Complexation of Organic Dyes by Peptides Built of Lysine and Glutamic Acid Amides

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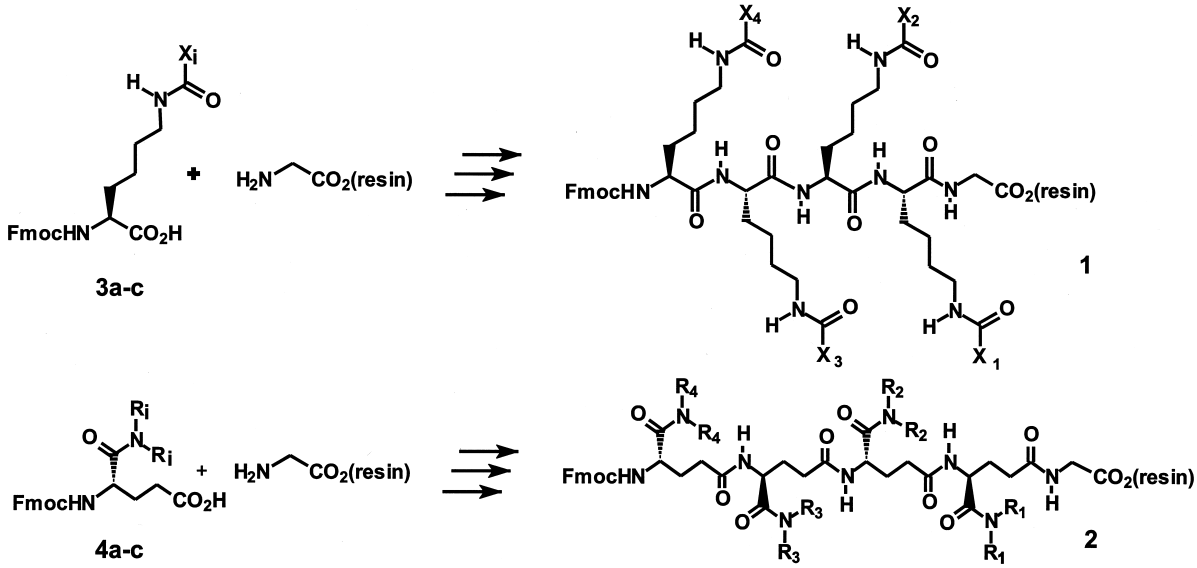
Two amide libraries, Fmoc-[Lys(ac_i)]$_4$-Gly-resin (**1**) (ac_i = 2-naphthylcarbonyl, 1-adamantylcarbonyl and benzyloxycarbonyl) and Fmoc-[δ-Glu(α-$amide_i$)]$_4$-Gly-resin (**2**) ($amide_i$ = morpholineamide, piperidineamide, (N′-phenyl)-piperazineamide), have been synthesized from the corresponding Fmoc-protected amino acid derivatives. Beads of the libraries complex organic dyes (crystal violet and Sudan black) differently according to the sequence of residues in **1** or **2**. The results are considered a step towards artificial receptors for small organic molecules build from linear oligoamides.

Nature uses linear oligomers to construct functional hosts as the enzymes, antibodies or ribozymes. The specificity and functionality of these compounds is determined by a sequence of monomeric units – only a few monomers (amino acids, nucleic acids) are sufficient to produce the complex world of natural receptors. Artificial receptors may be constructed by the same building recipe [1] but the monomeric units should then be as simple as possible and free from all the other structural and informational features associated with the chemistry of life.

Here, we report the synthesis of two small amide libraries **1** and **2**, containing sequences of α-amides of glutamic acid and ε-amides of lysine terminated by glycine (Scheme 1). The structure of these molecules is very similar to the structure of natural peptides. However, amide substituents



Scheme 1. Lysine derivatives **3a–c** and glutamic acid amides **4a–c** build the resin bound oligoamides **1** and **2** (see Scheme 2 for the structures of **3a–c** and **4a–c**.).

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R_i and X_i are introduced as a tool for chemists to tune the properties of the oligoamides. The complexation of an azo-dye and of crystal violet is used to test whether the sequence of lysine or glutamine derivatives in the pseudo peptides determines specific intermolecular interactions.

The synthesis of the monomers **3a–c** and **4a–c** is outlined in Scheme 2. The ε-amine of Fmoc–Lys–OH **5** is acylated using different carboxylic acid chlorides yielding **3a** and **3b** (**3c** is accessible via this route but is also commercially available). The hexafluoroacetone adduct **6** of glutamic acid [2] serves as the entry to the α-amides of glutamic acids (Scheme 2).

The lysine and glutamic acid amides **3a–c** and **4a–c** were used in solid phase syntheses of the modified peptides **1** and **2**. The peptides were built on the Gly-Wang resin [3] by the PyBOP-DiPEA protocol [4] in DMF. Piperidine was used to remove the Fmoc-groups. The usual split and combine technique [5] leads to libraries **1** and **2** each containing 81 different oligoamides. The libraries were also resynthesized on a parallel synthesizer [6a].

For a first demonstration of selectivity in complexation, the library **1** was treated with a solution of crystal violet (**7**) in chloroform and subsequently washed with methanol, then acetone and chloroform [6b]. The beads remain differently colored, so different sequences in the pseudo peptide complex the dye differently (Fig. 1).

One expects that the number of aromatic groups in each amide chain will have some influence on the complexation of the aromatic dye **7**. Indeed, there is a weak correlation of the number of aromatic units with the color density of the beads after washing (see Fig. 2). However, the interactions are also sequence specific – many sequences

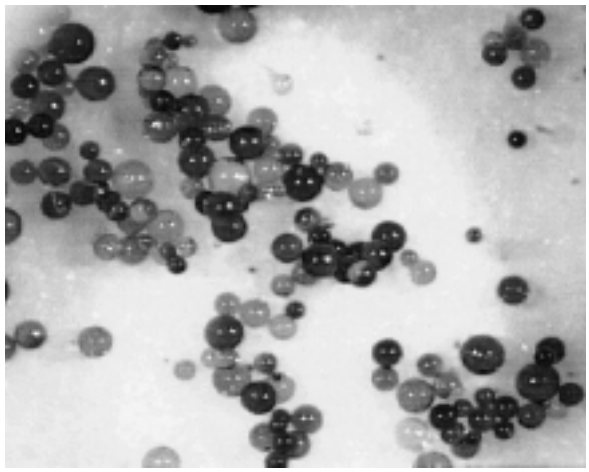
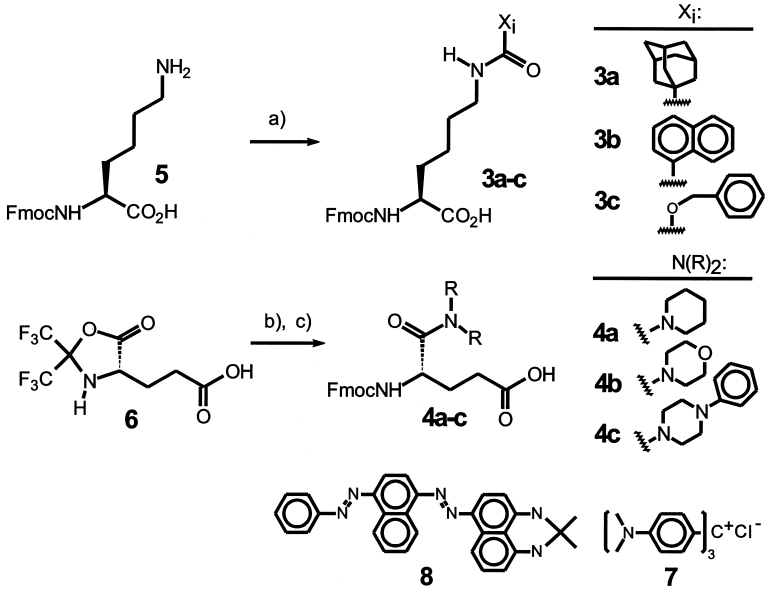


Fig. 1. Beads of a sublibrary of **1** (containing **3a** in the N-terminal position) after treatment with crystal violet (**7**) and washing with methanol, then acetone and chloroform.



Scheme 2. Preparation of the lysine and glutamic acid derivatives **3a–c** and **4a–c** (a) X_i–COCl (82. – 84%); b) HNR_2 (52–71%); c) Fmoc–Cl (74. – 88%)) and structure of crystal violet (**7**) and Sudan black (**8**).

which do not differ in the number of their aromatic units differ strongly in their complexation properties.

Sequences of **1** which result in highly colored beads (line above o.d. 45 in Fig. 2), contain the aromatic side chain with the naphthyl group (**3b**) relatively often at positions 4 and 2 and the benzoyl group (**3c**) at position 3. If the adamantyl (**3a**) group is present in position 1, close to the resin support, it does not hinder complexation. Further washing of the beads with chloroform/dichloromethane results in nearly complete decolorization of all beads. Beads with the sequence **3b–3b–3b–3c–**Gly-resin stay colored longest.

Similar observations were made when the glutamine library **2** is treated with a solution of the azo dye Sudan black (**8**) in dichloromethane and washed with methanol, acetone and dichloromethane. This library contains only one type of aromatic unit (**2c**) but again different resin bound sequences with the same number of aromatic units fix the dye differently. If **2** is treated with crystal violet **7** and washed several times with dichloromethane only two sequences with four and three aromatic units (**2c–2c–2c–2c–**Gly-resin and **2c–2c–2a–2c–**Gly-resin) remain colored.

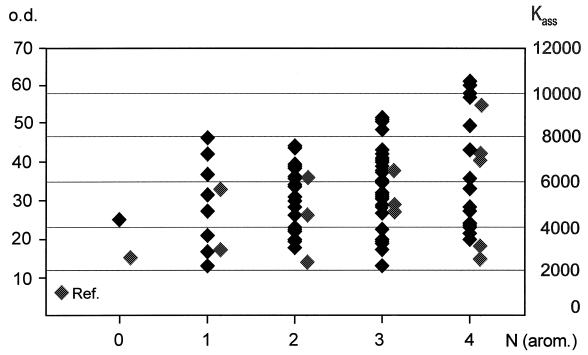


Fig. 2. Association behaviour of crystal violet (**7**) on the oligoamide surface of the library **1**. The data are sorted by the number of aromatic units (N (arom.)) in the modified lysine side chains. The grey signs mark the association constant K_{ass} [l/mol] for the binding of crystal violet on the surface of **1** in acetone. The black rhombic signs mark values of a densitometric analysis of the beads after treatment with crystal violet followed by several washing steps with ethanol, methanol, ethyl-acetate and acetone. The beads on the washing frit were photographed with a digital camera, the picture was converted to grey scale values and the optical densities of the beads corrected by the background density were calculated (o.d., arbitrary units, left scale).

A few beads of the library **1** were used to measure quantitatively the uptake of the dye on the bead surface. The data in Table 1 were obtained in acetone solution assuming a one to one equilibrium between the surface bounded (pseudo-)peptide and the crystal violet molecules (see experimental section).

Table 1 proofs that different sequences differ in their energy of complexation and a strong complex is associated with deeply colored beads. Even the lysine peptides with four aromatic side chains have significantly different association constants (entry 10 to 15 in Table 1). It is not expected that the used short sequences of (pseudo)peptides give high complexation constants but the enhancement over the background complexation (entry 16 in Table 1) is significant.

Conclusions

The resin bound oligoamides **1** or **2**, built from derivatives of lysine and glutamine, complex organic dyes selectively. Sequences of **1** and **2** with a

Table 1. Association of crystal violet in acetone on the "peptide" surface of selected beads of the library **1**. The numbers refer to the sequence of Fmoc-protected lysine derivatives used to build the oligoamides **1**. K_{ass} and ΔG_{298} were calculated from the decrease in the extinction coefficient of the dye in solution (see Experimental Section). The optical density of the bead surfaces (shown in Fig. 2) is also given for comparison.

Entry	Sequence-(G-resin)	$K_{ass} \cdot 10^{-3}$ [l·mol^{-1}]	o.d. in Fig. 2	ΔG_{298} [kcal/mol]
1	**3a3a3a3a–**	2.38 ± 0.34	26.3	–4.60
2	**3a3c3a3a–**	2.94 ± 0.48	21.6	–4.73
3	**3a3a3b3a–**	5.70 ± 0.84	47.6	–5.12
4	**3b3b3a3a–**	4.56 ± 0.74	45.2	–4.99
5	**3b3c3a3a–**	6.08 ± 0.45	40.5	–5.16
6	**3c3a3a3b–**	2.42 ± 0.39	20.0	–4.61
7	**3b3c3b3a–**	4.93 ± 0.62	52.9	–5.04
8	**3b3b3c3a–**	6.21 ± 0.33	52.0	–5.17
9	**3b3a3b3b–**	4.89 ± 0.52	49.7	–5.03
10	**3b3c3c3b–**	2.46 ± 0.39	24.7	–4.62
11	**3b3b3c3b–**	2.56 ± 0.49	33.7	–4.64
12	**3c3c3c3b–**	3.16 ± 0.61	22.1	–4.77
13	**3b3b3b3c–**	7.00 ± 0.52	58.2	–5.24
14	**3b3c3c3c–**	6.93 ± 0.94	62.6	–5.23
15	**3c3c3c3c–**	9.66 ± 0.38	59.4	–5.43
16	**reference**[a]	1.15 ± 0.14	–	–4.17

[a] Data for the Fmoc-glycine-Wang-resin listed as reference for K_{ass} and ΔG_{298} in acetone.

high number of aromatic units fix the dyes better than sequences with a low content of aromatic units. However, different sequences with the same number of aromatic units still differ significantly in their complexation ability. It is difficult to rationalize the selectivity – the role of the solid polystyrene support is unknown and the orientation of the π-surfaces and the hydrogen bonds to the azo dye is presumably important. All these interactions can be modulated by the accessible conformations of the oligoamide. Such conformational effects are probably enhanced if the α- and γ-peptide backbones in **1** and **2** adopt defined secondary structures usually expected in longer peptide chains. It is interesting to note in this context, that defined helical structures have been found in β- and γ-peptides even at relatively short sequence lengths [7].

Lysine and glutamine derivatives have been used in the past to construct peptide dendrimers [8], to orient helical nanotubes [9], to alter and modulate biochemical functions [10], or to demonstrate the principle of uniform, pulse counting protecting groups [11]. The purpose of the present work is different: The building principle of biological receptors is used to construct weak but selective receptors for small organic molecules. Stronger binding and higher selectivity for molecular guests may be found in more rigid oligoamides. Systems based on amino steroids, amino carbohydrates or biaryl-"amino acids" as well as oligoamides of the type **1** or **2** equipped with more strong binding functions are currently investigated.

Experimental Section

Measurements of the association constants, K

The association of the dye on the bead surface was modeled as an 1/1 complex of the dye on the peptide parts of the bead surface. With *P* (conc. of uncomplexed peptides on the bead surface), *C* (conc. of the dye in solution) and *PC* (formal conc. of the dye peptide complex on the surface) the association equilibrium was written as $P + C \leftrightarrow PC$. The measured data were *C* (by UV/VIS), C° (*C* before equilibration) and P° (*P* before equilibration, calculated from the weight of the resin). Hence the equilibrium constant *K* can be expressed as:

$$K = (C^\circ - C)/((P^\circ - (C^\circ - C))*C)$$

or reformulated to:

$$C = -(P^\circ - C^\circ + 1/K)/2 + \mathrm{sqrt}(((P^\circ - C^\circ + 1/K)**2)/4 + C^\circ/K)$$

Estimates of *K* were obtained by a non linear regression of the data sets *C*, C° and P° [12]. The dye concentration C° varied between $5\cdot10^{-5}$ and $15\cdot10^{-5}$ mol/l; beads of weight 0.1–0.3 mg were used in acetone solution (1.5 ml).

General synthetic procedure a) (see Scheme 2)

Fmoc–LysOH **5** (1.00 g, 2.7 mmol) was dissolved in a mixture of dioxane (11 ml) and 25% aqueous potassium carbonate (11 ml). The carboxylic acid chloride (2.7 mmol) dissolved in dioxane was added dropwise. The mixture was stirred for 2 days, diluted with twice the amount of water, extracted with *tert*-butylmethylether and acidified to pH = 4–5 with HCl. The mixture was extracted with chloroform, the organic phase dried over sodium sulfate and the solvents were removed in vacuo.

General synthetic procedure b) (see Scheme 2)

An excess of the amine component (9 mmol) dissolved in 15 ml ethyl acetate was added to ca. 500 mg (1.7 mmol) of **6** [2b], dissolved in dry diethyl ether (20 ml). The mixture was heated to reflux for 4 h and stirred for 12 h at r.t. The solvent was removed in vacuo, the remaining oil was taken up in ethyl acetate, the formed white precipitate was washed with ethyl acetate, dried in vacuo and used in the following step (c) .

General synthetic procedure c) (see Scheme 2)

The precipitate obtained in step b) was dissolved in 25% aqueous potassium carbonate solution (25 ml). The solution was cooled to 0 °C and an equimolar amount of Fmoc–Cl dissolved in dioxane (15 ml) was added. The mixture was stirred for 4 h, 90 ml of cold water are added and the solution was extracted with diethyl ether (150 ml). The aqueous phase was acidified with $NaHSO_4$ to pH = 2 and extracted with ethyl acetate (300 ml). The organic layer was dried (Na_2SO_4), the solvent was removed in vacuo and the remaining solid was used without further purification.

(L)-N_α-Fmoc-N_ε-(1-adamantyl-carbonyl)-lysine (**3a**)

Procedure a), yield: 1.21 g (84%), white powder. M.p. 152 °C. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.17 (s, 1 H, CO_2H), 7.28 (t, 1 H, ε-NH Lys),

7.88, 7.71, 7.41, 7.32 (m, 8 H, ar.-H (Fmoc)), 7.55 (d, 1 H, α-NH Lys), 4.25–4.20 (m, 3 H, CH–CH_2 (Fmoc)), 3.89 (m, 1 H, Lys-α), 3.01 (m, 2 H, Lys-ε), 1.78–1.62 (m, 17 H, Lys-β and Ada.), 1.37 (m, 2 H, Lys-δ), 1.29 (m, 2 H, Lys-γ). – High-resolution MS (FAB, *p*-nitrobenzyl alcohol): *m/z* = 531.28520 [$(M+H)^+$], calcd. 531.28590 ($C_{32}H_{39}N_2O_5$). – $C_{32}H_{38}N_2O_5$(0.5 $C_4H_8O_2$, 0.5 H_2O): calcd. C 69.96, H 7.42, N 4.80; found: C 69.55, H 7.28, N 4.79.

[L]-N_α-Fmoc-N_ε-(1-naphthyl-carbonyl)-lysine (**3b**)

Procedure a), yield: 1.16 g (82%), white powder. M.p. 163 °C. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.31 (s, 1 H, CO_2H), 8.48 (t, 1 H, ε-NH Lys), 8.18, 7.94, 7.47–7.60 (m, 7 H, Naphth.), 7.88, 7.71, 7.40, 7.31 (m, 8 H, ar.-H (Fmoc)), 7.60 (d, 1 H, α-NH Lys), 4.27–4.21 (m, 3 H, CH–CH_2 (Fmoc)), 3.95 (m, 1 H, Lys-α), 3.32 (m, 2 H, Lys-ε), 1.73 (m, 2 H, Lys-β), 1.58 (m, 2 H, Lys-δ), 1.45 (m, 2 H, Lys-γ). – $[\alpha]_D$ = +4.7° (c = 0.1, methanol). – High-resolution MS (FAB, *p*-nitrobenzyl alcohol): *m/z* = 523.22390 [$(M+H)^+$], calcd. 523.22330 ($C_{32}H_{31}N_2O_5$). – $C_{32}H_{30}N_2O_5$(0.25 $C_4H_8O_2$): calcd. C 72.78, H 5.92, N 5.14; found: C 72.80, H 6.11, N 5.02.

[4S]-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-5-(piperidin-1-yl)-5-oxo-pentanoic acid (**4a**)

Yields: procedure b) 52%, procedure c) 88%. M.p. 105 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66, 7.51, 7.30, 7.20 (m, 8 H, ar.-H), 6.12 (d, 1 H, NH–Glu), 4.64 (1 H, Glu-α), 4.27, 4.11 (m, 3 H, CH–CH_2 (Fmoc)), 3.43 (m, 4 H, pip.), 2,40 (m, 2 H, Glu-γ), 2.00, 1.75 (m, 2 H, Glu-β), 1.53–1.51 (m, 6 H, pip.). – $[\alpha]_D$ = +3.9° (c = 0.1, $CHCl_3$). – High-resolution MS (FAB, *p*-nitrobenzyl alcohol): *m/z* = 437.20760 [$(M+H)^+$], calcd. 437.20764 ($C_{25}H_{29}N_2O_5$).

[4S]-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-5-(morpholin-4-yl)-5-oxo-pentanoic acid (**4b**)

Yields: procedure b) 71%, procedure c) 74%. M.p. 153 °C. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.17 (s, 1 H, CO_2H), 7.94, 7.77, 7.46, 7.37 (m, 8 H, ar.-H), 7.65 (d, 1 H, NH–Glu), 4.53 (m, 1 H, Glu-α), 4.32–4.27 (m, 3 H, CH–CH_2 (Fmoc)), 3.7–3.4 (m, 8 H, morph.), 2,36 (m, 2 H, Glu-γ), 1.88–1.77 (m, 2 H, Glu-β). – $[\alpha]_D$ = +10.5° (c = 0.1, $CHCl_3$). – High-resolution MS (FAB, *p*-nitrobenzyl alcohol): *m/z* = 439.18780 [$(M+H)^+$], calcd. 439.18691 ($C_{24}H_{27}N_2O_6$). – $C_{24}H_{26}N_2O_6$(1.5 H_2O): calcd. C 61.93, H 6.28, N 6.02; found: C 62.29, H 6.06, N 5.78.

[4S]-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-5-(4-phenyl-piperazin-1-yl)-5-oxo-pentanoic acid (**4c**)

Yields: procedure b) 61%, procedure c) 80%. M.p. 95 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72–6.80 (m, 13 H, ar.-H), 6.11 (d, 1 H, NH-Glu), 4.84 (m, 1 H, Glu-α), 4.35–4.18 (m, 3 H, CH–CH_2 (Fmoc)), 3.75, 3.14 (2 m, 8 H, pip.), 2,45 (m, 2 H, Glu-γ), 2.06–1.79 (m, 2 H, Glu-β). – $[\alpha]_D$ = +11.6° (c = 0.1, $CHCl_3$). – MS (FAB): *m/z* (%) = 514(25) ($[M+H]^+$). – High-resolution MS (FAB, *p*-nitrobenzyl alcohol): m/z = 514.23270 [$(M+H)^+$], calcd. 514.23419 ($C_{30}H_{32}N_3O_5$). – $C_{30}H_{31}N_3O_5$ (1 H_2O): calcd. C 67.78, H 6.26, N 7.90; found: C 67.29, H 6.06, N 7.89.

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[6] a) A selfconstructed parallel synthesizer with a teflon reactor block, syphon technique, vortex mixing and a pipette robot has been used for the parallel synthesis of the libraries **1** and **2**. Approximately one third of the sequences were checked by MALDI-TOF MS; the expected molecular mass was found in all cases; b) The beads of the parallel syntheses were placed in 81 wells drilled into a frit. The frit was covered with an additional plain frit to allow washing by diving. After each washing procedure (ca. 10 minutes), the solvent was removed by suction.

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