

Synthesis and preliminary antifungal evaluation of a library of phytosphingolipid analogues†

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A library of 64 phytosphingolipid analogues resulting from the systematic variation of the C1, C3, C4, and the *N*-acyl moiety of phytosphingosine (PHS) has been prepared from common scaffolds derived from the chiral pool and Sharpless asymmetric dihydroxylation reactions. Library members have been evaluated as growth inhibitors of the yeast *Saccharomyces cerevisiae*. In addition, 1-amino-*N*-pivaloyl PHS analogues were also tested as IPC synthase inhibitors, in comparison with the natural product khafrefungin.

Introduction

Fungi are eukaryotic organisms that present a rigid cell wall together with an ergosterol-rich cytoplasmatic membrane. They grow as yeasts or moulds, and species such as *Candida*, *Aspergillus* and *Cryptococcus* are among the most common human pathogens. However, new pathogenic fungal species are emerging that can give rise to a wide variety of fungal infections, from superficial to deeply invasive and disseminated systemic ones.¹

In recent years, the number of serious fungal opportunistic infections has risen dramatically due to the increased number of immune-suppressed patients, either by HIV,^{2,3} cancer chemotherapy^{4,5} or organ allograft transplantation.^{6–8}

The increase of fungal resistance to standard treatments^{9,10} has stimulated the search for new therapeutic agents in this field. One of the current strategies relies on the discovery of new pharmacological targets.¹¹ In this context, exploitation of the metabolic differences between fungal and mammalian sphingolipid (SL) metabolism represents an attractive approach. SLs are essential components in eukaryotic cells that are also involved in cell growth regulation and communication processes.^{12,13} Since they present significant differences in mammalian and fungal cells, they are attractive new targets for fungal intervention, as evidenced by several natural products that have been discovered in recent years as selective inhibitors of fungal sphingolipid biosynthesis.¹⁴ One of the most significant differences in SL biosynthesis between fungi and mammals is the hydroxylation of dihydrosphingosine (DHS) to phytosphingosine (PHS) by means of a C4 hydroxylase.¹⁵ Moreover, PHS can be acylated to phytoceramide (PHC), which, in turn, can be transformed into complex fungal SLs via its initial conversion into inositolphosphoryl ceramide (IPC) by the

action of inositolphosphoryl ceramide synthase (IPC synthase) (Scheme 1). It has been reported that the reaction catalyzed by IPC synthase plays a role in the transition from the G1 to the S phase of the cell cycle¹⁶ and is essential for sphingolipid biosynthesis in fungi. Therefore specific IPC synthase inhibitors, such as the natural product khafrefungin (Scheme 1), are attractive targets for the design of new, nontoxic antifungal agents.^{14,17,18}

In this context, we are currently interested in the search of new, selective, modulators of fungal SL metabolism based on the design and synthesis of SL analogues. In particular, we wish to report on a series of new phytosphingolipid (PHSL) analogues shown in Fig. 1. They have been designed according to three different structural criteria: a) Modification of the C3–C4 dihydroxy framework present in natural PHC; b) replacement of the C1-OH group with amino and azido functionalities, and c) variations on the *N*-acyl substitution.

Preliminary results on antifungal activity as *Saccharomyces cerevisiae* growth inhibitors is reported in this work, as well as the inhibitory properties against IPC synthase for the most active analogues.

Results and discussion

Chemistry

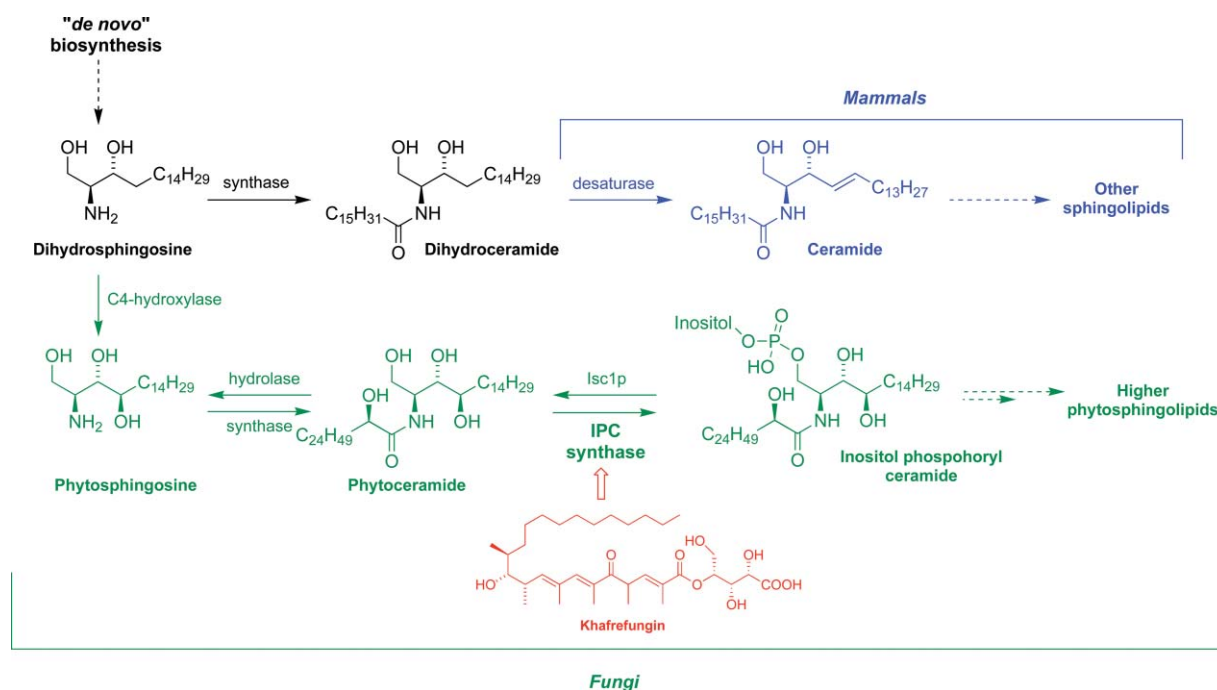
Synthesis of PHSL analogues started from *N*-Boc olefins (*E*)- and (*Z*)-**13b** (Fig. 1), obtained from Garner aldehyde through slight modifications of reported protocols.^{19–21} Azido olefins (*E*)- and (*Z*)-**14b** were obtained from (*E*)- and (*Z*)-**13b** following standard mesylation to (*E*)- and (*Z*)-**16b** and azide displacement. Sharpless asymmetric dihydroxylation (AD) of olefins (*E*)- and (*Z*)-**13b** with AD-mix- α or - β catalysts was carried out as described in the literature²⁰ to give the expected triols **1b–4b**. Application of the same protocol to azido olefins (*E*)- and (*Z*)-**14b** afforded the corresponding azido diols **5b–8b** in good yields and diastereoselectivities (Table 1).

As expected from the Kishi and Sharpless empirical rules for the AD reaction,^{22,23} the stereochemical course from olefins **13** and **14** was not affected by the presence of the azide group, as evidenced by the close similarities found in the ¹HNMR spectra for each equivalent isomeric pair (**1b/5b**, **2b/6b**, **3b/7b**,

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Scheme 1 Divergent biosynthetic pathways in mammals and fungi.

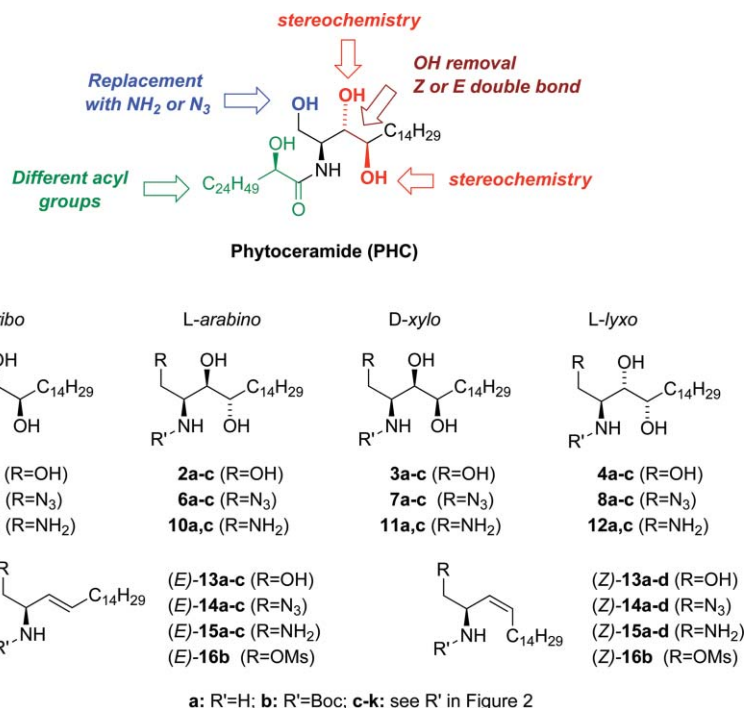
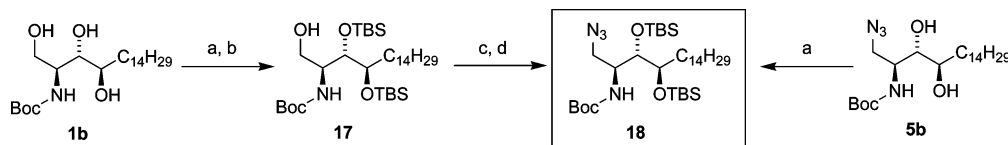


Fig. 1 General structures of the library of PHSL analogues described in this study.

4b/8b). In addition, we further confirmed the above assumption by chemical correlation. Thus, **1b** and **5b** afforded the same bis-OTBS derivative **18** by simple functional group manipulations, as shown in Scheme 2.

Despite the identical stereochemical course observed for azido and hydroxy olefins in the AD reaction, some reactivity differences are remarkable. Thus, a higher stereoselectivity arose from azido olefin (**Z**)-**14b** on reaction with AD-mix- β , compared with the

corresponding hydroxy counterpart (**Z**)-**13b** (entries 1 and 5, Table 1). Interestingly, an opposite trend was observed on reaction with AD-mix- α (entries 2 and 6). In addition, dihydroxylation of azido olefins in the presence of AD-mix- α led to incomplete conversions and lower yields than the corresponding hydroxy olefins (compare entries 2 and 4 with entries 6 and 8). This trend can be attributed to a presumed unfavourable interaction of the azido group with the chiral ligand.



Scheme 2 Reagents and conditions: a) TBSOTf, 2,6-lutidine, DCM, 0 → 25 °C; b) HF·pyr, THF, 0 → 25 °C; c) MsCl, TEA, DCM, 0 → 25 °C; d) NaN₃, DMF, 65 °C.

Table 1 Sharpless asymmetric dihydroxylation (AD) of olefins (*Z*)- and (*E*)-**13b** and (*Z*)- and (*E*)-**14b**

Entry	Olefin ^a	AD-mix	Yield (%)	Major isomer ^a	Isomeric ratio
1	(<i>Z</i>)- 13b	β	96	1b (D-ribo)	87 : 13 (1b/2b)
2	(<i>Z</i>)- 13b	α	82	2b (L-arabino)	13 : 87 (1b/2b)
3	(<i>E</i>)- 13b	β	94	3b (D-xylo)	85 : 15 (3b/4b)
4	(<i>E</i>)- 13b	α	93	4b (L-lyxo)	15 : 85 (3b/4b)
5	(<i>Z</i>)- 14b	β	94	5b (D-ribo)	92 : 8 (5b/6b)
6	(<i>Z</i>)- 14b	α	49 ^b	6b (L-arabino)	29 : 71 (5b/6b)
7	(<i>E</i>)- 14b	β	96	7b (D-xylo)	88 : 12 (7b/8b)
8	(<i>E</i>)- 14b	α	62 ^c	8b (L-lyxo)	12 : 88 (7b/8b)

^a See Fig. 1. ^b 39% recovered starting material. ^c 33% recovered starting material.

N-Boc removal of **1b–8b** and **13b**, **14b** (*E* and *Z* isomers) under classical acidic conditions^{19,20} set the stage for amide formation to gain access to PHC analogues. This was carried out by selective coupling the resulting amino derivatives **1a–8a** and **13a**, **14a** (*E* and *Z* isomers) with some of the acyl derivatives **c–k** shown in Fig. 2.

Acylating agents were chosen in order to compare the biological activities of the resulting PHC analogues with those of related ceramide analogues currently under evaluation in our group.²⁴ Acyl chlorides **c–j** reacted in good to moderate yields under Schotten–Baumann conditions (THF, 50% aq. NaOAc) to afford

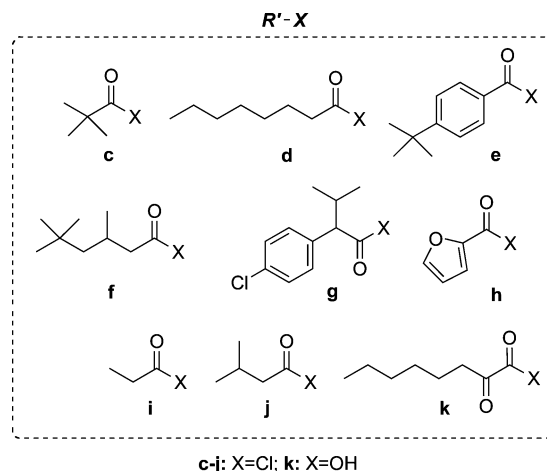
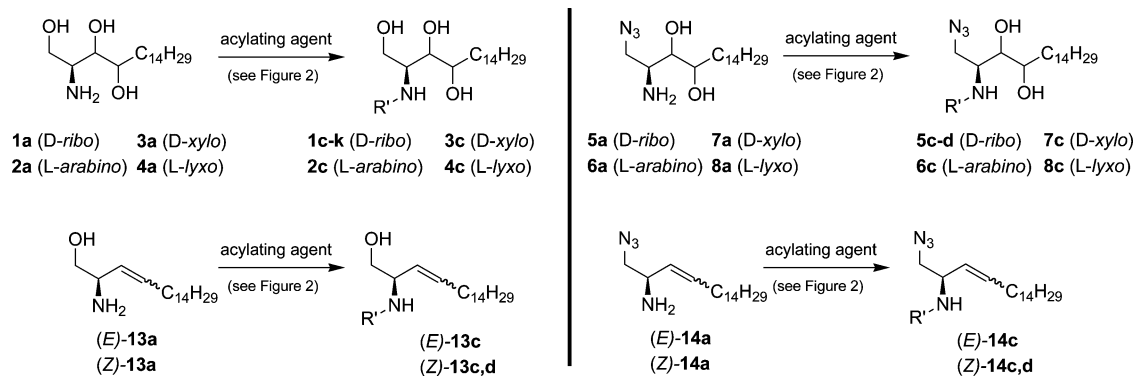


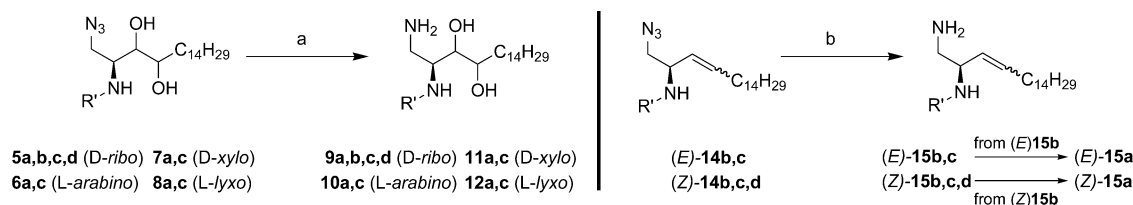
Fig. 2 Acylating agents used for the synthesis of PHC analogues.

the corresponding amides shown in Scheme 3. Carboxylic acid **1k** was coupled in the presence of HOBt–EDC in THF.²⁵

1-Amino PHSL analogues were obtained by reduction of the corresponding azide precursors (Scheme 4). Catalytic hydrogenation was the method of choice for **9–12**, whereas azide reduction *via* Staudinger reaction was required for azido olefins **14** to preserve the double bond integrity of the final analogues.



Scheme 3 PHC analogues synthesized in this study. **a**: R' = H; **b**: R' = Boc; **c–k**: see Fig. 2.



Scheme 4 Synthesis of 1-amino PHC analogues; **a**: R' = H; **b**: R' = Boc; **c,d**: see Fig. 2. Reagents and conditions: (a) H₂, Pd/C, THF, 20 h, rt; (b) Ph₃P, THF–H₂O (9 : 1), 60–72 h, 25 °C.

Yeast growth inhibition

S. cerevisiae, whose SL metabolism and signalling functions are well known,^{26,27} has also been considered as a pathogen model for antifungal activity.²⁸ In this context, PHS has been shown to inhibit *S. cerevisiae* growth through ubiquitin-dependent proteolysis of different nutrient permeases.²⁹ Along this line, the PHS analogues described in this work (**1a–12a**) were evaluated as *S. cerevisiae* growth inhibitors in order to determine the influence of the sphingoid chain configuration at the C3–C4 positions and the nature of the functional group at C1 (Table 2 and Fig. 3).

All four PHS diastereomers ($R = OH$, **1a–4a**, Fig. 1) were good growth inhibitors, especially the unnatural isomers **2a–4a** (IC_{50} 90–110 μM). Analogues resulting from the replacement of C1-OH with a primary amine ($R = NH_2$, **9a–12a**, Fig. 1) were devoid of activity, an indication of the importance of C1-OH in natural PHS. On the other hand, striking differences on growth inhibitory properties were found among azide derivatives ($R = N_3$, **5a–8a**, Fig. 1), where the naturally configured *D-ribo* analogue **5a** and *L-arabino* analogue **6a** (IC_{50} 150 and 135 μM , respectively) were the most active ones. It is worth noting that the stereoselectivity observed in the azide analogues was not found in the amino ($R = NH_2$) or hydroxy ($R = OH$) counterparts. Since the simple aliphatic alcohol (1-octadecanol), amine (1-octadecylamine) or azide (1-azidooctadecane) were totally devoid of activity in this assay, and unsaturated alcohols (*(E)*- and *(Z)*-**13a** and *(E)*- and *(Z)*-**15a**) showed a similar inhibition profile (IC_{50} 98–145 μM), it is reasonable to assume that the observed effects for our PHS analogues are the result of specific, albeit yet undisclosed, cellular effects rather than unspecific membrane-disrupting mechanisms. It is accepted that effective incorporation of *D-ribo* PHS into phytosphingolipids requires both phosphorylation and subsequent dephosphorylation. These reactions serve to properly localize the sphingoid base and to allow for efficient action of downstream enzymes, since yeasts with a deleted phosphatase gene (*LCB3*) are more resistant to PHS treatment.³⁰ Although additional experiments are required along this line, targets of natural PHS, such as AGC-type protein kinases³¹ and/or nutrient permeases^{29,32} seem reasonable candidate targets for PHS stereoisomers **1a–4a**.

Table 2 Inhibition of yeast growth by PHSL analogues at 400 μM

Compound	Inhibition (%) (mean \pm SD)	$IC_{50}/\mu M$
1a	77 \pm 2	280
2a	84 \pm 3	110
3a	92 \pm 1	98
4a	100 \pm 2	90
5a	98 \pm 1	150
6a	85 \pm 2	135
7a	40 \pm 4	—
8a	n/a	—
(<i>Z</i>)- 13a	79 \pm 3	145
(<i>E</i>)- 13a	83 \pm 4	128
(<i>Z</i>)- 15a	80 \pm 6	102
(<i>E</i>)- 15a	82 \pm 5	98
1b	23 \pm 5	—
2b	n/a	—
3b	64 \pm 3	—
4b	6 \pm 6	—
5b	6 \pm 7	—
9b	90 \pm 7	176
(<i>Z</i>)- 13b	43 \pm 6	—
1c	59 \pm 5	—
9c	43 \pm 5	—
10c	100 \pm 5	72
11c	83 \pm 5	98
12c	92 \pm 3	71
(<i>Z</i>)- 15c	50 \pm 5	—
(<i>E</i>)- 15c	70 \pm 6	—
(<i>Z</i>)- 15d	4 \pm 2	—
1e	n/a	—
1f	55 \pm 4	—
1g	44 \pm 3	—
1h	n/a	—
1i	3 \pm 8	—
1j	n/a	—
1k	n/a	—

Preliminary experiments on PHC analogues were first carried out from *N*-acyl derivatives of natural *D-ribo* stereochemistry (**1c–k**, Table 2, Fig. 1 and 2). Branched α - or β -*N*-acyl substituents led to the most potent growth inhibitors (compounds **1c**, **1f**, and **1g**).³³ These promising data led us to explore other *N*-pivaloyl derivatives arising from the systematic variation of the C1 functionality and/or the stereochemistry around the C3–C4

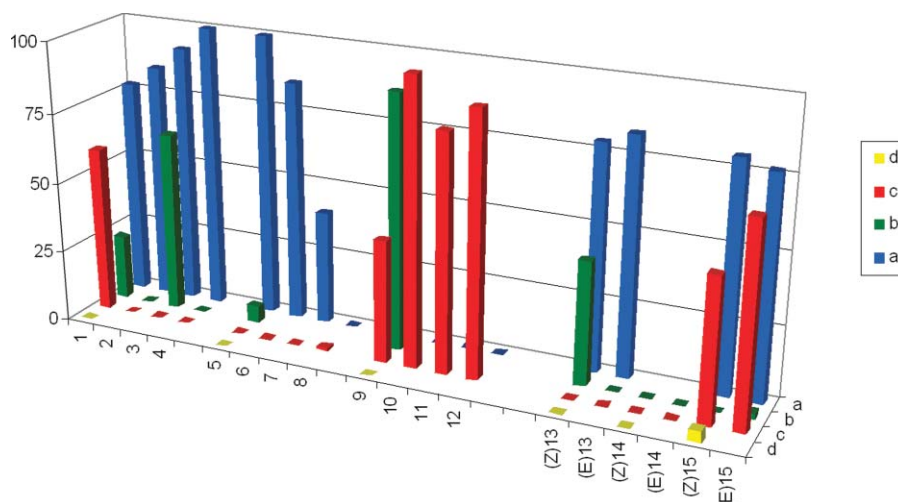


Fig. 3 Yeast growth inhibition (%) in the presence of PHSL analogues at 400 μM .

moiety of the sphingoid backbone. Thus, replacement of C1-OH with an azido group (**5c–8c**, Fig. 1 and 2), as well as the presence of a non-natural stereochemistry on the sphingoid base (**2c–4c**) was detrimental for growth inhibition. However, this trend was not observed for the C1-NH₂ analogues (**9c–12c**, Fig. 1 and 2), where the non-naturally configured **10c–12c** were the most potent growth inhibitors (IC₅₀ 71–98 μM, Table 2 and Fig. 3). Unsaturated analogues ((*E*)- and (*Z*)-**15c**), bearing the C1-NH₂ and *N*-pivaloyl moieties, showed growth inhibitory properties comparable to those of the corresponding *D*-ribo analogue **9c**, this stressing the importance of an unnatural stereochemistry at C3–C4 for the activity of these kinds of PHC analogues.³⁴ Finally, although *N*-octanoyl analogue **1d** was inactive, the inhibitory activity elicited by the C1-NH₂ analogues **9c–12c** and (*E*)- and (*Z*)-**15c** prompted us to test the corresponding C1-NH₂ *N*-octanoyl derivatives **9d** and (*Z*)-**15d**. However, none of them led to yeast growth inhibition.³⁵

Different C1-functionalized *N*-Boc PHS (**1b–5b** and **9b**, Fig. 1 and 2) and olefin isomers ((*Z*)- and (*E*)-**13b**, **14b**, and **15b**) were also tested (Table 2 and Fig. 3). As a general trend, the activity of C1-OH analogues (**1b–4b**) was lower than that of the corresponding free sphingoid base (**1a–4a**). However, some of them exhibited higher inhibitory activity than the corresponding *N*-pivaloyl analogues (compare, for example **3b/3c** and (*Z*)-**13b**/*(Z)*-**13c**, Fig. 3). Among the *N*-Boc C1-NH₂ analogues tested, the *D*-ribo compound **9b** was the most active one, exhibiting an IC₅₀ value of 176 μM (Table 2). With the exception of olefin (*Z*)-**13b**, the remaining olefin isomers were inactive as yeast growth inhibitors.

IPC synthase inhibition

As mentioned above, specific IPC synthase inhibitors are attractive targets for the design of new, nontoxic antifungal agents. However, only some structurally complex natural products have been reported so far.¹⁴ Attempts to obtain simplified analogues have found limited success so far, as reported for galbonolide³⁶ and khafrefungin (Scheme 1).^{37,38} The complex chemical structures of the above compounds and the lack of significant activity found for simplified analogues indicate the striking difficulty of finding new IPC synthase inhibitors. Along this line, several of our PHC analogues were assayed against IPC synthase using khafrefungin as a reference inhibitor.

Using a membrane preparation from *S. cerevisiae* obtained as described by Aeed *et al.*³⁹ and an HPLC-fluorimetric assay to assess the formation of fluorescent inositol phosphoryl C6-NBD ceramide (C6-NBD-Cer),⁴⁰ we were able to determine a *K_m* of 4.3 μM for C6-NBD-Cer, a similar value to that reported for IPC synthase from *Candida albicans*.³⁹ This assay was optimized and routinely used, as described in the Experimental section.

Based on the above analytical protocol, *N*-pivaloyl PHC analogues **1c–12c** were evaluated *in vitro* as IPC synthase inhibitors in comparison with khafrefungin.^{17,37,41} For comparative purposes, the *D*-ribo *N*-octanoyl analogues **1d** and **9d**, as well as *N*-Boc analogues **9b**, (*Z*)-**15b** and (*E*)-**15b**, were studied. Compounds were evaluated at 30 μM against the fluorescent substrate C6-NBD-Cer at 10 μM. The results are shown in Table 3.

As a general trend, C1-azido analogues (**5c–8c**) were weaker IPC synthase inhibitors than their corresponding C1-OH (**1c–4c**) or C1-NH₂ (**9c–12c**) counterparts. All C1-OH analogues were

Table 3 Inhibition of IPC synthase by PHSL analogues

Compound	Inhibition (%) (mean ± SD)
1c	35.4 ± 3
2c	31.7 ± 3
3c	29.2 ± 6
4c	48.1 ± 9
5c	29.3 ± 0.5
6c	24.9 ± 0.6
7c	14.3 ± 2
8c	28.2 ± 2
9c	45.3 ± 9
10c	25.4 ± 3
11c	24.1 ± 5
12c	22.0 ± 2
(<i>Z</i>)- 13c	34.0 ± 2
(<i>E</i>)- 13c	31.2 ± 0.4
(<i>Z</i>)- 15c	26.9 ± 5
(<i>E</i>)- 15c	5.3 ± 3
9b	10.6 ± 1
(<i>Z</i>)- 15b	18.5 ± 4
(<i>E</i>)- 15b	8.0 ± 5
1d	33.3 ± 2
9d	11.8 ± 0.4

moderate inhibitors, the *L*-lyxo compound **4c** being the most potent (around 48% inhibition, see Table 3). Both the *D*-ribo analogue **1c**, and the corresponding *N*-octanoyl analogue **1d**, having the natural configuration of the sphingoid backbone, as well as the non-natural **2c** and **3c** analogues, afforded similar results (30–35% inhibition). These data seem to indicate that the stereochemistry of the C3–C4 moiety in this series is irrelevant for the inhibitory activity, since the inhibition shown by both (*Z*)- and (*E*)-**13c** was comparable to that of the above analogues. Replacement of the C1-OH with an amino group led to more striking differences. Thus, *D*-ribo analogue **9c** was the most potent inhibitor of this series (45% inhibition), while stereochemical variations around the C3–C4 moiety led to the markedly less potent inhibitors **10c–12c** (around 20–25% inhibition). Likewise, replacement of the *N*-pivaloyl group with the *N*-octanoyl (**9d**) or *N*-Boc (**9b**) groups led to an even more pronounced loss of activity (around 10% inhibition). In contrast to the C1-OH series, replacement of the C3–C4 dihydroxy moiety with a double bond led to a substantial loss of activity, as found in (*Z*)-**15b**, (*E*)-**15b**, and (*E*)-**15c**.

For the most active compounds (**4c** and **9c**), incubations at different concentrations were carried out in comparison with khafrefungin (Fig. 4). Calculated IC₅₀ values of 33, 35 and

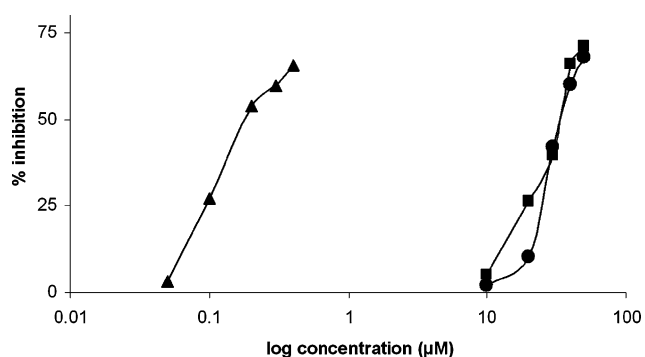


Fig. 4 Inhibition of IPC synthase by khafrefungin (▲) and phytoceramide analogues **4c** (●) and **9c** (■).

0.19 μM , respectively, were obtained. Although the inhibitory activity of our PHC analogues was two orders of magnitude lower than that of khafrefungin, their structural simplicity will make possible the design of focused libraries for further screening in the search of more potent IPC synthase inhibitors.

Conclusions

A library of 64 PHSL analogues resulting from the systematic variation of the C1, C3, C4, and the *N*-acyl moiety of phytosphingosine has been prepared. The antifungal activity of the library has been evaluated based mainly on its growth inhibitory properties against the yeast *S. cerevisiae*. PHS analogues ($R' = \text{H}$, Fig. 1) bearing the natural C1-OH functional group were potent growth inhibitors, irrespective of the nature or the stereochemistry of the C3–C4 moiety of the sphingoid backbone. In contrast, only C1- NH_2 PHS analogues with a C3–C4 double bond showed significant growth inhibition. Among PHC analogues ($R' = \text{acyl}$, Fig. 1), C1- NH_2 *N*-pivaloyl derivatives were good growth inhibitors, once again irrespective of the nature of the C3–C4 moiety. Attempts to correlate growth inhibition with IPC synthase inhibition in *N*-pivaloyl analogues were unsuccessful, since the most active enzyme inhibitors (**4c** and **9c**) were inactive or weakly active, respectively, in the growth inhibition assay. Nevertheless, the promising results on IPC synthase inhibition obtained with some of our PHC analogues will help to define new structural modifications for future research along this line.

Experimental

Inhibition assays

Biological assays. The isogenic *S. cerevisiae* strain W303a (*leu2, ura3, trp1, ade2, his3*) was grown in YPD medium, composed of 1% yeast extract, 2% peptone and 2% glucose. The yeasts were grown with aeration at 30 °C and growth was followed turbidimetrically at 600 nm. Yeast suspension on YPD medium to a final concentration of 0.15 OD in 0.1 mL was prepared. Tested compounds were dispensed into the wells of a 96-well polystyrene microtiter plate to a final concentration of 0.4 mM and inoculated with 0.1 mL of yeast suspension. Growth in liquid YPD with or without 0.5% tertitol in the presence of synthesized compounds was monitored over a 16 h period. All experiments were repeated at least three times. The IC_{50} values were determined by plotting percent activity *versus* $\log[\text{I}]$, using at least five different inhibitor concentrations.

IPC synthase assay. IPC synthase activity was determined using detergent-treated microsomal membranes from *S. cerevisiae*, prepared as described by Aeed *et al.*³⁹ The assay was based on an HPLC analysis of the conversion of the fluorescent C6-NBD-Cer to inositol phosphoryl C6-NBD-Cer.⁴⁰ Chromatographic conditions: column: Kromasil 100, C18, 5 μm , 15 \times 0.40 cm; flow rate 1 mL min^{-1} , $\text{CH}_3\text{CN}-\text{H}_2\text{O}$, gradient from 50 : 50 to 70 : 30 in 20 min; detection at 530 nm (excitation at 450 nm); retention times: inositol phosphoryl C6-NBD-Cer (7.1 min); C6-NBD-Cer (21.3 min). The protein was incubated with different concentrations of C6-NBD-Cer, and the K_m value was calculated by a Lineweaver–Burk plot. Yeast microsomes were incubated in the presence or absence of potential inhibitors for 1 h at 37 °C. Can-

didates were tested at 30 μM whereas the fluorescent substrate was used at 10 μM . The IC_{50} values were determined by plotting percent activity *versus* the inhibitor concentration, expressed as $\log[\text{I}]$, using at least five different inhibitor concentrations.

Chemistry

General methods. Garner aldehyde was obtained by oxidation of the corresponding primary alcohol,⁴² prepared according to reported methods.^{43,44} Solvents were distilled prior to use and dried by standard methods.⁴⁵ FT-IR spectra are reported in cm^{-1} . ^1H and ^{13}C NMR spectra were obtained in CDCl_3 solutions at 300 MHz (for ^1H) and 75 MHz (for ^{13}C), respectively. Chemical shifts are reported in δ units, in parts per million (ppm) relative to the singlet at 7.24 ppm of CDCl_3 for ^1H , and in ppm relative to the center line of a triplet at 77.0 ppm of CDCl_3 for ^{13}C . $[\alpha]_D$ values are given in $10^{-1} \text{deg cm}^2 \text{g}^{-1}$. HPLC-MS analyses were obtained on a Hewlett Packard MSD system. ESI/HRMS spectra were recorded on a Waters LCT Premier Mass spectrometer at CID–CSIC (Barcelona) and IQAC (Tarragona).

Synthesis of mesylates (*E*)- and (*Z*)-16b from alcohols (*E*)- and (*Z*)-13b. To 1.3 mmol of the corresponding alcohol (*E*)- or (*Z*)-13b were added 13 mL of anhydrous DCM under argon atmosphere, and the reaction was cooled to 0 °C. The reaction mixture was next treated with 0.36 mL (2.6 mmol) of freshly distilled TEA and, after stirring for 10 min at 0 °C, 0.12 mL (1.6 mmol) of freshly distilled MsCl was also added. The reaction mixture was stirred at 0 °C for 2 h until no starting material was observed by TLC. The mixture was then diluted with DCM (15 mL) and sequentially washed with 3 \times 15 mL of 1 N HCl, sat. aq. NaHCO_3 , and brine. The organic phase was dried over MgSO_4 , filtered, and the solvent evaporated *in vacuo* to give the required mesylate.

(2*R*,3*Z*)-Methanesulfonic acid 2-(*tert*-butoxycarbonylamino)-octadec-3-enylester ((*Z*)-16b). Obtained as a white solid (524 mg, 1.1 mmol, 89% from (*Z*)-13b), which was used in the next step without further purification. Mp 58–59 °C; $[\alpha]_D^{25} +6.5$ (*c* 1.10, CHCl_3); IR (film): 3368, 2926, 2855, 1708, 1513, 1463, 1361, 1245, 1174, 1054, 963, 829. ^1H NMR (CDCl_3 , 500 MHz): 5.64 (dt, $J = 10.8, 7.5$ Hz, 1H), 5.32 (dd, $J = 11, 9$ Hz, 1H), 4.67 (br s, 2H), 4.26 (m, 1H), 4.16 (dd, $J = 10, 5$ Hz, 1H), 3.02 (s, 3H), 2.11 (m, 2H), 1.44 (s, 9H), 1.36 (m, 2H), 1.25 (m, 22H), 0.88 (t, $J = 7$ Hz, 3H). ^{13}C NMR (CDCl_3 , 75 MHz): 155.1, 136.0, 124.5, 80.1, 71.3, 47.4, 37.6, 37.4, 32.0, 30.8, 29.8, 29.6, 29.5, 29.4, 28.5, 28.4, 28.1, 27.3, 22.8, 14.3, 14.1 (rotamers observed). ESI-MS m/z 484 [$\text{M} + \text{Na}$], 500 [$\text{M} + \text{K}$].

(2*R*,3*E*)-Methanesulfonic acid 2-(*tert*-butoxycarbonylamino)-octadec-3-enyl ester ((*E*)-16b). Obtained from 125 mg (0.33 mmol) of alcohol (*E*)-13b as a white solid (126 mg, 0.27 mmol, 81%), which was used in the next step without further purification. Mp 64–65 °C; $[\alpha]_D^{25} +3.0$ (*c* 1.15, CHCl_3); IR (film): 3344, 2917, 2849, 1685, 1525, 1463, 1353, 1280, 1247, 1168, 1054, 965, 836. ^1H NMR (CDCl_3 , 500 MHz): 5.73 (dtd, $J = 15.5, 6.5, 1$ Hz, 1H), 5.38 (dd, $J = 15.5, 6$ Hz, 1H), 4.72 (br s, 2H), 4.38 (m, 1H), 4.27 (dd, $J = 9.5, 4$ Hz, 1H), 4.18 (dd, $J = 10, 5$ Hz, 1H), 3.02 (s, 3H), 2.03 (m, 2H), 1.44 (s, 9H), 1.36 (m, 2H), 1.23–1.32 (m, 22H), 0.87 (t, $J = 7$ Hz, 3H). ^{13}C NMR (CDCl_3 , 75 MHz): 155.1, 135.1, 125.0, 80.1, 71.3, 51.5, 37.5, 32.4, 32.0, 29.8, 29.7,

29.6, 29.5, 29.2, 29.0, 28.4, 22.8, 14.2. ESI-MS m/z 484 [M + Na], 946 [2M + Na].

General procedure for the synthesis of azido olefins 14b. A solution of the required starting mesylate **16b** (346 mg, 0.75 mmol) in DMF (12 mL) was treated with NaN₃ (122 mg, 1.87 mmol). The reaction mixture was heated at 65 °C and stirred for 20 h. The mixture was then diluted with 40 mL of H₂O and 40 mL of Et₂O. The organic phase was washed with 1 N HCl (3 × 25 mL), dried over MgSO₄, filtered and the solvent removed under reduced pressure. The resulting crude was purified by flash chromatography (hexane–EtOAc 9 : 1) to give the final azide.

(2*R*,3*Z*)-[1-(Azidomethyl)heptadec-2-enyl]carbamic acid *tert*-butyl ester ((*Z*)-14b). Obtained in 94% yield (288 mg, 0.70 mmol) as a white solid from 346 mg (0.75 mmol) of (*Z*)-**16b**. Mp 55–56 °C; $[\alpha]_D^{25} +3.5$ (*c* 0.80, CHCl₃); IR (film): 3359, 2921, 2853, 2102, 1756, 1687, 1525, 1462, 1372, 1301, 1241, 1168, 1109, 852. ¹H NMR (CDCl₃, 500 MHz): 5.59 (dt, *J* = 10, 7.5 Hz, 1H), 5.31 (dd, *J* = 10, 9 Hz, 1H), 4.61 (br s, 1H), 4.55 (br s, 1H), 3.41 (m, 1H), 3.34 (dd, *J* = 12, 4.5 Hz, 1H), 2.11 (m, 2H), 1.44 (s, 9H), 1.36 (m, 2H), 1.25 (m, 22H), 0.88 (t, *J* = 7 Hz, 3H). ¹³C NMR (CDCl₃, 75 MHz): 155.0, 135.2, 126.3, 79.9, 55.4, 48.1, 32.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 28.5, 28.1, 22.8, 14.2. ESI-MS m/z 431 [M + Na], 840 [2M + Na].

(2*R*,3*E*)-[1-(Azidomethyl)heptadec-2-enyl]carbamic acid *tert*-butyl ester ((*E*)-14b). Obtained in 77% yield (557 mg, 1.36 mmol) as a white solid from 815 mg (1.77 mmol) of (*E*)-**16b**. Mp 49–50 °C; $[\alpha]_D^{25} +8.1$ (*c* 1.05, CHCl₃); IR (film): 3341, 2920, 2851, 2100, 1683, 1525, 1463, 1392, 1364, 1293, 1246, 1169. ¹H NMR (CDCl₃, 500 MHz): 5.59 (dtd, *J* = 15.5, 6.5, 1.5 Hz, 1H), 5.38 (dd, *J* = 15.5, 6.5 Hz, 1H), 4.67 (br s, 1H), 4.27 (m, 1H), 3.42 (dd, *J* = 12, 5 Hz, 1H), 3.34 (dd, *J* = 12, 4.5 Hz, 1H), 2.03 (m, 2H), 1.45 (s, 9H), 1.36 (m, 2H), 1.23–1.32 (m, 22H), 0.88 (t, *J* = 7 Hz, 3H). ¹³C NMR (CDCl₃, 75 MHz): 155.1, 134.2, 126.8, 79.9, 55.2, 52.3, 32.4, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 29.1, 28.5, 22.8, 14.2. ESI-MS m/z 431 [M + Na], 841 [2M + Na].

(1*S*,2*S*,3*R*)-[2,3-Bis-(*tert*-butyldimethylsilyloxy)-1-(hydroxymethyl)heptadecyl]carbamic acid *tert*-butyl ester (17**).** A solution of **1b** (152 mg, 0.36 mmol) in DCM (10 mL) at 0 °C was sequentially treated at 0 °C with neat TBSOTf (418 μL, 1.82 mmol) and 2,6-lutidine (0.64 mL). The reaction mixture was stirred at 0 °C for 30 min, and then warmed to 25 °C and stirred at this temperature for 20 h. The reaction was quenched with CH₃OH (5 mL) and stirred for 10 min. The solvent was removed at reduced pressure and the residue taken up in Et₂O and washed with H₂O, aq. NaHCO₃, and brine. The organic phase was dried over MgSO₄, filtered and evaporated to give a residue, which was purified by flash chromatography to afford 178 mg (0.23 mmol, 65%) of the tris-OTBS derivative as a colourless oil. $[\alpha]_D^{25} +6.3$ (*c* 0.50, CHCl₃); IR (film): 3453, 2960, 2930, 2858, 1723, 1495, 1468, 1386, 1365, 1254, 1172, 1063, 1008, 941, 838, 778. ¹H NMR (CDCl₃, 500 MHz): 4.91 (d, *J* = 9 Hz, 1H), 3.79 (dd, *J* = 10.2, 4.2 Hz, 1H), 3.74 (m, 2H), 3.65 (dd, *J* = 10, 4.5 Hz, 1H), 3.58 (m, 1H), 1.57 (m, 1H), 1.48 (m, 1H), 1.42 (s, 9H), 1.24–1.32 (m, 24H), 0.90 (br s, 6H), 0.89 (m, 6H), 0.89 (m, 6H), 0.86 (m, 12H), 0.12 (s, 2H), 0.07 (s, 2H), 0.06 (s, 2H), 0.05 (s, 2H), 0.04 (s, 4H), 0.01 (d, *J* = 0.5 Hz, 6H). ¹³C NMR (CDCl₃, 75 MHz): 155.5, 78.9, 75.7, 75.6, 62.0, 54.3, 32.4, 32.1, 30.0, 29.9, 29.8, 29.8, 29.5, 28.6,

26.5, 26.3, 26.2, 26.0, 26.0, 25.9, 25.6, 22.9, 18.5, 18.4, 18.3, 18.3, 14.3, –2.8, –3.5, –3.7, –4.6, –5.0, –5.1, –5.4. ESI-MS m/z 783 [M + Na].

A solution of the above tris-OTBS derivative (95 mg, 0.12 mmol) in THF (2 mL) under N₂ at 0 °C was treated with HF·pyridine (0.41 mL of a ~70% solution, 15.8 mmol) in 2.5 mL of a 65 : 35 mixture of THF–pyridine. The reaction mixture was stirred for 30 min at 0 °C and allowed to warm to rt. After 1 h, the mixture was quenched by addition of sat. aq. NaHCO₃ (15 mL) and stirred for 10 min. The reaction mixture was extracted with EtOAc and the organic phases were washed with brine, filtered and evaporated to dryness. The residue was flash chromatographed to afford 57 mg (0.09 mmol, 70%) of **17** as a colourless oil. $[\alpha]_D^{25} -11.6$ (*c* 1.93, CHCl₃); IR (film): 3446, 2929, 2857, 1699, 1501, 1467, 1387, 1368, 1253, 1171, 1054, 936, 836, 776. ¹H NMR (CDCl₃, 500 MHz): 5.22 (d, *J* = 8.5 Hz, 1H), 4.10 (m, 1H), 3.87 (m, 1H), 3.76 (dt, *J* = 6.3, 3 Hz, 1H), 3.72 (dd, *J* = 7.5, 3.8 Hz, 1H), 3.62 (ddd, *J* = 8.5, 8.1, 3.3, 1H), 2.97 (br d, *J* = 11.5 Hz, 1H), 1.53 (m, 2H), 1.44 (s, 9H), 1.34 (m, 2H), 1.25–1.30 (m, 22H), 0.92 (s, 9H), 0.90 (s, 9H), 0.88 (t, *J* = 7 Hz, 3H), 0.10 (s, 3H), 0.10 (s, 6H), 0.08 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz): 155.5, 79.3, 77.6, 76.1, 63.6, 52.2, 34.2, 32.1, 29.8, 29.7, 29.7, 29.5, 28.6, 26.2, 26.1, 25.9, 22.8, 18.3, 18.3, 14.3, –3.6, –3.9, –4.4, –4.7. ESI-MS m/z 547 [M – Boc + H], 669 [M + Na].

(2*S*,3*S*,4*R*)-[1-Azidomethyl-2,3-bis-(*tert*-butyldimethylsilyloxy)heptadecyl]carbamic acid *tert*-butyl ester (18**).** Method A: From silylation of **5b**: A solution of azide **5b** (64 mg, 0.14 mmol) in DCM (2 mL) under argon was cooled to 0 °C and treated with neat TBSOTf (111 μL, 0.48 mmol). After stirring for 10 min at 0 °C, 2,6-lutidine (168 μL, 1.45 mmol) was added to the mixture. Stirring was maintained for 1 h at 0 °C and for 2 h at 25 °C. The reaction mixture was quenched with CH₃OH (1 mL) and the solvent was removed under reduced pressure. The residue was flash chromatographed (hexane–EtOAc 98 : 2) to afford 60 mg (0.10 mmol) of **18** as a colourless oil.

Method B: From alcohol **17**: Following the mesylation–azidation protocol described above for the synthesis of azides **14b** from alcohols **13b**, alcohol **17** afforded azide **18** in 45% combined yield. $[\alpha]_D^{25} +1.7$ (*c* 0.87, CHCl₃); IR (film): 2928, 2857, 2102, 1710, 1498, 1466, 1371, 1254, 1169, 835. ¹H NMR (CDCl₃, 500 MHz): 4.78 (d, *J* = 9.5 Hz, 1H), 3.80 (m, 1H), 3.67 (m, 2H), 3.58 (m, 2H), 1.57 (m, 1H), 1.47 (m, 2H), 1.44 (s, 9H), 1.37 (m, 1H), 1.22–1.32 (m, 22H), 0.91 (s, 9H), 0.90 (s, 9H), 0.88 (t, *J* = 7 Hz, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H), 0.05 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): 155.2, 79.7, 76.5, 75.5, 52.1, 51.9, 33.1, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.5, 28.5, 26.2, 26.2, 26.1, 24.9, 22.8, 18.5, 18.3, 14.3, –3.5, –3.8, –4.5, –5.1. ESI-MS m/z 572 [M – Boc + H]⁺, 694 [M + Na].

General method for *N*-Boc removal. A solution of 0.50 mmol of the starting *N*-Boc-protected amine in 5 mL of a 95 : 5 TFA–H₂O mixture was stirred for 20 min at rt. The reaction mixture was evaporated to dryness under a stream of N₂ and the residual solid was washed with aq. NaHCO₃, filtered and thoroughly washed with H₂O. The white residue was purified by flash chromatography (92 : 8 : 1 DCM–CH₃OH–NH₄OH) to afford analytically pure samples of the target amino alcohols. Compounds **1a–8a** and **13–15a** (*E* and *Z* isomers) from **1b–8b** and **13–15b** (*E* and *Z*

isomers) were obtained following this protocol (see Electronic Supplementary Information†).

General method for acylation reactions with acyl chlorides c–j. A solution of the starting amine (0.1 mmol) in 5 mL of a 1 : 1 mixture of THF and 50% aq. NaOAc was cooled to 0 °C and stirred vigorously at this temperature for 15 min. The corresponding acyl chloride (0.19 mmol) was added dropwise to the reaction mixture, which was allowed to warm to 25 °C and stirred for an additional 20 h. The mixture was extracted with EtOAc (3 × 10 mL) and the combined organic phases were washed with 1 N NaOH and brine, dried over MgSO₄, filtered and evaporated. The resulting solid was purified by flash chromatography to give the final amide. Compounds **1c–j**, **2c–8c**, (*E*) and (*Z*)–**13c**, (*E*) and (*Z*)–**14c**, **5d**, and (*Z*)–**13d** were obtained following this protocol (see Electronic Supplementary Information†).

(1'S,2'S,3'R)-N-[2,3-Dihydroxy-1-(hydroxymethyl)heptadecyl]-2-oxooctanamide (1k). A solution of 37 mg (0.23 mmol) of 2-oxooctanoic acid, 45 mg (0.23 mmol) of EDC and 32 mg (0.23 mmol) of HOBt in THF (5 mL) under Ar was stirred for 40 min. A solution of **1a** (56 mg, 0.16 mmol) in THF (5 mL) was added dropwise and the reaction mixture was stirred at rt for 24 °C. The resulting viscous liquid was evaporated to dryness and taken up with 10 mL of a 1 : 1 H₂O–DCM mixture. The organic phase was separated and washed with aq. NaHCO₃, filtered and evaporated to dryness. The residue was purified by flash chromatography to afford 16 mg (0.03 mmol, 15%) of **1k** as a white waxy solid. [α]_D²⁵ +5.4 (*c* 1.14, CHCl₃); IR (film): 3308, 2919, 2851, 1707, 1669, 1541, 1468, 1212, 1184, 1067. ¹H NMR (CDCl₃, 500 MHz): 7.66 (d, *J* = 8 Hz, 1H, NH), 4.13 (m, 1H, H2), 3.95 (dd, *J* = 11.5, 3 Hz, 1H, H1a), 3.89 (dd, *J* = 11.5, 5.5 Hz, 1H, H1b), 3.66–3.70 (m, 2H, H3, H4), 2.91 (t, *J* = 7.4 Hz, 2H, H2'), 1.69 (m, 1H), 1.60 (m, 2H), 1.49 (m, 2H), 1.23–1.36 (m, 29H), 0.88 (t, *J* = 7 Hz, 3H), 0.87 (t, *J* = 7 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz): 198.9, 160.6, 75.9, 75.4, 73.0, 73.0, 61.5, 61.2, 52.3, 52.1, 37.0, 33.2, 32.1, 31.6, 29.8, 29.8, 28.8, 29.7, 29.7, 29.5, 28.7, 25.8, 23.2, 22.8, 14.3, 14.2. ESI-MS *m/z* 458 [M + H]⁺, 938 [2M + Na]; HPLC: >95% pure (H₂O–CH₃CN 20 : 80, *t*_R = 8.96 min).

C1-Amino PHC analogues from azides by catalytic hydrogenation. A solution of the starting azide **5a–d**, **6a**, **6c**, **7a**, **7c**, **8a** or **8c** (0.06 mmol) in THF (2 mL) at rt was hydrogenated (1 atm) in the presence of Degussa Schultz® Pd/C (9 mg). After 20 h, the solvent was removed under a stream of N₂ and the resulting black waxy crude solid was dissolved in CH₃OH (10 mL), filtered through a pad of Celite®, and the clean solution was evaporated to dryness to afford the desired products as white solids. Compounds **9a–d**, **10a**, **10c**, **11a**, **11c**, **12a**, **12c** were obtained following this protocol (see Electronic Supplementary Information†).

C1-Amino PHC analogues from azides by Staudinger reaction. A solution of the starting azide (*Z*)–**14b–d** or (*E*)–**14b,c** (0.62 mmol) in 20 mL of a 9 : 1 THF–H₂O mixture was treated with PPh₃ (400 mg, 1.52 mmol) and stirred for 72 h at rt. After consumption of the starting material (TLC analysis), the solvent was removed under reduced pressure and the residue was dried under high vacuum. The resulting solid was purified by flash chromatography (96 : 4 : 1 DCM–CH₃OH–NH₄OH) and fractions containing the desired product were collected and evaporated to dryness after

identification by ninhydrin staining on the TLC. Compounds (*Z*)–**15b–d**, (*E*)–**15b,c** were obtained following this protocol (see Electronic Supplementary Information†).

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References and notes

- 1 J. Ponton, R. Ruchel, K. V. Clemons, D. C. Coleman, R. Grillot, J. Guarro, D. Aldebert, P. Ambrose-Thomas, J. Cano, A. J. Carrillo-Munoz, J. Gene, C. Pinel, D. A. Stevens and D. J. Sullivan, *Med. Mycol.*, 2000, **38**(suppl. 1), 225–236.
- 2 L. P. Samaranayake, P. L. Fidel, J. R. Naglik, S. P. Sweet, R. Teanpaisan, M. M. Coogan, E. Blignaut and P. Wanzala, *Oral Dis.*, 2002, **8**(suppl. 2), 151–160.
- 3 N. M. Ampel, *Emerging Infect. Dis.*, 1996, **2**, 109–116.
- 4 E. Castagnola, S. Cesaro, M. Giacchino, S. Livadiotti, F. Tucci, G. Zanazzo, D. Caselli, I. Caviglia, S. Parodi, R. Rondelli, P. E. Cornelli, R. Mura, N. Santoro, G. Russo, R. De Santis, S. Buffardi, C. Viscoli, R. Haupt and M. R. Rossi, *Pediatr. Infect. Dis. J.*, 2006, **25**, 634–639.
- 5 E. J. Bow, *Br. J. Haematol.*, 1998, **101**(suppl. 1), 1–4.
- 6 J. M. Brown, *Curr. Opin. Infect. Dis.*, 2004, **17**, 347–352.
- 7 K. A. Marr and R. A. Bowden, *Transpl. Infect. Dis.*, 1999, **1**, 237–246.
- 8 J. A. Fishman, *Transpl. Infect. Dis.*, 2002, **4**(suppl. 3), 3–11.
- 9 M. E. Klepser, *Pharmacotherapy*, 2006, **26**, 68S–75S.
- 10 G. Chamilos and D. P. Kontoyiannis, *Drug Resist. Updates*, 2005, **8**, 344–358.
- 11 M. E. Klepser, *Curr. Treat. Options Infect. Dis.*, 2003, **5**, 489–494.
- 12 T. S. Worgall, *Curr. Opin. Clin. Nutr. Metab. Care*, 2007, **10**, 149–155.
- 13 J. Yang, Y. Yu, S. Sun and P. J. Duerksen-Hughes, *Cell Biochem. Biophys.*, 2004, **40**, 323–350.
- 14 N. H. Georgopapadakou, *Expert Opin. Invest. Drugs*, 2000, **9**, 1787–1796.
- 15 D. Haak, K. Gable, T. Beeler and T. Dunn, *J. Biol. Chem.*, 1997, **272**, 29704–29710.
- 16 J. Cheng, T. S. Park, A. S. Fischl and X. S. Ye, *Mol. Cell. Biol.*, 2001, **21**, 6198–6209.
- 17 Y. Sugimoto, H. Sakoh and K. Yamada, *Curr. Drug Targets: Infect. Disord.*, 2004, **4**, 311–322.
- 18 A. Delgado, J. Casas, A. Llebaria, J. L. Abad and G. Fabrias, *ChemMedChem*, 2007, **2**, 580–606.
- 19 O. Shirota, K. Nakanishi and N. Berova, *Tetrahedron*, 1999, **55**, 13643–13658.
- 20 R. Imashiro, O. Sakurai, T. Yamashita and H. Horikawa, *Tetrahedron*, 1998, **54**, 10657–10670.
- 21 *E* isomers were better obtained following the recent modification of Oh *et al.* for non-stabilised ylides: J. S. Oh, B. H. Kim and Y. G. Kim, *Tetrahedron Lett.*, 2004, **45**, 3925–3928.
- 22 H. C. Kolb, M. S. Vannieuwenhze and K. B. Sharpless, *Chem. Rev.*, 1994, **94**, 2483–2547.
- 23 J. K. Cha, W. J. Christ and Y. Kishi, *Tetrahedron*, 1984, **40**, 2247–2255.
- 24 Unpublished results.
- 25 G. Appendino, A. Minassi, L. Berton, A. S. Moriello, M. G. Cascio, L. De Petrocellis and V. Di Marzo, *J. Med. Chem.*, 2006, **49**, 2333–2338.
- 26 R. C. Dickson and R. L. Lester, *Biochim. Biophys. Acta-Mol. and Cell Biology of Lipids*, 2002, **1583**, 13–25.
- 27 K. Liu, X. Zhang, C. Sumanasekera, R. L. Lester and R. C. Dickson, *Biochem. Soc. Trans.*, 2005, **33**, 1170–1173.
- 28 A. L. Goldstein and J. H. McCusker, *Genetics*, 2001, **159**, 499–513.

- 29 N. C. Chung, C. Mao, J. Heitman, Y. A. Hannun and L. M. Obeid, *J. Biol. Chem.*, 2001, **276**, 35614–35621.
- 30 J. L. Brace, R. L. Lester, R. C. Dickson and C. M. Rudin, *Genetics*, 2007, **175**, 65–76.
- 31 K. Liu, X. Zhang, R. L. Lester and R. C. Dickson, *J. Biol. Chem.*, 2005, **280**, 22679–22687.
- 32 M. S. Skrzypek, M. M. Nagiec, R. L. Lester and R. C. Dickson, *J. Biol. Chem.*, 1998, **273**, 2829–2834.
- 33 Compounds **1f** and **1g** were tested as mixture of diastereomers, since the corresponding acylating agents (see Fig. 2) were used as racemic mixtures.
- 34 Other unsaturated analogues tested in this study ((*E*)- and (*Z*)-**13c** and (*E*)- and (*Z*)-**14c**) were not active as growth inhibitors.
- 35 The corresponding *N*-octanoyl azide (**5d** and (*Z*)-**14d**) and hydroxy ((*Z*)-**13d**) analogues were also inactive as yeast growth inhibitors.
- 36 H. Sakoh, Y. Sugimoto, H. Imamura, S. Sakuraba, H. Jona, R. Bamba-Nagano, K. Yamada, T. Hashizume and H. Morishima, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 143–145.
- 37 M. Nakamura, Y. Mori, K. Okuyama, K. Tanikawa, S. Yasuda, K. Hanada and S. Kobayashi, *Org. Biomol. Chem.*, 2003, **1**, 3362–3376.
- 38 P. Serrano, J. Casas, A. Llebaria, M. Zucco, G. Emeric and A. Delgado, *J. Comb. Chem.*, 2007, 635–643.
- 39 P. A. Aeed, A. E. Sperry, C. L. Young, M. M. Nagiec and A. P. Elhammer, *Biochemistry*, 2004, **43**, 8483–8493.
- 40 W. Zhong, D. J. Murphy and N. H. Georgopapadakou, *FEBS Lett.*, 1999, **463**, 241–244.
- 41 S. M. Mandala, R. A. Thornton, M. Rosenbach, J. Milligan, M. Garcia-Calvo, H. G. Bull and M. B. Kurtz, *J. Biol. Chem.*, 1997, **272**, 32709–32714.
- 42 M. Ocejo, J. L. Vicario, D. Badia, L. Carrillo and E. Reyes, *Synlett*, 2005, 2110–2112.
- 43 A. McKillop, R. J. K. Taylor, R. J. Watson and N. Lewis, *Synthesis*, 1994, 31–33.
- 44 A. Dondoni and D. Perrone, *Synthesis*, 1997, 1512–1512.
- 45 D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, 3rd edn, Pergamon Press, Oxford, 1988.