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GLYCO-*S*-NITROSOTHIOLS, A NOVEL CLASS OF NO DONOR COMPOUNDS

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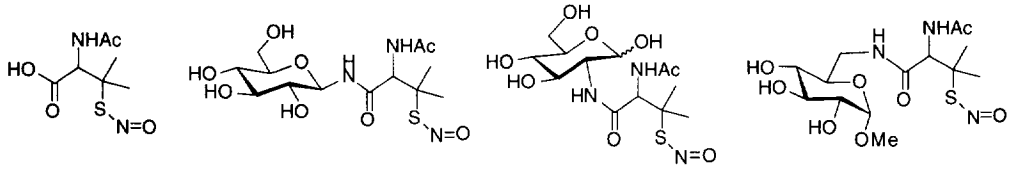
Abstract: Three novel glyco-*S*-nitrosothiol NO donor compounds **1**, **2**, and **3** have been synthesized. These compounds outperform *S*-nitroso-*N*-acetylpenicillamine (SNAP) in aqueous solubility and stability, with and without EDTA and in the presence or absence of Cu^{2+}. Copyright © 1996 Elsevier Science Ltd

Due to its multiple physiological functions,[1] nitric oxide (NO), a simple gas with free radical chemical properties synthesized in vivo from arginine by nitric oxide synthase (NOS),[2] has been intensively studied. NO is a mediator in blood vessel relaxation[3] and a messenger whereby macrophages exert their tumoricidal and bactericidal effects.[4] NO (or a close derivative) has been identified as the endothelial-derived relaxing factor (EDRF), which is responsible for diffusing to adjacent smooth muscle cells and eliciting relaxation.[5] NO also acts as a messenger in the brain, where it has been detected in cerebellar neuronal cultures,[6] and in brain extracts and slices.[7] To capitalize on these crucial roles, a series of NOS inhibitors and NO donors have been developed. NO donors have great potential as providers of NO in the treatment of an array of circulatory diseases, such as ischmia-reperfusion of the heart and splanchnic organs,[8] hypertension,[9] atherosclerosis,[10] and congestive heart failure[11] in which NO levels have declined to subphysiological levels.[12]

S-nitrosothiols, the most common type of NO donor currently available, have been shown to either undergo homolytically decomposition in solution to give the disulfide and NO,[13] or heterolytic cleavage resulting in transnitrosation reactions in which a nitrosonium group (NO^+) is transferred.[14] These compounds therefore are promising therapeutic agents. The issue to be addressed now is how to design NO-releasing *S*-nitrosothiols with better pharmacokinetic properties and specificities. Cardioglycosides, such as digitalis a class of natural compounds used in the treatment of heart disease,[15] provide an attractive model to mimic. These compounds are formed by an aglycone unit conjugated with one to four molecules of sugar. The aglycone moiety provides the pharmacological activity, whereas the carbohydrate unit enhances water solubility, cell penetration, drug-receptor interaction and influences the dose-response relationships. Inspired by this natural design, this work presents the synthesis of the first series of glyco-*S*-nitrosothiols (**1**, **2**, and **3**), where a *S*-nitroso-*N*-acetylpenicillamine moiety acts as an NO-donor and the sugar fragment provides the desired hydrophilicity and specificity due to intra/intercellular carbohydrate-protein recognition processes.[16] The stabilities of these *S*-nitrosothiols are compared to that of SNAP[17] in a variety of conditions. Future work will feature the synthesis of polydigitoxose and sialyl Lewis X-containing *S*-nitrosothiols. The latter should specifically target activated endothelial cells.[18]

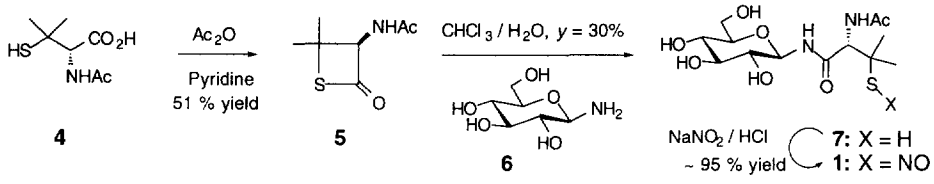
Glyco-*S*-nitrosothiols



SNAP **1, Glyco-SNAP-1** **2, Glyco-SNAP-2** **3, Glyco-SNAP-3**

Synthesis: The cyclic 3-acetamido-4,4-dimethylthietan-2-one *N*-acetyl penicillamine **5** was synthesized by treatment of *N*-acetyl penicillamine **4** with acetic anhydride and pyridine, followed by recrystallization from ethanol.[19] 1-Amino glycoside **6** was synthesized by reacting glucose with a concentrated aqueous ammonia solution.[20] Coupling of **5** with the aminated sugars (Scheme 1) was conveniently carried out in either a chloroform/water two-phase reaction system or in pyridine.[19] *S*-nitrosation of the resulting conjugate[17] afforded the desired glyco-*S*-nitrosothiol **1**. Compounds **2** and **3** were synthesized following similar procedures. This synthetic route is reliable and can be readily scaled up to support extensive biomedical use.

Scheme 1. Synthesis of glyco-*S*-nitrosothiol **1**



4 **5** **6**

Stability: It is well known that water soluble *S*-nitrosothiols decompose spontaneously in aqueous solution. Kinetic studies revealed that the decomposition of SNAP was first order with respect to the substrate concentration. In this study, all experiments were done in a phosphate buffer at pH 7.4, and room temperature. UV absorbance at 339 nm (tertiary thionitrite) was monitored as a function of time. All half-life values were calculated assuming pseudo first-order kinetics. UV-Visible spectrophotometric experiments show that the glyco-*S*-nitrosothiols are more stable than SNAP in aqueous solution in the absence or presence of EDTA (λ = 339 nm, Figure 1). It was determined that whereas the half-life of SNAP in phosphate buffer without EDTA is around 10 h, the half-lives of all three new donors are nearly 30 h (Table 1). SNAP has been reported to have a half-life ($t_{1/2}$) value of 5 h at pH 7 and 37 °C.[21] It is important to note that these stability experiments are susceptible to many factors such as temperature, aeration, buffer preparation, the presence of metal impurities and light. The half-life data for SNAP obtained in our experiments are reasonably larger than what has been reported elsewhere, which can be attributed to the room temperature conditions used.

Table 1. Half-lives ($t_{1/2}$) of **SNAP**, **1**, **2**, and **3**

Compound	SNAP	1	2	3
Without EDTA	10.3	30.2	27.4	28.2
With EDTA	15.9	28.5	27.5	30.2

These glyco-*S*-nitrosothiols are extremely soluble in water. In 50 mM sodium phosphate buffer at room temperature, the solubility of the parent SNAP is about 2 mg/mL, whereas those of **1**, **2**, and **3** were over 1g/mL. The hydrophilicity of the glucose moiety is responsible for this remarkable solubility.

Effect of Cu^{2+}: The catalytic effect of Cu^{2+} ions on the NO-release of *S*-nitrosothiols has been studied.[13] Williams and co-workers recently found that the actual species in the Cu^{2+}-catalyzed decomposition of *S*-nitrosothiols is Cu^{+}, which is generated from the reduction of Cu^{2+} by thiols.[22] This Cu^{2+}-promoted decomposition can be halted by EDTA. In this work we found that the rate of decomposition of SNAP was drastically enhanced in the presence of Cu^{2+} (50 μM), while this enhancement is attenuated for glyco-*S*-nitrosothiols, especially in the case of **3** (Figure 2).

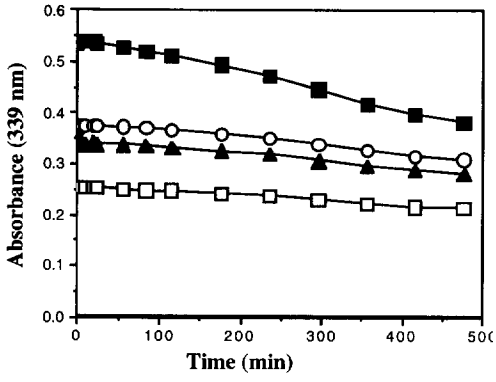


Figure 1. Stability of 0.40 mM solutions of **SNAP** (■), **1** (○), **2** (▲), and **3** (❑) in sodium phosphate buffer (50 mM, pH 7.40) with 1 mM EDTA.

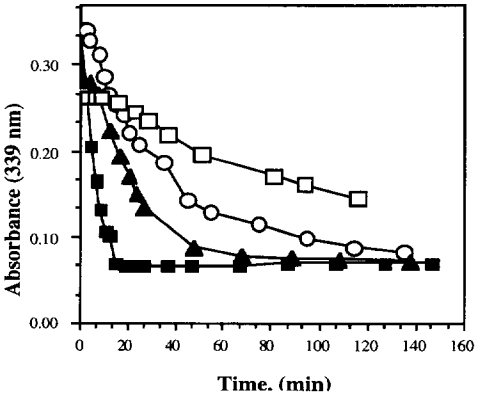


Figure 2. Effect of Cu^{2+} ion (50 μM) on the decomposition rates of 0.4 mM solutions of **1** (○), **2** (▲), **3** (❑), and **SNAP** (■) in 1 mM EDTA and 50 mM sodium phosphate buffer (pH 7.4), at room temperature.

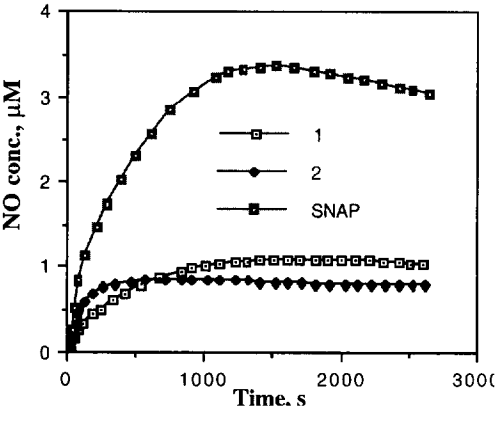


Figure 3. NO generation in 0.3 mM solutions of **SNAP**, **1**, and **2** in a phosphate buffer (pH 7.4).

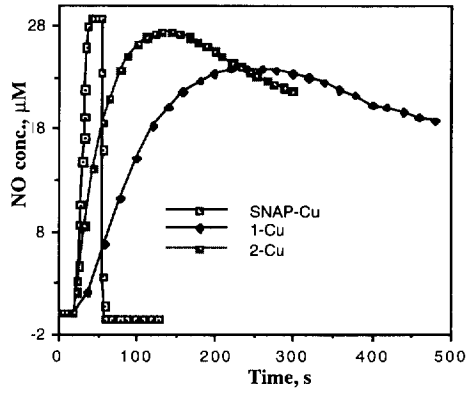


Figure 4. NO generation in 0.3 mM solutions of **SNAP**, **1**, and **2** in the presence of Cu^{2+} (50 μM) and a phosphate buffer (pH 7.4)

Nitric Oxide Measurements: A series of experiments to verify and quantify the NO concentration generated by 0.3 mM aqueous solutions of SNAP, **1** and **2** in a sodium phosphate buffer (pH 7.4) with and without the addition of Cu^{2+} (50 μM) were carried out using a NO-specific electrochemical analyzer. The results indicated that in the absence of Cu^{2+}, **1** and **2** generated around 30% of the NO concentration that equimolar amounts of SNAP (Figure 3). The experiment, run for 45 min. clearly indicates that these two glyco *S*-nitroso thiols are more stable than SNAP under these conditions. At the end of the experiment compounds **1** and **2** continued to deliver NO at a constant rate while the NO emission of SNAP was already decreasing. In the presence of Cu^{2+}, glyco-*S*-nitrosothiols **1** and **2** generated NO for a much longer time than SNAP (Figure 4).

Experimental

General. All unspecified reagents were the purest commercially available. The UV-Vis spectra were recorded in Hewlett Packard 8452A Diode Array Spectrophotometer. The ^{1}H NMR spectra were obtained with a 400 MHz Varian VRX400 instrument. NO measurements were carried out in an Electrochemical ISO-NO Mark II Isolated Nitric Oxide Meter, using a Duo.18 Interface & Software to process the data, both manufactured by World Precision Instruments, Inc (Sarasota, Florida). The samples were injected into the sealed O_2-free (N_2 atmosphere) aqueous buffer solutions dissolved in small amounts of methanol. $CuCl_2 \cdot H_2O$ was used as the source of Cu^{2+}.

Synthesis of the aminated sugars: 1-Amino glucose **6** was obtained by treatment of glucose with a concentrated ammonia solution consisting of commercially available concentrated (15 M) aqueous ammonia solution and 1 equiv. of sodium bicarbonate) at 45 °C for 24 h. 2-Amino glucose was generated in situ from the neutralization of commercial glucosamine hydrochloride with sodium hydroxide. 6-Amino glucose was prepared following several functional group transformations, including tosylation of the 6-hydroxy group of glucose, formation of the azido derivative by treatment with sodium azide and finally hydrogenation. **1-amino glucose** (**5**): ^{1}H NMR (D_2O) δ 3.06 (t, 1H, J = 8.0 Hz), 3.20-3.48 (m), 3.58-3.61 (m), 3.77-3.82 (m), 3.99 (d, 1H, J = 8.4). **6-amino 1-methyl glucoside**: ^{1}H NMR (D_2O) δ 2.61 (d, 1H, J = 7.6 Hz), 2.64 (d, 1H, J = 8.0 Hz), 2.75 (s, 1H), 2.856 (d, 1H, J = 2.4 Hz), 2.89 (d, 1H, J = 2.4 Hz), 3.13-3.26 (m), 3.21 (s, 3H), 3.41-3.53 (m).

3-acetamido-4,4,-dimethylthietan-2-one, 5:[19] *N*-Acetyl-*D*-penicillamine (6.50 g, 3.75 mmol) was added to a solution of dry pyridine (40 mL) and acetic anhydride (20 mL). The reaction mixture was allowed to stir overnight. The solvent was evaporated to give an oily residue. Chloroform (300 mL) was added to the residue and then the solution was washed with 1 N HCl (100 mL), water (100 mL) and brine (100 mL). The solution was dried over anhydrous magnesium sulphate and after evaporation of the solvents, the yellowish residue obtained was washed with petroleum ether (30 mL × 3) and recrystallized from ethanol. White crystals of **5** (3.3 g) were collected with a yield of 51%. ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 3H), 1.97 (s, 3H), 2.16 (s, 3H), 5.79 (d, J = 8.0 Hz, 1H), 7.45 (br, 1H); MS (*m/e*) 174 (M+1).

Coupling of 5 to aminated sugars. Synthesis of compound 7: An aminated sugar (1.2 mmol) dissolved in distilled water (2 mL) was added to a solution of 3-acetamido-4,4,-dimethylthietan-2-one **5** (173 mg, 1 mmol) in chloroform (0.5 mL). The two-phase system was shaken for 2 h after which the aqueous phase was separated and washed twice with chloroform (2 mL) and evaporated until dryness. The solid was purified via column chromatography eluting with ethyl acetate-ethanol (5/1) to afford the desired free thiols (white powders). An alternate procedure involves dissolving **5** and the amino glucose in pyridine, stirring for several hours. [*N*-(β-D-Glucopyranosyl)-N^2-acetyl-*S*-nitroso-D,L-penicillamine amide] (**G1SH**) is then peracetylated to facilitate its purification (column eluting with hexane-ethyl acetate 1:1). Deprotection with methanol/sodium methoxide followed by treatment with acid resin (Dowex 50w) afforded the free pure thiol **7** with a yield of 30%. **G1SH** (**7**): ^{1}H NMR (CD_3OD) δ 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.96 (s, 3H, Ac), 3.15-3.32 (m, 4H, glc.), 3.50-3.58 (m, 2H, glc.), 3.74 (dd, 1H, J_1 = 2.0 Hz, J_2 = 12.0 Hz), 4.5 (s, 1H, CH adj. C=O). ^{13}C NMR (CD_3OD) δ 22.55 (CH_3, NAc), 29.90 (tert. C), 30.41 (CH_3), 30.65 (CH_3), 46.77 (CH adj. C=O), 62.85 (CH_2), 71.47 (CH, glc.), 74.03 (CH, glc.), 79.25 (CH, glc.), 79.87 (CH, glc.), 81.03 (CH, glc.), 172.25 (C=O, NAc), 172.96 (C=O, amide). MS (*m/e*) 352 (M^+). [*N*-(2-deoxy-α,β-D-Glucopyranose-2-)-N^2-acetyl-*S*-nitroso-D,L-penicillamine amide] (**G2SH**): ^{1}H NMR (CD_3OD) δ 1.29 (s, 3H), 1.38 (s, 3H), 1.94 (s, 3H), 3.50 (m, 2H), 3.61 (m, 3H), 3.71 (m, 4H), 4.48 (s, 1H), 5.03 (s, 1H); FABMS (*m/e*) 353 ($M+H^+$). [*N*-(6-deoxy-α,β-D-Glucopyranose-6-)-N^2-acetyl-*S*-nitroso-D,L-penicillamine amide] (**G6SH**): ^{1}H NMR (CD_3OD) δ 1.29 (s, 3H), 1.36 (s, 3H), 1.93 (s, 3H), 3.03

(m, 1H), 3.27 (m, 2H), 3.29 (s, 3H), 3.52 (m, 3H), 4.44 (s, 1H), 4.55 (d, J = 4.0 Hz, 1H); FABMS (*m/e*) 367 ($M+NH_4^+$).

General method for the nitrosation of glyco-N-acetyl-penicillamines. Syntheses of 1, 2, and 3: Glyco-*N*-acetyl-penicillamine (1 mmol) was dissolved in methanol (5 mL), and 1 M hydrochloric acid (2 mL). Then sodium nitrite (140 mg) in water (0.5 mL) was added dropwise. After stirring for 30 min, the green solution was pumped till dryness. Cold ethanol (2 mL) was added to precipitate the salt. After filtration and solvent removal the glyco-*S*-nitrosothiols were obtained (green powders) in almost quantitative yields (~95%). **1**: ^{1}H NMR (D_2O) δ 1.85 (s, 3H), 1.88 (s, 3H), 1.94 (s, 3H), 3.29 (m, 2H), 3.38 (m, 2H), 3.58 (dd, J = 12.4, 4.2 Hz, 1H), 3.72 (m, 1H), 4.81 (d, J = 9.2 Hz, 1H), 5.11 (s, 1H). ^{13}C NMR (CD_3OD) δ 22.37 (CH_3, Ac. group), 25.24 (CH_3), 27.80 (CH_3), 59.82 (CH adj. to SNO), 61.50 (CH adj. to C=O), 62.71 (CH_2 glc.), 71.42 (CH glc.), 73.90 (CH glc.), 79.18 (CH glc.), 79.75 (CH glc.), 81.07 (CH glc.), 171.37 (C=O), 172.99 (C=O). MS (*m/e*) 382 (M^+), 352 (M-SH). IR, ν_{max} (cm^{-1}) 671 (C-S vib.); 1022 (C-N vib.); 1411, 1449 (N=O str.); 1660 (C=O); 2833, 2941 (C-H, str.); 3330 (O-H, str.). UV, λ_{max} (nm) 342, 596. mp: dec. at ~70 °C. **2**: ^{1}H NMR (CD_3OD) δ 1.85 (s, 3H), 1.93 (s, 3H), 1.98 (s, 3H), 3.20 (m, 2H), 3.61 (m, 3H), 3.72 (m, 4H), 4.50 (m, 1H), 5.12 (m, 1H). ^{13}C NMR (CD_3OD) δ 22.41 (CH_3, Ac. group), 25.78 (CH_3), 27.48 (CH_3), 56.06 (CH adj. to SNO), 57.05 (CH adj. to C=O), 59.90 (CH_2 glc.), 62.72 (CH glc.), 72.97 (CH glc.), 75.84 (CH glc.), 77.88 (CH glc.), 92.31 (CH anomeric), 170.62 (C=O), 173.15 (C=O). IR, ν_{max} (cm^{-1}) 661 (C-S vib.); 1028 (C-N vib.); 1417, 1449 (N=O); 1660 (C=O); 2833, 2941 (C-H str.); 3341 (O-H str.). UV, λ_{max} (nm) 342, 596. FABMS (*m/e*) 382 (M^+). mp: dec. at ~70 °C. **3**: ^{1}H NMR (CD_3OD) δ 1.87 (s, 3H), 1.92 (s, 3H), 1.96 (s, 3H), 3.04 (m, 1H), 3.26 (s, 3H), 3.29 (m, 2H), 3.51 (M, 3H), 4.55 (d, J = 4.0 Hz, 1H), 5.22 (S, 1H).

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References and Notes

1. Williams, R. J. P. *Chem. Soc. Rev.* **1990**, 77. *Encyclopedia of Inorganic Chemistry*; King, R. B., Ed.; Wiley: N. Y., 1994; Vol. 5, p 2482. Feldman, P. L.; Griffith, O. W.; Stuehr, D. J. *C & EN*, Dec. 20 **1993**, 26. Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev.* **1993**, 233. Culotta, E.; Koshland, D. E. Jr. *Science* **1992**, Vol. 258, 1862. Schmidt, H. H. H. W.; Walter, U. *Cell* **1994**, Vol. 78, 919.

2. Palmer, R. M. J.; Ashton, D. S.; Moncada, S. *Nature (London)* **1988**, *333*, 664. Garthwaite, J. *Trends Neurosci.* **1991**, *14*, 60. Marletta, M. A. *J. Biol. Chem.* **1993**, *268*, 12231.

3. Furchgott, R. F.; Zawadzki, J. V. *Nature (London)* **1980**, *288*, 373.

4. Hibbs, J. B. Jr.; Taintor, R. R.; Vavrin, Z.; Rachlin, E. M. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 87. Hibbs, J. B. Jr.; Vavrin, Z.; Taintor, R. R. *J. Immunol.* **1987**, *38*, 550. Marletta, M. A.; Yoon, P. S.; Iyengar, R.; Leaf, C. D.; Wishnok, J. S. *Biochemistry* **1988**, *27*, 8706. Nathan, C. F.; Hibbs, J. B. Jr. *Curr. Opin. Immunol.* **1991**, *3*, 65. Stuehr, D. J.; Nathan, C. F.; *J. Exp. Med.* **1989**, *169*, 1543.

5. Snyder, S. H. *Science* **1992**, *257*, 494. Ignarro, L. J. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 535. Furchgott, R. F.; Vanhoutte, P. M. *FASEB J.* **1989**, *3*, 2007. Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109. Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature (London)* **1987**, *327*, 524. Ignarro, L. J.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9265. Furchgott, R. F. In *Mechanisms of Vasodilation*; Vanhoutte, P. M., Ed., Raven: N. Y., 1987; Vol IV.

6. Garthwaite, J.; Charles, S. L.; Chess-Williams, R. *Nature (London)* **1988**, *336*, 385.

7. Bredt, D. S.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9030.

8. Lefer, A. M.; Lefer, D. J. *Ann. Rev. Pharmacol. Toxicol.* **1993**, *33*, 71.

9. Luscher, T. F.; Raij, L.; Vanhoutte, P. M. *Hypertension* **1987**, *9*, 157.

10. Lefer, A. M.; Sedar, A. W. *Pharmacol. Res.* **1991**, *23*, 1.

11. Drexler, H.; Hayoz, D.; Munzel, T.; Hornig, B.; Just, H.; Brunner, H.; Zelis, R. *Am. J. Cardiol.* **1992**, *69*, 1596.

12. Lefer, A. *J. Cardio. Pharmac.* **1993**, *22*, S1.

13. Askew, S. C.; Barnett, D. J.; McAninly, J.; Williams, D. L. H. *J. Chem. Soc. Perk. Trans. 2* **1995**, 741. Barnett, D. J.; Rios, A.; Williams, D. L. H. *J. Chem. Soc. Perk. Trans. 2* **1995**, 1279.

14. Stamler, J. S. *Cell* **1994**, *78*, 931.

15. *Digitalis*; Fisch, C.; Surawicz, B., Eds. Grune & Stratton: N.Y., 1969.

16. Sharon, N.; Lis, H. *Scientific American* **1993**, 82.

17. Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. E. *J. Chem. Soc., Chem. Comm.* **1978**, 249

18. Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7390.

19. Moynihan, H. A.; Roberts, S. M. *J. Chem. Soc. Perk. Tr. 1* **1994**, 797.

20. Lubineau, A.; Augé, J.; Drouillat, B. *Carb. Res.* **1995**, 211. Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carb. Res.* **1986**, *146*, C1.

21. Roy, B.; d'Hardemare, A.-M.; Fontecave, M. *J. Org. Chem.* **1994**, *59*, 7019.

22. Williams, D. L. H. *J. Chem. Soc., Chem. Comm.* **1996**, 1085.

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