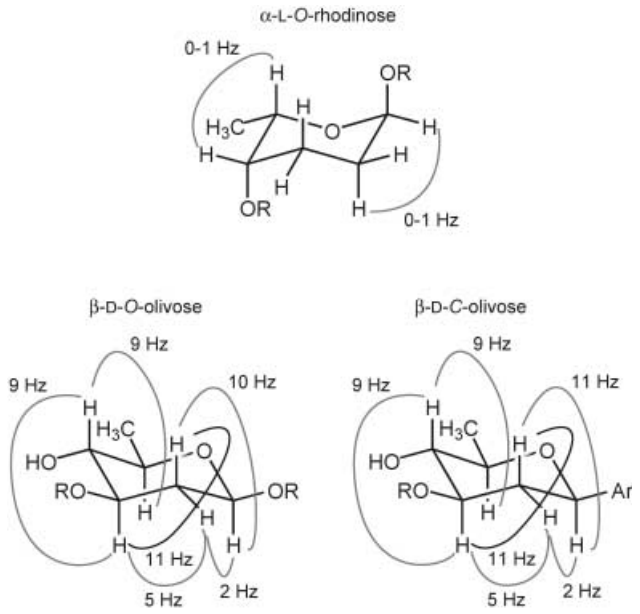

Figure 2. *$^{3}J_{H\text{-}H}$ coupling pattern of the sugar moieties found in **4** and **5**.*

Keywords: biosynthesis · drug design · enzymes · glycosyltransferase · urdamycin

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Expanding the Diversity of Unnatural Cell-Surface Sialic Acids

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Novel chemical reactivity can be introduced onto cell surfaces through metabolic oligosaccharide engineering.[1, 2] This technique exploits the substrate promiscuity of cellular biosynthetic enzymes to deliver unnatural monosaccharides that bear bioorthogonal functional groups into cellular glycans. For example, derivatives of *N*-acetylmannosamine (ManNAc) are converted by the cellular biosynthetic machinery into the corresponding sialic acids and subsequently delivered to the cell surface in the form of sialoglycoconjugates (Scheme 1 A). Analogues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) are also metabolized and incorporated into cell-surface glycans, probably through the sialic acid or GalNAc salvage pathways.[3–6] Furthermore, GlcNAc analogues can be incorporated into nucleocytoplasmic proteins in place of β-*O*-GlcNAc residues.[7] These pathways have been exploited in order to integrate unique electrophiles such as ketones and azides into the target glycoconjugate class. These functional groups can be further elaborated in a chemoselective fashion by condensation with hydrazides[8] or by Staudinger ligation,[9] respectively, thereby introducing detectable probes onto the cell (shown schematically in Scheme 1 B).

We have previously demonstrated that *N*-levulinoylmannosamine (ManLev, **1a**; Scheme 2) is metabolized by cells to *N*-levulinoyl sialic acid (SiaLev, **2a**), which is then appended to glycoconjugates that are ultimately expressed on the cell surface.[3, 8] Increasing the length or steric bulk of the *N*-acyl side chain of **1a** decreases cell-surface expression of the corresponding sialic acids.[10] A rate-determining step in the de novo biosynthesis of unnatural sialic acids appears to be the phosphorylation of ManNAc at the 6-OH by ManNAc 6-kinase.[10] Accordingly, Reutter and co-workers were able to introduce a

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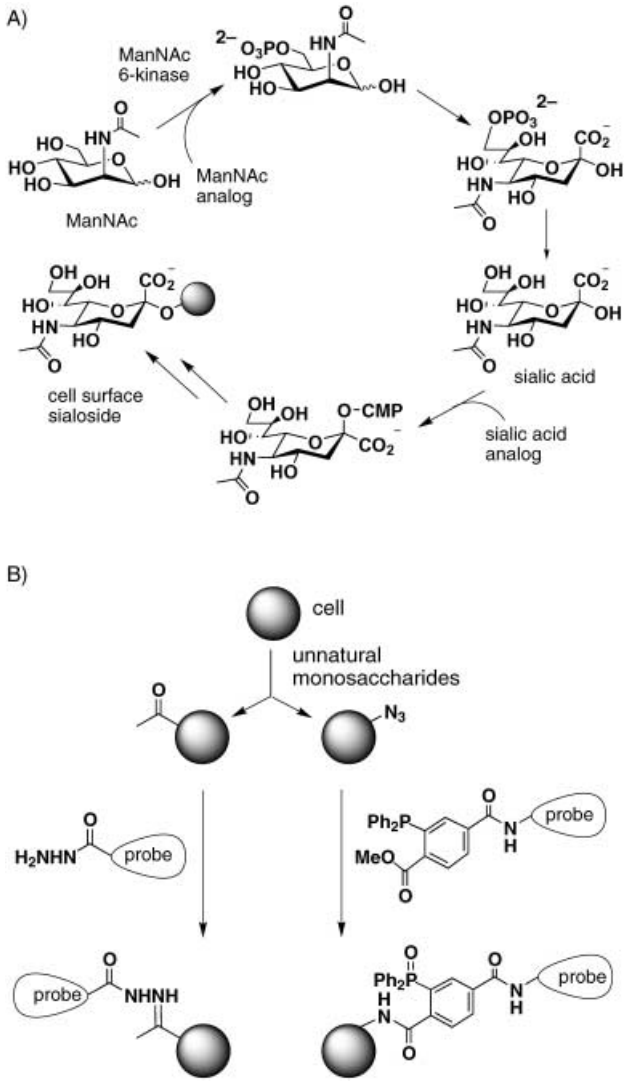
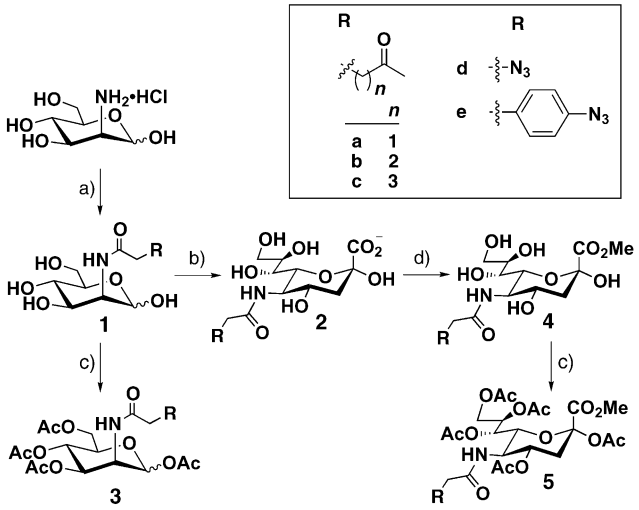


Scheme 1. *Unnatural monosaccharides can deliver novel functional groups to cell-surface glycans. A) Conversion of ManNAc to cell-surface sialic acid and points of interception with unnatural substrates. B) Schematic of metabolic oligosaccharide engineering and cell-surface modification.*



Scheme 2. *Synthesis of mannosamine and sialic acid analogues. a) Acylation with unnatural side chain; b) sodium pyruvate, 10% NaN_3, NeuAc aldolase, potassium phosphate (pH 7.2), 37 °C, overnight; c) acetic anhydride, pyridine, RT, overnight; d) cat. acetyl chloride, MeOH, RT, overnight.*

broader spectrum of sialic acid analogues into cellular glycans by feeding the sialic acid derivatives directly to the cells, thereby bypassing the bottleneck enzyme.[11] These observations suggest that sialic acid analogues might be more efficient delivery vehicles for bio-orthogonal functional groups than their mannosamine precursors. To this end, we synthesized a series of sialic acid derivatives and evaluated the efficiency of their metabolism to cell-surface sialosides. In most cases, the sialic acids were superior to the corresponding mannosamines. These analogues also permitted cell-surface expression of novel groups that would not be tolerated by the de novo pathway, including an aryl azide with potential photocrosslinking capability.

We synthesized a panel of sialic acids with ketone side chains of various length at the *N*-acyl position, a site known to be tolerant of unnatural modifications in the context of the mannosamine precursor (i.e. ManLev, **1a**). Sialic acid analogues **2a**–**c** were synthesized by the neuraminic acid (NeuAc) aldolase-catalyzed condensation[12] of pyruvate and the corresponding mannosamine derivatives[10](**1a**–**c**, Scheme 2). Compounds **1a**–**c** and **2a**–**c** were incubated with the human T-cell lymphoma Jurkat for three days to evaluate their metabolic incorporation into cell-surface glycans. Subsequent treatment of the cells with biotin hydrazide followed by treatment with FITC-labeled (FITC = fluorescein isothiocyanate) avidin allowed analysis of cell-surface ketone expression by flow cytometry.[3] As depicted in Figure 1, keto sialic acids produced a higher level of cell-surface ketones than the corresponding keto mannosamine analogues. The most significant difference was observed with

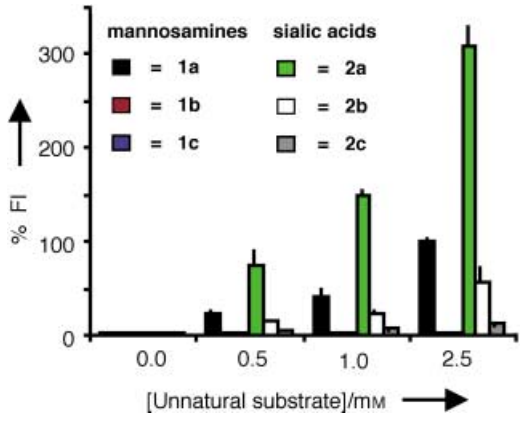


Figure 1. *Metabolism of keto sugars by Jurkat cells. Cells were incubated with the indicated compounds at various concentrations for three days, then treated with biotin hydrazide followed by FITC-avidin according to ref. [3]. The cells were then analyzed by flow cytometry. The data are reported as fluorescence intensity (%FI) relative to cells treated with* ***1a*** *(2.5 mM, 100%), and error bars represent the standard deviation of at least two replicated experiments.*

the levulinoyl analogues **2a** and **1a**; the sialic acid analogue produced three times more cell-surface ketones than the mannosamine analogue. The metabolic efficiency of longer side-chain analogues was reduced in both the mannosamine and sialic acid series, but the 5-oxohexanoyl analogue **2b** was integrated into cell-surface glycans at significant levels, unlike its mannosamine congener. Even the further extended homologue **2c** was expressed on the cell surface at levels significantly above background.

We next synthesized the azide-containing sialic acid derivative **2d** for direct comparison with its previously studied mannosamine counterpart **1d**[9] (Scheme 2). Compounds **2d** and **1d** were incubated with Jurkat cells and subsequently labeled with a phosphine reagent conjugated to the FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys; phosphine-FLAG).[4] Treatment of cells with a FITC-labeled anti-FLAG antibody enabled analysis of cell-surface azide expression by flow cytometry. In contrast to the trend observed with the ketone derivatives, compounds **1d** and **2d** showed similar metabolic efficiency (Figure 2A). In this case, phosphorylation by ManNAc 6-kinase may not impede conversion of **1d** to **2d**, and a more downstream step may be rate-limiting. Notably, previous work has shown that compound **1d** is processed by sialoside biosynthetic enzymes more efficiently than ManLev (**1a**),[4] a result that might reflect a more efficient phosphorylation step for **1d**.

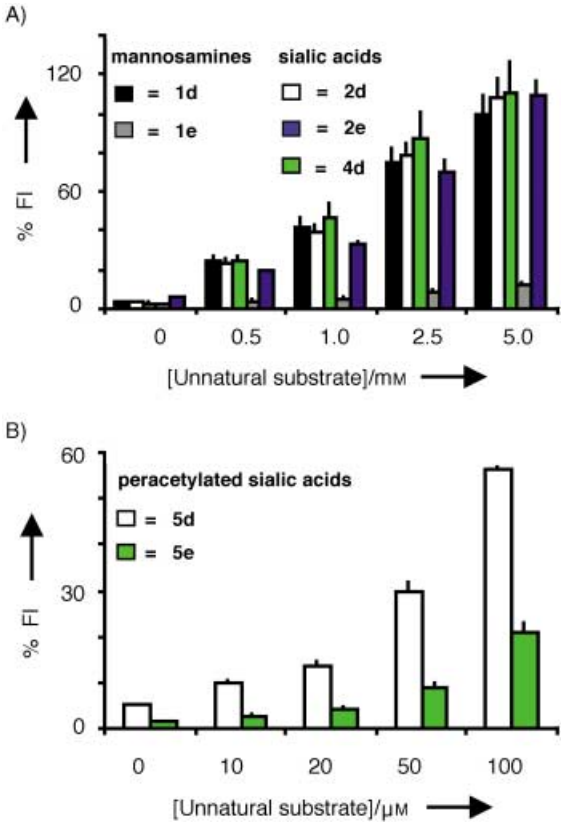


Figure 2. *Metabolism of azido sugars in Jurkat cells. Cells were incubated with the indicated compounds for three days, then treated with phosphine-FLAG followed by a FITC-labeled anti-FLAG antibody, and analyzed by flow cytometry. The data are reported as fluorescence intensity (% FI) relative to cells treated with A)* ***1d*** *(5 mM, 100%) and B)* ***3d*** *(20 μM, 100%). In both panels, error bars represent the standard deviation of at least three replicated experiments. Experimental details are given in the Supporting Information.*

Having demonstrated that larger acyl substituents can be escorted to the cell surface by sialic acid precursors, we sought to extend metabolic oligosaccharide engineering to novel functionalities such as photocrosslinkers. Thus, aryl azide analogues **1e** and **2e** were synthesized (Scheme 2) and subsequently incubated with Jurkat cells for three days. The cells were treated with phosphine-FLAG, treated with the FITC-labeled anti-FLAG antibody and analyzed by flow cytometry (Figure 2A). As expected, treatment of cells with mannosamine analogue **1e** produced cell-surface azides at minimal levels. This compound is likely to be a poor substrate for the enzymes in the de novo sialic acid biosynthetic pathway owing to its large *N*-acyl side chain.[10] Remarkably, however, sialic acid analogue **2e** yielded a robust cell-surface azide signal, which indicates that the aryl azide functionality was tolerated by the downstream enzymes. To our knowledge, this is the first example of the metabolic introduction of a photoactivatable cross-linking agent to mammalian cell-surface glycans.

Previous work in several labs has shown that protection of polar functional groups on sugars can increase their cellular uptake by permitting passive diffusion through membranes.[13–15] Esters have been particularly useful as they can be readily cleaved by cytosolic esterases, this permits further metabolic conversion. Indeed, peracetylated mannosamine derivatives can be fed to cells at concentrations 200-fold lower than the free sugars while achieving the same number of cell-surface products.[13] Reutter and co-workers previously demonstrated that the methyl ester of sialic acid is metabolized with similar efficiency to the free form; this suggests that the ester is cleaved inside cells.[11]

To further improve the metabolic efficiency of our analogues, we investigated protected derivatives. Compound **2d** was protected as its methyl ester **4d**, and this derivative was tested for incorporation into cell-surface glycans. As shown in Figure 2A, compound **4d** was converted to the cell-surface sialoside with the same efficiency as compound **2d**. We further protected the hydroxy groups to provide peracetylated sialic acid analogues **5d** and **5e**. Analysis of their cell-surface products showed that the compounds are efficiently metabolized at extracellular concentrations in the micromolar range (Figure 2B). Overall, we found the fully protected sialic acids to be utilized by cells between eight- and 20-fold more efficiently than their unprotected counterparts.

In conclusion, sialic acid derivatives are efficient vehicles for the delivery of bulky functional groups to cell surfaces, and masking their hydroxy groups improves their cellular uptake and utilization. Furthermore, the successful introduction of photoactivatable aryl azides into cell-surface glycans opens up new avenues for studying sialic acid-binding proteins and elucidating the role of sialic acid in essential processes such as signaling and cell adhesion.

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Keywords: azides · glycans · glycoconjugates · ketones · oligosaccharides · sialic acid · Staudinger ligation

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Lighting Up Biochemiluminescence by the Surface Self-Assembly of DNA – Hemin Complexes

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The discovery of catalytic RNAs (ribozymes) has sparked scientific activities directed to the preparation of new biocatalysts and raised the suggestion that these biomolecules participated in the evolutionary process as preprotein catalysts.[1, 2] Analogously, deoxyribozymes, catalytic DNAzymes, are not found in nature but extensive research efforts have demonstrated the successful synthesis of catalytic deoxyribozymes for many chemical transformations.[3, 4] One interesting example of a catalytic DNA that reveals peroxidase-like activity includes a supramolecular complex between hemin and a single-stranded guanine-rich nucleic acid (aptamer).[5] This complex was reported to catalyze the oxidation of 2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) by H_2O_2, a common reaction used as an assay for peroxidase activity. It was suggested[6] that the supramolecular docking of the guanine-quadruplex layers facilitates the intercalation of hemin into the complex and the formation of the biocatalytically active hemin center.

Enzymes[7] and, specifically, horseradish peroxidase (HRP)[8, 9] are used as biocatalytic labels for the amplified detection of DNA-sensing events. The electrochemical amplified detection of DNA has been accomplished in the presence of different enzymes[7, 8] and the chemiluminescent analysis of DNA in the presence of HRP has been reported.[9] The integration of a DNA biocatalyst into DNA-detection schemes could provide a new method for the detection of nucleic acids that might reveal important advantages: 1) The catalytic DNA may substitute the protein-based biocatalysts, and thus eliminate nonspecific binding phenomena; 2) Tailoring of the DNA biocatalyst as part of the labeled nucleic acid might reduce the number of analytical steps for DNA detection. Here we report that two separated nucleic acids that include the segments A and B—constituting the single-stranded peroxidase deoxyribozyme, which forms a layered G-quadruplex structure (see Scheme 1)[10]—self-assemble in the presence of hemin to form a biocatalyst for the generation of chemiluminescence in the presence of H_2O_2 and luminol. The effect of hybridization with the DNAzyme compounds on the resulting biochemiluminescence is discussed. We also demonstrate the self-assembly of biocatalytic, supramolecular hemin – nucleic acid complexes on gold electrodes in monolayer configurations, and describe the biocatalytic and bioelectrocatalytic formation of chemiluminescence at the

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