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ON THE USE OF CARBOXAMIDOMETHYL ESTERS (CAM ESTERS) IN THE SYNTHESIS OF MODEL PEPTIDES. SCOPE AND LIMITATIONS

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Abstract—The usefulness of carboxamidomethyl esters (CAM esters) as a carboxyl protecting group for peptide synthesis was demonstrated. The synthesis of the chemotactic peptide For–Met–Leu–Phe–OH as well as the synthesis of Met–enkephalin using CAM ester as carboxyl terminal protection were performed. These esters showed good stability during acidolytic removal of BOC N-protecting group, during hydrogenolysis of Z N-protecting group and during removal of FMOC N-protecting group. CAM esters were selectively and rapidly cleaved by NaOH 0.5 N or by Na_2CO_3. However, we did not succeed in removing selectively the CAM ester when β-benzyl ester of aspartic acid was present in the sequence.

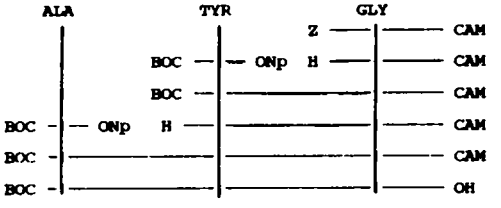
Among the carboxyl protecting groups actually useful in peptide synthesis,[1] no one fulfilled completely the requirements for their use in orthogonal strategy.†

The carboxamidomethyl esters (CAM esters) were reported[2] as carboxyl protecting groups which may be useful in the synthesis of peptides. The CAM esters appeared to present interesting properties. They are easily and readily prepared from commercially available compounds; are inert to the functional groups of peptides; are not affected under conditions of removal of common other protecting groups (BOC,[3] Z,[4] FMOC,[5] t-butyl-esters . . .) and they are easily and cleanly removed without affecting other protecting groups (BOC, Z, t-butyl-esters. . .). As examples we would like to report our experience in using CAM esters for the protection of the terminal carboxyl function during the synthesis of various peptides.

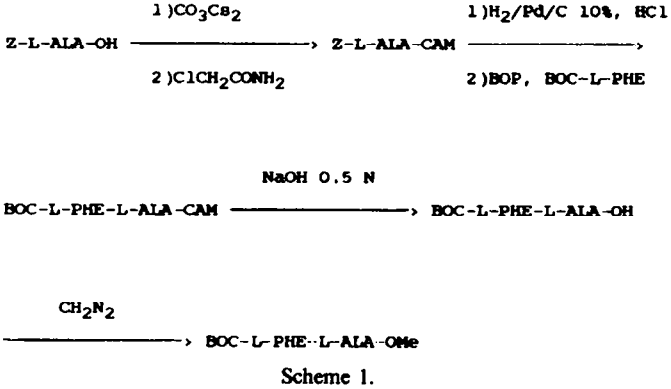
RESULTS AND DISCUSSION

The dipeptide BOC–Phe–Ala–CAM **2** was synthesized according to Scheme 1. This peptide was then hydrolyzed by 0.5 N NaOH and esterified by diazomethane to yield BOC–Phe–Ala–OMe **4**. Comparison of physical values of **4** with those given in the literature[6] (α_D and m.p. particularly) seem to show that no racemization had occurred during deblocking of the CAM ester. The CAM ester in this synthesis was not affected during the removal of the carbobenzoxy groups by hydrogenolysis or during coupling with BOP reagent.[7]

The preparation of the tripeptide BOC–Ala–Tyr–Gly–CAM **7**, representing a portion in the sequence in the gastrin family was performed according to Scheme 2. In this synthesis, CAM ester was compatible with BOP reagent in couplings, and during acylation with active esters.[8] Stability of CAM ester during acidolytic removal of BOC groups by TFA was unambiguous. Hydrolysis of **7** by Na_2CO_3 yielded the tripeptide fragment BOC–Tyr–Ala–Gly–OH **8**, which could be used for fragment condensation.



Scheme 2.



Scheme 1.

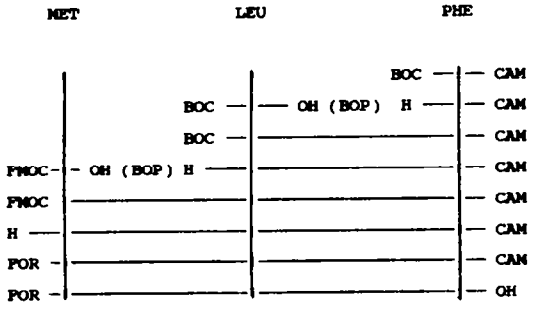
†An orthogonal system was defined as a set of completely independent of at least three classes of protecting groups, such that each one can be removed in any order and in the presence of all others (G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.* **99**, 7363 (1977)).

The chemotactic peptide,[9] For-Met-Leu-Phe-OH **13** was also obtained successfully with the aid of CAM ester as C-terminal protection. Synthesis of this peptide was carried out according Scheme 3. Again, CAM ester showed its stability in TFA during removal of α-amino BOC protecting groups. Methionine was introduced as its FMOC derivative. Removal of the FMOC N-protecting group did not affect the CAM ester.

Formylation of the tripeptide TFA, Met-Leu-Phe-CAM by using trichlorophenyl-formate[10] yielded For-Met-Leu-Phe-CAM **12**. Hydrolysis of **12** by Na_2CO_3 produced the chemotactic peptide For-Met-Leu-Phe-OH in really good conditions.

As another example of the use of the CAM ester for the protection of the C-terminal amino acid residue, we prepared the peptide BOC-Phe-Asp(OBzl)-Thr-(Bzl)-Gly-CAM **17**, which represent the amino acid sequence in the active site of most acid proteases and could after removal of the CAM ester, constitute an interesting fragment. This synthesis was carried out according to Scheme IV. No major problem was encountered during the growth of the peptide chain, neither during acidolytic removal of the BOC group, nor during coupling with BOP reagent or active esters. However, we could not remove selectively the CAM ester. In the presence of Na_2CO_3, $NaHCO_3$ or NaOH, the β-benzyl ester of aspartic acid was first removed, probably via the aspartimide formation.[11] It is indeed, well known that β-benzyl aspartyl protection is damaged in basic medium.[12] ^1H NMR spectroscopy clearly showed the disappearance of signals of the benzyl moiety. Hydrolysis of the CAM ester occurred, but we did not succeed in removing selectively the CAM ester.

In connection with the use of CAM ester, we would like to present the synthesis of BOC-Met-Enkephalin,[13] BOC-Tyr-Gly-Gly-Phe-Met-OH (Scheme 5). BOC-Met-Enkephalin was prepared stepwise, using BOP as condensing reagent. BOC-tyrosine was introduced as its N-hydroxysuccinimide ester[14] to yield BOC-Tyr-Gly-Gly-Phe-Met-CAM **24** which was completely deblocked and identified by TLC and by amino acid analysis to an authentic sample. In this synthesis, again, no major problem arose from the use of CAM ester as carboxyl terminal protection.



Scheme 3.

CONCLUSION

From our preliminary study concerning CAM esters as carboxyl protecting groups useful in peptide synthesis, some conclusions can be drawn.

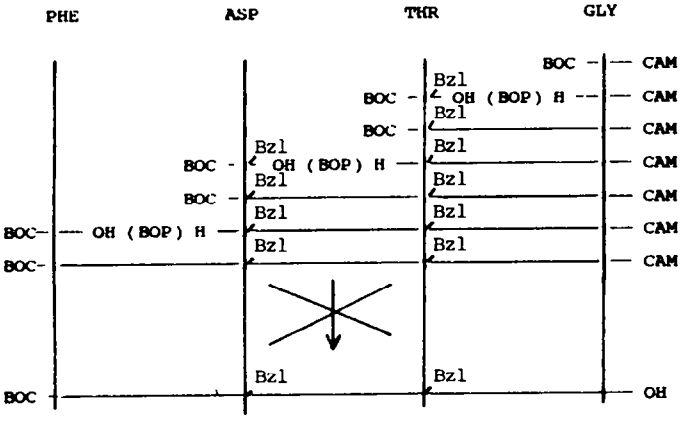
CAM esters are unchanged in TFA during few hours. They are not affected during hydrogenolysis of carbobenzoxy groups and during the removal of FMOC groups by diethylamine.

CAM esters are stable during coupling with BOP reagent, and active esters and can be used in peptide synthesis with BOC, Z and FMOC N-protecting groups.

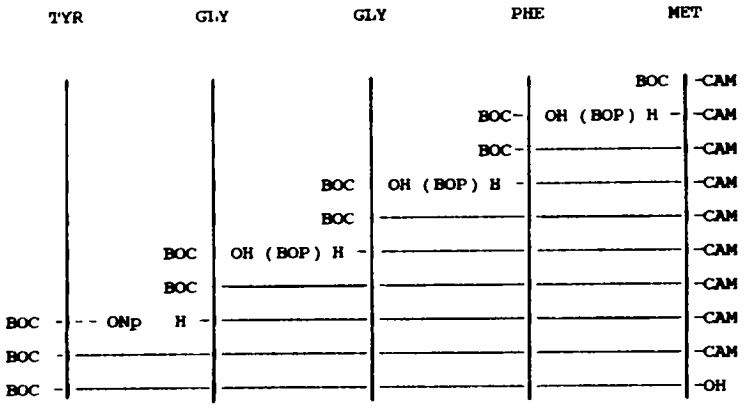
CAM esters are readily, and cleanly removed by 0.5 N NaOH or Na_2CO_3. Racemization does not seem to occur during this removal. It appears possible, using CAM ester as carboxy-terminal protection to obtain peptide fragments which may be used for fragment condensation by selective removal of the CAM ester. However, some concern may be felt when β-benzyl aspartyl residue is present in the sequence. On the other hand, no major problem was encountered in the synthesis of methionyl-peptides.

CAM esters are readily prepared from commercially available material and are crystalline compounds.

More results on the use of the CAM esters will be reported. They may offer some advantages in peptide synthesis, especially in designing orthogonal strategies. The experience gained in the application of CAM esters for the synthesis of some peptides encourages us to carry out the synthesis of longer chains by this procedure.



Scheme 4.



Scheme 5.

EXPERIMENTAL

Abreviations used are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature. *Biochemistry* **11**, 1726 (1972). Other abreviations used are: BOC, t-butyloxycarbonyl; Z, benzyloxycarbonyl; FMOC, 9-fluorenylmethyloxycarbonyl; CAM, carboxamidomethyl-ester; OMe, methyl ester; BOP, benzotriazolyloxytris-(dimethylamino) phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; For, formyl; Bzl, benzyl; AcOEt, ethyl acetate; AcOH, acetic acid; MeOH, methanol; EtOH, ethanol; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DIEA, ethyl-N,N, diisopropylamine; ONp, p-nitrophenyl ester; Otcp. 2, 4, 5-trichlorophenyl ester; OSu, N-hydroxysuccinimide ester; All m.p. were reported uncorrected. Proton spectra were taken on Brucker 360 MHz instrument with Me_4Si as internal standard. Thin layer chromatograms were run on commercial plates of silica gel (Merck, GF 264) in the solvent system A: AcOEt–Hexane (7:3); B: AcOEt; C: AcOEt–Pyridine–AcOH–H_2O (80:20:6:10); D: AcOEt–MeOH–AcOH (9:0.5:0.5). Spots were detected by UV absorption and by charring. For amino acid analysis, samples were hydrolyzed with constant boiling HCl in evacuated sealed ampules at 110° for 16 hr. Elemental analysis were performed by "Le Service de Microanalyse du CNRS", ENSCM, Montpellier.

Z–L–Ala–CAM **1**. To a cooled soln of Z–L–Ala (4.46 g, 20 mmoles) in a mixture of EtOH–water (100 ml) (7:3) was added a soln of cesium carbonate in water until pH ≃ 6.5. The soln was then concentrated to dryness *in vacuo*. Water was almost completely eliminated by addition of EtOH and benzene and azeotropically distilled. The residue was then dried *in vacuo* over P_2O_5. It was dissolved in DMF (150 ml) in the presence of α-chloroacetamide (2.82 g, 30 mmoles) and the mixture was stirred during 24 hr. The solvent was removed *in vacuo* and the residue dissolved in EtOAc (200 ml), washed with cold $NaHCO_3$aq (1 × 50 ml), water (2 × 50 ml), 10% citric acid soln (1 × 50 ml) and water (2 × 50 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was triturated with an ether–hexane soln to give crystals, yield 4.5 g (80%). R_FA 0.2; R_FB 0.48; m.p. 58–64°; $(\alpha)_D^{20} = -13.5$; (c 2.1 DMF); NMR (DMSO D_6) 1.35 (d, CH_3), 4.24 (m, CH), 4.46 (s, $COOCH_2$), 5.05 (s, ϕCH_2), 7.31 (d, $CONH_2$), 7.37 (s, C_6H_5), 7.81 (d, NH): Anal. ($C_{13}H_{16}N_2O_5$) C.H.N.

BOC–L–Phe–L–Ala–CAM **2**. Compound **1** (2.8 g, 10 mmoles) was hydrogenated in 95% EtOH (100 ml) containing acetic acid (5 ml) in the presence of Pd/C 10% as catalyst (0.3 g). The mixture was filtered, concentrated *in vacuo*, triturated with ether and dried *in vacuo*. The residue was dissolved in DMF (20 ml) in the presence of BOC–L–Phe (2.94 g, 11 mmoles) and BOP (3.98 g, 9 mmoles). After cooling the soln, DIEA (20 mmoles, 3.44 ml) was added and the mixture was stirred overnight and then concentrated *in vacuo* (t < 40°). The residue was dissolved in EtOAc (200 ml); the organic layer was washed with $NaHCO_3$aq (1 × 50 ml), water (2 × 50 ml), citric acid soln (1 × 50 ml), water (2 × 50 ml), dried over Na_2SO_4 and concentrated *in vacuo*. We obtained a white powder after trituration with an EtOAc–hexane mixture. R_FA 0.12; R_FB 0.41. m.p. 80° dec. $(\alpha)_D^{20} = -5.8$ (c 2.05 DMF). Anal. ($C_{19}H_{27}O_6N_3$) C.H.N.

BOC–L–Phe–L–Ala–OMe **4**. Compound **2** (1.97 g, 5 mmoles) was dissolved in DMF (20 ml). A soln of 0.5 N NaOH was added (14 ml, 7 mmoles) followed by water until a clear soln remained. After 15 mn stirring, hydrolysis was over. The mixture was then neutralized with 0.5 N citric acid and concentrated *in vacuo*. The oily residue was dissolved in sat $NaHCO_3$ (50 ml) and washed with EtOAc (2 × 20 ml). After cooling of the aqueous layer, solid citric acid was added (pH 2.5) and the ppt extracted with EtOAc (100 ml). The organic layer was washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to yield BOC–L–Phe–Ala–OH **3**, (1.35 g, 86%).

Compound **3** (0.6 g) was treated with a soln of diazomethane in ether until a yellow color remained. After 2 hr AcOH was added and the mixture was concentrated *in vacuo*. The crystalline residue was dissolved in EtOAc (50 ml) and washed with $NaHCO_3$aq, N citric acid, H_2O, dried over Na_2SO_4, and concentrated *in vacuo* to yield **4**. R_FA 0.73, m.p. 100–101°. $(\alpha)_D^{20} = -16.8$ (c 1 MeOH), m.p. lit[6] 98.99°. $(\alpha)_D$lit[6] − 18.0 (c 1 MeOH).

Z–Gly–CAM **5**. This compound was prepared from Z–Gly (4.2 g, 20 mmoles) as described for **1**. Crystals were washed with ether, yield 85%. R_FA 0.8, m.p. 105–108°. NMR (DMSO D_6) 3.91 (d, CH_2), 4.46 (s, $COOCH_2$), 5.06 (s, ϕCH_2), 7.31–7.42 (C_6H_5 and $CONH_2$), 7.70 (t, NH). Anal. ($C_{12}H_{14}O_5N_2$) C.H.N.

BOC–L–Tyr–Gly–CAM **6**. A sample of **5** (2.66 g, 10 mmoles) was hydrogenated in 95% EtOH (100 ml) containing 10 ml of 2N HCl in the presence of 10% Pd/C as catalyst. After 4 hr, the mixture was filtered, and concentrated to dryness *in vacuo*. The residue was triturated with ether, filtered washed with ether and dried *in vacuo*. Yield 1.6 g (95%). This compound (1.6 g, 9.5 mmoles) was dissolved in DMF and was treated with BOC–L–Tyr–ONp[15] (9.1 mmoles, 3.66 g) and DIEA (10 mmoles, 1.72 ml). After overnight at room temp. the solvent was removed *in vacuo*, the residue was dissolved in EtOAc, the soln washed with N citric acid, water, cold N $NaHCO_3$aq and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue triturated with ether and dried, yield 2.95 g (82%); R_FB 0.2, m.p. 112–114°. $(\alpha)_D^{20} = -7.8$ (c 2.05 DMF). Anal. ($C_{18}H_{25}N_3O_7$) C.H.N.

BOC–L–Ala–L–Tyr–Gly–CAM **7**. A sample of **6** (1.97 g, 5 mmoles) was treated with trifluoroacetic acid (10 ml) during 30 mn. Addition of ether (130 ml) under stirring yielded the TFA salt of the peptide which was filtered, washed several times with ether and dried *in vacuo*. This salt was

dissolved in DMF (10 ml), followed by the addition of BOC-L-Ala-ONp[16] (1.49 g, 4.8 mmoles) and DIEA (5.1 mmoles, 0.88 ml). After stirring, overnight at room temp, the mixture was treated as described for **6**. The compound crystallized by trituration with ether, yield 1.74 g (78%). R_F 0.3 (MeOH 0.5, AcOEt 9.5), m.p. 115° dec. $(\alpha)_D^{20} = -21.1$ (c 1.75 DMF). Anal. ($C_{21}H_{30}N_4O_8$) C.H.N.

BOC-L-Ala-L-Tyr-Gly-OH **8**. A sample of **7** (0.466 g, 1 mmole) in DMF (10 ml) was treated with Na_2CO_3 (0.070 g) in water (5 ml) under stirring. Water (2 ml) was added in order to get a clear soln. After 2 hr the mixture was neutralized with 0.5 N citric acid and the solvents were concentrated to dryness *in vacuo*. The residue is then treated as described for **3**, and crystallization was obtained by trituration with ether, yield 0.32 g (79%). R_FD 0.4; R_FC 0.4; m.p. 125° dec. $(\alpha)_D^{20} = -20.6$ (c 1.45 DMF). Anal. ($C_{19}H_{27}N_3O_7$) C.H.N.

BOC-L-Phe-CAM **9**. Prepared from BOC-L-Phe (20 mmoles, 5.28 g) and α-chloroacetamide (30 mmoles, 2.82 g) as described for **1**. Recrystallized in a mixture of AcOEt–hexane, yield 5.2 g (81%). R_FA 0.5 m.p. 113–116° $(\alpha)_D^{20} = -16.6$ (c 2.95 EtOH). NMR (DMSO D_6), 1.33 (s, $(CH_3)_3$), 2.87 and 3.11 (ϕCH_2), 4.29 (m, CH), 4.46 (s, $COOCH_2$), 7.18 to 7.39 (NH, C_6H_5, $CONH_2$). Anal. ($C_{16}H_{22}N_2O_5$) C.H.N.

BOC-L-Leu-L-Phe-CAM **10**. BOC-L-Phe-CAM (3.21 g, 10 mmoles) was treated with TFA as already described. The resulting salt was dissolved in DMF (20 ml) containing BOC-L-Leu, H_2O (2.49 g, 10 mmoles) and BOP (4.2 g, 9.5 mmoles), followed, after cooling to 0° by addition of DIEA (20 mmoles, 3.5 ml). After standing all night at room temp, the mixture was treated as usual. The compound was crystallized in a mixture of AcOEt–hexane, yield 3.54 g (86%). R_FA 0.5, R_FB 0.58, m.p. 99–100°. $(\alpha)_D^{20} = -13.9$ (c 1.15 DMF). Anal. ($C_{22}H_{33}N_3O_6$) C.H.N.

FMOC-L-Met-L-Leu-L-Phe-CAM **11**. Compound **10** (1.09 g, 2.5 mmoles) was partially deprotected in TFA (30 mn) as previously described. The salt (2.5 mmoles) was dissolved in DMF (10 ml) and FMOC-L-Met (0.93 g, 2.5 mmoles) (Bachem) was added, followed by BOP (0.97 g, 2.2 mmoles). After cooling (0°), the soln was treated with DIEA (0.86 ml, 5 mmoles), stirred overnight at room temp and treated as described for **6**. Yield 1.27 g (84%), R_FA 0.3; R_FB 0.6; m.p. 180–182°. $(\alpha)_D^{20} = -22.9$ (c 1.35 DMF). Anal. ($C_{37}H_{44}O_7N_4S$) C.H.N.

For-L-Met-L-Leu-L-Phe-CAM **12**. A sample of **11** (1.24 g, 1.8 mmoles) was dissolved in a 10% soln of diethylamine in DMF (15 ml).[12] After 2 hr, the mixture was evaporated to dryness *in vacuo* to remove the solvent and the secondary amine. The residue was treated with For-OTcp[10] (0.40 g, 1.8 mmoles) and DIEA (1.7 mmoles, 0.29 ml) in DMF (10 ml). After the reaction was complete (TLC), the solvent was removed *in vacuo* and the residue treated as described for **6**. Yield 0.76 g (86%). R_FB 0.4, m.p. 133.136°. $(\alpha)_D^{20} = -30.8$ (c 1 DMF). Anal. ($C_{23}H_{33}N_4O_6S$) C.H.N.

For-L-Met-L-Leu-L-Phe-OH **13**. Compound **12** (0.494 g, 1 mmole) was dissolved in DMF (10 ml) and treated with Na_2CO_3 (0.212 g, 2 mmoles) in water (6 ml). The soln was kept clear by either addition of DMF or water. After 90 mn, no more ester could be detected by TLC, 0.5 N citric acid was added (pH ≃ 7) and the solvents were concentrated *in vacuo*. The residue was dissolved in sat $NaHCO_3$ (10 ml) and washed with EtOAc (10 ml). Acidification of the aqueous layer at 0° yielded a white ppt which was filtered, rinsed with water and dried *in vacuo*, yield 0.34 g (77%). A second crop was recovered from the aqueous layer (0.060 g, 14%); m.p. 218–219° (m.p. lit[10] 220°). $(\alpha)_D^{20} = -9.2$ (c 0.95 AcOH). $(\alpha)_D$lit[10] − 10.0 (1.04 AcOH); R_F 0.6 (AcOEt 9, MeOH 1, AcOH 1), R_F 0.7 (AcOEt 80, Pyr 20, AcOH 6, H_2O 10). Anal. ($C_{21}H_{31}N_3O_5S$) C.H.N.

BOC-Gly-CAM **14**. Prepared as for **1** from BOC-Gly. Yield 81%. m.p. 64–67°; ^{1}HNMR (DMSO D_6) 1.37 (s, $(CH_3)_3$), 3.82 (d, CH_2), 4.45 (s, $COOCH_2$), 7.29 (t, NH), 7.35 (d, $CONH_2$), R_FA 0.12. Anal. ($C_9H_{16}O_5N_2$) C.H.N.

BOC-L-Thr(Bzl)-Gly-CAM **15**. A sample of **14** (2.55 g, 11 mmoles) was partially deprotected in TFA as previously described and treated with BOC-Thr (Bzl)-OH (Bachem) (3.40 g, 10 mmoles), BOP (4.42 g, 10 mmoles), DIEA (3.6 ml, 21 mmoles) in DMF (30 ml) at 0°. After overnight at room temp, the mixture was treated as described for **2**. Yield 3.73 g (88%), R_FA 0.2; R_FB 0.6, m.p. 63–67°. $(\alpha)_D^{20} = -5.58$ (c 2.1 DMF). Anal. ($C_{20}H_{29}N_3O_7$) C.H.N.

BOC-L-Asp(Bzl)-L-Thr(Bzl)-Gly-CAM **16**. Compound **15** (3 g, 7.1 mmoles) was partially deprotected in TFA. The resulting trifluoroacetate salt was dissolved in DMF (30 ml) in the presence of BOC-L-Asp(Bzl)-OH (2.29 g, 7.1 mmoles) (Bachem) and BOP (3.05 g, 6.9 mmoles) and cooled to 0°. DIEA (2.4 ml, 14.2 mmoles) was added and the mixture was allowed to stand at room temp overnight and then treated as previously described for **2**. Yield 2.95 g (68%). A second crop was recovered from ether of washing 0.52 g (12%). R_FA 0.2, R_FB 0.4, m.p. 88–91° dec. $(\alpha)_D^{20} = -5.7$ (c 1.4 DMF). Anal. ($C_{31}H_{40}N_4O_{10}$) C.H.N.

BOC-L-Phe-L-Asp(OBzl)-L-Thr(Bzl)-Gly-CAM **17**. Prepared from BOC-L-Phe-OH (1.32 g, 5 mmoles), BOP (2.12 g, 4.8 mmoles), DIEA (1.72 ml, 10 mmoles) and the TFA salt of compound **16** (obtained as usually, 5 mmoles) in DMF (20 ml) as previously described. After standing overnight at room temp, the solvent was concentrated *in vacuo*. The residue was treated with an EtOAc–ether mixture (100 ml, 1:2) to yield a white ppt. It was filtered and rinsed several times with ether then with water and again with ether. Yield 2.46 g (66%). Another crop was recovered from the EtOAc–ether mixture 0.45 g (12%), R_FA 0.2, R_FB 0.6, m.p. 154–157°. $(\alpha)_D^{20} = -1.5$ (c 1.95 DMF). Anal. ($C_{40}H_{49}N_5O_{11}$) C.H.N.

Hydrolysis of BOC-L-Phe-L-Asp(OBzl)-Gly-CAM. A sample of **17** (0.388 g, 0.5 mmoles) was dissolved in DMF (5 ml) and treated with Na_2CO_3 (1 mmole) in water 3 ml. After 3 hr, the mixture was treated as described for **13**. The obtained crystalline compound showed on TLC two spots (R_F0.2 for compound **18**, $R_F = 0.3$ for **19**, in AcOEt 80, Pyridine 20, acetic acid 6, H_2O 10). **18** and **19** were separated by chromatography and identified by ^{1}HNMR spectrocopy, **18** showed no more signals for the benzyl of the aspartyl residue, and **19** showed no more signals for the benzyl of the aspartyl residue, or for the CAM group. Hydrolysis with $NaHCO_3$ gave same results. In our hands, we could not obtain selective removal of the CAM ester.

BOC-L-Met-CAM **20**. Prepared from BOC-L-Met as already described, yield 55%; R_FA 0.5, R_FB 0.8, m.p. 78–81°. $(\alpha)_D^{20} = -29.2$ (c 2.7 DMF). Anal. ($C_{12}H_{22}N_2O_5S$) C.H.N.

BOC-L-Phe-L-Met-CAM **21**. A sample of **20** (3.4 g, 11 mmoles) was partially deprotected in TFA, 30 mn, as already described. The resulting salt was allowed to react with BOC-L-Phe (2.9 g, 11 mmoles), BOP (4.42 g, 10 mmoles) and DIEA (3.5 ml, 20 mmoles) in DMF (30 ml) at 0°. After overnight at room temperature, the reaction mixture was treated as already described, yield 4.0 g (88%), R_FA 0.3; R_FB 0.7, m.p. 137–140°. $(\alpha)_D^{20} = -13.9$ (c 1.15 DMF). Anal. ($C_{21}H_{31}N_3O_6S$) C.H.N.

BOC-Gly-L-Phe-L-Met-CAM **22**. Prepared from BOC-Gly (8 mmoles, 1.40 g), BOP (3.31 g, 7.5 mmoles), DIEA (16 mmoles, 2.75 ml) and the TFA salt of L-Phe-L-Met-CAM (8 mmoles, 3.63 g) obtained as already described, in DMF (30 ml). The mixture was treated as usually. The compound need to be purified by chromatography on a silica gel column using EtOAc as eluent, yield 2.87 g (75%). R_FA 0.3; R_FB 0.7, m.p. 115–117°. $(\alpha)_D^{20} = -16.2$ (c 0.8 DMF). Anal. ($C_{23}H_{34}N_4O_7S$) C.H.N.

BOC-Gly-Gly-L-Phe-L-Met-CAM **23**. Prepared from BOC-Gly (5.2 mmoles, 0.91 g), BOP (4.7 mmoles, 2.07 g), DIEA (10 mmoles, 1.72 ml) and the TFA salt of **22** (4.8 mmoles, 2.45 g), in DMF (30 ml). The mixture was treated as usual, yield 2.16 g (81%), R_FA 0.3; R_FB 0.7, m.p. 129–134° dec. $(\alpha)_D^{20} = -18.6$ (c 0.75 DMF). Anal. ($C_{25}H_{37}N_5O_8S$) C.H.N.

BOC-L-Tyr-Gly-Gly-L-Phe-L-Met-CAM **24**. A sample of **23** (3 mmoles, 1.70 g) was deprotected in TFA

(30 mn) as described. The TFA salt was dissolved in DMF (20 ml) and treated with BOC–L–Tyr–OSu[14] (2.9 mmoles, 1.096 g) and DIEA (3 mmoles, 0.52 ml). After 4 hr, the solvent was concentrated *in vacuo* and the residue triturated with a mixture of EtOAc–ether, 0.5:1 (100 ml). The ppt was filtered off, washed with ether, 10% citric acid, water, ether, and dried under vacuo, yield (82%), R_FA 0.1; R_FB 0.5, m.p. = 215–216 dec. $(\alpha)_D^{20} = -19.2$ (c 1.3 DMF). Anal. ($C_{34}H_{46}N_6O_{10}S$) C.H.N.

BOC–L–Tyr–Gly–Gly–L–Phe–L–Met–OH **25**. A sample of **24** (0.731 g, 1 mmole) was dissolved in DMF (10 ml) and treated with Na_2CO_3 (0.16 g, 1.5 mmoles) in water (6 ml), the mixture being kept clear by addition of DMF or water if necessary. After 90 mn, no more ester could be detected (TLC). The pH of the soln was brought to 7 by 0.5 N citric acid and the solvents were concentrated *in vacuo*. The residue was dissolved in sat $NaHCO_3$ (20 ml) and washed with EtOAc (2 × 10 ml). The aqueous layer was cooled (0°) and acidified by solid citric acid (pH 3). The ppt which formed was extracted in EtOAc (2 × 20 ml). The organic layer was washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to dryness. Trituration of the residue with ether gave a white powder, yield 0.53 g (79%). R_FC 0.52; R_FD 0.25, m.p. 110–120° dec. $(\alpha)_D^{20} = -13.6$ (c 1.25 DMF). Anal. ($C_{32}H_{43}N_5O_9S$) C.H.N.

Tyr–Gly–Gly–Phe–Met–OH **26**. Prepared from **25** as described in the lit.[17] The compound obtained showed correct aminoacid analysis as well as same RF as an authentic sample.

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