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Substituted Guanidines: Introducing Diversity in Combinatorial Chemistry

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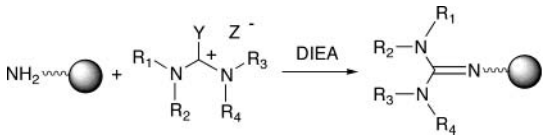
ABSTRACT



The guanidine moiety is an important motif present in many biologically active compounds. Fully substituted guanidines are of key importance for the development of bioactive molecules. The present paper reports on an efficient procedure for the direct solid-phase conversion of amines to fully substituted guanidines under very mild conditions.

Guanidine functions are an important motif often present in natural products as well as in many compounds having therapeutic activity.[1] This moiety is fully protonated under physiological conditions due to its strongly basic character, a fact which is crucial for specific ligand−receptor interactions.[2]

Consequently, procedures that allow for the preparation of guanidine-derived products in high yield under mild conditions are of great interest in medicinal chemistry.

Although fully substituted guanidines are not common, their presence can facilitate binding to complex receptors and therefore can be of key importance for the development of bioactive molecules.[2c,3] Many methods have been described for the preparation of functionalized guanidines either in solution[4] or by solid-phase methods,[1f−h,2c,3c,5] but they do not allow the formation of pentasubstituted guanidines.

The present paper reports on a new and convenient method for the solid-phase preparation of pentasubstituted guanidines which involves the use of aminium/uronium salt-based reagents. These compounds have been used mainly as

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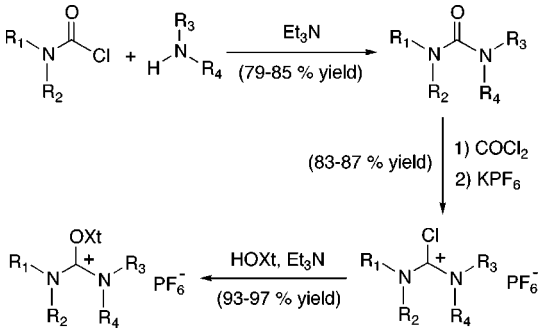
coupling reagents in peptide synthesis by activating the carboxyl group of the amino acid.[6] However, during the much slower activation of hindered amino acids, protected peptide segments, or carboxylic acids involved in cyclization, the aminium/uronium salt may undergo reaction with the amino component to give the corresponding guanylated derivative.[7,8]

We have taken advantage of this side reaction and used it for the synthesis of pentasubstitued guanidines. By treatment of an amine function with one of these aminium/uronium salts under basic conditions, the guanidine derivatives are obtained in high yields and purities.

The synthesis of the various guanylating agents bearing different substituents (R_n) can be performed by modification of standard methods previously described for the corresponding HBTU and HATU analogues (Scheme 1).[9−11] This

Scheme 1. General Procedure for the Synthesis of Guanylating Agents

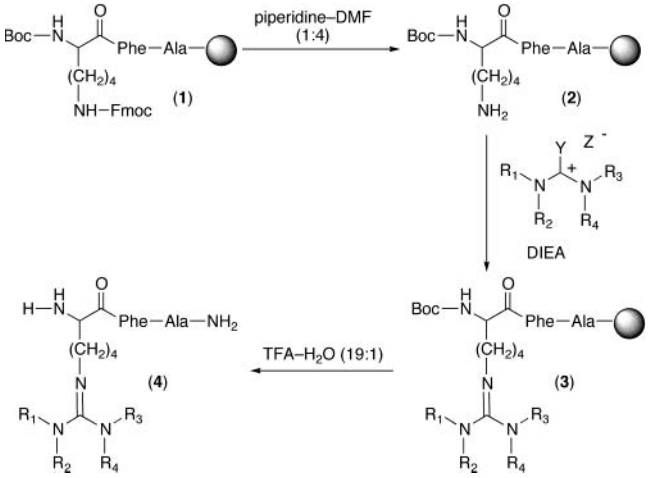


method allows the preparation of a broad range of reagents.

As a model target, the tripeptide Boc-Lys(Fmoc)-Phe-Ala-amide-linker-resin was chosen.[12] After removal of the Fmoc group with piperidine, the ϵ-amino function of lysine was converted to its guanylated form (Scheme 2). The guanylation reaction was first performed under various reaction conditions with commercial HATU in order to establish the efficiency of these compounds as guanylating agents (Table 1).

When treated with 1.2 equiv of the reagent for 2 h (entry 1, Table 1), a 92% purity was achieved with a very clean HPLC profile and the correct mass as shown by MALDI-

Scheme 2. Synthesis of the Guanylated Peptides



TOF-MS. With 5 equiv of the reagent (entry 3), a slightly higher purity (96%) was obtained but increasing the reaction time from 0.5 to 2 h (entries 3 vs 4) gave no change in the yield or the HPLC profile. Because of the known instability of some of these reagents under basic conditions, an experiment in which fresh reagent was added after 1 h of reaction time was also performed, but no major changes were detected (entries 2 and 5).

From these preliminary experiments, it is clear that the reaction occurs with very high efficiency, since a very high yield of pure product is obtained with only 1.2 equiv of reagent. Second, the series of guanylating agents shown in Figure 1was used to test the versatility of the reaction. This series includes reagents with different substituents in the dialkyl amino function (R_n) and different leaving groups (Y). The purity yields for this series of reactions are shown in Table 2. Formation of the guanylated derivative proceeded in almost quantitative yields in all cases, and no remarkable

Table 1. Study of the Number of Equivalents of HATU and the Reaction Time for the Guanylation of Boc-Lys-Phe-Leu-AM-MBHA

entry	reagents (equiv)	solvent	time, h	purity of **4** (%)[a]
1	HATU (1.2) DIEA (2.4)	DMF	2	92
2	HATU (1.2 + 1.2) DIEA (2.4)	DMF	1 + 1[b]	94
3	HATU (5) DIEA (10)	DMF	0.5	96
4	HATU (5) DIEA (10)	DMF	2	96
5	HATU (5 + 5) DIEA (10)	DMF	1 +1[b]	96
6	HATU (5 + 5) DIEA (10)	DMF	overnight	96

[a] As determined from integration of the HPLC chromatogram. [b] The resin was first treated with the guanylating reagent and DIEA and allowed to react for 1 h, and then fresh guanylating reagent was added.

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(8) Chloroformamidinium salts were used by Barton et al. (Barton, D. H. R.; Elliot, J. D.; Gero, S. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2085−2090) for the preparation of sterically hindered guanidine bases in a strategy similar to that the described in this work. The use of the aminium/uronium salts derived from either HOBt or HOAt has the advantage of the great shelf stability of these reagents, which allows them to be stored for long periods of time.

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(10) Among these reagents HATU is commercially available.

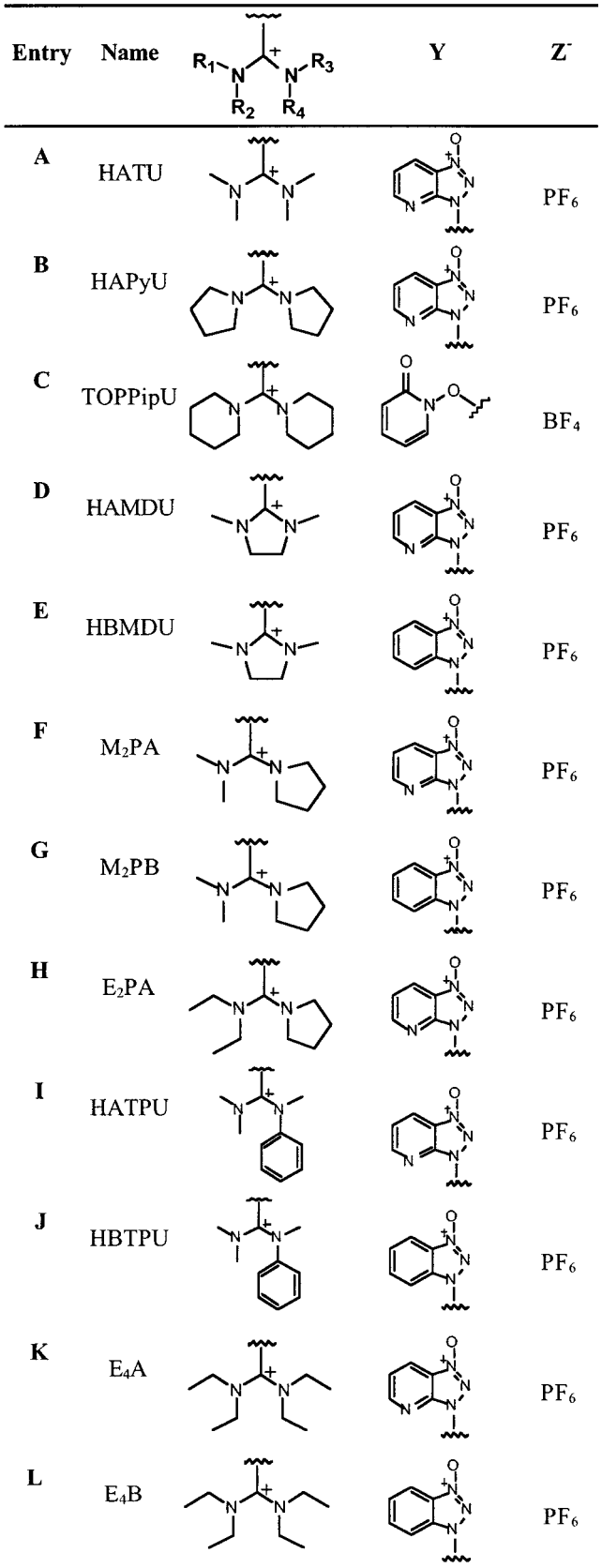

Figure 1. Structures of aminium/uronium salt-based reagents.

Table 2. Study of Guanylation by Reagents Other Than HATU[a]

entry	reagents (equiv)	solvent	purity of **4** (%)[b]
1	HAPyU (5) DIEA (10)	DMF	97
2	TOPPipU[c](5) DIEA (10)	DMF	91
3	HAMDU (5) DIEA (10)	DMF	92
4	HBMDU (5) DIEA (10)	DMF	91
5	M_2PA (5) DIEA (10)	DMF	99.9
6	M_2PB (5) DIEA (10)	DMF	90
7	E_2PA (5) DIEA (10)	DMF	93
8	HATPU (5) DIEA (10)	DMF	93
9	HBTPU (5) DIEA (10)	DMF	96
10	E_4A (5) DIEA (10)	DMF	97
11	E_4B (5) DIEA (10)	DMF	94

[a] All reactions were carried out overnight by adding the solvent and the base to the resin and adding the guanylating agent as a solid. [b] As determined from integration of the HPLC chromatogram. All products were charaterized by MALDI-TOF-MS. [c] This reagent is known to be less reactive than others; therefore the reaction was performed by adding HOAt as an additive.

difference is observed when changing the leaving group (Y). Most efficient appeared to be the reagent M_2PA (entry 5, Table 2), but the generality of this effect for the dimethylamino/pyrrolidinyl reagent will require further study.

(11) Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC−IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983. The following additional abbreviations are used: Boc, *tert*-butyloxycarbonyl; DIEA, *N*,*N*′-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; E_4A, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetraethyluronium hexafluorophosphate; E_4B, *O*-(benzotriazol-1-yl)-1,1,3,3-tetraethyluronium hexafluorophosphate; E_2PA, *O*-(7-azabenzotriazol-1-yl)-1,1-diethyl-3,3-dimethyleneuronium hexafluorophosphate; Fmoc, 9-fluorenylmethoxycarbonyl; HAMDU, *O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate; HAPyU, 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HATPU, *O*-(7-azabenzotriazol-1-yl)-1,1,3-trimethyl-3-phenyluronium hexafluorophosphate; HBMDU, *O*-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate; HBTPU, *O*-(benzotriazol-1-yl)-1,1,3-trimethyl-3-phenyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; Linker AM, *p*-[*R*,*S*]-α-[1-(9*H*-fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid; MALDI-TOF MS, matrix-assisted laser desorption ionization/time-of-flight mass spectrometry; TFA, trifluoroacetic acid; M_2-PA, *O*-(7-azabenzotriazol-1-yl)-1,1-dimethyl-3,3-dimethyleneuronium hexafluorophosphate; M_2PB, *O*-(benzotriazol-1-yl)-1,1-dimethyl-3,3-dimethyleneuronium hexafluorophosphate; TOPPipU, 2-[2-oxo-1(2*H*)-pyridyl]-1,1,3,3-bis(pentamethylene)uronium tetrafluoroborate. Amino acid symbols denote L-configuration unless indicated otherwise.

(12) The first two amino acids were introduced using a standard Fmoc/*t*Bu protocol. Fmoc-amide-linker-resin was washed with DMF (5 × 0.5 min), and Fmoc removal was accomplished with piperidine−DMF [(1: 4), 1 × 1 min, 2 × 5 min], followed by washes with DMF (5 × 0.5 min).

In summary, a new synthetic method for the preparation of pentasubstituted guanidines is presented. The reaction consists of an attack of an amino function on the guanylating agent. The reaction occurs under mild conditions and without producing any significant side products.

Fmoc-amino acids (5 equiv) were activated with DIPCDI (5 equiv) in the presence of HOBt (5 equiv) in DMF, and the coupling was carried out for 2 h at 25 °C. In all cases, the Kaiser test (Kaiser, E.; Colescott, R. L.; Bosinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595−598) was negative. The last amino acid was introduced in its N^{α}-Boc, N^{ϵ}-Fmoc form following the same procedure. After removal of the Fmoc group of Lys, DIEA (2 equiv with respect to the guanylating agent) was added in DMF and then the aminium/uronium salt. The reaction was also followed by the Kaiser test and after the reaction the test was negative. After all reactions, the resin was washed with DMF (5 × 0.5 min) and DCM (5 × 0.5 min). Finally, treatment of the resin with TFA−H_2O (19:1) caused simultaneous elimination of the Boc group and cleavage of the peptide from the resin.

As part of our solid-phase combinatorial library synthesis program, we are currently applying this methodology to the preparation of libraries of solid phases based on 2,5-diketopiperazine and hydantoins as scaffolds.

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