NMR Determination of Absolute Configuration of Butenolides of Annonaceous Type

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Abstract: We report herein the first determination of the absolute configuration of the annonaceous butenolides by a NMR method. This technique uses a chiral solvating agent (CSA), the so-called Pirkle's reagent, at *low temperature* and *low concentration*, allowing one to apply this method to other natural products as well. Indeed, the presence of basic sites (e.g. tetrahydrofuran, hydroxyl) did not interfere with the major solvation of the reagent with the lactone moiety. A new model is proposed which allowed us to confirm the (*S*) absolute configuration of the butenolide of annonaceous acetogenins. Furthermore this method can be successfully applied to the measure of the diastereomeric (or enantiomeric) excess of the same butenolide containing compounds.

Keywords: lactones · natural products · NMR spectroscopy

Introduction

The first determination of the absolute configuration of the annonaceous butenolides by a NMR method is reported. Numerous natural products with potent biological profile bear a γ-methyl butenolide moiety at one terminus such as ancepsenolides,[1a–c] acaterine,[1d] hamabiwalactones,[1e] akolactones,[1f] butenolides from *Hortonia* species,[1g] and acetogenins of *Annonaceae* are typical examples of such compounds.[1h–j] For the structural determinations of the (>350) known acetogenins of the *Annonaceae*, extensive spectroscopic methods have been used; however, only scarce reports have been dealing with the absolute configuration determination of the butenolide part (based on specific rotation comparisons, circular dichroism, or analyzes of degradation metabolites such as lactic acid).[1h] Furthermore, total syntheses have been reported, and most of the strategies used thereof are based on an aldolisation, lactonisation and dehydration sequence,[2] although we,[3] and others,[4, 5] have reported the difficulties in performing these three chemical transformations without epimerisation of the chiral center of the butenolide. We wish to report the first applicable NMR method for the determination of the absolute configuration of the butenolide (without derivatisation or immolation of the substrate).[6]

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Results and Discussion

Theoretically, a chiral solvating agent (CSA) should form stable complexes with enantiomers under question, and should also possess strongly anisotropic group. Thus the structure of the diastereomeric complex should be well suited to produce selective shielding effects on protons of substrate moiety. In the case of butenolide containing products this means that orientation of aromatic system should be asymmetric in respect with the butenolide ring plane in (*R*)- and (*S*)-CSA-substrate diasteromeric complexes (Figure 1). Then, provided the absolute stereochemistry of CSA is known, the absolute configuration of substrates can be deduced from respective high/low field chemical shifts of substituents at a chiral center, which are on different sides of the lactone ring plane. Therefore to start with, we analyzed several aryl alcohols and amines to meet the above-mentioned requirements. We carried out calculations (MM, PM3 and HF/6-31G) on complexes of simple 3-ethyl-5-methyl-furan-2(5*H*)-one **A** with alcohols and amines **1**–**4** (Figure 1).

According to calculations, only in the case of 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), **1**, both stability of the CSA-substrate complex and its geometry may lead to selective shielding effects: geometry of the complex is almost asym-

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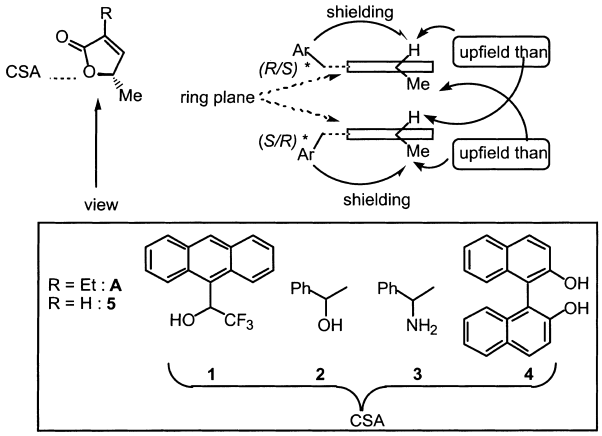


Figure 1. CSA **1**–**4** used for theoretical study with butenolide **A**.

metric with respect to the lactone ring plane (Figure 2). Aromatic shielding effects of anthryl ring should lead to upfield shifts for 5-Me and 5-H protons in (*R*,*S*) and (*S*,*S*) complexes, respectively, and this can be used to establish an absolute configuration at this chiral center.

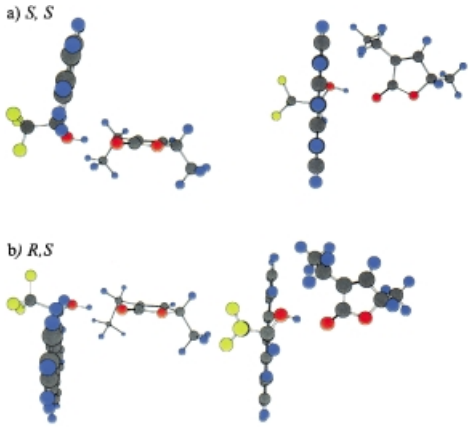


Figure 2. PM3 geometries of stable complexes of TFAE **1** with 3-ethyl-5-methylfuran-2(5*H*)-one **A**.

In the Seventies of the last century Pirkle,[7] and then Epstein[8] reported the use of 2,2,2-trifluoro-1-(9-anthryl)ethanol, **1**, the so-called Pirkle's reagent, as CSA[9] for the determination of the enantiomeric excess, as well as for absolute configuration determination of several compounds amongst others also lactones.[9, 10] NMR differentiation was explained on the basis of a model where stabilization of solvate structures was attributed to "two-point only" interactions. Later additional ("three-point") interactions were invoked to generalize the "two-point" model. In fact, the geometry of our complexes differs from the one proposed by Pirkle. He stressed "two-point" interactions between OH and H protons of CSA and carbonyl and carbinyl oxygen atoms, respectively, of a lactone that stabilize the proposed complex. According to our analysis, while OH proton can be involved in intermolecular hydrogen bonding, the distance between H of CSA and carbonyl oxygen of the lactone is about 5.6 Å, which does not suggest that there is any close contact between these atoms.

In order to prove this hypothesis we carried out NMR experiments at low temperature because larger stabilization of the complex can be expected at those conditions. Indeed, the simplest racemic 5-methylfuran-2(5*H*)one (**5**; 1.8 mg, 37 mM in $CDCl_3$) was analyzed by ^1H NMR in the presence of 4.3 equivalents of the (*R*)-reagent **1** at 300 K. We observed that H-5 appeared as a single signal whereas the methyl appeared as two overlapped doublets. However, at 223 K, we were pleased to observe that both the methyl ($\delta = 1.378$ and 1.331 ppm) and H-5 ($\delta = 5.095$ and 5.054 ppm) signals were split into two signals, corresponding to the (*R*,*S*) and (*R*,*R*) solvates. Furthermore as expected, the chemical shift of the methyl in the (*R*,*S*) solvate is shifted more upfield than in the (*R*,*R*) solvate, as shown by selective irradiation, (opposite to the H-5 signal, see below) and predicted by our model. Irradiation of the methyl signals allowed us to integrate the H-5 peaks and thus to further evidence the 50:50 mixture of both enantiomers.

Moreover, NOE experiments[11] at low temperature (223 K) allowed us to directly demonstrate interaction between HO proton of CSA and lactonic H-3 proton (see Figure 3). These experiments showed that the NOE effect between OH proton and H-3 proton (Figure 3c) is of the same magnitude as between 5-Me and H-3 proton (Figure 3b). At the same time, no intermolecular interactions between H or anthryl protons of CSA and protons of the lactone were observed in these experiments.

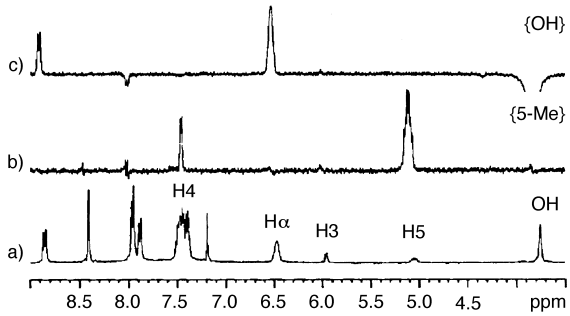
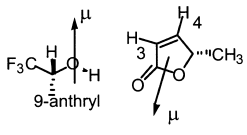


Figure 3. Low field fragment of NOE spectra of racemic 5-methylfuran-2(5*H*)-one **5** (37 mM in $CDCl_3$, at 223 K) in the presence of 4.3 equiv (*R*)-reagent **1**. DPFGNOE method was used with a mixing time of 800 ms; a) without irradiation, b) 5-Me irradiation, c) OH irradiation.

These results are in agreement with the calculations predicting a one-point interaction and in disagreement with the model proposed by Pirkle, according to which distance between H of CSA and substrate protons has to be shorter and, as a result, NOE effect between these protons should be expected. This is not the case.

The structure observed is most probably stabilized by both the O-H···O=C intermolecular hydrogen bonding and the dipole–dipole interactions between the CSA fragment and the substrate moiety (Scheme 1).

As soon as this model was established the absolute configuration determination should be straightforward. But, in practice, there is an obstacle, that is high CSA and substrate



Scheme 1. Proposed model.

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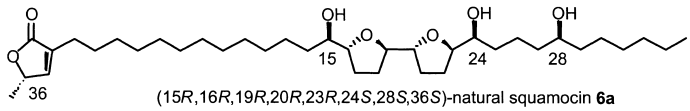
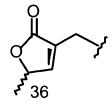
concentrations are required to get reliable $\Delta\delta^{RS}$ values, and multiple "basic" sites on the molecule which could interfere. As an alternative to the concentration problem we decided to tune the population of the complex by variation of the temperature. As temperature has an effect on $\Delta\delta$ values[12] we thought that the recording of the NMR data at lower temperatures should increase these values based on the notion that a preferred conformer of the two diastereomeric complexes is being increasingly populated as the temperature is lowered. Furthermore, we envisioned that low concentrations (around 1 mM) of acetogenins in $CDCl_3$ (instead of the usual 0.2–0.02 M solutions[7, 8]) should be tested,[13] using a ratio of Pirkle reagent **1**/acetogenin superior to what was already reported (3:1).[7a]

In a first set of experiments, we used squamocin **6a**, isolated in our laboratory from *Annona reticulata*,[14] and studied the behavior of Pirkle's reagent **1** as chiral solvating agents (CSA) using by ^{1}H NMR (400 MHz) spectroscopy in view of the preliminary results obtained above. In order to evaluate such a reagent for the discrimination between (*R*)- and (*S*)-butenolides, we treated squamocin **6a** with Et_2NH for 12 h at room temperature, as already described,[3] where it was used for epimerizing the stereogenic center at C-36 of the butenolide, affording **6b** as a mixture of diastereomers (Scheme 2). The NMR experiments were carried out by adding pure CSA **1** to a solution (1.6–3.2 mM) of the substrate which was going to be analyzed. Then, several spectra were recorded at low temperatures, and CH-36 was observed after irradiation of the methyl at C-36 of the butenolide (at $\delta =$ 1.40 ppm) for a better resolution. In fact, in the acetogenins the signal of methyl at C-36 is partially overlapped by the $(CH_2)_n$ of the alkyl chain.



(15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S*)-natural squamocin **6a**



(36*RS*)-epimerized squamocin **6b**

Scheme 2. Natural squamocin **6a** and its epimerized diastereomer **6b**.

The first experiment was performed with **6b** (0.5 mg, 1.6 mM) and 3 equiv (*S*)-**1** (0.7 mg) at 213 K in $CDCl_3$ and irradiation at $\delta = 1.40$ ppm; under these conditions the signal for H-36 appears as a broad singlet at $\delta = 5.059$ ppm. With 6 equiv (*S*)-**1**, the signal shifted without discrimination, but with 8 equivalents a large shouldered peak appeared at $\delta =$ 5.023 ppm. However, under the same conditions, but with 17 equiv (*S*)-**1**, we observed a split of this signal into two separated peaks at $\delta = 5.014$ (minor) and 4.975 ppm (major), corresponding to a $\Delta\delta = 0.039$. Finally, with 27 equiv (*S*)-**1**, under the same conditions, the two signals ($\delta = 4.991$ and 4.934 ppm, $\Delta\delta = 0.057$) are well separated, allowing their integration (28:72, respectively, showing that epimerisation partially occurred). It is also interesting to note that the temperature has an influence; at 223 K in the presence of 27 equiv (*S*)-**1**, in $CDCl_3$, the separation of the two peaks decreased (minor peak appearing now at $\delta = 4.986$ and the major one at $\delta = 4.940$ ppm, corresponding to $\Delta\delta = 0.046$, compare with $\Delta\delta = 0.057$ at 213 K) (Figure 4).

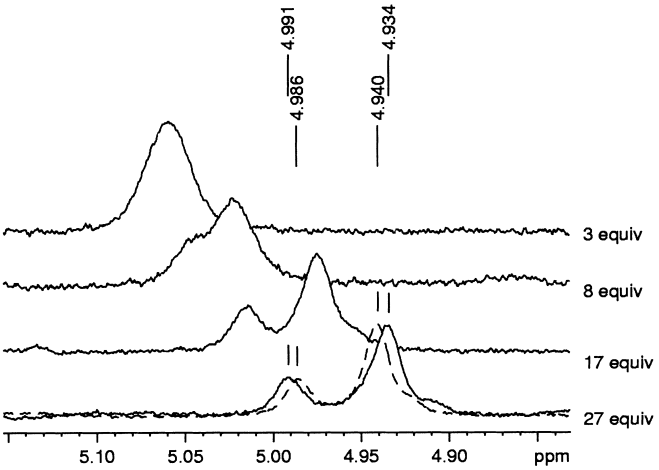


Figure 4. Squamocin **6b** with (*S*) reagent **1** at 213 K in $CDCl_3$ (dotted line: at 223 K).

We also observed a solvent effect, since when we analyzed **6b** (1 mg, 3.2 mM) in CD_2Cl_2 with 6 equiv (*S*)-**1**, at 223 K and after irradiation at $\delta = 1.40$ ppm, the signal for H-36 appears as two peaks at $\delta = 4.904$ (minor) and 4.886 ppm (major), ($\Delta\delta = 0.018$, compare with results in $CDCl_3$ in Figure 5, showing a single peak). However, under the same conditions, but at 213 K, the two peaks were well separated (at $\delta = 4.898$ (minor) and 4.865 ppm (major), corresponding to $\Delta\delta = 0.033$). At 203 K, the two signals were observed at $\delta = 4.887$ (minor) and 4.839 ppm (major), corresponding to $\Delta\delta = 0.048$ (Figure 5).

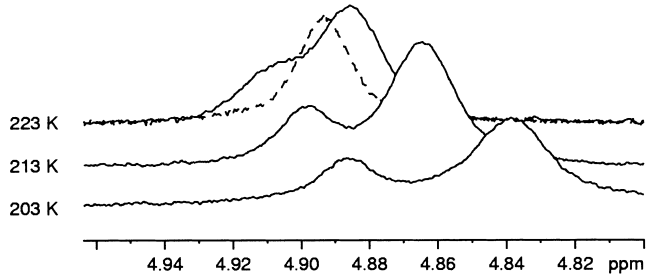


Figure 5. Squamocin **6b** with 6 equiv (*S*) reagent **1** in CD_2Cl_2 (dotted line: in $CDCl_3$ with a shifted scale).

Then we analyzed natural squamocin **6a** (1 mg, 3.2 mM, possessing the 36*S* configuration[6]) with 6 equiv (*R*)-**1** at 223 K in $CDCl_3$. After irradiation at $\delta = 1.40$ ppm, we observed a single signal at $\delta = 5.024$ ppm. Whereas when the natural squamocin **6a** (1 mg, 3.2 mM) was analyzed under the same conditions with 6 equiv (*S*)-**1**, the signal appeared at $\delta =$ 4.994 ppm ($\Delta\delta = 0.030$) (Figure 6). It is worth of note that with only 3 equiv either (*S*)-**1** or (*R*)-**1**, both chemical shifts for H-36, in these two experiments, were almost identical ($\delta =$ 5.039, 5.044 ppm, respectively, corresponding to $\Delta\delta = 0.005$). We can further conclude that when (36*S*)-squamocin **6a** is

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solvated with (*S*)-**1**, the H-36 signal appears upfield compared with (36*S*)-squamocin **6a** solvated with (*R*)-**1**. In other words, we can predict that the H-36 signal for (*S*,*S*) or (*R*,*R*) solvates appears upfield compare with the (*R*,*S*) or (*S*,*R*) solvates. This implies that if $\Delta(\delta\text{H-36}_{R\text{-}1} - \delta\text{H-36}_{S\text{-}1}) > 0$, then the absolute configuration of the butenolide is (*S*). We repeated the experiments with epimerized squamocin **6b** (0.5 mg, 1.6 mM in $CDCl_3$ at 213 K) with 25 equiv (*R*)-**1** and observed that two peaks for H-36 appeared with the major one at lower field ($\delta = 4.990$, corresponding to the (*S*,*R*) solvate) and the minor one at $\delta = 4.956$ (corresponding to the (*R*,*R*) solvate). Whereas at 213 K with 27 equiv (*S*)-**1** the minor one is at $\delta = 4.991$ and the major one at $\delta = 4.934$ ppm ($\Delta\delta = 0.057$) (Figure 6). It is noteworthy that the H-36 chemical shifts in the solvates are in accord with our model shown above.

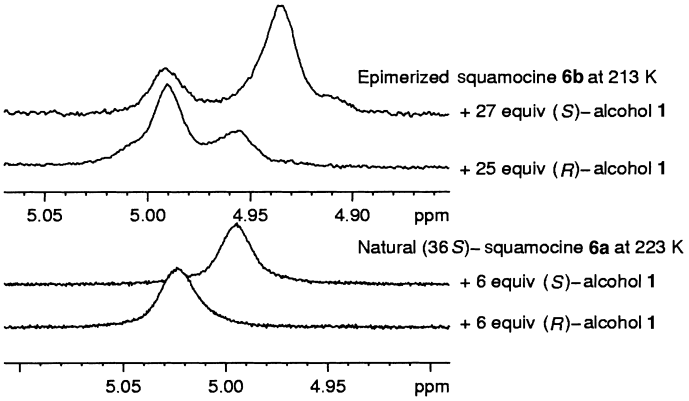
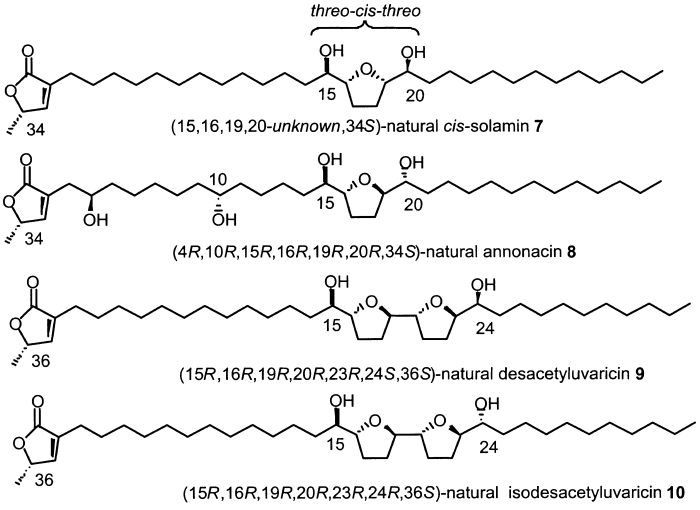


Figure 6. Squamocin **6b** and squamocin **6a** with variable amount of either (*R*)- or (*S*)-reagent **1**.

We then decided to check if this method could be generalized and thus performed a series of two experiments with mixtures of natural acetogenins with either (*S*) or (*R*) reagent **1** (Scheme 3, and Table 1). For instance, *cis*-solamin **7**[15] (0.5 mg, 1.7 mM) was dissolved in $CDCl_3$ together with 20 equiv (*R*)-**1**. The ^{1}H NMR spectrum was recorded at 213 K



Scheme 3. Natural *cis*-solamin **7**, annonacin **8**, desacetyluvaricin **9** and isodesacetyluvaricin **10**.

Table 1. Chemical shift δ of H-34 or δH-36 of **7**–**10** with reagent **1** at 213 K.

Compound	With (*n* equiv) of (*R*)-**1**	With (*n* equiv) of (*S*)-**1**	$\Delta(\delta_R - \delta_S)$
solamin **7**	4.995 (20)	4.955 (20)	+0.040
annonacin **8**	4.859 (35)	4.772 (35)	+0.087
desaceyluvaricin **9**	5.015 (14)	4.949 (14)	+0.066
isodesaceyluvaricin **10**	5.012 (9)	4.959 (12)	+0.053

after irradiation at $\delta = 1.40$ ppm. Under these conditions the signal for H-34 appears as a broad singlet at $\delta = 4.995$ ppm, whereas under the same conditions but 20 equiv (*S*)-**1**, H-34 appears at $\delta = 4.955$ ppm ($\Delta\delta = +0.040$). We can thus conclude that *cis*-solamin **3** has the (34*S*) absolute configuration (as confirmed by our enzymatic method[6]).

In the case of annonacin **8**,[16] it has been shown by enzymatic method that the absolute configuration at C-34 was (*S*); the ^{1}H NMR method described above confirms such findings (at 213 K, in the presence of 35 equiv (*R*)-**1**, δ H-34 = 4.859, whereas with 35 equiv (*S*)-**1** δ H-34 = 4.772 ppm, corresponding to $\Delta\delta = +0.087$). It is noteworthy that the hydroxyl group at the 4-position did not interfere with the major solvating of the lactone ring by reagent **1**. Desacetyluvaricin **9**[17] was analyzed next. Again, we observed two different signals (at 213 K, in the presence of 14 equiv (*R*)-**1**, δ H-36 = 5.015, whereas with 14 equiv (*S*)-**1** δ H-36 = 4.949 ppm, corresponding to $\Delta\delta = +0.066$). The results allow us to assume the (*S*) absolute configuration at C-36. With isodesacetyluvaricin **10**[17] the absolute configuration was again C-36 (*S*) as shown by NMR data (at 213 K, in the presence of 9 equiv (*R*)-**1**, δ H-36 = 5.012, whereas with 12 equiv (*S*)-**1**, δ H-36 = 4.959 ppm corresponding to $\Delta\delta = +0.053$).

In conclusion we describe herein a very efficient NMR method for the determination of the absolute configuration of the butenolide moiety of annonaceous acetogenins. This technique uses Pirkle's reagent at *low temperature and low concentration*, and allows one to apply this method to other natural products as well. Indeed, the presence of basic sites (e.g. tetrahydrofuran, hydroxyl) did not interfere with the major solvation of the reagent with the lactone moiety. We propose a new model which allowed us to confirm the (*S*) absolute configuration of the butenolide of annonaceous acetogenins (further confirmed by enzymatic method[18]). Furthermore, this method can be successfully applied to the measure of the diastereomeric excess of the same containing butenolide compounds, making this method a very useful tool in total synthesis of such interesting bioactive products.[19] Of course this can also be applied to the determination of the enantiomeric excess of butenolides containing a single stereogenic center.

Experimental Section

NMR spectra were recorded at 400.13 MHz for ^{1}H. The temperature was controlled to 0.1 °C. Chemical shifts are reported in parts per million (ppm) relative to internal TMS. Butenolide containing product (0.5 to 1 mg, 0.8 to 1.6 μmol), (*S*)- or (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (3 to 35 equiv),

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and 0.5 mL of solvent ($CDCl_3$ or CD_2Cl_2) were placed in a 5 mm NMR tube and the spectrum recorded at the required temperature with (or without) irradiation at $\delta = 1.40$ ppm (45 Db). Molecular mechanics (employing the MM2 force field) and PM3 molecular orbital calculations were performed by the CS Chem3D Ultra 6.0 on a AuthenticAMD Athlon(Im) computer. Ab initio electronic structure calculations (at the restricted Hartree-Fock level of theory) were performed using GAUSSIAN 98.

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[19] When squamocin **6a** was protected as a trisilylated ether (TBDMSOTf, pyridine, 4-DMAP), the trisilyl ether **6c** was, after purification, analyzed as described above for (*S*)-**1**. Surprisingly, we observed two peaks at $\delta = 4.997$ and 4.950 ppm in a 15/85 ratio. We can conclude that the protection step occurred with partial epimerization of the butenolide.

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