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Cyclic dipeptides as building blocks for combinatorial libraries. Part 2: Synthesis of bifunctional diketopiperazines

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Abstract—Twenty-four bifunctional diketopiperazines, cyclo(-Aax-Bbx-) consisting of glutamic or aspartic acid (Aax) and lysine, ornithine or diaminobutyric (Dab) acid (Bbx) were synthesized. D-Dab moiety was incorporated as D-Gln followed by Hoffman-type rearrangement induced by $PhI[OC(O)CF_3]_2$. Cyclization conditions for the related linear dipeptide precursors allowing racemization to be kept at a minimum were determined.

Recent advances in the rapidly-growing area of combinatorial chemistry caused dramatic acceleration of the drug discovery process, making 10^4–10^5-membered oligopeptide libraries readily available for biomedical screening.[1a–c] However, the direct medicinal use of naturally occurring bioactive peptides and their analogs consisting only of coded L-amino acids is hampered by their intrinsic proteolytic instability leading to short lifetime in vivo, low oral bioavailability and other unfavorable pharmacokinetic properties of peptides. At the molecular level, some of these features are believed to result from high inherent conformational flexibility of 'normal' oligopeptide chains. Therefore, the actual progress in the field of peptide libraries depends upon the availability of advanced building blocks preferably with limited conformational mobility that are usually referred to as 'peptide or small molecule mimetics'.[2a–c] These allow the above limitations to be overcome by reproducing (mimicking) structural properties of a peptide lead in an artificial way. Since only a small number of such building blocks are commercially available, related synthetic studies remain a 'hot' area of modern bioorganic synthesis.

With this paper we continue[3] reporting the results of our studies on utilization of common trifunctional aminoacids in constructing a variety of bifunctional building blocks which can be classified as dipeptide mimetics since all of them possess certain non-typical 'unnatural' structural features. The high potential of trifunctional amino acids to produce variously constrained 'non-proteinogenic' structures is illustrated in Fig. 1.

As exemplified below for lysine and glutamic acid, six different structural types of isomeric monocyclic dipeptides could be designed by combining a dicarboxylic aminoacid Aax with diaminoacid Bbx. Each of **I**–**VI** is represented by four possible stereoisomers which increase the total number of individual monocycles in the (Lys,Glu)-group up to 24. Further extension of this 'combinatorial' approach to aspartic acid, ornithine and diaminobutyric acid results in the 144-membered list of monocyclic dipeptides whose ring dimensions vary from 6 to 12 atoms.

We reason that this family of variously constrained and closely related bifunctional cyclopeptides presents a useful toolset for those pursuing research in the areas of combinatorial biomedical chemistry[1a–c] and de novo design of proteins and functional molecular devices.[4]

In fact, compounds **I**–**VI** could be viewed as peptidomimetic building blocks suitable for introducing a broad range of specific structural constraints or conformational preferences into biomolecules of pharmaceutical interest. Indeed, four representatives of **IV** and **V** have been utilized earlier by Manesis and Goodman in constructing an isolated β-turn tetrapeptide.[5] Recently, bifunctional diketopiperazines (DKPs) have been successfully utilized as templates in the synthesis of loop mimetics.[6]

The present paper describes synthetic procedures for, and physical properties of, the 24-membered family of bifunctional DKPs **1**–**24** which are of interest as molecular templates, scaffolds or spacers in modern molecular design (Table 1).

Keywords: combinatorial chemistry; piperazine-2,5-diones; 2,5-dioxopiperazines; cyclopeptide; peptidomimetic; racemization.

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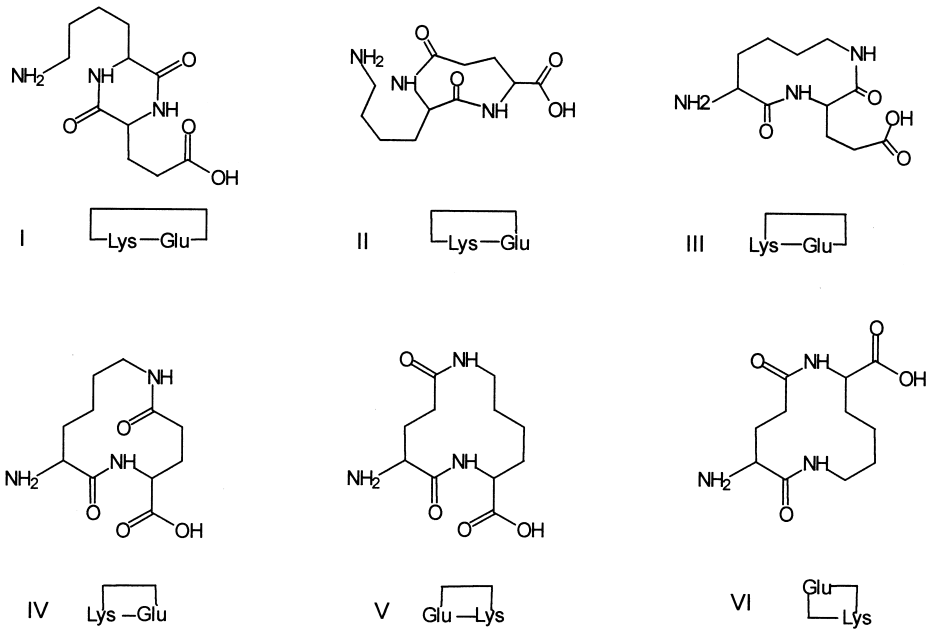


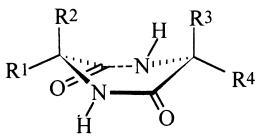
Figure 1. Isomeric monocyclic dipeptides.

Only a single representative of these compounds, cyclo(-Lys-Asp-) hydrobromide has been described earlier[7] as an intermediate in the chemical synthesis of bicyclopeptide antibiotic cairomycin B (Fig. 2).

The fact that all but one of cyclopeptides **1**–**24** have not been described yet in the literature seems to justify biomedical screening of the DKPs family as such. In particular, the DKPs derived from Glu and/or Dab, whose structures incorporate the fragment of γ-aminobutyric acid, GABA (Fig. 3), are worth evaluation as potential nootropic agents.

A number of fairly efficient synthetic procedures for diketopiperazines (DKPs) have been described.[8] After preliminary practical evaluation of these methods, the approach reported by Suzuki et al.[9] was selected for the production of DKPs **1**–**24** on a multi-gram scale

Table 1. Structures of diketopiperazines **1**–**24**



No.	Compound	R^1	R^2	R^3	R^4
1	Cyclo(-Lys-Glu-)	H	$-(CH_2)_4NH_2$	$-CH_2CH_2COOH$	H
2	Cyclo(-Lys-D-Glu-)	H	$-(CH_2)_4NH_2$	H	$-CH_2CH_2COOH$
3	Cyclo(-D-Lys-Glu-)	$-(CH_2)_4NH_2$	H	$-CH_2CH_2COOH$	H
4	Cyclo(-D-Lys-D-Glu-)	$-(CH_2)_4NH_2$	H	H	$-CH_2CH_2COOH$
5	Cyclo(-Lys-Asp-)	H	$-(CH_2)_4NH_2$	$-CH_2COOH$	H
6	Cyclo(-Lys-D-Asp-)	H	$-(CH_2)_4NH_2$	H	$-CH_2COOH$
7	Cyclo(-D-Lys-Asp-)	$-(CH_2)_4NH_2$	H	$-CH_2COOH$	H
8	Cyclo(-D-Lys-D-Asp-)	$-(CH_2)_4NH_2$	H	H	CH_2COOH
9	Cyclo(-Orn-Glu-)	H	$-(CH_2)_3NH_2$	$-CH_2CH_2COOH$	H
10	Cyclo(-Orn-D-Glu-)	H	$-(CH_2)_3NH_2$	H	$-CH_2CH_2COOH$
11	Cyclo(-D-Orn-Glu-)	$-(CH_2)_3NH_2$	H	$-CH_2CH_2COOH$	H
12	Cyclo(-D-Orn-D-Glu-)	$-(CH_2)_3NH_2$	H	H	$-CH_2CH_2COOH$
13	Cyclo(-Orn-Asp-)	H	$-(CH_2)_3NH_2$	$-CH_2COOH$	H
14	Cyclo(-Orn-D-Asp-)	H	$-(CH_2)_3NH_2$	H	$-CH_2COOH$
15	Cyclo(-D-Orn-Asp-)	$-(CH_2)_3NH_2$	H	$-CH_2COOH$	H
16	Cyclo(-D-Orn-D-Asp-)	$-(CH_2)_3NH_2$	H	H	$-CH_2COOH$
17	Cyclo(-Dab-Glu-)	H	$-(CH_2)_2NH_2$	$-CH_2CH_2COOH$	H
18	Cyclo(-Dab-D-Glu-)	H	$-(CH_2)_2NH_2$	H	$-CH_2CH_2COOH$
19	Cyclo(-D-Dab-Glu-)	$-(CH_2)_2NH_2$	H	$-CH_2CH_2COOH$	H
20	Cyclo(-D-Dab-D-Glu-)	$-(CH_2)_2NH_2$	H	H	$-CH_2CH_2COOH$
21	Cyclo(-Dab-Asp-)	H	$-(CH_2)_2NH_2$	$-CH_2COOH$	H
22	Cyclo(-Dab-D-Asp-)	H	$-(CH_2)_2NH_2$	H	$-CH_2COOH$
23	Cyclo(-D-Dab-Asp-)	$-(CH_2)_2NH_2$	H	$-CH_2COOH$	H
24	Cyclo(-D-Dab-D-Asp-)	$-(CH_2)_2NH_2$	H	H	$-CH_2COOH$

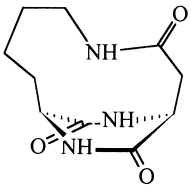


Figure 2. Cairomycin B.

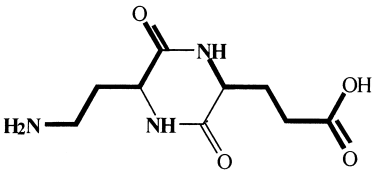
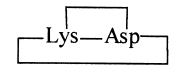
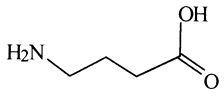


Figure 3. Cyclo(-Dab-Glu-).

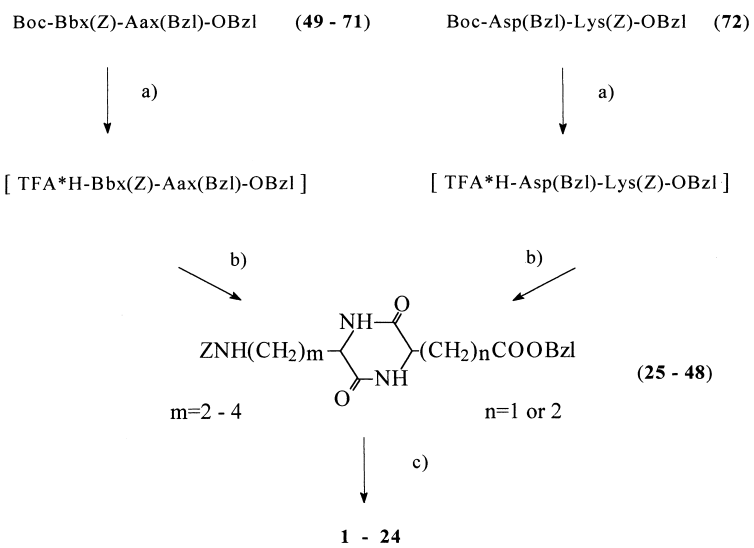




GABA

(Scheme 1). That procedure is based on the well-known side reaction in peptide synthesis, namely, the weak acid-catalyzed cyclization of dipeptide-esters. The reaction proceeds spontaneously under very mild conditions when one of the amino acids is proline or glycine. In a similar way, large numbers of dipeptide alkyl and benzyl esters were shown to undergo high-yield cyclization without detectable racemization when refluxed in 0.1N CH_3COOH/*sec*-BuOH for 3 h.[9] However, according to our experience, the precursors of DKPs **25**–**28** (Scheme 1) appeared to be less reactive, so that no less than 10 h of reflux were required to achieve acceptable cyclization yields.

Moreover, analysis of crude products and reaction mixtures has shown that formation of by-products having chromatographic properties very similar to the expected DKPs seriously complicates cyclization of diesters **25**–**28**. These



Scheme 1. (a) TFA-AcOH, 3:2; (b) 1 N AcOH/ROH; (c) PdO/C, 10% HCOOH in AcOH-i-PrOH.

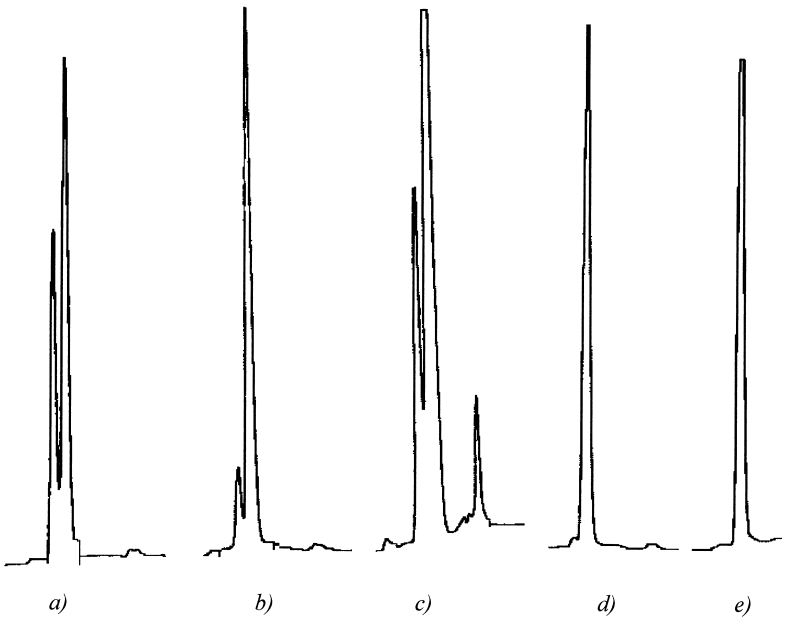


Figure 4. Analytical high-resolution ion-exchange chromatography of DKPs following conditions listed in Section 1.2: (a) resolution of diastereomers **5**, **6** synthesized from racemic H-Asp(Bzl)-OBzl; (b) and (c) detection of racemization product **5** in the crude **6** and related mother liquors accordingly (cyclization in refluxing *sec*-BuOH); (d) and (e) crude **6** and crude **5** obtained via optimized cyclization procedure.

Table 2. Physical properties of protected diketopiperazines **25**–**48**

No.	Compound	Yield (%)	Mp (°C)	R_f (A)	HRMS
25	Cyclo[-Lys(Z)-Glu(OBzl)-]	78	174–177[a]	0.59	482
26	Cyclo[-Lys(Z)-D-Glu(OBzl)-]	57	175–178	0.65	482
27	Cyclo[-D-Lys(Z)-Glu(OBzl)-]	59	175–180	0.65	482
28	Cyclo[-D-Lys(Z)-D-Glu(OBzl)-]	80	177–179	0.59	482
	Calculated for $C_{26}H_{31}N_3O_6$				481.6
29	Cyclo[-Lys(Z)-Asp(OBzl)-]	60	186–189	0.70	468
30	Cyclo[-Lys(Z)-D-Asp(OBzl)-]	58	184–185	0.61	468
31	Cyclo[-D-Lys(Z)-Asp(OBzl)-]	45	182–185	0.61	468
32	Cyclo[-D-Lys(Z)-D-Asp(OBzl)-]	79	185–187	0.70	468
33	Cyclo[-Orn(Z)-Glu(OBzl)-]	72	191–193	0.2	468
34	Cyclo[-Orn(Z)-D-Glu(OBzl)-]	66	187–189	0.35	468
35	Cyclo[-D-Orn(Z)-Glu(OBzl)-]	57	188–190	0.35	468
36	Cyclo[-D-Orn(Z)-D-Glu(OBzl)-]	56	190–192	0.2	468
	Calculated for $C_{25}H_{29}N_3O_6$				467.5
37	Cyclo[-Orn(Z)-Asp(OBzl)-]	67	195–200	0.32	454
38	Cyclo[-Orn(Z)-D-Asp(OBzl)-]	65	201–205	0.42	454
39	Cyclo[-D-Orn(Z)-Asp(OBzl)-]	60	200–204	0.42	454
40	Cyclo[-D-Orn(Z)-D-Asp(OBzl)-]	82	197–200	0.32	454
41	Cyclo[-Dab(Z)-Glu(OBzl)-]	78	170–171	0.45	454
42	Cyclo[-Dab(Z)-D-Glu(OBzl)-]	69	172–173	0.45	454
45	Cyclo[-D-Dab(Z)-Glu(OBzl)-]	58	171–172	0.43	454
46	Cyclo[-D-Dab(Z)-D-Glu(OBzl)-]	63	169–170	0.46	454
	Calculated for $C_{24}H_{27}N_3O_6$				453.5
43	Cyclo[-Dab(Z)-Asp(OBzl)-]	71	171–172	0.52	440
44	Cyclo[-Dab(Z)-D-Asp(OBzl)-]	59	179–180	0.50	440
47	Cyclo[-D-Dab(Z)-Asp(OBzl)-]	57	176–177	0.49	440
48	Cyclo[-D-Dab(Z)-D-Asp(OBzl)-]	61	171–172	0.52	440
	Calculated for $C_{23}H_{25}N_3O_6$				439.5

[a] Lit.[7]: mp 182–188°C.

by-products could result from the participation of the side chain ester group giving rise to regioisomeric bislactam II (Fig. 1). However, NMR spectra revealed near-symmetrical NH-pattern typical of DKPs. Independent synthesis of the four possible stereoisomeric Lys-Asp DKPs **5**–**8** led to identification of the by-product as diastereomer **5**. It was concluded therefore that considerable racemization takes place under the conditions recommended by Suzuki et al.[9] High-resolution ion-exchange chromatography on Biotronik Amino Acid Analyzer (Fig. 4) was found to be a convenient tool for detection of racemization when applied to free DKPs obtained after quantitative side chain deprotection of the samples of crude **25**–**48**. Formation of up to 20–30% of diastereomeric by-product we ascribe to fast thermal racemization of DKP.

To avoid deleterious overheating of the DKP already formed and keep racemization at minimum the reaction mixture was cooled after each 3–4 h of heating and the precipitated DKP was filtered off.

The cyclization step was further improved by using boiling methanol instead of *sec*-butanol and reducing the quantity of acetic acid catalyst to 1 equiv. with respect to dipeptide. These modifications directed towards keeping racemization of DKPs at minimum were prompted by observations of Izumiya et al.[15] Optically pure DKPs **17**–**24** were isolated in higher yields in this way, with less laborious purification procedure being used at the cost of an increasing the reaction duration to ca. 230 h. Normally, all crops of protected DKPs thus obtained were subjected to hydrogenolysis separately and the products were combined depending on their chiral integrity assayed either chromatographically (Fig. 4(d) and (e)) or by ^{1}H NMR spectroscopy.

A 3:2 mixture of trifluoroacetic and acetic acids was used for the selective removal of the Boc-group from the dipeptides **49**–**72** to minimize the well-known premature loss of carbobenzoxy protecting groups from lysine and

Table 3. Physical properties of diketopiperazines **1**–**24**

No.	Yield (%)	Mp (°C)	$[\alpha]_D^{23}$ (°)	Retention time (min)	R_f (B)	HRMS
1	70	264–265	−31.3	50.2	0.62	258
2	71	237–238	−2.8	51.3	0.56	258
3	55	240–243	+2.4	51.3	0.58	258
4	72	262–263	+31.3	50.6	0.64	258
	Calculated for $C_{11}H_{19}N_3O_4$					257.3
5[a]	78	228–229	−31.5	49.04	0.52	244
6	53	212–214	−30.7	50.17	0.54	244
7	51	236–237	+31.0	50.11	0.54	244
8	77	230–231	+31.7	49.06	0.52	244
9	80	225–226	−33.0	49.12	0.56	244
10	56	247–248	−6.0	51.92	0.49	244
11	51	249–250	+6.0	52.02	0.52	244
12	83	229–230	+33.5	49.00	0.56	244
	Calculated for $C_{10}H_{17}N_3O_4$					243.3
13	80	229–230	−31.2	47.75	0.52	230
14	56	167–169	−33.5	48.09	0.50	230
15	60	169–170	+33.7	48.09	0.50	230
16	81	231–233	+31.2	47.72	0.52	230
17	70	208–209	−18.0	52.3	0.54	230
18	65	204–207	−8.6	54.6	0.51	230
19	63	205–207	+7.5	54.5	0.50	230
20	51	203–205	+18.0	52.3	0.55	230
	Calculated for $C_9H_{15}N_3O_4$					229.2
21	78	201–202	−10.2	48.2	0.46	216
22	60	217–219	−27.0	48.5	0.41	216
23	47	207–208	+25.0	48.5	0.42	216
24	52	209–210	+9.8	48.1	0.48	216
	Calculated for $C_8H_{13}N_3O_4$					215.2

[a] Lit.[7] for hydrobromide: mp 252–254°C, $[\alpha]_D^{25}=-24.1$ (c=0.5, DMF).

Table 4. ^{1}H NMR assignments of diketopiperazines **1**–**24**

No.	pH	Bbx Aax	$\delta_{C(O)NH}$	$^3J_{HNC_\alpha H}$	$\delta_{C_\alpha H}$	$^3J_{HC_\alpha C_\beta HH'}$		H-C_β-H'		H-C_γ-H'		$\delta_{C_\delta H_2}$	$\delta_{C_\epsilon H_2}$	$\delta_{N_\omega H_2}$
						$J(H_\beta)$	$J(H'_\beta)$	δ_H	$\delta_{H'}$	δ_H	$\delta_{H'}$			
1	3.05	Lys	8.338	1.9	4.158	5.2	5.2	1.883	1.883	1.430	1.501	1.705	3.012	7.528
		Glu	8.338	1.9	4.197	5.2	5.2	2.145	2.145	2.495	2.495	–	–	–
2	3.00	Lys	8.321	1.5	4.238	5.1	5.1	1.830	1.943	1.359	1.474	1.684	3.993	7.507
		D-Glu	8.329	1.5	4.284	5.2	5.2	2.137	2.200	2.446	–	–	–	–
3	2.97	D-Lys	8.323	n.d.	4.237	5.2	5.2	1.829	1.942	1.358	1.474	1.684	2.993	7.500
		Glu	8.323	n.d.	4.285	5.1	5.1	2.146	2.200	2.453	–	–	–	–
4	3.05	D-Lys	8.338	1.8	4.158	5.1	5.1	1.881	1.881	1.429	1.500	1.706	3.011	7.519
		D-Glu	8.338	1.8	4.194	5.2	5.2	2.140	2.140	2.478	2.478	–	–	–
5	3.07	Lys	8.269	<1.5	4.292	4.4	5.0	1.854	1.933	1.415	1.509	1.696	3.012	7.507
		Asp	8.279	<1.5	4.447	4.4	5.9	2.874	2.940	–	–	–	–	–
6	3.27	Lys	8.287	<2	4.223	4.9	4.9	1.836	1.947	1.387	1.503	1.693	2.995	7.510
		D-Asp	8.287	<2	4.426	4.0	4.9	2.843	2.968	–	–	–	–	–
7	3.11	D-Lys	8.312	1.8	4.224	n.d.	n.d.	1.837	1.954	1.395	1.504	1.693	3.010	7.513
		Asp	8.312	1.8	4.443	4.3	4.9	2.873	3.005	–	–	–	–	–
8	3.01	D-Lys	8.279	1.8	4.220	4.2	6.5	1.850	1.930	1.407	1.508	1.694	3.009	7.500
		D-Asp	8.279	1.8	4.445	4.3	6.1	2.871	2.945	–	–	–	–	–
9	3.15	Orn	8.360	<2	4.209	4.9	4.9	1.926	1.926	1.726	1.805	3.048	–	7.615
		Glu	8.383	<2	4.209	5.5	5.5	2.145	2.145	2.475	2.475	–	–	–
10	3.07	Orn	8.359	<2	4.287	5.2	5.2	1.874	1.996	1.669	1.778	3.032	–	7.590
		D-Glu	8.386	<2	4.297	5.1	5.1	2.154	2.206	2.465	–	–	–	–
11	2.82	D-Orn	8.355	<2	4.280	4.4	4.4	1.865	1.984	1.661	1.770	3.024	–	7.585
		Glu	8.380	<2	4.287	4.4	4.4	2.145	2.195	2.455	–	–	–	–
12	2.90	D-Orn	8.361	<2	4.208	n.d	n.d.	n.d.	n.d	n.d.	n.d.	3.046	–	7.600
		D-Glu	8.382	<2	4.208	n.d	n.d	2.145	2.145	2.477	–	–	–	–
13	3.40	Orn	8.282	2.2	4.268	7.1	4.7	1.882	1.991	1.709	1.813	3.032	–	7.612
		Asp	8.282	2.0	4.428	4.3	6.1	2.834	2.907	–	–	–	–	–
14	2.20	Orn	8.366	<2	4.266	4.0	4.9	1.876	1.996	1.687	1.796	3.040	–	7.581
		D-Asp	8.366	<2	4.467	4.9	4.9	2.900	3.044	–	–	–	–	–
15	3.32	D-Orn	8.310	1.8	4.275	6.7	4.9	1.883	2.002	1.687	1.802	3.040	–	7.598
		Asp	8.325	1.2	4.433	4.3	4.9	2.946	2.971	–	–	–	–	–
16	3.07	D-Orn	8.314	<2	4.279	n.d.	n.d.	1.894	1.992	1.703	1.818	3.034	–	7.590
		D-Asp	8.314	<2	4.446	5.2	4.2	2.862	2.972	–	–	–	–	–
17	3.02	Dab	8.389	2	4.247	5.1	6.6	2.133	2.133	3.130	3.164	–	–	n.d.
		Glu	8.423	2	4.199	5.9	–	2.209	2.209	2.455	2.455	–	–	–
18	2.89	Dab	8.374	2	4.349	5.2	–	2.209	2.155	3.143	3.075	–	–	7.702
		D-Glu	8.430	2	4.241	5.2	–	2.209	2.155	2.455	2.455	–	–	–
19	2.90	D-Dab	8.370	<2	4.361	5.2	5.2	2.18	2.22	3.093	3.162	–	–	7.728
		Glu	8.428	<2	4.252	5.2	5.2	2.18	2.22	2.202	2.202	–	–	–
20	3.05	D-Dab	8.395	<2.2	4.272	5.9	7.4	2.232	2.232	3.155	3.190	–	–	7.813
		D-Glu	8.433	<2.2	4.225	5.5	6.6	2.161	2.161	2.490	2.490	–	–	–
21	3.12	Dab	8.324	<2.2	4.347	–	–	2.236	2.225	3.115	3.194	–	–	n.d.
		Asp	8.357	<2.2	4.419	4.4	5.1	2.830	2.989	–	–	–	–	–
22	3.06	Dab	8.335	2	4.338	5.1	5.9	2.208	2.252	3.084	3.172	–	–	n.d.
		D-Asp	8.373	2	4.422	4.4	5.15	2.837	2.992	–	–	–	–	–
23	3.22	D-Dab	8.341	<2	4.355	5.1	5.1	2.215	2.271	3.193	3.111	–	–	7.736
		Asp	8.377	<2	–	4.4	5.2	2.856	3.005	–	–	–	–	–
24	3.19	D-Dab	8.317	<2	4.346	5.5	5.5	2.238	2.263	3.115	3.189	–	–	7.725
		D-Asp	8.350	<2	4.420	4.4	5.1	2.830	2.989	–	–	–	–	–

ornithine side chains.[10] Trifluoroacetates thus obtained were directly used in cyclization according to modified Suzuki procedure[9] and subsequent quantitative removal of side-chain protecting groups from protected cyclic dipeptides **25**–**48** was carried out using Pd-catalyzed transfer hydrogenation.[11] Overall yields of the free DKPs **1**–**24** ranging from 50 to 70% (from dipeptides **49**–**72**) were reproducibly obtained. All cyclopeptides were characterized by satisfactory microanalytical data and correct HRMS. Properties of the DKPs and their precursors are given in Tables 2–6. The absence of racemization was judged from the data offered by high-resolution ion-exchange chromatography on amino acid analyzer (Fig. 4(d) and (e)) and ^{1}H NMR spectroscopy.

Linear protected dipeptides **49**–**67** were synthesized starting from aspartic and glutamic acid dibenzyl esters *p*-toluenesulfonates according to Scheme 2.

As specified above, the dipeptide precursor (**72**) of cyclo(-Asp-Lys-) (**5**) has been obtained by coupling the alternative pair of readily available derivatives: Boc-Asp(Bzl)-OH and H-Lys(*Z*)-OBzl*TosOH.

The particular approach (Scheme 3) was employed in the syntheses of dipeptides **68**–**71** containing D-Dab which is not commercially available. The approach utilizes D-glutamine as D-Dab precursor and involves two additional steps. Initially, Boc-D-Gln-OH was coupled with the aforementioned H-Aax(Bzl)-OBzl via mixed carbonic anhydride method at −10°C in the presence of NMM to produce the corresponding dipeptides (**73**–**76**) in high yields and essentially free of the usual by-products arising from dehydration of the Gln side chain amido-function. 'Unmasking' of the D-Dab-moiety, i.e. the $-CH_2C(O)NH_2 \rightarrow -CH_2NH_2$ conversion was effected by

Table 5. Physical properties of dipeptides **49**–**76**

No.	Compound	Yield, (%)	Mp (°C)	R_f (A)	HRMS
49	Boc-Lys(*Z*)-Glu(OBzl)-OBzl	78	86–87	0.76	690
50	Boc-Lys(*Z*)-D-Glu(OBzl)-OBzl	91	95–96	0.75	690
51	Boc-D-Lys(*Z*)-Glu(OBzl)-OBzl	87	89–90	0.75	690
52	Boc-D-Lys(*Z*)-D-Glu(OBzl)-OBzl	73	87–89	0.76	690
	Calculated for $C_{38}H_{47}N_3O_9$				689.8
72	Boc-Asp(OBzl)-Lys(*Z*)-OBzl	93	99–100	0.84	676
53	Boc-Lys(*Z*)-D-Asp(OBzl)-OBzl	92	101–102	0.74	676
54	Boc-D-Lys(*Z*)-Asp(OBzl)-OBzl	87	95–96	0.74	676
55	Boc-D-Lys(*Z*)-D-Asp(OBzl)-OBzl	90	108–110	0.76	676
56	Boc-Orn(*Z*)-Glu(OBzl)-OBzl	93	114–115	0.79	676
57	Boc-Orn(*Z*)-D-Glu(OBzl)-OBzl	95	100–101	0.73	676
58	Boc-D-Orn(*Z*)-Glu(OBzl)-OBzl	77	105–106	0.73	676
59	Boc-D-Orn(*Z*)-D-Glu(OBzl)-OBzl	75	120–121	0.79	676
	Calculated for $C_{37}H_{45}N_3O_9$				675.8
60	Boc-Orn(*Z*)-Asp(OBzl)-OBzl	88	86–88	0.73	662
61	Boc-Orn(*Z*)-D-Asp(OBzl)-OBzl	89	95–96	0.75	662
62	Boc-D-Orn(*Z*)-Asp(OBzl)-OBzl	83	96–97	0.75	662
63	Boc-D-Orn(*Z*)-D-Asp(OBzl)-OBzl	85	89–91	0.73	662
64	Boc-Dab(*Z*)-Glu(OBzl)-OBzl	92	91–92	0.79	662
65	Boc-Dab(*Z*)-D-Glu(OBzl)-OBzl	87	107–108	0.75	662
68	Boc-D-Dab(*Z*)-Glu(OBzl)-OBzl	85	103–105	0.74	662
69	Boc-D-Dab(*Z*)-D-Glu(OBzl)-OBzl	82	88–89	0.80	662
	Calculated for $C_{36}H_{43}N_3O_9$				661.8
73	Boc-D-Gln-Asp(OBzl)-OBzl	83	129–130	0.48	542
74	Boc-D-Gln-D-Asp(OBzl)-OBzl	84	131–132	0.42	542
	Calculated for $C_{28}H_{35}N_3O_8$				541.6
75	Boc-D-Gln-Glu(OBzl)-OBzl	89	117–118	0.40	556
76	Boc-D-Gln-D-Glu(OBzl)-OBzl	86	102–103	0.48	556
	Calculated for $C_{29}H_{37}N_3O_8$				555.6
66	Boc-Dab(*Z*)-Asp(OBzl)-OBzl	91	91–92	0.83	648
67	Boc-Dab(*Z*)-D-Asp(OBzl)-OBzl	85	127–128	0.77	648
70	Boc-D-Dab(*Z*)-Asp(OBzl)-OBzl	93	127–128	0.77	648
71	Boc-D-Dab(*Z*)-D-Asp(OBzl)-OBzl	83	89–90	0.83	648
	Calculated for $C_{35}H_{41}N_3O_9$				647.7

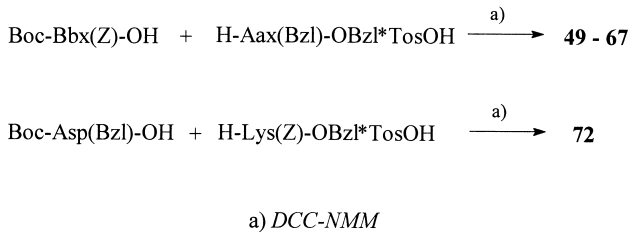
bis(trifluoroacetoxy)iodobenzene $PhI[OC(O)CF_3]_2$ (PIFA), a reagent of choice for performing Hoffman-type degradation of primary amides under mild conditions.[12] PIFA is not only exceptionally selective towards primary amides in the presence of secondary and tertiary ones but also is fully compatible with major protecting groups adapted for use in peptide chemistry (Fmoc, Alloc-, Boc, Z, etc.). Hopefully, PIFA and the new synthetic possibilities it offers will soon receive adequate attention in the area of combinatorial chemistry.

In our hands, freshly prepared PIFA induced clean transformations of peptides **73**–**76** into the expected trifluoroacetates that were then proceeded to the carbobenzoxylation step without isolation. The protected dipeptides **68**–**71** were thereby isolated in overall yields of 50–60% starting from Boc-D-Gln-OH.

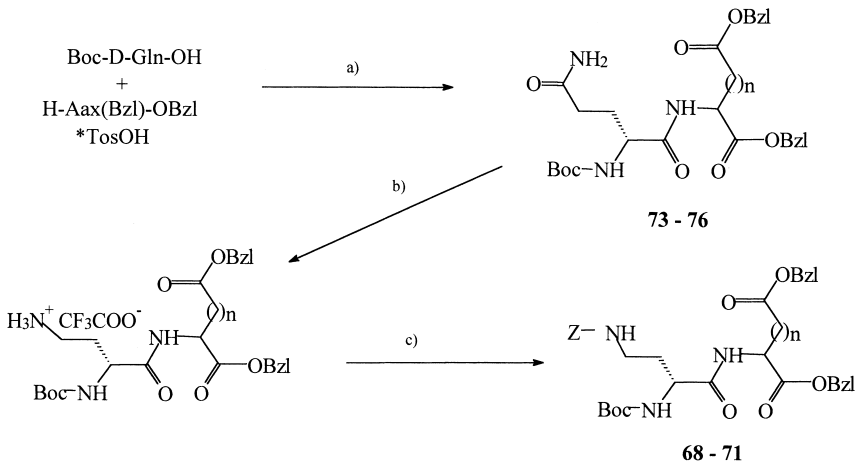
The last point worth noting is almost total racemization experienced in attempted preparation of dibenzyl esters of Asp and Glu (common starting materials in the present

Table 6. Microanalytical data for **1**–**76**

No.	Formula	Calculated (%)			Data obtained for this group (%)		
		C	H	N	C	H	N
1–**4**	$C_{11}H_{19}N_3O_4$	51.35	7.44	16.33	50.8–51.6	7.2–7.5	16.1–16.7
5–**12**	$C_{10}H_{17}N_3O_4$	49.37	7.04	17.27	48.9–49.7	6.9–7.2	16.9–17.4
13–**20**	$C_9H_{15}N_3O_4$	47.16	6.60	18.33	46.8–47.6	6.5–6.8	18.0–18.7
21–**24**	$C_8H_{13}N_3O_4$	44.65	6.09	19.53	44.3–45.1	5.9–6.2	19.3–19.9
25–**28**	$C_{26}H_{31}N_3O_6$	64.85	6.49	8.73	64.3–65.1	6.4–6.7	8.4–8.9
29–**36**	$C_{25}H_{29}N_3O_6$	64.23	6.25	8.99	63.9–64.6	6.1–6.5	8.7–9.1
37–**42**, **45**, **46**	$C_{24}H_{27}N_3O_6$	63.57	6.00	9.27	63.2–63.9	5.9–6.1	9.1–9.6
43, **44**, **47**, **48**	$C_{23}H_{25}N_3O_6$	62.86	5.73	9.56	62.6–63.3	5.6–5.9	9.5–9.9
49–**52**	$C_{38}H_{47}N_3O_9$	66.17	6.87	6.09	65.8–66.6	6.7–7.0	6.0–6.1
53–**59**, **72**	$C_{37}H_{45}N_3O_9$	65.76	6.71	6.22	65.4–66.2	6.5–6.8	6.0–6.3
60–**65**, **68**, **69**	$C_{36}H_{43}N_3O_9$	65.34	6.55	6.35	64.8–65.4	6.4–6.6	6.3–6.5
73, **74**	$C_{28}H_{35}N_3O_8$	62.10	6.51	7.76	61.8–62.3	6.6–6.7	7.6–7.8
75, **76**	$C_{29}H_{37}N_3O_8$	62.69	6.71	7.56	62.2–62.5	6.7–6.8	7.7–7.9
66, **67**, **70**, **71**	$C_{35}H_{41}N_3O_9$	64.90	6.38	6.49	64.6–65.3	6.3–6.6	6.4–6.7



Scheme 2.



Scheme 3. (a) i-BuOC(O)Cl-NMM, −10°C; (b) PIFA-pyridine; (c) Z-Cl/NMM.

syntheses) via the conventional procedure of TosOH-catalyzed esterification with azeotropic water removal.[13] DKPs **1** and **5** synthesized from Glu and Asp derivatives produced in this way were shown to be largely racemized (see Fig. 4(a)). Optical rotation measurements indicated that the above esterification yielded racemic mixtures in case of both diacids, while optically pure lysine derivative, H-Lys(*Z*)-OBzl toluenesulfonate, was isolated under the same reaction conditions. Finally, enantiomerically pure dibenzyl esters were obtained in high yields when the newer esterification procedure was employed in which TosCl at 80°C is utilized as dehydrating agent.[14]

1. Experimental

1.1. Chemicals

All solvents, trifluoroacetic acid, *N*-methylmorpholine, benzyl alcohol, *p*-toluensulfonic acid monohydrate, *p*-toluenesulfonylchloride obtained from REAKHIM (Russia) were purified before use according to standard methods.[15] *Z*-Cl, DCC, D-Glu, D-Lys were purchased from Peptide Institute Inc. (Japan). L-Asp, D-Asp, Lys(*Z*), L-Orn, D-Orn, Boc-Lys(*Z*)-OH*DCHA were obtained from Reanal (Hungary). 20% Palladium hydroxide on carbon, Z-OSu, Boc_2O were supplied by Fluka (Switzerland). Dibenzyl-esters *p*-toluenesulfonates of Asp and Glu (both enantiomers) were synthesised according to Arai and Muramatsu.[14] Boc-D-Lys(*Z*)-OH, Boc-D-Orn(*Z*)-OH and Boc-L-Orn(*Z*)-OH were synthesised using conventional procedures.[16]

1.2. Instrumentation

Proton NMR spectra were obtained on a Brucker WM-500 spectrometer. Spectra were taken in D_2O at sample concentrations of 2 mg/ml; pHs of solutions are indicated in Table 4. Peak positions are reported in ppm downfield from tetramethylsilane. Coupling constants are given in Hertz (Hz). Optical rotations of DKPs **1**–**24** were determined on a Perkin–Elmer 141M polarimeter using a 10 cm water-jacketed cell at 23°C. All substances were dissolved in 1N acetic acid at c=2.0. Mass spectra were taken on Kratos MS50TS (UK) using the fast atom bombardment method. TLC was performed on Merck F_{254} silica gel G plates in solvent systems: (A) $CHCl_3$–MeOH (9:1); (B) *i*-PrOH–H_2O (1:1). Spots were detected by 1% ninhydrin in acetone or UV-radiation. Chemical and optical purity of all free cyclic dipeptides was estimated on Biotronik High Performance Amino Acid Analyzer LC 5001 using Standard Hydrolysate Program and 200 mm column packed with BTC 2710 resin. Retention times of diketopiperazines **1**–**24** which were eluted between leucine and histidine (retention times 46.7 and 55.5 min, respectively) are summarized in Table 3. Melting points were determined on a Kofler melting point apparatus (VEB Analytik, Germany) and are uncorrected. Microanalyses were performed on a Hewlett–Packard 185B CHN-analyzer.

1.3. Synthetic procedures

1.3.1. Protected linear dipeptides (49–67, 72). A solution of Boc-amino acid (63 mmol) in 100 ml of acetonitrile was added to a solution of amino acid benzyl ester *p*-toluene-sulfonate (70.5 mmol) containing one equivalent of NMM in 100 ml of acetonitrile. The mixture was cooled to 0°C and DCC (70.5 mmol) was added in one portion with stirring. 18 hours later the *N*,*N*′-dicyclohexylurea was removed by filtration and the solution was evaporated under reduced pressure to give the crude product. The product was redissolved in 1.5 l of EtOAc and washed several times with 2 M aqueous sodium bisulfate and saturated sodium bicarbonate until no starting materials can be detected in the solution by TLC. Finally the solution was washed with brine

and dried over magnesium sulfate. The drying agent was filtered and washed with EtOAc (3×75 ml) and the combined organic solutions were concentrated under reduced pressure to give a solid residue, which was crystallized from ether or ether/hexane.[17] The pure product was collected and washed with cold ether: yields 80–95% (Table 5).

1.3.2. Protected dipeptides Boc-D-Gln-Aax(Bzl)-OBzl (73–76). The title compounds are prepared using a standard mixed anhydride method starting from Boc-D-Gln-OH (57 mmol) in 100 ml of DMF,[18] freshly distilled isobutylchloroformate (57 mmol), NMM (57+57 mmol) and *p*-toluenesulfonate of dibenzyl ester of Asp or Glu (57 mmol) dissolved in 100 ml of DMF. Temperature of the reaction mixture is kept at −10°C throughout dropwise addition of the reagents and during the next 2 h. After a standard work-up and crystallization from EtOAc–Et_2O–hexane peptides **73–76** (Table 5) homogeneous by TLC are obtained in 82–89% yields.

1.3.3. Boc-D-Dab(*Z*)-Aax(Bzl)-OBzl (68–71). To a stirred solution of freshly prepared PIFA (85 mmol) in DMF–H_2O (500 ml, 1:1 v/v), Boc-D-Gln-Aax(Bzl)-OBzl **73–76** (55 mmol) is added in one portion at room temperature. After 15 min pyridine (110 mmol) is added, and stirring is continued for 3 h.[12] The solvents are evaporated in vacuum and the residue is dissolved in ethyl acetate (700 ml). The solution is washed extensively with water, 10% sodium bicarbonate, brine and dried over $MgSO_4$. Solvent is removed in vacuum and crude product Boc-D-Dab(*TFA)-Aax(Bzl)-OBzl thus obtained is used in the next step without further purification. To a stirred solution of the latter salt (55 mmol) in acetonitrile (300 ml) at 0°C, *N*-methylmorpholine (55 mmol) and *Z*-Cl (55 mmol) are added consequently in three portions.[17] After stirring for 1 h at 0°C, the reaction mixture is left overnight at room temperature. The solvent is removed by evaporation under reduced pressure and the residue is redissolved in EtOAc (700 ml) and the solution is washed with 10% sodium bicarbonate, 5% sodium hydrosulfate, water, brine and is dried over $MgSO_4$. After solvent removal in vacuum, the crude dipeptides **68–71** (Table 5) are crystallized from EtOAc–Et_2O–hexane. Yields: 80–84%.

1.3.4. Protected diketopiperazines (25–48). Boc-dipeptide esters **49–72** (50 mmol) were treated with a 3:2 mixture of TFA–AcOH (100 ml) for 2 h at room temperature[10] and the acids were removed by repeated (×5) evaporation with benzene in vacuum. Dipeptide trifluoroacetates thus obtained were dissolved in 0.1 M AcOH–*s*-BuOH (750 ml), and NMM (5.5 ml, 50 mmol) was added.[9] The resulting weakly acidic solution was refluxed for total 9 h. Usually, diketopiperazines **25–48** began to crystallize out from the solution on cooling already after 2 h of heating. The reaction mixture was cooled after each 2–3 h of heating and precipitated protected DKP was separated by filtration. Each crop of cyclic peptide was collected on a filter separately, washed with methanol and ether and then recrystallized from *s*-BuOH. Total yields of **25–48** were in the range 50–80% (Table 2).

1.3.5. Diketopiperazines (1–24). A protected diketopiperazine **25–48** (30 mmol) was dissolved in 10% HCOOH–AcOH (200 ml). An equal weight of 20% palladium hydroxide on charcoal was added carefully and the reaction mixture was stirred overnight.[11] The catalyst was filtered and washed with 2×10 ml of 50% acetic acid. The combined filtrate and washes were evaporated in vacuum to oil. This was further re-evaporated with water (5×20 ml) to ensure complete removal of acids. The residue was dissolved in water (50 ml) and left over activated charcoal for 12 h. The charcoal was removed by filtration and washed with water (3×20 ml); the combined aqueous solutions were neutralized with 5% solution of ammonium hydroxide and concentrated in vacuum to a small volume. As a rule, crystallization of the product started spontaneously at this stage, otherwise the process was initiated by addition of methanol and after keeping the solution at +4°C overnight, crystalline **1–24** were obtained 83–47% yields (Tables 3 and 4). Samples for analytical purposes were recrystallized from water–methanol and vacuum dried in Fisher apparatus over KOH pellets.

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