MINI REVIEW

Biochemical engineering of the N-acyl side chain of sialic acid: biological implications

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N-Acetylneuraminic acid is the most prominent sialic acid in eukaryotes. The structural diversity of sialic acid is exploited by viruses, bacteria, and toxins and by the sialoglycoproteins and sialoglycolipids involved in cell–cell recognition in their highly specific recognition and binding to cellular receptors. The physiological precursor of all sialic acids is N-acetyl D-mannosamine (ManNAc). By recent findings it could be shown that synthetic N-acyl-modified D-mannosamines can be taken up by cells and efficiently metabolized to the respective N-acyl-modified neuraminic acids in vitro and in vivo. Successfully employed N-acyl-modified D-mannosamines can be incorporated into cell surface sialoglycoconjugates replacing in a cell type–specific manner 10–85% of normal sialic acids. Application of these compounds to different biological systems has revealed important and unexpected functions of the N-acyl side chain of sialic acids, including its crucial role for the interaction of different viruses with their sialylated host cell receptors. Also, treatment with ManNAc-prop, which contains only one additional methylene group compared to the physiological precursor ManNAc, induced proliferation of astrocytes, microglia, and peripheral T-lymphocytes. Unique, chemically reactive ketone and azido groups can be introduced biosynthetically into cell surface sialoglycans using N-acyl-modified sialic acid precursors, a process offering a variety of applications including the generation of artificial cellular receptors for viral gene delivery. This group of novel sialic acid precursors enabled studies on sialic acid modifications on the surface of living cells and has improved our understanding of carbohydrate receptors in their native environment. The biochemical engineering of the side chain of sialic acid offers new tools to study its biological relevance and to exploit it as a tag for therapeutic and diagnostic applications.

Key words: sialic acid/N-acyl modified D-mannosamines/biochemical engineering/ligand–receptor interactions

Introduction

The incorporation of unphysiological sialic acids into living cells provides an opportunity to study specific contributions of sialic acid and its N-acyl side chains to sialic acid–dependent ligand–receptor interactions at a submolecular level. Direct exogenous transfer of cytidine-monophosphate (CMP)–activated sialic acid analogues by purified sialyltransferases onto the enzymatically desialylated cell surface glycoconjugates of living cells has been successfully carried out (Gross et al., 1996; Bergler et al., 1997) but is less efficient and expensive. This review focusses on a recently developed alternative strategy for structurally modifying a high percentage of sialic acids on surface glycoconjugates of living cells (Kaysen et al., 1992a; Keppler et al., 1995) that takes advantage of N-acyl modified forms of mannosamine (Figure 1).

Metabolism of D-mannosamines

The most abundant sialic acid, N-acetylneuraminic acid (NeuAc), is synthesized in vivo from N-acytelylated D-mannosamine (ManNAc) or D-glucosamine (GlcNAc). NeuAc and its activated form, CMP-NeuAc, are biosynthesized in rat liver by five consecutive reactions: UDP-N-acetylgalactosamine (UDP-GlcNAc) → N-acetylmannosamine (ManNAc) → ManNAc 6-phosphate → NeuAc 9-phosphate → NeuAc → CMP-NeuAc (Figure 2). CMP-NeuAc is transported into the Golgi apparatus and, with the aid of specific sialyltransferases, added onto nonreducing positions on oligosaccharide chains of glycoproteins and glycolipids. At the beginning of our studies we asked if N-acyl-modified hexosamines would be metabolized in a same manner as the physiological N-acetylated sialic acid precursors. We could demonstrate that a broad spectrum of mammalian cells can take up synthetic N-substituted D-glucosamine (Kaysen et al., 1993) and D-mannosamine derivatives, metabolize them in the sialic acid biosynthetic pathway, and incorporate structurally altered sialic acids with substituted N-acyl side chains into
Fig. 1. Structures of N-acyl modified mannosamines and biochemically engineered sialic acids.

Various glycoconjugates (Figure 2). The biosynthetic incorporation of unphysiological sialic acids into both sialoglycoproteins and sialoglycolipids has been demonstrated in vitro and in vivo (Kayser et al., 1992a,b; Keppler et al., 1995, 1998; Wieser et al., 1996; Herrmann et al., 1997; Schmidt et al., 1998; Stehling et al., 1999; Collins et al., 2000).

The supplementation of culture medium with ManNProp or ManNBuT resulted in the efficient biosynthesis and incorporation of NeuProp and NeuBuT, respectively, into the human B lymphoma cell line, BJA-B. In contrast, the same concentrations of NeuProp and NeuBuT into the human B ManNBut resulted in the efficient biosynthesis and incorporation into different organs of rats after intravenous administration (Kayser et al., 1992a). Differences in the ability of GlcN and ManN derivatives to serve as sialic acid precursors may be related to tissue-specific differences in cellular uptake of these epimers or to differences in the intracellular conversion of GlcN derivatives to ManN derivatives either by GlcNAc 2-epimerase or UDP-GlcNAc 2-epimerase.

D-Mannosamine derivatives even at millimolar concentrations are generally well tolerated in in vitro cultures, and long-term treatment of Jurkat cells with ManNLev did not affect cell viability (Wieser et al., 1996; Yarem et al., 1998). ManNProp was also well tolerated by both human lymphoid cell lines and fibroblast cell lines from different species (Keppler et al., 1995, 1998; Herrmann et al., 1997) as well as by primary cultures of rat neurons and glia cells (Schmidt et al., 1998). The analogues of ManNBuT and ManNPent reduced the growth rate of certain cell cultures by up to 40% (Keppler et al., 1995). In vivo, the administration of 200 mg per kg body weight ManNProp to rats for a period of 3 weeks had no apparent side effects in Wistar rats (unpublished data). The incorporation of NeuProp showed an organ-specific distribution with highest incorporation rates in liver, lung, and kidney when radiolabeled ManNProp was used as precursor in rats (Kayser et al., 1992a).

The cellular machinery for intracellular transport and metabolism of sialic acid and its precursor analogues seems to be remarkably promiscuous for N-acetyl side chain modifications. In contrast, even minor modifications of the sialic acid N-acetyl side chain, such as the introduction of an uncharged, hydrophobic methylene group at C5, had pronounced effects on specific cell surface recognition functions, including sialylated virus receptors, cell surface differentiation markers, and regulation of cell proliferation, as will be discussed below.

Once taken up by cells, structurally modified D-mannosamines are thought to compete with physiological intracellular ManNAc pools for entry into the sialic acid biosynthetic pathway. Initial phosphorylation is catalyzed by either the bifunctional UDP-GlcNAc 2-epimerase/ManNAc kinase (Hinderlich et al., 1997; Effertz et al., 1999) or, as recently shown (Hinderlich et al., 2001), by the GlcNAc kinase. In most experimental studies, fairly high D-mannosamine analogue concentrations (5–30 mM) in tissue culture had to be applied to achieve structural modification of 15–70% of cell surface glycoprotein-associated sialic acids (Table I and references therein). Recently, subclones of BJA-B cells have been described that are constitutively hyposialylated (Keppler et al., 1999b), may thus be particularly valuable for studying the effect of sialic acid N-acetyl modifications on different sialic acid–dependent ligand–receptor interactions.

Sarkar and colleagues have demonstrated that peracetylation, which has been previously shown to greatly enhance uptake and anabolic utilization of carbohydrates (Sarkar et al., 1995, 1997), increased the conversion of peracetylated ManNGc into NeuGc in a rodent neuroblastoma/glioma cell line by up to 100-fold (Collins et al., 2000). Similarly, preliminary studies indicate that peracetylation of ManLev enhances its potency as a precursor approximately 200-fold (Lee et al., 1999). Peracetylation and other chemical modifications of D-mannosamine analogues may thus greatly improve their bioavailability and increase the fraction of structurally modified sialic acids both in in vitro and in vivo systems.

Engineering of cell surface sialic acid interferes with virus infections

Recognition of and binding to an appropriate receptor on the surface of the host cell by a virus is the first step of viral
Despite the ubiquity of sialic acid on the cell surface, sialylated oligosaccharides appear to be an essential receptor component for many animal viruses from different virus families, such as influenza A and C viruses (orthomyxoviruses), Newcastle disease virus (paramyxovirus), cardioviruses (picornaviruses), and murine and primate polyomaviruses (papovaviruses) (Burness, 1981). No complete natural receptor for any of these viruses has been unambiguously identified. On the
other hand, the binding of sialic acid to virus surface proteins is well characterized to the extent that the three-dimensional structures of two viral attachment proteins complexed with simple, monovalent sialylated saccharides, specifically, influenza A virus hemagglutinin (Weis et al., 1988; Watowich et al., 1994) and complete mouse polyomavirus (Stehle et al., 1994; Stehle and Harrison, 1996), have been solved. When 18–64% of the sialic acids on host cells were modified by treatment with the respective analogues (usually ManNProp), binding and/or infection of different primate polyomaviruses that depend on cell surface sialic acids for entry were markedly altered (Keppler et al., 1995; Herrmann et al., 1997). For human polyomavirus BK the elongation of the N-acyl side chain by one methylene group (from N-acetyl to N-propanoyl) resulted in up to sevenfold enhancement of infection, whereas further elongations to N-pentanoyl drastically reduced infection. Binding and infection by the African green monkey B-lymphotropic papovavirus were decreased about fivefold and more than 10-fold, respectively, by incorporation of the N-propanoyl side chain. In contrast, the sialidase-resistant

Table I. Biochemical engineering of the \(N\)-acyl-side chain of sialic acids: biological implications.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Species and Cell Type</th>
<th>% Incorporation</th>
<th>Biological Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ManNProp,</td>
<td>Human B-lymphoma (BJA-B)</td>
<td>45–55</td>
<td>4- to 8-fold loss of virus binding and 10- to 20-fold reduction of infection of lymphotropic papovavirus</td>
<td>Keppler et al., 1995</td>
</tr>
<tr>
<td>ManNBut,</td>
<td>Monkey kidney epithelium (Vero)</td>
<td>44–64</td>
<td>5-fold increase (ManNProp) or 4-fold decrease (ManNPent) of human polyomavirus BK infection</td>
<td>Keppler et al., 1995</td>
</tr>
<tr>
<td>ManNPent</td>
<td>Mouse fibroblasts (NIH-3T6)</td>
<td>ca. 35</td>
<td>2 to 7-fold reduction of binding and 3 to 4-fold reduction of infection of murine polyomavirus</td>
<td>Herrmann et al., 1997</td>
</tr>
<tr>
<td>ManNProp,</td>
<td>Dog kidney epithelium (MDCK-II)</td>
<td>18–35</td>
<td>Up to 5-fold reduction of influenza A virus infection</td>
<td>Keppler et al., 1998</td>
</tr>
</tbody>
</table>

2. Stimulation of neural cells

| ManNProp   | Rat brain cells                        | ca. 35          | Induction of proliferation of microglia and astrocytes, A2B5 epitope induction on neurons Prolonged expression of the A2B5 epitope as a marker for developing oligodendrocytes Calcium oscillation upon stimulation with GABA | Schmidt et al., 1998, 2000 |
| ManNGcPA   | Rodent neuroblastoma/glioma (NG108–15), human T-lymphoma (Jurkat) cell line | 70–90           | Abrogation of myelin-associated glycoprotein (MAG) binding to NeuGc expressing cells | Collins et al., 2000 |

3. Stimulation of T-lymphocytes

| ManNProp   | Human B-lymphoma (BJA-B), myeloid leukemia (HL-60) | 85              | Stimulation of proliferation, IL-2 secretion Increased expression of IL-2 receptor and transferrin receptor | Mantey et al. (forthcoming) |
| ManNBut    | Human T-lymphocytes                        | 10              | Stimulation of proliferation, IL-2 secretion Increased expression of IL-2 receptor and transferrin receptor | Reutter et al., 1999 |

4. Biochemical introduction of a reactive group

| ManNLev    | Mouse fibroblasts (NIH-3T3), human T-lymphoma (Jurkat), myeloid leukemia (HL-60), cervix carcinoma cell line (HeLa), primary human umbilical vein endothelial cells | Not determined (FACS analysis) | Ketone as cell surface substrate for selective chemical addition of ligands | Mahal et al., 1997, Yarema et al., 1998, Lee et al., 1999 |
| ManNAc-Azido | Jurkat, HeLa                             | Not determined (FACS analysis) | Azide as cell surface substrate for selective chemical modification (Staudinger reaction) | Saxon and Bertozzi, 2000 |

5. Immunotargeting tumor cells

| ManNProp   | Rat leukemic cells (RBL-3H3), mouse leukemic cells (RMA) | Not determined (FACS analysis) | Engineered polysialic acids as targets for complement kill \textit{in vivo}, reduction of metastasis formation | Liu et al., 2000 |

6. Modulation of contact-dependent cell growth

| ManNProp,  | Human diploid lung fibroblasts           | Not determined      | Abolishing of contact-dependent inhibition of cell growth | Wieser et al., 1996 |

| ManNBut,   |                                      |                  |                                                        |                    |
| ManNPent   |                                      |                  |                                                        |                    |

that depend on cell surface sialic acids for entry were markedly altered (Keppler et al., 1995; Herrmann et al., 1997). For human polyomavirus BK the elongation of the \(N\)-acyl side chain by one methylene group (from \(N\)-acetyl to \(N\)-propanoyl) resulted in up to sevenfold enhancement of infection, whereas further elongations to \(N\)-pentanoyl drastically reduced infection. Binding and infection by the African green monkey B-lymphotropic papovavirus were decreased about fivefold and more than 10-fold, respectively, by incorporation of the \(N\)-propanoyl side chain. In contrast, the sialidase-resistant
infection of closely related simian virus 40 was, as expected, unaffected (Keppler et al., 1995).

The three-dimensional crystal structures of influenza A virus and murine polyomavirus complexed with sialylactose provide a basis for a submolecular analysis of the interactions of unphysiologically sialic acids with these viruses, both in molecular modeling studies and in living cells carrying structurally altered sialylated virus receptors. For murine polyomavirus infection, NeuAc in α2,3-linkage with galactose has been identified as an essential receptor component (Cahan et al., 1983; Stehle et al., 1994). Studies on the biosynthetic modification of sialic acid on permissive 3T6 mouse fibroblasts have shown that the addition of a single methylene group to the N-acetyl side chain results in loss of binding and infection by murine polyomavirus (Herrmann et al., 1997). The biosynthetic incorporation of NeuProp into 3T6 cells resulted in a 30% reduction of high-affinity receptors, pointing to a very close interaction of the N-acetyl side chain with the virus surface and the efficient disruption of this interaction by the presence of the additional methylene group. This experimental observation agrees well with the crystal structure data: Molecular modeling suggested that the aliphatic elongation of the N-acetyl side chain leads to steric hindrance with the peptide backbone of a loop walling the tip of the shallow sialic acid binding groove in the viral capsid protein VP1.

Incorporation of only 18–35% biochemical-engineered sialic acids using ManNBut- and ManNPent as precursors led to an inhibition of influenza A virus infection by up to 80% in MDCK-II cells (Keppler et al., 1998). Molecular modeling showed that elongated N-acetyl side chains cause an increase in the total energy of the complex of over 20 kcal/mol, indicating a reduction in binding affinity towards the influenza hemagglutinin of several orders of magnitude. The experimental data also suggest a polyvalent interaction of the virus particle with several sialic acid–bearing oligosaccharides as part of a receptor complex, so that modification of one in five sialic acids would be sufficient to render such a receptor complex nonfunctional. In analogy with murine polyomavirus, virus–cell interaction is apparently disturbed by the steric hindrance of aliphatically elongated N-acetyl groups in the cellular sialoglycoconjugate receptor.

Biosynthetically modified sialic acids represent a potent experimental tool for further analyzing the high affinity interaction of influenza viruses with structurally modified virus receptors and for the more detailed investigation of the contribution of specific sialic acid groups. This may also aid the development, elucidation, and optimization of antiviral strategies, including the use of sialic acid analogues that have been shown to potently inhibit influenza A virus receptor binding both in vitro and in vivo (von Itzstein et al., 1993; Monto et al., 1999).

The N-acetyl group is a critical determinant for the binding and infection of three different mammalian polyoma viruses (Keppler et al., 1995; Herrmann et al., 1997), small DNA tumor viruses with nonenveloped icosahedral capsids, and a human member of the orthomyxovirus genus, a family of large enveloped RNA viruses (Keppler et al., 1998). Apart from their structural differences, these viruses also use quite distinct modes of infection. The N-acetyl group has also been previously implicated in the host range determination of *Escherichia coli* with strain K99 fimbriae (Teneberg et al., 1990). This provides evidence that the evolutionary conservation of the N-acetyl group of sialic acid is important for the specific interaction of microorganisms with their sialylated receptors.

**Engineering chemical reactivity on cell surfaces by metabolic delivery of ketone and azide groups to sialic acid residues**

The apparent promiscuity of the cellular sialic acid biosynthetic pathway for N-acetyl modified D-mannosamines has also been exploited to introduce chemically reactive ketone or azide groups into cell surface sialoglycoconjugates of primary human cell cultures and cell lines from different species.

Bertozzi and colleagues have shown that the introduction of a N-levulinoyl side chain bearing a ketone group (ManNLev) is tolerated by the biosynthetic pathway for sialylated glycoconjugates (Mahal et al., 1997; Yarema et al., 1998; Lee et al., 1999). Although ketone groups are abound inside cells in the form of metabolites, such as pyruvic acid and oxaloacetate, they are typically absent from cell surfaces; thus, glycoconjugates displaying a ketone group at the surface can be chemoselectively ligated with hydrdrazine, hydroxylamine, or thiosemicarbazide groups. This allows, for example, for chemotargeting of drugs.

Biosynthetic incorporation of the levulinate residue into surface glycoconjugates has been used as a means to build an artificial receptor for adenoviral gene transfer in otherwise nonpermissive target cells, for example, murine NIH-3T3 fibroblasts. Biotin hydrazine was covalently bound to the uniquely generated ketone group. Next, a NeutrAvidin–conjugated anti-adenovirus antibody, specific for the adenoviral fiber protein, was bound to pretreated cells, which facilitated adenoviral uptake and gene transfer into NIH-3T3 cells (Lee et al., 1999).

More recently, the same laboratory demonstrated chemoselective cell surface engineering using another set of reactive groups. Azides were installed within cellular glycoconjugates by metabolism of synthetic N-azidocetylmannosamine (ManNAc-azido). Displayed at the cell surface, azides were reacted with biotinylated triarylphosphine to produce a stable cell surface adduct (Saxon and Bertozzi, 2000). The azide and phosphine are both abiotic and essentially unreactive toward cellular biomolecules identifying their reaction as a cell-compatible chemoselective ligation which could proceed both inside and on the outside of cells. These novel approaches exemplify the potential application of biochemically engineered cell surfaces displaying abiotic reactive groups through the use of N-acetyl-modified D-mannosamines.

**Immunotargeting tumor cells expressing unnatural sialic acids**

Immunotargeting of tumor cells by creating vaccines based on cancer-specific cell surface glycoconjugate antigens has long been proposed, but many tumors fail to express unique markers and are more frequently characterized by different population densities of cell surface antigens compared to normal cells. Vaccines based on such common antigens are thus poorly immunogenic. Using ManNProp, Liu and colleagues have applied biochemical engineering of PSA expressed on leukemic tumors to render them more susceptible to a monoclonal antibody–mediated cell killing (Liu et al., 2000). The overexpression engineering of the N-acetyl side chain of sialic acid
of PSA is found on a number of cancers, including small cell lung carcinomas and Wilms’ tumors (Troy, 1992; Fukuda, 1996), and is thought to promote tumor cell metastasis (Michalides et al., 1994; Scheidegger et al., 1994). PSA is ubiquitous on fetal tissues, but in adults it is expressed only in specific regions of the brain. Interestingly, incorporation of ManLev into PSA did not seem to impede the neurite outgrowth of NT2 neurons (Charter et al., 2000). Also, PSA with N-propanoyl groups is highly immunogenic and a monoclonal antibody, 13D9, which specifically recognizes PSA, has been raised (Pon et al., 1997). Liu et al. (2000) demonstrate that the incorporation of NeuProp into leukemic cells renders them susceptible to killing mediated by monoclonal antibody 13D9. Furthermore, in an in vivo study this antibody effectively controlled metastasis of a solid tumor model in mice in which ManNProp had been coadministered. Future work will have to address the applicability of this strategy to the immunonegativity of PSA-expressing human cancers and assess the potential adverse effects of ManNProp for physiological sialic acid–dependent ligand–receptor interactions in normal human tissues.

N-Acyl-modified sialic acids can stimulate neural cell growth and alter the interaction of neural cells with myelin-associated glycoprotein

Modified sialic acids also have striking biological effects on the individual cell types of the mammalian nervous system. ManNProp was metabolized by primary glial cells in culture and was expressed as NeuProp in glycoproteins on the cell membrane (Schmidt et al., 1998). ManNProp induced the proliferation of astrocytes and microglia but not of oligodendrocyte progenitor cells, whereas oligodendrocytes showed increased signs of a nonmature cell stage when ManN-Prop was applied, as shown by the expression of the neural ganglioside epitope A2B5. The A2B5 epitope is regarded as a specific marker for a subset of rat oligodendrocyte progenitor cells. Oligodendrocyte progenitor cells are proliferative and migratory, properties that are thought to play an important role in the regeneration of the adult nervous system. They develop constitutively into myelin-forming cells in vitro and in vivo. Oligodendrocytes are functionally impaired in a number of severe neurological diseases such as multiple sclerosis.

Because the A2B5 epitope is considered to be a functional marker of cells of the early oligodendrocyte lineage, ManN-Prop has to be considered as a potent regulator of the lineage progression of oligodendrocytes at early stages of their development. These results also underline the important role of biochemically engineered sialic acid in neuronal development and regeneration. Although cerebellar neurons do not express the A2B5 epitope under the culture conditions of our experiments, surprisingly they start to express this epitope on their neurites after application of ManNProp (Schmidt et al., 1998). Furthermore, the incorporation of N-propanoylneuraminic acid, followed by the application of γ-amino butyric acid (GABA), leads to calcium oscillations in oligodendrocytes. It has been proposed that biosynthetic modifications of the acyl side chain of sialic acid in conjunction with the activation of GABA receptors modulate the intracellular calcium concentration in oligodendrocytes (Schmidt et al., 2000).

Further data underlining the potency of biochemically engineered sialic acid in myelination have been recently reported by Collins and colleagues, who demonstrated that the conversion of neuronal sialic acids from N-acetyl- to N-glycolylneuraminic acid using ManNGcPA as a synthetic precursor resulted in an inhibition of the binding of myelin-associated glycoprotein (MAG) to neuronal cells (Collins et al., 2000). This interaction of MAG with nerve cells is implicated in the inhibition of nerve regeneration after injury. Thus, a means of interfering with MAG binding to nerve cells might enhance the possibility of posttraumatic nerve regeneration. Collins and colleagues demonstrated that in vitro up to 80% of the ganglioside-associated sialic acids and 70% of the protein-associated sialic acids were converted to NeuGc. A prior in vivo study found only a low incorporation of intraperitoneally injected ManNProp into sialic acids in the rat brain relative to incorporation rates in other organs (Kayser et al., 1992a). Peracylation of ManNGc or alternative routes of in vivo delivery may increase the conversion of NeuAc to NeuGc in the central nervous system, hopefully leading to the evaluation of synthetic D-mannosamines as therapeutic agents in nerve regeneration.

In this context, it should be noted that in human lung fibroblasts, the contactinhibin-regulated cell growth is influenced by chemically modified N-acyl mannosamines. By treatment of these cells with ManNProp, ManNBut, or ManNPent for 7 days the density-dependent inhibition of growth was abolished (Wieser et al., 1996).

Activation of human T-lymphocytes by ManNProp

NeuAc has been implicated in the differentiation and maturation of lymphocytes (Varki, 1993; Schauer et al., 1995). Surprisingly, pretreatment of human T-cells with ManNProp stimulated their proliferation in a dose-dependent manner. At 10 mM ManNProp the proliferative response of T-cells was in the same range as that observed with the commonly used toxic plant lectins concanavalin A or wheat germ agglutinin. ManN-Prop, however, did not induce cytotoxicity, even at high concentrations. In addition to increasing the proliferation rate of T-cells, ManNProp also induced the secretion of interleukin-2 (IL-2) and the expression of the IL-2-receptor α-chain, which are all hallmarks of T-cell activation (for review, see Reutter et al., 1999).

Biosynthetic engineering of cell surface sialic acids has enabled us to perform a controlled and efficient “biochemical microsurgery” of sialoglycoconjugates on living cells. The biological properties of native sialic acid in a variety of sialylated cell surface receptors can now be studied in more detail, with particular reference to its N-acyl side chain. In combination with cell lines deficient in UDP-GlcNAc 2-epimerase activity and thus in the absence of competing endogenous sialic acids, this method of biochemical engineering allows the generation of cells with nearly homogenous populations of modified sialic acids, which should further improve the studies of sialic acid–dependent ligand–receptor interactions. In the future, therapeutic and diagnostic applications may be found for synthetic D-mannosamine derivatives.
Abbreviations

GABA, γ-aminobutyric acid; MAG, Myelin-associated glycoprotein; ManNAc, N-acetyl-D-mannosamine; ManNAc-azido, N-azidoacetyl-D-mannosamine; ManNProp, N-propanoyl-D-mannosamine; ManNBut, N-butanoyl-D-mannosamine; ManNPent, N-pentanoyl-D-mannosamine; ManNHex, N-hexanoyl-D-mannosamine; ManNCrot, N-crotonoyl-D-mannosamine; ManNLev, N-levulinoyl-D-mannosamine; ManNGc, N-glycolyl-D-mannosamine; NCAM, neural cell adhesion molecule; PA, peracetylated; PSA, alpha2,8-poly-sialic acid.

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