solution was heated to reflux for 24 h, poured into 500 ml of water, acidified to pH 1, and extracted with 2 × 100 ml of methylene chloride. The aqueous layer was then made basic (pH 9) and extracted with 2 × 100 ml of methylene chloride; the extracts were dried ($MgSO_4$) and evaporated, then chromatographed to give an off-white crystalline substance (0.6 g, 31% yield).

II. 1-Benzylimidazolidin-2-one Oxime from a Diamine and 7. To a refluxing solution of *N*-benzylethylenediamine (0.75 g, 0.005 mol) and triethylamine (1.01 g, 0.01 mol) in 100 ml of chloroform was added dropwise over 5 h 1.0 g (0.0051 mol) of **7** in 10 ml of chloroform. After the solution was heated at reflux for 4 h, the solvent was evaporated and the residue hydrolyzed with 20 ml of 1 N HCl on a steam bath for 1 h. The workup is the same as part I (from acidification step). The yield was 0.55 g (58%).

Registry No.—**7**, 59812-90-7; **8**, 24248-83-7; **10** (n = 2), 59812-91-8; dihydropyran, 110-87-2; phosgene oxime, 1794-86-1; *N*-benzylethylenediamine, 4152-09-4.

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Fully Automated Solid Phase Synthesis of Protected Peptide Hydrazides on Recycling Hydroxymethyl Resin

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A fully automated solid phase synthesis of Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (I) on hydroxymethyl resin (II) is described. All of the operations, including esterification of the first amino acid residue to the resin, deprotection of α-amino protecting group followed by coupling reaction with the next amino acid residue, as well as hydrazinolytic cleavage of I from the solid support, have been automated. The regenerated resin II was reused several times for the synthesis of the same compound to give automatically several batches of I. Results of this process are compared with results of other solid phase and classical syntheses of the Gly-Phe-Phe-Tyr-Thr sequence.

In solid phase peptide synthesis,[1] the process of assembling the peptide chain anchored to a polymer support has been quite effectively automated.[2] However, the attachment of the first amino acid residue to the resin and the cleavage of the anchoring linkage in order to release the products from the solid support have to be carried out individually in separate vessels.[3-5] In the following, a completely automatic recycled synthesis of the protected pentapeptide hydrazide Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$[6] (I) on the hydroxymethyl resin[7,8] (II) is described. The results of five consecutive syntheses of I on the same batch of resin II are presented and compared with results of several experiments in which the same pentapeptide sequence Gly-Phe-Phe-Tyr-Thr was prepared by different procedures.

For the fully automated synthesis of I, a Beckman Model 990 peptide synthesizer[9] was programmed to perform all operations described below within the same reaction vessel. Boc-Thr(Bzl)-OH was esterified to resin II by the 4-dimethylaminopyridine catalyzed dicyclohexylcarbodiimide (DCC) procedure.[8] After benzoylation to block remaining unreacted alcoholic functions on the resin, Boc-Tyr(Bzl)-OH, Boc-Phe-OH, Boc-Phe-OH, and Boc-Gly-OH were sequentially coupled to the growing peptide chain on the resin according to general principles of the solid phase method.[1-5] In each coupling cycle, the *tert*-butyloxycarbonyl group was removed by a 20-min treatment with 33% trifluoroacetic acid in CH_2Cl_2 and the coupling reaction was effected with 2.5-fold excess each of Boc-amino acid and DCC for 2 h. Upon completion of the chain assembly the pentapeptide resin was stirred with 10% H_2NNH_2 in DMF for 16 h. Product I released from the polymer support was obtained in crystalline form after evaporation of the reaction and wash fluids collected from the vessel outlet. The hydrazinolysis reaction served also to regenerate resin II which remained in the reaction vessel. It was recycled four times through the entire synthetic protocol to give a total of five batches of I, which was purified by recrystallization. Overall yields from each run were approximately 60% with no sign of decreasing (see Table I). The resin particles survived all operations as evident from inspection of the beads before and after these experiments under a microscope. There was no indication of any disintegration of resin particles. The completeness of the hydrazinolytic cleavage was checked after each run by ir spectrophotometry.[8] The rate of hydrazinolysis was found to be surprisingly rapid with a half-life of about 45 min.

Thus, with the possible exception of aspartic or glutamic acid containing peptides, the process described above appears to be rather versatile and generally applicable to rapid synthesis of protected oligopeptide hydrazides. These are useful intermediates for polypeptide synthesis by the azide method[10] allowing effective combination of solid phase techniques and classical procedures[11,12] with retention of the best features of each.[4]

In Table II, the results of recycled automated synthesis of I are compared with those of other processes for the synthesis of the same sequence.[13] A dramatic increase in speed, efficiency, and simplicity can be noted.

The manual solid phase synthesis of I on hydroxymethyl

Table I. Recycled Automated Synthesis of Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ on $HOCH_2$-C_6H_4-Resin[a]

Run no.	Product wt, g	Yield, %[b]	Mp, °C	$[\alpha]^{25}D$, deg		Anal.		
					Calcd	67.30 C	6.63 H	10.56 N
1	1.80	54.0	229–230	−2.24		67.10	6.68	10.43
2	2.09	62.4	228–230	−2.12		67.05	6.73	10.32
3	2.01	60.2	229–230	−2.35		67.32	6.76	10.39
4	2.04	61.1	229–230	−2.38		67.04	6.63	10.43
5	2.05	61.4	229–230	−2.16		67.47	6.52	10.33

[a] Hydroxymethylated copolystyrene–1% divinylbenzene (6.0 g, 0.70 mmol/g) was used. The degree of substitution was 0.55 mmol Thr/g. [b] Theoretical yield, 3.6 mmol = 3.34 g.

Table II. Synthesis of Gly-Phe-Phe-Tyr-Thr Sequence by Solid Phase Techniques and Classical Method

Compd	Synthetic method	Time consumed	Overall yield, %
Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (I) (mp 229–230 °C, cryst)	$HOCH_2$–Resin(automated)	30 h	59.2[a]
Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (I) (mp 227–229 °C, cryst)	$HOCH_2$–Resin(manual)[b]	5 days	61.5
Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (III) (mp 215–218 °C, cryst)	$ClCH_2$–Resin	6 days	34.0
Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OH (IV) (mp 205–208 °C, cryst)	$HOCH_2$–OCH_2–Resin	4 days	61.2
Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (V) (mp 196–198 °C, cryst)	H_2NNH–COO–C(CH_3)(CH_3)–CH_2–CH_2–OCH_2–Resin[b]	4 days	36.1
Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (VI) (mp 241–243 °C, cryst)	Classical synthesis (3 + 2)	40 days[c]	33.0

[a] Average of five synthetic runs. [b] For experimental details, see ref 8. [c] The time consumed includes purification and characterization of intermediates. The actual time spent on the synthetic operations was about 15 days.

resin as well as the preparation of Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (V) on 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin have already been described previously.[8] Preparation of Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (III) by the standard Merrifield technique[14] on chloromethyl resin gave the desired compound in 34% overall yield. This synthesis was started by refluxing Boc-Thr(Bzl)-OH triethylamine salt with $ClCH_2$-C_6H_4-resin. The pentapeptide chain was subsequently built up in the usual manner[3,14] with Boc-Tyr(Bzl)-OH, Boc-Phe-OH, Boc-Phe-OH, and Z-Gly-OH. The low yield of this process probably is attributable to the fact that the clevage product is heavily contaminated with hydrazine hydrochloride which tends to reduce the recovery of the desired compound by crystallization.

For the synthesis of Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OH (IV), Bpoc-Thr(Bzl)-OH was esterified to *p*-alkoxybenzyl alcohol resin[15] through the 4-dimethylaminopyridine catalyzed DCC method.[8] The synthesis was then continued by sequential incorporation of Bpoc-Tyr(Bzl)-OH, Bpoc-Phe-OH, Bpoc-Phe-OH, and Z-Gly-OH into the resin under the conditions identical with those described above except that the deblocking of the Bpoc groups was effected by 0.5% TFA in CH_2Cl_2 (10 min). Product IV was obtained in 61.2% overall yield as a pure, crystalline solid after cleavage from the polymer support by 50% TFA (30 min) and crystallization.

For a classical synthesis of Z-Gly-Phe-Phe-Tyr(Bzl)-Thr-$HNNH_2$ (VI), a 3 + 2 fragment condensation approach was chosen. Boc-Tyr(Bzl)-OH was coupled to H-Thr-OCH_3 by the DCC procedure[16] to provide Boc-Tyr(Bzl)-Thr-OCH_3 which on treatment with HCl–THF gave the dipeptide ester salt HCl·H-Tyr(Bzl)-Thr-OCH_3. Reaction of phenylalanine with Boc-Phe-OSu yielded Boc-Phe-Phe-OH, which was treated with TFA and the resulting dipeptide H-Phe-Phe-OH was subsequently acylated with Z-Gly-OSu to give Z-Gly-Phe-Phe-OH. This tripeptide was then condensed with the dipeptide ester HCl·Tyr(Bzl)-Thr-OCH_3 obtained above by the DCC–HOBT procedure[17] to afford the pentapeptide methyl ester Z-Gly-Phe-Phe-Tyr(Bzl)-Thr-OCH_3. On hydrazinolysis, the desired product VI was obtained in 33% overall yield. A total of 40 days were required for completing this synthesis, including the time spent on purification, crystallization, and analytical characterization of the intermediates and the product. It is obvious that the time requirement would be appreciably reduced if the classical synthesis were to be repeated, and it could readily be scaled up. However, the method is far less adaptable to automation than solid phase synthesis.

Reuse of the same hydroxymethyl resin (II) after synthesis of one compound for he preparation of another is also demonstrated. The resin II that had been used as the polymer support for synthesis I was utilized in solid phase synthesis of Z-Gly-His(Tos)-Lys(Z)-OCH_2-C_6H_4-resin. Ammonolysis of this material produced crystalline pure Z-Gly-His-Lys(Z)-

NH_2 in 64.2% overall yield. The *p*-toluenesulfonyl protecting group of the histidine side chain was cleanly removed at the same time during ammonolytic cleavage.

Experimental Section

Melting points are uncorrected. Thin layer chromatography was carried out on precoated silica gel plates (Merck, F-254) using the solvent system *n*-BuOH–HOAc–H_2O (4:1:1), *n*-BuOH–pyridine–HOAc–H_2O (15:10:3:12), and *n*-BuOH–EtOAc–HOAc–H_2O (1:1:1:1). Elemental analyses, amino acid analyses, and other physicochemical measurements (uv, ir, NMR, specific rotation) were performed by the Physical Chemistry Department.

Merrifield resin (chloromethylated copolystyrene–1% divinylbenzene beads, 200–400 mesh, 0.70 mmol Cl/g) was purchased from Lab Systems, Inc., San Mateo, Calif. Hydroxymethyl resin was prepared from Merrifield resin as described previously.[8] Boc-amino acids were obtained from Bachem Inc., Marina Del Ray, Calif., or from Beckman Instruments, Inc. Bpoc-amino acids were prepared according to the literature.[18,19] Other chemicals and solvents used were all of reagent grade materials available form commercial sources.

Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-HNNH$_2$ (I). Hydroxymethyl resin II (6.0 g, 4.2 mmol) was placed in the reaction vessel of a Beckman Model 990 peptide synthesizer and the machine was programmed to perform the following steps automatically with 100-ml portions of solvents or reagents: (1) three washings with CH_2Cl_2, (2) stir 120 min with 10 mmol each of 4-dimethylaminopyridine, Boc-Thr(Bzl)-OH, and DCC in CH_2Cl_2, (3) three washings with CH_2Cl_2,[20] (4) stir 15 min with 4.5 mmol each of pyridine and benzoyl chloride in CH_2Cl_2, (5) three washings with CH_2Cl_2, (6) prewash with 33% TFA in CH_2Cl_2, (7) stir 20 min with 33% TFA in CH_2Cl_2, (8) three washings each with 33% dioxane in CH_2Cl_2, CH_2Cl_2 (9) prewash with 10% Et_3N in CH_2Cl_2, (10) stir 10 min with 10% Et_3N in CH_2Cl_2, (11) three washings with CH_2Cl_2, (12) stir 120 min with 10 mmol each of Boc-Tyr(Bzl)-OH and DCC in CH_2Cl_2, (13) three washings each with CH_2Cl_2, DMF, MeOH, (14) repeat steps 5–13 using Boc-Phe-OH (10 mmol) in step 12 instead of Boc-Tyr(Bzl)-OH, (15) repeat steps 5–13 with Boc-Phe-OH (10 mmol) in 12th step, (16) repeat steps 5–13 with Boc-Gly-OH (10 mmol) in 12th step, (17) three washings with CH_2Cl_2, (18) stir 990 min in 10% anhydrous H_2NNH_2 in DMF, collect the filtrate, (19) rinse with DMF, collect the filtrate, (20) rerun steps 1–19 four more times.

The filtrates from steps 18 and 19 in each run wer separately evaporated to dryness and the residue treated with ether. The crude solid material obtained was triturated with boiling MeOH and crystallized from DMF (60 ml) and EtOH (120 ml). The materials obtained from five runs were analyzed and then results listed in Table I.

Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-HNNH$_2$ (III). Boc-Thr(Bzl)-OCH$_2$-C$_6$H$_4$-resin (25 g, 3.5 mmol) prepared from chloromethyl resin (0.70 mmol Cl/g, 1% DVB) and Boc-Thr(Bzl)-OH (4.7 g) according to the literature procedure[3] was deprotected (50% TFA, 30 min), neutralized (10% Et_3N, 10 min) and coupled with Boc-Tyr(Bzl)-OH (2.7 g, 8.7 mmol) in the presence of DCC (1.81 g) in CH_2Cl_2 (120 min). The synthetic cycle was repeated with 8.7 mmol each of Boc-Phe-OH (2.3 g), Boc-Phe-OH, and Z-Gly-OH (1.83 g) to give Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OCH$_2$C$_6$H$_4$-resin (26.2 g). It was suspended in DMF and stirred with H_2NNH_2 (8 ml) for 66 h. The peptide in the filtrate was then concentrated to near dryness and treated with ether, whereupon 5.7 g of solid precipitated. The crude material was triturated in MeOH and crystallized from DMF and EtOH: yield, 1.15 g (34%); mp 215–218 °C; $[\alpha]^{25}D$ −3.94 ° (*c* 1, DMF); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{55}H_{59}N_7O_9$ (962.1): C, 68.66; H, 6.18; N, 10.19. Found: C, 68.67; H, 6.16; N, 9.91.

Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OH (IV). Bpoc-Thr(Bzl)-OCH$_2$-C$_6$H$_4$-OCH$_2$-C$_6$H$_4$-resin (1.1 g, 0.43 mmol) prepared from Bpoc-Thr(Bzl)-OH, HOCH$_2$-C$_6$H$_4$-OCH$_2$-C$_6$H$_4$-resin[15] by the dimethylaninopyridine catalyzed DCC procedure[8] was deprotected (0.5% TFA in CH_2Cl_2, 10 min), neutralized (10% Et_3N in CH_2Cl_2), and coupled (120 min) with Bpoc-Try(Bzl)-OH (0.55 g, 1.08 mmol) in the presence of DCC (0.277 g). The coupling cycle was repeated with 1.08 mmol each of Bpoc-Phe-OH (0.44 g), Bpoc-Phe-OH, and Z-Gly-OH (0.26 g) to give Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OCH$_2$-C$_6$H$_4$-OCH$_2$-C$_6$H$_4$-resin (1.21 g). During the synthesis, 0.5% TFA in CH_2Cl_2 (10 min) was utilized as deprotecting agent for Bpoc group. The peptide was released from the resin with 50% TFA in CH_2Cl_2 (30 min) and isolated as an amorphous solid when the solvents were removed and the residue was treated with ether. The compound was crystallized from MeOH: yield, 0.25 g (61.2%); mp 205–208 °C; $[\alpha]^{25}D$ +13.97° (*c* 1, HOAc); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{55}H_{57}N_5O_{10}$ (948.0): C, 69.68; H, 6.06; N, 7.39. Found: C, 69.39; H, 5.90; N, 7.35.

H-Thr-OCH$_3$. A suspension of L-threonine (50 g) in 500 ml of MeOH was nearly saturated with HCl gas. The mixture was then refluxed at 85 °C for 30 min. The solvent was evaporated at 40 °C and the residue taken up in 300 ml of fresh MeOH which was again evaporated. After two repetitions of the entire process, 85 g of a clear oil was obtained. It was dispersed in $CHCl_3$ (280 ml) and treated with an equal volume of NH_3 saturated $CHCl_3$ at 0 °C. A white precipitate of NH_4Cl formed which was filtered off. Concentration at 15 °C provided a heavy oil which solidified on standing. Crystalization from ether and petroleum ether yielded 44.4 g (79%) of the desired compound: mp 68–70 °C (lit.[21] mp 70–72 °C); $[\alpha]^{25}D$ +14.61° (*c* 1, MeOH).

Anal. Calcd for $C_{15}H_{11}NO_3$ (133.15): C, 45.11; H, 8.33; N, 10.52. Found: C, 45.01; H, 8.40; N, 10.46.

Boc-Tyr(Bzl)-Thr-OCH$_3$. Boc-Tyr(Bzl)-OH (15.8 g) was stirred with H-Thr-OCH$_3$ (5.67 g) and DCC (9.65 g) at 0 °C for 1 h and at 25 °C for 2 h. After removal of the insoluble by-products the solvent was evaporated to form a syrup. It was taken up in 200 ml of ethyl acetate, filtered, and evaporated again to an oil. The compound crystallized when stored under petroleum ether. Recrystallized from THF and petroleum ether: yield 17.2 g (83%); mp 110–112 °C; $[\alpha]^{25}D$ −2.72° (*c* 1, MeOH).

Anal. Calcd for $C_{26}H_{34}N_2O_7$ (486.6); C, 64.18; H, 7.04; N, 5.76. Found: C, 64.40; H, 7.08; N, 5.71.

H-Tyr(Bzl)-Thr-OCH$_3$·HCl. The above compound (11.4 g) was dissolved in 500 ml of freshly prepared 2.6 N HCl in THF. After standing for 90 min with occasional shaking, the accumulated solid product was collected and washed with ether. The crude product was recrystallized from MeOH and ether: yield 7.0 g (71%); mp 232–234 °C; $[\alpha]^{25}D$ +2.09° (*c* 1, MeOH).

Anal. Calcd for $C_{21}H_{26}N_2O_5$·HCl (422.9): C, 59.64; H, 6.44; N, 6.62. Found: C, 59.43; H, 6.42; N, 6.54.

Boc-Phe-Phe-OH. L-Phenylalanine (13.2 g) was ground in a motar and pestle and suspended in 250 ml of DMF. It was stirred with 29 g of Boc-Phe-OSu for 24 h in the presence of 9.5 g of tetramethylguanidine. The reaction mixture was then partitioned between 600 ml of 2% citric acid and 800 ml of ethyl acetate. The organic layer was washed with 2% citric acid followed by three washings with water, dried (Na_2SO_4), and evaporated to an oil which solidified gradually. The compound was crystallized from ethyl acetate by addition of petroleum ether: yield 17.5 g (53%); mp 145–146 °C; $[\alpha]^{25}D$ −2.67° (*c* 1, MeOH).

Anal. Calcd for $C_{23}H_{28}N_2O_5$ (412.5): C, 66.98; H, 6.84; N, 6.79. Found: C, 66.93; H, 6.81; N, 6.79.

Z-Gly-Phe-Phe-OH. Boc-Phe-Phe-OH (12.5 g) was dissolved in 120 ml of TFA and left standing for 15 min. After evaporation of the solvents, the residue was treated with ether upon which the dipeptide salt precipitated as white solid. It was collected and washed with ether and then stirred with 9.5 g of Z-Gly-OSu for 24 h in the presence of 6.5 ml of Et_3N. The product was worked up as usual ad crystallized from ethyl acetate: yield 12.2 g (80%); mp 180–182 °C; $[\alpha]^{25}D$ +16.74° (*c* 1, HOAc).

Anal. Calcd for $C_{28}H_{29}N_3O_6$ (503.6): C, 66.79; H, 5.81; N, 8.34. Found: C, 66.72; H, 5.69; N, 8.34.

Z-Gly-Phe-Phe-Tyr(Bzl)-Thr-OCH$_3$. The dipeptide salt H-Tyr(Bzl)-Thr-OCH$_3$·HCl (6.25 g) and the tripeptide Z-Gly-Phe-Phe-OH (7.43 g) were dissolved in 120 ml of DMF and cooled to −10 °C when 1.66 ml of *N*-methylmorpholine, 4.0 g of HOBT, and 3.7 g of DCC were added. The mixture was stirred at −10 °C for 4 h and then at 25 °C for 48 h. Removal of the insoluble by-product and evaporation of the solvent (40 °C) left a solid mass. It was triturated with ethyl acetate and crystallized from MeOH: yield 8.5 g (66%); mp 181–184 °C; $[\alpha]^{25}D$ −16.58° (*c* 1, DMF).

Anal. Calcd for $C_{49}H_{53}N_5O_{10}$ (871.99): C, 67.49; H, 6.13; N, 8.03. Found: C, 66.97; H, 6.11; N, 8.08.

Z-Gly-Phe-Phe-Tyr(Bzl)-Thr-HNNH$_2$ (VI). The pentapeptide methyl ester above (8.5 g) was stirred in 10% H_2NNH_2 in DMF (140 ml) for 24 h. Upon dilution with 1500 ml of MeOH a heavy white precipitate formed. The product was crystallized from DMF (115 ml) and MeOH (250 ml): yield 6.8 g (78%); mp 241–243 °C; $[\alpha]^{25}D$ −16.73° (*c* 1, DMF); NMR spectrum agreed with the structure. Amino acid analysis after hydrogenation and digestion with leucine amino peptidase: Gly, 1.02, Thr, 1.01; Tyr, 0.97; Phe, 2.11.

Anal. Calcd for $C_{48}H_{53}N_7O_9$ (871.96): C, 66.11; H, 6.12; N, 11.24. Found: C, 65.95; H, 6.19; N, 11.09.

Z-Gly-His-Lys(Z)-nh$_2$ Resin II (5 g) that had been used for the

synthesis of I as described[8] was allowed to react with Boc-Lys(Z)-OH (1.95 g), pyridine (0.4 ml), and DCC (1.1 g) in CH_2Cl_2 for 2 h to give 6.0 g of Boc-Lys(Z)-OCH_2-C_6-H_4-resin (3.24 mmol). After benzoylation[15] at 0 °C for 15 min with 0.83 ml of pyridine and 0.98 ml of benzoyl chloride in 60 ml of CH_2Cl_2 the resin was deprotected (50% TFA in CH_2Cl_2, 30 min), neutralized (10% Et_3N in CH_2Cl_2, 10 min), and coupled (120 min) with Boc-His(Tos)-OH·DCHA (5.4 g, 8.1 mmol)[22] in the presence of DCC (1.69 g). The synthetic cycle was repeated again with 8.1 mmol each of Z-Gly-OH (1.7 g) and DCC (1.69 g) to give 6.9 g of Z-Gly-His(Tos)-Lys(Z)-OCH_2-C_6H_4-resin. Ammonolysis in 450 ml of NH_3-saturated MeOH for 70 h provided a partially crystalline precipitate. It was concentrated to a smaller volume, diluted with an equal volume of DMF, filtered to remove the resin particles, and evaporated to a solid mass. Crystallization from DMF with MeOH gave 1.31 g (64%) of the desired compound: mp 210–212 °C; $[\alpha]^{25}D$ −8.51° (*c* 1, DMF); the NMR spectrum agreed with the structure.

Anal. Calcd for $C_{30}H_{37}N_7O_7$ (607.6): C, 59.30; H, 6.14; N, 16.14. Found: C, 59.24; H, 6.09; N, 16.16.

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Registry No.—I, 54276-64-1; III, 59790-71-5; IV, 54647-58-4; V, 54276-67-4; VI, 57471-75-7; Boc-Thr(Bzl)-OH, 15260-10-3; Boc-Tyr(Bzl)-OH, 2130-96-3; Boc-Phe-OH, 13734-34-4; Boc-Gly-OH, 4530-20-5; Z-Gly-OH, 1138-80-3; Bpoc-Tyr(Bzl)-OH, 25692-91-5; Bpoc-Phe-OH, 40099-50-1; H-Thr-OCH_3, 59790-72-6; H-Thr-OH, 72-19-5; Boc-Tyr(Bzl)-Thr-OCH_3, 3373-59-9; H-Tyr(Bzl)-Thr-OCH_3·HCl, 57471-73-5; Boc-Phe-Phe-OH, 13122-90-2; H-Phe-OH, 63-91-2; Boc-Phe-OSu, 3674-06-4; Z-Gly-Phe-Ph-OH, 57471-71-3; Z-Gly-OSu, 2899-60-7; Z-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-Thr-OCH_3, 57471-74-6; Z-Gly-His-Lys(Z)-NH_2, 59790-73-7; Boc-Lys(Z)-OH, 2389-45-9; copolystyrene–divinylbenzene, 9003-70-7.

References and Notes

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Solid Phase Synthesis of Protected Peptides via Photolytic Cleavage of the α-Methylphenacyl Ester Anchoring Linkage

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Photolysis of α-methylphenacyl esters was adapted to solid phase peptide synthesis. Cleavage of the peptide to resin α-methylphenacyl ester anchoring bond by irradiation at 350 nm provided protected peptides in good yields. The process is examplified by the synthesis of Z-Lys(Z)-Phe-Phe-Gly-OH. For comparison, the same peptide was also prepared through photolytic cleavage of the *o*-nitrobenzyl ester anchoring linkage.

Studies on several photolyzable protecting groups that are potentially useful in peptide chemistry have recently been described in the literature.[1-11] Among these, the α-methylphenacyl ester[8] is of particular interest since it can readily be introduced into polymer matrices[12] and thus serve as an anchoring linkage between peptide chain and polymer support in solid phase synthesis.[13-17] Photolytic cleavage of this bond would therefore provide protected peptide intermediates that could subsequently be utilized in the synthesis of polypeptides by fragment condensation.[18-21]

In this report, the development of an efficient and convenient procedure for the preparation of protected peptides based on photolysis of the polymer linked α-methylphenacyl ester bond is described. A similar process involving photolytic cleavage of peptides from the *o*-nitrobenzyl ester resin[13] has recently been outlined.[10]

2-Bromopropionyl chloride was allowed to react with copolystyrene–2% divinylbenzene beads (200–400 mesh) in the presence of $AlCl_3$ as catalyst to form 2-bromopropionyl resin $BrCH(CH_3)CO$-C_6H_4-resin (I). The product contained 0.94 mmol of Br per gram of resin according to microanalysis. It showed an intense absorption band at 1685 cm^{-1} in the ir spectrum. The incorporation of Boc amino acids[22] into the resin was achieved by stirring I with a slight excess of Boc amino acid cesium salt[23] in dimethylformamide. The resultant Boc-HN-CHR-COO-CH-(CH_3)-CO-C_6H_4-resin (II) showed strong absorption bands at 1750 and 1725 cm^{-1} in addition to that at 1685 cm^{-1} in the ir spectrum. The degree of substitution is normally in the range of 0.5–0.7 mmol/g. There was practically no residual Br remaining after this treatment.

As outlined in Scheme I, Boc-Gly-OCH(CH_3)-CO-C_6H_4-resin (II) was deprotected, neutralized, and coupled with Boc-Phe-OH. The synthetic cycle was repeated with Boc-Phe-OH and then again with Z-Lys(Z)-OH. The