A MODIFIED BENZHYDRYLAMINE — A USEFUL HANDLE REAGENT FOR Fmoc BASED SOLID PHASE SYNTHESIS OF PEPTIDE AMIDES*

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Dedicated to the memory of Dr Karel Bláha.

Usefulness of a dimethoxybenzhydrylamine derivative, 3-(3-(Fmoc-amino-4-methoxyphenylmethyl)-4-methoxyphenyl)propionic acid, for Fmoc-based solid phase synthesis of peptide amides was demonstrated by preparation of three biologically active peptide amides, i.e. tetragastrin, neuromedin B and [8-arginine]vasopressin. 1M trimethylsilyl bromide–thioanisole (molar ratio 1 : 1) in trifluoroacetic acid was recommended as a deprotecting reagent for releasing the peptide amides from the resin.

Recently, 9-fluorenylmethyloxycarbonyl group** introduced by Carpino and Han[1,2] as a base-labile protecting group, has been successfully applied for automated solid phase synthesis of peptides[3] in combination with acid-labile side-chain protecting groups, such as Boc and tBu ester, which can be cleaved in the final step of the synthesis by mild TFA treatment. Its characteristic ultraviolet absorption spectra enable to monitor the progress of reactions, i.e. condensation and N^{α}-deprotection on a polymer support[4]. This Fmoc-based strategy simplified the synthesis of peptides containing Met and Cys as well as of some peptides containing acid-sensitive Trp. However, so far, the procedure for the preparation of peptide amides by the Fmoc strategy seems not to be established sufficiently. It is necessary to explore suitable precursors of the amide function, preferably precursors more acid-labile than those employed hitherto in the Merrifield's solid phase synthesis[5–8]. At the present, several resins based on benzylamine[9–11] or benzhydrylamine[12–15] have been in-

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** Unless stated otherwise, all amino acids with the exception of Gly are of L-configuration. The following abbreviations are used: Fmoc, 9-fluorenylmethyloxycarbonyl; Boc, tert-butoxycarbonyl; tBu, tert-butyl; Acm, acetamidomethyl; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulphonyl; DCC, dicyclohexylcarbodiimide; HOBt, N-hydroxybenzotriazole; OSu, N-succinimido-oxy; DMF, dimethylformamide.

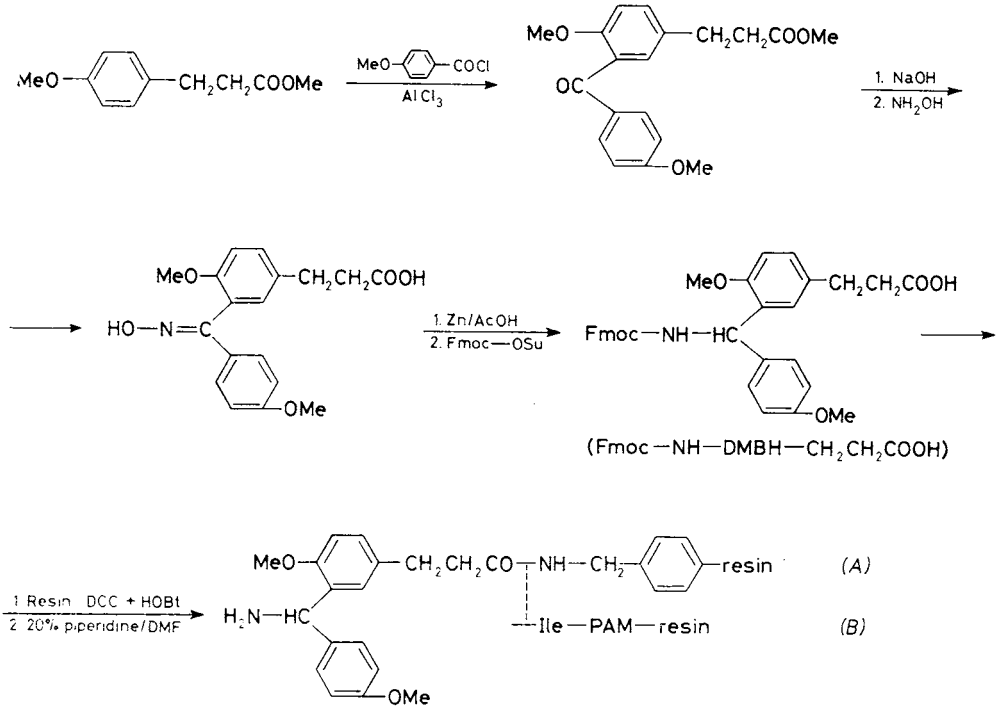
troduced for this purpose. However, chloromethylated polystyrene resin has to be manipulated in order to prepare these resins. In comparison with such laborious manipulation, application of a handle reagent which can be directly introduced onto a commercially available polystyrene resin, is attractive for practical purposes. Albericio and Barany[16] have introduced a modified benzylamine reagent, 5-[(2′ or 4′)--Fmoc-aminomethyl]-3′,5′-dimethoxyphenoxyvaleric acid preparable in 15% overall yield in a 7-step reaction as a suitable candidate. We have modified benzhydrylamine moiety in such manner that two methoxy groups were introduced at the both aromatic rings to make the C—N linkage acid-labile, and propionic acid group was added as an attachment site onto the aminomethylated polystyrene resin[17].

Our modified dimethoxybenzhydrylamine derivative, 3-(3-(Fmoc-amino-4-methoxyphenylmethyl)-4-methoxyphenyl)propionic acid (Fmoc-NH-DMBH-CH_2CH_2--COOH), was simply prepared in five successive reactions, starting with methyl 3-(*p*-methoxyphenyl)propionate, the total yield being 68% (Scheme 1): *1*) Friedel–-Crafts condensation with *p*-methoxybenzoyl chloride; *2*) saponification of the methyl ester; *3*) hydroxylamine treatment; *4*) reduction of the oxime with Zn in AcOH; *5*) Fmoc-OSu (refs[18–20]) treatment. The resulting Fmoc handle reagent was loaded on the aminomethylpolystyrene resin by condensation with DCC in the presence of HOBt (ref.[21]), until the resin became negative to Kaiser test[22]. The Fmoc group was removed by treatment with 20% piperidine in DMF (refs[1,2]) before use. Thus, 2,4′-dimethoxybenzhydrylamine resin anchored through the propionyl linkage (named A-resin) was prepared readily. Afterwards, we condensed this Fmoc handle reagent with H-Ile-PAM-resin[23], and then the Fmoc group was removed as described above. Ile in this resin (named B-resin) served for monitoring the progress of the reactions after acid hydrolysis.

First, the properties of the two above mentioned amide-precursor resins were examined by using Fmoc-amino acids. Condensation of Fmoc-Phe-OH with the resins (A or B) via the corresponding pentafluorophenyl esters[24] were completed within 60 min. After Fmoc cleavage, H-Phe-NH_2 was liberated almost quantitatively when the resin was treated with 1M thioanisole–TFA at 25°C for 60 min. When the treated Phe-resin-B was subjected to 6M-HCl hydrolysis, the amount of Phe detected by an amino acid analyzer was negligible, indicating that Phe was completely cleaved from the resin by the thioanisole-mediated TFA deprotection[25]. The role of thioanisole was remarkable during this deprotection since Phe was liberated from Phe-resin-B in only 2% yield by the treatment with TFA–CH_2Cl_2 (1 : 1) at 25°C for 60 min. The above results indicate that the Fmoc handle reagent serves well as an amide precursor for amino acids, 1M thioanisole–TFA being employed as a cleavage reagent.

Next, usefulness of these new supports for solid phase synthesis of the amides was examined by preparation of three biologically active peptides. First, tetragastrin[26] was synthesized by using the resin-B. Fmoc-Trp-Met-Asp(OtBu)-Phe-resin-B,

prepared manually according to the principles of Fmoc-strategy was treated with 20% piperidine in DMF, and then with 1M thioanisole–TFA at 28°C for 60 min. After purification by means of high performance liquid chromatography (HPLC), the product with an identical retention time in HPLC with that of an authentic sample of the synthetic tetragastrin was obtained in 41% yield (based on Ile on the resin-B – Fig. 1). Thus, tetragastrin, a C-amidated peptide was synthesized according to the Fmoc strategy without masking the functional groups of Trp and Met. However, acid hydrolysis of the above treated resin still indicated the presence of almost 39% of uncleaved peptide on the resin. This result indicates that longer TFA-treatment than that employed for the cleavage of amino acid amides from the resin seems to be required for cleavage the peptide amides from the resin.



Next, neuromedin B (refs[27,28]), a porcine spinal cord peptide amide was synthesized. Fmoc-Gly-Asn-Leu-Trp-Ala-Thr(tBu)-Gly-His(Boc)-Phe-Met-resin-B, prepared manually, was treated with 20% piperidine in DMF, and then with 1M thioanisole–TFA at 28°C for 60 min as described above. After HPLC purification, the product was obtained in 21% yield (see Fig. 2). Here again, a peptide amide containing Trp and Met has been synthesized without masking their functions. However, acid hydrolysis of the treated resin indicated that the peptide was cleaved

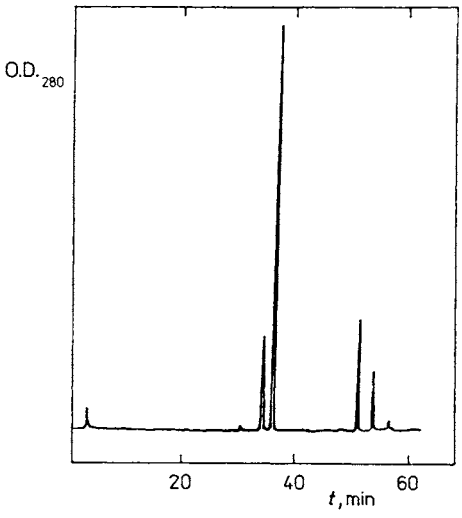


FIG. 1

HPLC of crude tetragastrin prepared by the Fmoc-based solid phase procedure (retention time 37·2 min)

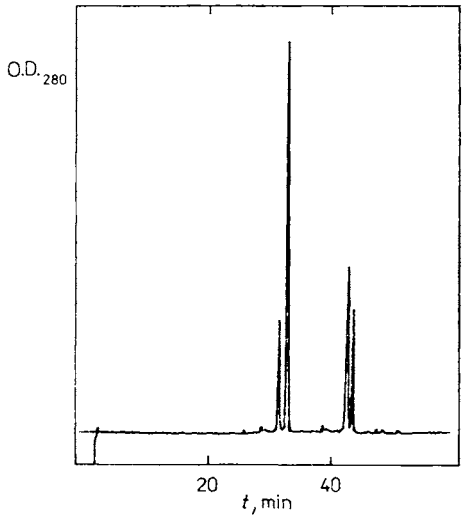


FIG. 2

HPLC of crude neuromedin B prepared by the Fmoc-based solid phase procedure (retention time 33·0 min)

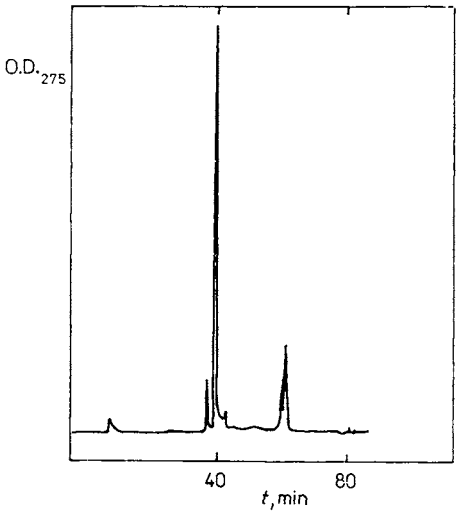


FIG. 3

HPLC of crude [8-arginine]vasopressin prepared by the Fmoc-based solid phase procedure (retention time 40·2 min)

from the resin in only 43% yield. The results suggested also that, for longer peptide amides, longer TFA-treatment is necessary. We have been able to demonstrate that the peptide amide could be cleaved from the resin, together with the cleavage of other protecting groups employed, by the treatment with 1M trimethylsilylbromide (TMSBr)–thioanisole–TFA (ref.[29]) (0°C, for 60 min) more effectively than with the former reagent. The yield was improved from 21% to 36% and the cleavage of the peptide amide reached 72%.

Finally, [8-arginine]vasopressin[30,31] has been synthesized by combination of our handle reagent with a newly introduced TMSBr–TFA deprotecting reagent. During this solid-phase synthesis, we were able to establish a disulphide bond on the resin by the treatment with thallium trifluoroacetate[32] and to deprotect Arg(Mtr) (ref.[33]) satisfactorily with 1M TMSBr–thioanisole–TFA. Fmoc-Cys(Acm)-Tyr(tBu)-Phe-Gln--Asn-Cys(Acm)-Pro-Arg(Mtr)-Gly-resin-A, prepared manually according to the Fmoc strategy was first treated with 20% piperidine–DMF, then with thallium trifluoroacetate–TFA in an ice-bath for 60 min and, finally, with 1M TMSBr–thioanisole––TFA in an ice-bath for 60 min. HPLC examination of the crude product revealed the presence of a main peak possessing an identical retention time as an authentic sample of [8-arginine]vasopressin (Fig. 3). Its HPLC isolation yield was 30%. It has been claimed that Arg(Mtr), when involved in the peptide chain, could not be deprotected satisfactorily by TFA–thioanisole treatment[34,35]. Our model experiments indicated the rate of the cleaving reaction of the Mtr with 1M TMSBr–thioanisole––TFA to be much faster than that using 1M thioanisole–TFA. From the above experiments we draw a conclusion that the thioanisole-mediated TMSBr–TFA deprotection procedure is useful for the solid phase synthesis of Arg-peptides.

As demonstrated above, an easily preparable handle reagent introduced here may offer wide application for solid phase synthesis of C-terminal peptide amides. The use of TMSBr–thioanisole–TFA as an alternative deprotecting reagent to thioanisole––TFA may open the possibility of application of a variety of amino acid derivatives for the Fmoc-based solid phase peptide synthesis, as this reagent is able to cleave various protecting groups currently employed in peptide synthesis under relatively mild conditions.

EXPERIMENTAL

The Fmoc-based solid phase synthesis was carried out according to the principle of Atherton et al.[36].

Fmoc cleavage: The resin was suspended in 20% piperidine in DMF (10 ml/g resin) at room temperature for 20 min. The resin was collected by filtration and washed with DMF and CH_2Cl_2 (20 ml/g resin, 3 times each).

Condensation: The Fmoc-cleaved resin was suspended in DMF (20 ml/g resin), the corresponding Fmoc-amino acid pentafluorophenyl (Pfp) ester (5 eq) and HOBt (5 eq) were added

and the suspension was shaken at room temperature for 60 min. The resin was collected by filtration and washed with DMF and CH_2Cl_2 (20 ml/g resin, 3 times each).

Chromatography was performed on a thin layer of silica gel (Merck) in systems: $CHCl_3$ (S1), $CHCl_3$–MeOH–H_2O 8 : 3 : 1 (S2). ^1H NMR spectra were taken with a JEOL FX-200 spectrometer using tetramethylsilane as an internal standard. Mass spectra were taken with a JEOL JMS-01SG-2 spectrometer. IR absorption was measured with a Hitachi 215 spectrophotometer. HPLC was conducted with a Waters 204 compact model.

Methyl 3-(4-Methoxybenzoyl)-4-methoxyphenylpropionate

$AlCl_3$ (26·7 g, 200 mmol, divided into 6 portions) was added to ice-chilled solution of methyl 3-(*p*-methoxyphenyl)propionate (10·8 g, 55·6 mmol) and *p*-methoxybenzoyl chloride (11·4 g, 66·8 mmol) in nitrobenzene (200 ml) during a period of 3 h, and stirring was continued for another 1 h. The mixture was poured on crushed ice to which 120 ml of 1M-HCl was added. The resulting precipitate was dissolved in ethyl acetate. The organic phase was washed with saturated $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography using 30% ethyl acetate–hexane to give an oily product. Yield: 17·8 g (97%). R_F 0·14 (S1); MS: 328·1 (calculated for $C_{19}H_{20}O_5$: 328·1). NMR ($CDCl_3$): 2·61 t, 2 H (J = 7·8); 2·92 t, 2 H (J = 7·8); 3·66 s, 3 H; 3·71 s, 3 H; 3·86 s, 3 H; 6·91 dt, 2 H (J = 9·0 and 2·3); 6·91 d, 1 H (J = 8·5); 7·14 d, 1 H (J = 2·2); 7·28 dd, 1 H (J = 8·5 and 2·2); 7·79 dt, 2 H (J = 9·0 and 2·3). IR (neat, cm^{-1}): λ_{max} 2 920, 1 723, 1 648, 1 590, 1 492, 1 416, 1 300, 1 250, 1 162, 1 022, 840.

Methyl 3-(3-(Hydroxyimino-4-methoxyphenylmethyl)-4-methoxyphenyl)propionate

Sodium hydroxide (5·0 g, 125 mmol) was added to an ice-chilled solution of the above ketone (17·3 g, 52·7 mmol) in CH_3OH (130 ml) and water (50 ml) and the mixture was stirred for 1 h. The resulting acid (R_F 0·84 (S2)) was allowed to react with hydroxylamine hydrochloride (33·0 g, 9·0 eq) in the presence of an additional portion of NaOH (13·8 g, 6·6 eq) under reflux for 7 h. The solvent was removed by evaporation and the residue, after being treated with citric acid, was extracted with ethyl acetate. The organic phase was washed with H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was recrystallized from ethyl acetate and 1-hexane; yield 15·2 g (88%), colorless needles, m.p. 177–178°C, R_F 0·75 (S2). ^1H NMR (CD_3OD): 2·59 t, 2 H (J = 7·4); 2·89 t, 2 H (J = 7·4); 3·57 s, 3 H; 3·79 s, 3 H; 6·85 dt, 2 H (J = 9·0 and 2·4); 6·9 d, 1 H (J = 8·3); 7·16 d, 1 H (J = 2·4); 7·25 dd, 1 H (J = 8·3 and 2·4); 7·52 dt, 2 H (J = 9·0 and 2·4). For $C_{18}H_9NO_5$ (329·4) calculated: 65·64% C, 5·81% H, 4·25% N; found: 65·66% C, 5·84% H, 4·26% N. IR (KBr, cm^{-1}): λ_{max} 3 170, 2 995, 1 702, 1 602, 1 512, 1 420, 1 248, 1 178, 1 021, 952, 830.

3-(3-(Amino-4-methoxyphenylmethyl)-4-methoxyphenyl)propionic Acid

The above oxime (5·0 g, 15·2 mmol) in acetic acid (140 ml) was treated with Zn powder (10 g) in an ice-bath. The mixture was stirred at room temperature for 1 h and filtered. The filtrate was concentrated and the residue was triturated with ethyl acetate. The resulting solid was recrystallized from ethanol; yield 3·84 g (80%). R_F 0·38 (S2); m.p. 191·5–192·5°C. ^1H NMR (CD_3OD): 2·41 t, 2 H (J = 6·8); 2·85 t, 2 H (J = 6·8); 3·78 s, 6 H; 5·63 s, 1 H; 6·88–6·98 m, 1 H; 6·93 d, 2 H (J = 8·7); 7·12–7·32 m, 2 H; 7·29 d, 2 H (J = 8·7). IR (KBr, cm^{-1}): λ_{max} 3 640, 3 370, 2 945, 1 635, 1 610, 1 565, 1 532, 1 510, 1 400, 1 245, 1 034. MS: 315·1 (calculated for $C_{18}H_{21}NO_4$: 315·1). For $C_{18}H_{21}NO_4.1{\cdot}5\ H_2O$ (342·4) calculated: 63·14% C, 7·06% H, 4·09% N; found: 63·38% C, 7·04% H, 3·88% N.

3-(3-(Fmoc-amino-4-methoxyphenylmethyl)-4-methoxyphenyl)propionic Acid

Fmoc-OSu (1·28 g, 1·2 eq) was added to a solution of the above amine (1·0 g, 3·17 mmol) in DMF (5 ml) containing triethylamine (0·88 ml, 2 eq) and the mixture was stirred at room temperature for 1 h. The solvent was removed by evaporation and the residue was treated with 5% citric acid and ether. The resulting powder was recrystallized from methanol and ether; yield 1·70 g (100%); m.p. 174–175°C, R_F 0·73 (S2). For $C_{33}H_{31}NO_6$ (537·6) calculated: 73·72% C, 5·81% H, 2·61% N; found: 73·47% C, 5·83% H, 2·59% N.

Preparation of NH_2-DMBH-CH_2CH_2-CO-aminomethylpolystyrene Resin (Resin-A)

DCC (326 mg, 1·1 eq) and HOBt (243 mg, 1·1 eq) were added to a solution of the above acid (774 mg, 1·44 mmol), the mixture was stirred at room temperature for 1 h, and filtered. The filtrate was mixed with the aminomethylpolystyrene resin (1 g, amino content 0·72 mmol/g), prewashed with DMF, and the suspension was gently shaken for 10 h, until the resin became negative to the Kaiser test. Further, the resin was treated with 20% acetic anhydride in DMF (20 ml) for 1 h in order to mask the amino function completely. Then, the resin was washed with DMF and CH_2Cl_2 (twice each) and dried in vacuo; yield 1·28 g. Before use, the resin thus prepared was treated with 20% piperidine in DMF (5 ml/200 mg resin) for 5 min. This treatment was repeated once more 15 min and the resin was washed with DMF (5 × 5 ml).

Preparation of NH_2-DMBH-CH_2CH_2-CO-Ile-PAM-resin (Resin-B)

Fmoc acid (269 mg, 0·5 mmol) in DMF (5 ml) was treated with DCC (103 mg, 1 eq) and HOBt (77 mg, 1·0 eq) as stated above. The filtrate was mixed with H-Ile-PAM-resin (139 mg, Ile content 0·76 mmol/g), prewashed with DMF, and the suspension was shaken for 3 h, until the resin became negative to the Kaiser test. The resin was washed with DMF and further treated with 20% acetic anhydride–DMF (5 ml) for 1 h. The resin was washed with DMF and with CH_2Cl_2 (3 times each) and dried under vacuo; yield 185 mg. Before use, the resin was treated with 20% piperidine–DMF as stated above.

Cleavage of H-Phe-NH_2 from the Resin-B

Fmoc-Phe-OPfp (0·5 mmol, 5 eq) and HOBt (0·5 mmol, 5 eq) were added to a suspension of the resin-B (prepared from 185 mg, 0·1 mmol of the Fmoc-derivative) in DMF (5 ml) and the mixture was shaken for 1 h. The resin was successively washed with DMF (5 × 5 ml), then treated with 20% piperidine in DMF (5 ml) for 5 min and with fresh 20% piperidine in DMF (5 ml) for another 15 min. The resin was washed with DMF (5 × 5 ml) and CH_2Cl_2 (2 × 5 ml) and dried in vacuo; yield 179 mg. Dried resin (5 mg) was treated with TFA–CH_2Cl_2 (1 : 1, 100 μl)–*m*-cresol (10 μl) or 1M thioanisole–TFA (100 μl)–*m*-cresol (10 μl) at 28°C for 60 min. H-Phe-NH_2 liberated was determined by a TLC scanner. The latter treated resin was subjected to 6M-HCl hydrolysis. No Phe was detected by an amino acid analyser.

H-Trp-Met-Asp-Phe-NH_2 (Tetragastrin)

Starting with the resin-B (185 mg, 0·1 mmol), the Fmoc-based solid phase synthesis was carried out manually according to Atherton et al.[36]. Treatment with 20% piperidine–DMF (5 ml, 5 min, and 5 ml, 15 min) followed by washing with DMF (5 × 5 ml); condensation of Pfp esters of the corresponding Fmoc amino acids (5 eq) in the presence of HOBt (5 eq) in DMF (5 ml) for 60 min, followed by washing with DMF (5 × 5 ml). H-Trp-Met-Asp(OtBu)-Phe-resin-B was

obtained (239 mg); amino acid ratios in a 6M-HCl hydrolysate: Asp 1·12, Met + Met(O) 0·52, Phe 1·10, Trp N. D., Ile 1·00 (internal standard). The peptide resin thus obtained (100 mg, 41·8 μmol) was treated with 1M thioanisole–TFA (5 ml) in the presence of 1,2-ethanedithio (293 μl) and *m*-cresol (293 μl) at 28°C for 1 h. The resin was removed by filtration and washed with TFA. The filtrate and the washing were combined, and TFA was removed by evaporation in vacuum. The powder resulting after the addition of dry ether was collected by centrifugation and washed with ether; yield 14·9 mg. After subjecting the treated resin to 6M-HCl hydrolysis, the ratio of Phe and Ile was 0·39 : 1·00, indicating a cleavage of 61% of the peptide from the resin under the above conditions. The crude peptide was purified by HPLC on a Cosmosil 5C18 ST column (4·6 × 150 mm) employing a gradient elution with acetonitrile (10–60%, for 50 min) in 0·1% aqueous TFA at a flow rate of 1 ml/min (retention time 37·2 min, identical with that of the synthetic tetragastrin); yield 10·2 mg (41%). Amino acid ratios after 4M-MSA hydrolysis: Asp 1·01, Met 0·72, Phe 1·00, Trp 0·82 (recovery of Phe 76%).

Neuromedin B

Starting with the resin-B (185 mg, amino content 0·1 mmol), H-Gly-Asn-Leu-Trp-Ala-Thr(tBu)--Gly-His(Boc)-Phe-Met-resin-B (291 mg) was synthesized utilizing the Fmoc-based solid phase synthesis, as stated above. Deprotection was carried out in two ways.

Thioanisole–TFA cleavage: The peptide resin (100 mg) was treated with 1M thioanisole–TFA (5 ml) in the presence of 1,2-ethanedithiol and *m*-cresol (293 μl each) at 28°C for 1 h. The resin was removed by filtration and washed with TFA (5 ml). The filtrate and the washing were combined and TFA was removed by evaporation in vacuo. The residue was collected by centrifugation and lyophilized from 50% acetic acid; yield 17·3 mg. Amino acid ratios in a 6M-HCl hydrolysate: Asp 1·02, Thr 1·04, Gly 2·13, Ala 2·05, Met 0·80, Leu 1·02, Phe 1·00, His 1·13, Trp N.D., Ile (diagnostic amino acid) 1·00. The treated resin subjected to 6M-HCl hydrolysis exhibited an amino acid ratio of Ile and Phe 1·00 : 0·57, indicating a cleavage yield 43% under the above conditions.

TMSBr–thioanisole–TFA cleavage: The peptide resin (100 mg) was treated with 1M-TMSBr––thioanisole–TFA in the presence of 1,2-ethanedithiol and *m*-cresol (295 μl, each) in an ice-bath for 1 h. The resin was removed by filtration and washed with TFA (5 ml). The filtrate and the washing were combined, TFA was removed by evaporation in vacuo as stated above, and dry ether was added. The resulting powder was collected by centrifugation and lyophilized from 50% acetic acid; yield 20·3 mg. Amino acid ratios of Ile and Phe in the treated resin were 1·00 : 0·28, indicating a cleavage yield of 72%. The crude product was purified by HPLC on a Cosmosil 5C18 ST column (4·6 × 150 mm) using a gradient of acetonitrile (10–60%, 50 min) in 0·1% aqueous TFA at a flow rate of 1 ml/min (the retention time 32·9 min, identical with that of an authentic sample of neuromedin B); yield 14·1 mg (36%, based on Met loaded on the resin). Amino acid ratios in a 4M-MSA hydrolysate: Asp 1·07, Ser 1·03, Gly 2·17, Ala 1·15, Met 0·89, Leu 1·06, Phe 1·00 , His 1·00, Trp 0·80 (recovery of Phe 83%).

[8-Arginine]vasopressin

Starting with the resin-B (178 mg, 0·1 mmol), H-Cys(Acm)-Tyr(tBu)-Phe-Gln-Asn-Cys(Acm)--Pro-Arg(Mtr)-Gly-resin-B (280 mg) was obtained utilizing the Fmoc-based solid phase method as stated above. Amino acid ratios of the peptide resin: Asp 0·84, Glu 0·83, Gly 1·00, Tyr 0·71, Pro 0·86, Phe 0·81, Arg 0·83. This peptide resin (100 mg, 26·5 μmol) was suspended in TFA (5 ml) and treated with $Tl(CF_3COO)_3$ (79·5 μmol, 3 eq) in the presence of anisole (0·5 ml) in an ice-bath for 60 min, with TMSBr and thioanisole (to a final concentration of 1 mol l^{-1}, each) for additional 60 min. The resin was washed with TFA (3 × 5 ml). The filtrate and the washing

were combined and TFA and TMSBr were removed by evaporation under vacuo. The residue was treated with dry ether and the resulting powder was lyophilized from H_2O and purified by gel-filtration on Sephadex G-10 (1·8 × 70 cm, elution with 0·2M acetic acid). The desired fractions (21—35, 3 ml each, monitored by UV absorption at 280 nm) were pooled and the solvent was removed by lyophilization; yield 13·5 mg (45%, based on Gly loaded on the resin). This crude sample was further purified by HPLC on a Cosmosil 5C18 column (1·0 × 25 cm) utilizing gradient elution wth acetonitrile (15—30%, 48 min) in 0·1% aqueous TFA at a flo w rate of 1·8 ml per min (retention time 20·5 min, identical with that of an authentic sample of [8-arginine]vasopressin); yield 8·8 mg (overall yield 30%, based on Gly loaded on the resin). Amino acid ratios in a 6M-HCl hydrolysate: Asp 0·97, Glu 0·96, Gly 1·00, Cys 0·48, Tyr 0·89, Phe 0·97, Arg 0·90, Pro 0·90 (recovery of Gly 100%).

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