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Tetraphenylethylene-based glycoclusters with aggregation-induced emission (AIE) properties as high-affinity ligands of bacterial lectins†

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Tetraphenylethylene (TPE) is fluorescent through aggregation induced emission (AIE) in water. Herein, TPE was used as the core of glycoclusters that target the bacterial lectins LecA and LecB of *Pseudomonas aeruginosa*. Synthesis of these TPE-based glycoclusters was accomplished by using azide–alkyne "click" chemistry. The AIE properties of the resulting glycoclusters could be readily verified, but imaging could not be pursued due to the overlap of the fluorescence signals from cells and bacteria. Nonetheless, the glycoclusters displayed nanomolar affinities toward LecA and LecB. Further evaluation in a cell-based anti-adhesive assay highlighted a limited decrease in adhesion (20%) for the fucosylated glycocluster. This confirmed that these TPE-based glycoclusters are indeed LecA and LecB high-affinity ligands. Nevertheless, the hypotheses involving their application in imaging or anti-adhesive therapy could not be verified.

Introduction

Glycoclusters are composed of a multivalent core conjugated with a single copy of an oligosaccharide on each branching point. This perfect control of the molecular structure allows for an exact valency and provides a single and homogeneous molecule, in contrast to glycopolymers or glyconanoparticles, for which the valency is not fully controlled. While this statement is true for valency, controlling the exact tridimensional shape of glycoclusters can sometimes be more difficult. Glycoclusters are designed with a large series of aromatic cores,[1] among several inherently fluorescent scaffolds.[2–12] We recently applied perylenediimide (PDI) in an anti-adhesive strategy[13] against *Pseudomonas aeruginosa* (PA) and evaluated successful PDI-based glyco-dots in the context of cancer cell detection.[14] As a continuation of these investigations, we report here tetraphenylethylene(TPE)-based glycoclusters as multivalent lectin ligands. We also evaluated their anti-adhesive properties against PA. The aggregation-induced emission (AIE) properties of TPE were investigated to capitalize on its fluorescence properties toward chemical biology applications and imaging of cells. Previous reports have used such TPE-based glycoclusters functionalized with different carbohydrates, such as mannose,[15,16] glucosamine,[17] and 6′-sialyllactose,[18] for the detection of lectins, alkaline phosphatase and the influenza virus, respectively.

Multivalency is a major strategy used for the design of lectin ligands with high affinity.[19] The host–pathogen interaction of PA with the human host cell is mediated by LecA and LecB. These two soluble lectins bind galactosides and fucosides, respectively. While a large series of multivalent ligands of these lectins has been designed,[19] very few incor-

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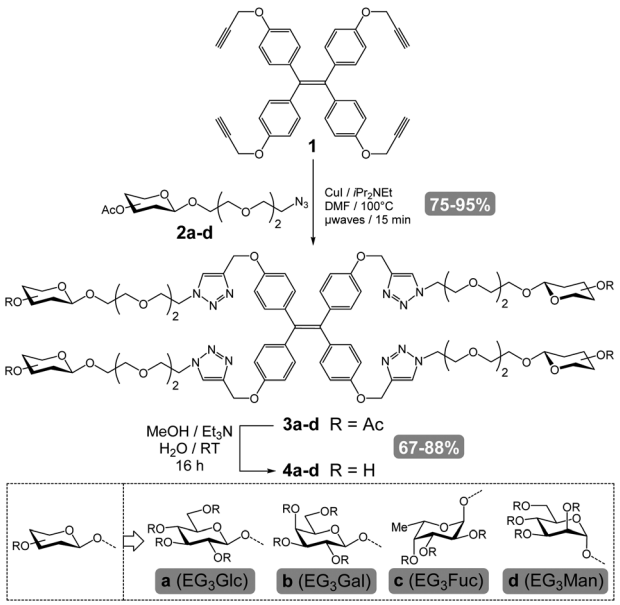
porate a fluorescent core for further chemical biology applications.

Results and discussion

Synthesis of the TPE-based glycoclusters

The synthesis of the known tetrapropargylated tetraphenylethylene (TPE) core **1** was achieved through the McMurry reaction of 4,4′-dimethoxybenzophenone,[20–22] followed by demethylation with boron tribromide to the tetra-phenol,[20,23] and then propargylation to the desired core **1**.[20,23] Four azido-functionalized carbohydrates **2a–d** [24–27] were then conjugated to the tetra-alkynylated TPE core **1** under Meldal's conditions[28,29] (CuI, iPr_2NEt) using microwave activation (Scheme 1). The resulting acetylated glycoclusters **3a–d** were then deacetylated to afford the desired TPE-based glycoclusters **4a–d** in good isolated yields.



Scheme 1 Synthesis of the TPE-based glycoclusters.

ITC binding studies

The affinity of the designed TPE-based glycoclusters was measured by isothermal titration microcalorimetry (ITC) towards LecA or LecB with the corresponding galactosylated or fucosylated glycoclusters (Table 1). The glucosylated glycocluster **4a** was used as a negative control of affinity toward LecA and LecB. The high affinity of the galactosylated TPE-based glycocluster **4b** was evidenced by an apparent dissociation constant (K_d) of 80 nM toward LecA. Moreover, the relative potency (β = 875) is among the best reported for such LecA ligands.[19] The stoichiometry of the complex (N = 0.275–1/4) implies that each of the four galactoside epitopes is involved in a binding interaction with the lectin. The fucosylated glycocluster **4c** displayed a nanomolar apparent K_d value, so binding properties somewhat similar to the monovalent methyl fucoside used as a reference with relative potency (β = 5). Nevertheless, the cluster effect for multivalent LecB ligands is much more difficult to obtain given the tetrahedral geometry of the multimeric protein.[19] Again, all four fucoside epitopes could interact simultaneously with a LecB binding site (N = 0.197–1/4 or even 1/5).

Both glycoclusters **4b** and **4c** displaying nanomolar affinities for their lectins therefore appear as very promising candidates for an anti-adhesive strategy against PA.

Photophysical properties

UV-vis spectra of the TPE-based glycoclusters (Fig. 1a) displayed an absorbance peak at *ca.* 340 nm, which is characteristic of TPE. Upon excitation at 340 nm, while we observed minimal fluorescence in organic solvents, including DMF, DMSO, MeOH and EtOH, the glycoclusters exhibited a much stronger emission in pure water and phosphate buffered saline (0.05 M, pH 7.4). This is in agreement with their typical AIE properties,[32] because the amphiphilic TPE-based glycoclusters could form aggregates in water, as was demonstrated by increasing the water fraction of a DMSO solution of the TPE-based glycoclusters **4a–d** (Fig. 1c and d). The difference in the fluorescence enhancement of the glycoclusters might be due

Table 1 ITC measurements for the binding of TPE-based glycoclusters **4a–c** to LecA or LecB

Ligand	Lectin	N^a	$-\Delta H$ (kJ mol^{-1})	$-T\Delta S$ (kJ mol^{-1})	K_d [b] (nM)	β^c
TPE-$(EG_3Glc)_4$ (**4a**)	LecA	No binding observed (see ESI Fig. S3)				
TPE-$(EG_3Gal)_4$ (**4b**)	LecA	0.275 ± 0.005	111.0 ± 0.5	70.5	80 ± 5	875
TPE-(EG3Glc)4 (**4a**)	LecB	No binding observed (see ESI Fig. S4)				
TPE-(EG3Fuc)4 (**4c**)	LecB	0.197 ± 0.001	119 ± 2	79	84 ± 5	5

[a] Stoichiometry of binding defined as the number of glycoclusters per monomer of LecB. [b] The present dissociation constants are presented for possible aggregates of the TPE-based glycoclusters and therefore must be considered as apparent K_d values. [c] Relative potency: calculated using methyl β-D-galactopyranoside (β-GalOMe) as a monovalent reference with K_d = 70 μM for LecA,[30] or methyl α-L-fucopyranoside (α-FucOMe) as a monovalent reference with K_d = 0.43 μM for LecB.[31]

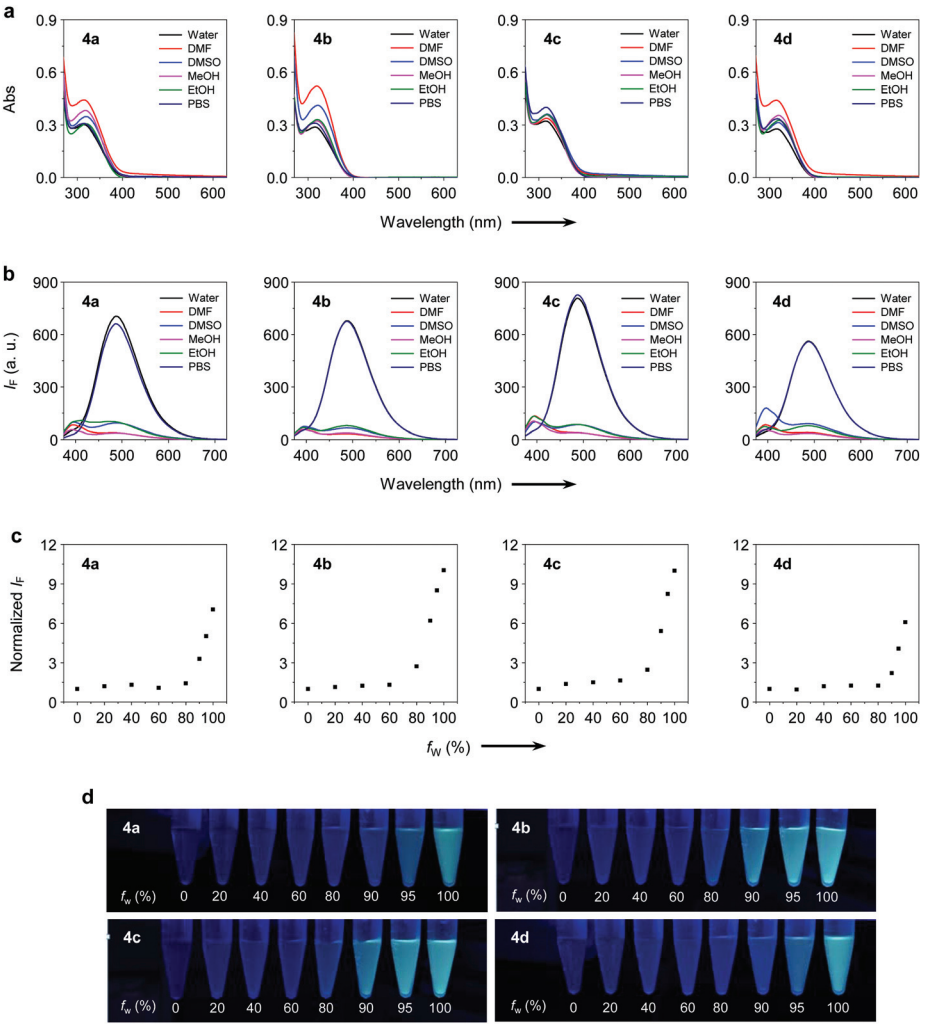


Fig. 1 Photophysical properties of **4a–c**. (a) Stacked UV-vis absorbance spectra of **4a–d** (60 μM) in different organic solvents (DMSO, DMF, EtOH, MeOH), pure water and phosphate buffered saline (0.05 M, pH 7.4). The UV-vis absorbance measurements were carried out at room temperature on a Varian Cary 60 UV-vis spectrophotometer. (b) Stacked fluorescence spectra of **4a–d** (60 μM) in different organic solvents (DMSO, DMF, EtOH, MeOH), pure water and phosphate buffered saline (0.05 M, pH 7.4) with an excitation wavelength of 340 nm. The fluorescence measurements were carried out at room temperature on an Agilent Cary Eclipse fluorescence spectrophotometer. (c) Plot of the fluorescence intensity of **4a–d** (60 μM) in DMSO as a function of water fraction (f_w%); the original intensities of the glycoclusters are normalized as 1.0. (d) AIE of **4a–d** in DMSO with increasing water fraction (f_w%) photographed with a camera.

to the structural difference of the carbohydrate epitopes conjugated on the TPE core.

The galactosylated glycocluster **4b** was investigated using dynamic light scattering (DLS) and high-resolution transmission electron microscopy (HR-TEM) to better understand the aggregation of such TPE-based glycoclusters (Fig. 2). The fluorescence intensity of the glycocluster increased drastically from 10 to 90 μM due to the AIE effect (Fig. 1a). Then fluorescence quenching occurred above these concentrations (from 120 to 600 μM) due to the aggregation-caused quenching (ACQ) effect, bringing the intensity back almost to its initial point (Fig. 2a). The DLS data collected for compound **4b** also confirmed the formation of larger aggregates upon increasing concentrations with typically 20 nm aggregates at 20 μM, 40 nm aggregates at 60 μM, 60 nm aggregates at 180 μM and 150 nm aggregates at 540 μM (Fig. 2b). These observations could be further confirmed by HR-TEM images (Fig. 2c), in which large aggregates can be observed at 180 or 540 μM.

PDI-based glycoclusters have previously been shown to provide weak fluorescence in cell adhesion assays, but their fluorescence is not strong enough for them to be used as imaging agents.[13] TPE-based glycoclusters could also not be applied in this context, since their fluore-

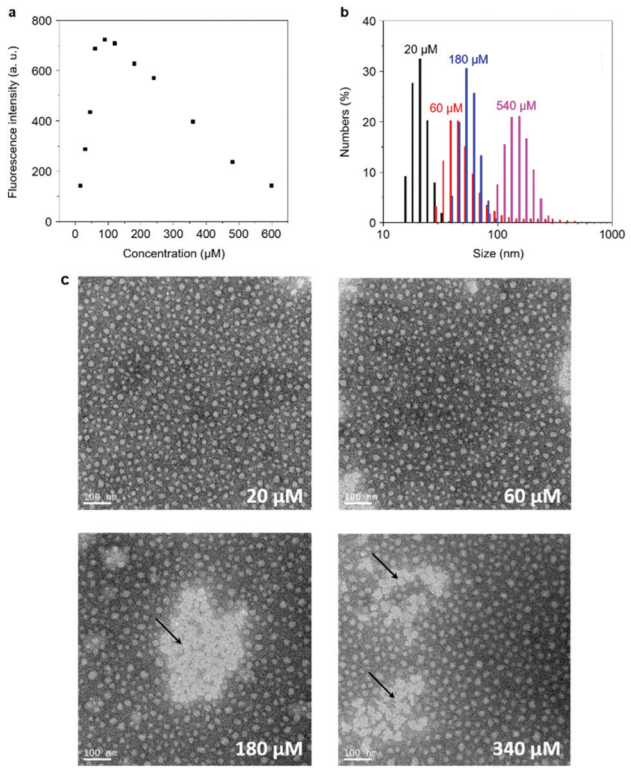


Fig. 2 Concentration-dependent aggregation of the TPE-based glycocluster **4b**. (a) Plot of the fluorescence intensity (at 488 nm) of **4b** as a function of its increasing concentration in deionized water. (b) Dynamic light scattering (DLS) of **4b** at different concentrations in deionized water. (c) Transmission electron microscopy images of **4b** at different concentrations in deionized water. The black arrows indicate **4b** aggregates at higher concentrations.

scence in the blue range overlaps with the intrinsic fluorescence of PAO1.

Cell adhesion assays

The involvement of PA lectins, namely LecA and LecB, in binding TPE-based glycoclusters to host cells was further investigated in an *in vitro* cell culture model using A549 lung epithelial cells. Inhibition of adhesion reaching 20% with fucosylated glycocluster **4d** was clearly observed while a non-significant inhibition of adhesion was demonstrated with galactosylated glycocluster **4b** (Fig. 3a). Adhesion assays performed with both *lecA* or *lecB* mutant strains (Fig. 3b and c) confirmed the lack of inhibition of the galactosylated glycocluster **4b**. The fucosylated glycocluster **4c** had no inhibitory effect on the adhesion of *lecA* or *lecB* mutant strains, highlighting that its inhibition is independent of both LecA and LecB. These results suggest a lack of specificity in the interaction of fucose with LecA, probably due to non-specific interactions with LecA.

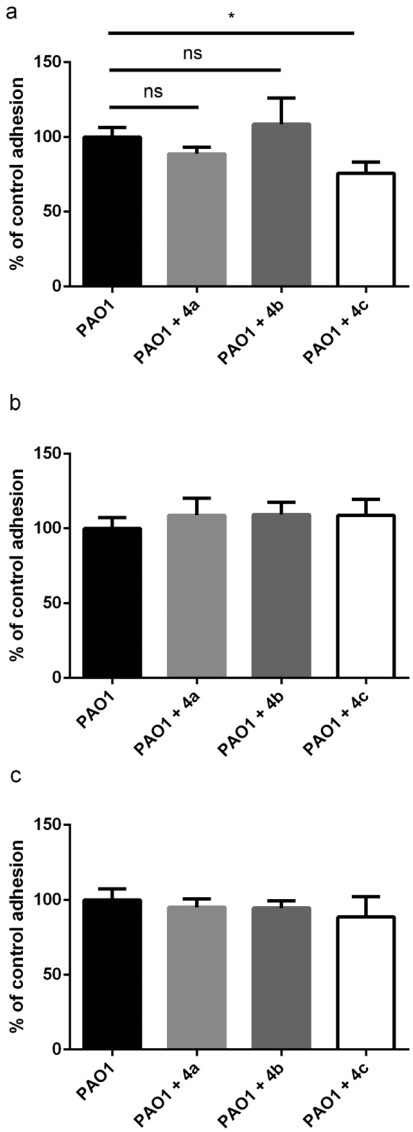


Fig. 3 Bacterial adhesion to A549 cells (3 h infection, MOI 10) in the presence or absence of different TPE-based glycoclusters at increasing concentrations (μM) after washing off (5 times) excess non-adherent bacteria with PBS. (a) Inhibition of adhesion of wild-type PAO1. All results are compared to the first column without adhesion inhibitors (PAO1, positive control). (b) Inhibition of adhesion of Δ*lecA* strain. All results are compared to the second column without adhesion inhibitors (*PAO1ΔlecA*), and the first column showing the PAO1 wild-type strain remains as a control. (c) Inhibition of adhesion of Δ*lecB* strain. All results are compared to the second column without adhesion inhibitors (*PAO1ΔlecB*), and the first column showing the PAO1 wild-type strain remains as a control. All experiments were performed in triplicate. Results are mean ± SEM. (*$p < 0.05$); ns: non-significant.

Conclusions

TPE was incorporated as the core of glycoclusters to take advantage of its intrinsic fluorescence properties caused by the AIE effect. The synthesis of TPE-based glycoclusters could be performed by azide–alkyne "click" chemistry and the result-

ing glycoclusters displayed the expected AIE effect. However, the blue fluorescence from cells and bacteria overlapped, preventing the application of the TPE-based glycoclusters as imaging agents. ITC binding studies with LecA and LecB as the two proteins involved in the adhesion of *Pseudomonas aeruginosa* to host cells provided strong binding properties towards both lectins with K_d values in the nanomolar range. Further evaluation of the TPE-based glycoclusters as anti-adhesive agents proved less encouraging, since only a very limited decrease of adhesion could be observed (20%) for the fucosylated glycocluster. These TPE-based glycoclusters therefore appear as high-affinity ligands of the LecA and LecB bacterial lectins, yet their AIE fluorescence properties could not be exploited for further biomedical applications. Nevertheless, improvement of the biological properties of such glycoclusters can be readily performed through modifying the valency to increase the affinity and anti-adhesive properties. Improvements can also be achieved by modifying the functional groups on the TPE scaffold to provide fluorescent probes with different emission properties that do not overlap with biological samples through a red-shifting strategy.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 Y. M. Chabre and R. Roy, *Chem. Soc. Rev.*, 2013, **42**, 4657–4708.
2 K.-R. Wang, H.-W. An, Y.-Q. Wang, J.-C. Zhang and X.-L. Li, *Org. Biomol. Chem.*, 2013, **11**, 1007–1012.
3 C. Xue, S. P. Jog, P. Murthy and H. Liu, *Biomacromolecules*, 2006, **7**, 2470–2474.
4 K.-R. Wang, Y.-Q. Wang, H.-W. An, J.-C. Zhang and X.-L. Li, *Chem. – Eur. J.*, 2013, **19**, 2903–2909.
5 R. L. Phillips, I.-B. Kim, L. M. Tolbert and U. H. F. Bunz, *J. Am. Chem. Soc.*, 2008, **130**, 6952–6954.
6 Y. Huang, J. Wang and Z. Wei, *Chem. Commun.*, 2014, **50**, 8343–8345.
7 X. Wang, O. Ramström and M. Yan, *Anal. Chem.*, 2010, **82**, 9082–9089.
8 D. Grünstein, M. Maglinao, R. Kikkeri, M. Collot, K. Barylyuk, B. Lepenies, F. Kamena, R. Zenobi and P. H. Seeberger, *J. Am. Chem. Soc.*, 2011, **133**, 13957–13966.
9 Y. Wang, J. C. Gildersleeve, A. Basu and M. B. Zimmt, *J. Phys. Chem. B*, 2010, **114**, 14487–14494.
10 K. Petkau, A. Kaeser, I. Fischer, L. Brunsveld and A. P. H. J. Schenning, *J. Am. Chem. Soc.*, 2011, **133**, 17063–17071.
11 K.-R. Wang, H.-W. An, F. Qian, Y.-Q. Wang, J.-C. Zhang and X.-L. Li, *RSC Adv.*, 2013, **3**, 23190–23196.
12 M. L. Lepage, A. Mirloup, M. Ripoll, F. Stauffert, A. Bodlenner, R. Ziessel and P. Compain, *Beilstein J. Org. Chem.*, 2015, **11**, 659–667.
13 M. Donnier-Maréchal, N. Galanos, T. Grandjean, Y. Pascal, D.-K. Ji, L. Dong, E. Gillon, X.-P. He, A. Imberty, E. Kipnis, R. Dessein and S. Vidal, *Org. Biomol. Chem.*, 2017, **15**, 10037–10043.
14 Y. Liu, D.-K. Ji, L. Dong, N. Galanos, Y. Zang, J. Li, S. Vidal and X.-P. He, *Chem. Commun.*, 2017, **53**, 11937–11940.
15 T. Sanji, K. Shiraishi, M. Nakamura and M. Tanaka, *Chem. – Asian J.*, 2010, **5**, 817–824.
16 J.-X. Wang, Q. Chen, N. Bian, F. Yang, J. Sun, A.-D. Qi, C.-G. Yan and B.-H. Han, *Org. Biomol. Chem.*, 2011, **9**, 2219–2226.
17 Q. Chen, N. Bian, C. Cao, X.-L. Qiu, A.-D. Qi and B.-H. Han, *Chem. Commun.*, 2010, **46**, 4067–4069.
18 T. Kato, A. Kawaguchi, K. Nagata and K. Hatanaka, *Biochem. Biophys. Res. Commun.*, 2010, **394**, 200–204.
19 S. Cecioni, A. Imberty and S. Vidal, *Chem. Rev.*, 2015, **115**, 525–561.
20 X.-M. Hu, Q. Chen, J.-X. Wang, Q.-Y. Cheng, C.-G. Yan, J. Cao, Y.-J. He and B.-H. Han, *Chem. – Asian J.*, 2011, **6**, 2376–2381.
21 Q. Qi, Y. Liu, X. Fang, Y. Zhang, P. Chen, Y. Wang, B. Yang, B. Xu, W. Tian and S. X.-A. Zhang, *RSC Adv.*, 2013, **3**, 7996–8002.
22 C. Li, X. Luo, W. Zhao, C. Li, Z. Liu, Z. Bo, Y. Dong, Y. Q. Dong and B. Z. Tang, *New J. Chem.*, 2013, **37**, 1696–1699.
23 J. Wu, S. Sun, X. Feng, J. Shi, X.-Y. Hu and L. Wang, *Chem. Commun.*, 2014, **50**, 9122–9125.
24 S. Wang, N. Galanos, A. Rousset, K. Buffet, S. Cecioni, D. Lafont, S. P. Vincent and S. Vidal, *Carbohydr. Res.*, 2014, **395**, 15–18.
25 S. Cecioni, D. Goyard, J.-P. Praly and S. Vidal, *Methods Mol. Biol.*, 2012, **808**, 57–68.
26 S. Cecioni, M. Almant, J.-P. Praly and S. Vidal, Synthesis of Azido-Functionalized Carbohydrates for the Design of Glycoconjugates, in *Carbohydrate Chemistry: Proven Synthetic Methods*, ed. P. Kováč, CRC Press, Boca Raton, 2012, vol. 1, pp. 175–180.
27 J. L. Xue, S. Cecioni, L. He, S. Vidal and J.-P. Praly, *Carbohydr. Res.*, 2009, **344**, 1646–1653.
28 M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952–3015.

29 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
30 S. Cecioni, R. Lalor, B. Blanchard, J.-P. Praly, A. Imberty, S. E. Matthews and S. Vidal, *Chem. – Eur. J.*, 2009, **15**, 13232–13240.
31 C. Sabin, E. P. Mitchell, M. Pokorná, C. Gautier, J.-P. Utille, M. Wimmerová and A. Imberty, *FEBS Lett.*, 2006, **580**, 982–987.
32 R. Hu, N. L. C. Leung and B. Z. Tang, *Chem. Soc. Rev.*, 2014, **43**, 4494–4562.