

Practical and Highly Efficient Synthesis of Remdesivir from GS-441524

Tianwen Hu, Fuqiang Zhu, Li Xiang, Jingshan Shen, Yuanchao Xie,* and Haji A. Aisa*

Cite This: *ACS Omega* 2022, 7, 27516–27522

Read Online

ACCESS |



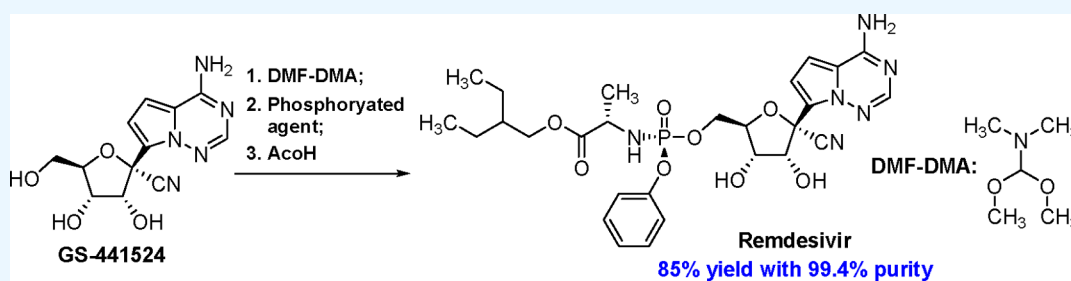
Metrics & More



Article Recommendations



Supporting Information



ABSTRACT: A three-step sequence for preparing remdesivir, an important anti-SARS-CoV-2 drug, is described. Employing *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) as a protecting agent, this synthesis started from (2*R*,3*R*,4*S*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydro-furan-2-carbonitrile (GS-441524) and consisted of three reactions, including protection, phosphoramidation, and deprotection. The advantages of this approach are as follows: (1) the protecting group could be removed under a mild deprotection condition, which avoided the generation of the degraded impurity; (2) high stereoselectivity was achieved in the phosphorylated reaction; (3) this synthesis could be performed successively without purification of intermediates. Moreover, the overall yield of this approach on a gram scale could be up to 85% with an excellent purity of 99.4% analyzed by high-performance liquid chromatography (HPLC).

INTRODUCTION

Remdesivir (**1**), an inhibitor of RNA-dependent RNA polymerase of the SARS-CoV-2 virus developed by Gilead Sciences,^{1–4} is the first drug to treat hospitalized patients with COVID-19.^{5–7} Recent study showed that the risk of hospitalization or death of COVID-19 outpatients with high risk could be reduced by 87% after receiving a three-day course of remdesivir.⁸ Moreover, the FDA authorized its emergency use in Covid-19 nonhospitalized patients based on the encouraging result of the clinical trial (NCT04501952).⁹ Therefore, enough supply of remdesivir is crucial to conquer the global health crisis caused by SARS-CoV-2.

Structurally, remdesivir is a monophosphoramidate prodrug of a C-nucleoside analogue GS-441524. Given the great clinical demand, the synthesis of this antiviral prodrug has attracted chemists' interest.^{10–15} The gram-scale synthesis of remdesivir from 2,3,5-tri-*O*-benzyl-*D*-ribose (6) was reported by Gilead's chemists (Scheme 1) and consisted of a six-step sequence.¹⁶ The low 48% overall yield of the last two reactions, including the phosphoramidation and the deprotection, might be the Achilles' heel for the sufficient supply of remdesivir. Researchers have been studying the problem to address it. Recently, Zhang's group reported the stereoselective synthesis of remdesivir by the coupling of phosphoramidoyl chloride (7) and acetonide-protected nucleoside 4 catalyzed by chiral imidazole derivatives with 73% overall yield (Scheme 2),

which avoided the preparation of enantiomerically pure phosphorylated agent.¹⁷ Soon after, Hung's group reported a similar approach to remdesivir with 70% yield by a one-pot method.¹⁸ Nonetheless, the instability of 7 posed a challenge to its purification and storage according to the study of phosphoramidate derivatives reported previously,¹⁹ which might limit its application for commercial production.

As far as our group was concerned, intensive efforts were devoted to investigate those two reactions. In our study, methylmagnesium chloride (MeMgCl) could be used instead of MgCl₂ and DIPEA in the phosphorylation reaction; moreover, the *p*-nitrophenylphosphoramidate 3 could be replaced with the pentafluorophenyl phosphoramidate 10 that had been used in the preparation of remdesivir in the literature.^{20,21} In the deprotection of acetonide, many reaction conditions, such as the concentration of hydrochloric acid, the solvents used, and the temperature, were screened, but no better result was obtained. Meanwhile, we found that the monophosphate impurity 8 was gradually produced before the compound 2 was consumed

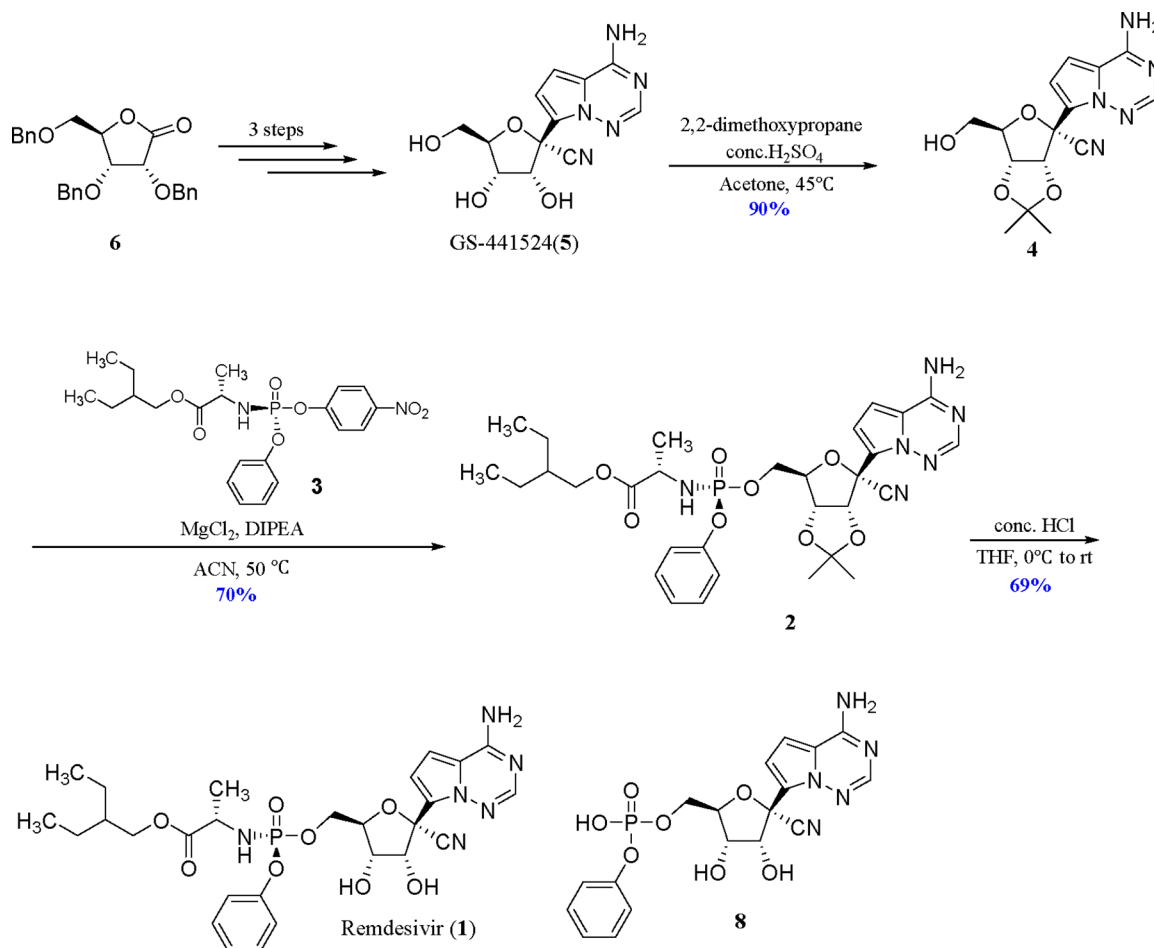
Received: May 7, 2022

Accepted: June 21, 2022

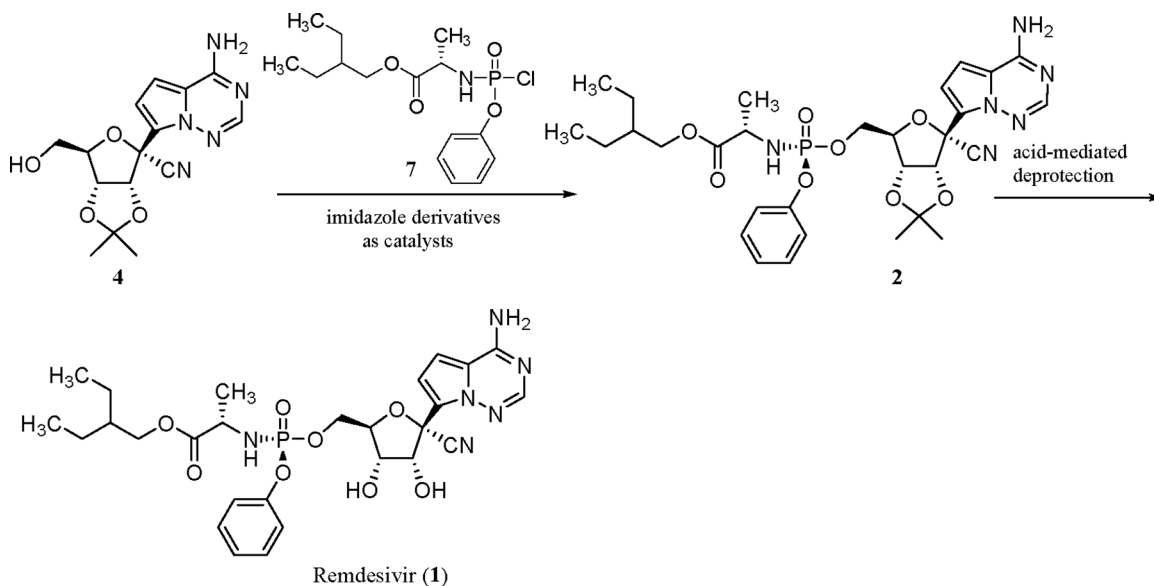
Published: July 27, 2022



Scheme 1. Gram-Scale Synthesis of Remdesivir Reported by Gilead's Chemists and Acid-Induced Degraded Impurity (8)



Scheme 2. Imidazole-Derivative-Catalyzed Asymmetric Synthesis of Remdesivir

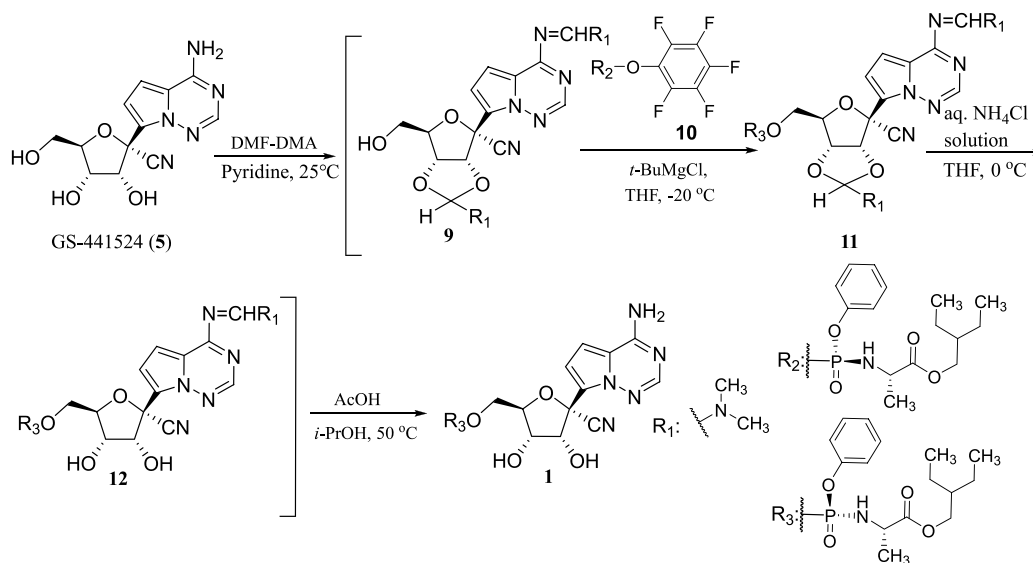


completely, which resulted from the hydrolysis of the phosphoramidate moiety of **1** in the presence of hydrochloric acid. Clearly, the side reaction caused by the harsh reaction condition resulted in the moderate 69% yield of this reaction. Therefore, a suitable protecting group at 2',3'-dihydroxyls is key

to achieve the high-efficient synthesis of remdesivir from GS-441524.

According to our previous study, *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) is a good protecting agent that could mask 2',3'-dihydroxyls and C4-amine of cytidine selectively to facilitate the esterification at 5'-hydroxyl group,

Scheme 3. Synthesis of Remdesivir from GS-441524 with DMF-DMA as the Protecting Agent



furthermore, the formed dimethylaminomethylene (DMAM) group at the 2',3'-dihydroxyls could be deprotected easily in the protic solvents, such as ethanol and isopropanol.²² Hence, employing DMF-DMA as a protecting agent, instead of 2,2-dimethoxypropane, might avoid the production of the by-product **8**. Herein, we developed a highly efficient approach for preparing remdesivir from GS-441524 (Scheme 3).

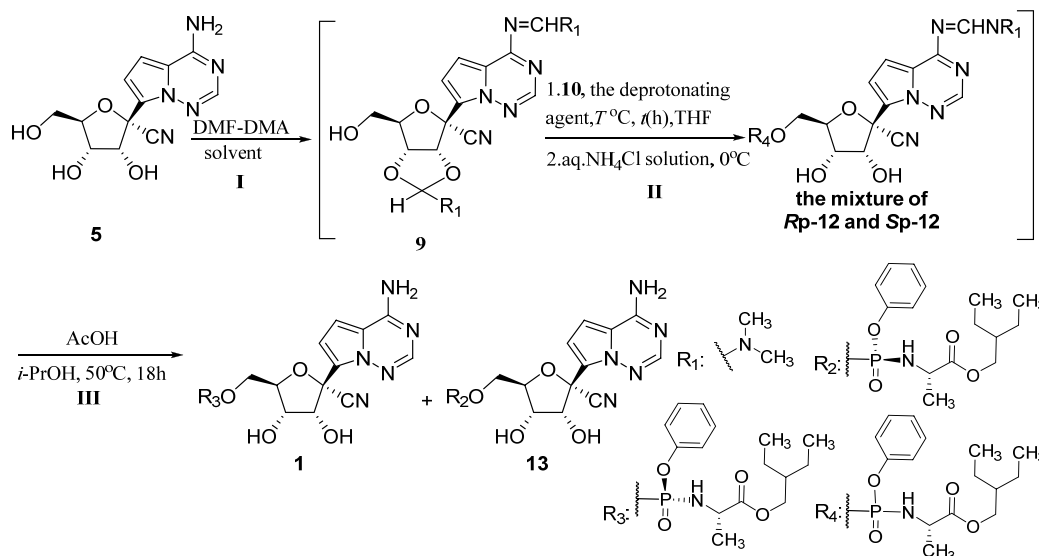
RESULTS AND DISCUSSION

The study started with the synthesis of the DMAM-protected nucleoside analogue **9**. This intermediate was synthesized from the compound **5** with a quantitative yield in the presence of DMF-DMA (4.0 equiv.) in pyridine at 25 °C for 18 h. In view of the instability of the DMAM group at the 2',3'-dihydroxyls, the progress of this reaction was indicated by the consumption of the starting material (monitored by thin layer chromatography (TLC)); to prepare the TLC sample, the reaction mixture was added to methanol and the resulting solution was used as the sample). Meanwhile, compound **9** was used in the next step directly after concentration under reduced pressure. Subsequently, using methylmagnesium chloride (MeMgCl) (1.5 equiv.) as the deprotonating agent, the **9** was reacted with **10** (1.1 equiv.) to furnish the unstable intermediate **11**. When the phosphorylation reaction was quenched with saturated NH₄Cl solution, the DMAM group at 2',3'-dihydroxyls of the compound **11** was concomitantly decomposed to provide the stable intermediate **12** with an 85% isolated yield, and the hydrolyzed impurity **8** was not observed by TLC analysis. With **12** in hand, the deprotection of the DMAM group at C6-amine was performed with AcOH (20 equiv.) in alcohol for 18 h at 50 °C and the product was afforded in 90% yield by chromatography purification. Overall, the yield of product **1** prepared from compound **5** was up to 76%, which was much higher than that of 43% reported by Gilead.¹⁶

Encouraged by the favorable outcome, we tried to optimize this new approach. Given the risk of the ester-transfer reaction in the amino acid ester moiety of compound **12** or **1** in ethanol, the deprotection reaction was conducted in isopropanol with lower reactivity, which proceeded smoothly as well and provided nearly the same result as using ethanol as the solvent. To simplify this synthesis, we quenched the phosphorylation reaction with a

solution of AcOH (20 equiv.) in THF or isopropanol and the resulting mixture was warmed to 50 °C to remove the DMAM protection; however, this reaction progressed much slower than that using the pure compound **12** as the starting material. We speculated that the existence of magnesium ion (Mg²⁺) might affect the speed of the deprotection reaction. According to this hypothesis, the reaction mixture was extracted with ethyl acetate (EA) to remove Mg²⁺ after the phosphorylation reaction was quenched with saturated aq. NH₄Cl solution. As expected, the reaction of crude **12** obtained from the workup of extraction and concentration with AcOH was continued for 18 h in isopropanol. Additionally, the protected nucleoside **9**, because of its instability, was directly subjected to the next reaction after simple workup. Therefore, this synthesis could be conducted successively, which not only avoided the purification of intermediates but also greatly improved the synthetic efficiency.

Having the simplified synthesis in hand, the optimizations of reaction conditions were investigated. Because of the stability of product **1** and its isomer **13** in the conditions of the deprotection reaction, the ratio of two *P*-chiral isomers could be used to indicate the stereoselectivity of the phosphorylation reaction. To reduce the solvent type in the synthesis, we tried to replace pyridine with THF in the first step. The condensation reaction of compound **5** and DMF-DMA was performed in THF at 60 °C for 3 h. When the starting material **5** was consumed completely, the solvent was removed under reduced pressure and the resultant mixture was processed by solvent exchange with toluene once to remove the unreacted DMF-DMA and the methanol generated from the condensation reaction. The coupling of the obtained crude **9** and **10** (1.2 equiv.) in the presence of MeMgCl (1.5 equiv.) was conducted at -10 °C for 2 h. After simple posttreatment, the obtained **12** was used with AcOH in the deprotection reaction at 50 °C for 18 h. Disappointedly, this attempt provided the final product **1** with much lower d.r. (Table 1, entry 2, 36/1 d.r.) than that using pyridine as the solvent of the condensation reaction (entry 1, 161/1 d.r.). The obvious decrease in the stereoselectivity indicated that the remained pyridine in the crude **9** might exert a special effect on the phosphorylation reaction of **10** and **9**. Subsequently, 1 equiv. of pyridine was added to the phosphorylation reaction but the ratio of **1** and **13** was only

Table 1. Condition Optimization of the Condensation Reaction (I) and the Phosphorylation Reaction (II)^a

entry	I		II			III	
	solvent	pyridine (equiv.)	deprotonating agent	<i>T</i> (°C)	<i>T</i> (h)	LCAP of 1 (%) ^d	d.r. ratio ^e (1 / 13)
1	pyridine ^b		MeMgCl	-10	2	96.7	161/1
2	THF ^c		MeMgCl	-10	2	93.0	36/1
3	THF ^c	1.0	MeMgCl	-10	2	92.7	42/1
4	pyridine ^b		MeMgCl	-20	2	92.9	232/1
5	pyridine ^b		<i>t</i> -BuMgCl	-20	2	94.1	783/1

^aGeneral procedure: the condensation reaction of compound **5** (500 mg, 1.72 mmol, 1 equiv.) and DMF-DMA (820 mg, 6.88 mmol, 4.0 equiv.) afforded compound **9**. The coupling of the obtained crude **9** and compound **10** (1.02 g, 2.06 mmol, 1.2 equiv.) was performed in the presence of the deprotonating agent (2.58 mmol, 1.5 equiv.). When crude **9** disappeared, the phosphorylation was quenched with aq. NH₄Cl solution and provided compound **12**. After simple aftertreatment, crude **12** was subjected to the solution of acetic acid (2.06 g, 34.4 mmol, 20.0 equiv.). Finally, product **1** was obtained. ^bThe condensation was conducted at 25 °C for 3 h. ^cThe condensation was conducted at 60 °C for 3 h. ^dLCAP: the area (%) of compound **1** determined by HPLC in the final reaction mixture. ^eThe d.r. ratio was determined by HPLC.

improved slightly (entry 3, LCAP of 92.7%, 42/1 d.r.). Nonetheless, the role of pyridine in the two reactions, including the condensation reaction and the phosphorylation reaction, needed to be studied further. Although pyridine was an infrequently used solvent, it was still chosen as the solvent of the condensation reaction in consideration of its benefit to the stereoselectivity in the synthesis.

Next, we studied the effects of the reaction temperature and the deprotonating agent on the phosphorylation reaction. When the reaction was conducted at -20 °C (entry 4), the LCAP of **1** slightly decreased to 92.9% and the value of d.r. was improved to 232/1, whereas at lower temperature (-40 °C), it took more than 8 h to finish the transformation of compound **12** to compound **1**. Notably, the lower reaction temperature resulted in the lower reactivity. In addition, using *t*-butylmagnesium chloride (*t*-BuMgCl) as the deprotonating agent was studied (entry 5). Surprisingly, the stereoselectivity was significantly improved to 783/1 with comparable LCAP of 94.1%, and the reaction was finished in only 2 h.

On the basis of the optimized conditions, we investigated the feasibility for the synthesis of remdesivir on the gram-scale. In a 5 g scale synthesis, compound **5** was reacted with DMF-DMA to provide crude **9**, and then **9** was phosphorylated by compound **10** in the presence of *t*-BuMgCl followed by quenching with saturated NH₄Cl solution. After extraction and concentration, the obtained **12** was deprotected in the solution of AcOH in the

isopropanol to furnish product **1** with 85% yield in 99.9:0.1 d.r. by chromatography purification.

CONCLUSIONS

In summary, facilitated by DMF-DMA as the protecting agent, a highly efficient synthesis of remdesivir from GS-441524 was developed. Compared to the 43% overall yield reported in the literature,¹⁶ this method provided remdesivir with 85% overall yield through a three-step sequence. Moreover, this synthesis could avoid the generation of the degraded impurity and be conducted successively without purification of intermediates. These advantages improved the synthetic efficiency of remdesivir greatly.

EXPERIMENTAL SECTION

General Information. (2*R*,3*R*,4*S*,5*R*)-2-(4-Aminopyrrolo-[2,1-*f*][1,2,4]triazin-7-yl)-3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-carbonitrile **5** and 2-ethylbutyl ((*S*)-(perfluorophenoxy)(phenoxy)phosphoryl)-*L*-alaninate **10** were obtained from Topharman Shanghai Co., Ltd. Other chemical reagents and solvents, such as DMF-DMA, 3.0 M methylmagnesium chloride solution in tetrahydrofuran (THF), 1.7 M *t*-butylmagnesium chloride solution in tetrahydrofuran, pyridine, THF, isopropanol, acetic acid, and ethyl acetate, were commercially available. ¹H NMR, ¹³C{¹H} NMR, and ³¹P NMR spectra were recorded on a Bruker 500 Hz instrument. Low-resolution mass spectra were measured on a Finnigan LTQ mass

spectrometer (Thermo Fisher), and the corresponding high-resolution were measured on a Q-TOF mass spectrometer (Agilent G6520). HPLC spectra were obtained with an Agilent 1100 HPLC system.

(2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl Phenyl Hydrogen Phosphate (**8**). To a solution of **2** (0.50 g, 0.78 mmol), prepared from **5** according to the reported method, in tetrahydrofuran (10.0 mL) was slowly added 37% aq. hydrochloric acid (0.80 mL) at 0 °C. The reaction mixture was then warmed to 25 °C and stirred for 5 h. When TLC showed compound **2** had disappeared, the pH of the reaction mixture was adjusted to 8 by the addition of saturated aq. NaHCO₃ solution. The resulting mixture was processed by reverse-phase HPLC using a YMC*GEL ODS column with gradient elution using acetonitrile and water. Finally, **8** was obtained with 99.6% purity as a white solid (54 mg, 15% yield). ¹H NMR (500 MHz, DMSO) δ 8.12–7.71 (m, 3H), 7.18–7.14 (m, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 6.94–6.89 (m, 2H), 6.80 (d, *J* = 4.5 Hz, 1H), 6.29 (s, 1H), 5.60 (s, 1H), 4.62 (d, *J* = 5.0 Hz, 1H), 4.15 (dd, *J* = 9.5, 5.4 Hz, 1H), 3.98–3.92 (m, 2H), 3.85 (dt, *J* = 11.6, 5.8 Hz, 1H). ¹³C{¹H} NMR (126 MHz, DMSO) δ 155.6, 153.9, 153.9, 147.8, 128.7, 124.0, 121.7, 120.0, 120.0, 117.2, 116.4, 110.4, 100.9, 83.3, 83.3, 78.3, 74.4, 70.3, 64.6, 64.6. ³¹P NMR (202 MHz, DMSO) δ –5.64. HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₁₈H₁₉N₅O₇P⁺ 448.1017, found 448.1019.

2-Ethylbutyl((*R*)-((2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**13**). Compound **4** (1.2 g, 3.6 mmol) and racemic **10** (Rp/Sp, 1/1) (2.0 g, 4.0 mmol) were dissolved in THF (20 mL). The resultant solution was cooled to –10 °C and 3.0 M methylmagnesium chloride solution in tetrahydrofuran (1.8 mL, 5.4 mmol) was added dropwise under a N₂ atmosphere. After that, the reaction mixture was stirred for 1 h at –5 °C. When TLC indicated **10** was consumed completely, the reaction was quenched with acetic acid and then the solvent was removed under reduced pressure. The obtained mixture was redissolved in ethyl acetate. The resultant solution was washed with water, saturated aq. NaHCO₃ solution and brine orderly, dried over Na₂SO₄, and concentrated. The crude **2**, obtained from the above phosphorylated reaction, was added to the mixture of 37% aq. hydrochloric acid solution (3.6 mL) and tetrahydrofuran (25 mL) at 0 °C. After the reaction temperature rose to 25 °C, stirring was continued for 5 h. When TLC showed completion of this reaction, saturated aq. NaHCO₃ solution was added until the pH of the reaction mixture was 8. The reaction mixture was concentrated to remove tetrahydrofuran and the resultant solution was extracted with ethyl acetate twice. The combined organic phase was washed with brine, dried over Na₂SO₄, and evaporated. The obtained concentrate was processed by a Daicel AD-H chiral column with isocratic elution by solution containing 70% hexane and 30% ethanol. Compound **13** was afforded with 92.9% purity as a white solid (585 mg, 27% yield). ¹H NMR (500 MHz, DMSO) δ 7.97–7.80 (m, 3H), 7.32 (t, *J* = 7.9 Hz, 2H), 7.17–7.12 (m, 3H), 6.91 (d, *J* = 4.5 Hz, 1H), 6.84 (d, *J* = 4.5 Hz, 1H), 6.30 (d, *J* = 6.1 Hz, 1H), 6.02 (dd, *J* = 12.9, 10.1 Hz, 1H), 5.38 (d, *J* = 5.5 Hz, 1H), 4.65 (t, *J* = 5.5 Hz, 1H), 4.31–4.24 (m, 2H), 4.17–4.09 (m, 1H), 4.01–3.87 (m, 3H), 3.82–3.72 (m, 1H), 1.46–1.39 (m, 1H), 1.28–1.22 (m, 4H), 1.16 (d, *J* = 7.1 Hz, 3H), 0.80 (t, *J* = 7.5 Hz, 6H). ¹³C{¹H} NMR (126 MHz, DMSO) δ 173.3, 173.3, 155.6, 150.7, 150.6, 147.9, 129.5, 124.5, 123.5, 120.2, 120.1, 116.9, 116.6, 110.4, 100.8,

82.2, 82.2, 78.8, 74.1, 69.9, 66.1, 65.2, 65.1, 49.9, 22.6, 22.5, 19.6, 19.6, 10.8, 10.8. ³¹P NMR (202 MHz, DMSO) δ 3.66. HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₂₇H₃₆N₆O₈P⁺ 603.2327, found 603.2330.

2-Ethylbutyl ((*S*)-((2*R*,3*S*,4*R*,5*R*)-5-cyano-5-(4-((dimethylamino)methylene)amino)pyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)-(phen-oxy)phosphoryl)-L-alaninate (**12**). DMF-DMA (1.64 g, 13.7 mmol) was added in one portion to the suspension of **5** (1.00 g, 3.42 mmol) in pyridine (5.0 mL) at 25 °C. The reaction mixture was stirred for 6 h at that temperature. When TLC indicated the full conversion of **5** (a sample was taken from the reaction mixture and quenched with methanol), the mixture was concentrated under reduced pressure. The obtained crude **9** and the phosphorylated agent **10** (1.86 g, 3.76 mmol) were dissolved in THF (25.0 mL). When the resultant solution was cooled to –10 °C, 3.0 M methylmagnesium chloride solution in tetrahydrofuran (1.7 mL, 5.13 mmol) was added dropwise under a N₂ atmosphere and the reaction temperature was not higher than –5 °C during the process of dropping. When TLC indicated the phosphorylated reaction was completed, the reaction mixture was poured into saturated aq. NH₄Cl solution and then concentrated to remove solvent. The obtained mixture was extracted with ethyl acetate twice. The organic phase was washed with water and brine orderly, dried over Na₂SO₄, and evaporated. The crude product was purified by chromatography purification with a gradient elution by solution containing CH₂Cl₂ and methanol (the ratio of CH₂Cl₂ and methanol was ranged from 80/1 to 30/1) to afford **12** with 97.5% purity as a foamy solid (1.91 g, 85% yield). ¹H NMR (500 MHz, DMSO) δ 8.94 (s, 1H), 8.15 (s, 1H), 7.37–7.32 (m, 2H), 7.21–7.14 (m, 3H), 6.94 (d, *J* = 4.5 Hz, 1H), 6.80 (d, *J* = 4.5 Hz, 1H), 6.33 (d, *J* = 6.2 Hz, 1H), 6.02 (dd, *J* = 13.0, 10.1 Hz, 1H), 5.40 (d, *J* = 5.6 Hz, 1H), 4.72–4.66 (m, 1H), 4.29–4.21 (m, 2H), 4.10 (dt, *J* = 12.3, 6.2 Hz, 1H), 3.99 (dd, *J* = 10.9, 5.6 Hz, 1H), 3.95 (dd, *J* = 10.9, 5.9 Hz, 1H), 3.86 (dd, *J* = 10.9, 5.8 Hz, 1H), 3.84–3.77 (m, 1H), 3.24 (s, 3H), 3.18 (s, 3H), 1.45–1.37 (m, 1H), 1.28–1.18 (m, 7H), 0.79 (t, *J* = 7.5 Hz, 6H). ¹³C{¹H} NMR (126 MHz, DMSO) δ 173.2, 173.2, 160.2, 158.1, 150.8, 150.7, 147.4, 129.6, 124.5, 123.4, 122.6, 120.1, 120.1, 116.8, 111.6, 101.8, 82.4, 82.4, 78.8, 74.0, 70.0, 66.1, 65.5, 65.4, 49.7, 41.1, 34.9, 22.6, 22.5, 19.8, 19.7, 10.8, 10.7. ³¹P NMR (202 MHz, DMSO) δ 3.68. HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₃₀H₄₁N₇O₈P⁺ 658.2749, found 658.2739.

2-Ethylbutyl ((*S*)-((2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**1**). AcOH (1.8 g, 30.0 mmol) was added to the solution of **12** (1.0 g, 1.5 mmol) in ethanol (15.0 mL) in one portion at 25 °C. After that, the reaction mixture was heated to 50 °C and stirred for 18 h under a N₂ atmosphere. When TLC showed that **12** was consumed completely, the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate then washed with saturated aq. NaHCO₃ solution, water and brine. The obtained organic phase was dried over Na₂SO₄ and evaporated. The crude product was subjected to the silica gel column with CH₂Cl₂ and methanol as the elution (CH₂Cl₂: methanol = 50:1 to 20:1) to provide **1** with 99.8% purity as a foamy solid (813 mg, 90% yield). ¹H NMR (500 MHz, DMSO) δ 8.00–7.81 (m, 3H), 7.38–7.31 (m, 2H), 7.22–7.15 (m, 3H), 6.90 (d, *J* = 4.5 Hz, 1H), 6.83 (d, *J* = 4.5 Hz, 1H), 6.33 (d, *J* = 6.1 Hz, 1H), 6.03 (dd, *J* = 13.0, 10.1 Hz, 1H), 5.37 (d, *J* = 5.8 Hz, 1H), 4.68–4.62 (m, 1H), 4.29–4.22 (m, 2H), 4.13–4.07 (m,

1H), 3.95 (dd, $J = 10.9, 5.8$ Hz, 2H), 3.90–3.78 (m, 2H), 1.45–1.37 (m, 1H), 1.28–1.19 (m, 7H), 0.79 (t, $J = 7.5$ Hz, 6H). $^{13}\text{C}\{^1\text{H}\}$ (126 MHz, DMSO) δ 173.2, 173.2, 155.6, 150.8, 150.7, 147.9, 129.6, 124.5, 123.5, 120.1, 120.0, 116.9, 116.6, 110.4, 100.8, 82.1, 82.0, 79.0, 74.0, 69.9, 66.1, 65.4, 65.4, 49.7, 22.6, 22.5, 19.8, 19.7, 10.8, 10.7. ^{31}P NMR (202 MHz, DMSO) δ 3.69. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{36}\text{N}_6\text{O}_8\text{P}^+$ 603.2327, found 603.2328.

Preparation of 1 from 5 without Purification of Intermediates on a 5 g Scale. To a suspension of **5** (5.0 g, 17.2 mmol) in pyridine (20.0 mL) was added DMF-DMA (8.2 g, 68.8 mmol). The reaction mixture was stirred for 6 h at 25 °C. When the condensation reaction of **5** and DMF-DMA was finished, pyridine was removed under reduced pressure. The obtained **9** and **10** (10.2 g, 20.6 mmol) were dissolved in THF (50.0 mL). When the resultant solution was cooled to –20 °C, 1.7 M *t*-butylmagnesium chloride solution in tetrahydrofuran (15.2 mL, 25.8 mmol) was added dropwise under a N_2 atmosphere and the temperature was kept under –15 °C. When the coupling reaction of **9** and **10** was completed, the reaction mixture was poured into saturated aq. NH_4Cl solution. After that, the resultant solution was concentrated and then redissolved in ethyl acetate. The obtained solution was washed with water and brine orderly, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was added to the mixture of AcOH (20.6 g, 0.34 mol) and isopropanol (100.0 mL). The temperature then rose to 50 °C and stirring was continued for 18 h. When the deprotection reaction was completed, the reaction mixture was evaporated. The obtained mixture was dissolved in ethyl acetate and neutralized with saturated aq. NaHCO_3 solution. When the pH of the mixture was 8, the organic phase was separated, washed with water and brine orderly, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column using the mixture of CH_2Cl_2 and methanol (CH_2Cl_2 : methanol = 50:1–20:1) to give **1** with 99.4% purity as a foamy solid (8.8 g, 85% yield).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c02835>.

^1H , $^{13}\text{C}\{^1\text{H}\}$, and ^{31}P NMR spectra of compounds and HRMS spectrum (PDF)

AUTHOR INFORMATION

Corresponding Authors

Yuanchao Xie – Lingang Laboratory, Shanghai 200031, P. R. China; orcid.org/0000-0001-9875-887X; Email: xieyuanchao@lglab.ac.cn

Haji A. Aisa – State Key Laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, P. R. China; University of Chinese Academy of Sciences, Beijing 100049, P. R. China; orcid.org/0000-0003-4652-6879; Email: haji@ms.xjb.ac.cn

Authors

Tianwen Hu – State Key Laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy

of Sciences, Urumqi 830011, P. R. China; University of Chinese Academy of Sciences, Beijing 100049, P. R. China
Fuqiang Zhu – Toppharman Shanghai Co., Ltd., Shanghai 201203, P.R. China; orcid.org/0000-0001-9075-6474
Li Xiang – Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China; Nanjing University of Chinese Medicine, Nanjing 210023, P. R. China
Jingshan Shen – Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China; University of Chinese Academy of Sciences, Beijing 100049, P. R. China

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.2c02835>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financed by the grant from Shanghai Science and Technology Committee in China (21S11903100).

REFERENCES

- (1) Yin, W.; Mao, C.; Luan, X.; Shen, D. D.; Shen, Q.; Su, H.; Wang, X.; Zhou, F.; Zhao, W.; Gao, M.; Chang, S.; Xie, Y. C.; Tian, G.; Jiang, H. W.; Tao, S. C.; Shen, J.; Jiang, Y.; Jiang, H.; Xu, Y.; Zhang, S.; Zhang, Y.; Xu, H. E. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* **2020**, *368*, 1499–1504.
- (2) Gordon, C. J.; Tchesnokov, E. P.; Woolner, E.; Perry, J. K.; Feng, J. Y.; Porter, D. P.; Gotte, M. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* **2020**, *295*, 6785–6797.
- (3) Shannon, A.; Le, N. T.; Selisko, B.; Eydoux, C.; Alvarez, K.; Guillemot, J. C.; Decroly, E.; Peersen, O.; Ferron, F.; Canard, B. Remdesivir and SARS-CoV-2: Structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. *Antiviral Res.* **2020**, *178*, 104793.
- (4) Wakchaure, P. D.; Ghosh, S.; Ganguly, B. Revealing the Inhibition Mechanism of RNA-Dependent RNA Polymerase (RdRp) of SARS-CoV-2 by Remdesivir and Nucleotide Analogues: A Molecular Dynamics Simulation Study. *J. Phys. Chem. B* **2020**, *124*, 10641–10652.
- (5) Wang, Y.; Zhang, D.; Du, G.; Du, R.; Zhao, J.; Jin, Y.; Fu, S.; Gao, L.; Cheng, Z.; Lu, Q. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* **2020**, *395*, 1569–1578.
- (6) Goldman, J. D.; Lye, D. C. B.; Hui, D. S.; Marks, K. M.; Bruno, R.; Montejano, R.; Spinner, C. D.; Galli, M.; Ahn, M. Y.; Nahass, R. G.; et al. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. *N. Engl. J. Med.* **2020**, *383*, 1827–1837.
- (7) FDA's approval of Veklury (remdesivir) for the treatment of COVID-19—the science of safety and effectiveness. U.S. Food and Drug Administration. <https://www.fda.gov/drugs/news-events-human-drugs/fdas-approval-veklury-remdesivir-treatment-covid-19-science-safety-and-effectiveness>.
- (8) Gottlieb, R. L.; Vaca, C. E.; Paredes, R.; Mera, J.; Webb, B. J.; Perez, G.; Oguchi, G.; Ryan, P.; Nielsen, B. U.; Brown, M.; et al. Early Remdesivir to Prevent Progression to Severe Covid-19 in Outpatients. *N. Engl. J. Med.* **2022**, *386*, 305–315.
- (9) EUA 046 Gilead Remdesivir LOA Outpatients. U.S. Food and Drug Administration. <https://www.fda.gov/media/137564/download>.
- (10) Paymode, D. J.; Cardoso, F. S. P.; Agrawal, T.; Tomlin, J. W.; Cook, D. W.; Burns, J. M.; Stringham, R. W.; Sieber, J. D.; Gupton, B. F.; Snead, D. R. Expanding Access to Remdesivir via an Improved Pyrrolotriazine Synthesis: Supply Centered Synthesis. *Org. Lett.* **2020**, *22*, 7656–7661.

- (11) Xie, Y.; Hu, T.; Zhang, Y.; Wei, D.; Zheng, W.; Zhu, F.; Tian, G.; Aisa, H. A.; Shen, J. Weinreb Amide Approach to the Practical Synthesis of a Key Remdesivir Intermediate. *J. Org. Chem.* **2021**, *86*, 5065–5072.
- (12) Von Keutz, T.; Williams, J. D.; Kappe, C. O. Flash Chemistry Approach to Organometallic C-Glycosylation for the Synthesis of Remdesivir. *Org. Process Res. Dev.* **2021**, *25*, 1015–1021.
- (13) Vieira, T.; Stevens, A. C.; Chtchemelinine, A.; Gao, D.; Badalov, P.; Heumann, L. Development of a Large-Scale Cyanation Process Using Continuous Flow Chemistry En Route to the Synthesis of Remdesivir. *Org. Process Res. Dev.* **2020**, *24*, 2113–2121.
- (14) Xue, F.; Zhou, X.; Zhou, R.; Zhou, X.; Xiao, D.; Gu, E.; Guo, X.; Xiang, J.; Wang, K.; Yang, L.; et al. Improvement of the C-glycosylation Step for the Synthesis of Remdesivir. *Org. Process Res. Dev.* **2020**, *24*, 1772–1777.
- (15) Kumar Palli, K.; Ghosh, P.; Krishna Avula, S.; Sridhara Shanmukha Rao, B.; Patil, A. D.; Ghosh, S.; Sudhakar, G.; Raji Reddy, C.; Mainkar, P. S.; Chandrasekhar, S. Total synthesis of remdesivir. *Tetrahedron Lett.* **2022**, *88*, 153590.
- (16) Warren, T. K.; Jordan, R.; Lo, M. K.; Ray, A. S.; Mackman, R. L.; Soloveva, V.; Siegel, D.; Perron, M.; Bannister, R.; Hui, H. C.; et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* **2016**, *531*, 381–385.
- (17) Wang, M.; Zhang, L.; Huo, X.; Zhang, Z.; Yuan, Q.; Li, P.; Chen, J.; Zou, Y.; Wu, Z.; Zhang, W. Catalytic Asymmetric Synthesis of the anti-COVID-19 Drug Remdesivir. *Angew. Chem., Int. Ed. Engl.* **2020**, *59*, 20814–20819.
- (18) Gannedi, V.; Villuri, B. K.; Reddy, S. N.; Ku, C. C.; Wong, C. H.; Hung, S. C. Practical Remdesivir Synthesis through One-Pot Organocatalyzed Asymmetric (S)-P-Phosphoramidation. *J. Org. Chem.* **2021**, *86*, 4977–4985.
- (19) Ross, B. S.; Ganapati Reddy, P.; Zhang, H.-R.; Rachakonda, S.; Sofia, M. J. Synthesis of diastereomerically pure nucleotide phosphoramidates. *J. Org. Chem.* **2011**, *76*, 8311–8319.
- (20) Chun, B. K.; Clarke, M. O.; Doerffler, E.; Hui, H. C.; Jordan, R.; Mackman, R. L.; Ray, A. S.; Siegel, D. Methods for Treating Filoviridae Virus Infections. WO2016069826, 2016.
- (21) Hu, T.; Xie, Y.; Zhu, F.; Gong, X.; Liu, Y.; Xue, H.; Aisa, H. A.; Shen, J. One-Pot Synthesis of Molnupiravir from Cytidine. *Org. Process Res. Dev.* **2022**, *26*, 358–364.
- (22) Hu, T.; Xie, Y.; Liu, Y.; Xue, H.; Zhu, F. A.; Aisa, H.; Shen, J. A Convenient and Cost Efficient Route Suitable for “One-Pot” Synthesis of Molnupiravir. *ChemRxiv*, 2021. DOI: [10.26434/chemrxiv.14208206.v1](https://doi.org/10.26434/chemrxiv.14208206.v1)