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Macrocyclic peptides, 4[a)]

Preparations and enantioface-differentiating abilities of 27- and 36-membered ring peptides containing *N,N'*-ethylene-bridged dipeptides and glycine

Tetsushi Yamashita, Joji Maruo, Atsuko Fujimoto, Kozo Shibata, Yoshitane Kojima, Akio Ohsuka*

Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan

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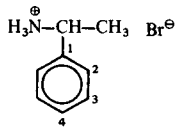
SUMMARY:

27- and 36-membered ring peptides were prepared using glycine, (2*S*, 3'*S*)-4-methyl-2-(2'-oxo-3'-isobutyl-1'-piperazinyl)pentanoic acid (**2**) and (2*S*,3'*S*)-3-phenyl-2-(2'-oxo-3'-benzyl-1'-piperazinyl)propanoic acid as the units of peptides. The interactions of 1-phenylethylammonium (**1**) and *p*-methoxy-1-phenylethylammonium bromides (**11**) with these cyclic peptides were studied by ^1H and ^{13}C NMR measurements in $CDCl_3$. It was found from these results that the peptides distinguish the enantiomers (*R*- and *S*-isomers) of the substrates. Furthermore, it was shown that the enantioface-differentiating abilities of 36-membered ring peptides are superior to those of 27-membered rings.

Introduction

In the preceding communication[1)], the interactions of (*R*)- and (*S*)-1-phenylethylammonium bromides **1** with 24-membered ring peptides containing glycine (Gly), sarcosine (Sar), (2*S*,3'*S*)-2-(2'-oxo-3'-methyl-1'-piperazinyl)propanoic acid (H-EAA-OH) and (2*S*,3'*S*)-4-methyl-2-(2'-oxo-3'-isobutyl-1'-piperazinyl)pentanoic acid (H-ELL-OH) (**2**) were examined by ^{13}C NMR measurements in $CDCl_3$. In the study, the methyl group carbon and one (C^1) of the phenyl group carbons of **1** were differentiated, but the other carbons were hardly distinguished.



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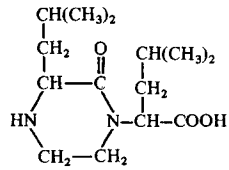

2 (H–ELL–OH)

[a)] Part 3: cf. [1)].

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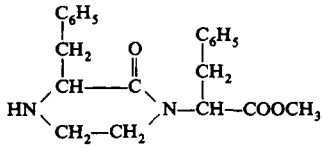
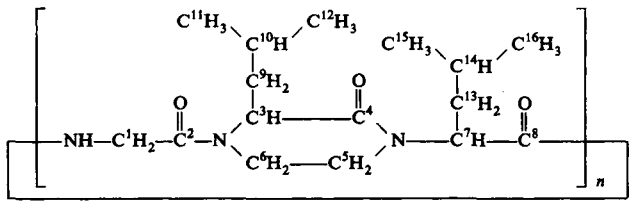
In the present work, a new dipeptide, methyl (2*S*, 3′*S*)-3-phenyl-2-(2′-oxo-3′-benzyl-1′-piperazinyl)propanoate (H-EFF-OCH_3) (**3**), was prepared by cyclization of dimethyl (2*S*, 7*S*)-2,7-dibenzyl-3,6-diazaoctanedionate derived from (*S*)-phenylalanine, and was used as a unit of cyclic peptides.



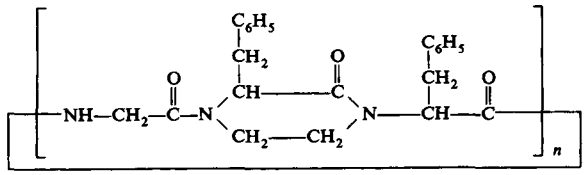
3 (H-EFF-OCH_3)

This paper describes the preparations (*Scheme I*) of four types of 27- and 36-membered ring peptides containing Gly and ELL or EFF as the peptides units, in which the ratios of Gly to ELL or EFF are 1 : 1, respectively [cyclo(Gly-ELL)$_3$ = **4a**, cyclo(Gly-ELL)$_4$ = **4b**, cyclo(Gly-EFF)$_3$ = **5a** and cyclo(Gly-EFF)$_4$ = **5b**]. The cavities of these peptides are larger and surrounded more densely by high walls such as ELL and EFF than those of 24-membered rings[1] reported earlier. Therefore, these cyclic peptides are expected to include and distinguish substrates more effectively than 24-membered rings.

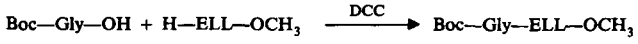
Scheme I:

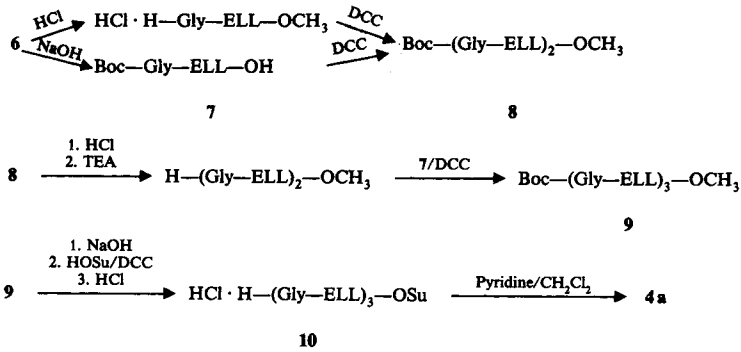


4a : n = 3
4b : n = 4



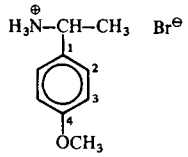
5a : n = 3
5b : n = 4





Boc: *t*-butoxycarbonyl
DCC: dicyclohexylcarbodiimide
HOSu: *N*-hydroxysuccinimide
TEA: triethylamine
HCl: 4 N HCl in 1,4-dioxane
NaOH: 1 N NaOH in methanol/water, vol. ratio 1/1

The interactions of **1** and *p*-methoxy-1-phenylethylammonium bromide (**11**)[2] with these preptides were studied in $CDCl_3$ by NMR measurements in order to examine the enantioface-differentiating abilities of these peptides.



11

Experimental part

Preparation of methyl (2S,3'S)-3-phenyl-2-(2'-oxo-3'-benzyl-1'-piperazinyl)propanoate (H-EFF-OCH$_3$): (2*S*,7*S*)-2,7-Dibenzyl-3,6-diazaoctanedioic acid, prepared by the method of Schoenberg et al.[3], was allowed to react with thionyl chloride in dry methanol. The dimethyl ester dihydrochloride thus obtained was transformed to the free base by sodium hydrogen carbonate in dry xylene. The xylene solution was refluxed for several days. The amino ester obtained as an oily material, yield: 66%. The hydrochloride was crystallized from ethanol; m. p. 129–134 °C; $[\alpha]_D^{20} = -192$ (conc.: 10 g · dm^{-3}; in ethanol).

$C_{21}H_{25}ClN_2O_3$ (388,9)	Calc.	C 64,86	H 6,48	N 7,20
	Found	C 64,92	H 6,49	N 7,20

Preparations of cyclic peptides (**4a, 4b, 5a** *and* **5b**): A method for the synthesis of **4a** is outlined in *Scheme 1*. The intermediates obtained by the DCC method proved to be sufficiently pure by IR, NMR and TLC. The cyclization was carried out in pyridine/dichloromethane (vol. ratio 1 : 4) at a definite concentration (1 g · dm^{-3}) for a weak at room temperature. Crude **4a** was purified by silica gel column chromatography (chloroform/methanol) and successive Sephadex LH-20 column chromatography (methanol). The method for the preparation of **4b** was similar to that of **4a**.

The intermediates of **5a** and **5b** were obtained by the HOBT—DCC method (HOBT; 1-hydroxybenzotriazole), and the cyclization manner was similar to that of **4a**. The analytical data of cyclic peptides **4a**, **4b**, **5a** and **5b** are shown in Tab. 1, and their cyclization yields and physical data are summarized in Tab. 2.

Interactions of substrates with cyclic peptides: The investigations were done in $CDCl_3$ at 36 °C by ^1H and ^{13}C NMR measurements using TMS (^1H) and $CDCl_3$ (^{13}C, 77,03 ppm) as internal standards. The estimated instrumental uncertainties are ±0,001 and ±0,020 ppm for ^1H and ^{13}C NMR data, respectively. The concentrations were 0,040 mol · dm^{-3} for cyclic peptides and 0,020–0,240 mol · dm^{-3} for substrates, respectively.

Tab. 1. Analytical data of cyclic peptides

4a	$C_{48}H_{81}N_9O_9 \cdot 3/2\ H_2O$ (955,2)	Calc.	C 60,35	H 8,86	N 13,19
		Found	C 60,34	H 8,95	N 13,11
4b	$C_{64}H_{108}N_{12}O_{12} \cdot 3/2\ H_2O$ (1264,7)	Calc.	C 60,78	H 8,84	N 13,29
		Found	C 60,73	H 8,87	N 13,15
5a	$C_{66}H_{69}N_9O_9 \cdot CHCl_3 \cdot 3\ H_2O$ (1305,7)	Calc.	C 61,63	H 5,87	N 9,65
		Found	C 61,70	H 6,03	N 9,84
5b	$C_{88}H_{92}N_{12}O_{12} \cdot 4/5\ CHCl_3$ (1605,3)	Calc.	C 66,44	H 5,83	N 10,47
		Found	C 66,20	H 5,93	N 10,57

Tab. 2. Physical data and cyclization yields of cyclic peptides

	m.p. in °C	$[\alpha]_D^{20}$ a)	Yield in % b)	MS: *m/e*
4a	205–208	−41	22	927
4b	185–190	−63,8	10	1 236
5a	133–136	+6,3	26	1 131
5b	179–182	+46,8	20	1 508

a) The specific rotations of **4a**, **4b**, **5a** and **5b** were measured in ethanol.

b) The cyclization yields were calculated from the free carboxylic acids of Boc-nona- or -dodeca-peptides.

Instruments: A Jasco IRA-1 (for IR spectra), a Hitachi R-90H and a Jeol GX-400 (for NMR spectra), a Jasco DIP-4 (for optical rotation, length of sample 10 cm) and a Jeol JMS-HX-100 (for mass spectra) were used for the measurements.

Results and discussion

Tab. 3 shows the values (in ppm) of the chemical shifts of the (*R*)- and (*S*)-isomers of **1** using **4a**, **4b**, **5a** and **5b** as the hosts. As shown in Tab. 3, a 27-membered ring peptide **4a** distinguishes the methyl group carbon and one (C^1) of the phenyl group carbons of **1**, and these results are comparable to those of 24-membered ring peptides reported earlier[1]. Another 27-membered ring peptide (**5a**) can also differentiate the methyl group and the carbon of **1**, but the signals of the other carbons could not be assigned because of the overlapping of the signals of the host.

Tab. 3. ^{13}C NMR data[a] of 1-phenylethylammonium bromide (1)[b]

Host	Form of 1	Chemical shifts δ in ppm CH_3	—CH—	C^1	C^2	C^3	C^4
No host[c]		20,55	52,34	137,21	129,24	127,00	129,24
4a[d]	*R*	20,80	52,00	138,01	129,24	126,84	129,12
	S	20,50	c)	137,83	c)	c)	c)
4b[d]	*R*	20,66	51,93	138,17	129,04	127,05	128,89
	S	20,49	51,84	138,04	129,00	127,09	c)
5a[d]	*R*	20,55	e)	138,08	e)	e)	e)
	S	20,47	e)	138,01	e)	e)	e)
5b[d]	*R*	20,59	51,87	138,18	e)	127,02	e)
	S	20,43	51,72	138,04	e)	126,87	e)

a) ^{13}C NMR data (in ppm) were obtained by a Jeol GX-400 in $CDCl_3$ at 36,0 ± 0,3 °C. Mole ratios of cyclic peptide/substrates are shown in[d].

b) The numbers of the atoms refer to formula **1**.

c) No splitting of signal.

d) Host: *R*-form: *S*-form = 1,5 : 1,0 : 0,5.

e) The signal of the guest could not be assigned because of the overlapping of the signals of the hosts.

Tab. 3 also indicates that a 36-membered ring peptide (**4b**) splits all signals of the carbons of **1** except that of the C^4 carbon, which is remote furthest from the ammonium group of **1**. Another 36-membered ring peptide **5b** can distinguish the methyl, methine, C^1 and C^3 carbons of **1**, though there are the signals of two carbons (C^2 and C^4) being not assigned because of the overlapping of the signals of the host. It is found from these results that the enantioface-differentiating abilities of these 36-membered ring peptides are superior to those of the previous 24-membered rings, implying that the sizes of their cavities are more suitable for **1**.

In this article, (*R*)- and (*S*)-isomers of **11** were also used as guests for **4a**, **4b**, **5a** and **5b**, respectively. The ^{13}C NMR measurements were carried out for **11** as for **1**.

Tab. 4 shows the ^{13}C NMR data of **11** obtained for the different hosts. The methyl groups of (*R*)- and (*S*)-**11** are distinguished by all of the hosts. The methine groups can be differentiated by **4b**, **5a** and **5b**. The signal of the C^1 of the phenyl group also splits in case of **4a**, **4b** and **5a**, but it should not be assigned in the case of **5b** because of the overlapping with the signals of the host. Only **4a** can distinguish the methoxy and C^4 remote furthest from the ammonium group of **11**.

Among the cyclic peptides prepared in this experiment, **4a** shows simple ^1H and ^{13}C NMR spectra, suggesting that **4a** has a C_3-symmetry conformation. Tab. 5 shows the values (in ppm) of the chemical shifts of each carbon of **4a**. The chemical shifts were assigned to the respective atoms by a selective decoupling method. Only two methine (C^{10} and C^{14}) and four methyl carbons (C^{11}, C^{12}, C^{15} and C^{16}) of the isobutyl group of **4a** could not be assigned because they show only one and two peaks, respectively.

Tab. 4. ^{13}C NMR data of *p*-methoxy-1-phenylethylammonium bromide (**11**)[a]

Host	Form of **11**	Chemical shifts δ in ppm CH_3	—CH—	$-OCH_3$	C^1	C^2	C^3	C^4
No host		20,44	51,82	55,30	129,27	128,48	114,55	160,18
4a[b]	*R*	20,62	51,46	55,36	129,97	128,33	114,63	160,18
	S	20,53	[c]	55,39	129,84	[c]	[c]	160,21
4b[b]	*R*	20,55	51,42	55,27	130,07	128,35	114,39	160,00
	S	20,33	51,26	[c]	129,94	128,39	[c]	[c]
5a[b]	*R*	20,37	51,42	55,28	129,97	[d]	114,41	160,06
	S	20,33	51,34	[c]	129,89	[d]	[c]	[c]
5b[b]	*R*	20,43	51,37	55,30	[d]	[d]	114,47	160,08
	S	20,31	51,27	[c]	[d]	[d]	[c]	[c]

[a] The numbers of the atoms refer to formula **11**.
[b] Host: *R*-form : *S*-form = 1,5 : 1,0 : 0,5.
[c] No splitting of signal.
[d] The signal of the guest could not be assigned because of the overlapping of signals of the hosts.

Tab. 5. Chemical shifts in ppm of the carbon atoms of **4a**

C^1: 41,65
C^2: 165,78
C^3: 52,66
C^4: 168,44
C^5: 41,78
C^6: 36,81
C^7: 55,23
C^8: 169,37
C^9: 40,03
C^{10} and C^{14}: 25,03
C^{13}: 40,44
C^{11}, C^{12}, C^{15} and C^{16}: 22,87 and 22,33

The numbers of the atoms refer to formula **4a**.

In order to examine the stoichiometry of substrate-cyclic peptide complexes, titration of **4a** with **1** was carried out by ^1H and ^{13}C NMR measurements. At the complex formation, two peaks of four methyl carbons and one peak of two methine carbons of the isobutyl group split to four and two peaks[4], respectively. Furthermore, the ^{13}C NMR data show that the downfield shifts of **4a** are marked in the amide carbonyl carbons, especially in that of the glycine amide carbonyl group (C^2). The up- or downfield shifts of the other carbons of **4a** are relatively small. These results suggest that the formation of substrate **4a** complexes is mainly attributed to the hydrogen bonds between the amide carbonyl groups of **4a** and the ammonium group of **1**.

It is also apparent that hydrogen bonds are important in the formation of **11**-**4a** complexes because the amide carbonyl carbons of **4a** show a remarkable downfield shift. The values (in ppm) of the chemical shifts of C^2, C^4 and C^8-carbons are 166,73, 169,05 and 169,83, respectively, at a mole ratio **4a**: (*R*)-**11** : (*S*)-**11** = 1,5:1,0:0,5.

Fig. 1 shows the changes of the chemical shifts of the amide carbonyl carbons (C^2, C^4 and C^8) of **4a** (0,04 mol · dm^{-3}) against (*R*)-**1**. It is found from the titration curves that the downfield shifts of the amide carbonyl carbons are marked at mole ratios of 0,5 and 1,0, and the saturation is attained at a mole ratio of 2,0. The saturation was further ascertained from negligible changes of the chemical shifts at the further addition of **1** to **4a** (to a mole ratio of 8,0).

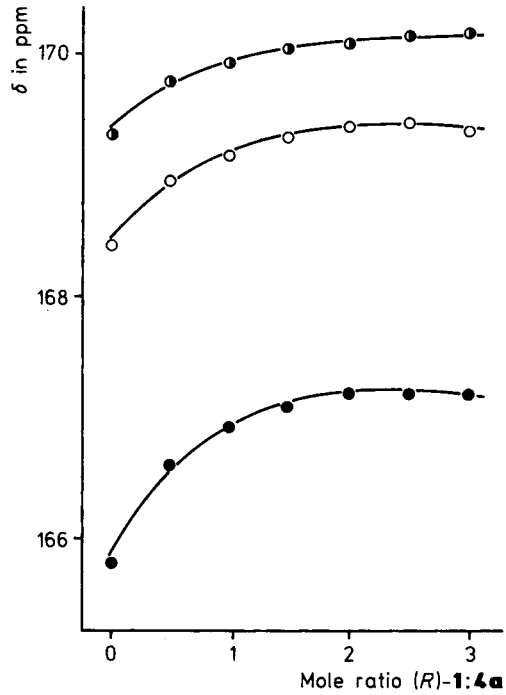


Fig. 1. Amine salt titration of cyclo(Gly-ELL)$_3$ (^{13}C NMR) (●): C^2, (○): C^4, (◑): C^8. The numbers of the atoms refer to formula **4a**

Previously, Blout et al.[5] carried out proline benzyl ester hydrochloride titration of cyclo(Pro-Gly)$_4$ by ^{13}C NMR measurement in $CDCl_3$, in which proline and glycine amide carbonyl carbons of the host were examined. The titration curves showed monotonous downfield shifts and were saturated at a mole ratio of 2,0 so that the stoichiometry of the guest-host complex was not clear. The titration curves, shown in Fig. 1, are similar to those reported by Blout et al. Therefore, the stoichiometry between (*R*)-**1** and **4a** was not established definitely. (*S*)-**1** titration of **4a** was also tried, and the titration curves were similar to those for (*R*)-**1**. Fig. 2 shows the changes of the ^{1}H chemical shifts of the asymmetric proton at the C^7 carbon adjacent to an amide carbonyl carbon (C^8) against (*R*)-**1**. This ^{1}H titration curve shows a biphasic change similar to that obtained by Blout et al.[5] with ^{13}C NMR of valine or phenylalanine methyl ester hydrochlorides-cyclo(Pro-Gly)$_4$ complexes. They described that the stoichiometries between amino acid ester salts and cyclo(Pro-Gly)$_4$ were 1 : 1 and 2 : 1.

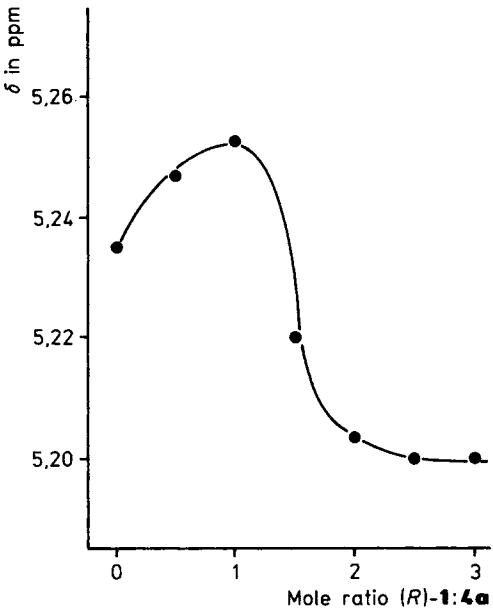


Fig. 2. Amine salt titration of cyclo(Gly-ELL)$_3$ (^{1}H NMR)

Comparing our results with those of Blout et al., a possibility that **1** forms both 1:1 and 2:1 salt-peptide complexes with **4a** cannot be excluded, though their data were obtained by ^{13}C NMR measurements. A titration curve similar to that of **4a** against (*R*)-**1** was also obtained in case of **4a** and (*S*)-**1**.

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2) H. O. Berhard, I. Kompis, S. Johne, D. Groger, M. Hesse, H. Schmid, *Helv. Chim. Acta* **56**, 1266 (1973)
3) L. N. Schoenberg, D. W. Cooke, C. F Liu, *Inorg. Chem.* **7**, 2386 (1968)
4) In case of a mole ratio **1** : **4a** = 1:1, the values in ppm of the chemical shifts of C^{11}, C^{12}, C^{15} and C^{16} change from 22,33 and 22,87 (no guest) to 21,92, 22,37, 22,78 and 23,03, and those of C^{10} and C^{14} vary from 25,03 (no guest) to 24,97 and 25,07, respectively
5) V. Madison, C. M. Deber, E. R. Blout, *J. Am. Chem. Soc.* **99**, 4788 (1977)