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N-BENZHYDRYL-GLYCOLAMIDE ESTERS (OBg ESTERS) AS CARBOXYL PROTECTING GROUPS IN PEPTIDE SYNTHESIS

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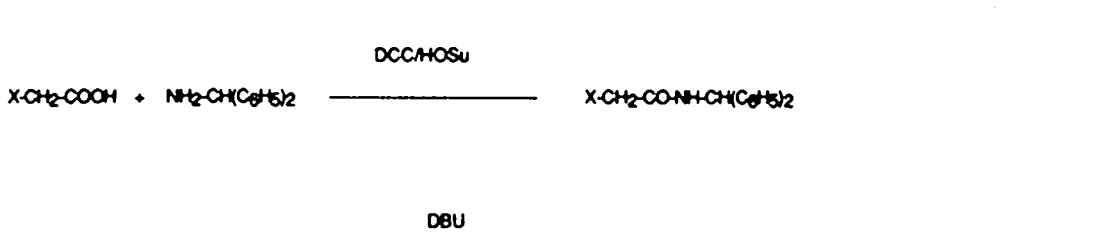
Abstract: N-Benzhydryl-glycolamide esters (OBg esters) of various N-protected amino acids have been synthesized. In order to demonstrate their usefulness in peptide chemistry, the syntheses of For-Met-Leu-Phe-OH (chemiotactic peptide) and Pro-Leu-Gly-NH_2 (MIF) have been carried out. OBg esters are compatible with commonly used protecting groups and are cleanly and selectively removed in mild alkaline conditions without any side reaction, except for β-benzyl aspartyl containing sequences.

We demonstrated a few years ago the usefulness of carboxamidomethyl esters (CAM esters) in peptide synthesis[1,2]. CAM esters showed good stability during removal of usual N-protecting groups and were cleanly and selectively removed in mild alkaline media (i.e. aqueous sodium carbonate). However, CAM esters of some N-protected amino acids or short peptide sequences showed a high water solubility, a property which has been used in enzymatic peptide syntheses[3], but which sometimes led to poor yields during workup of reaction mixtures. We therefore decided to investigate a CAM ester analogue bearing a large hydrophobic group on the amide, i.e. the benzhydryl group, in order to increase the liposolubility of the synthetic fragments. In this work, we describe a series of N-benzhydryl-glycolamide esters (OBg esters) of N-protected amino acids and investigate their use in peptide synthesis.

RESULTS AND DISCUSSION

N-Benzhydryl-glycolamide esters (OBg esters) are easily obtained by esterification of N-protected amino acids by a N-benzhydryl-haloacetamide in refluxing benzene or acetonitrile in the presence of 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU). This esterification method, described by Ono et al.[4] proved to be very efficient and racemization free, since it does not involve activation of the carboxyl moiety. N-Benzhydryl-chloroacetamide 1 and N-benzhydryl-bromoacetamide 2 were obtained in good yields by DCC coupling of chloro- or bromoacetic acid with benzhydrylamine in the presence of N-hydroxysuccinimide. Esterification was complete in less than 1 hour from the bromo derivative, and in about 3 hours from the chloro analogue (Scheme 1).



Y = Boc, Z. X = Cl, Br.

Scheme 1

Most of the N-protected amino acid derivatives were obtained as solids. Physical data of N-benzhydryl-glycolamide esters (OBg esters) of some Boc- and Z-amino acids are listed in table 1 (compounds 3 to 22). Their structure was ascertained by ^1H NMR spectroscopy (some examples are listed in the experimental part). Fmoc-amino acid N-benzhydryl-glycolamide esters could not be obtained by this method since DBU rapidly cleaved the Fmoc group. However, Fmoc-amino acid N-benzhydryl-glycolamide esters can be obtained from their cesium salt derivative, in the presence of N-benzhydryl bromoacetamide (or N-benzhydryl chloroacetamide), according to the method described by Gisin[14]. Alternatively, they can be prepared by a N,N dicyclohexylcarbodiimide / 4-(N,N-dimethylamino)pyridine (DCC/DMAP) mediated esterification with N-benzhydryl glycolamide, as described by Gilon and Klausner[15]. As an example, the synthesis of Fmoc-Nle-OBg 23, is described.

Ester Derivatives	React. solvent	X**	Cryst. solvent	Yield %	mp °C	$[\alpha]_D$ (c, DMF)	Rf*	Anal C,H,N
Boc-Ala-OBg 3	benzene	Cl	Et_2O/hexane	78	75-77	-17.4 (1,07)	A: 0.13; B: 0.59	$C_{23}H_{28}N_2O_5$
Boc-Arg(NO2)-OBg 4	acetonitrile	Br	Et_2O/hexane	75	103	-10.2 (1.09)	C: 0.31; D: 0.60	$C_{26}H_{34}N_6O_7$
Boc-Asn-OBg 5	acetonitrile	Br	Et_2O/hexane	68	65	-15.1 (1.25)	C: 0.47; D: 0.62	$C_{24}H_{29}N_3O_6$
Boc-Asp(OBzl)-OBg 6	benzene	Br	foam	88	42-45	-13.5 (1.16)	B: 0.62; C: 0.94	$C_{31}H_{34}N_2O_7$
Boc-Gln-OBg 7	acetonitrile	Br	Et_2O/hexane	82	163-167	-11.8 (1.02)	C: 0.49; D: 0.65	$C_{25}H_{31}N_3O_6$
Boc-Gly-OBg 8	benzene	Br	Et_2O/hexane	97	91-94		B: 0.59; C: 0.77	$C_{22}H_{26}N_2O_5$
Boc-Leu-OBg 9	benzene	Br	Et_2O/hexane	81	110	-19.7 (1.10)	B: 0.77; C: 0.94	$C_{26}H_{34}N_2O_5$
Boc-Lys(Z)-OBg 10	benzene	Br	AcOEt/hexane	85	117	-11.9 (1.09)	B: 0.47; C: 0.82	$C_{34}H_{41}N_3O_7$
Boc-Met-OBg 11	benzene	Br	CH_2Cl_2/hexane	85	74-76	-17.9 (1.03)	B: 0.67; C: 0.91	$C_{25}H_{32}N_2O_5S$
Boc-Phe-OBg 12	benzene	Cl	Et_2O/pentane	82	98-99	-18.9 (1.03)	A: 0.13; B: 0.69	$C_{29}H_{32}N_2O_5$
Boc-Phe-OBg 12	acetonitrile	Br	Et_2O/pentane	93	97-98	-16.7 (1.20)	A: 0.13; B: 0.69	$C_{29}H_{32}N_2O_5$
Boc-Pro-OBg 13	benzene	Br	AcOEt/hexane	75	114-116	-39.4 (1.09)	B: 0.60; C: 0.86	$C_{25}H_{30}N_2O_5$
Boc-Ser-OBg 14	benzene	Br	foam	88	58-60	-18.5 (1.01)	B: 0.39; C: 0.72	$C_{28}H_{28}N_2O_6$
Boc-Trp-OBg 15	benzene	Br	foam	78	63-65	-14.8 (0.94)	B: 0.37; C: 0.77	$C_{31}H_{33}N_3O_5$
Boc-Val-OBg 16	benzene	Br	Et_2O/pentane	85	107-110	-10.7 (1.15)	A: 0.22; B: 0.75	$C_{25}H_{32}N_2O_5$
Z-Ala-OBg 17	benzene	Br	Et_2O/hexane	75	94-96	-11.5 (1.01)	B: 0.43; C: 0.85	$C_{26}H_{26}N_2O_5$
Z-Asp(OBut)-OBg 18	benzene	Br	foam	89	44-46	-12.8 (1.11)	B: 0.57; C: 0.91	$C_{31}H_{34}N_2O_7$
Z-Glu(OBut)-OBg 19	benzene	Br	oil	85			B: 0.58; C: 0.92	
Z-Gly-OBg 20	benzene	Br	Et_2O/hexane	84	140-142		B: 0.31; C: 0.85	$C_{25}H_{24}N_2O_5$
Z-Thr-OBg 21	benzene	Br	foam	79	45-48	-10.1 (1.08)	B: 0.66; C: 0.94	$C_{27}H_{28}N_2O_6$
Z-Val-OBg 22	benzene	Br	CH_2Cl_2/hexane	94	121-122	- 4.4 (1.11)	B: 0.65; C: 0.92	$C_{28}H_{30}N_2O_5$
Fmoc-Nle-OBg 23	DMF	Br	Et_2O/hexane	80	107-109	-15.5 (1.03)	A: 0.28; B: 0.75	$C_{36}H_{36}N_2O_5$

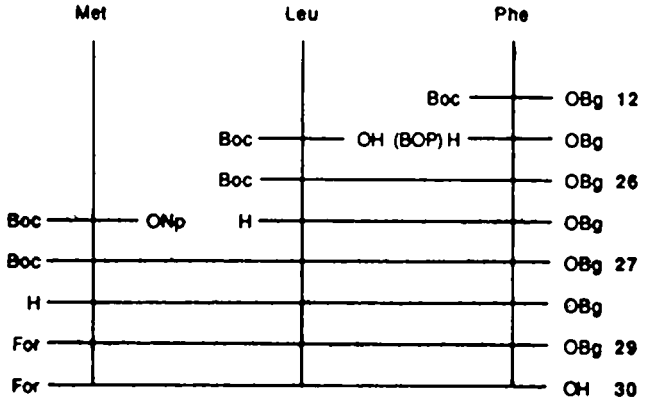
Table 1: Physical and analytical data of N-protected amino acid OBg esters. Experimental values of elemental analyses are within 0.4% of the calculated values. * TLC solvent systems: A: AcOEt/hexane 3:7; B: AcOEt/hexane 5:5; C: AcOEt/hexane 7:3; D: AcOEt. ** Cl-CH_2-CO-NH-CH-$(C_6H_5)_2$, Br-CH_2-CO-NH-CH-$(C_6H_5)_2$.

N-benzhydryl-glycolamide esters (OBg esters) are also easily and cleanly synthesized from polyfunctionnal N-protected amino acids such as Boc-Ser, Z-Thr, Boc-Arg(NO_2), etc. They are not affected under conditions of removal of usual protecting groups (Z, Boc, Fmoc, t-butyl esters,...) and are rapidly cleaved without affecting other protecting groups, by potassium carbonate hydrolysis in a mixture of DMF and water. The reaction by-product, identified as N-benzhydryl-glycolamide, is discarded by ether or ethyl acetate washings.

The stability of OBg esters derivatives has been demonstrated : Boc-Ala-OBg remained unaffected in the hydrogenolysis conditions usually used for the removal of benzyloxycarbonyl (Z) or benzyl (Bzl) protecting groups (e.g. H_2, 10% Pd/C as catalyst, EtOH 95%).

In order to prove the usefulness of this protecting group in peptide synthesis, we report syntheses of some model peptides. TFA deprotection of Boc-Ala-OBg **3** and coupling to Z-Ala-OH in the presence of BOP[5] afforded Z-Ala-Ala-OBg **24**. This compound was also obtained with a similar yield from Z-Ala-OBg. Potassium carbonate mediated hydrolysis led to the N-protected dipeptide Z-Ala-Ala-OH **25** whose analytical and physical data are in good agrement with those reported in the litterature[6].

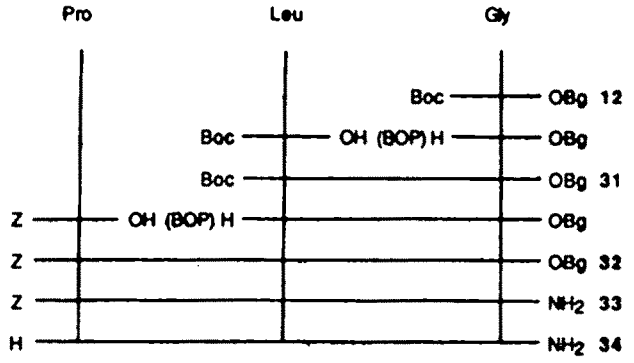
The chemotactic peptide For-Met-Leu-Phe-OH[7] **30** was also successfully synthesized according to scheme 2. Intermediate compound For-Met-Leu-Phe-OBg **29** was alternatively synthesized from Fmoc-Met-Leu-Phe-OBg **28**, obtained by BOP[5] mediated coupling of Fmoc-Met-OH with the partially deprotected dipeptide H-Leu-Phe-OBg. Again, OBg esters showed their stability during various reaction conditions of peptide synthesis: acidolytic cleavage of Boc or β-elimination of Fmoc by diethylamine. The formyl group was introduced by the means of 2,4,5-trichlorophenyl formate as described earlier[8]. Removal of the OBg ester was complete in less than 30 min.



Scheme 2

Another example of the usefulness of OBg esters in peptide chemistry is given with the synthesis of MIF[9] of sequence Pro-Leu-Gly-NH_2, that was carried out according to scheme 3.

This synthesis demonstrates the interesting possibility of generating a C-terminal amide in very mild conditions by reaction with NH_4OH. Physical data of the N-protected peptide amide **33**, obtained from Boc-Gly-OBg **12** through TFA deprotections, couplings with BOP[5] and ammonolysis, are in excellent agreement with those reported in the litterature[10]. After final hydrogenolysis, the tripeptide Pro-Leu-Gly-NH_2 **34**, isolated as its hemihydrate, was identical to an authentic commercial sample (Bachem, Switzerland).



Scheme-3

N-benzhydryl-glycolamide esters (OBg esters) undergo rapid hydrazinolysis in very mild conditions, as demonstrated by the synthesis of Boc-Ala-NH-NH_2 35 from Boc-Ala-OBg 3. This property could lead to a new improvement in the development of segment assembling through azide couplings.

We finally investigated the possibility of removing OBg esters in the presence of other ester protections in a peptidic sequence. It is well known that aspartyl peptides undergo rapid succinimide formation from their β-carboxyl esters when exposed to alkaline conditions[11]. This side reaction was effectively observed during an attempt of OBg ester cleavage from the sequence Boc-Asp(OBzl)-Phe-OBg 37. However, hydrolysis of the OBg ester of Boc-Glu(OBzl)-Phe-OBg 37 and Z-Asp(OBut)-Phe-OBg 39 led to the expected compounds Boc-Glu(OBzl)-Phe-OH 38 and Z-Asp(OBut)-Phe-OH 40 respectively. It is worth mentioning here that compound 38 was contaminated with traces only of Boc-Glu-Phe-OH. All those compounds were identified by comparison with authentic samples unequivocally synthesized by other means.

CONCLUSION

This study on the use of N-benzhydryl-glycolamide esters (OBg esters) in peptide synthesis led us to the following conclusions: - OBg esters are inert in the conditions used for the removal of Z, Boc and Fmoc groups (H_2-Pd/C, neat TFA, diethylamine/DMF). Therefore, they can be used in peptide synthesis in combination with these protecting groups. They are stable during coupling with BOP and active esters. - OBg esters are removed in mild alkaline conditions, typically by potassium carbonate in a mixture of DMF and water; no side reaction was encountered in these conditions except for β-benzyl aspartyl containing sequences. - OBg esters can be cleanly and rapidly converted into their amide or hydrazide derivatives. - OBg esters provide an interesting improvement of their CAM analogues by increasing their solubility in organic solvents.

We intend to apply this new methodology in the syntheses of larger peptides, such as cholecystokinin and gastrin.

EXPERIMENTAL

Melting points were taken on a Büchi apparatus in open capillary tubes. Optical rotations were determined at 20°C with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by "Le Service de Microanalyses de l'ENSCM" (Montpellier, France); experimental values are within 0.4% of the theoretical values. Ascending TLC was performed on precoated plates of silica gel 60 F 254 (Merck) using the following solvent systems (by volume): A, AcOEt/hexane, 3:7; B, AcOEt/hexane, 5:5; C, AcOEt/hexane, 7:3; D, AcOEt, E, chloroform/methanol/acetic acid 120:10:5, F, chloroform/methanol/acetic acid 85:10:5. Peptide derivatives were located with UV light (254 nm), charring reagent or ninhydrin. ^1H NMR were run on a Brücker 360 instrument at 20°C. Amino acids and derivatives were purchased from Bachem (Switzerland). All reagents and solvents were of analytical grade. BOP was recrystallized

from acetone and ether. The following abbreviations were used: DMF, dimethylformamide; DME, 1,2-dimethoxyethane; HOSu, N-hydroxysuccinimide; DIEA, N,N-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; NMM, N-methylmorpholine; BOP, benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate; DCC, dicyclohexylcarbodiimide. Other abbreviations used were those recommended by the IUPAC-IUB Commission (Eur. J. Biochem. 1984, 138, 9-37).

N-Benzhydryl-chloroacetamide 1: To a cold (0°C) solution of chloroacetic acid (9.45 g, 0.1 mol) and N-hydroxysuccinimide (12.6 g, 0.11 mol) in 1,2-dimethoxyethane (DME) (100ml), was added DCC (20.6 g, 0.1 mol). After 3 h stirring at 0°C, the precipitated DCU was filtered off, and the filtrate treated at 0°C with benzhydrylamine (19 ml, 0,11 mol), and the mixture was stirred for 1 h at room temperature. The expected compound precipitated upon addition of the reaction mixture into a well stirred 0.1 M aqueous solution of potassium hydrogenosulfate. It was collected by filtration, washed with 1 M aqueous potassium hydrogenosulfate, water, saturated aqueous sodium bicarbonate, water to neutrality and dried in vacuo over phosphorous pentoxide. It was recrystallized from a mixture of ethyl acetate and hexane. Yield 20.5 g (79%); mp 121-122°C; ^{1}H NMR(DMSO-d^6): δ ppm 9.10 (d, 1 H, ^{3}J = 8.1 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 6.10 (d, 1 H, ^{3}J = 8.1 Hz, CH), 4.17 (s, 2 H, CH_2); Anal $C_{15}H_{14}ClNO$ (259.74); Calc. C, 69.36; H, 5.43; N, 5.39; Found, C, 69.12; H, 5.38; N, 5.12.

N-Benzhydryl-bromoacetamide 2: Synthesized as described above from bromoacetic acid. Yield 70%; mp 140-141°C; ^{1}H NMR(DMSO-d^6): δ ppm 9.17 (d, 1 H, ^{3}J = 8.6 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 6.08 (d, 1 H, ^{3}J = 8.6 Hz, CH), 3.96 (s, 2 H, CH_2); Anal $C_{15}H_{14}BrNO$ (304.19); Calc. C, 59.23; H, 4.64; N, 4.60; Found, C, 58.94; H, 4.56; N, 4.39.

General procedure for the preparation of Boc- or Z-protected amino acid N-benzhydryl-glycolamide esters: To a solution/suspension of a N-protected amino acid (10 mmol) in benzene or acetonitrile (10 ml) was added DBU (1.49 ml, 10 mmol), followed by the appropriate N-benzhydryl-haloacetamide (9 mmol), and the mixture was stirred under reflux (typically 1h with N-benzhydryl-bromoacetamide, 3h with N-benzhydryl-chloroacetamide). Ethyle acetate (100 ml) was then added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford the expected ester that was crystallized (or recrystallized) in the appropriate solvent. Physical and analytical data of the amino acid derivatives are listed in Table 1.

^{1}H NMR data of some Boc- or Z-protected amino acid OBg esters: (DMSO-d^6):

Boc-Ala-OBg 3: δ ppm 8.76 (d, 1 H, ^{3}J = 8.3 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 7.32 (m, 1 H, NH-Boc), 6.13 (d, 1 H, ^{3}J = 8.5 Hz,CH-Φ_2), 4.67, 4.59 (AB, 2 H, ^{2}J = 14.7 Hz, CH_2), 4.09 (m, 1 H, ^{3}J = 7.6 Hz, CHα), 1.34 (s, 9 H, Boc), 1.27 (d, 3 H, ^{3}J = 7.3 Hz, CH_3).

Boc-Arg(NO$_2$)-OBg 4: δ ppm 8.74 (d, 1 H, ^{3}J = 8.3 Hz, NH), 7.4 to 7.2 (m, 11 H, arom. and NH-Boc), 6.13 (d, 1 H, ^{3}J = 8.5 Hz,CH-Φ_2), 4.68, 4.61 (AB, 2 H, ^{2}J = 14.7 Hz, CH_2), 4.04 (m, 1 H, CHα), 1.8 to 1.5 (m, 4 H, CH2 β and γ), 1.35 (s, 9 H, Boc).

Boc-Asn-OBg 5: δ ppm 8.77 (d, 1 H, ^{3}J = 8.7 Hz, NH), 7.4 to 7.2 (m, 11 H, arom. and 1 CO-NH$_2$), 7.12 (m, 1 H, NH-Boc), 6.94 (s, 1 H, 1 CO-NH$_2$), 6.15 (d, 1 H, ^{3}J = 8.7 Hz,CH-Φ_2), 4.69, 4.59 (AB, 2 H, ^{2}J = 14.8 Hz, CH_2), 4.49 (m, 1 H, ^{3}J = 7.6 Hz, CHα), 2.62 and 2.52 (ABX, 2 H, ^{3}J = 5.4 Hz and ^{3}J = 7.8 Hz, ^{2}J = 15.7 Hz, CH_2β), 1.35 (s, 9 H, Boc).

Boc-Ser-OBg 14: δ ppm 8.74 (d, 1 H, ^{3}J = 9.0 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 7.05 (m, 1 H,^{3}J = 7.4 Hz, NH-Boc), 6.15 (d, 1 H, ^{3}J = 8.8 Hz,CH-Φ_2), 5.03 (t, 1 H, ^{3}J = 6.3 Hz, OH), 4.70, 4.64 (AB, 2 H, ^{2}J = 14.9 Hz, CH_2), 4.18 (m, 1 H, ^{3}J = 7.6 Hz, CHα), 3.67 (m, 2 H, CH_2β),1.37 (s, 9 H, Boc).

Z-Ala-OBg 17: δ ppm 8.79 (d, 1 H, ^{3}J = 8.5 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 6.14 (d, 1 H, ^{3}J = 8.5 Hz, CH-Φ_2), 4.97 (m, 2 H, CH_2-Z), 4.69, 4.61 (AB, 2 H, ^{2}J = 14.7 Hz, CH_2), 4.19 (m, 1 H, ^{3}J = 7.3 Hz, CHα), 1.32 (d, 3 H, ^{3}J = 7.3 Hz, CH_3).

Z-Thr-OBg 21: δ ppm 8.80 (d, 1 H, ^{3}J = 8.5 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 6.16 (d, 1 H, ^{3}J = 8.5 Hz, CH-Φ_2), 5, 05 (m, 2 H, CH_2-Z), 5.01 (d, 1 H, ^{3}J = 6.5 Hz, OH), 4.69 (m, 2 H, CH_2), 4.22 (m, 1 H, CHα), 4. 12 (m, 1 H, CHβ), 1.12 (d, 3 H, ^{3}J = 6.4 Hz, CH_3).

Fmoc-Nle-OBg 23 : To a solution of Fmoc-Nle (0.5 g, 1.41 mmol) in DMF (5 ml) was added cesium carbonate (0.23 g, 0.65 mmol) followed by N-benzhydryl bromoacetamide (0.395 g, 1.30 mmol). After stirring for 2 h. at room temperature, the precipitate was

filtered, and the solvent was concentrated *in vacuo* at t < 40°C. The resulting residue was dissolved in ethyl acetate (100 ml) and washed with a 1% sodium bicarbonate solution (3 x 70 ml), water (80 ml), 1N potassium hydrogenosulfate (80 ml), and water (80 ml). The organic layer was dried over sodium sulfate, and concentrated *in vacuo*. The resulting residue crystallized upon trituration with a mixture of ether and hexane. It was recrystallized in a mixture of ether and hexane. Yield 0.38g. (80%). Physical and analytical data are reported in table 1.

Cleavage of a N-benzhydryl-glycolamide ester (OBg ester): Typical cleavage is illustrated with the following experiment: to a solution of Boc-Ala-OBg (825 mg, 2 mmol) in DMF (10 ml) was added a solution of potassium carbonate (552 mg, 4 mmol) in water (10 ml) and the mixture stirred for 30 min at room temperature. Water (50 ml) was added, and the solution was extracted with ether (3 x 50 ml). Upon acidification of the aqueous layer with 1 M aqueous potassium hydrogenosulfate, a white solid precipitated. It was collected, washed with water and dried in vacuo. Yield 337 mg (89%), identical to an authentic sample of Boc-alanine. Drying over magnesium sulfate and concentration of the ether extracts afforded a white solid which was recrystallized in a mixture of ethyle acetate and hexane and identified as N-benzhydryl-glycolamide. Yield 420 mg (87 %); mp 88-90°C; NMR(DMSO-d^6): δ ppm 8.34 (d, 1 H, ^3J = 8.8 Hz, NH), 7.4 to 7.2 (m, 10 H, arom.), 6.17 (d, 1 H, ^3J = 8.8 Hz, CH), 5.40 (t, 1 H, ^3J = 6.0 Hz, OH), 3.92 (d, 2 H, ^3J = 6.0 Hz, CH_2); Anal. $C_{15}H_{15}NO_2$ (241.29); Calc. C, 74.67; H, 6.27; N, 5.80; Found, C, 74.49; H, 6.22; N, 5. 59.

Z-Ala-Ala-OBg 24: *method A*: Boc-Ala-OBg 3 (412 mg, 1 mmol) was treated with TFA (2 ml) for 30 min at room temperature. Evaporation of TFA and trituration in ether afforded the TFA salt of alanine N-benzhydryl-glycolamide ester. Yield 390 mg (92%). It was added to a solution in DMF (5 ml) of Z-Ala-OH (223 mg, 1 mmol) and BOP[5] (442 mg, 1 mmol), followed by NMM (0.21 ml, 1.91 mmol). After 30 min stirring at room temperature, the expected compound precipitated upon addition of a 5% aqueous solution of sodium bicarbonate (100 ml). It was collected, washed with water, 1 M aqueous potassium hydrogenosulfate, water to neutrality, ether, and dried in vacuo. Yield 395 mg (84%); mp 178-181°C; Rf(C) 0.63; Rf(D) 0.73; $[\alpha]_D$ = -10.6 (c 1, DMF); Anal. $C_{29}H_{31}N_3O_6$. (517.58); Calc. C, 67.30; H, 6.04; N, 8.12; Found, C, 67.01; H, 6.02; N, 7.95.

method B: Compound 24 was obtained as described above, from Z-Ala-OBg 17, by hydrogenolysis of the Z group and subsequent coupling. Physical and analytical data of this compound were identical to those of the product obtained by method A.

Z-Ala-Ala-OH 25: Compound 24 (300 mg, 0.58 mmol) was hydrolyzed as already described in a mixture of DMF and water in the presence of potassium carbonate. The N-protected dipeptide was recrystallized in a mixture of chloroform and hexane. Yield 150 mg (88%); mp 147-148°C (mp litt[6] 150°C); $[\alpha]_D$ = -33.0 (c 0.96, methanol); $[\alpha]_D$litt.[6] = -35 (c 1, methanol); Rf(E) 0.29; Rf(F) 0.40; NMR(DMSO-d^6) (Z-Ala$_1$-Ala$_2$-OH): δ ppm 8.06 (d, 1 H, ^3J = 7.3 Hz, NH Ala$_2$), 7.37 (d, 1 H, ^3J = 7.3 Hz, NH Ala$_1$), 7.35 (m, 5 H, arom.), 5.01 (m, 2 H, CH_2), 4.18 (m, 1 H, CHα Ala$_2$), 4.07 (m, 1 H, CHα Ala$_1$), 1.27 (d, 3 H, ^3J = 7.3 Hz, CH_3 Ala$_2$), 1.20 (d, 3 H, ^3J = 7.3 Hz, CH_3 Ala$_1$); Anal. $C_{14}H_{18}N_2O_5$ (294.31); Calc. C, 57.13; H, 6.16; N, 9.52; Found, C, 56.86; H, 6.09; N, 9.23.

Boc-Leu-Phe-OBg 26: Boc-Phe-OBg 12 (2.0 g, 4.09 mmol) was partially deprotected with TFA as already described. The TFA salt was added to a solution in DMF (15 ml) of Boc-Leu-OH, H_2O (1.1 g, 4.4 mmol) and BOP[5] (1.95 g, 4.4 mmol), followed by DIEA (1.46 ml, 8.49 mmol). After 30 min stirring at room temperature, ethyl acetate (200 ml) was added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford 26 which was crystallized in a mixture of ether and hexane. Yield 2.22 g (90%); mp 97-99°C; Rf(B) 0.63; Rf(C) 0.86; $[\alpha]_D$ = -15.7 (c 1.14, DMF); Anal. $C_{35}H_{43}N_3O_6$ (601.74); Calc. C, 69.86; H, 7.20; N, 6.98; Found, C, 69.75; H, 7.11; N, 6.84.

Boc-Met-Leu-Phe-OBg 27: Boc-Leu-Phe-OBg 26 (2.1 g, 3.49 mmol) was partially deprotected with TFA as already described in a quantitative yield. The TFA salt was added to a solution in DMF (15 ml) of Boc-Met-ONp[12] (1.11 g, 3.0 mmol) and HOBt (0.405g, 3.0 mmol), followed by DIEA (0.60 ml, 3.49 mmol). After 30 min stirring at room temperature, ethyle acetate (200 ml) was added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford 27 which was crystallized upon trituration with ether. Yield 2.05 g (93%); mp 91-93°C; Rf(B) 0.52; Rf(C) 0.79; $[\alpha]_D$ = -19.1 (c 1.02, DMF); Anal.

$C_{40}H_{52}N_4O_7S$ (732.94): Calc. C, 65.55; H, 7.15; N, 7.64; Found, C, 65.28; H, 7.09; N, 7.47.

***Fmoc-Met-Leu-Phe-OBg* 28**: Boc-Leu-Phe-OBg 26 (600 mg, 1.0 mmol) was partially deprotected with TFA as already described in a quantitative yield. The TFA salt was added to a solution in DMF (8 ml) of Fmoc-Met-OH (351 mg, 0.99 mmol) and BOP[6] (440 mg, 0.95 mmol), followed by NMM (0.21ml, 1.95 mmol). After 30 min stirring at room temperature, ethyle acetate (100 ml) was added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford 28 which was crystallized in a mixture of ether and hexane. Yield 740 mg (91%); mp 170-172°C; Rf(B) 0.55; Rf(C) 0.79; $[\alpha]_D$ = -14.7 (c 1.09, DMF); Anal. $C_{50}H_{54}N_4O_7S$ (855.06); Calc. C, 70.23; H, 6.37; N, 6.55; Found, C, 69.98; H, 6.20; N, 6.39.

***For-Met-Leu-Phe-OBg* 29**: *method A*: Compound 27 (1.9 g, 2.59 mmol) was partially deprotected with TFA as already described in a quantitative yield. The TFA salt was added to a solution in DMF (10 ml) of 2,4,5-trichlorophenyl formate[8] (0.63 g, 2.8 mmol), followed by DIEA (0.46 ml, 2.59 mmol). After 15 min stirring at room temperature, the solvent was concentated to 2 ml, and compound 29 precipitated upon addition of ether (100 ml). It was collected, washed with small portions of ethyl acetate (3 x 3 ml), ether, and dried in vacuo. Yield 1.58 g (92%); mp 172-174; Rf(C) 0.28; Rf(D) 0.64; $[\alpha]_D$ = -19.0 (c 1.10, DMF); Anal. $C_{36}H_{44}N_4O_6S$ (660.83); Calc. C, 65.43; H, 6.71; N, 8.48; Found, C, 65.19; H, 6.32; N, 8.48.

method B: Compound 28 (500 mg, 0.58 mmol) was treated with a solution of diethylamine (0.6 ml) in DMF (6 ml) for 30 min at room temperature. The reaction mixture was then concentrated to dryness and the residue triturated in a mixture of ether and hexane (v/v; 80 ml) to afford the partially deprotected peptide. It was collected, washed with a mixture of ether and hexane (v/v) and dried in vacuo. Yield 350 mg (95%). It was added to a solution in DMF (4 ml) of 2,4,5-trichlorophenyl formate[8] (135 mg, 0.6 mmol). After 15 min stirring at room temperature, the reaction mixture was treated as described above. Yield 330 mg (86%). Physical and analytical data of this compound were identical to those of the product obtained by method A.

***For-Met-Leu-Phe-OH* 30**: Compound 29 (1.40 g, 2.11 mmol) in DMF (10 ml) was treated by a solution of potassium carbonate in water (10 ml), and the mixture was stirred for 30 min at room temperature. The solvent was then evaporated under reduced pressure, the residue dissolved in water (80 ml) and the solution extracted with ethyl acetate (3 x 50 ml). Upon acidification of the aqueous layer with 1 M potassium hydrogenosulfate, compound 30 precipitated. It was collected, washed with water and a mixture of ether and hexane and dried in vacuo. Yield 843 mg (91%); mp 212-214°C (litt.[2] 218-219°C); $[\alpha]_D$ = -8.9 (c 1.05, acetic acid); $[\alpha]_D$litt.[2] = -9.2 (c 0.95, acetic acid); Rf(E) 0.26; Rf(F) 0.36; Anal. $C_{21}H_{31}N_3O_5S$ (437.56); Calc. C, 57.65; H, 7.14; N, 9.60; Found, C, 57.32; H, 7.08; N, 9.33.

***Boc-Leu-Gly-OBg* 31**: Boc-Gly-OBg 8 (2.7 g, 6.78 mmol) was partially deprotected with TFA as already described in a quantitative yield. The TFA salt was added to a solution in DMF (25 ml) of Boc-Leu-OH, H_2O (1.8 g, 7.2 mmol) and BOP[5] (3.18 g, 7.2 mmol), followed by NMM (1.51 ml, 13.75 mmol). After 30 min stirring at room temperature, ethyl acetate (200 ml) was added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford 31 which was crystallized in a mixture of ether and hexane. Yield 2.80 g (84%); mp 89-91°C; Rf(B) 0.41; Rf(C) 0.64; $[\alpha]_D$ = -15.1 (c 0.93, DMF); Anal. $C_{28}H_{37}N_3O_6$ (511.62); Calc. C, 65.73; H, 7.29; N, 8.21; Found, C, 65.66; H, 7.23; N, 7.89.

***Z-Pro-Leu-Gly-OBg* 32**: Compound 31 (2.7 g, 5.27 mmol) was partially deprotected with TFA as already described in a quantitative yield. The TFA salt was added to a solution in DMF (25 ml) of Z-Pro-OH (1.4 g, 5.6 mmol) and BOP[5] (2.46 g, 5.6 mmol), followed by NMM (1.18 ml, 10.71 mmol). After 30 min stirring at room temperature, ethyl acetate (200 ml) was added, and the resulting solution was washed with 1 M aqueous potassium hydrogenosulfate (3 x 100 ml), water, saturated aqueous sodium bicarbonate (3 x 100 ml), brine, dried over magnesium sulfate and concentrated under reduced pressure to afford 32 which was crystallized in a mixture of ether and hexane. Yield 2.80 g (83%); mp 158°C; Rf(C) 0.36; Rf(C) 0.64; $[\alpha]_D$ = -42.9 (c 1.05, DMF); Anal. $C_{36}H_{42}N_4O_7$ (642.75); Calc. C, 67.27; H, 6.59; N, 8.72; Found, C, 66.95; H, 6.51; N, 8.62.

Z-Pro-Leu-Gly-NH2 33: To a solution of compound 32 (2.75g, 3.89 mmol) in DMF (20 ml) was added concentrated NH_4OH (8 ml), and the mixture was stirred for 15 min at room temperature. The solvent was concentrated in vacuo and the residue triturated with ether to afford a white solid. It was collected, washed with ether and dried in vacuo. Yield 1.41 g (86%); mp 156-157°C (mp litt.[12] 163-163.5°C); Rf(D) 0.12; Rf(F) 0.34; $[\alpha]_D$ = -76.0 (c 2.04, 95% ethanol); ; $[\alpha]_D$litt.[10] = -73.3 (c 2, 95% ethanol); Anal. $C_{21}H_{30}N_4O_5$ (418.49); Calc. C, 60.27; H, 7.23; N, 13.39; Found, C, 59.95; H, 7.18; N, 13.02.

H-Pro-Leu-Gly-NH2, hemihydrate 34: Compound 33 (1.0 g, 2.39 mmol) was hydrogenated for 3 h in 95% ethanol (80 ml) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The solvent was concentrated to dryness and the expected compound crystallized upon trituration in ether in the presence of two drops of water. It was collected, washed with ether and dried in vacuo. Yield 655 mg (94%); mp 119-122°C; Rf(F) 0.06; $[\alpha]_D$ = -48.9 (c 1.2, DMF); identical to a commercial sample.

Boc-Ala-NH-NH2 35: To a solution of Boc-Ala-OBg 3 (412 mg, 1 mmol) in DMF (3 ml) was added hydrazine hydrate (0.2 ml). After 30 min standing at room temperature, the mixture was concentrated to dryness and the residue triturated in ether to afford the hydrazide 35 as a white solid. Yield 180 mg (88%); mp 110-111°C (mp litt.[13] 112-113°C); $[\alpha]_D$ = -44.4 (c 1.0, 1 N HCl); $[\alpha]_D$litt.[13] = -45 (c 1.0, 1 N HCl).

Boc-Asp(OBzl)-Phe-OBg 36: Synthesized in the usual way from Boc-Phe-OBg 12 and Boc-Asp(OBzl) through a coupling with BOP[5]. Recrystallisation from a mixture of ethyl acetate and hexane. Yield 81%; mp 132-133°C; Rf(B) 0.62; Rf(C) 0.80; $[\alpha]_D$ = -14.9 (c 0.94, DMF); Anal. $C_{40}H_{43}N_3O_8$ (693.80); Calc. C, 69.25; H, 6.25; N, 6.06; Found, C, 69.01; H, 6.17; N, 5.82.

Boc-Glu(OBzl)-Phe-OBg 37: Synthesized in the usual way from Boc-Phe-OBg 12 and Boc-Glu(OBzl) through a coupling with BOP[5]. Recrystallisation from a mixture of ethyl acetate and hexane. Yield 75%; mp 88-91°C; Rf(B) 0.59; Rf(C) 0.81; $[\alpha]_D$ = -7.2 (c 0.97, DMF); Anal. $C_{41}H_{45}N_3O_8$ (707.82); Calc. C, 69.57; H, 6.41; N, 5.94; Found, C, 69.44; H, 6.36; N, 5.66.

Boc-Glu(OBzl)-Phe-OH 38: Obtained through the usual method of OBg esters hydrolysis. Yield 86%; identified by TLC comparison with an authentic sample. The reaction mixture was contaminated with about 5% of Boc-Glu-Phe-OH, identified by TLC comparison with an authentic sample.

Z-Asp(OBut)-Phe-OBg 39: Synthesized in the usual way from Boc-Phe-OBg 12 and Z-Asp(OBut) through a coupling with BOP[5]. Recrystallisation from a mixture of ethyl acetate and hexane. Yield 79%; mp 114°C; Rf(B) 0.59; Rf(C) 0.85; $[\alpha]_D$ = -15.8 (c 1.07, DMF); Anal. $C_{40}H_{43}N_3O_8$ (693.80); Calc. C, 69.25; H, 6.25; N, 6.06; Found, C, 68.86; H, 6.15; N, 5.79.

Z-Asp(OBut)-Phe-OH 40: Obtained through the usual method of OBg esters hydrolysis. Yield 78%; identified by TLC comparison with an authentic sample.

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