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Highly Substituted Cyclopentane–CMP Conjugates as Potent Sialyltransferase Inhibitors

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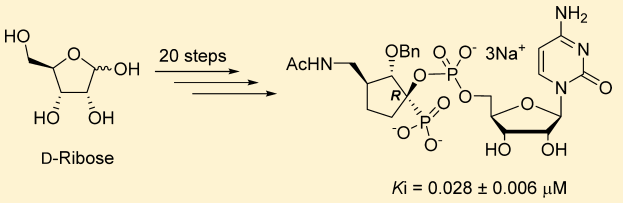
Supporting Information

ABSTRACT: Sialylconjugates on cell surfaces are involved in many biological events such as cellular recognition, signal transduction, and immune response. It has been reported that aberrant sialylation at the nonreducing end of glycoconjugates and overexpression of sialyltransferases (STs) in cells are correlated with the malignance, invasion, and metastasis of tumors. Therefore, inhibitors of STs would provide valuable leads for the discovery of antitumor drugs. On the basis of the transition state of the enzyme-catalyzed sialylation reaction, we proposed that the cyclopentane skeleton in its two puckered conformations might mimic the planar structure of the donor (CMP-Neu5Ac) in the transition state. A series of cyclopentane-containing compounds were designed and synthesized by coupling different cyclopentane α-hydroxyphosphonates with cytidine phosphoramidite. Their inhibitory activities against recombinant human ST6Gal-I were assayed, and a potent inhibitor **48*l*** with a K_i of 0.028 ± 0.006 μM was identified. The results show that the cyclopentanoid-type compounds could become a new type of sialyltransferase inhibitors as biological probes or drug leads.



INTRODUCTION

Sialic acids (in which the most abundant being Neu5Ac, known as *N*-acetylneuraminic acid) are nine-carbon α-keto aldonic acids, typically located at the nonreducing terminus of glycans presented in glycoproteins and glycolipids of vertebrates.[1,2] They are also the components of lipooligosaccharides or capsular polysaccharides of pathogenic bacteria.[3] The sialylconjugates play important roles in a variety of physiological or pathological processes including cell differentiation and signaling, immunological regulation, bacterial and viral infection, cancer metastasis, etc.[4] In the human body, the biosynthesis of sialylconjugates is predominantly controlled by 20 sialyltransferases. It is reported[5] that sialyltransferases overexpression and sialylated antigen overpresentation on cell surfaces are correlated with cancer metastasis and poor prognosis in different types of carcinomas such as human colorectal cancer,[6] malignant glioma,[7] breast carcinoma,[8] cervical carcinoma,[9] melanoma,[10] and leukemia.[11]

Independent of their origins and subtypes, all sialyltransferases share the same donor substrate, cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac), and transfer the sialic acid residue onto the terminal nonreducing site of sugar chains in glycoproteins and glycolipids. Currently, all sialyltransferase inhibitors[12−15] can be classified into donor analogues,[16−21] acceptor analogues,[22−26] bisubstrate analogues,[27−29] and transition-state analogues[30−36] and also some others can be found or modified from natural products.[37−41] Up to now, the most potent sialyltransferase inhibitor in vitro is the transition-state analogue **1*h*** (K_i of 0.029 ± 0.006 μM against rat α(2−6)-sialyltransferase) reported by Schmidt et al. in 2002 (Figure 1A),[35] which is consistent with Pauling's statement in 1946 that inhibitors closely imitating the structure of the transition state in an enzyme-catalyzed process could bind more tightly to the active site of the enzyme than the ground-state substrates.[42] Sialyltransferase inhibitors in vivo such as KI-8110,[16] soyasaponin I,[43] and Lith-O-Asp[44] (Figure 1B) could effectively attenuate the total sialylation on cancer cell surfaces and suppress tumor cell metastasis. Although some advances in sialyltransferase inhibitors have been achieved, it is still highly desirable to seek and develop more efficient sialyltransferase inhibitors to elucidate the biological functions of sialylation and even provide valuable leads for antitumor drug development.

The cyclopentane moiety is widespread in natural products and has been a valuable scaffold in drug discovery.[45] Successful examples include the anti-HIV drug abacavir,[46] the orally active and selective sphingosine 1-phosphate receptor-subtype 1 (S1P1) agonist VPC01091,[47] the experimental antiviral drug peramivir,[48] and so on. Generally, the cyclopentane ring tends to adopt the half-chair or envelope conformation, but it also interconverts between different half-chair and envelope conformations as well as intermediate ones due to the low energy barrier between these conformations (Figure 1C).[49]

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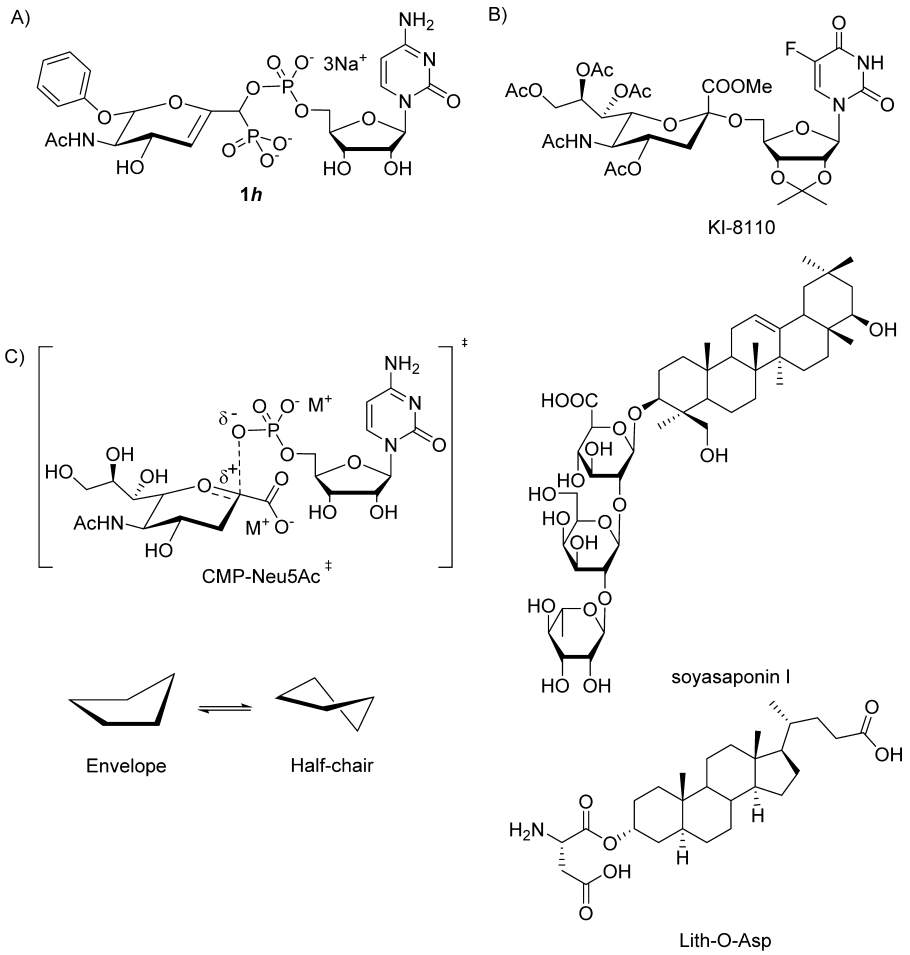
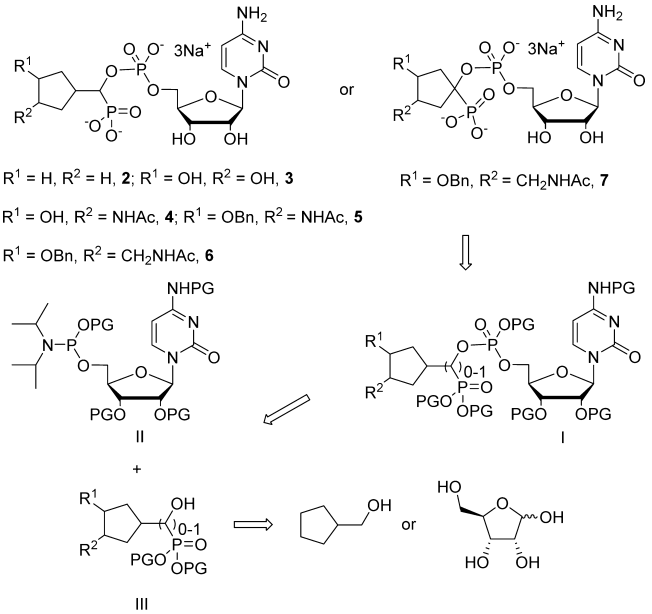


Figure 1. (A) Transition-state analogue inhibitor **1*h***. (B) Sialyltransferase inhibitors applied in vivo. (C) Transition state of sialylation reaction and two puckered conformations of the cyclopentane ring.

According to the proposed S_N1-like mechanism of the sialyltransferase-catalyzed sialylation reactions,[50−52] the donor in the transition state may involve the partial dissociation of the CMP moiety and the formation of a trigonal planar oxocarbenium ion (Figure 1C). Therefore, with great flexibility and adaptability of its conformations, the substituted cyclopentane ring might mimic the planar structure of the donor in the transition state and could potentially serve as a sialyltransferase inhibitor. Herein we want to report the synthesis of cyclopentane-merged compounds as sialyltransferase inhibitors and the studies on their structure−activity relationships.

On the basis of the previous studies by Schmidt group[15,31,32,53] and Horenstein group,[34] there are several valuable clues for designing potent transition-state-based sialyltransferase inhibitors: (1) the CMP moiety is very important to maintain the affinity to the enzyme,[53] (2) the Neu5Ac residue could be modified or even replaced by other different scaffolds,[31,34,36] (3) a planar structure close to the cleavage site to mimic the conformation of the oxocarbenium ion is fundamental for the inhibitory activity,[31,33] (4) two adjacent negative charge centers are preferred as in CMP-Neu5Ac,[31,36] (5) the phosphonate is a better choice than the carboxylate as the negative charge center,[36] and (6) an increased distance between the planar anomeric carbon and the CMP leaving group benefits to mimic the slightly longer C−O bond in the transition state.[31] Thus, in our design (Scheme 1), the Neu5Ac moiety was replaced by cyclopentyl α-hydroxyphosphonates and the effects of different substituents

Scheme 1. Design of Cyclopentanoid Inhibitors and the Retrosynthetic Analysis



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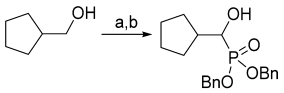
on the cyclopentane ring were investigated. At the 3-position of the cyclopentane ring, the hydrophilic hydroxyl group (**3** vs **2**) and the hydrophobic benzyloxy group (**5** vs **4**) were introduced to investigate their inhibitory activities. At the 4-position of the cyclopentane ring, acetylamino group (**4** and **5**) was chosen as that in the Neu5Ac, and the acetylaminomethyl group (**6**) with one more carbon was introduced to compare the difference. Additionally, the difference of diastereoisomers and the effect of the distance between the cyclopentane ring and the CMP leaving group (**7** vs **6**) were also considered.

As shown in Scheme 1, the target compounds **2**–**7** could be prepared from the masked compounds **I**, which were retrosynthetically disconnected into two units: the cytidine phosphoramidite building block (**II**) and the substituted cyclopentanoid α-hydroxyphosphonates (**III**). Intermediates **III** could be obtained via transformations starting from cyclopentanemethanol or D-ribose. After the synthetic work was completed, the inhibitory activities of these target molecules against $\alpha(2-6)$-sialyltransferase would be evaluated.

RESULTS AND DISCUSSION

1. Synthesis of α-Hydroxyphosphonates. According to the retrosynthetic analysis, the key part for obtaining target molecules is the construction of α-hydroxyphosphonates (**III**) that are derived from differently substituted cyclopentanoid alcohols. At first, as shown in Scheme 2, the commercially

Scheme 2. Synthesis of α-Hydroxyphosphonate 8[a]



8

[a]Reagents and conditions: (a) DMSO, oxalyl chloride, CH_2Cl_2, −72 °C, 2 h, then triethylamine, 1 h; (b) dibenzyl phosphite, triethylamine, CH_2Cl_2, rt, overnight, 44% over two steps.

available cyclopentanemethanol was converted to the volatile aldehyde by Swern oxidation, which was followed by H-phosphonate addition,[31] affording α-hydroxyphosphonate **8** as enantiomers in moderate yield (44%).

Because all the substituted cyclopentanoid alcohols could be synthesized from a common intermediate **17**, we developed an efficient method to prepare **17** from commercially available D-ribose based on the Dieckmann condensation.

As shown in Scheme 3, starting from D-ribose, the known enol ether **11** was obtained according to the previous reports[54,55] with some modifications in 74% overall yield over three steps (see Supporting Information). The ozonolysis of **11** gave the lactone, which was followed by the acidic methanolysis and subsequent Wittig reaction[56] to afford olefin **12** in 65% yield over three steps. The hydrogenation of the double bond in **12** provided compound **13**, which was transformed to cyclopentene **14** in 73% yield by the Dieckmann condensation.[57] The reduction of enol **14** by $NaBH_4$ and subsequent benzoylation of the free hydroxyl group yielded cyclopentane **16** as epimers, which were separated carefully in this step and their absolute configurations were ascertained by NMR analysis (NOESY). The cross-peaks between the proton at δ_H 3.16 (H1) and the proton at δ_H 4.70 (H3) in **16*R*** confirmed that they were cofacially oriented, while in **16*S*** there was no correlation between the proton at δ_H 3.33 (H1) and the proton at δ_H 4.87 (H3) (Figure 2). After treatment of **16** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), both diastereoisomers were smoothly converted to unsaturated ester **17** via β-elimination.

With compound **17** in hand, the synthesis of α-hydroxyphosphonate **20** was carried out (Scheme 4). Compound **17** was subjected to hydrogenation to provide methyl carboxylate **18**, which was further reduced to alcohol **19** with $LiBH_4$[58] in 83% yield. Compound **19** was oxidized by (diacetoxyiodo) benzene (BAIB) and catalytic amount of tetramethylpiperidinooxy (TEMPO) to afford the corresponding aldehyde, which underwent H-phosphonate addition to produce α-hydroxyphosphonate **20** as enantiomers.

Previous studies demonstrated the importance of a hydrophobic group in the inhibitors,[35] so we decided to synthesize some cyclopentane-containing molecules with hydrophobic substituents (Scheme 5).

Acidic cleavage of the isopropylidene functionality in compound **17** provided diol **21**, which was selectively benzylated at the allylic position with Ag_2O[59] to produce compound **22**. The absolute configuration of **22** was confirmed by its NMR analysis (HMBC). In the HMBC spectrum, the methylene protons at δ_H 4.68 (PhCH_2) showed correlation to the carbon at δ_C 83.0 (C3) and also the proton at δ_H 4.52 (H3) was correlated with the carbon at δ_C 72.7 (PhCH_2) (Figure 3). Next, selective reduction of the double bond in **22** was performed by using nickel borohydride generated in situ from $NiCl_2{\cdot}6H_2O$ and $NaBH_4$,[60] affording two diastereoisomers **23*R*** and **23*S*** (*dr* = 3:1) in 92% yield. The absolute configuration of the newly formed chiral center was assigned by the NMR analysis (NOESY). The cross-peaks between the proton at δ_H 2.75 (H1) and the proton at δ_H 3.80 (H3) in **23*R*** confirmed that they were cofacially oriented (Figure 3).

Subsequently, the free hydroxyl groups of both diastereoisomers were respectively subjected to tosylation followed by treatment with sodium azide to provide **24*R*** and **24*S*** in high yields. Under the conditions of thioacetic acid in pyridine,[61,62] the azides **24*R*** and **24*S*** were smoothly converted to compounds **25*R*** and **25*S***. Then by using the similar procedures as the preparation of **20**, α-hydroxyphosphonates **27*R*** and **27*S*** were easily synthesized.

The preparation of α-hydroxyphosphonate **34** containing acetylaminomethyl group at the 4-position of the cyclopentane ring was shown in Scheme 6, starting from the major diastereoisomer **23*R***. After reduction with $LiBH_4$, the newly formed primary hydroxyl in **28** was selectively protected with the *tert*-butyldiphenylsilyl group[63] and the free secondary hydroxyl was tosylated to afford compound **30**. Then replacing the tosylate by a cyano group was tried. Using KCN as the reagent, conditions such as different solvents (DMF, CH_3CN, DMSO), temperatures, and additives (18-crown-6 or 4 Å molecular sieves) were screened. Unfortunately, only elimination products were observed. Switching to Bu_4NCN as the cyano source, cyanide **31** was finally obtained in 48% yield at 85 °C in toluene along with some elimination byproduct. The cyano group in **31** was then reduced with $NaBH_4/CoCl_2$ to amine,[64] which was followed by acetylation to produce compound **32**. Removal of the TBDPS group with tetra-*n*-butylammonium fluoride yielded alcohol **33** quantitatively. Then it was subjected to Swern oxidation followed by treatment with dibenzyl phosphite and NaH to produce α-hydroxyphosphonate **34** as a diastereoisomeric mixture in 67% yield.

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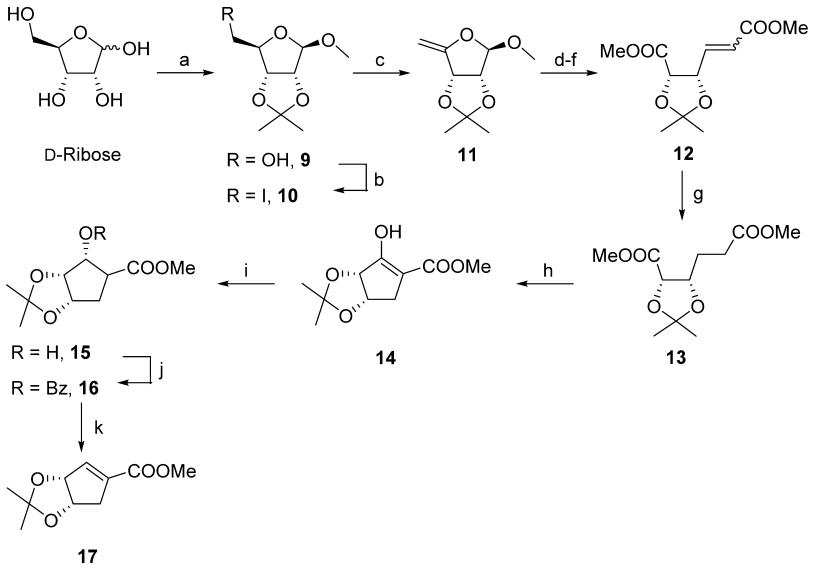
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis of the Common Intermediate 17[a]



[a]Reagents and conditions: (a) H_2SO_4, HCl(g), MeOH/acetone, rt, 8 h, 85%; (b) triphenylphosphine, imidazole, I_2, toluene, 70 °C, 2 h, 92%; (c) NaH, DMF, 0 °C to rt, overnight, 94%; (d) O_3, CH_2Cl_2/MeOH (1:2), −72 °C, 1 h, then dimethyl sulfide, 6 h; (e) *p*-toluenesulfonic acid, MeOH, rt; (f) $Ph_3P{=}CHCOOMe$, THF, rt, overnight, 65% over three steps; (g) Pd/C, H_2, ethyl acetate, rt, 24 h, 99%; (h) NaH, MeOH, toluene, 0−90 °C, 1 h, 73%; (i) $NaBH_4$, MeOH/CH_2Cl_2 (2:1), −72 °C, 5 h, 95%; (j) benzoyl chloride, 4-dimethylaminopyridine (DMAP), pyridine, 0 °C to rt, 4 h, 92%; (k) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), THF, 0 °C to reflux; for **16*R***, 1.5 h, 92%; for **16*S***, 4 h, 81%.

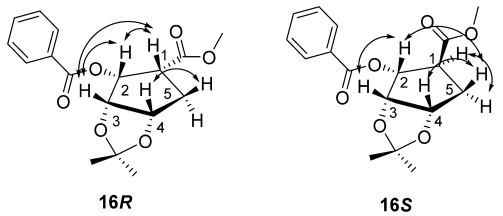
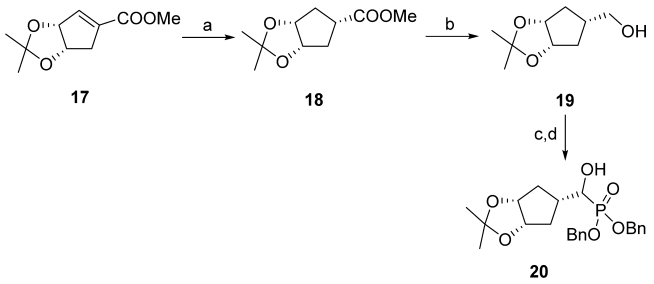


Figure 2. Key NOESY correlations of **16*R*** and **16*S***.

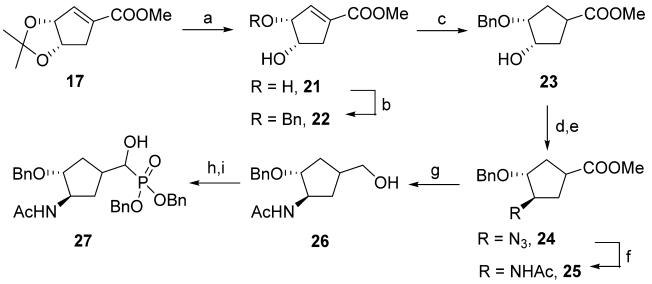
Scheme 4. Synthesis of α-Hydroxyphosphonate 20[a]



[a]Reagents and conditions: (a) Pd/C, H_2, ethyl acetate, rt, 15 h, 92%; (b) $LiBH_4$, MeOH, THF, 0 °C, 3 h, 83%; (c) (diacetoxyiodo)benzene, 2,2,6,6-tetramethylpiperidinooxy, CH_2Cl_2, 0 °C to rt, 6 h; (d) dibenzyl phosphite, triethylamine, CH_2Cl_2, rt, overnight, 71% over two steps.

Next, the cyclopentanone α-hydroxyphosphonate **43** was prepared. As shown in Scheme 7, starting from intermediate **17**, the methyl ester functionality was reduced by diisobutylaluminum hydride (DIBAL-H)[65] to afford alcohol **35**, which was followed by tosylation with TsCl/NaOH in ether[66] to give compound **36** in high yield. The tosyl group in **36** was easily replaced by the azido group at room temperature. After extraction, the volatile azide was directly treated under standard hydroboration-oxidation conditions[67] to generate compound **37** with a secondary hydroxyl *trans* to the vicinal protected diol,

Scheme 5. Synthesis of α-Hydroxyphosphonates 27*R* and 27*S*[a]



[a]Reagents and conditions: (a) 90% (v/v) acetic acid, 90 °C, 2 h, 85%; (b) Ag_2O, benzyl bromide, tetrabutylammonium iodide, CH_2Cl_2, rt, 6 h, 74%; (c) $NaBH_4$, $NiCl_2{\cdot}6H_2O$ (4% in MeOH), MeOH, 0 °C, 2.5 h, 92% (**23*R*:23*S*** = 3:1); (d) tosyl chloride, DMAP, triethylamine, CH_2Cl_2, 0 °C to rt, overnight; (e) NaN_3, DMF, 90 °C, overnight, **24*R*** 96% over two steps, **24*S*** 97% over two steps; (f) thioacetic acid, pyridine, rt, **25*R*** 36 h, 88%, **25*S*** 48 h, 86%; (g) $LiBH_4$, MeOH, THF, 0 °C, **26*R*** 1 h, 88%, **26*S*** 4 h, 98%; (h) (diacetoxyiodo)benzene, 2,2,6,6-tetramethylpiperidinooxy, CH_2Cl_2, 0 °C to rt, 17 h for **26*R***, 11 h for **26*S***; (i) dibenzyl phosphite, triethylamine, CH_2Cl_2, rt, overnight, **27*R*** 91% over two steps, **27*S*** 87% over two steps.

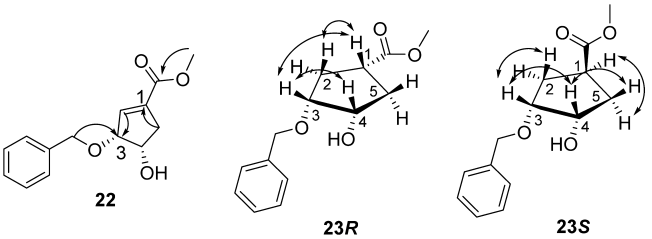


Figure 3. Key HMBC correlations of **22** and key NOESY correlations of **23*R*** and **23*S***.

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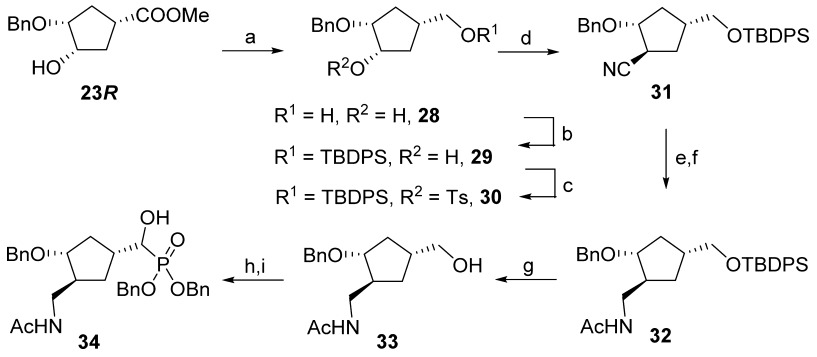
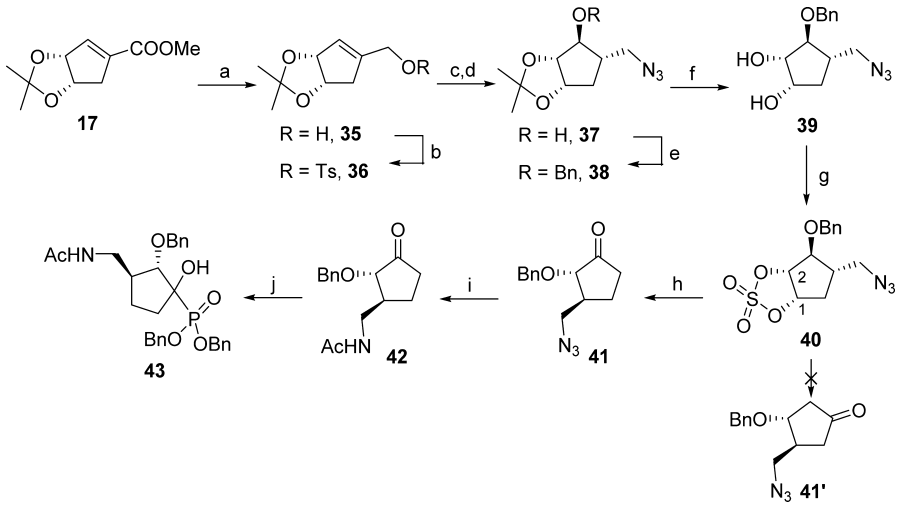
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 6. Synthesis of α-Hydroxyphosphonate 34[a]



[a]Reagents and conditions: (a) $LiBH_4$, MeOH, THF, 0 °C, 1.5 h, 98%; (b) *tert*-butyldiphenylchlorosilane, DMAP, triethylamine, CH_2Cl_2, 0 °C to rt, 36 h, 88%; (c) tosyl chloride, DMAP, pyridine/CH_2Cl_2 (3/1), 0 °C to rt, 24 h, 89%; (d) Bu_4NCN, toluene, 85 °C, 24 h, 48%; (e) $NaBH_4$, $CoCl_2$, MeOH, 0 °C, 3 h; (f) acetic anhydride, triethylamine, CH_2Cl_2, rt, overnight, 60% over two steps; (g) 1 M tetra-*n*-butylammonium fluoride in THF, THF, rt, overnight, quantitative; (h) DMSO, oxalyl chloride, CH_2Cl_2, −72 °C, 2 h, then triethylamine, 1 h; (i) dibenzyl phosphite, NaH, THF, 0 °C to rt, overnight, 67% over two steps.

Scheme 7. Synthesis of α-Hydroxyphosphonate 43[a]



[a]Reagents and conditions: (a) diisobutylaluminum hydride (DIBAL-H), toluene, −78 °C, 4 h, 98%; (b) TsCl, NaOH, ether, 0 °C to rt, 24 h, 97%; (c) NaN_3, DMF, rt, overnight; (d) 1 M B_2H_6·THF, THF, 0 °C, 5 h, then 3 N NaOH, 27% H_2O_2 (aq), 0−40 °C, overnight, 77% over two steps; (e) BnBr, NaH, DMF, 0 °C to rt, overnight, 87%; (f) 90% (v/v) acetic acid (aq), 70 °C, 5 h, 98%; (g) Im_2SO_2, NaH, THF, −20 °C, 45 min, 80%; (h) *t*BuOK, THF, rt, 40 min, 5% H_2SO_4, rt, overnight, 90%; (i) thioacetic acid, pyridine, rt, 4 h, 74%; (j) dibenzyl phosphite, NaH, THF, 0 °C to rt, overnight, 88%.

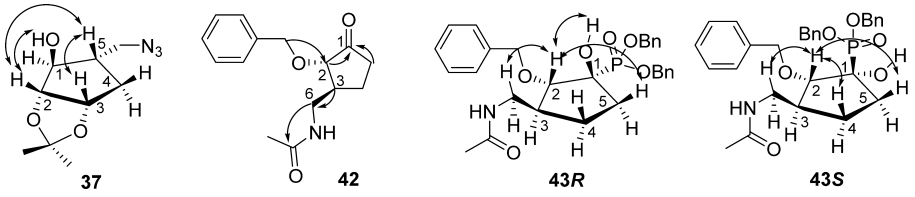


Figure 4. Key NOESY correlations of **37**, **43*R***, **43*S***, and key HMBC correlations of **42**.

whose absolute configuration was assigned by its NMR analysis (NOESY). The cross-peaks between the proton at δ_H 2.18 (H5) and the protons at δ_H 4.40 (H2) and 4.71 (H3) in **37** demonstrated that they were cofacially oriented. In addition, the proton at δ_H 2.51 (OH) was correlated with the proton at δ_H 4.40 (H2), therefore the hydroxyl group was believed to be *β*-oriented (Figure 4).

Benzylation of **37** and subsequent removal of the isopropylidene group produced diol **39**, which was converted to cyclic sulfate **40** with sulfonyl diimidazole (Im_2SO_2)[68] and sodium hydride. Compound **40** was then treated with *t*BuOK to give the vinyl sulfate intermediate, which was tautomerized in acidic conditions.[69] However, it provided **41** instead of the desired ketone **41′** in 90% yield with the carbonyl group on the 2-position. Reductive acetylation of azide **41** with thioacetic acid afforded cyclopentanone **42** in 74% yield, and the absolute configuration was confirmed by the NMR analysis (H−H COSY, HSQC and HMBC). As in the HMBC spectrum, the

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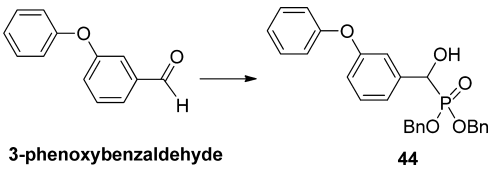
methylene protons at δ_H 5.12 and 4.67 ($PhCH_2$) were correlated with the carbon at δ_C 84.5 (C2), so the doublet proton at δ_H 3.62 (H2) showed that the carbonyl group was on the position near the benzyloxy group (Figure 4). H-Phosphonate addition to ketone **42** produced α-hydroxyphosphonate **43** as a mixture of two diastereoisomers (***R*:*S*** = 1:1.6, determined by ^1H NMR). The mixture could be partially separated, and their absolute configuration was confirmed by the NMR analysis (NOESY). The cross-peaks between the proton at δ_H 3.54 (OH) and the proton at δ_H 3.84 (H2) in **43*R*** demonstrated that they were cofacially oriented, while in **43*S*** the proton at δ_H 3.26 (OH) did not show any correlations with the proton at δ_H 3.79 (H2) (Figure 4).

Because of its good inhibitory activity against rat α(2−6)-sialyltransferase, the 3-phenoxybenzyl-derived compound **49*l*** prepared by Schmidt et al.[36] was selected as the control compound for the following biological assay. Thus, 3-phenoxybenzaldehyde was transformed to α-hydroxyphosphonate **44** as enantiomers by treatment with dibenzyl phosphite and triethylamine in THF (Scheme 8).

Scheme 8. Synthesis of α-Hydroxyphosphonate 44[a]

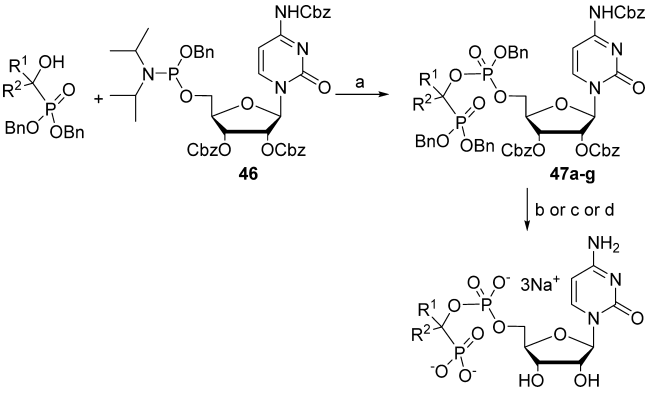


[a]Reagents and conditions: dibenzyl phosphite, triethylamine, THF, rt, overnight, 84%.

2. Synthesis of Cytidine Phosphoramidite. After finishing the preparation of various α-hydroxyphosphonates, the benzyl and benzyloxycarbonyl (Cbz) protected cytidine phosphoramidite **46** was obtained based on the previous report.[70] The Cbz-protected cytidine **45** was prepared from cytidine according to the literature procedure.[71] Compound **45** was condensed with benzyloxybis(diisopropylamino)-phosphine, which was prepared from bis(*N*,*N*-diisopropylamino)chlorophosphine and benzyl alcohol,[72] affording **46** in high yield (Scheme 9).

3. Synthesis of Target Molecules. With α-hydroxyphosphonates and building block **46** in hand, the coupling reaction was performed (Scheme 10). Condensation of **8** with cytidine phosphoramidite **46** in the presence of *H*-tetrazole and

Scheme 10. Synthesis of Target Molecules[a]

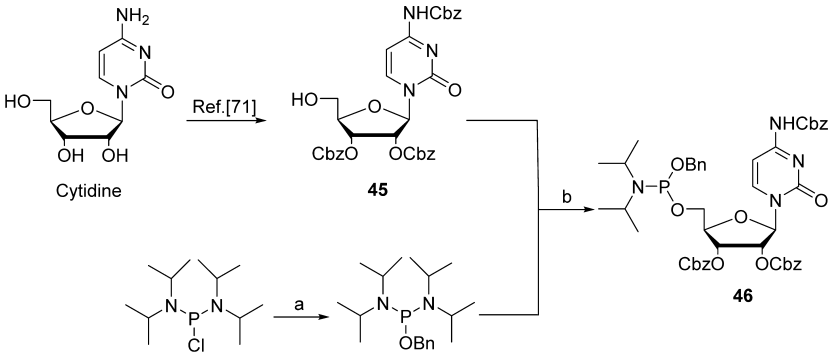


[a]Reagents and conditions: (a) *H*-tetrazole, CH_2Cl_2, rt, then *tert*-butyl hydroperoxide, rt; (b) (i) 10% Pd/C, 1,4-cyclohexadiene, DMF, rt, (ii) RP-HPLC, (iii) IR 120 Na^+; (c) (i) 1 M BCl_3 in heptane, CH_2Cl_2, 0 °C to rt, (ii) RP-HPLC, (iii) IR 120 Na^+; (d) (i) 10% Pd/C, 1,4-cyclohexadiene, EtOH, rt, (ii) RP-HPLC, (iii) IR 120 Na^+.

subsequent oxidation of the phosphite with *tert*-butyl hydroperoxide provided benzyl- and Cbz-protected target molecule **47a** as a mixture of four diastereoisomers. The global deprotection of **47a** was achieved by using 10% Pd/C and 1,4-cyclohexadiene in DMF.[71] After filtration and purification by C18 reverse-phase column chromatography ($H_2O \rightarrow H_2O$/MeOH 5:1), the crude product **2** was obtained in 75% yield. The two diastereoisomers were further separated by preparative RP-HPLC, converted to their corresponding sodium salt by ion-exchange (IR 120 Na^+), and lyophilized from water, yielding the final products **2*s*** and **2*l*** as white powder (***s*** stands for the component with a shorter retention time while ***l*** stands for the component with a longer retention time) (Table 1, entry 1).

Following the same procedure as the preparation of **47a**, masked target molecules **47b−g** were successfully synthesized. To obtain products **3*s*** and **3*l***, the isopropylidene, benzyl, and Cbz protective groups in **47b** were all removed smoothly by treatment with BCl_3 in CH_2Cl_2 (Table 1, entry 2).[73,74] The full deprotection of the Cbz and benzyl groups in **47c** was realized by catalytic transfer hydrogenation in DMF, affording **4*S*** in 79% yield (Table 1, entry 3). When the solvent was changed from DMF to EtOH,[75] the reaction showed some selectivity between the ether benzyl and ester benzyl, thus compound **5*S*** was obtained in moderate yield (Table 1, entry 3). Finally,

Scheme 9. Synthesis of Cytidine Phosphoramidite 46[a]



[a]Reagents and conditions: (a) BnOH, triethylamine, ether, 0 °C to rt, 1 h; (b) *H*-tetrazole, CH_2Cl_2, 0 °C to rt, 4 h, 92% over two steps.

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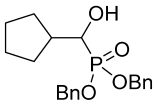
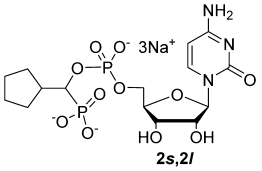
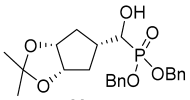
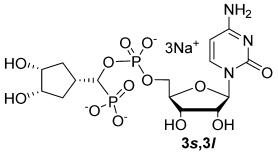
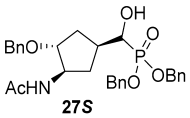
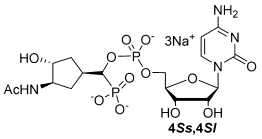
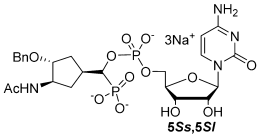
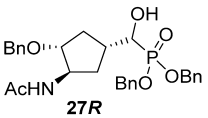
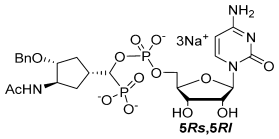
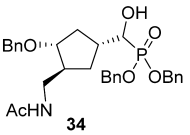
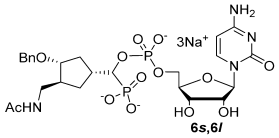
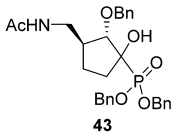
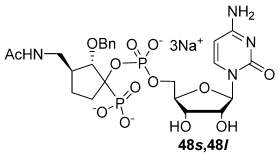
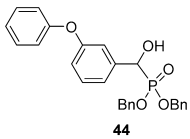
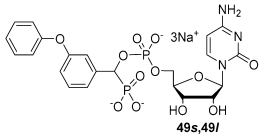
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. Coupling of Different α-Hydroxyphosphonates and the Deprotection

Entry	α-Hydroxyphosphonate	Coupling product	Final product	Deprotection[a]
1	 8	**47a**: 72%	 **2s,2l**	b: 75%
2	 20	**47b**: 90%	 **3s,3l**	c: 91%
3	 **27S**	**47c**: 76%	 **4Ss,4Sl**	b: 79%
			 **5Ss,5Sl**	d: 44%
4	 **27R**	**47d**: 79%	 **5Rs,5Rl**	d: 50%
5	 34	**47e**: 66%	 **6s,6l**	d: 52%
6	 43	**47f**: 76%	 **48s,48l**	d: 73%
7[36]	 44	**47g**: 81%	 **49s,49l**	b: 60%

[a]The data are the isolated yields of the deprotection procedures.

using the same procedures mentioned above, compounds **47d–g** were transformed to their corresponding target molecules.

4. Biological Assay. Measurement of the inhibition data and kinetic constants of K_i against recombinant human ST6Gal-I (aa 44-406)[76] was carried out based on the UV/RP-HPLC method developed by Schmidt group[30] with some modifications. CMP-Neu5Ac and *p*-nitrophenyl LacNAc were used as the donor and acceptor, and the product trisaccharide was detected by analytical RP-HPLC at 300 nm. Compound **49*l*** with known K_i[36] was selected as the control (Figure 5).

In our assay, when the acceptor *p*-nitrophenyl LacNAc was set at 1 mM, the K_m for CMP-Neu5Ac was determined to be 41.63 ± 6.66 μM (see Supporting Information, Figure S1), which is comparable to the reported K_m data ranging from 4.77[41] to 18 μM.[77] The inhibition rates of all the target compounds were first obtained at 10 μM level, then the K_i values were determined for the inhibitors whose inhibitory rates are superior to 50%. The Lineweaver–Burk double reciprocal plots as well as comparison among different inhibition modes of the inhibitors in GraphPad Prism 6 suggest competitive inhibition of the inhibitors (see Supporting Information, K_i data in Figures S2–S10). As shown in Table 2, the nonsubstituted cyclopentyl inhibitor **2*s*** exhibits about 7-fold stronger affinity to the enzyme rhST6Gal-I than the natural

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J. Med. Chem. XXXX, XXX, XXX–XXX

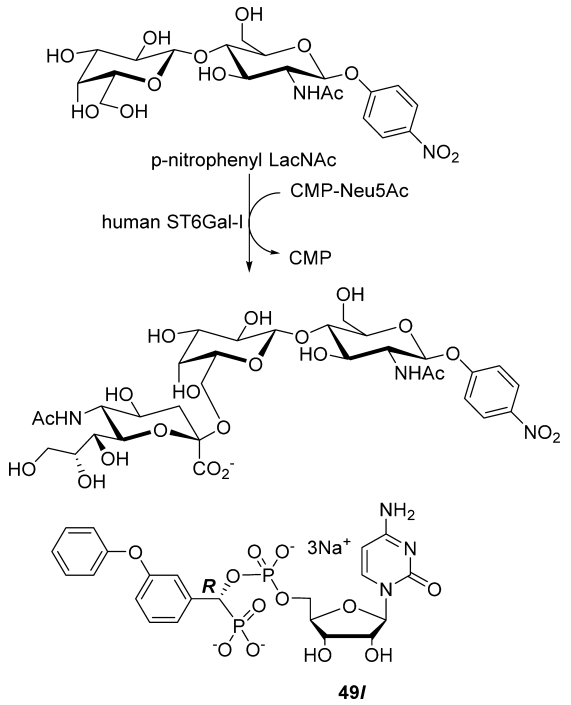

Figure 5. Enzymatic sialylation of acceptor *p*-nitrophenyl LacNAc and the structure of control compound **49*l***.

Table 2. Affinity of CMP-Neu5Ac (K_m) to Recombinant Human ST6Gal-I and Inhibition Data of the Cyclopentanoid Inhibitors[a]

compd [α]	inhibition at 10 μM (%)	K_m or K_i (μM)	K_m/K_i
CMP-Neu5Ac		41.63 ± 6.66	
2*s* (+17.0)	43.25	5.852 ± 0.908	7.1
2*l* (+0.9)	12.82		
3*s* (+6.7)	30.99		
3*l* (+2.9)	12.98		
4S*s* (+4.0)	50.30	4.314 ± 0.968	9.6
4S*l* (−3.9)	9.99		
5S*s* (−1.7)	63.86	1.629 ± 0.285	25.6
5S*l* (+0.5)	75.29	0.436 ± 0.117	95.5
5R*s* (+0.7)	65.87	1.510 ± 0.334	27.6
5R*l* (−3.7)	22.08		
6*s* (−3.6)	70.83	0.865 ± 0.141	48.1
6*l* (−7.1)	46.27		
48*s* (−22.2)	74.13	0.477 ± 0.087	87.3
48*l* (−11.5)	97.50	0.028 ± 0.006	1486.8
49*l*[36]	98.12	0.019 ± 0.005	2191.0

[a]For details of the procedures, see Supporting Information.

substrate CMP-Neu5Ac, indicating that the cyclopentane ring could mimic the Neu5Ac residue or its planar structure in the transition state. It seems that the hydrophilic hydroxyl group on the cyclopentane ring does not contribute to the inhibition and even results in erosion of the activity (**3*s***, **3*l*** vs **2*s***, **2*l***). Comparing **4S*s*** and **4S*l*** with **5S*s*** and **5S*l***, the hydrophobic benzyl group on the 3-position of the cyclopentane ring is really appreciated, which may possess some useful interaction with the hydrophobic area in the catalytic site of the enzyme and it is also consistent with the previous results reported by Schmidt et al. in 2002.[35] On the 4-position of the cyclopentane ring, adding one more carbon is somewhat useful for the activity (**6*s***, **6*l*** vs **5R*s***, **5R*l***). As **5S*s*** and **5S*l*** are better inhibitors than **5R*s*** and **5R*l***, the configuration of the C-1 position of the cyclopentane ring is also an important factor, and the ***S*** isomer might be a better choice. To our delight, the cyclopentanoid inhibitor **48*l*** that is the surrogate of the designed target molecule **7*l*** shows the best inhibitory activity (K_i = 0.028 ± 0.006 μM), which is 15-fold stronger than the best cyclopentanemethyl-containing inhibitor **5S*l*** and is almost equivalent to the control inhibitor **49*l*** (Ki = 0.019 ± 0.005 μM). Perhaps the cyclopentane ring with a quaternary carbon center could adopt a more planar conformation, thus better mimicking the Neu5Ac residue in the transition state.

5. Configuration Assignment of 48*l*. Since the new stereogenic center in **43*R*** was assigned, the condensation of the pure compound **43*R*** with **46** was carried out, which was followed by the oxidation under the same conditions as mentioned above to produce **47f*R***. After selective removal of the ester benzyl groups in **47f*R***, purification, and conversion to the corresponding sodium salt, the final product **48*R*** was obtained. It was found that the ^{1}H NMR spectrum of **48*R*** was the same as that of **48*l***. Therefore, the absolute configuration of the new stereogenic center in **48*l*** was determined to be ***R***.

CONCLUSIONS

In conclusion, with the proposal that the comformations of cyclopentane ring may mimic the planar character of the oxocarbenium ion in the transition state of the enzyme-catalyzed sialylation, we designed and synthesized 14 cyclopentane-containing compounds by coupling the highly substituted cyclopentane α-hydroxyphosphonates with cytidine phosphoramidite. The synthetic compounds were evaluated against the sialyltransferase (recombinant human ST6Gal-I), and several compounds with inhibitory activities were identified. Especially, among them, a potent transition state-based inhibitor **48*l*** with K_i of 0.028 ± 0.006 μM was obtained. These results show that the cyclopentane moiety could be introduced as a key structural motif for the discovery of new sialyltransferase inhibitors. Further structural modifications and their applications in the development of antitumor leads and synthesis of biological probes are now under investigation.

EXPERIMENTAL SECTION

General. All chemicals purchased were reagent grade and used without further purification unless otherwise stated. Dichloromethane (CH_2Cl_2) and pyridine were distilled over calcium hydride (CaH_2). *N*,*N*-Dimethylformamide (DMF) was stirred with CaH_2 and distilled under reduced pressure. Methanol and ethanol were distilled from magnesium and iodine. Toluene, tetrahydrofuran (THF), and ether were distilled over sodium/benzophenone. All reactions were carried out under anhydrous conditions using flame-dried glassware with freshly distilled solvents unless otherwise noted. Reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel 60 F_{254} precoated on aluminum plates (E. Merck). Spots were detected under UV (254 nm) light and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 40 °C (water bath). Column chromatography was performed on silica gel (200−300 mesh) or C18 reverse phase silica gel. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and 2D NMR spectra were recorded on an Avance III Bruker-600 or a Varian INOVA-500 or an Avance DRX Bruker-400 spectrometer at 25 °C. Chemical shifts (in ppm) were calibrated with the solvent residual peak. Mass spectra were recorded by using a Waters Xevo G2 Q-TOF spectrometer. Elemental analysis data were recorded on a Vario EL-III elemental analyzer. Optical rotation was determined by a Rudolph Research Analytical Autopol IV polarimeter. Preparative HPLC was performed with Waters 2545 autopurification system and detected at 254 nm.

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J. Med. Chem. XXXX, XXX, XXX−XXX

Columns: (A) SunFire Prep C18 OBD (Waters, 5 μm, 19 mm × 150 mm), (B) XBridge Prep C18 OBD (Waters, 5 μm, 19 mm × 150 mm). The purity of all the final compounds was determined to be ≥95% (see Supporting Information Table S1) by HPLC analysis, which was performed on an Agilent 1260 Infinity system equipped with VWD detector and the data were collected at 254 nm.

*Dibenzyl α-Hydroxycyclopentylmethylphosphonate (**8**).* To a solution of DMSO (176.2 mg, 160 μL, 2.25 mmol) in CH_2Cl_2 (6.0 mL) was added oxalyl chloride (253.2 mg, 174 μL, 2.00 mmol) dropwise at −78 °C under argon atmosphere. After stirring at the same temperature for 30 min, a solution of cyclopentanemethanol (50.0 mg, 54 μL, 0.50 mmol) in CH_2Cl_2 (4.0 mL) was slowly added. After 2 h, triethylamine (455.4 mg, 626 μL, 4.50 mmol) was added and the reaction mixture was stirred at −78 °C for another 1 h. Then the mixture was partitioned between CH_2Cl_2 and H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (3.0 mL), and dibenzyl phosphite (261.1 mg, 0.22 mL, 0.90 mmol, 90%) and triethylamine (101.8 mg, 0.14 mL, 1.00 mmol) were added. The reaction mixture was stirred overnight and concentrated. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 3:1) to give **8** (78.7 mg, 44% yield) as white solid. R_f = 0.37 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.27 (m, 10H), 5.11−5.01 (m, 4H), 4.09 (dd, *J* = 6.8, 4.4 Hz, 1H), 3.82 (td, *J* = 7.2, 4.8 Hz, 1H), 2.32−2.24 (m, 1H), 1.90−1.87 (m, 1H), 1.75−1.70 (m, 1H), 1.60−1.38 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.52 (d, *J* = 2.0 Hz), 136.46 (d, *J* = 2.0 Hz), 128.6, 128.4, 128.0, 71.5 (d, *J* = 156.0 Hz), 68.0 (d, *J* = 7.0 Hz), 67.9 (d, *J* = 7.0 Hz), 41.4 (d, *J* = 1.0 Hz), 29.4 (d, *J* = 6.0 Hz), 28.8 (d, *J* = 10.0 Hz), 25.6, 25.3. ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.96. HRMS (ESI) calcd for $C_{20}H_{26}O_4P$ $[M + H]^+$, 361.1569; found, 361.1564.

*Dimethyl (4S,5S)-4,5-Isopropylidenedioxy-2-hexene-1,6-dioate (**12**).* To a solution of enol ether **11** (see the Supporting Information) (10.18 g, 54.67 mmol) in MeOH (100 mL) and CH_2Cl_2 (50 mL) was bubbled with O_3 at −72 °C until the starting material was completely consumed. Then the O_3 dissolved in the reaction mixture was replaced by argon, and the reaction was quenched with dimethyl sulfide (12 mL) at the same temperature for 6 h. After the reaction mixture was concentrated, the residue was dissolved in MeOH (470 mL) and followed by adding *p*-toluenesulfonic acid (470.1 mg, 2.73 mmol). Then the reaction was stirred at room temperature until the starting material was completely consumed and quenched with triethylamine (0.46 mL). The solvent was evaporated under reduced pressure, and the residue was dissolved in THF (150 mL) under argon atmosphere. $Ph_3P{=}CHCOOMe$[78,79] (21.93 g, 65.60 mmol) was added, and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 8:1) to give **12** (8.72 g, 65% yield) as colorless oil. The *Z*-isomer: R_f = 0.59 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +195.0 (*c* = 0.4, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.20 (dd, *J* = 11.5, 7.0 Hz, 1H, H3), 5.95 (dd, *J* = 11.5, 1.5 Hz, 1H, H2), 5.85 (td, *J* = 7.5, 2.0 Hz, 1H, H4), 4.94 (d, *J* = 7.0 Hz, 1H, H5), 3.76 (s, 3H, OMe), 3.66 (s, 3H, OMe), 1.64 (s, 3H, CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 165.7, 143.7, 122.3, 111.6, 77.1, 75.1, 51.8, 51.6, 26.8, 25.5. HRMS (ESI) calcd for $C_{11}H_{16}O_6Na$ $[M + Na]^+$, 267.0845; found, 267.0841. The *E*-isomer: R_f = 0.49 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +44.6 (*c* = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (dd, *J* = 15.6, 5.2 Hz, 1H, H3), 6.15 (dd, *J* = 15.6, 1.2 Hz, 1H, H2), 4.97 (ddd, *J* = 6.8, 5.6, 1.2 Hz, 1H, H4), 4.79 (d, *J* = 7.2 Hz, 1H, H5), 3.74 (s, 3H, OMe), 3.69 (s, 3H, OMe), 1.66 (s, 3H, Me), 1.42 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 166.2, 140.9, 123.2, 112.1, 77.6, 76.5, 52.2, 51.9, 27.0, 25.7. HRMS (ESI) calcd for $C_{11}H_{16}O_6Na$ $[M + Na]^+$, 267.0845; found, 267.0842.

*Dimethyl (2S,3S)-2,3-Isopropylidenedioxy-1,6-hexanedioate (**13**).* To a solution of **12** (9.27 g, 37.95 mmol) in dry ethyl acetate (30 mL) was added Pd/C (404.4 mg, 0.38 mmol, 10%), and the reaction mixture was stirred at room temperature for 24 h under hydrogen atmosphere (4 atm). Then the mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 8:1) to give **13** (9.25 g, 99% yield) as colorless oil. R_f = 0.40 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +1.2 (*c* = 0.8, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (d, *J* = 6.5 Hz, 1H), 4.38−4.34 (m, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 2.56−2.43 (m, 2H), 1.94−1.88 (m, 1H), 1.74−1.66 (m, 1H), 1.59 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 170.4, 110.6, 77.0, 76.5, 52.0, 51.6, 30.5, 27.0, 25.6, 25.5. HRMS (ESI) calcd for $C_{11}H_{18}O_6Na$ $[M + Na]^+$, 269.1001; found, 269.0993.

*Methyl (3S,4S)-2-Hydroxy-3,4-isopropylidenedioxy-1-cyclopentene-1-carboxylate (**14**).* To a solution of **13** (3.86 g, 15.67 mmol) in toluene (77 mL) were added NaH (1.88 g, 47.00 mmol, 60% in mineral oil) in portions and methanol (0.4 mL) at 0 °C under argon atmosphere. After 10 min, the mixture was stirred at 90 °C for about 1 h. Then the reaction mixture was concentrated and diluted with CH_2Cl_2 (30 mL). After the pH of the solution was adjusted to 5−6 with 1 N HCl, the mixture was extracted with CH_2Cl_2 then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10:1) to give **14** (2.44 g, 73% yield) as colorless oil. R_f = 0.44 (petroleum ether/ethyl acetate = 2/1); $[\alpha]_D^{25}$ = +37.8 (*c* = 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H, OH), 4.97 (d, *J* = 6.0 Hz, 1H, H3), 4.69 (t, *J* = 5.2 Hz, 1H, H4), 3.79 (s, 3H, OMe), 2.70 (dd, *J* = 15.6 Hz, 5.2 Hz, 1H, H5α), 2.62 (d, *J* = 16.0 Hz, 1H, H5β), 1.45 (s, 3H, CH_3), 1.37 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.8, 111.4, 99.9, 81.5, 75.0, 51.6, 32.3, 27.4, 25.6. Anal. Calcd for $C_{10}H_{14}O_5$: C, 56.09; H, 6.57. Found: C, 56.11; H, 6.50. HRMS (ESI) calcd for $C_{10}H_{15}O_5$ $[M + H]^+$, 215.0919; found, 215.0920.

*Methyl (2R,3R,4S)-2-Hydroxy-3,4-isopropylidenedioxy-cyclopentane-1-carboxylate (**15**).* To a solution of **14** (1.17 g, 5.46 mmol) in methanol (30 mL) and CH_2Cl_2 (15 mL) was added $NaBH_4$ (0.62 g, 16.38 mmol) in portions at −72 °C under argon atmosphere. After stirring at the same temperature for 5 h, 1 N HCl was added to adjust the pH to 7. Then the mixture was concentrated and extracted with CH_2Cl_2, the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10:1 to 4:1) to give **15** (1.12 g, 95% yield) as colorless oil. R_f = 0.31 (petroleum ether/ethyl acetate 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (t, *J* = 5.2 Hz, 0.8H, H4′), 4.62 (td, *J* = 6.0, 2.4 Hz, 1H, H4), 4.51 (t, *J* = 5.6 Hz, 0.8H, H3′), 4.49 (dd, *J* = 6.4, 5.2 Hz, 1H, H3), 4.12 (dt, *J* = 7.6, 5.2 Hz, 1H, H2), 4.04 (td, *J* = 9.6, 5.6 Hz, 0.8H, H2′), 3.91 (d, *J* = 7.6 Hz, 1H, OH), 3.74 (s, 2.4H, OMe′), 3.73 (s, 3H, OMe), 2.86−2.78 (m, 1.8H, H1 and H1′), 2.60 (d, *J* = 10.0 Hz, 0.8H, OH′), 2.42 (ddd, *J* = 14.4, 6.0, 2.8 Hz, 1H, H5α), 2.07 (dd, *J* = 14.4, 6.4 Hz, 0.8H, H5α′), 1.92 (ddd, *J* = 14.0, 7.6, 6.0 Hz, 1H, H5β), 1.74 (ddd, *J* = 14.4, 12.8, 4.8 Hz, 0.8H, H5β′), 1.50 (s, 2.4H, *Me*′), 1.45 (s, 3H, *Me*), 1.34 (s, 2.4H, *Me*′), 1.33 (s, 3H, *Me*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 173.0, 112.1, 110.5, 79.4, 78.6, 78.2, 78.0, 76.0, 72.8, 52.0, 51.8, 47.5, 45.4, 32.0, 30.8, 25.9, 25.3, 24.0. Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.55; H, 7.46. Found: C, 55.40; H, 7.21. HRMS (ESI) calcd for $C_{10}H_{16}O_5Na$ $[M + Na]^+$, 239.0890; found, 239.0891.

*Methyl (2R,3S,4S)-2-Benzoyloxy-3,4-isopropylidenedioxy-cyclopentane-1-carboxylate (**16**).* To a solution of **15** (5.36 g, 24.79 mmol) in pyridine (80 mL) were added DMAP (0.60 g, 4.96 mmol) and benzoyl chloride (5.20 g, 4.3 mL, 37.18 mmol) dropwise at 0 °C under argon atmosphere. After stirring at room temperature for 4 h, the reaction mixture was concentrated and partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10:1 to 8:1) to give **16*R*** (3.91 g, 49% yield) as white solid and **16*S*** (3.44 g, 43% yield) as colorless oil. **16*R***: R_f = 0.29 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +45.0 (*c* = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08−8.06 (m, 2H, Ar), 7.58−7.54 (m, 1H, Ar), 7.46−7.42 (m, 2H, Ar), 5.57 (t, *J* = 4.4 Hz, 1H, H2), 4.77 (td, *J* = 6.8 Hz, 4.8 Hz 1H, H4), 4.70 (dd, *J* = 6.8, 4.4 Hz, 1H, H3), 3.62 (s, 3H, OMe), 3.16 (ddd, *J* = 11.6, 7.6, 4.4 Hz, 1H, H1), 2.56 (ddd, *J* = 14.0, 11.6, 4.8 Hz, 1H, H5α), 2.38 (dt, *J* = 14.0, 7.2

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J. Med. Chem. XXXX, XXX, XXX−XXX

Hz, 1H, H5β), 1.29 (s, 3H, CH_3), 1.21 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 165.5, 133.2, 130.0, 129.9, 128.5, 114.4, 80.6, 78.6, 73.5, 52.1, 46.9, 31.6, 25.4, 24.8. Anal. Calcd for $C_{17}H_{20}O_6$: C, 63.73; H, 6.29. Found: C, 63.74; H, 6.10. HRMS (ESI) calcd for $C_{17}H_{20}O_6Na$ $[M + Na]^+$, 343.1158; found, 343.1164. **16*S***: R_f = 0.44 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +114.6 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08−8.06 (m, 2H, Ar), 7.58−7.54 (m, 1H, Ar), 7.46−7.42 (m, 2H, Ar), 5.10 (dd, J = 10.8, 5.6 Hz, 1H, H2), 4.87 (t, J = 5.6 Hz, 1H, H3), 4.72 (t, J = 4.8 Hz, 1H, H4), 3.70 (s, 3H, OMe), 3.33 (ddd, J = 12.8, 10.4, 6.8 Hz, 1H, H1), 2.22 (dd, J = 14.4, 6.8 Hz, 1H, H5α), 1.86 (ddd, J = 14.0, 12.8, 4.8 Hz, 1H, H5β), 1.46 (s, 3H, CH_3), 1.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 166.0, 133.2, 130.0, 129.9, 128.4, 111.0, 78.4, 77.3, 76.9, 52.2, 44.5, 31.9, 26.1, 24.4. HRMS (ESI) calcd for $C_{17}H_{21}O_6$ $[M + H]^+$, 321.1333; found, 321.1338.

*Methyl (3R,4S)-3,4-Isopropylidenedioxy-1-cyclopentene-1-carboxylate (**17**).* To a solution of **16*R*** (3.89 g, 12.14 mmol) in THF (40 mL) was added DBU (3.67 g, 3.6 mL, 24.29 mmol) at 0 °C under argon atmosphere. After refluxing for 1.5 h, the reaction mixture was concentrated and partitioned between ethyl acetate and saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 9:1) to give **17** (2.22 g, 92% yield) as colorless oil. R_f = 0.53 (petroleum ether/ethyl acetate = 2/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (dd, J = 3.6, 1.6 Hz, 1H), 5.19−5.17 (m, 1H), 4.82−4.80 (m, 1H), 3.76 (s, 3H), 2.79−2.77 (m, 2H), 1.40 (s, 3H), 1.35 (s, 3H). The spectroscopic data coincide with the previous report.[80] Compound **17** was also prepared from **16*S*** in the same manner as described above. Quantities: **16*S*** (3.15 g, 9.83 mmol), DBU (2.96 g, 2.9 mL, 19.66 mmol), THF (40 mL), 4 h, affording **17** (1.58 g, 81% yield).

*Methyl c-3,c-4-Isopropylidenedioxy-r-1-cyclopentanecarboxylate (**18**).* To a solution of **17** (115.7 mg, 0.58 mmol) in dry ethyl acetate (5 mL) was added Pd/C (15.6 mg, 0.02 mmol, 10%), and the reaction mixture was stirred at room temperature for 15 h under hydrogen atmosphere (4 atm). Then the mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 6:1) to give **18** (108.1 mg, 92% yield) as colorless oil. R_f = 0.38 (petroleum ether/ethyl acetate 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65−4.61 (m, 2H), 3.70 (s, 3H), 2.81 (tt, J = 8.4, 2.8 Hz, 1H), 2.47 (dd, J = 14.4, 2.8 Hz, 2H), 1.91−1.85 (m, 2H), 1.38 (s, 3H), 1.27 (s, 3H). The spectroscopic data coincide with the previous report.[81]

*c-3,c-4-Isopropylidenedioxy-r-1-cyclopentylmethanol (**19**).* To a solution of **18** (196.5 mg, 0.98 mmol) in THF (10 mL) were added $LiBH_4$ (85.3 mg, 3.92 mmol) and methanol (0.2 mL) at 0 °C under argon atmosphere. After stirring at the same temperature for 3 h, 1 N HCl was added to adjust the pH to 7. Then the mixture was extracted with ethyl acetate, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:1) to give **19** (140.9 mg, 83% yield) as colorless oil. R_f = 0.38 (petroleum ether/ethyl acetate 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66−4.65 (m, 2H), 3.70 (d, J = 6.8 Hz, 2H), 2.29−2.20 (m, 1H), 1.93−1.79 (m, 4H), 1.70 (brs, 1H), 1.49 (s, 3H), 1.30 (s, 3H). The spectroscopic data coincide with the previous report.[82]

*Dibenzyl α-Hydroxy-(c-3,c-4-isopropylidenedioxy-r-1-cyclopentylmethyl)-phosphonate (**20**).* To a solution of **19** (88.6 mg, 0.51 mmol) in CH_2Cl_2 (2 mL) were added TEMPO (8.0 mg, 0.05 mmol) and BAIB (199.7 mg, 0.62 mmol) at 0 °C under argon atmosphere. After stirring at room temperature for 6 h, the reaction was quenched with saturated $Na_2S_2O_3$ solution and partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (4 mL), and dibenzyl phosphite (297.2 mg, 250 μL, 1.02 mmol, 90%) and triethylamine (103.2 mg, 142 μL, 1.02 mmol) were added. The reaction mixture was stirred overnight and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 1:2) to give **20** (157.2 mg, 71% yield) as colorless oil. R_f = 0.19 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.30 (m, 12H), 5.14−5.01 (m, 4.8H), 4.63−4.57 (m, 2.4H), 4.06 (dd, J = 12.8, 5.6 Hz, 1H), 3.86 (dd, J = 11.6, 6.0 Hz, 0.2H), 3.37 (dd, J = 8.8, 6.0 Hz, 1H), 3.29 (t, J = 5.2 Hz, 0.2H), 2.67−2.58 (m, 0.2H), 2.55−2.44 (m, 1H), 2.18−2.13 (m, 1H), 2.10−2.09 (m, 0.2H), 2.01−1.88 (m, 3.6H), 1.47 (s, 3H), 1.40 (s, 0.6H), 1.29 (s, 3H), 1.27 (s, 0.6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.60−25.36 (m), 25.00−24.77 (m). HRMS (ESI) calcd for $C_{23}H_{30}O_6P$ $[M + H]^+$, 433.1780; found, 433.1771.

*Methyl (3R,4S)-3,4-Dihydroxy-1-cyclopentene-1-carboxylate (**21**).* Compound **17** (100.1 mg, 0.50 mmol) was dissolved in 90% (v/v) acetic acid solution (2 mL) and stirred at 90 °C for 2 h. After the starting material was completely consumed, the mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:3) to give **21** (67.6 mg, 85% yield) as white solid. R_f = 0.36 (petroleum ether/ethyl acetate 1:5); $[\alpha]_D^{25}$ = −66.8 (c = 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (dd, J = 3.6, 2.0 Hz, 1H), 4.71 (brs, 1H), 4.39 (t, J = 5.6 Hz, 1H), 3.76 (s, 3H), 3.65 (brs, 1H), 3.42 (brs, 1H), 2.81−2.74 (m, 1H), 2.64−2.59 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 141.8, 135.8, 76.6, 71.3, 52.0, 38.8. Anal. Calcd for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: C, 53.25; H, 6.49. HRMS (ESI) calcd for $C_7H_{14}NO_4$[M + $NH_4]^+$, 176.0917; found, 176.0918.

*Methyl (3R,4S)-3-Benzyloxy-4-hydroxy-cyclopentene-1-carboxylate (**22**).* To a solution of **21** (220.2 mg, 1.39 mmol) in CH_2Cl_2 (80 mL) were added Ag_2O (354.3 mg, 1.53 mmol), BnBr (285.6 mg, 0.2 mL, 1.67 mmol), and TBAI (102.7 mg, 0.28 mmol) under argon atmosphere, and the reaction bottle was protected from the daylight. After stirring at room temperature for 6 h, the mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to give **22** (255.0 mg, 74% yield) as colorless oil. R_f = 0.41 (petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{28}$ = −43.3 (c = 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.31 (m, 5H, Ar), 6.68 (dd, J = 3.6, 2.0 Hz, 1H, H2), 4.68 (s, 2H, PhCH_2), 4.52 (ddd, J = 5.2, 3.2, 1.2 Hz, 1H, H3), 4.43 (td, J = 5.6, 3.2 Hz, 1H, H4), 3.75 (s, 3H, OMe), 2.80 (brs, 1H, OH), 2.73 (dddd, J = 16.8, 5.6, 2.4, 1.2 Hz, 1H, H5α), 2.65 (ddt, J = 17.2, 2.8, 1.6 Hz, 1H, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3 (CO_2Me), 139.2 (C2), 137.4 (C1), 137.1 (Ar), 128.8 (Ar), 128.3 (Ar), 128.1 (Ar), 83.0 (C3), 72.7 (PhCH_2), 70.7 (C4), 51.9 (OMe), 39.1 (C5). HRMS (ESI) calcd for $C_{14}H_{20}NO_4$[M + $NH_4]^+$, 266.1387; found, 266.1383.

*Methyl (3R,4S)-3-Benzyloxy-4-hydroxy-cyclopentane-1-carboxylate (**23**).* To a solution of **22** (531.7 mg, 2.14 mmol) in methanol (25 mL) was added a solution of $NiCl_2·6H_2O$ in methanol (21 mL, 3.64 mmol, 4% in methanol) at 0 °C under argon atmosphere. After 30 min, $NaBH_4$ (323.8 mg, 8.56 mmol) was added in portions. After stirring at 0 °C for another 2 h, the reaction was quenched with a drop of acetic acid and the mixture was filtered through a pad of Celite and concentrated. The residue was partitioned between ethyl acetate and saturated $NaHCO_3$ solution, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to give **23*R*** (371.7 mg, 69%) and **23*S*** (121.4 mg, 23%) both as colorless oil. **23*R***: R_f = 0.40 (petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{25}$ = −23.1 (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.27 (m, 5H, Ar), 4.63 (d, J = 11.6 Hz, 1H, PhCH_2), 4.52 (d, J = 11.6 Hz, 1H, PhCH_2), 4.08 (dt, J = 10.0, 5.2 Hz, 1H, H4), 3.80 (td, J = 6.8, 4.8 Hz, 1H, H3), 3.68 (s, 1H, OMe), 2.75 (ddd, J = 16.4, 9.2, 7.2 Hz, 1H, H1), 2.63 (d, J = 5.6 Hz, 1H, OH), 2.21 (td, J = 14.0, 7.6 Hz, 1H, H2α), 2.15−2.02 (m, 3H, H2β, H5α, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9 (CO_2Me), 137.9 (Ph), 128.6 (Ph), 127.9 (Ph), 127.8 (Ph), 80.2 (C3), 72.1 (C4), 71.4 (PhCH_2), 52.1 (OMe), 38.5 (C1), 34.7 (C5), 31.2 (C2). HRMS (ESI) calcd for $C_{14}H_{22}NO_4$[M + $NH_4]^+$, 268.1543; found, 268.1538. **23*S***: R_f = 0.47 (petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{25}$ = +3.1 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.30 (m, 5H, Ar), 4.61 (d, J = 11.6 Hz, 1H, PhCH_2), 4.54 (d, J = 11.6 Hz, 1H, PhCH_2), 4.22 (dt, J = 8.0, 3.6 Hz, 1H, H4), 3.99 (td, J = 6.8, 4.0 Hz, 1H, H3), 3.67 (s, 3H, OMe), 3.20−3.11 (m, 1H, H1), 2.50 (d, J = 2.8 Hz, 1H,

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J. Med. Chem. XXXX, XXX, XXX−XXX

OH), 2.18−2.02 (m, 3H, H2α, H2β, H5α), 1.95 (ddd, J = 13.6, 7.6, 5.6 Hz, 1H, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 137.9, 128.7, 128.1, 127.9, 80.9, 72.1, 72.0, 52.0, 39.1, 35.1, 31.7. HRMS (ESI) calcd for $C_{14}H_{22}NO_4[M + NH_4]^+$, 268.1543; found, 268.1539.

*Methyl (1R,3R,4R)-3-Benzyloxy-4-azido-cyclopentane-1-carboxylate (**24R**).* To a solution of **23R** (96.1 mg, 0.38 mmol) in CH_2Cl_2 (3 mL) were added TsCl (217.3 mg, 1.14 mmol), DMAP (23.2 mg, 0.19 mmol), and triethylamine (115.4 mg, 158 μL, 1.14 mmol) at 0 °C under argon atmosphere. After stirring overnight at room temperature, the reaction mixture was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in DMF (2 mL), and NaN_3 (247.1 mg, 3.80 mmol) was added. After stirring at 90 °C for 12 h, the reaction mixture was partitioned between ethyl acetate and H_2O, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 15:1) to give **24R** (101.2 mg, 96% yield) as colorless oil. R_f = 0.41 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = −19.6 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.27 (m, 5H), 4.57 (d, J = 12.0 Hz, 1H), 4.52 (d, J = 12.0 Hz, 1H), 4.01−3.97 (m, 1H), 3.85−3.81 (m, 1H), 3.68 (s, 3H), 2.92 (dt, J = 16.4, 8.0 Hz, 1H), 2.39−2.27 (m, 2H), 2.01 (ddd, J = 13.6, 7.6, 5.6 Hz, 1H), 1.90 (ddd, J = 14.0, 8.8, 4.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 138.0, 128.6, 127.9, 127.8, 83.6, 71.7, 66.0, 52.1, 39.6, 33.6, 32.3. HRMS (ESI) calcd for $C_{14}H_{17}N_3O_3Na$ [M + Na]$^+$, 298.1162; found, 298.1166.

*Methyl (1S,3R,4R)-3-Benzyloxy-4-azido-cyclopentane-1-carboxylate (**24S**).* This compound was prepared in the same manner as described in the preparation of **24R**. Quantities: **23S** (28.5 mg, 0.11 mmol), CH_2Cl_2 (1 mL), TsCl (41.9 mg, 0.22 mmol), DMAP (6.7 mg, 0.055 mmol), triethylamine (22.3 mg, 31 μL, 0.22 mmol), NaN_3 (71.5 mg, 1.10 mmol), DMF (1 mL), and eluent (petroleum ether/ethyl acetate = 20:1), affording **24S** (30.4 mg, 97% yield) as colorless oil. R_f = 0.45 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = −16.4 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.28 (m, 5H), 4.54 (s, 2H), 3.92−3.88 (m, 2H), 3.70 (s, 1H), 3.03 (dt, J = 16.8, 8.4 Hz, 1H), 2.37 (ddd, J = 13.6, 8.8, 6.8 Hz, 1H), 2.22 (ddd, J = 14.0, 8.0, 6.0 Hz, 1H), 2.06−2.00 (m, 1H), 1.96−1.89 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 137.9, 128.6, 128.0, 127.8, 83.6, 71.8, 66.1, 52.2, 39.8, 33.4, 32.8. HRMS (ESI) calcd for $C_{14}H_{17}N_3O_3Na$ [M + Na]$^+$, 298.1168; found, 298.1165.

*Methyl (1R,3R,4R)-3-Benzyloxy-4-acetamido-cyclopentane-1-carboxylate (**25R**).* To a solution of **24R** (32.7 mg, 0.12 mmol) in pyridine (1.5 mL) was added thioacetic acid (191.8 mg, 0.18 mL, 2.52 mmol) under argon atmosphere. After stirring at room temperature for 36 h, the reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 1:5) to give **25R** (30.5 mg, 88% yield) as colorless oil. R_f = 0.13 (petroleum ether/ethyl acetate 1:5); $[\alpha]_D^{25}$ = +31.9 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.26 (m, 5H), 5.42 (d, J = 5.2 Hz, 1H), 4.59 (s, 2H), 4.24−4.18 (m, 1H), 3.86 (dd, J = 10.4, 5.6 Hz, 1H), 3.67 (s, 3H), 2.91 (dt, J = 16.8, 8.4 Hz, 1H), 2.48 (dt, J = 14.0, 7.6 Hz, 1H), 2.27 (ddd, J = 14.4, 8.8, 6.8 Hz, 1H), 2.04 (ddd, J = 13.2, 7.2, 5.6 Hz, 1H), 1.94 (s, 3H), 1.79 (ddd, J = 13.6, 8.8, 4.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 169.9, 138.5, 128.5, 127.8, 127.7, 83.5, 71.4, 55.7, 52.1, 40.1, 33.8, 33.1, 23.6. HRMS (ESI) calcd for $C_{16}H_{21}NO_4Na$ [M + Na]$^+$, 314.1368; found, 314.1368.

*Methyl (1S,3R,4R)-3-Benzyloxy-4-acetamido-cyclopentane-1-carboxylate (**25S**).* This compound was prepared in the same manner as described in the preparation of **25R**. Quantities: **24S** (102.7 mg, 0.37 mmol), pyridine (5.5 mL), and thioacetic acid (704.1 mg, 0.66 mL, 9.25 mmol), 48 h, eluent (petroleum ether/ethyl acetate = 1:2), affording **25S** (93.7 mg, 86% yield) as white solid. R_f = 0.10 (petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{25}$ = +24.0 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.28 (m, 5H, Ar), 6.40 (d, J = 6.4 Hz, 1H, NH), 4.67 (d, J = 11.6 Hz, 1H, PhCH_2), 4.56 (d, J = 12.0 Hz, 1H, PhCH_2), 4.42 (t, J = 6.8 Hz, 1H, H4), 3.94 (d, J = 5.2 Hz, 1H, H3), 3.71 (s, 3H, OMe), 3.15−3.08 (m, 1H, H1), 2.42 (ddd, J = 14.4, 9.6, 6.8 Hz, 1H, H5α), 2.14 (dd, J = 14.0, 8.8 Hz, 1H, H2α), 2.02 (ddd, J = 14.8, 6.8, 5.6 Hz, 1H, H2β), 1.96 (s, 3H, CH_3), 1.73 (dt, J = 14.4, 2.8 Hz, 1H, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3 (CO_2Me), 169.5 (CH_3CO), 138.5 (Ph), 128.5 (Ph), 127.8 (Ph), 127.7 (Ph), 84.7 (C3), 71.2 (PhCH_2), 55.0 (C4), 52.4 (OMe), 40.7 (C1), 35.4 (C2), 33.4 (C5), 23.6 (CH_3). Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81; Found: C, 65.97; H, 7.13; N, 4.65. HRMS (ESI) calcd for $C_{16}H_{22}NO_4$ [M + H]$^+$, 292.1549; found, 292.1558.

*(1R,3R,4R)-3-Benzyloxy-4-acetamido-1-cyclopentylmethanol (**26R**).* This compound was prepared in the same manner as described in the preparation of **19**. Quantities: **25R** (319.7 mg, 1.10 mmol), $LiBH_4$ (95.8 mg, 4.40 mmol), and methanol (0.6 mL), THF (30 mL), 1 h, eluent (petroleum ether/acetone = 1:1), affording **26R** (254.6 mg, 88% yield) as white solid. R_f = 0.21 (petroleum ether/acetone 1:2); $[\alpha]_D^{25}$ = +6.7 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.25 (m, 5H, Ar), 5.70 (d, J = 6.4 Hz, 1H, NH), 4.62 (d, J = 12.4 Hz, 1H, PhCH_2), 4.58 (d, J = 12.0 Hz, 1H, PhCH_2), 4.25 (ddd, J = 11.6, 7.6, 4.4 Hz, 1H, H4), 3.81 (dd, J = 10.4, 4.8 Hz, 1H, H3), 3.55 (d, J = 6.0 Hz, 2H, H6α, H6β), 2.66 (s, 1H, OH), 2.34−2.23 (m, 1H, H1), 2.18−2.10 (m, 1H, H2α), 2.01 (dt, J = 14.4, 7.2 Hz, 1H, H5α), 1.93 (s, 3H, Ac), 1.60 (ddd, J = 13.6, 8.4, 4.8 Hz, 1H, H5β), 1.48 (dt, J = 13.6, 5.6 Hz, 1H, H2β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 138.5, 128.5, 127.8, 127.7, 84.2, 71.3, 66.7, 55.5, 38.0, 33.8, 33.0, 23.5. HRMS (ESI) calcd for $C_{15}H_{22}NO_3$ [M + H]$^+$, 264.1594; found, 264.1593.

*(1S,3R,4R)-3-Benzyloxy-4-acetamido-1-cyclopentylmethanol (**26S**).* This compound was prepared in the same manner as described in the preparation of **19**. Quantities: **25S** (380.3 mg, 1.30 mmol), $LiBH_4$ (132.4 mg, 6.08 mmmol), and methanol (0.46 mL), THF (23 mL), 4 h, eluent (petroleum ether/acetone = 1:1), affording **26S** (337.4 mg, 98% yield) as colorless oil. R_f = 0.28 (petroleum ether/acetone 1:2); $[\alpha]_D^{25}$ = +13.6 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.25 (m, 5H), 6.70 (d, J = 7.2 Hz, 1H), 4.63 (d, J = 12.0 Hz, 1H), 4.54 (d, J = 12.4 Hz, 1H), 4.31−4.27 (m, 1H), 3.83−3.82 (m, 1H), 3.66−3.57 (m, 2H), 2.80 (brs, 1H), 2.49−2.42 (m, 1H), 2.35 (ddd, J = 13.2, 10.4, 7.2 Hz, 1H), 1.90 (s, 3H), 1.88−1.75 (m, 2H), 1.36 (dt, J = 13.2, 4.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 138.8, 128.4, 127.8, 127.6, 85.1, 70.9, 64.9, 54.6, 37.5, 32.9, 32.4, 23.6. HRMS (ESI) calcd for $C_{15}H_{22}NO_3$ [M + H]$^+$, 264.1594; found, 264.1595.

*Dibenzyl [α-Hydroxy-(1R,3R,4R)-3-benzyloxy-4-acetamido-1-cyclopentylmethyl]-phosphonate (**27R**).* This compound was prepared in the same manner as described in the preparation of **20**. Quantities: **26R** (89.5 mg, 0.34 mmol), BAIB (164.3 mg, 0.51 mmol), TEMPO (5.3 mg, 0.034 mmol), and CH_2Cl_2 (2.2 mL), 17 h; dibenzyl phosphite (209.8 mg, 177 μL, 0.68 mmol, 85%), triethylamine (68.8 mg, 95 μL, 0.68 mmol), and CH_2Cl_2 (2 mL), overnight, eluent (petroleum ether/acetone = 1:1), affording **27R** (161.5 mg, 91% yield) as colorless oil. R_f = 0.44 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.26 (m, 15H), 6.07−6.00 (m, 1H), 5.10−4.97 (m, 4H), 4.66−4.56 (m, 2H), 4.29−4.27 (m, 1H), 4.21−4.08 (m, 1H), 3.94−3.85 (m, 1H), 3.82−3.79 (m, 1H), 2.72−2.69 (m, 1H), 2.31−1.95 (m, 3H), 1.89 (s, 3H), 1.73−1.57 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.83, 24.56. HRMS (ESI) calcd for $C_{29}H_{35}NO_6P$ [M + H]$^+$, 524.2202; found, 524.2200.

*Dibenzyl [α-Hydroxy-(1S,3R,4R)-3-benzyloxy-4-acetamido-1-cyclopentylmethyl]-phosphonate (**27S**).* This compound was prepared in the same manner as described in the preparation of **20**. Quantities: **26S** (40.2 mg, 0.15 mmol), BAIB (73.9 mg, 0.23 mmol), TEMPO (2.3 mg, 0.015 mmol), and CH_2Cl_2 (1.0 mL), 11 h; dibenzyl phosphite (96.1 mg, 81 μL, 0.31 mmol, 85%), triethylamine (31.4 mg, 43 μL, 0.31 mmol), and CH_2Cl_2 (1.0 mL), overnight, eluent (petroleum ether/acetone = 1:1), affording **27S** (69.3 mg, 87% yield) as white solid. R_f = 0.44 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.26 (m, 15H), 6.52 (brs, 1H), 5.10−4.94 (m, 4H), 4.89 (brs, 1H), 4.65−4.58 (m, 1H), 4.54−4.48 (m, 1H), 4.30−4.26 (m, 1H), 3.98−3.74 (m, 2H), 2.75 (brs, 1H), 2.46−2.21 (m, 1H), 1.96−1.91 (m, 2H), 1.87−1.85 (m, 3H), 1.74−1.39 (m, 1H). ^{31}P

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J. Med. Chem. XXXX, XXX, XXX−XXX

NMR (162 MHz, $CDCl_3$) δ 25.23, 25.17. HRMS (ESI) calcd for $C_{29}H_{35}NO_6P$ $[M + H]^+$, 524.2202; found, 524.2198.

*(1S,2R,4R)-2-Benzyloxy-4-hydroxymethyl-1-cyclopentanol (**28**).* This compound was prepared in the same manner as described in the preparation of **19**. Quantities: **23*R*** (270.9 mg, 1.08 mmol), $LiBH_4$ (94.3 mg, 4.33 mmmol), methanol (0.25 mL), and THF (12 mL), 1.5 h, eluent (petroleum ether/acetone = 3:2), affording **28** (235.0 mg, 98% yield) as colorless oil. R_f = 0.31 (petroleum ether/acetone 1:1); $[\alpha]_D^{25}$ = −12.0 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.30 (m, 5H), 4.60 (d, J = 12.0 Hz, 1H), 4.53 (d, J = 11.6 Hz, 1H), 4.08 (dt, J = 7.6, 3.6 Hz, 1H), 3.81 (ddd, J = 8.0, 7.2, 4.4 Hz, 1H), 3.56 (brs, 2H), 2.84 (s, 1H), 2.77 (s, 1H), 2.24−2.16 (m, 1H), 2.06−1.92 (m, 2H), 1.69 (dt, J = 13.2, 8.0 Hz, 1H), 1.56 (ddd, J = 14.4, 4.8, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.6, 128.0, 127.9, 81.5, 72.0, 71.7, 66.6, 36.2, 34.1, 30.4. HRMS (ESI) calcd for $C_{13}H_{22}NO_3$ $[M + NH_4]^+$, 240.1594; found, 240.1597.

*(1S,2R,4R)-2-Benzyloxy-4-tert-butyldiphenylsilyloxymethyl-1-cyclopentanol (**29**).* To a solution of **28** (89.6 mg, 0.40 mmol) in CH_2Cl_2 (7 mL) were added DMAP (9.8 mg, 0.08 mmmol), TBDPSCl (164.9 mg, 155 μL, 0.60 mmmol), and triethylamine (81.0 mg, 111 μL, 0.80 mmol) at 0 °C under argon atmosphere. After stirring at room temperature for 36 h, the reaction mixture was partitioned between CH_2Cl_2 and H_2O, and then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 12:1) to give **29** (164.4 mg, 88% yield) as colorless oil. R_f = 0.33 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = −4.2 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67−7.64 (m, 4H), 7.43−7.29 (m, 11H), 4.58 (d, J = 11.6 Hz, 1H), 4.51 (d, J = 11.6 Hz, 1H), 4.08 (dt, J = 10.4, 5.2 Hz, 1H), 3.79 (ddd, J = 7.2, 6.8, 4.4 Hz, 1H), 3.64 (dd, J = 9.6, 6.4 Hz, 1H), 3.59 (dd, J = 10.0, 7.2 Hz, 1H), 2.55 (d, J = 5.6 Hz, 1H), 2.19−2.07 (m, 1H), 2.03−1.93 (m, 2H), 1.63 (dt, J = 13.2, 7.6 Hz, 1H), 1.50 (ddd, J = 13.6, 6.8, 4.8 Hz, 1H), 1.05 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 135.7, 134.04, 134.02, 129.7, 128.6, 127.9, 127.85, 127.75, 81.0, 72.2, 71.4, 68.6, 36.6, 34.6, 31.5, 27.0, 19.4. Anal. Calcd for $C_{29}H_{36}O_3Si$: C, 75.61; H, 7.88. Found: C, 75.56; H, 8.01. ESI-MS m/z: 461 $[M + H]^+$.

*(1S,2R,4R)-2-Benzyloxy-4-tert-butyldiphenylsilyloxymethyl-1-cyclopentyl Tosylate (**30**).* To a solution of **29** (1.19 g, 2.58 mmol) in pyridine (32 mL) were added DMAP (0.63 g, 5.16 mmmol) and a solution of TsCl (2.46 g, 12.90 mmol) in CH_2Cl_2 (10 mL) at 0 °C under argon atmosphere. After stirring at room temperature for 24 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and H_2O, then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 15:1) to give **30** (1.42 g, 89% yield) as colorless oil. R_f = 0.44 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = +15.0 (c = 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 7.8 Hz, 4H), 7.42−7.22 (m, 13H), 4.88 (dd, J = 10.0, 5.6 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.38 (d, J = 12.0 Hz, 1H), 3.78 (td, J = 6.8, 4.4 Hz, 1H), 3.55 (d, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.15−2.04 (m, 1H), 1.98−1.90 (m, 2H), 1.76 (dt, J = 14.4, 6.0 Hz, 1H), 1.63 (dt, J = 13.6, 7.6 Hz, 1H), 1.01 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5, 138.3, 135.7, 134.6, 133.92, 133.88, 129.8, 129.7, 128.4, 127.9, 127.8, 127.64, 127.61, 81.4, 79.1, 71.5, 68.1, 35.7, 32.1, 31.6, 26.9, 21.8, 19.4. Anal. Calcd for $C_{36}H_{42}O_5SSi$: C, 70.32; H, 6.88; Found: C, 70.25; H, 7.04. HRMS (ESI) calcd for $C_{36}H_{46}NO_5SSi$ $[M + NH_4]^+$, 632.2866; found, 632.2860.

*(1S,2R,4R)-2-Benzyloxy-4-tert-butyldiphenylsilyloxymethyl-1-cyclopentyl Carbonitrile (**31**).* To a solution of **30** (256.4 mg, 0.42 mmol) in toluene (25 mL) was added Bu_4NCN (225.5 mg, 0.84 mmol) under argon atmosphere. After stirring at 85 °C for 24 h, the reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 25:1) to give **31** (93.0 mg, 48% yield) as colorless oil. R_f = 0.47 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = −13.9 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64−7.62 (m, 4H), 7.44−7.29 (m, 11H), 4.58 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 4.17 (dd, J = 12.4, 6.4 Hz, 1H), 3.60 (dd, J = 10.4, 6.0 Hz, 1H), 3.54 (dd, J = 10.0, 6.4 Hz, 1H), 2.83 (td, J = 8.0, 6.0 Hz, 1H), 2.40−2.29 (m, 1H), 2.18 (dt, J = 14.0, 7.6 Hz, 1H), 2.06−1.95 (m, 2H), 1.58−1.51 (m, 1H), 1.05 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 135.7, 133.6, 129.9, 128.6, 128.0, 127.9, 127.8, 122.0, 83.3, 72.1, 66.5, 38.7, 34.7, 34.4, 31.5, 27.0, 19.4. HRMS (ESI) calcd for $C_{30}H_{36}NO_2Si$ $[M + H]^+$, 470.2515; found, 470.2512.

*(1S,2R,4R)-2-Benzyloxy-4-tert-butyldiphenylsilyloxymethyl-1-cyclopentylmethylacetamide (**32**).* To a solution of **31** (69.3 mg, 0.15 mmol) in methanol (4.5 mL) were added $CoCl_2$ (58.4 mg, 0.45 mmol) and then $NaBH_4$ (85.1 mg, 2.25 mmol) in portions at 0 °C under argon atmosphere. After stirring at the same temperature for 3 h, the reaction was quenched with 2 M HCl (0.9 mL). While the black precipitates disappeared, 2 M $NH_3{\cdot}H_2O$ (1.4 mL) was added. After 5 min, the reaction mixture was concentrated and the residue was dissolved in CH_2Cl_2 (15 mL) under argon atmosphere. To this solution were added triethylamine (3.4 mL) and acetic anhydride (1.8 mL). After stirring overnight at room temperature, the reaction mixture was concentrated and the residue was partitioned between ethyl acetate and H_2O, then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 3:2) to give **32** (45.6 mg, 60% yield) as colorless oil. R_f = 0.41 (petroleum ether/ethyl acetate 1:2); $[\alpha]_D^{25}$ = −16.6 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 6.8 Hz, 4H, Ar), 7.44−7.30 (m, 11H, Ar), 6.05 (brs, 1H, NH), 4.58 (d, J = 11.6 Hz, 1H, PhCH_2), 4.39 (d, J = 11.6 Hz, 1H, PhCH_2), 3.66 (dd, J = 14.0, 7.6 Hz, 1H, H2), 3.57 (d, J = 6.0 Hz, 2H, H7α, H7β), 3.45 (dt, J = 12.8, 6.0 Hz, 1H, H6α), 3.03 (ddd, J = 13.2, 9.6, 3.6 Hz, 1H, H6β), 2.24−2.15 (m, 2H, H4, H3α), 2.09−1.98 (m, 1H, H1), 1.85 (s, 3H, CH_3), 1.71 (ddd, J = 13.6, 8.8, 4.8 Hz, 1H, H5α), 1.43−1.32 (m, 2H, H5β, H3β), 1.06 (s, 9H, tBu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 138.5, 135.7, 134.0, 129.7, 128.7, 128.0, 127.9, 127.8, 85.4, 71.8, 67.8, 44.0, 43.2, 37.5, 34.7, 29.7, 27.0, 23.3, 19.4. HRMS (ESI) calcd for $C_{32}H_{42}NO_3Si$ $[M + H]^+$, 516.2934; found, 516.2927.

*(1S,2R,4R)-2-Benzyloxy-4-hydroxymethyl-1-cyclopentylmethylacetamide (**33**).* To a solution of **32** (126.1 mg, 0.24 mmol) in THF (14 mL) was added TBAF (1 M in THF, 0.98 mL, 0.98 mmol) under argon atmosphere. After stirring overnight at room temperature, the reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/acetone = 2:1) to give **33** (68.1 mg, 100% yield) as colorless oil. R_f = 0.32 (petroleum ether/acetone 1:2); $[\alpha]_D^{25}$ = −31.0 (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.30 (m, 5H, Ar), 6.05 (s, 1H, NH), 4.59 (d, J = 11.2 Hz, 1H, PhCH_2), 4.42 (d, J = 11.6 Hz, 1H, PhCH_2), 3.70 (dd, J = 13.6, 6.8 Hz, 1H, H2), 3.56 (d, J = 6.0 Hz, 2H, H7α, H7β), 3.40 (dt, J = 13.2, 5.6 Hz, 1H, H6α), 3.07 (ddd, J = 13.2, 9.6, 4.0 Hz, 1H, H6β), 2.29−2.09 (m, 3H, H1, H4, H3α), 1.96 (brs, 1H, OH), 1.87 (s, 3H, CH_3), 1.72 (ddd, J = 13.6, 8.4, 5.2 Hz, 1H, H5α), 1.48−1.40 (m, 2H, H5β, H3β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (CH_3CO), 138.2 (Ph), 128.7(Ph), 128.0 (Ph), 127.9 (Ph), 84.8 (C2), 71.7 (PhCH_2), 67.1 (C7), 43.7 (C6), 43.6 (C1), 37.7 (C4), 34.9 (C3), 29.9 (C5), 23.3 (CH_3). HRMS (ESI) calcd for $C_{16}H_{24}NO_3$ $[M + H]^+$, 278.1751; found, 278.1750.

*Dibenzyl [α-Hydroxy-(1R,3R,4S)-3-benzyloxy-4-acetamidomethyl-1-cyclopentyl-methyl]-phosphonate (**34**).* To a solution of DMSO (93.8 mg, 85 μL, 1.20 mmol) in CH_2Cl_2 (3.0 mL) was added oxalyl chloride (135.0 mg, 93 μL, 1.06 mmol) dropwise at −78 °C under argon atmosphere. After stirring at the same temperature for 30 min, a solution of **33** (37.0 mg, 0.13 mmol) in THF (2.0 mL) was slowly added. After 2 h, triethylamine (333 μL, 2.39 mmol) was added and the reaction mixture was stirred for another 1 h. Then the mixture was partitioned between CH_2Cl_2 and H_2O, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. To a solution of dibenzyl phosphite (614.9 mg, 518 μL, 2.00 mmol, 85%) in THF (2.0 mL) was added NaH (53.2 mg, 1.33 mmol, 60% in mineral oil) at 0 °C under argon atmosphere. After 30 min, a solution of the above residue in THF (2.0 mL) was added to the reaction bottle. The mixture was stirred overnight and quenched with saturated NH_4Cl solution. Then the mixture was partitioned between CH_2Cl_2 and H_2O and the combined organic layer was washed

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J. Med. Chem. XXXX, XXX, XXX−XXX

with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/acetone = 1:2) to give **34** (48.2 mg, 67% yield) as colorless oil. R_f = 0.23 (petroleum ether/acetone 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.28 (m, 15H), 5.83−5.78 (m, 1H), 5.10−4.99 (m, 4H), 4.56−4.49 (m, 1H), 4.39−4.34 (m, 1H), 3.84−3.83 (m, 1H), 3.67−3.62 (m, 1H), 3.27−3.00 (m, 3H), 2.51−2.42 (m, 1H), 2.22−2.01 (m, 2H), 1.97−1.88 (m, 1H), 1.86 (s, 3H), 1.63−1.60 (m, 1H), 1.49−1.41 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.03, 24.66. HRMS (ESI) calcd for $C_{30}H_{37}NO_6P$ $[M + H]^+$, 538.2358; found, 538.2360.

*(3R,4S)-3,4-Isopropylidenedioxy-1-cyclopentene-1-methanol (**35**).* To a solution of **17** (100.5 mg, 0.51 mmol) in toluene (10 mL) was slowly added DIBAL-H (1.3 mL, 1.53 mmol, 1.2 M in toluene) at −78 °C under argon atmosphere. After stirring at the same temperature for 4 h, the reaction was quenched with H_2O and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 3:1) to give **35** (84.5 mg, 98% yield) as colorless oil. R_f = 0.15 (petroleum ether/ethyl acetate 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (td, J = 3.6, 2.0 Hz, 1H), 5.13−5.11 (m, 1H), 4.82 (td, J = 6.0, 1.2 Hz, 1H), 4.26−4.15 (m, 2H), 2.62−2.55 (m, 1H), 2.50−2.44 (m, 1H), 1.56 (t, J = 6.0 Hz, 1H), 1.43 (s, 3H), 1.35 (s, 3H). The spectroscopic data coincide with the previous report.[80]

*(3R,4S)-3,4-Isopropylidenedioxy-1-cyclopentenemethyl Tosylate (**36**).* To a solution of **35** (386.3 mg, 2.27 mmol) in ether (10 mL) were added TsCl (1298.3 mg, 6.81 mmol) and NaOH (408.8 mg, 10.22 mmol) at 0 °C under argon atmosphere. After stirring at room temperature for 24 h, the reaction mixture was partitioned between ethyl acetate and H_2O, then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to give **36** (712.2 mg, 97% yield) as colorless oil. R_f = 0.33 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{28}$ = −17.8 (c = 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 5.72 (brs, 1H), 5.04 (d, J = 5.6 Hz, 1H), 4.74 (t, J = 5.6 Hz, 1H), 4.61 (d, J = 12.4 Hz, 1H), 4.55 (d, J = 12.8 Hz, 1H), 2.56−2.50 (m, 1H), 2.45 (s, 3H), 2.39 (d, J = 17.6 Hz, 1H), 1.36 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.1, 138.8, 133.1, 130.0, 129.4, 128.1, 110.1, 84.9, 78.0, 68.2, 38.7, 27.5, 25.6, 21.8. HRMS (ESI) calcd for $C_{16}H_{20}O_5SNa$ $[M + Na]^+$, 347.0929; found, 347.0928.

*(1S,2R,3S,5S)-2,3-O-Isopropylidene-5-azidomethyl-1,2,3-cyclopentanetriol (**37**).* To a solution of **36** (353.3 mg, 1.09 mmol) in DMF (2 mL) was added NaN_3 (354.4 mg, 5.45 mmol) under argon atmosphere. After stirring overnight at room temperature, the reaction mixture was partitioned between ethyl acetate and H_2O, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in THF (25 mL) under argon atmosphere, and then B_2H_6·THF (3.3 mL, 3.27 mmol, 1 M) was added at 0 °C. After stirring at the same temperature for 5 h, the reaction was quenched with methanol (8.2 mL). After 10 min, 3 N NaOH (2 mL, 6.00 mmol) and H_2O_2 (aq) (0.69 mL, 6.00 mmol, 27%) were added at 0 °C. After stirring overnight at 40 °C, the solvent was evaporated and the residue was partitioned between CH_2Cl_2 and H_2O, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to give **37** (179.6 mg, 77% yield) as colorless oil. R_f = 0.27 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = +6.5 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (td, J = 6.4, 3.2 Hz, 1H, H3), 4.40 (dd, J = 6.8, 2.8 Hz, 1H, H2), 4.03 (dt, J = 4.8, 3.2 Hz, 1H, H1), 3.51 (dd, J = 12.4, 7.6 Hz, 1H, H6α), 3.43 (dd, J = 12.4, 6.8 Hz, 1H, H6β), 2.51 (d, J = 3.2 Hz, 1H, OH), 2.24 (dt, J = 14.0, 7.6 Hz, 1H, H4α), 2.18 (dt, J = 12.8, 6.8 Hz, 1H, H5), 1.72 (ddd, J = 13.6, 6.0, 3.2 Hz, 1H, H4β), 1.49 (s, 3H, CH_3), 1.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.8, 87.3, 79.2, 79.1, 53.3, 46.5, 33.4, 26.7, 24.2. HRMS (ESI) calcd for $C_9H_{19}N_4O_3$ $[M + NH_4]^+$, 231.1452; found, 231.1457.

*(1S,2R,3S,5S)-1-Benzyl-2,3-O-isopropylidene-5-azidomethyl-1,2,3-cyclopentane-triol (**38**).* To a solution of **37** (197.0 mg, 0.92 mmol) in DMF (3 mL) were added NaH (110.8 mg, 2.77 mmol, 60% in mineral oil) and BnBr (236.0 mg, 164 μL, 1.38 mmol) at 0 °C under argon atmosphere. After stirring overnight at room temperature, the reaction was quenched with H_2O and extracted with ethyl acetate. Then the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 20:1) to give **38** (243.0 mg, 87% yield) as colorless oil. R_f = 0.49 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = −19.1 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.28 (m, 5H, Ar), 4.71 (td, J = 6.4, 3.2 Hz, 1H, H3), 4.66 (d, J = 11.6 Hz, 1H, PhCH_2), 4.54−4.52 (m, 2H, H2, PhCH_2), 3.76 (dd, J = 4.8, 2.0 Hz, 1H, H1), 3.47 (dd, J = 12.2, 6.8 Hz, 1H, H6α), 3.37 (dd, J = 12.4, 7.2 Hz, 1H, H6β), 2.32 (td, J = 14.0, 7.2 Hz, 1H, H5), 2.21 (dt, J = 14.4, 6.4 Hz, 1H, H4α), 1.74 (ddd, J = 14.0, 6.0, 2.8 Hz, 1H, H4β), 1.48 (s, 3H, CH_3), 1.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0 (Ph), 128.6 (Ph), 127.9 (Ph), 111.7 ($C(CH_3)_2$), 85.6 (C1, C2), 79.5 (C3), 71.7 (PhCH_2), 53.3 (C6), 44.7 (C5), 33.6 (C4), 26.8 (CH_3), 24.2 (CH_3). HRMS (ESI) calcd for $C_{16}H_{25}N_4O_3$$[M + NH_4]^+$, 321.1927; found, 321.1927.

*(1S,2R,3S,5S)-1-Benzyl-5-azidomethyl-1,2,3-cyclopentanetriol (**39**).* Compound **38** (324.6 mg, 1.07 mmol) was dissolved in 90% (v/v) acetic acid solution (15 mL). After stirring at 70 °C for 5 h, the reaction mixture was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 1:1) to give **39** (275.2 mg, 98% yield) as colorless oil. R_f = 0.26 (petroleum ether/ethyl acetate 1:2); $[\alpha]_D^{25}$ = −14.1 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.28 (m, 5H), 4.71 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 11.6 Hz, 1H), 4.15 (dt, J = 10.4, 4.8 Hz, 1H), 4.00 (dd, J = 10.4, 5.2 Hz, 1H), 3.66 (t, J = 5.6 Hz, 1H), 3.46 (dd, J = 12.4, 6.0 Hz, 1H), 3.38 (dd, J = 12.0, 6.0 Hz, 1H), 2.38 (d, J = 5.6 Hz, 1H), 2.24 (d, J = 4.8 Hz, 1H), 2.23−2.10 (m, 2H), 1.55−1.50 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 128.6, 128.0, 86.4, 78.3, 72.4, 71.6, 54.8, 41.0, 33.4. HRMS (ESI) calcd for $C_{13}H_{21}N_4O_3$ $[M + NH_4]^+$, 281.1608; found, 281.1610.

*(1S,2R,3S,4S)-3-Benzyl-4-azidomethyl-1,2,3-cyclopentanetriol 1,2-Cyclicsulfate (**40**).* To a solution of **39** (275.2 mg, 1.04 mmol) in THF (14 mL) was added NaH (166.4 mg, 4.16 mmol, 60% in mineral oil) at −20 °C under argon atmosphere. After 15 min, Im_2SO_2 (247.8 mg, 1.25 mmol) was added. After stirring at the same temperature for 30 min, the reaction was quenched with saturated NH_4Cl solution. The mixture was extracted with ethyl acetate, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 6:1) to give **40** (270.8 mg, 80% yield) as colorless oil. R_f = 0.56 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = −34.7 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.32 (m, 5H), 5.22 (td, J = 7.2, 6.0 Hz, 1H), 5.04 (dd, J = 7.6, 4.0 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 4.02 (dd, J = 8.4, 4.4 Hz, 1H), 3.51 (dd, J = 12.4, 4.8 Hz, 1H), 3.40 (dd, J = 12.4, 6.4 Hz, 1H), 2.48 (dt, J = 14.0, 7.2 Hz, 1H), 2.25−2.15 (m, 1H), 2.00 (ddd, J = 14.4, 10.8, 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.6, 128.8, 128.6, 128.2, 87.5, 83.0, 81.3, 72.8, 51.4, 41.2, 32.1. HRMS (ESI) calcd for $C_{13}H_{19}N_4O_5S$ $[M + NH_4]^+$, 343.1071; found, 343.1075.

*(2S,3S)-2-Benzyloxy-3-azidomethyl-cyclopentanone (**41**).* To a solution of **40** (13.3 mg, 0.04 mmol) in THF (0.9 mL) was added tBuOK (18.0 mg, 0.16 mmol) under argon atmosphere. After stirring at room temperature for 40 min, 5% H_2SO_4 (0.43 mL) was added and the reaction was stirred overnight at room temperature. The mixture was partitioned between ethyl acetate and H_2O, then the combined organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10:1) to give **41** (9.0 mg, 90% yield) as colorless oil. R_f = 0.56 (petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{25}$ = −41.9 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.30 (m, 5H), 5.05 (d, J = 11.6 Hz, 1H), 4.69 (d, J = 11.6 Hz, 1H), 3.66 (d, J = 11.2 Hz, 1H), 3.55 (dd, J = 12.4, 3.6 Hz, 1H), 3.40 (dd, J = 12.4, 6.0 Hz, 1H), 2.38

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J. Med. Chem. XXXX, XXX, XXX−XXX

(dd, J = 19.6, 9.2 Hz, 1H), 2.33−2.19 (m, 2H), 2.10−2.02 (m,1H), 1.70−1.58 (m,1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.9, 137.6, 128.6, 128.4, 128.2, 81.7, 72.9, 52.7, 42.1, 34.9, 20.8. HRMS (ESI) calcd for $C_{13}H_{19}N_4O_2$ $[M + NH_4]^+$, 263.1503; found, 263.1509.

*(2S,3S)-2-Benzyloxy-3-acetamidomethyl-cyclopentanone (**42**).* To a solution of **41** (45.5 mg, 0.18 mmol) in pyridine (3 mL) was added thioacetic acid (342.5 mg, 0.32 mL, 4.50 mmol) under argon atmosphere. After stirring at room temperature for 4 h, the reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/acetone = 2:1) to give **42** (35.9 mg, 74% yield) as white solid. R_f = 0.58 (petroleum ether/acetone 1:2); $[\alpha]_D^{25}$ = −24.2 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.34 (m, 5H, Ar), 5.62 (s, 1H, NH), 5.12 (d, J = 11.2 Hz, 1H, PhCH_2), 4.67 (d, J = 11.2 Hz, 1H, PhCH_2), 3.62 (d, J = 11.6 Hz, 1H, H2), 3.52 (dt, J = 13.6, 6.0 Hz, 1H, H6α), 3.24 (ddd, J = 12.4, 7.6, 4.4 Hz, 1H, H6β), 2.38 (dd, J = 19.6, 9.2 Hz, 1H, H5α), 2.24 (dd, J = 20.0, 10.4 Hz, 1H, H5β), 2.21−2.13 (m, 1H, H3), 2.09−2.02 (m, 1H, H4α), 1.82 (s, 3H, CH_3), 1.51 (dt, J = 22.0, 11.6, 1H, H4β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.1 (C1), 170.3 (CH_3CO), 137.7 (Ar), 128.8 (Ar), 128.7 (Ar), 128.4 (Ar), 84.5 (C2), 72.9 (PhCH_2), 42.8 (C6), 41.7 (C3), 35.1 (C5), 23.2 (CH_3CO), 21.2 (C4). HRMS (ESI) calcd for $C_{15}H_{19}NO_3Na$ $[M + Na]^+$, 284.1257; found, 284.1257.

*Dibenzyl [α-Hydroxy-(2S,3S)-2-benzyloxy-3-acetamidomethyl-1-cyclopentyl]-phosphonate (**43**).* To a solution of dibenzyl phosphite (712.2 mg, 600 μL, 2.31 mmol, 85%) in THF (4.0 mL) was added NaH (61.6 mg, 1.54 mmol, 60% in mineral oil) at 0 °C under argon atmosphere. After 30 min, a solution of **42** (40.2 mg, 0.15 mmol) in THF (4.0 mL) was added slowly to the reaction bottle. The reaction was stirred overnight at room temperature and quenched with saturated NH_4Cl solution. Then the mixture was partitioned between CH_2Cl_2 and H_2O, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/acetone = 1:2) to give **43** (70.6 mg, 88% yield, ***R*:*S*** = 1:1.6) as colorless oil. R_f = 0.19 (petroleum ether/acetone 1:2); **43*R***: $[\alpha]_D^{20}$ = −13.3 (c = 0.03, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.20 (m, 15H, Ar), 5.93 (t, J = 4.8 Hz, 1H, NH), 5.06 (s, 1H, PhCH_2), 5.04 (s, 1H, PhCH_2), 4.99 (dd, J = 11.6, 7.2 Hz, 1H, PhCH_2), 4.94 (dd, J = 12.0, 8.4 Hz, 1H, PhCH_2), 4.69 (d, J = 11.6 Hz, 1H, PhCH_2), 4.57 (d, J = 11.2 Hz, 1H, PhCH_2), 3.84 (dd, J = 7.6, 4.8 Hz, 1H, H2), 3.54 (s, 1H, OH), 3.32−3.21 (m, 2H, H6), 2.39−2.29 (m, 2H, H3, H4α), 1.96−1.84 (m, 2H, H5α, H4β), 1.82 (s, 3H, CH_3), 1.60−1.50 (m, 1H, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3CO), 137.9 (Ar), 136.62 (d, J = 3.0 Hz Ar), 136.56 (d, J = 3.0 Hz Ar), 128.7 (Ar), 128.63 (Ar), 128.59 (Ar), 128.53 (Ar), 128.46 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 90.2 (d, J = 2.0 Hz C2), 82.8 (d, J = 165.0 Hz C1), 72.6 (PhCH_2), 68.4 (d, J = 7.0 Hz PhCH_2), 68.1 (d, J = 8.0 Hz PhCH_2), 44.1 (d, J = 7.0 Hz C3), 42.8 (C6), 34.7 (d, J = 6.0 Hz C4), 25.2 (d, J = 12.0 Hz C5), 23.3 (CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.87. HRMS (ESI) calcd for $C_{29}H_{35}NO_6P$ $[M + H]^+$, 524.2202; found, 524.2200. **43*S***: $[\alpha]_D^{20}$ = −38.0 (c = 0.05, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37−7.27 (m, 15H, Ar), 5.18 (t, J = 5.4 Hz, 1H, NH), 5.15−5.09 (m, 3H, PhCH_2), 5.04 (dd, J = 12.0, 7.2 Hz, 1H, PhCH_2), 4.80 (d, J = 11.4 Hz, 1H, PhCH_2), 4.54 (d, J = 10.8 Hz, 1H, PhCH_2), 3.79 (t, J = 8.4 Hz, 1H, H2), 3.26 (s, 1H, OH), 3.20−3.11 (m, 2H, H6), 2.56−2.16 (m, 2H, H4β, H3), 1.91−1.80 (m, 2H, H4α, H5α), 1.73 (s, 3H, CH_3), 1.24−1.18 (m, 1H, H5β). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (CH_3CO), 137.6 (Ar), 136.4 (d, J = 6.0 Hz Ar), 136.3 (d, J = 5.0 Hz Ar), 128.85 (Ar), 128.81 (Ar), 128.78 (Ar), 128.7 (Ar), 128.4 (Ar), 128.32 (Ar), 128.28 (Ar), 84.5 (d, J = 8.0 Hz C2), 77.2 (d, J = 170.0 Hz C1), 73.7 (PhCH_2), 68.8 (d, J = 7.0 Hz PhCH_2), 68.3 (d, J = 7.0 Hz PhCH_2), 43.3 (d, J = 12.0 Hz C3), 41.4 (C6), 33.2 (d, J = 7.0 Hz C4), 23.6 (d, J = 11.0 Hz C5), 23.2 (CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.36.

*Dibenzyl α-Hydroxy-(3-phenoxy)phenylmethyl-phosphonate (**44**).* To a solution of 3-phenoxybenzaldehyde (219.0 mg, 1.10 mmol) in THF (4.0 mL) were added dibenzyl phosphite (681.3 mg, 574 μL, 2.21 mmol, 85%) and triethylamine (223.6 mg, 308 μL, 2.21 mmol) under argon atmosphere. The reaction mixture was stirred overnight at room temperature and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:1) to give **44** (429.0 mg, 84% yield) as white solid. R_f = 0.20 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.18 (m, 14H), 7.12 (dd, J = 4.0, 2.0 Hz, 1H), 7.08 (t, J = 7.4 Hz, 1H), 6.96−6.94 (m, 3H), 5.03−4.88 (m, 5H), 3.56 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.24 (d, J = 3.0 Hz), 157.20, 138.7 (d, J = 1.0 Hz), 136.2 (d, J = 4.0 Hz), 136.1 (d, J = 3.0 Hz), 129.8, 129.7 (d, J = 2.0 Hz), 128.6 (d, J = 4.0 Hz), 128.4 (d, J = 5.0 Hz), 128.0 (d, J = 5.0 Hz), 123.3, 122.2 (d, J = 5.0 Hz), 118.9, 118.6 (d, J = 3.0 Hz), 117.9 (d, J = 6.0 Hz), 70.9 (d, J = 158.0 Hz), 68.8 (d, J = 7.0 Hz), 68.5 (d, J = 7.0 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.66. HRMS (ESI) calcd for $C_{27}H_{25}O_5NaP$ $[M + Na]^+$, 483.1337; found, 483.1338.

*5′-[Benzyl-N,N-bis(1-methylethyl)phosphoramidite]-N^4-2′,3′-O-tris-benzyloxy-carbonyl Cytidine (**46**).* To a solution of bis(*N*,*N*-diisopropylamino)chlorophosphine (1.35 g, 5.06 mmol) in ether (10 mL) was added a mixture of benzyl alcohol (0.55 g, 0.52 mL, 5.06 mmol) and triethylamine (0.51 g, 0.70 mL, 5.06 mmol) in ether (2.5 mL) at 0 °C under argon atmosphere. After stirring for 30 min at the same temperature, the reaction mixture was stirred for another 30 min at room temperature and then cold hexane (15 mL) was added at 0 °C. After 10 min, the mixture was filtered through a pad of Celite and concentrated under argon atmosphere to afford the benzyloxybis(*N*,*N*-diisopropylamino)phosphine. To a solution of the residue in CH_2Cl_2 (8.5 mL) was added a mixture of **45** (1.63 g, 2.53 mmol) and *H*-tetrazole (194.9 mg, 2.78 mmol) in CH_2Cl_2 (8.5 mL) under argon atmosphere. After stirring at room temperature for 4 h, the reaction mixture was concentrated under argon atmosphere and the residue was purified by column chromatography on silica gel (petroleum ether/acetone = 7:2, containing 1% triethylamine) to give **46** (2.06 g, 92% yield) as colorless foam. R_f = 0.52 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (brs, 1H), 7.38−7.20 (m, 21H), 7.12 (brs, 1H), 6.31−6.26 (m, 1H), 5.38−5.31 (m, 2H), 5.23−5.17 (m, 2H), 5.14−5.04 (m, 4H), 4.80−4.75 (m, 1H), 4.71−4.66 (m, 1H), 4.39 (brs, 1H), 4.06−3.81 (m, 2H), 3.69−3.59 (m, 2H), 1.25−1.16 (m, 12H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.13, 148.80. HRMS (ESI) calcd for $C_{46}H_{52}N_4O_{12}P$ $[M + H]^+$, 883.3314; found, 883.3343.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-cyclopentylmethyl]-phosphate (**47a**).* To a solution of **8** (58.2 mg, 0.16 mmol) in CH_2Cl_2 (6.0 mL) were added **46** (282.5 mg, 0.32 mmol) and *H*-tetrazole (22.4 mg, 0.32 mmol) under argon atmosphere. After stirring at room temperature for 2 h, TBHP (116 μL, 0.64 mmol, 5.5 M in decane) was added and the reaction mixture was stirred for another 3 h. Then the solvent was evaporated and the crude product was purified by column chromatography on silica gel (petroleum ether/acetone = 2:1 to 3:2) to give **47a** (135.2 mg, 72% yield) as colorless oil. R_f = 0.45 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87−7.85 (m, 1H), 7.36−7.23 (m, 31H), 7.13−7.11 (m, 1H), 6.05−6.02 (m, 1H), 5.37−5.04 (m, 14H), 4.78−4.76 (m, 1H), 4.33−4.13 (m, 3H), 2.40−2.38 (m, 1H), 1.82 (brs, 2H), 1.58−1.49 (m, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.50−20.23 (m), −0.13 − −1.16 (m). HRMS (ESI) calcd for $C_{60}H_{61}N_3O_{17}P_2Na$ $[M + Na]^+$, 1180.3368; found, 1180.3330.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-c-3,c-4-isopropylidenedioxy-r-1-cyclopentylmethyl]-phosphate (**47b**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **20** (42.8 mg, 0.10 mmol), **46** (180.1 mg, 0.20 mmol), *H*-tetrazole (14.0 mg, 0.20 mmol), CH_2Cl_2 (5.0 mL), 3 h, and TBHP (73 μL, 0.40 mmol, 5.5 M in decane), 6 h, eluent (petroleum ether/acetone = 3:2), affording **47b** (109.2 mg, 90% yield) as colorless oil. R_f = 0.29 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89−7.84 (m, 1H), 7.10 (brs, 1H), 7.38−7.22 (m, 30H), 7.16−7.03 (m, 1H), 6.13−6.02 (m, 1H), 5.36−4.90 (m, 15H), 4.63−4.09 (m, 5H), 2.57−2.44 (m, 1H), 2.26−1.78 (m, 4H), 1.54−1.51 (m, 3H), 1.29−1.22 (m, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.52−20.09 (m), 0.12 − −0.76 (m). HRMS (ESI) calcd for $C_{63}H_{65}N_3O_{19}P_2$ $[M + Na]^+$, 1252.3580; found, 1252.3522.

Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-(1S,3R,4S)-3-benzyloxy-4-acetamido-1-cyclopen-

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J. Med. Chem. XXXX, XXX, XXX−XXX

*tylmethyl]-phosphate (**47c**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **27*S*** (69.3 mg, 0.13 mmol), **46** (233.1 mg, 0.26 mmol), and *H*-tetrazole (18.5 mg, 0.26 mmol), 1 h; TBHP (96 μL, 0.53 mmol, 5.5 M in decane), eluent (petroleum ether/acetone = 1:1), affording **47c** (132.6 mg, 76% yield) as colorless oil. R_f = 0.17 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.66 (m, 2H), 7.37−7.16 (m, 35H), 7.14−6.80 (m, 1H), 6.01−5.68 (m, 1H), 5.50−5.27 (m, 2H), 5.18−4.99 (m, 12H), 4.83−4.69 (m, 1H), 4.61−4.49 (m, 2H), 4.37−4.03 (m, 5H), 3.84−3.76 (m, 1H), 2.83−2.75 (m, 1H), 2.39−2.00 (m, 2H), 1.93−1.89 (m, 3H), 1.73−1.60 (m, 1H), 1.49−1.38 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.73−19.35 (m), −0.27 − −1.56 (m). HRMS (ESI) calcd for $C_{69}H_{70}N_4O_{19}P_2Na$ $[M + Na]^+$, 1343.4002; found, 1343.4027.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-(1R,3R,4R)-3-benzyloxy-4-acetamido-1-cyclopentylmethyl]-phosphate (**47d**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **27*R*** (67.8 mg, 0.13 mmol), **46** (229.6 mg, 0.26 mmol), *H*-tetrazole (18.2 mg, 0.26 mmol), CH_2Cl_2 (5.0 mL), TBHP (95 μL, 0.52 mmol, 5.5 M in decane), and eluent (petroleum ether/acetone = 1:1), affording **47d** (134.9 mg, 79% yield) as colorless oil. R_f = 0.10 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (brs, 1H), 7.82−7.76 (m, 1H), 7.36−7.20 (m, 35H), 7.13−7.02 (m, 1H), 6.03−6.00 (m, 1H), 5.90−5.66 (m, 1H), 5.35−5.21 (m, 2H), 5.18−4.89 (m, 12H), 4.85−4.71 (m, 1H), 4.61−4.48 (m, 2H), 4.34−4.02 (m, 4H), 3.83−3.74 (m, 1H), 2.72−2.60 (m, 1H), 2.31−2.06 (m, 2H), 1.99−1.89 (m, 3H), 1.84−1.66 (m, 2H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.73−19.54 (m), −0.32 − −1.16 (m). HRMS (ESI) calcd for $C_{69}H_{70}N_4O_{19}P_2Na$ $[M + Na]^+$, 1343.4002; found, 1343.4050.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-(1R,3R,4S)-3-benzyloxy-4-acetamidomethyl-1-cyclopentylmethyl]-phosphate (**47e**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **34** (43.1 mg, 0.08 mmol), **46** (141.3 mg, 0.16 mmol), *H*-tetrazole (11.2 mg, 0.16 mmol), CH_2Cl_2 (2.0 mL), TBHP (58 μL, 0.32 mmol, 5.5 M), and eluent (petroleum ether/acetone = 1:2), affording **47e** (70.8 mg, 66% yield) as colorless oil. R_f = 0.30 (petroleum ether/acetone 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00−7.74 (m, 1H), 7.38−7.25 (m, 36H), 7.14−7.07 (m, 1H), 6.21−5.92 (m, 2H), 5.40−4.96 (m, 14H), 4.79−4.71 (m, 1H), 4.52−4.10 (m, 5H), 3.58−3.49 (m, 1H), 3.34−3.24 (m, 1H), 3.12−3.96 (m, 1H), 2.53−2.42 (m, 1H), 2.29−2.20 (m, 1H), 2.05−1.95 (m, 3H), 1.84−1.82 (m, 3H), 1.70−1.58 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.83−19.36 (m), −0.10 − −1.06 (m). HRMS (ESI) calcd for $C_{70}H_{72}N_4O_{19}P_2Na$ $[M + Na]^+$, 1357.4158; found, 1357.4200.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-1-((2S,3S)-2-benzyloxy-3-acetamidomethyl-cyclopentyl)]-phosphate (**47f**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **43** (62.9 mg, 0.12 mmol), **46** (211.9 mg, 0.24 mmol), *H*-tetrazole (16.8 mg, 0.24 mmol), CH_2Cl_2 (3.0 mL), TBHP (87 μL, 0.48 mmol, 5.5 M in decane), and eluent (petroleum ether/acetone = 1:2), affording **47f** (121.4 mg, 76% yield) as colorless oil. R_f = 0.24 (petroleum ether/acetone 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86−7.74 (m, 1H), 7.56 (brs, 1H), 7.38−7.20 (m, 35H), 7.14−7.11 (m, 1H), 6.07−5.79 (m, 2H), 5.52−5.22 (m, 2H), 5.20−4.92 (m, 12H), 4.80−4.44 (m, 2H), 4.33−4.06 (m, 3H), 3.90−3.73 (m, 1H), 3.38−3.07 (m, 2H), 2.51−2.27 (m, 3H), 1.98−1.82 (m, 1H), 1.82−1.71 (m, 3H), 1.24−1.11 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.08−19.25 (m), −5.94 − −6.69 (m). HRMS (ESI) calcd for $C_{69}H_{71}N_4O_{19}P_2$ $[M + H]^+$, 1321.4182; found, 1321.4223.

*Benzyl (N^4-2′,3′-O-Tris-benzyloxycarbonylcytidin-5′-yl)-[(dibenzylphosphonato)-(3-phenoxy)phenylmethyl]-phosphate (**47g**).* This compound was prepared in the same manner as described in the preparation of **47a**. Quantities: **44** (52.5 mg, 0.11 mmol), **46** (201.3 mg, 0.23 mmol), *H*-tetrazole (16.0 mg, 0.23 mmol), CH_2Cl_2 (2.0 mL), TBHP (83 μL, 0.46 mmol, 5.5 M in decane), and eluent (petroleum ether/acetone = 2:1), affording **47g** (116.3 mg, 81% yield) as colorless oil. R_f = 0.31 (petroleum ether/acetone 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.75 (m, 1H), 7.49 (brs, 1H), 7.38−6.91 (m, 40H), 6.04−5.99 (m, 1H), 5.69−5.62 (m, 1H), 5.32−5.28 (m, 1H), 5.20−4.76 (m, 13H), 4.19−4.04 (m, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 16.96−16.37 (m), −0.44 − −1.14 (m). HRMS (ESI) calcd for $C_{67}H_{61}N_3O_{18}P_2Na$ $[M + Na]^+$, 1280.3323; found, 1280.3300.

*Trisodium Cytidin-5′-yl-(cyclopentylphosphonatomethyl)-phosphate (**2**).* To a solution of **47a** (135.2 mg, 0.117 mmol) in DMF (2.0 mL) were added Pd/C (135.2 mg, 10%) and 1,4-cyclohexadiene (593.8 mg, 0.68 mL, 7.188 mmol, 97%) under argon atmosphere. The reaction mixture was stirred at room temperature until TLC showed complete consumption of the starting material. Then the mixture was filtered and concentrated. The residue was purified by column chromatography on RP-18 silica gel (H_2O → H_2O/MeOH = 5:1) to give crude product **2** (42.4 mg, 75% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+) and lyophilized from water to give **2*s*** and **2*l*** as white powder. **2*s***: RP-HPLC, column A, 3.5% CH_3CN, 0.05 M TEAB (triethylammonium bicarbonate (pH 7.2−7.5)) buffer, 15 mL/min flow; t_R = 7.27 min; $[\alpha]_D^{30}$ = +17.0 (*c* = 1.5, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 8.09 (d, *J* = 7.8 Hz, 1H), 6.22 (d, *J* = 7.8 Hz, 1H), 5.94 (d, *J* = 4.2 Hz, 1H), 4.35−4.31 (m, 2H), 4.28−4.26 (m, 2H), 4.24−4.17 (m, 2H), 2.31−2.24 (m, 1H), 1.82−1.73 (m, 2H), 1.62−1.42 (m, 6H). ^{13}C NMR (151 MHz, D_2O) δ 163.5, 154.0, 143.5, 96.64, 90.3, 83.8 (d, *J* = 9.1 Hz), 77.8 (dd, *J* = 155.5, 7.6 Hz), 75.0, 69.8, 65.0 (d, *J* = 4.5 Hz), 42.0, 30.1 (d, *J* = 7.6 Hz), 28.9 (d, *J* = 7.6 Hz), 25.7, 25.5. ^{31}P NMR (162 MHz, D_2O) δ 16.94, 0.61. HRMS (ESI) calcd for $C_{15}H_{24}N_3O_{11}P_2$ $[M - H]^-$, 484.0886; found, 484.0895. **2*l***: RP-HPLC, column A, 3.5% CH_3CN, 0.05 M TEAB (triethylammonium bicarbonate (pH 7.2−7.5)) buffer, 15 mL/min flow; t_R = 9.03 min; $[\alpha]_D^{30}$ = +0.9 (*c* = 1.3, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 8.08 (d, *J* = 7.8 Hz, 1H), 6.21(d, *J* = 7.8 Hz, 1H), 5.95 (d, *J* = 3.6 Hz, 1H), 4.36 (t, *J* = 5.4 Hz, 1H), 4.33 (t, *J* = 4.2 Hz, 1H), 4.28−4.15 (m, 4H), 2.30−2.25 (m, 1H), 1.81−1.73 (m, 2H), 1.59−1.45 (m, 6H). ^{13}C NMR (151 MHz, D_2O) δ 163.6, 154.1, 143.4, 96.6, 90.2, 83.8 (d, *J* = 9.1 Hz), 77.9 (dd, *J* = 155.5, 7.6 Hz), 74.9, 69.8, 64.7 (d, *J* = 4.5 Hz), 42.0, 30.1 (d, *J* = 7.6 Hz), 28.9 (d, *J* = 6.0 Hz), 25.6, 25.5. ^{31}P NMR (162 MHz, D_2O) δ 16.54 (d, *J* = 13.0 Hz), 0.47 (d, *J* = 13.0 Hz). HRMS (ESI) calcd for $C_{15}H_{24}N_3O_{11}P_2$ $[M - H]^-$, 484.0886; found, 484.0882.

*Trisodium Cytidin-5′-yl-(c-3,c-4-dihydroxy-r-1-cyclopentylphosphonatomethyl)-phosphate (**3**).* To a solution of **47b** (11.0 mg, 0.009 mmol) in CH_2Cl_2 (1.0 mL) was added BCl_3 (108 μL, 0.108 mmol, 1 M in heptane) at 0 °C under argon atmosphere. After stirring at room temperature for 30 min, the reaction was quenched with methanol and the mixture was concentrated. The residue was purified by column chromatography on RP-18 silica gel (H_2O) to afford the crude product **3** (4.2 mg, 91% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+), and lyophilized from water to give **3*s*** and **3*l*** as white powder. **3*s***: RP-HPLC, column A, 0−5 min linear gradient 0−1.0% CH_3CN, H_2O (0.1% TFA), 15 mL/min flow, t_R = 4.20 min; $[\alpha]_D^{30}$ = +6.7 (*c* = 1.1, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.98 (d, *J* = 7.8 Hz, 1H), 6.14 (d, *J* = 7.2 Hz, 1H), 5.97 (d, *J* = 4.2 Hz, 1H), 4.35 (t, *J* = 5.4 Hz, 1H), 4.31 (d, *J* = 4.2 Hz, 1H), 4.29−4.19 (m, 4H), 4.00−3.96 (m, 2H), 2.41−2.33 (m, 1H), 2.17−2.08 (m, 2H), 1.77−1.69 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 166.2, 157.5, 142.5, 97.2, 90.2, 83.5 (d, *J* = 9.1 Hz), 77.0 (dd, *J* = 157.0, 9.1 Hz), 75.0, 73.4, 73.3, 69.9, 65.1 (d, *J* = 4.5 Hz), 35.6, 34.6 (d, *J* = 7.6 Hz), 33.7 (d, *J* = 6.0 Hz). ^{31}P NMR (162 MHz, D_2O) δ 15.85, 0.41. HRMS (ESI) calcd for $C_{15}H_{24}N_3O_{13}P_2$ $[M - H]^-$, 516.0784; found, 516.0777. **3*l***: RP-HPLC, column A, 0−5 min linear gradient 0−1.0% CH_3CN, H_2O (0.1% TFA), 15 mL/min flow, t_R = 4.43 min; $[\alpha]_D^{30}$ = +2.9 (*c* = 0.9, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 7.2 Hz, 1H), 6.12 (d, *J* = 7.8 Hz, 1H), 5.98 (d, *J* = 4.2 Hz, 1H), 4.37 (t, *J* = 4.8 Hz, 1H), 4.30 (t, *J* = 4.8 Hz, 1H), 4.27−4.19 (m, 4H), 3.99−3.95 (m, 2H), 2.41−2.33 (m, 1H), 2.15−2.07 (m, 2H), 1.78−1.70 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 166.9, 158.5, 142.2, 97.3, 90.0, 83.4 (d, *J* = 9.1 Hz), 77.1 (dd, *J* = 155.5, 7.6 Hz), 75.0, 73.4, 73.3, 69.8, 64.9 (d, *J* = 4.5 Hz), 35.6, 34.5 (d, *J* = 7.6 Hz), 33.7 (d, *J* = 6.0 Hz). ^{31}P NMR (162 MHz,

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J. Med. Chem. XXXX, XXX, XXX−XXX

D_2O) δ 15.6, 0.31. HRMS (ESI) calcd for $C_{15}H_{24}N_3O_{13}P_2$ [M − H]$^-$, 516.0784; found, 516.0779.

*Trisodium Cytidin-5′-yl-[(1S,3R,4R)-3-hydroxy-4-acetamido-1-cyclopentyl-phosphonato-methyl]-phosphate (**4S**).* This compound was prepared in the same manner as described in the preparation of **2**. Quantities: **47c** (53.3 mg, 0.04 mmol), Pd/C (53.3 mg, 10%), 1,4-cyclohexadiene (198.2 mg, 234 μL, 2.40 mmol, 97%), DMF (1.0 mL), and eluent (H_2O), affording crude product **4S** (17.7 mg, 79% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+) and lyophilized from water to give **4Ss** and **4Sl** as white powder. **4Ss**: RP-HPLC, column A, 0−5 min linear gradient 0−1.0% CH_3CN, H_2O (0.1% TFA), 15 mL/min flow, t_R = 4.28 min; $[\alpha]_D^{30}$ = +4.0 (*c* = 1.9, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 8.07 (d, *J* = 7.8 Hz, 1H), 6.20 (d, *J* = 7.8 Hz, 1H), 5.94 (d, *J* = 3.0 Hz, 1H), 4.32−4.18 (m, 6H), 4.05−4.00 (m, 1H), 3.97−3.94 (m, 1H), 2.69−2.67 (m, 1H), 2.24 (dt, *J* = 13.8, 7.2 Hz, 1H), 2.06 (dt, *J* = 13.8, 7.8 Hz, 1H), 1.96 (s, 3H), 1.80 (ddd, *J* = 13.8, 9.0, 4.8 Hz, 1H), 1.61 (dt, *J* = 13.2, 9.6 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.6, 163.6, 154.0, 143.5, 96.6, 90.3, 83.7 (d, *J* = 9.1 Hz), 77.0, 76.6 (dd, *J* = 158.6, 9.1 Hz), 75.0, 69.8, 65.1 (d, *J* = 4.5 Hz), 58.2, 37.0, 35.7 (d, *J* = 9.1 Hz), 31.9 (d, *J* = 4.5 Hz), 22.7 ppm. ^{31}P NMR (162 MHz, D_2O) δ 15.66, 0.56. HRMS (ESI) calcd for $C_{17}H_{27}N_4O_{13}P_2$ [M − H]$^-$, 557.1050; found, 557.1055. **4Sl**: RP-HPLC, column A, 0−5 min linear gradient 0−1.0% CH_3CN, H_2O (0.1% TFA), 15 mL/min flow, t_R = 4.80 min; $[\alpha]_D^{30}$ = −3.9 (*c* = 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 7.8 Hz, 1H), 6.12 (d, *J* = 7.2 Hz, 1H), 5.99 (d, *J* = 4.2 Hz, 1H), 4.36 (t, *J* = 5.4 Hz, 1H), 4.30 (t, *J* = 4.2 Hz, 1H), 4.27−4.23 (m, 3H), 4.19 (ddd, *J* = 11.4, 4.2, 2.4 Hz, 1H), 4.03 (dt, *J* = 7.8, 5.4 Hz, 1H), 3.92 (dd, *J* = 15.0, 7.2 Hz, 1H), 2.71−2.65 (m, 1H), 2.20 (dt, *J* = 13.2, 7.8 Hz, 1H), 2.16 (dt, *J* = 14.4, 8.4 Hz, 1H), 1.93 (s, 3H), 1.75 (ddd, *J* = 13.8, 9.0, 4.8 Hz, 1H), 1.54 (dt, *J* = 12.6, 9.6 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.6, 166.2, 157.6, 142.4, 97.2, 89.9, 83.5 (d, *J* = 9.1 Hz), 77.1, 76.4 (dd, *J* = 158.6, 9.1 Hz), 74.9, 69.9, 64.9 (d, *J* = 6.0 Hz), 58.1, 36.9, 33.8 (d, *J* = 10.6 Hz), 33.3 (d, *J* = 3.0 Hz), 22.6. ^{31}P NMR (162 MHz, D_2O) δ 15.65, 0.20. HRMS (ESI) calcd for $C_{17}H_{27}N_4O_{13}P_2$ [M − H]$^-$, 557.1050; found, 557.1069.

*Trisodium Cytidin-5′-yl-[(1S,3R,4R)-3-benzyloxy-4-acetamido-1-cyclopentyl-phosphonatomethyl]-phosphate (**5S**).* To a solution of **47c** (117.7 mg, 0.089 mmol) in EtOH (2.7 mL) were added Pd/C (235.4 mg, 10%) and 1,4-cyclohexadiene (847.0 mg, 1.0 mL, 10.68 mmol, 97%) under argon atmosphere. The reaction mixture was stirred at room temperature until TLC showed complete consumption of the starting material. Then the mixture was filtered and concentrated. The residue was purified by column chromatography on RP-18 silica gel (H_2O → MeOH/H_2O = 1:1) to give crude product **5S** (25.2 mg, 44% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+) and lyophilized from water to give **5Ss** and **5Sl** as white powder. **5Ss**: RP-HPLC, column B, 0−18 min linear gradient 7.0−8.8% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 15.72 min; $[\alpha]_D^{30}$ = −1.7 (*c* = 0.7, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.94 (d, *J* = 7.2 Hz, 1H), 7.42−7.35 (m, 5H), 6.08 (d, *J* = 7.8 Hz, 1H), 5.97 (d, *J* = 4.2 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.32 (t, *J* = 5.4 Hz, 1H), 4.27−4.07 (m, 6H), 3.87 (dt, *J* = 7.2, 4.8 Hz, 1H), 2.68−2.60 (m, 1H), 2.20 (dt, *J* = 12.6, 7.8 Hz, 1H), 2.12 (dt, *J* = 13.8, 9.0 Hz, 1H), 1.94−1.90 (m, 1H), 1.89 (s, 3H), 1.50 (dt, *J* = 12.6, 10.2 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.0, 166.5, 157.9, 142.2, 138.1, 129.4, 129.1, 128.9, 97.1, 89.9, 84.2, 83.3 (d, *J* = 9.1 Hz), 76.7 (dd, *J* = 157.0, 7.6 Hz), 75.0, 71.8, 69.7, 64.7 (d, *J* = 4.5 Hz), 56.4, 37.6, 34.3 (d, *J* = 9.1 Hz), 32.3 (d, *J* = 4.5 Hz), 22.6 ppm. ^{31}P NMR (162 MHz, D_2O) δ 15.55, 0.11. HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ [M − H]$^-$, 647.1519; found, 647.1513. **5Sl**: RP-HPLC, column B, 0−18 min linear gradient 7.0−8.8% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 16.70 min; $[\alpha]_D^{30}$ = +0.5 (*c* = 0.4, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 7.8 Hz, 1H), 7.42−7.36 (m, 5H), 6.11 (d, *J* = 7.2 Hz, 1H), 5.94 (d, *J* = 3.6 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.29−4.24 (m, 5H), 4.19−4.16 (m, 1H), 4.14−4.10 (m, 1H), 3.91 (dt, *J* = 6.6, 4.8 Hz, 1H), 2.70−2.62 (m, 1H), 2.25 (dt, *J* = 13.8, 7.2 Hz, 1H), 2.08−2.02 (m, 1H), 1.92 (s, 3H), 1.92−1.89 (m, 1H), 1.58 (dt, *J* = 13.2, 9.6 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.1, 165.7, 156.9, 142.6, 138.2, 129.4, 129.1, 128.9, 97.0, 90.3, 84.5, 83.4 (d, *J* = 9.1 Hz), 76.4 (dd, *J* = 155.5, 7.6 Hz), 75.0, 71.8, 69.8, 65.2 (d, *J* = 4.5 Hz), 56.4, 37.5, 34.0 (d, *J* = 10.6 Hz), 32.3 (d, *J* = 4.5 Hz), 22.7. ^{31}P NMR (162 MHz, D_2O) δ 15.52, 0.48. HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ [M − H]$^-$, 647.1519; found, 647.1525.

*Trisodium Cytidin-5′-yl-[(1R,3R,4R)-3-benzyloxy-4-acetamido-1-cyclopentyl-phosphonatomethyl]-phosphate (**5R**).* This compound was prepared in the same manner as described in the preparation of **5S**. Quantities: **47d** (210.7 mg, 0.16 mmol), Pd/C (421.4 mg, 10%), 1,4-cyclohexadiene (1.61 g, 1.9 mL, 19.08 mmol, 97%), EtOH (4.8 mL), and eluent (H_2O → MeOH/H_2O = 1:1), affording crude product **5R** (51.8 mg, 50% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+), and lyophilized from water to give **5Rs** and **5Rl** as white powder. **5Rs**: RP-HPLC, column B, 0−16 min linear gradient 8−9.5% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 11.95 min; $[\alpha]_D^{30}$ = +0.7 (*c* = 0.3, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 7.8 Hz, 1H), 7.41−7.35 (m, 5H), 6.09 (d, *J* = 7.8 Hz, 1H), 5.93 (d, *J* = 3.6 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 1H), 4.31 (t, *J* = 5.4 Hz, 1H), 4.28−4.17 (m, 5H), 4.10−4.07 (m, 1H), 3.91 (dt, *J* = 7.2, 6.6 Hz, 1H), 2.52−2.48 (m, 1H), 2.38 (dt, *J* = 12.6, 6.6 Hz, 1H), 2.12 (dt, *J* = 13.8, 9.6 Hz, 1H), 1.92 (s, 3H), 1.68−1.62 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 174.0, 166.9, 158.3, 142.1, 138.2, 129.4, 129.2, 128.9, 97.1, 90.4, 85.3, 83.3 (d, *J* = 9.1 Hz), 76.4 (dd, *J* = 152.5, 15.1 Hz), 75.2, 72.1, 69.4, 64.8, 55.4, 37.6, 34.2 (d, *J* = 7.6 Hz), 33.8 (d, *J* = 4.5 Hz), 22.7. ^{31}P NMR (162 MHz, D_2O) δ 14.68, 0.56. HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ [M − H]$^-$, 647.1519; found, 647.1537. **5Rl**: RP-HPLC, column B, 0−16 min linear gradient 8−9.5% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 12.58 min; $[\alpha]_D^{30}$ = −3.7 (*c* = 0.4, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.98 (d, *J* = 7.8 Hz, 1H), 7.41−7.34 (m, 5H), 6.09 (d, *J* = 7.8 Hz, 1H), 5.90 (d, *J* = 3.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 11.4 Hz, 1H), 4.32 (t, *J* = 5.4 Hz, 1H), 4.28−4.19 (m, 5H), 4.05 (dt, *J* = 9.6, 4.8 Hz, 1H), 3.91 (dt, *J* = 8.4, 6.6 Hz, 1H), 2.50 (brs, 1H), 2.27 (dt, *J* = 12.6, 6.0 Hz, 1H), 2.17 (dt, *J* = 13.8, 10.2 Hz, 1H), 1.89 (s, 3H), 1.68−1.62 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 173.9, 166.9, 158.3, 142.0, 138.2, 129.3, 129.1, 128.9, 97.0, 90.3, 85.0, 83.0 (d, *J* = 9.1 Hz), 77.2 (d, *J* = 157.0 Hz), 75.2, 72.1, 69.2 (d, *J* = 7.6 Hz), 64.3, 55.3, 37.3, 35.0 (d, *J* = 9.1 Hz), 32.6 (d, *J* = 6.0 Hz), 22.6. ^{31}P NMR (162 MHz, D_2O) δ 14.95, 0.36. HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ [M − H]$^-$, 647.1519; found, 647.1525.

*Trisodium Cytidin-5′-yl-[(1R,3R,4S)-3-benzyloxy-4-acetamido-methyl-1-cyclopentyl-phosphonatomethyl]-phosphate (**6**).* This compound was prepared in the same manner as described in the preparation of **5S**. Quantities: **47e** (68.4 mg, 0.051 mmol), Pd/C (136.8 mg, 10%), 1,4-cyclohexadiene (508.2 mg, 0.60 mL, 6.120 mmol, 97%), EtOH (2.2 mL), and eluent (H_2O → MeOH/H_2O = 1:1), affording crude product **6** (17.6 mg, 52% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+) and lyophilized from water to give **6s** and **6l** as white powder. **6s**: RP-HPLC, column B, 0−16 min linear gradient 8−11.5% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 12.65 min; $[\alpha]_D^{30}$ = −3.6 (*c* = 0.2, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 8.01 (d, *J* = 7.8 Hz, 1H), 7.41−7.37 (m, 5H), 6.08 (d, *J* = 7.8 Hz, 1H), 5.93 (d, *J* = 3.6 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.46 (d, *J* = 11.4 Hz, 1H), 4.33 (t, *J* = 6.0 Hz,, 1H), 4.26−4.17 (m, 5H), 3.75 (dt, *J* = 8.4, 6.6 Hz, 1H), 3.20 (dd, *J* = 13.8, 7.2 Hz, 5H), 3.12 (dd, *J* = 13.8, 7.2 Hz, 1H), 2.47 (dt, *J* = 12.6, 6.6 Hz, 1H), 2.41 (brs, 1H), 2.18−2.12 (m, 1H), 1.99−1.93 (m, 1H), 1.92 (s, 3H), 1.64 (td, *J* = 11.4, 9.0 Hz, 1H), 1.54 (ddd, *J* = 12.6, 7.8, 4.8 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.7, 166.9, 158.3, 142.2, 138.2, 129.4, 129.3, 128.9, 97.1, 90.5, 84.1, 83.4 (d, *J* = 9.1 Hz), 79.0 (d, *J* = 149.5 Hz), 75.3, 71.9, 69.2, 64.5 (d, *J* = 4.5 Hz), 43.6, 43.1, 38.4, 35.1 (d, *J* = 4.5 Hz), 31.5 (d, *J* = 7.6 Hz), 22.6. ^{31}P NMR (162 MHz, D_2O) δ 14.38, 0.70. HRMS (ESI) calcd for $C_{25}H_{35}N_4O_{13}P_2$ [M − H]$^-$, 661.1676; found, 661.1675. **6l**: RP-HPLC, column B, 0−16 min linear gradient 8−11.5% CH_3CN, 100 mM

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J. Med. Chem. XXXX, XXX, XXX−XXX

NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 13.77 min; $[\alpha]_D^{30}$ = −7.1 (*c* = 0.3, H_2O). ^1H NMR (400 MHz, D_2O) δ 7.98 (d, *J* = 7.6 Hz, 1H), 7.45−7.35 (m, 5H), 6.11 (d, *J* = 7.6 Hz, 1H), 5.94 (d, *J* = 3.6 Hz, 1H), 4.56 (d, *J* = 11.2 Hz, 1H), 4.45 (d, *J* = 11.2 Hz, 1H), 4.33 (t, *J* = 5.6 Hz, 1H), 4.30−4.22 (m, 5H), 3.76 (dt, *J* = 8.4, 6.4 Hz, 1H), 3.21 (dd, *J* = 13.2, 6.8 Hz, 1H), 3.10 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.43 (brs, 1H), 2.32 (dt, *J* = 12.4, 6.4 Hz, 1H), 2.10−2.07 (m, 1H), 2.04−1.96 (m, 1H), 1.91 (s, 3H), 1.68−1.62 (m, 1H), 1.59−1.55 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.6, 166.9, 158.3, 142.2, 138.3, 129.3, 129.1, 128.9, 97.1, 90.3, 83.8, 83.1 (d, *J* = 9.1 Hz), 78.1 (d, *J* = 154.0 Hz), 75.2, 72.0, 69.0, 64.0, 43.7, 43.2, 37.9, 36.5 (d, *J* = 7.6 Hz), 29.1, 22.6. ^{31}P NMR (162 MHz, D_2O) δ 14.36, 0.42. HRMS (ESI) calcd for $C_{25}H_{35}N_4O_{13}P_2$ $[M - H]^-$, 661.1676; found, 661.1702.

*Trisodium Cytidin-5′-yl-[phosphonato-1-((2S,3S)-2-benzyloxy-3-acetamido-methyl-cyclopentyl)]-phosphate (**48**).* This compound was prepared in the same manner as described in the preparation of **5S**. Quantities: **47f** (16.1 mg, 0.012 mmol), Pd/C (16.1 mg, 10%), 1,4-cyclohexadiene (59.3 mg, 70 μL, 0.720 mmol, 97%), EtOH (0.5 mL), and eluent (H_2O → MeOH/H_2O = 1:1), affording crude product **48** (5.8 mg, 73% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+), and lyophilized from water to give **48*s*** and **48*l*** as white powder. **48*s***: RP-HPLC, column A, 6.0% CH_3CN, 0.05 M TEAB (triethylammonium bicarbonate (pH 7.2−7.5)) buffer, 15 mL/min flow; t_R = 6.13 min; $[\alpha]_D^{30}$ = −22.2 (*c* = 0.9, H_2O). ^1H NMR (600 MHz, D_2O) δ 7.98 (d, *J* = 7.8 Hz, 1H, H6′), 7.37−7.33 (m, 5H, Ar), 6.11 (d, *J* = 7.8 Hz, 1H, H7′), 5.77 (d, *J* = 1.8 Hz, 1H, H1′), 4.92 (d, *J* = 11.4 Hz, 1H, PhCH_2), 4.54 (d, *J* = 11.4 Hz, 1H, PhCH_2), 4.41 (dd, *J* = 7.2, 4.8 Hz, 1H, H3′), 4.34−4.28 (m, 2H, H5′*α*, H5′*β*), 4.12−4.10 (m, 1H, H4′), 3.91 (ddd, *J* = 9.6, 5.4, 4.2 Hz, 1H, H2), 3.50−3.49 (m, 1H, H2′), 3.28 (dd, *J* = 13.2, 5.4 Hz, 1H, H6*α*), 3.13 (dd, *J* = 13.8, 6.6 Hz, 1H, H6*β*), 2.43−2.33 (m, 2H, H3, H4*α*), 2.27 (td, *J* = 15.6, 9.0 Hz, 1H, H4*β*), 2.00−1.94 (m, 1H, H5*α*), 1.87 (s, 3H, CH_3), 1.37−1.31 (m, 1H, H5*β*). ^{13}C NMR (151 MHz, D_2O) δ 174.7 (CH_3CO), 164.7 (C8′), 155.2 (C9′), 142.8 (C6′), 138.9 (Ph), 129.2 (Ph), 129.1 (Ph), 128.8 (Ph), 96.4 (C7′), 90.7 (C1′), 86.0 (dd, *J* = 157.0, 7.6 Hz C1), 85.9 (dd, *J* = 6.0, 3.0 Hz C2), 82.7 (d, *J* = 6.0 Hz C4′), 75.1 (C2′), 74.0 (PhCH_2), 68.2 (C3′), 64.2 (d, *J* = 6.0 Hz C5′), 43.1 (d, *J* = 9.1 Hz C3), 42.1 (C6), 32.2 (d, *J* = 6.0 Hz C4), 23.3 (d, *J* = 9.1 Hz C5), 22.6 (CH_3). ^{31}P NMR (162 MHz, D_2O) δ 17.21, −2.48. HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ $[M - H]^-$, 647.1519; found, 647.1522. **48*l***: RP-HPLC, column A, 6.0% CH_3CN, 0.05 M TEAB (triethylammonium bicarbonate (pH 7.2−7.5)) buffer, 15 mL/min flow; t_R = 10.55 min; $[\alpha]_D^{30}$ = −11.5 (*c* = 0.5, H_2O). ^1H NMR (600 MHz, D_2O) δ 7.94 (d, *J* = 7.8 Hz, 1H, H6′), 7.44−7.35 (m, 5H, Ar), 6.02 (d, *J* = 7.8 Hz, 1H, H7′), 5.92 (d, *J* = 3.6 Hz, 1H, H1′), 4.99 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.61 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.35 (t, *J* = 5.4 Hz, 1H, H3′), 4.31−4.29 (m, 1H, H5′*α*), 4.26 (t, *J* = 4.2 Hz, 1H, H2′), 4.24−4.19 (m, 3H, H4′, H2, H5′*β*), 3.16 (dd, *J* = 13.8, 6.0 Hz, 1H, H6*α*), 3.08 (dd, *J* = 13.8, 7.2 Hz, 1H, H6*β*), 2.44−2.34 (m, 2H, H4*α*, H4*β*), 2.33−2.28 (m, 1H, H3),1.84 (s, 3H, CH_3), 1.83−1.77 (m, 1H, H5*α*), 1.55−1.48 (m, 1H, H5*β*). ^{13}C NMR (151 MHz, D_2O) δ 174.6 (CH_3CO), 166.8 (C8′), 158.3 (C9′), 142.1 (C6′), 139.0 (Ph), 129.6 (Ph), 129.2 (Ph), 128.8 (Ph), 97.2 (C7′), 90.4 (C2), 90.4 (d, *J* = 160.1, 9.1 Hz C1), 90.2 (C1′), 83.1 (d, *J* = 9.1 Hz C4′), 75.0 (C2′), 73.9 (PhCH_2), 69.4 (C3′), 64.5 (d, *J* = 6.0 Hz C5′), 42.7 (C3), 42.4 (C6), 32.0 (C4), 24.6 (d, *J* = 6.0 Hz C5), 22.5 (CH_3). ^{31}P NMR (162 MHz, D_2O) δ 15.60 (d, *J* = 25.9 Hz), −3.45 (d, *J* = 25.9 Hz). HRMS (ESI) calcd for $C_{24}H_{33}N_4O_{13}P_2$ $[M - H]^-$, 647.1519; found, 647.1511.

*Trisodium Cytidin-5′-yl-[(3-phenoxy)phenylphosphonato-methyl]-phosphate (**49**).* This compound was prepared in the same manner as described in the preparation of **2**. Quantities: **47g** (61.2 mg, 0.05 mmol), Pd/C (61.2 mg, 10%), 1,4-cyclohexadiene (485.3 mg, 573 μL, 5.88 mmol, 97%), DMF (1.0 mL), and eluent (H_2O → MeOH/H_2O = 1:1), affording crude product **49** (17.0 mg, 60% yield). The diastereoisomers were further separated by preparative RP-HPLC, converted to the form of sodium salt by ion-exchange (IR 120 Na^+), and lyophilized from water to give **49*s*** and **49*l*** as white powder. **49*s***: RP-HPLC, column B, *λ* = 260 nm, 0−12 min linear gradient 4−8% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 10.10 min. **49*l***: RP-HPLC, column B, *λ* = 260 nm, 0−12 min linear gradient 4−8% CH_3CN, 100 mM NH_4HCO_3 buffer (pH = 8.4), 15 mL/min flow; t_R = 10.80 min. ^1H NMR (400 MHz, D_2O) δ = 7.66 (d, *J* = 7.6 Hz, 1H), 7.34−7.29 (m, 3H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.15 (brs, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.98−6.96 (m, 2H), 6.84−6.82 (m, 1H), 6.03 (d, *J* = 7.6 Hz, 1H), 5.80 (d, *J* = 3.6 Hz, 1H), 5.02 (dd, *J* = 13.2, 10.4 Hz, 1H), 3.97−3.95 (m, 2H), 3.90−3.86 (m, 2H), 3.70 ppm (d, *J* = 12.0 Hz, 1H). The spectroscopic data coincide with the previous report.[36]

RP-HPLC Based Sialyltransferase Assay. The assay was performed according to the UV/RP-HPLC method developed by Schmidt group.[30] For details of the procedures, see Supporting Information.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01181.

Procedures for preparing compounds **9**, **10**, and **11**, HPLC purity of the target compounds, details of biological assay, and NMR spectral data (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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