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Efficient synthesis of diverse heterobifunctionalized clickable oligo(ethylene glycol) linkers: potential applications in bioconjugation and targeted drug delivery†

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Herein we describe the sequential synthesis of a variety of azide-alkyne click chemistry-compatible heterobifunctional oligo(ethylene glycol) (OEG) linkers for bioconjugation chemistry applications. Synthesis of these bioorthogonal linkers was accomplished through desymmetrization of OEGs by conversion of one of the hydroxyl groups to either an alkyne or azido functionality. The remaining distal hydroxyl group on the OEGs was activated by either a 4-nitrophenyl carbonate or a mesylate (–OMs) group. The –OMs functional group served as a useful precursor to form a variety of heterobifunctionalized OEG linkers containing different highly reactive end groups, *e.g.*, iodo, $–NH_2$, –SH and maleimido, that were orthogonal to the alkyne or azido functional group. Also, the alkyne- and azide-terminated OEGs are useful for generating larger discrete poly(ethylene glycol) (PEG) linkers (*e.g.*, PEG_{16} and PEG_{24}) by employing a Cu(I)-catalyzed 1,3-dipolar cycloaddition click reaction. The utility of these clickable heterobifunctional OEGs in bioconjugation chemistry was demonstrated by attachment of the integrin ($\alpha_v\beta_3$) receptor targeting peptide, cyclo-(Arg-Gly-Asp-D-Phe-Lys) (cRGfKD) and to the fluorescent probe sulfo-rhodamine B. The synthetic methodology presented herein is suitable for the large scale production of several novel heterobifunctionalized OEGs from readily available and inexpensive starting materials.

Introduction

PEGylation of drug molecules plays an important role in the delivery of certain therapeutic agents. PEG-drug conjugates exhibit more favorable *in vivo* behavior than the unconjugated forms. They are less susceptible to degradation by metabolic enzymes, and they exhibit prolonged circulation times in the blood stream. PEGylation can also reduce or even eliminate antigenicity and immunogenicity.[1–3]

Short-chain PEGs or oligo(ethylene glycol)s (OEGs) have found widespread use as spacers or linkers for targeted drug delivery systems because they are inexpensive, water soluble, biostable, and available in a wide range of molecular weight distributions.[4,5] A targeted drug delivery system requires two distinct reactive termini on the linker, one for attaching the therapeutic payload, and the other for attaching a targeting ligand (*e.g.*, peptides, proteins, or antibodies).[6] Most commercially available OEGs are monofunctional, containing only a single reactive hydroxyl, amine, thiol, aldehyde or carboxylic acid terminal group or activated variants of these. However, few heterobifunctional OEGs are commercially available containing two different reactive groups at the distal ends. Therefore, there is a need to develop efficient and diverse methods to synthesize heterobifunctionalized OEGs with highly reactive end groups (Fig. 1). These termini must additionally be reactive under mild, aqueous reaction conditions to permit conjugation of delicate peptide and antibody targeting moieties.

Although the synthesis of heterobifunctional PEGs with azide or alkyne groups at their termini has been accomplished using a ring-opening polymerization of ethylene oxide, this method employed polydisperse PEGs.[7,8] Although polydisperse PEGs are suitable for macromolecular drug delivery systems based on polymers, micelles, liposomes, or nanoparticles, they have limited use in the synthesis of small, discrete, multimodal theranostic agents. In our ongoing research on the utility of *closo*-boranes as multifunctional targeted drug delivery scaffolds,[9,10] we needed a diverse collection of monodisperse

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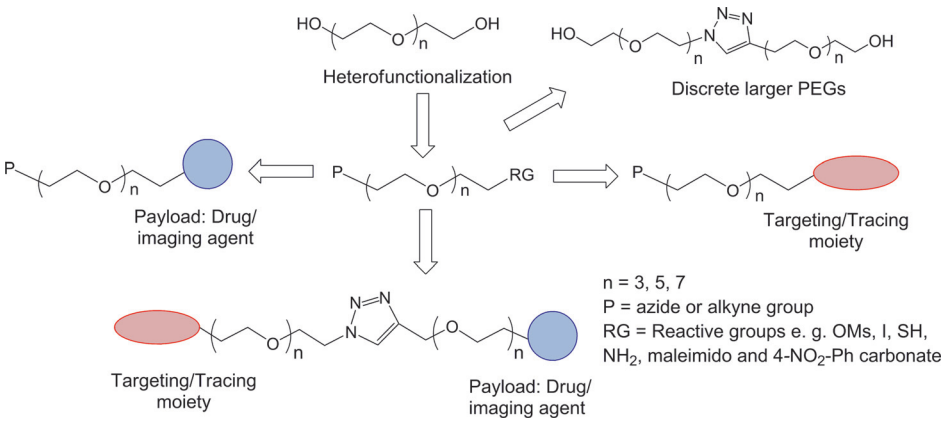
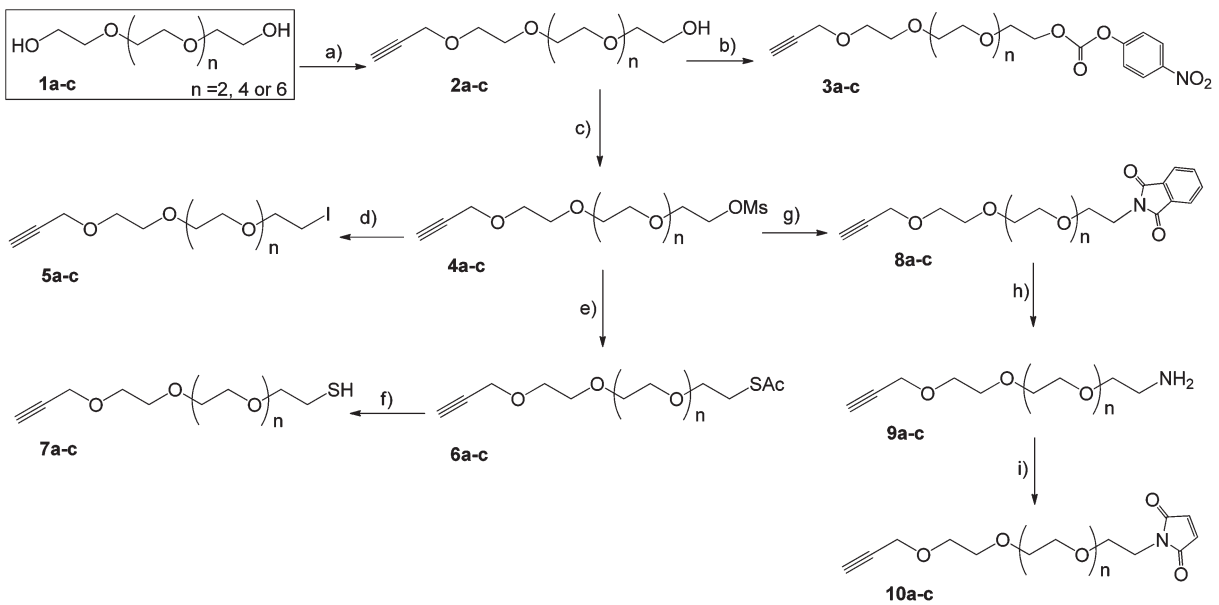


Fig. 1 Schematic illustration of the synthesis of clickable heterbifunctionalized OEGs and their potential applications in targetable/traceable delivery of drugs or imaging agents.



Scheme 1 Synthesis of alkyne terminated heterobifunctionalized OEG linkers. *Reagents and conditions*: (a) Propargyl bromide, NaH, THF, 0 °C–RT–60 °C, 15 h; (b) 4-nitrophenyl chloroformate, pyridine, MeCN, RT, 15 h; (c) MsCl, Et_3N, DCM, 0 °C–RT, 3.5 h; (d) NaI, acetone, 65 °C, 15 h; (e) KSAc, DMF, RT, 15 h; (f) LAH, THF, −10 °C–RT, 3 h; (g) Potassium phthalimide, DMF, 110 °C, 15 h; (h) Hydrazine, EtOH, 60 °C, 3 h; (i) *N*-methoxycarbonyl maleimide, Sat. aq $NaHCO_3$, 0 °C–RT, 2 h.

heterobifunctional OEG linkers having the ability to undergo click reactions. Therefore, the objective of this study was to find simple and efficient synthetic routes to generate a diverse library of heterobifunctional OEG linkers with one terminal alkyne or azide functionality, which would render them suitable for biocompatible azide-alkyne [3 + 2] dipolar cycloaddition reactions with bioactive ligands, leaving the remaining reactive terminus available for subsequent conjugation to payloads such as drugs and imaging probes (Scheme 1).[11–14]

Results and discussion

A crucial requirement while working with homobifunctional OEGs is the ability to differentiate the reactivity of two chemically equivalent terminal diols. Many previous studies addressed the desymmetrization of homofunctional OEGs using the Williamson ether synthesis,[15,16] in which the benzyl and *p*-methoxybenzyl ether groups were most commonly used for the monoprotection of symmetrical OEGs. However, reports on the synthesis of alkyne-terminated heterobifunctionalized OEGs are rare.[17] In one such report, Gill *et al.*[18] synthesized propargyl-PEG_6–OH in 54% yield from hexa(ethylene glycol) (OH–PEG_6–OH) using equimolar amounts of sodium hydride (NaH) and propargyl bromide. In our case, tetra(ethylene glycol) (OH–PEG_4–OH, **1a**), hexa(ethylene glycol) (OH–PEG_6–OH, **1b**) and octa(ethylene glycol) (OH–PEG_8–OH, **1c**) were used to synthesize **2a–c** in moderate yields (37–69%) using a similar procedure. In contrast to traditional monoalkylations, propargyl bromide was added to **1b** and **1c** at room

temperature to prevent the solidification of the starting materials at 0 °C in THF. Minor amounts (~5%) of dialkylated OEGs were also formed, but they were easily separated by silica gel column chromatography. Unreacted starting OEGs (**1a–c**) were recovered from the aqueous layer during the workup procedures. The ^{1}H NMR spectra of OEGs **2a–c** exhibited a very characteristic triplet for one proton at ~δ 2.4 ppm assigned to the propargyl group.

To construct useful heterobifunctional OEG linkers, the remaining hydroxyl group on **2a–c** was converted into several highly reactive, ready-to-use functional groups (Scheme 1 and Table 1). First, the hydroxyl group was converted into an amine-reactive 4-nitrophenyl carbonate (PNPC) group using 4-nitrophenyl chloroformate under basic conditions to produce **3a–c**. Yields were greater than 71%, and the ^{1}H NMR spectra of **3a–c** exhibited distinct doublets for the four protons assigned to the 4-nitrophenyl moiety. PNPC-functionalized PEG linkers are useful for biomedical applications because they can readily react with the amine functionality of amino acids present in bioactive peptides or antibodies to form carbamate bonds under very mild reaction conditions.

To the best of our knowledge, no alkyne-terminated OEG derivatives of **1a–c** with aryl or alkyl carbonates at their distal ends have been reported previously in the literature.

The remaining hydroxyl group on **2a–c** was converted to a mesylate (–OMs) group using 1.5 eq. of methanesulfonyl chloride and 2.0 eq. of Et_3N in DCM, giving mesylates **4a–c** in quantitative yields. The structures of **4a–c** were analyzed using ^{1}H, ^{13}C NMR and HRMS. The ^{1}H NMR spectra of the products showed a characteristic triplet for one proton at ~δ 2.4 ppm, which was assigned to the propargyl group, and a sharp singlet at ~δ 3.0 ppm, which was assigned to the three –OMs protons.

This is the first report of the synthesis of **4b–c**. Although the synthesis of **4a** has been previously published,[19] the method reported here requires fewer steps and gives a 1.5-fold higher yield. It should be noted that **4a–c** required no further purification for their use in the subsequent reactions described below.

Table 1 Yields of alkyne-terminated heterobifunctionalized OEG linkers **2–10**

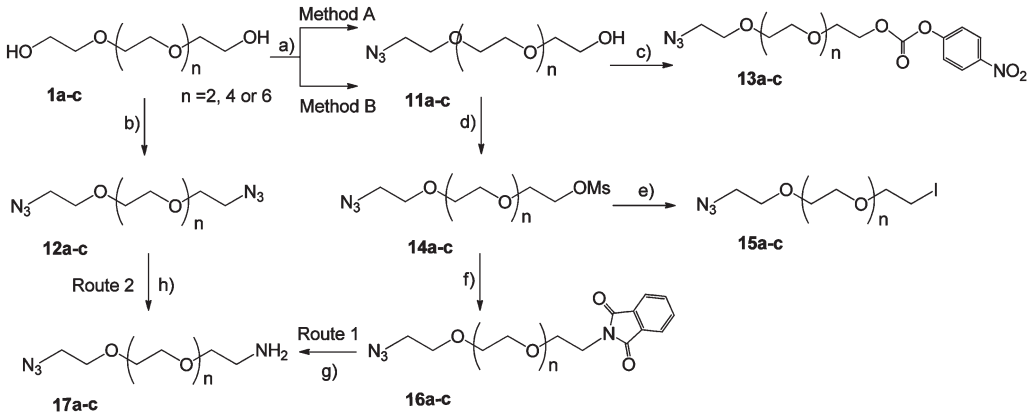
Compound	*n*	Yield (%)	Compound	*n*	Yield (%)
2a	2	58	**6c**	6	65
2b	4	37	**7a**	2	92
2c	6	69	**7b**	4	83
3a	2	75	**7c**	6	98
3b	4	71	**8a**	2	99
3c	6	87	**8b**	4	92
4a	2	100	**8c**	6	97
4b	4	100	**9a**	2	99
4c	6	99	**9b**	4	99
5a	2	74	**9c**	6	99
5b	4	78	**10a**	2	49
5c	6	98	**10b**	4	63
6a	2	99	**10c**	6	42
6b	4	97			

Because the mesylate group is an effective leaving group in nucleophilic substitution reactions, the alkyne–OEG–OMs compounds **4a–c** served as starting materials for the synthesis of several heterobifunctional OEG linkers commonly used in bioconjugation reactions. For example, the reaction of NaI with **4a–c** in refluxing acetone produced **5a–c**, which have alkyne and iodo-groups at their distal ends. The iodo-terminated OEG linkers **5a–c** were produced in quantitative yields and were readily purified by a simple silica gel filtration column. ^{1}H NMR, ^{13}C NMR, and HRMS were used to verify their structures. Absence of the characteristic sharp –OMs singlet at ~δ 3.0 ppm from the ^{1}H NMR spectra of **5a–c** confirmed conversion of the mesylate groups. This is the first published report of the synthesis of **5b–c**. Synthesis of **5a** has been previously reported; however, with a lower yield (52%) than our method (74%).[20]

Mesylates **4a–c** also were reacted with potassium thioacetate (KSAc) in dimethylformamide (DMF) at room temperature to afford nearly quantitative yields of previously unpublished thioacetates **6a–c**. These compounds were reduced to the novel set of OEG thiols **7a–c** following a reduction procedure reported by Stefanko *et al.*[21] Thioacetates **6a–c** and thiols **7a–c** were characterized using ^{1}H NMR, ^{13}C NMR, and HRMS. ^{1}H-NMR spectral analysis confirmed the conversion of –OMs to a thioacetate group by the appearance of a singlet at ~δ 2.3 ppm attributed to the three –SCOCH_3 protons along with the absence of the characteristic –OMs peak at ~δ 3.0 ppm. ^{1}H-NMR analysis of **7a–c** showed loss of the singlet for the three –SCOCH_3 protons at ~δ 2.3 ppm and the appearance of a triplet at ~δ 1.5 ppm assigned to the –SH functional group. Thiol-terminated heterobifunctional OEG linkers have frequently been used to form disulfide linkages with peptides, antibodies, and other bioactive molecules.[22,23] Moreover, the alkyne–OEG–SH linkers **7a–c** are excellent substrates for dual click reactions in which a Cu(I)-catalyzed alkyne-azide cycloaddition[4] is followed by a thiol-maleimide click reaction.[24]

The utility of mesylate intermediates **4a–c** was further demonstrated by the synthesis of alkyne–OEG–NH_2 compounds **9a–c** *via* the Gabriel reaction. The mesylate groups on **4a–c** were first transformed into phthalimides **8a–c** in nearly quantitative yields, and were then deprotected using hydrazine in ethanol to produce **9a–c** in excellent yields (≥95% over two steps). The structures of **8a–c** and **9a–c** were confirmed using ^{1}H, ^{13}C NMR and HRMS.

To the best of our knowledge, no OEG linkers with dual alkyne- and amino-functionalities at their distal ends have been produced from **1a–c** *via* Gabriel synthesis. Other investigators have synthesized alkyne-OEG-amines using the Staudinger reduction of an azide, but their yields for **9a–b** were 3- and 4-fold lower than what we were able to obtain.[25–27] Purification of **9a–b** on a multi-gram scale is difficult when using Staudinger reduction, and thus Gabriel synthesis has proven to be a much more efficient means of obtaining these molecules in high purity. Yields for **9c** *via* Staudinger reduction are potentially higher; however, no synthetic route has been reported starting from **1c** for accurate comparison.



Scheme 2 Sequential synthesis of azide terminated heterobifunctionalized OEG linkers. *Reagents and conditions*: (a) Method A: (i) MsCl, Et_3N, DCM, 0 °C–RT, 3 h; (ii) NaN_3, DMF, 65 °C, 15 h; Method B: (i) MsCl, Ag_2O, DCM, 0 °C–RT, 24 h; (ii) NaN_3, DMF, 65 °C, 15 h; (b) (i) MsCl, Et_3N, DCM, 0 °C–RT, 4 h; (ii) NaN_3, DMF, 65 °C, 15 h; (c) 4-nitrophenyl chloroformate, pyridine, MeCN, RT, 15 h; (d) MsCl, Et_3N, DCM, 0 °C–RT, 3.5 h; (e) NaI, acetone, 65 °C, 15 h; (f) potassium phthalimide, DMF, 110 °C, 15 h; (g) Hydrazine, EtOH, 60 °C, 3 h; (h) Ph_3P, Et_2O-5% aq HCl, RT, 24 h.

Finally, another set of dual click-compatible OEG linkers, alkyne–OEG–maleimides **10a–c**, were prepared in good yield by reacting **9a–c** with *N*-methoxycarbonyl maleimide using a recently reported procedure.[28] The ^{1}H NMR spectra of OEG linkers **10a–c** exhibited a very characteristic sharp singlet at ~δ 6.6 ppm, which was assigned to the two equivalent protons of the maleimido moiety.

Azido-terminated heterobifunctionalized OEG linkers were also prepared (Scheme 2 and Table 2) by using a synthetic strategy that paralleled the one used to produce the alkyne-terminated heterobifunctionalized OEG linkers (Scheme 1). First, the desymmetrization of OEGs **1a–c** was achieved by formation of the monoazide products **11a–c** in moderate yields (Method A, 36–46%). During this transformation, a small amount of diazido OEGs **12a–c** was also formed, but they were easily separated by silica gel chromatography. The mono-azido products **11a–c** were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The ^{1}H NMR spectra showed a characteristic triplet at ~δ 3.1 ppm for the $-CH_2-$ bound to the azide functional group.‡

In order to determine the best reaction conditions for the formation of **11a–c**, an alternative Method B was employed in which silver oxide (Ag_2O) was used during the monomesylation step.[16,29,30] Use of Ag_2O resulted in significantly higher yields of **11a–c** (51–69%) compared to Method A.

Functionalization of the remaining hydroxyl group on **11a–c** was achieved through a series of reactions similar to those used for the alkyne-terminated OEGs **2a–c**. The carbonates **13a–c** were prepared in high yield (87–95%). ^{1}H NMR verified the formation of the carbonates by the presence of aromatic doublets at ~δ 8.30 and 7.40 ppm. All three compounds **13a–c** are novel and have not been previously reported in the literature, nor have any aryl or alkyl carbonates of OEGs with 4, 6, or 8 ethylene glycol units.

Table 2 Yields of various azido-heterobifunctionalized OEG linkers

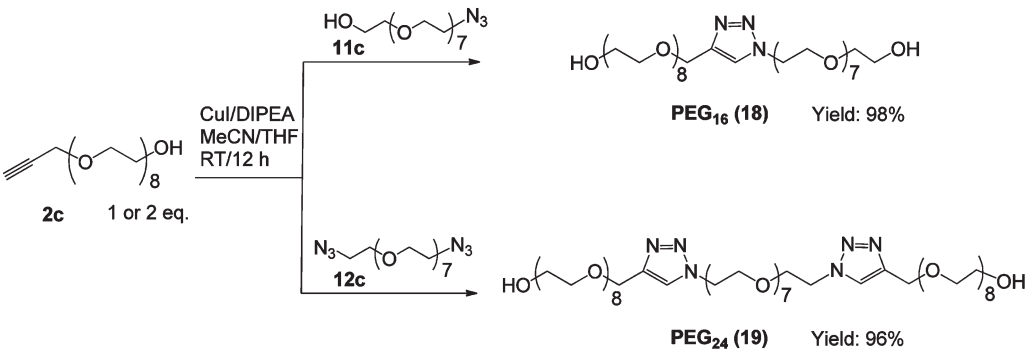
Compound	*n*	Yield (%)	Compound	*n*	Yield (%)
11a	2	36(69)[a]	**14c**	6	99
11b	4	37(54)[a]	**15a**	2	96
11c	6	46(51)[a]	**15b**	4	61
12a	2	100	**15c**	6	94
12b	4	100	**16a**	2	96
12c	6	100	**16b**	4	99
13a	2	94	**16c**	6	98
13b	4	87	**17a**	2	92(88)[b]
13c	6	95	**17b**	4	93(86)[b]
14a	2	99	**17c**	6	91(83)[b]
14b	4	99			

[a] Yields in parentheses are obtained using Method B. [b] Yields in parentheses are obtained using Route 2.

Nucleophilic substitution of the mesylate groups on the azide-substituted OEGs occurred just as readily as it did for the mesylate groups of the alkyne-substituted OEGs. Compounds **14a–c** were readily converted to iodo-substituted products **15a–c**; likewise, the mesylate group could also readily be replaced by phthalimide to give **16a–c** in high yield (96–99%). ^{1}H NMR analysis confirmed the formation of the phthalimide derivatives **16a–c** by the presence of characteristic aromatic peaks at ~δ 7.80 and 7.70 ppm. Reacting **16a–c** with hydrazine produced the amine derivatives **17a–c** in appreciable yields (91–93%). Although preparation of **17a** *via* the Gabriel synthesis has been reported by others,[31] the synthesis of **17b–c** from **1b–c** has not.

The di-azide OEGs **12a–c** were obtained as minor products (15–20%) during the synthesis of **11a–c**. However, increasing the amount of MsCl to 3.0 eq. in the reaction with **1a–c** gave **12a–c** in 100% yield. We used the di-azide products to investigate whether **17a–c** could be produced by selective reduction of one of the azido groups to an amine *via* Staundinger

‡Synthetic procedures and characterization details for the heterofunctionalized OEG linkers obtained from tetraethylene glycol and hexaethyelene glycols are included in the ESI.†



Scheme 3 Synthesis of discrete PEGs *via* alkyne-azide click reactions.

reduction in a biphasic reaction medium (Et_2O-5% aq HCl).[32] Interestingly, this attempt produced **17a–c** from **1a–c** in a more facile manner than the five-step method using the Gabriel synthesis described above. The overall yield of **17a–c** from **1a–c** was also considerably higher, with yields ranging from 83–88% when using the **12a–c** route compared to 30–37% when using the **11a–c** route. Formation of **17a–c** introduces heterofunctionality that allows the OEG linkers to be readily used in bioconjugation reactions. Moreover, the multiple pathways for producing **17a–c** demonstrate the versatility of these synthetic schemes for addition of both alkyne and azide functionalities on discretely sized OEGs.

The OEGs produced by these various methods make them useful for azide-alkyne click reactions. An example of this utility is demonstrated in high-yield production of discrete long-chain PEGs. Although expensive, PEGs having 6 or 8 ethylene glycol units are commercially available in 85–95% purity. However, long-chain PEGs (*e.g.*, with 16 or 24 ethylene glycol units), are extremely difficult to acquire and are usually of low purity. We overcame this barrier by developing a very simple and efficient methodology to synthesize discrete PEG_{16} (**18**) and PEG_{24} derivatives (**19**), in high yield by employing a Cu(I)-catalyzed azide-alkyne click reaction (Scheme 3). The PEG_{16} derivative **18** was synthesized in 98% yield by reacting 1.0 eq. of alkyne-terminated OEG **2c** with 1.0 eq. azide-functionalized OEG **11c**. The PEG_{24} derivative **19** was synthesized by reacting 2.0 eq. of alkyne-terminated OEG **2c** with 1.0 eq of diazide **12c** in 96% yield. PEG products **18** and **19** were characterized using ^1H, ^{13}C NMR and HRMS. The ^1H NMR spectra of both compounds showed a characteristic singlet at $\sim\delta$ 7.6 ppm that was assigned to the –CH– of the triazole ring formed *via* the azide-alkyne [3 + 2] dipolar cycloaddition reaction.

The utility of the clickable OEG linkers in bioconjugation reactions was demonstrated by their successful attachment to the peptide cyclo-(Arg-Gly-Asp-D-Phe-Lys) (cRGDfK, **20**) and the fluorophore sulfo-rhodamine B (**21**) (Scheme 4). The peptide cRGDfK is able to selectively target integrin receptors ($\alpha_v\beta_3$) that are commonly present in the neovasculature of a variety of tumor types and has been used successfully in tumor-targeting strategies.[33,34] Fluorescent dyes such as sulfo-rhodamine B are of increasing interest in biomedical research because of their ability to be traced *in vivo*.[35]

Conjugation of the peptide cRGDfK to **3c** *via* a carbamate linkage was achieved by reaction of the amino group on the lysine moiety of cRGDfK with the 4-nitrophenyl carbonate moiety of **3c** in the presence of Et_3N in DMF. A high yield (86%) of the OEG-cRGDfK conjugate **20** was obtained after size-exclusion chromatography on a lipophilic Sephadex LH-20 column. The alkyne-terminated OEG-fluorophore conjugate **21** was synthesized by reacting sulfo-rhodamine B acid chloride with **9c** in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in THF. Sulfo-rhodamine B conjugate **21** was obtained in good yield (71%) following alumina column chromatography. The structures of both conjugates were verified by ^1H NMR and mass spectrometry.

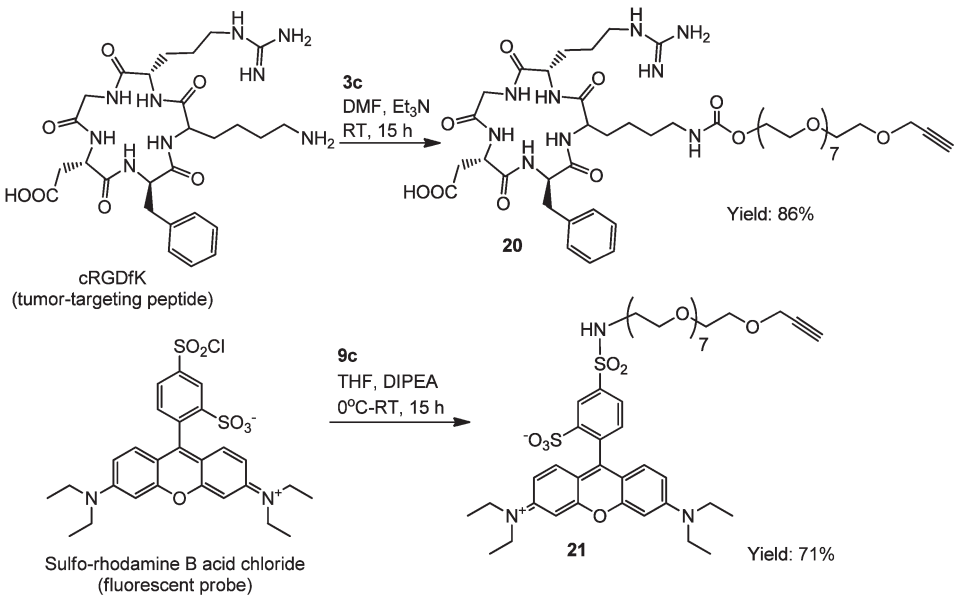
Conclusions

This manuscript describes the efficient and high-yield synthesis of a variety of discretely sized heterobifunctional oligo(ethylene glycol) linkers useful for click chemistry applications. To demonstrate the broad utility of these heterobifunctional linkers in bioconjugate chemistry, we developed a reliable, high-yield protocol for the synthesis of linker conjugates with a tumor-targeting peptide (cRGDfK) and the fluorophore sulfo-rhodamine B. The rapid covalent linkage of two dissimilar components under biocompatible conditions is useful for the creation of bifunctional agents for diagnostic and therapeutic applications.[36,37] The methods presented here will be useful for achieving diverse and efficient bioconjugation strategies in biomedical and drug discovery research. Specific biological applications of bioconjugates produced by the methods described here will be reported in future publications.

Experimental section

Materials and methods

Common reagents and chromatographic solvents were purchased from commercial suppliers (Sigma-Aldrich, Fisher Scientific and Acros Organics) and used without any further purification. Lipophilic Sephadex LH-20 was obtained from GE Healthcare. NMR spectra were recorded on Bruker Avance 400



Scheme 4 Conjugation of alkyne-terminated OEGs with the tumor-targeting peptide cRGDfK and the fluorescent probe, sulfo-rhodamine B.

and 500 MHz spectrometers. All NMR chemical shifts (δ) are reported in parts per million (ppm). The high-resolution mass spectrometry analysis was performed using Applied Biosystems Mariner ESI-TOF.

General procedure for the preparation of 2

A mixture of NaH (0.7 eq.) in THF (30 mL) was prepared in a two-neck round-bottom flask with extensive stirring, and a solution of OEG **1** (1.0 eq.) in THF (40 mL) was added over 30 min. The reaction was stirred for 1 h at room temperature, and a solution of propargyl bromide (1.0 eq.) in THF (30 mL) was then added by syringe pump over 1 h. The solution was stirred for 1 h at room temperature and then an additional 15 h at 60 °C. The reaction mixture was quenched with 3% HCl (100 mL), and the organic layer was removed by rotary evaporation. The crude product was extracted from the acidic aqueous layer with DCM (100 mL) and washed with brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to obtain the crude product as a colorless oil. The pure product was obtained by silica gel column chromatography (gradient eluent: 0–1–2–3–4–5% MeOH in DCM). The OEG starting material was easily recovered by extraction from the concentrated aqueous layer with diethyl ether.

Synthesis of 2c. By using the general procedure described above with OEG **1c** (2.00 g, 5.40 mmol), NaH (0.91 g, 3.78 mmol), and propargyl bromide (0.64 g, 5.40 mmol), the pure product was obtained as a colorless oil (1.07 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.21 (d, J = 2.4 Hz, 2H, HCCCH_2O–), 3.74–3.60 (m, 30H, –OCH_2CH_2O–), 3.10 (m, 1H, HCCCH$_2$O–), 2.45 ppm (t, J = 2.0 Hz, 2H, –CH$_2$CH_2OH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 80.47 (1C), 75.33 (1C), 73.44 (1C), 71.40–71.31 (11C), 71.19 (1C), 71.08 (1C), 69.91 (1C), 62.50 (1C), 59.19 ppm (1C). HRMS (ESI): m/z calcd for $C_{19}H_{36}O_9$ + Na^+ $[M + Na]^+$ 431.2257; found 431.2579.

General procedure for the preparation of 3

A stirred mixture of 4-nitrophenyl chloroformate (1.5 eq.) and pyridine (2.0 eq.) in acetonitrile was allowed to cool to 0 °C for 15 min. A solution of **2** (1.0 eq.) in acetonitrile was added slowly to the mixture. The mixture was allowed to warm to room temperature and reacted for 15 h. Then, the reaction mixture was concentrated to dryness, re-dissolved in DCM, and washed with brine. The organic layer was concentrated and dried *in vacuo* to give the crude product as a yellow oil. The pure product was then obtained by size-exclusion chromatography on lipophilic Sephadex LH-20 using acetonitrile as the mobile phase.

Synthesis of 3c. By using the general procedure above with **2c** (0.50 g, 1.22 mmol), 4-nitrophenyl chloroformate (0.37 g, 1.84 mmol), and pyridine (0.19 g, 2.45 mmol), the pure product was obtained as a yellow oil (0.61 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.20 (d, J = 9.20 Hz, 2H, Ar–H–NO$_2$), 7.32 (d, J = 9.20 Hz, 2H, Ar–H–OCO–), 4.36 (t, J = 4.4 Hz, 2H, –O–CH$_2$CH_2–OCO–), 4.11 (d, J = 2.0 Hz, 2H, HCCCH_2O–), 3.74 (m, 2H, –O–CH_2CH$_2$–OCO–), 3.61–3.57 (m, 28H, –OCH_2CH_2O–), 2.42 ppm (m, 1H, HCCCH$_2$O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 156.28 (1C), 153.18 (1C), 146.10 (1C), 126.02 (2C), 122.58 (2C), 80.47 (1C), 75.45 (1C), 71.41–71.29 (12C), 71.10 (1C), 69.81 (1C), 69.32 (1C), 69.06 (1C), 59.07 ppm (1C). HRMS (ESI): m/z calcd for $C_{26}H_{39}O_{13}$ + Na^+ $[M + Na]^+$ 596.2319; found 596.0696.

General procedure for the preparation of 4

A mixture of Et_3N (2.0 eq.) and **2** (1.0 eq.) in DCM (35 mL) was stirred at 0 °C, and a solution of MsCl (1.5 eq.) in DCM (20 mL) was added slowly over 30 min. After the addition was complete, the reaction was allowed to proceed at 0 °C for 90 min and then at room temperature for an additional 2 h.

The reaction mixture was concentrated to dryness, re-dissolved in DCM (100 mL), and washed with 3% HCl (100 mL) and brine. The organic layer was separated, dried over Na_2SO_4 and then concentrated *in vacuo* to give the pure product as a pale yellow oil.

Synthesis of 4c. By using the general procedure above with **2c** (1.45 g, 3.54 mmol), MsCl (0.61 g, 5.31 mmol), and Et_3N (0.72 g, 7.08 mmol), the pure product was obtained as a pale yellow oil (1.71 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.31 (m, 2H, HCCCH_2O–), 4.13 (d, J = 2.40 Hz, 2H, –OCH$_2$CH_2–SO$_2$CH$_3$), 3.70 (m, 2H, –OCH_2CH$_2$–SO$_2$CH$_3$), 3.64–3.57 (m, 28H, –O–CH_2CH_2–O–), 3.02 (s, 3H, –S–(CH_3), 2.43 ppm (m, 1H, HCCCH$_2$O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 80.47 (1C), 75.44 (1C), 71.35–71.23 (12C), 71.11 (1C), 70.15 (1C), 69.83 (1C), 69.73 (1C), 59.09 (1C), 38.44 ppm (1C). HRMS (ESI): m/z calcd for $C_{20}H_{38}O_{11}S$ + Na^+ $[M + Na]^+$ 509.2033; found 509.3040.

General procedure for the preparation of 5

A mixture of **4** (1.0 eq.) and NaI (4.0 eq.) in acetone was stirred for 15 h at 65 °C. The reaction mixture was concentrated to dryness and filtered over celite with DCM. The filtrate was concentrated and purified by silica gel chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) to obtain the product as a pale yellow oil.

Synthesis of 5c. By using the general procedure above with **4c** (0.60 g, 1.22 mmol) and NaI (0.73 g, 4.90 mmol), the pure product was obtained as a pale yellow oil (0.62 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.98 (d, J = 2.4 Hz, 2H, HCCCH_2O–), 3.54 (t, 2H, J = 6.8 Hz, –OCH_2CH$_2$I), 3.48–3.43 (m, 28H, –OCH_2CH_2–O), 3.06 (t, J = 6.8 Hz, 2H, –OCH$_2$CH_2I), 2.38 ppm (t, J = 2.4 Hz, 1H, HCCCH$_2$O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 80.43 (1C), 75.72 (1C), 72.52 (1C), 71.26–71.20 (11C), 70.99 (1C), 70.82 (1C), 69.68 (1C), 58.96 (1C), 4.08 ppm (1C). HRMS (ESI): m/z calcd for $C_{19}H_{35}IO_8$ + H^+ $[M + H]^+$ 519.1455; found 519.1453.

General procedure for the preparation of 6

A mixture of **4** (1.0 eq.) and KSAc (1.5 eq.) in DMF was stirred for 15 h at room temperature. The reaction mixture was washed with a saturated aqueous solution of NH_4Cl, extracted with DCM, dried over Na_2SO_4, and concentrated to dryness *in vacuo*. The pure product was then obtained by silica gel column chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) as a colorless oil.

Synthesis of 6c. By using the general procedure above with **4c** (0.24 g, 0.49 mmol) and KSAc (0.08 g, 0.74 mmol), the pure product was obtained as a colorless oil (0.15 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.17 (d, J = 2.4 Hz, 2H, HCCCH_2O–), 3.67–3.55 (m, 30H, –OCH_2CH_2O–), 3.06 (t, J = 6.4 Hz, 2H, –OCH$_2$CH_2–SCOCH$_3$), 2.44 (t, J = 2.4 Hz, 1H, HCCCH$_2$O–), 2.31 (s, 3H, –OCH$_2$CH$_2$–SCOCH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 196.21 (1C), 80.46 (1C), 75.36 (1C), 71.41–71.29 (13C), 71.17 (1C), 71.09 (1C), 70.52 (1C), 69.88 (1C), 59.15 ppm (1C). HRMS (ESI): m/z calcd for $C_{21}H_{38}O_9S$ + Na^+ $[M + Na]^+$ 489.2129; found 489.2032.

General procedure for the preparation of 7

In a dry round bottom flask, LAH (4.0 eq.) and THF were combined under cooling in an NH_4Cl ice bath (−10 °C). A solution of **6** (1.0 eq.) in THF was added slowly to the reaction vessel. The reaction mixture was stirred for 3 h allowing it to come to room temperature. The mixture was then quenched with a saturated aqueous solution of NH_4Cl. The crude product was extracted with dichloromethane, dried over sodium sulfate, and concentrated *in vacuo*. The pure product was obtained by silica gel column chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) as a pale yellow oil.

Synthesis of 7c. By using the general procedure above with **6c** (0.09 g, 0.19 mmol) and LAH (0.29 g, 0.77 mmol), the pure product was obtained as a pale yellow oil (0.08 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.20 (d, J = 2.4 Hz, 2H, HCCCH_2O–), 3.70–3.60 (m, 30H, –OCH_2CH_2O–), 2.70 (m, 2H, –OCH$_2$CH_2SH), 2.45 (t, J = 2.4 Hz, 1H, HCCCH$_2$O–), 1.60 ppm (t, J = 8.2 Hz, 1H, –OCH$_2$CH$_2$SH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 80.47 (1C), 75.35 (1C), 73.68 (1C), 71.43–71.33 (11C), 71.20 (1C), 71.03 (1C), 69.91 (1C), 59.18 (1C), 25.05 ppm (1C). HRMS (ESI): m/z calcd for $C_{19}H_{36}O_8S$ + Na^+ $[M + Na]^+$ 447.2023; found 447.1960.

General procedure for the preparation of 8

Compound **4** (1.0 eq.) was dissolved in DMF with potassium phthalimide (1.5 eq.) and reacted at 110 °C for 15 h. The mixture was concentrated under high-vacuum to remove DMF, and the resulting solid was dissolved in diethyl ether. This solution was filtered with a sintered funnel and concentrated to give the pure product as a yellow oil.

Synthesis of 8c. By using the general procedure above with **4c** (2.68 g, 5.51 mmol) and potassium phthalimide (1.53 g, 8.27 mmol), the pure product was obtained as a yellow oil (2.87 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.87 (m, 2H, Ar–H), 7.74 (m, 2H, Ar–H), 4.22 (d, J = 2.0 Hz, 2H, HCCCH_2O–), 3.92 (t, J = 5.6 Hz, 2H, –OCH_2CH_2–NPhth), 3.77–3.60 (m, 30H, –OCH$_2$CH$_2$O–), 2.46 ppm (t, J = 2.4 Hz, 1H, HCCCH$_2$O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 169.05 (2C), 134.90 (2C), 132.97 (2C), 124.20 (2C), 80.49 (1C), 75.31 (1C), 71.41–71.36 (11C), 71.22 (1C), 70.91 (1C), 69.93 (1C), 68.71 (1C), 59.21 (1C), 38.08 ppm (1C). HRMS (ESI): m/z calcd for $C_{27}H_{39}NO_{10}$ + Na^+ $[M + Na]^+$ 560.2466; found 560.2863; for $C_{27}H_{39}NO_{10}$ + K^+ $[M + K]^+$ 576.2206; found 576.2664.

General procedure for the preparation of 9

Hydrazine (17.0 eq.) was added to a solution of **8** (1.0 eq.) in ethanol, and the mixture was reacted for 3 h at 60 °C. The reaction mixture was concentrated to dryness, and the product was isolated from the resulting phthalyl hydrazide by filtering over celite and washing with diethyl ether. The solution was then concentrated to give the pure product as a pale yellow oil.

Synthesis of 9c. By using the general procedure above with **8c** (2.77 g, 5.16 mmol) and hydrazine (2.77 g, 86.5 mmol), the pure product was obtained as a pale yellow oil (2.08 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.23 (m, 2H, HCCCH_2O–),

3.73–3.66 (m, 30H, –OCH_2CH_2O–), 3.53 (m, 2H, –OCH_2CH_2-NH_2), 2.88 (m, 2H, –OCH_2C$H_2$$NH_2$), 2.45 ppm (m, 1H, –*H*CCCH_2O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 75.31 (1C), 74.30 (1C), 71.40 (12C), 71.22 (1C), 71.12 (1C), 69.93 (1C), 59.21 (1C), 42.65 ppm (1C). HRMS (ESI): *m*/*z* calcd for $C_{19}H_{37}NO_8$ + H^+ $[M + H]^+$ 408.2592; found 408.2108.

General procedure for the preparation of 10

Compound **9** (1.0 eq.) was dissolved in a saturated aqueous solution of $NaHCO_3$ at 0 °C, and *N*-methoxycarbonyl maleimide (1.0 eq.) was added slowly. The mixture was reacted for 1 h at 0 °C and then for an additional 1 h at room temperature. The product was extracted from the aqueous mixture with DCM, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The pure product was then obtained by silica gel column chromatography (gradient eluent: 0–1–2–3% MeOH in DCM) as a pale yellow oil.

Synthesis of 10c. By using the general procedure above with **9c** (1.00 g, 2.45 mmol) and *N*-methoxycarbonyl maleimide (0.38 g, 2.45 mmol), the pure product was obtained as a pale yellow oil (0.50 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.67 (s, 2H, Mal-H), 4.14 (d, *J* = 2.4 Hz, 2H, –HCCCH_2O–), 3.68–3.53 (m, 32H, –OCH_2CH_2O–), 2.42 ppm (t, *J* = 2.4 Hz, 1H, *H*CCCH_2O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 170.62 (2C), 134.15 (2C), 79.65 (1C), 74.60 (1C), 70.56–70.49 (11C), 70.35 (1C), 69.99 (1C), 69.06 (1C), 67.75 (1C), 58.34 (1C), 37.07 ppm (1C). HRMS (ESI): *m*/*z* calcd for $C_{23}H_{37}NO_{10}$ + Na^+ $[M + Na]^+$ 510.2310; found 510.2936.

General procedure for the preparation of 11 – Method A

A solution of MsCl (0.9 eq.) in DCM (20 mL) was added by syringe pump over 1 h to a mixture of Et_3N (1.5 eq.) and OEG **1** (1.0 eq.) in DCM (35 mL) stirring at 0 °C. After addition, the reaction was allowed to proceed at 0 °C for 1 h, and then at room temperature for a further 2 h. The reaction mixture was concentrated to dryness and washed first with 3% HCl (100 mL) and then with brine (100 mL), extracting with DCM (100 mL). This procedure was repeated thrice and the organic layer was dried over Na_2SO4 and then *in vacuo* to give a mixture of di- and mono-mesylated polyethylene glycol. This mixture was reacted with NaN_3 (5.0 eq.) in DMF for 15 h at 65 °C. The crude product was concentrated to dryness and filtered over celite, washing with Et_2O. The mixture was then concentrated and purified *via* silica gel chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) as a pale yellow oil.

General procedure for the preparation of 11 – Method B

Note: For the reaction with hexa(ethylene glycol), KI was used as an additive. KI was not required for tetra(ethylene glycol) or octa-(ethylene glycol). MsCl (1.1 eq.) was added over a period of 30 min with a syringe pump (0.333 mL min^{-1}) to a solution of OEG **1** (1.0 eq.) and Ag_2O (1.5 eq.) in DCM at 0 °C. The mixture was then allowed to slowly warm to room temperature and react for 24 h. The reaction mixture was filtered over celite, washing with ethyl acetate. The organic phase was concentrated and thoroughly dried *in vacuo*. This crude mixture was then reacted with NaN_3 (5.0 eq.) in DMF for 12 h at 65 °C. The mixture was again filtered over celite, concentrated, dried *in vacuo*, and then purified *via* silica gel chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) as a pale yellow oil.

Synthesis of 11c. By using the general procedure above for Method A with OEG **1c** (4.98 g, 13. 5 mmol), MsCl (1.39 g, 12.1 mmol), Et_3N (2.04 g, 20.2 mmol), and NaN_3 (4.37 g, 67.3 mmol), the pure product was obtained as a pale yellow oil (2.20 g, 46%). By using the general procedure above for Method B with OEG **1c** (1.00 g, 2.70 mmol), Ag_2O (938 mg, 4.05 mmol), and MsCl (340 mg, 2.97 mmol), the pure product was obtained as a pale yellow oil (545 mg, 51%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 3.56–3.50 (m, 28H, –OCH_2CH_2O–), 3.24 (t, *J* = 5.2 Hz, 2H, –CH_2CH_2OH), 3.07 ppm (t, *J* = 5.9 Hz, 2H, N_3CH_2CH_2O–). ^{13}C (100.6 MHz, $CDCl_3$) δ = 73.29 (1C), 71.30–71.21 (11C), 70.99 (1C), 70.67 (1C), 62.21 (1C), 51.32 ppm (1C). HRMS (ESI): *m*/*z* calcd for $C_{16}H_{33}N_3O_8$ + Na^+ $[M + Na]^+$ 418.2160; found 418.3255.

General procedure for the preparation of 12

A solution of MsCl (3.0 eq.) in DCM (15 mL) was added slowly over 30 min to a mixture of Et_3N (3.0 eq.) and OEG **1** (1.0 eq.) in DCM (15 mL) stirring at 0 °C. After addition was complete, the reaction was allowed to proceed at 0 °C for 2 h and then at room temperature for a further 2 h. The reaction mixture was concentrated to dryness and washed first with 3% HCl (100 mL) and then brine (100 mL), extracting with DCM (100 mL). This procedure was repeated thrice and the organic layer was dried over Na_2SO_4 and then *in vacuo.* The resulting dimesylated product was reacted with NaN_3 (5.0 eq.) in DMF at 65 °C for 15 h. DMF was removed *in vacuo* and then excess NaN_3 removed *via* filtration over celite, washing with Et_2O to give the pure product as a pale yellow oil.

Synthesis of 12c. By using the general procedure above with OEG **1c** (500 mg, 1.35 mmol), MsCl (466 mg, 4.05 mmol), Et_3N (409 mg, 4.05 mmol), and NaN_3 (438 mg, 6.75 mmol), the pure product was obtained as a pale yellow oil (568 mg, 100%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 3.63 (m, 28H, –OCH_2CH_2O–), 3.36 ppm (t, *J* = 5.2 Hz, 4H, N_3C$H_2$$CH_2$O–). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ = 71.46–71.36 (12C), 70.79 (2C), 51.46 ppm (2C). HRMS: *m*/*z* calcd for $C_{16}H_{32}N_6O_7$ + Na^+ $[M + Na]^+$ 443.2225; found 443.2072.

General procedure for the preparation of 13

A stirring mixture of 4-nitrophenyl chloroformate (1.5 eq.) and pyridine (2.0 eq.) in MeCN was cooled to 0 °C for 15 min, and a solution of **11** (1.0 eq.) in MeCN was added slowly. The mixture was allowed to warm to room temperature and then react for 15 h. The reaction mixture was then concentrated to dryness, washed with brine, and extracted with DCM. The organic layer was concentrated and dried *in vacuo* to give the crude product as a pale yellow oil. Pure product was obtained by size-exclusion chromatography on LH-20 using MeCN as the mobile phase.

Synthesis of 13c. By using the general procedure above with **11c** (100 mg, 0.25 mmol), 4-nitrophenyl chloroformate

(76.5 mg, 0.38 mmol), and pyridine (40.0 mg, 0.51 mmol), the pure product was obtained as a pale yellow oil (135 mg, 95%): ^{1}H-NMR: (500 MHz, $CDCl_3$) δ = 8.33 (d, J = 5.0 Hz, 2H, Ar–H–NO_2), 7.45 (d, J = 5.0 Hz, 2H, Ar–H–OCO–), 4.49 (m, 2H, –O–CH_2CH_2–OCO–), 3.87 (m, 2H, –O–CH_2CH_2–OCO–), 3.72 (m, 26H, –OCH_2CH_2O–), 3.40 ppm (t, J = 5.0 Hz, 2H, $N_3CH_2CH_2O$–). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ = 155.51 (1C), 152.45 (1C), 145.35 (1C), 125.28 (2C), 121.80 (2C), 70.68–70.55 (12C), 70.00 (1C), 68.59 (1C), 68.31 (1C), 50.65 ppm (1C). HRMS (ESI): m/z calcd for $C_{23}H_{36}N_4O_{12}$ + Na^+ $[M + Na]^+$ 583.2222; found 583.1295.

General procedure for the preparation of 14

A solution of MsCl (1.5 eq.) in DCM (20 mL) was added slowly over 30 min to a mixture of Et_3N (2.0 eq.) and **11** (1.0 eq.) in DCM (35 mL) stirring at 0 °C. After this, the reaction was allowed to proceed at 0 °C for 90 min, and then at room temperature for a further 2 h. The reaction mixture was concentrated to dryness and washed first with 3% HCl (100 mL) and then brine (100 mL), extracting with DCM (100 mL). This procedure was repeated thrice and the organic layer was dried over Na_2SO4 and then *in vacuo.* The organic layer was dried over Na_2SO4 and then *in vacuo* to give the pure product pale yellow oil.

Synthesis of 14c. By using the general procedure above with **11c** (2.39 g, 6.05 mmol), MsCl (1.04 g, 9.07 mmol), and Et_3N (1.22 g, 12.1 mmol), the pure product was obtained as a pale yellow oil (2.83 g, 99%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.34 (m, 2H, –OCH_2CH_2OMs), 3.73 (m, 2H, $N_3CH_2CH_2O$–), 3.63 (m, 26H, –OCH_2CH_2O–), 3.35 (m, 2H, $N_3CH_2CH_2O$–), 3.06 ppm (m, 3H, –OSO_2CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ = 71.40–71.26 (11C), 70.76 (1C), 70.16 (1C), 69.76 (1C), 51.44 (1C), 38.46 (1C), 32.38 ppm (1C). HRMS: m/z calcd for $C_{17}H_{35}N_3O_{10}S$ + Na^+ $[M + Na]^+$ 496.1935; found 496.1485.

General procedure for the preparation of 15

A mixture of **14** (1.0 eq.) and sodium iodide (4.0 eq.) in acetone was stirred for 15 h at 65 °C. The reaction mixture was then concentrated to dryness and filtered over celite with DCM. The filtrate was concentrated and purified by silica gel chromatography (gradient-eluent: 0–1–2–3–4% MeOH in DCM) to obtain the product as a pale yellow oil.

Synthesis of 15c. By using the general procedure above with **14c** (1.85 g, 3.91 mmol) and sodium iodide (2.34 g, 15.6 mmol), the pure product was obtained as a pale yellow oil (1.86 g, 94%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 3.67 (t, J = 6.8 Hz, 2H, –OCH_2CH_2I), 3.58 (m, 26H, –OCH_2CH_2O–), 3.31 (t, J = 5.2 Hz, 2H, $N_3CH_2CH_2O$–), 3.18 ppm (t, J = 6.8 Hz, 2H, –OCH_2CH_2I). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ = 72.68 (1C), 71.41–71.32 (11C), 70.95 (1C), 70.76 (1C), 51.41 (1C), 3.87 ppm (1C). HRMS (ESI): m/z calcd for $C_{16}H_{32}IN_3O_7$ + Na^+ $[M + Na]^+$ 528.1177; found 528.0790.

General procedure for the preparation of 16

Compound **14** (1.0 eq.) was dissolved in DMF with potassium phthalimide (1.5 eq.) and reacted at 110 °C for 15 h. The mixture was concentrated under high vacuum to remove the DMF. The resulting solid was filtered through a sintered funnel, washing with Et_2O, and then concentrated to give the pure product as a yellow oil.

Synthesis of 16c. By using the general procedure above with **14c** (400 mg, 0.85 mmol) and potassium phthalimide (234 mg, 1.27 mmol), the pure product was obtained as a yellow oil (434 mg, 98%): ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7. 85 (m, 2H, Ar–H), 7.74 (m, 2H, Ar–H), 3.91 (t, J = 10.0 Hz, 2H, –OCH_2CH_2NPhth), 3.75 (t, J = 5.0 Hz, 2H, –OCH_2CH_2NPhth), 3.68 (m, 26H, –OCH_2CH_2O–), 3.40 ppm (t, J = 5.0 Hz, 2H, $N_3CH_2CH_2O$–). ^{13}C-NMR (125.8 MHz, $CDCl_3$) δ = 168.15 (2C), 133.90 (2C), 132.06 (2C), 123.16 (2C), 70.61–70.45 (11C), 70.02 (1C), 69.96 (1C), 67.82 (1C), 50.61 (1C), 37.19 ppm (1C). HRMS: m/z calcd for $C_{24}H_{36}N_4O_9$ + Na^+ $[M + Na]^+$ 547.2374; found 547.0832; for $C_{24}H_{36}N_4O_9$ + K^+ $[M + K]^+$ 563.2114; found 563.0738.

General procedure for the preparation of 17 from 16 – Route 1

Hydrazine (17.0 eq.) was added to a solution of **16** (1.0 eq.) in ethanol, and the mixture was reacted for 3 h at 60 °C. The reaction mixture was concentrated to dryness, and the pure product was separated from the formed phthalyl hydrazide by filtration over celite, washing with Et_2O. The solution was then concentrated to give the pure product as a yellow oil.

General procedure for the preparation of 17 from 12 – Route 2

To **12** (1.0 eq.), 5% HCl was added with vigorous stirring at room temperature. A solution of triphenyl phosphine (0.9 eq.) in Et_2O was added to this mixture over 3 hours. The mixture was then allowed to react at room temperature for a further 24 hours. The reaction mixture was washed with ethyl acetate (50 mL × 3) to remove the unreacted starting materials and triphenylphosphine oxide that was formed during the reaction. The aqueous layer was collected, cooled to 0 °C, and concentrated potassium hydroxide was added to it until the pH of the solution was basic (~12). The product was extracted as a pale yellow oil by washing the aqueous layer with DCM thrice, drying over Na_2SO_4 and then *in vacuo*.

Synthesis of 17c. By using the general procedure above for Route 1 with **16c** (410 mg, 0.78 mmol) and hydrazine (410 mg, 12.8 mmol), the pure product was obtained as a yellow oil (281 mg, 91%). By using the general procedure above for Route 2 with **12c** (2.00 g, 4.76 mmol), triphenyl phosphine (1.12 g, 4.28 mmol), 5% HCl (19 mL), and Et_2O (14 mL), the pure product was obtained as a pale yellow oil (1.55 g, 83%): ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 3.62 (m, 26H, –OCH_2CH_2O–), 3.49 (t, J = 5.0 Hz, 2H, $N_3CH_2CH_2O$–), 3.36 (t, J = 5.0 Hz, 2H, –$OCH_2CH_2NH_2$), 2.85 ppm (t, J = 5.0 Hz, 2H, $N_3CH_2CH_2O$–). ^{13}C-NMR (125.8 MHz, $CDCl_3$) δ = 73.03 (1C), 70.57–70.43 (11C), 70.17 (1C), 69.92 (1C), 50.56 (1C), 41.56 ppm (1C). HRMS (ESI): m/z calcd for $C_{16}H_{34}N_4O_7$ + H^+ $[M + H]^+$ 395.2500; found 395.1928.

Synthesis of PEG_{16} (18) (click reaction). In an oven-dry 50 mL round-bottom flask, **2c** (0.21 g, 0.51 mmol), **11c** (0.20 g, 0.51 mmol) and copper(I) iodide (0.10 g, 0.51 mmol) were

dissolved in a 50 : 50 mixture of THF and MeCN (15 mL). DIPEA (0.07 g, 0.51 mmol) was added and the reaction mixture was vigorously stirred at room temperature for 12 h under an argon atmosphere. The reaction mixture was concentrated to dryness, re-dissolved in ethyl acetate, and filtered through a celite pad. The filtrate was concentrated, and the pure product was obtained by silica gel column chromatography as a colorless oil (0.40 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.68 (s, 1H, *CH*-triazole), 4.57 (s, 2H, N–CH_2–O–), 4.44 (t, J = 5.2 Hz, 2H, –NCH_2–CH_2O–), 3.77 (t, J = 5.2 Hz, 2H, –NCH_2–CH_2O–), 3.63–3.48 (m, 60H, –OCH_2CH_2O–), 3.19 (m, 2H, –HOCH_2CH_2O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 145.56 (1C), 124.56 (1C), 73.33 (1C), 71.29–71.02 (27C), 70.33 (1C), 70.16 (1C), 65.26 (1C), 62.28 (1C), 50.91 ppm (1C). HRMS (ESI): m/z calcd for $C_{35}H_{69}N_3O_{17}$ + Na^+ $[M + Na]^+$ 826.4519; found 826.5635.

Synthesis of PEG_{24} (19) (click reaction). In an oven-dry 50 mL round-bottom flask, **2c** (0.49 g, 1.19 mmol), **12c** (0.25 g, 0.59 mmol) and copper(I) iodide (0.23 g, 1.19 mmol) were dissolved in a 50 : 50 mixture of THF and MeCN (20 mL). DIPEA (0.15 g, 1.19 mmol) was added, and the reaction mixture was vigorously stirred at room temperature for 12 h under an argon atmosphere. The reaction mixture was concentrated to dryness, re-dissolved in ethyl acetate, and filtered through a celite pad. The filtrate was concentrated, and the pure product was obtained by silica gel column chromatography as a colorless oil (0.71 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.67 (s, 2H, 2-*CH*-triazole), 4.59 (s, 4H, 2-N–CH_2–O–), 4.44 (t, J = 4.8 Hz, 4H, 2-NCH_2–CH_2O–), 3.78 (t, J = 5.2 Hz, 4H, 2-NCH_2–CH_2O–), 3.63–3.48 (m, 88H, –OCH_2CH_2O–), 2.99 (m, 2H, –HOCH_2CH_2O–). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 145.59 (1C), 124.51 (1C), 73.29 (1C), 71.31–71.06 (46C), 70.34 (1C), 70.17 (1C), 65.29 (1C), 62.32 (1C), 50.91 ppm (1C). HRMS (ESI): m/z calcd for $C_{54}H_{104}N_6O_{25}$ + H^+ $[M + H]^+$ + Na^+ 1260.7027; found 1260.7263.

Synthesis of 20. In an oven-dry 50 mL round-bottom flask, c(RGDfK) (10.0 mg, 16.5 μmol; Peptides International, Inc., Cat. PCI-3661-PI), and **3c** (11.4 mg, 19.8 μmol) were dissolved in 10 mL DMF. Et_3N (50.0 μl, excess) was added and the mixture stirred for 15 h at room temperature under an argon atmosphere. The reaction mixture was concentrated under reduced pressure, and the pure product was obtained as a pale yellow oil by size-exclusion chromatography on lipophilic Sephadex LH-20 using MeOH as the mobile phase. Yield: 15.0 mg (86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29–7.23 (m, 5H), 4.66 (t, J = 4.0 Hz, 1H), 4.57 (t, J = 6.4 Hz, 1H), 4.49 (t, J = 6.0 Hz, 1H), 4.30 (d, J = 15.2 Hz, 1H), 4.19 (m, 5H), 4.10 (dd, J = 4.8 and 9.6 Hz, 1H), 3.75 (m, 38H), 3.43 (d, J = 14.8 Hz, 1H), 3.29–2.96 (m, 6H), 2.88 (m, 1H), 2.67 (m, 1H), 2.56 (m, 1H), 1.89 (m, 1H), 1.78–1.73 (m, 2H), 1.68–1.50 (m, 3H), 1.41 (m, 2H), 1.15 (t, J = 6.8 Hz, 1H), 1.05 ppm (m, 2H). HRMS (ESI): m/z calcd for $C_{47}H_{74}N_8O_{18}$ + Na^+ $[M + Na]^+$ 1061.5008; found 1061.3796.

Synthesis of 21. In an oven-dry 100 mL round-bottom flask, sulfo-rhodamine B acid chloride (0.30 g, 0.51 mmol) was dissolved in 10 mL THF followed by the addition of DIPEA (0.20 g, 1.56 mmol). The mixture was cooled to 0 °C, and **9c** (0.28 g, 0.77 mmol) in 10 mL THF was added slowly. The reaction mixture was gradually warmed to room temperature and stirred for 15 h under an argon atmosphere. The reaction mixture was concentrated *via* rotary evaporation, and the pure product was obtained as a pink oil by column chromatography on alumina(IV) using MeOH/DCM gradient as the mobile phase. Yield: 0.35 g (71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.66 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 9.2 Hz, 2H), 6.76 (d, J = 9.2 Hz, 2H), 6.65 (s, 2H), 4.13 (d, J = 2.4 Hz, 2H), 3.62–3.48 (m, 39H), 3.16 (t, J = 5.2 Hz, 1H), 2.43 (s, 1H), 1.24 ppm (t, J = 6.8 Hz, 12H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 158.65, 158.53, 156.31, 147.78, 143.08, 134.39, 133.70, 130.87, 128.20, 127.67, 114.92, 114.34, 96.44, 80.22, 75.42, 71.18, 71.13, 71.03, 70.96, 70.53, 69.76, 59.05, 46.57, 43.64, 13.18 ppm. HRMS (ESI): m/z calcd for $C_{46}H_{65}N_3O_{14}S_2$ + Na^+ $[M + Na]^+$ 970.3800; found 970.4205; for $C_{46}H_{65}N_3O_{14}S_2$ + $2Na^+$ $[M + 2Na^+]^{2+}$ 496.6849; found 496.7605.

Abbreviations

MeCN	Acetonitrile
NH_4Cl	Ammonium chloride
DCM	Dichloromethane
Et_2O	Diethyl ether
DIPEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*′-dimethylformamide
HCl	Hydrochloric acid
LAH	Lithium aluminum hydride
MsCl	Methanesulfonyl chloride
MeOH	Methanol
KSAc	Potassium thioacetate
NaN_3	Sodium azide
NaI	Sodium iodide
Na_2SO_4	Sodium sulfate
THF	Tetrahydrofuran
Et_3N	Triethylamine

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