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Communications

Effect of Tertiary Bases on *O*-Benzotriazolyluronium Salt-Induced Peptide Segment Coupling†

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Summary: Collidine activation of *O*-(7-azabenzotriazolyl)-uronium salts leads to relatively low levels of racemization in the coupling of peptide segments, especially with reagents derived from 1,1-carbonyldipyrrolidine.

Although tertiary organic bases are often essential components of peptide coupling processes whenever the amino reactant is taken as a hydrochloride or other salt or whenever phosphonium- (BOP, etc.) or uronium- (HBTU, etc.) based coupling reagents are adopted, it appears that a systematic study of the effect of base structure on coupling efficiency has never been conducted. During an examination of the loss of chirality which accompanies segment coupling in the presence of a new class of additives based on 1-hydroxy-7-azabenzotriazole (HOAt), it was found[1] that the bases currently in favor are in fact inadequate for general use. From the scattered studies reported in the literature,[2] two aliphatic amines, diisopropylethylamine (DIEA) and *N*-methylmorpholine (NMM), have come to be almost universally selected for both solution- and solid-phase syntheses, irrespective of whether the key step involves stepwise or segment condensation.

Conspicuously absent from published studies are the pyridine bases with the exception of pyridine itself and its highly basic 4-dimethylamino derivative (DMAP). This lack of attention to base structure has persisted even as the BOP-, PyBOP-, and HBTU-style reagents, with their specific need for a basic reaction environment, have become ever more widely adopted.[3] The problem is not limited to segment coupling since even some especially sensitive urethane-protected amino acids, e.g., those derived from histidine, are at risk.[4] In addition it is not clear to what extent the multitude of byproducts accompanying the solid

† Abbreviations used: Aib = α-aminoisobutyric acid; B = base; BOP = (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CR = coupling reagent; DIEA = diisopropylethylamine; DMAP = 4-(dimethylamino)pyridine; DMF = dimethylformamide; 2,4-DMP = 2,4-dimethylpyridine = 2,4-lutidine; 2,6-DMP = 2,6-dimethylpyridine = 2,6-lutidine; 3,4-DMP = 3,4-dimethylpyridine = 3,4-lutidine; HAMDU = *O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate; HAPyU = 7-HAPyU = *O*-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorophosphate; 4-HAPyU = *O*-(4-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorophosphate; HATU = 7-HATU = *O*-(7-azabenzotriazol-1-yl)-1,1:3,3-tetramethyluronium hexafluorophosphate; HBMDU = *O*-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate; HBPyU = *O*-(benzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorophosphate; HBTU = *O*-(benzotriazol-1-yl)-1,1:3,3-tetramethyluronium hexafluorophosphate; HOAt = 7-HOAt = 7-aza-1-hydroxybenzotriazole; 4-HOAt = 4-aza-1-hydroxybenzotriazole; HOBt = *N*-hydroxybenzotriazole; NMI = *N*-methylimidazole; NMM = *N*-methylmorpholine; PCA = *p*-chloroaniline; PS = Proton Sponge = (1,8-bis(*N*,*N*-dimethylamino)naphthalene); py = pyridine; TFA = trifluoroacetic acid; TMP = collidine = 2,4,6-collidine = 2,4,6-trimethylpyridine; Z = benzyloxycarbonyl.

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

(2) Perhaps the most extensive study was carried out in connection with optimization of the mixed anhydride coupling procedure by Anderson and co-workers [Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012]. See also: (a) Sakakibara, S.; Itoh, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 656. (b) Kisfaludy, L.; Nyeki, O.; Szirtes, T. In *Peptides 1971. Proceedings of the 11th European Peptide Symposium*; Nesvadba, H., Ed.; North Holland: Amsterdam, 1973; p 54. (c) Williams, A. W.; Young, G. T. *J. Chem. Soc., Perkin Trans. I* **1972**, 1194. (d) Bodanszky, M.; Bodanszky, A. *J. Chem. Soc., Chem. Commun.* **1967**, 591. (e) Steinauer, R.; Chen, F. M. F.; Benoiton, N. L. *Int. J. Pept. Prot. Res.* **1989**, *34*, 295.

(3) (a) Le Nguyen, D.; Castro, B. *Peptide Chemistry 1987. Proceedings of the Japanese Symposium on Peptide Chemistry*; Shiba, T., Sakakibara, S., Eds.; Protein Research Foundation: Osaka, 1988; p 231. (b) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617. (c) Fournier, A.; Wang, C.-T.; Felix, A. M. *Int. J. Pept. Prot. Res.* **1988**, *86*, 31. (d) Gausepohl, H.; Boulin, C.; Kraft, M.; Frank, R. W. *Pept. Res.* **1992**, *5*, 315. (e) Gausepohl, H. G.; Kraft, M.; Frank, R. In *Peptides 1988. Proceedings of the 20th European Peptide Symposium*; Jung, G., Bayer, E., Eds.; de Gruyter: Berlin, 1989; p 241. (f) Fields, C. G.; Lloyd, D. H.; Macdonald, R. L.; Otteson, K. M.; Noble, R. L. *Pept. Res.* **1991**, *4*, 95. (g) Schnölzer, M.; Kent, S. B. H. *Science* **1992**, *256*, 221.

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Table 1. Approximate Halftimes for the Disappearance of PCA via Reaction with Z-Aib-OH in the presence of DIEA (Eq 1)[a]

coupling reagent	$t_{1/2}$(DMF-d_7)	$t_{1/2}$($CDCl_3$)[b]
HBTU	10–11 h	3–3 1/2 h
HATU	35–40 min	20–30 min
HBPyU	12–13 h	3 1/2 h
4-HAPyU		75–90 min
HAPyU	35 min	20 min
HBMDU		4–4 1/3 h
HAMDU	45 min	20–30 min

[a] To a solution of 0.2 mmol of Z-Aib-OH, 0.2 mmol of PCA, and 0.4 mmol of DIEA in 0.5 mL of $CDCl_3$ or DMF-d_7 was added 0.2 mmol of the appropriate uronium salt. Integration of the ^1H NMR peaks at δ 5.3 (acid) and 5.08 (amide) as the reaction progressed at the NMR probe temperature (ca. 37 °C) allowed for rough determination of the relative rates. In the case of $CDCl_3$ the intermediate active ester (δ 5.2) could also be seen. The results given are the averages of at least two runs. A preparative run gave the amide,[5] mp 157–158 °C, in 82% yield. [b] If in the case of $CDCl_3$ as solvent, DIEA is replaced by collidine, halflives for HATU, HAPyU, and HAMDU were approximately 120–135, 90, and 35–40 min, respectively. For 2,6-lutidine as base, the corresponding figures for HAPyU and HAMDU were 120–130 and 60–70 min, respectively. These results correlate with the progressively decreasing basicities of the three amines (DIEA > collidine > 2,6-lutidine).

phase assembly of long peptides (>50 amino acids) might include diastereomeric failure sequences.

In light of the deficiencies in the published record, a thorough examination of the effect of base structure on coupling efficiency is now underway in our laboratory. As the first results of that study we report the effects of a series of bases on a group of model coupling reactions, one of which was used previously[1] in a comparison of HOAt and *N*-hydroxybenzotriazole (HOBt) as auxiliary nucleophiles. In addition, another model reaction used previously (eq 1) to compare relative rates of coupling processes

$$\underset{\mathbf{1}}{\text{Z-Aib-OH}} \xrightarrow[\text{PCA}]{\text{CR/B/solvent}} \underset{\mathbf{2a},\ X = A;\ \mathbf{b},\ X = B}{[\text{Z-Aib-OXt}]} \rightarrow \underset{\mathbf{3}}{\text{Z-Aib-PCA}} \quad (1)$$

effected by carbodiimide reagents was extended to the uronium analogs. In this test, reagents derived from HOAt fall in one category and those from HOBt in another, less reactive, category with less significant differences being found among the members of each group. Previously this test had been carried out only in DMF-d_7, in which solvent the protons of the benzylic CH_2 unit of **1** and those of the intermediate active ester **2** overlap. Upon switching to $CDCl_3$ as solvent, it was found that all three species involved could be distinguished: **1** (δ 5.3), **2** (δ 5.2), and **3** (δ 5.08). Assignment of the peak at δ 5.2 to labile intermediate **2a** or **2b** was confirmed by authentic syntheses of these two compounds.[5] Results of the halftime studies are collected in Table 1.[6] When authentic **2a** was treated with *p*-chloroaniline (PCA) under the same conditions, disappearance of half of the amine in $CDCl_3$ and DMF-d_7 required 5–7 and 15 min, respectively.

Table 2. Effect of the Identity of Base and Uronium Salt on Racemization during Formation in DMF of Z-Phe-Val-Ala-OMe[a]

	LDL isomer (%) in presence of base listed				
uronium salt	DIEA	NMM	PS[b]	Py[c]	TMP[c]
HBTU		5.6	4.6		2.9
HBPyU	10.7	5.7	1.9	7.7	0.6
HBMDU	14.9		1.3		1.0
4-HAPyU	14.5	10.6	1.8		0.9
HATU		2.7	0.6		0.1
HAMDU	2.2		0.4		0.1
HAPyU	2.3	1.7	0.4	6.2	0.1

[a] Test couplings were carried out as noted previously (Table I, ref 1). Because of discrepancies in racemization levels when using different samples of commercial tertiary amines, for the studies reported here, DIEA (Aldrich, 99%) and NMM (Aldrich, 99%) were distilled first from ninhydrin and then from CaH_2 (bp 126 °C and 114–116 °C, respectively) and stored over molecular sieves. TMP (Eastman Kodak, 97%) was distilled from CaH_2 (bp 170–172 °C) and stored over molecular sieves. Other bases were treated similarly. Untreated DIEA, which may have contained various amounts of primary or secondary amines (positive ninhydrin test), led to enhanced racemization (2–3%). On the other hand TMP (Aldrich, 99%), taken directly from the bottle, gave racemization levels comparable to those obtained with material distilled over CaH_2. DMF (Fisher HPLC grade) was aspirated with a stream of N_2 for 15 min and stored over molecular sieves. The percentages given are the averages of two runs, as determined by HPLC using a Waters Delta Pak Column (5 μm, C_{18}, 100 Å, 3.9 × 150 mm). For detection a Waters 996 photodiode array detector (PDA) was used for all analyses reported in this communication. In order to verify the capability of the system to quantify the minor diastereomer, authentic samples of mixtures were prepared and analyzed under the conditions adopted for routine use. As an example 106 μg of pure Z-D-Phg-L-Pro-NH_2 was dissolved in 100 mL of CH_3CN in a volumetric flask and to 1 mL of this solution was added 1063 μg of Z-L-Phg-L-Pro-NH_2 (calcd 0.101% DL). Analysis of the resulting solution in the normal manner using the PDA detector (injection volume 10 μL) gave 0.10% of the DL form (average of two injections). Three other solutions containing known amounts of the two diastereomers were examined similarly (% DL according to weight ratios/% DL as determined by instrument): 0.184/0.19; 0.256/0.29; 0.386/0.43. [b] Racemization in the presence of Proton Sponge (PS; for a review, see Staab, H. A.; Saupe, T. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 865) was consistently less than that observed with DIEA or NMM. Use of PS was potentially attractive in view of its reputation as a very strong base which is nevertheless of low kinetic activity toward C–H deprotonation (Alder, R. W.; Goode, N. C.; Miller, N.; Hibbert, F.; Hunte, K. P. P.; Robbins, H. J. *J. Chem. Soc., Chem.* **1978**, 89). As an example the rate of proton transfer from α,4-dinitrotoluene to PS is 1/25 of that to triethylamine (Jarczewski, A.; Pruszynski, P.; Leffek, K. T. *J. Chem. Soc., Perkin Trans. II* **1977**, 814). Unfortunately although obtained in high yields, the crude coupling products obtained in the presence of PS were sometimes contaminated by unidentified impurities. The nature of these contaminants and the question of their relevance to the practical use of this base in coupling processes are under continuing investigation. [c] In the case of HAPyU other pyridine bases were also examined: DMAP 10.35% LDL; 2,6-lutidine 0.64% LDL; 2,6-di-*tert*-butylpyridine, none of the tripeptide was formed, the highly hindered pyridine derivative being too weakly basic to activate the carboxyl function; pyridine, apparently because of its low basicity, reaction was inefficient and in addition to significant loss of chirality led to only 40% of the tripeptide.

The first racemization test examined involved the segment coupling outlined in eq 2. Results on the extent of racemization arising under various conditions are gathered in Table 2.

$$\underset{\mathbf{4}}{\text{Z-Phe-Val-OH}} \xrightarrow[\text{CR/B/DMF}]{\text{H-Ala-OMe·HCl}} \underset{\mathbf{5}}{\text{Z-Phe-Val-Ala-OMe}} \quad (2)$$

(4) (a) Forest, M.; Fournier, A. *Int. J. Pept. Prot. Res.* **1990**, *35*, 89. (b) Seyer, R.; Aumellas, A.; Caraty, A.; Rivaille, P.; Castro, B. *Int. J. Pept. Prot. Res.* **1990**, *35*, 465.

(5) All new compounds gave correct C, H, N values (±0.3%) and appropriate IR and NMR spectral data.

(6) The increased reactivity observed in the less polar solvent $CDCl_3$ is in agreement with previous work,[3a] although the nature of the reactivity of these compounds is also related to the question of *O*- vs *N*-acyl forms of the HOAt esters [cf. (a) Barlos, K.; Papaioannou, D.; Voliotis, S.; Prewo, R.; Bieri, J. H. *J. Org. Chem.* **1985**, *50*, 696; (b) Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *Int. J. Pept. Prot. Res.* **1984**, *23*, 300]. Infrared studies show that solution of **2a** in chloroform gives evidence of both *O*- and *N*-acyl species (1820, 1720 cm^{-1}) whereas in DMF only the latter peak (1720 cm^{-1}), characteristic of the *N*-acyl isomer, is observed, also in line with the reduced reactivity observed in DMF. It may be noted that for HOAt esters the pyridine N-atom represents a fourth possible site for N-acylation.

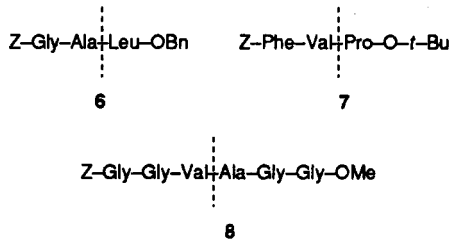
Table 3. Effect of Excess Base on Racemization during the Formation of Z-Gly-Ala-Leu-OBn in DMF via [2 + 1]Coupling[a,b]

coupling reagent	base (equiv)	yield, %	mp, °C	LDL, %[c]
HBTU	DIEA (3), (4), (6)	81.2, 80.0, 78.9	94–6, 93–5, 93–4	1.78, 2.01, 3.03
HBTU	TMP (3), (4), (6)	84.0, 71.4, 74.8	94–6, 93–5, 93–5	1.14, 1.44, 1.71
HATU	DIEA (3), (4), (6)	79.8, 84.2, 81.3	94–6, 95–7, 95–7	0.64, 1.06, 1.62
HATU	TMP (3), (4), (6)	89.1, 84.0, 89.1	95–7, 95–7, 94–6	0.24, 0.68, 0.89
HAMDU	TMP (3), (4), (6)	89.1, 79.8, 82.7	96–8, 95–7, 95–7	0.68, 0.82, 1.27
HAPyU	TMP (3), (4), (6)	89.1, 86.6, 79.8	96–8, 96–7, 96–7	0.23, 0.46, 0.68

[a] The method followed that of Table 2, ref *a*. In the last three columns the figures for the results observed with 3, 4, and 6 equiv of the base used are presented in the same sequence. [b] The purified tripeptide had mp 101–2 °C, α^{23}_D −30.9° (*c*, 1, EtOAc), lit.[8] mp 102 °C, α^{20}_D −31.6° (*c*, 1, EtOAc). [c] The values given are the averages of two runs. Analysis was carried out on a Waters Nova Pak column (4 μm, C_{18}, 3.9 × 150 mm) using as mobile phase 65/35 MeOH/H_2O, 0.1% TFA with detection at 220 nm.

Regardless of the amine used, the three uronium salts based on HOAt (HAPyU,[7] HATU,[1] and HAMDU[7]) stand out as being particularly effective in avoiding racemization. Equally dramatic is the performance of collidine relative to that of the other tertiary bases examined. With collidine, racemization is significantly reduced relative to that observed with the classic bases DIEA or NMM, even in the case of uronium salts derived from the long-known additive HOBt.

In order to begin the long task of generalizing these results to other systems, three additional segment couplings were examined, leading to tripeptides **6** and **7** and hexapeptide **8**, with the coupling position shown by the



broken vertical line. Tripeptide **6** had previously been used by Izumiya[8] to examine the effect of increasing excesses of added tertiary base on racemization. In a similar study, the results of which are outlined in Table 3, a gradual increase in racemization is observed as the amount of base is increased. Although the differences are small, of the various combinations, HAPyU-TMP provides the greatest protective effect.

Tripeptide **7** incorporates a classically difficult valine/proline coupling[9] and here the HAPyU–TMP combination stands alone in allowing coupling without significant racemization (Table 4). Finally, analogous results were obtained supporting the exceptional efficiency of the HAPyU–TMP pair for the [3 + 3] coupling leading to hexapeptide **8**[5] (Table 5).

Further studies are required to provide a consistent rationale for the unique performance of the HAPyU–TMP combination observed in this work. With regard to the amine component of the mixture it is likely that both intrinsic basicity and steric influences play a role. *N*-Methylmorpholine (aqueous pK_a 7.38[10]), *N*-methylimidazole (7.13[11]), and collidine (7.43[10]) show nearly the same basicity although only collidine is expected to exhibit any pronounced steric hindrance toward attack by the amino group on, for example, the α-hydrogen atom of the activated carboxyl component or the derived oxazolone, thus leading to reduced racemization.[12] In fact, as is clear from the data collected in Tables 2 and 4, of these three bases only collidine provides a safe coupling environment. In the case of DIEA the expected steric protection against racemization may be swamped by its significantly greater basicity. However, measurements of the basicity of amines in this category have been carried out in organic solvent/water mixtures[13] rather than under conditions which would allow direct comparison with those of other simple amines.

Table 4. Effect of the Identity of Base and Uronium Salt on Racemization during Formation of Z-Phe-Val-Pro-O-*t*-Bu in DMF via [2 + 1] Coupling[a,b]

coupling reagent	base	yield, %	mp, °C	LDL, %
HBTU	DIEA	80.6	85–87	25.6
HBTU	TMP	88.7	91–92	11.9
HBPyU	DIEA	80.6	85–88	26.6
HBPyU	NMI	72.6	84–88	23.9
HBPyU	TMP	88.7	90–92	13.7
HAMDU	DIEA	79.0	89–91	7.8
HAMDU	TMP	82.3	92–93	2.3
HATU	DIEA	79.8	89–90	6.5
HATU	TMP	85.5	90–92	1.7
HAPyU	PS	96.7	85–89	3.3
HAPyU	DIEA	80.6	87–89	6.3
HAPyU	NMM	81.2	88–91	5.9
HAPyU	NMI	80.6	86–89	9.4
HAPyU	3,4-DMP	88.7	87–90	13.4
HAPyU	2,4-DMP	88.1	87–90	12.3
HAPyU	2,6-DMP	88.7	89–91	2.3
HAPyU	TMP	92.7	92–94	<0.1

[a] The method followed that of Table 2, ref *a*. [b] The purified tripeptide *tert*-butyl ester had mp 110–112 °C (lit.[9] mp 115–116 °C). For HPLC analysis the crude tripeptide *tert*-butyl ester was dissolved in 1 mL of TFA and the solution left for 2 h after which TFA was removed by rotary evaporation and the crude residue was dissolved in ether and precipitated by hexane in order to remove traces of residual TFA which interfered with the HPLC analysis. It was demonstrated that all of the tripeptide acid was precipitated and the filtrate contained none of the LLL- or LDL-peptide acid. The crude precipitated solid was examined for racemization by HPLC using a Waters Nova Pak Column (4 μm, C_{18}, 3.9 × 150 mm) using as mobile phase 40/60 CH_3CN/H_2O, 0.1% TFA with detection at 220 nm. The results given are the averages of two runs. A purified sample of the tripeptide acid had mp 79–81 °C (lit.[9] mp 80–82 °C).

(7) HAPyU was prepared from 1,1-carbonyldipyrrolidine by a method analogous to that described for the synthesis of HBPyU [(a) Chen, S.; Xu, J. *Tetrahedron Lett.* **1992**, 647; (b) Coste, J.; Frérot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, 1967]. The chlorouronium hexafluorophosphate gave the azabenzotriazole-substituted uronium salt in 87.8% yield, mp 118–120 °C dec: ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 2.0–2.3 (m, 8, CH_2), 3.8–4.1 (m, 8, CH_2), 8.0 (dd, 1, β-H), 8.45 (dd, 1, γ-H), 8.95 (dd, 1, α-H). HAMDU was obtained in an analogous manner [cf. Kiso, Y.; Fujiwara, Y.; Kimura, T.; Nishitani, A.; Akaji, K. *Int. J. Pept. Prot. Res.* **1992**, *40*, 308], yield 79.4% from the chloroimidazolidinium hexafluorophosphate, mp 165–167 °C dec: ^{1}H NMR (DMSO-d_6) δ 3.15 (s, 6, CH_3), 4.17 (s, 4, CH_2), 8.05 (dd, 1, β-H), 8.5 (dd, 1, γ-H), 8.95 (dd, 1, α-H).

(8) Izumiya, N.; Muraoka, M.; Aoyagi, H. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3391.

(9) Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147.

(10) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.

(11) Takeuchi, Y.; Kirk, K. L.; Cohen, L. A. *J. Org. Chem.* **1978**, *43*, 3570.

Table 5. Effect of the Identity of Base and Uronium Salt on Racemization during Formation of Hexapeptide 8 in DMF via [3 + 3] Segment Condensation[a]

coupling reagent	base	yield, %	mp, °C	DL, %
HBPyU	DIEA	78.0	195–200	16.9
HBPyU	TMP	83.1	192–198	7.9
HAMDU	DIEA	70.9	193–200	8.7
HAMDU	TMP	92.1	198–201	3.7
HAPyU	DIEA	78.1	197–201	5.3
HAPyU	TMP	85.1[b]	198–202	<0.1

[a] Z-Gly-Gly-Val-OH was obtained from Bachem, Inc. and used as received. The hydrochloride of H-Ala-Gly-Gly-OMe, mp 171–172 °C (lit. mp 175 °C), was obtained by standard Fischer esterification [Fischer, E. *Chem. Ber.* **1906**, *39*, 2893]. The DL isomer, mp 150–152 °C (lit. mp 151–153 °C), was made similarly [Sluyterman, L. A.; Veenendaal, H. J. *Rec. Trav. Chim.* **1954**, *73*, 1001]. Coupling followed the method described in Table 2, ref *a*. The crude hexapeptide methyl ester was isolated by evaporation of solvent and direct column chromatography using as eluant $CH_3OH/CHCl_3/HOAc$ (3/7/0.1). The crude material from the column was examined by HPLC using a Waters Delta Pak column (5 μm, C_{18}, 100 Å, 3.9 × 150 mm) using as mobile phase 20/80 CH_3CN/H_2O, 0.1% TFA with detection at 214 nm. Retention times for the LL and DL isomers were 19.77 and 22.85 min, flow rate 1 mL/min. [b] After recrystallization from methanol–ether the HAPyU/TMP run gave in 75.1% yield the pure hexapeptide, mp 199–204 °C dec; amino acid analysis (Waters ACCQ-TAG) Gly 4.3 (4), Ala 1.0 (1), Val 1.1 (1); HRMS (FAB) *m/e* calcd for ($C_{25}H_{36}N_6O_9$ + H) 565.2622, found 565.2640.

Coupling processes involving protected peptide segments are rarely carried out in purely aqueous media and while it might be expected that relative basicities of a series of amines in a solvent such as DMF, in which these test reactions have been conducted, might parallel those determined in water, this need not necessarily be the case.[2a]

Only a limited number of references were located citing the specific use of amines such as collidine or 2,6-lutidine in peptide coupling reactions. One example involved the anchoring of FMOC amino acids to hydroxyl resins where it was found that both pyridine and 2,6-lutidine were inferior to NMM.[14] The use of 2,6-lutidine in connection with the mixed anhydride technique of segment coupling using 1-oxo-1-chlorophospholane has been mentioned briefly without comparison to other bases or experimental detail.[15] Collidine was also cited, again without comparison to any other base, in connection with a difficult coupling effected by an FMOC amino acid chloride.[16] It was success with such bases in effecting acylation via FMOC amino acid chlorides[17] and fluorides[18] which directly inspired the present application of α-alkylated pyridines to peptide coupling reactions.

The examples provided here represent only a limited selection and further work will be required in order to generalize these results. Still, the data are of sufficient promise to justify the recommendation that other investigators consider the substitution of DIEA and NMM by collidine for peptide coupling in general and test the HAPyU–TMP combination in a range of other segment couplings. Mechanistic studies are in progress.

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