Catalysis Communications 121 (2019) 73–77

Contents lists available at ScienceDirect

Catalysis Communications

journal homepage: www.elsevier.com/locate/catcom

Short communication

N-Acetylneuraminic acid aldolase-catalyzed synthesis of acyclic nucleoside analogues carrying a 4-hydroxy-2-oxoacid moiety

Mariano J. Nigro[a,1], Martín A. Palazzolo[a,1,2], Diego Colasurdo[b], Adolfo M. Iribarren[a], Elizabeth S. Lewkowicz[a,*]

[a] Biocatalysis & Biotransformations Laboratory, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, 1876 Bernal, Buenos Aires, Argentina
[b] Grupo de determinación de estructuras y mecanismos de reacción GEM, CEDECOR, CIC-BA, Facultad de Ciencias Exactas, Departamento de Química, CONICET, Universidad Nacional de La Plata, 47 y 115, 1900 La Plata, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
N-acetylneuraminic acid aldolase
Acyclic nucleosides
Aldol addition

ABSTRACT

N-Acetylneuraminic acid aldolase (NeuAcA) (EC 4.1.3.3) is a pyruvate-dependent class I aldolase that catalyzes the reversible aldol cleavage of *N*-acetylneuraminic acid to form *N*-acetyl-D-mannosamine and pyruvate. The synthetic activity of this enzyme has been applied to the preparation of many sialic acid analogues. In this report, we demonstrate the ability of NeuAcA from *Clostridium perfringens* to use 2-oxoethyl substituted nucleobases as unusual acceptor substrates to perform aldol additions. In this way, novel acyclic nucleoside analogues bearing a further derivatizable 4-hydroxy-2-oxo butyrate skeleton were prepared in good yields.

1. Introduction

Nucleoside analogues are a valuable source of antiviral agents, acting as enzyme inhibitors or chain terminators in RNA or DNA biosynthesis [1]. In particular, those containing alkyl chains that mimic open sugar moieties, give rise to a wide range of biologically active molecules. Since the discovery of acyclovir in 1978, acyclic nucleosides (ANs) have become the target of intense research. As a consequence, a number of outstanding drugs have been marketed to treat viral diseases including herpes, hepatitis and AIDS [2]. The chemical synthesis of ANs often comprises multi-step reactions and toxic reagents [3–5]; instead, biocatalysis offers an efficient, green, and chemo-, regio- and stereoselective alternative [6]. In this sense aldolases, which are carbon-carbon lyases (EC 4.1), proved to be useful enzymes for the stereoselective C−C bond formation. These enzymes catalyze the reversible and stereospecific aldol addition between a carbonyl compound (nucleophilic donor) and an aldehyde (electrophilic acceptor). All aldolases are strongly dependent on their donor but admit a broad diversity of aldehydes as acceptor substrates. In addition, two classes of aldolases can be distinguished depending on the interactions between the donor and the enzyme [7].

Pyruvate-dependent aldolases are usually Class I aldolases that reversibly catalyze the aldol addition of pyruvate to a variety of polyhydroxylated aldehydes to produce α-keto-acids. Between others, 2-keto-3-deoxy-6-phosphogalactonate (KDPGal) aldolase catalyzes the aldol addition using a broad range of electrophilic substrates containing polar functionalities at C2, C3, or C4 affording substituted-4-hydroxy-2-oxobutyrate products [8].

Class II pyruvate-dependent aldolases containing Mg^{2+} have demonstrated a broader substrate specificity for both acceptors and donors. Among them, *E. coli* K-12 2-keto-3-deoxy-L-rhamnonate aldolase (YfaU) was employed for the synthesis of amino acid derivatives of proline, 4-hydroxypipecolic acid, and pyrrolizidine-3-carboxylic acid by addition of pyruvate to a variety of unsubstituted and α-substituted *N*-Cbz-amino aldehyde derivatives [9].

In particular, *N*-acetylneuraminic acid aldolase (NeuAcA, also known as sialic acid aldolase, EC 4.1.3.3), catalyzes *in vivo* the reversible decondensation of α-*N*-acetylneuraminic acid to yield pyruvate and *N*-acetyl-D-mannosamine in a wide variety of organisms. NeuAcA has been thoroughly used in the carbohydrate field to synthesize a range of sugar mimics displaying different biological activities [7]. Among other applications, the preparation of L-sugars, polyhydroxylated alkaloids and melanoma antigens can be cited [10,11]. The first studied NeuAcAs did not recognize aldehydes with two or

* Corresponding author.
E-mail address: elewko@unq.edu.ar (E.S. Lewkowicz).
[1] The authors contributed equally to the present work.
[2] Present address: Instituto de Investigaciones en Tecnología Química (INTEQUI), Universidad Nacional de San Luis, CONICET, Almirante Brown 1455, 5700 San Luis, Argentina.

https://doi.org/10.1016/j.catcom.2018.12.013
Received 1 October 2018; Received in revised form 17 December 2018; Accepted 19 December 2018
Available online 21 December 2018

three carbons as acceptors and only admitted pyruvate as the donor [12]. However, NeuAcAs with unusual specificities were later discovered from different sources. Hader and Watts [13] prepared, through a chemoenzymatic route, four new difluorinated sialic acid derivatives with potential biological activity using NeuAcA and sodium fluoropyruvate as donor. NeuAcA from *Peptoclostridium difficile* not only accepted a variety of aldoses as acceptors but also aliphatic aldehydes such as acetaldehyde, propanal and butanal [14]. The enzyme activity decreased as the number of carbons of the aldehyde acceptor increased. On the other hand, NeuAcA from *Dyadobacter fermentas* was used to synthesize many 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) analogues from different monosaccharides, which proved to be promising scaffolds for the development of anti-influenza drugs [15]. NeuAcA from *Pasteurella multocida* P-105 has shown flexible substrate specificity, using 5-*O*-methyl ManNAc as substrate to produce the corresponding C8-modified sialic acids [16].

In view of the persistent need to develop new drugs with increased potency, bioavailability and selectivity, we have recently employed rabbit muscle aldolase (RAMA) in a novel procedure to prepare acyclic analogues of arabinonucleosides such as vidarabine, a drug currently used against herpes simplex and varicella zoster viruses [17]. The strategy proposed here makes use of NeuAcA to bring an interesting alternative for the synthesis of new AN analogues due to its relaxed specificity for the acceptor aldehyde, especially those that contain polar groups at C3. Having this in mind, in the present work we explored the preparation of novel nucleoside derivatives bearing a further derivatizable 4-hydroxy-2-oxo butyrate skeleton by means of a commercially available NeuAcA, employing a cheap donor such as pyruvate and a variety of nucleobase-substituted acetaldehydes as acceptor substrates.

2. Experimental

2.1. General

N-acetylneuraminic acid aldolase from *Clostridium perfringens* (NeuAcA, EC 4.1.3.3) was purchased from MPBio (Solon, OH, USA). All chemicals were acquired from Sigma Aldrich (Saint Louis, MO, USA) as reagent grade and used without further purification unless otherwise stated. Solvents were purchased from Biopack and dried, when necessary, following standard procedures before their use.

^{1}H and ^{13}C NMR experiments were recorded on a Bruker Avance II 500 spectrometer (Madison, WI, USA) at 500 MHz and 125 MHz respectively, using D_2O as solvent. TLC analyses were performed using silica gel 60 F254 aluminum plates from Merck (Darmstadt, Germany) and $CHCl_3$:MeOH (85:15) as mobile phase. Phenomenex C18 silica gel cartridges (Torrance, CA, USA) were used for solid phase extraction.

HPLC-UV analyses were carried out with a Gilson chromatograph (321 Pump, 156UV/VIS detector and 234 Autoinjector Series) (Middleton, WI, USA), using a Grace Apollo C18 column (5 μ, 150 mm × 4.6 mm) (Deerfield, IL, USA). HPLC-MS analyses were run in a Thermo Finnigan Surveyor chromatograph coupled to a LCQ Advantage Max detector (San Jose, CA,USA), using a Merck LiChroCART 55–2 Purospher START RP-18 e (3 μ, 55 mm × 2 mm) column and an isocratic mixture of ACN: NH_4OH 50 mM pH 10.8 (3:97) with a flow rate of 0.25 mL/min at room temperature. The MS detection was set to explore a range of 150–400 *m/z* in positive mode. ESI-MS spectra were recorded by direct injection (4 kV ion spray voltage, 350 °C capillary temperature), in positive mode.

2.2. Aldehydes

Compounds **2a-d** were prepared from nucleobases **1a-d** according to our previous report [18]. Briefly, the nucleobases (3 mmol) and K_2CO_3 (6 mmol) in anh DMF (30 mL) were stirred at 50 °C during 1 h and then 2-bromo-1,1-dimethoxyethane (6 mmol) was added at 90 °C. After 23 h, the reactions were filtered and the solvent removed under reduced pressure. The crude mixtures were purified by silica gel column chromatography with $MeOH/CH_2Cl_2$ or EtOAc/hexane as mobile phases to yield the corresponding 9-(2,2-dimethoxyethyl)-purine or 1-(2,2-dimethoxyethyl)-pyrimidines in 40–60% isolated yields. Aldehydes **2a-d** were obtained after hydrolysis of the corresponding dimethylacetals with HCl (1 N) at 90 °C and their identities were determined by NMR and ESI-MS analysis [17].

2.3. Analytical-scale standard biotransformation

Mixtures containing aldehydes **2a-d** (40 mM) and sodium pyruvate (20 mM) were incubated with NeuAcA (10 U) in potassium phosphate buffer (30 mM pH 7.5, 2 mL) and shaken at 37 °C and 200 rpm. Samples were withdrawn during a 48-h period and analyzed by HPLC-UV using as mobile phase isocratic triethylammonium acetate (TEAA): ACN (97:3) with a flow rate of 0.9 mL/min at room temperature. The UV detector was set at 254 nm (t_R (min): **2a** 5.7, **3a** 9.3, **2b** 2.0, **3b** 2.9, **2c** 4.6, **3c** 7.7, **2d** 4.3, **3d** 7.0).The identity of products **3a-d** was confirmed by HPLC-MS. Reactions without the addition of enzymes were performed as chemical controls following the same procedure.

2.4. Synthesis of 4-hydroxy-2-oxo-5-thyminylpentanoic acid (3c)

A solution of compound **2c** (40 mM), sodium pyruvate (80 mM) and NeuAcA (10 U) in potassium phosphate buffer (20 mM pH 7.5, 1 mL) was stirred at 37 °C and 200 rpm. After 3 h the reaction afforded the maximum conversion (78%), calculated by HPLC using isocratic 99:1 trifluoroacetic acid (TFA)(0.1% aq. *v/v*) pH 3:ACN as mobile phase (t_R (min): **2c** 8.0, **3c** 13.1). After centrifugation, the supernatant was applied onto a C18 reverse phase silica column and eluted with TFA (1% aq. *v/v*). Fractions containing **3c** were collected and lyophilized, affording a pure white powder (37.2% yield). ^{1}H NMR (500 MHz, D_2O) δ (ppm): 7.39 (d, J = 1.2 Hz, 1H H_{11}); δ 4.38–4.31 (m, 1H, H_4), 3.85 (dd, J = 14.3, 3.7 Hz, 1H, H_{5a}); 3.69 (dd, J = 14.3, 8.4 Hz, 1H, H_{5b}); 2.97 (dd, J = 17.4, 4.7 Hz, 1H, H_{3a}); 2.90 (dd, J = 17.4, 8.0 Hz, 1H, H_{3b}); 1.80 (d, J = 1.2 Hz, 3H, H_{12}). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 202.63 (C2); 172.3 (C9); 169.04 (C7); 167.07 (C1); 143.76 (C11); 110.37 (C10); 64.97 (C4); 53.21 (C5); 30.22 (C3); 11.24 (C12).

2.5. Theoretical calculations

Theoretical calculations were carried out following several steps. The molecules under study were first subjected to geometry optimizations using the DFT [19,20]. To this end, the B3LYP hybrid exchange-correlation functional [21,22] together with the 6-31G(d,p) basis set as implemented in the Gaussian 03 package were used [23]. All geometrical parameters were optimized without constraints. The minimum energy structures obtained from this first optimization were re-optimized using 6-31G++(d,p) basis. Isotropic chemical shifts for carbon and hydrogen atoms were calculated. In this case, the isotropic magnetic shielding tensor was obtained at the B3LYP/6-31G++(d,p) level of theory. The reported shifts are relative to tetramethylsilane (TMS). The absolute isotropic shieldings of TMS were also calculated using the B3LYP/6-31G++(d,p) model.

3. Results and discussion

It is well known that the chirality of the side chain plays an important role in ANs activity. Among the chiral ANs approved as antivirals, cidofovir is a representative example; only the *S*-enantiomer shows antiviral activity [24]. Different asymmetric synthetic strategies can be used for the preparation of chiral ANs and among them, the alkylation of nucleobases by chiral electrophilic alkylating agents is the most employed [25]. As an alternative, we developed a chemoenzymatic strategy consisting of a first alkylation step to introduce an aldehyde moiety in a nucleobase and a second step where the chiral

linear chain is constructed. Aldol addition is the most used and versatile reaction for C−C bond formation and particularly, organocatalysis and biocatalysis provide efficient strategies to perform it in an asymmetric manner. Following this direction, we have previously explored the synthesis of different AN analogues using pyrrolidine as organocatalyst [18] or RAMA (a dihydroxyacetone phosphate, DHAP, dependent aldolase) as biocatalyst [17] and a range of 2-oxoethyl purinic and pyrimidinic compounds as aldehyde acceptors.

In order to synthesize these substrates, *N*-alkylation of nucleobases was carried out using bromoacetaldehyde dimethyl acetal in presence of K_2CO_3. 9-(2,2-Dimethoxyethyl)-purine or 1-(2,2-dimethoxyethyl)-pyrimidines were regioselectively prepared in good yields. The corresponding aldehydes were obtained by acid treatment just before use.

Aldolases are highly specific for their corresponding donors and consequently, using different aldolases, diverse side chains can be built. Besides, it is well established that aldolases have a high level of stereocontrol at the formed chiral centres being their configurations highly predictable. For instance, AN analogues prepared using RAMA [17] showed the usual (3*S*,4*R*) stereochemistry with respect to the carbonyl group as a consequence of the nucleophilic attack of the enamine intermediate formed between DHAP and the enzyme on the *si*-face of the acceptor aldehyde. As mentioned before, NeuAcA, a pyruvate dependent aldolase has relaxed specificity for the acceptor, being capable of using a wide variety of aldehydic substrates, preferentially polyhydroxylated, including tetroses, pentoses, hexoses of the D and L series, amino sugars and disaccharides, among others [26–29].

In order to explore the synthesis of new AN analogues, aldehydes **2a-d** were screened as substrates of commercially available NeuAcA from *Clostridium perfringens* for the aldol reaction with pyruvate. Due to its catabolic role in Nature, the equilibrium of the reaction that NeuAcA catalyzes *in vivo* favours the decondensation of α-*N*-acetylneuramic acid. However, for synthetic purposes, such equilibrium can be shifted to the opposite direction by using excess substrates. Having in mind this consideration and that the experiments using ratios close to 1:1 afforded low conversions, 2:1 **2a-d**: pyruvate molar ratio was employed. Conversion to 4-hydroxy-2-oxo-5-(*N*9-purinyl or *N*1-pyrimidyl)pentanoic acids, **3a-e** (Scheme 1), was followed by HPLC for 48 h. It was observed that NeuAcA efficiently accepted the unusual substrates **2a-d**, since products were successfully obtained in up to 94% conversions (Table 1). Maximum conversions were observed between 24 and 30 h. A slight decrease occurred at longer times, probably due to retroaldol reactions. Besides, blank reactions without enzyme were carried out under the same experimental conditions with no evidence of product formation.

These results are remarkable since, as far as we know, NeuAcA from *Clostridium perfringens* was expected to accept only polyhydroxylated aliphatic aldehydes that are subsequently cyclized. Other NeuAcA sources or evolved enzymes were used in order to carry out the biotransformation employing different substrates [14,30,31]. However, we propose that the interactions of the heteroatoms present in the nucleobases might resemble those of the traditional sugar substrates.

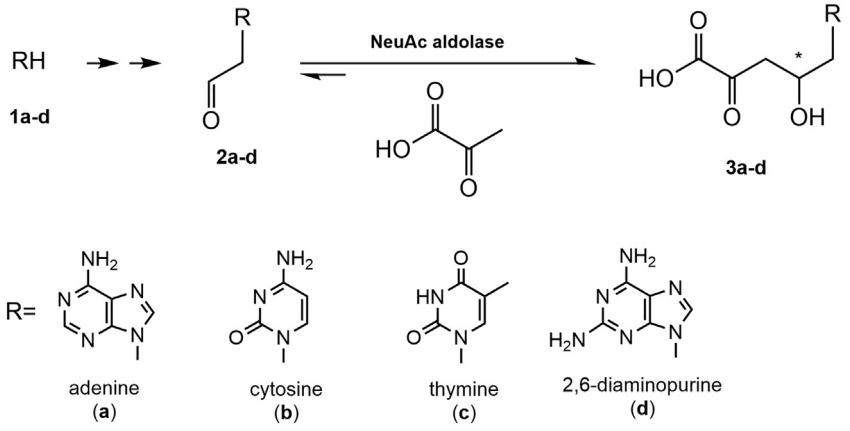
Table 1
3a-d maximal conversions obtained using 2:1 **2a-d**: pyruvate molar ratio.

Aldehyde acceptor	Aldol product	Conversion (%)[a,b]	Reaction time (h)
2a	**3a**	94	24
2b	**3b**	91	24
2c	**3c**	87	30
2d	**3d**	71	24

[a] Conversions are based on the limiting reagent pyruvate.
[b] Determined by HPLC.

For further studies, compound **3c** was selected as model. Since the enzyme is capable of performing the synthetic reaction with excess of either the heterocyclic aldehydes or pyruvate, we also tested a **2c**:pyruvate 1:2molarM ratio. Although a little decrease in **3c** conversion was observed applying these conditions (78% in contrast to 87%), the total amount of product obtained starting from the same amount of aldehyde was higher (31.2 mM *versus* 17.4 mM) and the reaction time was reduced to 3 h. In addition, reaction costs in terms of reagents and purification steps could be lowered.

Compound **3c** was isolated by centrifugation of the reaction medium and purification of the supernatant by reverse-phase column chromatography. The identity of the product was verified by NMR spectrometry. By comparing the ^{1}H NMR data of **3c** with those of the corresponding AN analogues previously prepared by us using another aldolases and also organocatalysts, a similar signal profile of the acyclic chain was observed. However, chemical shifts (δ) appear downfield by the effect of the carboxylic group at C1. Both 3- and 5-methylene signals show diasterotopic behaviour. Coupling constants with H4 are consistent with different environments as result of the chirality of C4 and with a probably hindered chain rotation. ^{13}C NMR spectrum also matched with the expected profile, with the only exception of the chemical shift of C7 in thymine, which shifted from 153 to 169 ppm. Patterson-Elenbaum et al. [32] have demonstrated, by computational and experimental techniques, that the presence of intramolecular hydrogen bonds can deshield carbonyl carbons in ^{13}C NMR spectra. Thus, in order to analyse the existence of such electronic effect and explain our experimental data, theoretical calculations were performed. As it can be observed in Fig. 1, the optimized structure of **3c** shows the presence of a hydrogen bond between CO(7) and the OH at C4 of the acyclic chain. This structure shows a 0.62 Kcal/mol additional stability in comparison with the other minimum, in which the OH at C4 of the acyclic chain forms the hydrogen bond with the carboxylic group. The signal assignment in ^{13}C NMR spectrum of **3c** was also performed by theoretical calculations. Using B3LYP at 6-31G++(d,p) level of theory,



Scheme 1. NeuAcA catalyzed synthesis of AN analogues **3a-d**.

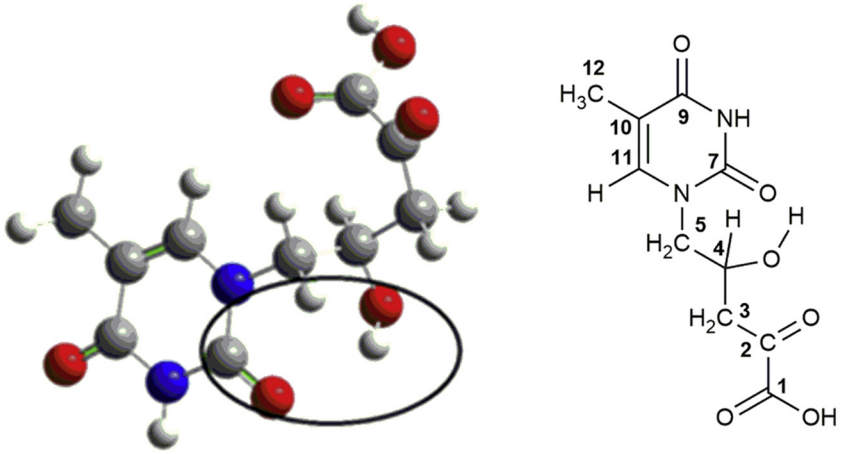


Fig. 1. Optimized structure of **3c** calculated using B3LYP at 6-31G + + (d,p) level of theory. Atoms involved in a hydrogen bond are indicated.

the calculated δ value for C7 were 163.9 and 156.6 ppm with and without the formation of the proposed hydrogen bond, respectively.

Unlike the strict enzymatic control of the stereochemistry usually observed for most aldolases, the stereoselectivity of the reaction catalyzed by NeuAcA depends on the structure of the involved acceptor aldehyde. Fitz et al. [26] proposed that the reaction is under thermodynamic control and that the configuration of the aldehyde substituent of C3 is critical for the selectivity. For sialic acid type-substrates, the attack occurs on the *si* face so that most of the products have *S* configuration. This was verified by Smith et al. [33] through molecular modelling of the enaminic intermediate based on X-ray analysis of NeuAcA co-crystallized with inhibitors. As the aldehydes used in this work lack a chiral carbon at position 3, according to the above mentioned Fitz model, NeuAcA would be unable to distinguish between *re* and *si* faces of the acceptor substrate. For this reason, it is expected that products **3** are obtained as racemic mixtures. Nevertheless, further studies to confirm this assumption are in progress.

4. Conclusions

In contrast to previous reports, the results reported herein demonstrate that NeuAcA from *Clostridium perfringens* is capable of catalysing the aldol addition of polyfunctionalized acceptor substrates which are not polyhydroxylated compounds. Moreover, the stabilization of the aldol **3c** by hydrogen bonding between the hydroxyl of the alkyl chain and the carbonyl group in the position 7 of the base was confirmed by theoretical and spectroscopic studies.

Novel acyclic nucleosides carrying a 4-hydroxy-2-oxoacid unit were synthesized through a green and scalable procedure. The obtained products are structurally related to *S*-adenosylhomocysteine hydrolase inhibitors, such as eritadenine and (*S*)-9-(2,3-dihydroxy-propyl)adenine which are broad spectrum antiviral agents. In addition, the range of structural and biological activity diversity could be increased by further (bio)chemical transformations of the obtained acyclic chain.

Acknowledgments

This study was supported by grants of Universidad Nacional de Quilmes, CONICET (PIP 2012-271) and ANPCyT (PICT 2012–0811). MAP, ESL and AMI are research members of CONICET. MJN is a CONICET fellow.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catcom.2018.12.013.

References

[1] S. Chaudhuri, J.A. Symons, J. Deval, Innovation and trends in the development and approval of antiviral medicines: 1987–2017, Antivir. Res. 155 (2018) 76–88.
[2] E. De Clercq, G. Li, Approved antiviral drugs over the past 50 years, Clin. Microbiol. Rev. 29 (2016) 695–747.
[3] L. Liang, M.S. Xie, T. Qin, M. Zhu, G.R. Qu, H.M. Guo, Regio- and enantioselective synthesis of chiral pyrimidine acyclic nucleosides via rhodium-catalyzed asymmetric allylation of pyrimidines, Org. Lett. 19 (2017) 5212–5215.
[4] M. Krečmerová, P. Jansa, M. Dračínský, P. Sázelová, V. Kašička, J. Neyts, J. Auwerx, E. Kiss, N. Goris, G. Stepan, Z. Janeba, 9-[2-(*R*)-(Phosphonomethoxy)propyl]-2,6-diaminopurine (*R*)-PMPDAP and its prodrugs: Optimized preparation, including identification of by-products formed, and antiviral evaluation in vitro, Bioorg. Med. Chem. 21 (2013) 1199–1208.
[5] J.P. Li, H.R. Tuo, M.S. Xie, B. Kang, G.R. Qu, H.M. Guo, Synthesis of chiral acyclic pyrimidine nucleosides with a sulfur containing side chain via enantioselective tandem conjugate addition/protonation, Asian J. Org. Chem. 7 (2018) 128–132.
[6] S. Friedrich, F. Hahn, Opportunities for enzyme catalysis in natural product chemistry, Tetrahedron 71 (2015) 1473–1508.
[7] P. Clapés, W.D. Fessner, G.A. Sprenger, A.K. Samland, Recent progress in stereoselective synthesis with aldolases, Curr. Opin. Chem. Biol. 14 (2010) 154–167.
[8] M.C. Shelton, I.C. Cotterill, S.T.A. Novak, R.M. Poonawala, S. Sudarshan, E.J. Toone, 2-Keto-3-deoxy-6-phosphogluconate aldolases as catalysts for stereocontrolled carbon-carbon bond formation, J. Am. Chem. Soc. 118 (1996) 2117–2125.
[9] K. Hernandez, A. Gomez, J. Joglar, J. Bujons, T. Parella, P. Clapés, 2-Keto-3-deoxy-L-rhamnonate aldolase (YfaU) as catalyst in aldol additions of pyruvate to amino aldehyde derivatives, Adv. Synth. Catal. 359 (2017) 2090–2100.
[10] C. Augé, C. Gautheron, S. David, A. Malleron, B. Cavayé, B. Bouxom, Sialyl aldolase in organic synthesis: from the trout egg acid 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) to branched-chain higher ketoses as possible new chirons, Tetrahedron 46 (1990) 201–214.
[11] P. Zhou, H. Salleh, J.F. Honek, Facile chemoenzymic synthesis of 3-(hydroxymethyl)-6-epicastanospermine, J. Org. Chem. 58 (1993) 264–266.
[12] M.J. Kim, W.J. Hennen, H.M. Sweers, C.H. Wong, Enzymes in carbohydrate synthesis: *N*-acetylneuraminic acid aldolase catalyzed reactions and preparation of *N*-acetyl-2-deoxy-D-neuraminic acid derivatives, J. Am. Chem. Soc. 110 (1988) 6481–6486.
[13] S. Hader, A.G. Watts, The synthesis of a series of deoxygenated 2,3-difluoro-*N*-acetylneuraminic acid derivatives as potential sialidase inhibitors, Carbohydr. Res. 374 (2013) 23–28.
[14] Q. Chen, L. Han, X. Chen, Y. Cui, J. Feng, Q. Wu, D. Zhu, A sialic acid aldolase from *Peptoclostridium difficile* NAP08 with 4-hydroxy-2-oxo-pentanoate aldolase activity, Enzyme Microb. Tech. 92 (2016) 99–106.
[15] P. Laborda, S.Y. Wang, A.M. Lu, M.H. Xu-Chu, D.Y.J. Qian, Y.S.J. Liu, J. Voglmeir, 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. 18 (2017) 3120–3125.
[16] H. Yu, H. Cao, V.K. Tiwari, Y. Li, X. Chen, Chemoenzymatic synthesis of C8-modified sialic acids and related α2–3- and α2–6-linked sialosides, Bioorg. Med. Chem. Lett. 21 (2011) 5037–5040.
[17] M.A. Palazzolo, M. Nigro, A.M. Iribarren, E.S. Lewkowicz, A chemoenzymatic route to prepare acyclic nucleoside analogues, Eur. J. Org. Chem. (5) (2016) 921–924.
[18] M.A. Palazzolo, M. Pérez-Sánchez, A.M. Iribarren, E.S. Lewkowicz, P. Domínguez de María, Organocatalytic synthesis of novel purine and pyrimidine acyclic nucleosides, Tetrahedron Lett. 53 (2012) 6797–6800.
[19] W. Kohn, L.J. Sham, Self-consistent equations including exchange and correlation effects, Phys. Rev. 140 (1965) 1133–1138.
[20] R.G. Parr, W. Yang, Density-functional Theory of Atoms and Molecules, Oxford University Press, New York, 1989.

[21] D. Becke, Density-functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652.
[22] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B 37 (1988) 785–789.
[23] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.B. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K. Kudin, J. Burant, J. Millam, S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. Petersson, H. Nakatsuji, M. Hada, M. Ehara, R. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. Knox, H. Hratchian, J. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. Stratmann, A. Yazyev, R. Austin, C. Cammi, J.W. Pomelli, P.Y. Ochterski, K. Ayala, G.A. Morokuma, P. Voth, J.J. Salvador, V.G. Dannenberg, S. Zakrzewski, A.D. Dapprich, M.C. Daniels, O. Strain, D.K. Farkas, A.D. Malick, K. Rabuck, J.B. Raghavachari, J.V. Foresman, Q. Ortiz, A. G. Cui, S. Baboul, J. Clifford, B.B. Cioslowski, G. Stefanov, A. Liu, P. Liashenko, I. Piskorz, R.L. Komaromi, D.J. Martin, T. Fox, M.A. Keith, C.Y. Al-Laham, A. Peng, M. Nanayakkara, P.M.W. Challacombe, B. Gill, W. Johnson, M.W. Chen, C. Wong, R. Gonzalez, J.A. Pople, Gaussian 03, Revision B.03, Gaussian, Inc., Pittsburgh PA, 2003.
[24] X. Ming-Shenge, N. Hong-Ying, Q. Gui-Rong, G. Hai-Ming, The development for the synthesis of chiral acyclic nucleosides and their phosphonates, Tetrahedron Lett. 55 (2014) 7156–7166.
[25] H.M. Guo, S. Wu, H.Y. Niu, G. Song, G.R. Qu, Chemical synthesis of acyclic nucleosides, in: P. Merino (Ed.), Hoboken, New Jersey, 2013, pp. 103–162.
[26] W. Fitz, J.R. Schwark, C.H. Wong, Aldotetroses and C(3)-modified aldohexoses as substrates for *N*-acetylneuraminic acid aldolase: a model for the explanation of the normal and the inversed stereoselectivity, J. Org. Chem. 60 (1995) 3663–3670.
[27] U. Kragl, A. Gödde, C. Wandrey, N. Lubin, C. Augé, New synthetic applications of sialic acid aldolase, a useful catalyst for KDO synthesis. Relation between substrate conformation and enzyme stereoselectivity, J. Chem. Soc. Perkin Trans. 10 (1994) 119–124.
[28] H. Yu, X. Chen, Aldolase-catalyzed synthesis of β-D-Gal p-(1→9)-D-KDN: a novel acceptor for sialyltransferases, Org. Lett. 8 (2006) 2393–2396.
[29] S. Huang, H. Yu, X. Chen, Disaccharides as sialic acid aldolase substrates: synthesis of disaccharides containing a sialic acid at the reducing end, Angew. Chem. Int. Ed. 46 (2007) 2249–2253.
[30] M.J. Walters, E.J. Toone, Pyruvate aldolases in chiral carbon–carbon bond formation, Nat. Protoc. 2 (2007) 1825–1830.
[31] P. Baker, S.Y.K. Seah, Rational design of stereoselectivity in the class II pyruvate aldolase BphI, J. Am. Chem. Soc. 134 (2012) 507–513.
[32] S. Patterson-Elenbaum, J.T. Stanley, D.K. Dillner, S. Lin, D. Traficante, 13C NMR chemical shifts of carbonyl groups in substituted benzaldehydes and acetophenones: substituent chemical shift increments, Magn. Reson. Chem. 44 (2006) 797–806.
[33] B.J. Smith, M.C. Lawrence, J.A.R.G. Barbosa, Substrate-assisted catalysis in sialic acid aldolase, J. Org. Chem. 64 (1999) 945–949.