Carbohydrate Research, 239 (1993) 133–142
Elsevier Science Publishers B.V., Amsterdam

Photolysis of 2-azi-2-deoxy-D-*arabino*-hexitol and analogous hexitols; the ineffectiveness of certain carbohydrate diazirines as photoaffinity labels

Jochen Lehmann and Stefan Petry

Institut für Organische Chemie und Biochemie der Universität Freiburg, Albertstraße 21, D-7800 Freiburg i.Br. (FRG)

(Received January 18th, 1992; accepted in final form July 21st, 1992)

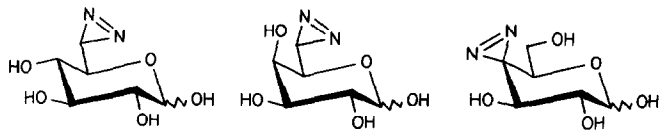
ABSTRACT

Three diazirines 2-azi-2-deoxy-D-*arabino*-hexitol (**4**), its 1-deoxy analogue (**5**), and 3-azi-3,6-dideoxy-L-*xylo*-hexitol (**9**) were synthesised and their products of photolysis analysed by TLC. Diazirine **4** gave exclusively 2-deoxy-D-*arabino*-hexose (**12**), **5** gave predominantly 1,2-dideoxy-D-*erythro*-3-hexulose (**10**) and 1-deoxy-D-glucitol (**11**), and **9** did not yield any main product. Carrying out the irradiation of **4** in D_2O gave selectively (2*S*)-2-deoxy-D-*arabino*-(2-^{2}H)hexose (**12a**). The results indicate that photolysis of a diazirine flanked by a hydroxymethyl group, as in compound **4**, leads to a rapid and stereoselective intramolecular reaction of the intermediate. This may be an explanation of why compound **4** is ineffective as a photoaffinity reagent for mannitol permease (D-mannitol-specific enzyme II) of the *E. coli* phosphotransferase system for which it is a substrate. A secondary hydroxymethylene group has a less pronounced effect and still allows some reaction with the medium.

INTRODUCTION

Derivatives of monosaccharides carrying photolabile diazirino groups, such as 6-azi-6-deoxy-D-glucose (**1**) and -D-galactose (**2**), as well as 4-azi-4-deoxy-D-*xylo*-hexose (**3**), have been prepared in order to label chemically the hexose transporting system in red blood cells[1]. All three compounds were specifically transported into and out of the red blood cells and had inhibition constants comparable to those of the natural hexoses[2]. Although radiolabelled **1** was found to label membrane protein in red blood cells specifically, though weakly, compound **3** was totally ineffective [3].

Correspondence to: Professor Dr. J. Lehmann, Institut für Organische Chemie und Biochemie der Universität Freiburg, Albertstraße 21, D-7800 Freiburg i.Br., Germany.



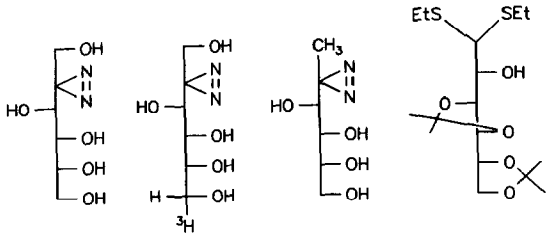
1 2 3

Recently, we prepared 2-azi-2-deoxy-D-*arabino*-hexitol (**4**) for affinity labelling of the mannitol transporting system in *E. coli*. Although the compound is transported effectively[4], its radioactive analogue **4a** failed to react with the transporting system on irradiation. Many efficient chemical modifications of different carbohydrate-binding proteins have been achieved using diazirines of carbohydrate derivatives[5], so the failure in two cases seems especially surprising. Comparing the structure of all diazirines that we have so far successfully applied[5] with those that failed (**3** and **4**), the latter share a structural element not present in all the others. Compounds **3** and **4** carry a free hydroxyl group on a carbon atom adjacent to the diazirino group. By preparing, in addition to compound **4**, two more diazirines of hexitols and analysing the products of photolysis, we planned to show why compounds like **4** are incapable of reacting with a receptor protein after activation by photolysis.

RESULTS AND DISCUSSION

2-Azi-2-deoxy-D-*arabino*-hexitol (**4**) was synthesised from D-fructose diethyl dithioacetal, via its 5,6-*O*-isopropylidene derivative. Liberation of the keto group was followed by its conversion into a diazirino group[6]. Deblocking yielded compound **4** in good yield.

The 1-deoxy analogue of **4**, 2-azi-1,2-dideoxy-D-*arabino*-hexitol (**5**) can be derived from 3,4:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal (**6**), which is a byproduct of the synthesis[7] of 2,3:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal (**7**). The dithioacetal **6**, after reductive desulfurisation by Raney nickel and subsequent oxidation of the 2-hydroxyl group, yields 1-deoxy-3,4:5,6-di-*O*-isopropylidene-D-fructose which can be converted into the diazirine **8**; deblocking then gives the diazirine **5**. Similarly, **7** is the starting material for the preparation of 3-azi-3,6-dideoxy-L-*xylo*-hexitol (**9**), a positional isomer of **5**.



4 4a 5 6

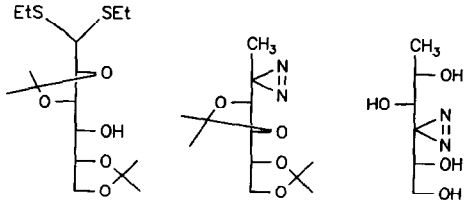
Whereas the diazirine **4** competitively inhibits D-mannitol transport in *E. coli*, **5** is only a feeble inhibitor and **9** does not inhibit at all. None of these compounds (**4**, **5**, and **9**) irreversibly deactivates D-mannitol transport on irradiation. Equally, no radioactivity was incorporated into the proteins of the transporting system when **4a** was used as photoaffinity reagent[4].

This ineffectiveness as a photoaffinity label is not surprising with compound **9**, since it has no affinity for the transporting system. However, it is surprising that compounds **4** and **5**, being competitive inhibitors, do not effect any chemical modification.

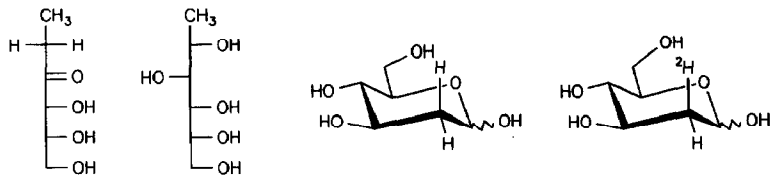
Although several pathways for the transformations of an irradiated diazirine are possible[8], the products in a protic solvent like water are always of the same type and well-defined. With *C*-alkylated diazirines, two kinds of main reaction products are formed in water: unsaturated compounds derived from intramolecular rearrangements of a carbene as a likely primary intermediate of photolysis, or alcohols as intermolecular insertion products[9].

The latter type of reaction can, in photoaffinity labelling, lead to chemical modification of a receptor protein if there is sufficient proximity between reactants. The reason why compound **4** fails as a photoaffinity label could be an exclusive and very rapid intramolecular reaction of the carbene.

When compounds **4**, **5**, and **9** are irradiated in water, the half-life of photolytic decay is similar in all three cases. TLC analysis of the resulting solutions, however, reveals striking differences. Compound **9** yields a very complex mixture of at least 5 products, none of which could be isolated. Compound **5** gave 7 detectable products, but two main products **10** and **11** as shown by ^{1}H NMR spectra. Compound **4** was quantitatively converted into 2-deoxy-D-*arabino*-hexose (**12**), identified by comparison with an authentic sample. This chemical specificity is accompanied by a stereoselective incorporation of one deuterium into the 2-position of **12** when photolysis of **4** is carried out in deuterated water.



7 8 9



10* 11 12 12a

It is apparent from this result that an intramolecular reaction assisted by the neighbouring primary hydroxyl group is kinetically dominating. This excludes any intermolecular reaction, except the addition of a proton which could well be stereoselectively donated by a hydroxyl group of the substrate. The assistance of a neighbouring hydroxyl group in the rapid reaction of a carbene is also demonstrated by the formation of **10** from compound **5**. The two major reaction products derived from compound **5** exclude the participation of the methyl group to any significant extent. Since no primary hydroxyl group is present, the secondary hydroxyl group in **5** assists the reaction and is converted into a ketone group, analogous to the formation of an aldehyde group from the primary hydroxyl group neighbouring the carbene derived from compound **4**. Part of the carbene reacts by insertion to yield compound **11**. From these findings, it appears that hydroxyl groups flanking photochemically produced carbenes favour an intramolecular reaction. Kinetic assistance by a primary hydroxyl group seems to be preferred, so that any intermolecular insertions are suppressed.

EXPERIMENTAL

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck). Flash chromatography[10] used ICN-silica 32-63 (ICN Biomedicals). HPLC was performed with an LKB HPLC controller, two pumps, a variable wavelength monitor, and a Shimadzu C-R2AX integrator. Radioactive material was detected either radioautographically (Agfa-Geveart Curix X-ray film) or with a Berthold automatic TLC-linear analyzer LB 2821. Radioactive samples in solution were assayed in a Berthold BF-815 liquid scintillation counter, using Quickszint 501 (Zinsser). Melting points were measured with a Büchi apparatus and are uncorrected. Optical rotations were obtained with a Schmidt & Haensch Polartronic I polarimeter. Photolysis of compounds **4**, **5**, and **9** was performed with a Rayonet-RPR-100-reactor equipped with 16 RPR 3500 A lamps. UV spectra and extinction coefficients were recorded with a Zeiss PMQ II spectrophotometer. ^1H NMR spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CDCl_3$ (internal Me_4Si). IR spectra were recorded with a Perkin–Elmer 1320 spectrophotometer. Elemental analyses were obtained with a Perkin–Elmer 240 analyser.

* May exist in equilibrium with cyclic forms, but can be converted under mild conditions into the acyclic triacetate

5,6-O-Isopropylidene-D-fructose diethyl dithioacetal (**13**).—D-Fructose diethyl dithioacetal[11] (35 g, 0.107 mol) was dissolved in DMF (500 mL). After addition of *p*-toluenesulfonic acid monohydrate (1.2 g, 5.8 mmol) to the stirred solution, 2-methoxypropene (10.53 g, 0.14 mol) was added at 25°C dropwise over a period of 15 min. The reaction mixture was left for 2 h, then poured into cold satd aq $NaHCO_3$ (500 ml), and **13** was extracted from the aqueous solution with ether (4 × 200 mL). The organic layer was dried (Na_2SO_4) and, after filtration, the solvent was evaporated in vacuo to yield an oily residue, which was purified by flash chromatography (3 : 1 cyclohexane–EtOAc). Chromatographically uniform **13**, R_f 0.62 (5 : 1 EtOAc–MeOH), crystallised from MeOH–ether (27.8 g, 70%); mp 58–60°C; $[\alpha]_D^{23}$ −128° (*c* 1.1, $CHCl_3$); ^{1}H NMR data: δ 1.27 (t, 6 H, CH_2CH_3), 1.35, 1.43 (2 s, 6 H, CMe_2), 2.72 (m, 4 H, S-CH_2), 3.22 (d, 1 H, $J_{3,4}$ 6.5 Hz, H-3), 3.59 (s, 3 H, OH), 3.91 (m, 2 H, H-4,5), 4.10 (m, 4 H, H-1a,1b,6a,6b). Anal. Calcd for $C_{13}H_{26}O_5S_2$: C, 47.85; H, 7.98; S, 19.63. Found: C, 47.66; H, 8.23; S, 19.83.

5,6-O-Isopropylidene-D-fructose (**14**).—The dithioacetal **13** (6 g, 18.4 mmol) was dissolved in acetonitrile (40 mL) and HgO (7.8 g, 36 mmol) was added to this solution. With stirring, a solution of $HgCl_2$ (9.8 g, 36 mmol) in acetonitrile (40 mL) was then added slowly. Stirring was continued (~ 2 h) until no more starting material could be detected by TLC. After removing insoluble material by filtration, the filtrate and washings were evaporated in vacuo. The residue was purified by flash chromatography (5 : 1 EtOAc–MeOH) to give **14** as a colourless oil (2.4 g, 59%), which decomposed rapidly and had to be converted without further purification; R_f 0.52 (5 : 1 EtOAc–MeOH); ν_{max}^{film} 1740 (C=O) cm^{-1}.

2-Azi-2-deoxy-5,6-O-isopropylidene-D-arabino-hexitol (**15**).—Into a solution of **14** (1.0 g, 4.55 mmol) in dry MeOH (100 mL), dry NH_3 was condensed at −20°C until the volume of the solution had increased by ~ 20%. A solution of hydroxylamine-*O*-sulfonic acid (750 mg, 7.7 mmol) in dry MeOH (25 mL) was then added dropwise. Stirring at −20°C was continued for 2 h and the mixture then allowed to reach 25°C slowly overnight. Precipitated ammonium sulfate was removed by filtration. Excess of NH_3 was removed by evaporating the solution in vacuo to a volume of ~ 25 mL. This solution was then made up with dry MeOH to 50 mL and triethylamine (5 mL) was added. Oxidation of the diaziridine was then carried out by adding iodine at 0°C until the red-brown colour persisted for at least 15 min. After stirring at 25°C for 30 min, the solvent was removed by evaporation in vacuo. Flash chromatography (1 : 2 cyclohexane–EtOAc) of the dark-red residue yielded compound **15** (428 mg, 40.5%), isolated as a colourless oil; R_f 0.6 (5 : 1 EtOAc–MeOH).

1,3,4-Tri-O-acetyl-2-azi-2-deoxy-5,6-O-isopropylidene-D-arabino-hexitol (**16**).—Compound **15** (500 mg, 2.16 mmol) was acetylated and worked up in the conventional way, using pyridine (10 mL) and acetic anhydride (5 mL). Flash chromatography (5 : 1 cyclohexane–EtOAc) of the residue gave **16** as a colourless oil (527 mg, 68%); R_f 0.49 (1 : 1 cyclohexane–EtOAc); ^{1}H NMR data: δ 1.32, 1.43 (2 s, 6 H, CMe_2), 2.10, 2.13, 2.17 (3 s, 9 H, 3 OAc), 3.81 (dd, 1 H, $J_{5,6a}$ 5, $J_{6a,6b}$ 9 Hz, H-6a),

3.97 (m, 3 H, H-1a,1b,6b), 4.10 (m, 1 H, $J_{4,5}$ 7.5 Hz, H-5), 5.19 (d, 1 H, $J_{3,4}$ 3 Hz, H-3), 5.42 (dd, 1 H, H-4).

2-Azi-2-deoxy-D-arabino-*hexitol* (**4**).—Compound **15** (486 mg, 2.09 mmol) was dissolved at 25°C in aq 30% acetic acid and the hydrolysis monitored by TLC. After 10 h, the starting material had disappeared. Solvent was removed by codistillation with toluene in vacuo. The remaining oil was purified by flash chromatography (5 : 1 EtOAc–MeOH). Compound **4** crystallised from EtOH–ether (200 mg, 50%); R_f 0.26 (5 : 1 EtOAc–MeOH), 0.49 (7 : 2 : 1 EtOAc–MeOH–H_2O); mp 102–105°C, $[\alpha]_D^{23}$ −104° (*c* 1.1, H_2O); λ_{max} 335 nm (ϵ 53); $t_{1/2}$ 12 min (decay on irradiation at 350 nm). Anal. Calcd for $C_6H_{12}N_2O_5$: C, 37.55; H, 6.30; N, 14.57. Found: C, 37.59; H, 6.33; N, 14.46.

1,3,4,5,6-Penta-O-*acetyl-2-azi-2-deoxy*-D-arabino-*hexitol* (**17**).—Compound **4** (15 mg, 0.05 mmol) was acetylated and worked up as described for compound **16**. Final purification of crude **17** was carried out by flash chromatography (2 : 1 cyclohexane–EtOAc) to yield a colourless oil (15 mg, 74.6%); R_f 0.26 (2 : 1 cyclohexane–EtOAc); ^{1}H NMR data: δ 2.06 (s, 3 H, OAc), 2.07 (2 s, 6 H, 2 OAc), 2.13, 2.20 (2 s, 6 H, 2 OAc), 3.85 (d, 1 H, $J_{1a,1b}$ 13.5 Hz, H-1a), 4.10 (d, 1 H, H-1b), 4.17 (m, 2 H, $J_{5,6a}$ 3, $J_{5,6b}$ 4.5, $J_{6a,6b}$ 12 Hz, H-6a,6b), 5.13 (ddd, 1 H, $J_{4,5}$ 9 Hz, H-5), 5.19 (d, 1 H, $J_{3,4}$ 3 Hz, H-3), 5.63 (dd, 1 H, H-4).

1,3,4-Tri-O-*acetyl-2-azi-2-deoxy*-D-arabino-*hexitol* (**18**).—Compound **16** (500 mg, 1.4 mmol) was submitted to mild hydrolysis at 25°C in 30% aq acetic acid (10 mL). The stirred suspension eventually became clear. After 48 h, water (20 mL) was added and acetic acid was carefully neutralised by addition of solid $NaHCO_3$. Then the aqueous mixture was extracted with CH_2Cl_2 (3 × 30 mL), the organic layer was washed with water (30 mL), dried (Na_2SO_4), and evaporated in vacuo, and the residue was purified by flash chromatography (1 : 2 cyclohexane–EtOAc) to yield **18** as a colourless oil (369 mg, 83%); R_f 0.2 (1 : 2 cyclohexane–EtOAc). Since there is danger of acetyl-migration, the compound cannot be stored.

1,3,4,5-Tetra-O-*acetyl-2-azi-2-deoxy-6*-O-*triphenylmethyl*-D-arabino-*hexitol* (**19**). —Compound **18** (550 mg, 1.57 mmol) was dissolved in pyridine (10 mL) containing triphenylmethyl chloride (1.1 g, 4 mmol) and stirred at 40°C. After ~ 4 h, no more starting material could be detected by TLC, and pyridine was then removed by codistillation with toluene in vacuo. Flash chromatography (2 : 1 cyclohexane–EtOAc) of the residue yielded a colourless oil, R_f 0.64 (1 : 2 cyclohexane–EtOAc), which was immediately acetylated and worked up as described for compound **16**. Final purification by flash chromatography (3 : 1 cyclohexane–EtOAc) yielded **19** as a colourless oil (580 mg, 64%); R_f 0.37 (3 : 1 cyclohexane–EtOAc).

1,3,4,5-Tetra-O-*acetyl-2-azi-2-deoxy*-D-arabino-*hexitol* (**20**).—Compound **19** (250 mg, 0.43 mmol) was dissolved in dry acetonitrile (5 mL). Sodium iodide (226 mg, 1.5 mmol) and chlorotrimethylsilane (0.195 mL) were added at 0°C under stirring. After 15 min, ice-cold water (10 mL) and 10% aq Na_2SO_3 (10 mL) were added, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The dried (Na_2SO_4) organic solution was evaporated in vacuo and the residue purified by flash

chromatography (1:1 cyclohexane–EtOAc) to yield **20** as a colourless oil (100 mg, 65%). Acetylation, as described for **16**, yielded a product which was identical with compound **17**.

1,3,4,5,6-Penta-O-acetyl-2-azi-2-deoxy-D-arabino-(6-^{3}H)hexitol (**17a**).—Compound **20** (200 mg, 0.6 mmol) was dissolved in dry CH_2Cl_2 and stirred overnight at 25°C with pyridinium chlorochromate. The black reaction mixture was submitted to flash chromatography (1:1 cyclohexane–EtOAc), and the resulting product was immediately taken up in dry MeOH (16 mL). Part of the solution (2 mL) was treated with methanolic M NaOMe (3 drops) and subsequently added to a fresh sample of NaB^3H_4 (~ 100 mCi; 69.6 Ci/mmol). The reaction mixture was left for 30 min, then, after evaporation in vacuo, submitted to flash chromatography (5:1 EtOAc–MeOH). The product associated with the main radioactivity (~ 30 mCi) was reacetylated and worked up in the usual manner. The radioactive compound **20** was uniform by TLC and indistinguishable from **17** obtained by per-*O*-acetylation of **4**; R_f 0.26 (2:1 cyclohexane–EtOAc). Equally, the deacetylated (Zemplén method) **4a** was uniform and indistinguishable by TLC from **4**; R_f 0.26 (5:1 EtOAc–MeOH). Compound **4a** was used as such for photoaffinity labelling experiments.

Determination of purity of radioactive compound **4a** *by cocrystallisation with* **4**.—Pure, crystalline **4** (50 mg) was dissolved in MeOH (0.5 mL) containing **4a** (11.9×10^7 cpm). Crystallisation was induced by careful addition of ether. Crystals were collected (40 mg) and the radioactivity of an aliquot was determined. The crystalline, radioactive sample (38 mg) was again submitted to crystallisation and again an aliquot of the crystalline sample counted for radioactivity. Radioactivity per 50 mg of **4** was, after subsequent crystallisations: 0, 11.9×10^7 cpm; 1, 3.6×10^7 cpm; 2, 3.6×10^7 cpm. Therefore, only 30.3% of the total radioactivity was associated with **4**. It is likely that the rest is associated with epimers of **4** formed by isomerisation of oxidised compound **20** under the basic conditions used in the reduction with NaB^3H_4.

1-Deoxy-3,4:5,6-di-O-isopropylidene-D-*glucitol* (**21**).—A solution of 3,4:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal[7] (**6**; 4 g, 10.62 mmol) in EtOH (150 mL) was treated with Raney nickel (100 mL) for 3 h under reflux. The mixture was filtered and the residue extracted with hot EtOH (100 mL). Filtrate and washings were combined and the solvent was removed in vacuo. Flash chromatography (1:2 cyclohexane–EtOAc) of the residue yielded **21**, isolated as a colourless oil (2.4 g, 91%); R_f 0.26 (1:2 cyclohexane–EtOAc); $[\alpha]_D^{23}$ +17.5° (*c* 1, EtOAc); ^{1}H NMR data: δ 1.27 (d, 3 H, $J_{1,2}$ 6 Hz, CH_3), 1.34, 1.38 (2 s, 6 H, CMe_2), 1.42 (d, 6 H, 2 CMe_2), 2.43 (d, 1 H, OH), 3.87 (m, 3 H, H-4,6a,6b), 3.96 (dd, 1 H, H-5). Anal. Calcd for $C_{12}H_{22}O_5$: C, 58.50; H, 9.00. Found: C, 58.07; H, 8.88.

1-Deoxy-3,4:5,6-di-O-isopropylidene-D-*fructose* (**22**).—Under anhydrous conditions, dicyclohexylcarbodiimide (3.5 g, 17 mmol), H_3PO_4 (180 mg, 1.4 mmol), and 4A molecular sieve were added to a solution of compound **21** (1.4 g, 5.6 mmol) in Me_2SO (50 mL). The mixture was left for 15 h at 25°C. After addition of water

(100 mL), **22** was extracted with $CHCl_3$ (3 × 50 mL). The organic layers were combined and dried (Na_2SO_4). After filtration and evaporation of the solvent, the remaining yellow oil was purified by flash chromatography (1:3 cyclohexane–EtOAc) to yield **22** as a colourless oil (1 g, 73%); R_f 0.45 (2:1 cyclohexane–EtOAc); $[\alpha]_D^{23}$ +6.5° (*c* 1.1, EtOAc); ^{1}H NMR data: δ 1.34, 1.37, 1.43, 1.47 (4 s, 12 H, 2 CMe_2), 2.31 (s, 3 H, CH_3), 3.98 (ddd, 1 H, H-6a), 4.08–4.21 (m, 3 H, H-4,5,6b), 4.35 (d, 1 H, $J_{3,4}$ 6 Hz, H-3). Anal. Calcd for $C_{12}H_{20}O_5$: C, 58.99; H, 8.25. Found: C, 59.14; H, 8.47.

*2-Azi-1,2-dideoxy-3,4:5,6-di-*O*-isopropylidene-*D*-arabino-hexitol* (**8**).—A solution of **22** (850 mg, 3.5 mmol) in MeOH (100 mL) was treated with NH_3 as described for compound **15**. A solution of hydroxylamine-*O*-sulfonic acid (600 mg, 5.3 mmol) in MeOH (25 mL) was added dropwise. After oxidation with I_2, the resulting red oil was finally purified by flash chromatography (10:1 cyclohexane–EtOAc) to yield **8** as a colourless oil (471 mg, 52.2%); R_f 0.55 (2:1 cyclohexane—EtOAc); ^{1}H NMR data: δ 1.13 (s, 3 H, CH_3), 1.30, 1.32, 1.37, 1.53 (4 s, 12 H, 2 CMe_2), 3.73 (m, 2 H), 3.93–4.10 (m, 2 H), 4.15 (m, 1 H, H-3); λ_{max} 340 nm.

*3,4,5,6-Tetra-*O*-acetyl-2-azi-1,2-dideoxy-*D*-arabino-hexitol* (**23**).—Compound **8** (471 mg, 2.69 mmol) was hydrolysed at 25°C with 80% trifluoroacetic acid (6 mL). The reaction was monitored by TLC (27:2:1 EtOAc–MeOH–H_2O). After 4 h, only one product (R_f 0.3) could be detected. Trifluoroacetic acid was removed in vacuo and the residue was acetylated and worked up as described for **16**. The resulting oil (R_f 0.76) was purified by flash chromatography (3:1 cyclohexane–EtOAc) to yield a colourless oil (480 mg, 79.7%), which crystallised from diethyl ether (3 mL) to give **23** (350 mg, 58%); mp 76°C; ^{1}H NMR data: δ 1.11 (s, 3 H, CH_3), 2.04 (s, 3 H, OAc), 2.08 (2 s, 6 H, 2 OAc), 2.24 (s, 3 H, OAc), 4.2 (dd, 2 H, H-6a,6b), 4.95 (d, 1 H, *J* 3 Hz, H-3), 5.14 (m, 1 H, H-5), 5.63 (dd, 1 H, H-4). Anal. Calcd for $C_{14}H_{20}N_2O$: C, 48.84; H, 5.86; N, 8.13. Found: C, 48.40; H, 5.77; N, 7.63.

*2-Azi-1,2-dideoxy-*D*-arabino-hexitol* (**5**).—Compound **23** (320 mg, 0.93 mmol) was dissolved in dry MeOH (10 mL) and three drops of methanolic M NaOMe were added. After 1 h, the reaction mixture was homogeneous by TLC (R_f 0.30; 27:2:1 EtOAc–MeOH–H_2O) and the solution was filtered through silica gel (MeOH). The solvent was evaporated in vacuo to yield an oil (143 mg, 87%) which crystallised from EtOH (1 mL) to give **5** as colourless crystals (98 mg, 60%); $[\alpha]_D^{23}$ −24° (*c* 0.9, MeOH); mp 140°C; λ_{max} 340 nm (ϵ 47); $t_{1/2}$ 3 min (decay on irradiation at 350 nm).

*1-Deoxy-2,3:5,6-di-*O*-isopropylidene-*D*-glucitol* (**24**).—A solution of 2,3:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal[7] (**7**; 2 g, 5.32 mmol) in EtOH (150 mL) was treated with Raney nickel (50 mL) for 1 h under reflux. The mixture was filtered and worked up as described for **21**. The resulting yellow oil was purified by flash chromatography (3:1 cyclohexane–EtOAc) to yield **24** as a light-yellow oil (1 g, 76%); R_f 0.25 (2:1 cyclohexane–EtOAc); $[\alpha]_D^{23}$ −13° (*c* 1.1, $CHCl_3$); ^{1}H NMR data: δ 1.31 (d, 3 H, $J_{1,2}$ 3 Hz, CH_3), 1.36 (s, 3 H, CMe_2), 1.40 (s, 6 H, CMe_2), 1.46 (s, 3 H, CMe_2), 2.49 (d, 1 H, OH), 3.40 (ddd, 1 H, H-4), 3.77 (dd, 1 H, H-6a),

3.97–4.2 (m, 4 H, H-2,3,5,6b). Anal. Calcd for $C_{12}H_{22}O_5$: C, 58.50; H, 9.00. Found: C, 58.17; H, 8.87.

*6-Deoxy-1,2:4,5-di-O-isopropylidene-*L*-xylo-3-hexulose* (**25**).—A solution of compound **24** (9.5 g, 38.2 mmol), H_3PO_4 (1 g, 10 mmol), dicyclohexylcarbodiimide (23.7 g, 115 mmol), and 4A molecular sieve in Me_2SO (250 mL) was stirred at 25°C for 48 h under anhydrous conditions. A solution of oxalic acid (6.9 g, 76.6 mmol) in MeOH (50 mL) was added dropwise over a period of 45 min. After addition of water (300 mL), 1,3-dicyclohexylurea was separated by filtration and the filtrate worked up as described for **22**. The residue was purified by flash chromatography (3:1 cyclohexane–EtOAc) to yield **25** as a colourless oil (5 g, 51%); R_f 0.44 (2:1 cyclohexane–EtOAc); $[\alpha]_D^{23}$ +19° (*c* 1, EtOAc); ^{1}H NMR data: δ 1.41 (d, 3 H, $J_{1,2}$ 3 Hz, CH_3), 1.44 (s, 6 H, CMe_2), 1.45, 1.49 (2 s, 6 H, CMe_2), 4.02 (dd, 1 H, $J_{1a,2}$ 6, $J_{1a,1b}$ 9 Hz, H-1a), 4.15 (m, 2 H, H-4,5), 4.33 (dd, 1 H, *J* 7.5 Hz, H-1b), 4.9 (dd, 1 H, H-2). Anal. Calcd for $C_{12}H_{20}O_5$: C, 58.99; H, 8.25. Found: C, 57.50; H, 8.24.

*6-Deoxy-*L*-xylo-3-hexulose* (**26**).—Compound **25** (4 g, 16.4 mmol) was hydrolysed (15 h) with trifluoroacetic acid (80%, 10 mL) and worked up as described for **23**. Flash chromatography (5:1 EtOAc–MeOH) of the residue yielded **26** as a colourless oil (1.7 g, 62%); R_f 0.5 (7:2:1 EtOAc–MeOH–H_2O); ν_{max}^{film} 3500–3000 (OH), 1720 (C=O) cm^{-1}.

*1,2,4,5-Tetra-*O*-acetyl-3-azi-3,6-dideoxy-*L*-xylo-hexitol* (**27**).—A solution of **26** (1.5 g, 9.14 mmol) in MeOH (100 mL) was cooled to −40°C and NH_3 was condensed until the volume increased by ~20%. At −25°C a solution of hydroxylamine-*O*-sulfonic acid (1.58 g, 14 mmol) in MeOH (25 mL) was added dropwise. The mixture was treated as described for compound **15** and the residue acetylated with 2:1 acetic anhydride–pyridine (30 mL). Workup was carried out as described for **16**. Flash chromatography (3:1 cyclohexane–EtOAc) of the residue gave **27** as a colourless oil (0.9 g, 34%); R_f 0.32 (2:1 cyclohexane–EtOAc); ^{1}H NMR data: δ 1.27 (d, 3 H, $J_{5,6}$ 6 Hz, 3 H-6), 2.07 (2 s, 6 H, 2 OAc), 2.1 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 3.76 (dd, 1 H, $J_{1a,1b}$ 12, $J_{1a,2}$ 7.5 Hz, H-1a), 4.14 (dd, 1 H, $J_{1b,2}$ 4.5 Hz, H-1b), 4.88–5.02 (m, 2 H, $J_{4,5}$ 3 Hz, H-4,5), 5.29 (dd, 1 H, H-2).

*3-Azi-3,6-dideoxy-*L*-xylo-hexitol* (**9**).—Compound **27** (500 mg, 1.45 mmol) was deacetylated as described for compound **5** to obtain a colourless oil, which was purified by chromatography on Sephadex G 25-M and freeze-dried to yield **9** as a colourless oil (214 mg, 83%); R_f 0.30 (27:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +27° (*c* 1.1, MeOH); λ_{max} 330 nm (ϵ 42); $t_{1/2}$ 11 min (decay on irradiation at 350 nm). Anal. Calcd for $C_6H_{12}N_2O_4$: C, 40.91; H, 6.86; N, 15.90. Found: C, 39.50; H, 6.84; N, 14.66.

*Irradiation of 2-azi-2-deoxy-*D*-arabino-hexitol* (**4**).—A solution of **4** (25 mg, 0.13 mmol) in H_2O (2 mL) was irradiated for 1 h. The reaction was monitored by TLC (7:2:1 EtOAc–MeOH–H_2O). Compound **4** (R_f 0.49) was converted into a single new product (R_f 0.43). After evaporation in vacuo, the residue was purified by flash chromatography (5:1 EtOAc–MeOH) to yield a colourless oil (17 mg, 77%), which was indistinguishable by TLC (5:1 EtOAc–MeOH; 27:2:1 EtOAc–

MeOH–H_2O; 7:2:1 EtOAc–MeOH–H_2O) from 2-deoxy-D-*arabino*-hexopyranose. The product was acetylated as described for compound **16**, and the ^1H NMR data were compared with those of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranose, derived from commercial 2-deoxy-D-*arabino*-hexopyranose by acetylation. The ^1H NMR data corresponded.

When **4** was irradiated in D_2O, one deuterium was incorporated into 2-deoxy-D-*arabino*-hexose in the axial position 2 to give (2*S*)-2-deoxy-D-*arabino*-(2-^2H)hexose (**12a**), as shown by ^1H NMR data of the corresponding acetylated product: δ 2.1 (m, 12 H, 4 OAc), 2.30 (m, 1 H, H-2), 3.76 (m, 1 H, H-5), 4.08 (dd, 1 H, $J_{5,6a}$ 2, $J_{6a,6b}$ 12 Hz, H-6a), 4.32 (dd, 1 H, $J_{5,6b}$ 4.5 Hz, H-6b), 5.07 (m, 2 H, H-3,4), 5.8 (d, 0.5 H, $J_{1,2}$ 2 Hz, H-1β), 6.27 (d, 0.5 H, $J_{1,2}$ 1.5 Hz, H-1α).

Irradiation of 2-azi-1,2-dideoxy-D-arabino-*hexitol* (**5**) *and 3-azi-3,6-dideoxy*-L-xylo-*hexitol* (**9**).—As described for compound **4**, solutions of **5** (50 mg, 0.28 mmol) and **9** (50 mg, 0.28 mmol) in H_2O (2 mL) were irradiated at 350 nm. After irradiation, **9** gave five products, none of which was predominant enough to be isolated. Compound **5** gave seven products (R_f: 0.49, 0.45, 0.33, 0.26, 0.21, 0.14, and 0.1) detectable by TLC (27:2:1 EtOAc–MeOH–H_2O). The water was evaporated in vacuo and the products were then separated by HPLC (Hypersil ODS 5 μm, 250 × 8 mm, 98:2 MeOH–H_2O). The two principal products **10** (R_f 0.49, 20 mg) and **11** (R_f 0.14, 6 mg) were isolated. Both products were separately acetylated as described for compound **16**. The structures of **10** and **11** could be determined by the ^1H NMR spectra of the corresponding acetylated products.

^1H NMR data of acetylated compound **10**: δ 1.08 (t, 3 H, $J_{1,2}$ 7.5 Hz, CH_3), 2.05, 2.10, 2.19 (3 s, 9 H, 3 OAc), 2.57 (dd, 2 H, $J_{2a,2b}$ 12 Hz, H-2a,2b), 4.25 (dd, 2 H, H-6a,6b), 5.34 (d, 1 H, $J_{4,5}$ 4.2 Hz, H-4), 5.46 (dt, 1 H, $J_{4,5}$ 6 Hz, H-5).

^1H NMR data of acetylated compound **11**: δ 1.27 (d, 3 H, CH_3), 2.05 (s, 3 H, OAc), 2.09 (3 s, 9 H, 3 OAc), 2.14 (s, 3 H, OAc), 4.13 (dd, 1 H, $J_{5,6a}$ 6, $J_{6a,6b}$ 12 Hz, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 3 Hz, H-6b), 5.05 (m, 2 H, H-5,2), 5.25 (dd, 1 H, *J* 3.8, *J* 7.5 Hz, H-3), 5.44 (dd, 1 H, *J* 3.8, *J* 7.5 Hz, H-4).

ACKNOWLEDGMENT

We thank the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1 G. Kurz, J. Lehmann, and R. Thieme, *Carbohydr. Res.*, 136 (1985) 125–133.
2 P.J.W. Midgley, B.A. Parkar, G.D. Holman, R. Thieme, and J. Lehmann, *Biochim. Biophys. Acta*, 812 (1985) 27–32.
3 R. Thieme, PhD Thesis, University of Freiburg, 1986.
4 R. Robillard, personal communication.
5 J. Lehmann and L. Ziser, *Carbohydr. Res.*, 225 (1990) 83–97; C.P.J. Glaudemans, J. Lehmann, and M. Scheuring, *Angew. Chem. Int. Ed. Engl.* 28 (1989) 1669–1670; J. Lehmann and M. Scheuring, *Liebigs Ann. Chem.*, (1990) 271–275.
6 R.F.R. Church and M.J. Weiss, *J. Org. Chem.*, 35 (1970) 2465–2471.
7 O.T. Schmidt, *Methods Carbohydr. Chem.*, 2 (1963) 318–325.
8 M.T. Liu, *Chemistry of Diazirines*, CRC Press, Boca Raton, FL, Vol. I, 1987, pp 120–134.
9 Ref. 8, Vol. II, 1987, p. 78.
10 W.C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, 43 (1978) 2923–2925.
11 M.L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, 56 (1934) 880–882.