Studies of Unusual Amino Acids and Their Peptides. XIII. The Chemistry of *N*-(Carboxymethyl)amino Acids. II. The Synthesis of Peptides Containing *N*-(Carboxymethyl)amino Acids

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The basic problems in the synthesis of peptides with *N*-carboxymethyl(Cm-)amino acids were investigated. Several kinds of *N*-protected derivatives of a free Cm-amino acid and the *N*-benzyloxycarbonyl (Z) derivative of its monoester were obtained by the conventional methods used for the parent amino acid. The peptide-bond formation at either carboxyl group of a Cm-amino acid proved to be achievable by the usual methods, such as the carbodiimide, dicyclohexylcarbodiimide–1-hydroxybenzotriazole, or mixed anhydride method, as illustrated by the examples using the Z–Cm-amino acid derivatives as the carboxyl components. On the other hand, a peptide using a Cm-amino acid diester as the amino component could not be prepared by the usual methods mentioned above. The desired compound was eventually obtained by the acid chloride method. A peptide containing a Cm-amino acid with the free imino group was also prepared by the condensation of a bromoacetylated amino acid ester with an amino acid or peptide ester.

In the previous paper,[1] the author has reported on the preparation and properties of a number of *N*-carboxymethyl(Cm-)amino acids and their mono- and diesters. Such polyfunctional amino acids are incorporated in most of the known biologically active peptides. The additional functional groups always pose special problems for the synthesis of peptides and necessitate detailed studies of the amino acids in question. However, nothing is known about the chemistry of peptides containing Cm-amino acids, though bottromycin, an antibiotic, has recently proved to be an example of such a peptide occurring in nature.[2] The present paper will deal with the basic problems in the preparation of peptides containing Cm-amino acids.

Protection of the Carboxyl Groups. The use of a Cm-amino acid[3] for peptide synthesis may involve many types of its *C*-protected derivatives, according to the synthetic routes. Among them, the diester carrying two selectively removable *C*-protecting groups may be the most useful intermediate. The direct preparation of this type of diester from the parent amino acid ester is preferable to the ordinary approach, *i.e.*, the esterification of the free Cm-amino acid prepared beforehand. Thus, as described previously,[1] any derivative of a Cm-amino acid carrying two different ester groups ($R^1 \neq R^2$) can be prepared by condensing an amino acid ester with a haloacetic acid ester (Scheme 1). On the other hand, the esterification of a Cm-amino acid, for example with an alcohol–thionyl chloride, tends to afford its dialkyl ester ($R^1=R^2$). The diester of this type may be utilized for the preparation of a peptide having a Cm-amino acid at the *C*-terminal position.

$$\text{H–AA–OR}^1 + \text{XCH}_2\text{CO}_2\text{R}^2 \longrightarrow \text{H–}\underset{\displaystyle \text{OR}^2}{\underset{|}{\text{C}}}\text{mAA–OR}^1$$

(X = Br or Cl; R^1, R^2 = Me (Et), Bzl, or Bu^t)

Scheme 1.

Protection of the Imino Group. As described in the previous paper,[1] Cm-amino acids are eluted from the strongly acidic cation-exchange resin with water as an eluent. Since this indicates the low basicity of the imino group of a Cm-amino acid, the acylation of this group is presumed to be difficult. Actually, Abderhalden and Haase[4] took advantage of this poor reactivity for the purification of Cm-valine contaminated with valine: Cm-valine was isolated unchanged even after treating the reaction mixture with benzoyl chloride. No other reports concerning the acylation of a free Cm-amino acid have been found. But the *N*-ethylthiocarbonothioyl (ETCT) derivatives of Cm-amino acids were accessible, as described previously.[1] Consequently, the applicability of the conventional methods[5] of *N*-protection to the Cm-amino acid was examined. Cm-alanine, Cm-phenylalanine, and Cm-valine were chosen as model compounds for this purpose.

Contrary to the results reported by Abderhalden and Haase, the benzyloxycarbonyl (Z) group could be introduced by the Schotten-Baumann reaction using the corresponding chloride, though the yields were somewhat poorer than those with the parent amino acids. The *p*-toluenesulfonyl (Tos) derivatives were obtained by the usual method using *p*-toluenesulfonyl chloride as an acylating agent, though in rather low yields. The *o*-nitrobenzenesulfenyl (Nps) derivatives could also be obtained by the use of the corresponding chloride under the Schotten-Baumann conditions. As another type of acylating agent, ethyl trifluoroacetate–1,1,3,3-tetramethylguanidine[6] was tried in the introduction of the trifluoroacetyl (CF_3CO) group. In this case, however, the acylation proceeded quite slowly compared with the case of the parent amino acid and with that of the acylation using a reagent of the acyl chloride type. Interestingly, the formylation using formic acid–acetic anhydride was an exceptional case: the reaction was accomplished in quite a short time. The yields and physical constants of the *N*-protected Cm-amino acids thus obtained are summarized in Table 1. The above examples show that the *N*-protection of a Cm-amino acid can be achieved without any greater difficulty than expected.

Most of the *N*-protected Cm-amino acids were obtained at first as oily substances, and crystallized as the bis(dicyclohexylamine) or bis(cyclohexylamine) salts. Repeated recrystallization and/or prolonged heating of the DCHA salts caused the unsatisfactory

TABLE 1. *N*-PROTECTED Cm-AMINO ACIDS

Compound	Yield %	Salt	Mp[c] °C	$[\alpha]_D^{25}$ (*c* 1.0, MeOH)	Found (Calcd) (%) C	H	N
Z–CmAla	74	2DCHA	150—151 (A)	−3.7°	68.41 (69.01)	9.61 9.55	6.33 6.53)
Z–CmPhe	76	2CHA	189.5—190 (B)	−77.6°	66.98 (67.00)	8.12 8.16	7.26 7.56)
Z–CmVal	50	2CHA	169.5—171 (C)	−27.2°	63.60 (63.88)	9.10 8.94	8.06 8.28)
Tos–CmAla	49	—[b]	157.5—159 (A)	−33.7°[d]	47.77 (47.84)	5.07 5.02	4.59 4.65)
Tos–CmPhe	53	—[b]	173.5—174.5 (A)	0.0°[e]	57.39 (57.29)	5.10 5.08	3.69 3.71)
Tos–CmVal	29	—[b]	179.5—181 (D)	−55.6°	50.95 (51.05)	5.81 5.81	4.23 4.25)
Nps–CmAla	56[a]	2DCHA	159.5—160.5 (E)	+81.6°	63.64 (63.42)	8.73 8.82	8.59 8.45)
Nps–CmPhe	57[a]	2DCHA	174.5—175 (E)	+41.6°	65.97 (66.64)	8.66 8.46	7.30 7.58)
Nps–CmVal	52	—[b]	140—143 (D)	+96.7°[f]	47.89 (47.56)	5.01 4.91	8.35 8.53)
CF_3CO–CmAla	69[a]	2DCHA	168—168.5 (A)	−8.6°	60.98 (61.47)	8.87 8.98	6.82 6.94)
CF_3CO–CmPhe	81	2DCHA	174—174.5 (A)	−46.1°	64.99 (65.17)	8.63 8.57	6.08 6.16)
CF_3CO–CmVal	90[a]	2DCHA	175—175.5 (A)	−18.0°	62.27 (62.53)	9.22 9.22	6.52 6.63)
HCO–CmAla	73[a]	2CHA	173—175 (F)	−14.6°	57.35 (57.88)	9.32 9.45	11.08 11.25)
HCO–CmPhe	64	—[b]	177.5—178 (G)	−63.3°	57.71 (57.37)	5.57 5.22	5.68 5.58)
HCO–CmVal	81[a]	2DCHA	175.5—176 (D)	−10.2°	67.75 (67.92)	10.46 10.51	7.40 7.43)

a) Yield of the salt based on the Cm-amino acid used. b) Physical and analytical data for the free *N*-protected compound. c) Solvents for recrystallization: A, EtOAc; B, EtOH; C, $CHCl_3$–petroleum ether; D, EtOAc–petroleum ether; E, $CHCl_3$–ether; F, EtOH–ether; G, H_2O. d) *c* 0.82. e) ORD: $[\phi]_{300}^{23}$ +184° (*c* 0.34, MeOH). f) *c* 0.40.

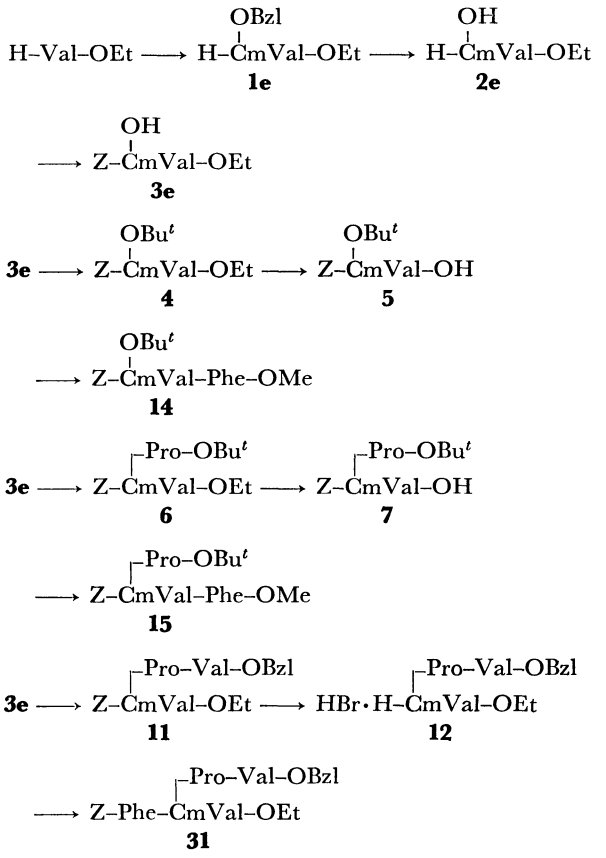
results in elemental analyses, probably due to the partial release of the amine. The melting point of an *N*-protected Cm-amino acid is higher than that of the corresponding derivative of the parent amino acid, while the reverse is the case with their DCHA salts.[7]

The *N*-protection of the monoester of a Cm-amino acid was examined next. A typical example is the benzyloxycarbonylation of the monoester (**2e**) of Cm-valine, prepared from ethyl valinate according to Scheme 2. To avoid the hydrolysis of the ester group, the method using Z–Cl and sodium hydrogencarbonate was chosen. Since the addition of the total amount of the chloride at the beginning had not as usual afforded a good result, its portion by portion addition was tried. By the excessive use of the chloride by 20%, the desired compound (**3e**) was obtained in a yield comparable to the cases of the monoesters of the usual monoamino dicarboxylic acids.

To shorten the synthetic route to the desired compound (**3e**), the direct benzyloxycarbonylation of the reaction mixture obtained by the condensation of ethyl valinate with bromoacetic acid was attempted. (As described previously,[1] the isolation of the monoester (**2e**) from the above mixture was unsuccessful.) The overall yield by this route, however, was no more than 28%; consequently, the route shown in Scheme 2 is more desirable.

The Z derivatives of other Cm-amino acid monoesters could likewise be obtained. These also showed no tendency to crystallize and were characterized as the DCHA or CHA salts (Table 2). As can be seen from Table 2, the DCHA salts of Z derivatives with the free carboxyl group at the glycine moiety (**3a**·DCHA and **3e**·DCHA) have lower melting points than those of their isomers (**3b**·DCHA and **3f**·DCHA).[8]

Formation of the Peptide Bond at the Carboxyl Groups. The peptide-bond formation at the two sorts of carboxyl groups of a Cm-amino acid was investigated, using Cm-valine as a model compound. First, in order to try the coupling at the carboxyl group in the glycine moiety, Z–CmVal–OEt (**3e**) obtained above was used as the carboxyl component, and H–Pro–OBu^t, H–Phe–OBzl,



Scheme 2.

H–Gly–Gly–OEt, H–Phe–Phe–OMe, H–Pro–Val–OBzl, or H–Pro–Val–Val–OBzl as the amino component. The desired di- or tripeptides, or tetrapeptide could be obtained without too much difficulty by the carbodiimide, DCC–HOBt, or mixed anhydride method.[5] All the products were isolated as viscous oils by preparative TLC on silica gel.

The coupling at the carboxyl group in the valine moiety was tried next. Two sorts of carboxyl components (**5** and **7**) were prepared for this purpose, as shown in Scheme 2. Z–CmVal–OEt (**3e**) was converted into the *t*-butyl ester (**4**) with isobutylene in the presence of sulfuric acid. Then the ethyl ester group was cleaved by saponification, to afford **5**. Similarly, the selective removal of the ethyl ester group from the dipeptide **6** afforded **7**. By the DCC–HOBt or mixed anhydride method the desired di- and tripeptide (**14** and **15**)[9] could be obtained, using H–Phe–OMe as the amino component.

When a Z–Cm-amino acid listed in Table 1 was used as the carboxyl component, as illustrated in the case of Z–Cm-phenylalanine, the amino acid ester reacted at both the carboxyl groups and afforded a tripeptide, though in a rather low yield. Cm-proline itself also gave a similar tripeptide (**18**). The yield was better than those with Z–Cm-phenylalanine, though the reaction was heterogeneous, due probably to some remaining zwitter-ionic character of the amino acid.

As illustrated by the above examples, the peptide-bond formation at either carboxyl group of a Cm-amino acid proved to be achievable by the conventional methods. The peptides obtained here are listed in Table 3.

Formation of the Peptide Bond at the Imino Group. As already stated, the acylation of a free Cm-amino acid or its monoester was achieved without too much difficulty. The applicability of the usual coupling methods[5] to the peptide-bond formation at the imino group of a Cm-amino acid was investigated. However, the attempted couplings of Z–Phe–OH with the diester (**1e**) of Cm-valine by such common methods as the mixed anhydride, carbodiimide, DCC–HOBt, or *p*-nitrophenyl ester method all ended in failure. Both the diphenyl phosphorazidate (DPPA) method (a modified azide method) and the carbonyldiimidazole (CDI) method were unsuccessful. This was also the case with the phosphoryl chloride method, or with the phosphazo method, which involves the activation of the

Table 2. *N*-Benzyloxycarbonyl derivatives (**3**) of Cm-amino acid monoesters

Compound	Yield %	Mp[a] °C	$[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$)	Found (Calcd) (%) C	H	N
Z–CmAla–OMe·DCHA (**3a**·DCHA)	78	99—100 (A)	−14.9°	65.65 (65.52	8.62 8.46	5.62 5.88)
Z–CmAla–OH·DCHA (**3b**·DCHA)	81	155—155.5 (B)	−12.5°	65.25 (65.52	8.72 8.46	5.86 5.88)
Z–CmPhe–OMe·CHA (**3c**·CHA)	72	155—156 (C)	−56.0°	66.34 (66.36	7.38 7.28	5.89 5.95)
Z–CmPhe–OH·DCHA (**3d**·DCHA)	58	137—138 (B)	−28.3°	69.26 (69.54	8.18 8.02	5.06 5.07)
Z–CmVal–OEt·DCHA (**3e**·DCHA)	79	109.5—110 (D)	−39.8°	66.95 (67.15	9.15 8.94	5.07 5.40)
Z–CmVal–OH·DCHA (**3f**·DCHA)	68	142—143 (D)	−29.6°	67.12 (67.15	9.21 8.94	5.39 5.40)

a) Solvents for recrystallization: A, acetone–hexane; B, acetone; C, EtOAc; D, hexane.

TABLE 3. PREPARATION OF PEPTIDES USING Cm-AMINO ACID DERIVATIVES AS THE CARBOXYL COMPONENTS

Compound	Carboxyl component; Amino component	(Coupling method[a])	Yield[h] %	$[\alpha]_D^{25}$	TLC, R_f[i]	Found (Calcd) (%) C	H	N
Z-CmVal-Pro-OBu^t (**6**)	**3e**; Pro-OBu^t	(A)	82	−86.7° (*c* 1.0, MeOH)	0.48	63.53 (63.65	7.77 7.80	5.96 5.71)
Z-CmVal-Phe-OBzl (**8**)	**3e**·DCHA; Phe-OBzl·TosOH	(B)[b]	53 (E)	−24.8° (*c* 1.5, $CHCl_3$)	0.59	69.10 (68.97	6.75 6.67	4.89 4.88)
	3e·DCHA; Phe-OBzl·TosOH	(C)	70 (E)	−24.2° (*c* 0.95, $CHCl_3$)	0.59			
	3e; Phe-OBzl·TosOH[c]	(D)	55 (E)	−23.7° (*c* 2.1, $CHCl_3$)	0.59			
Z-CmVal-Gly-Gly-OEt (**9**)	**3e**·DCHA; Gly-Gly-OEt·HBr[d]	(C)	66 (F)	−23.7° (*c* 1.0, MeOH)	0.10	57.56 (57.61	7.16 6.94	8.56 8.76)
Z-CmVal-Phe-Phe-OMe (**10**)	**3e**; Phe-Phe-OMe·HCl[c,e]	(C)	47 (E,G)	−26.6° (*c* 0.90, $CHCl_3$)	0.47	67.08 (66.96	6.78 6.71	6.58 6.51)
Z-CmVal-Pro-Val-OBzl (**11**)	**3e**·DCHA; Pro-Val-OBzl·HBr	(C)	51 (H)	−94.7° (*c* 1.1, $CHCl_3$)	0.49	65.23 (65.47	7.39 7.27	6.72 6.74)
Z-CmVal-Pro-Val-Val-OBzl (**13**)	**3e**·DCHA; Pro-Val-Val-OBzl·HBr[f]	(C)	37 (H)	−92.6° (*c* 0.99, $CHCl_3$)	0.25	64.39 (64.80	7.71 7.53	7.85 7.75)
Z-CmVal-Phe-OMe (**14**)	**5**; Phe-OMe·HCl[c]	(A)	69 (G)	+6.1° (*c* 1.4, $CHCl_3$)	0.63	66.17 (66.14	7.51 7.27	5.34 5.32)
	5; Phe-OMe·HCl[c]	(D)	52 (E)	+4.9° (*c* 1.9, $CHCl_3$)	0.63			
Z-CmVal-Phe-OMe (**15**)	**7**;[g] Phe-OMe·HCl[c]	(A)	46 (H)	−31.8° (*c* 1.6, $CHCl_3$)	0.40	65.91 (65.47	7.43 7.27	6.20 6.74)
Z-CmPhe-Ala-OBzl (**16**)	Z-CmPhe·2CHA; Ala-OBzl·TosOH	(B)[b]	30 (G)	−74.0° (*c* 0.90, $CHCl_3$)	0.56	69.03 (68.91	6.41 6.08	5.90 6.18)
Z-CmPhe-Val-OBzl (**17**)	Z-CmPhe; Val-OBzl·TosOH	(B)	25 (I)	−75.5° (*c* 1.1, $CHCl_3$)	0.63	70.37 (70.19	6.84 6.71	5.65 5.71)
CmPro-Phe-OBzl (**18**)	CmPro; Phe-OBzl·TosOH[c]	(A)	57 (F,G)	−49.0° (*c* 1.7, MeOH)	0.22	71.79 (72.31	6.43 6.38	6.12 6.49)

a) A, DCC–HOBt method; B, EDC method; C, DCC method; D, mixed anhydride method (using isobutyl chloroformate). b) An equimolar amount of TosOH·H_2O to EDC was added. c) An equimolar amount of TEA was added. d) Ref. 18. e) Prepared by the debenzyloxycarbonylation of Z–Phe–Phe–OMe[19] with 5% Pd–C in MeOH containing concd HCl. f) Prepared by the debenzyloxycarbonylation of Z–Pro–Val–Val-OBzl.[21] g) Prepared by the saponification of **6**. h) Solvents for preparative TLC: E, benzene–EtOAc (9 : 1); F, $CHCl_3$–EtOAc (1 : 1); G, benzene–EtOAc (3 : 1); H, benzene–EtOAc (1 : 1); I, $CHCl_3$–EtOAc (9 : 1). i) Benzene–EtOAc (1 : 1).

amino component.

The acid chloride is considered to be the most reactive acylating agent, and has often been employed in the coupling with *N*-methylamino acids, in the synthesis of peptolides, and in the cases where other methods give only poor yields. Fortunately, by the use of Z–Phe–Cl[10] in the presence of *N*-ethyldiisopropylamine (or TEA), the desired dipeptide (**27**) was obtained also in the present case, though in a moderate yield (41%) after several successive purification procedures. In the preparation of dehydropeptides, which has recently been reported,[11] the isolation of an acid chloride was omitted and the reaction mixture was directly used for the coupling with the pyridine solution of the amino component. In the present case, however, the yield by this convenient procedure was no more than 17%. This was also the case with the related method using *N*,*N*-dimethylchloroformimidium chloride, the yield being only 13%. Recently the peptide synthesis using 2-fluoro-1,3,5-trinitrobenzene has been reported:[12] the acid fluoride was supposed to be the reactive acylating agent, and the method was applied to the condensation of amino acids containing rather bulky substituents. The coupling in question, however, resulted in a much poorer yield (12%).

A number of other dipeptides could be obtained by the acid chloride method using the diester of Cm-alanine, Cm-phenylalanine, or Cm-valine as the amino component and the phthalyl (Pht) or Z derivative of glycine, alanine, phenylalanine, or valine as the carboxyl component. The results are summarized in Table 4. A considerable amount of the unchanged amino component remaining even after acid washings was removed by treatment with the cation-exchange resin

Table 4. Preparation of dipeptides using Cm-amino acid diesters as the amino components

Compound	Carboxyl component; Amino component[b,c]	Yield[g] %	$[\alpha]_D^{25}$ ($CHCl_3$)	TLC, R_f[h]	Found (Calcd) (%) C	H	N
Pht-Gly-CmAla-OMe (**19**)	Pht-Gly-Cl; **1a**·TosOH[d]	48 (A)	−33.4° (*c* 2.1)	0.30	62.48 (63.01)	4.99 (5.06)	6.14 (6.39)
Pht-Gly-CmVal-OEt (**20**)[a]	Pht-Gly-Cl; **1e**[e]	44	−16.5° (*c* 1.0)	0.46	65.19 (64.99)	5.87 (5.87)	5.58 (5.83)
Pht-Val-CmAla-OMe (**21**)	Pht-Val-Cl; **1a**·TosOH[d]	58 (A)	−131.6° (*c* 2.2)	0.38	64.55 (64.99)	5.87 (5.87)	5.59 (5.83)
Pht-Val-CmVal-OEt (**22**)	Pht-Val-Cl; **1e**[e]	22 (B)	−186.5° (*c* 1.0)	0.47	66.72 (66.65)	6.77 (6.56)	5.17 (5.36)
Z-Ala-CmPhe-OMe (**23**)	Z-Ala-Cl; **1c**·TosOH[d]	28 (B)	−67.9° (*c* 0.68)	0.44	67.62 (67.65)	6.18 (6.06)	5.10 (5.26)
Z-Ala-CmVal-OEt (**24**)	Z-Ala-Cl; **1e**·TosOH[d]	21 (B)	−52.6° (*c* 1.0)	0.48	65.02 (65.04)	7.04 (6.87)	5.43 (5.62)
Z-Phe-CmAla-OMe (**25**)	Z-Phe-Cl; **1a**·TosOH[d]	29 (A,B)	−30.0° (*c* 1.0)	0.43	67.79 (67.65)	6.26 (6.06)	5.06 (5.26)
Z-Phe-CmPhe-OMe (**26**)	Z-Phe-Cl; **1c**·TosOH[d]	28 (B)	−57.2° (*c* 0.72)	0.52	71.14 (71.03)	6.14 (5.96)	4.31 (4.60)
Z-Phe-CmVal-OEt (**27**)	Z-Phe-Cl; **1e**[f]	41 (B)	−43.9° (*c* 1.1)	0.57	68.95 (68.97)	6.87 (6.67)	4.60 (4.88)
Z-Val-CmAla-OMe (**28**)	Z-Val-Cl; **1a**·TosOH[d]	13 (B)	−33.2° (*c* 0.88)	0.43	64.74 (64.45)	6.86 (6.66)	5.42 (5.78)
Z-Val-CmPhe-OMe (**29**)	Z-Val-Cl; **1c**·TosOH[d]	8 (B)	−71.0° (*c* 0.62)	0.52	68.60 (68.55)	6.65 (6.47)	4.81 (5.00)
Z-Val-CmVal-OEt (**30**)	Z-Val-Cl; **1e**·TosOH[d]	7 (C)	−66.7° (*c* 0.54)	0.55	66.52 (66.14)	7.50 (7.27)	4.83 (5.32)

a) Mp 112.5—114 °C (benzene). b) **1a**, H–CmAla(OBzl)–OMe; **1c**, H–CmPhe(OBzl)–OMe. c) An equimolar amount of *N*-ethyldiisopropylamine or TEA was added when the amino component was used in the form of salt. d) Molar ratio of the carboxyl component to the amino component, 2 : 1. e) Molar ratio, 1.1 : 1. f) Molar ratio, 1 : 1. g) Solvents for preparative TLC: A, benzene–EtOAc (3 : 1); B, benzene–EtOAc (9 : 1); C, benzene–EtOAc (99 : 1). h) Benzene–EtOAc (3 : 1).

Table 5. Preparation of peptides containing Cm-amino acids with the free imino group

Compound	Starting materials	Yield[d] %	$[\alpha]_D^{25}$ (MeOH)	TLC, R_f[e]	Found (Calcd) (%) C	H	N
H–CmVal(Phe-OBzl)–OMe (**32**)[a]	$BrCH_2CO$–Phe–OBzl, Val–OMe·TosOH	30 (A)	−37.5° (*c* 0.88)	0.55	67.87 (67.58)	7.25 (7.09)	6.36 (6.57)
H–CmVal(Val-OBzl)–OMe (**33**)[a]	$BrCH_2CO$–Val–OBzl, Val–OMe·TosOH	47 (A,B)	−50.3° (*c* 1.5)	0.54	63.31 (63.47)	8.16 (7.99)	7.18 (7.40)
H–CmPhe(Phe-OBzl)–Phe–OMe (**34**)[b]	$BrCH_2CO$–Phe–OBzl, Phe–Phe–OMe·HCl	28 (A,C)	−21.3° (*c* 0.46)	0.31	71.44 (71.48)	6.33 (6.32)	6.51 (6.76)
H–CmPhe(Val-OBzl)–Phe–OMe (**35**)[c]	$BrCH_2CO$–Val–OBzl, Phe–Phe–OMe·HCl	40 (A,C)	−30.1° (*c* 2.6)	0.34	69.45 (69.09)	7.04 (6.85)	7.19 (7.33)

a) Syrup. b) Glassy. c) Mp 104—106 °C (with sintering at *ca.* 78 °C) (acetone). d) Solvents for preparative TLC: A, $CHCl_3$–EtOAc (9 : 1); B, benzene–EtOAc (3 : 1); C, $CHCl_3$–EtOAc (3 : 1). e) $CHCl_3$–EtOAc (3 : 1).

(Amberlyst 15). The final stage of purification required successive chromatographic separations on silica gel, to afford the desired compound as a viscous oil, except **20**. In the cases where the acid chlorides used were relatively stable, the desired compounds were obtained in moderate yields. On the other hand, the yields of the couplings using Z–Val–Cl[13] were extremely unsatisfactory. This is presumably attributable to both the

instability and the bulkiness of the chloride. In general, the difficulty in the separation of the desired product from the starting Cm-amino acid diester was another factor which affected the yield.

The elongation of the peptide chain from a peptide having a Cm-amino acid at the *N*-terminal position was examined next. As an example, the tetrapeptide **31** could be obtained in a yield comparable to those of the above dipeptides by the reaction of Z–Phe–Cl with **12**, prepared by the debenzyloxycarbonylation of the tripeptide **11** with hydrogen bromide in acetic acid, as shown in Scheme 2.

As an example of the coupling using a Cm-amino acid with the free carboxyl group as the amino component, the reaction of Z–Phe–Cl with the DCHA salt of the monoester (**2e**) of Cm-valine in dichloromethane was attempted.[14] The desired dipeptide was found to be produced in a *ca.* 20% yield. However, the isolation of the purified product was extremely troublesome, and this approach had to be abandoned.

Preparation of Peptides Containing Cm-amino Acids with the Free Imino Group. As an extension of the method for preparing Cm-amino acid diesters shown in Scheme 1, the condensation of a bromoacetylated amino acid ester or peptide ester with the other amino acid ester or peptide ester might be employed for preparing a peptide containing a Cm-amino acid in the middle of the molecule. In practice, the imino-free di- and tripeptides listed in Table 5 were prepared by this approach, though the yields were lower than those of the preparation of the Cm-amino acid diesters. Benzyl *N*-(bromoacetyl)phenylalaninate and benzyl *N*-(bromoacetyl)valinate were prepared by treatment of the corresponding amino acid benzyl esters with bromoacetyl bromide in the presence of TEA. The condensation with an amino acid or peptide ester was conducted in the presence of TEA at room temperature. The products did not form crystalline salts with *p*-toluenesulfonic or picrolonic acid, unlike the diesters of Cm-amino acids, but they changed to a similar blue coloration when sprayed with cobalt(II) thiocyanate.[15]

In conclusion, the results obtained here indicate that, in the synthesis of peptides containing Cm-amino acids, such routes starting from the parent amino acids as shown in Scheme 2 are highly recommendable. In the case where a new peptide bond is to be built at the imino group of a Cm-amino acid, the acid chloride method or an alternative, equally potent method should be employed.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter, and ORD curves recorded on a JASCO ORD/UV-5 spectropolarimeter. TLC and preparative TLC were performed on Merck Kieselgel 60 F_{254} and Kieselgel GF_{254} (Type 60), respectively. The amino acids and their derivatives used here are all of the L-configuration.

Preparation of N-*Protected Cm-amino Acids.* The introduction of each *N*-protecting group is illustrated by a typical example below. When the desired compound was obtained as a syrup, it was crystallized as the DCHA or CHA salt. The results are summarized in Table 1.

Z–CmPhe: To a solution of Cm-phenylalanine (1.00 g) in 2 M NaOH (4.5 ml) were added Z–Cl (0.85 g) in ether (3 ml) and 2 M NaOH (2.5 ml) simultaneously with vigorous stirring at 0—5 °C, and the mixture was stirred at room temperature overnight. The turbid reaction mixture was washed with ether and acidified to pH 2 with 2 M HCl. The precipitated oil was extracted with EtOAc, and the extract was washed with water and dried over Na_2SO_4. Evaporation of the solvent afforded a syrup; yield, 1.22 g (76%). Upon the addition of double molar amounts of CHA the corresponding salt separated out; mp 189.5—190 °C (EtOH).

Nps–CmVal: To a solution of Cm-valine (250 mg) in 2 M NaOH (1.4 ml) and dioxane (2 ml) were added *o*-nitrobenzenesulfenyl chloride (300 mg) and 2 M NaOH (0.85 ml) simultaneously with vigorous stirring, and the mixture was stirred at room temperature overnight. After addition of water and filtration, the reaction mixture was acidified to pH 2 with 0.5 M H_2SO_4. The precipitated oil was treated in a manner similar to that described for the preparation of Z–CmPhe, to afford crystals; yield, 244 mg (52%); mp 140—143 °C (EtOAc–petroleum ether).

Tos–CmPhe: To a solution of Cm-phenylalanine (1.00 g) in 1 M NaOH (13.5 ml) was added a solution of *p*-toluenesulfonyl chloride (1.29 g) in ether (6 ml) in five portions at intervals of 1 h, and the mixture was stirred at room temperature overnight. The reaction mixture was washed with ether and acidified to Congo Red with 2 M HCl. (The unchanged amino acid occasionally separated out at a lower pH.) The precipitated oil was extracted with EtOAc, and the extract was washed with 2 M HCl and water, and dried over Na_2SO_4. Evaporation of the solvent afforded crystals; yield, 895 mg (53%) (33%, in a run where an equimolar amount of the chloride was used); mp 173.5—174.5 °C (EtOAc).

CF_3CO–CmAla: A suspension of Cm-alanine (200 mg), ethyl trifluoroacetate (970 mg), and 1,1,3,3-tetramethylguanidine (390 mg) was stirred for 3 d (until an almost clear solution was obtained). After evaporation of excess ethyl trifluoroacetate, the residue was dissolved in water and acidified to pH 2 with 4 M HCl. The precipitated oil was treated in a manner similar to that described for the preparation of Z–CmPhe. Upon the addition of double molar amounts of DCHA to the residual syrup the corresponding salt separated out; yield, 569 mg (69%); mp 168—168.5 °C (EtOAc).

HCO–CmPhe: To a chilled solution of Cm-phenylalanine (500 mg) in 99% formic acid (4.7 ml) was added acetic anhydride (1.6 ml) at 0—3 °C, and the mixture was stirred at this temperature for 30 min and then at room temperature for *ca.* 4 h. After the addition and successive evaporation of water to the reaction mixture had been repeated, the residual crystals were filtered, and washed with water; yield, 360 mg (64%); mp 177.5—178 °C (H_2O).

Preparation of the Z Derivatives of Cm-amino Acid Monoesters. A typical example illustrating the preparation of these compounds is shown below.

Z–CmVal–OEt (3e): a) To a stirred solution of H–CmVal–OEt (**2e**) (2.03 g) and $NaHCO_3$ (2.10 g) in water (17.5 ml) was added a solution of Z–Cl (2.70 g) in ether (3 ml) portion by portion, after each consumption, over a period of 4—6 h. Then the mixture was stirred below 5 °C overnight. The reaction mixture was filtered, and the filtrate acidified to pH 2 with 4 M HCl. The deposited oil was extracted with EtOAc, and the extract was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residual syrup was treated with DCHA (1.81 g), to afford the corresponding

salt; yield, 4.08 g (79%); mp 109.5—110 °C (hexane).

b) A mixture of H–Val–OEt·TosOH (3.17 g), bromoacetic acid (1.53 g), and TEA (2.12 g) was refluxed in THF (30 ml) for 50 h with occasional filtration of the precipitates. After the solvent had been removed under reduced pressure, the residue was mixed with the above precipitates, and benzyloxycarbonylated using Z–Cl and $NaHCO_3$ as described above, to afford 0.95 g of the desired compound (**3e**) (28% yield based on H–Val–OEt·TosOH).

Other Z derivatives were obtained in the same manner as used in Method a), and crystallized as the DCHA or CHA salts. The results are summarized in Table 2.

Z–CmVal(OBu^t)–OEt (**4**). A mixture of Z–CmVal–OEt (**3e**) (recovered from the DCHA salt by treatment in EtOAc with 1.5 M HCl as usual) (667 mg), liquid isobutylene (*ca.* 2 ml), concd H_2SO_4 (0.02 ml), and dichloromethane (4 ml) was allowed to stand in a pressure vessel at room temperature overnight. The syrup (662 mg), obtained by the usual work-up,[16] was chromatographed on preparative layers of silica gel with benzene–EtOAc (98 : 2); yield, 566 mg (73%); syrup, $[\alpha]_D^{25}$ −48.2° (*c* 1.0, MeOH). TLC: R_f 0.52 (benzene–EtOAc (9 : 1)). Found: C, 64.15; H, 8.16; N, 3.32%. Calcd for $C_{21}H_{31}NO_6$: C, 64.10; H, 7.94; N, 3.56%.

Preparation of Peptides Using Cm-amino Acid Derivatives as the Carboxyl Components. The desired protected peptides were obtained as syrups. The final purification of all of these compounds, except **6**, was performed by preparative TLC on silica gel. The results are summarized in Table 3. Typical examples illustrating the procedures for the preparation of these compounds are shown below.

Z–CmVal(–Pro–OBu^t)–OEt (**6**), *by the DCC–HOBt Method*: Z–CmVal–OEt (**3e**) (650 mg), H–Pro–OBu^t (prepared through the debenzyloxycarbonylation of Z–Pro–OBu^t [16,17] (650 mg) by hydrogenation), and HOBt (270 mg) were dissolved in THF (7 ml). To the chilled solution was added DCC (400 mg), and the resulting mixture was stirred at 0 °C for 2.5 h and then at room temperature overnight. After *N,N'*-dicyclohexylurea had been removed by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc, washed successively with 5% citric acid, water, 5% $NaHCO_3$, and water, and dried over Na_2SO_4. Removal of the solvent afforded a pure product as a syrup; yield, 776 mg (82%); $[\alpha]_D^{25}$ −86.7° (*c* 1.0, MeOH).

Z–CmVal(–Phe–OBzl)–OEt (8), by the EDC Method: To a chilled mixture of Z–CmVal–OEt·DCHA (**3e**·DCHA) (311 mg), H–Phe–OBzl·TosOH (256 mg), TosOH·H_2O (114 mg), and dioxane (6 ml) was added EDC (93 mg). The resulting mixture was stirred at 0 °C for *ca.* 2 h and then at room temperature overnight. After the mixture had been evaporated under reduced pressure, the residue was distributed between EtOAc and water, and the organic layer was further washed with 2% (or 5%) HCl, water, 5% $NaHCO_3$, and water again, and dried over Na_2SO_4. Removal of the solvent afforded a syrup (305 mg), which was chromatographed on preparative layers of silica gel with benzene–EtOAc (9 : 1); yield, 183 mg (53%); syrup, $[\alpha]_D^{25}$ −24.8° (*c* 1.5, $CHCl_3$).

The tripeptides (**16** and **17**) were prepared using double molar amounts of the amino component and EDC in a manner similar to that described above.

Z–CmVal(–Pro–Val–OBzl)–OEt (11), by the DCC Method: To a chilled mixture of Z–CmVal–OEt·DCHA (**3e**·DCHA) (1036 mg), H–Pro–Val–OBzl·HBr (prepared by the debenzyloxycarbonylation of Z–Pro–Val–OBzl[20] (876 mg) with 25% HBr in AcOH (2.64 g) as usual), and CH_3CN (24 ml) was added DCC (415 mg) at 0 °C. The reaction mixture was stirred at this temperature for 2 h and then at room temperature overnight. After filtration of *N,N'*-dicyclohexylurea and a work-up similar to that described for the preparation of **8**, the residual syrup was chromatographed on preparative layers of silica gel with benzene–EtOAc (1 : 1); yield, 640 mg (51%); syrup, $[\alpha]_D^{25}$ −94.7° (*c* 1.1, $CHCl_3$).

Z–CmVal(OBu^t)–Phe–OMe (14), by the Mixed Anhydride Method: To a chilled solution of **5** (obtained by the saponification of **4** with 0.5 M NaOH in aq EtOH) (417 mg) and TEA (115 mg) in THF (3 ml) was added isobutyl chloroformate (156 mg) with vigorous stirring below −10 °C. After 5 min a mixture of H–Phe–OMe·HCl (246 mg) and TEA (115 mg) in $CHCl_3$ (3 ml) was added at *ca.* −10 °C. The resulting mixture was stirred below 0 °C for *ca.* 3 h and then at room temperature overnight. After a work-up similar to that described for the preparation of **6**, the residual syrup was chromatographed on preparative layers of silica gel with benzene–EtOAc (9 : 1); yield, 311 mg (52%); syrup, $[\alpha]_D^{25}$ +4.9° (*c* 1.9, $CHCl_3$).

Z–CmPro(–Phe–OBzl)–Phe–OBzl (18), by the DCC–HOBt Method: To a chilled suspension of Cm-proline (157 mg), H–Phe–OBzl·TosOH (778 mg), TEA (184 mg), and HOBt (245 mg) in $CHCl_3$–THF–DMF (6 ml each) was added DCC (374 mg), and the resulting mixture was stirred at 0 °C for *ca.* 2 h and then at room temperature for 2 d. After *N,N'*-dicyclohexylurea had been removed by filtration, the filtrate was treated in a manner similar to that described for the preparation of **8**, to afford a syrup (502 mg). It was chromatographed on preparative layers of silica gel with $CHCl_3$–EtOAc (1 : 1) and then with benzene–EtOAc (3 : 1); yield, 333 mg (57%); syrup, $[\alpha]_D^{25}$ −49.0° (*c* 1.7, MeOH).

Preparation of Dipeptides Using Cm-amino Acid Diesters as the Amino Components. All the desired protected peptides were prepared by the acid chloride method. They were obtained as syrups, except **20**. The final purification of all of these compounds, except **20**, was performed by preparative TLC on silica gel. The results are summarized in Table 4. The carboxyl components, Pht–Gly–Cl,[22] Pht–Val–Cl,[23] Z–Ala–Cl,[24] and Z–Phe–Cl,[10] were prepared according to the literature. Z–Val–Cl[13] was prepared in a manner similar to the case of Z–Ala–Cl as follows: to a stirred solution of Z–Val–OH (350 mg) in dry Et_2O (3 ml) was added PCl_5 (310 mg) portion by portion at 0 °C. After 25 min the resulting solution was filtered, and the filtrate concentrated under reduced pressure below 10 °C. The residual syrup was washed well with dry petroleum ether, and dissolved in dry THF (1.5 ml). The solution was directly used for the coupling reaction. Typical examples are shown below.

Z–Phe–CmVal(OBzl)–OEt (27): To a stirred solution of **1e** (980 mg) and *N*-ethyldiisopropylamine (430 mg) (or TEA (337 mg)) in dry THF (5 ml) was added Z–Phe–Cl (freshly prepared by the reaction of Z–Phe–OH (1.00 g) with PCl_5 (800 mg) in dry ether) in dry THF (2 ml) at 0 °C. The mixture was stirred at this temperature for *ca.* 3 h and then at room temperature for 2 d. The precipitates were filtered off, and the filtrate evaporated under reduced pressure. The residue was distributed between EtOAc (or Et_2O) and water, and the organic layer washed successively with 10% (w/w) HCl, water, 5% $NaHCO_3$, and water. After the dried (over

Na_2SO_4) solution had been evaporated under reduced pressure, the residue was treated batchwise with Amberlyst 15 (*ca.* 2 g) in MeOH, and then chromatographed on preparative layers of silica gel with benzene–EtOAc (9 : 1), to afford a syrup; yield, 787 mg (41%); $[\alpha]_D^{25}$ −43.9° (*c* 1.1, $CHCl_3$).

OBzl
|
Pht–Gly–CmVal–OEt (20): A mixture of **1e** (2.03 g), *N*-ethyldiisopropylamine (0.89 g), Pht–Gly–Cl (1.70 g), and dry THF (16 ml) was stirred at 0 °C for a few hours and then at room temperature for 2 d. After washing with 10% (w/w) HCl and 0.5 M $NaHCO_3$ the crude product (2.31 g) was obtained as crystals; mp 107—113 °C. It was recrystallized from benzene; yield, 1.44 g (44%); prisms, mp 112.5—114 °C, $[\alpha]_D^{25}$ −16.5° (*c* 1.0, $CHCl_3$).

OBzl
|
Z–Ala–CmPhe–OMe (23): A solution of **1c**·TosOH (350 mg) and TEA (142 mg) in dry THF (5 ml) was allowed to react with a solution of Z–Ala–Cl (freshly prepared by the reaction of Z–Ala–OH (310 mg) with PCl_5 (310 mg) in dry ether as a syrup and then dissolved in dry THF (1.5 ml)) in a manner similar to that described for the preparation of **27**. The final purification by preparative TLC with benzene–EtOAc (9 : 1) afforded the desired compound (**23**) as a syrup; yield, 104 mg (28%); $[\alpha]_D^{25}$ −67.9° (*c* 0.68, $CHCl_3$).

–Pro–Val–OBzl
|
Z–Phe–CmVal–OEt (31). The tripeptide **11** (310 mg) was treated with 25% HBr in AcOH (0.7 g) for 1 h, and worked up as usual, to afford **12** in a 72% yield. This product (204 mg) was mixed with *N*-ethyldiisopropylamine (107 mg) in dry THF (3 ml), and allowed to react with Z–Phe–Cl (freshly prepared from Z–Phe–OH (238 mg) as described above), at 0 °C for 5 h and then at room temperature for 2 d. After washing in a manner similar to that described for the preparation of **27**, the residual syrup (174 mg) was chromatographed on preparative layers of silica gel with benzene–EtOAc (1 : 1); yield, 84 mg (30%); syrup, $[\alpha]_D^{25}$ −126.1° (*c* 0.62, $CHCl_3$). TLC: R_f 0.09 (benzene–EtOAc (3 : 1)), 0.46 (benzene–EtOAc (1 : 1)). Found: C, 66.34; H, 7.12; N, 7.24%. Calcd for $C_{43}H_{54}N_4O_9$: C, 66.99; H, 7.06; N, 7.27%.

Preparation of Peptides Containing Cm-amino Acids with the Free Imino Group. The final purification of the desired peptides was performed by preparative TLC on silica gel. The results are summarized in Table 5. The general procedure for the preparation of these compounds is illustrated by the following example.

–Val–OBzl
|
H–CmVal–OMe (33): To an ice-chilled solution of H–Val–OBzl·TosOH (3.75 g) and TEA (2.00 g) in THF (30 ml) was added bromoacetyl bromide (2.00 g) in THF (10 ml) drop by drop. Then the mixture was stirred below 5 °C overnight. The usual work-up afforded benzyl *N*-(bromoacetyl)valinate as a syrup; yield, 2.85 g (88%). (Benzyl *N*-(bromoacetyl)phenylalaninate was prepared in a similar manner in an 89% yield.) This product was used for the next reaction without further purification.

To a stirred solution of H–Val–OMe·TosOH (750 mg) and TEA (500 mg) in THF (6 ml) was added the bromoacetylated compound (870 mg) obtained above in THF (4 ml) drop by drop. Then the reaction mixture was stirred at room temperature for a week, with occasional filtration of the precipitates. After the solvent had been removed under reduced pressure, the residue was distributed between EtOAc and water, and the organic layer was washed with water and dried over Na_2SO_4. Removal of the solvent afforded a syrup, which was chromatographed on preparative layers of silica gel successively with $CHCl_3$–EtOAc (9 : 1) and benzene–EtOAc (3 : 1); yield, 442 mg (47%); syrup, $[\alpha]_D^{25}$ −50.3° (*c* 1.5, MeOH).

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21) This compound was prepared from Z–Pro–OH and H–Val–Val–OBzl·HBr (prepared by the debenzyloxycarbonylation of Z–Val–Val–OBzl[25]) with 25% HBr in AcOH) by the mixed anhydride method using ethyl chloroformate in an 84% yield; mp 108—110 °C (EtOAc–petroleum ether), $[\alpha]_D^{25}$ −95.4° (*c* 1.0, MeOH). Found: C, 67.03; H, 7.48; N, 7.78%. Calcd for $C_{30}H_{39}N_3O_6$: C, 67.02; H, 7.31; N, 7.82%.
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