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Relative and Absolute Stereochemistry of the Didemnaketals, Metabolites of a Palauan Ascidian, *Didemnum* sp.

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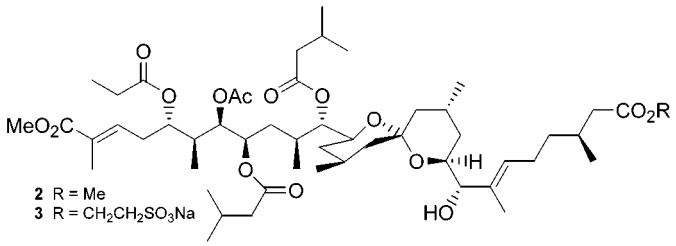
ABSTRACT



The absolute stereochemistry of the heptaprenoids didemnaketals B (2) and C (3), isolated from a Palauan ascidian, was determined using a combination of degradation and derivatization experiments, chiral shift methods, and comparison of fragments to known compounds.

In 1991, Potts et al. reported that an undescribed Palauan ascidian of the genus *Didemnum* contained two polyisoprenoids, didemnaketals A (**1**) and B (**2**), both of which inhibited the activity of HIV-1 protease.[1] Although the didemnaketals were of limited interest as protease inhibitors due to the instability of esters under physiological conditions, we made a fresh collection of the organism in 1993. The new sample did not contain ketals **1** and **2**. Instead, the major secondary metabolite was the isoethonic ester, didemnaketal C (**3**), which was hydrolyzed in MeOH to obtain didemnaketal B (**2**).[2] It was therefore assumed that didemnaketals A (**1**) and B (**2**) had been produced from didemnaketal C (**3**) during prolonged storage in MeOH by mechanisms involving oxidation and methanolysis, respectively. Unfortunately, didemnaketal C (**3**) did not inhibit HIV-1 in a peptidolysis assay.[3]

We then turned our attention to determination of the stereochemistry of didemnaketals B (**2**) and C (**3**), each of which contain 13 chiral centers. The relative stereochemistry of the bicyclic ketal region from C-12 to C-20 had been established by interpretation of ^{1}H NMR data,[1] but the relative stereochemistry of the eight remaining centers and the absolute stereochemistry of the molecules remained to be determined. In this paper, we report the complete stereochemistry of didemnaketals B (**2**) and C (**3**) as 5*S*,6*S*,7*R*,8*R*,10*S*,11*S*,12*S*,14*S*,16*S*,18*R*,20*S*,21*S*,26*S*.

Isolation and Methanolysis of Didemnaketal C (3). A fresh sample of *Didemnum* sp., which was collected in shallow water (∼1 m) at Topkukau in Palau, was extracted and chromatographed, as before,[2] to obtain a sample of

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(2) Pika, J.; Faulkner, D. *Nat. Prod. Lett.* **1995**, *7*, 291−296.
(3) Hyland, L. J.; Dayton, B. D.; Moore, M. L.; Shu, A. Y. L.; Heys, J. R.; Meek, T. D. *Anal. Biochem.* **1990**, *188*, 408−415.

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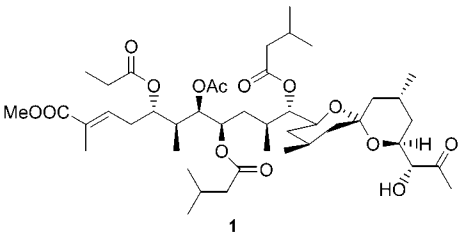

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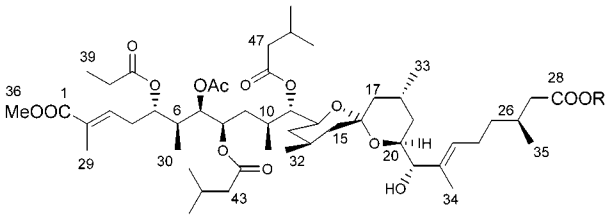
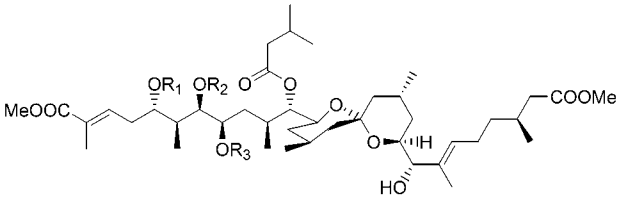


2 R = Me[52]
3 R = -$CH_2CH_2SO_3^-$ Na^+[53]



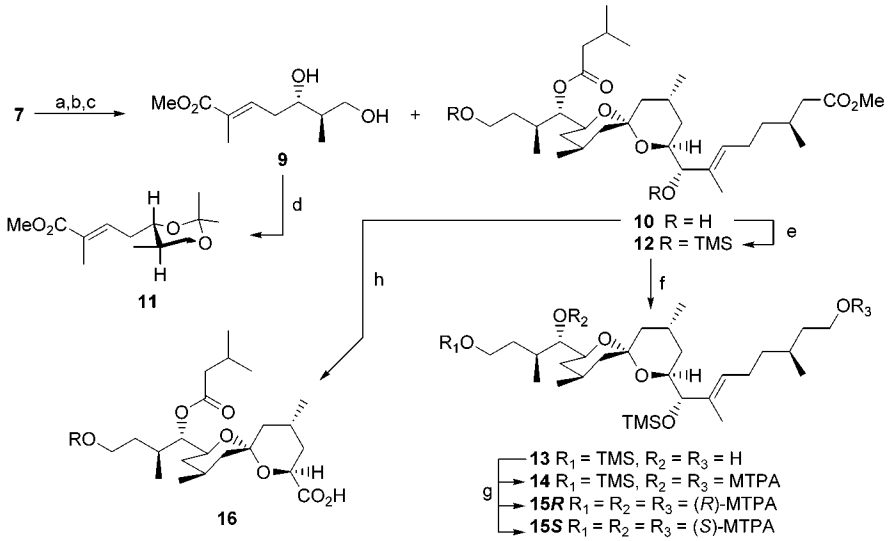
4 R_1 = $COCH_2CHMe$; R_2 = Ac; R_3= COEt
5 R_1 = R_2 = H; R_3= $COCH_2CHMe_2$
6 R_1 = $COCH_2CHMe_2$; R_2 = R_3= H
7 R_1 = R_2 = R_3 = H

didemnaketal C (**3**) of >80% purity, contaminated primarily with polar lipids. Aliquots of the semipurified material were then treated with sodium methoxide in MeOH at room temperature for 3−20 h, depending on the desired product mixture, to obtain didemnaketal B (**2**), iso-didemnaketal B (**4**), two isomeric triols **5** and **6**, and the tetraol **7**, all of which were purified by HPLC. The locations of the ester groups were determined by analysis of the chemical shifts of the −C*H*OR− protons, and in the case of isodidemnaketal B (**4**), the identity of each ester group was determined by analysis of HMBC data. The triols **5** and **6** and tetraol **7** were characterized by analysis of spectroscopic data and then used in reactions designed to determine the relative or absolute stereochemistry at each center.

Isodidemnaketal B (**4**) was an isomer of didemnaketal B (**2**) that had the molecular formula $C_{52}H_{86}O_{15}$ (*m*/*z* 973.5864 $[M + Na]^+$). The ^{1}H NMR spectrum of iso-didemnaketal B (**4**) was very similar to that of didemnaketal B, but careful analysis of the COSY experiment allowed assignment of all signals from H-3 to H-12. The ^{13}C NMR spectrum was assigned by interpretation of the HMQC spectrum. HMBC correlations between the isovalerate carbonyl signal at δ 172.8 and both the H-5 proton (δ 4.75) and the isovalerate methine signal at δ 2.05 and between the propionate carbonyl signal at δ 173.3 and both H-8 (δ 5.18) and the propionate methyl signal at δ 1.06 revealed that the isovalerate and propionate esters had switched positions in isodidemnaketal B (**4**) with respect to didemnaketal B (**2**).

The triols **5** and **6** both had the same molecular formula $C_{47}H_{80}O_{13}$, which required the loss of the acetate and propionate esters from didemnaketal B (**2**) and iso-didemnaketal B (**4**), respectively. Analysis of the COSY spectra of **5** and **6** allowed the determination of the position of the esters. The tetraol **7** had the molecular formula $C_{42}H_{72}O_{12}$ (*m*/*z* 791.4921 $[M + Na]^+$), which corresponds to the loss of the acetate, propionate, and isovalerate ester functions at C-5, C-7, and C-8, respectively. The position of the remaining isovalerate ester group at C-11 was determined by analysis of the ^{1}H NMR chemical shift data and ultimately confirmed by degradation of **7** to the acid **16**, the structure of which was determined by X-ray analysis.

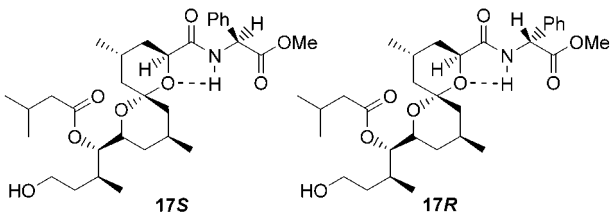
Scheme 1. Degradation and Derivatization of Didemnaketal B[a]



[a] Reaction Conditions: (a) $NaIO_4$, H_2O/CH_3CN; (b) $NaHSO_3$; (c) $NaBH_4$, MeOH; (d) 2,2-dimethoxypropane, *p*-TSA/DCM; (e) TMSCl, NEt_3, DCM; (f) $LiAlH_4$, THF; (g) DMAP, DCC, (*R*)- or (*S*)-MTPA, DCM; (h) OsO_4, $NaIO_4$, H_2O/CH_3CN.

Oxidative Degradation of Tetraol 7. Oxidative cleavage of the 7,8-diol of tetraol **7** with sodium periodate in 1:1 acetonitrile−water, followed by the quenching of excess reagent with sodium bisulfite and reduction of the resulting aldehyde groups with sodium borohydride, gave the diol ester **9** and diol diester **10**, which were easily separated by HPLC (Scheme 1). The diol ester **9** was treated with 2,2-dimethoxypropane and a trace of *p*-toluenesulfonic acid in DCM to obtain the acetonide **11** (Scheme 1). A coupling constant of $J_{5,6} = 12$ Hz indicated that H-5 and H-6 were trans-diaxial and that the relative stereochemistry at C-5 and C-6 in the didemnaketals was 5*S**,6*S**.

A subsample of the diol diester **10** was treated with trimethylsilyl chloride and triethylamine in DCM at room temperature to obtain the bis-TMS ether **12**, which was reduced with lithium aluminum hydride in THF to obtain the diol **13** (Scheme 1). An aliquot of diol **13** was esterified with (*R*)- or (*S*)-methoxytrifluoromethyl-phenylacetic acid (MTPA) in an attempt to obtain the (*R*)- and (*S*)-MTPA esters (**14**).[4] However, during esterification, the terminal TMS group was hydrolyzed, resulting in the formation of the tri-MTPA derivatives **15*R*** and **15*S*** (Scheme 1). Although some caution is necessary in analyzing the proton shift differences when two MTPA esters are relatively close together, comparison of the ^{1}H NMR chemical shifts of protons adjacent to the C-11 MTPA ester group indicated that the absolute stereochemistry at C-11 of the didemnaketals was *S*.



Further oxidation of a portion of the diol diester **10** with osmium tetroxide and sodium periodate resulted in an initial oxidation of the C-22 olefinic bond followed by oxidative cleavage of the C-21/C-22 bond to form the acid **16** (Scheme 1). A single crystal of acid **16** was obtained from isopropyl ether/hexane solution, and its structure was determined by X-ray crystallography. The relative stereochemistry resulting from the X-ray crystallographic study, coupled with the known absolute stereochemistry at C-11, required that the didemnaketals possess the 10*S*,11*S*,12*S*,14*S*,16*S*,18*R*,20*S* absolute stereochemistry. The absolute stereochemistry of acid **16** could also be determined by derivatization of the acid group with (*R*)- and (*S*)-phenylglycine methyl esters (PGME) to obtain the amides **17*S*** and **17*R***, once it had been realized that the resulting amides adopt an anomalous conformation.[5] Molecular modeling using PC Model suggested that the amide-NH proton is strongly hydrogen bonded to the oxygen of the adjacent pyran ring. A positive $\Delta\delta$ value (+4 and +56 ppb) for the protons at C-12 therefore indicated the absolute stereochemistry at C-12 (corresponding to C-20 in didemnaketal B) to be *S*.

7,8-Acetonide of Tetraol 7. The tetraol **7** was treated with dimethoxypropane in dichloroethane containing a catalytic amount of *p*-toluenesulfonic acid to obtain a single five-membered acetonide **18**, in which the 2,3-olefinic bond had partially isomerized to give a 1:3 mixture of *E* and *Z* geometrical isomers. While annoying, this result did not interfere with the determination of the absolute stereochemistry at C-5. The two remaining hydroxyl groups at C-5 and C-21 were sufficiently far apart to allow simultaneous determination of the absolute stereochemistry at both centers using the modified Mosher method. Comparison of the ^{1}H NMR chemical shift differences between the 5,21-bis-MTPA esters **19*R*** and **19*S***, which were prepared from the acetonide **18** under the standard conditions reported above, revealed that the absolute configurations at C-5 and C-21 were both *S*. Having previously determined the relative stereochemistry of the acetonide **11**, the absolute stereochemistry at C-6 must be *S*. Furthermore, analysis of the ROESY correlations about the 7,8-acetonide ring of either MTPA ester **19*R*** or **19*S*** (see Figure 1) provided strong evidence for the 7*R*,8*R* stereochemistry.

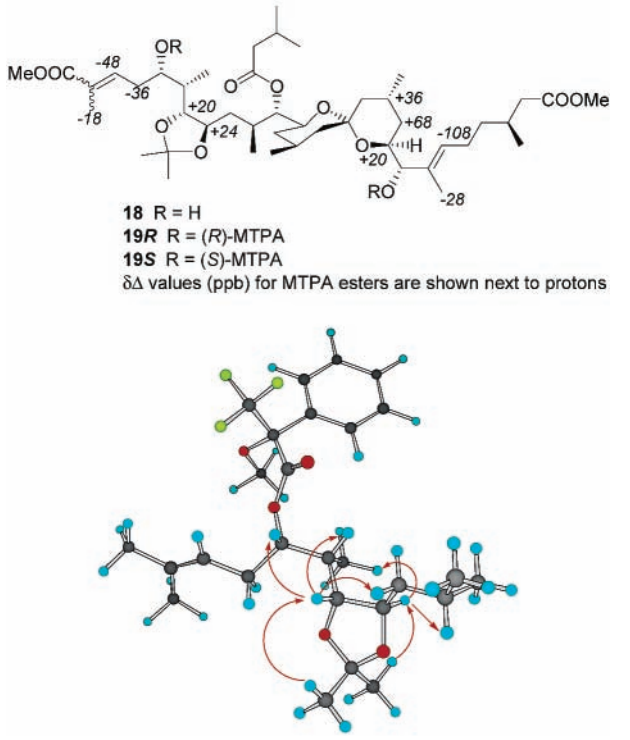


Figure 1. ROESY correlations of acetonide methyl protons.

Moshers Esters Prepared from Triol 6. Treatment of the triol **6** with (*R*)- or (*S*)-MTPA using the standard conditions gave mixtures of esters. The mixtures were separated to obtain the 8,21-bis-MTPA esters **21*R*** and **21*S***, which were major constituents of the mixtures. Analysis of

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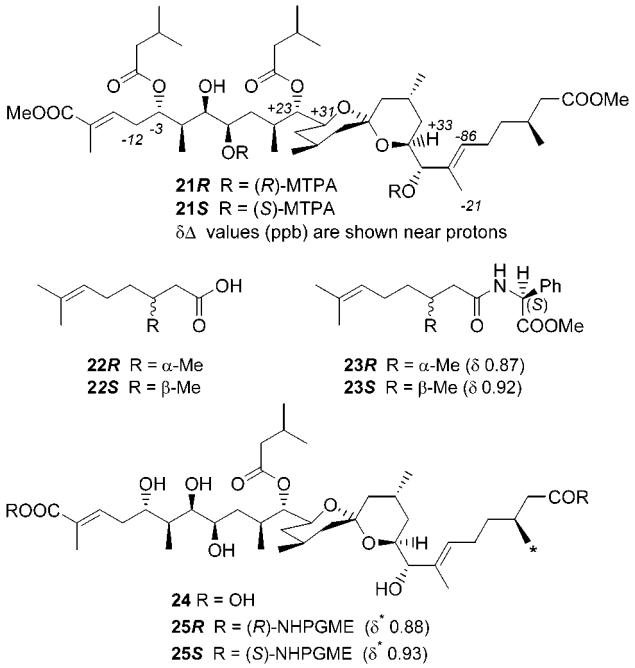
the chemical shift differences for relevant protons in MTPA esters **21*R*** and **21*S*** confirmed the 8*R* absolute stereochemistry.

Absolute Stereochemistry at C-26. Since there has been some concern expressed regarding the reliability of using the chiral amide method to determine the absolute stereochemistry at the carbon β to a carboxylic acid, we decided to use model compounds to confirm the signs of the chemical shift differences predicted by employing molecular modeling. The model compounds employed were racemic citronellic acid (**22*R,S***) and (3*R*)-citronellic acid (**22*R***), both of which are commercially available. Reaction of acids **22*R,S*** and **22*R*** with (*S*)-PGME, PyBOP, 1-hydroxy-benzotriazole (HOBT), and *N*-methyl-morpholine in DMF solution gave a mixture of enantiomers **23*R,S*** from the racemic acid **22*R,S*** and a pure amide **23*R*** from (3*R*)-citronellic acid (**22*R***). In the NMR spectrum of amide **23*R***, the 9-methyl signal was at δ 0.83 (d, 3 H, $J = 7$ Hz), whereas the mixture of enantiomers contained two methyl signals at δ 0.87 (d, 3 H, $J = 7$ Hz) and 0.92 (d, 3 H, $J = 7$ Hz). Mild hydrolysis of tetraol **7** in 1 N sodium hydroxide solution at room temperature for 3 h gave, after chromatography, the acid **24**, which was in turn converted into the (*R*)- and (*S*)-PGME amides **25*R*** and **25*S***. The signals for the secondary methyl groups β to the amide were located by analysis of the HMQC spectra and found to be at δ 0.93 (d, 3 H, $J = 7$ Hz) in **25*S*** and 0.88 (d, 3 H, $J = 7$ Hz) in **25*R***, thus defining the stereochemistry at C-26 as *S*. The complete stereochemistry of didemnaketals B (**2**) and C (**3**) is 5*S*, 6*S*,7*R*,8*R*,10*S*,11*S*,12*S*,14*S*,16*S*,18*R*,20*S*,-21*S*,26*S*.

The determination of the absolute configuration of the didemnaketals is an important step toward understanding the structure−activity relationship of the compounds as potent protease inhibitors. Didemnaketal A and B were found to be active against HIV-1 protease with IC_{50} values of 2 and 10 μM, respectively.[1] Although the lability of the esters under physiological conditions precluded further development as a clinical drug, several synthetic studies of the didemnaketals have been conducted to determine the structural components necessary for activity.[6] Fan et al. synthesized eight pentaester analogues of C-1−C-11 containing three chiral centers and found that the 5*S*,7*R*,8*S* analogue was



the most potent with a K_i value of 2.1 μM, comparable to that of didemnaketal A.[6] Interestingly, our assignment of the didemnaketals in that region 5*S*,7*R*,8*R* is opposite at the C-8 center and would correspond to the analogue with a K_i of 10 μM, which is exactly the activity reported for didemnaketal B. Knowledge of the complete stereochemistry of the didemnaketals will allow further development of stable analogues that may provide a unique and potent mode of HIV-1 protease inhibition.

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Supporting Information Available: ^{1}H NMR and mass spectral data for selected compounds and the crystal structure for compound (**16**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Fan, X. D.; Flenke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1998**, *120*, 8893−8894.