((9-Fluorenylmethyl)oxy)carbonyl Amino Acid Chlorides in Solid-Phase Peptide Synthesis[1]

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FMOC amino acid chlorides, previously shown to be rapid-acting coupling agents in two-phase systems or in homogeneous solution, did not prove to be directly applicable to solid-phase peptide synthesis due to sluggish reactivity. The problem was traced to prior conversion of the acid chlorides by necessary basic co-reactants (DIEA, NMM, etc.) to the corresponding oxazolones. If, in their place, 1:1 mixtures of these bases and HOBt were used, rapid solid-phase acylations were possible via the intermediate formation of the corresponding HOBt esters. The 1:1 base–HOBt mixtures also were shown to enhance generally the reactivity of FMOC amino acid active esters over that caused by HOBt alone. Peptides assembled from acid chlorides by using these techniques included leucine enkephalin, the ACP decapeptide (65–74), prothrombin (1–9), several substance P analogues, and eledoisin. Where particular acid chlorides could not be used due to their instability, the corresponding pentafluorophenyl esters or mixtures of the acids and BOP reagent were used.

Recently the use of FMOC amino acid chlorides as convenient, inexpensive coupling agents for peptide synthesis in solution has been reported.[2] Among other examples, these rapid-acting acylating agents have been used in a quick sequential method for the synthesis of short peptide segments without isolation of intermediates. Coupling reactions involving acid chlorides were over in a few minutes under both two-phase[2] (H_2O/CH_2Cl_2/$NaHCO_3$ or Na_2CO_3) and one-phase[3] (CH_2Cl_2/DIEA or NMM) conditions. Based on these results, it seemed likely that such acid chlorides might also serve as convenient acylating species for solid-phase syntheses. Major advantages of such reagents over mixtures of protected amino acids and various coupling reagents would be the potential low cost of the chlorides, their high solubility, and the expected rapidity of the coupling process.

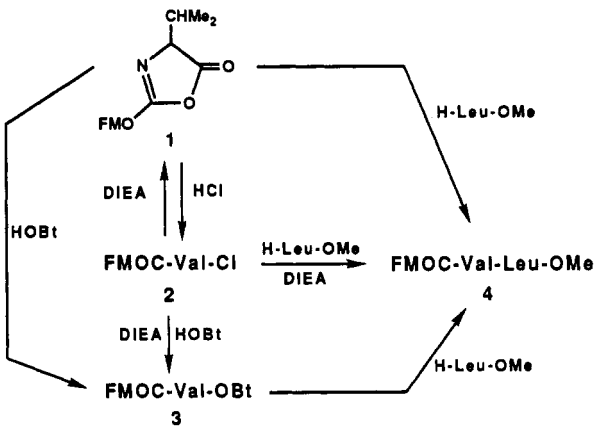
Oxazolone Formation as a Rationale for Anomalously Slow Solid-Phase Acylations by FMOC Amino Acid Chlorides. In pursuing this work model reactions using leucine-loaded resins of the Sheppard KA type[4] as well as regular 1% cross-linked polystyrene resins were examined. Surprisingly these solid-phase coupling reactions were far more sluggish than expected on the basis of analogous reactions in solution. When a polymeric ester bearing a free amino group was treated with 4 equiv of FMOC-phenylalanine chloride in DMF (0.1 M) in the presence of 4 equiv of a tertiary amine such as pyridine or DIEA, conditions appropriate for practical syntheses, reactions proved to be incomplete according to the Kaiser test even after 10 min.

Table I. Extent of Reaction between FMOC-Val-Cl and H-Leu-Pepsyn KA Resin in DMF in the Presence of Basic Reagents[a]

base	coupling time (min)	degree of coupling (%)
pyridine	10	61.9
2,6-di-*tert*-butylpyridine	10	49.3
DIEA	10	89.9

[a] Resin loaded with the amino acid at the level of 0.1 mequiv/g was treated with 4 equiv of the acylating agent and amine in DMF. For exact conditions, see Experimental Section.

Scheme I



Quantitative data, collected in Table I, using the model acylating agent FMOC-Val-Cl, confirmed the unexpected sluggishness of these reactions. The results prompted an examination of the infrared spectra of solutions prepared from various tertiary bases and FMOC-Val-Cl. Spectra were generally obtained in methylene dichloride rather than the preferred reaction solvent DMF in order to avoid interfering solvent absorptions, although in some cases reactions were repeated in the latter solvent. Upon addition of DIEA or NMM to a solution of FMOC-Val-Cl in

(1) A number of abbreviations are used in this paper. Those for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follows: TFA = trifluoroacetic acid, FMOC = [(9-fluorenylmethyl)-oxy]carbonyl, DCC = dicyclohexylcarbodiimide, HOBt = *N*-hydroxybenzotriazole, DMF = dimethylformamide, DBF = dibenzofulvene, DMAP = 4-(dimethylamino)pyridine, NMM = *N*-methylmorpholine, DIEA = diisopropylethylamine, TEA = triethylamine, NKT = negative Kaiser test, HOOBt = 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine, HOPy = 1-hydroxy-2-pyridone, HOODHQ = 3-hydroxy-4-oxo-3,4-dihydroquinazoline, Pfp = pentafluorophenyl, HOSu = *N*-hydroxysuccinimide, HOPht = *N*-hydroxyphthalimide, BOP = (benzotriazolyloxy)-tris(dimethylamino)phosphonium hexafluorophosphate, MBHA = *p*-methylbenzhydrylamine. As used here the term "binary mixture" refers to a 1:1 mixture of an *N*-hydroxy compound and a tertiary amine.

(2) (a) Carpino, L. A.; Cohen, B.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J. H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732. (b) Beyermann, M.; Bienert, M.; Niedrich, H.; Carpino, L. A.; Sadat-Aalaee, D. *J. Org. Chem.* **1990**, *55*, 721. (c) Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673.

(3) Carpino, L. A. *J. Org. Chem.* **1988**, *53*, 875.

(4) (a) Dryland, A.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 125. (b) Arshady, R.; Atherton, E.; Clive, D. L. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. I* **1981**, 529.

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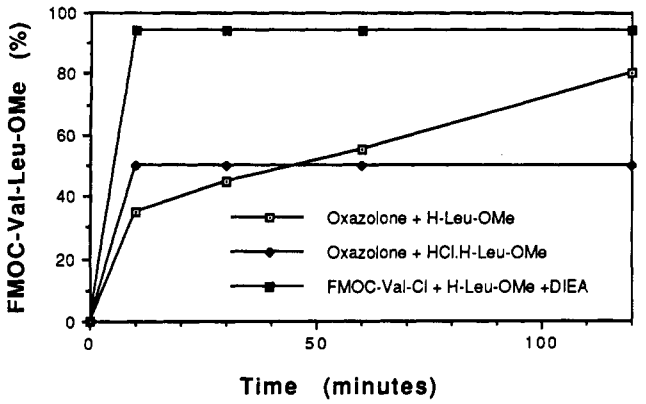


Figure 1. Time course for the formation of FMOC-Val-Leu-OMe via oxazolone in the presence and absence of HCl and via the acid chloride. To a solution of 0.2 mmol of H-Leu-OMe or H-Leu-OMe·HCl in 2 mL of CH_2Cl_2 was added 1 equiv of oxazolone. Quenching of an aliquot at various times was followed by HPLC determinatiom of the ratio of FMOC-Val-Leu-OMe to FMOC-Val-OMe: t_R 15.33 min and 10.48 min, respectively, f = 1.5 mL/min, 40/60 H_2O (0.1% TFA)/CH_3CN (0.08% TFA). For FMOC-Val-Cl, 1 equiv of DIEA was added to scavenge HCL.

methylene dichloride, all acid chloride disappears immediately and the oxazolone 1 of FMOC-Val-OH is formed[5] as evidenced by replacement of the acid chloride carbonyl absorption at 1790 cm^{-1} by the characteristic oxazolone absorptions at 1835 and 1685 cm^{-1} (Scheme I). Oxazolone 1 has been isolated, purified, and fully characterized. Previously Benoiton reported the isolation of this oxazolone in an impure state.[6]

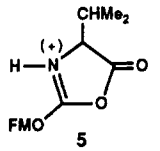
With pyridine added in place of the tertiary aliphatic amines, oxazolone 1 was again the major product, although reaction was incomplete as indicated by a weak band at 1790 cm^{-1} believed to arise from unreacted acid chloride. With DMAP, oxazolone was again the major product but none of the acid chloride remained. Instead a weak absorption at 1770 cm^{-1} appeared, which can be attributed to the acylammonium cation.[7] With 2,6-di-*tert*-butylpyridine, only a small amount of oxazolone appeared, the major species present being the unreacted acid chloride. These rapid preliminary conversions to oxazolone as noted here can explain the low rate of reaction between FMOC amino acid chlorides and resin-bound amino acid esters. Oxazolone formation can compete due to the lesser reactivity of the polymer-bound amine relative to its soluble counterpart. It has been noted that under the conditions ($CH_2Cl_2/H_2O/NaHCO_3$ or Na_2CO_3) of the recently described FMOC/polyamine rapid, continuous two-phase soluton synthesis of short peptides[2] FMOC amino acid chlorides react directly (2–3 min) with amino acid esters. This is clear from the fact that simulation of the coupling step in the absence of the amino acid ester leads over a period of about 10 min to the formation of oxazolone 1 in the case of valine.[8] If at this point the amino acid ester is added, the subsequent coupling reaction is slow and incomplete after 5 h. These results suggest that normal acylation proceeds via reaction of the acid chloride itself

Table II. Extent of Reaction of FMOC-Val-Cl with H-Leu-Pepsyn KA Resin in DMF in the Presence of Organic Bases and *N*-Hydroxy Compounds[a]

binary mixture (1:1)	coupling time (min)	extent of coupling (%)
HOSu/DIEA	3	14.8
HOPht/DIEA	3	19.5
HOBt/DIEA	1	97.3
HOBt/DIEA	2	NKT
HOOBt/DIEA	1	87.0
HOOBt/DIEA	2	93.7

[a] Carried out as described in footnote a of Table I with 0.16 M binary salt solution used in place of the tertiary amine solution.

with the amino acid esters, the inorganic base present in the aqueous phase serving to maintain a high concentration of free amino acid ester in the organic phase. It was possible to follow these reactions clearly by HPLC techniques. Upon addition of leucine methyl ester hydrochloride to a solution of oxazolone 1 in methylene dichloride rapid reaction occurs[9] within 10 min, after which it levels off with the formation of a mixture composed of 50% FMOC-Val-Leu-OMe, 50% FMOC-Val-Cl, and 50% H-Leu-OMe·HCl. If the oxazolone is treated under the same conditions with free leucine methyl ester, about 45 min is required for the formation of 50% of the dipeptide. Finally, if the acid chloride 2 is used in the presence of 1 equiv of DIEA, conversion to dipeptide reaches 95% after 10 min. The results are compared in Figure 1. It is not certain whether the protonated form 5 of oxazolone 1 has



5

an independent existence in the presence of a reactive nucleophile.[10] As noted previously, reaction with chloride ion leads to immediate formation of acid chloride.[8] Similarly treatment with *p*-toluenesulfonic acid, pivalic acid, or HOBt generates the corresponding mixed anhydride or *N*-hydroxybenzotriazole ester. If instead of hydrogen chloride the oxazolone is treated with an equivalent of the hydrochloride of an amine, an equilibrium is set up with the amount of acid chloride formed depending on the basicity of the amine. Thus, according to infrared examination, no reaction occurs between oxazolone 1 and the hydrochlorides of DIEA, NMM, or 2,6-dimethyllutidine, whereas with pyridine hydrochloride and 2,6-di-*tert*-butylpyridine hydrochloride increasing amounts of acid chloride are formed.

Avoidance of in Situ Oxazolone Formation via Salts of Weak Acids. In a search for more suitable bases for effecting solid-phase couplings via FMOC amino acid

(5) Dehydrochlorination of urethane-protected amino acid chlorides to 2-alkoxyoxazolones was first encountered by Miyoshi [Miyoshi, M. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 212, 1489; **1970**, *43*, 3321] as clarified by Jones [Jones, J. H.; Witty, M. J. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3203.

(6) Paquet, A.; Chen, F. M. F.; Benoiton, N. L. *Can. J. Chem.* **1984**, *62*, 1335.

(7) Upon complexation with DMAP the infrared carbonyl absorption of acetyl chloride drops from ca. 1785 to 1755 cm^{-1}. See: Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569.

(8) Sadat-Aalaee, D., Ph.D. Thesis, University of Massachusetts, Amherst, MA 01003, 1990.

(9) For previous examples of the greater reactivity of oxazolones in the presence of acid catalysts, see: (a) Carter, H. E.; Handler, P.; Stevens, C. M. *J. Biol. Chem.* **1941**, *138*, 619. (b) Weygand, F.; Steglich, W.; Barocio de la Lama, X. *Tetrahedron Suppl 8, Part I* **1966**, 9. (c) Rodriquez, H.; Chuaqui, C.; Atala, S.; Marquez, A. *Tetrahedron* **1971**, *27*, 2425. (d) Curran, T. C.; Farrar, C. R.; Niazy, O.; Williams, A. *J. Am. Chem. Soc.* **1980**, *102*, 6828. (e) Wipf, P.; Heimgartner, H. *Helv. Chim. Acta* **1986**, *69*, 1153; **1988**, *71*, 140. (f) Obrecht, D.; Heimgartner, H. *Helv. Chim. Acta* **1990**, *73*, 221.

(10) The stability of protonated oxazolones is dependent on the 2-substituent. Thus 2-aryl derivatives yield stable hydrochlorides, perchlorates, or tetrafluoroborates. See: Boyd, G. V.; Wright, P. H. *J. Chem. Soc., Perkin Trans. 1* **1972**, 909.

(11) Beyermann, M.; Granitza, D.; Bienert, M.; Haussner, M.; Carpino, L. A. In *Peptides 1988. Proceedings of the 20th European Peptide Symposium*; Jung, G., Bayer, E., Eds.; Walter de Gruyter: Berlin, 1989; p 28.

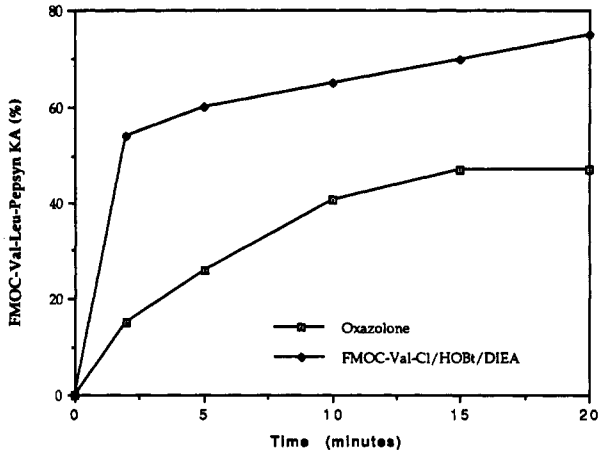


Figure 2. Time course for the formation of FMOC-Val-Leu-Pepsyn KA resin via oxazolone or HOBt ester. FMOC-Leu-Pepsyn KA resin (250 mg, 0.08 mequiv/g substitution level) was treated with 2 mL of 20% piperidine in DMF for 7 min. After washing by DMF, the resin was acylated with 4 equiv of the acylating agent (isolated oxazolone or a 1:1 mixture of FMOC-Val-Cl and DIEA–HOBt) in 2 mL of DMF. An aliquot of the resin (10–15 mg) was removed at various times, washed, dried, and subjected to UV analysis.

chlorides, salts of weak acids such as 1:1 mixtures of triethylamine, DIEA, or NMM and pivalic acid or *N*-hydroxybenzotriazole were tested. Contrary to the case where tertiary amines alone were used, very rapid solid-phase acylation reactions ensued. For example under conditions similar to those specified in Table I, the binary mixture HOBt/DIEA led to 97.3% coupling within 1 min. Nearly as effective was the combination HOOBt/DIEA; but far less effective were binary mixtures containing *N*-hydroxysuccinimide or *N*-hydroxyphthalimide in place of *N*-hydroxybenzotriazole. For the results, see Table II.

Infrared studies such as those mentioned above showed that upon treatment of FMOC-Val-Cl with the binary mixtures HOBt/DIEA or HOBt/NMM, the HOBt ester of the FMOC amino acid is formed immediately and is the species responsible for the rapid acylation process. For comparison of the reactivity of an HOBt ester and an oxazolone, see Figure 2. The efficiency of such esters in peptide coupling is well known,[12] these species being recognized as active reagents in a number of common coupling techniques (DCC/HOBt;[12] BOP,[13] etc.). In situ reaction of acid chlorides with the binary mixture may represent one of the simplest, cleanest routes to these intermediates. The question of whether the *O*-acyl derivative formed under these conditions is accompanied by the corresponding *N*-acyl isomer or whether subsequent *O*- to *N*-acyl isomerization occurs was not examined. Such effects may have some bearing on the inherent reactivity of these compounds.[14]

It should be noted that König and Geiger[15] long ago showed that HOBt catalyzes the reaction of amino acid esters with trichlorophenyl and *p*-nitrophenyl esters, and more recently the same effect has been demonstrated for pentafluorophenyl esters.[16] Indeed, the early investigators demonstrated the likelihood of an equilibrium between an active aryl ester and the HOBt ester (development of an IR band at 1815 cm^{-1}). Standard protocol for the routine use of pentafluorophenyl esters via continuous flow techniques involves the presence of 1 equiv of HOBt. It is likely that the HOBt anion, generated by reaction of added HOBt and the free amino group of the resin-attached amino acid, is the active catalytic species, and since reaction is expected to slow down as the amino group is consumed, deliberate addition of a source of HOBt anion is expected to maximize the rate. Recently Horiki[17] showed that the potassium salt of HOBt in the presence of a crown ether also effected marked catalysis of peptide coupling processes. Table III compares the speed of these anion-catalyzed reactions of active esters and shows that reactions are nearly as rapid as those involving acid chlorides. These data suggest that for best results a 1:1 mixture of HOBt and a tertiary base should routinely be used to catalyze pentafluorophenyl ester acylations unless

Table III. Reaction of FMOC-Val-OPfp with H-Leu-Pepsyn KA Resin in DMF in the Presence of Various Binary Salt Mixtures[a]

binary mixture	time (min)	extent of reaction (%)
HOODHQ/DIEA	10	65
	20	82.7
HOPy/DIEA	10	81
	20	83
HOOBt/DIEA	10	93
	20	94
HOBt/DIEA[b,c]	10	92
	20	94
HOBt/DIEA (1:2)	10	92.6
	20	95.5
HOBt/DIEA (2:1)	10	85.4
	20	88.0

[a] Resin (200 mg) loaded with the first amino acid at the level of 0.1 mequiv/g was treated with 2 equiv of the acylating agent in 1 mL of DMF solution added to 1 mL of a DMF solution containing 0.04 M binary salt. After rotation at room temperature for the period given, UV analysis for the FMOC function showed the extent of coupling. Relative to the conditions of Tables I and II, more dilute solutions were used in order to allow easier discrimination among the various mixtures tested. [b] Results were indistinguishable when NMM was substituted for DIEA. [c] Under conditions described in Table I with H-Ile-Pepsyn KA, treatment with FMOC-Ile-OPfp in the absence and presence of HOBt/DIEA for 3 min gave 36.9% and >99% coupling, respectively.

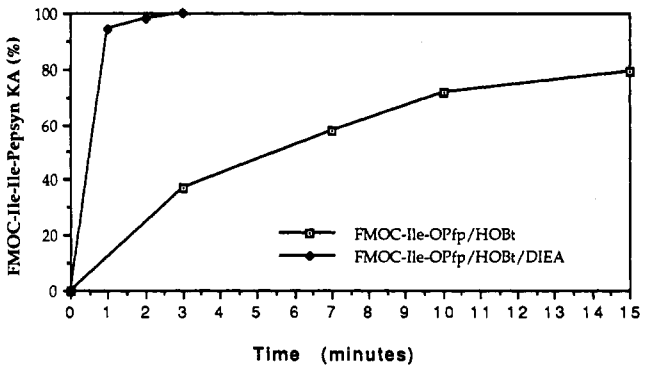
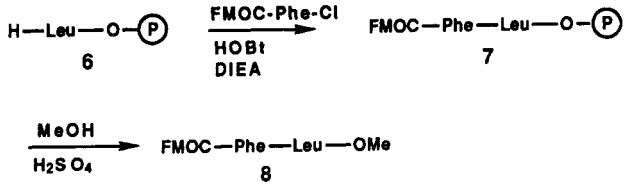


Figure 3. Time course for the formation of FMOC-Ile-Ile-Pepsyn KA resin via FMOC-Ile-OPfp and HOBt in the presence and absence of DIEA. FMOC-Ile-Pepsyn KA (200 mg, 0.1 mequiv/g substitution level) was treated with 2 mL of 20% piperidine in DMF for 7 min. After washing by DMF, the resin was acylated with 4 equiv of FMOC-Ile-OPfp and 4 equiv of HOBt without added DIEA in one case and with 4 equiv of DIEA in 1 mL of DMF in the other. An aliquot of the resin (10–15 mg) was removed at various times, washed, dried, and subjected to UV analysis.

(12) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(13) Castro, B.; Dormoy, J.-R.; Evin, G.; Selve, C. *J. Chem. Res., Synop.* **1977**, 182.
(14) Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *Int. J. Pept. Prot. Res.* **1984**, *23*, 300.
(15) König, W.; Geiger, R. *Chem. Ber.* **1973**, *106*, 3626.
(16) (a) Atherton, E.; Cameron, L. R.; Sheppard, R. C. *Tetrahedron* **1988**, *44*, 843. (b) Dryland, A.; Sheppard, R. C. *Tetrahedron* **1988**, *44*, 859.
(17) Horiki, K.; Murakami, A. *Heterocycles* **1989**, *28*, 615.

Scheme II



it can be shown in any particular case that the tertiary base–HOBt combination leads to byproduct formation or racemization.[18,19] For a graphic comparison, see Figure 3.

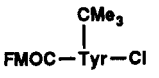
The presence of a tertiary base could complicate matters if any premature deblocking of the FMOC function occurred during the acylation step. Although such side reactions do indeed occur with strong unhindered bases (NEt_3, DMAP, etc.), either hindered (DIEA) or weak bases (NMM) are suitable. For example, binary mixtures derived from either DIEA or NMM caused no deblocking of the FMOC function of FMOC-Leu (or Ile)-Pepsyn KA for periods up to 4 h.

Racemization Tests. Regarding the question of possible racemization accompanying the use of acid chlorides, studies carried out on a model system showed no significant loss of chirality. For preliminary studies the reactions outlined in Scheme II were carried out. Following coupling in the presence of the binary mixture, dipeptide resin 7 was subjected to transesterification with methanol in the presence of a catalytic quantity of concentrated sulfuric acid. The resulting dipeptide 8 was analyzed by HPLC, the crude material showing less than 0.1% of the DL diastereomer. A second example involved the coupling of FMOC-Phe-Cl with phenylalanylglycine bound to a hydroxyacetamide resin[20] followed by detachment by hydroxide ion. With a binary mixture of pivalic acid and TEA (1:1), less than 0.1% racemization occurred. Under similar conditions with other bases (NMM, DMAP, or DIEA) in the absence of HOBt, there was also no significant racemization, although coupling was incomplete after 10 min. Increasing the base concentration speeded up the coupling reaction but also led to significant racemization (1.1–1.4% for DMAP or pyridine). Two cases demonstrating the lack of racemization during practical peptide syntheses are described below.

1:1 HOBt–Base Mixture as an Acid Chloride Coupling Promoter in Solid-Phase Assembly. Various model studies of this type were followed by syntheses of several short peptides using manual and automated techniques on instruments of both the continuous flow and batch types. The simple pentapeptide leucine enkephalin (**9**)[21] was chosen as a first target. Initial syntheses were

H-Tyr-Gly-Gly-Phe-Leu-OH
9

carried out manually and on a continuous flow instrument. For the latter, using a cycle time of 25 min (acylation time 10 min) with 5 equiv of the acid chloride along with 5 equiv of the binary mixture (HOBt/DIEA), a 69% yield of crude product (purity 93%) was obtained. An analogous run using pentafluorophenyl esters along with the binary mixture gave the peptide in 75% yield, purity 99%. No significant amount (<0.1%) of the D-Phe[4] diastereomer was detected in either case. Hydrolysis of the pentapeptides followed by GC analysis on a chiral column showed no significant racemization above the background level.[22] The only significant difference between these two syntheses was the longer time needed to dissolve the pentafluorophenyl esters, which are significantly less soluble than the corresponding acid chlorides. In the first synthesis all amino acids were introduced as acid chlorides, including tyrosine, thus demonstrating that at least some FMOC amino acids bearing side chains protected by *tert*-butyl functions can be converted to isolable acid chlorides. In previous work with FMOC amino acid chlorides, only benzyl-substituted side chains have been successfully used. It should be pointed out, however, that the crystalline tyrosine derivative **10** was used soon after



10

preparation and as expected slow loss of the *tert*-butyl ether function was observed on storage. The related *tert*-butyl ethers of FMOC serine and threonine were obtained only as oils[8] rather than crystalline solids as was the case for **10** and most other FMOC amino acid chlorides. Because "mixed" syntheses using both acid chlorides and pentafluorophenyl esters can be executed without any change in protocol with the exception of the initial time for dissolution of the reagent, it is convenient to substitute pentafluorophenyl or other stable esters for all those amino acids bearing *tert*-butyl-protected side chains.

Moving to more complex models, we studied the assembly of the substance P[23] analogue **11**, ACP decapeptide

H-Lys-Pro-Lys-Pro-Gly-Gly-Phe-Phe-Gly-Leu-Nle-NH_2
11

(65–74) **12**,[19b] and prothrombin (1–9) (**13**).[24] In the case of **11** acid chlorides were used throughout the synthesis with lysine being introduced as the ϵ-benzyloxycarbonyl derivative. The assembly was carried out in CH_2Cl_2 manually, coupling being checked after each step by the Kaiser test.[25] After incorporation of the final amino acid, the FMOC group was removed and the washed resin Z-deblocked and cleaved from the resin by use of HF/Me_2S/anisole. A similar synthesis was carried out with DMF as solvent, a technique also used to obtain the D-Phe[7] analogue. Since the two diastereomers could be readily separated by HPLC, it was demonstrated that no significant racemization (<0.1%) occurred at the phenylalanine[7] residue in any of the syntheses.

In the case of the ACP decapeptide **12**, pentafluorophenyl esters were substituted for acid chlorides in the case

H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH
12

(18) For a review of earlier work, see: Bodanszky, M.; Bednarek, M. A. *J. Prot. Chem.* **1989**, *8*, 461.
(19) For the effect of tertiary bases on BOP coupling, see (a) Gausepohl, H.; Kraft, M.: Frank, R. In *Peptides 1988. Proceedings of the 20th European Peptide Symposium*; Jung, G., Bayer, E., Eds.; Walter de Gruyter: Berlin, 1989; p 241. (b) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617.
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(22) Compare: Meienhofer, J. *Biopolymers* **1981**, *20*, 1761.
(23) For a general discussion of this type of peptide and references to earlier work see: Bienert, M.; Köller, G.; Wohlfeil, R.; Mehlis, B.; Bergmann, J.; Niedrich, H.; Kraft, R. *J. Prakt. Chem.* **1979**, *321*, 721.
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of Gln, Asp, Tyr, and Asn, and it became clear immediately that the binary mixture, HOBt/DIEA, caused decomposition of both FMOC-Asn-OPfp and FMOC-Gln-OPfp, although both these esters can be coupled successfully in the presence of HOBt alone.[16] This was not unexpected, since the free carboxamide function is known to be sensitive to cyclization and dehydration. Such reactions, which occur slowly on storage of the esters in DMF alone, are speeded up by the HOBt anion. The problem can be avoided by use of the less reactive *p*-nitrophenyl esters or by amide protection using the bis-4-methoxybenzhydryl function.[26] Alternatively Asn and Gln could be introduced as the simple pentafluorophenyl esters with HOBt alone and the binary mixture used for all other coupling steps. However, some degradation of the carboxamide function is to be expected under these conditions. FT-IR and HPLC studies confirmed the difficulties of clean coupling of unprotected asparagine derivatives. For example coupling of FMOC-Asn-OPfp with a glycine Pepsyn KA resin gave no evidence for the nitrile by-product, whereas in the presence of HOBt alone or when coupling the acid via the BOP reagent 4–6% and 16.7% of the nitrile by-product were observed, respectively.

Assembly of prothrombin (1–9) (**13**) proceeded without difficulty whether Pfp esters (HOBt/DIEA for all amino

H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-OH
13

acids except Asn where HOBt alone was used) or acid chlorides (Leu, Phe, Gly) pentafluorophenyl (Glu, Lys) and *p*-nitrophenyl (Asn) esters were used. The syntheses of **9**, **12**, and **13** discussed above were completed on a continuous flow instrument using polyamide resins. Synthesis of eledoisin (**14**)[27] on an automated batch synthesizer[28] was

pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
14

carried out using Polyhipe resins.[29a] Acid chloride substitutes were (a) BOP reagent in the cases of Ser, Lys, and Asp and (b) Pfp ester in the case of pyroglutamic acid. In all of the solid-phase syntheses reported in this work, HPLC studies showed the desired peptide to be the major product, although it was accompanied by small amounts of other materials. Work in progress aims at the identification of these byproducts and their comparison with related minor products obtained under common conditions (DCC, active ester, etc.).

Conclusions. Because FMOC-protected amino acid chlorides appear to be shelf-stable reagents and are among the least expensive potential peptide coupling agents, strong incentives exist for their incorporation into chain-assembly protocols. Following previous success with solution syntheses,[2] it is shown in this work that FMOC amino acid chlorides are also applicable to solid-phase syntheses by the simple expedient of in situ conversion to the appropriate *N*-hydroxybenzotriazole ester via reaction with a 1:1 mixture of HOBt and an appropriate tertiary amine. In cases where acid chlorides are inaccessible due to side-chain incompatibility, pentafluorophenyl esters or BOP-induced couplings represent convenient spot substitutes.[29b]

Experimental Section

General. Normal workup from an organic solvent involved drying over $MgSO_4$ and rotary evaporation. Melting points (hot plate or capillary) are uncorrected. Amino acid analyses were carried out after hydrolysis in 6 N HCl for 20 h at 110 °C. GC data were obtained on a flame ionization detector and a 25-m Chirasil-Val-L capillary column to resolve derivatized enantiomeric amino acid esters. TLC was effected with silica gel on precoated glass plates, using as mobile phases the following solvent systems: (1) chloroform/ethanol, 10:1; (2) toluene/acetic acid, 10:1; (3) ethyl acetate/pyridine/acetic acid/water, 120:20:6:11; (4) ethyl acetate/pyridine/acetic acid/water, 60:20:6:11; (5) ethyl acetate/pyridine/acetic acid/water, 30:20:6:11. Detection of TLC spots was by UV lamp or after visualization by ninhydrin or Cl_2/benzidine. Analytical HPLC characterization was carried out on LiChrosorb-RP-18, 10 μm, column dimensions 250 × 4.6 mm using the following mobile phases: (1) 40% acetonitrile, 60% 0.01 M NaH_2PO_4 + 0.15 M $NaClO_4$, pH 2.2 (isocratic): (2) 35% acetonitrile, 65% 0.01 M NaH_2PO_4 + 0.15 M $NaClO_4$, pH 2.2; (3) MeOH/0.1% TFA in H_2O (65/35) at flow rates of 1.0 mL/min. Preparative HPLC was carried out with a C_{18}-300Å, 15 μm column (7.8 × 30 cm) using as eluent H_2O (0.1% TFA)/CH_3CN (62/38). Purification of some protected peptides was carried out with silica gel (40–63 μm) in columns of dimension 300 × 20 mm. Free peptides were purified by ion-exchange chromatography on carboxymethyl cellulose on columns of dimensions 200 × 20 mm with elution by a linear gradient of 0.01–0.5 M ammonium acetate, pH 6.0. Dimethylformamide was distilled in a vacuum and stored over molecular sieves. Chloroform and methylene dichloride were distilled first over phosphorus pentoxide and finally from Na_2CO_3. The syntheses of 9-fluorenylmethanol,[30] FMOC-Cl,[31] FMOC-ONSu,[32] FMOC-ONB,[33] and FMOC amino acid chlorides[2a] proceeded according to published descriptions. Automated peptide syntheses were carried out on Milligen 9050 continuous flow- or Protein Technologies, Inc., PS-3 batch-style synthesizers. Manual syntheses of substance P analogues were carried out batchwise in a vessel fitted with a glass frit for the introduction and removal of reagents and solvents. All figures in Tables I–III are the average of two determinations. In order to provide a wider spread of reactivity, the conditions adopted for comparison in Table III are more dilute than would normally be chosen for actual peptide syntheses, which more nearly approximate those of Tables I and II.

General Protocol for Test Coupling of FMOC Amino Acid Chlorides with a Leucine-Loaded Polyamide Resin. The FMOC-Leu-resin was a sample of Sheppard KA resin (0.1 mequiv/g) provided by Milligen Division, Millipore Corp. Test reactions were carried out manually in small syringes with mixing being accomplished by brief stirring by hand. The syringe was attached to a vacuum manifold to effect rapid removal of reagents and solvents. The FMOC-leucine resin was first washed with DMF, deblocked with piperidine, and then acylated according to a standard nine-step protocol [(1) DMF wash, 3 mL (1 min) × 2; (2) 20% piperidine wash, 2 mL (5 min) × 2; (3) DMF wash, 3 mL (1 min) × 3; (4) HOBt/DIEA wash, 2 mL (1 min) × 1; (5) acylation (3 min); (6) DMF wash, 3 mL (1 min) × 5; (7) CH_2Cl_2 wash, 3 mL (1 min) × 2; (8) MeOH wash, 3 mL (1 min) × 1; (9) ether wash, 3 mL (1 min) × 3]. In each case for 200 mg of FMOC-Leu-resin there was used 4 equiv of FMOC-AA-Cl and 4 equiv of either a tertiary amine or 4 equiv of a 1:1 mixture of HOBt/amine (0.16 M, 0.5 mL) in DMF. The acid chloride was dissolved in 0.3 mL of DMF to give a final concentration of 0.1 M. Results are recorded in Tables I and II. For a test of racemization, 200 mg of FMOC-Phe-Leu-resin obtained as described

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(28) The instrument (PS-3) was made available by Protein Technologies, Inc., Tucson, AZ.
(29) (a) The resins were made available by National Starch and Chemical Corporation, Plainfield, NJ. (b) **Note Added in Proof.** In a companion paper that has recently appeared, it is shown that FMOC amino acid *fluorides*, which have acylating properties similar to the chlorides, differ from the latter in being available (and shelf-stable) with *tert*-butyl-based side-chain protecting groups and in not undergoing conversion to oxazolones in the presence of tertiary organic bases. See: Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 965.
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was suspended in 5 mL of dry MeOH containing a few drops of concd. H_2SO_4. After being refluxed for 4 h, the mixture was allowed to cool to room temperature and filtered, and the solvent was removed in vacuo. The residue was dissolved in 10 mL of EtOAc and washed with 10% $NaHCO_3$ (3 × 10 mL) and then saturated NaCl solution. Removal of solvent gave the FMOC-Phe-Leu-OMe as a white solid in 85–90% yield along with a small amount of an unidentified impurity (TLC). A sample (2 mg) of the solid was dissolved in 5 mL of EtOAc and 5 μL of this solution was injected onto a Waters Radialpak 10-μm silica gel column (0.8 × 10 cm) with elution by 99.2/0.8% hexane/*i*-PrOH, f = 2, t_R(LL) = 17.5 ± 1 min/[8] and t_R(DL) = 22.0 ± 1 min.[8] Couplings of FMOC-Phe-Cl effected by HOBt in the presence of either NEt_3, NMM, or DIEA showed less than 0.1% of the DL isomer.

Racemization Test for Coupling of Resin-Bound Phenylalanylglycine with FMOC-Phe-Cl. The resin (H-Phe-Gly-OCH_2CO-MBHA[20]) was prepared by coupling of bromoacetic acid (1 g) in 5 mL of CH_2Cl_2 with DCC (0.6 g) to 1 g of deprotonated MBHA resin (0.56 mequiv/g; Peninsula Laboratories, Inc., 1% DVB) for 1 h (NKT). Several washes with CH_2Cl_2 were followed by reaction with 3 equiv of BOC-Gly-OCs in 6 mL of DMF for 15 h at 60 °C.[34] After extensive washing of the resin (DMF, DMF/H_2O, DMF, CH_2Cl_2), the BOC group was deblocked (4 N HCl in dioxane, 30 min) and the resin washed with CH_2Cl_2, 10% TEA/CH_2Cl_2, and CH_2Cl_2. The resulting resin was treated with FMOC-Phe-OH (1.5 mmol) and DCC (0.75 mmol) in 5 mL of CH_2Cl_2 for 1 h to give a resin, which analyzed (UV method)[35] for 0.5 mequiv/g of dipeptide. For racemization studies 100 mg of the resin was deblocked by 20% piperidine/DMF, washed, and coupled with FMOC-Phe-Cl under varying conditions. As an example, 1 mL of a 0.375 M solution of FMOC-Phe-Cl in CH_2Cl_2 was added to 100 mg of the deblocked resin (0.5 meq/g) in 1.0 mL of CH_2Cl_2 containing a 1:1 mixture of pivalic acid and TEA (0.375 mmol). After 10 min the resin was washed with DMF, FMOC-deblocked (10 min, 20% piperidine/DMF), washed with DMF, and detached from the polymer (0.1 M NaOH in isopropyl alcohol), and the product was examined by HPLC on a 5-μm Hypersil ODS column (100 × 0.21 mm) using a mobile phase consisting of 17% CH_3CN and 83% 0.1 M NaH_2PO_4–0.15 M $NaClO_4$, pH 2.2, detection at 215 nm, flow rate 1 mL/min: t_R(L,L) 1.2 min, t_R(DL) 4.1 min. Less than 0.1% of the DL form of H-Phe-Phe-Gly-OH was observed.

FMOC-Tyr(*t*-Bu)-Cl. To a solution of 1 g of FMOC-Tyr-(*t*-Bu)-OH in 15 mL of THF at 0 °C was added 1.5 equiv of $SOCl_2$ and 0.2 equiv of tetramethylurea. The resulting solution was stirred at 0 °C for 40 min, and solvent was removed in vacuo with the aid of vacuum pump to give a solid. Recrystallization from CH_2Cl_2–hexane gave 670 mg (66.7%) of the still impure acid chloride as a white solid, mp 78–82 °C: HPLC f = 2, solvent MeOH/H_2O 0.1% TFA 75:25, FMOC-Tyr-Cl (as methyl ester) t_R = 5.33 (0.53%), FMOC-Tyr(*t*-Bu)-OH t_R = 11.40 (16%), FMOC-Tyr(*t*-Bu)-Cl (as methyl ester (t_R = 14.53 (80%); IR (KBr) 3320 (NH), 1780 (COCl), 1685 (OCON) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (s, 9, CMe_3), 3.2 (d, $CH_2C_6H_4$), 4.2 (t, 1, $CHCH_2O$), 4.4 (m, 2, $CHCH_2O$), 4.85 (m, 1, NCHCO), 5.2 (d, 1, NH), 6.8-7.8 (m, 12, aryl). In view of the presence of significant amounts of the free acid, for elemental analysis a sample (478 mg) was added to 10 mL of 5% pyridine in MeOH. After 10 min the solution was evaporated and the residue chromatographed on silica gel with elution by 25% EtOAc in Skelly B to give 354 mg (75%) of the **methyl ester** as a white solid, mp 96–97 °C (Et_2O–hexane).

Anal. Calcd for $C_{29}H_{31}NO_5$: C, 73.55; H, 6.60; N, 2.96. Found: C, 73.81; H, 6.58; N, 2.94.

Leucine Enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH, 9). From Acid Chlorides via a Continuous Flow Synthesizer (Milligen 9050). The synthesis was carried out on 1 g of Pepsyn KA resin loaded with 0.1 mequiv/g of FMOC-leucine using FMOC-Phe-Cl, 203 mg; FMOC-Gly-Cl, 158 mg (twice); FMOC-Tyr(*t*-Bu)-Cl, 300 mg; and 1.52 mL of the binary mixture DIEA/HOBt in DMF solution (0.33 M). The acylation time was 10 min. After completion of the synthesis the resin was treated with TFA (20 mL, 2 h), to give 46 mg of the crude peptide TFA salt, approximately 93% pure (HPLC), GC analysis[22] on a chiral column 10.67 (Gly), 12.53 (D-Leu, 1.4%), 13.55 (Leu), 23.10 (Phe) 28.13 (Tyr); analogous hydrolysis and GC analysis of the commercially available sample of the leucine resin used showed 1.4% D-Leu; amino acid analysis, Gly 1.9 (2), Phe 0.8 (1), Tyr 0.8 (1), Leu 0.9 (1); MS/FAB $(M + H)^+$ 556, calcd 555 (M). A second synthesis was carried out in the same way except that near-racemic phenylalanine was used and tyrosine was added as Pfp ester rather than acid chloride. After treatment with TFA (20 mL, 2 h) there was obtained 50 mg of the crude peptide TFA salt. The L-Phe and D-Phe diastereomers were present in the ratio 45/53 (HPLC); f = 1, (MeOH/H_2O 0.1% TFA, 50–50), t_R(L) 10.26, t_R(D) 17.90.

Lys[1]-Gly[5,6]-Nle[11]-substance P (11). (A) Synthesis in Methylene Dichloride. A norleucine resin was prepared by coupling of BOC-Nle-OH to an MBHA resin (Fluka, 1–1.2 mequiv/g, 1% DVB) with DCC/HOBt (3 equiv) in DMF. Deblocking by 4 N HCl in dioxane for 30 min and neutralization with 10% TEA in $CHCl_3$ followed by washing with CH_2Cl_2 gave the loaded resin. Assembly was initiated with 500 mg of resin, coupling being carried out with 5 mL of CH_2Cl_2 containing 4 mmol each of HOBt and TEA. The FMOC amino acid chloride was dissolved in 5 mL of CH_2Cl_2, which was then added to the resin, the resulting concentration of the acid chloride being 0.2 M at the start of the reaction. Completeness of coupling was checked by the ninhydrin test: Leu (5 min), Gly (10 min), Phe (30 min), Phe (after 35 min slight blue, therefore acetylation carried out with 1 mL of Ac_2O and 2 mL of pyridine in 7 mL of CH_2Cl_2 for 10 min), Gly (5 min), Gly (3 min), Pro (15 min), Lys (Z) (10 min), Pro (10 min), Lys (Z) (45 min). After incorporation of the last amino acid, the FMOC group was removed and the resin washed with DMF, CH_2Cl_2, HOAc, MeOH, and CH_2Cl_2. After drying, the resin was treated with 10 mL of HF containing 0.5 mL of Me_2S and 0.5 mL of anisole for 1 h at 0 °C, yield 300 mg. Amino acid analysis: Lys 1.9 (2), Pro 1.7 (2), Gly 3.4 (3), Leu 1.0 (1), Phe 2.1 (2), Nle 1.3 (1).

(B) Synthesis in Dimethylformamide. In this case the 1:1 mixture of HOBt/DIEA was used to obtain the D-Phe[7] analogue. DMF was used exclusively for all reactions and washes. The synthesis was initiated with 100 mg of the Nle-resin with HOBt (0.3 mmol) and DIEA (0.3 mmol) dissolved in 0.5 mL of DMF added to the resin followed by 0.3 mmol of the FMOC amino acid chloride dissolved in 0.5 mL of DMF. Couplings were carried out for 10 min except in the case of Gly (3 × 10 min), Lys(Z) (2 × 10 min), and Pro (2 × 10 min). The synthesis was completed as above, yield 165 mg. Amino acid analysis: Pro 1.9 (2), Gly 3.0 (3), Lys 1.8 (2), Ph 1.8 (2), Nle 1.0 (1), Leu 0.8 (1). A third synthesis in DMF of the normal compound gave 200 mg of the peptide. For the peptides prepared in DMF MS/FAB data confirmed the structures. In both cases the M + 1 peak was found at 1159.9 (calcd M 1158.7). By HPLC analysis on a 5-μm C-18 Nucleosil column (25 × 0.4 cm), detection at 220 nm, flow rate 0.7 mL/min with eluent 35% CH_3CN/65% 0.01 M NaH_2PO_4–0.15 M $NaClO_4$, no contamination due to racemization at the Phe[7] residue could be detected (<0.1%), t_R(all L) 17.9 min, t_R (D-Phe[7] analogue) 22.6 min. For the HPLC trace of the D-Phe[7] isomer, see Figure 5.

Acyl Carrier Protein Decapeptide (ACP 65–74, H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH, 12). The synthesis was carried out manually as previously described using 1 g of FMOC-Gly-Pepsyn KA resin bearing 0.1 mequiv/g of glycine with 4 quiv of the acylating agent (acid chlorides in all cases except Asn and Gln, where the corresponding *p*-nitrophenyl esters were used and Tyr and Asp where the Pfp esters were used. In each case 5 mL of 0.08 M HOBt/DIEA was used for the coupling reaction (15-min couplings except for Asn (30 min)), Ala (2 × 15 min), Gln (60 min), and Val (2 × 45 min)). The Kaiser test was still positive for the final coupling even after double treatment. There was obtained 94.4 mg of the peptide as the TFA salt: amino acid analysis, Asx 2.0 (2), Glx 1.0 (1), Gly 1.0 (1), Ala 2.0 (2), Tyr 1.0 (1), Val 0.8 (1), Ile 2.1 (2); MS/FAB $(M + Na^+)$ 1085, $(M + K^+)$ 1101, calcd 1062 (M). For the HPLC trace of the crude product, see Figure 4a.

Prothrombin (1–9, H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-OH, 13). The synthesis was carried out manually as previously described, using 1.5 g of FMOC-Val-Pepsyn KA resin bearing 0.14 mequiv/g of valine with 4 equiv of the penta-

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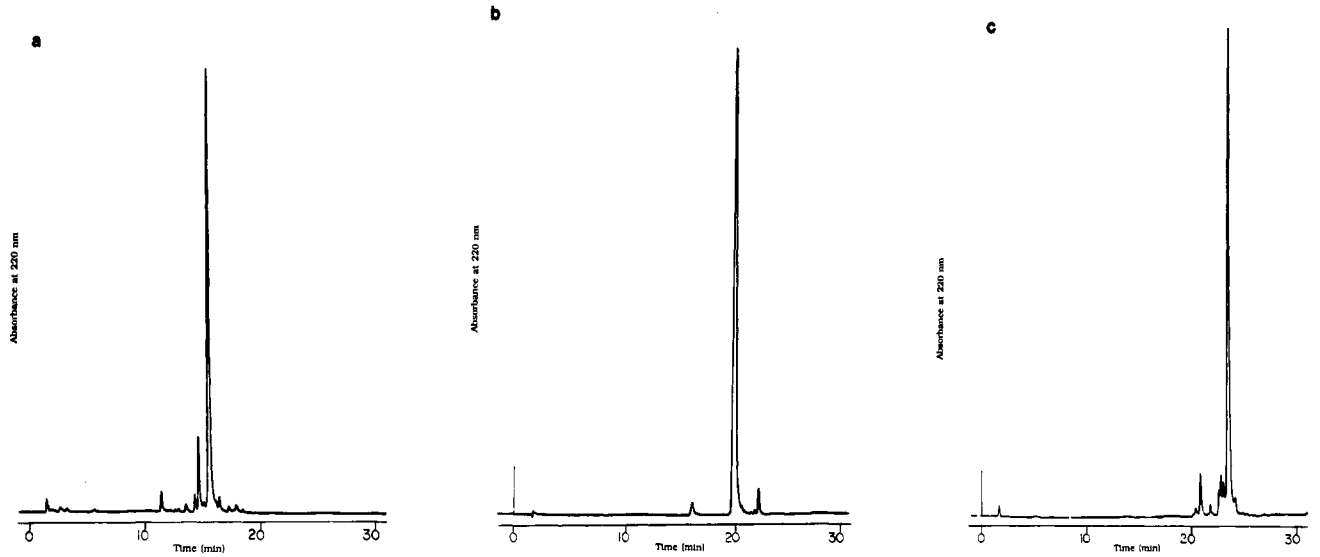

Figure 4. HPLC traces of crude peptides obtained via acid chlorides or pentafluorophenyl esters. Waters Deltapak C-18, 0.30 × 15 cm, 10 μm, f = 1 mL/min, 220 nm, eluent A 0.1% (v/v) TFA in H_2O, B 0.08% (v/v) TFA in CH_3CN containing 5% A, gradient 5–26% B in 30 min, linear. (a) ACP decapeptide (65–74); (b) prothrombin (1–9; (c) eledoisin.

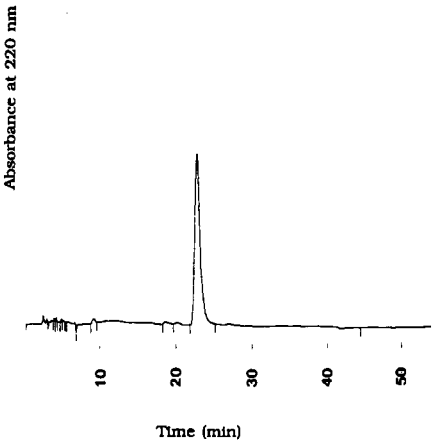

Figure 5. HPLC trace of crude substance P D-Phe[7] analogue 11 obtained via acid chlorides. Nucleosil C-18, 4 × 250 mm, 5 μm, f = 0.7 mL/min, 220 nm, 65% 0.01 M NaH_2PO_4, 0.15 M $NaClO_4$, pH 2.2–35% CH_3CN.

fluorophenyl ester. In each case 6 mL of 0.1 M HOBt/DIEA in DMF was used except for FMOC-Asn-OPfp, where HOBt alone was used. Coupling times were 15 min except for Asn (30 min). There was obtained 120 mg of the peptide as the di-TFA salt: amino acid analysis, Asx 0.9 (1); Gln 2.0 (2), Gly 1.1 (1), Ala 1.0 (1), Val 1.1 (1), Leu 0.9 (1), Phe 1.1 (1), Lys 0.9 (1). A second run with 1 g of resin using acid chlorides (Leu, Phe, Gly, Ala), pentafluorophenyl esters (Glu, Lys), and p-nitrophenyl esters (Asn) was carried out with the binary mixture HOBt/DIEA (10 min couplings in all cases except FMOC-Asn-ONp where two 30-min couplings were needed). There was obtained 74 mg of the di-TFA salt: amino acid analysis, Asx 0.9 (1), Glu 2.0 (2), Gly 1.1 (1), Ala 1.0 (1), Val 1.1 (1), Leu 1.0 (1), Phe 1.1 (1), Lys 0.8 (1); MS/FAB (M + H^+) 1006, calcd 1005 (M). For the HPLC trace of the crude product, see Figure 4b.

Eledoisin (pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2, 14). In this case the synthesis was completed automatically on a batch synthesizer,[28] using 100 mg of Polyhipe P-1000 resin[29] bearing a Rink linker[36] (0.61 mequiv/g). Acid chlorides were used as coupling agents in all cases except for Ser, Lys, and Asp, where the dry mixture of acid plus BOP reagent was used, and pyroglutamic acid, where the pentafluorophenyl ester was used. Coupling times were 30 min. The yield of crude eledoisin as TFA salt was 45 mg, the peptide being identified by HPLC co-elution with an authentic sample provided by Bachem, Inc.: MS/FAB (M + H^+) 1188, calcd 1187 (M). For the HPLC trace of the crude product, see Figure 4c.

Extent of Coupling in the Presence of Selected Tertiary Amines and Mixtures with *N*-Hydroxy Compounds. For these tests 200 mg of the appropriate FMOC-AA-Pepsyn KA resin (0.1 mequiv/g) was used in the presence of 4 equiv of the acylating agent and 0.5 mL of 0.16 M tertiary amine or binary salt. The acylating agent was dissolved in 0.3 mL of DMF so that the final concentration was 0.1 M. After the desired time, the resin was washed as follows: DMF (3 × 10 mL); MeOH (3 × 10 mL); HOAc (1 × 10 mL); Et_2O (5 × 10 mL); dried in vacuo overnight and subjected to UV analysis. For the results, see Tables I and II.

Infrared Studies of Active Species Formed from Standard Coupling Agents under the Conditions of Peptide Coupling. A solution of 71.6 mg of FMOC-Val-Cl in 2 mL of methylene dichloride was treated with 258 mg (0.358 mL) of DIEA, and the solution was examined immediately on an FT-IR instrument. The strong acid chloride peak at 1790 cm^{-1} was completely replaced by peaks at 1835 and 1685 cm^{-1} due to the corresponding oxazolone. The oxazolone was available as a pure crystalline compound (see below) and a solution of this compound in methylene dichloride gave a spectrum identical with that observed in the reaction of the acid chloride and DIEA. The same result was observed when DIEA was replaced by NMM. Other bases were examined similarly.

Esterification of Polyhipe Resins. A 100-mg sample of Polyhipe P-1000 resin (0.76 meq/g, HMPA linker)[29] in a small test tube was treated with 1 mL of DMF. After preswelling for 10 min, 1 mL of a DMF solution containing 283 mg (10 equiv, 0.76 mmol) of FMOC-Leu-Cl, 116.4 mg (10 equiv, 0.76 mmol) of HOBt·H_2O, and 167.12 μL (20 equiv, 1.52 mmol) of NMM was added, and the resulting mixture was allowed to stand at room temperature for 2 h. The resin was washed with DMF (3 × 30 mL), MeOH (3 × 30 mL), and ether (3 × 30 mL), dried in vacuo overnight, and subjected to UV analysis. Two separate runs showed substitution levels of 0.47 and 0.44 mequiv/g. The degree of substitution was determined on a weighed sample of resin (2–3 mg), which was treated with 20% piperidine in DMF for 15 min, and the solution was subjected to UV analysis using the fluorene chromophore of the dibenzofulvene–piperidine adduct at 301 nm (ϵ = 7800).

2-[(9-Fluorenylmethyl)oxy]-4-isopropyl-5(4*H*)-oxazolone. A solution of 359 mg of FMOC-Val-Cl in 10 mL of CH_2Cl_2 was stirred at room temperature with 10 mL of a saturated $NaHCO_3$ solution for 15 min. Removal of solvent and recrystallization from Skelly B (cooling in freezer) gave 200 mg (62.3%) of the oxazolone as a white solid, mp 92 °C, α^{23}_D −25.6° (c 0.5, CH_2Cl_2), lit.[8] mp 82–84 °C (polymorphic modification?), lit.[8] α^{23}_D −29.6° (c 0.5, CH_2Cl_2).

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Registry No. 1, 91613-96-6; 2, 103321-53-5; 3, 124007-51-8; 4, 132438-33-6; 5, 132409-91-7; 8, 82361-40-8; 9, 58822-25-6; 10, 132409-92-8; 11, 132409-93-9; 12, 66851-75-0; 13, 106083-79-8; 14, 69-25-0; HOBt, 2592-95-2; Fmoc-Phe-Cl, 103321-57-9; BOC-Gly-OCs, 42538-64-7; Fmoc-Phe-OH, 35661-40-6; $BrCH_2COOH$, 79-08-3; Fmoc-Tyr(*t*-Bu)-OH, 71989-38-3; Fmoc-Tyr(*t*-Bu)-OMe, 132409-94-0; Fmoc-Gly-Cl, 103321-49-9; BOC-Nle-OH, 6404-28-0; Fmoc-Leu-Cl, 103321-59-1; H-Leu-OMe, 2666-93-5.

Regiospecific and Highly Stereoselective Electrophilic Addition to Furanoid Glycals: Synthesis of Phosphonate Nucleotide Analogues with Potent Activity against HIV

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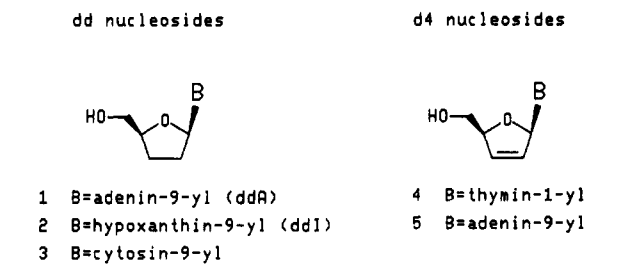
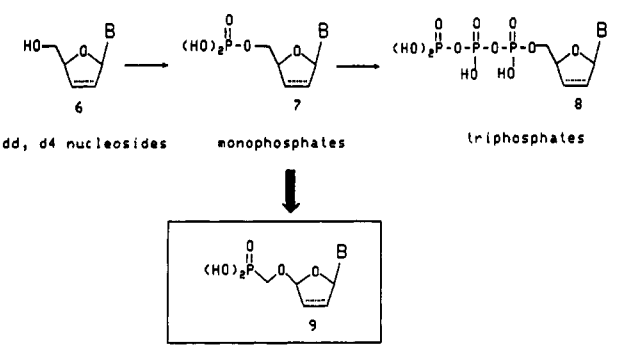
Regiospecific and highly stereoselective electrophilic addition to furanoid glycals has been used as a key step in the synthesis of phosphonate isosteres of nucleoside monophosphates. Using this methodology, phosphonate analogues of 1 (ddA), 4 (d4T), and 5 (d4A) monophosphates have been prepared. Present studies have also led to the development of a scheme for the synthesis of the phosphonate isostere of adenosine monophosphate. Despite the acetal structure, phosphonate derivatives 27 and 28 were substantially more acid stable than the corresponding nucleosides 1 and 5 with respect to glycosidic bond cleavage. The phosphonates 22 and 27 exhibited a potent antiretroviral activity comparable to that of 4 (d4T).

Most therapeutically useful antiviral agents selectively inhibit target enzymes produced in virus-infected cells. This selective inhibition of viral enzymes is the most important factor for the present wave of rapid development of novel nucleoside analogues as antiviral agents. Since the discovery of human immunodeficiency virus (HIV) as the causative agent of acquired immunodeficiency syndrome (AIDS),[1] there has been intense effort to find compounds that can selectively block the replication of HIV. Because 3′-azido-3′-deoxythymidine (AZT), which is currently the only licensed drug for the treatment of AIDS patients,[2] gives only limited prolongation of survival and suffers from some adverse toxicological effects,[3] there is an urgent need for other compounds that are at least as efficacious but less toxic in humans. In this regard two series of nucleoside analogues have emerged as a new class of potent and selective HIV inhibitors. They are characterized as 2′,3′-dideoxy (dd)[4] and 2′,3′-didehydro-2′,3′-dideoxy (d4)[5] nucleosides (Chart I). Several of these com-

Chart I



Scheme I



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