Asymmetric Total Synthesis of C9'-epi-Sinefungin

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The natural nucleoside (+)-sinefungin, structurally similar to cofactor S-adenosyl-L-methionine (SAM), inhibits various SAM-dependent methyltransferases (MTs). Access to sinefungin analogues could serve as the basis for the rational design of small-molecule methyltransferase inhibitors. We developed a route to the unnatural C9' epimer of sinefungin that employed a diastereoselective Overman rearrangement to install the key C6' amino stereocenter. The ability for late stage modification is highlighted, opening an avenue for the discovery of new MTs inhibitors.

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Asymmetric Total Synthesis of C9'-*epi*-Sinefungin

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ABSTRACT: The natural nucleoside (+)-sinefungin, structurally similar to cofactor *S*-adenosyl-*L*-methionine (SAM), inhibits various SAM-dependent methyltransferases (MTs). Access to sinefungin analogues could serve as the basis for the rational design of small-molecule methyltransferase inhibitors. We developed a route to the unnatural C9' epimer of sinefungin that employed a diastereoselective Overman rearrangement to install the key C6' amino stereocenter. The ability for late stage modification is highlighted, opening an avenue for the discovery of new MTs inhibitors.

S-Adenosyl-*L*-methionine (SAM)-dependent methyltransferases (MTs)^{1,2} represent a large class of enzymes which use SAM (1, Figure 1), the second most ubiquitous cofactor,³ to methylate a wide variety of biomolecules, including DNA, proteins and small molecules, yielding *S*-adenosyl-*L*-homocysteine (SAH) as the byproduct. The methyl transfer from SAM to these various substrates plays a fundamental role in many biological processes including embryo development,⁴ epigenetic regulation of gene expression,^{5,6} cellular signaling, and metabolism.⁷ Methyltransferases have also emerged as disease-relevant targets⁸⁻¹⁰ with abnormal expression implicated in neurological disorders,^{10,11} inflammatory diseases and a variety of cancers.¹²⁻¹³

(+)-Sinefungin (2), a naturally occurring nucleoside isolated from broths of *Streptomyces griseolus*¹⁴ and *Streptomyces incarnatus*, presents a structure highly homologous to the structure of SAM, with an amino methylene unit in place of the methyl sulfonium. Sinefungin is known to act as a competitive inhibitor of multiple SAM-dependent methyltransferases^{15,16} and to exhibit antitumor,¹⁷ antiviral,^{18,19} and antiparasitic activity.²⁰ Recently, sinefungin was shown to prevent myofibroblast activity in cell culture and *in vivo*, and to reduce myofibroblast activity in tumor stroma and metastasis burden in lungs in a mouse cancer model.²¹ Sinefungin has also emerged as a privileged scaffold in the development of potent and selective inhibitors of SAM-dependent MTs.^{22,23} Structural modification of sinefungin led to the development of a selective N-alkyl inhibitor (3) of SETD2,²⁴ a tumor-suppressing protein lysine methyltransferase (PKMT) whose aberrant activity is associated with multiple developmental pathologies and cancers.²⁵ The elaboration of a cyclohexyl substituted analog of sinefungin (4) displaying inhibitory properties towards the PKMTs EHMT1 and EHMT2 with good selectivity was also reported.²⁶

Inspired by the promising use of sinefungin and congeners to target SAM-dependent methyltransferases, herein we report the synthesis of C9'-*epi*-sinefungin. This study provides an efficient solution to the formation of the key C6' amine of the sinefungin scaffold. The reported synthesis is modular and amenable to the generation of sinefungin analogs to facilitate further investigation of sinefungin-derived MT inhibitors.

Figure 1. Structures of SAM (1), sinefungin (2) and analogs (3, 4).

The biochemical activity of sinefungin have made it an attractive target for the synthetic community (Figure 2). Since its first synthesis in 1982 by Mock and co-workers,²⁷ existing literature has highlighted the C6' stereocenter as the main synthetic challenge en route to sinefungin. This amine was either accessed in a racemic fashion to give a mixture of C6' epimers most often separable by chromatographic methods,^{28,29,30} or through stereoselective transformations. Such examples include the alkylation of a chiral imide auxiliary by Ghosh et al.³¹ or the diastereose-

Chemical reaction scheme showing the synthesis of compound 10 from compound 9. The scheme includes five intermediate structures with retrosynthetic arrows and labels for the transformations:

- Structure 1 (Top):** A complex molecule featuring a 4,6-O-isopropylidene-2-deoxyribose derivative. The C2' position is substituted with an NHBz group. The C5' position is linked to a side chain containing a nitro group (NO₂) and a hydroxyl group (OH). The C6' position is substituted with a CbzHN group and a BnO group. A blue arrow points to the C6' position, labeled "nitroaldol condensation".
- Structure 2:** A similar molecule to Structure 1, but the C6' position is substituted with a diethyl phosphonate group (EtO₂P=O) and a cyano group (CN). A blue arrow points to the C6' position, labeled "phosphonate alkylation".
- Structure 3:** A molecule where the C6' position is substituted with a benzylidene-protected aldehyde (Ph₂CHO) and a hydroxyl group (OH). The C5' position is linked to a side chain containing a nitro group (NO₂) and a hydroxyl group (OH). The C2' position is substituted with an NHBoc group. A blue arrow points to the C6' position, labeled "nitroaldol condensation".
- Structure 4:** A molecule where the C6' position is substituted with a tert-butyl ester (t-BuO) and an NHTs group. The C5' position is linked to a side chain containing a hydroxyl group (OH) and a methoxy group (OMe). The C2' position is substituted with an NHTs group. A blue arrow points to the C6' position, labeled "d.r. 92:8".
- Structure 5:** A molecule where the C6' position is substituted with a CbzHN group and a BnO group. The C5' position is linked to a side chain containing a hydroxyl group (OH) and a methoxy group (OMe). The C2' position is substituted with an NHTs group. A blue arrow points to the C6' position, labeled "radical addition".
- Structure 6:** A molecule where the C6' position is substituted with a CbzHN group and a BnO group. The C5' position is linked to a side chain containing a hydroxyl group (OH) and a methoxy group (OMe). The C2' position is substituted with an NHTs group. A blue arrow points to the C6' position, labeled "radical addition".
- Structure 7:** A molecule where the C6' position is substituted with a CbzHN group and a BnO group. The C5' position is linked to a side chain containing a hydroxyl group (OH) and a methoxy group (OMe). The C2' position is substituted with an NHTs group. A blue arrow points to the C6' position, labeled "d.r. 96:4".
- Structure 8 (Bottom):** A molecule where the C6' position is substituted with a CbzHN group and a BnO group. The C5' position is linked to a side chain containing a hydroxyl group (OH) and a methoxy group (OMe). The C2' position is substituted with an NHTs group. A blue arrow points to the C6' position, labeled "d.r. 96:4".

Our synthesis of C9'-*epi*-sinefungin (**5**) proceeded through key intermediate trichloroacetamide **7** (Scheme 1). Retrosynthetic analysis suggested that the adenine fragment could be installed by means of a Vorbrüggen glycosylation from triacetate **6**. The amino acid could derive from an aldehyde by a Horner-Wadsworth-Emmons olefination / rhodium-catalyzed asymmetric hydrogenation sequence. This strategy proved successful in our work on the development of high-affinity inhibitors of nicotinamide *N*-methyltransferase (NNMT).³³ Next, we envisioned that the key C6' stereocenter could be introduced by a COP-catalyzed (COP = cobalt oxazoline palladacycle) asymmetric Overman rearrangement of trichloroacetimidate **8** to provide the (*S*)-amine present in the target product.

Trichloroacetamide **7** was prepared in 6 scalable steps from acetonide **9**, commercially available or readily accessible from D-ribose^{4a} (Scheme 2). Triflation of acetonide **9** and subsequent alkylation upon treatment with the lithium acetylide derived from *t*-butyldimethyl(2-propynyloxy)silane yielded silyl protected propargyl alcohol **10** in quantitative yield. Cleavage of the TBS protecting group followed by reduction to the *E*-alkene with LiAlH₄ afforded known allylic alcohol **11**^{10b} in 63% yield over 2 steps. Alcohol **11** was converted to trichloroacetimidate **8** in the presence of trichloroacetonitrile and in excellent yield, giving access to the precursor required for the envisioned Overman rearrangement.

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Treatment of *E*-allylic trichloroacetimidate **8** in CH₂Cl₂ with Overman's [(*R*)-COP-Cl]₂³⁵ effected the desired [3,3]-sigmatropic rearrangement, delivering allylic trichloroacetamide **7** in 85% yield with a 14:1 *dr*. The minor diastereomer could be separated under standard silica gel chromatography conditions. While the extended reaction time proved necessary to reach the observed conversion, no erosion of *dr* was observed over the course of the reaction. The [(*R*)-COP-Cl]₂ catalyst was prepared in one step from [(*R*)-COP-OAc]₂ according to a procedure from Overman and co-workers.³⁶ Use of the related (*R*)-COP-hfacac catalyst (hfacac = hexafluoroacetylacetonate) did not prove beneficial and a slower conversion was observed with a comparable *dr*. An increased reaction temperature (50 °C) in 1,2-DCE did not improve the outcome, with similar yield and *dr* noted. Derivatization of compound **7** to the more crystalline carbamate **12** allowed for a small-molecule X-ray crystal structure to be obtained, confirming the (*R*) configuration of the C6' stereocenter. Interestingly, when [(*S*)-COP-Cl]₂ was used to catalyze the rearrangement, the C6' (*S*)-trichloroacetamide **13** was obtained in high diastereoselectivity to give the (*S*)-amide epimer in 29:1 *dr*, thus demonstrating that the stereochemical outcome of this rearrangement is largely under catalyst control. This result also suggests some degree of substrate control, but the catalyst choice largely overcomes this inherent substrate bias.

With this diastereoselective rearrangement providing an efficient solution to the installation of the stereocenter at C6', we turned our attention to the amino acid moiety (Scheme 3). Terminal alkene **7** was converted in good yield to aldehyde **14** under Wacker-Tsuji oxidation conditions using *t*-butyl nitrile as an organic redox cocatalyst.³⁷ It is worth noting that aldehyde **14** could represent a powerful handle to enable the synthesis of various analogs aimed at replacing the amino acid moiety. A Horner-Wadsworth-Emmons olefination with Cbz- α -phosphonoglycine trimethyl ester yielded enamide **15**. The C9' amino stereocenter was set using a rhodium-catalyzed asymmetric hydrogenation with (*S,S*)-Ph-BPE-Rh (BF₄ salt), a Burk-type 1,2-bis(phospholano)ethane (BPE) ligand,^{38,39} providing (*R*)-carbamate **16** as a single diastereomer and with no observed side reactions.

Scheme 3. Elaboration of the amino acid moiety. TMG = 1,1,3,3-tetramethylguanidine; BPE = bis(phospholano)ethane; nbd = norbornadiene.

With the C5'–C10' amino acid chain elaborated, the installation of the adenine moiety and subsequent global deprotection were undertaken (Scheme 4). To this end, acetone **16** was converted in two steps into triacetate **6**, as a 2:1 mixture of anomers at C1' (by ¹H NMR). Vorbrüggen glycosylation promoted by

2,6-di-*tert*-butyl-4-methylpyridinium triflate⁴⁰ provided nucleoside **17** in 79% yield and as a single diastereomer.

Scheme 4. Installation of the nucleobase and completion of the total synthesis. BSA = *N,O*-bis(trimethylsilyl)acetamide; DTBMP-OTf = 2,6-di-*tert*-butyl-4-methylpyridinium triflate.

With the entire carbon framework assembled, we finally examined the removal of the various protecting groups. We were particularly interested in implementing conditions which would selectively deprotect the C6' amine over the C9' one, therefore opening a practical avenue for the generation of sinefungin analogs at this position, similar to the work of the Luo group and their report on *N*-propyl sinefungin.²⁴ Treatment of trichloroacetamide **17** with aqueous potassium hydroxide delivered amine **18** in good yield and left the C9'-Cbz protected amine intact. Finally, cleavage of the remaining Cbz and Bz protecting groups by hydrogenolysis and subsequent addition of methanolic ammonia completed the synthesis of C9'-*epi*-sinefungin in a total of 15 steps with an overall yield of 7.6%.

Scheme 5. Functionalization of the C6' amine.

Finally, to illustrate the utility of this deprotection sequence, we subjected amine **18** to reductive amination conditions with 3-formyl-benzamide (Scheme 5). Benzamide **19**, directly inspired

by the structure of our high-affinity alkynyl inhibitor of nicotinamide *N*-methyltransferase³⁴ and related inhibitors,³⁴ was obtained in 81% yield, highlighting our ability to functionalize the C6' amine at a late stage.

In conclusion, an efficient asymmetric Overman rearrangement was developed to install the C6' amine present in the sinefungin scaffold, providing a tractable solution to this key challenge and avoiding multiple functional group interconversions as well as separation of diastereomers. The study was concluded by the synthesis of C9'-*epi*-sinefungin, featuring an asymmetric rhodium-catalyzed hydrogenation to form the amino acid stereo-center. The synthesis was designed to be modular and amenable to the elaboration of sinefungin analogs for further biological evaluation, as demonstrated by an illustrative functionalization of the C6' amine.

ASSOCIATED CONTENT

Supporting Information

Synthetic schemes, detailed experimental protocols, NMR spectra (PDF)

Small-molecule X-ray crystallography data for **12** (CIF)

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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

Notes

The authors declare no competing financial interests.

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Asymmetric Total Synthesis of C9'-*epi*-Sinefungin

Supporting Information: Experimental Protocols, Compound Characterization and NMR Spectra

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1 List of Abbreviations

Ac	acetyl
BPE	bis(phospholano)ethane
BSA	<i>N,O</i> -bis(trimethylsilyl)acetamide
Cbz	benzyloxycarbonyl
COP	cobalt oxazoline palladacycle
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DTBMP-OTf	2,6-di- <i>tert</i> -butyl-4-methylpyridinium triflate
equiv.	equivalent
M.S.	molecular sieves
MTBE	methyl <i>tert</i> -butyl ether
nbd	norbornadiene
Pyr	pyridine
rbf	round bottom flask
RT	room temperature
TCA	trichloroacetyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMG	1,1,3,3-tetramethylguanidine

2 General Procedures

Air and/or moisture sensitive reactions were performed under an atmosphere of nitrogen in a flame dried apparatus. Reactions were monitored by thin layer chromatography (TLC) using Merck 0.25 mm silica gel 60 covered glass backed plates F₂₅₄. TLC plates were visualized under UV light and/or exposure to an acidic solution of *p*-anisaldehyde, an aqueous solution of ceric ammonium molybdate or an aqueous solution of potassium permanganate, followed by heating on a hot plate. Reactions were also monitored by analytical LCMS using an Agilent 6120 Quadrupole LC/MS and an Agilent 1260 Infinity II LC system equipped with (1) a Phenomenex Kinetex F5 column (2.6 μm , 100 Å, 100 x 3.0 mm), (2) a Phenomenex Kinetex Biphenyl column (2.6 μm , 100 Å, 100 x 3.0 mm) or (3) a Thermo Scientific Accucore aQ C18 column (2.6 μm , 100 x 2.1 mm). Two methods were used: *method A* for columns 1, 2, 3: solvent A: 0.1% (v/v) formic acid in water, solvent B: 0.1% (v/v) formic acid in CH₃CN, gradient elution: 5% B for 1 min then 5% $\rightarrow$ 95% B over 8 min followed by 95% B for 2 min and reequilibration for 2 min, flow rate: 0.55 mL/min (1), 0.95 mL/min (2), 0.70 mL/min (3), column temperature: 50 °C, UV detection: 254 nm, and *method B* for columns 2, 3: solvent A: 0.1% (v/v) formic acid in water, solvent B: 0.1% (v/v) formic acid in CH₃CN, gradient elution: 0% B for 1 min, 0% $\rightarrow$ 40% B over 8 min, 40% $\rightarrow$ 95% B over 2 min followed by 95% B for 2 min and reequilibration for 2 min, flow rate: 0.95 mL/min (2), 0.70 mL/min (3), column temperature: 50 °C, UV detection: 254 nm. Flash column chromatography was performed using an Interchim PuriFlash 215 system with prepacked silica gel cartridges (Büchi FlashPure (35 to 45 μm), Büchi Reveleris HP (16 to 24 μm), Teledyne Isco RediSep Rf (35 to 70 μm) and Teledyne Isco RediSep Rf Gold (20 to 40 μm)). Preparative HPLC was performed at room temperature on an Agilent 1260 Infinity II Preparative LC system equipped with a Kromasil C18 column (10 μm , 100 Å, 21.2 x 250 mm) using solvents A (0.1% (v/v) TFA in water) and B (0.1% (v/v) TFA in acetonitrile) at flow rate 10 mL/min delivering gradients specified in individual experimental protocols and with UV detection at 254 nm.

3 Materials

Commercial reagents and solvents were used as received unless specified otherwise. The molarity of *n*-butyllithium solutions was determined by titration using *N*-benzylbenzamide (average of three determinations).¹

4 Instrumentation

¹H NMR spectra were recorded on Varian INOVA-600 or Varian INOVA-500 spectrometers at ambient temperature. Chemical shifts are reported in ppm (δ scale) relative to residual undeuterated solvent (CDCl₃: 7.26 (CHCl₃), CD₃CN: 1.94 (CD₂H₂CN), D₂O: 4.79 (HOD)). Data are reported as follows: chemical shift (δ ppm) (integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent, or a combination of these), coupling constant(s) *J* (Hz)). When a mixture of deuterated solvents is employed, the residual undeuterated solvent used for reference is indicated in bold. ¹³C NMR spectra were recorded on Varian INOVA-500, Varian MERCURY-400 and JEOL J400 spectrometers at ambient temperature. Data are reported as follows: chemical shifts (δ scale, ppm) relative to the carbon resonances of the solvent (CDCl₃: 77.16, CD₃CN: 1.32). When a mixture of deuterated solvents is employed,

¹ Burchat, A. F.; Chong, J.; Nielsen, N. *J. Organomet. Chem.* **1997**, 542, 281–283.

the solvent used for reference is indicated in bold. Infrared (FTIR) spectra were recorded on a Bruker Alpha FT-IR spectrophotometer. Data is reported in frequency of absorption (cm^{-1}). The IR spectrum of each compound (solid or liquid) was acquired directly on a thin layer at ambient temperature. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II or on an Agilent 6220 LC-TOF equipped with an electrospray ionization (ESI+). The structure of carbamate **12** was obtained with the assistance of Dr. Shao-Liang Zheng at the X-ray diffraction facility of the Department of Chemistry & Chemical Biology at Harvard University. The analytical characterization of C9'-*epi*-sinefungin was obtained with the assistance of Jennifer X. Wang at the small molecule mass spectrometry facility at Harvard University.

5 Synthetic Procedures & Compound Characterization

5.1 Trichloroacetimidate **8**

To a solution of known allylic alcohol **11**² (7.08 g, 29.9 mmol) in CH_2Cl_2 (200 mL) at $0\text{ }^\circ\text{C}$, were added DBU (0.87 mL, 5.8 mmol, 20 mol %) and trichloroacetonitrile (4.31 mL, 43.5 mmol, 1.5 equiv.) sequentially. The resulting mixture was allowed to warm up to RT over 30 min and was stirred at RT for an additional 1.5 h. Volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexanes/EtOAc, $0 \rightarrow 30\%$) to afford trichloroacetimidate **8** (10.3 g, 26.5 mmol, 91%) as a colorless oil.

$R_f = 0.34$ (hexanes/EtOAc, 80:20);

FTIR (thin film), cm^{-1} : ν_{max} 3343, 2989, 2937, 1662, 1442, 1381, 1373, 1286, 1209, 1088, 1058;

^1H NMR (600 MHz, CDCl_3): δ 8.29 (s, 1H), 5.91–5.85 (m, 1H), 5.83–5.76 (m, 1H), 4.95 (s, 1H), 4.81–4.74 (m, 2H), 4.61 (d, $J = 5.9$ Hz, 1H), 4.56 (dd, $J = 5.9, 1.1$ Hz, 1H), 4.25 (app td, $J = 7.8, 1.1$ Hz, 1H), 3.33 (s, 3H), 2.45 (dddd, $J = 14.4, 7.8, 6.4, 1.3$ Hz, 1H), 2.37–2.30 (m, 1H), 1.47 (s, 3H), 1.31 (s, 3H);

^{13}C NMR (126 MHz, CDCl_3): δ 162.4, 132.1, 126.2, 112.3, 109.6, 91.5, 86.2, 85.5, 83.5, 69.5, 55.0, 38.1, 26.5, 25.1;

HRMS (ESI⁺): calcd. for $[\text{C}_{14}\text{H}_{20}\text{Cl}_3\text{N}_1\text{O}_5\text{Na}]^+$ 410.0299, meas. 410.0292, Δ 1.7 ppm.

² K. Ghosh, A.; Wang, Y. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3597–3601.

5.2 (*R*)-Trichloroacetamide **7**

To a vial charged with trichloroacetimidate **8** (1.22 g, 3.14 mmol) and $[(R)\text{-COP-Cl}]_2$ ³ (115 mg, 78 μmol , 2.5 mol %) was added CH_2Cl_2 (9.2 mL). The vial headspace was purged with argon and the vessel was sealed. The resulting mixture was covered with foil and was stirred at 38°C for 50 h. Volatiles were removed *in vacuo*. ^1H NMR analysis of the crude mixture revealed a 14:1 *dr*. Crude material was purified by silica gel chromatography (hexanes/MTBE, 0 $\rightarrow$ 45%) to afford (*R*)-trichloroacetamide **7** (1.04 g, 2.68 mmol, 85%) as a single diastereomer, as a colorless oil. Trichloroacetamide **7** could be converted to carbamate **12** (*i.* 1,4-dioxane / 1 M NaOH aq. (1:1), rt; *ii.* ClCO_2Me (1.2 equiv.), 1,4-dioxane / sat. NaHCO_3 aq. (1:1), 0°C) which crystallized when stored as a neat oil at -20°C overnight. A small molecule X-ray structure was obtained, confirming its absolute configuration as shown in subsection 7.1.

$R_f = 0.27$ (hexanes/EtOAc, 80:20);

FTIR (thin film), cm^{-1} : ν_{max} 3298, 2980, 2938, 1711, 1691, 1531, 1381, 1372, 1274, 1260, 1236, 1209, 1191, 1160, 1105, 1091, 1058;

^1H NMR (500 MHz, CDCl_3): δ 7.65 (br d, $J = 7.9$ Hz, 1H), 5.83 (ddd, $J = 17.3, 10.3, 5.0$ Hz, 1H), 5.29–5.25 (m, 2H), 5.00 (s, 1H), 4.70–4.63 (m, 1H), 4.61 (d, $J = 6.0$ Hz, 1H), 4.56 (ddd, $J = 6.0, 1.6, 0.5$ Hz, 1H), 4.36 (ddd, $J = 11.4, 3.2, 1.6$ Hz, 1H), 3.40 (s, 3H), 2.05 (ddd, $J = 14.4, 11.4, 4.5$ Hz, 1H), 1.87 (dddd, $J = 14.4, 5.6, 3.2, 0.5$ Hz, 1H), 1.48 (s, 3H), 1.32 (s, 3H);

^{13}C NMR (126 MHz, CDCl_3): δ 161.4, 135.4, 116.5, 112.8, 110.3, 92.8, 84.9, 84.5, 83.9, 55.8, 51.6, 37.9, 26.6, 25.0;

HRMS (ESI+): calcd. for $[\text{C}_{14}\text{H}_{20}\text{Cl}_3\text{N}_1\text{O}_5\text{Na}]^+$ 410.0299, meas. 410.0299, Δ 0.2 ppm.

³ $[(R)\text{-COP-Cl}]_2$ (CAS: 612065-00-6) was prepared from $[(R)\text{-COP-OAc}]_2$ (CAS: 849592-74-1), commercially available from Millipore Sigma (catalog #: 661708), following the protocol outlined in *Org. Synth.* **2007**, 84, 148–155.

5.3 (*S*)-Trichloroacetamide **13**

To a vial charged with trichloroacetimidate **8** (101 mg, 0.260 mmol) and $[(S)\text{-COP-Cl}]_2^4$ (19 mg, 13 μmol , 5 mol %) was added CH_2Cl_2 (0.75 mL). The vial headspace was purged with argon and the vessel was sealed. The resulting mixture was covered with foil and was stirred at 38 $^\circ\text{C}$ for 48 h. Volatiles were removed *in vacuo*. ^1H NMR analysis of the crude mixture revealed a 29:1 *dr*. Crude material was purified by silica gel chromatography (hexanes/MTBE, 0 $\rightarrow$ 45%) to afford (*S*)-trichloroacetamide **13** (89 mg, 0.23 mmol, 88%) as a single diastereomer, as a colorless oil.

$R_f = 0.27$ (hexanes/EtOAc, 80:20);

FTIR (thin film), cm^{-1} : ν_{max} 3335, 2989, 2938, 1698, 1517, 1382, 1210, 1107, 1093, 1062;

^1H NMR (500 MHz, CDCl_3): δ 6.97 (br d, $J = 7.4$ Hz, 1H), 5.81 (ddd, $J = 17.2, 10.4, 6.5$ Hz, 1H), 5.34 (app dt, $J = 17.2, 1.0$ Hz, 1H), 5.27 (app dt, $J = 10.4, 1.0$ Hz, 1H), 4.98 (s, 1H), 4.62 (d, $J = 5.9$ Hz, 1H), 4.59–4.51 (m, 2H), 4.25 (ddd, $J = 10.7, 4.2, 1.3$ Hz, 1H), 3.43 (s, 3H), 1.99 (ddd, $J = 13.8, 10.7, 6.8$ Hz, 1H), 1.83 (ddd, $J = 13.8, 8.1, 4.2$ Hz, 1H), 1.48 (s, 3H), 1.31 (s, 3H);

^{13}C NMR (126 MHz, CDCl_3): δ 161.1, 135.5, 117.6, 112.7, 110.1, 92.8, 85.3, 84.5, 84.3, 55.7, 52.4, 39.4, 26.6, 25.1;

HRMS (ESI $^+$): calcd. for $[\text{C}_{14}\text{H}_{20}\text{Cl}_3\text{N}_1\text{O}_5\text{Na}]^+$ 410.0299, meas. 410.0304, Δ 1.2 ppm.

⁴ $[(S)\text{-COP-Cl}]_2$ (CAS: 612065-01-7) was prepared from $[(S)\text{-COP-OAc}]_2$ (CAS: 222400-03-5), commercially available from Millipore Sigma (catalog #: 661716), following the protocol outlined in *Org. Synth.* **2007**, 84, 148–155.

5.4 Aldehyde 14

To an oversized flask (250 mL rbf, in order to increase the surface area of exposed solvent) charged with $\text{Pd(PhCN)}_2\text{Cl}_2$ (99 mg, 0.257 mmol, 10 mol %), *t*-BuOH (36 mL) and *t*-BuONO (122 μL , 1.03 mmol, 40 mol %) was added a solution of alkene **7** (1.00 g, 2.57 mmol) in *t*-BuOH (4 mL). The flask headspace was purged with O_2 and the reaction mixture was stirred at RT under an atmosphere of O_2 (balloon) for 40 h. Volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (hexane/EtOAc, 0 $\rightarrow$ 100%) to yield aldehyde **14** (760 mg, 1.88 mmol, 73%) as a colorless oil, which was best used immediately in the subsequent reaction.⁵

$R_f = 0.31$ (cyclohexane/EtOAc, 50:50);

FTIR (thin film), cm^{-1} : ν_{max} 3325, 2988, 2937, 2835, 2734, 1694, 1518, 1382, 1373, 1210, 1160, 1092, 1059;

^1H NMR (500 MHz, CDCl_3): δ 9.79 (s, 1H), 7.60 (d, $J = 8.2$ Hz, 1H), 4.97 (s, 1H), 4.60 (d, $J = 5.9$ Hz, 1H), 4.53 (dd, $J = 6.0, 1.2$ Hz, 1H), 4.45 (ddt, $J = 13.4, 8.1, 5.3$ Hz, 1H), 4.27 (ddd, $J = 11.5, 3.4, 1.2$ Hz, 1H), 3.39 (s, 3H), 3.00 (ddd, $J = 18.3, 5.2, 1.3$ Hz, 1H), 2.85 (dd, $J = 18.3, 6.1$ Hz, 1H), 2.08 (ddd, $J = 14.5, 8.1, 3.4$ Hz, 1H), 1.89 (ddd, $J = 14.5, 11.6, 4.4$ Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H);

^{13}C NMR (126 MHz, CDCl_3): δ 200.1, 161.7, 112.9, 110.5, 85.1, 84.5, 83.9, 55.8, 46.7, 45.5, 37.7, 28.9, 26.6, 25.1;

HRMS (ESI⁺): calcd. for $[\text{C}_{14}\text{H}_{20}\text{Cl}_3\text{N}_1\text{O}_6\text{Na}]^+$ 426.0248, meas. 426.0240, Δ 2.0 ppm.

⁵ While in this protocol aldehyde **14** was purified by silica gel chromatography, it was found that subsection of the crude residue to the Horner-Wadsworth-Emmons olefination conditions reliably generated enamide **15** in 74% yield over two steps (oxidation/olefination) from 0.7 mmol of alkene **7**.

5.5 Enamide 15

To a stirred suspension of (±)-Cbz- α -phosphonoglycine trimethyl ester⁶ (747 mg, 2.25 mmol, 1.2 equiv.) in THF (10.0 mL) at $-78\text{ }^\circ\text{C}$, was added 1,1,3,3-tetramethylguanidine (0.26 mL, 2.1 mmol, 1.1 equiv.). The resulting mixture was stirred at $-78\text{ }^\circ\text{C}$ for 10 min, before the slow addition of a solution of aldehyde **14** (760 mg, 1.88 mmol) in THF (5.0 mL) over 3 min. The reaction mixture was stirred at $-78\text{ }^\circ\text{C}$ for 30 min and then placed in an ice/water bath and stirred for 10 min. The reaction was quenched by the addition of a 10% AcOH aq. solution (5 mL) and water (20 mL) was added. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 ($3 \times 40\text{ mL}$). The combined organic extracts were washed with water (25 mL) and brine (25 mL), passed through a phase separator (Biotage Isolute) and concentrated. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, $0 \rightarrow 75\%$) to afford *Z* enamide **15** (945 mg, 1.55 mmol, 83%) as a clear viscous oil.

$R_f = 0.42$ (hexanes/EtOAc, 50:50);

FTIR (thin film), cm^{-1} : ν_{max} 3315, 2952, 1699, 1514, 1438, 1382, 1374, 1234, 1159, 1089, 1055;

^1H NMR (500 MHz, CDCl_3): δ 8.05 (br s, 1H), 7.41–7.31 (m, 5H), 6.53 (br s, 1H), 6.52 (dd, $J = 10.0, 5.8$ Hz, 1H), 5.14 (s, 2H), 4.97 (s, 1H), 4.61 (s, 2H), 4.28 (dd, $J = 7.6, 7.1$ Hz, 1H), 4.26–4.19 (m, 1H), 3.76 (s, 3H), 3.36 (s, 3H), 2.65 (dt, $J = 15.0, 9.9$ Hz, 1H), 2.54 (dt, $J = 15.0, 5.9$ Hz, 1H), 1.86 (dd, $J = 8.1, 6.0$ Hz, 2H), 1.47 (s, 3H), 1.31 (s, 3H);

^{13}C NMR (151 MHz, CDCl_3): δ 164.6, 162.1, 154.2, 135.7, 130.9, 128.7, 128.5, 128.4, 112.6, 110.1, 92.8, 85.3, 84.4, 83.9, 67.8, 55.4, 52.8, 48.7, 39.9, 33.0, 26.5, 25.0;

HRMS (ESI⁺): calcd. for $[\text{C}_{25}\text{H}_{31}\text{Cl}_3\text{N}_2\text{O}_9\text{Na}]^+$ 631.0987, meas. 631.0997, Δ 1.6 ppm.

⁶ (±)-Cbz- α -phosphonoglycine trimethyl ester, CAS: 88568-95-0, was purchased from Chem-Impex International, Inc. (catalog #: 14125).

5.6 Protected Amino Acid 16

In a glove box, $[\text{Rh}(\text{nbd})_2]\text{BF}_4$ ⁷ (24 mg, 0.063 mmol, 19 mol %) and $(S,S)\text{-PhBPE}$ ⁸ (34 mg, 0.067 mmol, 20 mol %) were added to a vial containing a stir bar. The vial was capped with a septum, removed from the glovebox and placed under an atmosphere of nitrogen. MeOH (4 mL) was added and the red/orange solution was stirred for 15 min before being transferred to a pressure tube⁹ equipped with a side arm through which hydrogen was flowing freely. A solution of enamide **15** (203 mg, 0.333 mmol) in MeOH (4 mL) was added through the side arm and the side arm was sealed. The vessel was pressurized to 4 bar of hydrogen and the pressure was released to purge the vessel (3 ×). After the third purge, a hydrogen pressure of 8 bar was applied and the reaction mixture was stirred vigorously at RT for 15 min, before releasing the pressure. Volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 0 → 70%) to yield protected amino acid **16** (143 mg, 0.234 mmol, 70%) as an amorphous white solid. ¹H NMR analysis indicated the presence of only one diastereomer.

$R_f = 0.39$ (cyclohexane/EtOAc, 50:50);

FTIR (thin film), cm^{-1} : ν_{max} 3325, 2937, 1694, 1522, 1454, 1382, 1238, 1211, 1161, 1090, 1057;

¹H NMR (600 MHz, CDCl_3): δ 7.38–7.28 (m, 5H), 7.25 (br d, $J = 8.4$ Hz, 1H), 5.49 (br d, $J = 8.2$ Hz, 1H), 5.09 (s, 2H), 4.95 (s, 1H), 4.58 (d, $J = 5.9$ Hz, 1H), 4.52 (dd, $J = 5.9, 1.3$ Hz, 1H), 4.43–4.35 (m, 1H), 4.26 (ddd, $J = 11.2, 3.9, 1.3$ Hz, 1H), 4.11–4.04 (m, 1H), 3.72 (s, 3H), 3.34 (s, 3H), 1.98–1.89 (m, 1H), 1.84 (ddd, $J = 15.0, 10.9, 3.9$ Hz, 1H), 1.80–1.67 (m, 3H), 1.67–1.59 (m, 1H), 1.46 (s, 3H), 1.29 (s, 3H);

¹³C NMR (151 MHz, CDCl_3): δ 172.5, 161.8, 156.1, 136.2, 128.6, 128.3, 128.2, 112.8, 110.2, 92.9, 85.1, 84.5, 83.6, 67.2, 55.7, 53.8, 52.7, 49.6, 38.6, 30.0, 29.4, 26.6, 25.1;

HRMS (ESI⁺): calcd. for $[\text{C}_{25}\text{H}_{34}\text{Cl}_3\text{N}_2\text{O}_9]^+$ 611.1324, meas. 611.1323, Δ 0.3 ppm.

⁷ $[\text{Rh}(\text{nbd})_2]\text{BF}_4$, CAS: 36620-11-8, was obtained from Strem Chemicals, Inc. (catalog : 45-0230).

⁸ $(S,S)\text{-PhBPE}$ ligand, CAS: 824395-67-7, was obtained from Strem Chemicals, Inc. (catalog : 15-0474).

⁹ The pressure vessel, 60 mL, was obtained from Ace Glass (catalog : 8649-115).

5.7 Triacetate **6**

To a vial charged with acetone **16** (145 mg, 0.237 mmol) was added a 4:1 mixture of acetic acid and water (10 mL) at RT. The resulting mixture was stirred at 80 °C for 18 h. Volatiles were removed *in vacuo* and the residue was dried by azeotropic distillation with benzene (2 × 1.5 mL). Crude material was used directly in the next step without further purification.

To a vial charged with the crude triol was added a mixture of pyridine (1.53 mL, 19.0 mmol, 80 equiv.) and Ac₂O (0.90 mL, 9.5 mmol, 40 equiv.). The resulting mixture was stirred at RT for 1 h and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (2,2,4-trimethylpentane/EtOAc, 2 → 70%) to afford triacetate **6** (145 mg, 0.212 mmol, 89% over 2 steps) as a 2:1 mixture of diastereomers, as a white amorphous solid.

R_f = 0.36/0.45 (hexanes/EtOAc, 40:60);

FTIR (thin film), cm⁻¹: ν_{max} 3336, 2955, 1743, 1703, 1522, 1454, 1436, 1373, 1217, 1112, 1047, 1011;

¹H NMR (600 MHz, CDCl₃): δ 7.47 (br d, *J* = 8.8 Hz, 3H), 7.39–7.29 (m, 15H), 6.37 (d, *J* = 4.6 Hz, 1H), 6.18 (s, 2H), 5.49–5.41 (m, 3H), 5.29 (dd, *J* = 4.7, 0.8 Hz, 2H), 5.19 (dd, *J* = 6.9, 4.6 Hz, 1H), 5.12 (dd, *J* = 7.6, 4.7 Hz, 2H), 5.11–5.09 (m, 6H), 4.95 (dd, *J* = 6.9, 4.0 Hz, 1H), 4.40–4.31 (m, 6H), 4.17–4.11 (m, 2H), 4.11–4.06 (m, 1H), 3.73 (s, 6H), 3.73 (s, 3H), 2.13 (s, 6H), 2.10 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 6H), 2.05 (s, 6H), 1.98–1.90 (m, 3H), 1.82 (ddd, *J* = 14.9, 11.4, 3.6 Hz, 3H), 1.79–1.67 (m, 9H), 1.66–1.60 (m, 3H);

¹³C NMR (151 MHz, CDCl₃): δ 172.4, 170.3, 169.8, 169.5, 169.4, 169.4, 168.8, 161.8, 161.7, 156.1, 156.1, 136.2, 128.6, 128.6, 128.3, 128.2, 128.1, 98.0, 93.7, 92.8, 92.6, 80.5, 78.1, 77.2, 74.0, 73.7, 72.8, 69.6, 67.1, 53.8, 52.6, 49.5, 49.3, 37.7, 36.8, 29.6, 29.5, 29.3, 21.0, 21.0, 20.6, 20.5, 20.5, 20.3;

HRMS (ESI+): calcd. for [C₂₇H₃₃Cl₃N₂O₁₂K]⁺ 721.0731, meas. 721.0742, Δ 1.5 ppm.

5.8 Nucleoside 17

To a suspension of N⁶-benzoyladenine (168 mg, 0.702 mmol, 4.0 equiv.) in propionitrile (dried over 4 Å M.S., 4.0 mL) at RT, was added *N,O*-bis(trimethylsilyl)acetamide (0.22 mL, 0.88 mmol, 5.0 equiv.). The resulting mixture was stirred at 80 °C for 10 min, upon which complete solubilization of N⁶-benzoyladenine was observed. To a separate flask charged with triacetate **6** (dried by azeotropic distillation with benzene (4 ×), 120 mg, 0.175 mmol) and 2,6-di-*tert*-butyl-4-methylpyridinium triflate (31 mg, 88 μmol, 50 mol %) was added propionitrile (4.0 mL). The resulting mixture was stirred at RT for 5 min, until a clear solution was obtained, and the solution of silylated N⁶-benzoyladenine was added. The reaction mixture was stirred at 95 °C for 3 h, cooled to RT and the volatiles were removed *in vacuo*. Crude material was purified by silica gel chromatography (PhMe/MeCN, 0 → 100%) to afford nucleoside **17** (119 mg, 0.138 mmol, 79%) as a white amorphous solid.

R_f = 0.35 (EtOAc);

FTIR (thin film), cm⁻¹: ν_{max} 3315, 2956, 1747, 1702, 1611, 1583, 1518, 1455, 1329, 1244, 1221, 1097, 1048;

¹H NMR (600 MHz, CDCl₃): δ 8.77 (s, 1H), 8.11 (s, 1H), 8.06–8.02 (m, 2H), 7.64–7.59 (m, 1H), 7.55–7.51 (m, 2H), 7.38–7.28 (m, 4H), 7.11 (br d, *J* = 8.8 Hz, 1H), 6.13–6.09 (m, 2H), 5.56 (app t, *J* = 4.7 Hz, 1H), 5.46 (br d, *J* = 8.3 Hz, 1H), 5.10 (d, *J* = 12.9 Hz, 1H), 5.09 (d, *J* = 12.9 Hz, 1H), 4.40–4.33 (m, 2H), 4.14–4.07 (m, 1H), 3.72 (s, 3H), 2.32 (ddd, *J* = 14.6, 11.1, 3.4 Hz, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.11–2.05 (m, 1H), 1.95–1.86 (m, 1H), 1.77–1.62 (m, 3H);

¹³C NMR (126 MHz, CDCl₃): δ 172.5, 169.9, 169.6, 164.8, 161.8, 156.2, 153.0, 151.6, 150.0, 142.5, 136.2, 133.5, 133.0, 129.0, 128.7, 128.4, 128.3, 128.1, 124.1, 92.7, 87.7, 80.0, 74.0, 72.5, 67.3, 53.8, 52.7, 49.4, 36.9, 29.8, 29.6, 20.7, 20.5;

HRMS (ESI⁺): calcd. for [C₃₇H₃₉Cl₃N₇O₁₁]⁺ 862.1768, meas. 862.1753, Δ 1.7 ppm.

5.9 Amine 18

To a vial charged with nucleoside **17** (167 mg, 0.193 mmol) and cooled to 0 °C, was added a 2.0 M aq. KOH solution (5.32 mL, 10.6 mmol, 55 equiv.) precooled to 0 °C. The resulting mixture was stirred at 0 °C for 5 h and TFA (0.60 mL) was added dropwise until pH 7 was reached, at which point a white precipitate appeared. MeCN (1.5 mL) was added and the resulting clear solution was directly purified by preparative HPLC (gradient elution: 5% → 45% B over 30 min) to afford amine **18** (108 mg, 0.147 mmol, 76%) as the TFA salt.

^1H NMR (500 MHz, CD_3CN (350 μL)/ D_2O (350 μL)): δ 8.75 (s, 1H), 8.56 (s, 1H), 8.04–7.99 (m, 2H), 7.71–7.65 (m, 1H), 7.59–7.53 (m, 2H), 7.35–7.18 (m, 5H), 6.05 (d, J = 4.2 Hz, 1H), 5.04–4.96 (m, 2H), 4.71 (dd, J = 5.4, 4.2 Hz, 1H), 4.24 (dd, J = 5.7, 5.4 Hz, 1H), 4.17 (ddd, J = 9.7, 5.7, 3.0 Hz, 1H), 4.10–4.03 (m, 1H), 3.44–3.34 (m, 1H), 2.15 (ddd, J = 14.5, 9.7, 4.4 Hz, 1H), 2.09–2.01 (m, 1H), 1.90–1.79 (m, 1H), 1.78–1.63 (m, 3H);

^{13}C NMR (126 MHz, CD_3CN (350 μL)/ D_2O (350 μL)): δ 175.4, 169.1, 158.1, 152.4, 150.9, 149.0, 145.2, 137.4, 134.7, 133.2, 129.9, 129.5, 129.4, 129.1, 128.5, 123.1, 90.7, 81.1, 74.1, 74.1, 67.6, 54.4, 50.0, 35.4, 29.4, 27.8;

HRMS (ESI⁺): calcd. for $[\text{C}_{30}\text{H}_{34}\text{N}_7\text{O}_8]^+$ 620.2463, meas. 620.2468, Δ 0.8 ppm.

5.10 Amino Acid S1

To a solution of Cbz protected amino acid **18** (49 mg, 67 μmol) in MeOH (6.7 mL) at RT, was added $\text{Pd(OH)}_2/\text{C}$ (20 wt %, 19 mg, 27 μmol , 40 mol %). The reaction mixture was purged with hydrogen (3 $\times$), stirred vigorously at RT under a hydrogen atmosphere (balloon) for 3 h and filtered through a nylon syringe filter (13 mm, 0.22 μm). The solids were washed with MeOH (5×1 mL) and the filtrates were concentrated. Crude material was purified by preparative HPLC (injection volume: 3.0 mL (water/ CH_3CN , 0.1% (v/v) TFA, 97.5:2.5), gradient elution: 2.5% $\rightarrow$ 40% B over 65 min) to afford amino acid **S1** (35 mg, 49 μmol , 73%) as the TFA salt.

^1H NMR (500 MHz, CD_3CN (350 μL)/ D_2O (350 μL)): δ 8.70 (s, 1H), 8.44 (s, 1H), 8.02–7.96 (m, 2H), 7.68–7.62 (m, 1H), 7.57–7.52 (m, 2H), 6.03 (d, $J = 4.2$ Hz, 1H), 4.70 (dd, $J = 5.4, 4.2$ Hz, 1H), 4.27 (dd, $J = 6.0, 5.4$ Hz, 1H), 4.20–4.15 (m, 1H), 3.83 (app t, $J = 6.0$ Hz, 1H), 3.49–3.42 (m, 1H), 2.17 (ddd, $J = 15.4, 9.4, 4.2$ Hz, 1H), 2.10 (ddd, $J = 15.4, 7.5, 3.6$ Hz, 1H), 1.97–1.79 (m, 3H), 1.80–1.70 (m, 1H);

^{13}C NMR (126 MHz, CD_3CN (350 μL)/ D_2O (350 μL)): δ 172.1, 168.9, 152.5, 151.6, 149.4, 145.0, 134.5, 133.4, 129.9, 129.3, 123.7, 90.6, 80.7, 74.0, 74.0, 53.4, 49.7, 35.0, 28.5, 26.7;

HRMS (ESI $^+$): calcd. for $[\text{C}_{22}\text{H}_{28}\text{N}_7\text{O}_6]^+$ 486.2096, meas. 486.2098, Δ 0.5 ppm.

5.11 C9'-*epi*-sinefungin (**5**)

To a vial charged with Bz protected C9'-*epi*-sinefungin **S1** (35 mg, 49 μmol) was added a solution of ammonia in methanol (7 N, 5 mL) at RT. The resulting mixture was stirred for 5.5 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (injection volume: 3.0 mL (water), elution: 2.5% B over 20 min) to afford C9'-*epi*-sinefungin (**5**) (28 mg, 46 μmol , 94%) as the TFA salt. Analytical data is presented in section 6.

^1H NMR (500 MHz, D_2O (875 μL for 19 mg of material)): δ 8.46 (s, 1H), 8.46 (s, 1H), 6.14 (d, $J = 4.1$ Hz, 1H), 4.80 (dd, $J = 5.5, 4.1$ Hz, 1H), 4.39 (dd, $J = 6.0, 5.5$ Hz, 1H), 4.30 (ddd, $J = 9.6, 6.0, 3.7$ Hz, 1H), 3.90 (app t, $J = 5.9$ Hz, 1H), 3.62–3.56 (m, 1H), 2.32–2.19 (m, 2H), 2.07–1.82 (m, 4H);

^{13}C NMR (126 MHz, D_2O (875 μL for 19 mg of material)): δ 173.2, 150.3, 148.1, 145.0, 142.9, 119.1, 89.3, 79.7, 73.2, 73.1, 53.8, 48.8, 34.2, 27.7, 26.2;

HRMS (ESI⁺): calcd. for $[\text{C}_{15}\text{H}_{24}\text{N}_7\text{O}_5]^+$ 382.1833, meas. 382.1827, Δ 1.6 ppm.

5.12 Benzamide 19

To a solution of amine **18** (TFA salt, 50 mg, 0.068 mmol) in MeOH (4.0 mL) at RT, were added 3-formyl-benzamide (203 mg, 1.36 mmol, 20 equiv.) and AcOH (40 μ L, 0.68 mmol, 10 equiv.). The resulting mixture was stirred at RT for 1.5 h before the addition of NaBH₃CN (1.0 M in THF, 3.4 mL, 3.4 mmol, 50 equiv.). The reaction mixture was stirred for 4 h and the volatiles were removed *in vacuo*. Crude material was purified by preparative HPLC (gradient elution: 5% $\rightarrow$ 45% B over 60 min) to afford benzamide **19** (48 mg, 0.055 mmol, 81%) as the TFA salt.

¹H NMR (600 MHz, CD₃CN (350 μ L)/D₂O (350 μ L)): δ 8.54 (s, 1H), 8.44 (s, 1H), 8.02–7.99 (m, 2H), 7.69–7.65 (m, 1H), 7.62 (dt, J = 6.5, 2.0 Hz, 1H), 7.60–7.54 (m, 3H), 7.32–7.21 (m, 7H), 5.96 (d, J = 4.4 Hz, 1H), 5.02 (d, J = 14.6 Hz, 1H), 5.02 (d, J = 14.6 Hz, 1H), 4.66 (dd, J = 5.5, 4.4 Hz, 1H), 4.29 (dd, J = 6.1, 5.5 Hz, 1H), 4.21–4.18 (m, 1H), 4.14–4.10 (m, 1H), 4.11 (d, J = 13.5 Hz, 1H), 4.07 (d, J = 13.5 Hz, 1H), 3.51–3.44 (m, 1H), 2.31–2.18 (m, 2H), 1.91–1.70 (m, 4H);

¹³C NMR (126 MHz, CD₃CN (350 μ L)/D₂O (350 μ L)): δ 175.3, 171.1, 168.3, 158.1, 152.6, 152.1, 150.2, 144.8, 137.4, 134.4, 134.2, 133.9, 133.7, 132.0, 130.1, 129.8, 129.5, 129.4, 129.2, 129.1, 129.1, 128.6, 124.5, 90.4, 80.7, 73.7, 73.4, 67.6, 56.4, 54.3, 48.6, 32.0, 27.9, 26.7;

HRMS (ESI⁺): calcd. for [C₃₈H₄₁N₈O₉]⁺ 753.2991, meas. 753.3014, Δ 3.1 ppm.

6 Analytical Characterization of C9'-*epi*-Sinefungin

Method Description

Detection of various compounds by LC/MS were carried out on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Q Exactive Plus mass spectrometer system (Thermo Fisher Scientific Inc, Waltham, MA) equipped with an HESI-II electrospray ionization (ESI) source. Data were acquired with Chromeleon Xpress software for UHPLC and Thermo Xcalibur software version 3.0.63 for mass spectrometry, and processed with Thermo Xcalibur Qual Browser software version 4.0.27.19.

1 μ L sample was injected onto the UHPLC including an HPG-3400RS binary pump with a built-in vacuum degasser and a thermostated WPS-3000TRS high performance autosampler. A Coagent Diamond Hydride analytical column (2.1 x 150 mm, 4 μ m) from Microsolv Technology Corporation (Leland, NC) was used at the flow rate of 0.4 mL/min using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. The column temperature was maintained at room temperature. The following gradient was applied: 0-5 min: 95-75% B, 5-6 min: 75-60% B, 6-7 min: 60% B isocratic, 7-9 min: 60-50% B, 9-15 min: 50% B isocratic, 15-16 min: 50-95% B, 16-25 min, 95%B isocratic.

The MS conditions were as follows: positive ionization mode; full scan mass range, m/z 50 to 750; resolution, 70,000; AGC target, 1e6; maximum IT, 200 ms; PRM NCE, 20 for m/z 382.1833; resolution, 35,000; AGC target, 2e5; maximum IT, 100 ms; spray voltage, 3500 V; capillary temperature, 280 °C; sheath gas, 50; Aux gas, 12.5; probe heater temperature, 425 °C; S-Lens RF level, 50.00. A mass window of $\pm$ 5 ppm was used to extract the ion of $[M+H]^+$ for the compounds. Targets were considered detected when the mass accuracy was less than 5 ppm and there were a match of isotopic pattern between the observed and the theoretical ones.

RT: 0.00 - 25.01 SM: 7G

NL: 2.21E8
m/z=
382.1814-382.1852
F: FTMS + p ESI
Full ms
[50.00-750.00] MS
005462-3_001

C9'-*epi*-sinefungin

LC/MS Compound Detection

NL:
2.04E8
005462-3_001#1237
RT: 7.35 AV: 1 SB: 2
7.14 , 8.88 T: FTMS + p
ESI Full ms
[50.00-750.00]

NL:
8.17E5
C₁₅ H₂₃ N₇ O₅ +H:
C₁₅ H₂₄ N₇ O₅
pa Chrg 1

Hi-Res Mass Spectrometry

7 Small-Molecule X-Ray Crystallography

A crystal mounted on a diffractometer was collected data at 100 K. The intensities of the reflections were collected by means of a Bruker APEX DUO CCD diffractometer ($\text{Cu}_{\text{K}\alpha}$ radiation, $\lambda=1.54178$ Å), and equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 1.0° scans in ω at -30° , -55° , -80° , 30° , 55° , 80° and 115° in 2θ . Data integration down to 0.84 Å resolution was carried out using SAINT V8.37 A¹⁰ with reflection spot size optimization. Absorption corrections were made with the program SADABS¹⁰. The structure was solved by the Intrinsic Phasing methods and refined by least-squares methods again F^2 using SHELXT-2014¹¹ and SHELXL-2014¹² with OLEX 2 interface¹³. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were allowed to ride on the respective atoms. Crystal data as well as details of data collection and refinement are summarized in Table 1 for compound **12**. Geometric parameters are shown in Table 2, and hydrogen-bond parameters are listed in Table 3. The Ortep plots were produced with SHELXL-2014, and the other images were produced with Accelrys DS Visualizer 2.0¹⁴.

7.1 (*R*)-Carbamate **12**

Table 1: Experimental Details

Crystal Data	
Chemical Formula	$\text{C}_{14}\text{H}_{23}\text{NO}_6$
M_r	301.33
Crystal system, space group	Monoclinic, $C2$
Temperature (K)	100
a, b, c (Å)	26.9198 (7), 5.4862 (1), 10.6405 (3)
β (°)	99.4349 (14)
V (Å ³)	1550.21 (7)

¹⁰Bruker AXS APEX3, Bruker AXS, Madison, Wisconsin, 2015.

¹¹Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2015**, *71*, 3–8.

¹²Sheldrick, G. M. *Acta Crystallogr., Sect. C* **2015**, *71*, 3–8.

¹³Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Cryst.* **2009**, *42*, 339–341.

¹⁴Accelrys DS Visualizer v2.0.1, Accelrys Software Inc., 2007.

Z	4
Radiation type	Cu $K\alpha$
μ (mm ⁻¹)	0.84
Crystal size (mm)	0.18 $\times$ 0.12 $\times$ 0.06
Data collection	
Diffractometer	Bruker D8 goniometer with CCD area detector
Absorption correction	Multi-scan <i>SADABS</i>
$T_{\min}, T_{\max}$	0.724, 0.806
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	12976, 2622, 2557
R_{int}	0.027
$(\sin \theta / \lambda)_{\max}$ (Å ⁻¹)	0.596
Refinement	
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.033, 0.087, 1.05
No. of reflections	2622
No. of parameters	198
No. of restraints	1
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{\max}, \Delta\rho_{\min}$ (eÅ ⁻³)	0.39, -0.14
Absolute structure	Flack x determined using 1093 quotients [(I+)-(I-)]/[(I+)+(I-)] ¹⁵
Absolute structure parameter	-0.07 (17)

Computer programs: SAINT 8.37A (Bruker-AXS, 2015), SHELXT2014 (Sheldrick, 2015), SHELXL2014 (Sheldrick, 2015), Bruker SHELXTL (Sheldrick, 2015).

Table 2: Geometric parameters (Å, °)

O1–C1	1.426 (3)	C5–H5	1
O1–C2	1.433 (3)	C6–C7	1.533 (4)
O2–C3	1.418 (3)	C6–H6A	0.99
O2–C1	1.428 (3)	C6–H6B	0.99
O3–C4	1.408 (3)	C7–C8	1.504 (3)
O3–C5	1.450 (3)	C7–H7	1
O4–C4	1.412 (3)	C8–C9	1.311 (4)
O4–C12	1.427 (4)	C8–H8	0.95
O5–C13	1.354 (3)	C9–H9A	0.95
O5–C14	1.440 (4)	C9–H9B	0.95
O6–C13	1.210 (3)	C10–H10A	0.98

¹⁵ Parsons, S.; Flack, H. D.; Wagner, T. *Acta Crystallogr., Sect. B* **2013**, *69*, 249–259.

N1–C13	1.348 (4)	C10–H10B	0.98
N1–C7	1.469 (3)	C10–H10C	0.98
N1–H1	0.81 (4)	C11–H11A	0.98
C1–C11	1.503 (4)	C11–H11B	0.98
C1–C10	1.513 (4)	C11–H11C	0.98
C2–C5	1.530 (4)	C12–H12A	0.98
C2–C3	1.543 (4)	C12–H12B	0.98
C2–H2	1	C12–H12C	0.98
C3–C4	1.514 (4)	C14–H14A	0.98
C3–H3	1	C14–H14B	0.98
C4–H4	1	C14–H14C	0.98
C5–C6	1.522 (4)		
C1–O1–C2	107.8 (2)	C7–C6–H6B	109.4
C3–O2–C1	107.72 (18)	H6A–C6–H6B	108
C4–O3–C5	108.53 (17)	N1–C7–C8	110.3 (2)
C4–O4–C12	112.3 (2)	N1–C7–C6	109.1 (2)
C13–O5–C14	114.9 (2)	C8–C7–C6	113.2 (2)
C13–N1–C7	120.9 (2)	N1–C7–H7	108
C13–N1–H1	121 (2)	C8–C7–H7	108
C7–N1–H1	117 (2)	C6–C7–H7	108
O1–C1–O2	104.2 (2)	C9–C8–C7	124.2 (3)
O1–C1–C11	108.8 (2)	C9–C8–H8	117.9
O2–C1–C11	109.1 (2)	C7–C8–H8	117.9
O1–C1–C10	111.8 (2)	C8–C9–H9A	120
O2–C1–C10	110.6 (3)	C8–C9–H9B	120
C11–C1–C10	112.2 (3)	H9A–C9–H9B	120
O1–C2–C5	109.8 (2)	C1–C10–H10A	109.5
O1–C2–C3	104.21 (19)	C1–C10–H10B	109.5
C5–C2–C3	105.0 (2)	H10A–C10–H10B	109.5
O1–C2–H2	112.4	C1–C10–H10C	109.5
C5–C2–H2	112.4	H10A–C10–H10C	109.5
C3–C2–H2	112.4	H10B–C10–H10C	109.5
O2–C3–C4	109.10 (19)	C1–C11–H11A	109.5
O2–C3–C2	104.5 (2)	C1–C11–H11B	109.5
C4–C3–C2	104.5 (2)	H11A–C11–H11B	109.5
O2–C3–H3	112.7	C1–C11–H11C	109.5
C4–C3–H3	112.7	H11A–C11–H11C	109.5
C2–C3–H3	112.7	H11B–C11–H11C	109.5
O3–C4–O4	113.24 (19)	O4–C12–H12A	109.5
O3–C4–C3	106.11 (19)	O4–C12–H12B	109.5

O4–C4–C3	107.6 (2)	H12A–C12–H12B	109.5
O3–C4–H4	109.9	O4–C12–H12C	109.5
O4–C4–H4	109.9	H12A–C12–H12C	109.5
C3–C4–H4	109.9	H12B–C12–H12C	109.5
O3–C5–C6	110.78 (19)	O6–C13–N1	125.8 (2)
O3–C5–C2	104.39 (18)	O6–C13–O5	124.2 (2)
C6–C5–C2	115.5 (2)	N1–C13–O5	110.0 (2)
O3–C5–H5	108.7	O5–C14–H14A	109.5
C6–C5–H5	108.7	O5–C14–H14B	109.5
C2–C5–H5	108.7	H14A–C14–H14B	109.5
C5–C6–C7	111.1 (2)	O5–C14–H14C	109.5
C5–C6–H6A	109.4	H14A–C14–H14C	109.5
C7–C6–H6A	109.4	H14B–C14–H14C	109.5
C5–C6–H6B	109.4		
C2–O1–C1–O2	-32.3 (3)	O2–C3–C4–O4	-148.49 (18)
C2–O1–C1–C11	-148.5 (2)	C2–C3–C4–O4	100.2 (2)
C2–O1–C1–C10	87.1 (3)	C4–O3–C5–C6	92.0 (2)
C3–O2–C1–O1	34.1 (3)	C4–O3–C5–C2	-32.9 (2)
C3–O2–C1–C11	150.1 (2)	O1–C2–C5–O3	-93.7 (2)
C3–O2–C1–C10	-86.1 (3)	C3–C2–C5–O3	17.9 (2)
C1–O1–C2–C5	130.3 (2)	O1–C2–C5–C6	144.5 (2)
C1–O1–C2–C3	18.3 (3)	C3–C2–C5–C6	-104.0 (2)
C1–O2–C3–C4	-133.7 (2)	O3–C5–C6–C7	62.4 (2)
C1–O2–C3–C2	-22.4 (2)	C2–C5–C6–C7	-179.23 (19)
O1–C2–C3–O2	2.5 (2)	C13–N1–C7–C8	84.5 (3)
C5–C2–C3–O2	-113.0 (2)	C13–N1–C7–C6	-150.5 (2)
O1–C2–C3–C4	117.1 (2)	C5–C6–C7–N1	73.0 (3)
C5–C2–C3–C4	1.6 (2)	C5–C6–C7–C8	-163.7 (2)
C5–O3–C4–O4	-83.3 (2)	N1–C7–C8–C9	-114.5 (3)
C5–O3–C4–C3	34.5 (2)	C6–C7–C8–C9	122.9 (3)
C12–O4–C4–O3	-59.3 (3)	C7–N1–C13–O6	10.7 (4)
C12–O4–C4–C3	-176.2 (2)	C7–N1–C13–O5	-169.5 (2)
O2–C3–C4–O3	90.0 (2)	C14–O5–C13–O6	-2.5 (4)
C2–C3–C4–O3	-21.3 (2)	C14–O5–C13–N1	177.8 (2)

Table 3: Hydrogen-bond parameters

D – H ... A	D – H (Å)	H ... A (Å)	D ... A (Å)	D – H ... A (°)
C14–H14C...O1 ⁱ	0.98	2.77	3.136 (3)	102.7

Symmetry code(s): (i) -x+1, y-1, -z+1.

Figure 1: Perspective views showing 50% probability displacement.

Figure 2: Three-dimensional supramolecular architecture viewed along the *b*-axis direction.

8 NMR Spectra

8 Trichloroacetimidate	26
7 (<i>R</i>)-Trichloroacetamide	29
13 (<i>S</i>)-Trichloroacetamide	31
14 Aldehyde	33
15 Enamide	35
16 Protected Amino Acid	37
6 Triacetate	39
17 Nucleoside	41
18 Amine	43
S1 Amino Acid	45
5 C9'- <i>epi</i> -sinefungin	47
19 Benzamide	49

8, ^1H , 600 MHz, CDCl_3

8, ^{13}C , 126 MHz, CDCl_3

— 7.26 CDCl₃

7, ¹H, 500 MHz, CDCl₃

7, ^{13}C , 126 MHz, CDCl_3

— 7.26 CDCl₃

13, ¹H, 500 MHz, CDCl₃

13, ^{13}C , 126 MHz, CDCl_3

— 7.26 CDCl₃

14, ¹H, 500 MHz, CDCl₃

14, ^{13}C , 126 MHz, CDCl_3

15, ^1H , 500 MHz, CDCl_3

15, ^{13}C , 151 MHz, CDCl_3

— 7.26 CDCl₃

16, ¹H, 600 MHz, CDCl₃

16, ¹³C, 151 MHz, CDCl₃

— 7.26 CDCl₃

6, ¹H, 600 MHz, CDCl₃

6, ^{13}C , 151 MHz, CDCl_3

— 7.26 CDCl₃

17, ¹H, 600 MHz, CDCl₃

17, ^{13}C , 126 MHz, CDCl_3

18, ^1H , 500 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

18, ^{13}C , 126 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

S1, ^1H , 500 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

—1.94 CD_3CN

S1, ^{13}C , 126 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

5, ^1H , 500 MHz, D_2O

5, ^{13}C , 126 MHz, D_2O

— 173.21

— 150.30
— 148.09
— 144.97
— 142.94

— 119.12

— 89.33

— 79.66

73.22
73.05

— 53.75

— 48.82

— 34.24

27.66
26.21

19, ^1H , 600 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

19, ^{13}C , 126 MHz, $\text{CD}_3\text{CN}/\text{D}_2\text{O}$

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