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# Potent and specific sialyltransferase inhibitors: imino-linked 5a′-carbadisaccharides

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**Methyl 5a′-carba-β-lactoside, imino-linked, has been shown to possess potent and specific inhibitory activity ($IC_{50}$ = 185 μM) toward rat recombinant α2,3-sialyltransferase.**

In recent years, extensive studies on different functions of oligosaccharide chains on the cell surface have been carried out, attracting much attention. In particular, glycoconjugates containing sialic acid residues on non-reducing terminals have been shown to be involved in various cell interactions.[1] Sialyltransferases transfer a sialic acid residue to a non-reducing end of the sugar chain and development of specific inhibitors is of obvious interest since epitopes containing sialic acid glycoconjugates are intimately involved with many diseases.[2]

Design of candidate effective sialyltransferase inhibitors is therefore a high priority. It is desirable that sialyltransferase inhibitors should have high specificity for sialyltransferases and biological stability. A number of donor mimetic inhibitors prepared so far have been shown to possess high affinity for sialyltransferase,[3] but reports on acceptor-type inhibitors have been limited. Since some glycosyltransferases may not recognize acceptors which are partially deoxygenated,[4] design of acceptor-mimicking inhibitors is in fact complex. In addition, saccharide derivatives are likely to be promptly hydrolyzed and metabolized in organisms, so that persistence is a problem.

Carba-oligosaccharides,[5] belonging to a family of pseudo-oligosacchardies, are characteristically never metabolized by glycosidases, despite having structures very similar to the three-dimensional conformation of activated natural oligosaccharides. Therefore, they would be expected to be desirable sugar mimetics with potent medical effects. Biological evaluation of carba-oligosaccharides active against α-fucosyltransferases has been carried and some have thereby been recognized as good acceptors.[6] In the present paper,[7] four 5a′-carbadisaccharides were chosen and assayed for inhibitory activity against sialyltransferases.

The four carbadisaccharides were of two types: ether-linked methyl 5a′-carba-β-lactoside **1**, and imino-linked methyl 5a′-carba-β-lactoside **2**, methyl *N*-acetyl-5a′-carba-β-isolactosaminide **3**, and methyl *N*-acetyl-5a′-carba-β-lactosaminide **4**. Ether-linked 5a′-carbadisaccharides have previously been prepared by coupling of 1,2-anhydro-3-*O*-benzyl-4,6-*O*-benzylidene-5a-carba-β-D-mannopyranose[8] **5** and partially benzylated sugar acceptors in the presence of sodium hydride. On the other hand, 1,2-anhydro-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba-α-D-galactopyranose[9] **6** has successfully been employed to prepare imino-linked carbadisaccharides. Thus, the epoxide ring is readily cleaved at C-1 by protected amino sugars, selectively giving rise to coupling products. With these methods,[7] compounds **1**, **2**, and **4** have been synthesized as carbadisaccharide derivatives (Fig. 1).

We have newly prepared compound **3** by this convenient method. Coupling of the epoxide **6** with the protected amine[10] **7** prepared from *N*-acetyl-D-glucosamine was carried out at 120 °C in a sealed tube for 3 weeks to give the desired protected carbadisaccharide (24%), which was isolated, after some processing, as the peracetyl derivative[11] **8** (84%). Removal of the protecting groups gave the free carbadisaccharide **3** (Scheme 1). After purification, the carbadisaccharides were estimated for their ability to act as acceptors for both rat recombinant α2,3- and rat liver α2,6-sialyltransferases. The enzyme assay conditions using the fluorescent assay method were as previously reported.[12] However, these assays showed that the carbadisaccharides synthesized did not act as acceptors. Therefore, we investigated whether these might bind to the combining site for the two enzymes under the inhibition assay conditions.

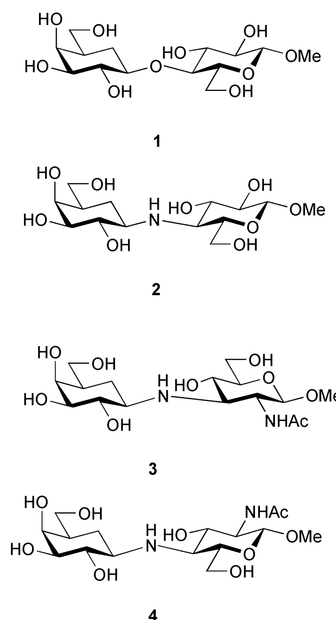


**Fig. 1** Four 5a′-carbaoligosaccharides.

Inhibitory activity of compounds **1**–**4** towards rat recombinant α2,3- and rat liver α2,6-sialyltransferases were investigated with 4-methylumbellipheryl-labeled LacNAc[13] as an acceptor substrate. The enzyme assay solution containing 4-methylumbellipheryl LacNAc (300 μM), CMP-Neu5Ac (50 μM), compounds **1**–**4** (0–1 mM), triton X-100 (0.5%), bovine serum albumin (9 μg), α2,3-sialyltransferase (0.07 mu) in 50 μM HEPES buffer (30 μL) was incubated at 37 °C for 30 min. In the case of α2,6-sialyltransferase, solution containing 4-methylumbellipheryl LacNAc (300 μM), CMP-Neu5Ac (50 μM), compounds **1**–**4** (0–1 mM), triton X-100 (0.5%), bovine serum albumin (9 μg), α2,6-sialyltransferase (0.08 mu) in 50 mM solution cacodylate buffer (30 μL) was employed for the assay.

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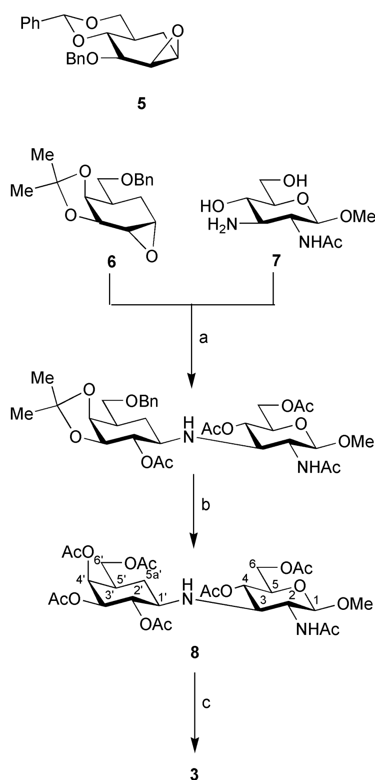
**Table 1** Enzyme inhibitory activity of 5a′-carbadisaccharides **1**–**4** against α2,3- and α2,6-sialyltransferases

| Compound | Inhibitory activity ($IC_{150}$/μM) | |
|---|---|---|
| | α2,3-Sialyltransferase | α2,6-Sialyltransferase |
| *O*-Linked 5a′C-β-Lac-OMe: **1** | 419 | 903 |
| *N*-Linked 5a′C-β-Lac-OMe: **2** | 185 | 533 |
| *N*-Linked 5a′C-β-IsolacNAc-OMe: **3** | 245 | 651 |
| *N*-Linked 5a′C-β-LacNAc-OMe: **4** | >1 mM | >1 mM |

$K_m$ values of 4-methylumbellipheryl LacNAc are 264 μM and 323 μM for α2,3-and α2,6-sialytransferases, respectively.



**Scheme 1** *Reagents and conditions*: a) **6** (1.5 molar equiv.), 2-propanol, 120 °C in a sealed tube, 3 weeks; b) 80% aq. AcOH, 110 °C, 0.5 h; $H_2$, Pd/C, EtOH; $Ac_2O$, pyridine; c) NaOMe, MeOH; Dowex 50 W × 2 ($H^+$) resin, 5% aq. $NH_3$.

Results of the enzyme-inhibition assays of compounds **1**–**4** are listed in Table 1. Types **2** and **3** showed more inhibition for α2,3-sialyltransferase than α2,6-sialyltransferase, the $IC_{50}$ values being similar to that for the acceptor ($K_m$ = 264 μM). The imino-linked carbadisaccharide **2** possessed greater inhibitory activity than ether-linked **1**. This suggests that the imino-function efficiently enhances affinity for sialyltransferases. Note that among compounds **1**,**2**, and **3**, the imino-linked lactoside **2** showed the highest inhibitory potential and specificity toward α2,3-sialyltransferase, and ether-linked lactoside **1** the lowest. We found that the 5a-carbadisaccharides[14] acted as acceptor substrates for sialyltransferases, but the 5a′-carbadisaccharides did not in a preceding study.[15] The data indicate that the enzymes recognize the ring oxygen atom of a galactose residue when transferring sialic acid. Carbadisaccharides containing a carbagalactose residue in the non-reducing terminal do not act as acceptor substrates, but they are inhibitors.

With the *N*-acetyl-lactosamine structure **4** surprising results were obtained. Although α2,3-sialyltransferase recognizes *N*-acetyl-lactosamine as well as lactose and *N*-acetyl-isolactosamine, α2,6-sialyltransferase recognizes only *N*-acetyl-lactosamine strictly.[16] At first we expect that compound **4** might inhibit both enzymes, but in fact neither proved susceptible. The inhibition with 1 mM of **4** was 46% (α2,3-sialyltransferase) and 29% (α2,6-sialyltransferase), and $IC_{50}$ values were estimated to be 1.10 mM and 1.56 mM respectively. The findings can probably be interpreted as follows: with sialyltransferase binding the nitrogen atom is strongly recognized by the reducing terminal of galactose, hence imino-linked compounds containing one nitrogen atom might have enhanced binding affinity. However, when two nitrogen atoms exist, the enzymes maintain an equilibrium of interaction between them. In the case of **3**, two nitrogen atoms are situated spacially close to each other and affinity would be fixed within a certain area. In contrast, when two nitrogen atoms are relatively far apart from each other, the structure required for the recognition is extensively warped, resulting in drastic reduction of binding affinity as found with compound **4**. On the other hand, it is also interpreted in a different way: the strange result may indicate that substrates and inhibitors do not bind the same way to the enzymes.

In conclusion, we have established that a carbagalactose residue in pseudosaccharides may bind to sialyltransferases, but without the transfer of sialic acid. This interesting substrate specificity might be worthy of consideration when designing specific inhibitors. Since carbadisaccharides are resistant to digestion by *exo*-glycosidases, they are likely to be suitable sialyltransferase inhibitors and warrant further research.

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10 For **7**: $^1$H NMR (300 MHz, $D_2O$): (*inter alia*) $\delta$ = 2.60 (dd, $J_{2,3}$ = 9.3, $J_{3,4}$ = 8.1 Hz, 1 H, 3-H), 3.16 (dd, $J_{4,5}$ = 9.8 Hz, 1 H, 4-H), 3.29 (ddd, $J_{5,6a}$ = 1.7, $J_{5,6b}$ = 2.0 Hz, 1 H, 5-H), 3.44 (ddd, $J_{1,2}$ = 8.5, $J_{2,NH}$ = 1.7 Hz, 1 H, 2-H), 4.27 (d, 1 H, NH).
11 for **8**: $^1$H NMR (300 MHz, $D_2O$): (*inter alia*) $\delta$ = 2.55 (dd, $J_{2,3}$ = 7.8, $J_{3,4}$ = 10.5 Hz, 1 H, 3-H), 2.98 (ddd, $J_{1',2'}$ = 10.3, $J_{1',5a'ax}$ = 11.2, $J_{1',5a'eq}$ = 4.0 Hz, 1 H, 1′-H), 3.69 (ddd, $J_{4,5}$ = 9.5, $J_{5,6a}$ = 4.4, $J_{5,6b}$ = 7.4 Hz, 1 H, 5-H), 4.79 (dd, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 2.4 Hz, 1 H, 3′-H), 5.04 (dd, $J_{1',2'}$ = 10.3 Hz, 1 H, 2′-H), 5.51 (d, $J_{2,NH}$ = 7.3 Hz, 1 H, NH).
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