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Water-Soluble Polyglycerol Dendrimers with Two Orthogonally Reactive Core Functional Groups for One-Pot Functionalization

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Abstract

The synthesis of a water-soluble polyglycerol dendrimer with two orthogonal functional groups at the core is reported. The two groups, an azide and amine group, are highly reactive towards alkyne and activated ester moieties, respectively. The orthogonality of the two chemical reactions is demonstrated by the ability to conjugate quantitatively either group, independent of the other and in either order. The orthogonal functionalization of the azide- and amine-cored dendrimer can be accomplished in a stepwise or a one-pot synthetic protocol. All resulting bifunctional dendrimers are fully soluble in water as the water-soluble dendritic scaffold decorated with 48 hydroxyl groups on the surface successfully solubilizes both the hydrophobic fluorophore and targeting group.

TOC Graphic

INTRODUCTION

Dendritic polyglycerols are polymeric structures that have found numerous biomedical applications in drug delivery, gene transfection, biomedical imaging, and diagnostics.^{1–6} Their wide-spread utility owes in large part to their excellent water-solubility, non-toxicity, and minimal nonspecific interactions in biological environments. Furthermore, and

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Supporting Information. ¹H NMR spectra of **2** and PGDs **4–11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

analogous to other dendritic polymers, the multiple peripheral hydroxyl groups serve as attaching points for further modification of the outer shell with a variety of functional groups such as amines, sulfates, and carboxylates.^{7–9} Convenient methods for preparing both polyglycerol dendrimers (PGDs) and hyperbranched polyglycerols on a large scale have been reported.^{10,11}

We and others^{12–17} have also demonstrated synthetic methods for incorporating a single functional group into the core of the dendritic polyglycerols. For example, Haag and co-workers have developed new synthetic pathways based on iterative allylation and dihydroxylation to obtain monofunctional PGDs that are useful as water-soluble building blocks.^{18,19} Similarly, we synthesized core-functionalized PGDs containing a single amine or azide, and utilized these PGDs to solubilize and protect hydrophobic fluorophores.^{20–22} The resulting dendritic fluorophores were fully water-soluble and exhibited enhanced properties in aqueous media. We also reported a one-step, large scale preparation of clickable hyperbranched polyglycerols functionalized with a single alkyne group and their application to gold nanoparticles and acid-labile nanocarriers.^{23,24}

Non-PGD dendrimers have been prepared with only one or two identically reactive groups on their periphery or within the branches,²⁵ but dendrimers with two orthogonally reactive functional groups at the core are largely unknown. We recently reported the preparation of a PGD scaffold with two differently protected amines at the core but the final conjugation to a BODIPY fluorophore required 7 days to reach completion.²² Moreover, stepwise deprotection and coupling steps were required for the introduction of each functionality, limiting the generality of the synthetic approach. Weck has described a trifunctional dendrimer core to which two different dendrons and a fluorophore were sequentially attached.²⁶ Building on this general approach, we present here a pentafunctional A₃BC core that can be used to assemble a new, universal PGD scaffold functionalized with two reactive groups that are completely orthogonal²⁷ to each other. This scaffold allows two different groups to be added to the core sequentially and in either order or for a one-pot, selective double conjugation.

EXPERIMENTAL SECTION

General Methods

All reagents were purchased from Advanced ChemTech, Alfa Aesar, or Sigma-Aldrich, and used without further purification unless otherwise noted. NMR spectra were recorded using a Varian Unity 400 or 500 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the corresponding residual proton in deuterated solvents. Mass spectral analyses were provided by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois, using ESI on a Waters Micromass Q-ToF spectrometer, or MALDI-TOF on an Applied Biosystems Voyager-DE STR spectrometer or a Bruker Daltonics UltrafleXtreme spectrometer. Dialysis was performed using dialysis tubing (Sigma-Aldrich MWCO 1200) against water at 25 °C. Compounds **1**,²⁸ **3**,¹⁴ **12**,³⁰ and **13**³¹ were synthesized according to previously published procedures.

Lysine Core (2)

1 (0.13 g, 0.54 mmol), Boc-Lys(Fmoc)-OH (0.30 g, 0.64 mmol), and HATU (0.27 g, 0.70 mmol) were dissolved in anhydrous DMF (3 mL) under a nitrogen atmosphere. DIPEA (0.23 mL) was added and the mixture was stirred at 25 °C for 15 h. After the solvent was removed under reduced pressure, water was added and the mixture was extracted with dichloromethane. The combined organic layers were washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using hexanes/ethyl acetate (5:3) as eluent to yield **2** (0.32 g, 87%). ¹H NMR (CDCl₃): δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 6.16 (s, 1H), 5.11 (br, 1H), 4.92 (br, 1H), 4.41 (d, *J* = 6.6 Hz, 2H), 4.21 (t, *J* = 6.6 Hz, 1H), 4.12 (s, 6H), 4.02 (br, 1H), 3.82 (m, 6H), 3.19 (m, 2H), 2.43 (s, 3H), 1.78 (m, 2H), 1.68–1.24 (m, 13H). ¹³C NMR (CDCl₃): δ 171.8, 156.5, 144.0, 141.3, 127.6, 127.0, 125.0, 119.9, 79.5, 74.7, 68.3, 66.5, 59.3, 58.6, 54.7, 47.3, 40.6, 32.4, 29.4, 28.3, 22.3. MS-ESI (*m/z*): [M+H]⁺ calcd for C₃₉H₄₈N₃O₈, 686.3441; found, 686.3450 [M+H]⁺.

PGD (4)

To a solution of compounds **2** (0.12 g, 0.18 mmol) and **3** (0.64 g, 0.73 mmol) in DMF (3 mL) were added CuSO₄·5H₂O (40 mg, 0.16 mmol) and sodium ascorbate (64 mg, 0.32 mmol). The mixture was stirred at 25 °C for 18 h and then filtered. Subsequently, the filtrate was concentrated under reduced pressure and precipitated into cold water. The precipitated crude product was purified by column chromatography on silica gel using dichloromethane/methanol (20:1) as eluent to afford **4** (0.59 g, 98%). ¹H NMR (CDCl₃): δ 7.78–7.23 (br, 11H), 6.76 (br, 1H), 5.96–5.70 (m, 25H), 5.42 (br, 1H), 5.32–5.04 (m, 48H), 4.63–3.30 (br, 169H), 3.14 (br, 2H), 1.84–1.22 (br, 15H). MALDI-TOF (*m/z*): [M]⁺ calcd for C₁₇₄H₂₇₂N₁₂O₅₀, 3329.9; found, 3352.6 [M+Na]⁺.

PGD (6)

To a solution of **4** (0.20 g, 60 μmol) in dichloromethane (10 mL) was added TFA (1.3 mL) at 0 °C. The mixture was stirred at 25 °C for 2 h and then evaporated under reduced pressure. The residue was redissolved in dichloromethane and *N*-methylmorpholine (0.2 mL) was added. The mixture was stirred at 25 °C for 10 min and evaporated under reduced pressure to obtain **5** which was used without further purification. To a solution of the crude **5** in DMF (3 mL) was added azidobutyric acid NHS ester (0.27 g, 1.2 mmol). After the mixture was stirred at 25 °C for 18 h, DMF was removed under reduced pressure, water was added and the mixture was extracted with dichloromethane. The combined organic layers were washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (30:1) as eluent to yield **6** (0.14 g, 70%). ¹H NMR (CDCl₃): δ 7.79–7.22 (br, 11H), 6.96 (br, 1H), 6.82 (br, 1H), 5.88 (m, 24H), 5.63 (br, 1H), 5.32–5.03 (m, 48H), 4.61–3.38 (br, 171H), 3.25 (br, 2H), 3.16 (br, 2H), 2.26 (br, 2H), 1.92–1.28 (br, 6H). MALDI-TOF (*m/z*): [M]⁺ calcd for C₁₇₃H₂₆₉N₁₅O₄₉, 3340.9; found, 3340.7 [M]⁺.

PGD (7)

To a solution of **6** (0.12 g, 36 μ mol) in acetone/water/*tert*-butanol (5:5:1) were added $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$ (catalytic amount), *N*-methylnmorpholine-*N*-oxide (0.40 g, 3.4 mmol), and citric acid (0.18 g, 0.86 mmol). The mixture was stirred at 25 °C for 15 h and excess Smopex-105 (an osmium scavenger) was added to the stirred solution. The mixture was stirred at 25 °C for 24 h, and filtered to remove the osmium scavenger. The filtrate was evaporated under reduced pressure to afford the crude **7** that was further purified by dialysis against water at 25 °C for 2 d (0.12 g, 82%). ^1H NMR (CD_3OD): δ 8.10–6.98 (br, 14H), 4.69–1.21 (br, 255H). MALDI-TOF (m/z): $[\text{M}]^+$ calcd for $\text{C}_{173}\text{H}_{317}\text{N}_{15}\text{O}_{97}$, 4157.0; found, 4156.8 $[\text{M}]^+$.

PGD (8)

To a solution of **7** (86 mg, 21 μ mol) in DMF (3 mL) was added piperidine (0.6 mL). After the mixture was stirred at 25 °C for 1 h, the solvent was removed under reduced pressure and the residue was dissolved in water and filtered off. The filtrate was dialyzed against water at 25 °C for 1 d to obtain **8** (58 mg, 71%). ^1H NMR (CD_3OD): δ 8.10–7.00 (br, 5H), 4.73–1.05 (br, 252H). MALDI-TOF (m/z): $[\text{M}]^+$ calcd for $\text{C}_{158}\text{H}_{307}\text{N}_{15}\text{O}_{95}$, 3935.0; found, 3934.6 $[\text{M}]^+$.

General Procedure for Amidation

The desired amount of **8** or **10** and **12** (25 equiv.) were dissolved in DMF (0.2 mL). The mixture was stirred at 25 °C for 18 h, concentrated under reduced pressure, and then precipitated into dichloromethane. The precipitate was dissolved in water and dialyzed against water at 25 °C for 1 d to afford **9** (87%) or **11** (94%).

General Procedure for 1,3-Dipolar Cycloaddition

To a solution of the desired amount of **8** or **9** and **13** (25 equiv.) in DMF (0.2 mL) were added $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2 equiv.) and sodium ascorbate (4 equiv.) in water (40 μ L). The mixture was stirred at 25 °C for 15 h and then filtered. Subsequently, the filtrate was concentrated under reduced pressure and precipitated into dichloromethane. The precipitate was dissolved in water and dialyzed against water at 25 °C for 1 d to obtain **10** (91%) or **11** (82%).

One-Pot Amidation and 1,3-Dipolar Cycloaddition

To a solution of **8** (3.0 mg, 0.76 μ mol), **12** (7.3 mg, 19 μ mol), and **13** (5.4 mg, 19 μ mol) in DMF (0.2 mL) were added $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.40 mg, 1.6 μ mol) and sodium ascorbate (0.60 mg, 3.0 μ mol) in water (40 μ L). The mixture was stirred at 25 °C for 18 h and filtered. The filtrate was concentrated under reduced pressure and precipitated into dichloromethane. The precipitate was dissolved in water and dialyzed against water at 25 °C for 1 d to afford **11** (3.0 mg, 87%).

PGD (9)

^1H NMR (CD_3OD): δ 8.40–7.20 (br, 15H), 4.68–1.23 (br, 258H). MALDI-TOF (m/z): $[\text{M}]^+$ calcd for $\text{C}_{178}\text{H}_{321}\text{N}_{15}\text{O}_{96}$, 4205.1; found, 4204.9 $[\text{M}]^+$.

PGD (10)

^1H NMR (CD_3OD): δ 8.20–7.20 (br, 7H), 4.76–1.18 (br, 267H). MALDI-TOF (m/z): $[\text{M}]^+$ calcd for $\text{C}_{171}\text{H}_{326}\text{N}_{18}\text{O}_{97}\text{S}$, 4216.1; found, 4239.1 $[\text{M}+\text{Na}]^+$.

PGD (11)

^1H NMR (CD_3OD): δ 8.40–7.20 (br, 17H), 4.78–1.20 (br, 273H). MALDI-TOF (m/z): $[\text{M}]^+$ calcd for $\text{C}_{191}\text{H}_{340}\text{N}_{18}\text{O}_{98}\text{S}$, 4486.2; found, 4510.1 $[\text{M}+\text{Na}]^+$.

RESULTS AND DISCUSSION

The key structural feature of the bifunctional PGD developed in this study is a lysine-based trifunctional core that contains two functional handles, an azide and amine group, and a third arm linked to three PG dendrons giving 48 hydroxyl peripheral groups. As outlined in Figure 1, the design is analogous to our previously reported system,²² but with the protected α -amino group of the lysine core replaced by an azide moiety introduced via an alkyl linker. Both the linker and high yielding click reaction were expected to successfully avoid the limitations mentioned above, thereby allowing any fluorophore to be linked to the PGD core together with a targeting moiety in a one-pot procedure.

The synthetic pathway is described in Scheme 1. The synthesis of the lysine-cored PGD began with HATU-mediated amide coupling of trialkyne-containing amine **1**²⁸ and *tert*-butoxycarbonyl (Boc)- and 9-fluorenylmethyloxycarbonyl (Fmoc)-protected lysine (Boc-Lys(Fmoc)-OH). This coupling affords the key pentafunctional A_3BC lysine core **2**, wherein a triple click reaction can rapidly assemble a macromolecular architecture (vide infra). The Boc-Lys(Fmoc)-OH starting material in Scheme 1 replaced the previously used Fmoc-Lys(Boc)-OH because the azide group introduced later at the α -position of the lysine core would be unstable under the Boc deprotection condition that required trifluoroacetic acid (TFA) to produce a free ε -amino group. The trialkyne product **2**, obtained in 87% yield, was reacted with azide-cored PG dendron **3**¹⁴ using copper-catalyzed 1,3-dipolar cycloaddition.²⁹ The resulting PGD **4** containing 24 allyl ether end-groups was readily purified by column chromatography, and the purity was determined by ^1H NMR spectroscopy and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Complete attachment of three PG dendrons was confirmed by a single main peak at 3352.6 m/z in the MALDI-TOF mass spectrum of **4** (Figure 2).

As shown in Scheme 1, PGD **4** was further modified to incorporate both azide and amine groups into the core as well as to confer aqueous solubility on the bifunctional PGD. First, the Boc group in **4** was selectively deprotected with TFA, and the resulting α -amino group of the lysine core in **5** was reacted with an excess of azide-containing *N*-hydroxysuccinimide (NHS) ester to afford **6** in 70% yield. The polyallylated PGD **6** was then dihydroxylated using 2 mol% of $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$ per alkene to obtain water-soluble PGD **7** with 48 hydroxyl end-groups. Subsequently, the Fmoc group in **7** was removed using piperidine to generate a free amine, thus affording water-soluble PGD **8** with the two orthogonally reactive functional groups. The synthesis and purification of PGDs **5**–**8** were straightforward. The polyallylated PGDs **5** and **6** could be easily purified by column chromatography, whereas the

dihydroxylated PGDs **7** and **8** were purified by dialysis against water. All of the synthesized PGDs **5–8** were characterized by a combination of ^1H NMR and MALDI-TOF mass spectra. For example, the ^1H NMR spectra revealed complete deprotection of Boc and Fmoc groups in **4** and **7**, respectively. Quantitative dihydroxylation of **6** to afford water-soluble **7** could also be verified by the complete disappearance of resonances corresponding to alkene protons at δ 5.9 and δ 5.3–5.0 ppm in the ^1H NMR spectrum of **7** (see the Supporting Information). In particular, comparisons of the MALDI mass spectra, which are the most useful means to monitor chemical reactions on dendrimers, confirmed quantitative conversions in each synthetic step, as shown by the complete shifts of molecular ion peaks from 3270.8 m/z for **5** to 3340.7 m/z for **6** to 4156.8 m/z for **7** to 3934.6 m/z for **8**. (Figure 2).

With water-soluble PGD **8** containing two reactive groups azide and amine, we investigated orthogonal functionalization strategies to produce monovalent dendritic fluorophores by employing pyrene and biotin as representative models of the fluorophore and targeting group, respectively. As outlined in Scheme 1, we first explored the synthesis of pyrene- and biotin-functionalized PGD **11** via stepwise strategies. We followed two distinct routes: one beginning with amide coupling between **8** and pyrene NHS ester **12**³⁰ followed by copper-catalyzed 1,3-dipolar cycloaddition using biotin alkyne **13**,³¹ and the other following the reverse order. Both of these routes could be followed by monitoring the ^1H NMR and MALDI mass spectra, which confirmed quantitative conversions in all cases (Figure 2 and Supporting Information).

In addition, we investigated our main goal, the one-pot preparation of the monovalent dendritic fluorophore **11** from the bifunctional **8** by simply mixing all reagents together required for the two transformations and reacting them at room temperature overnight. The synthesis of the final product **11** through the one-pot process was substantiated by the ^1H NMR and MALDI mass spectra of the product, which are identical to those obtained via the two stepwise routes, suggesting that the final products are identical. These results also indicate that there is no interference between the two chemical transformations, NHS ester-based amide coupling and 1,3-dipolar cycloaddition. Thus, the azide and amine groups are ideal functional handles to orthogonally incorporate any desired functionality into the polyglycerol dendrimers. Moreover, the final conjugate **11** is fully water-soluble despite the attachment of the water-insoluble pyrene and biotin moieties. The aqueous solubility allows for the easy and straightforward purification by dialysis against water. Overall, these novel orthogonal functionalization strategies successfully overcome the limitations described earlier and demonstrate the synthetic potential of our universal PGD backbone for conjugation to any molecule of interest.

CONCLUSION

We have developed a one-pot methodology for the preparation of monovalent, water-soluble dendritic fluorophores by employing two orthogonal chemical reactions, the amide coupling of amine with NHS ester groups and the copper-catalyzed 1,3-dipolar cycloaddition between azide and alkyne functionality. For this approach, a versatile pentafunctional A_3BC intermediate (**2**) was used to prepare a water-soluble PGD containing amine and azide

groups. Although the intermediate **2** was used to rapidly assemble the bifunctional dendritic architecture of interest here, we envision it as a useful building block for a wide-range of other macromolecular structures, including functional, three-arm star polymers.

The preparation of the PGDs described herein was straightforward as was their purification. All of the prepared PGDs could be readily characterized by ^1H NMR spectroscopy and MALDI-TOF mass spectrometry. Although NMR provided a rough indication that the dendrimers were pure (>95%), the MALDI-TOF is particularly good at showing the extent of functional group interconversions and in each case these appeared complete. To show utility of the orthogonally reactive PGD **8** we explored its orthogonal functionalization to produce monovalent dendritic fluorophores by both stepwise and one-pot synthetic strategies. All the investigated transformations proceed quantitatively in an orthogonal fashion under mild reaction conditions without any interference between the two chemical reactions. Furthermore, the polyglycerol dendrons allowed the targeted product to be fully water-soluble despite substitution with highly hydrophobic moieties. The new approach developed here provides an efficient synthetic pathway to fabricate highly functionalized water-soluble, dendritic scaffolds that may have potentially use in a range of biological and biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic representation of the synthetic strategies towards monovalent and water-soluble polyglycerol-dendronized fluorophores, as described previously (A) and developed in this study (B).

Figure 2.
MALDI-TOF mass spectra of **4–11**.

Scheme 1.
Synthesis of biotinylated PGD fluorophore **11**.