



HAL
open science

An artificial sodium ion channel from calix[4]arene in the 1,3-alternate conformation

Peter J. Cragg, Olasupo Lawal, Khayzuran Iqbal, Ali Mohamadi, Parichehr Razavi, Howard Dodd, Marcus Allen, Samreen Siddiqui, Flavia Fucassi

► **To cite this version:**

Peter J. Cragg, Olasupo Lawal, Khayzuran Iqbal, Ali Mohamadi, Parichehr Razavi, et al.. An artificial sodium ion channel from calix[4]arene in the 1,3-alternate conformation. *Supramolecular Chemistry*, 2009, 21 (01-02), pp.55-60. 10.1080/10610270802528307 . hal-00513548

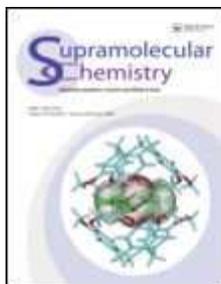
HAL Id: hal-00513548

<https://hal.science/hal-00513548>

Submitted on 1 Sep 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



An artificial sodium ion channel from calix[4]arene in the 1,3-alternate conformation

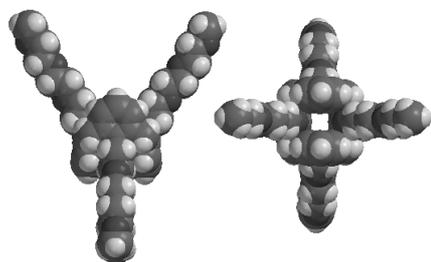
Journal:	<i>Supramolecular Chemistry</i>
Manuscript ID:	GSCH-2008-0074.R1
Manuscript Type:	Special Issue Paper
Date Submitted by the Author:	01-Oct-2008
Complete List of Authors:	Cragg, Peter; University of Brighton Lawal, Olasupo; University of Brighton, School of Pharmacy and Biomolecular Sciences Iqbal, Khayzuran; University of Brighton, School of Pharmacy and Biomolecular Sciences; University of Brighton, School of Pharmacy and Biomolecular Sciences Mohamadi, Ali; University of Brighton, School of Pharmacy and Biomolecular Sciences Razavi, Parichehr; University of Brighton, School of Pharmacy and Biomolecular Sciences Dodd, Howard; University of Brighton, School of Pharmacy and Biomolecular Sciences Allen, Marcus; University of Brighton, School of Pharmacy and Biomolecular Sciences Siddiqui, Samreen; University of Brighton, School of Pharmacy and Biomolecular Sciences Fucassi, Flavia; University of Brighton, School of Pharmacy and Biomolecular Sciences
Keywords:	Calixarenes, Artificial ion channels, Electrophysiology, Ion transport, Polyethers
<p>Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.</p>	
PJC Scheme 1.cdx	



For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Graphical Abstract



O. Lawal, K. S. J. Iqbal, A. Mohamadi, P. Razavi, H. T. Dodd, M. C. Allen, S. Siddiqui, F. Fucassi and P. J. Cragg

An Artificial Sodium Ion Channel from Calix[4]arene in the *1,3-Alternate* Conformation

Or Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

An artificial sodium ion channel from calix[4]arene in the *1,3-alternate* conformation

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

OLASUPO LAWAL, KHAYZURAN S. J. IQBAL, ALI MOHAMADI,
PARICHEHR RAZAVI, HOWARD T. DODD, MARCUS C. ALLEN, SAMREEN
SIDDIQUI, FLAVIA FUCASSI and PETER J. CRAGG*

45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

*School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft
Building, Moulsecoomb, Brighton BN2 4GJ, UK*

Abstract: The synthesis of a calix[4]arene with pendent polyether substituents is described. The compound was shown by NMR to bind Na⁺ when in the *1,3-alternate* conformation and computational simulations suggest metal binding within the calixarene annulus. ¹H NMR indicated that the complexed cation attracts water and the formation of a stable dihydrate was also indicated by *in silico* methods. Lipid bilayer experiments confirmed that the *1,3-alt* calixarene functions as an artificial transmembrane ion in the presence of Na⁺ but not K⁺.

Keywords: Calixarenes; Polyethers; Artificial ion channels; Electrophysiology; Ion transport

*Corresponding author. Tel.: +44 1273 642037. Fax: +44 1273 679333. E-mail:

P.J.Cragg@bton.ac.uk.

INTRODUCTION

From the earliest days of supramolecular chemistry researchers have exploited the biomimetic potential of macrocycles and related molecules. Similarities between crown ethers and the ionophores valinomycin and nonactin helped to explain the ion transporting ability of the former [1]. Likewise enterobactin, a highly selective bacterial siderophore that targets Fe^{III} in aqueous solution with a binding constant approaching 10^{50} , inspired a range of tripodal chelating agents [2]. On a broader front, calixarenes, cyclam derivatives and podands have all been considered as frameworks from which artificial metalloenzymes could be constructed [3].

Many analogues of naturally occurring macrocycles succeed because they bind guests strongly with high specificity, however, it is often just as important to facilitate highly specific binding that is environmentally reversible. In Nature examples can be found in transmembrane ion channels [4,5,6]. These are generally composed of large proteins having an affinity for cell surfaces with regions that can pass through the cellular phospholipid bilayer. The transmembrane region of the protein (or aggregation of proteins) forms a pore, through which ions travel, which must be continuous through the bilayer if ions are to flow continuously. This necessitates a concerted structure between 3 and 6 nm. The ion transport that results is controlled through two main mechanisms: filtering and gating.

Currently much of our detailed understanding ion channel protein behaviour relies on electrophysiological studies and the few high resolution crystal structures that are available. A vast majority of these are of proteins selective for simple cations [7] such

1
2
3 as the K^+ channels KcsA [8] and MthK [9], the Mg^{2+} transporter MgtE [10], Na^+/K^+
4 channels [11,12], and an acid sensing ion channel, Δ ASIC1 [13]. These structures in
5
6 turn often act as models for other, less well characterized, channel proteins such as the
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

as the K^+ channels KcsA [8] and MthK [9], the Mg^{2+} transporter MgtE [10], Na^+/K^+ channels [11,12], and an acid sensing ion channel, Δ ASIC1 [13]. These structures in turn often act as models for other, less well characterized, channel proteins such as the K^+ selective region of the protein product of the hERG (human *ether-á-go-go*) gene [14]. Despite the wealth of information concerning the physiological activity of natural transmembrane proteins there are extensive deficiencies our detailed knowledge of ion transport mechanisms. For example, structural and dynamic evidence from the voltage-gated K^+ channel KcsA indicates that as hydrated ions approach the entrance to the pore, which often resembles a funnel, they are stripped of their solvent shell as they reach a constrictive filter [8,15], yet whether this is a general principle remains unknown. The same deficiencies exist in our knowledge of the channel gating processes which control the flow of ions response to a range of external stimuli.

Synthetic ion channel models allow individual aspects of transmembrane transport to be probed, in particular the structural changes that can alter specificity or transport rate. Artificial analogues of cation channels based on crown ethers first started appearing in the 1980s [16] and have since been refined to include membrane-spanning substituents with high specificity [17,18] and even voltage-dependent gating [19]. Rigid macrocycles have also been employed in cation channel mimetic compounds. Resorcin[4]arenes with undecyl and heptadecyl substituents conduct both Na^+ and K^+ , with selectivity for K^+ over Na^+ by a factor of three, but only for derivatives with the longer substituents [20]. Similar results were reported for other resorcin[4]arenes [21] but calixarenes, in particular calix[4]arene, have latterly been seen as potential ion selective filters around which ionophore or channel frameworks

1
2
3 can be constructed [22,23]. Calix[4]arene-cholic acid conjugates have been shown to
4
5 transport Na⁺ across vesicle membranes with the rate constant for the *1,3-alt*
6
7 conformer an order of magnitude higher than the *cone* [24]. This agrees with other
8
9 examples that suggest *1,3-alt* calix[4]arenes exhibit enhanced ionophoricity [25]. In
10
11 this work we report on the synthesis and behaviour of an amphiphilic *1,3-alt*
12
13 calix[4]arene derivative designed to probe the influence of conformation on metal ion
14
15 selectivity and transmembrane transport.
16
17
18
19
20
21

22 **EXPERIMENTAL**

23
24
25
26
27 Unless otherwise stated, all materials and solvents are from Sigma-Aldrich
28
29 (Gillingham, Dorset, UK). NMR spectra were recorded on a Bruker AM-360
30
31 instrument at 360 and 90 MHz for ¹H and ¹³C respectively. Chemical shifts were
32
33 referenced to tetramethylsilane (TMS). Elemental analysis of polyether-containing
34
35 compounds is often inconsistent due to variable levels and composition of included
36
37 solvent. For this reason high resolution electrospray ionization mass spectrometry was
38
39 used to determine the compounds' identities as the technique is known to be a good
40
41 method of analysis for supramolecular complexes [26]. HRMS were recorded on a
42
43 Bruker micrOTOF instrument operating in the positive mode. Calix[4]arene (**1**) was
44
45 prepared from 4-t-butylcalix[4]arene according to the literature [27].
46
47
48
49
50
51
52

53 ***Triethylene glycol tosylate, monomethyl ether (2)***

54
55 Triethylene glycol monomethyl ether (16.1 ml, 16.4 g, 0.1 mol) was dissolved in a
56
57 mixture of tetrahydrofuran (50 ml) and aqueous sodium hydroxide (5.7 g, 0.14 mol in
58
59 30 ml H₂O) and stirred in an ice bath until the solution temperature fell below 5°C. A
60

1
2
3 solution of *p*-toluenesulfonyl chloride (20.7 g, 0.11 mol) in tetrahydrofuran (30 ml)
4
5 was added to the stirred solution at a rate that kept the temperature below 5°C. Once
6
7 the addition was complete, the solution was left to stir for a further 1 h below 5°C,
8
9 poured onto iced water (75 ml water, 75 g ice) and stirred until all the ice melted.
10
11 Most of the tetrahydrofuran was removed by rotary evaporation and the product
12
13 extracted into dichloromethane (3 x 30 ml). The organic extract was dried over
14
15 calcium chloride, filtered and the solvent removed by rotary evaporation. Triethylene
16
17 glycol tosylate, monomethyl ether, **2**, was obtained as a colourless oil. Yield: 27 g
18
19 (87%); ¹H NMR (CDCl₃) δ: 7.80 (d, *J* = 8.6, 2H ArH), 7.35 (d, *J* = 8.6, 2H ArH), 4.16
20
21 (t, 2H, -CH₂OTs), 3.50-3.75 (m, 10H, -CH₂CH₂O-), 3.39 (s, 3H, -OCH₃), 2.45 (s, 3H,
22
23 TsCH₃); ¹³C NMR (CDCl₃) δ: 144.8, 132.9, 129.5, 127.8, 72.4, 71.8, 71.0, 70.6, 70.1,
24
25 69.7, 58.8, 21.5; ESI HRMS *m/z* found: 341.1056, calculated: 341.1035 [M + Na]⁺.
26
27
28
29
30
31
32
33

34 ***Triethylene glycol iodide, monomethyl ether (3)*** [28]

35
36 Triethylene glycol tosylate, monomethyl ether, **2**, (10 g, 0.03 mol) was dissolved in
37
38 acetone (125 ml). Potassium iodide (25 g [5 equiv.]) was added and refluxed
39
40 overnight. The solution was cooled to room temperature and filtered to remove
41
42 inorganic salts. The solvent was removed under reduced pressure and the residue
43
44 dissolved in a mixture of ethyl acetate (75 ml) and distilled water (75 ml). The
45
46 mixture was separated and the ethyl acetate phase washed with water (30 ml) then
47
48 saturated sodium thiosulfate (50 ml). The organic phase was dried over magnesium
49
50 sulfate, filtered and solvent removed to give triethylene glycol iodide, monomethyl
51
52 ether, **3**, as a colourless oil. Yield: 5.2 g (61%); ¹H NMR (CDCl₃) δ: 3.76 (t, 2H, -
53
54 CH₂OCH₃), 3.65-3.70 (m, 6H, -CH₂CH₂O-), 3.54-3.60 (m, 2H, -OCH₂CH₂I), 3.39 (s,
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3H, -OCH₃), 3.26 (t, 2H, -OCH₂CH₂I); ¹³C NMR (CDCl₃) δ: 72.5, 71.6, 70.5 (x 2), 70.1, 59.0, 3.3; ESI HRMS m/z found: 296.9998, calculated: 296.9964 [M + Na]⁺.

Tetrakis(triethylene glycol monomethyl ether) calix[4]arene, 1,3-alternate conformer (1,3-alt 4)

Following the method of Verboom *et al.* [29] a suspension of calix[4]arene, **1**, (0.50 g, 1.15 mmol [98%]), anhydrous caesium carbonate (0.32 g, 2.3 mmol) and iodotriethylene glycol monomethyl ether, **3**, (1.26 g, 4.6 mmol) was refluxed in anhydrous acetonitrile (100 ml) under nitrogen for 7 days. After cooling to room temperature the solvent was removed under reduced pressure and the solids taken up in dichloromethane (30 ml). The suspension was washed with 1 M hydrochloric acid (2 x 10 ml) and brine (10 ml), the organic phase separated and solvent removed under reduced pressure. The crude material was subjected to flash chromatography on silica (BDH), eluting with ethanol, and fractions with an R_f of 0.84 were combined. Removal of solvent left a viscous residue that slowly solidified. The residue was dissolved in hot diethyl ether and left in a freezer overnight. A beige powder, isolated by filtration, was shown to be tetrakis(triethylene glycol monomethyl ether) calix[4]arene in the *1,3-alternate* conformation (*1,3-alt 4*). Solvent was removed from the filtrate to yield a mobile oil containing an intractable mixture of mono-, tri- and tetrasubstituted calix[4]arenes in the *cone* conformer. Yield of *1,3-alt 4*: 139 mg (12%); m.p.: 80.3-80.5°C; ¹H NMR (CDCl₃) δ: 7.05 (d, *J* = 7.56, 8H, ArH), 6.62 (t, *J* = 7.56, 4H, ArH), 3.85 (m, 8H, -CH₂OAr), 3.75 (s, 8H, ArCH₂Ar), 3.73-3.67 (m, 20H, -CH₂CH₂O-), 3.60-3.54 (m, 20H, -CH₂CH₂O-), 3.38 (s, 12H, -OCH₃); ¹³C NMR (CDCl₃) δ: 155.7, 133.5, 129.9, 121.7, 72.0, 71.2, 70.8, 70.7, 70.6, 70.4, 59.1, 35.0;

ESI HRMS m/z found: 527.2638, 1031.4727, calculated: 527.2621 $[M + 2 Na]^{2+}$,
1031.5344 $[M + Na]^+$.

NMR binding studies

Compound *1,3-alt* **4** was dissolved in $CDCl_3$ and the 1H and ^{13}C spectra recorded at $25^\circ C$. An excess of solid $NaPF_6$ was added and the spectra recorded again after 2 min, 27 min, 87 min and 18.5 h. An analogous experiment was undertaken with NH_4PF_6 .

Molecular modelling

Cone and *1,3-alt* conformers of **4** were drawn with ChemDraw and exported into Spartan '06 [30]. Full geometry optimization was undertaken using restricted HF/PM3 methods from energy minimized structures initially calculated using the MMFF94 molecular mechanics forcefield. The Na^+ complex of *1,3-alt* **4** and its dihydrate were constructed using options from the *Build* menu. For the initial dihydrate structures two water molecules were introduced in close proximity to the RHF/PM3 geometry optimized metal complexes prior to further geometric refinement by the RHF/PM3 method. In general 450 to 500 iterative cycles were required to optimize the $Na^+ \cdot 1,3-alt$ **4** RHF/PM3 model geometry with a further 130 cycles to optimize that of the dihydrate.

Planar bilayer experiments

The general method used is described elsewhere [31]. Pure synthetic lipids (Avanti Polar Lipids, Birmingham, AL USA) were dispersed in chloroform and stored at $-70^\circ C$ under nitrogen. Lipid bilayers were formed from a dispersion of 15 mg/ml 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE) and 15 mg/ml 1-palmitoyl-2-

1
2
3 oleoyl phosphatidylserine (POPS) in *n*-decane, which was drawn across a 0.25 mm
4 diameter hole in a polystyrene cup separating two solution filled chambers, designated
5
6 *cis* and *trans*. The *cis* chamber (to which the calixarene was added) was held at
7
8 ground, and the *trans* chamber was clamped at -50 mV using a BLM-120 patch
9
10 clamp amplifier (Bio-Logic) equipped with a 10GB (10 G Ω) bilayer headstage
11
12 (Warner Instruments). The sign of the membrane potential refers to the *trans*
13
14 chamber, and currents are defined as positive when cations flow from *trans* to *cis*.
15
16 Transmembrane currents were low pass filtered at 300 Hz (4 pole Bessel) digitized at
17
18 10 kHz and recorded directly to disk via a CED Micro 1401 Mark II AD interface.
19
20 Membrane capacitance was measured by differentiating a triangular wave input of 0.2
21
22 kHz. Only bilayers that had a resting conductance of less than 10 pS and an initial
23
24 capacitance of at least 150 pF were used. For cation transport studies bilayers were
25
26 bathed in symmetrical Ringer's solutions containing either 150 mM NaCl or 150 mM
27
28 KCl; 10 mM HEPES; 1 mM EGTA; 1.05 mM CaCl₂; 1 mM MgCl₂; 50 μ M free
29
30 calcium. All recordings were made at room temperature and analyzed offline using
31
32 WinEDR v2.3.9 software (Strathclyde electrophysiological software).
33
34
35
36
37
38
39
40
41
42

43 RESULTS AND DISCUSSION

44
45
46
47
48 We have previously reported model compounds for a sodium cation filter [32] and
49
50 transmembrane channel [33] that have threefold symmetry. Both encapsulate Na⁺
51
52 within a cavity defined by six oxygen atoms, presumably due to a preference for an
53
54 octahedral coordination environment, and cations in the filter model are additionally
55
56 linked by coordinated water. Interestingly, this trimeric motif, unique so far in ion
57
58 channel architecture, has recently been found in the high resolution structure of an H⁺-
59
60

1
2
3 activated ion channel [13]. In the case of K^+ , both binding [34] and transport [21]
4
5 appear to require ligands with eight binding sites in the cubic arrangement also found
6
7 in the filter region of the K^+ -selective transmembrane protein KcsA [8]. To date, well
8
9 known examples of metal transporting calix[4]arenes and related resorcin[4]arenes
10
11 are almost exclusively restricted to K^+ .
12
13
14

15
16
17 Calixarene *1,3-alt* **4** was originally prepared through reaction of the tosylated
18
19 polyether, **2**, with de-t-butylated calix[4]arene, **1**, using the appropriate metal
20
21 carbonate; however the easily synthesised iodo derivative, **3**, was found to give a
22
23 cleaner reaction (Scheme 1). Initially we attempted to synthesize both *cone* **4** and *1,3-*
24
25 *alt* **4** using potassium and caesium carbonate, respectively. Workup of the reaction
26
27 mixtures isolated solid *1,3-alt* **4** in both cases. In addition to mass spectral evidence,
28
29 the NMR spectra showed a singlet at 3.75 ppm in the 1H spectrum which correlated
30
31 with a peak at 35.0 ppm in the ^{13}C spectrum in agreement with literature values for
32
33 *1,3-alt* calixarenes [35]. Mass spectra of the remaining oily residues confirmed them
34
35 to be mixtures of mono-, tri- and tetrasubstituted calix[4]arenes with the
36
37 corresponding *m/z* values of 593.2, 885.4 and 1031.5 a.u for their sodium salts. NMR
38
39 confirmed that these compounds were in the *cone* conformer: a pair of doublets at
40
41 4.45 and 4.25 ppm in the 1H spectrum correlated with a peak at 31.9 ppm in the ^{13}C
42
43 spectrum. Given the difficulty in separating the *cone* components attention was
44
45 focused on *1,3-alt* **4**.
46
47
48
49
50
51
52
53
54

55 To our surprise Na^+ , but not K^+ , inclusion in *1,3-alt* **4** was inferred from changes in
56
57 the 1H and ^{13}C NMR spectra (Fig. 1 and Table S1 in the Supporting Information). The
58
59 most significant feature of the 1H spectrum was the deshielding of the aromatic
60

1
2
3 protons by 0.16 and 0.05 ppm. This effect was mirrored in the ^{13}C spectrum where the
4 associated carbons moved by 1.17 and 0.35 ppm. The shielding was increased for the
5 phenolic carbon (-0.34 ppm) together with the protons on the methylene bridge (-0.03
6 ppm) and those of the first carbon of the lower rim substituents. The methylene bridge
7 gave rise to a singlet at 3.75 ppm in the absence of Na^+ . Over the course of an 18 h
8 experiment the peak was initially shielded (-0.11 ppm) before coalescing with
9 ethyleneoxy resonances at 3.71 ppm. Upon addition of NaPF_6 , a new peak appeared
10 as a broad feature between 7.9 and 7.5 ppm (Fig. S10 in the Supporting Information).
11 It is probable that this was from trace water present in the Na^+ salt. The peak became
12 progressively deshielded until, after 18 h, it had sharpened and was centred at 10.5
13 ppm. Integration indicated that it corresponded to four protons for each calixarene,
14 consistent with two water molecules to every $\text{Na}^+ \cdot 1,3\text{-alt } \mathbf{4}$ complex. Taken together
15 these changes suggested that the cation was not associated with the polyether region
16 of the molecule but resided inside the annulus of the calixarene initially interacting
17 most strongly with the aryl carbons and protons on the methylene bridge. Over time
18 two water molecules coordinate to the bound cation down the axis of the calixarene.
19
20 Hydration was evident within two minutes of the salt being added but took almost 20
21 h to reach completion. As a control, the experiment was repeated using NH_4PF_6 over a
22 96 h period. Protons on the methylene bridge and, crucially, all those associated with
23 the polyether region of the molecule were deshielded by 0.05 to 0.07 ppm as would be
24 expected if the cation was interacting with the polyether substituents. No evidence of
25 bound water was observed.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

In the absence of crystallographic evidence a computer simulation was used to gain some insight into the probable position of a Na^+ guest within the calixarene host. We

1
2
3 have previously shown that even simple molecular mechanics geometry optimizations
4 can give models of host-guest complexes that compare favourably with information
5 drawn from NMR binding studies [36] and that gas phase PM3 calculations can
6 provide good models for inclusion phenomena in solution [37].
7
8
9

10
11
12
13
14
15 Computer models of *1,3-alt* **4** and *cone* **4**, generated as described in the Experimental
16 section, suggested stable extended structures in both cases with *cone* **4** in the
17 ‘flattened cone’ conformation having C_{2v} symmetry and *1,3-alt* **4** in the expected *1,3-*
18 *alternate* S_4 conformation as shown in Fig. 2. Molecular modelling of the Na^+
19 complex was undertaken using semiempirical methods (restricted HF/PM3) where
20 two starting positions were considered for Na^+ . In these the cation was either placed
21 close to the macrocyclic annulus or near the ends of two polyether substituents. In the
22 former case (structure I) the cation was drawn into the macrocyclic annulus with no
23 polyether-cation interactions. In the latter case (structure II) the cation bound by the
24 macrocycle was also enfolded by one polyether substituent to give a coordination
25 environment reminiscent of a cation-calixcrown complex. The heats of formation for
26 the two structures ($-2120 \text{ kJ mol}^{-1}$ for I vs. $-2169 \text{ kJ mol}^{-1}$ for II), illustrated in Fig. 3,
27 were within 2.5%. Structure I had closest contacts between Na^+ and four coplanar
28 carbon atoms at the lower rim in good agreement with density functional calculations
29 for the $Na^+ \cdot 1,3-alt$ calix[4]arene complex [38]. More importantly it was consistent
30 with the NMR shifts in the bridging methylene and aromatic protons following the
31 addition of Na^+ . Geometry optimized dihydrate structures were generated from
32 structures I and II of the $Na^+ \cdot 1,3-alt$ **4b** complex as shown in Fig. 3. Structure I
33 optimized with water molecules along the axis of the calixarene at distances of 2.7 to
34 2.8 Å from the cation. Na^+ had other close contacts from phenolic ether oxygen atoms
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 at 2.6 Å and two aromatic centroids at 2.7 Å. In structure II one water molecule
4 adopted an axial position 2.8 Å from the cation while the other displaced the
5 polyether and bridged between the ether oxygen atoms and the cation. The heats of
6 formation were within 2% of each other (-2625 kJ mol⁻¹ for structure I vs. -2678 kJ
7 mol⁻¹ for structure II). Perhaps surprisingly, these calculations indicated that even if
8 polyether interactions were important in the formation of Na⁺·*1,3-alt 4b* complexes,
9 water molecules could displace the more weakly bound ethers in the cation's primary
10 coordination sphere.
11
12
13
14
15
16
17
18
19
20
21
22
23
24

25 Given that *1,3-alt 4* approaches 3 nm in length when fully extended its potential to act
26 as an artificial transmembrane ion channel was investigated using a planar lipid
27 bilayer method. Solutions of *1,3-alt 4* were added to a planar lipid bilayer bathed in
28 biomimetic Ringer's solution containing either K⁺ or Na⁺ at a concentration of 150
29 mM [39]. In total five recordings were made with K⁺ and three with Na⁺ using a new
30 bilayer in each case. Insertion of *1,3-alt 4* was indicated by a perturbation of the
31 membrane shortly after the compound was introduced though no occurrences of the
32 membrane busting were observed as a result. Fig. 4 shows a typical example of
33 bilayer activity following insertion of *1,3-alt 4*. All recordings of experiments
34 involving K⁺ showed the level of membrane activity increasing with time as in the
35 upper trace of Fig. 4 though no features corresponding to channels were observed. In
36 two of the experiments where Na⁺ was present channel-like features were recorded: in
37 a third an increasing current exhibiting some stepped features was observed but the
38 bilayer burst without giving a long lasting channel-like response. Where channel
39 formation was observed it usually appeared 10 seconds after the addition of *1,3-alt 4*
40 and lasted for up to 60 s. Several examples of these stepped features were detected
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 during the course of both successful experiments. The lower trace in Fig. 4 shows a
4 section from a typical experiment involving Na^+ . The steps were on the scale of 100
5 pS implying insertion of several calixarenes in a manner that we have described with
6 regard to a calix[6]arene derivative [33]. The integrity of the bilayer held throughout
7 the experiments.
8
9

17 CONCLUSIONS

20 The synthesis of *1,3-alt* **4** has been described and the compound shown by NMR to
21 bind Na^+ . Over time the cation also attracted water, present in trace amounts in the
22 Na^+ salt, to form a dihydrate. Computational simulations agree with the NMR data in
23 predicting Na^+ binding within the annulus of the calixarene. The models indicate that
24 this may be facilitated by interactions with oxygen atoms in a polyether substituent.
25 Simulation of a hydrated complex mirrored the observation that two water molecules
26 were incorporated in the complex during the NMR experiment performed in CDCl_3 .
27 The process of metal binding and hydration would be expected to be significantly
28 faster when *1,3-alt* **4** was inserted in a bilayer bathed by water on both sides.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 Lipid bilayer experiments confirmed that *1,3-alt* **4** functions as an artificial
46 transmembrane ion channel in the presence of Na^+ but not K^+ . Why does this
47 calixarene act as a Na^+ channel when other compounds related to calix[4]arenes are
48 K^+ selective? The answer lies in its conformation. Shinkai has proposed that the *1,3-*
49 *alternate* conformer of calix[4]arene provides an extended electron rich environment
50 that is attractive to monovalent cations [40]. Evidence from NMR experiments and
51 molecular simulations suggests that Na^+ is in its preferred six-coordinate environment
52
53
54
55
56
57
58
59
60

1
2
3 in *1,3-alt 4* interacting with two water molecules, two phenolic oxygens and two
4 aromatic rings. This is consistent with current thinking on cation channel mechanisms
5 which indicate that conductance occurs as water-linked chains of partially dehydrated
6 cations pass through channel selectivity filters [8,15]. Calix[4]arene *1,3-alt 4* is
7 therefore ideally suited as a synthetic ion channel for Na⁺.
8
9
10
11
12
13
14
15
16
17

18 Supporting Information

19
20 NMR spectra for compounds **2**, **3**, *1,3-alt 4* and *cone 4*, *1,3-alt 4*·Na⁺·2 H₂O together
21 with HRMS spectra for *1,3-alt 4* and *cone 4* are available as supplementary
22 information.
23
24
25
26
27
28

29 Acknowledgements

30
31 We thank the University of Brighton for financial support (for K. S. J. I. and F. F.)
32
33
34
35

36 References

- 37
38 [1] Takaki, U.; Hogen Esch, T. E.; Smid, J. *J. Am. Chem. Soc.*, **1971**, *93*, 6760;
39
40
41 McLaughlin, S. G. A.; Szabo, G.; Ciani, S.; Eisenman, G. *J. Membrane Biol.*, **1972**, *9*,
42
43
44 3.
45
46 [2] Raymond, K. N.; Isied, S. S.; Brown, L. D.; Fronczek, F. R.; Nibert, J. H. *J. Am.*
47
48 *Chem. Soc.*, **1976**, *98*, 1767.
49
50 [3] Kirby, A. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 707.
51
52 [4] Cragg, P. J. *Science Progress*, **2002**, *85*, 219.
53
54
55 [5] Liang, X.; Campopiano, D. J.; Sadler, P. J. *Chem. Soc. Rev.*, **2007**, *36*, 968.
56
57 [6] Insight: ion channels, in *Nature*, **2006**, *440*, pp. 439-489.
58
59 [7] Zhorov, B. S.; Tikhonov, D. B. *J. Neurochem.*, **2004**, *88*, 782.
60

- 1
2
3 [8] Doyle, D.; Morais Cabral, J.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S.
4 L.; Chait, B. T.; MacKinnon, R. *Science*, **1998**, *280*, 69.
5
6
7
8 [9] Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B. T.; MacKinnon, R. *Nature*,
9
10 **2002**, *417*, 515.
11
12 [10] Hattori, M.; Tanaka, Y.; Fukai, S.; Ishitani, R.; Nureki, O. *Nature*, **2007**, *448*,
13 1072.
14
15
16
17 [11] Shi, N.; Ye, S.; Alam, A.; Chen, L.; Jiang, Y. *Nature*, **2006**, *440*, 570.
18
19 [12] Morth, J. P.; Pedersen, B. P.; Toustrup-Jensen, M. S.; Sørensen, T. L.-M.;
20 Petersen, J.; Andersen, J. P.; Vilsen, B.; Nissen, P. *Nature*, **2007**, *450*, 1043.
21
22 [13] Jasti, J.; Furukawa, H.; Gonzales, E. B.; Gouaux, E. *Nature*, **2007**, *449*, 316.
23
24 [14] Farid, R.; Day, T.; Friesner, R. A.; Pearlstein, R. A. *Bioorg. Med. Chem.*, **2006**,
25 *14*, 3160.
26
27 [15] Noskov, S. Y.; Bernèche, S.; Roux, B. *Nature*, **2004**, *431*, 830.
28
29 [16] Dock, A. C.; Moras, D.; Behr, J. P.; Lehn, J.-M. *Acta Cryst. C*, **1983**, *39*, 1001.
30
31 [17] Fyles, T. M.; James, T. D.; Kaye, K. C. *J. Am. Chem. Soc.*, **1993**, *115*, 12315.
32
33 [18] Gokel, G. W. *Chem. Commun.*, **2000**, 1.
34
35 [19] Hall, C. D.; Kirkovits, G.J.; Hall, A.C. *Chem. Commun.*, **1999**, 1897
36
37 [20] Tanaka, Y.; Kobuke, Y.; Sokabe, M. *Angew. Chem. Int. Ed. Engl.*, **1995**, *34*, 693
38
39 [21] Wright, A. J.; Matthews, S. E.; Fischer, W. B.; Beer, P. D. *Chem. Eur. J.*, **2001**,
40 *7*, 3474.
41
42 [22] Iqbal, K. S. J; Cragg, P. J. *Dalton Trans.*, **2007**, 26.
43
44 [23] de Mendoza, J.; Cuevas, F.; Prados, P.; Meadows, E. S.; Gokel, G. W. *Angew.*
45 *Chem. Int. Ed. Engl.*, **1998**, *37*, 1534.
46
47 [24] Kobuke, Y.; Nagatani, T. *J. Org. Chem.*, **2001**, *66*, 5094.
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [25] Baklouti, L.; Harrowfield, J.; Pulpoka, B.; Vicens, J. *Mini Rev. Org. Chem.*,
4
5
6 **2006**, *3*, 355.
7
8 [26] Schalley, C. A. *Mass Spectrom. Rev.*, **2001**, *20*, 253.
9
10 [27] Gutsche, C. D.; Levine, J. A.; Sujeeth, P. K. *J. Org. Chem.*, **1985**, *50*, 5802.
11
12 [28] General method from Loiseau, F. A.; Hill, A. M.; Hii, K. K. *Tetrahedron*, **2007**,
13
14 *63*, 9947.
15
16 [29] Verboom, W.; Datta, S.; Asfari, Z.; Harkema, S.; Reinhoudt, D. N. *J. Org.*
17
18 *Chem.*, **1992**, *57*, 5394.
19
20
21 [30] Wavefunction, Inc., Irvine, CA, USA.
22
23
24 [31] Hardy, S. P.; Richie, C.; Allen, M. C.; Ashley, R. H.; Granum, P. E. *BBA*
25
26 *Biomembranes*, **2001**, *1515*, 38.
27
28 [32] Cragg, P. J.; Allen, M. C.; Steed, J. W. *Chem. Commun.*, **1999**, 553.
29
30
31 [33] Iqbal, K. S. J.; Allen, M. C.; Fucassi, F.; Cragg, P. J. *Chem. Commun.*, **2007**,
32
33 3951.
34
35 [34] Matthews, S. E.; Schmitt, P.; Felix, V.; Drew, M. G. B.; Beer, P. D. *J. Am.*
36
37 *Chem. Soc.*, **2002**, *124*, 1341.
38
39 [35] Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. D.; Sánchez, C. *J. Org. Chem.*,
40
41 **1991**, *56*, 3372.
42
43 [36] Marcos, P. M.; Ascenso, J. R.; Cragg, P. J. *Supramol. Chem.*, **2007**, *19*, 199.
44
45
46 [37] Sheehan, R.; Cragg, P. J. *Supramol. Chem.*, **2007**, *20*, 437.
47
48
49 [38] Macias, A.; Norton, J. E.; Evanseck, J. D. *J. Am. Chem. Soc.*, **2003**, *125*, 2351.
50
51 [39] A similar bilayer experiment in Na⁺ rich media carried out using the crude
52
53 mixture of *cone* compounds, including *cone 4*, also demonstrated insertion into the
54
55 lipid membrane but no transmembrane ion current was observed [data not shown]. As
56
57
58
59
60

1
2
3 the *cone* compounds are *ca.* 1.7 nm when fully extended it is probable that they are
4
5 unable to span the bilayer and therefore cannot form conducting channels.
6
7

8 [40] Ikeda, A.; Shinkai, S. *J. Am. Chem. Soc.*, **1994**, *116*, 3102.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

List of figures

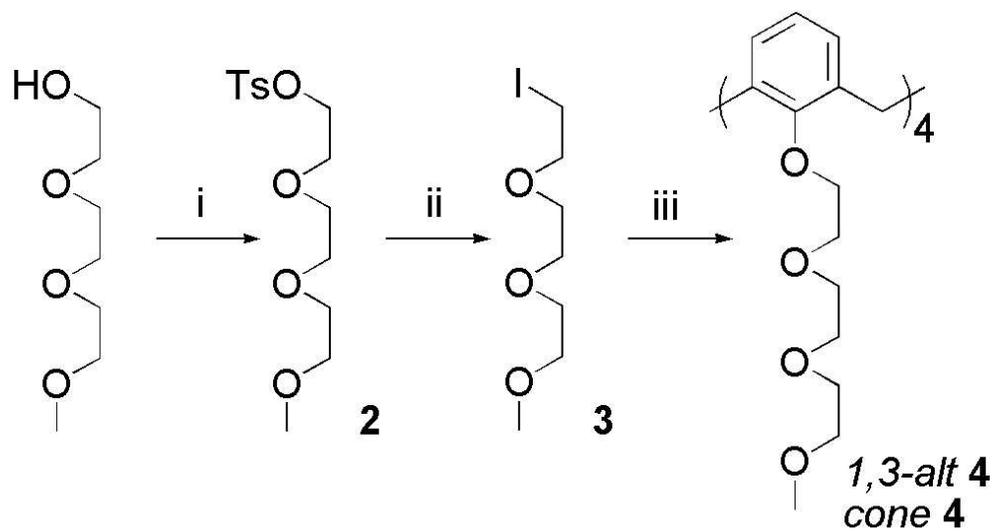
SCHEME 1. Synthesis of calixarenes: i) TsCl, NaOH_(aq), 1,4-dioxane, 0°C; ii) KI, acetone, reflux; iii) calix[4]arene, K₂CO₃ or Cs₂CO₃, CH₃CN, reflux.

FIGURE 1. Time dependent changes in the ¹H spectrum of *1,3-alt 4* following addition of NaPF₆ (▲ = aromatic protons in the 3 and 5 positions; ■ = aromatic protons in the 4 position; ● = methylene bridge protons; x = chloroform).

FIGURE 2. Simulated ligand structures: a) *1,3-alt 4* (side and top); b) *cone 4* (side and top).

FIGURE 3. Simulated complex structures: a) *1,3-alt 4*·Na⁺, structure I; b) *1,3-alt 4*·Na⁺·2 H₂O, structure I; c) *1,3-alt 4*·Na⁺, structure II; d) *1,3-alt 4*·Na⁺·2 H₂O, structure II.

FIGURE 4. Transmembrane activity of *1,3-alt 4*: with K⁺ (upper trace) and Na⁺ (lower trace, with minor signal dropout).



SCHEME 1. Synthesis of calixarenes: i) TsCl, NaOH(aq), 1,4-dioxane, 0°C; ii) KI, acetone, reflux; iii) calix[4]arene, K₂CO₃ or Cs₂CO₃, CH₃CN, reflux.
86x46mm (300 x 300 DPI)

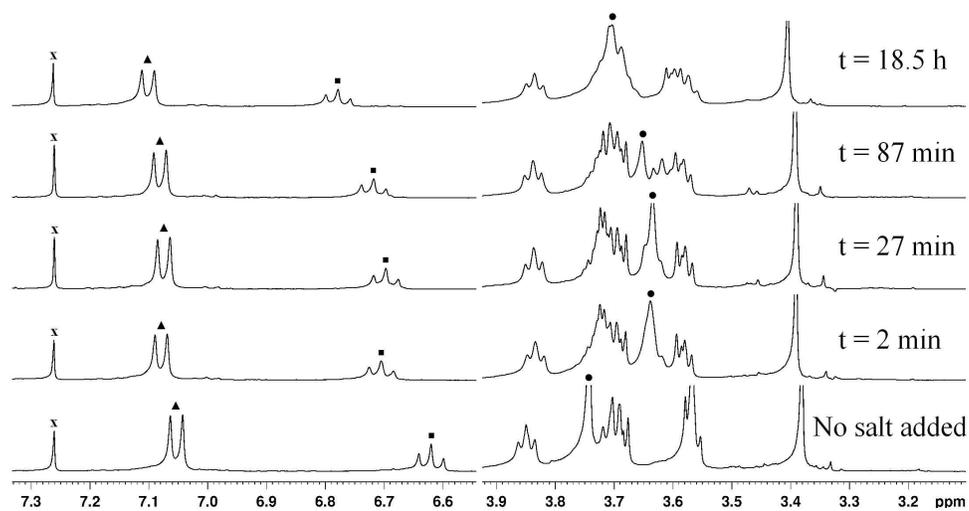


FIGURE 1. Time dependent changes in the ^1H spectrum of 1,3-alt 4 following addition of NaPF_6 (\blacktriangle = aromatic protons in the 3 and 5 positions; \blacksquare = aromatic protons in the 4 position; \bullet = methylene bridge protons; \times = chloroform).
597x345mm (96 x 96 DPI)

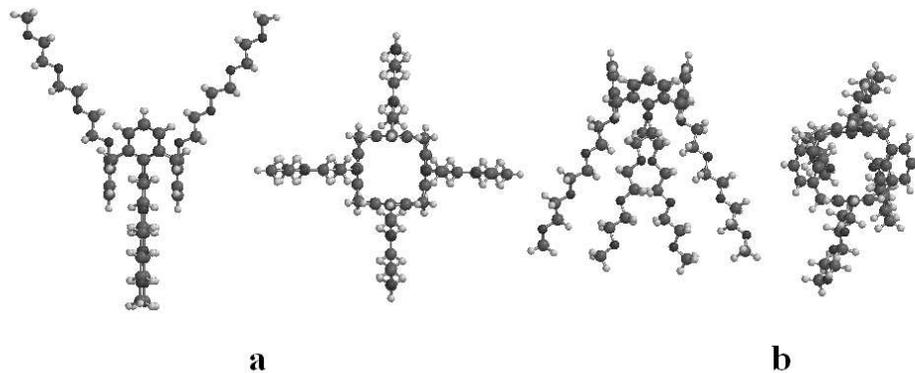


FIGURE 2. Simulated ligand structures: a) 1,3-alt 4 (side and top); b) cone 4 (side and top).
268x123mm (96 x 96 DPI)

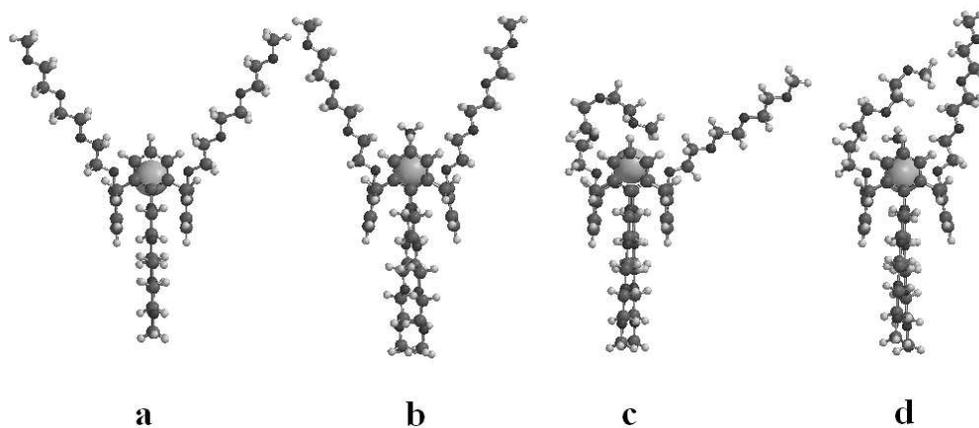


FIGURE 3. Simulated complex structures: a) 1,3-alt 4Å□Na+, structure I; b) 1,3-alt 4Å□Na+·2H₂O, structure I; c) 1,3-alt 4Å□Na+, structure II; d) 1,3-alt 4Å□Na+·2H₂O, structure II.
343x215mm (75 x 75 DPI)

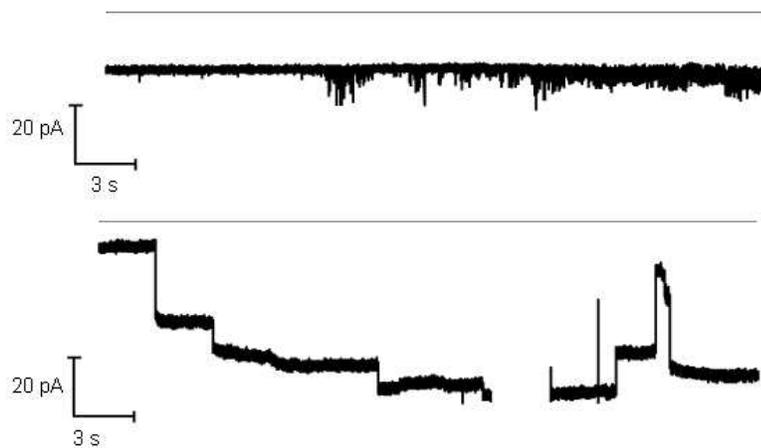


FIGURE 4. Transmembrane activity of 1,3-alt 4: with K⁺ (upper trace) and Na⁺ (lower trace, with minor signal dropout).
189x103mm (96 x 96 DPI)

1
2
3 **An artificial sodium ion channel from calix[4]arene in the *1,3-alternate***
4 **conformation**
5
6

7
8 O. Lawal, K. S. J. Iqbal, A. Mohamadi, P. Razavi, H. T. Dodd, M. C. Allen, S.

9
10 Siddiqui, F. Fucassi and P. J. Cragg
11
12

13
14
15 **Supporting Information**
16
17

18
19
20 **Figure S1.** ^1H NMR for **2**.
21

22 **Figure S2.** ^{13}C NMR for **2**.
23

24 **Figure S3.** ^1H NMR for **3**.
25

26 **Figure S4.** ^{13}C NMR for **3**.
27

28 **Figure S5.** ^1H NMR for *1,3-alt 4*.
29

30 **Figure S6.** ^{13}C NMR for *1,3-alt 4*.
31

32 **Figure S7.** ^1H NMR for crude *cone 4*.
33

34 **Figure S8.** ^{13}C NMR for crude *cone 4*.
35

36 **Figure S9.** Mass spectra for *1,3-alt 4* and crude *cone 4*.
37

38 **Figure S10.** ^1H NMR for *1,3-alt 4*·Na⁺·2 H₂O (18.5 h after addition of NaPF₆).
39

40 **Table S1.** Time dependent shifts (ppm) for selected nuclei after addition of NaPF₆.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

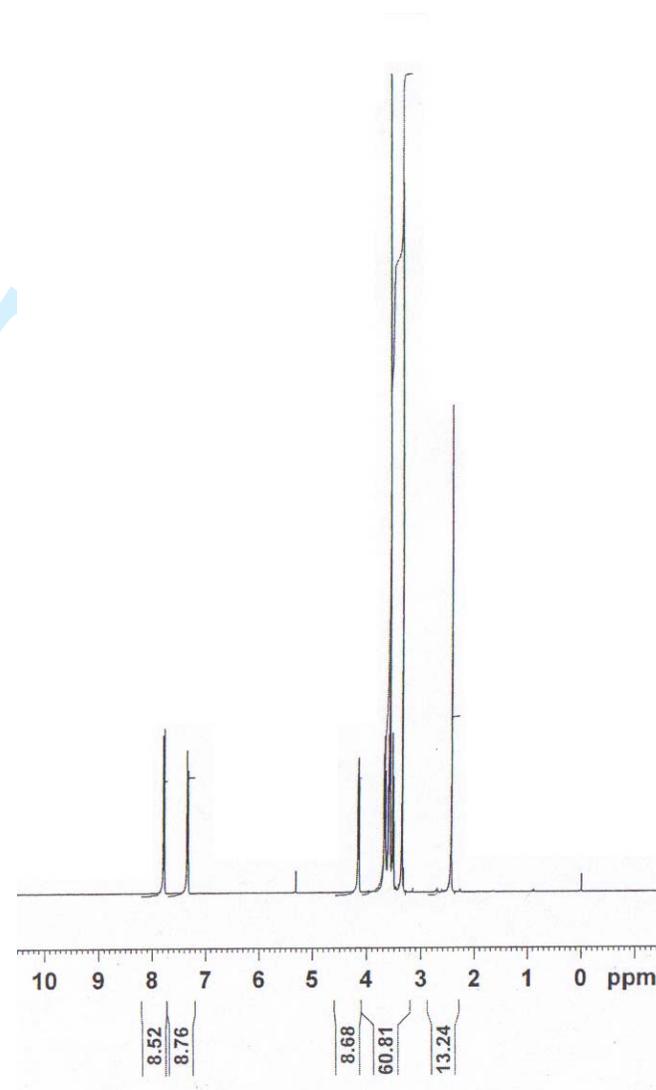


Figure S1. ^1H NMR for **2**.

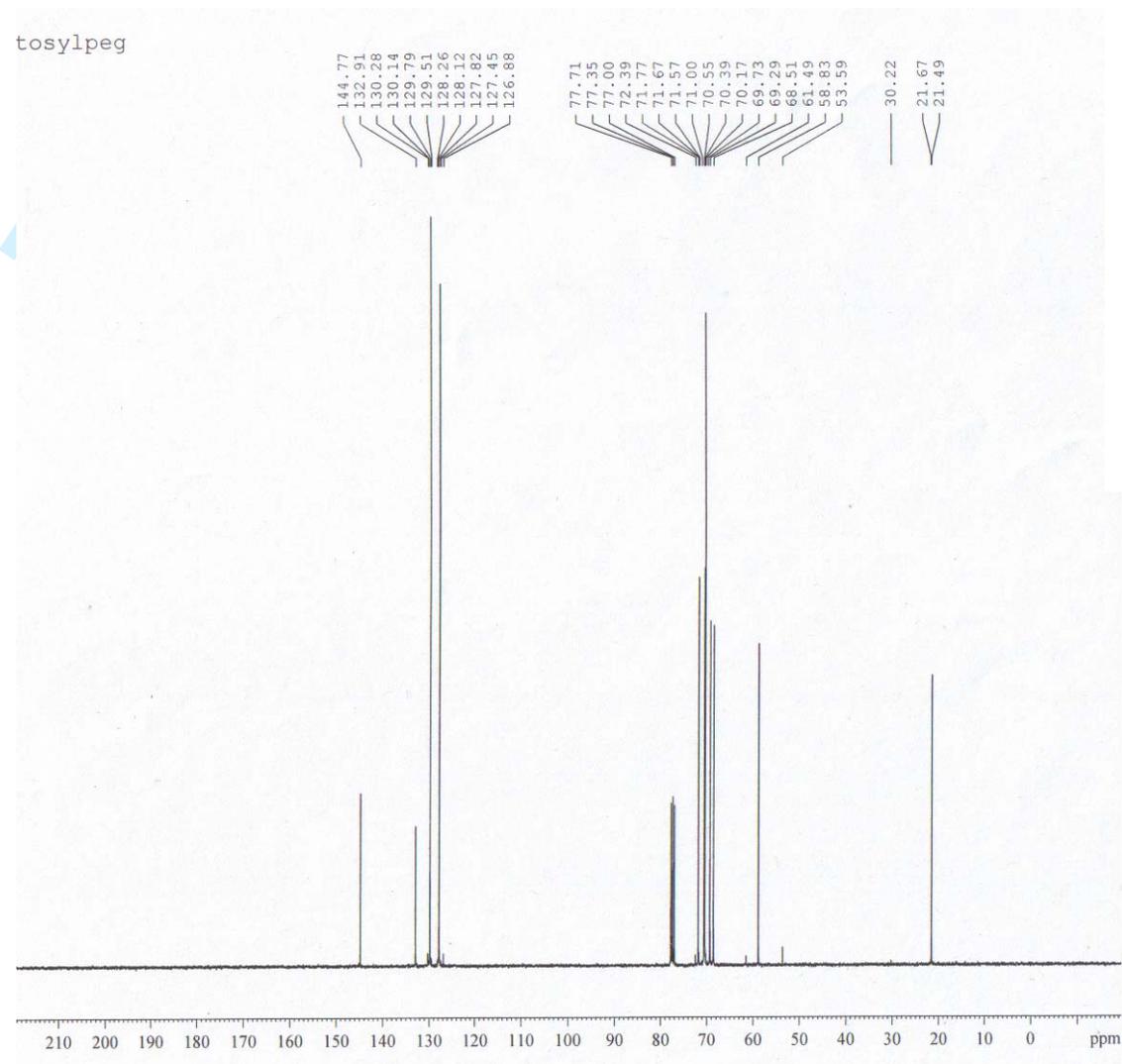


Figure S2. ^{13}C NMR for 2.

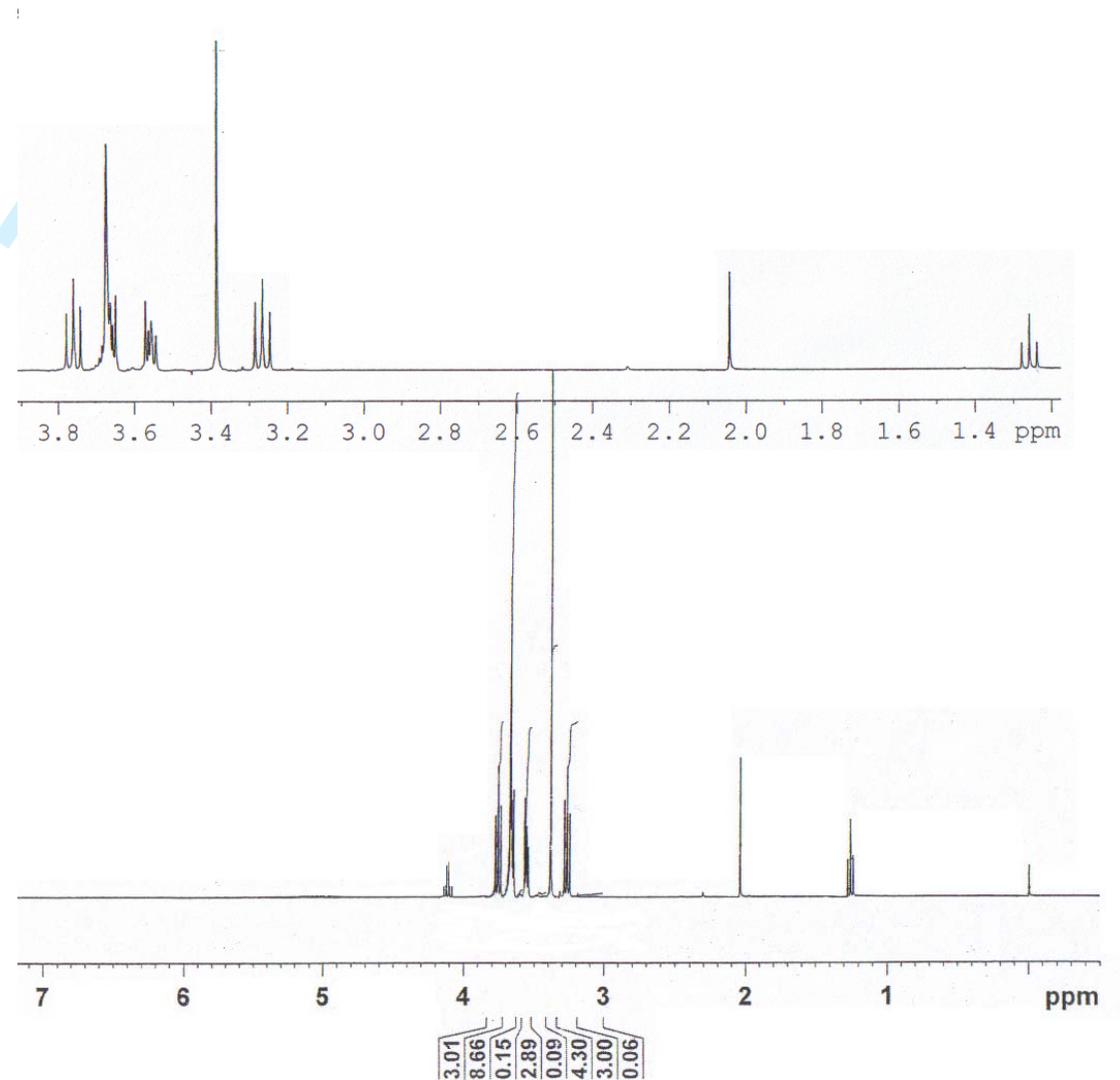


Figure S3. ^1H NMR for **3** (trace of ethyl acetate present).

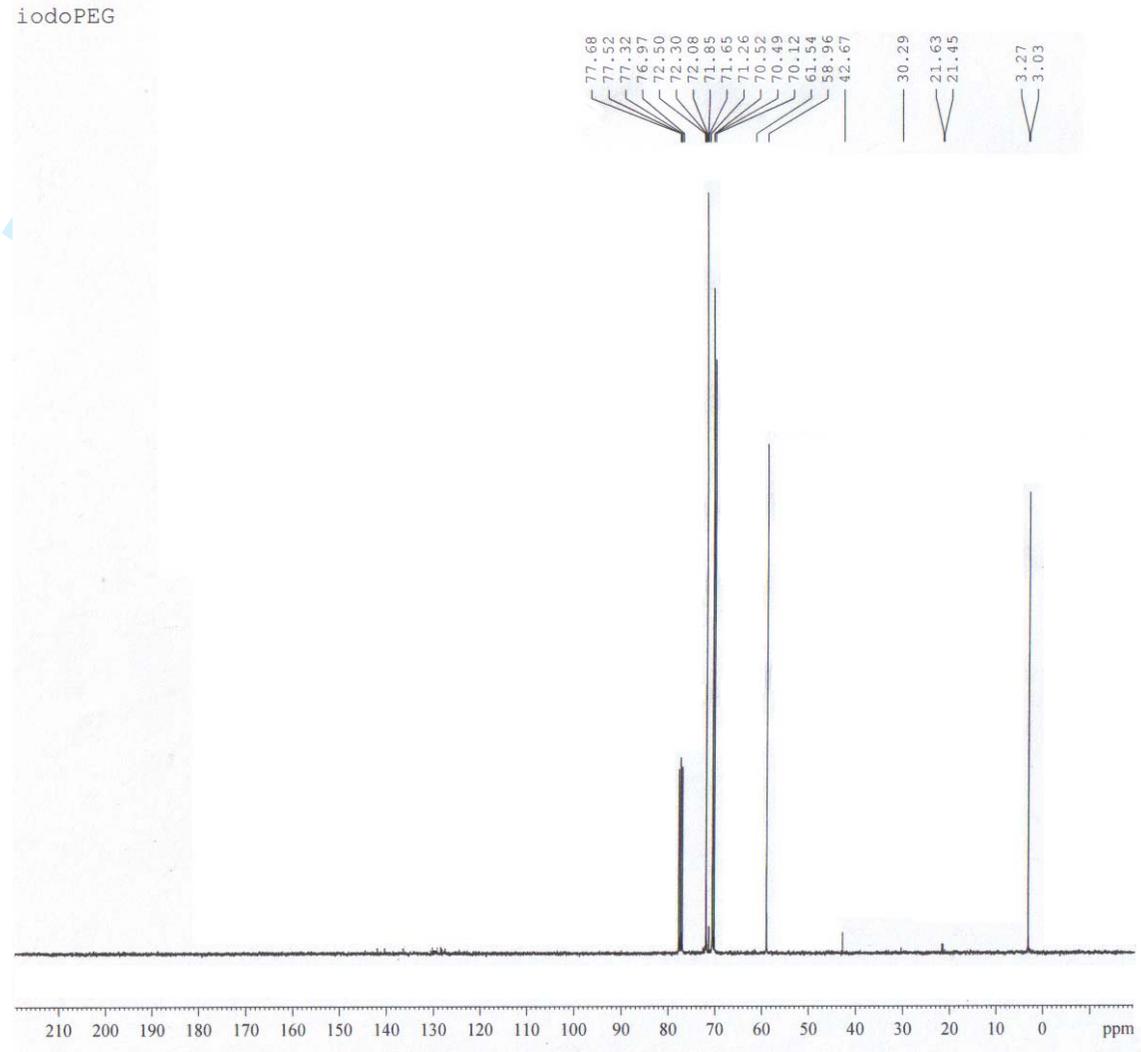


Figure S4. ^{13}C NMR for **3** (trace of ethyl acetate present).

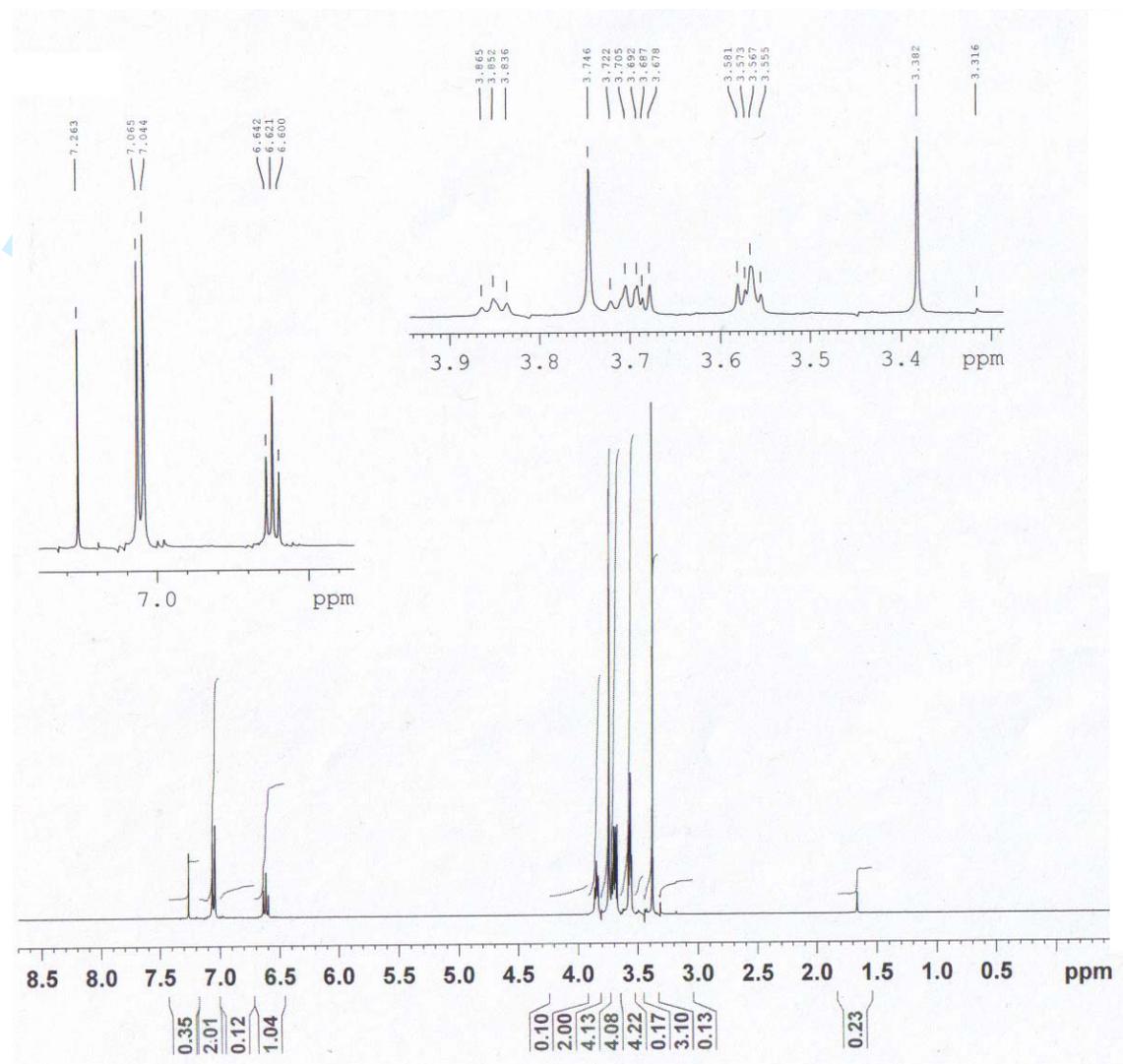


Figure S5. ^1H NMR for *1,3-alt 4*.

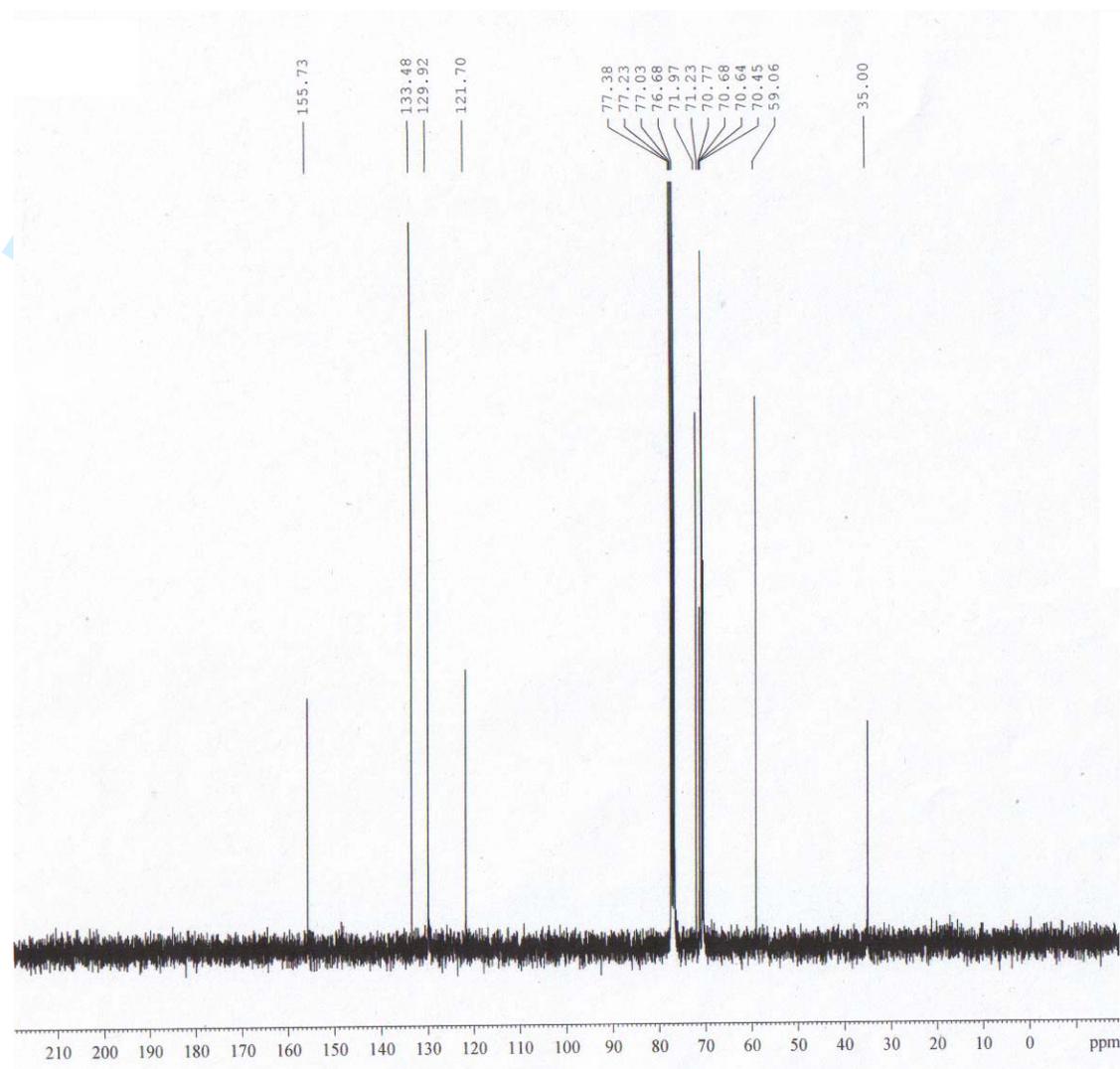


Figure S6. ^{13}C NMR for *1,3-alt 4*.

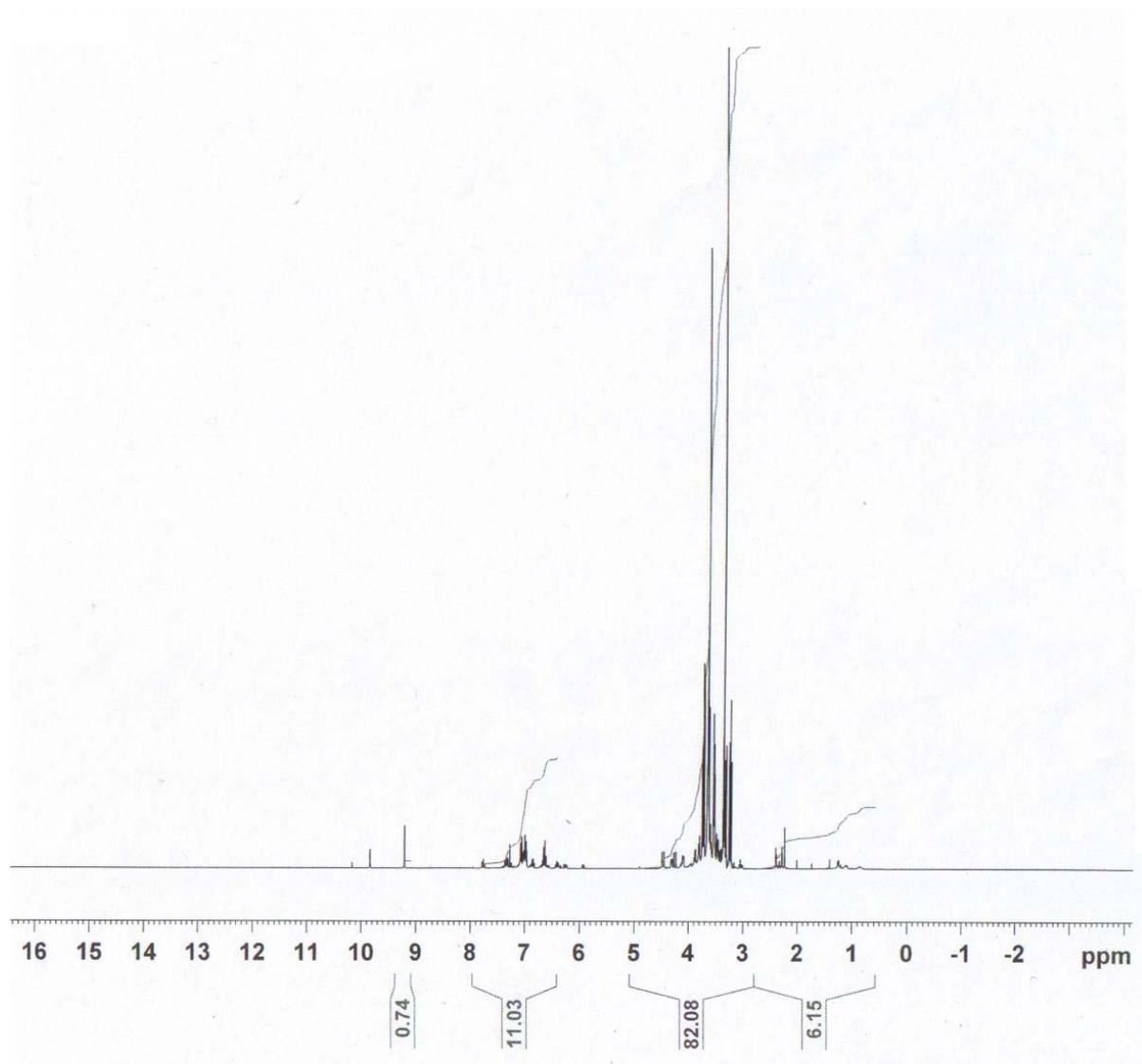


Figure S7. ^1H NMR for crude *cone 4*.

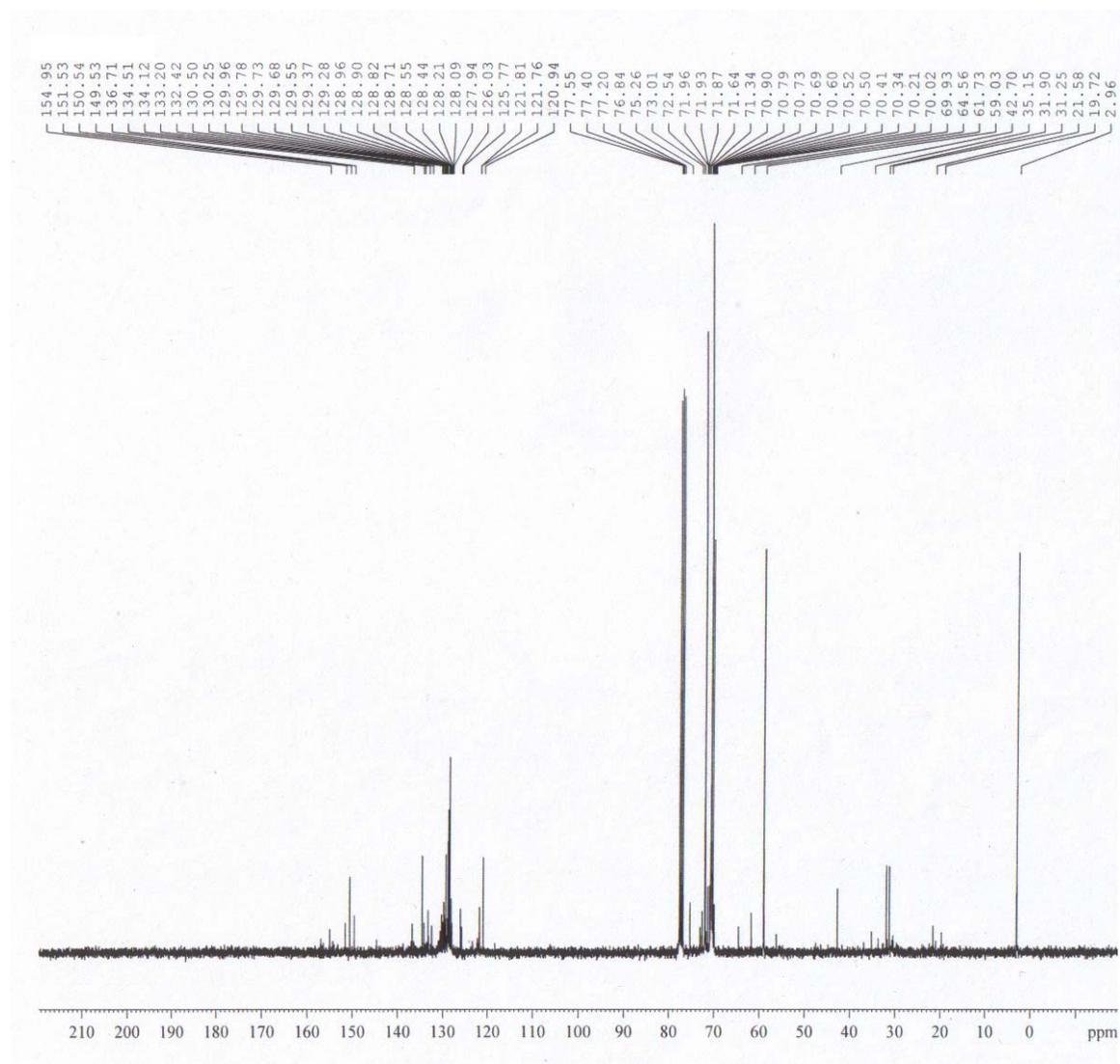


Figure S8. ^{13}C NMR for crude *cone 4*.

Mass Spectrum Molecular Formula Report

Analysis Info Acquisition Date 6/13/2008 9:33:00 AM
Analysis Name D:\Data\htd\130608000001.d
Method DI_50_1500_pos.m Operator Administrator
Sample Name Instrument micrOTOF 141
Comment Solvent: Methanol

Acquisition Parameter
Source Type ESI Ion Polarity Positive Set Corrector Fill 32 V
Scan Range n/a Capillary Exit 120.0 V Set Pulsar Pull 396 V
Scan Begin 50 m/z Hexapole RF 150.0 V Set Pulsar Push 396 V
Scan End 1500 m/z Skimmer 1 40.0 V Set Reflector 1300 V
Hexapole 1 22.0 V Set Flight Tube 9000 V
Set Detector TOF 2000 V

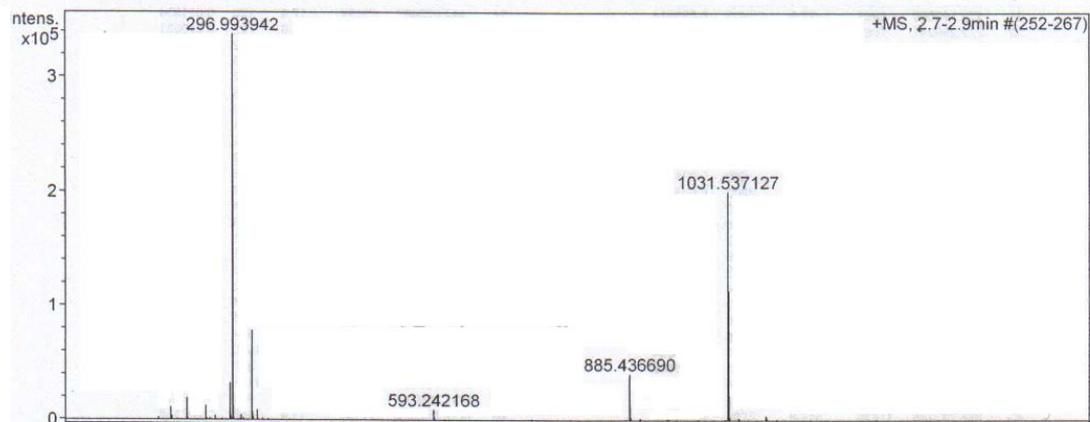
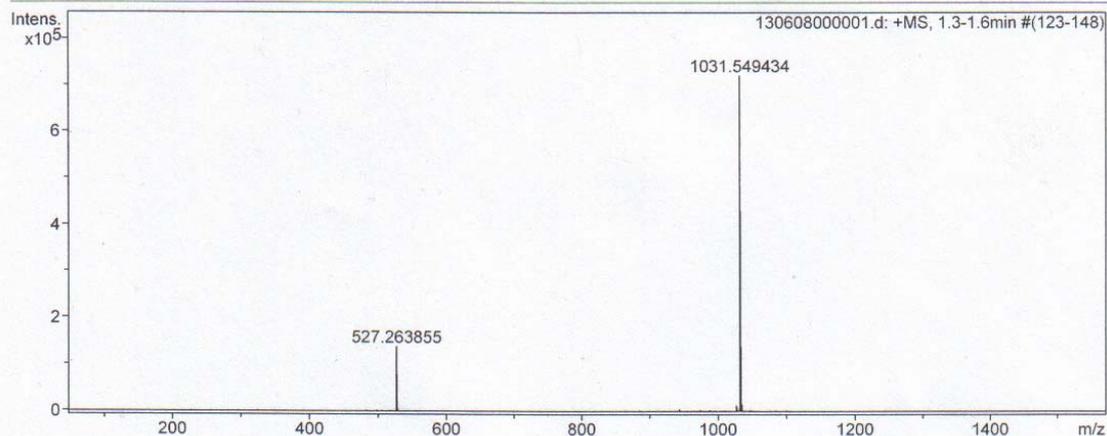


Figure S9. Mass spectra for *1,3-alt 4* (upper scan) and crude *cone 4* (lower scan)

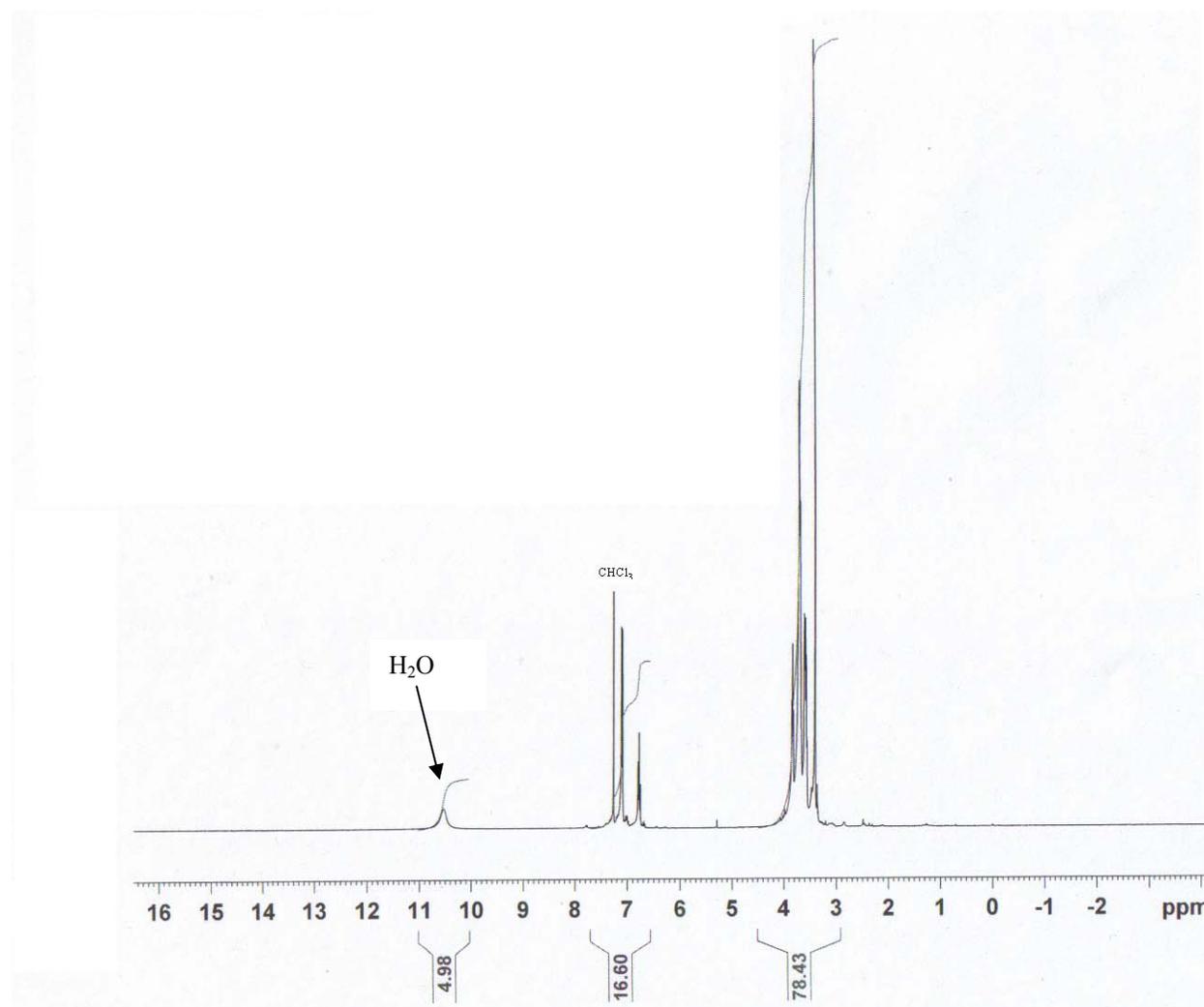
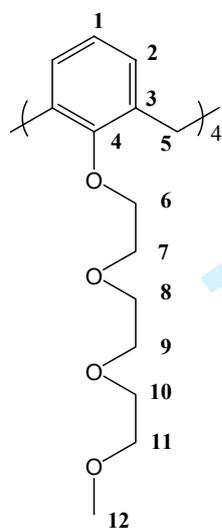


Figure S10. ^1H NMR for $1,3\text{-alt } 4 \cdot \text{Na}^+ \cdot 2 \text{H}_2\text{O}$ (18.5 h after addition of NaPF_6).



30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

C	0 min	18.5 h
1	121.7	122.9
2	133.5	133.8
3	129.9	130.2
4	155.7	155.4
5	35.0	36.2

H	0 min	2 min	27 min	84 min	18.5 h
1	6.62	6.70	6.70	6.72	6.78
2	7.05	7.08	7.07	7.08	7.10
5	3.75	3.64	3.63	3.65	3.69
6	3.85	3.83	3.84	3.84	3.84
H₂O	-	7.77	8.60	9.25	10.50

Table S1. Time dependent shifts (ppm) for selected nuclei after addition of NaPF₆.