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Synthesis of New (Difluoromethylphosphono)Azadisaccharides Designed as Bisubstrate Analogue Inhibitors for GlcNAc:β-1,4 Glycosyltransferases

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Abstract—We report here the design, synthesis and antifungal evaluation of a new model of bisubstrate analogue inhibitor for glycosyltransferases. The synthetic strategy relies on the reductive amination between the aldehyde derived from an *N*-allylphosphono-pyrrolidine and an aminosugar.

Introduction

Glycosyl transferase inhibitors have recently become the focus of particular interest because of their value in basic biochemical research[1] and their therapeutic potential as antifungal, antibacterial, antiviral, antimetastatic or anti-adhesive agents.[2]

The knowledge of the active site of glycosyl transferases is rather limited.[3] Putative mechanisms for the enzymatic process involved in glycosyl transfer reaction using sugar nucleotides, UDP-galactose, UDP-*N*-acetylglucosamine and other so-called Leloir donors have been put forward and subsequently used in the design of potent transition state and bisubstrate analogues of different enzymes of this class.[4] To improve the selectivity and inhibitory potency of such inhibitors, tricomponent bisubstrates containing both the donor and the acceptor molecules involved in the enzymatic glycosyl transfer reaction of the target enzyme, have recently been developed.[5]

As a part of a research programme aimed at designing novel inhibitors of chitin synthetase (an enzyme which catalyses polymerisation of *N*-acetyl-D-glucosamine starting from UDP-GlcNAc), we have become interested in finding a simple, versatile and efficient synthetic route for novel bisubstrate analogues for glycosyl transferases which use GlcNAc as acceptor and different nucleoside diphosphate sugars as donors. To mimic the proposed transition state possibly involved in this class of glycosyl transferases (Fig. 1), we designed **I** as a bisubstrate analogue prototype which includes the following structural features:

1. The five-membered ring azasugar, stereochemically apparented to L-xylose, would mimic the half chair conformation of the glycosyl cation and a possible hydrogen bonded contact of the *N*-acetyl group with enzymes.[3a]
2. Incorporation of a difluoromethylphosphonate group, mimicking the first portion of the pyrophosphate moiety of the donor, might provide favourable contribution to recognition by glycosyltransferases.
3. Insertion of an amino group in the spacer linking the azasugar and GlcNAc glycon could introduce additional electrostatic interaction between the 4-position of the acceptor and enzymes.[6]

Synthesis

The synthetic strategy to reach bisubstrate analogues of type **I** relies on the reductive amination between the aldehyde **11** derived from *N*-allylphosphonopyrrolidines **4** and the 2-acetamido-4-amino-2,4-dideoxy-D-glucopyranoside derivative **9** (Scheme 3). We have already shown the possibility of reaching *N*-benzyl-difluoromethylphosphono-pyrrolidines by condensation of (diethylphosphinoyl)-difluoromethyllithium with *N*-benzylglycosylamines.[7] We used the same methodology (Scheme 1) to prepare the corresponding *N*-allyl derivatives **4**, precursors for

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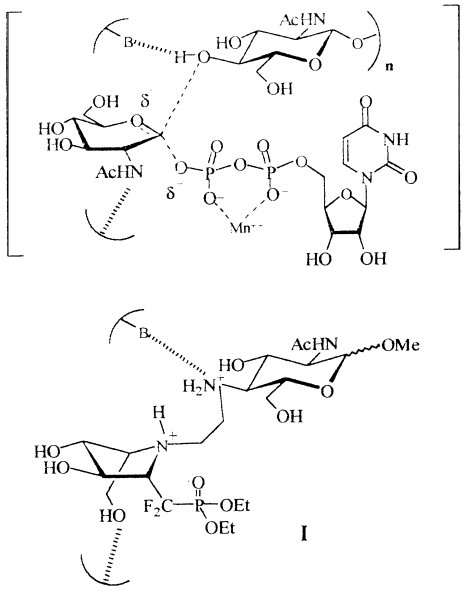
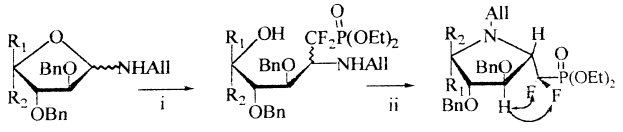
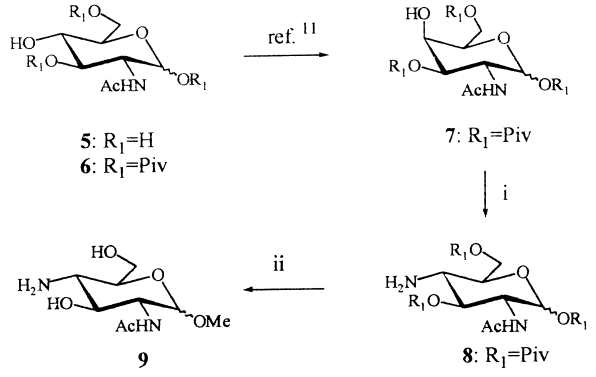


Figure 1.



2a: R_1=CH_2OBn, R_2=H
2b: R_1=H, R_2=CH_2OBn

Scheme 1. (i) $HCF_2P(O)(OEt)_2$, LDA, THF–hexane, −78 °C; (ii) Methanesulfonyl chloride, pyridine.



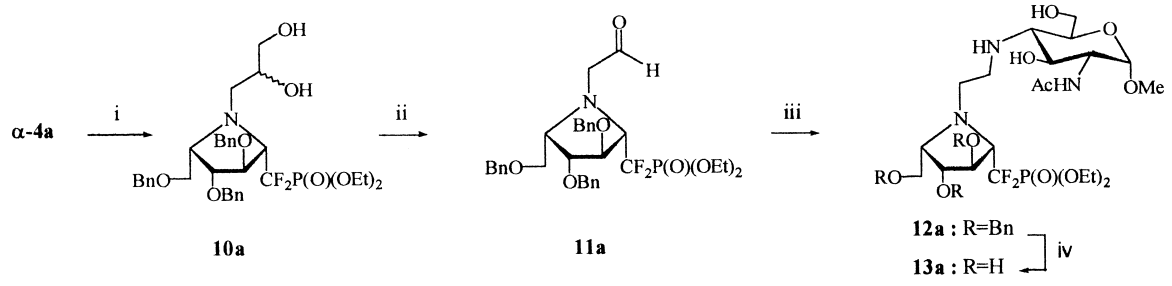
Scheme 2. (i) (a) Tf_2O, pyridine; (b) NaN_3, DMF; (c) H_2, Pd-C, MeOH; (ii) (a) CH_3COCl, MeOH; (b) MeONa, MeOH.

the target aldehydes: *N*-allylfuranosylamines[8] **2a,b** (prepared from commercial 2,3,5-Tri-*O*-benzyl-D-arabinose and from 2,3,5-Tri-*O*-benzyl-L-xylose[9] by reaction with allylamine) were reacted with (diethylphosphinoyl) difluoromethyllithium.

In each case, both possible diastereoisomers **3** were formed in good yields: the reaction favors the formation of the *threo* adduct in a 50% d.e., confirming the stereochemical control by the neighbouring C-2 carbon, as previously postulated.[10] The configuration at the newly created stereocentre was firmly assigned after conversion of **3** to pyrrolidines **4** with methanesulfonylchloride: a substantial nuclear Overhauser effect was observed on ^{19}F NMR signals by saturation of H-3 for minor α isomers **α-4a,b**, suggesting the *cis* relationship between H-3 and the difluoromethylphosphonate moiety in these compounds. Much weaker NOE were measured for major 1-β configurated epimers (not shown on Scheme 1).

The 2-acetamido-4-amino-2,4-dideoxy-D-glucopyranose derivative **9** can be prepared starting from GalNAc as described by Field.[6] Our synthesis used *N*-acetylglucosamine **5** as starting material (a cheap commercial compound) and required selective protection of 1-OH, 3-OH and 6-OH (**6**, Scheme 2) which could be achieved in one step with *N*-pivaloyl imidazole as the acylating agent.[11] After inversion of the 4-hydroxyl function, nucleophilic displacement of the corresponding galactotriflate with NaN_3 followed by catalytic hydrogenation of the azide, led to amine **8**. The *gluco* configuration of **8** was assessed by the 10 Hz coupling constant between H-3 and H-4, confirming the *trans*-diaxial position of these atoms. Formation of the methyl glycoside with in situ-generated HCl in MeOH, followed by classical deprotection of pivaloyl functions with MeOH/MeONa, led to fully deprotected amine **9**.

Coupling of **9** with the *N*-allylphosphonopyrrolidine **4a** relies on a one-pot sequence including: conversion of *N*-allylpyrrolidine to the corresponding aldehyde, addition of **9** and reduction of the resulting imine (Scheme 3). Unexpected ozonolysis of **4a** (as its sulfate salt) gave unsatisfactory results. The aldehyde **11a** was obtained after osmylation followed by the oxidative cleavage of glycol **10a** in CH_2Cl_2 with silica gel-supported $NaIO_4$ as the oxidant.[12] Unstable aldehyde **11a** was condensed without further purification with the amine **9**. Reduction of the in situ generated imine with $NaBH_3CN$ led



Scheme 3. (i) OsO_4, NMO, acetone–water; (ii) $NaIO_4/SiO_2$, CH_2Cl_2; (iii) **9**, $MgSO_4$, EtOH, then $NaBH_3CN$; (iv) HCO_2NH_4, Pd/C, refluxing EtOH.

to the protected bisubstrate analogue **12a** in a 50% overall yield from **4a**. Hydrogenolysis of the *O*-benzyl protecting groups was fairly difficult, and their removal was best achieved by Pd(0)-catalysed transfer hydrogenation affording the hydrosoluble compound **13a**.[13]

This synthetic route has been applied to **α-4b** and **β-4a,b** isomers to reach the corresponding bisubstrate analogues.

Biological Evaluation

Antifungal assays: as **13a** can be considered as a valuable model of bisubstrate analogue inhibitor for chitin synthetase (an important target for antifungal agents)[14] its activity was tested on a panel of several pathogenic fungi[15] (*Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*). Compound **13a** showed no activity up to the maximum tested concentration of 128 μg/mL, whereas the standard compound terbinafine showed the normal activity expected.[17] To explain this lack of cellular activity it may be advanced that **13a** does not penetrate the fungal cell envelope. In order to check this hypothesis, we evaluated the enzymatic inhibitory potency of **13a** on the activity of chitin synthetase from *Saccharomyces cerevisiae*.

For the inhibition studies, enzymatic assays were performed using *Saccharomyces cerevisiae* cells permeabilized with toluene–ethanol according to the procedure described by Masson et al.[18] The activation of enzyme present as zymogen was performed essentially as described by Cabib.[19] Chitin synthetase was assayed by measuring the rate of formation of (^{14}C)-chitin from [UDP^{14}C]-GlcNAc according to the general method reported previously.[20]

Using our enzymatic preparation, K_m value for UDP-*N*-acetylglucosamine was 0.78 mM. Nikkomycin Z, used as reference in our inhibition studies, strongly inhibited chitin synthetase preparation ($K_i = 0.6$ μM), whereas **13a**, tested in the same conditions, and for concentrations up to 500 μM did not inhibit chitin synthetase activity.

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13. ^{1}H NMR (D_2O, 500 MHz, δ ppm, *J* Hz) 4.62 (d, 1H, $J_{1,2}$ 3.5, H-1); 4.24 (m, 4H, $2CH_2CH_3$); 4.17 (dd, 1H, $J_{3',4'}$ 7.0, $J_{3',2'}$ 6.0, H-3′); 4.08 (dd, 1H, $J_{4',3'} = J_{4',5'}$ 7.0, H-4′); 3.79 (dd, 1H, $J_{2,3}$ 10.4, H-2); 3.73 (dd, 1H, $J_{6a,6b}$ 12.1, $J_{6a,5}$ 2.4, H-6a); 3.66–3.55 (m, 4H, H-6b, H-5, H-3, H-6′a); 3.36 (dd, 1H, $J_{6'a,6'b}$ 11.4, $J_{6'b,5'}$ 7.5, H-6′b); 3.28–3.19 (m, 4H, H-2′, CH_3O); 3.02 (m, 1H, H-5′); 2.90 (m, 1H, *N*-CH_2-); 2.78–2.65 (m, 3H, *N*-CH_2); 2.55 (bt, 1H, $J_{4,5} = J_{4,3}$ 10.0, H-4); 1.9 (s, 3H, C(O)CH_3); 1.26 (t, 6H, *J* 7.1, $2CH_3CH_2$). ^{13}C NMR (D_2O, 125 MHz) 174.4 (C=O); 122.2 (dt, $^1J_{C\text{-}F} = 262$, $J_{C\text{-}P} = 210$, CF_2); 98.0 (C-1); 75.1 (C-4′); 75.0 (C-3′); 70.5 (C-2′); 70.3 (C-5); 66.4 (m, CH_2CH_3); 65.3 (C-5′); 65.2 (C-3); 61.0 and 60.6 (C-6′, C-6); 59.6 (C-4); 56.3 (*N*-CH_2); 55.0 (CH_3O); 54.1 (C-2); 45.5 (*N*-CH_2); 21.7 ($COCH_3$); 15.6 (m, CH_2CH_3). ^{19}F NMR (D_2O, 235 MHz) 111.9 ppm (ddd, 1F, $^1J_{Fa,Fb}$ 309, $^2J_{F,P}$ 110, $^3J_{F,H}$ 7); 114.8 ppm (ddd, 1F, $^1J_{Fa,Fb}$ 309, $^2J_{F,P}$ 109, $^3J_{F,H}$ 21). ^{31}P NMR (D_2O 101 MHz) 10.4 ppm (t).
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The MIC was defined as the lowest concentration of the drug causing 80% inhibition of fungal growth in comparison with untreated controls.
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