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Effect of Crown Ether Ring Size on Binding and Fluorescence Response to Saxitoxin in Anthracylmethyl Monoaza Crown Ether Chemosensors†

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Abstract

Convenient macrocyclization synthetic routes for the preparation of different-sized monoaza anthracylmethyl crown ether chemosensors (15-crown-5, 18-crown-6, 21-crown-7, 24-crown-8 and 27-crown-9) are described. Evaluation of these crowns as chemosensors for saxitoxin revealed that the larger crowns have moderately higher binding constants, with the 27-crown-9 chemosensor having the largest binding constant: $2.29 \times 10^5 \text{ M}^{-1}$. Fluorescence enhancements of 100% were observed at saxitoxin concentrations of $5 \mu\text{M}$, which is close to the detection limit in mouse bioassay.

Keywords

Anthracene; Crown ethers; Saxitoxin; PSP; binding constants; chemosensors

Harmful algal blooms (HABs) are linked to many cases of human poisoning each year. Economic losses and costs to the fishing industry, public health, and tourism are estimated to be \$40,000,000 annually, as of the late 1990s¹. The toxins produced by HABs, as well as many other analytes of interest to members of the biomedical community are “small molecules”. Saxitoxin (STX) (Figure 1) is such a small molecule because of its neurotoxicity, and is the most toxic component of the paralytic shellfish poisons (PSPs).² Its activity is manifested through the binding of the toxin to voltage-gated sodium channels and blocking sodium ion transport across neuronal membranes. In 1996, Saxitoxin was included on the United States government’s list of “Select Agents” (potential terrorist weapons). It is one of only 3 small molecules on the list; the rest are viruses (e.g., ebola), bacteria (e.g., *Yersenia pestis*), and proteins (e.g., ricin, abrin). Mouse bioassay is the current method used by government agencies to detect saxitoxin and its derivatives (known collectively as paralytic shellfish poisons, or PSPs),³ but for both ethical and economic reasons, an alternative would be highly beneficial. We have been working for several years to develop fluorescent chemosensors for the detection of saxitoxin, and have recently made significant advances. Specifically, we have shown that arylmethylcrowns are selective for the detection of saxitoxin over sodium, potassium, and calcium ions,⁴ as well as several organic analytes,⁵ including tetrodotoxin (Figure 1).⁶ The latter point is clinically relevant since saxitoxin and

†This paper is dedicated to Alfred Bader, in recognition of his many contributions, both personal and professional, to the chemical enterprise.

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Supporting Information Available. Proton and Carbon NMR spectra for compounds, **3–8** and **12–18**, and typical binding isotherms for titration of crowns **1–4**.

tetrodotoxin bind, competitively, to the same site on voltage-gated sodium channels, and produce the same clinical symptoms.^{7,8}

Until now we have focussed on the 18-crown-6 monoaza or diaza crown ethers, with anthracene, coumarin, or acridine fluorophores. In early work, Cram, showed that 27-crown-9 crown ether is the optimum size to host a guanidinium guest.^{9,10} Since saxitoxin (Figure 1) is a bis-guanidinium dication, we explored larger crown sizes to determine the effect on binding, using the anthracene fluorophore as sensor. Thus, crown ethers **1–5** were prepared and evaluated for fluorescence response to saxitoxin: 15-crown-5 (**1**, $n=1$), 18-crown-6 (**2**, $n=2$), 21-crown-7 (**3**, $n=3$), 24-crown-8 (**4**, $n=4$) and 27-crown-9 (**5**, $n=5$) (Figure 1).

Synthesis

Anthracylmethyl crown ethers **1** and **2** were originally prepared by de Silva.¹¹ They are made by alkylation of monoaza crowns with 9-(chloromethyl)anthracene. Anthracylmethyl crowns **3–5** were prepared similarly, by alkylation of monoaza crowns **6–8**, as shown in Scheme 1. The diols **9** and **10** are commercially available (Scheme 2). Diol **11** was prepared in three steps from diethylene glycol and triethylene glycol according to a literature procedure¹² with some modifications: using benzyl instead of allyl as the protecting group. Ditosylation of polyethylene glycols **9**, **10**, **11** afforded ditosylates **12**,¹³ **13**¹⁴ and **14**, which were cyclized with *N*-benzyldiol **15** using sodium hydride to afford the *N*-benzyl crowns **16–18** in modest yields. Hydrogenolysis of **16–18** to crowns **6–8** proceeded smoothly, and alkylation with chloromethyl anthracene afforded the sensors **3–5**. The synthetic steps all proceeded in excellent yields, with the exception of the macrocyclization, which is typically low-yielding.¹⁰

Titrations

A solution of each crown ether in methanol was titrated against saxitoxin using the fluorescence response of anthracene at excitation wavelength of 372 nm and emission wavelength of 420 nm. The concentration of the crown ethers was held constant at 10^{-6} M, while the concentration of the toxin was varied by successive dilutions with a 10^{-6} M solution of the crown ether. Beginning with 150 μ L of a solution of 1 μ M crown sensor (**1–5**) and a starting STX concentration of 100 μ M, binding isotherms were obtained by successive removal of 50 μ L aliquots and replacement with 50 μ L of a 1 μ M crown solution. Fluorescence measurements for each dilution were made after 4 minutes equilibration. Binding constants for each anthracylmethyl crown ether were obtained by curve fitting of the intensity at 420 nm to a standard rectangular hyperbolic equation for 1:1 binding.¹⁵ The binding constant is determined according to the equation:

$$\frac{F}{F_0} = \frac{1 + \left(\frac{k_{11}}{k_{\text{crown}}}\right) K_{11} [\text{STX}]}{1 + K_{11} [\text{STX}]},$$

where F and F_0 are the observed fluorescence intensities in the presence and absence of STX, respectively; k_{crown} and k_{11} are constants related to fluorescence intensities of the crown and the 1:1 crown-STX complex, respectively; K_{11} is the binding constant for the 1:1 complex; and $[\text{STX}]$ is the equilibrium concentration of unbound saxitoxin.¹⁵ The equation describes a hyperbolic binding isotherm, and the binding constant, K_{11} , is obtained by a nonlinear least-squares curve fitting program. One of the isotherms for saxitoxin binding to **5** is shown in Figure 2. The binding constants (K_{11}) for the five crown sensors are listed in

Table 1. Each value is the average of at least two runs, and each run had a correlation coefficient for the least-squares fit of ≥ 0.98 .

Discussion

The sensing mechanism is illustrated by the following equilibrium:

where K_{11} is the binding (equilibrium) constant for formation of the 1:1 Toxin-Crown complex. In sensors such as **1–5**, the fluorescent chromophore having a benzylic nitrogen is only weakly fluorescent due to photoinduced electron transfer, PET.¹⁶ Upon complexation of the toxin, PET is turned off, and the chromophore fluoresces normally. The usual mechanism invoked for this type of PET quenching is complexation of a ligand to the benzylic nitrogen lone pair. The binding model that we believe is operative in saxitoxin sensing involves hydrogen bonding of the crown ether to one of the guanidiniums (C-8, Figure 1), as shown in Figure 3.⁵ Monte Carlo searching of possible docked structures failed to identify a low energy structure having a hydrogen bond to the benzylic nitrogen.⁵ Note that the second guanidinium appears to π -stack with the anthracene fluorophore. We suggest that this π -stacking perturbs the relative energies of the chromophore HOMO and the nitrogen lone pair, thus “turning off” the PET.⁵ This hypothesis is supported by the fact that sodium, potassium, calcium,⁴ guanidinium,^{5,17} and ammonium ions,^{5,17} all of which are known to bind to crown ethers, produce no fluorescence enhancement with these sensors in alcohol solvents containing small amounts of water. Further support is found in the failure of tetrodotoxin (Figure 1), a toxin having many hydrogen bond donors but only one guanidinium, and which binds competitively to saxitoxin in sodium channels, to produce any fluorescence enhancement.⁶

Three factors can contribute to an increase in fluorescence intensity upon binding of the toxin: (1) a large binding constant, which increases the relative concentration of the fluorescing Toxin-Crown complex over the unbound Crown; (2) a chromophore having a high molar absorptivity (extinction coefficient) and (3) a high fluorescence quantum yield, such that a high fraction of incident light is absorbed, and a high fraction of absorbed light is emitted when PET is turned off. Anthracene has one of the highest fluorescence quantum yields known (~ 0.4 – 0.8 , depending on conditions). The fluorescence enhancement by saxitoxin in these sensors has not shown significant dependence on the chromophore (anthracene,^{5,17} coumarin,⁴ or acridine⁶), so our efforts have focussed primarily on increasing the binding constant by making modifications to the crown ether.

In a full paper,⁵ we reported the synthesis and binding constants of eleven anthracylmethyl 18-crown-6 ethers, ten of which were diaza crowns with additional substituents added to the crown ring – opposite the anthracylmethyl group – in the hope of increasing the binding constant. Although replacement of one oxygen with a nitrogen doubled the binding constant, further substitution failed to produce any improvement.

In early work on the binding of guanidinium ions to crown ethers, molecular models were used to postulate six hydrogen bonds between guanidinium ion and 27-crown-9 ethers.^{9,10} The hypothesis was supported by the solubilization of (otherwise insoluble) guanidinium ion in chloroform by benzo-27-crown-9. The binding model invoked hydrogen bonding between the six guanidinium N-H's and six of the nine oxygens of the crown ether. Although this binding model cannot be employed for saxitoxin, the large number of hydrogen bond donors in the toxin prompted the question whether a larger crown might show enhanced binding.

Interestingly, neither ammonium ion nor guanidinium ion produce any fluorescence enhancement in **2** in ethanol.^{5,17} The results of the current study reveal increased binding as the size of the crown ring increases. A control experiment shows that guanidinium ion fails to enhance the fluorescence of **5**; if anything, a slight suppression is observed in methanol.

Summary

Anthracylmethyl crown ether chemosensors show enhanced binding over smaller crowns. This may be due to the fact that there are more heteroatoms in the crown, and/or that the larger crown is simply more flexible. Either effect could offer more sites for hydrogen bonding to the toxin. Our results show that simple chemosensors such as these could be developed for the detection of saxitoxin and possibly other PSP toxins, at concentrations comparable to those at which mice are sensitive.

Experimental Section

General methods

IR spectra were recorded as thin films between NaCl plates or as KBr pellets. ¹H and ¹³C NMR were recorded at 300 MHz for ¹H and 75 MHz for ¹³C. ¹H and ¹³C chemical shifts are reported in ppm relative to residual chloroform; coupling constants are reported in Hz. ESI mass spectra were obtained by flow injection on a quadrupole ion trap mass spectrometer with methanol as the carrier solvent. High Resolution ESI mass spectra (HRMS/ESI) were obtained by using direct flow injection on a 9.4 Tesla Fourier transform mass spectrometer. Dry solvents were freshly distilled before use: dichloromethane was distilled from calcium hydride, and THF from sodium-benzophenone. Methanol, acetonitrile, and ethyl acetate were used as received. Hexane used in column chromatography was distilled before use. Water refers to high purity water that was obtained from the Milli-Q purification system. All reactions were performed under a nitrogen atmosphere.

N-Benzyl-monoaza-21-crown-7 (**16**)

To a solution of pentaethylene glycol (5.00 g, 21.0 mmol) in CH₂Cl₂ (120 mL) was added Et₃N (8.8 mL, 63.0 mmol), DMAP (1.28 g, 10.5 mmol) and TsCl (8.41 g, 44.1 mmol) at 0°C. After stirring at RT for 1 h, NH₄Cl (sat. aq. sol., 50 mL) was added. The reaction mixture was extracted three times with CH₂Cl₂ (3 × 20 mL); the organic phase was dried with MgSO₄; evaporated under reduced pressure. The residue was purified on silica gel using EtOAc/hexane (50%/50%), which gave **12** as an oil (11.2 g, 97%).

To a solution of bistosylate **12** (11.2 g, 20.5 mmol) in anhydrous THF (150 mL) was added *N*-benzyl-diol **15** (4.00 g, 20.5 mmol) and NaH (2.46 g, 61.4 mmol; 60% dispersion in mineral oil, the oil was removed from the product chromatographically). The mixture was heated at 65 °C for 18 hours. After cooling to RT, NH₄Cl (sat. aq. sol., 100 mL) was added.

The reaction mixture was extracted three times with CH_2Cl_2 (3×30 mL). The organic phase was dried with MgSO_4 ; evaporated under reduced pressure; the residue was purified on alumina using EtOAc/hexane (50%/50%), which gave an oil (2.69 g, 33%). IR (CHCl_3) ν_{max} 3523, 2870, 1643, 1453, 1124 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 2.78 (t, 4H, $J = 5.8$ Hz, CH_2), 3.57–3.68 (m, 26H, CH_2), 7.19–7.33 (m, 5H, CH-Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 53.78 (CH_2), 59.66 ($\text{CH}_2\text{-Bn}$), 70.00, 70.63, 70.78, 70.83, 70.90 (CH_2), 126.78, 128.11, 128.83 (CH-Ar), 139.71 (C-Ar); MS m/e $\text{M}^+ + 1$: 398; HRMS calcd for $\text{C}_{21}\text{H}_{36}\text{NO}_6$ (MH^+) 398.2542, found 398.2531; Anal. Calcd for $\text{C}_{21}\text{H}_{35}\text{NO}_6$: C, 63.45; H, 8.87. Found C, 63.29; H, 8.88.

1-Aza-21-crown-7 (6)

A solution of **16** (2.69 g, 6.76 mmol) in MeOH (50 mL), containing Pd-C (10%, 200 mg), was stirred under 1 atm H_2 (balloon) overnight. The catalyst was filtered through Celite and washed with MeOH (5×10 mL). After evaporation, the residue was purified on alumina using MeOH/EtOAc (15%/85%), which gave a colorless oil (1.94 g, 93%). IR (CHCl_3) ν_{max} 3504, 2874, 1648, 1460, 1353, 1110 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 2.71 (t, 4H, $J = 4.9$ Hz, CH_2), 2.90 (1H, br, NH), 3.51–3.59 (m, 24H, CH_2); ^{13}C NMR (75 MHz, CDCl_3) δ 49.25 (CH_2), 70.53, 70.57, 70.69, 70.75 (CH_2); MS m/e $\text{M}^+ + 1$: 308; HRMS calcd for $\text{C}_{14}\text{H}_{30}\text{NO}_6$ (MH^+) 308.2073, found 308.2061.

1-(Anthracen-9-ylmethyl)-aza-21-crown-7 (3)

To a solution of **16** (0.301 g, 0.976 mmol) in anhydrous CH_3CN (30 mL) was added 9-(chloromethyl)anthracene (0.221 g, 0.976 mmol) and Cs_2CO_3 (0.954 g, 2.93 mmol). The mixture was heated at 80°C for 2 h. After cooling to RT, NH_4Cl (sat. aq. sol., 30 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3×20 mL), and the organic phase was dried with MgSO_4 , and evaporated under reduced pressure. The residue was purified on SiO_2 using EtOAc/MeOH (90%/10%), to give an oil (0.432 g, 89%). IR (CHCl_3) ν_{max} 3487, 2868, 1630, 1451, 1117 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 2.90 (t, 4H, $J = 5.7$ Hz, CH_2), 3.56–3.73 (m, 24H, CH_2), 4.64 (s, 2H, CH_2), 7.44–7.55 (m, 4H, CH-Ar), 8.00 (d, 2H, $J = 8.9$ Hz, CH-Ar), 8.41 (s, 1H, CH-Ar), 8.60 (d, 2H, $J = 8.9$ Hz, CH-Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 51.75 (CH_2), 53.86 (CH_2), 70.10, 70.44, 70.85 (CH_2), 124.80, 125.35, 125.52, 127.41, 128.89 (CH-Ar), 130.57, 131.41, 131.43 (C-Ar); MS m/e $\text{M}^+ + 1$: 498; HRMS calcd for $\text{C}_{29}\text{H}_{40}\text{NO}_6$ (MH^+) 498.2855, found 498.2838.

Hexaethylene glycol ditosylate (13)

To a solution of hexaethylene glycol (3.36 g, 11.9 mmol) in CH_2Cl_2 (50 mL) at 0°C was added Et_3N (5.0 mL, 35.7 mmol), DMAP (1.45 g, 5.96 mmol) and TsCl (5.68 g, 29.78 mmol). After stirring at RT for 30 min, NH_4Cl (sat. aq. sol., 20 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3×20 mL), and the combined organic

phase was dried with MgSO_4 , condensed, and the residue was purified on silica gel using EtOAc/hexane (80%/20%) to EtOAc (100%), which gave a colorless oil (6.34 g, 90%). IR (CHCl_3) ν_{max} 2957, 2871, 1596, 1450, 1361, 1181 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 2.41 (s, 6H, $\text{CH}_3\text{-Ts}$), 3.54 (s, 8H, CH_2), 3.56–3.62 (m, 8H, CH_2), 3.63–3.66 (m, 4H, CH_2), 4.10–4.13 (m, 4H, CH_2), 7.30–7.33 (m, 4H, CH-Ts), 7.74–7.77 (m, 4H, CH-Ts); ^{13}C NMR (75 MHz, CDCl_3) δ 21.61 ($\text{CH}_3\text{-Ts}$), 68.60, 69.31, 70.45, 70.50, 70.55, 70.66 ($6 \times \text{CH}_2$), 127.92 and 129.84 ($8 \times \text{CH-Ar}$), 132.90 and 144.84 ($4 \times \text{C-Ar}$); MS m/e $\text{M}^+ + 1$: 591; Anal. Calcd for $\text{C}_{26}\text{H}_{38}\text{O}_{11}\text{S}_2$: C, 52.87; H, 6.48. Found C, 52.75; H, 6.59.

1-aza-24-crown-8 (7)

To a solution of bistosylate **13** (19.56g, 33.1 mmol) in anhydrous THF (150 mL) was added *N*-benzyl-diol **15** (6.45 g, 33.1 mmol) and NaH (3.97 g, 99.3 mmol, 60% dispersion in mineral oil, the oil was removed from the product chromatographically). The mixture was heated at 65 °C for 18 hours. After cooling to RT, NH_4Cl (sat. aq. sol., 100 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3×30 mL); the combined organic phase was dried with MgSO_4 , condensed, and the residue was purified on alumina using EtOAc/hexane (80%/20%), which gave compound **17** as an oil product (3.85 g, 26%).

A solution of **17** (3.85 g, 8.71 mmol) in MeOH (50 mL) containing Pd-C (10%, 200 mg) was stirred under 1 atm H_2 (balloon) overnight. The catalyst was filtered through Celite and washed with MeOH (5×10 mL). After evaporation, the residue was purified on alumina using MeOH/EtOAc (15%/85%), which gave a colorless oil product (2.91 g, 95%). IR (CHCl_3) ν_{max} 3490, 2872, 1649, 1461, 1352, 1109 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 2.39 (s, br, NH), 2.72 (t, 4H, $J = 5.1$ Hz, CH_2), 3.51–3.60 (m, 28H, CH_2); ^{13}C NMR (75 MHz, CDCl_3) δ 49.09 (CH_2), 70.44, 70.56, 70.66, 70.67, 70.70, 70.80 (CH_2); MS m/e $\text{M}^+ + 1$: 352; HRMS calcd for $\text{C}_{16}\text{H}_{34}\text{NO}_7$ (MH^+) 352.2335, found 352.2326.

1-(Anthracen-9-ylmethyl)-aza-24-crown-8 (4)

To a solution of **7** (0.837 g, 2.38 mmol) in anhydrous CH_3CN (50 mL) was added 9-(chloromethyl)anthracene (0.450 g, 1.98 mmol) and Cs_2CO_3 (1.29 g, 3.96 mmol). The mixture was heated at 80°C for 2 h. After cooling to RT, NH_4Cl (sat. aq. sol., 30 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3×20 mL); and the combined organic phase was dried with MgSO_4 , evaporated, and purified on alumina using EtOAc/hexane (60%/40%), which gave an oil (0.903 g, 84%). IR (CHCl_3) ν_{max} 3486, 2868, 1636, 1452, 1119 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 2.90 (t, 4H, $J = 5.7$ Hz, CH_2), 3.55–3.69 (m, 28H, CH_2), 4.64 (s, 2H, CH_2), 7.43–7.54 (m, 4H, CH-Ar), 8.00 (d, 2H, $J = 8.9$ Hz, CH-Ar), 8.40 (s, 1H, CH-Ar), 8.58 (d, 2H, $J = 8.9$ Hz, CH-Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 51.84 (CH_2), 53.79 (CH_2), 70.08, 70.46, 70.80, 70.82, 70.84, 70.87 (CH_2), 124.81, 125.36, 125.52, 127.41, 128.89 (CH-Ar), 130.58, 131.41, 131.43 (C-Ar); MS m/e $\text{M}^+ + 1$: 542; HRMS calcd for $\text{C}_{31}\text{H}_{44}\text{NO}_7$ (MH^+) 542.3118, found 542.3099.

1-((2-(2-(2-(2-(2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)methyl)benzene

To a solution of triethylene glycol bistosylate (synthesized according to literature procedure¹³ with some modifications) (5.02 g, 10.93 mmol) in anhydrous THF (100 mL) was added 2-(2-(benzyloxy)ethoxy)ethanol (4.50 g, 22.96 mmol) and NaH (2.20 g, 54.68 mmol, 60% dispersion in mineral oil, the oil was removed from the product chromatographically). The mixture was heated at 65°C for 1.5 h. After cooling to RT, NH_4Cl (sat. aq. sol., 100 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3×30 mL); the combined organic phase was dried with MgSO_4 , evaporated and the residue was purified on SiO_2 using EtOAc, which gave the product as an oil (4.825 g, 87%). IR (CHCl_3) ν_{max} 3572, 2870, 1454, 1106 cm^{-1} ; ^1H NMR (300MHz, CDCl_3) δ 3.62–3.71 (m, 28H, CH_2), 4.58 (s, 4H, CH_2), 7.26–7.34 (m, 10H, CH-Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 69.44, 70.59, 70.61, 70.66, 73.23 (CH_2), 127.59, 127.74, 128.36 (CH-Ar), 138.29 (C-Ar); MS m/e $\text{M}^+ + 1$: 507; Anal. Calcd for $\text{C}_{28}\text{H}_{42}\text{O}_8$: C, 66.38; H, 8.36. Found C, 66.09; H, 8.30.

Heptaethylene glycol ditosylate (14)

The bis-benzyl ether prepared above (4.825 g, 9.52 mmol) was dissolved in MeOH (50 mL) containing Pd-C (10%, 300 mg), and stirred under 1 atm H₂ pressure (balloon) overnight. The catalyst was filtered through Celite and washed with MeOH (5 × 20 mL). After evaporation, the product (heptaethylene glycol) was obtained as a colorless oil (2.52 g, 81%), and used without further purification or characterization.

To the above heptaethylene glycol (2.52 g, 7.73 mmol) in CH₂Cl₂ (50 mL) was added Et₃N (3.2 mL, 23.2 mmol), DMAP (0.472 g, 3.87 mmol) and TsCl (3.09 g, 16.23 mmol) at 0°C. After stirring at RT for 1 h, NH₄Cl (sat. aq. sol., 20 mL) was added. The reaction mixture was extracted three times with CH₂Cl₂ (3 × 30 mL); the combined organic phase was dried with MgSO₄, evaporated and the residue was purified on silica gel using EtOAc, to afford a colorless oil (4.71 g, 96%). IR (CHCl₃) ν_{max} 3525, 2872, 1598, 1457, 1182 cm⁻¹; ¹H NMR (300MHz, CDCl₃) δ 2.43 (s, 6H, CH₃-Ts), 3.56–3.68 (m, 24H, CH₂), 4.12–4.15 (m, 4H, CH₂), 7.32–7.34 (m, 4H, CH-Ts), 7.76–7.79 (m, 4H, CH-Ts); ¹³C NMR (75 MHz, CDCl₃) δ 21.63 (CH₃-Ts), 68.63, 69.28, 70.48, 70.52, 70.54, 70.58, 70.70 (CH₂), 127.95 and 129.84 (CH-Ar), 132.95 and 144.82 (C-Ar); MS *m/e* M⁺+1:635; Anal. Calcd for C₂₈H₄₂O₁₂S₂: C, 52.98; H, 6.67. Found C, 53.16; H, 6.59.

The image shows the chemical structure of a 12-membered macrocyclic crown ether. The ring consists of six oxygen atoms and six ethylene groups. One of the ethylene groups is substituted with a benzyl group (N-Bn). The structure is drawn in a circular fashion, with the nitrogen atom and its benzyl substituent positioned on the right side of the ring.

1-Aza-27-crown-9 (8)

A solution of **18** (0.369 g, 0.759 mmol) in MeOH (15 mL) containing Pd-C (10%, 50 mg) was stirred under 1 atmosphere of H₂ (balloon) overnight. The catalyst was filtered through Celite and washed with MeOH (5 × 10 mL). After evaporation, the residue was purified on alumina using MeOH/EtOAc (15%/85%), which gave a colorless oil (0.274 g, 91%). IR (CHCl₃) ν_{max} 3452, 2915, 1649, 1461, 1103 cm⁻¹; ¹H NMR (300MHz, CDCl₃) δ 2.35 (s, br, NH), 2.80 (t, 4H, *J* = 5.0 Hz, CH₂), 3.58–3.69 (m, 32H, CH₂); ¹³C NMR (75 MHz, CDCl₃) δ 49.16 (CH₂), 70.52, 70.60, 70.71, 70.75 (CH₂); MS *m/e* M⁺+1:396; HRMS calcd for C₁₈H₃₈NO₈ (MH⁺) 396.2597, found 396.2580; Anal. Calcd for C₁₈H₃₇NO₈:C, 54.66; H, 9.43. Found C, 54.38; H, 9.48.

1-(Anthracen-9-ylmethyl)-aza-27-crown-9 (**5**)

To a solution of **8** (0.210 g, 0.530 mmol) in anhydrous CH₃CN (20 mL) was added 9-(chloromethyl)anthracene (0.109 g, 0.482 mmol) and Cs₂CO₃ (0.314 g, 0.964 mmol). The mixture was heated at 80 °C for 2 h. After cooling to RT, NH₄Cl (sat. aq. sol., 30 mL) was added. The reaction mixture was extracted three times with CH₂Cl₂ (3 × 20 mL); the combined organic phase was dried with MgSO₄, evaporated and the residue was purified on alumina using EtOAc/hexane (60%/40%), which gave an oil (0.221 g, 78%). IR (CHCl₃) ν_{max} 3449, 2870, 1646, 1108 cm⁻¹; ¹H NMR (300MHz, CDCl₃) δ 2.89 (t, 4H, *J* = 5.8 Hz, CH₂), 3.50–3.67 (m, 32H, CH₂), 4.64 (s, 2H, CH₂), 7.44–7.54 (m, 4H, CH-Ar), 7.99 (d, 2H, *J* = 8.8 Hz, CH-Ar), 8.41 (s, 1H, CH-Ar), 8.58 (d, 2H, *J* = 8.8 Hz, CH-Ar); ¹³C NMR (75 MHz, CDCl₃) δ 51.94 (CH₂), 53.79 (CH₂), 70.02, 70.32, 70.51, 70.69 (CH₂), 124.82, 125.33, 125.53, 127.42, 128.89 (CH-Ar), 130.52, 131.39, 131.42 (C-Ar); MS *m/e* M⁺+1:586; HRMS calcd for C₃₃H₄₈NO₈ (MH⁺) 586.3380, found 586.3354.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Figure 2.

Typical binding isotherm for titration of saxitoxin from against anthracylmethyl 27-crown-9 chemosensor **5** (10^{-6} M). A 50% fluorescence enhancement is observed at 1 μ M [STX], and 100% enhancement is observed at 5 μ M [STX].

Figure 3. Molecular mechanics model of STX docked to anthracylmethyl 18-crown-6 (global minimum.⁵ Note the absence of a hydrogen bond to the benzylic nitrogen.

Table 1

Binding Constants of Saxitoxin to Crown Sensors **1–5** in methanol. Each K_{11} is the average of at least 2 runs. Correlation coefficients of ≥ 0.98 were calculated for each fit.

Crown Sensor	Binding Constant, K_b , M^{-1}
1	4.93×10^4
2	5.3×10^4
3	4.68×10^4
4	1.07×10^5
5	2.29×10^5