutathione-related enzymes, and nitrosamine-activating enzymes *ex vivo* in the rat liver and compared to those of free dithiocarbamates. Nitrosamine dealkylase activity was assayed *in vitro*, and the mode of enzyme inhibition is discussed. Furthermore the effect of our compounds on nitrosamine metabolism in the rat measured as <sup>14</sup>CO<sub>2</sub> exhalation was examined by the use of [<sup>14</sup>C]NDEA. The influence of a selected compound on NDEA-induced genotoxicity in the rat liver was evalu-



**Figure 1.** High-performance liquid chromatographic profile of the incubates of **5a** in phosphate buffer (pH 7.4) in the presence of  $\beta$ -glucosidase (A) and  $\alpha$ -glucosidase (B) after 6 days of incubation at 25 °C. Aliquots of incubates were filtered through a 0.45  $\mu$ m filter and applied to a  $\mu$ Bondapak C18 column (300  $\times$  3.9 mm). The column was eluted with acetonitrile-H<sub>2</sub>O, 52:48, at a rate of 1 mL/min, and the eluate was detected at 254 nm.

ated using the alkaline elution technique. The pharmacokinetic behavior of SDTCs was investigated in rats.

## Biological Results

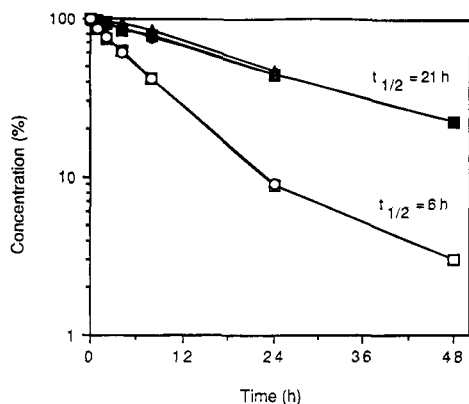
**Enzymatic Hydrolysis of SDTCs.** The O-glycosidic bond in the 1-4 linkage of two sugar molecules in disaccharides is very susceptible to the action of the corresponding glycosidases. In contrast, all the S-glycoside bonds tested were very stable under the conditions *in vitro*, showing, e.g., for **5a** in the presence of  $\beta$ -glucosidase at pH 7.4 a half-life of 6-12 days (Table 1). Under these conditions, DDTC and glucose were detected using HPLC (Figure 1) and TLC (data not shown). This compound was also degraded by  $\alpha$ -glucosidase, showing a half-life of 9-18 days. But in this case, neither DDTC nor glucose were detectable in our assay system. The hydrolytic effect of these enzymes increased at pH 4.8 (Figure 2). The rate of degradation of the compounds was relatively slow in rat liver cytosol ( $t_{1/2}$  = 2.3-6.3 days). They did not undergo nonenzymatic degradation by bovine serum albumin (BSA) or GSH. Thioglycosidase, traditionally used for hydrolyzing glucosinolates, and esterase failed to degrade the compounds.

**Biochemical Parameters.** The influence of **5a** on the dealkylating activity of liver microsomal enzyme

**Table 1.** Enzymatic Hydrolysis of SDTCs in Phosphate Buffer (pH 7.4) by Bacterial Glycolytic Enzymes

substance	enzyme	bond <sup>a</sup>	$t_{1/2}$ <sup>b</sup>	hydrolysate	detection
<b>5a</b>	buffer		stable		
	$\beta$ -glucosidase	1-S	6-12 days	glucose DDTC	TLC HPLC
	$\alpha$ -glucosidase	ni <sup>c</sup>	9-18 days	ni	
<b>5b</b>	buffer		stable		
	$\beta$ -galactosidase	1'-4	<10 min <sup>d</sup>	<b>5a</b>	HPLC
	$\beta$ -galactosidase + $\beta$ -glucosidase	1-S	9-11 days <sup>e</sup>		
<b>5c</b>	buffer		stable		
	$\beta$ -glucosidase	1'-4	<10 min <sup>d</sup>	<b>5a</b>	HPLC
		1-S	8-10 days <sup>e</sup>		

<sup>a</sup> Chemical bond cleaved by corresponding enzymes. <sup>b</sup> Values represent the range of the results of three different experiments. <sup>c</sup> Not identified. <sup>d</sup> The  $t_{1/2}$  value applies to the hydrolysis of the terminal sugar molecules of disaccharides. <sup>e</sup> The  $t_{1/2}$  value applies to the hydrolysis of **5a** which is formed from **5b,c** by  $\beta$ -galactosidase and  $\beta$ -glucosidase, respectively.



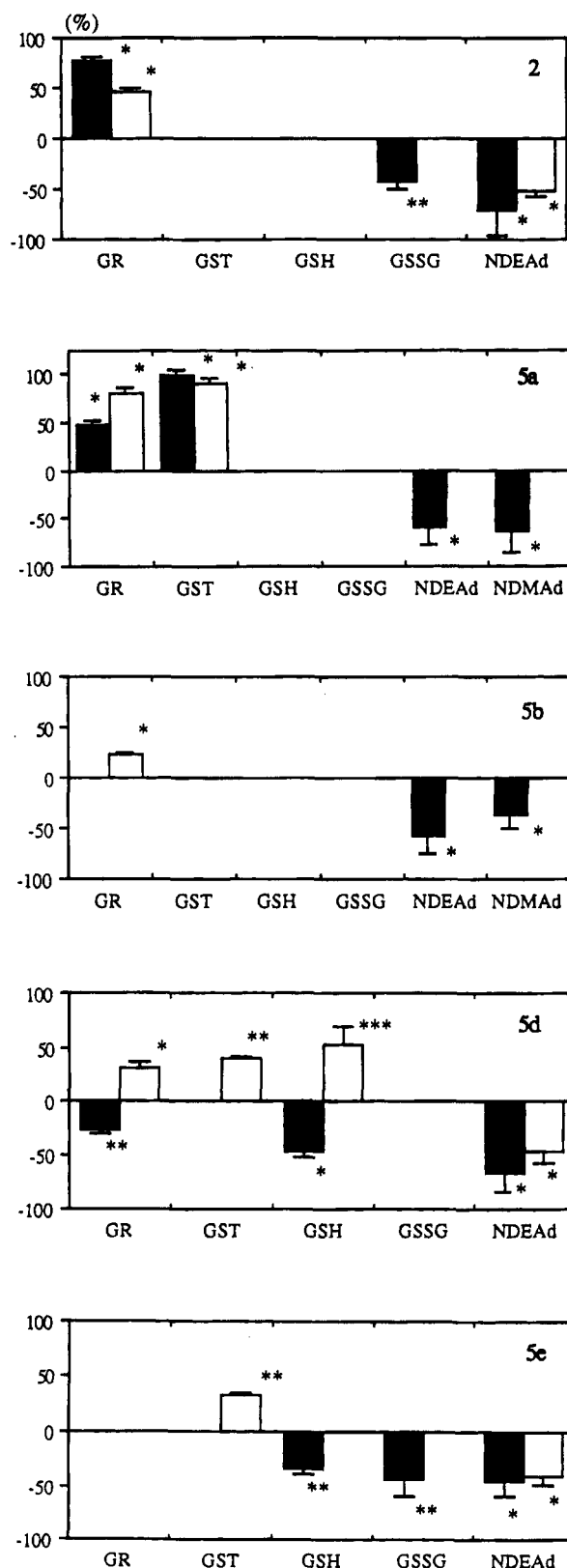
**Figure 2.** Enzymatic hydrolysis of SDTCs by glycolytic enzymes in acetate buffer (pH 4.8). **5a** (■), **5b** (△), **5c** (●), **5d** (□), and **5e** (○) were incubated in the presence of corresponding glycosidase, and the concentration was determined at the time points indicated using HPLC. Aliquots of incubates were filtered through a 0.45  $\mu$ m filter and applied to a APS Hypersil column (250  $\times$  4.6 mm with a 10 mm guard cartridge). The column was eluted with acetonitrile–H<sub>2</sub>O, 90:10, at a rate of 1.5 mL/min, and the eluate was detected at 245 nm.

**Table 2.** Effects of **5a** and DDTC on the NDMA Demethylase Activity in Vitro<sup>a</sup>

inhibitor	conc (mM)	NDMA (mM)	formaldehyde (nmol/mg of protein)	acetaldehyde (nmol/mg of protein)
<b>5a</b>	0	0.1	4.6 $\pm$ 1.1	0
	2	0.1	1.2 $\pm$ 1.2	10.9 $\pm$ 2.2
	2	0.5	9.0 $\pm$ 1.3	8.5 $\pm$ 1.3
	2	1	6.2 $\pm$ 0.6	4.3 $\pm$ 1.1
	0	2	15.0 $\pm$ 13	0
	2	2	14.7 $\pm$ 0.3	13.8 $\pm$ 0.9
	5	2	11.9 $\pm$ 0.8	25.9 $\pm$ 1.1
	20	2	1.5 $\pm$ 0.3	31.5 $\pm$ 4.1
DDTC	0	0.1	4.8 $\pm$ 1.3	0
	2	0.1	0.6 $\pm$ 0.2	2.2 $\pm$ 0.7
	2	0.5	0.3 $\pm$ 0.04	0.6 $\pm$ 0.001
	2	1	0	0.06 $\pm$ 0.04

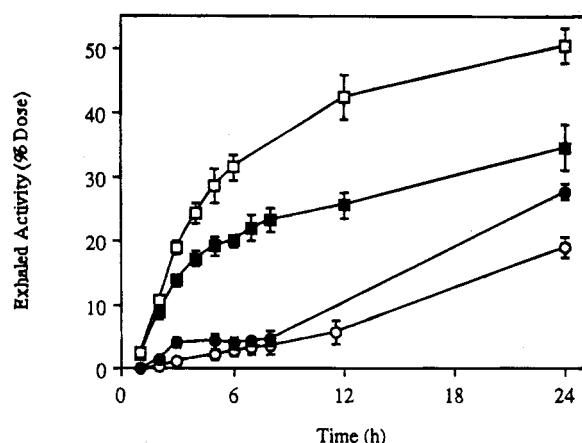
<sup>a</sup> Values represent mean  $\pm$  SD of the results of three separate experiments.

was studied in vitro. Compound **5a** (2–20 mM) or DDTC (2 mM) and NDMA (0.1–1 mM) were incubated with rat liver microsomal fraction in the presence of a NADPH-generating system. Formaldehyde generated in the reaction was measured using (2,4-dinitrophenyl)-hydrazine. DDTC inhibited the formaldehyde formation at all nitrosamine concentrations tested (0.1–1 mM), whereas **5a** showed an inhibitory effect only at low nitrosamine concentration (0.1 mM). Interestingly, a concentration-dependent formation of acetaldehyde was observed up to 20 mM of **5a**, which was not the case with DDTC (Table 2). The treatment of rats with **2** and **5a,b,d,e** affected various biochemical parameters, which are summarized in Figure 3. Compound **2** enhanced 4 and 24 h after application glutathione reductase (GR) activity to  $77 \pm 3.1\%$  ( $p < 0.001$ ) and  $46.6 \pm 3.1\%$  ( $p < 0.001$ ) of the control, respectively. The activation of this enzyme led to a  $42.8 \pm 1.4\%$  ( $p < 0.01$ ) decrease in GSSG content in liver which was normalized after 24 h. The oxidative dealkylation of NDEA was completely inhibited by **2** until 24 h after treatment. The activity of GST and GSH content were not significantly changed by the treatment with **2**. Concerning GST and GR, a significant increase in activity was observed in the **5a**-treated group. Compound **5a** activated GR by  $47.9\% \pm 4.9\%$  ( $p < 0.001$ ) (4 h) and  $82.0\% \pm 5.3\%$  ( $p < 0.001$ ) (24 h) compared to the control group and led to a 2-fold increase in GST activity. GSH content was decreased



**Figure 3.** Percent decrease or increase of glutathione reductase (GR) and glutathione S-transferase (GST) activity in cytosol, NDEA deethylase (NDEAd) and NDMA demethylase (NDMAAd) activity in microsomes, and glutathione (GSH and GSSG) content in the liver 4 h (closed bar) and 24 h (open bar) after ip administration of compounds **2** and **5a,b,d,e**, to rats (1.7 mmol/kg). Each bar represents the mean SD of results obtained from five rats. (\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.05$ ). For details of assay methods, see the Experimental Section.

by  $47.4 \pm 5.6\%$  ( $p < 0.001$ ) 4 h after treatment with **5d** but increased up to  $52.4 \pm 16.2\%$  ( $p < 0.05$ ) after 24 h



**Figure 4.** Cumulative exhalation profile of  $^{14}\text{CO}_2$  in rats treated with various inhibitors (1.5 mmol/kg, ip) and [ $^{14}\text{C}$ ]-NDEA (28 mg/kg, 5  $\mu\text{Ci/kg}$ ). Inhibitors were administered 1 h before and 5 h after treatment with nitrosamine ( $\square$ , NaCl + NDEA;  $\circ$ , 2 + NDEA;  $\bullet$ , 5a + NDEA;  $\blacksquare$ , 5b + NDEA). Exhaled  $^{14}\text{CO}_2$  was trapped in 1 N NaOH solution and analyzed for radioactivity. Each time point represents the mean of results from two experiments.

**Table 3.** Exhalation of  $^{14}\text{CO}_2$  and Urinary Excretion of Radioactivity and Unchanged NDEA 24 h after Administration of [ $^{14}\text{C}$ ]NDEA in Combination with Inhibitors

treatment <sup>a</sup>	$^{14}\text{CO}_2^b$		radioactivity in urine <sup>b</sup>		NDEA in urine <sup>b</sup>	
	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2
NaCl/NDEA	47.3	53.1	17.6	17.2	0.4	0.4
DDTC/NDEA	17.2	20.5	20.7	17.8	15.7	
5a/NDEA	26.3	28.8	15.0	22.7	7.9	7.1
5b/NDEA	30.8	38.1	17.0	17.8	2.1	0.7

<sup>a</sup> Inhibitors (1.5 mmol/kg, ip) were administered 1 h before and 5 h after the treatment of [ $^{14}\text{C}$ ]NDEA (28 mg/kg, 5  $\mu\text{Ci/kg}$ , ip).  
<sup>b</sup> Values represent percentage of the given dose.

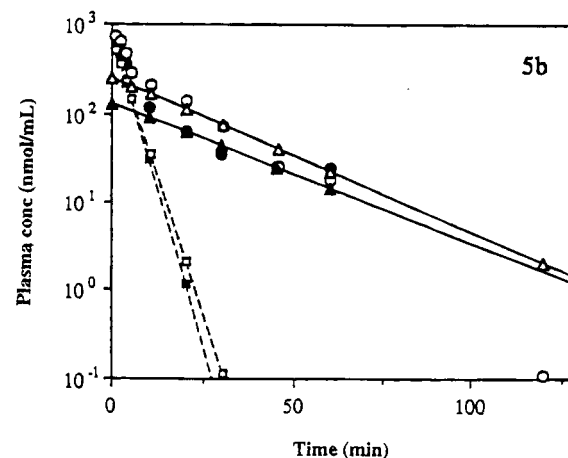
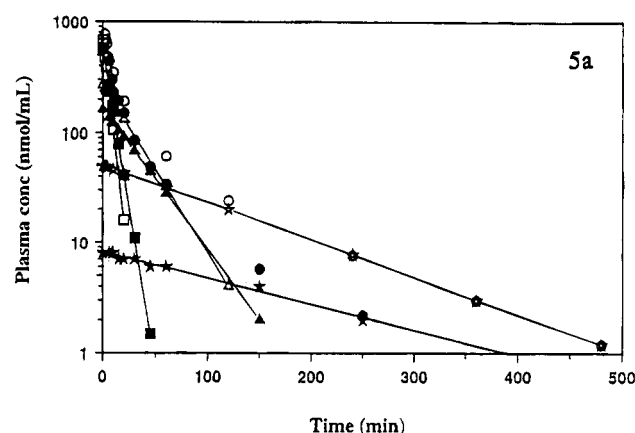
compared to that of the control. Surprising effects of 5a,b on the activity of nitrosamine-metabolizing enzymes were found. A decrease of the nitrosamine-activating enzyme activity was observed only 4 h after application, whereas after 24 h the activity was completely restored. In the 5d,e group, the inhibition lasted for 24 h.

**NDEA Metabolism and DNA Single-Strand Breaks.** The exhalation profiles of  $^{14}\text{CO}_2$  after the combined administration of [ $^{14}\text{C}$ ]NDEA and inhibitors are shown in Figure 4. DDTC and 5a completely suppressed the oxidative metabolism of [ $^{14}\text{C}$ ]NDEA to  $^{14}\text{CO}_2$  up to 8 h. In 5a-treated animals, a slightly faster metabolism of NDEA was found after 8 h, compared to that of DDTC treatment. The radioactivity in  $^{14}\text{CO}_2$  and urine, as well as the urinary excretion rates of unchanged NDEA, is summarized in Table 3. The total amounts of radioactivity in urine were not changed by the inhibitor treatment, but urinary excretion of NDEA was increased 20-fold by 5a treatment. Compound 5b showed only moderate effects concerning  $^{14}\text{CO}_2$  exhalation and NDEA excretion. NDEA (12.5 mmol/kg) induced distinct DNA single-strand breaks or alkaline-labile sites in DNA which were selectively eluted from the polycarbonate filters.  $C - T$  values of 36 and 21 were obtained in two separate experiments (Table 4). Pretreatment (2 h) of rats with 5a (0.5 mmol/kg) totally inhibited the genotoxic effects of NDEA on liver DNA. The percentages of DNA that remained on the filters after the end of elution were even higher than those of control filters (negative  $C - T$  value).

**Table 4.** Ex Vivo Analysis of DNA Single-Strand Breaks in Primary Rat Hepatocytes following Oral Treatment of NDEA Alone and in Combination with 5a

exp	treatment <sup>a</sup>	viability in % <sup>b</sup> (abs/rel)	DNA on the filter <sup>c</sup>	$C - T^d$
1	NaCl/NaCl	60/100	67 $\pm$ 7	—
	5a/NaCl	82	60 $\pm$ 8	7
	NaCl/NDEA	77	31 $\pm$ 5	36
	5a/NDEA	92	74 $\pm$ 3	-7
2	NaCl/NaCl	62/100	67 $\pm$ 8	—
	5a/NaCl	100	58 $\pm$ 5	9
	NaCl/NDEA	92	46 $\pm$ 5	21
	5a/NDEA	106	77 $\pm$ 7	-10

<sup>a</sup> Second gavage followed 2 h after first gavage; 0.5 mmol/kg 5a and 1.4 mg/kg NDEA both dissolved in 10 mL of NaCl/kg.  
<sup>b</sup> Absolute/relative viability of hepatocytes (control = 100%).  
<sup>c</sup> Values represent mean  $\pm$  SD ( $n = 6$ ).  
<sup>d</sup> Percentage of DNA retained on filter, control minus treated values.



**Figure 5.** Semilogarithmic plots of drug concentrations in plasma following iv bolus injection of 100 mg/kg 5a or 5b to rats. The data of individual rats are presented by open and closed symbols;  $\circ$  and  $\bullet$  represent the measured data. The fit lines for  $\alpha$ -phase ( $\square$ ),  $\beta$ -phase ( $\triangle$ ), and  $\gamma$ -phase ( $\star$ ) are drawn with the calculated values from nonlinear regression analysis.

**Pharmacokinetics.** The plasma eliminations of 5a,b obtained following iv bolus injection of 100 mg/kg to rats are shown in Figure 5. A two-compartment model was used as best fit for the calculation of the pharmacokinetic parameters of these compounds (Table 5). The half-lives of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -phase were 3.7 (5.2), 19.3 (23.6), and 88.8 (128.9) min for 5a and 2.1 (2.4) and 18.9 (17.2) min for 5b, respectively. The volume of distribution at steady state ( $V_{ss}$ ) of 5a,b approaches total body water for the animals, which would be expected for small amphiphilic compounds. Clearance

**Table 5.** Pharmacokinetic Parameters of **5a,b** after Intravenous Bolus Administration of 100 mg/kg to Rats

parameter (unit)	<b>5a</b>		<b>5b</b>	
	rat 1	rat 2	rat 3	rat 4
body weight (g)	140	150	143	165
AUC ( $\mu\text{mol min/L}$ )	17.6	11.4	6.0	8.6
MRT (min)	59.8	43.8	17.4	19
CL ( $\text{mL/kg min}$ )	25.5	42.1	35.1	24.4
$V_{ss}$ ( $\text{mL/kg}$ )	152.6	184.8	610.4	462.6
half-life $\alpha$ (min)	3.7	5.2	2.1	2.4
half-life $\beta$ (min)	19.3	23.6	18.9	17.2
half-life $\gamma$ (min)	88.8	128.9		

**Table 6.** Cumulative Urinary Excretion of Parent Drug and Metabolite following a Single Intraperitoneal and Intravenous Bolus Dose of 100 mg/kg **5a,b** to Rats<sup>a</sup>

substance	cumulative urinary excretion (% of dose)				recovered as
	ip		iv		
	0-8 h	8-24 h	0-8 h	8-24 h	
<b>5a</b>	31.2 ± 16.4	2.2 ± 1.4	34.5 ± 3.0	1.5 ± 1.5	<b>5a</b>
<b>5b</b>	54.3 ± 16.0	2.6 ± 1.4	50.8 ± 10.4	2.8 ± 1.0	<b>5b</b>
	21.0 ± 6.9	1.0 ± 0.5	13.1 ± 3.0	1.2 ± 0.7	<b>5a</b>

<sup>a</sup> Values represent mean  $\pm$  SD of the results from five different rats.

rate from total body for compounds **5a,b** were calculated to be 20.7 (27.2) and 35.1 (24.4) mL/kg min.

Following ip or iv administration of **5a**, 30% of the dose was excreted in the urine within the first 8 h. An average of 50% of the dose of **5b** was excreted unchanged in the urine and 10–20% as the corresponding monosaccharide from **5a** over 8 h. The urinary excretion of both compounds after 8 h was negligible (Table 6).

## Discussion

Diethyldithiocarbamate (**1**) is readily oxidized to its disulfide form, tetraethylthiuram disulfide (disulfiram). Compound **2**, which contains two allyl groups in the molecule, is also unstable against oxygen. Therefore preparation and storage of this compound need careful attention. As was expected, the two dithiocarbamates are stabilized by the glycosyl group. First, the enzymatic cleavage of the S–glycoside linkage of the compounds was tested. As it was expected that the enzymes also hydrolyze the terminal sugar molecule of disaccharides, differential quantitation of mono- and disaccharides was necessary. HPLC analysis using an amino-propyl column proved to be successful for the separation. All the SDTCs tested above were, like many other thioglucosides, difficult to hydrolyze with glucosidase.<sup>28,29</sup> A steric hindrance during the interaction of enzymes and aglycone parts of the thioglucosides could be suggested. In spite of this aspect, it remains difficult to explain the poor susceptibility of the thioglucosides versus thioglucosidase which usually hydrolyzes S–glycoside bonds of the natural and synthetic thioglycoside.<sup>30</sup> The possibility that the sugar conjugates of DDTC are hydrolyzed to DDTC, which might inactivate the enzyme activity, was excluded by an additional treatment with the same enzyme after 7 days of incubation, which showed the same activity as in the beginning (data not shown). The terminal sugar molecules in the disaccharides are hydrolyzed very rapidly which means that the inhibitory effects of the SDTCs on these enzymes are not due to the intact compounds. In spite of the stability of **5a** against b-glucosidase, HPLC analysis revealed that DDTC is formed by enzymatic

hydrolysis and a portion of DDTC is oxidized to disulfiram (Figure 1). Moreover, a nonenzymatic degradation catalyzed by BSA or GSH did not take place. These results suggest that the hydrolysis of this compound is an enzymatic process, although the rate is very slow. The relatively rapid hydrolysis of SDTCs by b-glucosidase at pH 4.8, an optimal pH value for this enzyme, supports this assumption. It is reasonable, however, to regard these sugar conjugates as other new compounds rather than as prodrugs of DDTC or **2**.

In in vitro experiments, the inhibitory effect of **5a** on nitrosamine dealkylase was studied and compared to that of DDTC. In contrast to DDTC, which showed at the lowest concentration (2 mM) a maximal inhibition of the enzyme activity at all substrate concentrations (0.1–1 mM), **5a** inhibited the formation of formaldehyde, as a degree of NDMA metabolism, only at 0.1 mM of nitrosamine. According to their  $K_m$  values, at least three different NDMA-demethylating isozymes are present in control microsomes.<sup>31</sup> Among these, the ethanol- or acetone-inducible cytochrome P450 IIE1 is most efficient in catalyzing the demethylation of NDMA with an apparent  $K_m$  of 10–20 mM. Other cytochrome P450s, such as the phenobarbital-inducible P450 IIB1, showed substantial activities only at high substrate concentration, suggesting higher  $K_m$  values.<sup>32</sup> Our results indicate that **5a** inhibits only the low- $K_m$  value isozyme of NDMA demethylase. This is believed to be important for the in vivo situation because animals or humans are seldom exposed to high concentrations of nitrosamine. The formation of acetaldehyde regardless of the substrate (NDMA) concentration is a remarkable feature of SDTCs on this enzyme. Acetaldehyde was formed in a dose-dependent manner from **5a** (Table 2) and **5c** (unpublished data). Lee et al.<sup>33</sup> postulated that the alkyl side chains of nitrosamines are the most important factor for determining the binding affinity to the active site of cytochrome P450 enzymes. Therefore, it is assumed that the ethyl groups of **5a** or **5c** are recognized by cytochrome P450 enzyme and metabolized to acetaldehyde and corresponding products. Why this is not the case with DDTC remains unclear.

The most striking effect on the metabolism of glutathione was found in the **5a**-treated rat. Unlike other compounds of this series which were tested in our institute,<sup>34,35</sup> **5a** significantly increased the GST and GR activity, while **5b** had only a marginal effect on this enzyme system. This is remarkable because the in vitro data on enzymatic degradation showed a rapid hydrolysis of **5b** to **5a**. It is suggested that this reaction is not significant in the in vivo situation. Pharmacokinetic data of both compounds should enable us to explain this result. Compound **5b** has a very short elimination half-life, and within 8 h, the major amount of the dose is excreted intact in urine. After iv injection of **5b**, compound **5a** was detectable in plasma only in the first 1–2 min, which possibly means that the rate of elimination of **5b** is so fast that hydrolysis of the terminal sugar molecule and formation of **5a** do not take place.

Although several reports indicate that there is a correlation between the induction of GST by and the chemopreventive potential<sup>12,36,37</sup> of anticarcinogens, it is not yet clear whether the induction of GST is directly responsible for the inhibition of neoplasms or whether it represents only a marker enzyme. Two contradictory reports exist regarding a possible involvement of GSH

and GST in the detoxification of NDEA.<sup>33,38</sup> The low concentration of DDTC resulting from slow degradation of the S-glycoside bond might be the reason why **5a,b** showed an inhibitory effect on nitrosamine metabolizing enzymes only 4 h after the treatment. In addition to this, another inhibitory mechanism may exist when we take into consideration that **5d,e**, two corresponding allyl derivatives, showed a lasting inhibitory effect up to 24 h after treatment. Further studies on the mechanism are needed for the explanation of the mode of enzyme inhibition.

On the basis of the results obtained on biochemical parameters, we have tested the effect of the sugar-linked dithiocarbamates on nitrosamine metabolism in vivo. It is generally accepted that nitrosamines are oxidized to  $\alpha$ -hydroxynitrosamine by cytochrome P450 enzymes followed by spontaneous degradation to aldehyde and monoalkylnitrosamine. The monoalkylnitrosamine reacts to an alkylcarbenium ion<sup>39</sup> which can react with DNA, resulting in alkylation and ultimately in tumor formation, or with water to yield the corresponding alcohol. Alcohol, as well as aldehyde, is enzymatically oxidized to CO<sub>2</sub>. Using radioactively labeled nitrosamine, the exhaled amount of <sup>14</sup>CO<sub>2</sub> can be measured. The sum of exhaled <sup>14</sup>CO<sub>2</sub> and radioactivity in the urine represent a measure of metabolism and excretion of nitrosamine. Neither the metabolism nor the toxicity of NDEA in the rat are completely inhibited by disulfiram or related compounds,<sup>9,34</sup> but they delay the metabolism of nitrosamine. Therefore multiple doses of inhibitors were applied, 1 h before and 5 h after the treatment of NDEA. As shown in Figure 4, DDTC and **5a** completely suppressed <sup>14</sup>CO<sub>2</sub> exhaled from [<sup>14</sup>C]NDEA for the first 8 h period. After that, the <sup>14</sup>CO<sub>2</sub> exhalation curve in the **5a**-treated rat runs steeper than in the DDTC group. This is probably connected with the finding that the activity of NDEA deethylase is inhibited only 4 h after treatment with **5a**. Although **5a,b** showed a quite similar profile regarding deethylase activity, **5b** has only a slight inhibitory effect on the oxidative metabolism of NDEA. This result suggests that the inhibition of nitrosamine-activating enzymes might be one of the reasons for the reduction of nitrosamine toxicity by sulfur compounds, but other factors like glucuronide formation and GSH content could also play an important role in its antitoxic effects. As was pointed out earlier,<sup>34</sup> reduction of the <sup>14</sup>CO<sub>2</sub> exhalation by antioxidants may be caused not only by their influence on NDEA metabolism but also by a suppressed oxidation of NDEA metabolites such as acetaldehyde or ethanol to CO<sub>2</sub>. Therefore, we suggested that the amount of unchanged nitrosamine excreted in the urine is a better indicator for the assessment of an inhibitory effect than <sup>14</sup>CO<sub>2</sub> exhalation.<sup>40</sup> Although the amount of radioactivity in the urine was almost the same in the control and treated groups, SDTCs influenced the urinary excretion of NDEA to a different extent. The influences of our compounds on the deethylase activity parallel partly with the effects on the urinary excretion of NDEA.

In order to elucidate the correlation between the aforementioned positive effects of SDTCs on several biochemical parameters and the genotoxicity induced by NDEA, we focused on the influence of **5a** on the NDEA-induced DNA single-strand breaks in rat liver cells. The inhibitory effect of **5a** on DNA single-strand

breaks is documented in Table 4. There is a good agreement among decreased deethylase activity, decreased NDEA metabolism in vivo, and reduced DNA single-strand breaks in **5a**-treated animals.

In view of the positive effects of SDTC like **5a** on glutathione-related detoxifying system, on nitrosamine metabolism, and on nitrosamine-induced DNA damage, it seems reasonable to assume that this class of compounds possesses anticarcinogenic properties and represents an improved concept for chemoprevention. The potential anticarcinogenic effects of the SDTCs, however, remain to be further investigated.

## Experimental Section

**Materials.** All chemicals used were of analytical grade supplied by either Merck (Darmstadt, FRG) or Boehringer (Mannheim, FRG). [<sup>14</sup>C]NDEA was synthesized by nitrosating [<sup>14</sup>C]diethylamine with NaNO<sub>2</sub> in our laboratory. TLC was performed on Merck F<sub>254</sub> silica gel plates containing a fluorescent indicator. The plates were developed with petroleum ether-EtOAc-MeOH, 6:2:1 (system A), CHCl<sub>3</sub>-MeOH-hexane, 6:2:1 (system B), or CHCl<sub>3</sub>-MeOH, 2:1 (system C). Column chromatography was done on silica gel 60 (240–400 mesh) from Merck. Melting points were observed on the apparatus according to Dr. Tottoli and are uncorrected. <sup>1</sup>H NMR (90 and 500 MHz) spectra were obtained on Bruker WH 90 and AM 500 spectrometers using TMS as an internal standard. Elemental analyses were obtained from the microanalytic laboratory of Max-Planck Institute in Heidelberg.

**N,N-Diallyldithiocarbamate Sodium Salt (2).** Diallylamine (29.2 g, 0.3 mol) in 12% NaOH (100 mL) was cooled to –5 °C, and CS<sub>2</sub> (25.1 g, 0.3 mol) was added dropwise at –5–0 °C. The mixture was stirred at 25 °C for 1 h, and the solvent was evaporated in vacuo. Recrystallization of the residue from Et<sub>2</sub>O gave dithiocarbamate **2** (15.8 g, 27%). Because of the instability against oxidation in the air and hygroscopy, this compound was handled in a glovebag filled in nitrogen: mp 72–74 °C; <sup>1</sup>H NMR (90 MHz in DMSO-*d*<sub>6</sub>)  $\delta$  4.68 (d, 4H, 2NCH<sub>2</sub>), 4.93–5.03 (m, br, 4H, 2C=CH<sub>2</sub>), 5.67–6.09 (m, br, 2H, 2C=CH). Anal. (C<sub>7</sub>H<sub>11</sub>NS<sub>2</sub>Na) C, H, N, S.

**S-(2',3',4',6'-Tetra-O-acetyl- $\beta$ -D-glucopyranosyl) N,N-Diethylthiocarbamate (4a).** To a solution of acetylated glucosyl bromide **3a** (5 g, 12 mmol), obtained by Merck, in 50 mL of CH<sub>3</sub>CN was added finely grounded dithiocarbamate **1** (2.7 g, 12 mmol) and the mixture stirred at 25 °C for 1 h. The reaction mixture was diluted with CHCl<sub>3</sub>, washed twice with H<sub>2</sub>O, and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated, and the residue was chromatographed on silica gel (petroleum ether-EtOAc-MeOH, 70:25:5) to yield **4a** (3.62 g, 63%): mp 77–78 °C; TLC (system A) *R*<sub>f</sub> 0.42; <sup>1</sup>H NMR (500 MHz in CDCl<sub>3</sub>)  $\delta$  1.271 (tt, 6H, 2CH<sub>3</sub> DDTC), 2.015, 2.029, 2.035 and 2.074 (s, 3H, OAc), 3.574–3.647 (m, br, 1H, N-CH<sub>2</sub>a DDTC), 3.736–3.809 (m, br, 1H, N-CH<sub>2</sub>b DDTC), 3.906 (ddd, 1H, *J*<sub>4,5</sub> = 10.5 Hz, *J*<sub>5,6a</sub> = 4.5 Hz, *J*<sub>5,6b</sub> = 2 Hz, H5), 3.897–3.967 (m, br, 1H, N-CH<sub>2</sub>c DDTC), 4.049–4.114 (m, br, 1H, N-CH<sub>2</sub>d DDTC), 4.134 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6a</sub> = 2 Hz, H6a), 4.287 (dd, 1H, *J*<sub>6a,6b</sub> = 12 Hz, *J*<sub>5,6b</sub> = 4.5 Hz, H6b), 5.133 (dd, 1H, *J*<sub>1,2</sub> = 10 Hz, *J*<sub>2,3</sub> = 9 Hz, H2), 5.295–5.375 (m, 2H, H3 and H4), 5.871 (d, 1H, *J*<sub>1,2</sub> = 10 Hz, H1). Anal. (C<sub>19</sub>H<sub>29</sub>NO<sub>9</sub>S<sub>2</sub>) C, H, N, S.

**S-( $\beta$ -D-Glucopyranosyl) N,N-Diethylthiocarbamate (5a).** **4a** (2 g, 4 mmol) was dissolved in 20 mL of NH<sub>4</sub>OH-MeOH and stirred at 25 °C for 1 h. The reaction mixture was evaporated in vacuo and subsequently lyophilized. Recrystallization of the lyophilisate from EtOAc-hexane gave **5a** (1.87 g, 72%): mp 109–110 °C; TLC (system B) *R*<sub>f</sub> 0.53; <sup>1</sup>H NMR (500 MHz in D<sub>2</sub>O)  $\delta$  1.265 (t, 3H, CH<sub>3</sub>a DDTC), 1.304 (t, 3H, CH<sub>3</sub>b DDTC), 3.477 (dd, 1H, *J*<sub>1,2</sub> = 10 Hz, *J*<sub>2,3</sub> = 9 Hz, H2), 3.598 (ddd, 1H, *J*<sub>4,5</sub> = 9.9 Hz, *J*<sub>5,6a</sub> = 5.3 Hz, *J*<sub>5,6b</sub> = 2.3 Hz, H5), 3.611–3.693 (m, 2H, H3 and H4), 3.734 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6a</sub> = 5.3 Hz, H6a), 3.797–3.873 (m, br, 2H, N-CH<sub>2</sub>a DDTC), 3.848 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 2.3 Hz, H6b), 4.013–4.093 (m, br, 2H, N-CH<sub>2</sub>b DDTC), 5.722 (d, 1H, *J*<sub>1,2</sub> = 10.2 Hz, H1). Anal. (C<sub>11</sub>H<sub>21</sub>NO<sub>5</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(2',3',4',6'-Tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl] N,N-Diethyldithiocarbamate (4b).** Compound **4b** was prepared analogous to **4a** from **3b** (5.5 g, 7.9 mmol) and **1** (1.8 g, 10.5 mmol) in 30 mL of acetone. Recrystallization from CHCl<sub>3</sub>-hexane and again from EtOAc gave **4b** (3.1 g, 51%): mp 132–133 °C; TLC (system A) *R<sub>f</sub>* 0.28; <sup>1</sup>H NMR (500 MHz in CDCl<sub>3</sub>) δ 1.262 (tt, 6H, 2CH<sub>3</sub> DDTC), 1.964, 2.026, 2.047, 2.066, 2.073, 2.106 and 2.158 (s, 3H, OAc), 3.560–3.632 (m, br, 1H, N-CH<sub>2a</sub> DDTC), 3.719–3.791 (m, br, 1H, N-CH<sub>2b</sub> DDTC), 3.819–3.883 (m, 3H, H5' and H6'), 3.900–3.954 (m, br, 1H, N-CH<sub>2c</sub> DDTC), 4.039–4.157 (m, 4H, N-CH<sub>2d</sub> DDTC, H4, H5 and H6a), 4.458 (d, 1H, *J*<sub>1,2</sub> = 8 Hz, H1'), 4.463 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 1.5 Hz, H6b), 4.943 (dd, 1H, *J*<sub>2,3</sub> = 10.5 Hz, *J*<sub>3,4</sub> = 3.5 Hz, H3'), 5.119 (dd, 1H, *J*<sub>1,2</sub> = 8 Hz, *J*<sub>2,3</sub> = 10.5 Hz, H2'), 5.239 (dd, 1H, *J*<sub>1,2</sub> = 10.5 Hz, *J*<sub>2,3</sub> = 9.5 Hz, H2), 5.329 (t, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9 Hz, H3), 5.352 (dd, 1H, *J*<sub>3,4</sub> = 3.5 Hz, *J*<sub>4,5</sub> = 1 Hz, H4'), 5.813 (d, 1H, *J*<sub>1,2</sub> = 10.5 Hz, H1). Anal. (C<sub>31</sub>H<sub>45</sub>NO<sub>17</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(β-D-Galactopyranosyl)-β-D-glucopyranosyl] N,N-Diethyldithiocarbamate (5b).** Compound **5b** was prepared analogous to **5a** from **4b** (2 g, 2.6 mmol) and 30 mL of NH<sub>4</sub>OH-MeOH. The crude product was chromatographed on silica gel (CHCl<sub>3</sub>-EtOH, 1:1) and subsequently recrystallized from EtOH to yield **5b** (580 mg, 47%): mp 210 °C; TLC (system C) *R<sub>f</sub>* 0.34; <sup>1</sup>H NMR (500 MHz in D<sub>2</sub>O) δ 1.266 (t, 3H, CH<sub>3</sub> DDTC), 1.305 (t, 3H, CH<sub>3</sub> DDTC), 3.572 (dd, 1H, *J*<sub>1,2</sub> = 7.7 Hz, *J*<sub>2,3</sub> = 10 Hz, H2'), 3.677 (dd, 1H, *J*<sub>2,3</sub> = 10 Hz, *J*<sub>3,4</sub> = 3.4 Hz, H3'), 3.715–3.887 (m, 10H, H2, H3, H4, H5, H6a, H5', H6a', H6b', and N-CH<sub>2a</sub> DDTC), 3.939 (dd, 1H, *J*<sub>3,4</sub> = 3.4 Hz, *J*<sub>4,5</sub> = 0.8 Hz, H4'), 3.962 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 1.5 Hz, H6b), 4.027–4.092 (m, br, 2H, N-CH<sub>2b</sub> DDTC), 4.486 (d, 1H, *J*<sub>1,2</sub> = 7.8 Hz, H1'), 5.740 (d, 1H, *J*<sub>1,2</sub> = 10.2 Hz, H1). Anal. (C<sub>17</sub>H<sub>31</sub>NO<sub>10</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl] N,N-Diethyldithiocarbamate (4c).** Compound **4c** was prepared analogous to **4b** from **3c** (5.5 g, 7.9 mmol) and **1** in 30 mL of acetone (3.4 g, 56%): mp 209–210 °C; TLC (system A) *R<sub>f</sub>* 0.26; <sup>1</sup>H NMR (500 MHz in CDCl<sub>3</sub>) δ 1.240–1.280 (m, 6H, 2CH<sub>3</sub> DDTC), 1.982, 2.008, 2.021, 2.024, 2.057, 2.096 and 2.110 (s, 3H, OAc), 3.557–3.629 (m, br, 1H, N-CH<sub>2a</sub> DDTC), 3.654 (ddd, 1H, *J*<sub>4,5</sub> = 9.9 Hz, *J*<sub>5,6a</sub> = 2 Hz, *J*<sub>5,6b</sub> = 4.5 Hz, H5), 3.715–3.789 (m, br, 1H, N-CH<sub>2b</sub> DDTC), 3.805–3.820 (m, 2H, 2H6'), 3.884–3.953 (m, br, 1H, N-CH<sub>2c</sub> DDTC), 4.048 (dd, 1H, *J*<sub>6a,6b</sub> = 12 Hz, *J*<sub>5,6a</sub> = 2 Hz, H6a), 4.042–4.091 (m, 1H, N-CH<sub>2d</sub> DDTC), 4.109–4.143 (m, 1H, H5'), 4.361 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 4.5 Hz, H6b), 4.491 (dd, 1H, *J*<sub>3,4</sub> = 12.2 Hz, *J*<sub>4,5</sub> = 1.3 Hz, *J*<sub>4,5</sub> = 1.3 Hz, H4), 4.492 (d, 1H, *J*<sub>1,2</sub> = 8 Hz, H1'), 4.935 (dd, 1H, *J*<sub>1,2</sub> = 8 Hz, *J*<sub>2,3</sub> = 9.3 Hz, H2'), 5.062 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, *J*<sub>4,5</sub> = 10 Hz, H4'), 5.135 (t, 1H, *J*<sub>2,3</sub> = 9.3 Hz, H3'), 5.238 (dd, 1H, *J*<sub>1,2</sub> = 10.5 Hz, *J*<sub>2,3</sub> = 9.2 Hz, H2), 5.312 (t, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9 Hz, H3), 5.800 (d, 1H, *J*<sub>1,2</sub> = 10.5 Hz, H1). Anal. (C<sub>31</sub>H<sub>45</sub>NO<sub>17</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(β-D-Glucopyranosyl)-β-D-glucopyranosyl] N,N-Diethyldithiocarbamate (5c).** Compound **5c** was prepared analogous to **5a** from **4c** (2 g, 2.6 mmol) and 30 mL of NH<sub>4</sub>OH-MeOH and precipitated from EtOH after lyophilization (603 mg, 49%): mp 250 °C; TLC (system C) *R<sub>f</sub>* 0.4; <sup>1</sup>H NMR (500 MHz in D<sub>2</sub>O) δ 1.266 (t, 3H, CH<sub>3</sub> DDTC), 1.305 (t, 3H, CH<sub>3</sub> DDTC), 3.343 (dd, 1H, *J*<sub>1,2</sub> = 8 Hz, *J*<sub>2,3</sub> = 9.3 Hz, H2'), 3.432 (dd, 1H, *J*<sub>3,4</sub> = 9 Hz, *J*<sub>4,5</sub> = 10 Hz, H4'), 3.501 (ddd, 1H, *J*<sub>4,5</sub> = 10 Hz, *J*<sub>5,6a</sub> = 2.5 Hz, *J*<sub>5,6b</sub> = 6 Hz, H5'), 3.539 (t, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9 Hz, H3'), 3.709–3.798 (m, 5H, H6a', H2, H5, H6a, and H6b), 3.813–3.887 (m, br, 3H, H3 and N-CH<sub>2a</sub> DDTC), 3.918–3.976 (m, 2H, H6b' and H4), 4.015–4.091 (m, br, 2H, N-CH<sub>2b</sub> DDTC), 4.545 (d, 1H, *J*<sub>1,2</sub> = 8 Hz, H1'), 5.736 (d, 1H, *J*<sub>1,2</sub> = 10.3 Hz, H1). Anal. (C<sub>17</sub>H<sub>31</sub>NO<sub>10</sub>S<sub>2</sub>) C, H, N, S.

**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) N,N-Diallyldithiocarbamate (4d).** Compound **4d** was prepared analogous to **4a** from **3a** (8.2 g, 19.9 mmol) and **2** in 40 mL of acetone. The crude product was chromatographed on silica gel (CHCl<sub>3</sub>-EtOH, 9:1) (5.7 g, 57%): mp 97.5–98.5 °C; TLC (system A) *R<sub>f</sub>* 0.43; <sup>1</sup>H NMR (500 MHz in CDCl<sub>3</sub>) δ 2.013, 2.014, 2.034 and 2.071 (s, 3H, OAc), 3.891 (ddd, 1H, *J*<sub>4,5</sub> = 10 Hz, *J*<sub>5,6a</sub> = 2.5 Hz, *J*<sub>5,6b</sub> = 4.5 Hz, H5), 4.126 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6a</sub> = 2.5 Hz, H6a), 4.165 (dd, br, 1H, N-CH<sub>2a</sub> DATC),

4.285 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 4.5 Hz, H6b), 4.348 (dd, br, 1H, N-CH<sub>2b</sub> DATC), 4.486 (dd, br, 1H, N-CH<sub>2c</sub> DATC), 4.761 (dd, br, 1H, N-CH<sub>2d</sub> DATC), 5.127 (dd, 1H, *J*<sub>1,2</sub> = 10.2 Hz, *J*<sub>2,3</sub> = 9 Hz, H2), 5.205 (dd, br, 2H, C=CH<sub>2a</sub> DATC), 5.265 (d, br, 2H, C=CH<sub>2b</sub> DATC), 5.280–5.369 (m, 2H, H3 and H4), 5.723–5.801 (m, br, 1H, C=CHa DATC), 5.846 (d, 1H, *J*<sub>1,2</sub> = 10.5 Hz, H1), 5.831–5.910 (m, br, 1H, C=CHb DATC). Anal. (C<sub>21</sub>H<sub>29</sub>NO<sub>9</sub>S<sub>2</sub>) C, H, N, S.

**S-(β-D-Glucopyranosyl) N,N-Diallyldithiocarbamate (5d).** Compound **5d** was prepared analogous to **5a** from **4d** (2 g, 4 mmol) and 30 mL of NH<sub>4</sub>OH-MeOH. The crude product was chromatographed on silica gel (CHCl<sub>3</sub>-EtOH, 4:1) and subsequently recrystallized from EtOAc-hexane to give **5d** (0.7 g, 56%): mp 112–113 °C; TLC (system B) *R<sub>f</sub>* 0.62; <sup>1</sup>H NMR (500 MHz in D<sub>2</sub>O) δ 3.473 (dd, 1H, *J*<sub>1,2</sub> = 10 Hz, *J*<sub>2,3</sub> = 9 Hz, H2), 3.388 (ddd, 1H, *J*<sub>4,5</sub> = 10 Hz, *J*<sub>5,6a</sub> = 5.5 Hz, *J*<sub>5,6b</sub> = 2.5 Hz, H5), 3.604–3.681 (m, 2H, H3 and H4), 3.730 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6a</sub> = 5.5 Hz, H6a), 3.892 (dd, 1H, *J*<sub>6a,6b</sub> = 12.5 Hz, *J*<sub>5,6b</sub> = 2.5 Hz, H6b), 4.404–4.502 (m, br, 2H, N-CH<sub>2a</sub> DATC), 4.649–4.728 (m, br, 2H, N-CH<sub>2b</sub> DATC), 5.224 (ddd, 1H, C=CH<sub>2a</sub> DATC), 5.240 (ddd, 1H, C=CH<sub>2b</sub> DATC), 5.297 (ddd, 1H, C=CH<sub>2c</sub> DATC), 5.3177 (ddd, 1H, C=CH<sub>2d</sub> DATC), 5.687 (d, 1H, *J*<sub>1,2</sub> = 10 Hz, H1), 5.854–5.938 (m, 2H, 2C=CH). Anal. (C<sub>13</sub>H<sub>21</sub>NO<sub>5</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(2',3',4',6'-Tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl] N,N-Diallyldithiocarbamate (4e).** Compound **4e** was prepared analogous to **4a** from **3b** (9.4 g, 13.5 mmol) and **2** (2.6 g, 13.5 mmol) in 50 mL of acetone. The crude product was chromatographed on silica gel (CHCl<sub>3</sub>-EtOAc, 9:1) and subsequently recrystallized from EtOAc-hexane to give **4e** (3.5 g, 33%): mp 185–186 °C; TLC (system A) *R<sub>f</sub>* 0.34; <sup>1</sup>H NMR (500 MHz in CDCl<sub>3</sub>) δ 1.963, 2.011, 2.045, 2.064, 2.071, 2.103 and 2.156 (s, 3H, OAc), 3.790–3.886 (m, 3H, H5', H6a', and H6b'), 4.074–4.180 (m, 4H, H4, H5, H6a, and N-CH<sub>2a</sub> DATC), 4.334 (dd, br, 1H, N-CH<sub>2b</sub> DATC), 4.455 (dd, 1H, *J*<sub>6a,6b</sub> = 12 Hz, *J*<sub>5,6b</sub> = 1.5 Hz, H6b), 4.460 (d, 1H, *J*<sub>1,2</sub> = 8 Hz, H1'), 4.480 (dd, br, 1H, N-CH<sub>2c</sub> DATC), 4.752 (dd, br, 1H, N-CH<sub>2d</sub> DATC), 4.940 (dd, 1H, *J*<sub>2,3</sub> = 10.5 Hz, *J*<sub>3,4</sub> = 3.5 Hz, H3'), 5.117 (dd, 1H, *J*<sub>1,2</sub> = 8 Hz, *J*<sub>2,3</sub> = 10.5 Hz, H2'), 5.172–5.269 (m, br, 4H, 2C=CH<sub>2</sub> DATC), 5.222 (dd, 1H, *J*<sub>1,2</sub> = 10.5 Hz, *J*<sub>2,3</sub> = 9 Hz, H2), 5.325 (t, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9 Hz, H3), 5.351 (dd, 1H, *J*<sub>3,4</sub> = 3.5 Hz, *J*<sub>4,5</sub> = 1.1 Hz, H4'), 5.715–5.791 (m, br, 1H, C=CHa DATC), 5.789 (d, 1H, *J*<sub>1,2</sub> = 10.5 Hz, H1), 5.824–5.905 (m, br, 1H, C=CHb DATC). Anal. (C<sub>33</sub>H<sub>46</sub>NO<sub>17</sub>S<sub>2</sub>) C, H, N, S.

**S-[4-(β-D-Galactopyranosyl)-β-D-glucopyranosyl] N,N-Diallyldithiocarbamate (5e).** Compound **5e** was prepared analogous to **5a** from **4e** (2 g, 2.5 mmol) and 30 mL of NH<sub>4</sub>OH-MeOH. The crude product was chromatographed on silica gel (CHCl<sub>3</sub>-MeOH, 1:1) and subsequently recrystallized from EtOH to give **5e** (420 mg, 34%): mp 204–205 °C; TLC (system C) *R<sub>f</sub>* 0.4; <sup>1</sup>H NMR (500 MHz in D<sub>2</sub>O) δ 3.571 (dd, 1H, *J*<sub>1,2</sub> = 7.8 Hz, *J*<sub>2,3</sub> = 10 Hz, H2'), 3.675 (dd, 1H, *J*<sub>2,3</sub> = 10 Hz, *J*<sub>3,4</sub> = 3.5 Hz, H3'), 3.634–3.839 (m, 8H, H2, H3, H4, H5, H6a, H5', H6a', and H6b'), 3.937 (dd, 1H, *J*<sub>3,4</sub> = 3.4 Hz, *J*<sub>4,5</sub> = 0.8 Hz, H4'), 3.958 (dd, 1H, *J*<sub>6a,6b</sub> = 12 Hz, *J*<sub>5,6b</sub> = 1.7 Hz, H6b), 4.411–4.499 (m, 2H, N-CH<sub>2a</sub> DATC), 4.486 (d, 1H, *J*<sub>1,2</sub> = 8 Hz, H1'), 4.691 (d, br, 2H, N-CH<sub>2b</sub> DATC), 5.226 (ddd, 1H, C=CH<sub>2a</sub> DATC), 5.242 (ddd, 1H, C=CH<sub>2b</sub> DATC), 5.299 (ddd, 1H, C=CH<sub>2c</sub> DATC), 5.330 (ddd, 1H, C=CH<sub>2d</sub> DATC), 5.708 (d, 1H, *J*<sub>1,2</sub> = 10 Hz, H1), 5.856–5.940 (m, 2H, 2C=CH DATC). Anal. (C<sub>19</sub>H<sub>31</sub>NO<sub>10</sub>S<sub>2</sub>) C, H, N, S.

**Enzymatic Hydrolysis of SDTCs.** SDTCs were incubated with α- or β-glucosidase, β-galactosidase, esterase, or cytosol from rat liver at pH 7.4 and 4.8 and with thioglucosidase at pH 5.8 at 25 or 37 °C for a maximum of 28 days. In order to test the possibility of nonenzymatic hydrolysis, substances were incubated with BSA or GSH. Quantification of the substances was carried out with HPLC by injecting known volumes of deproteinized incubates. Chromatographic separation was performed on a 5 μm APS-Hypersil column (250 × 4.6 mm with 10 mm guard cartridge), with a mobile phase consisting of CH<sub>3</sub>CN-H<sub>2</sub>O, 90:10, and the compounds were monitored by UV absorption at 245 nm. At a flow rate of 1.5 mL/min, retention times (*t<sub>R</sub>*) of **5a–e** were 4.7, 20.5, 17.1, 3.7, and 17.3 min, respectively. For the detection of **1**, which is formed by hydrolysis of **5a–c**, samples were separated on a



$\mu$ Bondapak C18 column (300  $\times$  3.9 mm). The mobile phase employed was a mixture of CH<sub>3</sub>CN–H<sub>2</sub>O, 52:48, with a flow rate of 1 mL/min, and the eluent was detected at 254 nm. The  $t_R$  was 20.0 min for **1** and 12.0 min for **DSF**.

**Determination of Biochemical Parameters.** Male Sprague–Dawley rats weighing 150–250 g were used for all experiments. Five animals of each group were fasted overnight and treated with the compounds **2** and **5a,b,d,e** (1.7 mmol/kg, ip). Control groups received the same volume of physiological saline. After 4 or 24 h, animals were killed and microsomal and cytosolic fractions of the liver cells were isolated as described earlier.<sup>7</sup> The determination of protein content was performed according to the method of Lowry et al.<sup>41</sup> GSH content was determined in the liver by the recycling assay.<sup>42</sup> The rate of formation of 2-nitro-5-thiobenzoic acid was measured in a system containing 5,5'-dithiobis(2-nitrobenzoic acid), GR, NADPH, phosphate buffer, and sample. Cytosolic GST activity with 1-chloro-2,4-dinitrobenzene as a substrate was determined by the method of Habig et al.<sup>43</sup> The rate of oxidation of NADPH by GSSG was used as a measure of GR activity.<sup>44</sup> Determination of nitrosamine dealkylase activity was carried out in accordance with the method described elsewhere<sup>45</sup> using 1 mM NDMA or NDEA as substrates, measuring formaldehyde and acetaldehyde as the (2,4-dinitrophenyl)hydrazones.<sup>46</sup> In the *in vitro* experiments, where the liver microsomal fraction of untreated rats was used, various concentrations of substances were added to the assay system.

**NDEA Metabolism.** Two rats in each group were fasted overnight and treated first with the inhibitor **1**, **5a**, or **5b** (1.5 mmol/kg, ip) or the saline. Later (1 h) the <sup>14</sup>C-labeled NDEA (28 mg/kg, 5  $\mu$ Ci/kg) was given ip, and 6 h later a second dose of the inhibitor was administered. After application of labeled NDEA, the animals were placed for 24 h into an all-glass metabolism cage. Air was pumped through the cage at 32 L/h, and exhaled air was dried in H<sub>2</sub>SO<sub>4</sub>. <sup>14</sup>CO<sub>2</sub> was trapped in 1 N NaOH, and the urine was collected in an ice-cold flask, containing 0.5 mL of 4 N NaOH. Radioactivity in the NaOH solution was measured at the time points indicated in Figure 4. At the end of the experiment, urinary excretion of total radioactivity and of unchanged [<sup>14</sup>C]NDEA was determined.<sup>46</sup> An aliquot of 1 mL of urine was spiked with 20  $\mu$ g of *N*-nitrosodiisopropylamine as an internal standard and extracted on an Extrelut column (120  $\times$  15 mm; Merck) with 20 mL of CH<sub>2</sub>Cl<sub>2</sub>. The extract was concentrated to 1 mL and analyzed by GC on a Carbowax 20M TPA column (2.2 m  $\times$  2 mm) with a thermal energy analyzer.

**DNA Single-Strand Breaks.** DNA single-strand breaks (SSB) were determined to assess a possible antigenotoxic effect of the sugar-linked DTCs. Rats were divided into four groups and fasted overnight. They received by a first gavage **5a** (0.5 mmol/kg) or saline. After 2 h, a second gavage followed with NDEA (1.4 mg/kg) or saline. One hour after the second gavage, the animals were anesthetized with metophane and a two-step *in situ* perfusion was performed to prepare a hepatocyte suspension according to Pool et al.<sup>47</sup> A modified version of the alkaline filter elution technique developed by Kohn et al.<sup>48</sup> was used to analyze DNA single-strand breaks.<sup>47</sup> Primary hepatocytes isolated from treated and control animals were loaded onto polycarbonate filters (pore size of the filter, 2  $\mu$ m; 1.5  $\times$  10<sup>6</sup> cells/filter; Nucleopore, Tübingen, FRG) and lysed at pH 10 with a buffer containing sodium dodecyl sulfate and proteinase K. Ten 2 mL elution fractions and the filter fraction were collected, and the DNA contents were determined fluorimetrically using Hoechst 33258.<sup>49</sup>

**Pharmacokinetics.** Two rats in each group were anesthetized with chloral hydrate and surgically fitted with catheters in the jugular vein for drug administration and blood sampling. The catheter was attached to the lid of the cage through a long steel spring to enable the rats to move around freely. Compound **5a** or **5b** (100 mg/kg) was administered through the catheter, and the catheter was rinsed with 500  $\mu$ L of saline. Blood samples (250–300  $\mu$ L) were drawn at the times indicated in Figure 5, and the catheter was rinsed immediately with 250  $\mu$ L of saline. The blood samples were transferred to heparinized tubes and centrifuged. For urine collection, five rats in each group injected iv or ip were placed individually into metabolism cages. Urine samples were

collected in an ice bath at 8 and 24 h. Plasma and urine samples were prepared for HPLC analysis using solid-phase extraction. Samples were passed through Diol columns (500 mg/3 mL; J.T. Baker Inc.), which were previously conditioned with CHCl<sub>3</sub>. After washing of the column with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  1 mL), compounds were extracted with CHCl<sub>3</sub>–MeOH, 10:1 (2  $\times$  1 mL), for **5a** or with MeOH (2  $\times$  2 mL) for **5b**. The extracts were evaporated to dryness under a stream of nitrogen, reconstituted in MeOH, filtered through a Millipore filter (0.45  $\mu$ m), and analyzed by HPLC. HPLC conditions were the same as mentioned above except for the composition of the mobile phase (CH<sub>3</sub>CN–H<sub>2</sub>O, 92:8) and the flow rate (1 mL/min for **5a**,  $t_R$  7.5 min; 1.5 mL/min for **5b**,  $t_R$  19.4 min).

**Pharmacokinetic Analysis.** Pharmacokinetic data were subjected to nonlinear regression analysis using the computer program Proc. NLIN of SAS.<sup>50</sup> A linear three-compartment model (eq 1) was fitted using the extended least squares modeling program MKMODEL.<sup>51</sup> The area under the curve (AUC) was determined using eq 2. The mean residence time (MRT) was calculated from eq 3, and the total body clearance (CL) was estimated from eq 4. Equation 5 was used to calculate the apparent volume of distribution at steady state ( $V_{ss}$ ).

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad (1)$$

$$AUC = A/\alpha + B/\beta + C/\gamma \quad (2)$$

$$MRT = \frac{A/\alpha^2 + B/\beta^2 + C/\gamma^2}{A/\alpha + B/\beta + C/\gamma} \quad (3)$$

$$CL = \text{dose}/AUC \quad (4)$$

$$V_{ss} = CL \times MRT \quad (5)$$

**Acknowledgment.** We thank Dr. R. Port (Department for Perinatal Toxicology, German Cancer Research Center) for calculating the pharmacokinetic data and Mr. G. Pougialis and Mr. K. A. Klokow for their assistance in animal experiments, biochemical assays, and HPLC measurements. We also thank Ms. M. Mann for typing the manuscript.

## References

- Wattenberg, L. W. Chemoprevention of cancer. *Cancer Res.* **1985**, *45*, 1–8.
- Talalay, P.; De Long, M. J.; Prochaska, H. J. Molecular mechanisms in protection against carcinogenesis. In *Cancer Biology and Therapeutics*; Cory, J. G., Szentivanyi, A., Eds.; Plenum Press: New York, 1987; pp 197–216.
- Hartman, P. E.; Shankel, D. M. Antimutagens and anticarcinogens: A survey of putative interceptor molecules. *Environ. Mol. Mutagen.* **1990**, *15*, 145–182.
- Wattenberg, L. W. Inhibitors of chemical carcinogenesis. *Adv. Cancer Res.* **1978**, *26*, 197–226.
- Schmähl, D.; Krüger, F. W.; Habs, M.; Diehl, B. Influence of disulfiram on the organotropy of the carcinogenic effect of dimethylnitrosamine and diethylnitrosamine in rats. *Z. Krebsforsch.* **1976**, *85*, 271–276.
- Zemaitis, M. S.; Greene, F. E. Impairment of hepatic microsomal drug metabolism in the rat during daily disulfiram administration. *Biochem. Pharmacol.* **1976**, *25*, 1355–1360.
- Bertram, B.; Schuhmacher, J.; Frei, E.; Frank, N.; Wiessler, M. Effects of disulfiram on mixed function oxidase system and trace element concentration in the liver of rats. *Biochem. Pharmacol.* **1982**, *31*, 3613–3619.
- Hadjiolov, D.; Frank, N.; Mundt, D.; Spiegelhalter, B.; Wiessler, M.; Schmähl, D. Effects of disulfiram on the metabolism of nitrosodiethylamine during liver carcinogenesis. *J. Cancer Res. Clin. Oncol.* **1984**, *108*, 281–285.
- Hadjiolov, D.; Frank, N.; Schmähl, D. Inhibition of diethylnitrosamine-induced strand breaks in liver DNA by disulfiram. *Z. Krebsforsch.* **1977**, *90*, 107–109.
- Abanobi, S. E.; Popp, J. A.; Chang, S. K.; Harrington, G. W.; Lotlikar, P. D.; Hadjiolov, D.; Levitt, M.; Rajalakshmi, S.; Serma, D. S. R. Inhibition of dimethylnitrosamine-induced strand breaks in liver DNA and liver cell necrosis by diethyldithiocarbamate. *J. Natl. Cancer Inst.* **1977**, *58*, 263–271.



- (11) Bertram, B.; Frei, E.; Wiessler, M. Influence of disulfiram on glutathione, glutathione S-transferase, and on nitrosamine dealkylases of liver, kidney and esophagus of the rat. *Biochem. Pharmacol.* **1985**, *34*, 387–388.
- (12) Benson, A. M.; Barretto, P. B. Effects of disulfiram, diethyldithiocarbamate, bisethylxanthogen, and benzyl isothiocyanate on glutathione transferase activities in mouse organs. *Cancer Res.* **1985**, *45*, 4219–4223.
- (13) Marselos, M.; Lang, M.; Törrönen, R. Modifications of drug metabolism by disulfiram and diethyldithiocarbamate II. D-glucuronic acid pathway. *Chem.-Biol. Interact.* **1976**, *15*, 277–287.
- (14) Bertram, B.; Tacchi, A. M.; Pool, B. L.; Wiessler, M. *In vitro* formation of methyldiethyldithiocarbamate after the reaction of nitrosoacetoxymethylmethylamine or methylnitrosourea with disulfiram. *Carcinogenesis* **1982**, *3*, 1361–1366.
- (15) Tacchi, A. M.; Bertram, B.; Wiessler, M. Chemical interaction of disulfiram with nitrosodimethylamine after *in vitro* enzymatic activation. *Carcinogenesis* **1984**, *5*, 217–220.
- (16) Belman, S. Onion and garlic oils inhibit tumor promotion. *Carcinogenesis* **1983**, *4*, 1063–1065.
- (17) Spornins, V. L.; Barany, G.; Wattenberg, L. W. Effects of organosulfur compounds from garlic and onions on benzo(a)-pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. *Carcinogenesis* **1988**, *9*, 131–134.
- (18) Maurya, A. K.; Singh, S. V. Differential induction of glutathione transferase isoenzymes of mice stomach by diallyl sulfide, a naturally occurring anticarcinogen. *Cancer Lett.* **1991**, *57*, 121–129.
- (19) Brady, J. F.; Li, D.; Ishizaki, H.; Yang, C. S. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer Res.* **1988**, *48*, 5937–5940.
- (20) Wargovich, M. J.; Woods, C.; Eng, V. W. S.; Stephens, L. C.; Gray, K. Chemoprevention of N-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. *Cancer Res.* **1988**, *48*, 6872–6875.
- (21) Namane, A.; Gouyette, C.; Fillion, M.-P.; Fillion, G. and Huynh-Dinh, T. Improved brain delivery of AZT using a glycosyl phosphotriester prodrug. *J. Med. Chem.* **1992**, *35*, 3039–3044.
- (22) Fisher, J. F.; Harrison, A. W.; Bundy, G. L.; Wilkinson, K. F.; Rush, B. D.; Ruwart, M. J. Peptide to glycopeptide: Glycosylated oligopeptide renin inhibitors with attenuated *in vivo* clearance properties. *J. Med. Chem.* **1991**, *34*, 3140–3143.
- (23) Negre, E.; Chance, M. L.; Hanboulia, S. Y.; Monsigny, M.; Roche, A. C.; Mayer, R. M.; Hommel, M. Antileishmanial drug targeting through glycosylated polymers specifically internalized by macrophage membrane lectins. *Antimicrob. Agents Chemother.* **1992**, *2228*–2232.
- (24) Fülling, G.; Schröder, D.; Franck, B. Wasserlösliche, photosensibilisierend wirkende Porphyrindiglycoside. (Watersoluble, photosensitizing porphyrine glycosides.) *Angew. Chem.* **1989**, *101*, 1550–1552.
- (25) Driaf, K.; Krausz, P.; Verneuil, B. Glycosylated cationic porphyrins as potential agents in cancer phototherapy. *Tetrahedron Lett.* **1993**, *34*, 1027–1030.
- (26) Tejima, S.; Ishiguro, S. Thiosugars. X. Studies on glycosyl N,N-dialkyldithiocarbamates. *Chem. Pharm. Bull.* **1967**, *15*, 255–263.
- (27) Schmezer, P.; Pool, B. L.; Lefevre, P. A.; Callander, R. D.; Ratpan, F.; Tinwell, H.; Ashby, J. Assay-specific genotoxicity of N-nitrosobenzylamine to the rat liver *in vivo*. *Environ. Mol. Mutagen.* **1990**, *15*, 190–197.
- (28) Wagner, G.; Metzner, R. Über die Spaltung von Arylthiogluco-siden und Arylselenogluco-siden durch  $\beta$ -Glucosidase des Mandelemulsins. (Cleavage of arylthiogluco-sides and arylselenogluco-sides by  $\beta$ -glucosidase from almondemulsin.) *Naturwissenschaften* **1965**, *52*, 61–62.
- (29) Dutton, G. J.; Illing, H. P. A. Mechanism of biosynthesis of thio- $\beta$ -D-glucuronides and thio- $\beta$ -D-glucosides. *Biochem. J.* **1972**, *129*, 539–550.
- (30) Kjaer, A.; Skrydstrup, T. Selenogluco-sinolates: Synthesis and enzymatic hydrolysis. *Acta Chem. Scand.* **1987**, *B41*, 29–33.
- (31) Tu, Y. Y.; Peng, R.; Chang, Z. F.; Yang, C. S. Induction of a high affinity nitrosamine demethylase in rat liver microsomes by acetone and isopropanol. *Chem.-Biol. Interact.* **1983**, *44*, 247–260.
- (32) Yang, C. S.; Yoo, J. S. H.; Ishizaki, H.; Hong, J. Cytochrome P450IIE1: Roles in nitrosamine metabolism and mechanisms of regulation. *Drug Metab. Rev.* **1990**, *22*, 147–159.
- (33) Lee, M.; Ishizaki, H.; Brady, J. F.; Yang, C. S. Substrate specificity and alkyl group selectivity in the metabolism of N-nitrosodialkylamines. *Cancer Res.* **1989**, *49*, 1470–1474.
- (34) Frank, N.; Bertram, B.; Frei, E.; Hadjiolov, D.; Wiessler, M. Influence of thiocompounds on the metabolism of N-nitrosodiethylamine. *Carcinogenesis* **1988**, *9*, 1303–1306.
- (35) Frank, N.; Bertram, B.; Scherf, H. R.; Wiessler, M. Influence of dithiocarbamates on the metabolism and toxicity of N-nitrosodimethylamine in rats. *Carcinogenesis* **1990**, *11*, 199–203.
- (36) Spornins, V. L.; Venegas, P. L.; Wattenberg, L. W. Glutathione S-transferase activity: Enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. *J. Natl. Cancer Inst.* **1982**, *68*, 493–496.
- (37) Benson, A. M.; Batzinger, R. P.; Ou, S. L.; Bueding, E.; Cha, Y. N.; Talalay, P. Elevation of hepatic glutathione S-transferase activities and protection against mutagenic metabolites of benzo(a)pyrene by dietary antioxidants. *Cancer Res.* **1978**, *38*, 4486–4495.
- (38) Frei, E.; Bertram, B.; Wiessler, M. Reduced glutathione inhibits the alkylation by N-nitrosodimethylamine of liver DNA *in vivo* and microsomal fraction *in vitro*. *Chem.-Biol. Interact.* **1985**, *55*, 123–137.
- (39) Magee, P. N. Nitrosamines and Human Cancer: Some implications of Basic Research. In *Cancer Biology and Therapeutics*; Cory, J. G., Szentivanyi, A., Eds.; Plenum Press: New York, 1987; pp 183–195.
- (40) Frank, N.; Wiessler, M. Inhibition of the metabolism of N-nitrosoacetoxymethylmethylamine in the rat by disulfiram. *Carcinogenesis* **1986**, 755–758.
- (41) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. J. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- (42) Griffith, O. W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **1980**, *106*, 207–212.
- (43) Habig, W. H.; Pabst, M. J.; Jacoby, W. B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139.
- (44) Carlberg, I.; Mannervik, B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J. Biol. Chem.* **1975**, *250*, 5475–5480.
- (45) Janzowski, C.; Pool, B. L.; Preussmann, R.; Eisenbrand, G. Fluoro-substituted N-nitrosamines. 2. Metabolism of N-nitrosodiethylamine and of fluorinated analogs in liver microsomal fractions. *Carcinogenesis* **1982**, *3*, 155–159.
- (46) Spiegelhalter, B.; Eisenbrand, G.; Preussmann, R. Volatile N-nitrosamines in beer and other beverages by direct extracting using a kieselgur column. In *Environmental Carcinogens: Selected methods of analysis. Volume 6. N-Nitroso Compounds*; Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalter, B., Bartsch, H., Eds.; IARC Sci. Publ.: Lyon, 1983; No. 45, pp 135–142.
- (47) Pool, B. L.; Brendler, S. Y.; Liegibel, U. M.; Tompa, A.; Schmezer, P. Employment of adult mammalian primary cells in toxicology: *In vivo* and *in vitro* genotoxic effects of environmentally significant N-nitrosodialkylamines in cells of the liver, lung, and kidney. *Environ. Mol. Mutagen.* **1990**, *15*, 24–35.
- (48) Kohn, K. W.; Ewing, R. A. G.; Erickson, L. C.; Zwelling, L. A. Measurement of strandbreaks and cross-links by alkaline elution. In *DNA repair, a laboratory manual of research procedures*; Friedberg, E.C., Hanawalt, P. C., Eds.; Marcel Dekker: New York, 1981; pp 379–401.
- (49) Sterzel, W.; Bedford, P.; Eisenbrand, G. Automated determination of DNA using the fluorochrome Hoechst 33258. *Anal. Biochem.* **1985**, *147*, 462–467.
- (50) SAS-STAT User's Guide, Version 6; SAS Institute Inc.: Cary, 1989.
- (51) Holford NHG: MKMODEL. An Extended Least Squares Modelling Program; Biosoft: Cambridge, U. K., 1988.