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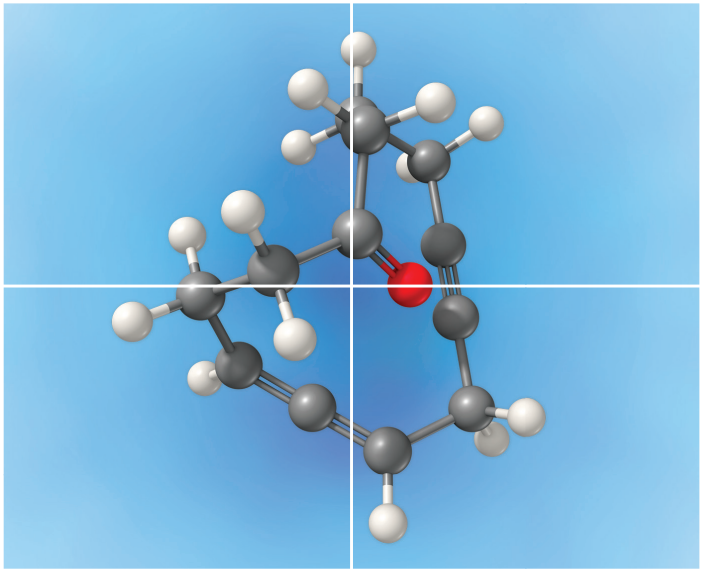

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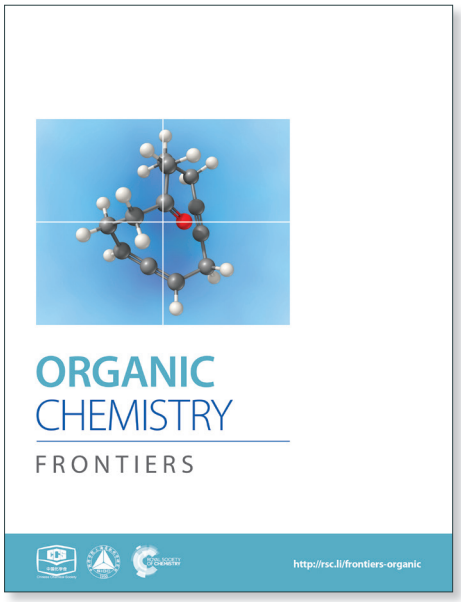

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ARTICLE

Glycosylation with *N*-acetyl glycosamine donors using catalytic iron(III) triflate: from microwave batch chemistry to a scalable continuous-flow process†‡

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Efficient and highly selective glycosylation reactions of peracetylated β-D-*N*-acetyl gluco- and galactosamine are described using catalytic iron (III) triflate under microwave conditions or in a continuous flow process. Simple β-glycosides and β-(1→6), β-(1→2) and β-(1→3) linked disaccharides bearing various protecting groups were obtained in high yields. Insights into the glycosylation mechanism are discussed.

Introduction

Numerous natural glycoconjugates (oligomeric structures and small molecules)[1,2] contain *N*-acetylated-D-glucosamine residues connected through a 1,2-*trans* linkage. They are implicated in important biological systems, such as structural polysaccharides (chitin), circulating signaling molecules (chitooligosaccharides[3] and lipo-chitooligosaccharides[4,5]), tumor markers (sialyl-Lewis X), anticoagulants (heparin), glycoproteins (multiantennary complex type *N*-glycans)[6] or as an essential part of small molecules for various bioactivities.[7] In all cases these structures are difficult to obtain from natural sources. The main challenge of these syntheses is the glycosidic linkage formation through glycosylation, one of the most studied reactions in organic synthesis,[8] especially in the case of *N*-acetyl D-glucosamine.

Under glycosylation conditions, the 2-acetamido group in sugar donors bearing various leaving groups at C-1 forms a rather stable 1,2-*O,N*-oxazoline, which must be opened by acceptors under appropriate conditions to give the *trans* glycoside.

Numerous β-selective glycosylation methods[8, 9] have been developed using elaborated glucosamine donors possessing temporary participating groups[10-12] of the 2-amino function such as the well-known phthaloyl,[13, 14] trichloroethoxycarbonyl,[15] trichloro- and trifluoroacetyl (TCA and TFA) groups,[16, 17] and more recently the *N*-acetyl-2,3-oxazolidinone group.[18, 19] The appropriate leaving groups at C-1 are the trichloroacetimidate,[20] phosphite,[21] or thio groups.[22] The reactions generally proceed at low temperature with high yields but require separated steps for the introduction of the protecting groups and the post-coupling conversion to the 2-acetamido substituent found in natural products. To date, these methods have been the most commonly used for the synthesis of glycoconjugates bearing an *N*-acetylated-D-glucosamine residue.

Glycosylation with glycosyl acetate donors[23] is a straightforward alternative to the above methods using donors bearing complex leaving groups at the anomeric position.[9] It involves a direct acid-catalyzed exchange of the anomeric oxygen to provide the glycosidic acetal. Recently, stoichiometric cupric salts ($CuCl_2$, $CuBr_2$),[24] 30 mol% $Yb(OTf)_3$,[25] 15 mol% rare earth metal triflates [$Sc(OTf)_3$, $Sm(OTf)_3$, $La(OTf)_3$, $Dy(OTf)_3$, $Nd(OTf)_3$],[26, 27] H_2SO_4-silica under microwave conditions,[28] and TsOH[29] were used as promotors in the synthesis of glycosides of *N*-acetyl D-glucosamine (GlcNAc), directly or via the isolated 1,2-*O,N*-oxazolines. Activation using $FeCl_3$ was also previously described for anomeric ester donors incorporating a C-2 amide functionality (*N*-acetyl, *N*-phthaloyl, *N*-chloroacetyl glycosyl acetate donors)[30, 31] via the oxazolinium cations. It was also reported for other donors having a C-2 ester participatory group[32-34] that react via the 1,2-acyloxonium ion. It involved a large excess of both $FeCl_3$ and glycosyl donors producing, in the case of fluorogenic and serine acceptors, rather the α-anomer under anomerization conditions.[35]

Mild conditions using triflates of rare earth metals were previously reported.[9, 26, 27] Iron[36-39] has a number of advantages over other metals typically used in catalysis since it is cheap, non-toxic, environmentally friendly and abundant. In carbohydrate chemistry,[40] iron(III) triflate has only been utilized in a few instances: oxidative C-C bond cleavage,[41] thioglycosylation of peracetylated glycosides[42] and type I Ferrier rearrangement of 2,4,6-tri-*O*-acetyl-D-glucal.[43] Over the

past years, our laboratory has developed several step-saving options that have significantly shortened the synthetic route to bioactive glycoconjugates.[44-49] Along these lines, we recently communicated[50] the glycosylation of the stable and commercially available glucosaminyl donor **1β** using, as the activator, catalytic amounts of stable and non-hygroscopic $Fe(OTf)_3$•6.2DMSO.[51] We present here a full account of this glycosylation: the catalysis design, the scope and limitations of the method, the scale-up using flow chemistry and some mechanistic elements.

Results and Discussion

Catalytic system design

For optimization conditions of the glycosylation glucose derivative **3**[52] was selected as a test sugar acceptor and the results are presented in Table 1. The glycosylation reaction of donor **1β**, prepared by acetylation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[53] was very slow at r.t. (Entries 1, 2, Table 1, 12-38%) and required heating under refluxing conditions for 84 hours in CH_2Cl_2 to furnish **4** in good yields with both $Fe(OTf)_3$•6.2DMSO and $Fe(OTf)_3$ (Entries 5, 6, Table 1, 86-87%). The same range of yields was also obtained using microwave irradiation at 110 °C for 45 min (Entries 7, 8, Table 1, 89-93%). At r.t., the catalyst $Fe(OTf)_3$•6.2DMSO was less efficient than $Fe(OTf)_3$ (Entries 1, 2, Table 1) and addition of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) blocked the reaction (Entries 3, 4, Table 1). This did not occur under microwave irradiation at 110 °C (Entries 7, 8, Table 1). In previous experiments,[50] we established that glycosylation of oxazoline **2** produced, similarly, the β-1,6 disaccharide using the $Fe(OTf)_3$ solvate (15 mol%) under microwave irradiation and that no reaction occurred with the more stable donor **1α** (Fig. 1).

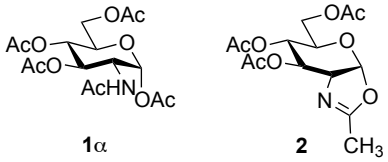
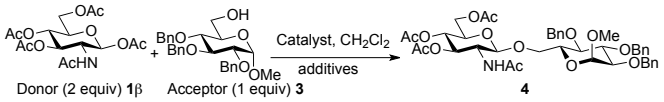


Fig. 1 Glucosaminyl donor 1a and oxazoline 2.

Table 2 shows that $Fe(OTf)_3$•6.2DMSO or $Fe(OTf)_3$ was superior to other Fe(III) salts (FeI_3, $FeCl_3$, $Fe(NTf_2)_3$•6.2DMSO) (Entries 1, 4 *vs.* 3, 5, 13, Table 2, 90-92% *vs.* 31-59%,), $Sc(OTf)_3$ (Entry 2, Table 2, 62% in our hands,),[26] and acidic conditions (TfOH) (Entries 6, 7, Table 2, 47-70%). The addition of TTBP (2 equiv) (Entries 7-8, Table 1 and entry 8, Table 2, 89-98%) optimized the procedure. Using another base such as 2,6-lutidine with $Fe(OTf)_3$•6.2DMSO was inefficient to carry out the transformation. It is interesting to note that in dichloromethane, the $Fe(OTf)_3$ solvate was not soluble at the onset of the reaction while the complex became soluble in the final medium. The dissolving of the Fe(III) salts occurred in acetonitrile but the yield of glycosylation decreased (Entries 9 *vs.* 8, Table 2, 43 *vs.* 89-98%). The use of a mixture of CH_2Cl_2/CH_3CN (7:3) or $CHCl_3/CH_3CN$ (7:3) provided a soluble mixture all along the reaction course with only a slight decrease in the glycosylation yield (Entries 10, 11 *vs.* 8, Table 2, 76-80 *vs.* 89-98%). This enabled the development of the reaction using a microfluidic device (see below). Interestingly, under our optimized conditions, $Bi(OTf)_3$, an alternative cheap, non-toxic, environmentally friendly and abundant metal complex[54-56] already described for the glycosylation of sialyl acetates,[57] proved to be as effective as $Fe(OTf)_3$•6.2DMSO (Entry 12 *vs.* entry 8, Table 2, 88 *vs.* 89-98%).

Table 1 Optimization of iron triflate-catalyzed glycosylation using donor 1β and acceptor 3 with $Fe(OTf)_3$•6.2DMSO and $Fe(OTf)_3$.



Donor (2 equiv) **1**β Acceptor (1 equiv) **3** **4**

Entry	Catalyst (15 mol-%)	TTBP[a]	Temperature, time	Yield[b] (product **4**)
1	$Fe(OTf)_3$•6.2DMSO	-	r.t., 96 h	12%
2	$Fe(OTf)_3$	-	r.t., 96 h	38%
3	$Fe(OTf)_3$•6.2DMSO	2 equiv	r.t., 96 h	nr[d]
4	$Fe(OTf)_3$	2 equiv	r.t., 96 h	nr[d]
5	$Fe(OTf)_3$•6.2DMSO	-	reflux, 84 h	87%
6	$Fe(OTf)_3$	-	reflux, 84 h	86%
7	$Fe(OTf)_3$•6.2DMSO	2 equiv	110 °C[c], 45 min	89%
8	$Fe(OTf)_3$	2 equiv	110 °C[c], 45 min	93%

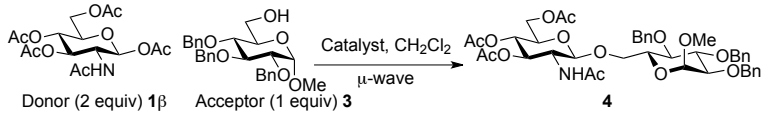
[a] TTBP = 2,4,6-tri-*tert*-butylpyrimidine. [b] Yield after silica gel chromatography. [c] Microwave irradiation (Anton Paar device). [d] No reaction.

Using an excess of the reactive benzyl alcohol acceptor with the commercially available $Fe(OTf)_3$ without TTBP (Entry 5, Table 3), a large amount of α-anomer **6**[35] was produced (α/β, 3:7). This was also observed but to a lesser extent with $Fe(OTf)_3$•6.2DMSO (Entry 7,Table 3, α/β 1:9). Proceeding with an excess of the donor (2 equiv) and/or adding TTBP with the catalyst $Fe(OTf)_3$ or $Fe(OTf)_3$•6.2DMSO prevented this α-anomerization to occur[26] (Entries 1-4 and 6, Table 3).

Iron triflate-catalyzed glycosylation under microwave irradiation

The scope of the β-glycosylation was evaluated with different acceptors using glycosyl donor **1β** (Table 4). Our conditions

Table 2 Comparison of the iron triflate-catalyzed glycosylation of glucosaminyl donor (D) 1β with other catalytic systems under microwave irradiation.

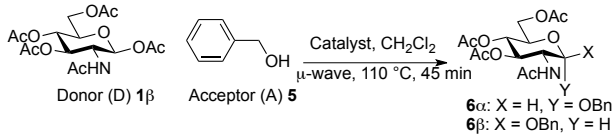


Donor (2 equiv) **1**β Acceptor (1 equiv) **3** **4**

Entry	Catalyst (mol-%)	TTBP(equiv)	Solvent	Time	Yield%[c]
1	$Fe(OTf)_3$•6.2DMSO [a] (15)	-	CH_2Cl_2	30 min	92%
2	$Sc(OTf)_3$[a] (15)	-	CH_2Cl_2	180 min	62% (75%)[26]
3	Fe/I_2[a] (20)	-	CH_2Cl_2	60 min	59%
4	$Fe(OTf)_3$ [a] (15)	-	CH_2Cl_2	30 min	90%
5	$FeCl_3$[a] (15)	-	CH_2Cl_2	30 min	31%
6	TfOH[a] 0.45 equiv	-	CH_2Cl_2	30 min	47%
7	TfOH[a] 0.45 equiv	2	CH_2Cl_2	30 min	70%
8	$Fe(OTf)_3$•6.2DMSO [a or b] (15)	2	CH_2Cl_2	45 min	89-98%[d]
9	$Fe(OTf)_3$•6.2DMSO [b] (15)	2	CH_3CN	45 min	43%
10	$Fe(OTf)_3$•6.2DMSO [b] (15)	2	CH_2Cl_2/CH_3CN 7:3	45 min	80%
11	$Fe(OTf)_3$•6.2DMSO [b] (15)	2	$CHCl_3/CH_3CN$ 7:3	45 min	76%
12	$Bi(OTf)_3$[a] (15)	2	CH_2Cl_2	45 min	88%
13	$Fe(NTf_2)_3$•6.2DMSO [a] (15)	-	CH_2Cl_2	30 min	51%

[a] Under microwave irradiation at 80 °C (CEM Discover). [b] Under microwave irradiation at 110 °C (Anton Paar device). [c] Yield after silica gel chromatography. [d] The yield varies with the used device (CEM device 80 °C or Anton Paar device 110 °C).

Table 3 Comparison of the $Fe(OTf)_3$ and $Fe(OTf)_3$•6.2DMSO catalysts in the glycosylation of benzyl alcohol 5.



Donor (D) **1**β Acceptor (A) **5**

6α: X = H, Y = OBn
6β: X = OBn, Y = H

Entry	D : A (equiv)	Catalyst (15 mol-%)	TTBP (equiv)	Yield%[b] (ratio α/β)[c]
1	2 : 1	$Fe(OTf)_3$•6.2DMSO [a]	2	95% (<5:95)
2	2 : 1	$Fe(OTf)_3$•6.2DMSO [a]	-	96% (<5:95)
3	2 : 1	$Fe(OTf)_3$ [a]	2	95% (<5:95)
4	2 : 1	$Fe(OTf)_3$ [a]	-	89% (5:95)
5	1 : 2	$Fe(OTf)_3$ [a]	-	79% (30:70)
6	1 : 2	$Fe(OTf)_3$ [a]	2	97% (<5:95)
7	1 : 2	$Fe(OTf)_3$•6.2DMSO [a]	-	77% (10:90)

[a] Under microwave irradiation with an Anton Paar device. [b] Yield after silica gel chromatography. [c] Ratio determined by ^1H NMR.

led to an efficient glycosylation with highly reactive acceptors BnOH (Entries 1, 3 and 6, Table 3, 95-97%) and 4-ClBnOH (Entry 1, Table 4, 95%). Glycosylation of 2-chloroacetic acid provided a poor yield of the α/β-anomeric esters **10** (Entry 2, 21%, α/β, 4/1, Table 4). The use of TTBP enabled the glycosylation of silylated or benzylidene acceptors (compounds **11**, **13**, **15** and **17**) without degradation (Entries 3-6, Table 4) with recovered acceptor. For instance, the β(1→3) linked disaccharide **14** was obtained in 74% yield from donor **1β** (Entry 4, Table 4). This method could also be applied to the efficient formation of β(1→2) linked disaccharides **16** and **18** (Entries 5-6, Table 4, 61-53%) with almost quantitative recovery of the acceptor. The reaction was tested in the synthesis of a β-1,4-glycosidic linkage between two D-glucopyranosyl units (donor **1β** and acceptors of the glucose and glucosamine series **19**, **21**, **23**, Entries 7-10, Table 4). Very moderate yields were obtained (20–26%) (Entry 7, 9-10, Table 4), with although a quantitative recovery of the acceptor (Entry 7, Table 4). In the glucosaminyl series with a phthaloyl group at the C2 position, a 3-*O*-acetyl (compound **21**) or 3-*O*-benzyl group (compound **23**) (Entries 9 and 10, Table 4) furnished the same amount of β-1,4-disaccharide **22** or **24** (23-25%). The *N*-acetyl-2,3-oxazolidinone[58] acceptor **25** or the 1,6-anhydro acceptor **27**[59] (Entries 11 and 12, Table 4), developed to enhance the nucleophilicity of the hydroxyl group at the C4 position, gave only traces of the glycosylation product **26** or no glycosylation. This could be due to the degradation of these acceptors or products in the reaction mixture. However, optimization by proceeding at higher concentration (0.65 M in acceptor) led to a slight increase in the yield of glycoside **20** to 37% (Entry 8, Table 4).

The glycosylation scope was then evaluated with various *N*-acetyl-D-glucosamine donors (**10**, **2**, **29**, **32**) in the formation of β-1,6 and β-1,4-glycosidic linkages between two D-glucopyranosyl units using glycosyl acceptors **3**, **19** and **21** (Table 5). Compared with donor **1β**, the replacement of the anomeric acetate group by a chloroacetate group or the acetates at the 3,4,6-positions by benzyl groups had no significant effect on the glycosylation (Entries 1-3, Table 5). However, the benzylidene donor **32** failed to give the expected β-(1→6) linked disaccharide (Entry 4, Table 5). This result is in accordance with the stereoelectronic effect of the 4,6-*O*-benzylidene acetals of pyranosides stabilizing the C-O bond at the anomeric center.[60] Oxazoline **2** furnished the β-(1→4) linked disaccharide **20** in poor yield (13%) (Entry 5, Table 5). Glycosylation with the commercially available *N*-acetyl D-galactosamine donor **33** gave results similar to those of the *N*-acetyl D-glucosamine donor **1β** in the formation of β-1,6; β-1,3; β-1,2 and β-1,4-glycosidic

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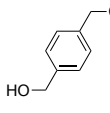
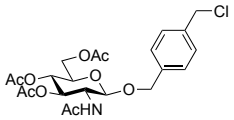
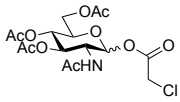
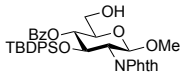
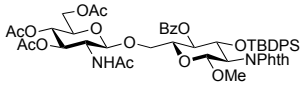
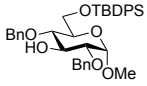
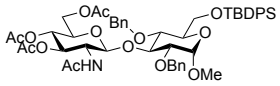
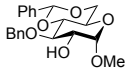
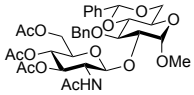
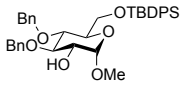
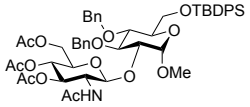
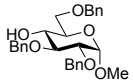
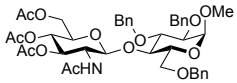
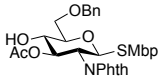
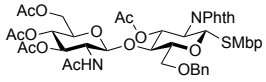
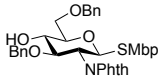
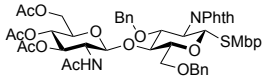
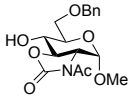
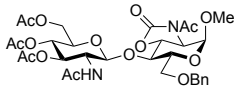
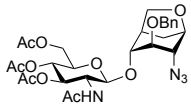
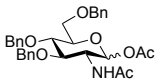
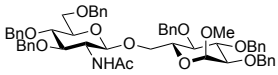
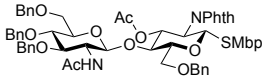
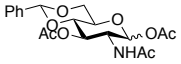
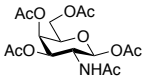
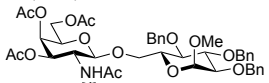
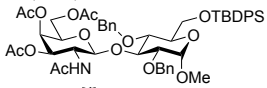
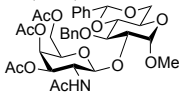
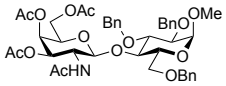
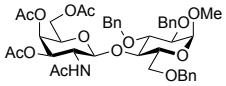
Table 4 Scope of the acceptor for the iron triflate-catalyzed glycosylation using donor (D) 1β with 15 mol-% of $Fe(OTf)_3$•6.2DMSO and 2 equiv of TTBP in CH_2Cl_2 under microwave irradiation.

Entry	Acceptor (A)		Product		D : A	[A] (M)	Time	Yield%[a]
1		**7**		**8**	2 : 1	0.065	30 min	95%[b]
2		**9**		**10**	2.5 : 1	0.065	45 min	21% (α/β 8:2) [c], [d]
3		**11**		**12**	2 : 1	0.065	45 min	76%[c]
4		**13**		**14**	2 : 1	0.065	45 min	74%[b]
5		**15**		**16**	2 : 1	0.065	1 h	61%[b] (90%)[e]
6		**17**		**18**	2 : 1	0.065	1 h	53%[b]
7		**19**		**20**	2 : 1	0.065	3 h	20-26% [b], [c], [f] (>95%)[e]
8		**19**		**20**	2 : 1	0.65	3 h	37%[b] (75%)[e]
9		**21**		**22**	5 : 1	0.065	11 h	23%[c], [g]
10		**23**		**24**	1 : 2	0.065	3 h	25%[c], [g]
11		**25**		**26**	2 : 1	0.065	3 h	7%[c]
12		**27**		**28**	2 : 1	0.065	45 min	0%[c]

[a] Yield after silica gel chromatography. [b] 110 °C (Anton Paar device). [c] 70-80 °C (CEM device); for details see Supporting information. [d] 20 mol-%, $Fe(OTf)_3$; no TTBP. [e] Yield based on recovered acceptor. [f] Donor was recovered as a mixture of anomers (**1α**/**1β** = 1/1; 24% combined yield). [g] No TTBP.

Table 5 Scope of *N*-acetyl D-glycosamine donors (2 equiv) for the iron triflate-catalyzed glycosylation under microwave irradiation.

Entry	Donor	Acceptor	Product (yield%)[a]
1	**10** 	**3**	**4** (73%)[b] 
2	**29** (α/β 1/2)	**3**	**30** (86%)[b] 
3	**29** 	**21**	**31** (14%)[b]
4	**32** (α/β 1/1)	**3**	nr[b], [c]
5	**2** 	**19**	**20** (13%)[b] 
6	**33**	**3**	**34** (95%)[d] 
7	**33**	**13**	**35** (75%)[d] 
8	**33**	**15**	**36** (63%)[d] (>95%)[e] 
9	**33**	**17**	**37** (55%)[d] 
10	**33**	**19**	**38** (26%)[d] (>95%)[e]

[a] Yield after silica gel chromatography. 15 mol-% of $Fe(OTf)_3$•6.2DMSO and TTBP (2 equiv) in CH_2Cl_2. [b] 45-180 min, 80 °C, CEM Discover®; for details see Supporting information. [c] no reaction. [d] 30-180 min, 110 °C, Anton Paar Monowave 300®; for details see Supporting information. [e] Yield based on recovered acceptor.

linkages (95-26%, Entries 6-10, Table 5, *versus* (89-98%)-(20-26%), Entry 8, Table 2 and Entries 4-7, Table 4) with a quantitative recovery of acceptors. Under our harsh reaction conditions (microwave irradiation at 80 - 110 °C), variations of the oxygen protecting group at the 1, 3, 4 and 6 positions in donor or acceptor had no effect on the disaccharide yield and the course of the reaction. With our device for microwave irradiation, the iron triflate-catalyzed glycosylation scale-up was limited to the use of a 30-mL reactor *versus* a 10-mL reactor. This change induced a slight decrease in the yield (77% *vs.* 89% for **4**, and 89% *vs.* 95% for **6β**) probably as a consequence of the impaired heat transfer.

Flow chemistry

The above limitation can be overcome by transposing the reaction in flow chemistry.[61, 62] It has been demonstrated that micro- or minifluidic flow devices fitted with a backpressure regulator mimic high temperatures and pressures attainable in a sealed-vessel microwave chemistry batch experiment. Flow chemistry has already been used for glycosylation with success.[63-65] The major limitation was the low solubility of donor **1β** that required the use of a mixture of solvents (CH_2Cl_2/acetonitrile or $CHCl_3$/acetonitrile) which induced a yield decrease under microwave irradiation (76-80% *vs.* 89-98%, Entries 10-11 *vs.* Entry 8, Table 2). In this study a Vapourtec R4-Unit was used as a millifluidic system. This system suppressed the tendency to block and does not limit the flow capacity observed with micro reactors when preparing substantial amounts of the product.[66] The formation of disaccharides **4**, **20** and benzyl glycosides **6β** and **8** was studied using donor **1β** and acceptor **3**, **5**, **7** or **19**.

The use of TTBP dramatically slowed down the process and decreased the yield of the reaction (Entries 1 *vs.* 2, **Table 6**, 25 *vs.* 62%). A slight decrease of the yield was also observed with a decrease of the loading of $Fe(OTf)_3$•6.2DMSO (Entries 3 *vs.* 2, Table 6, 51 *vs.* 62%). The optimized temperature of the reactor was 110 °C (Entries 2 *vs.* 4, Table 6, 62 *vs.* 44%) and higher temperatures increased degradation. A higher pressure (33 *vs.* 25 bar) associated with a longer residence time (70 *vs.* 45 min) and a more concentrated reaction mixture in acceptor (0.15 M) with an excess of donor **1β** gave a 78% yield of **4** with high recovery of the unreacted acceptor. This was also obtained with the commercially available $Fe(OTf)_3$ which provided a 75% yield of **4** (Entry 8, Table 6). The same yield range was obtained for benzyl glycosides **6β** (77%, Entry 9, Table 6) and **8** (75%, Entry 12, Table 6). An excess of benzyl alcohol **5** (2 equiv/**1β**) decreased the yield of **6β** (62%, Entry 10, Table 6) without the formation of **6α** as observed under microwave heating. A residence time of only 30 min, more practical for a g-scale production, allowed to maintain an acceptable yield of **6β** (73%, Entry 11, Table 6) as well with the $Fe(OTf)_3$ catalyst (77%). Our conditions were ineffective for the formation of the β-1,4-glycosidic linkage (< 10%, Entry 13, Table 6).

Extending the glycosylation reaction to a continuous flow process without further changes proceeded with good yields (75-78%) using chloroform instead of dichloromethane. Due to its high volatility, dichloromethane was not suitable with our flow chemistry device for long injection times. This procedure delivered 2 g (2.52 mmol) of disaccharide **4** with a 50-mL injected volume (Fig. 2).

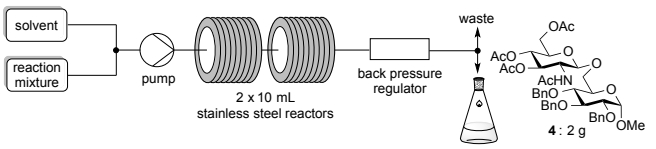


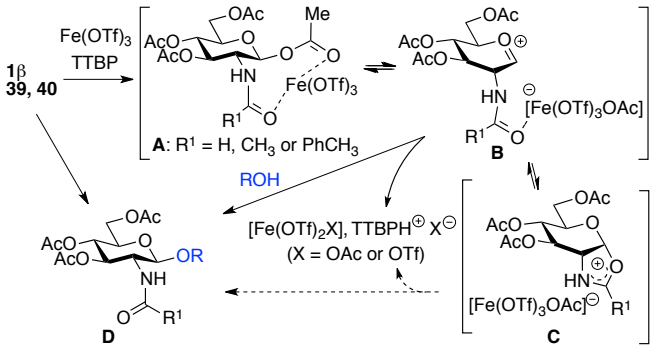
Fig. 2 Flow system used for glycosylation reactions after optimization conditions.

Mechanistic studies

Starting from GlcNAc glycosyl donors for the one-step synthesis of β-D-GlcNAc glycopyranosides, oxazolinium ion **C** (Scheme 1) is expected to be the intermediate, justifying the high β-stereoselectivity.[1, 9] However, the glycosylation results with the less nucleophilic 4-OH acceptor **19** and oxazoline **2** compared to β-acetate **1** (13% yield, Entry 5, Table 5 *vs.* 20-26% yield, Entry 7, Table 4) were different. This suggested that the reaction may not proceed via this intermediate and another route to the glycoside may operate. To study this possibility, glycosylation with iron(III) triflate was examined by modulating the electronic and/or the steric properties of the *N*-substituent in D-glucosaminyl donors **39**-**47**. This was done by choosing the glycosylation of primary alcohol **3** under the

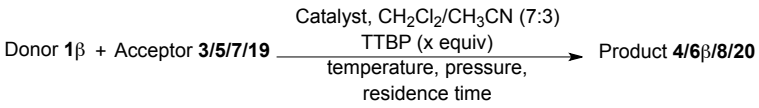
optimized conditions (Entry 1, Table 7) as a reference glycosylation reaction. Similarly to **1β**, formyl amide **39** and tolyl amide **40** (Entries 3, 4, Table 7) provided the expected glycosides **48** and **49**, while carbamate **41**, trichloroacetamide **42**, trifluoroacetamide **43**, phthalimide **46** or pivaloyl amide **45** (Entries 6, 7, 9, 13, 11, Table 7) were completely ineffective or significantly less effective (chloroacetamide **44,** Entry 10, Table 7). In the case of the diacetamide **47**, one acetyl group was transferred to the acceptor providing **58**, without detecting the formation of the disaccharide (Entries 14, Table 7). These negative results should be compared with 2-deoxy-2-trichloroacetamido[17] and 2-deoxy-2-trifluoroacetamido[10, 67, 68] derivatives equipped with a good leaving group at the anomeric carbon (e.g., trichloroacetimidate). When activated with appropriate promoters (e.g., Me_3SiOTf), they are known to be good glycosyl donors through the formation of the oxazolinium ion intermediate.[17] In the absence of nucleophile, oxazolines **2**, **56** and **57** (Entries 2, 8, 12, Table 7) were not detected except for oxazoline **55** from the tolylamide **40** (Entry 5, Table 7). These experiments suggest that glycosylation would proceed through an alternative intermediate and not necessarily through the oxazolinium ion. Glycosylation may require a pre-complexation of the catalyst by a proper amide group such as the acetamide present in **1β** (see **A**, Scheme 1), the tolyl amide in **40** or the formyl amide in **39** before the activation of the anomeric acetate occurs. Effective amide pre-complexation of $Fe(OTf)_3$•6.2DMSO may be partially or totally prevented for electronic reasons (NHTCA, donor **42**; NHTFA, donor **43**; NHAcCl, donor **44**) or steric grounds (NHPiv, donor **45**; NPhth, donor **46**) thus preventing glycoside formation as experimentally observed. Alpha-ionic pair **B** from **1β**, instead of oxazolinium ion **C**, would then encourage the glycosylation to take place from the β face by shielding the α face.

It is noteworthy that the reaction scale-up in the preparation of disaccharide **4** allowed the isolation of a small amount of oxazoline **2** suggesting a partial contribution of the oxazolinium ion **C** in the formation of the glycoside. A possible equilibrium between **B** and **C** could be envisioned depending on the nature of the group R^1 favoring one or the other.



Scheme 1 Possible mechanism for the iron triflate-catalyzed glycosylation.

Table 6 Optimization conditions of the iron triflate-catalyzed glycosylation in flow chemistry using injection loop.



Entry	Acceptor (equiv)	Catalyst (mol-%)	TTBP	**1β:** equiv; concentration	Pressure	Temperature	Residence time	Product (yield%)[a]
1	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	2 equiv	2; 0.1 M	25 bar	110 °C	45 min	**4** (25%)
2	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.1 M	25 bar	110 °C	45 min	**4** (62%)
3	**3** (1)	$Fe(OTf)_3$•6.2DMSO (10)	-	2; 0.1 M	25 bar	110 °C	45 min	**4** (51%)
4	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.1 M	25 bar	100 °C	45 min	**4** (44%)
5	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.1 M	33 bar	110 °C	45 min	**4** (70%)
6	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	1; 0.1 M	33 bar	110 °C	45 min	**4** (45%)
7	**3** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.15 M	33 bar	110 °C	70 min	**4** (74-78%) (86%)[b]
8	**3** (1)	$Fe(OTf)_3$ (15)	-	2; 0.15 M	33 bar	110 °C	70 min	**4** (75%)
9	**5** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.15 M	33 bar	110 °C	45 min	**6β** (77%)
10	**5** (2)	$Fe(OTf)_3$•6.2DMSO (15)	-	1; 0.15 M	33 bar	110 °C	45 min	**6β** (62%)
11	**5** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.15 M	33 bar	110 °C	30 min	**6β** (73%)
12	**7** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.15 M	33 bar	110 °C	45 min	**8** (75%)
13	**19** (1)	$Fe(OTf)_3$•6.2DMSO (15)	-	2; 0.15 M	33 bar	110 °C	70 min	**20** (<10%)

[a] Yield after silica gel chromatography. [b] Yield based on recovered acceptor.

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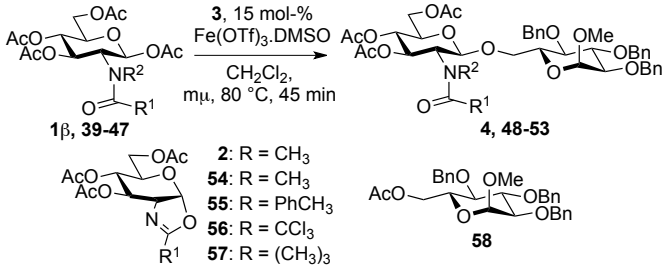
Table 7 Iron triflate-catalyzed glycosylation of 3 using 2 equiv of donors 1β and 39-47 under microwave irradiation.



Entry	Donor	Acceptor	Product (yield%)[a]
1	**1β**	**3**	**4** (98%)[b]
2	**1β**	None	**1α-1β** (100%)[c]
3	**39** $R^1 = R^2 = H$	**3**	**48** (50%)[b]
4	**40** $R^1 = PhCH_3$, $R^2 = H$	**3**	**49** (82%)[b]
5	**40** $R^1 = PhCH_3$, $R^2 = H$	None	**55** (62%)[b]
6	**41** $R^1 = OCH_2Ph$, $R^2 = H$	**3**	nr[b], [d]
7	**42** $R^1 = CCl_3$, $R^2 = H$	**3**	**50** (<5%)[b]
8	**42** $R^1 = CCl_3$, $R^2 = H$	None	nr[b], [d]
9	**43** $R^1 = CF_3$, $R^2 = H$	**3**	nr[b], [d]
10	**44** $R^1 = CH_2Cl$, $R^2 = H$	**3**	**51** (30%)[b], [e]
11	**45** $R^1 = C(CH_3)_3$, $R^2 = H$	**3**	**52** (<5%)[b], [f]
12	**45** $R^1 = C(CH_3)_3$, $R^2 = H$	None	nr[b], [d]
13	**46** $R^1 = R^2 = Phth$	**3**	**53** (<5%)[f], [g]
14	**47** $R^1 = CH_3$, $R^2 = COCH_3$	**3**	**58**[69] (40%)[b]

[a] Yield after silica gel chromatography. [b] Reaction performed in the presence of TTBP (2 equiv) with 15 mol-% of Fe(OTf)$_3$•6.2DMSO in CH_2Cl_2/CEM device 80 °C, 45-60 min. [c] **1β**/**1α** ratio of 4/1. [d] No reaction. [e] Inseparable mixture with the donor, conversion determined by ^{1}H NMR. [f] Traces detected by UPLC-MS/DAD. [g] Reaction performed in the presence of TTBP (2 equiv)/Anton Paar device 110 °C, 45 min.

Conclusion

This novel catalytic glycosylation using peracetylated β-GlcNAc **1β** and β-GalNAc **33** as glycosyl donors with Fe(III) triflate and TTBP is effective in the direct synthesis of β-GlcNAc and β-GalNAc glycosides but has not yet been efficient using less nucleophilic sugar acceptors. Our results suggest a possible mechanism which proceeds mostly by intermediates not involving the unique oxazolinium ion. We have demonstrated that the Fe(III) triflate glycosylation conducted under microwave irradiation is amenable to flow chemistry without requiring the presence of TTBP.

Experimental Section

Typical procedure for microwave-assisted glycosylation:
The donor **1β** (50 mg, 0.128 mmol, 2 equiv), TTBP (32 mg, 0.129 mmol, 2 equiv) and Fe(OTf)$_3$•6.2DMSO (10 mg, 0.010 mmol, 15 mol-%) were added to the acceptor **3** (30 mg, 0.065 mmol, 1 equiv) in an oven-dried, argon-purged microwave vial equipped with a magnetic stirring bar. Everything was flushed under argon and dry CH_2Cl_2 (1 mL) was added. After sealing the vial, the reaction mixture was heated to 110 °C under microwave irradiation for 45 min (1 minute ramp time from room temperature to 110 °C and 45 min hold time at 110 °C, stirring set at 800 rpm). The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3×10 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 5:5 to 0:1) to afford the pure product **4** (46 mg, 89 %, white amorphous solid).

Typical procedure for glycosylations under continuous flow conditions:
The donor **1β** (2.92 g, 7.50 mmol, 2 equiv) and Fe(OTf)$_3$•6.2DMSO (556 mg, 0.56 mmol, 15 mol-%) were added to the acceptor **3** (1.74 g, 3.75 mmol, 1 equiv) in an oven-dried, argon purged vial equipped with a magnetic stirring bar. A dry mixture of chloroform/acetonitrile 7:3 (50 mL) was added and the reaction mixture was stirred and sonicated for a few minutes (until complete homogenisation). After setting up and drying the whole flow system with dry chloroform/acetonitrile 7:3, the pump was primed and the reaction mixture (contained in an argon overpressured vial) is pumped into two 10 mL-stainless steel reactors in series, heated at 110 °C with a flow rate of 0.286 mL/min (corresponding to a residence time of 70 min). The system pressure, controlled with a back pressure regulator, was fixed at 33 bars and the reaction mixture was finally collected into a single receptor. The reaction mixture was diluted with dichloromethane (250 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (4×100 mL) and the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 5:5 to 0:1) to afford the pure product **4** (2.00 g, 78 %, white amorphous solid).

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Notes and references

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† Dedicated to Professor Max Malacria on the occasion of his 65th birthday

‡ Electronic Supplementary Information (ESI) available: Preparation, ^{1}H and ^{13}C NMR spectra for novel compounds. See DOI: 10.1039/b000000x/

1. P. Sinaÿ, B. Ernst and G. Hart, *Carbohydrates in Chemistry and Biology*, Wiley VCH edn., 2000.
2. A. Varki and J. D. E. R. D. Cummings, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler, *Essentials of Glycobiology*, 2nd edition Cold Spring Harbour Laboratory Press, Cold Spring Harbor (NY) edn., 2009.
3. N. Shibuya and E. Minami, *Physiol. Mol. Plant Pathol.*, 2001, **59**, 223-233.
4. J. Dénarié, F. Debellé and J. C. Promé, *Annu. Rev. Biochem.*, 1996, **65**, 503-535.
5. F. Maillet, V. Poinsot, O. Andre, V. Puech-Pagès, A. Haouy, M. Gueunier, L. Cromer, D. Giraudet, D. Formey, A. Niebel, E. A. Martinez, H. Driguez, G. Bécard and J. Dénarié, *Nature*, 2011, **469**, 58-63.
6. Z. Wang, Z. S. Chinoy, S. G. Ambre, W. Peng, R. McBride, R. P. de Vries, J. Glushka, J. C. Paulson and G. J. Boons, *Science*, 2013, **341**, 379-383.
7. M. E. Jung and P. Koch, *Org. Lett.*, 2011, **13**, 3710-3713.
8. X. M. Zhu and R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 2009, **48**, 1900-1934.
9. A. V. Demchenko, *Handbook of Chemical Glycosylation*, Wiley-VCH edn., WILEY-VCH, 2008.
10. A. F. G. Bongat and A. V. Demchenko, *Carbohydr. Res.*, 2007, **342**, 374-406.
11. R. Enugala, L. C. Carvalho, M. J. Dias Pires and M. M. Marques, *Chem. Asian J.*, 2012, **7**, 2482-2501.
12. Y. Yang and B. Yu, *Tetrahedron*, 2014, **70**, 1023-1046.
13. R. U. Lemieux, T. Takeda and B. Chung, *Abstr. Pap. Am. Chem. Soc.*, 1976, 6-6.
14. J. Banoub, P. Boullanger and D. Lafont, *Chem. Rev.*, 1992, **92**, 1167-1195.
15. W. Dullenkopf, J. C. CastroPalomino, L. Manzoni and R. R. Schmidt, *Carbohydr. Res.*, 1996, **296**, 135-147.
16. M. L. Wolfrom and H. B. Bhat, *J. Org. Chem.*, 1967, **32**, 1821-1823.
17. G. Blatter, J.-M. Beau and J.-C. Jacquinet, *Carbohydr. Res.*, 1994, **260**, 189-202.
18. Y. Geng, L.-H. Zhang and X.-S. Ye, *Chem. Commun.*, 2008, 597-599.
19. J. D. M. Olsson, L. Eriksson, M. Lahmann and S. Oscarson, *J. Org. Chem.*, 2008, **73**, 7181-7188.
20. M. R. E. Aly, E.-S. I. Ibrahim, E. S. H. El Ashry and R. R. Schmidt, *Carbohydr. Res.*, 2001, **331**, 129-142.
21. R. Arihara, S. Nakamura and S. Hashimoto, *Angew. Chem. Int. Ed.*, 2005, **44**, 2245-2249.
22. S. Yamago, T. Yamada, T. Maruyama and J. Yoshida, *Angew. Chem. Int. Ed.*, 2004, **43**, 2145-2148.
23. B. Helferich and E. Schmitz-Hillebrecht, *Ber. Dtsch. Chem. Ges.* , 1933, **66**, 378-383.
24. V. Wittmann and D. Lennartz, *Eur. J. Org. Chem.*, 2002, 1363-1367.
25. C. F. Crasto and G. B. Jones, *Tetrahedron Lett.*, 2004, **45**, 4891-4894.
26. H. Christensen, M. S. Christiansen, J. Petersen and H. H. Jensen, *Org. Biomol. Chem.*, 2008, **6**, 3276-3283.
27. J. Krag, M. S. Christiansen, J. G. Petersen and H. H. Jensen, *Carbohydr. Res.*, 2010, **345**, 872-879.
28. S. Mandal, N. Sharma and B. Mukhopadhyay, *Synlett*, 2009, 3111-3114.
29. Y. Cai, C.-C. Ling and D. R. Bundle, *Org. Lett.*, 2005, **7**, 4021-4024.
30. M. Kiso and L. Anderson, *Carbohydr. Res.*, 1985, **136**, 309-323.
31. F. Dasgupta and L. Anderson, *Carbohydr. Res.*, 1990, **202**, 239-255.
32. S. K. Chatterjee and P. Nuhn, *Chem. Commun.*, 1998, 1729-1730.
33. S. Koto, M. Hirooka, T. Tashiro, M. Sakashita, M. Hatachi, T. Kono, M. Shimizu, N. Yoshida, S. Kurasawa, N. Sakuma, S. Sawazaki, A. Takeuchi, N. Shoya and E. Nakamura, *Carbohydr. Res.*, 2004, **339**, 2415-2424.
34. J. Seibel, L. Hillringhaus and R. Moraru, *Carbohydr. Res.*, 2005, **340**, 507-511.
35. G. H. Wei, X. Lv and Y. Du, *Carbohydr. Res.*, 2008, **343**, 3096-3099.
36. B. D. Sherry and A. Fürstner, *Acc. Chem. Res.*, 2008, **41**, 1500-1511.
37. D. D. Diaz, P. O. Miranda, J. I. Padron and V. S. Martin, *Curr. Org. Chem.*, 2006, **10**, 457-476.
38. C. Bolm, J. Legros, J. Le Paih and L. Zani, *Chem. Rev.*, 2004, **104**, 6217-6254.
39. E. B. Bauer, *Curr. Org. Chem.*, 2008, **12**, 1341-1369.
40. J.-M. Beau, Y. Bourdreux, F.-D. Boyer, S. Norsikian, D. Urban, G. Doisneau, B. Vauzeilles, A. Gouasmat, A. Lemetais, A. Mathieu, J.-F. Soulé, A. Stévenin and A. Xolin, in *Carbohydrate Chemistry*, The Royal Society of Chemistry, 2014, vol. 40, pp. 118-139.
41. S. Ichikawa, I. Tomita, A. Hosaka and T. Sato, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 513-520.
42. S. S. Weng, *Tetrahedron Lett.*, 2009, **50**, 6414-6417.
43. P. Chen and S. Wang, *Tetrahedron*, 2012, **68**, 5356-5362.
44. N. Grenouillat, B. Vauzeilles, J. J. Bono, E. Samain and J. M. Beau, *Angew. Chem. Int. Ed.*, 2004, **43**, 4644-4646.
45. A. Français, D. Urban and J.-M. Beau, *Angew. Chem. Int. Ed.*, 2007, **46**, 8662-8665.
46. Y. Bourdreux, A. Lemétais, D. Urban and J.-M. Beau, *Chem. Commun.*, 2011, **47**, 2646-2648.
47. J.-F. Soulé, A. Mathieu, S. Norsikian and J.-M. Beau, *Org. Lett.*, 2010, **12**, 5322-5325.
48. S. Zameo, B. Vauzeilles and J. M. Beau, *Angew. Chem., Int. Ed.*, 2005, **44**, 965-969.
49. A. Malapelle, Z. Abdallah, G. Doisneau and J. M. Beau, *Angew. Chem., Int. Ed.*, 2006, **45**, 6016-6020.
50. A. Stévenin, F.-D. Boyer and J.-M. Beau, *Eur. J. Org. Chem.*, 2012, 1699-1702.
51. S. Antoniotti and E. Dunach, *Chem. Commun.*, 2008, 993-995.

52. T. Ishikawa, Y. Shimizu, T. Kudoh and S. Saito, *Org. Lett.*, 2003, **5**, 3879-3882.
53. H. Myszka, D. Bednarczyk, M. Najder and W. Kaca, *Carbohydr. Res.*, 2003, **338**, 133-141.
54. H. Gaspard-Iloughmane and C. Le Roux, *Eur. J. Org. Chem.*, 2004, 2517-2532.
55. V. Mandadapu, F. Wu and A. I. Day, *Org. Lett.*, 2014, **16**, 1275-1277.
56. J. R. Desmurs, M. Labrouillère, C. Le Roux, H. Gaspard, A. Laporterie and J. Dubac, *Tetrahedron Lett.*, 1997, **38**, 8871-8874.
57. K. Ikeda, Y. Torisawa, T. Nishi, J. Minamikawa, K. Tanaka and M. Sato, *Bioorg. Med. Chem.*, 2003, **11**, 3073-3076.
58. D. Crich and A. U. Vinod, *J. Org. Chem.*, 2005, **70**, 1291-1296.
59. D. Tailler, J. C. Jacquinet and J. M. Beau, *J. Chem. Soc., Chem. Commun.*, 1994, 1827-1828.
60. H. H. Jensen, L. U. Nordstrom and M. Bols, *J. Am. Chem. Soc.*, 2004, **126**, 9205-9213.
61. T. N. Glasnov and C. O. Kappe, *Chem. Eur. J.*, 2011, **17**, 11956-11968.
62. K. S. Elvira, X. Casadevall i Solvas, R. C. Wootton and A. J. deMello, *Nature Chem.*, 2013, **5**, 905-915.
63. D. M. Ratner, E. R. Murphy, M. Jhunjhunwala, D. A. Snyder, K. F. Jensen and P. H. Seeberger, *Chem. Commun.*, 2005, 578-580.
64. F. R. Carrel, K. Geyer, J. D. C. Codee and P. H. Seeberger, *Org. Lett.*, 2007, **9**, 2285-2288.
65. D. T. McQuade and P. H. Seeberger, *J. Org. Chem.*, 2013, **78**, 6384-6389.
66. J. Wegner, S. Ceylan and A. Kirschning, *Chem. Commun.*, 2011, **47**, 4583-4592.
67. L. G. Weaver, Y. Singh, J. T. Blanchfield and P. L. Burn, *Carbohydr. Res.*, 2013, **371**, 68-76.
68. D. J. Silva, H. Wang, N. M. Allanson, R. K. Jain and M. J. Sofia, *J. Org. Chem.*, 1999, **64**, 5926-5929.
69. M. Giordano, A. Iadonisi and A. Pastore, *Eur. J. Org. Chem.*, 2013, 3137-3147.