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# Synthesis of orthogonal end functionalized oligoethylene glycols of defined lengths

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**Abstract**—The synthesis of oligoethylene glycols of defined lengths possessing different end functionalities is described. The utility of these molecules towards the development of a generic membrane anchor is demonstrated.


Oligoethylene glycols (OEG) of defined lengths are important molecules as they serve as starting materials in the syntheses of a variety of compounds, among them crown ethers,[1] calixarenes,[2] spacer molecules for drug-conjugates,[3] surfactants,[4] specialty polymers,[5] interlocking rotaxanes[6] and molecular 'necklaces'.[7] We were interested in these 'bioneutral' molecules as spacers for sensing devices, as oligoethylene glycol modification of surfaces or self-assembled monolayers inhibit nonspecific adsorption of proteins and biofouling agents.[8] Specifically, we have been involved in the development of a membrane based sensor platform that allows proximity induced fluorescence resonance transfer in response to multivalent binding events.[9] In the course of the development of the biosensor, we realized that relevant recognition elements preferentially be separated by spacers, optimized in length, from the fluid membrane surface for improved binding efficiency. Our synthetic strategy in developing a generic membrane anchor for biosensor applications (shown in Fig. 1), necessitated the development of a flexible and practical method towards oligoethylene glycols of defined lengths with orthogonal end functionalities.

We tried to circumvent stepwise introduction of the amine groups and subsequent protection/deprotection steps in reported syntheses[10] to allow for a fast and convenient access to various length oligoethylene glycol spacers. Previous syntheses start with the conversion of one of the terminal hydroxyl groups of OEG's to the monomesylate derivative. This reaction was shown to be inefficient because of the concomitant generation of the bismesylate product and minor amounts of unprotected starting material. The separation of the monomesylate derivative is generally performed using tedious column chromatography. The monomesylate derivative is converted to the monoazide, subsequently reduced and protected using $BOC_2O$ or FMOC-Cl. Repeating the mesylation on the remaining hydroxyl and introduction of the amine yields the desired mono-N-protected diamino OEG derivative. To avoid this multi-step time consuming procedure, we developed a straightforward method towards orthogonal end-functionalized oligoethylene glycols yielding directly and in fewer steps, the mono-N-protected diamino OEG's, which can be easily incorporated into our synthetic strategy.

The synthesis of the bisazide derivatives of commercially available tetraethylene glycol and hexaethylene glycol is shown in Scheme 1. Briefly, tetra or hexaethylene glycol is converted to the bismesyl derivative in 90% yield using 2.2 equiv of methane sulfonyl chloride in the presence of triethylamine as base. Conversion of the bismesyl derivatives to the bisazide in 75% yield is achieved by reacting with 2.5 equiv of sodium azide in dry DMF at 75 °C. The crucial monoreduction step was achieved by reacting the bisazide with 1.05 equiv of triphenylphosphine in a 5:1 ethyl acetate: 1 M HCl biphasic system. Monoreduction of one of the azide groups is followed by rapid protonation; this water soluble polar OEG monoamine derivative migrates to the water layer and subsequent reduction is hindered. In fact, even a slight excess, ca. 1.3 equiv of triphenylphosphine *does not*

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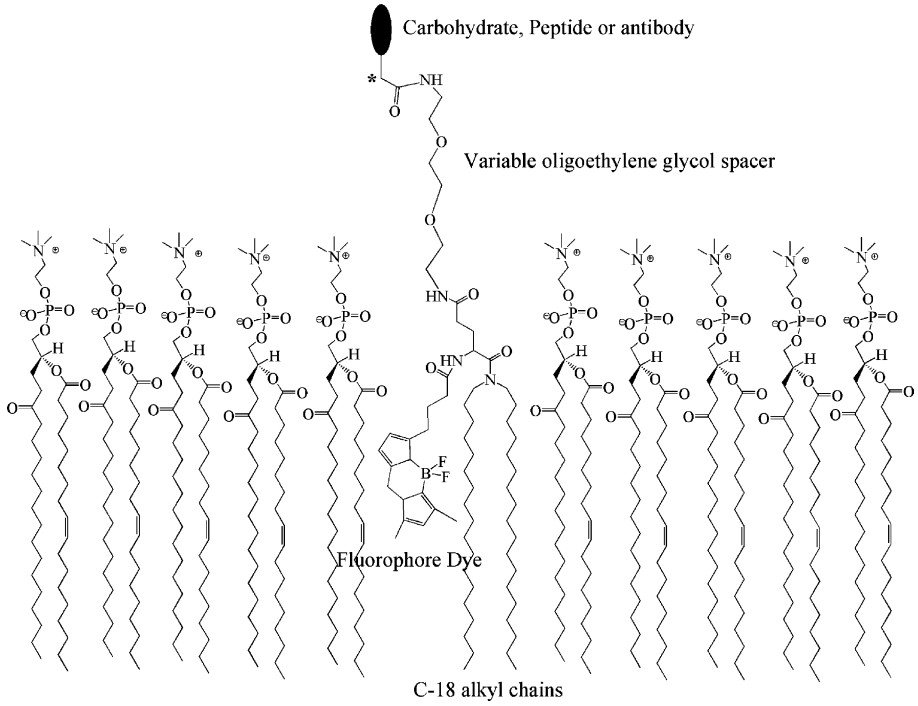
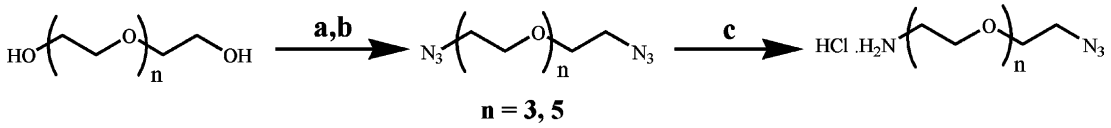


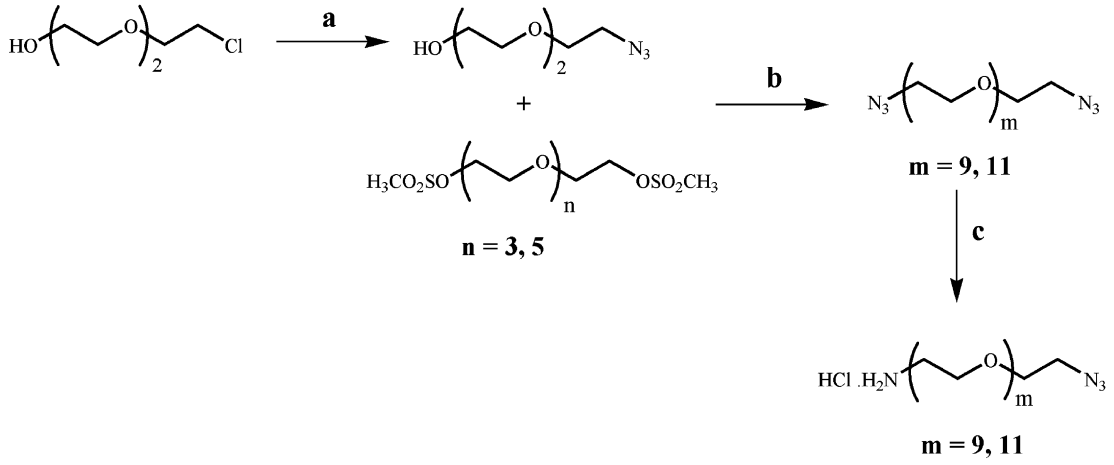

**Figure 1.** Schematic representation of the membrane biosensor. The generic membrane anchor consists of C18-alkyl chains to effect stable insertion in the POPC lipid layer, the BODIPY dye for fluorescence resonance energy transfer and the recognition element coupled via the variable 'bioneutral' oligoethylene chain for multivalent binding events.



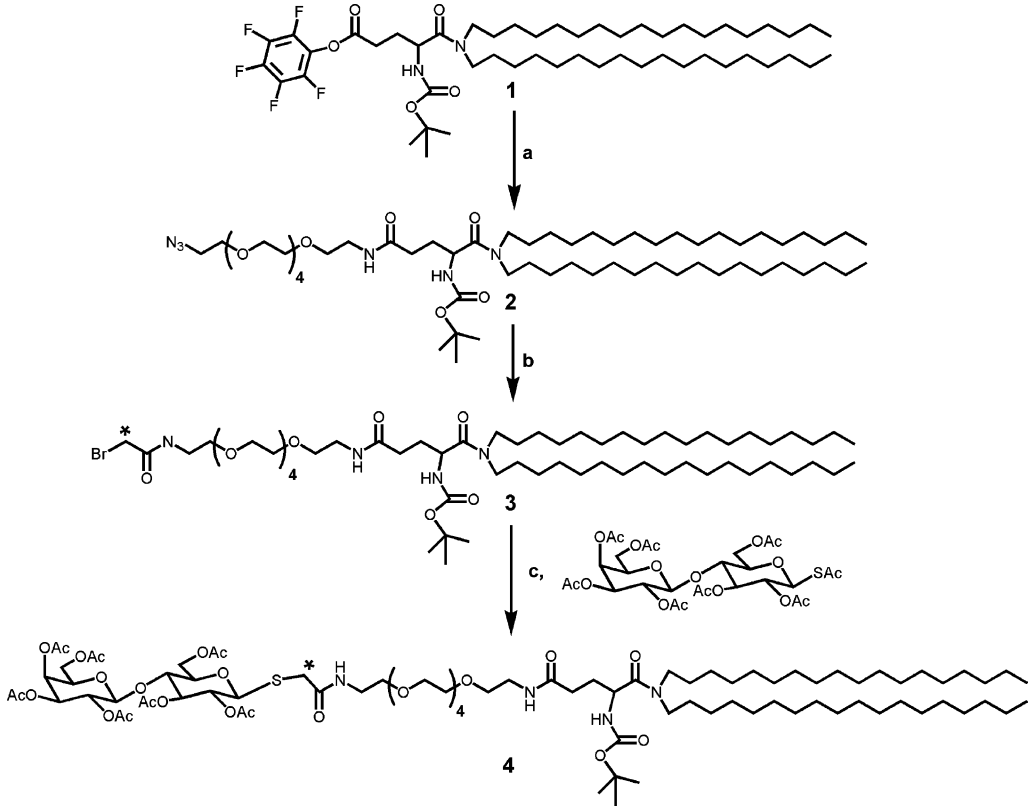

**Scheme 1.** Reagents and conditions: (a) $CH_3SO_2Cl$, $NEt_3$, 12 h, 25 °C, 90%; (b) $NaN_3$, DMF, 6 h, 80 °C, 75%; (c) $PPh_3$, EtOAc, 1 M HCl, 12 h, 25 °C, 65%.

*generate* the bisamine derivative. After the reaction is complete, the organic layer is discarded and the water layer is washed with ethyl acetate to remove triphenylphosphine oxide. Final purification steps involve passing the aqueous layer through a commercial solid phase extraction C-18 column to yield the desired product in 65% yield.[11] Thus, we have been able to take advantage of the water soluble nature of the OEG monoamine to achieve controlled partial reduction and employ a facilitated work up procedure to obtain the end differentiated ethyleneglycols. In order to test the applicability of this synthetic strategy towards longer OEG's such as deca, dodeca, hexadeca and octadeca OEG's, which we found not to be commercially available, we had to design a synthetic route towards these molecules (shown in Scheme 2). Briefly, commercially available chlorotriethylene glycol was converted to the azido triethylene glycol in 85% yield by reacting it with sodium azide in dry DMF at 75 °C. Reaction of the azidotriethylene glycol with tetra or hexaethylene glycol bismesylate in the presence of sodium hydride results in the formation of deca and dodeca bisazido OEG's in good to moderate yields. Similarly, reaction of the azidohexaethylene glycol with TEG or HEG bismesylate results in the formation of hexadeca and octadeca bisazidoethylene glycols. As described before, the reaction of the OEG bisazides with triphenylphosphine in an ethyl acetate/dil HCl media and subsequent workup resulted in the desired products.

Further elaborations of the monoamine monoazido OEG's was carried out using standard organic chemistry techniques. Briefly, the pentafluorophenyl activated ester of the trifunctional glutamic acid derivative possessing two $C_{18}$ alkyl chains was reacted with the monoamine monoazidohexaethylene glycol to yield the membrane anchor derivative with a hexaethylene glycol, **2** as shown in Scheme 3. Reduction of the free azide and subsequent derivatization with a $^{13}C$ labelled bromoacetamide was carried out in the presence of 2 equiv of diisopropylamine. The labelled carbon is of particular importance as it provides an NMR handle to monitor the progress of the conjugation of biomolecules possessing a free thiol group to the bromoacetamide. Indeed, reaction of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose with the bromoacetamide derivative, **3** in the presence of diethylamine yields results **4**. The




**Scheme 2.** Reagents and conditions: (a) $NaN_3$, DMF, 4 h, 80 °C, 85%; (b) NaH, 12 h, 60%; (c) $PPh_3$, EtOAc, 1 M HCl, 12 h, 25 °C, 65%.




**Scheme 3.** Reagents and conditions: (a) DIPEA, $H_2N(CH_2CH_2O)_5CH_2CH_2N_3$, 4 h, 0 °C → 25 °C, 70%; (b) i. $Pd(OH)_2$, $H_2$, 2 h, 25 °C, 90%; ii. $C_6F_5OC(=O)^{13}CH_2Br$, DIPEA, 3 h, 0 °C → 25 °C, 75%; (c) DMF, DIEA, 12 h, 25 °C, 60%.

$^{13}C$ NMR shift of the labelled carbon from 29 to 33 ppm can be used directly to monitor and confirm the formation of **4**. Analysis by ESI-MS is in accordance with the product.[11]

In conclusion, a simple, convenient and general method towards the synthesis of monoamine monoazide OEG's has been developed, that not only avoids several protection/deprotection steps but provides facilitated work up of the desired products. Studies to observe the effect of OEG chain length towards the binding efficiency of relevant receptor molecules is currently underway and will be reported soon.

## Acknowledgements

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11. All compounds gave satisfactory NMR and mass spectral data. Selected spectral data for some of the compounds are given below. For $N_3(CH_2CH_2O)_5CH_2CH_2N_3$: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 3.69 (m, 5H, $CH_2CH_2O$), 3.39 (t, 1H, $J = 5.1$ Hz, $CH_2N_3$) $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 70.9, 70.8, 70.7, 70.2, 50.8 ($CH_2N_3$). EIMS calcd for $C_{12}H_{24}N_6O_5$: 333.136, found: 333.188. For $N_3(CH_2CH_2O)_{11}CH_2CH_2N_3$: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 3.69–3.65 (m, br, 11H, $CH_2CH_2O$), 3.41–3.77 (m, 1H, $CH_2N_3$) $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 72.1, 70.8, 70.2, 50.9 ($CH_2N_3$). EIMS calcd for $C_{24}H_{48}N_6O_{11}Na$: 619.428, found: 619.467. For $N_3(CH_2CH_2O)_{15}CH_2CH_2N_3$: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 3.69–3.65 (m, br, 15H, $CH_2CH_2O$), 3.39 (t, 1H, $J = 5.1$ Hz, $CH_2N_3$) $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 72.7, 70.8, 70.2, 50.8 ($CH_2N_3$). EIMS calcd for $C_{32}H_{64}N_6O_{15}Na$: 795.433, found: 795.400. For $N_3(CH_2CH_2O)_3CH_2CH_2NH_3Cl$: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.30 (s, br, 1H, N*H*), 3.85 (t, 1H, $J = 5.7$ Hz), 3.68 (m, 5H, $CH_2CH_2O$), 3.46 (t, 1H, $J = 4.8$ Hz, $CH_2N_3$) $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 70.5, 70.3, 70.1, 66.9, 50.9 ($CH_2N_3$), 39.9 ($CH_2NH_3$). EIMS calcd for $C_8H_{19}N_4O_3$ ($M^+$): 219.146, found: 219.166. For $N_3(CH_2CH_2O)_5CH_2CH_2NH_3Cl$: $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 3.71–3.68 (m, br, 10H), 3.48 (t, 1H, $J = 4.8$ Hz, $CH_2N_3$), 3.08–3.04 (m, 1H) $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 69.4, 69.0, 68.2, 67.8, 49.9 ($CH_2N_3$), 39.1 ($CH_2NH_3$). EIMS calcd for $C_{12}H_{27}N_4O_3$ ($M^+$): 307.198, found: 307.267. For $N_3(CH_2CH_2O)_{11}CH_2CH_2NH_3Cl$: $^1H$ NMR (300 MHz, 10% $CD_3OD$ in $CDCl_3$) $\delta$ 3.61–3.40 (m, br, 22H), 3.48 (br, 1H), 2.96 (br, 1H) $^{13}C$ NMR (75 MHz, 10% $CD_3OD$ in $CDCl_3$) $\delta$ 71.9, 70.7, 70.5, 70.4, 67.1, 59.1, 51.1 ($CH_2N_3$), 39.8 ($CH_2NH_3$). EIMS calcd for $C_{24}H_{51}N_4O_{11}Na$: 593.337, found: 593.267. For $N_3(CH_2CH_2O)_{15}CH_2CH_2NH_3Cl$: $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 3.69–3.56 (m, br, 30H), 3.30 (m, 1H), 3.18 (m, 1H) $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 73.7, 71.6, 68.1, 62.3, 51.9 ($CH_2N_3$), 40.8 ($CH_2NH_3$). EIMS calcd for $C_{32}H_{67}N_4O_{15}$ ($M^+$): 747.460, found: 747.333. For **2**: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.91 (s, 1H, N*H*), 5.52 (d, 1H, $J = 4.2$ Hz), 4.53 (t, 1H, $J = 3.0$ Hz), 3.66–3.63 (m, br, 16H, $CH_2CH_2O$), 3.48–3.27 (m, br, 4H), 3.18 (m, 2H), 3.07 (m, 2H), 2.31 (m, 2H), 2.05 (s, 1H), 1.75 (m, br, 1H), 1.42 (s, br, 9H, $(CH_3)_3CO$), 1.25 (s, br, 68H, $CH_3(CH_2)_{17}N$), .87 (t, 6H, $J = 6.9$ Hz, $CH_3(CH_2)_{17}N$). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 70.7 ($CH_2CH_2O$), 70.4 ($CH_2CH_2O$), 50.8 ($CH_2N_3$), 29.9 ($CH_3(CH_2)_{17}N$), 22.9 (($CH_3)_3CO$) EIMS calcd for $C_{58}H_{115}N_6O_9$: 1039.872, found: 1039.835. For **3**: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.02 (s, 1H, N*H*), 6.70 (s, 1H), 5.56 (d, 1H, $J = 7.8$ Hz), 4.54 (t, 1H, $J = 6.9$ Hz), 4.15 (s, 1H), 3.64–3.32 (m, br, 17H, $CH_2CH_2O$), 3.14 (m, 1H), 3.06 (m, 1H), 2.29 (vt, 2H, $J = 6.3$ Hz), 2.02 (m, 1H), 1.75 (m, 2H), 1.56 (m, 4H), 1.44 (s, 9H, $(CH_3)_3CO$), 1.26 (s, br, 68H, $CH_3(CH_2)_{17}N$), 0.91 (t, 6H, $J = 6.9$ Hz, $CH_3(CH_2)_{17}N$). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 70.6 ($CH_2CH_2O$), 29.9 ($CH_3(CH_2)_{17}N$), 29.3 ($NH(C{=}O)^{13}CH_2Br$ labelled carbon) 22.9 (($CH_3)_3CO$). EIMS calcd for $C_{60}H_{117}N_4O_{10}Br$: 1156.807, found: 1156.793. For **4**: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.10 (s, 1H, N*H*), 6.67 (s, br, 1H, N*H*), 5.60–5.45 (m, 1H), 5.23 (m, 1H), 5.11 (m, 1H), 5.00–4.91 (m, 2H), 4.53 (d, 1H, $J = 9.9$ Hz, H1), 4.50 (d, 1H, $J = 8.1$ Hz, H1′), 4.17–4.07 (m, 2H), 3.90 (m, 1H), 3.68–3.57 (m, br, 22H, $CH_2CH_2O$), 3.52–3.34 (m, br, 7H), 3.20 (m, 2H), 2.30 (vt, 1H, $J = 6.3$ Hz), 2.19 (s, 3H, $CH_3CO$), 2.18 (s, 3H, $CH_3CO$), 2.20–2.10 (m, 2H), 2.10 (s, 3H, $CH_3CO$), 2.09 (s, 3H, $CH_3CO$), 2.07 (s, 6H, $CH_3CO$), 2.02 (s, 3H, $CH_3CO$), 1.59–1.53 (m, 3H), 1.46 (s, 9H, $(CH_3)_3CO$), 1.28 (s, br, 68H, $CH_3(CH_2)_{17}N$), .93 (t, 6H, $J = 6.9$ Hz, $CH_3(CH_2)_{17}N$). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 101.3 (C1′), 83.2 (C1), 70.7 ($CH_2CH_2O$), 70.4 ($CH_2CH_2O$), 33.8 ($NH(C{=}O)^{13}CH_2S$), 29.9 ($CH_3(CH_2)_{17}N$), 22.9 (($CH_3)_3CO$), 20.8 ($CH3CO_2$ (acetate)). EIMS calcd for $C_{86}H_{153}N_4O_{27}SNa$: 1729.264, found: 1729.267.