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Minireview: Bacterial Sialyltransferases for Carbohydrate Synthesis

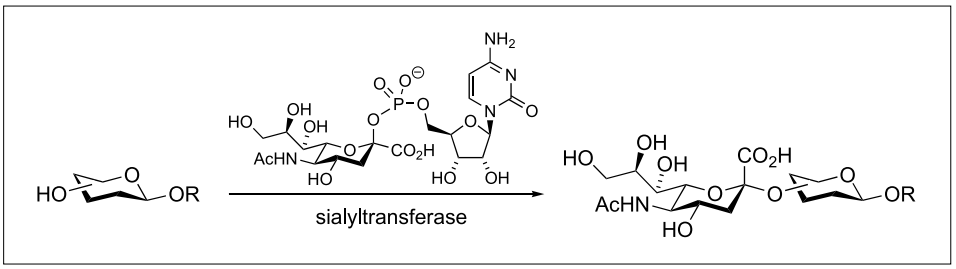
Oliver Schwardt[§*], Tamara Visekruna, Said Rabbani, and Beat Ernst
[§]SCS Poster Prize Winner

Abstract: Sialylation catalyzed by sialyltransferases is one of the most interesting enzymatic glycosyl transfer reactions, since chemical sialylations usually give only low yields and lead to poor stereoselectivities. In the last decade, several bacterial sialyltransferases were identified and found to exhibit broader substrate specificity than their mammalian counterparts. This suggests the potential usefulness of bacterial sialyltransferases in chemo-enzymatic synthesis of natural and non-natural sialooligosaccharides.

Keywords: Bacterial sialyltransferase · Chemo-enzymatic synthesis · Substrate specificity

Introduction

Sialic acid, often found in glycoproteins, glycolipids or polysaccharides, is associated with many important biological functions [1], which open up interesting therapeutic possibilities. In order to investigate these functions, and to develop novel carbohydrate-based therapeutics, libraries of diversified natural and modified sialooligosaccharides have to be synthesized and screened. Although recent progress in chemical sialylation reactions has facilitated the synthesis, it still remains one of the most difficult glycosylation reactions because of the hindered tertiary anomeric center, which often leads to unacceptably low yields, and the lack of a participating group to control the stereochemical outcome [2–5]. A convenient alternative is the use of sialyltransferases (STs) (EC 2.4.99.-) [6][7], which catalyze the transfer of a sialic acid moiety from cytidine-5'-monophospho-*N*-acetylneuraminic acid (CMP-Neu*N*Ac) α-selectively to the 3- or 6-position of a terminal galactose residue or to the hydroxyl group in the 8- or 9-position of a terminal sialic acid unit (Scheme 1).



Scheme 1. Enzymatic sialylation of carbohydrates using sialyltransferases and CMP-Neu*N*Ac

The high substrate specificity of mammalian STs, however, limits their usefulness for the synthesis of oligosaccharide libraries. In addition, the expression of STs in mammalian expression systems is prohibitively expensive. Recently, initial representatives of bacterial STs have been identified [8–10]. They accomplish transformations analogous to mammalian enzymes, and are of high interest because they are available by inexpensive large-scale expression in bacterial systems. Furthermore, studies clearly revealed their broader substrate specificity compared to their mammalian counterparts [9–11]. Because of their extended substrate specificity, the EC-numbers of most bacterial STs have not yet been determined [12].

Most microbial strains expressing STs are pathogens like *Neisseria gonorrhoeae*, *Neisseria meningitides* or *Campylobacter jejuni*. It has been shown that their surface lipooligosaccharides contain sialooligosaccharides identical to the structures found in mammalian glycolipids. This is presumably a form of molecular mimicry enabling the pathogens to evade the host's immune response [8][10].

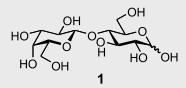
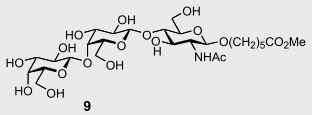
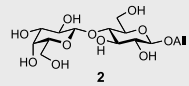
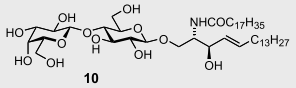
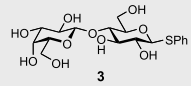
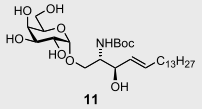
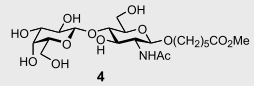
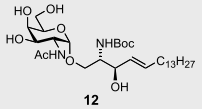
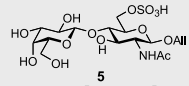
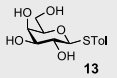
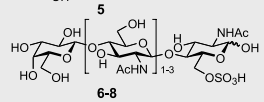
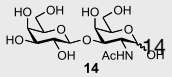
In this minireview, we focus on the donor and acceptor specificity of bacterial STs and their application for the chemo-enzymatic synthesis of sialooligosaccharides.

α(2→3)-Sialyltransferase from *Neisseria gonorrhoeae*

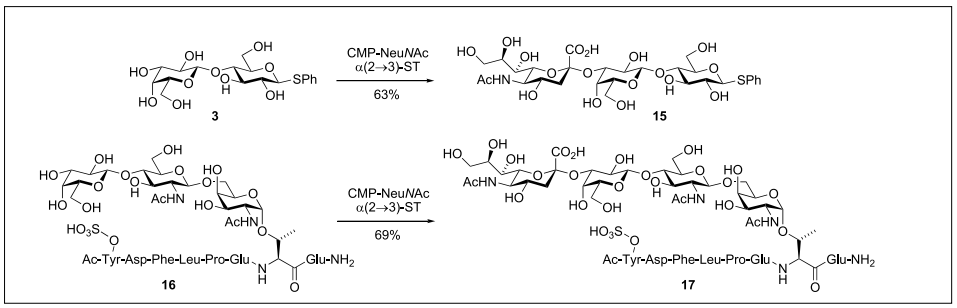
The *lst* gene, encoding for α(2→3)-ST (EC 2.4.99.4) from *Neisseria gonorrhoeae* immunotype F62 (ATCC33084), was cloned and over-expressed in *E. coli* using a *C*-terminal His_6-tag permitting an easy purification by Ni/NTA-affinity chromatography [13]. The acceptor specificity of the recombinant sialyltransferase was explored using synthetic oligosaccharides, sulfated oligosaccharides and glycolipids (Table 1) [13].

*Correspondence: Dr. O. Schwardt
University of Basel
Institute of Molecular Pharmacy
Klingelbergstrasse 50
CH-4056 Basel
Tel.: +41 61 267 1557
Fax: + 41 61 267 1552
E-Mail: oliver.schwardt@unibas.ch

Table 1. Acceptor specificity of the recombinant α(2→3)-ST from *N. gonorrhoeae* F62 (EC 2.4.99.4) [13]

Entry	Acceptor	Relative Rate[a]	Entry	Acceptor	Relative Rate[a]
1	 **1**	1	7	 **9**	0.24
2	 **2**	0.84	8	 **10**	0.28
3	 **3**	0.51	9	 **11**	< 0.1[b]
4	 **4**	2.33	10	 **12**	0.24
5	 **5**	1.40[b]	11	 **13**	0.29
6	 **6-8**	≈ 1.4[b]	12	 **14**	0.26

[a]Based on an isotope assay using CMP-[^{14}C]-Neu*N*Ac; [b]based on TLC and/or ESI-MS analysis.



Scheme 2. Preparative sialylations with recombinant *N. gonorrhoeae* F62 α(2→3)-ST (EC 2.4.99.4) [13][14]

Table 2. Acceptor specificity of the recombinant *N. meningitides* MC58(L3) α(2→3)-ST (EC 2.4.99.4) [15]

Entry	Acceptor	Concentration [mM]	Product detected
1	Galα(1→4)Glc*N*Acβ-O-AP-FCHASE (**18**)	0.5	+[a]
2	Galα(1→4)Glc*N*Acβ-O-TMR (**19**)	0.5	+[b]
3	Galα(1→3)Glc*N*Acβ-O-TMR (**20**)	0.5	+[b]
4	Galα(1→4)Glc*N*Acβ-S-AH-FCHASE (**21**)	0.8	+[a]
5	Galα(1→4)Glcβ-O-AP-FCHASE (**22**)	0.5	+[a]
6	Galα-O-AP-FCHASE (**23**)	0.5	+[a]
7	Galα-S-AH-FCHASE (**24**)	1.0	+[a]
8	Galα-O-AP-FCHASE (**25**)	0.5	+[a]
9	Galα(1→4)Galβ(1→4)Glcβ-O-AP-FCHASE (**26**)	0.5	+[a]
10	Galα(1→4)[Fucα1→3)]Glc*N*Acβ-O-TMR (Le^x, **27**)	0.5	-[b]
11	Galα(1→3)[Fucα(1→4)]Glc*N*Acβ-O-TMR (Le^a, **28**)	0.25	-[b]

[a]Analyzed by capillary electrophoresis; [b]analyzed by TLC.
AH = aminohexyl; AP = *p*-aminophenyl; FCHASE = 6-(5-fluoresceincarboxamido)-hexanoic acid succidimidyl ester; TMR = tetramethylrhodamine.

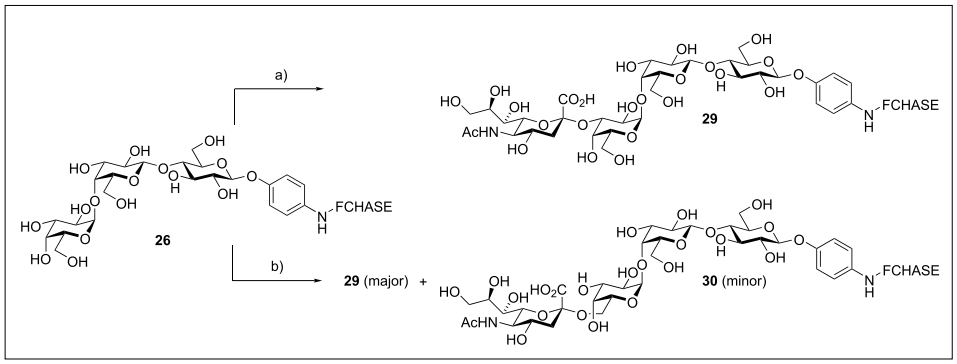
Lactose (**1**) and its allyl and phenylthio β-glycosides **2** and **3** turned out to be excellent substrates (entries 1–3). Lac*N*Acβ-O(CH$_2$)$_5$CO$_2$Me (**4**, entry 4) as well as sulfated oligosaccharides like 6-*O*-sulfo-Lac*N*Acβ-OAll (**5**, entry 5) and Galβ(1→4)[Glc*N*Acβ(1→4)]$_{1\text{-}3}$-6-*O*-sulfo-Glc*N*Ac (**6**-**8**, entry 6) even showed improved relative rates compared to **1**. This is a major difference to the commercially available rat α(2→3)-ST (rST3Gal III, EC 2.4.99.6), which accepts only Galβ(1→4)[Glc*N*Acβ(1→4)]$_3$-6-*O*-sulfo-Glc*N*Ac (**8**) from the sulfated oligosaccharides **5**-**8**. However, there are also substrates, like 4'-galactosyl-Lac*N*Ac (**9**), lactosyl ceramide (**10**), the *N*-Boc-sphingosines **11** and **12**, Galβ-STol (**13**) as well as Galβ(1→3)Gal*N*Ac (**14**) (entries 7–12), which are sialylated by the ST from *N. gonorrhoeae* with strongly reduced rates. Overall, the α(2→3)-ST from *N. gonorrhoeae* F62 transfers sialic acid to lactose and Lac*N*Ac derivatives and tolerates substitution with sulfate. However, glycolipids were sialylated with a reduced rate [11]. Investigations on the donor specificity of the enzyme with several CMP-Neu derivatives like CMP-Neu*N*Ac, CMP-Neu*N*Gc and CMP-KDN revealed that the enzyme is quite specific for CMP-Neu*N*Ac [13].

In a preparative sialylation experiment of Lacβ-SPh (**3**) on a 10 mg-scale using the α(2→3)-ST from *N. gonorrhoeae*, Neu*N*Acα(2→3)Lacβ-SPh (**15**) was obtained in 63% yield (Scheme 2). The phenylthio group at the reducing end is not only useful as an activating group for subsequent chemical transformations, *e.g.* for a conversion into glycolipids, but also facilitates the isolation of **15** by solid phase extraction [13]. Under identical conditions, the PSGL-1 glycopeptide **16**, carrying a sulfotyrosine residue, was transferred to **17** in 69% (Scheme 2) [14].

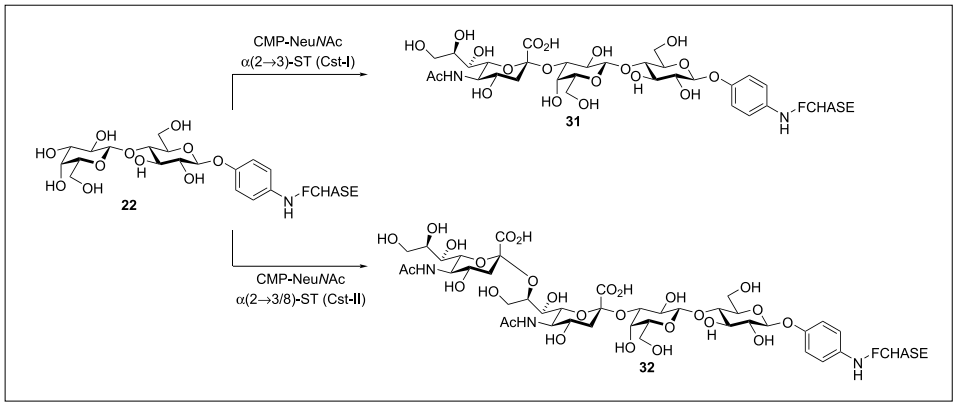
Sialyltransferases from *Neisseria meningitidis*

In 1996, the Wakarchuk group reported the cloning of the *lst* gene encoding the α(2→3)-ST involved in lipooligosaccharide biosynthesis from the human pathogen *Neisseria meningitides* immunotype MC58(L3) [10]. Again, the α(2→3)-ST gene was successfully over-expressed in *E. coli*. The acceptor specificity of the recombinant enzyme (Lst, EC 2.4.99.4) was examined by capillary electrophoresis or TLC using fluorescent-labeled saccharides (Table 2) [15].

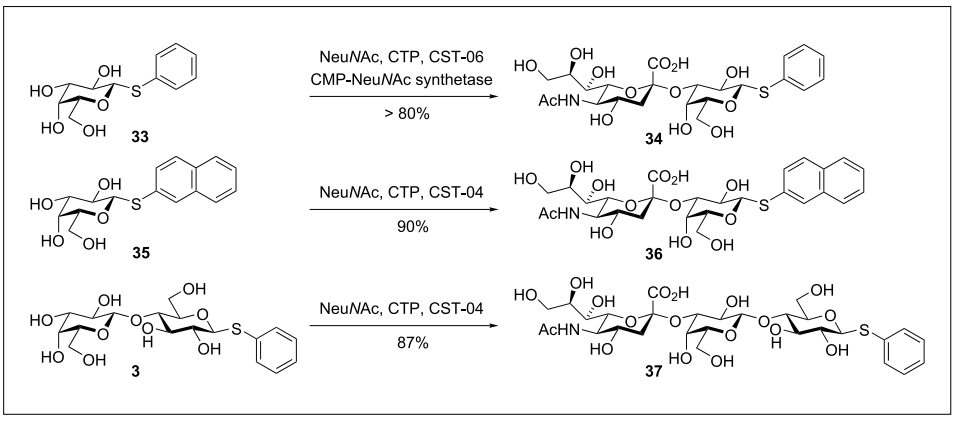
Detailed kinetic studies for some of these acceptors revealed that the sialic acid transfer by the enzyme to a terminal Galβ(1→4) moiety is about 10-fold better than that to a terminal Galβ(1→3)



Scheme 3. Preparative sialylation of P^k-FCHASE **26** with CMP-Neu*N*Ac and a) recombinant α(2→3)-ST from *N. meningitides* MC58(L3) [15]. b) bifunctional α(2→3/6)-ST from *N. meningitides* 126E(L1) [16].



Scheme 4. Sialylation of Lac-FCHASE **22** with Cst-I and Cst-II from *C. jejuni* OH4384 [18]



Scheme 5. Preparative syntheses of sialylated arylthio galactosides and lactoside with recombinant α(2→3)-ST (Cst-I) from *C. jejuni* OH4384 (EC 2.4.99.-) [19]

unit. However, both Galβ(1→4)Glc*N*Ac (compounds **18** and **19**; entries 1 & 2) and Galβ(1→3)Glc*N*Ac (**20**, entry 3) were accepted by the ST. The Lac*N*Ac-thioglycoside **21** (entry 4) and lactoside **22** (entry 5) were also sialylated. The enzyme tolerated the β-galactoside **23** as well as the Galβ-thioglycoside **24** (entries 6 & 7). In addition, the α-galactosides **25** and **26** (entries 8 & 9) were found to be substrates. On the other hand, neither of the trisaccharides **27** (Le^x) and **28** (Le^a) were sialylated (entries 10 & 11), exhibiting a behavior common to all known STs.

It was also shown that the activity of the recombinant α(2→3)-ST from *N. meningitides* MC58(L3) can be utilized for preparative sialylations (→ **29**, Scheme 3a) [15].

In another study the same group reported on the ST activity from the cell-free extract of *Neisseria meningitidis* 126E(L1) [16]. The 126E(L1) α(2→3)-ST (Lst, EC 2.4.99.4) was assayed with P^k-FCHASE (**26**) as acceptor, and two products were detected by capillary electrophoresis. The major product was identified as the α(2→3)-sialylated product **29**, whereas the minor fraction was found to be Neu*N*Acα(2→6)P^k-FCHASE (**30**) (Scheme 3b). A sequence alignment of the two Lst's from *N. meningitidis* strains 126E(L1) and MC58(L3) revealed a difference in only six amino acids. By site-directed mutagenesis (Gly168Ile) in the MC58(L3)-Lst, the α(2→6)-ST activity could be successfully improved, thus creating a new bifunctional α(2→3/6)-ST (EC not determined).

One of the major drawbacks of preparative enzymatic sialylations is the cost and the lability of the donor CMP-Neu*N*Ac. One possibility to overcome this handicap is the *in situ* formation of the sugar nucleotide from sialic acid and cytidine-5-triphosphate (CTP) by fusion enzymes with CMP-Neu*N*Ac synthetase and sialyltransferase activity. This methodology was successfully applied for the 100 g-scale synthesis of 3'-sialyllactose from lactose using the recombinant fusion protein CMP-Neu*N*Ac synthetase/*N. meningitidis* MC58 (L3) α(2→3)-ST [17].

Sialyltransferases from *Campylobacter jejuni*

Two ST genes were identified in the *Campylobacter jejuni* strain OH4384 by Wakarchuk and coworkers. [18]. These genes were designated as *cst-I* and *cst-II*, and then cloned and over-expressed in *E. coli*. The *cst-I* gene product transformed Lac-FCHASE **22** into Neu*N*Acα(2→3)Lac-FCHASE **31** (Scheme 4), indicating that the Cst-I (EC 2.4.99.-) is an α(2→3)-ST. The *cst-II* gene product turned out to be a bifunctional α(2→3/8)-ST (EC 2.4.99.-) transforming Lac-FCHASE **22** into Neu*N*Acα(2→8)Neu*N*Acα(2→3)Lac-FCHASE **32**.

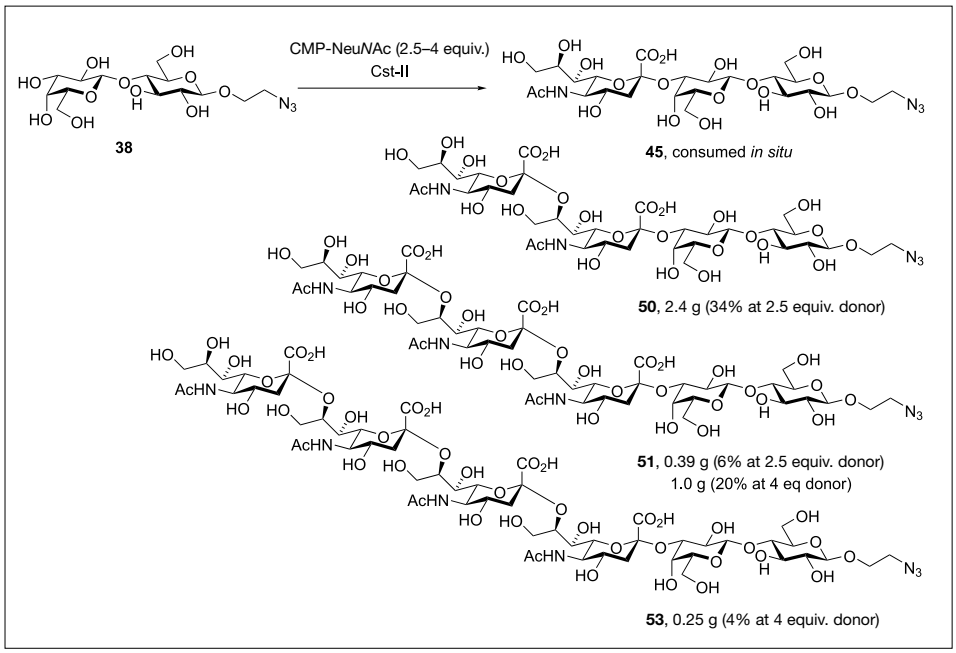
The recombinant Cst-I (EC 2.4.99.-) was used for the synthesis of thioacetals of 3-sialylgalactoside and 3'-sialyllactoside on a 100 mg–1 g-scale (Scheme 5) [19]. Sialylation of phenylthio β-galactoside (**33**) with CST-06, a fusion protein of Cst-I and maltose-binding protein (MBP), gave 3-sialylgalactoside **34** in >80% yield. The MBP was used as an *N*-terminal tag allowing the easy purification of the enzyme by amylose affinity chromatography. Sialylation of 2-naphtylthio β-galactoside (**35**) and phenylthio β-lactoside (**3**) with CST-04, a fusion protein of Cst-I and *N. meningitidis* CMP-Neu*N*Ac-synthetase, gave the corresponding sialosides **36** and **37** in 90% and 87% yield, respectively.

In 2004, the X-ray structure of Δ32Cst-II, a truncated form of the *C. jejuni* OH4384 Cst-II, co-crystallized with the inert donor substrate analog CMP-3-F-Neu*N*Ac was published [20]. A careful analysis of the protein structure suggested a random point mutation (Ile53Ser) that would lead to the stabilization of the Δ32Cst-II construct by interacting with the acceptor substrates. It was further expected that the mutation would affect the relative ratios of α(2→

Table 3. Relative acceptor substrate specificities of *C. jejuni* OH4384 Cst-II (EC 2.4.99.-) [21]

Entry	Acceptor	Relative specific activity (%)[a] α(2→3)	α(2→8)
1	Galβ(1→4)Glc (**1**)	**100**	
2	Galβ(1→4)Glcβ-R^1 (**38**)	123	
3	Galβ(1→4)Glc*N*Ac (**39**)	64	
4	Galβ(1→4)Glc*N*Acβ-R^1 (**40**)	88	
5	Galβ(1→4)Glc*N*Acβ(1→4)Galβ(1→4)Glcβ-R^1 (**41**)	27	
6	Galβ-R^1 (**42**)	38	
7	Galβ-$SCH(CH_3)_2$ (**43**)	<1	
8	Gal*N*Acβ-R^1 (**44**)	0	
9	Neu*N*Acα(2→3)Galβ(1→4)Glcβ-R^2 (**45**)		**100**
10	Neu*N*Acα(2→3)Galβ-R^2 (**46**)		63
11	Neu*N*Acα(2→3)Galβ(1→4)Glc*N*Acβ-R^2 (**47**)		199
12	Neu*N*Acα(2→6)Galβ(1→4)Glcβ-R^2 (**48**)		116
13	Neu*N*Acα(2→6)Galβ(1→4)Glc*N*Acβ-R^2 (**49**)		43
14	Neu*N*Acα(2→8)Neu*N*Acα(2→3)Galβ(1→4)Glcβ-R^2 (**50**, GD3)		12
15	Neu*N*Acα(2→8)Neu*N*Acα(2→8)Neu*N*Acα(2→3)Galβ(1→4)Glcβ-R^2 (**51**, GT3)		5
16	Neu*N*Gcα(2→3)Galβ(1→4)Glc*N*Acβ-R^2 (**52**)		104

[a]Relative specific activity for each compound is calculated as a percentage of the reference compound (**bold**).
$R^1 = O(CH_2)_2N_3$; $R^2 = O(CH_2)_2NHFmoc$.



Scheme 6. Preparative synthesis of GD3 (**50**), GT3 (**51**) and poly-sialylated oligosaccharides with recombinant α(2→3/8)-ST (Cst-II) from *C. jejuni* OH4384 (EC 2.4.99.-) [21]

3)- and α(2→8)-activities and enhance the α(2→8)-specificity.

Recently, the relative α(2→3)- and α(2→8)-activities of the mutated Cst-II (Ile53Ser) were evaluated (Table 3) [21]. To minimize α(2→8)-sialylation, the Cst-II α(2→3)-activity was measured using high substrate concentrations and short incubation times.

Lactose (**1**) and lactoside **38** (entries 1 & 2) had the highest α(2→3)-activity of all compounds tested. *N*-Acetyllactosamines (compounds **39** and **40**, entries 3 & 4) also demonstrated excellent activity, which is still sufficient for preparative applications. Extended Lac*N*Ac **41** (entry 5), however, showed a substantially reduced activity. Interestingly, the capability of β-galactosides **42**–**44** (entries 6–8) to act as acceptors was influenced dramatically by the reducing end substituent. Although **42** could be easily transformed into the corresponding sialoside, isopropylthio β-galactoside (**43**) and Gal*N*Ac **44** were not tolerated by the enzyme.

The α(2→8)-activity study showed that both α(2→3)-sialosides (**45**–**47**, entries 9–11) and α(2→6)-sialosides (**47**, **48**; entries 12 & 13) were excellent substrates for Cst-II. The larger oligosaccharides GD3 (**50**) and GT3 (**51**) (entries 14 & 15) showed significantly reduced acceptor qualities. It is noteworthy, however, that the *C. jejuni* OH4384 Cst-II is also able to synthesize polysialosides. In addition, Neu*N*Gcα(2→3)Lac*N*Ac (**52**) (entry 16), containing a glycolated sialic acid, turned out to be an excellent substrate.

Finally, the *C. jejuni* OH4384 Cst-II was also used for the preparative synthesis on a gram-scale of ganglioside derivatives starting from lactoside **38** (Scheme 6). The originally formed 3'-sialyllactoside **45** was completely consumed and converted to GD3 (**50**) and GT3 (**51**). Even a small amount of tetrasialyl-lactoside **53** could be isolated. In addition, traces of higher sialylated fractions were also detected but not purified. With increasing amounts of CMP-Neu*N*Ac, the formation of α(2→8)-polysialosides could be significantly raised [21].

Recently, we over-expressed the truncated *cst-II* gene from *C. jejuni* strain ATCC43438 encoding for Δ32Cst-II (EC not determined) in *E. coli*, again using a *C*-terminal His_6-tag for affinity chromatography purification [22]. The α(2→3)- and α(2→8)-specificity of the enzyme was assayed using the type I and II disaccharides **54** and **56**, as well as the corresponding mono-sialylated trisaccharides **55** and **57** (Table 4). All reactions were run on a preparative scale and the products analyzed by ^1H and ^{13}C NMR.

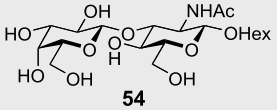
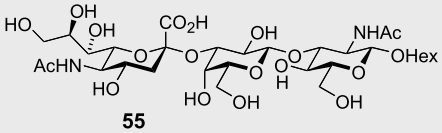
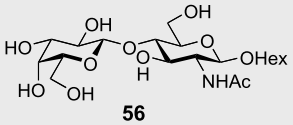
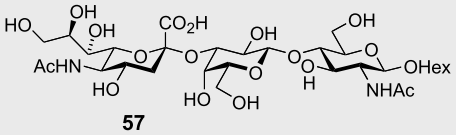
The α(2→3)-activity of the wild-type Δ32Cst-II was significantly higher for the type I disaccharide **54** (entry 1) than for Lac*N*Ac (**56**, entry 3). On the other hand, no α(2→8)-activity was observed with the type I sugars **54** and **55** (entries 1 & 2). The type II substrates **56** and **57** (entries 3 & 4), however, were partly converted into the corresponding bis-sialylated tetrasaccharide Neu*N*Acα(2→8)Neu*N*Acα(2→3)Galβ(1→4)Glc*N*Acβ-OHex, but with a much lower rate as described for the *C. jejuni* OH4384 Cst-II [21].

The sequences of the Cst-IIs from *C. jejuni* strains OH4384 and ATCC43438 differ by only six amino acids (97.3% sequence identity). The analysis of the X-ray struc-

ture of the OH4384 Δ32Cst-II [20] revealed two neutral amino acids, *i.e.* Asn177 and Asn182 close to the binding site, whereas the same positions in the ATCC43438 Cst-II enzyme are occupied by a negatively charged Asp177 and a positively charged Arg182. Based on molecular modeling studies, it was assumed that replacement of these charged residues might increase the α(2→8)-activity of the enzyme. Therefore, the mutant Δ32Cst-II (S53N177N182) was generated by site-directed mutagenesis (Gly53Ser, Asp177Asn, Arg182Asn), and the specificity of this enzyme evaluated again with carbohydrates **54**–**57** [22].

The mutant Δ32Cst-II (S53N177N182) acted as a bifunctional α(2→3/8)-sialyltransferase with both types of acceptors (Table 4). In the case of the type I disaccharide **54** (entry 1), the α(2→3)-activity remained unchanged compared to the wild-type enzyme, but the mutant showed additional α(2→8)-activity. Surprisingly, no significant change of either the α(2→3)- or the α(2→8)-activity was found for the type II substrate **56** (entry 3). In addition, the mutant accepted both trisaccharides **55** and **57** as substrates with a slightly higher activity for the type II acceptor **57** (entry 4). The conversion rates for both substrates did not exceed 50%. Running the reactions with higher amounts of CMP-Neu*N*Ac as well as adding more donor or transferase during the incubation did not influence this outcome. In contrast to findings by Blixt *et al.* [21] with the *C. jejuni* OH4384 Cst-II, formation of multi-sialylated products could not be observed.

Table 4. Sialylation of type I and II saccharides with recombinant α(2→3/8)-ST (Δ32Cst-II) from *C. jejuni* ATCC43438 [22]

Transferase		Δ32Cst-II (wild-type)			Δ32Cst-II (S53N177N182) (mutant)		
		Isolated Yield [%][a]			Isolated Yield [%][a]		
Entry	Acceptor	Di-	Tri-	Tetra-saccharide	Di-	Tri-	Tetra-saccharide
1	 **54**	11	80	-	11	72	15
2	 **55**		98	-		52	48
3	 **56**	36	45	13	39	42	15
4	 **57**		49	48		46	49

[a]Isolated yields after separation by RP-18 chromatography. All reactions were run on a 10 mg-scale with 2.5 equiv. of donor CMP-Neu*N*Ac for disaccharides and 1.5 equiv. for trisaccharides. Hex = *n*-hexyl.

Table 5. Transfer assay of the *P. damsela* α(2→6)-ST (EC 2.4.99.1) with PA-labeled oligosaccharides [24]

Entry	Acceptor	Transfer yield[a] [%]
1	Neu*N*Acα(2→3)Galβ(1→4)Glc-PA (**58**)	28
2	Neu*N*Gcα(2→3)Galβ(1→4)Glc-PA (**59**)	78
3	Neu*N*Acα(2→8)Neu*N*Acα(2→3)Galβ(1→4)Glc-PA (**60**)	76
4	Fucα(1→2)Galβ(1→4)Glc-PA (**61**)	57
5	Galβ(1→4)Glc*N*Acβ(1→3)Galβ(1→4)Glc-PA (**62**)	44
6	Galβ(1→4)[Fucα(1→3)]Glc*N*Acβ(1→3)Galβ(1→4)Glc-PA (**63**, Le^x)	< 5
7	Galβ(1→3)[Fucα(1→4)]Glc*N*Acβ(1→3)Galβ(1→4)Glc-PA (**64**, Le^a)	< 5
8	Galβ(1→3)Gal*N*Acβ(1→4)[Neu*N*Acα(2→3)]Galβ(1→4)Glc-PA (**65**)	< 5
9	Neu*N*Acα(2→3)Galβ(1→3)Gal*N*Acβ(1→4)[Neu*N*Acα(2→3)]Galβ(1→4)Glc-PA (**66**)	< 5
10	Galβ(1→3)Gal*N*Acβ(1→4)[Neu*N*Acα(2→8)Neu*N*Acα(2→3)]Galβ(1→4)Glc-PA (**67**)	< 5

[a]Yields were estimated by HPLC. PA = 2-pyridylamino

α(2→3)-Sialyltransferase from *Haemophilus influenzae*

The ST gene of the human pathogen *Haemophilus influenzae*, responsible for linking Neu*N*Ac in an α(2→3)-manner to lactose, was identified in 2001 [23]. It was cloned and over-expressed in *E. coli* and the α(2→3)-ST (EC not determined) activity determined by TLC or capillary electrophoresis using Lac-FCHASE as substrate. The acceptor specificity of the enzyme was examined using FCHASE-labeled acceptors, and ranked in the following manner: Lac-FCHASE (**22**) = Lac*N*Ac-FCHASE (**18**) > Galα-FCHASE (**25**) > Galβ-FCHASE (**23**). P^k-FCHASE (**26**) was not tolerated by the enzyme [23].

α(2→6)-Sialyltransferase from *Photobacterium damsela*

The purification of an ST from the marine bacterium *Photobacterium damsela* JT0160 was reported by a group from Japan Tobacco Inc. [9]. The enzyme was found to exhibit α(2→6)-activity. In a screening study with various mono- and disaccharides using ^{14}C-labeled CMP-Neu*N*Ac as donor, Galα/β-OMe, Gal*N*Acα-OMe, Lac*N*Acβ-OMe and lactose were found to be good substrates for the *P. damsela* JT0160 α(2→6)-ST (Bst, EC 2.4.99.1). The K_m value for lactose was almost the same as for Lac*N*Ac, whereas that of methyl β-galactoside was about 25 times higher.

A transfer assay with the *P. damsela* JT0160 α(2→6)-ST using 2-pyridylamino (PA)-labeled oligosaccharides revealed a remarkable ability of this enzyme to sialylate the 6-position of terminal, but also mono- and bis-sialylated galactose moieties (Table 5) [24].

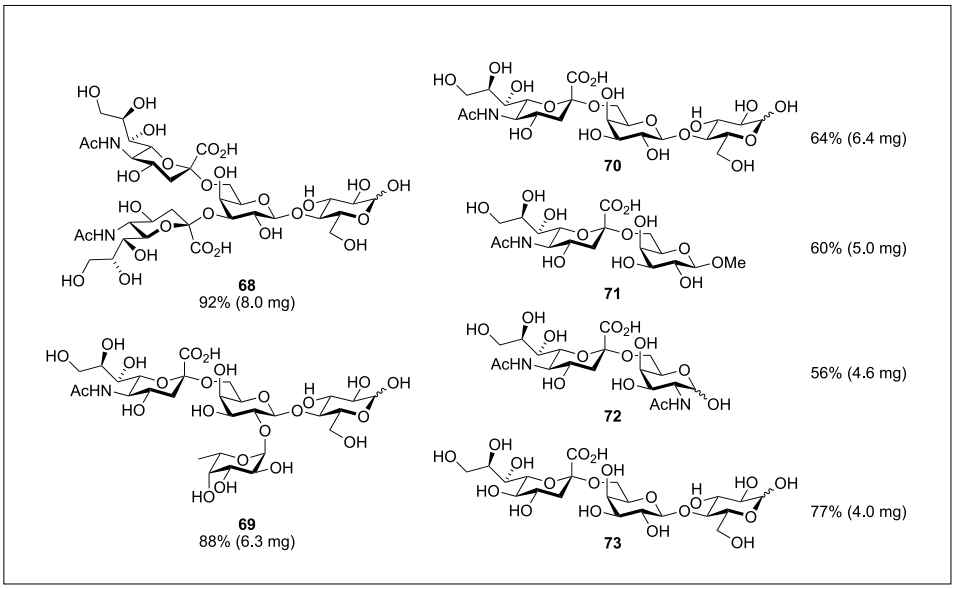
Whereas 3'-sialyllactoside **58** (entry 1) was a moderate substrate, its *N*-glycolyl-

neuraminyl-analogue **59** (entry 2) and the bis-sialylated lactoside **60** (entry 3) gave excellent transfer results. 2'-Fucosyllactoside **61** (entry 4) and extended Lac*N*Ac **62** (entry 5) were also accepted by the enzyme. The K_m values of 3'-sialyllactoside **58** and 2'-fucosyllactoside **61** were found to be similar to those of lactose and *N*-acetyllactosamine [24]. However, Le^x (**63**, entry 6), Le^a (**64**, entry 7) and the oligosaccharides **65**–**67** (entries 8–10), which contain β(1→3)-linked galactose, were not sialylated.

For the application of the enzyme in preparative synthesis, a large-scale production of the α(2→6)-ST was established by cultivating *P. damsela* JT0160 cells in culture media containing galactose and beef extract at pH 8. The crude enzyme extract obtained in the supernatant proved to be sufficiently pure for synthetic applications [25a]. The ST gene (*bst*) was also cloned and over-expressed in *E. coli* MV1184, and the recombinant gene product gave a soluble form of the enzyme [25b].

The following oligosaccharides were synthesized in 50–90% yield and fully characterized by ^{1}H and ^{13}C NMR (Scheme 7) [25]: 3',6'-Disialyllactose (**68**) and 2'-fucosyl-6'-sialyllactose (**69**) were synthesized on a 6–8 mg scale. 6-Sialylated lactose (**70**), methyl β-galactoside (**71**) and *N*-acetylgalactosamine (**72**) were obtained on a *ca*. 5 mg scale using the crude enzyme extract. 6'-KDN-lactose (**73**) was also synthesized using CMP-KDN as donor.

Recently, the α(2→6)-ST from *P. damsela* was also applied for the enzymatic sialylation of Gal*N*Ac, α-linked either to a serine or threonine residue of the T_N-glycopeptides **74** and **75** (Scheme 8) [26]. In contrast to the mammalian sialyltransferase ST6Gal*N*Ac I (EC 2.4.99.3), which sialylates only the Gal*N*Acα-Thr moiety to give **76**, the bacterial enzyme also accepts Gal*N*Acα-Ser as a substrate (→ **77**).



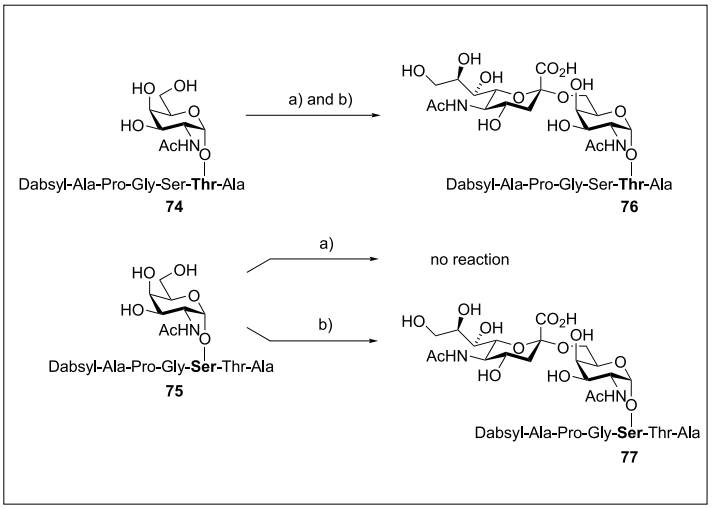
Scheme 7. Natural and non-natural sialooligosaccharides synthesized with *P. damsela* α(2→6)-ST (EC 2.4.99.1) [25]

Synthesis of Sialooligosaccharides Using Metabolically Engineered Whole Cells

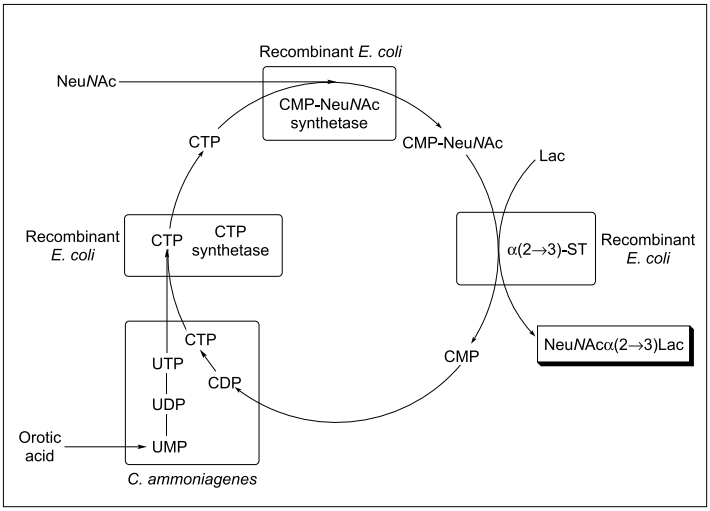
Sialooligosaccharides and CMP-Neu*N*Ac were also synthesized on a large scale using metabolically engineered whole cells as reactors. For the production of CMP-Neu*N*Ac, two recombinant *E. coli* strains (*E. coli* K1), over-expressing CMP-Neu*N*Ac synthetase (EC 2.7.7.43) and CTP synthetase (EC 6.3.4.2), were generated [27] and combined in a reaction vessel with *Corynebacterium ammoniagenes*, which is responsible for the production of UTP from orotic acid. In this system, consisting of three cell lines, CMP-Neu*N*Ac was accumulated after 1 d of incubation up to a concentration of 27 mM (17 g/l). When another *E. coli* strain, over-expressing the *N. gonorrhoeae* α(2→3)-ST gene, was added to this system, 3'-sialyllactose was produced up to a maximal concentration of 52 mM (33 g/l) after 11 h of reaction. Incubation on different scales (30 ml and 2 l) gave comparable results (Scheme 9) [27].

Neu*N*Acα(2→3)Gal*N*Ac was also obtained on a 1 g-scale by coupling two bacterial expression systems (the CMP-Neu*N*Ac production system and an *E. coli* strain over-expressing *P. damsela* α(2→6)-ST) [28].

Recently, the oligosaccharide moieties of the gangliosides GM3 (3'-sialyllactose) and GD3 [Neu*N*Acα(2→8)Neu*N*Acα(2→



Scheme 8. Preparative sialylations of T_N-glycopeptides **74** and **75** [26] with CMP-Neu*N*Ac and a) mammalian ST6Gal*N*Ac I (EC 2.4.99.3). b) *P. damsela* α(2→6)-ST (EC 2.4.99.1). Dabsyl = 4-dimethylaminoazobenzene-4'-sulfonyl



Scheme 9. Bacterial system consisting of four components for the synthesis of 3'-sialyllactose [27]

3)Galβ(1→4)Glc] were synthesized by incubating lactose and sialic acid for 9 h with a metabolically engineered *E. coli* strain (*E. coli* K12) over-expressing *N. meningitides* CMP-Neu*N*Ac synthetase (EC 2.7.7.43) and the bifunctional *C. jejuni* ATCC43438 α(2→3/8)-ST (Cst-II) [29]. From 1 l of culture 49 mg of GM3 and 98 mg of GD3 could be isolated. By a prolongation of the incubation time to 24 h, GD3 was partially further sialylated to yield 15 mg of tri-sialylated product GT3 [Neu*N*Acα(2→8)Neu*N*Acα(2→8)Neu*N*Acα(2→3)Galβ(1→4)Glc].

Future Perspectives

Enzymatic sialylations with sialyltransferases have substantially improved the glycosylation repertoire. They are superior to chemical sialylation in terms of stereoselectivity and overall yield. Additional advantages are reduced cost and labor, since there is no need for multiple protection-deprotection steps and cumbersome separation of isomers. Drawbacks of the enzymatic approach, however, are the difficulty of over-expression of mammalian enzymes and the prohibitively expensive sugar nucleotide CMP-Neu*N*Ac. Since mammalian STs usually exhibit high substrate specificities, their use is often limited to the synthesis of natural products.

The herein-reviewed STs from bacterial sources, however, frequently show broader substrate specificity for both acceptors and donors, suggesting their usefulness not only in the chemo-enzymatic synthesis of natural, but also non-natural sialooligosaccharides and glycoconjugates, such as sulfated oligosaccharides or thioglycosides. Initial examples clearly demonstrated that the substrate tolerance can be further broadened by simple point mutations, offering the unique chance to engineer STs with tailor-made specificities. In addition, the large-scale production of catalysts in bacterial expression systems is considerably less expensive than the expression in mammalian or insect cells.

A new era in enzymatic sialylation became possible with the fusion protein technology and the utilization of whole cells as production systems of sialoglycans. Finally, the search for additional bacterial enzymes may provide new catalysts with surprising substrate specificities.

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