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Application of a Janus aglycon with dual function in benzyl-free synthesis of spacer-armed oligosaccharide fragments of polysaccharides from rhizobacterium *Azospirillum brasilense* sp7

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ABSTRACT

Both protective and pre-spacer features of 4-(2-chloroethoxy)phenyl (CEP) aglycon, which belong to the class of Janus aglycons, were engaged in a benzyl-free synthesis of oligosaccharide fragments of polysaccharides from rhizobacterium *Azospirillum brasilense* sp7. Introduction of α-1,4-linked L-fucose residue was performed using 3,4-di-*O*-benzoyl-2-*O*-triisopropylsilyl-α-L-fucopyranosyl *N*-phenyltrifluoroacetimidate in excellent stereoselectivity and high yields. The obtained deprotected di-, tri- and tetrasaccharides contain 4-(2-azidoethoxy) phenyl (AEP) spacer aglycon, which allows straightforward preparation of neoglycoconjugates that will be used for the study of the role of lipopolysaccharide of rhizobacterium *A. brasilense* sp7 in plant–microbe symbiosis. The intermediate protected oligosaccharide building blocks with cleavable CEP/AEP aglycons have a strong potential for further application in the synthesis of more complex oligosaccharides.

1. Introduction

1.1. Neoglycoconjugates and spacer-armed oligosaccharides

Carbohydrates (glycans) and glycoconjugates are important natural compounds with highly complex structures that play many important roles in numerous biological processes [1]. At the same time, it is well known that in many cases behavior of natural glycoconjugates can be successfully modeled by neoglycoconjugates comprising only small (usually terminal) oligosaccharide fragments of the parent glycans [2,3]. However, various strategies [4–10], which have been developed for the assembly of large complex oligosaccharides [11–21], cannot always be *directly* applied to the synthesis of the corresponding neoglycoconjugates since the latter are usually prepared from spacer-armed oligosaccharides equipped with a functionalized aglycon.[1]

There are two fundamentally different approaches to the synthesis of spacer-armed oligosaccharides [2]: (a) a spacer aglycon is introduced after the assembly of the oligosaccharide by an additional glycosylation reaction, (b) a spacer aglycon (or, more commonly, its more inert precursor called pre-spacer aglycon[2]) is present in the carbohydrate fragment from the very beginning of the synthesis of a spacer-armed oligosaccharide.

The first approach usually makes use of temporary blocking group at the anomeric position. After the assembly of the required oligosaccharide, the anomeric protection is cleaved and the resulting hemiacetal is converted into a suitable glycosyl donor[3,4] and then to the glycoside with a functionalized spacer aglycon. Despite the many obvious advantages of this approach, which allows the use of the full power of modern methods of chemical glycosylation [5,6,8,9,22,23] and strategies for oligosaccharide synthesis including block synthesis

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[1] See below a detailed discussion of this issue.

[2] The need for a more stable pre-spacer aglycon stems from the often-observed limited orthogonality of an *extra* protective group, which is required to block a functional group in the aglycon, under conditions used during oligosaccharide assembly and manipulations with *other* protective groups. The use of a pre-spacer obviates the need for this extra protective group.

[3] Such oligosaccharide-based glycosyl donors can also be used in the block synthesis of even more complex oligosaccharides [7].

[4] Note that many modern strategies for the oligosaccharide assembly [7] including pre-activation based glycosylation approaches [10,21,22] allow one to get an oligosaccharide already equipped with a leaving group ready for subsequent glycosylation.

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[5–9], it has a very serious limitation[5] associated with the often-problematic (from the viewpoint of efficiency and stereoselectivity) introduction of a (pre)spacer into a fairly complex oligosaccharide at the last stages of the synthesis. Indeed, it is known that in some cases glycosidation of complex oligosaccharides by simple (functionalized) alcohols may lead [24] to the mixtures of anomers, which are difficult to separate due to their close chromatographic mobilities, while the presence of a (protected) functional group[6] in the (pre)spacer aglycon of a (mono/oligo)saccharide glycosyl acceptor may dramatically (in some cases negatively) influence [25,26] stereochemical outcome or efficiency of glycosylation.[7]

For this reason, an alternative pre-spacer approach [2], which would avoid such problems in the synthesis of the target oligosaccharide, is commonly used. This approach is based on the preparation of protected oligosaccharide as a pre-spacer glycoside that can further be transformed into a fully functional spacer glycoside ready for conjugation with a carrier to give the target neoglycoconjugate. Unfortunately, further application of spacer glycosides in the synthesis of more complex oligosaccharides is met with difficulties since such glycosides normally cannot be transformed to glycosyl donors that could be used in block synthesis of oligosaccharides.

1.2. Janus aglycons

The use of an aglycon with dual function that would simultaneously possess protective and pre-spacer properties thus combining the best features of both mentioned approaches (a Janus aglycon),[8] in our opinion, would be more effective for the assembly of complex oligosaccharides as glycosides with functionalized spacer aglycons since it leaves room for the use of block synthesis of oligosaccharides. To date, several Janus aglycons have already been identified and used in the synthesis of well-defined glycans and neoglycoconjugates thereof. One has to mention allyl glycosides [25,46–48], 3-aminopropyl glycosides [49–53], pentenyl glycosides [54,55], as well as the “extended” 2-(trimethylsilyl)ethyl (SE, TMSEt) glycosides [56] with the cleavable 2-[(hex-5-enyl)dimethylsilyl]ethyl [57] and 2-[(4-methoxycarbonylbutyl)dimethylsilyl]ethyl [58] spacer aglycons.

We have been using 4-(2-chloroethoxy)phenyl (CEP) [35,37,59–64] aglycon,[9] another Janus aglycon, for the preparation of oligosaccharides and neoglycoconjugates (Scheme 1). Like other Janus aglycons, CEP aglycon can be further modified by replacement of the chlorine atom with an azido group (as with ω-chloroalkyl aglycons [66–70]) to give 4-(2-azidoethoxy)phenyl (AEP) aglycon [60,61,63,64]. The azide in AEP aglycon can be directly converted into amine or, alternatively, it can be used in click chemistry approaches [71] for the preparation of a wide range of neoglycoconjugates [2,60,61,63]. Importantly, both CEP and AEP aglycons can be easily removed under oxidative conditions (as with 4-methoxyphenyl aglycon that is known to be a good anomeric blocking group[10] useful in block synthesis of oligosaccharides [11,73–79]) with further application for the synthesis of more complex oligosaccharides.

This possibility to cleave CEP/AEP aglycons amenable to further selective functionalization opens another putative application of Janus aglycons. We envisage that high sensitivity of glycosylation outcome to the nature of aglycon in glycosyl acceptor (see section 1.1 above) may be used to an advantage if glycosyl acceptor was initially designed as a glycoside with a CEP or AEP aglycon. Rational modification of the CEP/AEP aglycon in glycosyl acceptor by introduction of protective/functional groups differing in ability to participate in hydrogen bonding (as donors or acceptors) may favor formation of reaction solutions with different structure hence modulating the outcome of glycosylation and improving stereoselectivity or yield [28]. A similar approach has successfully been used for optimization of a glycosylation reaction by modifying N-protective groups both in glycosyl donor and glycosyl acceptor (though not in aglycon) [80,81]. A possibility to introduce a variety of such remote “stereodirecting” or “activating” groups[11] in the aglycon without modifying protective group patterns on sugar moieties of glycosyl acceptor and glycosyl donor is very promising since it would allow one to optimize a problematic glycosylation step without resorting to changing or even completely abandoning the initially adopted strategy for oligosaccharide assembly, which usually determines the choice of protective groups on sugar moieties. After being used for fine-tuning glycosylation outcome, such modified aglycon could be cleaved leading to the hemiacetal and then to a glycosyl donor that will be used in further synthetic steps as described above (Scheme 1).

1.3. Oligosaccharide fragments of Azospirillum polysaccharides

Bacteria of the genus *Azospirillum* are gram-negative bacteria belonging to plant growth promoting rhizobacteria [82]. Most of their strains were isolated from the rhizosphere or internal tissues of various plants with which they form a symbiotic association. Due to their ability to fix atmospheric nitrogen, produce phytohormones, increase the solubility of phosphates, and act as antagonists against many phytopathogens, azospirilla have a number of promising applications in agricultural biotechnology.

Synthesis of the different fragments of chemical repeating units of *Azospirillum* polysaccharides and their subsequent use for the inhibition of the biological activity of *Azospirillum* lipopolysaccharide may provide a deeper insight into the processes in plant–microbe symbiosis and will enable the identification and characterization, at the molecular level, of biomacromolecules of plant and bacterial cells, which are responsible for the success of interactions of the organisms [83]. At the present time, this approach is the only viable alternative to the bioinformatics approach for the determination of the monosaccharide sequence (biological repeating unit structure) in O-specific polysaccharides, which was successfully employed for representatives of enterobacteria [84,85] but have limited value for representatives of the genus *Azospirillum* for various reasons discussed by us earlier [83].

Recently, the structures of O-specific polysaccharide of lipopolysaccharide of *A. brasilense* sp7 and chemically identical O-glycan of the polar flagellum flagellin of *A. brasilense* sp7 have been established [86–88]. Linear tetrasaccharide corresponding to the OPS of lipopolysaccharide of *A. brasilense* sp7 was synthesized as 2-aminoethyl glycoside [89]. An isomeric branched tetrasaccharide of O-glycan chain of

[5] The problem is not widely recognized in the carbohydrate community although practitioners in the neoglycoconjugate field have been encountering it as follows from the variety of examples of Janus aglycons already suggested to circumvent this issue (see below).

[6] Including an azido group often used as a synthetic equivalent of amino group.

[7] This sensitivity of glycosylation reaction to the nature of remote functional/protective groups is apparently associated, as we have been arguing for some time [27], with their ability to participate in intermolecular interactions (e.g., hydrogen bonding) favoring formation of reaction solutions with different structure [28] hence modulating the outcome of glycosylation, in which fundamentally different supramolecular assemblies of reagents (supramers) are involved [27–40] and the reaction apparently proceeds along alternative pathways within S_N1–S_N2 continuum [41]. For more examples of the influence of nature of glycosyl acceptor (including protective groups) on the yield and stereoselectivity of glycosylation see Refs. [35,42–44].

[8] Here we propose to call such aglycons the *Janus aglycons* (and the corresponding glycosides – *Janus glycosides*) in analogy to the well-known Janus particles [45], which are special types of nanoparticles whose surfaces have two or more distinct physical properties hence two different types of chemistry may occur on the same particle.

[9] Glycosides with homologous 4-(3-chloropropoxy)phenyl (CPP) aglycon with similar properties have recently been described [65].

[10] It is worth noting that CEP/AEP glycosides might be directly transformed into various glycosyl donors in a way similar to that described for 4-methoxyphenyl glycosides [72].

[11] Such groups should rather be called “solvent structure forming” groups since it is solvent structure changes that influence outcome of glycosylation, according to the supramer approach [28].