JOC Article

Organophosphorus and Nitro-Substituted Sulfonate Esters of 1-Hydroxy-7-azabenzotriazole as Highly Efficient Fast-Acting Peptide Coupling Reagents†

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Organophosphorus esters **9**, **10**, **14**, and **15** prepared via reaction of diethyl- and diphenylphosphoryl chloride, di(*o*-tolyl)phosphinyl chloride, and 2,8-dimethylphenoxaphosphinyl chloride with HOAt are excellent coupling reagents for peptide synthesis which are generally superior to their uronium/guanidinium analogues and HOBt- or HODhbt-derived phosphate ester counterparts in minimizing loss of configuration during segment coupling. The phosphinyl analogues are more shelf-stable than the phosphoryl systems. The new reagents have been tested in segment couplings leading to two tripeptides (**20**, **21**) and a hexapeptide **22**. Outstanding utility is also shown for the solid-phase assembly of the ACP decapeptide. Similar results were obtained with the 2- and 4-nitro- and 2,4-dinitrophenylsulfonyl esters derived from HOAt.

Preventing loss of configuration is one of the major challenges in peptide synthesis.[1] A common method of minimizing loss of configuration during segment coupling is to include in the coupling mixture an additive such as 1-hydroxybenzotriazole (HOBt)[2] or less often, but usually more effectively, 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HODhbt),[3] both described in 1970 by König and Geiger. The former is also used in many protocols for stepwise peptide assembly, either in conjunction with carbodiimides[4] or active esters[5] or by being incorporated into stand-alone reagents such as phosphonium[6] or uronium/guanidinium salts.[7]

Recently, 1-hydroxy-7-azabenzotriazole (HOAt) and its derived phosphonium and uronium/guanidinium salts[8] have been described as more favorable coupling additives or reagents for both solution-[9] and solid-phase syntheses.[10] These compounds enhance coupling yields and reduce the loss of chiral integrity during segment coupling.

While investigating the efficiency of various types of coupling reagents, a literature survey disclosed several reports describing the use of a number of organophosphorus derivatives of HOBt and HODhbt. These included diethoxyphosphinyloxybenzotriazole (DepOBt, **1**),[11] 3-(diethoxyphosphinyloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazine (DepODhbt, **2**),[12] 3-[*O*-(2-oxo-1,3,2-dioxaphosphori-

† Abbreviations: ACP = acyl carrier protein decapeptide 65−74; Aib = α-aminoisobutyric acid; Bs = benzenesulfonyl; DB (DMAP) = 2,6-di-*tert*-butyl-4-(*N*,*N*-dimethylamino)pyridine; DIEA = diisopropylethylamine; DmppOAt = 1-(2,8-dimethylphenoxaphosphinyloxy)-7-azabenzotriazole; DNBs = 2,4-dinitrobenzenesulfonyl; DpopOAt = 1-(diphenoxyphosphoryloxy)-7-azabenzotriazole; DpopODhbt = 3-(diphenoxyphosphinyloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazene; DpopOBt =1-(diphenoxyphosphinyloxy)benzotriazole; DpopCl = diphenoxyphosphoryl chloride; DppCl = diphenylphosphinyl chloride; DtpOAt = 1-[di(*o*-tolyl)-phosphinyloxy]-7-azabenzotriazole; DtpOBt = 1-[di(*o*-tolyl)phosphinyloxy]benzotriazole; DtpODhbt = 3-di(*o*-tolyl)phosphinyloxy]-3,4-dihydro-4-oxo-1,2,3-benzotriazine; HAPyU = 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridinylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDTU = *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt = 7-aza-1-hydroxybenzotriazole; 2NBs =2-nitrobenzenesulfonyl; 4NBs = 4-nitrobenzenesulfonyl; Ns =2-naphthalenesulfonyl; TCM = trichloromethane = chloroform; TIPA = triisopropylamine; TFE = trifluoroethanol; TMP = 2,4,6-collidine

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(1) For a recent review, see: Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton, 1997. See also: (a) Kemp, D. S. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 315. (b) Kovacs, J. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, p 485. Benoiton, N. L. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.;, Academic Press: New York, 1983; Vol. 5, p 217.

(2) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(3) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024, 2034.

(4) Rich, D. H.; Singh, J. In The *Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 250.

(5) (a) König, W.; Geiger, R. *Chem. Ber.* **1973**, *106*, 3626. (b) Bodanszky, M. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 143.

(6) (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *14*, 1219. (b) Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.

(7) (a) Dourtoglou, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, *15*, 1269. (b) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C.; *Synthesis* **1984**, 572. (c) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

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

(9) For a recent illustration of the use of HATU in rapid peptide synthesis in solution, see: Carpino, L. A.; Ismail, M.; Truran, G.; Mansour, E. M. E.; Iguchi, S.; Ionescu, D.; El-Faham, A.; Riemer, C.; Warrass, R. *J. Org. Chem.* **1999**, *64*, 4324.

(10) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

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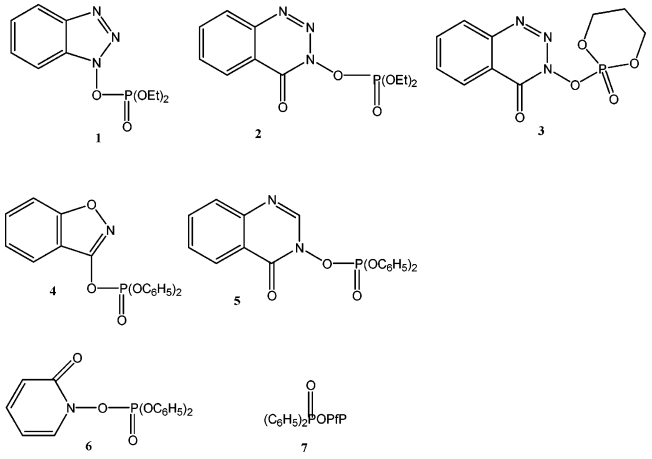
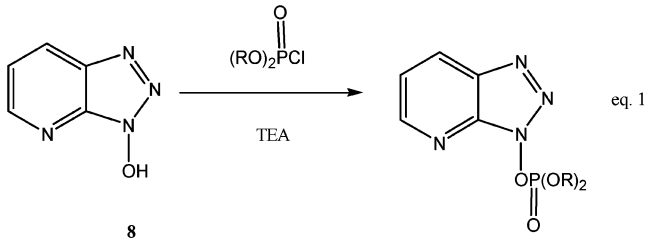
nanyl)oxy]-3,4-dihydro-4-oxo-1,2,3-benzotriazine (DopODhbt, **3**),[12] 1,2-benzisoxazol-3-yl diphenyl phosphate **4**,[13] 3-(diphenoxyphosphinyloxy)-3,4-dihydro-4-oxo-1,3-quinazoline **5**,[14] 1-diphenoxylphosphinyloxy-2-oxopyridine **6**,[15] and pentafluorophenyl diphenylphosphinate (FDPP, **7**).[16] While the present work was underway a report on the remarkable properties of **2** appeared.[17] Although our own studies on DepODhbt(DEPBT) **2** showed that this reagent did not always outperform HATU, we were struck by how much better DepOBt **1** and DepODhbt **2** performed in terms of configurational control than their uronium/guanidinium counterparts HBTU and HDTU, respectively. These findings led us to an examination of new organophosphorus reagents built upon HOAt, for in this case the neighboring group effects[8] believed to be important in the properties of HOAt would be superimposed on whatever effects enhance the efficiency of the phosphorus moiety. Based on the results described here, we believe that these effects are related to the greater speed with which protected amino acids are converted to their active esters by the phosphorus derivatives.



1 2 3 4 5 6 7

Diethoxyphosphinyloxy-7-azabenzotriazole (DepOAt) **9** and diphenoxyphosphinyloxy-7-azabenzotriazole (DpopOAt) **10** were obtained from diethyl phosphorochloridate or the diphenyl analogue and HOAt in the presence of triethylamine in dry benzene under a nitrogen or argon atmosphere at 0 °C (eq 1). Recrystallization of **9** from dry chloroform-hexane and **10** from DCM−hexane gave analytically pure reagents as colorless blocklike crystals. The crude products obtained in this way could be used without further purification, and in the tests



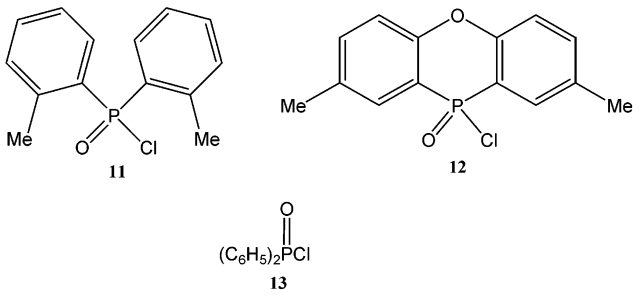
8 eq. 1

9, R = Et

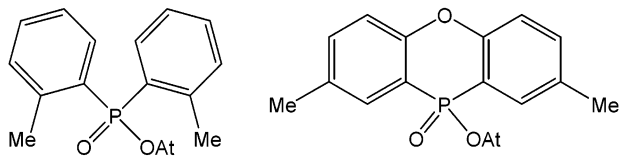
10, R = C_6H_5

reported here freshly prepared material was always used. Long-term storage, unfortunately, required great care due to the hydrolytic sensitivity of these materials. Because these new phosphate esters were clearly superior to the older uronium/guanidinium reagents for segment coupling and under certain conditions for solid-phase peptide assembly as well, it was considered essential to search for reagents of this type, which exhibited greater shelf stability.

Having available from other ongoing studies a number of phosphinyl chlorides such as **11**[18] and **12**[19] which were easy to handle and not particularly moisture-sensitive relative to the parent model, diphenylphosphinyl chloride **13**,[20] we investigated the conversion of **11** and **12** to the corresponding OAt esters. Indeed, as expected the resulting esters **14** and **15** proved to be significantly more stable toward moisture in the air than the corresponding phosphate esters yet showed the same excellent character as coupling reagents.



11 **12** **13**



14 **15**

Because the greatest stability was found in the Dtp derivative **14**, this system was extended to the corresponding OBt and ODhbt esters **16** and **17**, respectively. The phosphinyl esters proved generally to be as efficient as the phosphate analogues and in view of their extended shelf stability are to be recommended for general use.

(11) Kim, S.; Chang, A.; Ko, Y. K. *Tetrahedron Lett.* **1985**, *26*, 1341. Previously the abbreviation BDP was used for **1**. In the present work, abbreviations have been used which more easily allow **1**, **2**, and other members of the series to be distinguished from one another.

(12) Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455. Previously, the abbreviation DEPBT was used for **2**. See the comment in ref 11 regarding abbreviations.

(13) Ueda, M.; Oikawa, H. *J. Org. Chem.* **1985**, *50*, 760.

(14) Horiki, K. In *Peptides: Chemistry, Structure and Biology*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, The Netherlands, 1990; p 907.

(15) Kim, S.; Kim, S. S. *J. Chem. Soc., Chem. Commun.* **1986**, 719.

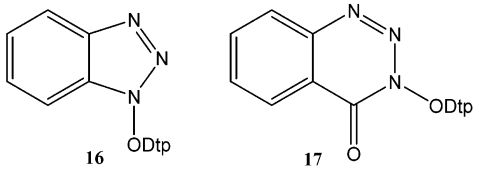
(16) (a) Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711. (b) Dudash, J., Jr.; Jiang, J.; Mayer, S. C.; Joullie, M. M. *Synth. Commun.* **1993**, *23*, 349.

(17) Li, H.; Xiaohui, J.; Ye, Y.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91.

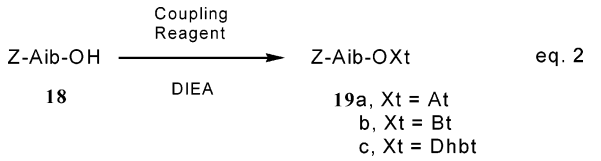
(18) Harger, M. J. P. *J. Chem. Soc, Perkin Trans. 2* **1980**, 154.

(19) Granoth, I.; Kalir, A.; Pelah, Z.; Bergmann, E. D. *Tetrahedron* **1970**, *26*, 813.

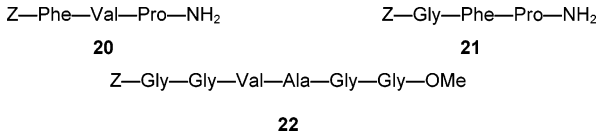
(20) Hunt, B. B.; Saunders: B. C. *J. Chem. Soc.* **1957**, 2413.



Active Ester Formation. It is generally believed that during peptide coupling via uronium/guanidinium or phosphonium salts, the N-protected carboxylic acid first reacts with the coupling reagent to form an active ester, which then reacts with the amino component to give the corresponding amide. Thus, the speed of formation of such an active ester is one of the important factors in evaluating the efficiency of a coupling reagent. The model chosen for study here involved conversion of *N*-benzyloxycarbonyl-α-aminoisobutryic acid (Z-Aib-OH, **18**) to the corresponding active ester in both DMF and $CDCl_3$ (eq 2). The benzylic CH_2 units of acid **18** (δ 5.09) and active ester **19** (δ 5.20) were monitored by proton NMR analysis. Assignment of the peak at δ 5.20 to **19a**−**c** was confirmed by authentic syntheses of these compounds.[21] Because of the sterically hindered carbonyl group of Aib, activation in the sense of eq 2 is slow relative to the cases of the proteinogenic amino acids allowing different coupling reagents to be more easily differentiated. Results of the halftime determinations are collected in Table 1. In DMF, it was found that all of the tested reagents, except for DtpOBt and DepODhbt, led to rapid formation of the active esters. Upon switching to the less polar solvent $CDCl_3$, noticeable reactivity differences were observed, especially for DtpOBt and HBTU. The case of HDTU stands out as an exception: the rapid activation caused by this reagent is not matched by exemplary results in either segment coupling or peptide assembly.[22] Although not yet completely understood, similar results have been cited previously and ascribed to the low reactivity of the active ester.[23] These data show that coupling reactions will be completed much more quickly for the OAt relative to the ODhbt or OBt systems with the natural high reactivity of the OAt ester enhanced by its greater ease of formation.



To test for configurational control using the new phosphorus-based reagents, three previously studied[22,24] model systems **20**−**22** were examined. A summary of the results for the various coupling reagents is given in Table 2. A more extensive listing is presented in the Supporting Information section (Tables 10 − 12).



It is clear that the new phosphorus-based OAt derivatives are more effective in preserving configuration than any of the other tested reagents, including HATU. The best of the previously described uronium/guanidinium salts (HAPyU)[21,24] sometimes equals the results of the new phosphorus esters, but where differences are observed, the latter have proved more effective in every case examined to date. Best results were generally, but not always obtained when 1 equiv of HOXt was included in the reaction mixture. Since results were best when using the base 2,4,6-collidine, only results with this base are included in the summary table. Results for a wide variety of other bases are found in the Supporting Information.

Among the results gleaned from these tables, similar to an earlier case[24] involving HAPyU, it was found that for the new reagents DepOAt and DpopOAt a 1 equiv excess of proline serving as base gave the lowest epimerization levels yet observed for tripeptide **20** in DMF (0.5% LDL-isomer). Upon switching to other solvents, even

TABLE 1. Approximate Halftimes for the Formation of Z-Aib-OXt

coupling reagent	$t_{1/2}$ (DMF) (min)	$t_{1/2}$ ($CDCl_3$) (min)
DepOAt, **9**	<2	2−3
DpopOAt, **10**	<2	2−3
DepODhbt, **2**	7−8	45−47
DpopODhbt[a]	<2	<2
DtpOBt, **16**	65−70	11−12 h
HATU[a]	<2	14−15
HAPyU[a]	<2	<2
HDTU[a]	<2	<2
HBTU[a]	<2	>24 h

[a] See list of abbreviations not defined in text.

TABLE 2. Effect of Coupling Reagent on Extent of Epimerization during [2 + 1] Coupling Leading to Z-FVP-NH_2 and Z-GFP-NH_2 and [3 + 3] Coupling Leading to Z-GGVAGG-OMe in DMF with 2 equiv of TMP as Base[a,b]

coupling reagent	Z-FVP-NH_2	Z-GFP-NH_2	Z-GGVAGG-OMe
DepOAt, **9**	0.9 (0.9)	<0.1	<0.1
DmppOAt, **15**	3.6 (2.0)	0.3	
DtpOAt, **14**	2.9 (1.4)	0.4	
HATU[c]	5.0 (1.8)	1.1 (0.9)	2.4
DepODhbt, **2**	3.5	0.3	2.4
DtpODhbt, **17**	4.3 (3.6)		
HDTU[c]	8.5 (4.0)		3.3
DtpOBt, **16**	11.4	2.2	
HBTU[c]	14.2	3.6	8.2

[a] All figures are given as percent of the LDL- or DL-form as observed by HPLC analysis. [b] Figures in parentheses refer to identical runs but with 1 equiv of the appropriate HOXt added. [c] See list of abbreviations not defined in text.

(21) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695. Although the term "active ester" is used in the present work, this term should be understood to refer to the mixture of species, often including the corresponding "active amide", obtained upon activation of the acid. See: Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 441. The latter reference also describes the synthesis of the O-forms of HATU, HAPyU, and HBTU. In the present work, all studies of these reagents involved the N-forms.

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

(23) (a) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678. (b) See, however, ref 8 and for a more extensive compilation of pK_a data in this series, see: Koppel, I.; Koppel, J.; Leito, I.; Pihl, V.; Grehn, L.; Ragnarsson, U.; *J. Chem. Res., Synop.* **1993**, 446.

(24) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.

TABLE 3. Distribution of Products, Including Various Deletion Peptides, According to HPLC Analysis[a] for the Assembly of ACP (65−74) via HOAt-Derived and Related Coupling Reagents

entry	coupling method	equiv of reagents[b]	preactivation time (min)	coupling (min)	ACP (%)	−2Ile (%)	−Ile^{72} (%)	−Ile^{69} (%)	−Val (%)	−Ala (%)	−Asn (%)
1	DepOAt, **9**	1.5	7	1.5	84		2	2	1	4	5
2	DpopOAt, **10**	1.5	7	1.5	85		2	1	2	6	3
3	DepODhbt, **2**	1.5	7	1.5	6	9	13	19	3		1
4	DpopOBt[c]	1.5	7	1.5	23	21	26	19	1	1	2
5	HATU[c]	1.5	7	1.5	85		1	1	3		10
6	HDTU[c]	1.5	7	1.5	38	15	15	26			5
7	DepOAt, **9**	1.5	0	1.5	86		4	2	2	3	1
8	DpopOAt, **10**	1.5	0	1.5	81		4	1	1	7	
9	DepODhbt, **2**	1.5	0	1.5	< 1[d]						
10	DpopOBt[c]	1.5	0	1.5	29	17	25	17	2		3
11	HATU[c]	1.5	0	1.5	87		3	1	2		6
12	HDTU[c]	1.5	0	1.5	30	15	19	22	3		4

[a] A reversed-phase C-18 column was used with elution by a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% TFA from 1:19 to 1:1, flow rate 1.0 mL/min. [b] Couplings were carried out in DMF in the presence of 2 equiv of DIEA per equivalent of Fmoc-amino acid/coupling reagent. [c] See list of abbreviations not defined in text. [d] Only a trace of the desired product was obtained.

greater differences were found between the new phosphorus reagents and the related uronium/guanidinium salts. For example, in the special structure-breaking combination solvent trifluoroethanol/trichloromethane (TFE/TCM, 1:3)[25] 12.2% of the LDL-form was obtained for DpopOAt/TMP as opposed to 38.5% for HATU/TMP. In DCM in the presence of TMP, 2.2% (DepOAt) and 2.9% (DpopOAt) were clearly better than values observed for guanidinium reagents HATU (9.3%) or HAPyU (5.3%). In the case of a 2:1 mixture of DMF/DCM as solvent in the presence of TMP, the epimerization figures for various coupling reagents were found to be less than for either DMF or DCM alone. For example, in the case of **20,** coupling via HATU/TMP in DMF/DCM the amount of the LDL-form is 2.6% compared to 5.0% in the case of DMF and 9.3% for DCM.

To check on the coupling efficiency of diphenyl phosphorochloridate (DpopCl), various coupling conditions were used. It was noted that without additive, DpopCl gave only a very small amount of the desired peptide for both DIEA and TMP, results which are in sharp contrast to those of Quibell and co-workers[26] on the use of diphenylphosphinyl chloride (DppCl). If 1 equiv of HOAt was present, the results were acceptable. Indeed, the mixture DpopCl/HOAt/Base, which contains DpopOAt as the active species, gave results which are comparable to those obtained with the isolated reagent DpopOAt.

Tripeptide **20** was also chosen as a model to study loss of configuration associated with use of the new organophosphorus reagents under solid-phase conditions. In comparison with results obtained in solution, the data show how much more difficult it is to maintain configuration in the solid-phase mode.[27] The system involved overnight coupling of four equivalents of Z-Phe-Val-OH onto H-Pro-PAL-PEG-PS in the presence of 8 equiv of TMP in DMF, cleavage of the tripeptide from the resin via TFA-H_2O (9:1) over a period of 1 h, and separation of the diastereomers as described for the solution system. Although extensive loss of configuration occurs in all cases, the data show that the effectiveness of the various coupling reagents follows the same order as in solution, thus coupling reagent/LDL (%): DepOAt/11.6, HAPyU/13.0, HATU/13.6, DpopODhbt/19.1, DepODhbt/19.5, HDTU/24.2, HBTU/29.8.

ACP Assembly via Stepwise Coupling on Solid Phase. To demonstrate the suitability of the new organophosphorus-based coupling reagents and compare their performance with that of the corresponding uronium/guanidinium analogues in solid-phase syntheses, several syntheses of the ACP decapeptide segment 65−74 (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2) were carried out by Fmoc chemistry according to a method described previously[10] in which coupling times are shortened and excesses of reagents are reduced in order to bring out differences among the various reagents studied. Under these conditions, incomplete incorporations were detected for Asn onto Gly, Ile^{72} onto Asn, Ile^{69} onto Asp, Val onto Gln, and Ala onto Ala or Asp. The results are collected in Table 3. Analysis of the chromatograms indicated that the new HOAt-based organophosphorus reagents **9** and **10** are as effective as HATU under these so-called "1.5 × 1.5" conditions with or without preactivation. Under normal coupling conditions such as 4 eqs. excess amino acid/30 min. coupling time, all reagents worked well with the exception of HDTU.

Esters of sulfonic acids and HOBt related to those described for the phosphorus series have also been used for peptide coupling.[28] The reactivity of such sulfonate esters was shown to be directly related to the presence of electron-withdrawing substituents in both the HOBt and the sulfonic acid moieties.[29]

This methodology has seen little practical application however, since, depending on the basicity or reactivity of the amino component of the coupling process, the use of such sulfonate esters for in situ coupling is often com-

(25) Kurada, H.; Chen, Y.-N.; Kimura, T.; Sakakibara, S. *Int. J. Pept. Protein Res.* **1992**, *40*, 294.

(26) Quibell, M.; Packman, L. C.; Johnson, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1219.

(27) See also: Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279.

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TABLE 4. Sulfonate Esters of HOXt[a]

compd	mtd	yield (%)	mp (°C)	^{1}H NMR[b] δ	mol formula	anal.data: calcd/found C	H	N
Ts-OAt	A	89.9	133−134	2.51 (s, 3), 7.41−7.49 (m, 3), 7.87−7.90 (m, 2), 8.36−8.41 (dd, 1), 8.78−8.79 (dd, 1)	$C_{12}H_{10}N_4O_3S$	49.65/49.42	3.47/3.31	19.30/19.08
Ts-OBt	A	69.6	80−81	2.50 (s, 3), 7.38−7.48 (m, 3), 7.55−7.68 (m, 2), 7.76−7.80 (m, 2), 7.99−8.03 (m, 1)	$C_{13}H_{11}N_3O_3S$	53.97/53.94	3.83/3.66	14.52/14.40
Ts-4-OAt	A	72.7	102−103 dec	2.51 (s, 3), 7.39−7.44 (dd, 2), 7.56−7.62 (q, 1), 7.73−7.77 (m, 2), 8.10−8.15 (dd, 1), 8.82−8.84 (dd, 1)	$C_{13}H_{12}N_4O_3S$	49.65/49.43	3.47/3.25	19.30/19.33
Ts-4-Me-OAt	A	80.5	120−121	2.51 (s, 3), 2.82 (s, 3), 7.21−7.24 (dd, 1), 7.41−7.45 (d, 2), 7.86−7.90 (d, 2), 8.59−8.61 (d, 1)	$C_{12}H_{10}N_4O_3S$	51.30/51.23	3.98/3.95	18.45/18.47
Bs-OAt	A	88.2	108−109	7.43−7.49 (q, 1), 7.61−7.69 (m, 2), 7.80−8.04 (m, 3), 8.37−8.42 (dd, 1), 8.75−8.78 (dd, 1)	$C_{11}H_8N_4O_3S$	47.82/47.54	2.92/2.64	20.28/19.99
Bs-OBt	A	77.6	84−85	7.41−7.49 (m, 1), 7.55−7.67 (m, 4), 7.78−7.87 (m, 1), 7.90−7.95 (m, 2), 7.99−8.04 (m, 1)	$C_{12}H_9N_3O_3S$	52.35/52.20	3.30/3.25	15.26/15.13
Ms-OAt	A	86.2	76−78	3.69 (s, 3), 7.48−7.55 (q, 1), 8.43−8.48 (dd, 1), 8.81−8.84 (dd, 1)	$C_6H_6N_4O_3S$	33.64/33.45	2.82/2.56	26.16/25.92
Ms-OBt	A	70.2	89−90	3.61 (s, 3), 7.44−7.53 (m, 1), 7.60−7.73 (m, 2), 8.05−8.10 (m, 1)	$C_7H_7N_3O_3S$	39.43/39.29	3.31/3.17	19.7/19.43
2-NBs-OAt	A	88.1	135−136 dec	7.46−7.52 (q, 1), 7.81 (m, 1), 8.00−8.14 (m, 3), 8.39−8.44 (dd, 1), 8.73−8.76 (dd, 1)	$C_{11}H_7N_5O_5S$	41.12/40.97	2.20/2.03	21.80/21.74
4-NBs-OAt	A	78.4	160−161 dec	7.47−7.53 (q, 1), 8.23−8.28 (m, 2), 8.40−8.52 (m, 3), 8.75−8.78 (dd, 1)	$C_{11}H_7N_5O_5S$	41.12/41.17	2.20/2.25	21.80/21.81
4-NBs-OBt	A	72.2	138−139 dec	7.45−7.54 (m, 1), 7.63−7.72 (m, 2), 8.01−8.18 (m, 3), 8.45−8.50 (m, 2)	$C_{12}H_8N_4O_5S$	45.00/45.25	2.52/2.56	17.49/17.56
Ns-OAt	A	93.6	140−141	7.5−8.15 (m, 6), 8.5 (dd, 1), 8.65 (dd, 2), 8.74 (dd, 1)	$C_{15}H_{10}N_4O_3S$	55.21/55.06	3.07/3.12	17.18/17.09
Ns-4-OAt	A	93.1	135 dec	7.2−8.3 (m, 7), 8.65 (d, 2), 8.84 (dd, 1)	$C_{15}H_{10}N_4O_3S$	55.21/55.12	3.07/3.06	17.18/17.25
Ns-ODhbt	A	92.1	139 dec	7.40−8.4 (m, 9), 8.65 (d, 2)	$C_{17}H_{11}N_3O_4S$	57.78/57.66	3.12/3.06	11.89/11.95
2-NBs-4-OAt	A	78.4	105 dec	7.6−7.7 (m, 1), 7.8−7.92 (m, 1), 8.0−8.2 (m, 4), 8.82 (dd, 1)	$C_{11}H_7N_5O_5S$	41.12/41.01	2.18/2.10	21.81/21.74
4-NBs-4-OAt	A	83.5	114 dec	7.2−7.6 (m, 2), 8.0−8.2 (m, 2), 8.24−8.70 (m, 2), 8.9 (dd, 1)	$C_{11}H_7N_5O_5S$	41.12/41.05	2.18/2.18	21.81/21.61
2,4-DNBs-4-Me-OAt	B	75.2	156−157 dec	2.83 (s, 3), 7.29 (s, 1), 8.36−8.40 (d, 1), 8.54−8.66 (m, 2), 8.84−8.50 (d, 1)	$C_{12}H_8N_6O_7S$	37.90/37.71	2.12/2.13	22.10/21.95
2,4-DNBs-OAt	B	89.9	147−148 dec	7.51−7.58 (q, 1), 8.30−8.35 (d, 1), 8.48−8.59 (m, 2), 8.64−8.66 (dd, 1), 8.84−8.85 (d, 1)	$C_{11}H_6N_6O_7S$	36.07/36.06	1.65/1.58	22.95/23.00

[a] For abbreviations in this table and in Tables 5−9 for the sulfonate moiety see the list of abbreviations not cited in the text. For the method see the Experimental Section. [b] Solvent $CDCl_3$.

promised by formation of a sulfonamide byproduct.[28a,29b] Such byproduct formation which, among amino acid derivatives,[30] is particularly prominent with proline derivatives, can be avoided by preactivation of the reactive carboxylic acid component.

On the other hand, in the use of these reagents for segment coupling, especially if such conversions are slow, use of a preactivation step is counterproductive since loss of configuration at the C-terminal carboxylic acid residue directly increases with preactivation time.[31] As demonstrated in other systems, the substitution of 1-hydroxy-7-azabenzotriazole (HOAt) for HOBt is expected to reduce the extent of configurational loss, although the advantages of HOAt are lost for long preactivation times. In the case of application to model tripeptide, **20** which involves coupling to proline amide, esters **23a** or **23b** gave **20** in only low yield (ca. 15%), the major product being sulfonamide **24**. For the case involving collidine as base,

(30) For specific use of the methanesulfonate of HOBt as a selective N-mesylating agent, see: Kim, S. Y.; Sung, N.; Choi, J.; Kim, S. S. *Tetrahedron Lett.* **1999**, *40*, 117.

(31) For the deleterious effect of long preactivation times in other systems see: (a) ref 27. (b) Han, Y.; Albericio, F.; Barany, G. *J. Org. Chem.* **1997**, *62*, 4307.

TABLE 5. Effect of Coupling Reagent and Base on the Extent of Inversion at Valine during Formation of Z-Phe-Val-Pro-NH_2 via [2 + 1] Coupling in DMF[a]

	DIEA		TMP		DB(DMAP)	
coupling reagent	LDL, % (yield, %)	side peak,[b] %	LDL, % (yield, %)	side peak,[b] %	LDL, % (yield, %)	side peak,[b] %
NsOAt	10.8 (92.1)	nd	4.0 (89.9)			
NsOAt[c]	37.5 (93.4)	nd	33.1 (91.2)			
NsOBt	21.2 (91.2)	nd	12.6 (89.3)			
NsODhbt[d]	29.1 (93.1)	nd	16.4 (89.9)			
2-NBsOAt	19.2 (53.5)	25.8	5.6 (66.4)	8.0		
2-NBsOAt/HOAt	8.1 (59.4)	10.2	6.2 (68.5)	9.1		
4-NBsOAt	21.0 (70.4)	18.9	4.4 (79.3)	11.1	2.5 (80.4)	13.9
4-NBsOAt/HOAt	7.2 (82.4)	14.7	4.4 (81.4)	10.8	1.4 (63.7)	7.5
4-NBsOAt[e]	35.7 (72.5)	0	4.7 (81.7)	7.6		
4-NBsOAt/HOAt[e]	12.8 (84.1)	0	3.1 (73.4)	7.2		
4-NBsOBt	31.0 (70.3)	11.4	12.6 (78.8)	6.1	9.6 (85.4)	6.8
DNBsOAt	25.5 (47.7)	33.2	3.9 (71.9)	21.9	4.4 (43.1)	37.8
DNBsOAt/HOAt	14.9 (34.7)	37.1	2.3 (48.5)	35.6	1.6 (35.01)	38.1

[a] Couplings were carried out following published procedures[24] with 2 equiv of base being used unless otherwise specified. For HPLC conditions and retention times for the various reaction components see the references cited. [b] The side peak represents the arenesulfonyl derivative of proline amide formed as a byproduct. In the case of NsOAt, the amide was isolated and characterized (see the Experimental Section). As noted in previous publications (refs 28a and 29b), such side products are observed only with the more basic amines, e.g., β-phenylethylamine and proline; nd = not determined. [c] The dipeptide acid was preactivated at 0 °C for 30 min and added to a solution of H-Pro-NH_2 and 1 equiv of base in DMF. [d] HPLC analysis in this case showed 20−25% of unreacted starting material. [e] In these runs, H-Pro-NH_2 (instead of the coupling reagent) was added last to the reaction mixture (preactivated for 3 min).

TABLE 6. Effect of Coupling Reagent and Base on the Extent of Inversion at Phenylalanine during Formation of Z-Gly-Phe-Pro-NH_2 in DMF[a]

coupling reagent	base	yield (%)	LDL (%)	side peak[b] (%)
4-NBs-OAt	DIEA (2)	30.4	9.9	22.6
4-NBs-OAt/HOAt	DIEA (2)	51.6	5.5	9.4
4-NBs-OAt	TMP (2)	45.3	2.6	11.3
4-NBs-OAt/HOAt	TMP (2)	48.1	1.1	11.0

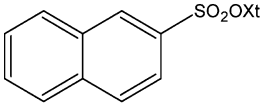
[a] Coupling conditions and method of HPLC analysis followed the description given in footnote a, Table 5. [b] Arenesulfonyl-Pro-NH_2.

TABLE 7. Effect of Coupling Reagent and Base on the Extent of Inversion at α-Phenylglycine during Formation of Z-Phg-Pro-NH_2 in DMF[a]

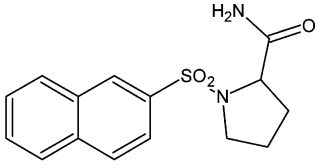
coupling reagent	base	yield(%)	LDL (%)	side peak[b] (%)
4-NBs-OAt	DIEA (2)	61.1	1.2	9.0
4-NBs-OAt/HOAt	DIEA (2)	63.5	1.1	9.5
4-NBs-OAt	TMP (2)	67.4	3.3	6.9
4-NBs-OAt/HOAt	TMP (2)	73.5	3.0	6.6

[a] Coupling conditions and method of HPLC analysis followed the description given in footnote a, Table 5. [b] Arenesulfonyl-Pro-NH_2.

the extent of epimerization was 12.6% for the OBt ester and 3.9% for the OAt ester. Preactivation for 30 min in the latter case provided a good yield of **20** but loss of configuration increased drastically from 3.9 to 33.1%. Thus, this technique is completely unsuitable for practical application in any case where sulfonamide formation is competitive with amide formation.



23a, X=B
23b, X=A



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Curiously, in the sulfonamidation of proline amide, there did not appear to be a clear difference between the reactivity of the sulfonyl esters of HOAt and those of HOBt as has previously been observed in the case of the corresponding acyl esters where the OAt esters are significantly more reactive than the OBt esters.[8] Indeed, results varied depending on the particular system under study. Thus, whereas the 2-naphthalenesulfonyl and 4-tosyl OBt esters gave the sulfonamides more quickly than the OAt esters, in the case of the mesyl and 4-nitrobenzenesulfonyl cases the OAt derivatives were more reactive. Either neighboring group effects are not important in the sulfonyl systems or other factors take precedence.[32] Indeed sulfonate esters derived from 4-HOAt were the most reactive of those examined in the present studies. Currently we have no consistent explanation for the high reactivity of the 4-OAt sulfonates, since, if neighboring group effects are not important, it is expected that the order of reactivity would be RSO_2-4-OAt > RSO_2-7-OAt > RSO_2OBt in view of the reported pK_a data.[8,23] While this could explain the high reactivity of the 4-HOAt derivatives relative to those of 7-HOAt it cannot account for the inconsistency between the figures for the 7-HOAt and HOBt derivatives.

Of the various sulfonate esters of HOAt, which were examined (Tables 4−7) only the nitrosubstituted benzenesulfonyl esters proved to be useful in reducing epimerization levels equal to or lower than those attained via the common reagent HATU. For example, whereas TsOAt/HOAt and BsOAt/HOAt gave in the case of model tripeptide **20**, in the presence of collidine 5.4% and 7.2% of the LDL-form, respectively, in the case of 4-NBs-OAt/

(32) It is not known whether sulfonate esters are involved in an equilibrium analogous to that of the O-acyl/N-acyl type which has been firmly established by X-ray crystallography for the carboxylic acid derivatives.[33] The asymmetric and symmetric stretching vibrations for the sulfonyl residue measured for $MeSO_2OAt$ (1390, 1190 cm^{-1}) or $MeSO_2OBt$ (1387, 1190 cm^{-1}) are more in line with quoted positions[34] for sulfonate esters (1420−1330, 1200−1195 cm^{-1}) than for sulfonamides (1370−1330, 1180−1160 cm^{-1}). 1-(Methylsulfonyl)benzotriaole shows the corresponding absorptions at 1382 and 1178^{-1}.[35] X-ray crystallographic analysis of $MeSO_2OBt$ has confirmed the sulfonate ester structure.[36]

TABLE 8. Extent of Reaction over Time for the Formation of Active Ester via Reaction with Z-Aib-OH in the Presence of DIEA[a]

coupling reagent	yield (%) (Z-Aib-OAt or Z-Aib-OBt)						
	2 min	5 min	10 min	20 min	30 min	1 h	24 h
TsOAt		44.2	52.6	63.3	66.8	81.2	100
TsOBt		56.3	66.7	78.6	82.5	89.9	98.9
Ts-4-OAt[b]	77.1	88.1	92.9	96.4	98.1	99.8	100
BsOAt		73.8	82.5	89.8	92.3	96.8	
BsOBt		79.8	86.9	93.4	95.6	98.8	
2-NBsOAt	88.0	93.3	95.1	96.0	97	97.8	100
4-NBsOAt	99.1	100	100	100	100	100	100
4-NBsOBt	99.4	99.5	99.5	100	100	100	100
4-NBs-4-OAt[c]	97.1	100	100	100	100	100	100
MsOAt		86.8	90.2	93.6	94.9	97	100
MsOBt		86.2	88.9	91.7	93.9	97	

[a] To a solution of 0.1 mmol of Z-Aib-OH and 0.1 mmol of DIEA in 1 mL of DMF was added 0.1 mmol of RSO_2OAt (or RSO_2OBt). Then 40 μL of the reaction mixture was dissolved in 2.5 mL of CH_3CN and directly analyzed by HPLC. For HPLC separation, a gradient system was used involving 5−60% CH_3CN in H_2O/0.1% TFA in 20 min, flow rate 1 mL/min, $\lambda_{254\ nm}$, t_R: Z-Aib-OAt, 20.1 min; Z-Aib-OBt, 23.1 min; TsOAt, 19.97 min; TsOBt, 22.62 min; BsOAt, 18.22 min; BsOBt, 21.12 min; 2-NBsOAt, 17.97 min; 4-NBsOAt, 19.38 min; 4-NBsOBt, 21.68 min; MsOAt, 11.09 min; MsOBt, 15.74 min. [b] For HPLC separation, a gradient system was used involving 5−40% CH_3CN in H_2O/0.1% TFA in 20 min, flow rate 1 mL/min, $\lambda_{254\ nm}$, t_R: Ts-4-OAt, 25.83 min; Z-Aib-4-OAt, 26.17 min. [c] For HPLC separation, a gradient system was used involving 5−60% CH_3CN in H_2O/0.1% TFA in 20 min, flow rate 1 mL/min, $\lambda_{254\ nm}$, t_R: 4-NBs-4-OAt, 18.73 min; Z-Aib-4-OAt, 19.8 min. For all separations in this table the column was a Waters C_{18} Novapak, 60 Å, 4 μm column of dimensions 3.9 × 150 mm.

HOAt the corresponding figure was 3.1% and for DNBs-OAt/HOAt, 2.3%. If in this system collidine is replaced by DB(DMAP)[24] these figures drop to 1.4% and 1.6%, respectively. It is to be expected that even lower levels of configurational loss and lower levels of sulfonamide byproduct formation will be observed on application of this methodology to coupling at amino acids other than proline. For comparison, with HATU in the presence of 1−4 equiv of DB(DMAP) and 1 equiv of HOAt the range of LDL-isomer formation is 1.5−2.2%.[24]

Part of the reason for these low epimerization levels may be the exceptionally rapid rate of activation of the carboxylic acid component of the reaction induced by these sulfonate esters. Thus, for conversions of Z-Aib-OH to Z-Aib-OAt in the presence of DIEA up to 99.1% of the OAt ester is formed within 2 min with 4-NBs-OAt, whereas with Ts-OAt at 5 min the comparable figure is 44.2% and even after 1 h only 81.2% of the acid has reacted. For selected examples, see Tables 8 and 9.

With such rapid reactions one also expects highly effective stepwise peptide assembly. In fact, this proved to be the case for the model decapeptide ACP upon assembly according to the "1.5 × 1.5" scheme, i.e., with a 1.5-fold excess of protected amino acid, a 1:1 mixture of DIEA and collidine as base, and a coupling time of 1.5 min.[10] With both 4-NBs-7-OAt and 4-NBs-4-OAt, this led to syntheses comparable to that achieved by HATU (Figure 1, Supporting Information). Such excellent results were not achieved if the mixed base system is replaced by 2 equiv of DIEA. As noted in earlier studies, excess DIEA may lead to poorer syntheses due to increased oxazolone formation and various degradation reactions including hydrolysis of the active esters.[37]

TABLE 9. Rate of Formation of Z-Aib-OXt via Reaction of NsOXt[a]

preactivation time (min)	NsOAt	NsOBt	Ns-4-OAt	NsODhbt
1	39.6	52.6	96.5	3.2
2	82.2	81.1	100	8.6
5	85.6	90.5	100	16.7
30	94.1	99.5	100	37.5

[a] The rate of formation was followed by reaction of 0.125 mmol of NsOXt with 0.125 mmol of Z-Aib-OH in 1 mL of DMF. At the various preactivation times 50 μL was picked up and diluted to 3 mL with a mixture of CH_3CN/H_2O (1:2) and 10 μL was injected onto the HPLC column using a linear gradient of 5−65% CH_3CN, in H_2O/ 0.1% TFA in 20 min, C_{18} Nova Pak column (4 μm, 60 Å, 3.9 × 150 mm). The reaction was followed by the rate of decrease of the starting material (NsOXt) at t_R 21.8, 24, 20.8, 23.4 min for NsOAt, NsOBt, Ns(4OAt), and NsODhbt, respectively, and the rate of formation of Z-Aib-OXt at t_R 19.4, 21.6, 18.8, 21.5 min for Z-Aib-OAt, Z-Aib-OBt, Z-Aib-4-OAt, and Z-Aib-ODhbt, respectively.

Conclusions

A new class of phosphorus-based coupling reagents incorporating the HOAt moiety is described which is superior to the uronium/guanindinium counterparts in terms of retention of configuration during segment coupling. Similarly effective sulfonate esters are obtainable from nitro substituted sulfonic acids. In contrast to the uronium/guanindinium counterparts, the new reagents are soluble in many organic solvents including a nonpolar solvent such as DCM.

Experimental Section

Diethoxyphosphinyloxy-7-azabenzotriazole (DepOAt, 9). A solution of HOAt (1.36 g, 10 mmol) and TEA (1.39 mL, 10 mmol) in 15 mL of dry benzene was cooled in an ice bath. To the solution was added dropwise with stirring a solution of diethyl chlorophosphate (1.72 g, 1.45 mL, 10 mmol) in 10 mL of dry benzene under N_2. The addition was completed in 10 min, and stirring was continued at ice-bath temperature for 1 h and then at room temperature for an additional 4 h. The reaction mixture was cooled to 5−10 °C, and TEA·HCl was removed quickly by filtration. The colorless clear solution was evaporated to dryness while the temperature was kept below 35 °C. Dry hexane was added to the oily residue, and the whole mixture was tightly capped under N_2 and placed in a refrigerator (−20 °C) for 2 h. The oily residue solidified as a white solid, which was then recrystallized from dry CH_2Cl_2−hexane to give 1.74 g (63%) of the ester as colorless plates: mp 48−50 °C; ^{1}H NMR($CDCl_3$) δ 8.77 (dd, 1), 8.40 (dd, 1), 7.44 (dd, 1), 4.57 (m, 4), 1.47 (2t, 6); IR (film NaCl) 2987 (m), 1395 (m), 1306 (s), 1026 (vs), 838 (m), 775 (s), 699 (m) cm^{-1}. Anal. Calcd for $C_9H_{13}N_4O_4P$: C, 39.71; H, 4.81; N, 20.58. Found: C, 39.84; H, 4.72; N, 20.54.

Diphenoxyphosphinyloxy-7-azabenzotriazole (DpopOAt, 10). The synthesis was carried out as described for **9**, HOAt (1.36 g, 10 mmol) being reacted with diphenyl chlorophosphate (Dpop−Cl) (2.68 g, 2.1 mL, 10 mmol) in the presence of TEA (1.39 mL, 10 mmol) for 2−3 h to give an oily residue. Dry hexane was added to the oil, and the whole

(33) For example: (a) O-acyl: Vlassi, M.; Germain, G.; Barlos, K.; Mamos, P.; Refaat, L. S. *Z. Kristallagr.* **1990**, *192*, 59. (b) N-acyl: Barlos, K.; Papaioannou; Voliotis, S.; Prewo, R.; Bieri, J. M. *J. Org. Chem.* **1985**, *50*, 696.

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(37) For studies of the stability of HATU in the presence of DIEA, see: (a) See ref 23a. (b) Gausephol, H.; Behn, C. Peptides 1996. In *Abstracts of the 24th European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower Scientific, Ltd.: Kingswinford, UK, 1998; p 409.

mixture was placed at −20 °C for 2 h. The mixture was triturated until the oil solidified. Recrystallization of the solid from dry chloroform−hexane gave 2.32 g (63%) of the ester as colorless blocklike crystals: mp 62−64 °C; ^{1}H NMR ($CDCl_3$) δ 8.77 (dd, 1), 8.41 (dd, 1), 7.48−7.26 (m, 11); IR (KBr) 3056 (w), 1594 (sh, s), 1492 (s), 1394 (m), 1328 (s), 1184 (tr, s), 1026 (s), 984 (s), 844 (s), 775 (s), 685 (s) cm^{-1}. Anal. Calcd for $C_{17}H_{13}N_4O_4P$: C, 55.43; H, 3.55; N, 15.21. Found: C, 55.20; H, 3.53; N, 15.28.

3-(Diphenoxyphosphinyloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazine (DpopODhbt). The preparation was carried out as described for **9**, HODhbt (1.63 g, 10 mmol) being reacted overnight with diphenyl chlorophosphate (2.06 g, 1.73 mL, 12 mmol) in the presence of TEA (1.67 mL, 12 mmol) in 40 mL of dry CH_2Cl_2. The resulting pale yellow solution was washed with cold water (3 × 15 mL) and dried over $MgSO_4$. A pale yellow oil was obtained after removing the solvent. Dry hexane was added to the oil and the whole mixture was placed at −20 °C for 2 h. After trituration with a spatula the oil solidified. Recrystallization from dry chloroform-hexane gave 3.2 g (80%) of the ester as pale yellow crystals: mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (ddd, 1), 8.21 (ddd, 1), 7.99(m,1), 7.83 (m, 1), 7.44−7.25 (m, 10); IR (KBr) 1718 (vs), 1586 (m), 1488 (s), 1319 (s), 1179 (s), 1017 (m), 945 (s), 836 (s), 746 (s), 677 (m) cm^{-1}. Anal. Calcd for $C_{19}H_{14}N_3O_5P$: C, 57.72; H, 3.57; N, 10.63. Found: C, 57.58; H, 3.49; N, 10.58.

4,4-Dimethyldiphenyl Ether. *p*-Cresol (32 mL, 0.3052 mol) and 19.7 g (1.02 equiv) of potassium hydroxide were added to a 250-mL three-neck flask fitted with a magnetic stirrer, thermometer, air condenser, and an oil bath. Upon warming the mixture to 160 °C for 20 min it became dark brown. The mixture was cooled to 100 °C, and to the solution were slowly added 1.1 g of very fine copper powder and 36.7 mL (0.2982 mol) of *p*-bromotoluene, and the mixture was heated at 230−240 °C for 2 h. After the reaction mixture was cooled to room temperature, 100 mL of ether was added and the mixture was filtered using additional ether (60 mL) to wash the inorganic solid which was precipitated. The combined filtrates were washed with 2 N aqueous sodium hydroxide (2 × 75 mL), water, and saturated NaCl (50 mL) and dried over anhydrous magnesium sulfate. Evaporation of ether by means of a water aspirator and subsequent fractional distillation (bp 151−156 °C/17 mm) gave di-*p*-tolyl ether as a colorless oil which quickly solidified. Recrystallization from MeOH gave 41 g (69.3%) of the ether as white crystals: mp 50−51 °C (lit.[38] mp 50 °C); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 6), 6.80 and 7.20 (AA′, BB′ system).

2,8-Dimethylphenoxaphosphinic Acid. By modification of literature procedures,[39] 24.8 g of di-*p*-tolyl ether, 44 mL of phosphorus trichloride and 21 g of anhydrous aluminum chloride were placed in a three-neck flask equipped with a magnetic stirrer and a reflux condenser protected with a $CaCl_2$ drying tube. The mixture was stirred and refluxed overnight. The reaction mixture was cooled in an ice bath and treated slowly with 500 g of crushed ice, which caused the precipitation of a white solid. The solid was removed by filtration and washed thoroughly with water. The white solid was suspended in 300 mL of 5% aqueous sodium hydroxide solution in a beaker containing a magnetic stirrer and treated very slowly with 20 mL of 30% H_2O_2. The oxidation reaction was so exothermic that ice bath cooling was needed. After 15 min the resulting clear, hot solution was filtered. The filtrate was cooled in an ice bath and acidified slowly with concentrated HCl to cause precipitation of 31.62 g (97.2%) of the phosphinic acid as a white solid: mp >280 °C (lit.[39] mp > 300 °C); yield 73%; ^{1}H NMR (60 MHz, TFA) δ 2.43 (s, 6), 7.14−7.78 (m, 6); IR (KBr) broad 2600, 2250, 1650 (P−OH), 1145 (P═O) cm^{-1}.

2,8-Dimethylphenoxaphosphinic Chloride (DmppCl).[19] Dimethylphenoxaphosphinic acid (14.53 g 55.84 mmol) was added portionwise to 55 mL of thionyl chloride while cooling in an ice bath. The mixture was refluxed under a $CaCl_2$ drying tube for 3 h. After removal of excess thionyl chloride by a water aspirator in a hood, the solid residue was recrystallized from CH_2Cl_2−hexane to give 15.22 g (97.7%) of the phosphinic acid chloride as an off-white solid: mp 174−176 °C; ^{1}H NMR (200 MHZ, $CDCl_3$) δ 2.44 (s, 6), 7.18−7.50 (m, 4), 7.87 (d, 2); IR (KBr) 1133 (P═O) cm^{-1}. Previously the chloride was made by the same method[19] but it was not isolated or characterized. Alternatively, the corresponding phosphine-10-oxide, mp 161−163 °C, could be directly treated with $SOCl_2$ by exactly the same method to give the chloride in 90.2% yield.

2,8-Dimethylphenoxaphosphinyloxy-7-azabenzotriazole (DmppOAt, 15). Method A. To a suspension of 0.42 g (3.054 mmol) of HOAt in 20 mL of anhydrous CH_2Cl_2 was added 0.43 mL (1 equiv) of triethylamine with magnetic stirring. The resulting clear yellow solution was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 0.85 g (1 equiv) of 2,8-dimethylphenoxaphosphinic chloride (**12**). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. After dilution with 30 mL of CH_2Cl_2, the organic phase was washed with H_2O and saturated aqueous NaCl (30 mL) and dried over $MgSO_4$. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH_2Cl_2−hexane to give 0.65 g (56.3%) of the phosphinic ester as white crystals: mp 164−166 °C dec; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.47 (s, 6), 7.22−7.56 (m, 5) 8.09 (d, 2), 8.34 (d, 1), 8.71 (d, 1); I(KBr) 1128 (P═O) cm^{-1}; ^{31}P NMR (300 MHz, $CDCl_3$/85%H_3PO_4) δ 23.3 ppm; HREIMS calcd for $C_{19}H_{15}N_4O_3P$, M^+ 378.0882, found 378.0877.

Method B. To a suspension of 1.25 g of HOAt in 20 mL of anhydrous CH_2Cl_2 was added 0.623 g (1 equiv) of imidazole with magnetic stirring. The resulting white suspension was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 2.56 g (1 equiv) of 2,8-dimethylphenoxaphosphinic chloride **12**. The reaction mixture was stirred at 0 °C for 30 min, at room temperature for 2 h, and diluted with 30 mL of CH_2Cl_2. The reaction mixture was filtered in a sintered glass funnel over anhydrous $MgSO_4$ under an atmosphere of N_2. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH_2Cl_2−hexane to give 2.86 g (82.3%) of the phosphinic ester as white crystals, for which the melting point and NMR data agreed with the data reported above.

Di-*o*-tolylphosphine Oxide. Magnesium turnings (13.96 g) were added to 100 mL of anhydrous ether in a three-neck flask fitted with a condenser, magnetic stirrer, and a dropping funnel kept under an atmosphere of nitrogen. *o*-Bromotoluene (100 g, 0.579 mol) in 100 mL of ether was slowly added to the mixture. During the addition, the Grignard reaction was initiated and became so vigorous that ice bath cooling was needed frequently. After the addition was complete (40 min), the reaction mixture was refluxed for 15 min and then cooled with an ice bath and treated slowly with 30.8 mL (0.232 mol) of diethyl phosphite in 40 mL of ether. The mixture was refluxed again for 15 min and cooled with an ice bath. Two hundred and fifty milliliters of 10% HCl and 200 mL of water were added slowly to the cooled mixture with magnetic stirring. Ether was evaporated, and the insoluble phosphine oxide was collected by filtration and recrystallized from CH_2Cl_2-hexane (a few drops of methanol may be added to help dissolve the solid) to give 39.47 g (73.9%) of the phosphine oxide as a pale yellow solid: mp 94 °C (lit.[40] mp 93−94 °C); yield 57%; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.38 (s, 6), 4.23 (s, 1), 7.19−7.94 (m, 8); IR (KBr) 2369 (P−H), 1168 (P═O) cm^{-1}.

Di-*o*-tolylphosphinic Acid (Dtp-OH). By modification of a literature[18,40] procedure, to a suspension of 15.04 g of di-*o*-

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tolylphosphine oxide in 80 mL of 5 N aqueous NaOH was treated with 40 mL of 30% H_2O_2 all at once, and the resulting mixture was heated on a steam-bath for 20 min. A clear solution resulted and was filtered while hot. The filtrate was cooled in an ice bath and acidified slowly with concentrated HCl, which caused the precipitation of a white solid which was recrystallized from MeOH−ether to give 13.4 g (83.3%) of the phosphinic acid: mp 174−176 °C (lit.[40] mp 175−177 °C); yield 58−74%; ^{1}H NMR (60 MHz, TFA) δ 2.37 (s, 6), 7.24−8.12 (m, 8); IR (KBr) 1143 (P═O) cm^{-1}.

Di-*o*-tolylphosphinic Chloride (DtpCl, 11).[18,41] Di-*o*-tolylphosphinic acid (13.5 g) was slowly added to 50 mL of thionyl chloride with cooling in an ice bath. The mixture was refluxed under a $CaCl_2$ drying tube for 3 h. After removal of excess thionyl chloride by a water aspirator in a hood, the oily residue was fractionally distilled to give 13.55 g (93.4%) of the phosphinic chloride as a colorless oil (bp 158−165 °C/0.1 mmHg) which solidified quickly (lit.[18] bp 150−160 °C/0.08 mmHg): mp 65−66 °C; yield 80.5%; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.45 (s, 6), 7.05−8.08 (m, 8); IR (KBr) 1220 (P═O) cm^{-1}.

Di-*o*-tolylphosphinyloxy-7-azabenzotriazole (DtpOAt, 14). The preparation was carried out as described for DmppOAt (method A) to give the ester as a white solid (83.7%): mp 170−172 °C; ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 6), 7.27−7.60 (m, 7), 7.93−8.04 (m, 2), 8.32 (d, 1), 8.74 (d, 1); ^{31}P NMR (300 MHz, $CDCl_3$/85% H_3PO_4) δ 46.6; HRIEMS calcd for $C_{19}H_{17}N_4O_2P$, M^+ 364.1089, found 364.1106. Anal. Calcd for $C_{19}H_{17}N_4O_2P$: C, 62.64; H, 4.7; N, 15.38. Found: C, 62.23; H, 4.72; N, 15.47.

Di-*o*-toylphosphinyloxybenzotriazole (DtpOBt, 16). The preparation was carried out as described for **14**, HOBt (0.1351 g, 1 mmol) being reacted with Dtp-Cl (0.2647 g, 1 mmol) in the presence of DIEA (0.21 mL, 1.2 mmol) for 5 h in 10 mL of dry CH_2Cl_2 to give 0.24 g (66%) of white solid after workup. Recrystallization from CH_2Cl_2−hexane gave 0.18 g of an analytically pure sample of the ester as colorless crystals: mp 198−200 °C; ^{1}H NMR ($CDCl_3$) δ 7.84−8.06 (m, 3), 7.50−7.59 (m, 3), 7.30−7.42 (m, 6), 2.62 (s, 6); IR (KBr) 3065 (w), 1593 (s), 1457 (s), 1362 (m), 1278 (m), 1230 (vs), 1151 (s), 1084 (sh, s), 812 (vs), 774 (sh, vs), 704 (m) cm^{-1}. Anal. Calcd for $C_{20}H_{18}N_3O_2P$: C, 66.10; H, 4.92; N, 11.56. Found: C, 65.75; H, 4.97; N, 11.41.

DtpODhbt (17) was made according to the method described for DmppOAt (method A) from HODhbt and di-*o*-tolylphosphinic chloride and obtained as white crystals (76.5%) after recrystallization from ethyl acetate-ether: mp 178−179 °C dec; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (s, 6), 7.28−7.39 (m, 4), 7.49−7.57 (m, 2), 7.75−7.83 (m, 1), 7.90−8.15 (m, 4), 8.35 (dd, 1); IR (KBr) 1709 (C═O), 1240 (P═O) cm^{-1}. Anal. Calcd for $C_{21}H_{18}N_3O_3P$: C, 64.45; H, 4.64; N, 10.74. Found: C, 64.49; H, 4.54; N, 10.69.

Active Ester Formation. To a solution of 0.1 mmol of Z-Aib-OH **18** and 0.1 mmol of the appropriate coupling reagent in 0.5 mL of $CDCl_3$ or DMF was added 0.1 mmol of DIEA. The mixture was immediately transferred to an NMR tube which was placed in the probe of a Hitachi R-1200 (60 MHz) NMR instrument. Integration of the ^{1}H NMR peaks at δ 5.1 (acid) and 5.2 (active ester **19**) as the reaction progressed at the probe temperature (~37 °C) allowed for rough determination of the relative rates. The results given in Table 1 are the averages of at least two runs.

Assembly of ACP (65−74) via Stepwise Solid-Phase Coupling.[10] Run 2 of Table 3 is taken as an example to demonstrate the standard protocol used: 150 mg of Fmoc-Gly-PAL-PEG-PS resin (0.19 mmol/g, 0.0285 mmol) in a 10 mL disposable syringe fitted with a Teflon filter was washed with CH_2Cl_2 (3 × 10 mL) and DMF (3 × 10 mL) and deprotected with 20% piperidine in DMF (10 mL) for 7 min. The deprotected resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), and again DMF (3 × 10 mL). Preactivation was carried out for 7 min using 25.5 mg (0.04 mmol, 1.5 equiv) of Fmoc-Asn(Trt)-OH, 15.75 mg (0.04 mmol, 1.5 equiv) of DpopOAt, and 14.89 μL (0.09 mmol, 3 equiv) of DIEA in 0.3 mL of DMF in a 4 mL vial. Following the requisite preactivation period (7 min), the solution of the activated amino acid was added to the resin. The small vial was washed with 0.1 mL of DMF, and the washing was also added to the above resin. The resulting resin mixture was allowed to react at room temperature for 1.5 min. The loaded resin was washed with DMF (3 × 10 mL), and the Fmoc group was deblocked with 10 mL of 20% of piperidine in DMF for 7 min. Washing the deblocked resin with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), and DMF (3 × 10 mL) was followed by an analogous coupling step with Fmoc-Ile-OH. Other amino acids were coupled similarly, and after the last coupling with Fmoc-Val-OH and deblocking of the Fmoc group with 20% piperidine in DMF, the loaded resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), EtOH (5 mL), and ether (5 mL). The resin was then treated with 10 mL of 90% aqueous TFA for 2 h, filtered, and washed on the filter with 10 mL of 10% TFA in CH_2Cl_2 and 10 mL of CH_2Cl_2. The combined filtrates were evaporated to dryness. The crude product was washed four times with anhydrous ether and separated by centrifugation. The yield was calculated by the weight of the crude product. For analysis, 1 mg of the crude product was dissolved in 1 mL of 0.1% aqueous TFA and injected directly onto the HPLC column for analysis.

Model Test Coupling Reactions. All test coupling reactions were carried out as previously described with the phosphorus reagents simply substituted for the uronium/guanidinium salts: Z-Phe-Val-Pro-NH_2,[22,24] Z-Phg-Pro-NH_2,[42] Z-Gly-Phe-Pro-NH_2,[22] and Z-Gly-Gly-Val-Ala-Gly-Gly-OMe.[21,24]

General Methods for the Synthesis of Sulfonate Esters. Method A. To a suspension of HOAt (1 g, 7.35 mmol) or HOBt (0.99 g, 7.35 mmol) in 30 mL of anhydrous CH_2Cl_2 was added 1.04 mL (1 equiv) of triethylamine with magnetic stirring. The resulting clear yellow solution was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 1 equiv of RSO_2Cl. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. After dilution with 30 mL of CH_2Cl_2, the organic phase was washed with water and saturated aqueous NaCl (30 mL) and dried over $MgSO_4$. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH_2Cl_2−hexane to give the sulfonate esters collected in Table 4.

Method B. To a suspension of HOAt (1 g, 7.35 mmol) or HOBt (0.99 g, 7.35 mmol) in 30 mL of anhydrous CH_2Cl_2 was added 0.51 g (1 equiv) of imidazole with magnetic stirring. The resulting pale yellow suspension was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 1 equiv of RSO_2Cl. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h and diluted with 30 mL of CH_2Cl_2. The reaction mixture was filtered in a sintered glass funnel over anhydrous $MgSO_4$ under an atmosphere of N_2. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from anhydrous CH_2Cl_2−hexane to give the sulfonate esters collected in Table 4.

***N*-2-Naphthalenesulfonylproline Amide 24.** A solution of Ns-OAt (0.5 mmol) and proline amide (0.5 mmol) in 2 mL of DMF was stirred at rt overnight. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with 1 M HCl, 1 M $NaHCO_3$, and satd NaCl (2 × 10 mL each) and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was recrystallized from methylene chloride and hexane (1:4) as white crystals in 89.1% yield: mp 130−131 °C; IR (KBr) 3446 (NH), 1675 (CO, amide) 1337, 1184 (SO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.8−2.2 (m, 4), 3.2 (t,2), 4.3 (m, 1), 5.6−5.8 (m, 2) 7.4−8.2 (m, 5) 8.5 (d, 2). Anal. Calcd for $C_{15}H_{16}NO_3S$: C, 59.21; H, 5.26; N, 9.20. Found: C, 58.96; H, 5.34; N, 9.12.

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Note Added after Proof. After the submission of this paper, we located a reference (Hoffmann, F.; Jäger, L.; Griehl, C. *Phosphorus, Sulfur Silicon* **2003**, *178*, 299) which describes the synthesis of a number of phosphorus derivatives of *N*-hydroxy compounds, including our compound **10**. These authors cite ^{31}P NMR data which suggests that mixtures of O- and N-phosphorylated derivatives were formed. In our work, we assumed the formation of O-substituted species. For the previously described compound **2** the O-structure has been confirmed by X-ray crystallography (ref 17).

Supporting Information Available: Figure 1 and tables detailing results on segment coupling reactions, appropriate spectral data for new compounds, and HPLC curves for various samples of the synthetic ACP decapeptide. This material is available free of charge via the Internet at http://pubs.acs.org.

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