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Enantiomerical excess determination, purification and biological evaluation of (3S) and $(3R) \alpha,\beta$ -butenolide analogues of isobenzofuranone

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Abstract—The asymmetric synthesis of isobenzofurane analogues, new potential antiviral agents, is reported. High performance liquid chromatography (HPLC) was the technique chosen to separate the enantiomers. We describe this chiral separation and then determine the enantiomerical excess. The biological results of each tested enantiomer are given. © 2004 Elsevier Ltd. All rights reserved.

For several years there has been an intensive search focused on the synthesis of nucleosides and analogues in the therapy of viral diseases. A number of nucleoside analogues have been found to possess antiviral activities against human immunodeficiency virus (HIV)¹ such as AZT,² ddC,³ ddI,⁴ d4T,⁵ 3TC⁶ and ABC.⁷ The use of L-nucleosides has greatly increased due to their potent biological activity and lower toxicity compared to their D-nucleoside analogues.⁸ However, the toxicity and emergence of mutant and resistant viral strains have been a critical problem in using these chemotherapeutic agents. Consequently the obvious emphasis is to design drugs with a higher therapeutic index. Among the compounds approved by the US Food and Drugs Administration for the treatment of HIV infection, d4T shows selective anti-HIV activity comparable to that of AZT in vitro.⁶ However, d4T is less toxic and less inhibitory to mitochondrial DNA replication than AZT¹⁴ (Fig. 1).

Novel nucleoside analogues based on 1,3-dihydrobenzo[c]furan glycone **BcF** have been described as thymine analogues of d4T.^{9–13} Unfortunately, all of the benzo[c]furan derivatives synthesized in our work have shown



Figure 1. Potent anti-HIV drugs.

no activity against HIV reverse transcriptase except for the uracil derivative having a benzovl group in 5'-position. One reason for the inactivity of the benzo[*c*]furan nucleosides in general might be due, simply, to them not being good substrates for HIV reverse transcriptase. However, the activity found in the one exception might have arisen from the lactone 10S resulting from a glycosidic bond cleavage and not from the parent nucleoside. This hypothesis has prompted us to investigate the synthesis of compounds related to α,β -butenolides. The chiral derivatives such as (S)-benzovloxymethyl- α , β -butenolide have served as key intermediates for the elaboration into various natural products and analogues.¹⁴⁻¹⁶ α , β -Butenolide derivatives are widely present in secondary metabolites, which show interesting physiological activities as exemplified by the aglycon of ranunculin,^{17,18} namely, (S)-hydroxymethyl- α , β -butenolide (Fig. 2). Herein we describe an alternative synthesis of the 3-benzoyloxymethylisobenzofuranone 10S and

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10*R*,¹⁹ which are potential metabolites of the corresponding 5'-benzoylated nucleoside **BcF** and are benzoylated derivatives of hydroxymethyl- α , β -butenolide to make available a library of novel compounds for biological evaluation.

To synthesize the target compounds 3-benzoyloxymethylisobenzofuranone 10S and 10R, retrosynthesis analysis was examined to be the best way for this novel aromatic family. The lactone was obtained starting from the phthalaldehyde (1) as depicted in Scheme 1. Compound 1 was firstly protected using an achiral group such as propane-1,3-diol to give the benzaldehyde derivative 2. Homologation of the remaining formyl group was effected by a Wittig reaction using methyltriphenylphosphonium bromide and potassium *t*-butoxide in





anhydrous toluene to afford the styrene derivative 3. Asymmetric dihydroxylation (AD) using AD-mix- α and AD-mix- β gave a mixture of two diols 4 and 5.^{20–23} A selective protection of the primary hydroxyl group was required to selectively obtain only the isobenzofurane derivative. Reaction of the diol 4 with benzoyl chloride afforded the intermediate 6 with a free secondary hydroxyl group. Removal of the acetal from compound 6 in an acidic medium (acetone, water, PTSA) gave the desired benzo[c] furan 8 obtained using RuCl₃-NaIO₄²⁴ to give solely 10S in 50% yield. Compound 5 gave the enantiomer 10R in a similar yield. Because the AD of the styrene derivatives with AD-mix- α and AD-mix- β were key reactions in the synthesis of the lactone 10S and 10R, a reliable method for the determination of the ee of the products was required. The absolute configuration was determined by comparison of the samples obtained through an unambiguous stereoselective pathway.¹⁹

The enantiomerical excess determination of the lactones 10S and 10R was developed in the case of the first strategy. HPLC studies have been effected by modification of different parameters such as the concentration of mobile phase modifier, the structure of polysaccharide derivatives of the chiral stationary phases (CSPs). The effect



Scheme 1. Reagents: (i) PTSA, propan-1,3-diol, toluene; (ii) *tert*-BuOK, $CH_3(C_6H_5)_3PBr$, toluene; (iii) AD-mix- α , *tert*-BuOH, H_2O ; (iv) AD-mix- β , *tert*-BuOH, H_2O ; (v) BzCl, (C_2H_5)_3N, toluene; (vi) PTSA, acetone, H_2O ; (vi) NaIO₄, RuCl₃, acetonitrile, ethyl acetate, H_2O .

of concentration of alcohol modifier (from 5% to 30%) on retention k' and resolution R_s factors was investigated using ethanol. It can be seen that an increase of the polar modifier concentration in the mobile phase, from eluent A–D, leads to a decrease of retention and resolution factors k' and R_s , for the four types of CSPs (Table 1).

Nowadays it is well known, that chiral recognition process results from several interactions of: (i) different magnitude involving hydrophobic, hydrogen, dipoledipole interactions between the electronegative atoms of the solute and the -COO and the -NHCO of the CSPs; (ii) π - π interaction, between the aromatic ring of the solute and the substituted phenyl moiety of the CSP. Chiral recognition process results also from inclusion phenomenon of the solute into the chiral cavity of the CSP. This last contribution was due to the regular higher order structure of the chiral sites of the CSP: a left handed threefold (3:2) helicoidal chain conformation for modified cellulose and a (4:1) helicoidal chain conformation for modified amylose. This point was illustrated by the best results obtained with Chiralpak AD towards Chiralcel OD-H, though these two CSPs had the same chiral selector. The results of the investigation of the effect of alcoholic modifier on retention and resolution showed that for a same eluent (C) cellulose OD seems better appropriated than cellulose OJ (Table 1). Comparing the two types of amylose CSPs, amylose AD was the best one to resolve those enantiomers with an $R_{\rm s}$ of about 20 (Table 1). Here the carbamate residue appears to be the most important adsorbing site of the phenylcarbamate derivatives of polysaccharide. It is notable that the enantiomer 10R eluted first on cellulose tris-3,5dimethylphenylcarbamate (OD-H), cellulose tris-methylbenzoate (OJ) and on amylose tris-(S)-1-phenylethylcarbamate (AS) but the enantiomer 10S eluted first on amylose tris-3,5-dimethylphenylcarbamate (AD). The reversal of the elution order of the two enantiomers, on this amylose-based CSP has often been reported and was generally related to an alteration in the steric environment of the chiral cavities (Table 1).²⁵ After optimization, the chiral purity of each enantiomer obtained after asymmetric synthesis has been evaluated using the four CSPs, with a mobile phase composed of *n*-hexane/ethanol (80:20) (eluent C). The purity was determined by the relative percentages of peaks areas. The lactone **10***S* was found with 98.50% and the enantiomer **10***R* with 97.50% (Fig. 3).

After optimization, Chiralpak AD analytical column was chosen in order to purify each enantiomer and to obtain an analytical sample through repetitive injections (200 μ L; 30 mM). The mobile phase was composed of a mixture of *n*-hexane and ethanol (60:40) for enantiomer **10***R* and (70:30) for the enantiomer **10***S*, the eluent rate was 2.5 mL min⁻¹ in both cases. The purity was determined on the four CSPs to be greater than 99% for each



Figure 3. CLHP analysis of enantiomer 10R in the presence of enantiomer 10S as impurity after asymmetric synthesis, on various polysaccharide CSPs and eluents.

CSP	Eluent	k'_1	α	$R_{ m s}$	First eluted isomer
Cellulose OJ	А	10.97	1.15	1.94	10 <i>R</i>
	В	6.00	1.17	1.55	10 <i>R</i>
	С	3.74	1.16	1.53	10 <i>R</i>
Cellulose OD	В	2.90	1.27	2.48	10 <i>R</i>
	D	0.73	1.15	1.03	10 <i>R</i>
Amylose AS	В	4.32	1.30	3.72	10 <i>R</i>
	С	2.23	1.27	2.77	10 <i>R</i>
Amylose AD	С	3.42	5.99	22.96	10 <i>S</i>
	D	2.13	5.65	20.50	10 <i>S</i>

Table 1. Retention factors (k') enantioselectivities (α) and resolution factors (R_s) of enantiomers 10S and 10R on different CSPs

Eluents A: *n*-hexane/ethanol: 95:5; B: *n*-hexane/ethanol: 90:10; C: *n*-hexane/ethanol: 80:20; D: *n*-hexane/ethanol: 70:30. The flow-rate was 1 mL min⁻¹, $\lambda = 200$ and 226 nm, T = 30 °C, $t_0 = 3.5$ min, 20 µL.

Chiralpak AD column (amylose tris-3,5-dimethylphenylcarbamate), Chiralpak AS column (amylose tris-(S)-1-phenylethylcarbamate; 250×4.6 mm i.d.; 10μ m), Chiralcel OD-H column (cellulose tris-3,5-dimethylphenylcarbamate; 250×4.6 mm i.d.; 5μ m), Chiralcel OJ column (cellulose tris-methylbenzoate; 250×4.6 mm i.d.; 10μ m) were purchased from Daicel Chemical Industries, Baker France.



Figure 4. Extended scale chromatogram of CLHP analysis of the two enantiomers on Chiralpak AS (eluent C) after purification through preparative CLHP.

enantiomer. Chromatograms of each enantiomer obtained after purification are given as examples on Chiralpak AS (Fig. 4).

Two methods have been developed to give the lactones 10S and 10R via dihydroxylation. The first one was effected using D-xylose derivatives as chiral auxiliaries.¹⁹ Starting from the chiral styrene AD using AD-mix- α (de 99%) and AD-mix- β (de 98%) or dihydroxylation using OsO_4 (de 0%), permitted the preparation of the corresponding diastereoisomerically pure intermediates easily after flash chromatography. In this case the presence of a stereogenic protecting group has achieved the desired objective by allowing the effective resolution of the stereocentre of the lactone 10S and 10R. The second one was effected using achiral substrate 3. Starting from this achiral styrene 3 AD using AD-mix- α (ee 98.5%) and AD-mix- β (ee 97.5%), permitted the preparation of the corresponding enantiomerically pure lactones 10S and 10R after HPLC purification using chiral stationary phase. Both methods afforded the lactone 105 $([\alpha]_{D}^{28} + 35 \ (c \ 0.8, \ CHCl_{3}))$ and the lactone **10***R* $([\alpha]_{D}^{28}$ -34 (c 0.8, CHCl₃)) with the same physical data.

In conclusion the benzo[*c*]furanones **10***S* and **10***R* having one asymmetric carbon atom were obtained in good yields starting from the achiral phthalaldehyde. The key step of this route was the use of the asymmetric dihydroxylation developed by Sharpless. In anti-HIV studies, compounds **10***S* and **10***R* were evaluated for their inhibitory effects on the replication of HIV-1²⁶ in human T4-lymphoblastoid cells, CEM-SS and MT-4. Compounds **10***S* and **10***R* have been found inactive against HIV-1 replication at concentrations up to 10 μ M.

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