RECENT ADVANCES IN CHEMISTRY AND APPLICATIONS OF SUBSTITUTED POLY(ETHYLENE GLYCOL)S

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Poly(ethylene glycol)s are well defined and easily accessible macromolecules with suitable properties for applications in chemistry, biotechnologies and medicine. The terminal hydroxy groups of poly(ethylene glycol)s can easily be converted into reactive functional groups by a number of routine reactions of organic chemistry. The chemical reagents or catalysts anchored to poly(ethylene glycol) chains were used in a number of syntheses including the enantioselective ones. Poly(ethylene glycol)s serve as carriers in combinatorial syntheses in the liquid phase. Coupling of poly(ethylene glycol)s with other polymers was used to prepare series of block copolymers having numerous applications. From the point of view of medical applications it is significant that substituted poly(ethylene glycol)s are non-toxic and resistant to recognition by the immunity system. That is why they are often used as carriers of many low-molecular-weight as well as high-molecular-weight medical drugs (drug delivery systems). In the conjugates with drugs their biological activity increases and their toxicity decreases. A review with 41 references.

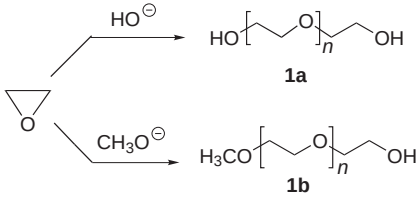
Keywords: Poly(ethylene glycol); Poly(ethylene oxide); Poly(oxyethylene); PEG; PEGylation; Enantioselective synthesis; Combinatorial synthesis; Biomineralization; Carriers of medical drugs; Polymer supported reagents; Polymer supported ligands.

1. INTRODUCTION

The present dramatic development in the area of biotechnology and gene manipulations[1] has caused a considerable shift in the chemistry of substituted poly(ethylene glycol)s. Poly(ethylene glycol)s are well defined, simple and easily synthesised polymers with unique properties[2], which make them useful for extensive applications both in basic research and for particular practical purposes. In general, the methods are referred to as "PEGylations" and "PEGylation" technologies[2,3]. It has to be emphasised in the beginning that the examples given in this review are not, and cannot be, exhaustive; they represent only certain aspects of this broad area. The following examples indicate and document the trends in recent years. Basic and detailed information on poly(ethylene glycol)s themselves can be found in monographs[2].

Poly(ethylene glycol)s possess a number of unique properties: insoluble molecules covalently bound to them are rendered water-soluble, colloid particles are stabilised, they can change properties of surfaces. Poly(ethylene glycol)s are very well soluble both in polar and non-polar solvents, i.e. in water and most organic solvents, being insoluble in only few solvents[2] such as diethyl ether, disopropyl ether, petroleum ether and ethylene glycol. They form complexes of various stability[2a] with a number of metals, resembling thus crown ethers. Thanks to their helical structure they easily crystallise from some solvents. Appropriately substituted poly(ethylene glycol)s can be used as carriers of functional groups, specific ligands or "large" molecules. At present, two basic linear types of poly(ethylene glycol)s exist: poly(ethylene glycol) (**1a**; PEG), ($HO–(CH_2–CH_2–O)_n–CH_2–CH_2–OH$), and methoxy poly(ethylene glycol) (**1b**; mPEG) ($CH_3O–(CH_2–CH_2–O)_n–CH_2–CH_2–OH$). Disubstituted, i.e. α-activated-ω-methoxy poly(ethylene glycol)s derived from **1b** are used in the cases where it is necessary to prevent mutual linking of molecules or particles (crosslinking). For some applications, the commercial poly(ethylene glycol) (**1b**) must be free of diol **1a** present as impurity[4a]. Both types of poly(ethylene glycol)s **1a**, **1b** are synthesised by polymerisation of oxirane using nucleophilic catalysis either with hydroxide anion or methoxide anion; the modern technologies used at present provide products of high quality and low polydispersity ($M_w/M_n \leq 1.1$) (Scheme 1). The most current molecular weights commercially available in pharmaceutical purity or purity for biomedical applications include 500, 1000, 2000, 5000, 10 000, 15 000, 20 000. Poly(ethylene glycol)s of molecular weights from 200 to 600 are viscous liquids, above 1000 waxes, and above 6000 powders. The products with molecular weights

above 20 000 are referred to as poly(ethylene oxide) (PEO), poly(oxyethylene) (POE) or polyoxirane (PO).

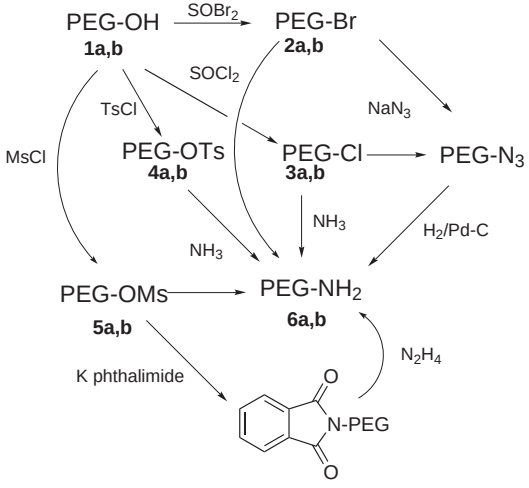


SCHEME 1
Synthesis of PEGs by nucleophilic polymerisation of oxirane

2. PREPARATION OF ACTIVATED POLY(ETHYLENE GLYCOL)S

The end groups of poly(ethylene glyco)s (**1a**, **1b**) can easily be transformed into other, more reactive functional groups by routine reactions of organic chemistry. The activation to nucleophiles can be achieved by introducing[3] the functional groups X onto the poly(ethylene glycol) chain (**a**, X–(CH_2–CH_2–O)$_n$–CH_2–CH_2–X; **b**, CH_3O–(CH_2–CH_2–O)$_n$–CH_2–CH_2–X; **c**, HO–(CH_2–CH_2-O)$_n$–CH_2–CH_2–X): Br (**2a**, **2b**), Cl (**3a**, **3b**), $OSO_2C_6H_4CH_3$ (lit.[4a,4c]) (**4a**, **4b**, **4c**), OSO_2CH_3 (lit.[3a]) (**5a**, **5b**), OSO_2CF_3 (lit.[4d]), CHO (lit.[4e]), CO_2R, etc. For instance, the reactions with thionyl bromide and thionyl chloride give the bromo and chloro derivatives, respectively. For preparation of **2a**, **2b** the authors[5] used the thionyl bromide–triethylamine system so as to prevent cleavage of ether linkage by the hydrogen bromide released (degradation of poly(ethylene glycol)). The activation of hydroxy group of poly(ethylene glycol)s to nucleophiles can be accomplished not only by their reaction with tosyl chloride[4b] or trifluoromethanesulfonic anhydride[4d], but also by the Mitsonobu reaction[6]. The mentioned derivatives possessing good leaving groups can be further activated for electrophiles, e.g., by transformation into amino derivatives[3,6] (**6a**, **6b**) by substitution reactions with ammonia, or azide and subsequent reduction, or phthalimide and subsequent hydrazinolysis. The amino end group can also by introduced by the reaction of mesylated poly(ethylene glycol)s with potassium salt of ethanolamine[3b] (Scheme 2).

Literature[3,7] describes several methods of preparation of poly(ethylene glycol) carboxylic acids (**7a**, **7b**), the carboxylic group serving for linking ligands, mostly with amide or ester linkages. This is often used especially in pharmaceutical conjugates[2,3,8]. The most frequent and most reliable

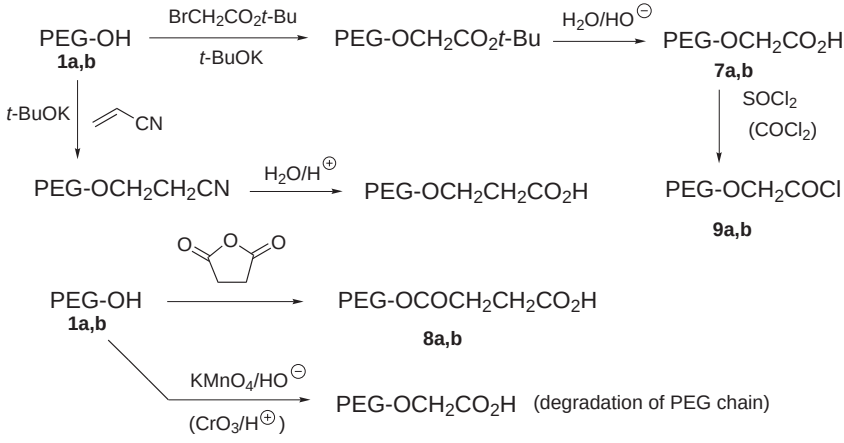


SCHEME 2
Synthetic pathways leading to activated PEGs

method is the reaction of poly(ethylene glycol) in alkaline medium with esters of bromo- or chloroacetic acid and subsequent hydrolysis[3,6,7]. The reaction[8a] of poly(ethylene glycol)s with succinic acid anhydride leads to ester-acids (**8a**, **8b**). Another possibility[3] is nucleophilic addition of anion of poly(ethylene glycol) to acrylonitrile with subsequent hydrolysis of the nitrile group. Reduction[3a] of the nitrile group can also provide amino derivatives (**6a**, **6b**). Likewise, literature[3a,7d] describes introduction of the carboxylic group by oxidation of terminal $-CH_2OH$ groups of poly(ethylene glycol) with potassium permanganate[3a] in alkaline medium, or with chromium(VI) oxide[7d] in acid medium. Although these oxidation methods seem elegant and simple, they are not reliable. The oxidation is accompanied by degradation[3a,7d] of the poly(ethylene glycol) chain (Scheme 3).

The carboxylic group must be activated[7c,8] before subsequent reactions. Its transformation into acid chloride[7c] is not very frequent (Scheme 2); with regard to better handling and stability, it is more convenient to use active esters with *N*-hydroxysuccinimide[8a] (**10a**, **10b**), 1-hydroxybenzotriazole[8b] (**11a**, **11b**), 4-nitrophenol[8c] (**12a**, **12b**) or pentafluorophenol[8d] (**13a**, **13b**) (Fig. 1).

For some purposes it is suitable to transform[9] poly(ethylene glycol)s into reactive derivatives of carbonic acid: chloroformates[9a] (**14a**, **14b**) succinimidyl carbonates[9b,9c] (**15a**, **15b**) benzotriazol-1-yl carbonates[9a] (**16a**, **16b**), or 4-nitrophenyl carbonates[9a,9c] (**17a**, **17b**). The product of reaction of PEG



SCHEME 3
Introduction of a carboxylic group onto PEGs

with 1,1′-carbonyldiimidazole is the imidazo1-1-lylcarbonyloxy derivatives (**18a**, **18b**) possessing a marked reactivity to amino group[3a,9a] (Fig. 1).

Poly(ethylene glycol) reacts[10] with cyanuric chloride to give substituted 1,3,5-triazines (**19a**, **19b**) with reactive chlorine substituents. The two-step reaction of poly(ethylene glycol) with epichlorohydrin is an easy and reli-

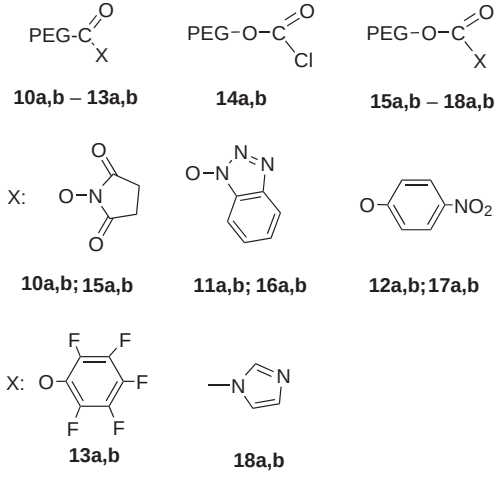
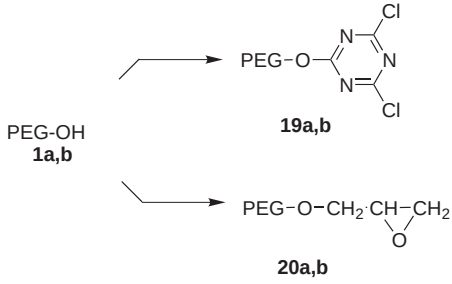


FIG. 1
Reactive derivatives of PEGs

able way to glycidyl ether[7c,11] (**20a**, **20b**), which can be reacted with a number of nucleophilic groups (Scheme 4).



SCHEME 4
Activation of PEGs to nucleophiles

Substituted poly(ethylene glycol)s can be purified by several methods. The most frequent is precipitation by a change in solvent system[2]. Crystallisations from ethanol, propan-2-ol or 1,2-dimethoxyethane proved highly advantageous in a number of preparations. Ion-exchange chromatography is sometimes useful in separating[4a,12] various α,ω-disubstituted poly(ethylene glycol)s. Dialysis is especially suitable[7c,11b] for removal of "small" molecules of the reagents used in excess in the precedent reaction(s). Routine physical-chemical methods (^{1}H, ^{13}C NMR, FT-IR, MS-MALDI-TOF, GPC, HPLC) are suitable for characterisation of substituted poly(ethylene glycol)s.

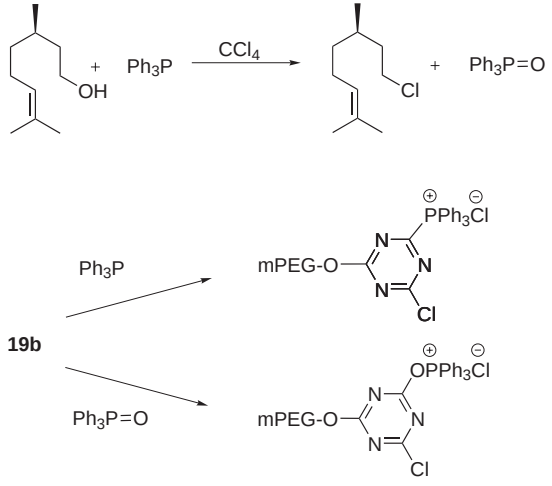
In the future, it can be anticipated that with the activation of terminal hydroxy groups of PEGs newly described reaction will be applied which proceed practically quantitatively under mild conditions like e.g. the above-cited[6] Mitsunobu reaction. Also such reactions will be applied which allow introduction of several functional groups at the ends of poly(ethylene glycol) chain, which will make it possible to attach a larger number of ligands. Further development of polymerisation techniques will make the α,ω-substituted PEGs with different terminal substituents (e.g. NH_2–PEG–CO_2H) commercially available.

3. SUBSTITUTED POLY(ETHYLENE GLYCOL)S IN ORGANIC SYNTHESIS

3.1. Poly(ethylene glycol)s as Carriers of Chemical Reagents or Chiral Ligands

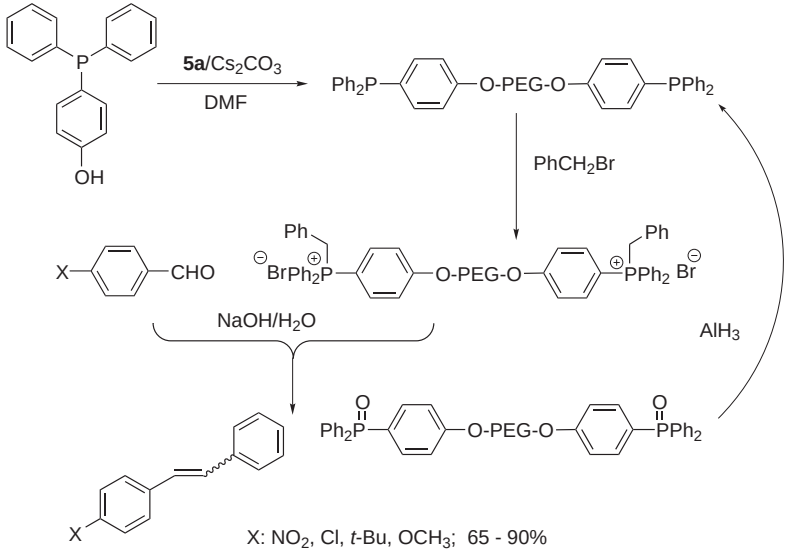
Besides the applications of low-molecular-weight poly(ethylene glycol)s as solvents[13] or cheap substitutes[14] of crown ethers, poly(ethylene glycol)s are

adopted in organic syntheses as carriers of specific molecules, The basic poly(ethylene glycol) skeleton dominantly predetermines the properties[2] associated with solubility of the modified reagents or catalysts. The starting poorly soluble ligands are rendered soluble in numerous solvents including water after substitution with poly(ethylene glycol). The subsequent reactions can then be carried out in homogeneous media, which is much more advantageous[15] from the point of view of diffusion of reactants as compared with the reactions proceeding in heterogeneous systems. This, in general, leads to shortening of reaction times and increase[15] in conversions and yields of reactions. Another considerable advantage lies in the fact that the reagents or catalysts anchored to poly(ethylene glycol) carrier can easily be isolated or regenerated, as the case may be, by a change of solvent system after the reaction proper ("green chemistry"). For instance, the reaction[10b] of citronellol with triphenylphosphine and tetrachloromethane gives chlorocitronellol and triphenylphosphine oxide. After the reaction it is very difficult to remove both the excess of triphenylphosphine and the produced triphenylphosphine oxide. The problem was solved by using the triazine derivative **19b** as a scavenger of nucleophiles, which removes the said substances from the reaction mixture in the form of polymeric salts insoluble in hexane (Scheme 5).



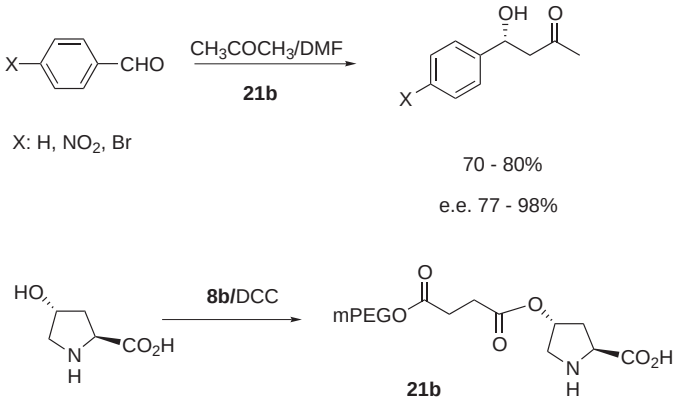
SCHEME 5
Using of substituted PEGs as a nucleophile scavengers after synthesis of chlorocitronellol

An elegant solution[16] to the problem of removal and recycling of triphenylphosphine oxide after the Wittig reaction is presented in Scheme 6. First, (4-hydroxyphenyl)diphenylphosphane was linked to activated poly(ethylene glycol) **5a** by means of ether linkage. The "polymeric" triphenylphosphine prepared in this way then reacts in a known way: first with benzyl bromide to give the phosphonium salt (source of ylide), and then with substituted benzaldehydes in aqueous phase under catalysis with sodium hydroxide. The reaction provides the corresponding stilbenes in the yields 65–95%. The isolation of products consists in extraction of aqueous phase with dichloromethane. The "polymeric" triphenylphosphine oxide is precipitated from the dichloromethane phase with ether. The products remain in solution, and the "polymeric" triphenylphosphine oxide is subsequently reduced with alumane, whereby the "polymeric" triphenylphosphine is recovered.



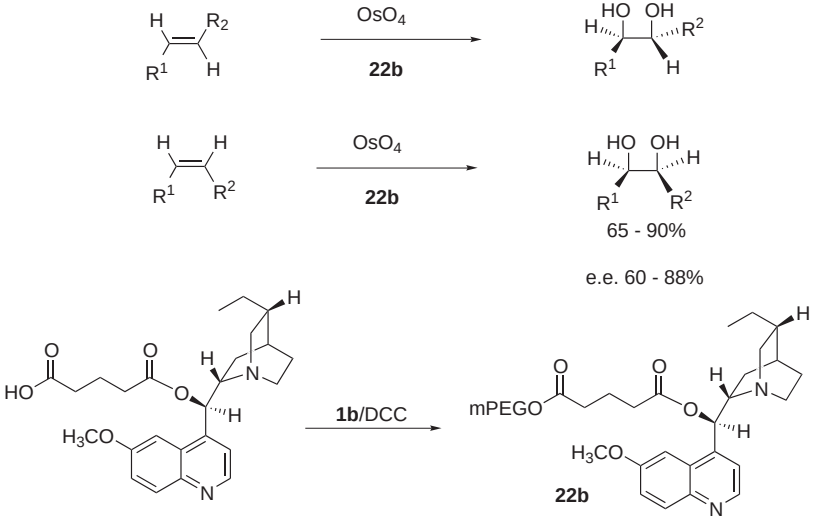
SCHEME 6
Liquid-phase Wittig synthesis using PEG-supported phosphonium salt

The following examples[17] document the applications of substituted poly(ethylene glycol)s as carriers of chiral ligands in asymmetric syntheses. Scheme 7 shows an aldolisation of substituted benzaldehydes with acetone, the yields of the respective aldols being 70–80% and the optical yields ranging from 77 to 98%. The chiral catalyst used[17a,17b] was optically pure 4-hydroxyproline linked to poly(ethylene glycol) by means of ester linkage.



SCHEME 7
Enantioselective aldolisation catalysed by PEG-supported proline

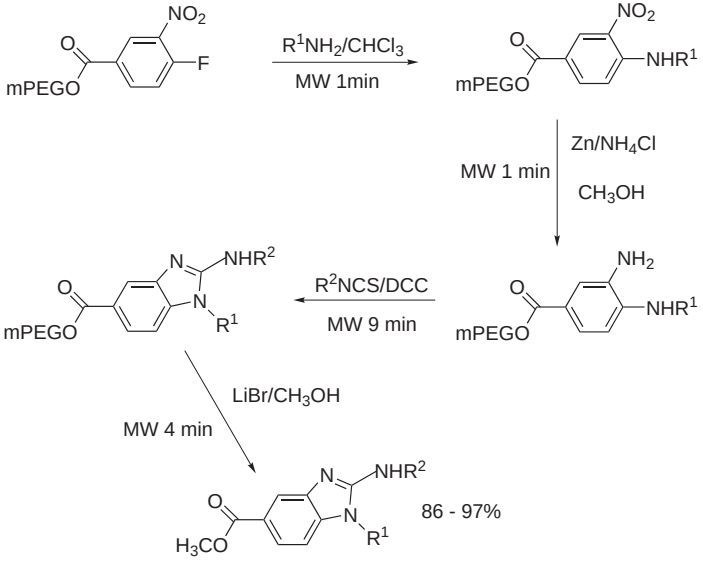
The second example[17c] adopts anchored dihydroquinine as an enantioselective catalyst. In asymmetrical dihydroxylation of olefins, the corresponding diols were obtained with yields of 65–90%, the maximum optical yield being 88% (Scheme 8).



SCHEME 8
Enantioselective olefin dihydroxylation catalysed by PEG-supported dihydroquinine

3.2. Combinatorial Synthesis in Liquid Phase

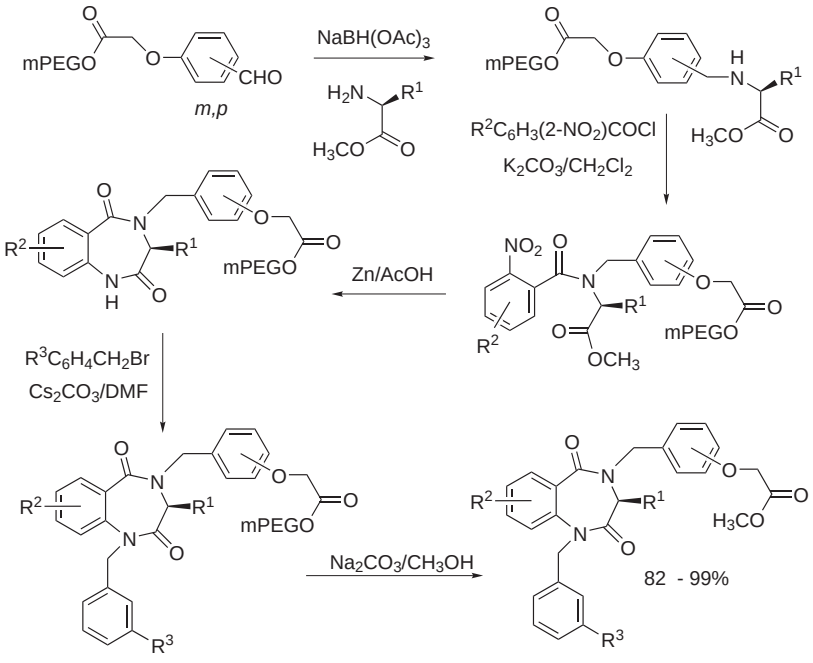
Most combinatorial syntheses are realised on solid carriers[18], which brings numerous evident advantages. The mentioned drawbacks[15] concerning diffusion of reactants are partially overcome[19] by transversal bridging of polystyrene with ethylene glycol bridges (JANDAJEL™), or modification[20] of Merrifield resins with poly(ethylene glycol)s. The direct realisation of the reactions in solution[15] on substituted poly(ethylene glycol)s, which solves this problem, must, however, be associated with regeneration of the solvents. The application of substituted poly(ethylene glycol)s in combinatorial synthesis can be documented by several examples[15,21]. The first were syntheses of peptide libraries[15,21a,21b] by the liquid-phase combinatorial synthesis (LPCS). In these cases, poly(ethylene glycol) not only acts as a solubilising agent but also protects the terminal carboxylic group of the peptide. The resulting peptide anchored to methoxy poly(ethylene glycol) is purified by precipitation with diethyl ether and recrystallisation from ethanol. In the conclusion of the synthesis, the methoxy poly(ethylene glycol) is detached by the action of potassium cyanide in methanol, giving methyl ester of the peptide. The whole procedure was carried out in an automated apparatus and by simple varying the N-protected amino acids it allowed obtaining a library of peptides[21a,21b] in the yields exceeding 98%. Similarly, it was possible to create an arenesulfonamide library[21a] with overall yields 95–97%. Methoxy poly(ethylene glycol) (**1b**) reacts in the first step with 4-isocyanatobenzene-1-sulfonyl chloride, and in the second step the sulfonyl chloride reacts with amines. The last reaction step is base-catalysed hydrolysis of carbamate linkage, giving a set of the corresponding arenesulfonamides. An example of creation of a library of 2-(arylamino)-benzimidazoles[21c] by the LPCS method is presented in Scheme 9. The methoxy poly(ethylene glycol) acylated with 4-fluoro-3-nitrobenzoic acid first undergoes nucleophilic aromatic substitution of fluorine with substituted amines. In the second step, the nitro group is reduced to the amino group, which is followed by the reaction with substituted isothiocyanates in the presence of dicyclohexylcarbodiimide (DCC). The thiourea formed is cyclised due to the nucleophilic effect of the neighbouring alkylamino group to give benzimidazole cycle with concomitant splitting off of dicyclohexylthiourea. The final detachment of 2-(arylamino)benzimidazoles is achieved by re-esterification with methanol catalysed with lithium bromide. All the reaction steps were distinctly accelerated by application of microwaves. The library of 2-(arylamino)benzimidazoles was created with overall yields of 86–97% (Scheme 9).



SCHEME 9
Microwave-assisted liquid-phase combinatorial synthesis of 2-substituted benzimidazoles

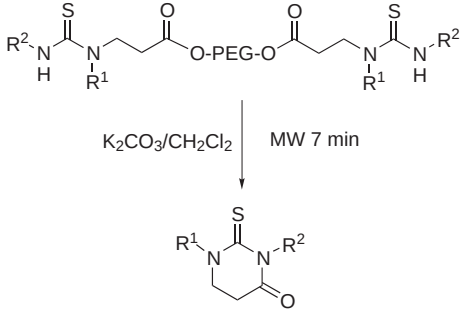
Some combinatorial syntheses in liquid phase carried out on poly(ethylene glycol)s can be very well monitored[21d] by application of conventional ^{1}H NMR spectroscopy. Out of them, 4,5-dihydro-1,2,4-oxadiazoles[21e], thiohydantoins[21f], 1,4-benzodiazepin-2,5-diones[21g] (Scheme 10), 2,3-dihydropyridin-4-ones[21h], β-lactams[21h], or 2-thioxotetrahydropyrimidin-4-ones[21i] can be mentioned (Scheme 11).

The direction of further development in applications of substituted PEGs to organic synthesis indicates an increase in number of papers dealing with LPCS syntheses, which have another great advantage as compared with syntheses on solid carriers, viz. in the possibility of direct monitoring of the reaction advancement by means of NMR. As for new reaction media, it can be expected e.g. that ionic liquids modified with PEG will be adopted. One of the new ways of increasing the efficiency of syntheses and decreasing environmental loads consists in utilisation of PEGylated catalysts, which can easily be recycled.



SCHEME 10

Liquid-phase combinatorial synthesis of substituted 1,4-benzodiazepine-2,5-diones

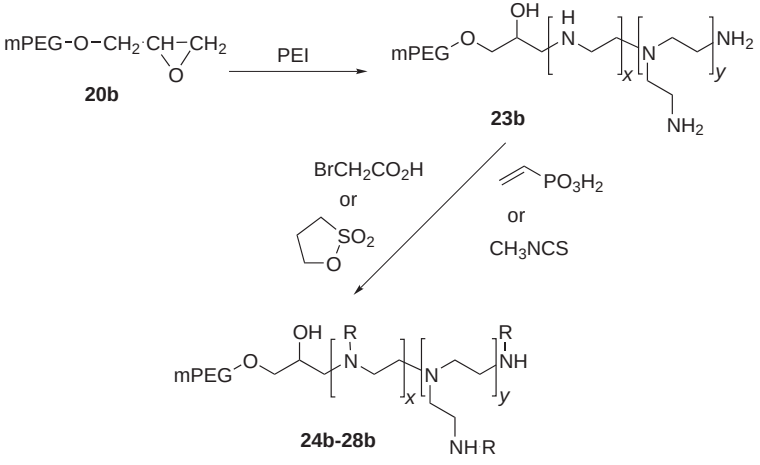


SCHEME 11

Microwave-assisted liquid-phase combinatorial synthesis of substituted 2-thioxotetrahydropyrimidin-4-ones

4. BLOCK COPOLYMERS OF POLY(ETHYLENE GLYCOL)S AND THEIR APPLICATIONS

This paper presents only selected examples involving formation of block copolymers by reactions of activated terminal group of poly(ethylene glycol) with other functional group of polymers (coupling). The examples in which poly(ethylene glycol) acts as ionic or radical macroinitiator in polymer reactions can be found in recent articles and reviews[22]. Some typical syntheses can be given as examples of coupling of activated PEG with other polymers: PEG-*block*-poly(methacrylic acid)[23], PEG-*block*-polyspermine[24], PEG-*block*-poly(aspartate)[7c], PEG-*block*-poly(β-benzyl L-aspartate)[25]. In the last case mentioned[25], a relatively low yield was obtained (54–74%). A general problem of these syntheses is the completeness of conversion, which depends on the collision probability of two mutually reacting groups located on relatively large molecules. However, in the couplings of activated poly(ethylene glycol) with another polymer, it needs not always be statistically favourable if the other polymer contains many reactive groups of the same kind. Such type of polymer is branched poly(ethylenimine) (PEI), which on reaction of its terminal amino groups with chloride of methoxy poly(ethylene glycol)carboxylic acid (**9b**) or α-glycidyl-ω-methoxypoly(ethylene glycol) (**20b**) gives the copolymer poly(ethylene glycol)-*block*-poly(ethylenimine)[7c,11b] (mPEG-*b*-PEI) (**23b**) (Scheme 12).



R: **24b**: CH_2CO_2H, **25b**: $(CH_2)_2PO(OH)_2$, **26b**: $(CH_2)_3SO_3H$, **27b**: $CH_3N{=}C(SH)$-

SCHEME 12
Synthesis of block copolymers PEG-*b*-PEI modified by different functional groups

After the coupling reaction with the reactant ratio 1:1, it was possible to detect by ^{1}H NMR and to determine by analytical ultracentrifugation of the reaction product, substituted methoxy poly(ethylene glycol)-*block*-poly(ethylenimine)-*block*-methoxy poly(ethylene glycol) (mPEG-*b*-PEI-*b*-mPEG). For coupling of a single PEG block with a single PEI block, it is necessary to use an excess of PEI (1:9), and the unreacted PEI must finally be removed by dialysis[11b]. Such a simple block copolymer **23b** forms complexes with oligonucleotides or with DNA [26], which is utilised in gene manipulations and gene therapy[1]. On the other hand, the block copolymer PEG-*b*-PEI (**23b**) forms complexes[27] with metal salts such as $AuCl_3$, $PdCl_2$ or H_2PtCl_6. A controlled synthesis[27] of nanoparticles of the said metals is performed by reducing these complexes with hydrogen or hydrazine. The amino groups of the block copolymer PEG-*b*-PEI can further be easily modified[7c,11b] by attaching other functional groups (–CO_2H, –SO_3H, –PO_3H_2, –SH) (**24b**–**28b**) or ligands. This series of block copolymers was adopted as a polymer template[7c,11b] for biomimetic growth of mineral crystals[28] in aqueous medium. It was possible to observe[7c,11b,23a,28b] a considerable effect of substitution of PEG-*b*-PEI with functional groups on the morphology of the formed crystals of $CaCO_3$ or $BaSO_4$. Other poly(ethylene glycol)-*block*-poly(ethylenimine)s modified with chiral ligands: quinine (**29b**), (*S*)-proline (**30b**), (*S*)-histidine (**31b**), (*S*)-ascorbic acid (**32b**) and D-gluconate (**33b**) were used in controlled crystallisations[29] of racemic ammonium or calcium tartrates. In some cases resolution of the racemates was achieved (Fig. 2).

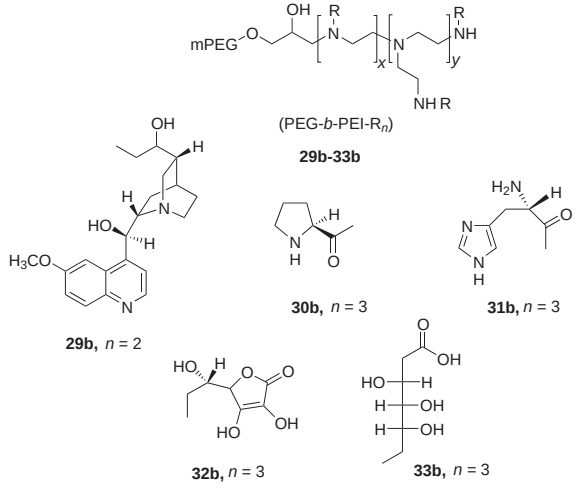


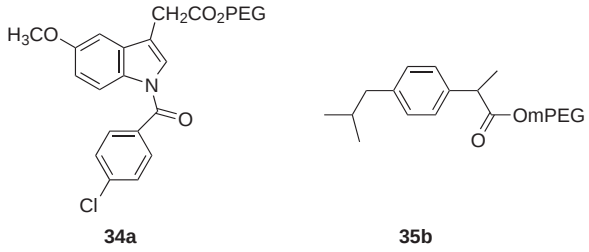
FIG. 2
Block copolymers of PEG-*b*-PEI modified by chiral ligands

Due to the low conversions in coupling reactions between PEG and synthetic polymers we cannot expect any distinct increase in these syntheses in the construction of new block copolymers. Other methods[22] appear much more advantageous: the substituted PEGs operate as macroinitiators for building another polymeric block. However, on the other hand, we can anticipate an increase in applications of coupling reactions in the preparation of block copolymers between PEGs and natural macromolecules, such as proteins, particularly for use in medicine. In the field of application of PEGylated block copolymers we can expect inter alia an increase in new crystallisation technologies. Appropriately substituted PEGs used as additives will be able to solve the problems of polymorphs in pharmaceutical production. Other new possibilities consist in separation[29] of racemates by crystallisations in the presence of optically pure block copolymers.

5. SUBSTITUTED POLY(ETHYLENE GLYCOL)S IN MEDICAL APPLICATIONS

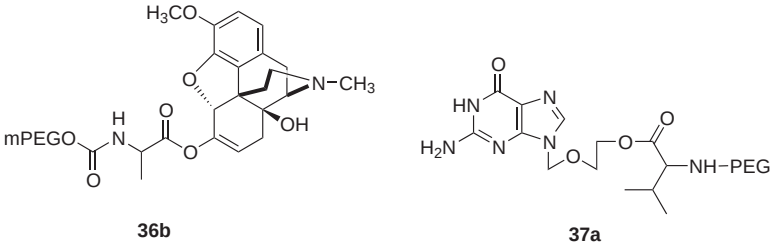
For medical applications of substituted poly(ethylene glycol)s the following properties are most important[2]: they are non-toxic for molecular weights above 400, non-immunogenic and non-antigenic, i.e. resistant to recognition by the immunity system of organism. The proteins modified with poly(ethylene glycol) do not produce immunological reactions in the organism, which is used in modifications[30,31] of some hormones and enzymes and factors intended for therapeutic purposes (interferon[31a,31b], (Pegasys®, Peg-intron®), erythropoietin[31c], somatotropin[9a] (Somavert®), protamin[31d], etc.). Surfaces of some tools and instruments coming into contact with blood are also modified with poly(ethylene glycol) to ensure their biocompatibility[2]. In parenteral administration, the PEGylation significantly affects[32] the pharmacokinetics of medical drugs, which means a reduced rate of elimination of the drugs by kidneys. However, for the purposes of increasing the circulation lifetime it is necessary[31c] to use poly(ethylene glycol)s with higher molecular weight (20 000–40 000). For the above-mentioned reasons[2,30–32], poly(ethylene glycol)s are used as drug delivery systems. The drugs bound by covalent bonds are referred to as covalent conjugates, those with bonds of other types are non-covalent conjugates[30]. An example is the matrix of suitably substituted poly(ethylene glycol) for preparation of liposomes[33]. Literature[32a,34] quotes numerous papers concerning low-molecular-weight medical drugs attached to poly(ethylene glycol)s. At present the already classical examples include antiphlogistics: indomethacin–PEG [34a] (**34a**), ibuprofen–PEG [34b] (**35b**) (Fig. 3). More recent

examples are the opioid oxycodone–PEG [34c] (**36b**) and antivirotic drug acyclovir–PEG [34d] (**37a**) (Fig. 4), or conjugates releasing nitrogen(II) oxide[34e].



34a **35b**

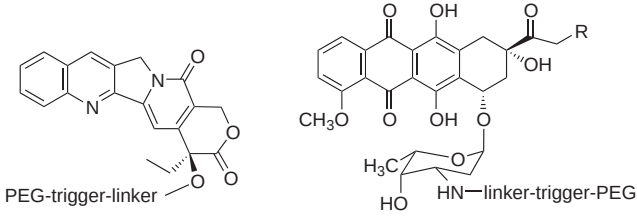
FIG. 3
Structures of conjugates: indomethacin–PEG (**34a**) and ibuprofen–PEG (**35b**)



36b **37a**

FIG. 4
Structures of conjugates: oxycodone–PEG (**36b**) and acyclovir–PEG (**37a**)

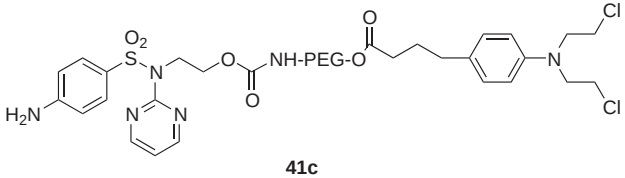
However, the most frequently discussed[30c,30d] antineoplastics in literature are the camptothecin–PEG [35] (Prothecan®) (**38a**), daunorubicin–PEG [32c] (**39a**), doxorubicin–PEG [9b,32c] (Doxil®) (**40a**) (Fig. 5). Recently, new types of anticancer conjugates have appeared[36], which are based upon non-



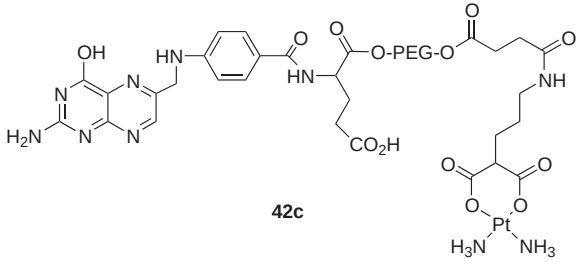
38a **39a,** R = H; **40a,** R = OH

FIG. 5
Structures of PEG-spacer conjugates: camptothecin–PEG (**38a**), daunorubicin–PEG (**39a**) and doxorubicin–PEG (**40a**)

symmetrically α,ω-disubstituted poly(ethylene glycol)s. In order to achieve a drug-targeting effect of chlorambucil conjugate, the second end of poly(ethylene glycol) chain was linked to sulfadiazine (sulfadiazine–PEG–chlorambucil, **41c**). Such drug-targeting effect of the conjugate is based on the finding[36a] that tumour cells tend to concentrate sulfadiazine. An analogous principle was used[36b] in the system of folic acid–PEG–carboplatin (**42c**) (Fig. 6).



41c

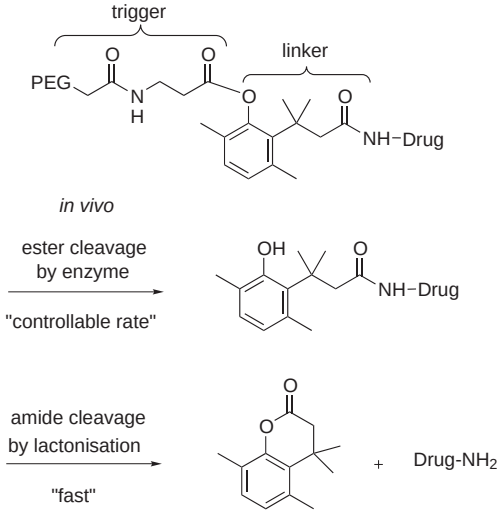


42c

FIG. 6
Structures of unsymmetrical conjugates: sulfadiazine–PEG–chlorambucil (**41c**) and folic acid–PEG–carboplatin (**42c**)

In newer types of conjugates, the molecules of pharmaceuticals are bound by a system of labile bonds[32,37], trigger-linker (Scheme 13). These bonds between carrier and drug are constructed so as to be gradually broken, thereby ensuring a constant level of the drug in organism. The cascade decomposition of linker is initiated first by enzymatic reactions. For instance, blood hydrolases split ester linkages to produce a prodrug, which is subsequently decomposed into the final active substance by the influence of pH. The role of pH is also vital in numerous other cases. For example, the pH values inside a tumour cell significantly differ from those in a healthy cell, which enables a goal-directed decomposition[37b] of the bond of prodrug or conjugate and release of the effective substance at the required site. A number of strategies were suggested and developed for release of active pharmaceuticals, making use of elimination[37c] or cyclisation[37a,37d,37e] reac-

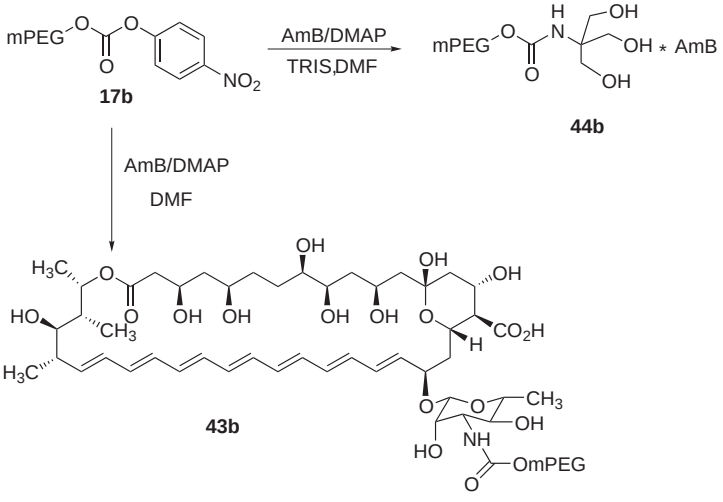
tions (Scheme 13). In future, it can be expected that other known reactions[38] whose course is affected by pH of the medium will be utilised.



SCHEME 13
Cyclisation prodrug strategy: two-step drug release from PEG carrier

The last generation of conjugates enabling the targeted therapy are constructed as block copolymers whose middle section is formed by a polyfunctional macromolecule with attached molecules of the active substance. One end of this carrier is linked to with poly(ethylene glycol) and the other to a monoclonal antibody having affinity to the tumour cell[37b,39a]. As therapeutically highly advantageous have also proved conjugates of macrocyclic antibiotics for treatment of fungal infections in immunosuppressed patients, especially after transplantations of organs, in the case of acquired immunodeficiency (AIDS), and malignities of organs[40]. An example is amfotericin B (AmB) – a polyene macrocyclic antibiotic that has been saving lives for more than 40 years in cases where other antimycotics fail[40b]. At present clinically used are liposomal drug forms[40c] of amfotericin B (Abelcet®), which are non-covalent complexes with modified poly(ethylene glycol)s; however, they are not suitable for peroral administration. We used the reaction of **17b** with AmB with addition of tris(hydroxymethyl)ethylamine (TRIS) to synthesise (and characterise) a new type of water-soluble conjugate, in which AmB was linked to methoxy poly(ethylene glycol) by covalent carbamate bond (45 mole %) (**43b**), the

rest (55 mole %) being linked by non-covalent bonds (**44b**) (hydrogen bonds and/or π–π interactions)[9c,40d] (Scheme 14).



SCHEME 14
Synthesis of covalent (**43b**) and non-covalent (**44b**) amphotericin B–PEG conjugates

The conjugate prepared in this way exhibits a similar spectrum of effects those of liposomal and deoxycholate formulations. However, in some cases we observed as high as ten-fold increase in activity accompanied by marked drop (ten-fold) of toxicity[40d]. Our work was continued by Greenwald's team[40e], who prepared and characterised an analogous conjugate PEG-AmB in which AmB is bound by means of labile carbamate bond. They observed[40e] a six-fold drop in toxicity.

The above-given examples show a privileged position of substituted PEGs in medicinal chemistry, which is particularly true of newly constructed therapeutic systems. The anticipated marked development in research and production of bio-pharmaceuticals[41a,41c] is unthinkable without PEGylation technologies allowing modification of peptides, proteins and segments of nucleic acids obtained from natural sources or by the methods of genetic engineering. Apart from preparation of new pharmaceuticals, further pharmaceutical innovations will consist in PEGylations of classical or new low-molecular-weight medical drugs, which should increase their therapeutic index. For instance, α,ω-substituted PEGs with different terminal substituents will be utilised in preparation of non-symmetrical conjugates that will combine therapeutic effects of two drugs. The non-symmetrically

α,ω-substituted PEGs will possibly be utilised besides in targeted therapy also for the purposes of diagnosis. Particularly oncology[41a] will see a more massive application of the drug delivery systems, which will determine commercial success of pharmaceutical industry in the future.

6. CONCLUSION

One of the rapidly developing areas on the boundary of chemical and biological sciences is the chemistry of substituted poly(ethylene glycol)s. In organic synthesis, substituted poly(ethylene glycol)s are used particularly in preparation of new catalytic systems or serve as carriers in combinatorial syntheses. Conjugates of poly(ethylene glycol)s and lower-molecular-weight pharmaceuticals increase the therapeutic index of the latter[41]. In biopharmacy[1,41a,41c], modification of surfaces of high-molecular-weight protein drugs enables their therapeutic applications. They have considerable importance in synthesis of block copolymers with a wide spectrum of applications. In medical applications of substituted poly(ethylene glycol)s we can expect an increase in utilisation of poly(ethylene glycol)s in construction of new systems designed for targeted therapy. Newly described reactions will be used for further modifications of poly(ethylene glycol)s. In future, we can expect a further increase in number of published papers in all the areas mentioned.

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