A convergent synthesis of carbocyclic sinefungin and its C5 epimer

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Abstract

A convergent synthesis of carbocyclic sinefungin 2 and its C5 epimer 3 is described. The key features in our synthesis include the use of commercial available L-methionine and readily available (1R, 4S)-4-hydroxy-2-cyclopentenyl acetate as starting materials, cross-metathesis coupling, enzymatic kinetic resolution and Staudinger reduction. The current synthesis is flexible and therefore provides convenient access to the synthesis of various carbocyclic SIN analogues for biological evaluation.

Keywords

sinefungin; synthesis; methyltransferase; enzyme resolution; carbocyclic derivative

Introduction

Sinefungin (1, SIN, Figure 1) was isolated from the fermentation broth of Streptomyces griseoleus NRRL 3739 at Lilly research Laboratories in 1973.[1] It is structurally similar to S-adenosyl-methionine (AdoMet) and S-adenosyl-homocysteine (AdoHcy). It is a highly potent and competitive inhibitor of AdoMet dependent methyltransferase (MTase).[2] Preliminary bioassays of sinefungin displayed antifungal,[3] antitumor,[4] antiviral,[5] and antiparasitic activity.[6] However, toxicological studies showed that the therapeutic and lethal dose of SIN are very close in goats, causing severe nephrotoxic side effects.[7] Sinefungin showed a short plasma half-life.[8] Intravenous administration of SIN in rats showed that the blood clearance time was less than 270 min and 50% of the SIN dose was eliminated by excretion in the urine.[9] Schneller and co-workers synthesized carbocyclic SIN (Figure 1), which replaced the ribosyl oxygen of SIN to its isosteric methylene unit.[10] Such modification may alleviate metabolic instability associated with sinefungin.

Recently, Li and co-workers reported the X-ray crystal structure of the flavivirus MTase in complex with SIN.[11] Analysis of the SIN-MTase complex structure revealed an additional pocket that is adjacent to the AdoMet-binding pocket. Based upon this molecular insight, it
has been proposed that modification of the adenine base of SIN may show enhanced binding affinities, thereby leading to more potent and specific inhibitors of flavivirus MTase.\[11\]

We therefore, planned to design and synthesize a series of carbocyclic SIN derivatives in which different substitution will be introduced to the adenine base of SIN. We sought to develop an efficient and flexible synthesis of carbocyclic SIN for analog preparation. Herein, we report convergent syntheses of carbocyclic SIN and its C5 epimer 3. The present synthesis utilized commercial available L-methionine 7 and readily available (1R, 4S)-4-hydroxy-2-cyclopentenyl acetate 8 as starting materials. Other key reactions include cross-metathesis, enzymatic resolution and Staudinger reduction.

**Results and Discussion**

Our retrosynthetic analysis of carbocyclic SIN and its derivatives is outlined in Scheme 1. We planned to introduce the adenine base in a late stage via SN2 substitution of 4a-b. These derivatives could be synthesized utilizing the cross-metathesis of the segment 5 and olefin 6a-b. The fragment 5 would be prepared from L-methionine 7. The alcohol 6a-b could be derived from (1R, 4S)-4-hydroxy-2-cyclopentenyl acetate 8.

The synthesis of vinyl glycine derivative 5 is shown in scheme 2. L-methionine 7 was converted to methyl ester 9 using thionyl chloride in methanol according to reported literature procedure.\[12\] Protection of the amine group as Cbz-derivative followed by oxidation of the sulfide in the presence of NaIO4 provided sulfoxide 10.\[13\] Boc-protected sulfoxide 11 was prepared by following similar course of reactions. For the subsequent elimination in mesitylene at 170 °C, the Boc-protected derivative 11 provided a complex mixture of unidentified products along with a trace amount of olefin 5. The Cbz-protected derivative 10 however, provided the desired terminal alkene 12 in 60% yield.\[14\] We then converted the Cbz-protected derivative 12 to Boc-protected derivative 5.

The synthesis of segment 6a-b is shown in Scheme 3. It began with (1R, 4S)-4-hydroxy-2-cyclopentenyl acetate 8 which is readily available in high enantiomeric purity on a large scale from cyclopentadiene according to literature procedures.\[15\] As shown in Scheme 3, coupling of 8 with methyl chloroformate, followed by selective palladium-catalyzed nucleophilic substitution in the presence of sodium hydride providing the cis-adduct 13.\[16\] Decarboxylation of 13 in DMF in the presence of KI at 150 °C afforded ester 14. Dihydroxylation with OsO4 and subsequent protection of cis-diol with 2,2-dimethoxypropane in the presence of a catalytic amount of p-TsOH afforded isopropylidene derivative 15.\[17\] Saponification of 15 with potassium hydroxide in methanol and THF furnished alcohol 16. The (R)-alcohol 16 was inverted to (S)-alcohol 17 upon oxidation with Dess-Martin reagent and selective reduction with sodium borohydride. Treatment of the (S)-alcohol 17 with PMBCl and sodium hydride yielded the PMB-protected derivative 18. Reduction of 18 with DIBAL followed by treatment of the resulting aldehyde with vinylmagnesium chloride resulted in allyl alcohol 19 as a mixture of diastereomers (1:1). This mixture could not be separated by silica gel chromatography. Oxidation of allyl alcohol 19 with Dess–Martin periodinane afforded enone 20, which was then subjected to Corey-Bakshi-Shibata reduction using (R)-2-Me-CBS and borane dimethylsulfide complex (BMS)
affording segment 6a in excellent distereoselectivity (15:1 dr, by $^1$H and $^{13}$C NMR analysis).\textsuperscript{[18]} Allylic alcohol 6a was obtained in 55% yield along with olefin-reduced by product. We also investigated this reaction using borane tetrahydrofuran complex or catecholborane instead of BMS, however, the yield was not improved.

In an alternative route, we attempted the separation of diastereomeric alcohol 19 by enzymatic kinetic resolution.\textsuperscript{[19]} As shown in Scheme 4, alcohol 19 was treated with Amano lipase in the presence of vinyl acetate in dimethoxyethane. The resolution afforded (R)-isomer 6b (13:1 dr, by $^1$H and $^{13}$C NMR analysis) and acetate derivative 21 (15:1 dr, by $^1$H and $^{13}$C NMR analysis) in a good yield and distereoselectivity. The stereochemistry can be predicted by Kazlauskas’ rule,\textsuperscript{[20]} and the result is consistent with that of Corey-Bakshi-Shibata reduction. Acetate derivative 21 was hydrolyzed by treatment with aqueous NaOH to afford the desired (S)-isomer 6a.

With segment 5 and 6a-b in hand, we then turned our attention to construct the C3-C4 bond of the target compound. As shown in Scheme 5, cross-metathesis of these two fragments proceeded smoothly in the presence of Grubbs’ second-generation catalyst to afford alkene 22a-b.\textsuperscript{[21]} A homodimer was also formed from 6a-b in this reaction, even when the ratio of 6a-b to 5 was decreased to 1 : 4. The unreacted homodimer was recycled under the same conditions affording alkene 22a-b in 75-78% total yields. Mesylation of alkene 22a-b followed by treatment with sodium azide did not provide the expected azide derivatives 23a-b, instead resulted in the elimination of the hydroxyl group along with other byproduct.

To circumvent this problem, we first carried out hydrogenation of alkene 22a-b to provide saturated alkane 24a-b. Mesylation of the respective alcohol to mesylate followed by displacement of mesylate with azide proceeded well to afford azide 25a-b. Deprotection of the PMB group on azide 25a-b with DDQ in DCM afforded cyclopentanol 4a-b as shown in Scheme 6.

Treatment of cyclopentanol 4a-b with triflic anhydride and pyridine in DCM followed by SN$_2$ substitution afforded the protected carbocyclic SIN 26a-c. However, the SN$_2$ substitution using NaH as base following literature procedure gave a low yield.\textsuperscript{[10a]} We found that carbocyclic SIN 26a-c could be obtained in good yield using K$_2$CO$_3$ and 18-Crown-6 in DMF [at 50 °C.\textsuperscript{[22]} Staudinger reduction of 26a-c converted the azide to an amine.\textsuperscript{[23]} The removal of the acetonide and Boc protecting group by exposure to trifluoroacetic acid provided the corresponding amine. Saponification of methyl ester with aqueous LiOH furnished carbocyclic SIN 2 and 3.

Conclusions

In summary, a convergent synthesis of carbocyclic sinefungin 2 and its C5 epimer 3 is described. The key features in our syntheses include the use of commercially available L-methionine and readily available (1R, 4S)-4-hydroxy-2-cyclopentenyl acetate 8 as starting materials, cross-metathesis coupling, enzymatic kinetic resolution and Staudinger reduction. The current synthesis is flexible and therefore provides a convenient access to the synthesis of various carbocyclic SIN analogues for biological evaluation. Further study of the
chemistry and biology of carbocyclic SIN derivatives is an active area of research in our laboratory.

**Experimental Section**

**Aminoester 5.** A solution of 12 (2.49 g, 10 mmol) in hydrobromic acid solution (4 mL) was stirred for 2 h at room temperature, then diluted with DCM and condensed under reduced pressure to get a crude product as a brown oil. The above crude product was dissolved in 1, 4-dioxane (20 mL) and H₂O (15 mL), then slowly adjusted to pH 12 with NaHCO₃ at 0 °C. A solution of Boc₂O (4.36 g, 20 mmol) in 1, 4-dioxane (10 mL) was added at the same temperature. The above mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was treated with H₂O (15 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via silica gel chromatography (15:1 hexanes/EtOAc) to afford 5 (1.39 g, 65%, 2 steps) as a colorless oil. [a]²⁰_D +3.2 (c 1, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 5.89 (m, 1H), 5.35 (d, J = 17.2, 1H), 5.26 (d, J = 10.4 Hz, 1H), 5.23 (brs, 1H), 4.87 (brs, 1H), 3.76 (s, 3H), 1.45 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) 171.10, 154.86, 132.55, 117.27, 79.92, 55.64, 52.42, 28.14; HRMS (ESI), m/z (M+Na)⁺ Calcd for C₁₀H₁₇O₄Na: 238.1055, found 238.1059.

**Compound 13.** To a stirred solution of 8 (1.00 g, 7.0 mmol) in anhydrous DCM (20 mL) was added pyridine (0.85 mL, 10.5 mmol) and methyl chloroformate (0.82 mL, 10.5 mmol) at 0 °C. The mixture was stirred for 1 h at the same temperature, and quenched with 5% HCl (10 mL). The resulting mixture was extracted by DCM (3 × 20 mL), and the combined extracts were dried over anhydrous Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified via silica gel chromatography (10:1 hexanes/ EtOAc) to afford a colorless oil.

To a suspension of NaH (60% in mineral oil, 840 mg, 21 mmol) in THF (20 mL) was added dropwise a solution of dimethyl malonate (1.60 mL, 14 mmol) in THF (20 mL) at 0 °C, and the mixture was stirred for 30 min at room temperature. This reaction mixture was added to a solution of Pd(Ph₃P)₄ (404 mg, 0.35 mmol) and the above oil in THF, and then stirred for 3 h at room temperature. Then the reaction mixture was quenched with 5% HCl (20 mL). The resulting mixture was extracted by DCM (3 × 20 mL), and the combined extracts were dried over anhydrous Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified via silica gel chromatography (10:1 hexanes/ EtOAc) to afford compound 13 (1.49 g, 83%) as a colorless oil. [a]²⁰_D +17.5 (c 1.01, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 6.02-6.00 (m, 1H), 5.89-5.87 (m, 1H), 5.63-5.59 (m, 1H), 3.74 (s, 3H), 3.73 (s, 3H), 3.36-3.26 (m, 2H), 2.58 (dt, J = 14.5, 7.5 Hz, 1H), 2.02 (s, 3H), 1.56 (dt, J = 14.5, 4.6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 170.63, 168.51, 168.44, 137.21, 131.36, 78.71, 56.75, 52.44, 52.38, 43.45, 34.56, 21.09; LRMS (ESI), m/z 279.1 (M+Na)⁺

**Acetate derivative 14.** To a stirred solution of 13 (240 mg, 0.94 mmol) in DMF (15 mL) was added KI (468 mg, 2.82 mmol) and H₂O (1.5 mL) at room temperature under argon. The mixture was heated to 150 °C and stirred for 8 h, then cooled to room temperature and diluted with water. The mixture was extracted with Et₂O, and the combined extracts were
dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The residue was purified via silica gel chromatography (10:1 hexanes/EtOAc) to afford compound 14 (130 mg, 70%) as a colorless oil. [α]$_{D}^{20}$ +23.3 (c 1.05, CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$) δ 6.01 (d, J = 6.0 Hz, 1H), 5.86-5.82 (m, 1H), 5.65-5.60 (m, 1H), 3.73 (s, 3H), 3.08-3.01 (m, 1H), 2.59 (dt, J = 14.5, 7.6 Hz, 1H), 2.48 (dd, J = 15.6, 7.0 Hz, 1H), 2.38 (dd, J = 15.6, 8.2 Hz, 1H), 2.07 (s, 3H), 1.46 (dt, J = 14.1, 4.4 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.57, 170.71, 139.43, 130.07, 79.32, 51.50, 40.40, 40.34, 36.34, 21.17; HRMS (ESI), m/z (M+Na)$^+$ Calcd for C$_{10}$H$_{14}$O$_4$Na: 221.0790, found 221.0786.

Isopropylidene derivative 15. To a stirred solution of 14 (110 mg, 0.56 mmol) in acetone (5 mL) was added N-methylmorpholine-N-oxide (195 mg, 1.67 mmol) and OsO$_4$ (4% in water, 25 μL) at 0 °C, and the resulting mixture was stirred for 6 h at room temperature. To the reaction mixture was added NaHSO$_3$, and stirred for additional 30 minutes. The mixture was diluted with EtOAc (10 mL), filtered and concentrated. The residue was dissolved in 0.5 M H$_2$SO$_4$ (10 mL), extracted with EtOAc (3 × 20 mL). The combined extracts were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to get a brown oil. The above oil was dissolved in 2, 2-dimethoxypropane (3 mL) at room temperature. To the mixture was added TsOH (10 mg, 0.06 mmol), and stirred for 2 h. The reaction mixture was quenched by saturated aqueous NaHCO$_3$ solution, and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over MgSO$_4$, filtered, and concentrated. The residue was purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford 15 (102 mg, 68%, 2 steps) as a colorless oil. [α]$_{D}^{20}$ −6.7 (c 1, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ 5.03 (d, J = 3.0 Hz, 1H), 4.52 (d, J = 5.6 Hz, 1H), 4.44 (d, J = 5.5 Hz, 1H), 3.68 (s, 3H), 2.65-2.31 (m, 4H), 2.04 (s, 3H), 1.58 (d, J = 16.1 Hz, 1H), 1.44 (s, 3H), 1.27 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.35, 169.79, 110.94, 84.78, 84.53, 79.54, 51.61, 41.01, 37.41, 33.98, 26.45, 24.08, 21.06; LRMS (ESI), m/z (M+Na)$^+$ Calcd for C$_{13}$H$_{20}$O$_6$Na: 295.1158, found 295.1163.

Alcohol 16. To a stirred solution of 15 (500 mg, 1.8 mmol) in MeOH (10 mL) and THF (10 mL) was added KOH (110 mg, 2.0 mmol) at room temperature, and the resulting mixture was stirred for 30 min. The reaction mixture was adjusted to pH 6.0 with 5% HCl, diluted with water (15 mL), and extracted by EtOAc (3 × 30 mL). The combined organic extracts were dried over MgSO$_4$, filtered, and concentrated. The residue was purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford 16 (400 mg, 95%) as a colorless oil. [α]$_{D}^{20}$ +1.4 (c 1, CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$) δ 4.48-4.45 (m, 2H), 4.24-4.22 (m, 1H), 3.69 (s, 3H), 2.70-2.45 (m, 3H), 2.29 (dt, J = 13.8, 6.2 Hz, 1H), 1.53 (d, J = 14.0 Hz, 1H), 1.43 (s, 3H), 1.28 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.02, 110.54, 87.10, 85.02, 77.47, 51.54, 41.24, 37.96, 36.49, 26.51, 24.13; LRMS (ESI), m/z 253.1 (M+Na)$^+$ Calcd for C$_{11}$H$_{14}$O$_4$Na: 295.1158, found 295.1163.

Compound 17. To a stirred solution of 16 (40 mg, 0.17 mmol) in DCM (3 mL) was added Dess-Martin periodinane (110 mg, 0.26 mmol) at room temperature. The mixture was stirred for 3 h at the same temperature, and quenched with saturated aqueous NaHCO$_3$ (3 mL) and Na$_2$S$_2$O$_4$ (3 mL) solutions. The resulting mixture was extracted by DCM (3 × 20 mL), and the combined extracts were dried over anhydrous Na$_2$SO$_4$, and filtered. The filtrate was
concentrated under reduced pressure. The residue was purified via silica gel chromatography (3:1 hexanes/EtOAc) to afford ketone as a colorless oil.

To a stirred solution of the above oil in THF (3 mL) was added sodium borohydride (10 mg, 0.26 mmol) at 0 °C. The mixture was stirred for 20 min at the same temperature, quenched with saturated aqueous NH₄Cl solution, and extracted by DCM. The combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified via silica gel chromatography (2:1 hexanes/EtOAc) to afford 17 (37 mg, 93%) as a colorless oil.

[a]²⁰_D −7.6 (c 0.8, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 5.75-5.65 (t, J = 5.7 Hz, 1H), 4.38 (dd, J = 6.1, 1.5 Hz, 1H), 4.09-4.03 (m, 1H), 3.68 (s, 3H), 2.55-2.48 (m, 1H), 2.40 (d, J = 5.3 Hz, 1H), 2.31 (dd, J = 15.5, 7.9 Hz, 1H), 2.25 (dd, J = 15.5, 7.8 Hz, 1H), 1.95 (dt, J = 13.3, 7.7 Hz, 1H), 1.50 (s, 3H), 1.33 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.21, 111.84, 84.10, 79.05, 70.98, 51.65, 37.93, 36.63, 36.49, 25.97, 24.25; LRMS (ESI), m/z 231.3 (M+H)+.

Compound 18. To a stirred solution of NaH (6 mg, 0.15 mmol) in DMF (2 mL) was added a solution of 17 (30 mg, 0.13 mmol) in DMF (1 mL) and 4-Methoxybenzyl chloride (20 μL, 0.15 mmol) at 0 °C under argon. The mixture was stirred for 3 h, quenched with saturated aqueous NH₄Cl solution (5 mL), and extracted by DCM (3 × 10 mL). The combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified via silica gel chromatography (3:1 hexanes/EtOAc) to afford 18 (40 mg, 88%) as a colorless oil. [α]²⁰_D −16.1 (c 0.9, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.29 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 4.60 (d, J = 11.9 Hz, 1H), 4.55-4.51 (m, 1H), 4.28 (d, J = 5.6 Hz, 1H), 3.80 (s, 3H), 3.78-3.74 (m, 1H), 3.66 (s, 3H), 2.46-2.41 (m, 1H), 2.22 (dd, J = 15.3, 8.3 Hz, 1H), 2.21-2.09 (m, 2H), 1.63-1.60 (m, 1H), 1.52 (s, 3H), 1.31 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 172.29, 159.15, 130.17, 129.41, 113.66, 111.08, 83.74, 78.33, 71.26, 55.17, 51.67, 37.85, 37.04, 32.45, 29.59, 26.21, 24.26; HRMS (ESI), m/z (M+Na)+ Calcd for C₁₉H₂₆O₆Na: 373.1627, found 373.1630.

Alcohol 19. To a stirred solution of 18 (50 mg, 0.14 mmol) in DCM (3 mL) was added dropwise DIBAL (1 M in hexanes, 0.16 ml) under argon at −78 °C. The reaction mixture was stirred for 10 min, then treated with H₂O (1 mL), and warmed to room temperature. The mixture was diluted with DCM (10 mL) and washed with water (4 mL) and brine (4 mL), then dried over anhydrous Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified via silica gel chromatography (10:1 hexanes/EtOAc) to afford aldehyde as a colorless oil, which was subject to the next step immediately.

To a stirred solution of the above colorless oil in anhydrous THF (5 mL) was added dropwise vinylmagnesium chloride (1 M in THF, 0.16 mL) over 5 min at 0 °C. The reaction mixture was stirred for 1 h at the same temperature, quenched with saturated aqueous NH₄Cl solution (20 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford 19 (37 mg, 75%, 2 steps) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) 7.30 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 5.90-5.79 (m, 1H), 5.27-5.20 (m, 1H), 5.13-5.10 (m, 1H), 4.70-4.48 (m, 3H), 4.37-4.38 (m, 1H), 4.15-4.13 (m, 1H), 3.80 (s, 3H), 3.80-3.78 (m, 1H), 2.07-2.02 (m, 3H), 1.78-1.50 (m, 1H), 1.50-1.38 (m, 1H); ¹³C NMR (500 MHz, CDCl₃) δ 172.24, 160.34, 139.52, 130.63, 123.34, 112.62, 81.83, 79.55, 71.28, 40.96, 37.92, 37.03, 32.56, 29.69, 26.21, 24.26; HRMS (ESI), m/z (M+Na)+ Calcd for C₁₉H₂₆O₆Na: 373.1627, found 373.1630.

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2H), 1.52 (s, 3H), 1.32 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 159.10, 140.72, 140.61, 130.39, 129.37, 129.33, 115.07, 114.97, 113.65, 111.22, 84.79, 84.49, 78.60, 77.50, 72.04, 71.46, 71.30, 55.16, 39.85, 39.77, 38.32, 37.60, 33.49, 26.26, 24.43; HRMS (ESI), m/z (M + Na)$^+$ Calcd for C$_{20}$H$_{28}$O$_5$Na: 371.1834, found 371.1837.

Enzymatic kinetic resolution of compound 19. To a stirred solution of 19 (0.53 g, 1.52 mmol) in dimethoxyethane (30 mL) was added vinyl acetate (2.80 mL, 30.4 mmol) and Amano lipase (2.65 g) at room temperature. The reaction mixture was stirred for 24 h, and filtered. The filtrate was concentrated, and the residue was purified via silica gel chromatography (4:1 to 3:2 hexanes/EtOAc) to afford 6b (2.49 g, 47%) and 21 (2.91 g, 48%) as colorless oils.

Compound 6b, [a]$^{20}$D −40.3 (c 1.01, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.27 (d, $J$ = 8.4 Hz, 2H), 6.84 (d, $J$ = 8.4 Hz, 2H), 5.79 (ddd, $J$ = 17.2, 10.4, 6.8 Hz, 1H), 5.19 (d, $J$ = 17.2 Hz, 1H), 5.07 (d, $J$ = 10.0 Hz, 1H), 4.59 (d, $J$ = 12.0 Hz, 1H), 4.53-4.45 (m, 2H), 4.32 (d, $J$ = 6.0 Hz, 1H), 4.10-4.08 (m, 1H), 3.77 (s, 3H), 3.77-3.75 (m, 1H), 2.18-2.04 (m, 3H), 1.64-1.50 (m, 2H), 1.49 (s, 3H), 1.29 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 159.09, 140.82, 130.39, 129.36, 114.85, 113.64, 111.13, 84.78, 78.51, 77.38, 71.87, 71.27, 55.14, 39.83, 38.20, 33.40, 26.25, 24.43; LRMS (ESI), m/z 371.2 (M + Na)$^+$.

Compound 21, [a]$^{20}$D −34 (c 1.02, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.29 (d, $J$ = 8.4 Hz, 2H), 6.86 (d, $J$ = 8.4 Hz, 2H), 5.79 (ddd, $J$ = 17.2, 10.4, 6.8 Hz, 1H), 5.26-5.16 (m, 3H), 4.61 (d, $J$ = 12.0 Hz, 1H), 4.53-4.45 (m, 2H), 4.25-4.23 (m, 1H), 3.78 (s, 3H), 3.77-3.75 (m, 1H), 2.15-2.04 (m, 6H), 1.64-1.50 (m, 2H), 1.49 (s, 3H), 1.29 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.04, 159.12, 135.74, 130.36, 129.40, 117.46, 113.65, 110.98, 84.47, 78.20, 77.49, 73.40, 71.31, 62.07, 55.15, 37.27, 36.89, 32.67, 26.15, 24.26, 21.12; HRMS (ESI), m/z (M + Na)$^+$ Calcd for C$_{22}$H$_{30}$O$_6$Na: 413.1940, found 413.1944.

Alcohol 6a. To a solution of 21 (273 mg, 0.70 mmol) in THF (4 mL) and H$_2$O (4 mL) was added NaOH (84 mg, 2.10 mmol) at room temperature. The reaction mixture was stirred for 4 h at the same temperature, poured into H$_2$O (8 mL). The aqueous phase was extracted with DCM (3 × 10 mL). The combined organic extracts were dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The residue was purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford 6a (238 mg, 98%) as a colorless oil. [a]$^{20}$D −25.8 (c 1.00, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.29 (d, $J$ = 8.4 Hz, 2H), 6.86 (d, $J$ = 8.4 Hz, 2H), 5.79 (ddd, $J$ = 17.2, 10.4, 6.8 Hz, 1H), 5.26-5.16 (m, 3H), 4.61 (d, $J$ = 12.0 Hz, 1H), 4.53-4.45 (m, 2H), 4.25-4.23 (m, 1H), 3.78 (s, 3H), 3.77-3.75 (m, 1H), 2.15-2.04 (m, 6H), 1.64-1.50 (m, 2H), 1.46 (s, 3H), 1.29 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 159.11, 140.60, 135.74, 130.36, 129.40, 117.46, 113.65, 110.98, 84.47, 78.20, 77.49, 73.40, 71.31, 62.07, 55.15, 37.27, 36.89, 32.67, 26.15, 24.26, 21.12; LRMS (ESI), m/z (M + Na)$^+$.

Compound 22a. To a stirred solution of 6a (174 mg, 0.50 mmol) in anhydrous DCM (10 mL) was added 5 (537 mg, 2.50 mmol) and Grubbs 2nd (26 mg, 0.03 mmol) at room temperature under argon. The reaction mixture was stirred for 24 h at the same temperature, and then concentrated. The residue was purified via silica gel chromatography (1:1 hexanes/EtOAc).
EtOAc) to afford 22a (201 mg, 75%) as a colorless oil. [a]20D −3.1 (c 1, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.28 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.74-5.68 (m, 2H), 5.27 (brs, 1H), 4.82 (brs, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.52-4.45 (m, 2H), 4.23 (d, J = 5.3 Hz, 1H), 4.17 (brs, 1H), 3.79 (s, 3H), 3.77-3.75 (m, 1H), 3.73 (s, 1H), 2.18-2.08 (m, 2H), 1.58-1.54 (m, 1H), 1.49 (s, 3H), 1.39-1.36 (m, 2H), 1.27 (s, 3H). 13C NMR (100 MHz, CDCl3) δ 171.18, 159.10, 154.89, 133.83, 130.36, 129.33, 125.27, 113.65, 111.32, 84.36, 80.12, 78.71, 77.43, 71.31, 70.31, 55.16, 54.77, 52.55, 39.78, 37.67, 33.80, 28.20, 26.27, 24.42; HRMS (ESI), m/z (M + Na)+ Calcd for C28H41O9NNa: 558.2679, found 558.2683.

Compound 22b was obtained from 6b and 5 in a similar manner for the preparation of 22a as a colorless oil in 78% yield. It was used directly in the next step.

Compound 24a. To a stirred solution of 22a (54 mg, 0.10 mmol) in anhydrous methanol (5 mL) was added Pd/C (10% palladium on activated charcoal, 6 mg). The mixture was stirred under an atmosphere of H2 at room temperature for 1 h, filtered, and concentrated. The residue was purified via silica gel chromatography (1:1 hexanes/EtOAc) to afford 24a (46 mg, 85%) as a colorless oil. [a]20D −17.2 (c 1, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.28 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.20 (brs, 1H), 4.62 (d, J = 11.8 Hz, 1H), 4.53-4.48 (m, 2H), 4.33 (brs, 1H), 4.25 (m, 1H), 3.84 (s, 3H), 3.79-3.74 (m, 1H), 3.66 (s, 3H), 3.65-3.63 (brs, 1H), 2.46-1.95 (m, 4H), 1.70-1.52 (m, 3H), 1.51 (s, 3H), 1.50-1.48 (m, 2H), 1.42 (s, 9H), 1.24 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 173.05, 159.08, 155.46, 130.45, 129.29, 113.64, 111.47, 85.14, 79.96, 78.94, 77.44, 71.33, 69.38, 55.16, 52.99, 52.21, 40.55, 38.02, 34.08, 32.86, 29.37, 28.20, 26.32, 24.51; LRMS (ESI), m/z 560.5 (M + Na)+.

Compound 24b. The title compound was obtained from 22b in a similar manner for the preparation of 24a as a colorless oil in 80% yield. [a]20D −11.6 (c 1, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.29 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 5.13 (brs, 1H), 4.63 (d, J = 11.7 Hz, 1H), 4.54 (t, J = 5.2 Hz, 1H), 4.48 (d, J = 11.7 Hz, 1H), 4.30 (brs, 2H), 3.79 (s, 3H), 3.78-3.74 (m, 1H), 3.73 (s, 3H), 3.69-3.64 (brs, 1H), 2.38-1.90 (m, 4H), 1.85-1.55 (m, 3H), 1.51 (s, 3H), 1.50-1.44 (m, 2H), 1.43 (s, 9H), 1.24 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 173.15, 159.09, 155.40, 130.48, 129.29, 113.65, 111.66, 85.14, 79.81, 79.09, 71.40, 70.16, 55.15, 53.16, 52.17, 40.30, 39.10, 33.90, 32.97, 28.82, 28.20, 26.32, 24.60; LRMS (ESI), m/z 560.4 (M + Na)+.

Azide 25a. To a stirred solution of 24a (40 mg, 0.074 mmol) in anhydrous DCM was added triethylamine (103 μL, 0.74 mmol) and methanesulfonyl chloride (29 μL, 0.37 mmol) at 0 °C. The mixture was stirred for 5 min and then quenched with water. The aqueous phase was extracted with DCM. The combined extracts were dried over MgSO4, filtered, and concentrated to give the crude product as a colorless oil.

The above crude product was dissolved in DMF (5 mL), and then NaN3 (24 mg, 0.37 mmol) was added. After being stirred at 45 °C for 3.5 h, the solution was allowed to cool on ice-water bath. Then, the reaction mixture was diluted with Et2O (10 mL) and quenched with water (5 mL). The aqueous phase was extracted with Et2O (3 × 10 mL), and the combined organic phase was washed with water (2 × 5 mL). The organic phase was dried over
MgSO₄, filtered and concentrated. The residue was purified via column chromatography (2:1 hexanes/EtOAc) to give the desired azide compound 25a (32 mg, 77%, for two steps) as a colorless oil. [α]D 𝑛 −6.4 (c 1, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.28 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 5.07 (brs, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.58-4.48 (m, 2H), 4.31 (brs, 1H), 4.23 (d, J = 5.8 Hz, 1H), 3.79 (s, 3H), 3.76-3.60 (m, 4H), 3.28-3.22 (m, 1H), 2.40-1.60 (m, 7H), 1.51 (s, 3H), 1.50-1.46 (m, 2H), 1.43 (s, 9H), 1.30 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.72, 159.15, 155.26, 130.30, 129.35, 113.67, 111.29, 84.64, 79.98, 78.40, 77.37, 71.37, 60.28, 55.16, 52.78, 52.35, 38.31, 37.14, 32.55, 30.04, 29.28, 28.19, 26.21, 24.37; HRMS (ESI), m/z (M + Na)+ Calcd for C₂₈H₄₂O₈N₄Na: 585.2900, found 585.2903.

Azide 25b. The title compound was obtained from 24b in a similar manner for the preparation of 25a as a colorless oil in 80% yield. [α]D 𝑛 −14.5 (c 1, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.28 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 5.08 (brs, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.58-4.48 (m, 2H), 4.31-4.18 (m, 2H), 3.82 (s, 3H), 3.75-3.70 (m, 4H), 3.15-3.12 (m, 1H), 3.11 (s, 3H), 2.18-1.95 (m, 3H), 1.80-1.50 (m, 6H), 1.52 (s, 3H), 1.43 (s, 9H), 1.32 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.71, 159.14, 155.22, 130.30, 129.34, 113.68, 111.45, 84.23, 79.99, 78.66, 77.32, 71.36, 60.75, 55.16, 53.05, 52.34, 38.45, 37.30, 33.47, 30.18, 29.10, 28.19, 26.24, 24.41; LRMS (ESI), m/z 585.4 (M + Na)+.

Alcohol 4a. To a stirred solution of 25a (30 mg, 0.053 mmol) in anhydrous DCM (4 mL) was added DDQ (18 mg, 0.080 mmol) at room temperature. The mixture was stirred for 3 h at the same temperature, and then quenched with saturated NaHCO₃ solution (5 mL). The aqueous phase was extracted with DCM. The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was then purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford 4a (24 mg, 92%) as a colorless oil. [α]D 𝑛 +10.2 (c 1, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 5.08 (brs, 1H), 4.48 (t, J = 6.2 Hz, 1H), 4.32-4.30 (brs, 2H), 3.80 (brs, 1H), 3.74 (s, 3H), 3.33 (brs, 1H), 2.47 (brs, 1H), 2.24-2.20 (m, 1H), 2.01-1.56 (m, 8H), 1.52 (s, 3H), 1.43 (s, 9H), 1.32 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.71, 155.25, 112.23, 85.06, 79.97, 79.24, 70.51, 60.61, 52.80, 52.36, 38.67, 36.95, 30.04, 29.21, 28.20, 26.03, 24.35; LRMS (ESI), m/z 443.2 (M + H)+.

Alcohol 4b. The title compound was obtained from 25b in a similar manner for the preparation of 4a as a colorless oil in 88% yield. It was used directly in the next step.

Compound 26a. To a stirred solution of 4a (20 mg, 0.04 mmol) in anhydrous DCM (5 mL) was added pyridine (36 μL, 0.45 mmol) and triflic anhydride (30 μL, 0.18 mmol) at 0 °C. The mixture was stirred for 5 min and then quenched with water. The aqueous phase was extracted with DCM. The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was then purified via silica gel chromatography (3:2 hexanes/EtOAc) to afford a colorless oil which was subjected to the next step immediately.

To a stirred solution of above oil in anhydrous DMF (4 mL) was added adenine (10 mg, 0.07 mmol), K₂CO₃ (48 mg, 0.35 mmol) and 18-Crown-6 (4 mg, 0.015 mmol) at room temperature. The resulting mixture was heated to 50 °C, and stirred for 5 h. The mixture was allowed to cool on ice-water bath, diluted with Et₂O (10 mL) and quenched with water.
mL). The aqueous phase was extracted with Et$_2$O (3 × 10 mL), and the combined organic phase was washed with water (2 × 5 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated. The residue was purified via column chromatography (2:1 hexanes/EtOAc) to give the compound 26a (15 mg, 75%) as a colorless oil. [α]$^{20}$D +5.2 (c 1, CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.35 (s, 1H), 7.85 (s, 1H), 5.76 (s, 2H), 5.17 (brs, 1H), 5.13 (dd, J = 5.7, 6.9 Hz, 1H), 4.74 (ddd, J = 11.7, 6.6, 5.1 Hz, 1H), 4.56 (t, J = 6.5 Hz, 1H), 4.37 (brs, 1H), 3.79 (s, 3H), 3.42 (brs, 1H), 2.50-2.40 (m, 1H), 2.35-2.30 (m, 1H), 2.02-1.55 (m, 8H), 1.59 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.76, 155.43, 152.61, 149.83, 140.06, 120.44, 114.00, 84.29, 83.26, 79.99, 61.67, 60.45, 52.81, 52.36, 41.16, 37.91, 36.69, 29.99, 29.10, 28.20, 27.42, 25.05. HRMS (ESI) calefd for C$_{25}$H$_{38}$N$_9$O$_6$ (M + H)$^+$: 560.2945, found 560.2949.

Compound 26b. The title compound was obtained from 4b and adenine in a similar manner for the preparation of 4a as a colorless oil in 68% yield. [α]$^{20}$D +0.8 (c 1, CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.34 (s, 1H), 7.84 (s, 1H), 5.84 (s, 2H), 5.22 (d, J = 10.1 Hz), 5.13 (dd, J = 5.8, 6.9 Hz, 1H), 4.74 (ddd, J = 11.8, 6.4, 5.0 Hz, 1H), 4.52 (t, J = 10.1 Hz, 1H), 4.37 (brs, 1H), 3.79 (s, 3H), 3.53 (m, 1H), 2.50-2.30 (3H, m), 2.07-2.03 (m, 1H), 1.94-1.60 (m, 5H), 1.59 (s, 3H), 1.47 (s, 9H), 1.32 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.78, 155.47, 152.62, 149.81, 140.04, 120.43, 114.16, 84.51, 83.56, 79.98, 61.49, 61.16, 53.08, 52.34, 41.70, 38.31, 37.10, 31.06, 29.24, 28.20, 27.36, 25.13. HRMS (ESI) calefd for C$_{25}$H$_{38}$N$_9$O$_6$ (M + H)$^+$: 560.2945, found 560.2944.

Compound 26c. The title compound was obtained from 4b and N-(3-methylbenzyl)-9H-purin-6-amine in a similar manner for the preparation of 4a as a colorless oil in 63% yield. [α]$^{20}$D −2.4 (c 0.5, CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.38 (s, 1H), 7.72 (s, 1H), 7.24-7.15 (m, 1H), 7.09 (d, J = 7.3 Hz, 1H), 6.06 (brs, 1H), 5.13-5.08 (m, 2H), 4.82 (brs, 2H), 4.73-4.67 (m, 1H), 4.54-4.48 (m, 1H), 4.34 (brs, 1H), 3.55-3.48 (m, 1H), 2.50-2.40 (m, 2H), 2.37 (s, 3H), 2.36-2.35 (m, 1H), 1.75-1.60 (m, 5H), 1.52 (s, 3H), 1.43 (s, 9H), 1.29 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.75, 155.22, 154.65, 152.79, 139.34, 138.31, 138.18, 128.50, 128.35, 128.17, 124.68, 120.59, 114.15, 84.53, 83.61, 80.02, 61.49, 61.18, 60.28, 53.09, 52.36, 41.73, 38.34, 37.18, 31.06, 29.29, 28.21, 27.37, 25.14. HRMS (ESI) calefd for C$_{33}$H$_{43}$N$_9$O$_6$Na (M + Na$^+$): 686.3390, found 686.3396.

Compound 2. To a stirred solution of 26a (10 mg, 0.02 mmol) in anhydrous methanol (3 mL) was added triphenylphosphine at room temperature. The mixture was stirred for 4 h at the same temperature, and then trifluoroacetic acid (1.5 mL) was added. The resulting mixture was stirred for additional 2 h, and concentrated. The residue was dissolved in THF (1.5 mL), and then slowly adjusted to pH 12 with LiOH solution (1 M) at 0 °C. The mixture was stirred overnight at room temperature, adjusted to pH 7 with HCl solution (1 M), and concentrated. The residue was purified with preparation TLC (5:3:1 DCM/MeOH/CH$_3$OH) to afford carbocyclic sinefungin 2 (4 mg, 60%) as an amorphous solid. [α]$^{20}$D +3.4 (c 1, H$_2$O); $^1$H NMR (500 MHz, D$_2$O) δ 8.15 (s, 1H), 8.14 (s, 1H), 4.45 (t, J = 7.1 Hz, 1H), 3.96 (t, J = 6.1 Hz, 1H), 3.73 (t, J = 6.0 Hz, 1H), 3.37 (td, J = 12.7, 6.6 Hz, 1H), 2.47-2.43 (m, 1H), 2.15-2.11 (m, 1H), 1.96-1.69 (m, 7H); $^{13}$C NMR (125 MHz, D$_2$O) δ 174.42, 155.52, 152.28, 149.18, 140.89, 118.83, 74.55, 74.48, 59.91, 54.09, 50.00, 39.48, 36.06, 31.80, 28.18, 26.18; HRMS (MALDI) calefd for C$_{16}$H$_{26}$N$_7$O$_4$ (M + H)$^+$: 380.2046, found 380.2049.

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Compound 3a was obtained from 26b in a similar manner for the preparative of 2 as an amorphous solid in 58% yield. [α]20D −5.6 (c 0.35, H2O); 1H NMR (500 MHz, 313 K, D2O) δ 8.10 (s, 1H), 8.06 (s, 1H), 4.68-4.65 (m, 1H), 4.39 (dd, J = 8.0, 6.5 Hz, 1H), 3.89 (t, J = 6.1 Hz, 1H), 3.38-3.34 (m, 1H), 3.11-3.08 (m, 1H), 2.42-2.36 (m, 1H), 2.08-2.02 (m, 1H), 1.88-1.57 (m, 6H), 1.52-1.42 (m, 1H); 13C NMR (125 MHz, 313 K, D2O) δ 155.88, 152.71, 149.60, 141.20, 119.21, 75.02, 74.92, 60.26, 55.65, 49.55, 39.81, 39.28, 32.48, 29.49, 26.65. HRMS (ESI) calcd for C16H26N7O4 (M + H)+: 380.2046, found 380.2051.

Compound 3b was obtained from 26c in a similar manner for the preparation of 2 as an amorphous solid in 75% yield. [α]20D −3.1 (c 0.38, H2O); 1H NMR (500 MHz, 313 K, D2O) δ 8.38 (s, 1H), 8.35 (s, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.39-7.31 (m, 3H), 5.00-4.92 (m, 3H), 4.67 (t, J = 5.9 Hz, 1H), 4.18 (t, J = 5.6 Hz, 1H), 3.72 (t, J = 5.9 Hz, 1H), 3.44-3.36 (m, 1H), 2.71-2.65 (m, 1H), 2.46 (s, 3H), 2.40-2.31 (m, 1H), 2.16-1.75 (m, 7H); 13C NMR (125 MHz, 313 K, D2O) δ 152.70, 140.58, 139.09, 138.79, 128.90, 128.12, 127.66, 124.16, 74.91, 74.78, 60.17, 55.30, 49.65, 39.60, 38.23, 32.35, 29.76, 28.65, 20.54. HRMS (ESI) calcd for C24H33N7O4 (M + H)+: 484.2672, found 484.2666.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


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Figure 1.
Design of new carbocyclic SIN derivatives.
Scheme 1.
Retrosynthesis of carbocyclic SIN and its derivatives.
Scheme 2.
Synthesis of segment 5.
Scheme 3.
Synthesis of segment 6a.
Scheme 4.
Enzymatic kinetic resolution of alcohol 19.
Scheme 5.
Cross coupling and examination of azide substitution
Scheme 6.
Synthesis of azides 4a-b.
Scheme 7.
Synthesis of carbocyclic SIN 2 and 3.