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Total Synthesis of Anti-Influenza Agents Zanamivir and Zanaphosphor via Asymmetric Aza-Henry Reaction

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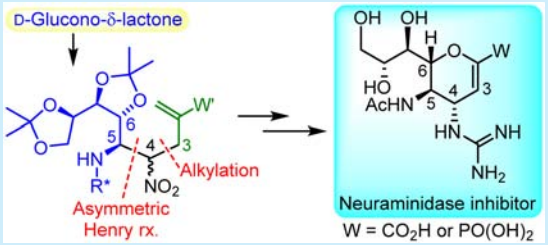
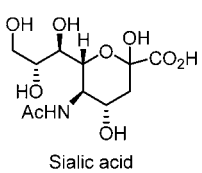
Supporting Information

ABSTRACT: The potent anti-influenza agents, zanamivir and its phosphonate congener, are synthesized by using a nitro group as the latent amino group at C4 for asymmetric aza-Henry reaction with a chiral sulfinylimine, which is derived from inexpensive D-glucono-δ-lactone to establish the essential nitrogen-containing substituent at C5. This method provides an efficient way to construct the densely substituted dihydropyran core of zanamivir and zanaphosphor without using the hazardous azide reagent.



Seasonal influenza epidemics have been a serious health problem to humans. Due to their high genetic variability, influenza viruses may also mutate to unprecedented strains to cause global and cross-species infections, thus claiming a vast number of lives and causing huge economic losses. In addition to using vaccines to prevent influenza infections, an effective medical treatment for infected patients is to administer neuraminidase (NA) inhibitors, such as zanamivir (**1**),[1,2] oseltamivir,[3,4] peramivir,[5,6] and laninamivir.[7,8] Neuraminidase is responsible for breaking the linkage between the influenza virus and the sialo receptor of host cells, so that the newly formed virus particles can be released to infect other cells. At present, the orally available oseltamivir is the most popular anti-influenza drug; however, emergence of oseltamivir-resistant viruses may limit its clinic use.[9] In contrast, the influenza viruses are rarely resistant to zanamivir because it carries a glycerol side chain, as that in the structure of sialic acid (also known as *N*-acetylneuraminic acid, Neu5Ac), for essential binding of influenza hemagglutinin with host cells.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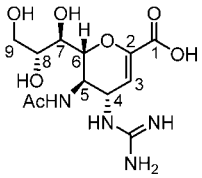
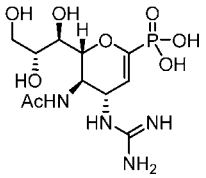

1, Zanamivir


2, Zanaphosphor

Although the nine-carbon monosaccharide structure of zanamivir is not particularly complex, its densely substituted dihydropyran core structure, with five consecutive stereogenic centers, still demonstrates a synthetic challenge. Zanamivir was first synthesized from sialic acid,[10] and this synthetic method has been modified for industrial manufacture.[11,12] Three synthetic methods without using the relatively expensive sialic acid as the starting material have also been explored.[13−15] In Yao's synthesis,[13] the C3−C4 linkage and C2 oxy group of zanamivir was simultaneously introduced by a 1,3-dipolar cycloaddition between methyl acrylate (C1−C3 fragment) and a chiral nitrone compound (C4−C9 fragment) that is derived from inexpensive D-glucono-δ-lactone. This synthetic route requires 17 steps to produce zanamivir in low yield (∼2%). In Shibasaki's synthesis,[14] the C5−C6 strategic bond of zanamivir is established by an asymmetric nitroaldol reaction (also known as Henry reaction) between 4-nitro-1-butene and (*E*)-4-methoxybenzyloxy-2-butenal, using the chiral heterobimetallic catalyst prepared from $Nd_3O(O\text{-}i\text{-}Pr)_{13}$, $NaN(SiMe_3)_2$, and a chiral amide-based ligand. Although the nitroaldol reaction is highly enantioselective, over 10 steps are required to convert the C2−C3 and C7−C8 double bonds to oxoacid and glycol moieties, respectively, to complete the synthesis of zanamivir. In Ma's synthesis,[15] an asymmetric Michael reaction of acetone to (*Z*)-*tert*-butyl (2-nitrovinyl)carbamate is carried out by the catalysis of chiral amine. The product containing the C1−C5 fragment is then subjected to asymmetric Henry reaction with an aldehyde (C6−C9 fragment), which is prepared from inexpensive D-araboascorbic acid. At the final stage, the C1 methyl group is oxidized to carboxylic acid to give zanamivir in 18% overall yield through 13 linear synthetic steps.

In our synthetic design of zanamivir (Figure 1), nitromethane was utilized as the pivotal C4 center to connect the C5−C9 fragment **E** and the C1−C3 fragment $\mathbf{D}_1$ (W′ = CO_2Et) via asymmetric aza-Henry reaction and alkylation reaction, respectively. The aza-Henry reaction would provide the two essential nitrogen-containing substituents at the C4 and C5 positions in the desired absolute configuration.[16] The nitro group is a latent amino group;[14−16] therefore, using a hazardous azide reagent is avoided in this synthetic route to zanamivir. The chiral imine **E** could be easily prepared from D-glucono-δ-lactone (**3**), a carbohydrate chiral pool. Furthermore, this

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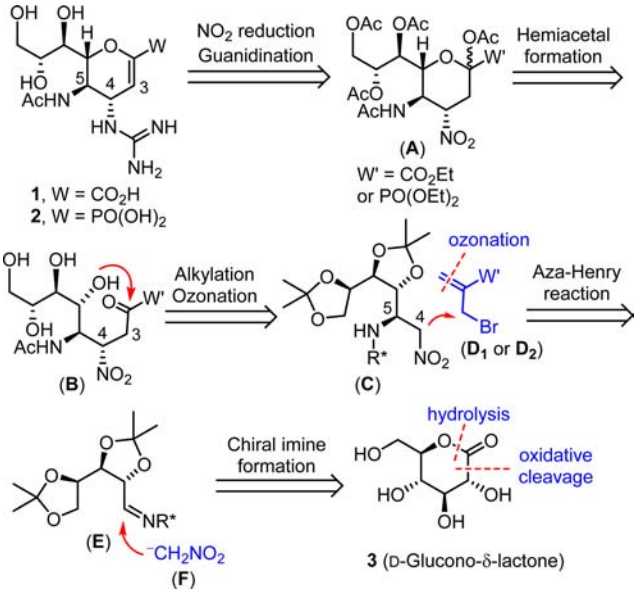


Figure 1. Retrosynthetic analysis of zanamivir (**1**) and zanaphosphor (**2**).

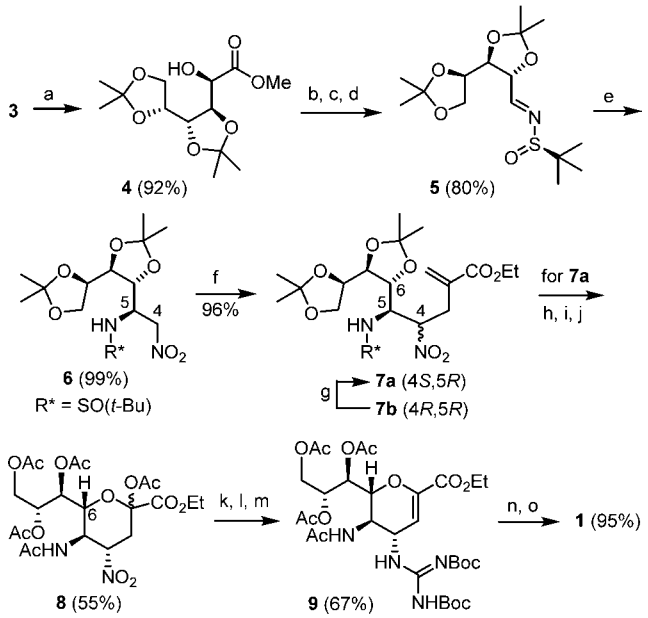
synthetic approach could be applied to the synthesis of zanaphosphor (**2**) by using the phosphonate reagent $\mathbf{D_2}$ [W′ = $PO(OEt)_2$] instead of the carboxylate reagent $\mathbf{D_1}$.

Phosphonate groups are generally employed as a bioisostere of carboxylate in drug design.[17,18] Zanaphosphor has been shown to exhibit higher anti-influenza activity than zanamivir[19] because the phosphonate ion has stronger electrostatic interactions with the three arginine residues (R118, R292, and R371) in the active site of influenza neuraminidase. So far, only one synthetic method of zanaphosphor has been accomplished by using sialic acid as the starting material.[19] Thus, our present method would represent the first synthesis of zanaphosphor without using sialic acid.

Scheme 1 shows our synthesis of zanamivir. According to the previously reported procedure,[20] D-glucono-δ-lactone (**3**) was first converted to a derivative **4** bearing acetal and ester groups. The ester group of **4** was reduced with $LiAlH_4$, and the diol product was subjected to oxidative cleavage with $NaIO_4$ to give an aldehyde intermediate, which reacted with (*R*)-*tert*-butylsulfinamide[21] in the presence of $Ti(OEt)_4$ to give the chiral sulfinylimine **5** in 80% overall yield after a simple chromatographic purification to remove the residual titanium and aluminum salts. The asymmetric aza-Henry reaction of **5** with nitromethane proceeded smoothly by the catalysis of tetrabutylammonium fluoride (TBAF) to afford 99% yield of the addition product **6** exclusively in the (5*R*)-configuration. The structure of **6** was confirmed by the X-ray diffraction analysis (Figure S1A, Supporting Information). The (*R*)-*tert*-butylsulfinamide appeared to be an excellent chiral auxiliary to render the remarkable stereoselectivity in the aza-Henry reaction.[16]

Another key step was to extend the carbon chain by alkylation of sulfinamide **6** with ethyl 2-(bromomethyl)acrylate ($\mathbf{D_1}$). The reaction was promoted by excess Et_3N (5.5 equiv) in THF solution (40 °C, 24 h) to give a high yield (96%) of the alkylation product that contained **7a** and **7b** in equal amounts.[22] By using the bulky base (−)-sparteine (2 equiv), instead of Et_3N, the alkylation reaction of **6** gave less **7a** epimer (**7a**/**7b** = 37:63). We also tested another possible approach to

Scheme 1. Synthesis of Zanamivir[a]



[a]Reagents and conditions: (a) $(MeO)_2CMe_2$, *p*-TsOH, Me_2CO, MeOH, rt, 9 h, 92%; (b) $LiAlH_4$, THF, rt, 4 h; (c) $NaIO_4$, $NaHCO_{3(aq)}$, CH_2Cl_2, rt, 5 h; (d) (*R*)-*tert*-butylsulfinamide, $Ti(OEt)_4$, THF, 65 °C, 9 h, 80% (three steps); (e) CH_3NO_2, TBAF (cat.), rt, 1 h, 99%; (f) $BrCH_2C(=CH_2)CO_2Et$, Et_3N, THF, 40 °C, 24 h, 96% (**7a**/**7b** = 1:1); (g) Et_3N, THF, 40 °C, 24 h; (h) 12 M HCl, MeOH, 50 °C, 30 min, then Ac_2O, NaOEt, pH ≈ 7, rt, 10 min; (i) O_3, MeOH, CH_2Cl_2, −78 °C, 15 min, then Me_2S, rt, 2 h; (j) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 24 h, 55% (three steps); (k) Zn, HOAc, EtOH, 80 °C, 30 min; (l) TMSOTf, CH_3CN, rt, 16 h; (m) MeS−C(=NBoc)NHBoc, $HgCl_2$, Et_3N, CH_2Cl_2, rt, 2 h, 67% (three steps); (n) $NaOH_{(aq)}$, MeOH, rt, 30 min; (o) TFA, CH_2Cl_2, rt, 1 h, 95% (two steps).

synthesize **7a**/**7b** by the aza-Henry reaction of **5** with ethyl 2-methylene-4-nitrobutanoate (**G**) (eq 1, SI). However, the reaction failed because reagent **G** decomposed under various conditions (e.g., 1 M $NaOH_{(aq)}$, K_2CO_3 in CH_3CN or THF, NaH in THF, and TBAF in THF).

Fortunately, the (4*S*)-isomer **7a** was readily extracted from the diastereomeric mixture by *n*-hexane, while the (4*R*)-isomer **7b** was retained as a solid residue. The X-ray diffraction analysis of **7b** confirmed its (4*R*,5*R*,6*R*,7*S*,8*R*)-configuration (Figure S1B, SI). In the presence of Et_3N, the strong electron-withdrawing nitro group rendered a base-catalyzed epimerization at the C4 stereocenter (Table 1). For example (entry 3), the recovered **7b** was treated with Et_3N (14 equiv) in THF at 40 °C for 48 h to give a mixture of **7a** and **7b** (57:43) as shown by ^1H NMR analysis. The H-4 of major isomer **7a** occurred at δ 5.12 (dt, J = 9.0, 4.5 Hz), whereas the corresponding proton of minor isomer **7b** exhibited at δ 5.06 (dt, J = 10.2, 3.2 Hz). The desired (4*S*)-epimer **7a** turned out to be the thermodynamically favored product after treatment with Et_3N for a prolonged period (entries 3−5). The epimer **7a** was simply separated by extraction with *n*-hexane, and the recycling of **7b** could be repeated to give additional product **7a**.

The acetal and sulfinylimine groups of **7a** were hydrolyzed under strongly acidic conditions (12 M $HCl_{(aq)}$ in MeOH), and the exposed amino group was trapped by acetylation. Without further purification, the product was subjected to ozonolysis to give an α-oxo ester, which was readily attacked by the C6 hydroxyl group to form a tetrahydropyran ring. All of the

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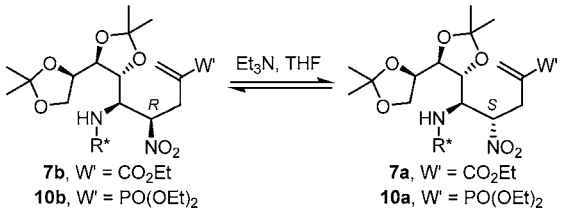
Table 1. Epimerization of 7b and 10b



entry	W′ =	Et_3N (equiv)	temp (°C)	time (h)	ratio of **a**/**b** epimers[a]
1[b]	CO_2Et	7	25	48	14:86
2[c]	CO_2Et	14	40	24	40:60
3[c]	CO_2Et	14	40	48	57:43
4[c]	CO_2Et	14	40	72	63:37
5[c]	CO_2Et	14	40	96	60:40
6[d]	$PO(OEt)_2$	16	40	24	41:59
7[d]	$PO(OEt)_2$	16	50	24	50:50
8[d]	$PO(OEt)_2$	16	50	48	50:50

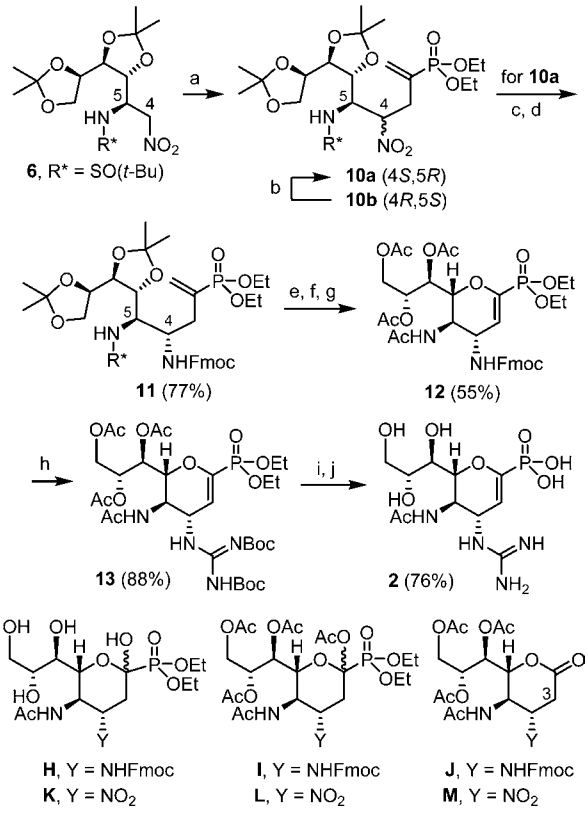
[a]The ratio of **7a**/**7b** was determined by ^1H NMR analysis, whereas the ratio of **10a**/**10b** was determined by ^{31}P NMR analysis. [b]Compound **7b** (50 mg, 0.1 mmol) and Et_3N (0.1 mL, 0.7 mmol) in THF (0.9 mL). [c]Compound **7b** (50 mg, 0.1 mmol) and Et_3N (0.2 mL, 1.4 mmol) in THF (0.8 mL). [d]Compound **10b** (50 mg, 0.09 mmol) and Et_3N (0.2 mL, 1.4 mmol) in THF (0.8 mL).

hydroxyl groups were then acetylated to provide the fully protected sialoside **8** with retention of the (4*S*)-configuration through the three-step reaction sequence. The nitro group in **8** was reduced with activated zinc powder in hot AcOH−EtOH, followed by elimination of an AcOH molecule on treatment with trimethylsilyl trifluoromethanesulfonate (TMSOTf), to form the dihydropyran core of zanamivir.[12] The intermediate amino compound was further reacted with 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea in the presence of $HgCl_2$ to afford the guanidination product **9** in 67% overall yield. Saponification of **9**, followed by removal of the *tert*-butoxycarbonyl (Boc) protecting groups with trifluoroacetic acid (TFA), thus culminated in the synthesis of zanamivir.[23] Starting from D-glucono-*δ*-lactone (**3**), we accomplished the total synthesis of zanamivir in 14 steps with 12% overall yield, which does not include the added yield from recycling **7b** to reobtain the (4*S*)-epimer **7a**.

In principle, the pivotal nitro compound **6** could be used to synthesize zanaphosphor by a similar approach (Scheme 2). Thus, the reaction of **6** with diethyl (3-bromoprop-1-en-2-yl)phosphonate (**D_2**) was carried out by the promotion of Et_3N to give the alkylation product that contained two isomers **10a** and **10b** in a ratio of 1:2. The stereoselectivity of the alkylation reaction was not improved by using either bulky base *i*-Pr_2NEt (Hünig base) or 1,4-diazabicyclo[2.2.2]octane (DABCO). The (4*S*)-epimer **10a** was isolated by extraction with *n*-hexane, and the residual solid (4*R*)-epimer **10b** could be recycled to afford **10a** by epimerization (Table 1, entries 6−8). For example, epimerization of **10b** was carried out by treatment with excess Et_3N in THF solution at 50 °C for 24 h to give equal amounts of **10a** and **10b**. The (4*R*,5*R*,6*R*,7*S*,8*R*)-configuration of **10b** was confirmed by X-ray crystallography (Figure S1C, SI).

The nitro group of **10a** was first reduced to an amino group and then protected as the 9-fluorenylmethoxycarbonyl (Fmoc) derivative **11**. By a procedure similar to that for conversion of **7a** to **8**, compound **11** was subjected to acid-catalyzed hydrolysis, followed by acetylation of the intermediate amine.

Scheme 2. Synthesis of Zanaphosphor[a]



[a]Reagents and conditions: (a) $BrCH_2C(=CH_2)PO(OEt)_2$, Et_3N, THF, 40 °C, 48 h, 72% (**10a**/**10b** = 1:2); (b) Et_3N, THF, 40 °C, 24 h; (c) Zn, AcOH, EtOH, 70 °C, 30 min; (d) 9-fluorenylmethyl chloroformate, $NaHCO_{3(aq)}$, CH_2Cl_2, rt, 9 h, 77% (2 steps); (e) 12 M HCl, MeOH, 50 °C, 30 min, then Ac_2O, NaOEt (pH ≈ 7), rt, 10 min; (f) O_3, MeOH, CH_2Cl_2, −78 °C, 30 min, then Me_2S, rt, 2 h; (g) Ac_2O, I_2 (cat.), 40 °C, 48 h, 55% (three steps); (h) Et_3N, $HgCl_2$, MeS−C(=NBoc)NHBoc, CH_2Cl_2, rt, 24 h, 86%; (i) TMSBr, CH_2Cl_2, 0 °C, 24 h; (j) MeONa, MeOH, rt, 1 h, 76% (two steps).

After ozonolysis of the double bond, the presumed *α*-oxophosphonate product proceeded to form a tetrahydropyran **H**. The subsequent peracetylation was best performed by using iodine as a mild Lewis acid catalyst, giving 55% yield of the desired dihydropyran product **12**, which was likely derived from an in situ elimination reaction of intermediate **I**. In comparison, treatment of **H** with Ac_2O and TMSOTf afforded less yield of **12** (37%), and an appreciable amount of lactone **J** was observed in the ^1H NMR spectrum, which showed the two diagnostic C3 protons at *δ* 3.08 (dd, *J* = 17.7, 6.6 Hz) and 2.55 (dd, *J* = 17.7, 9.9 Hz). Like Neu5Ac phosphonate esters,[19,24] tetrahydropyran **H**, that bears both hydroxyl and phosphonate groups at the C2 position, was unstable in both acids and bases (e.g., 1 M $HCl_{(aq)}$, cat. $HClO_4$, concentrated H_2SO_4, AcOH, TMSBr, $K_2CO_{3(aq)}$, and Et_3N), and it readily lost the phosphonate substituent as detected by the resonance of diethyl phosphite at 7.33 ppm in the ^{31}P NMR spectrum. Direct transformation of the nitro compound **10a** by a similar reaction sequence, including acidic hydrolysis, ozonolysis and peracetylation (steps e−g in Scheme 2), gave a complicated mixture that contained phosphonate **L** and lactone **M** derived from the intermediate **K**. Although elimination of an AcOH molecule from **L** could be achieved by using iodine or TMSOTf as the promoter, the dihydropyran product decomposed on reduction of the nitro

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group with Zn in AcOH. In contrast, the Fmoc protecting group in **12** was smoothly removed by Et_3N, and the in situ guanidination was carried out to give **13** in 86% yield. Zanaphosphor was thus synthesized from **13** in a one-pot operation by solvolysis of the phosphonate ester with TMSBr, followed by the removal of the Boc groups on workup with methanol (presumably effected by the in situ generated acid), and deacetylation with sodium methoxide.[19]

In conclusion, inexpensive D-glucono-*δ*-lactone was elaborated to a chiral sulfinylimine **5** for the asymmetric aza-Henry reaction with nitromethane by the catalysis of TBAF to give exclusively the addition product **6** in the (5*R*)-configuration. The subsequent alkylation with ethyl 2-(bromomethyl)acrylate ($\mathbf{D_1}$) or diethyl (3-bromoprop-1-en-2-yl)phosphonate ($\mathbf{D_2}$), followed by ozonolysis of the double bond, thus constructed the densely substituted dihydropyran core of both zanamivir and zanaphosphor with five consecutive stereogenic centers. The nitro group was used as a latent amino group, so that no hazardous azide reagent was required in this synthetic route. Although the alkylation reaction was not stereoselective, the desired (4*S*)-compounds **7a** and **10a** were easily separated from their (4*R*)-epimers by extraction with *n*-hexane. Furthermore, the (4*R*)-epimers could undergo epimerization in the presence of trimethylamine to provide more products of **7a** and **10a**. To solve the instability problem of the Neu5Ac phosphonate esters (e.g., **H** and **K**), iodine was utilized as a mild Lewis acid to promote the peracetylation and formation of the dihydropyran core. Thus, we accomplished the syntheses of the anti-influenza agent zanamivir and its phosphonate congener in reasonable yields by an efficient method. In particular, this method provides the first zanaphosphor synthesis without using sialic acid as the starting material.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02131.

Experimental procedures, NMR spectra, and X-ray crystallographic data (PDF)
X-ray data for compound **6** (CIF)
X-ray data for compound **7b** (CIF)
X-ray data for compound **10b** (CIF)

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Notes

The authors declare no competing financial interest.

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