JOC Article

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene†

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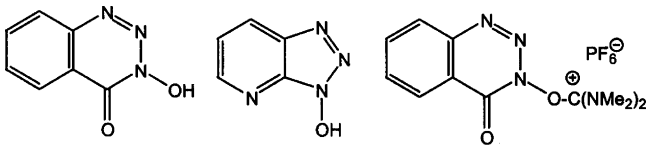
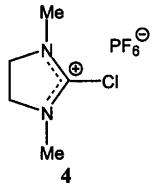
The known but long-neglected compound HODhat was shown to be in certain situations a useful peptide coupling additive. Uronium and phosphonium salts with HODhat built into the system were also useful stand-alone coupling reagents. Comparisons with related additives and coupling reagents showed that the new systems were sometimes more and sometimes less effective than previously described systems in the case of stepwise and segment couplings. Applications to assembly of the model decapeptide ACP showed that HDATU was far more effective than HDTU and more effective than HATU under some conditions.

Among a variety of peptide coupling additives which have been studied since 1970, beginning with the classic studies of König and Geiger,[1,2] 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt, **1**) proved to be generally superior to all other popular additives in terms of reactivity and coupling efficiency, with the exception of 1-hydroxy-7-azabenzotriazole (HOAt **2**), an additive developed only recently.[3] In addition, the use of HODhbt allows one to follow the progress of the reaction visually by a color change which occurs when acylation is complete.[4,5] Subsequent work on HODhbt, carried out by others,[6−10] has shown that the uronium salt HDTU **3** and combinations such as HODhbt/CIP **4**[11,12] or HODhbt/Fmoc-AA-Pfp esters[13] and, especially, the isolated amino acid esters (Fmoc-AA-ODhbt[4,14,15]) provide many attractive properties for solution- and solid-phase peptide synthesis.



1 **2** **3**



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Although mentioned briefly[16] in the original paper by Harrison and Smith which described the synthesis of HODhbt, 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-traizene (HODhat, **5**) was apparently overlooked by König and Geiger at the time they described the remarkable properties of HODhbt and to our knowledge has never again been cited in the literature. We took up the examination of **5** as a new peptide coupling additive because of its structural similarity to HODhbt and the consideration that the introduction of a nitrogen atom at the 5-position should enhance its reactivity due to the

† Abbreviations: Aib = α-aminoisobutyric acid; ACP = acyl carrier protein decapeptide (65−74); CIP = 2-chloro-1,3-dimethyl-2-imidazolidinium hexafluorophosphate; DCM = dichloromethane; DIC = diisopropylcarbodiimide; DIEA = *N*,*N*-diisopropylethylamine; EDC = 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide; HAPyU = 1-(l-pyrrolidinyl-l*H*-1,2,3-triazolo[4,5-*b*]pyridin-l-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-l-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU = *N*-[(1*H*-benzotriazol-l-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDTU = *O*-(3,4-dihydro-4-oxo-l,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate; Pfp = pentafluorophenyl; Phg = α-phenylglycine; PyBrOP = bromotris(pyrrolidino)phosphonium hexafluorophosphate; PyClU = bis(tetramethylene)-chlorofomamidinium hexafluorophosphate; TEA = triethylamine; TCFH = tetramethylchloroformamidinium hexafluorophosphate; TCM = trichloromethane = chloroform; TFE = trifluoroethanol; TMP = 2,4,6-trimethylpyridine (collidine)

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(1) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(2) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024, 2034.
(3) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(4) Atherton, E.; Cameron, L.; Meldal, M.; Sheppard, R. C. J. *Chem. Soc., Chem. Commun.* **1986**, 1763.
(5) Cameron, L. R.; Holder, J. L.; Meldal, M.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2895.
(6) (a) Atherton, E.; Holder, J. L.; Meldal, M.; Sheppard, R. C.; Valerio, R. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2887. (b) Meldal, M.; Bielfeldt, T.; Peters, S.; Jensen, K.; Paulsen, H.; Bock, K. *Int. J. Pept. Protein Res.* **1994**, *43*, 3561.
(7) Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561.
(8) Kuroda, H.; Chen, Y.-N.; Kimura, T.; Sakakibara, S. *Int. J. Pept. Protein Res.* **1992**, *40*, 294.
(9) (a) Spetzler, J. C.;-Meldal, M.; Felding, J.; Vedso, P.; Begtrup, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1727. (b) Jakobsen, M. H.; Buchardt, O.; Holm, A.; Meldal, M. *Synthesis* **1990**, 1008.
(10) Mostafavi, H.; Austermann, S.; Forssmann, W.-G.; Adermann, K. *Int. J. Pept. Protein Res.* **1996**, *48*, 200.
(11) Akaji, K.; Tamai, Y.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.
(12) Akaji, K.; Tamai, Y.; Kiso, Y. *Tetrahedron* **1997**, *53*, 567.
(13) (a) Peters, S.; Bielfeldt, T.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1163. (b) Meinjohanns, E.; Vargas-Berenguel, A.; Meldal, M.; Paulsen, H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2165.
(14) Cameron, L.; Meldal, M.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1987**, 270.
(15) Jakobsen, M. H.; Buchardt, O.; Engdahl, T.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 6199.
(16) (a). Harrison, D.; Smith, A. C. B. *J. Chem. Soc.* **1960**, 2157. (b) *Dictionary of Organic Compounds*, 6th ed.; Chapman and Hall: London, 1996; Vol. 4, p 3833.

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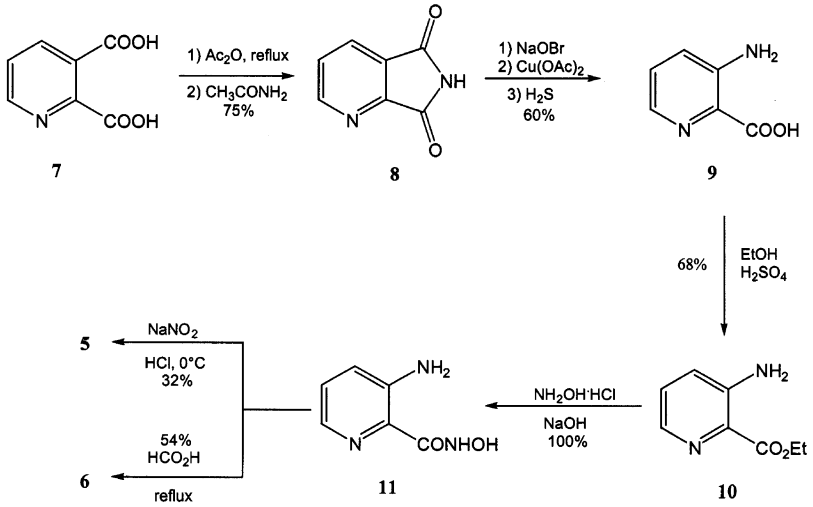
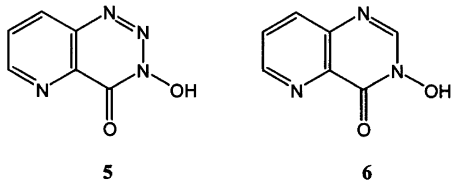
SCHEME 1. Synthesis of HODhat 5 and HODhad 6



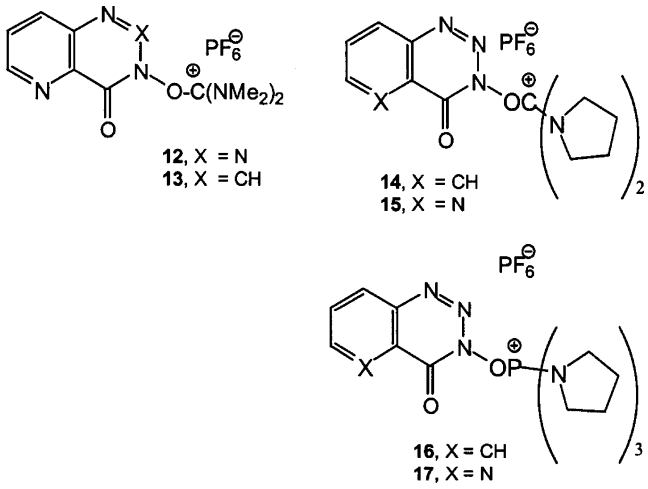
electron-withdrawing effect of the pyridine N-atom. Although related to HODhbt in the same way that HOAt is related to 1-hydroxybenzotriazole (HOBt), it should be noted that esters of HODhat are not able to participate in the type of neighboring group effects commonly thought to enhance the effectiveness of HOAt derivatives relative to those of HOBt. Any neighboring group effect occurring in either case would involve the carbonyl function or the adjacent nitrogen atom. The related 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-l,3-diazene (HODhad, **6)** was also examined.



Synthesis of HODhat 5 and HODhad 6. Although the Harrison and Smith paper cited above briefly outlined the preparation of HODhat **5**, no experimental details were given. Syntheses of **5** and **6** are outlined in Scheme 1.[17−19]

To study the reactivity of these compounds, conversion to model active esters, as well as uronium and phosphonium salts, was examined. Peptide coupling additives HOXt (HOAt **2,** HOBt, HODhbt **1**, HODhat **5**, and HODhad **6**) were treated with *N*-benzyloxycarbonyl α-aminoisobutyric acid (Z-Aib-OH) in the presence of EDC·HCl to give in good yield the active esters Z-Aib-OAt, Z-Aib-OBt, Z-Aib-ODhbt, Z-Aib-ODhat, and Z-Aib-ODhad. Carpino and El-Faham[20] had previously recorded the synthesis of Z-Aib-OAt and Z-Aib-OBt from Z-Aib-F. Pivaloyl esters Me_3CCO-OAt, Me_3CCO-OBt, Me_3CCO-ODhbt, Me_3CCO-ODhat, and Me_3CCO-ODhad were synthesized similarly by reaction of pivaloyl chloride with HOXt in the presence of TEA. Characterization data for both types of esters are given in Table 1 in the Supporting Information.

By methods analogous to those used for HODhbt,[7,21] the uroniumn reagents *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-l,1,3,3-tetramethyluronium hexafluorophosphate (HDATU, **12**) and *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyluroniumn hexafluorophosphate (HDADU, **13**) and the pyrrolidine analogues *O*-(3,4-dihydro-4-oxo-l,2,3-benzotriazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HDPyU, **14)** and *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate(HDAPyU, **15**) were obtained.



Phosphonium salts [(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyDOP, **16**[22]) and [3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyDAOP, **17**) were prepared similarly.

The use of isolated Dhbt esters of Fmoc amino acids has previously been described as being advantageous for

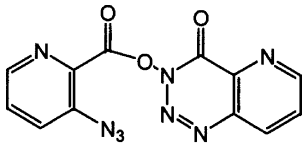
(17) Sucharda, E. *Chem. Ber.* **1925**, *58*, 1727.
(18) (a) Nakadate, M.; Takano, Y.; Hirayama, T.; Sakaizana, S.; Hirano, T.; Okamoto, K.; Hirano, K.; Kawamura, T.; Kimura, M. *Bull. Chem. Pharm. Jpn.* **1965**, *13*, 113. (b) Oakes, V.; Pascoe, R.; Rydon, H. N. *J. Chem. Soc.* **1956**, 1045.
(19) Harrison, D.; Smith, A. C. B. *J. Chem. Soc.* **1959**, 3157.
(20) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.
(21) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
(22) Hoeg-Jensen, T.; Olsen, C. E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257.

TABLE 2. Approximate Halftimes for Disappearance of Z-Aib-OXt in $CDCl_3$ in the Presence of *p*-Chloroaniline

Z-Aib-OXt	$t_{1/2}$ (min)
Z-Aib-ODhat	8−9
Z-Aib-OAt	9−10
Z-Aib-ODhbt	12−13
Z-Aib-ODhad	70
Z-Aib-OBt	210

peptide synthesis.[4,14,15,23−25] Because of the higher reactivity expected for the corresponding HODhat esters, these compounds were also investigated in the present work. As an example, Fmoc-Ile-ODhat was prepared in good yield and purity by refluxing HODhat with Fmoc isoleucine and thionyl chloride.[15] During its preparation, no azido side product was formed. The method is easy, fast, and efficient and should be well suited for the synthesis of other ODhat esters of Fmoc amino acids.

As described by König and Geiger,[2] during HODhbt/DCC-mediated peptide couplings, side products may be formed due to a competing ring-opening reaction, whereby 2 mol of HODhbt and 1 mol of DCC react to give the *o*-azidobenzoyl active ester, which can then react with a free amino residue during peptide assembly, leading to chain termination. A similar side reaction occurred in the case of HODhat by reaction with DCC or EDC·HCl. In both cases, 3-(3′-azidopicolinoyloxy)-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine **18** was obtained.



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During peptide coupling via uronium or phosphonium salts, the N-protected carboxylic acid first reacts with the coupling reagent to give an active ester, which then reacts with the amino component to give the corresponding amide. The latter step is rate-limiting and responsible for loss of configuration. The inherent reactivity of the active ester intermediates is a critical aspect of the value of particular uronium or phosphonium salts.

As a first model, reactions of the hindered active esters Z-Aib-OXt with *p*-chloroaniline (PCA) were studied in $CDCl_3$. Approximate halftimes were determined by proton NMR analysis according to disappearance of the benzylic CH_2 unit (δ 5.2) of the active esters and appearance of the benzylic CH_2 residue (δ 5.5) of product **19**. Results are collected in Table 2.

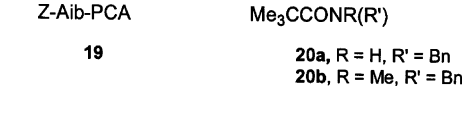

TABLE 3. Approximate Halftimes for Disappearance of $Me_3CCOOXt$ in $CDCl_3$

$Me_3CCOOXt$	$t_{1/2}$ (min) ($PhCH_2NH_2$)	$t_{1/2}$ ($PhCH_2NHMe$)
$Me_3CCOODhat$	<1	<2 min
$Me_3CCOOAt$	<1	7−8 min
$Me_3CCOODhbt$	<1	18−20 min
$Me_3CCOODhad$	<1	35−40 min
$Me_3CCOOBt$	<1	4.5 h

TABLE 4. Approximate Halftimes for the Disappearance of [Z-Aib-OXt] in Various Solvent Systems in the Presence of *p*-Chloroaniline

coupling reagent	$t_{1/2}$ ($CDCl_3$)	$t_{1/2}$ (CD_3CN)	$t_{1/2}$ (DMF)	$t_{1/2}$ (DMF/$CDCl_3$)[a]
HDATU, **12**	<3 min	70−75 min	2−2.5 hr	30−40 min
HATU[b]	18−24 min	90−95 min	75−85 min	40−45 min
HDTU, **3**	20−25 min	4.5−5 h	6−7 h	2.5−3 h
HBTU[b]	3.5−4 h		10−11 h[c]	

[a] 1:1 mixture of DMF/$CDCl_3$. [b] See list of abbreviations not defined in text. [c] In this case, the halftime is determined by the disappearance of acid *Z*-Aib-OH and appearance of both intermediate active ester and amide **19**.

It was found that the ODhat ester is slightly more reactive even then the OAt ester, which was previously found to be the most reactive derivative among these esters. Interestingly, despite the structural similarity between HODhat **5** and HODhad **6**, the reactivities of the corresponding active esters are very different. This may be due to the presence or absence of additional neighboring group effects promoted by the presence or absence of a nitrogen atom substituted at the 2-position. On the other hand, comparison of the OBt and ODhad esters demonstrates the importance of the neighboring carbonyl group.

As a second model to test the reactivity of these active esters, the pivaloyl esters were treated with benzylamine and *N*-methylbenzylamine, which led to the formation of amides **20a** and **20b**, respectively. Approximate halftimes for these reactions were determined by proton NMR analysis, according to the disappearance of the methyl peak (δ 1.5) for pivaloyl-OXt and the appearance of the methyl peak for products **20a** (δ 1.2) or **20b** (δ 1.3). Results are collected in Table 3.

In the case of benzylamine, all reactions were rapid whereas in the case of the more hindered *N*-methyl derivative, clear reactivity differences were seen according to the following order: ODhat> OAt> ODhbt> ODhad> OBt. Again the greater reactivity of the HODhat ester relative to that derived from HOAt is seen.

A related model system used to compare relative rates of coupling processes involves reaction of Z-Aib-OH with *p*-chloroaniline (PCA) in the presence of a coupling agent. Because formation of intermediate Z-Aib-OXt is usually very fast, halftimes are determined by disappearance of the benzylic CH_2 residue (δ 5.2) of the active ester and appearance of the benzylic CH_2 unit (δ 5.05) of the product **19**, unless otherwise noted. Approximate halftimes are collected in Table 4. In this case, various solvent systems were examined.

Interestingly, in all solvent systems examined except for DMF, the new coupling reagent was found to be more reactive than HATU. In $CDCl_3$, HDATU is at least six

(23) Meldal, M.; Holm, C. B.; Bojesen, G.; Jakobsen, M. H.; Holm, A. *Int. J. Pept. Protein Res.* **1993**, *41*, 250.
(24) Reimer, K. B.; Meldal, M.; Kusumoto, S.; Fukase, K.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 925.
(25) Knapp, D. R.; Oatis, Jr., J. E.; Papac, D. J. *Int. J. Pept. Protein Res.* **1993**, *42*, 259.

TABLE 5. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 21 via [1 + 1] Coupling

coupling reagent	additive	base (equiv)	solvent	yield (%)	DL (%)
HDATU, **12**		DIEA (2)	DMF	83.9	4.8
HDTU, **3**		DIEA (2)	DMF	78.4	12.8
HATU[a]		DIEA (2)	DMF	71.7	2.8
HBTU[a]		DIEA (2)	DMF	81.3	6.3
HDATU, **12**		TMP (2)	DMF	87.5	6.0
HDTU, **3**		TMP (2)	DMF	80.7	16.0
HATU[a]		TMP (2)	DMF	90.8	3.8
HBTU[a]		TMP (2)	DMF	85.4	8.7
DCC	HODhat (l)	TMP (1)	TFE/TCM[b]	74.8	0.4
DCC	HODhbt (1)	TMP (1)	TFE/TCM[b]	71.8	0.8
DCC	HOAt (1)	TMP (1)	TFE/TCM[b]	69.2	0.3

[a] See list of abbreviations not defined in text. [b] In this case, 1.3 mL of trifluoroethanol−chloroform (1:3 v/v) was used as solvent.

times as reactive as HATU and about eight times as reactive as HDTU. So far, in every case tested HDATU was shown to be significantly more reactive than HDTU.

To test the configuration-retention effectiveness of the new additives HODhat **5** and HODhad **6**, and the new coupling reagents HDATU **12**, HDADU **13**, HDAPyU **15**, HDPyU **14**, PyDAOP **17**, and PyDOP **16**, several previously studied model peptide systems (**21**−**24**) and a system **25** previously studied by Sakakibara[8] were examined. These involve a [1 + 1] stepwise coupling and three [2 + 1] and one [3 + 3] segment couplings.

Z-Phg-Pro-NH_2 **21** Z-Phe-Val-Pro-NH_2 **22** Z-Gly-Phe-Pro-NH_2 **23**

Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2 **24** H-Gly-Leu-Phe-OBzl **25**

For the sensitive coupling of the urethane-protected Z-Phg-OH to H-Pro-NH_2 to give **21**, HDATU was more effective in preserving configuration than HDTU and HBTU, but not better than HATU. Curiously with this system, use of the base diisopropylethylamine (DIEA) proved more satisfactory than collidine (TMP), a result that is rarely observed in the case of the corresponding segment couplings. Results are collected in Table 5 and its more extensive version in the Supporting Information section.

With carbodiimide in the so-called "structure-breaking" combination solvent TFE/TCM recommended as the best solvent for use with HODhbt by Sakakibara, HODhat was even more effective than HODhbt. Thus, EDC/HODhat gave 0.5% of the DL-isomer, whereas EDC/HODhbt led to 1.3% of the same form. For DCC/HODhat and DCC/HODhbt in the presence of 1 equiv of TMP, the figures were 0.4% and 0.8%, respectively.

For the well-studied segment coupling of Z-Phe-Val-OH to H-Pro-NH_2 leading to tripeptide **22**, the 5-aza derivative of HDTU was generally less effective than the parent system. Best results were generally obtained with HATU and other HOAt-derived reagents, except for the case of carbodiimide reagents in the combination solvent TFE/TCM according to the Sakakibara technique. The results are collected in Table 6 and its more extensive version in the Supporting Information section..

For the rather insensitive case of the segment coupling of Z-Gly-Phe-OH− to H-Pro-NH_2 the results paralleled those for **22**. Results are presented in Table 7 in the Supporting Information section. In contrast to the case of **22,** for tripeptide **23** HDATU was similar to or even slightly more effective than HATU.

TABLE 6. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 22 via [2 + 1] Coupling

coupling reagent	additive	base (equiv)	solvent	yield (%)	LDL (%)
HDATU, **12**		DIEA (2)	DMF	85.4	15.1
HDTU, **3**		DIEA (2)	DMF	81.0	13.3
HDADU, **13**		DIEA (2)	DMF	72.4	27.6
HATU[a]		DIEA (2)	DMF	81.2	12.7
HBTU[a]		DIEA (2)	DMF	89.6	27.4
HDATU, **12**		TMP (2)	DMF	88.8	8.7
HDTU, **3**		TMP (2)	DMF	86.4	8.5
HDADU, **13**		TMP (2)	DMF	83.8	18.6
HATU[a]		TMP (2)	DMF	80.1	5.0
HBTU[a]		TMP (2)	DMF	81.2	14.2
HDATU, **12**	HODhat (l)	TMP (2)	DMF	68.0	7.3
HDTU, **3**	HODhbt (1)	TMP (2)	DMF	65.0	4.0
HATU[a]	HOAt (1)	TMP (2)	DMF	62.7	1.8

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

TABLE 8. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 25 via [2 + 1] Coupling

coupling reagent	solvent	yield (%)	LDL (%)
EDC/HODhat	DMF	76.2	0.20.
EDC/HODhbt	DMF	88.	0.25
EDC/HOAt	DMF	90.6	0.33
EDC/HOBt	DMF	77.4	0.43[a]
EDC/HODhat	TFE/TCM[b]	98.6	<0.1
EDC/HODhbt	TFE/TCM[b]	96.2	<0.1
EDC/HOAt	TFE/TCM[b]	98.2	<0.1
EDC/HOBt	TFE/TCM[b]	90.0	0.20[c]

[a] Sakakibara reports[8] under the same conditions 3.6% of the LDL-isomer. [b] Combination solvent trifluoroethanol−chloroform (1:3 v/v) was used. [c] Sakakibara reports[8] under the same conditions 0.5% of the LDL-isomer.

The test tripeptide **25** was prepared according to the procedure of Sakakibara.[8] Thus, coupling of H-Phe-OBzl·TosOH with Boc-Gly-Leu-OH in the presence of EDC/additive (HOXt) in various solvents gave a product Boc-Gly-Leu-Phe-OBzl which was BOC-deblocked via 50% TFA/CH_2Cl_2 to give the crude tripeptide, which was directly analyzed by HPLC.

In the EDC-mediated synthesis of **25** carried out in trifiuoroethanol/chloroformn (1:3 v/v), the three additives HODhat, HODhbt, and HOAt were found equally effective with less than 0.1% epimerization being observed. Upon switching to DMF as solvent, differences, although small, could be noted. Results are collected in Table 8.

Following preliminary studies with simple di- and tripeptide models **21**−**23** and **25**, a test hexapeptide **24** was assembled. The coupling-of Z-Gly-Gly-Val-OH to H-Ala-Gly-Gly-OMe had previously been shown[20,26] to be a sensitive- test for the nature of both coupling reagent and base. Results for the reaction in DMF, in the presence of collidine, are gathered in Table 9. HDATU was found to be more effective in preventing loss of configuration at valine than HATU and other coupling reagents. Epimerization levels up to 8.2% of the DL-form were noted according to the order: HDATU < HATU < HDTU < HBTU.

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

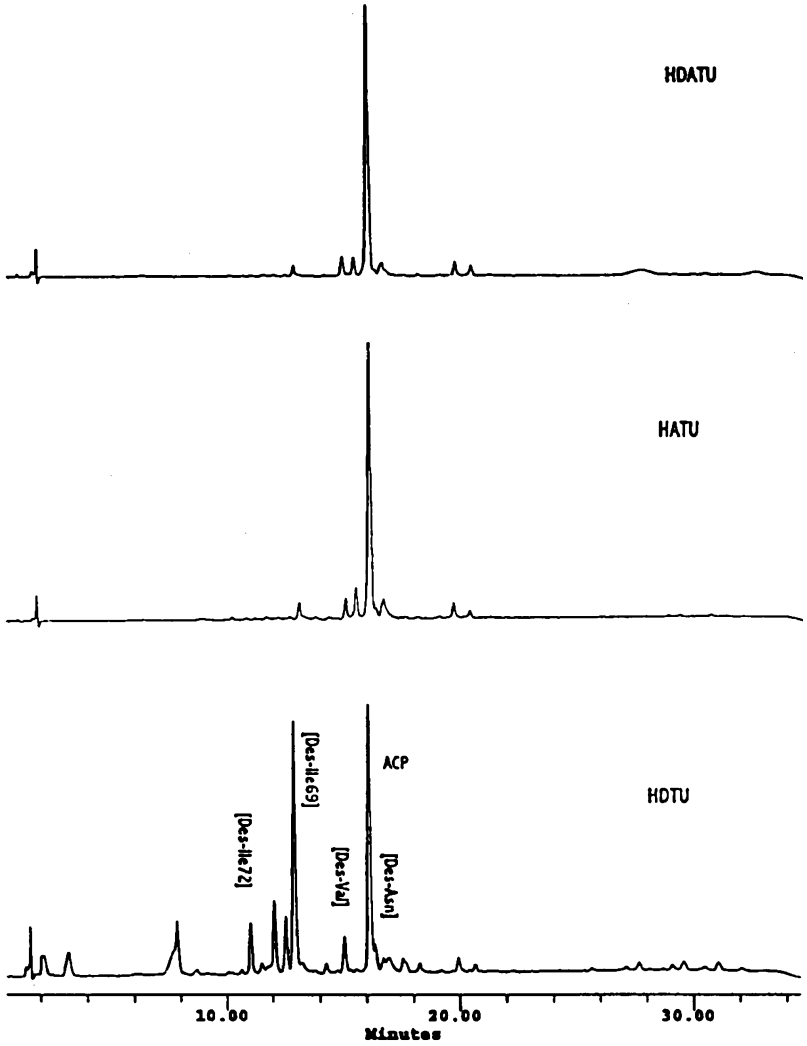

FIGURE 1. Comparison of HPLC traces of ACP decapeptide assembled in methylene chloride via HDATU (ACP 86%), HATU (78%), and HDTU (31%). All syntheses carried out using 4 equiv of the Fmoc amino acid and coupling reagent, 7 min preactivation time, and 3 min coupling time in the presence of 8 equiv of DIEA.

TABLE 9. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 24 via [3 + 3] Coupling

coupling reagent	base	solvent	yield (%)	LDL (%)
HDATU, **12**	TMP (3)	DMF	98.4	0.8
HDTU, **3**	TMP (3)	DMF	95.0	3.3
HATU[a]	TMP (3)	DMF	96.6	2.4
HBTU[a]	TMP (3)	DMF	85.6	8.2

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

To demonstrate the suitability of the new HODhat-based coupling reagent HDATU and compare its performance with that of the corresponding guanidinium/uronium analogues HATU and HDTU in solid-phase syntheses, 30 syntheses of the ACP segment H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-lle-Asn-Gly-NH_2 were carried out by an Fmoc/*tert*-butyl protection scheme. Poly(ethylene glycol)-polystyrene (PEG-PS) resin bearing Fmoc-glycine was used as solid support. Peptide elongation was performed manually, coupling times being shortened and excesses of reagents being reduced in order to bring out the differences among the various coupling reagents studied. The methodology has been described previously.[27] Under these conditions, incomplete incorporations were detected for Asn onto Gly, Ile onto Asn, Ile onto Asp, and Val onto Gln. Peptide purity was judged by reverse-phase HPLC analysis, after cleavage from the resin with TFA−H_2O (9:1) for 2 h at room temperature. The results are collected in Table 10 in the Supporting Information.

Analysis of the chromatograms indicated that HDATU is far more effective than HDTU under all conditions examined and more effective even than HATU under some conditions. Methylene chloride was found to be a particularly suitable solvent for HDATU-mediated ACP synthesis. Thus, under so-called "1.5 × 1.5" conditions in DCM, HADTU gave the decapeptide in a putity of 47%, whereas HATU and HDTU led to only 21% and 4% of the desired product, respectively. When a 4-equiv excess of reagents and a 3-min coupling time were used, 86% of ACP was obtained for HDATU, compared with 78% and 31% for HATU and HDTU, respectively (Figure 1).

Although in DMF under "1.5 × 1.5" conditions, the performance of HDATU was not as efficient as HATU

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

with or without preactivation, HDATU was found to be the better reagent under normal conditions. Thus, while using a 2 equiv excess of reagents without preactivation for a 5 min coupling, ACP was obtained in 97% purity by HDATU, whereas the corresponding figures were 94 and 81% for HATU and HDTU. With 4-equiv/30 min coupling conditions with a 7-min preactivation time, excellent purity (95%) was obtained for HDATU, whereas with HATU and HDTU, the ACP purity was only 86 and 62%, respectively.

When DIC/HODhat was used as a coupling reagent, satisfactory results were also obtained. Although not suitable under stringent conditions ("1.5 × 1.5"), HODhat could be used as an excellent catalyst and indicator in Fmoc-amino acid pentafluorophenyl (Pfp) ester couplings under normal conditions. A bright yellow-to-orange-red color change was noted which is much clearer than the color change from bright yellow to pale yellow observed with HODhbt. In DMF under conditions involving 3 equiv of Pfp-ester and a 30-min coupling time both HODhat and HODhbt gave the desired ACP product in a purity of over 85%.

For model pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 **26**,[27,28] which incorporates the highly hindered Aib-Aib unit, whether in DCM or DMF, the new reagent HDATU was not able to equal the results obtained with HATU. For example with 4 equiv of excess acid, 7 min preactivation, and 30 min coupling time, HDATU gave in DMF a peptide of 31% purity, whereas with HATU the purity was 91%.

In conclusion, the long-known but neglected hydroxybenzotriazene derivative HODhat represents a useful, fast-acting coupling additive for both solution- and solid-phase peptide syntheses, with which one can follow the progress of the reaction visually by a color change from bright yellow to orange-red. For stepwise coupling of a urethane-protected amino acid, HDATU was more effective than HDTU, although less effective than HATU. For segment coupling, results were mixed, depending on the system in question. For solid-phase assembly of model peptide ACP, HDATU was shown to be more effective than even HATU under a number of conditions.

Experimental Section

General Methods. Boc-Gly-Leu-OH was prepared by a literature[8] method. TCFH and PyClU were synthesized according to published procedures.

For model peptide H-Gly-Leu-Phe-OBzl **25**,[8] 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 using a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% aqueous TFA from 1:9 to 11:9. An authentic sample of the DL-isomer was prepared from Boc-Gly-D-Leu-OH and H-Phe-OBzl-TsOH via EDC/HOAt coupling in DMF by following the standard protocol for **25**. Retention times for the LL- and DL-isomers are 17.3 and 17.9 min. respectively, under the conditions specified above.

Other model peptides (Z-Phg-Pro-NH_2 **21**,[29] Z-Phe-Val-Pro-NH_2 **22**,[7] Z-Gly-Phe-Pro-NH_2 **23**,[7] and Z-Gly-Gly-Val-Ala-Gly-Gly-OMe **24**[20,26]) were analyzed according to the procedures previously described. For analysis of pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 **26** see footnote a of Table 10 (Supporting Information).[27]

Ethyl 3-Aminopicolinate, 10. The procedure given is an improvement over that described previously. A mixture of 3-aminopicolinic acid **9** (5.07 g, 36 mmol), absolute ethanol (20 mL), and concentrated H_2SO_4 (6 mL) was refluxed for 48 h. After cooling, the mixture was concentrated to about 15 mL and poured into 15 g of ice. The mixture was basified with concentrated aqueous ammonia to pH 8−9 with cooling in an ice bath, and the white precipitate that separated was collected by filtration. The filtrate was extracted with ether (4 × 50 mL), and the ether layer washed with brine (4 × 50 mL) and dried over $MgSO_4$. Evaporation of the ether afforded a solid which was treated with decolorizing carbon and recrystallized from benzene-hexane to give 4.05 g (68%) of the ester as white needles: mp 126−127 °C (lit.[18] 131−133 °C, yield 42%); ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 1),7.23 (dd, 1), 7.04 (dd, 1), 5.76 (s, 2), 4.46 (q, 2), 1.45 (t, 3).

3-Amino-2-picolinehydroxamic Acid, 11. Experimental details were not previously given for this compound. Hydroxylamine hydrochloride (16.3 g, 0.233 mol) was added slowly with stirring and cooling to 110 mL of an aqueous NaOH solution prepared from 18.7 g (0.467 mol) of NaOH. To the solution was added 19.4 g (0.116 mol) of ester **10** portionwise followed by 110 mL of methanol, and the mixture was stirred for 48 h. The solution was concentrated under reduced pressure to about 100 mL and neutralized with cooling to pH 5−6 with 25% HCl. The white precipitate was filtered, washed with a small amount of cold water, and dried over P_20_5 in vacuo to give 17.8 g (100%) of the acid **11** as a white solid, which was pure enough for the next step. An analytical sample was obtained in 90% yield after two recrystallizations from $MeNO_2$−MeOH−EtOAc as white blocklike crystals: mp 131−133 °C (lit.[19] mp 143−145 °C, yield 49%); ^{1}H NMR(DMSO-d_6/$CDCl_3$) δ 10.91 (s, 1), 8.89 (s, 1), 7.74 (t, 1), 7.16 (d, 2), 6.71 (s, 2); IR (KBr) 3443(m), 3334 (s), 1660 (s, CON), 1606 (s), 1262 (w), 1017 (w), 805 (m) cm^{-1}.

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene (HODhat, 5). Experimental details not previously given. To a suspension of finely powdered **11** (7.3 g, 47.6 mmol) in 28 mL of water was added 8.5 mL of concentrated HCl with stirring. While the mixture cooled in an ice bath, a cold solution of $NaNO_2$ (4.93 g, 71.4 mmol) in 5 mL of water was added dropwise and the temperature was maintained below 5 °C. Following addition, stirring in the ice bath was continued for another 30 min, and the solid was filtered, washed with a small amount of cold water, and air dried to give 2.52 g (32%) of the triazene **5** as a yellow solid: mp 195 °C (explodes) [lit.[16] mp 195 °C (explodes)]. The analytical sample was obtained by recrystallization from EtOH−water (9:1 v/v) as light orange-yellow needles: mp 203 °C (explodes); ^{1}H. NMR (DMSO-d_6) δ 9.13 (dd, 1), 8.65 (dd, 1), 8.08 (dd, ^{1}H); IR (KBr) 2600 (broad, OH), 1713 (vs, CON), 1574 (s), 1420 (m), 1230 (sh, s), 1185 (s), 1066 (sh, s), 974 (sh, m), 794 (m) cm^{-1}.

3-Hydroxypicolinic acid (1.6 g, 20%) was also isolated from the mother liquor as light pink needles: mp 222−224 °C. Anal. Calcd for $C_6H_5NO_3$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.52; H, 3.58; N, 9.98.

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazene (HODhad, 6). A mixture of 1.224 g (8 mmol) of hydroxamic acid **11** and 3 mL of 98% formic acid was heated under reflux for 15 min, after which time 8 mL of water was added and the whole mixture was boiled for 15 min and cooled to rt. The precipitate was collected by filtration and washed with water (2 × 5 mL). After being boiled with ethanol twice, 0.71 g (55%) of **6** was obtained, which in an analytically pure form was a yellow solid: mp 318.5−320 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.15 (br, 1), 8.85 (dd, 1), 8.69 (s, 1), 8.17 (dd, 1) 7.84 (dd, 1); IR (KBr) 2625 (broad, OH), 1683 (sh, vs, CON), 1600 (w), 1446 (m), 1410 (s), 1359 (s), 1223 (s), 990 (s), 902 (m), 791 (s) cm^{-1}. Anal. Calcd for $C_7H_5N_3O_2$: C, 51.53; H, 3.09; N, 25.76. Found: C, 51.46; H, 3.00; N, 25.80.

(28) Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853.

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

Z-Aib-ODhat. In an ice bath, 0.3168 g of EDC-HCl (1.65 mmol) was added with stirring to a suspension of Z-Aib-OH (0.3555 g; 1.5 mmol) and HODhat **5** (0.246 g, 1.5 mmol) in 10 mL of THF and 5 mL of DMF. The resulting mixture was stirred at rt for 3 h. Solvents were removed in vacuo, and the oily residue was extracted with 40 mL of EtOAc. The EtOAc solution was washed with 5% aqueous citric acid (3 × 10 mL), 10% $NaHCO_3$ solution (3 × 10 mL), and brine (3 × 10 mL) and dried over $MgSO_4$. Evaporation of the solvent gave a yellow oily residue, which solidified after drying in vacuo over P_20_5 overnight. The crude solid was purified by flash chromatography with EtOAc as eluent to give 0.46 g (80%) of the ester as a cream yellow solid. For characterization data see Table 1, Supporting Information. Other Aib esters were made similarly.

Me_3CCOODhat. Under an atmosphere of dry N_2, TEA (0.42 mL, 3 mmol) was added to a suspension of HODhat **5** (0.3282 g, 2 mmol) in 10 mL of dry methylene chloride. The resulting mixture was cooled to 0 °C, and a solution of pivaloyl chloride (0.27 mL, 2.2 mmol) in 5 mL of dry methylene chloride was introduced dropwise with stirring. The stirring was continued for 30 min in an ice bath, and the temperature was allowed to rise to rt. After 4 h, the mixture was diluted with 30 mL of CH_2Cl_2, and the whole mixture was washed with saturated $NaHCO_3$ (3 × 20 mL), brine (2 × 20 mL), and water (2 × 20 mL) and finally dried over anhydrous $MgSO_4$. Evaporation of solvent gave a pale yellow solid, which was recrystallized from EtOAc−hexane to give 0.31 g (61%) of the analytically pure ester as colorless needles. For characterization data see Table 1, Supporting Information. Other pivaloyl esters were made similarly.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HDATU, 12).** Under an atmosphere of dry N_2, 0.22 mL (1.65 mmol) of TEA was added to a suspension of HODhat, **5** (0.246 g, 1.5 mmol), in 10 mL of dry CH_2Cl_2. After being stirred for 5 min, the resulting clear light yellow solution was cooled to 0 °C in an ice bath and 0.4209 g (1.5 mmol) of TCFH was introduced protionwise with stirring. The stirring was continued for 30 min in an ice bath and then at rt for 1.5 h. The precipitate was collected by filtration, washed twice with methylene chloride, and recrystallized twice from MeCN−ether to give 0.42 g (69%) of analytically pure hexafluorophosphate **12** as a white solid: mp 152 °C (explodes); ^{1}H NMR (CD_3CN) δ 9.19 (dd, l), 8.69 (dd, 1), 8.13 (dd, l), 3.21 (s, 12); IR (KBr) 1738 (vs), 1702 (vs) cm^{-1}. Anal. Calcd for $C_{11}H_{15}N_6O_2PF_6$: C, 32.36; H, 3.70; N, 20.58. Found: C, 32.14; H, 3.79; N, 20.47.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HDADU, 13).** As described for HDATU **12**, diazine HODhad **6** (0.2447 g, 1.5 mmol) was treated with TCFH (0.4209 g, 1.5 mmol) in 10 ml of dry CH_2Cl_2 in the presence of TEA (0.30 mL, 2.1 mmol) to give 0.55 g (90%) of the hexafluorophosphate **13** as a white solid, which was recrystallized twice from MeCN−ether to give 0.48 g (79%) of analytically pure salt as a white solid: mp 203−205 °C dec; ^{1}H NMR (CD_3CN) δ 8.91 (dd, l), 8.64 (s, 1), 8.22 (dd, 1), 7.88 (dd, 1), 3.17 (s, 12); IR (KBr) 1701(vs) cm^{-1}. Anal. Calcd for $C_{12}H_{16}N_5O_2PF_6$: C, 35.38; H, 3.96; N, 17.19. Found: C, 35.51; H, 3.86; N, 17.33.

***O*-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium Hexafluorophosphate (HDPyU, 14).** To a mixture of 0.8157 g (5 mmol) of HODhbt **1** and 0.70 mL (5 mmol) of TEA in 25 mL of dry CH_2Cl_2 at 0 °C was added 1.6633 g (5 mmol) of PyClU portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight, and 50 mL of ether was added causing an oily material to separate. The mixture was stored at −20 °C for 2 days until the oil solidified. The yellow solid was collected by filtration and washed with ice-cold water (4 × 5 mL) to remove TEA·HCl and the residue recrystallized from MeCN−ether to give 1.1 g (44%) of the uronium salt **14** as cream yellow crystals: mp 124 °C (explodes); ^{1}H NMR (CD_3CN) δ 8.34 (m, 2), 8.17 (m, 1), 8.00 (m,1), 3.74 (t, 8), 1.97 (m, 8); IR (KBr) 1716 (vs), 1676 (vs) cm^{-1}. Anal. Calcd for $C_{16}H_{20}N_5O_2PF_6$: C, 41.83; H, 4.39; N, 15.25. Found: C, 41.64; H, 4.27; N, 15.16.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-y)-1,1,3,3-bis(tetramethylene) uronium Hexafluorophosphate (HDAPyU, 15).** To a mixture of 0.4923 g (3 mmol) of HODhat **5** and 0.46 mL (3.3 mmol) of TEA in 25 mL of dry CH_2Cl_2 at 0 °C was added 1.0 g (3 mmol) of PyClU portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 50 mL, washed with ice-cold water (2 × 15 mL), and dried over $MgSO_4$. The solvent was removed, the oily residue was dissolved in 5 mL of MeCN to which 30 mL of ether was added, and the whole mixture was stored at −20 °C for several days until the oil solidified. The solid was collected by filtration and redissolved in 20 mL of CH_2Cl_2, and the solution was washed with ice-cold water (2 × 5 ml) and dried over $MgSO_4$. Removal of the solvent gave a pink-yellow solid which was recrystallized from MeCN−ether to give 0.42 g (30%) of the uronium salt **15** as off-white crystals: mp 136.5 °C (explodes); ^{1}H NMR (CD_3CN) δ 9.17 (dd, 1), 8.67 (dd, 1), 8.11 (dd, 1), 3.75 (t, 8), 1.97 (m, 8); IR (KBr) 1734 (vs), 1679 (vs) cm^{-1}. Anal. Calcd for $C_{15}H_{19}N_6O_2PF_6$: C, 39.13; H, 4.16; N, 18.26. Found: C, 38.94; H, 4.08; N, 18.30.

[1-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)oxy]-tris(pyrrolidino)phosphoninm Hexafluorophosphate (PyDAOP, 17). To a mixture of 0.2462 g (1.5 mmol) of HODhat **5** and 0.24 mL (1.65 mmol) of TEA in 10 mL of dry CH_2Cl_2 at 0 °C was added 0.6993 g (1.5 mmol) of PyBrOP portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 25 mL, and the solution was washed with ice-cold water (2 × 10 mL) and dried over $MgSO_4$. The resulting clear light yellow solution was treated with 50 mL of ether, and the solid which separated was collected by filtration to give 0.45 g (54%) of analytically pure phosphoniumn salt **17** as a white solid after recrystallization from MeCN−ether: mp 149 °C dec; ^{1}H NMR (CD_3CN) δ 9.20 (dd, 1), 8.67 (dd, 1), 8.13 (dd, 1), 3.42 (td, 12), 1.96 (td, 8); IR (KBr) 1742 (vs), 1566 (m), 1462 (sh, w) cm^{-1}. Anal. Calcd for $C_{18}H_{27}N_7O_2P_2F_6$: C, 39.34; H, 4.95; N, 17.84. Found: C, 39.36; H, 5.09; N, 17.90.

Synthesis of Fmoc-Ile-ODhat. Method A.[15] Under an atmosphere of dry N_2, a suspension of Fmoc-Ile-OH (0.3534 g, 1 mmol), HODhat **5** (0.1805 g, 1.1 mmol), and $SOCl_2$ (0.73 mL, 10 mmol) in 8 mL of dry CH_2Cl_2 was refluxed overnight. Evaporation of CH_2Cl_2 and the excess of $SOCl_2$ gave a yellow solid which was purified by flash chromatography through a short silica gel column with a mixture of EtOAc−CH_2Cl_2 (1:1 v/v) as eluent to give, after two recrystallizations from CH_2Cl_2−benzene−ether−hexane, 0.42 g (81%) of the analytically pure ester as a white solid: mp 160.5−162 °C; ^{1}H NMR ($CDCl_3$) δ 9.15 (dd, 1), 8.58 (dd, 1), 7.96 (dd, 1); 7.76 (dd,2), 7.61 (dd, 2), 7.27−7.44 (m, 4), 5.20 (d, 1), 4.88 (q, 1), 4.49 (d, 2), 4.26 (t, 1), 2.21 (m, 1), l.70 (m, 1), 1.34 (m, 1), 1.15 (d,3), 1.05 (t, 3); IR (KBr) 1811 (s, COO), 1738 (vs, CONN), 1692 (vs, NHCO) cm^{-1}. Anal. Calcd for $C_{27}H_{25}N_5O_5$: C, 64.91; H, 5.04; N, 14.02. Found: C, 64.77; H, 5.23; N, 13.94.

Method B. Under an atmosphere of dry N_2, 0.1854 g (0.5 mmol) of Fmoc-Ile-Cl was added with stirring to a solution of HODhat **5** (0.0821 g, 0.5 nmol) and DIEA (95.8 μL, 0.55 mmol) in 10 mL of CH_2Cl_2 at 0 °C. Stirring was continued at 0 °C for 30 min and then at rt for 5 h. The resulting light yellow solution was diluted to 30 mL with CH_2Cl_2 and washed quickly with ice-cold brine (2 × 15 mL). After drying over $MgSO_4$ and removing the solvent, the light yellow sticky solid was recrystallized twice from CH_2Cl_2 ether−hexane to give the analytically pure ester as a white solid: mp 161−162 °C. NMR and IR spectra were identical with those of the sample obtained by method A.

Ring-Opening Reaction: Synthesis of 3-(3′-Azidopicolinoyloxy)-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine 18. To a solution of HODhat **5** (0.3282 g, 2 mmol) in 5 mL of DMF was added 0.2063 g (1 mmol) of DCC portionwise, and the resulting mixture was stirred at rt overnight. The side product DCU was removed by filtration, and the filtrate was evaporated to dryness. The residual solid was recrystallized from $MeNO_2$−EtOAc−hexane to give 0.22 g (65%) of analytically pure **18** as cream yellow needles: mp 153 °C dec; ^{1}H NMR (DMSO-d_6/$CDCl_3$) δ 9.13 (dd, 1), 8.64 (dd, 1) 8.40 (dd, 1), 8.06 (dd, 1), 7.85 (dd, 1), 7.60 (dd, 1); IR (KBr) 1796 (vs, COO), 1733 (vs). Anal. Calcd for $C_{12}H_6N_8O_3$: C, 46.45; H, 1.95; N, 36.12. Found: C, 46.18; H, 1.97; N, 36.28.

Reactivity of HODhat Derivatives.

Z-Aib-OXt Esters. The reaction of Z-Aib-ODhat with PCA is taken as an example to demonstrate the standard method used in order to follow aminolysis via an NMR protocol: To a solution of 47.9 mg (0.125 mmol) of Z-Aib-ODhat in 0.5 mL of $CDCl_3$ was added 15.6 mg (0.125 mmol) of *p*-chloroaniline (PCA). The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ^{1}H NMR peaks at δ 1.7 (CH_3 residue of ester Z-Aib-ODhat) and 1.57 (CH_3 residue of amide **19**) [or peaks at δ 5.2 (benzylic CH_2 unit of ester Z-Aib-ODhat) and 5.05 (benzylic CH_2 unit of amide **19**)] as the reaction progressed at the NMR probe temperature (~37 °C) allowed for rough determination of the relative rates. The results given in Table 2 are the average of at least two runs.

Me_3CCOOXt Esters. As in the case with Z-Aib esters, the reaction of pivaloyl ester with *N*-methylbenzylamine is taken here as an example to demonstrate the methodology used: to a solution of 31.0 mg (0.125 mmol) of Me_3CCOODhat in 0.5 mL of $CDCl_3$ was added 15.1 mg (16.1 μL, 0.125 mmol) of $PhCH_2NHMe$. The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ^{1}H NMR peaks at δ 1.5 (CH_3 residue of ester Me_3CCOODhat and 1.3 (CH_3 residue of amide **20b**) as the reaction progressed at the NMR probe temperature (~37 °C) allowed for rough determination of the relative rates. The results given in Table 3 are the average of at least two runs.

Model Segment Coupling Reactions. Test couplings were carried out as described previously for Z-Phg-Pro-NH_2, Z-Phe-Val-Pro-NH_2, Z-Gly-Phe-Pro-NH_2, and Z-Gly-Gly-Val-Ala-Gly-Gly-OMe. For Boc-Gly-Leu-Phe-OBzl, 60.6 mg (0.21 mmol) of Boc-Gly-Leu-OH, 85.45 mg (0.20 mmol) of H-Phe OBzl·TsOH, and 0.22 mmol of an appropriate coupling additive (HOXt) were dissolved in 1 mL of DMF or TFE/TCM (1:3 v/v). To the mixture was added a solution of 34.2 mg (0.22 mmol) of EDC in 1 mL of DMF or TFE/TCM, and the whole mixture was stirred at rt overnight. The resulting mixture was diluted with 25 mL of EtOAc, washed with 1 N HCl (2 × 10 mL), 10% $NaHCO_3$ (2 × 10 mL), and brine (2 × 10 mL), and dried over $MgSO_4$. After removal of solvent, the solid was weighed to determine the yield. The solid was then stirred with 2 mL of 50% TFA in a methylene chloride solution for 2 h to deblock the BOC-group. The TFA and CH_2Cl_2 were then removed in vacuo, 20 mL of anhydrous ether was added to the oily residue, and the mixture was stored overnight. The white precipitate which had separated was collected by filtration and washed with ether. About 5 mg of the crude product, containing both LL- and DL-forms of **25**, was dissolved in 4 mL of MeCN and directly analyzed by HPLC using a reversed-phase Waters C_{18} column, with elution by a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% aqueous TFA from 1:9 to 11:9, at a flow rate of 1.0 mL/min. The retention times for the LL- and DL-forms of **25** were 17.3 and 17.9 min, respectively.

Solid-Phase Assembly of ACP under Stringent Conditions. The standard method previously described according **to** the so-called "1.5 × 1.5" protocol[27,28] was followed. See Table 10, Supporting Information, and Figure 1.

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Supporting Information Available: Table 1, Table 7, and Table 10 and more extensive versions of Tables 5 and 6 and confirming IR and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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