



(51) International Patent Classification:

C07D 245/02 (2006.01) A61K 31/395 (2006.01)

C07K 5/078 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/US2023/019733

(22) International Filing Date:

25 April 2023 (25.04.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/334,625 25 April 2022 (25.04.2022) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(54) Title: SYRBACTIN MACROLACTAMS AND UNNATURAL ANALOGS AS PROTEASOME INHIBITORS FOR THE TREATMENT OF MULTIPLE MYELOMA AND CHEMOENZYMATIC SYNTHESIS THEREOF

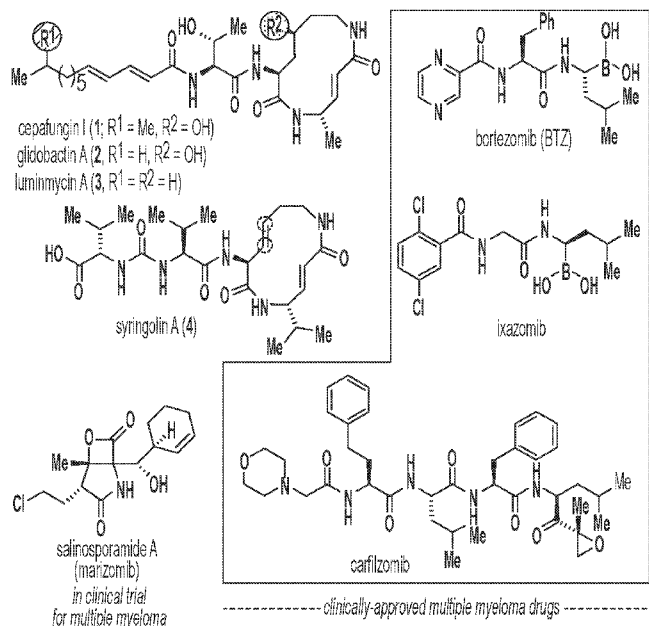


FIG. 1

(57) Abstract: One aspect of the invention is any compound, or salt thereof, described herein. Another aspect is a method of treating a disease, disorder, or symptom thereof, in a subject, comprising administration to the subject of a compound, or salt thereof, herein. Another aspect is a method of inhibiting a proteasome in a subject, comprising administration to the subject of a compound, or salt thereof, herein. Another aspect is a method of making a compound, or salt thereof, described herein using one or more reagents, chemical transformations, or chemical intermediate compounds as described herein.

(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

**SYRBACTIN MACROLACTAMS AND UNNATURAL ANALOGS AS
PROTEASOME INHIBITORS FOR THE TREATMENT OF MULTIPLE
MYELOMA AND CHEMOENZYMATIC SYNTHESIS THEREOF**

GOVERNMENT SUPPORT

This invention was made with government support under R35 GM128895 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Background of the present disclosure can be found in Amatuni, A. and Renata, H., *Org. Biomol. Chem.* 2019 February 13; 17(7): 1736-1739 and Amatuni, A. *et al.*, *Cell Chem. Biol.* 2020 October 15; 27(10): 1318-1326.e18, which are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further described below with reference to the following non-limiting examples and with reference to the following figures, in which:

FIG. 1 depicts representative structures of syrbactin natural products and clinical proteasome inhibitors.

FIG. 2 depicts a chemoenzymatic route to cepafungin I.

FIG. 3 depicts a preliminary series of 1 analogs and comparison to prior syringolin analogs.

FIG. 4 depicts the bound crystal structure of cepafungin I at $\beta 5$ subunit of yeast 20S proteasome. PDB ID: 4FZC.⁸ Dashed lines indicate polar contacts. The catalytic $\beta 5$ N-terminal threonine covalently bonded to the cepafungin macrocycle is labeled "T1". $\beta 5$ residues involved in polar contacts are Gly47, Ala49, Thr21 and Asp126. A hydrophobic channel for the tail fragment involves $\beta 6$ subunit residues Pro127, Val128, Pro104, Tyr106 and Tyr5.

FIG. 5 depicts synthesis of cepafungin analogs 40–51.

FIGs. 6A-6E depict biological evaluation of cepafungin and analogs in RPMI 8226 cells. FIG. 6A depicts proteasome inhibition screening at 30 nM compound concentration with subunit-specific fluorogenic proteasome substrates Suc-LLVY-AMC ($\beta 5$) and Ac-RLR-AMC ($\beta 2$). FIG. 6B depicts cytotoxicity screening at 30 nM and 100 nM compound concentration. FIG. 6C depicts EC₅₀ measurement of 50 in comparison to 1. FIG. 6D depicts proteasome subunit IC₅₀ measurements of 50 in comparison to 1. FIG. 6E depicts a comparison of phenyl cepafungin 50 and bortezomib in human MM (RPMI8226, U266, H929) and MCL (JeKo-1, HBL2, Mino) cell lines.

FIG. 7 depicts cytotoxicity EC₅₀ measurements for 50 and bortezomib (BTZ) in MM and MCL cell lines.

FIG. 8 depicts cytotoxicity EC₅₀ measurements for phenyl cepafungin (50) and hybrid analog (S39) in RPMI 8226.

FIG. 9 depicts the bound crystal structure of cepafungin I at β 2 subunit of yeast 20S proteasome. PDB ID: 4FZC.^{S3} Dashed lines indicate polar contacts. The catalytic β 2 N-terminal threonine covalently bonded to the cepafungin macrocycle is labeled "T1". β 2 residues involved in polar contacts are Gly47, Ala49, Thr21 and Asp124. A hydrophobic channel for the tail fragment involves β 3 subunit residues Leu125, Ile126, Pro101 and Phe103.

DETAILED DESCRIPTION

5.1 – Introduction

Considering that cepafungin I (**1**) is the most potent proteasome inhibitor among the syrbactins, has low nanomolar inhibitory activity towards both β 2 and β 5 subunits, and engages the 20S proteasome with exceptional selectivity in a MM cell line, this natural product was selected as a promising scaffold for further SAR optimization towards a new anticancer agent.

Syntheses of syringolins A (**4**) and B have been reported by Kaiser,¹ Stephenson,² Pirrung³ and Ichikawa,⁴ and Schmidt in 1992 achieved the first total synthesis of glidobactin A (**2**).⁵ Early structure-activity relationships (SAR) were established with semi-synthetic glidobactin analogs harboring unnatural tail fragments, though the syrbactins' mechanism of action had not been elucidated at the time.⁶ More recent studies on the syringolins and unnatural analogs have elucidated their activity in the context of proteasome inhibition. Collectively, these studies have revealed some key factors contributing to the syrbactins' potent bioactivity. The presence of longer lipophilic tails seen in glidobactins, cepafungins and synthetic analogs versus those of natural syringolins has been shown to result in greater proteasome inhibition in vitro, likely by increased hydrophobic interactions distal to the active site.^{4,7} Notably, a single methyl branching at the end of the cepafungin I (**1**) fatty acid leads to ca. 5-fold improvement of β 5-subunit inhibition compared to glidobactin A (**2**).⁸ Further exploration of hydrophobic functionalities at this position may further improve the proteasome binding affinity at one or more subunits. Larger hydrophobic residues in place of the macrocycle-adjacent threonine are tolerated by the S3 subsite and can improve binding affinity.⁴ However, other β -hydroxy amino acids in place of threonine have not been explored, nor has there been a direct comparison

between threonine and its non-hydroxylated counterpart. Interestingly, several bacteria produce glidobactin-like syrbactins, and several other bacteria have bioinformatically been shown to harbor syrbactin-like gene clusters.^{9,10} While several lysine hydroxylases are found in nature, syrbactins with different lysine hydroxylation patterns have not been found, except for minor amounts of desoxy-glidobactins known as “luminmycins” from *Photorhabdus laumondii*, such as luminmycin A (**3**).¹¹ Given the cepafungins’ potent bioactivity and the current gap in SAR data, we sought to utilize our chemoenzymatic strategy (FIG. 2) to access analogs harboring modifications at all regions of the scaffold. Unique in our approach is the utilization of several enzymes for site-selective C-H hydroxylation of amino acids, facilitating rapid exploration of chemical space around the cepafungin macrocycle and tail fragment linker. This approach has resulted in the first comprehensive SAR analysis of cepafungin’s proteasome inhibitory activity and five unnatural analogs with improved bioactivity.

5.2 – Considerations for Analog Design

Early syntheses of the syringolins ultimately led to the development of various lipophilic analogs with improved cytotoxicity and proteasome inhibition. Ichikawa’s analog **36** and Pirrung’s analog **37** both benefited from replacement of one or more C-terminal Val residues in the syringolin A tail (FIG. 1).^{4,12} Ichikawa reported that a Phe macrocycle linker was found to increase $\beta 5$ inhibition 15-fold compared to syringolin A, likely by improved binding at the hydrophobic pocket of the S3 subsite, although this analog still exhibited low cytotoxicity. Exploration of cell-permeable lipid tails led to the discovery of analog **36** which exhibited low nanomolar cytotoxicity. Likewise, Pirrung reported a syringolin A analog **37** containing a dodecanoyl-ureido-Val tail fragment. This analog had inhibitory activity comparable to ixazomib, displays lower resistance index in MM cell lines than bortezomib, and prolonged survival in MM mouse xenografts.¹² However, prior SAR studies on syrbactins have not explored analogs that are more cepafungin/glidobactin-like.

Our preliminary series of analogs (FIG. 3), including the natural product glidobactin A (**2**), were synthesized by largely the same route (FIG. 2).¹³ Desoxycepafungin (**38**) was synthesized from Boc-Lys(Boc)-OH to assess the role of the macrocycle hydroxylation in **1** towards proteasome inhibition. The saturated cepafungin analog **39** was designed to address the possible role of rigidity in the tail fragment induced by the diene seen in many natural cepafungins and glidobactins. An analog bearing unsaturation at its macrocyclic lysine was envisioned to provide a head-to-head comparison between the cepafungin and syringolin A (**4**)

macrocyclic core. However, dehydration conditions on the hydroxylated macrocycle resulted in decomposition and inseparable mixtures of olefin isomers. Proteasome subunit $\beta 5$ IC_{50} values were determined in situ in RPMI 8226 cells (FIG. 3). In close agreement with prior measurement of $\beta 5$ IC_{50} for natural **1** and **2** in purified yeast proteasome,⁸ in our hands the additional branching methyl in **1** indeed leads to ca. 5-fold improved IC_{50} compared to **2** in RPMI 8226 cells. Notably, the macrocyclic hydroxyl group in **1** leads to ca. 11-fold improved $\beta 5$ inhibition compared to its “desoxy-cepafungin” analog **38**. Likewise, saturation of the fatty acid fragment in **39** causes ca. 14-fold decrease in $\beta 5$ inhibition. This preliminary series of analogs highlighted that the macrocyclic hydroxylation, fatty acid unsaturation, and terminal lipid functionality are critical for potent proteasome inhibitory activity.

To gain a more comprehensive understanding of cepafungin's SAR, we designed a broader series of analogs guided by a previous crystal structure of yeast proteasome: cepafungin complex⁸ (FIG. 4), insights from prior SAR studies on related syrbactins,^{4,6,7,14,15} and our preliminary SAR data. Based on these insights, four divergence points were identified, namely the oxygenation pattern of the macrocycle, the vinylogous amino acid residue in the macrocycle, the side-chain of the β -OH amino acid linker, and the terminal functionality of the lipid tail. Regarding the first point, we envisioned L-lysine (**8**) as the key divergence point in analog generation (FIG. 2). That desoxycepafulgin (**38**) has ca. 11-fold higher IC_{50} than **1** just from omission of the lysine hydroxylation raises the question whether the reduced potency arises from a general decrease in polarity of the macrocycle or from a more specific electrostatic interaction in the active site. Notably, no hydrogen bonds or water molecules are seen near the hydroxy group of **1** and the $\beta 5$ subunit of yeast proteasome in the crystal structure (FIG. 4). To probe the relevance of this hydroxylation, we sought to compare analogs containing different lysine oxidation patterns. The (2*S*,4*R*) diastereomer of **10** could be prepared via KDO3, an Fe/ α KG enzyme from *Flavobacterium johnsoniae* that performs regio- and stereoselective (*R*)-C4-hydroxylation on free lysine. Elaboration to the macrocycle would then follow the general route outlined in FIG. 2. Additionally, the (2*S*,3*S*)-3-hydroxylysine regioisomer could be prepared with KDO1, an Fe/ α KG enzyme from *Catemulispora acidiphila* that performs selective (*S*)-C3-hydroxylation on free lysine.¹⁶ As this monomer is not expected to undergo spontaneous lactonization upon amine protection, the protected 3-hydroxy amino acid could be directly coupled to an Ala derivative for elaboration to the macrocycle.

The second divergence point is the vinylogous amino acid residue in the macrocycle (FIG. 2). The naturally occurring syringolins incorporate an L-Val residue in place of the L-

Ala seen in the glidobactins and cepafungins, and in both cases these sidechains occupy the S1 subsite at the $\beta 5$ subunit. However, a direct comparison between cepafungin and the Val-substituted macrocycle analog has yet to be reported. The third divergence point is the β -OH amino acid adjacent to the macrocycle, which for cepafungins and glidobactins is canonically occupied by an L-Thr residue. Late-stage introduction of this residue in the tail fragment synthesis facilitates the incorporation of other β -OH amino acids. Notably, the hydroxy group of Thr is involved in hydrogen bonding with Asp126 at the S3 subsite, which comprises a prominent pocket adjacent to the methyl group of Thr and can accommodate larger functional groups (FIG. 4). Ichikawa's syringolin analogs incorporated fatty-acyl-Phe residues in place of the canonical bis-ureido-Val (FIG. 3) and resulted in greater proteasome inhibition.⁴ However, direct comparison of cepafungin I against analogs containing desoxy-Thr or other β -OH amino acids has not been performed. Such β -OH amino acids can be prepared by enzymatic or chemical methods.

Finally, our preliminary SAR results (FIG. 3) indicate that the diene functionality in the fatty acid as well as the terminal methyl branching are very important for bioactivity. The diene may assist in orienting the lipid tail along a hydrophobic patch on the adjacent $\beta 6$ subunit and between residues Pro127 and Val128 (FIG. 4). Additionally, early cytotoxicity assays and more recent studies on glidobactin-like natural products highlight that C₁₂ fatty acid chains in the tail fragment are the optimal length for potent bioactivity.^{6,11} Therefore, additional analogs were designed to keep the C₁₂ chain length and the diene moiety but explore alternate groups in place of the terminal isopropyl in **1**. Substitutions can readily be made by variations of the Grignard reagent during the initial Kochi coupling to 6-bromo-1-hexanol. Notably, the cepafungin lipid terminates near $\beta 6$ subunit residues Tyr5 and Tyr106, which appear poised to facilitate potential pi-stacking interactions (FIG. 4). Thus, we sought an analog with a phenyl ring at the lipid terminus to induce pi-stacking with either of these residues. Larger hydrophobic groups also include tert-butyl, cyclopentyl and cyclohexyl. Additionally, as trifluoromethyl groups may prefer interactions with Phe, Met, Leu and Tyr residues compared to the corresponding methyl-substituted compound,¹⁷ an analog containing a CF₃-substituted lipid fragment was sought. Direct adaptation of our original synthetic route to this analog would require the introduction of a terminal hexafluoroisopropyl moiety by Wittig olefination.¹⁸ Due to operational hazards involved in working with highly volatile and toxic hexafluoroacetone, we opted to instead make the CF₃-substituted lipid fragment via photocatalytic trifluoromethylation of 5-hexen-1-ol,¹⁹ followed by further elaboration based on our synthetic

route to cepafungin. Taken together, this expanded series of analogs spans modifications of the macrocycle, tail fragment β -OH amino acid, and fatty acid fragments. Importantly, the modularity of our synthesis would readily allow combinations of fragment building blocks after initial screening of mono-substitutions to identify any synergistic effects.

5.3 – Syntheses of 13 Novel Cepafungin Analogs

In our initial attempts to hydroxylate lysine with KDO3, full consumption of starting material was observed with 2 equivalents of α KG co-substrate, but no desired hydroxylysine product could be isolated by ion-exchange resin or by preparative TLC after Fmoc derivatization. NMR and LCMS analysis indicated primarily decomposition of the starting material. With 1.25 equivalents of α KG co-substrate, LCMS analysis indicated cleaner full conversion to the desired hydroxylated product. Upon scale-up, performing the reaction with 20 mM Lys at pre-lysis $OD_{600} = 10$ revealed the overoxidized keto lysine **S13** as the major product (32%), and (2*S*,4*R*)-4-hydroxylysine **S11** (11%) and lactone **S12** (6%) as minor products. To our knowledge, this reactivity pattern for a lysine hydroxylase was not described before in the initial disclosure of KDO3. To favor mono-oxidation, the reaction was performed with 40 mM lysine substrate, 1.25 equivalents of α KG and pre-lysis $OD_{600} = 4.3$ (see Table 5.S1 for optimization). On gram scale, these conditions provided a combined 42% 2-step yield of (2*S*,4*R*)-4-hydroxylysine **S11** and its spontaneously lactonized counterpart **S12**, and 12% of keto-lysine byproduct **S13** after Boc protection (Scheme 5.S1). Lactone **S12** was carried forward as in FIG. 2 to access the epi-4-OH cepafungin analog **40** (FIG. 5).

While the keto-lysine product **S13** was unexpected, it raised a question of whether a cepafungin macrocycle harboring a C4-ketone in place of a hydroxyl would be beneficial. We envisioned that the ketone sp^2 center could introduce additional strain to the 12-membered ring, potentially making the α,β -unsaturated amide more reactive. Additionally, this substitution would swap a hydrogen bond donor for an acceptor. The protected keto-lysine byproduct could be coupled directly to an Ala derivative towards the keto-macrocycle analog, though we opted instead to directly oxidize intermediate **S17c** from the natural product synthesis. Subsequent deprotection and macrocyclization proceeded in similar yields as in cepafungin I, despite the additional sp^2 center. KDO1 was used for selective C3 oxidation of lysine as described in the literature.²⁰ After peptide coupling to **31**, subsequent steps followed those of the natural product synthesis to provide **41** in good yield. The valinyl cepafungin analog **43** was prepared by aminolysis of lactone **30** with a dimethylaluminum amide reagent generated from Val

derivative **S14b**. Gratifyingly, the aminolysis conditions developed in the total synthesis tolerated the bulkier Val sidechain and gave excellent yield of dipeptide **S15c** on gram scale.

For diversification of the β -OH amino acid linker, the desoxy-Thr variant **44** was synthesized from commercial (*S*)-ethylglycine. Towards β -OH-Phe **45**, Franck's auxiliary-based aldol chemistry²¹ readily allowed the union of a chiral glycine equivalent to benzaldehyde. The addition product was directly subjected to methanolysis in one pot to cleave the auxiliary and form methyl ester **S34** in 66% yield over 2 steps. Catalytical hydrogenation and subsequent couplings to the unsaturated fatty acid and core macrocycle completed the synthesis of β -OH-Phe cepafungin **45**. Next, we targeted β -OH-Leu to compare the natural product against both larger aromatic and aliphatic β -OH amino acids at the S3 subsite. Unfortunately, the Franck aldol reaction did not produce any of the desired adduct with either isobutyraldehyde or methacrolein. Instead, we turned to the Fe/ α KG dioxygenase SadA, which performs stereoselective β -hydroxylation on a variety of aliphatic N-succinyl amino acids, as well as LasA, an N-desuccinylase enzyme from the same producing organism for a cascade synthesis of β -hydroxy-Leu.²² While the original report for the enzymes describes a one-pot transformation with both enzymes, we opted for flash C18 purification of intermediate **S36** to facilitate optimization of the LasA reaction that had initially failed to produce the desired product or with only minimal conversion. Ultimately, 10 mM loading of N-succinyl- β -hydroxy-Leu substrate and 20 °C reaction with LasA at pre-lysis OD₆₀₀ = 20 promoted nearly complete conversion to free β -hydroxy-Leu, isolated in 81% yield as the Fmoc derivative over two steps. Fatty acid analogs **47–50** were prepared by Kochi couplings of 6-bromo-1-hexanol with the corresponding Grignard reagents, then carried forward following the cepafungin I route. Finally, the CF₃ fatty acid derivative **51** was prepared by way of alcohol **S24e**, made via photocatalytic trifluoromethylation of **52** with Togni reagent II (**53**).¹⁹ In total, 12 analogs were synthesized in this campaign.

5.4 – In Vitro Biological Evaluation

Cellular assays were performed with human multiple myeloma cell line RPMI 8226. First, relative inhibition of proteasome subunits β 5 and β 2 for all analogs and the natural product was measured at 30 nM compound concentration. Following incubation of the analogs in cell culture for 6 hours, cells were lysed and fluorogenic subunit-specific peptide substrates Suc- LLVY-AMC (β 5) or Ac-RLR-AMC (β 2) were incubated with the lysates, and fluorescence readings provided relative proteasome activity levels with respect to DMSO (FIG.

6A). Next, cytotoxicity screening was performed at 30 nM and 100 nM concentrations for better coverage of dynamic range (FIG. 6B). From these results, cytotoxicities track closely with relative proteasome inhibition, suggesting that proteasome inhibition is a primary cause of cytotoxicity. Notably, compounds **43**, **47**, **48**, and **50** are more potent than the parent natural product **1**, with phenyl cepafungin **50** being the most potent. EC₅₀ measurement in RPMI 8226 revealed that **50** has ca. 4–5 fold greater cytotoxicity than the parent cepafungin I **1** with an EC₅₀ of 3 nM (FIG. 6C). Proteasome subunit activity measurements revealed that **50** has 7-fold lower IC₅₀ than **1** for the β 5 subunit and roughly similar IC₅₀ for the β 2 subunit (FIG. 6D). Therefore, the improved cytotoxicity of phenyl cepafungin **50** likely results from a more pronounced loss of β 5 function in RPMI 8226 cells. This drastic increase in proteasome inhibition may be due to pi-stacking of the lipid tail phenyl ring with the β 6 subunit residues Tyr106 and/or Tyr5. The β 2 subunit-bound natural product has residues Tyr102 and Phe103 on the adjacent β 3 subunit oriented further away from the terminal lipid methyl groups, and presumably would not facilitate pi-stacking to the extent possible at the adjacent β 6 subunit (see FIG. 4, FIG. 9). The tert-butyl and cyclopentyl cepafungins **47** and **48**, respectively, also have improved cytotoxicity relative to **1** albeit to a lesser extent than **50**, suggesting the aromatic moiety in the lipid tail is favored over large aliphatic groups. Cyclohexyl cepafungin **49** appears to have roughly similar activity as the natural product. Interestingly, valinyl cepafungin **43** displayed slightly greater cytotoxicity than the natural product and prompted the synthesis of a hybrid analog (**S39**) containing the phenyl cepafungin tail fragment and valinyl cepafungin macrocycle. These combined modifications were made in hopes that the effects of both substitutions would be additive. While this hybrid analog was significantly more potent than the natural product with an EC₅₀ of 4.5 nM against RPMI 8226, its cytotoxicity did not surpass that of **50** (FIG. 8). Of the other modified macrocycle analogs, only keto cepafungin **42** displayed potency approaching that of the natural product, while epi-4-OH (**40**) and 3-OH cepafungin (**41**) were nearly inactive at both concentrations. This result indicates that the regio- and stereochemistry of lysine hydroxylation in the cepafungin and glidobactin biosyntheses is critical for potent proteasome inhibition. The presence of a Thr residue next to the cepafungin macrocycle appears to be favored over the ethyl sidechain of desoxy-Thr analog **44** as indicated by cytotoxicity and hydrogen-bonding seen in the cepafungin bound crystal structure (Figure 3, 4B). However, the β -OH-Phe analog **45** was substantially weaker, while the β -OH-Leu analog **46** approached the potency of the natural product. The trifluoromethyl analog **51** was essentially inactive at both concentrations tested.

Finally, we sought to compare the new lead analog (**50**) against the clinical drug bortezomib. It has been shown that in addition to MM, mantle cell lymphoma (MCL) is also sensitive to proteasome inhibition. Indeed, bortezomib itself is FDA-approved for the treatment of MCL.²³ Therefore, we compared **50** and bortezomib in 3 different cell lines of both MM and MCL (FIG. 6E, FIG. 7). Gratifyingly, phenyl cepafungin displayed similarly low-nanomolar cytotoxicity in all cell lines as bortezomib except for U266, for which it has an EC₅₀ of ~20 nM.

5.5 – Conclusion

The chemoenzymatic strategies described herein enabled the first synthesis of cepafungin I. Early route exploration examined the use of the fungal Fe/ α KG FoPip4H for site-selective C-H hydroxylation of L-pipecolic acid, which was followed by functional group interconversions to generate a 4-hydroxylysine surrogate. Unfortunately, all attempts at macrocyclization failed to provide the desired 12-membered macrolactam and instead resulted in the formation of a 6-membered cyclization product in low yield. This result underscored the challenge of forming this 12-membered ring and prompted a more biomimetic protecting group-free macrolactamization strategy, which led to our study on the glidobactin biosynthetic gene cluster to discover and characterize the native lysine hydroxylase. The Fe/ α KG from the cluster, GlbB, hydroxylates L-lysine with exquisite regio-/stereoselectivity and very high total turnover, allowing for easy scale-up whereby ~7 g of free L-lysine could be hydroxylated with 1 L of clarified lysate. By leveraging the high efficiency of this biocatalytic reaction, we completed the first synthesis of cepafungin I in 7.9% yield over 9 longest linear steps.

Prior to our work, a major knowledge gap in the bioactivity of cepafungin was its engagement of the proteasome in human MM cells and selectivity in targeting the proteasome over a multitude of other proteases. To address this, an alkyne-tagged probe analog was synthesized by our chemoenzymatic route and used for classical chemoproteomics studies. A combination of in-gel competitive profiling and in situ competitive LC-MS/MS based chemoproteomics demonstrated cepafungin's exceptional selectivity towards several proteasome subunits. Moreover, global proteomics experiments with **1** and bortezomib indicated a high degree of overlap in upregulated protein expression, suggesting a similar mechanism of action between the two molecules.

The modularity of our chemoenzymatic route enables facile modification of all regions of the natural product scaffold to gain comprehensive SAR data and its efficiency

allows for routine scale up to provide ≥ 40 mg of final compounds. By taking advantage of these features, modifications to the natural product were made at three distinct regions of the scaffold, guided by proteasome-bound crystal structure and preliminary SAR data. The regio- and stereochemistry of lysine hydroxylation is critical for potent proteasome inhibition, whereby the configuration found in the parent natural product was found to be optimal for proteasome inhibition, despite a lack of obvious binding interactions in the crystal structure of yeast 20S proteasome:cepa fungin complex. The S3 subsite appears to prefer aliphatic β -OH amino acids linked to the macrocycle, and a larger aliphatic residue proximal to the macrocycle's reactive electrophile is tolerated. The diene moiety in the tail fragment is also essential for potent inhibition, as is the terminal functionality of the fatty acid. This work constitutes the most comprehensive SAR study on the cepa fungin series to date, undoubtedly made possible by the high modularity of our chemoenzymatic route. One fatty acid analog, phenyl cepa fungin (**50**), has 7-fold greater $\beta 5$ inhibitory activity and exhibits similar cytotoxicity to the clinically approved drug bortezomib in several MM and MCL cell lines. Potent $\beta 5$ and $\beta 2$ co-inhibitory activity, as seen in **50** and other analogs, may serve as a viable means of overcoming bortezomib and carfilzomib resistance in multiple myeloma. In vivo mouse studies, evaluation against PI-resistant cancers, and further structural refinements on **50** are ongoing in our laboratories.

5.6 – References

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5.7 – Experimental

General Materials and Methods

Unless otherwise noted, all chemicals and reagents for chemical reactions were purchased at the highest commercial quality and used without further purification. Reactions were monitored by thin layer chromatography (TLC). TLC was performed with 0.25 mm E. Merck silica plates (60F-254) using short-wave UV light as the visualizing agent and KMnO₄, ninhydrin, vanillin, bromocresol green, iodine and heat as developing agents. LC/HRMS was performed on a Thermo Vanquish UHPLC coupled to an Orbitrap Exploris 120 (HRESI) equipped with an Accucore C18 column (100 mm × 2.1 mm), or on an Agilent 1260 Infinity system equipped with Poroshell 120 EC-C18 column (4.6 x 50 mm, 2.7 μm) and an Agilent G6230B TOF LC/MS with water and acetonitrile buffered with 0.1% formic acid as mobile phases. LCMS analysis was performed on an Agilent 1260 Infinity System coupled to an Agilent 6100 Series Single Quadrupole LCMS equipped with Poroshell 120 EC-C18 column (3.0 x 50 mm, 2.7 μm). Preparative HPLC purification was performed on an Agilent 1260 Infinity system equipped with a Zorbax Eclipse XDB-C18 PrepHT column (21.2 x 250 mm, 7 μm) with water and acetonitrile buffered with 0.1% formic acid as mobile phases. NMR spectra

were recorded on a Bruker AVANCE AV400 (400 MHz and 101 MHz) or Bruker AVANCE AV600 (600 MHz and 151 MHz) at 23 °C unless otherwise noted. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research Analytical). Sonication was performed using a Qsonica Q500 sonicator. Biochemicals and media components were purchased from standard commercial sources. Expression vectors were obtained via DNA synthesis from Twist Bioscience and were used directly with electrocompetent *E. coli* BL21(DE3). Electrocompetent *E. coli* BL21(DE3) strains were purchased from Lucigen. All *E. coli* strains generated in this work are stored as glycerol stocks at –80 °C.

Proteasome Inhibition Screening

RPMI 8226 cells were seeded in a 6-well plate (1,000,000 cells/well) in cell culture media supplemented with **30 nM of indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for 6 hours. Cells were then collected and lysed. Lysates (1 mg/mL, 25 µL) were dispensed into 100 µL assay buffer containing 100 µM proteasome substrate **Suc-LLVY-AMC** (β5) or **Ac-RLR-AMC** (β2). The plates were incubated at 37 °C for one hour and fluorescence was read at A360ex/A460em. (*n* = 3)

RPMI8226 Cytotoxicity Screening

RPMI 8226 cells were seeded in a 96-well plate (20,000 cells/well) in cell culture media supplemented with **10 or 30 nM of indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for **48 hours**. Toxicity was determined using the WST-1 assay (Roche) according to the protocol of the manufacturer. (*n* = 3)

RPMI 8226 Cytotoxicity EC₅₀ Measurements

RPMI 8226 cells were seeded in a 96-well plate (20,000 cells/well) in cell culture media supplemented with **indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for **48 hours**. Toxicity was determined using the WST-1 assay (Roche) according to the protocol of the manufacturer. (*n* = 3)

Proteasome Inhibition IC₅₀ Measurements

RPMI 8226 cells were seeded in a 6-well plate (1,000,000 cells/well) in cell culture media supplemented with **indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for 6 hours. Cells were then collected and lysed. Lysates (1 mg/mL, 25 µL) were dispensed into 100 µL assay buffer containing 100 µM proteasome substrate **Suc-LLVY-AMC**

($\beta 5$) or **Ac-RLR-AMC** ($\beta 2$). The plates were incubated at 37 °C for one hour and fluorescence was read at A_{360ex}/A_{460em}. ($n = 3$)

MM and MCL Cytotoxicity EC₅₀ Measurements

Cells were seeded in a 96-well plate (20,000 cells/well) in cell culture media supplemented with **indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for **48 hours**. Toxicity was determined using the WST-1 assay (Roche) according to the protocol of the manufacturer. ($n = 3$)

Protein and DNA Sequences

Protein sequence of KDO3 (UniProt accession code: A5FF23)

MKSQSLIEDEIPVKENYAYQIPTSLIVEVTPQERNILSNVGALLEKAFKSYENPDYIEA
LHLYSFQLLPERIARILSRFGTDFSDQYGAIIFRGLLEVDQDHLGPTPANWQSADYS
KLNKYGFICSLHGA VPSKPVQYYAQRKGGGILHAVIPDEKMAATQTGSGSKTNLY
VHTEDAFLHQA DFLSFLYLRNEERVSTLYSVRSHGKVNKIMEKLFDPIYQCPKDA
NYQEEINDGPLASVLYGNKKLPFIRFDAAEQIFNENAGQTPEALYNLTEFWNEAKELI
NSDYIPDSGDVIFVNNHLC AHGRSAFTAGQKEENGKLVPCERRQMLRMMSKTSLIHI
RSMTHTD DPYFVMEEHLGKVFDQA

DNA sequence of KDO3 (codon optimized by Genscript)

AAGTCTCAGAGCTTAATTGAAGACGAAATCCCGGTGAAGGAGAATTATGCATAC
CAGATTCCTACGTCTCCTCTCATTGTCTGAAGTGACGCCACAAGAACGCAATATCC
TGTCAAATGTAGGTGCGCTCTTGAGAGAAGGCGTTTAAATCGTACGAGAATCCTGA
TTATATCGAGGCCCTTCACCTCTACTCATTCCAATTGTTGCCGGAGCGCATCGCGC
GTATTCTCTCTCGTTTCGGCACGGATTTCTCCGCTGATCAGTACGGCGCAATCATT
TTCCGCGGCCTGCTTGAGGTGATCAGGACCACTTAGGCCCGACCCCGCGAACT
GGCAAAGCGCTGACTACAGCAAACCTGAATAAGTATGGTTTCATCTGTAGCCTCTT
ACACGGTGCCGTGCCAAGTAAACCCGTGCAATACTACGCACAACGCAAGGGCGG
CGGTATCCTGCATGCGGTGATTCCGGACGAGAAGATGGCAGCTACCCAAACTGG
GTCCGGAAGTAAACTAATCTGTACGTTTCATACGGAAGATGCCTTCCTGCTGCAT
CAAGCCGACTTCTTAAGCTTTCTTTATCTGCGCAACGAAGAACGCGTGCCTTCAA
CATTGTATTCGGTTCGCTCTCACGGTAAGGTAAACAAGATTATGGAGAAGTTGTT
CGACCCGATTTATCAATGTCCTAAGGATGCGAACTACCAAGAGGAAATCAACGA
CGGCCCGCTGGCCTCAGTTCTGTATGGTAATAAGAAGTTGCCCTTCATTCGCTTC

GATGCCGCGGAACAGATCTTTAACGAGAACGCAGGTCAGACCCCGGAAGCCTTG
 TACAACTTAACAGAGTTCTGGAATGAGGCGAAAGAGTTGATCAACAGTGACTAT
 ATCCCTGACAGTGGGGACGTCATTTTCGTTAATAACCACCTCTGCGCACATGGCC
 GCTCGGCTTTCACCGCTGGTCAGAAAGAGGAAAATGGTAAGCTGGTCCCATGTG
 AGCGCCGTCAAATGTTACGTATGATGAGCAAGACGAGTTTGATCCATATTCGCAG
 TATGACTCACACTGATGACCCTTATTTTGTTATGGAAGAGCACTTGGGAAAGGTC
 TTTGATCAAGCCTAA

Expression vectors for KDO1^{S1} and SadA/LasA^{S2} were constructed as described in previously reported procedures. For the construction of expression vectors, each of the above sequences was inserted between the NdeI and BamHI restriction sites within the commercial pET28a(+) vector. The resulting expression vector was used directly to transform electrocompetent *E. coli* strain BL21(DE3). Variants were stored as glycerol stocks at -80°C .

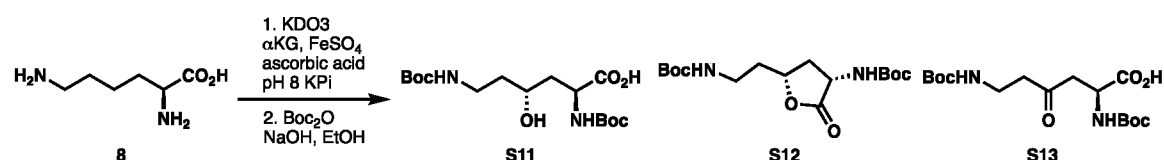
Hybrid analog (S39) EC₅₀ determination

Cytotoxicity of phenyl cepafungin (**50**) and hybrid analog (**S39**) in RPMI 8226

RPMI 8226 cells were seeded in a 96-well plate (20,000 cells/well) in cell culture media supplemented with **indicated compounds** (1,000x stock in DMSO) or DMSO and cultured for **48 hours**. Toxicity was determined using the WST-1 assay (Roche) according to the protocol of the manufacturer. (n = 3)

Synthetic Procedures

KDO3 Reaction Optimization:



Scheme 5.S1. Hydroxylation of L-lysine **8** with clarified lysate of *E. coli* expressing KDO3 and in situ Boc protection of the reaction supernatant.

Standard conditions:

A glycerol stock of *E. coli* BL21(DE3) cells harboring pET-28a(+)-KDO3 plasmid was used to inoculate an overnight culture of LB media (4 mL) containing 50 µg/mL kanamycin. 0.5 mL of this culture was used to inoculate 100 mL TB media containing 50 µg/mL kanamycin

in a 500 mL non-beveled Erlenmeyer flask. The culture was shaken at 250 rpm at 37 °C for 2.5 h or until an $OD_{600} = 0.6$ was reached. The culture was cooled on ice (15 min), induced by adding IPTG to final concentration of 25 μ M, then allowed to continue shaking at 250 rpm/22 °C for another 22 h. Cells were harvested by centrifugation (4 °C, 15 min, 4200 rpm), then resuspended in 50 mM pH = 8 KPi buffer (ca. 25 mL) to a final $OD_{600} = 30$. The cell suspension was lysed by sonication at 50% amplitude for 3 min (1 s on, 4 s off) in an ice bath. The cell debris was pelleted by centrifugation (4 °C, 15 min, 4200 rpm), and the clarified lysate supernatant was diluted with 50 mM pH = 8 KPi and added to a non-beveled Erlenmeyer flask ($\geq 80\%$ headspace) containing L-lysine (1 equiv), α -ketoglutaric acid (1.25 equiv), ascorbic acid (0.5 equiv) and $FeSO_4 \cdot 7H_2O$ (0.1 equiv). The reaction mixture was then shaken for 10 h at 200 rpm/23 °C in the open Erlenmeyer flask. The reaction was then quenched by addition of 6 M HCl to final pH = 2 and centrifuged (4 °C, 15 min, 4200 RPM). The supernatant was directly subjected to the next reaction.

The supernatant was basified with 6 M NaOH to pH = 11. With rapid stirring, Boc_2O (4 equiv) dissolved in EtOH (final 2:1 H_2O :EtOH) was slowly added at rt. After 30 min, the mixture was adjusted back to pH = 10 with additional 6 M NaOH and allowed to stir overnight. The mixture was again adjusted back to pH = 10 with 6 M NaOH and Boc_2O (2 equiv) dissolved in minimal EtOH was slowly added at rt. The mixture was allowed to stir overnight or until completion as judged by LCMS. Ethanol was removed from the reaction mixture *in vacuo*. The aqueous phase was adjusted to pH = 1 with 6 M HCl and diluted with 75 mL EtOAc. The aqueous layer was separated and extracted with an additional 3 x 50 mL EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4 , filtered, and concentrated. The crude material was purified by flash chromatography (97:3:1 DCM:MeOH:AcOH) followed by two evaporations from toluene to provide compounds **S11–13** as white solids.

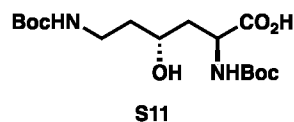
Table 5.S1. KDO3 reaction optimization. Clarified lysates generated from 100 mL cell culture at pre-lysis $OD_{600} = 30$ were diluted with varying volumetric equivalents of 50 mM pH 8 KPi and reacted with varying final concentrations of L-lysine substrate. KDO3 reactions in entries 1, 2 and 5–8 were performed under standard conditions. Co-substrate ratios and buffer conditions in entries 3 and 4 (products not isolated) are adapted from the procedure reported by Baud.^{S4}

Entry	Conditions ^a	NMR ratio S11:S13	Yield S11^b	Yield S12^b	Yield S13^b	Yield (S11+S12)^b
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1	20 mM Lys, lysate diluted 2x	1.0:2.9	11%	6%	32%	17%
2	40 mM Lys, lysate diluted 2x	1.1:1.0	32%	7%	17%	39%
3	20 mM Lys, 1.5 eq α KG, 0.25 eq ascorbate, 0.1 eq FeSO_4 . pH 7.5 HEPES. Lysate diluted 1x	Only S13 + byproducts	-	-	-	-
4	40 mM Lys, 1.5 eq α KG, 0.25 eq ascorbate, 0.1 eq FeSO_4 . pH 7.5 HEPES. Lysate diluted 1x	0.4:1.0	-	-	-	-
5	40 mM Lys, lysate diluted 3x	1.0:1.3	24%	17%	19%	41%
6	40 mM Lys, lysate diluted 4x	1.0:0.9	5%	30%	17%	35%
7	40 mM Lys, lysate diluted 5x	1.0:0.8	17%	22%	14%	39%
8	40 mM Lys (gram scale), lysate diluted 6x	1.0:0.5	20%	22%	12%	42%

^a Standard conditions: (1) 1.25 eq. α KG, 0.1 eq. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 eq. ascorbic acid, 50 mM pH 8 KPi, 200 RPM/23 °C, 10 h; HCl quench. (2) 6 eq. Boc_2O , NaOH to pH = 11, 2:1 H_2O :EtOH

^b Isolated yield after purification by flash chromatography as described above



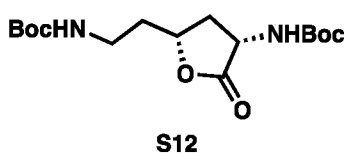
(2*S*,4*R*)-2,6-bis((*tert*-butoxycarbonyl)amino)-4-hydroxyhexanoic acid (S11). Prepared on gram-scale following standard conditions in entry 8, Table 5.S1. L-lysine **8** (1.02 g, 7 mmol, 1 equiv), α -ketoglutaric acid (1.98 g, 8.75 mmol, 1.25 equiv), ascorbic acid (616 mg, 3.5 mmol, 0.5 equiv), $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (195 mg, 0.7 mmol, 0.1 equiv) and Boc_2O (9.17 g, 10.5 mmol, 6 equiv) were used to afford **S11** (500 mg, 20% over 2 steps) as a white solid.

^1H NMR (400 MHz, Methanol- d_4): δ 4.33 – 4.13 (m, 1H), 3.78 – 3.65 (m, 1H), 3.15 (td, J = 6.8, 2.7 Hz, 2H), 1.91 – 1.79 (m, 1H), 1.79 – 1.67 (m, 1H), 1.65 – 1.53 (m, 2H), 1.44 (d, J = 3.9 Hz, 18H).

^{13}C NMR (151 MHz, Methanol- d_4): δ 176.6, 158.8, 158.4, 80.5, 80.0, 66.5, 52.3, 39.8, 39.0, 38.1, 28.8, 28.7.

HRMS (ESI): calculated for $\text{C}_{16}\text{H}_{31}\text{N}_2\text{O}_7^+$ ($[\text{M}+\text{H}]^+$) 363.2126, found 363.2125

$$[\alpha]_D^{24} = -3.8 \text{ (c 0.7, MeOH)}$$



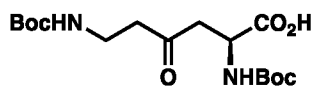
tert-butyl (2-((2*R*,4*S*)-4-((*tert*-butoxycarbonyl)amino)-5-oxotetrahydrofuran-2-yl)ethyl)carbamate (S12). Prepared on gram-scale following standard conditions in entry 8, Table 5.S1. L-lysine **8** (1.02 g, 7 mmol, 1 equiv), α -ketoglutaric acid (1.98 g, 8.75 mmol, 1.25 equiv), ascorbic acid (616 mg, 3.5 mmol, 0.5 equiv), $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (195 mg, 0.7 mmol, 0.1 equiv) and Boc_2O (9.17 g, 10.5 mmol, 6 equiv) were used to afford **S12** (515 mg, 22% over 2 steps) as a white solid.

^1H NMR (400 MHz, Methanol- d_4): δ 4.54 – 4.24 (m, 2H), 3.28 – 3.12 (m, 2H), 2.61 (ddd, J = 12.1, 8.8, 5.5 Hz, 1H), 2.03 – 1.78 (m, 3H), 1.44 (d, J = 6.2 Hz, 18H).

^{13}C NMR (151 MHz, Methanol- d_4): δ 177.2, 158.5, 157.6, 80.8, 80.1, 77.0, 52.2, 37.7, 36.6, 35.8, 28.8, 28.7.

HRMS (ESI): calculated for $\text{C}_{16}\text{H}_{29}\text{N}_2\text{O}_6^+$ ($[\text{M}+\text{H}]^+$) 345.2020, found 345.2015

$$[\alpha]_D^{27} = +12.7 \text{ (c 0.5, MeOH)}$$

**S13**

(S)-2,6-bis((tert-butoxycarbonyl)amino)-4-oxohexanoic acid (S13). Prepared on gram-scale following standard conditions in entry 8, Table 5.S1. L-lysine **8** (1.02 g, 7 mmol, 1 equiv), α -ketoglutaric acid (1.98 g, 8.75 mmol, 1.25 equiv), ascorbic acid (616 mg, 3.5 mmol, 0.5 equiv), $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (195 mg, 0.7 mmol, 0.1 equiv) and Boc_2O (9.17 g, 10.5 mmol, 6 equiv) were used to afford **S13** (292 mg, 12% over 2 steps) as a white solid.

^1H NMR (600 MHz, Methanol- d_4): δ 4.53 – 4.37 (m, 1H), 3.28 (t, J = 6.5 Hz, 2H), 3.02 – 2.84 (m, 2H), 2.74 – 2.58 (m, 2H), 1.43 (d, J = 10.2 Hz, 18H).

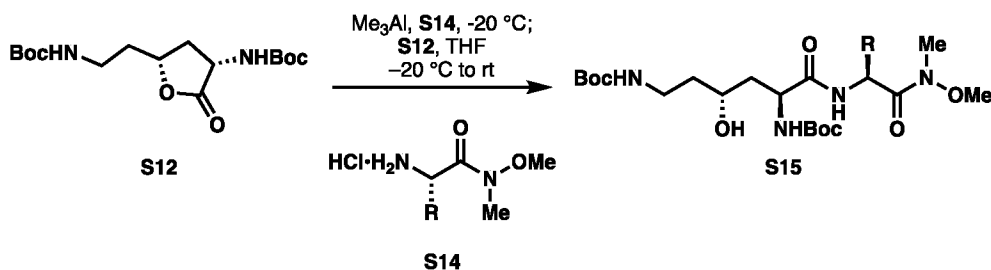
^{13}C NMR (151 MHz, Methanol- d_4): δ 208.16, 175.09, 158.35, 157.83, 80.65, 80.13, 50.60, 45.08, 43.62, 36.29, 28.75, 28.70.

HRMS (ESI): calculated for $\text{C}_{16}\text{H}_{29}\text{N}_2\text{O}_7^+$ ($[\text{M}+\text{H}]^+$) 361.1969, found 361.1967

$$[\alpha]_D^{24} = -2.2 \text{ (c 1.0, MeOH)}$$

General Procedures for Analog Syntheses

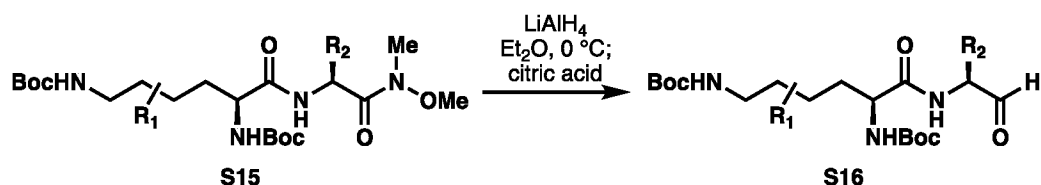
General Procedure A: aminolysis of 4-hydroxylysine lactones



Amine hydrochloride **S14** (5 equiv) was set stirring in anhydrous THF (0.4 M). The mixture was cooled to $-20\text{ }^\circ\text{C}$, then a 2 M solution of trimethylaluminum (4.95 equiv) in anhydrous toluene was added dropwise. The reaction was stirred under argon at $-20\text{ }^\circ\text{C}$ for 30 minutes to provide a clear, light-yellow solution. Lactone **S12** (0.4 M, 1 equiv) in anhydrous THF was added dropwise at $-20\text{ }^\circ\text{C}$. The reaction was stirred to room temperature over 28 h or until completion by TLC. The reaction was cooled to $0\text{ }^\circ\text{C}$ and quenched by dropwise addition of 2 M NaHSO_4 solution. The mixture was adjusted to $\text{pH} = 1$ with 1 M HCl , then concentrated *in vacuo* to remove THF. The aqueous phase was extracted three times with ethyl acetate. The combined organic layers were then washed with H_2O , NaHCO_3 , H_2O , brine and dried over

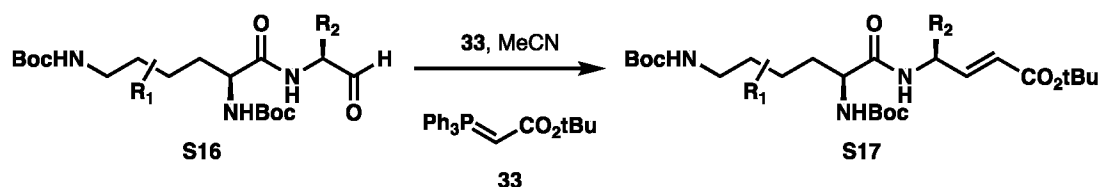
Na₂SO₄, filtered and concentrated. The residue was purified by flash chromatography using a step gradient of hexanes/EtOAc/MeOH mixture as eluent to provide **S15** as a white solid.

General Procedure B: reduction of Weinreb amides



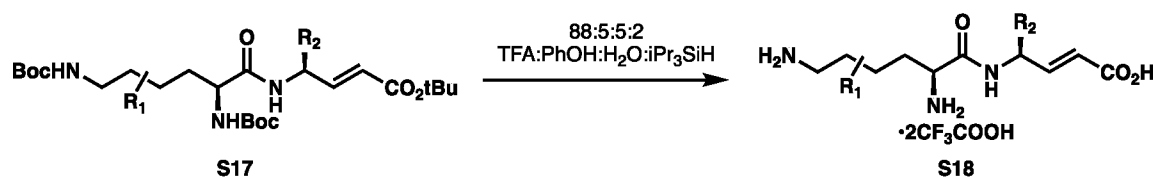
Weinreb amide **S15** (1 equiv, 0.1 M) was set stirring in anhydrous Et₂O and the solution was cooled to 0 °C. 1 M LiAlH₄ solution (2.5 equiv) in Et₂O was added dropwise. The mixture was stirred at 0 °C under argon for 30 minutes or until completion by TLC, then quenched at 0 °C by dropwise addition of 2 M citric acid (2.5 equiv). The mixture was diluted with 10% v/v H₂O and stirred for 5 min. The mixture was diluted with additional H₂O and Et₂O, then the layers were separated. The aqueous layer was extracted three times with Et₂O, and the combined organic layers were washed with H₂O, NaHCO₃, brine and dried over Na₂SO₄, filtered and concentrated to provide **S16** as white solid (d.r. ≥ 98:2) used immediately without further purification.

General Procedure C: Wittig olefination



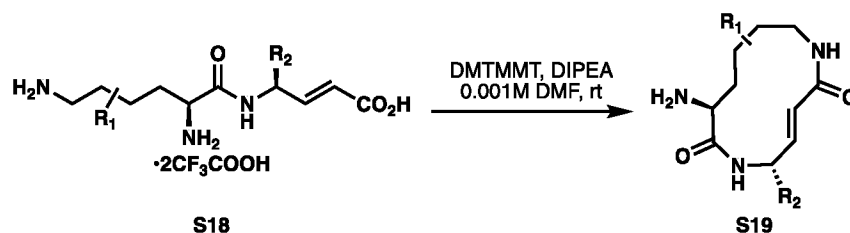
Ylide **33** (1.25 equiv) was dissolved in freshly-distilled anhydrous acetonitrile (0.2 M), then a solution of aldehyde **S16** (1 equiv) in acetonitrile (0.2 M) was added dropwise at room temperature. The reaction was stirred for 30 minutes or until completion by TLC, and then concentrated to dryness. Purification by silica flash chromatography using a mixture of DCM/EtOAc/MeOH as eluent provided **S17** as an off-white solid.

General Procedure D: global deprotection



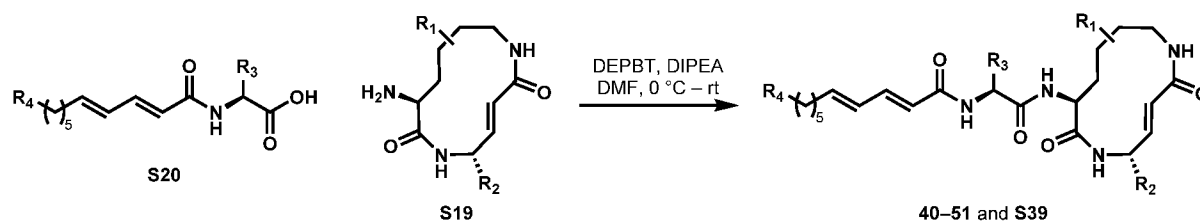
Dipeptide **S17** (1 equiv) was dissolved (0.04 M) in a freshly prepared solution of “Reagent B” (88:5:5:2 TFA:PhOH:H₂O:*i*Pr₃SiH) and the mixture was stirred for 1 hour at rt or until completion by LCMS. The reaction was diluted with toluene and concentrated *in vacuo* to dryness, then evaporated twice more from 30 mL toluene. The residue was dissolved in minimal MeOH and triturated into diethyl ether at 0 °C. The solids were collected by centrifugation (4 °C, 10 min, 4200 rpm), then triturated into ether and centrifuged as above once more, then dried from MeOH to provide pure **S18** as an off-white solid.

General Procedure E: macrolactamization



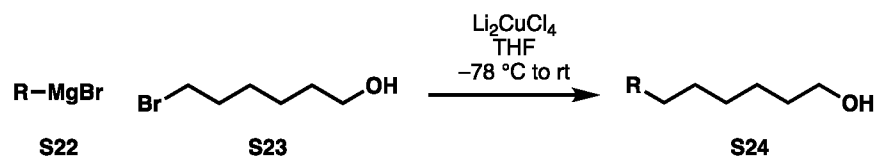
Linear macrocycle precursor **S18** (1 equiv) was dissolved anhydrous DMF (0.001 M) and treated with diisopropylethylamine (4 equiv) then 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate (DMTMMT) (1.50 equiv) at rt. The reaction was stirred under argon for 36 hours, then quenched with 10% v/v H₂O and concentrated *in vacuo* to dryness. The residue was dissolved in minimal methanol and triturated into diethyl ether with rapid stirring. The solids were collected by centrifugation (4 °C, 15 min, 4200 rpm) and dried from MeOH to provide **S19** as a tan solid. Yield was determined by ¹H NMR analysis with 4-toluenesulfonamide as internal standard.

General Procedure F: fragment coupling



Carboxylic acid **S20** (2 equiv.) and crude macrocycle **S19** (1 equiv as judged by ^1H NMR analysis) were dissolved in anhydrous DMF (0.08 M) and cooled to 0 °C. To the mixture was added diisopropylethylamine (6 equiv), followed by DEPBT (2 equiv). The reaction was warmed to room temperature and stirred overnight or until completion by LCMS. The reaction was quenched at 0 °C 30 % v/v H_2O then concentrated *in vacuo* to dryness. The residue was triturated into 50 mL Et_2O at 0 °C from minimal MeOH, and the resulting solids were collected by centrifugation (4 °C, 15 min, 4200 rpm). The solids were dissolved in DMSO, filtered through a 0.2 mm PTFE membrane filter, then purified by preparative reversed-phase HPLC using a gradient of MeCN/ H_2O /0.1% formic acid mixtures over 35 minutes to provide **40-51** and **S39** as fluffy white powders after lyophilization.

General Procedure G: Kochi coupling

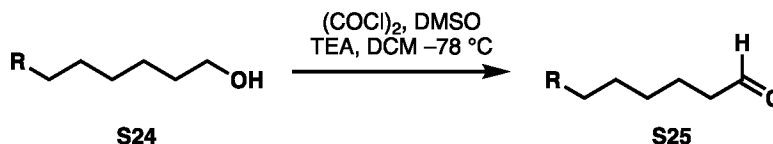


Following a procedure adapted from Melaugh et al.,^{S5} lithium chloride (2.05 equiv) was flame-dried in a round-bottom flask under high vacuum and purged with argon (3x). Solid copper(II) chloride (1 equiv) was added under argon. The combined reagents were then dissolved in anhydrous THF (0.7 M with respect to CuCl_2) with rapid stirring to provide a dark red solution of Li_2CuCl_4 .

Grignard reagent **S22** was used as provided from commercial sources or prepared as described previously.^{S6} A flame-dried flask was charged with **S22** (6.5 equiv) in anhydrous THF (1 M). The mixture was cooled to -78 °C and **S23** (1 equiv) was added in anhydrous THF (7 M), followed by the above solution of Li_2CuCl_4 (1.1 equiv with respect to **S24**). Upon complete addition, the reaction was removed from its cooling bath and allowed to warm to room temperature. Reaction progress was monitored by TLC until completion at 1 h. The reaction was then carefully quenched at 0 °C with saturated NH_4Cl and filtered through a sintered glass funnel. The filtrate was concentrated *in vacuo* to remove most of the THF, then

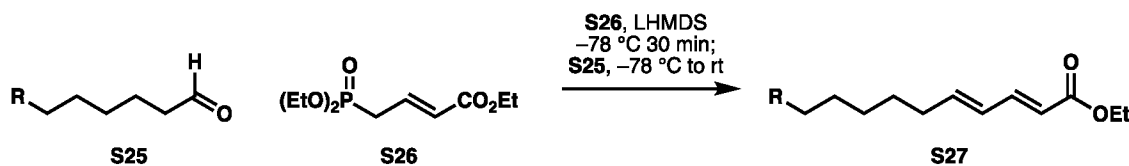
diluted with ethyl acetate and 1 M HCl to a final pH = 1. The layers were separated, and the aqueous phase was extracted three times with ethyl acetate. The combined organic layers were washed with H₂O, NaHCO₃ and brine, then dried over Na₂SO₄, filtered and concentrated. Purification by silica flash chromatography using hexanes/EtOAc as eluent provided pure **S24** as a colorless liquid.

General Procedure H: Swern oxidation



Oxalyl chloride (1.5 equiv) was dissolved in anhydrous DCM (1.3 M) at -78°C . A solution of anhydrous DMSO (3 equiv) in anhydrous DCM (7 M) was added dropwise and the resulting mixture was stirred at -78°C for 15 minutes. A solution of **S24** (1 equiv) in anhydrous DCM (1 M) was then added dropwise over 10 minutes. The mixture was stirred at -78°C for 1 hour then treated with triethylamine (5 equiv) dropwise. The resulting thick slurry was diluted with additional DCM to aid stirring, and reaction progress was monitored by TLC until completion at 1 hour. The reaction was quenched at -78°C with H₂O and allowed to stir to room temperature. The layers were separated, and the aqueous layer was extracted three times with DCM. The combined organic layers were washed with H₂O, brine, then dried over Na₂SO₄ and filtered through a plug of silica with ether as eluent. Concentration *in vacuo* provided **S25** as a colorless oil which was used immediately in the next reaction (General Procedure I) without further purification.

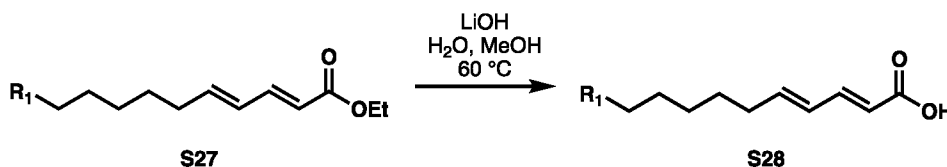
General Procedure I: Allylic HWE



In a procedure adapted from Imker et al.,^{S7} **S26** (1.5 equiv) in anhydrous THF (0.5 M) was cooled to -78°C and treated with lithium hexamethyldisilazide (1 M THF, 1.4 equiv) dropwise, then allowed to stir under argon at -78°C for 30 min. Aldehyde **S25** (1 equiv) in anhydrous THF (1.4 M) was added dropwise at -78°C and the mixture was allowed to stir to

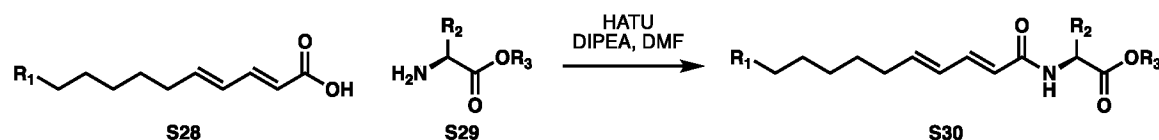
room temperature overnight or until completion by TLC. The reaction was quenched at 0 °C with saturated NH₄Cl solution. The layers were separated and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with H₂O, NaHCO₃, brine and dried over Na₂SO₄, filtered and concentrated. Purification by silica flash chromatography using hexanes/Et₂O as eluent provided **S27** as a light yellow oil.

General Procedure J: ester hydrolysis



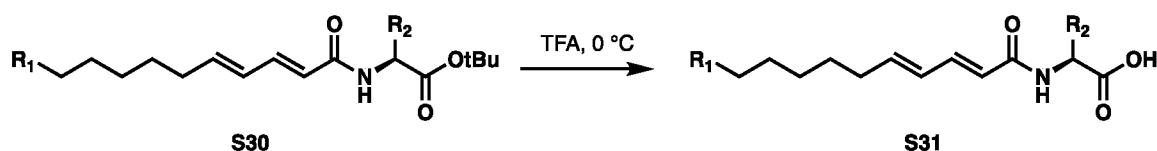
Ester **S27** (1 equiv) was dissolved in MeOH (0.2 M). With rapid stirring, an aqueous solution of lithium hydroxide (1 M, 5 equiv) was added, and the reaction was heated to 60 °C for 5 hours or until completion by TLC. Methanol was evaporated *in vacuo*, then the mixture was adjusted to pH 1 with 1 M HCl and diluted with 20 mL DCM. The layers were separated, and the aqueous layer was extracted three times with DCM. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated to provide pure **S28** as a waxy white solid.

General Procedure K: amide coupling



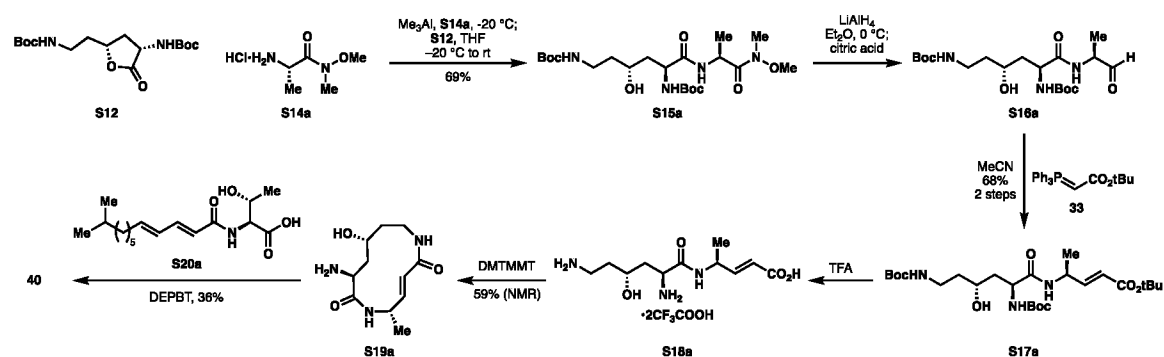
Fatty acid **S28** (1 equiv) was combined with amine **S29** (1–1.5 equiv) in anhydrous DMF (0.2 M). Diisopropylethylamine was added (1–3 eq), followed by HATU (1.05 equiv). The reaction was stirred at room temperature under argon for 4 hours or until completion by LCMS. The reaction was then quenched with 10% v/v H₂O and concentrated *in vacuo*. The residue was dissolved in ethyl acetate and washed with 1M HCl (2x), H₂O, NaHCO₃, and brine, then dried over Na₂SO₄, filtered and concentrated. Purification by flash chromatography using hexanes:EtOAc as eluent provided **S30** as a colorless oil.

General Procedure L: tail fragment deprotection

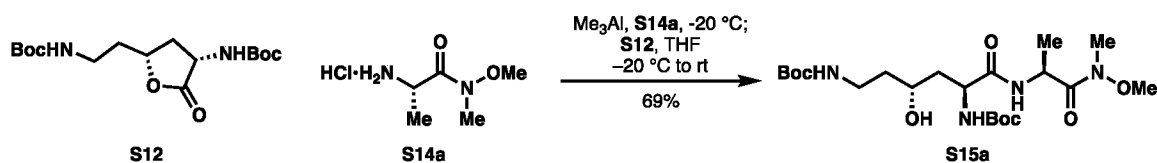


Amide **S30** (1 equiv) was dissolved in TFA (0.1 M) at 0 °C and stirred for 5 min, then stirred at rt for 1 hour. The reaction was then diluted with toluene and concentrated *in vacuo* to dryness. The residue was evaporated twice more from toluene to provide **S31** as a pale yellow resin.

Scheme 5.S2 Synthesis route to epi-4-OH cepafungin (**40**)



di-tert-butyl ((3*R*,5*S*)-3-hydroxy-6-(((*S*)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S15a**)**



Lactone **S12** was prepared as described above in Table 5.S1, entry 8. Amine **S14a** was prepared as described in a previous report.^{S6} Following General Procedure A: **S12** (638 mg, 1.85 mmol, 1 equiv), **S14a** (1.56 g, 9.26 mmol, 5 equiv) and Me₃Al (4.58 mL 2 M toluene, 9.16 mmol, 4.95 equiv) in THF (28 mL) were used to produce **S15a** (609 mg, 69%) as a white solid.

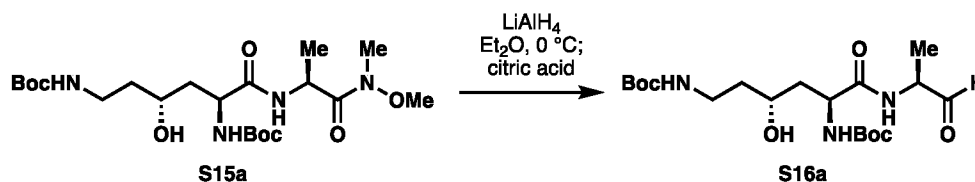
¹H NMR (600 MHz, CDCl₃): δ 7.10 – 6.92 (m, 1H), 6.01 – 5.72 (m, 1H), 4.95 – 4.84 (m, 1H), 4.36 (q, *J* = 6.3, 5.8 Hz, 1H), 3.76 (s, 4H), 3.41 (qd, *J* = 8.5, 5.5, 4.6 Hz, 1H), 3.20 (s, 3H), 3.10 (dt, *J* = 14.2, 5.4 Hz, 1H), 2.96 (s, 1H), 1.79 (t, *J* = 6.5 Hz, 2H), 1.64 – 1.49 (m, 2H), 1.46 – 1.40 (m, 19H), 1.34 (d, *J* = 6.9 Hz, 3H).

¹³C NMR (151 MHz, CDCl₃): δ 172.8, 171.4, 157.0, 156.6, 80.4, 79.6, 66.5, 61.7, 52.3, 46.0, 40.9, 37.8, 37.5, 32.3, 28.5, 28.4, 18.4.

HRMS (ESI): calculated for C₂₁H₄₁N₄O₈⁺ ([M+H]⁺) 477.2919, found 477.2920

$$[\alpha]_D^{27} = -24.4 (c\ 1.4, \text{MeOH})$$

di-*tert*-butyl ((3*R*,5*S*)-3-hydroxy-6-oxo-6-(((*S*)-1-oxopropan-2-yl)amino)hexane-1,5-diyl)dicarbamate (S16a)

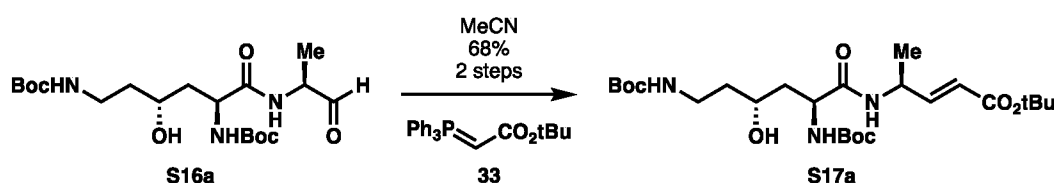


Following General Procedure B: **S15a** (300 mg, 0.630 mmol, 1 equiv) and LAH (60 mg, 1.57 mmol, 2.5 equiv) in Et₂O (7.9 mL) were used to provide **S16a** (323 mg) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 9.53 (s, 1H), 7.24 (d, J = 5.0 Hz, 1H), 6.12 (d, J = 7.4 Hz, 1H), 4.91 (s, 1H), 4.68 (s, 1H), 4.45 – 4.34 (m, 2H), 3.84 – 3.78 (m, 1H), 3.13 – 3.01 (m, 1H), 1.98 – 1.88 (m, 1H), 1.87 – 1.73 (m, 2H), 1.64 – 1.48 (m, 2H), 1.43 (d, J = 7.0 Hz, 18H), 1.35 (d, J = 7.4 Hz, 3H).

HRMS (ESI): calculated for C₁₉H₃₆N₃O₇⁺ ([M+H]⁺) 418.2548, found 418.2553

***tert*-butyl (*S*,*E*)-4-((2*S*,4*R*)-2,6-bis((*tert*-butoxycarbonyl)amino)-4-hydroxyhexanamido)pent-2-enoate (S17a)**



Following General Procedure C: **S16a** (94 mg, 0.225 mmol, 1 equiv), **33** (106 mg, 0.281 mmol, 1.25 equiv) in MeCN (3.2 mL) were used to provide **S17a** (80 mg, 68% over 2 steps) as a white solid.

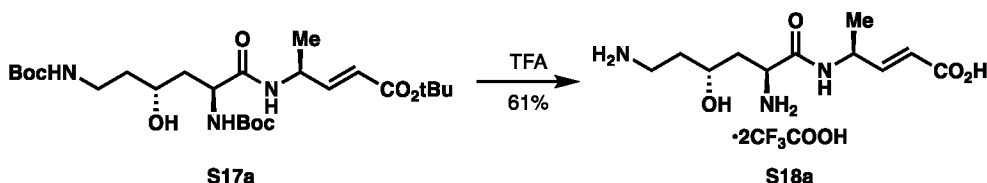
¹H NMR (600 MHz, Methanol-*d*₄): δ 6.77 (dd, J = 15.7, 4.9 Hz, 1H), 5.81 (dd, J = 15.7, 1.7 Hz, 1H), 4.63 – 4.56 (m, 1H), 4.27 – 4.15 (m, 1H), 3.75 – 3.66 (m, 1H), 3.15 (td, J = 6.8, 4.6 Hz, 2H), 1.79 – 1.68 (m, 2H), 1.63 – 1.55 (m, 2H), 1.48 – 1.42 (m, 27H), 1.28 (d, J = 7.1 Hz, 3H).

¹³C NMR (151 MHz, Methanol-*d*₄): δ 174.88, 167.38, 158.82, 157.96, 149.24, 122.79, 81.72, 80.76, 80.03, 66.53, 53.70, 46.91, 40.42, 39.11, 38.11, 28.83, 28.77, 28.35, 19.91.

HRMS (ESI): calculated for C₂₅H₄₆N₃O₈⁺ ([M+H]⁺) 516.3279, found 516.3289

$$[\alpha]_D^{27} = -18.2 \text{ (c 1.1, MeOH)}$$

(*S,E*)-4-((2*S*,4*R*)-2,6-diamino-4-hydroxyhexanamido)pent-2-enoic acid (S18a)



Following General Procedure D: **S17a** (336 mg, 0.652 mmol, 1 equiv) was used to provide **S18a** (194 mg, 61%) as a white solid.

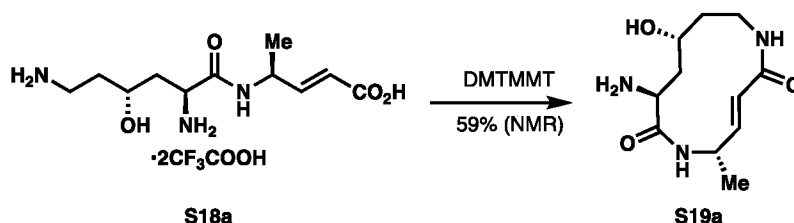
¹H NMR (400 MHz, Methanol-*d*₄): δ 6.84 (dd, *J* = 15.7, 5.3 Hz, 1H), 5.86 (dd, *J* = 15.7, 1.6 Hz, 1H), 4.70 – 4.61 (m, 1H), 4.08 (t, *J* = 5.7 Hz, 1H), 3.94 – 3.84 (m, 1H), 3.15 – 3.00 (m, 2H), 2.08 – 1.98 (m, 2H), 1.92 – 1.75 (m, 2H), 1.33 (d, *J* = 7.0 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 170.0, 169.3, 149.3, 122.5, 66.9, 52.4, 47.4, 38.7, 38.0, 35.8, 19.7.

HRMS (ESI): calculated for C₁₁H₂₂N₃O₄⁺ ([M+H]⁺) 260.1605, found 260.1606

$$[\alpha]_D^{27} = -17.4 \text{ (c 0.5, MeOH)}$$

(5*S*,8*S*,10*R*,*E*)-8-amino-10-hydroxy-5-methyl-1,6-diazacyclododec-3-ene-2,7-dione (S19a)



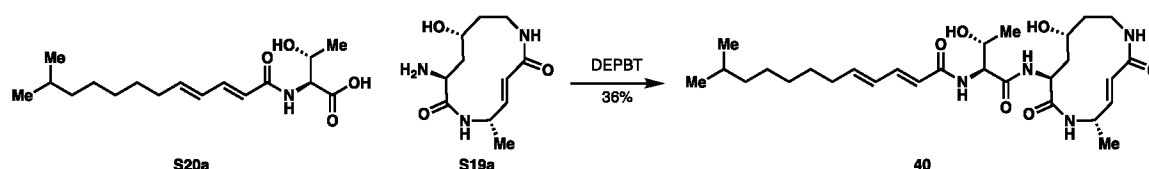
Following General Procedure E: **S18a** (163 mg, 0.335 mmol, 1 equiv) was used to provide **S19a** (276 mg, 59%) as a tan solid. Yield was determined by ¹H NMR analysis of a 6.2 mg sample of crude **S19a** and 5.1 mg 4-toluenesulfonamide added as internal standard.

¹H NMR (400 MHz, Methanol-*d*₄): 4-toluenesulfonamide: δ 7.85 – 7.70 (m, 2H), 7.41 – 7.28 (m, 2H), 2.42 (s, 3H). Compound **S19a**: δ 7.07 (ddd, *J* = 15.3, 11.8, 4.8 Hz, 1H), 6.29 (ddd, *J* = 15.4, 7.8, 1.3 Hz, 1H), 4.69 – 4.60 (m, 1H), 4.56 (q, *J* = 6.5 Hz, 1H), 3.49 – 3.35 (m, 2H),

2.49 (dd, $J = 16.1, 3.7$ Hz, 1H), 2.11 – 1.96 (m, 1H), 1.87 (ddd, $J = 15.7, 10.5, 3.4$ Hz, 1H), 1.80 – 1.58 (m, 3H).

HRMS (ESI): calculated for $C_{11}H_{20}N_3O_3^+$ ($[M+H]^+$) 242.1499, found 242.1495

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*R*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11-methyldodeca-2,4-dienamide (40)



Following General Procedure F: Carboxylic acid **S20a** was synthesized as described previously.² **S20a** (38 mg, 0.121 mmol, 2 equiv), **S19a** (85 mg, 0.0604 mmol by ^1H NMR analysis, 1 equiv), DEPBT (36 mg, 0.121 mmol, 2 equiv) and DIPEA (63 μL , 0.362 mmol, 6 equiv) were used to provide **40** as a fluffy white solid (11.6 mg, 36%).

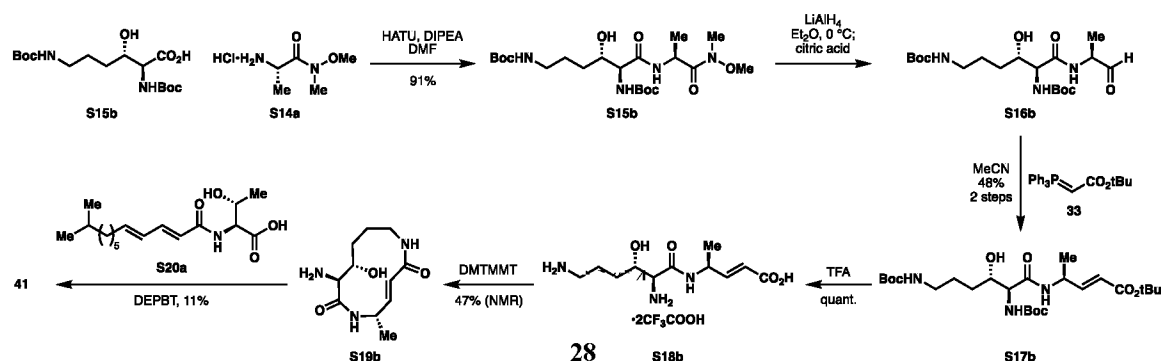
^1H NMR (600 MHz, DMSO- d_6): δ 8.42 (d, $J = 7.8$ Hz, 1H), 8.07 (d, $J = 7.7$ Hz, 1H), 7.92 (d, $J = 8.6$ Hz, 1H), 7.42 (dd, $J = 8.0, 6.1$ Hz, 1H), 6.99 (dd, $J = 15.1, 10.7$ Hz, 1H), 6.79 (dd, $J = 15.3, 4.7$ Hz, 1H), 6.22 – 6.04 (m, 4H), 4.97 (d, $J = 3.3$ Hz, 1H), 4.84 (d, $J = 5.7$ Hz, 1H), 4.48 (dt, $J = 7.7, 3.8$ Hz, 1H), 4.38 (tdd, $J = 12.3, 6.3, 3.8$ Hz, 1H), 4.26 (dd, $J = 8.6, 4.0$ Hz, 1H), 4.03 – 3.93 (m, 1H), 3.55 – 3.47 (m, 1H), 3.20 – 3.10 (m, 1H), 3.02 – 2.92 (m, 1H), 2.43 – 2.33 (m, 1H), 2.12 (q, $J = 7.2$ Hz, 2H), 1.58 – 1.47 (m, 3H), 1.47 – 1.41 (m, 1H), 1.41 – 1.35 (m, 2H), 1.28 – 1.22 (m, 4H), 1.20 (d, $J = 7.1$ Hz, 3H), 1.16 – 1.11 (m, 2H), 1.03 (d, $J = 6.3$ Hz, 3H), 0.84 (d, $J = 6.6$ Hz, 6H).

^{13}C NMR (151 MHz, DMSO- d_6): δ 170.9, 169.3, 165.5, 165.4, 147.3, 142.0, 139.7, 128.6, 123.0, 118.3, 66.5, 64.4, 58.2, 51.8, 45.7, 41.1, 38.4, 37.9, 34.8, 32.2, 28.9, 28.4, 27.4, 26.6, 22.5, 19.4, 18.3.

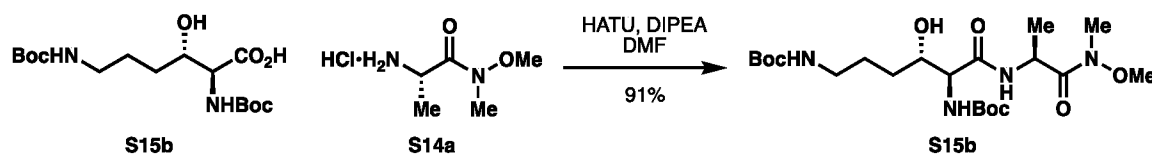
HRMS (ESI): calculated for $C_{28}H_{47}N_4O_6^+$ ($[M+H]^+$) 535.3490, found 535.3474

$$[\alpha]_D^{27} = -51.5 \text{ (c 0.1, MeOH)}$$

Scheme 5.S3 Synthesis route to 3-OH-cepafungin (**41**).



di-*tert*-butyl ((4*S*,5*S*)-4-hydroxy-6-(((*S*)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (**S15b**)



Compound **S15b** was prepared as described in a previously reported procedure using lysine hydroxylase KDO1.^{S1} Amine **S14a** was prepared as described previously.^{3x} Following General Procedure K: **S15b** (1.00 g, 2.76 mmol, 1 equiv), **S14a** (698 mg, 4.14 mmol, 1.5 equiv), HATU (1.10 g, 2.90 mmol, 1.05 equiv) and DIPEA (1.44 mL, 8.28 mmol, 3 equiv) were used to provide **S15b** (1.20 g, 91%) as a white solid.

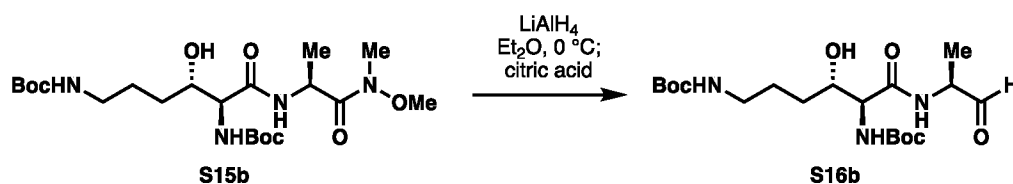
¹H NMR (400 MHz, Methanol-*d*₄): δ 4.83 (d, *J* = 7.2 Hz, 1H), 4.11 – 3.92 (m, 1H), 3.82 (s, 3H), 3.76 – 3.68 (m, 1H), 3.21 (s, 3H), 3.14 – 2.98 (m, 2H), 1.71 – 1.49 (m, 3H), 1.49 – 1.37 (m, 19H), 1.34 (d, *J* = 7.1 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 174.7, 173.1, 158.6, 157.8, 80.8, 79.8, 72.4, 62.1, 60.4, 47.5, 41.1, 32.5, 31.2, 28.9, 28.7, 27.1, 17.1.

HRMS (ESI): calculated for C₂₁H₄₁N₄O₈⁺ ([M+H]⁺) 477.2919, found 477.2916

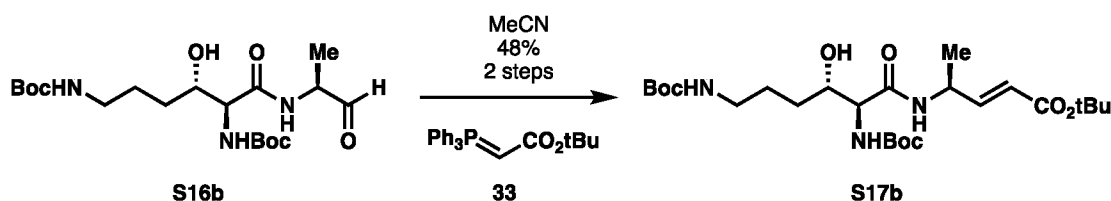
[α]_D²⁷ = −28.1 (*c* 0.5, MeOH)

di-*tert*-butyl ((4*S*,5*S*)-4-hydroxy-6-oxo-6-(((*S*)-1-oxopropan-2-yl)amino)hexane-1,5-diyl)dicarbamate (**S16b**)



Following General Procedure B: **S15b** (500 mg, 1.05 mmol, 1 equiv) and LAH (149 mg, 3.94 mmol, 3.75 equiv) were used to provide **S16b** (332 mg) as a white solid used without further purification.

***tert*-butyl (*S,E*)-4-((2*S*,3*S*)-2,6-bis((*tert*-butoxycarbonyl)amino)-3-hydroxyhexanamido)pent-2-enoate (**S17b**)**



Following General Procedure C: **S16b** (332 mg) and **33** (374 mg, 0.994 mmol, 1.25 equiv) were used to provide **S17b** (262 mg, 48% over 2 steps) as a white solid.

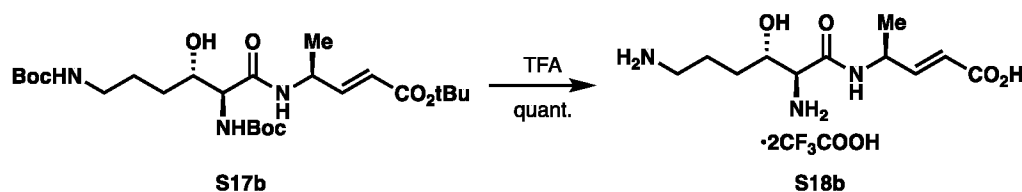
¹H NMR (400 MHz, Methanol-*d*₄): δ 6.78 (dd, J = 15.6, 4.8 Hz, 1H), 5.89 (dd, J = 15.7, 1.8 Hz, 1H), 4.66 – 4.57 (m, 1H), 4.00 (d, J = 6.9 Hz, 1H), 3.74 (ddd, J = 9.7, 6.9, 2.6 Hz, 1H), 3.05 (td, J = 6.6, 3.9 Hz, 2H), 1.74 – 1.49 (m, 4H), 1.47 (s, 9H), 1.46 (s, 9H), 1.43 (s, 9H), 1.29 (d, J = 7.1 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 172.7, 167.5, 158.6, 157.7, 149.2, 122.9, 81.7, 80.8, 79.9, 72.1, 60.9, 47.0, 41.2, 31.7, 28.8, 28.7, 28.4, 27.2, 19.9.

HRMS (ESI): calculated for C₂₅H₄₆N₃O₈⁺ ([M+H]⁺) 516.3279, found 516.3277

$$[\alpha]_D^{27} = -33.5 \text{ (} c \text{ 1.1, MeOH)}$$

(*S,E*)-4-((2*S*,3*S*)-2,6-diamino-3-hydroxyhexanamido)pent-2-enoic acid (S18b**)**



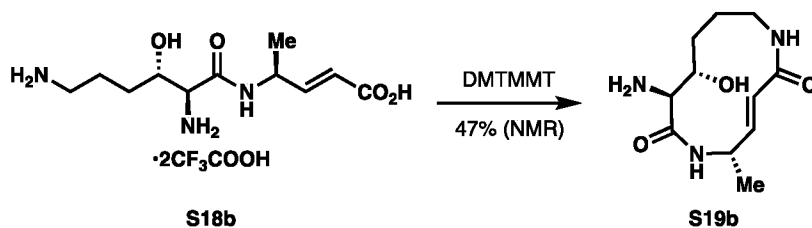
Following General Procedure D: **S17b** (100 mg, 0.194 mmol, 1 equiv) was used to provide **S18b** (100 mg, ~quant.) as a white solid.

¹H NMR (400 MHz, Methanol-*d*₄): δ 6.87 (dd, J = 15.7, 5.3 Hz, 1H), 5.88 (dd, J = 15.7, 1.6 Hz, 1H), 4.67 (qdd, J = 7.0, 5.1, 1.7 Hz, 1H), 4.07 – 3.99 (m, 1H), 3.93 (d, J = 4.9 Hz, 1H), 2.97 (ddt, J = 8.9, 6.7, 3.5 Hz, 2H), 2.00 – 1.87 (m, 1H), 1.82 – 1.69 (m, 1H), 1.55 – 1.48 (m, 2H), 1.33 (d, J = 7.0 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 169.6, 167.0, 149.8, 122.0, 70.0, 59.2, 47.4, 40.5, 29.6, 25.4, 19.6.

HRMS (ESI): calculated for C₁₁H₂₂N₃O₄⁺ ([M+H]⁺) 260.1605, found 260.1611

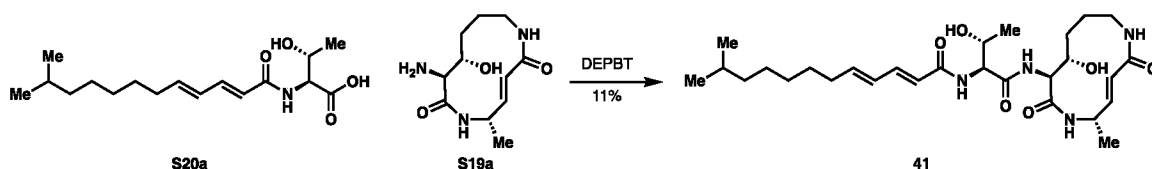
$$[\alpha]_D^{24} = -6.6 \text{ (} c \text{ 1.2, MeOH)}$$

(5*S*,8*S*,9*S*,*E*)-8-amino-9-hydroxy-5-methyl-1,6-diazacyclododec-3-ene-2,7-dione (S19b)

Following General Procedure E: **S18b** (80 mg, 0.164 mmol, 1 equiv) was used to provide **S19b** (57 mg, 47%) as a tan solid. Yield was determined by ^1H NMR analysis of a 6.2 mg sample of crude **S18b** and 6.5 mg of 4-toluenesulfonamide added as internal standard.

^1H NMR (400 MHz, DMSO- d_6): 4-toluenesulfonamide: δ 7.70 (d, J = 7.8 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H), 7.27 (br s, 2H), 2.37 (s, 3H). Compound **S19b**: δ 8.61 (d, J = 7.4 Hz, 1H), 6.78 (d, J = 14.2 Hz, 1H), 6.19 (d, J = 15.1 Hz, 1H), 4.98 – 4.72 (m, 2H), 4.45 – 4.26 (m, 2H), 3.06 – 2.92 (m, 1H), 1.73 (d, J = 11.4 Hz, 1H), 1.56 – 1.46 (m, 1H).

HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{20}\text{N}_3\text{O}_3^+$ ($[\text{M}+\text{H}]^+$) 242.1499, found 242.1497

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,9*S*,*E*)-9-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11-methyldodeca-2,4-dienamide (41)

Following General Procedure F: Carboxylic acid **S20a** was synthesized as described previously.^{S6} **S20a** (39 mg, 0.125 mmol, 2 equiv), **S19a** (38 mg, 0.0626 mmol as judged by ^1H NMR, 1 equiv), DEPBT (37 mg, 0.125 mmol, 2 equiv) and DIPEA (65 μL , 0.375 mmol, 6 equiv) were used to provide **41** (3.6 mg, 11%) as a fluffy white solid.

^1H NMR (600 MHz, DMSO- d_6): δ 8.61 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.35 (dd, J = 8.3, 6.0 Hz, 1H), 6.99 (dd, J = 15.1, 10.8 Hz, 1H), 6.78 (dd, J = 15.3, 4.7 Hz, 1H), 6.21 – 6.13 (m, 3H), 6.12 – 6.06 (m, 1H), 4.97 (d, J = 4.8 Hz, 1H), 4.80 (d, J = 6.5 Hz, 1H), 4.70 (dd, J = 7.9, 3.9 Hz, 1H), 4.44 (dd, J = 8.4, 4.3 Hz, 1H), 4.39 – 4.32 (m, 1H), 4.31 – 4.22 (m, 1H), 4.03 – 3.90 (m, 1H), 3.03 – 2.90 (m, 1H), 2.12 (q, J = 7.2 Hz, 2H), 1.71 (td, J = 12.4, 4.5 Hz, 1H), 1.55 – 1.45 (m, 1H), 1.38 (p, J = 7.2 Hz, 2H), 1.34 – 1.28 (m,

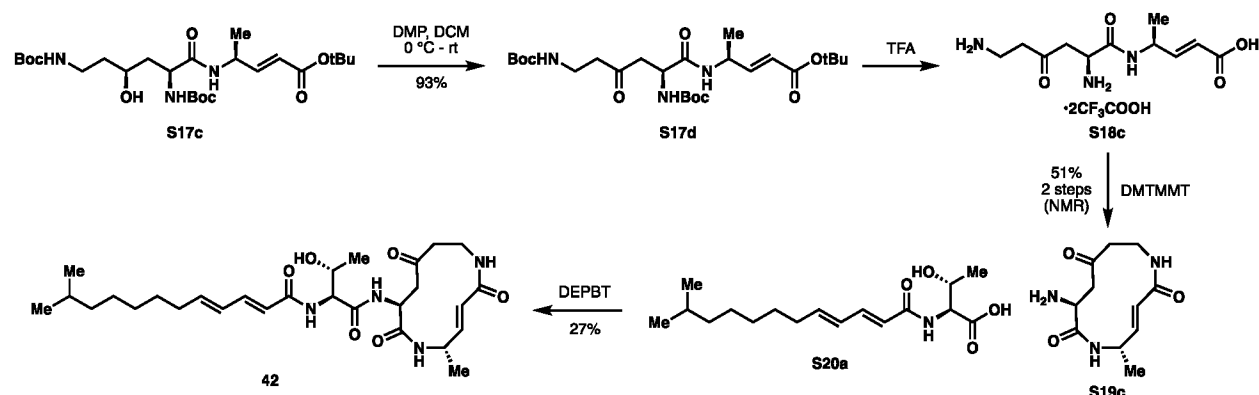
2H), 1.27 – 1.23 (m, 4H), 1.19 (d, $J = 7.1$ Hz, 3H), 1.16 – 1.12 (m, 3H), 1.04 (d, $J = 6.3$ Hz, 3H), 0.84 (d, $J = 6.6$ Hz, 6H).

^{13}C NMR (151 MHz, DMSO- d_6): δ 170.7, 169.2, 165.6, 165.4, 146.9, 142.0, 139.7, 128.6, 123.1, 118.7, 67.5, 67.0, 58.0, 57.7, 45.9, 38.4, 37.8, 32.2, 28.9, 28.4, 27.4, 26.6, 26.4, 25.0, 22.5, 19.1, 18.2.

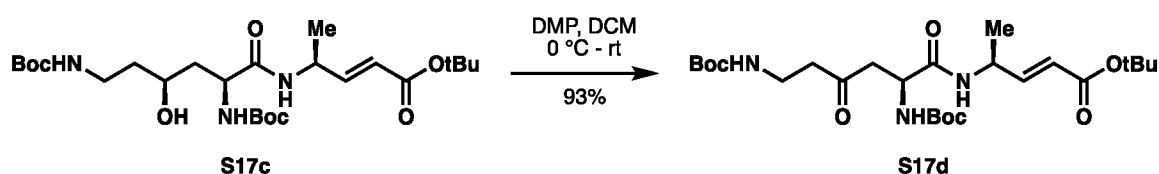
HRMS (ESI): calculated for $\text{C}_{28}\text{H}_{47}\text{N}_4\text{O}_6^+$ ($[\text{M}+\text{H}]^+$) 535.3490, found 535.3488

$$[\alpha]_D^{27} = -32.2 \text{ (c 0.1, MeOH)}$$

Scheme 5.S4. Synthesis route to keto-cepafungin 42.



tert-butyl (S,E)-4-((S)-2,6-bis((*tert*-butoxycarbonyl)amino)-4-oxohexanamido)pent-2-enoate (S17d)



Compound **S17c** was synthesized as previously reported.^{S6} A 50 mL round-bottom flask was charged with **S17c** (171 mg, 0.332 mmol, 1 equiv), dissolved in 17 mL anhydrous DCM, and cooled to 0 °C. Dess-Martin Periodinane (169 mg, 0.398 mmol, 1.2 equiv) was added and the reaction was stirred under argon to rt overnight. The mixture was diluted with 6 mL Et₂O, filtered through a plug of celite and concentrated. The residue was purified by flash chromatography (70:29:1 DCM:EtOAc:MeOH) to provide **S17d** (159 mg, 93%) as a white solid.

^1H NMR (400 MHz, Methanol- d_4): δ 6.77 (dd, $J = 15.7, 4.8$ Hz, 1H), 5.83 (dd, $J = 15.7, 1.8$ Hz, 1H), 4.62 – 4.52 (m, 1H), 4.42 (d, $J = 6.6$ Hz, 1H), 3.28 (t, $J = 7.1$ Hz, 2H), 2.87 (tt, $J =$

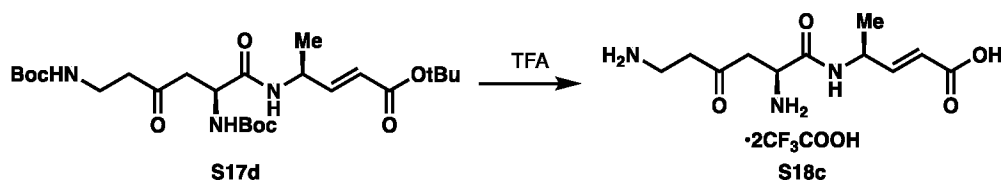
17.4, 9.5 Hz, 2H), 2.65 (t, $J = 6.5$ Hz, 2H), 1.47 (s, 9H), 1.46 (s, 9H), 1.42 (s, 9H), 1.28 (d, $J = 7.1$ Hz, 3H).

^{13}C NMR (101 MHz, Methanol- d_4): δ 208.0, 173.5, 167.4, 158.3, 157.7, 149.1, 122.9, 81.7, 80.9, 80.1, 52.0, 47.1, 45.0, 43.7, 36.4, 28.8, 28.7, 28.4, 19.8.

HRMS (ESI): calculated for $\text{C}_{25}\text{H}_{44}\text{N}_3\text{O}_8^+$ ($[\text{M}+\text{H}]^+$) 514.3123, found 514.3122

$[\alpha]_D^{27} = -17.3$ (c 1.5, MeOH)

(*S,E*)-4-((*S*)-2,6-diamino-4-oxohexanamido)pent-2-enoic acid (S18c**)**

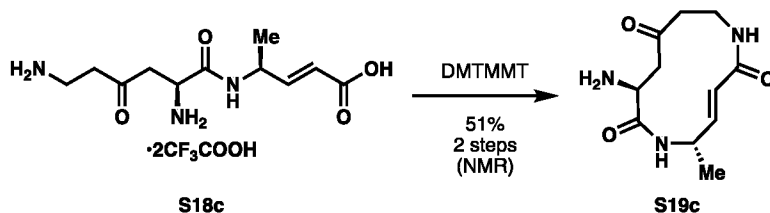


Following General Procedure D: **S17d** (76 mg, 0.148 mmol, 1 equiv) was used to provide **S18c** (71 mg) as a white solid used without further purification.

^1H NMR (600 MHz, Methanol- d_4): δ 6.81 (dd, $J = 15.7, 5.1$ Hz, 1H), 5.84 (dd, $J = 15.7, 1.7$ Hz, 1H), 4.62 (ddt, $J = 10.5, 7.1, 3.6$ Hz, 1H), 4.25 – 4.19 (m, 1H), 3.26 – 3.10 (m, 4H), 2.98 (q, $J = 6.1$ Hz, 2H), 1.57 (dd, $J = 11.8, 7.0$ Hz, 1H), 1.32 (d, $J = 7.1$ Hz, 3H).

HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{20}\text{N}_3\text{O}_4^+$ ($[\text{M}+\text{H}]^+$) 258.1448, found 258.1447

(*5S,8S,E*)-8-amino-5-methyl-1,6-diazacyclododec-3-ene-2,7,10-trione (S19c**)**

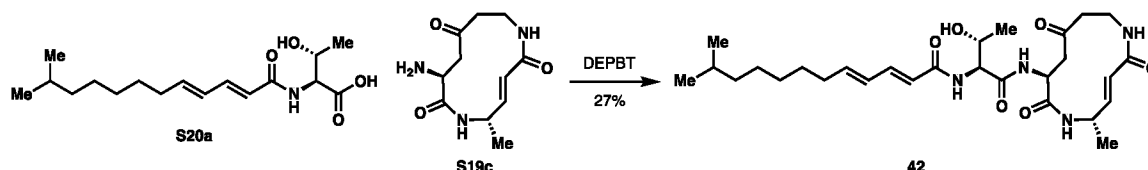


Following General Procedure E: **S18c** (58 mg, 0.119 mmol, 1 equiv) was used to provide **S19c** (72 mg, 51% over 2 steps) as a tan solid. Yield was determined by ^1H NMR analysis of a 2.4 mg sample of crude **S19c** and 2.8 mg of 4-toluenesulfonamide added as internal standard.

^1H NMR (400 MHz, Methanol- d_4): 4-toluenesulfonamide: δ 7.86 – 7.63 (m, 2H), 7.41 – 7.26 (m, 2H), 2.42 (s, 3H). Compound **S19c**: δ 6.86 (dd, $J = 15.5, 5.1$ Hz, 1H), 6.00 – 5.92 (m, 1H), 4.67 – 4.56 (m, 1H), 3.16 – 3.11 (m, 1H), 3.03 – 2.92 (m, 1H), 1.54 (s, 1H), 1.31 (d, $J = 7.2$ Hz, 3H).

HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{18}\text{N}_3\text{O}_3^+$ ($[\text{M}+\text{H}]^+$) 240.1343, found 240.1333

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,*E*)-5-methyl-2,7,10-trioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11-methyldodeca-2,4-dienamide (42)



Following General Procedure F: Compound **S20a** was synthesized by a previously reported procedure.^{S6} **S20a** (38 mg, 0.122 mmol, 2 equiv), **S19c** (69 mg, 0.0612 mmol as judged by ¹H NMR analysis, 1 equiv), DEPBT (37 mg, 0.122 mmol, 2 equiv) and DIPEA (64 μ L, 0.367 mmol, 6 equiv) were used to provide **42** (4.2 mg, 27%) as a fluffy white solid.

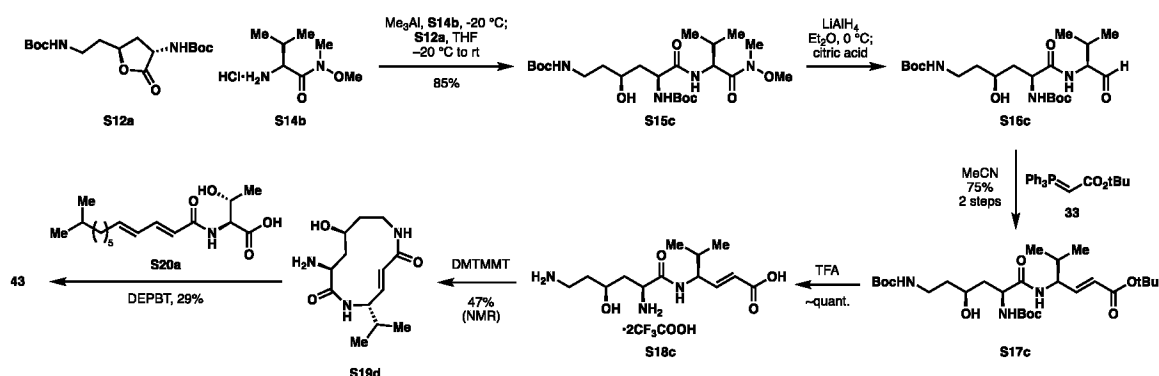
¹H NMR (600 MHz, DMSO-*d*₆): δ 8.60 (d, *J* = 7.1 Hz, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.51 (dd, *J* = 7.1, 5.4 Hz, 1H), 7.00 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.61 (dd, *J* = 15.4, 4.9 Hz, 1H), 6.22 – 6.06 (m, 3H), 5.72 (dd, *J* = 15.4, 1.4 Hz, 1H), 4.93 (d, *J* = 5.1 Hz, 1H), 4.66 (ddd, *J* = 10.2, 7.9, 4.0 Hz, 1H), 4.37 – 4.26 (m, 2H), 4.00 – 3.91 (m, 1H), 3.62 (dq, *J* = 14.9, 4.7 Hz, 1H), 3.04 – 2.95 (m, 1H), 2.95 – 2.82 (m, 2H), 2.67 (dd, *J* = 13.9, 4.0 Hz, 1H), 2.39 (dt, *J* = 18.4, 4.4 Hz, 1H), 2.13 (q, *J* = 7.2 Hz, 2H), 1.53 – 1.46 (m, 1H), 1.38 (p, *J* = 7.2 Hz, 2H), 1.29 – 1.22 (m, 4H), 1.17 – 1.11 (m, 5H), 1.00 (d, *J* = 6.3 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 6H).

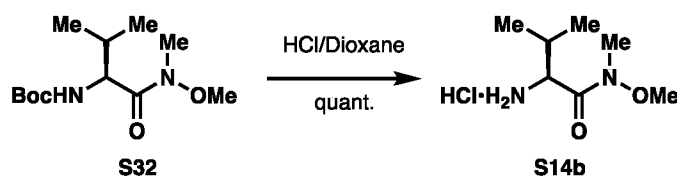
¹³C NMR (151 MHz, DMSO-*d*₆): δ 207.3, 169.7, 169.3, 166.3, 165.5, 146.0, 142.1, 139.8, 128.6, 123.0, 119.5, 66.7, 58.2, 50.4, 46.8, 45.7, 45.6, 38.4, 34.3, 32.2, 28.8, 28.4, 27.4, 26.6, 22.5, 19.8, 18.2.

HRMS (ESI): calculated for C₂₈H₄₅N₄O₆⁺ ([M+H]⁺) 533.3334, found 533.3321

$$[\alpha]_D^{27} = -81.5 (c\ 0.1, \text{MeOH})$$

Scheme 5.S5 Synthetic route to valinyl cepafungin **43**



(S)-2-amino-N-methoxy-N,3-dimethylbutanamide (S14b)

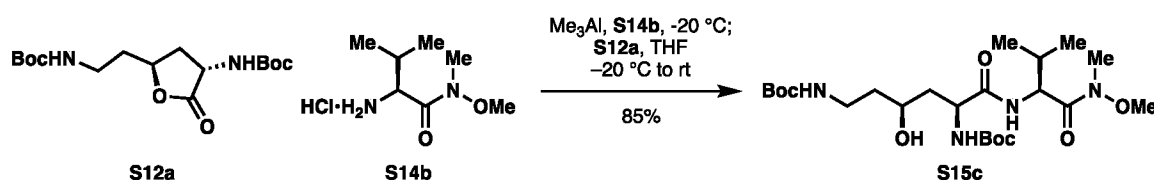
Following a previously reported procedure,^{S6} a flame-dried 100 mL round-bottom flask was charged with **S32** (3.85 g, 14.8 mmol, 1 equiv) and dissolved in 24 mL 4 M HCl/dioxane solution. The mixture was stirred under argon at rt for 1 h, then concentrated in vacuo to dryness. The residue was then evaporated from 50 mL 1:1 MeOH:toluene, then twice again from 50 mL toluene to provide **S14b** (3.19 g, quant.) as a clear, colorless solid used without further purification.

¹H NMR (400 MHz, Methanol-*d*₄): δ 4.22 (d, J = 5.3 Hz, 1H), 3.80 (s, 3H), 3.27 (s, 3H), 2.28 (dhept, J = 7.1, 5.4 Hz, 1H), 1.05 (dd, J = 22.6, 7.0 Hz, 6H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 169.8, 62.3, 56.9, 32.3, 30.8, 19.2, 17.4.

HRMS (ESI): calculated for C₇H₁₇N₂O₂⁺ ([M+H]⁺) 161.1285, found 161.1277

$$[\alpha]_D^{27} = -0.9 \text{ (} c \text{ 1.2, MeOH)}$$

di-tert-butyl ((3*S*,5*S*)-3-hydroxy-6-(((*S*)-1-(methoxy(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S15c)

Compound **S12a** was synthesized as described previously.^{S6} Following General Procedure A: **S12a** (1.02 g, 2.96 mmol, 1 equiv) and **S14b** (3.19 g, 14.8 mmol, 5 equiv) were used to provide **S15c** (1.27 g, 85%) as a white solid.

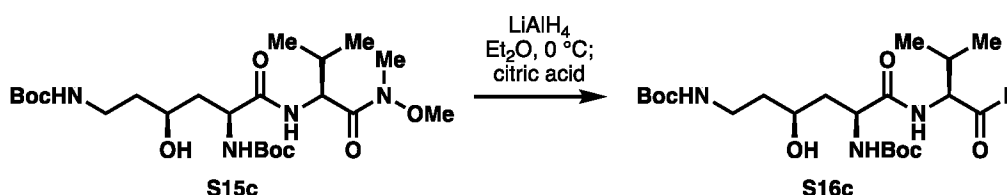
¹H NMR (400 MHz, Methanol-*d*₄): δ 4.81 (d, J = 6.5 Hz, 1H), 4.20 (t, J = 7.4 Hz, 1H), 3.81 (s, 3H), 3.72 – 3.63 (m, 1H), 3.21 (s, 3H), 3.18 – 3.11 (m, 2H), 2.07 (hept, J = 6.8 Hz, 1H), 1.93 – 1.78 (m, 1H), 1.72 – 1.60 (m, 2H), 1.57 – 1.50 (m, 1H), 1.48 – 1.39 (m, 18H), 0.95 (t, J = 6.6 Hz, 6H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 174.9, 173.6, 158.6, 157.7, 80.7, 80.0, 67.3, 62.1, 55.4, 53.7, 40.6, 38.4, 38.1, 32.2, 32.1, 28.8, 28.7, 19.6, 18.4.

HRMS (ESI): calculated for $C_{23}H_{45}N_4O_8^+$ ($[M+H]^+$) 505.3232, found 505.3231

$$[\alpha]_D^{27} = -37.7 \text{ (c 0.6, MeOH)}$$

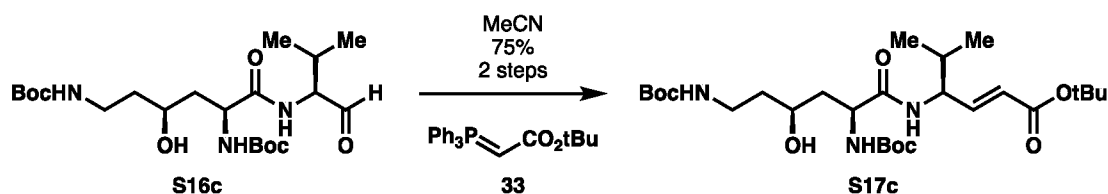
di-tert-butyl ((3*S*,5*S*)-3-hydroxy-6-(((*S*)-3-methyl-1-oxobutan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (S16c)



Following General Procedure B: **S15c** (600 mg, 1.19 mmol, 1 equiv) and LAH (169 mg, 4.46 mmol, 3.75 equiv) were used to provide **S16c** (519 mg) as a white solid that was directly subjected to the next reaction.

¹H NMR (400 MHz, CDCl₃): δ 9.61 (d, *J* = 4.0 Hz, 1H), 7.25 – 7.09 (m, 1H), 5.87 – 5.57 (m, 1H), 4.92 – 4.78 (m, 1H), 4.55 – 4.39 (m, 1H), 4.38 – 4.12 (m, 2H), 3.84 – 3.72 (m, 1H), 3.51 – 3.40 (m, 1H), 3.17 – 3.05 (m, 1H), 2.39 – 2.25 (m, 1H), 2.03 – 1.93 (m, 1H), 1.84 – 1.77 (m, 1H), 1.62 – 1.55 (m, 2H), 1.46 – 1.41 (m, 18H), 1.02 – 0.93 (m, 6H).

tert-butyl (*S*,*E*)-4-((2*S*,4*S*)-2,6-bis((*tert*-butoxycarbonyl)amino)-4-hydroxyhexanamido)-5-methylhex-2-enoate (S17c)



Following General Procedure C: **S16c** (519 mg) was used to provide **S17c** (487 mg, 75% over 2 steps) as a white solid.

¹H NMR (400 MHz, Methanol-*d*₄): δ 6.79 (dd, *J* = 15.7, 5.9 Hz, 1H), 5.86 (dd, *J* = 15.7, 1.6 Hz, 1H), 4.34 (t, *J* = 6.3 Hz, 1H), 4.18 (t, *J* = 7.4 Hz, 1H), 3.70 – 3.61 (m, 1H), 3.16 (dd, *J* = 7.7, 6.0 Hz, 2H), 1.96 – 1.82 (m, 2H), 1.74 – 1.63 (m, 2H), 1.61 – 1.51 (m, 1H), 1.50 – 1.40 (m, 27H), 0.97 – 0.90 (m, 6H).

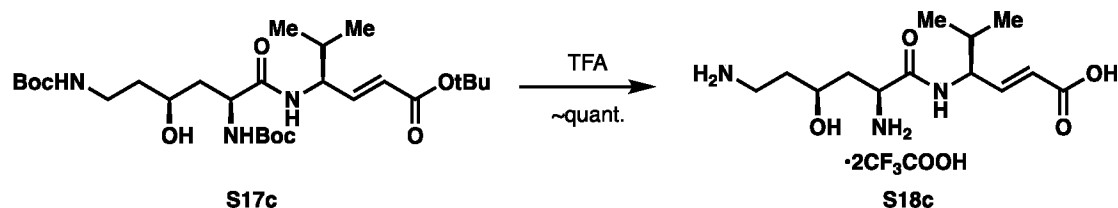
¹³C NMR (101 MHz, Methanol-*d*₄): δ 174.7, 167.2, 158.7, 157.7, 147.1, 124.4, 81.8, 80.7, 80.0, 67.6, 56.9, 54.2, 40.5, 38.4, 38.1, 33.4, 28.8, 28.8, 28.4, 19.6, 18.7.

HRMS (ESI): calculated for $C_{27}H_{50}N_3O_8^+$ ($[M+H]^+$) 544.3592, found 544.3591

$$[\alpha]_D^{27} = -35.3 \text{ (c 0.5, MeOH)}$$

(*S,E*)-4-((2*S*,4*S*)-2,6-diamino-4-hydroxyhexanamido)-5-methylhex-2-enoic acid (S18c**)**

Following General Procedure D: **S17c** (150 mg, 0.276 mmol, 1 equiv) was used to provide



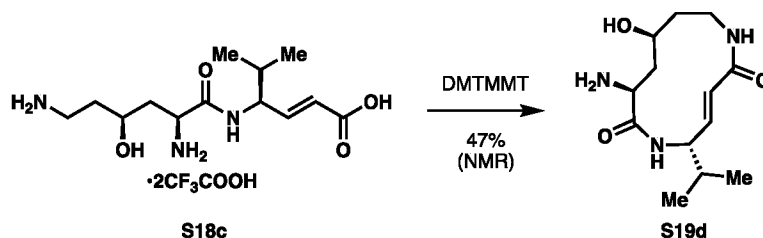
S18c (143 mg, quant.) as a white solid.

¹H NMR (400 MHz, Methanol-*d*₄): δ 6.81 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.90 (dd, *J* = 15.7, 1.4 Hz, 1H), 4.44 – 4.30 (m, 1H), 4.08 (dd, *J* = 8.1, 5.2 Hz, 1H), 4.00 – 3.86 (m, 1H), 3.15 – 2.99 (m, 2H), 2.06 – 1.97 (m, 1H), 1.97 – 1.72 (m, 4H), 1.03 – 0.92 (m, 6H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 169.9, 169.8, 146.9, 124.3, 68.4, 57.7, 53.6, 39.2, 37.8, 36.0, 33.2, 19.3, 19.0.

HRMS (ESI): calculated for C₁₃H₂₆N₃O₄⁺ ([M+H]⁺) 288.1918, found 288.1917

$$[\alpha]_D^{27} = +2.6 \text{ (c 0.4, MeOH)}$$

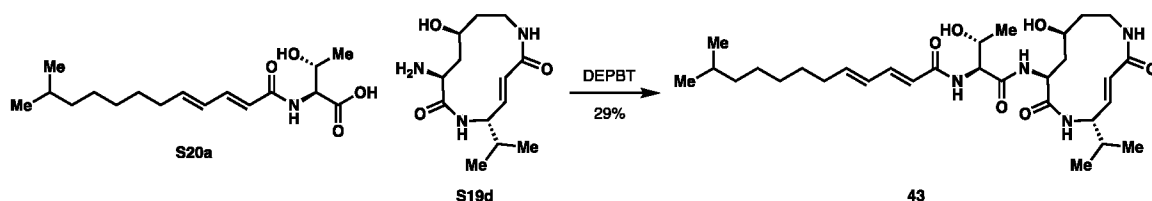
(5*S*,8*S*,10*S*,*E*)-8-amino-10-hydroxy-5-isopropyl-1,6-diazacyclododec-3-ene-2,7-dione (S19d**)**

Following General Procedure E: **S18c** (131 mg, 0.254 mmol, 1 equiv) was used to provide **S19d** (138 mg, 47%). Yield was determined by ¹H NMR analysis of a 8.6 mg sample of crude **S19d** and 7.1 mg of 4-toluenesulfonamide added as internal standard.

¹H NMR (400 MHz, Methanol-*d*₄): 4-toluenesulfonamide: δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 2.41 (s, 3H). Compound **S19d**: δ 6.59 (dd, *J* = 16.1, 6.9 Hz, 1H), 6.40 (dd, *J* = 16.1, 10.6 Hz, 1H), 4.36 – 4.24 (m, 1H), 3.98 – 3.88 (m, 4H), 2.26 – 2.14 (m, 1H), 1.97 – 1.75 (m, 3H), 1.68 (s, 2H), 1.03 (dd, *J* = 13.4, 6.7 Hz, 6H).

HRMS (ESI): calculated for C₁₃H₂₄N₃O₃⁺ ([M+H]⁺) 270.1812, found 270.1815

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-isopropyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11-methyldodeca-2,4-dienamide
(43)

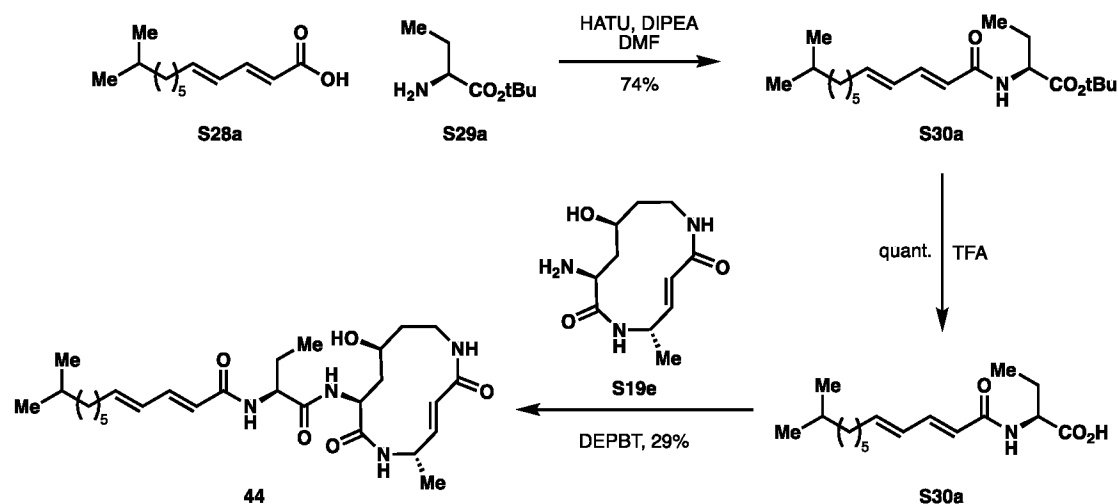
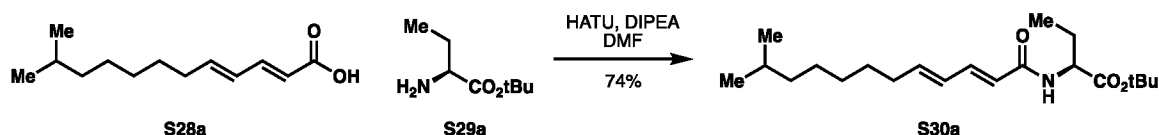


Compound **S20a** was synthesized by a previously reported procedure.^{S6} Following General Procedure F: **S19d** (60 mg, 0.0573 mmol as judged by ¹H NMR analysis, 1 equiv) and **S20a** (36 mg, 0.115 mmol, 2 equiv) were used to provide **43** (9.2 mg, 29%) as a fluffy white powder. **¹H NMR (600 MHz, DMSO-*d*₆):** δ 8.48 (d, *J* = 8.6 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 6.3 Hz, 1H), 6.99 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.32 (dd, *J* = 15.8, 6.7 Hz, 1H), 6.24 – 6.01 (m, 4H), 4.85 (br s, 1H), 4.67 (br s, 1H), 4.37 (t, *J* = 9.9 Hz, 1H), 4.28 (dd, *J* = 8.8, 4.5 Hz, 1H), 4.05 (q, *J* = 8.0 Hz, 1H), 3.98 – 3.82 (m, 1H), 3.62 – 3.52 (m, 1H), 3.05 – 2.92 (m, 2H), 2.13 (q, *J* = 7.2 Hz, 2H), 1.86 (td, *J* = 12.5, 6.8 Hz, 1H), 1.81 – 1.69 (m, 1H), 1.57 (d, *J* = 13.3 Hz, 1H), 1.50 (hept, *J* = 6.6 Hz, 1H), 1.46 – 1.34 (m, 4H), 1.29 – 1.22 (m, 4H), 1.17 – 1.11 (m, 2H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 6H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.5, 169.4, 167.8, 165.4, 142.0, 140.8, 139.7, 128.6, 124.8, 123.1, 66.9, 66.8, 58.2, 55.6, 51.2, 42.2, 38.4, 32.2, 30.6, 28.8, 28.4, 27.4, 26.6, 22.5, 19.8, 19.6, 19.1.

HRMS (ESI): calculated for C₃₀H₅₁N₄O₆⁺ ([M+H]⁺) 563.3803, found 563.3804

$$[\alpha]_D^{27} = -135.3 \text{ (} c \text{ 0.2, MeOH)}$$

Scheme 5.S6. Synthetic route to desoxy-Thr cepafungin **44**.*tert*-butyl (*S*)-2-((*2E,4E*)-11-methyldodeca-2,4-dienamido)butanoate (**S30a**)

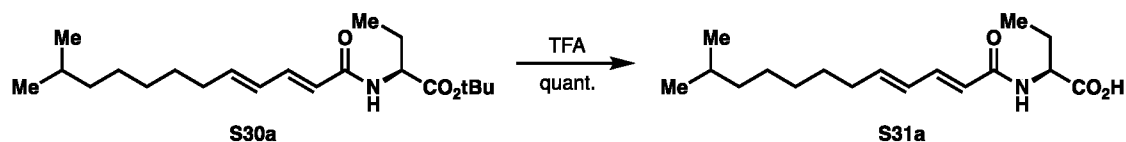
Compound **S28a** was synthesized as described previously.^{S6} Following General Procedure K: **S28a** (500 mg, 2.38 mmol, 1 equiv), **S29a** (698 mg, 3.57 mmol, 1.5 equiv), HATU (949 mg, 2.50 mmol, 1.05 equiv) and DIPEA (1.24 mL, 7.13 mmol, 3 equiv) were used to provide **S30a** as a colorless resin.

¹H NMR (400 MHz, CDCl₃): δ 7.18 (dd, J = 15.0, 10.1 Hz, 1H), 6.21 – 5.99 (m, 3H), 5.79 (d, J = 15.1 Hz, 1H), 4.57 (dt, J = 8.0, 5.8 Hz, 1H), 2.14 (q, J = 7.0 Hz, 2H), 1.90 (dq, J = 15.1, 7.5, 5.4 Hz, 1H), 1.79 – 1.69 (m, 1H), 1.55 – 1.48 (m, 1H), 1.47 (s, 9H), 1.40 (q, J = 6.8 Hz, 2H), 1.30 – 1.23 (m, 4H), 1.18 – 1.11 (m, 2H), 0.89 (t, J = 7.4 Hz, 3H), 0.85 (d, J = 6.6 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 172.0, 165.9, 143.7, 141.9, 128.3, 121.5, 82.2, 53.7, 39.0, 33.1, 29.5, 28.9, 28.2, 28.1, 27.3, 26.0, 22.8, 9.3.

HRMS (ESI): calculated for C₂₁H₃₈NO₃⁺ ([M+H]⁺) 352.2846, found 352.2845

$$[\alpha]_D^{27} = -40.6 (c\ 0.6, \text{MeOH})$$

(S)-2-((*2E,4E*)-11-methyldodeca-2,4-dienamido)butanoic acid (**S31a**)

Following General Procedure L: **S30a** (60 mg, 0.171 mmol, 1 equiv) was used to provide **S31a** (57 mg, quant.) as a clear, colorless resin.

¹H NMR (400 MHz, CDCl₃): δ 7.25 – 7.18 (m, 1H), 6.37 (br s, 1H), 6.21 (d, *J* = 7.5 Hz, 1H), 6.17 – 6.06 (m, 2H), 5.82 (d, *J* = 15.0 Hz, 1H), 4.63 (td, *J* = 7.1, 5.5 Hz, 1H), 2.15 (q, *J* = 7.0 Hz, 2H), 2.06 – 1.94 (m, 1H), 1.88 – 1.75 (m, 1H), 1.57 – 1.45 (m, 1H), 1.41 (p, *J* = 7.0 Hz, 2H), 1.30 – 1.24 (m, 4H), 1.18 – 1.11 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 6H).

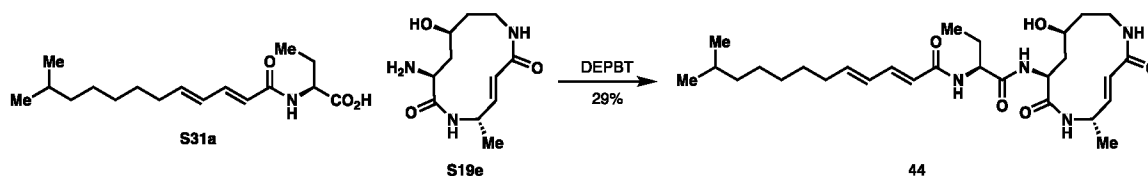
¹³C NMR (101 MHz, CDCl₃): δ 175.3, 167.3, 145.0, 143.3, 128.2, 120.4, 53.9, 39.0, 33.2, 29.6, 28.9, 28.1, 27.3, 25.3, 22.8, 9.7.

HRMS (ESI): calculated for C₁₇H₃₀NO₃⁺ ([M+H]⁺) 296.2220, found 296.2225

$$[\alpha]_D^{26} = -15.7 (c\ 0.4, \text{MeOH})$$

(2*E*,4*E*)-*N*-((*S*)-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11-methyldodeca-2,4-dienamide (44)

Compound **S19e** was synthesized as previously described.^{S6} Following General Procedure F:



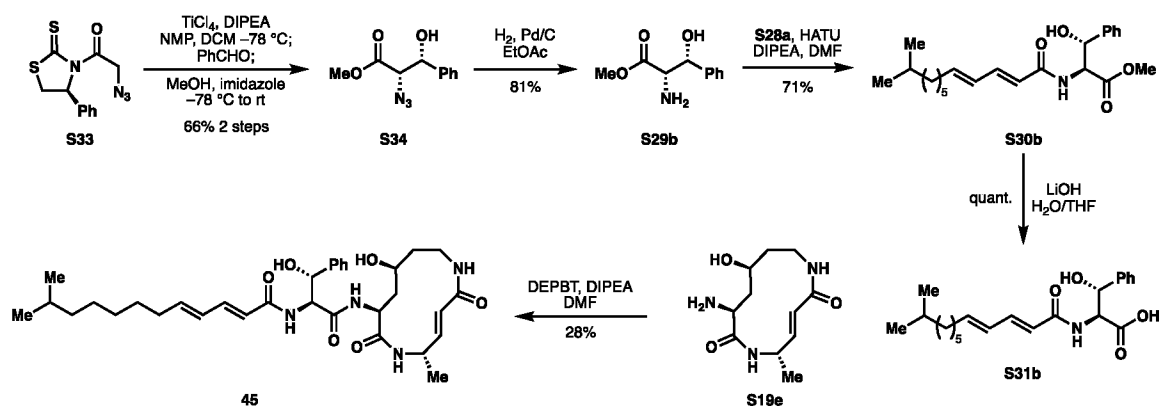
S31a (50 mg, 0.169 mmol, 2 equiv) and **S19e** (102 mg, 0.847 mmol as judged by ¹H NMR analysis, 1 equiv) were used to provide **44** (12.8 mg, 29%) as a fluffy white powder.

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.80 – 8.40 (m, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.41 (t, *J* = 6.2 Hz, 1H), 6.98 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.40 (d, *J* = 14.1 Hz, 1H), 6.18 (dt, *J* = 15.2, 5.0 Hz, 2H), 6.11 – 6.00 (m, 2H), 4.82 – 4.51 (m, 1H), 4.38 (s, 1H), 4.34 – 4.20 (m, 2H), 3.56 (s, 1H), 3.07 – 2.94 (m, 2H), 2.12 (q, *J* = 7.2 Hz, 2H), 1.97 – 1.77 (m, 1H), 1.67 – 1.57 (m, 1H), 1.57 – 1.42 (m, 4H), 1.41 – 1.34 (m, 3H), 1.31 – 1.22 (m, 5H), 1.22 – 1.18 (m, 2H), 1.14 (q, *J* = 6.9 Hz, 2H), 0.84 (d, *J* = 6.6 Hz, 6H), 0.81 (t, *J* = 7.4 Hz, 3H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.1, 170.8, 167.6, 165.1, 143.2, 142.0, 139.6, 128.6, 123.2, 123.0, 67.0, 53.6, 51.2, 44.8, 42.4, 38.4, 32.2, 28.8, 28.4, 27.4, 26.6, 25.5, 22.5, 18.6, 10.2.

HRMS (ESI): calculated for C₂₈H₄₇N₄O₅⁺ ([M+H]⁺) 519.3541, found 519.3544

$$[\alpha]_D^{27} = -125.7 (c\ 0.2, \text{MeOH})$$

Scheme 5.S7 Synthetic route to β -OH-Phe cepafungin (**45**)**methyl (2*S*,3*R*)-2-azido-3-hydroxy-3-phenylpropanoate (**S34**)**

Compound **S33** was prepared from (*S*)-2-amino-2-phenylethan-1-ol following previously reported procedures.^{S8} Following a procedure adapted from Patel et al.,^{S9} in a flame-dried 100 mL round bottom flask **S33** (1.00 g, 3.59 mmol, 1 equiv) was dissolved in 36 mL anhydrous DCM and cooled to -78°C . Neat TiCl_4 (414 μL , 3.77 mmol, 1.05 equiv) was added dropwise, followed by DIPEA (688 μL , 3.95 mmol, 1.1 equiv). The mixture was stirred for 1 h under argon at -78°C . Then, N-methylpyrrolidone (693 μL , 7.19 mmol, 2 equiv) was added and stirred a further 15 min. A solution of benzaldehyde (548 μL , 5.39 mmol, 1.5 equiv) in 2 mL anhydrous DCM was added dropwise, and the reaction was stirred under argon at -78°C for 6 h or until complete consumption of **S33** as judged by TLC. At -78°C , a solution of imidazole (4.89 g, 71.9 mmol, 20 equiv) dissolved in 31 mL anhydrous MeOH was slowly added and the mixture was warmed to $\sim 35^\circ\text{C}$ over 1.5 h. The reaction was then quenched with 50 mL H_2O and the organics were removed in vacuo. The aqueous phase was diluted with 100 mL H_2O and extracted with 5 x 30 mL DCM. The combined organic layers were washed with 30 mL 1M HCl, H_2O , NaHCO_3 , and brine, dried over Na_2SO_4 , filtered and concentrated. The residue was precipitated into 45 mL cyclohexane from 5 mL DCM, and the solids were collected by centrifugation (4°C , 10 min, 4200 rpm). The supernatant was concentrated and purified by flash chromatography (100% DCM) to provide **S34** (527 mg, 66% over 2 steps) as an oil.

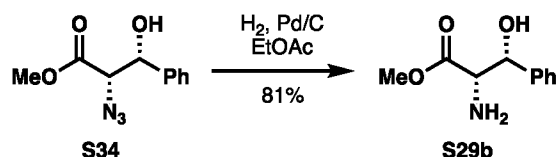
^1H NMR (400 MHz, CDCl_3): δ 7.42 – 7.31 (m, 5H), 5.21 (d, $J = 4.3$ Hz, 1H), 4.05 (d, $J = 4.3$ Hz, 1H), 3.77 (s, 3H), 2.67 (s, 1H).

^{13}C NMR (101 MHz, CDCl_3): δ 169.17, 139.27, 128.75, 128.66, 126.23, 74.56, 67.78, 52.98.

HRMS (ESI): calculated for $C_{10}H_{11}N_3NaO_3^+$ ($[M+Na]^+$) 244.0693, found 244.0692

$$[\alpha]_D^{27} = -77.2 (c\ 2.4, \text{MeOH})$$

methyl (2*S*,3*R*)-2-amino-3-hydroxy-3-phenylpropanoate (S29b)



In a 25 mL reaction vial, azide **S34** (200 mg, 0.904 mmol, 1 equiv) was dissolved in 6 mL EtOAc, then 10% Pd/C (40 mg, 20 wt % relative to **S34**) was added. The mixture was sparged with argon for 25 min, then with H_2 for 15 min, then stirred under an atmosphere of H_2 overnight. An additional 10 wt % Pd/C were added twice over the course of 2 days. At 72 h, the reaction was filtered through a plug of celite then purified by flash chromatography (95:5 DCM:MeOH) to provide **S29b** (118 mg) as a colorless oil.

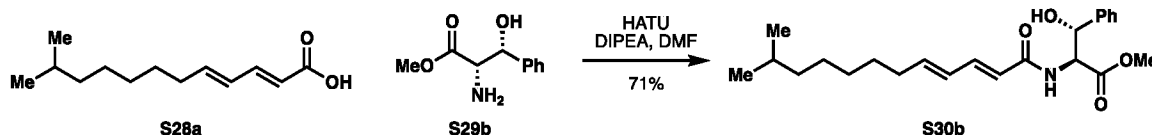
1H NMR (400 MHz, $CDCl_3$): δ 7.38 – 7.27 (m, 5H), 4.97 (d, $J = 5.4$ Hz, 1H), 3.86 – 3.81 (m, 1H), 3.61 (s, 3H), 3.01 (br s, 2H), 1.25 (d, $J = 1.9$ Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.4, 140.4, 128.6, 128.2, 126.3, 73.8, 60.5, 52.6.

HRMS (ESI): calculated for $C_{10}H_{14}NO_3^+$ ($[M+H]^+$) 196.0968, found 196.0969

$$[\alpha]_D^{27} = +12.8 (c\ 0.3, \text{MeOH})$$

methyl (2*S*,3*R*)-3-hydroxy-2-((2*E*,4*E*)-11-methyldodeca-2,4-dienamido)-3-phenylpropanoate (S30b)



Following General Procedure K: **S28a** (95 mg, 0.451 mmol, 1 equiv), **S29b** (80 mg, 0.496 mmol, 1.1 equiv), HATU (180 mg, 0.474 mmol, 1.05 equiv) and DIPEA (94 μ L, 0.541 mmol, 1.2 equiv) were used to provide **S30b** (124 mg, 71%) as a clear, colorless resin.

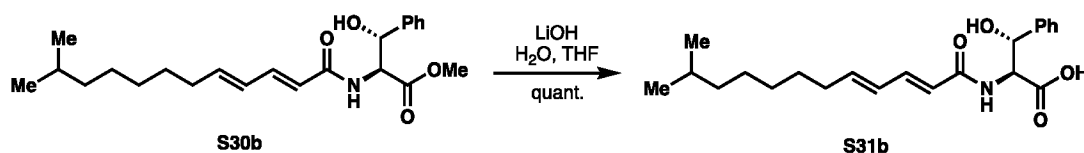
1H NMR (400 MHz, $CDCl_3$): δ 7.38 – 7.26 (m, 5H), 7.12 (dd, $J = 15.0, 9.8$ Hz, 1H), 6.24 (d, $J = 8.6$ Hz, 1H), 6.15 – 5.99 (m, 2H), 5.75 (d, $J = 15.0$ Hz, 1H), 5.28 (d, $J = 3.4$ Hz, 1H), 4.95 (dd, $J = 8.5, 3.5$ Hz, 1H), 3.72 (s, 3H), 2.13 (q, $J = 6.7$ Hz, 2H), 1.57 – 1.45 (m, 1H), 1.46 – 1.34 (m, 2H), 1.30 – 1.22 (m, 4H), 1.19 – 1.09 (m, 2H), 0.86 (d, $J = 6.7$ Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 171.2, 166.7, 144.4, 142.8, 139.7, 128.6, 128.3, 128.2, 126.0, 120.6, 74.2, 58.4, 52.8, 39.0, 33.1, 29.6, 28.9, 28.1, 27.3, 22.8.

HRMS (ESI): calculated for C₂₃H₃₄NO₄⁺ ([M+H]⁺) 388.2482, found 388.2474

$$[\alpha]_D^{27} = -50.9 \text{ (c 0.6, MeOH)}$$

(2*S*,3*R*)-3-hydroxy-2-((2*E*,4*E*)-11-methyldodeca-2,4-dienamido)-3-phenylpropanoic acid (S31b)



Following General Procedure J: **S30b** (88 mg, 0.227 mmol, 1 equiv), LiOH (11 mg, 0.454 mmol, 2 equiv) and 22 mL of 1:1 THF:H₂O reaction solvent were used to provide **S31b** (95 mg, quant.) as a clear, colorless resin.

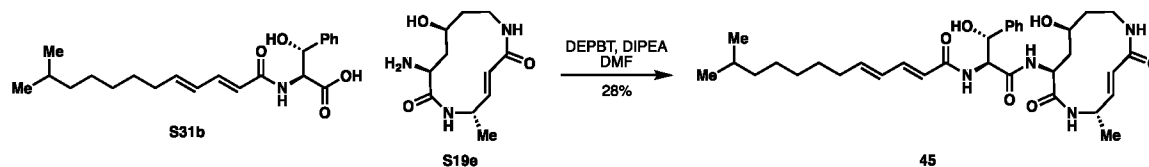
¹H NMR (400 MHz, CDCl₃): δ 7.37 – 7.26 (m, 5H), 7.14 – 7.02 (m, 1H), 6.61 (d, *J* = 7.8 Hz, 1H), 6.13 – 5.96 (m, 2H), 5.72 (d, *J* = 15.0 Hz, 1H), 5.46 (d, *J* = 2.9 Hz, 1H), 4.91 (dd, *J* = 7.7, 2.9 Hz, 1H), 2.18 – 2.08 (m, 2H), 1.56 – 1.45 (m, 1H), 1.38 (p, *J* = 6.6 Hz, 2H), 1.29 – 1.22 (m, 5H), 1.18 – 1.11 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 173.0, 168.4, 145.3, 143.8, 139.0, 128.7, 128.2, 128.1, 125.9, 119.9, 72.2, 58.5, 39.0, 33.2, 29.6, 28.9, 28.1, 27.3, 22.8.

HRMS (ESI): calculated for C₂₂H₃₂NO₄⁺ ([M+H]⁺) 374.2326, found 374.2327

$$[\alpha]_D^{27} = -29.2 \text{ (c 0.4, MeOH)}$$

(2*E*,4*E*)-*N*-((1*R*,2*S*)-1-hydroxy-3-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-oxo-1-phenylpropan-2-yl)-11-methyldodeca-2,4-dienamide (45)



Compound **S19e** was synthesized as previously described.^{S6} Following General Procedure F: **S31b** (43 mg, 0.116 mmol, 2 equiv) and **S19e** (70 mg, 0.0580 mmol as judged by ¹H NMR analysis, 1 equiv) were used to provide **45** (9.7 mg, 28%) as a fluffy white powder.

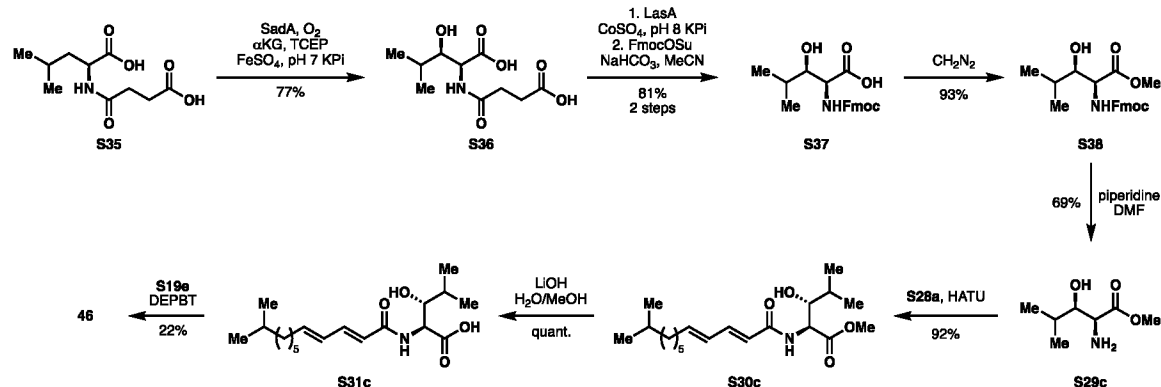
¹H NMR (600 MHz, DMSO-*d*₆): δ 8.87 – 8.42 (m, 1H), 8.01 (d, J = 9.2 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.42 (t, J = 6.4 Hz, 1H), 7.35 (d, J = 7.4 Hz, 2H), 7.25 (t, J = 7.6 Hz, 2H), 7.20 – 7.15 (m, 1H), 6.84 (dd, J = 15.2, 10.8 Hz, 1H), 6.42 (d, J = 15.7 Hz, 1H), 6.19 (d, J = 15.8 Hz, 1H), 6.16 – 5.99 (m, 3H), 5.72 (d, J = 4.7 Hz, 1H), 5.09 (t, J = 3.6 Hz, 1H), 4.70 (s, 1H), 4.55 (dd, J = 9.3, 3.0 Hz, 1H), 4.41 (br s, 2H), 3.57 (s, 1H), 3.09 – 2.93 (m, 2H), 2.10 (q, J = 7.2 Hz, 2H), 1.86 (br s, 1H), 1.72 – 1.55 (m, 1H), 1.54 – 1.41 (m, 2H), 1.41 – 1.32 (m, 3H), 1.28 – 1.18 (m, 7H), 1.16 – 1.10 (m, 2H), 0.84 (d, J = 6.6 Hz, 6H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 170.8, 168.9, 167.7, 165.3, 143.0, 142.5, 142.1, 139.7, 128.5, 127.6, 126.9, 126.2, 123.3, 122.9, 72.3, 67.2, 58.7, 51.2, 44.8, 42.9, 38.4, 32.2, 28.8, 28.4, 27.4, 26.6, 22.5, 18.5.

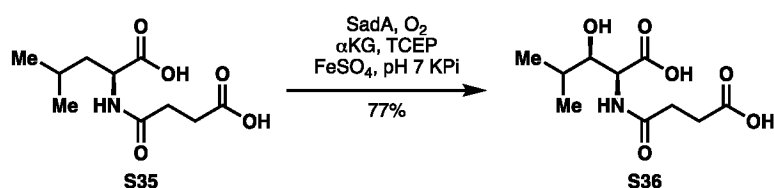
HRMS (ESI): calculated for C₃₃H₄₉N₄O₆⁺ ([M+H]⁺) 597.3647, found 597.3639

$$[\alpha]_D^{27} = -94.6 \text{ (c 0.1, MeOH)}$$

Scheme 5.S8 Synthetic route to β -OH-Leu cepafungin **46**.



(2*S*,3*R*)-2-(3-carboxypropanamido)-3-hydroxy-4-methylpentanoic acid (S36)



Compound **S35** was prepared from L-leucine as described in the literature.^{S10} Following a procedure adapted from Hibi et al.,^{S11} a glycerol stock of *E. coli* BL21(DE3) cells harboring pET-28a(+)-SadA plasmid was used to inoculate an overnight culture of LB media (6 mL) containing 50 μ g/mL kanamycin. 1.0 mL of this culture was used to inoculate each of 4 x 200 mL TB media containing 50 μ g/mL kanamycin in a 1 L non-beveled Erlenmeyer flask. The

cultures were shaken at 250 rpm/37 °C for 2.5 h or until an $OD_{600} = 0.6$ was reached. The culture was cooled on ice (15 min), induced by adding IPTG to final concentration of 25 μ M, then allowed to continue shaking at 250 rpm/23 °C for another 21 h. Cells were harvested by centrifugation (4 °C, 15 min, 4200 rpm), then resuspended in 50 mM pH = 7 KPi buffer (ca. 45 mL per 200 mL culture) to a final $OD_{600} = 30$. The cell suspension was lysed in 45 mL batches by sonication at 50% amplitude for 5 min (1 s on, 4 s off) in an ice bath. The cell debris was pelleted by centrifugation (4 °C, 15 min, 4200 rpm), and the clarified lysate supernatant was diluted 1:1 with 50 mM pH = 7 KPi and added to a non-beveled Erlenmeyer flask ($\geq 80\%$ headspace) containing **S35** (833 mg, 3.6 mmol, 1 equiv, 10 mM final concentration), α -ketoglutaric acid (1.22 g, 5.4 mmol, 1.5 equiv), TCEP (206 mg, 0.720 mmol, 0.2 equiv) and $FeSO_4 \cdot 7H_2O$ (200 mg, 0.720 mmol, 0.2 equiv). The reaction mixture was then shaken at 250 rpm/30 °C in an open Erlenmeyer flask overnight or until completion by LCMS. The reaction was then quenched by addition of 6 M HCl to final pH = 2 and centrifuged (4 °C, 15 min, 4200 RPM). The supernatant was concentrated in vacuo to a final volume of ~ 25 mL and centrifuged. The resulting pellet was resuspended in ~ 5 mL H_2O , centrifuged, and the supernatants were combined and purified by C18 flash chromatography (gradient 0–50% MeOH in H_2O over 10 column volumes, then 3 column volumes to 100% MeOH) to provide **S36** (681 mg, 77%) as an off-white solid after lyophilization.

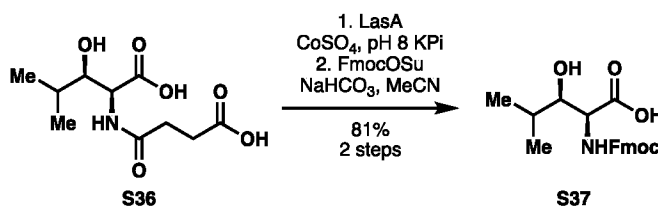
1H NMR (400 MHz, DMSO- d_6): δ 12.24 (br s, 2H), 7.76 (d, $J = 9.1$ Hz, 1H), 4.88 (br s, 1H), 4.44 (dd, $J = 9.1, 2.7$ Hz, 1H), 3.51 (dd, $J = 8.9, 2.6$ Hz, 1H), 2.48 – 2.31 (m, 4H), 1.64 – 1.49 (m, 1H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.76 (d, $J = 6.7$ Hz, 3H).

^{13}C NMR (101 MHz, DMSO- d_6): δ 173.8, 172.9, 171.4, 76.2, 54.4, 30.7, 29.9, 29.2, 19.2, 18.9.

HRMS (ESI): calculated for $C_{10}H_{18}NO_6^+$ ($[M+H]^+$) 248.1129, found 248.1136

$$[\alpha]_D^{27} = +20.4 (c\ 1.6, MeOH)$$

(2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-hydroxy-4-methylpentanoic acid (S37)



Following a procedure adapted from Hibi et al.,^{S11} a glycerol stock of *E. coli* BL21(DE3) cells harboring pET-28a(+)-LasA plasmid was used to inoculate an overnight culture of LB media (4 mL) containing 50 µg/mL kanamycin. 0.5 mL of this culture was used to inoculate each of 2 x 100 mL TB media containing 50 µg/mL kanamycin in 2 x 500 mL non-beveled Erlenmeyer flasks. The cultures were shaken at 250 rpm/37 °C for 2.5 h or until an OD₆₀₀ = 0.6 was reached. The culture was cooled on ice (15 min), induced by adding IPTG to final concentration of 25 µM, then allowed to continue shaking at 250 rpm/23 °C for another 20 h. Cells were harvested by centrifugation (4 °C, 15 min, 4200 rpm), then resuspended in 50 mM pH = 8 KPi buffer (ca. 50 mL per 100 mL culture) to a final OD₆₀₀ = 20. The combined cell suspensions were lysed by sonication at 50% amplitude for 2 x 3 min (1 s on, 4 s off) in an ice bath. The cell debris was pelleted by centrifugation (4 °C, 15 min, 4200 rpm), and the clarified lysate supernatant was added to a non-beveled 500 mL Erlenmeyer flask containing **S36** (247 mg, 1 mmol, 1 equiv, 10 mM final concentration) and CoSO₄•7H₂O (2.81 mg, 0.0100 mmol, 0.01 equiv). The mixture was shaken at 200 rpm/20 °C overnight or until completion by LCMS. The reaction was then directly treated with 10 mL saturated NaHCO₃ solution and FmocOSu (1.35 g, 4 mmol, 4 equiv) dissolved in 30 mL MeCN and stirred at rt for 4 h or until completion by LCMS. The mixture was centrifuged (4 °C, 15 min, 4200 rpm) and the supernatant was concentrated in vacuo to remove MeCN. The aqueous phase was adjusted to pH = 1 with 6 M HCl and extracted with 4 x 50 mL EtOAc. The combined organic layers were washed with 1:1 brine:1 M HCl mixture and dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash chromatography (95:5:1 DCM:MeOH:AcOH) and evaporated twice from toluene to provide **S37** (294 mg, 81% over 2 steps).

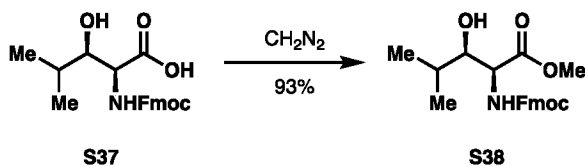
¹H NMR (400 MHz, Methanol-*d*₄): δ 7.80 (d, *J* = 7.6 Hz, 2H), 7.70 – 7.65 (m, 2H), 7.44 – 7.25 (m, 5H), 4.44 – 4.36 (m, 3H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.68 (dd, *J* = 9.3, 2.4 Hz, 1H), 1.73 – 1.62 (m, 1H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (151 MHz, Methanol-*d*₄): δ 174.90, 158.81, 145.37, 145.06, 142.60, 142.58, 128.78, 128.77, 128.17, 128.14, 126.26, 126.24, 120.92, 120.90, 78.37, 68.00, 48.42, 32.36, 26.26, 19.62, 19.48.

HRMS (ESI): calculated for C₂₁H₂₄NO₅⁺ ([M+H]⁺) 370.1649, found 370.1646

$$[\alpha]_D^{24} = +2.4 \text{ (c 0.5, MeOH)}$$

methyl (2*S*,3*R*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-hydroxy-4-methylpentanoate (S38)



Diazomethane was prepared from *N*-methyl-*N*-nitrosourea as described in the literature.^{S12} Compound **S37** (50 mg, 0.135 mmol, 1 equiv) was dissolved in 1.4 mL of a 1.5:1 mixture of Et₂O:MeOH and cooled to 0 °C. A solution of diazomethane (ca. 10 eq) was added dropwise, and the reaction was stirred at 0 °C for 20 min or until completion by TLC. The reaction was quenched at 0 °C with 0.5 mL AcOH, diluted with 30 mL Et₂O, washed with 2 x 50 mL NaHCO₃, then brine and dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash chromatography (70:30 Hexanes:EtOAc) to provide **S38** (48 mg, 93%) as a colorless oil.

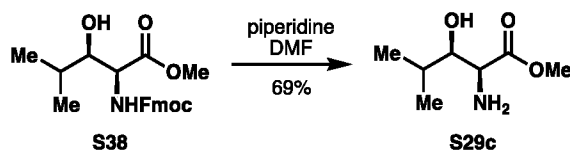
¹H NMR (400 MHz, Methanol-*d*₄): δ 7.82 – 7.75 (m, 2H), 7.70 – 7.62 (m, 2H), 7.45 – 7.22 (m, 5H), 4.45 (d, *J* = 2.5 Hz, 1H), 4.39 (d, *J* = 6.7 Hz, 2H), 4.22 (t, *J* = 6.9 Hz, 1H), 3.74 (s, 3H), 3.64 (dd, *J* = 9.2, 2.5 Hz, 1H), 1.73 – 1.60 (m, 1H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 173.62, 158.78, 145.30, 145.03, 142.61, 142.59, 128.77, 128.16, 128.13, 126.22, 126.19, 120.93, 120.91, 78.17, 68.00, 58.12, 52.81, 48.41, 32.24, 19.56, 19.38.

HRMS (ESI): calculated for C₂₂H₂₆NO₅⁺ ([M+H]⁺) 384.1805, found 384.1807

$$[\alpha]_D^{27} = -3.7 \text{ (} c \text{ 0.4, MeOH)}$$

methyl (2*S*,3*R*)-2-amino-3-hydroxy-4-methylpentanoate (S29c)



A flame-dried 25 mL reaction vial was charged with **S38** (123 mg, 0.321 mmol, 1 equiv) dissolved 6.4 mL anhydrous DMF and cooled to 0 °C. Piperidine (1.6 mL, 20% v/v) was added, and the mixture was stirred at 0 °C for 1 hour or until completion by TLC. The mixture was diluted with 10 mL toluene and concentrated to dryness to provide **S29c** (36 mg, 69%) as a colorless liquid.

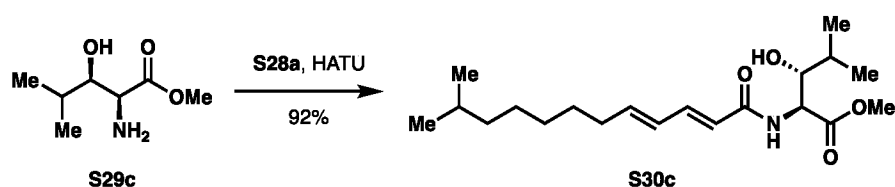
¹H NMR (400 MHz, Methanol-*d*₄): δ 3.73 (s, 3H), 3.57 (d, *J* = 3.1 Hz, 1H), 3.48 (dd, *J* = 8.6, 3.1 Hz, 1H), 1.79 (dhept, *J* = 8.6, 6.7 Hz, 1H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (101 MHz, Methanol-*d*₄): δ 176.3, 79.0, 57.6, 52.5, 31.7, 19.7, 19.3.

HRMS (ESI): calculated for C₇H₁₆NO₃⁺ ([M+H]⁺) 162.1125, found 162.1127

[α]_D²⁷ = +9.4 (*c* 0.5, MeOH)

methyl (2*S*,3*R*)-3-hydroxy-4-methyl-2-((2*E*,4*E*)-11-methyldodeca-2,4-dienamido)pentanoate (S30c)



Following General Procedure K: **S29c** (29 mg, 0.180 mmol, 1 equiv), **S28a** (38 mg, 0.180 mmol, 1 equiv), HATU (68 mg, 0.180 mmol, 1 equiv) and DIPEA (31 μL, 0.180 mmol, 1 equiv) were used to provide **S30c** (59 mg, 92%) as a colorless oil.

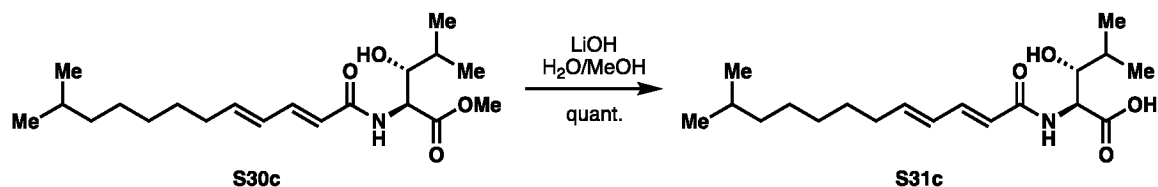
¹H NMR (600 MHz, Methanol-*d*₄): δ 7.16 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.27 – 6.21 (m, 1H), 6.14 (dt, *J* = 15.0, 6.9 Hz, 1H), 6.06 (d, *J* = 15.2 Hz, 1H), 4.81 – 4.77 (m, 1H), 3.74 (s, 3H), 3.69 (dd, *J* = 9.0, 2.5 Hz, 1H), 2.21 – 2.16 (m, 2H), 1.68 – 1.59 (m, 1H), 1.57 – 1.50 (m, 1H), 1.45 (p, *J* = 7.2 Hz, 2H), 1.34 – 1.30 (m, 4H), 1.21 – 1.17 (m, 2H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.91 – 0.88 (m, 9H).

¹³C NMR (151 MHz, Methanol-*d*₄): δ 173.3, 169.3, 144.8, 143.2, 129.8, 122.4, 78.1, 56.4, 52.8, 40.1, 34.0, 32.4, 30.6, 30.0, 29.1, 28.3, 23.0, 19.5, 19.4.

HRMS (ESI): calculated for C₂₀H₃₆NO₄⁺ ([M+H]⁺) 354.2639, found 354.2635

[α]_D²⁷ = +10.9 (*c* 0.2, MeOH)

(2*S*,3*R*)-3-hydroxy-4-methyl-2-((2*E*,4*E*)-11-methyldodeca-2,4-dienamido)pentanoic acid (S31c)



Following General Procedure J: **S30c** (42 mg, 0.1189 mmol, 1 equiv) was used to provide **S31c** (43 mg, quant.) as a colorless resin.

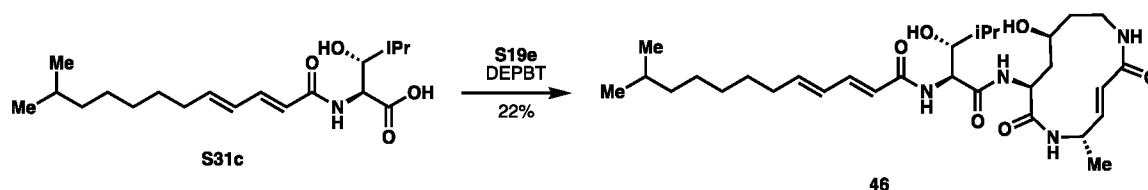
¹H NMR (600 MHz, Methanol-*d*₄): δ 7.16 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.24 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.16 – 6.10 (m, 1H), 6.06 (d, *J* = 15.1 Hz, 1H), 4.76 (d, *J* = 2.4 Hz, 1H), 3.73 (dd, *J* = 9.1, 2.4 Hz, 1H), 2.21 – 2.16 (m, 2H), 1.69 – 1.60 (m, 1H), 1.57 – 1.49 (m, 1H), 1.45 (p, *J* = 7.3 Hz, 2H), 1.35 – 1.30 (m, 4H), 1.21 – 1.16 (m, 2H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 6H).

¹³C NMR (151 MHz, Methanol-*d*₄): δ 174.6, 169.2, 144.6, 143.1, 129.8, 122.5, 78.2, 56.1, 40.1, 34.0, 32.5, 30.6, 30.0, 29.1, 28.3, 23.0, 19.5, 19.4.

HRMS (ESI): calculated for $\text{C}_{19}\text{H}_{34}\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 340.2482, found 340.2478

$$[\alpha]_D^{27} = +28.9 (c\ 0.2, \text{MeOH})$$

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-4-methyl-1-oxopentan-2-yl)-11-methyldodeca-2,4-dienamide (46)



Following General Procedure F: **S31c** (31 mg, 0.0913 mmol, 1.5 equiv), **S19e** (73 mg, 0.0609 mmol as judged by ¹H NMR analysis, 1 equiv), DEPBT (27 mg, 0.0913 mmol, 1.5 equiv) and DIPEA (48 μL, 0.2740, 4.5 equiv) were used to provide **46** (7.6 mg, 22%).

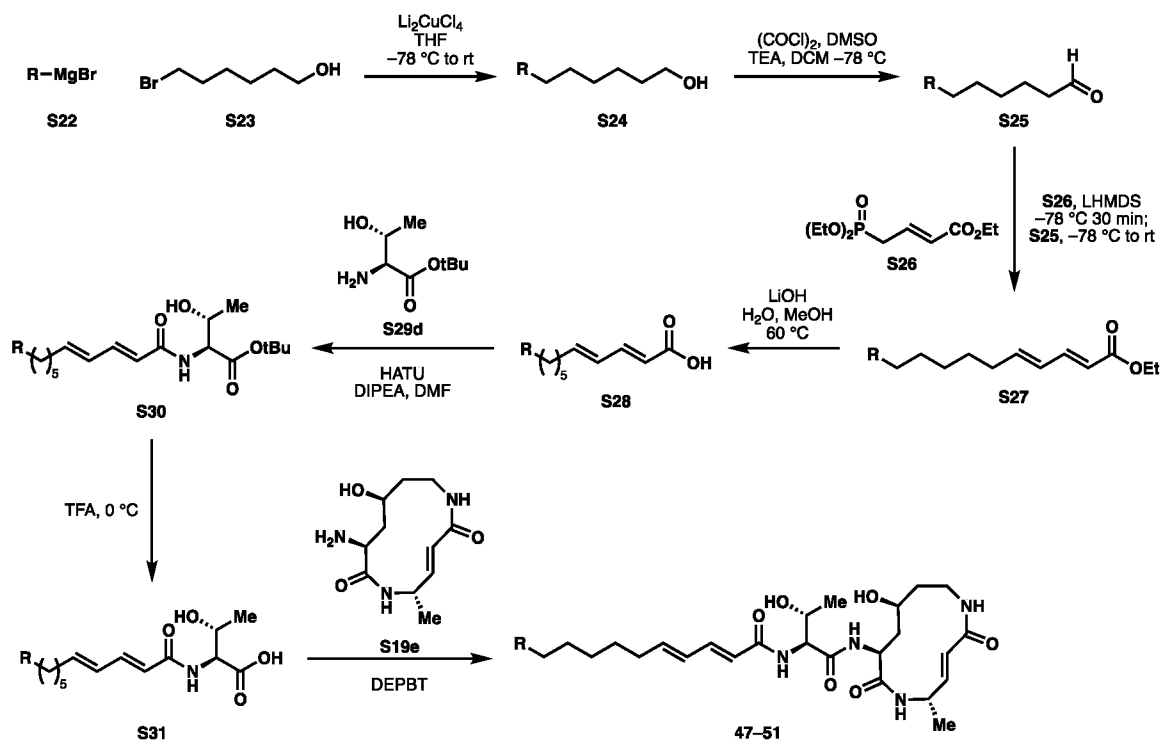
¹H NMR (600 MHz, DMSO-*d*₆): δ 8.70 (br s, 1H), 7.93 (d, *J* = 9.3 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.42 (t, *J* = 6.4 Hz, 1H), 7.00 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.40 (d, *J* = 13.4 Hz, 1H), 6.22 – 6.16 (m, 2H), 6.15 – 6.07 (m, 2H), 4.82 (d, *J* = 6.7 Hz, 1H), 4.68 (br s, 1H), 4.48 (dd, *J* = 9.4, 2.7 Hz, 1H), 4.38 (br s, 2H), 3.62 – 3.46 (m, 2H), 3.09 – 2.92 (m, 2H), 2.13 (q, *J* = 7.2 Hz, 2H), 1.84 (br s, 1H), 1.60 (d, *J* = 13.1 Hz, 1H), 1.53 – 1.41 (m, 3H), 1.41 – 1.34 (m, 3H), 1.31 – 1.17 (m, 7H), 1.16 – 1.11 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 6H), 0.77 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.0, 170.1, 167.7, 165.4, 143.0, 142.3, 139.9, 128.6, 123.3, 123.0, 76.0, 67.0, 55.2, 51.2, 44.8, 42.6, 38.4, 32.3, 30.8, 28.8, 28.4, 27.4, 26.6, 22.5, 19.1, 19.0, 18.5.

HRMS (ESI): calculated for $C_{30}H_{51}N_4O_6^+$ ($[M+H]^+$) 563.3803, found 563.3801

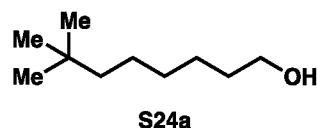
$$[\alpha]_D^{27} = -75.0 \text{ (c 0.1, MeOH)}$$

Scheme 5.S9 General synthetic route to fatty acid analogs 47–51.



Synthesis of *tert*-butyl cepafungin (47):

7,7-dimethyloctan-1-ol (S24a)

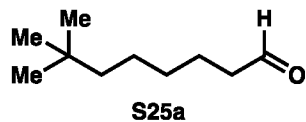


Following General Procedure G: *tert*-butylmagnesium chloride **S22a** was used to provide **S24a** (3.90 g, 89%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 3.63 (t, *J* = 6.7 Hz, 2H), 1.62 – 1.51 (m, 2H), 1.42 – 1.10 (m, 9H), 0.85 (s, 9H).

¹³C NMR (101 MHz, CDCl₃): δ 63.2, 44.3, 32.9, 30.5, 30.4, 29.5, 25.9, 24.6.

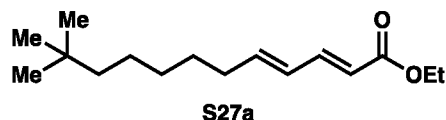
7,7-dimethyloctanal (S25a)



Following General Procedure H: **S24a** (2.0 g, 12.6 mmol, 1 equiv) was used to provide **S25a** (2.59 g), used without further purification.

¹H NMR (400 MHz, CDCl₃): δ 9.76 (t, J = 1.9 Hz, 1H), 2.42 (td, J = 7.4, 1.9 Hz, 2H), 1.70 – 1.57 (m, 2H), 1.33 – 1.13 (m, 6H), 0.86 (s, 9H).

ethyl (2*E*,4*E*)-11,11-dimethyldodeca-2,4-dienoate (S27a)



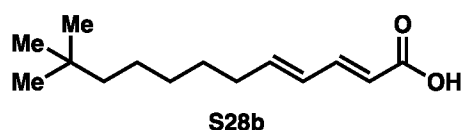
Following General Procedure I: **S25a** (2.59 g) was used to provide **S27a** (1.75 g, 55% over 2 steps) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.31 – 7.20 (m, 1H), 6.21 – 6.07 (m, 2H), 5.77 (d, J = 15.5 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 2.16 (td, J = 7.3, 5.8 Hz, 2H), 1.49 – 1.37 (m, 2H), 1.31 – 1.22 (m, 7H), 1.18 – 1.11 (m, 2H), 0.85 (s, 9H).

¹³C NMR (101 MHz, CDCl₃): δ 167.5, 145.3, 144.9, 128.5, 119.3, 60.3, 44.3, 33.2, 30.4, 30.3, 29.5, 28.9, 24.5, 14.5.

HRMS (ESI): calculated for C₁₆H₂₉O₂⁺ ([M+H]⁺) 253.2162, found 253.2159

(2*E*,4*E*)-11,11-dimethyldodeca-2,4-dienoic acid (S28b)



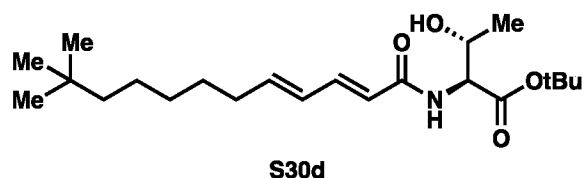
Following General Procedure J: **S27a** (700 mg, 2.77 mmol, 1 equiv) was used to provide **S28b** (648 mg, quant.) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 7.41 – 7.29 (m, 1H), 6.25 – 6.14 (m, 2H), 5.78 (d, J = 15.3 Hz, 1H), 2.23 – 2.13 (m, 2H), 1.49 – 1.40 (m, 2H), 1.31 – 1.22 (m, 4H), 1.15 (ddt, J = 12.2, 5.3, 2.6 Hz, 2H), 0.86 (s, 9H).

¹³C NMR (101 MHz, CDCl₃): δ 172.7, 147.7, 146.5, 128.4, 118.3, 44.3, 33.2, 30.4, 30.3, 29.5, 28.8, 24.5.

HRMS (ESI): calculated for C₁₄H₂₅O₂⁺ ([M+H]⁺) 225.1849, found 225.1849

***tert*-butyl ((2*E*,4*E*)-11,11-dimethyldodeca-2,4-dienoyl)-*L*-threoninate (S30d)**



Following General Procedure K: **S28b** (320 mg, 1.43 mmol, 1 equiv), **S29d** (495 mg, 2.14 mmol, 1.5 equiv), HATU (569 mg, 1.50 mmol, 1.05 equiv) and DIPEA (745 μ L, 4.28 mmol, 3 equiv) were used to provide **S30d** (303 mg, 49%) as a colorless oil.

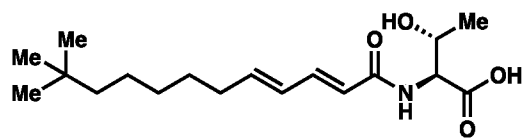
^1H NMR (400 MHz, CDCl_3): δ 7.21 (dd, J = 15.0, 10.3 Hz, 1H), 6.23 – 6.00 (m, 3H), 5.87 (d, J = 15.0 Hz, 1H), 4.49 (dd, J = 9.2, 2.1 Hz, 1H), 4.21 (qd, J = 6.3, 2.2 Hz, 1H), 2.22 – 2.07 (m, 2H), 1.49 – 1.37 (m, 11H), 1.30 – 1.21 (m, 4H), 1.19 – 1.11 (m, 14H), 0.85 (s, 9H).

^{13}C NMR (101 MHz, CDCl_3): δ 170.2, 166.6, 143.6, 141.9, 128.4, 121.6, 82.0, 73.9, 67.6, 58.4, 44.3, 33.1, 30.4, 30.2, 29.5, 29.0, 28.8, 28.3, 24.5, 21.0.

HRMS (ESI): calculated for $\text{C}_{22}\text{H}_{40}\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 438.3578, found 438.3573

$$[\alpha]_D^{27} = +5.3 \text{ (c 0.6, MeOH)}$$

((2*E*,4*E*)-11,11-dimethyldodeca-2,4-dienoyl)-*L*-threonine (S31d**)**



S31d

Following General Procedure L: **S31d** (108 mg, 0.247 mmol, 1 equiv) was used to provide **S31d** (96 mg, quant.) as a clear, colorless resin.

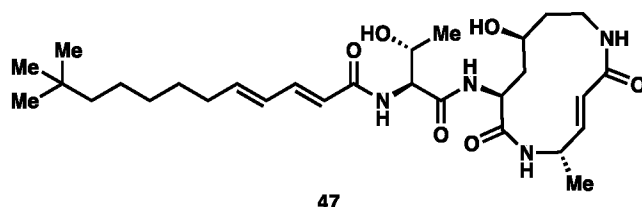
^1H NMR (400 MHz, CDCl_3): δ 7.25 – 7.19 (m, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.14 (s, 2H), 5.93 (d, J = 15.1 Hz, 1H), 4.68 – 4.59 (m, 1H), 4.54 – 4.45 (m, 1H), 2.15 (d, J = 6.7 Hz, 2H), 1.46 – 1.38 (m, 2H), 1.30 – 1.20 (m, 8H), 1.18 – 1.12 (m, 2H), 0.86 (s, 9H).

^{13}C NMR (101 MHz, CDCl_3): δ 173.6, 168.8, 145.6, 144.0, 128.2, 120.0, 67.7, 57.9, 44.3, 33.3, 30.4, 30.4, 29.6, 29.0, 24.5, 19.3.

HRMS (ESI): calculated for $\text{C}_{18}\text{H}_{32}\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 326.2326, found 325.2329

$$[\alpha]_D^{27} = +12.6 \text{ (c 0.3, MeOH)}$$

(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-11,11-dimethyldodeca-2,4-dienamide (47**)**



47

Following General Procedure F: **S31d** (38 mg, 0.116 mmol, 2 equiv) and **S19e** (70 mg, 0.0580 mmol as judged by ^1H NMR analysis, 1 equiv) were used to provide **47** (9.6 mg, 30%) as a fluffy white solid.

^1H NMR (600 MHz, DMSO- d_6): δ 8.66 (br s, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.41 (t, J = 6.4 Hz, 1H), 7.00 (dd, J = 15.1, 10.8 Hz, 1H), 6.40 (d, J = 14.1 Hz, 1H), 6.23 – 6.05 (m, 4H), 4.86 (d, J = 4.9 Hz, 1H), 4.67 (s, 1H), 4.50 – 4.31 (m, 2H), 4.28 (dd, J = 8.8, 4.1 Hz, 1H), 3.96 (s, 1H), 3.57 (s, 1H), 3.07 – 2.93 (m, 2H), 2.13 (q, J = 7.2 Hz, 2H), 1.85 (s, 1H), 1.58 (d, J = 13.1 Hz, 1H), 1.49 – 1.34 (m, 4H), 1.28 – 1.18 (m, 7H), 1.16 – 1.11 (m, 2H), 1.00 (d, J = 6.3 Hz, 3H), 0.85 (s, 9H).

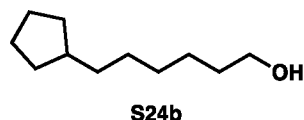
^{13}C NMR (151 MHz, DMSO- d_6): δ 171.0, 169.4, 167.6, 165.4, 143.1, 142.1, 139.7, 128.6, 123.3, 123.1, 67.0, 66.7, 58.1, 51.2, 44.7, 43.7, 42.5, 32.3, 30.1, 29.6, 29.3, 28.4, 23.9, 19.9, 18.5.

HRMS (ESI): calculated for $\text{C}_{29}\text{H}_{49}\text{N}_4\text{O}_6^+$ ($[\text{M}+\text{H}]^+$) 549.3645, found 549.3645

$$[\alpha]_D^{27} = -86.4 \text{ (c 0.1, MeOH)}$$

Synthesis of cyclopentyl cepafungin (**48**)

6-cyclopentylhexan-1-ol (**S24b**)

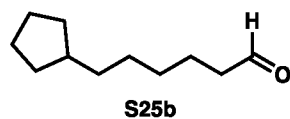


Following General Procedure G: Cyclopentylmagnesium bromide **S22b** was used to provide **S24b** (4.18 g, 89%) as a colorless oil.

^1H NMR (400 MHz, CDCl_3): δ 3.61 (t, J = 6.7 Hz, 2H), 1.77 – 1.66 (m, 3H), 1.65 – 1.40 (m, 7H), 1.40 – 1.22 (m, 8H), 1.12 – 0.95 (m, 2H).

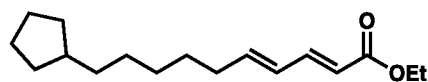
^{13}C NMR (101 MHz, CDCl_3): δ 63.1, 40.3, 36.3, 32.9, 32.8, 29.8, 28.9, 25.9, 25.3.

6-cyclopentylhexanal (**S25b**)



Following General Procedure H: **S24b** (2.50 g, 14.7 mmol, 1 equiv) was used to provide **S25b** (3.12 g), used without further purification.

^1H NMR (400 MHz, CDCl_3): δ 9.76 (t, J = 1.9 Hz, 1H), 2.41 (td, J = 7.4, 1.9 Hz, 2H), 1.77 – 1.68 (m, 3H), 1.65 – 1.45 (m, 6H), 1.35 – 1.25 (m, 7H), 1.09 – 1.00 (m, 2H).

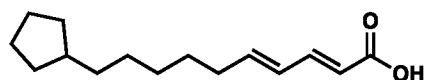
ethyl (2E,4E)-10-cyclopentyldeca-2,4-dienoate (S27b)**S27b**

Following General Procedure I: **S25b** (3.12 g) was used to provide **S27b** (1.44 g, 37%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.30 – 7.19 (m, 1H), 6.22 – 6.06 (m, 2H), 5.77 (d, *J* = 15.4 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.15 (td, *J* = 7.4, 5.9 Hz, 2H), 1.78 – 1.66 (m, 4H), 1.60 – 1.55 (m, 2H), 1.52 – 1.46 (m, 2H), 1.45 – 1.38 (m, 2H), 1.30 – 1.26 (m, 8H), 1.09 – 0.99 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 167.5, 145.3, 144.9, 128.4, 119.3, 60.3, 40.2, 36.3, 33.1, 32.8, 29.6, 28.9, 28.7, 25.3, 14.4.

HRMS (ESI): calculated for C₁₇H₂₉O₂⁺ ([M+H]⁺) 265.2162, found 265.2160

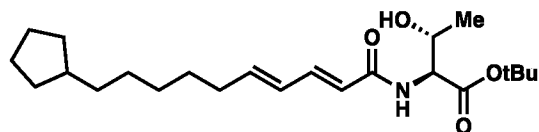
(2E,4E)-10-cyclopentyldeca-2,4-dienoic acid (S28c)**S28c**

Following General Procedure J: **S27b** (718 mg, 2.72 mmol, 1 equiv) was used to provide **S28c** (645 mg, quant.) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 7.35 (ddd, *J* = 15.4, 6.8, 3.2 Hz, 1H), 6.25 – 6.13 (m, 2H), 5.78 (d, *J* = 15.3 Hz, 1H), 2.23 – 2.13 (m, 2H), 1.78 – 1.67 (m, 3H), 1.63 – 1.54 (m, 2H), 1.54 – 1.47 (m, 2H), 1.47 – 1.40 (m, 2H), 1.32 – 1.26 (m, 6H), 1.10 – 1.00 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 172.9, 147.7, 146.5, 128.3, 118.3, 40.3, 36.3, 33.2, 32.9, 29.6, 28.8, 28.7, 25.3.

HRMS (ESI): calculated for C₁₅H₂₅O₂⁺ ([M+H]⁺) 237.1849, found 237.1847

tert-butyl ((2E,4E)-10-cyclopentyldeca-2,4-dienoyl)-L-threoninate (S30e)**S30e**

Following General Procedure K: **S28c** (350 mg, 1.48 mmol, 1 equiv), **S29d** (514 mg, 2.22 mmol, 1.5 equiv), HATU (591 mg, 1.56 mmol, 1.05 equiv) and DIPEA (774 μL, 4.44 mmol, 3 equiv) were used to provide **S30e** (457 mg, 69%) as a colorless oil.

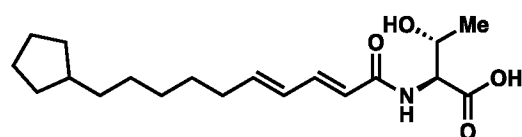
¹H NMR (400 MHz, CDCl₃): δ 7.20 (dd, *J* = 15.0, 10.3 Hz, 1H), 6.20 – 6.01 (m, 3H), 5.87 (d, *J* = 15.0 Hz, 1H), 4.49 (dd, *J* = 9.2, 2.1 Hz, 1H), 4.21 (qd, *J* = 6.3, 2.2 Hz, 1H), 2.14 (q, *J* = 7.0 Hz, 2H), 1.77 – 1.67 (m, 3H), 1.62 – 1.47 (m, 4H), 1.45 (s, 9H), 1.43 – 1.36 (m, 2H), 1.30 – 1.24 (m, 6H), 1.18 – 1.13 (m, 12H), 1.10 – 0.99 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 170.2, 166.6, 143.6, 141.9, 128.4, 121.6, 82.0, 73.9, 67.6, 58.4, 40.2, 36.3, 33.1, 32.9, 29.6, 29.0, 28.9, 28.7, 28.3, 25.3, 21.0.

HRMS (ESI): calculated for C₂₃H₄₀NO₄⁺ ([M+H]⁺) 450.3578, found 450.3589

$$[\alpha]_D^{27} = +5.5 \text{ (c 0.4, MeOH)}$$

((2*E*,4*E*)-10-cyclopentyldeca-2,4-dienoyl)-*L*-threonine (S31e)



S31e

Following General Procedure L: **S30e** (62 mg, 0.138 mmol, 1 equiv) was used to provide **S31e** (57 mg, quant.) as a clear, colorless resin.

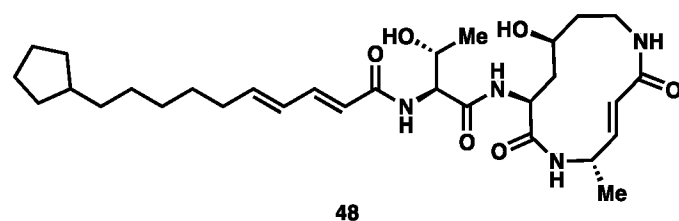
¹H NMR (400 MHz, CDCl₃): δ 7.25 – 7.14 (m, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 6.59 (br s, 1H), 6.21 – 6.06 (m, 2H), 5.91 (d, *J* = 15.0 Hz, 1H), 4.61 (d, *J* = 7.8 Hz, 1H), 4.54 – 4.43 (m, 1H), 2.21 – 2.08 (m, 2H), 1.79 – 1.66 (m, 3H), 1.63 – 1.45 (m, 4H), 1.45 – 1.36 (m, 2H), 1.34 – 1.25 (m, 7H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.10 – 0.99 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 173.4, 168.6, 145.4, 143.8, 128.2, 120.2, 67.5, 57.8, 40.3, 36.3, 33.3, 32.9, 29.7, 28.9, 28.7, 25.3, 19.2.

HRMS (ESI): calculated for C₁₉H₃₂NO₄⁺ ([M+H]⁺) 338.2326, found 338.2327

$$[\alpha]_D^{27} = +12.4 \text{ (c 0.3, MeOH)}$$

(2*E*,4*E*)-10-cyclopentyl-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)deca-2,4-dienamide (48)



48

Following General Procedure F: **S31e** (41 mg, 0.121 mmol, 2 equiv) and **S19e** (73 mg, 0.0603 mmol as judged by ^1H NMR analysis, 1 equiv) were used to provide **48** (12.7 mg, 38%) as a fluffy white solid.

^1H NMR (600 MHz, DMSO- d_6): δ 8.77 – 8.40 (m, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.84 – 7.64 (m, 1H), 7.41 (t, J = 6.4 Hz, 1H), 7.00 (dd, J = 15.2, 10.7 Hz, 1H), 6.40 (d, J = 15.6 Hz, 1H), 6.23 – 6.04 (m, 4H), 4.89 (s, 1H), 4.68 (s, 1H), 4.51 – 4.31 (m, 2H), 4.28 (dd, J = 8.9, 4.1 Hz, 1H), 3.96 (s, 1H), 3.57 (s, 1H), 3.12 – 2.87 (m, 2H), 2.13 (q, J = 7.1 Hz, 2H), 1.84 (br s, 1H), 1.75 – 1.66 (m, 3H), 1.63 – 1.50 (m, 3H), 1.50 – 1.41 (m, 3H), 1.41 – 1.33 (m, 3H), 1.32 – 1.23 (m, 7H), 1.23 – 1.18 (m, 2H), 1.07 – 1.02 (m, 2H), 1.00 (d, J = 6.3 Hz, 3H).

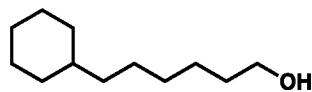
^{13}C NMR (151 MHz, DMSO- d_6): δ 171.1, 169.4, 167.6, 165.4, 143.1, 142.1, 139.7, 128.6, 123.3, 123.1, 67.0, 66.7, 58.1, 51.2, 44.7, 42.5, 35.6, 32.3, 28.9, 28.4, 28.0, 24.7, 19.9, 18.5.

HRMS (ESI): calculated for $\text{C}_{30}\text{H}_{49}\text{N}_4\text{O}_6^+$ ($[\text{M}+\text{H}]^+$) 561.3647, found 561.3655

$$[\alpha]_D^{27} = -98.4 (c\ 0.2, \text{MeOH})$$

Synthesis of cyclohexyl cepafungin (**49**)

6-cyclohexylhexan-1-ol (S24c)



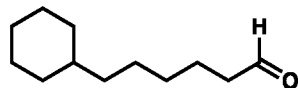
S24c

Following General Procedure G: Cyclohexylmagnesium bromide **S22c** was used to provide **S24c** (4.58 g, 90%) as a colorless oil.

^1H NMR (400 MHz, CDCl_3): δ 3.61 (t, J = 6.7 Hz, 2H), 1.73 – 1.59 (m, 6H), 1.58 – 1.50 (m, 2H), 1.38 – 1.23 (m, 6H), 1.23 – 1.05 (m, 6H), 0.90 – 0.77 (m, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 63.1, 37.8, 37.6, 33.6, 32.9, 29.9, 26.9, 26.9, 26.6, 25.9.

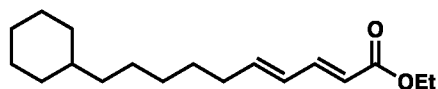
6-cyclohexylhexanal (S25c)



S25c

Following General Procedure H: **S24c** (2.50 g, 13.6 mmol, 1 equiv) was used to provide **S25c** (3.30 g), used without further purification.

^1H NMR (400 MHz, CDCl_3): δ 9.76 (t, J = 1.9 Hz, 1H), 2.41 (td, J = 7.4, 1.9 Hz, 2H), 1.71 – 1.58 (m, 8H), 1.35 – 1.26 (m, 5H), 1.18 – 1.12 (m, 4H), 0.85 – 0.82 (m, 2H).

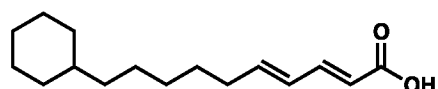
ethyl (2E,4E)-10-cyclohexyldeca-2,4-dienoate (S27c)**S27c**

Following General Procedure I: **S25c** (3.30 g) was used to provide **S27c** (1.62 g, 43% over 2 steps) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.30 – 7.21 (m, 1H), 6.22 – 6.05 (m, 2H), 5.77 (d, J = 15.3 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 2.15 (td, J = 7.5, 6.0 Hz, 2H), 1.72 – 1.62 (m, 5H), 1.46 – 1.37 (m, 2H), 1.28 (t, J = 7.1 Hz, 7H), 1.23 – 1.12 (m, 6H), 0.90 – 0.79 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 167.5, 145.3, 144.9, 128.4, 119.3, 60.3, 37.8, 37.5, 33.6, 33.1, 29.6, 28.9, 26.9, 26.8, 26.6, 14.5.

HRMS (ESI): calculated for C₁₈H₃₁O₂⁺ ([M+H]⁺) 279.2319, found 279.2318

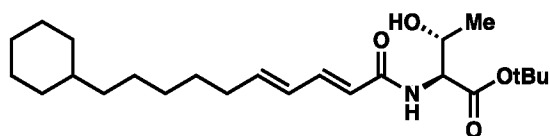
(2E,4E)-10-cyclohexyldeca-2,4-dienoic acid (S28d)**S28d**

Following General Procedure J: **S27c** (800 mg, 2.87 mmol, 1 equiv) was used to provide **S28d** (717 mg, quant.) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 7.35 (ddd, J = 15.3, 6.8, 3.2 Hz, 1H), 6.26 – 6.12 (m, 2H), 5.78 (d, J = 15.3 Hz, 1H), 2.23 – 2.13 (m, 2H), 1.73 – 1.59 (m, 5H), 1.43 (s, 2H), 1.32 – 1.24 (m, 4H), 1.24 – 1.13 (m, 6H), 0.91 – 0.79 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 172.8, 147.6, 146.4, 128.2, 118.2, 37.6, 37.4, 33.4, 33.1, 29.5, 28.7, 26.8, 26.6, 26.5.

HRMS (ESI): calculated for C₁₆H₂₇O₂⁺ ([M+H]⁺) 251.2006, found 251.2004

tert-butyl ((2E,4E)-10-cyclohexyldeca-2,4-dienoyl)-L-threoninate (S30f)**S30f**

Following General Procedure K: **S28d** (350 mg, 1.40 mmol, 1 equiv), **S29d** (485 mg, 2.10 mmol, 1.5 equiv), HATU (558 mg, 1.47 mmol, 1.05 equiv) and DIPEA (731 μ L, 4.19 mmol, 3 equiv) were used to provide **S30f** (582 mg, 90%).

¹H NMR (400 MHz, CDCl₃): δ 7.20 (dd, J = 15.0, 10.4 Hz, 1H), 6.22 – 5.99 (m, 3H), 5.87 (d, J = 15.0 Hz, 1H), 4.49 (dd, J = 9.2, 2.1 Hz, 1H), 4.21 (qd, J = 6.2, 2.2 Hz, 1H), 2.14 (q, J = 7.1

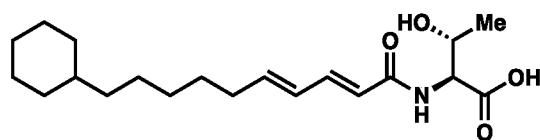
Hz, 2H), 1.71 – 1.59 (m, 5H), 1.45 (s, 9H), 1.43 – 1.35 (m, 2H), 1.30 – 1.20 (m, 5H), 1.20 – 1.11 (m, 17H), 0.90 – 0.78 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 170.2, 166.6, 143.6, 141.9, 128.4, 121.6, 82.0, 73.9, 67.6, 58.4, 37.8, 37.6, 33.6, 33.1, 29.6, 29.0, 28.8, 28.2, 26.9, 26.8, 26.6, 21.0.

HRMS (ESI): calculated for C₂₄H₄₂NO₄⁺ ([M+H]⁺) 464.3734, found 464.3733

$$[\alpha]_D^{27} = +5.5 (c\ 0.7, \text{MeOH})$$

((2E,4E)-10-cyclohexyldeca-2,4-dienoyl)-L-threonine (S31f)



S31f

Following General Procedure L: **S30f** (60 mg, 0.129 mmol, 1 equiv) was used to provide **S31f** (61 mg, quant.).

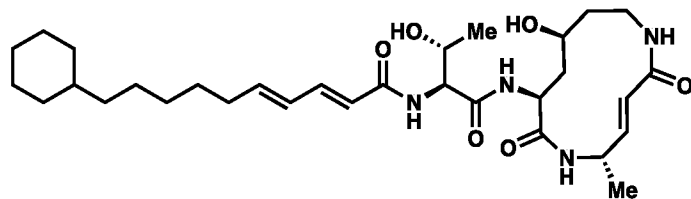
¹H NMR (400 MHz, CDCl₃): δ 7.26 – 7.15 (m, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.32 (br s, 1H), 6.19 – 6.07 (m, 2H), 5.91 (d, *J* = 15.0 Hz, 1H), 4.61 (dd, *J* = 8.1, 2.3 Hz, 1H), 4.55 – 4.40 (m, 1H), 2.15 (q, *J* = 6.7 Hz, 2H), 1.73 – 1.59 (m, 5H), 1.45 – 1.36 (m, 2H), 1.33 – 1.20 (m, 8H), 1.20 – 1.12 (m, 5H), 0.91 – 0.79 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 173.4, 168.5, 145.3, 143.7, 128.2, 120.2, 67.5, 57.8, 37.8, 37.6, 33.6, 33.3, 29.7, 29.0, 26.9, 26.8, 26.6, 19.2.

HRMS (ESI): calculated for C₂₀H₃₄NO₄⁺ ([M+H]⁺) 352.2482, found 352.2483

$$[\alpha]_D^{27} = +13.4 (c\ 0.4, \text{MeOH})$$

(2E,4E)-10-cyclohexyl-N-((2S,3R)-3-hydroxy-1-(((5S,8S,10S,E)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)deca-2,4-dienamide (49)



49

Following General Procedure F: **S31f** (49 mg, 0.139 mmol, 2 equiv) and **S19e** (84 mg, 0.0693 mmol as judged by ¹H NMR analysis, 1 equiv) were used to provide **49** (16.5 mg, 41%) as a fluffy white solid.

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.85 – 8.35 (m, 1H), 7.97 – 7.85 (m, 1H), 7.82 – 7.65 (m, 1H), 7.41 (t, *J* = 6.3 Hz, 1H), 7.00 (dd, *J* = 15.1, 10.8 Hz, 1H), 6.40 (d, *J* = 14.6 Hz, 1H), 6.24 – 6.02 (m, 4H), 4.88 (s, 1H), 4.68 (s, 1H), 4.51 – 4.30 (m, 2H), 4.28 (dd, *J* = 8.8, 4.1 Hz, 1H), 4.03 – 3.88 (m, 1H), 3.64 – 3.51 (m, 1H), 3.13 – 2.85 (m, 2H), 2.12 (q, *J* = 7.2 Hz, 2H), 1.96 – 1.73 (m, 1H), 1.69 – 1.54 (m, 6H), 1.49 – 1.41 (m, 1H), 1.40 – 1.34 (m, 3H), 1.29 – 1.10 (m, 13H), 1.00 (d, *J* = 6.3 Hz, 3H), 0.88 – 0.78 (m, 2H).

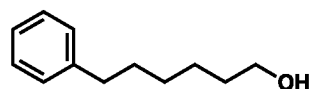
¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.1, 169.4, 167.6, 165.4, 143.1, 142.1, 139.7, 128.6, 123.3, 123.1, 66.9, 66.7, 58.1, 51.2, 44.7, 42.5, 37.0, 36.9, 32.9, 32.2, 28.9, 28.4, 26.2, 26.1, 25.9, 19.9, 18.5.

HRMS (ESI): calculated for C₃₁H₅₁N₄O₆⁺ ([M+H]⁺) 575.3803, found 575.3808

$$[\alpha]_D^{27} = -93.1 (c\ 0.2, \text{MeOH})$$

Synthesis of phenyl cepafungin (**50**)

6-phenylhexan-1-ol (**S24d**)



S24d

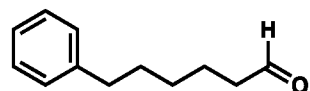
Following Procedure G: Phenylmagnesium bromide **S22d** was used to provide **S24d** (3.95 g, 80%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.34 – 7.12 (m, 5H), 3.62 (t, *J* = 6.6 Hz, 2H), 2.67 – 2.55 (m, 2H), 1.68 – 1.52 (m, 5H), 1.44 – 1.33 (m, 4H).

¹³C NMR (101 MHz, CDCl₃): δ 142.8, 128.5, 128.4, 125.7, 63.1, 36.0, 32.8, 31.5, 29.2, 25.7.

HRMS (ESI): calculated for C₁₂H₁₉O⁺ ([M+H]⁺) 179.1430, found 179.1430

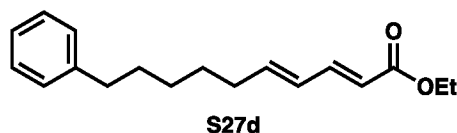
6-phenylhexanal (**S25d**)



S25d

Following General Procedure H: **S24d** (2.00 g, 11.2 mmol, 1 equiv) was used to provide **S25d**, used immediately without further purification.

¹H NMR (400 MHz, CDCl₃): δ 9.76 (t, *J* = 1.8 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.20 – 7.15 (m, 3H), 2.64 – 2.59 (m, 2H), 2.45 – 2.40 (m, 2H), 1.71 – 1.60 (m, 4H), 1.42 – 1.33 (m, 2H).

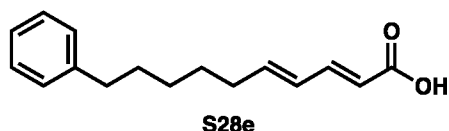
ethyl (2E,4E)-10-phenyldeca-2,4-dienoate (S27d)

Following General Procedure I: **S25d** was used to provide **S27d** (1.08 g, 35% over 2 steps) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.31 – 7.21 (m, 3H), 7.20 – 7.15 (m, 3H), 6.21 – 6.06 (m, 2H), 5.78 (d, *J* = 15.4 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.65 – 2.56 (m, 2H), 2.16 (q, *J* = 7.0 Hz, 2H), 1.68 – 1.58 (m, 2H), 1.51 – 1.42 (m, 2H), 1.40 – 1.33 (m, 2H), 1.29 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (101 MHz, CDCl₃): δ 167.5, 145.2, 144.6, 142.8, 128.6, 128.5, 128.4, 125.8, 119.4, 60.3, 36.0, 33.0, 31.4, 28.9, 28.7, 14.5.

HRMS (ESI): calculated for C₁₈H₂₅O₂⁺ ([M+H]⁺) 273.1849, found 273.1848

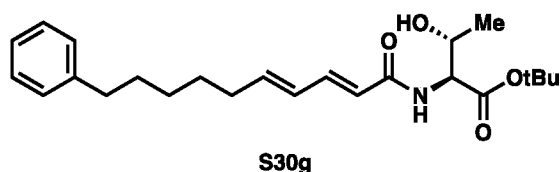
(2E,4E)-10-phenyldeca-2,4-dienoic acid (S28e)

Following General Procedure J: **S27d** (500 mg, 1.84 mmol, 1 equiv) was used to provide **S28e** (474 mg, quant.) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 7.38 – 7.31 (m, 1H), 7.30 – 7.25 (m, 2H), 7.21 – 7.15 (m, 3H), 6.24 – 6.12 (m, 2H), 5.79 (d, *J* = 15.2 Hz, 1H), 2.64 – 2.58 (m, 2H), 2.18 (q, *J* = 7.0 Hz, 2H), 1.68 – 1.59 (m, 2H), 1.47 (tt, *J* = 8.0, 6.7 Hz, 2H), 1.40 – 1.33 (m, 2H).

¹³C NMR (101 MHz, CDCl₃): δ 172.92, 147.65, 146.21, 142.72, 128.51, 128.44, 128.41, 125.80, 118.44, 35.95, 33.09, 31.37, 28.91, 28.61.

HRMS (ESI): calculated for C₁₆H₂₁O₂⁺ ([M+H]⁺) 245.1536, found 245.1535

tert-butyl ((2E,4E)-10-phenyldeca-2,4-dienoyl)-L-threoninate (S30g)

Following General Procedure K: **S28e** (237 mg, 0.970 mmol, 1 equiv), **S29d** (337 mg, 1.46 mmol, 1.5 equiv), HATU (387 mg, 1.02 mmol, 1.05 equiv) and DIPEA (507 μL, 2.91 mmol, 3 equiv) were used to provide **S30g** (253 mg, 57%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 7.32 – 7.21 (m, 2H), 7.22 – 7.14 (m, 4H), 6.20 – 6.00 (m, 3H), 5.88 (d, *J* = 15.0 Hz, 1H), 4.50 (dd, *J* = 9.2, 2.1 Hz, 1H), 4.22 (qd, *J* = 6.3, 2.2 Hz, 1H), 2.64 –

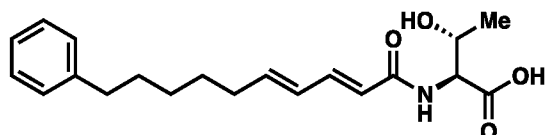
2.57 (m, 2H), 2.15 (q, $J = 7.0$ Hz, 2H), 1.67 – 1.58 (m, 2H), 1.50 – 1.41 (m, 11H), 1.39 – 1.32 (m, 2H), 1.20 – 1.14 (m, 12H).

^{13}C NMR (101 MHz, CDCl_3): δ 170.21, 166.60, 143.26, 142.82, 141.82, 128.53, 128.40, 125.77, 121.78, 81.98, 73.94, 67.60, 58.45, 35.99, 32.97, 31.40, 28.91, 28.87, 28.79, 28.27, 21.01.

HRMS (ESI): calculated for $\text{C}_{28}\text{H}_{44}\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 458.3265, found 458.3263

$$[\alpha]_D^{27} = +4.6 \text{ (c 0.4, MeOH)}$$

((2E,4E)-10-phenyldeca-2,4-dienoyl)-L-threonine (S31g)



S31g

Following Procedure L: **S30g** (101 mg, 0.221 mmol, 1 equiv) was used to provide **S31g** (93 mg, quant.) as a clear, colorless resin.

^1H NMR (400 MHz, CDCl_3): δ 7.29 – 7.27 (m, 1H), 7.25 – 7.14 (m, 5H), 6.91 (d, $J = 8.0$ Hz, 1H), 6.52 (br s, 1H), 6.18 – 6.05 (m, 2H), 5.91 (d, $J = 15.0$ Hz, 1H), 4.62 (dd, $J = 8.1, 2.4$ Hz, 1H), 4.53 – 4.44 (m, 1H), 2.63 – 2.55 (m, 2H), 2.14 (q, $J = 6.7$ Hz, 2H), 1.66 – 1.57 (m, 2H), 1.49 – 1.29 (m, 5H), 1.22 (d, $J = 6.3$ Hz, 3H).

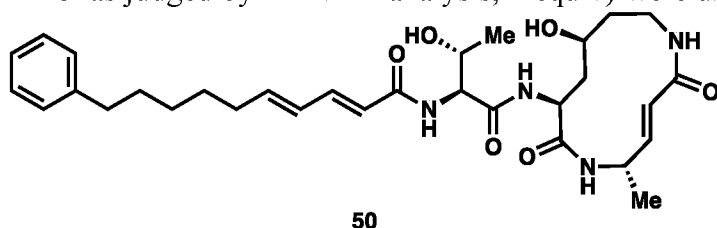
^{13}C NMR (101 MHz, CDCl_3): δ 173.4, 168.5, 145.1, 143.7, 142.7, 128.5, 128.4, 128.3, 125.8, 120.3, 67.5, 57.8, 36.0, 33.1, 31.4, 29.0, 28.8, 19.3.

HRMS (ESI): calculated for $\text{C}_{20}\text{H}_{28}\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 346.2013, found 346.2014

$$[\alpha]_D^{27} = +11.4, \text{ (c 0.3, MeOH)}$$

(2E,4E)-N-((2S,3R)-3-hydroxy-1-(((5S,8S,10S,E)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-10-phenyldeca-2,4-dienamide (50)

Following General Procedure F: **S31g** (189 mg, 0.546 mmol, 2 equiv) and **S19e** (70 mg, 0.0580 mmol as judged by ^1H NMR analysis, 1 equiv) were used to provide **50** (44.3 mg, 29%) as a



50

fluffy white powder.

^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 8.75 – 8.43 (m, 1H), 7.93 (d, $J = 8.7$ Hz, 1H), 7.75 (d, $J = 6.5$ Hz, 1H), 7.41 (t, $J = 6.2$ Hz, 1H), 7.28 – 7.24 (m, 2H), 7.20 – 7.14 (m, 3H), 6.99 (dd, $J =$

15.1, 10.8 Hz, 1H), 6.40 (d, $J = 15.8$ Hz, 1H), 6.22 – 6.05 (m, 4H), 4.89 (br s, 1H), 4.68 (br s, 1H), 4.53 – 4.31 (m, 2H), 4.28 (dd, $J = 8.8, 4.1$ Hz, 1H), 4.02 – 3.89 (m, 1H), 3.61 – 3.53 (m, 1H), 3.07 – 2.94 (m, 2H), 2.56 (t, $J = 7.7$ Hz, 2H), 2.13 (q, $J = 7.1$ Hz, 2H), 1.95 – 1.76 (m, 1H), 1.62 – 1.53 (m, 3H), 1.49 – 1.34 (m, 4H), 1.33 – 1.26 (m, 2H), 1.25 – 1.15 (m, 3H), 1.00 (d, $J = 6.3$ Hz, 3H).

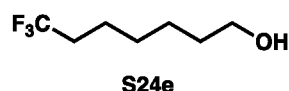
^{13}C NMR (151 MHz, DMSO- d_6): δ 171.05, 169.38, 167.63, 165.43, 143.11, 142.26, 142.02, 139.72, 128.62, 128.27, 128.23, 125.61, 123.29, 123.12, 66.95, 66.72, 58.13, 51.19, 44.74, 42.46, 35.07, 32.18, 30.78, 28.19, 28.17, 19.93, 18.55.

HRMS (ESI): calculated for $\text{C}_{31}\text{H}_{45}\text{N}_4\text{O}_6^+$ ($[\text{M}+\text{H}]^+$) 569.3334, found 569.3338

$$[\alpha]_D^{27} = -103.3 \text{ (c 0.1, MeOH)}$$

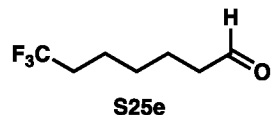
Synthesis of trifluoromethyl cepafungin (**51**)

7,7,7-trifluoroheptan-1-ol (S24e)



Compound **S24e** was prepared as described by Pitre et al.^{S13}

7,7,7-trifluoroheptanal (S25e)

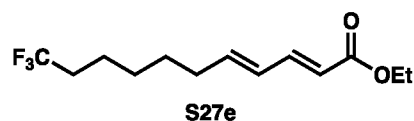


Following General Procedure H: **S24e** (308 mg, 1.81 mmol, 1 equiv) was used to provide **S25e**, used immediately without further purification.

^1H NMR (400 MHz, CDCl_3): δ 9.76 (t, $J = 1.6$ Hz, 1H), 2.52 – 2.38 (m, 2H), 2.22 – 1.99 (m, 2H), 1.82 – 1.49 (m, 4H), 1.45 – 1.22 (m, 2H).

HRMS (ESI): calculated for $\text{C}_7\text{H}_{12}\text{F}_3\text{O}^+$ ($[\text{M}+\text{H}]^+$) 169.0835, found 169.0843

ethyl (2E,4E)-11,11,11-trifluoroundeca-2,4-dienoate (S27e)



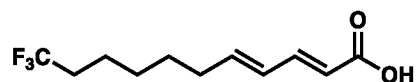
Following General Procedure I: **S25e** was used to provide **S27e** (120 mg, 25% over 2 steps).

^1H NMR (400 MHz, CDCl_3): δ 7.25 (dd, $J = 15.4, 10.3$ Hz, 1H), 6.23 – 6.02 (m, 2H), 5.79 (d, $J = 15.5$ Hz, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 2.18 (q, $J = 7.0$ Hz, 2H), 2.14 – 1.95 (m, 2H), 1.62 – 1.51 (m, 2H), 1.50 – 1.32 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H).

^{13}C NMR (101 MHz, CDCl_3): δ 167.4, 144.9, 143.9, 128.9, 119.7, 60.3, 53.6, 32.8, 33.8 (q, J = 28.4 Hz), 28.4, 28.3, 21.8 (q, J = 2.9 Hz), 14.4.

HRMS (ESI): calculated for $\text{C}_{13}\text{H}_{20}\text{F}_3\text{O}_2^+$ ($[\text{M}+\text{H}]^+$) 265.1410, found 265.1409

(2E,4E)-11,11,11-trifluoroundeca-2,4-dienoic acid (S28f)



S28f

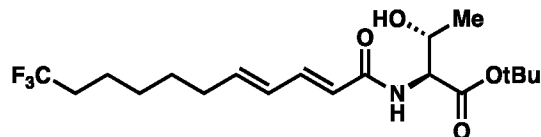
Following General Procedure J: **S27e** (95 mg, 0.359 mmol, 1 equiv) was used to provide **S28f** (77 mg, 91%).

^1H NMR (600 MHz, CDCl_3): δ 7.34 (dd, J = 15.3, 10.3 Hz, 1H), 6.24 – 6.13 (m, 2H), 5.80 (d, J = 15.3 Hz, 1H), 2.23 – 2.18 (m, 2H), 2.12 – 2.01 (m, 2H), 1.62 – 1.52 (m, 2H), 1.52 – 1.44 (m, 2H), 1.43 – 1.35 (m, 2H).

^{13}C NMR (151 MHz, CDCl_3): δ 172.7, 147.4, 145.5, 128.7, 118.7, 33.8 (q, J = 28.4 Hz), 32.8, 28.3, 21.9 (q, J = 2.9 Hz).

HRMS (ESI): calculated for $\text{C}_{11}\text{H}_{16}\text{F}_3\text{O}_2^+$ ($[\text{M}+\text{H}]^+$) 237.1097, found 237.1089

***tert*-butyl ((2E,4E)-11,11,11-trifluoroundeca-2,4-dienoyl)-*L*-threoninate (S30h)**



S30h

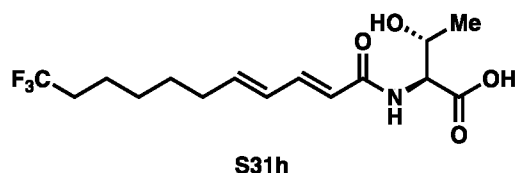
Following General Procedure K: **S28f** (66 mg, 0.279 mmol, 1 equiv), **S29d** (97 mg, 0.419 mmol, 1.5 equiv), HATU (112 mg, 0.293 mmol, 1.05 equiv) and DIPEA (49 μL , 0.279 mmol, 1 equiv) were used to provide **S30h** (98 mg, 78%).

^1H NMR (600 MHz, CDCl_3): δ 7.21 (dd, J = 15.0, 10.8 Hz, 1H), 6.22 – 6.10 (m, 2H), 6.04 (dt, J = 14.7, 6.9 Hz, 1H), 5.89 (d, J = 15.0 Hz, 1H), 4.49 (dd, J = 9.2, 2.1 Hz, 1H), 4.21 (qd, J = 6.2, 2.1 Hz, 1H), 2.21 – 2.14 (m, 2H), 2.12 – 1.99 (m, 2H), 1.61 – 1.51 (m, 2H), 1.51 – 1.41 (m, 11H), 1.41 – 1.33 (m, 2H), 1.20 – 1.14 (m, 12H).

^{13}C NMR (151 MHz, CDCl_3): δ 170.2, 166.5, 142.6, 141.7, 128.8, 122.0, 82.0, 73.9, 67.6, 58.5, 33.8 (q, J = 28.4 Hz), 32.7, 28.9, 28.5, 28.3, 28.3, 21.8 (q, J = 2.9 Hz), 21.0.

HRMS (ESI): calculated for $\text{C}_{23}\text{H}_{39}\text{F}_3\text{NO}_4^+$ ($[\text{M}+\text{H}]^+$) 450.2826, found 450.2815

$$[\alpha]_D^{27} = +4.6 \text{ (c 0.3, MeOH)}$$

((2E,4E)-11,11,11-trifluoroundeca-2,4-dienoyl)-L-threonine (S31h)

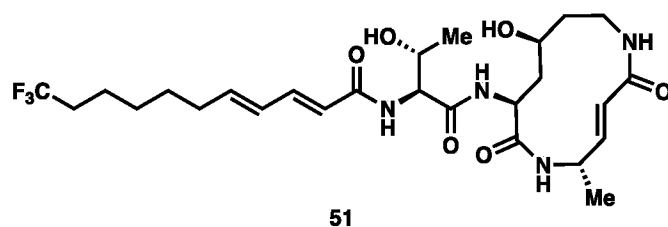
Following General Procedure L: **S30h** (45 mg, 0.100 mmol, 1 equiv) was used to provide **S31h** (43 mg, quant.)

¹H NMR (600 MHz, CDCl₃): δ 7.26 – 7.18 (m, 1H), 6.86 (s, 1H), 6.21 – 6.06 (m, 2H), 5.93 (d, *J* = 14.8 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 4.49 (s, 1H), 2.21 – 2.14 (m, 2H), 2.12 – 2.01 (m, 2H), 1.56 (p, *J* = 8.1 Hz, 2H), 1.49 – 1.42 (m, 2H), 1.41 – 1.35 (m, 2H), 1.31 – 1.17 (m, 5H).

¹³C NMR (151 MHz, CDCl₃): δ 173.3, 168.5, 144.4, 143.5, 128.6, 120.5, 67.4, 57.8, 33.8 (q, *J* = 28.3 Hz), 32.8, 29.9, 28.4, 28.3, 21.8 (q, *J* = 2.9 Hz), 19.3.

HRMS (ESI): calculated for C₁₅H₂₃F₃NO₄⁺ ([M+H]⁺) 338.1574, found 338.1583

$$[\alpha]_D^{27} = +8.1 (c\ 0.3, \text{MeOH})$$

(2E,4E)-11,11,11-trifluoro-N-((2S,3R)-3-hydroxy-1-(((5S,8S,10S,E)-10-hydroxy-5-methyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)undeca-2,4-dienamide (51)

Following General Procedure F: **S31h** (34 mg, 0.101 mmol, 2 equiv) and **S19e** (60 mg, 0.0501 mmol as judged by ¹H NMR analysis, 1 equiv) were used to provide **51** (8.9 mg, 32%) as a fluffy white solid.

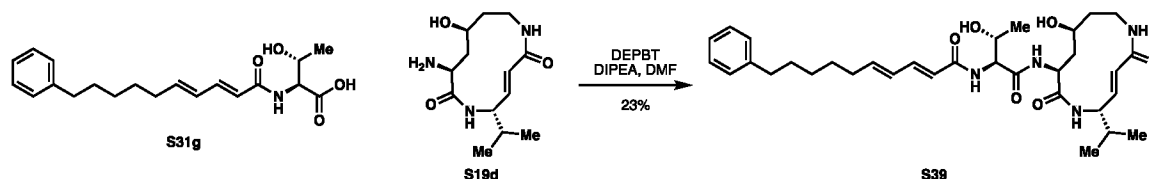
¹H NMR (600 MHz, DMSO-*d*₆): δ 8.67 (s, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 6.2 Hz, 1H), 7.00 (dd, *J* = 15.1, 10.9 Hz, 1H), 6.40 (d, *J* = 14.4 Hz, 1H), 6.24 – 6.05 (m, 4H), 4.87 (d, *J* = 4.6 Hz, 1H), 4.67 (s, 1H), 4.49 – 4.30 (m, 2H), 4.28 (dd, *J* = 8.8, 4.1 Hz, 1H), 4.04 – 3.88 (m, 1H), 3.57 (s, 1H), 3.08 – 2.92 (m, 2H), 2.28 – 2.18 (m, 2H), 2.14 (q, *J* = 6.9 Hz, 2H), 1.85 (s, 1H), 1.58 (d, *J* = 13.2 Hz, 1H), 1.51 – 1.45 (m, 2H), 1.45 – 1.38 (m, 3H), 1.38 – 1.31 (m, 3H), 1.26 – 1.14 (m, 3H), 1.00 (d, *J* = 6.3 Hz, 3H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.0, 169.4, 167.6, 165.4, 143.1, 141.8, 139.7, 128.7, 123.3, 123.2, 67.0, 66.7, 58.1, 51.2, 44.8, 42.5, 32.3 (q, J = 27.3 Hz), 32.0, 27.9, 27.5, 21.3 (q, J = 2.9 Hz), 19.9, 18.5.

HRMS (ESI): calculated for C₂₆H₄₀F₃N₄O₆⁺ ([M+H]⁺) 561.2894, found 561.2901

$$[\alpha]_D^{27} = -80.6 \text{ (c 0.2, MeOH)}$$

Synthesis of hybrid phenyl-valinyl cepafungin (**S39**)



(2*E*,4*E*)-*N*-((2*S*,3*R*)-3-hydroxy-1-(((5*S*,8*S*,10*S*,*E*)-10-hydroxy-5-isopropyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)-10-phenyldeca-2,4-dienamide (S39**)**

Following General Procedure F: **S31g** (38 mg, 0.109 mmol, 2.04 equiv) and **S19d** (56 mg, 0.0535 mmol as judged by ¹H NMR analysis, 1 equiv) were used to provide **S39** (7.5 mg, 23%) as a fluffy white solid.

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.48 (d, J = 8.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.42 (t, J = 6.3 Hz, 1H), 7.29 – 7.24 (m, 2H), 7.19 – 7.14 (m, 3H), 6.99 (dd, J = 15.1, 10.8 Hz, 1H), 6.33 (dd, J = 15.9, 6.6 Hz, 1H), 6.23 – 6.03 (m, 4H), 4.84 (d, J = 5.1 Hz, 1H), 4.67 (d, J = 5.0 Hz, 1H), 4.45 – 4.32 (m, 1H), 4.28 (dd, J = 8.8, 4.5 Hz, 1H), 4.10 – 3.99 (m, 1H), 3.99 – 3.84 (m, 1H), 3.65 – 3.49 (m, 1H), 3.05 – 2.93 (m, 2H), 2.56 (t, J = 7.7 Hz, 2H), 2.12 (q, J = 7.1 Hz, 2H), 1.86 (td, J = 12.3, 6.8 Hz, 1H), 1.75 (dq, J = 13.6, 6.9 Hz, 1H), 1.61 – 1.53 (m, 3H), 1.45 – 1.35 (m, 4H), 1.32 – 1.26 (m, 2H), 0.99 (d, J = 6.3 Hz, 3H), 0.94 – 0.86 (m, 6H).

¹³C NMR (151 MHz, DMSO-*d*₆): δ 171.47, 169.37, 167.77, 165.38, 142.27, 141.98, 140.82, 139.68, 128.64, 128.28, 128.24, 125.62, 124.76, 123.16, 66.88, 66.79, 58.17, 55.64, 51.21, 42.25, 35.09, 32.19, 30.80, 30.58, 28.20, 28.18, 19.77, 19.63, 19.12.

HRMS (ESI): calculated for C₃₃H₄₉N₄O₆⁺ ([M+H]⁺) 597.3647, found 597.3643

$$[\alpha]_D^{27} = -70.0 \text{ (c 0.1, MeOH)}$$

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INCORPORATION BY REFERENCE

The present application refers to various issued patent, published patent applications, scientific journal articles, and other publications, all of which are incorporated herein by reference. The details of one or more embodiments of the invention are set forth herein. Other features, objects, and advantages of the invention will be apparent from the Detailed Description, the Figures, the Examples, and the Claims.

EQUIVALENTS AND SCOPE

In the articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Embodiments or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

The compounds provided herein may also contain linkages (e.g., carbon-carbon bonds) wherein bond rotation is restricted about that particular linkage, e.g. restriction resulting from the presence of a ring or double bond. Accordingly, all cis/trans and E/Z isomers are expressly included in the present invention. The compounds herein may also be represented in multiple tautomeric forms, in such instances, the invention expressly includes all tautomeric forms of the compounds provided herein, even though only a single tautomeric form may be represented. All such isomeric forms of such compounds herein are expressly included in the present invention. All crystal forms and polymorphs of the compounds provided herein are expressly included in the present invention. All hydrate and solvate forms of the compounds provided herein are expressly included in the present invention. Also embodied are extracts and fractions comprising compounds provided herein. The term “isomers” is intended to include diastereoisomers, enantiomers, regioisomers, structural isomers, rotational isomers, tautomers, and the like. All such isomers of such compounds herein are expressly included in the present invention. For compounds which contain one or more stereogenic centers, e.g., chiral compounds, the methods provided herein may be carried out with an enantiomerically enriched compound, a racemate, or a mixture of diastereomers.

Furthermore, the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claims that is dependent on the same base claim. Where elements are presented as lists, e.g., in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements and/or features, certain embodiments of the disclosure or aspects of the disclosure consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that the terms “comprising” and “containing” are intended to be open and permits the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

A group is optionally substituted unless expressly provided otherwise. The term “optionally substituted” refers to being substituted or unsubstituted. In certain embodiments, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups are optionally substituted. “Optionally substituted” refers to a group which is substituted or unsubstituted (*e.g.*, “substituted” or “unsubstituted” alkyl, “substituted” or “unsubstituted” alkenyl, “substituted” or “unsubstituted” alkynyl, “substituted” or “unsubstituted” heteroalkyl, “substituted” or “unsubstituted” heteroalkenyl, “substituted” or “unsubstituted” heteroalkynyl, “substituted” or “unsubstituted” carbocyclyl, “substituted” or “unsubstituted” heterocyclyl, “substituted” or “unsubstituted” aryl or “substituted” or “unsubstituted” heteroaryl group). In general, the term “substituted” means that at least one hydrogen present on a group is replaced with a permissible substituent, *e.g.*, a substituent which upon substitution results in a stable compound, *e.g.*, a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a “substituted” group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term “substituted” is contemplated to include substitution with all permissible substituents of organic compounds, and includes any of the substituents described herein that results in the formation of a stable compound. The present invention contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety. The invention is not limited in any manner by the exemplary substituents described herein.

Exemplary carbon atom substituents include halogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{N}_3$, $-\text{SO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{OH}$, $-\text{OR}^{\text{aa}}$, $-\text{ON}(\text{R}^{\text{bb}})_2$, $-\text{N}(\text{R}^{\text{bb}})_2$, $-\text{N}(\text{R}^{\text{bb}})_3^+\text{X}^-$, $-\text{N}(\text{OR}^{\text{cc}})\text{R}^{\text{bb}}$, $-\text{SH}$, $-\text{SR}^{\text{aa}}$, $-\text{SSR}^{\text{cc}}$, $-\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{CO}_2\text{H}$, $-\text{CHO}$, $-\text{C}(\text{OR}^{\text{cc}})_2$, $-\text{CO}_2\text{R}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{R}^{\text{aa}}$, $-\text{OCO}_2\text{R}^{\text{aa}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{CO}_2\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{NR}^{\text{bb}})\text{R}^{\text{aa}}$, $-\text{C}(=\text{NR}^{\text{bb}})\text{OR}^{\text{aa}}$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{R}^{\text{aa}}$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{OR}^{\text{aa}}$, $-\text{C}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{O})\text{NR}^{\text{bb}}\text{SO}_2\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{SO}_2\text{R}^{\text{aa}}$, $-\text{SO}_2\text{N}(\text{R}^{\text{bb}})_2$, $-\text{SO}_2\text{R}^{\text{aa}}$, $-\text{SO}_2\text{OR}^{\text{aa}}$, $-\text{OSO}_2\text{R}^{\text{aa}}$, $-\text{S}(=\text{O})\text{R}^{\text{aa}}$, $-\text{OS}(=\text{O})\text{R}^{\text{aa}}$, $-\text{Si}(\text{R}^{\text{aa}})_3$, $-\text{OSi}(\text{R}^{\text{aa}})_3$, $-\text{C}(=\text{S})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{C}(=\text{S})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{S})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{OR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{R}^{\text{aa}}$, $-\text{P}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{P}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{OP}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{OP}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{P}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{OP}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{R}^{\text{aa}})_2$,

$-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{P}(\text{R}^{\text{cc}})_2$, $-\text{P}(\text{OR}^{\text{cc}})_2$, $-\text{P}(\text{R}^{\text{cc}})_3^+\text{X}^-$,
 $-\text{P}(\text{OR}^{\text{cc}})_3^+\text{X}^-$, $-\text{P}(\text{R}^{\text{cc}})_4$, $-\text{P}(\text{OR}^{\text{cc}})_4$, $-\text{OP}(\text{R}^{\text{cc}})_2$, $-\text{OP}(\text{R}^{\text{cc}})_3^+\text{X}^-$, $-\text{OP}(\text{OR}^{\text{cc}})_2$, $-\text{OP}(\text{OR}^{\text{cc}})_3^+\text{X}^-$,
 $-\text{OP}(\text{R}^{\text{cc}})_4$, $-\text{OP}(\text{OR}^{\text{cc}})_4$, C_{1-20} alkyl, C_{1-20} perhaloalkyl, C_{1-20} alkenyl, C_{1-20} alkynyl,
hetero C_{1-20} alkyl, hetero C_{1-20} alkenyl, hetero C_{1-20} alkynyl, C_{3-10} carbocyclyl, 3-14 membered
heterocyclyl, C_{6-14} aryl, and 5-14 membered heteroaryl, wherein X^- is a counterion;

or two geminal hydrogens on a carbon atom are replaced with the group $=\text{O}$, $=\text{S}$,
 $=\text{NN}(\text{R}^{\text{bb}})_2$, $=\text{NNR}^{\text{bb}}\text{C}(=\text{O})\text{R}^{\text{aa}}$, $=\text{NNR}^{\text{bb}}\text{C}(=\text{O})\text{OR}^{\text{aa}}$, $=\text{NNR}^{\text{bb}}\text{S}(=\text{O})_2\text{R}^{\text{aa}}$, $=\text{NR}^{\text{bb}}$, or $=\text{NOR}^{\text{cc}}$;
wherein:

each instance of R^{aa} is, independently, selected from C_{1-20} alkyl, C_{1-20}
perhaloalkyl, C_{1-20} alkenyl, C_{1-20} alkynyl, hetero C_{1-20} alkyl, hetero C_{1-20} alkenyl,
hetero C_{1-20} alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-
14 membered heteroaryl, or two R^{aa} groups are joined to form a 3-14 membered
heterocyclyl or 5-14 membered heteroaryl ring;

each instance of R^{bb} is, independently, selected from hydrogen, $-\text{OH}$, $-\text{OR}^{\text{aa}}$,
 $-\text{N}(\text{R}^{\text{cc}})_2$, $-\text{CN}$, $-\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{cc}})_2$, $-\text{CO}_2\text{R}^{\text{aa}}$, $-\text{SO}_2\text{R}^{\text{aa}}$, $-\text{C}(=\text{NR}^{\text{cc}})\text{OR}^{\text{aa}}$,
 $-\text{C}(=\text{NR}^{\text{cc}})\text{N}(\text{R}^{\text{cc}})_2$, $-\text{SO}_2\text{N}(\text{R}^{\text{cc}})_2$, $-\text{SO}_2\text{R}^{\text{cc}}$, $-\text{SO}_2\text{OR}^{\text{cc}}$, $-\text{SOR}^{\text{aa}}$, $-\text{C}(=\text{S})\text{N}(\text{R}^{\text{cc}})_2$,
 $-\text{C}(=\text{O})\text{SR}^{\text{cc}}$, $-\text{C}(=\text{S})\text{SR}^{\text{cc}}$, $-\text{P}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{P}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{P}(=\text{O})(\text{N}(\text{R}^{\text{cc}})_2)_2$, C_{1-20}
alkyl, C_{1-20} perhaloalkyl, C_{1-20} alkenyl, C_{1-20} alkynyl, hetero C_{1-20} alkyl, hetero C_{1-20}
alkenyl, hetero C_{1-20} alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14}
aryl, and 5-14 membered heteroaryl, or two R^{bb} groups are joined to form a 3-14
membered heterocyclyl or 5-14 membered heteroaryl ring;

each instance of R^{cc} is, independently, selected from hydrogen, C_{1-20} alkyl, C_{1-20}
perhaloalkyl, C_{1-20} alkenyl, C_{1-20} alkynyl, hetero C_{1-20} alkyl, hetero C_{1-20} alkenyl,
hetero C_{1-20} alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-
14 membered heteroaryl, or two R^{cc} groups are joined to form a 3-14 membered
heterocyclyl or 5-14 membered heteroaryl ring.

In certain embodiments, each carbon atom substituent is independently halogen,
substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C_{1-6} alkyl, $-\text{OR}^{\text{aa}}$,
 $-\text{SR}^{\text{aa}}$, $-\text{N}(\text{R}^{\text{bb}})_2$, $-\text{CN}$, $-\text{SCN}$, $-\text{NO}_2$, $-\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{CO}_2\text{R}^{\text{aa}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{O})\text{R}^{\text{aa}}$,
 $-\text{OCO}_2\text{R}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{CO}_2\text{R}^{\text{aa}}$, or $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$. In
certain embodiments, each carbon atom substituent is independently halogen, substituted
(*e.g.*, substituted with one or more halogen) or unsubstituted C_{1-10} alkyl, $-\text{OR}^{\text{aa}}$, $-\text{SR}^{\text{aa}}$,
 $-\text{N}(\text{R}^{\text{bb}})_2$, $-\text{CN}$, $-\text{SCN}$, $-\text{NO}_2$, $-\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{CO}_2\text{R}^{\text{aa}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{O})\text{R}^{\text{aa}}$,
 $-\text{OCO}_2\text{R}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{CO}_2\text{R}^{\text{aa}}$, or $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$,

wherein R^{aa} is hydrogen, substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C₁₋₁₀ alkyl, an oxygen protecting group (*e.g.*, silyl, TBDPS, TBDMS, TIPS, TES, TMS, MOM, THP, *t*-Bu, Bn, allyl, acetyl, pivaloyl, or benzoyl) when attached to an oxygen atom, or a sulfur protecting group (*e.g.*, acetamidomethyl, *t*-Bu, 3-nitro-2-pyridine sulfenyl, 2-pyridine-sulfenyl, or triphenylmethyl) when attached to a sulfur atom; and each R^{bb} is independently hydrogen, substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C₁₋₁₀ alkyl, or a nitrogen protecting group (*e.g.*, Bn, Boc, Cbz, Fmoc, trifluoroacetyl, triphenylmethyl, acetyl, or Ts). In certain embodiments, each carbon atom substituent is independently halogen, substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C₁₋₆ alkyl, -OR^{aa}, -SR^{aa}, -N(R^{bb})₂, -CN, -SCN, or -NO₂. In certain embodiments, each carbon atom substituent is independently halogen, substituted (*e.g.*, substituted with one or more halogen moieties) or unsubstituted C₁₋₁₀ alkyl, -OR^{aa}, -SR^{aa}, -N(R^{bb})₂, -CN, -SCN, or -NO₂, wherein R^{aa} is hydrogen, substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C₁₋₁₀ alkyl, an oxygen protecting group (*e.g.*, silyl, TBDPS, TBDMS, TIPS, TES, TMS, MOM, THP, *t*-Bu, Bn, allyl, acetyl, pivaloyl, or benzoyl) when attached to an oxygen atom, or a sulfur protecting group (*e.g.*, acetamidomethyl, *t*-Bu, 3-nitro-2-pyridine sulfenyl, 2-pyridine-sulfenyl, or triphenylmethyl) when attached to a sulfur atom; and each R^{bb} is independently hydrogen, substituted (*e.g.*, substituted with one or more halogen) or unsubstituted C₁₋₁₀ alkyl, or a nitrogen protecting group (*e.g.*, Bn, Boc, Cbz, Fmoc, trifluoroacetyl, triphenylmethyl, acetyl, or Ts).

The term “proteasome” as used herein includes, without limitation, those proteasomes that are expressed as “standard” or “constitutive” proteasomes, immunoproteasomes, or thymoproteasomes.

This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the embodiments. Because such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the invention can be excluded from any embodiment, for any reason, whether or not related to the existence of prior art.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The

scope of the present embodiments described herein is not intended to be limited to the above Description, but rather is as set forth in the appended embodiments. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

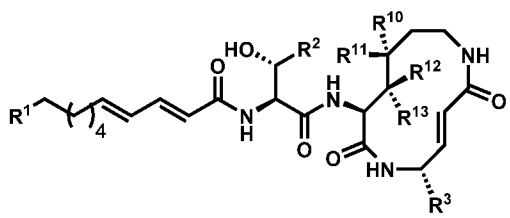
EMBODIMENTS

One aspect of the invention is any compound, or salt thereof, described herein. Another aspect is a method of treating a disease, disorder, or symptom thereof, in a subject, comprising administration to the subject of a compound, or salt thereof, herein. Another aspect is a method of inhibiting a proteasome in a subject, comprising administration to the subject of a compound, or salt thereof, herein. Another aspect is a method of making a compound, or salt thereof, described herein using one or more reagents, chemical transformations, or chemical intermediate compounds as described herein.

Further embodiments include:

1. A compound, or salt thereof, that is selected from compounds 40-51 or S11-S39, or salt thereof.
2. A compound, or salt thereof, that is selected from compounds 40-51 or S39, or salt thereof.
3. A pharmaceutical composition comprising a compound of any of compounds 40-51 or S11-S39, or salt thereof, and a pharmaceutically acceptable excipient.
4. A pharmaceutical composition comprising a compound of any of compounds 40-51 or S39, or salt thereof, and a pharmaceutically acceptable excipient.
5. A method of treating cancer in a subject, comprising administering any of compounds 40-51 or S11-S39, or salt thereof, to the subject.
6. A method of treating cancer in a subject, comprising administering any of compounds 40-51 or S39, or salt thereof, to the subject.
7. The method of any one of embodiments 5 or 6, wherein the cancer is multiple myeloma or solid tumor.
8. A method of inhibiting a proteasome in a subject, comprising administering any of compounds 40-51 or S11-S39, or salt thereof, to the subject.
9. A method of inhibiting a proteasome in a subject, comprising administering any of compounds 40-51 or S39, or salt thereof, to the subject.

10. The method of any one of embodiments 8 or 9, wherein the proteasome is the yeast core particle (CP) β 5 subunit.
11. The method of any one of embodiments 8 or 9, wherein the proteasome is the yeast core particle (CP) β 2 subunit.
12. The method of any one of embodiments 8 or 9, wherein the proteasome is the yeast core particle (CP) β 5 subunit and the yeast core particle (CP) β 2 subunit.
13. A method of inhibiting a proteasome, comprising contacting a compound of any of compounds 40-51 or S11-S39, or salt thereof, with a proteasome.
14. A method of inhibiting a proteasome, comprising contacting a compound of any of compounds 40-51 or S39, or salt thereof, with a proteasome.
15. A method of making compound 40-51 and S39, comprising treating compound S17 with TFA, and the resulting product S18 with DMTMMT, and the resulting product S19 with compound S20, to provide compound 40-51 and S39.
16. A compound of formula:



or salt thereof, wherein:

R^1 is optionally substituted alkyl, optionally substituted cycloalkyl, or optionally substituted aryl;

R^2 is optionally substituted alkyl or optionally substituted aryl;

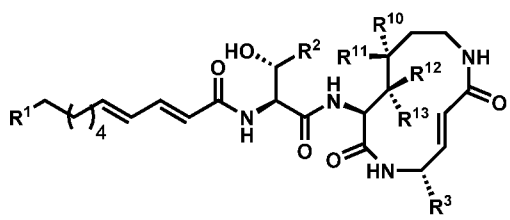
R^3 is optionally substituted alkyl; and

each of R^{10} , R^{11} , R^{12} , and R^{13} is hydrogen or $-OR'$, or R^{10} and R^{11} are taken together to form $=O$, or R^{12} and R^{13} are taken together to form $=O$, wherein each instance of R' is independently hydrogen, optionally substituted alkyl, or optionally substituted acyl;

provided that if R^2 is $-CH_3$, R^3 is $-CH_3$, R^{10} is hydrogen, R^{11} is hydrogen or $-OH$, R^{12} is hydrogen, and R^{13} is hydrogen, R^1 is not $-CH(CH_3)_2$ or $-CH_2CH_3$.

CLAIMS

1. A compound, or salt thereof, that is selected from compounds 40-51 or S11-S39, or salt thereof.
2. The compound, or salt thereof, of claim 1, wherein the compound, or salt thereof, is selected from compounds 40-51 or S39, or salt thereof.
3. A method of treating cancer in a subject, comprising administering any of compounds 40-51 or S11-S39, or salt thereof, to the subject.
4. A method of inhibiting a proteasome in a subject, comprising administering any of compounds 40-51 or S11-S39, or salt thereof, to the subject.
5. A method of inhibiting a proteasome, comprising contacting a compound of any of compounds 40-51 or S11-S39, or salt thereof, with a proteasome.
6. A method of making compound 40-51 and S39, comprising treating compound S17 with TFA, and the resulting product S18 with DMTMMT, and the resulting product S19 with compound S20, to provide compound 40-51 and S39.
7. A compound of formula:



or salt thereof, wherein:

R^1 is optionally substituted alkyl, optionally substituted cycloalkyl, or optionally substituted aryl;

R^2 is optionally substituted alkyl or optionally substituted aryl;

R^3 is optionally substituted alkyl; and

each of R^{10} , R^{11} , R^{12} , and R^{13} is hydrogen or $-OR'$, or R^{10} and R^{11} are taken together to form $=O$, or R^{12} and R^{13} are taken together to form $=O$, wherein each instance of R' is independently hydrogen, optionally substituted alkyl, or optionally substituted acyl;

provided that if R^2 is $-CH_3$, R^3 is $-CH_3$, R^{10} is hydrogen, R^{11} is hydrogen or $-OH$, R^{12} is hydrogen, and R^{13} is hydrogen, R^1 is not $-CH(CH_3)_2$ or $-CH_2CH_3$.

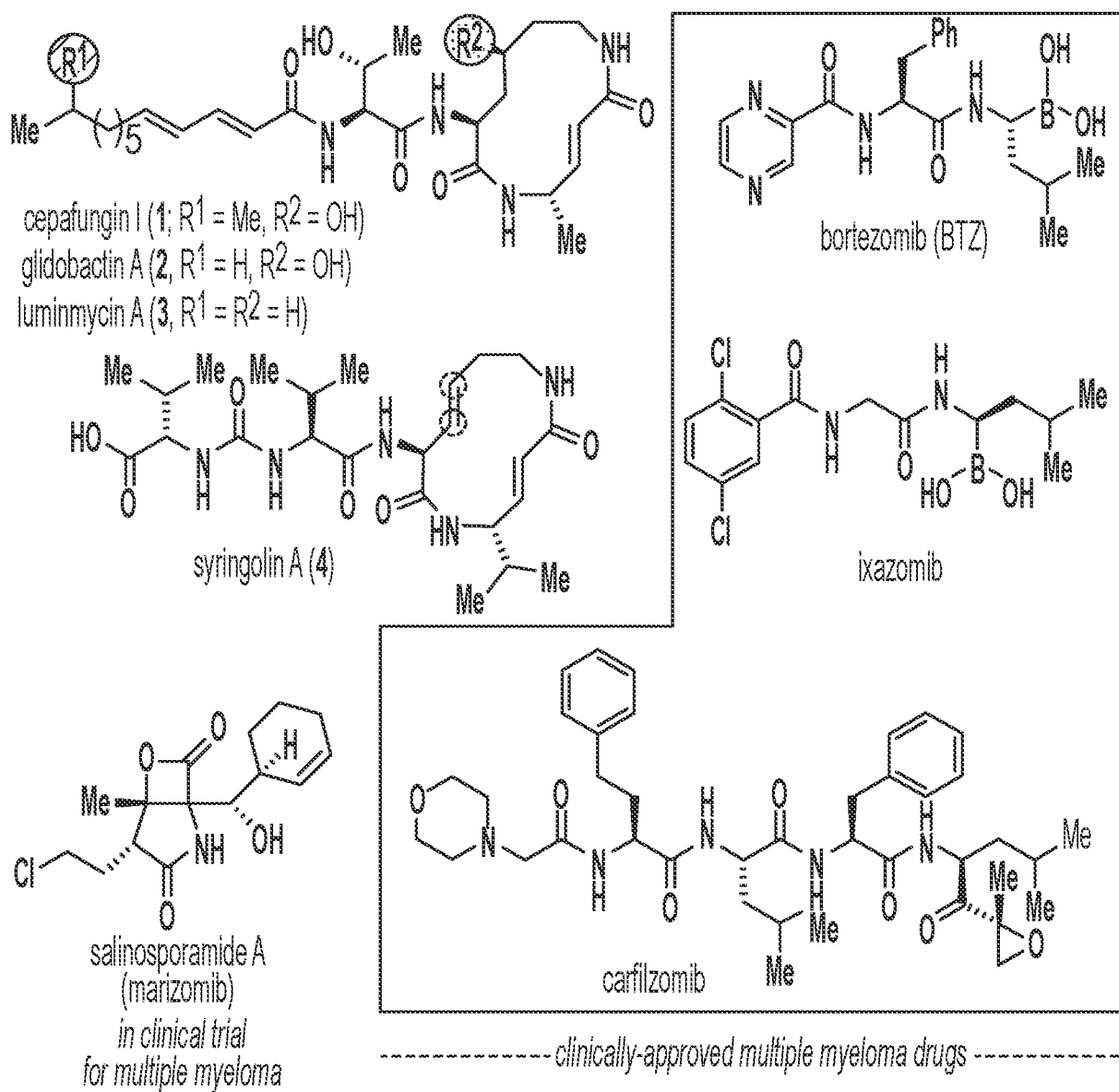


FIG. 1

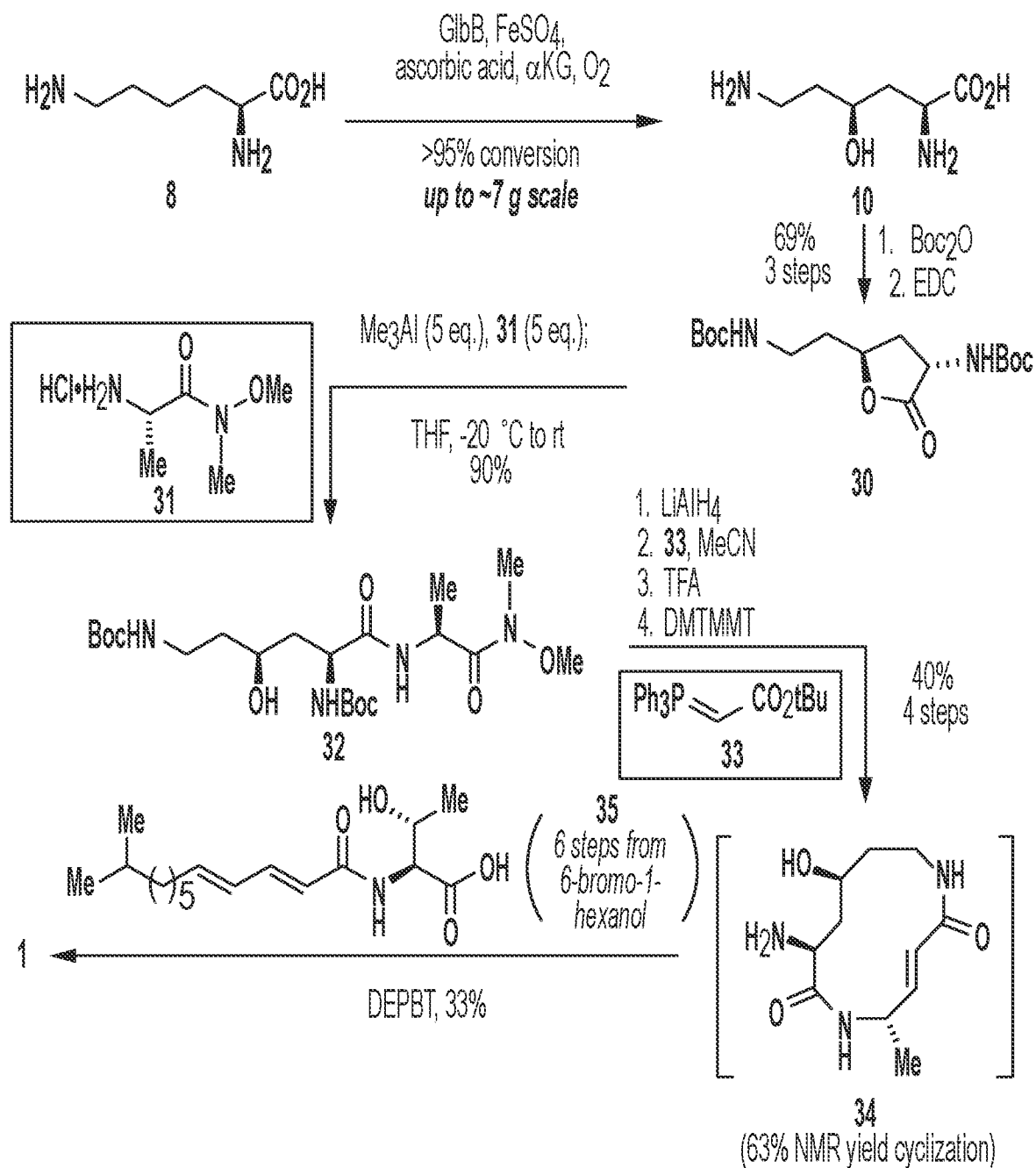


FIG. 2

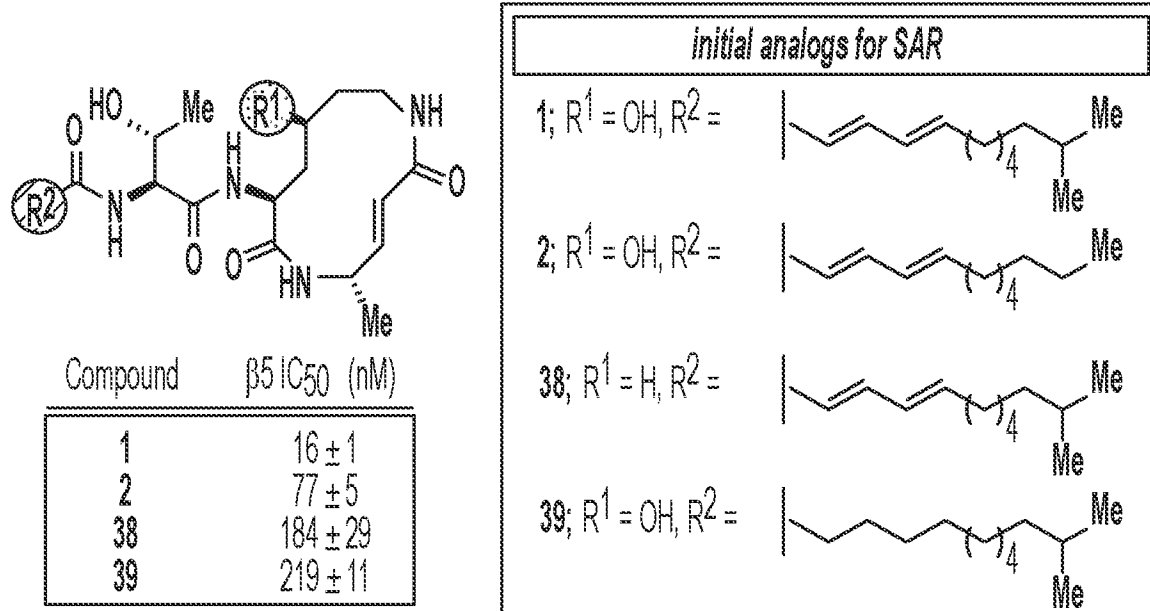
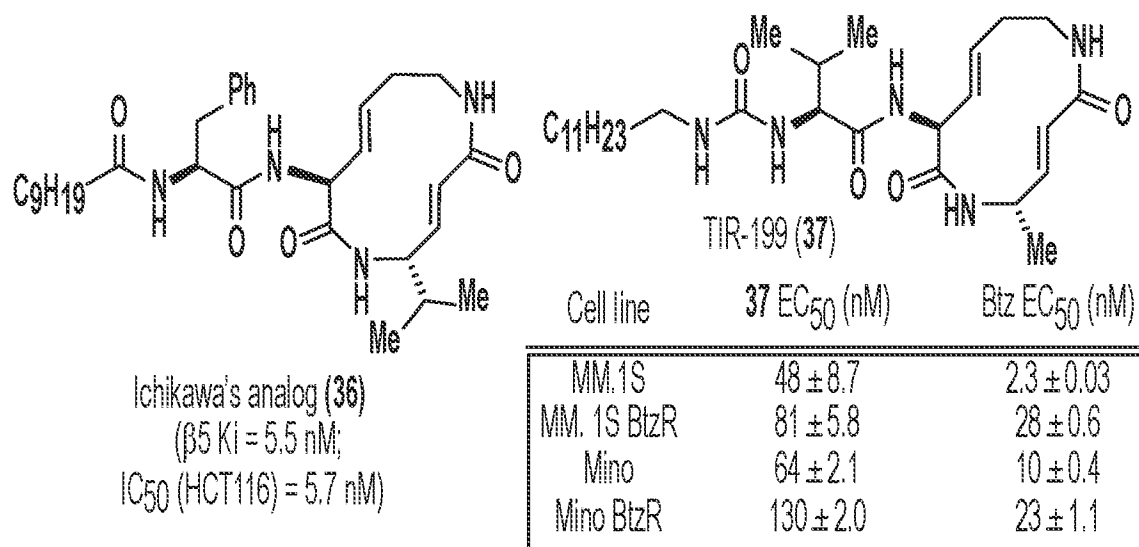


FIG. 3

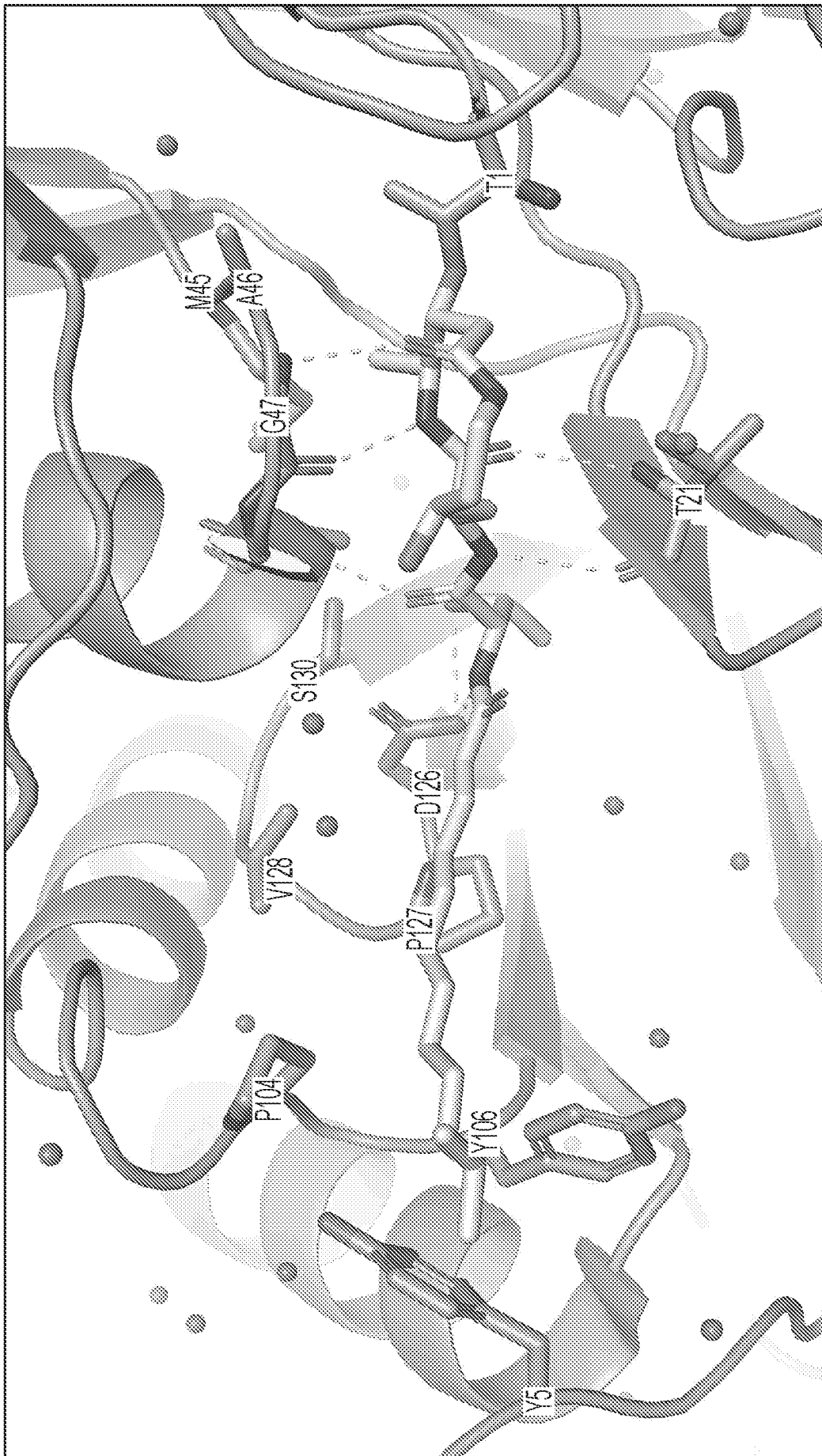
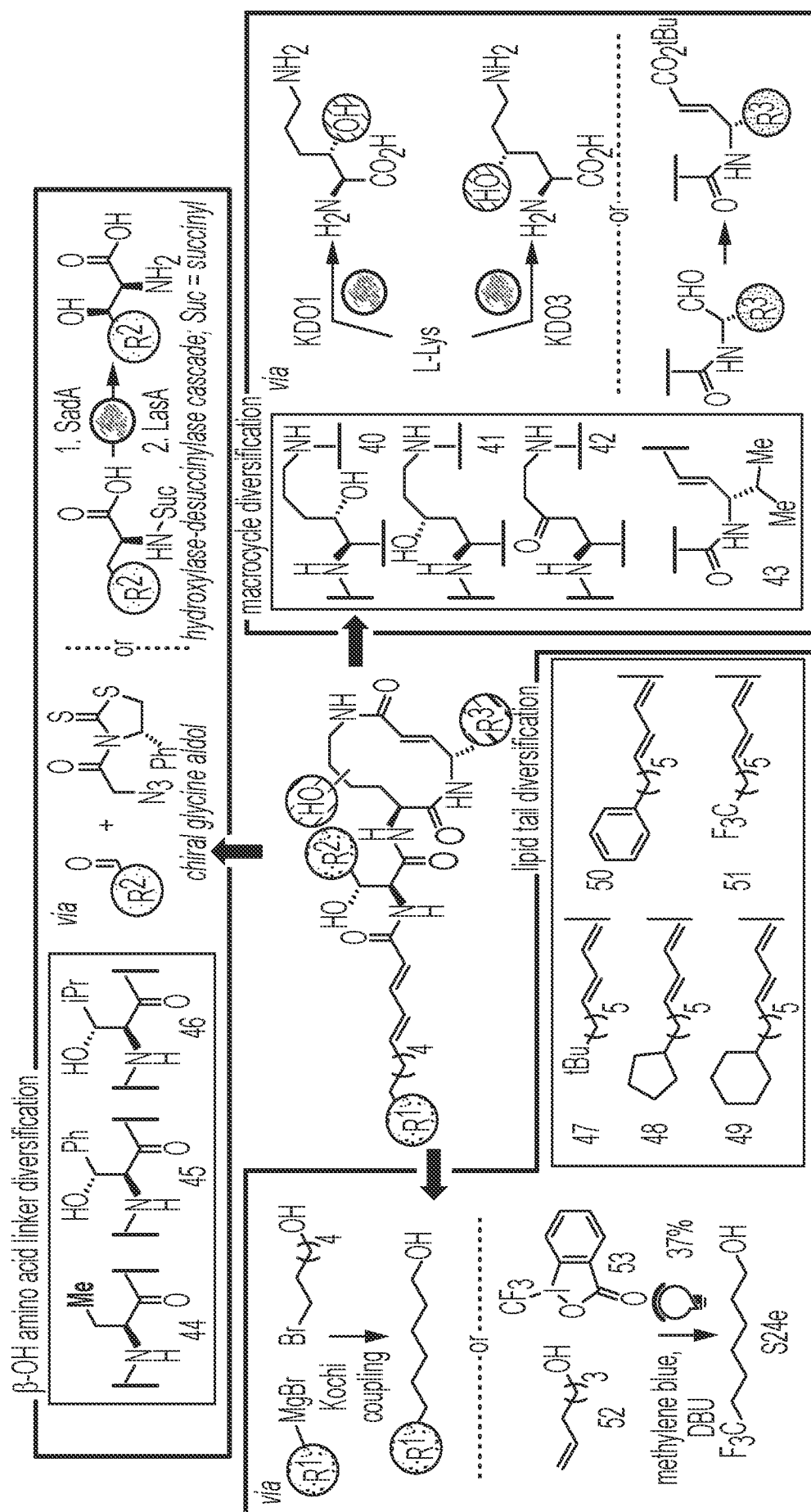


FIG. 4





 DEPARTMENT OF HEALTH AND HUMAN SERVICES

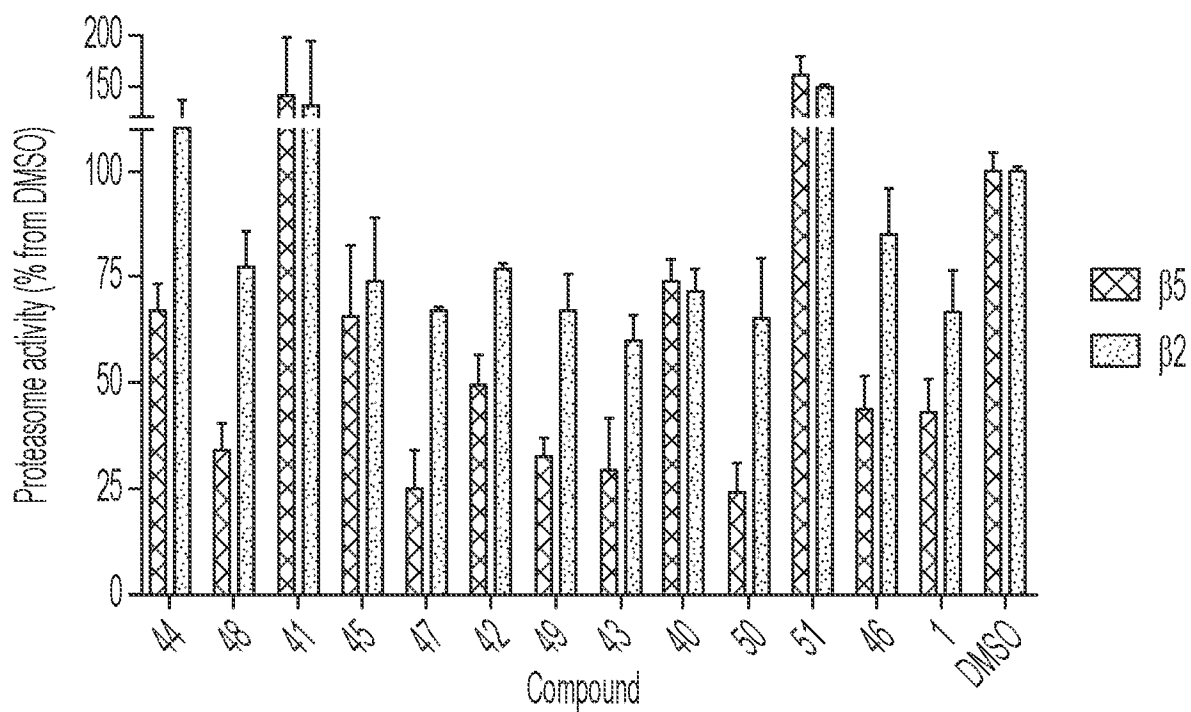


FIG. 6A

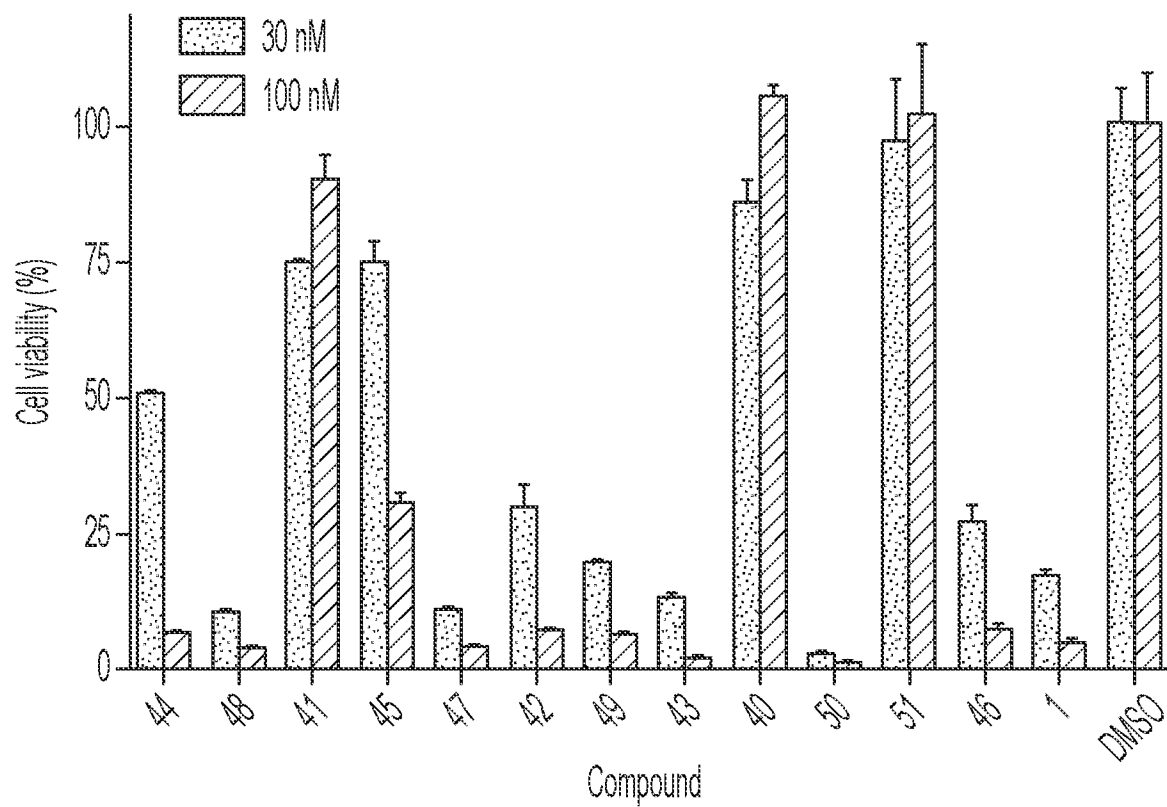


FIG. 6B

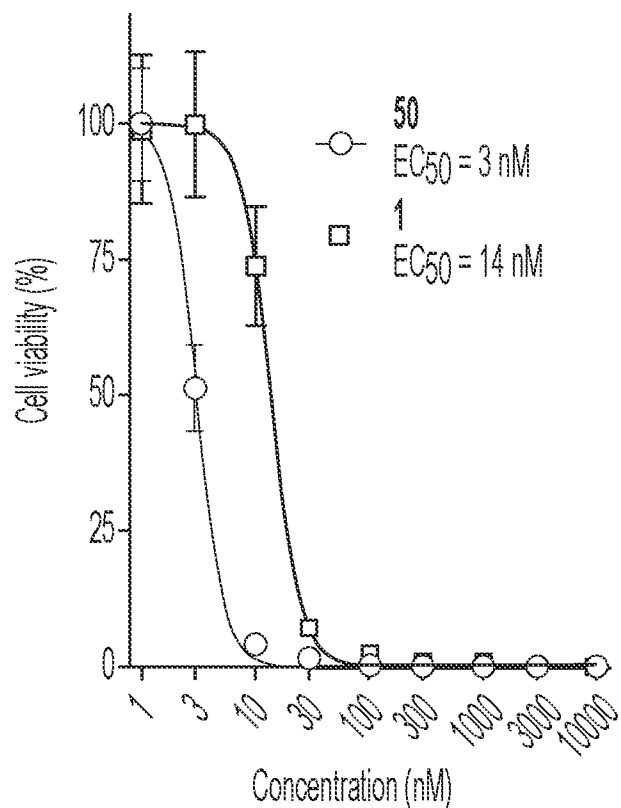


FIG. 6C

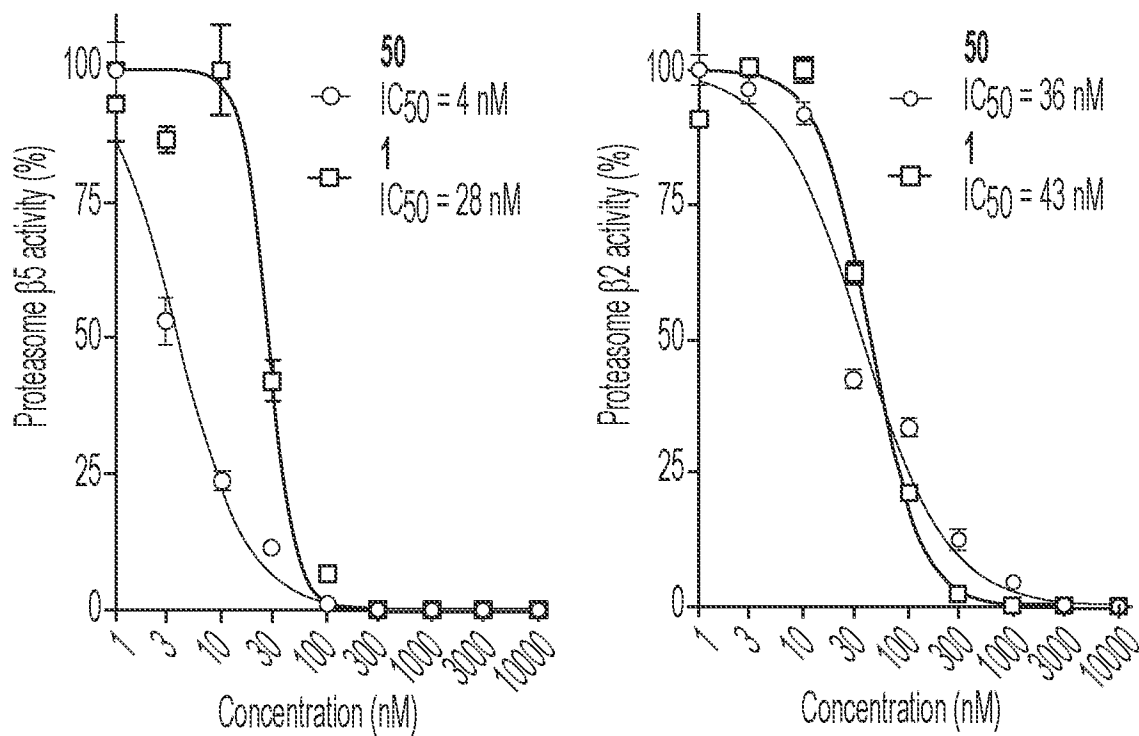


FIG. 6D

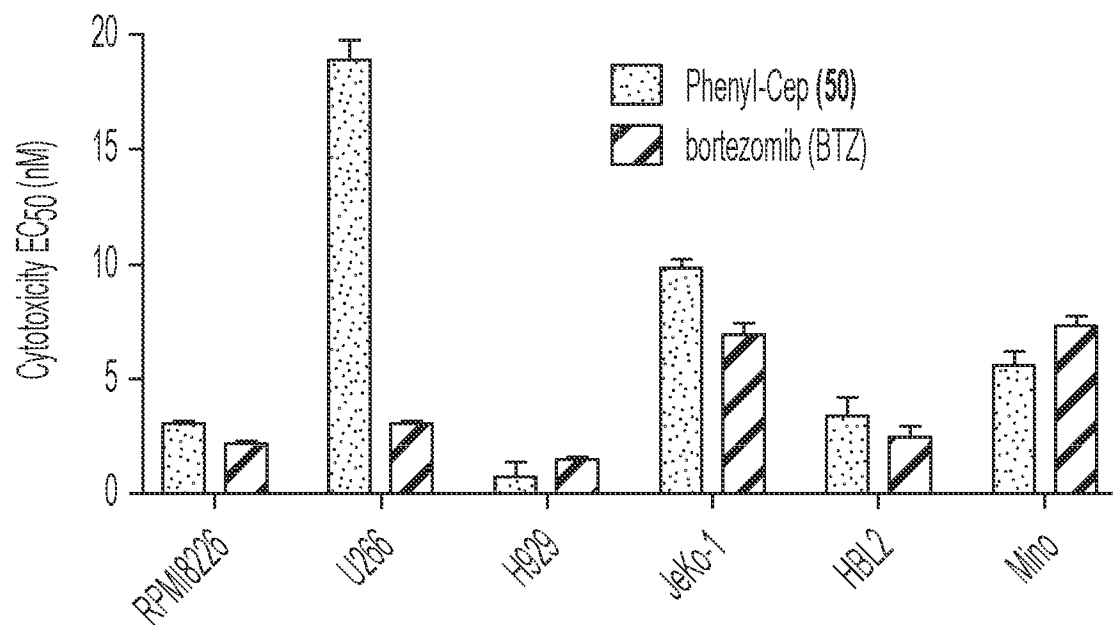


FIG. 6E

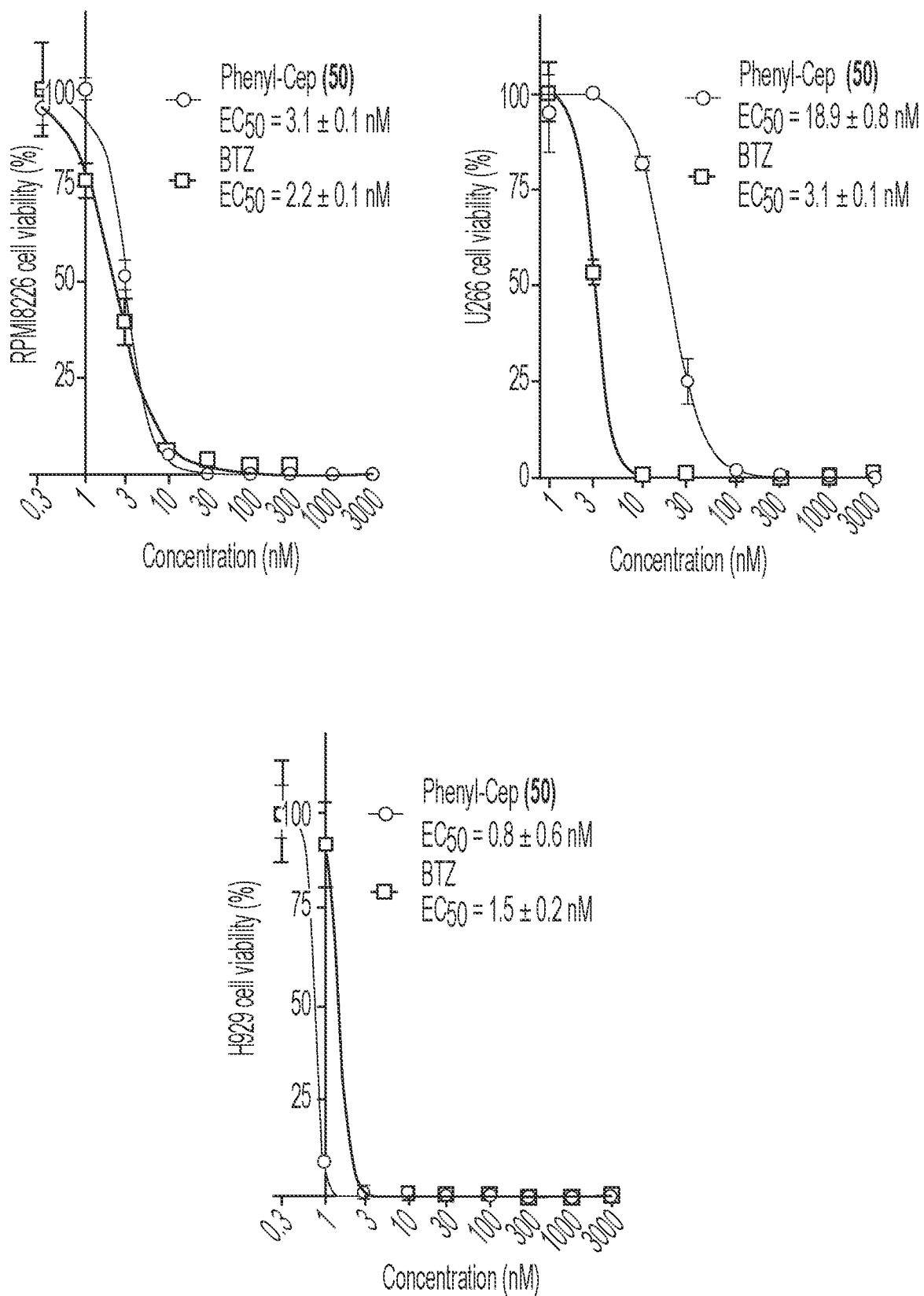


FIG. 7

SUBSTITUTE SHEET (RULE 26)

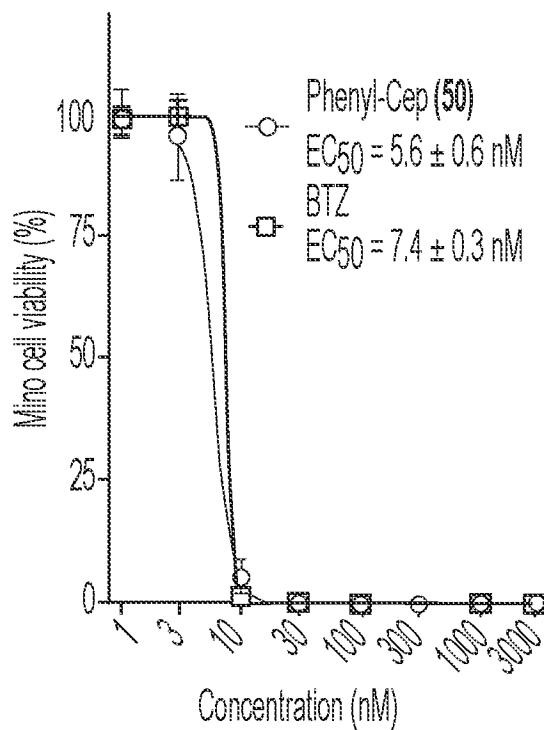
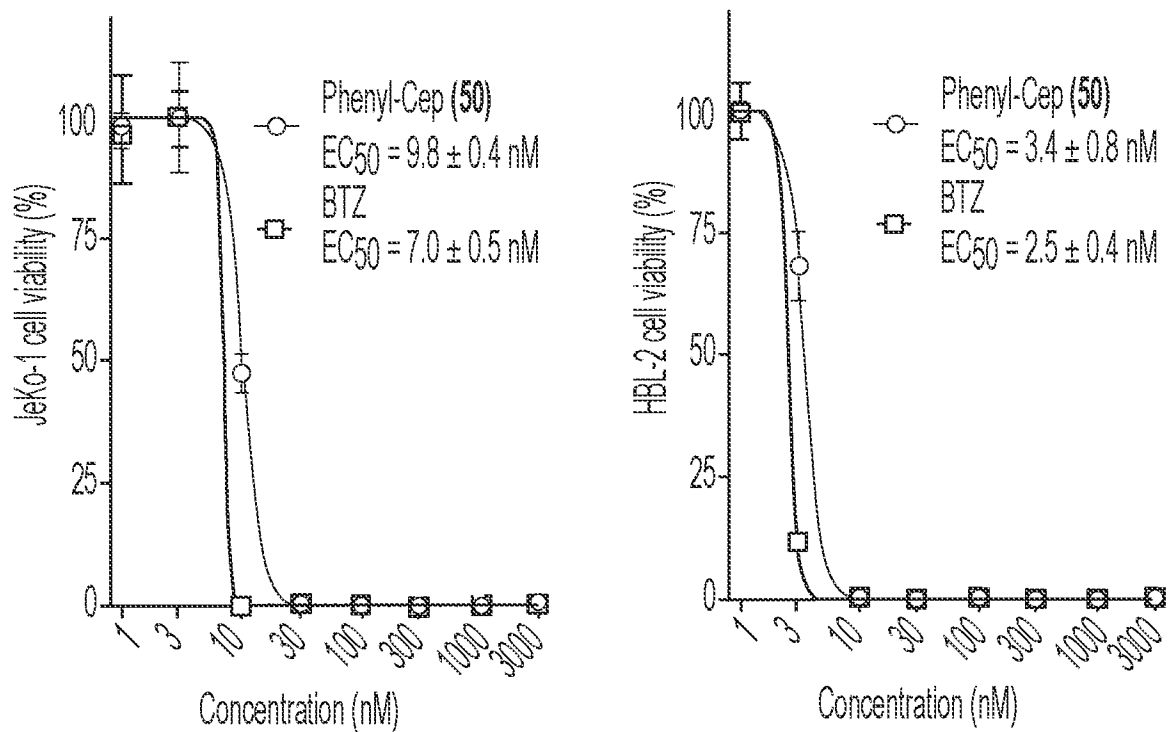


FIG. 7
CONTINUED

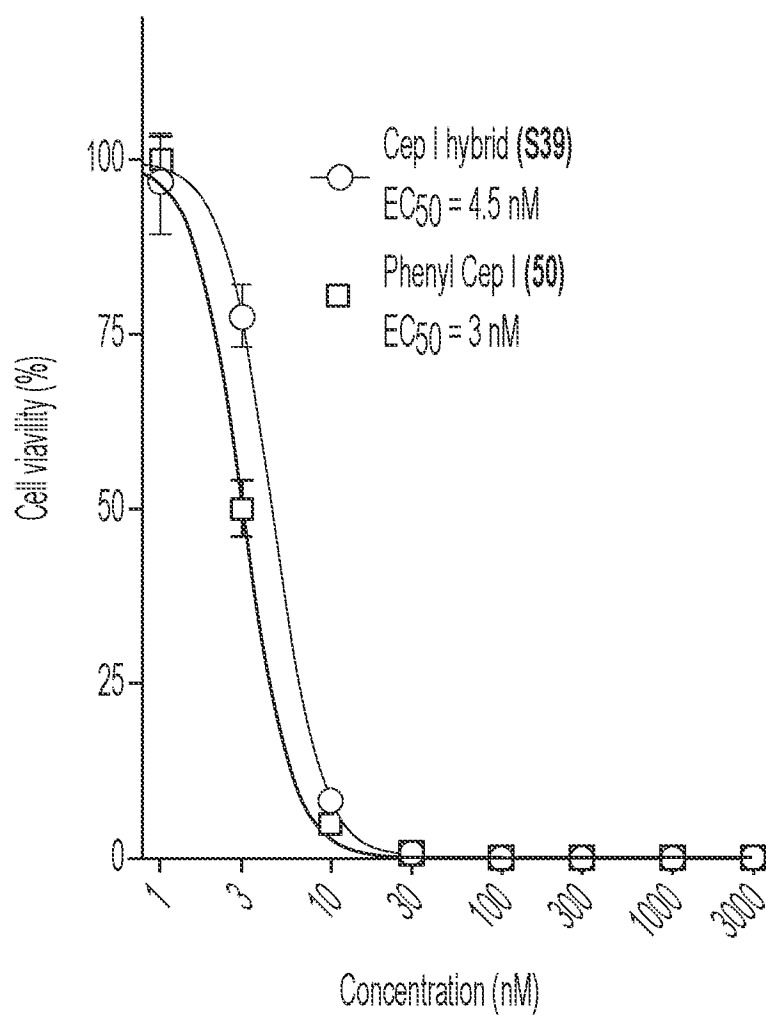


FIG. 8

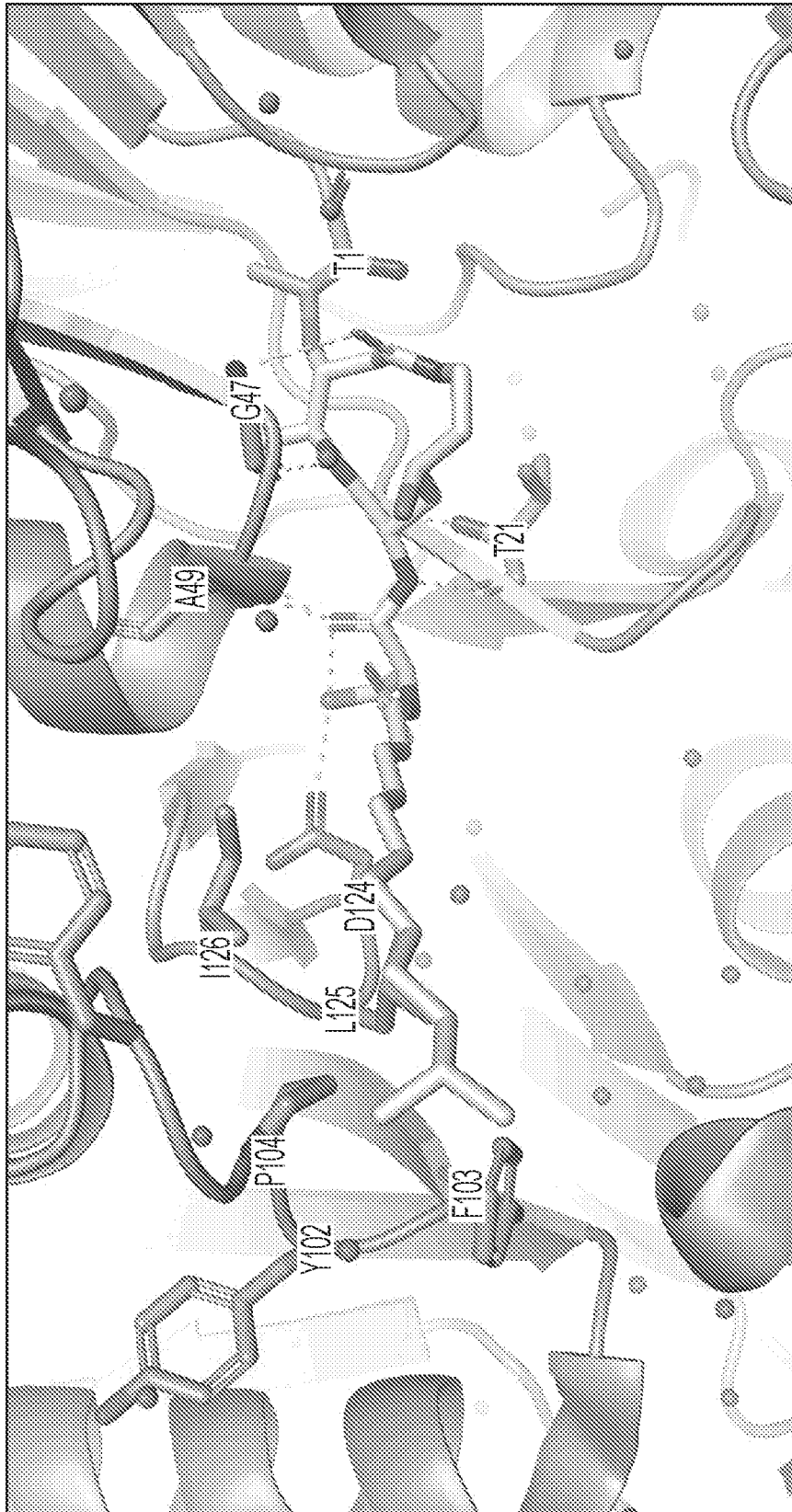


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/19733

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(see supplemental page)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/19733

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07D 245/02 (2023.01)

ADD. C07K 5/078, A61K 31/395, A61P 35/00 (2023.01)

CPC - INV. C07D 245/02

ADD. C07K 5/06139, A61K 31/395, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Amatuni et al. "Concise Chemoenzymatic Total Synthesis and Identification of Cellular Targets of Cepafungin I" Cell Chemical Biology. 15 October 2020 (15.10.2020) vol 27, pg. 1318-1326; pg. 1320, Figure 2C, . 1322, Figure 4A-B	1-2
A	WO 2009/065090 A2 (UNIVERSITY OF HAWAII) 22 May 2009 (22.05.2009); entire document	1-2
A	WO 2014/008204 A2 (PONO CORPORATION) 09 January 2014 (09.01.2014); entire document	1-2
A	US 9,399,627 B2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 26 July 2016 (26.07.2016); entire document	1-2



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 August 2023 (25.08.2023)

Date of mailing of the international search report

SEP 25 2023

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/19733

--continued from Box No. III--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-2 and 7, directed to a compound of claim 1, a compound selected from compounds 40-51 or S11-S39. The compound of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by a compound which is compound 40. It is believed that claims 1-2 read on this first named invention, and thus these claims will be searched without fee. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound which is compound 41 (i.e., claims 1-2 and 7).

Group II: Claims 3-5, directed to a method of treating cancer in a subject / inhibiting a proteasome.

Group III: Claim 6, directed to a method of making compound 40-51 and S39.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound of claim 1, which is not required by any other invention of Group I+.

Group II includes the technical feature of a method of treating cancer in a subject / inhibiting a proteasome, which is not required by any other invention of Group I+ or III.

Group III includes the technical feature of a method of making compound 40-51 and S39, which is not required by any other invention of Group I+ or II.

Common technical features:

The inventions of Groups I+ share the technical feature of a compound selected from compounds 40-51 or S11-S39.

The inventions of Groups I+ and II share the technical feature of a compound selected from compounds 40-51 or S11-S39.

The inventions of Groups I+ and II-III share the technical feature of a compound selected from compounds 40-51 or S39 or S17-S20.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by a document entitled "Concise Chemoenzymatic Total Synthesis and Identification of Cellular Targets of Cepafungin I" to Amatuni et al. (hereinafter Amatuni). Amatuni discloses a compound which is S20 (see instant specification, pg. 28, para 1: S20a) (pg. 1320, Figure 2C: compound 14).

As said compound was known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+ or II-III. The inventions of Group I+ and II-III thus lack unity under PCT Rule 13.

Note: Claims 1-6 lack clarity regarding the structure of formula S20, as compound S20 (instant specification, pg. 22, para 1) comprises an R group which is not further defined; for the purpose of this ISR, compound S20 is assumed to be compound S20a (instant specification, pg. 28, para 1).