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Expedient synthesis of trifunctional oligoethyleneglycol-amine linkers and their use in the preparation of PEG-based branched platforms†

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We designed a convergent synthesis pathway that provides access to trifunctional oligoethyleneglycol-amine (OEG-amine) linkers. By applying the reductive coupling of a primary azide to bifunctional OEG-azide precursors, the corresponding symmetrical dialkylamine bearing two homo-functional end chain groups and a central nitrogen was obtained. These building blocks bear minimal structural perturbation compared to the native OEG backbone which makes them attractive for biomedical applications. The NMR investigations of the mechanism process reveal the formation of nitrile and imine intermediates which can react with the reduced free amine form. Additionally, these trifunctional OEG-amine linkers were employed in a coupling reaction to afford branched multifunctional PEG dendrons which are molecularly defined. These discrete PEG-based dendrons (n = 16, 18 and 36) could be useful for numerous applications where multivalency is required.

Introduction

Polyfunctional molecularly defined short-chain PEGs called oligoethylene glycols (OEG) are becoming an increasingly important class of linkers. Thanks to their particular biophysical characteristics (*i.e.*, being uncharged, water soluble, biostable, and biocompatible), these building blocks are omnipresent in every application where biological systems have to interact with exobiotic, synthetic moieties. For example, they are considered as the privileged linker backbone for immobilizing biomolecules on various surfaces,[1–3] designing a drug delivery system,[4] or preparing neutral surfactants and water/bio-compatible polymers. Regarding pharmaceutical applications, engineered OEGs with controlled molecular structures are often used as spacers for the conjugation of small drugs to antibodies[5,6] and nanoparticles.[7] Because of this pivotal role, intensive research efforts have been dedicated to developing a variety of OEGs, which differ in their shapes and functionalities. Hence, a large panel of reactive functional groups has been introduced at the opposite end of these linear OEGs such as activated carboxylic esters, free or protected amines, azides, alkynes, or thiols for example.[8] In order to achieve the multivalency required by most biological applications, these linear hetero-bifunctional OEGs are assembled *via* stepwise chemical coupling onto a multi-reactive core structure, such as a triazine heterocycle,[9] a glycerol, a 2-(2-aminoethoxy) ethanol,[10] a bicine,[11] a trimethylolpropane allyl ether[12] or a lysine structure[13–15] (Fig. 1a). In addition, due to often being tedious, this synthetic strategy loses the intrinsic benefit of using an OEG backbone by introducing a central structural motif that can lead to biological instability, pH sensitivity, or non-specific interaction with the biological media.

Thus, on one hand, our goal was to devise a straightforward access to trifunctional OEG bearing minimal structural pertur-

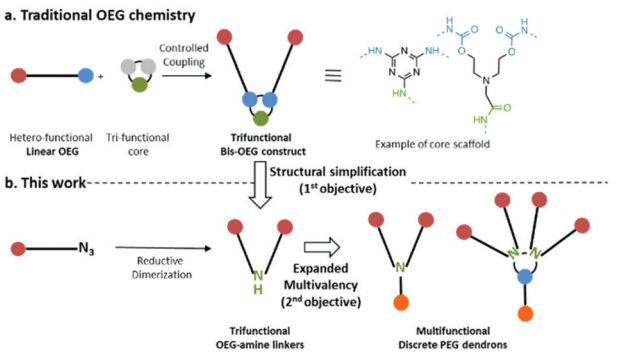

Fig. 1 (a) Illustration of current strategies to access trifunctional bis-OEG constructs. (b) The proposed alternative approach toward unaltered trifunctional OEG-amine linkers and their use for the preparation of discrete multifunctional PEG-based dendrons.

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bation compared to the native OEG backbone (Fig. 1, first objective: structural simplification). We hypothesized that introducing a central nitrogen atom in the OEG skeleton would create a molecularly defined analogue bearing an additional derivatization center. These OEG linkers could then replace the traditional bifunctional building blocks for biomedical applications. On the other hand, due to their trifunctional properties, these linkers can also be incorporated into the traditional multifunctional cores to increase the multivalency of the resulting chemical platforms offering molecularly defined PEG-based dendrons (Fig. 1b, second objective: expanded multivalency).

Examination of the literature showed that such molecules have only been scarcely described. For instance, bis-chloro-OEG_3-amine has been synthesized by alkylation of *N*-Boc diethanolamine with 1,1′-dichloro ethyl ethers[16] and converted to the corresponding bis-azide derivative after NaN_3 treatment.[17] This synthetic approach appears limited, due to the low availability of bis-chloro OEG derivatives and its narrow scope.

In order to design a versatile synthesis of such a highly functionalized hydrosoluble compound in a limited number of steps, we turned our attention to the reductive coupling of primary azides, a reaction first described by M. Lange *et al.*[18] Interestingly, this reaction seemed to be compatible with the ethylene glycol motif as demonstrated by the synthesis of a simple amino bis-OEG_6 albeit in a poor 30% yield.[19] We thus decided to investigate this key reaction further and evaluate its application to the synthesis of *N*,*N*-bis-oligoethyleneglycol-amines by testing it on a readily available azido OEG. In order to further demonstrate the potency of these new trifunctional OEG-amine linkers we engaged them in the preparation of new branched multifunctional discrete PEG-dendrons.

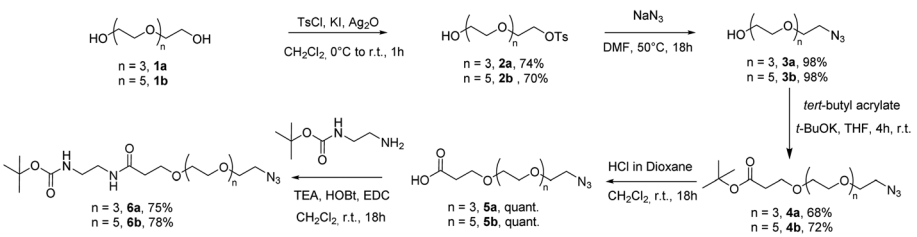
Results & discussion

We first synthesized the required linear bifunctional OEG building blocks bearing an azide group and either an ester or an amino group on the opposite end of the chain. To this end, mono-activation of one of the hydroxyl-ends of the parent OEG was performed according to the procedure of Bouzide A. and Sauvé G.[20] The use of a stoichiometric amount of tosyl chloride in the presence of silver(I) oxide and a catalytic amount of potassium iodide led selectively to the monotosyl derivatives in good yields (≥70%). Thus, tetra-ethylene glycol **1a** and hexa-ethylene glycol **1b** gave mono-tosyl-OEGs **2a** and **2b**, respectively, which were converted quantitatively to their corresponding azido-OEGs **3a** and **3b** (Scheme 1). The *tert*-butyl esters **4a** and **4b** were subsequently obtained by alkylation of OEG azides **3a** and **3b** with *tert*-butyl acrylate. After quantitative acidic deprotection, the resulting carboxylic acids **5a** and **5b** were reacted with commercially available *N*-Boc ethylene diamine, using EDC and HOBt as coupling reagents, cleanly delivering bifunctional OEGs **6a** and **6b** in 75 and 78% yields, respectively.

The optimization of the reductive azide dimerization step was then performed using the hetero-bifunctional OEG_4 **4a** as the model substrate. In 2004, I. H. An *et al.* reported a study on the reductive dimerization of 3-phenyl propyl azide and showed that this process was preferred over the simple reduction, especially at elevated temperature and high concentrations.[21] With these considerations in mind, we screened several parameters, including the catalyst loading, the reactant concentration, the temperature, and the solvent, and compared by ^{1}H-NMR spectroscopy the ratios between the expected dimer **8a** and the amine side-product **7a** resulting from the simple reduction (see ESI Table 1†). Briefly, the reductive dimerization reaction was found to be dependent on the catalyst loading, reaction temperature and substrate concentrations. For instance **8a** was obtained in 42% yield when 5 mol% of Pd/C was used, as opposed to only 18% with a 1 mol% Pd loading. Also the yield of the desired product increased when the substrate concentration increased from 100 mM to 500 mM. As previously reported for 3-phenyl propyl azide, we also found that the dimerization process was more efficient at elevated temperatures with yields of 39% at 0 °C and 79% at 60 °C. Finally, ethyl acetate, 1,4-dioxane or THF gave a better yield (90–93%) than EtOH (79%).

Applying these optimized conditions to precursors **4a–b** and **6a–b** (500 mg, 5% Pd/C, 500 mM in dioxane at 60 °C), we were able to synthesize the desired *N*,*N*-bis-OEG-amines **8a**, **8b**, **9a** and **9b** in one step and in good isolated yields (72–84%) (Scheme 2). It is noteworthy that these reactions were performed at a 2 g scale and they proved to be highly reproducible affording high yields (>70%) each time.

The reductive azide dimerization was also directly applied to azido-OEG_4-alcohol **3a**, affording the corresponding bis-OEG-amine **10**. The resulting secondary amine was then Boc-protected before the alcohol groups were converted to their



Scheme 1 Synthesis of bifunctional OEG azides.

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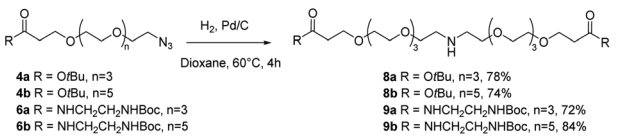
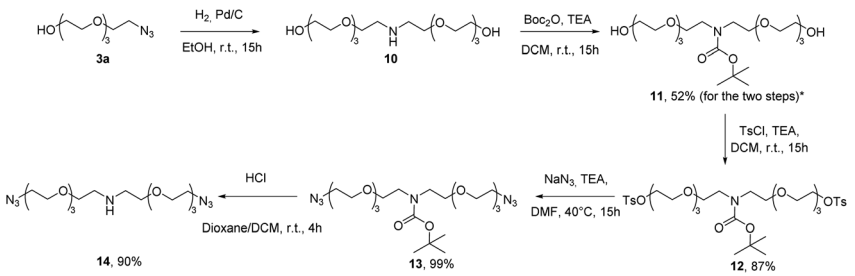


Scheme 2 Reductive dimerization of OEG azides to bis-OEG amines.

corresponding tosyl derivatives (Scheme 3). The bis-azido-OEG_4-*N*-Boc amine **13** was finally obtained after treatment with sodium azide and was subsequently deprotected under acidic conditions to afford bis-azido-OEG-amine **14**. It is worth mentioning that this clickable linker was prepared on a 3 g scale, demonstrating the robustness of our synthetic strategy.

We then turned our attention towards the investigation of the mechanism of this reaction. In their early work, Lange *et al.* proposed as a first catalytic act the formation of an imine intermediate that is complexed with palladium *via* the expulsion of N_2.[18] This intermediate undergoes on-site reduction leading to a primary amine complexed with Pd. Ultimately this amine would react with a neighboring imine Pd complex to form an aminal intermediate which is further reduced to afford the final dimerized compound. In our reactions, we had sometimes observed traces of a nitrile derivative. Since other reports describe that an azide can be converted into a nitrile, and that the nitrile can be reduced into an amine, we wonder whether this pathway could be predominant in azide dimerization of an OEG-based substrate. To evaluate this alternative mechanism, the reduction of azide-OEG **4a** was monitored by ^{1}H NMR spectroscopy. The reaction was performed at room temperature, for reducing the kinetics of the reaction and allowing isolation and characterization of the intermediates. Aliquots were taken from the reaction mixture at regular intervals (5 min, 30 min, 1 h, 3 h and 22 h), filtered through a Celite pad, concentrated under reduced pressure and analyzed by ^{1}H NMR spectroscopy (Fig. 2). The complete conversion of **4a** was observed after 1 h leading first, as suspected, to the formation of the corresponding nitrile **15** and primary amine **7a**



Scheme 3 Synthesis of a bis-azide OEG_4 amine. * Isolated yield after dimerization and *N*-Boc protection.

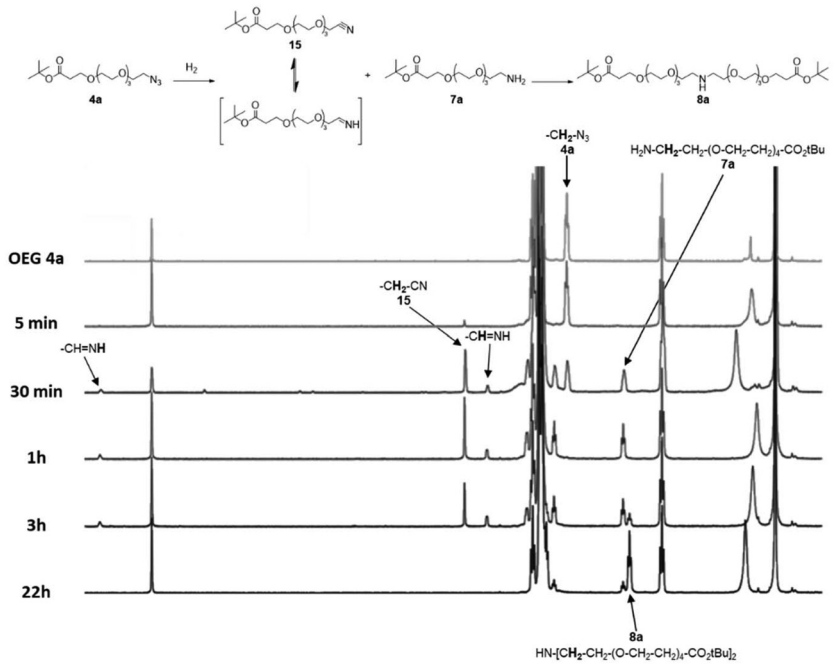


Fig. 2 ^{1}H NMR monitoring of reductive azide dimerization.

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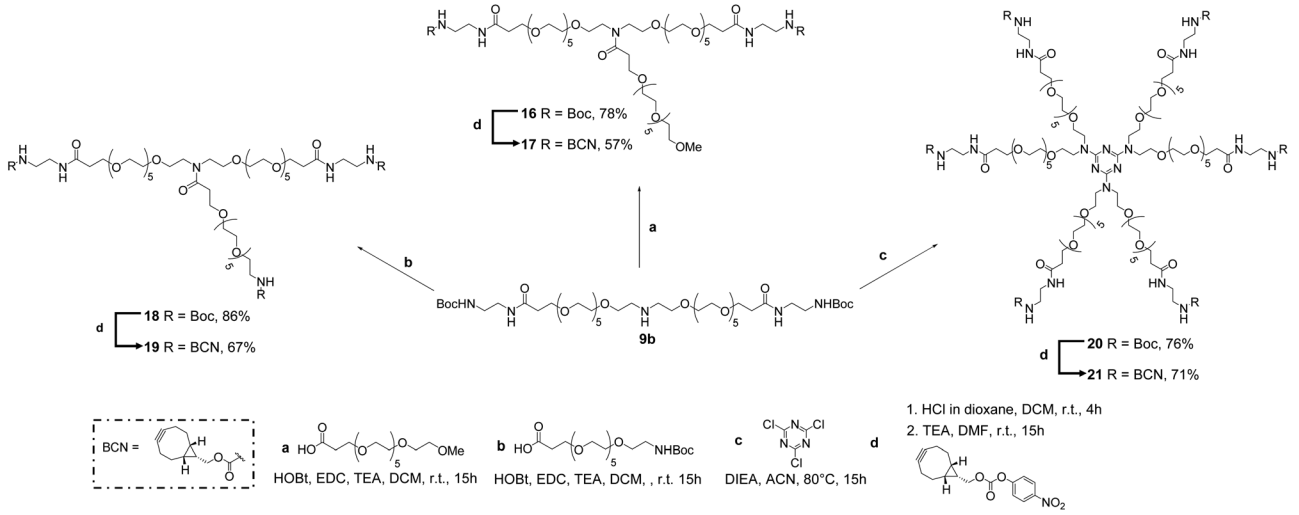
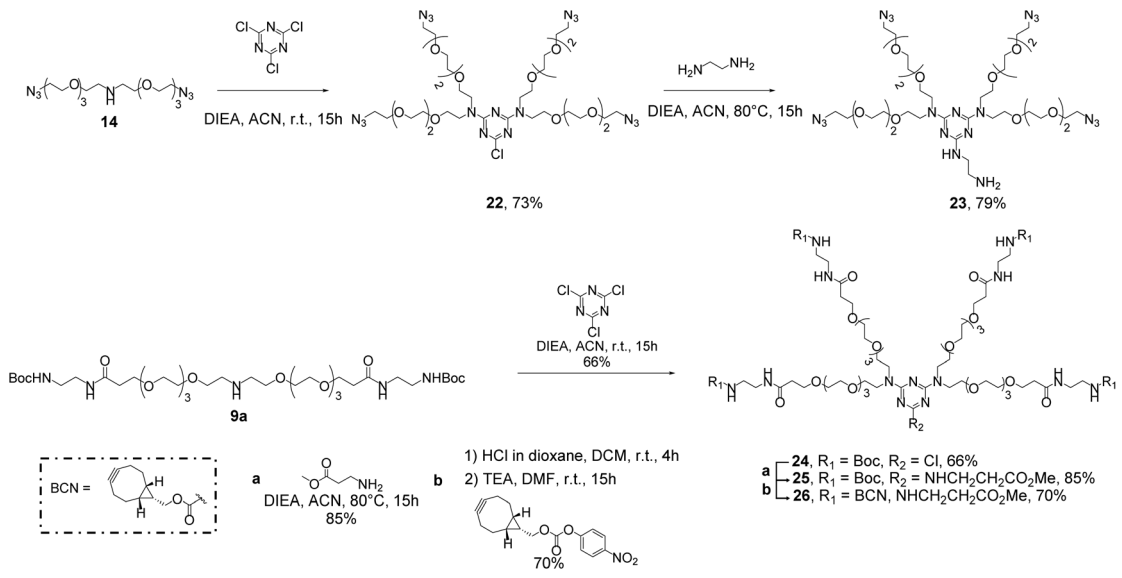
derivatives, which were isolated and characterized. With prolonged reaction times, we observed the formation of the bis-alkylamine **8a**, while the concentration of both nitrile and primary amine decreased, suggesting that the reductive azide dimerization involves indeed a nitrile substrate as an intermediate. A transient compound whose chemical shifts match those of the imine derivative could also be observed. This study thus suggests that the reductive dimerization involves the formation of a nitrile and an imine intermediate, which can then react with the reduced free amine derivative to form the corresponding symmetrical dialkylamine compound. Interestingly, the ratio between the nitrile and imine forms remains constant during the reaction suggesting an equilibrium between the two intermediates. These observations are in agreement with Díez-González's work describing the synthesis of nitrile and imine compounds from the corresponding azide derivatives using Pd catalysts.[22] Moreover the direct synthesis of secondary or tertiary amines *via* catalytic reduction of nitriles has been widely described in the literature affording secondary[23–29] and tertiary amines.[30]

To assess this hypothesis, the isolated nitrile derivative **15** was subjected to the reductive dimerization conditions. We were delighted to notice the formation of the corresponding bis-alkyl amine **8a** that was isolated in 50% yield, thus demonstrating that the nitrile derivative is a key intermediate in this reaction.

Finally, we demonstrated that these trifunctional OEG-amine linkers can be used for the preparation of poly-functional platforms increasing the multivalency of chemical platforms particularly when these building blocks are introduced on multi-functional cores such as triazine (Scheme 4). Starting



Scheme 4 Preparation of compact tri- and hexa-functional OEG platforms.



Scheme 5 Preparation of hetero-functional OEG platforms.

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from bis-*N*-Boc-OEG_6-amine **9b**, an additional OEG chain was introduced on the central secondary amine by coupling it with various linear OEG_6 carboxylic acids. The corresponding tri-OEG derivatives **16** and **18** were isolated in 78% and 86% yields, respectively. With the aim of obtaining clickable OEG platforms, the *N*-Boc derivatives **16** and **18** were deprotected to yield the corresponding free amines, which were immediately reacted with an activated BCN[31] carbonate to give the tri-OEG_6 scaffolds **17** and **19** bearing two or three stained alkyne moieties in 57% and 67% yields, respectively. Amine **9b** was then reacted with cyanuric chloride to afford the homo hexa-functional OEG platform **20** in 76% yield (Scheme 4). Subsequent *N*-Boc deprotection delivered the hexa-OEG_6 platform **21**, bearing six strained alkyne moieties, in a good 71% yield.

In order to obtain hetero-tetra-functionalized derivatives, double addition of bis-OEG amine on cyanuric chloride was performed by controlling temperature conditions (Scheme 5). Cyanuric chloride was thus disubstituted at room temperature with either bis-azide OEG **14** or bis-NHBoc-OEG **9a** to yield tetra-azide-OEG **22** or tetra-NHBoc-OEG **24**, respectively, in 73 and 66% yields. These tetra-OEG platforms were then subjected to a third nucleophilic substitution at a higher temperature (80 °C). The introduction of ethylene diamine into **22** afforded the tetra-azide-OEG amine **23** in 79% yield. On the other hand, OEG-platform **24** was reacted with β-alanine methyl ester, giving access to the tetra-NHBoc-OEG-methyl ester **25** in 85% yield. After *N*-Boc deprotection, the resulting free amines were coupled to activated BCN derivatives to deliver tetra-BCN-OEG-methyl ester **26** in 70% yield. Interestingly, the use of our new bis-functional OEG amine instead of the classical heterobifunctional OEG enabled the doubling of the density of the reactive groups per generation. These hetero-functionalized OEG platforms are suitable for both click reaction and peptidic coupling and could find various applications in bioconjugation and surface functionalization processes.

Conclusions

In this study, we showed that the reductive azide dimerization is a straightforward reaction for the preparation of trifunctional OEG-amine linkers. After reaction condition optimization we were able to obtain these key compounds in up to 80% yield at the multigram scale in just 2 steps starting from readily available OEG. Our mechanistic investigations by ^{1}H-NMR spectroscopy showed that this coupling reaction proceeds *via* a nitrile and an imine intermediate which can further undergo reductive coupling with a free amine. From these bis-OEG amines, which bear minimal structural perturbation as compared to a native OEG backbone, we developed a series of trifunctional reagents. We further illustrated the usefulness of these original bis-OEG amines by preparing a collection of compact first generation of multifunctional discrete PEG-dendrons ready to serve in materials science, nanochemistry or bioconjugation.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 J. L. Zimmermann, T. Nicolaus, G. Neuert and K. Blank, *Nat. Protoc.*, 2010, **5**, 975–985.
2 H. Chen, Y. Chen, H. Sheardown and M. A. Brook, *Biomaterials*, 2005, **26**, 7418–7424.
3 C. Boozer, J. Ladd, S. Chen, Q. Yu, J. Homola and S. Jiang, *Anal. Chem.*, 2004, **76**, 6967–6972.
4 K. Knop, R. Hoogenboom, D. Fischer and U. S. Schubert, *Angew. Chem., Int. Ed.*, 2010, **49**, 6288–6308.
5 R. Y. Zhao, S. D. Wilhelm, C. Audette, G. Jones, B. A. Leece, A. C. Lazar, V. S. Goldmacher, R. Singh, Y. Kovtun, W. C. Widdison, J. M. Lambert and R. V. J. Chari, *J. Med. Chem.*, 2011, **54**, 3606–3623.
6 R. P. Lyon, T. D. Bovee, S. O. Doronina, P. J. Burke, J. H. Hunter, H. D. Neff-LaFord, M. Jonas, M. E. Anderson, J. R. Setter and P. D. Senter, *Nat. Biotechnol.*, 2015, **33**, 733–735.
7 E. C. Dreaden, S. C. Mwakwari, Q. H. Sodji, A. K. Oyelere and M. A. El-Sayed, *Bioconjugate Chem.*, 2009, **20**, 2247–2253.
8 L. N. Goswami, Z. H. Houston, S. J. Sarma, S. S. Jalisatgi and M. F. Hawthorne, *Org. Biomol. Chem.*, 2013, **11**, 1116–1126.
9 H. Wada, I. Imamura, M. Sako, S. Katagiri, S. Tarui, H. Nishimura and Y. Inada, *Ann. N. Y. Acad. Sci.*, 1990, **613**, 95–108.
10 A. Martinez, A. Pendri, J. Xia and R. B. Greenwald, *Macromol. Chem. Phys.*, 1997, **198**, 2489–2498.
11 H. Zhao, K. Yang, A. Martinez, A. Basu, R. Chintala, H.-C. Liu, A. Janjua, M. Wang and D. Filpula, *Bioconjugate Chem.*, 2006, **17**, 341–351.
12 Z. Li and Y. Chau, *Polym. Chem.*, 2011, **2**, 873–878.
13 C. Monfardini, O. Schiavon, P. Caliceti, M. Morpurgo, J. M. Harris and F. M. Veronese, *Bioconjugate Chem.*, 1995, **6**, 62–69.
14 P. Bailon, A. Palleroni, C. A. Schaffer, C. L. Spence, W. J. Fung, J. E. Porter, G. K. Ehrlich, W. Pan, Z. X. Xu, M. W. Modi, A. Farid, W. Berthold and M. Graves, *Bioconjugate Chem.*, 2001, **12**, 195–202.
15 K. Rajender Reddy, M. W. Modi and S. Pedder, *Adv. Drug Delivery Rev.*, 2002, **54**, 571–586.
16 C. P. Mandl and B. König, *Synth. Commun.*, 2004, **34**, 3573–3578.
17 A. V. Dix, L. Fischer, S. Sarrazin, C. P. H. Redgate, J. D. Esko and Y. Tor, *ChemBioChem*, 2010, **11**, 2302–2310.

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18 M. Lange, A. L. Pettersen and K. Undheim, *Tetrahedron*, 1998, **54**, 5745–5752.
19 H. Zhu, D. M. Coleman, C. J. Dehen, I. M. Geisler, D. Zemlyanov, J. Chmielewski, G. J. Simpson and A. Wei, *Langmuir*, 2008, **24**, 8660–8666.
20 A. Bouzide and G. Sauvé, *Org. Lett.*, 2002, **4**, 2329–2332.
21 I.-H. An, H.-R. Seong and K.-H. Ahn, *Bull. Korean Chem. Soc.*, 2004, **25**, 420–422.
22 L. Martínez-Sarti and S. Díez-González, *ChemCatChem*, 2013, **5**, 1722–1724.
23 A. Galan, J. De Mendoza, P. Prados, J. Rojo and A. M. Echavarren, *J. Org. Chem.*, 1991, **56**, 452–454.
24 C. Bianchini, P. Barbaro, V. D. Santo, R. Gobetto, A. Meli, W. Oberhauser, R. Psaro and F. Vizza, *Adv. Synth. Catal.*, 2001, **343**, 41–45.
25 S. M. Islam and C. R. Saha, *J. Mol. Catal. A: Chem.*, 2004, **212**, 131–140.
26 K. Rajesh, B. Dudle, O. Blacque and H. Berke, *Adv. Synth. Catal.*, 2011, **353**, 1479–1484.
27 S. Lu, J. Wang, X. Cao, X. Li and H. Gu, *Chem. Commun.*, 2014, **50**, 3512–3515.
28 Y. Sato, Y. Kayaki and T. Ikariya, *Organometallics*, 2016, **35**, 1257–1264.
29 Z. Shao, S. Fu, M. Wei, S. Zhou and Q. Liu, *Angew. Chem., Int. Ed.*, 2016, **55**, 14653–14657.
30 J. Shares, J. Yehl, A. Kowalsick, P. Byers and M. P. Haaf, *Tetrahedron Lett.*, 2012, **53**, 4426–4428.
31 J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chem., Int. Ed.*, 2010, **49**, 9422–9425.

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