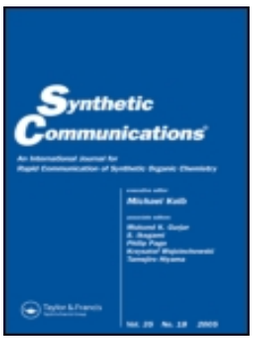


Taylor & Francis
Taylor & Francis Group

Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

An Efficient Synthesis of ^{15}N-Hydroxyurea

Genichiro Yasaki , Yueping Xu & S. Bruce King

To cite this article: Genichiro Yasaki , Yueping Xu & S. Bruce King (2000) An Efficient Synthesis of ^{15}N-Hydroxyurea, Synthetic Communications, 30:11, 2041-2047, DOI: 10.1080/00397910008087253

To link to this article: http://dx.doi.org/10.1080/00397910008087253

Published online: 04 Dec 2007.

SYNTHETIC COMMUNICATIONS, 30(11), 2041–2047 (2000)

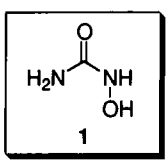
AN EFFICIENT SYNTHESIS OF ^{15}N-HYDROXYUREA

Genichiro Yasaki, Yueping Xu, and S. Bruce King*

Department of Chemistry, Wake Forest University
Winston-Salem, NC 27109
336-758-5774/kingsb@wfu.edu

Abstract: Treatment of trimethylsilyl isocyanate with ^{15}N-hydroxylamine hydrochloride produces ^{15}N-hydroxyurea, a valuable pharmacological tool, in an efficient, one-pot procedure in 74% yield. Recrystallization of the crude product yields analytically pure material in 47% overall yield.

Hydroxyurea (1) possesses a diverse pharmacology and long therapeutic history in humans. Hydroxyurea has recently been used as an effective treatment for sickle cell anemia based on its ability to stimulate the production of fetal hemoglobin.[1] Hydroxyurea also displays activity against various tumors and has been found effective for the treatment of a number of cancers.[2] These biological effects appear to arise mainly from the ability of hydroxyurea to inhibit ribonucleotide reductase, the enzyme responsible for the conversion of ribonucleotides to the deoxyribonucleotides required for DNA synthesis.[2] This



1

*To whom correspondence should be addressed.

inhibition of DNA synthesis provided the basis for studies that demonstrated the inhibition of human immunodeficiency virus (HIV) replication by hydroxyurea, making hydroxyurea a possible candidate for AIDS therapy.[3]

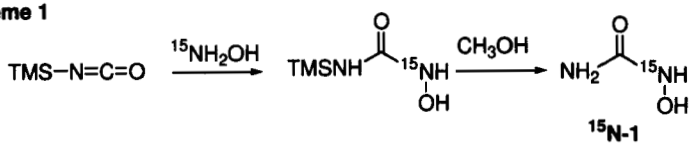
While the precise molecular mechanisms for the biological actions of hydroxyurea are not fully understood, evidence implicating the biologically important messenger molecule nitric oxide (NO) in a portion of the activities of this drug is accumulating. Chemically, the treatment of hydroxyurea with hydrogen peroxide and copper (II) sulfate produces an "NO-like" species capable of N-nitrosating morpholine.[4] Both hydroxyurea and nitric oxide inhibit ribonucleotide reductase and quench the catalytically essential tyrosyl radical of the M2 sub-unit of this enzyme.[5] In the presence of hydrogen peroxide, copper-zinc superoxide dismutase catalyzes the production of nitric oxide from hydroxyurea.[6] Hydroxyurea reacts with oxyhemoglobin (both normal and sickle cell) to produce methemoglobin, a methemoglobin-hydroxyurea complex, nitrosyl hemoglobin (Fe^{+2}-NO) and a nitroxyl radical ($NH_2CONHO^{\cdot}$).[7] Further evidence indicates that this nitroxyl radical ($NH_2CONHO^{\cdot}$) decomposes to both nitric oxide (NO) and nitroxyl (HNO), the one-electron reduced from of nitric oxide.[8] The formation of nitrosyl hemoglobin from this reaction may be of physiological importance to the sickle cell patient as nitrosylated hemoglobins play an important role in blood pressure control.[9] Both in vitro and in vivo electron paramagnetic resonance (EPR) experiments using ^{15}N-hydroxyurea have successfully demonstrated that the NO group of the nitrosyl hemoglobin formed in these

reactions derives from the NHOH group of hydroxyurea.[8a,10] These results indicate that ^{15}N-hydroxyurea represents a valuable biochemical tool for determining the source of biologically relevant nitrogen monoxides from hydroxyurea. We wish to report a simple and efficient synthesis of ^{15}N-hydroxyurea from commercially available starting materials.

Treatment of a toluene solution of trimethylsilyl (TMS) isocyanate (9.55 mmol, Aldrich) with a slight excess of a methanolic solution of ^{15}N-hydroxylamine, prepared by free-basing the commercially available hydrochloride salt (Cambridge), produced white crystals of ^{15}N-hydroxyurea in 74% yield (Scheme 1). This synthetic route directly derives from a previous synthesis of N-hydroxyurea by the addition of O-TMS hydroxylamine to TMS-isocyanate followed by silyl group hydrolysis.[11] The crude material was contaminated by a small amount (~2%) of ^{15}N-hydroxylamine hydrochloride as judged by ^{15}N-NMR spectroscopy (δ = 82.8 ppm for ^{15}N-hydroxylamine hydrochloride in deuterated dimethyl sulfoxide). Recrystallization of this crude solid from hot methanol yielded analytically pure ^{15}N-hydroxyurea (340 mg, 47% yield overall, 62% recrystallization yield) as determined by elemental analyses, ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy and high resolution chemical ionization mass spectrometry. In comparison, the previously reported yield for the synthesis of ^{15}N-hydroxyurea was 20% from the condensation of ^{15}N-hydroxyurea with potassium cyanate followed by recrystallization.[10] Removal of hydroxylamine from the crude hydrdroxyurea is important for any experiments involving heme proteins, as

hydroxylamine reacts faster with heme proteins than hydroxyurea to produce similar products.[12]

Scheme 1



In summary, an efficient and improved one-pot synthesis of analytically pure ^{15}N-hydroxyurea from commercially available materials has been described. In addition, previously unreported spectral data for this compound have been presented. Given the variety of available analytical techniques capable of distinguishing ^{14}N from ^{15}N and the current interest in hydroxyurea as a treatment for sickle cell disease, the further use of ^{15}N-hydroxyurea as a biochemical tool for studying the pharmacology of this fascinating molecule is anticipated.

Synthesis of ^{15}N-Hydroxyurea--A warm solution of potassium hydroxide (0.662 g, 11.8 mmol) in methanol (2.5 mL) was slowly added to a warm solution of ^{15}N hydroxylamine hydrochloride (Cambridge, 0.805 g, 11.5 mmol) in methanol (7.0 mL). The resulting solution was cooled (ice bath) to ensure the precipitation of the produced potassium chloride. This mixture was directly filtered into a flask containing a solution of trimethylsilyl isocyanate (Aldrich, 1.1 g, 9.55 mmol) in toluene (10 mL). White crystals formed at room temperature within one hour and the flask was transferred to a freezer. After 24 hrs, the crystals were collected by

filtration to give ^{15}N-hydroxyurea (0.55 g, 74% yield): mp 143-146 °C, (144-146 °C, Acros Catalog of Fine Chemicals); TLC R_f 0.13 (EtOAc, $FeCl_3$ stain positive); ^{1}H-NMR (200 MHz, d_6-DMSO) 8.81 (s, 1H), 8.26 (d, 1H, J = 89.5 Hz), 6.18 (s, 2H); ^{13}C-NMR (50 MHz, D_2O, CH_3OH internal reference) 164.65 (d, J = 15.7 Hz), (d_6-DMSO) 162.81 (d, J = 13.0 Hz); ^{15}N-NMR (30 MHz, d_6-DMSO) 143.00 (J = 91.2 Hz for proton coupled spectra), HRMS (CI, isobutane reagent gas) m/z 78.0314 calculated for $CH_5O_2{}^{15}N^{14}N$ 78.0321 ($M + H^+$); Anal. Calcd. For $CH_4{}^{15}N^{14}NO_2$: C, 15.59; H, 5.23; N, 36.36. Found: C, 15.65; H, 5.25; N, 36.64.

Acknowledgement: This work was supported by grants (9630310N, SBK) from the American Heart Association and the American Chemical Society Petroleum Research Fund (Type G, SBK). Mass spectrometry was performed by the Nebraska Center for Mass Spectrometry. The authors wish to thank Professor Eric R. Johnston of the University of North Carolina at Greensboro and Mr. Roy W. Ware, Jr. (Wake Forest University) for performing ^{15}N NMR spectroscopy.

References:

1. Charache, S.; Terrin, M.L.; Moore, R.D. *N. Engl. J. Med.* **1995**, *332*, 1037-45.

2. Yarbro, J.W. *Semin Oncol.* **1992**, *19*, 1-10.

3. Lori, F.; Malykh, A.; Cara, .A.; Sun, D.; Weinstein, J.N.; Lisziewicz, J.; Gallo, R.C. *Science* **1994**, *266*, 801-805.

4. Kwon, N.S.; Stuehr, D.J.; Nathan, C. *J. Exp. Med.* **1991**, *174*, 761-767.

5. (a) Lepoiver, M.; Flaman, J.M; Bobe, P.; Lemaire, G.; Henry, Y. *J. Biol. Chem.* **1994**, *269*, 21891-21897. (b) Liermann, B.; Lassmann, G.; Langen, P. *Free Radical Biology and Medicine* **1990**, *9*, 1-4.

6. Sato, K.; Akaike, T.; Sawa, T.; Miyamoto, Y.; Suga, M.; Ando, M.; Maeda, H. *Jpn. J. Cancer Res.* **1997**, *88*, 1199-1204.

7. (a) Kim-Shapiro, D.B.; King, S.B.; Bonifant, C.L.; Kolibash, C.P.; Ballas, S.K. *Biochimica et Biophysica Acta* **1998**, *1380*, 64-74. (b) Stolze, K.; Nohl, H. *Biochem. Pharmacol.* **1990**, *40*, 799-802.

8. (a) Xu, Y.; Mull, C.D.; Bonifant, C.L.; Yasaki, G.; Palmer, E.C.; Shields, H.; Ballas, S.K.; Kim-Shapiro, D.B.; King, S.B. *J. Org. Chem.* **1998**, *63*, 6452-6453. (b) Pacelli, R.; Taira, J.; Cook, J.A.; Wink, D.A.; Krishna, M.C. *The Lancet* **1996**, *347*, 900.

9. (a) Gow, A.J.; Stamler, J.S. *Nature* **1998**, *391*, 169-173. (b) Stamler, J.S.;

Jia, L.; Eu, J.P.; McMahon, T.J.; Demchenko, I.T.; Bonaventura, J.; Gernert, K.; Piantadosi, C.A. *Science* **1997**, *276*, 2034-2037.

10. Jiang, J.; Jordan, S.J.; Barr, D.P.; Gunther, M.R.; Maeda, H.; Mason, R.P. *Molecular Pharmacology* **1997**, *52*, 1081-1086.

11. Kozyukov, V.P.; Muzovskaya, E.V.; Mironov, V.F. *J. Gen. Chem. USSR (Engl. Transl.)* **1985**, *55*, 947-953

12. Stolze, K.; Nohl, H. *Biochem.. Pharmacol.* **1995**, *49*, 1261-1267.

Received in the USA 8/19/99