SPIROCYCLIC DIPEPTIDES OF 1-AMINO-1-CYCLOHEXANECARBOXYLIC ACID

Jarmila VINSOVA[a], Karel KOSAR[b] and Evzen KASAFIREK[c]

[a] *Department of Organic Chemistry, Faculty of Pharmacy, Charles University, 501 65 Hradec Kralove, The Czech Republic*
[b] *Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, 501 65 Hradec Kralove, The Czech Republic*
[c] *Research Institute for Pharmacy and Biochemistry, 130 60 Prague 3, The Czech Republic*

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Dedicated to Assoc. Prof. Dr Milan Celadnik on the occasion of his 70th birthday.

Spirocyclic cyclodipeptides with 1-amino-1-cyclohexanecarboxylic acid of the general formula cyclo(-Ach-A-), where A is Gly, L-Ala, D-Ala, L-Val, D-Val, L-Leu, D-Leu, D-Pgl, L-Phe or D-Phe, have been prepared by cyclization of the corresponding linear dipeptide methyl esters. The peptides cyclo(-L- or D-Ala-Ach-), cyclo(-L-Val-Ach-) and cyclo(-D-Leu-Ach-) show higher activity in the Chick Embryotoxicity Screening Test when compared with derivatives containing 1-amino-1-cyclopentanecarboxylic acid or 1-amino-1-cyclobutanecarboxylic acid.

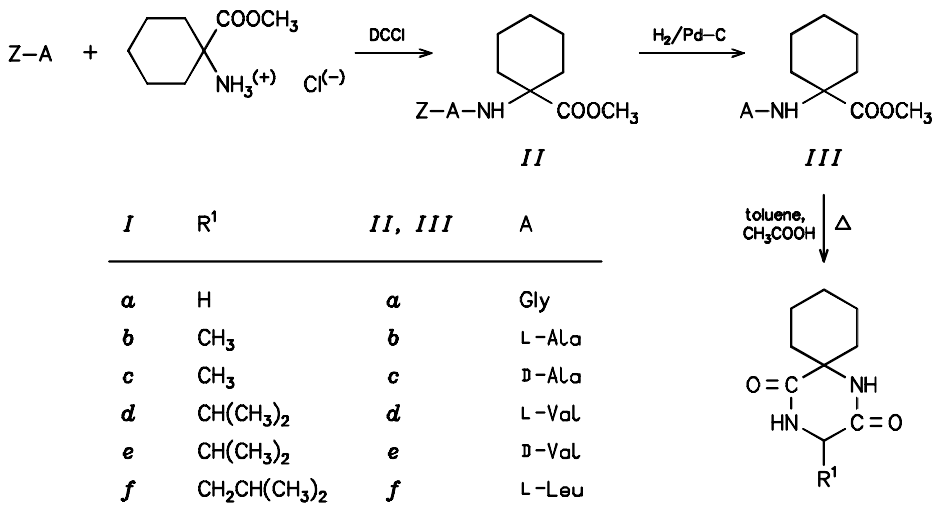
Recently increased attention has been paid to the study of spirocyclic cyclodipeptide substances derivated from 1-amino-1-cyclobutane- and 1-amino-1-cyclopentanecarboxylic acids mainly for their ability to influence the memory of experimental animals[1–3]. Spirocyclic cyclodipeptide cyclo(-alanyl-1-amino-1-cyclopentanecarbonyl-) (VUFB-15754; ALAPTIDE) is a potential drug with nootropic activity. It beneficially affects the growth of the diploid cell structure of human embryonic lungs and stimulates epidermal regeneration[4]. To study relations between chemical structure and biological activity we synthesized a series of spirocyclic cyclodipeptides containing 1-amino-1-cyclopentanecarboxylic acid[5] (Acp)*, 1-amino-1-cyclobutanecarboxylic acid[7] (Acb)

* The symbols for amino acids and peptides are according to the suggestions of the IUPAC-IUB Commission of Biochemical Nomenclature[6]. Acb, 1-amino-1-cyclobutanecarboxylic acid; Acp, 1-amino-1-cyclopentanecarboxylic acid; Ach, 1-amino-1-cyclohexanecarboxylic acid; Pgl, phenylglycine; DMSO, dimethyl sulfoxide; DCCI, *N*,*N*′-dicyclohexylcarbodiimide; CMS, caudal morphogenetic system.

and in the present paper we describe the synthesis of cyclodipeptide derivatives with 1-amino-1-cyclohexanecarboxylic acid (Ach).

The synthesis of these asymmetrically substituted spirocyclic 2,5-piperazinediones started with *N*-benzyloxycarbonyl amino acids which were condensed with methyl ester of 1-amino-1-cyclohexanecarboxylic acid[8] by *N*,*N*′-dicyclohexylcarbodiimide methods[9]. The protected dipeptide methyl esters *IIa – IIj* listed in Table I were hydrogenolyzed in the presence of 5% palladium on carbon in methanol. Free dipeptide methyl esters *IIIa – IIIj* were cyclized without being isolated in anhydrous conditions in the presence of 0.1 mol of acetic acid in toluene. The resulting cyclodipeptides *Ia – Ij* were isolated and purified by recrystalization. Their characteristics are summarized in Table II.

The Chick Embryotoxicity Screening Test (CHEST I)[10,11] was employed with regard to the possibility of an ultimate comparison of the antiproliferative effects of newly synthesized spirocyclic dipeptides.



I	R^1	*II, III*	A
a	H	*a*	Gly
b	CH_3	*b*	L-Ala
c	CH_3	*c*	D-Ala
d	$CH(CH_3)_2$	*d*	L-Val
e	$CH(CH_3)_2$	*e*	D-Val
f	$CH_2CH(CH_3)_2$	*f*	L-Leu
g	$CH_2CH(CH_3)_2$	*g*	D-Leu
h	C_6H_5	*h*	D-Pgl
i	$CH_2C_6H_5$	*i*	L-Phe
j	$CH_2C_6H_5$	*j*	D-Phe

SCHEME 1

EXPERIMENTAL

The melting points were determined on a Koffler block and were not corrected. The melting points of subliming compounds were determined in a sealed tube. The samples for analyses were dried in vacuo at 70 Pa over phosphorus pentoxide at 78 °C. The optical rotation measurements were carried out on Polamat A (Zeiss Jena, Germany) polarimeter in a 10 cm tube at 25 °C. The evaporation of solvents from samples was carried out generally at reduced pressures. Thin-layer chromatography was carried out on Silufol UV 254 nm in system petroleum ether–ethyl acetate 1 : 1. The measurement of the inhibition of proliferative activity was read using a stereomicroscope. Dimethyl sulfoxide of analytical grade was subjected to vacuum fractional distillation before use.

TABLE I
Properties of protected linear dipeptides *II*

Compound	M.p., °C Yield, %	$[\alpha]_D$ c^a	Formula M.w.	Calculated/Found			$R_F{}^b$
				% C	% H	% N	
IIa[c,d]	98 – 100		$C_{18}H_{24}N_2O_5$ 348.4	–	–	–	
IIb	oil 95	–22.7 1.0	$C_{19}H_{26}N_2O_5$ 362.4	–	–	–	0.449
IIc	oil 93	+24.6 0.9	$C_{19}H_{26}N_2O_5$ 362.4	–	–	–	0.457
IId	120 – 122 78	–20.8 1.1	$C_{21}H_{30}N_2O_5$ 390.5	64.60 64.82	7.74 7.92	7.17 7.28	0.807
IIe	122 – 126 95	+21.6 1.1	$C_{21}H_{30}N_2O_5$ 390.5	64.60 64.58	7.74 8.03	7.17 7.07	0.828
IIf	oil 75	–19.8 1.2	$C_{22}H_{32}N_2O_5$ 404.5	–	–	–	–
IIg	oil 64	+24.3 1.1	$C_{22}H_{32}N_2O_5$ 404.5	–	–	–	–
IIh	oil 74	–79.0 0.4	$C_{24}H_{28}N_2O_5$ 424.5	–	–	–	–
IIi	97.5 – 98.5 75	–13.4 1.0	$C_{25}H_{30}N_2O_5$ 438.5	68.47 68.53	6.90 6.92	6.39 6.48	0.816
IIj	94 – 96 82	+15.1 0.9	$C_{25}H_{30}N_2O_5$ 438.5	68.47 68.02	6.90 6.90	6.39 6.49	0.812

[a] In methanol. [b] Petroleum ether–ethyl acetate 1 : 1. [c] Ethyl ester. [d] Refs[13,14].

N-Benzyloxycarbonylpeptide Methyl Esters *IIb – IIj*. General Procedure

To a stirred suspension of methyl 1-amino-1-cyclohexanecarboxylate hydrochloride (0.58 g, 3 mmol) in methylene chloride (15 ml) was added 1-ethylpiperidine (3 mmol) and after 5 min *Z*-amino acid (3 mmol). The suspension was cooled to −15 °C and *N,N′*-dicyclohexylcarbodiimide (3.3 mmol) was added. The mixture was stirred at −15 °C for 1 h and at 4 °C for 24 h. Excess of DCCI was destroyed by acetic acid (0.2 ml). *N,N′*-Dicyclohexylurea was removed by filtration and the filtrate was diluted by methylene chloride (30 ml). The solution was evaporated in a vacuum (at 40 °C max.). The semi-solid residue was dispersed in ethyl acetate (40 ml) and filtered again to remove the remains of *N,N′*-dicyclohexylurea. The filtrate was washed with 5% hydrochloric acid (10 ml), 5% sodium carbonate (10 ml) and water (10 ml) and dried over sodium sulfate. The solution was evaporated and

TABLE II
Properties of spirocyclic cyclodipeptides *I*

Compound	M.p.[a], °C Yield, %	$[\alpha]_D$ *c*[b]	Formula M.w.	Calculated/Found		
				% C	% H	% N
Ia[c]	307 – 308	–	$C_9H_{14}N_2O_2$ 182.2	–	–	–
Ib	350 – 352 47	−18.8 0.3	$C_{10}H_{16}N_2O_2$ 196.3	61.20 61.11	8.22 8.31	14.27 13.90
Ic	354 – 356 67	+19.4 0.3	$C_{10}H_{16}N_2O_2$ 196.3	61.20 61.30	8.22 8.08	14.27 14.47
Id	344 – 346 20	−16.7 0.8	$C_{12}H_{20}N_2O_2$ 224.3	64.26 64.57	8.99 8.76	12.49 12.35
Ie	355 –358 48	+18.0 0.8	$C_{12}H_{20}N_2O_2$ 224.3	64.26 64.16	8.99 8.97	12.49 12.73
If	323 – 325 38	−19.2 0.8	$C_{13}H_{22}N_2O_2$ 238.3	65.52 65.62	9.30 8.96	11.75 11.70
Ig	323 – 326 39	+17.3 0.8	$C_{13}H_{22}N_2O_2$ 238.3	65.52 66.02	9.30 9.36	11.75 11.92
Ih	293 – 295 35	−31.4 1.0	$C_{15}H_{18}N_2O_2$ 258.3	69.75 69.68	7.02 7.19	10.84 10.64
Ii	279 – 281 32	+48.6 0.9	$C_{16}H_{20}N_2O_2$ 272.3	70.56 70.59	7.40 7.30	10.29 10.16
Ij	283 – 285 29	−46.2 0.6	$C_{16}H_{20}N_2O_2$ 272.3	70.56 70.52	7.40 7.35	10.29 10.19

[a] Melting points were measured in sealed capillary tube. [b] In acetic acid. [c] Refs[13,14].

the residue was recrystallized from ethyl acetate–petroleum ether. The yields and analytical data are listed in Table I.

In IR spectra of all dipeptides expected maxima were found: 3 395 (NH), 1 705 (CO, ester), 1 660 (CO, amide-I), 1 494 (CO, amide-II).

Spirocyclic Cyclodipeptides *Ib – Ij*. General Procedure

N-Benzyloxydipeptide methyl ester *IIb – IIj* (2 mmol) was dissolved in methanol and 5% palladium on carbon (0.5 g) was added. The suspension was hydrogenated at 40 °C for 2 – 6 h. The catalyst was removed by filtration and the clear filtrate was evaporated to dryness. The crude oily linear dipeptides *IIIb – IIIj* were cyclized by heating for 2 h in dry toluene (120 ml) and acetic acid (0.2 ml), with continuous removal of toluene–water azeotrope. The crude products were recrystallized from 2-propanol. The yields and analytical data are listed in Tables II and III.

Antiproliferative Activity Test

The experiments were performed on chick embryos of White Leghorn stock (breeding station Dobrenice, The Czech Republic) incubated for 40 h in a forced-draft incubator at 37.5 °C and at a relative humidity of 60 – 70%. The substances were administered subgerminally to the embryos which were all in the Hamburger–Hamilton developmental stages 10 and 11 (ref.[12]) representing the critical stage interval of the embryo's trunk development. By the routine window technique a single dose (3 μl) was administered to 8 – 10 embryos using a calibrated glass micropipette. The solvent used, 10% aqueous dimethyl sulfoxide, had no effect on chick embryo development. The dose range was selected on the basis of preliminary tests starting with the usual concentration of most substances tested.

Twenty four hours after the administration and subsequent incubation, the length of the newly formed portion of the trunk was measured as an indicator of CMS function. The distance between the vitelline arteries and the tip of the trunk which were measured by an ocular micrometer were transformed to millimeters by a simple algorithm. The lower limit of normal growth of CMS within 24 h was 2.3 mm. A decrease of the mean value below this level indicated an interaction of the compound tested with the function of the CMS. This state was regarded as growth retardation, i.e. inhibition of proliferation.

TABLE III
Characteristic IR frequencies (cm^{-1}) for cyclodipeptides *I*

Compound	Band A	Band B	Amide-I	Amide-II	Amide-III	CH_2
Ib	3 170	3 040	1 655	1 440	1 315	1 475
Ic	3 165	3 035	1 650	1 438	1 315	1 475
Id	3 190	3 080	1 650	1 440	1 292	1 465
Ie	3 200	3 100	1 665	1 445	1 297	1 470
If	3 180	3 060	1 655	1 440	1 310	
Ig	3 180	3 060	1 658	1 440	1 310	
Ih	3 180	3 050	1 655	1 437	1 295	1 490
Ii	3 175	3 040	1 650	1 440	1 315	1 475
Ij	3 175	3 040	1 652	1 440	1 315	1 478

RESULTS AND DISCUSSION

The result of the CHEST I for the highest concentration ($1 . 10^{-3}$ mol l^{-1}) cannot be considered reliable because of the insolubility of the majority of the dipeptides tested. The only response obtained for *Ia* represents the significant antiproliferative effect. Following the adminitration of the concentration of $1 . 10^{-4}$ mol l^{-1}, the strongest interaction with the developing CMS was recorded in peptides *Ic* and *Ie*. The considerable antiproliferative effect was shown by peptides *Ia*, *Ib*, *Id* and *Ig*. The insignificant growth inhibition was caused by *Ih*. Compound *If* showed no antiproliferative effect. At the lowest concentration tested ($1 . 10^{-5}$ mol l^{-1}) no antiproliferative effects were proved for most of the dipeptides used. The very strong antiproliferative effect was shown by *Ie* again. Cyclodipeptide *Ij* cannot be tested in the concentration spectrum selected for absolute insolubility (see Table IV).

To compare the antiproliferative effects of cyclo(-A-Acb-), cyclo(-A-Acp-) and cyclo(-A-Ach-) *Ia – Ij* (Table V), the middle concentration was chosen. It is evident that the interference with CMS which resulted in a significant antiproliferative effect was almost identical in all the representatives from the series of cyclo(-A-Ach-). The only exceptions are cyclo(-L-Leu-Ach-) and cyclo(-D-Pgl-Ach-). The presence of L-Leu and surprisingly also D-Pgl in spirocyclic cyclodipeptides which contain all three carboxylic acids, seems to be favourable and does not inhibit the proliferation of CMS in cell populations.

TABLE IV
Antiproliferative activity of cyclic dipeptides. Length of embryo trunk (in mm) 24 h after treatment (mean ± SE)

Compound	*c*, mol l^{-1}		
	$1 . 10^{-3}$	$1 . 10^{-4}$	$1 . 10^{-5}$
Ia	1.6 ± 0.7	1.75 ± 0.8	2.5 ± 0.2
Ib	[a]	1.8 ± 1.0	2.4 ± 0.4
Ic	[a]	1.5 ± 1.0	2.25 ± 0.4
Id	[a]	1.6 ± 1.0	2.4 ± 0.1
Ie	[a]	1.5 ± 0.9	1.6 ± 0.8
If	[a]	2.5 ± 0.2	2.6 ± 0.25
Ig	[a]	1.6 ± 0.5	1.75 ± 0.8
Ih	[a]	1.8 ± 0.85	2.1 ± 0.3
Ii	[a]	2.1 ± 0.4	2.25 ± 0.4
Ij	[a]	[a]	[a]

[a] Insoluble.

On the basis of our results it can be concluded that at the concentration of 1 . 10^{-4} mol l^{-1} the highest antiproliferative activity was observed in those spirocyclic cyclodipeptides that contain the amino acids of D-series, D-Phe, D-Val and D-Ala, regardless on the structure of cycloalkanecarboxylic acid. On the contrary, spirocyclic dipeptides of all three series containing L-Leu and D-Pgl exhibited none or minimal interference with proliferation.

TABLE V
Comparison of the length of the trunk 24 h after treatment with cyclopeptides cyclo(-A-Acb-), cyclo(-A-Acp-) and cyclo(-A-Ach-) in concentration 1 . 10^{-4} mol l^{-1}

A	Cyclo(-A-Acb-)	Cyclo(-A-Acp-)	Cyclo(-A-Ach-)
Gly	2.46 ± 0.4	1.7 ± 0.9	1.75 ± 0.8
L-Ala	2.23 ± 0.3	2.6 ± 0.24	1.8 ± 1.0
D-Ala	1.93 ± 0.6	1.6 ± 0.9	1.5 ± 1.0
L-Val	1.8 ± 0.6	2.4 ± 0.2	1.6 ± 1.0
D-Val	2.1 ± 0.5	1.23 ± 0.8	1.5 ± 0.9
L-Leu	2.3 ± 0.3	2.1 ± 0.3	2.5 ± 0.2
D-Leu	2.0 ± 0.5	2.25 ± 0.7	1.6 ± 0.5
D-Pgl	2.3 ± 0.35	[a]	2.1 ± 0.4
L-Phe	1.8 ± 0.8	2.5 ± 0.2	[a]
D-Phe	1.4 ± 0.95	1.38 ± 0.8	1.8 ± 0.85

[a] Insoluble.

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