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Facile triflic acid-catalyzed α -1,2-*cis*-thio glycosylations: scope and application to the synthesis of *S*-linked oligosaccharides, glycolipids, sublancin glycopeptides, and T_N/T_F antigens

Catalytic triflic acid cleanly knits together glycan donors with thiol acceptors to form glycosyl linkages to peptides, sugars and lipids with excellent α -selectivity. This back cover art was generated from images downloaded from the Protein Data Bank's NGL viewer. (Rose *et al.*, *Bioinformatics* **2018**, *34*, 3755–3758.)



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Facile triflic acid-catalyzed α -1,2-*cis*-thio glycosylations: scope and application to the synthesis of *S*-linked oligosaccharides, glycolipids, sublancin glycopeptides, and T_N/T_F antigens†

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Studies of *S*-linked glycoconjugates have attracted growing interest because of their enhanced chemical stability and enzymatic resistance over *O*-glycoside counterparts. We here report a facile approach to access α -1,2-*cis*-*S*-linked glycosides using triflic acid as a catalyst to promote the glycosylation of a series of thiols with *D*-glucosamine, galactosamine, glucose, and galactose electrophiles. This method is broadly applicable for the stereoselective synthesis of *S*-linked glycopeptides, oligosaccharides and glycolipids in high yield and excellent α -selectivity. Many of the synthetic limitations associated with the preparation of these *S*-linked products are overcome by this catalytic method.

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Introduction

Protein glycosylation, one of the most ubiquitous post-translational modifications, typically involves the attachment of carbohydrate chains to proteins through the hydroxyl group of serine or threonine (*O*-glycans) or the amido group of asparagine (*N*-glycans).^{1–5} The resulting glycoproteins exhibit a diverse array of biological functions such as cell adhesion, protein folding, signal transduction, and immune response.^{1–5} However, naturally occurring glycoproteins exist as mixtures of glycoforms, and their isolation as homogeneous species is a complicated process.⁶ As such, there is a great demand for methods to efficiently access structurally defined glycoproteins. Recently, replacement of the anomeric oxygen of *O*-linked glycosides with a sulfur atom to generate *S*-linked glycosides has attracted considerable attention because of the enhanced resistance of the latter to chemical and enzymatic hydrolysis.^{7,8} In addition, *S*-linked glycosides exhibit similar conformational preferences and equal or even improved biological activities compared to their native *O*-glycoside counterparts.^{9–11} In this context, *S*-linked glycan analogs could be utilized as structural mimetics and serve as powerful tools for the biological study of the natural *O*-linked substrates. The recent discovery of *S*-glycosylation, the addition of carbohydrate residues to the sulfur atom of cysteine on bacterial peptides,^{12–14} suggests that

naturally existing *S*-linked glycoproteins may be more widespread than was previously thought and may lead to the development of new therapeutics.

Given the significant importance of thiol-containing carbohydrate molecules, methods that enable access to the challenging α -1,2-*cis* thiol glycosidic linkages are of high synthetic value. Formation of β -1,2-*trans* glycosides can be readily accomplished by employing electrophiles with C(2)-participatory groups.¹⁵ The stereoselective formation of α -1,2-*cis* glycosides, however, has proven challenging, and a mixture of α - and β -glycosides is often obtained.¹⁵ A variety of strategies have been reported for the synthesis of mucin-related α -thiol-containing GalNAc glycopeptide mimetics.^{16–24} Representative methods include stereoselective preparation of α -GalNAc thiols followed by (1) an S_N2 displacement with β -bromoalanine-containing peptides,^{16,17} (2) site-selective conjugation with aziridine-containing peptides,^{18,19} or (3) conjugate addition to dehydroalanine-containing peptides.²⁰ S_N2 reaction of α -glycosyl thiols with 4-axial triflate glycosides^{21,22} 6-iodinated glycosides,^{23–25} enzymatic glycosylation,^{26–29} and metal-catalyzed cross coupling,^{30,31} have also been reported to generate α -1,2 *cis* *S*-linked oligosaccharides and glycopeptides. Despite these important advances over the past decades, numerous challenges remain, including the need for highly specialized coupling partners and the multistep preparation of bromoalanine-, aziridine-, and dehydroalanine-containing amino acid residues or peptides.³² In addition, most of the current methods are limited to specific classes of thiol nucleophiles. To date, there is only one reported method that is applicable for a variety of sulfur nucleophiles, and it uses glycosyl stannane to promote *S*-linked glycoside formation.³¹

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We recently found that triflic acid, released from nickel triflate, can effectively promote the glycosylations of serine/threonine amino acids and hydroxyl groups of carbohydrates with the *C*(2)-*N*-*ortho*-(trifluoromethyl)benzylideneamino *N*-phenyl trifluoroacetimidates.^{33–36} This catalytic system features several advantages such as mild conditions, short reaction time, good yields and excellent levels of α -selectivity. However, the question remains whether it will be suited for *S*-glycosylations as Lewis acid-promoted reactions are known to be incompatible with thiol nucleophiles.^{37–40} Lewis acid-promoted *S*-glycosylations were underutilized with a few limited examples.^{37–40} The reactions generally proceed to provide the coupling products with low yields and require stoichiometric amount of promoter. An inherent issue with the *S*-nucleophiles is their tendency to undergo oxidation to form disulfide and other side products.³⁸ They also react with the TMSOTf catalyst during the glycosylation process.⁴⁰ All those factors make it more challenging to handle *S*-nucleophiles than their *O*-counterparts.

Herein we report a distinct approach to *S*-linked glycosylations: a triflic acid catalyst is shown to enable the facile α -1,2-*cis* synthesis of thiol-oligosaccharides, glycopeptide of antimicrobial sublancin, *S*-linked tumor-associated T_N/T_F antigens, and thiol-glycolipids. This strategy obviates the need for substrate prefunctionalization and can proceed by direct coupling of thiol-containing molecules or cysteine-containing peptides with *N*-phenyl trifluoroacetimidates.

Results and discussions

To test our hypothesis, we initiated our study by examining the glycosylation of *N*-Cbz cysteine methyl ester **2** with *C*(2)-*N*-*ortho*-(trifluoromethyl)benzylidene glucosamine *N*-phenyl trifluoroacetimidate **1** under previously established conditions (Table 1).³⁶ To our delight, the coupling of **2** with **1** was

successful using 15 mol% nickel triflate, Ni(OTf)₂, (entry 1). As we expected based on the previously reported conditions,³⁶ the reaction with 5 mol% triflic acid, TfOH, proceeded to completion within 1 h at 35 °C (entry 2) to afford the desired thiol-linked glycoconjugate **4** in 68% yield with excellent selectivity (α : β > 20 : 1). This result is consistent with our recently reported mechanism of the triflic acid-catalyzed α -selective 1,2-*cis* glycosylation with *N*-phenyl trifluoroacetimidate electrophiles.³⁶ Specifically, triflic acid engages in the activation of electrophile **1** to generate a glycosyl triflate intermediate, which then undergoes equilibration from the stable α -anomer to the more reactive β -anomer. Subsequent S_N2-like displacement of the reactive β -anomer of glycosyl triflate by **2** results in the formation of **4** with exclusive α -configuration.

We observed that while the reaction proceeded to completion faster with use of triflic acid, the glycol elimination product derived from donor **1** was also detected (see Fig. S1† for details). Lowering the reaction temperature (entry 3) and catalyst loading (entry 4) provided a similar outcome (Table 1). To address the problem of **1** from undergoing elimination, the cysteine residue **2** was utilized as the limiting reagent in the presence of 1.5 equiv. of donor **1**. This modification improved the yield to 76% (entry 5). Further increasing the donor **1** to two equivalents gave the desired coupling product **4** in an enhanced 81% yield while maintaining high levels of selectivity (entry 6). Most notably, the glycosylation reached completion within 1 h at 25 °C with use of 5 mol% TfOH to provide **4** with comparable yield and α -selectivity (entry 7). The cysteine residue **3** with *N*-Fmoc protection, commonly utilized in the solid-phase peptide synthesis (SPPS), also displayed good efficiency (entry 8) to provide the desired glycoconjugate **5** (Table 1) in good yield and α -selectivity.

With the optimized conditions in hand, we evaluated the scope of the triflic acid-catalyzed *S*-glycosylations (Fig. 1) using

Table 1 Optimization of the reaction conditions^a



Entry	1 (equiv.)	2 or 3 (equiv.)	Catalyst	Temp. (°C)	Time (h)	4 or 5 yield (α : β)
1	1	2 (1.5)	15 mol% Ni(OTf) ₂	35	16	4 : 66% (>20 : 1)
2	1	2 (1.5)	5 mol% TfOH	35	1	4 : 64% (>20 : 1)
3	1	2 (1.5)	5 mol% TfOH	25	2	4 : 68% (>20 : 1)
4	1	2 (1.5)	1 mol% TfOH	25	20	4 : 67% (>20 : 1)
5	1.5	2 (1.0)	3 mol% TfOH	25	3	4 : 76% (>20 : 1)
6	2	2 (1.0)	3 mol% TfOH	25	3	4 : 81% (>20 : 1)
7	2	2 (1.0)	5 mol% TfOH	25	1	4 : 80% (>20 : 1)
8	2	3 (1.0)	5 mol% TfOH	25	1	5 : 78% (>20 : 1)

^a The reaction was conducted with 0.1–0.2 mmol of donor **1**. Yields of the isolated product averaged two runs. The (α / β) ratios were determined by ¹H NMR analysis.



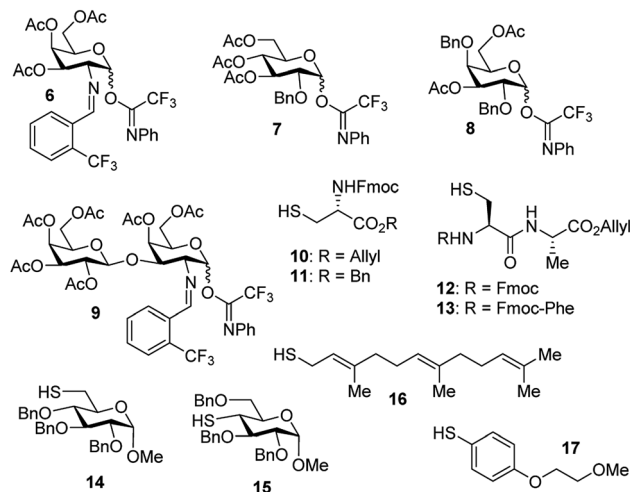


Fig. 1 Carbohydrate donors and thiol-containing acceptors.

glycosyl *N*-phenyl trifluoroacetimidate donors (**1**, **6–9**) and thiol-containing molecules (**10–17**). Based on our previous study,³⁶ we anticipated that *N*-phenyl trifluoroacetimidates **1**, **6** and **7** would exhibit high α -selectivity because their C(2)-*N*-benzylidene and C(2)-benzyl ether can modulate the electronic properties of the anomeric carbon, facilitating isomerization of the glycosyl triflate intermediate. Indeed, both donors **1** and **6** were effectively glycosylated to cysteine amino acids **10** and **11** to provide **1a**, **1b**, and **6a**, respectively, in good yield and with excellent α -selectivity (Table 2). There are several underlying factors that could influence the stereochemical outcome of the coupling event. To confirm that the α -selectivity arise from the directing ability of the C(2)-*N*-benzylidene group, we also coupled C(2)-azido donors **18** and **19** with cysteine residue **10** under standard conditions (Scheme 1). The desired products **18a** and **19a** were obtained with poor levels of α -selectivity (α : β = 1.6 : 1 to 2 : 1).

These results in Scheme 1 support the importance of the C(2)-*N*-benzylidene group in the triflic acid-catalyzed stereoselective α -1,2-*cis* *S*-linked glycosylation. Next, we expanded nucleophilic scope from single cysteine amino acid to more complex peptides. Both dipeptide **12** and tripeptide **13** (Fig. 1) performed well under the standard conditions with **1** and **6** to afford glycopeptides **1c**, **6b**, and **6c** (Table 2), presaging the potential utility of this transformation for access *S*-linked glycopeptides of tumor-associated mucin T_N and T_F antigens (*vide infra*).^{11,44}

In addition to C(2)-*N*-benzylidene electrophiles **1** and **6**, the C(2)-*O*-benzyl protecting group is tolerated. Glucose donor **7** (Table 2) was an effective electrophilic partner, providing the coupling products **7a–7d** in excellent yields (87–95%) with exclusive α -configuration. More importantly, donor **7** does not generate a glycal elimination product. Employing the axial 4-*O*-benzyl protected donor **8** in place of the equatorial 4-*O*-acetyl group (**7**) slightly diminished the α -selectivity (**8a**: α : β > 20 : 1, **8b**: α : β = 4.5 : 1). Nevertheless, this result illustrates the ability of this catalytic system to overturn the inherent bias of *D*-galactose donors whose axial C(4)-*O*-benzyl protecting group

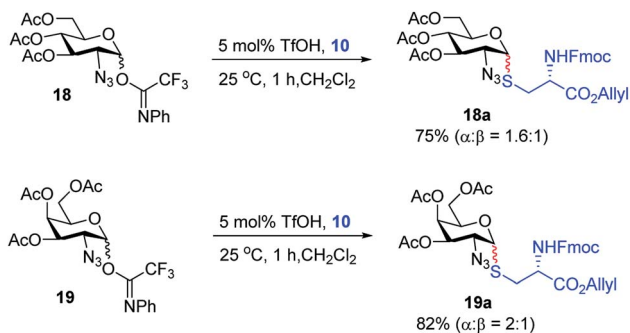
Table 2 Scope with respect to cysteine-containing peptides^a



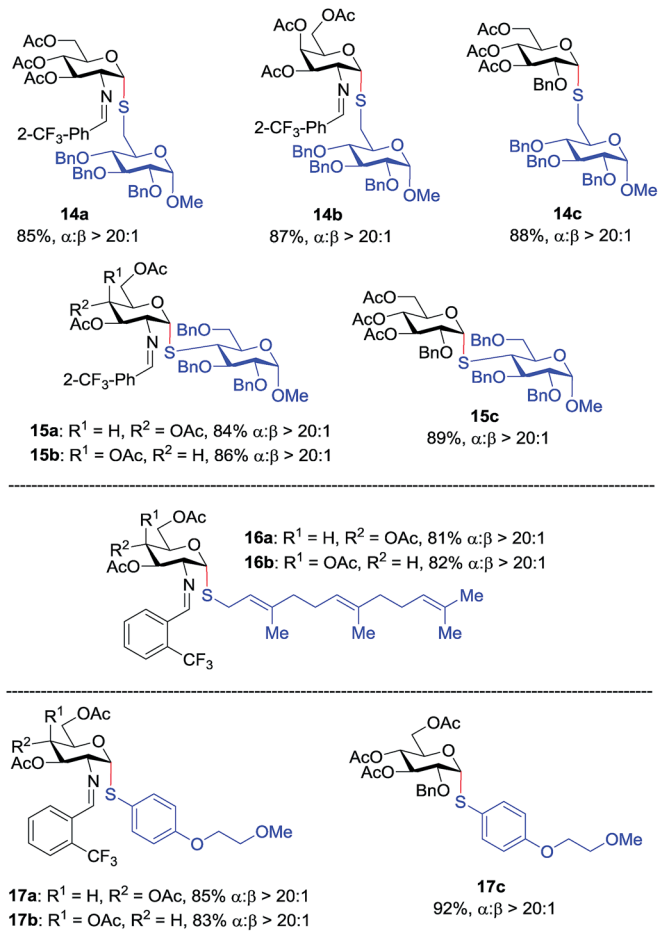
^a All reactions were conducted with donor (2 equiv.), acceptor (1 equiv.) and 5 mol% TfOH in CH₂Cl₂ at 25 °C for 1 h. Yields of isolated *S*-linked glycopeptides averaged two runs. The (α / β) ratios were determined by ¹H NMR analysis.

has been reported to favor β -products.⁴¹ The difference in α -selectivity between the coupling products **8a** and **8b** could be explained by the relative rate for anomerization of the α -to the β -glycosyl triflate intermediate generated from the reaction of glycosyl electrophile **8** with triflic acid. Since a dipeptide is



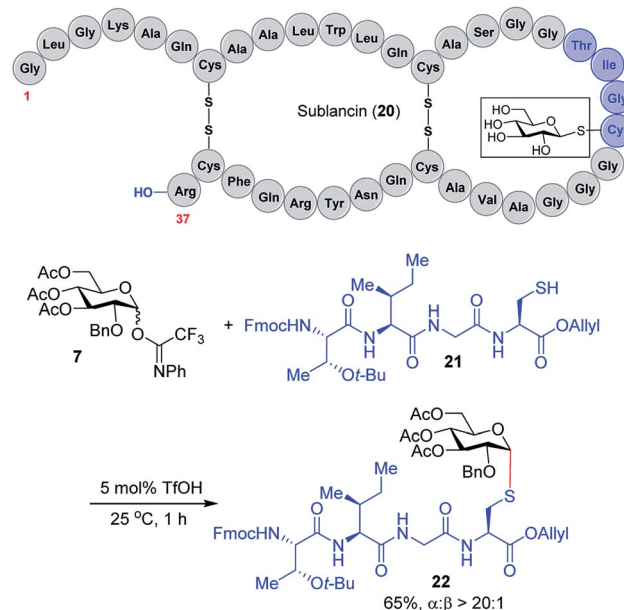
Scheme 1 Glycosylation with C(2)-azido donors **18** and **19**.

a more reactive nucleophile than a cysteine amino acid residue, less time is allowed for anomerization of the α -triflate to the more reactive β -triflate, resulting in decreasing the α -selectivity observed in the coupling product **8b**. Finally, disaccharide donor **9**, a carbohydrate motif of T_F antigen,⁴² was readily coupled to provide the corresponding 1,2-*cis* *S*-glycoconjugates **9a** and **9b** (63–69%, $\alpha : \beta > 20 : 1$).

Table 3 Scope with respect to thiol-containing acceptors^a^a See Table 2.

Inspired by the above discovery, we next examined the scope with respect to nucleophilic coupling partners **14**–**17**. As illustrated in Table 3, the glycosylations of primary (**14**) and secondary (**15**) thiol acceptors with donors **1**, **6**, and **7** proceeded smoothly to produce the desired *S*-linked disaccharides **14a**–**c** and **15a**–**c** in good yields (84–89%) with exclusive α -selectivity. The *trans,trans*-farnesyl mercaptan **16** was also a suitable nucleophile (**16a**, **b**), revealing the potential utility of this method for preparation of *S*-linked glycolipids. Recently, Mes-saudi and co-workers reported the Pd-mediated cross coupling of aryl iodides with β -glycosyl thiols to generate *S*-linked glycosides with exclusive β -configuration.³⁰ For comparison, we examined the coupling efficiency of our method with aryl thiol **17**. To our delight, the reaction compared favorably with excellent α -stereoselectivity and yield (**17a**–**c**).

We expect that the triflic acid-catalyzed α -1,2-*cis* *S*-linked glycosylation method will be particularly useful when applied to the synthesis of bioactive glycopeptides. To establish the potential of a late-stage glycosylation approach, we prepared the tetrapeptide sequence surrounding the β -linked D-glucose unit in sublancin **20** (Scheme 2). This *S*-linked glycosyl unit is essential for antimicrobial activity.¹² SunS, the recently discovered *S*-glycosyl transferase enzyme, is responsible for the unusual glycosylation of cysteine residues with carbohydrates.¹² It has a relaxed substrate specificity and is able to glycosylate other hexose sugars, which has allowed its use in the preparation of sublancin analogs bearing other β -linked glycans.^{12,13} However, to the best of our knowledge, the activity of α -1,2-*cis* *S*-linked sublancin analogs has not been investigated,¹⁰ presumably because of the difficulty of α -1,2-*cis* *S*-glycosylations. Our current catalytic method would provide an ideal approach for the assembly of such analogs. Accordingly, we investigated the coupling of tetrapeptide **21** with glucose donor **7** in the presence of 5 mol% triflic acid. The



Scheme 2 Synthesis of the sublancin glycopeptide fragment.



- 12 T. J. Oman, J. M. Boettcher, H. Wang, X. N. Okalibe and W. A. van der Donk, *Nat. Chem. Biol.*, 2011, **7**, 78.
- 13 S. Biswas, C. V. Garcia De Gonzalo, L. M. Repka and W. A. van der Donk, *ACS Chem. Biol.*, 2017, **12**, 2965.
- 14 C. Wu, S. Biswas, C. V. Garcia De Gonzalo and W. A. van der Donk, *ACS Infect. Dis.*, 2019, **5**, 454.
- 15 M. J. McKay and H. M. Nguyen, *ACS Catal.*, 2012, **2**, 1563.
- 16 C. F. Liang, M. C. Yan, T. C. Chang and C. C. Lin, *J. Am. Chem. Soc.*, 2009, **131**, 3138.
- 17 X. M. Zhu and R. R. Schmidt, *Chem.–Eur. J.*, 2004, **10**, 875.
- 18 D. P. Galonic, N. D. Ide, W. A. van der Donk and D. Y. Gin, *J. Am. Chem. Soc.*, 2005, **127**, 7359.
- 19 D. P. Galonic, W. A. van der Donk and D. Y. Gin, *J. Am. Chem. Soc.*, 2004, **126**, 12712.
- 20 M. I. Gutierrez-Jimenez, C. Aydilho, C. D. Navo, A. Avenzoza, F. Corzana, G. Jimenez-Oses, M. M. Zurbano, J. H. Busto and J. M. Peregrina, *Org. Lett.*, 2016, **18**, 2796.
- 21 L. Lazar, M. Csavas, M. Herczeg, P. Herczegh and A. Borbas, *Org. Lett.*, 2012, **14**, 4650.
- 22 S. Mandal and U. J. Nilsson, *Org. Biomol. Chem.*, 2014, **12**, 4816.
- 23 G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. El Oualid, T. D. W. Claridge and B. G. F. Davis, *Angew. Chem., Int. Ed.*, 2008, **47**, 2244–2247.
- 24 X. M. Zhu, T. Haag and R. R. Schmidt, *Org. Biomol. Chem.*, 2004, **2**, 31.
- 25 E. Calce, G. Digilio, V. Menchise, M. Saviano and S. De Luca, *Chem.–Eur. J.*, 2018, **24**, 6231.
- 26 J. R. Rich, A. Szpacenko, M. M. Palcic and D. R. Bundle, *Angew. Chem., Int. Ed.*, 2004, **43**, 613.
- 27 J. R. Rich, W. W. Wakarchuk and D. R. Bundle, *Chem.–Eur. J.*, 2006, **12**, 845.
- 28 G. Tegl, J. Hanson, H. M. Chen, D. H. Kwan, A. G. Santana and S. G. Withers, *Angew. Chem., Int. Ed.*, 2019, **58**, 1632.
- 29 H. Wang, T. J. Oman, R. Zhang, C. V. G. De Gonzalo, Q. Zhang and W. A. van der Donk, *J. Am. Chem. Soc.*, 2014, **136**, 84.
- 30 D. Montoir, M. Amoura, Z. E. Ababsa, T. M. Vishwanatha, E. Yen-Pon, V. Robert, M. Beltramo, V. Piller, M. Alami, V. Aucagne and S. Messaoudi, *Chem. Sci.*, 2018, **9**, 8753.
- 31 F. Zhu, E. Miller, S. Q. Zhang, D. Yi, S. O'Neill, X. Hong and M. A. Walczak, *J. Am. Chem. Soc.*, 2018, **140**, 18140.
- 32 K. Pachamuthu and R. R. Schmidt, *Chem. Rev.*, 2006, **106**, 160.
- 33 E. A. Mensah and H. M. Nguyen, *J. Am. Chem. Soc.*, 2009, **131**, 8778.
- 34 E. A. Mensah, F. Yu and H. M. Nguyen, *J. Am. Chem. Soc.*, 2010, **132**, 14288.
- 35 F. Yu, M. S. McConnell and H. M. Nguyen, *Org. Lett.*, 2015, **17**, 2018.
- 36 E. T. Sletten, Y.-J. Tu, H. B. Schlegel and H. M. Nguyen, *ACS Catal.*, 2019, **9**, 2110.
- 37 C. A. De Leon, G. Lang, M. I. Saavedra and M. R. Pratt, *Org. Lett.*, 2018, **20**, 5032.
- 38 E. Repetto, V. E. Manzano, M. L. Uhrig and O. Varela, *J. Org. Chem.*, 2012, **77**, 253.
- 39 A. Noel, B. Delpuch and D. Crich, *Org. Lett.*, 2012, **14**, 4138.
- 40 G. H. Xin and X. M. Zhu, *Tetrahedron Lett.*, 2012, **53**, 4309.
- 41 S. Chatterjee, S. Moon, F. Hentschel, K. Gilmore and P. H. Seeberger, *J. Am. Chem. Soc.*, 2018, **140**, 11942.
- 42 S. Nath and P. Mukherjee, *Trends Mol. Med.*, 2014, **20**, 332.
- 43 H. Coelho, T. Matsushita, G. Artigas, H. Hinou, F. J. Canada, R. Lo-Man, C. Leclerc, E. J. Cabrita, J. Jimenez-Barbero, S. Nishimura, F. Garcia-Martin and F. Marcelo, *J. Am. Chem. Soc.*, 2015, **137**, 12438.
- 44 R. Lo-Man, S. Vichier-Guerre, R. Perraut, E. Deriaud, V. Huteau, L. BenMohamed, O. M. Diop, P. O. Livingston, S. Bay and C. Leclerc, *Cancer Res.*, 2004, **64**, 4987.

