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Studies of Unusual Amino Acids and Their Peptides. XII. The Chemistry of *N*-(Carboxymethyl)amino Acids. I. The Preparation, Properties, and Characterization of *N*-(Carboxymethyl)amino Acids and Their Esters

Toshifumi Miyazawa

Department of Chemistry, Faculty of Science, Kōnan University, Okamoto, Higashinada-ku, Kobe 658

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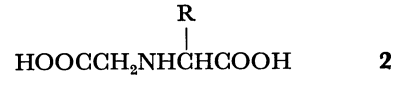
N-Carboxymethyl(Cm-)amino acids and their mono- and diesters were prepared by several routes, and their physical and chiroptical properties were compared with those of the parent amino acids and their esters. The isolation procedure was improved for the preparation of the free Cm-amino acids obtained from the corresponding amino acids and bromoacetic acid; the aimed-at compounds were also obtained by the catalytic hydrogenolysis of the dibenzyl esters. The isomeric pairs of monoalkyl esters of Cm-amino acids were similarly accessible via the benzyl alkyl esters. The diesters proved to be suitable for the GLC and MS analyses of Cm-amino acids. All the free Cm-L-amino acids and the dicyclohexylammonium salts of their *N*-ethylthiocarbonothioyl derivatives show positive Cotton effects at 200—225 nm and at 335—350 nm respectively in CD measurements; therefore, these correlations can be used for the configurational assignments of Cm-amino acids.

Up to the present, various kinds of unusual amino acids have been found in nature. Among them there is a group which can be represented by the general formula **1**. Some typical examples of such amino

1

acids are octopine (R=CH_3, R′=$(CH_2)_3NHC(=NH)NH_2$),[1] lysopine (R=CH_3, R′=$(CH_2)_4NH_2$),[2] and nopaline (R=$(CH_2)_2CO_2H$, R′=$(CH_2)_3NHC(=NH)NH_2$).[3] The importance of this group seems to have been becoming increasingly great, for the occurrence of *N*-carboxymethyl(Cm-)amino acids (**2**), simple-structured members of this group, has recently been reported.



2

Cm-*t*-leucine[4] has been found in a peptide antibiotic, bottromycin A_2, and Cm-alanine has been isolated from an extract of *Strombus gigas* as a fish attractant and designated as strombine.[5]

Unfortunately, though synthetic Cm-amino acids have been referred to in the literature, with regard to their physical properties and chemical behavior little has yet been investigated systematically, except for their coordination chemistry.[6] For some of the synthetic Cm-amino acids even their melting points and/or optical rotations remain undetermined. In particular, nothing is known about the chemistry of peptides containing Cm-amino acids. In connection with the synthetic work of bottromycin[7] the present author felt an interest in this kind of unusual amino acids and started a systematic investigation of them.

Preparation of Cm-amino Acids and Their Esters. The Cm-amino acids[8] listed in Table 1 were prepared by condensing the corresponding amino acids with bromoacetic acid according to the method of Snyder and Angelici.[6a] Even after 4 h, the consumption of the starting amino acid was always incomplete, as determined by following its ninhydrin reaction;[14] nevertheless, an appreciable amount of the *N,N*-biscarboxymethylated derivative was produced in some cases. For the isolation of the Cm-amino acid from the reaction mixture, ion-exchange chromatography, recommended by the above authors, was useful, but elution with aq ammonia did not always give a satisfactory separation. In general, water proved to be a better eluent; through a short column of cation-exchange resin the *N,N*-biscarboxymethylated amino acid was eluted first, and the Cm-amino acid next, while the unchanged amino acid was usually immobile. The rate of elution was quite fast for such Cm-amino acids as Cm-alanine, Cm-aspartic acid, Cm-proline, and Cm-serine. On the other hand, such Cm-amino acids with a low solubility as Cm-phenylalanine and Cm-*t*-leucine could be purified merely by recrystallizing the crude products from water.

As alternative routes[15] to the aimed-at compounds the following reactions were also attempted: the condensations of (i) an amino acid with a haloacetic ester, (ii) an amino ester with a haloacetic acid, and (iii) an amino ester with a haloacetic ester. Though the resulting esters must be hydrolyzed (or hydrogenolyzed) to obtain free Cm-amino acids, they can be used as intermediates for peptide synthesis. Especially by Method (iii) it is possible to prepare derivatives of a Cm-amino acid carrying selectively removable *C*-protecting groups.

However, Methods (i) and (ii) were of no practical use, because of the low rates of conversion, probably because of the heterogeneity of the reaction (Method (i)), or because of the difficulty in the isolation of the desired products (Methods (i) and (ii)). On the other hand, Method (iii) always gave a satisfactory result. In this method, the formation of the *N,N*-biscarboxymethylated derivative was not observed. After the crude mixture had been washed with aq citric acid to remove the unchanged amino ester, it was further purified by extracting in acid. If the product obtained here was not pure enough, it was necessary to resort to column or thin-layer chromatography. The dimethyl or diethyl esters were also accessible from the free Cm-amino acids by the usual method. The diesters (**3**) of Cm-amino acids thus obtained are listed in Table 2.

TABLE 1. *N*-(CARBOXYMETHYL)AMINO ACIDS (**2**)

Compound	Yield (%)	Mp[i] (°C)	$[\alpha]_D^{25}$ (*c* 1.0)		^{1}H-NMR, δ (-NHC$\underline{H}_2$CO-)[q]	Elemental analysis Found (Calcd)		
			H_2O	1 M HCl		C%	H%	N%
L-CmAbu	65	205.5—207 (A)	+14.5°	+9.8°	E 4.36 F 3.22	44.94 (44.71	7.04 6.88	8.81 8.69)
L-CmAla[a]	66	203.5—204(dec) (B)	+6.3°	+3.7°	E 4.34 F 3.65	40.81 (40.81	6.23 6.17	9.66 9.52)
L-CmAsp[b]	44	185—186(dec) (A)	+11.8°	+15.6°	E 4.49 F 3.38	37.60 (37.70	4.58 4.75	7.16 7.33)
L-CmIle[c]	49	189—189.5(dec) (C)	+16.0° [j]	+13.9°	E 4.31 F 3.05	50.62 (50.78	7.99 7.99	7.12 7.40)
L-CmLeu[d]	44	200.5—201.5(dec) (A)	+10.3°	+12.5° [n]	E 4.35 F 3.07	50.53 (50.78	8.17 7.99	7.55 7.40)
L-CmLys(Tos)	66	171—172.5(dec) (A)	—[k]	+14.6°	E 4.38 F 3.08	50.22 (50.27	6.23 6.19	7.82 7.82)
L-CmPhe[e]	72	223—223.5(dec) (A)	—[k]	+15.7°	E 4.24 F 3.29	58.94 (59.18	5.98 5.87	6.19 6.28)
D-CmPhg	51	227—229(dec) (A)	—[k]	−149.0° [o]	E 4.16 F 3.05	57.29 (57.41	5.03 5.30	6.52 6.70)
L-CmPro[f]	37	217.5—219.5(dec) (D)	−59.9°	−58.2°	E 4.46 F 2.91 and 3.32[r]	48.81 (48.55	6.48 6.40	8.21 8.09)
L-CmSer[g]	22	178—182(dec) (A)	+0.5°	+5.4°	E 4.55 F 3.15	36.63 (36.81	5.45 5.56	8.87 8.59)
L-CmThr	34	165.5—168.5(dec) (A)	−26.7°	−27.8°	E 4.51 F 3.12	40.38 (40.68	6.10 6.26	7.61 7.91)
L-CmTle	75	247—250(dec) (A)	−14.2° [l]	−23.3°	E 4.33[s] F 3.32 and 3.44[t]	50.83 (50.78	8.03 7.99	7.66 7.40)
DL-CmTle	71	227—229(dec) (A)				50.68 (50.78	8.11 7.99	7.36 7.40)
L-CmTyr[h]	30	243.5—245.5(dec) (A)	—[k]	+14.5° [p]	E 4.27 F 3.05	55.08 (55.23	5.36 5.48	5.58 5.86)
L-CmVal	64	212—213(dec) (C)	+6.8° [m]	+1.8°	E 4.32 F 3.51 and 3.56[r]	47.75 (47.99	7.65 7.48	7.71 8.00)

a) Lit,[9] mp 220 °C. b) Lit,[10] mp 198—199 °C, $[\alpha]_D^{27}$ +4.1° (H_2O), $[\alpha]_D^{27}$ +11° (1 M HCl). c) Lit,[11] mp 197—198 °C. d) Lit,[12] mp 201 °C (dec), $[\alpha]_D^{24}$ +8.42° (*c* 0.95, 0.2 M HCl). e) Lit,[12] mp 243 °C, $[\alpha]_D^{26}$ +15.67° (*c* 1.85, 1 M HCl); lit,[10] mp 228—233 °C (dec). f) Lit,[10] mp 220 °C (dec). g) Lit,[13] mp 167 °C, $[\alpha]_{578}$ +3° (*c* 2.2, H_2O). h) Lit,[12] mp 247 °C (dec), $[\alpha]_D^{25}$ +10.11° (*c* 0.89, 0.2 M HCl). i) Solvents for recrystallization: A, H_2O; B, aq EtOH; C, aq acetone; D, aq *i*-PrOH. j) *c* 0.77. k) Insufficiently soluble; not measured. l) *c* 0.54. m) *c* 0.80. n) $[\alpha]_D^{24}$ +11.4° (*c* 0.95, 0.2 M HCl). o) *c* 0.52. p) $[\alpha]_D^{25}$ +14.6° (*c* 0.89, 0.2 M HCl). q) E, in TFA; F, in D_2O-NaOD (pH 12). r) ABq, J=15 Hz. s) Broad. t) ABq, J=16 Hz.

To obtain a free Cm-amino acid, the diester was heated in 6 M HCl (1 M=1 mol dm^{-3}) at 110 °C for *ca.* 15 h. Even on heating with a more dilute acid or with 6 M HCl for a few hours, the diester disappeared completely, but there remained an appreciable amount of the monoester(s). Under these conditions, the formation of neither glycine nor the other amino acid was observed.[16] The rate of the acidic hydrolysis of a benzyl ester, especially of a dibenzyl ester, was quite slow compared with that of a dimethyl (or diethyl) ester.

On the other hand, its debenzylation by catalytic hydrogenolysis proceeded quite smoothly; thus, the Cm-amino acid was obtained from the corresponding dibenzyl ester in a good yield. Similarly, the monoalkyl esters (**4** and **5**) of Cm-amino acids were obtained from the corresponding benzyl alkyl esters, which are listed in Table 3. In the preparation of **5b**, the crude benzyl ethyl ester (**3f**), contaminated by benzyl valinate, was also used, because the desired compound could easily be separated from the reduction mixture. This is not the case with its isomer (**4b**).

Properties of Cm-amino Acids and Their Esters. As can be seen from Table 1, the melting (or decomposition) point of a Cm-amino acid is much lower than that of the parent amino acid. The optical rotation of a sample of Cm-phenylalanine prepared by the condensation of phenylalanine with bromoacetic acid was in good agreement with that of a different sample prepared by the hydrogenolysis of the dibenzyl ester (**3o**) or by the acidic hydrolysis of the dimethyl ester (**3l**), obtained by the condensation of a phenylalanine ester with a bromoacetic ester (see Experimental). The coincidence of the optical rotations of the Cm-amino acid prepared in these different ways apparently means that little or no racemization occurred during

Table 2. Diesters (**3**) of Cm-L-amino acids

Compound	Yield (%)	n_D^t (t, °C)	IR (cm^{-1})[b] ν_{NH} / $\nu_{C=O}$	^{1}H-NMR, δ[c]	Salt[d]	Mp[e] (°C)	$[\alpha]_D^{25}$ (c 1.0, MeOH)	Elemental analysis (%) Found	Calcd
OMe CmVal-OMe (**3a**)	58[a]	1.4410 (21)	3310 1743	0.96(6H, d, J=6.5 Hz), 1.97(1H, m), 2.09(1H, s), 3.04(1H, d, J=5.5 Hz), 3.35(2H, s), 3.69(6H, s)	**t**	123—125 (A)	+3.5°	C: 51.25 H: 6.78 N: 3.72	51.19 6.71 3.73
OEt CmVal-OEt (**3b**)	85	1.4330 (24)	3320 1736	0.96(6H, d, J=6.5 Hz), 1.26(6H, t, J=7 Hz), 1.98(1H, m), 2.02(1H, s), 3.02(1H, d, J=5.5 Hz), 3.33(2H, s), 4.17(4H, q)	**p**	120—121 (B)	+1.6°	C: 50.74 H: 5.83 N: 14.26	50.91 5.90 14.13
OBzl CmVal-OMe (**3c**)	75	1.4902 (24)	3310 1740	0.96(6H, d, J=6.5 Hz), 1.97(1H, m), 2.02 (1H, s), 3.05(1H, d, J=5.5 Hz), 3.40(2H, s), 3.68(3H, s), 5.12(2H, s), 7.31(5H, s)	**p**	150—151 (B)	+2.0°	C: 55.20 H: 5.58 N: 12.86	55.25 5.38 12.88
OMe CmVal-OBzl (**3d**)	62	1.4802 (24)	3330 1737	0.95(6H, d, J=6.5 Hz), 2.00(1H, m), 2.05 (1H, s), 3.07(1H, d, J=6 Hz), 3.35 (2H, s), 3.67(3H, s), 5.13(2H, s), 7.31(5H, s)	**p**	119—120 (B)	−12.3°	C: 55.13 H: 5.56 N: 12.87	55.25 5.38 12.88
OBzl CmVal-OEt (**3e**)	76	1.4849 (25)	3300 1737	0.97(6H, d, J=6.5 Hz), 1.26(3H, t, J=7 Hz), 1.98(1H, m), 2.14(1H, s), 3.05(1H, d, J=5.5 Hz), 3.42(2H, s), 4.17(2H, q, J=7 Hz), 5.13 (2H, s), 7.31(5H, s)	**t**	87.5—89 (C)	−12.4° [f]	C: 59.03 H: 6.65 N: 2.81	59.34 6.71 3.01
OEt CmVal-OBzl (**3f**)	61	1.4864 (26)	3330 1732	0.94(6H, d, J=6.5 Hz), 1.23(3H, t, J=7 Hz), 2.00(1H, m), 2.11(1H, s), 3.08(1H, d, J=6 Hz), 3.33(2H, s), 4.14(2H, q, J=7 Hz), 5.13 (2H, s), 7.31(5H, s)	**t**	117—118.5 (C)	−21.1° [f]	C: 59.09 H: 6.83 N: 2.93	59.34 6.71 3.01
OBzl CmVal-OBzl (**3g**)	72	1.5329 (19)	3320 1732	0.93(6H, d, J=6.5 Hz), 1.97(1H, m), 2.04 (1H, s), 3.07(1H, d, J=6 Hz), 3.39(2H, s), 5.10(4H, s), 7.28(10H, s)	**p**	114.5—116 (B)	−8.2°	C: 60.13 H: 5.32 N: 11.36	60.09 5.37 11.30
OBu^t CmVal-OMe (**3h**)	62	1.4410 (20)	3320 1738	0.97(6H, d, J=6.5 Hz), 1.44(9H, s), 1.97 (1H, m), 2.02(1H, s), 3.02(1H, d, J=5.5 Hz), 3.23(2H, s), 3.68(3H, s)	**p**	153—153.5 (B)	+3.8°	C: 51.75 H: 6.27 N: 13.71	51.86 6.13 13.75
OMe CmAla-OMe (**3i**)	67[a]	1.4292 (31)	3310 1735	1.33(3H, d, J=6.5 Hz), 2.11(1H, s), 3.40 (2H, s), 3.40(1H, q, J=6.5 Hz), 3.69(6H, s)	**p**	134.5 —135.5 (B)	+9.5°	C: 46.56 H: 4.83 N: 15.75	46.47 4.82 15.94

Table 2. (Continued)

Compound	Yield (%)	n_D^t (t, °C)	IR (cm^{-1})[b] ν_{NH} $\nu_{C=O}$	^{1}H-NMR, δ[c]	Salt[d]	Mp[e] (°C)	$[\alpha]_D^{25}$ (c 1.0, MeOH)	Elemental analysis (%) Found	Calcd
OBzl CmAla-OMe (**3j**)	63	1.5035 (25)	3320 1739	1.31(3H, d, J=7 Hz), 2.17(1H, s), 3.40(1H, q, J=7 Hz), 3.43(2H, s), 3.66(3H, s), 5.11 (2H, s), 7.29(5H, s)	**t**	156—157 (B)	−1.2°	C: 56.66 H: 5.91 N: 3.04	56.73 5.95 3.31
OMe CmAla-OBzl (**3k**)	64	1.5015 (25)	3290 1745	1.33(3H, d, J=7 Hz), 2.14(1H, s), 3.39(2H, s), 3.43(1H, q, J=7 Hz), 3.66(3H, s), 5.12 (2H, s), 7.30(5H, s)	**t**	159—161 (D)	−9.2°	C: 56.98 H: 6.06 N: 3.29	56.73 5.95 3.31
OMe CmPhe-OMe (**3l**)	84[a]	1.5009 (27)	3320 1741	2.06(1H, s), 2.88—3.00(2H, three peaks, J_{vic}=6 Hz and 7 Hz), 3.33(2H, s), 3.45, (1H, o), 3.60 and 3.61(6H, each s), 7.18 (5H, s)	**t**	108—108.5 (A)	+21.8°	C: 56.59 H: 6.07 N: 3.25	56.73 5.95 3.31
OBzl CmPhe-OMe (**3m**)	57	1.5426 (26)	3320 1740	2.09(1H, s), 2.91—3.02(2H, three peaks, J_{vic}=6 Hz and 7 Hz), 3.36(2H, s), 3.45 (1H, o), 3.55(3H, s), 5.05(2H, s), 7.16 (5H, s), 7.25(5H, s)	**t**	147.5—149 (A)	+18.3°	C: 62.47 H: 5.77 N: 2.65	62.51 5.85 2.80
OMe CmPhe-OBzl (**3n**)	66	1.5173 (29)	3330 1736	2.10(1H, s), 2.96(2H, d-like, $J\fallingdotseq$6.5 Hz), 3.34(2H, s), 3.50(1H, o), 3.60(3H, s), 5.04(2H, s), 7.14(5H, s), 7.22(5H, s)	**p**	149.5 —150.5 (B)	+7.1°	C: 58.88 H: 5.12 N: 11.79	58.88 4.94 11.84
OBzl CmPhe-OBzl (**3o**)	65	1.5587 (27)	3330 1737	2.24(1H, s), 2.95(2H, d-like, $J\fallingdotseq$7 Hz), 3.37 (2H, s), 3.61(1H, t-like, $J\fallingdotseq$7 Hz), 5.00 and 5.04(4H, each s), 7.12—7.24(15H, m)	**t**	123.5 —124.5 (A)	+5.2°	C: 66.74 H: 5.87 N: 2.23	66.77 5.78 2.43
OMe CmLeu-OBzl (**3p**)	56	1.4861 (28)	3310 1738	0.91(6H, bd, J=5.5 Hz), around 1.55(3H, m), 2.02(1H, s), 3.36(1H, o), 3.36(2H, s) 3.65 (3H, s), 5.12(2H, s), 7.30(5H, s)	**p**	114—116 (B)	−4.6°	C: 55.81 H: 5.79 N: 12.36	56.01 5.60 12.56
OBzl CmLeu-OBzl (**3q**)	62	1.5183 (30)	3320 1730	0.88(6H, bd, J=5.5 Hz), around 1.55(3H, m), 1.97(1H, s), 3.35(1H, o), 3.39(2H, s), 5.09 (4H, s), 7.26(10H, s)	**t**	132—133.5 (D)	−3.5°	C: 64.30 H: 6.53 N: 2.70	64.31 6.51 2.59

a) By the direct esterification of Cm-amino acids. b) Neat. c) In $CDCl_3$. The signal attributable to -NHC$\underline{H_2}$CO- is underlined. d) **t**: p-toluenesulfonate; **p**: picrolonate. e) Solvents for recrystallization: A, AcOEt; B, EtOH; C, AcOEt-petroleum ether; D, EtOH-Et_2O. f) c 1.0, AcOEt.

Table 3. Monoesters (**4** and **5**) of Cm-L-amino acids

Compound	Yield (%)	Mp[b] (°C)	$[\alpha]_D^{25}$ (c 1.0)[c]	TLC, R_f[e]	^{1}H-NMR, δ[f]	Elemental analysis (%) Found	Calcd
OH CmVal-OMe (**4a**)	42	139—140	A −0.9° B −2.7°	C 0.18 D 0.54 E 0.52	1.07(3H, d, J=6.5 Hz), 1.09(3H, d, J=6.5 Hz), 2.42(1H, m), 3.64 and 3.66 (2H, each s-like), 3.85(3H, s), 4.06(1H, d, J=4 Hz)	C: 50.62 H: 8.17 N: 7.42	50.78 7.99 7.40
OMe CmVal-OH (**5a**)	51	170—171	A −1.5° B +1.2°	C 0.15 D 0.47 E 0.53	1.06(6H, d, J=7 Hz), 2.31(1H, m), 3.58(1H, d, J=4.5 Hz), 3.81(3H, s), 4.00(2H, s)	C: 51.01 H: 8.21 N: 7.69	50.78 7.99 7.40
OH CmVal-OEt (**4b**)	81	149—150	A −7.2° B −9.2°	C 0.23 D 0.56 E 0.69	0.92(3H, d, J=7 Hz), 0.95(3H, d, J=7 Hz), 1.27 (3H, t, J=7 Hz), 1.90(1H, m), 3.08(2H, s), 3.15(1H, d, J=5 Hz), 4.20(2H, q, J=7 Hz)	C: 52.97 H: 8.66 N: 6.62	53.19 8.43 6.89
OEt CmVal-OH (**5b**)	68[a]	167—168	A +1.2° B +0.6°	C 0.27 D 0.54 E 0.71	0.91(6H, d, J=6.5 Hz), 1.23(3H, t, J=7 Hz), 1.85(1H, m), 2.81(1H, d, J=5.5 Hz), 3.33(2H, s), 4.17(2H, q, J=7 Hz)	C: 53.02 H: 8.48 N: 6.92	53.19 8.43 6.89
OH CmAla-OMe (**4c**)	82	167—169	A −2.4° B −3.6°	C 0.07 D 0.38 E 0.19	1.28(3H, d, J=7 Hz), 3.12(2H, s), 3.45(1H, q, J=7 Hz), 3.71(3H, s)	C: 44.48 H: 6.85 N: 8.65	44.71 6.88 8.69
OMe CmAla-OH (**5c**)	79	186—189	A +1.4° B +1.8°	C 0.06 D 0.36 E 0.17	1.29(3H, d, J=7 Hz), 3.31(1H, q, J=7 Hz), 3.56(2H, s), 3.74(3H, s)	C: 44.61 H: 6.97 N: 8.63	44.71 6.88 8.69
OH CmPhe-OMe (**4d**)	82	157.5—159	A +15.8° B +17.2°	C 0.28 D 0.57 E 0.72	2.91—3.03(2H, three peaks, J_{vic}=6 Hz and 7 Hz), 3.11(2H, s), 3.60(3H, s), 3.64(1H, o), 7.27(5H, s)	C: 60.82 H: 6.38 N: 6.03	60.75 6.37 5.90
OMe CmPhe-OH (**5d**)	80	207—209 (dec)	A —[d] B +15.4°	C 0.24 D 0.56 E 0.67	2.84—2.97(2H, three peaks, J_{vic}=6 Hz and 8 Hz), 3.35(2H, s), 3.37(1H, o), 3.69(3H, s), 7.29(5H, s)	C: 60.74 H: 6.39 N: 6.05	60.75 6.37 5.90

a) Based on H-L-Val-OBzl·TosOH. See Experimental. b) Solvent for recrystallization: EtOH. c) A, in H_2O; B, in 1 M HCl. d) Insufficiently soluble; not measured. e) C, on silica gel, *n*-BuOH-AcOH-H_2O (4:1:2); D, on silica gel, *n*-PrOH-H_2O (64:36); E, on cellulose, *n*-BuOH-AcOH-H_2O (4:1:2). f) In D_2O-K_2CO_3. The signal attributable to -NHCH_2CO- is underlined.

the preparation by the former method.

The diesters of Cm-amino acids were oily substances with a low viscosity. They were far more stable than the free esters of the parent amino acids; they could be kept at room temperature for a week without any detectable change, though in a few months they had gradually changed into a solid mass.[17] The diesters formed crystalline substances with *p*-toluenesulfonic or picrolonic acid (Table 2). In general, the picrolonate crystallized more easily than the *p*-toluenesulfonate. These crystals were practically non-hygroscopic, and they could be stored for a long time.

Though the monoesters of Cm-amino acids have zwitter-ionic structures, they have low and sharp melting points and are highly soluble in hot ethanol.

Characterization of Cm-amino Acids and Their Esters. The detection of Cm-amino acids with ninhydrin was unsuccessful in most cases, while ninhydrin–formic acid[18] could generally be used for this purpose. The diesters of Cm-amino acids gave a distinct blue spot on a pink background when sprayed with cobalt(II) thiocyanate.[19] Such coloration was specific for the diesters, for neither the monoesters nor the Cm-amino acids themselves gave this coloration. In contrast to the free Cm-amino acids and the diesters, the monoesters gave a distinct coloration with ninhydrin. The initial color (on TLC) was purple for the monoesters (**4**), while it was yellow for the monoesters (**5**).

In general, a Cm-amino acid and the parent amino acid could not be satisfactorily separated by thin-layer or paper chromatography, while paper electrophoresis was useful for this purpose. The Cm-amino acid migrated a fairly long distance toward the anode in an acidic medium.

The ^{1}H-NMR spectrum of a Cm-amino acid showed a signal attributable to the methylene protons in the carboxymethyl group near δ 4.4 in trifluoroacetic acid (TFA) and near δ 3.2 in D_2O–NaOD. For Cm-proline, Cm-*t*-leucine, and Cm-valine, it appeared as an AB quartet with J=15—16 Hz in the latter solvent (Table 1).

The R_f value on TLC of the diester of a Cm-amino acid was much larger than that of the corresponding parent amino ester, so their separation was extremely easy.

It is worth noting that GLC (or even TLC) could be used to distinguish among various kinds of Cm-amino acid diesters, including the isomeric pairs (Table 4). Consequently, the diesters of Cm-amino acids seem to be an excellent derivative for the analysis of these amino acids by GLC; they were volatile and stable enough for the purpose.

The diesters of Cm-amino acids could also be well analyzed by mass spectrometry. For example, in the mass spectra of the isomeric benzyl ethyl esters (**3e** and **3f**) of Cm-valine, the benzyl fragment and the fragment resulting from the removal of the alkoxycarbonyl group of the valine moiety appeared strongly, as expected. The discrimination of the isomers was quite easy.

In the ^{1}H-NMR spectra of the isomeric pair of diesters (*e.g.*, **3e** and **3f**), there was a tendency for a signal attributable to the methylene protons in the benzyloxycarbonylmethyl group of one isomer (*e.g.*, **3e**) to appear in a slightly lower field than that in the methoxy- or ethoxycarbonylmethyl group of the other (*e.g.*, **3f**) (Table 2). On the other hand, the ^{13}C-NMR method proved to be ineffective for their discrimination.

TABLE 4. R_f AND t_R VALUES ON TLC AND GLC FOR THE DIESTERS OF Cm-L-VALINE

Compound	TLC, R_f[a]		GLC, t_R (min)[b]
	A	B	
3a	0.18	0.35	0.5
3b	0.26	0.46	0.8
3c	0.36	0.60	4.4
3d	0.34	0.61	4.2
3e	0.38	0.63	5.3
3f	0.33	0.56	4.9
3g	0.44	0.69	34.9

a) Solvent systems: A, benzene : AcOEt=9 : 1; B, $CHCl_3$: AcOEt=9 : 1. b) Column temp, 180 °C; injector temp, 250 °C; carrier gas, N_2 60 ml/min.

For the monoesters, though it was difficult to differentiate between a pair of isomers (*e.g.*, **4a** and **5a**) by TLC, they could clearly be distinguished by the difference in the chemical shifts in the ^{1}H-NMR of the methylene protons in the two types of carboxymethyl groups; a signal attributable to the methylene protons in the alkoxycarbonylmethyl group of one isomer (*e.g.*, **5a**) appeared in a lower field than that in the free carboxymethyl group of the other (*e.g.*, **4a**) (Table 3).

Configurational Assignments of Cm-amino Acids. The chiroptical behavior of α-amino acids has been widely used for their configurational assignments. Concerning the Cm-amino acids, however, no useful data are available for this purpose, except for a few reports on their metal complexes.[6c]

As can be seen from Table 1, the Clough-Lutz-Jirgensons rule[20] for α-amino acids could not be applied to the Cm-amino acids. This is also the case with the monoesters of Cm-amino acids listed in Table 3, though the absolute values of their optical rotations are generally small.

The ORD and CD techniques have been employed for the correlations of the configurations of α-amino acids,[21] and recently such methods have also been applied to *N*-methyl-[22] and *N*,*N*-dimethylamino acids.[23] Table 5 shows the ORD and CD data for Cm-L-amino acids and their monoesters, recorded in water and in a hydrochloric acid solution. Every aliphatic Cm-L-amino acid thus far examined shows one positive CD Cotton effect both in water (at 200—206 nm) and in an acidic solution (at 206—210 nm). The CD maxima appear at wavelengths approximately identical with those of the parent α-amino acids, but the intensities are somewhat enhanced. This is also the case with the wavelengths of the first extremum and the molecular rotations in ORD measurements. The relatively small changes on the carboxymethyla-

Table 5. ORD and CD data for Cm-L-amino acids and their monoesters[a]

Compound	ORD (H_2O)		CD (H_2O)		ORD (1 M HCl)		CD (1 M HCl)	
	λ(nm)	$[\phi]$	λ(nm)	$\Delta\varepsilon$	λ(nm)	$[\phi]$	λ(nm)	$\Delta\varepsilon$
CmAbu	221	+2350	202	+1.73	224	+2490	210	+1.61
CmAla	219	+1400	202	+1.26	226	+2000	208	+1.22
CmAsp	220	+2020	200	+1.62	224	+1870	206	+0.98
CmIle	219	+3260	200	+2.38	224	+4170	207	+2.38
CmLeu	220	+2760	202	+2.22	226	+2840	210	+1.85
CmPro	222	−240	206	+0.82	223	+220	208	+1.04
CmSer	221	+2000	200	+1.57	225	+2410	209	+1.63
CmThr	224	+1070	200	+1.37	227	+2190	210	+1.95
CmTle	223	+1810	200	+1.80	225	+2410	209	+1.84
CmVal	215	+3270	201	+2.21	225	+3680	207	+2.12
CmPhe[b]		—[d]		—[d]	226	+8530	216	+4.92
CmPhg[b,c]		—[d]		—[d]	225	+17900	216	+12.5
CmTyr[b]		—[d]		—[d]	234	+3540	226	+3.87
5a	214	+2080	199	+2.27	224	+1930	209	+1.86
5b	212	+3730	200	+2.28	224	+3210	210	+2.02
5c	213	+1670	202	+1.44	222	+1730	209	+1.24
5d[b]	224	+5140	215	+4.30	224	+9010	215	+5.41
4a	221	+3600	206	+2.21	220	+3490	208	+2.10
4b	221	+2850	207	+1.99	223	+3290	210	+2.16
4c	218	+2790	206	+1.24	221	+1600	207	+1.15
4d[b]	224	+6940	217	+4.40	224	+8380	215	+5.02

a) *c* 0.5—1, *l*=5 mm (ORD) or 1 mm (CD), unless otherwise noted. b) *c* 0.02—0.05, *l*=5 mm (ORD) or 1 mm (CD). c) The D-isomer was used for measurements. d) Insufficiently soluble; not measured.

tion of the amino group suggest the same transition, *i.e.*, the n→π^* transition of the carbonyl group, and a similar predominant conformation[24] may be responsible for the observed Cotton effect.

Similarly, the aromatic Cm-L-amino acids exhibit a positive CD band at 216 nm (Cm-phenylalanine and Cm-phenylglycine) or at 226 nm (Cm-tyrosine) in an acidic solution, the intensity being somewhat enhanced.[25]

Thus, the dominant positive Cotton effect generally observed between 200 and 225 nm can be utilized for the configurational assignments of Cm-amino acids.

Even when the carboxymethyl group is replaced by the methoxycarbonylmethyl or ethoxycarbonylmethyl group, the wavelength where the Cotton effect appears and its intensity are little affected either in water or in an acidic solution (*e.g.*, Cm-valine, **5a**, and **5b**) (Table 5). This result suggests that neither the electronic state nor the bulkiness of the carboxymethyl group has a profound influence on the predominant conformation of a Cm-amino acid.

In addition to the strong positive maximum at 210—218 nm, the CD spectra of the diesters of Cm-L-amino acids show a very weak negative band at 236—246 nm in 95% ethanol (Table 6); the occurrence of such a band has previously been reported on the free esters of some amino acids and interpreted in terms of the existence of an alternative conformation.[26] This band could not be observed in an aqueous solution of such Cm-amino acid esters as **4a** listed in Table 5.

For a preferable approach to the configurational assignments of amino acids, a number of their derivatives have been recommended which exhibit the Cotton effects at longer wavelengths. The *N*-ethylthiocarbonothioyl (ETCT) derivative of an α-amino acid has been used as one of the most suitable compounds for this purpose,[27] and this approach has recently been applied to β-amino acids also.[28] In the ETCT derivatives of these amino acids, the solvents influence the Cotton effects in a striking manner. Thus, in certain cases, the sign of the Cotton effect is inverted by changing the solvent.

The Cm-amino acids (except Cm-proline) listed in Table 1 were converted into the ETCT derivatives, and their Cotton effects examined in four different solvents (methanol, dioxane, chloroform, and benzene).

Table 6. CD data for the diesters of Cm-L-amino acids in 95% EtOH[a]

Compound	λ(nm)	$\Delta\varepsilon$	λ(nm)	$\Delta\varepsilon$
3a	215	+1.18	243	−0.08
3b	213	+1.27	242	−0.06
3c[b]	214	+2.46	242	−0.11
3d[b]	218	+1.44	244	−0.20
3h	212	+1.52	242	−0.14
3i	210	+0.73	236	−0.20
3l[b]	217	+3.32	246	−0.20

a) *c* 0.45—1, *l*=1 mm, unless otherwise noted. b) *c* 0.035—0.045, *l*=1 mm and 5 mm.

Table 7. ORD and CD data for ETCT-Cm-L-amino acids in MeOH[a]

ETCT derivative of	ORD[b] λ(nm)	ORD [φ]	CD λ(nm)	CD Δε
CmAbu	366	+1120	346	+0.93
CmAla	365	+2120	345	+1.41
CmAsp	364	+240	345	+0.21
CmIle	362	−2480	347	−0.47
CmLeu	367	+1900	347	+1.28
CmLys(Tos)	364	+1090	347	+0.90
CmPhe	374	−730	348	+0.29
CmPhg[c]	362	+5720	345	+2.63
CmSer	365	+3500	345	+2.29
CmThr	366	+3750	347	+1.95
CmTle	369	−3120	353	−1.38
CmTyr	376	−790[d]	350	+0.20
CmVal	366	−3320	348	−0.95

a) c 0.2—0.4, l=5 mm. b) 1st extremum. c) The D-isomer was used for measurements. d) Shoulder.

Table 8. Effects of solvents on the CD Cotton effect of ETCT-Cm-L-valine[a]

Solvent	λ(nm)	Δε
Methanol	348	−0.95
Ethanol	348	−0.77
Acetonitrile	346	−0.31
	378	+0.02
Acetone	348	−0.65
1,2-Dichloroethane	350	−0.60
Dichloromethane	351	−0.83
Chloroform	353	−1.42
Ethyl acetate	350	−0.72
Tetrahydrofuran	349	−0.85
Dioxane	352	−0.51
Diethyl ether	350	−0.69
Benzene	353	−1.25
Carbon tetrachloride	357	−1.81

a) c 0.25, l=5 mm.

The results obtained by measurement in methanol are summarized in Table 7. All the ETCT derivatives of Cm-L-amino acids show a CD maximum at 345—370 nm and the ORD first extremum at 360—380 nm in the above solvents, similarly to those of the parent α-amino acids. In contrast to the latter, however, the sign of the Cotton effect is independent of the solvents, though the intensity fluctuated somewhat depending on the solvents used. This is also the case with many more solvents of widely differing polarities, as is illustrated with ETCT-Cm-L-valine (Table 8). The Cotton effects of the ETCT derivatives of prolines, pipecolic acid, and *N*-methylalanine have been reported to be little influenced by solvents.[27] Consequently, the insusceptibility to solvent effects may be regarded as a feature common to the ETCT derivatives of imino acids. As can be seen from Table 8, there is a tendency for the CD maximum to be bathochromically shifted upon a decrease in the solvent polarity.

Table 9. Effects of added amines on the CD Cotton effect of ETCT-Cm-L-valine in $CHCl_3$[a]

Amine[b]		λ(nm)	Δε
N-Ethyldiisopropylamine	(10.4)	349	+1.68
N-Ethylmorpholine	(7.6)	352	+1.58
Triethylamine	(7.5)	350	+1.53
Dicyclohexylamine	(6.3)	349	+1.50
Tributylamine	(13.3)	351	+1.23
N-Ethylpiperidine	(8.6)	347	+1.01
Diisopropylamine	(13.7)	348	+0.63
Diethylamine	(14.4)	336	−0.56
None		353	−1.42
Ammonia		344	−1.99
Butylamine	(10.1)	344	−2.06
Piperidine	(9.2)	341	−2.34
Morpholine	(9.4)	343	−2.67
Isopropylamine	(10.3)	345	−2.72
Cyclohexylamine	(10.7)	344	−2.76
t-Butylamine	(7.3)	345	−3.19

a) c 0.38, l=2 mm. b) The number in the parentheses indicates the molar ratio of the amine to ETCT-L-CmVal.

Table 10. ORD and CD data for ETCT-Cm-L-amino acids in the presence of dicyclohexylamine in MeOH[a]

ETCT derivative of	ORD[b] λ(nm)	ORD [φ]	CD λ(nm)	CD Δε
CmAbu	365	−48[c]	343	+0.15
CmAla	361	+480	340	+0.56
CmAsp	361	−170[c]	344	+0.01
CmIle	363	−680	341	+0.16
CmLeu	360	+320	339	+0.45
CmLys(Tos)	366	−73[c]	341	+0.16
CmPhe	365	−470	349	+0.22
CmSer	361	+300	341	+0.21
CmThr	363	+400	344	+0.65
CmTle	367	−850[c]	339	+0.32
CmVal	363	+71	340	+1.24

a) c 0.2—0.4, l=5 mm. b) 1st extremum. c) Shoulder.

This is also suggestive of the same transition as in the case of the ETCT derivative of the parent α-amino acid, *i.e.*, the $n \rightarrow \pi^*$ transition of the NCS_2 chromophore.[27]

Most of the ETCT derivatives of Cm-L-amino acids show a positive Cotton effect, while those of Cm-L-valine, Cm-L-isoleucine, and Cm-L-*t*-leucine show a negative one; there could be recognized no simple correlation between the sign of the Cotton effect and the configuration of the parent amino acid. In the case of the ETCT derivatives of α-amino acids[27] or β-amino acids,[28] an empirical rule correlating the sign of the Cotton effect with the configuration has been achieved through the salt formation with such a base as cyclohexylamine or dicyclohexylamine (DCHA). Table 9 shows the effects of added amines

on the Cotton effect of ETCT-Cm-L-valine. The effects are surprisingly great,[29] and some kinds of amines such as DCHA invert the sign of the Cotton effect. Therefore, the Cotton effect of all the ETCT derivatives were reexamined in the presence of three molar amounts of DCHA in the same four solvents as above. The results obtained by measurement in methanol are summarized in Table 10.

All the DCHA salts of ETCT-Cm-L-amino acids show a positive Cotton effect (with a CD maximum at 335—350 nm and the ORD first extremum at 360—370 nm) in all the solvents used, except in some cases where the insolubility of the salts made the measurements impossible. Therefore, the correlation observed here can also be used for the configurational assignments of Cm-amino acids.

Experimental

All the melting points are uncorrected. The optical rotations were measured with a JASCO DIP-4 polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Hitachi R-24B spectrometer and a JEOL JNM-PS-100 spectrometer respectively. The chemical shifts are given in δ values with respect to TMS or DSS as an internal standard. The IR spectra were recorded on a Hitachi EPI-S2 spectrophotometer, and the mass spectra (MS) on a JEOL-OIS instrument at an ionization energy of 75 eV. The ORD and CD measurements were carried out with a JASCO ORD/UV-5 spectropolarimeter with its CD attachment. The TLC and preparative TLC were performed on Merck Kieselgel 60 F_{254} and Kieselgel GF_{254} (Type 60) respectively. Merck Kieselgel 60 was used for column chromatography, and Amberlite CG-120 (Type I) in the H^+ form for ion-exchange chromatography. The GLC analyses were performed on a Hitachi 063 Gas Chromatograph equipped with a flame-ionization detector using a ϕ3 mm×1 m 10% SE-30 column. Paper electrophoresis was carried out on a Yanagimoto EC-10 instrument, using a buffer with this composition: pyridine:AcOH:H_2O=25:1:225 (pH 6.4).

Benzyl bromoacetate (bp 112.5—113 °C/3 mmHg)[30] and *t*-butyl bromoacetate (bp 73—75 °C/25—25.5 mmHg)[31] were prepared according to the literature. The optical rotations of the less common amino acids used here as starting materials are as follows: L-Abu,[32] $[\alpha]_D^{16}$ +20.8° (*c* 4.8, 6 M HCl); D-Phg,[33] $[\alpha]_D^{20}$ −111.1° (*c* 1.0, 1 M NaOH); L-Tle,[34] $[\alpha]_D^{25}$ +30.0° (*c* 1.0, AcOH).

General Procedure for the Preparation of N-*(Carboxymethyl)-amino Acids (**2**).* N-*Carboxymethyl-L-2-aminobutyric Acid (L-CmAbu)*: H-L-Abu-OH (2.23 g) was allowed to react with bromoacetic acid (3.01 g) at pH 11 and at *ca.* 50 °C according to the method of Snyder and Angelici.[6a] After 4 h the reaction mixture was neutralized with 6 M HCl to pH 7. Then the solution was concentrated to some extent, loaded onto a column of cation-exchange resin (ϕ 3 cm×13 cm), and eluted with water. The front of the zone containing the desired product had clearly been observed throughout the elution. The pH of the eluate first went steeply down to 1, then rose gradually near 7, became acidic again, and finally reached *ca.* 7. The second acidic eluate with pH 2—4 (1.8 l), which showed only one spot in the paper electrophoresis, was evaporated to dryness under reduced pressure; yield, 2.28 g (65%). Recrystallization from water afforded large plates; mp 205.5—207 °C, $[\alpha]_D^{25}$ +9.8° (*c* 1.0, 1 M HCl).

N-*Carboxymethyl-L-t-leucine (L-CmTle)*: H-L-Tle-OH (786 mg) was allowed to react with bromoacetic acid (840 mg) for 4 h in a manner similar to that described above. Then the reaction mixture was acidified with concd HCl to pH 2. The deposited crystals were collected, and the concentration of the filtrate afforded a second crop. They were combined and recrystallized from water to afford prisms; yield, 850 mg (75%); mp 247—250 °C (dec), $[\alpha]_D^{25}$ −23.3° (*c* 1.0, 1 M HCl). Paper-electrophoresis showed only one spot.

Cm-DL-*t*-leucine and Cm-L-phenylalanine were likewise prepared in this way.

*Preparation of the Diesters (**3**) of* N-*(Carboxymethyl)amino Acids.* To a stirred mixture of an amino ester *p*-toluenesulfonate (or hydrochloride) (10 mmol), and TEA (20 mmol) in dry THF (30 ml), we added, drop by drop, a solution of a bromoacetic ester (12 mmol) in dry THF (10 ml). Then the mixture was stirred for 4—7 d at room temperature, with an occasional filtration of the precipitates (TEA salts). After the solvent had been removed under reduced pressure, the residual syrup was distributed between AcOEt and water, and the organic layer was washed successively with water, 10% citric acid, and water. After the solvent had been removed under reduced pressure again, 4—6 M HCl was added to the residue and the resulting solution was washed with AcOEt.[35] The aqueous layer was basified with solid K_2CO_3, and the liberated oil was extracted into AcOEt. The extracts were combined, washed with water, and dried over Na_2SO_4. The subsequent removal of the solvent yielded a syrup, the purity of which was checked by TLC, GLC, or ^{1}H-NMR. If the product thus obtained was not pure enough, it was further purified by column chromatography or preparative TLC, as will be described in the examples below.

For characterization, the diester was converted into the crystalline derivatives, the *p*-toluenesulfonate and/or the picrolonate. Even when the *p*-toluenesulfonate was difficult to crystallize, the corresponding picrolonate easily became crystalline.

Some typical examples of the procedures for the isolation of the diesters will be given below.

Ethyl N-*Benzyloxycarbonylmethyl-L-valinate (**3e**)*: H-L-Val-OEt·TosOH (3.17 g) was allowed to react with benzyl bromoacetate for 7 d. After the work-up described above, a colorless liquid was obtained in a pure state; yield, 2.24 g (76%); n_D^{32} 1.4825, $[\alpha]_D^{25}$ −18.5° (*c* 1.0, MeOH). Found: C, 65.56; H, 7.71; N, 4.52%. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.78%. *p*-Toluenesulfonate, mp 87.5—89 °C; picrolonate, mp 120—122 °C (from EtOH).

When the reaction mixture was heated under reflux for 40 h, a similar work-up yielded the diester (**3e**) in a little poorer yield (71%).

The free diester was recovered from the *p*-toluenesulfonate as follows. The salt was distributed between Et_2O (or AcOEt) and 10% K_2CO_3, and the organic layer was further washed with the alkali and water, and then dried over Na_2SO_4. The subsequent removal of the solvent, followed by the addition of $CHCl_3$ to the residue and its successive evaporation, gave the diester (**3e**); n_D^{25} 1.4849.

Methyl N-*Benzyloxycarbonylmethyl-L-phenylalaninate (**3m**)*: H-L-Phe-OMe·HCl (2.16 g) was allowed to react with benzyl bromoacetate for 4 d. After the work-up described above, the residual syrup (3.25 g) was chromatographed on a silica-gel column, using $CHCl_3$–AcOEt (9:1) as the eluent. The fractions which indicated coloration (on TLC) with $Co(SCN)_2$ and not with ninhydrin were collected and then evaporated under reduced pressure; yield, 1.86 g (57%). *p*-Toluenesulfonate, mp 147.5—149 °C; picrolonate, 168—169 °C (from EtOH).

Benzyl N-*Benzyloxycarbonylmethyl*-L-*phenylalaninate (**3o**)*: H-L-Phe-OBzl·TosOH (915 mg) was allowed to react with benzyl bromoacetate for 7 d. The syrup (752 mg) obtained by the work-up described above was chromatographed on preparative TLC, using benzene–AcOEt (9:1) as the developing solvent; yield, 563 mg (65%). *p*-Toluenesulfonate, mp 123.5—124.5 °C; picrolonate, mp 153—153.5 °C (from EtOH).

General Procedure for the Direct Esterification of N-*(Carboxymethyl)amino Acids. Methyl* N-*Methoxycarbonylmethyl*-L-*alaninate (**3i**)*: To a chilled solution of $SOCl_2$ (0.6 ml) in dry MeOH (5 ml), Cm-L-alanine (500 mg) was added, after which the mixture was stirred at room temperature for 2 d. After it had then been refluxed for 4 h, it was evaporated to dryness under reduced pressure. The residue was dissolved in water and basified with solid K_2CO_3 to pH 9; the mixture was extracted with AcOEt. The organic extracts were combined, washed with water, and dried over Na_2SO_4. The subsequent removal of the solvent afforded **3i** as a liquid in a pure state; yield, 397 mg (67%); $[\alpha]_D^{25}$ −24.4° (*c* 1.0, MeOH). Picrolonate, mp 134.5—135.5 °C.

Preparation of N-*(Carboxymethyl)amino Acids (**2**)* via *Their Diesters (**3**)*. N-*Carboxymethyl*-L-*phenylalanine (*L-*CmPhe), a) by the Hydrogenolysis of the Dibenzyl Ester (**3o**)*: The dibenzyl ester (**3o**) (270 mg) was hydrogenolyzed in AcOH–H_2O (2:1) (30 ml) in the presence of 5% Pd–C (60 mg). After the absorption of hydrogen had ceased (*ca.* 2 h), the catalyst was filtered off and the filtrate was evaporated under reduced pressure, affording a white crystalline mass in a quantitative yield. It was then recrystallized from water; $[\alpha]_D^{25}$ +15.8° (*c* 1.0, 1 M HCl).

*b) By the Acidic Hydrolysis of the Dimethyl Ester (**3l**)*: The dimethyl ester (**3l**) (710 mg) was heated in 20% HCl (7 ml) at 100—110 °C. The progress of the hydrolysis was followed by TLC on cellulose or by circular paper chromatography. Within 2 h the spot due to the starting material disappeared, while those spots corresponding to the monoester(s) (**4d** and/or **5d**) and the desired compound appeared on the chromatograms. After *ca.* 10 h, the color of the former faded considerably, but it did not completely vanish even after a further 7 h. Even at this time, though, the formation of neither glycine nor phenylalanine could be detected. The reaction mixture was evaporated under reduced pressure. After the addition of water to the residue and its successive evaporation has been repeated, the residual solid mass was recrystallized from water; yield, 565 mg (90%); $[\alpha]_D^{25}$ +15.9° (*c* 1.0, 1 M HCl).

*General Procedure for the Preparation of the Monoesters (**4** and **5**) of* N-*(Carboxymethyl)amino Acids. Methyl* N-*Carboxymethyl*-L-*phenylalaninate (**4d**)*: The benzyl methyl ester (**3m**) (290 mg) was hydrogenolyzed in AcOH–H_2O (2:1) (10 ml) in the presence of 5% Pd–C (90 mg). After the absorption of hydrogen had ceased (*ca.* 3.5 h), the catalyst was filtered off and the filtrate was evaporated under reduced pressure, affording a crystalline mass (210 mg); mp 145—153 °C. It was recrystallized from EtOH; yield 139 mg (66%); mp 157.5—159 °C, $[\alpha]_D^{25}$ +17.2° (*c* 1.0, 1 M HCl).

N-*Ethoxycarbonylmethyl*-L-*valine (**5b**)*: The crude benzyl ethyl ester (**3f**) (3.96 g), obtained from 5.36 g of H-L-Val-OBzl·TosOH and contaminated by the unchanged starting ester, was hydrogenolyzed in a manner similar to that described above. The catalyst was filtered off, and the filtrate evaporated under reduced pressure. After it had been dried sufficiently, the residue was treated with hot EtOH and the insoluble materials (valine) were filtered off. From the filtrate, the desired product (**5b**) was obtained; yield, 1.94 g (68%, based on H-L-Val-OBzl·TosOH); mp 167—168 °C, $[\alpha]_D^{25}$ +0.6° (*c* 1.0, 1 M HCl).

^{13}C-NMR and MS Spectra of the Isomeric Diesters of N-*Carboxymethyl*-L-*valine, **3e** and **3f***. **3e**: ^{13}C-NMR ($CDCl_3$): δ 14.33 (q, $-OCH_2\underline{C}H_3$), 18.40 and 19.17 (each q, $-CH(\underline{C}H_3)_2$), 31.56 (d, $-\underline{C}H(CH_3)_2$), 49.80 (t, $-NH\underline{C}H_2CO-$), 60.49 (t, $-O\underline{C}H_2CH_3$), 66.45 (t, $-O\underline{C}H_2C_6H_5$), 66.70 (d, $-NH\underline{C}HCO-$), 128.30 (d, C2,6* and C4 of C_6H_5), 128.53 (d, C3,5* of C_6H_5), 135.69 (s, C1 of C_6H_5), 171.60 (s, $-CH_2\underline{C}O-$), 174.03 (s, $=CH\underline{C}O-$). An alternative assignment is probable for the peak marked with an asterisk.

MS *m/e* (rel intensity): 293 (M^+, 0.5), 250 ($M^+-(CH_3)_2CH$, 5.2), 221 ($250^+-C_2H_5$, 9.2), 220 ($M^+-COOC_2H_5$, 63.2), 202 ($M^+-C_6H_5CH_2$, 1.6), 158 (202^+-CO_2, 7.6), 130 ($221^+-C_6H_5CH_2$, 4.6), and 91 ($C_6H_5CH_2^+$, base peak).

3f: ^{13}C-NMR ($CDCl_3$): δ 14.18 (q, $-OCH_2\underline{C}H_3$), 18.36 and 19.19 (each q, $-CH(\underline{C}H_3)_2$), 31.60 (d, $-\underline{C}H(CH_3)_2$), 49.83 (t, $-NH\underline{C}H_2CO-$), 60.77 (t, $-O\underline{C}H_2CH_3$), 66.45 (t, $-O\underline{C}H_2C_6H_5$), 66.77 (d, $-NH\underline{C}HCO-$), 128.31 (d, C2, 6* of C_6H_5), 128.56 (d, C3,5* and C4 of C_6H_5), 135.74 (s, C1 of C_6H_5), 171.74 (s, $-CH_2\underline{C}O-$), 174.10 (s, $=CH\underline{C}O-$).

MS *m/e* (rel intensity): 293 (M^+, 0.4), 278 (M^+-CH_3, 0.2), 250 ($M^+-(CH_3)_2CH$, 5.4), 221 ($250^+-C_2H_5$, 0.8), 220 ($M^+-COOC_2H_5$, 5.4), 202 ($M^+-C_6H_5CH_2$, 0.9), 158 (202^+-CO_2, base peak), 130 ($221^+-C_6H_5CH_2$, 2.6), and 91 ($C_6H_5CH_2^+$, 58.3).

Measurements of the ORD and CD Spectra. All the measurements were carried out at room temperature. The sample solution concentration and the length of the cell, l, are given in the footnotes of each table.

The Cm-amino acids (**2**) listed in Table 1 were converted into the corresponding ethyl dithiocarbamates according to the methods in the literature.[27] Their ORD and CD spectra were measured in several solvents, with or without the addition of three molar amounts of DCHA.

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