The Solid State and Solution Structure of HAPyU†,‡,§

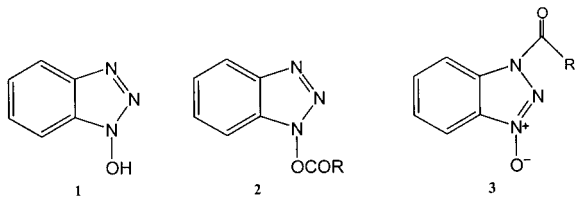
Louis A. Carpino,*,|| Peter Henklein,*,⊥ Bruce M. Foxman,*,# Iman Abdelmoty,# Burkhard Costisella,▽ Victor Wray,○ Tobias Domke,○ Ayman El-Faham,|| and C. Mügge⊥

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, Institute of Biochemistry, Humboldt University, 10098 Berlin, Germany, Department of Chemistry, Brandeis University, Waltham, Massachusetts 02454, Institut für Angewandte Chemie, Berlin-Adlershof, 12489 Berlin, Germany, and Gesellschaft für Biotechnologische Forschung, 38124 Braunschweig, Germany

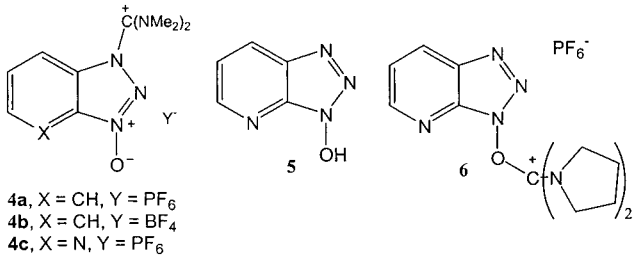
carpino@chem.umass.edu

Received November 13, 2000

In their classic study of additives for peptide coupling, König and Geiger[1] reported that the addition of *N*-hydroxybenzotriazole **1** to DCC-mediated coupling reactions caused the suppression of various side reactions,



in particular, the loss of configuration at the reactive carboxylic acid site via the intermediate formation of an active ester. Depending on the case, such active esters exist either as the simple ester **2** or as mixtures of, or in equilibrium with, the corresponding 3-acyl-1-oxide **3**.[1,2] Subsequently, coupling reagents HBTU[3] **4a** and TBTU[4] **4b** were shown to generate the same intermediates upon treatment of the appropriate carboxylic acid with a



4a, X = CH, Y = PF_6
4b, X = CH, Y = BF_4
4c, X = N, Y = PF_6

tertiary amine. Later, the 7-aza analogues **5** and **4c** were shown to be even more effective replacements for **1** and **4a**.[5,6] Then, on the basis of reports that the pyrrolidine analogues of BOP reagent and HBTU were more effective than BOP reagent and HBTU, respectively,[8] it was shown that the same held true for HAPyU relative to HATU, especially in controlling the loss of configuration during segment coupling.[9] This seemed unusual, considering that both HATU and HAPyU should lead to the formation of the same active ester/amide mixture of **2** and **3**, and it was speculated that this difference might be due to differences in the structures of the two coupling reagents, with HAPyU perhaps existing as a uronium species **6** in contrast to HATU for which structure **4c** was established.

Now we show that HAPyU, according to X-ray structure determination, also crystallizes in the guanidinium form **7** (Figure 1). Three separate crystal forms were obtained, two solvates and one solvent-free form. In each case, the structure corresponds to the guanidinium form shown in **7**. According to the ^{15}N NMR spectrum, the same structure is maintained in solution. Thus, in DMSO-d_6, five signals are observed at 327.4, 316.8, 285.2, 167.9, and 128.6 ppm relative to ammonia. The signals are of approximately equal intensity except for the signal at 128.6 ppm that, being twice as intense, is assigned to the five-membered ring nitrogen atom. The three re-

† A portion of this work was reported in preliminary form at the 24th European Peptide Symposium. See: Henklein, P.; Costisella, B.; Wray, V.; Domke, T.; Carpino, L. A.; El-Faham, A.; Kates, S. A.; Abdelmoty, I.; Foxman, B. M. In *Peptides 1996. Proceedings of the 24th European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower Scientific, Ltd.: Kingswinford, U.K., 1998; p 465.

‡ Full details of the X-ray crystallographic structure determination for all three crystalline modifications of HAPyU will be provided in a separate communication (B.M.F and I.A.).

§ Abbreviations: BOP = (benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium hexafluorophosphate; DCC = dicyclohexylcarbodiimide; DMSO = dimethyl sulfoxide; 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]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBPyU = 1-(1-pyrrolidinyl-1*H*-benzotriazol-1-ylmethylene)-pyrrolidinium hexafluorophosphate *N*-oxide; HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt = 7-aza-1-hydroxybenzotriazole; HOBt = 1-hydroxybenzotriazole; TBTU = *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide.

|| University of Massachusetts.

⊥ Humboldt University.

Brandeis University.

▽ Institut für Angewandte Chemie.

○ Gesellschaft für Biotechnologische Forschung.

(1) König, W.; Geiger, R. *Ber. Dtsch. Chem. Ges.* **1970**, *103*, 788.

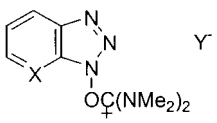
(2) For references to the X-ray crystallographic determination of structures of both types, see: (a) Crisma, M.; Moretto, F.; Formaggio, F.; Toniolo, C. *Z. Kristallogr.* **1999**, *214*, 766. (b) Vlassi, M.; Germain, G.; Barlos, K.; Mamos, P.; Refaat, L. S. *Z. Kristallogr.* **1990**, *192*, 59.

(3) (a) Dourtoglou, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, *19*, 1269. (b) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Ziodrou, C. *Synthesis* **1984**, 572.

(4) Knorr, R.; Trzeciak, A.; Bannworth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

(5) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(6) Initially, HBTU[3a] and HATU[5] were assigned uronium salt structures **i**, but later



i, X = CH, N; Y = PF_6, BF_4

X-ray crystallographic analysis established that, at least in the crystalline state, the structures were of the guanidinium type **4a**−**c**.[7]

(7) Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57.

(8) (a) Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205. (b) Chen, S.; Xu, J. *Tetrahedron Lett.* **1992**, *33*, 647.

(9) (a) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695. (b) Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561. (c) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460. (d) Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831.

10.1021/jo001616+ CCC: $20.00
Published on Web 07/04/2001

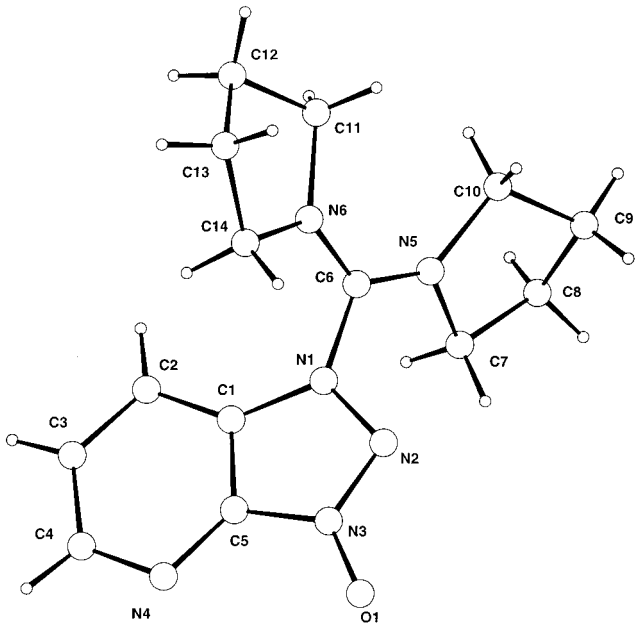
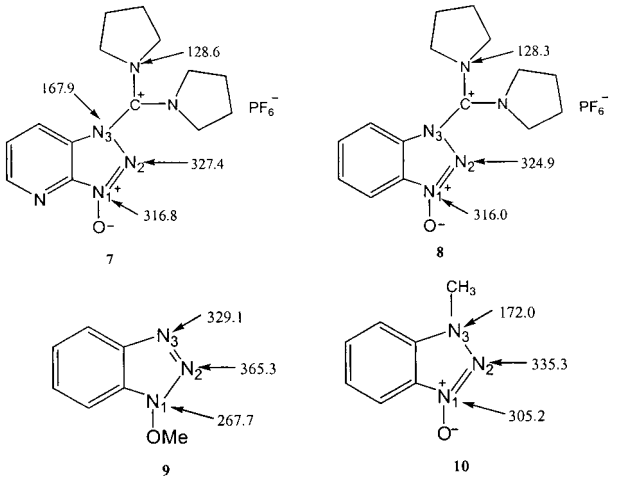


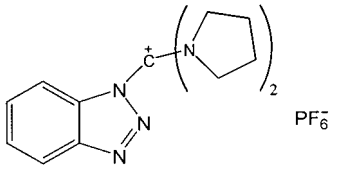
Figure 1. Molecular structure of HAPyU. The anion is not shown. For the full CIF report see the Supporting Information.

Scheme 1. ^{15}N NMR Assignments for HAPyU and HBPyU



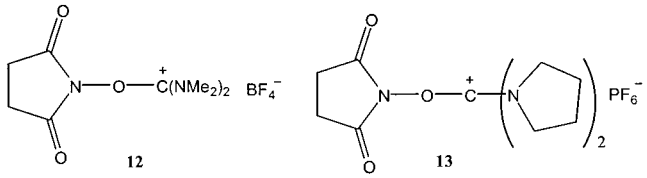
maining signals were then assigned to the benzotriazole system as a guanidium *N*-oxide upon comparison with previously reported data of benzotriazole analogues **9** and **10** (Schilf et al.,[10] Scheme 1): N1, 316.8 ppm; N2, 327.4 ppm; N3, 167.9 ppm. The ^{15}N data for HAPyU **7**, its HOBt analogue **8**, and the known model species **9** and **10** are shown in Scheme 1. Since HBPyU **8** was relatively insoluble in DMSO-d_6, the ^{15}N NMR data were incomplete, with N3 being only equivocally detected.

In confirmation of the ^{15}N NMR results, the ^{13}C NMR spectra of HAPyU in the solution and solid state are basically the same (Table 1, Supporting Information). The position for the carbenium carbon atom (δ 144.2 ppm) is similar to that for HBPyU (δ 144.3 ppm), confirming the guanidinium structure in that case also. The model, non-oxygenated species PBPH **11** shows its



11

carbenium carbon at 146.3 ppm. For related salts[4] **12** and **13**, which can exist only in the uronium form, the



12 **13**

carbenium carbon appears at 160.7 and 155.1 ppm, respectively.[11] For both structural types (O- or N-linked carbenium species) the bispyrrolidinyl systems are generally found ca. 5 ppm lower than the dimethylamino systems, e.g., for the two guanidinium systems 6-Me-HATU (δ 150.1 ppm) vs 6-Me-HAPyU (δ 144.4 ppm).[12]

In summary, it is shown that for the pyrrolidine analogues of HATU and HBTU, the guanidinium form is preferred in both solid state and solution. Recently, the N-form has been calculated to be more stable than the O-form by about 4.9 kcal/mol.[13,14] Thus, the reactivity differences between HATU and HAPyU must be sought in other effects, perhaps in the existence of intermediates preceding the formation of the active ester/amide.[15,16]

Experimental Section

Synthesis of HAPyU. The synthesis followed an earlier description.[9a] The sample had a mp of 152−155 °C, possibly a polymorphic form of that previously observed (mp: 118−120 °C dec). The ^{1}H NMR data were unchanged.

1-(1-Pyrrolidinyl-1*H*-benzotriazol-1-ylmethylene)pyrrolidinium Hexafluorophosphate (PBPH, 11). Benzotriazole (119.1 mg, 1 mmol) was dissolved in 5 mL of dry CH_2Cl_2, and 0.124 mL (0.5 mmol) of *N*,*O*-bis(trimethylsilyl)acetamide was added. After stirring at room temperature for 10 min, 322.6 mg (1 mmol) of chloro-*N*,*N*,*N*′,*N*′-bis(tetramethylene)formamidinium hexafluorophosphate was added. The mixture was stirred for 3 h at room temperature, the solvent was removed, and the residue was recrystallized from acetonitrile/ether to give 365 mg (88%) of the salt as white crystals. Mp: 227−229 °C. ^{13}C NMR (DMSO-d_6): δ 146.3, 144.7, 131.1, 130.2, 126.4, 120.6, 111.7, 52.5, 47.3, 25.7, 23.8. Anal. Calcd for $C_{15}H_{20}N_5PF_6$: C, 43.38; H, 4.85; N, 16.86. Found: C, 43.24; H, 4.85; N, 16.77.

Crystal Growth of HAPyU. HAPyU **7** was obtained from Perseptive Biosystems, Inc. Three forms of **7** were obtained using

(10) Schilf, W.; Stefaniak, L.; Witanoski, M.; Webb, G. A. *Magn. Reson. Chem.* **1985**, *23*, 181.

(11) For chemical shift positions for carbenium ions bearing donor substituents, see: Kalinovsky, H.-O.; Berger, S.; Braun, S. In *^{13}C NMR Spektroskopie*; Thieme Verlag: Stuttgart, 1984; pp 370−371.

(12) Henklein, P.; Mügge, C.; Costisella, B.; Wray, V.; Domke, T.; El-Faham, A.; Lee, Y.; Kates, S. A.; Carpino, L. A. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Ed.; Collected Papers of the 5th International Symposium; Mayflower Scientific, Ltd.: Birmingham, 1999; p 309.

(13) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.-I.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710.

(14) Compare: Gund, P. *J. Chem. Educ.* **1972**, *49*, 100.

(15) For cases where HOBt/carbodiimide and HBTU/base activation procedures lead to differing results that are indicative of differing mechanistic details, see: Zhang, Y.; Boyer, R.; Sun, X.; Paschal, J.; Chen, S.-H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 775.

(16) For additional mechanistic speculation regarding the activation process involving the intermediacy of *O*-acyluronium salts, see: Li, P.; Xu, J.-C. *Tetrahedron* **2000**, *56*, 4437.

three crystallization techniques: (i) An amount of 1.0 g (2.24 mmol) of **7** was dissolved in 20 mL of acetone. Transparent needles of form **I** (**7-I**), containing water of crystallization, were formed upon slow evaporation. (ii) An amount of 1.0 g (2.24 mmol) of **7** was dissolved in 20 mL of a 1:1 acetone/ethyl acetate solution. Upon slow evaporation, transparent platelets of form **II** (**7-II**), containing ethyl acetate of crystallization, were formed. (iii) An amount of 1.0 g (2.24 mmol) of **7** was dissolved in 10 mL of acetonitrile. Small transparent platelets of form **III** (**7-III**), a solvent-free phase, appeared upon slow evaporation. X-ray diffraction studies were carried out on all three "pseudopolymorphic" forms. Full details of all three structures will be reported in a separate paper. Here we report the structure of form **III**; the other two forms contain guanidinium cations, PF_6^- anions, and the solvent of crystallization.

X-ray Crystal Structure of HAPyU, Form III. $C_{14}H_{19}ON_6PF_6$, M = 432.31, tetragonal, space group $P\bar{4}2_1c$ (no. 114), colorless plates, a = 15.767(4), c = 14.856(1) Å; Z = 8; V = 3694.3 Å^3; ρ_o = 1.57(2) g/cm^3, ρ_c = 1.556 g/cm^3; T = 294 K. Data were collected on a Nonius CAD-4U diffractometer ($\lambda_{CuK\alpha}$ = 1.54178 Å); transmission factors ranged from 0.953−1.0. Full-matrix least-squares refinement (based on $|F|$) of positional and anisotropic displacement parameters for all non-hydrogen atoms, with H atoms at fixed geometric positions (total parameters = 254), led to R = 0.0503 and R_w = 0.0640 using 1423 data for which I > 1.96σ(I); maximum residual ρ = 0.43 e/Å^3. A full report on form **III** has been deposited as a CIF file in Supporting Information.

^{15}N NMR. Natural abundance ^{15}N NMR spectra of HAPyU and HBPyU were measured at 300 K on a Bruker AVANCE DMX 600 MHz spectrometer equipped with a 5 mm broadband probehead. A sample of HAPyU (500 mg) was dissolved in DMSO-d_6 (0.7 mL), and a spectrum (sweep width of 400 ppm) was recorded with 90° impulses and 4 s delay times during which weak ^{1}H irradiation was applied to afford nuclear Overhauser enhancement. A satisfactory spectrum showing all quaternary nitrogen signals was obtained after 20 h. ^{15}N chemical shifts were indirectly referenced to NH_3. For HBPyU, due to its lesser solubility and tendency to crystallize out during long measurement times, the determination of a complete data set was not possible, with the signal near 168 ppm being only ambiguously identified. See Figures 1 and 2 in the Supporting Information.

^{13}C NMR. The ^{13}C NMR spectra were measured in a DMSO-d_6 solution on a Varian Unity plus 500 MHz spectrometer with a 5 mm broadband probehead. Assignments of the carbon atoms were carried out by various two-dimensional methods (COSY, HSQC, and HMBC) using indirect detection and gradient techniques. The solid state ^{13}C MAS spectra were measured on a Varian Unity plus 300 MHz spectrometer with a Doty probehead using a polarization technique. The spinning frequency of the 5 mm rotors was 5000 Hz. See Figures 3−6 and Table 1 in the Supporting Information.

Acknowledgment. We are indebted to the National Science Foundation (NSF CHE-9003192), the National Institutes of Health (GM-09706), and Perseptive Biosystems, Inc., for support of the work carried out in Amherst and the National Science Foundation (DMR-0089257) for support of the work at Brandeis.

Supporting Information Available: ^{15}N NMR spectra of HAPyU and HBPyU, ^{13}C NMR spectra (solution and solid state) of HAPyU with two-dimensional NMR spectra allowing ^{13}C assignments of chemical shift data, X-ray structural diagrams, and a CIF file for HAPyU, form **III**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO001616+