Pergamon

Tetrahedron, Vol. 53, No. 40, pp. 13883-13896, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00877-6

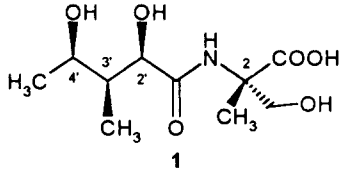
Synthesis of Conagenin Analogs Modified at 3'-Carbon Atom

Árpád Kovács-Kulyassa, Pál Herczegh and Ferenc Sztaricskai*

Research Group for Antibiotics of the Hungarian Academy of Sciences
Lajos Kossuth University, P. O. Box 70., H-4010 Debrecen, Hungary

Abstract: Three conagenin analogs modified at 3'-carbon centre and their diastereoisomers at position C-2 were synthesized. The synthesis of the acylating carboxylic acids **13**, **22** and **33** having stereotriads were elaborated starting from D-xylose performing stereoselective reactions.

Screening for substances that modulate immune responses in conjunction with T cells is of great importance in physiology[1]. Most immunomodulators act primarily on macrophages and consequently stimulate T cells. However it is known that macrophage activation produces inflammatory mediators, which may cause non-specific augmentation in the host immune system. The low molecular weight immunomodulator conagenin (CNG, **1**, Fig. 1), which was isolated from the fermentation broth of *Streptomyces roseosporus* MI696-AF3[2], exhibits specific action on T cells. In an *in vivo* study it was shown that CNG enhanced lymphokine production and generation of antitumor effector cells in tumor-bearing mice; the cytotoxic T lymphocyte activity was maintained on high level, whereas this activity was reduced without CNG according to tumor growth. In studies on the effect of CNG on myelosuppression, which is induced by antitumor agents and is a limiting factor for cancer chemotherapy, was found that CNG improves the reduced platelet counts in peripheral blood of mice treated by cyclophosphamide. The antitumor efficacy of some antitumor agents in tumor-bearing mice treated with CNG was improved. As CNG is not toxic for higher organism, it is suggested a valuable tool in cancer chemotherapy[3-6].



1

Fig. 1

The total synthesis of the naturally occuring conagenin was published last year by a Japanese group[7]. We are interested in the synthesis of conagenin analogs, which could be suitable for structure-activity relationship studies[8]. Herein we wish to report on the construction of conagenin analogs modified at 3'-carbon atom (**2**, **4**, **6**) and their 2*R*-diastereoisomers (**3**, **5**, **7**, Fig. 2).

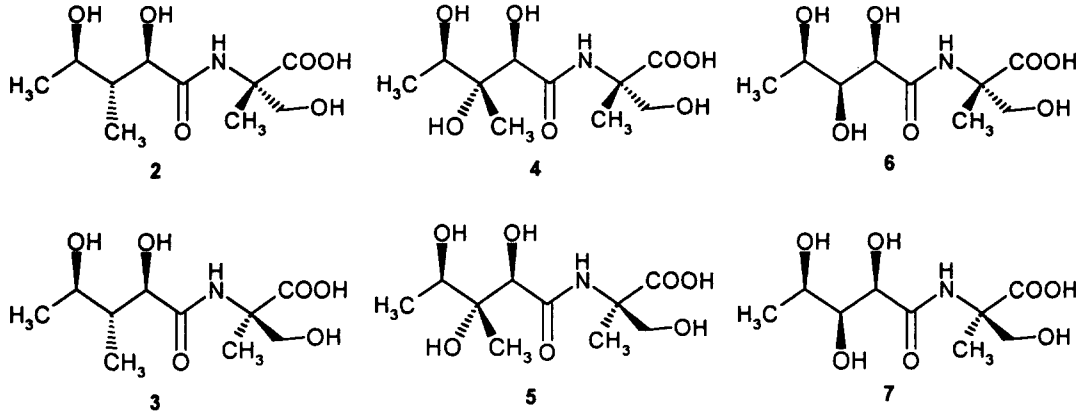


Fig. 2

In all three cases D-xylose was chosen as starting material. For the synthesis of compounds **2**, **3**, **4** and **5** the known 5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose[9] (**8**) was synthesized first. The oxidation of the free secondary OH-group[10] followed by a Wittig reaction with methylene triphenylphosphorane gave the exomethylene derivative **9** (Fig. 3). Saturation of the C-C double bond resulted in 3,5-dideoxy-3-C-methyl-D-*ribo* sugar **10**, which had identical spectroscopical data as of those synthesized by Tronchet et al. *via* a different route[11]. Removal of the isopropylidene group and the mercaptalization underwent in a one pot reaction giving rise to compound **11**. The two secondary OH-groupings had been protected as benzyl ethers before the formyl group of **12** was released by mercury-(II) salt promoted demercaptalization. The resulted unstable aldehyde was oxidized with the aid of the Corey methodology[12]. The syrupy D-ribonic acid derivative **13** was then coupled with (*S*)-O-benzyl-2-methylserine methyl ester[13] by the use of the active ester method affording compound peptide-like **14**. The protective groups were removed *lege artis* leading to 3'-epiconagenin (**2**). To obtain **3** the same procedures were applied for compound **13** and (*R*)-O-benzyl-2-methylserine methyl ester[14].

Compound **10**, a key intermediate in the reaction sequence above, could be synthesized *via* an alternative route (Fig. 4). After oxidation of the 5-deoxy sugar derivative **8** the resulting 3-ulose reacted with methyl magnesium iodide giving rise to the branched-chain compound **18**. The configuration of C-3 was established by NOE measurement and **18** proved to be a D-*ribo* derivative as it could be expected[15]. In order to achieve a radical deoxygenation reaction the tertiary hydroxy group was converted into its xanthate form. The reduction step was carried out under Barton condition[16] to afford **10**. The stereochemical outcome of the reductive deoxygenation can be explained by the shielding of the C-3 position of **19** from the α-side by the bulky dioxolane grouping. Therefore, the intermediate radical can be attacked by the hidrogen atom only from the β-side.

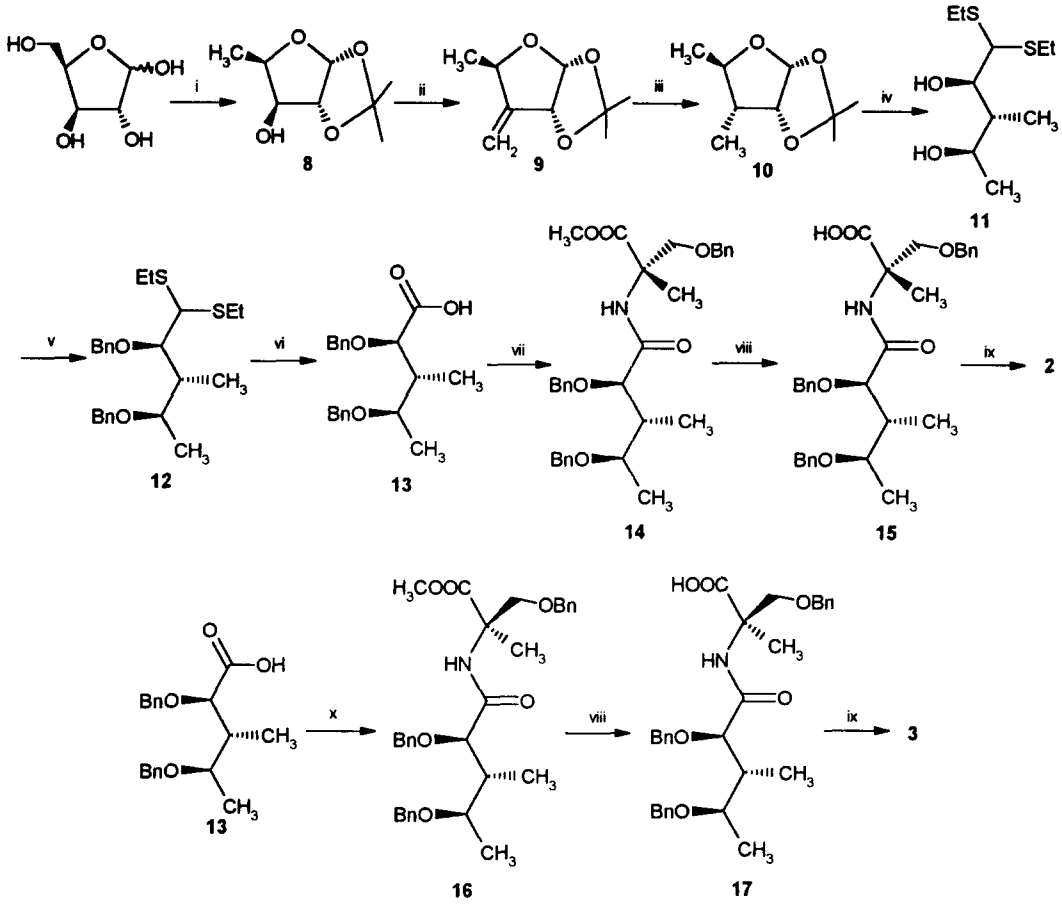


Fig. 3

Conditions: i) ref. 9; ii) 1) $CrO_3 \cdot 2pyr$, Ac_2O, CH_2Cl_2, 2) Ph_3PCH_3Br, nBuLi, THF; iii) cat. H_2, EtOAc; iv) EtSH, cc. HCl; v) BnBr, NaH, Bu_4NI, THF; vi) 1) $HgCl_2$, $CdCO_3$, acetone/water, 2) PDC, DMF; vii) HOBT, DCC, (*S*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2; viii) 1M KOH, dioxane; ix) cat. H_2, MeOH/THF; x) HOBT, DCC, (*R*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2

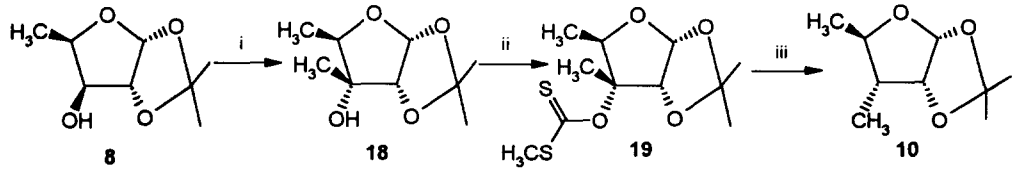


Fig. 4

Conditions: i) 1)$CrO_3 \cdot 2pyr$, Ac_2O, CH_2Cl_2, 2) MeMgI, abs. ether; ii) NaH, CS_2, THF then MeI; iii) Bu_3SnH, AIBN, toluene, Δ

Compound **18** provided the synthesis of branched-chain conagenin analogs (Fig. 5). Mercaptalization with 1,2-ethanedithiol gave better results then ethanethiol. The cyclic mercaptal **20** was treated with benzaldehyde dimethyl acetal in the presence of p-toluenesulfonic acid to yield the benzylidene acetal **21**. Removal of the dithioacetal group with the use of mercury(II) chloride did not work, while careful treatment with boron trifluoride etherate in the presence of mercury(II) oxide[17] released the formyl group and the acetal function survived this condition. Oxidation of the intermediate aldehyde with using of hypochlorite-TEMPO reagent[18] afforded the desired carboxylic acid **22** in moderate yield. Compound **22** was then coupled with both of the protected methylserine enantiomers and the resulted adducts were deprotected under similar conditions as it was described above to give **4** and **5** respectively.

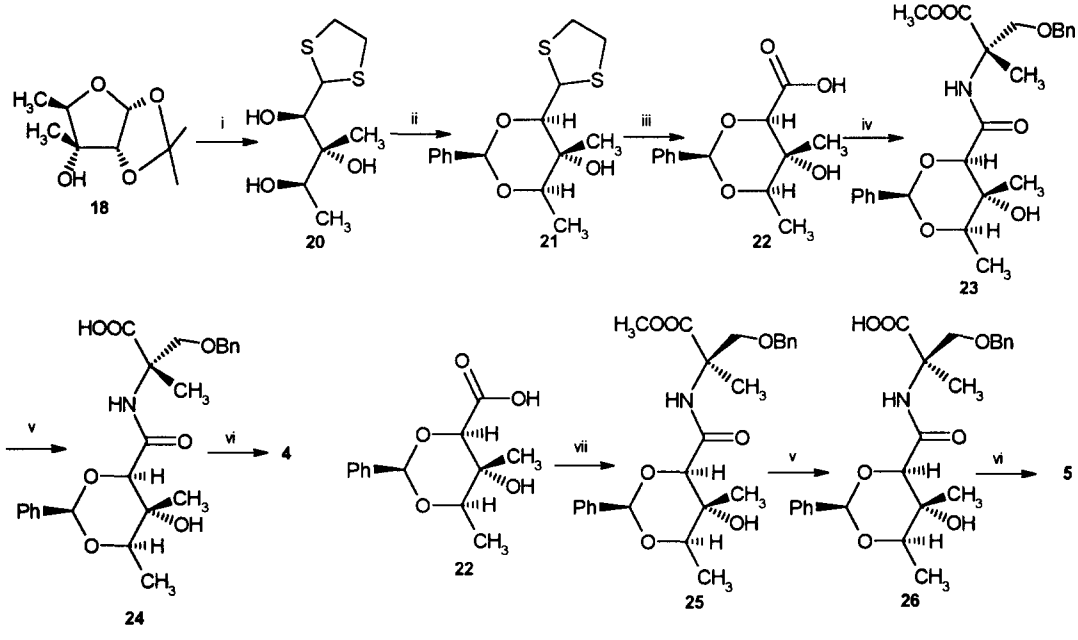


Fig. 5

Conditions: i) $HS(CH_2)_2SH$, cc. HCl, CH_2Cl_2; ii) $PhCH(OCH_3)_2$, pTsOH, CH_2Cl_2; iii) 1) red HgO, $BF_3{\cdot}Et_2O$, THF/water, 2) TEMPO, NaOCl, KBr, CH_2Cl_2/sat. $NaHCO_3$ soln.; iv) HOBT, DCC, (*S*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2; v) 1M KOH, dioxane; vi) cat. H_2, MeOH; vii) HOBT, DCC, (*R*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2

As the yields of the aldehyde→carboxylic acid conversions in both cases were not satisfying, we attempted to perform this step in the very first stage of the reaction sequence. Of course the protective group strategy had to be modified (Fig. 6). Thus, D-xylose was oxidized under electrolytic conditions described by Zinner[19] giving rise to calcium D-xylonate (**27**). The xylonate salt reacted with dimethoxy propane in the presence of methanol and sulfuric acid[20] and the methyl xylonate derivative **28** could be isolated. After selective hydrolysis of the terminal dioxolane ring of **28** the primary OH-group was exchanged with bromine atom[21] affording compound **30**. The reductive debromination of **30** with Raney nickel in hydrogen atmosphere worked

smoothly. The only free hydroxy function in **31** was protected as tetrahydropyranyl ether. Alkaline hydrolysis of the ester group led to the carboxylic acid **33**. Coupling of acid **33** and the amino acids with free base gave rise to compounds **34** and **35**. Removal of the different protective groups was achieved in one pot in the following order: alkaline hydrolysis of the ester, acidic hydrolysis of the isopropylidene acetal and the THP-ether, catalytic hydrogenolysis of the benzyl group. Conagenin analogs **6** and **7** were purified by column chromatography.

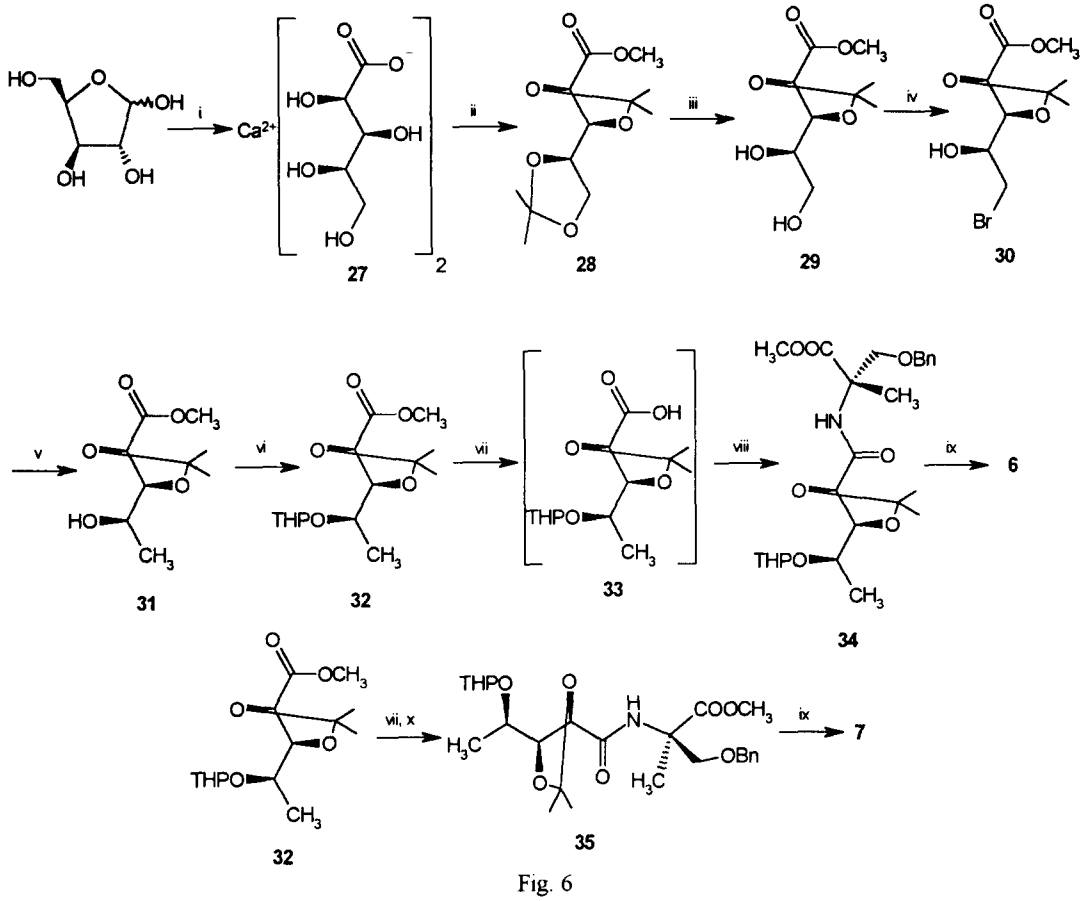


Fig. 6

Conditions: i) ref 19; ii) acetone dimethylacetal, acetone, MeOH, H_2SO_4; iii) 80% AcOH, 60°C; iv) CBr_4, PPh_3, pyridine; v) Raney-Ni, H_2; vi) dihydropyran, pyridinium tosylate, CH_2Cl_2; vii) 1M KOH, dioxane; viii) HOBT, DCC, (*S*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2; ix) 1) 1M KOH, dioxane, 2) 75% TFAA, dioxane, 3) cat. H_2, MeOH; x) HOBT, DCC, (*R*)-O-benzyl-2-methylserine methyl ester, CH_2Cl_2

Compounds **2**, **3** and **7** have been tested for T cell proliferation and lymphokine production. The preliminary results show that **2** (3'-epiconagenin) seems to be more active than conagenin itself, but **3** and **7** have similar activity as of the natural parent compound[22]. Biological investigations of the conagenin analogs prepared by us are in the way in the frame of a joint research program and will be published later in details.

Acknowledgements. This work was supported by grants OTKA 19512 and 19338. The authors thanks to Drs. Z. Dinya, J. Jekő, M. Lipták and P. T. Szabó for the MS measurements and to Dr. Gy. Batta for 2D NMR spectra. One of us (Á. K.-K.) is indebted to the Lajos Kossuth University, Debrecen and the Hungarian Academy of Sciences for fellowships.

EXPERIMENTAL SECTION

Organic extracts were dried over magnesium sulphate. Solutions were concentrated at 35-40 °C (bath) at ca. 17 mmHg. Melting points were determined in capillary tubes and are reported uncorrected. For TLC precoated aluminium-backed plates (Silica gel 60 F_{254}, Merck, layer thickness: 0.2 mm) was used. Compounds were visualized by charring with 5% sulfuric acid in ethanol or spraying with 7% ammonium molibdate in 5% sulfuric acid and heating. Column chromatography: Merck silica gel 60 (0.062-0.200 mm). Preparative TLC was carried out on Merck silica gel 60 F_{254} plates, layer thickness 0.25 mm. The following eluent mixtures were used: A) hexane-ether 9:1; B) hexane→hexane-acetone 50:1; C) hexane-ethyl acetate 1:1; D) hexane-acetone 49:1→19:1; E) hexane-ethyl acetate 4:1 then 1:1, containing 0.5% of acetic acid; F) hexane-ethyl acetate 9:1→7:3; G) hexane-ethyl acetate 1:1, 1% of acetic acid; H) dichloromethane-methanol 1:1, 0.1% of acetic acid; I) hexane-ethyl acetate 85:15; J) hexane→hexane-ethyl acetate 4:1; K) hexane-ethyl acetate 4:1→1:1; L) hexane-ethyl acetate 4:1→3:2; M) hexane-ethyl acetate 4:1; N) hexane-ethyl acetate 19:1→4:1; O) hexane-ethyl acetate 8:2 then 7:3; P) dichloromethane-methanol 4:1→3:7. IR spectra (KBr discs) were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. Specific rotations were measured on a Perkin-Elmer 141 MC polarimeter. ^1H and ^{13}C NMR spectra were recorded on Bruker WP 200 SY, Gemini 200 and Bruker AM 360 instruments; TMS as internal standard. EI and CI (gas: isobutane) mass spectra were obtained on a VG-7035 spectrometer. Thermospray (TSP) mass spectra were recorded on a VG TRIO-2 instrument connected with a Waters 501 HPLC pump in an isocratic mode; samples were dissolved in a 0.1M ammonium acetate buffer/methanol mixture (1:1) and injected into the same solvent system at a flow rate of 1 mL/min; PSP tip interface temperature 210 °C. FAB mass spectra were measured on a VG-7070 spectrometer (matrix: glycerol; gas: Xe). Electrospray (ESP) mass spectrometric measurements were run on FINNIGAN TSQ 7000 triple quadrupole mass spectrometer, samples were introduced into an acetonitrile-water 1:1 solution containing 0.01% trifluoroacetic acid.

3,5-Dideoxy-1,2-O-isopropylidene-3-methylene-α-D-*erythro*-pentofuranose 9

To a solution of dry dichloromethane (50 ml) and dry pyridine (3.76 ml, 46.6 mmol) 2.30 g of CrO_3 (23.0 mmol) was added. The suspension was stirred under exclusion of humidity ($CaCl_2$) for 5 min and a solution of alcohol **8** (0.99 g, 5.69 mmol) in 10 ml of dry dichloromethane then 2.20 ml of acetic anhydride (23.3 mmol) were added dropwise to the suspension. After 20 min the alcohol consumed. The dichloromethane was evaporated and the chromium salt was filtered off on a short silica column eluting with ethyl acetate. The organic solvent was evaporated and the resulting syrup was coevaporated with dry toluene. The crude ulose was dissolved in THF (10 ml) and the solution was added to an ylide suspension prepared previously from 6.18 g of $MePh_3PBr$ (17.3 mmol) in dry THF (75 ml) and 10.0 ml of 1.6 M nBuLi in hexane (16.0 mmol). The reaction mixture was warmed to 55 °C for 2 h under N_2 and quenched after cooling to rt with sat. NH_4Cl solution. The mixture was extracted with ethyl acetate, the organic layer was dried, the solvent was evaporated and the residue was chromatographed with eluent A; 0.56 g of volatile liquid was collected (58%). $[\alpha]_D^{23}$= +127.1 (*c*0.63, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 1.34 (3H, d, $J_{4,5}$= 6 Hz, H-5), 1.37 and 1.53 (2x3H, 2 s, isopropylidene), 4.76 (1H, q, H-4), 4.88 (1H, d, $J_{1,2}$= 4 Hz, H-2), 5.08 and 5.33 (2x1H, 2 d, *J*= 2 Hz, $=CH_2$), 5.82 (1H, d, H-1).

3,5-Dideoxy-1,2-O-isopropylidene-3-methyl-α-D-ribofuranose 10

a) 10% Pd on charcoal (0.10 g) was suspended in ethyl acetate (5 ml) under N_2. Compound **9** (0.83 g, 4.88 mmol) was added to the suspension. The mixture was stirred for 2 h under hydrogen atmosphere. The catalyst was then filtered off and the residue was purified on silica (eluent A) resulting in 0.73 g (87%) of **10** as a

colourless volatile liquid. $[\alpha]_D^{23}$= +33.3 (*c*0.98, $CHCl_3$); lit.[11] $[\alpha]_D$= +36.1 (*c*1.2, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 1.03 (3H, d, $J_{3,Me}$= 6.5 Hz, C(3)-*CH*$_3$), 1.22 (3H, d, $J_{4,5}$= 6 Hz, H-5), 1.31 and 1.59 (2x3H, 2 s, isopropylidene), 1.56 (1H, m, H-3), 3.82 (1H, dq, $J_{3,4}$= 10 Hz, H-4), 4.53 (1H, dd, $J_{2,3}$= 3.5 Hz, H-2), 5.78 (1H, d, $J_{1,2}$= 3.5 Hz, H-1).

b) To a solution of compound **19** (1.05 g, 3.78 mmol) in 25 ml of abs. toluene Bu_3SnH (1.52 ml, 4.83 mmol) and α,α'-azoisobutyronitrile (30 mg) were added. The solution was heated to reflux under N_2 for 2.5 hours. After cooling to rt the toluene was evaporated, the residue was subjected to a short column and eluted with eluent B to yield 563 mg of a colourless volatile liquid (87%), whose spectroscopical data were identical of those described above; $[\alpha]_D^{23}$= +32.9 (*c*0.78, $CHCl_3$).

3,5-Dideoxy-3-methyl-D-ribose diethyl dithioacetal 11

To 3 ml of ethanethiol compound **10** (380 mg, 2.21 mmol) in 1 ml of THF then 0.20 ml of cc. HCl were added. The mixture was stirred for 1 h at rt and was allowed to stand in the refrigerator for 3 days. After diluting with dichloromethane (5 ml) the acid was neutralized with solid Na_2CO_3. The solvents were evaporated and the residue was purified on column (eluent C); colourless syrup (440 mg, 84%). $[\alpha]_D^{23}$= -49.2 (*c*2.25, $CHCl_3$); FAB-MS m/z = 238 $(M)^+$; ^1H NMR (200 MHz, $CDCl_3$ + D_2O): δ 0.87 (3H, d, $J_{3,Me}$= 7 Hz, C(3)-*CH*$_3$), 1.20 (3H, d, $J_{4,5}$= 6 Hz, H-5), 1.28 (6H, t, J= 7.5 Hz, -CH_2-*CH*$_3$), 2.00 (1H, m, H-3), 2.65-2.75 (4H, m, -*CH*$_2$-CH_3), 3.68 (1H, dd, $J_{2,3}$= 8.5 Hz, H-2), 3.86 (1H, dq, $J_{3,4}$= 8 Hz, H-4), 4.03 (1H, d, $J_{1,2}$= 3.5 Hz, H-1); Anal. calcd. for $C_{10}H_{22}O_2S_2$ (238.403) C: 50.38%, H: 9.30%, S: 26.90%, found C: 49.92%, H: 9.22%, S: 26.98%.

2,4-Di-O-benzyl-3,5-dideoxy-3-methyl-D-ribose diethyl dithioacetal 12

To a suspension of NaH (0.35 g, 50% in oil, 7.3 mmol, previously washed with hexane) in 5 ml of dry THF compound **11** (400 mg, 1.68 mmol) in dry THF (8 ml) was dropped under N_2. After stirring for 1 h at rt 0.60 ml of benzyl bromide (5.05 mmol) was added drop by drop followed by 20 mg of tetrabutylammonium iodide. After stirring for 2 days the excess NaH was carefully hydrolized with water and the mixture was extracted with ethyl acetate. The organic solution was dried, the ethyl acetate was evaporated and the residue was chromatographed (eluent D) yielding 682 mg of **12** as a syrup (97%). $[\alpha]_D^{23}$= +15.1 (*c*1.07, $CHCl_3$); EI-MS m/z = 357 $(M+H\text{-}SEt)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 0.95 (3H, d, $J_{3,Me}$= 7 Hz, C(3)-*CH*$_3$), 1.08 (3H, d, $J_{4,5}$= 6 Hz, H-5), 1.23 and 1.27 (2x3H, 2t, *J*= 7 Hz, -CH_2-*CH*$_3$), 2.51 (1H, m, H-3), 2.65-2.75 (4H, m, -*CH*$_2$-CH_3), 3.60 (1H, dd, $J_{2,3}$= 7.5 Hz, H-2), 3.87 (1H, dq, $J_{3,4}$= 4.5 Hz, H-4), 4.05 (1H, d, $J_{1,2}$= 3.5 Hz, H-1), 4.54 (2H, d, -*CH*$_2$Ph), 4.55 and 4.95 (2H, ABq, *J*= 11 Hz, -*CH*$_2$Ph), 7.20-7.40 (10H, m, phenyl); Anal. calcd. for $C_{24}H_{34}O_2S_2$ (418.652) C: 68.86%, H: 8.19%, S: 15.32%, found C: 68.56%, H: 8.16%, S: 15.17%.

2,4-Di-O-benzyl-3,5-dideoxy-3-methyl-D-ribonic acid 13

Compound **12** (1.18 g, 2.82 mmol) was dissolved in a mixture of acetone (18 ml) and water (2 ml). $CdCO_3$ (1.60 g) and $HgCl_2$ (1.60 g, 5.9 mmol) were added to the solution. The suspension was stirred for 4 h at rt. The solid was filtered off, the filtrate was diluted with sat. $NaHCO_3$ solution and the acetone was carefully evaporated. The residue was extracted with ethyl acetate and the combined organic extracts were washed with 10% NaI and sat. $Na_2S_2O_3$ solutions. After drying the ethyl acetate was removed and 0.88 g (2.82 mmol) of syprupy crude aldehyde was obtained. To the solution of the aldehyde in dry DMF (15 ml) pyridinium dichromate (3.18 g, 8.46 mmol) was added under N_2. After stirring for 16 h at rt the dark brown solution was poured into a mixture of ethyl acetate (50 ml) and brine (50 ml). The phases were separated and the water phase was washed with ethyl acetate. The combined organic solutions were extracted with 0.5M NaOH solution and the water phases were acidified with cc. HCl solution to pH= 3. The acidic water phase was extracted with ethyl acetate. Purification on silica gel (eluent E) and coevaporation of the fractions with abs. toluene gave 0.51 g of syrupy acid (55%). $[\alpha]_D^{23}$= +3.38 (*c*0.71, $CHCl_3$); FTIR $\nu_{C=O}$= 1714 cm^{-1}; EI-MS m/z = 329 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$ + D_2O): δ 0.95 (3H, d, $J_{3,Me}$= 7 Hz, C(3)-*CH*$_3$), 1.13 (3H, d, $J_{4,5}$= 6 Hz, H-5), 2.20 (1H, m, H-3), 3.70 (1H, dq, $J_{3,4}$= 8 Hz, H-4), 4.10 (1H, d, $J_{2\ 3}$= 3.5 Hz, H-2), 4.45 and 4.55 (2H, ABq, *J*= 11 Hz, -*CH*$_2$Ph), 4.48 and 4.70 (2H, ABq, *J*= 12 Hz, -*CH*$_2$Ph),7.20-7.40 (10H, m, 2 phenyl); Anal. calcd. for $C_{20}H_{24}O_4$ (328.408) C: 73.15%, H: 7.37%, found C: 73.02%, H: 7.30%.

General procedure for acylating of (S)- and (R)-O-benzyl-2-methylserine methyl esters with acids 13, 22 and 33

Typical procedure: Acid **13** (0.70 mmol) was dissolved in 5 ml of dichloromethane and to this solution 1- hydroxybenzotriazole (0.81 mmol) and 1,3-dicyclohexylcarbodiimide (0.78 mmol) were added. After stirring for 2 h a solution of (*S*)-O-benzyl-2-methylserine methyl ester (1.70 mmol) in dichloromethane (4 ml) was dropped to the reaction mixture. The reaction time was 3 days at rt. The precipitate was filtered off, the filtrate was diluted with dichloromethane and was extracted with 10% $NaHSO_4$, sat. $NaHCO_3$ solutions and brine. Purification on silica column (eluent F) yielded syrupy **14** (83%).

(2*S*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dibenzyloxy-3'-methylpentanoyl]-O-benzyl-2-methylserine methyl ester 14: $[\alpha]_D^{23}$= +20.0 (*c*0.69, $CHCl_3$); FTIR ν= 1746, 1682, 1520, 1504, 1454 cm^{-1}; FAB-MS m/z = 534 $(M)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 0.93 (3H, d, $J_{3',Me}$= 7 Hz, C(3')-*CH*$_3$), 1.10 (3H, d, $J_{4',5'}$= 6 Hz, H-5'), 1.54 (3H, s, C(2)-*CH*$_3$) 2.15 (1H, m, H-3'), 3.72 (3H, s, $COOCH_3$), 3.74 and 3.98 (2H, ABq, *J*= 9 Hz, CH_2-OBn), 3.75 (1H, m, H-4'), 3.95 (1H, d, $J_{2',3'}$= 5.5 Hz, H-2'), 4.43 and 4.59 (2H, ABq, *J*= 11.5 Hz, -CH_2Ph), 4.46-4.52 (4H, m, -CH_2Ph), 7.20-7.35 (15H, m, phenyl), 7.46 (1H, s, CONH); Anal. calcd. for $C_{32}H_{39}NO_6$ (533.663) C: 72.02%, H: 7.37%, N: 2.62%, found C: 71.90%, H: 7.41%, N: 2.55%.

(2*R*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dibenzyloxy-3'-methylpentanoyl]-O-benzyl-2-methylserine methyl ester 16: chromatography: eluent F; yield: 76%; $[\alpha]_D^{23}$= +22.7 (*c*0.93, $CHCl_3$); FTIR ν= 1742, 1682, 1510, 1454 cm^{-1}; EI-MS m/z = 535 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 0.92 (3H, d, $J_{3',Me}$= 7 Hz, C(3')-*CH*$_3$), 1.08 (3H, d, $J_{4',5'}$= 6 Hz, H-5'), 1.55 (3H, s, C(2)-*CH*$_3$) 2.18 (1H, m, H-3'), 3.73 (3H, s, $COOCH_3$), 3.70-3.80 (3H, m, CH_2-OBn and H-4'), 3.97 (1H, d, $J_{2',3'}$= 5.5 Hz, H-2'), 4.40-4.66 (6H, m, -CH_2Ph), 7.20-7.30 (15H, m, phenyl), 7.38 (1H, s, CONH); Anal. calcd. for $C_{32}H_{39}NO_6$ (533.663) C: 72.02%, H: 7.37%, N: 2.62%, found C: 71.91%, H: 7.34%, N: 2.68%.

(2*S*)N-(2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribonyl)-O-benzyl-2-methylserine methyl ester 23: chromatography: eluent L; yield: 77%; $[\alpha]_D^{23}$= +12.8 (*c*0.72, $CHCl_3$); FTIR ν= 1742, 1666, 1530, 1454 cm^{-1}; MS (ESP) m/z 458 $(M+H)^+$; ^1H NMR (360 MHz, $CDCl_3$): δ 1.26 (3H, d, H-5', $J_{4',5'}$= 6.5 Hz), 1.29 (3H, s, C_3-CH_3), 1.59 (3H, s, C_2-CH_3), 3.73 and 3.82 (2H, ABq, CH_2-OBn, *J*= 9.1 Hz), 3.75 (3H, s, $COOCH_3$), 3.84 (1H, q, H-4'), 4.10 (1H, s, H-2'), 4.49 (2H, s, Ph-CH_2O-), 5.68 (1H, s, PhC*H*), 7.18-7.72 (11H, m, 2 phenyl, N*H*). Anal. calcd. for $C_{25}H_{31}NO_7$ (457.523) C: 65.63%, H: 6.83%, N: 3.06%, found C: 65.50%, H: 6.75%, N: 2.98%.

(2*R*)N-(2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribonyl)-O-benzyl-2-methylserine methyl ester 25: chromatography: eluent L; yield: 74%; $[\alpha]_D^{23}$= +31.8 (*c*0.51, $CHCl_3$); FTIR ν= 1742, 1662, 1526, 1454 cm^{-1}; MS (ESP) m/z 458 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28 (3H, d, H-5', $J_{4',5'}$= 6.2 Hz), 1.29 (3H, s, C_3-CH_3), 1.59 (3H, s, C_2-CH_3), 3.72 and 3.81 (2H, ABq, CH_2-OBn, *J*= 9.3 Hz), 3.76 (3H, s, $COOCH_3$), 3.86 (1H, q, H-4'), 4.10 (1H, s, H-2'), 4.50 (2H, s, Ph-CH_2O-), 5.68 (1H, s, PhC*H*), 7.16-7.55 (11H, m, 2 phenyl, N*H*). Anal. calcd. for $C_{25}H_{31}NO_7$ (457.523) C: 65.63%, H: 6.83%, N: 3.06%, found C: 65.55%, H: 6.90%, N: 3.22%.

(2*S*)N-(5-Deoxy-2,3-O-isopropylidene-4-O-tetrahydropyranyl-D-xylonyl)-O-benzyl-2-methylserine methyl ester 34: acid **33** was first prepared *in situ* form ester **32** using 1M KOH solution; chromatography: eluent O; yield: 57% from ester **32**; FTIR ν= 1742, 1682, 1514, 1454 cm^{-1}; CI-MS m/z 480 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$,): [1:1 mixture of diasteroisomers] δ 1.23 and 1.29 (6H, 2d, H-5', $J_{4',5'}$= 6.3 and 6.5 Hz), 1.40-1.81 (24H, m, 2 isopropylidenes, THP), 1.60 (6H, s, 2 C_2-CH_3), 3.52 (2H, m, THP), 3.74 (6H, s, 2 $COOCH_3$), 3.72-4.58 (16H, m, H-2', H-3', H-4', CH_2Ph, CH_2OBn, THP), 4.78 and 4.95 (2H, 2m, THP), 7.23-7.35 (10H, m, Ph), 7.54 (2H, br, N*H*); Anal. calcd. for $C_{25}H_{37}NO_8$ (479.569) C: 62.61%, H: 7.78%, N: 2.92%, found C: 62.49%, H: 7.68%, N: 2.84%.

(2*R*)N-(5-Deoxy-2,3-O-isopropylidene-4-O-tetrahydropyranyl-D-xylonyl)-O-benzyl-2-methylserine methyl ester 35: acid **33** was first prepared *in situ* form ester **32** using 1M KOH solution; chromatography: eluent O; yield: 57% from ester **32**; FTIR ν= 1742, 1680, 1524, 1456 cm^{-1}; CI-MS m/z 480 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$,): [1:1 mixture of diasteroisomers] δ 1.23 and 1.34 (6H, 2d, H-5', $J_{4',5'}$= 6.3 and 6.5 Hz), 1.41-1.82 (24H, m, 2 isopropylidenes, THP), 1.62 and 1.68 (6H, 2s, 2 C_2-CH_3), 3.50 (2H, m, THP), 3.76 and 3.77 (6H, 2s, 2 $COOCH_3$), 3.70-4.08 (10H, m, H-3', H-4', CH_2OBn, THP), 4.22 and 4.54 (2H, 2d, H-2', $J_{2',3'}$= 6.8 and 7.0 Hz), 4.49 (4H, ABq, CH_2Ph), 4.74 and 4.81 (2H, 2m, THP), 7.23-7.36 (10H, m, Ph), 7.57 and 7.59 (2H, 2s,

N*H*); Anal. calcd. for $C_{25}H_{37}NO_8$ (479.569) C: 62.61%, H: 7.78%, N: 2.92%, found C: 62.46%, H: 7.68%, N: 2.82%.

(2*S*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dibenzyloxy-3'-methylpentanoyl]-O-benzyl-2-methylserine 15

Compound **14** (300 mg, 0.56 mmol) was dissolved in dioxane (2 ml) and 0.56 ml of 1M KOH solution was added. After 1.5 h stirring at rt the same amount of base was added again. After 16 h the dioxane was evaporated, the residue was dissolved in water (10 ml) and was extracted with ethyl acetate. The water phase was acidified with cc. HCl to pH= 3 and was extracted three times with warm dichloromethane. After drying the residue was crystallized from hexane-dichloromethane and 160 mg of white powder was obtained. The mother liquor was purified by chromatography (eluent G) and 78 mg of product was collected, altogether 238 mg (82%). Mp: 152-153 °C; $[\alpha]_D^{23}$= +4.0 (*c*0.62, $CHCl_3$); FTIR ν= 3428, 1628, 1508, 1456 cm^{-1}; FAB-MS m/z 520 $(M)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 0.93 (3H, d, $J_{3',Me}$= 7.1 Hz, C(3')-*CH*$_3$), 1.13 (3H, d, $J_{4',5'}$= 6.2 Hz, H-5'), 1.58 (3H, s, C(2)-*CH*$_3$) 2.21 (1H, ddq, H-3'), 3.74 (1H, dq, $J_{3',4'}$= 6.2 Hz, H-4'), 3.74 and 3.77 (2H, ABq, *J*= 9.5 Hz, CH_2-OBn), 4.11 (1H, d, $J_{2',3'}$= 4.2 Hz, H-2'), 4.47-4.57 (6H, m, -CH_2Ph), 7.24-7.33 (15H, m, phenyl), 7.57 (1H, s, CONH); Anal. calcd. for $C_{31}H_{37}NO_6$ (519.636) C: 71.65%, H: 7.18%, N: 2.70%, found C: 71.38%, H: 7.14%, N: 2.74%.

(2*S*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dihydroxy-3'-methylpentanoyl]-2-methylserine 2

Compound **15** (220 mg, 0.42 mmol) was taken up in a mixture of dry methanol (10 ml) and dry THF (3 ml) then 120 mg of 10% Pd/C was added under N_2. The mixture was stirred for 3 days under H_2 atmosphere. After filtering off the catalyst and evaporation of the solvents the residue was purified on preparative TLC (eluent H). After coevaporation with abs. toluene 69 mg of product could be obtained (67%). Mp 105-106 °C (dec.); $[\alpha]_D^{23}$= +25.8 (*c*1.25, MeOH); MS (TSP) m/z 250 $(M+H)^+$; ^1H NMR (400 MHz, MeOD): δ 0.94 (3H, d, $J_{3',Me}$= 7.1 Hz, 3'-*CH*$_3$), 1.16 (3H, d, $J_{4',5'}$= 6.4 Hz, 5'-H), 1.43 (3H, s, 2-*CH*$_3$), 1.95 (1H, m, 3'-H), 3.82 (1H, dq, $J_{3',4'}$= 7.1 Hz, 4'-H), 3.83 and 4.04 (2H, ABq, *J*= 10.9 Hz, *CH*$_2$OH), 4.08 (1H, d, $J_{2',3'}$= 4.0 Hz, 2'-H); ^{13}C NMR (90 Hz, MeOD): δ 180.5 and 175.9 (carbonyl and amide), 75.0 (2'-C), 69.8 (4'-C), 66.7 (CH_2OH), 63.1 (2-C), 45.8 (3'-C), 21.4 (5'-C), 20.5 (2-CH_3), 13.1 (3'-*C*H_3); Anal. calcd. for $C_{10}H_{19}NO_6$ (249.263) C: 48.19%, H: 7.68%, N: 5.62%, found C: 47.98%, H: 7.40%, N: 5.50%.

(2*R*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dibenzyloxy-3'-methylpentanoyl]-O-benzyl-2-methylserine 17

A similar method was performed as at compound **15** starting from 300 mg (0.56 mmol) of **16**; 240 mg of white powder (82%). Mp: 137-138 °C; $[\alpha]_D^{23}$= +22.0 (*c*0.75, $CHCl_3$); FTIR ν= 3444, 1636, 1558, 1456 cm^{-1}; EI-MS m/z = 428 $(M-Bn)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 0.92 (3H, d, $J_{3',Me}$= 7.0 Hz, C(3')-*CH*$_3$), 1.12 (3H, d, $J_{4',5'}$= 6.1 Hz, H-5'), 1.41 (3H, s, C(2)-*CH*$_3$) 2.18 (1H, m, H-3'), 3.61 and 3.83 (2H, ABq, *J*= 9.3 Hz, CH_2-OBn), 3.73 (1H, dq, $J_{3',4'}$= 7.8 Hz, H-4'), 4.08 (1H, d, $J_{2',3'}$= 4.3 Hz, H-2'), 4.45-4.56 (6H, m, -CH_2Ph), 7.20-7.30 (15H, m, phenyl), 7.50 (1H, s, CONH) Anal. calcd. for $C_{31}H_{37}NO_6$ (519.636) C: 71.65%, H: 7.18%, N: 2.70%, found C: 71.51%, H: 7.08%, N: 2.62%.

(2*R*)N-[(2'*R*,3'*R*,4'*R*)2',4'-Dihydroxy-3'-methylpentanoyl]-2-methylserine 3

Compound **17** (210 mg, 0.40 mmol) in 10 ml of dry methanol was similarly hydrogenolized and purified as it was described at **2**; 95 mg of product (95%). Mp 168-170 °C (subl.); $[\alpha]_D^{23}$= +6.0 (*c*1.06, MeOH); FAB-MS m/z 250 $(M+H)^+$; ^1H NMR (200 MHz, D_2O): δ 0.92 (3H, d, $J_{3',Me}$= 7.1 Hz, 3'-C*H*$_3$), 1.17 (3H, d, $J_{4',5'}$= 6.4 Hz, 5'-H), 1.42 (3H, s, 2-C*H*$_3$), 2.01 (1H, m, 3'-H), 3.83 and 3.94 (2H, ABq, *J*= 11.3 Hz, C*H*$_2$OH), 3.91 (1H, dq, $J_{3',4'}$= 7.1 Hz, 4'-H), 4.10 (1H, d, $J_{2',3'}$= 4.8 Hz, 2'-H); ^{13}C NMR (90 MHz, D_2O): δ 180.2 and 175.4 (carbonyl and amide), 74.9 (2'-C), 69.2 (4'-C), 65.9 (*C*H_2OH), 63.2 (2-C), 44.4 (3'-C), 20.3 (5'-C), 20.2 (2-*C*H_3), 12.3 (3'-*C*H_3); Anal. calcd. for $C_{10}H_{19}NO_6$ (249.263) C: 48.19%, H: 7.68%, N: 5.62%, found C: 48.05%, H: 7.70%, N: 5.47%.

5-Deoxy-1,2-O-isopropylidene-3-C-methyl-α-D-ribofuranose 18

Compound **8** (2.00 g, 11.49 mmol) was oxidized with CrO_3·2pyr complex as it was described in the case of **9**. The crude ulose dissolved in 20 ml of abs. ether was allowed to react with MeMgI (prepared from Mg (1.4 g, 7.6 mmol) and MeI (2.40 ml, 38.5 mmol) in 50 ml of abs. ether previously). After 20 min. the reaction was carefully quenched with sat. NH_4Cl solution. The phases were separated and the water solution was washed 3 times with ether. After drying, chromatographic purification (eluent I) and recrystallization from hexane 1.27 g (59%) of white needles could be isolated. Mp 114-115 °C; $[\alpha]_D^{23}$ = +27.5 (*c*0.61, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.13 (3H, s, C_3-C*H*$_3$), 1.19 (3H, d, H-5, $J_{3,4}$= 6.5 Hz), 1.36 and 1.58 (6H, 2s, isopropylidene), 2.51 (1H, s, O*H*), 3.88 (1H, q, H-4), 4.13 (1H, d, H-2, $J_{1,2}$= 4 Hz), 5.73 (1H, d, H-1); Anal. calcd. for $C_9H_{16}O_4$ (188.223) C: 57.43%, H: 8.57%, found C: 57.48%, H: 8.70%.

5-Deoxy-1,2-O-isopropylidene-3-C-methyl-3-O-methylthio(thiocarbonyl)-α-D-ribofuranose 19

To a suspension of NaH (0.62 g, ≈60% in oil, 15.5 mmol, previously washed with hexane) in THF (5 ml) a solution of compound **18** (1.17 g, 6.22 mmol) in 13 ml of THF was added under N_2. After 30 min. CS_2 (2.0 ml, 33.3 mmol) was dropped to the reaction mixture and after 1 h of MeI (1.0 ml, 16.0 mmol) was added. The reaction was quenched with several drops of methanol, the suspension was diluted with water and extracted with dichloromethane. The organic phase was washed with 10% $NaHSO_4$, sat. $NaHCO_3$ solutions and brine. Purification by column chromatography (eluent J) afforded 1.55 g (90%) of **19**. Mp. 93-94 °C (hexane); $[\alpha]_D^{23}$= +142 (*c*0.59, $CHCl_3$); FAB-MS m/z 279 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.27 (3H, d, H-5 $J_{4,5}$= 6.5 Hz), 1.32 and 1.54 (6H, 2s, isopropylidene), 1.58 (3H, s, C_3-C*H*$_3$), 2.52 (3H, s, SC*H*$_3$), 4.30 (1H, q, H-4), 5.16 (1H, d, H-2, $J_{1,2}$= 4 Hz), 5.72 (1H, d, H-1); Anal. calcd. for $C_{11}H_{18}O_4S_2$ (278.381) C: 47.46%, H: 6.52%, S: 23.03%, found C: 47.46%, H: 6.42%, S: 22.52%.

5-Deoxy-3-C-methyl-D-ribose ethylene dithioacetal 20

Compound **18** (0.96 mg, 5.11 mmol) was dissolved in 15 ml of dichloromethane then 1,2-ethanedithiol (1.0 ml, 11.9 mmol) and cc. HCl (0.6 ml) were added. The emulsion was allowed to stand in refrigerator for a weekend. The acid was then neutralized with OH-form resin, the solvent was evaporated and the residue was purified by column chromatography (eluent K) to give 1.06 g of colourless syrup (92%). $[\alpha]_D^{23}$= -15.0 (*c*0.93, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$ + D_2O): δ 1.25 (3H, s, C_3-C*H*$_3$), 1.26 (3H, d, H-5, $J_{4,5}$= 6 Hz), 3.20-3.30 (4H, m, ethylene), 3.70 (1H, d, H-2, $J_{1,2}$= 4 Hz), 3.83 (1H, q, H-4), 5.03 (1H, d, H-1); Anal. calcd. for $C_8H_{16}O_3S_2$ (224.332) C: 42.83%, H: 7.19%, S: 28.58%, found C: 42.84%, H: 7.15%, S: 25.77%.

2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribose ethylene dithioacetal 21

To a solution of compound **20** (0.80 g, 3.57 mmol) in 15 ml of dichloromethane benzaldehyde dimethyl acetal (0.59 ml, 3.93 mmol) and catalytic amount of toluene-4-sulfonic acid were added. The reaction mixture was allowed to stand at rt for 20 hours. The acid was neutralized with one drop of triethylamine, the mixture was concentrated and purified by column chromatography (eluent K) to afford 0.95 g of white solid (85%). Mp 107-108 °C (hexane); $[\alpha]_D^{23}$= -98.7 (*c*1.13, $CHCl_3$); MS (ESP) m/z 313 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (3H, d, H-5, $J_{4,5}$= 6.3 Hz), 1.31 (3H, s, C_3-C*H*$_3$), 3.10-3.37 (5H, m, ethylene, O*H*), 3.60 (1H, d, H-2, $J_{1,2}$= 9.5 Hz), 3.73 (1H, q, H-4), 4.59 (1H, d, H-1), 5.64 (1H, s, PhC*H*), 7.34-7.52 (5H, m, phenyl). Anal. calcd. for $C_{15}H_{20}O_3S_2$ (312.441) C: 57.66%, H: 6.45%, S: 20.52%, found C: 57.90%, H: 6.40%, S: 21.25%.

2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribonic acid 22

To a suspension of red HgO (1.13 g, 5.22 mmol) in 15 ml of THF and 0.5 ml of water BF_3·Et_2O (0.66 ml, 5.24 mmol) was dropped under ice-water cooling. A solution of mercaptal **22** (740 mg, 2.37 mmol) in 5 ml of THF was added dropwise to the vigorous stirred orange suspension. The cooling bath was then removed and the reaction mixture was allowed to warm to rt. After 6 h BF_3·Et_2O (0.10 ml, 0.79 mmol) was added again and the mixture was stirred overnight. After dilution with sat. $NaHCO_3$ solution white precipitation appeared which

was filtered off. The filtrate was diluted with ethyl acetate (100 ml) and washed with 10% NaI and sat. $Na_2S_2O_3$ solutions. After drying the crude aldehyde was purified on a short column (hexane-ethyl acetate 4:1→3:2) in order to remove a small amount of the by-product benzaldehyde yielding 0.49 g of syrup. The syrupy aldehyde was taken up in 8 ml of dichloromethane then sat. $NaHCO_3$ solution (6 ml), KBr (12 mg, 10.2 μmol), TEMPO (21 mg, 13.5 μmol) and at ice-water cooling Clorox® (Henkel, 6 ml) were added. After 1 h stirring at rt the solution was extracted with ethyl acetate and the water phase was acidified with citric acid to pH= 3-4. The acidic solution was extracted with dichloromethane 10 times (10 ml each). The combined organic layers were dried, concentrated and the residue was recrystallized from hexane-dichloromethane to give 310 mg of white solid (52%). Mp. 127-128 °C; $[\alpha]_D^{23}$= -0.7 (*c*1.23, MeOH); FTIR $\nu_{C=O}$= 1718 cm^{-1}; MS (ESP) m/z 253 $(M+H)^+$; ^1H NMR (360 MHz, $CDCl_3$): δ 1.32 (3H, d, H-5, $J_{4,5}$= 6.3 Hz), 1.33 (3H, s, C_3-CH_3), 3.90 (1H, q, H-4), 4.33 (1H, s, H-2,), 5.72 (1H, s, PhC*H*), 7.40-7.54 (5H, m, phenyl). Anal. calcd. for $C_{13}H_{16}O_5$ (252.266) C: 61.90%, H: 6.39%, found C: 61.71%, H: 6.29%.

(2*S*)N-(2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribonyl)-O-benzyl-2-methylserine 24

Compound **23** (150 mg, 0.33 mmol) in 3 ml of dioxane was hydrolized with 1M KOH solution (1.0 ml) as at compound **15** and 104 mg of white solid (71%) was obtained. Mp. 56-57 °C; $[\alpha]_D^{23}$= +11.9 (*c*0.57, $CHCl_3$); MS (ESP) m/z 444 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28 (3H, s, C_3-CH_3), 1.29 (3H, d, H-5', $J_{4',5'}$= 6.2 Hz), 1.59 (3H, s, C_2-CH_3), 3.74 and 3.83 (2H, ABq, CH_2-OBn, *J*= 9.2 Hz), 3.87 (1H, q, H-4'), 4.16 (1H, s, H-2'), 4.49 and 4.57 (2H, ABq, Ph-CH_2O-, *J*= 12.1 Hz), 5.68 (1H, s, PhC*H*), 7.16-7.53 (11H, m, 2 phenyl, N*H*); Anal. calcd. for $C_{24}H_{29}NO_7$ (443.496) C: 65.00%, H: 6.59%, N: 3.16%, found C: 64.85%, H: 6.51%, N: 3.10%.

(2*S*)N-[(2'*R*,3'*R*,4'*R*)2',3',4'-Trihydroxy-3'-methylpentanoyl]-2-methylserine 4

Compound **24** (60 mg, 13.5 μmol) was dissolved in 3 ml of abs. methanol and hydrogenolized over 20 mg of Pd/C for 60 hours. The catalyst was filtered off and the solvents were evaporated. The residue was partitioned between dist. water and ether and the water phase was liophylized to afford 31 mg (87%) of a very hygroscopic white solid. $[\alpha]_D^{23}$= +24.7 (*c*1.03, H_2O); MS (ESP) m/z 266 $(M+H)^+$; ^1H NMR (200 MHz, D_2O): δ 1.20 (3H, d, H-5', $J_{4',5'}$= 6.0 Hz), 1.21 (3H, s, C_3-CH_3), 1.47 (3H, s, C_2-CH_3), 3.83-3.95 (3H, m, CH_2-OH and H-4'), 4.10 (1H, s, H-2'); ^{13}C NMR (50 MHz, D_2O): δ 177.3 and 174.1 (carbonyl and amide), 76.6 (C-3'), 75.5 (C-2'), 70.9 (C-4'), 64.9 (CH_2OH), 61.4 (C-2), 19.9 (C_2-CH_3), 17.6 and 16.6 (C_3-CH_3 and C-5').

(2*R*)N-(2,4-O-Benzylidene-5-deoxy-3-C-methyl-D-ribonyl)-O-benzyl-2-methylserine 26

Compound **25** (68 mg, 14.9 μmol) in 2 ml of dioxane was hydrolized with 1M KOH solution (0.5 ml) as at compound **24** and 55 mg of white solid (83%) was isolated. Mp. 177-178 °C; $[\alpha]_D^{23}$= +27.8 (*c*1.15, $CHCl_3$); MS (ESP) m/z 444 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 1.26 (3H, s, C_3-CH_3), 1.28 (3H, d, H-5', $J_{4',5'}$= 6.3 Hz), 1.62 (3H, s, C_2-CH_3), 3.80 (2H, s, CH_2-OBn), 3.84 (1H, q, H-4'), 4.17 (1H, s, H-2'), 4.52 (2H, s, Ph-CH_2O-), 5.69 (1H, s, PhC*H*), 7.18-7.54 (11H, m, 2 phenyl, N*H*). Anal. calcd. for $C_{24}H_{29}NO_7$ (443.496) C: 65.00%, H: 6.59%, N: 3.16%, found C: 65.11%, H: 6.66%, N: 3.22%.

(2*R*)N-[(2'*R*,3'*R*,4'*R*)2',3',4'-Trihydroxy-3'-methylpentanoyl]-2-methylserine 5

Compound **26** (53 mg, 12.0 μmol) in 5 ml of methanol was hydrogenolized over 20 mg of Pd/C as it at compound **4**. Liophylization gave 27 mg (85%) of a hygroscopic white solid. $[\alpha]_D^{23}$= +12.6 (*c*2.49, H_2O); MS (ESP) m/z 266 $(M+H)^+$; ^1H NMR (200 MHz, D_2O): δ 1.19 (3H, d, H-5', $J_{4',5'}$= 6.1 Hz), 1.21 (3H, s, C_3-CH_3), 1.48 (3H, s, C_2-CH_3), 3.88 (3H, m, CH_2-OH and H-4'), 4.10 (1H, s, H-2'); ^{13}C NMR (50 MHz, D_2O): δ 177.2 and 174.2 (carbonyl and amide), 76.6 (C-3'), 75.4 (C-2'), 71.0 (C-4'), 65.2 (CH_2OH), 61.5 (C-2), 19.8 (C_2-CH_3), 17.7 and 16.6 (C_3-CH_3 and C-5').

Methyl 2,3:4,5-di-O-isopropylidene-D-xylonate 28

Calcium D-xylonate[19] (**27**, 70.0 g, 18.9 mmol) was suspended in a mixture of acetone (500 ml), dimethoxy propane (300 ml) and methanol (70 ml) then 12 ml of cc. H_2SO_4 was added to the suspension drop by drop under vigorous stirring. After stirring at rt for 3 days the acid was carefully neutralized by adding of solid Na_2CO_3, the precipitated salts were filtered off, the filtrate was concentrated and the resulting crude syrup was dissolved in ethyl acetate (300 ml). This solution was extracted with sat. $NaHCO_3$, dried and the organic solvent was evaporated. Vacuum distillation (100-105 °C/0.5 mmHg) gave 63.0 g of a syrup (64%). $[\alpha]_D^{23}$= -20.0 (*c*6.01, acetone), lit.[23] $[\alpha]_D^{20}$= -36.3 (6.67 acetone); FTIR $\nu_{C=O}$= 1760 cm^{-1}; MS (TSP) m/z 261 $(M+H)^+$, 278 $(M+NH_4)^+$; ^1H NMR (360 MHz, $CDCl_3$): δ 1.40, 1.45 and 1.50 (12H, 3s, 2 isopropylidene), 3.81 (3H, s, $COOCH_3$), 3.92 (1H, dd, H-5a, $J_{4,5a}$= 6.8 Hz, $J_{5a,5b}$= 8.5 Hz), 4.09 (1H, dd, H-3, $J_{3,4}$= 4.6 Hz), 4.29 (1H, dt, H-4), 4.44 (1H, d, H-2, $J_{2,3}$= 7.2 Hz); Anal. calcd. for $C_{12}H_{20}O_6$ (260.286) C: 55.37%, H: 7.74%, found C: 55.26%, H: 7.83%.

Methyl 2,3-O-isopropylidene-D-xylonate 29

A solution of compound **28** (63.0 g, 24.2 mmol) in acetic acid (300 ml) and water (100 ml) was heated at 60 °C for 1.5 hours. The solvents were then evaporated and the residue was coevaporated with toluene three times. Recrystallization from hexane-dichloromethane then hexane-ether afforded 29.0 g of a solid material (55%). Mp. 95-96 °C; $[\alpha]_D^{23}$= -16.1 (*c*1.04, $CHCl_3$); FAB-MS m/z 221 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$ + D_2O): δ 1.44 and 1.50 (6H, 2s, isopropylidene), 3.75-3.85 (6H, m, $COOCH_3$, H-4, H-5a, H-5b), 4.23 (1H, dd, H-3, $J_{3,4}$= 2.5 Hz), 4.62 (1H, d, H-2, $J_{2,3}$= 7.5 Hz); Anal. calcd. for $C_9H_{16}O_6$ (220.221) C: 49.09%, H: 7.32%, found C: 48.95%, H: 7.21%.

Methyl 5-bromo-5-deoxy-2,3-O-isopropylidene-D-xylonate 30

Compound **29** (10.0 g, 45.5 mmol) and triphenylphosphin (29.7 g, 11.3 mmol) was dissolved in 300 ml of dry pyridine and the mixture was stirred with molecular sieves (≈10 g) under N_2 for 1 hour. The suspension was then cooled to about 0 °C and carbon tetrabromide (37.7 g, 11.4 mmol) was added. The reaction mixture was allowed to warm to rt then was heated at 55 °C for 3.5 hours. The excess of reagent was destroyed by adding of methanol (10 ml), pyridine was evaporated (codistillation with toluene). The residue was taken up in ether and the precipitate was filtered off. Chromatographic purification (eluent M) yielded 11.7 g (91%) of a colourless syrup. $[\alpha]_D^{23}$= -20.2 (*c*1.03, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 and 1.50 (6H, 2s, isopropylidene), 2.35-2.50 (1H, br, O*H*), 3.50 (2H, d, CH_2Br), 3.82 (3H, s, $COOCH_3$), 3.99 (1H, m, H-4), 4.38 (1H, dd, H-3, $J_{3,4}$=2 Hz), 4.56 (1H, d, H-2, $J_{2,3}$= 7 Hz); Anal. calcd. for $C_9H_{15}BrO_5$ (283.119) C:38.18%, H: 5.34%, Br: 28.22%, found C: 37.96%, H: 5.26%, Br: 27.64%.

Methyl 5-deoxy-2,3-O-isopropylidene-D-xylonate 31

The mixture of compound **30** (9.2 g, 32.5 mmol), prewashed wet Raney-nickel (≈22 g), methanol (250 ml) and triethylamine (5.0 ml, 35.9 mmol) was stirred under H_2 for 2.5 days. The solid was filtered off, the filtrate was concentrated and the residue was partitioned between ethyl acetate and brine. The organic layer was dried and 5.5 g of syrup (83%) could be isolated after column chromatography (eluent M). $[\alpha]_D^{23}$= -15.3 (*c*0.78, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 1.30 (3H, d, H-5, $J_{4,5}$= 6.5 Hz), 1.44 and 1.50 (6H, 2s, isopropylidene), 2.05-2.15 (1H, br, O*H*), 3.81 (3H, s, $COOCH_3$), 3.90 (1H, m, H-4), 4.06 (1H, dd, H-3, $J_{3,4}$=4.5 Hz), 4.47 (1H, d, H-2, $J_{2,3}$= 7 Hz); Anal. calcd. for $C_9H_{16}O_5$ (204.222) C: 52.92%, H: 7.90%, found C: 52.70%, H: 7.94%.

Methyl 5-deoxy-2,3-O-isopropylidene-4-O-tetrahydropyranyl-D-xylonate 32

Compound **31** (130 mg, 0.64 mmol) was dissolved in a mixture of dichloromethane (2.5 ml) and dihydropyran (0.12 ml, 1.32 mmol). Catalytic amount of pyridinium tosylate was then added and the mixtured was stirred at rt for overnight. The catalyst was neutralized with one drop of triethylamine and the solvents were evaporated. Purification by column chromatography (eluent N) gave 170 mg of syrupy **32** (92%). EI-MS m/z 273 $(M-CH_3)^+$; ^1H NMR (200 MHz, $CDCl_3$,): [1:1 mixture of diasteroisomers] δ 1.23 and 1.30 (6H, 2d, H-5,

$J_{4,5}$= 6.5 Hz in both cases), 1.40-1.80 (24H, m, 2 isopropylidenes, THP), 3.50 (2H, m, THP), 3.78 (6H, s, 2 $COOCH_3$), 3.80-4.10 (6H, m, H-4, THP), 4.25 (2H, m, H-3), 4.38 and 4.65 (2H, 2d, H-2, $J_{2,3}$= 7 Hz in both cases), 4.78 (2H, m, THP); Anal. calcd. for $C_{14}H_{24}O_6$ (288.340) C: 58.32%, H: 8.39%, found C: 58.20%, H: 8.31%.

(2*S*)N-[(2'*R*,3'*S*,4'*R*)2',3',4'-Trihydroxypentanoyl]-2-methylserine 6

Compound **34** (423 mg, 0.88 mmol) was hydrolyzed with 1M KOH solution (2 ml) in dioxane (4 ml) at rt for 20 hours. The intermidiate crude acid was treated with 67% trifluoroacetic acid solution (2.0 ml) in 3 ml of dioxane under ice-water cooling. The mixture was allowed to warm to rt and stirred for 8 hours. The solvents were then evaporated and the residue was codistilled with toluene three times. The resulting material was hydrogenolized in methanol (5 ml) over 100 mg of Pd/C for a weekend. Chromatographic purification on silica (eluent P) afforded 112 mg of hygroscopic product (53%). $[\alpha]_D^{23}$= +14.9 (*c*1.12, H_2O); MS (ESP) m/z 266 $(M+H)^+$; ^{1}H NMR (200 MHz, D_2O): δ 1.24 (3H, d, H-5', $J_{4',5'}$= 6.3 Hz), 1.43 (3H, s, C_2-CH_3), 3.72 (1H, d, H-4', $J_{3',4'}$= 7.2 Hz), 3.87 and 4.02 (2H, ABq, C_2-CH_2OH, *J*= 11.3 Hz), 3.92 (1H, m, H-4'), 4.22 (1H, s, H-2'); ^{13}C NMR (50 MHz, D_2O): δ 179.1 and 173.4 (carbonyl and amide), 75.6 (C-3'), 72.2 (C-2'), 68.4 (C-4'), 64.3 (CH_2OH), 62.5 (C-2), 19.7 (C_2-CH_3), 18.1 (C-5').

(2*R*)N-[(2'*R*,3'*S*,4'*R*)2',3',4'-Trihydroxypentanoyl]-2-methylserine 7

The protective groups of compound **35** (128 mg, 0.27 mmol) were removed in the same order under similar conditions as it was described at compound **6** to yield 44 mg of hygroscopic product (65%). $[\alpha]_D^{23}$= +9.2 (*c*1.70, H_2O); MS (ESP) m/z 266 $(M+H)^+$; ^{1}H NMR (200 MHz, D_2O): δ 1.24 (3H, d, H-5', $J_{4',5'}$= 6.4 Hz), 1.44 (3H, s, C_2-CH_3), 3.73 (1H, dd, H-4', $J_{3',4'}$= 7.1 Hz), 3.85 and 3.97 (2H, ABq, C_2-CH_2OH, *J*= 11.4 Hz), 3.91 (1H, m, H-4'), 4.22 (1H, d, H-2', $J_{2',3'}$= 1.9 Hz); ^{13}C NMR (90 MHz, D_2O): δ 178.9 and 173.4 (carbonyl and amide), 75.5 (C-3'), 72.1 (C-2'), 68.3 (C-4'), 64.6 (CH_2OH), 62.5 (C-2), 19.3 (C_2-CH_3), 18.0 (C-5').

REFERENCES AND NOTES

1. Ishizuka, M.; Kawatsu, M.; Yamashita, T.; Ueno, M.; Takeuchi, T. *Int. J. Immunopharmac.* **1995**, *17*, 133-139.
2. Yamashita, T.; Iijima, M.; Nakamura, H.; Isshiki, K.; Naganawa, H.; Hattori, S.; Hamada, M.; Ishizuka, M.; Takeuchi, T.; Iitaka, Y. *J. Antibiotics* **1991**, *44*, 557-559.
3. Kawatsu, M.; Yamashita, T.; Osono, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiotics* **1993**, *46*, 1687-1691.
4. Kawatsu, M.; Yamashita, T.; Osono, M.; Masuda, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiotics* **1993**, *46*, 1692-1698.
5. Kawatsu, M.; Yamashita, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiotics* **1994**, *47*, 1123-1129.
6. Kawatsu, M.; Yamashita, T.; Ishizuka, M.; Takeuchi, T. *J. Antibiotics* **1995**, *48*, 222-225.
7. Hatakeyama, S.; Fukuyama, H.; Mukugi, Y.; Irie, H. *Tetrahedron Lett.* **1996**, *37*, 4047-4050.
8. Kovács-Kulyassa, Á.; Herczegh, P.; Sztaricskai, F. *Tetrahedron Lett.* **1996**, *37*, 2499-2502.
9. Snyder, J. R.; Serianni, A. S. *Carbohydr. Res.* **1987**, *163*, 169-188.
10. Garegg, P. J.; Samuelsson, B. *Carbohydr. Res.* **1978**, *67*, 267-270.
11. Tronchet, J. M. J.; Graf, R. *Helv. Chim. Acta* **1972**, *55*, 1141-1150.
12. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399-402.

13. Mickos, H.; Sundberg, K.; Lüning, B. *Acta Chem. Scand.* **1992**, *46*, 989-993.
14. Groth, U.; Chiang Y.-c.; Schöllkopf, U. *Liebigs Ann. Chem.* **1982**, 1756-1757.
15. a) Nutt, R. F,; Dickinson, R. J.; Holly, F. W.; Walton, E. *J. Org. Chem.* **1968**, *33*, 1789-1795.
    b) Horwitz, J. P.; Mody, N.; Gasser, R. *J. Org. Chem.* **1970**, *35*, 2335-2339.
    c) Rosenthal, A.; Sprinzl, M. *Carbohyd. Res.* **1971**, *16*, 337-342.
16. a) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574-1585.
    b) Barton, D. H. R.; Parekh, S. I.; Tse, C.-L. *Tetrahedron Lett.* **1993**, *34*, 2733-2736.
17. Vedejs, E.; Fuchs, P. L. *J. Org. Chem.* **1971**, *36*, 366-367.
18. e. g. Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181-1184 and references cited therein.
19. Zinner, H.; Voigt, H.; Voigt, J. *Carbohyd. Res.* **1968**, *7*, 38-55.
20. Regeling, H.; de Rouville, E.; Chittenden, G. J. F. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 461-464.
21. Whistler, R. L.; Anisuzzaman, K. M. *Methods in Carbohydrate Chemistry, Vol. VIII*, **1980**, 227-231.
22. Professor M. Ishizuka, personal communication
23. Zinner, H.; Nehring, H. *Z. Chem.* **1970**, *10*, 394-395.

(*Received in UK* 9 *July* 1997; *accepted* 31 *July* 1997)