Glycomics & Lipidomics

Extended Abstract

Glycolipids and glycopeptides: Minimally competent Lewis acid catalysis produces surfactants for use ex vivo and in vivo

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Abstract

Use of minimally competent main group Lewis acids such as InBr3 and Bi (OSO2CF3)3 permit the formation of glycosides in high yield and purity from simple sugar per-acetates at room temperature or above with remarkable α/β-selectivity. Classical reactions rely on very reactive glycosyl donors in conjunction with metal promoters (Hg++, Ag+ etc). Newer methods have used trichloroacetimidates and strong Bronsted or Lewis acids or thioglycosides in conjunction with oxidative or thiophilic activators. Use of the more stable glycosyl per acetates in conjunction with stoichiometric amounts of strong Lewis acids (BF3.Et2O, FeBr3 etc) has been explored with limited success. The use of "minimally competent" Lewis acids has allowed us to perform glycosidation reactions with catalytic amounts of InBr3 or Bi (OTfl)3 well above room temperature and without significant decomposition of the glycoside products. We will discuss the exploitation of these methods to produce glycolipid surfactants from renewable resources as well as glycopeptide drugs that penetrate the blood-brain barrier (BBB). These "biousian glycopeptides" are not subject to "Lipinski's rules" that would otherwise eliminate them as CNS drug candidates. Glycosylated amphipathic helices or "address segments" have been used to target G-protein coupled receptors (GPCRs) in the brain after intravenous administration. The addition of glycosides to endogenous peptide neurotransmitters and peptide hormones imparts favorable pharmacokinetic and pharmacodynamics (PK/PD) properties and enables penetration of the BBB which is not constrained by molecular weight.

Here we report a method for the preparation of anomerically pure β-S-glycopyranosides (1,2-trans-glycosides) from the corresponding peracetate donors. S-glycosylation was performed in CHCl3 at reflux in the presence of a catalytic amount of InBr3. Deacylation of the intermediate peracetates were achieved under Zemplén conditions. Five pyranose examples, monosaccharides D-glucose and D-galactose and disaccharides cellobiose, maltose, and lactose, were used as donors, and five thiols including an α/ω dithiol and Fmoc-L-cysteine were used as acceptors. Melting points, high res MS, [α]D and NMR data ((1)H and (13)C, including COSY, HSQC and HMBC) are reported for compounds not previously described.

A simplified method for the preparation of Fmoc-serine and Fmoc-threonine glycosides for use in O-linked glycopeptide synthesis is described. Lewis acids promote glycoside formation, but also promote undesired reactions of the glycoside products. Use of 'minimally competent' Lewis acids such as InBr(3) promotes the desired activation catalytically, and with greatly reduced side products from sugar peracetates.

Conventional solution-phase synthesis of thioglycosides from glycosyl acetates and thiols in the presence of In(III) triflate as reported for benzyl thioglucoside failed when applied to the synthesis of phenolic and alkyl thioglycosides. But, it was achieved in high efficiency and diastereospecificity with ease by solvent-free grinding in a ball mill.

The acetates in turn were also obtained by the homogenization of free sugars with stoichiometric amounts of acetic anhydride and catalytic In(OTf)3 in the mill as neat products. Per-O-benzylated thioglycosides on grinding with an acceptor sugar in the presence of In(OTf)3 yield the corresponding O-glycosides efficiently. The latter in the case of a difficult secondary alcohol was nearly exclusive (>98%) in 1,2-cis-selectivity. In contrast, the conventional methods for this purpose require use of a coreagent such as NIS along with the Lewis acid to help generate the electrophilic species that actually is responsible for the activation of the thioglycoside donor in situ. The distinctly different self-assembling features of the peracetylated octadecyl 1-thio-α- and β-D-galactopyranosides observed by TEM could be rationalized by molecular modeling.

Catalytic or stoichiometric amounts of Lewis acids were found to be very effective α-directing additives in the stereoselective glycosylations of diverse 2,3-O-carbonate-protected glucose and galactose thioglycoside donors by preactivation protocol. The poor stereoselectivities of 4,6-di-O-acetyl-2,3-O-carbonate protected thioglycoside donors in glycosyl coupling reactions were greatly improved, and excellent α-stereoselectivities were achieved by the addition of 0.2 equiv of BF(3)·OEt(2). On the other hand, the β-selectivities of 4,6-di-O-benzyl-2,3-O-carbonate-protected thioglucoside donor toward glycosylations were reversed completely to the α-selectivities by the use of 1 equiv of SnCl(4), making the stereoselectivity controllable. Furthermore, the poor stereoselectivities of 4,6-di-O-benzyl-2,3-O-carbonate-protected thiogalactoside donor in glycosylations were also improved by using SnCl(4) as additive.

Glycan recognition is typically studied using free glycans, but glycopeptide presentations represent more physiological conditions for glycoproteins. To facilitate studies of glycopeptide recognition, we developed Glyco-SPOT synthesis, which enables the parallel production of diverse glycopeptide libraries at microgram scales. The method uses a closed system for prolonged reactions required for coupling Fmoc-protected glycoamino acids, including O-, N-, and S-linked glycosides, and release conditions to prevent side reactions.

To optimize reaction conditions and sample reaction progress, we devised a biopsy testing method. We demonstrate the efficient utilization of such microscale glycopeptide libraries to determine the specificity of glycan-recognizing antibodies (e.g., CTD110.6) using microarrays, enzyme specificity on-array and in-solution (e.g., ST6GalNAc1, GCNT1, and T-synthase), and binding kinetics using fluorescence polarization. We demonstrated that the glycosylation on these peptides can be expanded using glycosyltransferases both in-solution and on-array. This technology will promote the discovery of biological functions of peptide modifications by glycans.

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