Compounds 3c,e-k were prepared as described previously[5] by procedures B, B, A, B, B, B, A, and A, respectively.

***N*-[*p*-(Dimethylamino)benzoyl]-*P*,*P*,*P*-triphenylphospha-λ⁵-azene (3a).** Procedure B was used. From 0.27 g of *p*-(dimethylamino)benzoyl azide (1.4 mmol) and 0.38 g (1.4 mmol) of triphenylphosphine in 10 mL of dry THF was obtained, after recrystallization from chloroform-hexane, 0.20 g (34%) of product; mp 135-137 °C. IR (KBr): $\bar{\nu}$ (cm^{-1}) 3051, 2889 (C-H), 1601, 1582 (C=O), 1331 (P=N). ^1H NMR ($CDCl_3$): δ 3.01 (s, 6 H, NCH_3), 6.69 (d, 2 H, Ar H, J = 8.8 Hz), 7.4-7.7 (m, 9 H, Ar H), 7.8-8.0 (m, 6 H, Ar H), 8.25 (d, 2 H, Ar H, J = 8.8 Hz). Anal. Calcd for $C_{27}H_{25}N_2OP$: C, 76.40; H, 5.94; N, 6.60. Found: C, 76.37; H, 5.96; N, 6.47.

***N*-(*p*-Butoxybenzoyl)-*P*,*P*,*P*-triphenylphospha-λ⁵-azene (3b).** Procedure B was used. From 1.10 g (5.1 mmol) of *p*-butoxybenzoyl azide and 1.31 g (5.0 mmol) of triphenylphosphine in 15 mL of dry THF was obtained 2.15 g (95%) of **3b**, mp 152-155 °C. After recrystallization from benzene-petroleum ether, 1.90 g (84%) of **3b** was obtained, mp 154-156 °C. IR (KBr): $\bar{\nu}$ (cm^{-1}) 3060, 2960, 2920 (C-H), 1592 (C=O), 1328 (P=N). ^1H NMR ($CDCl_3$): δ 0.96 (t, 3 H, CH_3, J = 6 Hz), 1.43 (m, 2 H, CH_2), 1.73 (m, 2 H, CH_2), 3.98 (t, 2 H, OCH_2, J = 5 Hz), 6.91 (d, 2 H, Ar H, J = 8 Hz), 7.46 (m, 9 H, Ar H), 7.85 (m, 6 H, Ar H), 8.32 (d, 2 H, Ar H, J = 8 Hz). Anal. Calcd for $C_{29}H_{28}NO_2P$: C, 76.80; H, 6.22; N, 3.09. Found: C, 77.06; H, 6.42; N, 3.00.

***N*-(*p*-Ethylbenzoyl)-*P*,*P*,*P*-triphenylphospha-λ⁵-azene (3d).** Procedure B was used. From 1.75 g (10.2 mmol) of *p*-ethylbenzoyl azide and 2.62 g (10.0 mmol) of triphenylphosphine in 25 mL of dry THF was obtained 3.88 g (95%) of **3d**, mp 133-136 °C. After recrystallization from methanol, 3.34 g (82%) of **3d** was obtained, mp 135-137 °C. IR (KBr): $\bar{\nu}$ (cm^{-1}) 3060, 2962 (C-H), 1592 (C=O), 1550 (Ar), 1325 (P=N). ^1H NMR ($CDCl_3$): δ 1.21 (t, 3 H, CH_3, J = 7.6 Hz), 2.64 (q, 2 H, CH_2, J = 7.6 Hz), 7.19 (d, 2 H, Ar H, J = 8.0 Hz), 7.42 (m, 9 H, Ar H), 7.80 (m, 6 H, Ar H), 8.29 (d, 2 H, Ar H, J = 8.1 Hz). Anal. Calcd for $C_{27}H_{24}NOP$: C, 79.20; H, 5.91; N, 3.42. Found: C, 79.44; H, 6.06; N, 3.32.

^{15}N-labeled 3 was prepared as above by using either ^{15}N-labeled benzamides[25] (procedure A) or ^{15}N-labeled aroyl azides (procedure B).[26] The yields and melting points of the labeled compounds are as follows: **3c**, R = OCH_3, 63%, 158-160 °C; **3e**, R = CH_3, 73%, 153-155 °C; **3f**, R = H, 36%, 190-195 °C; **3g**, R = F, 52%, 178-180 °C; **3h**, R = Cl, 53%, 146-150 °C; **3i**, R = Br, 61%, 145-146 °C; **3j**, R = CN, 62%, 178-179 °C; **3k**, R = NO_2, 74%, 201-205 °C.

Acknowledgment. We thank the Robert A. Welch Foundation of Houston, TX (Grant Y-0684), for financial support and Dr. Barbara T. Ziemnicka for the preparation of compounds **3b** and **3d**.

(25) Ott, D. G. *Syntheses with Stable Isotopes*; Wiley: New York, 1981; p 83.

(26) The labeled aroyl azides were made by standard methods from the aroyl chloride and ^{15}N-labeled sodium azide in THF/H_2O (2:1).[24]

Rapid Continuous Peptide Synthesis via FMOC Amino Acid Chloride Coupling and 4-(Aminomethyl)piperidine Deblocking

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The recently described FMOC/4-AMP technique for rapid continuous solution synthesis of peptides involving coupling by crystalline FMOC amino acid chlorides and deblocking via 4-(aminomethyl)piperidine (4-AMP) has been extended to the synthesis of a varied number of tachykinin peptides. Previously only the simple pentapeptide leucine enkephalin had been synthesized by this procedure. Continuous syntheses of peptides up to the heptapeptide stage have been executed in a single repetitive operation. Removal of excess acid chloride during the coupling step was simplified by use of 4-AMP for this purpose as well as for the deblocking step. Use of this modified technique gave purer crude products and higher yields. A method of avoiding complications due to the interfering separation of solid material during the washings with phosphate buffer to remove byproducts was developed based on the use of chloroform. Crude products obtained by the modified procedure were often of greater than 95% purity. Segments bearing proline at the C-terminal position required special attention due to diketopiperazine formation at the dipeptide stage. Such diketopiperazines were avoided with both proline amides and proline *tert*-butyl esters. Using the latter technique Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OH, **9**, was synthesized and coupled with H-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH_2, **6**, obtained via benzyl esters, to give the undecapeptide Substance P analogue **10**, which was also synthesized by solid-phase methods for comparison. MS/FAB and NMR data are presented for selected peptides synthesized by these new rapid procedures.

Recently we described in preliminary form a new technique for the rapid, multigram synthesis of short peptide segments.[1,2] Both coupling and deblocking steps were novel, the former being based on a new class of stable, highly reactive coupling agents, FMOC amino acid chlorides, and the latter involving reaction with 4-(aminomethyl)piperidine (4-AMP), a difunctional amine chosen to facilitate efficient removal of byproducts. The previous paper emphasized the preparation and characterization of the FMOC amino acid chlorides; here we provide experimental details along with a description of modifications of the basic technique leading to greater reliability and convenience. According to the original protocol (Table I, protocol A) excess acid chloride is scavenged following the initial rapid acylation reaction in a chloroform-water

(1) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S.-Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.

(2) For a discussion of other rapid, sequential solution syntheses, see: (a) Kisfaludy, L. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 2, p 417. (b) Nozaki, S.; Muramatsu, I. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2165. (c) Dölling, R.; Kaufmann, K.-D. *J. Prakt. Chem.* **1984**, *326*, 171.

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Table I. Continuous Peptide Assembly via the FMOC/4-AMP Technique

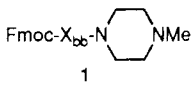
protocol A	protocol B
1 mmol of H-X_{aa}-OBn in 10 mL of $CHCl_3$/10 mL of 5% Na_2CO_3 plus 1.1 mmol of FMOC-X_{bb}-Cl in 10 mL of $CHCl_3$, 1–10 min	1 mmol of H-X_{aa}-OBn in 10 mL of $CHCl_3$/10 mL of 5% Na_2CO_3 plus 1.2–1.5 mmol of FMOC-X_{bb}-Cl in 10 mL of $CHCl_3$, 10 min
0.5 mL of NMP, 2 min	10 mL of 4-AMP, 30 min
extraction with 5% HCl	dilution with 80 mL of $CHCl_3$, extraction with phosphate buffer, pH 5.5 (5×), or as in protocol A with NaCl (2×) followed by buffer (2×)
3 mL of 4-AMP, 10–30 min	volume reduction to 10 mL
extraction with saturated NaCl (2×), followed by phosphate buffer, pH 5.5 (2×)	start of the next cycle
volume adjustment as necessary	
start of the next cycle	

Table II. Peptides and Protected Segments Synthesized by the FMOC/4-AMP Technique[a]

3	H-Phe-Phe-Gly-Leu-Nle-NH_2
4	H-Lys(Z)-Phe-Phe-Gly-Leu-Met-NH_2
5	H-Phe-Phe-Val-Gly-Leu-Met-NH_2
6	H-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH_2
7	BOC-Gln-Glu(OBn)-Phe-Phe-Gly-Leu-Nle-OBn[b]
8	H-Lys-Pro-Lys-Pro-$NHC_{12}H_{25}$-*n*
9	Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OH[b]
10	H-Arg-Pro-Lys-Pro-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH_2[b]

[a] Also obtained under the normal reaction conditions inadvertantly or deliberately were the following diketopiperazines: cyclo-[Lys(Z)-Pro] (11), cyclo(Leu-Nle) (12), and cyclo(Leu-Met) (13). [b] Obtained via amino acid or segment coupling at the bond indicated using pentafluorophenyl esters (7, 9) or the mixed anhydride method (10). All other peptides were synthesized in one continuous sequence via appropriate acid chlorides. See Experimental Section.

mixture by the addition of *N*-methylpiperazine (NMP). This results in the formation of piperazide 1, which can



1

Fmoc-Tyr(OBn)-Gly-Gly-Phe-Leu-OBn

2

be removed from the organic phase by extraction with aqueous hydrochloric acid. Subsequently 4-AMP is added to the organic phase causing rapid deblocking, which is accompanied by adduct formation between excess 4-AMP and the liberated dibenzofulvene (DBF). Phosphate buffer of pH 5.5 is then used to extract the DBF/4-AMP adduct and thus separate it from the less basic growing peptide ester, which remains in the organic phase and can immediately be extended by means of the next FMOC amino acid chloride.

This strategy allowed the rapid, large-scale synthesis of the relatively simple protected pentapeptide ester **2** within 3–4 h. In our further investigations we have considerably simplified the technique and extended it to other, more complex peptides. This paper explores and expands on some of the basic chemistry involved in this novel technique and demonstrates its applicability in the rapid preparation of C- and N-terminal Substance P and Neurokinin B sequences[3] (Table II).

Successful application of the FMOC/4-AMP technique requires adequate solubility of the growing peptide in a water-immiscible organic solvent. Since Substance P and other tachykinins bear a C-terminal amide function which greatly increases the hydrophilic character of the peptide, it is necessary to build the chain in the form of a more hydrophobic intermediate. The corresponding benzyl esters were chosen for this purpose with the final step involving ammonolysis to the desired peptide amide.

Table III. Relative Rate of Deblocking of FMOC-$NHC_6H_4NO_2$-*p* by Selected Amines[a]

amine	k_{rel}
4-AMP	234
NMP	19
DMAE[b]	1

[a] To a solution of 5 mL of 0.06 M urethane in $CHCl_3$ was added 0.5 mL of the appropriate amine. After 30 min the UV spectra were recorded and the absorption intensities at 400 nm were measured. [b] β-(*N*,*N*-Dimethylamino)ethanol.

Results and Discussion

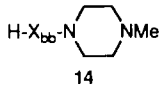
As a model with which to optimize the synthetic cycle the simple C-terminal pentapeptide **3** of Nle[11]-Substance P, available via an earlier conventional synthesis,[4] was chosen. In contrast to the high overall yield of the previously described synthesis of leucine enkephalin, use of protocol A (Table I) gave the new sequence in only 20–30% yield, albeit in high purity. Losses may have been associated with premature deblocking, inefficient separation of phases due to unpredictable precipitation of solids of unknown composition during some of the extractive procedures used for purification purposes, etc. Each step in the process was therefore examined in order to locate any correctable problems. Loss of peptide from the organic phase following FMOC deblocking via extraction into the phosphate buffer was excluded. For example according to UV analysis norleucine benzyl ester was not perceptibly removed from chloroform by five extractions with the buffer of pH 5.5. Even with a buffer of pH 4.7 the loss from the organic phase amounted to less than 1% per wash step.

In addition to possible extractive losses, partial hydrolysis of the benzyl ester catalyzed by 4-AMP or ammonolysis by 4-AMP was also excluded. On the other hand it was shown that treatment with NMP in order to scavenge excess acid chloride induced at the same time partial deblocking of the FMOC group to an extent which depended on the concentration of NMP used and the exact reaction conditions. Under the conditions specified in Table I(A), premature deblocking amounted to 5–6% within 5 min. Although this process leads to no impurities which accumulate during the synthesis, all peptide deblocked at this stage will be lost via the subsequent hydrochloric acid extraction step. These results were confirmed for various acid chloride scavenging amines by determination of relative cleavage rates using FMOC *p*-nitroaniline (FMOC-pNA) as model urethane (Table III). At the concentrations taken the rate for NMP was about one-tenth that for 4-AMP. Although the extent of unwanted deblocking could be reduced by lowering the NMP concentration, it could not be completely eliminated with this reagent. In addition the method was not completely general since piperazides 1 derived from hydrophobic amino acids, e.g., *O*-benzyltyrosine, are only inefficiently extracted from organic solvents by means of 5% hydrochloric

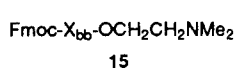
(3) (a) Kimura, S.; Ogawa, T.; Goto, K.; Sugita, Y.; Munekata, E.; Kanazawa, I. In *Substance P, Metabolism and Biological Actions*; Jordan, C. C., Oehme, P., Eds.; Taylor & Francis: London, 1985; p 33. (b) Sandberg, B. E. B. In *Substance P, Metabolism and Biological Actions*; Jordan, C. C., Oehme, P., Eds.; Taylor & Francis: London, 1985; p 65.

(4) (a) Bienert, M.; Niedrich, H.; Teplán, I.; Medzihradsky, K. *J. Lab. Cpds. Radiopharm.* **1978**, *14*, 577. (b) Bienert, M.; Klauschenz, E.; Ehrlich, A.; Katzwinkel, S.; Niedrich, H.; Tôth, G.; Teplán, I. *J. Lab. Cpds. Radiopharm.* **1979**, *16*, 673.

acid. This problem is self-correcting, however, since subsequent addition of 4-AMP to effect deblocking of the growing peptide chain also leads to deblocking of residual 1. The resulting deblocked piperazide 14 is sufficiently



14



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hydrophilic to be extracted readily by the phosphate buffer. An alternate amine considered as a possible scavenging agent for excess acid chloride was β-(*N*,*N*-dimethylamino)ethanol (DMAE). As a simple tertiary amine bearing an inductively electron-withdrawing substituent DMAE guarantees capture of acid chloride as the corresponding ester without significant FMOC deblocking taking place under the reaction conditions (Table III). Reaction of the hydroxyl function of DMAE with acid chlorides is rapid due to internal catalysis;[5] however, the ester **15** formed is partially hydrolyzed during its subsequent extraction with 5% hydrochloric acid, thus leading to retention of some free FMOC amino acid in the organic phase. The sensitivity of such esters to hydrolysis is well known.[6] The presence of free acid is irrelevant since it would be removed eventually by phosphate extraction as already noted for the *N*-methylpiperazide. Considering the implications of these results, the use of a separate acid chloride scavenging agent is clearly unnecessary since the standard deblocking amine (4-AMP) should be able to serve the same purpose. This was shown to be the case. An outline of the new protocol is diagrammed in Scheme I. Presumably the mixed piperidides **20a,b** are generated and washed out under these conditions.

With this new variant (protocol B, Table I), the yield of the intermediate for the synthesis of **3**, H-Phe-Phe-Gly-Leu-Nle-OBn, was raised from 20–30% to 80% with the purity of the crude product prior to chromatography being greater than 95%. Protocol B was therefore adopted for all subsequent chain extension processes.

A general problem always associated with the use of the FMOC amino-protecting group for solution peptide syntheses is the possible accumulation of the sensitive hydrocarbon DBF which is subject to unpredictable polymerization and consequent separation of insoluble polymer or gelation of the reaction mixture. Thus, if possible, quantitative conversion to the DBF adducts **21** with 4-AMP is desirable in order to allow for eventual complete removal of DBF from the organic phase during repetition of the synthetic cycle. Theoretically the amount of DBF remaining throughout a lengthy synthesis should not exceed the equilibrium value under the conditions chosen.[7] The build-up and removal of DBF during the deblocking process was investigated for a model urethane, 9-fluorenylmethyl *p*-nitrocarbanilate (FMOC-pNA). Following buffer extraction, ultraviolet examination at 258 nm[8] allowed determination of residual DBF after selected intervals. It was shown that with 5 mL of 4-AMP added to 10 mL of 0.05 M FMOC-pNA in chloroform, a period of 30 min was required to effect nearly quantitative extraction of DBF as its adduct with 4-AMP. In methylene dichloride[7b] or ethylene dichloride the process of deblocking/adduct formation proceeded distinctly faster (Table IV), but a disadvantage accompanying the use of these solvents, namely formation of a precipitate during phosphate extraction, interfered with clean-layer separation. It was not determined whether this precipitate consisted of adducts **21**, the corresponding phosphate salt, or some other unidentified material. The free base, prepared separately, was shown to be relatively insoluble in most solvents.[9] Eventually the precipitate disappeared

Table IV. Relative Rate of DBF/4-AMP Adduct Formation in Selected Solvents. Occurrence of Precipitates during Buffer Extraction[a]

solvent	k_{rel}	precipitate during washing
1. $CHCl_3$–EtOH	0.5	not tested
2. $CHCl_3$	1	–
3. $CHCl_3$–CH_2Cl_2 (1:1)	2	–
4. CH_2Cl_2	10	+
5. $ClCH_2CH_2Cl$	15	+

[a] For details see Experimental Section.

Table V. Formation of Diketopiperazines and Pyroglutamyl Peptides during FMOC Cleavage with 4-AMP[a]

peptide	cyclization product	time, min	yield, %
FMOC-Leu-X_{aa}-OBn	cyclo(Leu-X_{aa}) (**12**, **13**)	10	<5
(X_{aa} = Nle, Met)		30	10–20
FMOC-Lys(Z)-Pro-OBn	cyclo[Lys(Z)-Pro] (**11**)	1	80
		2	100
FMOC-Lys(Z)-Pro-O-*tBu*	–	30	0
FMOC-Glu(OBn)-Phe-Phe-Gly-Leu-Nle-OBn (**23**)	Pyr-Phe-Phe-Gly-Leu-Nle-OBn	5	20
		10	60
		30	>90

[a] Conditions involve 100 μL of a 0.1 M solution of the FMOC peptide in $CHCl_3$ with 50 μL of 4-AMP.

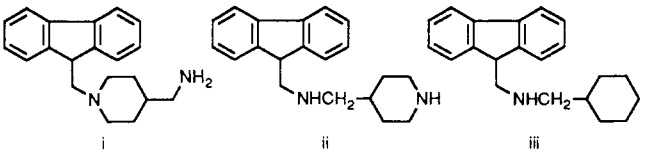
(5) Bogatkov, S. V.; Golovina, Z. P.; Flid, V. R.; Cherkasova, E. M. *J. Org. Chem. (USSR)* **1977**, 2116.

(6) (a) Barton, M. A.; Lemieux, R. U.; Savoie, J. Y. *J. Am. Chem. Soc.* **1973**, *95*, 4501. (b) Greben, A. E.; Martinov, V. F.; Tutov, M. I. *J. Gen. Chem. (USSR)* **1968**, *38*, 639.

(7) Compare: (a) Carpino, L. A.; Mansour, E. M. E.; Cheng, C. H.; Williams, J. R.; MacDonald, R.; Knapczyk, J.; Carman, M.; Lopusinski, A. *J. Org. Chem.* **1983**, *48*, 661. (b) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666.

(8) Neuenschwander, M.; Vögeli, R.; Fahrni, H.-P.; Lehmann, H.; Ruder, J.-P. *Helv. Chim. Acta* **1977**, *60*, 1073.

(9) Diagram **21** is used since the adduct may be a mixture of the primary, tertiary and secondary, secondary adducts i or ii with possibly



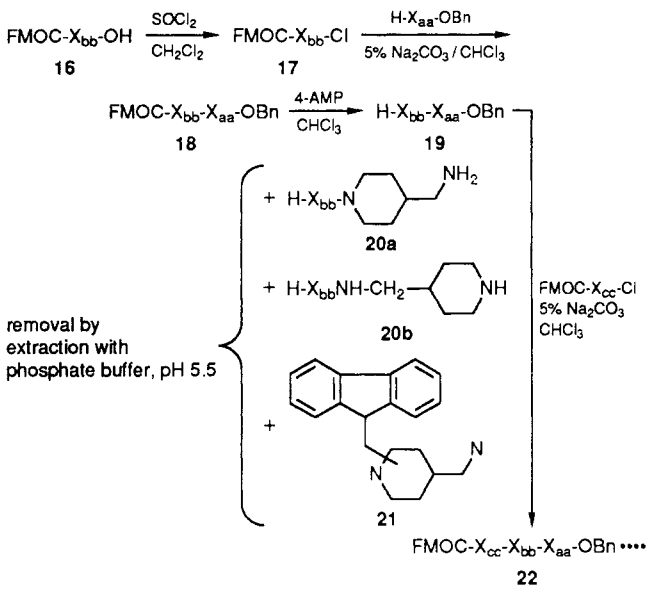
i ii iii

some of the bis-adduct present. Because of the ease of reaction of cyclic secondary amines with DBF derivatives,[10] we at first assumed conversion to structure i. More recently it has become clear that simple primary amines also readily yield adducts, especially when the amine is present at high concentration.[11] Thus, (aminomethyl)cyclohexane appears to give iii readily. However, as obtained, these adducts, especially those from 4-AMP, may separate with varying amounts of water of crystallization and are exceptionally insoluble even in solvents such as DMSO and thus difficult to analyze cleanly. We are indebted to Dr. Y.-Z. Lin for these preliminary observations. Full characterization of these materials is in progress. Regardless of the exact structure of the adducts, their ready acid solubility facilitates their exploitation for the present purposes. Simple NMR experiments reveal the speed of the deblocking–scavenging process. In one case a solution prepared from 21 mg of the FMOC derivative of *p*-chloroaniline (FMOC-PCA)[7a] and 70 mg of 4-AMP in 0.6 mL of $CDCl_3$ was first examined 5 min after its preparation. Small peaks for DBF (δ 6.02 s) and PCA (δ 6.50 d, upfield portion of AA'BB' system) slowly grew until they were maximized at 30 min. Within another 15 min a crystalline precipitate appeared in the NMR tube. After 24–36 h the DBF peak was no longer visible. A second experiment carried out with 50 mg of FMOC-PCA and 0.7 mL of 4-AMP in 0.2 mL of $CDCl_3$ showed at the first examination (5 min) that the PCA peak was already at its maximum. No DBF peak was visible, showing that under these conditions it had been nearly completely scavenged by this time. Traces of DBF remained as evidenced by TLC examination.

(10) Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748; *J. Org. Chem.* **1972**, *37*, 3404.

(11) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401.

Scheme I



on continued washing with buffer solution. With chloroform as solvent the precipitate was seldom observed and could often be avoided by dilution of the organic phase with chloroform prior to buffer extraction. If desired, one can cut the deblocking time in half by periodically evaporating completely the chloroform layer following buffer extraction and reaction with the next amino acid chloride and then substituting methylene dichloride for the deblocking step. Following deblocking or the subsequent acylation step, the methylene dichloride is removed and replaced by chloroform to continue the process.

For the use of our new technique on native tachykinin sequences, the inclusion of methionine was of special importance. At first, partial S-oxidation was feared during the many extractive separatory funnel shakings in the presence of air. Surprisingly, synthesis of the Neurokinin B hexapeptide **5** proceeded without any such oxidation. Hexapeptide **4** was obtained in high purity in a yield of 40% after a single chromatographic purification on silica gel.

Racemization was also not a problem. In agreement with earlier model experiments,[1] HPLC analysis of H-Leu-Nle-OBn and the separately prepared D-leucine analogue showed that the use of FMOC-protected amino acid chlorides in the acylation step and 4-AMP in the deblocking step cause no significant racemization.[12]

All peptides listed in Table II were synthesized by these new improved techniques. In spite of being slightly more time consuming (assembly of the benzyl ester precursor of **3** requires about 5 h), protocol B is clearly preferred. In particular cases prior determination of the optimum amount of excess acid chloride or the speed of the acylation reaction (TLC test) could conserve both reagents and time.

Diketopiperazine and Pyroglutamylpeptide Formation. So far unresolved is the problem of diketopiperazine formation at the dipeptide stage. Thus during the synthesis of **3**, cyclo(Leu-Nle) is formed at the deblocking step to the extent of 5–10% (Table V). Fortunately the presence of this byproduct does not influence further chain growth, and its separation is easily achieved at the pentapeptide stage either directly following the final FMOC deblocking or following the quantitative ammonolysis to **3**. In the case of proline-containing dipeptides, enhanced diketopiperazine formation can lead to severe losses.[13] For example in construction of the proline-containing tetrapeptide **9** via the corresponding benzyl ester the rate of ring closure was so great that following the normal 30-min treatment with 4-AMP, greater than 90% of cyclo[Lys-(Z)-Pro] (**11**) resulted from the corresponding FMOC derivative, and cyclization was not avoidable on reducing the period of deblocking (Table V). On the other hand the formation of **11** could be completely eliminated by substitution of proline *tert*-butyl ester in place of the benzyl derivative. Extension of the chain to tetrapeptide **9** then followed normally via FMOC-Pro-Cl and Z-Arg-(NO_2)-OPfp. Synthesis of **8**, a peptide amide of interest in connection with studies on the biological activity of Substance P, proceeds as expected without any diketopiperazine formation by successive treatment of proline–dodecyl amide with FMOC-Lys(Z)-Cl, FMOC-Pro-Cl, and FMOC-Lys(Z)-Cl. Eventual catalytic deblocking followed by ion-exchange chromatography gave pure **8**.

Less straightforward were the syntheses of glutamine-containing Substance P sequences. Following incorporation of the highly hydrophilic glutamine side chain, the solubility of the peptide in chloroform is lowered drastically, hindering further operations. Therefore, the glutamine unit at position 6 was substituted by the glutamic acid γ-benzyl ester residue. Following deblocking via 4-AMP the resulting hexapeptide was lengthened with BOC-Gln-OPfp[14] to the highly soluble BOC-heptapeptide dibenzyl ester **7**. In this case the use of a second equivalent of FMOC-Glu(OBn)-Cl is inadvisable since ammonolysis would then be accompanied by FMOC-deblocking and subsequent cyclization to the pyroglutamylpeptide. This side reaction is also not completely avoided during 4-AMP treatment of the FMOC-hexapeptide **23**, which leads to a product contaminated by varying amounts of the pyroglutamylpeptide (Table V). Conversion of Glu(OBn)-substituted peptides into the corresponding Glu(4-AMP) derivatives, as has been reported for the homologous Asp(OBn) sequences during FMOC deblocking with 20% piperidine to give the analogous piperidides,[15] was excluded by model studies on the tripeptide H-Glu(OBn)-Val-Tyr-OBn. However, in initial efforts to convert Boc-Glu-(OBn)-Phe-Phe-Gly-Leu-Nle-OBn into the corresponding diamide by treatment with ammonia in methanol, in addition to the expected reaction we obtained evidence for transpeptidation processes. On the other hand, Boc-Glu-(OBn)-Nle-OBn and Boc-Glu(OBn)-Val-Tyr-OBn were smoothly converted to the corresponding diamides.

Hexapeptide **6**, in which the two glutamine units are substituted by a single δ-amino valeric acid (δ-Ava) residue, was synthesized in a total yield of 40% and coupled via the mixed anhydride method with tetrapeptide **9**. Deprotection by hydrogenolysis gave decapeptide **10**, which represents a useful analogue with which to probe the role of glutamine residues on the biological and conformational properties of Substance P derivatives.[16] For comparison decapeptide **10** was also synthesized by a continuous

(12) (a) Beyermann, M.; Granitza, D.; Bienert, M.; Mehlis, B.; Niedrich, H.; Carpino, L. A. *Abstracts of the 10th American Peptide Symposium*; St. Louis, MO, 1987. (b) The coupling of FMOC-amino acid chlorides without racemization will be the subject of a separate detailed experimental report.

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(14) Kisfaludy, L.; Löw, M.; Nyéki, O.; Szirtes, T.; Schön, I. *Justus Liebigs Ann. Chem.* **1973**, 1421.

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(16) For a conventional synthesis of the methionine analogue of **6**, see: Torigoe, K.; Katayama, T.; Sofuku, S.; Murumatsu, I. *Chem. Lett.* **1982**, 563.

FMOC-based solid-phase technique.

Conclusions. The results described show that FMOC amino acid chlorides are very effective in the FMOC/4-AMP system for the synthesis of short- and medium-length peptides as well as partial sequences for subsequent segment condensation. The FMOC amino acid chlorides studied to date can be synthesized without problems and are crystalline, shelf-stable reagents. Even with only slight excesses of acid chloride (10–50%) quantitative acylations are possible under mild conditions within a few minutes. So far no significant racemization or other interferences have been encountered during the coupling step. Combining the use of such rapid-acting coupling agents with an equally rapid deblocking process involving 4-AMP followed by aqueous extractions for the removal of byproducts allows quick assembly of the desired peptide. Only simple laboratory glassware and relatively inexpensive reagents are used, thus making possible the facile synthesis of multigram quantities of material. Substance P pentapeptide benzyl ester could be obtained in 5 h and benzyl ester **7** and the benzyl esters of the hexapeptide amides **4–6** within 6–8 h.

Implications for Future Work. It is worth emphasizing that both of the novel techniques introduced in this work, namely the utility of FMOC amino acid chlorides and the 4-AMP deblocking/scavenging process are of general relevance with implications for peptide synthesis beyond the rapid, continuous synthesis described herein. For example FMOC amino acid chlorides can be coupled in homogeneous solution during conventional syntheses in place of active esters or mixtures of FMOC amino acids and coupling agents. Application of these acid chlorides to solid-phase syntheses is promising.[17] Similarly the use of 4-AMP in place of piperidine or diethylamine can add new versatility to the choice of appropriate workup conditions during conventional syntheses. Limitations on the applicability of FMOC-protected complex amino acid chlorides bearing moderately acid-sensitive side-chain protecting groups, e.g., *tert*-butyl-based functions, remain to be catalogued. In the meantime the corresponding pentafluorophenyl esters can be used as somewhat less reactive substitutes as shown in case of the syntheses of **7** and **9**.

Experimental Section

General. Melting points were determined on a micro hot plate according to Boetius or a Mel-Temp apparatus and are uncorrected. Optical rotations were measured with a VEB Carl Zeiss Jena Polamat A or Rudolph Autopol-III instrument, IR (KBr) and UV spectra on VEB Carl Zeiss instruments, Specord 71 IR and Specord M40, respectively, or on Perkin-Elmer 1310 or FT-1600 (IR) or Lambda 2 (UV) instruments. NMR determinations were carried out on Varian A-60A, Perkin-Elmer R12, and Varian XL-200 and XL-300 instruments. Amino acid analyses were carried out after hydrolysis in 6 N HCl for 20 h at 110 °C on Microtechna AAA (Prague) or Waters-Millipore instruments. TLC was effected with silica gel 60 F254 on precoated glass plates from Merck 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. Spot intensities were determined with a Shimadzu TLC scanner CS-920. 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) (Shimadzu LC-6A or a Waters system incorporating a 720 controller, 730 data module, U6K injector, 6000A pumps, 441 detector and Z-module radial compression unit), flow rate 1.0 mL/min. Preparative HPLC was carried out with a Waters Delta Prep unit using a C_{18}-300 Å, 1.5 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 60 (Merck, 40–63 μm) in columns of dimension 300 × 20 mm. Free peptides were purified by ion-exchange chromatography on carboxymethyl cellulose (Serva, 0.7 mg/g) on columns of dimension 200 × 20 mm with elution by a linear gradient of 0.01–0.5 M ammonium acetate, pH 6.0. 4-(Aminomethyl)piperidine (4-AMP) and *N*-methylpiperazine (NMP) were obtained from EGA-Chemie and Aldrich Chemical Co. Dimethylformamide was distilled in vacuum and stored over Zeosorb A4. Chloroform and methylene dichloride were distilled over phosphorus pentoxide. Amino acids were obtained from Reanal (Hungary) and FMOC amino acids and their derivatives from Milligen/Biosearch (Bedford, MA). The synthesis of 9-fluorenylmethanol,[18] FMOC-Cl,[10] FMOC-ONSu,[19] and FMOC-ONB[20] proceeded according to published descriptions. FMOC amino acids were prepared using the latter two reagents, in order to avoid contamination by oligopeptide derivatives.

Preparation of Amino Acid Chlorides. The FMOC amino acid (2 g) was suspended in 10 mL of CH_2Cl_2 (in the case of glycine the reaction solvent was a mixture of 8 mL of CH_2Cl_2 and 2 mL of THF), and after the addition of 4 mL of $SOCl_2$ the mixture was refluxed for 30 min. Evaporation in vacuo followed by addition of CH_2Cl_2 and reevaporation 2–3 times gave an oil or a solid free of excess $SOCl_2$. The residue was dissolved in a little ether or CH_2Cl_2, and hexane was added. The resulting crystals (yield 80–90%) were filtered and dried over P_4O_{10} in vacuo. Melting points and optical rotations correspond to the values recently published.[1] With the exception of FMOC-δ-Ava-Cl the acid chlorides could be stored for months in a desiccator at room temperature without perceptible decomposition. In the case of FMOC-δ-Ava-Cl, mp 54–63 °C, for which no attempt was made to obtain an analytically pure sample, the reflux period was limited to 10 min to avoid cyclization to 1-FMOC-2-piperidone.[21] Some samples of FMOC-Glu(OBn)-Cl contained traces of ninhydrin-positive byproducts, which could be extracted following the coupling reaction and did not interfere in the synthesis. [FMOC-Glu(OBn)-Cl, mp 102–3 °C. Anal. Calcd for $C_{27}H_{24}ClNO_5$: C, 67.85; H, 5.06; N, 2.93. Found: C, 67.33; H, 5.13; N, 2.57.] All compounds showed characteristic IR bands (KBr) at 1790–1810 cm^{-1}. The purity of the acid chlorides could be conveniently followed by methanolysis in dry methanol and subsequent TLC analysis of the methyl ester using the system 2 or preferentially by HPLC analysis[1] using HPLC-system (3). Depending on the case, 2–10% of free FMOC amino acid could be detected. In the case of FMOC-δ-Ava-Cl the amount of free acid present was about 20%. In order to ensure complete conversion of the amino component to the desired coupling product, the FMOC amino acid chloride was generally used in 10–50% excess.

9-Fluorenylmethyl *p*-Nitrocarbanilate (FMOC-pNA). From FMOC-Cl, *p*-nitroaniline, and pyridine in THF the urethane was obtained after recrystallization from MeOH (80% yield) as white crystals: mp 166–167 °C; UV (CH_2Cl_2) 267 (9725), 290 (6350), 301 (8180); ^1H NMR ($CDCl_3$) δ 4.25 (t, 1, C*H*CH_2), 4.65

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(d, 2, CH_2O), 6.95 (d, 1, NH), 7.3–7.7 (m, 8, aryl), 7.75–8.3 (AB q, 4, arylamine). Anal. Calcd for $C_{21}H_{15}N_2O_4$: C, 70.18; H, 4.21; N, 7.80. Found: C, 70.07; H, 4.46; N, 7.60.

Effect of Solvent on the Rate of Adduct Formation between Dibenzofulvene and 4-(Aminomethyl)piperidine. A 0.05 M solution of FMOC-pNA (15 mL) in the appropriate solvent (Table IV) was treated with 3 mL of 4-AMP, and the solution was let stand at room temperature. After 10, 20, 30, 40, or 50 min or longer a 3-mL portion of the solution was extracted with three 3-mL portions of phosphate buffer (pH 5.5), 10 μL of the organic phase was diluted to 10 mL with $CHCl_3$, and the UV spectrum was recorded. In the case of solutions in CH_2Cl_2 or $ClCH_2CH_2Cl$, in order to avoid formation of a precipitate during the phosphate washing, 2 mL of $CHCl_3$ was added prior to the extraction, and in these cases 20 μL of the organic phase was diluted to 10 mL with $CHCl_3$. Determination of DBF content was based on measurements at 258 and 279 nm [λ (ε) 257.5 (56300), 279 (10600); cyclohexane].[8] For the results see Table IV.

Determination of the Rate of Hydrolysis of FMOC-Gly-Cl in $CHCl_3$/5% Na_2CO_3. A solution of 4 mg of FMOC-Gly-Cl in 10 mL of $CHCl_3$ was treated with 10 mL of 5% Na_2CO_3 solution, and the mixture was stirred vigorously for 40 min. After phase separation, 1 mL of the organic phase was diluted with 5 mL of $CHCl_3$, and the UV spectrum was recorded at 290 nm. In a parallel experiment 4 mg of FMOC-Gly-OH was dissolved in 10 mL of $CHCl_3$ and treated similarly, although in this case the mixture was stirred for only 5 min. The optical density of the FMOC-Gly-Cl solution had dropped about 10% after 40 min whereas that of the free acid solution had dropped from 1.45 to 0.05.

General Method for Executing Rapid FMOC/4-AMP Peptide Synthesis (Table I, Protocol B). Five milliliters of a 0.1 M solution of the C-terminal starting amino acid ester, 5 mL of $CHCl_3$ containing 0.75 mmol of the appropriate FMOC-X_{aa}-Cl, and 5 mL of 5% Na_2CO_3 solution were combined, and the two-phase mixture was stirred vigorously for 10 min (end point determined by TLC analysis). The organic phase was separated and treated with 5 mL of 4-AMP. After 30 min (end point determined by TLC analysis) 40 mL of $CHCl_3$ was added, and the organic phase was washed with five 25-mL portions of phosphate buffer of pH 5.5 (prepared from 90 g of $NaH_2PO_4 \cdot H_2O$ and 32.7 g of Na_2HPO_4 in 500 mL of distilled water). Alternatively two 25-mL portions of saturated NaCl solution were used to remove excess 4-AMP prior to extraction with the phosphate buffer. The organic phase was concentrated in vacuo to a volume of 5 mL on a rotary evaporator, and the resulting $CHCl_3$ solution was used analogously for treatment with the next FMOC amino acid chloride.

Phe-Phe-Gly-Leu-Nle-NH_2·HCl (3·HCl). A solution of 197 mg (0.5 mmol) of Nle-OBn·TsOH in 5 mL of $CHCl_3$ was mixed in a 100-mL round-bottomed flask with 279 mg of FMOC-Leu-Cl (0.75 mmol) in 5 mL of $CHCl_3$, 5 mL of 5% Na_2CO_3 was added, and the mixture was stirred vigorously for 10 min. TLC analysis after 2 min showed that Nle-OBn was no longer detectable. The mixture was poured into a 100-mL separatory funnel, the aqueous phase was removed, the $CHCl_3$ phase was treated with 5 mL of 4-AMP, and the solution was stirred for 30 min, after which time 40 mL of $CHCl_3$ was added, and the solution was extracted with five 25-mL portions of phosphate buffer (pH 5.5). TLC analysis, system 1, showed, following ninhydrin detection, a strong spot of R_f 0.6 characteristic of the dipeptide H-Leu-Nle-OBn as well as a weak spot at the starting position (4-AMP). Visualization by Cl_2/benzidine showed an additional spot of R_f 0.45 (3) which corresponds to the position noted for an authentic sample of cyclo(Leu-Nle) (see below). Relative to the major product, the diketopiperazine amounts to about 10%. Under UV light an additional nonpolar material [R_f 0.9, (1)], not visible by ninhydrin or Cl_2/benzidine visualization, was assigned to residual DBF. The $CHCl_3$ solution was concentrated to 5 mL in vacuo with a rotary evaporator and in an analogous manner treated with 237 mg of FMOC-Gly-Cl and twice with 304-mg portions of FMOC-Phe-Cl. The resulting protected pentapeptide, FMOC-Phe-Phe-Gly-Leu-Nle-OBn [MS/FAB: 686.4 (MH^+), calcd 685.4 (M); ^1H NMR (DMSO-d_6) δ 0.85 (m, 9, CH_3), 1.26 (m, 4, CH_2), 1.4 (m, 1, CH_2CHMe_2), 1.65 (m, 4, CH_2CH), 2.9 (dd, 2, $CH_2C_6H_5$), 3.2 (dd, 2, $CH_2C_6H_5$), 3.8 (d, 2, CH_2CO), 3.9 (t, 1, CHCO), 4.2 (t, 1, CHCO), 4.4 (t, 1, CHCO), 4.6 (t, 1, CHCO), 5.2 (s, 2, $OCH_2C_6H_5$), 7.2–7.4 (m, 16, aryl, NH), 8.0 (m, 1, NH), 8.4 (m, 2, NH), 8.8 (m, 1, NH)], was deblocked without any prior chromatographic purification. Following the usual phosphate extractions the $CHCl_3$ was removed and the resulting oil taken up in 10 mL of ether, and the free amino peptide ester was precipitated as the trifluoroacetate by addition of 200 μL of trifluoroacetic acid. Precipitation was completed by addition of 10 mL of hexane; the peptide salt was filtered and dried to give 362 mg (90.5%) of the crude salt [R_f 0.55 (1); 0.73 (3)]. Both DBF and diketopiperazine were partially removed during the precipitation process. The crude salt was dissolved in 15 mL of 15% NH_3 in MeOH, the mixture was stirred at room temperature for 36 h, the solvent was removed in vacuo, the residue was dissolved in 5 mL of EtOH, and the pH was brought to 4 by the addition of 4 N HCl in EtOAc. The mixture was evaporated to dryness, and the residue was taken up in 5 mL of MeOH and precipitated by the addition of 20 mL of ether to give 250 mg (79%) of the hydrochloride as a white powder, mp 136–142 °C, $[\alpha]^{20}_D$ = −64° (c = 0.5, DMF) [lit.[4a] mp 138–144 °C; $[\alpha]^{20}_D$ = −65° (c = 0.25, DMF)]; amino acid analysis: Phe 2.10 (2), Gly 1.00 (1), Leu 0.97 (1), Nle 1.05 (1); R_f 0.43 (1), R_f 0.35 (3).

H-Lys(Z)-Phe-Phe-Gly-Leu-Met-NH_2·2HCl (4·2HCl). The precursor pentapeptide was obtained as described for **3** from 0.05 mmol of H-Met-OBn·TsOH [mp 119–20 °C; $[\alpha]^{25}_D$ = +2.2° (c = 1, MeOH); ^1H NMR ($CDCl_3$) δ 1.85 (s, 3, $CH_3C_6H_4$), 2.1 (m, 2, $CHCH_2CH_2$), 2.3 (s, 3, SCH_3), 2.45 (m, 2, CH_2S), 4.15 (br s, 1, NCHCO), 5.15 (q, 2, $CH_2C_6H_5$), 7.0–7.7 (m, 9, aryl), 8.4 (br s, 3, NH_3^+)]. Following coupling with FMOC-Lys(Z)-Cl the $CHCl_3$ phase was extracted with 5 mL of 0.1 N HCl to remove ninhydrin-positive byproducts. The intermediate, H-Lys(Z)-Phe-Phe-Gly-Leu-Met-OBn, was purified by silica gel chromatography using $CHCl_3$–EtOH (9:1) as eluant. The residue from the chromatography was precipitated from 5 mL of $CHCl_3$ by addition of 20 mL of hexane to give 305 mg (63%) of the pure peptide ester: R_f 0.45 (1); mp 130–136 °C; $[\alpha]^{20}_D$ = −39.4° (c = 0.5, MeOH). A solution of 130 mg of the ester was dissolved in 10 mL of 15% NH_3 in MeOH, the solution was stored at room temperature for 40 h, the solvent was removed in vacuo, and the hexapeptide amide was converted into its hydrochloride salt by addition of 2 mL of 0.1 N HCl to a solution of the peptide in 3 mL of DMF. By evaporation and final precipitation from DMF by addition of ether there was obtained 95 mg (73%) of the hydrochloride: R_f 0.10 (1), R_f 0.25 (3); mp 208–211 °C; $[\alpha]^{20}_D$ = −27.6° (c = 0.5, DMF); amino acid analysis Lys 0.91 (1), Phe 2.05 (2), Gly 1.00 (1), Met 0.86 (1); MS/FAB 875.4 (MH^+), calcd 874.5 (M); ^1H NMR (DMSO-d_6) δ 0.87 (t, 6, CH_3), 1.1–1.7 (m, 10, CH_2), 1.9 (m, 1, $CHMe_2$), 2.1 (s, 3, SMe), 2.45 (m, 4, CH_2NH, CH_2S), 2.9 (m, 4, $CH_2C_6H_5$), 3.51 (t, 1, CH_2NHCO), 3.75 (br s, 2, $NHCH_2CO$), 4.3 (m, 5?, NHCHCO), 4.6 (br s, 5?, NH), 5.0 (s, 2, $OCH_2C_6H_5$), 7.0–7.5 (m, 15, aryl), 8.0–8.5 (m, 5, NHCO, NH_2).

H-Phe-Phe-Val-Gly-Leu-Met-NH_2 (5). Obtained as described above for **3** and **4** from 0.5 mmol of H-Met-OBn·TsOH. Recrystallization from MeOH gave the hexapeptide amide in an overall yield of 29% as a white solid: mp 228–32 °C; $[\alpha]^{25}_D$ = −33.7° (c = 0.3, DMF); MS/FAB 712.5 (MH^+), calcd 711.4 (M); ^1H NMR (DMSO-d_6) δ 0.9 (m, 12, CH_3), 1.4 (m, 2, CH_2CH_2S), 1.6 (m, 1, CH_2CHMe_2), 1.8–2.05 (m, 6, $CHMe_2$, $CHCH_2CH$, SMe), 2.5 (m, 2, CH_2S), 2.85 (m, 2, $CH_2C_6H_5$), 3.0 (m, 2, $CH_2C_6H_5$), 3.4 (m, $NHCH_2CO$), 3.7 (s, 2, $CONH_2$), 4.1–4.4 (m, 5, NHCHCO), 4.7 (br s, 1, NH), 7.05 (s, 1, NH), 7.1–7.3 (m, 10, aryl), 7.9–8.3 (d, d, s, 5, NH); amino acid analysis Phe 1.98 (2), Val 1.08 (1), Gly 1.00 (1), Leu 1.06 (1), Met 0.91 (1).

δ(Ava)-Phe-Phe-Gly-Leu-Nle-NH_2 (6). Compound **6** was obtained as described above for **3** and **4** from 0.5 mmol of H-Nle-OBn and 1.0 mmol of FMOC-δ(Ava)-Cl. The peptide ester was purified via chromatography using $CHCl_3$/EtOH/HOAc/EtOAc (8/1/0.5/0.5) and treated with NH_3 in MeOH. The overall yield of amide amounted to 40%: R_f 0.22 (4); t_R = 22.5 min (1); amino acid analysis δAva 1.07 (1), Phe 2.11 (2), Gly 1.00 (1), Leu 0.97 (1), Nle 0.98 (1). Without further purification the amide was coupled with **9** to give **10**.

BOC-Gln-Glu(OBn)-Phe-Phe-Gly-Leu-Nle-OBn (7). From 0.5 mmol of H-Nle-OBn·TsOH the protected pentapeptide **3** was assembled as noted above, deblocked by treatment with 5 mL of 4-AMP (30 min) and extracted 5 times with phosphate buffer (pH 5.5). Following reduction of the volume of the $CHCl_3$ phase

to 5 mL the residual 0.35 mmol of pentapeptide ester was mixed with 223 mg (0.47 mmol) of FMOC-Glu(OBn)-Cl in 5 mL of $CHCl_3$ and 10 mL of 5% Na_2CO_3 solution, and the mixture was stirred for 15 min. After phase separation, which in this case was aided by the addition of saturated NaCl solution, 5 mL of 4-AMP was added, and after 30 min, dilution with 40 mL of $CHCl_3$ was followed by five extractions with phosphate buffer. TLC analysis showed that besides the deblocked hexapeptide [R_f 0.53 (3)] a ninhydrin-negative spot [R_f 0.62 (3)] was evident. The latter spot, amounting to about 15% of the mixture according to TLC scanning, showed the same R_f value as that of an authentic sample of Pyr-Phe-Phe-Gly-Leu-Nle-OBn prepared from Pyr-OPcp (for a method of reducing the cyclization see below). The $CHCl_3$ phase was reduced to 5 mL in vacuo and treated with 55 μL of *N*-methylmorpholine and 247 mg (0.6 mmol) of BOC-Gln-OPfp in 5 mL of DMF. The mixture was stirred for 80 min, whereby the heptapeptide partially precipitated. Removal of $CHCl_3$ in vacuo followed by trituration with ether gave a solid, which was filtered and washed in sequence with ether, 1% HOAc, and ether. Drying in vacuo gave 232 mg (47%) of the protected heptapeptide: R_f 0.55 (1) and 0.50 (1) (the latter R_f value representing about 15%, corresponding to Pyr-Phe-Phe-Gly-Leu-Nle-OBzl); amino acid analysis Glu 1.94 (2), Phe 1.96 (2), Gly 1.00 (1), Leu 1.00 (1), Nle 1.02 (1).

Cyclo[Lys(Z)-Pro] (11). In an attempted synthesis of H-Lys-Pro-Lys-Pro-OH, 60.5 mg (0.25 mmol) of H-Pro-OBn·HCl and 195 mg of FMOC-Lys(Z)-Cl (0.375 mmol) gave (TLC) only about 10% of the ninhydrin-positive dipeptide ester [R_f 0.60 (3)] and 90% of a ninhydrin-negative, Cl_2/benzidine-positive substance which showed the same R_f value [0.68 (3)] and other spectral and physical properties as authentic cyclo[Lys(Z)-Pro] synthesized in a conventional manner from H-Lys(Z)-Pro-OBn by heating at pH 6.0. After removal of $CHCl_3$, recrystallization from MeOH gave 72 mg (80%) of the crystalline diketopiperazine: mp 139–141 °C; R_f 0.68 (3), R_f 0.90 (4); $[\alpha]^{20}_D$ = −77.8° (*c* = 0.5, ethanol); ^{1}H NMR ($CDCl_3$) δ 1.5 (m, 4, CH_2), 1.65–2.4 (m, 6, cyclic $CHCH_2CH_2$, CH_2CH), 3.2 (m, 2, CH_2NH), 3.5 (m, 2, cyclic CH_2N), 3.9 (m, 2, $NHCHCO$), 5.1 (s, 2, $OCH_2C_6H_5$), 5.5 (m, 1, NHCO), 7.3 (s, 5, aryl), 7.45 (s, 1, $CHNHCO$).

Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.67. Found: C, 63.28; H, 7.09; N, 11.25.

Cyclo(Leu-Nle) (12). A solution of 300 mg (0.81 mmol) of H-Leu-Nle-OBzl·HCl in 5 mL of $CHCl_3$ and 2.5 mL of 4-AMP was kept at room temperature for 12 h. After addition of 10 mL of $CHCl_3$, the mixture was extracted four times with 5 mL of 1 N HCl and two times with water. From the organic layer was obtained 134 mg (73%) of the diketopiperazine from MeOH as white needles: R_f 0.38 (1), R_f 0.45 (3); mp 258–260 °C; $[\alpha]^{20}_D$ = −34.6° (*c* = 1, DMF); ^{1}H NMR ($CDCl_3$) δ 0.9 (m, 9, CH_3), 1.4 (m, 4, $CH_2CH_2CH_3$), 1.6–1.9 (m, 5, CH_2CHMe_2, CH_2CH), 4.0 (m, 2, $NHCHCO$), 6.1 (s, 2, NH).

Anal. Calcd for $C_{12}H_{22}N_2O_2$: C, 63.68; H, 9.80; N, 12.38. Found: C, 63.87; H, 9.81; N, 11.92.

Cyclo(Leu-Met) (13). The compound was prepared from 100 mg (0.25 mmol) of H-Leu-Met-OBzl·HCl as described for **12**. The isolated solid was recrystallized from hot 2-propanol/water (1:1) to give 42 mg (69%) of the diketopiperazine: R_f 0.35 (1), R_f 0.43 (3); mp 220–235 °C; $[\alpha]^{20}_D$ = −94.8° (*c* = 0.5, DMF); ^{1}H NMR ($CDCl_3$) δ 0.81 (m, 6, Me), 1.45 (q, 2, $CHCH_2CH_2$), 1.7 (m, 2, $CHCH_2CH$), 1.8–2.2 (m, 4, $CHMe_2$, SMe), 2.5 (t, 2, CH_2S), 3.8 (t, 1, $COCHNH$), 3.95 (t, 1, $COCHNH$), 7.35 (s, 1, NH), 7.65 (s, 1, NH).

Anal. Calcd for $C_{11}H_{20}N_2O_2S$: C, 54.07; H, 8.27. Found: C, 54,53; H, 8.21.

H-Lys-Pro-Lys-Pro-NHC$_{12}$H$_{25}$ (8). A solution of 319 mg (1 mmol) of H-Pro-$NHC_{12}H_{25}$·HCl in 10 mL of $CHCl_3$ was combined with a solution of 782 mg (1.5 mmol) of FMOC-Lys(Z)-Cl in 10 mL of $CHCl_3$ and 10 mL of 5% Na_2CO_3 solution, and the mixture was stirred vigorously for 10 min. Following layer separation, treatment in the normal manner with 4-AMP for 30 min (no diketopiperazine formation), extraction with phosphate buffer and reduction of the volume of $CHCl_3$ to 10 mL, chain extension was continued in the same manner with FMOC-Pro-Cl and FMOC-Lys(Z)-Cl. The intermediate H-Lys(Z)-Pro-Lys(Z)-Pro-$NHC_{12}H_{25}$ was isolated from the dried $CHCl_3$ layer by addition of 1 mL of 4 N HCl/ethyl acetate, evaporation of solvent, and precipitation from $CHCl_3$/petroleum ether to give 440 mg (47%) of the hydrochloride salt, R_f 0.28 (3). A solution of 218 mg of the intermediate in 15 mL of methanol/acetic acid/water, 2:1:1, was hydrogenated in the presence of 100 mg of palladium–charcoal (10%) for 4 h. The catalyst was removed by filtration; the filtrate was concentrated in vacuo, diluted with water, and lyophilized to give 178 mg (31.6%): R_f 0.50 (5), weak impurities detectable with R_f (5) values of 0.10, 0.80, 0.90. Of this material, 80 mg was purified by chromatography on carboxymethyl cellulose (15 × 1.5 cm, elution with a gradient of 0.01–0.5 M ammonium acetate pH 6.0). Pure fractions were collected and lyophilized, yielding 65 mg of the amide as a white powder: R_f 0.50 (5); $[\alpha]^{20}_D$ = −86° (*c* = 1.5, acetic acid); amino acid analysis Lys 2.00 (2), Pro 2.11 (2); MS/FAB 636.4 (MH^+), calcd 635.5 (M); ^{1}H NMR (DMSO-d_6) δ 0.9 (s, 3, CH_3), 1.1–2.2 (m, 40, $CH_2CH_2CH_2$), 2.6–3.0 (m, 6, $NHCH_2CH_2$), 3.2–3.7 (m, CH_2N), 4–4.2 (m, 4, NCHCO), 4.4 (br s, 2, NH), 7.9 (br s, 4, NH_2CH_2), 8.2 (br s, 2, NH_2CH).

Z-Arg(NO$_2$)-Pro-Lys(Z)-Pro-OH (9). H-Pro-Lys(Z)-Pro-O-*t*-Bu, obtained as described for **8** from 0.5 mmol of H-Pro-O-*t*-Bu, 0.75 mmol of FMOC-Pro-Cl, and 0.75 mmol of FMOC-Lys(Z)-Cl, was elongated by addition of 0.55 mmol (286 mg) of Z-Arg-(NO_2)-OPfp and 0.5 mmol (60 μL) of *N*-methylmorpholine to the $CHCl_3$ solution (5 mL) of the tripeptide. After 120 min the solution was diluted by addition of 20 mL of $CHCl_3$ and extracted with 0.1 N HCl (2×) and 5% Na_2CO_3 solution (2×). After the normal workup procedure the protected peptide, Z-Arg(NO_2)-Pro-Lys(Z)-Pro-O-*t*-Bu, was precipitated from $CHCl_3$/ether to give 291 mg (66%) of the protected tetrapeptide, *tert*-butyl ester, R_f 0.75 (3), R_f 0.30 (1), t_R = 37.6 min (1), purity (HPLC) 93%. To remove minor impurities, the peptide was purified by column chromatography on silica gel (40–63 μm, column size 30 × 2 cm, with elution by $CHCl_3$/ethyl acetate/ethanol, 2:1:0.3, yielding 240 mg of the peptide ester (99% by HPLC): mp 84 °C; $[\alpha]^{20}_D$ = −70.6° (*c* = 1, MeOH). [Anal. Calcd for $C_{42}H_{59}N_9O_{11}\cdot H_2O$: C, 57.01; H, 6.96; N, 14.25. Found: C, 57.43; H, 6.94; N, 13.76.] The *tert*-butyl ester (190 mg) was treated with 10 mL of TFA/CH_2Cl_2, 1:1, for 45 min at room temperature. The peptide acid was isolated by evaporation and final precipitation from $CHCl_3$/ether to give 150 mg (84%) of a white powder: mp 100–104 °C; TLC [R_f 0.53 (4)]; HPLC [t_R = 10.6 min (1), purity 98%]; $[\alpha]^{20}_D$ = −61° (*c* = 1, MeOH) [lit.[22] mp 101–103 °C; $[\alpha]^{20}_D$ = −63° (*c* = 0.8, MeOH)]; MS/FAB 810.6 (MH^+), calcd 809.4 (M); ^{1}H NMR ($CDCl_3$) δ 1.2–2.3 (m, 18, CH_2), 3.1 (m, 4, CH_2NH), 3.6 (m, 4, CH_2N), 3.9–4.8 (m, 8, NH, NCHCO, $NHCHCO$), 5.1 (s, 4, $CH_2C_6H_5$), 7.3 (br s, 10, aryl), 7.6 (br s, 2, NH_2).

H-Arg-Pro-Lys-Pro-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH$_2$ (10). To a solution of 138 mg of **9** (0.17 mmol) in 4 mL of DMF were added at −10 °C 20 μL of *N*-methylmorpholine (0.179 mmol) and 22 μL of isobutylchloroformate (0.17 mmol), and the mixture was stirred at −10 to −5 °C. After 10 min a solution of 117 mg (0.17 mmol) of **6** in 4 mL of hexamethylphosphoric acid triamide/DMF (1:1) and 20 μL of *N*-methylmorpholine was added at −10 °C. The mixture was stirred at −5 °C for 1 h and at 0 °C for 2 h. After partial removal of solvent by rotary evaporation the peptide was precipitated by the addition of 20 mL of 5% $KHSO_4$ solution. After standing at 4 °C for 2 h, the precipitate was filtered and washed with 5% $KHSO_4$ solution and water, followed by $CHCl_3$ to remove minor amounts of **9**, and finally with ether. There was obtained 153 mg (60.5%) of the protected amide: R_f 0.28 (4), impurity with R_f 0.31 (4) (compound **6**); $[\alpha]^{20}_D$ = −10.0° (*c* = 1, HOAc). The protected intermediate (85 mg) was deprotected by hydrogenolysis in 6 mL of acetic acid/methanol/water, 3:2:1, in the presence of 50 mg of 10% Pd–C. After 5 h the catalyst was separated by filtration, and the solution was evaporated to dryness. The residue was triturated with 5 mL of 0.1 N acetic acid. Impurity **6** was insoluble and could be removed by filtration. The clear solution gave 37 mg (57.6%) of **10** after lyophilization: R_f 0.23 (5); amino acid analysis Arg 0.87 (1), Lys 0.97 (1), Pro 2.12 (2), δAva 1.10 (1), Phe 1.90 (2), Gly 1.00 (1), Leu 0.99 (1), Nle 1.10 (1). The decapeptide was also assembled on a Milligen 9050 continuous flow peptide synthesizer using FMOC amino acid pentafluorophenyl esters as coupling agents with Mtr(Arg) and BOC(Lys) as side chain protectants. δ-Ava was introduced as the pentafluorophenyl ester (see below for preparation). The yield of the tris(trifluoroacetate) was 42%; MS/FAB 1172 (MH^+), calcd 1171.7 (M); ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 9, CH_3), 1–2 (m, 31,

CH_2CH), 2.8-3.0 (m, 8, $CH_2C_6H_5$, CH_2CO, CH_2NH_2), 3.2-4.6 (m, 24, CONHCHCO, $NHCH_2CO$, CH_2N, COCHN, CH_2NH), 7.2 (m, 10, aryl), 7.7-8.5 (m, 9, NH_2, =NH).

FMOC-δ-aminovaleric Acid. A mixture of 1.56 g of δ-aminovaleric acid, 3.2 g of $NaHCO_3$, 25 mL of H_2O, 5 g of FMOC-OSu, and 30 mL of dioxane was treated with Na_2CO_3 to bring the pH to 7.8 and stirred overnight. Addition of 80 mL of distilled water effected complete solution. Three washes with 50-mL portions of ether removed excess acylating agent, and the remaining aqueous solution was acidified with concentrated HCl to pH 8. The precipitate was washed three times with 30-mL portions of 1 N HCl and three 30-mL portions of water. The residue was dissolved in acetone; the solution was dried (Na_2SO_4), filtered, evaporated, and dried in a vacuum dessicator. The filtrate was acidified to pH 3, and the new precipitate was treated similarly. Both fractions were dissolved in 125 mL of acetone; the solution was dried (Na_2SO_4) and evaporated to give 3.5 g (84.5%) of the acid as a white solid, mp 136-7 °C. The analytical sample was recrystallized from EtOAc/hexane to give white crystals: mp 135-6 °C; IR (KBr) 3349 (NH), 1693 (COOH, OCONH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1-1.9 (m, 4, CH_2CH_2), 2.2 (t, 2, CH_2CO), 2.9 (m, 2, CH_2NH), 4.1-4.4 (m, 3, $CHCH_2O$), 5.6 (d, 1, NH), 7.3-8.0 (m, 8, aryl). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.77; H, 6.23; N, 4.12. Found: C, 70.55; H, 6.11; N, 3.96.

FMOC-δ-aminovaleric Acid Pentafluorophenyl Ester. To a solution of 824 mg of DCC and 736 mg of pentafluorophenol in 25 mL of CH_2Cl_2 was added after 5 min 1.24 g of FMOC-δ-Ava-OH. After adding 25 mL of CH_2Cl_2, stirring was continued for 10 h, and the precipitated urea was filtered and washed with another 25 mL of CH_2Cl_2. The filtrate gave after recrystallization from CH_2Cl_2-hexane 1.69 g (87%) of the ester: mp 118-9 °C; IR (KBr) 3387 (NH), 1790, 1699 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5-1.9 (m, 4, CH_2CH_2), 2.7 (t, 2, CH_2CO), 3.3 (q, 2, CH_2NH), 4.25 (t, 1, $CHCH_2O$), 4.45 (d, 2, CH_2O), 4.9 (m, 1, NH), 7.3-7.9 (m, 8, aryl). Anal. Calcd for $C_{26}H_{20}F_5NO_4$: C, 61.78; H, 3.96; N, 2.77. Found: C, 61.63; H, 3.96; N, 2.77.

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Registry No. 3·HCl, 68759-90-0; 4·2HCl, 123622-30-0; 5, 97559-40-5; 6, 123622-31-1; 7, 123622-32-2; 8, 111422-33-4; 9, 42001-58-1; 10, 123622-33-3; 10·3 TFA, 123622-35-5; 11, 78326-84-8; 12, 123622-34-4; 13, 61243-38-7; 4-AMP, 7144-05-0; Fmoc-PNA, 123622-29-7; $4\text{-}O_2NC_6H_4NH_2$, 100-01-6; H-Nle-OBn·TsOH, 63219-55-6; H-Met-OBn·TsOH, 68739-90-2; Fmoc-Gly-Cl, 103321-49-9; Fmoc-Leu-Cl, 103321-59-1; Fmoc-Phe-Cl, 103321-57-9; Fmoc-Glu(OBn)-Cl, 12362-36-6; BOC-Gln-OPfp, 50903-58-7; Fmoc-Lys(Z)-Cl, 103321-56-8; Fmoc-δ-Ava-Cl, 123622-37-7; Fmoc-Pro-Cl, 103321-52-4; Z-Arg(NO_2)-OPfp, 17543-52-1; H-Pro-OBu-*t*, 2812-46-6; H-Pro-$NHC_{12}H_{25}$·HCl, 123622-38-8; H-Leu-Nle-OBn, 123622-39-9; Fmoc-Phe-Phe-Gly-Leu-Nle-OBn, 123622-40-2; H-Lys(Z)-Phe-Phe-Gly-Leu-Met-OBn, 123622-41-3; H-Phyr-Phe-Phe-Gly-Leu-Nle-OBn, 123622-42-4; H-Lys(Z)-Pro-OBn, 68280-75-1; H-Leu-Nle-OBn·HCl, 123622-43-5; H-Leu-Met-OBn·HCl, 123622-44-6; H-Lys(Z)-Pro-Lys(Z)-Pro-$NHC_{12}H_{25}$·HCl, 123622-45-7; H-Pro-Lys(Z)-Pro-OBu-*t*, 123622-46-8; Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OBu^t, 123639-60-1; Z-Arg(NO_2)-Pro-Lys(Z)-Pro-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH_2, 123622-47-9; H-δ-Ava-OH, 660-88-8; Fmoc-δ-Ava-OH, 123622-48-0; Fmoc-δ-Ava-OPfp, 123622-49-1; Fmoc-Gly-OH, 29022-11-5; Fmoc-Leu-OH, 35661-60-0; Fmoc-Phe-OH, 35661-40-6; Fmoc-Glu(OBn)-OH, 123639-61-2; Fmoc-Lys(Z)-OH, 86060-82-4; Fmoc-Pro-OH, 71989-31-6; H-δ-Ava-Phe-Phe-Gly-Leu-Nle-OBn, 123622-50-4; H-Pro-OBn·HCl, 16652-71-4.

Synthesis of Novel Thiazole-Containing DNA Minor Groove Binding Oligopeptides Related to the Antibiotic Distamycin

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The synthesis of novel thiazole-bearing oligopeptides related to the antibiotic distamycin is described. These lexitropsins, or information reading agents, are designed to test the principle of DNA base site exclusion or avoidance as well as base pair acceptance in molecular recognition of oligopeptides for DNA. The first group of compounds, therefore, has the sulfur atom aligned inward to the DNA minor groove, whereas the second group of agents has the sulfur atom directed away from the DNA minor groove. All six compounds synthesized bind to double helical DNA, and their binding constants to calf thymus DNA and relative to distamycin and netropsin are reported.

Introduction

Because of current interest in the control of gene expression,[1] synthetic chemists have been attracted to the problem of developing DNA sequence-specific agents. Conceptually there are a number of approaches to this problem, e.g., using β-oligonucleotides[2-4] or their backbone-modified counterparts[5-8] which take advantage of inherent Watson-Crick base pairing to target single-strand sequences or with hybrid probes incorporating an intercalator.[9-11] Another approach is to take advantage of the ability of certain oligonucleotide sequences to form triplex

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