MultiPEGs: High Molecular Weight Multifunctional Poly(ethylene glycol)s Assembled by a Dendrimer-Like Approach

Maurizio Ballico,[a] Sara Drioli,[a] and Gian M. Bonora*[a]

Keywords: Dendrimers / Polymers / Poly(ethylene glycol) / Supported synthesis / Liquid-phase synthesis

New multifunctional, soluble and biocompatible polymeric systems exhibiting good physicochemical properties and good binding capacity have been obtained by assembly of smaller PEG components, selectively protected, purified and activated, by use of appropriate polyfunctional linkers. A general process for the synthesis of those derivatives was characterized by: a) selective derivatization of a PEG unit with a defined MW with an appropriate protecting group and activation of residual functional groups, and b) multiple condensation reactions of PEG units and linkers to provide multifunctional polyethylene glycol derivatives (MultiPEGs) of the desired size and complex structure. These complex-structured MultiPEGs have larger numbers of functional groups, and probably better biodegradability, than commercial PEGs of comparable molecular weight. They can be advantageously used as carriers or stabilizers of pharmacologically or biologically active substances, and also as soluble supports for reagents and catalysts in liquid-phase synthesis reactions.

Introduction

Polyethylene glycol (PEG) and the polymeric materials based on it are arousing great interest in view of their application in the biotechnological and pharmacological fields,[1] the major example of such application being the PEGylation of peptides and proteins.[2–3] This technology improves therapeutic efficacy, since the molecule attached to the PEG, while maintaining its original biological functions, has increased in vivo stability to degradation processes. In fact, the presence of PEG chains, especially those of high molecular weight, masks the protein surface, prevents attack by the antibodies or cells involved in the destruction of the active compound and reduces kidney ultrafiltration. Finally, PEG conveys its own physicochemical properties onto the attached molecules, thus improving their bioavailability and solubility and, in general, facilitating their administration.[4] Recent investigations also concerned the PEGylation of oligonucleotide chains capable of behaving as antisense and antigen[5] or ribozymes for the specific hydrolysis of RNA chains.[6] An increased stability to enzymatic degradation[7] and a longer retention time in the blood circulatory system—the higher the polymer molecular weight, the higher the effect—have been observed.

In addition to the above examples, numerous small-sized molecules in the form of PEG conjugates have been used for the purpose of improving the characteristics connected with pharmacological administration. It is also worth mentioning anticancer agents such as doxorubicin, taxol, antimalarial drugs and various enzymatic inhibitors.[8–10]

In addition to the above uses of PEG in the modification of biologically active molecules, it may also be applied to support of reagents, to their removal from solution and, obviously, to the transient support of the molecule in the assembling step.[11] In general, the polymers used to this end must satisfy some conditions, such as commercial availability, good chemical and mechanical properties, the presence of appropriate functional groups for the attachment of organic molecules, as well as the required solubilizing properties. Commercial PEGs show most of these characteristics, with the exceptions of reduced size homogeneity and a low functionalizing capacity, obviously limited to the two polymer chain ends only. Important fields of application of PEG as a soluble synthetic support are the production of peptides,[12] oligonucleotides[13] and oligosaccharides.[14] In addition, an ever increasing number of organic syntheses have been proposed, especially for combinatorial chemical processes.[15] PEG may also be used as a supporting agent with specific catalysts and reagents, conjugation of which allows ease of recovery and recycling of the reagent.[16]

With a view to increasing the loading capacity, which limits the use of PEG, a linear bifunctional derivative, modified by benzyl ether dendrons,[17] has been proposed, as well as the so-called PEG-star polymers.[18] Additionally, branched PEG, analogous to the linear form but with two monofunctional polymeric chains linked together through a polyfunctional molecule, has been used.[19] Very recently, new branched, dendrimeric poly(ethylene glycol)s with increased loading levels have been proposed.[20] Furthermore,

[a] Department of Chemical Science, University of Trieste,
Via Giorgieri 1, 34127 Trieste, Italy
Fax: +39-040-558-2400
E-mail: bonora@units.it

  DOI: 10.1002/ejoc.200400704

new hybrid dendritic macromolecules in which PEG is linked together with glycerol and succinic acid have recently been reported.[21]

A very promising class of high-capacity soluble polymeric supports consists of aliphatic dendritic polyethers and hyperbranched polyethers/polyols. These have been obtained by convergent synthesis from the condensation of propylene glycol and methallyl dichloride growing units, by divergent synthesis starting from a multifunctional core represented by glycerol, which binds glycidol growing units, or through a single-stage anionic polymerization.[22–25]

The present investigation was intended to provide a new class of PEG-based systems characterized by high molecular weight and the presence of several functional groups. These multifunctional polyethylene glycol derivatives (MultiPEGs) were obtained by repeated condensations of PEG units of the same average MW with appropriate linkers in a dendrimeric-like fashion. The final goal was the preparation of new PEG-based high molecular weight molecules that would preserve the typical physicochemical properties of the starting polymer but would offer an increased loading capacity in comparison with commercial PEGs of the same final size. Moreover, the introduction of different chemical bonds inside the final macromolecule would be likely to offer better biodegradability than the starting polyether backbone and this new feature could improve its use as profitable drug delivery system.

Results and Discussion

Multifunctional, branched polyethylene glycol derivatives (MultiPEGs) formed by the repetition of PEG units of the same average MW through successive condensation reactions were prepared by a process characterized by: a) selective derivatization with an appropriate protecting group and activation of the residual functional group of the protected PEG unit, and b) assembly by repeated condensation reactions between the activated functional group and a free functional group of one or more PEG derivatives.

The above procedure has so far been hindered by the need to produce adequate amounts of bifunctional polydisperse PEG, selectively protected at one of the two terminal ends, in a pure and controlled way. Indeed, only in this way is it possible to plan an effective assembly strategy that will allow, after the final removal of the protections still present, the obtainment of a MultiPEG with controlled structure, size and functionality, retaining all the physicochemical characteristics of the polyether skeleton. The monoderivatization of the starting PEG unit and the purification of the polydisperse PEG chains of high molecular weight are therefore the steps setting a limit on the whole synthesis process. Furthermore, while the introduction of new chemical bonds between the starting PEG molecules could allow the preparation of very large polymeric systems with convenient biodegradability properties, the right choice of short, polyfunctional molecules linking those PEG units together should permit the degree of functionalization of the final MultiPEG to be increased.

Depending on the complex structure desired, the synthesis has been exploited by two different, dendrimer-like procedures: one convergent and one divergent.

The described structures were synthesized to demonstrate the potential to assemble large polymeric systems from polydisperse products as starting materials. The ultimate goal was a dendrimeric-like assembly of final molecular constructions characterized by a larger molecular weight and an advantageous number of functional groups

For all these reasons, the introduction of simpler polyfunctional linkers was initially exploited and a low average molecular weight of the starting PEG was adopted to demonstrate the feasibility of the project.

In the first example, a large MultiPEG molecule was constructed by a convergent approach, monoprotected polymeric units of 3000 Da molecular weight being joined through an appropriate linker such as 2-aminopropane-1,3-diol and a number of PEG units being assembled by successive, repeated condensations. The synthetic approach is represented in Figure 1.

The following steps were envisaged.

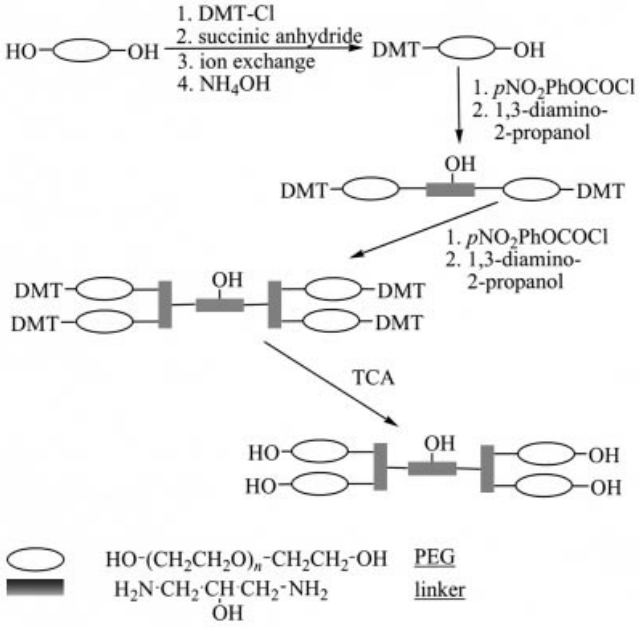


Figure 1. Scheme of synthesis of MultiPEG #1

Selective Monoprotection of the Starting PEG

Commercial PEG, polydisperse type, has two hydroxy groups at the polymer chain ends, and these may be modified by traditional organic synthesis processes. An acid-labile dimethoxytrityl group (DMT) is shown here. The problem of selective monoderivatization was solved by treatment with a suitable quantity of the reactive derivative of the protecting group and subsequent separation from all unreacted or doubly reacted polymer chains. Since said modification does not substantially alter the general characteristics of the polymer, the only possible means of purification is chroma-

tographic separation. A convenient ionizable group was therefore temporarily introduced to allow ion-exchange purification.[26] The successive removal of this group, after chromatographic separation, yielded the desired product.

Activation of the DMT–PEG-OH Unit

For the introduction of the successive linker molecule with a final chemically stable bond, the residual OH group of PEG should be activated under conditions that do not cause the removal of the protecting group. Of the numerous possible activating systems,[27] an ester carbonate, active for the final production of urethane bonds with the amino group of a linker molecule, was preferred.

Formation of a PEG Unit Comprizing Two Starting PEG Units

Treatment of two DMT–PEG-OH activated units obtained in the preceding step with the linker allowed PEG dimerization, without any evident formation of PEG-linker monomer in detectable amounts. At this point, the residual OH group on the linker represented the only still free functionality that could be activated for successive condensation step.

Formation of a PEG Unit Comprizing Four Starting PEG Units

Subsequent activation of the OH group of the linker on the obtained dimer and treatment with a second linker molecule, identical with the previous one, yielded a tetrameric PEG still possessing a residual reactive OH group on the linker. Final deprotection gave a PEG-derived polymer with a larger number of functionalizable OH groups than commercial PEG of the same size. Moreover, the new chemical bonds between the single polymeric units could provide some fragility under physiological conditions.

The chromatographic patterns of the crude and purified MultiPEG #1 are shown in Figure 2.

The MW, evaluated from the M_p from GPC analysis, was 15420 (calculated 12436, starting from a 3000 Da commercial PEG). The discrepancy can reasonably be ascribed to the differences from the standard, linear PEG polymers used for calibration purposes and to the modified hydrodynamic properties of this derivative.

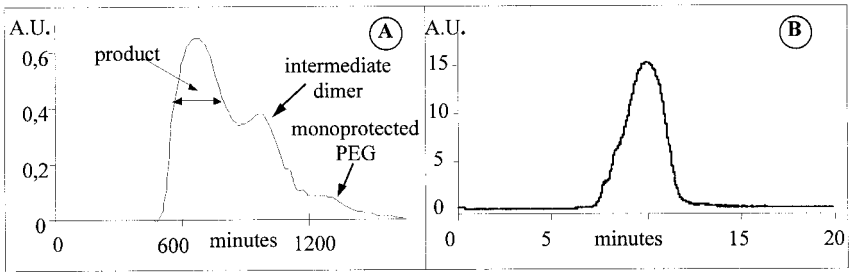
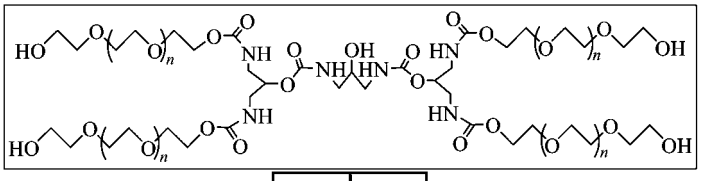


Figure 2. A) Purification of the crude MultiPEG #1. B) GPC analysis of the purified MultiPEG #1.



		Integral value (calcd.)	Integral value (found)
A	NH, urethanes	2	1.90
B	NH, urethanes	4	4.07
C	-NH-CH_2-CH(OH)-CH_2-NH-	1	1.3
D	-CH_2-CH_2-OH	4	3.92
E	PEG-CH_2-O-CO-NH- + -NH-CH_2-CH(O...)-CH_2-NH-	11	11.6
F	(-CH_2-CH_2-O)$_n$	1090.1	1090.1
G	-CH_2- linker	12	11.2

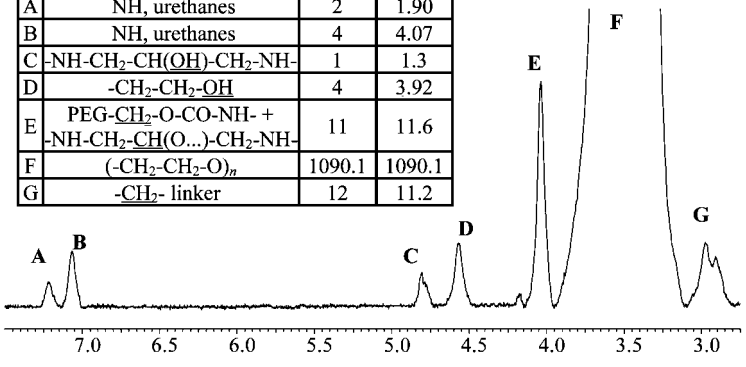


Figure 3. ^{1}H NMR spectrum of the MultiPEG #1 in [D_6]DMSO. The structure of the product is indicated in the insert.

The ^{1}H NMR spectrum was in agreement with the expected composition, as confirmed by the good correspondence between the integral values of the more significant proton signals, as shown in Figure 3.

Unfortunately, a further attempt aimed towards the preparation of the octaPEG$_{3000}$-(OH)$_9$ derivative based on repetition of the above conditions failed. A new procedure to overcome the observed drawbacks is now under evaluation.

In a second process, a divergent assembly was devised and a different branched structure was constructed. The use of two different linkers was required, both to add new functional groups to the starting PEG and to increase the reactivity of the monoprotected PEGs to be assembled onto the initial core. The synthesis is described in Figure 4.

A 2-amino-1,3-propanol linker was introduced into the starting, unprotected PEG unit. Subsequent branching was performed through the use of a monoprotected PEG of the same molecular weight modified with 1,3-diaminopropane. A further modification of the free hydroxy groups with the same starting trifunctional linker eventually doubled the number of functionalizable moieties.

The steps of the synthesis can be summarized as follows:

a) Activation of the OH functional groups of the PEG unit.
b) Introduction of 2-aminopropane-1,3-diol into the activated PEG unit.
c) Selective monoprotection of another PEG unit of the same molecular weight.
d) Modification of OH groups of the monoprotected PEG unit with a higher nucleophilic moiety.
e) Activation of the residual OH functional groups of the PEG units obtained in step b).
f) Assembly of more PEG units obtained in steps c) and d) on the multifunctional core obtained in step e).
g) Deprotection of the functional group of the PEG unit obtained in step f) and introduction of more linkers as in step b).

The chromatographic patterns of the crude and purified MultiPEG #2 are shown in Figure 5. Some small-size impurities originating from the commercial diamino linker were present, together with some unreacted starting PEG. A late-running peak, corresponding to higher molecular mass samples, was observed in the purified product due to some aggregation of product, as previously reported in the literature and as supported by its decreasing after dilution or warming of the sample.[28–29]

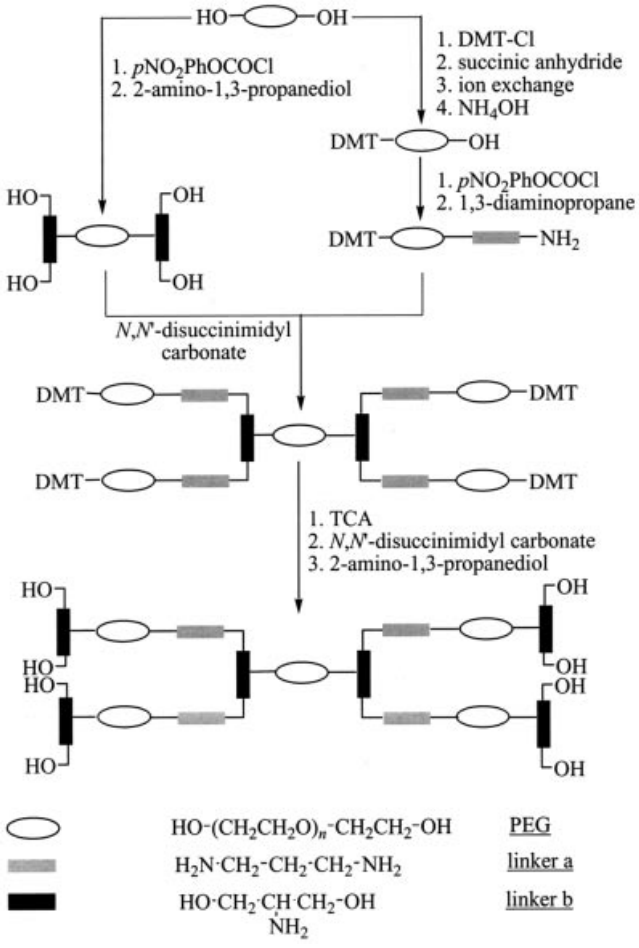


Figure 4. Scheme of synthesis of MultiPEG #2.

The evaluation of the MW, from the M_p of the purified product, gave a value close to that expected (found 9990, calculated 11143 starting from a 2000 Da PEG). As underlined previously, some divergence due to the (structurally different) standard PEGs used for calibration purposes is to be expected.

Again, the ^{1}H NMR spectrum was in good agreement with the expected derivative, as judged from comparison of the integral values shown in Figure 6.

To evaluate the synthetic properties of the MultiPEGs they were tested in a liquid-phase procedure, being treated

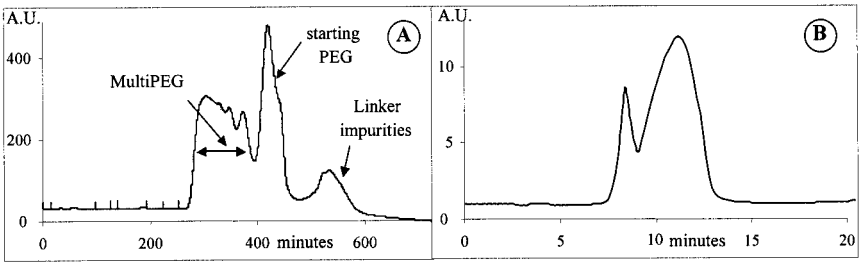
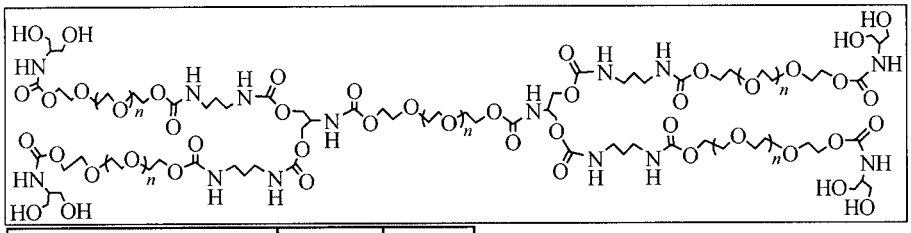


Figure 5. A) Purification of the crude MultiPEG #2. B) GPC analysis of the purified MultiPEG #2.



		Integral value (calcd.)	Integral value (found)
A	NH, urethanes	2	2.00
B	NH, urethanes	8	8.26
C	NH, urethanes	4	3.98
D	-CH(CH$_2$-O-CO-...)$_2$	2	1.83
E	OH	8	7.25
F	PEG-CH$_2$-O-CO-NH- + -CH(CH$_2$-OH)$_2$ + -CH(CH$_2$-O-...)$_2$	32	31.08
G	(-CH$_2$-CH$_2$-O)$_n$	909.1	909.10
H	-NH-CH$_2$-CH$_2$-CH$_2$-NH-	16	16.70
I	-NH-CH$_2$-CH$_2$-CH$_2$-NH-	8	8.23

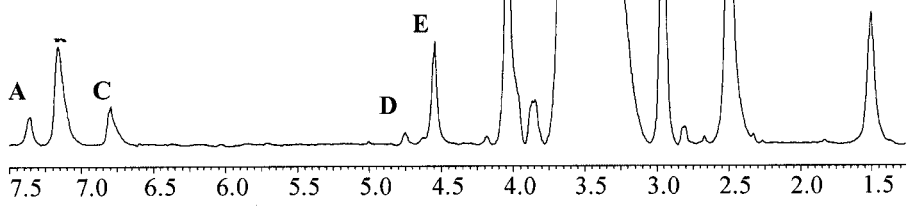


Figure 6. ^{1}H NMR spectrum of MultiPEG #2 in [D$_6$]DMSO. The structure of the product is indicated in the insert.

with a simple N-protected, activated amino acid (Fmoc-Gly-OH) and subsequently deblocked, as previously exploited in a classical peptide synthesis on a commercial PEG.[30] The UV absorption of the Fmoc N-protecting group allowed the spectrophotometric evaluation of the Gly derivatives introduced for each PEG unit and confirmed the presence of a higher number of functionalizable units relative to commercial PEGs of similar size. Fmoc deprotection then permitted a colorimetric TNBS test for the free NH_2 group, while NMR analysis gave a further estimation of the condensation reaction. The collected data, listed in Table 1, verified the increased loading values of the MultiPEGs, while the reaction proceedings suggested that the advantageous chemicophysical properties needed to perform a liquid-phase synthesis efficiently were essentially maintained.

Table 1. Evaluation of the loading values after derivatization of starting PEG polymer and new MultiPEGs with Fmoc-Gly-OH.

Polymer	μmol/g of Fmoc	μmol/g of NH_2	Gly residue (based on ^{1}H NMR integral values)
diOH–PEG$_{10000}$	189.4	197.7	2.0
MultiPEG$_{3000}$#1	390.0	392.4	4.7
MultiPEG$_{2000}$#2	700.8	796.5	7.8

Conclusions

In conclusion, we report the preparation of new soluble and biocompatible polymeric systems, named MultiPEGs, obtained by connecting smaller, polydispersed commercial PEG chains, selectively protected, purified and activated, through appropriate polyfunctional linkers. Analogous multi-arm PEGs are now commercially available,[31] but in such cases the PEG moieties are generally assembled all at once on simple supporting molecules, or multifunctional linker are added to the polyether chain, leaving the chemical and biological characteristics of the starting polymeric backbone mostly unmodified

The complex-structured MultiPEGs described here presented, as planned, larger numbers of functional groups than commercial PEGs of the same size, while the physicochemical properties were practically identical. In addition, it should be possible to devise the conjugation of different organic molecules by taking advantage of the free functional groups of linker molecules before the final deprotection of the other OH groups. The use of these MultiPEGs in liquid-phase systems appears very promising in the light of the first data collected from the simple activation, conjugation and deprotection procedures tested on sample N-protected amino acids. From our previous experience[32] we are confident that the same synthetic procedures can be adopted for these new polymeric systems.

The limiting aspect of our approach is undoubtedly given by the laborious steps required during the synthetic process. For this reason, most of our study is now addressed to acceleration of the overall procedure and to overcoming some of the tedious intermediate separations for the assembling of the final, crude MultiPEG by dialysis or ultrafiltration. In fact, even if the reported synthetic procedures can be performed on a gram scale, scaling up must be reasonably affordable if these MultiPEGs are to be produced on a larger commercial scale.

We have also ascertained that no toxicity was conferred by the chemical modification performed on the known atoxic PEGs, to demonstrate that together with higher loadings the new MultiPEGs should give an improved and safe pharmacodynamic character to their conjugates. Then, since the introduction of chemical bonds of different stability within the backbone could add a more degradable chemical structure, potentially useful for pharmacological purposes, extensive investigations in vitro and in vivo aimed towards evaluation of their degree of biostability are now in progress. Their application as new drug delivery systems on appropriate pharmacologically active molecules are now in progress with a new MultiPEG structure investigated for higher functionality and an easier assembling procedure.

Experimental Section

Abbreviations: DCE = dichloroethane; DCM = dichloromethane; AcCN = acetonitrile; DMF = *N*,*N*-dimethylformamide; DMAP = 4-dimethylaminopyridine; DMT = 4,4′-dimethoxytrityl; EDC = *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide; Fmoc = 9-fluorenylmetoxycarbonyl; MTBE = methyl *tert*-butyl ether; TCA = trichloroacetic acid; TEA = triethylamine; TNBS = 2,4,6-trinitrobenzensulfonic acid.

Materials and Methods: NMR spectra were recorded on a JEOL EX 400 spectrometer (400 MHz) in [D_6]DMSO and $CDCl_3$ with TMS as internal standard. The spectrophotometric UV/Vis analyses were performed on a Helios UNICAM spectrophotometer.

The GPC analyses were obtained on a PL Aquagel–OH 30–8 μm (30 × 0.75 cm) or by use of two columns connected in series (PL Aquagel-OH 30 + PL Aquagel-OH 40–8 μm (30 × 0.75 cm)) (Polymer Laboratories, Lab Service Analytica s.r.l., Bologna, Italy), with elution of samples with milliQ water. A Hewlett–Packard series 1100 HPLC system was fitted with a RI K-2301 refractive index detector (KNAUER, Berlin, Germany). A solution (20 μL) of each sample (2 mg) in milliQ water (1 mL) was injected into the column, with a flow of 0.6 mL min^{-1}.

The amount of NH_2 was calculated by colorimetric TNBS test as follows: TNBS (0.03 M in borate buffer at pH = 9.3, 250 μL) was added to the sample (1 mg). The solution was diluted to 10 mL with borate buffer (pH = 9.3), stirred and allowed to stand for 30 minutes at room temperature. Absorbance at 421 nm (ε = 12860) was measured.

1. Synthesis of TetraPEG$_{3000}$-(OH)$_5$ (MultiPEG #1)

1.1. Synthesis of the DMT-PEG$_{3000}$-OH

1.1.1. Dimethoxytritylation: OH–PEG$_{3000}$-OH was coevaporated twice from anhydrous pyridine in a 250-mL three-necked flask and dried for 30 min in the vacuum of a rotary pump. PEG was dissolved in the minimum amount of anhydrous pyridine. Argon was flushed through the side necks through needles. 4,4′-Dimethoxytrityl chloride (DMT-Cl, 1.2 equiv.) was added with stirring, followed by DMAP (1.0 equiv.) and TEA (4 equiv.). The mixture was allowed to react under argon with stirring at room temperature for 4 h.

The PEG derivative was precipitated by the slow addition of anhydrous MTBE (1 g in approx. 100 mL solvent) in an ice bath. The precipitate was filtered through a Gooch 4 under vacuum, washed with isopropyl alcohol and ether and then dried over KOH under vacuum.

The presence of DMT-Cl residue was checked by TLC (eluent $CHCl_3$/EtOH, 9:1, detection $HClO_4$(70%)/EtOH, 3:2). The product was recrystallized from EtOH. The degree of functionalization was estimated from the UV absorption (498 nm) of the trityl cation released by treating the compound with a solution of $HClO_4$ (70%) in EtOH (3:2). The functionalization was equal to 62% (344 μmol g^{-1} of DMT) and the recovery of the product 98%.

^{1}H NMR ($CDCl_3$): δ = 7.45 (d, 1.2 H (1), DMT), 7.40–7.20 (m, 7.8 H (8), DMT), 6.8 (d, 4.0 H (4), DMT), 3.78–3.38 (s, 272.7 H (272.7), $(CH_2CH_2O)_n$ (PEG)), 3.20 (t, 1.88 H (2), (PEG) CH_2–O–DMT) ppm.

1.1.2. Succinylation: The DMT derivative was coevaporated twice from anhydrous pyridine in a 250 mL three-necked flask and dried for 30 min under vacuum. The compound was dissolved in the minimum amount of anhydrous pyridine and placed into an ice bath with stirring. Succinic anhydride (5 equiv. with respect to the amount of OH still present) and DMAP (2.5 equiv.) were added and the solution was allowed to react under argon and with stirring at room temperature in the dark for 16 h. The DMT–PEG-succinate was precipitated by slow addition of MTBE in an ice bath. The precipitate was filtered through a Gooch 4 under vacuum, washed with isopropyl alcohol and ether, and dried over KOH and under vacuum. The product was recrystallized from EtOH. The yield was 100% and the recovery of the product 99%.

1.1.3. Purification: Purification of DMT–PEG$_{3000}$-succinate was carried out by anion exchange liquid chromatography on a QAE Sephadex A-50 resin and with a 20.0 × 2.5 cm column for approx. 0.5 g product. The resin was equilibrated with a 20 mM solution of 1,3-diaminopropane in bidistilled H_2O at pH 9.0. A flow of approx. 1.1 mL min^{-1} was maintained. The elution was monitored by reading the UV absorbance of the aromatic group (DMT, 260 nm) of the collected fractions (5 mL). The fractions containing the DMT–PEG-succinate were combined and the solvents were evaporated to dryness under vacuum

The product were extracted with AcCN and left stirring for 30 minutes. The solution was filtered and dehydrated over anhydrous Na_2SO_4. The AcCN solution was concentrated under vacuum and the product was precipitated with anhydrous ether in an ice bath, filtered through a Gooch 4, washed again with ether, and dried over KOH under vacuum.

^{1}H NMR ($CDCl_3$): δ = 7.42 (d, 1.0 H (1), DMT), 7.37–7.18 (m, 8.1 H (8), DMT), 6.8 (d, 4.0 H (4), DMT), 4.25 (m, 1.98 H (2), (PEG)CH_2–O–Succ.), 3.76–3.37 (s, 272.7 H (272.7), $(CH_2CH_2O)_n$ (PEG)), 3.20 (t, 1.97 H (2), (PEG)CH_2–O–DMT), 2.62 (m, 4.0 H (4), CH_2CH_2 (Succ.)) ppm.

1.1.4. Hydrolysis of Succinate: The combined DMT–PEG$_{3000}$-succinate samples were dissolved in conc. NH_4OH (50 ml; 30% v/v) in a 250-mL flask and stirred at room temperature for 4 h. Ammonia was evaporated to dryness by rotary evaporator and the residue

was taken up with AcCN. The mixture was stirred at room temperature for 30 minutes, the residue was filtered, and the AcCN solution was dried over Na_2SO_4. The solution was concentrated to approx. 5 mL, and the ice-cooled product was precipitated with anhydrous ether in an ice bath, filtered through a Gooch 4 under vacuum, washed with diethyl ether, and dried over KOH under vacuum.

The UV analysis of DMT groups was in agreement with the expected 50% level of functionalization. The yield was quantitative and the recovery of the product was 96%.

^{1}H NMR ($CDCl_3$): δ = 7.45 (d, 1.1 H (1), DMT), 7.40–7.20 (m, 8.0 H (8), DMT), 6.8 (d, 4.0 H (4), DMT), 3.78–3.38 (s, 272.7 H (272.7), $(CH_2CH_2O)_n$ (PEG)), 3.20 (t, 1.96 H (2), (PEG)CH_2–O–DMT) ppm.

1.2. Synthesis of $(DMT)_2$-diPEG_{3000}-OH

1.2.1. Activation of Monoprotected PEG: DMT–PEG_{3000}-OH (900 mg, 0.272 mmol) was coevaporated from anhydrous toluene (20 mL) in a 250-mL flask and dried for 30 minutes under vacuum. The residue was dissolved in an ice bath with the minimum amount of anhydrous DCM (3 mL). *p*NO_2phenyl chloroformate (109.9 mg, 0.545 mmol, 2.0 equiv.) and TEA (76 µL, 55.2 mg, 0.545 mmol, 2.0 equiv.) were added to the mixture with stirring. The ice bath was removed and the pH of the solution was monitored and brought to 8 with TEA. The mixture was allowed to react with stirring at room temperature for 16 h. The ice-cooled product (850 mg) was precipitated with MTBE, recovered by filtration through a Gooch 4 under vacuum, washed with propan-2-ol and ether and dried over KOH under vacuum. The product was recrystallized from EtOH. The yield was 98% and the recovery of the product was 97%.

^{1}H NMR ($CDCl_3$): δ = 8.25 (d, 1.88 H (2), *p*NO_2Ph), 7.55 (d, 1.78 H (2), *p*NO_2Ph), 7.45–7.17 (m, 8.67 H (9), DMT), 6.8 (d, 4.0 H (4), DMT), 4.45 (m, 1.91 H (2), (PEG)CH_2–O–CO–O-*p*NO_2Ph), 3.76–3.44 (s, 278.6 H (272.7), $(CH_2CH_2O)_n$ (PEG)), 3.20 (t, 1.94 H (2), (PEG)CH_2–O–DMT) ppm.

1.2.2. Formation of the Dimer: 1,3-Diaminopropan-2-ol (11.5 mg, 0.128 mmol) in a 1:1 mixture of anhydrous AcCN and DMF (2 mL) was dissolved in a 250-mL flask and stirred. The pH value was brought to 8.0 with 1 drop glacial acetic acid. DMT–PEG_{3000}-*p*NO_2phenyl carbonate (800 mg, 0,231 mmol, 1.8 equiv.) was added portionwise over 2 h, while the pH value was maintained at 8 with TEA. The mixture was stirred at room temperature for 72 h, the solution turning yellow due to the *p*NO_2-phenol released. The product (650 mg) was precipitated in an ice bath with anhydrous ethyl ether, washed with propan-2-ol and ether, and dried over KOH under vacuum. The compound was recrystallized from EtOH.

Analysis of the product with TNBS was unable to detect any free amino groups, confirming the absence of monoderivatized products. ^{1}H NMR analysis of the crude compound showed some presence of unreacted DMT–PEG. The yield was equal to 80%, as determined by GPC analysis, and the recovery of the product was 88%.

^{1}H NMR ([D_6]DMSO): δ = 7.45–7.17 (m, 17.97 H (18), DMT), 7.07 (m, 1.78 H (2), NH, urethanes), 6.88 (d, 8.06 H (8), DMT), 4.81 (d, 0.90 H (1), –O*H*), 4.45 (m, 0.97 H (1), *CH* linker), 4.04 (m, 3.84 H (4), (PEG)CH_2–O–CO–NH–), 3.70–3.38 (s, 545.5 H (545.5), $(CH_2CH_2O)_n$ (PEG)), 3.15 (t, 4.25 H (4), (PEG)CH_2–O–DMT), 3.05–2.80 (m, 3.88 H (4), CH_2 linker) ppm.

1.3. Synthesis of $(DMT)_4$-tetraPEG_{3000}-OH

1.3.1. Activation of the Dimer: $(DMT)_2$-diPEG_{3000}-OH (600 mg, 0.088 mmol) was activated by treatment with *p*NO_2phenyl chloroformate as described above. The product (520 mg) was precipitated by slow addition of anhydrous MTBE in an ice bath, recovered by filtration under vacuum, washed with propan-2-ol and ether, and dried over KOH under vacuum. The product was recrystallized from EtOH.

The yield was 99% and the recovery of the product 94%.

^{1}H NMR ($CDCl_3$): δ = 8.30 (d, 1.98 H (2), *p*NO_2Ph), 7.55 (d, 2.13 H (2), *p*NO_2Ph), 7.45–7.16 (m, 17.07 H (18), DMT), 6.80 (d, 8.0 H (8), DMT), 5,65 (m, 1.60 H (2), NH, urethane), 4.45 (m, 1.03 H (1), *CH* linker), 4.19 (m, 4.17 H (4), (PEG)CH_2–O–CO–NH–), 3.76–3.45 (s, 545.5 H (545.5), $(CH_2CH_2O)_n$ (PEG)), 3.20 (t, 4.15 H (4), (PEG)CH_2–O–DMT), 3.10–2.95 (m, 3.66 H (4), CH_2 linker) ppm.

1.3.2. Formation of the Tetramer: 1,3-Diaminopropan-2-ol (3.6 mg, 0.040 mmol) in a 1:1 mixture of AcCN and DMF (2 mL) was dissolved in a 250-mL flask and stirred. The pH value was brought to 8 with glacial acetic acid. $(DMT)_2$-diPEG_{3000}-*p*NO_2phenyl carbonate (500 mg, 0.072 mmol, 1.8 equiv.) was added portionwise over 2 h, while the pH value was maintained at 8 with TEA. The mixture was stirred at room temperature for 72 h; the solution turned yellow due to the *p*NO_2-phenol released. The product (350 mg) was precipitated with MTBE in an ice bath, recovered by filtration under vacuum, washed with propan-2-ol and ether, and then dried over KOH under vacuum. The compound was recrystallized from EtOH.

Analysis of the product with TNBS confirmed the absence of free amino groups.

The yield as determined by GPC analysis was equal to 80% and the recovery 89%.

1.3.3. Purification: Purification of crude $(DMT)_4$-tetraPEG_{3000}-OH was carried out by molecular exclusion chromatography on a Bio-Gel P 100 resin on a 2.5 × 55 cm column. The final crude sample (200 mg) was eluted with an aqueous solution at pH 9.0 and with a 0.15 mL min^{-1} flow rate. The fractions were collected on the basis of the absorption (260 nm) of the DMT groups. The chromatogram showed the presence of $(DMT)_4$-tetraPEG_{3000}-OH, along with some amount of lower mass derivatives.

From the fractions collected it was possible to obtain $(DMT)_4$-tetraPEG_{3000}-OH in a 60% yield with respect to the quantity applied to the column.

^{1}H NMR ($CDCl_3$): δ = 7.45–7.17 (m, 35.85 H (36), DMT), 6.80 (d, 16.05 H (16), DMT), 5.75 (m, 5.88 H (6), NH, urethanes), 4.45 (m, 2.85 H (3), *CH* linker), 4.19 (m, 8.02 H (8), (PEG)CH_2–O–CO–NH–), 3.77–3.44 (s, 1090.1 H (1090.1), $(CH_2CH_2O)_n$ (PEG)), 3.25–2.80 (m, 19.78 H (20), CH_2 linker + (PEG)CH_2–O–DMT) ppm.

1.4. Synthesis of TetraPEG_{3000}-$(OH)_5$: Purified $(DMT)_4$-tetra-$PEG_{(3000)}$-OH (30 mg, 2.2 µmol) was dissolved in anhydrous DCE (1 mL) in a 25-mL flask in an ice bath, and a solution of TCA in DCE (6% w/v, 120 µL) was added dropwise, with stirring. The mixture was allowed to react at room temperature for 30 minutes with stirring.

The final product (25 mg) was precipitated by slow addition of anhydrous MTBE in an ice bath, recovered by filtration under vacuum, washed with diethyl ether and dried over KOH under vacuum. The product was recrystallized from DCE/ether and dried over KOH under vacuum.

^{1}H NMR ([D_6]DMSO): δ = 7.21 (m, 1.90 H (2), NH, urethanes), 7.07 (m, 4.07 H (4), NH, urethanes), 4.82 (m, 1.30 H (1), secondary

O*H*), 4.57 (m, 3.92 H (4), primary OH), 4.08 (m, 11.6 H (11), (PEG)CH_2–O–CO–NH– + CH linker), 3.75–3.50 (s, 1090.1 H (1090.1), $(CH_2CH_2O)_n$ (PEG)), 3.05–2.87 (11.20 H (12), m, CH_2 linker) ppm.

2. Synthesis of PentaPEG$_{2000}$-(OH)$_8$ (MultiPEG #2)

2.1. Synthesis of DMT–PEG$_{2000}$-OH: The synthesis of the monoprotected PEG and its activation with *p*NO_2phenyl chloroformate were carried out by the same procedure as previously described for the corresponding compound with *MW* = 3000 Da. during the preparation of MultiPEG #1.

^{1}H NMR ([D$_6$]DMSO): δ = 7.48–7.15 (m, 8.4 H (9), DMT), 6.89 (d, 3.99 H (4), DMT), 4.56 (m, 0.89 H (1), –O*H*), 3.78–3.38 (s, 181.8 H (181.8), $(CH_2CH_2O)_n$ (PEG)), 3.16 (t, 1.86 H (2), (PEG) CH_2–O–DMT) ppm.

2.2. Synthesis of DMT–PEG$_{2000}$-propylamino: DMT–PEG$_{2000}$-*p*-NO_2phenyl carbonate (1.0 g, 0.42 mmol) was coevaporated twice from anhydrous DCM (20 mL) in a 250-mL flask and dried under vacuum. The residue was dissolved in the minimum amount of anhydrous DCM (3 mL), and 1,3-diaminopropane (110 μL, 95 mg, 1.3 mmol, 3.0 equiv.) was added. The mixture was allowed to react with stirring, at room temperature, for 24 h. The product (970 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration, washed with diethyl ether, and dried over KOH under vacuum. The product was recrystallized from DCM/diethyl ether.

The compound proved to contain DMT–PEG-DMT in trace amounts. The amount of free NH_2 was also monitored by the TNBS analysis and confirmed the identity of the product.

The yield was 98% and the recovery of the product 97%.

^{1}H NMR ($CDCl_3$): δ = 7.49–7.15 (m, 8.9 H (9), DMT), 6.80 (d, 3.96 H (4), DMT), 5.35 (m, 0.93 H (1), NH, urethane), 4.20 (m, 2.0 H (2), (PEG)CH_2–O–CO–NH), 3.75–3.37 (s, 181.8 H (181.8), $(CH_2CH_2O)_n$ (PEG)), 3.30–3.15 (m, 4.20 H (4), (PEG)CH_2–O–DMT + –NH–CH_2–CH_2–CH_2–NH_2), 2.75 (m, 2.02 H (2), –NH–CH_2–CH_2–CH_2–NH_2), 1.65 (m, 2.12 H (2), –NH–CH_2–CH_2–CH_2–NH_2) ppm.

2.3. Synthesis of PEG$_{2000}$-(OH)$_4$

2.3.1. Activation of OH–PEG-OH: Bifunctional PEG_{2000} (2.0 g, 1.0 mmol) was coevaporated twice from anhydrous dichloromethane (DCM) (20 mL) in a 250-mL flask and dried under vacuum for 30 minutes. The residue was dissolved in the minimum amount of anhydrous dichloromethane (5 mL), in an ice bath. *p*NO_2-phenyl chloroformate (807 mg, 4.0 mmol, 4.0 equiv.) and triethylamine (TEA) (558 μL, 404.6 mg, 4.0 mmol, 4.0 equiv.) were added with stirring. The ice bath was removed and the pH was brought to 8 with TEA. The mixture was allowed to react with stirring, at room temperature, for 16 h.

The product (2.131 g) was precipitated by slow addition of anhydrous ethyl ether (200 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, and dried over KOH under vacuum. The product was recrystallized from dichloromethane/ether.

The yield was 99.5% and the recovery of the product 98%.

^{1}H NMR ([D$_6$]DMSO): δ = 8.31 (d, 4.0 H (4), *p*NO_2Ph), 7.58 (d, 3.94 H (4), *p*NO_2Ph), 4.36 (m, 3.96 H (4), (PEG)CH_2–O–CO–O-*p*NO_2Ph), 3.71–3.35 (s, 181.8 H (181.8), $(CH_2CH_2O)_n$ (PEG)) ppm.

2.3.2. Reaction with 2-Aminopropane-1,3-diol: PEG_{2000}-(*p*NO_2-phenyl carbonate)$_2$ (2.13 g, 0.9 mmol) was coevaporated twice from anhydrous DCM (25 mL) in a 250-mL flask and dried under vacuum. The residue was dissolved in the minimum amount of anhydrous DCM (5 mL), and 2-aminopropane-1,3-diol (332 mg, 3.6 mmol, 4.0 equiv.) was added. The mixture was allowed to react with stirring, at room temperature, for 18 h. The product (1.98 g) was precipitated by slow addition of anhydrous ethyl ether (200 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, and dried over KOH under vacuum. The compound was recrystallized from DCM/ether, dissolved in water (5 mL), and extracted with DCM (100 mL). The organic phase was concentrated and dehydrated over anhydrous Na_2SO_4. The product was precipitated with anhydrous ether and recovered as described previously.

The yield was 99% and the recovery of the product 97%.

^{1}H NMR ([D$_6$]DMSO): δ = 6.78 (d, 1.80 H (2), NH, urethanes), 4.56 (t, 4.00 (4), –O*H*), 4.03 (m, 3.90 H (4), (PEG)CH_2–O–CO–NH–), 3.70 (t, 1.90 H (2), –NH–$CH(CH_2$–$OH)_2$), 3.65–3.41 (s, 181.8 H (181.8), $(CH_2CH_2O)_n$ (PEG)) ppm.

2.4. Synthesis of (DMT)$_4$-pentaPEG$_{2000}$

2.4.1. Activation of PEG$_{2000}$-(OH)$_4$: PEG_{2000}-$(OH)_4$ (630 mg, 0.28 mmol) was coevaporated twice from anhydrous DCM in a 250-mL flask and dried for 30 minutes under vacuum. The residue was dissolved in a mixture of anhydrous DCM, AcCN and pyridine in a 5:2:1 ratio (3 mL), and *N*,*N*′-disuccinimidyl carbonate (578 mg, 2.26 mmol, 8.0 equiv.) was added. The product was allowed to react with stirring, at room temperature, for 18 h. The product (620 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, and dried over KOH under vacuum. The product was recrystallized from dichloromethane/ether.

The yield was 97% and the recovery of the product 98%.

^{1}H NMR ($CDCl_3$): δ = 5.97 (m, 1.74 H (2), NH, urethanes), 4.56 (t, 4.00 (4), –O*H*), 4.60–4.40 (m, 7.36 H (8), –NH–CH(CH_2–O–CO–$OSu)_2$), 4.25–4.10 (m, 6.50 H (6), (PEG)CH_2–O–CO–NH– + –NH–$CH(CH_2$–O–CO–$OSu)_2$), 3.75–3.36 (s, 181.8 H (181.8), $(CH_2CH_2O)_n$ (PEG)), 2.85 (s, 15.73 H (16), CH_2CH_2 (OSu)) ppm.

2.4.2. Reaction with DMT–PEG$_{2000}$-propylamino: PEG_{2000}-$(OSu)_4$ (100 mg, 0.018 mmol) was coevaporated twice from anhydrous DCM (5 mL) in a 250-mL flask and dried under vacuum. The residue was dissolved in anhydrous DCM (2 mL), and DMT–PEG_{2000}-propylamino (378 mg, 0.079 mmol, 4.4 equiv.) was slowly added with stirring. The mixture was allowed to react with stirring, at room temperature, for 72 h.

The product (400 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, recrystallized from DCM/ether and dried over KOH under vacuum.

Analysis of the product with the TNBS test indicated the residual presence of free amino groups (<10%).

The yield, as determined by GPC analysis, was equal to 30% and the recovery of the product 89%.

^{1}H NMR ($CDCl_3$): δ = 7.47–7.15 (m, 33.87 H (36), DMT), 6.80 (d, 14.94 H (16), DMT), 5.80 (m, 9.10 H (10), NH, urethanes), 4.20 (m, 21.66 H (20), (PEG)CH_2–O–CO–NH– + –NH–CH(CH_2–O–)$_2$), 3.74–3.45 (s, 909.1 H (909.1), $(CH_2CH_2O)_n$ (PEG)), 3.30–3.01 (m, 25.06 H (24), (PEG)CH_2–O–DMT + –NH–CH_2–CH_2–CH_2–NH–), 1,64 (m, 7.83 H (8), –NH–CH_2–CH_2–CH_2–NH–) ppm.

2.5. Synthesis of PentaPEG$_{2000}$-(OH)$_4$: A solution of TCA in DCE (6% w/v, 10 mL) was slowly added dropwise with stirring to an ice-

cooled solution of $(DMT)_4$-pentaPEG$_{2000}$ (350 mg, 0.03 mmol) in anhydrous DCE (3 mL). Once the addition was complete, stirring was continued, at room temperature, for 30 minutes. The product (300 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether and dried over KOH under vacuum.

The product was recrystallized from DCE/ether and dried over KOH under vacuum.

The yield was 98% and the recovery of the product 99%.

^{1}H NMR ([D$_6$]DMSO): δ = 7.35 (m, 1.62 H (2), NH, urethanes), 7.15 (m, 7.35 H (8), NH, urethanes), 4.65 (m, 3.48 H (4), –O*H*), 4.04 (m, 18.75 H (20), (PEG)*CH*$_2$–O–CO–NH– + –NH–CH(*CH*$_2$–O–)$_2$), 3.72 (m, 1.57 H (2), –NH–*CH*(CH$_2$–O–)$_2$), 3.65–3.40 (s, 909.1 H (909.1), $(CH_2CH_2O)_n$ (PEG)), 2.85 (m, 15.88 H (16), –NH–*CH*$_2$–CH$_2$–*CH*$_2$–NH–), 1,88 (m, 8.85 H (8), –NH–CH$_2$–*CH*$_2$–CH$_2$–NH–) ppm.

2.6. Synthesis of PentaPEG$_{2000}$-(OH)$_8$

2.6.1. Activation of PentaPEG$_{2000}$-(OH)$_4$: PentaPEG$_{2000}$-(OH)$_4$ (250 mg, 0.0235 mmol) was coevaporated twice from anhydrous DCM in a 250-mL flask and dried for 30 min by rotary pump. The residue was dissolved in a mixture of anhydrous DCM, AcCN and pyridine in a 3:2:1 ratio (3 mL), and *N*,*N*′-disuccinimidyl carbonate (48.2 mg, 0.188 mmol, 8.0 equiv.) was added. The mixture was allowed to react with stirring, at room temperature, for 18 h. The product (200 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, and dried over KOH under vacuum. The product was recrystallized from dichloromethane/ether.

The yield was 99% and the recovery of the product 97%.

^{1}H NMR ($CDCl_3$): δ = 5.60 (m, 9.30 H (10), NH, urethanes), 4.45 (m, 7.96 H (8), (PEG)*CH*$_2$–O–CO–OSu), 4.20 (m, 20.98 H (20), (PEG)*CH*$_2$–O–CO–NH– + –NH–CH(*CH*$_2$–O–)$_2$), 3.77–3.38 (s, 909.1 H (909.1), $(CH_2CH_2O)_n$ (PEG)), 3.20 (m, 13.28 H (16), –NH–*CH*$_2$–CH$_2$–*CH*$_2$–NH-), 2.85 (m, 16.27 H (16), CH_2CH_2 (OSu)), 1,88 (m, 8.75 H (8), –NH–CH$_2$–*CH*$_2$–CH$_2$–NH–) ppm.

2.6.2. Reaction with 2-Aminopropane-1,3-diol: PentaPEG$_{2000}$-(OSu)$_4$ (200 mg, 0.02 mmol) was coevaporated twice from anhydrous DCM (5 mL) in a 250-mL flask and dried under vacuum. The residue was dissolved in anhydrous DCM (3 mL), and 2-aminopropane-1,3-diol (19 mg, 0.21 mmol, 12.0 equiv.) was added. The product was allowed to react with stirring, at room temperature, for 18 h. The product (177 mg) was precipitated by slow addition of anhydrous ethyl ether (100 mL) in an ice bath, recovered by filtration under vacuum, washed with diethyl ether, and dried over KOH under vacuum. The product was recrystallized from DCM/ethyl ether.

The yield was 98% and the recovery of the product 97%.

2.6.3. Purification: Purification was carried out by molecular exclusion chromatography on Superdex 75 resin, on a 2.6 × 58 cm column. Crude pentaPEG$_{(2000)}$-(OH)$_8$ (200 mg) was eluted with milliQ H_2O at a flow rate of 0.8 mL min^{-1}. The fractions (10 mL) were collected by monitoring refractive index. The fractions containing the pentaPEG$_{(2000)}$-(OH)$_8$ were combined and the solvents were evaporated to dryness under vacuum. The product were extracted with AcCN and stirred for 30 minutes, the solution was dehydrated over anhydrous Na_2SO_4, the AcCN solution was concentrated under vacuum, and the product (120 mg) was precipitated with anhydrous ether, filtered, washed with diethyl ether, and dried over KOH under vacuum.

^{1}H NMR ([D$_6$]DMSO): δ = 7.35 (m, 2.00 H (2), NH, urethanes), 7.17 (m, 8.26 H (8), NH, urethanes), 6.80 (m, 3.98 H (4), NH, urethanes), 4.75 (m, 1.83 H (2), –*CH*(CH$_2$–O–CO–)$_2$), 4.57 (m, 7.25 H (8), –OH), 4.04–3.80 (m, 31.08 H (32), (PEG)*CH*$_2$–O–CO–NH– + –NH–CH(*CH*$_2$–O–)$_2$ + –NH–*CH*(CH$_2$–OH)$_2$), 3.65–3.38 (s, 909.1 H (909.1), $(CH_2CH_2O)_n$ (PEG)), 2.94 (m, 15.48 H (16), –NH–*CH*$_2$–CH$_2$–*CH*$_2$–NH–), 1.50 (m, 8.75 H (8), –NH–CH$_2$–*CH*$_2$–CH$_2$–NH–) ppm.

3. Synthesis of MPEG-(Gly)$_n$

A) Use of MultiPEG$_{3000}$ #1

3.1. Synthesis of MPEG #1-(*N*-Fmoc-Gly)$_5$: TetraPEG$_{3000}$-(OH)$_5$ (150 mg) was coevaporated twice from anhydrous DCM (5 mL) in a 25-mL flask and dried under vacuum. The residue was dissolved in anhydrous DCM (5 mL), and Fmoc-Gly-OH (30 mg, 120 μmol, 10 equiv.) and DMAP (7.5 mg, 60 μmol, 5 equiv.) were added with stirring. The mixture was cooled to –20 °C, and DCC (25 mg, 120 μmol, 10 equiv.) was added with continuous stirring.

The mixture was allowed to react at room temperature, under argon and with stirring, for 18 h.

The DCU was filtered and the solution was concentrated under vacuum. MPEG #1-(*N*-Fmoc-Gly)$_5$ was precipitated in an ice bath by slow addition of anhydrous ether (50 mL).

The product was recovered by filtration, washed with diethyl ether and dried over KOH pellets under vacuum. MPEG #1-(*N*-Fmoc-Gly)$_5$ was recrystallized from DCM/ether. The degree of functionalization of the product was estimated from the UV absorption of the Fmoc protecting group in a MeOH solution (λ_{max} = 265 nm; ε = 13475). The functionalization was almost quantitative (390 mmol g^{-1}), and 140 mg of the product were recovered (93%).

^{1}H NMR ($CDCl_3$): δ = 7.75 (d, 10 H (9.70), Fmoc), 7.60 (d, 10 H (9.30), Fmoc), 7.50–7.20 (m, 20 H (21.80), Fmoc), 6.10–5.40 (m, 11 H (10.50), NH, urethanes), 4.46 (m, 10 H (9.72), –CO–C*H*$_2$–CH–Fmoc), 4.39–4.15 (m, 24 H (23.34), (PEG)*CH*$_2$–O–CO–NH + –CO–CH$_2$–*CH*–Fmoc + –O–*CH*(CH$_2$–NH–CO–)$_2$), 3.98 (m, 10 H (10.23), CH_2 (Gly)), 3.70–3.45 (s, 1090 H (1090), (–CH_2CH_2O)$_n$ (PEG)), 3.25 (m, 12 H (12.51), –O–CH(*CH*$_2$–NH–CO–)$_2$) ppm.

3.2. Synthesis of MPEG #1-(Gly-NH$_2$)$_5$: MPEG #1-(*N*-Fmoc-Gly)$_5$ (100 mg) was dissolved in a 20% solution of piperidine in anhydrous DMF (5 mL) in a 25-mL flask and the mixture was allowed to react at room temperature for 1 h with stirring. MPEG #1-(Gly-NH$_2$)$_5$ was precipitated by a slow addition of anhydrous ether in an ice bath. The product was recovered by centrifugation, washed with diethyl ether and dried over KOH pellets under vacuum. The amount of free NH_2 was monitored by the TNBS. The yield was quantitative (392 mmol g^{-1}), and 80 mg of product were recovered (80%).

B) Use of MultiPEG$_{2000}$ #2

3.3. Synthesis of MPEG #2-(*N*-Fmoc-Gly)$_8$: PentaPEG$_{(2000)}$-(OH)$_8$ (60 mg) was coevaporated twice from anhydrous DCM (5 mL) in a 50-mL flask and dried under vacuum. The residue was dissolved in anhydrous DCM (3 mL), and Fmoc-Gly-OH (21 mg, 86.1 μmol, 16 equiv.) and DMAP (5.2 mg, 43.0 μmol, 8 equiv.) were added with stirring. The mixture was cooled to –20 °C, and DCC (18 mg, 86.1 μmol, 16 equiv.) were added with continuous stirring.

The mixture was allowed to react at room temperature, under argon and with stirring, for 18 h.

The DCU was filtered off and the solution was concentrated under vacuum. MPEG #1-(*N*-Fmoc-Gly)$_5$ was precipitated by slow addition of anhydrous ether (25 mL) in an ice bath.

The product was recovered by filtration, washed with diethyl ether and dried over KOH pellets under vacuum. MPEG #2-(*N*-Fmoc-Gly) was recrystallized from DCM/ether. The degree of functionalization of the product was estimated from the UV absorption of the Fmoc protecting group in MeOH. The functionalization was almost quantitative (700.8 mmol g^{-1}), and 56 mg of product were recovered (93%).

^{1}H NMR ($CDCl_3$): δ = 7.78 (d, 16 H (16.60), Fmoc), 7.62 (d, 16 H (16.20), Fmoc), 7.50–7.20 (m, 32 H (31.70), Fmoc), 6.00–5.20 (m, 22 H (20.55), NH, urethanes), 4.46 (m, 16 H (15.72), –CO–C*H*$_2$–CH–Fmoc), 4.35–4.10 (m, 62 H (58.40), (PEG)*CH*$_2$–O–CO–NH + –CO–CH$_2$–*CH*–Fmoc + –O–*CH*(CH$_2$–NH–CO–)$_2$ + –NH–CH(*CH*$_2$–O–)$_2$), 4.00 (m, 16 H (17,20), CH$_2$ (Gly)), 3.67–3.40 (s, 909.1 H (909.1), (–CH$_2$CH$_2$O)$_n$ (PEG)), 3.25 (m, 16 H (16.51), –NH–*CH*$_2$–CH$_2$–*CH*$_2$–NH–), 1.67 (m, 8 H (8.50), –NH–CH$_2$–*CH*$_2$–CH$_2$–NH–) ppm.

3.4. Synthesis of MPEG #2-(Gly-NH$_2$)$_8$: MPEG #2-(*N*-Fmoc-Gly)$_8$ (50 mg) was dissolved in a 20% solution of piperidine in anhydrous DMF (2.5 mL) in a 25-mL flask. The mixture was allowed to react at room temperature for 1 h with stirring. MPEG #2-(Gly-NH$_2$)$_8$ was precipitated by slow addition of anhydrous ether in an ice bath. The product was recovered by centrifugation, washed with diethyl ether and dried over KOH pellets under vacuum. The amount of free NH$_2$ was monitored by the TNBS. The yield was quantitative (796.5 mmol g^{-1}), and 48 mg of product were recovered (95%).

Acknowledgments

This work was supported by financial contributions from the MIUR-Italy (PRIN2003) and the Regione Friuli Venezia-Giulia, L.R. 3/98.

[1] *Poly(ethylene glycol) Chemistry. Biotechnical and Biomedical Applications* (Ed.: M. J. Harris), Plenum Press, New York, **1992.**
[2] F. M. Veronese, *Biomaterials* **2001**, *22*, 405–417.
[3] M. J. Roberts, M. D. Bentley, J. M. Harris, *Adv. Drug Delivery Rev.* **2002**, *54*, 459–476.
[4] R. B. Greenwald, Y. H. Choe, J. McGuire, C. D. Conover, *Adv. Drug Delivery Rev.* **2003**, *55*, 217–250.
[5] G. M. Bonora, G. Tocco, S. Zaramella, F. M. Veronese, O. Pliasunova, A. Pokrovsky, E. Ivanova, V. Zarytova, *Il Farmaco* **1998**, *53*, 634–637.
[6] L. Gold, *J. Biol. Chem.* **1995**, *270*, 13581–13584.
[7] G. M. Bonora, E. Ivanova, V. Zarytova, B. Burcovich, F. M. Veronese, *Bioconjugate Chem.* **1997**, *8*, 793–797.
[8] R. B. Greenwald, A. Pendri, D. Bolikal, *J. Org. Chem.* **1995**, *60*, 331–336.
[9] R. B. Greenwald, *J. Controlled Release* **2001**, *74*, 159–171.
[10] T. Ooya, J. Lee, K. Park, *J. of Controlled Release* **2003**, *93*, 121–127.
[11] T. J. Dickerson, N. N. Reed, K. D. Janda, *Chem. Rev.* **2002**, *102*, 3325–3344.
[12] E. Bayer, M. Mutter, *Nature* **1972**, *237*, 512–513,.
[13] G. M. Bonora, G. Biancotto, M. Maffini,C. L. Scremin, *Nucleic Acids Res.* **1993**, *21*, 1213–1217.
[14] G. Nodosi, J. J. Krepinsky, *Synlett* **1996**, 159.
[15] C. M. Sun, *Combinat. Chem. & High Throughput Screening* **1999**, *2*, 299–315.
[16] P. Wentworth Jr., K. D. Janda, *Chem. Commun.* **1999**, 1917–1924.
[17] M. Benaglia, R. Annunziata, M. Cinquini, F. Cozzi, S. Ressel, *J. Org. Chem.* **1998**, *63*, 8628–8629.
[18] N. N. Reed, K. D. Janda, *Org. Lett.* **2000**, *2*, 1311–1313.
[19] C. Monfardini, O. Schiavon, P. Caliceti, M. Morpurgo, M. J. Harris, F. M. Veronese, *Bioconjugate Chem.* **1995**, *6*, 62–69.
[20] A. Fishman, M. E. Farrah, J.-H. Zhong, S. Paramanantham, C. Carrera, E. J. Lee-Ruff, *Org. Chem.* **2003**, *68*, 9843–9846.
[21] N. R. Luman, K. A. Smeds, M. W. Grinstaff, *Chemistry. A European Journal* **2003**, *9*, 5618–5626.
[22] M. Jayaraman, J. M. Fréchet, *J. Am. Chem. Soc.* **1998**, *120*, 12996–12997.
[23] R. Haag, A. Sunder, J.-F. Stumbé, *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955.
[24] H. Ihre, O. L. P. De Jesús, J. M. Fréchet, *J. Am. Chem. Soc.* **2001**, *123*, 5908–5917.
[25] A. Sunder, R. Mülhaupt, R. Haag, H. Frey, *Macromolecules* **2000**, *39*, 253–254.
[26] S. Drioli, F. Benedetti, G. M. Bonora, *Reactive and Functional Polymers* **2001**, *48*, 119–128.
[27] S. Zalipsky, *Adv. Drug Delivery Rev.* **1995**, *16*, 157–182.
[28] A. Mühlebach, B. Müller, C. Pharisa, M. Hofmann, B. Seiferling, D. Guerry, *J. Polymer Sci. Part A: Polymer Chemistry* **1997**, *35*, 3603–3611.
[29] R. Knischka, P. J. Lutz, *Macromolecules* **2000**, *33*, 315–320.
[30] I. Adamo, M. Ballico, P. Campaner, S. Drioli, G. M. Bonora, *Eur. J. Org. Chem.* **2004**, 2603–2609.
[31] a) NOF Corporation, Tokyo (Japan); b) SunBio, Anyang City (South Korea); c) Nektar Therapeutics, San Carlos (California, USA).
[32] S. Drioli, I. Adamo, M. Ballico, F. Morvan, G. M Bonora, *Eur. J.Org. Chem.* **2002**, 3473–3480.

Received: October 06, 2004

