CYCLIC DIPEPTIDES WITH 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID

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Received July 31, 1996
Accepted November 5, 1996

Cyclic dipeptides of the formula cyclo(-Acc-X-), where Acc = 1-aminocyclopropane-1-carboxylic acid, X = Gly, Ala, Val, Leu, Phe or Tyr, were synthesized. The prepared 2,5-piperazinediones showed no proliferative or antiproliferative activity on normal human embryo cells as well as on HL60 leukemia cells. Results of trace memory assays in mice were negative.
Key words: Dipeptides; 2,5-Piperazinedione derivatives; 1-Aminocyclopropane-1-carboxylic acid.

Cyclo(-1-amino-1-cyclopentanecarbonyl-L-alanyl-), (8(*S*)-methyl-6,9-diazaspiro[4,5]-decane-7,10-dione, VUFB-15754 (ALAPTIDE)), is an experimental drug[1] that shows pronounced effects on the central nervous, digestive, and endocrine systems[2]. It also influences the peripheral proliferative and antiproliferative activities. The structure of Alaptide was an impulse for the synthesis of its cyclohexane[3] or cyclobutane[4] analogues; however, no pharmacological effects of these compounds were observed[5]. The aim of the present paper is an extension of the series to analogues containing 1-aminocyclopropane-1-carboxylic acid (Acc).

The protected dipeptide esters **1a–1j** were prepared from the corresponding derivatives of Z-amino acids and methyl or ethyl esters of Acc (ref.[6]) by the method of mixed anhydrides (Scheme 1). The protected dipeptide esters were deprotected by hydrogenolysis on Pd/C and the obtained free dipeptide esters were converted into the corresponding 2,5-piperazinedione derivatives **2a–2f** by standing at room temperature for two days[3,4].

Analytical data of the protected dipeptides **1a–1j** are given in Table I and their NMR spectral data in Table II. Analytical data for the final substances, 2,5-piperazinedione

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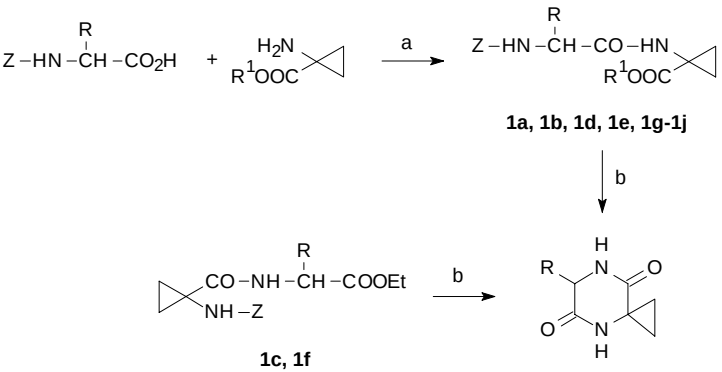
derivatives **2a–2f**, are listed in Table III and the corresponding NMR data in Table IV. The final substances **2a–2f** were assessed for the proliferative and antiproliferative activity in normal human embryo cells as well as in HL60 leukemia cells and they did not show any marked activity compared with Alaptide. *In vivo* trace memory studies in mice also showed activities insignificant compared with those of Alaptide.



a) EtOCOCl, *N*-ethylpiperidine; b) Pd/C, MeOH

1	R	R^1
a	H	CH_3
b	H	C_2H_5
c	H	-
d	CH_3	CH_3
e	CH_3	C_2H_5
f	CH_3	-
g	$CH(CH_3)_2$	CH_3
h	$CH_2CH(CH_3)_2$	C_2H_5
i	$C_6H_5CH_2$	CH_3
j	4-Z-$OC_6H_4CH_2$	C_2H_5

2	R
a	H
b	CH_3
c	$CH(CH_3)_2$
d	$CH_2CH(CH_3)_2$
e	$C_6H_5CH_2$
f	4-$HOC_6H_4CH_2$

SCHEME 1

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 70 Pa and 100 °C. Compounds melting below 120 °C were dried at room temperature. Optical rotations were determined on a Perkin–Elmer 141 photoelectric polarimeter in methanol, concentration 0.2 g/100 ml. Proton and ^{13}C NMR spectra (δ in ppm; J in Hz) were measured in $CDCl_3$ or $(CD_3)_2SO$ solutions on a DPX 250 (Bruker) apparatus, with TMS as the standard.

Synthesis of Dipeptides **1a–1j**. General Procedure

Ethyl chloroformate (6.8 ml, 68 mmol) was added at –10 °C to a solution of the *N*-benzyloxycarbonyl amino acid (67 mmol) and *N*-ethylpiperidine (9.6 ml, 67 mmol) in ethyl acetate (120 ml). After stirring at –10 °C for 15 min, a solution of methyl or ethyl 1-aminocyclopropane-1-carboxylate (67 mmol; prepared from the corresponding hydrochlorides and 9.6 ml (67 mmol) of *N*-ethylpiperidine) in ethyl acetate (100 ml) was added. The reaction mixture was stirred at 0 °C for 2 h and then set aside in a refrigerator for 12 h. The solvent was evaporated, the residue was dissolved in ethyl acetate and extracted with 1 M HCl, 5% sodium hydrogen carbonate and water. After drying over sodium sulfate, the solvent was evaporated and the product crystallized from ethyl acetate–light petroleum. For yields, melting points and analyses see Table I.

TABLE I
Properties of protected dipeptides **1**

Compound	M.p., °C Yield, %	$[\alpha]_D^{20,a}$	Formula M.w.	Calculated/Found		
				% C	% H	% N
1a	129–132	–	$C_{15}H_{18}N_2O_5$	58.82	5.92	9.15
	79		306.4	58.52	6.00	9.15
1b	78–79	–	$C_{16}H_{20}N_2O_5$	59.99	6.29	8.74
	69		320.4	59.88	6.34	8.45
1c	97[b]	–	$C_{16}H_{20}N_2O_5$	59.99	6.29	8.74
	71		320.4	60.30	6.30	8.59
1d	142–144	–6.3	$C_{16}H_{20}N_2O_5$	59.99	6.29	8.74
	68		320.4	59.59	6.31	8.50
1e	145–146	–20.5	$C_{17}H_{22}N_2O_5$	61.07	6.63	8.38
	65		334.4	60.65	6.67	8.46
1f	oil	–16.6	$C_{17}H_{22}N_2O_5$			
			334.4			
1g	174–176	–14.6	$C_{18}H_{24}N_2O_5$	62.05	6.94	8.04
	73		348.4	61.73	7.23	7.71
1h	112–113	–18.7	$C_{20}H_{28}N_2O_5$	62.97	7.23	7.73
	71		376.4	63.29	7.25	8.05
1i	145–147	–19.9	$C_{22}H_{24}N_2O_5$	66.65	6.10	7.07
	70		396.4	66.11	5.97	6.97
1j	166–167	–6.5	$C_{31}H_{32}N_2O_8$	66.42	5.75	5.00
	61		560.6	65.83	5.83	4.80

[a] In methanol. [b] Ref.[7] gives 97–98 °C.

TABLE II
^{13}C NMR data (δ, ppm) of compounds **1a**, **1b**, **1d**, **1e**, **1g–1j** in hexadeuteriodimethyl sulfoxide

Carbon	**1a**	**1b**	**1d**	**1e**	**1g**	**1h**	**1i**	**1j**
$H_2C—CH_2$ $\diagdown C \diagup$	17.45	17.00	17.27, 17.10	17.33	17.45	17.19, 17.04	17.54	17.14
	33.40	33.57	33.51	33.46	33.45	33.52	33.31	33.57
–**C**OOCH$_3$	172.64	171.92	173.40	173.59	172.46	173.24	172.41	171.69 or 171.67
–COO**C**H$_3$	52.53		52.23		52.43		52.46	
–COO**C**H$_2$CH$_3$		61.27		61.40		61.17		61.26
–COOCH$_2$**C**H$_3$		13.96		14.04		13.95		14.01
CH$_2$–O**C**O–NH–	156.72	156.58	156.01	156.04	156.48	156.23	156.03	155.95
C$_6$H$_5$**C**H$_2$–O–	67.16	67.03	66.91	67.00	67.09	66.92	67.03	67.04
C$_6$H$_5$-**C**-1	136.18	136.30	136.31	136.24	136.22	136.33	136.16	136.24
C-2	128.02	127.87	127.07	128.03	128.01	127.83	127.95	127.91
C-3	128.56	128.41	128.43	128.25	128.56	128.40	128.52	128.45
C-4	128.26	128.06	128.85	128.55	128.23	128.03	128.10	128.10
–CHR–**C**O–NH–	170.41	170.21	172.42	172.17	172.34	171.88	171.97	171.67 or 171.69
–**C**HR–CO–NH–	44.51	44.64	50.52	50.34	60.19	53.65	56.02	56.11
R = **C**H$_3$			18.21	18.52				
R = **C**H(CH$_3$)$_2$					31.11			
CH(**C**H$_3$)$_2$					19.09, 17.68			
R = **C**H$_2$CH(CH$_3$)$_2$						41.35		
CH$_2$**C**H(CH$_3$)$_2$						24.63		
CH$_2$CH(**C**H$_3$)$_2$						22.75, 22.02		
R = **C**H$_2$C$_6$H$_5$							38.57	
C$_6$H$_5$-**C**-1							136.16	
C-2,6							128.59, 128.52	
C-3,5							129.38	
C-4							126.97	
R = **C**H$_2$C$_6$H$_4$OCO								37.77
OCH$_2$C$_6$H$_5$ C$_6$H$_4$-**C**-1 or								134.92
C$_6$H$_5$-**C**-1								134.46
C$_6$H$_4$-**C**-2,6								130.34
C$_6$H$_4$-**C**-3,5								121.00
C$_6$H$_4$-**C**-4 or								153.47
CH$_2$C$_6$H$_4$O**C**OOCH$_2$C$_6$H$_5$								150.27
C$_6$H$_5$-**C**-2,6								128.31
C$_6$H$_5$-**C**-3,5								128.61
C$_6$H$_5$-**C**-4								128.64

Methyl N-benzyloxycarbonylglycyl-1-aminocyclopropane-1-carboxylate (**1a**). ^{1}H NMR spectrum: 1.54 m, 2 H and 1.13 m, 2 H (CH_2CH_2); 3.63 s, 3 H (OCH_3); 3.86 d, 2 H, J = 5.7 ($NHCH_2CO$); 5.10 s, 2 H ($ArCH_2OCO$); 5.76 bt, 1 H, J = 5.7 (OCONH); 7.01 bs, 1 H (CH_2CONH); 7.33 s, 5 H (arom. H).

Ethyl N-benzyloxycarbonylglycyl-1-aminocyclopropane-1-carboxylate (**1b**). ^{1}H NMR spectrum: 1.18 t, 3 H, J = 7.2 (CH_3); 1.13 m, 2 H (CH_2CH_2); 1.52 m, 2 H (CH_2CH_2); 3.85 d, 2 H, $J \approx 5.5$ ($NHCH_2CO$); 4.08 q, 2 H, J = 7.2 (OCH_2CH_3); 5.09 s, 2 H ($ArCH_2O$); 5.87 bt, 1 H, $J \approx 5.5$ (OCONH); 7.12 bs, 1 H (CH_2CONH); 7.32 m, 5 H (arom. H).

Methyl N-benzyloxycarbonyl-L-alanyl-1-aminocyclopropane-1-carboxylate (**1d**). ^{1}H NMR spectrum: 1.37 d, 3 H, J = 7.2 ($CHCH_3$); 1.51 m, 2 H and 1.10 m, 2 H (CH_2CH_2); 3.62 s, 3 H (OCH_3); 4.22 b quintet, 1 H ($CHCH_3$); 5.09 s, 2 H ($ArCH_2O$); 5.55 bd, 1 H, J = 7.5 ($NHCH(CH_3)CO$); 6.93 bs, 1 H ($CH(CH_3)CONH$); 7.31 s, 5 H (arom. H).

Ethyl N-benzyloxycarbonyl-L-alanyl-1-aminocyclopropane-1-carboxylate (**1e**). ^{1}H NMR spectrum: 1.20 t, J = 7.0, 3 H (OCH_2CH_3); 1.40 d, J = 7.0, 3 H ($CHCH_3$); 1.10 m, 2 H and 1.52 m, 2 H (CH_2CH_2); 4.10 q, J = 7.0, 2 H (OCH_2CH_3); 4.20 q, J = 7.0, 1 H ($CHCH_3$); 5.10 s, 2 H ($ArCH_2O$); 5.38 bd, 1 H ($NHCH(CH_3)$); 6.70 bs, 1 H ($CH(CH_3)CONH$); 7.30 s, 5 H (arom. H).

Methyl N-benzyloxycarbonyl-L-valyl-1-aminocyclopropane-1-carboxylate (**1g**). ^{1}H NMR spectrum: 0.96 d, 6 H, J = 6.9 and 0.99 d, J = 6.9 ($CH(CH_3)_2$); 1.54 m, 2 H and 1.12 m, 2 H (CH_2CH_2); 2.16 m, 1 H ($CH(CH_3)_2$); 3.64 s, 3 H (OCH_3); 3.94 dd, 1 H, J = 8.8 Hz, J = 6.0 ($CHCH(CH_3)_2$); 5.10 s, 2 H ($ArCH_2O$); 5.32 bs, 1 H, $J \approx 8.8$ ($OCONHCH(CH(CH_3)_2)$); 6.50 bs, 1 H ($CH(CH(CH_3)_2)CONHC$); 7.33 s, 5 H (arom. H).

TABLE III
Properties of 2,5-piperazinediones **2**

Compound	M.p., °C Yield, %	$[\alpha]_D^{20}$	Formula M.w.	Calculated/Found		
				% C	% H	% N
2a	280–282[a]	–	$C_6H_8N_2O_2$	51.42	5.75	19.99
	68		140.1	50.90	5.86	20.03
2b	285–286	+9.4[b]	$C_7H_{10}N_2O_2$	54.54	6.54	18.17
	75	+11.4[c]	154.2	53.96	648	18.04
2c	243–245	+18.1[b]	$C_9H_{14}N_2O_2$	59.32	7.74	15.37
	69		182.2	58.94	7.79	15.33
2d	228–230	+17.5[b]	$C_{10}H_{16}N_2O_2$	61.20	8.22	14.27
	66		196.3	61.25	8.47	13.95
2e	269–271	+35.7[b]	$C_{13}H_{14}N_2O_2$	67.81	6.13	12.17
	71		230.3	67.84	6.13	11.89
2f	277–278	+28.3[b]	$C_{13}H_{14}N_2O_3$	63.40	5.73	11.38
	70		246.3	62.69	5.71	10.78

[a] Ref.[7] gives m.p. >260 °C. [b] In methanol. [c] In 50% acetic acid.

Ethyl N-benzyloxycarbonyl-L-leucyl-1-aminocyclopropane-1-carboxylate (**1h**). ^{1}H NMR spectrum: 0.91 d, J = 6.3 and 0.93 d, 6 H, J = 6.3 ($CH(CH_3)_2$); 1.18 t, 3 H, J = 7.2 ($COOCH_2CH_3$); 1.08 m, 2 H (CH_2CH_2); 1.49 m, 3 H (CH_2CH_2 and $CHCH_2CH$); 1.70 m, 2 H ($CH_2CH(CH_3)_2$); 4.08 q, J = 7.2 and 4.18 m, 3 H (OCH_2CH_3 and $CHCH_2CH(CH_3)_2$); 5.08 ABq, 2 H ($ArCH_2O$); 5.51 d, J = 8.8, 1 H (OCONH); 6.93 bs, 1 H (CONHC); 7.30 m, 5 H (arom. H).

Methyl N-benzyloxycarbonyl-L-phenylalanyl-1-aminocyclopropane-1-carboxylate (**1i**). ^{1}H NMR spectrum: 1.46 m, 2 H and 0.95 m, 2 H (CH_2CH_2); 3.06 d, J = 7.9, 2 H ($CH_2C_6H_5$); 3.61 s, 3 H (OCH_3); 4.38 q, J = 7.9, 1 H ($CHCH_2C_6H_5$); 5.06 s, 2 H ($ArCH_2O$); 5.44 bd, J = 7.9, 1 H (OCONH); 6.43 bs, 1 H (CHCONH); 7.24 m, 10 H (arom. H).

Ethyl N,O-bis(benzyloxycarbonyl-L-tyrosyl-1-aminocyclopropane-1-carboxylate (**1j**). ^{1}H NMR spectrum: 1.18 t, J = 7.2, 3 H (OCH_2CH_3); 1.47 m, 2 H and 0.97 m, 2 H (CH_2CH_2); 3.06 bd, 2 H ($CHCH_2Ar$); 4.08 q, J = 7.2, 2 H (OCH_2CH_3); 4.37 m, 1 H ($CHCH_2Ar$); 5.06 ABq, 2 H ($ArCH_2O$); 5.25 s, 2 H (OCH_2Ar); 5.50 d, J = 8.5, 1 H (OCONHCH); 6.55 bs, 1 H (CHCONH); 7.07 d, J = 8.5, 2 H (3,5-H-Tyr); 7.20 d, J = 8.5, 2 H (2,6-H-Tyr); 7.34 m, 10 H (arom. H).

TABLE IV

^{13}C NMR data (δ, ppm) of 2,5-piperazinediones **2a–2f** in hexadeuteriodimethyl sulfoxide

Carbon	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**
C-8	168.10	169.38[a]	168.30[a]	169.09[a]	167.75[a]	167.82[a]
C-5	166.34	168.31[a]	167.73[a]	168.18[a]	167.44[a]	167.67[a]
C-6	44.85	50.57	60.65	53.80	56.21	56.48
C-3	36.07	36.18	35.94	35.91	35.66	35.11
C-1 and C-2	13.93	14.01	16.35	15.42	15.16	15.18
	13.90	13.89	13.02	12.46	12.69	12.65
CH_3		18.87				
$\mathbf{C}H_2CH(CH_3)_2$				42.37		
$CH_2\mathbf{C}H(CH_3)_2$				39.25		
$CH_2CH(\mathbf{C}H_3)_2$				22.55		
$CH_2CH(\mathbf{C}H_3)_2$				21.87		
$\mathbf{C}H(CH_3)_2$			32.35			
$CH(\mathbf{C}H_3)_2$			18.23			
$CH(\mathbf{C}H_3)_2$			17.02			
$\mathbf{C}H_2C_6H_5$					38.83	38.11
i-C_6H_5					135.85	130.73
o-C_6H_5					127.70	125.84
m-C_6H_5					129.82	114.69
p-C_6H_5					126.32	
						155.98

[a] Assignment uncertain, the order may be opposite.

Synthesis of 2,5-Piperazindiones **2**. General Procedure

A suspension of 5% Pd/C (2.5 g) in toluene (100 ml) was added to a solution of the dipeptide **1** (45 mmol) in methanol (200 ml) and the stirred mixture was hydrogenated at 2 MPa at room temperature in an autoclave. After 20 min, the catalyst was filtered off and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in methanol (50 ml) and the solution set aside at room temperature for 2 days. The deposited product was collected, washed with methanol and crystallized from methanol.

Cyclo(-1-amino-1-cyclopropanecarbonylglycyl-) (**2a**). ^{1}H NMR spectrum: 1.19 m, 2 H and 0.94 m, 2 H (CH_2CH_2); 3.87 s, 2 H ($NHCH_2CO$); 7.85 s, 1 H (NH-7); 8.11 bs, 1 H (NH-4).

Cyclo(-1-amino-1-cyclopropanecarbonyl-L-alanyl-) (**2b**). ^{1}H NMR spectrum: 1.21 m, 2 H and 0.95 m, 2 H (CH_2CH_2); 1.36 d, J = 6.9, 3 H (CH_3); 3.95 q, J = 6.9, 1 H (H-6); 7.97 bs, 8.05 bs, 2 H (H-4 and H-7).

Cyclo(-1-amino-1-cyclopropanecarbonyl-L-valyl-) (**2c**). ^{1}H NMR spectrum: 0.95 d, J = 7.2 and 0.98 d, J = 7.2 ($CH(CH_3)_2$); 1.11 and 1.32 m, 10 H (CH_2CH_2); 2.17 m, 1 H ($CH(CH_3)_2$); 3.67 d, J = 4.4, 1 H (H-6); 7.99 bs and 8.10 bs, 2 H (H-4, H-7).

Cyclo(-1-amino-1-cyclopropanecarbonyl-L-leucyl-) (**2d**). ^{1}H NMR spectrum: 0.94 d, J = 6.6 and 0.92 d, J = 6.6, 7 H ($CH(CH_3)_2$ and 1 H from CH_2CH_2); 1.30 m, 1 H, 1.08 m, 2 H and 0.87 m (overlapped CH_2CH_2); 1.63 t, 2 H, J = 6.6 (CH_2CH); 1.84 m, 1 H ($CH(CH_3)_2$); 3.82 dt, 1 H, J = 2.8, J = 6.6 (H-6); 8.07 bs, 1 H (H-4); 8.09 bs, 1 H (H-7).

Cyclo(-1-amino-1-cyclopropanecarbonyl-L-phenylalanyl-) (**2e**). ^{1}H NMR spectrum: 0.43 m, 1 H, 0.70 m, 1 H, 0.85 m, 1 H and 1.14 m, 1 H (CH_2CH_2); 3.07 m, 2 H ($CH_2C_6H_5$); 4.17 bt, 1 H (H-6); 7.23 m, 5 H (arom. H); 7.57 bs, 1 H and 7.70 bs, 1 H (H-4 and H-7).

Cyclo(-1-amino-1-cyclopropanecarbonyl-L-tyrosyl-) (**2f**). ^{1}H NMR spectrum: 1.11 m, 1 H, 0.82 m, 1 H, 0.63 m, 1 H and 0.34 m, 1 H (CH_2CH_2); 3.02 dd, J = 4.7, J = 13.5 and 2.83 dd, J = 5.0, J = 13.5, 2 H ($C_6H_4CH_2$); 4.10 m, 1 H (H-6); 6.67 d, J = 8.5, 2 H (2 × arom. 3,5-H); 6.97 d, J = 8.5, 2 H (2 × arom. 2,6-H); 7.88 s, 1 H (H-4); 7.93 s, 1 H (H-7); 9.03 bs, 1 H (OH).

The study was supported by the Grant Agency of the Czech Republic (grant No. 203/95/1483).

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