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# Chemo-Enzymatic Synthesis of Antagonists of the Myelin-associated Glycoprotein (MAG)

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*Abstract:* Ganglioside GQ1bα is the most potent antagonist of the Myelin-associated glycoprotein (MAG) identified so far. For the efficient synthesis of the partial structure of GQ1bα and derivatives thereof, a chemo-enzymatic strategy using the α(2→3)-sialyltransferase *ratST3Gal III* (EC 2.4.99.6) was applied. Besides the natural substrates Galβ(1→3)Glc*N*Ac (**19**) and Galβ(1→4)Glc*N*Ac (**20**), the disaccharides Galβ(1→3)Gal*N*TCAβ-OSE (**9**), Galβ(1→3)Gal*N*Acβ-OSE (**11**), and Galβ(1→3)Galβ-OSE (**14**) were also tolerated by the enzyme and were transformed to the target structures in preparative scale.

**Keywords:** Chemo-enzymatic synthesis · Myelin-associated glycoprotein (MAG) · *ratST3Gal III* · α(2→3)-Sialyltransferase

## Introduction

Axons of the adult mammalian central nervous system (CNS) do not regenerate after injury, largely due to inhibitors in the myelin sheath, an insulator that wraps around axons [1]. To date, the myelin-associated glycoprotein (MAG) [2] has been identified as one of the neurite outgrowth-inhibitory proteins, together with Nogo-A and the oligodendrocyte myelin glycoprotein (OMgp) [3]. All of them bind to the same receptor NgR, which interacts with $p75^{NTR}$ to transduce the inhibitory signal across the membrane (Fig. 1) [4]. In addition, MAG – the only member of the *siglec* (*s*ialic acid binding *i*mmuno*g*lobulin *lec*tin) family in the mammalian CNS – was found to bind with high specificity and affinity to brain gangliosides like GT1b, GD1a and GQ1bα [5].

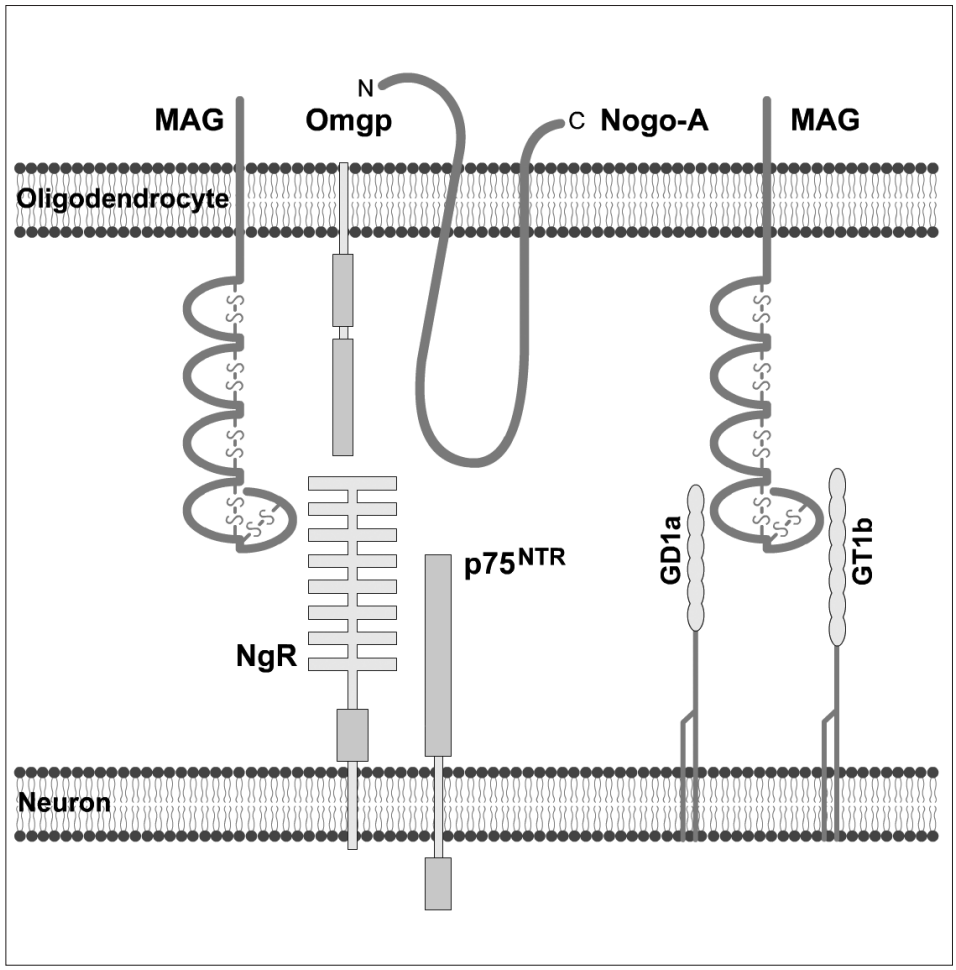


Fig. 1. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS

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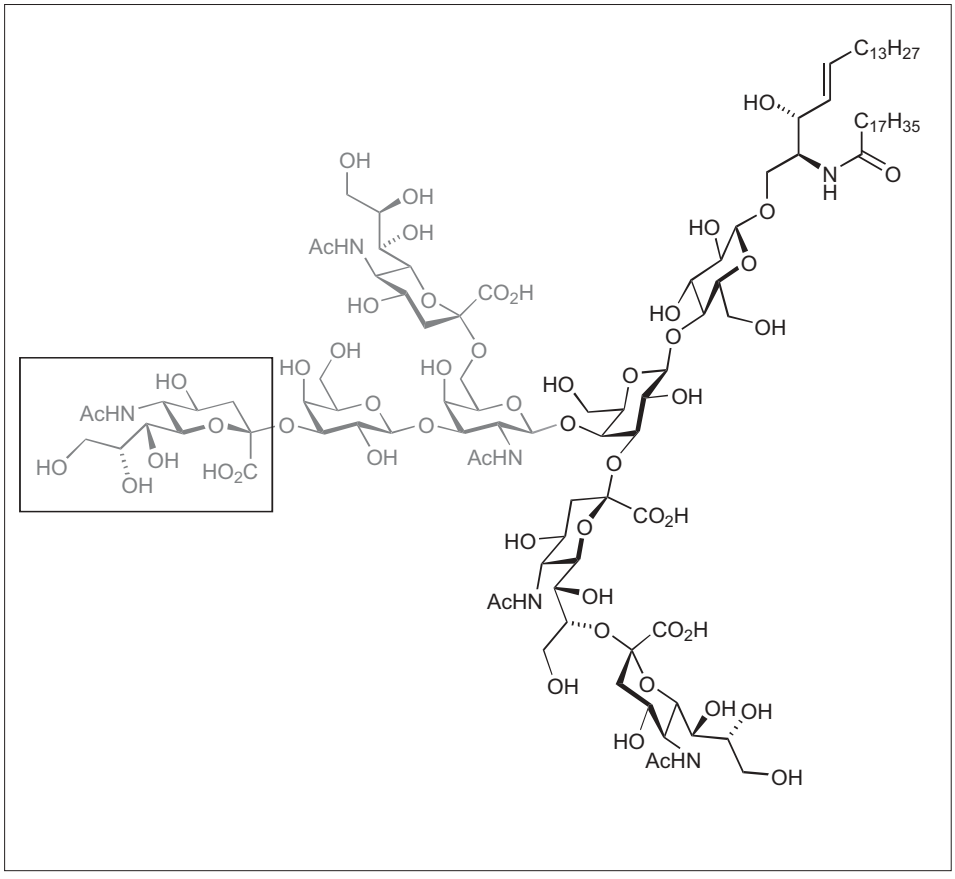
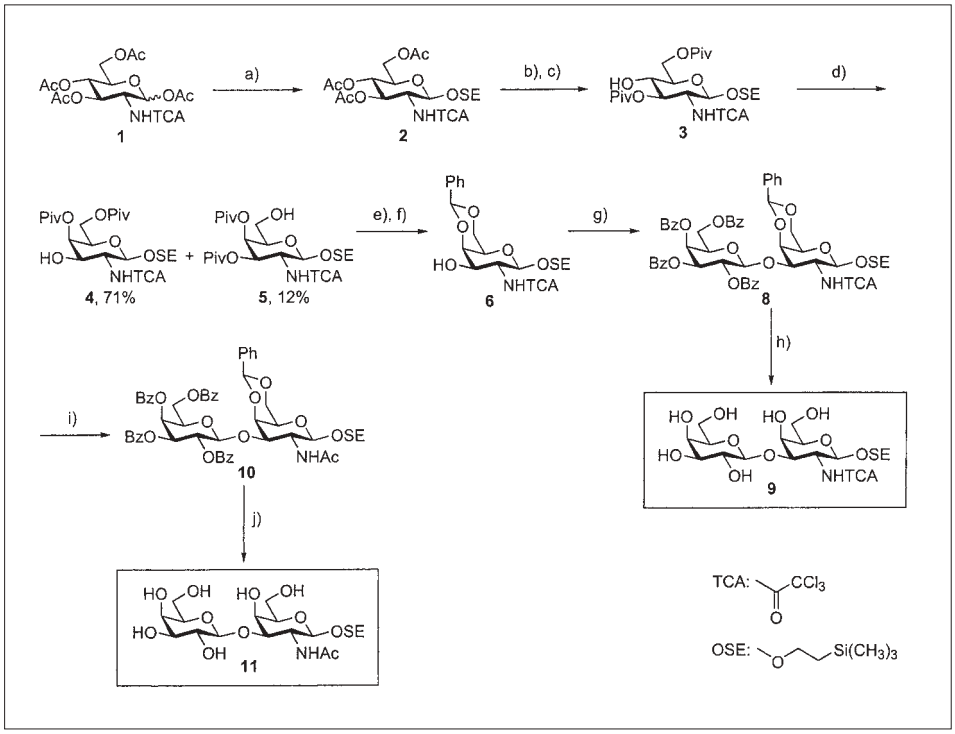


Fig. 2. The ganglioside GQ1bα



Scheme 1. a) i. HBr/AcOH, rt, 17 h, ii. HOSE, $Hg(CN)_2$, MS 3Å, toluene, rt, 3 d (89%); b) cat. NaOMe/MeOH, rt, 16 h (quant); c) 2.5 equiv. PivCl, cat. DMAP, pyridine, –30 °C, 20 h (89%); d) $Tf_2O$, DCE/pyridine 2:1, 0 °C, 4 h, then $H_2O$, 80 °C, 2 h (83%); e) 0.1 M NaOMe/MeOH, rt, 7 h, (96%); f) $PhCH(OMe)_2$, cat. *p*-TsOH, MeCN, rt, 4 h (86%); g) ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside (**7**), NIS, TfOH, $CH_2Cl_2$, –25 °C, 1.5 d (64% β); h) i. cat. NaOMe/MeOH, rt, 18 h, ii. 80% aq. AcOH, 80 °C, 2 h (46%); i) $Bu_3SnH$, AIBN, benzene, 80 °C, 3 h (93%); j) i. 80% aq. AcOH, 50 °C, 4.5 h, ii. cat. NaOMe/MeOH, rt, 21 h (64%).

The ganglioside GQ1bα (Fig. 2), which is the most potent MAG antagonist identified so far [5], consists of an octasaccharide and a ceramide at its reducing end. Based on a structure–activity relationship (SAR) study with numerous glycosphingolipids, the branched tetrasaccharide Neu5Acα–(2→3)Galβ(1→3)[Neu5Acα(2→6)]Gal*N*Ac (Fig. 2, in grey) was found to make the major contribution to MAG-binding, whereas the α(2→3)-linked sialic acid residue (Fig. 2, in box) was a prerequisite for affinity [5].

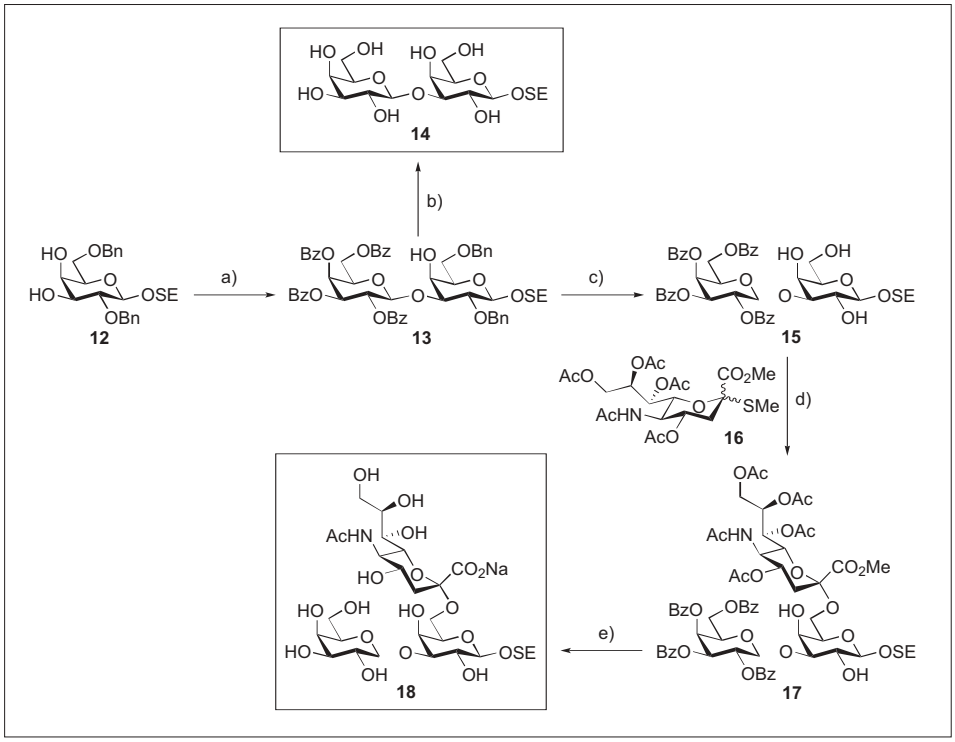
For a refined SAR study, we decided to synthesize modified sialylated oligosaccharides. Despite considerable recent progress [6], the stereoselective synthesis of α-sialosides by chemical sialylation remains a significant challenge due to the sterically hindered tertiary anomeric centre, the presence of an electron-withdrawing carboxylate and the lack of a participating neighbouring group in the 3-position of the sialic acid moiety [7]. Enzymatic syntheses employing sialyltransferases as biocatalysts offer an alternative approach to sialylated oligosaccharides with excellent stereoselectivity and generally good to excellent yields [8].

Previous studies showed that the α(2→3)-sialyltransferases *ST3Gal I* [9], *II* [10], and *IV* [11] are the physiological enzymes which transfer sialic acid residues to the 3-OH group of the terminal galactose moiety of the type III disaccharide Galβ(1→3)Gal*N*Ac. The natural substrates of *ST3Gal III* [12], however, are type I [Galβ(1→3)Glc*N*Ac] and type II [Galβ(1→4)Glc*N*Ac] disaccharides. In addition, it was shown that *ST3Gal III* accepts a wide variety of substituents on the glucosamine nitrogen [8b], as well as lactal, lactose and 2-O-pivaloyllactose [13]. Here, we report that *ratST3Gal III* (EC 2.4.99.6) can also be used for the enzymatic synthesis of partial structure of GQ1bα and derivatives thereof.

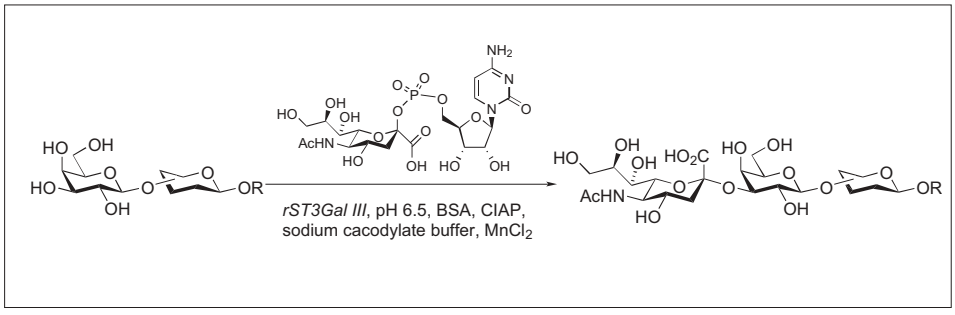
## Results and Discussion

The type III substrates Galβ(1→3)Gal*N* and Galβ(1→3)Gal were chemically synthesized as shown in Schemes 1 and 2 [14]. By using the trichloroacetyl (TCA) protecting group, Galβ(1→3)Gal*N*TCAβ-OSE (**9**) and Galβ(1→3)Gal*N*Acβ-OSE (**11**) could easily be obtained (Scheme 1).

For this purpose, per-O-acetylated N-trichloroacetylglucosamine (**1**) [15] was transferred into the (trimethylsilyl)ethyl glycoside **2** *via* the corresponding bromide. Subsequent O-deacetylation and selective 3,6-O-bis-pivaloylation gave **3** in 89% yield. After transformation of the 4-hydroxy group into the corresponding triflate, the galactosamides **4** and **5** were obtained in




Scheme 2. a) **7**, DMTST, $CH_2Cl_2$, 7 °C, 16 h (87% β); b) i. NaOMe, MeOH, rt, 2 h, ii. 10% Pd/C, $H_2$, MeOH, rt, 3 h (75%); c) 10% Pd/C, $H_2$ (4 bar), MeOH/dioxane, rt, 9 d (67%); d) **16**, NIS, TfOH, $CH_2Cl_2$, –30 °C, 16 h (45% α); e) NaOMe, MeOH, rt, 7 h, then aq. NaOH, rt, 16 h (90%).




Scheme 3. Enzymatic sialidation using *rST3Gal III* and CMP-Neu5Ac

a 6:1 ratio by epimerization at 80 °C. By transesterification, the pivaloates were removed and the 4- and 6-OHs were protected as benzylidene (→**6**). The subsequent coupling reaction with donor **7** [16] was carried out using NIS/TfOH [17] as promotor, resulting in pure β-disaccharide **8** in 64% yield. Removal of the benzoyl groups and subsequent cleavage of the benzylidene group gave Galβ(1→3)Gal*N*TCAβ-OSE (**9**) in 46% yield. From **8**, Galβ(1→3)Gal*N*Acβ-OSE (**11**) was obtained by reduction of the N-trichloroacetyl group with $Bu_3SnH$/AIBN in refluxing benzene (→**10**), followed by O-debenzoylation (→**11**).

The other two starting materials for the enzymatic sialylation experiments, Galβ(1→3)Galβ-OSE (**14**) and Galβ(1→3)[Neu5Acα(2→6)]Galβ-OSE (**18**), were synthesized starting from the galactose derivative **12** [18] (Scheme 2). For this purpose, **12** was coupled with donor **7** using dimethyl(methylthio)sulfonium triflate (DMTST) [19] as promoter to afford **13** in excellent yield and stereoselectivity. After removal of the benzoyl and benzyl protecting groups by transesterification and hydrogenolysis respectively, Galβ(1→3)Galβ-OSE (**14**) was isolated in 75% yield. **18** was obtained starting from **13**, which was partially deprotected by catalytic hydrogenation (→**15**), followed by coupling with the sialic acid donor **16** [20] in the presence of NIS/TfOH affording the corresponding α-sialoside **17** in 45% yield. Subsequent saponification gave trisaccharide **18** in 90%.

Following our standard sialylation protocol [12][21], the oligosaccharides **9**, **11**, **14** and **18**, and, as positive controls, the natural substrates **19** [22][23] and **20** [23][24] were then incubated with cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-Neu5Ac) and recombinant *rST3Gal III* (9 U/L) (Scheme 3) in preparative scale [14]. After 17–24 h of incubation, one additional aliquot of transferase was added (except for the natural substrates **19** and **20**). The addition of another aliquot of *rST3Gal III* and CMP-Neu5Ac did not further increase the yields.

The natural substrates **19** (type I) and **20** (type II) were converted quantitatively into the corresponding trisaccharides **21** and **22** [12] (entries 1 and 2 in the Table). In addition, the disaccharides **9**, **11** and **14** were also sialylated by *rST3Gal III* affording the corresponding trisaccharides **23**–**25** in acceptable yields (entries 3–5, Table). In all three cases, the unreacted substrates could be recovered almost quantitatively. The kinetic data for the sialylation reactions indicate that the activity of *rST3Gal III* towards the substrates **9**, **11** and **14** is reduced about 10-fold compared to the one for its natural substrates **19** and **20**. As expected, also the transfer efficiency $V_{max}/K_m$ is much lower for the unnatural substrates. This explains the incomplete, but still preparatively useful conversion of the type III disaccharides. Probably, due to the bulky substituent in the 6-position of galactose, the 6-O-sialylated trisaccharide **18** was not tolerated as substrate by *rST3Gal III*.

The introduction of a sialic acid unit was indicated by signals in $^{13}C$ NMR at approximately 100 ppm and 40 ppm, which are characteristic for the C(2) and C(3) of an α-linked sialic acid [12][13][24]. In addition, down-field shifts (~4 ppm) of the galactose C(3) in $^{13}C$ NMR and approximately 0.6 ppm of the galactose H(3) in $^1H$ NMR confirmed the regioselective introduction of sialic acid in the 3-position of the galactose moiety [12].

## Conclusion

The MAG antagonists **23**, **24**, and **25** were prepared by the chemical synthesis of the type III disaccharides **9**, **11**, and **14**, and subsequent enzymatic sialylation using *rST3Gal III* for the transfer of sialic acid from CMP-Neu5Ac. The determination of the MAG affinity in comparison with GQ1bα is currently ongoing.

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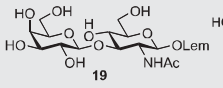
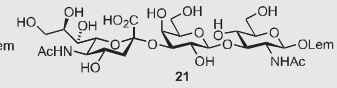
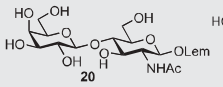
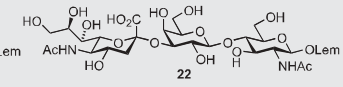
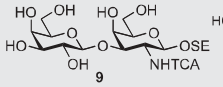
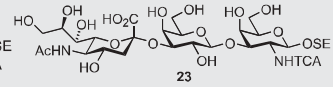
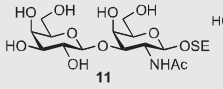
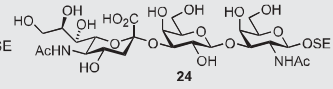
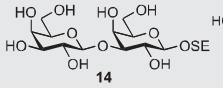
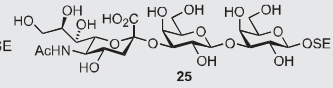
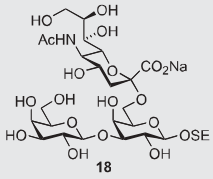
Table. Isolated yields and kinetic data of the enzymatic sialidations[a]

| Entry | Acceptor[b] | Product | Isolated Product [%][c] | Recovered Acceptor [%] | $V_{max}$ $\left[\frac{nmol}{ml \cdot min}\right]$ | $K_m$ [μM] |
|---|---|---|---|---|---|---|
| 1 |  **19** |  **21** | 90 | – | 3.42 | 66.08 |
| 2 |  **20** |  **22** | 76 | – | 3.19 | 87.61 |
| 3 |  **9** |  **23** | 36 | 62 | 0.89 | 811.64 |
| 4 |  **11** |  **24** | 56 | 44 | 1.11 | 995.55 |
| 5 |  **14** |  **25** | 59 | 40 | 0.43 | 990.51 |
| 6 |  **18** | no reaction | – | 95 | – | – |

[a]Substrates and CMP-Neu5Ac were incubated with *rST3Gal III* for 3–5 d at 37 °C in a mixture of 50 mM sodium cacodylate buffer (pH 6.5), 60 mM $MnCl_2$-solution and water containing BSA and CIAP (EC 3.1.3.1); b. **19**, **20**: 3 mg, **9**, **11**, **14**, **18**: ~10 mg; c. The course of the reactions was monitored by TLC (silica gel, DCM/MeOH/$H_2O$ 10:4:0.8). Isolated yields, recovered starting material and kinetic data ($V_{max}$ and $K_m$) are summarized in the Table [14]

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