Synthesis of Poly(ester-amide) Dendrimers based on 2,2-*Bis*(hydroxymethyl) Propanoic Acid and Glycine

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ABSTRACT: Water-soluble, biodegradable, and biocompatible poly(ester-amide) dendrimers with hydroxyl functional groups are synthesized from previously prepared AB_2 adduct of 2,2-*bis*(hydroxymethyl) propanoic acid (*bis*-MPA) and glycine as a repeating unit. Two esterification procedures using different coupling reagent/catalyst systems (DCC/DPTS or EDC/DMAP) are studied with respect to efficiency, ease of products purification, and quality of the final products. Both procedures have their own benefits and drawbacks, depending on dendrimer generation. The synthesized poly(ester-amide) dendrimers as well as commercially available *bis*-MPA dendrimers, poly(ester-amide) hyperbranched polymer, and poly(vinyl alcohol) are used for preparation of solid dispersions of sulfonylurea antidiabetic drug glimepiride to improve its poor water-solubility. *In vitro* dissolution studies show in comparison with pure glimepiride in crystalline or amorphous form, to the same extent improved glimepiride solubility for solid dispersions based on dendritic polymers, but not for poly(vinyl alcohol). The amount of glimepiride complexed with both dendrimer types increases with dendrimer generation. © 2014 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2014**, *52*, 3292–3301

KEYWORDS: dendrimers; drug delivery systems; esterification; hyperbranched; water-soluble polymers

INTRODUCTION Dendrimers with a precisely controlled branched structure reveal several advantages over their linear counterparts of comparable molar mass, that is, uniform molar mass distribution, multifunctionality, and in the case of amphiphilic nature of dendrimers also unimolecular micellar architecture.[1,2] These intrinsic properties of dendrimers translate to their unique chemical and physical properties (e.g., solubility, viscosity, chemical reactivity, etc.), which make them interesting as materials for biological application (scaffolds for drug delivery systems, MRI contrast agent, materials for tissue engineering, transfection).[3–13] Although many dendritic structures have been synthesized, only few of them possess adequate water-solubility and biocompatibility to have a potential for biomedical applications.[4,5,12–16] To optimize the chemical and physical properties of dendrimers for biomedical applications, various biocompatible monomers have been applied for their synthesis,[17–25] for example, natural metabolites (amino acids, sugars, α-hydroxy acids, fatty acids), chemical intermediates in metabolic pathways (succinic acid, fumaric acid, citric acid, pyruvic acid), and monomers currently used for the preparation of medical-grade linear polymers, that is, poly(ethylene glycol), poly(caprolactone), poly(trimethylene carbonate), etc. A group of neutral aliphatic polyester dendrimers based on 2,2-*bis*(hydroxymethyl) propanoic acid (*bis*-MPA) and their hybrids with poly(ethylene glycol), which are water soluble, nonimmunogenic, biodegradable, biocompatible and nontoxic,[26,27] have also been widely investigated as candidates for the development of anticancer drug delivery systems.[24,25,28–39]

The dendrimers based on *bis*-MPA can be prepared by either convergent, divergent, or double exponential approaches.[40–44] Esterification reaction for preparation of polyester dendrimers is most commonly accomplished by utilizing a carbodiimide coupling reagent (usually *N*,*N*'-dicyclohexylcarbodiimide, DCC) in the presence of a catalyst.[18,19,43–46] Unfortunately, the use of DCC has displayed multiple disadvantages related primarily to a side reaction of DCC with *bis*-MPA, resulting in formation of the *N*-acylurea that have to be removed to ensure good quality of end products. This is of course accompanied by significantly decreased reaction yields, especially when going to higher dendrimer generation.[41,44] To circumvent the aforementioned problems related to carbodiimide based esterification, a

Additional Supporting Information may be found in the online version of this article.

4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) has been utilized as a catalyst.[19,30,44,46]

Esterification reaction was performed also by a preactivation of the *bis*-MPA either in the form of acid chloride[40] or acid anhydride.[41,42,44] The *bis*-MPA chloride and the *bis*-MPA anhydride react with the hydroxyl groups of central core or dendron/dendrimer under a catalytic amount of 4-dimethylaminopyridine (DMAP) in dichloromethane (DCM) solution in the presence of triethylamine (TEA) and pyridine, respectively. The divergent synthetic approach using anhydride activated strategy belongs to one of the most robust approaches for preparation of *bis*-MPA dendrimers on a large scale, in high yields and with little byproducts that are simply purified by extraction and precipitation.

Another approach involving precipitation as simple purification method is a divergent synthetic route in which the *bis*-MPA dendrons are grown up on a linear polystyrene support in the form of dendritic hybrids.[47]

Recently, accelerated approaches involving highly selective and efficient click reactions (copper-catalyzed azide–alkyne cycloaddition (CuAAC), thiol-ene coupling (TEC), Diels–Alder cycloaddition (DA), thiol/acrylate Michael addition) with[48–50] or without[51,52] traditional esterification reaction have been proposed for the synthesis of *bis*-MPA type dendrimers with high yields and purity as well as for dendrimer functionalization.[2,22,53–55]

Our previous work revealed that poly(ester-amide) hyperbranched polymers enhance water-solubility of the poorly water-soluble antidiabetic drug glimepiride by preparation of solid dispersions[56] that were finally incorporated into the tablet formulation.[57] Experimental results showed that the improved glimepiride solubility is due to the formation of a complex between the glimepiride drug and the hyperbranched polymer which is stabilized by a hydrogen-bond interaction between the slightly acidic proton of NH group of the glimepiride sulfonylurea segment and the carbonyls of the amide and ester bonds of the hyperbranched polymers, while the hydroxyl groups did not seem to play a role in the complex formation.[58] This interaction allows molecularly dispersed glimepiride within the amorphous hyperbranched polymers and thereby glimepiride solubility and dissolution rate are significantly improved.

Since hyperbranched polymers show distribution in molar mass and branching pattern that can result in irreproducible quality of the material from batch to batch synthesis and, consequently, in variation of loading capacity and kinetics of drug release, dendrimers are preferred over hyperbranched polymers. Herein we thus report on a synthesis of different generation, water-soluble poly(ester-amide) dendrimers with hydroxyl functional groups from an AB_2 adduct of *bis*-MPA and glycine as a repeating unit and 1,1,1-*tris*(hydroxymethyl)propane as a core molecule. The synthesis of dendrimers was carried out by two parallel carbodiimide based esterification reactions to determine the efficiency of both coupling reagent/catalyst systems. The synthesized poly(ester-amide) dendrimers were tested as solubility enhancers for poorly water-soluble glimepiride drug and the results were compared with those obtained for the solid dispersions of glimepiride and commercially available *bis*-MPA based dendrimers as well as the solid dispersion of glimepiride and poly(vinyl alcohol) to explore the role of ester and hydroxyl groups in complex formation. Additionally, the cytotoxicity of the synthesized poly(ester-amide) dendrimers was evaluated.

EXPERIMENTAL

All chemicals and solvents were used as purchased if not stated otherwise. Commercial dendrimers (the third and the fourth generations) with hydroxyl functional groups, synthesized from *bis*-MPA as a repeating unit and 1,1,1-*tris*(hydroxymethyl)propane as a core, were obtained from Polymer Factory Sweden AB, Stockholm, Sweden. Poly(vinyl alcohol) (98–99% hydrolyzed) with the M_n of 2.1 kDa and molar mass dispersity of 2.2 was purchased from Sigma-Aldrich, Germany.

Detailed experimental procedures are available in the Supporting information. In general, two coupling methods either DCC/DPTS (method A) or EDC/DMAP (method B) were used for amidation reaction of benzyl protected glycine and acetonide protected *bis*-MPA to obtain the AB_2 adduct. The same two coupling methods were used for the esterification reaction to prepare the second-generation dendron as well as the first (AB_2 unit from the core), the second (the second-generation dendron to the core), the third (the second-generation dendron to the first-generation dendrimer) and the fourth (the second-generation dendron to the second-generation dendrimer) generation dendrimers. The acetonide protection group was removed by Dowex resin, whereas the benzyl protected focal group by catalytic hydrogenation.

^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on an Agilent Technologies DD2 spectrometer at 300 and 75 MHz, respectively and with delay time of 5 and 2 sec, respectively.

Analytical thin layer chromatography (TLC) was performed on Merck 60 F254 precoated silica gel plates. TLC plates were analyzed by short-wave UV light and/or by dipping in $KMnO_4$ solution. Flash column chromatography was performed using Zeochem Silica Gel (ZEOprep 60 Eco 40–63 μm).

The separations of protected and deprotected dendrimers by SEC were carried out using an Agilent 1260 HPLC pump and a PolarGel-L 8 μm analytical column (7.5 mm $\times$ 300 mm) with a precolumn (Polymer Laboratories, UK) in 0.1 M aqueous solution of $NaNO_3$ with added azide. The PolarGel-L column covers the molar masses up to 30 kDa. For the detection, we used a multi-angle light-scattering (MALS with 18 angles) detector (DAWN-HELEOS, Wyatt Technology

Corp.) and an interferometric refractive index (RI) detector (Optilab rEX, Wyatt Technology Corp.). The nominal eluent flow rate was 0.8 mL/min, the injection volume was typically 100 μL, and the mass of the samples injected onto the column was 150 μg. The determination of absolute M_w and the calculation of M_n values from MALS detector require a sample-specific refractive-index increment (dn/dc), which was determined from the RI response assuming 100% of sample mass recovery from the column. For the data acquisition and evaluation, Astra 5.3.4 software (Wyatt Technology Corp.) was utilized.

The mass spectra were acquired with a Bruker UltrafleXtreme MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with a nitrogen laser ($\lambda = 337$ nm). The linear positive ion mode was used to acquire the mass spectra. The calibration was made externally with a Peptide calibration standard (Bruker Daltonics). The matrices used were super-DHB (9/1 mixture of 2,5-dihidroxybenzoic acid and 2-hydroxy-5-methoxy benzoic acid) and trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), which were supplied by Sigma-Aldrich. Sodium trifluoroacetic acid was also supplied by Sigma-Aldrich. A 5 mg/mL water solution of deprotected dendrimer was mixed with a 20 mg/mL solution of sDHB matrix and 2.2 mg/mL of cationic agent NaTFA dissolved in a 50/50 mixture of acetonitrile/water. The volume ratio between the sample, matrix solution and cationic agent was 3/3/0.5. Then, 1 μL of the final solution was spotted on the target plate using the dried-droplet method. The protected dendrimers were dissolved in THF at concentration of 5.0 mg/mL and mixed with a 20 mg/mL solution of DCTB matrix in THF and a 2.2 mg/mL solution of NaTFA in THF. The volume ratio between the solutions of protected dendrimer, matrix and NaTFA was 3/3/0.5. Then, 0.5 μL of the final solution was spotted on the target plate using the dried-droplet method.

Preparation of Solid Dispersions

A conventional solvent evaporation method was used for the preparation of solid dispersions. The glimepiride substance in crystalline form together with the carrier (dendrimers, hyperbranched polymer, PVA) were weighted in a weight ratio of 5/95 %, w/w, and dissolved in a mutual solvent—ethanol at room temperature during continuous stirring with magnetic stirrer (300 rpm) for 4 h to assure transparent solutions. The solvent was then removed at slightly elevated temperature (40 °C) in vacuum. Such prepared solid dispersions were stored in a desiccated container until additional study.

Determination of Dendrimer Loading Capacity

Drug loading capacity (LC) of dendritic polymers was determined from the results of *in vitro* dissolution experiments using reversed phase HPLC and calibration curve. The amount of loaded glimepiride into carriers was determined using eq 1.

$$LC(\%) = \frac{c(\text{HPLC})}{c(\text{assay})} \times c(\text{SD}) \quad (1)$$

where LC: loading capacity in %, c(HPLC): concentration of glimepiride (in μg/mL) determined by HPLC after 60 min dissolution of solid dispersions in phosphate buffer (pH = 6.8), c(assay): theoretical 100% concentration of glimepiride (4.4 μg/mL), calculated using the assay result and volume of dissolution media, c(SD): concentration of glimepiride in solid dispersions (5%, w/w).

In Vitro Dissolution Studies

Dissolution studies were performed in phosphate buffer solution at pH = 6.8, physiologically relevant media, at 37 °C using an USP Dissolution Tester, Apparatus II (Paddle method) at a rotation rate of 75 rpm. The tested samples (glimepiride as well as solid dispersions) were added in the correct amount directly to a 900 mL phosphate buffer solution to achieve a final concentration of 4.4 μg/mL (glimepiride). The experiments were performed in triplicates. Aliquots, each of 2 mL, were withdrawn from the dissolution medium at a time interval of 60 min. The sample aliquots were withdrawn through a syringe and filtered through the Millipore filter (0.45 μm, PVDF). The sample aliquots were analyzed for the dissolved glimepiride content using reversed-phase HPLC method.

Assay

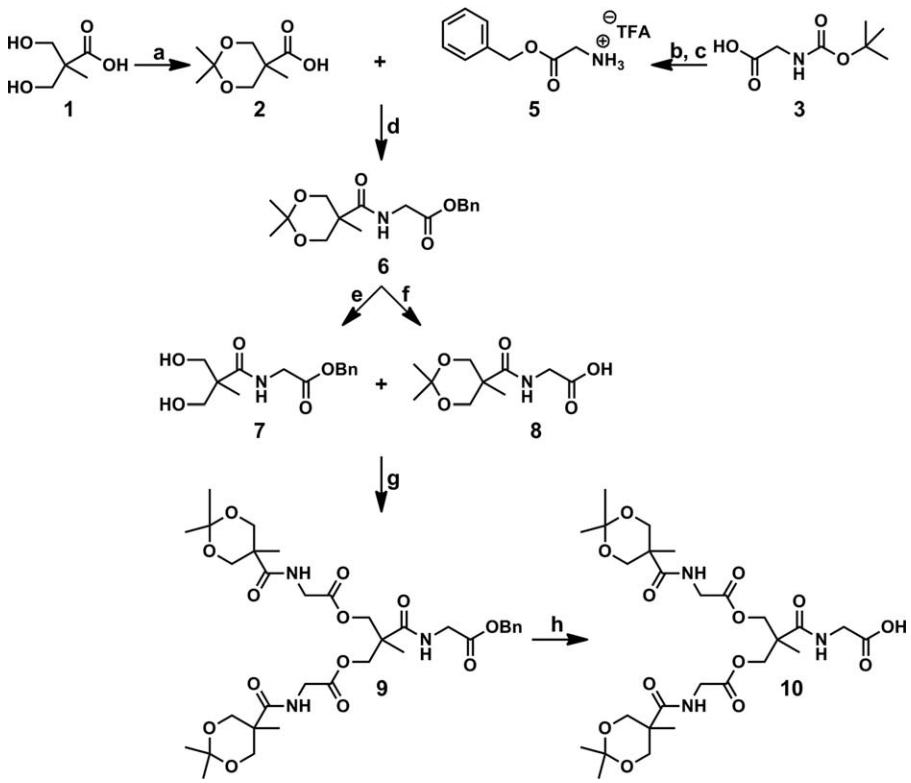
The tested samples were accurately weighted (5 mg) into a 10 mL volumetric flask. 5 mL of water/acetonitrile (20/80, V/V) mixture was then added. The solution was stirred for 5 min and then completed to volume with water/acetonitrile mixture.

HPLC Method

The assay analysis and amount of dissolved glimepiride was estimated by reversed-phase HPLC (Waters Alliance) in a binary mode with a photodiode array detector at 230 nm. The analyses were performed on a C18 column (150 × 4.6 mm, 3.5 μm) placed in a column oven at 30 °C and a mobile phase: A (phosphate buffer, pH = 2.5 : acetonitrile = 72 : 28) and B (phosphate buffer, pH = 2.5 : acetonitrile = 30 : 70) delivered at a flow-rate of 1.5 mL/min under the following gradient conditions: 0–6 min (100% A–0% A): 6–6.5 min (0% A–100% A). The column equilibration time was 5 min. Retention time of the glimepiride was 4.0 min. The concentration of dissolved glimepiride was determined from the area of glimepiride peak using a preformed calibration curve. Standard curve for glimepiride was measured over a range of 15 to 0.1 μg/mL and shown to be linear. The limit of detection was 0.005 μg/mL.

In Vitro Biocompatibility Testing—Hemolytic Activity

Freshly collected human blood samples supplemented with EDTA were centrifuged at 2000 g for 10 min. The sediment of red blood cells (RBC) was washed three times with 6 volumes of phosphate buffered saline (PBS) and each time centrifuged at 2000 g for 10 min. The buffy coat was removed with each wash according to previous reports. The retrieved



SCHEME 1 Synthesis of the AB_2 adduct from *bis*-MPA and glycine and the second-generation dendrons: Reagents and conditions: (a) 2,2-dimethoxypropane, acetone, 85%; (b) BnOH, DCC, DPTS, DCM, 87%; (c) TFA, DCM; (d) Method A: TEA, DCC, DPTS, DCM, 79%; Method B: TEA, EDC, DMAP, DCM, 99%; (e) Dowex, CH_3CN, 99%; (f) H_2, Pd/C, EtOAc, 99%; (g) Method A: DCC, DPTS, DCM, 90%; Method B: EDC, DMAP, DCM, 79%; (h) H_2, Pd/C, EtOAc, 99%.

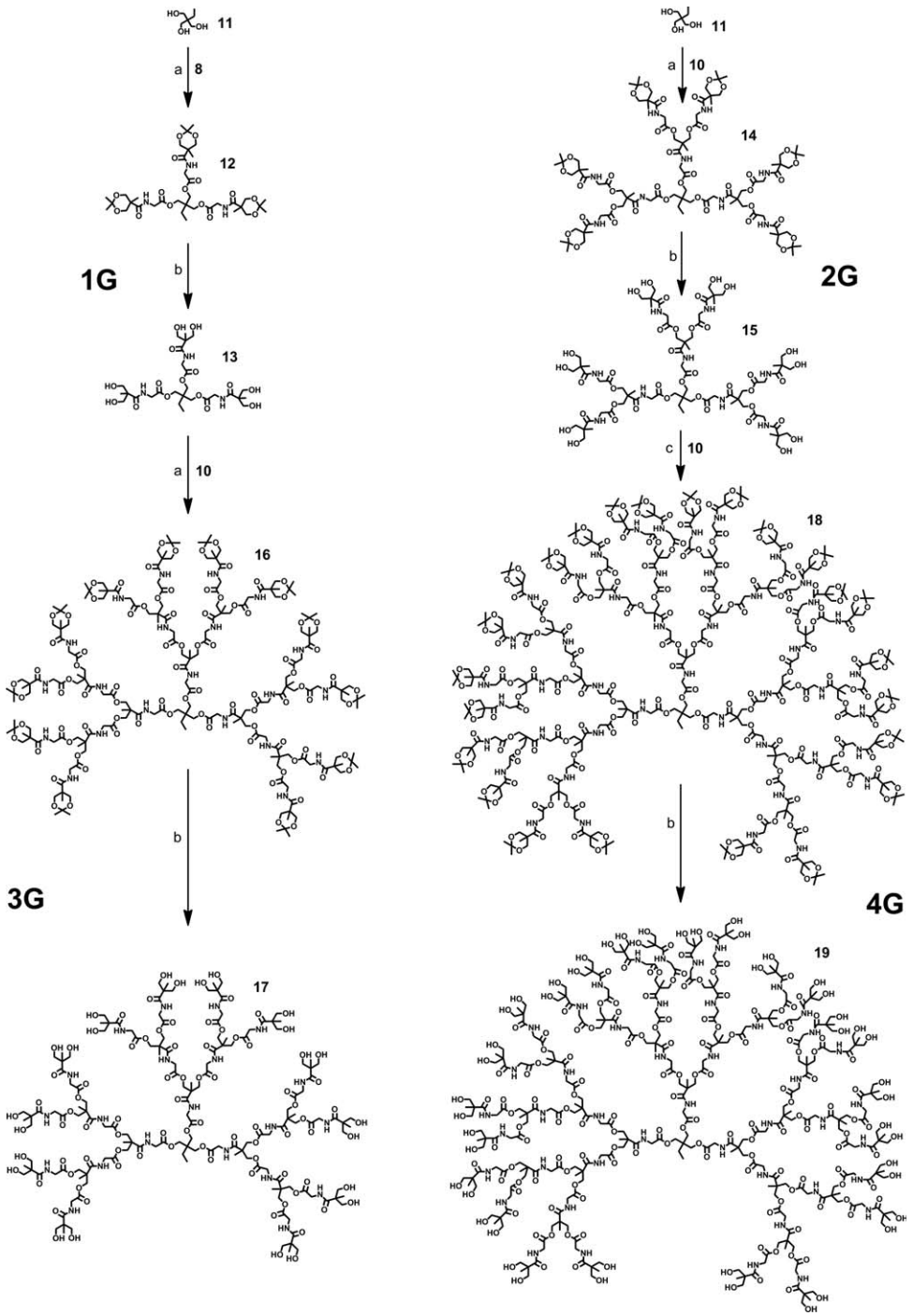
RBC was re-suspended in PBS at the dilution of 1:15. Dendritic polymers were added to the 96-well plate, followed by addition of RBC suspension resulting in final RBC dilution factor of 1 : 30. Then the plate was incubated for 3 h (37 °C, 400 rpm). After the incubation period, the plate was centrifuged at 2000 g for 10 min and 10 μL of supernatant was transferred into a 96-half well transparent plate, followed by 20-times dilution with PBS. Hemolysis degree was estimated from the extent of hemoglobin release into the supernatant as measured by absorbance at $\lambda = 450$ nm using Safire reader (Tecan, Switzerland). The results are expressed as percent hemolysis. The absorbance of the supernatant in the absence of tested compounds was taken as zero hemolysis (0%) and the total hemolysis (100%) was assigned when sodium lauryl sulphate (0.1%, w/v) was added to the RBC suspension.

RESULTS AND DISCUSSION

Synthesis of Poly(ester-amide) Dendrimers of Different Generation

First the AB_2 adduct of *bis*-MPA and glycine was prepared to easily introduce an amide bond into the dendrimer structure and to avoid a side reaction of an amine group with one of the many dendron/dendrimer ester groups that could take place in the case of direct amidation reaction. In order to obtain a good overall yield and highly pure dendron/dendrimers, it is essential that esterification as well as deprotection reactions proceed quantitatively and selectively. The synthesis of the *bis*-protected AB_2 repeating unit **6** alongside the synthesis of the second-generation dendron **10** is described in Scheme 1. The protected *bis*-MPA acid **2** and the TFA-salt of **5** were reacted at room temperature in DCM under basic conditions (TEA) according to two different procedures, either DCC/DPTS (Procedure A) or *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)/DMAP (Procedure B) to form the *bis*-protected AB_2 repeating unit **6**. Both procedures provided the desired product **6**, however, the **6** prepared by procedure A necessarily required purification by column chromatography, whereas the **6** prepared by procedure B required no additional product purification, only a simple extraction workup. Thus, the procedure B is less time consuming and resulted in significantly higher yield. Highly pure AB_2 adduct is necessary for preparation of well-defined dendron/dendrimer in the next step. The acetonide protected group was easily removed under mild conditions in the presence of acidic resin to provide the diol **7** in quantitative yield. The benzyl protected group was also quantitatively removed by catalytic hydrogenolysis utilizing 10% Pd/C to produce the acid **8**.

Subsequently, the diol **7** and the acid **8** were coupled again by two different coupling procedures to give the benzyl- and acetonide-protected second-generation dendron **9** (Scheme 1).



SCHEME 2 Schematic presentation of poly(ester-amide) dendrimers synthesis up to the fourth generation: reagents and conditions: (a) Method A: DCC, DPTS, DCM/CH_3CN, 50 °C (protected dendrimers: **12** (1G), 81%; **14** (2G), 81%; **16** (3G), 69%); Method B: EDC, DMAP, DCM/CH_3CN, rt (protected dendrimers: **12** (1G), 98%; **14** (2G), 99%; **16** (3G), 83%); (b) Dowex®, CH_3CN (deprotected dendrimers: **13** (1G), **15** (2G), **17** (3G), **19** (4G), 99%); (c) Method A: DCC, DPTS, DCM/CH_3CN, 50 °C (protected dendrimer: **18** (4G), 42%); Method B: EDC, DMAP, DCM/DMF, rt (protected dendrimer: **18** (4G), 45%).

Both coupling reactions were performed in DCM under similar mild conditions and both provided comparable results. Yet again, the procedure utilizing EDC coupling reagent proved to be less time consuming due to a simple extraction workup as the only purification method. After removing of 1-ethyl-3-(3-dimethyl aminopropyl)urea dihydrochloride (EDU) and excess reagents, the obtained product was analytically pure.

Since the preparation of the AB_2 adduct of *bis*-MPA and glycine, which is analogue of the *bis*-MPA monomer, requires four synthetic steps (two for benzyl protected glycine,

TABLE 1 Comparison between the DCC/DPTS and the EDC/DMAP Reaction Conditions

	Method A: DCC[a]		Method B: EDC[b]	
Compound	DPTS (Equiv)	Yield (%)[c]	DMAP (Equiv)	Yield (%)[c]
6	0.05	79[b]	0.25	99
9	0.2	90[b]	1	79
12	0.2	81	3	99
14	0.2	81	3	99
16	0.4	69	12	83
18	0.44	42	24	45

[a] Reactions were performed at 50 °C.
[b] Reactions were performed at room temperature.
[c] Isolated yield.

followed by coupling with protected *bis*-MPA and subsequent deprotection of benzyl group) we did not apply well established, robust anhydride activated strategy (reported for preparation of the *bis*-MPA dendrimers) for esterification reaction to avoid the loss of starting material. Instead, the syntheses of poly(ester-amide) dendrimers of the first (1G), the second (2G), the third (3G), and the fourth (4G) generations were carried out by the divergent, convergent, mixed divergent/convergent, and double stage convergent protocols,[59] respectively, using two different carbodiimide based esterification reactions (Scheme 2). The first synthetic procedure (A) was performed in DCM/acetonitrile mixture at 50 °C using DCC/DPTS coupling procedure, whereas the second procedure (B) was performed in the same solvent mixture but with EDC/DMAP coupling system at room temperature. In the case of procedure A, the reaction had to be carried out at 50 °C in order to achieve completion in a reasonable time. Additionally, the preparation of well-defined 3G and 4G dendrimers from the 1G and 2G dendrimers, regardless of the type of synthetic procedure, required isolation of the raw products with a quick workup in which the resulting urea was removed (filtration in procedure A and extraction in procedure B). The raw product was then redissolved and coupling reaction repeated with a small amount of acid **10** added.

The differences in yields and the amount of catalysts used for each generation dendrimer for both esterification procedures are summarized in Table 1. In both esterification procedures, the reaction time required for completion increases with increasing dendrimer molar mass. Although reaction preceded at a reasonable rate for lower generation dendrimers, it slowed down for higher generation dendrimers. By utilizing the DCC/DPTS esterification procedure, the protected 1G, 2G, 3G, and 4G poly(ester-amide) dendrimers were obtained in 81, 81, 69, and 42% yields, respectively. The purification of dendrimers by chromatographic techniques was required for all generations, and it got harder at higher dendrimer generation due to increase in dendrimer polarity. The reaction yield of the fourth generation dendrimer was significantly lower most probably due to steric congestion of hydroxyl functional groups resulting in their decreased accessibility.

The second esterification procedure utilized EDC/DMAP coupling at room temperature in DCM/acetonitrile mixture for the 1G–3G dendrimers and in DCM/DMF mixture for the 4G dendrimer. For the 4G dendrimer, the DMF instead of acetonitrile had to be used to ensure complete solubility of the 2G deprotected dendrimer at room temperature. This procedure offered very mild conditions with significantly reduced reaction time and showed considerably improved yields, that is, 98, 99, 83, and 45% for the 1G, 2G, 3G, and 4G dendrimers, respectively. For DCC/DPTS coupling method, the amount of catalyst did not change significantly with dendrimer generation, however, for EDC/DMAP coupling we had to significantly enlarge the amount of DMAP with dendrimer molar mass in order to achieve complete conversion. In EDC/DMAP esterification procedure, the simple aqueous workup method as the only purification procedure was enough to obtain 1G and 2G dendrimers of good quality. However, a detailed analysis of the raw 3G and 4G dendrimers prepared by this procedure showed some side products that were not able to be removed solely by extraction workup and thus a column chromatography had to be applied. Besides, the 4G dendrimer showed encapsulated

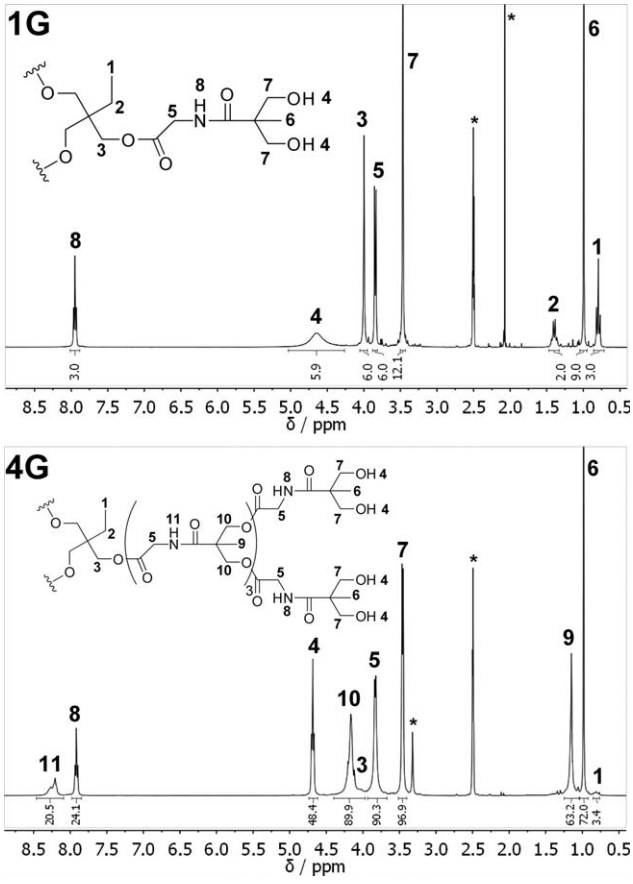


FIGURE 1 ^{1}H NMR spectra of deprotected 1G and 4G dendrimers with the assignation of signals.

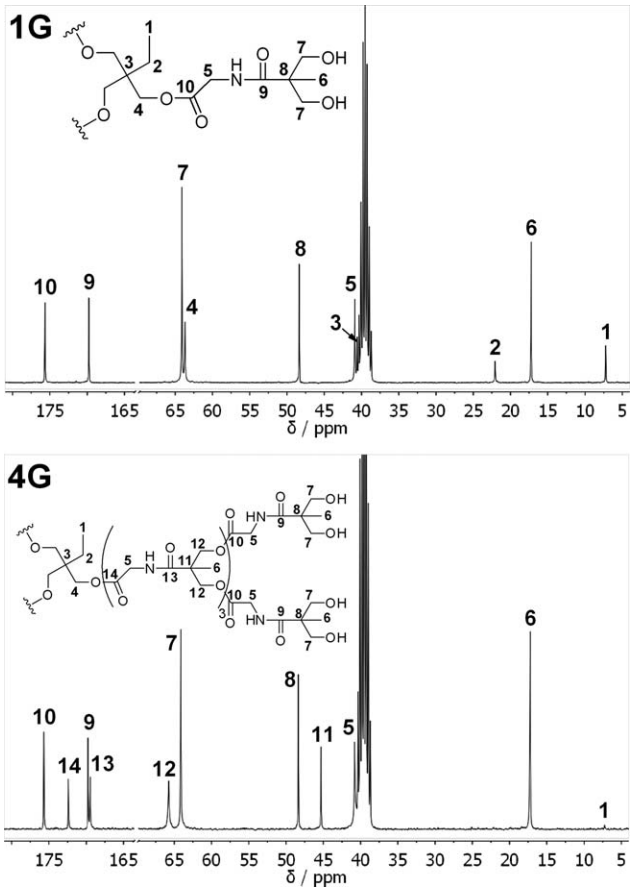


FIGURE 2 ^{13}C NMR spectra of deprotected 1G and 4G dendrimers with the assignation of signals.

DMAP, which was used in a large amount, in the dendrimer interior, and thus an additional acidic workup step was required to remove it. Encapsulation of DMAP in the dendrimer interior might be the reason for higher amount of catalyst needed for efficient reaction to higher generation dendrimers.

The structure of synthesized poly(ester-amide) dendrimers was investigated by ^{1}H and ^{13}C NMR and MALDI-TOF MS. Integration of the assigned signals in proton NMR spectra confirms high purity of dendrimers (Figs. 1 and 2, Supporting Information Figs. S1–S4). MALDI-TOF mass spectra of protected and deprotected 1G and 2G (Fig. 3, Supporting Information Fig. S5) confirm high structural uniformity of synthesized dendrimers. On the other hand, mass spectra of the 3G and 4G reveal some defect structures in trace amount for the protected 3G and somewhat higher amount for the protected 4G (Fig. 3). The content of defect structures is larger in deprotected 3G and 4G (Supporting Information Fig. S5). Since contrary the results of NMR and SEC-MALS measurements indicate highly pure dendrimers, it is possible that some degradation due to hydrolysis occurred during sample preparation for mass spectroscopic measurements rather than during hydroxyl group deprotection.

The molar mass characteristics of dendrimers were determined by SEC-MALS (Fig. 4, Table 2). SEC traces (responses of RI and LS detectors) together with flat curves, representing the molar mass as a function of elution volume confirmed uniform molar mass distributions of deprotected dendrimers, which are reflected in their low dispersity values ($Đ_M$), (Table 2). The determined molar mass values of dendrimers are very close to theoretical ones (Table 2).

Solid Dispersions of Glimepiride and Particular Polymer

Glimepiride is one of the third generation sulfonylurea drugs used for control of diabetis mellitus, type 2.[60] It shows poor water-solubility and slow dissolution rate, which can cause irreproducible clinical response or therapeutic failure due to

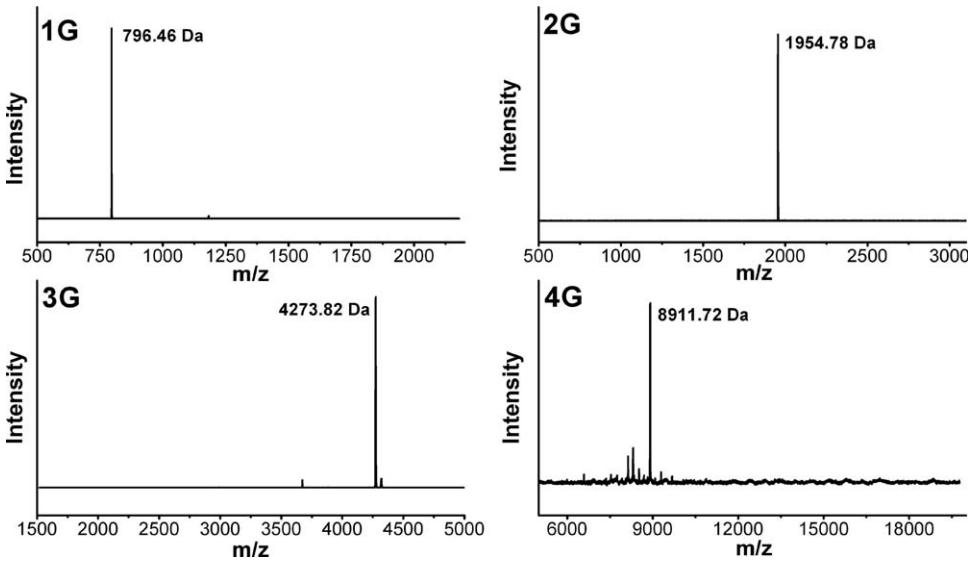


FIGURE 3 MALDI-TOF MS spectra of protected 1G–4G dendrimers. The calculated exact mass of 1G sodium adduct is 796.38 Da, and of 2G sodium adduct it is 1954.89 Da. The calculated molecular weight apex of 3G sodium adduct is 4273.99 Da, and of 4G sodium adduct it is 8911.19 Da.

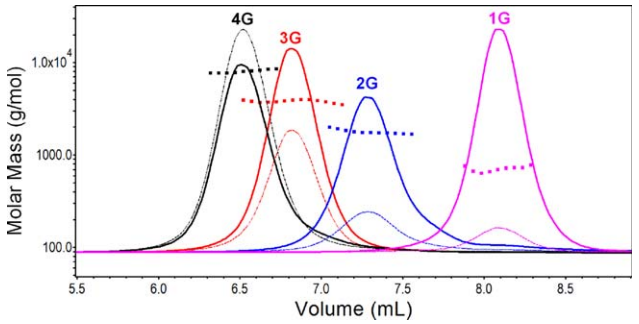


FIGURE 4 SEC-MALS chromatograms (solid curves = RI detector responses, dotted curves = LS detector responses at angle 90°) together with molar mass as a function of elution volume (spotted curves) for deprotected 1G–4G dendrimers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

sub therapeutic plasma drug levels. *In vitro* dissolution studies of solid dispersions of glimepiride and poly(ester-amide) dendrimers of different generations showed improved glimepiride solubility in aqueous media as compared with pure glimepiride in crystalline and amorphous form (Table 3). The glimepiride solubility increased with increasing dendrimer generation and reached the highest value when glimepiride had been incorporated in the solid dispersion with 4G poly(ester-amide) dendrimer (Table 3). After 60 min, this solid dispersion showed comparable glimepiride solubility as the glimepiride incorporated in solid dispersion with hyperbranched poly(ester-amide) polymer bearing hydroxyl functional groups (Table 3).[56]

In order to exactly evaluate the role of ester and amide bonds as well as hydroxyl functional groups in synthesized poly(ester-amide) dendrimers the *in vitro* dissolution studies were performed also for the solid dispersions of glimepiride and commercially available *bis*-MPA dendrimers (3G and 4G) containing ester bonds and hydroxyl functional groups as well as the solid dispersion of glimepiride and linear poly(vinyl alcohol) (PVA) bearing only hydroxyl groups in the structure (Table 3). Surprisingly, the 4G *bis*-MPA dendrimer was equally effective in improving the glimepiride solubility as the 4G poly(ester-amide) dendrimer or poly(ester-amide) hyperbranched polymer. These results reveal that carbonyl groups of the ester bonds of *bis*-MPA dendrimer are as effective in H-bond formation with slightly acidic proton of

TABLE 2 Molar Mass Characteristics (M_n, M_w, $Đ_M$) of 1G–4G Deprotected Dendrimers as Determined by SEC-MALS Measurements

Deprotected Dendrimers	M_{theor} (g/mol)	M_n (g/mol)	M_w (g/mol)	$Đ_M$
1G	653.7	710	770	1.08
2G	1692.7	1750	1780	1.02
3G	3770.7	3830	3860	1.01
4G	7926.7	7900	8060	1.02

TABLE 3 Solubility and Loading Capacity (LC) of Glimepiride in Synthesized Poly(ester-amide) Dendrimers Versus Poly(ester-amide) hyperbranched polymer,[56] *Bis*-MPA Dendrimers, and PVA

Sample	w/w (%)	c(HPLC) after 60 min (μg/mL)	c(theor.) (μg/mL)	LC (%)
Crystalline Glimepiride	/	0.2	/	/
Amorphous Glimepiride	/	0.5	/	/
Hybrane S1200	5	4.0	4.4	4.6
Dendrimer 2G	5	1.9	4.5	2.2
Dendrimer 3G	5	2.4	4.8	2.4
Dendrimer 4G	5	4.1	4.6	4.5
Bis-MPA dendrimer 3G	5	2.7	4.8	2.8
Bis-MPA dendrimer 4G	5	4.2	4.7	4.5
PVA	5	0.7	4.4	0.8

NH group of the glimepiride sulfonylurea segment as carbonyls of the ester and the amide groups in the structure of poly(ester-amide) dendritic polymers. On the contrary, the PVA proved to be ineffective. These results thus confirm the findings of our previous studies, which revealed that hydroxyl functional groups are not involved in complex formation between glimepiride and poly(ester-amide) hyperbranched polymer.[56,58]

From the results of *in vitro* dissolution measurements, we estimated the amount of glimepiride complexed with polymers in solid dispersions, that is, loading capacity (LC). LC increased with dendrimer generation in both dendrimer types and reached maximum values (4.5%, w/w) for the 4G dendrimers which is comparable to LC of poly(ester-amide) hyperbranched polymer (Table 3). On the other hand, the LC of PVA was significantly lower as expected (Table 3).

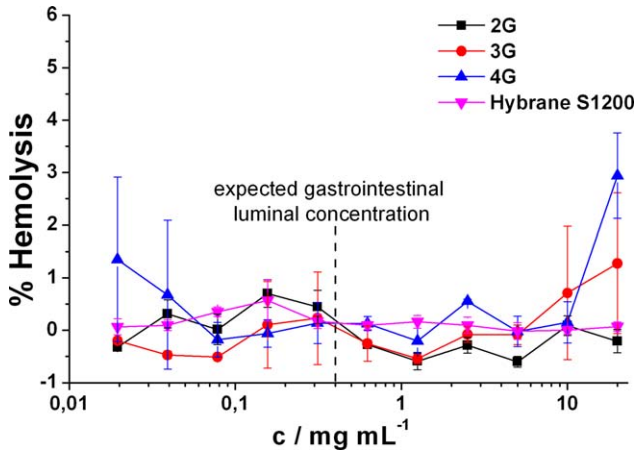


FIGURE 5 The degree of hemolysis for G2–G4 poly(ester-amide) dendrimers and Hybrane S1200 hyperbranched polymer at polymer concentrations between 0.02 and 20 mg/mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Glimepiride has molar mass of 491 g/mol and rather rigid structure and as such it is very unlikely to be encapsulated in the interior of 4G *bis*-MPA dendrimer containing small cavities. Since both dendrimer types reveal similar LC we infer that glimepiride is most probably not encapsulated neither into the interior of the 4G poly(ester-amide) dendrimer with larger cavities. Instead, glimepiride is more likely simply adsorbed on the dendrimers' surface, where it interacts with carbonyls of ester/amide bonds. Since the probability of ester/amide bonds to be exposed on the dendrimer's surface increases with dendrimer generation number as a consequence of greater probability for back-folding, the loading capacity of both dendrimer types increases in the same order.

Hemolytic Activity of Poly(ester-amide) Dendrimers

The red blood cell (RBC) lysis is a cytotoxicity assay that is based on hemoglobin release from RBC due to membrane rupture caused by investigated compounds. Although the mechanistic perspective of cell lysis is not investigated by such an approach, it gives a clear indication whether the investigated compounds possess a cytotoxic potential. The positive control solution of sodium lauryl sulphate (0.01%) caused a complete RBC lysis.

Hemolytic activity was tested at different concentration of poly(ester-amide) dendrimers in the range from 0.02 to 20 mg/mL. Since the use of synthesized 4G dendrimer is suggested for oral pharmaceutical products in the range of 100 mg/formulation/250 mL, the expected gastrointestinal luminal concentration is 0.4 mg/mL. At this concentration none of the poly(ester-amide) dendritic polymers exhibit hemolytic effect (Fig. 5). The 3G and the 4G poly(ester-amide) dendrimers caused marginal RBC lysis only at the highest tested concentration (20 mg/mL), which is 50-times higher from the expected gastrointestinal luminal concentration. Therefore, the occurrence of mediated cytotoxicity of these dendrimers is estimated to be very unlikely.

CONCLUSIONS

We synthesized poly(ester-amide) dendrimers from the AB_2 adduct of 2,2-*bis*(hydroxymethyl) propanoic acid (*bis*-MPA) and glycine using two different esterification procedures, either the DCC/DPTS or the EDC/DMAP coupling reagent/catalyst systems. The synthetic procedure using DCC/DPTS coupling was performed in DCM/acetonitrile at 50 °C, whereas the second one using EDC/DMAP was performed at room temperature in DCM/acetonitrile mixture for the 1G to 3G dendrimers and in DCM/DMF mixture for the 4G dendrimer. A comparison of both synthetic procedures reveals that EDC/DMAP coupling offers reduced reaction time and improved reaction yields, however, the amount of required catalyst is larger, especially for the synthesis of higher generation dendrimers. In EDC/DMAP esterification procedure, the simple aqueous workup method as the only purification procedure is enough to obtain the 1G and 2G dendrimers of good quality, however, for isolation of highly pure 3G and 4G dendrimers purification by column chromatography is necessary. The DCC/DPTS procedure requires purification by column chromatography for all generation dendrimers.

The synthesized poly(ester-amide) dendrimers as well as commercially available *bis*-MPA dendrimers, poly(ester-amide) hyperbranched polymer, and poly(vinyl alcohol) were used for preparation of solid dispersions of sulfonylurea antidiabetic drug glimepiride to improve its poor water-solubility. *In vitro* dissolution studies show in comparison with pure glimepiride in crystalline or amorphous form, significantly improved glimepiride solubility for all solid dispersions based on dendritic polymers, but not for poly(vinyl alcohol). These results indicate that the ester groups in dendrimer structure are equally effective as the amide groups in the formation of glimepiride/polymer complex, however, the hydroxyl groups do not play a role in complex formation. The amount of glimepiride complexed with both dendrimer types increases with dendrimer generation and reaches the maximum value for the solid dispersions of glimepiride with the fourth generation dendrimers, that is, 4.5%, w/w. From the red blood cell lysis test, the occurrence of mediated cytotoxicity of synthesized poly(ester-amide) dendrimers is estimated to be very unlikely.

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