A Convenient Preparation of Monosubstituted *N,N'*-di(Boc)-Protected Guanidines

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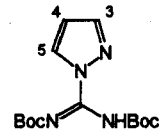
1-*H*-Pyrazole-1-[*N,N'*-bis(*tert*-butoxycarbonyl)]carboxamidine (**1**) reacts under mild conditions with a number of amines and amino acids to give the respective protected guanidines in moderate to high isolated yields.

Compounds containing carboxamidine functionality have been widely utilized in the area of pharmaceuticals or as probes for investigating biochemical processes, since the guanidino moiety often causes significant changes in the biological activity of organic molecules.[1] The change can be mainly attributed to the strongly basic carboxamidine group ($pK_a \sim 12$) and to the obvious charge interactions with acidic functionalities in proteins. As a consequence, the development of new methodologies for the preparation of proteinogenic, natural, and unnatural guanidino amino acids, or simple substituted guanidines has emerged as a highly significant and challenging synthetic endeavor.

The first syntheses of these compounds, involving the reaction of cyanamide with amines,[2] date back more than half a century. However, because of several drawbacks (e.g., high reaction temperatures, polymerization) the most widely used method for the introduction of such functionality is the reaction of amines with electrophilic amidine derivatives, such as *O*-methylisourea hydrogensulfate,[3] *S*-alkylisothiouronium salts,[4] aminoiminomethanesulfonic acid,[5] and salts of substituted 1*H*-pyrazole-1-carboxamidines.[6] The drawbacks inherent in using many of these reagents (toxicity, odor, necessity for strong bases, poor solubility of products) have led to the development of new methodologies and agents affording protected guanidines, though not all are devoid of these disadvantages. Verdini et al. reported the syntheses of N^{α}-Z (or N^{α}-Fmoc)-N^{ω},$N^{\omega'}$-di(Boc)-protected arginine derivatives starting from *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea and N^{α}-protected ornithine.[7] A report by Poss et al. describes a general synthesis of mono- and disubstituted *N,N'*-di(Boc)-protected guanidines using *N,N'*-bis(*tert*-butoxycarbonyl)thiourea.[8] Although both methods provide reasonable yields of the di(Boc)-protected guanidines under mild conditions, either the syntheses of guanylating agents are time consuming[9] or the yield is insufficient.[8]

For an ongoing project aimed at synthesis of protected guanidino derivatives of a variety of amines and amino acids we needed a steady supply of convenient guanylating agent. Based on the literature precedents,[6-9] we presumed that compound **1** should react similarly to the analogous 1*H*-pyrazole-1-carboxamidine, since the 1-pyrazolyl substituent represents a good leaving group which can stabilize the developing negative charge in the second step of a probable addition–elimination process. Furthermore, in such an amidine derivative, there are two electron-withdrawing Boc groups in positions conjugated with the reaction center, hence they should further increase the electrophilicity of the amidino carbon relative to the unprotected 1*H*-pyrazole-1-carboxamidines.[6] The guanylating agent **1** was readily synthesized in 95% yield from 1H-pyrazole-1-carboxamidine[6] by an one-pot reaction with di-*tert*-butyl dicarbonate in the presence of 3 equivalents of lithium hydride. The best results were obtained using 3 equivalents of the di-*tert*-butyl dicarbonate relative to the pyrazole derivative; attempts to use less of the di-*tert*-butyl dicarbonate or replace lithium hydride with sodium hydride resulted in lower yields. Thus, when 3 equivalents of the di-*tert*-butyl dicarbonate and sodium hydride were used, the final product was isolated in 76% yield. The structural assignment of **1** was made on the basis of ^{1}H NMR, ^{13}C NMR, and FAB-MS.



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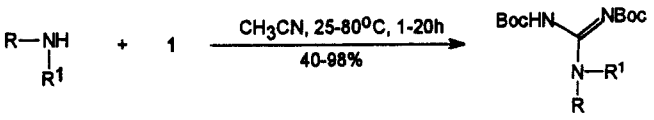
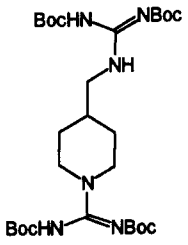


2a-u **3a-u**



3k

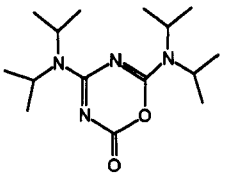
2–3	R[a]	R^1	2–3	R	R^1
a	Bu	H	**k**[b]	$-(CH_2)_2CH(CH_2NH_2)(CH_2)_2-$	
b	*t*-Bu	H	**m**	Gly	H
c	*c*-C_6H_{11}	H	**n**	(β)-Ala	H
d	Bn	H	**o**	(β)-Abu	H
e	Allyl	H	**p**	(ε)-Aca	H
f	Ph	H	**r**	Pro	
g	4-(OMe)C_6H_4	H	**s**	Fmoc-Lys	H
h	4-(NO_2)C_6H_4	H	**t**	Z-Orn	H
i	$-(CH_2)_5-$		**u**	(β)-Ala-OMe	H
j	$-(CH_2)_2O(CH_2)_2-$				

[a] (β)-Abu = 3-aminobutyric acid, (ε)-Aca = 6-aminocaproic acid, Fmoc = fluorenylmethoxycarbonyl, Z = benzyloxycarbonyl, Orn = ornithine.
[b] Structure of **3k** is shown separately.

Scheme

The reaction of a variety of amines with the reagent **1** (Scheme) has been examined and the results are summarized in Table 1 and Table 2. As regards the solvents, this reaction could be successfully performed in methanol, tetrahydrofuran, ethyl acetate, acetonitrile, chloroform, dimethyl sulfoxide and dimethylformamide, leading to the desired products in reasonable yields. Acetonitrile, however, appeared to be the solvent of general use for all studied reactions due to its appropriate physical properties (aprotic and high polar nature, easier removal during the workup). The course of the reaction was conveniently monitored by TLC analysis, and isolation of the product involved simple chromatography or crystallization depending on the product.[10]

The guanylated products, **3**, were formed in high yield from primary amines and **1** in all cases, except where an electron-withdrawing substituent was present (Table 1). Even with a poor nucleophilic amine such as 4-nitroaniline, the guanylated product, **3h**, was isolated in 37% yield. However, we found that this particular reaction is dependent on the solvent used and an effect was also observed in the reaction times. Thus, the highest yield of **3h** (37%) was obtained using chloroform as a solvent and stirring the reaction mixture for 3 days at room temperature. With secondary amines, the reaction also proceeded smoothly to give the corresponding derivatives **3** in good yields except for diisopropylamine where two isopropyl groups may sterically hinder the approach of the central amidine carbon, so that there was no detectable amount of the guanylated product **3**. Attempts to improve the yield by, for example, prolonging reaction times and heating the reaction mixture were unsuccessful and only 4,6-bis(diisopropylamino)[1,3,5]oxadiazin-2-one **(4)** was isolated from the reaction mixture in 52% yield.[11]



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In addition to the demonstrated utility for aminoguanylation of simple amines, this methodology also possesses considerable potential for aminoguanylation of amino acids. In this way, poor solubility of amino acids in many organic solvents appeared to be the major limitation. The reaction was very sluggish and not completed even after several days. Therefore, we carried out the guanylation reaction in various, mainly polar aprotic solvents in order to improve the yields of protected guanidines **3m–u**. Thus, with aqueous acetonitrile, satisfactory results were obtained in all cases except with the β-alanine where the yield was modest. In this case, increasing the amount of **1** substantially did not improve the yield of **3n**. However, when a similar reaction was carried out with β-alanine methyl ester, product **3u** was isolated in 89% yield.

In conclusion, the synthesis of monosubstituted di(Boc)-protected guanidines from amines using amidine derivative **1** represents an inexpensive and simple method with broad application for the ready preparation of these interesting compounds. This method may prove to be especially useful in cases where the insolubility of the unprotected guanido amino acids prevents their use in peptide synthesis.

All reagents and solvents are commercially available and used as received. Melting points were determined with MEL-TEMP II capillary melting point apparatus (Aldrich) and are uncorrected. Microanalyses were performed by Desert Analytics – Organic Microanalysis, Tucson, AZ. NMR spectra were recorded on a General Electric QE 300 spectrometer at 300 MHz. Chemical shifts are reported in ppm relative to internal TMS or to the proton resonance due to the residual protons in the deuterated NMR solvent. FAB-MS were measured on a ZAB-EQ spectrometer (VG Analytical Ltd., Manchester). Reversed-phase TLC was carried out with Bio-Si C_{18} silica (Bio-Rad, HL Silica, 40–63 μm). Merck silica gel 60 (230–400 mesh) was used for column chromatography.

1-*H*-Pyrazole-1-(*N*,*N*'-bis(*tert*-butoxycarbonyl)]carboxamidine (1): The di-*tert*-butyl dicarbonate (16.4 g, 75 mmol) was dissolved in anhydr. THF (200 mL) and treated with 95% LiH (0.95 g,

Table 1. Di(Boc)-Protected Derivatives **3** Prepared

Product[a]	Reaction Conditions		Yield (%)	mp (°C) (solvent)[b]
	Method	Time (h)/Temp. (°C)		
3a	A	1/r.t.	90	76–78 (Et_2O/hexane)
3b	A	3/r.t.	93	140–141.5 (Et_2O/hexane)
3c	A	16/r.t.	96	126–128
3d	A	3/1 r.t.	91	126–127
3e	A	1/r.t.	98	83–85
3f	A	4/reflux	71	125 (dec)
3g	A	16/r.t.	71	110.5–111.5 (EtOAc/hexane)
3h	A	4/reflux	5	188–190 (dec) (EtOAc/hexane)
	A	72/r.t.	7[c]	
3i	A	5/r.t.	92	117 (dec) (hexane)
3j	A	2/r.t.	86	115–116 (Et_2O/hexane)
3k	A	2/r.t.	67	260 (dec)
3l	A	24/reflux	0	–
3m	B	3/reflux	71	190 (dec) (EtOAc/hexane)
3n	B	2/reflux	38	126 (dec)
3o	B	20/reflux	70	135 (dec) (EtOAc/hexane)
3p	B	20/r.t.	76	95 (dec)
3r	B	16/r.t.	73	126 (dec)
3s	B	2/reflux	57	155 (dec)
3t	B	2/reflux	70	102–103 (dec)[d]
3u	B	3/r.t.	89	97–98 (EtOAc/hexane)

[a] For all new compounds satisfactory microanalyses obtained: C ± 0.25, H ± 0.30, N ± 0.30.

[b] Solvent given if product recrystallized.

[c] Yield depends on the solvent used: THF (17%, 3 d/r.t.); $CHCl_3$ (37%, 3 d/r.t.)

[d] Lit.[7] mp 120°C.

Table 2. Spectral Data of Di(Boc)-Protected Guanidines 3

Compound	^{1}H NMR ($CDCl_3$/TMS) δ, J (Hz)	MS (FAB)
3a	0.94 (t, 3H, J = 7.5), 1.33–1.62 (m, 4H), 1.49 (s, 9H), 1.51 (s, 9H), 3.42–3.52 (m, 2H), 8.38 (br s, 1H), 11.51 (s, 1H)	316 ($M^+ + 1$), 260, 216, 116
3b	1.44 (s, 9H), 1.48 (s, 9H), 1.49 (s, 9H), 8.25 (br s, 1H), 11.41 (br s, 1H)	316 ($M^+ + 1$), 260, 216, 204
3c	1.15–2.00 (m, 10H), 1.49 (s, 9H), 1.50 (s, 9H), 4.05 (br s, 1H), 8.33 (br s, 1H), 11.53 (s, 1H)	342 ($M^+ + 1$), 286, 242, 230
3d	1.48 (s, 9H), 1.49 (s, 9H), 4.68 (d, 2H, J = 4.9), 7.28–7.38 (m, 5H), 8.71 (br s, 1H), 11.55 (br s, 1H)	350 ($M^+ + 1$), 238, 194, 91
3e	1.49 (s, 18H), 3.95–4.11 (m, 2H), 5.13–5.27 (m, 2H), 5.80–5.95 (m, 1H), 8.41 (br s, 1H), 11.46 (br s, 1H)	300 ($M^+ + 1$), 244, 200, 144
3f	1.51 (s, 9H), 1.53 (s, 9H), 7.08–7.60 (m, 5H), 10.33 (br s, 1H), 11.64 (br s, 1H)	336 ($M^+ + 1$), 280, 236, 180
3g	1.49 (s, 9H), 1.53 (s, 9H), 3.70 (s, 3H), 6.85 (d, 2H, J = 9.0), 7.46 (d, 2H, J = 9.0), 8.86 (br s, 1H), 10.18 (br s, 1H), 11.64 (br s, 1H)	366 ($M^+ + 1$), 310, 254, 210, 192
3h	1.57 (s, 18H), 7.87 (d, 2H, J = 9.1), 8.24 (d, 2H, J = 9.1), 10.80 (br s, 1H), 11.64 (br s, 1H)	381 ($M^+ + 1$), 325, 269, 225, 181
3i	1.49 (s, 18H), 1.63–1.71 (m, 6H), 3.53 (br s, 4H), 10.18 (br s, 1H)	328 ($M^+ + 1$), 272, 216, 172, 128
3j	1.49 (s, 18H), 3.48–3.61 (m, 4H), 3.70–3.80 (m, 4H), 10.25 (br s, 1H)	330 ($M^+ + 1$), 274, 218, 174, 130
3k	1.48 (s, 9H), 1.49 (s, 27H), 1.20–1.38 (m, 2H), 1.49–1.51 (m, 2H), 2.75–2.81 (m, 2H), 2.91–2.99 (m, 2H), 3.31–3.40 (m, 2H), 4.05–4.22 (m, 1H), 8.42 (t, 1H, J = 5.2), 10.06 (br s, 1H), 11.49 (br s, 1H)	599 ($M^+ + 1$), 543, 399, 343, 299
3m	1.50 (s, 9H), 1.52 (s, 9H), 4.29 (s, 2H), 9.17 (br s, 1H), 11.41 (br s, 1H)	318 ($M^+ + 1$), 262, 218, 162
3n	1.49 (s, 18H), 2.72 (t, 2H, J = 6.1), 3.70 (t, 2H, J = 6.1), 8.86 (br s, 1H), 11.36 (br s, 1H)	332 ($M^+ + 1$), 276, 232
3o	1.38 (d, 3H, J = 7.0), 1.49 (s, 9H), 1.51 (s, 9H), 2.58 (dd, 1H, J = 15.8, J = 5.5), 2.78 (dd, 1H, J = 4.6), 4.30–4.35 (m, 1H), 8.74 (b, 1H), 11.42 (br s, 1H)	346 ($M^+ + 1$), 290, 246, 190
3p	1.34–1.73 (m, 6H), 1.49 (s, 18H), 2.36 (t, 2H, J = 6.5), 3.38–3.45 (m, 2H), 8.33 (br s, 1H), 11.38 (br s, 1H)	374 ($M^+ + 1$), 318
3r	1.51 (s, 9H), 1.53 (s, 9H), 1.79–1.86 (m, 2H), 2.09–2.21 (m, 1H), 2.40–2.53 (m, 1H), 3.01–3.12 (m, 1H), 4.00–4.12 (m, 1H), 5.20–5.32 (m, 1H), 11.53 (br s, 1H)	358 ($M^+ + 1$), 258, 158
3s	1.30–1.70 (m, 4H), 1.48 (s, 9H), 1.49 (s, 9H), 1.80–1.84 (m, 1H), 1.90–1.98 (m, 1H), 3.40–3.52 (m, 2H), 4.21 (t, 1H, J = 6.7), 4.39 (d, 2H, J = 6.7), 4.41 (br s, 1H), 5.55 (d, 1H, J = 7.9), 7.30–7.75 (m, 8H), 8.55 (br s, 1H), 11.53 (bs, 1H)	611 ($M^+ + 1$), 555, 511, 455, 411
3t	1.39 (s, 9H), 1.47 (s, 9H), 1.35–1.75 (m, 4H), 3.18–3.31 (m, 2H), 3.78–3.86 (m, 2H), 5.01 (s, 2H), 7.08 (br s, 1H), 7.25–7.40 (m, 5H), 8.25 (t, 1H, J = 5.1), 11.48 (br s, 1H)[a]	509 ($M^+ + 1$), 309
3u	1.48 (s, 9H), 1.49 (s, 9H), 2.61 (t, 2H, J = 6.2), 3.71 (s, 3H), 3.65–3.73 (m, 2H), 8.75 (br s, 1H), 11.45 (br s, 1H)	346 ($M^+ + 1$), 290, 246, 146

[a] NMR spectrum was measured in DMSO-d_6.

120 mmol). The mixture was vigorously stirred at reflux under N_2 and solid 1*H*-pyrazole-1-carboxamidine · HCl (4.4 g, 30 mmol) was added portionwise over the following 2 h. After 1 h, another portion of di-*tert*-butyl dicarbonate (3.2 g, 15 mmol) was added and the mixture was stirred at reflux for another 4–5 h while a suspension which is difficult to stir was formed. The reaction was monitored by RP TLC [R_f (**1**) ~ 0.5, R_f (mono-Boc) ~ 0.6; 30% aq acetone/MeOH (9:1)] and was quenched after completion with glacial AcOH (6.9 mL, 120 mmol) added dropwise to the cooled (10–15°C) mixture. Then sat. $NaHCO_3$ (10 mL) was added slowly and the mixture was stirred for 15 min. After separation of layers, the aqueous layer was extracted with hexane (3 × 20 mL), the combined organic phases were washed with brine (20 mL), dried ($MgSO_4$), and evaporated in vacuo. The crude product was purified by crystallization from hexane at −5°C to give **1** as a white solid; yield: 6.4 g (69%); mp 86–87°C.

Purification of the mother liquor by flash chromatography (eluent: 1. hexane, 2. hexane/EtOAc, 95:5) afforded an additional portion of **1**; 2.5 g (26%).

$C_{14}H_{22}N_4O_4$ (310.4) calc. C 54.18 H 7.14 N 18.05
found 54.47 7.36 18.17

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.51 (9H, s, Boc), 1.57 (9H, s, Boc), 6.44 (1H, dd, H-4, $J_{4,5}$ = 2.7 Hz, $J_{4,3}$ = 1.7 Hz), 7.64 (1H, d, H-3, $J_{3,4}$ = 1.7 Hz), 8.32 (1H, d, H-5, $J_{5,4}$ = 2.7 Hz), 8.94 (1H, br s, NH).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 27.79 (CH_3), 81.32 (CMe_3), 82.28 (CMe_3), 109.74 (C-4), 128.92 (C-3), 139.07 (NC=N), 142.68 (C-5), 149.32 (C=O), 157.30 (C=O).

FABMS: *m/z* (%) = 311 (43, M + H), 211 (20), 155 (100), 111 (98).

Di(Boc)-Protected Guanidines 3; General Procedure:

Method A: To a stirred solution of **1** (1.21 g, 3.9 mmol) in MeCN (20 mL) at r.t. the appropriate amine (1.2–1.5 equiv) was added in one portion. The mixture was stirred for the specified period of time at the temperature shown in Table 1. After the reaction was complete (TLC; hexane/EtOAc, 85:15), the solvent was removed under reduced pressure to yield the product which was purified either by crystallization and/or by column chromatography (Tables 1 and 2).

Method B: To a stirred solution of **1** (1.55 g, 5 mmol) and *i*-Pr_2NEt (1.05 mL, 6 mmol) in MeCN/H_2O (95:5, 25 mL) was added the appropriate amino acid (5 mmol) and the mixture was stirred for the specified period of time at the temperature shown in Table 1. After the reaction was complete (TLC; CH_2Cl_2/MeOH, 9:1), the solvent was removed under reduced pressure and the residue dissolved in EtOAc (50 mL). The organic phase was washed with 5% HCl (2 × 25 mL), brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the product which was purified either by crystallization and/or by column chromatography (Tables 1 and 2).

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(11) A more detailed study concerning the mechanism of the formation of compound **4** is in progress.