Synthesis of Novel *N*-Nitrosothioureas and Examination of Their Mechanisms of Formation by High-Field Nitrogen-15 and Carbon-13 Nuclear Magnetic Resonance Spectra of Specifically Labeled Compounds

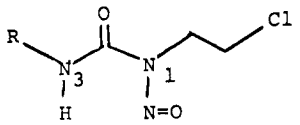
J. William Lown* and Shive M. S. Chauhan

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received May 11, 1982

An examination by ^{15}N NMR of $Na^{15}NO_2$ under various conditions of acidity led to the identification of several electrophilic nitrogen species. This permitted the selection of reaction conditions of low acidity which favor nitrosation at the nitrogen atom of thioureas rather than on the normally more reactive sulfur, leading to the isolation of novel *N*-nitrosothioureas. In contrast it was shown by employing ^{15}N and ^{13}C labeling that higher acidity conditions favor reaction via a detectable thionitrosyl intermediate.

The 1-(2-chloroethyl)-3-alkyl-1-nitrosoureas (CENUs, **1a–c**) which are clinically useful in the treatment of a range



1a, R = C_6H_{11}

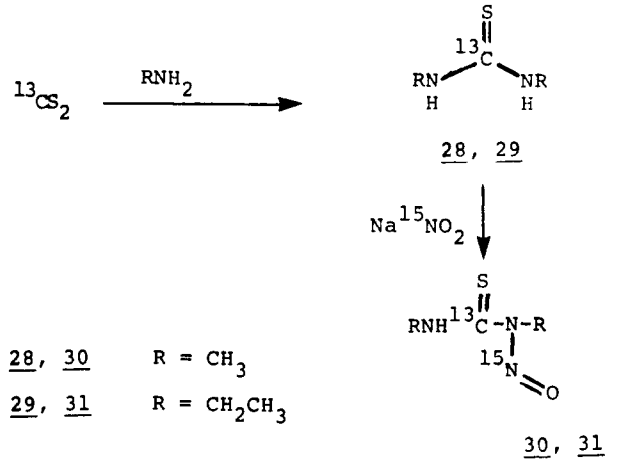
b, R = $4CH_3C_6H_6$-

c, R = $ClCH_2CH_2$-

of malignant diseases[1-4] decompose under physiological conditions to give, among other species, the 2-chloroethanediazohydroxide and the alkyl isocyanate.[5-11] The in vivo activity of CENUs correlates with their capacity to generate electrophiles which cause lesions in biological macromolecules, in particular alkylation and interstrand cross-linking of DNA.[10,11] In contrast, the isocyanate leads to carbamoylation of proteins, which reaction tends to correlate more with undesired toxic side effects.[12] Efforts to minimize this latter problem have included the synthesis of CENUs designed to trap the isocyanate intramolecularly.[13,14] We are exploring the alternative approach of synthesizing *N*-nitrosothioureas based on the premise that the isothiocyanates released should be less reactive than isocyanates[15] and, moreover, have been shown to exhibit antitumor properties in their own right.[16]

The synthesis of *N*-nitrosothioureas poses problems in the greater propensity of the sulfur of thioureas to undergo attack by electrophiles (e.g., NO^+) than the nitrogen.[15,17-22]

Scheme I



28, **30** R = CH_3

29, **31** R = CH_2CH_3

Only one *N*-nitrosothiourea had been reported hitherto.[18] Development of a useful general synthesis of *N*-nitrosothioureas necessitated an examination of the precise nature of the nitrosating species from sodium nitrite under different conditions and an examination of the alternative mechanistic pathways involved, which are reported herein. We report the general synthesis of *N*-nitrosothioureas and an examination of their mechanisms of formation using specifically ^{15}N- and ^{13}C-labeled nitrosothioureas.

Selection of Nitrosation Reaction Conditions Designed To Favor N-Nitrosation of Thioureas Permitting Isolation of *N*-Nitrosothioureas. The action of hydrochloric acid on sodium nitrite gives rise to a variety of electrophiles depending on the reaction conditions.[23-28] Dilute (0.07–0.1 N) acid conditions were selected so as to favor formation of unprotonated nitrous acid and N_2O_3. Since these species may be harder acids[29] than the nitro-

(1) Wheeler, G. D. *ACS Symp. Ser.* **1976**, *No. 30*, 87–119.
(2) Proceedings of the 7th New Drug Symposium on Nitrosoureas in: *Cancer Treat. Rep.* **1976**, *60*, 651–811.
(3) Montgomery, J. A. *J. Med. Chem.* **1980**, *23*, 1063.
(4) Hansch, C.; Leo, A.; Schmidt, C.; Jow, P. C. C.; Montgomery, J. A. *J. Med. Chem.* **1980**, *23*, 1095.
(5) Montgomery, J. A.; James, R.; McCaleb, G. D.; Kirk, M. C.; Johnston, J. P. *J. Med. Chem.* **1975**, *18*, 568.
(6) Brundrett, R. B. *J. Med. Chem.* **1980**, *23*, 1245.
(7) Lown, J. W.; Chauhan, S. M. S. *J. Med. Chem.* **1981**, *24*, 270.
(8) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* **1981**, *46*, 2479.
(9) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* **1981**, *46*, 5309.
(10) Kohn, K. W. *Cancer Res.* **1977**, *37*, 1450.
(11) Lown, J. W.; McLaughlin, L. M.; Chang, Y. M. *Bioorg. Chem.* **1978**, *7*, 17.
(12) Kann, H. E.; Schott, M. A.; Petkas, A. *Cancer Res.* **1980**, *40*, 50.
(13) Montgomery, J. A. *Cancer Treat. Rep.* **1976**, *60*, 651.
(14) Tsujihara, K.; Ozeki, M.; Morikawa, T.; Arai, Y. *Chem. Pharm. Bull.* **1981**, *29*, 2509.
(15) Lown, J. W.; Chauhan, S. M. S. *J. Chem. Soc., Chem. Commun.* **1981**, 651.
(16) Horakava, K.; Drobnica, L.; Nemeci, P. *Neoplasma* **1971**, *18*, 355.
(17) Werner, A. E. *J. Chem. Soc.* **1912**, 101, 2180.
(18) Johnston, T. P.; McCaleb, G. S.; Opliger, P. S.; Laster, W. R.; Montgomery, J. A. *J. Med. Chem.* **1971**, *14*, 600.
(19) Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1963**, *6*, 669.
(20) Al-Mallah, K.; Collings, P.; Stedman, G. *J. Chem. Soc., Dalton Trans.* **1974**, 2469.
(21) Collings, P.; Al-Mallah, K.; Stedman, G. *J. Chem. Soc., Perkin Trans. 2* 1975.
(22) Stedman, G. *Adv. Chem. Radiochem.* **1979**, *22*, 113.
(23) Bonnett, R.; Holleyhead, R.; Johnson, B. L.; Randall, E. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2261.
(24) Olah, G. A.; Balaram Gupta, B. C.; Narang, S. C. *J. Am. Chem. Soc.* **1979**, *101*, 5317.
(25) Park, N. J.; Krohn, K. A.; Mathis, C. A.; Chasko, J. H.; Greiger, K. R.; Peek, N. F. *Science* **1981**, *212*, 58.
(26) Mirvish, S. S. *Toxicol. Appl. Pharmacol.* **1975**, *81*, 325.
(27) Van Elten, R. L.; Risley, J. M. *J. Am. Chem. Soc.* **1981**, *103*, 5633.
(28) Oae, S.; Asai, N.; Fujimori, K. *J. Chem. Soc., Perkin Trans. 2* **1978**, 571.

0022-3263/83/1948-0507$01.50/0

Table I. ^{15}N Chemical Shifts of Sodium Nitrite and Accompanying Electrophilic Species Produced under Acidic Conditions

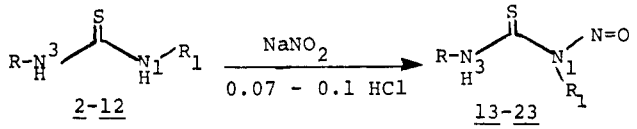
reactant (concn, mM)	reaction conditions and solvent[a]	^{15}N chemical shifts,[b] ppm
$Na^{15}NO_2$ (1)	50:50 H_2O + acetone, 0 °C	612.30[c]
$Na^{15}NO_2$ (2.0)	acetone (10 mL) ± 0.01 N HCl (10 mL), 0 °C	598.73[d] (br), 376.30[e]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 0.01 N HCl (10 mL), 0 °C	603.55[d] (br)
$Na^{15}NO_2$ (1.0)	acetone (10 mL) ± 0.05 N HCl (10 mL), 0 °C	586.46 (s), 376.20[e]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 0.1 N HCl (10 mL), −5 °C	582.53 (s), 376.0[e]
	acetone (10 mL) + 0.1 N HCl (10 mL) 0 °C	581.19 (s), 376.25[e]
	acetone (10 mL) + 0.1 N HCl (10 mL), 25 °C	580.23 (s), 376.17, 360.96[f]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 1.0 N HCl, 5 °C	580.35 (100.0), 412.95 (20), 376.20[e]
	acetone (10 mL) + 1.0 N HCl, 0 °C	580.35 (100.0), 412.95 (40.0)
	acetone (10 mL) + 1.0 N HCl, 25 °C	580.81 (5.0), 412.81 (100.0)[g]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 1.0 N HCl, 5 °C	570.50 (~1.0), 412.65 (100.0)[h]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 98% HCOOH, 0 °C	575.48 (100.0), 372.25 (40)
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + CH_3COOH (10 mL), 0 °C	576.60 (100.0), 564 (br)
n-butyl nitrite (10 mL)	acetone (10 mL), 0 °C	572.61[i]
$Na^{15}NO_2$ (1.0)	acetone (10 mL) + 48% HBr, 5 °C	372.08 (100.0), 354.5 (10), 309 (9)

[a] Total volume was 20 mL. [b] Relative to external anhydrous ammonia as a reference (0.0 ppm). [c] This value has been reported at δ 608.0 by: Lambert, J. B.; Roberts, J. D. *J. Am. ChemSoc.* **1965**, *87*, 4087. [d] The chemical shift of this peak depends upon the concentration of acid used. [e] The intensity of this peak was approximately 0.5–1.5% of the peak at δ 598.73–580.23. [f] This peak was reported at δ 354.0 in our previous paper.[15] [g] Additional peaks were also observed at δ 375.24 and 239.76. [h] Two minor peaks appeared at δ 370.40 and 231.56. [i] This peak has been reported in the reference in footnote *c* at δ 572.0.

sonium ion or its derivatives, they were anticipated to favor electrophilic attack at the harder nitrogen of thioureas rather than at the competing soft sulfur atom (see section on discussion of mechanisms).

In the event, when a suspension of the thioureas and sodium nitrite in dichloromethane or chloroform at −10 °C was treated dropwise with 1 equiv of dilute HCl (0.07–0.1 N), a yellow color developed slowly, and a normal workup permitted the isolation of a series of novel *N*-nitrosothioureas in 50–90% yield (Table I). These novel compounds proved to be sensitive to heat, light, and air oxidation.

In the case of the unsymmetrical nitrosothioureas selected for study (**18–23**) the R_1 group is cyclohexyl, which



2-12 **13-23**

2, **13**, R = R_1 = CH_3

3, **14**, R = R_1 = CH_2CH_3

4, **15**, R = R_1 = $CH_2CH_2CH_3$

5, **16**, R = R_1 = $CH_2CH_2OCH_3$

6, **17**, R = R_1 = CH_2CH_2F

7, **18**, R = C_6H_{11}, R_1 = CH_3

8, **19**, R = C_6H_{11}, R_1 = CH_2CH_3

9, **20**, R = C_6H_{11}, R_1 = $CH_2CH_2CH_3$

10, **21**, R = C_6H_{11}, R_1 = $CH_2CH_2OCH_3$

11, **22**, R = C_6H_{11}, R_1 = CH_2CH_2F

12, **23**, R = C_6H_{11}, R_1 = CH_2CH_2OH

was employed so that its steric bulk would promote regioselective nitrosation on the less hindered side. Specifically ^{15}N- (labeled NO) as well as ^{13}C=S-enriched nitrosothioureas (**30**, **31**) were synthesized according to Scheme I to facilitate the spectral assignments and mechanistic studies.

Spectral Characterization of *N*-Nitrosothioureas. The IR spectra of the *N*-nitrosothioureas in chloroform solution show NH stretching at 3410–3375 cm^{-1} and an NH or a CNH band at 1560–1510 cm^{-1}. The appearance of a new band at 1450–1435 cm^{-1} clearly indicates N-nitrosation and the strong band at 1395–1375 cm^{-1} is ascribed to the thioamide II band. The band at 1220–1150 cm^{-1} may be assigned to C=S stretch.[30] The IR spectra of nitrosothioureas obtained with progressively dilute solutions of 10^{-3}–10^{-4} M nitrosothiourea in carbon tetrachloride exhibited 10–20-cm^{-1} shifts in the NH stretching which militates against strong intramolecular hydrogen bonding.[9]

The UV absorption spectra of *N*-nitrosothioureas show characteristic bands at 266–270 [ε (1.1–1.2) × 10^4] and 353–364 nm [ε (1.0–1.6) × 10^2]. It may be noted that when thioureas are treated with $Na^{15}NO_2$ in 5–6 N HCl (under which conditions an $S^{15}NO$ species is detected by ^{15}N NMR; vide infra) a transient absorption band appeared at 242–246 nm which gradually decreased in intensity and may correspond to the thionitrosyl intermediate (see disscussion on mechanism).

The high-resolution mass spectrum was useful in characterizing *N*-nitrosothioureas. The molecular ion is seen together with M^+ − NO as the major fragmentation. Another major fragmentation is due to M^+ − HSNO (affording carbodiimides) which is in contrast to nitrosoureas where the C(O)N cleavage predominates, leading to diazohydroxides.[9] The generation of the latter species from nitrosoureas has been attributed to transfer of the NH proton to the N=O group in the energetically excited state which requires the groups in question to be coplanar or close to it.[31] This provides the first indication (substantiated by other methods, q.v.) that in contrast to the nitrosoureas the NH and N=O groups are not proximate in the nitrosothioureas.[31]

^{1}H NMR Spectra. In the ^{1}H NMR spectrum of symmetrical *N*-nitrosothioureas the alkyl group adjacent to the N=O group resonates downfield compared with the corresponding unnitrosated alkyl group, e.g., N_1,N_3-dimethyl-*N*-nitrosothiourea (**13**) shows a doublet at δ 3.37

(29) Parham, J. C.; Templeton, J. A. *J. Org. Chem.* **1982**, *47*, 652.

(30) Avram, M.; Matescu, G. D. "Organic Sulfur Compounds In Infrared Spectroscopy"; Robert E. Krieger Publishing Co.: Huntington, NY, 1978; Chapter 4, p 290.

(31) Bar-Shai, R.; Bortinger, A.; Sharvit, J.; Mandelbaum, A. *Isr. J. Chem.* **1980**, *20*, 137.

($^3J_{H-H}$ = 6.5 Hz) for N_3–CH_3 which became a singlet upon deuterium exchange while the N_1–CH_3 appears as a singlet at δ 3.53. The ^1H NMR data of other compounds (**14–17**) are given in the Experimental Section. The ^1H NMR spectral data are useful in assigning the structure of regioisomers in the case of the unsymmetrical *N*-nitrosothioureas **18–22**. For example in the case of **18** the singlet at δ 3.50 in $CDCl_3$ solution is assigned to the CH_3 adjacent to the N=O group because there is no detectable coupling to an NH proton. When the R_1 group is cyclohexyl and R_2 is a lower alkyl, nitrosation takes place regioselectively on the less hindered side. In the case of R_2 = methyl or ethyl, nitrosation occurs about 95% on the N_1 nitrogen, and in the cases where R^2 = propyl or methoxyethyl, N_1 nitrosation is preferred to the extent of 85–95%.

Mechanism of Formation of *N*-Nitrosothioureas. The nature of the species resulting from acid treatment of sodium nitrite was examined by ^{15}N NMR.[32,33] Sodium nitrite (^{15}N, 95% enrichment) dissolved in 1:1 aqueous acetone showed a resonance at δ 612.30 when acetone-d_6 was employed as a lock signal. A solution of 20 mmol of $Na^{15}NO_2$ in 20 mL of a 1:1 mixture of acetone and 0.01 N HCl at −5 °C showed a strong broad peak at δ 603.55 and new weaker resonance at δ 376.30. The intensities and positions of the ^{15}N resonances are dependent on factors including concentration and temperature (see Table I). A solution of 10 mmol of $Na^{15}NO_2$ in 20 mL of a 1:1 mixture of acetone and 0.1 N HCl at −5 °C showed a broad peak at δ 598.73. When the acidity was increased to 0.05 N HCl, the latter resonance moved to δ 586.46, and the additional peaks at δ 376.2 reappeared. A solution of 10 mmol of $Na^{15}NO_2$ in 20 mL of a 1:1 mixture of 0.1 N HCl and acetone at −5 °C showed a sharp and strong resonance at δ 582.52 together with the smaller peak at δ 376.28. The two distinct species represented by these two signals may be equilibrated upon maintaining the solution at 15–20 °C for 4 h and examination of the ^{15}N NMR spectrum at 20°. Under these conditions two additional sharp peaks appear at δ 360.96 and 360.63 (which correspond to the peaks at δ 354.0 from 10 mmol of $Na^{15}NO_2$ in 0.1 N HCl at 15 °C[15]).

When one increases the acidity to 1.0 N HCl (maintaining other conditions constant), one observes ^{15}N peaks at δ 580.35 (strong) and 412.95 at −4 °C while upon raising the temperature to 25 °C the δ 412.81 peak becomes stronger at the expense of the δ 580.81 peak. At 25 °C a new resonance appears at δ 239.76 in addition to the δ 375.24 peak. When 6 N HCl is used in the above reaction mixture, the δ 412.65 resonance persists and becomes stronger at the expense of weak signals at δ 570.50, 370.42, and 231.56 at −5 °C in 1:1 acetone/aqueous acid mixtures. It would appear that the species which predominates in 0.1 N HCl at −5 °C evidenced by the ca. δ 580.3 absorption is HONO. The resonance observed at δ 376.30 corresponds in position to that ascribed to $^{15}NO^+BF_4^-$ by Olah et al.[24] but under somewhat different reaction conditions. A more likely assignment is that it is due to $H^{15}NO_3$, and which was confirmed by independent measurements. $H^{15}NO_3$ can arise from the disproportionation of $H^{15}NO_2$ or from air oxidation[25] (eq 3). Previous work on nitrous acid at

$$O{=}NONa + HCl \rightleftharpoons O{=}NOH + NaCl \quad (1)$$

$$O{=}NOH \rightleftharpoons HON{=}O \quad (2)$$

$$2HON{=}O \rightleftharpoons HNO_3 + HNO \quad (3)$$

$$2HON{=}O \rightleftharpoons N_2O_3 + H_2O \quad (4)$$

$$HON{=}O + H^+ \rightleftharpoons H_2O^+{-}N{=}O \quad (5)$$

$$H_2O^+{-}N{=}O \rightleftharpoons H_2O + {}^+NO \quad (6)$$

$$^+NO + HCl \rightleftharpoons ClNO + {}^+H \quad (7)$$

comparable concentrations[26] and at 25 °C leads one to anticipate N_2O_3 as an intermediate. While its presence is implicated, experimental difficulties associated with ^{15}N natural-abundance measurements on N_2O_3 prevented confirmation of this point at present. Proton exchange with water (eq 5 and 6) is possible at ambient temperature but is likely to be slow at −5 °C and can take place either via the free nitrosonium ion[27] or by formation of a tetrahedral intermediate at the nitroso nitrogen.[28] The persistent stable peak at δ 412.65 in 12 N HCl is ascribed to nitrosyl chloride, $^{15}NOCl$ (eq 7). These data are in accord with the set of equilibria shown (eq 1–7).

Simultaneous accumulation of ^{15}N resonances during the treatment of thioureas **13** and **18** with $Na^{15}NO_2$ (1 molar equiv, 95% enrichment) in 0.07–0.1 N hydrochloric acid revealed three peaks at δ 588.1 ($HO^{15}NO$), 650.7 ($N{-}^{15}NO$), and δ 375 ($H^{15}NO_3$). The peak at δ 560.7 slowly increased at the expense of the δ 588.1 and 375 peaks which disappeared after 1 h. Thioureas may be considered as ambident species with different nucleophilic character at the sulfur and nitrogen atoms.[29,34] The observation of preferential N-nitrosation under the above reaction conditions may then reflect the preferred reaction of a hard acid (HNO_2) with the harder base according to the HSAB scheme.[29]

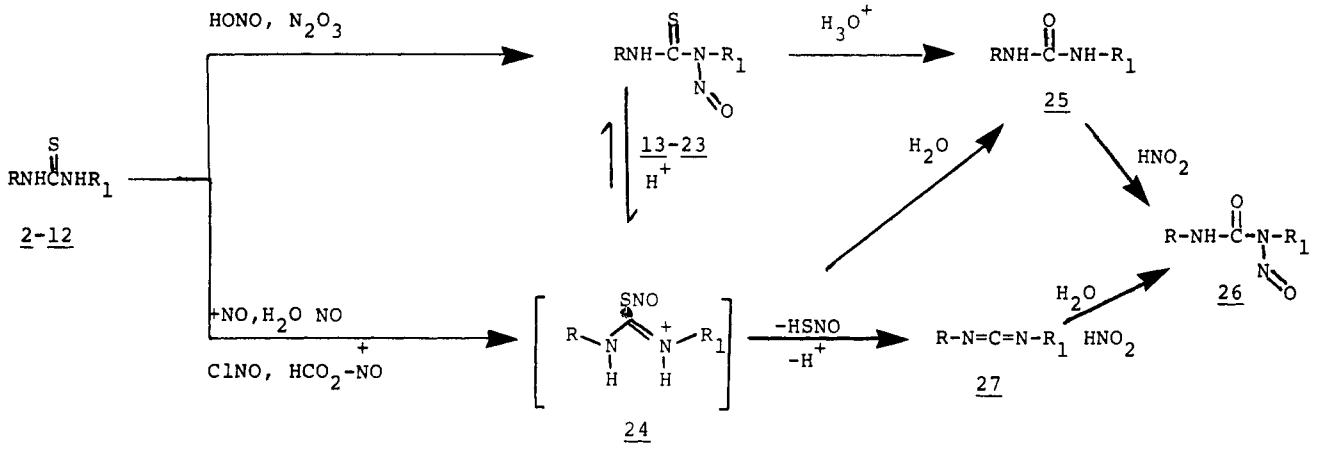
Catalysis of nitrosation reactions by thiocyanate under low acidity has been attributed to the formation of *N*-nitrosocyanate.[35] Studies of the reactions of certain nucleophiles (KNCS, Cl, Br, thioureas) with the protonated form of nitrosamines and comparison with the reactivity of the sulfur in the thioureas with iodide ion[36,37] are consistent with the idea that the preferred site of attack may be controlled by adjusting the reaction conditions.

N-Nitrosation may also be expected to depend on the relative ease of proton exchange and deprotonation of the NH group in thioureas. Recent evidence shows that the NH proton exchange depends upon the stereochemistry of the NH proton relative to the C=S bond.[38]

The nitrosation of the thioureas **13** and **18** were examined in 1.0 N hydrochloric acid in ethanol (to reduce the nucleophilicity of the medium) and at the lower temperature of −10 °C (to increase the lifetime of intermediates). Under these conditions the solution turned transient light red immediately, indicative of a thionitrosyl intermediate (NOSCN can be monitored at 460 nm[39]). At the same time a new ^{15}N peak appeared at δ 762.9 which is ascribed to the thionitrosyl species **24** since it corresponds closely in position to the δ 762.0 peak reported for the ethylthionitrosyl derivative.[15,23] The δ 762.9 peak disappeared after 30 min at −10 °C, and the δ 560.8 signal was not detected. This shows that under these conditions of higher acidity the *N*-nitrosothioureas were not formed since the corresponding ureas were the only products detected. A thionitrosyl intermediate similar to the species **24** impli-

(32) Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley-Interscience: New York, 1979.
(33) Lichter, R. L. "Determination of Organic Structure"; Academic Press: New York, 1977; Vol. 4, p 196.
(34) Gompper, R. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 560.
(35) Meyer, T. A.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1981**, 361.
(36) Williams, D. L. H. *J. Chem. Soc., Chem. Commun.* **1975**, 375.
(37) Hallett, G.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1980**, 624.
(38) Chun, Y.; Yavari, I.; Roberts, J. D. *Org. Magn. Reson.* **1982**, *18*, 74.
(39) Morgan, T. D. B.; Stedman, G.; Whincup, P. A. E. *J. Chem. Soc.* **1965**, 4813.

Scheme II



cated in the present reaction has been proposed to form at 0 °C during the reaction of NOCl with thioamides. In one instance, reaction under similar conditions but in the presence of HCl afforded HNSO which decomposed to give products which included elemental S and NO.[40] Thionitrosyl compounds that are somewhat more stable than intermediate **24** have been further characterized by UV and IR spectroscopy.[41] The transient nature of the intermediate **24** precludes such spectral examination and dictates that ^{15}N NMR at low temperatures is ideally suited for its detection.

Treatment of the *N*-nitrosothiourea **13** with anhydrous HCl in ether followed by addition of water gave the 1,3-dimethylurea **24** quantitatively. It may be noted that thioureas can form their oxygen counterparts in the presence of reactive oxygen species including superoxide anion[42] either by direct oxidation or via an intermediate disulfide.[43] Consequently, efforts were made to rigorously exclude oxygen during the above experiments. Reaction of the thiourea **2** with HCl and excess $NaNO_2$ gave nitrosourea **26** (R = R_1 = CH_3) in high yield.

Independent supporting evidence for the mechanism of formation of the *N*-nitrosothioureas was obtained from ^{13}C NMR studies employing ^{13}C=S-enriched thioureas. Treatment of **30** (which shows the ^{13}C=S signal at δ 182.02 in 1:1 acetone/water at 20 °C) with $Na^{15}NO_2$ in 0.1 N HCl at −10 °C and simultaneous accumulation of ^{13}C resonances revealed two broad peaks centered at δ 182.75 and 180.42. When the solution was maintained at 0 °C for 45 min, these peaks became sharper at δ 181.85 and 180.68, and at 15–20 °C these sharp peaks appear at δ 182.02 (thiourea) and 180.90 [$^2J(^{15}N-^{13}C)$ = 3.9 Hz, nitrosothiourea]. These peaks persisted at 25 °C without changing their chemical shifts within experimental error.

An experiment with ^{13}C-enriched thiourea **31** and an equimolar amount of $Na^{15}NO_2$ in acetone-d_6 treated with 0.5 N HCl at −10 °C and with simultaneous accumulation of ^{13}C resonances revealed peaks at δ 179.5 and a very broad peak at δ 165.09. After the sample warmed to 10 °C, the peak at δ 179.50 became sharp, and a new peak appeared at δ 171.88 (broad) and another at δ 162.50. After 1 h at 15 °C the spectrum showed the sharp peak at δ 179.50 [$^2J(^{15}N-^{13}C)$ = 2.8 ± 1.4 Hz] which is ascribed to the nitrosothiourea **14**, and the broad peak at δ 171.88 may be assigned to the thionitrosyl intermediate **24** (R = R_1 = CH_2CH_3) which is broadened due to resonance interactions.[44,45] The peak at δ 163.86 is assigned to the urea **25** (R = R^1 = CH_2CH_3), and a new peak appeared at δ 141.76 which from its position is tentatively ascribed to the carbodiimide (**27**)[43,46] that results from elimination of HSNO from **24** (Scheme II).

Under more extreme conditions of acidity (5–6 N HCl) and at −5 °C only a very small and broad ^{13}C peak was observed at δ 171.88 and a strong peak at δ 163.22 which is assigned to 1,3-diethylurea arising from **24** or the corresponding carbodiimide.

The distribution of products shows that the thionitrosyl intermediate is not subject to arrangement to the nitrosothioureas under these conditions but instead leads to ureas via elimination of HSNO to the carbodiimide and addition of water. Alternatively, ureas could arise from direct attack of water on the amide carbon with displacement of the SNO group. An analogous conversion of the C=S into a C=O group via S-protonation followed by nucleophilic attack at the adjacent carbon has been proposed.[47,48]

These results are in accord with Scheme II in which the nitrosothiourea is formed by direct N-nitrosation under conditions of low acidity (0.05–0.10 N HCl). It is conceivable but unlikely that a transient thionitrosyl intermediate may be formed and undergo rapid diffusion controlled rearrangement to the nitrosothiourea. If such an intermediate participates in this reaction it was below the limit of detection by either ^{15}N or ^{13}C NMR under these conditions.

The development of a general synthesis of *N*-nitrosothioureas now affords the opportunity to explore the effects of the sulfur atom on the direction of stereoelectronically controlled release of electrophiles under physiological conditions and the possibility of development of less toxic anticancer agents.

Experimental Section

The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal, sharply defined peaks were reported. The ^1H NMR spectra of the intermediates were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spec-

(40) Heyns, K.; Bebenburg, W. *Chem. Ber.* **1956**, *83*, 1303.
(41) Field, L.; Delts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. E. *J. Chem. Soc., Chem. Commun.* **1978**, 249 and references therein.
(42) Kalori, E.; Nagano, T.; Kuniedo, T.; Hirobe, M. *Chem. Pharm. Bull.* **1981**, *29*, 3075.
(43) Lecher, H. Z.; Gubermnaler, K. *J. Am. Chem. Soc.* **1953**, *75*, 1088.
(44) Kalinoski, H. O.; Kessler, H. *Org. Magn. Reson.* **1974**, *6*, 312.
(45) Kessler, H. *Angew. Chem., Int. Engl. Ed.* **1970**, *9*, 219.
(46) Williams, A.; Ibrahim, I. T. *Chem. Rev.* **1981**, *81*, 389.
(47) Cussans, N. J.; Ley, S. V.; Barton, D. H. R. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1650.
(48) Mikolajczyk, M.; Luczak, J. *J. Org. Chem.* **1978**, *43*, 2132.

trometers, and those of the final nitrosoureas were recorded on Bruker WG-200 and WH-400 spectrometers. The spectra were measured on approximately 5–10% (w/v) solutions, depending upon the spectrometers, in appropriate deuterated solvents with tetramethylsilane as an internal standard. The ^{13}C spectra of specifically ^{15}N-labeled compounds were recorded on a Bruker WH-200 spectrometer.

The ^{15}N spectra were recorded on a Bruker WH-200 spectrometer operating at 20.283 MHz. The spectra were recorded by using dimethylformamide as an external reference, and chemical shift values are reported relative to 10–20% ammonia at 0.0 ppm with the respective deuterated solvent as a lock signal. Most of the proton-decoupled natural-abundance ^{15}N spectra were obtained by using a 1 M solution with 0.05–0.1 M $Cr(acac)_3$ in a 20-mm-diameter tube after 80–86K scans. The spectra of specifically labeled compounds were recorded with 0.01 M solutions with or without $Cr(acac)_3$ (0.01–0.1 M) bu using approximately 1–4K scans.

Materials. The required alkylamines were obtained from Aldrich, and isothiocyanates were obtained from Trans World Chemicals. Ammonium-^{15}N chloride (95–99%), sodium nitrite-^{15}N (95–99%), potassium phthalimide-^{15}N (95–99%), carbon-^{13}C disulfide (90%), and ethylamine-^{15}N hydrochloride (95%) were obtained from Merck Sharp and Dohme.

General Method for the Preparation of *N*-Nitrosothioureas. A dilute solution of HCl (0.07–0.1 N, 200–500 mL) was added dropwise to a suspension of the thiourea (20–50 mmol) and sodium nitrite (20–50 mmol) in dichloromethane (200–400 mL) with mechanical stirring and cooling (−10 to −5 °C) in a nitrogen atmosphere during 1 h. After the addition was completed, the reaction mixture was warmed to 5 °C with stirring, the organic layer was removed, washed with water, and dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford the crude nitrosothioureas **13–23**, **30**, and **31** which were purified by either crystallization from petroleum or by chromatography over Florisil. Satisfactory combustion analytical data were obtained for **13–19**, **21**, and **23**.

1,3-Dimethyl-1-nitrosothiourea (13). Nitrosation of 1,3-dimethylthiourea (**2**)[49] in this way afforded **13**: 90% yield; mp 46 °C (lit.[19] mp 44–45 °C) IR ($CHCl_3$) ν_{max} 3375 (NH), 1540, 1440 (NO), 1375, 1185, 1060, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.37 (d, 3 H, $NHCH_3$), 3.53 (s, 3 H, CH_3), 10.00 (br m, 1 H, NH, exch); MS, *m/e* (relative intensity) 133.0313 (64.64, M^+), 103.0331 (100.00, $M^+ - NO$; calcd 103.0329).

1,3-Diethyl-1-nitrosothiourea (14). Nitrosation of 1,3-diethylthiourea (**3**)[49] similarly afforded **14**: 68% yield; an oil; IR ($CHCl_3$) ν_{max} 3325 (NH), 1525, 1445, 1375, 1185, 1055, 980 cm^{-1}; ^1H NMR ($CDCl_3$) 1.05 (t, 3 H, CH_2CH_3), 1.32 (t, 3 H, CH_2CH_3), 3.90 (octet, 2 H, CH_2CH_3), 4.25 (q, 2 H, CH_2CH_3), 8.56 (br m, 1 H, NH, exch); MS, *m/e* (relative intensity) 161.0627 (2.08, M^+; calcd 161.0623) 132.0722 (100.00, $M^+ - NO$; calcd 132.0722).

1,3-Dipropyl-1-nitrosothiourea (15). Nitrosation of 1,3-dipropylthiourea[50] (**4**) gave **15**: an oil; 77% yield; IR ($CHCl_3$) ν_{max} 3375 (NH), 1505, 1446, 1405, 1160, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H, CH_3), 1.12 (t, 3 H, CH_3), 1.50 (sextet, 2 H, CH_2), 1.65 (sextet, 2 H, CH_2), 3.98 (q, 2 H, CH_2), 4.20 (t, 2 H, CH_2), 8.80 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 189.0934 (18.19, M^+; calcd 189.0933), 159.0953 (68.43; calcd for $C_7H_{15}N_2S$, 159.0956), 88.0638 (8.63; calcd for $C_3H_8N_2O$, 88.0637), 58.0683 (100.0; calcd for C_3H_8N, 58.0657).

1,3-Bis(2-methoxyethyl)-1-nitrosothiourea (16). Nitrosation of 1,3-bis(2-methoxyethyl)thiourea (**5**)[51] afforded **16**: an oil; 67% yield; IR ($CHCl_3$) ν_{max} 3376 (NH), 1521, 1456, 1405, 1334, 1119, 1009 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.45 (s, 3 H, OCH_3), 3.50 (s, 3 H, OCH_3), 3.55 (t, 2 H, CH_2), 3.65 (q, 2 H, $NHCH_2$), 4.00 (t, 2 H, CH_2), 4.50 (t, 2 H, CH_2), 8.80 (br m, 1 H, NH, exch); MS, *m/e* (relative intensity) 221.0833 (23.70, M^+; calcd 221.0837), 191.0853 (100.0; calcd for $C_3H_{15}N_2O_2S$, 191.0854).

1,3-Bis(2-fluoroethyl)-1-nitrosothiourea (17). Reaction of free fluoroethylamine with 2-fluoroethyl isothiocyanate gave 1,3-bis(2-fluoroethyl)thiourea: 50% yield; mp 78–80 °C (dichloromethane/petroleum ether); IR ($CHCl_3$) 3228 (NH), 1440, 1324, 1019 cm^{-1}; ^1H NMR ($CDCl_3$) 3.95 (dq, 4 H, $^2J_{H-F}$ = 28.0 Hz, $^2J_{H-H}$ = 5.0 Hz), 4.60 (dt, 4 H, $2J_{H-F}$ = 47.5 Hz, $^2J_{H-H}$ = 5.0 Hz), 6.4 (br s, 2 H, NH, exch); ^{13}C NMR ($CDCl_3$) δ 44.6 (d, C_1, $^1J_{C-CF}$ = 19.1 Hz), 72.7 (d, C_2, $^1J_{^{13}C-^{19}F}$ = 66.2 Hz), 183.6 (C=S); MS, *m/e* (relative intensity) 168.0531 (100; calcd for $C_5H_{10}F_2N_2S$, 168.0533), 148.0470 (28.27; calcd for $C_5H_9FN_2S$, 148.0470). Anal. Calcd for $C_5H_{10}F_2N_2S$: C, 30.45, H, 4.56. Found: C, 30.10; H, 4.53.

Nitrosation of **6** gave **17**: 70% yield; mp 23 °C; IR ($CHCl_3$) ν_{max} 3373 (NH), 1523, 1436, 1396, 1339, 1153, 1022 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20 (dq, 2 H, CH_2, $^2J_{H-F}$ = 280 Hz, $^2J_{H-H}$ = 5.01 Hz), 4.50 (dt, 2 H, CH_2, $^1J_{H-F}$ = 46.0 Hz, $^2J_{H-H}$ = 5.0 Hz), 4.55 (dt, 2 H, CH_2, $^1J_{H-F}$ = 21.0 Hz, $^2J_{H-H}$ = 5.0 Hz), 4.75 (dt, 2 H, CH_2, $^1J_{H-F}$ = 50.2 Hz, $^2J_{H-H}$ = 5.0 Hz), 8.95 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 197.0431 (12.74, M^+; calcd 197.0435), 167.0453 (100.0; calcd for $C_2H_9N_2F_2S$, 167.0454), 94.0128 (17.94; calcd for C_2H_5FNS, 94.0127), 92.0384 (2.14; calcd for $C_2H_5N_2FO$, 92.0386).

3-Cyclohexyl-1-methyl-1-nitrosothiourea (18). Reaction of methylamine with cyclohexyl isothiocyanate gave 3-cyclohexyl-1-methylthiourea (**7**): mp 165–166 °C; IR ($CHCl_3$) ν_{max} 3240 (NH), 1559, 1527, 1440, 1225 (C=S), 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–2.20 (m, 10 H, CH_2), 3.00 (s, 3 H, CH_3), 3.95 (m, 1 H, CH), 6.75 (br m, 2 H, NH exch); MS, *m/e* (relative intensity) 172.1027 (44.04, M^+; calcd 172.1034).

Nitrosation of **7** gave **18**: 90% yield; mp 35–36 °C; IR ($CHCl_3$) ν_{max} 3320 (NH), 1520, 1450, 1375, 1180, 1020 cm^{-1}; ^1H NMR δ 1.20–2.20 (m, 10 H, CH_2), 3.50 (s, 3 H, CH_3), 4.20 (m, 1 H, H_1', axial), 8.37 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 201.0937 (10.94, M^+; calcd 201.0936), 171.0955 (72.75; calcd for $M^+ - NO$, 171.0955), 98.0969 (100.0; calcd for $C_6H_{12}O$, 98.0969).

3-Cyclohexyl-1-ethyl-1-nitrosothiourea (19). 3-Cyclohexyl-1-ethylthiourea (**8**) was prepared from cyclohexyl isothiocyanate and ethylamine as a solid: mp 104–105 °C; IR ($CHCl_3$) ν_{max} 3220 (NH), 1560, 1525, 1445, 1225, (C=S), 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–2.20 (m, 10 H, CH_2), 1.25 (t, 3 H, CH_2CH_3), 3.40 (octet, 2 H, CH_2CH_3), 3.95 (m, 1 H, CH), 6.15 (br m, 2 H, NH exch); MS, *m/e* (relative intensity) 186.1189 (100.00, M^+; calcd 186.1190).

Nitrosation of **8** afforded **19**: 83% yield; mp 42 °C; IR ($CHCl_3$) ν_{max} 3385 (NH), 1520, 1440, 1385, 1160, 1020, 960 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3 H, CH_2CH_3), 1.20–2.20 (m, 10 H, CH_2), 3.60 (m, 1 H, H_1', axial), 4.20 (q, 2 H, CH_2CH_3), 8.37 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 215.1095 (3.30, M^+; calcd 215.1093), 185.1113 (55.74; calcd for $M^+ - NO$, 185.1113), 98.0969 (100.00; calcd for $C_6H_{12}N$, 98.0969).

3-Cyclohexyl-1-propyl-1-nitrosothiourea (20). 3-Cyclohexyl-1-propylthiourea (**9**) was prepared from cyclohexyl isothiocyanate and propylamine as a solid: mp 81 °C; IR ($CHCl_3$) ν_{max} 3335 (NH), 1547, 1360, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (t, 3 H, CH_3), 1.95 (sextet, 2 H, $CH_2CH_2CH_3$), 1.00–2.20 (m, 10 H, CH_2), 3.45 (q, 2 H, $CH_2CH_2CH_2$), 3.95 (m, 1 H, CH), 5.75 (br m, 2 H, NH exch); MS, *m/e* (relative intensity) 200.1345 (100.00; calcd for $C_{10}H_{20}N_2S$, 200.1348), 98.0971 (63.86; calcd for $C_6H_{12}N$, 98.0970).

Nitrosation of **9** afforded **20**: 68% yield; mp 22–23 °C; IR ($CHCl_3$) ν_{max} 3375 (NH), 1502, 1451, 1403, 1364, 1160, 1017 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_3), 1.10–2.20 (m, 10 H, CH_2), 1.32 (sextet 2 H, CH_2), 4.10 (t, 2 H, CH_2), 4.35 (m, 1 H, H_1', axial), 8.60 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 229.1245 (3.60, M^+; calcd 229.1249), 199.1269 (54.45; calcd for $C_{10}H_{19}N_2S$, 199.1269), 98.0969 (100.0; calcd for $C_6H_{12}N$, 98.0969), 88.0640 (0.97; calcd for $C_3H_8N_2O$, 88.0644).

3-Cyclohexyl-1-(2-methoxyethyl)-1-nitrosothiourea (21). Cyclohexyl isothiocyanate (7.1 g, 50 mmol) was added dropwise to a solution of 2-methoxyethylamine (3.5 g, 50 mmol) in ether at room temperature and stirred at room temperature for 12 h. The solvent was removed under reduced pressure to produce a heavy oil of 3-cyclohexyl-1-(2-methoxyethyl)thiourea (**10**): 9.5 g (88% yield); IR ($CHCl_3$) 3290, 1557, 1450, 1342, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.00 (m, 10 H, CH_2), 3.20 (s, 3 H, OCH_3), 3.38 (br s, 4 H, CH_2CH_2), 3.84 (m, 1 H, OH), 6.58 (br m, 2 H, NH exchangeable); MS, *m/e* (relative intensity) 216.1294 (100.00, M^+; $C_{10}H_{20}N_2OS$, 216.13007); ^{13}C NMR ($CDCl_3$) 24.64 (C_3', C_5'), 25.56 (C_4'), 31.82 (C_2', C_6'), 44.61 (C_1), 53.12 (C_1'), 58.79 (OCH_3), 72.26 (C_2), 181.49 (C=S).

Nitrosation of **10** according to the above procedure gave **21**: 65% yield; an oil; IR ($CHCl_3$) ν_{max} 3276 (NH), 1547, 1450, 1342,

(49) Sullivan, R. H.; Price, E. *Org. Magn. Reson.* **1975**, *7*, 143.
(50) Hecht, O. *Chem. Ber.* **1890**, *23*, 285.
(51) Lewis, W. W., Leipest, D. U.S. Patent 3 168 560, 1965.

1257, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–2.20 (m, 10 H, CH_2), 3.35 (s, 3 H, OCH_3), 3.50 (t, 2 H, OCH_2), 4.50 (t, 2 H, CH_2), 4.50 (br m, 1 H, H_1', axial), 8.50 (br m, 1 H, NH exch); MS, *m/e* (relative intensity) 245.1196 (2.14, M^+; calcd 245.1198), 215.1215 (66.09; calcd for $C_{10}H_{19}N_2OS$ 215.1218), 104.0585 (0.69; calcd for $C_3H_8N_2O_2$, 104.0586), 98.0971 (100.0; calcd for $C_6H_{12}N$, 98.0972).

3-Cyclohexyl-1-(2-fluoroethyl)-1-nitrosothiourea (22). Nitrosation of 1-cyclohexyl-3-(2-fluoroethyl)thiourea gave **22**: 60% yield; mp 51–52 °C; IR ($CHCl_3$) ν_{max} 3375 (NH), 1508, 1445, 1375, 1160, 1005 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20–2.20 (m, 10 H, CH_2), 3.45 (m, 1 H, H_1', axial), 3.52 (dt, 2 H, CH_2CH_2F, $^1J_{H-F}$ = 47.0 Hz, $^2J_{H-H}$ = 5.0 Hz), 3.65 (dt, 2 H, CH_2CH_2F, $^2J_{H-H}$ = 20.0 Hz, $^2J_{H-H}$ = 5.0 Hz), 9.50 (br m, 1 H, NH, exch); MS, *m/e* (relative intensity) 233.0998 (1.04, M^+; calcd 233.0998), 203.1018 (10.0; calcd for $C_9H_{16}N_2FS$, 203.1018), 98.0968 (100.00; calcd for $C_6H_{12}N$, 98.0970), 92.0390 (0.11; calcd for $C_2H_5N_2OF$, 92.0386).

3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosothiourea (23). A solution of cyclohexyl isothiocyanate (25.0 g, 200 mmol) in ether (500 mL) was added dropwise to a stirred suspension of 2-aminoethanol (12.2 g, 200 mmol), and the reaction mixture was stirred for 12 h at room temperature. The precipitate which formed was collected and recrystallized from ethanol to afford white crystals of 3-cyclohexyl-1-(2-hydroxyethyl)thiourea (**12**): 34.0 g (85% yield); mp 120–121 °C; IR (KBr) 3300–3250 (br m, NH, OH), 1565, 1320, 1200, 1042 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12–2.20 (m, 10 H, CH_2), 3.30 (m, 2 H, NCH_2), 3.50 (m, 2 H, CH_2OH), 3.65 (br, m, 1 H, CH), 4.50 (br m, 1 H, OH exch), 5.45 (d, 1 H, NH exch), 5.85 (t, 1 H, NH exch); MS, *m/e* (relative intensity) 202.1138 (100.00, M^+; calcd for $C_9H_{18}N_2OS$, 202.1140); ^{13}C NMR (Me_2SO-d_6) δ 24.39 (C_3', C_5'), 25.18 (C_4'), 32.29 (C_2, C_6), 46.1 (C_1), 51.67 (C_1'), 59.75 (C_2'), 181.42 (C=S).

Nitrosation of **12** gave **23**: 65% yield; mp 55–57 °C; IR ($CHCl_3$) ν_{max} 3371 (NH, OH), 1509, 1451, 1406, 1364, 1150, 1019 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12–2.20 (m, 10 H, CH_2), 3.72 (t, 2 H, CH_2), 4.40 (m, 1 H, H_1', axial), 8.56 (br m, 1 H, NH, exch), 9.40 (br m, 0.5 H, OH, exch), 10.10 (br m, 0.5 H, OH, exch); MS, *m/e* (relative intensity) 231.1010 (1.82, M^+; calcd 231.1041), 230.0961 (11.97; calcd for $C_9H_{16}N_3O_2S$, 230.0962), 201.1060 (42.82; calcd for $C_6H_{16}N_2OS$, 201.1062), 89.0175 (3.45; calcd for $C_2H_5N_2S$, 89.0162), 98.0970 (100.0; calcd for $C_6H_{12}N$, 98.0970).

1,3-Dimethyl-1-(nitroso-^{15}N)thiourea-^{13}C (30). To a solution of $^{13}CS_2$ (500.0 mg, 6.5 mmol) and methylamine hydrochloride (935 mg, 14 mmol) in water (50 mL) was added a solution of sodium hydroxide (60 mg, 15 mmol) in water (15 mL) dropwise, and the reaction mixture was stirred at room temperature for 4 h and refluxed for 12 h. The solvent was removed under reduced pressure, the residue was extracted with chloroform, the extract was dried (Na_2SO_4), and then the solvent was removed to afford 1,3-dimethylthiourea-^{13}C (**39**), 400 mg (54% yield). The above thiourea (350 mg, 3.5 mmol) was nitrosated with $Na^{15}NO_2$ (300 mg, 4.5 mmol) and 50 mL of 0.1 N HCl at −10 °C in CH_2Cl_2 (50 mL) in the usual manner to afford 320 mg (68%) of 1,3-dimethyl-1-nitrosothiourea (**42**): MS, *m/e* (relative intensity) 135.0313 (58.32; calcd for $C_2{}^{13}CH_7N_2{}^{15}NOS$, 135.0314), 104.0365 (100.00; calcd for $C_2{}^{13}CH_7N_2S$, 104.0364), 72.0648 (21.22; calcd for $C_2{}^{13}CH_7N_2$, 72.0643).

Formation of Dimethylurea from 1,3-Dimethyl-1-nitrosothiourea (13). Dry HCl gas was passed through the solution of (**13**) (2.66 mg, 2 mmol) in ether (20 mL) for 30 min. After a few minutes a white crystalline solid separated. The ether and excess HCl gas were removed under reduced pressure, and the residue was dissolved in water and basified with dilute NaOH solution. The solution was extracted with chloroform and dried (Na_2SO_4), the solvent was removed, and the residue was triturated with petroleum ether to afford 100 mg (62% yield) of dimethylurea, mp 102 °C (lit.[5] mp 101–102 °C). The IR spectrum was superimposable with that of an authentic sample of dimethylurea.

Formation of 1,3-Dimethyl-1-nitrosourea from 1,3-Dimethylthiourea. Dry sodium nitrite (3.45 g, 50 mmol) was added in portions to a cooled solution of 1,3-dimethylthiourea (1.33 g, 10 mmol) in formic acid (20 mL). The reaction mixture was stirred for 2 h at 0 °C, water (20 mL) was added slowly, and the mixture was stirred for another 30 min. The solution was extracted with dichloromethane, washed with water, dried (Na_2SO_4), and concentrated. The residue was crystallized from ether/petroleum ether to afford 800 mg (67%) of 1,3-dimethyl-1-nitrosourea, mp 96–97 °C (lit.[5] mp 96–97 °C). The spectral properties were identical with those of an authentic sample.

Note: All nitrosothioureas should be handled with extreme care owing to their potential mutagenicity.

Acknowledgment. This investigation was supported by Grant 1-R01-CA20488-01 awarded by the National Cancer Institute, DHEW, and by grants from the National Foundation for Cancer Research and the Alberta Provincial Cancer Hospitals Board (to J.W.L.). S.M.S.C. acknowledges the award of an Alberta Heritage Foundation for Medical Research Post-Doctoral Fellowship. We thank Dr. Tom Nakashima and Mr. Glen Bigam and their associates for extensive NMR measurements.

Registry No. **2**, 534-13-4; **3**, 105-55-5; **4**, 26536-60-7; **5**, 1119-13-7; **6**, 84081-65-2; **7**, 13120-04-2; **8**, 32900-12-2; **9**, 70498-34-9; **10**, 84081-66-3; **11**, 33024-70-3; **12**, 81467-07-4; **13**, 79645-01-5; **14**, 79645-03-7; **15**, 84050-92-0; **16**, 84050-93-1; **17**, 84050-94-2; **18**, 79645-02-6; **19**, 79645-04-8; **20**, 84050-95-3; **21**, 84050-96-4; **22**, 79645-05-9; **23**, 84056-92-8; **24** (R = R_1 = CH_2CH_3), 84081-61-8; **25** (R = R_1 = CH_2CH_3), 623-76-7; **28**, 84081-67-4; **30**, 84081-63-0; $^{13}CS_2$, 30860-31-2; O=NONa, 7632-00-0; NOCl, 2696-92-6; HON=O, 7782-77-6; HNO_3, 7697-37-2; N_2O_3, 10544-73-7; H_2O^+–NO, 80094-82-2; ^+NO, 14452-93-8; fluoroethylamine, 406-34-8; methylamine, 74-89-5; ethylamine, 75-04-7; propylamine, 107-10-8; 2-methoxyethylamine, 109-85-3; 2-aminoethanol, 141-43-5; 2-fluoroethyl isothiocyanate, 84081-64-1; cyclohexyl isothiocyanate, 1122-82-3; methylamine hydrochloride, 593-51-1.