Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 22 Aug 2006.

To cite this article: A. Jirgensons , I. Kums , V. Kauss & I. Kalvins (1997) A Convenient Reagent for N-hydroxyguanylation, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 27:2, 315-322

To link to this article: http://dx.doi.org/10.1080/00397919708005034

SYNTHETIC COMMUNICATIONS, 27(2), 315-322 (1997)

A CONVENIENT REAGENT FOR N-HYDROXYGUANYLATION

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ABSTRACT: 1-Benzyloxy-3-benzyloxycarbonylthiourea was prepared and demonstrated as a useful reagent for direct N-hydroxyguanylation of various amines.

Regulation of endogenous nitric oxide (NO), a universal mediator is an active area of scientific research[1,2]. Endogenous NO is produced from (L)-arginine, which is a substrate for an enzyme NO synthase (NOS)[1,2]. Typically, N^G-substituted arginines are effective inhibitors of NOS[1,3]. Only N^G-hydroxyarginine liberates NO as the result of NOS action[3,4]. Therefore, analogs of N^G-hydroxyarginine have became very attractive substrates for NOS studies.

However, the synthesis of N-hydroxyguanidine moiety is a multistep procedure involving transformation of the amino functionality to active thiourea[5-7] or cyanamide intermediates[5,8] and subsequent reaction with protected hydroxylamine.

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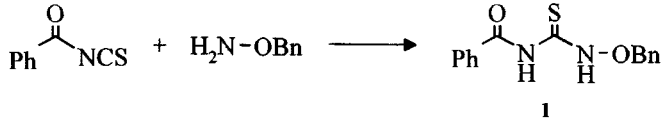
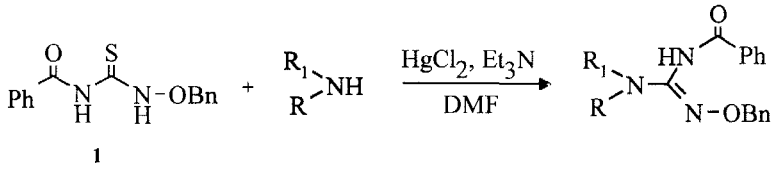


Scheme 1



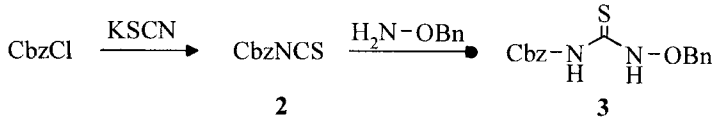
Scheme 2

To avoid this multistep procedure we suggest to use 1-benzyloxy-3-acyl thioureas as direct N-hydroxyguanylating agents in the presence of mercury (II) chloride. A similar principle has been used in the case of an effective guanylating reagent N,N'-bis-tert-butoxycarbonylthiourea[9] that acts through very reactive acyl carbodiimide generated in situ.

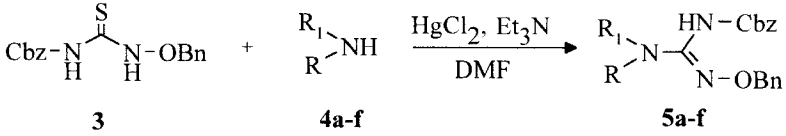
In the case of 1-benzyloxythioureas, 3-acyl substituent is also essential for the stability of N-hydroxythioureas, because 3-unsubstituted, 3-alkyl or 3-aryl-1-alkoxythioureas are unstable[5].

The method we offer was tried with 1-benzyloxy-3-benzoylthiourea (**1**), that was readily prepared from commercially available O-benzylhydroxylamine and benzoyl isothiocyanate (Scheme 1). Compound **1** reacted with various amines to give N,O-protected N-hydroxyguanidines (Scheme 2).

However, the utility of N-benzoyl group is limited because of harsh deprotection conditions. Therefore, we synthesized 1-benzyloxy-3-benzyloxy-carbonylthiourea (**3**) as shown in scheme 3.



Scheme 3

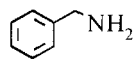
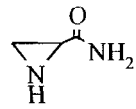
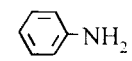
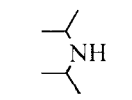
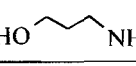
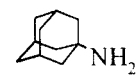


Scheme 4

Cbz group was chosen for N'- protection because the removal of both Cbz and Bn groups could be accomplished simultaneously under mild conditions. Unfortunately, when we used the procedure for preparation of benzyloxycarbonyl isothiocyanate (**2**) disclosed by Townsend et al.[10], we were able to reach only 10% yield of **3** in two steps from benzyl chloroformate. This prompted us to change the reaction conditions. When benzene was used instead of ethyl acetate and dibenzo-18-crown-6 was employed as phase transfer catalyst, we were able to increase the yield of **3** to 26% from benzyl chloroformate (47 % from O-benzylhydroxylamine) without isolation of **2**. Thus we have worked out a simple procedure for preparation of 1-benzyloxy-3-benzyloxycarbonylthiourea (**3**). Compound **3** is stable and can be stored unchanged in refrigerator for several months.

Reactions of hydroxythiourea **3** with various amines **4a-f** were studied in DMF using $HgCl_2$ for desulfurization of **3** and Et_3N as a base (Scheme 4).

Table 1.

 **4a-f**	Excess of reagent **3**, (%)	Reaction time, h	Hydroxy-guanidine	Yield of **5a-f**
	10	7	**5a**	47
	15	9	**5b**	34
	20	7,5	**5c**	54
	50	9	**5d**	34
	15	7	**5e**	35
	12	10.5	**5f**	67

All reaction conditions in scheme 4 were similar to guanylation with N,N'-bis-Boc thiourea[9]. The yields of protected hydroxyguanidines **5a-f** are given in Table 1. Compounds **5a-f** were characterized by PMR and mass spectra, and elemental analysis (see experimental section).

In conclusion, compound **3** presented here is the first reagent for direct one-step preparation of O,N'-protected N-hydroxyguanidines from different amines.

EXPERIMENTAL SECTION

Melting points were determined on a Boetius table and are uncorrected. All

^{1}H NMR spectra were recorded on a 90 MHz Bruker WH-90 spectrometer in $CDCl_3$ solutions with TMS as an internal standard. Mass spectra were recorded on MS 50 Ion Tech Fab 11 NF instrument. Microanalyses were obtained using a Carlo Erba 1106 element analyzer. All reactions were carried out under an argon atmosphere. TLC was carried out using Kieselgel 60 F_{254} (Merck) employing ethyl acetate in hexane as eluent.

1-Benzyloxy-3-benzyloxycarbonylthiourea

Benzyl chloroformate (6.6 ml, 7.9 g, 46 mmol) and dibenzo-18-crown-6 (0.84 g, 2.4 mmol) were added to a well stirred suspension of KSCN (5.4 g, 56 mmol) in benzene (80 ml). The mixture was stirred and refluxed for 3h, cooled and filtered into a dropping funnel. The resulting yellow solution was immediately added to a cold (5^0 C) solution of O-benzylhydroxylamine in benzene (prepared by stirring a suspension of O-benzylhydroxylamine hydrochloride (4.0 g, 25 mmol) in benzene (100 ml) with $NaHCO_3$ (4.3 g, 51 mmol) solution in H_2O (80 ml) for 2 h; the organic phase was washed 3 times with H_2O, dried over Na_2SO_4, filtered and concentrated to 25-30 ml). The suspension was allowed to reach room temperature under stirring. The white precipitate was filtered and washed twice with 20 ml of benzene. After drying on air, 3.8 g (47 % from O-benzylhydroxylamine hydrochloride) of **3** was obtained, m.p. 141-143^0 C.

Anal. Calcd. for $C_{16}H_{16}N_2O_3S$: C, 60.7; H, 5.1; N, 8.9; S, 10.1. Found: C, 60.6; H, 5.2; N, 8.8; S, 10.0. MS (FAB) m/z 316 [M^+].^{1}H NMR δ: 5.05 (s, 2H, CH_2O); 5.10 (s, 2H, CH_2O); 7.3-7.5 (m, 10H, $2C_6H_5$); 7.92 (br s, 1H, HNCO); 11.27 ppm (br s, 1H, HNO).

A typical procedure for the preparation of **5.**

1-(Benzyloxycarbonylamino-benzyloxyiminomethyl)-aziridine-2-carboxamide (**5b**).

$HgCl_2$ (0.43 g, 1.58 mmol) was added to a well stirred solution of **3** (0.5 g, 1.58 mmol), aziridine-2-carboxamide (**4b**) (0.12g, 1.39 mmol), and triethylamine (0.42 g, 4.16 mmol) in 4 ml of DMF. The reaction mixture was stirred for 8 h, diluted with 20 ml of EtOAc, and filtered. The filtrate was washed twice with 20 ml of water and 20 ml of brine. The organic phase was dried over Na_2SO_4. Filtration and concentration provided a dark oil which was purified on Kieselgel column (EtOAc). 0.33 g (34%) of **5b** was obtained as a viscous pale yellow oil.

Anal. Calcd. for $C_{19}H_{20}N_4O_4$: C, 62.0; H, 5.5; N, 15.2. Found: C, 62.0; H, 5,4; N, 15,0. MS (FAB) m/z 369 $[M^{+1}]$.^{1}H NMR δ: 2.32 (dd, J= 1Hz, 3Hz, 1H, aziridine CHCO); 2.76 (m, 2H, aziridine CH_2N); 4.91 (s, 2H, CH_2ON); 5.12 (s, 2H, CH_2OCO); 5.37 and 6.80 (br s, both < 1H, $CONH_2$); 7.31 (s, 5H, C_6H_5); 7.36 (s, 5H, C_6H_5); 7.62 ppm (br s,1H, NH).

5a: Anal. Calcd. for $C_{23}H_{23}N_3O_3$: C, 70.9; H, 5.9; N, 10.8. Found: C, 70.4; H, 5.9; N, 11.2. MS (FAB) m/z 390 $[M^{+1}]$. ^{1}H NMR δ: 4.25 (d, J=5.5 Hz, 2H, CH_2N); 4.84 (s, 2H, CH_2ON); 5.08 (s, 2H, CH_2OCO); 6.56 (br t, 1H, NH); 7.1- 7.5 (m, 15H, $3C_6H_5$); 7.90 ppm (br s, 1H, NH).

5c: Anal. Calcd. for $C_{22}H_{21}N_3O_3$: C, 70.4; H, 5.6; N, 11.2. Found: C, 69.9; H, 5.5; N, 10.9. MS (FAB) m/z 376 $[M^{+1}]$. ^{1}H NMR δ: 4.95 (s, 2H, CH_2ON); 5.12 (s, 2H, CH_2OCO); 6.91 (m, 1H, $H_{para}C_6H_5N$); 7.1-7.6 (m, 14H, Ar); 8.01 (br s, 1H, NH); 8.77 ppm (br s, 1H, NH).

5d: Anal. Calcd. for $C_{22}H_{29}N_3O_3$: C, 68.9; H, 7.6; N, 10.9. Found: C, 68.4; H, 7.9; N, 10.7. MS (FAB) m/z 384 [M^{+1}]. ^{1}H NMR δ: 1.60 (d, 7Hz, 12H, $2(CH_3)_2C$); 3.60 (m, 2H, $2CH(CH_3)_2$); 4.87 (s, 2H, CH_2ON); 5.10 (s, 2H, CH_2OCO); 6.54 (br s, <1H, NH); 7.1-7.4 ppm (m, 10H, $2C_6H_5$).

5e: Anal. Calcd. for $C_{19}H_{23}N_3O_4$: C, 63.8; H, 6.5; N, 11.8. Found: C, 63.6; H, 6.3; N, 11.6. MS (FAB) m/z 358 [M^{+1}]. ^{1}H NMR δ: 1.56 (m, 2H, -CH_2-); 3.16 (q, 2H, CH_2N); 3.49 (t, 2H, CH_2O); 3.79 (br s, 1H, OH); 4.73 (s, 2H, CH_2ON); 5.0 (s, 2H, CH_2OCO); 6.4 (br t, <1H, NH); 7.23 (s, 10H, $2C_6H_5$); 7.81 ppm (br s, 1H, NH-COO).

5f: Anal. Calcd. for $C_{26}H_{31}N_3O_3$: C, 72.0; H, 7.2; N, 9.7. Found: C, 71.4; H, 7.3; N, 9.5. MS (FAB) m/z 434 [M^{+1}]. ^{1}H NMR δ: 1.66 (br s, 6H, Adamantane); 1.8-2.1 (m, 9H, Adamantane); 4.82 (s, 2H, CH_2ON); 5.08 (s, 2H, CH_2OCO); 6.17 (br s, <1H, NH); 7.32 (s, 10H, $2C_6H_5$); 7.89 ppm (br s, 1H, NH).

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(Received in The Netherlands 16 July 1996)