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Semisynthetic Modifications of Hemiaminal Function at Ornithine Unit of Mulundocandin, Towards Chemical Stability and Antifungal Activity☆

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Abstract—Mulundocandin (**1**), is an echinocandin class of lipopeptide. It has wide spectrum of antifungal activity against *Candida* and *Aspergillus* species. Semisynthetic modification at Ornithine-5-hydroxyl (hemiaminal function) of **1** was carried out to improve solution stability and hence in vivo activity. Synthesis of ether (C-OR), thioether (C-SR) and C–N linkage at hemiaminal function have been described. All synthetic analogues were evaluated for their stability in aqueous solution and found to be more stable than mulundocandin. Antifungal activity of Orn-5 analogues was evaluated both in vitro against *Candida albicans* and *Aspergillus fumigatus* by agar well method and in vivo (oral and intraperitoneal) in *C. albicans* infected Swiss mice. Results of in vivo assays of analogues **2**–**9** by the oral route suggests that the introduction of either oxygen nucleophiles (-OR) or sulphur nucleophiles (-SR), at either Orn-5 or at both Orn-5 and HTyr-4 positions, results in retaining the activity of the parent compound with improved aqueous stability in most cases. Compound **9** has shown improved antifungal activity in comparison to mulundocandin by oral application in Swiss mice.
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Introduction

The prevalence of systemic fungal infections, especially disseminated systemic mycoses in immunodeficient hosts has increased significantly during the last two decades.[1] This increase is due to greater use of broad-spectrum antibiotics, immunosuppressive agents, hyperalimentation products and central venous catheters, intensive care of low birth weight infants, organ transplantation and the acquired immunodeficiency syndrome (AIDS) epidemic.[2] Prior to the development of echnocandins, the option for the treatment of fungal infections are limited to two class of compounds namely the polyenes and the azoles. Amphotericin B, used alone or in combination with 5-flucytosine, is a polyene antifungal that has broad-spectrum activity, but its utility is limited due to nephrotoxicity.[3] The other available agents are highly effective and widely used azoles.[4] However, since azoles are fungistatic agents, the concern of developing resistance to these drugs are quite high.[5] The need for a new antifungal agent is due to the alarming rise in the number of AIDS cases and the subsequent suppression of the immune system in these patients. Other reasons that have spurred the development of new systemic antifungal agents include the increase in the frequent use of antineoplastic agents and long term use of antibiotics. The polyenes and azoles, target the fungal cell membrane, a structure shared by both mammalian and fungal cells, and thus these drugs have inherent toxicity.

A newer class of cell wall active agents that have been developed to the point of seeing clinical candidates is the cyclic lipopeptides belonging to echinocandin family.[6] This group has the potential of providing a

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broad-spectrum fungicide with much lower toxicity than the current antifungal agents, through the inhibition of the biosynthesis of (1,3)-β-D-glucan, an essential component of the fungal cell wall.[7] This novel mode of action and potent antifungal activity has led to the development of several interesting drug candidates during the past several years. Caspofungin and micafungin are new echinocandin antifungals that have recently been introduced in the market for the treatment of invasive *Aspergillus* infections in patients unresponsive to or unable to receive amphotericin B.[8] Other echinocandins as antifungals are under development in attempts to provide improved therapy for fungal infections.[9]

Mulundocandin (**1**), an echinocandin class of antifungal lipopeptide, was isolated from the culture broth of a strain of *Aspergillus sydowi*.[10] It exhibited excellent in vitro activity against *Candida* species, especially against *C. albicans* and *C. glabrata* isolates.[11]

Naturally occurring echinocandins have reactive hemiaminal function due to Orn-5-hydroxyl group and is responsible for its unstability. It undergoes ring opening and rearrangement leading to biologically inactive compounds in acidic, basic and even neutral solutions.[12] Various approaches are reported in the literature for modifications at ornithine-5-position of echinocandins to obtain stable and water-soluble analogues.[13]

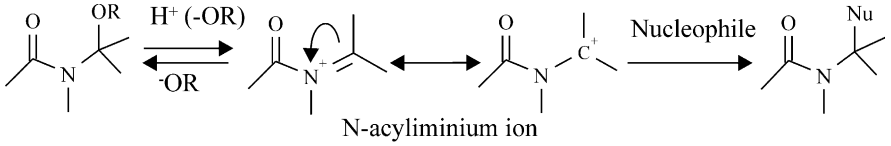
Mulundocandin also bear a hemiaminal function at C-5 of Orn residue and suffers from similar stability problem. We observed that water: acetonitrile (1:1) solution of mulundocandin in acidic (pH = 3) as well as in base (pH = 8.5) undergo rapid decomposition. Particularly in basic pH the $T_{1/2}$ was found to be less than 1 h. Even in neutral condition substantial decomposition was observed ($T_{1/2}$ = 4 days in phosphate buffer of pH 7.2 and $T_{1/4}$ = 5 days in sodium acetate buffer). In this work we describe the approach for the stabilization of mulundocandin in order to improve its stability and hence the in vivo antifungal activity, through formation of ether (C-OR), thioether (C-SR) and C–N linkage.[14] The crucial step during the present study involved the utilization of the very reactive *N*-acyliminium ion intermediate.

Chemistry

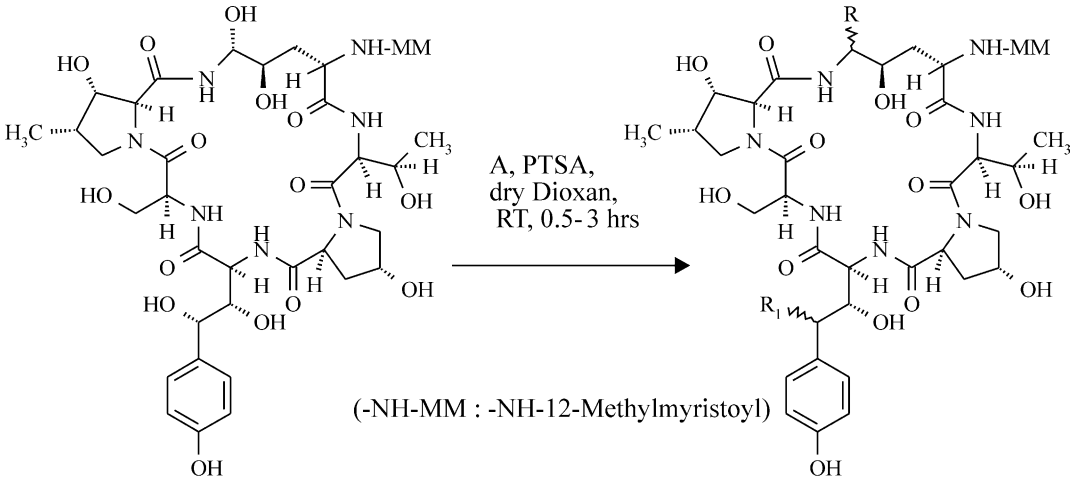
It is observed from literature that most of the reactions of *N*-acyliminium ions are of intermolecular type.[15] The presence of strong electron withdrawing carbonyl group makes *N*-acyliminium ions more electrophilic. The *N*-acyliminium ions are almost always generated insitu, in view of their limited stability and high reactivity. The mechanism of amidoalkylation reaction is shown in Scheme 1.

Formation of ether linkage at Orn-5-position of mulundocandin (Scheme 2)

Under acidic conditions, Orn-5-hydroxyl of mulundocandin is protonated, leading to the formation of *N*-acyliminium ion, which either reacts with an internal nucleophile to furnish a rearranged compound,[12] or with an external nucleophile to furnish an ether derivative or combination. The formation of the rearranged product could be suppressed by performing the reaction in the presence of a large excess of an external nucleophile. Thus, treatment of mulundocandin with excess of benzyl alcohol in the presence of catalytic amounts of *p*-toluene sulphonic acid at room temperature furnished two nonpolar products, monobenzylated **2** (67%) at Orn-5 position and dibenzylated **3** (13%) at Orn-5- and HTyr-4 positions, as shown in Scheme 2. The ratio of products **2** and **3** depends upon the amount of the acid used, the reaction temperature and duration. Compound **2** and **3** are purified by flash column chromatography over silica gel in >95% purity and are well characterized by using ^{1}H NMR, ^{13}C NMR, DEPT-135 and ESI MS spectra (see Experimental). Subsequent attempt was made to introduce smaller group like methyl ether so as to minimize the steric impact on the molecule, thereby retaining its original activity but possibly with increased stability. Thus, Orn-5-methoxymulundocandin (**4**) and Orn-5- and HTyr-4-dimethoxymulundocandin (**5**) were prepared in a manner similar to that used for compound **2** using excess methanol. Compound **4** was obtained, as the major product while compound **5** was the minor product formed in this reaction in 70 and 5% yields, respectively (Scheme 2). ^{1}H NMR of **5** showed two doublets at δ 7.25 and 7.15 for D_7-H and $D_{7'}$-H and two more doublets merged at δ 6.82 for D_8-H and $D_{8'}$-H. The effect due to stereoisomerisms at HTyr-4 position was apparent in the chemical shift of aromatic protons and the diastereomers are formed in the ratio of 1:1. In case of **3** also, two stereoisomers at HTyr-4 position could be detected based on TLC and HPLC analysis, but the aromatic protons of H-Tyr unit did not reveal any NMR differentiation, possibly because the benzyloxy group is away from the aromatic π cloud. We have not made any attempt to separate diastereomers arising out of the reactions unless the biological activity of the resulting compounds are significantly higher than mulundocandin and comparable to marketed product.



Scheme 1. Mechanism of amidoalkylation reaction.



Comp.No.	A	R	R_1
2	Benzyl alcohol	$-OCH_2Ph$	-OH
3	Benzyl alcohol	$-OCH_2Ph$	$-OCH_2Ph$
4	Methanol	$-OCH_3$	-OH
5	Methanol	$-OCH_3$	$-OCH_3$
6	Methylthioglycolate	$-SCH_2CO_2CH_3$	-OH
7	Methylthioglycolate	$-SCH_2CO_2CH_3$	$-SCH_2CO_2CH_3$
8	Thiophenol	-SPh	-OH
9	Thiophenol	-SPh	-SPh

Scheme 2. Formation of ether/thioether linkage at Orn-5 position of mulundocandin.

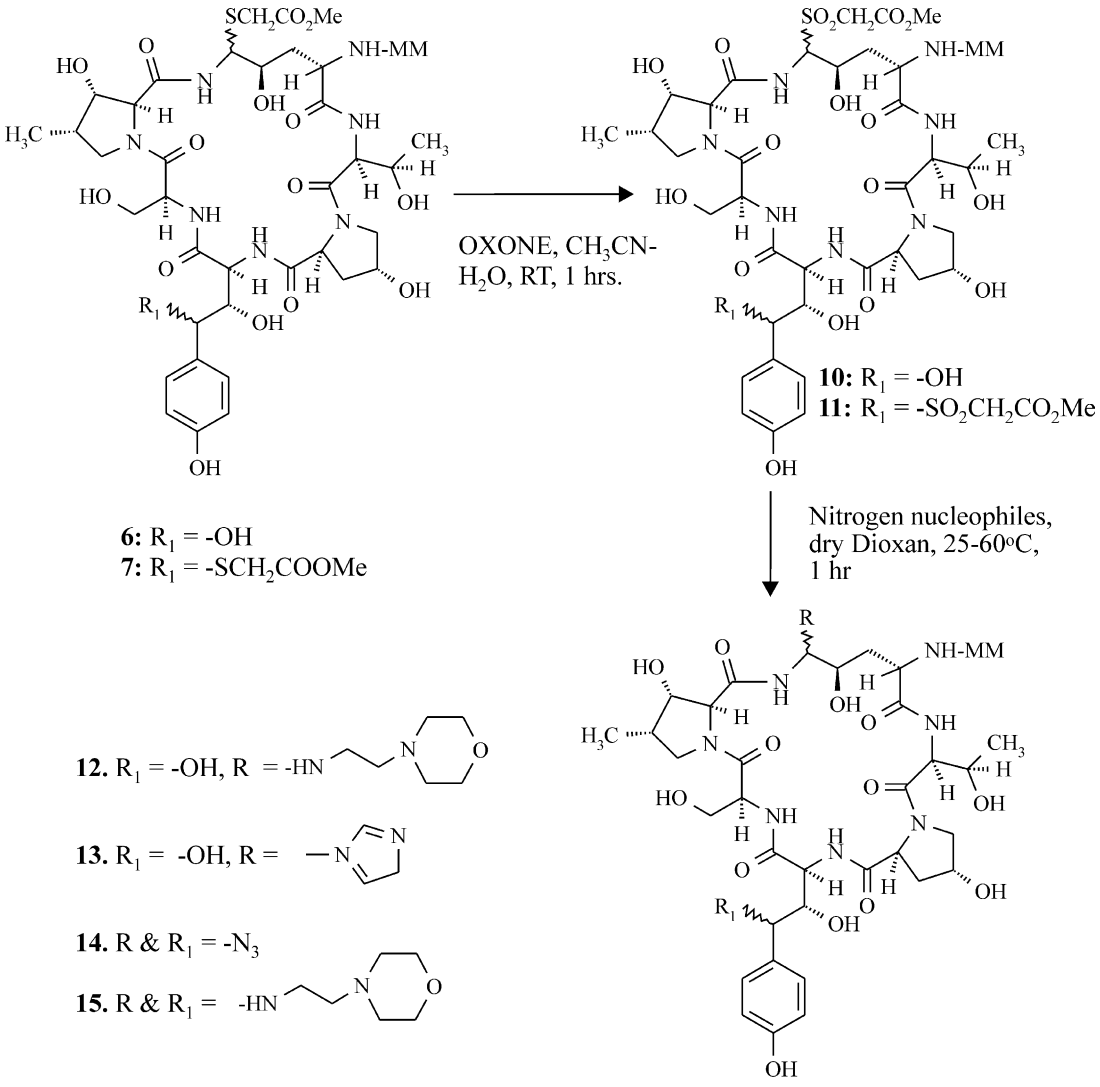
The strategy adopted for the introduction of C–C bond or C–N bond at Orn-5-position, was through introduction of thio-ether, which could be activated to sulfone (a good leaving group) by mild oxidation. An initial experiment utilizing ethyl mercaptan and PTSA combination led to the formation of Orn-5- and HTyr-4-di-thioether derivative as the major product (diastereomeric ratio = 2:3) while the desired mono-thioether derivative at Orn-5 position was obtained in very small yield. Presumably, the high reactivity of sulphur nucleophile is responsible for the observed low selectivity. Literature report[13f] indicated that the use of methyl thioglycolate leads to the formation of Orn-5-SCH_2COOCH_3 as the major product in pneumocandin Bo. Thus, on treatment with methyl thioglycolate in presence of catalytic amounts of *p*-toluene sulphonic acid, mulundocandin gave compounds **6** and **7** in 61 and 16% yields, respectively.

Orn-5- and HTyr-4-di-$SCH_2CO_2CH_3$ mulundocandin (**7**) also displayed stereoisomerisms at HTyr-4 position, which can be visualized by two doublets appearing at δ 7.25, 7.12 for D_7-H and $D_{7'}$-H with coupling constants 8.55 Hz and two additional doublets at δ 6.8 for D_8-H and $D_{8'}$-H in the ^{1}H NMR spectrum and the diastereomers were in the ratio of 1:3. The research group at Merck reported[13e] that SPh group at Orn-5 position could be replaced directly by primary or secondary amines, leading to *N*-substitution. Taking the lead Mulundocandin was treated with thiophenol in presence of catalytic amount of p-toluene sulfonic acid to give **8** in 50% yield and **9** in 18% yield.

The analogues **2–5** are useful starting materials for further modifications to improve the aqueous solubility and antifungal activity of mulundocandin.[14]

Formation of C–N linkage at Orn-5-position of mulundocandin (Scheme 3)

Another approach for the stabilization of hemiaminal function with desired antifungal activity of mulundocandin with additional advantage of water solubility could be achieved by formation of C–N linkage at Orn-5 position. Balkovec et al.,[13,16] reported nucleophilic displacement of activated sulfone by nitrogen nucleophile to get *N*-substituted ornithine analogues of echinocandins. Using similar approach compound **6** was subjected to oxidation[13,16] with OXONE®. The resultant reactive sulfone intermediate **10** was reacted with the potential nitrogen neuclophiles shown in Scheme 3. In the ^{1}H NMR spectrum of sulfone **10,** B_7-H appeared downfield at δ 5.6, in comparison to δ 5.39 in the thioether **6**, due to the deshielding nature of the sulfone ($-SO_2CH_2CO_2CH_3$) group. Subsequent treatment of sulfone intermediate **10** with 4-(2-aminoethyl) morpholine in anhydrous dioxan at 25–60 °C, gave the desired compound **12** in 70% yield. Compound **13** was synthesized similarly in 64% yield using imidazole as the nucleophile. The products could be purified by HPLC



Scheme 3. Formation of C–N linkage at Orn-5 position of mulundocandin.

on a C-18 column using 50–90% acetonitrile/water as the eluent. The compound (**13**) was characterized by an $(M+Na)^+$ peak at 1080 (calcd $M^+ = 1057$) and a base peak at 1011, corresponding to the loss of 68 units due to the elimination of imidazole moiety. The ^1H NMR spectrum of the compound was similar to that of mulundocandin but with singlets at δ 7.8 and 7.65, that were assigned to the three-imidazole protons.

Sulfone **11** was obtained from thioether **7**, following the same procedure as described above by treatment with OXONE® in acetonitrile–water mixture. This was then subjected to displacement reaction nitrogen nucleophiles as shown in Scheme 3. The diazide derivative **14** was thus prepared in 68% yield by the treatment of the sulfone **11** with sodium azide in anhydrous dioxan. Compound **14** displayed a characteristic IR band for azide group at 2100 cm^{-1} and the $(M+Na)^+$ peak at 1080 (calcd $M^+ = 1057$) in ESI-MS, with the base peak at 1037 resulting from the loss of the azide unit. The B_7 proton appeared downfield at δ 5.39 (d, 1H, $J = 1.86$ Hz) due to the deshielding nature of azide. Due to racemization at HTyr-4 position, the aromatic protons of H-Tyr unit appeared as two doublets at δ 7.28 and 7.14 with coupling constants 8.88 Hz and were assigned to D_7-H, $D_{7'}$-H and another pair of doublets centred at δ 6.83 were assigned to D_8-H, $D_{8'}$-H. Attempts to reduce the diazide **14,** to corresponding di-amino compound, using a variety of reported procedures, did not succeed. Disulfone **11** on treatment with 4-(2-aminoethyl) morpholine in dioxan gave **15** in 44% yield.

Some of the compounds as representative example were studied for their stability in water–acetonitrile (1:1) solution at neutral, acidic (pH = 3), and alkaline (pH = 8.5) conditions followed by HPLC analysis. We observed that compounds **2** and **4** were stable in both neutral and basic pH however in acidic pH $T_{1/2}$ was found to be ~24 h. On the other hand, compounds **6** and **9** were found to be stable in neutral and acidic pH while in basic pH $T_{1/2}$ was ~24 h. The extent of decomposition of compounds **12** and **13** were found to

less than 10% over a period of 5 days. Thus modification of aminal function of mulundocandin in the present study resulted in more stable mulundocandin analogues that can be exploited in future.

Biological studies of Mulundocandin analogues

In vitro. Mulundocandin analogues were screened in vitro by standard agar well method against *C. albicans* (IV) and *A. fumigatus* (AF-1), in Sabouraud agar bioassay plates.[17] The zones produced by the mulundocandin analogues were compared with the zone of mulundocandin and the results of the assay are given in Table 1.

From the Table 1, it is apparent that, the analogues modified at Orn-5 position (**2**–**15**) have retained in vitro activity comparable to that of mulundocandin (**1**). Replacement of Orn-5-hydroxyl by either benzyloxy (**2**) or methoxy (**4**) or methylthioglycolate (**6**), results in slight reduction of in vitro activity compared with mulundocandin. Compound **4** shows better in vitro activity than **2**, possibly because comparatively smaller methoxy group replaces Orn-5-hydroxyl. Compounds **3**, **5**, **7** and **9**, di-alkylated at Orn-5- and HTyr-4 positions showed lower in vitro activity than **2**, **4**, **6** and **8**, possibly, suggesting that HTyr-4 position may not be the ideal position for modification. Amongst the ether derivatives at Orn-5 position, analogue **4** show better in vitro activity against *C. albicans* and *A. fumigatus*. The activity of *N*-substituted compounds **12**–**15** was found to be much lower in comparison to *O*-ether and *S*-ether analogues. Compound **13** showed 22 and 20^{hd} mm zone size against *C. albicans* and *A. fumigatus*, respectively, at 1 mg/mL of concentration and showed improved aqueous stability than mulundocandin.

Table 1. In vitro activities of mulundocandin analogues against *C. albicans* (IV) and *A. fumigatus* (AF-1)

Compd	Concentration (mg/mL) in MeOH	Zone size (mm)	
		C. albicans	*A. fumigatus*
2	2	12	24^{h}
	5	16	26^{h}
3	2	11	18^{hd}
	5	14	20^{hd}
4	1	25	25^{hd}
	2	26^{h}	$24^{h}/37^{h}$
	5	27	35^{vhd}
5	2	18	30^{h}
	5	20	30^{vhd}
6	1	20	20^{hd}
	2	22	24^{vh}
	5	26	29^{hd}
7	2	10^{h}	20^{hd}
	5	19	26^{hd}
8	2	16	17^{hd}
	5	18^{h}	21^{hd}
9	2	15	15^{hd}
	5	18	17^{vhd}
10	1	21	20^{hd}
	2	23	21^{vh}
	5	24	27^{hd}
12	2	13	20^{hd}
	5	16	24^{hd}
13	1	22	20^{hd}
	2	24	24^{hd}
	5	24	30^{hd}
14	1	15	17^{hd}
	2	21	19^{hd}
	5	25	$8/25^{hd}$
15	2	17^{h}	13^{vh}
	5	19^{h}	16^{vhd}
1	0.1	24	24^{hd}
Mulundocandin	1.0	29	29^{hd}
	2.0	32	35^{vh}

h, hazy; vh, very hazy; hd, hazy diffused; vhd, very hazy diffused. (50 μL of solution used for testing).

In vivo testing of mulundocandin analogues against *C. albicans* using swiss mice model[18]

Antifungal activity of mulundocandin analogues was evaluated in vivo in *C. albicans* infected Swiss mice after oral and intraperitoneal administration. The colony formation unit (CFU) in kidney homogenate after administrating mulundocandin derivatives were determined and compared with mulundocandin, and fluconazole. The experimental details of activity evaluation are given below and the results are summarized in Tables 2 and 3.

Swiss mice, 4–6 weeks old and 18–22 g body weights were used for in vivo studies. The inoculum was prepared by culturing *C. albicans* on Sabouraud agar and cell density adjusted to 10^6 cells/mouse for non-lethal model. Mice were infected by injecting the conidia of *C. albicans* through the lateral tail vein, with 0.25 mL of the inoculum on D_0. Treatment was commenced at zero hr after infection and continued from D_0 to D_{+4}. Control groups (untreated) consisted of four mice infected but not treated as the negative control and mice infected and treated with the standard antifungal fluconazole as the positive control. A test sample of mulundocandin, mulundocandin analogues and fluconazole were prepared in tween 80 and distilled water and tested at 100, 80 and 50 mg/kg, respectively, administered by the oral route and at 20 mg/kg each by the intraperitoneal route. Dosing protocol consisted of drug administrating on D_0, D_{+1}, D_{+2}, D_{+3} and D_{+4} by either of the routes mentioned above. After the treatment was over, the animals were sacrificed after two days. Paired kidneys were removed aseptically and cultured for the presence of *C. albicans* in sterile saline. The kidneys of each group were homogenized and 10-fold serial dilutions were prepared. A 10-μL aliquot from each dilution was plated on Sabouraud agar plate, incubated at 30 °C for 24–48 h and the CFU's were determined. The number of CFU per gram of paired kidneys was calculated. Activity was interpreted by the difference shown in the load of organisms per gram kidney weights of treated group and untreated control group.

The results of in vivo assays of compounds **2**–**9,** after oral administration, suggests that both ether (-OR) and thioether (-SR) at either Orn-5 or at both Orn-5 and HTyr-4 positions are well tolerated in mulundocandin in its biological potency. Compound **9** showed improved activity corresponding to two-log reduction in CFU as compared with **1** by the oral route at 80 mg/kg and are comparable with fluconazole, the standard antifungal compound.

Table 2. Anti-*Candida* activity of mulundocandin (MCN) derivatives by oral application in Swiss mice

Compd	Activity of MCN derivatives	Activity of MCN	Activity of Fluconazole	Control (no treatment is given)
	(approx CFU count, per g kidney)/dose (mg/kg×5 per os)			
2	1.1×10^6 (80 mg)	6.8×10^7 (100 mg)	NT[a]	2.0×10^8
3	20.0×10^6 (80 mg)	6.8×10^7 (100 mg)	NT	2.0×10^8
4	8.4×10^7 (80 mg)	8.8×10^7 (100 mg)	1.5×10^3 (50 mg)	4.0×10^8
5	6.4×10^7 (80 mg)	8.8×10^7 (100 mg)	1.5×10^3 (50 mg)	4.0×10^8
6	2.0×10^6 (80 mg)	2.4×10^6 (100 mg)	1.5×10^4 (50 mg)	2.6×10^7
7	3.4×10^6 (80 mg)	$2.4\times x10^6$ (100 mg)	1.5×10^4 (50 mg)	2.6×10^7
8	2.5×10^7 (80 mg)	2.4×10^6 (100 mg)	1.5×10^4 (50 mg)	2.6×10^7
9	2.8×10^4 (80 mg)	2.4×10^6 (100 mg)	1.5×10^4 (50 mg)	2.6×10^7

[a]NT, not tested.

Table 3. Anti-*Candida* activity of mulundocandin (MCN) derivatives by intraperitoneal injection in Swiss mice

Compd	Activity of MCN derivatives	Activity of MCN	Activity of Fluconazole	Control (no treatment is given)
	(approx CFU count, per g kidney)/dose (mg/kg×5 per os)			
3	1.2×10^7 (20 mg)	4.8×10^6 (20 mg)	NT[a]	6.6×10^6
4	1.6×10^7 (20 mg)	7.2×10^7 (20 mg)	NT	5.9×10^7
5	1.1×10^8 (20 mg)	7.2×10^7 (20 mg)	NT	5.9×10^7
7	1.2×10^8 (20 mg)	1.25×10^7 (20 mg)	1.0×10^4 (20 mg)	6.7×10^7

[a]NT, not tested.

Conclusion

We have achieved our initial objective of stabilizing mulundocandin through ether, thioether or C–N linkage at Orn-5 position that is quite obvious from the comparison of the result of stability studies. Mulundocandin undergoes rapid degradation in acidic and basic pH particularly at pH 8.5 wherein $T_{1/2}$ was found to be <1 h while mulundocandin analogues showed much higher stability. Most significantly in neutral solution $T_{1/2}$ of mulundocandin was found to be 4 days while synthetic analogues shows very little sign of decomposition. The in vitro activity of these compounds suggest that introduction of ether or thioether at Orn-5 position maintained the antifungal activity of mulundocandin to a great extent while improving stability. However, the disubstituted analogues showed lower activity due to the blocking of Tyr-4 hydroxy group believed to be required for antifungal activity discussed earlier. Results of in vivo study of **2**–**9** through oral route also suggest the maintenance of activity. Compound **9** has shown improved activity (two log difference in CFU in comparison to mulundocandin), which is close to fluconazole. We cannot explain at this stage why **9** showed higher in vivo activity in comparison to ether or C-N compounds. One possibility could be the better stability of **9** in acidic pH in comparison to **2** and **4** surviving initial transport phase followed by oxidative hydrolysis at Orn-5 position releasing active substance. Further studies will be required to carry out to ascertain the exact reason. However the analogues **2** and **4** are useful starting material for further modification towards increased water solubility. This part of the work will be published elsewhere.

Experimental

General Remarks

All reagents and solvents were purchased from commercial supplier and were used as received unless otherwise stated. Yields reported are isolated yields of the materials. All mulundocandin analogues synthesized during this study were >95% pure based on HPLC analysis. Dioxan was freshly distilled over sodium prior to use. Melting points (mp) were recorded on a Kofler hot-plate apparatus and were uncorrected. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on 300 and 75 MHz, respectively, on a Brucker ACP-300 spectrometer, using CD_3OD as solvent, unless otherwise mentioned. Chemical shifts values are expressed in δ scale (parts per million) using TMS as internal standard. Coupling constant (*J*) values were reported in Hz. Alphabets B_7, D_7, $D_{7'}$, D_8, and $D_{8'}$ on structures of compounds **2**–**15** are used to identify the protons position (Fig. 1). ESI MS were recorded on a Fisons VG QUATTRO II instrument. IR spectra were recorded as KBr wafers on a Perkin-Elmer 782: Infrared spectrophotometer. UV spectra were recorded on Chemito 2500: UV–vis Recording spectrometer. TLC was performed on precoated silica gel aluminium plates containing a fluorescent indicator (1.05554, Silica gel 60 F_{254}, Merck). Flash chromatography was performed using Acme's silica gel (230–400 mesh) and MeOH–$CHCl_3$ mixtures as eluent. Visualization of the components on TLC plates was achieved either by exposure to iodine vapour or UV light or by spraying 50% H_2SO_4 followed by charring. Perkin-Elmer HPLC (Diode Array Detector 235, Binary LC pump 250) was used for purification using Knauer Eurosphere 100, RP C-18 column (size 250 mm×16 mm and particle size 10 μm) and CH_3CN/H_2O gradient as an eluent with a flow rate of 5–8 mL/min and was monitored at λ = 220 and 270 nm. The purity of compounds was checked on YMC-Pack, AQ-313 S-5 120A ODS, RP C-18 column (size, 250 mm×6 mm; particle size 5 μm; mobile phase, 70% CH_3CN/H_2O; flow rate 1 mL/min; monitored at λ = 220 and 270 nm).

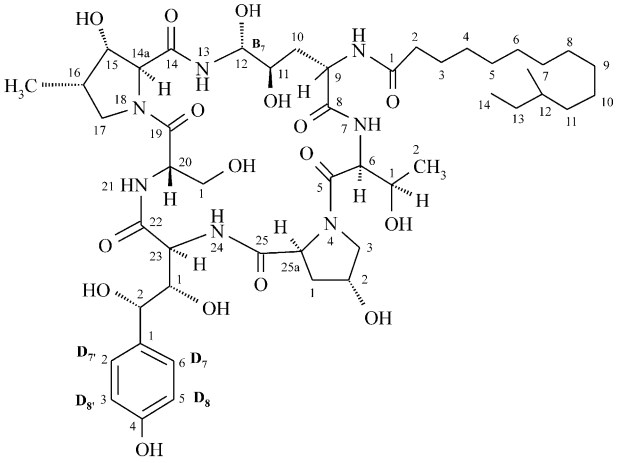


Figure 1. Mulundocandin (**1**).

Nomenclature of mulundocandin

*N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*,12*R*)-23-((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,12,15-tetrahydroxy-6-(1*R*)-1-hydroxyethyl)-20-hydroxymeth-yl-16-methyl-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl]-12-methyltetradecanamide.*

Experimental procedures

N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-12-benzyloxy-23-((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)-ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymeth-yl-16-methyl-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexa-azacyclohenicosin-9-yl]-12-methyltetradecanamide (2) and N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*, 20*S*,23*S*,25a*S*,2*R*,11*R*)-12-benzyloxy-23-((1*S*)-2-benzyloxy-1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl-5, 8,14, 19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/] [1,4,7,10,13,16] hexaazacyclohenic-osin-9-yl]-12-methyltetradecanamide (3). To a stirred solution of mulundocandin (5.2 g, 5.15 mmol) in anhydrous dioxan (150 mL), under nitrogen atmosphere, anhydrous benzyl alcohol (10.45 g, 96.6 mmol), and p-toluene sulfonic acid (0.32 g, 1.66 mmol) were added and the reaction mixture was stirred at ambient temperature for 1 h. After 1 h TLC (20% MeOH/$CHCl_3$) showed disappearance of starting compound. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ at 5–10 °C and evaporated to a smaller volume (25 mL). The crude mixture was diluted with water (250 mL), extracted with *n*-BuOH (3×150 mL), washed with water (200 mL) followed by brine (200 mL). Combined organic extract was dried over anhydrous Na_2SO_4. Evaporation of solvent under vacuum gave crude gummy product. Which was purified by flash column chromatography on silica gel with 0–15% MeOH/$CHCl_3$ as 5% step gradient eluent. Evaporation of the appropriate fractions gave white compound **2** (3.8 g, 67.1%; mp: 196–99 °C) and **3** (0.82 g, 13.3%; mp: 182–83 °C). **Compound 2:** ^{1}H NMR: δ 7.28–7.41 (m, 5H, –O$\underline{CH_2}$Ph). 7.17 (d, 2H, *J* = 8.41 Hz, $\underline{D_7}$ and $\underline{D_{7'}}$), 6.78 (d, 2H, *J* = 8.37 Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.32 (d, 1H, *J* = 1.60 Hz, $\underline{B_7}$ or iminol proton), 4.68 (s, 2H, –O$\underline{CH_2}$Ph), ^{13}C NMR (DMSO-d_6): 172.07, 171.51, 170.46, 170.27, 169.59, 168.14, 156.57, 138.78, 132.47, 128.19, 127.94, 127.35, 127.08, 114.65, 79.01, 75.19, 74.24, 73.19, 69.23, 68.99, 68.66, 68.04, 66.10, 62.27, 60.82, 56.29, 55.67, 53.49, 51.84, 51.28, 49.23, 37.26, 36.99, 35.99, 35.13, 34.72, 33.73, 29.36, 29.03, 28.90, 28.52, 26.45, 25.42, 19.38, 19.06, 11.19, 10.81. IR (KBr): ν_{max} 3350–3450, 2930, 1650, 1615, 1520, 1450, 1385, 1220, 1070 cm^{-1}. ESI-MS (ES+): for $C_{55}H_{83}N_7O_{16}$; Calculated: 1097; found: $(M+Na)^+ = 1120$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 225, 277 nm (14016, 1595). **Compound 3:** ^{1}H NMR: δ 7.24–7.31 (m, 10H, 2×O$\underline{CH_2}$Ph) 7.12 (d, 2H, *J* = 8.55 Hz, $\underline{D_7}$ and $\underline{D_{7'}}$), 6.74 (d, 2H, *J* = 8.54 Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.17 (d, 1H, *J* = 3.66

Hz, $\underline{B_7}$), 4.4–4.53 (2×s, 4H, –O$\underline{CH_2}$Ph). IR (KBr): ν_{max} 3350–3450, 2930, 1650, 1615, 1520, 1450, 1385, 1220, 1070 cm^{-1}. ESI-MS (ES+): for $C_{62}H_{89}N_7O_{16}$; calcd 1187; found: $(M+Na)^+ = 1210$ (base peak). UV (MeOH): λ_{max} (ε M^{-1} cm^{-1}): 228, 275 nm (14113, 1767).

N1 - [(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*) - 23 - ((1*S*,2*S*) - 1,2 - dihydroxy - 2 - (4 - hydrox - yphenyl)ethyl)2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl - 12 - methoxy - 16 - methyl - 5,8,14,19,22,25 - hexaoxoperhydrodiazolo[2,1 - c:2,1 - /][1,4,7,10,13,16]hexaaz-acyclohenicosin-9-yl]-12-methyltetradecanamide (4) and N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*, 20*S*,23*S*,25a*S*,2*R*,11*R*)-23-((1*S*)-1-hydroxy-2-(4-hydroxyphenyl)-2-methoxyethyl) -2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-12-methoxy-16-methyl-5,8,14,19, 22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexaazaclohenicosin-9-yl]-12-methyltetradecanamide (5). To a stirred solution of mulundocandin (2.2 g, 2.18 mmol) in anhydrous dioxan (50 mL), under nitrogen atmosphere, anhydrous MeOH (6.0 mL, 147.9 mmol) and *p*-toluene sulfonic acid (0.12 g, 0.624 mmol) were added and the reaction mixture was stirred at ambient temperature for 0.5 h. Reaction progress was monitored by TLC (20% MeOH/$CHCl_3$). The reaction workup and purification process is similar to the described for compound **2** and **3**. Evaporation of the appropriate fractions gave white compound **4** (1.55 g, 69.5%; mp: 188 °C dec.) and **5** (0.109 g, 4.8%; mp 172–75 °C). **Compound 4:** ^{1}H NMR: δ 7.19 (d, 2H, $J = 8.55$ Hz, $\underline{D_7}$ & $\underline{D_{7'}}$), 6.89 (d, 2H, $J = 8.55$ Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.12 (d, 1H, $J = 1.65$ Hz, $\underline{B_7}$), 3.38 (s, 3H, –OC$\underline{H_3}$). IR (KBr): ν_{max} 3300–3400, 2920, 1660, 1625, 1515, 1440, 1385 cm^{-1}. ESI MS (ES+): for $C_{49}H_{79}N_7O_{16}$; calcd 1021; found: $(M+Na)^+ = 1044$ (base peak); UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 223, 277 nm (8085, 557). **Compound 5:** ^{1}H NMR: δ 7.25, 7.15 (2×d, 2H, $J = 8.37$ Hz, $\underline{D_7}$ and $\underline{D_{7'}}$), 6.82 (2×d (merged), 2H, $J = 8.37$ Hz, $\underline{D_8}$ & $\underline{D_{8'}}$), 5.12 (br, 1H, $\underline{B_7}$), 3.42 (2×s, 6H, 2 × OC$\underline{H_3}$). IR (KBr): ν_{max} 3300–3400, 2915, 1650, 1630, 1520, 1445, 1390, 1240, 1080 cm^{-1}. ESI MS (ES+): for $C_{50}H_{81}N_7O_{16}$; calcd 1035; found: $(M+Na)^+ = 1058$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 223, 275 nm (5526, 506).

Methyl-2-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-23-((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl-9-(11-methyltridecylcarboxamido)-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:1/][1,4,7,-10,13,16]hexaazacyloenicosin-12-ylsulfanyl]acetate (6) and Methyl-2-[(6*S*, 9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-23-((1*S*)-1-hydroxy-2-(4-hydroxyphenyl)-2-methyloxycarbonylmethylsulfanylethyl)-20-hydroxymethyl-16-methyl-9-(11-methyltridecycarboxamido)-5,8,14,19,22,25-hexaoxoperhyd-rodiazolo[2,1-c:1-/][1,4,7,10,13,16]hexaazacyclohenicosin-12-ylsulfanyl]acetate (7). To a stirred solution of mulundocandin (4.8 g, 5.15 mmol) in anhydrous dioxan (150 mL), under nitrogen atmosphere, anhydrous methylthioglycolate (11.87 g, 111.83 mmol) and *p*-toluene sulfonic acid (0.338 g, 1.758 mmol) were added and the reaction mixture was stirred at ambient temperature for 1.5 h. The reaction was quenched by the addition of aqueous $NaHCO_3$ at 5–10 °C and evaporated to smaller volume (25 mL). The mixture was diluted with water (250 mL). The semisolid material was extracted with *n*-BuOH (3×150 mL), washed with water (200 mL), followed by brine (200 mL). Combined organic extract was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give crude gummy product. The crude material was purified by flash chromatography over silica gel with 0–15% MeOH/$CHCl_3$ as 5% step gradient eluent. Evaporation of the appropriate fractions gave white compound **6** (3.171 g, 60.7%; mp: 183–85 °C) and **7** (0.885 g, 15.7%; mp: 115–17 °C). **Compound 6:** ^{1}H NMR: δ 7.2 (d, 2H, $J = 8.54$ Hz, $\underline{D_7}$ & $\underline{D_{7'}}$), 6.8 (d, 2H, $J = 8.54$ Hz, $\underline{D_8}$ & $\underline{D_{8'}}$), 5.39 (br, 1H, $\underline{B_7}$), 3.75 (s, 3H,-SCH$_2$COO$\underline{CH_3}$), 3.45, 3.65 (2×d, 2H, $J = 15.78$ Hz, –S$\underline{CH_2}$COOCH$_3$). IR (KBr): ν_{max} 3350, 2920, 1730, 1660–1620, 1520, 1440, 1385 cm^{-1}. ESI MS (ES+): for $C_{51}H_{81}N_7O_{17}S$; calcd: 1095; found: $(M+Na)^+ = 1118$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 225, 277 nm (5769, 9428). **Compound 7:** ^{1}H NMR: δ 7.25, 7.12 (2×d, 2H, $J = 8.55$ Hz, $\underline{D_7}$ & $\underline{D_{7'}}$), 6.8 (2×d, 2H, $J = 8.55$ Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.41 (br, 1H, $\underline{B_7}$), 3.75 (s, 3H, –SCH$_2$COO$\underline{CH_3}$), 3.65, 3.8 (2×s, 3H, –SCH$_2$COO$\underline{CH_3}$), 3.45, 3.64 (2×d, 2H,-S$\underline{CH_2}$COOCH$_3$), 3.21–2.85 (m, 2H, –S$\underline{CH_2}$COOCH$_3$). IR (KBr): ν_{max} 3300–3400, 2930, 1740, 1680–1610, 1520, 1435, 1380, 1260, 1070 cm^{-1}. ESI–MS (ES+): for $C_{54}H_{85}N_7O_{18}S_2$; calcd: 1183; found: $(M+Na)^+ = 1206$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 227 nm (2421).

N1 - [(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*) - 23 - ((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11, 15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl--5,8,14,19,22,25-hexaoxo-12-phenylsulfanylperhydrodiazo-lo[2,1-c:2,1-/][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl]-12-methyltetradecanamide (8) and N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2R,11R) - 2,11,15 - trihydroxy-6-((1*R*)-1-hydroxyethyl)-23-((1*S*)-1-hydroxy-2-(4-hydroxyphenyl)-2-phenylsulfanylethyl)-20-hydroxymethyl-16-methyl-5,8, 14,19,22,25-hexaoxo-12-phenylsulfanylperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexa-azacyclohenicosin-9-yl]-12-methyltetradecanamide (9). To a stirred solution of mulundocandin (2.3 g, 2.28 mmol) in anhydrous dioxan (100 mL), under nitrogen atmosphere were added anhydrous thiophenol (4.29 g, 38.95 mmol), and *p*-toluene sulfonic acid (0.23 g, 1.196 mmol) and the reaction mixture was stirred at ambient temperature for 3 h. The workup and purification process were similar to that described for compounds **6** and **7**. Yield of the white solids **8** (1.241 g, 49.4%; mp: 189–90 °C dec.) and **9** (0.478 g, 17.5%; mp: 175 °C dec.). **Compound 8:** ^{1}H NMR: δ 7.58 (m, 2H,-S$\underline{Ph}$), 7.33 (t, 3H, $J = 2.63$ Hz,-S$\underline{Ph}$), 7.2 (d, 2H, $J = 8.39$ Hz, $\underline{D_7}$ & $\underline{D_{7'}}$), 6.8 (d, 2H, $J = 8.39$ Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.69 (br, 1H, $\underline{B_7}$). IR (KBr): ν_{max} 3400–3300, 2940, 1670, 1630, 1525, 1460, 1390, 1250, 1075 cm^{-1}. ESI MS (ES+): for $C_{54}H_{81}N_7O_{15}S$; calcd: 1099; found: $(M+Na)^+ = 1122$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 228, 265 nm (22,336, 4703). **Compound 9:** ^{1}H NMR: δ 7.58 (m, 2H, –S$\underline{Ph}$), 7.30 (t, 3H, $J = 3.3$ Hz,-S$\underline{Ph}$), 7.18–7.25 (m, 5H, –S$\underline{Ph}$), 6.91 (d, 2H, $J = 8.4$ Hz, $\underline{D_7}$ and $\underline{D_{7'}}$), 6.61 (d, 2H, $J = 8.4$ Hz, $\underline{D_8}$ and $\underline{D_{8'}}$), 5.69 (br, 1H, $\underline{B_7}$). IR

(KBr): ν_{max} 3400–3300, 2940, 1680–1620, 1520, 1450, 1380, 1240, 1075 cm^{-1}. ESI MS (ES+): for $C_{60}H_{85}N_7O_{14}S_2$; calcd 1191; found: $(M+Na)^+ = 1214$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 255 nm (4892).

Methyl-2-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-23-((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl-9-(11-methyltridecylcarboxamido)-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:1-/][1,4,7,10,13,16]hexaazacyclohenicosin-12-ylsulfonyl]acetate (10). To a stirred solution of thioether **6** (0.515 g, 0.47 mmol) in acetonitrile (35 mL) and water (35 mL) at ambient temperature, OXONE® (0.577 g, 0.94 mmol) was added. After a period of 1 h TLC in 20% MeOH/$CHCl_3$ showed complete conversion of **6** to a more polar product. Reaction mixture was evaporated under reduced pressure to smaller volume (25 mL). White solid precipitated out was filtered off, washed with water (25 mL) dried under high vacuum to yield sulfone **10** (0.45 g, 84.9%; purity 90% by HPLC). This was used as such without further purification. mp: 63–65 °C; ^{1}H NMR: δ 7.18 (d, 2H, $J = 8.58$ Hz, $\underline{D}_7$ & $\underline{D}_{7'}$), 6.8 (d, 2H, $J = 8.58$ Hz, $\underline{D}_8$ and $\underline{D}_{8'}$), 5.6 (br, 1H, $\underline{B}_7$), 3.92–4.08 (m, 2H, $-SO_2C\underline{H}_2CO_2CH_3$), 3.85 (s, 3H, $-SO_2CH_2-CO_2C\underline{H}_3$). IR (KBr): ν_{max} 3500–3400, 2920, 2890, 1680-1625, 1525, 1445, 1225, 1080 cm^{-1}. ESI–MS (ES+): for $C_{51}H_{81}N_7O_{19}S$; calcd 1127; found: $(M+Na)^+ = 1150$ (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 223, 276 nm (31366, 3587).

Methyl-2-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-23-((1*S*)-1-hydroxy-2-(4-hydroxyphenyl)-2-methyloxycarbonylmethylsul-fonylethyl)-20-hydroxymethyl-16-methyl-9-(11-methyltridecylcarboxamido)-5,8,14,19, 22,25-hexaoxoperhydrodiazolo[2,1-c:1-/][1,4,7,10,13,16]hexaazacyclohenicosin-12-ylsulfonyl]acetate (11). Compound **11** (Orn-5- and HTyr-4-disulfone mulundocandin) was prepared from di-thioether **7,** using the process outlined for preparation of Orn-5-sulfone **10** and was used immediately for further reactions without purification.

N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-23-((1*S*,2*S*)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl-12-(2-morpholinoethylamino)-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl]-12-methyltetradecanamide (12). To a stirred solution of Orn-5-sulfone **10** (0.1 g, 0.089 mmol) in anhydrous dioxan (10 mL) under nitrogen atmosphere, 4-(2-aminoethyl) morpholine (0.495 g, 3.8 mmol) was added and the reaction mixture was stirred at 55–60 °C for 1 h. The reaction mixture was diluted with water (150 mL), extracted with *n*-BuOH (3×100 mL). It was washed with water (150 mL) followed by brine (150 mL). Combined organic extract was dried over anhydrous Na_2SO_4, filtered and was concentrated under vacuum to give crude product. The crude product was purified by flash column chromatography over reverse-phase (RP, C-18) by using 50–90% acetonitrile/water as 10% step gradient eluent. Removal of organic solvent from appropriate fractions under vacuum followed by lyophilization provided product **12** (0.07 g, 70.5%). Yield is calculated from nearly 90% pure starting compound. ^{1}H NMR: δ 7.2 (d, 2H, $J = 8.55$ Hz, $\underline{D}_7$ and $\underline{D}_{7'}$), 6.8 (d, 2H, $J = 8.55$ Hz, $\underline{D}_8$ and $\underline{D}_{8'}$), 5.04 (br, 1H, $\underline{B}_7$), 3.7–3.8 (m, 4H, 2 × $O\underline{C}H_2$), 2.35–2.2 (m, 8H, 4 × $N\underline{C}H_2$). IR (KBr): ν_{max} 3300–3400, 2930, 1680–1620, 1520, 1435, 1380, 1260, 1070 cm^{-1}. ESI MS (ES+): for $C_{54}H_{89}N_9O_{16}$; calcd 1119; found: $(M+Na)^+ = 1142$ (base peak).

N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-12-(1H-1,3-diazolo-1-yl)-23-((1S,2S)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymethyl-16-methyl-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10, 13,16]hexaazacyclohenicosin-9-yl]-12-methyltetradecanamide (13). To a stirred solution of Orn-5-sulfone **10** (0.1 g, 0.089 mmol) in anhydrous dioxan (10 mL) under nitrogen atmosphere, imidazole (0.024 g, 0.356 mmol) was added and the reaction mixture was stirred at 55–60 °C for 1 h. Reaction progress was monitored by TLC (20% MeOH/$CHCl_3$). After 1 h the reaction mixture was diluted with water (100 mL), extracted with *n*-BuOH (3×50 mL). It was washed with water (100 mL) followed by brine (100 mL). Combined organic extract was dried over anhydrous Na_2SO_4 and was concentrated under vacuum to give a crude product. The crude product was purified in a similar manner as shown for compound **12,** to furnish compound **13** (0.06 g, 64%) Yield is calculated from nearly 90% pure starting compound. ^{1}H NMR: δ 7.8 (s, 1H, -Imidazole-$\underline{H}$), 7.65 (br s, 2H, -Imidazole-$\underline{H}$), 7.18, (d, 2H, $J = 8.55$ Hz, $\underline{D}_7$ and $\underline{D}_{7'}$), 6.8 (d, 2H, $J = 8.55$ Hz, $\underline{D}_8$ and $\underline{D}_{8'}$), 5.30 (br s, 1H, $\underline{B}_7$). IR (KBr): ν_{max} 3350–3400, 2931, 1650, 1620, 1520, 1455, 1390, 1225, 1065 cm^{-1}. ESI MS (ES+): for $C_{51}H_{79}N_9O_{15}$; calcd 1057; found: $(M+Na)^+ = 1080$, 1012 (base peak).

N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-12-azido-23-((1*R*)-2-azido-1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxyethyl)-20-hydroxymeth-yl-16-methyl-5,8,14,19,22,25-hexaoxoperhydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16] hexaazacyclohenicosin-9-yl]-12-methyltetradecanamide (14). Using the process outlined for the preparation of **12,** a solution of Orn-5 and HTyr-4-disulfone mulundocandin (**11**) (0.2 g, 0.16 mmol), anhydrous sodium azide (0.104 g, 1.6 mmol) in anhydrous dioxan (10 mL), was stirred at 45–50 °C for 2 h. After usual workup, the crude product was purified by HPLC using Semi-preparative RP-18 column (size, 250×16 mm; particle size 10 μ; mobile phase 70% acetonitrile/water; flow rate 8 mL/min; monitoring wavelength λ = 220 and 270 nm). Removal of organic solvent from appropriate fractions under vacuum followed by lyophilization provided compound **14** (0.115 g, 67.8%; mp: 175 °C). Yield is calculated from nearly 90% pure starting compound. ^{1}H NMR: δ 7.28, 7.14 (2×d, 2H, $J = 8.88$ Hz, $\underline{D}_7$ and $\underline{D}_{7'}$), 6.83 (dd merged, 2H, $J = 8.88$ Hz, $\underline{D}_8$ and $\underline{D}_{8'}$), 5.39 (d, 1H, $J = 1.86$ Hz, $\underline{B}_7$). IR (KBr): ν_{max} 3300–3400, 2930, 2100, 1650, 1620, 1515, 1440, 1240, 1070 cm^{-1}. ESI MS (ES+): for $C_{48}H_{75}N_{13}O_{14}$; Calculated: 1057; found:

$(M+Na)^+ = 1080$, 1037 (base peak). UV (MeOH) λ_{max} (ε M^{-1} cm^{-1}): 221, 275 nm (8266, 1985).

N1-[(6*S*,9*S*,14a*S*,15*S*,16*S*,20*S*,23*S*,25a*S*,2*R*,11*R*)-2,11,15-trihydroxy-6-((1*R*)-1-hydroxy-ethyl)-23-((1*R*)-1-hydroxy-2-(4-hydroxyphenyl)-2-(2-morpholinoethylamino)ethyl)-20-hydroxymethyl-16-methyl-12-(2-morpholinoethylamino)-5,8,14,19,22,25-hexaoxoper-hydrodiazolo[2,1-c:2,1-/][1,4,7,10,13,16]hexaazacyclohenicosin-9-yl]-12-methyl-tetrade-canamide (15). Using the process outlined for the preparation of **12,** a solution of Orn-5- and HTyr-4-disulfone mulundocandin (0.2 g; 0.16 mmol), 4-(2-aminoethyl)-morpholine (0.208 g, 1.6 mmol) in anhydrous dioxan (10 mL), was stirred at 45–50 °C for 2 h. The crude product was purified as described for compound **14**. Removal of organic solvent from appropriate fractions under vacuum followed by lyophilization provided compound **15** (0.093 g, 43.8%). Yield is calculated from nearly 90% pure starting compound. ^{1}H NMR: δ 7.26 (t, 2H, $J = 8.55$ Hz, $\underline{D}_7$ and $\underline{D}_{7'}$), 6.8 (d, 2H, $J = 8.55$ Hz, $\underline{D}_8$ and $\underline{D}_{8'}$), 5.04 (br, 1H, $\underline{B}_7$), 3.7–3.8 (m, 8H, $4\times O\overline{CH_2}$), 2.4–2.27 (m, 16H, $8\times N\overline{CH_2}$). IR (KBr): ν_{max} 3300–3400, 2930, 1680–1620, 1520, 1435, 1380, 1260, 1070 cm^{-1}. ESI–MS (ES+): for $C_{60}H_{101}N_{11}O_{16}$; calcd 1231; found: $(M+Na)^+ = 1254$ (base peak).

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References and Notes

1. (a) Walsh, T. J.; Jarrosinski, P. F.; Fromtling, R. A. *Diagn. Microbiol. Infect. Dis* **1990**, *13*, 37. (b) Samonis, G.; Bafaloukos, D. *In vivo* **1992**, *6*, 183. (c) Pizzo, P. A.; Young, L. S. *Am. J. Med.* **1984**, *76*, 101. (d) Walsh, T. J.; Gonzalez, C.; Lyman, C. A.; Chanock, S. J.; Pizzo, P. A. *Adv. Pediatr. Infect. Dis.* **1996**, *11*, 187.
2. (a) Walsh, T. *J. Hematol. Oncol. Clin. North Am.* **1993**, *7*, 1003. (b) Warnock, D. W. *J. Antimicrob. Chemother.* **1995**, *36* (Suppl B), 73. (c) Buchner, T.; Roos, N. *Ann. Hematol* **1992**, *65*, 153. (d) Dean, D. A.; Burchard, K. W. *Am. J. Surg.* **1996**, *171*, 374. (e) Khan, Z. K.; Gyanchandani, A. *PINSA-B: Proc. Indian Natl. Sci. Acad., Part B* **1998**, *64*, 1. (f) Powderly, W. G. *J. Phys. Assoc. AIDS Care* **1994**, 32.
3. Gallis, H. A.; Drew, R. H.; Pickard, W. W. *Rev. Inf. Dis.* **1990**, *12*, 308.
4. Bailey, E. M.; Drakovsky, D. J.; Ryback, M. J. *Pharmacotherapy* **1990**, *10*, 146.
5. (a) Fox, R.; Neal, K. R.; Leen, C. L. S.; Ellis, M. E.; Mandal, B. K. *J. Infect.* **1991**, *22*, 201. (b) Smith, D.; Boag, F.; Midgley, J.; Gazzard, B. *J. Infect.* **1991**, *23*, 345. (c) Hitchcock, C. A.; Pye, G. W.; Trok, F. J.; Johnson, E. M.; Warnock, D. W. *Antimicrob. Agents Chemother.* **1993**, *37*, 1962. (d) Sanglard, D.; Kuchler, K.; Ischer, F.; Pagani, J.-L.; Monod, M.; Bille, J. *Antimicrob. Agents Chemother.* **1995**, *39*, 2378. (e) Marybeth, F.; Barrett, J. F. *Expert Opin. Invest. Drugs* **1998**, *7*, 175 (a review with 221 refs).
6. (a) Debono, M.; Turner, W. W.; LaGrandeur, L.; Burkhardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zekner, D. J.; Gordee, R. S.; Tang, J.; Parr, T. R., Jr *J. Med. Chem.* **1995**, *38*, 3271. (b) Masurekar, P. S.; Fountoulakis, J. M.; Hallada, T. C.; Sosa, M. S.; Kaplan, L. *J. Antibiot.* **1992**, *45*, 1867. (c) Iwamoto, T.; Fujie, A.; Nitta, K.; Hashimoto, S.; Okuhara, M.; Kohsaka, M. *J. Antibiot.* **1994**, *47*, 1092.
7. Debono, M.; Gordee, R. S. *Ann. Rev. Microbiol.* **1994**, *48*, 471.
8. Pound, M. W.; Drew, R. H.; Perfect, J. R. *Curr. Opin. Infect. Dis.* **2002**, *15*, 183.
9. (a) Ablordeppey, S. Y.; Fan, P.; Ablordeppey, J. H.; Mardenborough, L. *Curr. Med. Chem.* **1999**, *6*, 1151 (A review with 537 refs). (b) Green, L. J.; Marder, P.; Mann, L. L.; Chio, L.-C.; Current, W. L. *Antimicrob. Agents Chemother.* **1999**, *43*, 830. (c) Tomishima, M.; Ohki, H.; Yanada, A.; Takasugi, H.; Maki, K.; Tawara, S.; Tanaka, H. *J. Antibiot.* **1999**, *52*, 674.
10. Roy, K.; Mukhopadhyay, T.; Reddy, G. C. S.; Desikan, K. R.; Ganguli, B. N. *J. Antibiot.* **1987**, *40*, 275.
11. Hawser, S.; Borgonovi, M.; Markus, A.; Isert, D. *J. Antibiot.* **1999**, *52*, 305.
12. Bouffard, F. A.; Hammond, M. L.; Arison, B. H. *Tetrahedron Lett.* **1995**, *36*, 1405.
13. (a) Balkovec, J. M.; Bouffard, F. A.; Dropinski, J. F. (Merck and Co. Inc., USA). PCT. Int. Appl. WO 9,613,272, 9 May 1996. (b) Balkovec, J. M.; Christian, R. M. (Merck and Co., Inc.). Eur. Pat. Appl. EP 459,564, 4 Dec. 1991. (c) Bouffard, F. A. (Merck and Co., Inc., USA). Eur. Pat. Appl. EP 538,002, 21 Apr. 1993. (d) Bouffard, F. A.; Dropinski J. F. (Merck and Co., Inc. USA). Eur. Pat. Appl. EP 539,088, 28 Apr. 1993. (e) Belyk, K. M.; Bender, D. R.; Black, R. M.; Hughes, D. L.; Leonard, W. (Merck and Co., Inc.). US 5,552,521, 03 Sep. 1996. (f) Boffard, F. A. (Merck and Co., Inc.). PCT, WO 96/22784, 01 Aug. 1996.
14. (a) Lal, B: Gund, V. G.; Gangopadhyay, A. K. (Aventis Pharma Deutschland G.m.b.H., Germany). PCT Int. Appl., WO 0107468 A2 200110201, 2001. (b) Gund, V. G. *Semisynthetic Studies On Mulundocandin: An Antifungal Lipopeptide*. PhD Thesis, Nov 2001, Indian Institute Of Technology, Bombay, India.
15. (a) Zaugg, H. E. *Synthesis* **1970**, *2*, 49. (b) Zaugg, H. E. *Synthesis* **1984**, 85. (c) Zaugg, H. E. *Synthesis* **1984**, 181. (d) Speckamp, W. N.; Hiemstra, H. *Tetrahedron* **1985**, *41*, 4367.
16. Bouffard, F. A.; Zambias, R. A.; Dropinski, J. F.; Balkovec, J. M.; Hammond, M. L.; Abruzzo, G. K.; Bartizal, K. F.; Marrinan, J. A.; Powels, M. A.; Schmatz, D. M. *J. Med. Chem.* **1994**, *37*, 222.
17. (a) Barry, A. L. Procedure and Therotical Considerations for Testing Antimicrobial Agents in Agar Media. In *Antibiotics in Laboratory Medicine*, 3rd ed., Lorian V. ed., Williams and Wilkins: Baltimore, MD, 1991; p 16. (b) Denning, D. W.; Radford, S. A.; Oakley, K. L.; Hall, L.; Johnson, E. M.; Warnock, D. W. *J. Antimicrob. Chemother.* **1997**, *40*, 401.
18. (a) Walsh, T. J.; Lee, J. W.; Kelly, P.; Bacher, J.; Lecciones, J.; Thomas, V.; Lyman, C.; Coleman, D.; Gordee, R.; Pizzo, P. A. *Antimicrob. Agents Chemother.* **1991**, *35*, 1321. (b) Nawada, R.; Amitani, R.; Tanaka, E.; Niimi, A.; Suzuki, K.; Murayama, T.; Kuze, F. *J. Clin. Microb.* **1996**, *34*, 1433. (c) Valentin, A.; Guennec, R. L.; Rodriguez, E.; Reynes, J.; Mallie, M.; Bastide, J.-M. *Antimicrob. Agents Chemother.* **1996**, *40*, 1342. (d) Bartizal, K.; Abruzzo, G.; Trainor, C.; Krupa, D.; Nollstadt, K.; Schmatz, D.; Hammond, M.; Balkovec, J.; Van Middlesworth, F. *Antimicrob. Agents Chemother.* **1992**, *36*, 1648.