Effect of crown ether ring size on binding and fluorescence response to saxitoxin in anthracylmethyl monoazacrown ether chemosensors[1]

Hua Mao, John B. Thorne, Jennifer S. Pharr, and Robert E. Gawley

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 (2.29×10^5 $(mol/L)^{-1}$). Fluorescence enhancements of 100% were observed at saxitoxin concentrations of 5 μmol/L, which is close to the detection limit in mouse bioassay.

Key words: anthracene, crown ethers, saxitoxin, paralytic shellfish poison (PSP), binding constants; chemosensors.

Résumé : On décrit des voies de synthèse pratiques à base de macrocyclisations pour la préparation de senseurs chimiques à base des monoaza anthracylméthyl éthers couronnes de tailles différentes, 15-courone-5, 18-couronne-6, 21-couronne-7, 24-couronne-8 et 27-couronne-9. L'évaluation de ces couronnes comme senseurs chimiques pour la saxitoxine révèle que les couronnes les plus grosses présentent les constantes de fixation les plus élevées, alors que le senseur chimique à base de 27-couronne-9 est associée à la constante de fixation la plus élevée, soit $2{,}29 \times 10^{-5}$ $(mol/L)^{-1}$. On a observé des augmentations de la fluorescence de 100 % à des concentrations de 5 μmol/L, valeur qui est proche de la limite de détection dans les bioessais à l'aide de souris.

Mots clés : anthracène, éthers couronnes, saxitonine, intoxication paralysante par les mollusques, constantes de fixation, senseurs chimiques.

[Traduit par la Rédaction]

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 000US annually as of the late 1990s (1). The toxins produced by HABs, as well as many other analytes of interest to members of the biomedical community, are "small molecules". Saxitoxin (STX) (Fig. 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 three 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 (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 (Fig. 1) (6). The latter point is clinically relevant since saxitoxin and tetrodotoxin bind, competitively, to the same site on voltage-gated sodium channels and produce the same clinical symptoms (7, 8).

Received 11 November 2005. Accepted 24 January 2006. Published on the NRC Research Press Web site at http://canjchem.nrc.ca on 20 July 2006.

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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[1]This article is part of a Special Issue dedicated to Dr. Alfred Bader.
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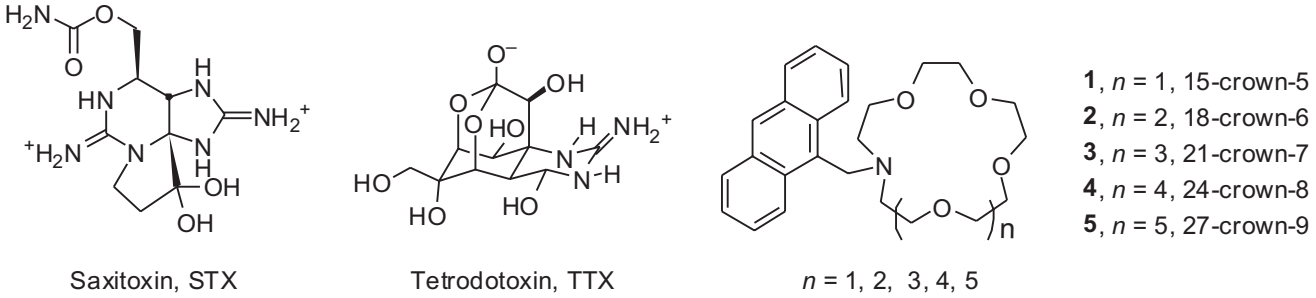
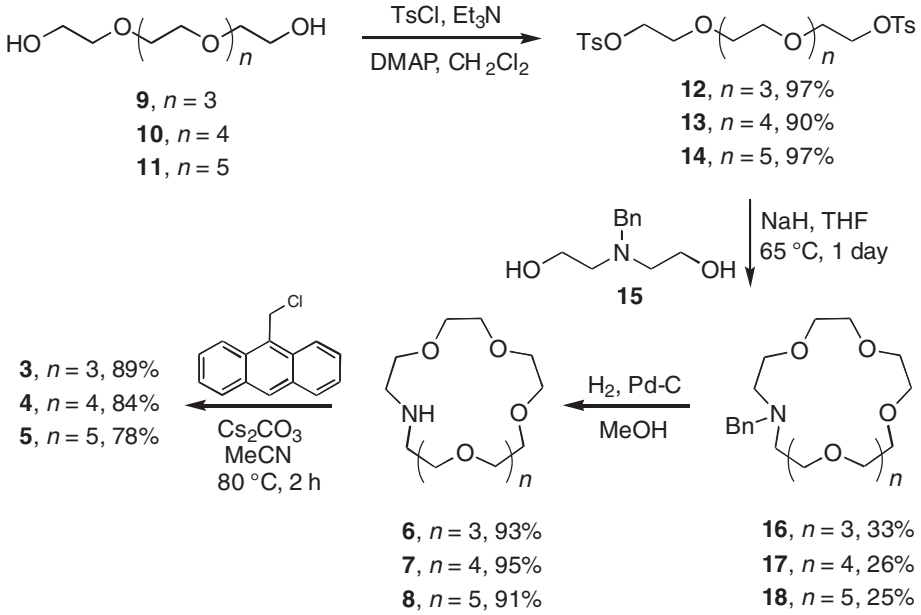
 doi:10.1139/V06-093

Fig. 1.



Scheme 1.



Until now we have focused on the 18-crown-6 monoaza- or diaza-crown ethers, with anthracene, coumarin, or acridine fluorophores. In early work, Cram and co-workers showed that 27-crown-9 crown ether is the optimum size to host a guanidinium guest (9, 10). Since saxitoxin (Fig. 1) is a bisguanidinium 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) (Fig. 1).

Synthesis

Anthracylmethyl crown ethers **1** and **2** were originally prepared by de Silva and co-workers (11). They were made by alkylation of monoazacrowns with 9-(chloromethyl)anthracene. Anthracylmethyl crowns **3**–**5** were similarly prepared by alkylation of monoazacrowns **6**–**8**, as shown in Scheme 1. The diols **9** and **10** are commercially available. Diol **11** was prepared in three steps from diethylene glycol and triethylene glycol according to a literature procedure (12) with some modifications (using benzyl instead of allyl as the protecting group). Ditosylation of polyethylene glycols **9**–**11** afforded ditosylates **12** (13), **13** (14), and **14**, respectively, 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).[3]

Titrations

A solution of each crown ether in methanol was titrated against saxitoxin using the fluorescence response of anthracene at an excitation wavelength of 372 nm and an emission wavelength of 420 nm. The concentration of the crown ethers was held constant at 10^{-6} mol/L, while the

[3] Supplementary data (^{1}H and ^{13}C NMR spectra for compounds **3**–**8** and **12**–**18** and typical binding isotherms for the titration of crowns **1**–**4**) for this article are available on the journal Web site (http://canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 5060. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

concentration of the toxin was varied by successive dilutions with a 10^{-6} mol/L solution of the crown ether. Beginning with 150 µL of a solution of 1 µmol/L crown sensor (**1**–**5**) and a starting STX concentration of 100 µmol/L, binding isotherms were obtained by successive removal of 50 µL aliquots and replacement with 50 µL of a 1 µmol/L crown solution. Fluorescence measurements for each dilution were made after a 4 min 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 was determined according to the equation

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

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 [STX] is the equilibrium concentration of unbound saxitoxin (15). The equation describes a hyperbolic binding isotherm and the binding constant (K_{11}) was obtained by a nonlinear least-squares curve-fitting program. One of the isotherms for saxitoxin binding to **5** is shown in Fig. 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

$$\text{Toxin} + \text{Crown} \underset{}{\overset{K_{11}}{\rightleftharpoons}} \text{Toxin·Crown}$$

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 owing 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, Fig. 1), as shown in Fig. 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

Fig. 2. Typical binding isotherm for titration of saxitoxin from 10^{-4} mol/L to 5×10−7 mol/L vs. anthracylmethyl 27-crown-9 chemosensor **5** (10^{-6} mol/L). A 50% fluorescence enhancement is observed at 1 µmol/L [STX] and 100% enhancement is observed at 5 µmol/L [STX].

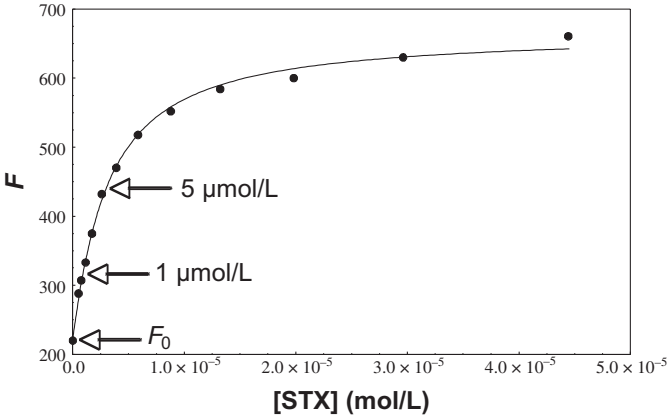
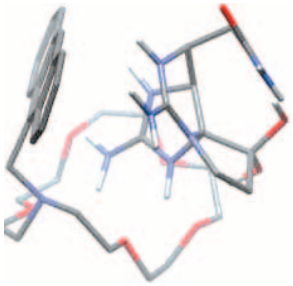


Table 1. Binding constants of saxitoxin to crown sensors **1**–**5** in methanol.

Crown sensor	Binding constant (K_b, $(mol/L)^{-1}$)
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

Note: Each K_{11} is the average of at least two runs. Correlation coefficients of ≥0.98 were calculated for each fit.

Fig. 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.



tetrodotoxin (Fig. 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 acridine (6)), 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 11 anthracylmethyl 18-crown-6 ethers, 10 of which were diazacrowns 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-Hs 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 produced 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

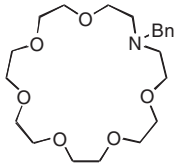
Anthracylmethyl crown ether chemosensors show enhanced binding over smaller crowns. This may be due to the fact that there are more heteroatoms in the crown and (or) that the larger crown is simply more flexible. Either effect could offer more sites for hydrogen bonding to the toxin. Our results show 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. ^{1}H and ^{13}C NMR were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C. ^{1}H and ^{13}C NMR 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 T 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. The 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.

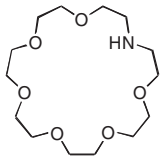
N-Benzyl monoaza-21-crown-7 (16)



To a solution of pentaethylene glycol (5.00 g, 21.0 mmol) in CH_2Cl_2 (120 mL) was added Et_3N (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, NH_4Cl (satd. aq. sol., 50 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 20 mL), the organic phase was dried with $MgSO_4$ and 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 anhydr. 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 h. After cooling to RT, NH_4Cl (satd. 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, and the residue was purified on alumina using EtOAc–hexane (50%:50%), which gave an oil (2.69 g, 33%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3523, 2870, 1643, 1453, 1124. ^{1}H NMR (300 MHz, $CDCl_3$) 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 (*C*H_2), 59.66 (*C*H_2-Bn), 70.00, 70.63, 70.78, 70.83, 70.90 (*C*H_2), 126.78, 128.11, 128.83 (*C*H-Ar), 139.71 (*C*-Ar). MS *m*/*e*: 398 [M^+ + 1]. HRMS calcd. for $C_{21}H_{36}NO6$ 398.2542 [MH^+]; found: 398.2531. Anal. calcd. for $C_{21}H_{35}NO_6$: C 63.45, H 8.87; found: C 63.29, H 8.88.

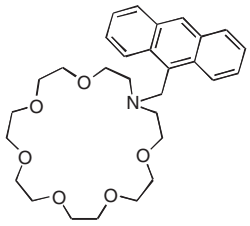
1-Aza-21-crown-7 (6)



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, 1 atm = 101.325 kPa) 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 ($CHCl_3$, cm^{-1}) ν_{max}: 3504, 2874, 1648, 1460, 1353, 1110. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.71

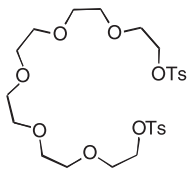
(t, 4H, J = 4.9 Hz, CH_2), 2.90 (1H, br, NH), 3.51–3.59 (m, 24H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 49.25 (CH_2), 70.53, 70.57, 70.69, 70.75 (CH_2). MS *m/e*: 308 [M^+ + 1]. HRMS calcd. for $C_{14}H_{30}NO_6$: 308.2073 [MH^+]; found: 308.2061.

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



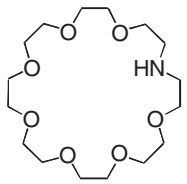
To a solution of **16** (0.301 g, 0.976 mmol) in anhydr. CH_3CN (30 mL) was added 9-(chloromethyl)anthracene (0.221 g, 0.976 mmol) and Cs_2CO_3 (0.954 g, 2.93 mmol). The mixture was heated at 80 °C for 2 h. After cooling to RT, NH_4Cl (satd. aq. sol., 30 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 20 mL), 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 ($CHCl_3$, cm^{-1}) ν_{max}: 3487, 2868, 1630, 1451, 1117. ^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 (*C*H-Ar), 130.57, 131.41, 131.43 (*C*-Ar). MS *m/e*: 498 [M^+ + 1]. HRMS calcd. for $C_{29}H_{40}NO_6$: 498.2855 [MH^+]; 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 (satd. 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 ($CHCl_3$, cm^{-1}) ν_{max}: 2957, 2871, 1596, 1450, 1361, 1181. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.41 (s, 6H, CH_3-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 (CH_3-Ts), 68.60, 69.31, 70.45, 70.50, 70.55, 70.66 (6 × CH_2), 127.92 and 129.84 (8 × *C*H-Ar), 132.90 and 144.84 (4 × *C*-Ar). MS *m/e*: 591 [M^+ + 1]. Anal. calcd. for $C_{26}H_{38}O_{11}S_2$: C 52.87, H 6.48; found: C 52.75, H 6.59.

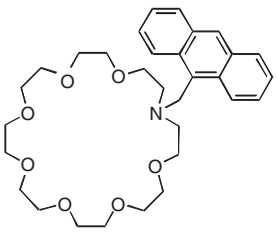
1-Aza-24-crown-8 (7)



To a solution of bistosylate **13** (19.56g, 33.1 mmol) in anhydr. 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 h. After cooling to RT, NH_4Cl (satd. aq. sol., 100 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 30 mL) and 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 (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 (2.91 g, 95%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3490, 2872, 1649, 1461, 1352, 1109. ^1H NMR (300 MHz, $CDCl_3$) 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*: 352 [M^+ + 1]. HRMS calcd. for $C_{16}H_{34}NO_7$: 352.2335 [MH^+]; found: 352.2326.

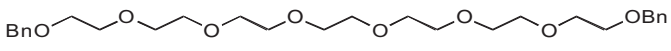
1-(Anthracen-9-ylmethyl)-aza-24-crown-8 (4)



To a solution of **7** (0.837 g, 2.38 mmol) in anhydr. 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 (satd. 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 ($CHCl_3$, cm^{-1}) ν_{max}: 3486, 2868, 1636, 1452, 1119. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.90 (t, 4H, J = 5.7 Hz, CH_2), 3.55–

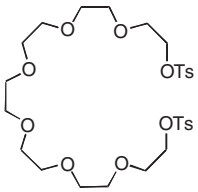
3.69 (m, 28H, CH_2), 4.64 (s, 2H, CH_2), 7.43–7.54 (m, 4H, CH-Ar), 8.00 (d, 2H, J = 8.9 Hz, CH-Ar), 8.40 (s, 1H, CH-Ar), 8.58 (d, 2H, J = 8.9 Hz, CH-Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 51.84 (*C*H_2), 53.79 (*C*H_2), 70.08, 70.46, 70.80, 70.82, 70.84, 70.87 (*C*H_2), 124.81, 125.36, 125.52, 127.41, 128.89 (*C*H-Ar), 130.58, 131.41, 131.43 (*C*-Ar). MS *m/e*: 542 [M^+ + 1]. HRMS calcd. for $C_{31}H_{44}NO_7$: 542.3118 [MH^+]; found: 542.3099.

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



To a solution of triethylene glycol bistosylate (5.02 g, 10.93 mmol, synthesized according to literature procedure (13) with some modifications) in anhydr. 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 (satd. aq. sol., 100 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 30 mL) and 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 ($CHCl_3$, cm^{-1}) ν_{max}: 3572, 2870, 1454, 1106. ^{1}H NMR (300 MHz, $CDCl_3$) 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 (*C*H_2), 127.59, 127.74, 128.36 (*C*H-Ar), 138.29 (*C*-Ar). MS *m/e*: 507 [M^+ + 1]. Anal. calcd. for $C_{28}H_{42}O_8$: C 66.38, H 8.36; found: C 66.09, H 8.30.

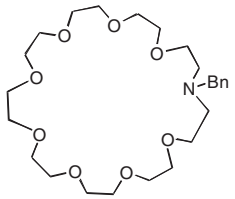
Heptaethylene glycol ditosylate (14)



The bisbenzyl 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 H_2 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.

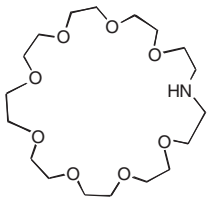
To this heptaethylene glycol (2.52 g, 7.73 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (3.2 mL, 23.2 mmol), DMAP (0.472 g, 3.87 mmol), and TsCl (3.09 g, 16.23 mmol) at 0 °C. After stirring at RT for 1 h, NH_4Cl (satd. aq. sol., 20 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 30 mL) and the combined organic phase was dried with $MgSO_4$, evaporated, and the residue was purified on silica gel using EtOAc to afford a colorless oil (4.71 g, 96%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3525, 2872, 1598, 1457, 1182. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (s, 6H, CH_3-Ts), 3.56–3.68 (m, 24H, CH_2), 4.12–4.15 (m, 4H, CH_2), 7.32–7.34 (m, 4H, CH-Ts), 7.76–7.79 (m, 4H, CH-Ts). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.63 (*C*H_3-Ts), 68.63, 69.28, 70.48, 70.52, 70.54, 70.58, 70.70 (*C*H_2), 127.95 and 129.84 (*C*H-Ar), 132.95 and 144.82 (*C*-Ar). MS *m/e*: 635 [M^+ + 1]. Anal. calcd. for $C_{28}H_{42}O_{12}S_2$: C 52.98, H 6.67; found: C 53.16, H 6.59.

***N*-Benzyl monoaza-27-crown-9 (18)**



To a solution of bistosylate **14** (2.164 g, 3.41 mmol) in anhydr. THF (50 mL) was added *N*-benzyl diol **15** (0.798 g, 4.09 mmol) and NaH (0.545 g, 13.6 mmol; 60% dispersion in mineral oil, the oil was removed from the product chromatographically). The mixture was heated at 65 °C for 18 h. After cooling to RT, NH_4Cl (satd. aq. sol., 50 mL) was added. The reaction mixture was extracted three times with CH_2Cl_2 (3 × 30 mL) and the combined organic phase was dried with $MgSO_4$, evaporated, and the residue was purified on alumina using EtOAc–hexane (80%:20%), which gave an oil (0.414 g, 25%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3525, 2871, 1644, 1456, 1113. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.79 (t, 4H, J = 5.9 Hz, CH_2), 3.52–3.71 (m, 34H, CH_2), 7.23–7.40 (m, 5H, CH-Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 53.79 (*C*H_2), 59.85 (*C*H_2-Bn), 69.93, 70.53, 70.75 (*C*H_2), 126.84, 128.15, 128.88 (*C*H-Ar), 139.45 (*C*-Ar). MS *m/e*: 486 [M^+ + 1]. HRMS calcd. for $C_{25}H_{44}NO_8$: 486.3067 [MH^+]; found: 486.3049. Anal. calcd. for $C_{25}H_{43}NO_8$: C 61.83, H 8.93; found: C 61.81, H 9.06.

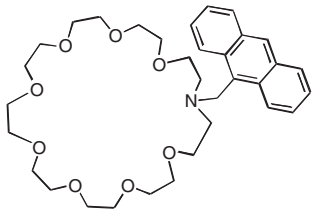
1-Aza-27-crown-9 (8)



A solution of **18** (0.369 g, 0.759 mmol) in MeOH (15 mL) containing Pd-C (10%, 50 mg) was stirred under 1 atm of 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 (0.274 g, 91%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3452, 2915, 1649, 1461, 1103. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.35 (s, br, NH), 2.80 (t, 4H, J = 5.0 Hz, CH_2), 3.58–3.69 (m, 32H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 49.16 (*C*H_2), 70.52, 70.60, 70.71, 70.75

(CH_2). MS *m/e*: 396 [M^+ + 1]. HRMS calcd. for $C_{18}H_{38}NO_8$: 396.2597 [MH^+]; found: 396.2580. Anal. calcd. for $C_{18}H_{37}NO_8$: 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 anhydr. CH_3CN (20 mL) was added 9-(chloromethyl)anthracene (0.109 g, 0.482 mmol) and Cs_2CO_3 (0.314 g, 0.964 mmol). The mixture was heated at 80 °C for 2 h. After cooling to RT, NH_4Cl (satd. 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 the residue was purified on alumina using EtOAc–hexane (60%:40%), which gave an oil (0.221 g, 78%). IR ($CHCl_3$, cm^{-1}) ν_{max}: 3449, 2870, 1646, 1108. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.89 (t, 4H, *J* = 5.8 Hz, CH_2), 3.50–3.67 (m, 32H, CH_2), 4.64 (s, 2H, CH_2), 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). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 51.94 (CH_2), 53.79 (CH_2), 70.02, 70.32, 70.51, 70.69 (CH_2), 124.82, 125.33, 125.53, 127.42, 128.89 (*C*H-Ar), 130.52, 131.39, 131.42 (*C*-Ar). MS *m/e*: 586 [M^+ + 1]. HRMS calcd. for $C_{33}H_{48}NO_8$: 586.3380 [MH^+]; found: 586.3354.

Acknowledgements

This work was supported by the US EPA Science to Achieve Results (STAR) program (R82959902 and GR83238201). We are also grateful to Dr. Sherwood Hall, US Food and Drug Administration, for a gift of saxitoxin. Core facilities were funded by the US National Institutes of Health (P20 R15569) and the Arkansas Biosciences Institute.

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