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Enantiomeric d4T analogues and their structural determination

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Abstract—Asymmetric synthesis of d4T analogues having a benzo[*c*]furan moiety with two asymmetric carbon atoms was realized using Sharpless asymmetric dihydroxylation as the key step in a synthesis starting from *o*-phthalaldehyde. Enantiomeric purities were determined by analytical chiral HPLC with an amylose-derived stationary phase, while the absolute configurations were established by X-ray crystallography. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

2′,3′-Dideoxynucleosides (ddNs) are the most important class of compounds active against HIV.[1–3] They act as DNA chain terminators and competitive inhibitors of viral reverse transcriptase (RT).[4] Currently, six drugs belonging to the ddNs family are approved by the FDA and are commercially available: zidovudine (AZT, Retrovir),[5] stavudine (d4T, Zerit),[6] zalcitabine (ddC, Hivid),[7] didanosine (ddI, Videx),[8] lamivudine (3TC, Epivir)[9] and abacavir (ABC, 1592U89, Ziagen).[10] d4T shows selective anti-HIV activity comparable to that of AZT in vitro.[11] However, d4T is less toxic and less inhibitory to mitochondrial DNA replication than AZT.[12] Novel nucleoside analogues based on a 1,3-dihydrobenzo[*c*]furan glycone and 1-phenylethan-1,2-diol, as exemplified by **1**–**5**, have been described as thymine analogues of d4T[13–15] (Fig. 1).

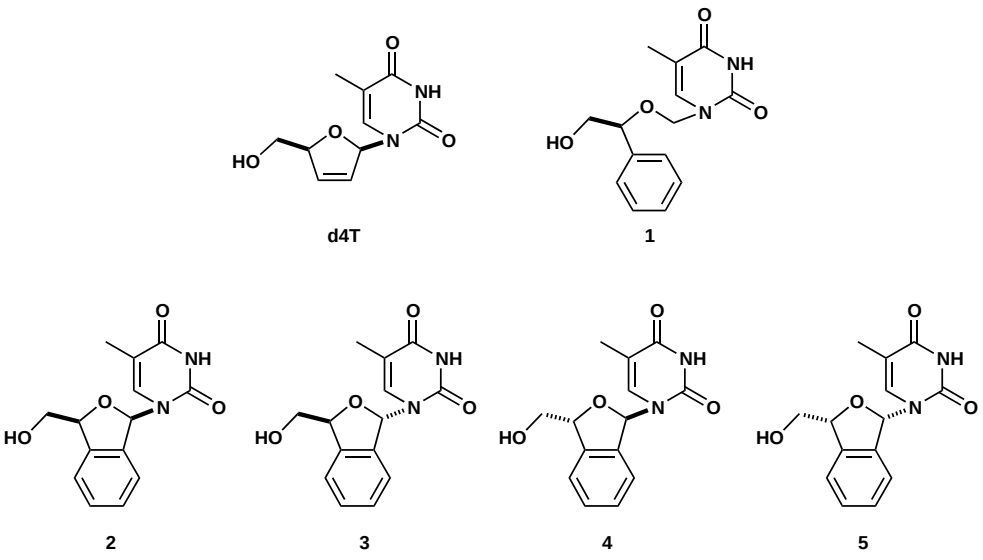

Figure 1.

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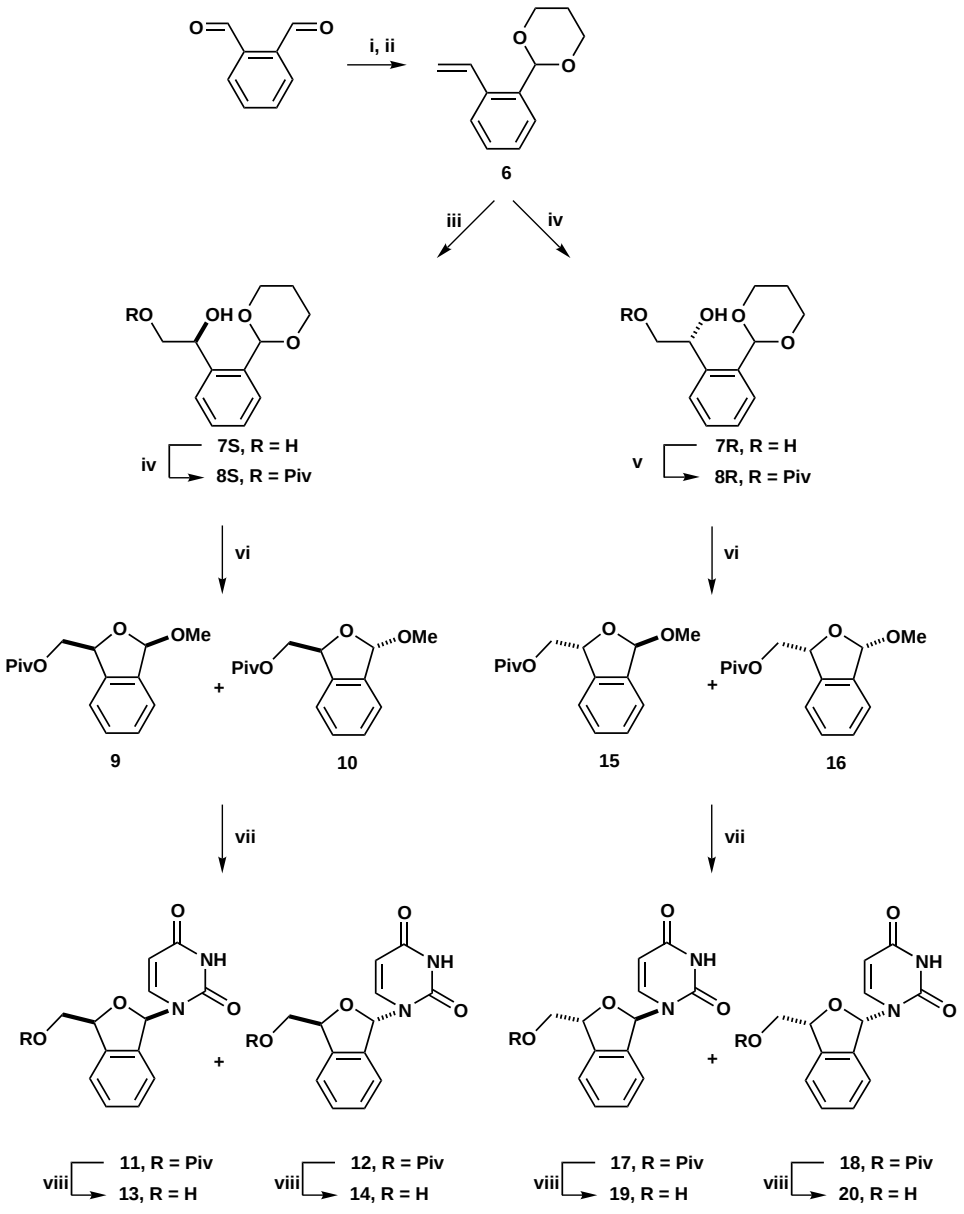
Various routes to the nucleosides **2**–**5** have been published starting in all cases from *o*-phthalaldehyde.[14,15] Two stereogenic carbon centers are created in the formation of the nucleosides leading to four stereoisomers in a racemic synthesis[14] or four diastereomerically pure stereoisomers via asymmetric synthesis.[15] The diastereomerically pure nucleosides have been prepared by application of the Sharpless asymmetric dihydroxylation (AD) methodology.[16] Starting from *o*-phthalaldehyde, the diols (*S*)-**7** and (*R*)-**7** having one stereogenic center were obtained using AD-mix α and AD-mix β, respectively. For the first time the absolute configuration of the carbon was assigned using the mnemonic device.[17] However, the literature[18,19] has shown that the enantioselectivity was opposite to that expected. Modeling studies based on molecular mechanic models have been completed and permit rationalization of the Sharpless' model.[19] To confirm the modeling part, NMR and X-ray studies have been developed which determine the absolute configuration of the two stereogenic centers of the nucleoside analogues.

2. Results and discussion

Synthesis of the diols (*S*)-**7** and (*R*)-**7** have been reported previously.[15] Selective protection of one aldehyde function of *o*-phthalaldehyde using propan-1,3-diol in the presence of *p*-TSA followed by homologation using classical Wittig chemistry gave the styrene derivative **6** in two steps in 58% yield overall (Scheme 1).



Scheme 1. *Reagents and conditions*: (i) propan-1,3-diol, *p*-TSA, toluene; (ii) $MePPh_3Br$, *n*-BuLi, THF, rt; (iii) AD mix α, *t*-BuOH, H_2O; (iv) AD mix β, *t*-BuOH, H_2O; (v) PivCl, $N(C_2H_5)_3$, toluene; (vi) MeOH/HCl (1%); (vii) silylated uracil, $SnCl_4$, $C_2H_4Cl_2$; (viii) NaOH, H_2O, dioxane.

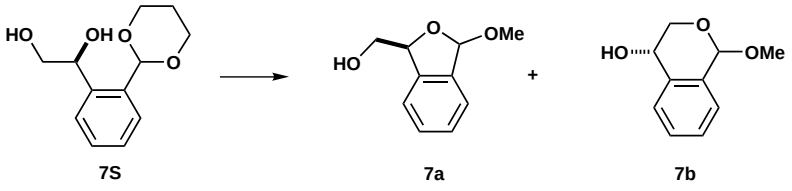
Asymmetric dihydroxylation of **6** with AD mix α and AD mix β afforded the diols (*S*)-**7** and (*R*)-**7** in 85 and 78% yields, respectively. Deprotection of the aldehyde group of diol (*S*)-**7** afforded a mixture of four compounds namely 3-hydroxymethyl-1-methoxy-1,3-dihydrobenzo[*c*]furan **7a** and the 4-hydroxy-1-methoxyisochromane **7b** in 38 and 4% yields, respectively with an epimeric mixture of **7a** and **7b** (1:1) (Scheme 2).

The protection of the primary hydroxyl group of (*S*)-**7** with pivaloyl chloride in a mixture of toluene and triethylamine gave (*S*)-**8** in 78% yield. The aldehyde functionality of (*S*)-**8** was selectively deprotected with HCl in methanol to afford the cyclic acetals **9** and **10** in 72% yield. The mixture **9** and **10** was condensed with silylated uracil and the two epimeric nucleoside products were separated by column chromatography to afford **11** and **12** in 35 and 38% yields, respectively. Deprotection of the pivaloyl group of **11** and **12** with 1N NaOH in dioxane then afforded the nucleosides **13** and **14** in 93% yield. Starting from the diol (*R*)-**7** the above strategy gave the derivatives (*R*)-**8**, **15**–**20** with similar yields. Because the AD reactions of the styrene derivative **6** with AD-mix α and AD-mix β were key reactions in the synthesis of the nucleosides **11**–**14** and **17**–**20**, a reliable method for the determination of the enantiomeric excess of the products was required.

Separation of enantiomers by chiral analytical HPLC is now an established preparative and analytical tool, with over 50 different chiral stationary phases (CSPs) commercially available. Among them cellulose and amylose ester and carbamate derivatives coated onto large pore silica gel supports had proven useful stationary phases when used in normal-phase mode.[20]

Table 1 shows the chromatographic data: retention, selectivity and resolution factors for the stereoisomers **11**, **12**, **17** and **18** using different mobile phases by changing the nature of the alcohol (ethanol or 2-propanol) and the percentage (10 or 20%) with the amylose-derived CSP Chiralpak AS. The alcohol modifier can affect the retention of the solutes in different ways: (i) by improving solvation in the mobile phase and/or (ii) by competing for the H-bonding sites in the stationary phase. We observed an increase in the retention factors k' of all compounds by changing the modifier from ethanol to 2-propanol, as generally expected, due to the higher polarity of the ethanol. The nucleosides **11**, **12**, **17** and **18** having a pivaloyl group showed significantly lower k' values than those when the pivaloyl group was changed to a benzyl or a benzoyl group.[21] This may support a possible role in the retention mechanism of π–π interactions between the phenyl groups of the stationary phases and the aromatic group of the R substituent of the solute.[21,22]

On this class of CSP the type and concentration of alcohol did not influence the elution order. It is noteworthy that the same elution order was observed between the diastereomers **17** (first eluted) and **18** (second eluted) corresponding to the (3′*R*) series in one part and between **12** (first eluted) and **11** (second eluted) corresponding to the (3′*S*) series in the other one. The same elution order was observed between the enantiomers **17** (first eluted) and **12** (second eluted) corresponding to the *trans* isomers in one part and between **18** (first eluted) and **11** (second eluted) corresponding to the *cis* isomers in the other one (Fig. 2). The good separation of the diastereomers makes this chromatographic method suitable to quantify their



Scheme 2. *Reagents and conditions*: MeOH/HCl (1%).

Table 1. Chromatographic resolution on the amylose-derived column (Chiralpak AS): retention factors (k') separation factor (α) and resolution (R_s) of diastereomers **11**, **12** **17** and **18**

Compounds	Eluent	k'_1	k'_2	α	R_s
17, **18** (**3′*R***)	A	1.83 (1′*R*,3′*R*)-**17**	2.23 (1′*S*,3′*R*)-**18**	1.28	1.92
	B	4.52 (1′*R*,3′*R*)-**17**	5.16 (1′*S*,3′*R*)-**18**	1.14	1.79
	C	2.90 (1′*R*,3′*R*)-**17**	3.65 (1′*S*,3′*R*)-**18**	1.26	2.51
	D	8.10 (1′*R*,3′*R*)-**17**	9.15 (1′*S*,3′*R*)-**18**	1.13	1.47
11, **12** (**3′*S***)	A	2.94 (1′*S*,3′*S*)-**12**	3.59 (1′*R*,3′*S*)-**11**	1.22	1.92
	B	7.45 (1′*S*,3′*S*)-**12**	8.53 (1′*R*,3′*S*)-**11**	1.14	2.16
	C	3.47 (1′*S*,3′*S*)-**12**	6.06 (1′*R*,3′*S*)-**11**	1.74	5.89
	D	1.03 (1′*S*,3′*S*)-**12**	1.54 (1′*R*,3′*S*)-**11**	1.49	5.09

Eluents A: *n*-hexane/ethanol: 80/20; B: *n*-hexane/ethanol: 90/10; C: *n*-hexane/2-propanol: 80/20; D: *n*-hexane/2-propanol: 90/10.

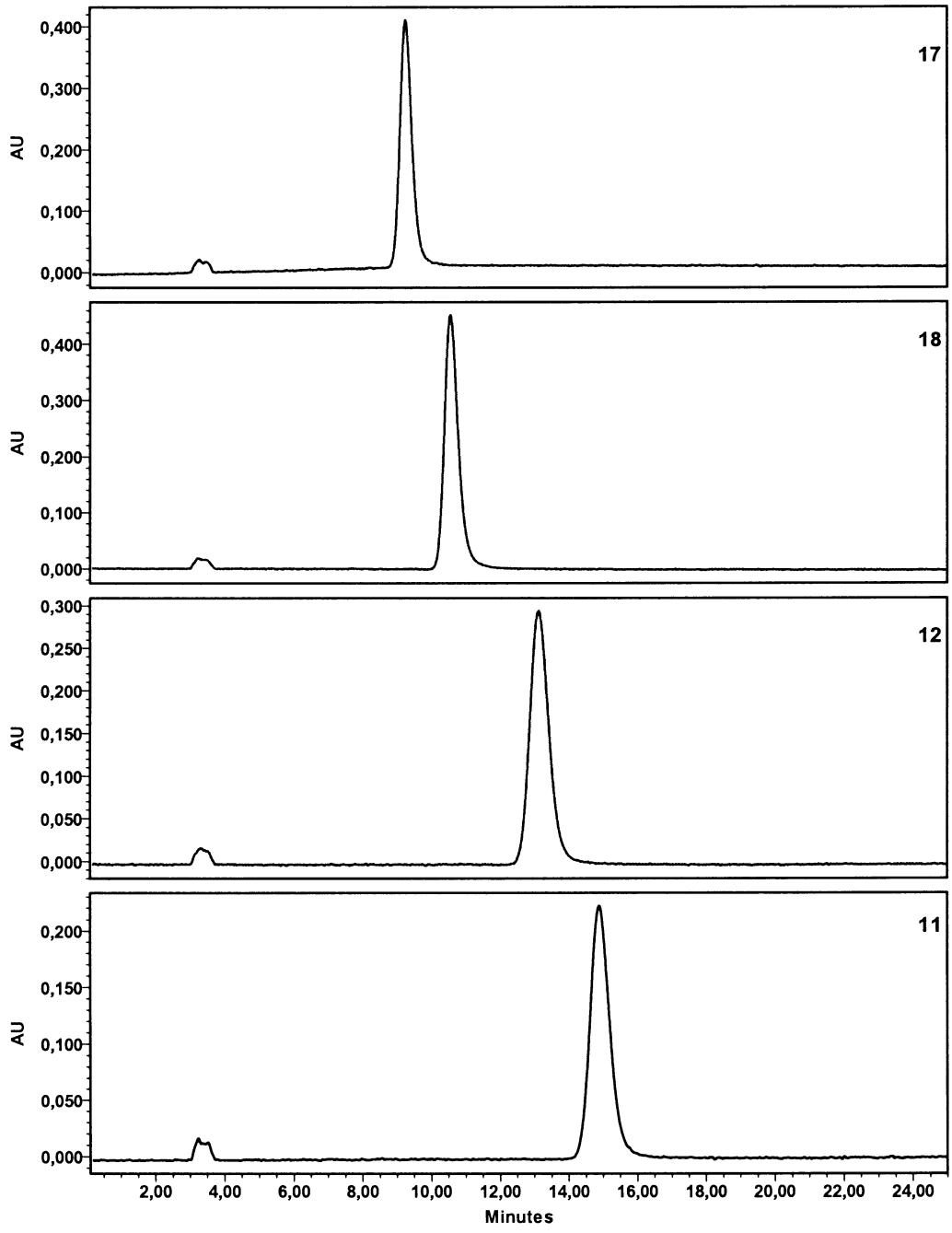
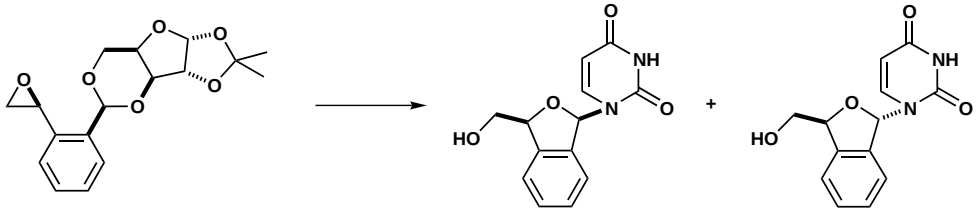


Figure 2. Stacking plot for the separations of (1′*R*,3′*R*)-**17**, (1′*S*,3′*R*)-**18**, (1′*S*,3′*S*)-**12** and (1′*R*,3′*S*)-**11** on Chiralpak AS at $l=200$ nm with 20% ethanol (eluent A).

purity which was greater than 99% (Fig. 2) and permitted to determine the enantiomeric excess (e.e. >99%) of the AD products (*S*)-**7** and (*R*)-**7**.

The absolute configuration of the carbon atoms C(1′) and C(3′) of the nucleoside analogues **11**–**14** and **17**–**20** was determined using NMR and X-ray spectroscopies. The NMR spectroscopy showed an NOE interaction between the C(1′)H and C(3′)H for compounds **11** and **18** confirming the *cis* configuration (1′*R*,3′*S*) or (1′*S*,3′*R*) and no interaction for the *trans* compounds **12** and **17** (1′*S*,3′*S*) or (1′*R*,3′*R*). It was notable that the $J_{1'3'}$ was not accessible for the *cis* configuration and the $J_{1'3'}$ of 2.5 Hz in the *trans* configuration.

The absolute configuration of the C(3′) carbon atoms was determined by X-ray spectroscopy. The 3,5-*O*-[2-((*S*)-oxiranyl)benzylidene]-1,2-*O*-isopropylidene-α-D-xylofuranose **21**[23] was an intermediate in the synthesis of benzo[*c*]furan derivatives and afforded unequivocally the nucleosides **2** and **3** having (3′*S*)-configuration (Scheme 3). The specific rotations of nucleosides **2** and **3** were identical starting from both the chiral oxirane **21** and the diol (*S*)-**7**. These results confirmed the stereo-



Scheme 3.

chemistry of the nucleosides **11**–**14** and **17**–**20** obtained with the present strategy.

The synthesis of benzo[*c*]furan nucleoside analogues was developed starting from styrene derivative **6** using an AD with high enantiomeric excess (e.e. >99%). The determination of the configuration of the two stereogenic carbon atoms was realized by NMR study and X-ray crystallography.

3. Experimental

3.1. General procedures

NMR spectra were recorded with a Lambda 400 spectrometer using standard conditions with a data point resolution of ca. 0.1 Hz. ^{1}H Chemical shifts were measured relative to Me_4Si and ^{13}C chemical shifts relative to $CDCl_3$ (77.0 ppm) or $(CD_3)_2SO$ (39.5 ppm). All coupling constants are given in hertz. Assignments of the ^{1}H spectra were made by detailed analysis using decoupling or correlation techniques where appropriate. Diastereoisomer ratios were determined from the integration of suitable peaks. Column chromatography was performed on silica gel (230–400 mesh; Prolabo) and TLC on silica gel 60, F_{254} (Merck) with detection by UV absorbance or phosphomolybdic acid. Optical rotation values are given in 10^{-1} deg cm^2 g^{-1}.

3.2. Syntheses

3.2.1. (*S*)- and (*R*)-1-(2-(1,3-Dioxan-2-yl)phenyl)ethan-1,2-diols, (*S*)-7 and (*R*)-7. AD-mix α or AD-mix β (19.98 g) in a mixture of *tert*-butyl alcohol (71.35 mL) and water (71.35 mL) was stirred at room temperature until both phases were clear. The mixture was cooled to 0°C and (2-(1,3-dioxan-2-yl)phenyl)ethene (2.71 g, 14.27 mmol) was added to the mixture at –10°C. The resulting slurry was stirred vigorously at 0°C for 1 h. Sodium sulfite (21.4 g) was added and the mixture stirred at 20°C for 30 min, then diluted with water (80 mL) and extracted with dichloromethane. This extract was worked up and the crude product purified by column chromatography (gradient of hexane:EtOAc, 3:7; then 2:8, then 1:9, then pure EtOAc) to give the diol as an oil. (Found: C, 63.03; H, 7.35, calcd for $C_{12}H_{16}O_4 \cdot 0.25\ H_2O$: C, 63.00; H, 7.27%); AD-mix α reagent gave the (*S*)-enantiomer (*S*)-**7**, (2.7 g, 85%), (e.e. >99% by comparison of NMR spectra with added chiral shift reagent) $[\alpha]_D^{22}$ +32.8 (*c* 3.86 in $CHCl_3$), AD-mix β gave the *R*-enantiomer (*R*)-**7**, (2.5 g, 78%), (e.e. >99% by comparison of NMR spectra with added chiral shift reagent), $[\alpha]_D^{22}$ –33.6 (*c* 3.42 in $CHCl_3$); both enantiomers had R_f 0.1 (hexane:ethyl acetate, 3:7); δ_H ($CDCl_3$): 1.46, 2.25 (2H, m, CH_2 dioxanyl), 2.7, 3.2 (2H, two br s, OH), 3.75 (2H, m, OCH_2), 3.89, 4.25 (4H, m, OCH_2 dioxanyl), 5.24 (1H, m, OCH), 5.81 (1H, s, dioxanyl), 7.40–7.65 (4H, m, aromatic H); δ_C ($CDCl_3$) 25.6 (1C, CH_2 dioxanyl), 67.2, 67.6 (1C, OCH_2 dioxanyl), 67.2 (1C, OCH), 70.5 (1C, OCH_2), 101.0 (1C, CH dioxanyl), 126.7, 126.9, 127.8, 129.3, 135.6, 139.0 (6C, aromatic C).

3.2.2. (*S*)- and (*R*)-1-*O*-Pivaloyl-1-(2-(1,3-dioxan-2-yl)phenyl)ethan-1,2-diols, (*S*)-8 and (*R*)-8. Pivaloyl chloride (4.90 mL, 40.13 mmol) was added to the diol (*S*)-**7** or (*R*)-**7** (7.5 g, 33.44 mmol) in a mixture of toluene (9 mL) and triethylamine (3.5 mL) at –20°C and the mixture stirred overnight at –10°C. Ice-water was added and the mixture stirred for 30 min and then extracted with toluene. This extract was worked up and the crude product purified by column chromatography (hexane:ethyl acetate, 7:3) to afford the protected diols (*S*)-**8** or (*R*)-**8** as an oil, (8.1 g, 78%). (Found: C, 66.21; H, 7.84, calcd for $C_{17}H_{24}O_5$: C, 66.15; H, 7.91%); *S*-enantiomer (*S*)-**8** $[\alpha]_D^{22}$ +35.0 (*c* 1.0 in $CHCl_3$), (*R*)-enantiomer (*R*)-**8** $[\alpha]_D^{22}$ –34.7 (*c* 1.0 in $CHCl_3$); both enantiomers had R_f 0.6 (hexane:ethyl acetate, 7:3); δ_H ($CDCl_3$) 1.22 (9H, s, CH_3), 1.45, 2.20 (2H, m, CH_2 dioxanyl), 4.01, 4.26 (4H, m, CH_2 dioxanyl), 3.1 (1H, m, OH), 4.20 (1H, dd, $J=3.7$, $J=11.4$, OCH_2), 4.43 (1H, dd, $J=8.0$, OCH_2), 5.41 (1H, m, OCH), 5.71 (1H, s, dioxanyl), 7.35–7.57 (4H, m, aromatic H); δ_C ($CDCl_3$) 26.0 (CH_2, dioxanyl), 27.6 (3C, CH_3), 39.2 (1C, $C(CH_3)_3$), 67.8 (2C, OCH_2, dioxanyl), 68.9 (1C, CH_2O), 69.0 (1C, CHOH), 101.1 (1C, dioxanyl), 127.2, 128.4, 129.6, 136.0, 138.6 (6C, aromatic C), 179.1 (CO).

3.2.3. (3*S*)- and (3*R*)-3-Pivaloyloxymethyl-1,3-dihydro-1-methoxybenzo[*c*]furans: 9, 10, 15, 16. To compound (*S*)-**8** or (*R*)-**8** (4.2 g, 13.6 mmol) was added a solution of methanolic HCl (1%, 90 mL) at 20°C for 1 h. The solution was concentrated to about 10 mL and the precipitate collected to give the 1,3-dihydro-1-methoxybenzo[*c*]furans **9**, **10** or **15**, **16** as oils, (2.6 g, 72%); R_f 0.2 (hexane:diethyl ether, 5:5). (Found: C, 68.53; H, 7.60, calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63%); (1*R*,3*S*)-isomer **9** and (1*S*,3*R*)-enantiomer **16** δ_H ($CDCl_3$) 1.18 (9H, s, CH_3), 3.50 (3H, s, OCH_3), 4.30 (1H, dd, $J=4.4$, $J=11.5$, OCH_2), 4.40 (1H, dd, $J=6.1$, OCH_2), 5.34

(1H, m, H-3), 6.12 (1H, s, H-1), 7.28-7.41 (4H, m, aromatic H); δ_C ($CDCl_3$) 27.4 (CH_3), 39.2 ($C(CH_3)_3$), 55.3 (OCH_3), 67.5 (C-8), 81.7 (C-3), 107.5 (C-1), 122.0, 123.4, 128.9, 129.7, 138.6, 139.7 (6C, aromatic C), 178.5 (CO); (1*S*,3*S*)-enantiomer **10** and (1*R*,3*R*)-enantiomers **15** δ_H ($CDCl_3$) 1.07 (9H, s, CH_3), 3.45 (3H, s, OCH_3), 4.33 (1H, dd, $J=3.6$, $J=11.8$, OCH_2), 4.50 (1H, dd, $J=4.6$, OCH_2), 5.55 (1H, m, H-3), 6.24 (1H, s, H-1), 7.28–7.41 (4H, m, aromatic H); δ_C ($CDCl_3$) 27.4 (CH_3), 39.2 ($C(CH_3)_3$), 54.8 (OCH_3), 66.0 (C-8), 81.7 (C-3), 107.5 (C-1), 122.0, 123.4, 128.9, 129.7, 138.6, 139.7 (6C, aromatic C), 178.5 (CO).

3.2.4. (1*R*,3*S*)-, (1*S*,3*S*)-, (1*R*,3*R*)- and (1*S*,3*R*)-1-(3-Pivaloyloxymethyl-1,3-dihydrobenzo[*c*]furan-1-yl)uracils 11, 12, 17 and 18. A suspension of uracil (1.1 g, 9.46 mmol) in hexamethyldisilazane (19 mL) and ammonium sulfate were refluxed with exclusion of moisture until a clear solution was obtained (3 h). Volatiles were removed by repeated co-evaporation with toluene to leave syrup. This syrup and the 1,3-dihydrobenzo[*c*]furan **9**, **10** or **15**, **16** (1.0 g, 3.78 mmol) were taken up in dry dichloroethane (24 mL) and $SnCl_4$ (890 μL, 9.46 mmol) added at –15°C. After stirring for 2 h at 0°C sat. $NaHCO_3$ solution (20 mL) was added, the mixture was stirred for 30 min and then extracted with CH_2Cl_2. This extract was worked up and the diastereoisomers **11**, **12** or **17**, **18** were separated by column chromatography (hexane:EtOAc, 7:3); the compound eluting first was the *cis* isomer (**11** or **18**), 460 mg (35%), mp 178°C (EtOH), R_f 0.56, (hexane:EtOAc, 1:1), isomer **11**. (Found: C, 62.65; H, 5.94; N, 8.16, calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13%); $[\alpha]_D^{22}$ +24.0 (*c* 1.0 in $CHCl_3$), enantiomer **18** (Found: C, 62.58; H, 5.85; N, 8.11, calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13%); $[\alpha]_D^{22}$ –23.8 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$): 1.07 (9H, s, CH_3), 4.37 (1H, m, $J=2.9$, $J=12.6$, CH_2O), 4.79 (1H, m, $J=4.5$, H-8b), 5.68 (1H, d, *J* 8.1, H-5), 5.51 (1H, $J=2.7$, H-3), 7.18 (1H, d, H-6), 7.47 (1H, d, H-1), 7.31–7.52 (4H, m, aromatic H); δ_C ($CDCl_3$) 27.4 (CH_3), 39.1 ($C(CH_3)_3$), 65.4 (C-8), 82.4 (C-3), 87.9 (C-1), 103.5 (C-5 uracil), 122.4, 123.3, 130.0, 130.6, 136.5, 138.9 (6C, aromatic C), 140.6 (C-6 uracil), 151.3 (C-2 uracil), 162.8 (C-4 uracil), 178.2 (CO). *trans* Isomer (**12** or **17**), 485 mg (38%), mp 121–122 °C (EtOH), R_f 0.47, (hexane:EtOAc, 1:1), enantiomer **12**. (Found: C, 62.77; H, 5.83; N, 8.16, calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13%); $[\alpha]_D^{22}$ –104.2 (*c* 1.0 in $CHCl_3$), enantiomer **17**. (Found: C, 62.79; H, 5.93; N, 8.10, calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13%); $[\alpha]_D^{22}$ +104.6 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$): 1.09 (9H, s, CH_3), 4.33 (1H, m, $J=3.3$, $J=12.0$, CH_2O), 4.53 (1H, m, $J=4.4$, H-8b), 5.67 (1H, d, *J* 8.1, H-5), 5.70 (1H, $J=2.7$, H-3), 6.81 (1H, d, H-6), 7.54 (1H, d, H-1), 7.28–7.52 (4H, m, aromatic H), 9.13 (1H, br s, NH); δ_C ($CDCl_3$) 27.4 (CH_3), 39.3 ($C(CH_3)_3$), 65.9 (C-8), 83.2 (C-3), 88.8 (C-1), 103.7 (C-5 uracil), 122.6, 123.3, 130.1, 130.7, 136.5, 139.2 (6C, aromatic C), 140.2 (C-6 uracil), 150.8 (C-2 uracil), 162.8 (C-4 uracil), 178.2 (CO).

3.2.5. (1*R*,3*S*)-, (1*S*,3*S*)-, (1*R*,3*R*)- and (1*S*,3*R*)-1-(3-Hydroxymethyl-1,3-dihydrobenzo[*c*]furan-1-yl)uracils 13, 14, 19 and 20. The protected nucleosides **11**, **12**, **17** or **18** (660 mg, 1.83 mmol) were dissolved in a mixture of dioxane (36 mL) and aqueous NaOH 1N (36 mL) and the mixture stirred for 2 h. After addition of acetic acid (pH 7) the evaporation of the solvent and column chromatography ($CHCl_3$:MeOH, 9:1) gave the nucleosides **13**, **14**, **19** or **20** (503 mg, 93%). In all cases the NMR data and the physical data were identical to those reported in a previous paper.[15]

3.3. General procedure for HPLC

Chromatography was carried out on a Chiralpak AS column (amylose tris-(*S*)-1-phenylethylcarbamate; 250× 4.6 mm i.d.; 10 μm) (Daicel Chemical Industries, Baker France) using a gradient Waters 600E metering pump model equipped with a Waters 996 photodiode array spectrophotometer. Chromatographic data were collected and processed on a Digital computer running with Millennium 2010. The column eluate was monitored at 200; 254 nm. The sample loop was 20 μL (Rheodyne 7125 injector). Mobile phase elution was made isocratically using *n*-hexane and a modifier (ethanol or 2-propanol) at various percentages. The flow-rate was 1.0 mL min^{-1}. The peak of the solvent front was considered to be equal to the dead time ($t_o=3.5$ min) and was taken from each particular run. Retention times were mean values of two replicate determinations. All separations were carried out at 30°C. The separation factor (α) was calculated as k'_2/k'_1 and retention factors (k') as $k'_1=(t_1-t_0)/t_0$ and $k'_2=(t_2-t_0)/t_0$ where t_1, t_2 refer to the retention times of the first and second enantiomers, respectively. The resolution factor (R_S) was calculated by the formula $R_S=2\ (t_2-t_1)/(w_1+w_2)$ where w_1 and w_2 are the peak widths for the first and second eluting enantiomer peaks, respectively. Reagents: ethanol, 2-propanol and *n*-hexane were HPLC grade from Merck or Baker. All the solutions were filtered (0.45 μm), degassed with a Waters in-line degasser apparatus. The mobile phases used were A: *n*-hexane/ethanol: 80/20; B: *n*-hexane/ethanol: 90/10; C: *n*-hexane/2-propanol: 80/20; D: *n*-hexane/2-propanol: 90/10. Compounds were chromatographed by dissolving them in the corresponding alcohol to a concentration of about 0.75 mM (which corresponds to 15 nmoles injected) and passed through a 0.45 μm membrane filter prior to loading the column.

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