ORGANIC LETTERS

2006
Vol. 8, No. 18
4035−4038

N^G-Hydroxyguanidines from Primary Amines

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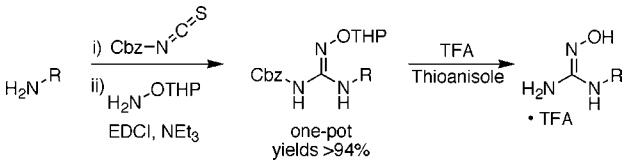
Received June 13, 2006

ABSTRACT



A concise and general method for the preparation of N^G-hydroxyguanidines from primary amines is reported. Using available and readily prepared materials, primary amines are converted to protected N^G-hydroxyguanidines in a one-pot procedure followed by deprotection under nonreducing conditions. The method has been successfully applied to a number of examples including a high-yielding preparation of N^G-hydroxy-L-arginine, the intermediate in the enzymatic conversion of L-arginine to nitric oxide and L-citrulline by nitric oxide synthase.

N^G-Hydroxyguanidines[1] have been of medicinal interest for some time having been shown to exhibit cytotoxicity in human leukemic cells and antitumor activity in vivo.[2,3] Recently, these compounds have also received attention as protective agents in ischemia-reperfusion,[4] as potential vasodilators, and for use in treatment of diseases such as pulmonary and cardiovascular disorders, hypoxia, sexual dysfunction, and even memory loss.[5] Our interest in this class of molecules lies in the intermediacy of N^G-hydroxy-L-arginine in the conversion of L-arginine to nitric oxide and L-citrulline via the action of nitric oxide synthase (Figure 1).[6]

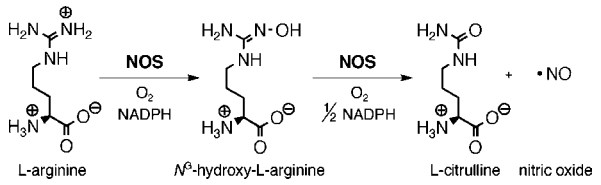


Figure 1. Two-step NOS reaction.

As part of our efforts to better characterize the mechanistic details of this process (more specifically, the enzymatic "second step") we recently undertook the synthesis of fluorinated analogues of L-arginine and N^G-hydroxy-L-arginine to serve as mechanistic probes (Figure 2). The

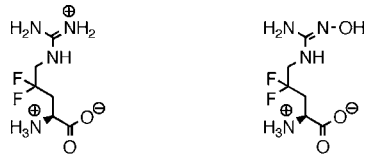


Figure 2. NOS substrate analogues 4,4-difluoro-L-arginine (**1**) and 4,4-difluoro-N^G-hydroxy-L-arginine (**2**).

synthesis of the difluorinated L-arginine analogue (**1**) was straightforward and based largely upon the work of Kim and

(1) For comprehensive reviews on the chemistry and biology of guanidines, N^G-hydroxyguanidines, and other NO donors, see: (a) Katritzky, A. R.; Rogovoy, B. V. *ARKIVOC* **2005**, *4*, 49. (b) Wang, P. G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk, A. J. *Chem. Rev.* **2002**, *102*, 1091.

(2) Everett, S. A.; Smith, K. A.; Patel, K. B.; Dennis, M. F.; Stratford, M. R. L.; Wardman, P. *Br. J. Cancer* **1996**, *74*, S172.

(3) Chern, J. W.; Leu, Y. L.; Wang, S. S.; Lou, R.; Lee, C. F.; Tsou, P. C.; Hsu, S. C.; Liaw, Y. C.; Lin, H. W. *J. Med. Chem.* **1997**, *40*, 2276.

(4) Dambrova, M.; Baumane, L.; Kiuru, A.; Kalvinsh, I.; Wikberg, J. E. S. *Arch. Biochem. Biophys.* **2000**, *377*, 101.

(5) Nitromed Inc, WO Patent 99/62509, 1999.

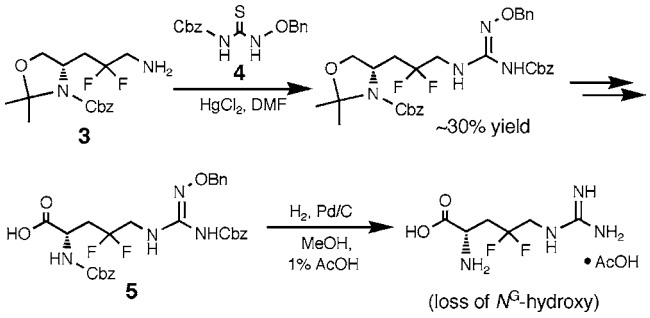
(6) Tayeh, M. A.; Marletta, M. A. *J. Biol. Chem.* **1989**, *264*, 19654.

10.1021/ol061454p CCC: $33.50
Published on Web 07/29/2006

co-workers.[7] The preparation of the difluorinated N^G-hydroxy-L-arginine analogue (**2**), however, was more challenging.

As a first attempt, the protected 4,4-difluoro-N^G-hydroxy-L-arginine species (**5**) was prepared in modest yield starting from Kim's precursor difluoroamine **3**[7] and employing the thiourea reagent (**4**) based method of Jirgensons and co-workers.[8] Disappointingly, the final deprotection step (under a variety of conditions) following this route led to complete loss of the N^G-hydroxyguanidine functionality by over-reduction (Scheme 1).[9]

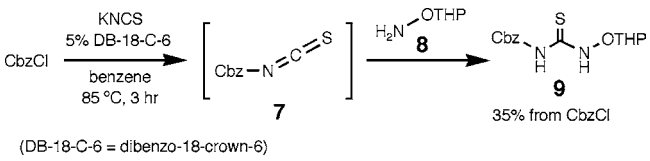
Scheme 1



The next approach toward **2** involved treatment of compound **3** with cyanogen bromide. The reaction of amines with cyanogen bromide can afford cyanamides in varying yields.[10] Cyanamides, while often unstable,[11] can be treated with amines to yield guanidines or with hydroxylamine to provide N^G-hydroxyguanidines.[12] Indeed this process has been used most often in the synthesis of N^G-hydroxy-L-arginine.[10] Treatment of **3** with cyanogen bromide, however, failed to yield the desired difluorocyanamide resulting instead in a complex mixture.

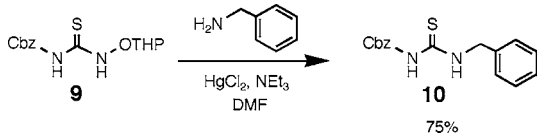
The failure of established methods to provide a route to the desired N^G-hydroxyguanidine prompted us to investigate alternate approaches. We reasoned that by using an acid-labile protecting group for the *N*-hydroxy moiety it might still be possible to make use of a thiourea-based reagent approach. To this end, the *N*-Cbz/OTHP protected N^G-hydroxythiourea **9** was prepared by reaction of Cbz-NCS (**7**) (formed in situ) and O-THP-protected hydroxylamine[13] (**8**) following the procedure of Jirgensons and co-workers (Scheme 2).[8]

Scheme 2



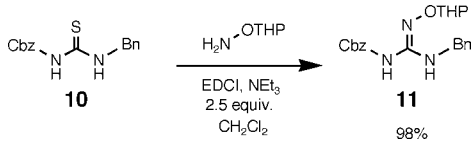
Next, as a model, benzylamine was treated with thiourea **9** along with $HgCl_2$ and triethylamine (Scheme 3). Surprisingly, this process yielded the exchanged thiourea product **10** in 75% yield with only trace amounts of the expected hydroxyguanidine product. Similar results were also obtained when using EDCI as an activating agent.[14] These observations suggest that O-protected N^G-hydroxythioureas are prone to a competitive exchange of the hydroxylamine moiety in the presence of another amine to yield a more stable thiourea. This may also explain the modest yields (34−67%) reported by Jirgensons and co-workers in their preparation of protected N^G-hydroxguanidines.[8]

Scheme 3



It was then reasoned that under forcing conditions (i.e., excess amounts of activating agent and O-protected hydroxylamine) it might be possible to generate the N^G-hydroxyguanidine moiety from a protected thiourea. Compound **10** was thus treated with EDCI, NEt_3, and THP-ONH_2. After 30 min, a new, more polar species was detected by TLC. Additional 0.5 equiv of EDCI, THP-ONH_2, and NEt_3, administered at 30-minute intervals, led to complete consumption of the thiourea after 1.5 h (2.5 total equivalents of activator, protected hydroxylamine, and amine base). Following workup and chromatography, the protected N^G-hydroxyguanidine **11** was obtained in 98% yield (Scheme 4).

Scheme 4



We next set out to optimize the preparation of the carbamoyl isothiocyanate required for production of the

(7) Kim, K. S.; Qian, L. *Tetrahedron Lett*. **1993**, *34*, 7195.

(8) Jirgensons, A.; Kums, I.; Kauss, V.; Kalvins, I. *Synth. Commun*. **1997**, *27*, 315.

(9) The published work[8] upon which this approach was based does not describe any conditions by which the Cbz/OBn-protected species might be deprotected to yield the desired product.

(10) Pufahl, R. A.; Nanjappan, P. G.; Woodard, R. W.; Marletta, M. A. *Biochemistry* **1992**, *31*, 6822.

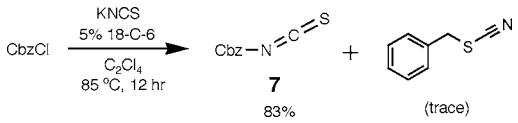
(11) Wagenaar, F. L.; Kerwin, J. F. *J. Org. Chem*. **1993**, *58*, 4331.

(12) Renodon-Corniere, A.; Dijols, S.; Perollier, C.; Lefevre-Groboillot, D.; Boucher, J.-L.; Attias, R.; Sari, M.-A.; Stuehr, D.; Mansuy, D. *J. Med. Chem*. **2002**, *45*, 944.

(13) Other O-protected hydroxylamines (MOM-ONH_2[13a] and ^{t}Bu-ONH_2[13b] were also prepared and could be used; however, THP−ONH_2 was deemed most convenient due to its ease of preparation[13c] and commercial availabilty. It should be noted that the use of O-THP-protected hydroxylamine does result in diastereomeric mixtures when a stereogenic centre is present in the starting material. (a) Ullrich, T.; Sulek, P.; Binder, D.; Pyerin, M. *Tetrahedron* **2000**, *56*, 3697. (b) Palandonken, H.; Bocian, C. M.; McCombs, M. R.; Nantz, M. H. *Tetrahedron Lett*. **2005**, *46*, 6667. (c) Haslanger, M. F.; Karanewsky, D. S. U.S. Patent 4 604 407, 1986.

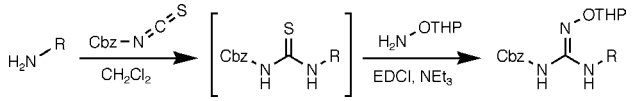
intermediate thioureas.[15] The ideal reagent in this case would be BocNCS allowing for a straightforward deprotection of the product N^G-hydroxyguanidine under mild acidic conditions. BocNCS,[16] however, is not readily prepared via standard procedures (numerous attempts at acylation of KNCS with Boc_2O or BocF[17] failed to yield BocNCS) and so we turned our attention to improving the preparation of CbzNCS. The reaction of benzyl chloroformate with KNCS has been previously described along with the problematic, competitive decarboxylation reaction to yield benzyl thiocyanate.[18] The thiocyanate anion is a bidentate nucleophile and the outcome of the acylation reaction is dependent upon solvent polarity. Analysis of the product distribution obtained using the benzene/crown ether conditions described by Jirgensons and co-workers for their in situ preparation of CbzNCS[8] revealed formation of the undesired benzyl thiocyanate in major proportion (ca. 2:1 relative to CbzNCS) also explaining our modest yield of compound **9** (Scheme 2). Other reports using ethyl acetate[18a] as solvent failed to give CbzNCS. A third preparation of CbzNCS has been described[18b] in which a solvent mixture of 20% acetonitrile/80% toluene was reported to yield the desired product. While this approach did show some selectivity for the formation of CbzNCS it was also difficult to reproduce from one attempt to the next and due to the lengthy reaction times (>5 days) was deemed undesirable. We therefore screened a number of solvents and solvent mixtures, with and without crown ether or phase transfer catalysts. This investigation revealed that the use of 5 mol % 18-crown-6 (relative to CbzCl) in tetrachloroethylene at 85 °C yields the desired CbzNCS product with only trace amounts (<5%) of benzyl thiocyanate (Scheme 5). Contrary to the reports of others[18a,b]

Scheme 5



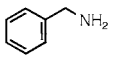
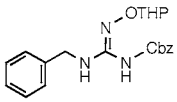
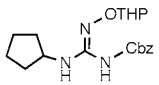
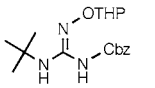
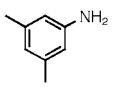
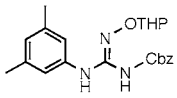
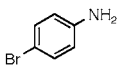
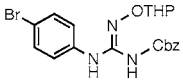
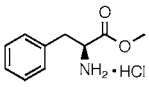
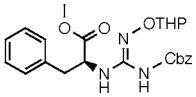
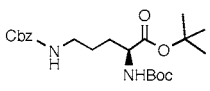
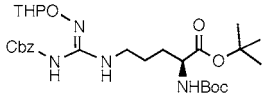
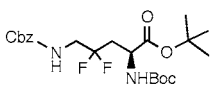
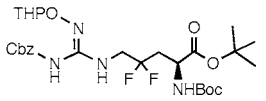
we found that CbzNCS is not stable to silica chromatography or purification by distillation resulting in both instances in decomposition to benzyl thiocyanate. Instead, the crude product mixture can be diluted with hexanes, filtered to remove salts, and after solvent removal, redissolved in dichloromethane (to a concentration of 0.5 M) to provide a stock solution of CbzNCS which can be stored at 4 °C with no appreciable decomposition.[19]

Scheme 6



The reaction of amines with carbamoyl isothiocyanates to yield thioureas is known to proceed rapidly and in high yield.[20] The direct activation of thioureas formed in situ, followed by treatment with an amine, has been recently applied to the one-pot synthesis of various substituted guanidines.[18b] We therefore employed a similar approach in

Table 1. Protected N^G-Hydroxyguanidines from Primary Amines[a]

entry	amine	product	yield (%)[b]
1			98
2			95
3			96
4			99
5			97
6[c]			98
7[d]			94[f]
8[e]			96[f]

[a] In a typical experiment, the amine (0.50 mmol) in CH_2Cl_2 (10.0 mL) was treated with an equimolar quantity of CbzNCS (administered as a 0.50 M solution in CH_2Cl_2). Once consumption of the amine was verified by TLC, the mixture was treated with EDCI (0.50 mmol), NEt_3 (0.50 mmol), and THP-ONH_2 (0.50 mmol). Additional half-equivalents of each reagent were then added at 15 min intervals until the intermediate thiourea was fully consumed (generally required 2.0−3.0 total equivalents). [b] Isolated yield based on amine starting material. [c] An equivalent of NEt_3 added to the amine hydrochloride prior to treatment with CbzNCS. [d] Protected ornithine species prepared by a published procedure.[10] [e] Prepared by method of Kim and co-workers.[7] [f] Yield for three-step process beginning with hydrogenolytic removal of side-chain amine Cbz-protecting group.

(14) Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z. *Tetrahedron Lett.* **1992**, *33*, 5933.

(15) Commercially available carbamoyl isothiyocyanates (FmocNCS and EtOCONCS) were deemed unsuitable for our application.

(16) A preparation of BocNCS has been reported via the decomposition of BocCONCS in liquid SO_2: Bunnenberg, R.; Jochims, J. C. *Chem. Ber.* **1981**, *114*, 1746.

(17) Dang, V. A.; Olofson, R. A. *J. Org. Chem.* **1990**, *55*, 1847.

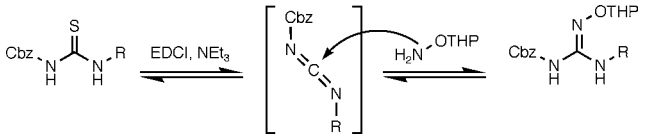
(18) (a) Groziak, M. P.; Townsend, L. B. *J. Org. Chem.* **1986**, *51*, 1277. (b) Linton, B. R.; Carr, A. J.; Orner, B. P.; Hamilton, A. D. *J. Org. Chem.* **2000**, *65*, 1566.

the preparation of protected N^G-hydroxyguanidines (Scheme 6).

A variety of amines were converted to their corresponding thioureas (formation of thiourea and concomitant consumption of amine monitored by TLC) which were then directly treated with EDCI, NEt_3, and THP−ONH_2 (typically 2−3 equiv) to give protected N^G-hydroxyguanindine products in high yield (Table 1).

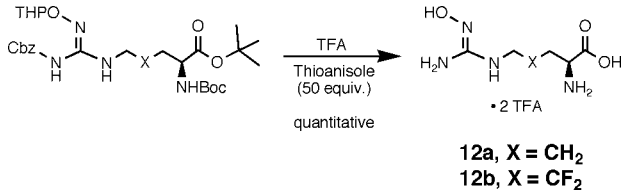
It should be noted that secondary amines are not amenable to this procedure. While both morpholine and diallyamine were rapidly converted to their thioureas after treatment with CbzNCS (based on TLC observation), none of the N^G-hydroxyguanidine product was formed upon treatment with EDCI, NEt_3, and THP-ONH_2. This result supports a mechanism whereby EDCI-mediated desulfurization is accompanied by double deprotonation of the thiourea to yield a transient carbodiimide[1a] which is subsequently attacked by the amine nucleophile (Scheme 7).

Scheme 7



Using the protected N^G-hydroxy-L-arginine species (entry 7, Table 1) as a model, we next examined potential deprotection conditions. As expected, hydrogenation was once again problematic giving, in the best case, a 1:1 mixture of the Cbz deprotected/O-THP protected product and the overreduced guanidine. As an alternative, the method of Kiso and co-workers,[21] using a mixture of TFA and thioanisole, provided the fully deprotected N^G-hydroxy-L-arginine product in quantitative yield. The process was equally successful in the global deprotection 4,4-difluoro-N^G-hydroxy-L-arginine (Scheme 8).

Scheme 8



12a, X = CH_2
12b, X = CF_2

In summary, we have developed a method allowing for the rapid preparation of N^G-hydroxyguanidines from primary amines. The method has been applied to a variety of examples including a much improved synthesis[10,22] of N^G-hydroxy-L-arginine and its 4,4-difluorinated analogue. Current work is underway evaluating the use of such analogues as mechanistic probes for the NOS reaction cycle.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Council of Canada and the Alberta Heritage Foundation for Medical Research (postdoctoral fellowships to N.I.M.).

Supporting Information Available: Experimental procedures for transformations that produced previously unknown compounds as well as their full spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) (a) While this process does not remove the trace amount of benzyl thiocyanate, it can be considered a "silent" impurity in the subsequent reactions and is readily separated in the final chromatography-based purification of the protected N^G-hydroxyguanidine product. (b) This improved preparation of CbzNCS should also be of general use in the preparation of substitued guanidines.[18b]

(20) Esmail, R.; Kurzer, F. *Synthesis* **1975**, 301.

(21) Kiso, Y.; Ukawa, K.; Akita, T. *J. Chem. Soc., Chem. Commun.* **1980**, *3*, 101.

(22) Feldman, P. L. *Tetrahedron Lett.* **1991**, *32*, 875.