Tetrahedron 65 (2009) 690–695

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Modular synthesis of di- and tripeptides of luminescent crown ether aminocarboxylic acids

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ARTICLE INFO

Article history:
Received 14 July 2008
Received in revised form 19 October 2008
Accepted 27 October 2008
Available online 31 October 2008

ABSTRACT

Luminescent benzo crown ether aminocarboxylic acids with ammonium ion affinity were prepared and converted into linear bis- and tris benzo crown ether amides using standard peptide coupling protocols. The affinities of the new crown ethers to ammonium ions and di- and tetrapeptides bearing ammonium ion moieties were determined by emission titration in methanol and buffered water.

1. Introduction

Many biologically important molecules have ammonium ions as functional groups. Typical examples are neutrotransmitters,[1,2] growth factors,[3] histamine[2,4] or peptides.[5] The development of synthetic receptors that allow the specific recognition of organic ammonium ions is therefore an area of ongoing interest and results have potential applications in medicinal diagnostics or chemosensors. Typical artificial binding sites for ammonium ions are crown ethers,[6] calixarenes,[7] phosphonate[8]—and oxazoline-based[9] receptors and porphyrine metal complexes; most examples have been reported for crown ether systems.[10] However, for many cases the binding strengths of a single ammonium ion–receptor site interactions are too weak in an aqueous environment to give stable and defined aggregates. The combination of several binding sites in one synthetic receptor can improve the situation, if their individual contributions are additive or even show positive cooperativity.

Several examples of oligovalent artificial cation receptors based on crown ethers have been reported: bis- and tris-crown ethers as host compounds for alkaline metal ions have been published by Huang,[11] and a carrier molecule composed of three crown ethers was used to mediate the transport of mercury ions through a chloroform phase.[12] Nolte[13] polymerized an isocyanide derivative of benzo-18-crown-6 and applied the polymer to liposome mediated copper-ion transport. Oligo- or poly[14] crown ethers have been used as chemical model systems for ion channels. A poly(crown) channel was constructed using the benzo-21-crown-7 derivative of phenylalanine and leucine.[15] Incorporation of the resulting helical peptide into a lipid bilayer leads to proton and sodium ion transport through a columnar crown ether stack. Gokel[16] developed a family of tris-macrocycles that permits the transport of sodium ions through membranes, using crown ethers, connected by hydrophobic alkane spacers, as channel head groups and cation entry portals.

For ammonium ion binding, only ditopic receptors have been reported so far. Phenolphtaleine bis-crown ethers have been used for the binding of diamines and dipeptides with a C-terminal Lys.[17] Two crown ether aminocarboxylic acids, which were incorporated into an oligo Ala peptide were used by Voyer[18] to bind α,ω-diammonium alkanes. We have recently reported the synthesis and dimerization of ammonium ion binding phthalimide crown ethers.[19] We now extend the approach and present here a modular synthetic route to functionalized, luminescent bis- and tris-crown ethers with ammonium ion affinity.

2. Results and discussion

2.1. Synthesis

The twofold ring closing substitution reaction of di-tosylate **1**[19] with primary amines **2** under basic conditions yields crown ether aminocarboxylic acids **3** in good yields (Scheme 1, Table 1). The presence of potassium ions favours the ring closing reaction by its template effect. No by-products from intermolecular reactions were observed under the experimental conditions (c=6×10^{-2} mol/L). Alkyne or azide functional groups (entries **g**, **h**) are tolerated and the resulting products are suitable substrates for Huisgen cycloaddition reactions. The reaction tolerates cbz- or Boc-protected amines (entries **a**–**d**).

The Boc-deprotection of compound **3a** and the ester hydrolysis of compound **3a** or **3e** allows the preparation of crown ether aminocarboxylic acid dipeptides **6**[20] in good yield using standard peptide coupling conditions (Scheme 2). A further extension to

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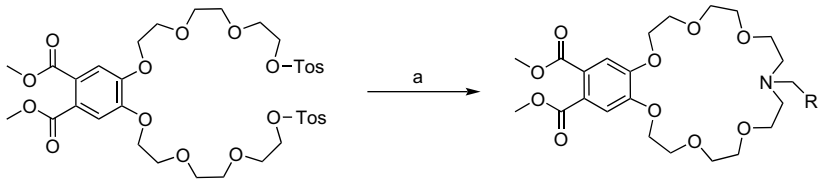
doi:10.1016/j.tet.2008.10.086



1 3

Scheme 1. Synthesis of a luminescent benzo azacrown ethers **3**: (a) H_2NCH_2R (**2**, see Table 1), K_2CO_3, KI, MeCN, H_2O, 80 °C, 63–86%.

Table 1
Synthesis of compounds **3a–i**

Entry	R	Yield **3** [%]
a[19]	NHBoc	79
b	NHCbz	68
c	NHBoc	72
d	NHBoc	63
e		86
f	NHBoc	76
g[a]	H	71
h[a]	N_3	69
i		78

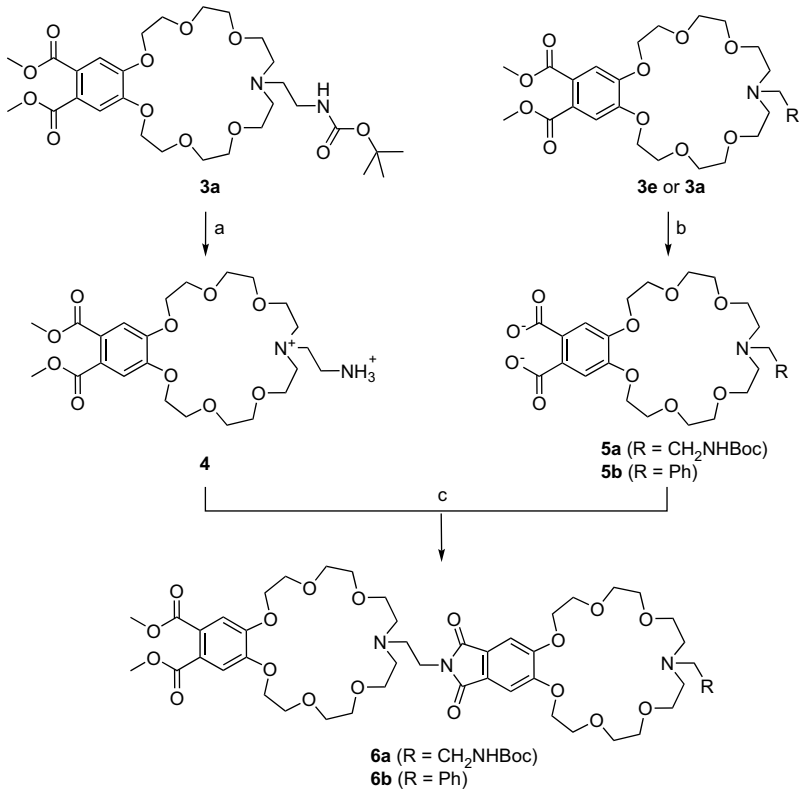
[a] In the dark.

compounds **7**, bearing three crown ether units, is possible by removal of the Boc-protecting group of **6a** yielding amine **6c**, and coupling with **5a** or **5e**, again, using standard peptide coupling conditions (Scheme 3). The purification of tripeptides **7** requires repeated column chromatography to remove by-products and reagents.

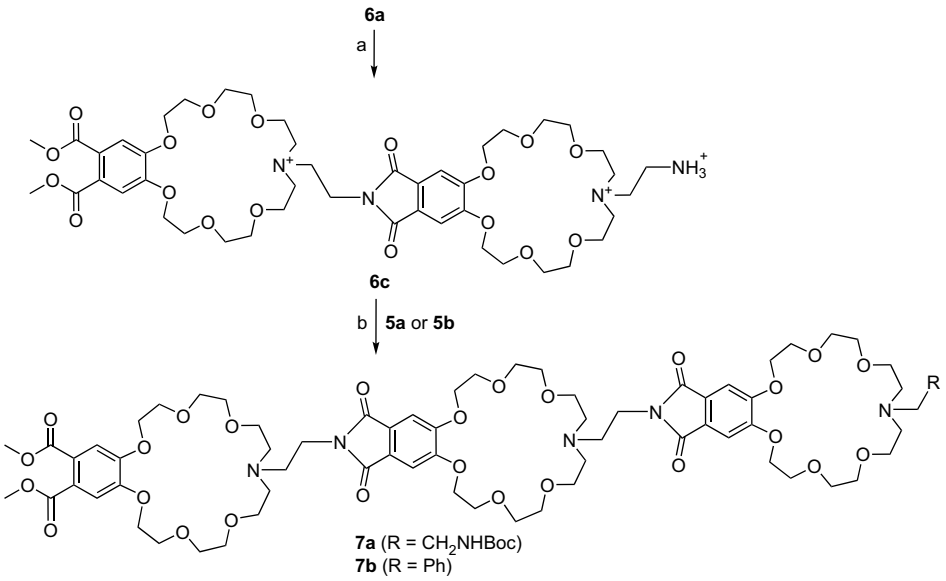
2.2. Photophysical properties

Compounds **3** show absorption maxima in methanol at 220 nm and 270 nm, and emit upon excitation at 390 nm with a quantum yield of about φ=0.1.[21] The absorption and emission properties are only marginally affected by the nature of the substituent R. Upon addition of KSCN or *n*-butyl ammonium chloride the emission intensity increases significantly and binding affinities were derived from emission titration experiments. Table 2 summarizes the results. The affinity for potassium ions is typically one order of magnitude larger than the ammonium ion binding, which is in accordance with earlier reports.[19,22] The cation affinity for all crown ethers **3** is very similar in methanol solution. In buffered aqueous solution (50 mM HEPES, pH 7.5; $c=2\times10^{-5}$ mol/L) no change in the emission intensity was observed, even if a large excess of KSCN or butyl ammonium chloride was used.

Upon the addition of the second crown ether moiety, as in compound **6**, the phthalic amide fluorophore is created and a new



Scheme 2. Preparation of crown ether aminocarboxylic acid dipeptides **6a** and **6b**: (a) HCl in Et_2O, DCM, 95%; (b) NaOH 1 M, MeOH, H_2O, 40 °C, quant.; (c) EDC, HOBt, DIPEA, DMF, $CHCl_3$, 70 °C, 79% (**6a**);[19] 76% (**6b**).



Scheme 3. Synthesis of crown ether aminocarboxylic acid tripeptides **7a** and **7b**: (a) HCl in Et_2O, DCM, 92%; (b) EDC, HOBt, DIPEA, DMF, $CHCl_3$, 70 °C, 35% (**7a**); 37% (**7b**).

Table 2
Binding affinities and emission intensity changes of compounds **3** and *n*-butyl ammonium chloride or KSCN in methanol

Compound	*n*-Butyl ammonium chloride		KSCN	
	log *K*	I_∞/I_0	log *K*	I_∞/I_0
3a	2.8±0.3	1.7	3.9±0.4	3.5
3b	2.8±0.2	1.7	3.8±0.3	3.5
3e	2.8±0.1	1.9	3.9±0.3	3.6
3d	2.7±0.2	1.8	3.8±0.4	3.4
3f	2.7±0.3	1.7	3.6±0.3	3.4
3g	2.6±0.2	1.6	—	—
3i	3.2±0.3	2.1	4.0±0.4	3.7

absorption band at 247 nm appears. The tris-crown ether compounds **7** bear two phthalimide chromophores, which results in a significant increase of the UV absorption intensity at 247 nm if compared to **6**. Excitation of the fluorophores at 309 nm stimulates emission at 386 nm with a shoulder at 488 nm (Fig. 1).

The binding properties of bis-crown ether **6b** to *n*-butyl ammonium chloride and lysine methyl ester hydrochloride were evaluated by fluorescence titration in methanol and buffered aqueous solution (50 mM HEPES, pH 7.5). The different optical properties of the phthalic ester and the phthalimide moiety of **6b** allow the individual monitoring of binding to the two-crown ether cation binding sites. Ammonium ions bind to both crown ethers in methanol with a stoichiometry of 1:1 and similar affinity in the order of log *K*=2. In aqueous solution no interaction between **6b** and ammonium ions can be detected. The affinity of lysine methyl ester hydrochloride reaches log *K* ~4 in methanol, due to the simultaneous interaction of both ammonium ions with the bis-crown compound. In buffered aqueous solution the interaction of Lys–HCl–**6b** is detectable via the phthalic ester emission change, but drops to log *K*<2. The emission of the phthalimide moiety is quenched in aqueous solution (Table 3).

Next, the trimeric crown ethers **7** were investigated for ammonium ion binding (Table 4). As expected, the affinity for ammonium ions in methanol solution of the crown ether moieties remain unchanged if compared to **6**. Lysyllysine methyl ester trihydrochloride was chosen as a guest with three ammonium groups to determine the binding to compound **7**. Although the aggregate of **7** and lysyllysine is potentially stabilized by three simultaneous ammonium ions–crown ether interactions, the determined affinity constants in methanol and buffered water only slightly exceed the values for the interaction of bis-crown ether **6** with a diammonium

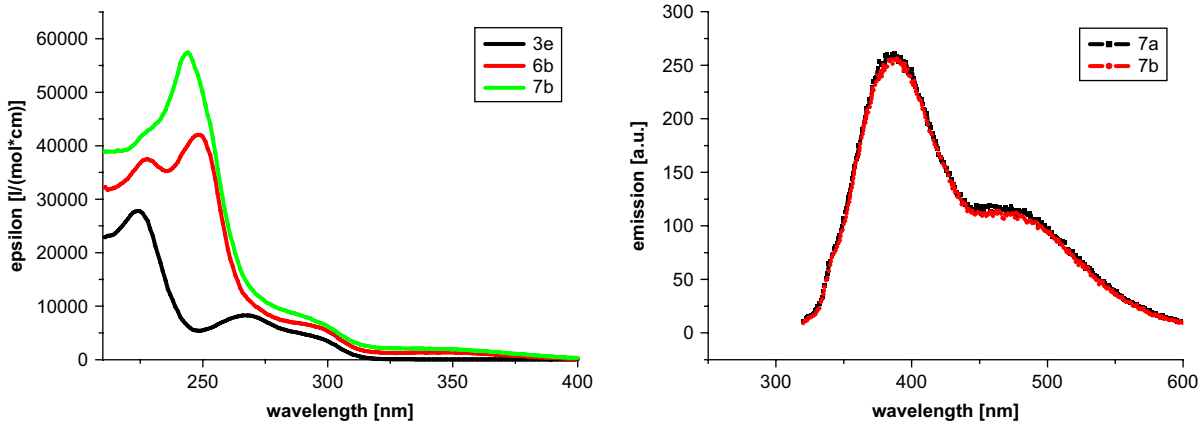


Figure 1. Absorption spectra of compounds **3e**, **6b**, and **7b** (in methanol, left) and emission spectra of compounds **7a** and **7b** (methanol, $c=2\times10^{-5}$ mol/L, excitation at 309 nm, right).

Table 3
Binding affinity and emission enhancement of compound **6b** in the presence of *n*-butyl ammonium chloride and lysine methyl ester hydrochloride in methanol and buffered aqueous solution

Solvent	Guest	Phthalic ester moiety of **6b**		Phthalimide moiety of **6b**	
		log *K*	I_∞/I_0	log *K*	I_∞/I_0
Methanol	Butyl ammonium chloride	2.2±0.4	1.3	2.6±0.3	4.7
	Lysine methyl ester hydrochloride	4.3±0.3	1.6	4.6±0.2	8.1
H_2O, HEPES (50 mM, pH 7.5, 2% MeOH)	Lysine methyl ester hydrochloride	1.8±0.2	1.7	—	—

compound. The differences of lysyllysine binding affinities of **7a** and **7b** are within the error margins of the measurements. For both interactions a stoichiometry of 1:1 is indicated by Job's plot analysis (see supporting information).

Although the extension of the ammonium ion receptor by one crown ether from **6** to **7** had little impact on the ammonium binding affinity, the additional chromophore leads to a stronger emission in the presence of ammonium ions, which becomes visible by the naked eye. In addition, the ability of compounds **7** to bind ammonium ions within small peptides was investigated using isomeric tetrapeptides bearing two lysines (K) and two glycines (G) in their sequence. H-K-K-G-G–NH_2 carrying two lysines at the N-terminus has the same ammonium ion pattern as H-K-K–OMe. In H-K-G-K-G–NH_2 one glycine and in H-K-G-G-K–NH_2 two glycines separate the lysines. All peptides were prepared on Rink amide resin (see Supplementary data) and their binding affinities as trihydrotriflates to compounds **7** were determined by titration in methanol and buffered water (see Supplementary data for data). The derived affinities are, within the errors of the experiments, identical to the values obtained for H-K-K–OMe.

In summary, luminescent aza benzo crown ethers and crown ether aminocarboxylic acids are available by an efficient ring closing reaction of bis-tosylates and amines. Using standard peptide coupling procedures, linear bis- and tris-crown ether peptides were obtained. Emission titrations in methanol or buffered aqueous solution revealed affinities of ammonium ions to the benzo crown ether moieties of approx. log *K*=2 in methanol, but no affinity in buffered water. The interaction of bis-crown ethers **6** and tris-crown ethers **7** with bis- or tris-ammonium ions, respectively, is of similar strengths in methanol (log *K* ~4.5) and in buffered water (log *K* ~2.5). This shows that the extension of bis- to tris-crown ethers does not lead to a significant increase of binding affinities. The flexible structure of the extended crown ethers and their peptidic guest molecules is a likely rational for the observation: the limited preorganization of the extended receptors binding sites prohibits an additive or cooperative action of the intermolecular interactions, and illustrates the importance of well balanced entropy and enthalpy contributions in the design of synthetic receptors.

Table 4
Affinity constants and emission enhancement of compounds **7** in the presence of *n*-butyl ammonium chloride and lysyllysine methyl ester in protic solvents

Solvent	Compound	Guest	Phthalic ester		Phthalimide	
			log *K*	I_∞/I_0	log *K*	I_∞/I_0
Methanol	**7a**	Butyl ammonium chloride	2.2±0.4	1.2	2.1±0.2	4.5
Methanol	**7a**	Lysyllysine methyl ester hydrochloride	4.6±0.5	2.6	4.4±0.1	6.8
H_2O, HEPES (50 mM, pH 7.5, 2% MeOH)	**7a**	Lysyllysine methyl ester hydrochloride	2.6±0.3	1.4	—	—
Methanol	**7b**	Butyl ammonium chloride	2.0±0.3	1.3	1.9±0.2	4.6
Methanol	**7b**	Lysyllysine methyl ester hydrochloride	4.2±0.3	2.0	4.3±0.1	6.8
H_2O, HEPES (50 mM, pH 7.5, 2% MeOH)	**7b**	Lysyllysine methyl ester hydrochloride	2.8±0.3	1.4	—	—

3. Experimental

3.1. General method for the preparation of aza-benzo-21-crown-7-ethers

Compound **1** (2 g, 2.5 mmol)[19] was dissolved in 40 mL of acetonitrile and 0.3 mL of H_2O. The corresponding amine or mono Boc-protected diamine (2.5 mmol), 610 mg (3.6 mmol) KI and 3.45 g (25 mmol) K_2CO_3 were added successively and the mixture was refluxed over night. After cooling to room temperature, the mixture was filtered over Celite and the solid residue washed with acetonitrile and dichloromethane. The solvent was evaporated and the crude product was purified by column chromatography. If not stated differently, silica gel, and ethyl acetate/ethanol; 3:1, as eluent was used.

The molecular structures for the assigned NMR data sets are given in the Supplementary data to this article.

3.2. 14-[3-(*tert*-Butyl)oxycarbonylamino-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12*H*-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (3c)

3-(*tert*-Butyl)oxycarbonylamino-propyl-amine (435 mg) was reacted according to the general procedure to yield product **3c** (910 mg, 58%) as a clear, yellow oil (R_f=0.08). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.33 (s, 9H, s), 1.57 (m, 2H, n), 2.51 (m, 2H, m), 2.65 (br s, 4H, l), 3.12 (m, 2H, o), 3.55–3.57 (m, 4H, k), 3.61–3.65 (m, 4H, i), 3.70–3.76 (m, 4H, h), 3.81 (s, 6H, a), 3.84–3.90 (m, 4H, g), 4.12–4.19 (m, 4H, f), 5.43 (br s, 1H, p), 7.15 (s, 2H, d); ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.6 (−, 1C, o), 28.5 (+, 3C, s), 38.3 (−, 1C, n), 52.5 (+, 2C, a), 53.2 (−, 1C, m), 53.9 (−, 2C, l), 68.9 (−, 2C, k), 69.4 (−, 4C, 6, g), 70.3 (−, 2C, i), 70.7 (−, 4C, h), 78.8 (C_{quat}, 1C, r), 114.0 (+, 2C, d), 125.7 (C_{quat}, 2C, c), 150.4 (C_{quat}, 2C, e), 156.3 (C_{quat}, 1C, q), 167.7 (C_{quat}, 2C, b); IR (KBr): ν (cm^{-1})=3393 (br m), 2939 (m), 2874 (m), 1708 (m), 1593 (m), 1517 (m), 1435 (m), 1352 (m), 1286 (m), 1250 (m), 1186 (m), 1126 (m), 1050 (m), 978 (m), 940 (m), 785 (m); UV (MeOH): λ (ε)=267 (7700), 224 (24,700); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=629.6 (100, MH^+); $C_{30}H_{48}N_2O_{12}$: calcd C 57.3, H 7.7, N 4.5, found: C 57.1, H 7.5, H 4.2.

3.3. 14-[4-(*tert*-Butyl)oxycarbonylamino-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12*H*-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (3d)

4-(*tert*-Butyl)oxycarbonylamino-butyl-amine (470 mg) was used as reactant in the procedure. A clear, yellow oil (847 mg, 53%) is obtained (R_f=0.05). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.40 (s, 9H, t), 1.44 (br s, 4H, o, p), 2.50 (m, 2H, m), 2.74 (m, 4H, l), 3.06 (m, 2H, n), 3.56–3.58 (m, 4H, k), 3.61–3.63 (m, 4H, i), 3.72–3.74 (m, 4H, h), 3.84 (s, 6H, a), 3.88–3.89 (m, 4H, g), 4.18–4.19 (m, 4H, f), 4.90 (br s, 1H, q), 7.17 (s, 2H, d); ^{13}C NMR (75 MHz, $CDCl_3$): δ=24.1 (−, 1C, o), 27.7 (−, 1C, p), 28.4 (+, 3C, t), 40.3 (−, 1C, n), 52.5 (+, 2C, a), 53.8 (−, 1C, m), 55.0 (−, 2C, l), 69.3 (−, 2C, k), 69.5 (−, 2C, f), 69.5 (−, 2C, g), 70.6 (−, 4C, i), 71.1 (−, 4C, h), 78.8 (C_{quat}, 1C, s), 113.7 (+, 2C, d), 125.4 (C_{quat}, 2C, c), 150.5 (C_{quat}, 2C, e), 156.0 (C_{quat}, 1C, r), 167.7 (C_{quat}, 2C, b); IR

(KBr): ν (cm^{-1})=3375 (br m), 2936 (m), 2870 (m), 1715 (m), 1589 (m), 1519 (m), 1436 (m), 1353 (m), 1284 (m), 1185 (m), 1126 (m), 1053 (m), 979 (m), 945 (m), 785 (m); UV (MeOH): λ (ε)=267 (8000), 224 (25,200); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=643.6 (100, MH^+); $C_{31}H_{50}N_2O_{12}$: calcd C 57.9, H 7.8, N 4.4, found: C 57.7, H 7.6, H 4.2.

3.4. 14-Propargyl-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12*H*-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (3g)

Propargylamine (140 mg) was allowed to react according to the general procedure. The reaction was performed under nitrogen atmosphere and protection from light. The product **3g** was obtained as a clear, yellow oil (910 mg, 71%, R_f=0.17). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.09 (m, 1H, o), 2.75 (m, 4H, l), 3.45 (m, 2H, m), 3.55–3.58 (m, 4H, k), 3.61–3.65 (m, 4H, i), 3.68–3.74 (m, 4H, h), 3.80 (s, 6H, a), 3.84–3.88 (m, 4H, g), 4.12–4.17 (m, 4H, f), 7.12 (s, 2H, d); ^{13}C NMR (75 MHz, $CDCl_3$): δ=31.1 (+, 1C, o), 43.3 (−, 1C, m), 52.5 (+, 2C, a), 53.3 (−, 1C, l), 69.4 (−, 2C, k), 69.5 (−, 2C, f), 70.6 (−, 4C, 9, g), 71.2 (−, 4C, h), 73.0 (C_{quat}, 1C, n), 113.5 (+, 2C, d), 125.3 (C_{quat}, 2C, c), 150.5 (C_{quat}, 2C, e), 167.8 (C_{quat}, 2C, b); IR (KBr): $\bar{\nu}$ (cm^{-1})=3310 (s), 2976 (m), 2880 (m), 1721 (s), 1599 (m), 1516 (m), 1438 (m), 1348 (m), 1282 (s), 1194 (m), 1123 (m), 1053 (m), 981 (m), 943 (m); UV (MeOH): λ (ε)=267 (7400), 224 (26,800); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=510.3 (100, MH^+); $C_{25}H_{35}NO_{10}$: calcd C 58.9, H 6.9, N 2.8, found: C 58.6, H 6.6, N 2.6.

3.5. 14-[2-Azido-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12*H*-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (3h)

2-Azido-ethyl-amine hydrobromide (250 mg) was added as amine component, nitrogen atmosphere was used. Column chromatography was performed with chloroform and methanol in a 10:1 ratio. A clear, yellow glass (934 mg, 69%) was obtained (R_f=0.42). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.76 (t, 2H, *J*=6.8 Hz, m), 2.80 (m, 4H, l), 3.26 (t, 2H, *J*=6.8 Hz, n), 3.59 (t, 4H, *J*=5.6 Hz, k), 3.66–3.69 (m, 4H, i), 3.72–3.74 (m, 4H, h), 3.86 (s, 6H, a), 3.91 (t, 4H, *J*=5.6 Hz, g), 4.20 (t, 4H, *J*=5.6 Hz, 6), 7.19 (s, 2H, d); ^{13}C NMR (75 MHz, $CDCl_3$): δ=49.4 (−, 1C, n), 52.6 (+, 2C, a), 54.5 (−, 2C, l), 54.7 (−, 1C, m), 69.3 (−, 2C, k), 69.5 (−, 2C, f), 70.0 (−, 2C, g), 70.7 (−, 4C, i), 71.2 (−, 4C, h), 113.6 (+, 2C, d), 125.3 (C_{quat}, 2C, c), 150.5 (C_{quat}, 2C, e), 167.8 (C_{quat}, 2C, b); IR (KBr): $\bar{\nu}$ (cm^{-1})=2971 (m), 2876 (m), 1719 (s), 1596 (m), 1521 (m), 1438 (m), 1348 (m), 1282 (s), 1195 (m), 1123 (m), 1049 (m), 983 (m), 942 (m); UV (MeOH): λ (ε)=267 (7800), 224 (27,300); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=541.4 (100, MH^+), 513.4 (11, $(M-N_2)H^+$); $C_{24}H_{36}N_4O_{10}$: calcd C 53.3, H 6.7, N 10.4, found: C 52.8, H 6.3, N 9.9.

3.6. Compound 6b

A solution of **4** (144 mg, 0.25 mmol) in chloroform (10 mL) containing *N*-hydroxybenzotriazol (HOBt) (68 mg, 0.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (79 mg, 0.5 mmol) was stirred for 15 min at 0 °C. A mixture of **5b** (141 mg, 0.25 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (104 mg, 0.80 mmol) in chloroform (10 mL) was added dropwise under cooling. The mixture was stirred 3 h at ambient temperature and then refluxed over night. After cooling, the suspension was filtered over Celite and the filter cake was washed with chloroform. The solvent was removed under reduced pressure and the residue was purified by column chromatography (chloroform/methanol, 6:1) to give a yellow glassy solid (193 mg, 76%, R_f ($CHCl_3$/MeOH, 6:1)=0.44). ^{1}H NMR (600 MHz, $CDCl_3$): δ=2.76–2.84 (m, 10H, l, m, l′), 3.55 (t, 4H, *J*=5.30 Hz, k′), 3.59–3.64 (m, 8H, i, k), 3.65–3.75 (m, 10H, h, n, i′), 3.78 (t, 4H, *J*=4.80 Hz, h′), 3.86 (s, 6H, a), 3.90 (t, 4H, *J*=4.56 Hz, g), 3.95 (t, 4H, *J*=4.56 Hz, g′), 4.19 (t, 4H, *J*=4.56 Hz, f), 4.23 (t, 4H, *J*=4.56 Hz, f′), 7.17 (s, 4H, d), 7.21–7.28 (m, 5H, o′, p′, q′), 7.33 (br s, 2H, d′); ^{13}C NMR (150 MHz, $CDCl_3$): δ=36.1 (−, 1C, n), 52.5 (+, 2C, a), 52.9 (−, 1C, m), 53.8 (−, 2C, l′), 54.3 (−, 2C, l), 59.8 (−, 1C, m′), 69.3 (−, 2C, f), 69.4 (−, 2C, g′), 69.5 (−, 2C, g), 69.6 (−, 2C, f′), 70.2 (−, 2C, k), 70.7 (−, 6C, i, i′, k′), 71.1 (−, 2C, h), 71.2 (−, 2C, h′), 107.0 (+, 2C, d′), 113.7 (+, 2C, d), 125.3 (C_{quat}, 2C, c′), 125.6 (C_{quat}, 2C, c), 126.9 (C_{quat}, 1C, n′), 128.2 (+, 3C, p′, q′), 128.9 (+, 2C, o′), 150.5 (C_{quat}, 2C, e), 153.4 (C_{quat}, 2C, e′), 167.8 (C_{quat}, 2C, b′), 168.4 (C_{quat}, 2C, b); IR (NaCl): $\bar{\nu}$ (cm^{-1})=3400 (br w), 2930 (m), 2880 (m), 1707 (s), 1599 (m), 1510 (m), 1436 (m), 1393 (m), 1352 (m), 1291 (s), 1196 (m), 1129 (s), 1056 (m), 980 (w), 945 (w); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=506.8 (100, $(M+2H^+)^{2+}$), 1012.6 (8, MH^+); UV (MeOH): λ (ε)=340 (1200), 248 (43,100), 227 (37,400); HRMS (PI-LSIMS, MeOH/glycerin): calcd for $C_{51}H_{68}N_3O_{18}\cdot H^+$: 1011.4576, found: 1011.4558.

3.7. Compound 7a

A solution of **5a** (132 mg, 0.24 mmol) in chloroform (10 mL) containing *N*-hydroxybenzotriazol (HOBt) (55 mg, 0.40 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (63 mg, 0.40 mmol) was stirred under nitrogen atmosphere for 30 min at 0 °C. A mixture of **6c** (215 mg, 0.2 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (156 mg, 1.20 mmol) in chloroform (10 mL) was added dropwise under cooling. The mixture was stirred 2 h under nitrogen at ambient temperature and refluxed over night. After cooling to room temperature the solution was washed with brine (3×10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by repeated column chromatography (chloroform/methanol, 6:1; 8:1→5:1, 6:1→5:1) yielding a yellow glass (106 mg, 35%). [R_f ($CHCl_3$/MeOH, 6:1)=0.4]. ^{1}H NMR (600 MHz, $CDCl_3$): δ=1.41 (s, 9H, r″), 2.68–2.93 (m, 14H, l, l′, l″, m″), 3.25 (m, 2H, n″), 3.48–3.80 (m, 40H, k, m, n, o, m′, k″), 3.82–3.96 (m, 14H, g, g′, n′, g″), 3.85 (s, 6H, a), 4.12–4.28 (m, 12H, f, f′, f″), 5.64 (br s, 1H), 7.15 (s, 2H, d), 7.19 (m, 2H, d′), 7.24 (br s, 2H, d″); ^{13}C NMR (150 MHz, $CDCl_3$): δ=28.5 (+, 3C, r″), 35.9 (−, 2C, n, n′), 52.5 (+, 2C, a), 52.8 (−, 1C, m), 53.0 (−, 1C, m″), 54.1 (−, 1C, m′), 54.2 (−, 2C, l′), 54.4 (−, 2C, l), 54.6 (−, 2C, l″), 69.1–69.5 (−, 12C, f, g, f′, g′, f″, g″), 70.0 (−, 2C, k), 70.6–70.8 (−, 10C, i, i′, k′, i″, k″), 71.0–71.2 (−, 6C, h, h′, h″), 80.0 (C_{quat}, 1C, q″), 106.7 (+, 4C, d′, d″), 113.7 (+, 2C, d), 125.3 (C_{quat}, 2C, c), 125.4 (C_{quat}, 2C, c′), 125.5 (C_{quat}, 2C, c″), 150.5 (C_{quat}, 2C, e), 153.2 (C_{quat}, 2C, e′), 153.3 (C_{quat}, 2C, e″), 156.2 (C_{quat}, 1C, p″), 167.7 (C_{quat}, 4C, b′, b″), 163.1 (C_{quat}, 2C, b); IR (KBr): (cm^{-1})=3458 (br m), 3018 (m), 2952 (m), 2881 (w), 1677 (s), 1599 (m), 1501 (m), 1438 (s), 1391 (s), 1299 (s), 1178 (s), 1116 (s), 1052 (m), 946 (m), 799 (m), 719 (m), 659 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *e*/*z* (%)=487.3 (63, $(M-t\text{-}Bu+3H^+)^{3+}$), 506.0 (80, $(M+3H^+)^{3+}$), 758.6 (100, $(M+2H^+)^{2+}$), 1516.1 (3, $MH^+)^+$; UV (MeOH): λ (ε)=247 (59,100), 224 (42,600).

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft (GRK 760) and the University of Regensburg for support of this work.

Supplementary data

General experimental procedures, experimental procedures, and spectral data for the synthesis of compounds **3b**, **3e**, **3f**, **3i**, **5b**, **6c**, **7a**, **7b**, of the peptide sequences for testing, as well as ^{1}H and ^{13}C NMR spectra for all new compounds; comparison of *n*-butyl ammonium binding to **6b**, **7a** and **7b**; selected fluorescence titration

curves and Job's plot analysis. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.10.086.

References and notes

1. Bohlen und Halbach, O. v.; Demietzel, R. *Neurotransmitters and Neuroregulators, Handbook of Receptors and Biological Effects*, 2nd ed.; Wiley-VCH: Weinheim, 2006.
2. Brunton, L. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 11th ed.; B&T: New York, NY, 2005.
3. (a) Jiang, W. G.; Matsumoto, K.; Nakamura, T. *Growth Factors and their Receptors in Cancer Metastasis*, 1st ed.; Springer: Netherland, 2001; (b) Nilsen, H. M.; Nilsen-Hamilton, M. *Growth Factors and Signal Transduction in Development*; Wiley-VCH: New York, NY, 1994.
4. Pertz, H. H.; Elz, S.; Schunack, W. *Mini-Rev. Med. Chem.* **2004**, *4*, 935.
5. (a) Kopple, K. D. *Peptides and Amino Acids*; W.A. Benjamin: New York, NY, 1966; (b) Konig, W. *J. Am. Chem. Soc.* **1995**, *117*, 1673; (c) Shafer, W. M. *Antimicrobial Peptides and Human Disease*, 1st ed.; Springer: Berlin, 2006; (d) Varani, G. *Acc. Chem. Res.* **1997**, *30*, 189; (e) Cuozzo, J. W.; Sahagian, G. G. *J. Biol. Chem.* **1994**, *269*, 14490; (f) Andersson, H.; Kappeler, F.; Hauri, H. P. *J. Biol. Chem.* **1999**, *274*, 15080.
6. (a) Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004**, *104*, 2723; (b) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **1995**, *95*, 2529; (c) Bradshaw, J. S.; Izatt, R. M.; Bordunov, A. V.; Zhu, C. Y. *Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, 1996; Vol. 1, p 35; (d) Gokel, G. W.; Abel, E. *Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, 1996; Vol. 1, p 511.
7. (a) Arduini, A.; Demuru, D.; Pochini, A.; Secchi, A. *Chem. Commun.* **2005**, 645; (b) Ito, K.; Ohba, Y. *Trends Heterocycl. Chem.* **2003**, *9*, 117; (c) Ito, K.; Noike, M.; Kida, A.; Ohba, Y. *J. Org. Chem.* **2002**, *67*, 7519; (d) Arena, G.; Casnati, A.; Contino, A.; Lombardo, G.; Sciotto, D.; Ungaro, R. *Chem.—Eur. J.* **1999**, *5*, 738.
8. (a) Werner, M.; Schrader, T.; Finochiaro, P.; Faila, S.; Consiglio, G. *Org. Lett.* **2000**, *2*, 605; (b) Park, C. E.; Jung, Y.-G.; Hong, J.-I. *Tetrahedron Lett.* **1998**, *39*, 2353; (c) Dougherty, D.; Stauffer, D. A. *Science* **1990**, *250*, 1558.
9. Ahn, K. H.; Kim, S. G.; Jung, J.; Kim, K. H.; Kim, J.; Chin, J.; Kim, K. *Chem. Lett.* **2000**, 170.
10. Imai, H.; Misawa, K.; Munakata, H.; Uemori, Y. *Chem. Lett.* **2001**, 688.
11. Huang, Z. B.; Kim, S. H.; Chang, S. H. *Bull. Korean Chem. Soc.* **2006**, *27*, 893.
12. Costero, A. M.; Andreu, C.; Monrabal, E.; Tortajada, A.; Ochando, L. E.; Amigó, J. M. *Tetrahedron* **1996**, *38*, 12499.
13. Roks, M. F. M.; Nolte, R. J. M. *Macromolecules* **1992**, *25*, 5398.
14. Gong, C.; Gibson, H. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 310; Zolotukhin, M. G.; Hernandez, M. C. G.; Lopez, A. M.; Fomina, L.; Cedillo, G.; Nogales, A.; Ezquerra, T.; Rueda, D.; Colquhoun, H. M.; Fromm, K. M.; Ruiz-Trevino, A.; Ree, M. *Macromolecules* **2006**, *39*, 4696; Xu, W. Y.; Roland, B.; Smid, J. *Macromolecules* **1985**, *18*, 2061; Smid, J.; Varma, A. J.; Shah, J. C. *J. Am. Chem. Soc.* **1979**, *101*, 5764.
15. Voyer, N.; Robataille, M. *J. Am. Chem. Soc.* **1995**, *117*, 6599; Voyer, N.; Potvin, L.; Rousseau, E. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1469.
16. Murray, C. L.; Gokel, G. W. *J. Supramol. Chem.* **2001**, *1*, 23; Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7665.
17. Tsubaki, K.; Kusumoto, T.; Hayashi, N.; Nuruzzaman, M.; Kinoshita, T.; Fuji, K. *J. Am. Chem. Soc.* **1999**, *121*, 3807.
18. Voyer, N.; Deschenes, D.; Bernier, J.; Roby. *J. Chem. Soc., Chem. Commun.* **1992**, 664.
19. Mandl, C. P.; König, B. *J. Org. Chem.* **2005**, *70*, 670.
20. Several other bis-crown ether amino acid dipeptides have been prepared in comparable yields: (a) Mandl, C. P. Dissertation, 2004, Universität Regensburg; (b) Späth, A. Diploma thesis, 2005, Universität Regensburg.
21. All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ=0.546).
22. (a) Kruppa, M.; Mandl, C. P.; Miltschitzky, S.; König, B. *J. Am. Chem. Soc.* **2005**, *127*, 3362; (b) Stadlbauer, S.; Riechers, A.; Späth, A.; König, A. *Chem.—Eur. J.* **2008**, *14*, 2536.