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SYNTHETIC STUDIES OF DIDEMNINS. I. REVISION OF THE STEREOCHEMISTRY OF THE HYDROXYISOVALERYLPROPIONYL (HIP) UNIT.

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Abstract: Synthetic and spectral evidence for the absolute configuration of the asymmetric centers of the hydroxyisovalerylpropionyl (HIP) unit present in the depsipeptides didemnins is discussed.

Didemnins are a new class of depsipeptides isolated from a Caribbean tunicate of the family Didemnidae, a species of the genus Tridemnum.[1] These cyclic peptides are reported to be highly active antiviral and antitumor agents.[2] They are potent inhibitors of the L1210 leukemia cells in vitro, and are also active in vivo against P388 leukemia and B16 melanoma.[3] Didemnin B was more potent that didemnin A and was found to be approximately twenty times more cytotoxic than didemnin A in vitro.[4] Didemnin B also exhibits potent immunosupressive activity both in vitro and in vivo.[5] Didemnins A,B, and C (Figure 1) all contain the same parent macrocycle.

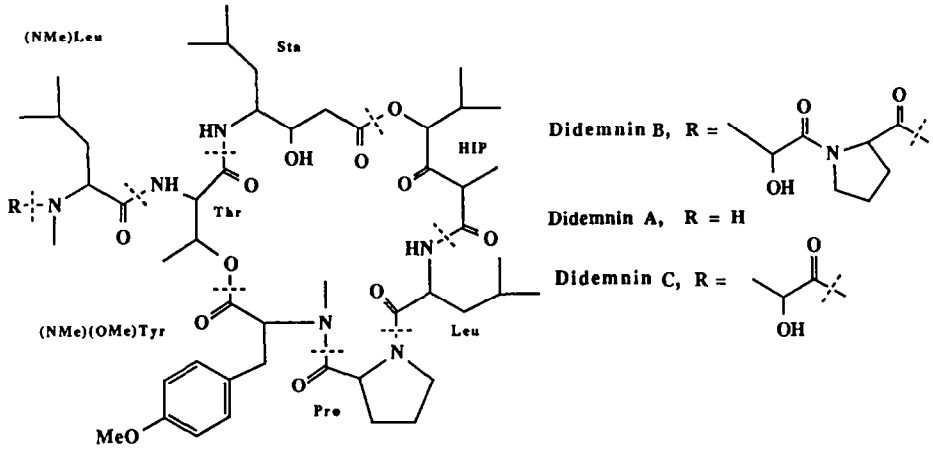


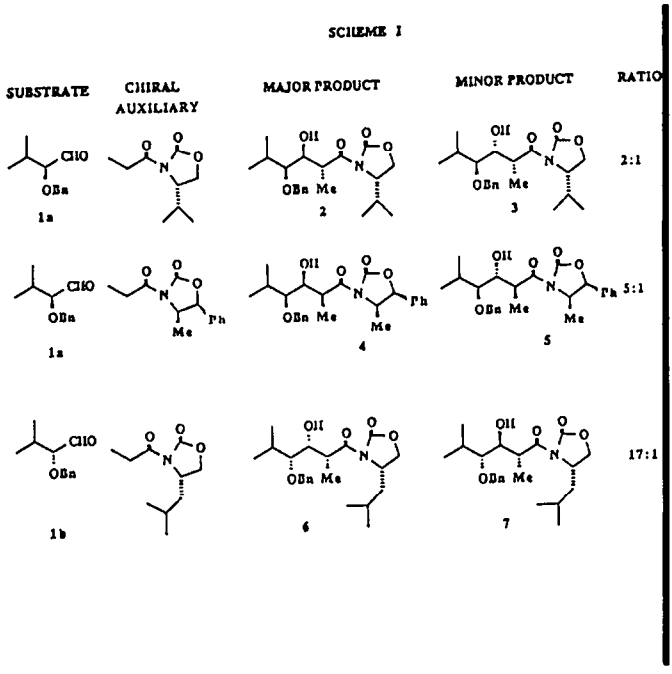
Figure 1. Didemnins A, B, and C.

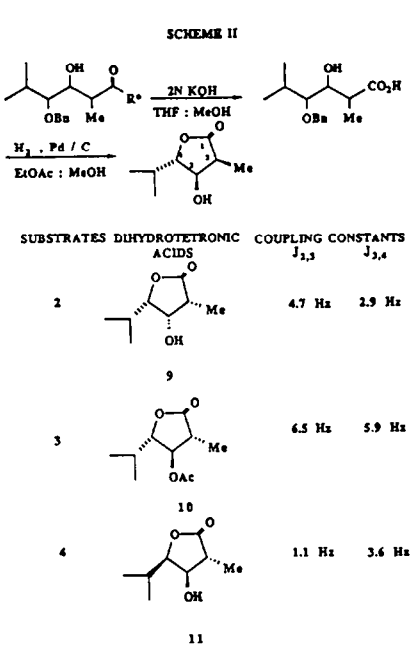
The ring system incorporates a new stereoisomer (3S,4R) of the amino acid statine, and a hydroxyisovalerylpropionyl unit abbreviated as HIP.

The structures of the didemnins were determined by spectroscopic and degradative studies.[1,6] The configurational assignment of statine was based on the value of its optical rotation and on circular dichroism studies. Coinjection of the isolated product with an authentic sample of the same statine diastereomer supported the spectral assignments.[6] The absolute stereochemistry of the HIP unit, however, was not determined unambiguously but was tentatively assigned as the 2R, 4R diastereomer.[6] Our synthetic studies in this area indicate that this stereochemistry should be revised to that of the 2S,4S diastereomer.

Degradation of didemnin A into its constituent units gave the HIP unit as the cyclized 2-methyl-4-isopropyltetronic acid. Also, treatment of didemnin A with sodium borohydride gave one stereoisomer, degradation of which yielded the cyclo-HIP-ol unit as 2R*, 3R*, 4R* isomer.[6] The assignment of the absolute configuration as 2R, 4R was made by comparison of the optical rotation of the 2-methyl-4-isopropyltetronic acid with the known 4-methyltetronic acid.[6] Hudson's lactone rule[7] was also used to support the configurational assignments.

With the reported data in hand, we decided to synthesize both the 2-methyl-4-isopropyltetronic acid and its corresponding dihydro derivative. We prepared several dihydrotetronic acids using Evans aldol condensation[8a-c] on 2-benzyloxy-3-methylbutanal (1), followed by hydrolysis and cyclization. Both the S and R isomer of 2-benzyloxy-3-methylbutanal (1a and 1b) were prepared according to a literature procedure.[9] The results of the Evans aldol condensations are shown in Scheme I. In all cases only two products were formed in significant amounts. The



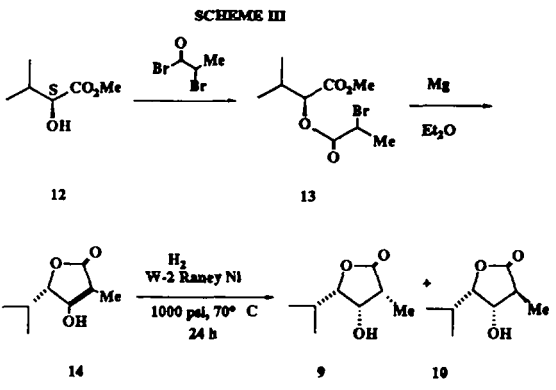


highest selectivity was obtained when the N-propionyloxazolidinone derived from L-leucine was used with the R isomer of the aldehyde (1b). Assignment of the stereocenters of the aldol products was based on the determined configurations of the corresponding dihydrotetronic acid derivatives. The aldol condensation of 1b with the N-propionyloxazolidinone derived from L-leucine gave the expected syn product (6) as the major diastereomer. In the case of 1a, reaction with the N-propionyloxazolidinone derived from L-valine gave the anti diastereomer (2) as the major product with the expected syn derivative (3) as the minor component.

Conversion of the aldol products to the corresponding dihydrotetronic acids was accomplished by treatment with aqueous potassium hydroxide in tetrahydrofuran, followed by debenzylation using Pd/C under a hydrogen atmosphere (Scheme II). The dihydrotetronic acid 9 was assigned the configuration 2R, 3S, 4S based on its coupling constants and on chemical evidence. Reduction of 2,4-dimethyltetronic acid using Raney nickel and high pressure was shown by Kelly and co-workers to give predominantly one diastereomer.[10] Attack by hydrogen occurred from the face opposite the 4-methyl group. Following the same protocol and starting with methyl (S)-2-hydroxy-3-methylbutanoate (12), compound 9 was obtained as the major diastereomer (94:6), confirming the previous structural assignment (Scheme III). Configurational assignment of compound 10 was based on the ^{1}H NMR of the acetate derivative as the spectrum of the compound with the free hydroxyl group exhibited overlapping signals for the H3 and H4 protons. These signals could not be resolved by decoupling. The acetate derivative, however, had coupling constants $J_{2,3}$ = 6.5Hz and $J_{3,4}$ = 5.9Hz, that agreed well with the values calculated by Font and co-workers for similar systems.[11]

Finally, compound 11 had a ^{1}H NMR comparable to that determined by Gloer.[6] Single crystal X-ray analysis of 11 (Figure 2) confirmed the assigned relative configuration 2R*, 3R*, 4R*.

SCHEME III



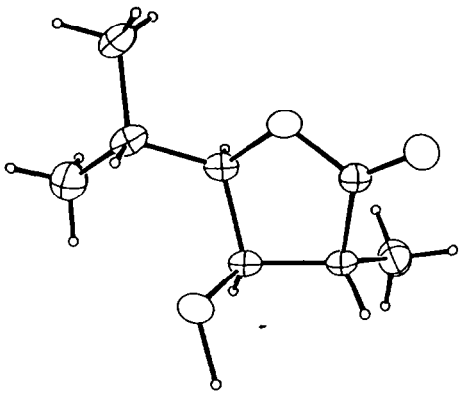


Figure 2. ORTEP Drawing of Compound 11.

Since this product was obtained from the (R)-2-benzyloxy-3-methylbutanal, the absolute stereochemistry of 11 must be 2R, 3R, 4R. The optical rotation of 11 was opposite in sign to the rotation reported by Gloer.[6] Oxidation of 11 using Swern conditions[12] gave (R)-2-methyl-4-isopropyltetronic acid. This compound also had an optical rotation opposite in sign to the reported one.[6] The (S)-2-methyl-4-isopropyltetronic acid (14) prepared as shown in Scheme III had an optical rotation of $[\alpha]_D^{26}$ = -61.6° agreeing with the literature value of $[\alpha]_D^{26}$ = -67.9°. Based on these results, the chiral center of the 2-methyl-4-isopropyltetronic acid obtained from the didemnins should be S and the absolute stereochemistry of the HIP unit found in the didemnins should be revised to 2S, 4S.

EXPERIMENTAL

General. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WM 250 (250 MHz) Fourier transform spectrometer. Chemical shifts are in parts per million (δ) relative to tetramethylsilane. Coupling constants (J values) are in Hertz (Hz). Multiplicities are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Infrared spectra (IR) were run on a Perkin-Elmer Model 281A or 281B spectrometers. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F-254 plates (0.2 mm). Visualization was effected with ultraviolet light and phosphomolybdic acid reagent (7% w/v) in 95% ethanol. Chromatography was performed on Merck silica gel (230-400 mesh) under a slight positive pressure. Optical rotations were recorded on a Perkin-Elmer Model 241 polarimeter, at the sodium D line, and ambient temperatures. High resolution mass spectra (HRMS) were obtained on a Hitachi-Perkin Elmer RMH-2 high resolution, double focusing, electron-impact spectrometer or a vacuum generator's V.G. 707H spectrometer interfaced with a Kratos DS-50-S data system. All solvents used were reagent grade. Tetrahydrofuran and diethyl ether were distilled from sodium and benzophenone; dichloromethane was distilled from calcium hydride.

Representative Procedure for the Aldol Condensations

Reaction of (S)-2-benzyloxy-3-methylbutanal (1a) with (S)-3-(1-oxopropyl)-4-(1-methylethyl)-2-oxazolidinone. (S)-3-(1-Oxopropyl)-4-(1-methylethyl)-2-oxazolidinone (0.5223 g; 3.015 mmole) was dissolved in dry methylene chloride (14 mL). To this solution at 0°C was added di-n-butylboron triflate (3.16 mL of 1M solution in CH_2Cl_2, 3.16 mmole) and diisopropylethylamine (0.61 mL; 3.44 mmole). The solution was stirred for 30 min at 0°C and then cooled to -78°C. To this freshly prepared and distilled (S)-2-benzyloxy-3-methylbutanal was added (0.5463 g; 2.872 mmole). The mixture was stirred for 30 min at -78°C, then at room temperature for 1.5 h. The boron aldolate complex was quenched with 15 mL of phosphate buffer (pH 7), and the reaction mixture treated with 30 mL of methanol and then oxidized with 15 mL of 30% H_2O_2. After stirring for 1 h, the solution was concentrated in vacuo to half of the total volume. It was then extracted with ether. The ether layer was dried (Na_2SO_4) and concentrated. Purification of the resulting oil by flash column chromatography using 15% ethyl acetate:petroleum ether gave 519.2 mg of 2 and 282.7 mg of 3, 70% total yield.

Major isomer (2) : (4S)-3-[2R,3R,4S)-4-benzyloxy-3-hydroxy-2,5-dimethyl-1-oxohexyl]-4-(1-methylethyl)-2-oxazolidinone. ^{1}H NMR 7.25-7.46 (5H,m, Ar), 4.69 (2H, AB pattern, $ArCH_2O$-), 4.45-4.49 (1H,m, NCH-), 4.19-4.36 (3H,m,-OCH_2CH and -C(O)CHMe), 3.77 (1H, t, J=10Hz,

CHOH) 3.20 (1H, d, J=7.2Hz, BnOCH-) 2.90 (1H, d, J=10.7Hz, OH, D_2O exchangeable), 2.41-2.46 (1H, m, Me_2CHCHN-), 2.02-2.10 (1H, m, Me_2CH) 0.88-1.07 (15H, m, Me and Me_2C); IR ($CHCl_3$): 3580, 1750, 1705, 1475, 1390, 1310, 1200, 1080, 695 cm^{-1}; $[\alpha]_D^{26}$ + 6.25 (c 5.26, $CHCl_3$). HRMS (CI), M^++1 calcd. for $C_{21}H_{32}O_5$, 378.2280, found 378.2241.

Minor isomer (3) : (4S)-3-[(2R,3R,4S)-4-benzyloxy-3-hydroxy-2,5-dimethyl-1-oxohexyl-4-(1-methylethyl)-2-oxazolidinone. ^1H NMR 7.27-7.43 (5H, m, Ar), 4.61 (2H, AB, $ArCH_2O$-), 4.38-4.44 (1H, m, NCH-), 4.18-4.31 (2H, m, OCH_2CH-), 4.03-4.08 (2H, m, $HOCHCHMe_2$), 3.30 (1H, dd, J=3.0Hz, 7.9Hz, BnOCH-), 3.10 (1H, d, J=2.6Hz, OH, D_2O exchangeable), 2.30-2.37 (1H, m, Me_2CH-), 2.10-2.16 (1H, m, Me_2CH), 1.24 (3H, d, J = 7.2Hz, CH_3), 1.04 (3H, dd, J = 7.0Hz, 20.8Hz, MeCHMe), 0.89 (3H, dd, J = 7.0Hz, 12.8Hz, MeCHMe); IR ($CHCl_3$): 3570, 1795, 1700, 1500, 1465, 1400, 1310, 1240, 1070, 1000 cm^{-1}; $[\alpha]_D^{26}$ +33.4 (c = 9.76, $CHCl_3$). HRMS (CI) M^++1 calcd. for $C_{21}H_{32}NO_5$, 378.2280, found 378.2327.

Reaction of (S)-2-benzyloxy-3-methylbutanal (1a) with (4S,5R)-3-(1-oxopropyl)-4-methyl-5-phenyl-2-oxazolidinone. The reaction conditions were the same as that described above. (S)-2-Benzyloxy-3-methylbutanal (257 mg) yielded 4 (302 mg) and 5 (87.3 mg), 68% total yield, which were separated by flash column chromatography using methylene chloride:hexane: ether in a ratio of 50:50:7.

Major isomer (4) : (4S,5R)-3-[(2S,3S,4S)-4-benzyloxy-3-hydroxy-2,5-dimethyl-1-oxohexyl]-4-methyl-5-phenyl-2-oxazolidinone. ^1H NMR 7.25-7.45 (10H, m, Ar), 5.68 (1H, d, J = 7.4Hz, OCHAr), 4.77-4.82 (1H, m, -NCH-), 4.60 (1H, AB, $ArCH_2O$), 4.00-4.05 (2H, m, $HOCHCHC(O)N$-), 3.10 (1H, dd, J = 2.2Hz, 5.2Hz, BnOCH-), 1.49 (1H, d, J = 6.8Hz, -OH, D_2O exchangeable), 2.00-2.06 (1H, m, Me_2CH), 1.34 (3H, d, J = 6.4Hz, $MeCH(O)N$), 1.02 (6H, dd, J = 2.4Hz, 6.8Hz, Me_2C), 0.85 (3H, d, J = 6.6Hz, -MeCHN-); IR ($CHCl_3$): 3560, 3120, 1790, 1710, 1465, 1380, 1360, 1200, 1130, 1080, 960, 700 cm^{-1}. $[\alpha]_D^{26}$ +52.9 (c 3.57, $CHCl_3$). HRMS (CI) M+1 calcd. for $C_{25}H_{32}NO_5$, 426.2280, found 426.2556.

Minor isomer (5) : (4S,5R)-3-[(2R,3S,4S)-4-benzyloxy-3-hydroxy-2,5-dimethyl-1-oxohexyl]-4-methyl-4-phenyl-2-oxazolidinone. ^1H NMR 7.20-7.43 (10H, m, Ar), 5.60 (1H, d, J = 7.2Hz, ArCHO-), 4.64-4.69 (1H, m, MeCHN-), 4.54 (2H, AB, ArCHO-) 4.26-4.30 (1H, m, -NC(O)CHC-), 3.77-3.90 (2H, m, -CHOH, D_2O exchangeable), 3.28-3.33 (1H, m, BnOCH-), 2.06-2.17 (1H, m, Me_2CH), 1.39 (3H, d, J = 7.2, $MeCHC(O)N$-), 1.00 (6H, dd, J = 6.5Hz, 11.2Hz (Me_2C-), 0.59 (3H, d, J = 6.6Hz, MeCHN-); IR ($CHCl_3$): 3540, 3120, 1795, 1685, 1465, 1395, 1380, 1355, 1200, 1130, 1080, 1000, 725 cm^{-1}. $[\alpha]_D^{26}$ +23.8 (c 1.75, $CHCl_3$). HRMS (CI) M+1 calcd. for $C_{25}H_{32}NO_5$, 426.2280, found 426.2323.

Reaction of (R)-2-benzyloxy-3-methylbutanal (1b) with (S)-3-(1-oxopropyl-4-(2-methylpropyl)-2-oxazolidinone. Reaction conditions and the reagents proportions were the same as those described for the representative procedure. (R)-2-Benzyloxy-3-methylbutanal (728.7 mg) yielded 1.2544 g of 6 and 74.4 mg of 7 (89% overall). The crude oil was separated by elution with 10% ethyl acetate:petroleum ether, followed by elution with 15% ethyl acetate:petroleum ether.

Major isomer (6) : (4S)-3-[(2R,3R,4R)-4-benzyloxy-3-hydroxy-2,5-dimethyl-1-oxohexyl]-4-(2-methylpropyl)-2-oxazolidinone. ^1H NMR 7.26-7.36 (5H, m, Ar), 4.61 (2H, AB, $ArCH_2O$-), 4.45-4.52 (1H, m, -CHN-), 4.35-4.41 (1H, m, $CH_2CHC(O)N$-), 4.08 (1H, dd, J = 3.2Hz, 8.5Hz, -CHOH), 3.94-3.98 (2H, m, $CH_2OC(O)$-), 3.10 (1H, dd, J = 3.2Hz, 4.5Hz, Bn-O-CH), 2.45-2.65 (1H, bs, OH), 2.00-2.08 (1H, m, Me_2CHCHO-), 1.31-1.72 (3H, m, Me_2CHCH_2), 1.26 (3H, d, J = 6.5Hz, -CHMe), 0.95-1.05 (12H, m, 2 Me_2CH-); IR ($CHCl_3$): 3560, 1790, 1710, 1465, 1400, 1250, 1100, 1080, 1030, 980 cm^{-1}. $[\alpha]_D^{26}$ +22.1 (c 1.82, $CHCl_3$). HRMS (CI) M+1 calcd. for $C_{22}H_{34}NO_5$, 392.2437, found 392.2349.

General Procedure for Hydrolysis of Aldol Adducts.

The samples were dissolved in a solution of 2 mL:1 mL:1 mL (MeOH:THF: 2N KOH) per 0.5mmol of starting material. The mixture was stirred at 0°C for 1.5 h. After this time the reaction was concentrated to 1/4 of its volume. The reaction mixture was extracted with 3 x 5 mL CH_2Cl_2. The extracts were combined and treated with 2 x 10 mL 50% NaCl solution. The aqueous layers were combined and adjusted to pH 1 with 3N HCl and then extracted with 3 x 10 mL EtOAc. The ethyl acetate layer was dried (Na_2SO_4) filtered and concentrated to yield the corresponding acids in yields ranging from 80-85%.

(2R,3S,4S)-4-Benzyloxy-2,6-dimethyl-3-hydroxyhexanoic acid obtained from (2). White solid recrystallized from ether:petroleum ether, mp 56-58°C; ^1H NMR 7.28-7.35 (5H, m, Ar), 4.63 (2H, AB, $ArCH_2O$-), 3.75 (1H, dd, J = 2.2Hz, 7.6Hz, -CHOH), 3.22 (1H, dd, J = 2.2Hz, 6.2Hz, -CHOBn) 2.60-2.65 (1H, m, MeCHCOOH), 2.04-2.12 (1H, m, Me_2CH-), 1.15 (3H, d, J = 7.0Hz, -CHMe), 1.01 (6H, d, J = 6.8Hz, Me_2CH-); IR ($CHCl_3$): 3580, 3400-2600, 1715, 1500, 1470, 1460, 1410, 1245, 1070, 1030, 700 cm^{-1}. $[\alpha]_D^{26}$ -15.9° (c 2.77, MeOH). HRMS (CI) M + 1 calcd. for $C_{15}H_{22}O_4$, 267.1596, found 267.1615

(2R,3R,4S)-4-Benzyloxy-2,6-dimethyl-3-hydroxyhexanoic acid obtained from (3). White solid recrystallized from ether:petroleum ether, mp 108-109°C; ^1H NMR 7.27-7.37 (5H, m,Ar), 4.60

(2H, AB, $ArCH_2$-O-), 4.09 (1H, dd, J = 3.3Hz, 7.8Hz, -CHOH), 3.28 (1H, dd, J = 3.3Hz, 7.8Hz, -CHOBn), 2.85 (1H, dq, J = 3.3Hz, 7.2Hz, -CHMe), 2.06-2.13 (1H, m, Me_2CH), 1.06 (3H, d, J = 7.0Hz, MeCHMe), 1.01 (3H, d, J = 7.0Hz, MeCHMe); IR (KBr): 3600 - 2400, 1680, 1460, 1415, 1370, 1275, 1130, 1090, 1075, 1035, 980, 910, 850, 735, 700, 640 cm^{-1}. $[\alpha]_D^{26}$ +11.3° (c 0.425, MeOH) HRMS (CI) M + 1 calcd. for $C_{15}H_{22}O_4$, 267.1596, found 267.1634.

(2R,3R,4R)-4-Benzyloxy-2,6-dimethyl-3-hydroxyhexanoic acid obtained from (6). ^1H NMR 7.28-7.36 (5H, m, Ar), 4.64 (2H, AB, $ArCH_2O$-), 3.88 (1H, dd, J = 2.9Hz, 7.2Hz, -CHOH), 3.19 (1H, dd, J = 2.9Hz, 5.8Hz, -CHOBn), 2.61-2.67 (1H, m, -CHMe), 2.00-2.04 (1H, m, Me_2CH-), 1.28 (3H, d, J = 7.1Hz, -CHMe), 1.01 (6H, d, J = 6.8Hz, Me_2CH); IR ($CHCl_3$): 3560, 3400-2500, 1720, 1510, 1475, 1410, 1300, 1260, 1170, 990, 930, 890, 870, 700, 660 cm^{-1}. $[\alpha]_D^{26}$ -31.0 (c 2.745, $CHCl_3$). HRMS (CI) M + 1 calcd. for $C_{15}H_{22}O_4$, 267.1596, found 267.1577.

General Procedure for the Synthesis of Dihydrotetronic Acids.

Each 1mmol of acid was dissolved in 4 mL of a mixture of 2:1 methanol: ethyl acetate. Activated 10% Pd/C (20% by weight) was added. The reaction mixture was shaken in an atmosphere of hydrogen on a Parr apparatus for 48 h. The mixture was then filtered through Celite and concentrated. The crude product was placed on a flash column and eluted with 20% ethyl acetate:petroleum ether followed by 30% ethyl acetate:petroleum ether. The yields of dihydrotetronic acids ranged from 65-75%.

(2R,3S,4S)-2,3-Dihydro-4-isopropyl-2-methyltetronic acid (9). White solid, recrystallized from ether:petroleum ether, mp 109-111°C, ^1H NMR 4.38 (1H, dd, J = 2.9Hz, 4.7Hz, -CHOH), 3.83 (1H, dd, J = 2.9Hz, 10.3Hz, Me_2CHCHO-), 2.72 (1H, dq, J = 4.7Hz, 7.2Hz, -CHMe), 2.10-2.19 (1H, m, Me_2CH-), 1.93 (1H, brs, OH), 1.27 (3H, d, J = 7.2Hz, -CHMe), 1.13 (3H, d, J = 6.6Hz, MeCHMe), 0.99 (3H, d, J = 6.6Hz, MeCHMe); IR ($CHCl_3$): 3650, 3480, 1780, 1485, 1465, 1400, 1380, 1180, 1150, 1130, 1020, 1013, 1005, 985, 970, 915, 900, 840, 620 cm^{-1}. $[\alpha]_D^{26}$ -47.0° (c 1.29, MeOH). HRMS (CI) M+1 calcd. for $C_8H_{14}O_3$, 159.1021, found 159.1037

(2R,3R,4S)-2,3-Dihydro-4-isopropyl-2-methyltetronic acid. White solid, recrystallized from ether:petroleum ether, mp 63-65°C; ^1H NMR 3.72-3.94 (2H, m, -CHOH and Me_2CHCHO, 2.60-2.66 (1H, m, CHMe), 1.90-2.05 (2H, m, -OH, Me_2CH), 1.32 (3H, d, J = 9.1Hz, -CHMe), 1.06 (3H, d, J = 5.4Hz, MeCHMe), 1.04 (3H, d, J = 5.1Hz, MeCHMe); IR ($CHCl_3$): 3480, 1785, 1480, 1470, 1405, 1350, 1240, 1180, 1135, 1080, 1020, 1000, 820, 640 cm^{-1}. $[\alpha]_D^{26}$ -45.7 (c 1.13, $CHCl_3$). HRMS (CI) M + 1 calcd. for $C_8H_{14}O_3$, 159.1021, found 159.1052

(2R,3R,4R) 2,3-Dihydro-4-isopropyl-2-methyltetronic acid (11). White solid, recrystallized from ether:petroleum ether, mp 120-121°C; ^1H NMR 4.17 (1H, dd, J = 1.1 Hz, 3.6Hz, -CHOH), 4.02 (1H, dd, J = 3.6Hz, 9.7Hz, Me_2CHCHO-), 2.65 (1H, dq, J = 1.1Hz, 7.8Hz, -CHMe), 2.04-2.25 (1H, m, Me_2CH-), 1.75-1.94 (1H, br s, -OH), 1.29 (3H, d, J = 7.7Hz, CHMe), 1.12 (3H, d, J = 6.6Hz, MeCHMe), 0.99 (3H, d, J = 6.6Hz, MeCHMe; IR ($CHCl_3$): 3620, 3480, 1780, 1470, 1395, 1340, 1280, 1235, 1185, 1005, 965, 920, 895, 810 cm^{-1}. $[\alpha]_D^{26}$ +89.7° (c 0.75, MeOH). HRMS (CI) M + 1 calcd. for $C_8H_{14}O_3$, 159.1021, found 159.1020.

(2R,3R,4S)-3-Acetoxy-4-isopropyl-2-methyl-2(3H)-furanone (10). To (2R,3R,4S)-2,3-dihydro-4-isopropyl-2-methyltetronic acid (30 mg, 0.19 mmole) in 1 mL CH_2Cl_2 was added triethylamine (0.03 mL, 0.22 mmole), acetic anhydride (0.02 mL, 0.21 mmole) and dimethylaminopyridine (5 mg, 0.04 mmole). The reaction was stirred at room temperature for 1 h, after which time the reaction was diluted with ether and transferred to a separatory funnel. The organic layer was extracted with 5% HCl, 5% $NaHCO_3$ and saturated NaCl. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo.[3] The resulting acetate derivative was obtained as a colorless oil[4] (34 mg, 90% yield; ^1H NMR 5.05 (1H, dd, J = 5.9Hz, 6.5Hz, -CHOAc), 4.08 (1H, dd, J = 5.9Hz, 6.5Hz, Me_2CHCHO-), 2.67-2.73 1H, m, -CHMe), 2.11 (3H,s,OC(O)Me), 1.89-1.97 1H, m, Me_2CH-), 1.37 (3H, d, J = 7.4 Hz, -CHMe), 1.01 (3H, d, J = 4.6Hz, MeCHMe), 0.99 (3H, d, J = 4.6Hz, MeCHMe); irradiation at 2.7ppm, 5.05 (d, J = 5.9Hz); IR ($CHCl_3$) 1780, 1740, 1480, 1385, 1280, 1180, 1130, 1030, 1000, 920 cm^{-1}.

Methyl 3-methyl-2S-[2-bromo-1-oxopropyloxy]-butanoate (13). To methyl 2S-hydroxy-3-methyl-butanoate (12), (3.35 g, 25.4 mmol) was added 2-bromopropionyl bromide (5.48g, 25.4mmole) under an argon atmosphere. The reaction mixture was stirred for 4 h at room temperature. After this time the mixture was distilled at 0.2 mmHg and the fraction distilling at 93-95°C was collected. The resulting colorless oil (4.43 g, 65% yield) was a diastereomeric mixture. ^1H NMR 4.91-4.98 (1H, m, -CHO-), 4.42-4.58 (1H, m, -CHBr) 3.78 (3H, s,-OMe), 2.23-2.40 (1H, m, Me_2CH), 1.83-1.92 (3H, m, CH_2CHBr), 0.98-1.10 (6H, m, Me_2CH); IR ($CHCl_3$): 1740, 1470, 1445, 1380, 1340, 1280, 1260, 1160, 1130, 1120, 1060, 1020, 980, 940, 900 630 cm^{-1}. HRMS (CI) calcd. for $C_9H_{15}O_4Br$, 267.0232, found 267.0267.

(S)-2-Methyl-4-isopropyltetronic acid (14). To a mixture of magnesium turnings (600 mg, 24.7 mmol) in 30 mL of ether, under argon, was added 12 (2.4 g, 8.98 mmol) in 20 mL of ether over a 2 h period. The mixture was stirred overnight. The solution was decanted into a separatory funnel and the flask rinsed with ether. The organic layer was extracted with 50mL of 1N NaOH. The combined aqueous layers were extracted with 2 x 25 mL $CHCl_3$. The $CHCl_3$ extracts were

combined and treated with 2 x 5 mL NaOH. The combined aqueous layers were adjusted to pH 1 using 6N HCl, then saturated with NaCl. The aqueous layer was then extracted 3 times with 25 mL $CHCl_3$. The combined organic layers were dried over Na_2SO_4, and concentrated yielding a crude oil. This oil could be recrystallized from ether:petroleum ether to afford 587mg (42% yield) of a white solid.

This compound was also prepared in 80% yield from the (S)-2,3-dihydrotetronic acids previously described via Swern oxidation using trifluoroacetic anhydride; mp 117-119°C. ^1H NMR 4.67 (1H, d, J = 2.9Hz, Me_2CHCHO-), 2.23-2.29 (1H, m, Me_2CH-), 1.75 (3H, s, -C=CMe), 1.10 (3H, d, J = 7.0Hz, MeCHMe), 0.81 (3H, d, J = 6.8Hz, MeCHMe); $[\alpha]_D^{26}$ -61.6° (c = 1.10, CH_2Cl_2); lit[6] $[\alpha]_D^{25}$ -67.9° (CH_2Cl_2). IR ($CHCl_3$) 3400-2600, 1730, 1660, 1470, 1410, 1380, 1360, 1310, 1180, 1030, 1010, 960 cm^{-1}. HRMS (CI) M + 1 calcd. for $C_8H_{12}O_3$, 157.0864, found 157.0857.

(2R,3S,4S)-2,3-Dihydro-4-isopropyl-2-methyltetronic acid (9). (S)-2-Methyl-4-isopropyltetronic acid (192 mg, 1.23 mmol) was placed in a Parr high pressure bomb and treated with 25 mL H_2O, and 0.5mL W-2 Raney Nickel (50% slurry in H_2O, pH 10). Hydrogen was added until 1000 psi was reached. The reaction vessel was heated to 70°C (pressure rose to 1150 psi) and stirred for 40 h. After this time, the mixture was filtered through Celite. The reaction vessel was washed with MeOH. The filtrate was concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2, dried (Na_2SO_4), filtered and concentrated. The product (9), 175.9 mg, 90.5% yield, had an ^1H NMR spectrum comparable to that of 9 prepared by the aldol condensation route and was contaminated with 6% of the 3-S-isomer of 11.

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REFERENCES

1. Rinehart, Jr., K.L.; Gloer, J.B.; Cook, Jr., J.C. J. Am. Chem. Soc. 1981, *103,* 1857.

2. (a) Rinehart, Jr., K.L.; Gloer, J.B.; Hughes, Jr., R.G.; Renis, H.E.; McGovren, P.J.; Swynenberg, E.G.; Stringellow, D.A.; Kuentzel, S.L.; Li, H.L. Science 1981, *212,* 933. (b) Jiang, T.L.; Liu, R.H.; Salmon, S.E. Cancer Chemother. Pharmacol. 1983, *11,* 1. (c) Canonico, P.G.; Pannier, W.L.; Huggins, J.W.; Rinehart, K.L. Antimicrob. Agents Chemother. 1982, *22,* 696. (d) Maldonado, E.; Lavergne, J.A.; Kraiselburd, E. P.R. Heath Sci. J., 1982, *1,* 22; C.A. 1982, *99:* 20563f. (e) Weed, S.D.; Stringfellow, D.A. Antiviral Res. 1983, *3,* 269. (f) Rinehart, Jr., K.L. U. S. Appl. 186932, 12 Sept. 1980; U.S. Appl. 217768, 18 Dec 1980; C.A., 1982, *97:* 133565n.

3. Rinehart, Jr., K.L.; Gloer, J.B.; Wilson, G.R.; Hughes, Hr., R.G.; Li, L.H.; Renis, H.E.; McGovren, J.P. Fed. Proc. Fed. Am. Soc. Exp. Biol. 1983, *42,* 87; C.A., 1983, *99:* 186j.

4. Crampton, S.L.; Adams, E.G.; Kuentzel, S.L.; Li, L.H.; Badiner, G.; Bhuyan, B.K. Cancer Research 1984, *44,* 1796.

5. Montgomery, D.W.; Zukowski, C.F. Transplantation 1985, *40,* 49.

6. Gloer, J.B., Ph.D. thesis, University of Illinois at Urbana-Champaign, 1983.

7. King, J.F. In "Elucidation of Structure by Physical and Chemical Methods"; Bentley, K.W. Ed.; Wiley: New York, 1963, Part 1, p396.

8. (a) Evans, David A. Aldrichimica Acta 1982, *15,* 23. (b) Evans, David, A.; Battroli, J.; Shih, T.L. J. Am. Chem. Soc. 1981, *103,* 2127. (c) Mukaiyama, T.; Imoue, T. Chem. Lett. 1976, 559.

9. Kock, P.; Nakatani, Y.; Luu, B.; Ourisson, G. Bull. Soc. Chim. Fr. 1983, 189.

10. Kelly, T.; Chandrakumar, N.S.; Cutting, D.; Goehring, R.; Weibel, R. Tetrahedron Lett. 1985, *26,* 2173. (b) Personal Communication from Professor Ross Kelly.

11. Jaime, C.; Ortuno, R.M.; Font, J. J. Org. Chem. 1986, 0000. Koch, P.; Nakatani, Y.; Luu, B.; Ourisson, G. Bull. Soc. Chim. Fr. 1983, 189.

12. Mancuso, A.J.; Swern, D. Synthesis 1981, 165.