N^1-Hydroxylated Derivatives of Chlorpropamide and Its Analogs as Inhibitors of Aldehyde Dehydrogenase in Vivo

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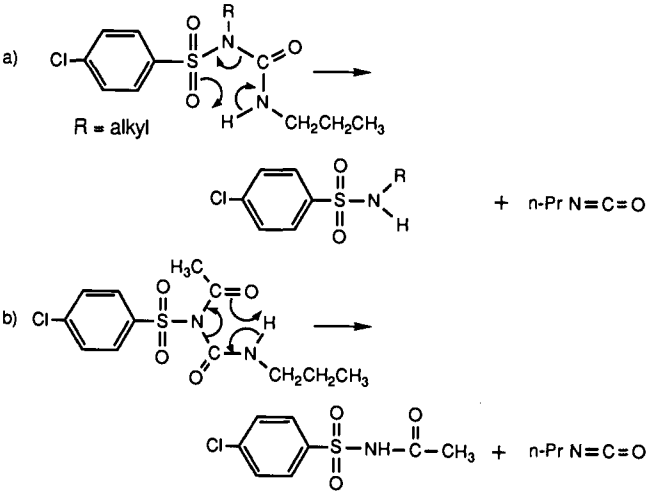
Received December 20, 1991

Certain (arylsulfonyl)urea hypoglycemic drugs exemplified by chlorpropamide (CP) are known to interact pharmacologically with alcohol (ethanol) to elicit a chlorpropamide–alcohol flushing (CPAF) reaction that is reminiscent of the disulfiram–ethanol reaction (DER). In the present structure–activity study, designed to elucidate the mechanism of inhibition of aldehyde dehydrogenase (AlDH) by CP, we discovered that the N^1-methoxy derivative of CP **2a** was a potent inhibitor of AlDH in vivo similar in activity to that of the N^1-ethyl derivative **2b**. Both **2a** and **2b** can release *n*-propyl isocyanate, a known inhibitor of AlDH, nonenzymatically. However, (arylsulfonyl)carbamates that are structurally analogous to **2a** were also active inhibitors of AlDH, whereas the corresponding (arylsulfonyl)carbamate analogs of **2b** were uniformly without activity. We propose a mechanism of bioactivation of **2a** and its analogs that involves initial O-demethylation followed by disproportionation and solvolysis of the intermediate formed to release nitroxyl, the putative inhibitor of AlDH.

Chlorpropamide (CP, 1), a sulfonylurea-type oral hypoglycemic agent, when taken in combination with alcoholic beverages even in moderate amounts, elicits an adverse physiological reaction manifested visibly by facial flushing in approximately 30% of individuals who use this drug to control blood sugar.[1] This chlorpropamide–alcohol flushing (CPAF) reaction is reminiscent of the disulfiram–ethanol reaction[2] (DER) and is caused by the inhibition of hepatic as well as erythrocyte aldehyde dehydrogenase (EC 1.2.1.3; AlDH), leading to the elevation of blood acetaldehyde (AcH) following ingestion of ethanol.[3] Since CP itself does not inhibit AlDH in vitro, we,[4] and others,[5] have postulated that it must undergo bioactivation in vivo to a reactive metabolite that inhibits this enzyme.

We had previously established that oxidative biotransformation of the *n*-propyl side chain of CP does not produce any metabolic species that inhibit AlDH.[4] However, based on chemical evidence that *alkylation* or *acetylation* of the N^1-position of CP led to products that displayed the propensity to eliminate the *n*-propyl side chain nonenzymatically as *n*-propyl isocyanate (Scheme I), the latter

Scheme I



a potent inhibitor of AlDH, we postulated that biological functionalization of CP at this N^1-position must be involved in its bioactivation mechanism.[6] In support of this, it was shown that N^3-methyl-CP (**3**), where the N^3-H is substituted with methyl group, or CP analogs, where O replaced this N^3-H bridge such that isocyanate elimination was blocked or made impossible, were devoid of any biological activity in vivo. Moreover, N^1-*tert*-butyl-CP (**4**) was found to be unstable at physiological pH and temperature, giving rise spontaneously to *N*-*tert*-butyl-4-chlorobenzenesulfonamide and, by deduction, *n*-propyl isocyanate.[6] Thus, these N^1-substituted analogs of CP (Scheme I) may be construed to represent *prodrugs* of *n*-propyl isocyanate that can release this enzyme inhibitor nonenzymatically in vivo.

Indeed, we have shown recently that *S*-(*n*-propylcarbamoyl)-L-cysteine and *S*-(*n*-propylcarbamoyl)-L-glu-

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(1) (a) Anonymous. Alcohol sensitivity to sulphonylureas. *Br. Med. J.* **1964**, *2*, 586–587. (b) Leslie, R. G. D.; Pyke, D. A. Chlorpropamide-alcohol flushing: a dominantly inherited trait associated with diabetes. *Brit Med. J.* **1978**, *2*, 1519–1521.

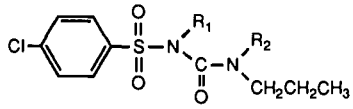
(2) Kitson, T. M. The disulfiram-ethanol reaction. *J. Stud. Alcohol* **1977**, *38*, 96–113.

(3) (a) Barnett, A. H.; Gonzalez-Auvert, C.; Pyke, D.; Saunders, J. B.; Williams, R.; Dickenson, C.; Rawlins, M. D. Blood concentrations of acetaldehyde during chlorpropamide-alcohol flush. *Brit. Med. J.* **1981**, *283*, 939–941. (b) Jerntorp, P.; Ohlin, H.; Bergstrom, B.; Almer, L.-O. Increase in plasma acetaldehyde: an objective indicator of the chlorpropamide alcohol flush. *Diabetes* **1981**, *30*, 788–791. (c) Ohlin, H.; Jerntorp, P.; Bergstrom, B.; Almer, L.-O. Chloropropamide-alcohol flushing, aldehyde dehydrogenase activity, and diabetic complications. *Brit. Med. J.* **1982**, *285*, 838–840. (d) Johnston, C.; Saunders, J. B.; Barnett, A. H.; Ricciardi, B. R.; Hopkinson, D. A.; Pyke, D. A. Chlorpropamide-alcohol flush reaction and isozyme profiles of alcohol dehydrogenase and aldehyde dehydrogenase. *Clin. Sci.* **1986**, *71*, 513–517.

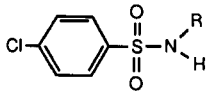
(4) Nagasawa, H. T.; DeMaster, E. G.; Kwon, C.-H.; Fraser, P. S.; Shirota, F. N. Structure vs. activity in the sulfonylurea-mediated disulfiram-ethanol reaction. *Alcohol* **1985**, *2*, 123–128.

(5) Little, R. G.; Petersen, D. R. Effects of tolbutamide and chloropropamide on acetaldehyde metabolism in two inbred strains of mice. *Toxicol. Appl. Pharmacol.* **1985**, *80*, 206–214.

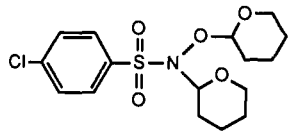
(6) (a) Nagasawa, H. T.; Elberling, J. A.; DeMaster, E. G.; Shirota, F. N. N^1-Alkyl-substituted derivatives of chlorpropamide as inhibitors of aldehyde dehydrogenase. *J. Med. Chem.* **1989**, *32*, 1335–1340. (b) Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N.; DeMaster, E. G. A nonhypoglycemic chlorpropamide analog that inhibits aldehyde dehydrogenase. *Alcoholism Clin. Exp. Res.* **1988**, *12*, 563–565.



	R_1	R_2
1	H	H
2a	OCH_3	H
2b	CH_2CH_3	H
3	H	CH_3
4	$C(CH_3)_3$	H
5	OH	H



	R
13a	OH
13b	OCH_3
13c	Et



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tathione, which are the S conjugates of *n*-propyl isocyanate with L-cysteine and glutathione, respectively, also function as prodrugs of *n*-propyl isocyanate and inhibit AlDH in rats by virtue of their chemical reversibility in vivo, raising ethanol-derived blood AcH several-fold higher than elicited by CP itself.[7]

In search of other biologically relevant substituents at this N^1-position of CP, we considered the possibility of the hydroxyl group even though we are unaware of any biological N-hydroxylation reactions on the sulfonamide nitrogen of sulfonamides or on sulfonylureas. We were unable to prepare N^1-hydroxy-CP (**5**) for pharmacological evaluation (vide infra), but did succeed in preparing the corresponding oxygen-masked N^1-methoxy-CP (**2a**). This latter CP analog turned out to be a highly potent in vivo inhibitor of AlDH in rats, as reflected by blood AcH levels after ethanol challenge of four times that seen with CP itself (Figure 1). However, since **2a** can be considered to be an isostere of N^1-ethyl-CP (**2b**) and could also release *n*-propyl isocyanate in vivo by the same mechanism depicted in Scheme Ia, other N^1-OMe analogs of CP were prepared, and systematic modifications were made not only in the side chain, but also in the functionality of the molecule, e.g., from a sulfonylurea to a sulfonylcarbamate (Chart I). We then conducted a detailed structure–activity study comparing the N^1-OMe with the N^1-Et-substituted sulfonylureas and the *N*-OMe with the *N*-Et-substituted sulfonylcarbamates. The results of these experiments to be described herein suggest that the mechanism of action of these N^1-OMe analogs of CP may be completely different from that depicted in Scheme I, viz., may, in fact, involve the release of nitroxyl (nitrosyl hydride, HN=O) instead of isocyanate as the putative inhibitor of AlDH.

(7) Shirota, F. N.; Elberling, J. A.; Nagasawa, H. T.; DeMaster, E. G. Failure of glutathione and cysteine prodrugs to block the chlorpropamide-induced inhibition of aldehyde dehydrogenase in vivo. *Biochem. Pharmacol.* **1992**, *43*, 916–918.

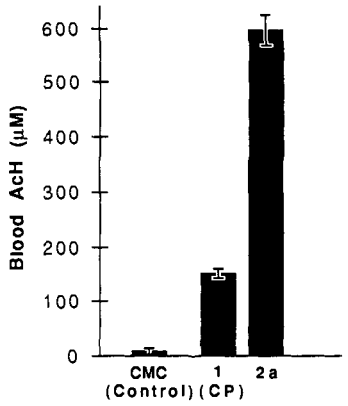
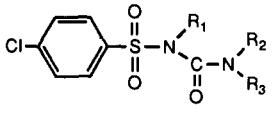


Figure 1. Effect of the N^1-methoxy derivative of chlorpropamide (**2a**) on ethanol-derived blood AcH in rats. The drug administration protocols and blood AcH assay methods are described in the Experimental Section. The results are given as mean ± SEM from duplicate determinations of blood AcH from three or more animals.

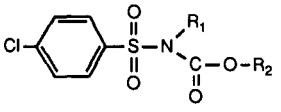
Chart I

a)



R_1 = OCH_3 Series	R_1 = Et Series	R_2	R_3
2a	2b	H	$CH_2CH_2CH_3$
6a	6b	CH_3	$CH_2CH_2CH_3$
7a	7b	H	CH_3

b)



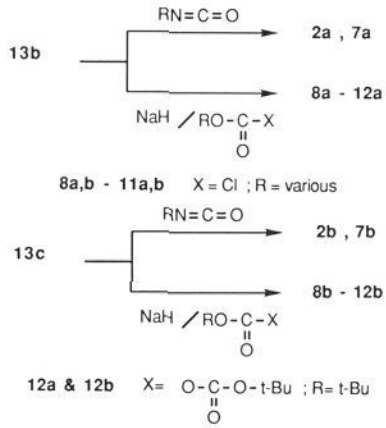
R_1 = OCH_3 Series	R_1 = Et Series	R_2
8a	8b	CH_3
9a	9b	CH_2CH_3
10a	10b	$CH_2CH_2CH_3$
11a	11b	$CH_2CH(CH_3)_2$
12a	12b	$C(CH_3)_3$

Chemistry

In efforts to prepare N^1-hydroxy-CP (**5**), we first sought to protect the hydroxyl function of the synthon 4-chloro-*N*-hydroxybenzenesulfonamide (**13a**) by selective O-acetylation in the manner described for *N*-hydroxybenzenesulfonamide.[8] However, the *N*,*O*-bis-acetyl compound was formed from **13a** under a variety of acetylation conditions. Use of pyruvonitrile[9] also did not result in selective O-acetylation. Attempted O-dihydropyranylation of **13a** also produced what we believe to be the *N*,*O*-bis-dihy-

(8) Smith, P. A. S.; Hein, G. E. The alleged role of nitroxyl in certain reactions of aldehydes and alkyl halides. *J. Am. Chem. Soc.* **1960**, 82, 5731–5740.

Scheme II



dropyran derivative **14**. For these reasons, we elected to proceed with our studies using the *O*-methyl derivative **13b** as synthon since the methyl group can theoretically be removed in vivo by enzymatic O-demethylation. Sulfonylation of methoxylamine (*O*-methylhydroxylamine) with 4-chlorobenzenesulfonyl chloride gave **13b** which was then condensed with *n*-propyl isocyanate to give the first of our target compounds **2a**. Various other carbamoylating and carboalkoxylating agents were also reacted with **13b** to give the corresponding compounds in the N^1-OMe series and with **13c** to give the compounds in the N^1-Et series (Chart I and Scheme II). For the preparation of **6a** and **6b**, *N*-methyl-*N*-propylcarbamoyl chloride was coupled with **13b** and **13c**, respectively. These reactions all proceeded without difficulty, and the target compounds were readily characterized by their IR and NMR spectra, and by elemental analyses.

Biological Results and Discussion

As alluded to earlier, the N^1-OMe derivative **2a** of CP was found to be a potent in vivo inhibitor of AlDH (Figure 1). Its structural similarity to the corresponding N^1-Et derivative **2b** suggests that the mechanism depicted in Scheme Ia may also be operative here. However, compound **6a** with an N^1-OMe substituent and where the N^3-H has been substituted with a methyl group was nearly as active as **2a** in elevating ethanol-derived blood AcH in rats, whereas the corresponding N^1-Et compound (**6b**) was much less active (Figure 2). Similarly, **7a**, where the N^1-substituent is OMe and N^3 is substituted with CH_3 *and* H, was highly active in vivo, whereas the N^1-Et analog **7b** had less activity. It is recalled that chlormethamide (CH_3 replaces *n*-propyl at N^3 in CP, structure not shown) was devoid of activity,[4] whereas N^1-substitution with the ethyl group to give compound **7b** restored the activity (Figure 2). Here again, if the mechanism of bioactivation were the same for **6a** and **6b**, or for **7a** and **7b**, the **a** and **b** series should have shown equivalent activity, just as equivalent activity was observed for **2a** and **2b**.

More compelling evidence that the active species from the N^1-OMe sulfonylureas must be different from that depicted for the N^1-Et sulfonylureas in Scheme Ia can be adduced from the results of the sulfonylcarbamate series

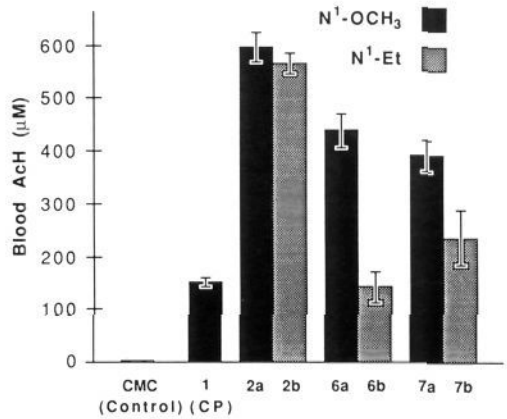


Figure 2. Effect of N^1-substituted (arylsulfonyl)ureas on ethanol-derived blood AcH in rats. See the legend to Figure 1 for further details.

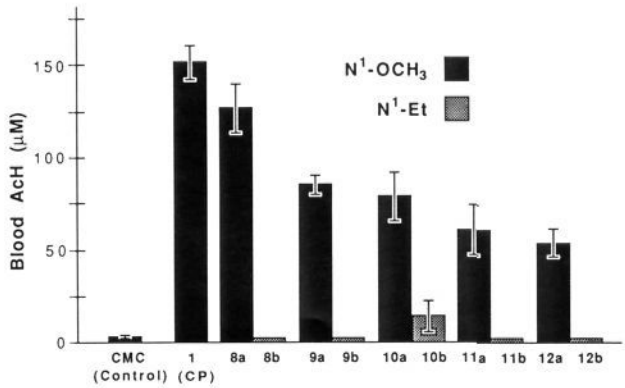


Figure 3. Effect of N^1-substituted N^1-(arylsulfonyl)carbamates on ethanol-derived blood AcH in rats. The synthesis of **10b** is described in ref 6a. See the legend to Figure 1 for further details.

(Chart Ib; Figure 3). It can be seen that regardless of the size of the alkyl group on the alkoxycarbonyl moiety, OMe substitution on the sulfonamide nitrogen gave rise to active compounds, whereas the corresponding compounds with the Et group on the sulfonamide nitrogen were uniformly devoid of any in vivo activity.

Our initial goal was to evaluate the AlDH inhibitory activity of compounds such as **5** bearing a free hydroxyl group on the sulfonamide nitrogen as well as their structural analogs, but because of synthetic difficulties, we compromised with the methoxy group at this position, reasoning that metabolic O-demethylation would transform **2a** to **5** in vivo. That the liver can in fact catalyze this O-demethylation reaction is shown in Figure 4. Liver microsomes from phenobarbital pretreated rats were incubated in the presence of cofactors with **2a**, as well as with the series of sulfonylcarbamates **9a–12a** (compound **8a** was omitted because of potential ambiguities), and the formaldehyde liberated after 30 min was measured. While sluggish compared to aminopyrine, the positive control where *N-demethylation* is involved, the data show that O-demethylation of such compounds shown in Chart I can be effected in vitro and therefore would be possible in vivo. However, when the N^1-OCH_3 compounds **10a**, **11a**, and **12a** were administered to phenobarbital pretreated rats, followed by ethanol challenge 2 h later, blood AcH levels *decreased* rather than increased (Figure 5). Increased AcH would have suggested that metabolic O-demethylation was enhanced in the phenobarbital treated animals. This decreased blood AcH observed with

(9) (a) Prabhakar, S.; Lobo, A. M.; Marques, M. M. Reactions between hydroxylamines and aroyl cyanides. *Tetrahedron Lett.* **1982**, *23*, 1391–1394. (b) Famulok, M.; Bosold, F.; Boche, G. Synthesis of N-acetoxy-2-aminonaphthalene, an ultimate carcinogen of the carcinogenic 2-naphthalamine, and its in vitro reactions with (bio)nucleophiles. *Tetrahedron Lett.* **1989**, *30*, 321–324.

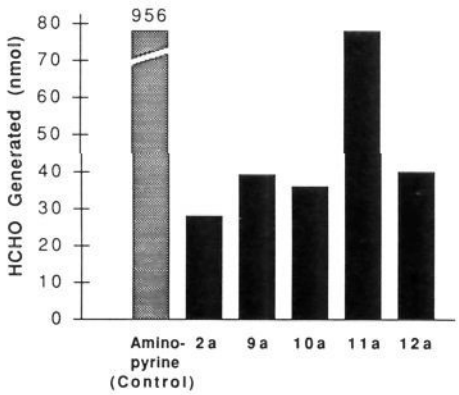


Figure 4. O-Demethylation of N^1-OMe analogs of CP in vitro by phenobarbital-induced rat liver microsomes measured as formaldehyde generated. The results are given as mean ± SEM, $n = 3$.

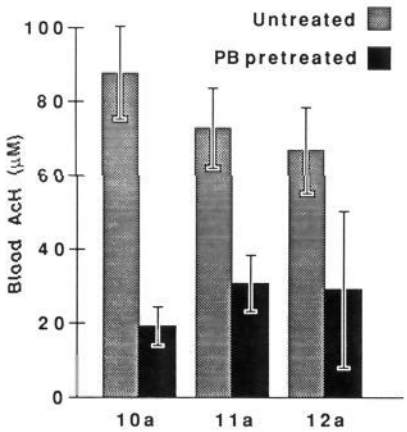


Figure 5. Effect of phenobarbital (PB) pretreatment of rats on ethanol-derived blood AcH by N^1-methoxy-N^1-(4-chlorobenzenesulfonyl)carbamates (**10a**, **11a**, and **12a**). See the legend to Figure 1 for further details.

phenobarbital pretreatment was not totally unexpected since similar data was seen with CP (**1**) itself.[4] Phenobarbital treatment can not only accelerate side chain oxidation, but is also known to induce the cytosolic AlDH of rat liver.[10]

Evidence that the bioactivation of **10a**, **11a**, and **12a** in vivo involved initial O-demethylation by the hepatic microsomal enzymes was provided by pretreatment of the rats with 1-benzylimidazole, an inhibitor of cytochrome P-450.[11] It can be seen from Figure 6 that 1-benzylimidazole pretreatment completely blocked the bioactivation of **10a**, **11a**, and **12a**, resulting in no inhibition of AlDH in vivo as reflected by no elevation of ethanol-derived blood AcH.

Summarizing the above data, we postulate that compounds such as **15** (X = NH; O) with a free hydroxyl group on the sulfonamide nitrogen are unstable and would disproportionate in vivo to give 4-chlorobenzenesulfinic acid (**16**) and an unstable nitrosocarbonyl compound (**17**) that can readily solvolyze to nitroxyl and, hence, N_2O as the final product (Scheme III). This unique mechanism

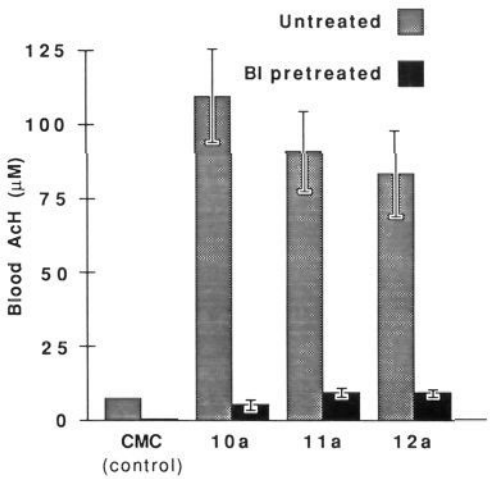


Figure 6. Effect of 1-benzylimidazole (BI) pretreatment of rats on ethanol-derived blood AcH by N^1-methoxy-N^1-(4-chlorobenzenesulfonyl)carbamates (**10a**, **11a**, and **12a**). See the legend to Figure 1 for further details.

is intriguing in that nitroxyl has also been postulated to be the active metabolite of *cyanamide*, a clinically used alcohol deterrent agent that inhibits AlDH.[12] This mechanism of disproportionation of **15** is similar to the decomposition mode of Piloty's acid (*N*-hydroxybenzenesulfonamide) in strong base.[13] Nitrosocarbonyl compounds exemplified by nitrosobenzoyl (PhCONO) are known to solvolyze readily to give the corresponding carboxylic acid and nitroxyl, with the latter dimerizing to hyponitrous acid ($H_2N_2O_2$) and ultimately decomposing to H_2O and N_2O.[14] We will provide further evidence for Scheme III and nitroxyl formation from similar compounds in a companion paper.

Experimental Section

Organic chemicals were purchased mostly from Aldrich Chemical Company, Milwaukee, WI, while enzymes and reagents for biological assays were purchased from Sigma Chemical Company, St. Louis, MO. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Infrared spectra were determined on a Beckman IR-10 or a Digilab FTS-40 infrared spectrophotometer. Some ^{1}H NMR spectra were obtained on a Varian T-60A (60 MHz) spectrometer, while other ^{1}H NMR and all ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz), AC-300 (300 MHz), or Gemini 300 (300 MHz) NMR spectrometers. For the 60-MHz spectra, Silanor C [$CDCl_3$ containing 1% tetramethylsilane (TMS) as a reference] was used as solvent, whereas for the 200- and 300-MHz spectra, $CDCl_3$ was used both as solvent and reference unless otherwise indicated. The reaction mixtures and chromatography effluents were monitored by TLC on silica gel GF (Analtech Inc., Newark, DE) using the solvent systems indicated.

General Procedure for the Preparation of [(4-Chlorophenyl)sulfonyl]ethyl(or methoxy)carbamic Acid Alkyl

(10) Petersen, D. R.; Collins, A. C.; Deitrich, R. A. Role of liver cytosolic aldehyde dehydrogenase isozymes in control of blood acetaldehyde concentrations. *J. Pharmacol. Exp. Ther.* **1977**, *201*, 471–481.

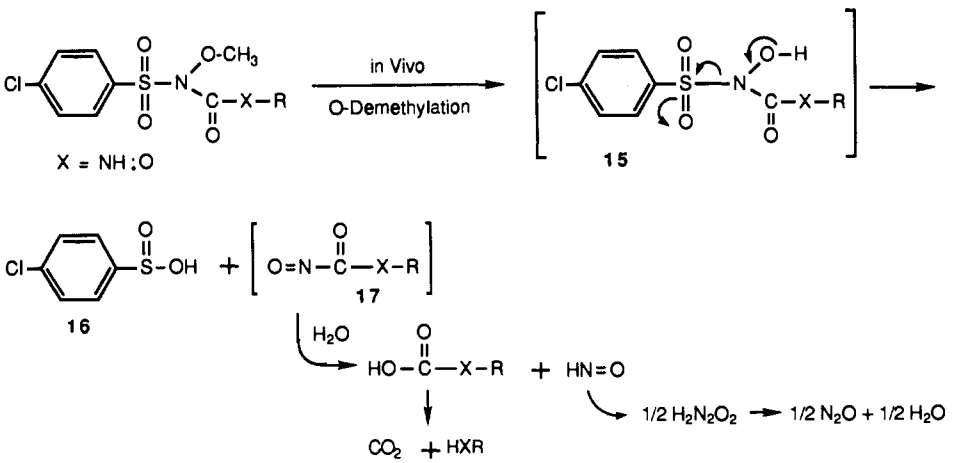
(11) (a) Shore, L. J.; Mogilevsky, W. S.; Smith, P. B. W.; Fenslau, C.; Odell, G. B. *In vitro* formation of glutathione conjugates of the dimethylester of bilirubin. *Biochem. Pharmacol.* **1991**, *42*, 1969–1976. (b) Testa, B.; Jenner, J. Inhibitors of cytochrome P-450s and their mechanism of action. *Drug Metabolism Rev.* **1981**, *12*, 1–117.

(12) (a) Shirota, F. N.; DeMaster, E. G.; Kwon, C.-H.; Nagasawa, H. T. Metabolism of cyanamide to cyanide and an inhibitor of aldehyde dehydrogenase (AlDH) by rat liver microsomes. *Alcohol Alcoholism* **1987**, *Suppl. 1*, 219–223. (b) Shirota, F. N.; DeMaster, E. G.; Nagasawa, H. T. Cyanide is a product of the catalase-mediated oxidation of the alcohol deterrent agent, cyanamide. *Toxicol. Lett.* **1987**, *37*, 7–12. (c) Nagasawa, H. T.; DeMaster, E. G.; Redfern, B.; Shirota, F. N.; Goon, D. J. W. Evidence for nitroxyl in the catalase-mediated bioactivation of the alcohol deterrent agent cyanamide. *J. Med. Chem.* **1990**, *33*, 3120–3122.

(13) Piloty, O. The oxidation of hydroxylamines by benzenesulfonyl chloride. *Ber. Dtsch. Chem. Ges.* **1896**, *29*, 1559–1567.

(14) Beckwith, A. L. J.; Evans, G. W. Reactions of alkoxy-radicals. Part III. Formation of esters from alkyl nitrites. *J. Chem. Soc.* 1962, 130–137.

Scheme III



Esters. [(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid Methyl Ester (8b). NaH (50% in oil, 0.96 g, 0.020 mol) was added to **13c**[15] (2.20 g, 0.010 mol) in dimethylacetamide (20 mL) and the mixture stirred for 30 min. Methyl chloroformate (1.89 g, 0.020 mol) was added, and the mixture was stirred for another 30 min. The suspension was then diluted with H_2O (50 mL), and the mixture was extracted with Et_2O (5 × 20 mL). Evaporation of the Et_2O extract gave a residue that was purified by flash chromatography on a silica gel column using hexane–THF (10:1) as eluant. The fractions were analyzed by TLC using hexane–THF (5:1) as solvent. Fractions containing **8b** were combined, and the solvent was evaporated. The residue was dissolved in Et_2O, the solution filtered, and the solvent evaporated. The product was recrystallized from Et_2O–hexane to give **8b**: colorless crystals; 1.49 g (31% yield); mp 53.1–53.3 °C; IR (KBr, cm^{-1}) 1738 (C=O), 1356 and 1157 (SO_2), 1089 (1,4-disubstituted benzene); ^1H NMR ($CDCl_3$, 60 MHz, δ) 7.72 (A_2B_2, q, 4 H, J = 9.0 Hz, $\Delta\nu$ = 25 Hz, Ar*H*), 3.93 (q, 2 H, J = 6.9 Hz, NCH_2CH_3), 3.72 (s, 3 H, OCH_3), 1.32 (t, ~3 H, J = 6.9 Hz, NCH_2CH_3). Anal. ($C_{10}H_{12}ClNO_4S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Methyl Ester (8a). This compound was prepared from **13b**[16] and methyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc–light petroleum (1:5). The product was recrystallized from EtOAc–light petroleum to give **8a**: colorless crystals (85% yield); mp 64–65.5 °C; IR (KBr, cm^{-1}) 1749 (C=O), 1383 and 1186 (SO_2), 1092 (1,4-disubstituted benzene); ^1H NMR (60 MHz, δ) 3.8 (s, 3 H, OCH_3), 4.0 (s, 3 H, OCH_3), 7.4–8.0 (q, 4 H, 1,4-disubstituted benzene). Anal. ($C_9H_{10}ClNO_5S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Ethyl Ester (9a). This compound was prepared from **13b** and ethyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc–light petroleum (1:5). Fractions (R_f 0.64 in EtOAc–hexane, 1:4) were collected to give a white solid (78% yield): mp 68–69.5 °C; IR (KBr, cm^{-1}) 1745 (C=O), 1382 and 1186 (SO_2); ^1H NMR (60 MHz, δ) 1.27 (t, 3 H, CH_2CH_3), 4.02 (s, 3 H, OCH_3), 4.22 (q, 2 H, OCH_2CH_3), 7.40–7.98 (q, 4 H, 1,4-disubstituted benzene). Anal. ($C_{10}H_{12}ClNO_5S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid Ethyl Ester (9b). This compound was prepared from **13c** and ethyl chloroformate according to the general procedure above. The yield of **9b** (oil) was 83%: IR (neat, cm^{-1}) 1734 (C=O), 1357 and 1163 (SO_2), 1090 (1,4-disubstituted benzene); ^1H NMR (300 MHz δ) 7.70 (A_2B_2, q, 4 H, J = 8.7 Hz, $\Delta\nu$ = 121 Hz, Ar*H*), 4.15 (q, 2 H, J = 7.1 Hz, OCH_2CH_3), 3.92 (q, 2 H, J = 7.0 Hz, NCH_2CH_3), 1.35 (t, 3 H, J = 7.0 Hz, NCH_2CH_3), 1.21 (t, 3 H, J = 7.1 Hz, OCH_2CH_3). Anal. ($C_{11}H_{14}ClNO_4S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Propyl Ester (10a). This compound was prepared from **13b** and *n*-propyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc–hexane (1:4). The yield of **10a** (oil) was 78.6%: ^1H NMR (60 MHz, δ) 0.93 (t, 3 H, CH_2CH_3), 1.60 (m, 2 H, $CH_2CH_2CH_3$), 4.05 (s, 3 H, OCH_3), 4.17 (t, 2 H, $OCH_2CH_2CH_3$), 7.45–8.05 (q, 4 H, 1,4-disubstituted benzene). Anal. ($C_{11}H_{14}ClNO_5S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid 2-methylpropyl Ester (11a). This compound was prepared from **13b** and isobutyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc–light petroleum (1:5). The yield of **11a** (oil) was 68%: ^1H NMR (200 MHz δ) 0.89 (d, 6 H, $CH(CH_3)_2$), 1.93 (m, 1 H, $CH_2CH(CH_3)_2$), 3.93 (d, 2 H, OCH_2), 4.01 (s, 3 H, OCH_3), 7.47–7.94 (q, 4 H, 1,4-disubstituted benzene); ^{13}C NMR ($CDCl_3$) 18.80 (2 C, $CH(CH_3)_2$), 27.77 ($CH(CH_3)_2$), 67.07 ($OCH_2CH(CH_3)_2$), 74.10 (OCH_3), 129.4 and 130.1 (4 C, aromatic), 135.1 and 141.2 (2 C, substituted aromatic), 152.5 (carbamate C=O). Anal. ($C_{12}H_{16}ClNO_5S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid 2-Methylpropyl Ester (11b). This compound was prepared from **13c** and isobutyl chloroformate according to the general procedure above, except that the crude product was distilled at 181–183 °C (2.2 mm). The yield of **11b** (oil) was 61%: IR (neat, cm^{-1}) 1732 (C=O), 1360 and 1152 (SO_2), 1091 (1,4-disubstituted benzene); ^1H NMR (300 MHz, δ) 7.70 (A_2B_2, q, 4 H, J = 8.7 Hz, $\Delta\nu$ = 121 Hz, Ar*H*), 3.93 (q, 2 H, J = 7.0 Hz, NCH_2CH_3), 3.88 (d, 2 H, J = 6.7 Hz, OCH_2CH), 1.88 (m, 1 H, $CH_2CH(CH_3)_2$), 1.36 (t, 3 H, J = 7.0 Hz, NCH_2CH_3), 0.87 (d, 3 H, J = 6.7 Hz, $CH(CH_3)_2$). Anal. ($C_{13}H_{18}ClNO_4S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid 1,1-Dimethylethyl Ester (12a). This compound was prepared from **13c** and di-*tert*-butylcarbonic anhydride according to the general procedure above. The flash chromatography eluant was THF–hexane (1:8). A clear oil was obtained (R_f = 0.78 in THF–hexane, 1:5), which crystallized when stored at −15 °C to give 1.05 g (65.2% yield) of **12a**: mp 63–65 °C; IR (KBr, cm^{-1}) 1744 (C=O), 1374, 1153 (SO_2), 1092 (1,4-disubstituted benzene); ^1H NMR (60 MHz, δ) 1.47 (s, 9 H, *tert*-butyl CH_3), 4.03 (s, 3 H, OCH_3), 7.45–8.03 (q, 4 H, aromatic H's, 1,4-disubstituted benzene). Anal. ($C_{12}H_{16}ClNO_5S$) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid 1,1-Dimethylethyl Ester (12b). This compound was prepared from **13c** and di-*tert*-butylcarbonic anhydride according to the general procedure above. The yield of **12b** (low melting solid) was 90%: IR (KBr, cm^{-1}) 1729 (C=O), 1353 and 1156 (SO_2), 1092 (1,4-disubstituted benzene); ^1H NMR (300 MHz, δ) 7.67 (A_2B_2, q, 4 H, J = 8.6 Hz, $\Delta\nu$ = 109 Hz, Ar*H*), 3.89 (q, 2 H, J = 7.0 Hz, NCH_2CH_3), 1.37 (s, ~9 H, $C(CH_3)_3$, 1.35 (t, ~3 H, NCH_2CH_3). Anal. ($C_{13}H_{18}ClNO_4S$) C, H, N.

General Procedure for the Preparation of 4-Chloro-*N*-ethyl-(or *N*-methoxy)-*N*-[(alkylamino)carbonyl]benzene-

(15) Hagen, V.; Morgenstern, E.; Gores, E.; Franke, R.; Sauer, W. Quantitative structure-activity relations in anticonvulsant benzenesulfonamides. *Pharmazie* **1980**, *35*, 183–185.

(16) Blackburn, G. M.; Mann, B. E.; Taylor, B. F.; Worrall, A. F. A nuclear-magnetic-resonance study of the binding of novel N-hydroxybenzenesulphonamide carbonic anhydrase inhibitors to native and cadmium-111-substituted carbonic anhydrase. *Eur. J. Biochem.* **1985**, *153*, 553–558.

sulfonamides. 4-Chloro-*N*-ethyl-*N*-[(methylamino)carbonyl]benzenesulfonamide (7b). A mixture of **13c** (1.10 g, 5.0 mmol), triethylamine (1.1 mL, 7.5 mmol), and methyl isocyanate (2.0 mL, 3.4 mmol) placed in each of two capped pressure tubes (5 mL) were heated for 20 h at 75 °C. The cooled solutions were stirred into 1 N HCl (110 mL) to hydrolyze the excess reagent, and after 1 h the mixture was extracted with EtOAc (4 × 20 mL). The extract was dried (Na_2SO_4), and the solvent was evaporated to give an oil. The oil was purified by flash chromatography on a silica gel column using hexane–THF (10:1) as eluant. The fractions were analyzed by TLC using hexane–THF (5:1) as solvent. Fractions containing **7b** were combined, and the solvent was evaporated to give an oil. The oil was repurified by prep TLC on 1-mm silica plates eluting with THF–hexane (1:5). The band containing **7b** was extracted with THF and evaporated to give **7b**: 0.70 g (25% yield); IR (KBr, cm^{-1}) 3411 (NH), 1697 (C=O), 1528 (amide II), 1350 and 1162 (SO_2), 1092 (1,4-disubstituted benzene); ^1H NMR (60 MHz, δ) 7.64 (A_2B_2, q, 4 H, J = 9.0 Hz, $\Delta\nu$ = 14 Hz, Ar*H*), 7.12 (b s, 1 H, N*H*), 3.68 (q, 2 H, J = 6.9 Hz, NCH_2CH$_3$), 2.90 and 2.82 (2 s, 3 H, NHCH_3), 1.18 (t, 3 H, J = 6.9 Hz, NCH$_2$CH_3). Anal. ($C_{10}H_{13}ClN_2O_3S$) C, H, N.

4-Chloro-*N*-methoxy-*N*-[(methylamino)carbonyl]benzenesulfonamide (7a). This compound was prepared from **13b** and methyl isocyanate according to the general procedure above. The product was not purified by flash chromatography, but was recrystallized from THF–hexane in 30% yield: mp 172–174 °C; ^1H NMR (60 MHz, δ) 2.85 (d, NHCH_3), 4.05 (s, OCH_3), 6.2 (broad s, N*H*), 7.45–8.0 (q, 4 H, 1,4-disubstituted benzene). Anal. ($C_9H_{11}ClN_2O_4S$) C, H, N.

4-Chloro-*N*-methoxy-*N*-[(propylamino)carbonyl]benzenesulfonamide (2a). This compound was prepared from **13b** and *n*-propyl isocyanate according to the general procedure above. The flash chromatography eluant was EtOAc–hexane (1:4). The product was recrystallized from EtOAc–light petroleum to yield white needles: 1.4 g (63.3% yield); mp 89–90 °C; IR (KBr, cm^{-1}) 3325 (N—H), 1708 (urea C=O), 1377 and 1180 (SO_2); ^1H NMR (90 MHz, δ) 0.82 (t, 3 H, CH_3CH$_2$), 1.45 (m, 2 H, CH$_3$CH_2CH$_2$), 3.14 (q, 2 H, CH$_2$CH_2NH), 3.98 (s, 3 H, OCH_3), 6.23 (broad s, 1 H, N*H*), 7.43–7.93 (q, 4 H, Ar*H*). Anal. ($C_{11}H_{15}ClN_2O_4S$) C, H, N.

Methylpropylcarbamoyl Chloride. A solution of methylpropylamine (10.0 g, 0.137 mol) and pyridine (10.8 g, 0.137 mol) in CH_2Cl_2 (200 mL) was placed in a flask cooled in an ice–salt bath and equipped with a dry ice condenser. A solution of triphosgene (13.5 g, 0.046 mol) was added with stirring at such a rate that the temperature was maintained at less than 4 °C. After 30 min, the ice bath was removed and the solution was allowed to stand overnight. The orange solution was evaporated to near dryness and the semisolid residue was diluted with Et_2O (100 mL) and the mixture was filtered. The filtrate was decolorized with charcoal and evaporated to give an amber liquid which was vacuum distilled. The fraction with bp 96.0–98.0 °C (26 mm) was collected to give a colorless liquid, 8.29 g (45% yield). IR (CCl_4, cm^{-1}): 1740 (C=O); ^1H NMR (60 MHz, δ) 0.93 (t, 3 H, CH_3CH$_2$), 1.65 (m, 2 H, CH_2CH$_2$), 3.05 and 3.12 (2 s, 3 H, CH_3N), 3.40 and 3.42 (2 t, 2 H, CH_2, N); n^{24}_D 1.4517. Anal. ($C_5H_{10}ClNO$) C: calcd, 44.29; found, 45.00; H, N. This material was used directly without further purification.

4-Chloro-*N*-ethyl-*N*-[(methylpropylamino)carbonyl]benzenesulfonamide (6b). NaH (50% in oil, 1.08 g, 0.023 mol) was added to **13c** (3.30 g, 0.015 mol) in dimethylacetamide (30 mL) and the mixture stirred for 120 min. Methylpropylcarbamoyl chloride (3.06 g, 0.023 mol) was added, and the mixture was stirred overnight. The suspension was then diluted with H_2O (70 mL), and the mixture was extracted with Et_2O (4 × 20 mL). Evaporation of the Et_2O extract gave a residue that was purified by flash chromatography on a silica gel column using hexane–THF (10:1) as eluant. Fractions containing **6b** were combined, and the solvent was evaporated. The residue separated into two phases. TLC showed the presence of two impurities. The oil was purified by flash chromatography using hexane–CH_2Cl_2 (4:1) as eluant. Fractions containing pure **6b** were combined, and the solvent was evaporated. The residue was dissolved in Et_2O, the solution filtered, and the solvent evaporated to constant weight to give a colorless oil: 1.49 g (31% yield); IR (neat, cm^{-1}) 1685 (C=O), 1353 and 1177 (SO_2), 1089 (1,4-disubstituted benzene); ^1H NMR (300 MHz, δ) 7.64 (A_2B_2, q, 4 H, J = 8.7 Hz, $\Delta\nu$ = 93, Ar*H*), 3.46 (t, 2 H, J = 7.5 Hz, NCH_2CH$_2$), 3.18 (q, 2 H, J = 7.2 Hz, NCH_2CH$_3$), 3.17 (s, 3 H, NCH_3), 1.67 (m, 2 H, NCH$_2$CH_2CH$_3$), 1.09 (t, 3 H, J = 7.2 Hz, NCH$_2$CH_3), 0.96 (t, 3 H, J = 7.4 Hz, NCH$_2$CH$_2$CH_3). Anal. ($C_{13}H_{19}ClN_2O_3S$) C, H, N.

4-Chloro-*N*-methoxy-*N*-[(methylpropylamino)carbonyl]benzenesulfonamide (6a). This compound was prepared from **13b** and methylpropylcarbamoyl chloride according to the procedure for **6b** above. The flash chromatography solvent was THF–hexane (1:10). The fractions containing product were further purified by prep TLC on 1-mm silica plates eluted with THF–hexane (1:5). CH, and N analysis indicated that this product retained hexane. The oil was therefore repurified by prep TLC on 1-mm silica plates eluting with CH_2Cl_2. The yield of **6a** (oil) was 39%: IR (neat, cm^{-1}) 1709 (C=O), 1365 and 1173 (SO_2), 1089 (1,4-disubstituted benzene); ^1H NMR (60 MHz, δ) 0.97 (t, 3 H, CH$_2$CH$_2$CH_3), 1.61 (m, 2 H, CH$_2$CH_2CH$_3$), 3.20 (s, 3 H, NCH_3), 3.50 (t, 2 H, NCH_2), 3.93 (s, 3 H, OCH_3), 7.45–7.91 (q, 4 H, 1,4-disubstituted benzene). Anal. ($C_{12}H_{17}ClN_2O_4S$) C, H, N.

Pharmacological Evaluation. These studies were performed in adherence with guidelines established in the *Guide for the Care and Use of Laboratory Animals* published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the Animal Study Committee of the Minneapolis VA Medical Center.

Drug Administration Protocol. The compounds were suspended in 2% aqueous (carboxymethyl)cellulose (CMC) and administered at a dose of 1.0 mmol/kg, ip, at zero time to fasted (24 h) male rats of Sprague-Dawley descent (BioLab, Inc., St. Paul, MN), weighing 185–225 g. Ethanol (2.0 g/kg, ip) was given at 2 h, and the animals were sacrificed 1 h subsequent to each ethanol dose for measurement of blood AcH. CP-treated animals served as positive controls. Phenobarbital as its sodium salt was administered as a 0.1% solution in the drinking water for 6 days, followed by water 24 h prior to the experiment. 1-Benzylimidazole (75 mg/kg, ip) was administered 2 h prior to test drug administration.

Blood Acetaldehyde Determination. Blood AcH levels in treated and control animals were measured 1 h after the administration of ethanol in treated and control animals as previously described.[17]

Determination of Formaldehyde Produced by Microsome-Catalyzed O-Demethylation.[18] The test compounds were dissolved in acetone (192 μmol/mL), and an aliquot (final concentration 2 mM) was added to a 50-mL Ehrlenmeyer flask and preincubated at 37 °C for 30 min, after which time 10 mL of the incubation mix [containing 80 mM potassium phosphate buffer (pH 7.4), 2 mM NADP, 8 mM nicotinamide, 2.5 mM glucose-6-phosphate, 4 mM semicarbazide (pH 7.0), 16.5 mM KCl, 4 mM $MgCl_2$, and H_2O] was added and the reaction was initiated by addition of 1.0 mL of glucose-6-phosphate dehydrogenase (40 units/mL) and 0.5 mL of rat liver microsomes. The reaction mixture was incubated at 37 °C for 30 min, and a 6-mL aliquot was taken and added to 2 mL of 15% $ZnSO_4$, followed by addition of 2 mL of saturated $Ba(OH)_2$ solution. The protein precipitate was sedimented by centrifugation, a 5-mL aliquot of the supernatant was removed, and 2 mL of Nash reagent (30 g NH_4OAc, 0.4 mL of acetylacetone brought up to 100 mL with H_2O) was added. The mixture was incubated at 55 °C for 30 min, and the absorbance at 412 nm was measured for CH_2O determination.

(17) Kwon, C.-H.; Nagasawa, H. T.; DeMaster, E. G.; Shirota, F. N. Acyl, N-protected α-aminoacyl, and peptidyl derivatives as prodrug forms of the alcohol deterrent agent cyanamide. *J. Med. Chem.* **1986**, *29*, 1922–1929.

(18) Mazel, P. Experiments Illustrating Drug Metabolism in Vitro. In *Fundamentals of Drug Metabolism and Drug Disposition*; La Du, B., Mandal, H., Way, E., Eds.; Williams and Wilkins: Baltimore, 1971; pp 548–550.

Acknowledgment. This work was supported in part by the Department of Veterans Affairs and in part by ADAMHA Grant 1 R01-AA07317. We thank Dr. E. G. DeMaster for his expert help in sacrificing the animals for blood collection.

Registry No. 1, 94-20-2; **2a**, 143065-09-2; **2b**, 117048-15-4; **3**, 98922-58-8; **4**, 120789-42-6; **5**, 143065-10-5; **6a**, 143065-11-6; **6b**, 143065-12-7; **7a**, 143065-13-8; **7b**, 143065-14-9; **8a**, 143065-15-0; **8b**, 143065-16-1; **9a**, 143065-17-2; **9b**, 143065-18-3; **10a**, 143065-19-4; **10b**, 120789-47-1; **11a**, 143065-20-7; **11b**, 143065-21-8; **12a**, 143065-22-9; **12b**, 143065-23-0; **13a**, 50695-53-9; **13b**, 143076-07-7; **13c**, 6318-34-9; **14**, 143076-08-8; **16**, 100-03-8; methyl chloroformate, 79-22-1; ethyl chloroformate, 541-41-3; *n*-propyl chloroformate, 109-61-5; isobutyl chloroformate, 543-27-1; di-*tert*-butylcarbonic anhydride, 34619-03-9; methyl isocyanate, 624-83-9; *n*-propyl isocyanate, 110-78-1; methylpropylamine, 627-35-0; methylpropylcarbamoyl chloride, 51493-02-8; aldehyde dehydrogenase, 9028-86-8.