"Clicked"-calix[4]pyrrole: a new membrane transporter for chloride†

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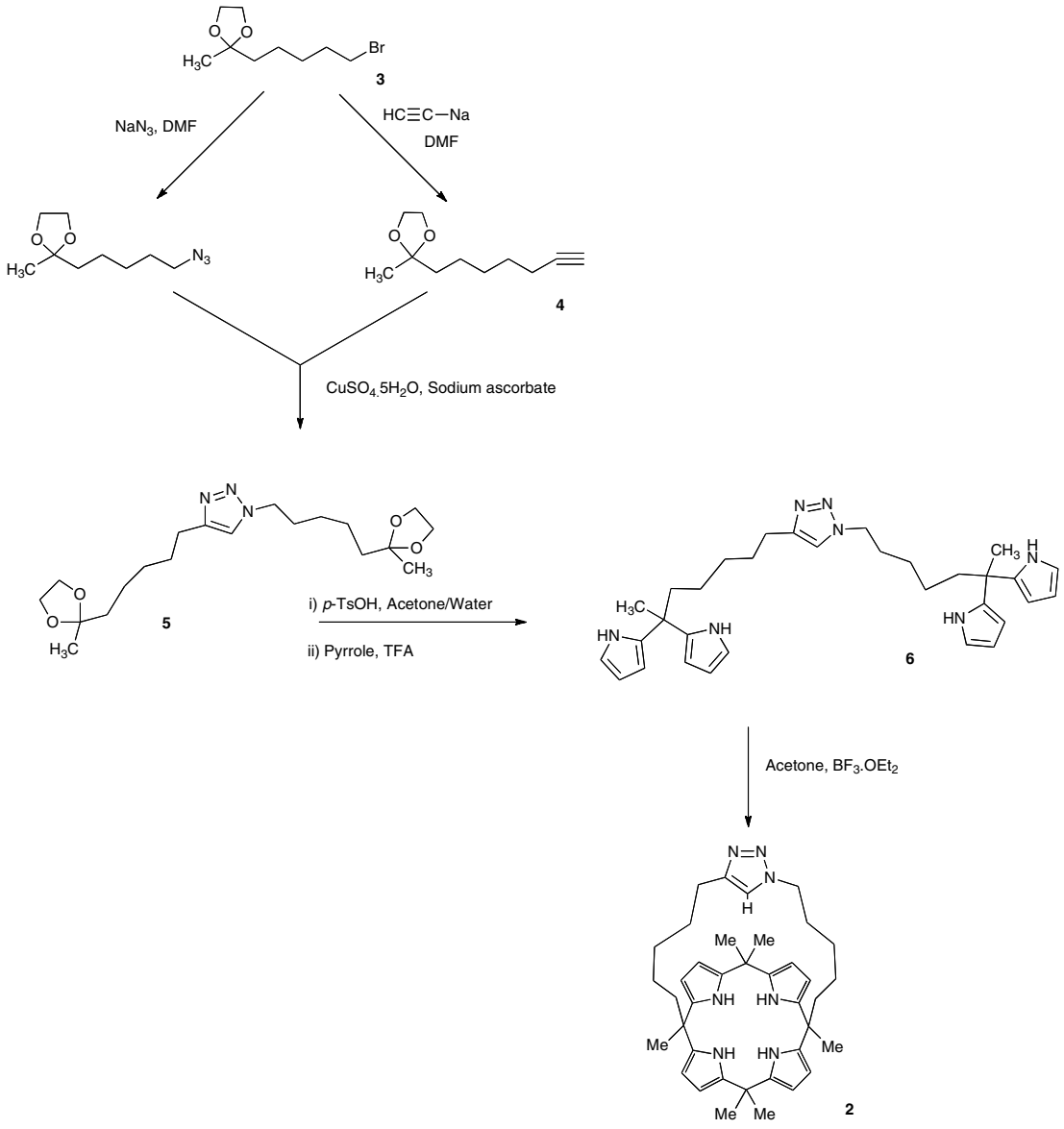
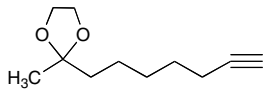
Electronic supplementary information



Scheme S1 - The synthesis of compound **2**.

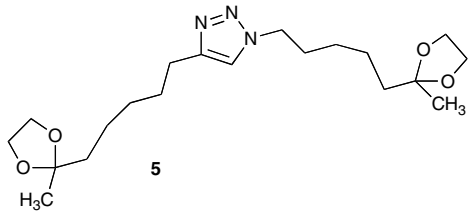
Synthesis summary:

2-(5-Bromopentyl)-2-methyl-1,3-dioxolane was prepared according to a literature procedure[1] and was then converted to 2-(hept-6-ynyl)-2-methyl-1,3-dioxolane by reaction with sodium acetylide in DMF at room temperature. The analogous azide was prepared by reaction of the bromoketal with sodium azide in DMF at 60˚C and was used *in situ* to prepare the triazole by subsequent addition of the alkyne to the reaction mixture together with copper sulfate (10 mol%) and sodium ascorbate (20 mol %) (Huisgen cycloaddition).[2] The triazole strap was deprotected using *cat. p*-TsOH in acetone/water to afford the diketone which was then converted to the bis-dipyrromethane by reaction with pyrrole in the presence of TFA. The bis-dipyrromethane was subsequently converted to the strapped calix[4]pyrrole **2** by reaction with acetone in the presence of $BF_3.Et_2O$.



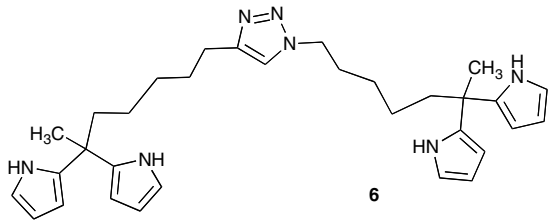
4

2-(Hept-6-ynyl)-2-methyl-1,3-dioxolane (4):- 2-(5-Bromopentyl)-2-methyl-1,3-dioxolane **3** was prepared by literature procedures.[3],[4] Compound **3** (4g, 0.017mol) was taken up in dry DMF (~15ml) and cooled to 0˚C under argon. To this solution was added a slurry of 18 wt. % sodium acetylide in xylene/light mineral oil (6.8ml). The resulting slurry was allowed to reach room temperature and stirred overnight. The reaction mixture was diluted with diethyl ether and quenched with water. The organic layer was then washed with 1M HCl, brine and water, dried over sodium sulfate and concentrated *in-vacuo*. The crude material was flash columned on silica gel eluted with hexanes:ethyl acetate (4:1) to leave a colourless oil (2.82g, 92%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ1.18 (s, 3H), δ1.29 (m, 4H), δ1.44 (m, 2H), δ1.52 (m, 2H), δ1.84 (t, 1H), δ2.06 (m, 2H), δ3.80 (m, 4H). $^{13}C\{^1H\}$-NMR ($CDCl_3$, 75MHz) δ19.8 (CH_2), δ25.1 (CH_2), δ25.2 (CH_2), δ30.0 (CH_2), δ30.4 (CH_2), δ40.6 (CH_2), δ66.1 (2xCH_2), δ69.8 (CH), δ86.0 (C), δ111.5 (C). LR-MS (CI) m/z Calcd: 182.3 Found: 183.2 $(M+H)^+$. HR-MS (EI+) m/z Calcd: 167.1072 $(M-CH_3)^+$ Found: 167.1076 $(M-CH_3)^+$. IR (cm^{-1}) 3309, 2929, 2865, 1376, 1252, 1218.



5

1,4-Bis(6-(2-methyl-1,3-dioxolan-2-yl)hexyl)-1*H*-1,2,3-triazole (5):- Compound **3** (3.8g, 0.016mol) and sodium azide (3.12g, 0.048mol) were stirred in DMF (15ml) overnight at 60 ^{o}C. The reaction was cooled to room temperature and compound **4** (2.75g, 0.015mol) added in DMF (5ml). Copper sulfate pentahydrate (400mg, 10mol %) and sodium ascorbate (634mg, 20mol %) were dissolved in water (5ml) and added to the reaction and the resulting mixture stirred overnight at room temperature. Water was added and the mixture extracted with ethyl acetate. The organic layer was washed with 5% aqueous sodium hydrogencarbonate and brine, dried over magnesium sulfate, concentrated and columned on silica gel eluted with 2% methanol/DCM. This yielded a colourless oil (2.94g, 50%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ1.17 (s, 6H), δ1.30 (m, 8H), δ1.56 (m, 6H), δ1.78 (m, 2H), δ2.58 (t, 2H), δ3.78 (m, 8H), δ4.17 (t, 2H), δ7.19 (s, 1H). ^{13}C{^{1}H}-NMR ($CDCl_3$, 100MHz) δ22.7 (CH_2), δ22.9 (2xCH_3), δ23.0 (CH_2), δ24.8 (CH_2), δ25.9 (CH_2), δ28.6 (CH_2), δ28.7 (CH_2), δ29.5 (CH_2), δ38.1 (CH_2), δ38.3 (CH_2), δ49.4 (CH_2), δ63.8 (4xCH_2), δ109.1 (C), δ109.3 (C), δ119.7 (CH), δ147.3 (C). LR-MS (ESI+) m/z Calcd: 381.5 Found: 382.4 $(M+H)^+$, 404.4 $(M+Na)^+$. HR-MS (ES+) m/z Calcd: 382.2706 $(M+H)^+$, 404.2525 $(M+Na)^+$ Found: 382.2694 $(M+H)^+$, 404.2515 $(M+Na)^+$. IR (cm^{-1}) 3398, 2936, 2862, 1709, 1360.

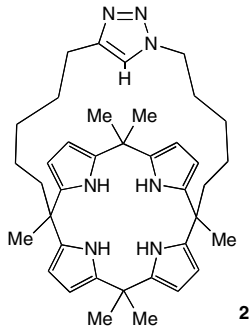


6

1,4-Bis(6,6-di(pyrrol-2-yl)heptyl)-1*H*-1,2,3-triazole (6):- Compound **5** (1.43g, 3.74mmol) was stirred in 10% water/acetone (50ml) with *para*-toluenesulfonic acid (700mg, ~1eqv.) for 20hrs at room temperature. The reaction mixture was concentrated, taken up with a little 5% aqueous sodium hydrogencarbonate solution, extracted with DCM, dried over sodium sulfate and concentrated to leave an off-

yellow oil in quantitative yield which was used in the next step without further purification or analysis.

The oil (1g, 3.4mmol) was taken up in pyrrole (9.46ml, 0.14mol) and TFA (141µl, 1.67mmol) was added with stirring. The resulting mixture was then stirred at 60^{o}C for 0.5hrs. The reaction was quenched with 0.1M NaOH and the solution extracted with DCM and dried over sodium sulfate. The crude material was gradient columned on silica gel, starting with hexanes:ethyl acetate (4:1) to remove pyrrole and two by-products and increasing to hexanes:ethyl acetate (1:1) to remove the product as an off-white foam (812mg, 45%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ1.22 (m, 8H), δ1.55 (s, 6H), δ1.62 (m, 2H), δ1.82 (m, 2H), δ1.92 (m, 4H), δ2.64 (t, 2H), δ4.22 (t, 2H), δ6.04 (s, 4H), δ6.11 (s, 4H), δ6.57 (s, 4H), δ7.17 (s, 1H), δ7.74 (s, 2H), δ7.78 (s, 2H). ^{13}C{^{1}H}-NMR (CD_2Cl_2, 75MHz) δ24.2 (CH_2), δ24.5 (CH_2), δ25.8 (CH_2), δ26.2 (CH_3), δ27.2 (CH_2), δ29.7 (CH_2), δ29.9 (CH_2), δ30.6 (CH_2), 39.3 (C), 41.3 (CH_2), 50.3 (CH_2), 104.7 (CH), 108.0 (CH), 117.1 (CH), 120.9 (CH), 138.4 (C), 138.6 (C), 148.4 (C). LR-MS (ESI+) m/z Calcd: 525.7 Found: 548.6 $(M+Na)^{+}$. HR-MS (ES+) m/z Calcd: 548.3478 $(M+Na)^{+}$ Found: 548.3466 $(M+Na)^{+}$. IR (cm^{-1}) 3304, 2938, 1648, 1554, 1241.



2

Clicked-calix[4]pyrrole (2):- Compound **6** (808mg, 1.54mmol) was dissolved in acetone (150ml) under argon and $BF_3.OEt_2$ (289µl, 1.5eqv.) was added. The resulting solution was stirred at room temperature for 3hrs and quenched with 0.1M NaOH. The solution was extracted with DCM exhaustively, dried over sodium sulfate and flash columned on silica gel eluted with hexanes:ethyl acetate (1:1) to leave a pink oil. This oil was triturated with methanol which produced fine white crystals which were collected and washed with ice-cold methanol to yield the pure title compound (41mg,

5%). ^{1}H-NMR (CD_3CN, 300MHz) δ1.05 (m, 5H), δ1.30 (m, 5H), δ1.36 (s, 6H), δ1.45 (s, 12H), δ1.57 (m, 3H), δ1.76 (m, 3H), δ2.61 (t, 2H), δ4.26 (t, 2H), δ5.83 (s, 8H), δ7.53 (s, 1H), δ7.95 (s, 4H). ^{13}C{^{1}H}-NMR (CD_3CN, 75MHz) δ24.4 (CH_2), δ24.7 (CH_2), δ26.1 (CH_2), δ26.2 (CH_2), δ27.0 (CH_2), δ28.2 (2xCH_3), δ28.8 (CH_2), δ30.2 (4xCH_3), 30.9 (CH_2), 31.6 (CH_2), 35.6 (CH_2), 41.4 (2xC), 41.5 (2xC), 50.7 (CH_2), 103.9 (4xCH), 105.1 (4xCH), 122.5 (CH), 138.3 (2xC), 138.4 (2xC), 138.7 (2xC), 138.8 (2xC), 149.1 (C). LR-MS (ESI+) m/z Calcd: 605.9 Found: 606.6 $(M+H)^+$, 628.6 $(M+Na)^+$. HR-MS (ES+) m/z Calcd: 606.4284 $(M+H)^+$ Found: 606.4271 $(M+H)^+$. IR (cm^{-1}) 3294, 2935, 1708, 1363.

Vesicle studies

A chloroform solution of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (22.32 mg/mL) (Genzyme) or a 7: 3 POPC–cholesterol mixture was evaporated using a rotary evaporator and the resulting lipid film was dried under high vacuum for at least 2 h. The lipid film was rehydrated in 1 mL of 488 mM MCl (M = Na, K, Rb, Cs) solution buffered to pH = 7.2 using 5 mM phosphate buffer solution per mL of lipid solution by vortexing. The lipid suspension was then subjected to nine freeze–thaw cycles and twenty-nine extrusions through a 200 nm polycarbonate nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.) to obtain unilamellar vesicles. The vesicles were dialysed against a $NaNO_3$ solution (488 mM $NaNO_3$ and 5 mM phosphate buffer, pH 7.2) or Na_2SO_4 (162 mM Na_2SO_4 and 5 mM phosphate buffer, pH 7.2) to remove unencapsulated MCl salts. The vesicles were diluted to 5 mL to obtain a solution with 5.87 mM of lipid.

Samples to assay the activity of **2** were prepared by diluting 0.8 mL of vesicles solution (at 5.87 mM of lipid) to 5 mL with either 488 mM $NaNO_3$ or 162 mM Na_2SO_4 buffered at pH 7.2 with 5 mM phosphate buffer to give a final lipid concentration of 1mM. A 20 uL aliquot of 10 mM **2** in DMSO was added and the chloride release from vesicles was monitored using an Accumet chloride selective electrode for 7 min. At 5 min, the vesicles were lysed with detergent (polyoxyethylene (8) lauryl ether) to release all chloride ions; the resulting value was the maximum amount of chloride in the system and was taken as the value for 100% chloride effluxed.

[1]W.-C. Zhang and C.-J. Li, *J. Org. Chem.* 2000, **65**, 5831-5833. H. Rudler and T. Durand-Reville, *J. Organometallic Chem.,* **2001**, *617*, 571-587; C.-H. Lee, J.-S. Lee, H.-K. Na, D.-W. Yoon, H. Miyaji, W.-S. Cho, and J.L. Sessler, *J. Org. Chem.,* 2005, **70**, 2067-2074.

[2]V.V. Rostovtsev, L.G. Green, V.V. Fokin and K.B. Sharpless, *Angew. Chem. Int. Ed.* 2002, **41**, 2596-2599.

[3] W.-C. Zhang and C.-J. Li, *J. Org. Chem.,* **2000**, *65*, 5831-5833.

[4] H. Rudler and T. Durand-Reville, *J. Organometallic Chem.,* **2001**, *617*, 571-587.

Crystallography. DMSO solvates.

Crystals from DMSO were obtained from two separate batches of receptor **2** by dissolving the compound in the minimum DMSO and allowing to crystallise. The structures are both DMSO solvates containing two and three molecules of solvent per calixpyrrole.

Compound **2**.$(DMSO)_2$

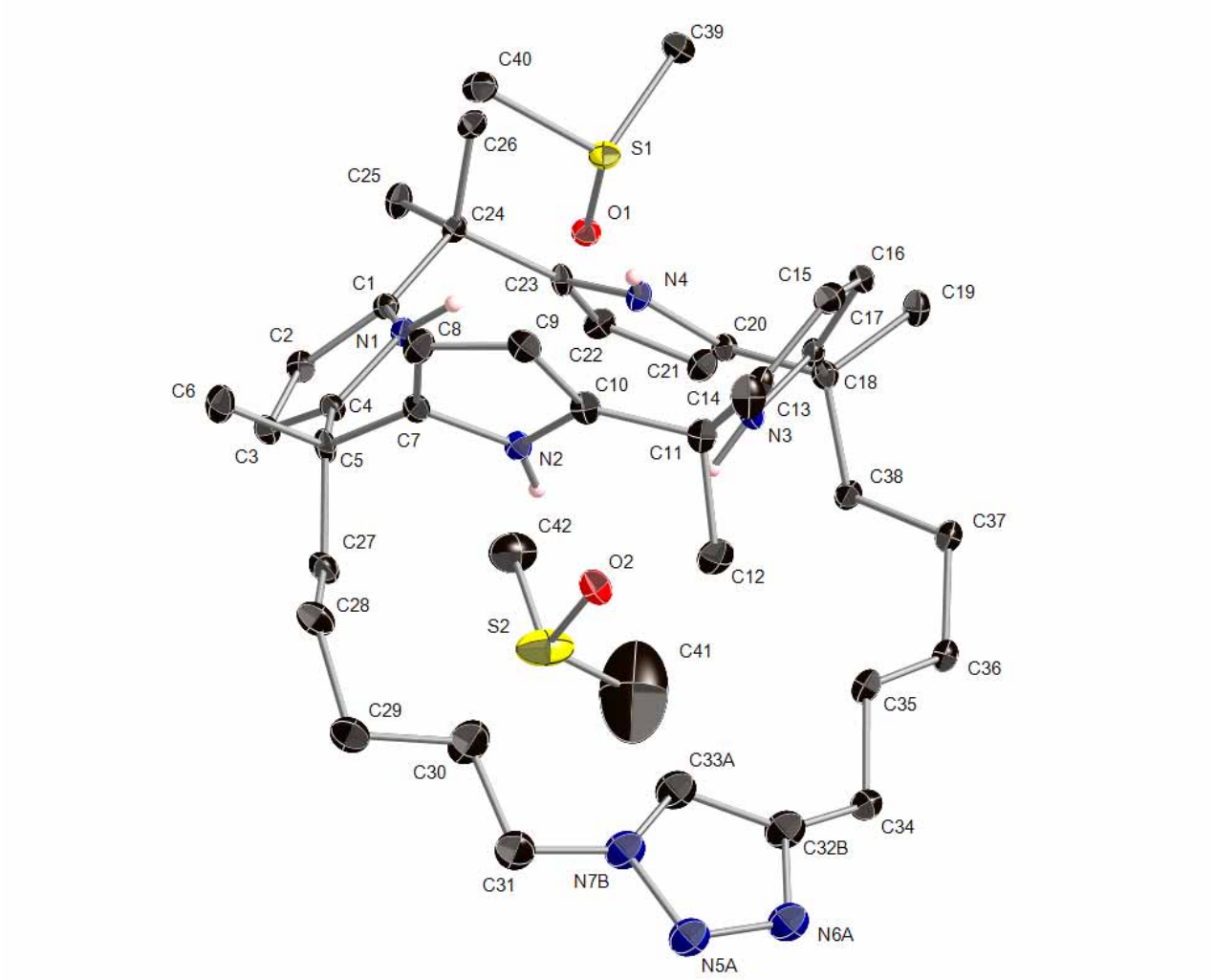


Figure S1 - Compound **2**·$(DMSO)_2$ Thermal ellipsoids drawn at the 35% probability level

Table S1. Crystal data and structure refinement details for compound **2**·(DMSO)$_2$

Identification code	**2009sot0158**
Empirical formula	$C_{42}H_{63}N_7O_2S_2$ $C_{38}H_{51}N_7$, 2(C_2H_6OS)
Formula weight	762.11
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*–1
Unit cell dimensions	a = 10.5958(3) Å α = 84.7310(10)° b = 11.3296(3) Å β = 89.8080(10)° c = 17.7452(4) Å γ = 76.5870(10)°
Volume	2063.03(9) Å^3
Z	2
Density (calculated)	1.227 Mg / m^3
Absorption coefficient	0.173 mm^{-1}
F(000)	824
Crystal	Fragment; Colourless
Crystal size	0.3 × 0.2 × 0.04 mm^3
θ range for data collection	3.01 – 27.48°
Index ranges	$-13 \le h \le 13$, $-14 \le k \le 14$, $-22 \le l \le 23$
Reflections collected	36159
Independent reflections	9378 [R_{int} = 0.0691]
Completeness to θ = 27.48°	99.2 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9931 and 0.9498
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9378 / 15 / 462
Goodness-of-fit on F^2	2.012
Final R indices [$F^2 > 2\sigma(F^2)$]	*R1* = 0.1338, *wR2* = 0.3323
R indices (all data)	*R1* = 0.1744, *wR2* = 0.3578
Largest diff. peak and hole	4.558 and –1.762 e Å^{-3}

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit*). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.10 S**tructure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). S**tructure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model.
One of the DMSO molecules is disordered and is surrounded by various significant electron density peaks. Various attempts were made to refine a number of DMSO PARTS using rigid body restraints. However, even with 4 PARTS there were still residual peaks. It was decided to revert to the original model as adding extra parameters had not improved the fit.

The triazole ring is disordered over the two possible configurations

Compound **2**.$(DMSO)_3$

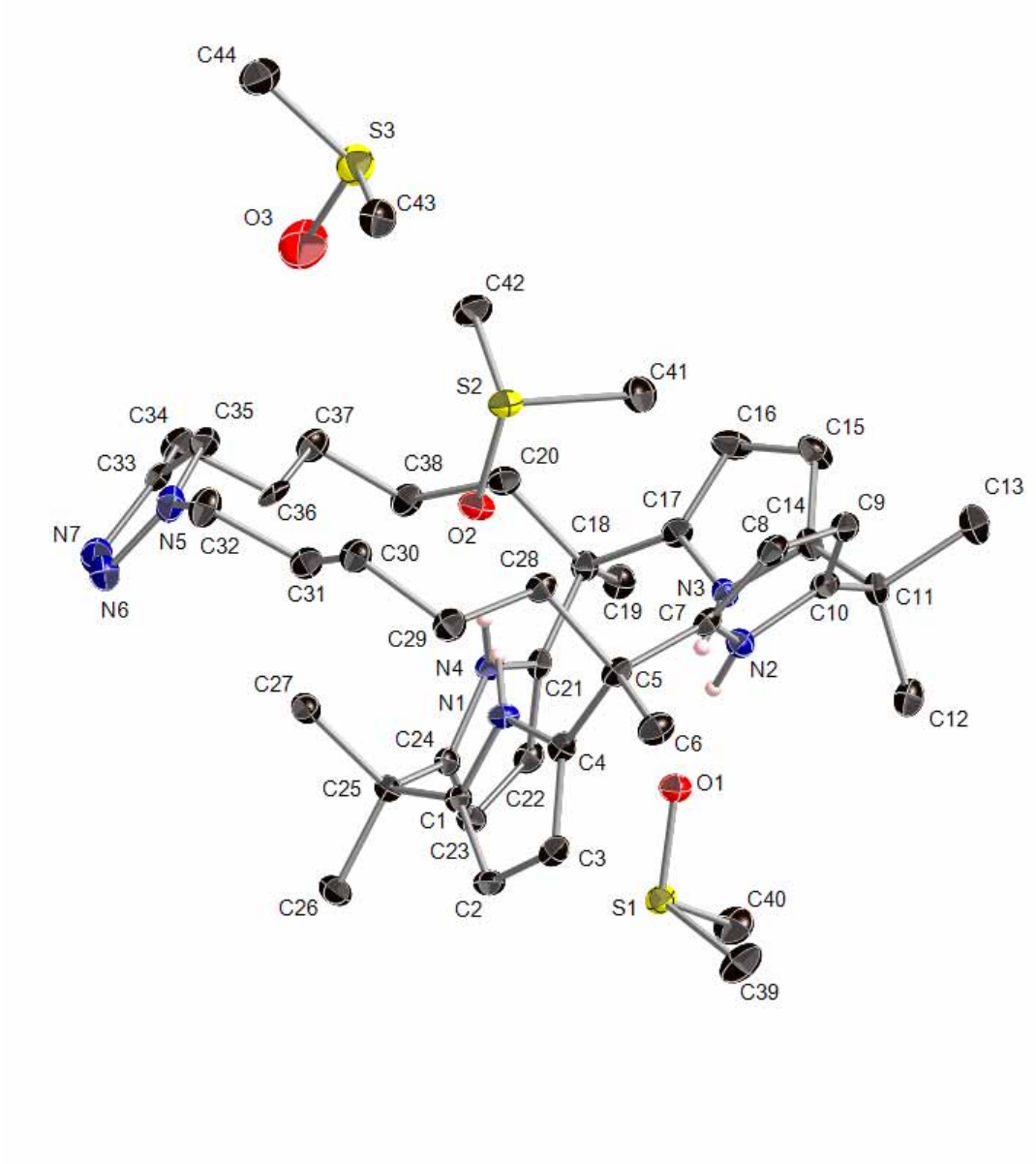


Figure S2 - Compound **2**·$(DMSO)_3$ Thermal ellipsoids drawn at the 35% probability level

Table S2. Crystal data and structure refinement details.

Identification code	**2009sot0155**
Empirical formula	$C_{44}H_{69}N_7O_3S_3$
	$C_{38}H_{51}N_7$, $3(C_2H_6OS)$
Formula weight	840.24
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	a = 10.4348(2) Å
	b = 22.9357(3) Å
	β = 100.8400(10)°
	c = 19.7522(3) Å
Volume	4642.93(13) Å^3
Z	4
Density (calculated)	1.202 Mg / m^3
Absorption coefficient	0.205 mm^{-1}
F(000)	1816
Crystal	Block; Colourless
Crystal size	0.2 × 0.2 × 0.2 mm^3
θ range for data collection	3.00 – 25.03°
Index ranges	$-12 \le h \le 12$, $-27 \le k \le 27$, $-23 \le l \le 23$
Reflections collected	44419
Independent reflections	8189 [R_{int} = 0.0721]
Completeness to θ = 25.03°	99.7 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9798 and 0.9501
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	8189 / 0 / 542
Goodness-of-fit on F^2	1.560
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0658, $wR2$ = 0.1078
R indices (all data)	$R1$ = 0.0950, $wR2$ = 0.1159
Largest diff. peak and hole	0.383 and –0.432 e Å^{-3}

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit*). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.10 S**tructure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). S**tructure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

Special details: All hydrogen atoms were located form the difference map and then placed in geometric positions and refined using a riding model, except those attached to nitrogen which were freely refined.

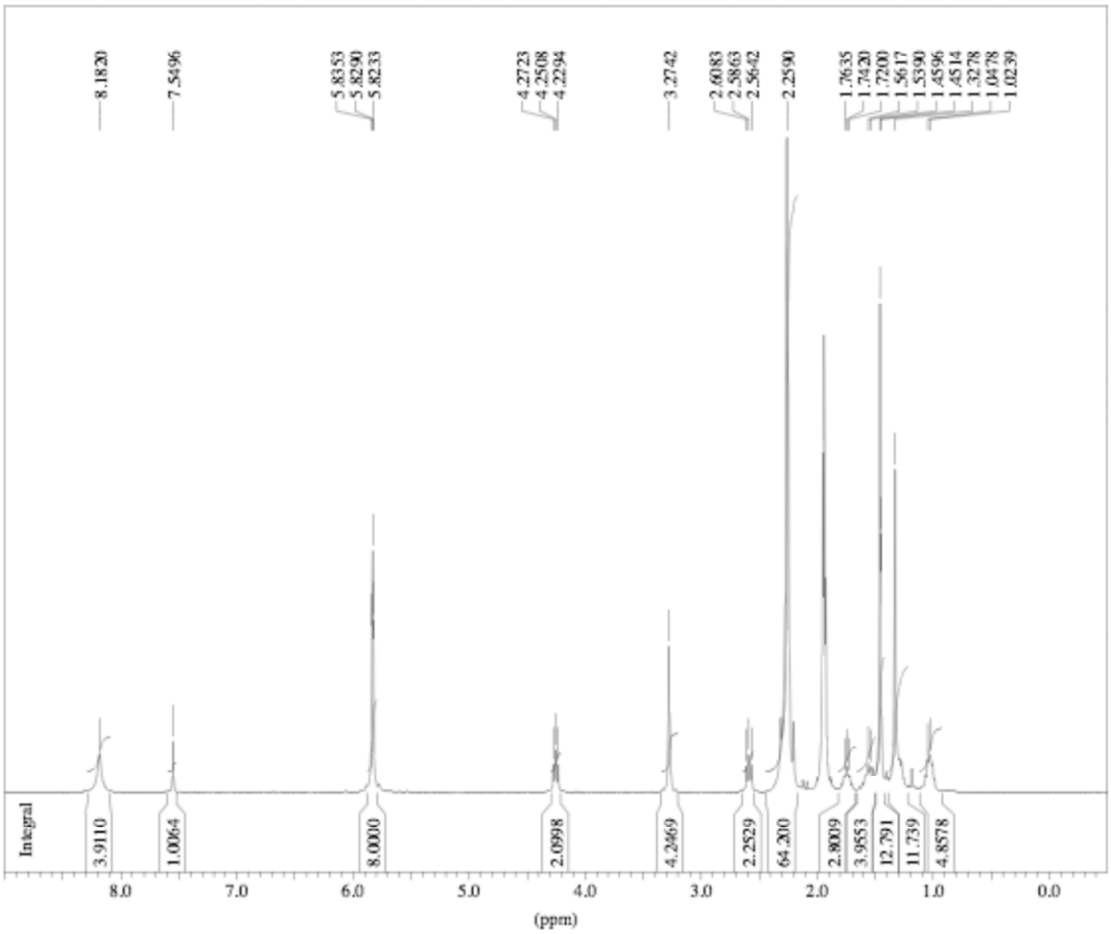


Figure S3 ^{1}H NMR of compound **2** in CD_3CN

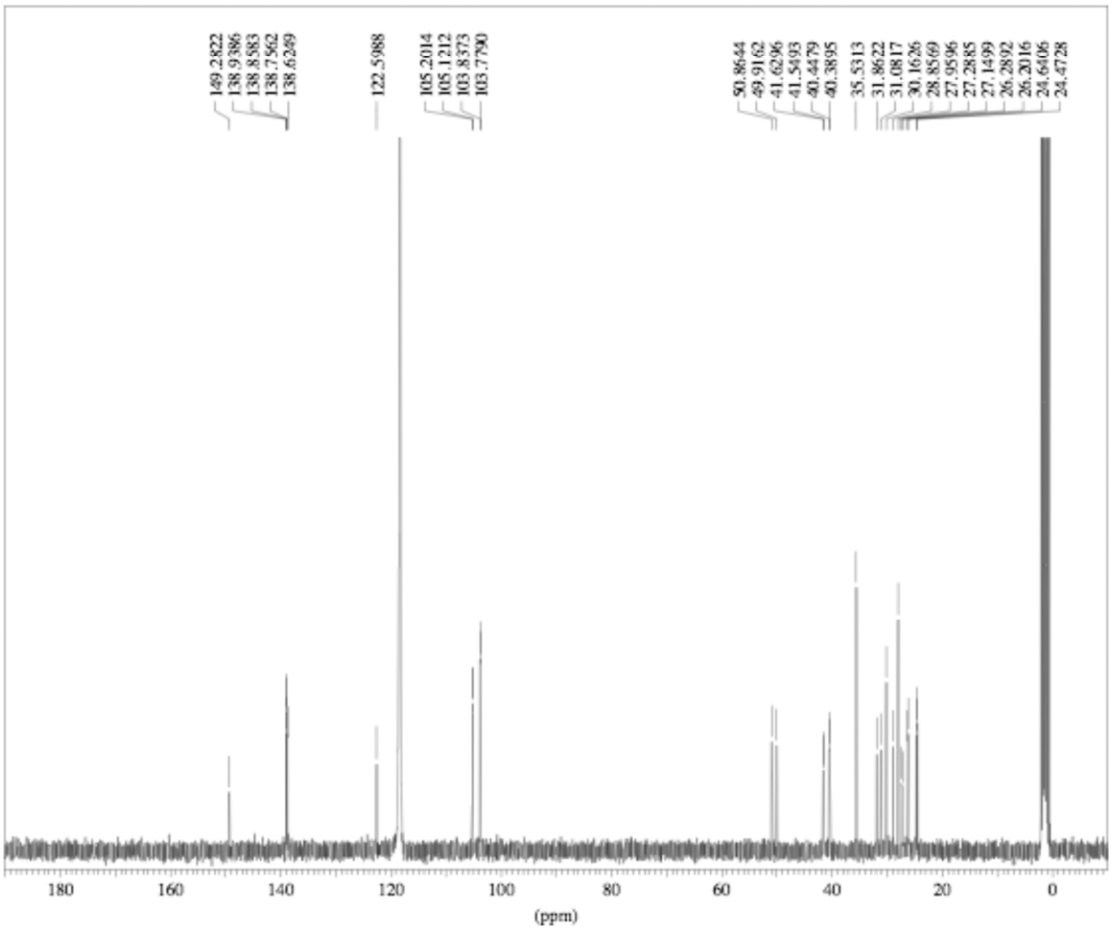


Figure S4 ^{13}C NMR of compound **2** in CD_3CN