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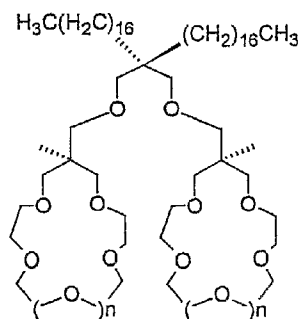
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(54) Title: A PROCESS FOR SYNTHESIZING IONOPHORES AND MOLECULES OBTAINED THEREFROM



(I)

(57) Abstract: Novel lipophilic metal cation ionophore comprising two cyclic polyether substituents having the following molecular formula is disclosed, wherein $n = 0, 1, 2$ which along with $2n$ number of methylene (CH_2) units, of each of the cyclic polyether substituent is varied to derive an ionophore having specific affinity to any one of lithium (Li^+), sodium (Na^+) and potassium (K^+) cations. Apart from intermediate compounds, a process is also disclosed for synthesizing the ionophore from pentaerythritol by first converting it to a protected diol with 2 tosylate leaving groups, converting it to a lipophilic protected diol with Grignard reaction before deprotecting the diol. A diol silyl ether is synthesized into cyclic polyether with a tosylate leaving group which is then reacted with the deprotected diol to give the desired lipophilic metal cation ionophore.

A process for synthesizing ionophores and molecules obtained therefrom

5 TECHNICAL FIELD

[001] This invention discloses lipophilic neutral ion carrier molecules, specifically, lipophilic metal ion ionophores. It may be used as a component in the cocktail preparation for forming a hydrophobic polymer membrane for selectively
10 detecting metal cations and which may be useful in ion-sensitive field effect transistor (ISFET) of ion-selective electrode (ISE) sensing devices. Process for forming these ionophores and certain intermediate compounds is also disclosed.

15

BACKGROUND ART

[002] An ionophore is typically a neutral lipophilic molecule found embedded in biological membrane systems for transporting ions across the membrane,
20 especially from where the ion would not be soluble, to the other side of the membrane across a lipid bilayer of the membrane. As ion carriers, ionophores are known to be ion-specific; hence, for example, monensin is known as a Na^+ carrier, ionomycin for Ca^{2+} , valinomycin for K^+ , and FCCP for H^+ to name but a few.

25

[003] Such ionic transportation features has been duplicated in non-biological systems and implemented as biosensor membrane. Typically, a polymeric membrane is doped with a specific ionophore to give the membrane chemical sensing ability to selectively bind desired ions and produce electrochemical
30 potential that can be related to activity of analyte. For example, in U.S. Patent No. 4,661,235 (Krull) granted and published on 28th April 1987 proposed an ion selective electrode of a lipid bilayer containing ionophores. Advances in semiconductor photolithography would naturally progress to have such

biosensing membranes fabricated at microchip level thus enabling such ion-selective features to be incorporated into metal oxide semiconductor (MOS)-type or ISFET-type of devices such as that disclosed in U.S. Patent No.

5,130,265 (Battilotti) published 14th July 1992.

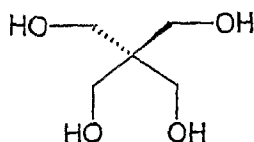
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[004] Crown ethers is known in the art as heterocycles that strongly solvate cations by coordinating the cation within its crown ring with its oxygen atoms. The size of the crown ring determines the size of the cation it can solvate. For example, in U.S. Patent No. **7,208,121** (Peper *et al.*) granted 24th April 2007

10discloses 5 ionophore molecules including 2 crown ethers, i.e. (i) BME-44 which has *bis* or two 15-crown-5 rings each suitable to solvate K⁺, and (ii) 6,6-dibenzyl-14-crown-4 which is suitable to solvate Li⁺. U.S. Patent No. **5,290,950** (Raban) discloses a wide range of tetrasubstituted cyclohexanes having ionophoric groups which may be a crown, podand or cryptand capable of
15complexing a metal or NH₄⁺ cation. In addition, Bartsch *et al.*, have also disclosed proton-ionizable lariat form of crown ethers as metal ion carriers. (Bartsch *et al.*, "Ionic recognition by proton-ionizable lariat ethers and their polymers", *Pure & Appl. Chem.*, vol. 65, No. 3, pp. 399 – 402, 1993).

20[005]In any event, an ionophore molecule having solvation for a specific cation has to be synthesised via elaborate and expensive processes. Hence, for conventional wafer level integration at volume scale production, ionophores have to be purchased at prohibitive costs.

25[006]In relation to another aspect of our invention, pentaerythritol is a known compound having a general formula as follows:



30[007] Pentaerythritol is a white, crystalline odorless solid. It is a polyol and can

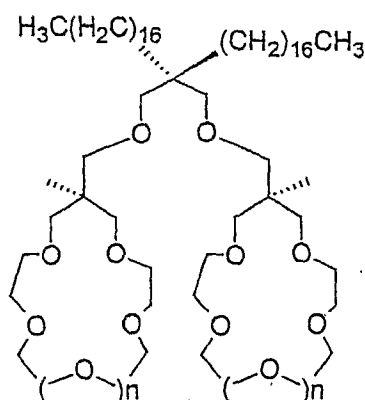
be procured in large quantity from Aldrich Chemical Co. (a member of Sigma-Aldrich Group) at economical cost. In the field of ionophore synthesis, pentaerythritol is known to be used in constituting the hydrophobic layer of synthetic lipid membranes such as disclosed in U.S. Patent No. **5,798,030** 5(Raguse) wherein tetra-alkylated pentaerythritol derivative is used. It is also known to be used as a cross-linking agent in the polymerization of membrane layers such as that disclosed in U.S. Patent No. **5,852,126** (Barnard). None of the prior art documents disclosed pentaerythritol in the synthesis of ionophores.

10

SUMMARY OF DISCLOSURE

[008]It is thus desirable that economical routes for preparing highly lipophilic carrier molecules capable of solvating metal cations such that these carrier 15 molecules may be synthesized in large quantities. It follows that our proposed neutral lipophilic carrier molecules may preferably be synthesized using economical starting materials, reagents and reaction conditions to be detailed hereinafter.

20[009]In the first aspect of our invention, certain molecules are claimed, including a lipophilic metal cation ionophore comprising two cyclic polyether substituents having a molecular formula as follows:



..... Formula V

25wherein $n = 0, 1, 2$.

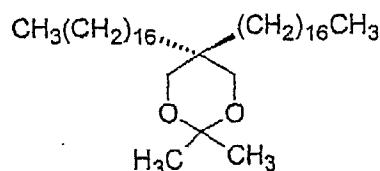
[0010] Preferably, the n number of oxygen atoms, along with $2n$ number of methylene (CH_2) units, of each of the cyclic polyether substituent is varied to derive an ionophore having specific affinity to any one of lithium (Li^+), sodium (Na^+) and potassium (K^+) cations.

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[0011] Another molecules claimed include intermediate compounds of our process, including the following 3 compounds:

- a lipophilic 1,3-dioxane-protected diol of Formula II:

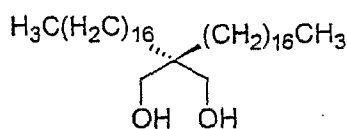
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..... Formula II

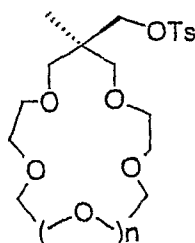
- a lipophilic diol of Formula IIa:

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..... Formula IIa

- and a cyclic polyether of Formula IV:



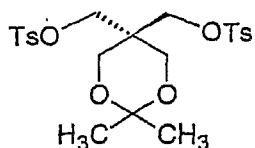
..... Formula IV

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[0012] In another aspect of our invention, a process is claimed for synthesising a lipophilic metal cation ionophore comprising the steps of:

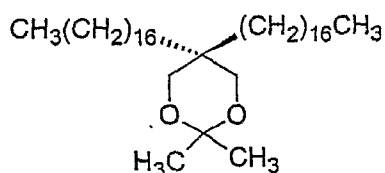
- converting pentaerythritol into a protected diol with two tosylate leaving groups having a general Formula I as follows:

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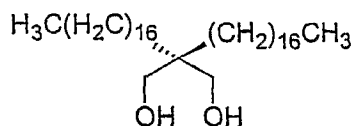
..... Formula I

- (ii) converting the protected diol with two tosylate leaving groups of Formula I via an organometallic reaction into a lipophilic protected diol having a Formula II as follows:



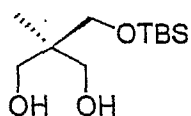
..... Formula II

- and deprotect said diol to produce corresponding lipophilic diol having a Formula IIa as follows:



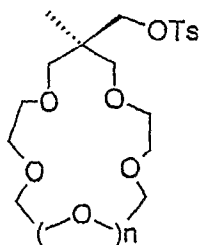
..... Formula IIa

- synthesizing from a diol silyl ether having a general Formula III as follows:



..... Formula III

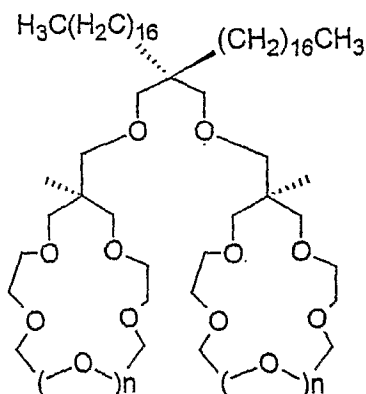
- into a cyclic polyether with a tosylate leaving group having a general Formula IV as follows:



..... Formula IV

wherein $n = 0, 1, 2$;

- (iv) reacting lipophilic diol of Formula IIa with cyclic polyether tosylate of
 5 Formula IV to synthesize a lipophilic metal cation ionophore having a
 general Formula V as follows:



..... Formula V

10[0013] Preferably, the derivation of the lipophilic protected diol of Formula I from
 pentaerythritol in step (i) above includes the steps:

- (a) protecting said diol's first two hydroxyl groups; and
- (b) converting said diol's two remaining hydroxyl groups to *p*-
 toluenesulfonate leaving group.

15

[0014] Preferably, the aforesaid organometallic conversion of the lipophilic
 protected diol of Formula I into a corresponding lipophilic diol of Formula IIa
 involves a Grignard reaction, including using hexadecylmagnesiumbromide
 $[\text{CH}_3(\text{CH}_2)_{15}\text{MgBr}]$ as the Grignard reagent. Preferably still, the protected diol of
 20 Formula II is a 1,3-dioxane, and which may be extracted from the reaction
 mixture and deprotected to provide the corresponding diol of Formula IIa.

[0015] In another preferred embodiment, the diol silyl ether of Formula III in step (iii) is obtained from direct silylation of a dioxane compound. Preferably, the diol silyl ether of Formula III is prepared from 2,2-bis(hydroxymethyl) propionic acid by protecting the two hydroxyl groups followed by reduction of the carboxylic group and silylation of the alcohol product. The reaction preferably takes place in THF and in an inert atmosphere with sodium hydride (NaH). Preferably, the number n of oxygen atoms and the number $2n$ of methylene units, in the resultant silylated cyclic polyether of Formula IV are predetermined in the synthesis by affinity to specific cation, including any one of lithium, sodium and potassium, desired of the subsequent-lipophilic carrier of Formula V.

[0016] In yet another preferred embodiment, the lipophilic diol of Formula IIa and the silyl cyclic polyether of Formula IV are each dissolved in THF and are added to each other in a proportion of 1:2. Preferably, the lipophilic metal cation ionophore is crystalized from resultant residue in methanol of the reaction mixture.

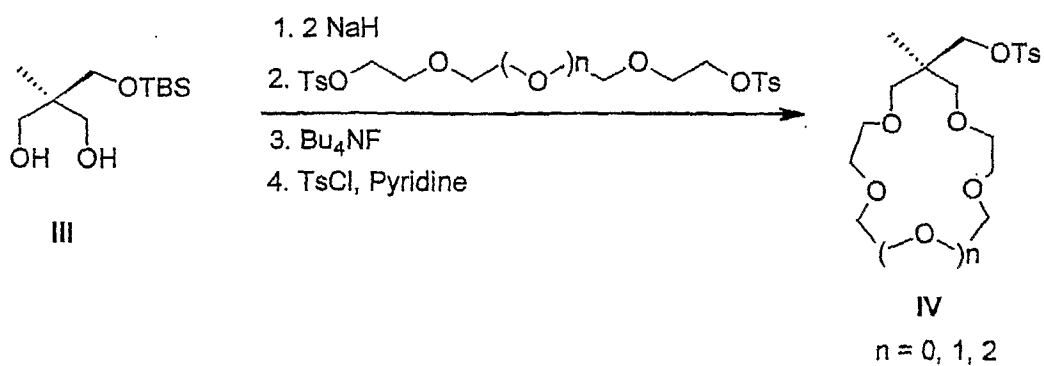
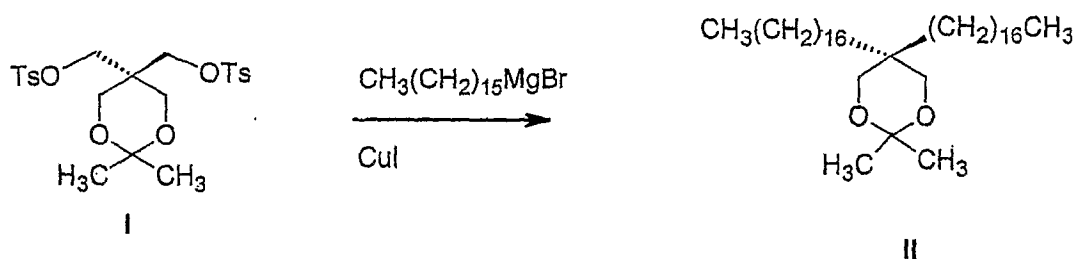
DETAILED DESCRIPTION SPECIFIC EMBODIMENTS

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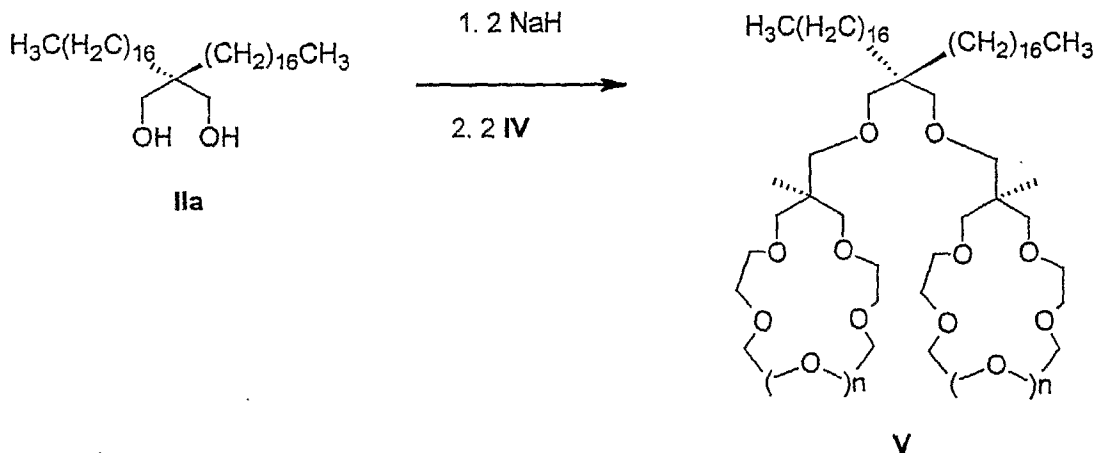
[0017] We shall now describe in detail processes comprising synthetic routes for preparation of novel neutral carrier molecules with high lipophilic characteristics for specific metal cations. These processes are suitable to be incorporated in the production of hydrophobic sensing membrane cocktails.

25

[0018] In brief, our proposed process may be conveniently described in the following 3 principal stages of synthesis as represented in the following reactions:



5



10[0019]The 3 stages may be conveniently captioned in the following as

- (i) synthesis of lipophilic protected diol;
- (ii) synthesis of hydroxymethyl crown ether; and
- (iii) synthesis of metal cation ionophore.

Each of these stages will be described in detail in the following.

Synthesis of Lipophilic Protected Diol

[0020] Briefly, this stage involves converting pentaerythritol, as a starting material, into a protected diol with two tosylate leaving group having a general Formula I, and converting it via an organometallic reaction into a lipophilic protected diol having a Formula II.

[0021] The protected diol of Formula I needs to be prepared first using standard preparative procedure with pentaerythritol as the starting material.

10 Pentaerythritol and common reagents used in our trials may be purchased from Fluka/Sigma-Aldrich and, in our trials, we were able to use them without further purification. Two hydroxyl groups are protected first followed by conversion of the two remaining hydroxyl groups to tosylate leaving groups. These standard conversion steps give protected diol I in high yields.

15

[0022] For the organometallic reaction, preferably Grignard reagent is used. Hexadecylmagnesium-bromide is prepared from hexadecyl-bromide using literature procedure. The alkyl bromide may be purchased from Sigma-Aldrich and is first freshly distilled under reduced pressure.

20

[0023] As for reaction vessel, we use a 500 ml, one-necked, round-bottomed flask which is equipped with septum, magnetic stirring bar, addition funnel and argon line. The flask is flame-dried and cooled to room temperature in a desiccator. An argon atmosphere is introduced and copper iodide (CuI) (1.89 g, 259.9 mmol) is added. Then 30 ml of tetrahydrofuran (THF) is added to the flask, stirring is initiated and the slurry is cooled to 0°C with ice-water bath. Dissolved hexadecylmagnesiumbromide (0.035 mol in 100 ml solution of THF) is added via a 50-ml syringe over a period of 20 min. and stirring is continued for an additional 30 min.

30

[0024] Protected diol of Formula I (0.033 mol in 100 ml THF) is added dropwise through the addition funnel over a period of 20 min. After the additional 30 min.

of stirring at 0°C, the ice-water bath is removed and the reaction mixture is allowed to warm to room temperature. After an additional 3- hour stirring at ambient temperature, the reaction mixture is quenched by slow addition of 200 ml of an aqueous saturated ammonium chloride solution while the flask is chilled with ice-water bath. Then the ice-water bath is removed, and the quenched mixture is stirred for 20 min:

[0025] The reaction mixture is then transferred to a 1-litre separatory funnel to have its layers separated. The aqueous layer is extracted four times with 50-ml of methylene chloride. The organic layer and extracts were then combined and dried with magnesium sulfate. The drying agent is separated by filtration followed by distillation of the solvent to give about 0.03 mol of yellowish oil. This material is to be used in the next step without further purification. The protected diol of Formula II is deprotected using a standard procedure to give the corresponding diol of Formula IIa to give high yield and used in the final stage of synthesizing the metal cation ionophore.

Synthesis of Hydroxymethyl Crown Ether

[0026] Diol silyl ether of Formula III can be prepared from direct silylation of commercially available dioxane compound. This diol silyl ether can be prepared from commercially available 2,2-bis(hydroxymethyl)propionic acid using well-known procedure, i.e. protection of the two hydroxyl groups followed by reduction of the carboxylic group and silylation of the alcohol product. These steps give good yield of the diol silyl ether.

[0027] To a 250-ml three-necked, round-bottomed flask equipped with a reflux condenser, addition funnel, magnetic stir bar and a syringe inlet is placed 50 ml of freshly distilled THF. The reaction chamber is kept flushed with a steady flow of argon and sodium hydride (NaH) (0.63 g, 26.25 mmol) is added into the flask. The flask is chilled with ice-water bath and 6.55 mmol of diol silyl ether of Formula III (prepared earlier) is added dropwise into the flask via the addition

funnel over a period of 30 min.

[0028] The ice-water bath is then removed and the reaction mixture stirred for additional 2 hours at room temperature. The reaction mixture is allowed to remain at room temperature and 7.0 mmol of ditosylate ($n = 0, 1, 2$) in 50-ml of anhydrous THF is added over 20 min via 50-ml syringe while keeping the temperature of the reaction mixture not to exceed 30°C. The reaction mixture is stirred for additional 12 hr at room temperature before the flask is immersed in ice-water bath. After the mixture is cooled to 0°C, ice-water is added slowly to destroy unreacted NaH.

[0029] About 100 ml of methylene chloride is first added to the residue. The organic layer is then dried over $MgSO_4$ and distilled at atmospheric pressure to remove the solvent. Crystallization of the residue from methanol gives about 156.0 mmol of silylated crown ether of Formula IV.

Synthesis of Metal Cation Ionophore

[0030] To a 250-ml three-necked, round-bottomed flask equipped with a reflux condenser, addition funnel, magnetic stir bar and a syringe inlet is placed about 50 ml of freshly distilled THF. The reaction chamber is kept flushed with a steady flow of argon and sodium hydride (NaH) (0.63g, 26.25 mmol) is added into the flask.

[0031] The flask is chilled with ice-water bath and 7 mmol of lipophilic diol of Formula IIa, prepared earlier, is added dropwise into the flask via the addition funnel over a period of 30 min. The ice-water bath is then removed and the reaction mixture stirred for additional 2 hours at room temperature. The reaction mixture is allowed to remain at room temperature while 14 mmol of silyl crown ether of Formula IV ($n = 0, 1, 2$) in 50 ml of anhydrous THF is added over 20 min via a 50-ml syringe. The temperature of the reaction mixture is not allowed to exceed 30°C.

[0032] The reaction mixture is stirred for additional 1 hour at room temperature followed by reflux for 2 hour. The progress of the reaction is monitored using thin layer chromatography. The flask is then immersed in ice-water bath. After the mixture is cooled to 0 °C, ice-water is added slowly to destroy unreacted NaH.

[0033] Finally, the THF solvent removal by distillation is described. Methylene chloride (100 ml) and 50 ml of 10% HCl is first added to the residue. The mixture is transferred to a 500-ml separatory funnel and the organic layer is washed twice with 50 ml of water. The organic layer is then dried over MgSO_4 and distilled at atmospheric pressure to remove the solvent. Crystallization of the residue from methanol gives 5.5 mmol of white solid which is the lipophilic metal cation ionophore of Formula V.

15

INDUSTRIAL APPLICABILITY

[0034] The ionophore molecules synthesized from the above process can be characterized using spectroscopic methods and other conventional analytical means to show that the particular molecule has been successfully synthesized. Bis-crown ether of Formula V is found to have good binding characteristics on metal cations. Depends on the number of oxygen atoms ($n = 0, 1$ or 2) of the crown ether group, it can be designed to recognize and specifically binds lithium, sodium and potassium.

[0035] The ionophores can be used to prepare cocktails of hydrophobic polymeric membrane. The prepared cocktails can be applied on ISE or ISFET surfaces such that the sensitivity and selectivity of the hydrophobic membrane can be characterized from the respective response and selectivity plots.

[0036] Our present invention can thus be applied to fabricate chemical sensor

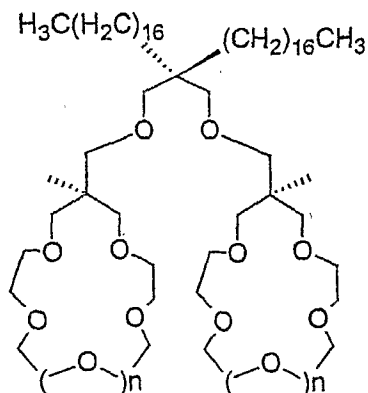
platform for the analysis of cations such as soil macronutrients, *in vivo* biochemical analysis of plant nutrient uptake and determination of the extent of contamination of ground water and river caused by fertilizers, natural causes or accidental spills, for example. With our present invention, economically viable routes are thus provided for the syntheses of highly lipophilic metal cations ionophores in large quantities or volume production using commercially available starting materials and chemical reagents that can be procured cheaply from various sources and at bulk quantities.

10[0037]It will be understood by a person having ordinary skill in the art that many of the materials, reagents, catalysts, reaction conditions and parameters described above may be substituted or replaced by an analog or similarly reactive substitutes. Some of the reaction routes may also be modified or varied according to conventional procedures or depending on the materials or
15reagents used, or carried out in similarly modified apparatuses. These modifications or variations are to be deemed to fall within the letter and scope of our invention as defined in the following claims.

CLAIMS

What we claim are:

51. A lipophilic metal cation ionophore comprising two cyclic polyether substituents having a molecular formula as follows:



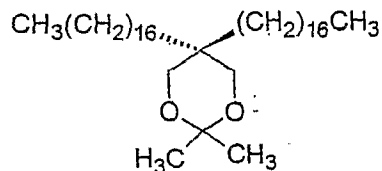
..... Formula V

wherein $n = 0, 1, 2$.

10

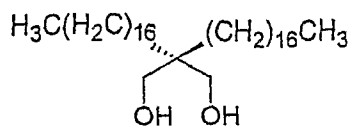
2. A lipophilic metal cation ionophore according to Claim 1 wherein the n number of oxygen atoms, along with $2n$ number of methylene (CH_2) units, of each of the cyclic polyether substituent is varied to derive an ionophore having specific affinity to any one of lithium (Li^+), sodium (Na^+) and potassium (K^+) cations.

3. A lipophilic 1,3-dioxane-protected diol having a molecular formula as follows:



..... Formula II

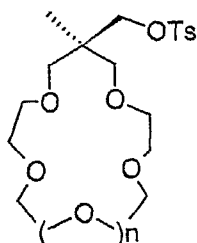
4. A lipophilic diol having a molecular formula as follows:-



..... Formula IIa

5. A cyclic polyether having a molecular formula as follows:

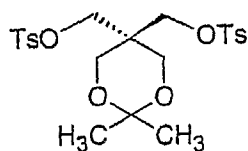
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..... Formula IV

6. A process for synthesising a lipophilic metal cation ionophore comprising the steps of:

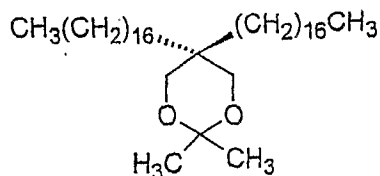
- (i) converting pentaerythritol into a protected diol with two tosylate leaving groups having a general Formula I as follows:



..... Formula I

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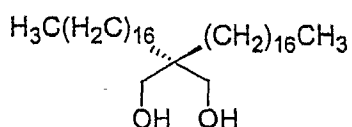
- (ii) converting the protected diol with two tosylate leaving groups of Formula I via an organometallic reaction into a lipophilic protected diol having a Formula II as follows:



..... Formula II

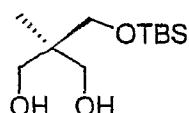
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and deprotect said diol to produce corresponding lipophilic diol having a Formula IIa as follows:



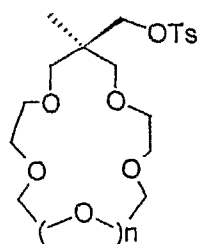
..... Formula IIa

- (iii) synthesizing from a diol silyl ether having a general Formula III as follows:



..... Formula III

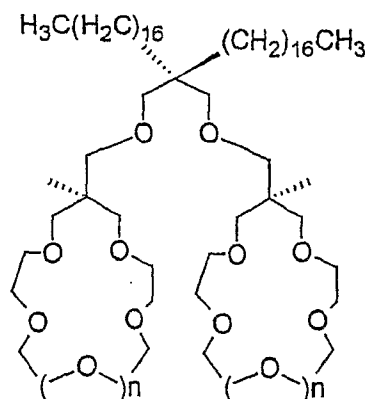
into a cyclic polyether with a tosylate leaving group having a general Formula IV as follows:



..... Formula IV

wherein $n = 0, 1, 2$;

- (iv) reacting lipophilic diol of Formula IIa with cyclic polyether tosylate of Formula IV to synthesize a lipophilic metal cation ionophore having a general Formula V as follows:



..... Formula V

7. A process for synthesising a lipophilic metal cation ionophore according to Claim 6 wherein the derivation of the lipophilic protected diol of Formula I from pentaerythritol in step (i) includes the steps:
- (a) protecting said diol's first two hydroxyl groups; and
 - 5(b) converting said diol's two remaining hydroxyl groups to *p*-toluenesulfonate leaving group.
8. A process for synthesising a lipophilic metal cation ionophore according to Claim 6 wherein the organometallic conversion of the lipophilic protected diol of Formula I into a corresponding lipophilic diol of Formula IIa involves a Grignard reaction.
159. A process according to Claim 8 wherein the Grignard reaction includes hexadecylmagnesiumbromide $[\text{CH}_3(\text{CH}_2)_{15}\text{MgBr}]$ as the Grignard reagent.
10. A process for synthesizing a lipophilic metal cation ionophore according to Claim 6 wherein the protected diol of Formula II is a 1,3-dioxane.
11. A process according to Claim 10 wherein the protected diol of Formula II is extracted from the reaction mixture and deprotected to provide the corresponding diol of Formula IIa.
12. A process for synthesising a lipophilic metal cation ionophore according to Claim 6 wherein the diol silyl ether of Formula III in step (iii) is obtained from direct silylation of a dioxane compound.
13. A process according to Claim 12 wherein the diol silyl ether of Formula III

is prepared from 2,2-bis(hydroxymethyl)propionic acid by protecting the two hydroxyl groups followed by reduction of the carboxylic group and silylation of the alcohol product.

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14. A process according to Claim 12 wherein the reaction takes place in THF in an inert atmosphere with sodium hydride (NaH).

1015. A process according to Claim 12 wherein the number n of oxygen atoms and the number $2n$ of methylene units, in the resultant silylated cyclic polyether of Formula IV are predetermined in the synthesis by affinity to specific cation, including any one of lithium, sodium and potassium, desired of the subsequent lipophilic carrier of Formula V.

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16. A process according to Claim 6 wherein the lipophilic diol of Formula IIa and the silyl cyclic polyether of Formula IV are each dissolved in THF and are added to each other in a proportion of 1:2

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17. A process according to Claim 6 wherein the lipophilic metal cation ionophore is crystalized from resultant residue in methanol of the reaction mixture.

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