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Organoaluminium Induced Ring-Opening of Epoxypyranosides. IV.[1] Synthesis and Structure of γ- Hydroxy-Isoleucine Stereoisomers and Their Corresponding Lactones.

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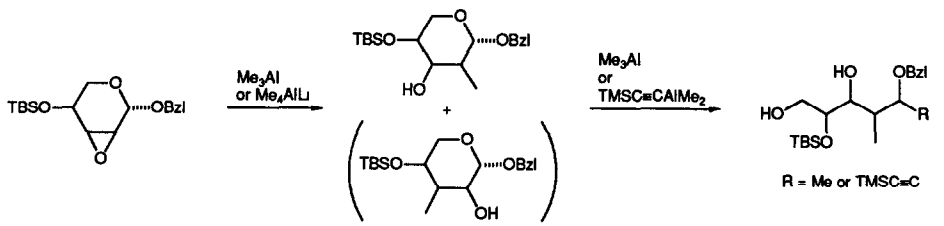
Abstract: Two γ- hydroxy-isoleucine stereoisomers 8 (2R, 3R, 4R), and 14 (2S, 3R, 4R) as well as their corresponding γ- lactones 9 and 15 were synthesized using a tandem, Me_3Al induced opening of the epoxide and pyranoside rings of benzyl 2,3-anhydro-4-O-(tert-butyldimethylsilyl)-β-L-ribopyranoside (1). The structure of the lactone hydrochloride 9 was confirmed by an X-ray crystal structure determination.

In an earlier work we found that benzyl 2-deoxy-2-*C*-methyl-4-*O*-(*tert*-butyldimethylsilyl)-pento-pyranosides underwent pyranoside ring-opening to give open-chain protected tetrols on treatment with trimethylaluminium or other aluminium reagents such as TMS-ethynyldimethylalane (Scheme 1).[2] In these reactions the carbohydrate carbon chain was elongated at C-1 by a methyl group originating from trimethylaluminum or a TMS-ethynyl group when TMS-ethynyldimethylalane was used. The benzyl glycosides with xylo configuration gave diastereomeric mixtures close to 1:1, while those of β-L- and α-D-arabino configuration gave synthetically useful mixtures of C-5-diastereoisomers (diastereomeric ratio >10:1 (methyl), > 20:1 (alkynyl)).



Scheme 1.[3]

In the β-L-arabino case the major stereochemical outcome using Me_3Al was suggested to be an inversion at C-1 based on a combination of molecular mechanics calculations and NOE measurements on an acetonide mixture obtained from the original reaction products. Similar experiments implied that the ring opening using the acetylenic alane resulted in retention. Curiously the reverse seemed to be true in the α-D-arabino case.

Although it was possible to draw the above conclusions from the NOE experiments we judged it desirable to more firmly determine the stereochemical result of the reactions by X-ray crystallographic structure determination of a well defined substance which is unambiguously related to some of the products of the pyranoside ring-opening reaction. We now have made such an investigation on the (2R,3R,4R)-γ-hydroxy-isoleucine lactone hydrochloride **9**, which was prepared from **3** (Scheme 3). In this connection lactone **15**, diastereomeric to **9**, was also prepared.

This work not only confirms our earlier conclusion, but also shows that anhydro-pentopyranosides, despite their higher flexibility as compared to the corresponding hexopyranosides, are well suited starting materials for enantiospecific synthesis of natural products. In this respect we believe that particularly the acetylenic derivatives would be suitable for further development. Moreover, some confusions in the literature concerning which stereoisomer of γ- hydroxy-isoleucine is formed or isolated under different circumstances have been sorted out.

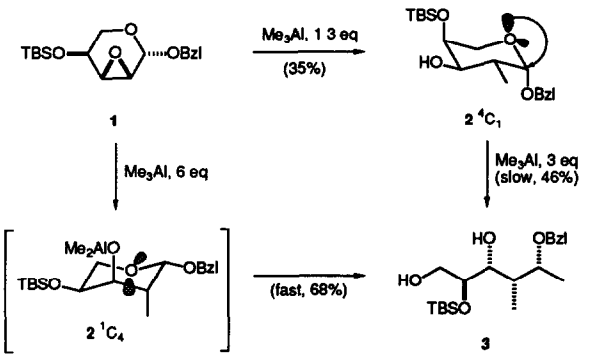
Syntheses of γ- hydroxy-isoleucine stereoisomers and related lactones have been reported. Thus, a synthesis involving photochlorination of D-allo-Ile (2R,3S) resulted in a difficultly separable diastereomeric mixture of isomers (< 5% of the RRR-lactone hydrochloride was obtained).[4] Photochlorination of L-Ile (2S,3S) gave a similar result with only 3% yield of the >95% pure (3S,4R,5R)-lactone hydrochloride.[5] An enantioselective synthesis aiming at the RRR-lactone hydrochloride gave a mixture of the (3R,4R,5R) and the (3R,4S,5S) diastereomers in a 6.6/1 ratio.[6] However, the stereochemical assignment of the (3R,4R,5R) lactone is erroneous since neither the ^1H NMR data nor the absolute optical rotations are in agreement with our data (see Experimental part, substance **9**).[7] Synthesis of racemic N-benzoylated γ- hydroxy isoleucine lactone (relative configuration not determined) has also been performed.[8]

Compound **14** ((2S,3R,4R)-γ- hydroxy-isoleucine) was believed to be present as a constituent in the peptide γ- amanitin[9] found in *Amanita phalloides* and in the spice plant *Trigonella foenum-graecum*[10] as well as in other species[11]. The amino acid component in γ- amanitin was later shown to be the (2S,3R,4S) stereoisomer.[5] Since the stereochemical assignment of the amino acid found in *Trigonella foenum-graecum* was made by comparison with the amino acid found in γ- amanitin, the former should also be of (2S,3R,4S) stereochemistry, which was indeed shown during this work.[12,13] It has been claimed that also the (2R,3R,4R)-isomer, **8**, is present in *Trigonella foenum-graecum*[10] but this is probably the (2R,3R,4S)-isomer[14]. Still another stereoisomer, (2S,3S,4R), has been isolated from *Quararibea funebris* where it is a constituent of the pyrrole alkaloid funebrine.[13,15]

Since our syntheses led to the (2R,3R,4R)- and the (2S,3R,4R)-stereoisomers we can confirm that neither compound is present in detectable amounts (300 MHz ^1H NMR) in extracts from *Trigonella foenum-graecum*.

RESULTS AND DISCUSSION.

Instead of using our earlier described step-wise procedure for the synthesis of **3**, we now have improved the yield by addition of 6 equiv. of Me_3Al to **1** (Scheme 2).



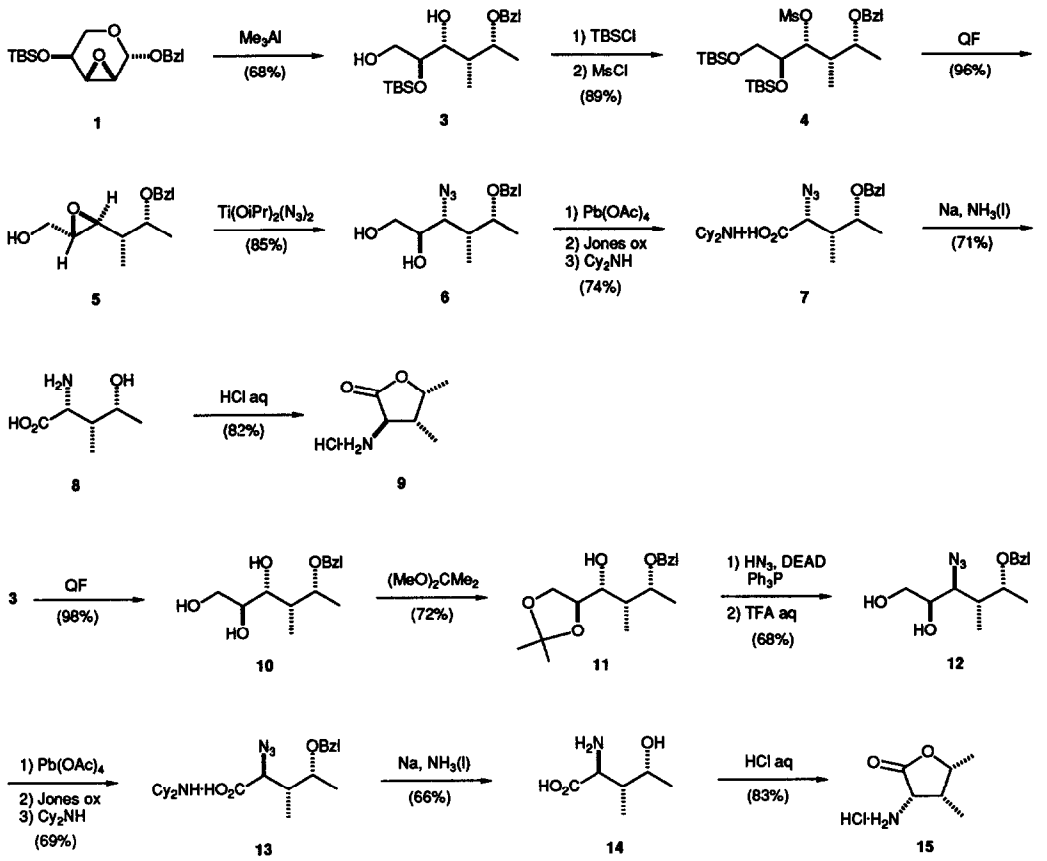
Scheme 2.[3]

In the step-wise procedure we noticed that the pyranoside ring-opening of **2** was rather slow using 3.0 equiv. of Me_3Al. The first product, when isolated as the alcohol **2**, is in the $^4C_1$ conformation as shown by NMR spectroscopy ($J_{2,3}$ = 11.2 Hz for the 3-O-acetate)[16] and it seems reasonable that this material, on reaction with Me_3Al, gives the alcoholate mainly in the same less reactive conformation with only minor amounts of the more reactive $^1C_4$ conformer present. It has been argued that the reason for this rate difference is due to the greater availability of the ring oxygen lone pair orbitals in the $^1C_4$ conformation for complexation with Lewis acids.[17] In this conformer the axial lone pair orbital of the ring oxygen has no overlap with the σ* orbital of the glycosidic C-O bond (*i.e.* no anomeric effect). In the one-pot procedure, on the other hand, compound **2** could not be detected (TLC) in hydrolysed samples during the reaction, and since the total yield and the reaction rate was higher than in the step-wise procedure it seems likely that the intermediate 2-C methyl alcoholate is rapidly attacked by the reagent before any ring-flip has occurred as indicated in Scheme 2. The new reaction conditions increased the yield of **3**, obtained as a C-5 diastereomeric mixture (10:1), from 16 to 68% (calculated from **1**).

The mesylate **4** (Scheme 3), obtained from **3** by selective silylation of the primary hydroxyl group followed by mesylation, gave the epoxide **5** on treatment with QF in THF. By using $Ti(OiPr)_2(N_3)_2$,[18] the azide ion regioselectively opened the epoxide ring of **5** at C-3 to give **6**. These operations resulted in a net retention of the configuration of the C-3 stereocenter of **3**.

Our planned oxidative cleavage of the diol moiety of **6** to give the corresponding carboxylic acid turned out to be more difficult than anticipated. The cleavage of the diol to give the azidoaldehyde by $Pb(OAc)_4$ in benzene worked satisfactorily but the labile azidoaldehyde[19] must be oxidized directly to the more stable azido carboxylic acid.[20] In order to avoid epimerization of the azidoaldehyde it was necessary to use neutral or slightly acidic conditions. The use of the permanganate based reagents $KMnO_4$/ tBuOH/ 5% NaH_2PO_4[21] and $QMnO_4$/benzene[22] resulted in incomplete oxidation and was accompanied by debenzylation and deterioration of the material while Ag_2O in benzene[23] resulted in a slow decomposition of the azido aldehyde.

It should be mentioned that other diol cleaving reagents such as $RuCl_3/H_5IO_6$[18] and $CrO_3/NaIO_4$[24], that would yield the azido acid directly, gave competing oxidation of the benzyl group and that $KMnO_4/NaIO_4$[25] resulted only in destruction of the aldehyde intermediate. No reaction was observed by the use of Pt/O_2 in hexane[26]. The successful cleaving conditions were found to be a combination of lead tetraacetate[27] and Jones reagent[28] to give the corresponding azido acid, isolated as its dicyclohexylammonium salt **7**.



Scheme 3.[3]

There are several methods for the selective reduction of an azido group to an amino group in the presence of a benzyl ether (e.g. H_2/Pd black, HCO_2NH_4/Pd-C, $LiAlH_4$).[29] However, in this case we wanted the benzyl protection removed in the same operation. This was accomplished by the use of sodium in liquid ammonia to give the amino acid **8**, which on treatment with 1 M aqueous HCl gave the crystalline lactone hydrochloride **9**.[30] The structure of **9** was confirmed by an X-ray crystal structure determination (see below).

The C-2 diastereomeric γ- hydroxy-isoleucine **14** and its corresponding lactone hydrochloride **15** were synthesized by a somewhat different route. The acetonide **11** was obtained from **3** after removal of the TBS protecting group by QF treatment, followed by camphorsulfonic acid catalysed acetalisation using 2,2-di-

methoxypropane. The selectivity in the formation of the acetonide **11** was not complete but the side products were easily removed by flash chromatography. Thus, we avoided extra protecting group chemistry by this direct acetalisation step.

A common way of introducing nitrogen functionalities is *via* nucleophilic substitution of sulfonates with azide ion.[29] Several such conditions were tried but resulted in low yields and/or formation of side products. Thus, LiN_3 or NaN_3 in DMF or DMSO, alternatively QN_3 and R_4PN_3 in cyclohexane applied on the mesylate of **11** gave large amounts of elimination products and less than 50% of the desired azide was obtained. The same set of reagents was also tested on the 1,2-OTBS mesylate **4** but gave very low yields of the azide. When the TBS groups were removed from **4** (using aq. HF in dioxane) the resulting mesylate gave a low yield of the epoxide **5** when treated with LiN_3 or NaN_3 in different solvents. It turned out that the Mitsunobu conditions (HN_3, DEAD, PPh_3) were the most effective and they are also known to give clean inversion of the reacting stereocenter.[31] The work-up procedure of this reaction mixture involved hydrolysis of the acetal to give the 1,2-diol, **12**, which was converted to **14** and its corresponding lactone hydrochloride **15**[30] in the same manner as already described. The ^1H NMR data of **15** were in good agreement with those reported but the optical rotation and the melting point disagreed with the literature data which, however, were measured on material that was only "> 95%" pure.[5]

X-ray crystal structure determination.

The structure of **9** was confirmed by a single crystal X-ray analysis. The crystal data are: $C_6H_{12}NO_2Cl$; M_r = 165.62; Monoclinic, spacegroup C2; unit cell parameters: a = 24.687(5), b = 10.722(2), c = 6.8622(9) Å, ß = 102.91(2)°, V = 1770.5(5) $Å^3$, Z = 8 (two independent molecules in the asymmetric unit); D_{calc} = 1.243(1) g cm^{-3}, F(000) = 704, μ = 3.78 cm^{-1}.

A stereo-view of one molecule with the atom-numbering scheme is shown in Fig. 1.[32]

Final atomic parameters for non-H atoms are listed in Table 1 and bond lengths and angles in Table 2.

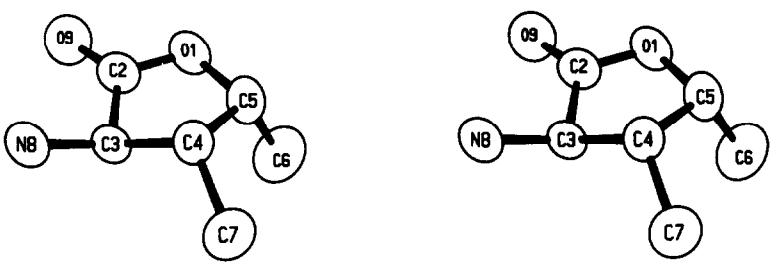


Fig 1. Stereoview of **9**.

The conformation and configuration of the two molecules of **9** in the asymmetric unit are identical, as well as distances and angles; largest deviation is 5σ. The five-membered ring in **9** has an envelope conformation and the puckering parameters[33] are Q_2/Å = 0.328, 0.391; Φ_2/° = 283.9, 281.3.

Data collection and refinement. Colorless crystals of **9** were grown from an EtOH/THF solution. A crystal having approximate dimensions 0.15x0.30x0.15 mm^3 was mounted on an Enraf-Nonius CAD4

diffractometer with graphite-monochromated MoK_α radiation (λ = 0.71069 Å). Cell constants were determined from 23 reflections in the range $32 \leq 2\theta \leq 48°$. Systematic absences ($h+k$ = $2n+1$), optical activity and the known configuration at C-3 and C-4 fixed the spacegroup to C2. A total of 2406 reflections in the range $6 \leq 2\theta \leq 54°$ were collected (h -31 → 31, k -13 → 0, l -8 → 0) with the $\omega/2\theta$-scan technique ($\Delta\omega$ = 0.8°+0.5°tanθ). Of these 2010 were unique (R_{int} = 0.027) and 1358 with $I > 3\sigma(I)$ were considered observed. Data were corrected for Lorentz, polarisation and absorption effects (numerical integration), transmission factor 0.89-0.95. Three standard reflections measured every hour showed no significant variation.

The structure was solved by Patterson methods and subsequent electron density maps. The coordinates of most of the hydrogen atoms were located from the final maps. The absolute configuration was identified from the known configuration at C-3 and C-4. Final refinement was on F with non-H atoms treated anisotropically, hydrogen atom parameters were fixed with isotropic temperature factors of 0.08 $Å^2$. The model was refined by full-matrix least-squares, minimizing $\Sigma w(|F_o|-|F_c|)^2$, to R = 0.042, R_w = 0.057 and S = 1.50, 180 parameters. The weighting scheme used was $w = [\sigma^2(F_o)+(0.03F_o)^2]^{-1}$. Maximum and minimum residual electron density in final maps were 0.26 and -0.22 $eÅ^{-3}$ respectively. Atomic scattering factors were taken from Ref [34] and the computer programs used are described in Ref [35].

Table 1. Final Atomic Parameters for the Non-H Atoms with e.s.d.'s in Parentheses.
($U_{eq} = 1/3\Sigma_i\Sigma_j U_{ij} a^*_i a^*_j \mathbf{a}_i \mathbf{a}_j$)

	x/a	y/b	z/c	$U_{iso}/Å^2$
Cl-1	0.0657(1)	0	0.7992(2)	0.0528(5)
Cl-2	0.9383(1)	0.3057(2)	0.7155(2)	0.0525(5)
O1	0.1893(1)	0.4820(4)	0.8192(6)	0.063(1)
C2	0.1635(2)	0.3743(6)	0.8397(8)	0.054(2)
C3	0.1041(2)	0.3998(5)	0.8465(7)	0.044(2)
C4	0.0924(2)	0.5252(5)	0.7442(7)	0.047(2)
C5	0.1508(2)	0.5875(5)	0.8014(9)	0.057(2)
C6	0.1630(3)	0.6604(8)	0.9994(11)	0.089(3)
C7	0.0443(2)	0.6006(7)	0.7969(12)	0.076(3)
N8	0.0674(2)	0.2978(5)	0.7508(6)	0.050(1)
O9	0.1864(2)	0.2723(4)	0.8421(7)	0.077(2)
O1'	0.3268(2)	0.2901(4)	0.5557(6)	0.059(1)
C2'	0.3799(2)	0.3287(5)	0.5991(8)	0.052(2)
C3'	0.3818(2)	0.4684(5)	0.6186(6)	0.041(2)
C4'	0.3275(2)	0.4949(5)	0.6860(7)	0.043(1)
C5'	0.2900(2)	0.3953(6)	0.5603(9)	0.061(2)
C6'	0.2624(3)	0.4337(8)	0.3472(11)	0.101(3)
C7'	0.3064(2)	0.6285(6)	0.6609(13)	0.078(3)
N8'	0.4332(1)	0.5116(5)	0.7511(8)	0.047(1)
O9'	0.4187(2)	0.2597(5)	0.6158(8)	0.080(2)

Table 2. Bond Distances (Å) and Angles(°) with e.s.d.'s in Parentheses. Primed and Unprimed Refers to the Two Crystallographically Independent Molecules in the Asymmetric Unit.

	Unprimed	Primed
O1 - C2	1.341(8)	1.343(7)
O1 - C5	1.465(7)	1.452(7)
C2 - C3	1.501(7)	1.503(7)
C2 - O9	1.230(8)	1.196(7)
C3 - C4	1.514(7)	1.540(6)
C3 - N8	1.478(7)	1.462(6)
C4 - C5	1.558(7)	1.544(8)
C4 - C7	1.544(8)	1.521(8)
C5 - C6	1.538(10)	1.526(10)
C2 - O1 - C5	111.0(4)	109.9(4)
O1 - C2 - C3	109.7(5)	109.7(4)
O1 - C2 - O9	122.6(5)	123.6(5)
C3 - C2 - O9	127.6(5)	126.8(5)
C2 - C3 - C4	103.6(4)	101.4(4)
C2 - C3 - N8	111.0(4)	112.2(4)
C4 - C3 - N8	114.6(4)	116.6(4)
C3 - C4 - C5	101.1(4)	99.1(4)
C3 - C4 - C7	115.7(4)	116.0(4)
C5 - C4 - C7	115.6(5)	116.2(4)
O1 - C5 - C4	103.7(4)	104.5(4)
O1 - C5 - C6	108.9(5)	109.7(5)
C4 - C5 - C6	115.4(5)	115.6(6)

EXPERIMENTAL SECTION.[3]

Column chromatography separations were performed with ethyl acetate/heptane (E/H) or with chloroform/methanol/water (C/M/W) mixtures as eluents using Merck SiO_2 60 (40-63 μm) silica gel. Merck SiO_2 60 F254 0.5 mm precoated glass plates were used for TLC analyses in solvent systems E/H or C/M/W, and the spots were visualized by charring with 10% H_2SO_4, with 5% molybdophosphoric acid in EtOH or with 0.3% ninhydrin in BuOH/HOAc 10/1. Melting points (uncorrected) were determined with a Reichert microscope. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded on a Perkin-Elmer 298 spectrometer. NMR spectra were recorded at 23°C with a Varian XL-300 spectrometer, using $CDCl_3$ ($CHCl_3$, 7.26 ppm) or, if indicated, d_6-DMSO (d_5-DMSO, 2.50 ppm) or D_2O ($TMS(CH_2)_3SO_3Na$, 0.00 ppm) as solvent. Organic solutions were dried with Na_2SO_4 and were

concentrated using a rotary evaporator (35°C, 15 mm Hg). Air and/or moisture sensitive reactions were carried out in oven-dried glassware under an argon atmosphere. Solvents were dried over 4Å molecular sieves.

Compounds **3-6** and **10-12** were isolated and characterized as C-5 10/1 diastereomeric mixtures; compounds **7** and **13** as C-4 10/1 diastereomeric mixtures. NMR data refer to the major diastereomers shown in Scheme 3.

(2S,3R,4R,5R)-5-O-Benzyl-4-methyl-2-O-(*tert*-butyldimethylsilyl)-1,2,3,5-hexanetetrol (3). Benzyl 2,3-anhydro-4-O-(*tert*-butyldimethylsilyl)-β-L-ribopyranoside (**1**)[16,36] (27.8 g, 82.6 mmol) was dissolved in dry hexane (325 mL). Me_3Al (248 mL, 2.0 M in hexanes, 496 mmol) was added during 5 minutes at room temperature. The mixture was refluxed for 19 h, cooled to room temperature and Et_2O (150 mL) was added to moderate the subsequent hydrolysis rate. The solution was injected, *via* a double tipped needle, into ice cooled 2M aq NH_4Cl (600 mL). The slurry was filtered and the solids were carefully washed with EtOAc. The aqueous phase was extracted with EtOAc (100 mL) and the combined organic phase was washed with H_2O (100 mL), dried and concentrated. Flash chromatography (E/H 1/8) gave **3** as a colourless oil (20.8 g, 68%).

The physical and spectral data of **3** were in full agreement with those previously reported.[2]

(2S,3R,4R,5R)-5-O-Benzyl-3-O-methanesulfonyl-4-methyl-1,2-di-O-(*tert*-butyldimethylsilyl)-1,2,3,5-hexanetetrol (4). Compound **3** (10.0 g, 27.1 mmol) and imidazole (4.61 g, 67.8 mmol) were dissolved in dry DMF (75 mL) and TBSCl (4.50 g, 29.8 mmol) was added. After being stirred for 3 h, the solution was diluted with CH_2Cl_2 (50 mL), washed with 1M aq. HCl (2x35 mL), sat. aq. $NaHCO_3$ (35 mL) and H_2O (35 mL), dried and concentrated (40°C, 0.08 mm Hg). The remaining oil was dissolved in dry pyridine (150 mL) and the flask was placed in an ice bath. Methanesulfonylchloride (6.32 mL, 81.3 mmol) was added and after being stirred at room temperature for 20 h, the solution was diluted with CH_2Cl_2 (200 mL), washed with H_2O (3x40 mL), dried and co-evaporated with toluene. Flash chromatography (E/H 1/10) gave **4** as a colourless oil (13.5 g, 89%): TLC (E/H 1/6) R_f = 0.40; $[\alpha]^{20}_D$ =-4.8° (c 1.26, $CHCl_3$); ^1H NMR δ 7.32 (m, 5H, C_6H_5), 4.94 (dd, 1H, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 6.5 Hz, H-3), 4.59, 4.39 (AB q, each 1H, J_{AB} = 11.6 Hz, CH_2Ph), 4.12 (m, 1H, H-2), 3.71, 3.55 (dAB q, each 1H, J_{AB} = 10.6 Hz, $J_{1,2}$, $J_{1',2}$ = 5.8, 6.0 Hz, H-1, H-1'), 3.64 (dq, 1H, $J_{4,5}$ = 4.2 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.01 (s, 3H, $MeSO_2$), 2.15 (m, 1H, H-4), 1.24 (d, 3H, H-6), 1.09 (d, 3H, $J_{Me,4}$ = 7.0 Hz, 4-Me), 0.90, 0.87 (2 s, each 9H, Me_3CSi), 0.07-0.05 (3s, 12H, Me_2Si).

Anal. Calcd for $C_{27}H_{52}SSi_2O_6$: C, 57.81; H, 9.34. Found: C, 58.16; H, 9.46.

(2S,3R,4R,5R)-2,3-Anhydro-5-O-benzyl-4-methyl-1,2,3,5-hexanetetrol (5). Compound **4** (5.00 g, 8.91 mmol) in dry THF (40 mL) was cooled in an ice bath and QF (5.62 g, 17.8 mmol), dissolved in dry THF (20 mL), was added. After being stirred at room temperature for 16 h, the solution was diluted with CH_2Cl_2 (150 mL), washed with 0.5 M aq. HCl (2x20 mL) and H_2O (30 mL), dried and concentrated. Flash chromatography (E/H 1/1) gave **5** as a colourless oil (2.02 g, 96%): TLC (E/H 1/1) R_f = 0.27; $[\alpha]^{20}_D$ =-50.7° (c 1.09, $CHCl_3$); ^1H NMR δ 7.35 (m, 5H, C_6H_5), 4.64, 4.47 (AB q, each 1H, J_{AB} = 11.7 Hz, CH_2Ph), 3.91 (dq, 1H, $J_{1,1'}$ = 12.5 Hz, $J_{1,2}$ = 2.6 Hz, $J_{1,OH}$ = 5.6 Hz, H-1), 3.68 (dq, 1H, $J_{4,5}$ = 4.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.60 (dq, 1H, $J_{1',2}$ = 4.2 Hz, $J_{1',OH}$ = 7.2 Hz, H-1'), 2.99 (dd, 1H, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 9.9 Hz, H-3),

2.96 (m, 1H, H-2), 1.77 (dd, 1H, OH), 1.51 (m, 1H, H-4), 1.24 (d, 3H, H-6), 0.98 (d, 3H, $J_{Me,4}$ = 7.1 Hz, 4-Me).

Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 70.98; H, 8.44.

(2S,3R,4R,5R)-3-Azido-5-O-benzyl-4-methyl-1,2,5-hexanetriol (6). Compound **5** (1.86 g, 7.87 mmol) was dissolved in dry benzene (125 mL). $Ti(Oi\text{-}Pr)_2(N_3)_2$[18] (3.04 g, 9.44 mmol) was added and the solution was stirred at 50°C for 5 h. The flask was placed in an ice bath and 1M aq. H_2SO_4 (125 mL) was added. After being rapidly stirred for 1 h, the aqueous phase was extracted with toluene (3x30 mL). The combined organic phase was washed with H_2O (2x25 mL), dried and concentrated. Flash chromatography (SiO_2 deactivated with 5% water, E/H 1/2) gave **6** as a colourless oil (1.88 g, 85%): TLC (E/H 2/1) R_f = 0.35; $[\alpha]^{20}_D$ = -34.2° (c 1.06, $CHCl_3$); IR(CCl_4) 2100 (N_3) cm^{-1}; ^1H NMR δ 7.34 (m, 5H, C_6H_5), 4.63, 4.44 (AB q, each 1H, J_{AB} = 11.5 Hz, CH_2Ph), 3.89-3.63 (m, 3H, H-1, H-1', H-2), 3.66 (m, 1H, H-5), 3.58 (dd, 1H, $J_{2,3}$, $J_{3,4}$ = 4.8, 7.4 Hz, H-3), 3.27 (br s, 1H, OH), 2.16 (br s, 1H, OH), 2.06 (m, 1H, H-4), 1.27 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.06 (d, 3H, $J_{Me,4}$ = 7.1 Hz, 4-Me).

Anal. Calcd for $C_{14}H_{21}N_3O_3$: C, 60.20; H, 7.58; N, 15.04. Found: C, 60.34; H, 7.60; N, 14.91.

The 1,2-di-O-acetate of **6** had: ^1H NMR δ 7.33 (m, 5H, C_6H_5), 5.34 (m, 1H, H-2), 4.59, 4.45 (AB q, each 1H, J_{AB} = 11.4 Hz, CH_2Ph), 4.44, 4.19 (ABX, each 1H, J_{AB} = 12.2 Hz, $J_{1,2}$, $J_{1',2}$ = 2.6, 6.8 Hz, H-1, H-1'), 3.79 (dd, $J_{2,3}$ = 5.6 Hz, $J_{3,4}$ = 6.5 Hz, H-3), 3.62 (dq, 1H, $J_{4,5}$ = 4.2 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 2.10, 2.02 (2s, each 3H, Ac), 1.81 (m, 1H, H-4), 1.27 (d, 3H, H-6), 1.01 (d, 3H, $J_{Me,4}$ = 6.9 Hz, 4-Me).

(2R, 3R, 4R)-2-Azido-4-benzyloxy-3-methyl-pentanoic acid dicyclohexylamine salt (7). Compound **6** (770 mg, 2.78 mmol), dissolved in dry benzene (8 mL), was added to a solution of $Pb(OAc)_4$ (1.48 g, 3.33 mmol, moistened with 2% acetic acid) in dry benzene (7 mL). The mixture was stirred for 35 min, filtered and frozen at -30°C. Cold (-30°C) acetone (30 mL) was added. To this homogeneous solution, a cold (0°C) solution of CrO_3 (370 mg, 3.70 mmol) in H_2O/H_2SO_4 3/1 (4 mL) was added dropwise over 10 minutes. The temperature was raised to -10°C (30 min) and i-PrOH (107 µL, 1.39 mmol) was added to destroy the excess of the oxidant. After 10 min, Et_2O and H_2O (15 mL each) were added. The aqueous phase was extracted with Et_2O (2x15 mL). The combined organic phase was washed with H_2O, and then extracted with sat. aq. $NaHCO_3$ (3x10 mL). After acidification to pH 3.5 with 6M aq. HCl, the aqueous solution was extracted with Et_2O (3x10 mL), dried and concentrated. The crude product was dissolved in petroleum ether (60-70) (5 mL) and Cy_2NH (448 µL, 2.25 mmol) was added. Recrystallisation from EtOAc/petroleum ether (60-70) gave **7** as thin colourless needles (910 mg, 74%): TLC (E/H 2/1 with 2% HOAc) R_f = 0.43; mp 118-119°C; $[\alpha]^{20}_D$ = +21.5° (c 1.40, $CDCl_3$); IR (CCl_4) 2095 (N_3), 1630 (C = O) cm^{-1}; ^1H NMR δ 7.35 (m, 5H, C_6H_5), 4.60, 4.48 (AB q, each 1H, J_{AB} = 11.5 Hz, CH_2Ph), 3.94 (d, 1H, $J_{2,3}$ = 4.2 Hz, H-2), 3.50 (dq, 1H, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 6.1 Hz, H-4), 2.99 (m, 2H, $(CH)_2N$), 2.26 (m, 1H, H-3), 2.10-1.15 (m, 2H, CH_2), 1.27 (d, 3H, H-5), 1.03 (d, 3H, $J_{Me,3}$ = 6.6 Hz, 3-Me).

Anal. Calcd for $C_{25}H_{40}N_4O_3$: C, 67.54; H, 9.07; N, 12.60. Found: C, 67.48; H, 9.15; N, 12.68.

(2R, 3R, 4R)-2-Amino-4-hydroxy-3-methyl-pentanoic acid (8). Compound **7** (600 mg, 1.35 mmol), dissolved in EtOAc (10 mL), was washed with 5% $KHSO_4$ (2x4 mL), dried and concentrated. The oil, dissolved in THF (8 mL) and EtOH (393 µL, 6.74 mmol), was added to liquid NH_3 (40 mL) at -40°C. Freshly

cut sodium pieces were added until the deep blue colour persisted for 2 minutes. NH_4Cl (776 mg, 13.5 mmol) was added and the solvents were evaporated. The residue was dissolved in H_2O and was applied on an ion exchange column (Dowex 50W X8). The column was washed with H_2O (50 mL) and then the amino acid was eluted with 1M NH_4OH. The ninhydrin positive fractions were lyophilized. Flash chromatography (C/M/W 10/5/1) of this ammonium salt gave the free amino acid. Recrystallisation from 90% aq. EtOH gave enantiomerically pure **8** (141 mg, 71%): TLC (C/M/W 10/5/1) R_f = 0.14; mp ~230°C (sublimation); $[\alpha]^{20}_D$ = -35.6° (c 1.12, D_2O); ^1H NMR (D_2O) δ 4.11 (dq, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 3.88 (d, 1H, $J_{2,3}$ = 2.7 Hz, H-2), 2.22 (m, 1H, H-3), 1.24 (d, 3H, $J_{4,5}$ = 6.5 Hz, H-5), 0.92 (d, 3H, $J_{Me,3}$ = 7.2 Hz, 3-Me); ^{13}C NMR (D_2O) δ 176.72 (C-1), 73.38 (C-4), 62.44 (C-2), 41.06 (C-3), 23.18 (C-5), 8.30 (3-Me).

Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.86; H, 9.11; N, 9.51.

(3R, 4R, 5R)-3-Amino-4,5-dimethyl-2-oxo-tetrahydrofuran hydrochloride (9). Compound **8** (50 mg, 340 μmol) dissolved in 1M aq. HCl (1 mL) was refluxed for 15 minutes. The solution was evaporated to dryness and the residue was recrystallized from EtOH(99.5%)/Et_2O to give **9** as colourless needles (46 mg, 82%). Slow crystallization from EtOH/THF gave crystals suitable for X-ray analysis: mp 203-218°C (sublimation); (lit.[4] mp 222°C); $[\alpha]^{20}_D$ = +96.4°, (c 0.97, MeOH);(lit.[4] $[\alpha]^{20}_D$ = +88.7° (c 1%, MeOH)); $[\alpha]^{20}_D$ = +88.3°, (c 0.73, D_2O);(lit.[6] $[\alpha]^{20}_D$ = +37.1° (c 1%, D_2O) cf. compound **15** and Ref [7]) The ^1H NMR spectrum (in d_6-DMSO) was in good agreement ($\Delta\delta \leq 0.15$ ppm, $\Delta J \leq 0.3$ Hz) with data given in Ref [4].

Anal. Calcd for $C_6H_{12}ClNO_2$: C, 43.51; H, 7.30; N, 8.46. Found: C, 43.50; H, 7.36; N, 8.44.

(2S,3R,4R,5R)-5-O-Benzyl-4-methyl-1,2,3,5-hexanetetrol (10). Compound **3** (6.97 g, 18.9 mmol) was dissolved in THF (45 mL) and QF (5.96 g, 18.9 mmol) in THF (30 mL) was added at room temperature. After 40 min, CH_2Cl_2 (100 mL) was added and the solution was washed with 0.5 M aq HCl (2x20 mL) and water (20 mL), dried and concentrated. Flash chromatography (SiO_2 deactivated with 5% water, E/H 8/1) gave **10** as a colourless oil (4.69 g, 98%): TLC (E/H 4/1) R_f = 0.18; $[\alpha]^{20}_D$ = -59.5° (c 0.91, $CHCl_3$); ^1H NMR δ 7.33 (m, 5H, C_6H_5), 4.66, 4.38 (AB q, each 1H, J_{AB} = 11.5 Hz, CH_2Ph), 3.87 (dq, 1H, $J_{4,5}$ = 2.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.81, 3.70 (ABX, each 1H, J_{AB} = 11.3 Hz, $J_{1,2}$, $J_{1',2}$ = 3.9, 5.2 Hz, H-1, H-1'), 3.73 (dd, 1H, $J_{2,3}$ = 7.4 Hz, $J_{3,4}$ = 2.0 Hz, H-3), 3.59 (m, 1H, H-2), 1.95 (m, 1H, H-4), 1.27 (d, 3H, H-6), 1.01 (d, 3H, $J_{Me,4}$ = 7.2 Hz, 4-Me).

Anal. Calcd for $C_{14}H_{22}O_4$: C, 66.12; H, 8.72. Found: C, 65.96; H, 8.78.

(2S,3R,4R,5R)-5-O-Benzyl-1,2-di-O-isopropylidene-4-methyl-1,2,3,5-hexanetetrol (11). Compound **10** (4.10 g, 16.1 mmol) was dissolved in dry CH_2Cl_2 (100 mL). Camphorsulfonic acid (~10 mg) was added and the flask was placed in an ice bath. A solution of 2,2-dimethoxypropane (1.98 mL, 16.1 mmol) in dry CH_2Cl_2 (25 mL) was added over 15 minutes. After 75 min, the solution was washed with sat. aq. $NaHCO_3$ (20 mL), dried and concentrated. Flash chromatography (E/H 1/4) gave **11** as a colourless oil (3.40 g, 72%): TLC (E/H 1/3) R_f = 0.40; $[\alpha]^{20}_D$ = -73.1° (c 0.86, $CHCl_3$); ^1H NMR δ 7.32 (m, 5H, C_6H_5), 4.65, 4.39 (AB q, each 1H, J_{AB} = 11.4 Hz, CH_2Ph), 4.11-3.92 (m, 3H, H-1, H-1', H-2), 3.85 (dq, 1H, $J_{4,5}$ = 2.7 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.72 (dd, 1H, $J_{2,3}$ = 7.9 Hz, $J_{3,4}$ = 1.9 Hz, H-3), 3.50 (br s, 1H, OH), 1.89 (m, 1H, H-4), 1.39, 1.34 (2 s, each 3H, $C(CH_3)_2$), 1.27 (d, 3H, H-6), 1.01 (d, 3H, $J_{Me,4}$ = 7.2 Hz, 4-Me).

Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.24; H, 8.83.

(2S,3S,4R,5R)-3-Azido-5-O-benzyl-4-methyl-1,2,5-hexanetriol (12). Compound **11** (3.00 g, 10.2 mmol) and Ph_3P (6.68 g, 25.5 mmol) were dissolved in dry benzene (55 mL) and the mixture was cooled in an ice bath. HN_3[37] (22.0 mL, 1.14 M in benzene, 25.5 mmol) and DEAD (4.01 mL, 25.5 mmol) were added. After 5 min, the solution was heated to reflux. After 30 min, the solution was concentrated and the residue was triturated with heptane/EtOAc 2/1. Flash chromatography (E/H 1/8) gave a product mixture, including the desired azidoacetal (TLC(E/H 1/3) R_f = 0.52), which was dissolved in 1,4-dioxane (30 mL) and H_2O (20 mL). TFA (2 mL) was added and the solution was stirred for 48 h, diluted with CH_2Cl_2 (50 mL), washed with sat. aq. $NaHCO_3$ (2x15 mL), dried and concentrated. Flash chromatography (SiO_2 deactivated with 5% water, E/H 1/1) gave **12** as a colourless oil (1.93 g, 68%): TLC (E/H 1/1) R_f = 0.27; $[\alpha]^{20}_D$ = -48.7° (c 1.10, $CHCl_3$); IR(CCl_4) 2100 (N_3) cm^{-1}; ^1H NMR δ 7.33 (m, 5H, C_6H_5), 4.63, 4.42 (AB q, each 1H, J_{AB} = 11.5 Hz, CH_2Ph), 3.93 (m, 1H, H-2), 3.91 (dq, 1H, $J_{4,5}$ = 2.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.78, 3.68 (ABX, each 1H, J_{AB} = 10.9 Hz, $J_{1,2}$, $J_{1',2}$ = 4.4, 7.5 Hz, H-1, H-1'), 3.37 (dd, 1H, $J_{2,3}$ = 1.7 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 2.38 (d, 1H, $J_{OH,2}$ = 6.4 Hz, 2-OH), 2.02 (br s, 1H, 1-OH), 1.98 (m, 1H, H-4), 1.28 (d, 3H, H-6), 1.03 (d, 3H, $J_{Me,4}$ = 7.0 Hz, 4-Me).

Anal. Calcd for $C_{14}H_{21}N_3O_3$: C, 60.20; H, 7.58; N, 15.04. Found: C, 60.12; H, 7.68; N, 14.96.

(2S, 3R, 4R)-2-Azido-4-benzyloxy-3-methyl-pentanoic acid dicyclohexylamine salt (13). Compound **12** (1.72 g, 6.20 mmol) was oxidized as described for the preparation of **7**. Recrystallisation from EtOAc/ petroleum ether (60-70) gave **13** as thin colourless needles (1.90 g, 69%): TLC (E/H 2/1 with 2% HOAc) R_f = 0.40; mp 106-107°C; $[\alpha]^{20}_D$ = -22.8° (c 1.40, $CDCl_3$); IR(CCl_4) 2090 (N_3), 1625 (C = O) cm^{-1}; ^1H NMR δ 7.34 (m, 5H, C_6H_5), 4.60, 4.44 (AB q, each 1H, J_{AB} = 11.5 Hz, CH_2Ph), 3.86 (dq, 1H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 6.3 Hz, H-4), 3.62 (d, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 2.99 (m, 2H, $(CH)_2N$), 2.10-1.10 (m, 2H, CH_2), 1.92 (m, 1H, H-3), 1.24 (d, 3H, H-5), 1.04 (d, 3H, $J_{Me,3}$ = 6.9 Hz, 3-Me).

Anal. Calcd for $C_{25}H_{40}N_4O_3$: C, 67.54; H, 9.07; N, 12.60. Found: C, 67.53; H, 9.17; N, 12.63.

(2S, 3R, 4R)-2-Amino-4-hydroxy-3-methyl-pentanoic acid (14). Compound **13** (1.20 g, 2.70 mmol) was reduced as described for the preparation of **8**. After flash chromatography on SiO_2, the crude amino acid was recrystallized twice from 90% aq. EtOH to give enantiomerically pure **14** (264 mg, 66%): TLC (C/M/W 10/5/1) R_f = 0.14; mp ~220°C (sublimation); $[\alpha]^{20}_D$ = -24.9° (c 1.17, D_2O); ^1H NMR (D_2O) δ 4.06 (dq, 1H, $J_{3,4}$ = 2.7 Hz, $J_{4,5}$ = 6.5 Hz, H-4), 3.81 (d, 1H, $J_{2,3}$ = 4.0 Hz, H-2), 2.13 (m, 1H, H-3), 1.21 (d, 3H, H-5), 1.06 (d, 3H, $J_{Me,3}$ = 7.3 Hz, 3-Me); ^{13}C NMR (D_2O) δ 176.55 (C-1), 71.20 (C-4), 62.03 (C-2), 41.20 (C-3), 22.76 (C-5), 11.47 (3-Me).

Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.88; H, 8.78; N, 9.49.

(3S,4R,5R)-3-Amino-4,5-dimethyl-2-oxo-tetrahydrofuran hydrochloride (15). Compound **14** (50 mg, 340 μmol) dissolved in 1M aq. HCl (1 mL) was refluxed for 15 minutes. The solution was evaporated to dryness and the residue was recrystallized from EtOH (99.5%) /Et_2O to give **15**, colourless needles (47.0 mg, 83%): mp 198-208°C (sublimation); (lit.[4] mp 255°C); $[\alpha]^{20}_D$ = +40.7 ° (c 0.77, MeOH);(lit.[4] $[\alpha]^{20}_D$ = +24.3° (c 1%, MeOH)); $[\alpha]^{20}_D$ = +37.3° (c 0.72, D_2O). The ^1H NMR spectrum (in d_6-DMSO) was in good agreement ($\Delta\delta \leq 0.07$ ppm, $\Delta J \leq 0.1$ Hz) with data given in Ref [4].

Anal. Calcd for $C_6H_{12}ClNO_2$: C, 43.51; H, 7.30; N, 8.46. Found: C, 43.45; H, 7.34; N, 8.38.

Extraction of *Trigonella foenum-graecum* (fenugreek). From 500 g of seed, 30 g ninhydrin-positive material was obtained after extraction and ion-exchange chromatography, following a literature procedure.[12] A 5.2 g portion of this dark brown oil was subjected to flash chromatography (SiO_2, C/M/W 10/5/1) to give a mixture of amino acids (TLC(C/M/W 10/5/1) R_f = 0.14) (438 mg). Several recrystallizations from hot 90% aq. EtOH gave (2S,3R,4S)-2-Amino-4-hydroxy-3-methyl-pentanoic acid (91mg): mp 205-215°C (sublimation) (lit.[12] mp 224-225°C); $[\alpha]^{20}_D$ = +35.9° (c 0.30, D_2O);(lit.[10] $[\alpha]^{20}_D$ = +31° (c 1, H_2O)); The ^1H NMR spectrum (in D_2O) was in excellent agreement ($\Delta\delta \leq 0.005$ppm, $\Delta J \leq 0.1$Hz) with data given in Ref [12].

The combined mother liquours were evaporated to dryness. The ^1H NMR spectrum of this amino acid mixture (20 mg in D_2O) showed that, within the detection limits, neither **8** nor **14** are present in fenugreek.

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REFERENCES AND NOTES

1. Part I: Ref 16 ; Part II: Ref 2; Part III: Inghardt, T.; Frejd, T. *Synthesis* **1990**, 285-291.
2. Inghardt, T.; Frejd, T. *J. Org. Chem.* **1989**, *54*, 5539-5543.
3. The following abbreviations are used: TBS = *tert*-butyldimethylsilyl; Cy_2NH = dicyclohexylamine; DEAD = diethylazodicarboxylate; Q = tetrabutylammonium; TFA = trifluoroacetic acid.
4. Hasan, M.; Georgopoulos, D.; Wieland, T. *Liebigs Ann. Chem.* **1976**, 781-787.
5. Gieren, A.; Narayanan, P.; Hoppe, W.; Hasan, M.; Michl, K.; Wieland, T.; Smith, H.O.; Jung, G.; Breitmaier, E. *Justus Liebigs Ann Chem.* **1974**, 1561-1569.
6. Gull, R.; Schöllkopf, U. *Synthesis* **1985**, 1052-1055.
7. The isomer determined by Gull and Schöllkopf (Ref 6) was claimed to be the RRR isomer is probably the SRR form since the optical rotation and NMR data in D_2O agree with our data for that stereoisomer (**15**).
8. Ben-Ishai, D.; Berler, Z.; Altman, J. *J. Chem. Soc., Chem. Commun* **1975**, 905-906.
9. Wieland, T.; Hasan, M.; Pfaender, P. *Justus Liebigs Ann. Chem.* **1968**, *717*, 205-214.
10. Fowden, L.; Pratt, H. M.; Smith, A. *Phytochemistry*, **1973**, *12*, 1707-1711.
11. Hardman, R.; Abu-Al-Futuh, I. M. *Phytochemistry*, **1976**, *15*, 325.
12. Alcock, N.W.; Crout, D.H.G.; Gregorio, M.V.M.; Lee, E.; Pike, G.; Samuel, C.J. *Phytochemistry* **1989**, *28*, 1835-1841.
13. The Cambridge Crystallographic Data Base contains structural information on this compound.
14. Hasan, M. In *New Trends in Natural Products Chemistry*; Atta-ur-Rahman and Le Quesne, P.W., Eds.; Elseiver: Amsterdam, 1986, pp 123-141.
15. Raffauf, R.F.; Zennie, T.M.; Onan, K.D.; Le Quesne, P.W. *J.Org. Chem.* **1984**, *49*, 2714-2718.
16. Inghardt, T.; Frejd, T.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 4542-4548.
17. Guindon, Y.; Anderson, P: C. *Tetrahedron Lett.* **1987**, *28*, 2485-2488.
18. Caron, M.; Carlier, P.R.; Sharpless, K.B. *J. Org. Chem.* **1988**, *53*, 5187-5189.
19. Kuzuhara, H.; Emoto, S. *Tetrahedron Lett.* **1975**, 1853-1856 and references cited herein.
20. Zaloom, J.; Roberts, D.C. *J. Org. Chem.* **1981**, *46*, 5173-5176.
21. Abiko, A.; Roberts, J.C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, *27*, 4537-4540.
22. Herriott, A.W.; Picker, D. *Tetrahedron Lett.* **1974**, 1511-1514.

23. Haines, A.H. *Methods for the Oxidation of Organic Compounds*; Academic Press: London, 1988; pp 251
24. See Ref 23, pp 300.
25. See Ref 23, pp 298.
26. Heyns, K.; Paulsen, H.; Rüdiger, G.; Weyer, J. *Fortschr. Chem. Forsch.* **1969**, *11*, 285-374.
27. See Ref 23, pp 287.
28. Bowden, K.; Heilbron, I.M.; Jones, E.R.H.; Weedon, B.C.L. *J. Chem. Soc.* **1946**, 39-45.
29. For a review on azide chemistry, see: Scriven, E.F.V.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297-368.
30. In order to check that no epimerisation had occurred under these acidic conditions, the lactone **9** (**15**) was opened with 1M aq. NH_4OH which gave **8** (**14**).
31. Loibner, H.; Zbiral, E. *Helv. Chim. Acta* **1976**, *59*, 2100-2113.
32. Full lists of hydrogen atom coordinates, anisotropic thermal parameters for non-H atoms torsion angles and calculated and observered structure factors are available as Supplementary Material and have been deposited at the Cambridge Crystallographic Data Centre.
33. Cremer, D. and Pople, J.A. *J. Am. Chem. Soc.* **1975**, *97* 1354-1358.
34. *International Tables for X-ray Crystallography*, Vol IV. : Kynoch Press: Birmingham, 1974 (Present Distributor Kluwer Academic Publisher, Dordrecht).
35. Lundgren, J.O. Rep. No. UUIC-B13-4-05. Univ. of Uppsala, Sweden 1982.
36. Compound **1** may be purified by distillation (152-5°C, 0.6 mm Hg).
37. Wolff, H. *Org. React.* **1955**, *3*, 307-336.