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Asymmetric dihydroxylation of chiral styrene derivatives: development of an analytical strategy for the determination of the diastereomeric excess

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Abstract—A new efficient route to novel nucleosides having a benzo[*c*]furan moiety is described. The key step in the synthesis was the asymmetric dihydroxylation of a diastereomerically pure styrene derivative. NMR, mass spectrometry (high resolution and MS/MS) and X-ray crystallography have been used for the structural characterisation and the determination of the absolute configuration of the new stereogenic carbon atom. A GC/MS method has been developed to quantify the diastereomeric excess of this oxidation with high accuracy. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Among the various structural classes of antiviral agents approved by the US Food and Drug Administration for the treatment of HIV infection, the dideoxynucleoside analogues AZT,[1] ddC,[2] ddI,[3] 3TC,[4] d4T,[5] ABC[6] are the most frequently used (Fig. 1). As compared to the anti-HIV drug AZT, d4T shows similar selective anti-HIV activity in vitro.[7,8] Moreover, d4T has been found to be less toxic to bone marrow stem cells than AZT and to be less inhibitory to mitochondrial DNA replication.[9] In a previous paper, we described the synthesis of d4T analogues such as (1*R*,3*S*)-1-(3-hydroxymethyl-1,3-dihydrobenzo[*c*]furan-1-yl)uracil **8Sb** having a benzo[*c*]furan core[10,11] via asymmetric dihydroxylation using AD-mix α and AD-mix β.[12]

To validate this multi-step strategy it was essential to control the selectivity of the oxidation step. We report herein the analytical procedures used to establish the diastereomeric excess and the absolute configuration of the asymmetric 1,2-dihydroxyethyl carbon atom of 3,5-*O*-[2-((*S*)-1,2-dihydroxyethyl)benzylidene]-1,2-*O*-isopropylidene-α-D-xylofuranose **4S** as an intermediate for the synthesis of **8Sb**.

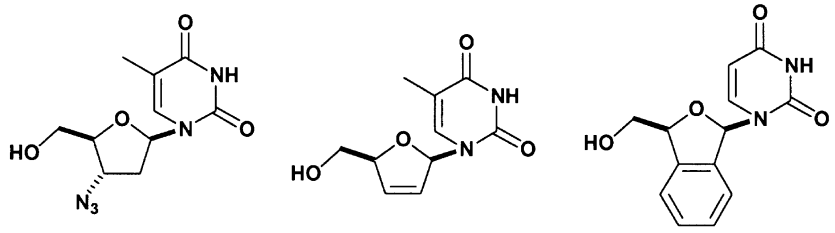

Figure 1. Potent anti-HIV drugs.

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2. Results and discussion

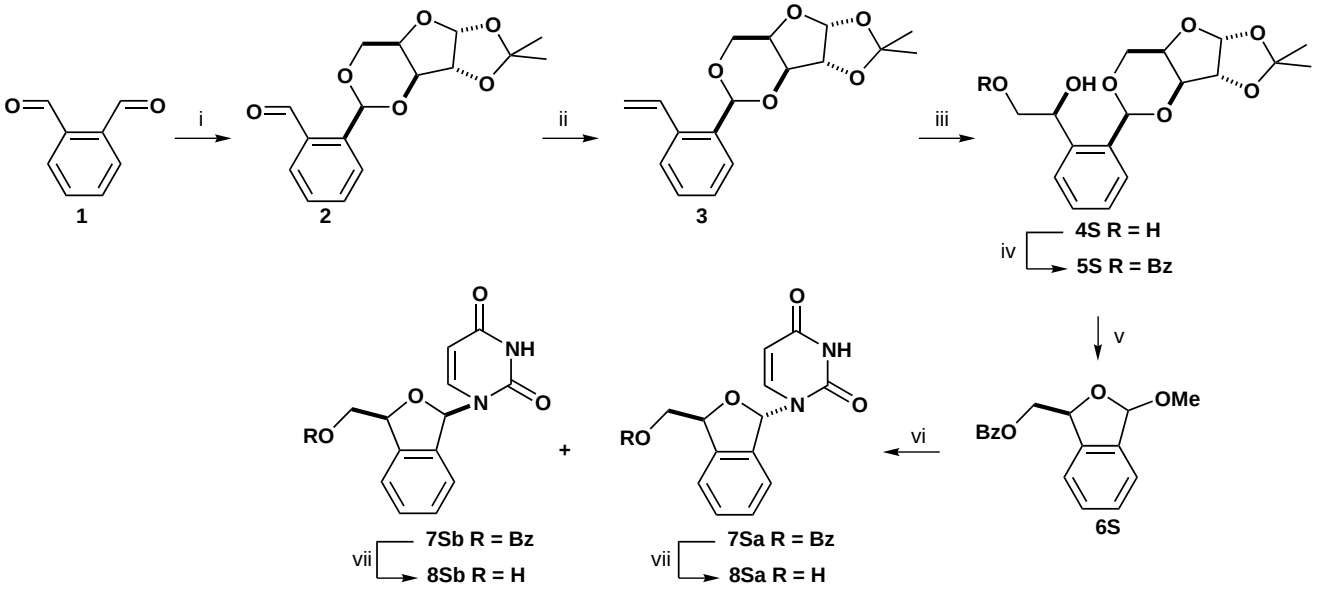
The synthesis of **8Sb** is described in Scheme 1. Starting from phthalaldehyde the formation of benzaldehyde acetal with 1,2-*O*-isopropylidene-α-D-xylofuranose afforded the benzylidene derivative **2**.[13] Despite the creation of one new stereogenic centre in the 1,3-dioxane ring only one diastereoisomer is formed. The NMR showed a strong NOE interaction between the three axial protons in this ring and confirmed both the equatorial position of the phenyl group and the (*S*)-configuration of the new stereogenic carbon atom. The key intermediate for estimation of the selectivity of this sequence was the diol **4S**, which was prepared using Sharpless asymmetric dihydroxylation (AD) of the styrene derivative **3** obtained via classical Wittig reaction starting from benzaldehyde derivative **2**. In general, the use of AD gave good enantiomeric excess starting from styrene derivatives, but the selectivity decreased when the olefin was attached to a glycone moiety.[14,15]
Starting from **1** the use of AD-mix α should afford diol intermediates for the synthesis of diastereomeric nucleoside analogues **8Sa** and **8Sb** (Scheme 1) and the use of AD-mix β should give intermediates for the analogues **8Ra** and **8Rb** (Scheme 2).

It was necessary to perform analytical measurements on the crude AD reaction product **4S** and **4R** to determine the diastereoselectivity of the dihydroxylation step. Different analytical methods have been developed for the characterisation and quantification of the asymmetric dihydroxylation products, such as TLC, NMR, mass spectrometry (high resolution and MS/MS), HPLC, GC/MS and X-ray crystallography. These analyses were carried out on the AD products and where necessary methods were developed as detailed below:

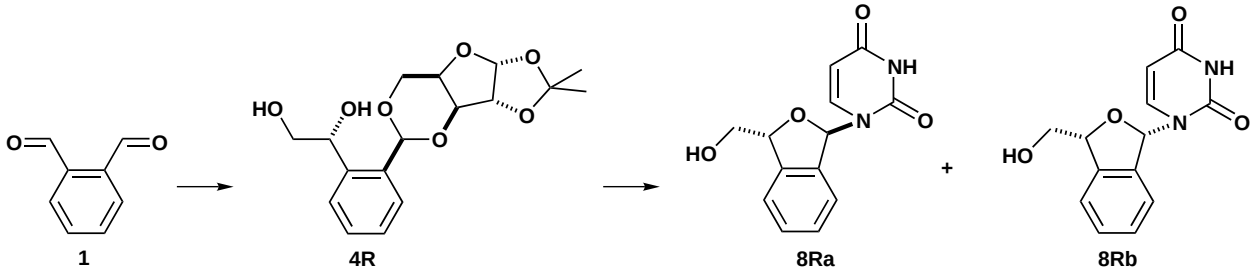
TLC: The crude products were determined to be two single components using a mobile phase of hexane–acetone (50:50) with R_f values of 0.67 for **4S** (AD-mix α) and 0.73 for **4R** (AD-mix β).

NMR: The ^1H and ^{13}C NMR spectra in $CDCl_3$ of the reaction mixtures leading to **4S** and **4R** gave different data corresponding to the proposed structures (Table 1). These analytical results showed that the diastereomeric excess of the product from the asymmetric dihydroxylation of **3** was >95%.

Mass spectrometry: Electrospray ionisation (ESI) experiments were performed on the two crude products **4S** and **4R**. As expected the two source mass spectra were



Scheme 1. (i) 1,2-*O*-Isopropylidene-α-D-xylofuranose, PTSA, toluene; (ii) $CH_3(C_6H_5)_3PBr$, *n*-BuLi, THF; (iii) AD-mix α, *t*-BuOH, H_2O; (iv) BzCl, toluene, $N(C_2H_5)_3$; (v) MeOH, HCl 1%; (vi) silylated uracil, $SnCl_4$, $C_2H_4Cl_2$; (vii) NH_3, MeOH.



Scheme 2.

Table 1. ^{1}H and ^{13}C NMR data of diol **4S** and **4R**

	H-1′	H-2′	H-3′	H-4′	H-5′a	H-5′b	CHOH	CH_2OH	CH_2OH	CH
4S	6.04	4.55	4.09	4.38	4.39	4.08	5.17	3.79	3.64	5.59
4R	6.19	4.59	4.12	4.38	4.39	4.10	5.39	3.69	3.63	5.56
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CHOH	CH_2OH	CH		
4S	105.6	83.7	71.9	78.8	66.5	70.8	67.5	98.8		
4R	105.7	83.8	71.9	78.9	66.7	70.7	67.8	100.2		

identical, showing an abundant $[M+Na]^+$ ion at m/z 361. It was not possible to displace this strong sodium adduct even after adding small amounts of formic acid. No impurity, except traces of *t*-butyl alcohol (showing a $[(t\text{-BuOH})_2+H]^+$ ion at m/z 149), was detected. The signal observed at m/z 699.2 corresponds to the $[MM+Na]^+$ adduct of the diol species. For illustration the electrospray mass spectra (MS and MS/MS) of diol **4R** are presented in Fig. 2.

To establish the elemental composition of **4S** and **4R**, accurate mass measurements of the $[M+Na]^+$ ions were performed using the high-resolution capabilities[16] of a quadrupole orthogonal time-of-flight mass spectrometer (Q-TOF). For lock mass correction, the sodium adduct ion of a reference compound (m/z 431, $C_{23}H_{25}FN_4O_2Na = 431.1859$) was used. The results of these measurements are presented in Table 2.

CID MS/MS experiments of the pseudomolecular ions $[M+Na]^+$ m/z 361 were performed in an effort to distinguish the two diastereomeric species. Unfortunately, due to the sodium cation charge localisation on the diol moiety the two collision spectra were similar (Fig. 2). It is noteworthy that the fragmentation pathway under collision induced dissociation is in agreement with the chemical structures of **4S** and **4R**.

HPLC: The HPLC profile of a 1/1 V/V mixture of the diastereoisomers **4S** and **4R** was investigated using a reverse phase column (Symmetry C18, Waters), an acetonitrile/water gradient and an evaporative light scattering detector (ELSD) due to the poor response of compounds **4S** and **4R** in UV detection. The best conditions gave two peaks with a resolution (R_S) of 1.2 and a valley of 15% (Fig. 3). These results were not sufficient to allow the unambiguous determination of the diastereomeric excess.

Table 2. Accurate mass measurements of $[M+Na]^+$ ions of **4S** and **4R**

Compounds	Mass	Calcd mass	mDa	Formula
4S	361.1249	361.1263	−1.4	$C_{17}H_{22}O_7Na$
4R	361.1264	361.1263	+0.1	$C_{17}H_{22}O_7Na$

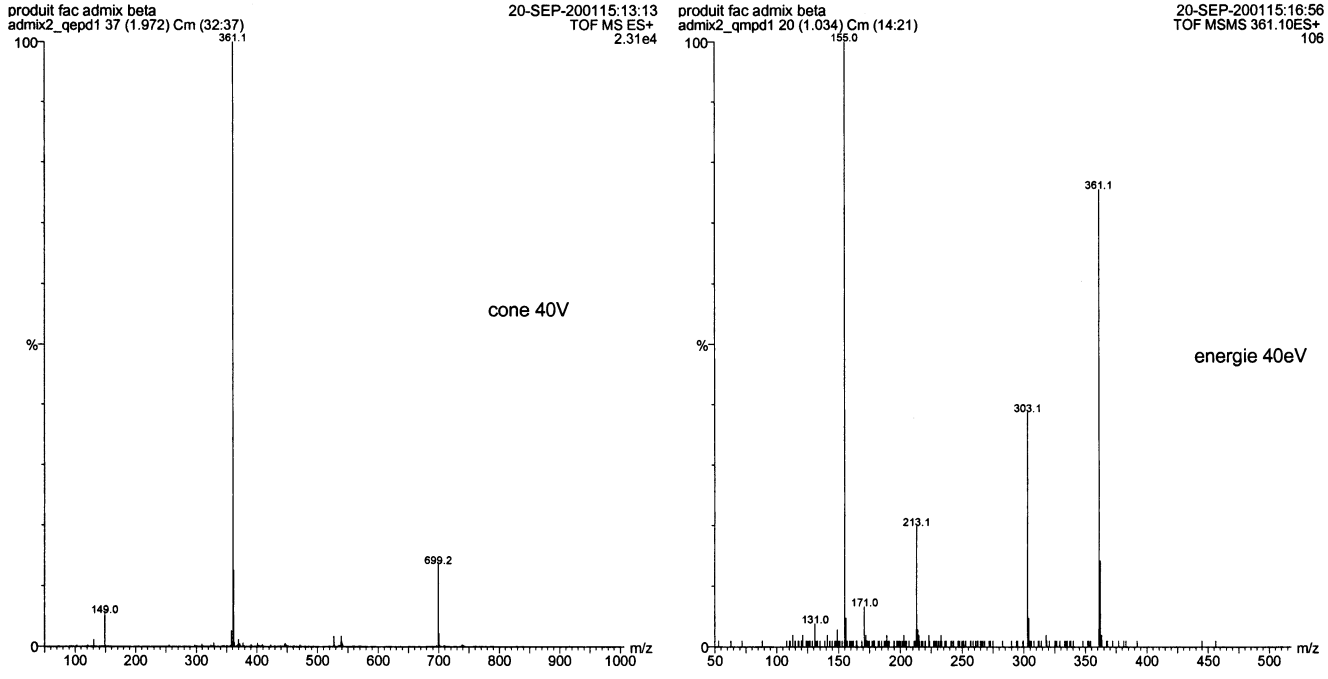


Figure 2. Electrospray MS (left) and MS/MS (right) mass spectra of **4R**.

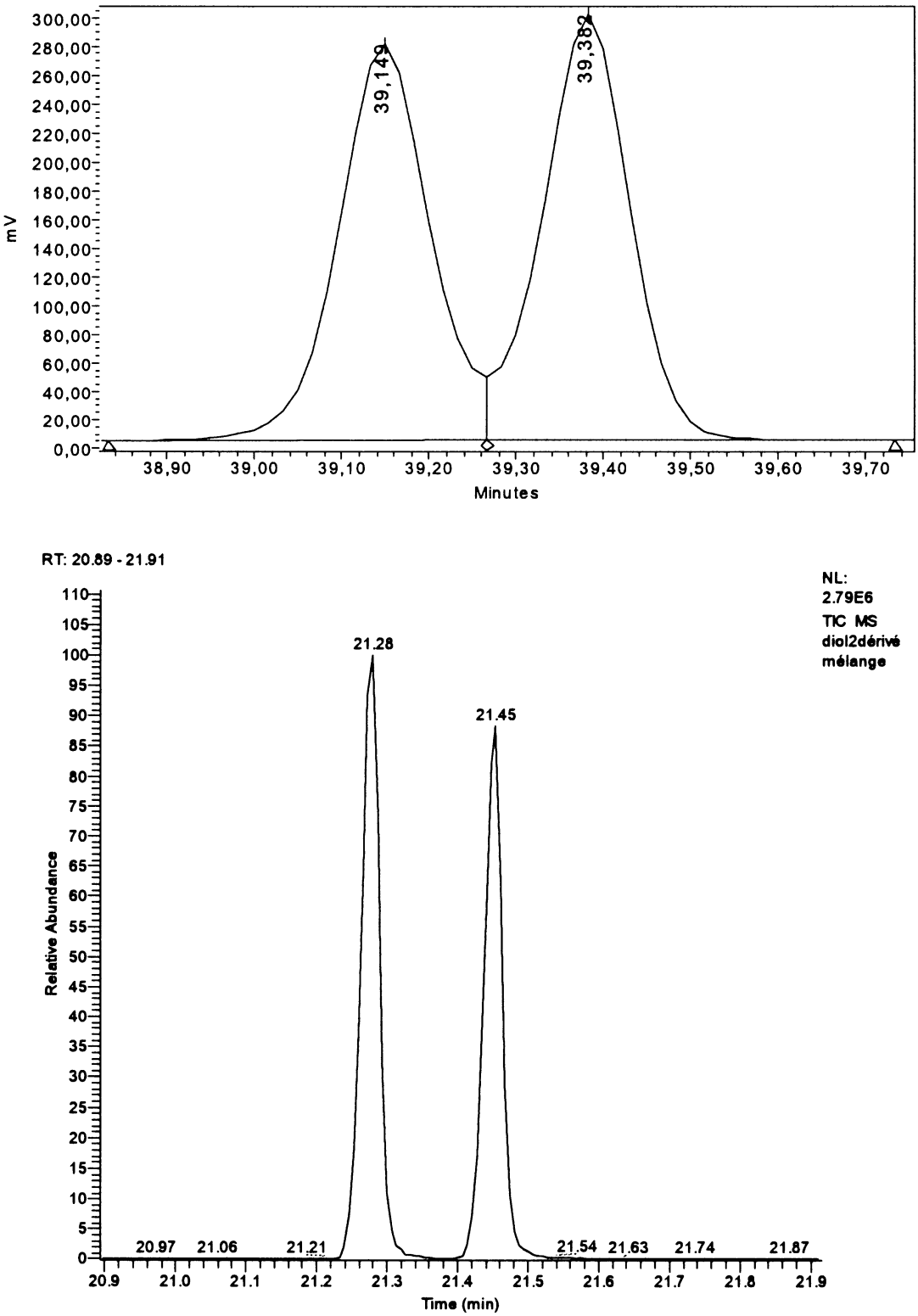


Figure 3. HPLC (ELSD) chromatogram of a 1:1 mixture of **4S** and **4R** (top). GC/MS (TIC) chromatogram of a 1:1 BSA derivatized mixture of **4S** and **4R** (bottom).

The solution could be the use of chiral cellulose-, amylose- or cyclodextrin-bonded phases.[17,18] However, chiral chromatography method development tends to be time-consuming and requires planning and careful experimentation. So we opted to develop a gas chromatography-mass spectrometry (GC/MS) method.

GC/MS: This hyphenated technique, which offers the advantages of being fast, specific and more sensitive[19] than conventional liquid chromatography detection methods (UV, ELSD) is in open access in our research team. The two diastereomeric diols **4S** and **4R** were silylated (BSA, pyridine, TMSCl) and analysed as a 1:1

V/V mixture by GC-MS (Restek, Rtx-1701 column) using EI and CI–NH_3 ionisation modes. A typical chromatogram obtained in EI (GC/EI) is presented Fig. 3. Under these conditions the two peaks were totally resolved ($R_S = 3.4$).

Using individual injections of the derivatized diols (Figs. 4 and 5) we could easily determine the corresponding diastereomeric excess (d.e.). The results are presented in Table 3.

The mass spectra of the derivatizated diols obtained under EI and CI–NH_3 ionisation conditions were characteristic of bis-silylated diols (+2$SiMe_3$, $m/z = 482$) and confirmed the integrity of the original molecular structures during the different steps of this analytical proce-

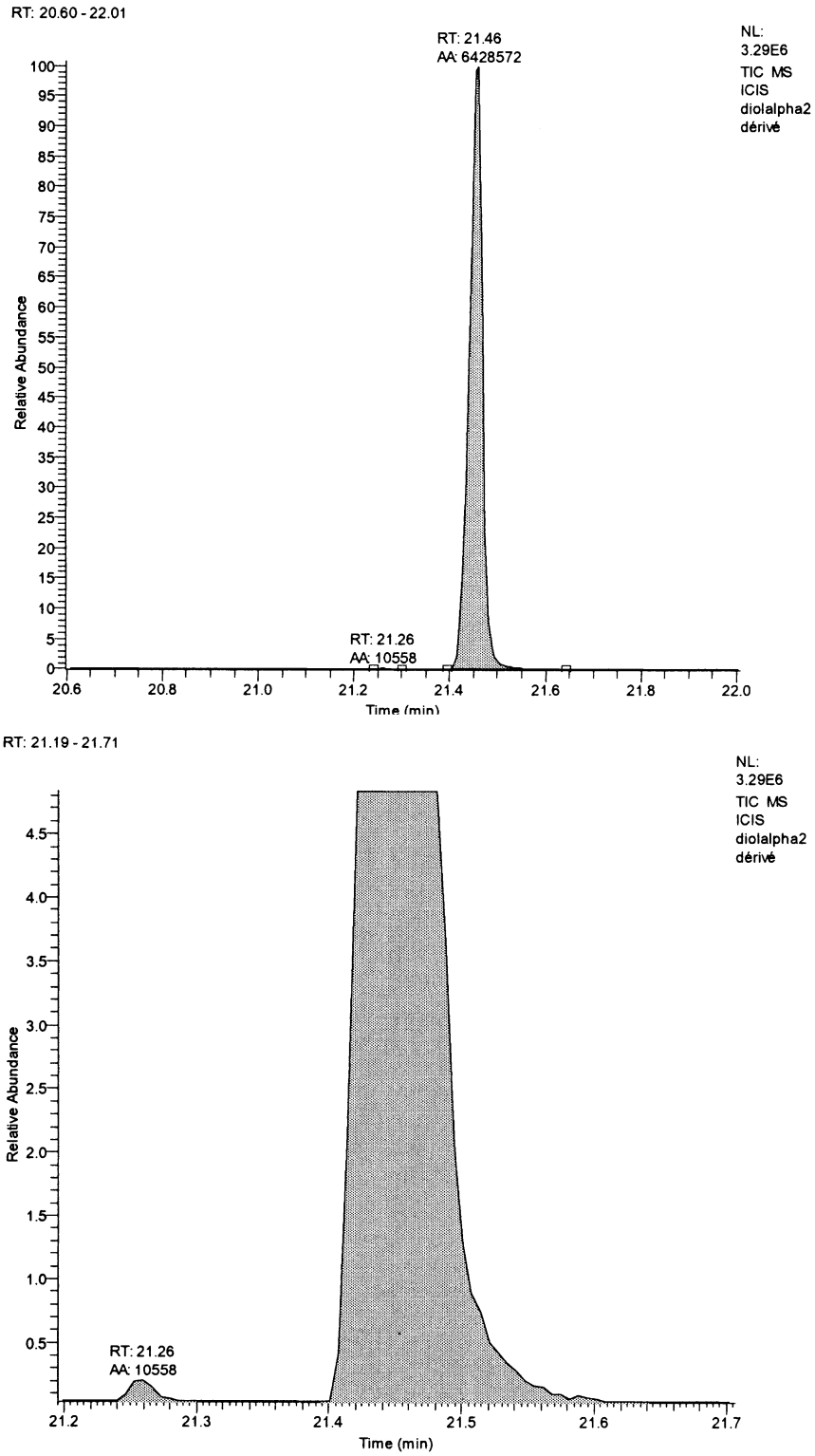


Figure 4. Bis-silylated **4S** GC/EI total ionic current (TIC).

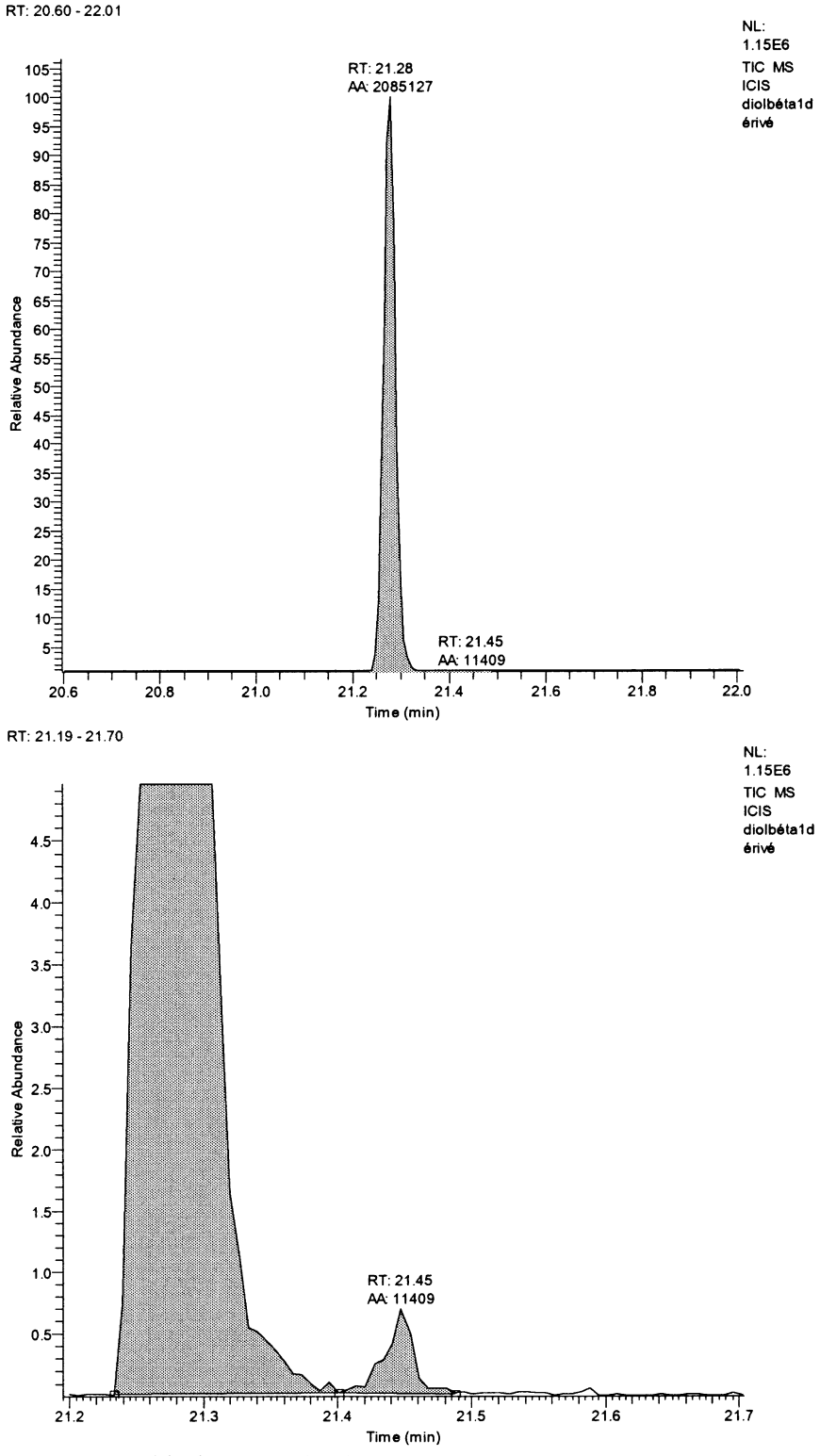


Figure 5. Bis-silylated **4R** GC/EI total ionic current (TIC).

dure (derivatisation reaction and gas chromatography experiment). As an illustration, the mass spectra of the bis-silylated diol **4S** are presented Fig. 6.

In EI mode a diagnostic fragment ion corresponding to the loss of the $(CH_3)_3SiOCH_2$ leads to a stable oxonium ion at m/z 379. For the CI ionisation mass spectrum using ammonia as the reagent gas (CI–NH_3) the protonated molecule was observed at m/z 483, the base peak at m/z 393 corresponds to the loss of $(CH_3)_3SiOH$ by β-elimination.

X-Ray crystallography: After the determination of the diastereomeric excess it was important to establish

Table 3. GC/MS (EI mode) diastereomeric excess (d.e.) obtained for two different dihydroxylation reactions

Compounds	D.e.	D.e.	D.e.	Average d.e.
4S[a]	99.7	99.6	99.7	99.7
4S[b]	97.5	97.6	97.9	98.7
4R[a]	98.9	98.0	98.0	98.3
4R[b]	96.3	96.9	96.4	96.5

[a] First dihydroxylation.
[b] Second dihydroxylation.

unequivocally the absolute configuration of the diols **4S** and **4R**. The X-ray crystallography structure of **4S**[20] (Fig. 7) confirms the configuration both of the carbon atoms created in the oxidation reaction and of the benzylidene carbon. These results show that the dihydroxylation with AD-mix α afforded the diol **4S** in accordance with the mnemonic rule[21] and with our previous results.[10,11]

3. Conclusion

We have developed a method for the characterisation and quantification of asymmetric dihydroxylation products. The structural characterisation was performed by ^{1}H and ^{13}C NMR, MS using electrospray ionisation (ESI-MS) and MS/MS experiments and X-ray crystallography. The quantification of the synthetic selectivity was obtained with a GC/MS method using the corresponding bis-silylated diols and gave excellent diastereomeric excess (96.3–99.7%).

4. Experimental

4.1. Materials and reagents

Technical grade D-xylose, phthalaldehyde were obtained from a commercial source (Sigma-Aldrich).

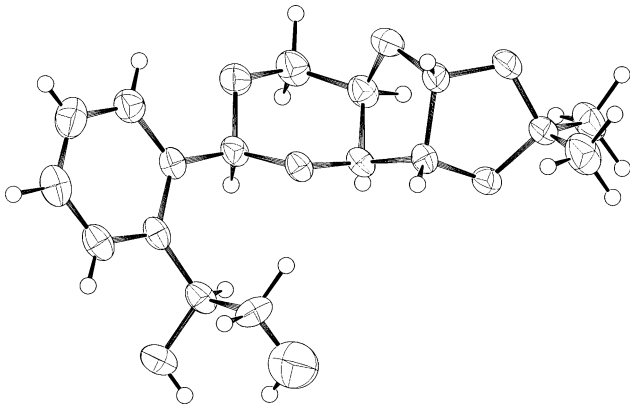


Figure 7. Configuration and conformation of diol **4S** as determined by X-ray crystallographic analysis.

HPLC grade solvents were used for chromatographic separations (Merck) and high purity reagents (Sigma-Aldrich) were used for preparation of the per-silylated diol solutions. Thin layer chromatography (TLC) was performed on Silica F_{254} (Merck) with detection by UV light at 254 nm or by charring with phosphomolybdic acid–H_2SO_4 reagents. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 (with tetramethylsilane (TMS) as internal standard), at 300.13 and at 75.47 MHz, respectively (Bruker, AM WB-300).

Electrospray MS: Infusion electrospray mass spectra in the positive ion mode were obtained on a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass). The source and desolvation temperatures were kept at 80 and 150°C, respectively. Capillary and cone voltages were 3.9 kV and 40 V, respectively, and the collision energy was set to 40 eV. Argon was used as collision gas at an indicated analyser pressure of 5×10^{-5} Torr. Calibration files were created with polyalanine and single lock mass correction was applied for the accurate mass measure-

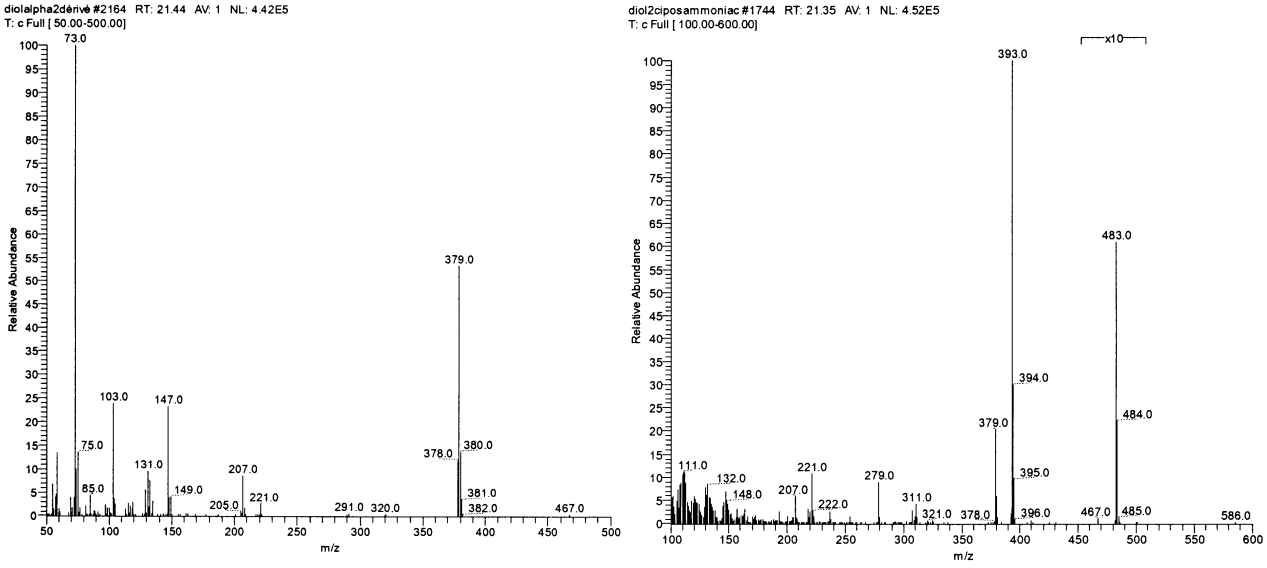


Figure 6. GC/EI (left) and GC/CI–NH_3 (right) mass spectra of **4S**.

ments. Samples were introduced through a Harvard syringe pump at 5 μL min^{-1}, from 0.01 mg mL^{-1} 50/50/0.2 V/V/V water/methanol/formic acid solutions. The mass range was 50–1050 Daltons and spectra were accumulated at 3 sec/scan at a resolution of 9000 FWHM. Data were obtained in the profile mode. Data acquisition and processing was performed with MassLynx V3.4 software.

Liquid chromatography: Liquid chromatography was performed with a Waters 600 series chromatography system (Waters). Crude extracts (0.2 mg) were dissolved in methanol (1 mL) and 20 μL injected onto a 150×4.6 mm symmetry (Waters) C_{18} column (particle size 5 μm) maintained at room temperature. Gradient elution was starting at 100 V water held for 20 min, then ramped to 1:1 V/V water/acetonitrile over 20 min and maintained at 1:1 V/V water/acetonitrile for 10 min. The flow rate was 0.8 mL min^{-1}. The eluted compounds were detected and quantified using an evaporative light scattering detector (Polymer Laboratories) mounted in series (nitrogen gas flow: 1.5 mL min^{-1}, nebulisation, temperature: 80°C, evaporation temperature: 90°C). Data acquisition and processing was performed with Millennium[32] software.

Gas chromatography and GC/MS: Gas chromatography and GC/MS analyses were performed on a GC 2000 Trace gas chromatograph (Thermo Finnigan) coupled with a quadrupole mass spectrometer (Finnigan Automass).

GC: An Rtx-1701 (Restek) capillary column coated with 14% cyanopropylphenyl and 86% dimethylpolysiloxane (30 m; 250 μm i.d.; 0.10 μm film thickness) was used. The initial oven temperature was 60°C, maintained for 2 min, followed by a temperature gradient of 10°C per min to 240°C. Samples were injected at a volume of 1 μL with a split ratio of 1:50 and at an injector temperature of 250°C. Helium was used as carrier gas with a flow rate of 1 mL min^{-1}.

GC/EI: Electron energy 70 eV, scan range 50–500 Daltons, scan time 400 ms, source temperature 150°C and interface temperature 250°C.

GC/CI–NH_3: Electron energy 150 eV, scan range 100–600 Daltons, scan time 500 ms, source temperature 150°C and interface temperature 250°C. Ammonia gas pressure 0.38 torr. Data acquisition and integration was performed with Xcalibur™ software.

4.2. Preparation of samples for gas chromatography/mass spectrometry (GC/MS) analysis

Preparation of per-silylated diols: Crude extracts (1–2 mg) were dissolved in anhydrous pyridine (140 μL) in small closed tubes and derivatized at room temperature for 30 min by exposure to *N*,*O*-bis-(trimethylsilyl)acetamide (BSA, 260 μL) and trimethylchlorosilane (TMSCl, 20 μL).

4.3. X-Ray crystallography

X-Ray diffraction measurements were performed on a Bruker AXS diffractometer with a CCD detector (λ Mo Kα = 0.71069 Å, graphite monochromator, T = 294 K, Ûω scans). Absorption correction was not necessary. Structure solution was obtained by direct methods (SHELXS-97), with refinement by the SHELXL-97 program. Hydrogen atoms were refined with isotropic thermal parameters.

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References

1. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D. P.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096–7100.
2. Cooney, D. A.; Dalal, M.; Mitsuya, H.; McMahon, J. B.; Nadkarni, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1986**, *35*, 2065–2068.
3. Ahluwalia, G.; Cooney, D. A.; Mitsuya, H.; Fridland, A.; Flora, K. P.; Hao, Z.; Dalal, M.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1986**, *35*, 3797–3800.
4. Coates, J. A. V.; Cammack, N. S.; Jenkinson, H. J.; Jowett, A. J.; Jowett, M. I.; Pearson, B. A.; Pen, C. R.; Rouse, P. L.; Viner, K. C.; Cameron, J. M. *Antimicrob. Agents Chemother.* **1992**, *36*, 733–739.
5. Balzarini, J.; Kang, G. J.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1987**, *32*, 162–167.
6. Crimmins, M. T.; King, B. W. *J. Org. Chem.* **1996**, *61*, 4192–4193.
7. Balzarini, J.; Kang, G. J.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1987**, *32*, 162–167.
8. Mansuri, M. M.; Starrett, J. E.; Ghazzouli, I.; Hitchcock, M. J. M.; Sterzycki, R. Z.; Brankovan, V.; Lin, T. S.; August, E. M.; Prusoff, W. H.; Sommadossi, J. P.; Martin, J. C. *J. Med. Chem.* **1989**, *32*, 461–466.
9. Simpson, M. V.; Chin, C. D.; Keilbaugh, S. A.; Lin, T. S.; Prusoff, W. H. *Biochem. Pharmacol.* **1989**, *38*, 1033–1036.
10. Ewing, D. F.; Fahmi, N.-E.; Len, C.; Mackenzie, G.; Ronco, G.; Villa, P.; Shaw, G. *Collect. Czech. Chem. Commun.* **1996**, *61*, S145–S147.
11. Ewing, D. F.; Fahmi, N.-E.; Len, C.; Mackenzie, G.; Ronco, G.; Villa, P.; Shaw, G. *Nucleosides Nucleotides* **1999**, *18*, 2613–2630.
12. Ewing, D. F.; Fahmi, N.-E.; Len, C.; Mackenzie, G.; Pranzo, A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3561–3565.

13. Ewing, D. F.; Len, C.; Mackenzie, G.; Ronco, G.; Villa, P. *Tetrahedron: Asymmetry* **2000**, *11*, 4995–5002.
14. Moitessier, N.; Chrétien, F.; Chapleur, Y. *Tetrahedron: Asymmetry* **1997**, *8*, 2889–2892.
15. Moitessier, N.; Maigret, B.; Chrétien, F.; Chapleur, Y. *Eur. J. Org. Chem.* **2000**, 995–1005.
16. Pilard, S.; Caradec, F.; Jackson, P.; Luijten, W. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2362–2366.
17. Belloli, E.; Len, C.; Mackenzie, G.; Ronco, G.; Bonte, J. P.; Vaccher, C. *J. Chromatogr. A* **2001**, *943*, 91–100.
18. Snyder, L. R.; Kirkland, J. J.; Glajch, J. L. *Practical HPLC Method Development*, 2nd ed.; John Wiley and Sons Inc: New York, 1997.
19. Eiceman, G. A.; Hill, H. H.; Gardea-Torresdey, J. *Anal. Chem.* **2000**, *72*, 137R–144.
20. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre (deposition number: CCDC 175311).
21. Kolb, H. C.; Andersson, P. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1994**, *116*, 1278–1291.