Advanced Synthesis & Catalysis

Accepted Article

Title: Diastereoselective One-step Synthesis of 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) Analogues as Templates for the Development of Influenza Drugs

Authors: Pedro Laborda, Su-Yan Wang, Ai-Min Lu, Meng He, Xu-Chu Duan, Ying-Juan Qian, Yong-Sam Jung, Li Liu, and Josef Voglmeir

To be cited as: *Adv. Synth. Catal.* 10.1002/adsc.201700678

Link to VoR: http://dx.doi.org/10.1002/adsc.201700678

10.1002/adsc.201700678

COMMUNICATION

DOI: 10.1002/adsc.201((will be filled in by the editorial staff))

Diastereoselective One-step Synthesis of 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) Analogues as Templates for the Development of Influenza Drugs

Pedro Laborda,[a] Su-Yan Wang,[a] Ai-Min Lu,[b] Meng He,[a] Xu-Chu Duan,[a] Ying-Juan Qian,[c] Yong-Sam Jung,[c] Li Liu,[a,d]* and Josef Voglmeir[a,e]*

[a] Glycomics and Glycan Bioengineering Research Center, College of Food Science and Technology, Nanjing Agricultural University, China
[b] College of Sciences, Nanjing Agricultural University, China
[c] Antiviral Unit, Department of Veterinary Medicine, Nanjing Agricultural University, China
[d*] Email: lichen.liu@njau.edu.cn, Tel: + 86-25-84399512, Fax: + 86-25-84399553
[e*] Email: josef.voglmeir@njau.edu.cn, Tel: + 86-150-6227-0463, Fax: + 86-25-84399553

Received: ((will be filled in by the editorial staff))

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201######.

Abstract. Novel sialic acid scaffolds have great significance in the development of influenza neuraminidase inhibitors. Here the enzymatic synthesis of a wide range of 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) analogues *via* aldol addition of pyruvate to D-mannose, D-glucose, D-galactose, 2-deoxy-D-glucose, D-arabinose, L-arabinose and L-rhamnose using a previously unstudied *N*-acetylneuraminic acid (Neu5Ac) aldolase derived from the bacterium *Dyadobacter fermentas* was exemplified. Several of the synthesized KDN analogues showed comparable or better inhibitory activity than unstudied Neu5Ac against the mutated influenza neuraminidases (A/California/04/2009 and A/Anhui/1/2005), which both show resistance to Neu5Ac-based neuraminidase inhibitors, demonstrating that these compounds are promising templates for the development of anti-influenza drugs.

Keywords: Aldol reaction; Diastereoselectivity; KDN; Antiviral agents, Sialic acids.

In addition to the five major flu pandemics of the last century, seasonal influenza outbreaks cause annual epidemics that lead in many cases to hospitalization and death, especially in children and the elderly.[1] Two major glycoproteins found on the influenza virus envelope are hemagglutinin, responsible for viral attachment to terminal *N*-acetylneuraminic acids (Neu5Ac, **1**) of cell surface receptors,[2] and a neuraminidase able to cleave off newly formed Neu5Ac-bound virions from the host cell to further enhance the viral mobility.[3] The treatment of influenza consist in the administration of neuraminidase inhibitors such as zanamivir (Relenza®), oseltamivir (Tamiflu®), laninamivir (Inavir®) or peramivir (Rapivab®, Rapiacta® or Peramiflu®).[4] Since the discovery that the Neu5Ac 2,3-didehydro derivative DANA (2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid) can inhibit neuraminidases,[5] Neu5Ac is used as the standard template for the development of effective small molecule neuraminidase inhibitors including the above-mentioned commercially available drugs (Figure 1). However, some naturally-occurring mutations of the influenza virus, such as the H274Y mutation in H1N1 and H5N1 virus strains, have shown significant levels of resistance towards these drugs generating the necessity to develop new synthetic inhibitors to compete against these reported resistances.[6] The creation of new sialic acid scaffolds as templates for the synthesis of new neuraminidase inhibitors may be a suitable way to overcome this limitation.

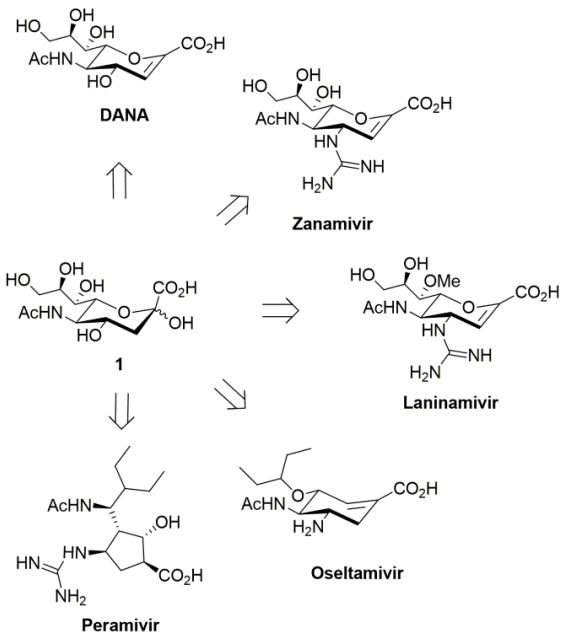
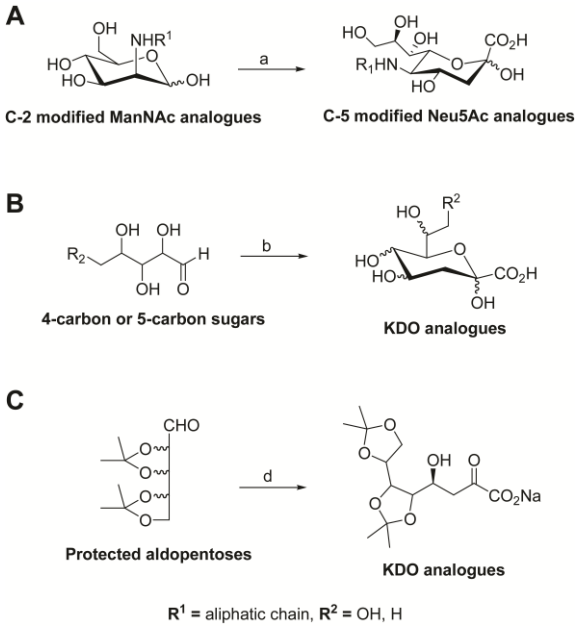


Figure 1. Current spectrum of influenza neuraminidase inhibitors.

10.1002/adsc.201700678

Aldolase enzymes play an important role in the chemoenzymatic synthesis of sialic acid analogues[7] (Scheme 1). Wong *et al.* reported the synthesis of KDO and KDO analogues from D-threose, D-ribose and D-erythrose using a KDO aldolase from *Aureobacterium barkerei*.[8] Furthermore, the activity of Neu5Ac aldolases from *E. coli* and *Peptoclostridium difficile* were previously studied towards a series of monosaccharides.[9] Later, Hilbert, Seeberger and coworkers reported the macrophomate synthase-catalyzed aldol addition of pyruvate enolate into several protected aldopentoses obtaining the corresponding sialic acids as diastereomeric mixtures (Scheme 1 C).[10]

Here, we report the synthesis of a wide range of 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN, **2**) analogues using a previously unstudied, highly promiscuous *N*-acetylneuraminate lyase cloned from the bacterium *Dyadobacter fermentas* and the potential of the synthetized compounds to inhibit mutated influenza neuraminidases in comparison.



Scheme 1. Reported chemoenzymatic routes to new sialic acid analogues. **A**: Enzymatic synthesis of C-5 modified Neu5Ac analogues.[7] **B:** Synthetic strategy to KDO analogues.[8] **C:** Synthetic route to KDO analogues.[10] Reaction conditions: [a] bacterial sialic acid aldolase, [b] KDO aldolase from *Aureobacterium barkerei*, [c] macrophomate synthase.

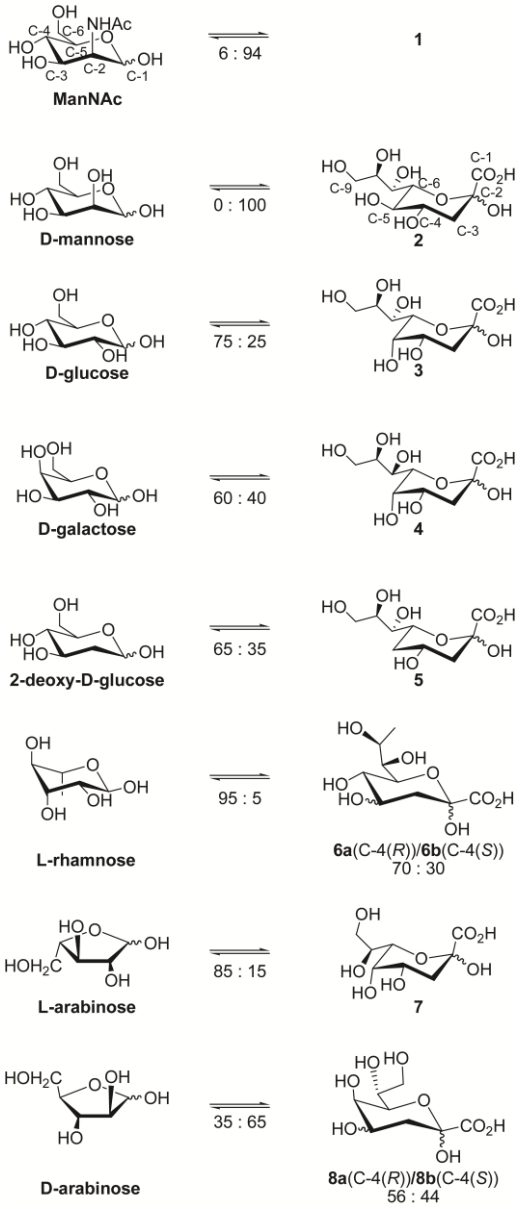
Recombinant *Dyadobacter fermentas N*-acetylneuraminic acid aldolase (DfNAL)[11] could successfully catalyze the retro-aldol reaction of Neu5Ac obtaining ManNAc and pyruvate. The photometric assay used for the detection of the DfNAL activity consisted in the selective *N*-acyl-D-mannosamine dehydrogenase-catalyzed oxidation of ManNAc to produce *N*-acetylmannominic acid in presence of NAD^+ which is reduced to NADH. The readout of formed NADH at 340 nm could be successfully applied to quantitatively determine the conversion of the retro-aldol reaction. Biochemical characterization of DfNAL (Supporting Figure S4) revealed that DfNAL showed the highest activity at pH 8.0 and 65 °C. No metal ions were essential for the enzymatic activity, but the presence of Cu^{II} could slightly decrease the enzyme's activity. Sodium dodecyl sulfate inhibited DfNAL activity whereas other additives, such as urea, triton X-100 or 2-mercaptoethanol, had no effect in the enzymatic activity. V_{max}, K_m and k_{cat} values were determined to be 3.1±0.4 µM/s, 10.1±1.8 mM and 36.5 s^{-1}, respectively.

Neu5Ac was successfully synthetized from ManNAc with 94% conversion when 5 equivalents of pyruvate were added in the reaction mixture. Surprisingly, DfNAL was also able to catalyze the aldol addition of pyruvate into mannose to provide sialic acid KDN (**2**) in a quantitative manner (Scheme 2, top), after 12 h reaction time when 5 equivalents of pyruvate were used. The ability to observe the enzymatic reaction product in a quantitative manner by NMR encouraged us to try the enzymatic aldol reaction using other hexoses than mannose (D-glucose and D-galactose), deoxyhexoses, (2-deoxy-D-glucose and L-rhamnose), and aldopentoses (L-arabinose and D-arabinose) as substrates obtaining the corresponding KDN derivatives (4*S*,5*S*,6*R*)-3-deoxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-hex-2-ulopyranosonic acid (**3**), (4*S*,5*S*,6*R*)-3-deoxy-6-[(1*S*,2*R*)-1,2,3-trihydroxypropyl]-hex-2-ulopyranosonic acid (**4**), (5*S*,6*R*)-3,5-dideoxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-hex-2-ulopyranosonic acid (**5**), (4*R*,5*S*,6*R*)-3-deoxy-6-[(1*S*,2*S*)-1,2-dihydroxypropyl]-hex-2-ulopyranosonic acid (**6a**), (4*S*,5*S*,6*R*)-3-deoxy-6-[(1*S*,2*S*)- 1,2-dihydroxypropyl]-hex-2-ulopyranosonic acid (**6b**), (4*S*,5*S*,6*S*)-3-deoxy-6-[(1*S*)-1,2-dihyroxyethyl]-hex-2-ulopyranosonic acid (**7**), KDO (**8a**) and *gluco*-KDO (**8b**) in different proportions (Scheme 2). DfNAL showed no activity towards other amino sugars apart from ManNAc, such as glucosamine, *N*-acetylglucosamine or kanosamine, or towards disaccharides, such as lactose or maltose. Despite DfNAL could catalyze the aldol reaction of D-arabinose or L-arabinose, no activity was observed when other aldopentoses like D-ribose or L-ribose were used as substrates.

KDN was discovered in 1986 and several synthetic routes to produce this sialic acid have been reported so far.[12] Three enzymatic routes to 2-keto-3-deoxy-D-manno-octonic acid (KDO, **8a**) using different aldolases have also been described.[8, 10, 13] **8b** has been synthetized starting from protected D-glucose derivatives.[13] This is the first time KDN analogues **3**, **4**, **5**, **6a** and **6b** are reported.

To analyze the diastereoselectivity of the aldol reactions, synthetized sialic acids were labeled with 1,2-diaminobenzene.[14] The HPLC-based analysis of the labeled sialic acids revealed that aldol reactions to sialic acids **2**, **3**, **4**, **5** and **7** occurs with complete diastereoselectivity whereas diastereomeric mixtures were obtained using L-rhamnose, **6a**/**6b** 70:30, and D-arabinose, **8a**/**8b** 56:44, as substrates (Supporting Figure S5). **2**, **3**, **4**, **5**, **7** and major diastereoisomers, **6a** and **8a**, were isolated by silica gel chromatography.

10.1002/adsc.201700678

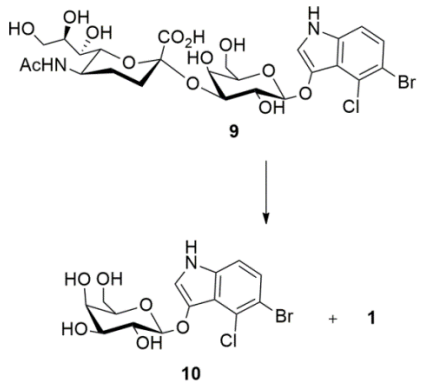


Scheme 2. DfNAL-catalyzed synthesis of Neu5Ac (**1**), KDN (**2**) and KDN analogues (**3**, **4**, **5**, **6a**, **6b**, **7**, **8a** and **8b**). Reaction conditions: commercial sugar (1.11 mmol), sodium pyruvate (5.55 mmol), 50 mM Na_3PO_4/citrate buffer pH 8.0, 37 °C, 12 h (reaction volume: 5 mL). Yields: **1** 89%, **2** 86%, **3** 21%, **4** 29%, **5** 32%, **6a** 2%, **7** 14%, **8a** 23%.

The configuration of the newly generated asymmetric center in C-4 of the purified compounds was elucidated by ^{1}H and ^{13}C NMR (Supporting Figures S6-S10). In the ^{1}H NMR spectrum of **2**, the signal corresponding with the proton linked to C-3 in axial position appears as a triplet at 1.72 ppm with coupling constants of 12.2 Hz due to the coupling between geminal and vicinal axial-axial protons indicating the (*S*) configuration of C-4 (Supporting Figure S11). Compound **3**, **4**, **5** and **7** adopt the same chair conformation in comparison with **2**. ^{1}H NMR spectra of these KDN derivatives showed that the signals corresponding to the proton linked to C-3 in axial position appear as a triplet with similar coupling constants as the ones observed for **2** demonstrating an (*S*) configuration on C-4 of these structures. KDN analogues **6a** and **8a** are in alternate chair conformation with the C-6-linked glycerol side-chain is located in equatorial position.[10, 13] Curiously, a triplet with coupling constants about 12 Hz could be also observed for the proton linked to C-3 in axial position in the ^{1}H NMR spectrum of **6a** indicating an (*R*) configuration on C-4 (Supporting Figure S12). The ^{1}H NMR spectrum of **8a** is in accordance with the previously described spectra of KDO confirming an (*R*) configuration on C-4 (Supporting Figure S13).[13]

The previously reported aldolase KdsA allowed the synthesis of sialic acids with (*R*) configuration on C-4,[12] whereas the macrophomate synthase reported permitted the synthesis of C-4 (*S*) diasteroisomer in a higher ratio.[10] Although the substrate promiscuity of other bacterial Neu5Ac aldolases towards D-glucose, 2-deoxy-D-glucose, D-galactose, 2-deoxy-D-galactose, D-allose, D-lyxose, D-arabinose, 2-deoxy-D-ribose, L-xylose and L-fucose was described with efficiencies previously, the produced sialic acids were not structurally characterized and the diastereoselectivity of the enzymatic reaction was not studied.[9a, 9c] Although the configuration on the generated asymmetric center is commonly conserved regardless of the acceptor substrate in enzymatic aldol reactions,[15] DfNAL was able to change the configuration of this center, presumably depending on the configuration of the substituents on the C-3 of the substrate. Substrates with (*R*) configuration on C-3 led to sialic acids with (*S*) configuration on C-4, whereas sialic acids possessing (*R*) configuration on C-4 were obtained with major proportion when substrates with (*S*) configuration on C-3 were used.

The inhibitory activity of the obtained KDN and KDN derivatives were tested against H274Y mutated H1N1 (A/California/04/2009) and H274Y mutated H5N1 (A/Anhui/1/2005) influenza A virus neuraminidases. A 5-bromo-4-chloro-indolyl-β-D-galacto-pyranosyl-sialoside, X-gal-α-2,3-Neu5Ac (**9**), was used as the influenza neuraminidase substrate, yielding 5-bromo-4-chloro-indolyl-β-d-galactopyranoside, which hydrolysis to X-gal (**10**) can be easily followed by HPLC analysis[16] (Scheme 3). **9** was synthetized according to the method described by Chen and co-workers.[17] All tested sialic acid analogues showed inhibitory activity against H274Y mutated H1N1 and H274Y mutated H5N1 neuraminidases (Figure 2), with **4** having the highest inhibitory activity. Although



Scheme 3. Neuraminidase inhibition assays were carried out using X-gal-α-2,3-Neu5Ac (**9**) as neuraminidase substrate yielding X-gal (**10**) and Neu5Ac (**1**).

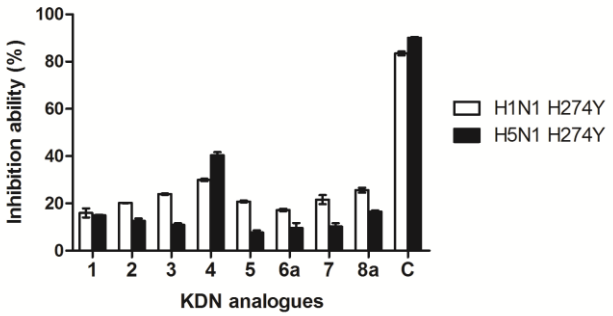


Figure 2. Screening of Neu5Ac (**1**) and synthetized KDN analogues (**2**, **3**, **4**, **5**, **6a**, **7** and **8a**) against H274Y mutant H1N1 neuraminidase (A/California/04/2009, in white) and H274Y mutant H5N1 neuraminidase (A/Anhui/1/2005, black) using oseltamivir as control (C). The neuraminidase inhibitor assays were performed at 1 mM concentrations.

KDN derivative **4** could significantly enhance the inhibitory activities compared with **1**, **4** showed lower inhibitory activities in comparison to the commercially neuraminidase inhibitor oseltamivir (Supporting Figure S14 and Figure S15).

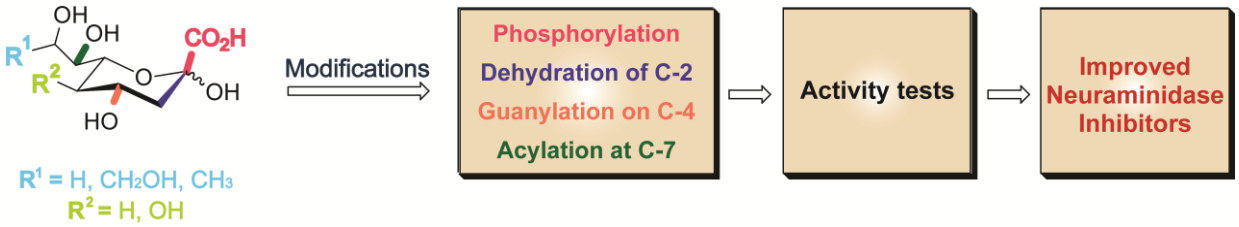
A number of modifications have been successfully carried out in **1** to enhance the inhibitory activity of this compound against influenza neuraminidases: the dehydratation of **1** led to DANA,[17] whereas the replacing the hydroxyl group at C-4 of DANA with a guanidine group led to zanamivir.[5, 18] Further replacing the carboxylic acid with a phosphonate led to Zanaphosphor.[19] Several new drugs with high inhibitory activity against influenza B strains could show modifications at the hydroxyl group at C-7 of zanamivir.[20] These alterations can be also a suitable and interesting way to increase the inhibitory activity of the herein presented KDN analogues in future (Scheme 4).

In summary, a previously uncharacterized bacterial *N*-acetylneuraminate lyase from *Dyadobacter fermentans* was applied in the diastereoselective one-step synthesis of sialic acids. KDN and KDN derivatives from D-glucose, D-galactose, 2-deoxy-D-glucose, L-rhamnose, D-arabinose and L-arabinose were successfully obtained. The recombinant enzyme allowed the synthesis of sialic acids with (*S*) configuration on C-4 using carbohydrates with (*R*) configuration on C-3 as the substrates, and *vice versa*. KDN and most KDN derivatives showed higher inhibitory activities against H274Y mutated H1N1 neuraminidase in comparison with that observed for Neu5Ac demonstrating the potential of these compounds as templates for the development of new drugs.

Experimental Section

Gene cloning and construction of the expression vector
The oligonucleotide primers for amplifying the target genes were designed based on the genomic DNA sequence of *D. fermentas* (GenBank number WP 015812487.1) sense primer: 5'-GGAATTCCATATGAACTTACATTTAGAGGGCCTCA TCG -3' (containing an underlined Nde*I* restriction site), anti-sense primer: 5'-CCGCTCGAGAAATGTATTTTTGGCCAAAACGG -3' (Xho*I* site underlined)). The genes were amplified by polymerase chain reaction (PCR) using Primestar HS DNA polymerase (Takara) and genomic DNA as templates according to the manufacturer's instructions. Briefly, the PCR amplification was performed using 35 PCR cycles consisting of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min. The PCR fragments were purified on an agarose gel and double-digested by the simultaneous use of the corresponding restriction endonucleases and ligated into a predigested pet-30a(+) expression vector containing a coding region for a C-terminal hexahistidine fusion tag. The recombinant vectors were transformed into *E. coli* Mach1 T1 competent cells (Invitrogen) and transformants were selected on Luria-Bertani (LB) agar containing 50 µg/mL kanamycin. Colonies containing the expected plasmid construct were screened by DNA sequencing. The extraction, endonuclease digestion, ligation and plasmid transformation were carried out using standard methods. The transformed strains containing the cloned genes were stored in 20% glycerol solutions at -80 °C until further use.

Expression and purification of recombinant DfNAL
The plasmid containing the expected DfNAL was transformed into the *E. coli* BL21 (DE3) expression host and grown on a LB solid medium (10 $g \cdot L^{-1}$ NaCl, 10 $g \cdot L^{-1}$ Trypton, 5 $g \cdot L^{-1}$ yeast extract and 5 $g \cdot L^{-1}$ agarose) containing 50$\mu g \cdot mL^{-1}$ kanamycin. A single colony was transferred into 5 mL fresh LB medium (10 $g \cdot L^{-1}$ NaCl, 10 $g \cdot L^{-1}$ Trypton and 5 $g \cdot L^{-1}$ yeast extract) supplemented with



Scheme 4. Possible structural modifications to enhance the inhibitory activity of KDN (**2**) and KDN analogues (**3**, **4**, **5**, **6a**, **7** and **8a**) against influenza. a) Dehydratation of C-2;[5, 18] b) introduction of a guanidine group;[5, 18] c) introduction of a phosphonate;[20] d) modifications at C-7.[20]

50 μg·mL^{-1} kanamycin. 5 mL of the culture was used to inoculate 400 mL of LB medium and shaking at 37 °C until optical density (OD_{600}) of the culture reached 0.5. Then, 0.4 mL of a 1 M IPTG (Isopropyl-β-D-thiogalactopyranoside) were added to the medium to induce the recombinant protein expression. After 16 h of induction at 18 °C, cells were harvested by centrifugation at 4,000 g for 10 min at 4 °C. The cell pellets were suspended in 10 mL lysis buffer (100 mM NaCl, 50 mM Tris, 1% Triton X-100, 1 mM PMSF, and pH 8.0) and disrupted by sonification for 20 min. Cellular debris was removed by centrifugation at 20,000 g for 20 min at 4 °C. The supernatant containing the recombinant enzymes were loaded onto a Ni-NTA column (Qiagen, Germany, 2 mL column volume). The column was washed with washing buffer (50 mM NaCl, 50 mM Tris, 10 mM imidazole, adjusted to pH 8.0 with HCl) to remove unbound proteins. Recombinant proteins were eluted using elution buffer (50 mM NaCl, 50 mM Tris, 500 mM imidazole, adjusted to pH 8.0 with HCl). Eluted fractions were partially desalted by gel chromatography using prepacked PD-10 cartridges (GE Healthcare).

The molecular mass and purity of the recombinant proteins were monitored by 12% SDS-PAGE acrylamide gels after Coomassie brilliant G-250 staining. The protein concentration was determined using the quantification method of Bradford with bovine serum albumin (BSA) as quantification standard. The purified enzyme was stored at -80 °C in a 20% glycerol solution for further use.

Synthesis of Neu5Ac, KDN and KDN analogues

Reaction mixtures (5 mL) containing 222 mM of D-mannose, D-glucose, D-galactose, 2-deoxy-D-glucose, L-rhamnose, D-arabinose, L-arabinose or *N*-acetyl-D-mannosamine, 1110 mM pyruvate, sodium phosphate/citrate buffer (50 mM, pH 8.0) and recombinant enzyme (DfNAL, 760 U), were incubated for at 37 °C 12 h reaction time. The corresponding sialic acids (**1**, **2**, **3**, **4**, **5**, **6a**, **7** and **8a**) were isolated by silica gel chromatography using a mixture AcOEt/MeOH/AcOH (2:1:1) as the eluent. Reactions were monitored by thin-layer chromatography (TLC). TLC was performed by spotting 1 μL from reaction mixture using AcOEt/MeOH/AcOH (2:1:1) as the eluent. Compounds were stained using an orcinol based stain (2 mg·mL^{-1} orcinol in 20% H_2SO_4).

Acknowledgements

This work was supported in part by the Natural Science Foundation of China (grant numbers 31471703, A0201300537 and 31671854 to J.V. and L.L.), and the 100 Foreign Talents Plan (grant number JSB2014012 to J.V.).

References

[1] a) T. Szucs, *J. Antimicrob. Chemoth.* **1999**, *44*, 11-15; b) R. E. Thomas, *Vaccine* **2014**, *32*, 6884-6901.

[2] X. L. Xiong, J. W. McCauley, D. A. Steinhauer, in *Influenza Pathogenesis and Control - Vol I, Vol. 385* (Eds.: R. W. Compans, M. B. A. Oldstone), **2014**, pp. 63-91.

[3] a) J. S. Rossman, R. A. Lamb, *Virology* **2011**, *411*, 229-236; b) X. J. Xu, X. Y. Zhu, R. A. Dwek, J. Stevens, I. A. Wilson, *J. Virol.* **2008**, *82*, 10493-10501.

[4] a) S. Chamni, W. De-Eknamkul, *Expert Opin. Ther. Pat.* **2013**, *23*, 409-423; b) A. J. Burnham, T. Baranovich, E. A. Govorkova, *Antivir. Res.* **2013**, *100*, 520-534.

[5] W. P. Burmeister, B. Henrissat, C. Bosso, S. Cusack, R. W. H. Ruigrok, *Structure* **1993**, *1*, 19-26.

[6] a) K. Thorlund, T. Awad, G. Boivin, L. Thabane, *BMC Infect. Dis.* **2011**, *11*; b) S. Ludwig, R. Zell, M. Schwemmle, S. Herold, *Int. J. Med. Microbiol.* **2014**, *304*, 894-901.

[7] a) S.-Y. Wang, P. Laborda, A.-M. Lu, X.-C. Duan, H.-Y. Ma, L. Liu, J. Voglmeir, *Catalysts* **2016**, *6*, 212; b) C. M. Deijl, J. F. Vliegenthart, *Biochem. Bioph. Res. Co.* **1983**, *111*, 668-674; c) H. Yu, X. Chen, *Org. Lett.* **2006**, *8*, 2393-2396.

[8] T. Sugai, G. J. Shen, Y. Ichikawa, C. H. Wong, *J. Am. Chem. Soc.* **1993**, *115*, 413-421.

[9] a) M. J. Kim, W. J. Hennen, H. M. Sweers, C. H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 6481-6486; b) C. C. Hsu, Z. Y. Hong, M. Wada, D. Franke, C. H. Wong, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9122-9126; c) Q. Chen, L. Han, X. Chen, Y. Cui, J. Feng, Q. Wu, D. Zhu, *Enzyme Microb. Tech.* **2016**, *92*, 99-106.

[10] D. G. Gillingham, P. Stallforth, A. Adibekian, P. H. Seeberger, D. Hilvert, *Nat. Chem.* **2010**, *2*, 102-105.

[11] The full-length open reading frame (ORF) encoding a putative *N*-acetylneuraminate lyase (EC 4.1.3.3) could be successfully cloned and was annotated as DfNAL (*Dyadobacter fermentans N*-acetylneuraminate lyase, Supporting information Figure S1). Homology search revealed that DfNAL (Uniprot ID C6VS55) shares 45% amino acid identity to *N*-acetylneuraminate lyases from *Escherichia coli* and *Staphylococcus carnosus*, 36% with *Pasteurella multocida* and 24% with *Staphylococcus aureus* isoforms. DfNAL shares also reasonable amino acid identities with *N*-acetylneuraminate lyases *Homo sapiens* (39%) and *Sus scrofa* (32%).

[12] L. S. Li, Y. L. Wu, *Curr. Org. Chem.* **2003**, *7*, 447-475.

[13] L. Q. Wen, Y. Zheng, T. H. Li, P. G. Wang, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2825-2828.

[14] H. L. Yao, L. P. Conway, M. M. Wang, K. Huang, L. Liu, J. Voglmeir, *Glycoconjugate J.* **2016**, *33*, 219-226.

[15] a) X. Garrabou, L. Gomez, J. Joglar, S. Gil, T. Parella, J. Bujons, P. Clapes, *Chem.-Eur. J.* **2010**, *16*, 10691-10706; b) P. Laborda, F. J. Sayago, C. Cativiela, T. Parella, J. Joglar, P. Clapes, *Org. Lett.* **2014**, *16*, 1422-1425; c) T. Gefflaut, C. Blonski, J. Perie, M. Willson, *Prog. Biophys. Mol. Bio.* **1995**, *63*, 301-340.

[16] Both **9** and **10** were detected by HPLC (absorption wavelength: 300 nm). Reaction conditions: neuraminidase inhibitor (1 mM), **9** (40 μM), $CaCl_2$ (5 mM), neuraminidase (H274Y mutated H1N1 10^{-5}

10.1002/adsc.201700678

U; H274Y mutated H5N1 1.3⁄ 10^{-5} U), 20 mM Tris⁄ HCl buffer pH 7.5, 37 °C, 20 min (reaction volume: 30 μL).

[17] H. Yu, S. S. Huang, H. Chokhawala, M. C. Sun, H. J. Zheng, X. Chen, *Angew. Chem. Int. Edit.* **2006**, *45*, 3938-3944.

[18] M. Von Itzstein, W. Y. Wu, B. Jin, *Carbohydr. Res.* **1994**, *259*, 301-305.

[19] A. Vasella, R. Wyler, *Helv. Chim. Acta* **1991**, *74*, 451-463.

[20] T. Honda, T. Masuda, S. Yoshida, M. Arai, Y. Kobayashi, M. Yamashita, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921-1924.

10.1002/adsc.201700678

COMMUNICATION

Diastereoselective One-step Synthesis of 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) Analogues as Templates for the Development of Influenza Drugs

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

Pedro Laborda, Su-Yan Wang, Ai-Min Lu, Meng He, Xu-Chu Duan, Ying-Juan Qian, Yong-Sam Jung, Li Liu,* Josef Voglmeir*