



Synthesis of Natural Product-Like Tricyclic Higher-Carbon Sugar Nucleosides

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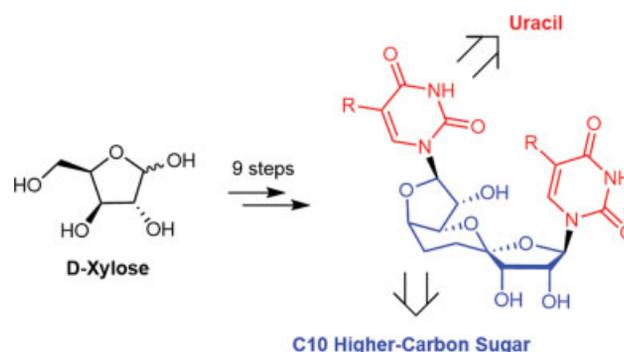
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Pharmaceut Fronts 2021;3:e18–e22.



Abstract

Keywords

- ▶ higher-carbon sugars
- ▶ nucleosides
- ▶ uracil
- ▶ antiproliferative activity

Because of the structural novelty and interesting biological profiles, the synthesis of higher-carbon sugars has been highly pursued. In this work, we first synthesized a series of structurally novel bis-uracil containing tricyclic higher-carbon sugar nucleosides (**4a–e**) using *D*-xylose as the starting material and the classical Vorbruggen glycosylation as the key synthetic step. The yields of the target compound were good. Unfortunately, despite the presence of pharmaceutically relevant uracil fragment, compounds **4a–e** were inactive against the proliferation of several cancer cell lines (EC109, EC9706, PC-3, and MGC-803). Whether and how **4a–e** functioned as anticancer agents would be further studied in our laboratory.

Introduction

The synthesis and biological evaluation of carbohydrates have been highly pursued in the fields of organic and medicinal chemistry, due to the structural novelty and

diverse bioactivities of these compounds,¹ including antimicrobial,² anticancer,^{3,4} antifatigue,⁵ and antioxidation.⁶ Higher-carbon sugars refer to carbohydrates bearing 7 or more continuous carbon atoms and are attractive synthetic targets because of the structural complexity and their existence in some microbially produced antibiotics.⁷ Therefore, several methods have been developed to access this class of structurally complex and unique carbohydrates.^{8–11}

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received
April 20, 2021
accepted
May 21, 2021

DOI <https://doi.org/10.1055/s-0041-1731300>
ISSN 2628-5088.

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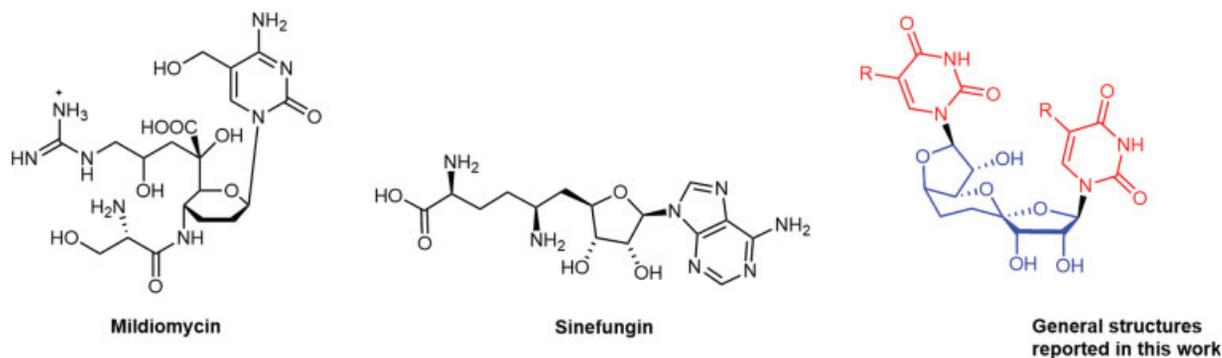


Fig. 1 Selected bioactive higher-carbon sugar nucleosides and the compounds reported in this work.

Higher-carbon sugars, isolated from nature or chemically modified, have proven to possess different bioactivities.¹² For example, peptidyl nucleoside milbemycin (►Fig. 1), produced by *Streptomyces rimofaciens*, shows antimicrobial activities through inhibiting protein synthesis.^{13,14} Sinefungin, the S-adenosyl-L-methionine analog, was proved to have inhibitory activities against fungi, viruses, and trypanosomal pathogens.^{15,16} In contrast, the anticancer properties of higher-carbon sugars have rarely been studied and may deserve further investigations for searching new chemotypes for cancer therapy.

Following our previous work on constructing higher-carbon sugars,¹⁷ we herein describe the synthesis and preliminary antiproliferative activity of a series of structurally novel sugar nucleosides, which feature the bis-uracil motif (highlighted in red) attached to the tricyclic higher-carbon sugars (highlighted in blue), albeit with low cytotoxic activity against the tested human cancer cell lines (►Fig. 1). The preliminary antiproliferative activity is also explored.

Materials and Methods

Chemistry

All reagents were of analytical grade and purchased from commercial sources. The anhydrous solvents were used in this work. Thin-layer chromatography was performed on glass plates coated with silica gel and visualized by heating with a heat gun or under ultraviolet light. The products were purified by column chromatography over silica gel. Melting points were determined on a Beijing Keyi XT4A (Beijing, China) apparatus and are uncorrected. All nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE DPX-400 spectrometer with tetramethylsilane (TMS) as the internal standard, and chemical shifts are given as δ values. High-resolution mass spectrometry (HRMS) data were recorded on a Waters Q-TOF micro-spectrometer.

Synthesis of Compound 1

Compound 1 was synthesized from *D*-xylose in an overall yield of 60% according to the procedure described previously.¹⁷

Compound 1, white solid, yield: 60%. m.p.: 102–104°C. ¹H NMR (400 MHz, CDCl₃) δ 5.98 (d, *J* = 4.0 Hz, 1H), 5.71 (d, *J* = 4.8 Hz, 1H), 4.83 (dd, *J* = 6.1, 4.1 Hz, 1H), 4.73 (dd, *J* = 5.7, 4.9 Hz, 1H), 4.08 (t, *J* = 5.9 Hz, 1H), 4.01 (dd, *J* = 5.7, 4.1 Hz, 1H), 3.86 (dd, *J* = 9.3, 4.1 Hz, 1H), 2.89 (d, *J* = 5.7 Hz, 1H),

2.16–1.92 (m, 4H), 1.64 (s, 3H), 1.58 (s, 3H), 1.45 (s, 3H), 1.40 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 115.70, 115.60, 109.82, 105.09, 105.04, 82.02, 80.17, 77.38, 77.06, 76.74, 74.44, 72.02, 70.45, 28.16, 27.63, 27.39, 26.89, 23.54, 22.64. HRMS (ESI): *m/z* calcd. for C₁₆H₂₅O₈ [M + H]⁺, 345.1549; found, 345.1522.

Synthesis of Compound 2

Compound 1 (4.0 g, 12 mmol) was added to a mixture of diluted H₂SO₄ (50 mL, 0.05 mol/L) and acetone (30 mL) at 40°C. After 4 hours, the pH of the reaction was adjusted to 7 to 8 with pyridine. The reaction mixture was then concentrated, and toluene (10 mL \times 2) was added and continued to concentrate under reduced pressure. The residue obtained was dried under vacuum at 35°C for 24 hours to obtain the deprotection intermediate, which was used without further purification. To a solution of the intermediate in pyridine (50 mL) was added 4-dimethylaminopyridine (DMAP; 0.03 g, 0.024 mmol) and acetic anhydride (25 mL) and the mixture was stirred at room temperature under a nitrogen atmosphere for 6 hours. After the completion of the reaction, the mixture was diluted with ethyl acetate (200 mL), washed with diluted hydrochloric acid (50 mL \times 3, 1 mol/L), saturated aqueous NaHCO₃ (50 mL \times 3) and brine (50 mL \times 3), dried with anhydrous Na₂SO₄, concentrated, and recrystallized from ethanol to give the white solid compound 2.

Compound 2, white solid, yield: 80%. m.p.: 113–115°C. ¹H NMR (400 MHz, CDCl₃) δ 6.26 (d, *J* = 3.6 Hz, 1H), 6.15 (d, *J* = 0.7 Hz, 1H), 5.26 (dd, *J* = 6.1, 1.0 Hz, 1H), 5.21–5.12 (m, 2H), 4.64 (dd, *J* = 4.7, 2.1 Hz, 1H), 4.29–4.23 (m, 1H), 2.20–2.04 (m, 19H). ¹³C NMR (100 MHz, CDCl₃) δ 170.26, 169.71, 169.67, 169.51, 168.96, 103.59, 99.84, 97.34, 79.67, 77.38, 77.06, 76.74, 74.39, 73.53, 72.69, 70.35, 72.15, 21.16, 20.98, 20.95, 20.53, 20.48, 20.45. HRMS (ESI): *m/z* calcd. for C₂₀H₂₆NaO₁₃ [M + Na]⁺, 497.1271; found, 497.1272.

General Procedure for the Synthesis of Compounds 3a–e

To a solution of uracil (8.92 mmol) in toluene (30 mL) were added catalytic (NH₄)₂SO₄ and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 4.67 mL, 22.3 mmol), then the reaction mixture was heated to 110°C under a nitrogen atmosphere until the solution became clear. The solution

was then concentrated under vacuum and dissolved in CH₃CN (5 mL), and the mixture was then added to a stirred solution of compound **2** (3.57 mmol) in CH₃CN (25 mL) containing SnCl₄ (1.04 mL, 8.92 mmol). This mixture was stirred at room temperature for 6 hours under the nitrogen atmosphere, and then neutralized with solid NaHCO₃, filtered, and extracted with ethyl acetate (500 mL). The organic layer was washed with saturated aqueous NaHCO₃ and brine 50 mL × 3, and dried over anhydrous MgSO₄. The crude product was purified by a short silica gel column chromatography to afford compounds **3a–e**, which were used for the next step directly.

General Procedure for the Synthesis of Compounds **4a–e**

Compound **3** (0.86 mmol) was dissolved to methanolic ammonia (25 mL) and stirred for 2 hours at room temperature. Upon completion of the reaction, the solution was dried under vacuum to give the residue, which was then purified by a short silica gel column chromatography to give the desired compounds **4a–e**.

Compound **4a**, white solid, yield: 90%. m.p.: 141–143°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.97 (d, *J* = 7.0 Hz, 1H), 7.83 (d, *J* = 6.5 Hz, 1H), 5.89 (d, *J* = 7.4 Hz, 1H), 5.74 (s, 1H), 5.22 (d, *J* = 11.6 Hz, 1H), 4.85 (t, *J* = 10.1 Hz, 2H), 4.39 (d, *J* = 11.8 Hz, 2H), 4.08–3.76 (m, 3H), 1.99–1.59 (m, 3H), 1.53–1.37 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 157.63, 157.50, 157.40, 157.27, 154.01, 153.29, 137.62, 137.51, 135.21, 135.10, 127.10, 126.79, 126.56, 126.26, 102.75, 91.52, 89.59, 76.25, 74.19, 72.82, 71.97, 71.33, 24.37, 21.47. HRMS (ESI): *m/z* calcd. for C₁₈H₁₉F₂N₄O₁₀ [M + H]⁺, 489.1069; found, 489.1373.

Compound **4b**, white solid, yield: 80%. m.p.: 132–134°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.23 (s, 1H), 8.11 (s, 1H), 5.95 (d, *J* = 7.5 Hz, 1H), 5.77 (d, *J* = 3.5 Hz, 1H), 5.41 (d, *J* = 11.4 Hz, 1H), 5.14–4.87 (m, 2H), 4.54 (d, *J* = 16.3 Hz, 2H), 4.10 (m, 3H), 2.00–1.70 (m, 3H), 1.59 (d, *J* = 10.0 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 159.01, 158.88, 150.19, 149.57, 139.29, 138.40, 108.12, 107.96, 103.01, 91.19, 88.82, 75.95, 73.95, 73.26, 71.32, 71.19, 24.22, 21.33. HRMS (ESI): *m/z* calcd. for C₁₈H₁₈Cl₂N₄NaO₁₀ [M + Na]⁺, 543.0298; found, 543.0298.

Compound **4c**, white solid, yield: 78%. m.p.: 115–117°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.21 (s, 1H), 8.11 (s, 1H), 5.88 (d, *J* = 7.5 Hz, 1H), 5.68 (d, *J* = 3.5 Hz, 1H), 5.33 (d, *J* = 11.5 Hz, 1H), 5.02 (d, *J* = 6.5 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.48 (d, *J* = 10.8 Hz, 2H), 4.17–3.90 (m, 3H), 1.95–1.65 (m, 3H), 1.51 (d, *J* = 9.9 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 159.07, 158.97, 150.37, 149.69, 141.82, 140.81, 103.01, 96.72, 96.53, 91.40, 88.81, 75.91, 73.96, 73.26, 71.25, 71.19, 24.24, 21.35. HRMS (ESI): *m/z* calcd. for C₁₈H₁₈Br₂N₄NaO₁₀ [M + Na]⁺, 630.9287; found, 630.9289.

Compound **4d**, white solid, yield: 82%. m.p.: 129–131°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.23 (s, 1H), 8.15 (s, 1H), 5.92 (d, *J* = 7.5 Hz, 1H), 5.72 (d, *J* = 3.5 Hz, 1H), 5.36 (d, *J* = 11.6 Hz, 1H), 5.05 (d, *J* = 6.5 Hz, 1H), 4.92 (d, *J* = 10.8 Hz, 1H), 4.54 (s, 2H), 4.23–3.95 (m, 3H), 1.98–1.74 (m, 3H), 1.55 (d, *J* = 10.1 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 160.49, 160.36, 150.74, 150.04, 146.53, 145.38, 102.96, 91.53, 88.66, 75.84, 74.00, 73.22, 71.17, 70.52, 70.32, 24.31, 21.38. HRMS (ESI):

m/z calcd. for C₁₈H₁₈I₂N₄NaO₁₀ [M + Na]⁺, 726.9010; found, 726.9010.

Compound **4e**, white solid, yield: 88%. m.p.: 134–136°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.65 (s, 1H), 7.49 (s, 1H), 5.96 (d, *J* = 7.6 Hz, 1H), 5.82 (d, *J* = 3.5 Hz, 1H), 5.36 (d, *J* = 11.5 Hz, 1H), 5.03 (dd, *J* = 27.6, 8.3 Hz, 2H), 4.48 (s, 2H), 4.12–3.92 (m, 3H), 2.01–1.70 (m, 9H), 1.56 (d, *J* = 12.5 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 163.69, 163.67, 151.07, 150.45, 137.37, 136.74, 110.19, 110.16, 102.75, 90.15, 88.07, 75.79, 74.20, 73.03, 71.44, 71.19, 24.37, 21.40, 11.96, 11.91. HRMS (ESI): *m/z* calcd. for C₂₀H₂₅N₄O₁₀ [M + H]⁺, 481.1571; found, 481.1565.

MTT Assay

The MTT assay was performed following our previously reported methods.¹⁸

Results and Discussion

Inspired by the fact that capecitabine, a prodrug consisting of the fluoropyrimidine and sugar moieties, has been used in the clinic for the treatment breast cancer, gastric cancer, and colorectal cancer, we thus speculated that introduction of the uracil group into the higher-carbon sugar may yield novel sugar nucleosides and unexpected bioactivities. In continuation with our ongoing interest in constructing higher-carbon sugars, we synthesized five structurally novel tricyclic higher-carbon sugar nucleosides, each of which possessed two uracil units attached to both ends of the tricyclic higher-carbon sugar core (→Fig. 2).

The synthetic route of higher-carbon sugar nucleosides **4a–e** is shown in Scheme 1. The C10 higher-carbon sugar **1** was synthesized from *D*-xylose in an overall yield of 60% following our previously reported methods.¹⁷ Treatment of compound **1** with diluted H₂SO₄ in acetone gave the deprotection intermediate, which was used directly without additional purification. Acetylation of the intermediate with acetic anhydride in pyridine afforded compound **2**. Uracil was treated with (NH₄)₂SO₄ and TMSOTf in toluene for 3 hours at 110°C, and then the solvent was removed by distillation under reduced pressure to give the silylated uracil, which then reacted with compound **2** in the presence

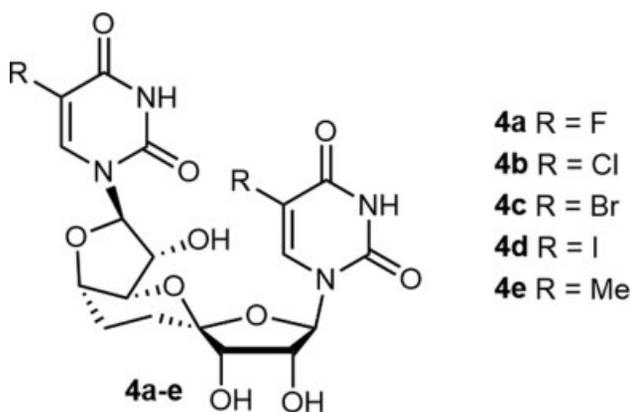
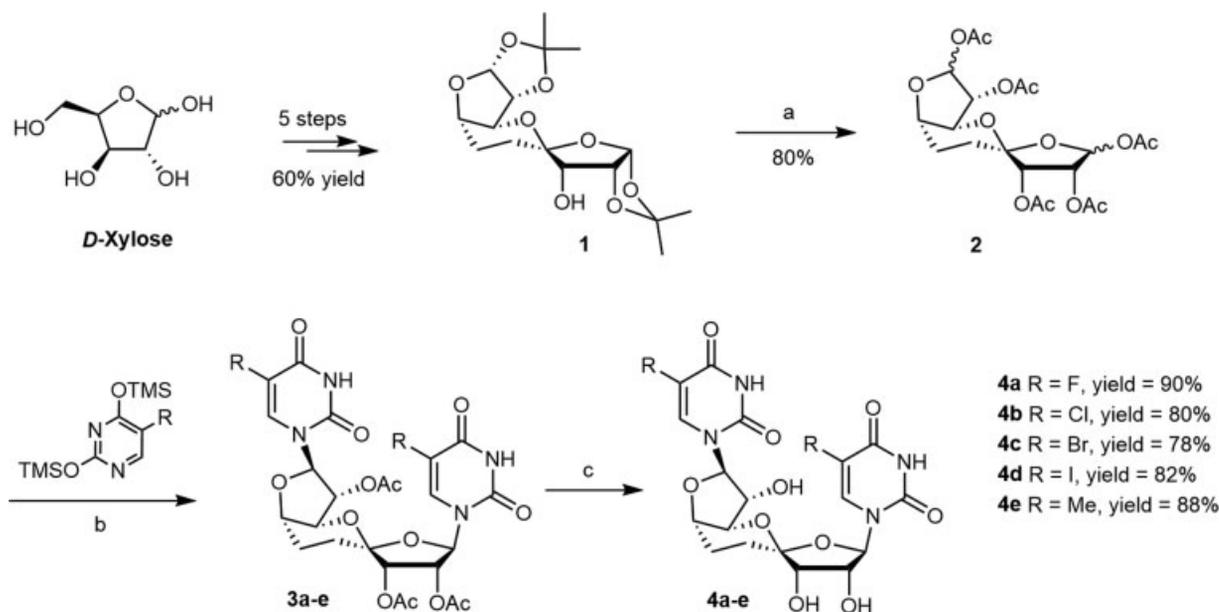


Fig. 2 Synthesized higher-carbon sugar nucleosides **4a–e**.



Scheme 1 Synthesis of higher-carbon sugar nucleosides **4a–e**. Reagents and conditions: (a) (i) H_2SO_4 , acetone, 40°C , 4 hours; (ii) Ac_2O , pyridine, DMAP, N_2 , r.t., 6 hours; (b) SnCl_4 , CH_3CN , N_2 , r.t., 6 hours; (c) NH_3 , CH_3OH , r.t., 2 hours.

of SnCl_4 under a nitrogen atmosphere, producing the bis-uracil substituted higher-carbon sugars **3a–e**. Treatment of compounds **3a–e** with saturated methanolic ammonia gave compounds **4a–e** in good yields (–Fig. 2). All final compounds were characterized by NMR and HRMS.

Inspired by the structural novelty and in continuation with our efforts toward the identification of potent anticancer agents,¹⁹ we next examined the antiproliferative activity of compounds **4a–e** against a panel of cell lines including EC109, EC9706, PC-3, and MGC-803 using the MTT assay. However, compounds **4a–e** were found to be inactive against the tested cancer cell lines with the IC_{50} values of more than $50 \mu\text{mol/L}$. The poor antiproliferative activity of these compounds may be attributed to the relatively poor permeability across the cell membrane. Interestingly, we found that our title compounds had similar structural features to capecitabine, an oral active chemotherapeutic agent, which is currently used in the clinic as a prodrug. Mechanistically, capecitabine is enzymatically hydrolyzed *in vivo* to release the active 5-fluorouracil (5-FU), thus exerting its *in vivo* anticancer efficacy. Whether our title compounds could exert the anticancer efficacy through similar mechanisms remains unclear and needs to be explored further.

Conclusion

In summary, based on our previous protocols, we have first synthesized a series of structurally novel bis-uracil containing tricyclic higher-carbon sugar nucleosides from *D*-xylose. The synthetic routes are straightforward, high-yielding, and could be utilized for accessing more analogs for biological screens. The SnCl_4 -mediated substitution reaction between the silylated uracil and compound **2** is the key to success. The

title compounds possess unique structural features and may provide reference for the design of other higher-carbon sugars with interesting biomedical activities. However, biological evaluation showed that this series of compounds weakly inhibited the proliferation of EC109, EC9706, PC-3, and MGC-803, and thus, whether these compounds could exert their *in vivo* anticancer activity like capecitabine would be further studied in our laboratory.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81773562) the Open Fund of State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, China (Grant No. KF-GN-201902), and the Open Project of State Key Laboratory of Natural Medicines (Grant No. SKLNMKF202005).

Conflict of Interest

The authors declare no conflict of interest.

References

- 1 Yue H, Zeng H, Ding K. A review of isolation methods, structure features and bioactivities of polysaccharides from *Dendrobium* species. *Chin J Nat Med* 2020;18(01):1–27
- 2 Kuorwel KK, Cran MJ, Sonneveld K, Miltz J, Bigger SW. Antimicrobial activity of biodegradable polysaccharide and protein-based films containing active agents. *J Food Sci* 2011;76(03):R90–R102
- 3 Ye H, Wang K, Zhou C, Liu J, Zeng X. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem* 2008;111(02):428–432
- 4 Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl Microbiol Biotechnol* 2002;60(03):258–274

- 5 Liu ZX, Yin H, Xiao LL, et al. Antifatigue and antioxidant activities and monosaccharide composition of polysaccharide from roots of kiwifruit. *Shipin Kexue* 2013;34(13):239–242
- 6 Yan H, Xie YP, Sun SG. Chemical analysis of *Astragalus mongholicus* polysaccharides and antioxidant activity of the polysaccharides. *Carbohydr Polym* 2010;82(03):636–640
- 7 Isono K. Nucleoside antibiotics: structure, biological activity, and biosynthesis. *J Antibiot (Tokyo)* 1988;41(12):1711–1739
- 8 Osuch-Kwiatkowska A, Zeng QD, Cieplak M, Jarosz S. A review on the stereoselective synthesis of higher carbon sugars with an eye to making higher alditols. *Curr Org Chem* 2014;18(03):327–340
- 9 Marco-Contelles J, de Opazo E, Arroyo N. Synthesis of higher-carbon sugars by addition of organometallic reagents to aldehydes or lactols derived from carbohydrates. *Tetrahedron* 2001;57(22):4729–4739
- 10 Danishefsky SJ, DeNinno MP. Totally synthetic routes to the higher monosaccharides. *Angew Chem Int Ed Engl* 1987;26(01):15–23
- 11 Popik O, Pasternak-Suder M, Baś S, Mlynarski J. Organocatalytic synthesis of higher-carbon sugars: efficient protocol for the synthesis of natural sedoheptulose and d-glycero-l-galacto-oct-2-ulose. *ChemistryOpen* 2015;4(06):717–721
- 12 Lin CI, McCarty RM, Liu HW. The biosynthesis of nitrogen-, sulfur-, and high-carbon chain-containing sugars. *Chem Soc Rev* 2013;42(10):4377–4407
- 13 Harada S, Mizuta E, Kishi T. Structure of mildiomycin, a new antifungal nucleoside antibiotic. *J Am Chem Soc* 1978;100(15):4895–4897
- 14 Harada S, Mizuta E, Kishi T. Structure of mildiomycin, a new antifungal nucleoside antibiotic. *Tetrahedron* 1981;37(07):1317–1327
- 15 Yadav MK, Park SW, Chae SW, Song JJ. Sinefungin, a natural nucleoside analogue of S-adenosylmethionine, inhibits *Streptococcus pneumoniae* biofilm growth. *BioMed Res Int* 2014;2014:156987
- 16 Zheng W, Ibáñez G, Wu H, et al. Sinefungin derivatives as inhibitors and structure probes of protein lysine methyltransferase SETD2. *J Am Chem Soc* 2012;134(43):18004–18014
- 17 Liu HM, Zou DP, Zhang FY, Zhu WG, Peng T. Stereoselective synthesis of new higher carbon sugars from D-xylose. *Eur J Org Chem* 2004;2004(10):2103–2106
- 18 Wang S, Zhao L, Shi XJ, et al. Development of highly potent, selective, and cellular active triazolo[1,5- a]pyrimidine-based inhibitors targeting the DCN1-UBC12 protein-protein interaction. *J Med Chem* 2019;62(05):2772–2797
- 19 Wang S, Shen DD, Zhao LJ, et al. Discovery of [1,2,4]triazolo [1,5-a]pyrimidine derivatives as new bromodomain-containing protein 4 (BRD4) inhibitors. *Chin Chem Lett* 2020;31(02):418–422