47. Synthesis of New Sialidase Inhibitors, 6-Amino-6-deoxysialic Acids

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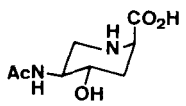
The synthesis of the 6-amino-6-deoxysialic-acid analogues **4**, **5**, and **6** is described. *Mitsunobu* reaction of the 1-*C*-nitroglycal **8** (PPh_3, HCOOH, DEAD) gave the formiate **10** with inversion of configuration at C(3) *(Scheme 2)*. Treatment of **10** with aq. NH_3 and subsequent protection of the amino function gave the imines **14** and **15** *(Scheme 3)*, which were transformed into the triflates **17**. Substitution by azide, deprotection, and *N*-acetylation gave the anomeric 2-acetamido-3-azido-1-deoxy-1-nitro-D-mannoses **16** and the enol ether **18**. Chain elongation of the nitro azides **16** followed by hydrolysis gave the nonulosonates **20**/**22**, which upon reduction yielded the diols **23** and **24**, respectively *(Scheme 4)*. The diol **23** was transformed into the sialic-acid analogues **5**, **6**, and **32** by ozonolysis, transfer hydrogenation, hydrogenolysis, and deprotection *(Scheme 5)*, and the diol **24** into **4** by a similar reaction sequence. The sialic-acid analogues **4** and **6** inhibit bacterial and viral sialidases competitively. The inhibitor constants for this enzyme from *Vibrio cholerae* are 0.12 mM for **4** and 0.19 mM for **6**, respectively. The activity of fowl plague virus sialidase was reduced by 17% and 36% under the influence of **4** and **6**, respectively, at a concentration of 0.1 mM. Compound **5** was inactive.

Introduction. – Sialidases [1–3] play an important role in the catabolism of sialooligosaccharides and sialoglycoconjugates, which are involved in many biological functions [3] [4] by hydrolytically releasing α-glycosidically bound sialic acids. Sialidases have been found in some viruses [2], in pathogenic and nonpathogenic bacteria [2] [5], in trypanosomes [6], and in mammalian tissues [2] [3] [7]. They have toxic effects when present in non-physiologically high amounts. Absence of these enzymes or their presence in insufficient quantities also leads to pathological consequences, such as some forms of mucolipidosis and sialidosis [1–3] [7–8]. Sialidases play a decisive role during viral infection by myxoviruses [9] and in the process of virus multiplication [10] [11]. In pneumococcal and clostridial infections, high concentrations of sialidases in wounds and body fluids have been found [12] [13]. The role of sialidases – particularly those of *Vibrio cholerae* – in cancer and cancer therapy is unclear and controversial [14]. Selective inhibition of sialidases might, therefore, be a target for biochemical studies and clinical applications, particularly in view of the preparation of antiviral [15], antibacterial, and antiprotozoal drugs.

Several sialidase inhibitors are known [16–20], *e.g.* *N*-acetyl-2-deoxyneur-2-enaminic acid ('2,3-dehydro-*N*-acetylneuraminic acid'; Neu2en5Ac) [21] [22], *N*-acetyl-2-deoxy-4-epineur-2-enaminic acid (4epiNeu2en5Ac) [22] [23], and *N*-acetyl-2-deoxy-4-oxoneur-2-enaminic acid (Neu2en5Ac4oxo) [24]. Many naturally occurring α- and/or β-glycosidase inhibitors [25–40] (*e.g.* nojirimycin and analogous piperidine derivatives) possess as basic



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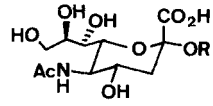

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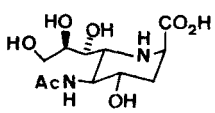

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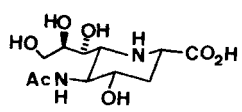

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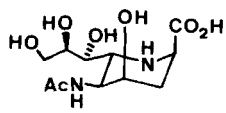

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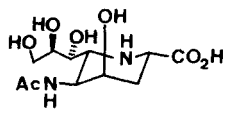

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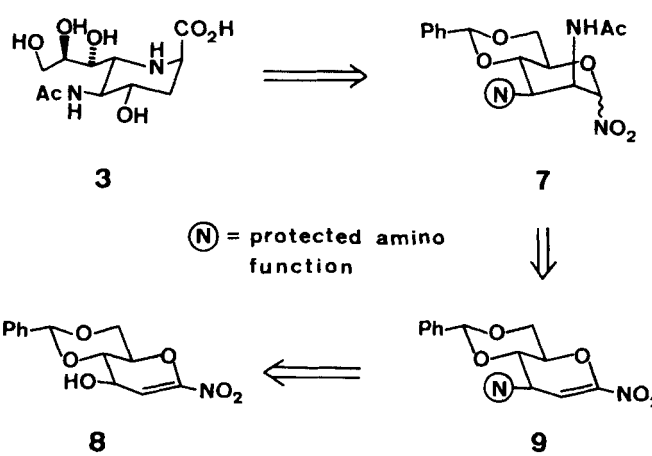
N-atom in a 5- or 6-membered ring. The pipecolinic-acid derivative **1** corresponds to a 2-deoxysialic acid in which the ring O-atom has been replaced by an amino function and where the 'axial' COOH group possesses the same orientation as the COOH group in the naturally occurring α-D-glycoside **2** of *N*-acetylneuraminic acid (Neu5Ac), but which lacks the glycerol side chain. It has been synthesized in view of its potential neuraminidase inhibitory activity and is indeed a competitive and selective sialidase inhibitor of bacterial ($K_i = 10^{-2}$ M), but not of mammalian sialidases [41]. Since analogues corresponding to **1**, but possessing the glycerol side chain ought to be better inhibitors, we planned to prepare the 6-amino-6-deoxysialic acids **3** and **4**. *Schauer* and coworkers [3] [42–45] and *Flashner et al.* [16] have shown the importance of the OH–C(4) group in sialidase activity, and the sialidase inhibitory activity of 4epiNeu2en5Ac. The 6-amino-6-deoxy-4-episialic acids **5** and **6** are thus also compounds of interest[1]).

Plan. – To take advantage of our synthesis of Neu5Ac and 4epiNeu5Ac [47] using the *Michael* addition of a nitro ether to a 2-(bromomethyl)acrylate as the chain elongation

Scheme 1



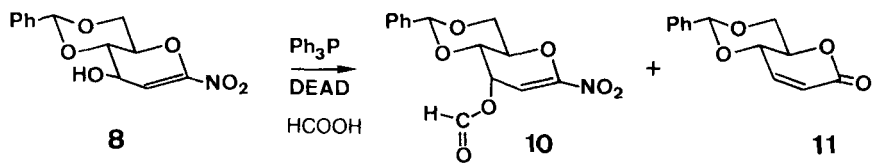
[1]) The importance of the OH–C(4) group also derives from the fact that *N*-acetyl-2,4-dideoxyneur-2-enaminic acid inhibits *V. cholerae* sialidase only weakly (50% inhibition at 1 mM concentration and 10% at 0.1 mM concentration) [46], while Neu2en5Ac inhibits this enzyme by 70% at a concentration of 0.1 mM.

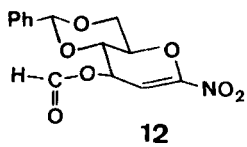
step, we required an 1-deoxy-1-nitro-mannosamine derivative such as **7**, with two different N-functions at C(2) and C(3) *(Scheme 1)*. Such a derivative might be obtained from the nitroglycal **8**. Replacement of the allylic OH–C(3) group (corresponding to C(6) of Neu5Ac) by a N-function with retention of configuration leads to a nitro-olefin **9**. A study of this transformation appeared interesting from the viewpoint of the general reactivity at C(3) of 1-*C*-nitroglycals. The stereoelectronically controlled β-addition of NH_3 to **9**, followed by acetylation of the amino function was expected to give **7**.

In the following, we report the syntheses of the 6-amino-6-deoxysialic-acid analogues **4**–**6** and their action on a bacterial and a viral sialidase.

Results. – Treatment of the nitro-olefin **8** with HCOOH according to the conditions of the *Mitsunobu* reaction gave, after chromatography, the D-*ribo*-configurated nitro-olefin **10** (56%) and the known δ-lactone **11** [48] (27%, *Scheme 2*).

Scheme 2





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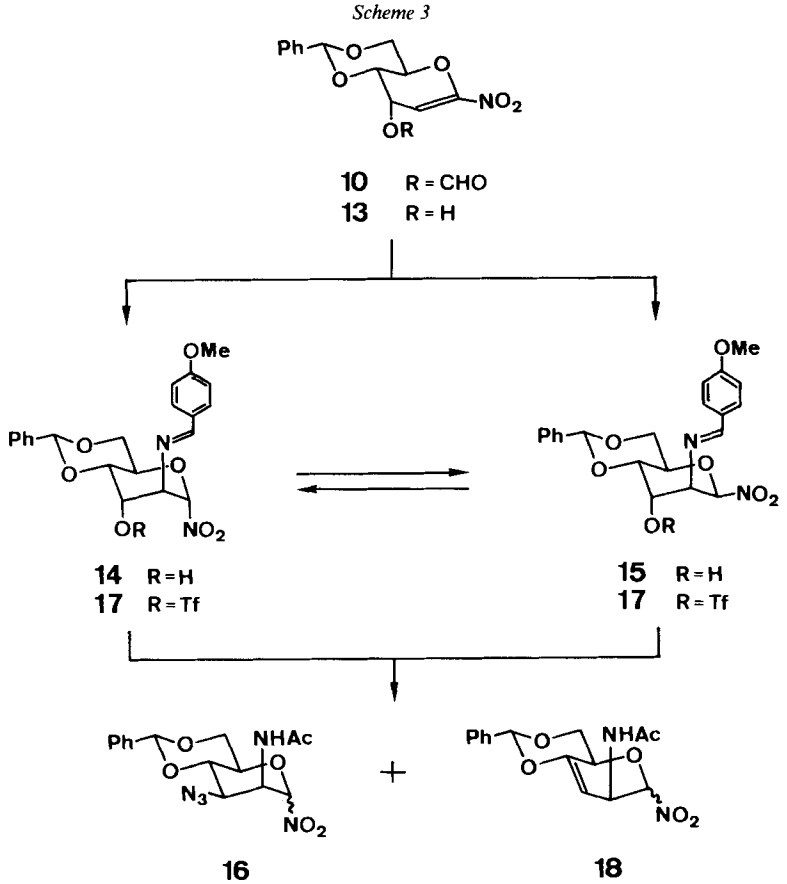
DEAD = Diethyl azodicarboxylate

$J(2,3) = 2.7$ Hz
$J(3,4) = 8.0$ Hz

The ^{1}H-NMR spectra of the nitro-olefin **10** differs from that of the C(3)-epimer **12** [49] only by a larger $J(2,3)$ (6.0 Hz for **10**, 2.7 Hz for **12**) and a smaller $J(3,4)$ value (4.0 Hz for **10**, 8.0 Hz for **12**), indicating inversion of the configuration at C(3). The δ-lactone **11** may be formed by an S_N1 or S_N2' process, *e.g.* from the expected phosphonium-salt intermediate of the *Mitsunobu* reaction, followed by solvolytic loss of the NO_2 group and elimination of HCOOH. *Dyong et al.* [50] have shown that *Mitsunobu* reactions [51] of allylic substrates with proper steric arrangement may predominantly follow a S_N2' mechanism.

Aqueous ammonia transformed **10** into **13** (0°; $J(2,3) = 5.8$ Hz, $J(3,4) = 3.8$ Hz) and, hence, into the addition products (r.t.), which were directly converted into a mixture of the anomeric D-*altro*-imines **14** and **15** (89% from **10**). Pure **14** was obtained by crystallization. Base-catalyzed equilibration of **14** (NEt_3, THF, r.t.) gave a 85:15 mixture (^{1}H-NMR) of **14** and **15** *(Scheme 3)*. The alcohol **13** was also obtained in a yield of 79% by treating the formiate **10** with NaOMe in THF/MeOH.

Scheme 3



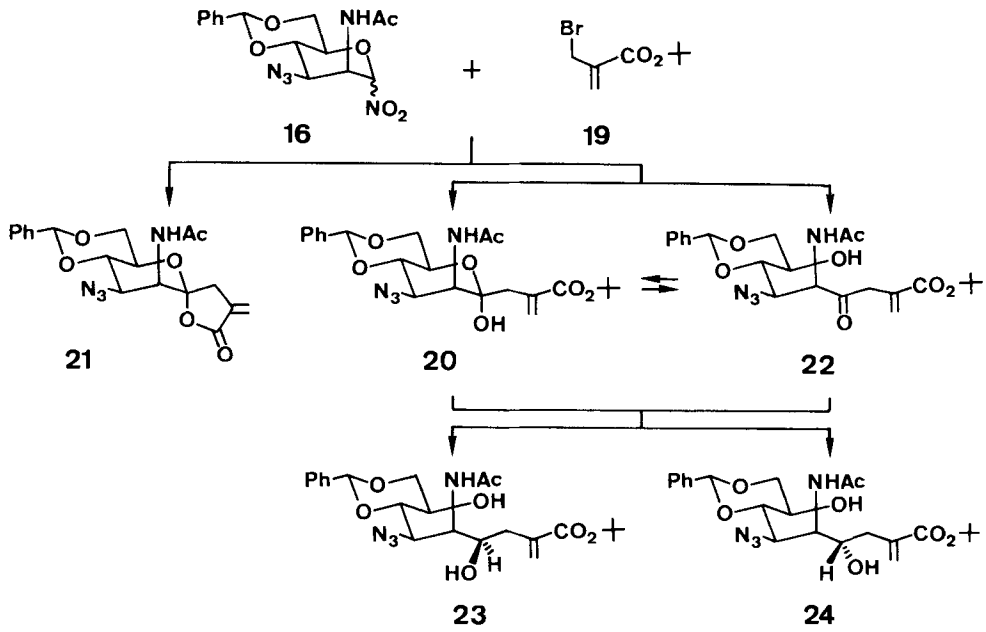
The imine **14** is characterized by a UV absorption at 283 nm (ε = 18810) typical [52] for the presence of the *N*-methoxybenzylidene group and by an IR absorption at 1632 cm^{-1} (conjugated C=N bond). The D-*altro*-configuration of the imines **14** and **15** is deduced from the ^3J values in their ^{1}H-NMR spectra (**14**: $J(1,2) = 0$, $J(2,3) = 4{,}0$, and $J(3,4) = 2.1$ Hz; **15**: $J(1,2) = 2.0$ Hz). Comparison of $J(1,2)$ of **14** and **15** with the corresponding coupling constants of the D-*manno*- and D-*altro*-configurated 1-deoxy-1-nitro-sugars [53] [54] confirm the postulated α-D-configuration of **14**.

The imine **14** was best transformed into the azides **16** without isolation of any intermediate. Treatment of **14** with trifluoromethanesulfonic acid anhydride in CH_2Cl_2/pyridine between −30° and 0° gave the anomeric triflates **17** (α/β = 4:1; 92%; *Scheme 3*). The triflates were transformed into the corresponding azides (LiN_3, benzene/HMPT, r.t.)[2]) [55] [56], which were deprotected (tosylhydrazide, AcOH, 0°, 20 h) [58] and acetylated (Ac_2O/EtOH) yielding the 2-acetamido-3-azido-nitro-sugars **16** (81% from **14**) and the enol ether **18** (13% from **14**)[3]).

[2]) Similar results were obtained from the reaction of **17** with $Bu_3PC_{16}H_{34}N_3$ in Et_2O at r.t. This method has the disadvantage that excess reagent must be removed by chromatography [57].

[3]) Neither the glycal **13** nor the imine **14** gave the corresponding azides under the conditions of the *Mitsunobu* reaction [59]. Treatment of **13** with $CF_3SO_3N_3$ [60] to cause direct substitution of the OH–C(3) group gave complex mixtures.

Scheme 4



The configurations of the anomeric 2-acetamido-3-azido-sugars **16** were assigned from the ^{1}H-NMR coupling constants (α-D-**16**: $J(1,2)$ = 1.2 Hz, $J(2,3)$ = 5.0 Hz, $J(3,4)$ = 10.0 Hz; β-D-**16**: $J(1,2)$ = 2.7 Hz, $J(2,3)$ = 5.2 Hz). IR absorptions at 2110, 1688, and 1560 cm^{-1} confirm the presence of the azido, acetamido, and nitro groups, respectively. The enol ether **18** does not show an azide band in its IR spectrum, and the expected absorption band of the enol ether function is obscured by a strong amide band. It is further characterized in the ^{13}C-NMR spectrum by a *doublet* at *ca.* 100 ppm for C(3) and a *singlet* for C(4) at 153.5 ppm. These values agree well with the proposed enol-ether structure.

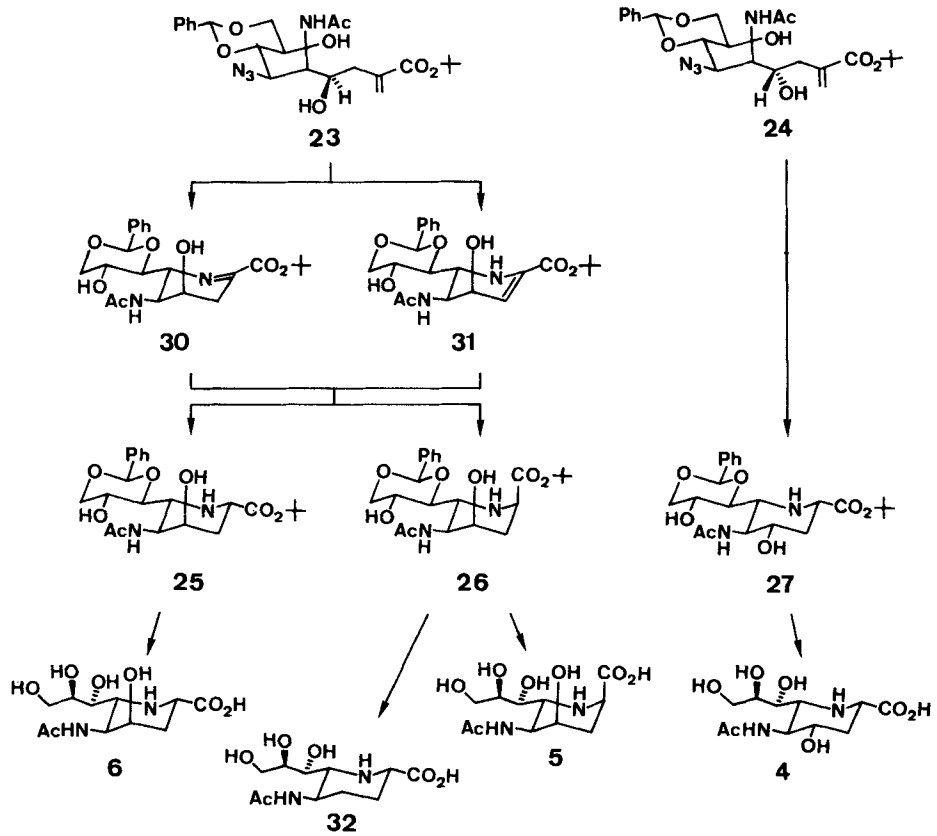
The *N*-acetylmannosamine derivatives **16** reacted at 0° in THF and in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with *tert*-butyl 2-(bromomethyl)prop-2-enoate (**19**) to an intermediate (89%), which was hydrolyzed in a mixture of CH_3CN and aqueous citrate buffer (pH 5.5) at r.t. to the crystalline *tert*-butyl 4-nonulosonate **20** and the γ-lactone **21** *(Scheme 4)*. While **20** did not tautomerize to **22** in solution in (D_6)acetone, it equilibrated within 5 min with **22** in (D_6)DMSO (**20**/**22** = 6:4).

In the ^{13}C-NMR spectrum, the pyranose **20** shows a *singlet* at 99.17 ppm for C(4) (anomeric centre), while the open-chain tautomer **22** shows a *singlet* for C(4) at 204.03 ppm. The ^{1}H- and ^{13}C-NMR spectra of the γ-lactone **21** show the absence of a *t*-Bu group and a *singlet* for C(4) at 105 ppm. An IR band at 1786 cm^{-1} [61] confirms the presence of a γ-lactone. In solution, the lactone **21** decomposes within *ca.* 14 days at r.t.; it is more stable in the solid state.

Reduction of the *tert*-butyl 4-nonulosonate **20**/**22** with $NaBH_4$ in the presence of AcOH [47] gave a 84:16 mixture of the diols **23** and **24**[4]). The configuration of the diols **23** and **24** was deduced by transformation of **23** into the 6-amino analogues **25** and **26** of 4epiNeu5Ac, and of the diol **24** into the 6-amino analogue **27** of Neu5Ac *(Scheme 5)*.

[4]) The ratio of the epimers **23**/**24** did not depend upon the solvent (protic, aprotic, aqueous, or anhydrous) and was always *ca.* 4:1 or larger.

Scheme 5

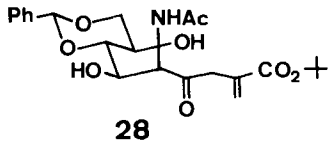
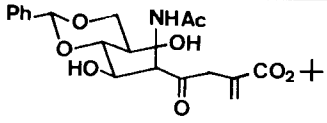
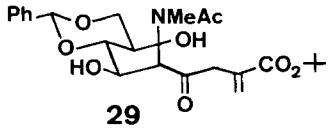
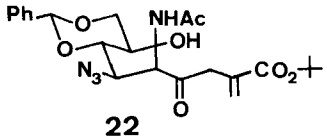


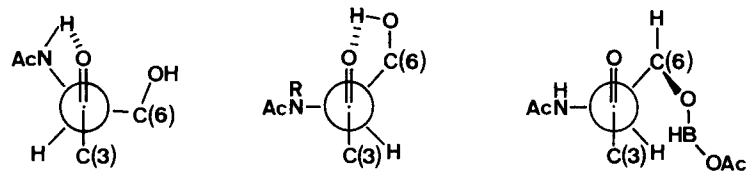
Although a disadvantage from the preparative viewpoint, this result contributes to an understanding of the influence of the reaction conditions upon the diastereoselectivity of the $NaBH_4$ reduction. The results of the diastereoselective reduction of various nonulosonic-acid derivatives are gathered in the *Table*.

The stereoselectivity of the $NaBH_4$ reduction of **28** in MeOH *(Entry 1)* may be explained by assuming a H-bond between the NHAc group and the 4-oxo group (conformation **A**). This conformation corresponds to the cyclic *'Cram'* model and favours the attack from the '*si*-side' giving predominatly the (4*R*)-configurated reduction product. Evidence for such a H-bond is found in the reduction of the *N*-methylated nonulosonic ester **29** *(Entry 3)* [63]. Under otherwise identical conditions (as in the reduction of **28**), the reduction of **29** produces predominantly the (4*S*)-configurated product. This result can be rationalized by assuming a conformation according to the *'Anh-Felkin'* model [62]. Conformation **B** is expected to be the most reactive one; it should be attacked from the '*re*-side'. While the $NaBH_4$ reduction of **28** in the presence of AcOH, however, gave predominantly the (4*S*)-configurated triol (*Entry 2;* see [47]), the azide **22** yielded mainly the (4*R*)-configurated compound **23** *(Entry 4)*, independently of the reaction conditions. This is only compatible with a participation of the OH–C(6) group in the reduction of **28**. The OH–C(6) group can either form a H-bond to the 4-oxo group, stabilizing conformation **B**, or react with the reducing agent and thus lead to an intramolecular hydride transfer to the '*re*-side'.

Ozonolysis of the diol **23** and subsequent reduction of the azido function by transfer hydrogenation (Pd/C, $HCOONH_4$ in MeOH) gave a 55:45 mixture of the imine **30** and

Table. *Stereoselective Reduction of Nonulosonate Derivatives*

Entry	Substrate	Conditions	Product [%]		Ref.
			Configuration at C(4)		
			(*R*)	(*S*)	
1	 **28**	$NaBH_4$, MeOH or $NaBH_4$, oxolane/H_2O 4:1	70	30	[47]
2	 **28**	$NaBH_4$/AcOH oxolane/H_2O 4:1	6	94	[47]
3	 **29**	$NaHB_4$, MeOH	33	67	[63]
4	 **22**	$NaBH_4$/AcOH oxolane/H_2O 4:1 or $NaBH_4$, MeOH	80	20	



28 R = H
29 R = Me

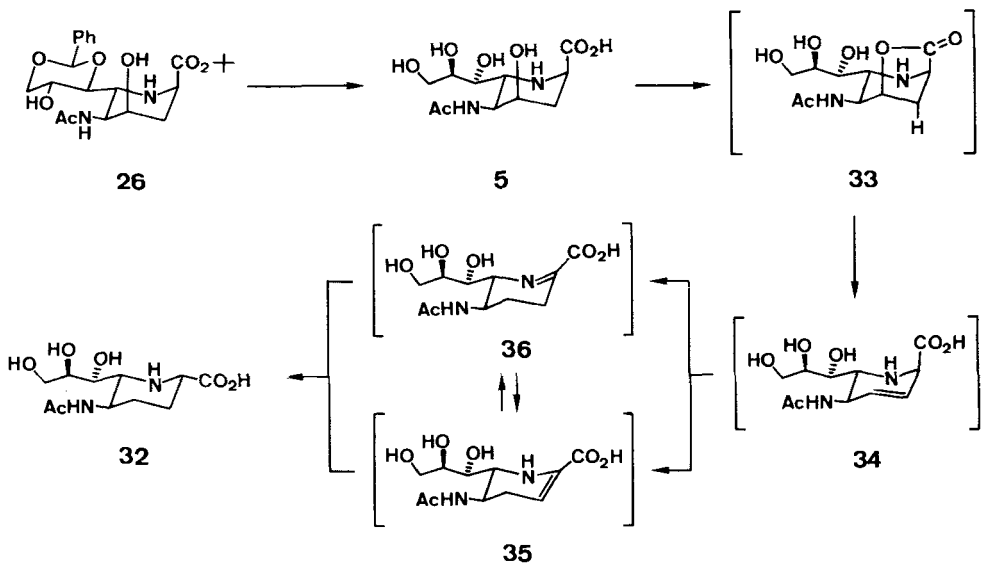
A **B** **C**

the enamine **31** (94% from **23**) [64] [65] *(Scheme 5)*. Conversion of the azido group into an amino group by the *Staudinger* reaction [66–68] followed by hydrolysis occurred only upon heating the mixture to 60° for 3 d and led also to a mixture of the imine **30** and the enamine **31** (68% from the diol **23**).

In the ^{13}C-NMR spectrum, the imine **30** is characterized by a *singlet* at 161.80 ppm for C(2) and a *triplet* at 36.34 ppm for C(3); and the enamine **31** by a *singlet* at 136.64 ppm for C(2) and a *doublet* for C(3) at 103.96 ppm. An absorption at 1710 cm^{-1} in the IR spectrum of **30/31** indicates the presence of an α,β-unsaturated ester.

Hydrogenation of a mixture of **30/31** (AcOEt/benzene, 1 d) in the presence of Pd/C yielded the D-*erythro*-L-*allo*-configurated piperidine derivative **25** (75%) and the D-*erythro*-L-*altro*-configurated piperidine derivative **26** (19%). The configuration of **25** and **26** was deduced from the ^{1}H-NMR spectra. (**25:** $J(2,3) = 3.0$ and 12.0 Hz,

Scheme 6



and $J(4,5) = 2.8$ Hz; **26:** $J(2,3) = 3.0$ and 6.0 Hz, and $J(4,5) = 2.8$ Hz.) All other coupling constants were very similar to those obtained for the corresponding 4epiNeu5Ac derivative (see [47]).

Deprotection of **25** with CF_3COOH followed by ion-exchange chromatography gave the amino acid **6** in a 72% yield as a colourless, microcrystalline solid. Under similar conditions, the piperidine derivative **26** did not give the amino acid **5** but the C(4) deoxygenated amino acid **32** (43%).

The formation of the deoxygenated product **32** is rationalized by assuming a conversion of the amino acid **5** into a γ-lactone **33** *(Scheme 6)* followed by an elimination to the unsaturated **34**. Isomerization of **34** to **35** and further to the imine **36**, and subsequent reduction of **36** with HCOOH during the ion-exchange chromatography gives **32**[5]).

The amino acid **32** is characterized in the ^{13}C-NMR spectrum by two signals at 31.87 and 27.71 ppm for the $CH_2(3)$ and $CH_2(4)$ groups, respectively. The ^{1}H-NMR spectrum of **32** is very similar to the one of 4-deoxy-Neu5Ac, particularly the chemical shifts of H_{ax}–C(3), H_{ax}–C(4), H_{eq}–C(4), and H_{eq}–C(3) are almost identical (see [69]). The expected configuration at C(5) is confirmed by $J(4,5) = 11.0$ and 4.2 Hz, and at C(2) by $J(2,3) = 13.0$ and 3.2 Hz, establishing the equatorial orientation of the COOH group.

The D-*erythro*-L-*altro*-configurated amino acid **5** was obtained from **26** by saponification of the *tert*-butyl ester with NaOH, followed by acidic debenzylidenation and a final purification by ion-exchange chromatography. The configuration at C(2) of **5** is deduced from the $J(2,3)$ values of 1.5 and 7.0 Hz, respectively.

Ozonolysis of the D-*glycero*-D-*galacto*-configurated diol **24** and subsequent reduction of the azido group (H_2, Pd/C) gave the benzylidene-protected amino ester **27** in a yield of 58% *(Scheme 5)*. An imine/enamine intermediate corresponding to **30**/**31** was observed neither under these conditions nor under conditions of transfer hydrogenation. The latter

[5]) The modest yield may be due to incomplete elution by HCOOH from the ion-exchanger column, as elution of **5** and **6** with aq. HCOOH was less efficient than with aq. HCl.

conditions gave a complex mixture, which was directly hydrogenated (H_2, Pd/C) yielding the amino ester **27**. The C(2)-epimer of **27** could not be isolated, although three minor by-products were observed on TLC. The D-*erythro*-L-*gluco*-configuration of **27** was deduced from the values of the vicinal coupling constants (J(2,3eq) = 2.8 Hz, J(2,3ax) = 11.8 Hz, and J(4,5) = 10.0 Hz). All other coupling constants were very similar to those of the corresponding Neu5Ac derivative (see [47]).

Similarly to **26**, the piperidine **27** was deprotected by saponification of the *tert*-butyl ester (NaOH), followed by acidic removal of the benzylidene group and an ion-exchange chromatography to give the amino acid **4** (98%). Apart from J(2,3) (13.0 and 3.0 Hz), the ^3J values were very similar to those of Neu5Ac [47] [70].

Sialidase Experiments. – Of the three substances tested, **4** and **6** were effective inhibitors of the bacterial sialidase, but weaker inhibitors of the viral enzyme. Substance **5** was inactive. At 0.1 M concentration, **4** reduced the *V. cholerae* sialidase activity by 43%, compound **6** by 55%, and Neu2en5Ac, used as a reference, by 70% (as compared with the enzyme reaction in the absence of an inhibitor). The inhibitor constants (K_i values) were calculated to be 0.12 mM for **4**, 0.19 mM for **6**, and 0.16 mM for Neu2en5Ac. At 0.1 mM concentration, **4** reduced the activity of the viral enzyme by only 17% and **6** by 36%. Preincubation (15 min) of the enzymes with the inhibitors did not significantly influence their effect.

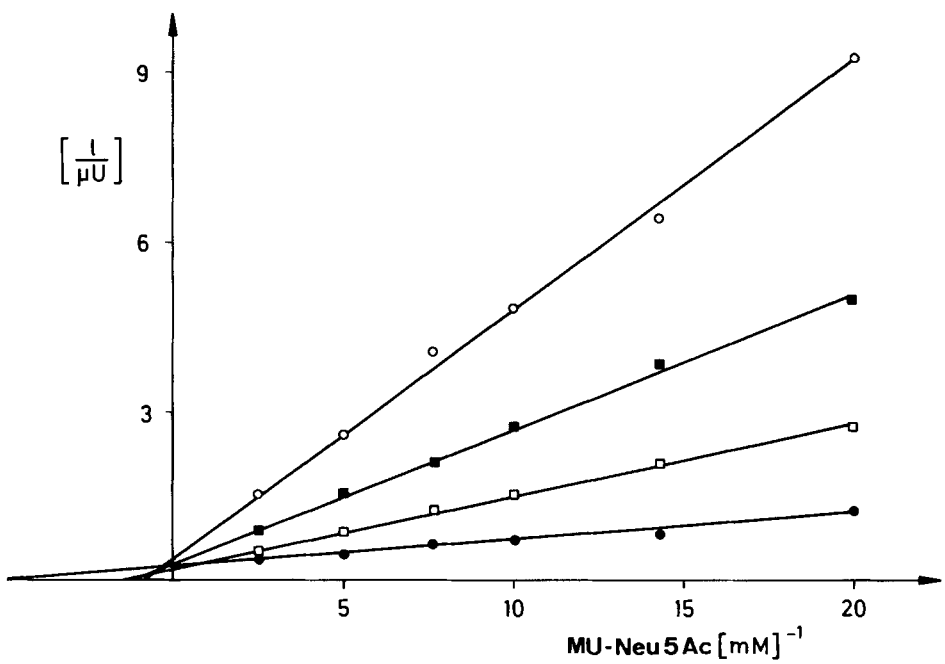


Figure. *Example of an experiment showing inhibition of the action of* Vibrio cholerae *sialidase on MU-Neu5Ac by various concentrations of substance* **4** *for determination of the inhibitor constant.* ●, no inhibitor added (K_m 0.20 mM); □, 0.1 mM **4** (K'_m 0.67 mM); ■, 0.25 mM **4** (K'_m 0.83 mM); ○, 0.5 mM **4** (K'_m 1.25 mM). The V_{max} value for all slopes is about 0.42 mU/0.1 ml. For experimental details, see *Exper. Part.*

Compounds **4** and **6** are competitive inhibitors, as can be delineated from the plots obtained by applying constant inhibitor and variable *N*-acetyl-2-(7-*O*-4-methylumbelliferyl)neuraminic acid (MU-Neu5Ac) concentrations for the calculation of the K_i values. An example is given in the *Figure* for **4**. It can be seen from the points of intersection with the ordinate that the V_{max} values of the reactions in the presence of various inhibitor concentrations are similar to those of the non-inhibited enzyme reaction (V_{max} 0.4 mU/0.1 ml), while the K_m values decrease with increasing inhibitor concentrations when compared with the K_m value of 0.18 mM for MU-Neu5Ac in the absence of the inhibitor. The data are mean values of 5 experiments and each measurement was made in duplicate. There was no significant variation between the individual experiments.

Discussion. – To assess the influence of the imino group upon the inhibitory effect of **4** and **6**, a comparison with the known inhibitory activity of *N*-acetyl-2-deoxy-4-epineuraminic acid is required. The reported inhibition of *Arthrobacter sialophilus* sialidase by the latter compound, presumably possessing the (2*S*)-configuration, appears to be significantly lower (K_i 12.1 mM) [71]. These results indicate the influence of the basic substituent at C(6). The (2*R*)-configurated compound **5**, possessing an axial COOH group was inactive. It should be noted that the pipecolinic acid **1** possessing an axial COOH group in the preferred $^2C_5$ conformation inhibited bacterial sialidases from *Vibrio cholerae* and *Arthrobacter ureafaciens* albeit only weakly [41]. The implications of these findings are the subject of further research.

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Experimental Part

General. See [47] [72]. *Amberlite IRA-93* was activated by washing it sequentially with 0.5N NaOH, H_2O (bidest), MeOH, and Et_2O, and drying it over P_2O_5 at 10^{-2} mbar. *Dowex 1X8* ($HCOO^-$) was washed according to [73]. $(ClCH_2CO)_2O$ was used without further purification. HCOOH was distilled from phthalic anhydride. FC = flash chromatography.

*4,6-O-Benzylidene-1,2-dideoxy-3-O-formyl-1-nitro-*D*-ribo-hex-1-enopyranose* (**10**) *and 4,6-O-Benzylidene-2,3-dideoxy-*D*-erythro-hex-2-eno-1,5-lactone* (**11**). A soln. of diethyl azodicarboxylate (1.41 ml, 9.0 mmol) and HCOOH (680 µl, 18.0 mmol) in THF (20 ml) was added during 48 h to a soln. of **8** (1.0 g, 3.60 mmol) and Ph_3P (2.35 g, 9.00 mmol) in THF (20 ml) at 5°[6]). When TLC (hexane/AcOEt 6:4) indicated the disappearance of **8**, the mixture was diluted with AcOEt (40 ml), extracted with ice-cold 5% $NaHCO_3$ soln. (50 ml), H_2O, and brine. The solvent was removed and the residue purified by FC (200 g of SiO_2). Hexane/Et_2O 7:3 eluted **10** (620 mg, 56%), hexane/Et_2O 1:1 gave **11** (230 mg, 27%). An anal. sample of **10** was obtained by recrystallization from AcOEt/hexane and one of **11** by recrystallization from AcOEt/Et_2O 1:2 and hexane.

Data of **10**. M.p. 143–144°. $[\alpha]_D^{25} = +223°$ (c = 1.0, $CHCl_3$). UV (CH_2Cl_2): 278 (3620). IR: 3110*w*, 3020*w*, 2940*w*, 2870*w*, 1730*s*, 1665*m*, 1552*s*, 1468*w*, 1452*w*, 1382*m*, 1340*s*, 1271*m*, 1147*s*, 1100*s*, 1023*m*, 937*m*. ^{1}H-NMR (200 MHz): 8.13 (*d*, J = 1.1, HCOO); 7.53–7.33 (*m*, 5 arom. H); 6.47 (*d*, J = 6.0, H–C(2)); 5.84 (*ddd*, J = 6.0, 4.0, 1.1, H–C(3)); 5.65 (*s*, ArC*H*); 4.69 (*dd*, J = 10.5, 5.3, H–C(6)); 4.49 (*ddd*, J = 10.5, 10.5, 5.3, H–C(5)); 4.09 (*dd*, J = 10.5, 4.0, H–C(4)); 4.04 (*dd*, J = 10.5, 10.5, H–C(6)). ^{13}C-NMR (50 MHz): 159.51 (*d*); 154.81 (*s*); 136.03 (*s*); 129.45 (*d*); 128.36 (*d*); 126.01 (*d*); 101.86 (*d*); 96.94 (*d*); 74.21 (*d*); 67.96 (*d*); 67.73 (*t*); 61.01 (*d*). Anal. calc. for $C_{14}H_{13}NO_7$ (307.27): C 54.73, H 4.26, N 4.56; found: C 54.49, H 4.39, N 4.82.

Data of **11**. M.p. 133.0–133.5° ([48]: 134–135°). $[\alpha]_D^{25} = +29.3°$ (c = 1.0, $CHCl_3$) ([48]: +26.5° (c = 1.0, $CHCl_3$)). ^{1}H-NMR (400 MHz, (D_6)benzene): 7.47–7.45 (*m*, 2 arom. H); 7.17–7.11 (*m*, 3 arom. H); 6.15 (br. *d*,

[6]) Shorter reaction times and/or higher reaction temp. as well as the addition of the nitro-olefin **8** to the mixture of the reagents [74] led to increased amounts of the by-product **11** (*Townsend et al.* [74] obtained better yields in *Mitsunobu* reactions by addition of the substrate to the mixture of the reagents than *vice versa*).

J = 9.9, H–C(3)); 5.47 (*dd*, J = 9.9, 2.6, H–C(2)); 5.01 (*s*, ArC*H*); 3.89 (*dd*, J = 10.3, 4.8, H–C(6)); 3.76 (*ddd*, J = 10.3, 10.2, 4.8, H–C(5)); 3.55 (*ddd*, J = 10.2, 2.6, 1.5, H–C(4)); 3.32 (*dd*, J = 10.3, 10.3, H–C(6)). ^{13}C-NMR (50 MHz): 161.65 (*s*); 146.85 (*d*, C(3)); 136.23 (*s*); 129.40 (*d*); 128.30 (*d*); 126.02 (*d*); 120.55 (*d*, C(2)); 102.18 (*d*); 73.70 (*d*); 72.58 (*d*); 67.98 (*t*).

*4,6-O-Benzylidene-1,2-dideoxy-1-nitro-*D-ribo*-hex-1-enopyranose* (**13**). To an ice-cold soln. of **10** (420 mg, 1.37 mmol) in THF (15 ml) and anh. MeOH (6 ml) was added a 0.5M NaOMe/MeOH soln. (50 µl). When TLC (hexane/AcOEt 6:4) indicated the disappearance of **10**, the mixture was poured onto ice water (20 ml) and extracted with AcOEt. The solvent was removed and **13** (220 mg, 56%) crystallized from AcOEt/hexane. FC (20 g of SiO_2, hexane/AcOEt 3:1) of the mother liquor gave further **13** (82 mg, 21%). M.p. 177–178°. $[\alpha]_D^{25}$ = +152.9° (c = 1.1, $CHCl_3$). UV (CH_2Cl_2): 279 (3475). IR: 3575*m*, 3105*w*, 3020*w*, 2940*w*, 2870*w*, 1665*m*, 1550*s*, 1468*w*, 1453*w*, 1402*w*, 1383*m*, 1346*s*, 1334*s*, 1272*m*, 1143*s*, 1122*s*, 1102*s*, 1090*s*, 1026*s*, 996*s*. ^{1}H-NMR (200 MHz): 7.60–7.37 (*m*, 5 arom. H); 6.47 (*d*, J = 5.8, H–C(2)); 5.72 (*s*, ArC*H*); 4.71 (*dd*, J = 10.5, 5.3, H–C(6)); 4.66–4.56 (*m*, H–C(3); addn. of D_2O: 4.62, *dd*, J = 5.8, 3.8); 4.52 (*ddd*, J = 10.2, 10.2, 5.3, H–C(5)); 4.04 (*dd*, J = 10.5, 10.2, H–C(6)); 3.93 (*dd*, J = 10.2, 3.8, H–C(4)); 2.70 (*d*, J = 1.8, OH). ^{13}C-NMR (50 MHz): 154.06 (*s*); 136.20 (*s*); 129.64 (*d*); 128.44 (*d*); 126.14 (*d*); 101.96 (*d*); 100.23 (*d*); 76.46 (*d*); 67.71 (*t*); 66.84 (*d*); 60.33 (*d*). Anal. calc. for $C_{13}H_{13}NO_6$ (279.26): C 55.91, H 4.69, N 5.02; found: C 56.14, H 4.57, N 4.95.

*2-[*N*-4-(Methoxybenzylidene)amino]-4,6-*O*-benzylidene-1,2-dideoxy-1-nitro-α-*D*-altro-pyranose* (**14**). To an ice-cold soln. of **10** (2.76 g, 9.0 mmol) in THF (135 ml) were added 34 ml of a 25% soln. of NH_3 in H_2O. The mixture was stirred at 0°, until **10** had disappeared (TLC: hexane/AcOEt 6:4, 4 h; giving first **13**) and then at r.t. overnight. THF was removed and the remaining aq. layer was freeze-dried. To a soln. of the residue in anh. MeOH (9 ml) and dry benzene (90 ml) was added 4-methoxybenzaldehyde (2.2 ml, 1.8 mmol). The mixture was stirred at r.t. overnight. The solvent was evaporated to 10–15 ml, and **14** (2.11 g, 57%) was crystallized by adding dry Et_2O[7]). The mother liquor was concentrated and purified by FC (100 g of SiO_2). Hexane/THF 8:1 separated excess 4-methoxybenzaldehyd, while hexane/THF 7:3 eluted **14/15** (1.20 g, 32%); 590 mg of **14** crystallized spontaneously upon removal of the solvent. A sample for analysis was obtained by recrystallization from AcOEt/hexane. M.p. 187–188° (dec.). $[\alpha]_D^{25}$ = –61.9° (c = 1.0, THF). UV (CH_2Cl_2): 283 (18810). IR (KBr): 3400*m* (br.), 1632*m*, 1602*s*, 1565*s*, 1513*m*, 1377*w*, 1363*m*, 1306*m*, 1263*s*, 1172*s*, 1102*s*, 1045*m*, 983*m*, 834*m*, 761*m*. ^{1}H-NMR ((D_8)THF, 200 MHz): 8.51 (*s*, N=CH); 7.84–7.73, 7.04–6.92 (2 *AA'BB'*, 4 arom. H); 7.54–7.24 (5 arom. H); 5.68 (*s*, ArC*H*); 5.36 (*s*, H–C(1)); 4.92 (*d*, J = 3.0, OH); 4.82 (*ddd*, J = 10.0, 10.0, 5.0, H–C(5)); 4.53 (br. *d*, J = 4.0, H–C(2)); 4.44 (*dd*, J = 10.0, 5.0, H–C(6)); 4.30 (*dd*, J = 10.0, 2.1, H–C(4)); 3.87 (*dd*, J = 10.0, 10.0, H–C(6)); 3.90–3.80 (*m*, H–C(3)); 3.83 (*s*, CH_3O). ^{13}C-NMR ((D_8)THF, 50 MHz): 165.14 (*d*); 163.48 (*s*); 139.02 (*s*); 130.98 (*d*); 129.60 (*s*); 129.26 (*d*); 128.43 (*d*); 127.13 (*d*); 114.65 (*d*); 104.12 (*d*); 102.67 (*d*); 77.33 (*d*); 73.90 (*d*); 70.38 (*d*); 69.43 (*t*); 63.52 (*d*); 55.57 (*q*). Anal. calc. for $C_{21}H_{22}N_2O_7$ (414.43): C 60.86, H 5.35, N 6.76; found: C 61.06, H 5.30, N 6.50.

Equilibration of **14** *into* **14/15**. A soln. of **14** (10 mg, 0.024 mmol) in anh. THF (1 ml) was treated with NEt_3 (3.3 µl, 0.024 mmol) and stored at 25° until $[\alpha]_D^{25}$ (–78.9°, c = 1.0) did not change any more (10 d). The solvent was removed and the residue dried for 2 h at 10^{-2} mbar. The ^{1}H-NMR of the residue indicated a 85:15 mixture **14/15**. ^{1}H-NMR of **15** (200 MHz, (D_8)THF): 8.23 (*s*, N=CH); 7.72–7.67, 6.95–6.90 (2 *AA'BB'*, 4 arom. H); 7.54–7.24 (5 arom. H); 5.97 (*d*, J = 2.0, H–C(1)); 5.70 (*s*, ArC*H*); 3.80 (*s*, CH_3); H–C(2) to 2 H–C(6) were overlapped by the signals of **14**.

*2-[*N*-4-(Methoxybenzylidene)amino]-4,6-*O*-benzylidene-1,2-dideoxy-1-nitro-3-*O*-(trifluoromethanesulfonyl)-*D*-altropyranose* (**17**). To a soln. of **14** (100 mg, 0.24 mmol) in anh. CH_2Cl_2 (3 ml) and pyridine (120 µl) at –30° was added trifluoromethanesulfonic acid anhydride (60 µl, 0.36 mmol). The mixture was slowly warmed to 0° and stirred (3 h) until TLC (CH_2Cl_2/MeOH 200:1) indicated the disappearance of **14**. Extractive workup (5% ice-cold $NaHCO_3$ soln., H_2O, brine) and purification of the residue by FC (15 g of SiO_2, CH_2Cl_2) gave **17** (121 mg, 92%, colourless foam) as a 4:1 mixture of the α/β-D-anomers (^{1}H-NMR). IR: 1631*m*, 1602*s*, 1567*s*, 1558 (sh), 1508*m*, 1418*m*, 1303*w*, 1250*m*, 1165*m*, 1142*s*, 1113*m*, 936*s*. ^{1}H-NMR (200 MHz): 8.50 (*s*, 0.8 N=CH); 8.23 (*s*, 0.2 N=CH); 7.86–7.70, 7.05–6.91 (2 *AA'BB'*, 4 arom. H); 7.52–7.34 (*m*, 5 arom. H); 5.86 (*d*, J = 2.0, 0.2 H–C(1)); 5.69 (*s*, ArC*H*); 5.34 (*s*, 0.8 H–C(1)); 5.28–5.22 (*m*, 0.2 H); 5.06–4.98 (*m*, 0.8 H–C(3)); 4.86 (*d*, J = 3.5, 0.8 H–C(2)); 4.75 (*ddd*, J = 10.0, 10.0, 5.0, 0.8 H–C(5)); 4.67–4.44 (*m*, 2.2 H; including a *dd* at 4.59, J = 10.0, 5.0 for 0.8 H–C(6) and a *dd* at 4.57, J = 10.0, 2.4 for 0.8 H–C(4)); 4.28 (*ddd*, J = 10.0, 9.0, 4.5, 0.2 H–C(5)); 4.14 (*dd*, J = 10.0, 10.0, 0.2 H–C(6)); 3.97 (*dd*, J = 10.0, 10.0, 0.8 H–C(6)); 3.89 (*s*, 0.8 CH_3); 3.86 (*s*, 0.2 CH_3).

*2-Acetamido-3-azido-4,6-*O*-benzylidene-1,2,3-trideoxy-1-nitro-*D*-mannopyranoses* (**16**) *and 2-Acetamido-4,6-*O*-benzylidene-1,2,3-trideoxy-1-nitro-*D*-hex-3-enopyranoses* (**18**). Similarly to **17**, **14** (1.00 g, 2.41 mmol) was treated

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[7]) Sometimes, the imine **14** precipitated directly from the reaction mixture.

with trifluoromethanesulfonic acid anhydride (600 µl) to give, after extractive workup, crude **17** (1.40 g). To a soln. of crude **17** in dry benzene (20 ml) was added LiN_3 (235 mg, 4.8 mmol). HMPT (2.0 ml) was added dropwise to the mixture (30 min), which was stirred at r.t. overnight. TLC (hexane/THF 6:4) indicated the disappearance of **17**. The orange-brown soln. was diluted with AcOEt (100 ml) and extracted with 2 × 5% $NaHCO_3$ soln. and brine. A soln. of the residue (1.17 g) in Et_2O/EtOH (99%) 1:3 (12.5 ml) at 0° was treated with AcOH (1 ml) and tosylhydrazide (900 mg). The mixture was stirred at 0° (3 h), stored in the refrigerator overnight, and treated with Ac_2O (2.5 ml). Ater 5 h, the excess of Ac_2O was destroyed by adding ice (2.0 g) and stirring for 1 h (0°). The mixture was diluted with AcOEt (200 ml), extracted with 2 × 5% $NaHCO_3$ soln. and brine, and purified by FC (150 g of SiO_2, hexane/THF 7:3 to 1:1) to give **16** (707 mg, 81%) as a slightly yellow foam and **18** (100 mg, 13%) as an oil.

Data of **16**. $[\alpha]_D^{25} = +58.2°$ (*c* = 1.1, DMSO). IR: 3435*w*, 2995*w*, 2925*w*, 2865*w*, 2110*s*, 1688*s*, 1560*s*, 1495*m*, 1370*s*, 1255*m*, 1160*m*, 1120*m*, 1092*s*, 1028*m*, 1005*m*. ^{1}H-NMR (400 MHz): α-D-anomer: 7.50–7.35 (*m*, 5 arom. H); 5.84 (*d*, *J* = 5.7, NH); 5.77 (*d*, *J* = 1.2, H–C(1)); 5.66 (*s*, ArC*H*); 5.23 (*ddd*, *J* = 5.7, 5.0, 1.2, H–C(2)); 4.46 (*dd*, *J* = 10.5, 4.5, H–C(6)); 4.08 (*dd*, *J* = 10.0, 5.0, H–C(3)); 3.97 (*ddd*, *J* = 10.0, 9.0, 4.5, H–C(5)); 3.87 (*dd*, *J* = 10.0, 9.0, H–C(4)); 3.84 (*dd*, *J* = 10.5, 10.0, H–C(6)); 2.13 (*s*, CH_3). β-D-anomer: 6.00 (*d*, *J* = 9.8, NH); 5.54 (*d*, *J* = 2.7, H–C(1)); 5.31 (*ddd*, *J* = 9.8, 5.2, 2.7, H–C(2)); 4.50 (*dd*, *J* = 10.5, 4.5, H–C(6)); 4.10–3.76 (*m*, H–C(3), H–C(4), H–C(5), H–C(6) overlapped by the signals of the α-D-anomer); 2.09 (*s*, CH_3). ^{13}C-NMR (50 MHz): 171.42 (*s*); 136.06 (*s*); 129.19 (*d*); 128.23 (*d*); 125.74 (*d*); 103.55 (*d*); 101.77 (*d*); 76.20 (*d*); 68.66 (*d*); 67.68 (*t*); 57.12 (*d*); 49.71 (*d*); 22.81 (*d*). CI-MS: 317 (20, $[M + 1]^+ - HNO_2$), 107 (100, $PhCH{=}OH^+$). Anal. calc. for $C_{15}H_{17}N_5O_6$ (363.35): C 49.58, H 4.72, N 19.28; found: C 49.44, H 4.95, N 19.01.

Data of **18**. IR: 3445*m*, 2985*w*, 2875*w*, 1685*s*, 1560*s*, 1486*s*, 1455*m*, 1382*s*, 1370*s*, 1176*s*, 1082*s*, 1010*m*, 980*m*, 965*m*. ^{1}H-NMR (200 MHz): 7.51–7.29 (*m*, 5 arom. H); 5.65–5.51 (*m*, NH, ArC*H*, H–C(1)); 5.38–5.28 (*m*, H–C(2), H–C(3)); 4.83 (*dd*, *J* = 10.5, 6.5, H–C(5)); 4.50 (*dd*, *J* = 10.5, 6.5, H–C(6)); 3.72 (*dd*, *J* = 10.5, 10.5, H–C(6)); 2.01 (*s*, CH_3). ^{13}C-NMR (50 MHz): 169.60 (*s*); 153.51 (*s*, C(4)); 135.49 (*s*); 129.84 (*d*); 128.48 (*d*); 126.14 (*d*); 103.54 (*d*); 102.75 (*d*); 99.99 (*d*); 69.38 (*t*); 63.91 (*d*); 45.46 (*d*); 23.05 (*q*).

*5-Acetamido-6-azido-7,9-*O*-benzylidene-2,3,5,6-tetradeoxy-2-methylidene-*α-D-manno*-4-nonulo-4,8-pyranosono-1,4-lactone* (**21**), tert-*Butyl 5-Acetamido-6-azido-7,9-*O*-benzylidene-2,3,5,6-tetradeoxy-2-methylidene-*D-manno*-nonulopyranosonate* (**20**), *and* tert-*Butyl 5-Acetamido-6-azido-7,9-*O*-benzylidene-2,3,5,6-tetradeoxy-2-methylidene-*D-manno*-4-nonulosonate* (**22**). To an ice-cold soln. of **16** (1.10 g, 3.05 mmol) and *tert*-butyl 2-(bromomethyl)prop-2-enoate (**19**; 1.01 g, 4.57 mmol) in THF (20 ml) was added dropwise DBU (960 µl, 1 h). After 2 h, TLC (AcOEt) indicated the disappearance of **16**. The mixture was diluted with AcOEt (20 ml) and extracted with H_2O, 5% $NaHCO_3$ soln., and brine. The residual oil was filtered through SiO_2 (150 g, hexane/AcOEt 1:4) to give the *Michael*-addition product (1.365 g, 89%), which was dissolved in CH_3CN (55 ml) and citrate buffer (14 ml, pH 5.5), and stirred at r.t. (3 d, in the dark)[8]. The mixture was diluted with AcOEt, extracted with H_2O, 5% $NaHCO_3$ soln., brine, and then treated with charcoal. The slightly yellow soln. was concentrated. Crystallization of the residue from AcOEt/hexane gave **20** (834 mg, 58% from **16**). FC of the mother liquor (20 g of SiO_2, acetone/hexane 1:3) gave further **20**/**22** (78 mg, 5%), and the by-product **21** (240 mg, 20%).

Data of **20**/**22**. An anal. sample of **20** was obtained by recrystallization from AcOEt/hexane. M.p. 132–135° (dec.) $[\alpha]_D^{25} = -5.9°$ (5 min) → −27.8° (48 h; *c* = 1.0, DMSO). IR (KBr): 3410*m*, 3270*m*, 2975*w*, 2870*w*, 2105*s*, 1682*s*, 1650*s*, 1630*m*, 1540*m*, 1372*s*, 1160*s*, 1098*s*, 1070*s*, 1020*s*. ^{1}H-NMR (400 MHz, (D_6)acetone): **20**: 7.50–7.34 (*m*, 5 arom. H); 7.31 (*d*, *J* = 10.5, NH); 6.16 (*d*, *J* = 1.6, 1 olef. H); 5.69 (br. *s*, 1 olef. H); 5.61 (*s*, ArC*H*); 5.60 (*s*, OH); 4.54 (*dd*, *J* = 10.5, 4.1, H–C(5)); 4.16 (*dd*, *J* = 10.5, 4.1, H–C(6)); 4.13–4.00 (*m*, H–C(7), H–C(8), H–C(9)); 3.74 (*dd*, *J* = 10.5, 9.0, H–C(9)); 2.73 (*d*, *J* = 14.3, H–C(3)); 2.68 (*d*, *J* = 14.3, H–C(3)); 1.97 (*s*, CH_3); 1.48 (*s*, *t*-Bu). ^{1}H-NMR (200 MHz, (D_6)DMSO; **20**/**22** = 6:4 (24 h)): **20**: 8.00 (*d*, *J* = 10.2, NH); 7.55–7.37 (*m*, 5 arom. H); 6.48 (*s*, OH); 6.09 (*d*, *J* = 1.9, 1 olef. H); 5.71 (br. *s*, 1 olef. H); 5.66 (*s*, ArC*H*); 4.44–3.56 (*m*, 8 H); 1.94 (*s*, CH_3); 1.46 (*s*, *t*-Bu); **22**: 8.51 (*d*, *J* = 8.0, NH); 6.14 (*d*, *J* = 1.6, 1 olef. H); 5.47 (br. *s*, 1 olef. H); 4.65 (*dd*, *J* = 9.0, 8.0, H–C(5)); 1.97 (*s*, CH_3); 1.40 (*s*, *t*-Bu); all other signals were overlapped by the signals of **20**. ^{13}C-NMR (50 MHz, (D_6)DMSO): **20**: 169.55 (*s*); 164.98 (*s*); 138.00 (*s*); 135.85 (*s*); 128.65 (*d*); 127.95 (*d*); 127.28 (*t*); 126.19 (*d*); 100.04 (*d*); 99.17 (*s*); 80.51 (*s*); 79.60 (*d*); 70.80 (*t*); 60.85 (*d*); 58.43 (*d*); 55.66 (*d*); 43.33 (*t*); 27.54 (*q*); 22.36 (*q*); **22**: 204.03 (*s*); 169.84 (*s*); 166.30 (*s*); 137.55 (*s*); 135.85 (*s*); 129.03 (*d*); 128.20 (*d*); 128.07 (*t*); 126.19 (*d*); 101.30 (*d*); 80.00 (*s*); 76.39 (*d*); 68.42 (*t*); 64.31 (*d*); 59.18 (*d*); 52.24 (*d*); 37.42 (*t*); 27.72 (*q*); 22.59 (*q*). CI-MS: 447 (38, $[M + 1]^+ - N_2$), 107 (100, $PhCH{=}OH^+$). Anal. calc. for $C_{23}H_{30}N_4O_7$ (474.54): C 58.22, H 6.37, N 11.81; found: C 58.48, H 6.38, N 11.60.

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[8]) The NO_2 group was not fully hydrolyzed after 4 days. Longer reaction times gave increased amounts of the by-product **21**.

Data of **21**. An anal. sample was obtained by FC (CH_2Cl_2/MeOH 98:2) and subsequent crystallization from CH_2Cl_2/Et_2O. The compound decomposed at *ca.* 220° without melting until 300°. $[\alpha]_D^{25} = +44.0°$ (*c* = 1.0, $CHCl_3$). IR: 3450*m*, 3005*w*, 2870*w*, 2120*s*, 1786*s*, 1690*s*, 1500*m*, 1372*m*, 1280*m*, 1260*m*, 1110*s*, 1098*s*, 1060*m*, 995*s*, 900*m*. ^{1}H-NMR (400 MHz): 7.52–7.33 (*m*, 5 arom. H); 6.34 (*dd*, *J* = 3.4, 2.0, 1 olef. H); 5.72 (*d*, *J* = 10.2, NH); 5.72 (*dd*, *J* = 3.0, 2.0, 1 olef. H); 5.62 (*s*, ArC*H*); 4.76 (*dd*, *J* = 10.2, 4.2, H–C(5)); 4.31 (*dd*, *J* = 10.5, 4.5, H–C(6)); 4.28 (*dd*, *J* = 10.5, 4.8, H–C(9)); 4.15 (*ddd*, *J* = 10.0, 10.0, 4.8, H–C(8)); 3.75 (*dd*, *J* = 10.5, 10.0, H–C(9)); 3.72 (*dd*, *J* = 10.5, 10.0, H–C(7)); 3.00 (*ddd*, *J* = 17.0, 3.4, 3.0, H–C(3)); 2.79 (*ddd*, *J* = 17.0, 2.0, 2.0, H–C(3)); 2.15 (*s*, CH_3); attribution by selective decoupling experiments. ^{13}C-NMR (50 MHz, (D_6)acetone): 206.22 (*s*); 138.12 (*s*); 133.56 (*s*); 129.57 (*d*); 128.70 (*d*); 126.84 (*d*); 123.88 (*t*); 105.58 (*s*); 102.44 (*d*); 77.18 (*d*); 68.64 (*t*); 67.20 (*d*); 59.55 (*d*); 53.32 (*d*); 37.74 (*t*); 22.68 (*q*). CI-MS: 401 (100, $[M + 1]^+$), 358 (61, $[M + 1]^+ - 43$). Anal. calc. for $C_{19}H_{20}N_4O_6$ (400.41): C 56.99, H 5.03, N 14.00; found: C 57.21, H 5.21, N 13.79.

tert-*Butyl* *5-Acetamido-6-azido-7,9-*O*-benzylidene-2,3,5,6-tetradeoxy-2-methylidene-*D-glycero-D-talo-*nononate* (**23**) *and* tert-*Butyl* *5-Acetamido-6-azido-7,9-*O*-benzylidene-*D-glycero-D-galacto-*nononate* (**24**). To an ice-cold soln. of **20**/**22** (500 mg, 1.045 mmol) and AcOH[9]) (150 µl) in THF/H_2O 4:1 (35 ml) was added $NaBH_4$ in small portions (*ca.* 10 mg each batch) until TLC (AcOEt/hexane 4:1) indicated the disappearance of **20**/**22** (2 h). The mixture was diluted with AcOEt (100 ml) and extracted with H_2O, aq. $NaHCO_3$ soln., and brine. HPLC (*Zorbax-Sil*, AcOEt/hexane 6:4) indicated a 84:16 mixture **23**/**24**. FC of the residue (30 g of SiO_2 impregnated with 2% $NaHCO_3$, elution with AcOEt/hexane 4:1) gave **23** (380 mg, 76%), and **24** (66 mg, 13%; elution with AcOEt) as colourless and relatively unstable oils. THF soln. of **23** and **24** were stable for several days when stored in the refrigerator.

Data of **23**. $[\alpha]_D^{25} = -8.4°$ (*c* = 1.1, $CHCl_3$). IR: 3420*m* (br.), 2975*m*, 2930*w*, 2860*w*, 2110*s*, 1678*s*, 1628*w*, 1507*m*, 1368*s*, 1148*s*, 1085*s*, 1072*s*, 1026*m*. ^{1}H-NMR (400 MHz): 7.53–7.36 (*m*, 5 arom. H); 6.51 (*d*, *J* = 9.4, NH); 6.17 (*d*, *J* = 1.3, 1 olef. H); 5.67 (br. *s*, 1 olef. H); 5.53 (*s*, ArC*H*); 4.51 (*ddd*, *J* = 9.4, 9.0, 4.0, H–C(5)); 4.34 (*dd*, *J* = 10.5, 5.2, H–C(9)); 4.26 (*dd*, *J* = 8.9, 3.5, H–C(7)); 4.11–4.03 (*m*, H–C(6), H–C(8)); 3.95 (br. *s*, 1 OH); 3.74–3.68 (*m*, H–C(4); addn. of D_2O: *ddd*, *J* = 9.0, 8.9, 2.2); 3.66 (*dd*, *J* = 10.5, 10.0, H–C(9)); 3.34 (br. *s*, 1 OH); 2.66 (*dd*, *J* = 14.2, 2.2, H–C(3)); 2.34 (*dd*, *J* = 14.2, 8.6, H–C(3)); 1.86 (*s*, CH_3); 1.47 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz): 170.95 (*s*); 167.28 (*s*); 138.03 (*s*); 137.11 (*s*); 129.33 (*d*); 128.37 (*d*); 127.77 (*t*); 125.93 (*d*); 101.12 (*d*); 81.69 (*d*); 81.32 (*s*); 71.17 (*t*); 61.59 (*d*); 58.46 (*d*); 55.47 (*d*); 37.60 (*t*); 27.90 (*q*); 23.25 (*q*). CI-MS: 448 ($[M + 1]^+ - N_2$).

Data of **24**. IR: 3425*m* (br.), 2990*w*, 2930*w*, 2855*w*, 2105*s*, 1678*s*, 1628 (sh), 1500*m*, 1369*s*, 1149 (sh), 1089*s*, 1071*s*, 1028*m*. ^{1}H-NMR (400 MHz): 7.56–7.30 (*m*, 5 arom. H); 6.38 (*d*, *J* = 9.2, NH); 6.15 (*d*, *J* = 1.3, 1 olef. H); 5.63 (*d*, *J* = 0.6, 1 olef. H); 5.47 (*s*, ArC*H*); 4.43 (*ddd*, *J* = 9.2, 7.2, 1.1, H–C(5)); 4.32 (*dd*, *J* = 10.5, 5.2, H–C(9)); 4.16–4.10 (*m*, H–C(4)); 4.02 (*ddd*, *J* = 10.0, 9.0, 5.2, H–C(8)); 3.96–3.86 (*m*, OH); 3.82 (*dd*, *J* = 9.0, 3.0, H–C(7)); 3.78 (*dd*, *J* = 7.2, 3.0, H–C(6)); 3.61 (*dd*, *J* = 10.5, 10.0, H–C(9)); 3.48–3.29 (*m*, OH); 2.49–2.41 (*m*, 2 H–C(3)); 2.03 (*s*, CH_3); 1.49 (*s*, *t*-Bu).

tert-*Butyl* *5-Acetamido-2-*N*-6-anhydro-7,9-*O*-benzylidene-2,3,5-trideoxy-2-imino-*D-glycero-D-talo-*nononate* (**30**) *and* tert-*Butyl* *5-Acetamido-2-amino-2-*N*-6-anhydro-7,9-*O*-benzylidene-2,3,5-trideoxy-*D-glycero-D-talo-*non-2-enonate* (**31**). To a soln. of **23** (380 mg, 0.797 mmol) in CH_2Cl_2 (50 ml) was added $NaHCO_3$ (38 mg). The mixture was cooled to −78° and ozonized until the colour turned blue. The soln. was purged with O_2 (2 min) and N_2 (10 min), and mixed with Ph_3P (167 mg, 0.8 eq.) dissolved in CH_2Cl_2 (1 ml). After warming to r.t. (30 min), the solvent was removed and a stirred soln. of the residue in MeOH (12 ml) treated with $HCOONH_4$ (243 mg) and Pd/C (10%, 110 mg); 10 min later, additional Pd/C (150 mg) was added, and stirring was continued for 30 min (r.t.), when TLC (CH_2Cl_2/MeOH 9:1) showed a main spot. The mixture was filtered through *Celite* and washed with AcOEt (50 ml). The filtrate was extracted with sat. Na_2CO_3 soln. and brine. FC (25 g SiO_2, CH_2Cl_2/MeOH 95:5) gave a mixture **30**/**31** (324 mg, 94%) as a slightly yellow foam. IR: 3420*m* (br.), 2985*m*, 2930*w*, 2860*w*, 1710*s*, 1665*s*, 1510*m*, 1394*m*, 1370*s*, 1285*s*, 1156*s*, 1072*s*, 1028*s*. ^{1}H-NMR (400 MHz, D_3COD): 7.49–7.27 (*m*, 5 arom. H); 5.50 (*d*, *J* = 5.0, 0.45 H–C(3) of **31**); 5.48 (*s*, 0.45 ArC*H* of **31**); 5.45 (*s*, 0.55 ArC*H* of **30**); 4.40–4.20 (*m*, 3.45 H); 4.09–4.05 (*m*, 0.55 H); 3.84–3.57 (*m*, 3.0 H); 2.78 (*ddd*, *J* = 19.0, 2.0, 2.0, 0.55 H–C(3) of **30**); 2.57 (*dddd*, *J* = 19.0, 3.5, 3.0, 1.0, H–C(3) of **30**); 2.03 (*s*, 0.55 CH_3 of **30**); 2.02 (*s*, 0.45 CH_3 of **31**); 1.51 (*s*, 0.55 *t*-Bu of **30**); 1.49 (*s*, 0.45 *t*-Bu of **31**). ^{13}C-NMR (50 MHz, D_3COD): 173.25 (*s*); 173.10 (*s*); 164.65 (*s*); 164.57 (*s*); 161.80 (*s*, C(2) of **30**); 139.28 (*s*); 138.13 (*s*); 136.64 (*s*, C(2) of **31**); 129.57 (*d*); 129.22 (*d*); 128.87 (*d*); 128.84 (*d*); 127.26 (*d*); 127.19 (*d*); 103.96 (*d*, C(3) of **31**); 102.23 (*d*); 101.94 (*d*); 83.41 (*s*); 82.74 (*s*); 82.24 (*d*); 81.11 (*d*); 72.31 (*t*); 65.33 (*d*); 64.07 (*d*); 62.18 (*d*); 61.32 (*d*); 58.36 (*d*); 50.99 (*d*); 49.64 (*d*); 47.57 (*d*); 36.34 (*t*, C(3) of **30**); 28.19 (*q*); 28.10 (*q*); 22.83 (*q*); 22.79 (*q*). CI-MS: 419, 417, 415, 358. Anal. calc. for $C_{22}H_{30}N_2O_7$ (434.50): C 60.82, H 6.96, N 6.45; found: C 60.59, H 7.20, N 6.28.

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[9]) Without AcOH, a strong UV-active by-product was formed.

tert-*Butyl 5-Acetamido-2-amino-2-*N*-6-anhydro-7,9-*O*-benzylidene-2,3,5-trideoxy-*D-erythro-L-allo-*nononate* (**25**) *and* tert-*Butyl 5-Acetamido-2-amino-2-*N*-6-anhydro-7,9-*O*-benzylidene-2,3,5-trideoxy-*D-erythro-L-altro-*nononate* (**26**). To a soln. of **30**/**31** (305 mg, 0.703 mmol) in AcOEt (15 ml) and benzene (3 ml) was added Pd/C (10%, 270 mg). The mixture was hydrogenated at r.t. for 17 h. Additional Pd/C (10%, 70 mg) was added and hydrogenation was continued for 5 h, when TLC (CH_2Cl_2/MeOH 95:5) indicated the disappearance of **30**/**31**. The mixture was filtered through *Celite*, washed with MeOH, and concentrated. FC (30 g of SiO_2, CH_2Cl_2/MeOH 95:5) gave **26** (59 mg, 19%), which crystallized from Et_2O, and **25** (229 mg, 75%), which solidified upon co-evaporation with benzene.

Data of **25**. $[\alpha]_D^{25} = -75.5°$ (c = 1.1, MeOH). IR: 3435*m*, 3320*m*, 2985*m*, 2935*m*, 2875*m*, 1729*s*, 1670*s*, 1505*m*, 1455*w*, 1371*s*, 1310*m*, 1292*m*, 1148*s*, 1090*s*, 1030*s*, 980*m*. ^{1}H-NMR (400 MHz, CD_3OD): 7.54–7.28 (*m*, 5 arom. H); 5.42 (*s*, ArC*H*); 4.23 (*dd*, J = 10.5, 5.3, H–C(9)); 4.08–4.05 (*m*, H–C(4)); 4.00 (*ddd*, J = 10.0, 9.5, 5.3, H–C(8)); 3.96 (*dd*, J = 10.8, 2.8, H–C(5)); 3.69 (*dd*, J = 12.0, 3.0, H–C(2)); 3.56 (*dd*, J = 9.5, 1.2, H–C(7)); 3.54 (*dd*, J = 10.5, 10.0, H–C(9)); 3.31 (*dd*, J = 10.8, 1.2, H–C(6)); 2.05 (*ddd*, J = 13.0, 3.5, 3.0, H_{eq}–C(3)); 2.01 (*s*, CH_3); 1.54 (*ddd*, J = 13.0, 12.0, 1.5, H_{ax}–C(3)); 1.45 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, CD_3OD): 174.70 (*s*); 173.10 (*s*); 139.49 (*s*); 129.71 (*d*); 128.98 (*d*); 127.40 (*d*); 102.32 (*d*); 82.45 (*s*); 80.97 (*d*); 72.47 (*t*); 67.75 (*d*); 61.07 (*d*); 53.84 (*d*); 51.63 (*d*); 50.54 (*d*); 38.85 (*t*); 28.25 (*q*); 22.81 (*q*). CI-MS: 437 (100, $[M + 1]^+$). Anal. calc. for $C_{22}H_{32}N_2O_7$ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.65, H 7.53, N 6.23.

Data of **26**. M.p. 210–211° (dec.). $[\alpha]_D^{25} = -92.0°$ (c = 1.1, MeOH). IR. 3420*m*, 3350 (sh), 2985*m*, 2935*w*, 2870*w*, 1725*s*, 1666*s*, 1510*m*, 1392*m*, 1371*s*, 1151*s*, 1080*s*, 1030*m*. ^{1}H-NMR (400 MHz, CD_3OD): 7.53–7.28 (*m*, 5 arom. H); 5.43 (*s*, ArC*H*); 4.25 (*dd*, J = 10.5, 5.3, H–C(9)); 4.10 (*dd*, J = 9.6, 2.8, H–C(5)); 4.01–3.98 (*m*, H–C(4)); 3.96 (*ddd*, J = 10.0, 9.5, 5.3, H–C(8)); 3.63 (*dd*, J = 9.6, 1.9, H–C(6)); 3.59 (*dd*, J = 9.5, 1.9, H–C(7)); 3.55 (*dd*, J = 10.5, 10.0, H–C(9)); 3.50 (*dd*, J = 6.0, 3.0, H–C(2)); 2.28 (*ddd*, J = 14.0, 4.6, 3.0, H_{eq}–C(3)); 1.99 (*s*, CH_3); 1.89 (*ddd*, J = 14.0, 6.0, 2.4, H_{ax}–C(3)); 1.48 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, CD_3OD): 174.86 (*s*); 172.91 (*s*); 139.47 (*s*); 129.56 (*d*); 128.88 (*d*); 127.38 (*d*); 102.24 (*d*); 82.02 (*s*); 81.43 (*d*); 72.54 (*t*); 67.83 (*d*); 61.53 (*d*); 53.32 (*d*); 50.68 (*d*); 50.57 (*d*); 35.60 (*t*); 28.27 (*q*); 22.84 (*q*). CI-MS: 437 (100, $[M + 1]^+$). Anal. calc. for $C_{22}H_{32}N_2O_7$ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.42, H 7.59, N 6.37.

tert-*Butyl 5-Acetamido-2-amino-2-*N*-6-anhydro-7,9-*O*-benzylidene-2,3,5-trideoxy-*D-erythro-L-gluco-*nononate* (**27**). Similarly to **23**, a soln. of **24** (130 mg, 0.272 mmol) in CH_2Cl_2 (18 ml) was ozonized, treated with PPh_3 (58 mg), and concentrated. A soln. of the residue in MeOH (15 ml) was hydrogenated in the presence of Pd/C (10%, 1.1 g)[10]. After 5–10 min, TLC (CH_2Cl_2/MeOH 9:1) showed a main spot, indicating the disappearance of the ozonolysis products. The mixture was filtered through *Celite*, washed with MeOH (20 ml), and evaporated. FC (20 g of SiO_2, CH_2Cl_2/MeOH 95:5) gave **27** (68 mg, 58%), which solidified on solvent evaporation. $[\alpha]_D^{25} = -43.5°$ (c = 1.0, MeOH). IR: 3430*m*, 3320*m*, 2980*m*, 2930*m*, 2870*m*, 1730*s*, 1655*s*, 1515*w*, 1455*m*, 1370*s*, 1150*s*, 1070*s*, 1030*m*. ^{1}H-NMR (400 MHz, D_3COD): 7.55–7.31 (*m*, 5 arom. H); 5.43 (*s*, ArC*H*); 4.23 (*dd*, J = 10.5, 5.0, H–C(9)); 3.97 (*ddd*, J = 10.5, 9.5, 5.0, H–C(8)); 3.75 (*dd*, J = 10.3, 10.0, H–C(5)); 3.64–3.56 (*m*, H–C(4)); 3.58 (*dd*, J = 9.5, 1.0, H–C(7)); 3.53 (*dd*, J = 10.5, 10.5, H–C(9)); 3.30 (*dd*, J = 11.8, 2.8, H–C(2)); 2.89 (*dd*, J = 10.3, 1.0, H–C(6)); 2.24 (*ddd*, J = 12.5, 4.5, 2.8, H_{eq}–C(3)); 2.02 (*s*, CH_3); 1.46 (*s*, *t*-Bu); 1.27 (*ddd*, J = 12.5, 11.8, 10.0, H_{ax}–C(3)); attribution by selective decoupling. ^{13}C-NMR (50 MHz, $CDCl_3$): 172.73 (*s*); 171.55 (*s*); 137.44 (*s*); 128.85 (*d*); 128.08 (*d*); 126.10 (*d*); 101.07 (*d*); 81.68 (*s*); 79.45 (*d*); 72.40 (*d*); 71.15 (*t*); 60.69 (*d*); 56.58 (*d*); 55.39 (*d*); 54.08 (*d*); 38.42 (*t*); 27.87 (*q*); 23.17 (*q*). CI-MS: 437 (100, $[M + 1]^+$). Anal. calc. for $C_{22}H_{32}N_2O_7$ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.58, H 7.59, N 6.27.

*5-Acetamido-2-amino-2-*N*-6-anhydro-2,3,5-trideoxy-*D-erythro-L-allo-*nononic Acid* (**6**). A soln. of **25** (100 mg, 0.230 mmol) in CF_3COOH (3 ml) was stirred at r.t., until TLC ($CHCl_3$/MeOH 9:1 and 3:1) indicated the disappearance of **25** (3 h). The solvent was removed and the residue partitioned between H_2O (10 ml) and CH_2Cl_2. The aq. layer was freeze-dried to give crude **6** (95 mg), which was dissolved in H_2O (1 ml), basified with 0.5N NaOH (pH 9), and chromatographed on *Dowex 1* × *8* ($HCOO^-$, 10 g, elution with 0–0.3N HCOOH, linear). Fractions containing **6** were combined and freeze-dried to give pure **6** (30 mg, 44%, 2 d at 10^{-5} mbar). The later eluted fractions (70 5-ml fractions) were combined, freeze-dried, and re-chromatographed on *Dowex 1* × *8* ($HCOO^-$) giving further **6** (20 mg, 28%). $[\alpha]_D^{25} = -74.9°$ (c = 1.0, H_2O). IR (KBr): 3700–2300*s*, 1630*m*, 1550*m*. ^{1}H-NMR (400 MHz, D_2O): 4.26 (*dd*, J = 11.0, 2.5, H–C(5));: 4.25–4.20 (*m*, H–C(4)); 4.03 (*dd*, J = 13.0, 3.0, H–C(2)); 3.94 (*ddd*, J = 6.0, 5.2, 4.5, H–C(8)); 3.83 (*d*, J = 6.0, H–C(7)); 3.79 (*dd*, J = 11.5, 4.5, H–C(9)); 3.75 (*d*, J = 11.0, H–C(6));

[10]) We suspect that a part of the catalyst was poisoned by the formed amine. Reduction of the azido function with $HCOONH_4$, Pd/C as described for **23** gave multi-component mixtures, which yielded **27** on succeeding hydrogenation (H_2, Pd/C).

3.67 (*dd*, *J* = 11.5, 5.2, H–C(9)); 2.39 (*ddd*, *J* = 15.0, 3.5, 3.0, H_{eq}–C(3)); 2.08 (*ddd*, *J* = 15.0, 13.0, 1.5, H_{ax}–C(3)); 2.07 (*s*, CH_3); attribution by selective decoupling. ^{13}C-NMR (50 MHz, D_2O): 174.39 (*s*); 173.65 (*s*); 72.46 (*d*); 65.91 (*d*); 64.91 (*d*); 62.15 (*t*); 54.00 (*d*); 53.81 (*d*); 48.00 (*d*); 32.19 (*t*); 22.00 (*q*). FAB-MS: 293 ([*M* + 1]$^+$). Anal. calc. for $C_{11}H_{20}N_2O_7 \cdot 1\,H_2O$ (310.32): C 42.58, H 7.15, N 9.03; found: C 42.30, H 7.18, N 9.01.

*5-Acetamido-2-amino-2-*N*-6-anhydro-2,3,4,5-tetradeoxy-*D-glycero-D-talo-*nononic Acid* (**32**). Similarly to **25**, **26** (40 mg, 0.092 mmol) was deprotected with CF_3COOH to give after ion-exchange chromatography (6 g *Dowex 1 × 8* ($HCOO^-$), **32** (11 mg, 43%). $[\alpha]_D^{25} = -38.8°$ (*c* = 1.0, H_2O). ^{1}H-NMR (400 MHz, D_2O): 4.14 (*ddd*, *J* = 11.0, 11.0, 4.2, H–C(5)); 3.93 (*ddd*, *J* = 5.5, 5.0, 4.0, H–C(8)); 3.87 (*dd*, *J* = 5.0, 1.0, H–C(7)); 3.77 (*dd*, *J* = 11.5, 4.0, H–C(9)); 3.75 (*dd*, *J* = 13.0, 3.2, H–C(2)); 3.65 (*dd*, *J* = 11.5, 5.5, H–C(9)); 3.44 (*dd*, *J* = 11.0, 1.0, H–C(6)); 2.34 (*dddd*, *J* = 14.0, 7.0, 3.5, 3.2, H_{eq}–C(3)); 2.18 (*dddd*, *J* = 13.0, 7.0, 4.2, 3.0, H_{eq}–C(4)); 2.03 (*s*, CH_3); 1.85 (*dddd*, *J* = 14.0, 13.0, 12.0, 3.0, H_{ax}–C(3)); 1.72 (*dddd*, *J* = 13.0, 12.0, 11.0, 3.5, H_{ax}–C(4)); attribution by selective decoupling. ^{13}C-NMR (100.6 MHz, D_2O): 177.20; 176.22; 75.35; 68.73; 64.93; 62.50; 62.03; 47.51; 31.87; 27.71; 24.85. FAB-MS (NOBA): 299 ([*M* + Na]$^+$), 277 ([*M* + 1]$^+$).

*5-Acetamido-2-amino-2-*N*-6-anhydro-2,3,5-trideoxy-*D-erythro-L-altro-*nononic Acid* (**5**). Similarly to **27**, **26** (50 mg, 0.115 mmol) was deprotected by treatment with aq. NaOH and then with aq. HCl to give, after (two) ion-exchange chromatographies, **5** (33 mg, 92%, 2 d at 10^{-5} mbar over P_2O_5) as the monohydrate. $[\alpha]_D^{25} = -41.0°$ (*c* = 1.0, H_2O). IR (KBr): 3700–2300*s*, 1630*s*, 1560*m*. ^{1}H-NMR (400 MHz, D_2O): 4.18 (*dd*, *J* = 11.5, 2.0, H–C(5)); 4.12 (*d*, *J* = 11.5, H–C(6)); 4.10–4.07 (*m*, H–C(4)); 3.99–3.93 (*m*, H–C(8), H–C(2)); 3.84 (*d*, *J* = 5.0, H–C(7)); 3.76 (*dd*, *J* = 11.5, 5.0, H–C(9)); 3.72 (*dd*, *J* = 11.5, 6.5, H–C(9)); 2.59 (*ddd*, *J* = 15.0, 4.0, 1.5, H_{eq}–C(3)); 2.17 (*ddd*, *J* = 15.0, 7.0, 2.2, H_{ax}–C(3)); 2.05 (*s*, CH_3). ^{13}C-NMR (50 MHz, D_2O): 174.45 (*s*); 174.05 (*s*); 73.60 (*d*); 65.67 (*d*); 64.82 (*d*); 62.59 (*t*); 52.76 (*d*); 51.21 (*d*); 48.33 (*d*); 30.48 (*t*); 22.24 (*q*). FAB-MS: 293 ([*M* + 1]$^+$). Anal. calc. for $C_{11}H_{20}N_2O_7 \cdot 1\,H_2O$ (310.32): C 42.58, H 7.15, N 9.03; found: C 42.43, H 7.21, N 8.98.

*5-Acetamido-2-amino-2-*N*-6-anhydro-2,3,5-trideoxy-*D-erythro-L-gluco-*nononic Acid* (**4**). A soln. of **27** (75 mg, 0.171 mmol) in MeOH (0.25 ml) and 0.5N NaOH (1.5 ml) was stirred over night at r.t. TLC ($CHCl_3$/MeOH 4:1 and i-PrOH/MeOH/0.3N HCOOH 6:1:3) indicated then the disappearance of **27**. The mixture was chromatographed on *Dowex 1 × 8* ($HCOO^-$, 9 g, elution with 0–0.3N HCOOH) and freeze-dried yielding 64 mg of a product, which was dissolved in 1M HCl (3 ml) and stirred at r.t. After 9 h, TLC indicated the formation of a new compound. The mixture was diluted with H_2O (3 ml) and extracted with CH_2Cl_2. The aq. layer was freeze-dried, the crude **4** was dissolved in 0.5N NaOH and purified by ion-exchange chromatography (12 g *Dowex 1 × 8* ($HCOO^-$), elution with 0–0.5N HCl[11])). Fractions containing the product were combined and freeze-dried to give **4** (52 mg, 98%, 2 d at 10^{-5} mbar) as the monohydrate. $[\alpha]_D^{25} = -20.3°$ (*c* = 0.7, H_2O). IR (KBr): 3700–2400*s*, 1630*s*, 1555*m*. ^{1}H-NMR (400 MHz, D_2O): 4.03 (*dd*, *J* = 11.0, 10.0, H–C(5)); 3.95–3.89 (*m*, H–C(4), H–C(8)); 3.85 (*dd*, *J* = 13.0, 3.0, H–C(2)); 3.85 (*d*, *J* = 6.0, H–C(7)); 3.78 (*dd*, *J* = 11.5, 4.5, H–C(9)); 3.65 (*dd*, *J* = 11.5, 5.2, H–C(9)); 3.49 (*d*, *J* = 11.0, H–C(6)); 2.60 (*ddd*, *J* = 13.0, 4.5, 3.0, H_{eq}–C(3)); 2.09 (*s*, CH_3); 1.86 (*ddd*, *J* = 13.0, 13.0, 11.0, H_{ax}–C(3)); attribution by selective decoupling. ^{13}C-NMR (50 MHz, D_2O): 175.48 (*s*); 172.48 (*s*); 72.89 (*d*); 69.23 (*d*); 66.16 (*d*); 62.36 (*t*); 57.98 (*d*); 57.56 (*d*); 51.65 (*d*); 33.20 (*t*); 22.49 (*q*). FAB-MS: 293 ([*M* + 1]$^+$). Anal. calc. for $C_{11}H_{20}N_2O_7 \cdot 1\,H_2O$ (310.32): C 42.58, H 7.15, N 9.03; found: C 42.32, H 7.40, N 9.25.

Methods for Sialidase Experiments. The incubation mixtures for testing the inhibitory potency of **4**, **5**, and **6** contained in a total volume of 0.1 ml of buffer (0.1M NaOAc, 0.154M NaCl, and 0.5 mM $CaCl_2$ at pH 5.5) 1 mU of *V. cholerae* sialidase (*Behringwerke*, Marburg) or 0.4 mU of fowl plague virus sialidase (provided by Prof. *R. Rott*, Giessen), and 0.2 mM MU-Neu5Ac as substrate. This substance was synthesized according to *Warner* and *O'Brien* [75] with a modification by *Berg et al.* [76]. The Na salts of **4**, **5**, or **6** were added to this mixture at concentrations varying between 0.01 mM and 1.0 mM. Control assays did not contain inhibitors. In the blanks, the enzyme was omitted. The mixtures were incubated for 15 min at 37°, and the enzyme reactions were terminated by the addition of 0.9 ml of 0.133M glycine, 0.042M Na_2CO_3 and 0.06M NaCl buffer at pH 10. The amounts of liberated 4-methylumbelliferone were determined fluorimetrically at 365 nm for excitation and 450 nm for emission [77]. The blank values were subtracted from the enzyme values before calculation of the moles Neu5Ac released. It was ascertained that during this time of incubation the rates of the enzyme reactions were linear. For estimation of the inhibitory constants of **4** and **6** with *V. cholerae* sialidase, various concentrations of MU-Neu5Ac were incubated in the presence of 0, 0.1, 0.25, or 0.5 mM of the inhibitors or Neu2en5Ac as reference. The formation of 4-methylumbelliferone was followed as described above and plotted as reciprocal values against the reciprocal Neu5Ac concentrations. The K_i values were calculated using the formula [78] $K_i = K_m \cdot i/K'_m - K_m$ where K'_m is the effective *Michaelis* constant in the presence of the inhibitor at the concentration *i*.

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[11]) Elution with aq. HCOOH instead of aq. HCl led to diminished yield of **4**, due to partially very slow release of **4** from the resin (*cf.* also **6**).

REFERENCES

[1] A. P. Corfield, J.-C. Michalski, R. Schauer, in 'Sialidases and Sialidoses, Perspectives in Inherited Diseases', Eds. G. Tettamanti, P. Durand, and S. DiDonato, Edi Ermes, Milan, 1981, Vol. 4, pp. 3–70.

[2] A. Rosenberg, C.-L. Schengrund, in 'Biological Roles of Sialic Acid', Plenum Press, New York, 1976, pp. 295–359.

[3] R. Schauer, *Adv. Carbohydr. Chem. Biochem.* **1982,** *40,* 131.

[4] W. Reutter, E. Köttgen, Ch. Bauer, W. Gerok, in 'Sialic Acids, Chemistry, Metabolism and Function', 'Cell Biology Monographs', Ed. R. Schauer, Springer Verlag, Wien–New York, 1982, Vol. 10, pp. 263–305.

[5] Y. Uchida, Y. Tsukada, T. Sugimori, *Biochim. Biophys. Acta* **1974,** *350,* 425.

[6] G. Reuter, R. Schauer, R. Prioli, M.E.A. Pereira, *Glycoconjugate J.* **1987,** *4,* 339.

[7] R. W. Veh, R. Schauer, in 'Enzymes of Lipid Metabolism', Eds. S. Gatt, L. Freysz, and P. Mandel, Plenum Press, New York, 1978, pp. 447–462.

[8] S. J. Mattingly, T. W. Milligan, A. A. Pierpant, D. C. Strans, *J. Clin. Microbiol.* **1980,** *12,* 633.

[9] R. T. C. Huang, R. Rott, K. Wahn, H. D. Klenk, T. Kohama, *Virology* **1980,** *107,* 313.

[10] T. H. Haskell, F. E. Peterson, D. Watson, N. R. Plessas, T. Culbertson, *J. Med. Chem.* **1970,** *13,* 697.

[11] P. Palese, J. L. Schulman, G. Bodo, P. Meindl, *Virology* **1974,** *59,* 490.

[12] R. Schauer, A. K. Shukla, C. Schroeder, E. Mueller, *Pure Appl. Chem.* **1984,** *56,* 907.

[13] R. Schauer, M. Sander-Wewer, G. H. M. Gutschker-Gdaniec, P. Roggentin, E. A. Randow, R. Hobrecht, *Clin. Chim. Acta* **1985,** *146,* 119.

[14] H. H. Sedlacek, in 'Tumor Therapy with Tumor Cells and Neuraminidase', 'Contributions to Oncology', Karger Verlag, Basel, 1987, Vol. 27.

[15] P. M. Coleman, J. N. Varghese, W. G. Laver, *Nature (London)* **1983,** *303,* 41.

[16] M. Flashner, J. Kessler, St. W. Tanenbaum, *Arch. Biochem. Biophys.* **1983,** *221,* 188.

[17] P. Meindl, H. Tuppy, *Z. Physiol. Chem.* **1969,** *350,* 1088.

[18] P. Meindl, G. Bodo, J. Lindner, H. Tuppy, *Z. Naturforsch., B* **1971,** *26,* 792.

[19] P. Palese, R. W. Compans, *J. Gen. Virol.* **1976,** *33,* 159.

[20] R. Schauer, A. P. Corfield, in 'Sialidases and Their Inhibitors', 'Medical Chemistry Advances', Eds. F. G. de las Heras and S. Vega, Pergamon Press, Oxford, 1981, pp. 423–434.

[21] P. Meindl, H. Tuppy, *Monatsh. Chem.* **1969,** *100,* 1295; *ibid.* **1970,** *101,* 639; *ibid.* **1973,** *104,* 402.

[22] V. Kumar, J. Kessler, M. E. Scott, B. H. Patwardhan, St. W. Tanenbaum, M. Flashner, *Carbohydr. Res.* **1981,** *94,* 123.

[23] V. Kumar, St. W. Tanenbaum, M. Flashner, *Carbohydr. Res.* **1982,** *101,* 155.

[24] V. Kumar, St. W. Tanenbaum, M. Flashner, *Carbohydr. Res.* **1982,** *103,* 281.

[25] a) S. Inouye, T. Tsuruoka, Y. Koaze, T. Niida, *Tetrahedron* **1968,** *23,* 2125; b) D. D. Schmidt, W. Frommer, L. Muller, E. Truscheit, *Naturwissenschaften* **1979,** *66,* 584.

[26] a) A. Vasella, R. Voeffray, *Helv. Chim. Acta* **1982,** *65,* 1134; b) H. Saeki, E. Ohki, *Chem. Pharm. Bull.* **1968,** *16,* 2477.

[27] S. Murao, S. Miyata, *Agric. Biol. Chem.* **1980,** *44,* 219; see also [25b].

[28] P. Lalegerie, G. Legler, J. M. Yon, *Biochemie* **1982,** *64,* 877.

[29] T. Niwa, T. Tsuruoka, H. Goi, Y. Kodama, J. Itoh, S. Inouye, Y. Yamada, T. Niida, M. Nobe, Y. Ogawa, *J. Antibiot.* **1984,** *37,* 1579.

[30] L. E. Fellows, E. A. Bell, D. G. Lynn, F. Pilkiewicz, I. Muira, K. Nakanishi, *J. Chem. Soc., Chem. Commun.* **1979,** 977.

[31] M. J. Eis, C. J. Rule, B. A. Wurzburg, B. Ganem, *Tetrahedron Lett.* **1985,** *26,* 5397.

[32] J. R. Winkler, H. L. Segal, *J. Biol. Chem.* **1984,** *259,* 15369.

[33] S. M. Colgate, P. R. Dorling, C. R. Huxtable, *Austr. J. Chem.* **1979,** *32,* 2258.

[34] G. Legler, E. Julich, *Carbohydr. Res.* **1984,** *128,* 61.

[35] U. Fuhrmann, E. Bause, G. Legler, H. Plough, *Nature (London)* **1984,** *307,* 755.

[36] K. S. Manning, D. G. Lynn, J. Shabanowitz, L. E. Fellows, M. Singh, B. D. Schrire, *J. Chem. Soc., Chem. Commun.* **1985,**127.

[37] I. Cenci di Bello, P. Dorling, L. E. Fellows, B. Winchester, *FEBS Lett.* **1984,** *176,* 61.

[38] R. C. Bernotas, B. Ganem, *Tetrahedron Lett.* **1985,** *26,* 4981.

[39] A. Buku, H. Faulstich, T. Wieland, J. Dabrowski, *Proc. Natl. Acad. Sci.* **1980,** *77,* 2370.

[40] C. J. Rule, B. A. Wurzburg, B. Ganem, *Tetrahedron Lett.* **1985,** *26,* 5379.

[41] K. Clinch, A. Vasella, R. Schauer, submitted to *Tetrahedron Lett.*

[42] J. M. Beau, R. Schauer, *Eur. J. Biochem.* **1980,** *106,* 531.
[43] R. Schauer, *Adv. Carbohydr. Chem. Biochem.* **1982,** *40,* 200 and ref. cit. therein.
[44] A. P. Corfield, M. Sander, R. W. Veh, M. Wember, R. Schauer, *Biol. Chem. Hoppe-Seyler* **1986,** *367,* 433.
[45] F. Baumberger, A. Vasella, R. Schauer, *Helv. Chim. Acta* **1986,** *69,* 1927.
[46] R. Schauer, unpublished results.
[47] F. Baumberger, A. Vasella, *Helv. Chim. Acta* **1986,** *69,* 1205.
[48] a) H. H. Baer, W. Rank, *Can. J. Chem.* **1969,** *47,* 2811; b) K. Furnichi, S. Yogai, T. Miwa, *J. Chem. Soc., Chem. Commun.* **1980,** 66.
[49] F. Baumberger, Ph. D. Thesis, University of Zürich, 1987.
[50] I. Dyong, J. Weigand, J. Thiem, *Anal. Chem.* **1986,** 577.
[51] a) O. Mitsunobu, *Synthesis* **1981,** 1; b) E. Slusarska, A. Zwierzak, *Anal. Chem.* **1986,** 402; c) H. Loibner, E. Zbiral, *Helv. Chim. Acta* **1976,** *59,* 2100.
[52] a) 'The Sadtler Handbook of UV Spectra', Ed. W. W. Simons, 1979, p. 229; b) E. Pretsch, T. Clerc, J. Seibl, W. Simon, 'Tabellen zur Strukturaufklärung organischer Verbindungen', Springer Verlag, Berlin, 1981, U40–U45.
[53] B. Aebischer, A. Vasella, H. P. Weber, *Helv. Chim. Acta* **1982,** *65,* 621.
[54] R. Julina, A. Vasella, *Helv. Chim. Acta* **1985,** *68,* 819.
[55] M. Kiso, H. Ishida, A. Kawaide, A. Hasegawa, *Carbohydr. Res.* **1984,** *127,* 129.
[56] S. De Bernardo, J. P. Tengi, G. J. Sasso, M. Weigele, *J. Org. Chem.* **1985,** *50,* 3457.
[57] K. Banert, W. Kirmse, *J. Am. Chem. Soc.* **1982,** *104,* 3766.
[58] G. W. Fleet, J. Fleming, *J. Chem. Soc. (C)* **1969,** 1758.
[59] a) H. H. Baer, B. Radutus, *Carbohydr. Res.* **1985,** *144,* 77; b) K. Clinch, A. Vasella, unpublished results.
[60] a) C. J. Cavender, V. J. Shiner, Jr., *J. Org. Chem.* **1972,** *37,* 3567; b) G. H. Hakimelahi, G. Just, *Synth. Commun.* **1980,** *10,* 429.
[61] a) W. Gajewski, G. Rücker, *Arch. Pharm.* **1983,** *316,* 256; b) G. Schlewer, J. L. Stampf, C. Benezra, *J. Med. Chem.* **1980,** *23,* 1031; c) N. Bensel, K. D. Klinkmüller, H. Marschall, P. Weyerstahl, *Liebigs Ann. Chem.* **1977,** 1572.
[62] a) N. T. Anh, *Topics Curr. Chem.* **1980,** *88,* 145; b) M. Cherest, H. Felkin, N. Prudent, *Tetrahedron Lett.* **1968,** *9,* 2199.
[63] M. Christen, Diplomarbeit, University of Zürich, 1985.
[64] T. Gartiser, C. Selve, J.-J. Delpuech, *Tetrahedron Lett.* **1983,** *24,* 1609.
[65] T. Sheradsky, in 'The chemistry of the azido group', Ed. S. Patai, Interscience, New York, 1971, pp. 333–338.
[66] H. Staudinger, *Helv. Chim. Acta* **1919,** *2,* 635; *ibid.* **1921,** *4,* 861, 887, 897.
[67] M. Vaultier, N. Knouzi, R. Carrie, *Tetrahedron Lett.* **1983,** *24,* 763.
[68] N. Knouzi, M. Vaultier, R. Carrie, *Bull. Soc. Chim. Fr.* **1985,** 815.
[69] F. Baumberger, A. Vasella, *Helv. Chim. Acta* **1986,** *69,* 1535.
[70] J. Haverkamp, H. van Halbeek, L. Dorland, J. F. G. Vliegenthart, R. Pfeil, R. Schauer, *Eur. J. Biochem.* **1982,** *122,* 305.
[71] J. Kessler, J. Heck, St. W. Tanenbaum, M. Flashner, *J. Biol. Chem.* **1982,** *257,* 5056.
[72] F. Baumberger, A. Vasella, *Helv. Chim. Acta* **1983,** *66,* 2210.
[73] T. G. Cooper, in 'Biochemische Arbeitsmethoden', Walter de Gruyter, Berlin, 1981, p. 126.
[74] C. A. Townsend, G. M. Salituro, L. T. Nguyen, M. J. DiNovi, *Tetrahedron Lett.* **1986,** *27,* 3819.
[75] T. G. Warner, J. S. O'Brien, *Biochemistry* **1979,** *18,* 2783.
[76] W. Berg, G. Gutschker-Gdaniec, R. Schauer, *Anal. Biochem.* **1985,** *145,* 339.
[77] M. Potier, L. Mameli, M. Belisle, L. Dallaire, S. B. Melançon, *Anal. Biochem.* **1979,** *94,* 287.
[78] M. Dixon, E. C. Webb, in 'Enzymes', 3rd edn., Longman, London, 1979, p. 348.