

Chemical Synthesis of the Anti-COVID-19 Drug Remdesivir

Mo Wang,^{1,2} Lu Zhang,² Xiaohong Huo,¹ Zhenfeng Zhang,²
and Wanbin Zhang^{1,2,3}

¹Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, Frontiers Science Center for Transformative Molecules, School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai, China

²School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China

³Corresponding author: wanbin@sjtu.edu.cn

Remdesivir has become an important compound for the treatment of COVID-19. Here, we describe the catalytic asymmetric synthesis of this anti-COVID-19 drug. First, the *P*-racemic phosphoryl chloride is synthesized in a facile procedure. Then, it is possible to obtain the protected remdesivir via the organocatalytic asymmetric phosphorylation of protected nucleoside GS441524 with *P*-racemic phosphoryl chloride catalyzed by chiral bicyclic imidazole. Finally, remdesivir is easily prepared by deprotection. © 2021 Wiley Periodicals LLC.

Basic Protocol 1: Synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac*-4

Basic Protocol 2: Synthesis of chiral bicyclic imidazole **Ad-DPI**

Basic Protocol 3: Synthesis of remdesivir

Keywords: anti-COVID-19 drug • asymmetric phosphorylation • chiral bicyclic imidazole • organocatalysis

How to cite this article:

Wang, M., Zhang, L., Huo, X., Zhang, Z., & Zhang, W. (2021). Chemical synthesis of the anti-COVID-19 drug remdesivir. *Current Protocols*, 1, e303. doi: 10.1002/cpz1.303

INTRODUCTION

Recently, remdesivir, developed by Gilead Sciences using ProTide technology, has become one of the most effective anti-COVID-19 drugs. Thus far, two synthetic methods for remdesivir have been established (Siegel et al., 2017; Warren et al., 2016), but both of these methods require chiral resolution and additional synthetic steps, leading to resource waste and low synthetic efficiency. In 2012, our group developed the first catalytic asymmetric synthesis of *P*-stereogenic phosphoric acid derivatives using chiral bicyclic imidazole organocatalysts (Liu et al., 2012). Inspired by this methodology for the construction of *P*-stereocenters, we developed the first catalytic asymmetric synthesis of remdesivir via chiral bicyclic imidazole-catalyzed phosphorylation (Wang et al., 2020).

The core strategy in this protocol is to generate the *S_P*-isomer of protected remdesivir via catalytic asymmetric phosphorylation. Basic Protocol 1 describes the synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac*-4. Basic Protocol 2 describes the synthesis of the chiral bicyclic imidazole **Ad-DPI**. Basic Protocol 3 describes the synthesis of remdesivir.

Figures and some textual material are taken from our recently published work (Wang et al., 2020) and rewritten as step-by-step protocols to support reproduction of the original work.

SYNTHESIS OF 2-ETHYLBUTYL (CHLORO(PHENOXY)PHOSPHORYL)-L-ALANINATE *rac*-4

This protocol describes the synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac*-4 (Mackman, Parrish, Ray, & Theodore, 2012; Meppen et al., 2009), the phosphorylation reagent, which is derived from 2-ethylbutan-1-ol (Fig. 1). The synthesis of *rac*-4 involves two steps: (1) esterification of 2-ethylbutan-1-ol **1** with L-alanine to prepare 2-ethylbutyl L-alaninate hydrochloride salt **2** and (2) phosphorylation of 2-ethylbutyl L-alaninate hydrochloride salt **2** by phenyl dichlorophosphate **3** to obtain 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac*-4.

NOTE: Anhydrous reaction conditions are required for the above two steps of this procedure. Glassware needs to be oven-dried, and anhydrous solvents should be prepared or purchased in Sure/Seal bottles.

Materials

- 2-Ethylbutan-1-ol (99.0%, Energy Chemical, A040495)
- Thionyl chloride (SOCl₂, 99.0%, Energy Chemical, W610261)
- L-alanine (99.0%, Energy Chemical, A070054)
- Petroleum ether (AR, General-Reagent, G84208C)
- Anhydrous dichloromethane (CH₂Cl₂, >99.5%, General-Reagent, G81014C)
- Dry nitrogen
- Phenyl dichlorophosphate (98%, Energy Chemical, A020205)
- Triethylamine (Et₃N, 99.5%, Energy Chemical, B010065)
- Anhydrous ethyl ether (Et₂O, >99.5%, Sinopharm Chemical Reagent, 10009318)

- 150-, and 200-ml two-necked round-bottomed flasks
- Magnetic stirring bar
- Magnetic stirrer
- Magnetic stirrer with heating
- 150-, and 200-ml round-bottomed flasks
- Rotary vacuum evaporator
- High-vacuum oil pump
- Syringe needle
- Ice bath
- Oil bath

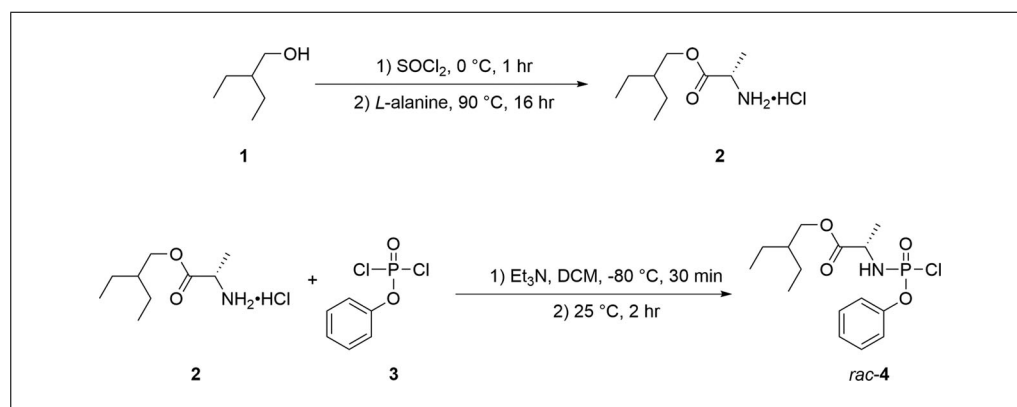


Figure 1 Synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac*-4.

Cryogenic cooling circulation pump
Vacuum filtration system
¹H, ¹³C, and ³¹P NMR equipment or facilities

Synthesis of 2-ethylbutyl L-alaninate hydrochloride salt 2

1. Add 75 ml (609.3 mmol) 2-ethylbutan-1-ol **1** to a dry 200-ml two-necked round-bottomed flask.
2. Add 10.9 ml (150.0 mmol) SOCl₂ dropwise over 10 min to the flask containing 2-ethylbutan-1-ol **1** at 0°C and stir the mixture for 1 hr at 0°C.
3. Weigh out 8.9 g (100.0 mmol) of L-alanine into the flask containing the mixture at 0°C.
4. Raise the temperature of the reaction mixture to 90°C, stir the mixture for 16 hr at 90°C, and then allow it to cool to 25°C.
5. Transfer the reaction mixture into a dry 200-ml round-bottomed flask.
6. Evaporate the reaction mixture using a rotary evaporator.
7. Wash the reaction mixture with 20 ml petroleum ether twice.
8. Dry the resulting white solid for 1 hr in vacuo to obtain 2-ethylbutyl L-alaninate hydrochloride salt **2**.

Synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate rac-4

9. Weigh out 3.15 g (15.0 mmol) of **2** into a dry 150-ml two-necked round-bottomed flask.
10. Add 50 ml anhydrous CH₂Cl₂ to the flask using a syringe needle.
11. Cool the temperature of reaction mixture to –80°C under dry nitrogen.
12. Add 2.24 ml (15.0 mmol) phenyl dichlorophosphate to the flask using a syringe needle.
13. Add 4.17 ml (30.0 mmol) Et₃N to the flask using a syringe needle over 10 min, and stir the mixture for 30 min.
14. Raise temperature of reaction mixture to 25°C and stir it for 2 hr.
15. Transfer the reaction mixture into a dry 150-ml round-bottomed flask.
16. Evaporate the reaction mixture using a rotary evaporator to obtain a white solid.
17. Add 50 ml anhydrous Et₂O to the flask and stir the mixture for 10 min.
18. Vacuum filter the mixture using a vacuum filtration system with the 150-ml round-bottomed flask to remove the solid.
19. Evaporate the filtrate using a rotary evaporator.
20. Dry the resulting colorless oil for 1 hr in vacuo to provide 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate *rac-4*.
21. Characterize the product by ¹H NMR, ¹³C NMR, and ³¹P NMR.

Product rac-4 is a colorless oil (4.9 g, 94% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.41-7.34 (m, 2H), 7.29-7.22 (m, 3H), 4.53-4.33 (m, 1H), 4.26-4.06 (m, 3H), 1.62-1.48 (m, 4H), 1.40-1.33 (m, 4H), and 0.95-0.87 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 173.0, 172.9, 172.8, 172.7, 149.9, 149.9, 149.9, 149.8, 149.8, 130.0, 129.9, 129.9, 129.9, 126.0, 126.0, 126.0, 120.6, 120.6, 68.0, 67.9, 50.9, 50.6, 50.6, 40.3, 23.3, 23.3, 23.3, 20.6, 20.6, 20.6, 20.6, 11.0, and 11.0. ³¹P NMR (162 MHz, CDCl₃): δ 8.04 (s) and 7.69 (s).

SYNTHESIS OF CHIRAL BICYCLIC IMIDAZOLE Ad-DPI

This protocol describes the synthesis of chiral bicyclic imidazole **Ad-DPI** (Wang et al., 2020; Wang, Zhang, Ling, Zhang, & Zhang, 2017; Zhang, Xie, Jia, & Zhang, 2010), the catalyst for asymmetric phosphorylation prepared from imidazole (Fig. 2). The synthesis involves three steps: (1) synthesis of (*rac*)-**HO-DPI** from acrolein and imidazole, (2) synthesis of (*S*)-**HO-DPI** via kinetic resolution catalyzed by Novozyme 435, and (3) synthesis of **Ad-DPI** from (*S*)-**HO-DPI**.

Materials

- Imidazole (99.0%, Energy Chemical, E020263)
 - 1,4-Dioxane (>99.5%, Sinopharm Chemical Reagent, 10008918)
 - Acetic acid (AcOH, >99.5%, Sinopharm Chemical Reagent, 10000218)
 - Acrolein (>98.0%, Senfeida, BXQ100500)
 - Silica gel (100-200 Mesh)
 - Ethyl acetate (EtOAc, >99.5%, General-Reagent, G23272D)
 - Methanol (MeOH, >99.5%, Sinopharm Chemical Reagent, 10014118)
 - Acetonitrile (MeCN, >99.0%, Sinopharm Chemical Reagent, 80000618)
 - Isopropenyl acetate (>98.0%, TCI, A0035)
 - Novozyme 435 (Shanghai Yuanye Bio-Technology Co., Ltd, S27516-10g)
 - Anhydrous tetrahydrofuran (THF, >99.0%, Sinopharm Chemical Reagent, 80124418)
 - Dry nitrogen
 - Sodium hydride (NaH, 60% dispersion in mineral oil, Energy Chemical, E060138)
 - 1-Isocyanatoadamantane (>98.0%, TCI, A1706)
 - Dichloromethane (CH₂Cl₂, >99.5%, General-Reagent, G81014C)
 - Sodium sulfate (Na₂SO₄, >99.0%, General-Reagent, G82667C)
 - Isopropanol (99.8%, Adamas-Beta, 75885G)
 - Hexane (99.0%, Adamas-Beta, 14153F)
- 100-, and 250-ml two-necked round-bottomed flasks
 Reflux condensers
 Magnetic stirring bar
 Magnetic stirrer
 Magnetic stirrer with heating
 50-, 100-, and 250-ml round-bottomed flasks
 Rotary vacuum evaporator
 Flash chromatography columns
 High-vacuum oil pump
 Vacuum filtration system
 Ice bath

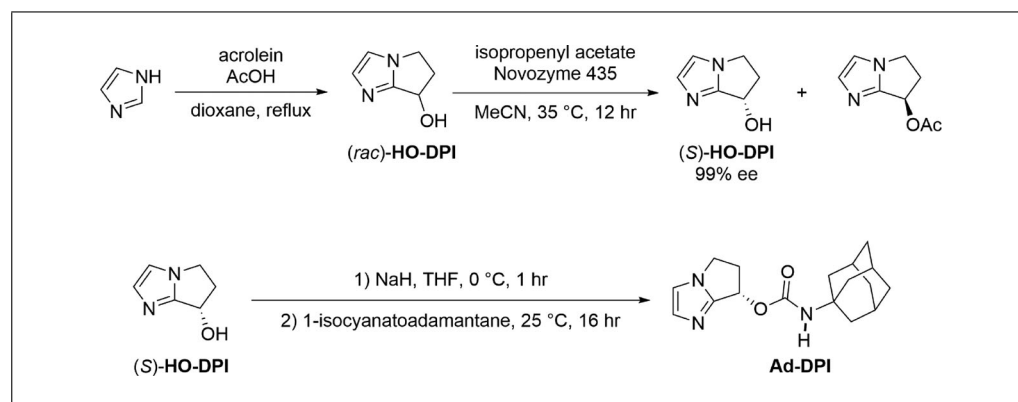


Figure 2 Synthesis of chiral bicyclic imidazole **Ad-DPI**.

Oil bath

Syringe needle

¹H and ¹³C NMR, HPLC, and high-resolution mass spectrometry (HRMS) equipment or facilities

Additional reagents and equipment for silica gel flash chromatography (see Current Protocols article: Meyers, 2001)

Synthesis of (rac)-HO-DPI

1. Weigh out 1.6 g (23.9 mmol) imidazole into a 100-ml two-necked round-bottomed flask.
2. Add 25 ml 1,4-dioxane to this flask.
3. Add 0.1 ml (1.7 mmol) AcOH to the flask.
4. Add 2.5 ml (36.7 mmol) acrolein to the flask and stir the mixture.
5. Reflux the reaction mixture for 36 hr.
6. Transfer the reaction mixture into a 100-ml round-bottomed flask.
7. Evaporate the reaction mixture using a rotary evaporator.
8. Purify the crude product by silica gel chromatography (100-200 Mesh; Meyers, 2001) using 3/1 (v/v) EtOAc/MeOH to obtain (rac)-HO-DPI.
9. Characterize the product by ¹H NMR, ¹³C NMR, HRMS, and HPLC.

The product (rac)-HO-DPI is a white solid (2.4 g, 81% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.07 (d, J = 1.2 Hz, 1 H), 6.85 (d, J = 1.2 Hz, 1 H), 5.23 (dd, J = 7.2 Hz, 3.2 Hz, 1 H), 4.24-4.16 (m, 1 H), 3.98-3.88 (m, 1 H), 3.00-2.88 (m, 1 H), and 2.64-2.54 (m, 1 H). ¹³C NMR (100 MHz, CDCl₃): δ 156.5, 132.5, 114.3, 63.6, 43.2, and 36.3. HRMS (ESI): m/z calculated for C₆H₉N₂O⁺ (M+H)⁺ 125.0709 was 125.0707. HPLC: Daicel CHIRALCEL OD-H, 25 cm × 4.6 μm; 0.5 ml/min isopropanol/hexane = 10/90, 210 nm; retention times: 19.1 min and 30.5 min.

Synthesis of (S)-HO-DPI

10. Weigh out 5.0 g (40.0 mmol) (rac)-HO-DPI into a 250-ml two-necked round-bottomed flask.
11. Add 150 ml of MeCN to the flask.
12. Add 21.8 ml (200.0 mmol) isopropenyl acetate to the flask.
13. Add 5.0 g Novozyme 435 to the flask.
14. Raise temperature of reaction mixture to 35°C and stir the mixture gently for 12 hr.
15. Vacuum filter the mixture using a vacuum filtration system with the 250-ml round-bottomed flask to remove the solid.
16. Evaporate the reaction mixture using a rotary evaporator to remove MeCN.
17. Purify the crude product by silica gel chromatography (100-200 Mesh; Meyers, 2001) using 10:1 v/v EtOAc/MeOH to yield (S)-HO-DPI.
18. Characterize the product by ¹H-NMR, ¹³C-NMR, and HPLC.

The product (S)-HO-DPI is a white solid (1.6 g, 32% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.07 (d, J = 1.2 Hz, 1 H), 6.85 (d, J = 1.2 Hz, 1 H), 5.23 (dd, J = 7.2 Hz, 3.2 Hz, 1 H), 4.24-4.16 (m, 1 H), 3.98-3.88 (m, 1 H), 3.00-2.88 (m, 1 H), and 2.64-2.54 (m, 1 H). ¹³C NMR (101 MHz, CDCl₃): δ 156.5, 132.5, 114.3, 63.6, 43.2, and 36.3. HPLC: 99.9% ee, Daicel CHIRALCEL OD-H, 25 cm × 4.6 μm; 0.5 ml/min, isopropanol/hexane = 10/90, 210 nm; retention times: 19.1 min (minor) and 30.5 min (major).

Synthesis of Ad-DPI

19. Weigh out 200 mg (1.6 mmol) (*S*)-**HO-DPI** into a dry 50-ml two-necked round-bottomed flask.
20. Add 20 ml THF to the flask.
21. Cool the reaction mixture to 0°C under dry nitrogen.
22. Add 77 mg NaH (60% in mineral oil, 1.9 mmol) in portions over 30 min to the flask and stir the mixture for 1 hr.
23. Add 428 mg (2.4 mmol) 1-isocyanatoadamantane to the flask.
24. Raise the temperature of the reaction mixture to 25°C and stir the mixture gently for 16 hr.
25. Add 30 ml water to the flask to quench the reaction.
26. Extract the mixture with 20 ml CH₂Cl₂ three times.
27. Dry the organic layer over Na₂SO₄ and remove the solid by filtration.
28. Evaporate the reaction mixture using a rotary evaporator to remove CH₂Cl₂.
29. Purify the crude product by silica gel chromatography (100-200 Mesh; Meyers, 2001) using 10/1 v/v EtOAc/MeOH to obtain **Ad-DPI**.
30. Characterize the product by ¹H NMR, ¹³C NMR, and HRMS.

The product **Ad-DPI** is a white solid (285 mg, 59% yield). ¹H NMR (500 MHz, CDCl₃): δ 7.18 (d, *J* = 1.3 Hz, 1H), 6.94 (d, *J* = 1.2 Hz, 1H), 5.86 (dd, *J* = 7.2, 2.7 Hz, 1H), 4.72 (s, 1H), 4.19-4.06 (m, 1H), 4.03-3.91 (m, 1H), 3.11-2.98 (m, 1H), 2.66-2.53 (m, 1H), 2.11-2.05 (m, 3H), 1.95-1.90 (m, 6H), 1.68-1.64 (m, 6H). ¹³C NMR (126 MHz, CDCl₃): δ 153.5, 151.7, 134.8, 115.5, 67.1, 51.0, 43.1, 42.6, 41.8, 36.6, 36.3, 35.4, 29.7, 29.5. HRMS (ESI): *m/z* calcd. for C₁₇H₂₄N₃O₂⁺ (*M*+*H*)⁺ 302.1863, found 302.1868.

BASIC PROTOCOL 3

SYNTHESIS OF REMDESIVIR

This protocol describes the synthesis of remdesivir (Wang et al., 2020), the ProTide pro-drug prepared from *rac*-**4** and nucleoside **5** (Fig. 3). The synthesis involves two steps: (1) catalytic asymmetric phosphorylation of nucleoside GS441524 **5** with 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate (*rac*-**4**) catalyzed by **Ad-DPI** to prepare

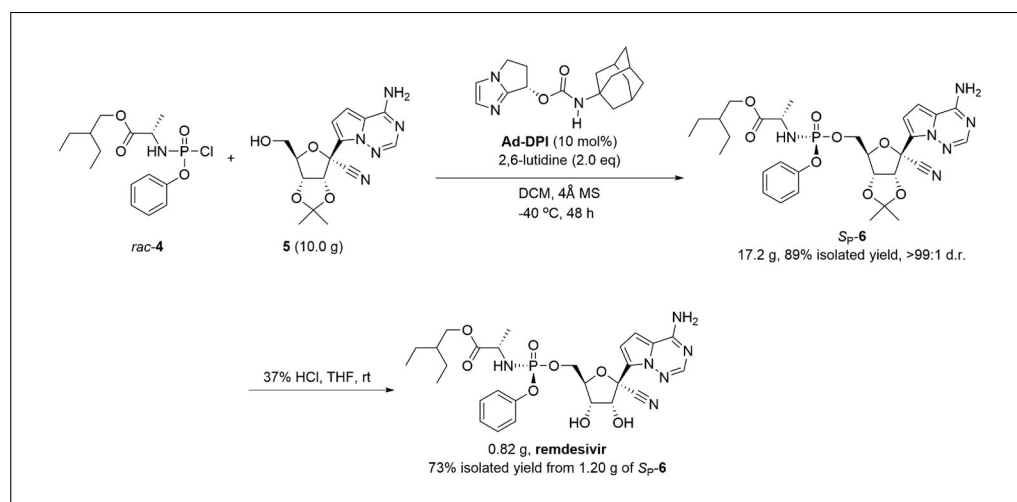


Figure 3 Synthesis of remdesivir.

protected remdesivir *S_P-6*. (2) deprotection of *S_P-6* with 37% HCl in THF to provide **remdesivir** (Warren et al., 2016).

Materials

GS441524 (**5**, 98.0%, Aikonchem, AK01FWCB)
(*S*)-6,7-Dihydro-5*H*-pyrrolo[1,2-*a*]imidazol-7-yl adamantan-1-ylcarbamate
(**Ad-DPI**, Basic Protocol 2)
4 Å molecular sieves
Dry nitrogen
Dichloromethane (CH₂Cl₂, >99.5%, General-Reagent, G81014C)
2,6-Lutidine (99.0%, Energy Chemical, W330004)
2-Ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate (*rac*-**4**; Basic Protocol 1)
Silica gel (100-200 Mesh)
Petroleum ether (AR, General-Reagent, G84208C)
Isopropyl ether (99.0%, Energy Chemical, W330208)
Ethyl acetate (EtOAc, >99.5%, General-Reagent, G23272D)
Isopropanol
Hexane
Tetrahydrofuran (THF, >99.0%, Sinopharm Chemical Reagent, 80124418)
37% aqueous hydrochloric acid solution (Sinopharm Chemical Reagent, 10011061)
Saturated aqueous sodium bicarbonate solution (99.0%, Adamas-Beta, 24073D)
Na₂SO₄ (>99.0%, General-Reagent, G82667C)

100-, and 1000-ml two-necked round-bottomed flasks
Magnetic stirring bar
Magnetic stirrer
Magnetic stirrer with heating
500-, and 1000-ml round-bottomed flasks
Rotary vacuum evaporator
High-vacuum oil pump
Flash chromatography columns
Preparative HPLC column: Daicel CHIRALCEL IE, 1 cm × 25 cm
Separatory funnels
Ice bath
Oil bath
Syringe needle
Cryogenic cooling circulation pump
Vacuum filtration system
¹H, ¹³C, and ³¹P NMR and HPLC and HRMS equipment or facilities

Additional reagents and equipment for silica gel flash chromatography (see Current Protocols article: Meyers, 2001)

Synthesis of protected remdesivir *S_P-6*

1. Add 10.0 g (30.2 mmol) **5** to a dry 1000-ml two-necked round-bottomed flask equipped with a magnetic stir bar.
2. Add 909.6 mg (3.0 mmol) **Ad-DPI** to the flask.
3. Add 11.0 g 4 Å molecular sieve to the flask.
4. Evacuate and backfill the flask with dry nitrogen three times.
5. Add 300 ml CH₂Cl₂ to the flask with the aid of a syringe needle.

6. Add 7.0 ml (60.4 mmol) 2,6-lutidine to the flask with the aid of a syringe needle.
7. Cool the temperature of reaction mixture to -40°C under dry nitrogen and stir the mixture for 10 min.
8. Add 15.7 g (45.3 mmol) *rac*-**4** to the flask with the aid of a syringe needle and stir the reaction mixture for 48 hr.
9. Add 30 ml water to the flask to quench the reaction.
10. Transfer the reaction mixture into a 1000-ml round-bottomed flask.
11. Evaporate the reaction mixture using a rotary evaporator and concentrate the residue in vacuo using a high-vacuum oil pump.
12. Analyze the crude product by ^{31}P NMR showing a d.r. of 21.2:1.
13. Purify the crude product by silica gel chromatography (100-200 Mesh) using 1/5 v/v petroleum ether/ethyl acetate and then recrystallize the product from dichloromethane/isopropyl ether at -30°C to provide protected remdesivir $S_{\text{p}}\text{-6}$ as a white solid (16.5 g, 85% yield, $>99:1$ d.r.).
14. Purify the residue from mother liquor by preparative HPLC (Daicel CHIRALCEL IE, 1 cm \times 25 cm, 10 ml/min, isopropanol/hexane = 30/70, 220 nm, collected from 49.2 to 63.9 min) to yield $S_{\text{p}}\text{-6}$ as a white solid (0.8 g, 4% yield, $>99:1$ d.r.).
15. Combine two parts of product providing 17.3 g $S_{\text{p}}\text{-6}$ (89% yield, $>99:1$ d.r.).
16. Characterize the product by ^1H NMR, ^{13}C NMR, ^{31}P NMR, HPLC, and HRMS.

*The product $S_{\text{p}}\text{-6}$ is a white solid (17.3 g, 89% yield, $>99:1$ d.r.). ^1H NMR (400 MHz, CD_3OD): δ 7.88 (s, 1H), 7.29-7.23 (m, 2H), 7.17-7.10 (m, 3H), 6.93-6.90 (m, 2H), 5.33 (d, $J = 6.7$ Hz, 1H), 4.98 (dd, $J = 6.6, 3.4$ Hz, 1H), 4.60-4.55 (m, 1H), 4.36-4.25 (m, 2H), 4.02 (dd, $J = 10.9, 5.8$ Hz, 1H), 3.91 (dd, $J = 10.9, 5.7$ Hz, 1H), 3.88-3.79 (m, 1H), 1.70 (s, 3H), 1.49-1.41 (m, 1H), 1.39 (s, 3H), 1.35-1.24 (m, 8H), and 0.85 (t, $J = 7.5$ Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 174.9, 174.8, 156.6, 152.0, 151.9, 147.6, 130.6, 126.0, 124.9, 121.3, 121.2, 118.0, 117.7, 116.9, 112.5, 103.0, 85.7, 84.8, 84.8, 83.0, 82.5, 68.1, 67.0, 66.9, 51.4, 41.6, 26.5, 25.6, 24.2, 24.1, 20.5, 20.4, 11.3, and 11.3. ^{31}P NMR (162 MHz, CD_3OD): δ 3.23 (s). **HPLC analysis:** $>99:1$ d.r. [Daicel CHIRALPAK IE column; 4.6 μm \times 25 cm; solvent system: isopropanol/hexane = 30/70; 1.0 ml/min; retention times: 21.2 min (minor) and 26.5 min (major)]. **HRMS (ESI):** m/z calculated for $\text{C}_{30}\text{H}_{40}\text{N}_6\text{O}_8\text{P}^+$ ($M+H$) $^+$ 643.2640 was 643.2609.*

Synthesis of remdesivir

17. Weigh out 1.2 g (1.8 mmol) $S_{\text{p}}\text{-6}$ into a 100-ml two-necked round-bottomed flask.
18. Add 24 ml THF to the flask.
19. Cool the reaction mixture to 0°C and stir the mixture.
20. Add 2.1 ml 37% aqueous hydrochloric acid solution slowly to the flask.
21. Raise temperature of reaction mixture to 25°C and stir the mixture for 8 hr.
22. Transfer the reaction mixture into a 500-ml round-bottomed flask and dilute the reaction mixture with 60 ml water.
23. Add saturated aqueous sodium bicarbonate solution to adjust the pH of reaction mixture to 8.
24. Extract the resulting mixture with 60 ml ethyl acetate three times.
25. Dry the combined organic phase over Na_2SO_4 and remove the solid by filtration.

26. Evaporate the reaction mixture using a rotary evaporator.
27. Purify the crude product by silica gel chromatography (100-200 Mesh) using 1/3 v/v) petroleum ether/ethyl acetate to provide **remdesivir**.
28. Characterize the product by ^1H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution mass spectrometry (HRMS).

The product **remdesivir** is a white solid (0.82 g, 73% yield). ^1H NMR (400 MHz, CD_3OD): δ 7.87 (s, 1H), 7.33-7.27 (m, 2H), 7.21-7.12 (m, 3H), 6.91 (d, $J = 4.6$ Hz, 1H), 6.88 (d, $J = 4.6$ Hz, 1H), 4.79 (d, $J = 5.4$ Hz, 1H), 4.44-4.35 (m, 2H), 4.33-4.25 (m, 1H), 4.20-4.15 (m, 1H), 4.02 (dd, $J = 10.9, 5.8$ Hz, 1H), 3.95-3.85 (m, 2H), 1.49-1.40 (m, 1H), 1.34-1.27 (m, 8H), 0.85 (t, $J = 7.5$ Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.0, 174.9, 157.1, 152.1, 152.1, 148.2, 130.7, 126.0, 125.5, 121.3, 121.3, 117.9, 117.6, 112.3, 102.7, 84.3, 84.2, 81.2, 75.6, 71.6, 68.1, 67.2, 67.1, 51.5, 41.6, 24.2, 24.2, 20.6, 20.5, 11.3, 11.3. ^{31}P NMR (162 MHz, CD_3OD): δ 3.52 (s). HRMS (ESI): m/z calcd. for $\text{C}_{27}\text{H}_{36}\text{N}_6\text{O}_8\text{P}^+$ ($M+H$) $^+$ 603.2327, found 603.2300.

COMMENTARY

Background Information

Remdesivir is one of the most effective treatments for COVID-19 (Holshue et al., 2020), which attracts researchers to develop an effective synthetic approach for this compound. Until now, two synthetic methods for remdesivir have been established (Siegel et al., 2017; Warren et al., 2016). Both of these methods require not only chiral resolution but also additional synthetic steps, which lead to the waste of resources and low synthetic efficiency. In 2010, a novel chiral bicyclic imidazole organocatalyst was developed by our group (Zhang et al., 2010). Over the past decade, this kind of organocatalyst was successfully applied in a number of asymmetric reactions (Liu et al., 2012; Wang et al., 2017; Wang et al., 2020; Wang, Zhang, Liu, Xie, & Zhang, 2014; Wang, Zhang, Xie, & Zhang, 2014; Wang, Zhou, Zhang, Zhang, & Zhang, 2020; Zhang et al., 2019; Zhang, Wang, Xie, Sun, & Zhang, 2014; Zhou et al., 2019; Zhou et al., 2021). In 2012, the first catalytic asymmetric synthesis of *P*-stereogenic phosphoric acid derivatives was developed by our group using the chiral bicyclic imidazole catalyst (Liu et al., 2012). Later, scientists at Merck & Co. utilized bicyclic imidazole catalyst in the asymmetric synthesis of nucleoside-based phosphoramidate prodrugs (DiRocco et al., 2017). Inspired by our previous work, we successfully developed the first catalytic asymmetric synthesis of remdesivir using the chiral bicyclic imidazole **Ad-DPI** (Wang et al., 2020).

Critical Parameters and Troubleshooting

In Basic Protocol 1, both steps are sensitive to water. In Basic Protocol 2, the step for syn-

thesis of **Ad-DPI** is sensitive to water. In Basic Protocol 3, the catalytic asymmetric synthesis of S_p -**6** step is sensitive to water. Anhydrous reaction conditions are required for the aforementioned steps. Glassware needs to be oven-dried, and anhydrous solvents should be prepared or purchased in Sure/Seal bottles. In the step for synthesis of S_p -**6** in Basic Protocol 3, 4 Å molecular sieves are necessary for the reaction.

Understanding Results

After the synthesis, the purified compounds are identified by ^1H -NMR, ^{13}C -NMR, and HRMS as shown in each synthesis. The d.r. value of crude S_p -**6** is analyzed by ^{31}P -NMR. The optically pure S_p -**6** is verified by ^{31}P -NMR and HPLC.

Time Considerations

Synthesis of 2-ethylbutyl (chloro(phenoxy)phosphoryl)-L-alaninate (*rac*-**4**) (Basic Protocol 1) can be completed within 28 hr. Synthesis of chiral bicyclic imidazole **Ad-DPI** (Basic Protocol 2) can be completed within 4 days. Synthesis of remdesivir (Basic Protocol 3) can be completed within 3 days.

Acknowledgments

This work was supported by National Natural Science Foundation of China (Nos. 91856106 and 21991112), National Key R&D Program of China (No. 2018YFE0126800), Medical-Engineering Cross Research Foundation of SJTU (YG2020YQ13), Shanghai Municipal Education Commission (No. 201701070002E00030), and Science and Technology Commission of Shanghai Municipality (19JC1430100).

Author Contributions

Mo Wang: investigation, writing original draft; **Lu Zhang:** investigation; **Xiaohong Huo:** writing review and editing; **Zhenfeng Zhang:** writing review and editing; **Wanbin Zhang:** project administration, writing review and editing.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data openly available in a public repository that issues datasets with DOIs.

Literature Cited

- DiRocco, D. A., Ji, Y., Sherer, E. C., Klapars, A., Reibarkh, M., Dropinski, J., ... Davies, I. W. (2017). A multifunctional catalyst that stereoselectively assembles prodrugs. *Science*, *356*, 426–430. doi: 10.1126/science.aam7936.
- Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., ... Washington State 2019-nCoV Case Investigation Team. (2020). First case of 2019 novel coronavirus in the United States. *The New England Journal of Medicine*, *382*, 929–936. doi: 10.1056/NEJMoa2001191.
- Liu, S., Zhang, Z., Xie, F., Butt, N. A., Sun, L., & Zhang, W. (2012). First catalytic enantioselective synthesis of *P*-stereogenic phosphoramides via kinetic resolution promoted by a chiral bicyclic imidazole nucleophilic catalyst. *Tetrahedron: Asymmetry*, *23*, 329–332. doi: 10.1016/j.tetasy.2012.02.018.
- Mackman, R. L., Parrish, J. P., Ray, A. S., & Theodore, D. A. (2012). Methods and compounds for treating Paramyxoviridae virus infections. U.S. Patent 20120027752 February, 2, 2012.
- Meppen, M., Pacini, B., Bazzo, R., Koch, U., Leone, J. F., Koeplinger, K. A., ... Gardelli, C. (2009). Cyclic phosphoramidates as prodrugs of 2'-*C*-methylcytidine. *European Journal of Medicinal Chemistry*, *44*, 3765–3770. doi: 10.1016/j.ejmech.2009.04.043.
- Meyers, C. L. F. (2001). Column chromatography. *Current Protocols in Nucleic Acid Chemistry*, *3*, A.3E.1–A.3E.7. doi: 10.1002/0471142700.nca03es03.
- Siegel, D., Hui, H. C., Doerffler, E., Clarke, M. O., Chun, K., Zhang, L., ... Mackman, R. L. (2017). Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-*f*][triazin-4-amino] adenine *C*-nucleoside (GS-5734) for the treatment of Ebola and emerging viruses. *Journal of Medicinal Chemistry*, *60*, 1648–1661. doi: 10.1021/acs.jmedchem.6b01594.
- Wang, M., Zhang, L., Huo, X., Zhang, Z., Yuan, Q., Li, P., ... Zhang, W. (2020). Catalytic asymmetric synthesis of the anti-COVID-19 drug remdesivir. *Angewandte Chemie International Edition*, *59*, 20814–20819. doi: 10.1002/anie.202011527.
- Wang, M., Zhang, X., Ling, Z., Zhang, Z., & Zhang, W. (2017). Direct enantioselective *C*-acylation for the construction of a quaternary stereocenter catalyzed by a chiral bicyclic imidazole. *Chemical Communications*, *53*, 1381–1384. doi: 10.1039/C6CC09451A.
- Wang, M., Zhang, Z., Liu, S., Xie, F., & Zhang, W. (2014). Enantioselective Black rearrangement catalyzed by chiral bicyclic imidazole. *Chemical Communications*, *50*, 1227–1230. doi: 10.1039/C3CC47455K.
- Wang, M., Zhang, Z., Xie, F., & Zhang, W. (2014). Cu-catalyzed amidation of halogenated imidazoles. *Chemical Communications*, *50*, 3163–3165. doi: 10.1039/C3CC49107B.
- Wang, M., Zhou, M., Zhang, L., Zhang, Z., & Zhang, W. (2020). A step-economic and one-pot access to chiral *C*^α-tetrasubstituted α -amino acid derivatives via a bicyclic imidazole-catalyzed direct enantioselective *C*-acylation. *Chemical Science*, *11*, 4801–4807. doi: 10.1039/D0SC00808G.
- Warren, T. K., Jordan, R., Lo, M. K., Ray, A. S., Mackman, R. L., Soloveva, V., ... Bavari, S. (2016). Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature*, *531*, 381–385. doi: 10.1038/nature17180.
- Zhang, L., Wang, M., Zhou, M., Zhang, Z., Muraoka, M., & Zhang, W. (2019). Chiral bicyclic imidazole-catalyzed direct enantioselective *C*-acylation for the synthesis of 2-oxindoles bearing a quaternary stereocenter. *Asian Journal of Organic Chemistry*, *8*, 1024–1028. doi: 10.1002/ajoc.201900294.
- Zhang, Z., Wang, M., Xie, F., Sun, H., & Zhang, W. (2014). Chiral bicyclic imidazole nucleophilic catalysts: Design, synthesis, and application to the kinetic resolution of arylalkylcarbinols. *Advanced Synthesis & Catalysis*, *356*, 3164–3170. doi: 10.1002/adsc.201400415.
- Zhang, Z., Xie, F., Jia, J., & Zhang, W. (2010). Chiral bicycle imidazole nucleophilic catalysts: Rational design, facile synthesis, and successful application in asymmetric Steglich rearrangement. *Journal of the American Chemical Society*, *132*, 15939–15941. doi: 10.1021/ja109069k.
- Zhou, M., Gridneva, T., Zhang, Z., He, E., Liu, Y., & Zhang, W. (2021). Chiral bicyclic imidazole-catalyzed acylative dynamic kinetic resolution for the synthesis of chiral phthalidyl esters. *Angewandte Chemie International Edition*, *60*, 1641–1645. doi: 10.1002/anie.202012445.
- Zhou, M., He, E., Zhang, L., Chen, J., Zhang, Z., Liu, Y., & Zhang, W. (2019). Development of a new bicyclic imidazole nucleophilic organocatalyst for direct enantioselective *C*-acylation. *Organic Chemistry Frontiers*, *6*, 3969–3972. doi: 10.1039/C9QO01025D.