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Sordaricin Antifungal Agents

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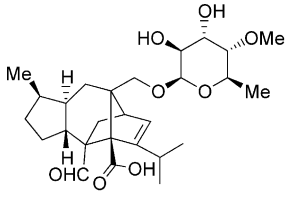
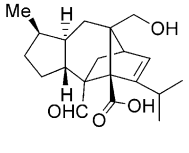
Abstract—Compounds based on sordaricin were prepared via organometallic addition onto a fully protected sordaricin aldehyde. The fungal growth inhibition profiles for these compounds were established and the results are presented here. The synthesis of homologated sordaricin as well as ether and ester derivatives is presented, and structural rearrangement products upon oxidation. These compounds were evaluated as agents to inhibit fungal growth.

The incidence of infections caused by opportunistic fungal pathogens has increased dramatically, especially among immunocompromised patient populations. As such, the search for effective antifungal agents with novel modes of action remains a high priority in anti-infective drug discovery. Protein synthesis has become an attractive target for antifungal chemotherapy as a result of discernible differences between fungal and mammalian translational pathways.



Sordarin



Sordaricin

The natural product sordarin,[1] its aglycone sordaricin, and its semi-synthetic derivatives have been discovered to selectively inhibit fungal protein synthesis by interfering with the translational complex containing co-factor Elongation Factor 2 (EF-2).[2,3] Several scientific reports[4] disclose the involvement of EF-2 in the fungal pathway. Despite the extensive EF-2 sequence homology among all eukaryotic genomes, the sordarins appear to specifically impair the fungal protein synthesis machinery by blocking translocation through binding with high affinity to the EF-2/ribosome complex.[5] Consequently, fueled by the novelty of this mode of action and the ready availability of sordarin via fermentation, the search for a potent and selective antifungal agent was undertaken.

Replacement of the sugar moiety with simpler aliphatic sidechains has been the primary focus of much research effort, and it was shown that addition of lipophilic side chains imparted products with excellent fungal growth inhibition properties.[6] We now wish to report our effort on the synthesis and biological activity of sordaricin derivatives bearing alkyl chains linked directly to the sordaricin framework, and where the hydroxymethyl moiety has been homologated. Moreover, a structural rearrangement product from attempted oxidation of the homologated alcohol is also presented.

The required starting material was obtained from sordarin in four steps (Scheme 1). Once the aldehyde **3** was obtained, the next step involved organometallic addition providing the alcohols **4** and **5** (Scheme 2). For compound **4**, the required organolithium reagents were conveniently prepared from the corresponding organobromides or iodides according to literature methods.[7] Alternatively, deprotonation of the terminal acetylene afforded the alkynyl lithium reagent which led to **5**.[8] As

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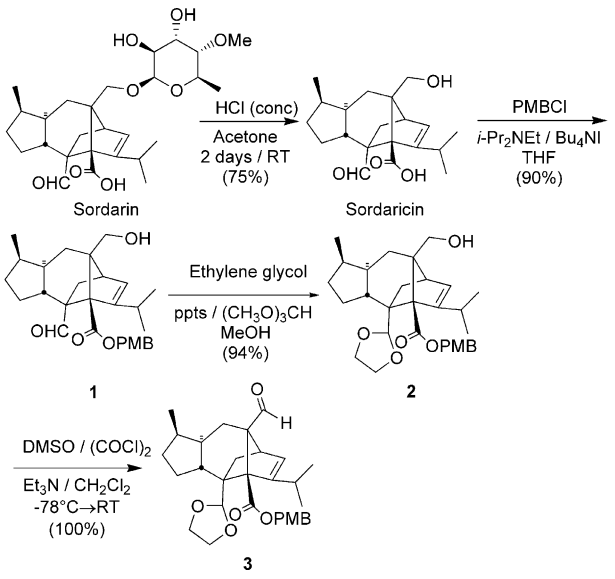


Scheme 1. Preparation of sordaricin aldehyde **3**.

can be seen in Table 1, the yields for these reactions are generally modest and the desired products isolated cleanly.

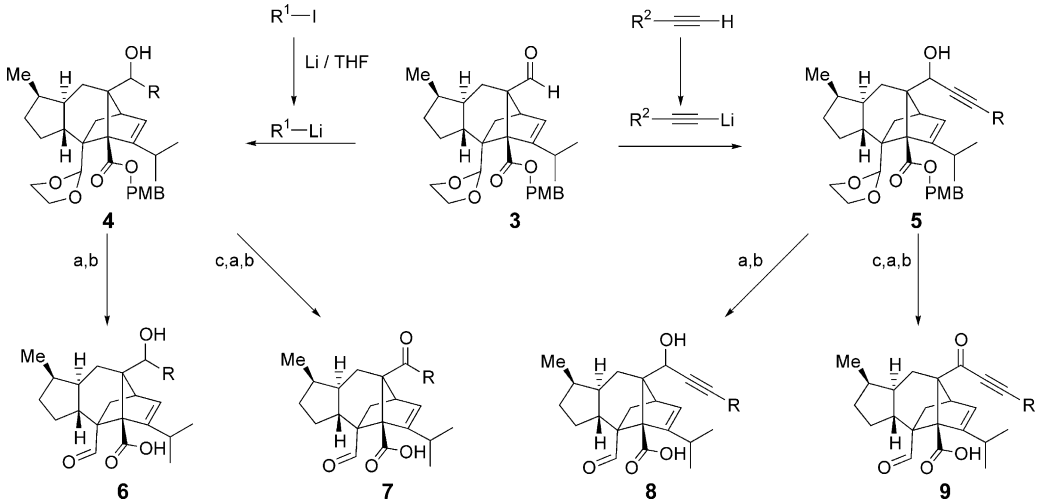
Deprotection of the alcohols **4** and **5** was accomplished by hydrolysis of the acetal (HCl/MeOH) followed by hydrogenation of the benzyl ester ($Pd(OH)_2/H_2$) in respectable yields (Table 1). Alcohols **6** and **8** were thus obtained and submitted for biological evaluation. In a parallel route, alcohols **4** and **5** were also subjected to oxidation conditions (Swern or Dess–Martin periodinane). The obtained fully protected ketones were then subjected to the afore mentioned deprotection conditions to provide the desired ketones ready for biological evaluation. In terms of yields, for the series leading to **7** the oxidation and deprotection sequence provided the compounds in good to excellent yields. However, for the acetylene analogues **9**, the yields of pure material were poor. Notwithstanding, the products were isolated and submitted.

The starting material for the preparation of the homologated analogue of sordaricin was the aldehyde **10**, prepared analogously to **3**.[9] The aldehyde was treated with the Tebbe reagent to provide the relatively unstable vinylic product **11** (Scheme 3). Hydroboration provided the desired homologated alcohol **12**. Hydrolysis of the acetal afforded **13**.

Alkylation was found to be possible only with the conditions shown in Scheme 4, that is using the alkylation agent as the solvent with silver oxide as the base. These, as opposed to typical NaH/RX conditions, prevented the undesired side product derived from lactonization. Esterification was found to be efficacious using the pyridinium iodide activating agent. After either functionalization, the ester was hydrolyzed with TBAF to provide the desired compounds ready for biological evaluation.

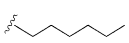
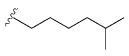
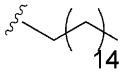
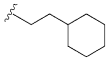
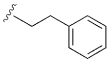
In an attempt to oxidize **12** to its corresponding aldehyde, an interesting rearrangement was observed. Treatment of **12** with the Dess–Martin periodinane did not provide the desired aldehyde but rather a novel and unexpected structurally rearranged compound (Scheme 5). Employing one equivalent of Dess–Martin reagent, the alcohol **14** was obtained as a single diastereomer while using excess reagent provided the ketone **15**, both in excellent yields.[10] As observed in the ^1H NMR spectra for these compounds, key structural changes were the disappearance of the signals for the olefinic hydrogen of the endocyclic double bond (found at 5.84 ppm as a doublet in **12**) and the isopropyl moiety (found at 3.12 ppm (hept, 1H), 1.24 ppm (d, 3H), 1.12 ppm (d, 3H) in **12**) and the appearance of two new singlets at 1.76 ppm and 1.75 ppm for **14**, and at 1.75 and 1.68 ppm for **15**. This reactivity of the double bond is precedented,[11] although the conditions for this transformation were more rigorous than those employed in this present example.

The reaction has the appearance of being an uncatalyzed intramolecular aldehyde ene reaction. It is believed that after the initial oxidation to the aldehyde **16**, the compound is perfectly juxtaposed for the ene

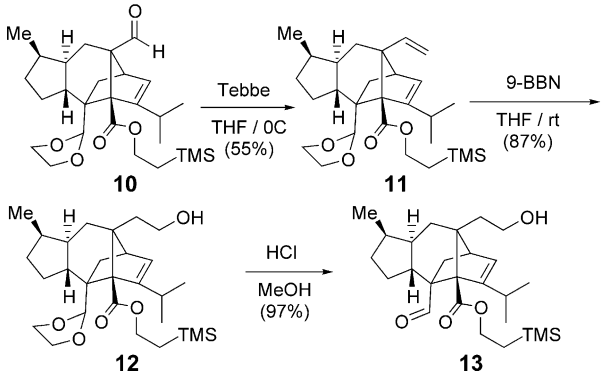


Scheme 2. (a) HCl/MeOH/rt; (b) $Pd(OH)_2/H_2$/EtOH: (c) DMSO/$(COCl)_2$/Et_3N/CH_2Cl_2/−78 °C or Dess–Martin periodinane/CH_2Cl_2/rt.

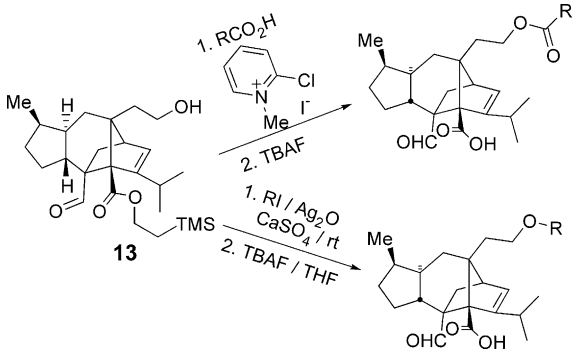
Table 1. Yields for compounds prepared

Compd	Structure	Product, yield (%)			Structure	Product, yield (%)		
		4	**6**	**7**		**5**	**8**	**9**
a		59	92	50		97	71	24
b		61	81	46		83	34	14
c		80	32	55		86	31	13
d		90	50	41		86	81	7
e		64	71	39		83	40	9
f		82	65	31		100	27	8
g		83	73	48				

reaction (Scheme 6). A rapid reaction ensued and the alcohol product **14** is thusly obtained as a single diastereomer. A type I[12] concerted reaction pathway may be envisioned for this novel transformation process.



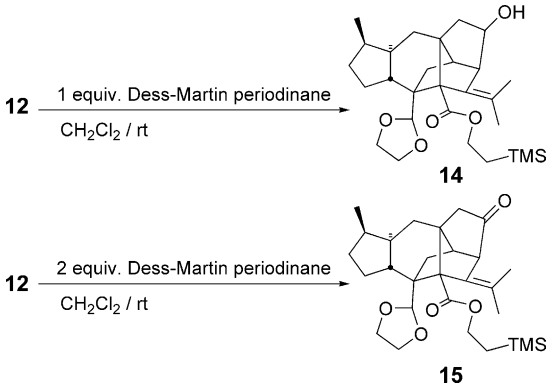
Scheme 3. Preparation of homosordaricin.



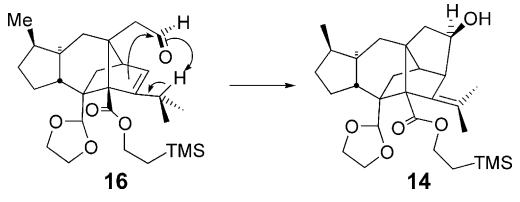
Scheme 4. Alkylation or acylation and deprotection.

With one equivalent of the oxidant only the alcohol **14**, and not the ketone **15**, is obtained and all the starting material is consumed. Consequently, it can be inferred that any subsequent oxidation of **14** to **15** is much slower than the initial formation of **14**. However, with excess oxidant, the alcohol **14** is oxidized to the ketone **15**.[13,14]

Compounds prepared were subjected to biological evaluation and the minimum inhibitory concentration (MIC_{90})[15] results tabulated (Tables 2 and 3).

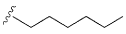
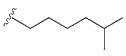
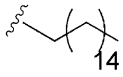
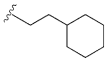
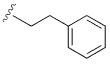


Scheme 5. Cyclization of homologated sordaricin.



Scheme 6. Proposed stereochemistry.

Table 2. In vitro activity of alcoholic (**6**,**8**) and ketonic (**7**,**9**) derivatives

Compd	R	MIC[a] (μg/mL)		CC_{50}[b] (μg/mL)	T.I.[c]
		C. albicans	*C. glabrata*		
6a		16	64	na[d]	–
7a		>128	1	2.5	<0.02
6b		4	32	2.4	0.6
7b		0.25	4	1.5	6
6c		1	32	1.2	1.2
7c		0.5	>128	2.5	5
6d		>128	>128	na	–
7d		>128	>128	na	–
6e		2	8	3.1	1.6
7e		0.5	1	1.8	3.6
6f		0.5	16	1.5	3
7f		0.5	>128	1.8	3.6
6g		>128	>128	na	–
7g		16	8	na	–
8a		128	128	na	–
9a		>128	>128	1.4	<0.01
8b		8	32	18.3	2.3
9b		<0.06	0.5	3.3	>55
8c		1	8	15.8	15.8
9c		<0.06	0.25	1.4	>23.3
8d		0.25	8	na	–
9d		>128	16	>12	0.09
8e		1	16	14.2	14.2
9e		<0.06	0.125	0.9	>15
8f		0.25	4	10.7	42.8
9f		<0.06	0.5	1.4	>23.3

[a]MIC value defined as the lowest drug concentration required to inhibit 90–100% visible growth relative to controls.
[b]CC_{50} value measured against Hep2 cells.
[c]In vitro therapeutic index (CC_{50} cytotoxicity/MIC *C. albicans*).
[d]Activation observed, IC_{50} could not be determined.

As can be gleaned from Table 2, in the alkyl series, analogues **6b**,**c**,**e**,**f** were roughly equipotent versus *Candida albicans* to their corresponding ketones (**7b**,**c**,**e**,**f**). The outliers included **6a** which was more potent over the ketone **7a** and **6g** being less potent than the ketone. For *Candida glabrata*, there does not appear to be a clear preference for the alcohols over the ketones as the MIC's varied considerably and no clear SAR can be observed. Generally for all strains tested, there seems to be a preference of medium length alkyl over shorter or longer chains (**6**,**7b**, **6**,**7c** vs **6**,**7d**, **6**,**7a**). This compares with what was observed in the literature.[6] Also, fully reduced cyclic substituents were more potent than the aromatic analogues (**6**,**7f** vs **6**,**7g**), perhaps suggesting a large hydrophobic pocket. Moreover, these compounds were shown to be toxic, exhibiting low potency values and therefore poor therapeutic indices.

The alkyne series showed clearer SAR with the ketones **8** invariably being more potent than the alcohols **9**, except for the *tert*-butyl analogue **8**,**9a** which were inactive and alcohol **8d** being more potent than the ketone **9d**. Potencies for the ketones **9b**,**c**,**e**,**f** versus *C. albicans* were excellent (<0.06 μg/mL). A potential explanation could be that the restraining effect of the alkynyl moiety orients the appended groups more favorably into the postulated large hydrophobic pocket. Also, these derivatives were more potent versus *C. glabrata* over their corresponding alcohols. In addition, these acetylene analogues generally exhibited less cytotoxicity over the alkyl derivatives. More specifically, the alcohols had higher CC_{50} values over the ketones which resulted in roughly equal therapeutic indices.

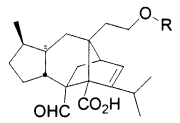
All derivatives also showed negligible activity against other fungi (*Candida parapsilosis, Cryptococcus neoformans, Aspergillus fumigatus* and *Aspergillus flavus*).

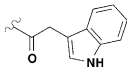
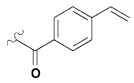
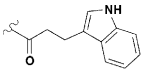
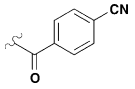
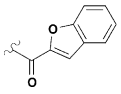
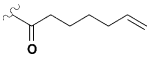
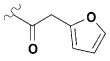
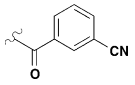
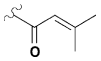
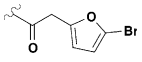
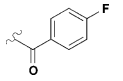
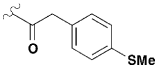
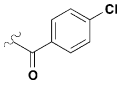
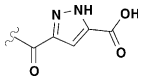
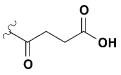
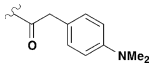
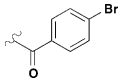
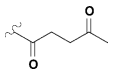
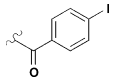
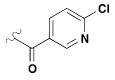
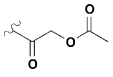
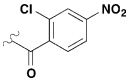
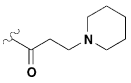
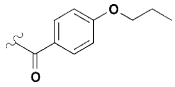
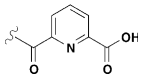
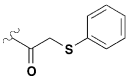
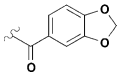
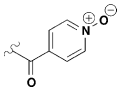
Notwithstanding the excellent activity of some of these compounds, their CYP inhibition profiles were less than satisfactory. The least inhibitory compound was **7d**, unsurprisingly a very lypophilic long chain analogue and unfortunately one of the least active antifungals (CYP3A4 IC_{50} = 19 μM). Alcohol and ketone analogues displayed similar inhibitory profiles (e.g., CYP3A4 IC_{50} **6c** = 9.3 μM, **7c** = 4.1 μM) except for acetylenic alcohol derivatives **8** which were potent CYP2D6 inhibitors over their corresponding ketones **9** (e.g., CYP2D6 IC_{50} **8f** = <0.046 μM, **9f** = 15 μM).

In the homologated sordaricin series, the parent compound (**17**) was found to be as inactive as sordaricin. However, when alkylated to give **18**, modest activity was obtained. This compares favorably well with non-homologated analogues.[16] This was, however, the sole alkylated compound prepared and therefore a thorough study into this class is still necessary. The remainder of the compounds prepared (**19**–**70**) were esters. The carboxylic acids **22**, **62**, **66** were not active. Benzoates as well as the heteroaromatic analogues for the most part displayed minimal activity. Functionality with polar moieties had a detrimental effect on potency. Only compounds bearing non-polar, lypophilic functionalities showed any promise. For example, the most potent analogues against *C. albicans* were **19**, **21** and **39**, further supporting the theory of a large hydrophobic pocket. These also proved to be the most potent against *C. glabrata* with the vast majority of the remaining compounds being inactive.

Interesting patterns appeared. For example, 4-ketopentanoate **23** was more potent than close analogues **22** and **24** bearing polar groups. Carbamates with free N–H's (**27**, **28**, and **29**) were inactive while **41** was the sole exception. Amines were also inactive (**25**, **31**, **32**, **57**, and **61**), except for **36**. Furthermore, the sulfonamide **56** was also inactive.

Table 3. MIC data for compounds prepared



Cmpd	R	MIC[a]	Compd	R	MIC[a]	Compd	R	MIC[a]	Compd	R	MIC[a]
17	H	>128/>128	**31**		na[b]/>128	**45**		4/>128	**58**		8/128
18		2/64	**32**		>128/>128	**46**		8/>128	**59**		>128/>128
19		<0.06/8	**33**		2/64	**47**		1/>128	**60**		>128/>128
20		0.5/16	**34**		2/64	**48**		4/>128	**61**		>128/>128
21		<0.06/4	**35**		0.5/>128	**49**		4/>128	**62**		>128/>128
22		<128/>128	**36**		1/>128	**50**		>128/>128	**63**		16/>128
23		8/>128	**37**		0.5/8	**51**		2/>128	**64**		4/>128
24		>128/>128	**38**		0.125/4	**52**		128/>128	**65**		4/>128
25		>128/>128	**39**		<0.06/8	**53**		128/>128	**66**		>128/>128
26		0.5/32	**40**		32/>128	**54**		2/>128	**67**		>128/>128
27		64/>128	**41**		4/>128	**55**		2/>128	**68**		4/>128
28		32/>128	**42**		>128/>128	**56**		128/>128	**69**		>128/>128
29		128/>128	**43**		16/128	**57**		>128/>128	**70**		>128/>128
30		8/>128	**44**		8/>128						

[a]MIC value defined as the lowest drug concentration required to inhibit 90–100% visible growth relative to controls. Data tabulated as *C. albicans*/*C. glabrata*.
[b]Compound was active, MIC unable to be determined.

Changing the *para*-halogens on benzoates (**48**–**51**) showed little effect on potency as little change (except for bromine **50**) of potency was observed.

Another trend was that the most favorable ring size was a six-membered, unsubstituted fully reduced cycloalkane (**39**>**38**>**37**) while the benzoate **40** exhibited a marked decrease in potency.

The deprotected analogues of pentacyclic derivatives **14** and **15**, when tested for fungal growth inhibition, were all inactive.

In summary, several compounds have been prepared that proved to be potent antifungal agents against *Candida* strains. Acetylenic ketones were the most potent, displayed modest therapeutic indices but, like all the derivatives tested, modest CYP inhibition profiles. Moreover, this study demonstrates the clear necessity of non-polar, medium sized functionality in this portion of the molecule. Potencies decrease as polar functionality is introduced as is the case with increasing size.

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References and Notes

1. Hauser, D.; Sigg, H. P. *Helv. Chim. Acta* **1971**, *54*, 1178.
2. (a) Dominguez, J. M.; Martin, J. J. *Antimicrob. Agents Chemother*. **1998**, *42*, 2274. (b) Capa, L.; Mendoza, A.; Lavandra, J. L.; Gomez de Las Heras, F.; Garcia-Bustos, J. F. *Antimicrob. Agents Chemother*. **1998**, *42*, 2279. (c) Justice, M. C.; Hsu, M. J.; Tse, B.; Ku, T.; Balkovec, J.; Schmatz, D.; Nielsen, J. *J. Biol. Chem*. **1998**, *273*, 3148.
3. (a) Odds, F. C. *Exp. Opin. Ther. Pat*. **2001**, *11*, 283. (b) Ziegelbauer, K.; Spaltmann, F. *Drugs Future* **2000**, *25*, 63. (c) Gargolla-Viola, D. *Curr. Opin. Anti-infective Invest. Drugs* **1999**, *1*, 297. (d) Herreros, E.; Almela, M. J.; Lozano, S.; Gomez de las Heras, F.; Gargallo-Viola, D. *Antimicrob. Agents Chemother*. **2001**, *45*, 3132 and references cited therein.
4. Rambelli, F.; Brigotti, M.; Zamboni, M.; Denaro, M.; Montanaro, L.; Sperti, S. *Biochem. J*. **1989**, *259*, 307.
5. Dominguez, J. M.; Gomez-Lorenzo, M. G.; Martin, J. J. *J. Biol. Chem*. **1999**, *274*, 22423.
6. Balkovec, J. M.; Tse, B. Sordarin Derivatives, WO 9815178, 1998.
7. Gilman, H.; Beel, J. A.; Brannen, C. G.; Bullock, M. W.; Dunn, G. E.; Miller, L. S. *J. Am. Chem. Soc*. **1949**, *71*, 1499. Bailey, W. F.; Punzalan, E. R. *J. Org. Chem*. **1990**, *55*, 5404.
8. Freshly prepared alkynyl lithium reagents were prepared by treatment of the appropriate alkyne with *s*-BuLi in THF or Et_2O at $-78\,^{\circ}C$ and were used directly.
9. Serrano-Wu, M. H.; St Laurent, D. R.; Mazzucco, C. E.; Stickle, T. M.; Barrett, J. F.; Vyas, D. M.; Balasubramanian, B. N. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 943.
10. Experimental conditions: For alcohol **14**: To a solution of **12** (920 mg, 1.88 mmol) in CH_2Cl_2 (40 mL) was added Dess–Martin periodinane (797 mg, 1.88 mmol) and the contents stirred at room temperature overnight. $NaHCO_3$ (10 mL, aq satd) and $Na_2S_2O_3$ (10 mL, 1 M) were added and stirred vigorously for 30 min. The reaction was diluted with CH_2Cl_2, the organic phase separated and dried ($MgSO_4$), filtered, concentrated and the residue purified by flash chromatography (4:1 hexane/EtOAc) to afford the product (770 mg, 84%) as a colourless oil: ^1H NMR (C_6D_6, 400 MHz) δ 4.91 (s, 1H), 4.33–4.17 (m, 3H), 3.71 (m, 1H), 3.66 (ddd, 1H, $J=12.1$, 12.1, 5.8 Hz), 3.46 (m, 1H), 3.40–3.29 (m, 2H), 2.79 (dd, 1H, $J=2.5$, 1.5 Hz), 2.13 (dd, 1H, $J=14.2$, 4.3 Hz), 2.07–1.88 (m, 4H), 1.76 (s, 3H), 1.75 (s, 3H), 1.53–1.40 (m, 2H), 1.33–1.22 (m, 3H), 1.13–1.03 (m, 2H), 0.98 (d, 3H, $J=6.8$ Hz), −0.15 (s, 9H). For ketone **15**: To a solution of **12** (266 mg, 0.542 mmol) in CH_2Cl_2 (10 mL) was added Dess–Martin periodinane (518 mg, 1.221 mmol) and the contents stirred at room temperature for 2 h. $NaHCO_3$ (4 mL, aq satd) and $Na_2S_2O_3$ (4 mL, 1M) were added and stirred vigorously for 30 min. The reaction was diluted with CH_2Cl_2, the organic phase separated and dried ($MgSO_4$), filtered, concentrated and the residue purified by flash chromatography (4:1 hexane/EtOAc) to afford the product (242 mg, 92%) as a white foam: ^1H NMR (C_6D_6, 400 MHz) δ 5.00 (s, 1H), 4.24 (m, 1H), 4.11 (m, 1H), 3.66 (m, 1H), 3.44 (m, 1H), 3.38–3.27 (m, 3H), 3.15 (d, 1H, $J=18.4$ Hz), 3.09 (s, 1H), 2.06–1.61 (m, 11H), 1.75 (s, 3H), 1.68 (s, 3H), 1.46–1.21 (m, 7H), 0.91 (d, 3H, $J=7.1$ Hz), −0.14 (s, 9H).
11. Arigoni, D.; Vasella, A.; Sharpless, K. B.; Jensen, H. P. *J. Amer. Chem. Soc*. **1973**, *95*, 7917.
12. Snider, B. B. In *Comprehensive Organic Synthesis*; Trost, B. M. Ed., Pergamon Press: New York, 1991; Vol. 2, p 527.
13. However, upon oxidation of **12** under Swern conditions, the ketone **15** is the only product isolated. Under the proposed Swern oxidation mechanism, the initially formed activated alcohol becomes oxidized upon addition of the base and therefore **14** is formed, even at −78° C. Interestingly, the rate of the following step appears much quicker than with the Dess–Martin reagent as the alcohol **14** is transformed to ketone **15** even at this low temperature before the reagent is destroyed. This dichotomy in the rates for the second step may be due to the greater steric encumbrance of the Dess–Martin periodinane over that of the activated DMSO for the Swern oxidation.
14. Alternatively, the reaction may be envisioned to proceed via initial acetate (from the Dess–Martin reagent) abstraction of the seemingly non-acidic allylic proton.
15. MICs were determined according to NCCLS standards against fungal strains obtained from the American Type Culture Collection.
16. Tse, B.; Balkovec, J. M.; Blazey, C. M.; Hsu, M. J.; Nielsen, J.; Schmatz, D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2269.