Direct C-11 functionalisation of anatoxin-a. Application to the synthesis of new ligand-based structural probes

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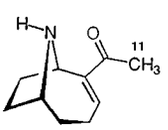
A variety of methods have been evaluated for the functionalisation of the C-11 methyl group of anatoxin-a. Reaction of *N*-Boc anatoxin-a 9 with PhI(OH)OTs (Koser's reagent) represents the method of choice and gives the synthetically versatile α-tosyloxy ketone 10. This intermediate provides a convenient vehicle for the attachment of spacer units to C-11 *via* a thioether linkage which has been applied to the synthesis of the dansylated [*N*-(5-dimethylamino-1-naphthylsulfonyl)] anatoxin-a derivatives. Preliminary biological data relating to the α-thiomethyl anatoxin-a derivative 16 and the dansylated ligands, 25 and 26, are also reported.

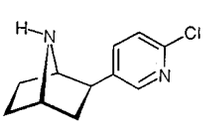
Introduction

The azabicyclic alkaloids, anatoxin-a **1**[1] and epibatidine **2**,[2] represent two of the most potent nicotinic agonists known and, as such, provide excellent starting points for the design of new molecular probes for the characterisation of neuronal nicotinic acetylcholine receptors.[3]

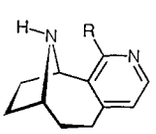
The total synthesis of these two natural products is recognised as an important challenge that has, as a consequence, stimulated much effort.[4,5] However, the pharmacological significance of these potent ligands cannot be ignored, and there is a need to shift some attention towards producing novel structural variants of these ligands that can be used to increase our understanding of the steric and electronic demands of the nicotinic receptor.[6] Total synthesis does, however, provide the only practical source of both **1** and **2** so any new programme aimed at extending the range of ligand analogues available must recognise that this represents an additional and substantial synthetic commitment.



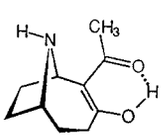
1 Anatoxin-a



2 Epibatidine



3 (R = H or CH_3)



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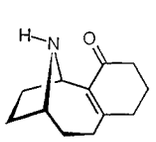

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In the anatoxin-a area, Rapoport and co-workers have described elegant synthetic routes to (+)-anatoxin-a [as well as the (−)-enantiomer and the racemate][7] and have also applied this chemistry to the production of a series of anatoxin-a analogues that have allowed a comprehensive structure–activity relationship to be established for this class of nicotinic ligand.[8]

In other related studies, the nicotine-anatoxin-a hybrid **3**[9] has been prepared and ligands **4**[10] and **5**,[11] conformationally constrained variants of the s-*cis* and s-*trans* enone conformers of anatoxin-a respectively, have been described.

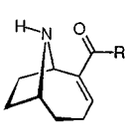
While these studies all aimed to address specific issues regarding the relationship between ligand structure/conformation and biological activity, those more traditional tools sought by the pharmacologist, such as radio-labelled and fluorescent derivatives based on anatoxin-a, have not yet been made available.[12] Such structural probes have obvious application in the study of receptor localisation and mechanism of action, however access to these tools requires that the appropriate synthetic chemistry is in place.

In this paper we describe a new method for activation and functionalisation at C-11 (the methyl residue of the acetyl side chain) of anatoxin-a. This chemistry allows for ready incorporation of thioether residues at this site and has also been applied to the synthesis of two dansyl (5-dimethylamino-1-naphthylsulfonyl)-containing fluorescent derivatives of anatoxin-a.

Results and discussion

When contemplating a significant structural modification, for example, the introduction of a spacer unit to an existing ligand template (such as anatoxin-a), there are two important questions that must be addressed. Firstly, which site (or sites) on the ligand skeleton will tolerate substitution but in the process retain an effective and useful biological profile? Secondly, have suitable synthetic methods been identified to allow for the selective chemical activation required at this preferred site on the ligand skeleton and what constraints, if any, does this methodology impose?

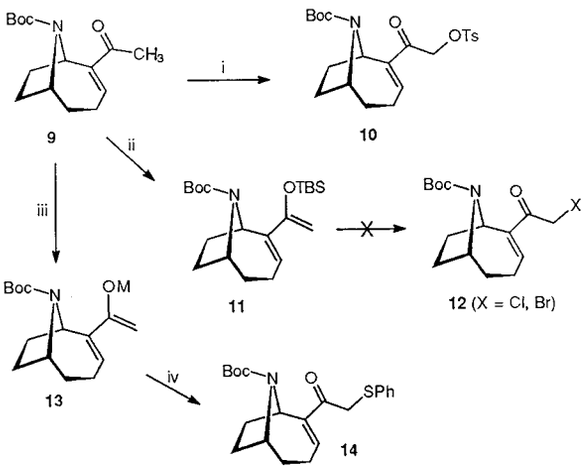
For anatoxin-a, both of these issues have been assessed. Anatoxin-a offers various options in terms of sites for substitution. The most obvious pathway, *via* direct *N*-alkylation, is known to result in loss of nicotinic potency[13] and, as a consequence, has not been pursued further. Modification at C-11 of anatoxin-a is, however, a viable alternative. Firstly, we have already demonstrated that extending the acetyl unit *via* alkylation of C-11 does not compromise biological activity.[14] The simple alkyl derivatives, homoanatoxin **6**[15,16] (an obvious candidate on which to base a tritiated ligand) and the propyl and



6 R = CH_2CH_3
7 R = $CH_2CH_2CH_3$
8 R = $CH(CH_3)_2$

isopropyl variants, **7** and **8** respectively, are all potent nicotinic ligands.[15] The choice of C-11 is also attractive from a synthetic perspective since this position should offer access to both nucleophilic (*via* an enolate) and electrophilic (*e.g. via* an α-halo ketone) reactivity profiles.

A number of options for activating C-11 of *N*-Boc anatoxin-a **9** (using the more readily available racemate) have been evaluated (see below), but the method of choice has involved use of hydroxy(tosyloxy)iodobenzene (Koser's reagent)[17] (Scheme 1).



Scheme 1 *Reagents and conditions:* i, PhI(OH)OTs, CH_2Cl_2, room temp., 68% based on recovered **9**; ii, KHMDS, then TBSCl, 88%; iii, MHMDS (M = Li or K); iv, PhSSPh, 55% (from **9** see text)

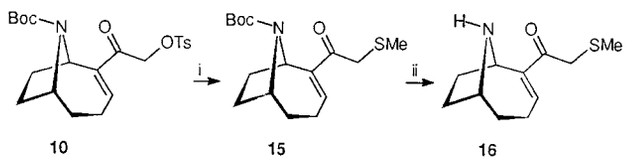
Treatment of (±)-**9** with 5 equiv. of the Koser reagent (in CH_2Cl_2 at room temp.) gave the α-tosyloxy ketone **10**, in 36% yield (68% based on recovered **9**). Using more extended reaction times in an attempt to increase the yield resulted in decomposition of **9** and/or **10**, and optimal conditions involved quenching the reaction prior to complete consumption of **9**. The desired product **10** was then readily separated from unreacted **9** and the latter was recycled.

We have also examined other options for functionalisation at C-11 of *N*-Boc-anatoxin-a **9**. Silyl enol ether **11**[7b] (TBS = Bu^t-Me_2Si) was prepared from **9**, but our attempts to convert this intermediate to an α-halo ketone **12** failed.† Enolate generation by direct deprotonation of the C-11 methyl group of **9** is achievable: methylation of enolate **13** (M = Li) occurs in a modest 40% yield and this chemistry has been used in a synthesis of (+)-homoanatoxin.[18] In this present study, enolate **13** (M = K) was trapped by diphenyl disulfide to furnish the α-phenylthio derivative **14** in 55% yield. However, we have encountered problems with this enolate-based chemistry; reactions (especially those involving alkylations) are capricious and the reactivity of enolate **13** (M = Li, Na or K) is limited in terms of the range of electrophiles that have been trapped. On balance, enolization of *N*-Boc-anatoxin-a **9** is not an attractive method for C-11 functionalisation.

Exploiting the electrophilic reactivity of 10

Since we planned to attach a fluorescent unit to anatoxin-a *via* a thioether linkage, it was necessary to evaluate the effect of this type of structural modification on biological activity. Exposure of the α-tosyloxy ketone **10** to sodium methanethiolate gave the α-thiomethyl derivative **15** which was deprotected to give the corresponding amine **16** in 82% overall yield (Scheme 2). While full details of the biological aspects of this study will be reported elsewhere, the racemic thiomethyl analogue **16**

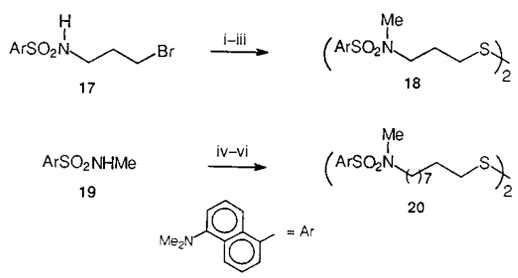
† A variety of halogenation methods were examined (for both ketone **9** and silyl enol ether **11**) based on direct halogenation or using *N*-halo reagents. In general, only decomposition products were observed.



Scheme 2 *Reagents and conditions:* i, NaSMe, THF, room temp. (91%); ii, CF_3CO_2H, CH_2Cl_2, then $NaHCO_3$, H_2O (90%)

inhibited [^{3}H]nicotine binding to brain membranes with an IC_{50} of 6.2 nM which is comparable to anatoxin-a itself.‡

Having established that introducing a simple thioether unit did not compromise the nicotinic activity associated with anatoxin-a, we set out to construct two fluorescent derivatives **25** and **26**; both targets constitute a dansyl unit connected *via* a methylene spacer arm and a thioether linkage to C-11 of the anatoxin-a skeleton. Using the dansyl amides **17** and **19**[19] the synthetic routes developed for the C_3-spacer **18** and C_9-spacer **20** units are shown in Scheme 3. The slightly different approaches for each series used simply reflect the options that were explored in this study rather than any specific problem that was encountered.



Scheme 3 *Reagents and conditions:* i, KSAc, DMF; ii, NaOMe then air; iii, NaH, MeI (94% overall yield for **18**); iv, NaH, $Br(CH_2)_9Br$; v, KSAc, DMF; vi, NaOMe then air (48% overall yield for **20**)

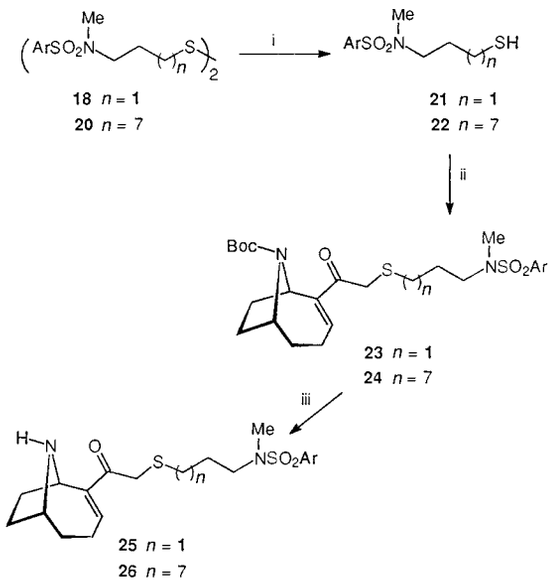
In each case, following cleavage of the intermediate thioacetates, the crude thiols **21** and **22** (which were isolable and characterised—see below) were exposed to air and converted to the corresponding disulfides **18** and **20** respectively. This was convenient to aid work-up, purification and storage.

The requisite thiol nucleophiles **21** and **22** were then regenerated by reductive cleavage of disulfides **18** and **20** respectively and were immediately coupled with α-tosyloxy ketone **10** to give the *N*-Boc-protected adducts **23** and **24** in 74 and 73% yields respectively (Scheme 4). Cleavage of the *N*-Boc moiety, which was essentially quantitative, gave the two dansylated anatoxin-a targets **25** and **26**.

While the thiomethyl analogue **16** was active, the two dansylated derivatives **25** and **26** did, however, show negligible potency at the [^{125}I]αbungarotoxin binding site (as compared to anatoxin-a itself). At the [^{3}H]nicotine binding site (which corresponds to the α4β2 nicotinic receptor), the simpler dansyl ligand **25** showed some affinity for the binding site but was more than two orders of magnitude less potent than anatoxin-a. Since the thioether linkage itself does not appear to contribute significantly to a reduction in nicotinic potency—the thiomethyl analogue **16** is active—it would seem that loss of activity is associated with the dansyl moiety.

While further work is needed to identify a viable fluorescent analogue of anatoxin-a, the α-tosyloxy ketone **10**, available in

‡ Competition binding assays were carried out using rat brain P2 membrane and [^{125}I]αbungarotoxin (1 nM) or [^{3}H]nicotine (10 nM) as described previously.[8a,8b,15] Ligands **16**, **25** and **26** were assayed over the concentration range 10^{-4}–10^{-9} M: the ligands were dissolved in DMSO to a concentration of 10 mM and diluted in assay buffer to yield the desired concentration. DMSO, diluted to correspond to the highest concentration of **25** and **26** examined, itself inhibited the binding of both radioligands by about 20%. Anatoxin-a was assayed in parallel as a positive control.



Scheme 4 *Reagents and conditions:* i, Zn, AcOH; ii, KHMDS, then **10** (**23**, 74%; **24**, 73% from **18** and **20** respectively); iii, CF_3CO_2H, CH_2Cl_2, then $NaHCO_3$, H_2O **25**, 100%; **26**, 100%)

one step from *N*-Boc-anatoxin-a, does represent a versatile synthon for exploring new structural variations and for constructing novel nicotinic ligands. Further studies in this area are underway to exploit the reactivity associated with **10** and, once viable ligands have been identified, then this chemistry can be applied to the synthesis of enantiomerically pure derivatives.

Experimental

General

All solvents and commercially available reagents were purified and dried as required, according to standard procedures. Light petroleum refers to that fraction boiling in the range 60–80 °C unless otherwise stated. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR Spectrophotometer by evaporation of a CH_2Cl_2 solution to produce a thin film on NaCl plates. ^{1}H NMR (270 MHz) spectra were recorded in $CDCl_3$ on a JEOL GNM FT270, with *J* values stated in Hz. Mass spectra were recorded with a Fisons/VG Analytical Autospec System. High resolution mass determinations were carried out on the same instruments as the corresponding low resolution spectra. Positive FAB spectra were obtained using a matrix of 3-nitrobenzyl alcohol.

(±)-9-(*tert*-Butoxycarbonyl)-2-[2-(tosyloxy)acetyl]-9-azabicyclo-[4.2.1]non-2-ene 10

To a solution of *N*-Boc-anatoxin-a **9** (120 mg, 0.45 mmol) in CH_2Cl_2 (8.5 cm^3) was added hydroxy(tosyloxy)iodobenzene (888 mg, 2.26 mmol). After stirring vigorously for 17 h, water (5 cm^3) was added, the organic phase separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 cm^3). The organic fractions were then combined and dried (Na_2SO_4), the solvent was removed *in vacuo*, and the residue was purified by flash chromatography (silica) to give unreacted *N*-Boc-anatoxin-a (56 mg) (15% EtOAc in light petroleum) followed by toluene-*p*-sulfonate **10** (71 mg, 36%, 68% based on recovered starting material) (using 50% EtOAc in light petroleum) as a colourless foam (Found: $M^+ + 1$, 436.1778. $C_{22}H_{30}NO_6S$ requires *M*, 436.1794); ν_{max}(film)/cm^{-1} 2972, 1688 and 1630; δ_H 1.20–2.42 (17 H, m), 2.45 (3 H, s), 4.28–4.42 (1 H, m), 4.84–5.30 (3 H, m), 6.71 (1 H, t, *J* 6.2), 7.36 (2 H, d, *J* 8.4) and 7.84 (2 H, d, *J* 8.4); *m*/*z* (CI) 436, 408, 380, 336 and 164.

(±)-9-(*tert*-Butoxycarbonyl)-2-[2-(phenylthio)acetyl]-9-aza-bicyclo[4.2.1]non-2-ene 14

A solution of *N*-Boc-anatoxin-a **9** (265 mg, 1 mmol) in THF (7 cm^3) was cooled to −78 °C under argon and treated with solid potassium hexamethyldisilazide (310 mg, 1.5 mmol) and the mixture was stirred for 25 min. After this time, diphenyl disulfide (545 mg, 2.5 mmol) was added and the mixture was stirred for 40 min. Water (15 cm^3) was added and, after warming to room temperature, the mixture was extracted with CH_2Cl_2 (3 × 15 cm^3). The combined extracts were washed with water (5 cm^3), dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 25% EtOAc in light petroleum) to give sulfide **14** (206 mg, 55%) as a colourless foam (Found: $M^+ + 1$, 374.1783. $C_{21}H_{28}NO_3S$ requires *M*, 374.1790); ν_{max}(film)/cm^{-1} 2974 and 1684; δ_H(as a mixture of amide rotomers) 1.35–2.40 (17 H, m), 4.00 (2 H, s), 4.24–4.43 (1 H, m), 5.14 (1 H, br t, *J* 8), 6.77 (1 H, t, *J* 7) and 7.20–7.43 (5 H, m); *m*/*z* (CI) 374, 318, 300 and 274.

(±)-9-(*tert*-Butoxycarbonyl)-2-[2-(thiomethyl)acetyl]-9-aza-bicyclo[4.2.1]non-2-ene 15

A solution of α-tosyloxy ketone **10** (50 mg, 0.11 mmol) in degassed THF (1.5 cm^3), under an atmosphere of argon, at room temperature was treated with sodium methanethiolate (16 mg, 0.23 mmol) and stirred for 30 min. After this time, saturated aqueous NH_4Cl (2 cm^3) was added, the product was extracted with CH_2Cl_2 (3 × 5 cm^3) and the combined extracts were dried (Na_2SO_4). Removal of solvent *in vacuo* and purification by flash chromatography (silica; 50% EtOAc in light petroleum) gave thioether **15** (32 mg, 91%) as a colourless oil (Found: $M^+ + 1$, 312.1637. $C_{16}H_{26}NO_3S$ requires *M*, 312.1633); ν_{max}(film)/cm^{-1} 1692 and 1683; δ_H(as a mixture of rotomers) 1.20–2.02 (17 H, m), 2.10/2.18 (3 H, 2 × s, SMe), 3.49–3.57 (2 H, m), 4.30–4.42 (1 H, m), 5.05–5.11 (1 H, m) and 6.82 (1 H, br t, *J* 6); *m*/*z* (CI) 312, 256, 210 and 164.

(±)-2-[2-(Thiomethyl)acetyl]-9-azabicyclo[4.2.1]non-2-ene 16

A solution of thioether **15** (25 mg, 0.08 mmol) in CH_2Cl_2 (2 cm^3) was treated with trifluoroacetic acid (0.4 cm^3) and the mixture was heated at reflux for 30 min. The mixture was then carefully treated with saturated aqueous sodium hydrogen carbonate (3 cm^3) and the product was extracted with CH_2Cl_2 (3 × 10 cm^3). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give amine **16** (15 mg, 90%) as a colourless oil; ν_{max}(film)/cm^{-1} 1690; δ_H 1.75–2.44 (8 H, m), 2.11 (3 H, SMe), 3.42 (1 H, d, *J* 14), 3.59 (1 H, d, *J* 14), 4.01 (1 H, m), 4.85 (1 H, br d, *J* 8.7) and 7.01 (1 H, br t, *J* 6.1); *m*/*z* (+FAB) 312 (M + 1), 197 and 165. This material, which was homogeneous by TLC, was not characterised further and was used directly for biological assay.

Preparation of spacer unit 18

***N*-(3-Bromopropyl)-5-dimethylaminonaphthalene-1-sulfonamide 17.** To a solution of 3-bromopropylamine hydrobromide (1.22 g, 5.55 mmol) and sodium hydrogen carbonate (800 mg) in water (20 cm^3) was added dansyl (5-dimethylaminonaphthalene-1-sulfonyl) chloride (1.25 g, 4.63 mmol) in acetone (40 cm^3). After 5 h, the reaction mixture was concentrated *in vacuo* and the aqueous phase extracted with EtOAc (3 × 50 cm^3). The organic extracts were combined, dried (Na_2SO_4) and concentrated to give the title compound **17** (1.67 g, 98%) as a yellow solid which was used without further purification (Found: M^+, 370.0349. $C_{15}H_{19}{}^{79}BrN_2O_2S$ requires *M*, 370.0351); ν_{max}(film)/cm^{-1} 3312 and 1572; δ_H 1.94 (2 H, quintet, *J* 6), 2.90 (6 H, s), 3.06 (2 H, q, *J* 6), 3.32 (2 H, t, *J* 6), 4.71 (1 H, br t, *J* 6), 7.20 (1 H, d, *J* 7), 7.28–7.61 (2 H, m), 8.26 (2 H, d, *J* 8) and 8.56 (1 H, d, *J* 8); *m*/*z* (EI) 372/370, 171 and 154.

***N*-(3-Acetylthiopropyl)-5-dimethylaminonaphthalene-1-sulfonamide.** To a solution of bromide **17** (1.64 g, 4.35 mmol) in DMF (20 cm^3) at 23 °C under nitrogen was added potassium thioacetate (522 mg, 4.57 mmol) and the mixture was heated at

40 °C for 1 h. The reaction mixture was then poured into water (250 cm^3) and extracted with Et_2O (2 × 125 cm^3). The organic extracts were combined, washed with water (250 cm^3) dried (Na_2SO_4), and the solvent removed *in vacuo* to give the title compound (1.60 g, 100%) as a yellow oil which was used without further purification (Found: $M^+ + 1$, 367.1143. $C_{17}H_{23}N_2O_3S_2$ requires *M*, 367.1150); ν_{max}(film)/cm^{-1} 3296 and 1688; δ_H 1.64 (2 H, quintet, *J* 7), 2.26 (3 H, s), 2.78 (2 H, t, *J* 7), 2.89 (6 H, s), 2.93 (2 H, q, *J* 7), 5.04 (1 H, m), 7.19 (1 H, d, *J* 7), 7.49–7.61 (2 H, m), 8.22–8.31 (2 H, m) and 8.53 (1 H, d, *J* 8.5); *m*/*z* (CI) 367, 325, 291 and 253.

***N*,*N*′-(4,5-Dithiaoctane-1,8-diyl)bis(5-dimethylaminonaphthalene-1-sulfonamide).** Under an argon atmosphere, a solution of *N*-(3-acetylthiopropyl)-5-dimethylaminonaphthalene-1-sulfonamide (1.30 g, 3.55 mmol) in methanol (10 cm^3) was added *via* a cannula to methanolic sodium methoxide [from sodium (408 mg, 17.76 mmol) and methanol (10 cm^3)] at room temperature. After 1 h, air was bubbled beneath the surface of the reaction mixture for 0.25 h, and after a further 4 h, saturated aqueous ammonium chloride (5 cm^3) followed by water (10 cm^3) was added to the mixture. The product was extracted with $CHCl_3$ (3 × 50 cm^3) and the organic extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 60% EtOAc in light petroleum) to give the title compound (1.14 g, 99%) as a yellow solid (Found: $M^+ + 1$, 647.1840. $C_{30}H_{39}N_4O_4S_4$ requires *M*, 647.1854); ν_{max}(film)/cm^{-1} 3296, 1588 and 1573; δ_H 1.68–1.78 (4 H, quintet, *J* 7), 2.49 (4 H, t, *J* 7), 2.89 (12 H, s), 2.97 (4 H, q, *J* 7), 4.89 (2 H, br t, *J* 7), 7.18 (2 H, d, *J* 7.5), 7.53 (4 H, m), 8.23–8.30 (4 H, m) and 8.54 (2 H, d, *J* 8.5); *m*/*z* (CI) 647, 615, 494 and 325.

***N*,*N*′-Dimethyl-*N*,*N*′-(4,5-dithiaoctane-1,8-diyl)bis(5-dimethylaminonaphthalene-1-sulfonamide) 18.** Under an argon atmosphere, a solution of *N*,*N*′-(4,5-dithiaoctane-1,8-diyl)-bis(5-dimethylaminonaphthalene-1-sulfonamide) (1.30 g, 2.01 mmol) in DMF (13 cm^3) was treated with NaH (60% dispersion in mineral oil; 201 mg, 5.03 mmol) and the mixture was heated at 60 °C. After 0.25 h, iodomethane (725 mg, 5.03 mmol) was slowly added to the reaction mixture, and after 0.5 h the mixture was cooled to room temperature and water (20 cm^3) was added. The product was extracted with $CHCl_3$ (3 × 50 cm^3) and the extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue purified by flash chromatography (silica; EtOAc) to give the title compound **18** (1.31 g, 97%) as a yellow solid (Found: $M^+ + 1$, 675.2178. $C_{32}H_{43}N_4O_4S_4$ requires *M*, 675.2167); ν_{max}(film)/cm^{-1} 2940, 2786 and 1572; δ_H 1.91 (4 H, quintet, *J* 7), 2.56 (4 H, t, *J* 7), 2.84 (6 H, s), 2.88 (12 H, s), 3.28 (4 H, t, *J* 7), 7.18 (2 H, d, *J* 7), 7.48–7.57 (4 H, m), 8.14–8.17 (2 H, m), 8.34 (2 H, d, *J* 8.5) and 8.54 (2 H, d, *J* 8.5); *m*/*z* (CI) 675, 339 and 171.

Preparation of spacer unit 20

***N*-Methyl-*N*-(9-bromononyl)-5-dimethylaminonaphthalene-1-sulfonamide.** Under an argon atmosphere, a solution of sulfonamide **19** [19] (440 mg, 1.66 mol) in DMF (4.5 cm^3) was treated with NaH (60% dispersion in mineral oil; 133 mg, 5.03 mmol) and the mixture was heated at 60 °C. After 0.5 h, 1,9-dibromononane (1.19 g, 4.16 mmol) was slowly added to the reaction mixture, and after 0.5 h the mixture was cooled to room temperature and water (20 cm^3) was added. The product was extracted with $CHCl_3$ (3 × 50 cm^3) and the extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 15% EtOAc in light petroleum) to give the title compound (505 mg, 65%) as a yellow oil (Found: $M^+ + 1$, 469.1503. $C_{22}H_{34}{}^{79}BrN_2O_2S$ requires *M*, 469.1524); ν_{max}(film)/cm^{-1} 2929, 2850, 2788 and 1572; δ_H 1.21–1.60 (12 H, m), 1.84 (2 H, quintet, *J* 7.5), 2.82 (3 H, s), 2.88 (6 H, s), 3.19 (2 H, t, *J* 7.5), 3.40 (2 H, t, *J* 6.5), 7.17 (1 H, d, *J* 7.5), 7.53–7.57 (2 H, m), 8.16 (1 H, d, *J* 8.5), 8.34 (1 H, d, *J* 8.5) and 8.52 (1 H, d, *J* 8.5); *m*/*z* (CI) 471/469, 389 and 171.

***N*-Methyl-*N*-(9-acetylthiononyl)-5-dimethylaminonaphthalene-1-sulfonamide.** Potassium thioacetate (136 mg, 1.19 mmol) was added to a solution of *N*-methyl-*N*-(9-bromononyl)-5-dimethylaminonaphthalene-1-sulfonamide (505 mg, 1.08 mmol) in DMF (9 cm^3) at room temperature and the reaction mixture was heated at 40 °C for 20 min. After this time water (50 cm^3) was added and the product was extracted with diethyl ether (3 × 40 cm^3). The organic extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 25% ethyl acetate in light petroleum) to give the title compound (481 mg, 96%) as a yellow oil (Found: $M^+ + 1$, 465.2245. $C_{24}H_{37}N_2O_3S_2$ requires *M*, 465.2246); ν_{max}(film)/cm^{-1} 2932, 2788, 1690 and 1572; δ_H 1.19–1.60 (14 H, m), 2.32 (3 H, s), 2.82–2.88 (2 H, m), 2.82 (3 H, s), 2.88 (6 H, s), 3.18 (2 H, t, *J* 7), 7.17 (1 H, d, *J* 7), 7.48–7.56 (2 H, m), 8.16 (1 H, d, *J* 8.5), 8.34 (1 H, d, *J* 8.5) and 8.52 (1 H, d, *J* 8.5); *m*/*z* (CI) 465, 423, 389 and 232.

***N*,*N*′-Dimethyl-*N*,*N*′-10,11-dithiaicosane-1,20-diyl)bis-(5-dimethylaminonaphthalene-1-sulfonamide) 20.** A solution of *N*-methyl-*N*-(9-acetylthiononyl)-5-dimethylaminonaphthalene-1-sulfonamide (550 mg, 1.19 mmol) in methanol (5 cm^3) was added *via* a cannula to methanolic sodium methoxide [from sodium (136 mg, 5.93 mmol) and methanol (5 cm^3)] at room temperature. After 1 h, the reaction mixture was poured into water (10 cm^3) and extracted with $CDCl_3$ (3 × 30 cm^3). The extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 20% EtOAc in light petroleum) to give the title compound (372 mg, 77%) as a yellow oil; ν_{max}(film)/cm^{-1} 2926, 2853, 2788, 1588 and 1573; δ_H 1.20–1.64 (30 H, m), 2.47–2.56 (4 H, m), 2.82 (6 H, s), 2.88 (12 H, s), 3.19 (2 H, t, *J* 7), 7.18 (2 H, d, *J* 7), 7.49–7.57 (4 H, m), 8.16 (2 H, d, *J* 8.5), 8.35 (2 H, d, *J* 8.5) and 8.53 (2 H, d, *J* 8.5); *m*/*z* (CI) 423. This disulfide was not characterised further but the corresponding thiol **22** was characterised.

***N*-Methyl-*N*-(3-mercaptopropyl)-5-dimethylaminonaphthalene-1-sulfonamide 21.** Disulfide **18** (230 mg, 0.34 mmol) was dissolved in acetic acid (4.6 cm^3) at room temperature and zinc dust (460 mg) was added. The reaction mixture was stirred vigorously for 6 h then poured into a solution of saturated aqueous sodium hydrogen carbonate (100 cm^3). The resulting mixture was extracted with ethyl acetate (3 × 75 cm^3), the extracts were combined, dried (Na_2SO_4), and the solvent removed *in vacuo*. The residue was purified by flash chromatography (silica; 50% EtOAc in light petroleum) to give thiol **21** (146 mg, 84% based on recovered disulfide **18**) as a yellow oil (Found: $M^+ + 1$, 339.1208. $C_{16}H_{23}N_2O_2S_2$ requires *M*, 339.1201); ν_{max}(film)/cm^{-1} 2620 and 1572; δ_H 1.36 (1 H, t, *J* 8.1), 1.78–1.88 (2 H, m), 2.45–2.53 (2 H, m), 2.84 (3 H, s), 2.89 (6 H, s), 3.31 (2 H, t, *J* 6.5), 7.18 (1 H, d, *J* 7.5), 7.50–7.58 (2 H, m), 8.16–8.19 (1 H, m), 8.34 (1 H, d, *J* 8.5) and 8.54 (1 H, d, *J* 8.5); *m*/*z* (CI) 339, 170 and 104.

(±)-9-(*tert*-Butoxycarbonyl)-2-({3-[*N*-methyl-*N*-(5-dimethylamino-1-naphthyl)sulfonamido]propylthio}acetyl)-9-azabicyclo-[4.2.1]non-2-ene 23. A solution of thiol **21** (70 mg, 0.21 mmol) and α-tosyloxy ketone **10** (60 mg, 0.14 mmol) in degassed THF (2.5 cm^3), under an atmosphere of argon, was cooled to 0 °C and potassium hexamethyldisilazide (39 mg, 0.19 mmol) added. After 20 min, water (2 cm^3) was added and the mixture was extracted with $CHCl_3$ (3 × 75 cm^3). The extracts were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by flash chromatography (silica; 50% EtOAc in light petroleum) to give thioether **23** (73 mg, 88%) as a yellow solid (Found: M^+, 601.2637. $C_{31}H_{43}N_3O_5S_2$ requires *M*, 601.2644); ν_{max}(film)/cm^{-1} 1694, 1683 and 1651; δ_H(as a mixture of rotomers) 1.37–2.55 (21 H, m), 2.84 (3 H, s), 2.88 (6 H, s), 3.29 (2 H, br t, *J* 6), 3.48 (2 H, br s), 4.41–4.44 (1 H, m), 5.07–5.10 (1 H, m), 6.87 (1 H, t, *J* 5.5), 7.17 (1 H, d, *J* 7.5), 7.49–7.57 (2 H, m), 8.15 (1 H, d, *J* 8.5), 8.33 (1 H, d, *J* 8.5) and 8.53 (1 H, d, *J* 8.5); *m*/*z* (EI) 601, 502 and 170.

(±)-2-({3-[*N*-Methyl-*N*-(5-dimethylamino-1-naphthyl)sulfonamido]propylthio}acetyl)-9-azabicyclo[4.2.1]non-2-ene 25. Thioether **23** (17 mg, 0.03 mmol) was dissolved in CH_2Cl_2 (1.7 cm^3) at room temperature and trifluoroacetic acid (0.3 cm^3) was added. The reaction mixture was warmed to 40 °C for 0.5 h and cooled and saturated aqueous sodium hydrogen carbonate (5 cm^3) was slowly added. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3). The organic extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo* to give the title compound **25** (14 mg, 100%) as a yellow solid; δ_H 0.78–2.38 (11 H, m), 2.41–2.46 (2 H, m), 2.76 (3 H, s), 2.81 (6 H, s), 3.21 (2 H, br t, *J* 7), 3.33 (1 H, d, *J* 14), 3.45 (1 H, d, *J* 14), 3.76–3.81 (1 H, m), 4.60 (1 H, d, *J* 8.8), 6.84 (1 H, t, *J* 5.5), 7.10 (1 H, d, *J* 7), 7.42–7.47 (2 H, m), 8.09 (1 H, d, *J* 8.4), 8.26 (1 H, d, *J* 8.4) and 8.46 (1 H, d, *J* 8.4); *m*/*z* (+FAB) 502 (M + 1), 459, 429, 281, 207 and 147. We were unable to obtain satisfactory high resolution mass or microanalytical data for this compound and this material, which was homogeneous by TLC, was used directly for biological assays.

***N*-Methyl-*N*-(9-mercaptononyl)-5-dimethylaminonaphthalene-1-sulfonamide 22.** Disulfide **20** (185 mg, 0.22 mmol) was dissolved in acetic acid (3.7 cm^3) and zinc dust (500 mg) was added. The reaction mixture was stirred vigorously for 6 h then poured into saturated aqueous sodium hydrogen carbonate (15 cm^3). The resulting mixture was extracted with $CHCl_3$ (3 × 15 cm^3) and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give thiol **22** (184 mg, 99%) as a yellow oil which was used without further purification (Found: $M^+ + 1$, 423.2122. $C_{22}H_{35}N_2O_2S_2$ requires *M*, 423.2139); ν_{max}(film)/cm^{-1} 2620 and 1572; δ_H 1.11–1.68 (15 H, m), 2.46–2.56 (2 H, m), 2.82 (3 H, s), 2.88 (6 H, s), 3.20 (2 H, br t, *J* 6.5), 7.17 (1 H, d, *J* 7.5), 7.48–7.57 (2 H, m), 8.17 (1 H, d, *J* 8.5), 8.35 (1 H, d, *J* 8.5) and 8.54 (1 H, d, *J* 8.5); *m*/*z* (CI) 423, 391 and 172.

(±)-9-(*tert*-Butoxycarbonyl)-2-({9-[*N*-methyl-*N*-(5-dimethylamino-1-naphthyl)sulfonamido]nonylthio}acetyl)-9-azabicyclo-[4.2.1]non-2-ene 24. A solution of thiol **22** (36 mg, 0.09 mmol) and α-tosyloxy ketone **10** (25 mg, 0.06 mmol) in degassed THF (2 cm^3), under an atmosphere of argon, was cooled to 0 °C and potassium hexamethyldisilazide (17 mg, 0.08 mmol) was added. After 20 min, water (4 cm^3) was added and the mixture was extracted with $CHCl_3$ (3 × 15 cm^3). The extracts were dried (Na_2SO_4) and concentrated *in vacuo* to afford a residue which was purified by flash chromatography (silica; 50% ethyl acetate in light petroleum) to give thioether **24** (29 mg, 74%) as a yellow oil (Found: M^+, 685.3573. $C_{37}H_{55}N_3O_5S_2$ requires *M*, 685.3583). ν_{max}(film)/cm^{-1} 1692; δ_H(as a mixture of rotomers) 1.20–2.47 (31 H, m), 2.53 (2 H, m), 2.82 (3 H, s), 2.88 (6 H, s), 3.19 (2 H, t, *J* 7), 3.51 (2 H, br s), 4.41–4.44 (1 H, m), 5.07–5.10 (1 H, m), 6.89 (1 H, t, *J* 5.9), 7.17 (1 H, d, *J* 7), 7.48–7.56 (2 H, m), 8.16 (1 H, d, *J* 8.5), 8.35 (1 H, d, *J* 8.5) and 8.52 (1 H, d, *J* 8.5); *m*/*z* (EI) 685, 658 and 423.

(±)-2-({9-[*N*-Methyl-*N*-(5-dimethylamino-1-naphthyl)sulfonamido]nonylthio}acetyl)-9-azabicyclo[4.2.1]non-2-ene 26. A solution of thioether **24** (22 mg, 0.03 mmol) in CH_2Cl_2 (2 cm^3) containing trifluoroacetic acid (0.4 cm^3) was warmed to 40 °C for 0.5 h. After cooling, saturated aqueous sodium hydrogen carbonate (10 cm^3) was slowly added and the organic layer separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3) and the organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give the title compound **26** (19 mg, 100%) as a yellow oil δ_H 0.85–2.55 (25 H, m), 2.82 (3 H, s), 2.88 (6 H, s), 3.19 (2 H, t, *J* 7.5), 3.45 (1 H, d, *J* 14), 3.55 (1 H, d, *J* 14), 3.79–3.83 (1 H, m), 4.62 (1 H, br d, *J* 8.5), 6.89 (1 H, br t, *J* 6.2), 7.17 (1 H, d, *J* 7.5), 7.48–7.56 (2 H, m), 8.16 (1 H, d, *J* 8.5), 8.34 (1 H, d, *J* 8.5) and 8.53 (1 H, d, *J* 8.5); *m*/*z* (+FAB) 586 (M + 1). We were unable to obtain satisfactory additional high resolution mass or microanalytical data for this compound and this material, which was homogeneous by TLC, was used directly for biological assays.

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