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Soluble polymers in organic chemistry
5. Preparation of carboxyl- and amino-terminal polyethylene glycol of low molecular weight*

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1. SUMMARY

The transformation of polyethylene glycol monomethyl ether (MPEG-OH), of molecular weight 550 to carboxyl- and amino-terminal derivatives is described. Applying polymer-analogous reactions for the functionalization, the final products are isolated by pH-controlled extraction procedures. The surprisingly high solubilizing power in water together with their convenient handling demonstrates, that reactive polyethylene glycols of low molecular weight have considerable potential for applications in organic and bioorganic chemistry.

2. INTRODUCTION

Polyethylene glycols (PEGs) prove to be rather exceptional and useful polymers with respect to their physicochemical, pharmacological and biomedical properties allowing a widespread range of applications (HARRIS 1985, HUNTER et al. 1967, POWELL 1980, SOEHRING et al. 1951). Due to the amphiphilic character of its monomer unit PEG exhibits high solubility in organic solvents as well as in water. This remarkable property is used for liquid-phase peptide synthesis, PEG acting as a solubilizing protecting group of the growing peptide chain (MUTTER and BAYER 1980, PILLAI and MUTTER 1982). Moreover, the pronounced tendency for crystallization of PEGs with molecular weight (MW) ≥1,500 facilitates the reaction cycle in repetitive syntheses. As shown below for the example of α-naphthylurethane derivatives, the solubilizing effect of PEG is not limited to crystalline fractions (MW ≥2,000) but is also found for medium sized liquid or wax-like PEGs (MW ≤1,000). The application of low molecular weight PEGs offers many attractive features; however, the accessibility of these polymers carrying reactive end groups requires alternative experimental procedures compared to crystallizable PEGs. In the present communication, we report on the functionalization of MPEG-OH (MW 550) to carboxyl- and amino-terminal derivatives to be used as solubilizing spezies in organic and bioorganic chemistry .

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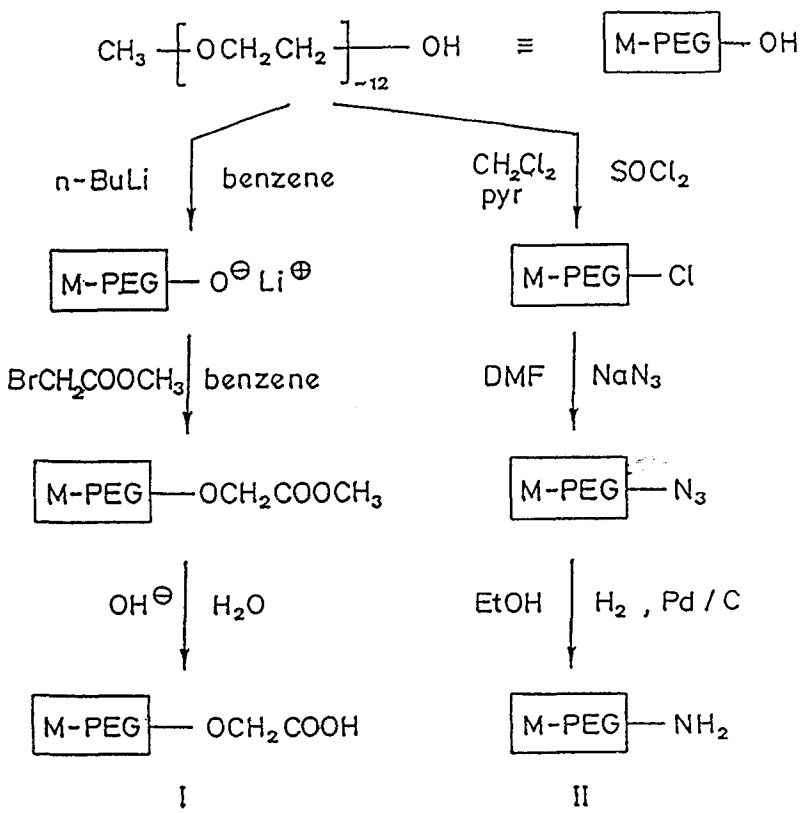
3. RESULTS AND DISCUSSION

The reaction steps for the transformation of the terminal hydroxyl group in MPEG-OH, MW 550, to either carboxyl or amino function is depicted in scheme 1.

Scheme 1



Compound I was prepared according to methods used for the modification of crystalline PEGs (ANZINGER and MUTTER 1982, GECKELER and BAYER 1979, VAN DEN BERGE et al. 1986). The lithium salt of MPEG-OH was obtained by titration of the extensively dried alcohol with butyl lithium. Reaction with methyl bromoacetate and subsequent saponification of the ester afforded acid I in an overall yield of 60%. The corresponding amine II was obtained essentially as described for high molecular weight PEGs (ZALIPSKY et al. 1983) by converting the alcohol to the chloride with thionyl chloride and pyridine as catalyst, substitution with NaN_3 in dimethyl formamide to the azide and subsequent catalytic hydrogenation in 40% overall yield. Contrary to methods described for crystalline PEGs, isolation and purification of both intermediates and products were performed by extraction procedures. An aqueous solution of the crude product was first washed with diethyl ether to remove organic by-products, the PEG-derivatives remaining in the aqueous layer. The polymer could

then be transferred almost quantitatively to dichloromethane by repeated extraction. Most notably, PEG-derivatives of different pK values, e.g. hydroxy-, carboxy- and amino-PEGs, may easily be separated by pH-controlled extraction; this allows for the removal of non-converted starting material from the desired product giving access to homogenous derivatives independent of the completeness of the functionalization. With regard to the application of medium-sized PEGs as solubilizing support in liquid-phase peptide synthesis, quantitative coupling yields in each step during the synthesis are no longer a methodological prerequisite. The unreacted amino component can be separated from the fully protected product by extraction procedures similar to conventional solution methods.

The extensively dried hygroscopic derivatives were characterized by ^{1}H-NMR and IR spectroscopy as well as by thin-layer chromatography. The first step in both reaction sequences was quantitative, judging by IR spectra (disappearance of the alcohol stretching frequency at 3500 cm^{-1}) . Similary, the IR band for the azido group at 2110 cm^{-1} permitted the monitoring of both the azide formation and its subsequent catalytic hydrogenation in the synthesis of the amino compound II. The synthesis of carboxy-PEG I was easily followed by observing the peak for the methyl ester (singlet, 3H, 3.72 ppm) and the newly introduced methylene group (-OCH_2CO-, singlet, 2H) in ^{1}H-NMR.

The final products showed quantitative functionalization as verified by micro titration. In view of the considerable potential in the application of medium-sized PEGs, the solubilizing power of these polymers in water was determined as a function of their molecular weight. To this end, the hydrophobic α-naphthyl group was attached as model substrate to monofunctional PEGs of various chain length via urethane bond. As shown in Table 1, the reference compund 1 proved to be insoluble in water, indicating that the polyether monomeric unit itself does not exert any solubilizing effect. However, the naphthyl-derivative resulting from the attachment of MPEG-OH, MW 550, showed unlimited miscibility with water at 10° C.

TABLE 1

Compound	$CH_3(OCH_2CH_2)_nOR$[a]	MW[b]	Solubility[c]
1	n = 1	76	-
2	≈12	550	+
3	≈16	750	0.74
4	≈112	5000	0.11

[a] R = -CO-NH-α-naphthyl; [b] MW of the MPEG-OH block;

[c] concentrations(mol · l^{-1}), of saturated solutions in water at 10° C:
- insoluble, + unlimited miscible with water.

Table 1: Solubilities of MPEG-O-α-naphthylurethanes, prepared from the corresponding polyethylene glycol monomethyl ether (MPEG-OH) and α–naphthylisocyanate (according to MUTTER 1978), for different chain lengths of the polymer. The concentrations of the thermostated saturated solutions were photometrically determined at E_{max} = 222 nm (for compounds 2 - 4: ε(222 nm)= 62,700 $M^{-1} \cdot cm^{-1}$)

Interestingly, the maximum molar solubilizing power of PEG is found to be in the range of about 10 monomer units (MW 500) for the investigated substrate as reflected in Table 1.
In conclusion we may note that the application of reactive PEG derivatives of medium chain length appears to represent a valuable alternative and completion to the use of crystalline PEGs for solubilizing substrates as drugs, peptides or proteins in organic and bioorganic chemistry. Some of these applications of compounds I and II are presently under investigation (MUTTER et al. in press).

4. EXPERIMENTAL PART

Abbrevations:
m: medium; MPEG-OH: polyethylene glycol monomethyl ether (MW 550); s: singlet; st: strong; t: triplet; w: weak.

Spectra were recorded on the following instruments: IR-spectra: Perkin Elmer Infrared Spectrophotometer 781; ^{1}H-NMR-spectra: Varian anaspect EM 360 NMR Spectrometer (60 MHz).

Micro titrations were carried out in a titrating apparatus from Metrohm (Potentiograph E 536, Dosimat E 535 and combined glass electrode), Herisau, Switzerland.
TLC was performed with precoated silica gel 60 F_{254} (200 µm) plates (Merck, Darmstadt, FRG) using the following solvent systems: (A) CH_2Cl_2 / methanol (7:1); (B) CH_2Cl_2 / methanol / pyridine (7:1:1). In the sequence mentioned below, the obtained chromatograms were detected by: UV (254 nm), J_2, ninhydrin (nin.) (FAHRNY et al. 1961) and Dragendorff-Bürger reagent (D.-B.) (THOMA et al. 1964).

All solvents were distilled before use; absolute solvents were obtained by standard proceedings described in the literature. MPEG-OH (MW 550, pract.), methyl bromoacetate (puriss.), thionyl chloride (puriss.), sodium azide (puriss.) and potassium hydroxide (puriss.) were purchased from Fluka (Buchs, Switzerland). Butyl lithium was obtained from Aldrich-Chemie (Steinheim, FRG) as a 1.6 M solution in hexane and titrated against butanol to determine its exact concentration. All PEG derivatives were never submitted to temperatures above 40° C and were well dried at 0.05 mbar (r.t.) over P_2O_5 before analysis and use, respectively.

α-Methoxycarbonylmethyl polyethylene glycol ω-methyl ether (MPEG-OCH_2COOCH_3)

MPEG-OH (28.67 g; 52.14 mmole) was placed in an oven-dried 750 ml four-necked flask, equipped with mechanical stirrer, dropping funnel (with gas inlet pipe) and a condenser protected by a calcium chloride tube and dissolved in 300 ml abs. benzene under dry nitrogen atmosphere. The solution was cooled to 5° C. Then a standardized 1.55 M butyl lithium solution (33.63 ml; 52.14 mmole) in hexane was injected. Freshly distilled methyl bromoacetate (40.0 ml; 430 mmole) was slowly added through the dropping funnel under good stirring. A clear yellow solution resulted. The mixture was stirred overnight at r.t. The precipitated lithium bromide was filtered off by suction and the solvent was evaporated under reduced pressure from the obtained filtrate. The oily residue was taken up in 300 ml H_2O; unreacted methyl bromoacetate separated as a second, yellow layer, which was washed off with 100 ml

diethyl ether. The remaining aqueous solution was further washed 3 times with 100 ml ether, then concentrated in vacuo to half of its volume and the polymer was extracted with five 150 ml portions of CH_2Cl_2. The combined CH_2Cl_2 layers were dried over anhydrous Na_2SO_4; the solvent was removed under reduced pressure yielding the oily product.

Yield: MPEG-OCH_2COOCH_3: 30.43 g; 48.92 mmole = 94%.
TLC: R_f(A): 0.58 (J_2, D.-B.); R_f(B): 0.87 (J_2, D.-B.);
IR(film): $\tilde{\nu}_{CO}$: 1755 cm^{-1} (m), 1740 cm^{-1} (shoulder);
missing: $\tilde{\nu}_{OH}$
^{1}H-NMR($CDCl_3$): new: δ= 3.72 ppm (s. 3H, -$COOCH_3$)
δ= 4.13 ppm (s, 2H, -OCH_2COOCH_3)

α-Carboxymethyl polyethylene glycol ω-methyl ether (MPEG-OCH_2COOH, I)

MPEG-OCH_2COOCH_3 (29.53 g; 47.5 mmole) was dissolved in 300 ml 5% aqueous KOH and stirred at r.t. for 4 h. The strong alkaline reaction solution was then washed with five 100 ml portions of CH_2Cl_2 to remove any non-acidic polymer (MPEG-OH, MPEG-OCH_2COOCH_3). The aqueous layer containing the desired acid was adjusted to pH 1 by the addition of 30 ml conc. HCl. The solution brightened up and was subsequently extracted 5 times with 100 ml CH_2Cl_2, which were combined and dried over anhydrous Na_2SO_4. The solvent was evaporated under diminished pressure. The dry acid was obtained as an oil.

Yield: I: 18.94 g; 31.15 mmole = 66%
m.p.: 16.1° - 18.6° C
TLC: R_f(A): 0.21 (J_2, D.-B); R_f(B): 0.12 (J_2, D.-B)
IR(film): $\tilde{\nu}_{OH}$: 3500 - 3300 cm^{-1} (w)
$\tilde{\nu}_{CO}$: 1760 cm^{-1}(m), 1745 cm^{-1} (shoulder)
^{1}H-NMR($CDCl_3$): new: δ= 4.18 ppm (s, 2H, -OCH_2COOH)
δ= 6.73 ppm (s, 1H, -COOH)
missing: δ= 3.72 ppm; δ= 4.13 ppm
micro titration: with standardized 1.03 · 10^{-2}M NaOH in abs. ethanol gave 1.64 mmole COOH groups per gram (= 100% functionalization).

α-Chloro polyethylene glycol ω-methyl ether (MPEG-Cl)

To a solution of MPEG-OH (25.00 g; 45.5 mmole) and dry pyridine (3.70 ml; 45.5 mmole) in 100 ml CH_2Cl_2 thionyl chloride (13.3 ml; 182 mmole) was added dropwise during 90 min. and the solution was refluxed for 4 days under gentle stirring. The precipitated pyridine hydrochlorid was then filtered off from the pale brown mixture; solvent and excess of thionyl chloride were removed under reduced pressure. After drying, the oily product was applied in the next step without any additional purification.

Yield: MPEG-Cl: 25.85 g; 45.5 mmole = 100%
TLC: R_f(A): 0.84 (J_2, D.-B.)
IR(film): missing: $\tilde{\nu}_{OH}$

α-Azido polyethylene glycol ω-methyl ether (MPEG-N_3)

MPEG-Cl (25.80 g; 45.4 mmole) and sodium azide (23.40 g; 360 mmole) were dissolved in 450 ml freshly distilled DMF and well stirred at 40° C for 4 days. The resultant cloudy mixture filtered by suction. The filtrate was left over aluminium chips (100g, activated at 120° C for 2 h) at r.t. for 2 h, in order to remove any traces of sulphur. Then the solvent was evaporated in vacuo, the oily residue was dried at 0.05 mbar (r.t.) and directly used for the following hydrogenation.

Yield: MPEG-N_3: 26.10 g; 45.4 mmole = 100%
TLC: R_f (A): 0.86 (J_2, D.-B.)
IR(film): $\tilde{\nu}_{N_3}$: 2110 cm^{-1} (st, sharp)

α-Amino polyethylene glycol ω-methyl ether (MPEG-NH_2, II)

Pd/C (10%; 3.00g) was suspended in 50 ml abs. ethanol. A solution of MPEG-N_3 (17.50 g; 30.4 mmole) in 150 ml abs. ethanol was added under nitrogen atmosphere and the mixture well shaken in a Parr low-pressure hydrogenation apparatus at 5 atm. H_2 for 20 h. The mixture was subsequently filtered over diatomaceous earth and the ethanol completely removed under diminished pressure. The oily residue was dissolved in 100 ml H_2O and washed with three 50 ml portions of ether. The aqueous layer was acidified (pH 1) by conc. HCl and treated 4 times with 100 ml CH_2Cl_2; next aqueous solution was neutralized (pH 6) by saturated $NaHCO_3$ and washed twice with 100 ml CH_2Cl_2 in order to separate any non-amino polymers. Finally, the aqueous layer containing II was adjusted to pH 10 by addition of saturated Na_2CO_3; the product was extracted by five 100 ml portions of CH_2Cl_2. After drying the combined CH_2Cl_2 layers over anhydrous Na_2SO_4, the solvent was evaporated under diminished pressure. The obtained product was dried at 0.05 mbar (r.t.).

Yield: II: 6.68 g; 12.2 mmole = 40%
m.p.: 15.5° - 18.0° C
TLC: R_f(A): 0.55 (J_2, nin., D.-B.)
IR(film): new: $\tilde{\nu}_{NH_2}$: 3370 cm^{-1}(m), 3305 cm^{-1} (shoulder)
missing: $\tilde{\nu}_{N_3}$
^{1}H-NMR ($CDCl_3$): new: δ= 1.90 ppm (s, 2H, -NH_2);
δ= 2,88 ppm (t, 2H,-CH_2-C$\underline{H_2}$-NH_2)
micro titration: with standardized 3.41 · 10^{-3}M $HClO_4$ in glacial acetic acid (II was dissolved in CH_2Cl_2/glacial acetic acid 10:1) gave 1.82 mmole amino groups per gram (= 100% functionalization).

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