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Artificial *N*-functionalized UDP-glucosamine analogues as modified substrates for *N*-acetylglucosaminyl transferases

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Dedicated to Professor András Lipták on the occasion of his 70th birthday

Abstract—Analogues of UDP-GlcNAc modified at the 2-acetamido group of the GlcNAc moiety were prepared in order to study their role in the mechanism of *N*-acetylglucosaminyl transferase mediated glycosylation reactions. The structural analogues with *N*-formyl-, *N*-propionyl-, *N*-butyryl- and *N*-isobutyryl-groups were synthesized, utilizing the morpholidate coupling method starting from D-glucosaminyl-1-phosphate after selective N-acylation of its amino group with the appropriate *N*-acyloxysuccinimide esters as well as a chlorinated formylformiate.

Keywords: GlcNAc-1-phosphate; Acylamidoglucose; UDP-glucosamine analogues; Morpholidate coupling; Carbohydrate-binding proteins

1. Introduction

Approaches for efficient syntheses of carbohydrate building blocks up to complex oligosaccharides still receive increasing attention. In addition to naturally occurring carbohydrate structures displayed on cell surfaces and involved in molecular recognition processes mediated by binding events[1–5] unnatural oligosaccharide target compounds became the goal of both chemical and enzymatic approaches.[6–13] Some of these compounds show potential as drug candidates since they interfere in cellular recognition either by modulation or inhibition and give rise to more effective artificial analogues than their natural substrates. A promising and still developing approach besides classical linear and convergent glycosylation paths involves the use of transferases that employ nucleoside diphosphohexoses (NDP hexoses) as activated carbohydrate donors.[14–22] Such transferases of the Leloir pathway display high stereospecificity and regioselectivity in directing an activated carbohydrate donor to a specific position of a recognized acceptor, eliminating the need for numerous protection and deprotection steps. However, this methodology suffers in the sense that these enzymes preclude the use of alternative carbohydrate donors that differ significantly in structure from their naturally occurring counterparts, making enzymatic synthesis of unnatural oligosaccharides a still challenging and difficult endeavour. By use of their naturally occurring glycosylation donor substrate *N*-acetylhexosaminyl transferases take part in the stepwise glycosylation towards glycoconjugates, among them sphingolipids and gangliosides, regulating the essential lipid metabolism in human nervous system cells. A number of disease phenotypes, based on deficient degradation of specific gangliosides are known, including the many well characterized hydrolases taking part in this process.[23] Their counter parts, the ganglioside generating and membrane bound transferases are so far less well investigated since they are not applicable to common methods such as X-ray crystallography and require alternative investigative techniques based on affinity labelling and enzyme–substrate interaction studies. Modified structural analogues of UDP-GlcNAc applicable for this purpose are described in this contribution.

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Natural and unnatural NDP-sugars have been synthesized by the classical method[24] and many more recent variations such as by Wittmann and Wong.[25] In our previous work we reported the syntheses of several structural analogues of UDP-GalNAc, displaying 2-amino-2-deoxy, 2-azido-2-deoxy, *N*-bromoacetyl, *N*-propionyl and *N*-butyryl functionalities by N-acylation of D-galactosaminyl-1-phosphate, in the latter three cases with corresponding *N*-hydroxysuccinimide esters with high *N*-selectivity and good yields.[26] This conception was transferred to D-glucosaminyl-1-phosphate giving rise to *N*-acylamidoglucosyl phosphates all of which were converted to uridine glucosaminyl nucleotides under morpholidate coupling conditions.

Recently in the UDP-GlcNAc series, UDP-*N*-trifluoracetylglucosamine[27] and 2-azido analogue of UDP-GlcNAc were prepared.[28] Attempts for their further processing by UDP-GlcNAc transferases showed success for the N-trifluoracetylated UDP derivative. However, neither the corresponding non-acylated UDP-2-aminoglucose nor the UDP-2-azidoglucose sugar nucleotides proved to be transferable by transferases. This indicates the presence of an acylamido group to be critical since it serves as a necessary recognition element in order to be processed by GlcNAc transferases. UDP-*N*-Formamido-, UDP-*N*-propionylamido-, UDP-*N*-butyramido- and UDP-*N*-isobutyramidoglucose, described in this paper are therefore expected to be potentially transferable in transferase reactions to give oligosaccharide analogues with potential bioactive properties.

2. Results and discussion

2.1. Syntheses of phosphates

The selective N-acylation of unprotected aminosugars with esters derived from *N*-hydroxysuccinimide as activated acylating agents has proven to be a versatile and general approach to acylamidosugars, since such esters display moderate reactivity making them ideal reactants for N-nucleophiles. A wide range of acyl groups can be introduced and many other functionalities such as labile phosphate groups at the anomeric position within the aminosugar are compatible to this reaction as we have shown in our recent work on the synthesis of selectively N-acylated galactosamines.[26]

For the syntheses of *N*-acylamidoglucopyranosyl phosphates and further towards their corresponding uridine diphospho derivatives D-glucosaminyl-1-phosphate (**4**) was chosen as the building block and proved to be a suitable common starting material for the structural analogues of UDP-GlcNAc reported in this paper. The synthesis of D-glucosaminyl-1-phosphate (**4**) started from perbenzylated D-glucal (**1**) and followed a pathway via azidonitration.[29,30] After regioselective introduction of the azide in equatorial manner into the 2-position the 2-azidoglucopyranosyl nitrate obtained was subjected to reductive hydrolysis yielding the benzylated 2-azidoglucose **2** after treatment with sodium nitrite in an aqueous dioxane solution. The following stereoselective phosphorylation was carried out employing dibenzyl-*N*,*N*′-di-*iso*-propylphosphoramidite in the presence of 1*H*-tetrazole as catalyst via an initially formed phosphate. This was oxidized with *meta*-chloroperbenzoic acid to the protected α-phosphate **3** in a one-pot reaction, in which no formation of the corresponding β-phosphate was observed. Complete debenzylation and reduction of the azide functionality was carried out by hydrogenolysis using 10% palladium on activated charcoal affording glucosaminyl-1-phosphate (**4**) in one step, which turned out to be the most critical of the whole synthetic protocol regarding yield and completion of deprotection. The same conditions [50 bar H_2 atmosphere, solvent system ethylacetate, methanol, water (1:2:1) and excessive amount of palladium catalyst] previously reported by us for the synthesis of galactosaminyl-1-phosphate by a similar synthetic route were applied leading to the unprotected amine **4** in 67% yield (Scheme 1).

The subsequent selective N-acylation of the aminophosphate **4** utilizing *N*-propionyl-oxysuccinimide, *N*-butyroxysuccinimide and *N*-isobutyrosuccinimide, respectively, gave the 2-acylamidophosphates **6**–**8** in yields ranging from 80% to 85% at pH 7.0 in a solvent system of THF and water (1:10) dissolving both the esters and the highly polar aminophosphate **4**.

No hydrolysis of the phosphate group and no side products arising from O-acylation at the unprotected 3-, 4- and 6-positions could be monitored as expected. The appropriate *N*-acyloxysuccinimide esters were easily prepared by reacting *N*-hydroxysuccinimide with either propionyl chloride, butyryl chloride and pivaloyl chloride in the presence of triethylamine followed by column chromatographic purification or recrystallization. N-Formylation of the aminophosphate **4** was carried out by use of 2,4,5-trichlorophenylformiate[31] as formylating reagent under improved conditions as described for the preparation of the phosphates **6**–**8**, thus leading to the *N*-formamido modified aminoglucosyl-1-phosphate **5** in 78% yield after Biogel P2 purification procedures and lyophilization in a DIPEA mediated transesterification step. Foregoing attempts to prepare the N-formylated glucosaminyl-1-phosphate by use of the more common formylating reagents such as formyl acetate or *p*-nitrophenyl formiate were unsuccessful or led to unsatisfactory yields and side products.

The acylamido phosphates **5**–**8** were isolated as ammonium salts due to size exclusion chromatography with 250 mM ammonium hydrogencarbonate solution and were converted to triethylammonium salts by ion