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AN ITERATIVE SYNTHESIS OF RADIOLABELLED POLYETHYLENE GLYCOL OLIGOMERS

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SUMMARY

A synthetic method has been developed for the iterative preparation of unlabelled and ^{3}H-polyethylene glycol (PEG) oligomers. The strategy involved alkylating the sodium anion of mono-tritylated ethylene glycol oligomers ($Ph_3C[OCH_2CH_2]_nOH$, n=1-28) with O-tosyl-O-allyl-triethylene glycol or O-tosyl-O-allyl-pentaethylene glycol. Subsequent ozonolysis of the terminal olefin followed by reduction with $NaBH_4$ or $NaB[^{3}H]_4$ provided the next higher mono-tritylated ethylene glycol oligomer which could be deprotected to the final glycol oligomer ($H[OCH_2CH_2]_nOH$, n=4-34) or carried on in the iterative process. The method provides discrete PEG oligomers of high specific activity (20.6-48.6 mCi/mmoL) in 96-98% radiochemical purity.

Keywords: tritium, [1-^{3}H]polyethylene glycols, permeability probes, paracellular transport.

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INTRODUCTION

The role of molecular size in the determination of intestinal absorption of orally administered drugs remains incompletely resolved. Polyethylene glycols (**1**, PEGs; $H[OCH_2CH_2]_nOH$) are commonly employed as size standards for probing the transport pathway of the intestinal epithelium (1-3). Developing a homologous series of uniquely characterized size probes allows determination of membrane permeability over a range of sizes (mw = 194-1515 gm/mole) relevant to absorption of both common xenobiotics and small peptides. Further, use of radiolabelled PEGs offers the opportunity to simplify and expedite sample analysis. Commercially available radiolabelled PEGs are a non-specifically labelled asymmetric distribution of molecular weights surrounding the average value. For example, ~95% of the mass of ^{3}H-PEG 900 is distributed between molecular weights of 100 and 2000 with the

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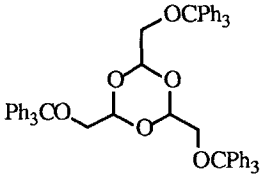
distribution skewed towards the lower molecular weights (4,5). Use of these mixtures would have made unequivocal interpretation of results very difficult because each fragment in the commercial sample would have an independent value for intestinal uptake. To resolve this impasse, we elected to develop an iterative synthesis which would produce discrete oligomers of PEGs in either a non-radiolabelled or radiolabelled form (6). Progress towards that end is reported in this paper.

DISCUSSION AND RESULTS

The Scheme summarizes the synthetic approach which was designed to introduce the radiolabel late in the synthesis of each discrete oligomer to avoid carrying the radiolabel through each iteration. This permits large quantities of material to be carried through several iterations to prepare the higher molecular weight oligomers without the concerns of radiochemical decomposition of intermediates or of handling large quantities of radioactivity. The building blocks **3b**, **6**, and **7** were prepared by routine synthetic methods as summarized in equations 1-3. It is noteworthy that the ozonolysis of **4b** must be carried out in the presence of sodium carbonate (0.1 equiv) to prevent the formation of the trimer **5** and that when the reduction is carried out with sodium borotritide ^{3}H-**6** is obtained and can be converted into ^{3}H-ethylene glycol (**1**, n=1)(7).



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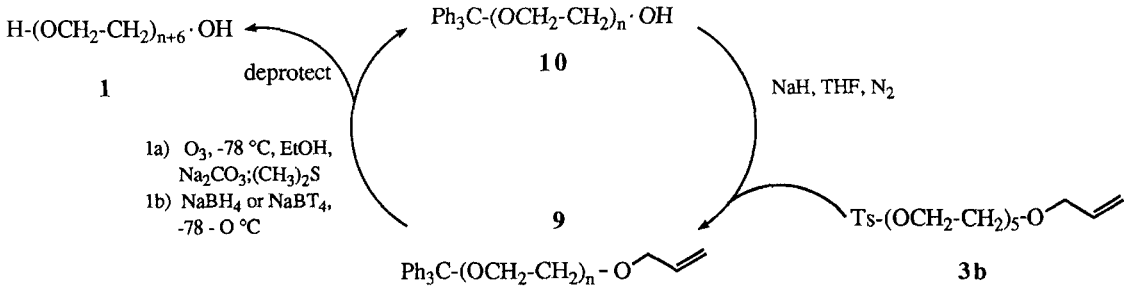
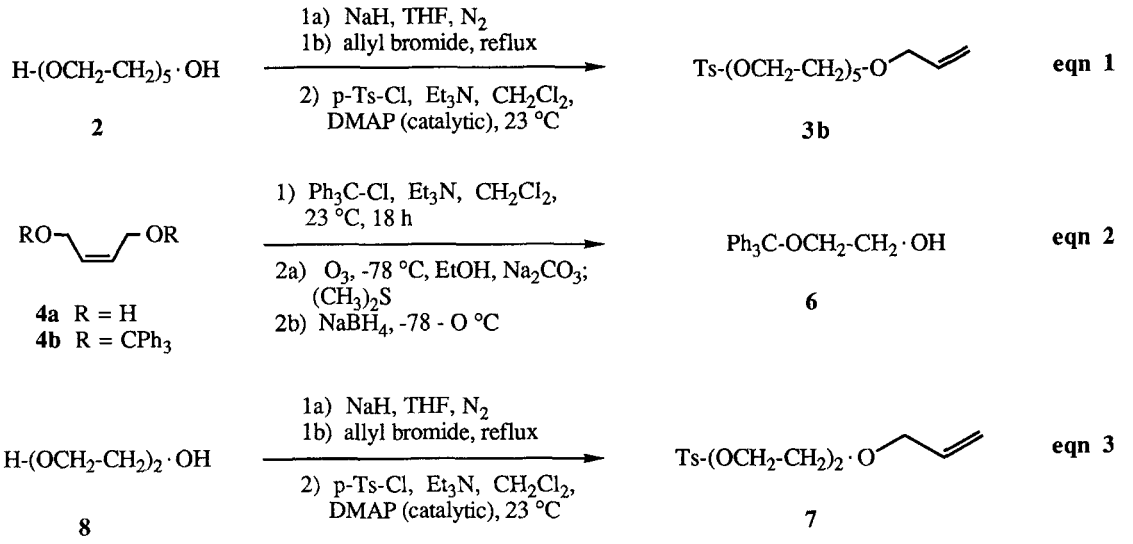
The iterative elongation began with the alkylation of the sodium anion of **6** (3.0 equiv NaH as an 80% dispersion in oil) with allyl tosylate **7** in refluxing tetrahydrofuran (THF) for three hours to provide **9** (n + 5 = 3) in a 93% yield. When the alkylation was carried out with 1.2 or 2.2 equivalents of sodium hydride, the reactions failed to give complete conversion even though **6** and **7** were detected by tlc analysis of a quenched aliquot from the reaction mixture. In each case, when the total amount of sodium hydride added to the reaction reached approximately 3 equivalents, complete consumption of **6** and **7** was observed. We have no explanation for the apparent consumption of sodium hydride in the alkylation step.

The radiolabel was introduced by ozonolysis of **9** in absolute ethanol at -78 °C in the presence of sodium carbonate (0.1 equiv). The resulting ozonide was reduced directly with a small excess of sodium borotritide at -78 °C to provide the mono-protected ^{3}H-oligomer **10a** (n = 3). The final ^{3}H-tetraethylene glycol **1** (n = 4) was obtained by removing the trityl protecting group through exposure to a 4M solution of hydrochloric acid in dioxane at 23 °C, neutralization of the solution with concentrated

SCHEME. ITERATIVE SYNTHETIC CYCLE





ammonium hydroxide, and purification by chromatography. Each of the subsequent oligomers, **9b-9f**, were processed in a similar fashion to provide each of the radiolabelled ethylene glycol oligomers tabulated in Table I. The final radiolabelled oligomers were obtained in 96-98% radiochemical purity as judged by radio-high performance liquid chromatography (8).

Table I. Yields and Specific Activities of ^{3}H-Polyethylene Glycols (1).

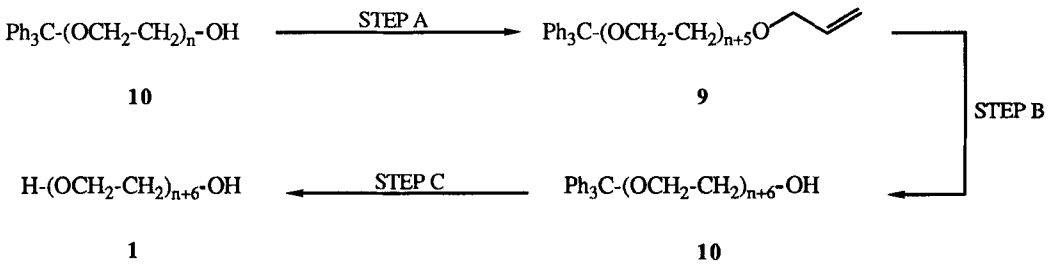
9 **10** **1**

n	starting olefin **9** (mmol)	NaB^3H_4 (mCi)	^{3}H-alcohol **10** (mCi), % yield[a]	^{3}H-PEG **1** (mCi), % yield[b]	^{3}H-PEG specific activity (mCi/mmol)
3	0.347	12.28[c,d]	5.65, 92	4.83, 86	20.6
9	0.287	23.69[d]	10.56, 89	6.95, 66	40.2
15	0.135	14.11[d]	3.10, 44	2.53, 81	33.2
21	0.113	12.70[d]	3.15, 50	1.90[f], 60	37.7
27	0.081	12.85[e]	2.53, 39	1.65, 65	40.1
33	0.080	13.70[e]	3.01, 44	1.54, 51	48.6

[a] Radiochemical yields are based on material of ≥97% purity by radio-TLC and are calculated from 50% of the total NaB^3H_4 radioactivity due to reduction of formaldehyde produced in ozonolysis. [b] Radiochemical yields are based on material of ≥97% purity by radio-TLC, ≥98% purity by radio-HPLC. [c] 0.26 mmol of unlabelled $NaBH_4$ added after 1.5 h. [d] 196 mCi/mmol. [e] 407 mCi/mmol. [f] Contained 8% of an unidentified radiochemical impurity. A second chromatography of a portion of this material provided 0.643 mCi of n = 21 homologue of 96% radiochemical purity by HPLC.

The subsequent oligomers were prepared by ozonolysis of the previous oligomer of **9** and direct reduction of the resulting ozonide with excess sodium borohydride to provide the corresponding oligomer of unlabeled **10** which served as pivotal intermediates in that they could be converted into the final ethylene glycol oligomers or elongated by alkylation with **3b** following the procedure described for the preparation of **9a**. Initially, the detritylations were carried out as described for the preparation of the radiolabelled series; later it was found that deprotection by hydrogenolysis with Pd black (formed in situ by treating $PdCl_2$ with hydrogen gas) and hydrogen gas for 1 h in absolute methanol provided superior results. The final unlabeled ethylene glycol oligomers were obtained in pure form by standard silica gel

Table II. Yields for the Iterative Synthesis of Unlabeled Polyethylene Glycol Oligomers



Starting	Step A		Step B		Step C	
Material (n)	Product	% Yield	Product	% Yield	Product	% Yield
10a (4)	**9b**	98	**10b**	91	**1b**	54
10b (10)	**9c**	85	**10c**	95	**1c**	64
10c (16)	**9d**	59	**10d**	88	**1d**	43
10d (22)	**9e**	79	**10e**	85	**1e**	36
10e (28)	**9f**	59	**10f**	82	**1f**	17

chromatography. Typically, 1 - 2 gms of each oligomer of **10** were deprotected and the balance of the material was carried forward to the next iteration. The yields for each iteration are tabulated in Table II.

In conclusion, we have presented a simple, iterative synthetic scheme which provides access to discrete ethylene glycol oligomers (**1**, n = 4 - 34; mw = 194 - 1515 gm/mole). The scheme also provides the opportunity to obtain these oligomers in either the non-radiolabelled or radiolabelled form through the reduction of a common ozonide intermediate with either sodium borohydride or sodium borotritide. The radiolabelled oligomers can be obtained in high specific activity (20.6 - 48.6 mCi/mmoL) and in 96-98% radiochemical purity. These discrete oligomers of known specific activity are being utilized in intestinal permeation studies to assess the role of molecular weight in intestinal uptake processes; these results will be reported in due course.

EXPERIMENTAL SECTION

Proton Magnetic Resonance spectra were obtained on a Nicolet QE-300 (300 MHz). Chemical shifts are reported as δ values (ppm) relative to Me_4Si as the internal standard. Mass spectra were obtained with Hewlett Packard HP5985 (CI, EIO, Varian CH7 (EI)), and Kratos MS50 (FAB, HRMS) spectrometers. Elemental analysis and the above determinations were performed by the Analytical Research Department at Abbott Laboratories, Abbott Park and North Chicago.

Thin-layer chromatography (TLC) was carried out using E. Merck precoated silica gel F-254 plates (thickness, 0.25 mm). TLC plates were visualized with iodine vapors or ultraviolet light (when possible). Radiochemical purity determinations using TLC plates were performed on a Radiomatic RS radio-thin-layer chromatography scanner. Chromatographic purification was carried out by either medium pressure liquid chromatography (MPLC) employing columns packed with EM Silica gel 60 (40-63 μM) or Merck 63-200 μM silica gel at 30-50 psi or by forced air chromatography (FAC) employing the previously described silical gel at 5-10 psi of air pressure in the noted solvent system. High performance liquid chromatography (HPLC) was carried out using a Waters 6000A solvent delivery system, a Rheodyne 7125 injector, and a Waters μBondapak C_{18} 3.9 x 30 cm column. An HPLC mobile phase of methanol/water 65:35 was used at a flow rate of 1.0 mL/min. This system was connected to a Radiomatic Flo-one Beta Model IC radioactive flow detector for HPLC radiochemical purity determinations. Liquid scintillation counting was performed with an LKB-Wallac 1214 Rackbeta Excel liquid scintillation counter and a Packard Insta-Gel scintillation cocktail.

Sodium boro[^{3}H]hydride was obtained from Amersham (196 mCi/mmol) and from New England Nuclear (407 mCi/mmol). Tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane was distilled from P_2O_5. All other solvents and reagents were reagent grade and used without further purification.

O-(Allyl)pentaethylene glycol tosylate (3b). A flame-dried, 2-necked, 1000 mL round-bottom flask was charged, under a nitrogen atmosphere, with 400 mL of freshly dried THF, sodium hydride (5.04 g, 0.17 mol, 80% dispersion in oil), and a magnetic stir bar. The flask was fitted with a side-arm pressure equalizing addition funnel, which was charged with pentaethylene glycol **2** (100 g, 0.42 mol) in dry THF (200 mL), and a reflux condenser with a nitrogen inlet and outlet. The pentaethylene glycol was added slowly to the stirred reaction mixture at 23 °C. When hydrogen gas evolution ceased, the addition funnel was recharged with allyl bromide (14.5 mL, 0.17 mol, passed through a neutral alumina pad just prior to use) and THF (50 mL); the resulting solution was added to the reaction mixture. The reaction was heated at 75 °C for 1 h, cooled to ~40 °C, the pH adjusted to ~pH = 6 with glacial acetic acid, filtered through a celite pad, and concentrated *in vacuo*. The resulting thick oil was plug filtered through a silica gel [sg] pad (450 gm) while eluting with 2 L of ethyl acetate then 1 L of 5% methanol/ethyl acetate. Fractions containing the product were combined and concentrated *in vacuo* to provide **3a** (44.37 g, 94%).

Tosylation of **3a** was achieved by combining **3a** (44.37 g, 0.16 mol), *p*-toluenesulfonyl chloride [Ts-Cl] (38.89 g, 0.20 mol), triethylamine (35.5 mL, 0.26 mol), 4-dimethylaminopyridine [DMAP] (4.15 g, 0.034 mol) and dichloromethane (650 mL) in a 1 L round-bottom flask and stirring overnight at 23 °C. The resulting reaction mixture was diluted with ethyl ether (1000 mL), washed successively (1 X, 500 mL 10% aqueous HCl; 1 X, 500 mL saturated aqueous $NaHCO_3$; 1 X, 500 mL brine), dried (anhydrous Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by forced-air chromatography (450 gm sg; 50% ethyl acetate in hexanes) provided **3b** (56.34 gm, 82%) as a thick faintly yellow oil. **3b**: ^1H NMR ($CDCl_3$, TMS) δ 7.8 (d, 2 H), 7.33 (d, 2 H), 5.92 (m, 1 H), 5.27 (d q, 1 H), 5.18 (d, q, 1 H), 4.17 (t, 2 H), 4.03 (t, 2 H), 3.58-3.70 (m, 21 H); Anal. calcd for $C_{20}H_{32}O_8S$: C, 55.54; H, 7.46. Found: C, 55.61; H, 7.42.

bis-O-Trityl cis-2-butene-1,4-diol (4b). A 500 mL round-bottom flask was charged with a magnetic stir bar, cis-2-butene-1,4-diol (7.06 g, 77.7 mmol), triethylamine (27.0 mL, 194 mmol), and dichloromethane (330 mL). To the reaction was added trityl chloride (48.6 g, 171 mmol) in large portions; a slight exotherm was observed. The reaction was stirred overnight at 23 °C. After removing the volatiles *in vacuo*, the resulting slurry was partitioned between ethyl ether and water. The aqueous layer was drawn off and back extracted with ethyl ether (2 X, 300 mL). The combined organic layers were washed (2 X, brine), dried (anhydrous $MgSO_4$), filtered, and concentrated *in vacuo* to provide **4b** as a sticky foam. Recrystallization from dichloromethane and hexanes provided

pure **4b** (35.79 g; 80.4 % from 2 crops. **4b**: m.p. 141-142 °C; ^{1}H NMR ($CDCl_3$, TMS) δ 7.13-7.50 (m, 30 H), 5.24 (t, 2 H), 3.50 (d, 4 H); Anal. calcd for $C_{42}H_{36}O_2$: C, 88.08; H, 6.34. Found: C, 88.07; H, 6.40.

O-trityl ethylene glycol (6). A 500 mL round-bottom flask was charged with **4b** (68.7 g, 120 mmol), THF (300 mL), and a magnetic stir bar. Gentle warming was required to completely dissolve **4b**. To this solution was added sodium carbonate (1.27 g, 12.0 mmol). After cooling to -78 °C, ozone was bubbled through the solution until a very faint blue color persisted. The excess ozone was purged from the reaction by bubbling N_2 gas through the solution until the blue color was removed. To the -78 °C solution was added absolute ethanol (250 mL) and methyl sulfide (26.7 mL, 360 mmol) and the reaction stirred for 5 minutes. A single portion of sodium borohydride (4.54 g, 120 mmol) was added, the cooling bath removed, and the reaction stirred overnight as it warmed to 23 °C. A portion of aqueous sodium hydroxide (250 mL, 2 N NaOH) was added, the reaction stirred 1 h at 23 °C, and the volatiles removed *in vacuo*. The resulting white slurry was partitioned between ethyl acetate (500 mL) and water (150 mL). The aqueous layer was drawn off and back extracted with ethyl acetate (2 X, 300 mL). The combined organic extracts were washed successively (1 X, saturated aqueous NH_4Cl; 1 X, water; 1 X, brine), dried (anhydrous $MgSO_4$), filtered, and concentrated *in vacuo* to provide **6** as a crystalline solid. Recrystallization from hexanes/dichloromethane provided pure **6** (47.34 g, 65%) collected in two crops. **6:** m.p. 106-107.5 °C. (lit. 95-96 (8) and 98-100 °C (9); ^{1}H NMR ($CDCl_3$, TMS) δ 7.2-7.5 (m, 15 H), 3.75 (br q, 2 H), 3.28 (t, 2 H), 1.97 (br t, 1 H).

3-(2-Tosyloxyethoxy)-1-propene (7). Following the procedure for the preparation of **3a**, diethylene glycol **8** (14.5 mL, 1.53 mol) was converted to the corresponding sodium salt with sodium hydride (15.3 g, 80% dispersion in oil, 0.51 mol) and alkylated with allyl bromide (44 mL, 0.51 mol) to provide the alkylation adduct (52.30 g, 70%) after forced air chromatography (450 g sg, 25% ethyl acetate/hexanes). Subsequent tosylation, as previously described, provided the title compound as a light yellow oil in a 93% yield. **7**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.8 (d, 2 H), 7.33 (d, 2 H), 5.88 (m, 1 H), 5.27 (d q, 1 H), 5.19 (d q, 1 H), 4.17 (t, 2 H), 3.98 (d, 2 H), 3.7 (t, 2 H), 3.51-3.63 (m, 4 H), 2.43 (5, 3 H); MS (DCI) $(M+NH_4)^+ = 318$, $(M + H)^+ = 301$.

Due to the iterative nature of this work, a typical procedure for each conversion is provided. Yields for each step in the subsequent iterations are tabulated in Table II. The corresponding conditions for purification and melting points of the final unlabelled ethylene glycol oligomers are

tabulated in Table III. The oligomers of **9** and **10** were amorphous waxy solids. Yields dropped for the larger oligomers due to lower recoveries from column chromatography. These losses could be minimized by packing the column using 2% of a low boiling amine (i.e. isopropylamine or dipropylamine) in the desired solvent system and increasing the percent of the polar solvent by 5-10% after the product started eluting from the column. Satisfactorily analytical results were obtained on the alkylation adducts **9** and the final ethylene glycol oligomers.

3-(O-trityl triethylene glycol)-1-propene 9a. The general procedure for the preparation of **3a** was followed when **6** (17.15 g, 56.34 mmol) was converted to the corresponding sodium anion with sodium hydride (5.1 g, 80% dispersion in oil, 169.0 mmol) and refluxed for 3 h in dry THF with **7** (22.0 g, 73.2 mmol). The reaction was cooled, the excess sodium hydride quenched by slowly adding excess methanol, and the resulting suspension was made homogeneous by adding water. The quenched reaction mixture was extracted (2 X, ethyl ether, 700 mL). The combined organic layers were washed (3 X, brine), dried (anhydrous Na_2SO_4), filtered, and concentrated *in vacuo*. The resulting gold oil was purified by chromatography (FAC; 450 gm sg; 25% ethyl acetate/hexanes) to provide the pure title compound **9a** (22.61 g, 93%) as a faintly yellow oil. **9a**: ^{1}H NMR ($CDCl_3$, TMS) δ 7.43-7.5 (m, 6 H), 7.20-7.32 (m, 9 H), 5.91 (m, 1 H), 5.27 (d q, 1 H), 5.16 (d q, 1 H), 4.02 (d t, 2 H), 3.58-3.72 (m, 10 H), 3.24 (t, 2 H); Anal. calcd for $C_{28}H_{32}O_4 \cdot 0.75H_2O$: C, 75.39; H, 7.56. Found: C, 75.65; H, 7.30.

This general procedure was employed to convert **10a** - **10e** to the next higher homologs **9b** - **9f**. For the conversions of **9c**, **d**, and **e** to the higher homologs, it was necessary to employ 2.0 equivalents of **7** to achieve complete conversion. The ^{1}H NMR data given for **9a** is representative of those obtained for **9b** - **9f** with the only difference being that the multiplet between δ= 3.6 - 3.7 increased relative to the other absorptions. In all cases, analytically pure samples were obtained either from a center cut fraction or by recrystallization. Refer to Table II for yields in the alkylation steps.

General Procedure for Ozonolysis of Olefin Precursor 9 and Tritium Reduction to [1-^{3}H] Trityl Alcohol 10. A solution of polyethylene olefin precursor (0.08 - 0.347 mmol) and sodium bicarbonate (0.1 equiv) in 8 mL of methanol, 4 mL of tetrahydrofuran, and 5 mL of dichloromethane (15 mL of absolute ethanol and 8 mL of tetrahydrofuran for n = 3 and 9) was stirred and cooled to -78 °C. Ozone (3 - 5 equiv) was passed through the solution followed by a 10 min nitrogen purge. Dimethyl sulfide (3 equiv) was added and the solution was stirred for 10 min.

Table III. Purification Conditions for Compounds in Each Iterative Cycle.

Starting Material **9** (n)	**10**	Purification[1] Method/Solvent(s)[2]	**9**	Purification[1] Method/Solvent(s)[2]	**1**	Purification[1] Method/Solvent(s)[2]
4	b	F/a	b	F/k	b	F/d
10	c	F/b	c	F/k	c	F/b, e
16	d	F/b	d	F/l	d	F/f
22	e	M/c	e	F/l	e	R/g; F/h, e
28	f	M/c	f	F/l	f	R/i; F/e, h, j

[1]F=forced-air chromatography; M=medium pressure liquid chromatography; R=recrystallization. [2]a) 1.5% MeOH/EtOAc b) 2% MeOH/EtOAc c) 5% MeOH/$CHCl_3$ d) 10% MeOH/EtOAc e) column packed with noted solvent system plus 2 - 5% isopropylamine f) EtOAc/Hexanes g) EtOAc h) 3% MeOH/$CHCl_3$ i) MeOH/EtOAc j) 2% MeOH/2% isopropylamine/$CHCl_3$ k) 5% MeOH/EtOAc l) 1:10:90 dipropylamine/ MeOH/ EtOAc

Sodium boro[^{3}H]hydride (196 mCi/mmol or 407 mCi/mmol, 12.85 - 23.69 mCi as listed in Table I) was suspended in a total of 4 - 5 mL of absolute ethanol and added with stirring to the reaction mixture. After warming to room temperature over 2 h, ca. 1 mL of water was added and the solution was concentrated under reduced pressure. The residue was redissolved in 25 mL of ethyl acetate and washed (2 x 10 mL of saturated sodium carbonate) The combined aqueous layers were extracted with 10 mL of ethyl acetate and the combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography (10% methanol in chloroform) of the residue afforded [1-^{3}H]trityl alcohol in 62 - 87% chemical yield (see Table I for radiochemical yields). Radio-TLC analysis of all products indicated radiochemical purities of ≥ 96% and an R_f range of 0.32 - 0.55.

In the case of the n = 3 precursor, 12.28 mCi (0.063 mmol) of sodium boro[^{3}H]hydride was added to the ozonolysis mixture as described above. After 1.5 h, 10 mg (0.26 mmol) of unlabelled sodium borohydride was added and the reaction mixture was stirred at room temperature an additional 30 min. Reaction workup and product isolation were then performed as usual. This procedure was subsequently found to be unnecessary for the incorporation of adequate amounts of radioactivity during the preparation of higher homologues.

Non-radiolabelled Procedure for the Conversion of the Olefin Precursors 9 to the Trityl Alcohols 10. A solution (THF: absolute ethanol: dichloromethane; 2:2:1) (1.0 equiv) of the desired oligomer of **9** and sodium carbonate (0.1 equiv) was cooled to -78 °C and ozone bubbled into the solution until starting material was consumed as judged by tlc analysis. Excess ozone was purged from the system by bubbling nitrogen gas through the reaction solution for 5 minutes. The ozonide was decomposed by adding methyl sulfide (3 equiv) to the -78 °C reaction solution and stirring for 5 minutes. Addition of sodium borohydride (1.0 equiv) to the -78 °C solution, removal of the cooling bath, warming of the reaction to 23 °C, and removal of the volatiles *in vacuo* provided a white partially solid residue from which the oligomers of **10** were obtained by FAC on silica gel. Yields for this step are reported in Table II and the purification conditions are tabulated in Table III.

General Procedure for Deprotection of [1-^{3}H] Trityl Alcohol 10 to [1-^{3}H] PEG 1. [1-^{3}H] Trityl alcohol (2.53 - 10.56 mCi) was added to a solution of 1 mL of 4 M hydrochloric acid in dioxane and 2 mL of methanol and stirred at room temperature for 25 min. The reaction solution was neutralized with concentrated ammonium hydroxide and concentrated *in vacuo*. The residue was taken up in 10% methanol in chloroform (significant amounts of ammonium chloride remained undissolved)

Table IV. Chemical Characterization of the Oligomers of Polyethylene Glycol.

$H(OCH_2\text{-}CH_2)_n\text{-}OH$

1

n	Cmpd	m.p. (°C)	rf	Elemental Analysis % Calcd C	% Calcd H	% Found C	% Found H	Formula
4	**11a**	oil	0.28[a]	48.79	9.37	48.79	9.15	$C_8H_{18}O_5 \cdot 0.15H_2O$
10	**1b**	oil	0.12[b]	50.41	9.30	50.21	8.86	$C_{20}H_{42}O_{11} \cdot 1.0H_2O$
16	**1c**	38.5-39.5	0.31[c]	53.17	9.20	53.25	9.22	$C_{32}H_{66}O_{17}$
22	**1d**	42-44	0.34[c]	53.54	9.19	53.70	9.09	$C_{44}H_{90}O_{23}$
28	**1e**	47-47.5	0.31[c]	53.75	9.18	53.78	9.22	$C_{56}H_{114}O_{29}$
34	**1f**	48-50.5	0.28[c]	53.88	9.18	53.54	9.08	$C_{68}H_{138}O_{35}$

a) 10% MeOH/$CHCl_3$, b) 7.5% MeOH/$CHCl_3$, c) 15% MeOH/$CHCl_3$.

and purified by chromatography (10% MeOH/$CHCl_3$). The fractions containing labelled product were combined, concentrated, filtered through decolorizing carbon (Darco), and concentrated under reduced pressure to furnish [1-^{3}H] PEG in 51 - 86% radiochemical and chemical yields (see Table I). Only the n = 4 product was obtained as an oil, all others were obtained as white solids after drying under high vacuum. Radio-HPLC analysis of the [1-^{3}H] PEG products indicated ≥ 97% radiochemical purity for each, with an R_f range of 0.19 - 0.40. Radio-HPLC analysis indicated radiochemical purities of ≥ 98%, except for the n = 22 homologue which was obtained in 96% radiochemical purity after a second chromatography to remove an unknown radiochemical impurity detected by HPLC (8% of total radioactivity after the first chromatography).

General Method for Deprotection of the Mono-trityl Ethylene Glycol Oligomers.

Method A. To a solution of **10a** (3.16 g, 7.25 mmol) in absolute methanol (20 mL) and treating the resulting solution with excess hydrochloric acid (45 mmol, 10 mL of a 4.5 M solution of HCl in dioxane) for 30 minutes at 23 °C. After cooling the reaction to ~0 °C, the reaction solution was made basic (pH ≥ 10) by adding 5 N aqueous sodium hydroxide, filtered, and the volatiles removed *in vacuo*. The resulting white cake was transferred to a prepacked column (50 g sg/gm starting material) and eluted with the solvent system noted in Table II to obtain pure tetraethylene glycol, **1a**. In a similar fashion **10b** was converted to **1b**.

Method B. A flask charged with a stir bar, $PdCl_2$ (195 mg, 1.09 mmol), and absolute methanol (2 mL) was exposed to hydrogen gas until the characteristic red color of $PdCl_2$ disappeared and was replaced by the characteristic black color of Pd black. To the resulting solution was added **10c** (1.85 g, 1.92 mmol) in absolute methanol (8 mL). The reaction was then exposed to 1 atm of hydrogen gas for 1 - 2 h when tlc analysis indicated complete reaction. The Pd black was removed by filtration through a pad of celite. The eluant was concentrated *in vacuo* and the residue purified as indicated in Table III. Table IV tabulates the chemical characterization of the final polyethylene glycol oligomers.

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FOOTNOTES AND REFERENCES

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4. This data was obtained by gel permeation chromatography on a sample of ^{3}H-PEG 900 obtained from New England Nuclear.

5. Unpublished results of Dr. D. Hoffman, Abbott Laboratories, D-46R, Abbott Park, Illinois 60064.

6. Examples of synthetic methods to prepare up to octaethylene glycols have been reported (see below). However, these methods would be unacceptable for preparing the higher molecular weight oligomers as purification is achieved through vacuum distillation. See: a. Nakatsuji, Y.; Kameda, N.; Okahara, M. - Synthesis: 280-1 (1987), b. Coudert, G.; Mpassi, M.; Guillamet, G.; Selve, C. - Synthetic Commun. 16 (1): 19-26 (1986).

7. The ozonolysis reduction and deprotection were carried out as described in the experimental section using the following amounts of reagents and chromatographic conditions for isolation:
A. Ozonolysis and reduction - 0.175 mmol **4b**, 25 mCi sodium borotritide (360 mCi/mmol) followed by 0.13 mmol sodium borohydride. Preparative TLC (hexane/ethyl acetate 50:50, 20 x 20 x 10 mm Analtech Uniplate silica gel GF precoated plate; ethyl acetate used to elute product from silica gel) afforded 4.876 mCi (37%) and 97.5 mg (92%) of [1-^{3}H] **6** as a white semisolid.
B. Deprotection - Preparative TLC (15% methanol in chloroform; ethyl acetate used to elute product from silica gel) gave 1.75 mCi (36%, 16.0 mCi/mmol) of [1-^{3}H] ethylene glycol as a clear oil. Radio-TLC analysis (15% methanol in chloroform, Rf = 0.65, $KMnO_4$ visualization) and radio-HPLC analysis (50% methanol in water, μBondapak C_{18} column, 1 mL/min) indicated ≥ 98% radiochemical purity.

8. Less than 3% of the total radioactivity was associated with impurities 1 or 2 monomeric units smaller than the designated oligomer. Radioactivity associated with the chromatographic solvent front accounted for less than 1% of the total and could be readily separated from the desired product.

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