Peptide Coupling in the Presence of Highly Hindered Tertiary Amines

Louis A. Carpino,* Dumitru Ionescu,† and Ayman El-Faham‡

Department of Chemistry, Box 34510, University of Massachusetts, Amherst, Massachusetts 01003-4510

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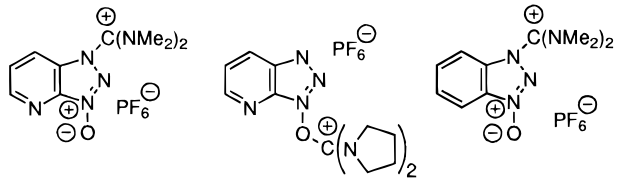
Previously, 2,4,6-trimethylpyridine (collidine), due to steric shielding around the N-atom, was found to be an efficient base for effecting peptide segment coupling via azabenzotriazole-based onium-style coupling reagents. A number of even more highly hindered bases, including 2,3,5,6-tetramethylpyridine, 2,6-di-*tert*-butyl-4-(dimethylamino)pyridine, triisopropylamine, and *N*-*tert*-butylmorpholine, have been compared with collidine in such reactions. Some of the newer bases showed advantages in terms of convenience in handling and maintenance of configuration during segment coupling processes, although dramatic differences based on steric effects were not observed. On the basis of results with a number of test peptides and many base-coupling reagent combinations, it was noted that most efficient results are obtained if 1 equiv of HOAt is present as an additive during the coupling process. For rapid activation of onium-style coupling reagents during stepwise solid-phase coupling reactions, the stronger base 2,6-di-*tert*-butyl-4-(dimethylamino)pyridine was more effective than collidine.

While we were examining the utility of a new class of coupling reagents based on 7-aza-1-hydroxybenzotriazole (HOAt)[1,2] such as *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU), **1**, and *O*-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)-uronium hexafluorophosphate (HAPyU), **2**, analogs of the well-known reagent *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU) **3**, it became apparent that



1 **2** **3**

the two bases most commonly recommended for peptide coupling reactions, diisopropylethylamine (DIEA) and *N*-methylmorpholine (NMM), often lead to unacceptable levels of inversion of configuration at the activated carboxyl function in the case of segment coupling. An examination of other bases was begun, and among those previously studied, 2,4,6-trimethylpyridine (collidine, TMP, aqueous pK_a 7.43),[3] an amine of lowered basicity relative to DIEA (pK_a 10.1) and enhanced steric shielding of the amino function relative to NMM (pK_a 7.38), gave the best results.[4] Classic mechanistic studies[5] of the effect of base structure on C−H proton abstraction rates provide a consistent rationale for the superiority of collidine in these reactions. 2,6-Dimethylpyridine (2,6-lutidine) gave results comparable to those of collidine, but other dimethylpyridines bearing the methyl groups at positions other than the α-position proved to be unsuitable.

In the present work the effect of further increases in the bulk of the pyridyl α-substituents has been examined. Model test peptides **4**−**8**,[4,6,7] all of which have been examined in earlier studies, were chosen for initial testing with these newer bases. For tripeptides the coupling position is shown by the vertical dotted line. Passing from the 2,6-dimethylpyridine to the 2,6-diethyl- ,[8] diisopropyl-,[8] and di-*tert*-butylpyridines[9] was

Model Peptides

Z−Phe-Val┆Pro−NH_2 **4**[6]

Z−Gly-Phe┆Pro−NH_2 **5**[6]

Z−Phe-Val┆Ala−OMe **6**[4]

Z−Gly-Phe┆Val-OMe **7**[6]

Z−Phg-Pro−NH_2 **8**[7]

† Fulbright Scholar. On leave of absence from the Department of Organic Chemistry, Faculty of Chemistry, University of Bucharest, 70346 Bucharest, Romania.

‡ On leave of absence from the Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt.

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

(2) For a list of some of these new reagents as well as current structural assignments and nomenclature, see: (a) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203. (b) Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Peptide Sci.* **1994**, *1*, 57−67. Whether HAPyU crystallizes in the form shown (**2**) or in the isomeric guanidinium form (comparable to structure **1** for HATU) is not yet known. In the absence of appropriate X-ray data structure **2** is arbitrarily retained in the present paper.

(3) Aqueous pK_a data, unless otherwise indicated, are taken from: Perrin, D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965; Supplement, 1972.

(4) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.

(5) (a) Gold, V. In *Progress in Stereochemistry*; de la Mare, P. B. D., Klyne, W., Eds.; Butterworths: Washington, 1962; Vol. 3, p 169. (b) Feather, J. A.; Gold, V. *Proc. Chem. Soc.* **1963**, 306; *J. Chem. Soc.* **1965**, 1752. (c) Covitz, F.; Westheimer, F. H. *J. Am. Chem. Soc.* **1963**, *85*, 1773. (d) Hine, J.; Houston, J. G.; Jensen, J. H.; Mulders, J. *J. Am. Chem. Soc.* **1965**, *87*, 5050. (e) Lewis, E. S.; Funderburk, L. H. *J. Am. Chem. Soc.* **1967**, *89*, 2322. (f) Gutsche, C. D.; Redmore, D.; Buriks, R. S.; Nowotny, K.; Grassner, H.; Armbruster, L. W. *J. Am. Chem. Soc.* **1967**, *89*, 1235. (g) Zollinger, H. *Helv. Chim. Acta* **1955**, *38*, 1623.

(6) Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561.

(7) Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1995**, *60*, 405.

(8) Balaban, A. T.; Nenitzescu, C. D. *Liebigs Ann. Chem.* **1959**, *625*, 74.

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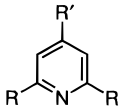
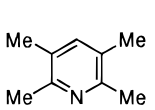
expected to lead to a progressive decrease in the extent of epimerization or racemization at the activated carboxylic acid residue of the protected dipeptide or amino acid involved in the reaction. Although such an effect was observed for the very bulky *tert*-butyl substituent, this was not the case for the ethyl or isopropyl derivatives. Thus, for coupling leading to tripeptide **4** neither 2,6-diethyl- nor 2,6-diisopropylpyridine derivatives **9a** or **9b**, respectively, proved to be more effective than TMP.



9a, R = Et, R′ = Me
9b, R = *i*-Pr, R′ = Me

These two bases, however, appear to be relatively less stable than TMP and become discolored on standing. Freshly distilled material was used in all experiments, but it is not certain that the results are unaffected by unknown contaminants.

For alkylated pyridines the addition of each new alkyl group increases the basicity by about 0.5−0.85 pK_a units.[10] 2,3,5,6-Tetramethylpyridine, **10** (TEMP, pK_a 7.90), and the analogous cycloalkyl derivative octahydroacridine, **11** (OHA, pK_a 8.09[11]), were examined along with pentamethylpyridine, **12** (pK_a 8.75), as somewhat more basic analogs of TMP. For these three amines best



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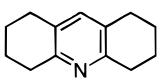

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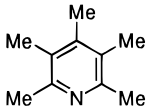

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results were obtained with TEMP and OHA which were about as efficient as TMP. In addition, TEMP and OHA have practical advantages over TMP in being stable odorless solids. Not yet commercially available, in contrast to OHA, TEMP was obtained for these studies via a multistep procedure, each step of which, however, proceeds readily.[12] For the limited number of cases examined, clear-cut differences among TMP, TEMP, and OHA were not evident. For TEMP the steric effects of the two α-methyl substituents might conceivably be "buttressed"[13] by the adjacent 3,5-dimethyl groups, and judging from quaternization rates on the lower homologs of **10** and **11** steric effects may be significantly less for the latter.[17]

For model peptides **4**, **6**, and **8**, coupling efficiency for pentamethylpyridine, **12**,[18] was slightly less than for TEMP, and in addition, this liquid base was difficult to handle under ordinary conditions due to its extremely hygroscopic nature. Use of the hydrate itself caused increased loss of configuration relative to the anhydrous amine. For example, in the case of tripeptide **5**, with coupling effected by HAPyU, **2**, the anhydrous amine and its hydrate gave 1.8 and 4.8% of the DL form of Z-Gly-Phe-Pro-NH_2 (Z = benzyloxycarbonyl), respectively. These results led to a brief study of the influence of water on the segment coupling process in general. It seems possible that with a strong highly hindered base the kinetically active base may not be the amine itself.[19] In the presence of water, hydroxide ion might play this role. In addition, in the presence of large amounts of water, the increase in solvent polarity may adversely affect the coupling process.[20]

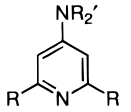
For most of the studies reported earlier,[1,4,6] a commercial grade of DMF (dimethylformamide, Fisher HPLC grade) dried for at least 3 days over molecular sieves (3 Å) at room temperature was routinely used as solvent for the test coupling reactions. This technique is known not to provide totally anhydrous DMF.[21] A more efficiently dried sample of DMF was obtained by a special procedure which involved distillation from P_2O_5 followed by treatment with molecular sieves (3 Å) which had been freshly activated by heating over a free flame. The resulting freshly prepared dry solvent was then used directly in various coupling reactions and subsequently diluted with increasing amounts of water for further tests. The tertiary amines were freshly dried as described previously.[4] In reactions leading to model peptides **4** and **6**, for a number of bases both hindered and nonhindered, the loss of configuration at valine first increased with the addition of small amounts of water, then decreased, and finally rose rapidly (Table 4, supporting information). In a sample of DMF which had simply been dried over ordinary molecular sieves, the extent of epimerization at valine was in most cases comparable to that at the point of reversal and thus lower than that observed in the carefully dried medium. It seems possible that these effects may be responsible for the discrepancies often observed between various runs before attempts were made to control more carefully the reaction conditions. While the increased loss of configuration observed in the presence of large amounts of water might be associated with the increased polarity of the reaction medium, a rationale for the effect of adding small amounts of water to the dried solvent is not obvious. Whatever the reason, the effect allows for simplification of the reaction protocol: DMF dried merely

(9) Brown, H. C.; Kanner, B. *J. Am. Chem. Soc.* **1966**, *88*, 986.
(10) Ikekawa, N.; Sato, Y.; Maeda, T. *Chem. Pharm. Bull.* **1954**, *2*, 205.
(11) Thummel, R. P.; Kohli, D. K. *J. Org. Chem.* **1977**, *42*, 2742.
(12) Tsuda, K.; Ikekawa, N.; Mishima, H.; Iino, A. *Chem. Pharm. Bull.* **1953**, *1*, 122.
(13) Kinetic examination of the Menschutkin reaction has often been used to compare the steric encumbrance around the N-atom of pyridine derivatives.[14] According to this criterion the effect of buttressing has been demonstrated by comparison of the 2,5- and 2,3-dimethylpyridines, the former being 2.5 as reactive as the latter.[15] On the other hand, the analogy between the Menschutkin reaction and steric effects toward the deprotonation of weak carbon acids may be inexact in view of the radical differences between the expected transition states for the two reactions. Furthermore, while a highly hindered base such as 2,6-di-*tert*-butylpyridine reacts with potent methylating agents only very slowly, it gives a stable *N*-methylpyridinium salt which has been shown not to be deformed according to X-ray crystal analysis.[16] Thus, it may not be unexpected that differences among α-substituted pyridines in the reactions examined here will be muted except for the most bulky substituents.
(14) Shug, J. C.; Viers, J. W.; Seeman, J. I. *J. Org. Chem.* **1983**, *48*, 4892.
(15) Seeman, J. I.; Schug, J. C.; Viers, J. W. *J. Org. Chem.* **1983**, *48*, 2399.
(16) Weber, H.; Pant, J.; Liedigk, M.; Wunderlich, H. *Chem. Ber.* **1981**, *114*, 1455.
(17) Seeman, J. I.; Galzerno, R.; Curtis, K.; Schug, J. C.; Viers, J. W. *J. Am. Chem. Soc.* **1981**, *103*, 5982.
(18) Balaban, A. T.; Bota, A.; Chiraleur, F.; Sliam, E.; Hanes, A.; Draghici, C. *Rev. Roum. Chim.* **1977**, *22*, 1003.
(19) An example of such an effect has been reported for proton sponge. See: Alder, R. W.; Goode, N. C.; Miller, N.; Hibbert, F.; Hunte, K. P. P.; Robbins, H. J. *J. Chem. Soc., Chem. Commun.* **1978**, 89.
(20) Although increases in solvent polarity are often associated with increased loss of configuration in peptide coupling the true nature of the effect is more complex. For a review see: Benoiton, N. L. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1983; Vol. 5, Part B, p 276.
(21) Burfield, D. R.; Smithers, R. H. *J. Org. Chem.* **1978**, *43*, 3966.

by standing over molecular sieves at room temperature has been adopted for all routine coupling tests.

Previously[4] it was reported that 2,6-di-*tert*-butylpyridine (pK_a 4.95[22,23]) was too weakly basic to induce coupling between Z-Phe-Val-OH and H-Ala-OMe via HAPyU, **2**. The unusually low basicity in water of this base has been the subject of many studies since the first observations of Brown and co-workers.[9] The effect seems best ascribed to steric effects on solvation and loss of entropy of the cation formed upon protonation in solution.[23] In contrast to effects observed in water, the gas phase basicity of 2,6-di-*tert*-butylpyridine is enhanced over that of pyridine as expected on the basis of the inductive effects of the two alkyl residues.[24]

Introduction of a 4-(dialkylamino) residue into such systems causes a significant increase in basicity: the pK_a (7.45) measured[25] for the 4-diethylamino derivative **13b**



13a, R = *t*-Bu; R′ = Me
13b, R = *t*-Bu; R′ = Et
13c, R = R′ = Me

in methoxyethanol/water (80/20) is only slightly lower than that observed for DMAP (7.70) under the same conditions. The latter corresponds to a pK_a of about 9.22 in water, suggesting a pK_a of about 9 in water for the analogous amine **13a**,[26] for which no pK_a measurements were performed during the course of the present work.

In spite of the relatively high basicity of these substituted DMAP derivatives the rate of proton transfer from the α-position of an activated amino acid may be expected to be less than for other pyridine bases including TMP and TEMP. Proton transfer rates between 2,6-di-*tert*-butylpyridine and hydronium ion are 50−70 times lower than for simple pyridines.[27]

2,6-Di-*tert*-butyl-4-(dimethylamino)pyridine [DB(DMAP), **13a**] was first synthesized by modification of the method described by Potts and Winslow[25] for the analog **13b**. A second, much more convenient synthesis was based on direct reaction of *tert*-butyllithium with DMAP.[26] This highly hindered amine effected rapid and complete coupling of Z-Phe-Val-OH with H-Pro-NH_2, and while retention of configuration was not complete, it was somewhat better than for other tertiary amines investigated in the case of this highly sensitive system (Table 1). In the case of HAPyU, **2**, yields were rather low unless reaction times were extended or 3 or more equiv of base was used, although normal yields were observed with HATU, **1**, and HBTU, **3**.

Since the amino acid derivative to which coupling is made, proline amide (pK_a 8.82) in the case of **4**, is itself basic enough to activate the coupling reagent, the reactions were also carried out in the absence of any added tertiary amine. As shown in Table 1, in the presence of a total of 2 or 3 equiv of proline amide, the extent of epimerization was 1.8 and 6.9%, respectively. With 1 or 2 equiv of DB(DMAP) in place of the extra proline amide, epimerization was reduced to 1.3 or 1.7%, respectively. The results were drastically different in the case of 2,6-dimethyl-4-(dimethylamino)pyridine (2,6-diMe-DMAP),[28] the collidine analog of DMAP, where 16.1% of the epimer was obtained in the presence of 2 equiv of this base. Other examples confirm that the high basicity of 2,6-diMe-DMAP outweighs any steric protection provided by the two α-methyl substituents (Tables 5 and 6, supporting information). Due to its low melting point and extreme hygroscopic nature, 2,6-diMe-DMAP was a difficult base with which to work. The even more basic (estimated pK_a about 14),[29] relatively unhindered guanidine derivative, *N*-*tert*-butyltetramethylguanidine (*t*-Bu-TMG), generally led to extensive loss of configuration. Even in the case of tripeptide **7**, shown in a previous study[6] to be quite insensitive, the use of HAPyU/*t*-Bu-TMG gave 16.1% of the LDL form. Generally, for this system less than 0.1−0.8% LDL isomer is obtained when coupling was effected by HAPyU, **2**, in the presence of DIEA, NMM, or the various other bases described here.

In addition to the pyridine bases studied here which were designed to push the collidine example to its limit, two more highly hindered aliphatic analogs of DIEA and NMM were also examined. Triisopropylamine is an amine of unique structure, being planar according to gas phase electron diffraction and $^{14}N/^{15}N$ NMR data.[30] While triisopropylamine proved generally to be more effective than DIEA for the systems examined (Table 1; Tables 5−8, supporting information; Table 2 of ref 4; and Tables 2 and 3 of ref 6), it was less efficient than TEMP. In the morpholine series the *N*-*tert*-butyl derivative[31] showed similar advantages over the commonly used NMM (Table 1; Tables 5−8, supporting information; and Table 2 of ref 4).

Following these preliminary studies with simple di- and tripeptide models **4**−**8**, a test hexapeptide **14** was assembled. The [3 + 3] coupling to give **14** had previously been shown[4] to be a sensitive test for the nature of both coupling reagent and base. Results for TEMP and DB(DMAP) are shown in Table 2. Both bases give

(22) Although 2,6-di-*tert*-butylpyridine is a weak base in water it is unusually weak in DMSO. In water it differs from pyridine by only 0.2 pK_a units whereas in DMSO it is 2.5 units less basic [Benoit, R. L.; Fréchette, M.; Lefebvre, D. *Can. J. Chem.* **1988**, *66*, 1159]. Although not examined in DMF, relative pK_a's are expected to be similar in solvents such as DMF and DMSO (Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456. Bordwell, F. G.; Branca, J. C.; Hughes, D. L.; Olmstead, W. N. *J. Org. Chem.* **1980**, *45*, 3305). A unique example of the difference which may be observed in water and organic solvents is that of the equilibrium between benzoic acid and triethylamine which is said to show complete ionization in water ($K = 10^{6.5}$) but little ionization in DMSO ($K = 10^{-2.1}$) (Ritchie, C. D. In *Physical Organic Chemistry*, 2nd ed.; Dekker: New York, 1990; p 209). Relationships of this type may be responsible for the poor activation of carboxylic acids sometimes observed in DMF which may show effects similar to those seen in DMSO.

(23) Hopkins, H. P., Jr.; Jahagirdar, D. V.; Moulik, P. S.; Aue, D. H.; Webb, H. M.; Davidson, W. R.; Pedley, M. D. *J. Am. Chem. Soc.* **1984**, *106*, 4341.

(24) Arnett, E. M.; Chawla, B. *J. Am. Chem. Soc.* **1979**, *101*, 7141.

(25) Potts, K. T.; Winslow, P. A. *Synthesis* **1987**, 839.

(26) Deutsch, E.; Cheung, N. K. V. *J. Org. Chem.* **1973**, *38*, 1123.

(27) Bernasconi, C. F.; Carré, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 2707.

(28) Although a number of references to the use (but not the preparation) of 2,6-dimethyl-DMAP *N*-oxide could be located (*e.g.* Dega-Szafran, Z.; Hrynio, A.; Szafran, M. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1161) no reference to 2,6-dimethyl-DMAP itself could be found. The method used here was adapted from that of related compounds as noted in the experimental section. The closest analog for which a pK_a has been reported is that of **13b**. For pK_a values of related amines see: Essery, J. M.; Schofield, K. *J. Chem. Soc.* **1961**, 3939.

(29) Barton, D. H. R.; Elliott, J. D.; Gero, S. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2085.

(30) (a) Kuffner, F.; Koechlin, W. *Monatsh. Chem.* **1962**, *93*, 476. (b) Bock, H.; Goebel, I.; Havlas, Z.; Liedle, S.; Oberhammer, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 187. (c) Wong, T. C.; Collazo, L. R.; Guziec, F. S., Jr. *Tetrahedron* **1995**, *51*, 649.

(31) Cook, M. J.; Katritzky, A. R. Maños, M. M. *J. Chem. Soc.* **1971**, 1330.

Table 1. Effect of Couping Reagent and Base on the Extent of Inversion at Valine During Formation of Z-Phe-Val-Pro-NH_2 in DMF[a]

		LDL, % (yield, %)[c,d]		
base[b]	additive[b]	HAPyU	HATU	HBTU
t-BuTMG (2)		38.1 (81.8)		
i-Pr_3N (2)		6.5 (93.5)	7.1 (89.5)	19.2 (98)
i-Pr_3N (2)	HOAt (1)	2.7 (89.1)	5.4 (86.8)	9.9 (94.7)
TMP (1)		3.5 (74.1)		
TMP (2)		3.3 (100)		
TMP (1)	HOAt (1)	3.4 (66)		
TMP (2)	HOAt (1)	2.8, 1.4, 1.3 (82.8)		
NMM (2)		12.2 (90.2)		
NMM (2)	HOAt (1)	3.3 (71.2)		
NBM (2)		5.5 (86.5)	8.9 (100)	17.6 (86.8)
NBM (2)	HOAt (1)	1.9 (70.9)	5.4, 5.3 (86.8)	6.7 (84.2)
TEMP (1)		2.9 (90.2)		
TEMP (2)		2.5, 3.5	3.5, 2.8 (76)	
TEMP (1)	HOAt (1)	3.7 (76.0)	3.7, 4.1 (70.4)	
TEMP (2)	HOAt (1)	1.3 (66.7)		
2,6-diEt-4-Me-Py (2)		4.7, 5.4 (84)	3.2, 3.6, 3.0 (100)	9.7, 8.3, 10.6 (94.7)
2,6-diEt-4-Me-Py (2)	HOAt (1)	1.9, 3.8 (79.6)	4.2, 4.8, 5.9 (84.2)	5.1 (73.7)
2,6-di-*i*-Pr-4-Me-Py (2)		4.9 (75.2)	5.6, 4.2 (89.5)	8.5 (81.6)
2,6-di-*i*-Pr-4-Me-Py (2)	HOAt (1)	2.7 (63.7)	8.8 (39.5)	6.1 (71)
OHA (2)		4.6 (88.5)		
OHA (2)	HOAt (1)	2.8 (54.8)		
Me_5Py (2)		3.9 (84.7)		
Me_5Py (2)	HOAt (1)	1.4 (67)		
$Me_5Py \cdot 1/2H_2O$ (2)		4.4 (90.9)		
DMAN (2)			3.3, 3.8 (100)	13.2 (100)
DMAN (2)	HOAt (1)		4.1, 5.1 (89.5)	4.2 (80.7)
DMAN (1)/TEMP (1)			3.6, 3.0 (100)	12.7 (94.8)
DMAN (1)/TEMP (1)	HOAt (1)		3.0, 3.7 (84.2)	6.5 (93.5)
iPr_3N (1)/TEMP (1)			3.9 (81.6)	17.6 (94.7)
iPr_3N (1)/TEMP (1)	HOAt (1)		5.4, 5.6 (89.5)	8.4 (84.2)
DB(DMAP) (3)		1.7 (76.1)	2.1, 2.9 (92.9)	11.4 (93)
DB(DMAP) (3)	HOAt (1)	0.9 (61.9)	1.8 (61.9)	3.1 (79.6)
DB(DMAP) (2)		1.7, 2.5 (70.8)	3.2, 3.5 (84.2)	15.9 (74.7)
DB(DMAP) (2)	HOAt (1)	1.1, 1.0, 0.9 (35.4)	2.2 (85)	3.6 (81.6)
DB(DMAP) (1)		1.3 (66.4)	2.7, 2.2 (100)	9.5, 9.8, 8.9 (97.4)
DB(DMAP (1)	HOAt (1)	0.9 (44.3)	2.2 (85)	3.7, 3.5 (73.7)
DB(DMAP) (0.5)		1.1 (36.9)		
DB(DMAP) (0.5)	HOAt (1)	1.6 (52.6)		
DB(DMAP) (4)		2.5, 1.9 (88.5)	2.3, 2.3 (99.5)	
DB(DMAP) (4)	HOAt (1)	1.0 (70.8)	1.5 (84)	
2,6-diMe-DMAP (2)		16.1 (79.7)		
H-Pro-NH_2 (1)[e]		1.7 (88.5)	3.2 (88.4)	
H-Pro-NH_2 (1)[e]	HOAt (1)	0.7 (60)	3.2 (73)	
H-Pro-NH_2 (2)[e]		6.9 (59.8)	4.9 (73.5)	

[a] Standard protocol: test couplings were carried out by adding the coupling reagent (0.125 mmol) to a stirred and ice-cooled solution of the protected dipeptide acid (0.125 mmol), H-Pro-NH_2 (0.125 mmol), and the chosen base (0.25 mmol) in 1 mL of DMF. After 1 h the ice bath was removed and stirring was continued for 2−3 h. In the case of reactions involving an amino acid ester hydrochloride instead of H-Pro-NH_2, 0.375 mmol of base was used and the volume of DMF was increased to 1.5 mL. The reaction mixture was diluted with 25 mL of EtOAc, and the organic layer was washed twice with 10-mL portions of 1 N HCl, twice with 10-mL portions of 10% $NaHCO_3$, and twice with 10-mL portions of saturated NaCl solution. The organic solution was dried ($MgSO_4$) and filtered, the solvent removed *in vacuo* at 30−35 °C, and the resulting residue dissolved in 50 mL of CH_3CN for direct injection onto a C-18 column for HPLC analysis of yield and extent of LDL isomer. The LLL and LDL forms of the test tripeptide have been described elsewhere.[6] [b] The number in parentheses refers to the number of equivalents of base or additive used. In some cases the amount of base was less than that used in the standard protocol. [c] The number in parentheses refers to the yield of tripeptide, generally based on HPLC examination. In many cases evaporation of solvent gave the crude peptide as a solid for which the yield data based on weight and that obtained by HPLC analysis were roughly comparable. [d] Where more than one number is given for % LDL, the results are of separate runs. The first number given is from the run for which the yield is cited. [e] In this case the figure in parentheses refers to the number of equivalents of H-Pro-NH_2 used in excess of the 1 equiv needed for the acylation reaction.

Z–Gly–Gly–Val–Ala–Gly–Gly–OMe

14

H–Pro–NH–PEG–PS $\xrightarrow[\text{coupling reagent, base}]{\text{Fmoc–Leu–OH}}$

15

Fmoc–Leu–Pro–NH–PEG–PS $\xrightarrow{\text{TFA}}$ Fmoc–Leu–Pro–NH_2

16 **17**

relatively little epimerization in contrast to the case involving DIEA (HAPyU/DIEA: 5.3% DL).[4]

Two final examples of the use of these new bases involved stepwise solid phase coupling reactions. One involved the solid phase coupling of Fmoc-Leu-OH onto proline which had been attached to a PAL-PEG-polystyrene resin **15**.

Following removal of the protected dipeptide amide from the resin by means of trifluoroacetic acid, analysis was carried out according to a method described previously for the coupling of Fmoc-Leu-F with proline amide in solution.[7] The results are presented in Table 3. In this case, the new coupling reagent, tetramethylfluoroformamidinium hexafluorophosphate (TFFH),[32] was ex-

Table 2. Effect of Coupling Reagent and Base on Loss of Configuration at Valine During Formation of Hexapeptide 14 in DMF [a]

coupling reagent	base	yield, %	DL, %
HATU	TEMP	81.2	0.5
HATU	DB(DMAP)	91.2	0.4
HAPyU	TEMP	84.6	0.4
HAPyU	DB(DMAP)	86.2	0.5

[a] Tripeptide segments needed for the assembly of **14** were obtained and coupling reactions performed as described in Table 5 of ref 4, footnote *a*.

Table 3. Effect of Coupling Reagent and Base on Racemization of Fmoc-Leu-OH upon Coupling onto H-Pro-PAL-PEG-PS in DMF

coupling reagent	base	DL, %
TFFH	DIEA	0.8
TFFH	TMP	<0.1
TFFH	TEMP	0.1
TFFH	DB(DMAP)	0.2

[a] Fmoc-Leu-OH was coupled to H-Pro-PAL-PEG-PS (0.2 mmol/g) using 3 equiv of protected amino acid, 3 equiv of coupling reagent, and 6 equiv of base. Solvent: DMF; preactivation time: 7 min; coupling time: 30 min. The protected dipeptide was removed from the resin by cleavage with TFA for 1 h and the analysis carried out according to the method described previously in Table 2, footnotes *a* and *b*, of ref 7.

amined. Results are comparable to those observed with HATU as coupling reagent (DIEA: 0.8% DL; TMP: 0.2% DL). Thus, in spite of its significantly greater basicity, DB(DMAP) is comparable to collidine in maintaining configuration during activation of the carboxylic acid residue.

A second example involved the stepwise assembly of tripeptide **4**. For practical solid phase syntheses quick activation of the amino acid is essential. Collidine is insufficiently basic for such purposes, leading in the case of **4** (TFFH as coupling reagent) to contamination by 39.5% of the des-Val product under conditions where the more basic amine, DB(DMAP), provides for a tripeptide of good quality.

Experimental Section

General. Coupling reactions were carried out as described in footnote *a* of Table 1. Bases were purified as noted in footnote *a*, Table 2, ref 4. Fmoc-PAL-PEG-PS resin, HATU, and HAPyU were obtained from Perseptive Biosystems, Inc. All model peptides have been reported previously. For Z-Phe-Val-Pro-NH_2,[6] Z-Gly-Phe-Pro-NH_2,[6] and Z-Phe-Val-Ala-OMe[4] HPLC analysis was carried out on a C-18, 4 μm Waters Novapak column, 3.9 × 150 mm, flow rate 1 mL/min, detection at 220 nm with a Waters 996 PDA detector using a linear gradient 25−50% CH_3CN/H_2O 0.1% TFA. For Z-Gly-Phe-Val-OMe[6] and Z-Phg-Pro-NH_2[7] all parameters were the same except that for separation isocratic systems of 40% CH_3CN/60% H_2O (0.1% TFA) and 25% CH_3CN/75% H_2O (0.1% TFA), respectively, were used. For retention times and data on characterization of the model peptides see the references cited. As a check on the ability of the system to quantify the minor diastereomer a method similar to that described in footnote *a*, Table 2, ref 4 was used. The system Z-Phe-Val-Pro-NH_2 was selected, and authentic samples of the diastereomeric mixture were weighed out and analyzed using the PDA detector: % LDL form via weight ratio/HPLC-determined figure 50.54/50.95; 1.19/1.20; 0.633/0.63; 0.477/0.49; 0.401/0.40; 0.358/0.36; 0.318/0.33; 0.256/0.26. For the system containing 0.401% by weight of the LDL isomer, for six runs the standard deviation of the collected data was found to be 0.03%. For the coupling reactions, data for which are collected in Tables 1−4, some scatter was noted in measuring the loss of configuration. Where separate runs were carried out the data are listed in Tables 1−4. Measured differences may be due to variable amounts of water present in solvents or reagents, inability to control the presence of basic impurities which might serve to increase racemization or epimerization, and the presence of extraneous materials which might overlap the regions of interest in the HPLC traces. On the basis of experience in carrying out several hundred such determinations, it appears that closer correspondence is not to be expected when the reactions are completed over a period of several months using different samples of solvents, reagents, tertiary amines, etc.

Triisopropylamine. The preparation followed that described.[30] In the synthesis of the intermediate, α-(diisopropylamino)propionitrile, it is important to avoid overheating in the presence of water which is difficult to remove completely from the crude product. In that case most of the propionitrile is converted to lactonitrile. Indeed, lactonitrile, and *i*-Pr_2NH could be converted to α-(diisopropylamino)propionitrile in 38.5% yield by heating in benzene at reflux temperature with removal of water as the azeotrope, followed by distillation at 70−79 °C (13 mm). Conversion of the nitrile to triisopropylamine by the method described gave a yield of 55% (overall yield 21.2%): bp 46−48 °C (14 mm) (lit.[30b] bp 47 °C (14 mm)); ^{1}H-NMR ($CDCl_3$) δ 0.99 (d, 18); 3.12 (sept, 3).

***N*-*tert*-Butylmorpholine**. The synthetic method followed the described[31] procedure which involved refluxing of bis(2-chloroethyl) ether and *tert*-butylamine in ethanol for 7 days: yield 31.8%; bp 175−176 °C (lit.[31] bp 174−176 °C); ^{1}H-NMR ($CDCl_3$) δ 1.06 (s, 9), 2.54 (t, 4), 3.72 (t, 4).

4-(*N*,*N*-Dimethylamino)-2,6-di-*tert*-butylpyridine (13a). (A) From Pinacolone. The method followed that of Potts and Winslow[25] except that dimethylamine was used instead of diethylamine for replacement of the methylthio group. Some discrepancies with the literature description were noted. Pinacolone (5 g, 0.05 mol) dissolved in 200 mL of anhydrous THF was stirred vigorously with 11 g (0.1 mol) of KO-*t*-Bu at room temperature for 1 h, and to the resulting orange-colored reaction mixture was added 10 g (0.05 mol) of 5,5-bis-(methylthio)-2,2-dimethyl-4-penten-3-one. Stirring was continued for 16 h, and the resulting crude orange crystalline potassium salt was filtered and used in the next step without purification. The crude salt (21 g, 0.071 mole), 470 mL of 50% aqueous HBF_4, and 80 mL of CH_2Cl_2 were stirred together at reflux temperature for 3 h. The aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), solvent evaporated, and the residue dissolved in a small amount of acetone. Addition of ether gave 5.7 g (24.6%) of 2,6-di-*tert*-butyl-4-(methylthio)pyrrylium tetrafluoroborate as a colorless crystalline mass, mp 113−114 °C. To a stirred solution prepared from 1.5−2 g (0.033−0.044 mole) of anhydrous dimethylamine in 50 mL of dry MeOH was added 3.5 g (0.01 mole) of the crystalline pyrrylium salt at a temperature of −30 °C. The reaction vessel was then closed, the dry ice/acetone bath removed, and the mixture stirred at room temperature for 24 h. Removal of MeOH *in vacuo* gave an orange crystalline residue which was triturated with ether. Filtration and washing with ether gave 3.4 g (97.4%) of the aminopyrrylium salt as colorless crystals, mp 147−148 °C. An analytical sample was obtained by crystallization from acetone−ether: ^{1}H NMR ($CDCl_3$) δ 6.67 (s, 2), 3.45 (s, 6), 1.39 (s, 18).

Anal. Calcd for $C_{15}H_{26}BF_4NO$: C, 55.73; H, 8.10; N, 4.33. Found: C, 55.90; H, 8.18; N, 4.39.

A solution of 7.5 g (0.023 mole) of the pyrrylium salt in 200 mL of MeOH was added dropwise to a stirred solution of 225 mL of 30% ammonium hydroxide with cooling in an ice bath. The resulting mixture was stirred at room temperature for 12 h and the crystalline product collected by filtration. Recrystallization from MeOH−H_2O gave 5 g (92.5%) of the amine as colorless needles: mp 65−66 °C (lit.[26] mp 65−66 °C); ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 18), 2.97 (s, 6), 6.38 (s, 2). The overall yield from pinacolone was 17.8%.

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(B) From DMAP. The described method[26] was followed using *tert*-butyllithium and led to a yield of 37.5%. The physical properties of the sample agreed with those described above.

***N,N*,2,6-Tetramethyl-4-aminopyridine (2,6-Dimethyl-DMAP, 13c)**. The method followed the latter steps of the procedure described under method A for DB(DMAP) except that the 2,6-dimethyl-4-methoxypyrrylium iodide (45%, mp 114 °C (lit.[33] mp 110 °C) was obtained from 2,6-dimethyl-4-pyrone.[34] Reaction of the pyrrylium iodide with dimethylamine gave 2,6-dimethyl-4-(dimethylamino)pyrrylium iodide (89.6%, mp 220 °C dec): ^{1}H NMR ($CDCl_3$) δ 2.6 (s, 6), 3.55 (s, 6), 7.25 (s, 2). Previously, the corresponding perchlorate salt (mp 184−185 °C dec) had been characterized.[33] Finally, treatment of the 4-(dimethylamino)pyrrylium salt with NH_3/$(NH_4)_2CO_3$[35] for 15 min gave **13c** in 78.4% yield, mp 38−39 °C, after recrystallization from hexane: ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6), 2.94 (s, 6), 6.21 (s, 2). Operations with 2,6-dimethyl-DMAP had to be carried out in a dry glove bag under N_2 due to its extreme hygroscopic nature.

Anal. Calcd for $C_9H_{14}N_2$: C, 71.95; H, 9.39; N, 18.65. Found: C, 71.91; H, 9.41; N, 18.61.

2,3,4,5,6-Pentamethylpyridine (9). The literature method[18] was followed exactly: yield 12.1%; bp 98−100 °C (10 mm); ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 9), 2.48 (s, 6). The corresponding half hydrate had: mp 49−50 °C; ^{1}H-NMR ($CDCl_3$) δ 2.18 (s, 9), 2.46 (s, 6).

2,6-Diethyl-4-methylpyridine. The literature method[8] was followed exactly: yield 17%; bp 94−95 °C (20 mm); ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 6), 2.28 (s, 3), 2.76 (q, 4), 6.79 (s, 2).

2,6-Diisopropyl-4-methylpyridine. The literature method[8] was followed exactly: yield 22%; bp 105 °C (20 mm); ^{1}H-NMR ($CDCl_3$) δ 1.27 (d, 12), 2.29 (s, 3), 2.99 (sept, 2), 6.79 (s, 2).

2,3,5,6-Tetramethylpyridine (7). The method followed exactly that described in the literature.[12,36] Although a multistep procedure, each step works well. The various intermediates were characterized by ^{1}H NMR analysis: (1) 2,6-Dimethyl-3,5-dicarbethoxypyridine ($CDCl_3$) δ 1.41 (t, 6), 2.84 (s, 6), 4.40 (q, 4), 8.68 (s, 1); (2) 2,6-dimethyl-3,5-bis(hydroxymethyl)pyridine ($CDCl_3$) δ 2.54 (s, 6), 4.73 (s, 4), 7.37 (s, 1); (3) 2,6-dimethyl-3,5-bis(chloromethyl)pyridine ($CDCl_3$) δ 2.61 (s, 6), 4.58 (s, 4), 7.56 (s, 1); (4) TEMP ($CDCl_3$) δ 2.19 (s, 6), 2.42 (s, 6); 7.1 (s, 1), 28.9% (overall from ethyl acetoacetate); mp 75−76 °C (lit.[12] mp 76 °C).

Assembly of Z-Phe-Val-Pro-NH_2 and Fmoc-Leu-Pro-NH_2 by Stepwise Solid Phase Coupling. A 10-g sample of amide resin (Fmoc-PAL-PEG-PS; 0.2 mmol/g) was washed with DMF (5 × 20 mL), deprotected with 20% piperidine in DMF (20 mL) for 7 min, washed with DMF (5 × 20 mL), and then treated with 10 mmol of Fmoc-Pro-OH, 20 mmol of DIEA, and 10 mmol of HBTU in 33 mL of CH_2Cl_2 for 2 h. The resulting resin was washed with CH_2Cl_2 (5 × 20 mL), 95% ethanol (1 × 20 mL), and anhydrous ether (2 × 20 mL) and dried *in vacuo*, and 200-mg portions were used in the sequential coupling via Fmoc-Val-OH and Z-Phe-OH. The resin (200 mg) was washed with DMF (5 × 5 mL), deprotected with 20% piperidine in DMF (5 mL) for 7 min, and washed with DMF (5 × 5 mL). Preactivation was carried out for 7 min using 40.7 mg of Fmoc-Val-OH and 56.3 mg of DB(DMAP) or 31.8 μL of TMP and 31.7 mg of TFFH in 0.4 mL of DMF. Following the requisite preactivation period (7 min), the solution of the activated amino acid was added to the resin and reaction allowed to proceed for 30 min. Washing and deblocking of the Fmoc group was followed by an analogous coupling step with Z-Phe-OH (39.5 mg). After the second coupling the resin was washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), 95% EtOH (1 × 5 mL), and anhydrous ether (2 × 5 mL) and dried for 30 min *in vacuo*. The dried resin was treated with TFA (4 mL) for 1 h, filtered, and washed on the filter with 2 mL of TFA and CH_2Cl_2 (2 × 5 mL). The combined filtrates were distilled *in vacuo* at room temperature, and the resulting residue was dissolved in 4 mL of CH_3CN and injected directly onto an HPLC column for analysis. The HPLC traces are shown in Figure 1 (supporting information). Yields as estimated by the intensities of the HPLC curves were DB(DMAP), 80%, and TMP, 65%. The HPLC conditions were as described[6] and showed that for DB(DMAP) and TMP the amount of the des-Val product (Z-Phe-Pro-NH_2, t_R = 12.4 min, confirmed by authentic synthesis) amounted to 3.1% and 35.9%, respectively. Scanning the same HPLC curve for the presence of the LDL or DLL diastereomers (t_R 16.9 and 16.2 min, respectively) showed that no significant stereomutation had occurred at either valine or phenylalanine (<0.2%) during the assembly of **4**. The coupling of Fmoc-Leu-OH to proline amide was examined in the same manner. See Table 3.

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Supporting Information Available: Additional data comparable to that of Table 1 detailing the effect of water, base, additive, and coupling reagent on model peptide coupling and HPLC data demonstrating rapid activation of a protected amino acid by TFFH in the presence of DB(DMAP) (6 pages). This material is contained in 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.

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