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Esterification of Polyethylene Glycols

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ABSTRACT

Esterification of polyethylene glycols by various carboxylic acids in high yield and high substitution levels is described. The esterification reaction is achieved by dicyclohexylcarbodiimide and catalyzed by dimethylaminopyridine. Rate measurements indicated that the reaction is complete after 2 h at room temperature.

INTRODUCTION

The physicochemical and biological properties of polyethylene glycols (PEG) and especially their water solubility and biocompatibility have made these polymers useful for producing conjugates with bioactive materials [1, 2]. The method of choice for attaching bioactive materials to PEG is by esterification [3-9]. This method can be divided into two main approaches: (a) activation of the hydroxy endgroup through transformation into a good leaving group and subsequent attack by the carboxylate component, and (b) activation of the carboxy component and subsequent attack by PEG hydroxy end groups. To approach (a) belong formation of PEG-isourea [3] and PEG-tosylate [4]. Esterification through carboxyl activation was performed by the acid chloride method [5], transesterification [4], or direct coupling by dicyclohexylcarbodiimide (DCC) [6]. The last method is the method of

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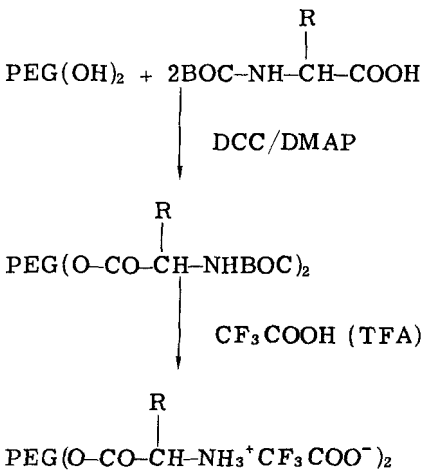
choice for attachment of N-protected amino acids to PEG [7-9]. The yields and degree of substitution achieved depend on the method employed, reaction conditions, and character of the carboxy component, and are usually low (e.g., Ref. 3). Elevated temperatures, prolonged reaction times, and large excess of reactant are often used to achieve a high degree of substitution and high yields, especially with sterically hindered carboxylic acids. Thus, for example, the maximal degree of substitution achieved by DCC coupling of sterically hindered N-tert-butoxycarbonyl (BOC)-amino acids was 60-80% [8] even though the above-mentioned drastic conditions were used.

We wish to report here an improved method for the esterification of PEG. This method employs one pot coupling of carboxyl component to PEG mediated by DCC and catalyzed by dimethyl amino pyridine (DMAP). The use of DMAP to catalyze acylation was recently reviewed by Höfle et al. [10]. The usefulness of DMAP to catalyze DCC-mediated esterification was demonstrated for low molecular weight alcohols and carboxylic acids [11-14] as well as for esterification of BOC amino acids to hydroxymethyl polystyrene resin [15, 16].

RESULTS AND DISCUSSION

As model compounds for the esterification of PEG by DCC/DMAP we have used N-carbobenzyloxy (Z-) and BOC-protected amino acids. We have chosen PEG 2000 and PEG 3000 since these two polymers were reported to give low substitution levels with BOC amino acids [7]. Also, the low molecular weight polymers allow fairly accurate determination of substitution levels by NMR and elemental analysis. The reaction is described in Scheme 1.



SCHEME 1.

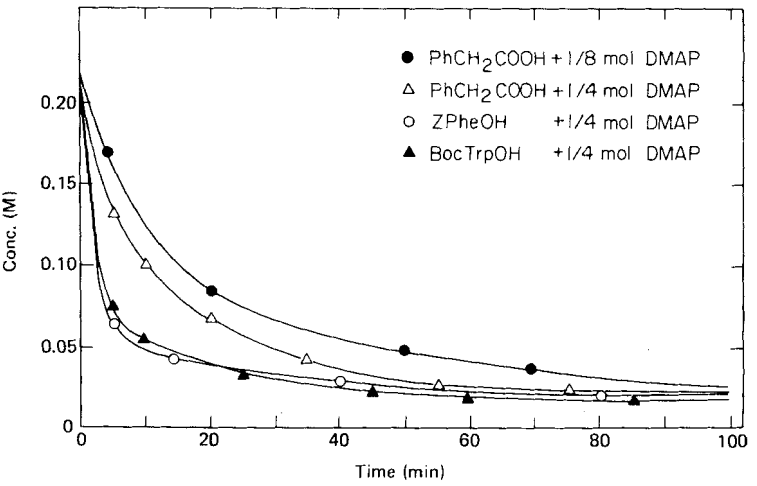


FIG. 1. Decrease in concentration of acid component during reaction with PEG-2000.

Reaction time and optimal catalyst concentrations were determined by monitoring free carboxyl component during the reaction. This was achieved by direct nonaqueous titration of aliquots from the reaction mixture [17]. The decrease in concentration of various carboxylic acids during the esterification is shown in Fig. 1. As can be seen, all reactions were completed after about 90 min. Comparing the amounts of free carboxyls left after 5 min reveals that increasing the amount of catalyst from 0.125 mol accelerated the reaction. As can also be seen from Fig. 1, protected amino acids reacted faster than phenyl acetic acid under the same conditions. (After 5 min, phenylacetic acid gave 43% conversion while Z-phenylalanine and BOC-tryptophane gave 78 and 74% conversion, respectively.) This is due to the negative inductive effect exerted by the NH group which renders the activated carboxyl of the protected amino acids more electronegative.

Table 1 shows data of PEG 3000 esters with seven BOC protected amino acids. According to Fig. 1, reaction times were 2 h at room temperature, since an equivalent amount of the carboxylic acid was consumed. All PEG diesters gave correct elemental analysis, were pure by TLC, and gave the right NMR integration ratio between PEG methylene (δ = 3.6) and BOC methyl (δ = 1.4) groups. PEG 3000 BOC tryptophane diester was checked quantitatively by UV absorbance and showed a purity of 99.3%. Table 1 also shows results of nonaqueous titration of PEG 3000 trifluoroacetyl amino acids diesters. The completeness of deprotonation was also followed by disappearance of the BOC methyl resonance at 1.4 ppm. It is evident from elemental analysis, NMR, nonaqueous titration, and UV analysis, that coupling of car-

TABLE 1. Yields and Substitution Levels of PEG-3000 Amino Acid Diesters

BOC amino acids[a]	Yields of BOC diesters (%)[b]	Yields of TFA salts (%)[b]	Molecular weight (for 1 equivalent weight)	
			Calculated	Observed[c]
Ser(OBzl)	89	80	1791	1731
His(im.Tos)	83	70	1905	2080
Arg(NO_2)	92	83	1815	1840
Tyr(2,6-diClBzl)	88	78	1936	1955
Trp	92	84	1800	1704
Asn	96	88	1728	1781
Gly	92	84	1671	1717

[a]Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (1967, 1970, 1972, 1975).
[b]Yields were determined after purification.
[c]By nonaqueous titration.

boxylic acid by the DCC/DMAP method gives highly substituted polymers. From these data we can approximate substitution levels above 98%. In conclusion, the DCC/DMAP method for coupling of carboxylic acids to PEG is fast, gives high yields of highly substituted pure materials, does not need a large excess of reactants, and can be performed under mild reaction conditions.

EXPERIMENTAL

Materials and Methods

PEG 3000 and PEG 2000 were purchased from Fluka; DCC from Fluka was distilled in vacuum. BOC amino acids were purchased from Bachem and DMAP from Aldrich. All solvents were dried and distilled prior to use. Nonaqueous titrations were performed with Triton B [17]. The deblocked PEG amino acid esters were determined by titration with perchloric acid in dioxane, using thymol blue as indicator. TLC was carried out on silica gel Polygram Sil N-HR/UV 254 from Macherey-Nagel Co., using the n-BuOH/AcOH/H_2O (4:1:1) system and visualized with ninhydrin after introduction to HCl vapors.

Reaction Rate Determination

The following materials were dissolved in 5 mL of dioxane in a 10-mL volumetric flask: PEG 2000 (2 g, 2 mmol OH), carboxylic acid component (2.18 mmol), and DMAP (61 mg, 0.5 mmol). To this mixture, DCC (300 mg, 2.5 mmol) dissolved in a minimum amount of dioxane was added, and the mixture was diluted to 10 mL with dioxane. The mixture was stirred in a 25°C water bath. Reaction time was measured from DCC addition. Aliquots of 1 mL were taken and titrated as above.

NMR

PEG diesters were dissolved in CCl_4, and integration ratios of the ether methylene group at $\delta = 3.6$ ppm and BOC methyl group at $\delta = 1.4$ ppm were observed. The theoretical ratio is 15.055. All PEG diesters gave this value within ±2%.

UV Quantitative Test

A standard solution (3×10^{-5} mol/L) of BOC-tryptophane in absolute EtOH was prepared, and its absorbance was measured at 222 nm. Solutions of PEG-BOC tryptophane diester were prepared and diluted until the tryptophane concentration was in the range of $\pm 10^{-6}$ mol/L, and their absorbance at 222 nm was measured. Correlation of the two measurements showed that the purity of the PEG 3000 BOC-tryptophane diester is 99.3%.

General Procedure for Esterification of PEG

The following materials were dissolved in 20 mL of either CH_2Cl_2, dioxane, or a CH_2Cl_2/DMF mixture: PEG (1 mmol OH), BOC amino acid (1.1 mmol), and DMAP (30 mg, 0.25 mmol). To this mixture a solution of DCC (300 mg, 1.2 mmol) in a minimal amount of solvent was added. The reaction mixture was kept 2 h at room temperature. Dicyclohexylurea (DCU) was filtered off and the filtrate evaporated to dryness. The residue was dissolved in acetone, cooled, and traces of DCU filtered off. The volume of the filtrate was reduced by vacuum and poured into a large excess of vigorously stirred cold ether. The polymer was collected after centrifugation, dissolved in either CH_2Cl_2, DMF, or EtOH, and precipitated again by ether as above. The product was dried overnight in vacuum. The PEG diesters were then subjected to elemental analysis, and integration ratio of the ether methylene and BOC methyl groups was measured by NMR. All compounds gave the correct elemental analysis and integration ratio. TLC did not show

any DCU or free BOC amino acid contaminants. Yields are shown in Table 1. Deprotection of the BOC protecting group was performed by dissolving the PEG-BOC amino acid diesters in TFA/CH_2Cl_2 (1:1). After 30 min at room temperature the solution was evaporated to dryness and the polymer precipitated by ether and centrifuged; reprecipitation was from EtOH-ether. The resulting polymer was dried in vacuum over NaOH granules overnight. The completeness of deprotection was checked by NMR (disappearance of BOC methyl at δ 1.4) and nonaqueous titration.

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