(hexane/ether, 4:1): mp 88–89 °C dec (lit.[2,7] mp 71–73 °C dec, 72–76 °C); $^1$H NMR ($CDCl_3$) δ 6.41 (d, $J$ = 1.7 Hz, 1 H), 7.1–7.3 (m, 3 H), 7.51 (dd, $J$ = 7.4, 1.1 Hz, 1 H), 8.0 (br s, 1 H) ppm; $^{13}$C NMR ($CDCl_3$) δ 100.7, 110.3, 119.8, 120.5, 122.2, 123.3, 128.1, 134.9 ppm.

**2,3-Diiodoindole (2).** The general method of Bocchi and Palla[4] was used. KOH (0.56 g, 10 mmol) was added to a solution of 2-iodoindole (0.972 g, 4.0 mmol) in DMF (20 mL). A solution of iodine (1.03 g, 4.05 mmol) in DMF (10 mL) was added dropwise during 8 min. After 30 min the reaction mixture was poured onto ice water (containing 4 mL of $NH_3$ and 200 mg of $K_2S_2O_5$), and the resulting fine suspension was extracted with ether (2 × 50 mL). The combined ether extracts were washed with water (5 × 20 mL) followed by brine, dried ($MgSO_4$), and evaporated. The crude product was recrystallized from hexane: yield 1.21 g (82%) of white needles; mp 130–131 °C (lit.[14] mp 220 °C); IR (KBr) 3361, 1434, 1396, 1334, 1302, 1221, 966, 747 cm$^{-1}$; mass spectrum, $m/z$ 369 ($M^+$, base peak); $^1$H NMR ($CDCl_3$) δ 7.2 (m, 2 H), 7.3 (m, 1 H), 7.4 (m, 1 H), 8.4 (br s, 1 H) ppm; $^{13}$C NMR ($CDCl_3$) δ 73.23, 87.13, 110.41, 121.1, 121.2, 123.4, 131.3, 138.8 ppm. Anal. Calcd for $C_8H_5NI_2$: C, 26.03; H, 1.37; N, 3.80; I, 68.81. Found: C, 25.65; H, 1.35; N, 3.70; I, 68.55.

**1-(Phenylsulfonyl)-2,3-diiodoindole.** To a solution of 2 (369 mg, 1 mmol) in dry DMF (5 mL) was added NaH (26 mg, 1.1 mmol) under nitrogen. The mixture was stirred for 30 min, whereafter benzenesulfonyl chloride (194 mg, 1.1 mmol) was added. The reaction mixture was stirred for 2 h, and the solvent was evaporated in vacuo. The residue was dissolved in $CH_2Cl_2$ (20 mL), and the organic phase was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (hexane/ether, 1:1): yield 380 mg (76%) of a whitish powder; mp 166–167 °C (lit.[6] mp 166–167 °C); IR (KBr) 1440, 1430, 1369, 1188, 1128, 1087, 1013, 742, 725, 682, 590, 565 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 7.0–7.6 (m, 6 H), 7.7–8.3 (m, 3 H) ppm.

**1-Methyl-2-iodoindole.** To a solution of 1a (673 mg, 2.77 mmol) in dry DMF (7.5 mL) was added NaH (73 mg, 3.05 mmol), and the mixture was stirred for 30 min. Methyl iodide (590 mg, 4.16 mmol) was then added, and the reaction mixture was stirred at 50 °C for 4 h. The solvent was evaporated, the oily residue was dissolved in ether (30 mL), and the organic phase was washed with water, dried ($MgSO_4$), and evaporated. Flash chromatography (hexane/ether, 4:1) of the crude product gave 640 mg (90%) of white crystals: mp 76 °C (lit.[12b] mp 76–77 °C); $^1$H NMR ($CDCl_3$) δ 3.75 (s, 3 H), 6.78 (s, 1 H), 7.0–7.2 (m, 2 H), 7.3 (m, 1 H), 7.5 (m, 1 H) ppm.

**Registry No.** 1a, 26340-49-8; 1b, 139409-34-0; 1c, 7135-31-1; 2, 139409-35-1; indole, 120-72-9; 1-(phenylsulfonyl)-2,3-diiodoindole, 80360-26-5; 1-methyl-2-iodoindole, 75833-63-5.

# 1*H*-Pyrazole-1-carboxamidine Hydrochloride: An Attractive Reagent for Guanylation of Amines and Its Application to Peptide Synthesis

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*Received November 26, 1991*

## Introduction

The possibility of preparing arginine-containing peptides by guanylation of the δ-amino groups of the appropriate ornithine-containing precursors has long been recognized.[1] Such a strategy is attractive for its potential to eliminate the many problems associated with the use of conventionally protected arginine starting materials in peptide synthesis.[2] It is also attractive because it allows for the preparation of various ornithine-derived analogues from a single common precursor peptide. Despite the potential advantages of this strategy, only a few examples of its successful use have appeared in the literature.[3] Presumably the commonplace use of this approach has been impeded by the lack of general availability of guanylating reagents with the appropriate reactivity and solubility characteristics. Preliminary studies in this laboratory have shown that the widely used commercially available guanylating reagents cyanamide,[4] *O*-methylisourea hydrogen sulfate,[3d,e] 2-ethyl-2-thiopseudourea hydrobromide,[5] and 3,5-dimethylpyrazole-1-carboxamidine nitrate[6] did not possess sufficient reactivity for practical and generalized use in the context of solid-phase peptide synthesis. Tian and Roeske have arrived at similar conclusions in a recent report noting the shortcomings of such reagents.[7] As a consequence of these observations, an interest in generalizing the "ornithine → arginine" strategy, and the potential medicinal chemistry applications of guanidines, some of the work in this laboratory was focused on the development and characterization of more suitable reagents for the conversion of amines to guanidines. In the course of this investigation it was found that 1*H*-pyrazole-1-carboxamidine hydrochloride[8] (1) (1-guanylpyrazole hydrochloride) was reactive enough to merit further study. Although 1 has been previously used to convert some amines and hydrazines to the corresponding guanidines (Figure 1) in good yields (using 2 mol of amine for each mole of 1 in refluxing tetrahydrofuran or dibenzyl ether, 160 °C in the case of aniline)[8] and has also been applied to the synthesis of Edeine B and F,[9] systematic studies designed to provide a better understanding of its relative reactivity and compatibility with other potentially nucleophilic functional groups, and detailed optimized synthetic procedures for its more generalized and widespread use as a guanylating reagent were lacking. Reported here are experiments designed to provide a better understanding of 1 with regard to its reactivity as well as to

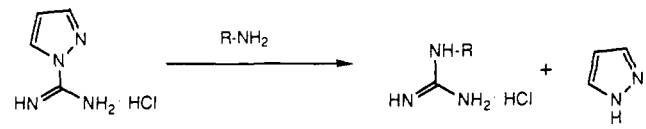


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**Figure 1.** Reaction of 1*H*-pyrazole-1-carboxamidine hydrochloride (1) with a primary amine.

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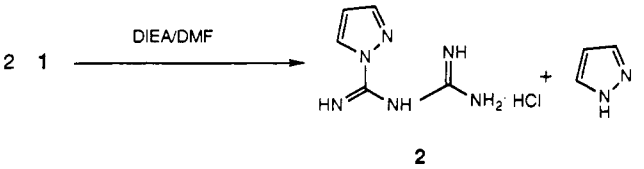


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**Figure 2.** Self-condensation of 1*H*-pyrazole-1-carboxamidine hydrochloride (1).

better define its synthetic scope and limitations, particularly as they might apply to conventional peptide synthesis procedures and conditions.

A more recently introduced method which produces monosubstituted guanidines from amines employs aminoiminomethanesulfonic acid as guanylating agent.[10] This reagent may also be valuable for application to the "ornithine → arginine" strategy of peptide synthesis. The use of this reagent in DMF,[11] the solvent of choice for solid-phase peptide synthesis, has not yet been described, and the zwitterionic nature of the compound led the authors to suspect that it may be insufficiently soluble in that solvent for general use in solid phase synthesis. In addition, the long-term stability of this reagent is questionable.[10c] These considerations have thus far prevented evaluation of this reagent for peptide synthesis in this laboratory; however, future investigation along these lines may prove to be valuable.

## Results and Discussion

1*H*-Pyrazole-1-carboxamidine hydrochloride (1) was conveniently and reproducibly prepared in high yield (93–95%) by the previously unreported reaction of pyrazole with 1 equiv of cyanamide in refluxing anhydrous HCl/*p*-dioxane. Under these conditions analytically pure 1 crystallizes from the reaction mixture, simplifying its direct isolation.

Compound 1 possesses desirable solubility properties for its use as a guanylating reagent. When 1 is neutralized by 1 equiv of diisopropylethylamine (DIEA) in dimethylformamide (DMF), it can be utilized at about 2 M concentration, conditions that are compatible with peptide synthesis, and in fact favor quantitative guanylation of peptidyl-polymeric resins (intermediates obtained by solid-phase synthesis) by virtue of high reactant concentration and good solvating (resin swelling) characteristics. DIEA neutralized 1 also has very good solubility in water as well as water-miscible organic solvents such as acetonitrile, acetone, alcohol, and tetrahydrofuran. Thus concentrated aqueous–organic solutions of 1 and the starting amine to be guanylated can be arrived at as dictated by the solubility of the amine. Furthermore, 1 exists as an easily handled, apparently nonhygroscopic powder that is stable (no detectable decomposition after 3 months) at room temperature.

The pH-dependent UV spectrum of 1 ($\lambda_{max}$ 252 nm, in 0.05 M $Na_2CO_3$ buffer, pH 9.8) allows for convenient spectral or chromatographic monitoring of its consumption by aminolysis or hydrolysis. In this manner it was found that 1 was remarkably stable in aqueous solution. Under more basic conditions, simple spectrophotometric monitoring of the stability of solutions of 1 is complicated by a slow self-condensation of 1 to form the diguanidine derivative 2[12] as depicted in Figure 2. No significant change in absorbance at 244 nm (a wavelength where the pyrazole leaving group has no absorbance) was observed after 2 h at room temperature when 1 was dissolved at 1 M in 1 M $Na_2CO_3$, indicating substantial stability toward hydrolysis even in aqueous base. The self-condensation reaction (Figure 2) is slow enough not to preclude the use of 1 to prepare guanidine derivatives of sufficiently reactive amines as shown in Figure 1 (data given in Table I). When 1 was intentionally allowed to self-condense in DMF containing 1 equiv of DIEA at approximately 2 M concentration for 64 h at room temperature, a 57% yield of pure 2 was obtained. In contrast, simple primary aliphatic amines in solutions of 1 under similar conditions were guanylated in higher yields after only 2–4 h (Table I).

**Table I. Guanidines Obtained by Reaction of 1 (Figure 1)**

| starting amine | guanidine product entry | yield (%)[a] | mp (°C)[b] | procedure[c] |
|---|---|---|---|---|
| cyclohexylamine | 4 | 84 | 228–29 | A |
| piperidine | 5 | 71 | 186–87 | A |
| 2-ethanolamine | 6 | 88 | 97–98 | A |
| glycine | 7 | 77 | 280 dec[d] | B |
| 4-methoxyaniline | 8 | 55 | 144–46 | C |
| | | 60 | 146–48 | D |
| aniline | 9 | 48 | 224–28[e] | E |

[a] Unoptimized yield of recrystallized product. [b] In general observed mp was in good agreement with those reported (see the Experimental Section for comparison). All NMR spectra (available as supplementary material) and MS data were consistent with product structures. [c] See the Experimental Section for details. [d] Isolated as zwitterionic species. [e] Isolated as picrate salt.

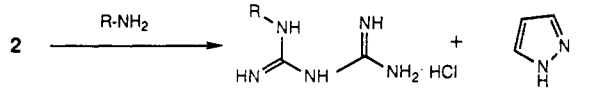


**Figure 3.** Formation of *N*-alkyldiguanidine from 2 and an alkylamine.

It has been reported that *N*-alkyldiguanidines can be prepared by reaction of 2 with unhindered amines (Figure 3) under appropriately forcing conditions (refluxing organic solvent, 4–24 h).[12] This reaction (Figure 3) must be considered as it could lead to the formation of *N*-alkyldiguanidine side products when 1 is used for amine guanylation (1 → 2 → *N*-alkyldiguanidine). Thus, an ornithine residue could possibly be converted to an undesired guanylarginine residue by reaction with side product 2 in the context of a peptide synthesis. It was found in a separate experiment that 2 did not react with cyclohexylamine under conditions that cyclohexylamine was rapidly guanylated by 1. Thus 1 was found to be much more reactive than 2 toward primary amines. This observation, along with the fact that formation of 2 from 1 was slow, and that *N*-alkyldiguanidine side products had not been isolated or observed in preliminary model studies, led to the tentative conclusion that this potential side reaction would not preclude the use of 1 for the synthesis of arginine-containing peptides. The insignificance of this possible side reaction was later demonstrated by the successful use of 1 for guanylation in the synthesis of an arginine-containing heptapeptide.

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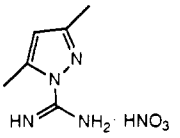
(11) Abbreviations for amino acids and peptide nomenclature follow the reccomendations of the IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are DIEA = diisopropylethylamine, DMF = dimethylformamide, Boc = *tert*-butyloxycarbonyl, Fmoc = [(9-fluorenylmethyl)oxy]carbonyl, Bom = benzyloxymethyl, 2-Cl-Z = 2-chlorobenzyloxycarbonyl, Bn = benzyl, pMBHA = *p*-methylbenzhydrylamine, CI = chemical ionization, FAB = fast atom bombardment, MS = mass spectrum (spectroscopy), dec = decomposes.

(12) (a) Schenker, E.; Hasspacher, K. U.S. Patent 3,503,984, 31 Mar 1970. (b) Schenker, E.; Hasspacher, K. U.S. Patent 3,519,644, 7 Jul 1970.

In order to evaluate the relative reactivity of potential guanylating reagents as they might be applied to solid-phase peptide synthesis, a simple model system was used which involves carrying out reactions of guanylating agent with aminoethyl-Pepsyn K resin (a cross-linked poly(dimethylacrylamide)-based kieselguhr composite used in continuous flow solid-phase peptide synthesis)[13] and determining the time required for complete consumption of resin amino groups as indicated by the Kaiser ninhydrin test[14] performed on periodic aliquots of resin samples. Use of this system led to the expected results, demonstrating that 1 was more reactive, and probably better suited for peptide synthesis, than its 3,5-dimethyl analogue, 3,5-dimethylpyrazole-1-carboxamidine nitrate 3, which has



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previously been used for satisfactory amine guanylation[6] and was subsequently employed in the solution synthesis of two peptides,[3a] but was not found to be suitable in a solid-phase peptide synthesis.[7] When 3 was allowed to react at room temperature (100 equiv relative to resin-bound amine at about 1 M concentration in DMF containing equimolar DIEA, conditions near solution saturation) with the amino-Pepsyn K resin (0.1 mmol amine/g), about 5 h were required to achieve a ninhydrin negative endpoint. When 1 was allowed to react similarly (except 1.8 M 1 was employed), only 1.5 h were required for complete amine consumption. When the above experiments were repeated using water as solvent (1 at 2 M, 3 at 1.8 M), 1 required about 30 min and 3 required about 1.5 h to complete their respective reactions. Although the reactivities of both 1 and 3 were enhanced with water as solvent, it is unclear as to whether this result is due to differences in stabilities of intermediates found along the reaction pathway, differences in mechanism, or simply better solvation and accessibility of the resin-bound amino groups. The enhanced reactivity in water could arise from combined contributions by several such effects. In any event, when conventional polystyrene-based peptide synthesis supports are employed, the use of pure water as solvent is not possible due to insufficient resin solvation. Nevertheless, the better reactivity and solubility characteristics of 1 compared to 3 as demonstrated in these experiments suggested that 1 may be practically used in DMF for generalized (solution or solid-phase synthesis using either polyamide or polystyrene-based supports) incorporation of arginine into peptides from ornithine-containing precursors.

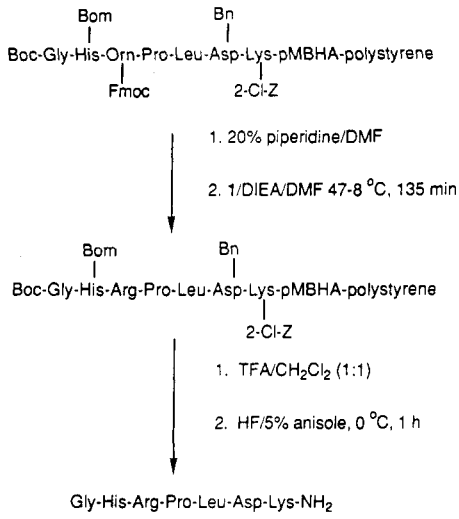
Before examining the use of 1 for guanylation of synthetic peptides, a better understanding of its general synthetic scope and limitations was gained by carrying out (or attempting to carry out) solution guanylation reactions (Figure 1) of structurally diverse amines and other potential nucleophiles (thiols, hydroxyl, carboxylate, and imidazole) that might be found in peptides or other natural products. The results (isolated yields) of these experiments, in cases where guanylation was readily achieved, are summarized in Table I. From this data it appears that simple, unhindered primary and secondary aliphatic amines (solubility permitting) can be readily converted to their guanidine hydrochloride derivatives by reaction with a stoichiometric amount of 1 and equimolar DIEA under mild conditions (room temperature) by a general procedure developed in the course of these studies. The pyrazole byproduct of the reaction is readily soluble in ether, facilitating its removal from the desired insoluble guanidine hydrochloride. This protocol for amine guanylation by 1 has clear advantages over that described for guanylation by commercially available 3,5-dimethylpyrazole-1-carboxamidine nitrate (3)[6] which involves the use of refluxing ethanol and at least 2 equiv of amine if satisfactory yields are to be obtained in a comparable reaction time. In addition, use of 3 requires the use of ion-exchange purification if crystalline guanidine hydrochloride salts are desired (pure nitrates were not crystallizable). Thus 1 can be used for guanylation under milder conditions than 3 and directly yields products than can be purified by simple crystallization. This procedure (procedure A, Experimental Section) also has clear advantages over the previously reported procedure[8] for the use of 1 for solution guanylation in that it requires only 1 equiv of amine and can be performed under milder conditions (room temperature), potentially allowing its use for the efficient guanylation of complex, thermally labile amines.

This procedure for guanylation by 1 does, however, have certain limitations. For example, guanidine derivatives of the sterically hindered, more basic secondary amines, dicylohexylamine, and diisopropylamine were not obtainable in this manner. In these cases the extremely insoluble hydrochloride salts of the amines rapidly and essentially quantitatively precipitate, leaving only the free base form of 1 in solution which slowly self-condenses to yield the diguanidine derivative 2 (Figure 2). Thus far, all attempts to prepare the guanidine derivatives of these nonnucleophilic secondary amines starting from 1 under a variety of conditions (different solvents, elevated temperatures up to 80 °C, use of the isolated free base of 1, and use of the tosylate salt of 1) have failed. In the course of such experiments it was found that the isolated free base of 1 at 2 M in DMF at room temperature failed to react even with 2 M cyclohexylamine (which reacts readily with hydrochloride 1 under these conditions). The implication of this observation is that a soluble proton source (or possibly Lewis acid complexation) is required in order for 1 to guanylate an amine. For this reason it can be assumed that the reaction involves attack by the unprotonated amine on protonated 1. This hypothesis is consistent with the behavior observed for more weakly basic aromatic amines (e.g. 4-methoxyaniline) which can be slowly guanylated in DMF at room temperature even in the absence of DIEA without observable formation of diguanidine 2. When equimolar DIEA in DMF at room temperature was used for guanylation of 4-methoxyaniline by 1, a substantial amount of diguanidine 2 is observed due to the long reaction time required. Thus with aromatic amines, it is preferential to carry out the reaction with 1 in the absence of DIEA and an equivalent amount of the amine to avoid formation of the side product 2. In the case of aniline or aromatic amines in which basicity and nucleophilicity are reduced by electron-withdrawing or resonance-stabilizing ring substituents (e.g. 2-aminonaphthalene, 4-nitroaniline), the use of DMF as solvent at room temperature for guanylation by 1 was not successful as guanylation products were not detectable by TLC even after 60 h. Aniline could be guanylated by 1 only under more forcing conditions of elevated temperature (e.g., refluxing nitrobenzene). Attempts to guanylate the more deactivated aromatic amines

(13) Dryland, A.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 125.

(14) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

**Scheme I**

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mentioned above using 1, even under such forcing conditions, have thus far failed.

The chemical specificity with which 1 reacts with sufficiently nucleophilic aliphatic amines or hydrazines at room temperature merits discussion. As previously noted, 1 is essentially stable in aqueous and alcoholic solutions. It was also observed that 1 failed to produce observable products with potentially nucleophilic hydroxyl, carboxyl, thiol, indole, and imidazole groups under conditions which readily guanylate unhindered amines. The implication of these findings is that 1 should be useful as a guanylating reagent for the specific conversion of ornithine to arginine residues in peptides even in the presence of unprotected Ser, Thr, Asp, Glu, Cys, His, and Trp side chains. More generally, it appears that 1 can be applied to the synthesis of complex guanidines containing a variety of functional groups using minimal protection strategies. Additionally, 1 may also be useful as a reagent for the specific modification of proteins.

In order to demonstrate the feasibility of using 1 for solid-phase peptide synthesis of arginine-containing peptides by the "ornithine → arginine" strategy, Boc-Gly-His(Bom)-Orn(Fmoc)-Pro-Leu-Asp(Bn)-Lys(2-Cl-Z)-pMBHA-polystyrene resin was prepared by conventional solid-phase synthesis from the appropriately protected $N^{\alpha}$-Boc-amino acids starting with (*p*-methylbenzhydryl)-aminopolystyrene resin (0.77 mmol $NH_2$/g). After specific cleavage of the $\delta$-amino Fmoc group from ornithine (Scheme I), a portion of the peptidyl-resin was guanylated using an excess of 1 and DIEA in DMF at 47–8 °C for 135 min. After deprotection and isolation, the desired crude peptide product, Gly-His Arg-Pro-Leu-Asp-Lys-amide 10, was obtained in 88% overall yield (based on starting resin substitution and assuming isolation of the diacetate salt). Comparative HPLC data (Figure 4) indicated clean and successful guanylation with no observable unguanylated Orn[3] starting peptide present. The crude product was easily purified to homogeneity by preparative HPLC, and the identity of the peptide product was confirmed by FAB MS, amino acid analysis, and comparison to authentic material. These results clearly demonstrate the feasible and practical use of 1 for the solid-phase synthesis of a relatively small but reasonably complex biologically active arginine-containing peptide by an "$N^{\alpha}$-Boc/$\omega$-benzyl-/$N^{\delta}$-Fmoc" protection strategy. The use of 1 for the solution synthesis of several smaller biologically active peptides using $N^{\alpha}$-Boc protection has also been successful.[15] Work is in progress to examine whether this technology using 1 can be extended and generalized to include synthesis of larger and more complex peptide sequences (for example, containing multiple arginine and/or tryptophan residues). The potential uses of 1 for protein, natural product, and aminopolymer modification remain to be examined.

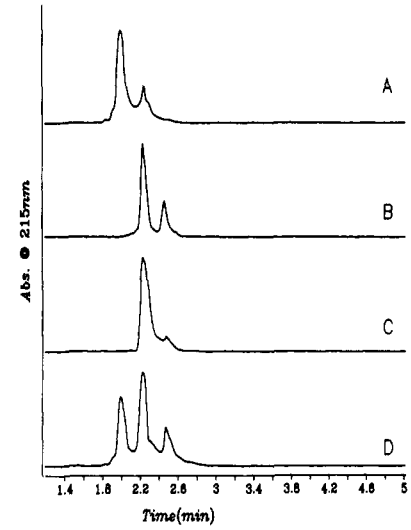


**Figure 4.** HPLC comparison of crude peptide products: (A) chromatogram of Gly-His-Orn-Pro-Leu-Asp-Lys-amide; (B) chromatogram of Gly-His-Arg-Pro-Leu-Asp-Lys-amide (10) prepared by guanylation with 1 of the same peptidyl resin used to produce the result shown in A (Orn precursor); (C) chromatogram of Gly-His-Arg-Pro-Leu-Asp-Lys-amide prepared by standard solid phase synthesis using Boc-Arg(Tos)-OH; (D) chromatogram of a mixture of the peptides used in A and B. See Experimental Section for details and chromatography conditions.

## Summary and Conclusions

1*H*-Pyrazole-1-carboxamidine hydrochloride (1) can be smoothly prepared in excellent yield from readily available starting materials and has stability, reactivity, and solubility properties desirable in a versatile reagent for the efficient and chemically specific guanylation of sterically unhindered primary and secondary aliphatic amines under mild conditions. Unless aromatic amines contain an activating substituent (e.g., 4-methoxyaniline), guanylation by 1 at room temperature is not possible. Other potentially nucleophilic functional groups (hydroxyl, carboxyl, thiol, imidazole) do not observably react with 1 under the mild conditions used for efficient guanylation of simple amines. The feasibility of the use of 1 for the practical synthesis of small peptides by an "ornithine → arginine" strategy has been demonstrated. Additional work is required on more complex peptide targets before the use of 1 for the synthesis of arginine-containing peptides can become generalized and commonplace, although preliminary results suggest the possibility of such an application. Applications of 1 to guanylation of proteins, drugs, drug candidates, natural products, aminopolymers, and in general organic synthesis strategies may also be important.

## Experimental Section

**General.** Melting points were obtained using a micro hot plate apparatus and are uncorrected. NMR spectra were obtained on instruments operating at either 300 or 400 MHz using tetramethylsilane as internal standard. Mass spectra were obtained on either CI, FAB, or ion spray instrumentation operating in house. Elemental analyses were performed on site at the analytical

(15) Bernatowicz, M. S. Unpublished results.

facility. Hydrolysis of peptides for amino acid composition analysis was according to the procedure of Liu and Boykins.[16] Amino acid analysis was carried out on a commercial instrument using ion-exchange chromatography, sodium citrate buffers, and postcolumn ninhydrin detection. Solid-phase peptide synthesis was conducted on an ABI 431A automated peptide synthesizer (Foster City, CA) using reagents and protocols specified by the vendor for the "Boc/benzyl" synthesis strategy. (*p*-Methylbenzhydryl)aminopolystyrene resin (0.77 mmol $NH_2$/g) and protected amino acids, except for Boc-Orn(Fmoc)-OH (Peninsula Laboratories, Belmont, CA), were purchased from ABI (Foster City, CA). Pyrazole, cyanamide, 3,5-dimethylpyrazole-1-carboxamidine nitrate, and nitrobenzene were purchased from Aldrich (Milwaukee, WI) and were used as supplied. Cyclohexylamine (Aldrich) was distilled under nitrogen before use. 4-Methoxyaniline (Aldrich) was recrystallized from hexanes. HCl (4 N) in dioxane was purchased from Pierce Chemical (Rockford, IL). DIEA, piperidine, 2-ethanolamine, and analine were purchased from Fluka (Buchs, Switzerland) and were used as supplied. Glycine was obtained from Sigma (St. Louis, MO). Sequencing-grade DMF was from Fisher Scientific (Fair Lawn, NJ) and was used without purification. All other solvents were analytical reagent grade or better and were used as supplied. TLC was performed on precoated silica gel 60 F254 plates which were developed using $CH_2Cl_2$–MeOH–$NH_4OH$ (40:10:2). Compounds on TLC plates were visualized with UV light, iodine, and 2% ninhydrin in EtOH. Analytical HPLC was performed using a YMC (Morris Plains, NJ) column (C18 bonded silica, 4 mm × 5 cm, 3-μm particle) and a commercial pumping system with high pressure mixing and 0.01-mL pump head volume. Peaks were observed with a photodiode array detector. Solvents used for HPLC elution were (A) $H_2O$ containing 0.1% TFA, (B) 95% acetonitrile in $H_2O$ containing 0.1% TFA. The column was eluted with a linear gradient of solvent A going to 21% solvent B in A over the course of 5 min at a flow rate of 2.0 mL/min. For preparative HPLC a Waters (Milford, MA) PrepPak (C18, 25 × 100-mm, 100-Å pore) column was eluted with the same gradient over the course of 16 min at 20.0 mL/min (230-nm detection).

**1*H*-Pyrazole-1-carboxamidine Hydrochloride (1).** To pyrazole (8.17 g, 0.12 mol) and cyanamide (5.05 g, 0.12 mol) in 120 mL of *p*-dioxane was added 31 mL of 4 N HCl in *p*-dioxane. The mixture was gently refluxed with stirring for 2 h under nitrogen. During the course of the reaction the product crystallizes. After cooling to room temperature, 30 mL of anhydrous ether was added and the mixture allowed to stand for 30 min. The white crystalline product was collected by filtration, washed with anhydrous ether, and dried to constant weight in vacuo to yield 16.7 g (95%): mp 167–68 °C (lit.[8] mp 165–66 °C); $^1H$ NMR (DMSO-$d_6$) δ 6.84 (t, 1), 8.13 (s, 1), 9.03 (d, 1), 9.75 (br s, 4); $^{13}C$ NMR (DMSO-$d_6$) δ 112, 132, 146, 153. MS/CI 111 $(M + H)^+$, calcd (as free base) 110 (M).

**N-Amidinopyrazole-1-carboxamidine Hydrochloride (2).** To 1 (0.293 g, 2.0 mmol) in 0.5 mL of DMF was added DIEA (0.348 mL, 2.0 mmol), and the mixture was stirred for 64 h at room temperature. Ether (15 mL) was added, and the resulting sticky solid product was collected, washed with ether, and dried in vacuo to yield 0.38 g (97%) of crude product. Recrystallization of the crude product from ethanol/ether yielded 0.22 g (57%) white crystalline solid: mp 150 °C (softens), 162–65 °C (lit.[12] mp 172–74 °C, reported as anhydrous salt); $^1H$ NMR (DMSO-$d_6$) δ 6.57 (s, 1), 7.85 (s, 1), 8.20 (br s, 4), 8.35–8.37 (m, 3); MS/FAB 153 $(M + H)^+$, calcd (as free base) 152 (M), 187 $(M + Cl)^-$, calcd 187 (M + Cl).

Anal. Calcd for $C_5H_8N_6 \cdot HCl \cdot H_2O$: C, 28.79; H, 5.42; N, 40.30. Found: C, 28.83; H, 5.15; N, 40.26.

**Procedure A.** This procedure is useful for guanylation of sterically unhindered primary and secondary aliphatic amines that are DMF soluble. To the amine, 1, and DIEA (2.0 mmol each), was added DMF sufficient to produce a final concentration of approximately 2 M reactants. The reaction mixture was stirred at room temperature under nitrogen while being monitored by TLC. After a few h, ether (10–15 mL) was added to complete precipitation of the crude product which was collected, washed with ether, and dried. The crude product was recrystallized from an appropriate solvent system and dried in vacuo.

**Procedure B.** This procedure was used for the guanylation of glycine and may be useful for other DMF-insoluble amines or amino acids that are soluble in aqueous base. A mixture of glycine (0.15 g, 2.0 mmol), 1 (0.293 g, 2.0 mmol), and 2.0 mL of 1.0 M $Na_2CO_3$ was stirred for 3 h at room temperature. The white solid product which separates was collected and washed with several small portions of MeOH/$H_2O$ (1:1). The product was dried to constant weight in vacuo to yield 178 mg (77%): mp 280 °C dec (lit.[17] mp 280–84 °C); MS/FAB 118 $(M + H)^+$, calcd 117 (M).

**Procedure C.** This procedure was used for the guanylation of 4-methoxyaniline and may be useful for other DMF-soluble aromatic amines with activating substituent(s). A mixture of 4-methoxyaniline (0.246 g, 2.0 mmol), 1 (0.308 g, 2.1 mmol), and DMF (0.81 mL) was stirred for 21 h at room temperature. The solvent was removed in vacuo under 40 °C, and the resulting oily residue was washed twice with 5 mL of warm ether. The residue was crystallized from acetonitrile/ether to give 361 mg (90%) of crude product. Recrystallization from EtOH/acetonitrile/ether provided 234 mg (58%) of white crystalline solid: mp 144–46 °C; MS/FAB 166 $(M + H)^+$, calcd (as free base) 165 (M).

Anal. Calcd for $C_8H_{11}N_3O \cdot HCl$: C, 47.65; H, 6.00; N, 20.84; Cl, 17.58. Found: C, 47.57; H, 5.98; N, 21.26; Cl, 17.67.

**Procedure D.** This procedure was used for the guanylation of 4-methoxyaniline and may be a useful alternative to procedure C for guanylation of aromatic amines with activating substituent(s). A solution of 4-methoxyaniline (0.271 g, 2.2 mmol), 1 (0.293 g, 2.0 mmol), DIEA (0.366 mL, 2.1 mmol), and 1.0 mL of $H_2O$/acetone (1:1) was stirred for 2.5 h at room temperature and then was warmed to 50 °C for 3 h. The reaction mixture was extracted three times with 10 mL ether and the aqueous phase lyophilized to provide 361 mg (90%) of crude product which was recrystallized from MeOH/ether to give 242 mg (60%): mp 146–48 °C; MS/FAB 166 $(M + H)^+$, calcd (as free base) 165 (M).

**Procedure E.** A mixture of aniline (0.365 mL, 4.0 mmol), 1 (0.586 g, 4.0 mmol), and 1.0 mL of nitrobenzene was refluxed for 5 h. After cooling to room temperature, 10 mL of ether was added, and the mixture was cooled to 4 °C. The crude product seperated as a brown-colored oil, the supernatant was removed by decantation, and the residue was dried in vacuo. An excess of moist picric acid in a minimum of EtOH was mixed with the crude product, and the yellow picrate salt of the product crystallized. The product was collected, washed with ether/EtOH (4:1), and dried in vacuo to provide 0.69 g (48%): mp 224–28 °C (lit.[8] mp 224–26 °C); MS/CI 136 $(M + H)^+$, calcd (as free base) 135 (M).

**Cyclohexylamine-*N*-carboxamidine Hydrochloride (4).** The title compound was prepared by procedure A (reaction time 4 h) and was obtained in 84% yield after recrystallization from EtOH/ethyl acetate: mp 228–29 °C, (lit.[6] mp 228–29 °C); MS/CI 142 $(M + H)^+$, calcd (as free base) 141 (M).

**Piperidine-*N*-carboxamidine Hydrochloride (5).** The title compound was prepared by procedure A (reaction time 4 h) and was obtained in 71% yield after recrystallization from EtOH/acetone: mp 186–87 °C, (lit.[6] mp 187–88.5 °C); MS/CI 128 (M + H)$^+$, calcd (as free base) 127 (M).

**2-Ethanolamine-*N*-carboxamidine Hydrochloride (6).** The title compound was prepared by procedure A (reaction time 8 h) and was obtained in 88% yield after recrystallization from MeOH/ether: mp 97–98 °C; MS/ion spray 104 $(M + H)^+$, calcd (as free base) 103 (M).

Anal. Calcd for $C_3H_9N_3 \cdot HCl$: C, 25.81; H, 7.22; N, 30.10; Cl, 25.40. Found: C, 25.90; H, 7.35; N, 29.98; Cl, 25.81.

**Glycine-*N*-carboxamidine (7).** Preparation and characterization of the title compound is given in procedure B.

**4-Methoxyaniline-*N*-carboxamidine Hydrochloride (8).** Preparation and characterization of the title compound is given in procedures C and D.

**Aniline-*N*-carboxamidine Picrate (9).** Preparation and characterization of the title compound is given in procedure E.

**Gly-His-Arg-Pro-Leu-Asp-Lys-amide (10).** Boc-Gly-His(Bom)-Orn(Fmoc)-Pro-Leu-Asp(Bn)-Lys(2-Cl-Z)-pMBHA-polystyrene resin (0.24 g, 0.1 mmol of peptide theoretical) was swelled

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and saturated with DMF and then treated twice with 20% piperidine in DMF (1 min, 7 min) at room temperature. After removal of excess reagent the peptidyl-resin was washed liberally with DMF and treated with 1 (0.405 g, 2.76 mmol) and DIEA (0.545 mL, 3.13 mmol) diluted to 1.5 mL with DMF, and the reaction was allowed to proceed at 47–8 °C for 135 min after which the Kaiser ninhydrin test[14] of a resin sample was negative. The resulting peptidyl-resin was washed four times each with DMF then $CH_2Cl_2$, and the Boc group cleaved with TFA/$CH_2Cl_2$ (1:1) for 15 min at room temperature. After removal of excess reagent the peptidyl-resin was washed successively with $CH_2Cl_2$, MeOH, and ether and dried in vacuo to yield 0.215 g of peptidyl-resin. The entire sample was treated at 0 °C with 10 mL of HF containing 0.5 mL of anisole, and the mixture was stirred for 1 h. After evaporation of HF, the resin was washed four times with ether and the crude product extracted with 10 mL of 5% aqueous acetic acid. Lyophilization of the extract furnished 83 mg of white powder (88% overall yield based on starting resin substitution and assuming isolation of the diacetate salt). HPLC data for the crude product is given in Figure 4. The crude product was purified by preparative HPLC as described in the General Experimental Section to yield 45 mg (43%, as the bis-TFA salt) of homogeneous product: MS/FAB 821 $(M + H)^+$, calcd (as free peptide) 820 (M); amino acid analysis, Asp 0.91 (1), Pro 1.16 (1), Gly 0.99 (1), Leu 1.05 (1), His 0.87 (1), Lys 0.97 (1), Arg 1.04 (1).

**Acknowledgment.** We are indebted to the Bristol-Myers Squibb Pharmaceutical Research Institute for support of this work. We thank Mr. Paul J. Reiss for amino acid analysis and HPLC work and Dr. H.-G. Chao for critically reading the manuscript as well as interesting discussions during the course of these studies. We are also grateful to Prof. M. Bodanszky for an interesting discussion at an early stage of this work regarding the feasibility of guanylating ornithine peptides.

**Supplementary Material Available:** $^1H$ NMR spectra of guanidine products 5–10 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

## Photochemistry of 4-Substituted (Phenylethynyl)triphenylborate Salts: Analysis of the Visible-Region Electronic Absorption Spectra of Tetraarylboratirene Anions

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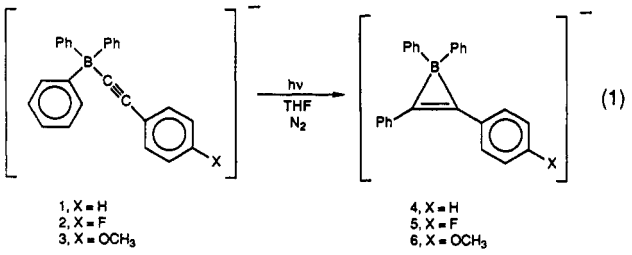
*Received November 19, 1991*

### Introduction

Interest in the chemistry of boron-containing substances has recently been boosted[1] by the discovery that highly reactive compounds containing a boron atom in a three-membered ring can be isolated and fully characterized.[2,3] One of the unusual features of these compounds is their relatively intense color. The boratanorcaradiene anions are deep red, and the boratirene anions are yellow.[3] For comparison, the hydrocarbon analogues of these compounds, norcaradienes and cyclopropenes, respectively, are colorless with strong absorptions only in the deep UV spectral region.[4] We have attributed the unusual absorption of the three-membered boron-containing anions to an electronic effect.[3,5] Others have suggested that the color is due to a small amount of ring-opened carbanion present in equilibrium with the ring-closed structure that is characterized by NMR spectroscopy and by X-ray crystallography.[6] We report herein the results of investigations designed to help resolve this issue. Irradiation of 4-substituted (phenylethynyl)triphenylborate anions (**1**, **2**, or **3**) gives substituted boratirenes **4**, **5**, **6**, respectively, as is shown in eq 1. The substituents were chosen so that



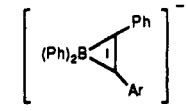
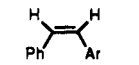

(1)

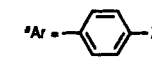
1, X = H
2, X = F
3, X = $OCH_3$

4, X = H
5, X = F
6, X = $OCH_3$

they might alter the amount of ring-opened anion present in equilibrium but, by analogy with other chromophores, should affect only slightly the absorption spectrum of the ring-closed borate anion.[7]

**Table I. Absorption Spectra in Acetonitrile Solvent**

| compd[a] | $\lambda_{max}$, nm ($\epsilon_{max}$, $M^{-1}$ $cm^{-1}$) H | OMe | F |
|---|---|---|---|
| $[(Ph)_3BC{\equiv}CAr]^-$ | 259 (19300)<br>266 (19200) | 260 (28000)<br>269 (28000) | 255–260<br>(19000) |
|  | 274 (16000)<br>325 (11500)<br>400 (2000) | 260 (20300)<br>340 (6000)<br>400 (1900) | 270 (20000)<br>330 (7000)<br>390 (1300) |
|  | 276 (10900) | 285 (14400) | 274 (11000) |

[a] Ar = 

### Results

The synthesis of borate **1** has already been reported,[3] borates **2** and **3** were prepared by an analogous procedure starting from (4-fluorophenyl)acetylene and (4-methoxyphenyl)acetylene. As potassium salts, borates **2** and **3** show single peaks in their $^{11}B$ NMR spectra at $\delta$ −12.6 and −12.7, respectively. Significantly, the UV spectra of borates **1**, **2**, and **3** are very similar both in band position and intensity. These data are summarized in Table I.

The irradiation of the potassium salt of borate **2** in THF solution at 254 nm was followed by $^{11}B$ NMR spectroscopy. As the reaction proceeds, the resonance due to starting material decreases and a new peak at $\delta$ −16.3 appears. A

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