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Stereoselective synthesis of fluorinated aminoglycosyl phosphonates†

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We describe the conjugate addition of lithiated difluoromethanephosphonates to a diverse range of nitroglycals as a convenient method for the highly stereoselective synthesis of fluorinated aminoglycosyl phosphonates. Our approach provides opportunities to produce hydrolytically stable mimics of biologically important aminoglycosyl phosphates.

The complexity of the wide variety of oligosaccharide structures found in nature is derived from a rational management of various enzymes. The formation of glycosidic linkages is typically achieved by glycosyl transferases *via* a controlled transfer of monosaccharide structures from an activated donor sugar substrate to acceptor substrates (*e.g.* oligosaccharides, lipids and proteins).[1,2] Donor sugar substrates are most commonly activated in the form of nucleoside diphosphate sugars (*e.g.* UDP-Gal, UDP-GlcNAc, *etc.*), which are generally referred to as Leloir donors.[3] However, nucleoside monophosphate sugars and lipid phosphates are also suitable for activation (Fig. 1A). The inhibition of this transfer reaction provides an excellent opportunity for the potential modulation of oligosaccharide biosynthesis and interference with biological signaling and metabolism. Accordingly, significant effort has been devoted to the synthesis of hydrolytically stable glycosyl phosphate mimics, such as C-glycoside analogs.[4–6] In particular the synthesis of 2-deoxy-2-acetamido derivatives is of special interest because of the widespread biological activity of various aminoglycoside phosphates.[7] For instance, sugars such as *N*-acetylglycosamine (GlcNAc) and *N*-acetyl-galactosamine (GalNAc) are involved in the biosynthesis of *N*-linked glycoproteins which are the key intermediates of many important cell–cell and cell-pathogen recognition phenomena.[8] In addition, GlcNAc 1-phosphate is a substrate for nucleotidyl

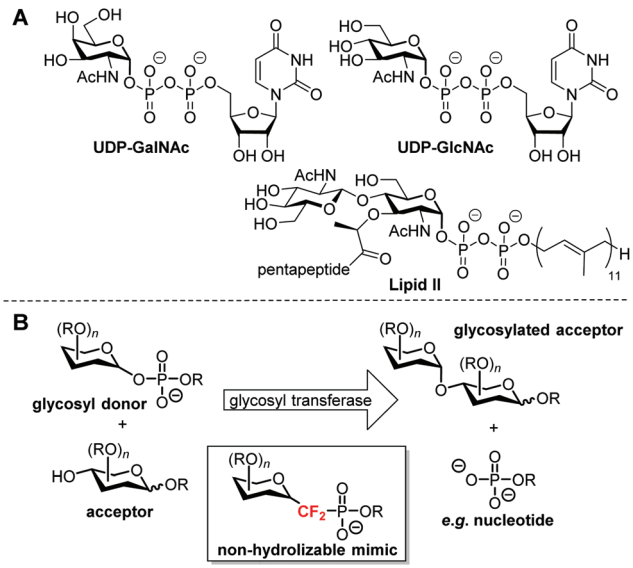

Fig. 1 (A) Structures of aminoglycosyl donors and a cell wall intermediate. (B) Generalized reaction scheme of glycosyl transferases and structure of non-hydrolyzable glycosyl donor mimic.

transferase enzymes in the assembly of the fungal cell wall to form the chitin layer.[9] Likewise, C55-isoprenyl diphosphate MurNAc(GlcNAc) pentapeptide (Lipid II, Fig. 1A) is an important intermediate in the biosynthesis of the rigid peptidoglycan layer of bacteria.[10,11] Several synthetic approaches toward 2-deoxy-2-acetamido glycosyl-CH_2-phosphonates are known.[5,12–15] However, such ground state analogues have shown to be moderate inhibitors of the corresponding aminoglycosyl transferases under physiological conditions.[13] In light of this observation, better bio-isosteres might be obtained by the introduction of fluorine substituents on the α-carbon of the phosphonate, because their electron-withdrawing properties will maintain the electronegativity of the oxygen.[16–18] In fact, the CF_2-methylene linkage closely resembles the oxygen linkage in terms of size, charge distribution and, consequently, the second dissociation constant of the phosphate

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moiety (pK_a^2).[19] Glycosyl CF_2-phosphonates are therefore attractive synthetic targets as probes for glycosyl transferases and related enzymes (Fig. 1B).

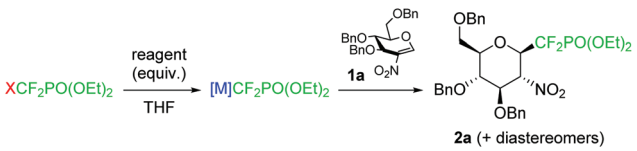
Although several interesting methods have been developed to replace the anomeric oxygen atom of carbohydrates by a difluoromethylene group,[20,21] reports on the synthesis of 2-deoxy-2-amino-CF_2-glycosides are relatively scarce. This might be explained by the difficulties associated with the incompatibility of neighboring nitrogen-based functional groups. Moreover, the installation of a glycosyl acceptor with the correct relative stereochemistry has proven difficult. The most common approach toward the synthesis of 2-deoxy-2-acetamido-C-glycosides is to employ other pyranose starting materials and install the acetamido group after the C–C(F_2) bond formation. These approaches typically require many steps and proceed in low overall yield.[22]

The poor control over stereochemistry and the long reaction sequences prompted us to develop an efficient procedure toward 2-deoxy-2-acetamido-CF_2-glycosyl phosphonates. Herein we report a novel approach to difluoromethanephosphonate C-glycoside bond formation based on conjugate addition to readily available 2-nitroglycals. We will discuss and illustrate our approach as a convenient method for the highly stereoselective synthesis of several fluorinated aminoglycosyl phosphonates. 2-Nitroglycals have been recognized as important building blocks in carbohydrate chemistry because glycosyl acceptors can easily be installed at the anomeric centre *via* conjugate addition, often proceeding with high diastereoselectivity.[23–25] We envisioned the conjugate addition of difluoromethanephosphonate to these readily available 2-nitroglycals could provide access to difluorophosphonate C-glycosides. Subsequent chemical manipulation of the 2-nitro group would afford the 2-acetamido group.

The proposed reaction was initially evaluated with 3,4,6-tri-*O*-benzyl-2-nitro-D-glucal as the model substrate, which is readily available by recently developed methods by Vankar *et al.*[26,27] We started screening the reactivity of several preformed metalated difluoromethanephosphonates toward benzylated nitroglycal.[28] The first set of experiments (Table 1, entries 1–5) did not provide satisfactory results. Only the lithiated reagent was able to undergo reasonable conjugate addition to the nitroglycal, whereas all other cases gave no or only trace amounts of product while the remainder of the starting material could be recovered. Pleasingly, the use of LDA in combination with difluoromethanephosphonate significantly increased the yield from 47% to 84% (entry 8). A lower temperature (−78 °C) is required as a result of the thermal instability of lithiated difluoromethanephosphonates.[29] Additional optimization revealed that the use of 1.2 equivalents of phosphonate is sufficient and that the reaction proceeds to completion within 30 minutes. Fluorinated C-glycosyl phosphonate **2a** was obtained in very high diastereomeric excess. We assigned the 1,2-*trans* configuration to the major isomer based on NMR analysis. The large H-1/H-2 and H-2/H-3 coupling constants ($J_{1,2}$ and $J_{2,3}$ ~ 10 Hz) observed in the ^{1}H NMR spectrum of **2a** indicated a diaxial relationship between the hydrogen atoms, which is consistent with a $^4C_1$ conformation of the glycosyl phosphonate. Since crucial signals in the ^{1}H NMR spectra of **2a** overlapped, which prevented us from collecting useful data from 2D-NMR experiments, the β-configuration was confirmed by NOESY correlation between H-1 and H-5 after debenzylation of **2a**.

After having defined the optimal conditions, the feasibility and protecting group compatibility of the reaction was explored and a series of fluorinated glycosyl phosphonates was synthesized (Scheme 1). Differentially protected nitroglycals were reacted with diethyl lithiodifluoromethanephosphonate to furnish **2a–2c** in good yields and with high diastereomeric ratios. Apparently silyl, methyl and benzyl ethers are tolerated, whereas tri-*O*-acetyl nitroglycal (**1d**) gave no product formation,

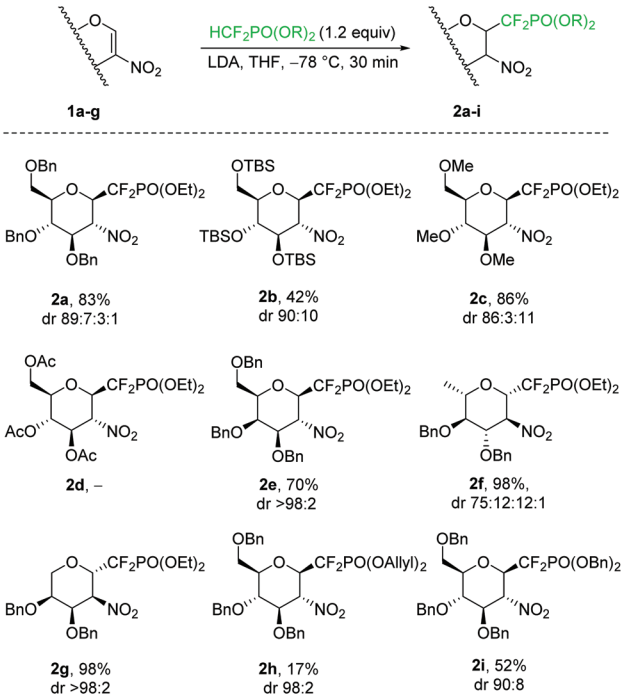
Table 1 Optimization of the reaction conditions



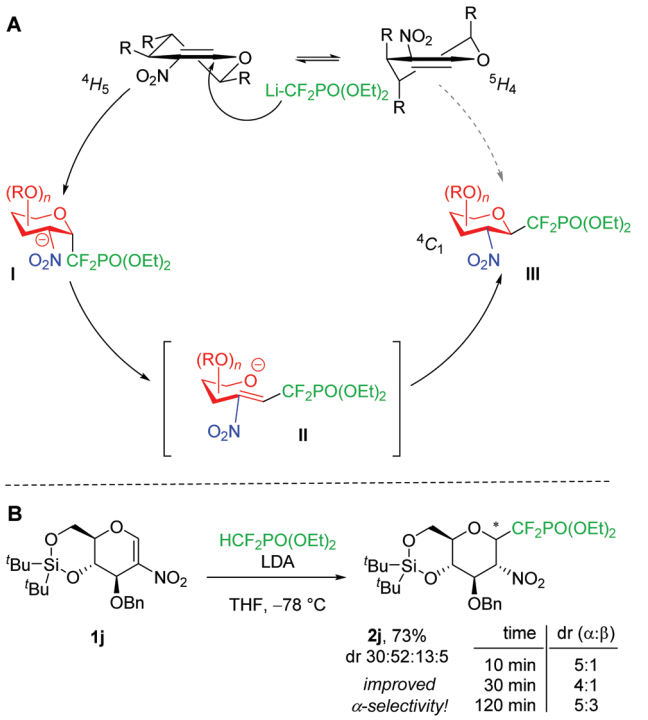
2a (+ diastereomers)

Entry	X	Reagent	T (°C)	[M]	Equivalents	Time (min)	Yield[a] (%)
1	$SiMe_3$	CsF	rt	Cs	2	120	6
2	Br	iPrMgCl	rt	MgCl	2	120	—
3	Br	Cd	rt	CdBr	2	120	—
4	Br	Zn	rt	ZnBr	2	120	—
5[b]	ZnBr	CuBr	rt	$CuZnBr_2$	2	120	—
6	Br	*n*BuLi	−78	Li	2	120	47
7	H	NaHMDS	−78	Na	2	120	—
8	H	LDA	−78	Li	2	120	84
9	H	LDA	−78	Li	1	120	62
10	H	LDA	−78	Li	1.2	120	83
11	H	LDA	−78	Li	1.2	30	83[c]

[a] Determined by ^{19}F NMR using α,α,α-trifluorotoluene as an internal standard. [b] DMF was used as solvent. [c] Diastereomeric ratio: 89 : 7 : 3 : 1.



Scheme 1 Scope of fluorinated glycosyl phosphonates. Yields determined by ^{19}F NMR using α,α,α-trifluorotoluene as an internal standard. Reaction conditions: **1** (0.50 mmol), $HCF_2PO(OR)_2$ (0.60 mmol, 1.2 equiv.), LDA (0.60 mmol, 1.2 equiv.), THF, –78 °C.



Scheme 2 (A) Rationalization of observed stereoselectivity; (B) Reduced equilibration in the formation of **2j**.

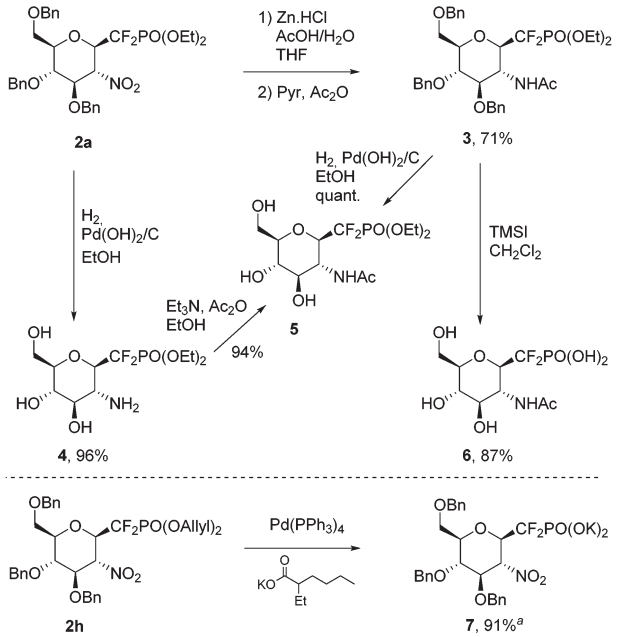
presumably due to the incompatibility of esters with the lithiated phosphonate. Also the tribenzylated galactal derivative **1e** reacted smoothly under these conditions. We then turned our attention to the rhamnal and arabinal derivatives **1f** and **1g**, which were also readily converted to the corresponding desired products **2f** and **2g** in good yield and with high dr's.

In order to introduce a set of orthogonal protection groups in the products, the difluoromethanephosphonate tolerance was examined next. For this reason, allyl and benzyl protected difluoromethanephosphonates were constructed by means of a Michaelis–Becker reaction between the corresponding phosphites and chlorodifluoromethane.[30] Unfortunately, reactions employing these phosphonates yielded the corresponding glycosyl derivatives **2h** and **2i** in poor to modest yield. The driving factor in the reduced coupling efficiency is most likely the instability of these lithiated phosphonates due to the good leaving group capacity of benzylic and allylic alkoxides. This was supported by the formation of a significant amount of 2-deoxy-2-nitro-1*O*-benzylglycoside as a side product in the reaction of **1a** with dibenzyl difluoromethanephosphonate.

Subsequently, we set out to rationalize the observed stereoselectivity. Glycals are known to be conformationally flexible and can exist either in the normal half chair $^4H_5$ or the inverted conformer $^5H_4$, with the predominant conformation being highly dependent on hydroxyl substitution.[31,32] Taking into account that this would affect the stereoselectivity of the conjugate reaction, it is not likely that the high value of diastereomeric excess should be attributed to the direct addition of the lithiated phosphonate. Thus, we hypothesized that the formation of the thermodynamically more stable 1,2-diequatorial 2-nitrophosphonate likely results from a ring opening/ring closure event (Scheme 2A), as observed previously.[33] We anticipated that the introduction of more rigidity by means of a $(t\mathrm{Bu})_2\mathrm{Si}$ protecting group creating a *trans*-fused bicyclic system would provide insight in the mechanism. The $^5H_4$ conformer is unattainable in case of the 4,6-*O*-di-*tert*-butylsilylene protected glycal **1j**, since the ring-fused bonds cannot adopt the axial positions. Consequently, attack from the α-face on the half-chair $^4H_5$ is highly preferred, as it proceeds *via* a chair-like transition state, while β-attack will give a twist-boat-like transition state which is much higher in energy. Thus, we anticipated to increase the preference for the (potentially biologically more relevant) α-anomer of **2j**.

To this end, the addition reaction was performed with **1j** and indeed the stereoselectivity of the phosphonate addition was found to be time dependent (Scheme 2B). Thus, the α/β ratio of **2j**, being 5 : 1 after 10 min, decreased to a 5 : 3 ratio after 120 min. This supports our hypothesis that the initially formed α-isomer **I** equilibrates to the β-isomer **III** *via* intermediate **II** under the basic reaction conditions.

Finally, we explored the conversion of 2-deoxy-2-nitroglycosyl phosphonates to fluorinated aminoglycosyl phosphonates (Scheme 3). Reduction of the nitro group of **2a** with Zn/HCl followed by acetylation of the resulting amine with Ac_2O/pyridine afforded **3** in 71% yield over two steps. Selective cleavage of the benzyl protective groups by catalytic hydrogenation provided



Scheme 3 Synthetic elaboration toward fluorinated aminoglycosyl phosphonates. [a] Determined by ^{19}F NMR using α,α,α-trifluorotoluene as the internal standard.

the partially deprotected aminoglycosyl phosphonate **5**. This compound could also be obtained when **2a** was first subjected to hydrogenation with $Pd(OH)_2/C$ followed by *N*-acetylation in 90% yield over two steps. Treatment of glycosyl phosphonate **3** with TMSI provided the target aminoglycosyl phosphonate **6**. Deprotection of the allyl esters of **2h** smoothly proceeded under mild conditions using $Pd(PPh_3)_4$ in the presence of potassium 2-ethylhexanoate, affording the dipotassium phosphonate **7**.

Conclusions

We have established a highly efficient and stereoselective route to fluorinated aminoglycosyl phosphonates starting from readily available nitroglycals.[34] We have shown that introducing rigidity in the carbohydrate framework could provide access to the biologically important α-anomer. The use of orthogonal protection strategies provides opportunities for post-modification of the carbohydrates or further functionalization of the phosphonates to obtain mimics of nucleotide diphosphate derivatives (*e.g.* UDP-GlcNAc) as competitive inhibitors of glycosyl transferases.

Acknowledgements

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Notes and references

1 L. L. Lairson, B. Henrissat, G. J. Davies and S. G. Withers, *Annu. Rev. Biochem.*, 2008, **77**, 521.
2 C. A. G. M. Weijers, M. C. R. Franssen and G. M. Visser, *Biotechnol. Adv.*, 2008, **26**, 436.
3 L. F. Leloir, *Science*, 1971, **172**, 1299.
4 H.-D. Junker and W.-D. Fessner, *Tetrahedron Lett.*, 1998, **39**, 269.
5 O. Gaurat, J. Xie and J.-M. J. Valéry, *Carbohydr. Chem.*, 2003, **22**, 645.
6 N. Auberger, C. Gravier-Pelletier and Y. Le Merrer, *Eur. J. Org. Chem.*, 2009, 3323.
7 R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683.
8 H. Schachter, *Glycoconjugate J.*, 2001, **17**, 465.
9 S. M. Bowman and S. J. Free, *Bioessays*, 2006, **28**, 799.
10 J. Van Heijenoort, *Microbiol. Mol. Biol. Rev.*, 2007, **71**, 620.
11 A. Bouhss, A. E. Trunkfield, T. D. H. Bugg and D. Mengin-Lecreulx, *FEMS Microbiol. Rev.*, 2008, **32**, 208.
12 F. Casero, L. Cipolla, L. Lay, F. Nicotra, L. Panza and G. Russo, *J. Org. Chem.*, 1996, **61**, 3428.
13 J. Hajduch, G. Nam, E. J. Kim, R. Fröhlich, J. A. Hanover and K. L. Kirk, *Carbohydr. Res.*, 2008, **343**, 189.
14 A. Dondoni, A. Marra and C. Pasti, *Tetrahedron: Asymmetry*, 2000, **11**, 305.
15 R. Chang, T.-T. Vo and N. S. Finney, *Carbohydr. Res.*, 2006, **341**, 1998.
16 K. Müller, C. Faeh and F. Diederich, *Science*, 2007, **317**, 1881.
17 S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320.
18 D. O'Hagan, *Chem. Soc. Rev.*, 2008, **37**, 308.
19 V. D. Romanenko and V. P. Kukhar, *Chem. Rev.*, 2006, **106**, 3868.
20 E. Leclerc, X. Pannecoucke, M. Ethève-Quelquejeu and M. Sollogoub, *Chem. Soc. Rev.*, 2013, **42**, 4270.
21 M.-C. Belhomme, T. Poisson and X. Pannecoucke, *Org. Lett.*, 2013, **15**, 3428.
22 F. Poulain, E. Leclerc and J.-C. Quirion, *Tetrahedron Lett.*, 2009, **50**, 1803.
23 R. R. Schmidt and Y. D. Vankar, *Acc. Chem. Res.*, 2008, **41**, 1059.
24 T. Delaunay, T. Poisson, P. Jubault and X. Pannecoucke, *Eur. J. Org. Chem.*, 2014, 7525.
25 During the preparation of this manuscript, Pannecoucke *et al.*, reported the β-selective addition of organolithium reagents to 2-nitroglycals; see: T. Delaunay, T. Poisson, P. Jubault and X. Pannecoucke, *Eur. J. Org. Chem.*, 2014, 3341.
26 P. K. Kancharla, Y. S. Reddy, S. Dharuman and Y. D. Vankar, *J. Org. Chem.*, 2011, **76**, 5832.
27 S. Dharuman, P. Gupta, P. K. Kancharla and Y. D. Vankar, *J. Org. Chem.*, 2013, **78**, 8442.
28 We chose to report yields and product distribution determined by ^{19}F NMR with an internal standard to provide an

accurate representation of the actual conversion and diastereoselectivity. Although the isolated yields of **2** after flash chromatography were somewhat lower, the products were typically further enriched in the major diastereomer. Further transformations of **2a** provided the products generally as single diastereomers after chromatographic purification (*cf.* **3**–**7**).

29 M. Obayashi, E. Ito, K. Matsui and K. Kondo, *Tetrahedron Lett.*, 1982, **23**, 2323.

30 D. B. Berkowitz and D. G. Sloss, *J. Org. Chem.*, 1995, **60**, 7047.

31 A. Nowacki, D. Walczak and B. Liberek, *Carbohydr. Res.*, 2012, **352**, 177.

32 A. Nowackia and B. Liberek, *Carbohydr. Res.*, 2013, **371**, 1.

33 K. Pachamuthu, I. Figueroa-Perez, I. A. I. Ali and R. R. Schmidt, *Eur. J. Org. Chem.*, 2004, 3959.

34 While our manuscript was under review, a similar approach to fluorinated aminoglycosyl phosphonates employing a silylated difuoromethanephosphonate was reported: T. Delaunay, T. Poisson, P. Jubault and X. Pannecoucke, *J. Fluorine Chem*, 2014, DOI: 10.1016/j.jfluchem.2014.10.001.