

## NMR and X-ray characterization of steroids with furospirostane side chains

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### Abstract

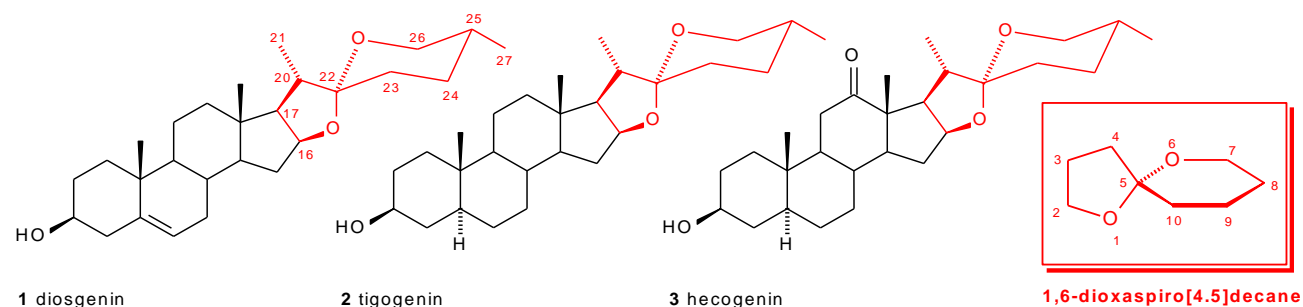
The synthesis and assignment of  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals and X-Ray characterization of steroids bearing furospirostane side chains is provided. The effects of the side chain modifications on the puckering parameters and NMR characteristics derived from the modification of the side chains are discussed.

**Keywords:** Steroids, furospirostanes, NMR, X-Ray structures, puckering

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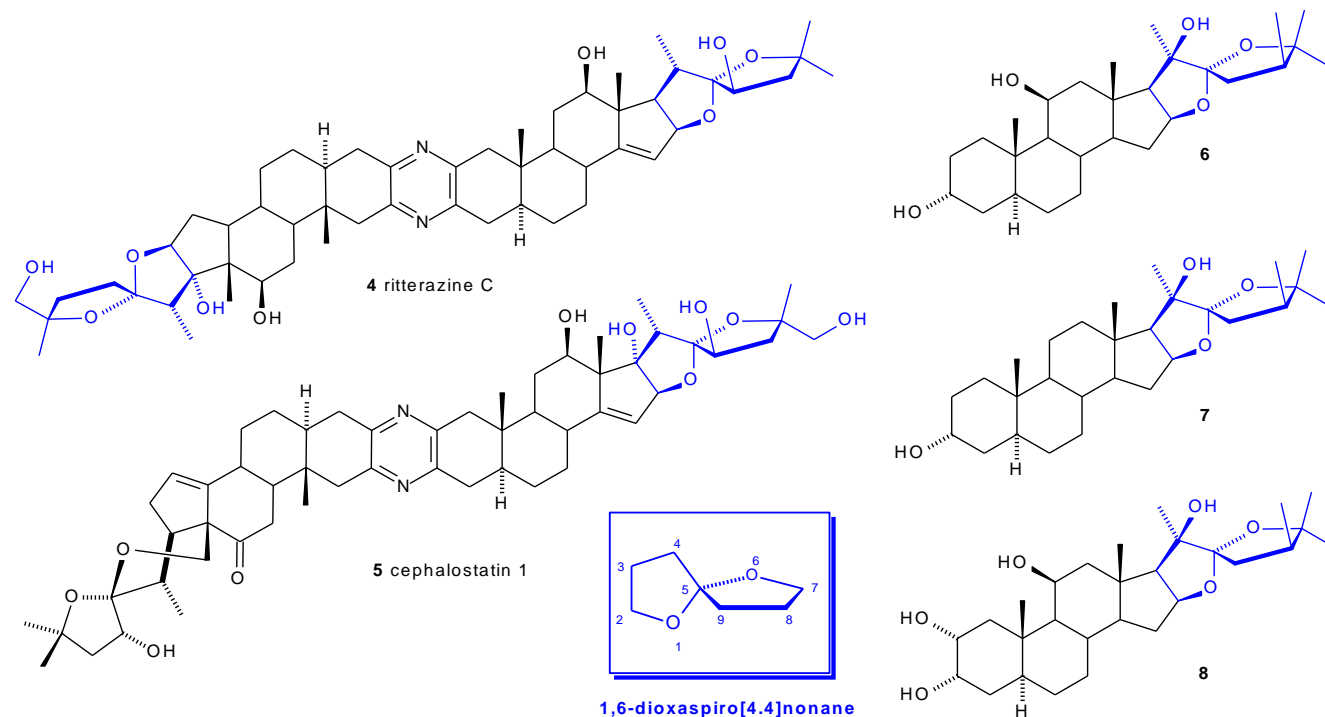
### Introduction

Steroids bearing oxygenated spiroketal side chains are widespread in both the natural and synthetic domains. Steroid sapogenins, which have produced a large variety of interesting reactions and rearrangements,<sup>1</sup> are characterized by the presence of a  $16\beta,22:22,26$ -diepoxy moiety in side chain and can be regarded as 1,6-dioxaspiro[4.5]decane derivatives (Figure 1). This kind of compound has been subject of much research due to their biological activity<sup>2</sup> and their usefulness as starting materials for the synthesis of different bioactive compounds as sexual or adrenocortical hormones,<sup>1a</sup> ecdysteroids,<sup>3</sup> plant growth stimulators,<sup>4</sup> and cytotoxic steroids<sup>5</sup> among others.



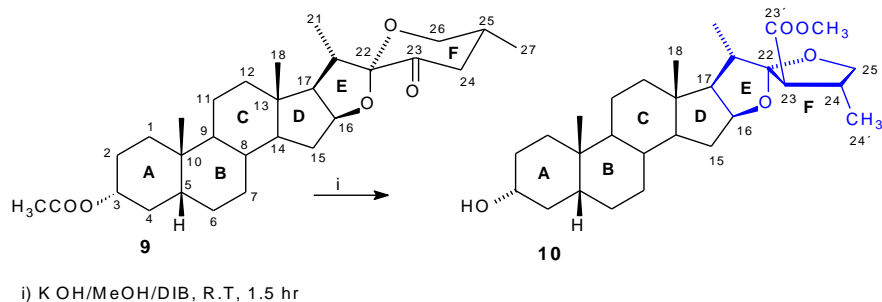
**Figure 1.** Some naturally occurring steroid sapogenins.

Furospirostanes, a smaller and relatively less known family of steroids bearing a  $16\beta,22:22,25$ -diepoxy moiety in the chain, may be considered 1,6-dioxaspiro[4.4]nonane derivatives, and include compounds with antitumor activity as the ritterazines **4**,<sup>5f</sup> cephalostatins **5**,<sup>5f</sup> or hippuristanols **6-8**,<sup>6</sup> among others (see Figure 2).



**Figure 2.** Some furospirostanes with cytotoxic activity against cancer cells.

As a part of our project to explore the reactivity of steroid bearing spiroketals side chains, we have recently focused our attention on the  $16\beta,22:22,25$ -diepoxy moiety derived from our recently reported hypervalent iodine-induced F-ring contraction (see Scheme 1).<sup>1f</sup>

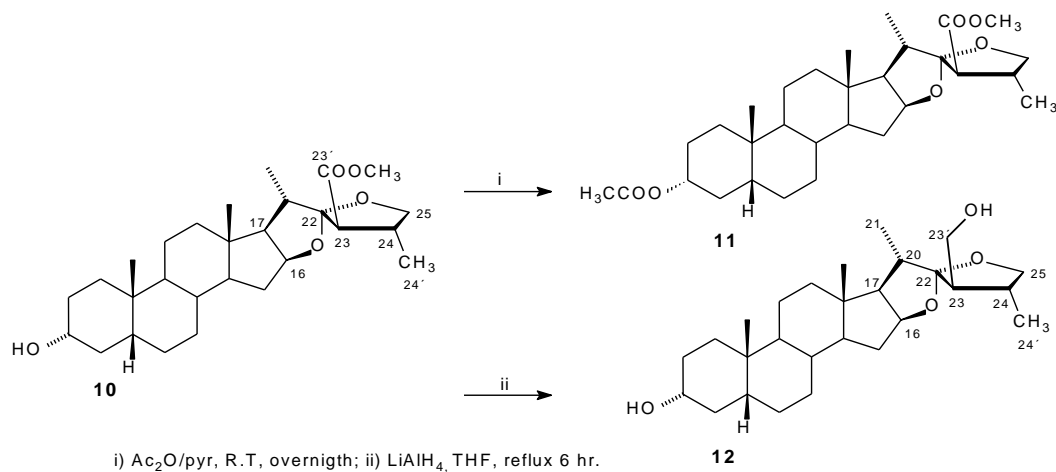


### Scheme 1

In this context and for structural reference purposes, we decided to carry out a detailed characterization of compounds bearing such functionality that serve as materials for our biological, synthetic and chemical reactivity studies. In this communication we describe the unambiguous assignment of all  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of spirocyclic steroids bearing the  $16\beta,22:22,25$ -diepoxy moiety in the side chain. In addition a detailed analysis of the crystal structures of such compounds is also provided.

### Results and Discussion

Since compound **10** persisted in crystallizing in needles not suitable for X-Ray studies, derivatization of **10** was necessary. Hence the acetate **11** was prepared in 74.8% yield by the standard  $\text{Ac}_2\text{O}$ /pyridine procedure.  $\text{LiAlH}_4$  reduction of **10** afforded the diol **12** in 84.9% yield (see Scheme 2).



### Scheme 2

**<sup>1</sup>H NMR Characteristic derived from the steroid nucleus.**

Several papers have dealt with the total assignment of protons attached to the steroid framework. In particular Kirk<sup>7a</sup> and Agrawal<sup>7b</sup> have provided excellent reference papers that also provided useful hints for the assignment of the <sup>1</sup>H signals of naturally occurring and synthetic steroids. Although we are providing the total assignment of the <sup>1</sup>H signals of all the studied compounds, the discussion will be focused in the <sup>1</sup>H signals associated to the 16 $\beta$ , 22:22,25-diepoxy side chain.

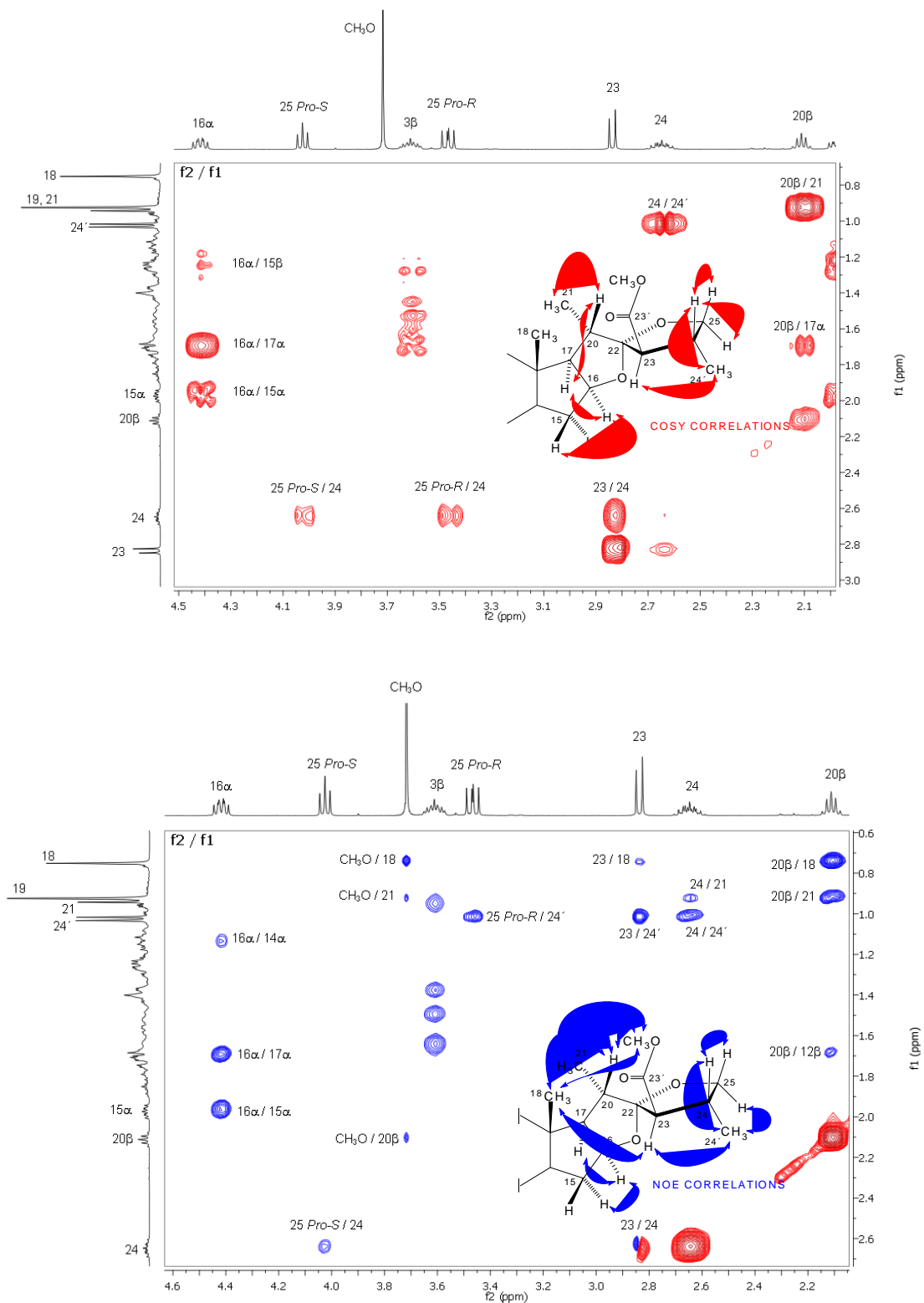
**<sup>1</sup>H NMR Characteristic derived from the 16 $\beta$ , 22:22,25-diepoxy side chain.**

The contraction of the F-ring produces significant changes on the signals of the protons placed in the side chain. In particular the furospirostane side chains of compounds **10** and **11** are characterized by the presence of a new deshielded signal corresponding to H-23 in  $\alpha$  position of both the spiroketal function at C-22 and the carboxymethyl group attached to C-23. The conversion of the F-ring into a 5-membered cycle also produces strong deshielding of H-25 *Pro-S* (formerly H-26 *Pro-S* in compound **9**) and shielding of H-25 *Pro-R*, (formerly H-26 *Pro-R* in **9**).

Similarly, upfield shift of H-20 and downfield shifts of both H-24 (formerly H-25 in **9**) and H-24' (formerly H-27 in **9**) are also associated to the F-ring contraction that gave place to compound **10**. Table 1 shows the key 2D homo- and heteronuclear correlations that allow the unambiguous assignment of most of the <sup>1</sup>H signals associated to the (23*R*)-23-carboxymethyl-16 $\beta$ , 22:22,25-diepoxy side chain of compound **10**, (see Figure 3 for key H-H correlations and supplementary information file for annotated spectra).

**Table 1.** Main hetero- and homonuclear correlations of the side chain of compound **10**

H	COSY	NOESY H-H	HMBC H-C	
	H-H		2 bonds	3 bonds
20 $\beta$	17 $\alpha$ , 21	12 $\beta$ , 18, 21, CH <sub>3</sub> O	17, 21, 22	13, 23
21	20 $\beta$	20 $\beta$ , 24, CH <sub>3</sub> O	20	17, 22
23	24	18, 24, 24'	22, 23', 24	24'
24	23, 25 <i>Pro-R</i> , 25 <i>Pro-S</i>	21, 23, 25 <i>Pro-S</i>	23, 25	23'
24'	24	23, 24, 25 <i>Pro-R</i>	24	23, 25
25 <i>Pro-R</i>	24	24'	24	23, 24'
25 <i>Pro-S</i>	24	24	24	22, 23



**Figure 3.** Selected H-H and NOE correlations in the side chain of compound 10.

The reduction of the carboxymethyl group in **10** to produce the diol **12** results in the downfield shift of H-20 and the upfield shift of H-23. The  $^1\text{H}$  spectrum of the reduced compound **12** also shows two new signals corresponding to the diastereotopic hydrogens attached to C-23' which shows the typical AB-X coupling pattern. Table 2 shows the key 2D homo- and heteronuclear correlations that allow the unambiguous assignment of most of the  $^1\text{H}$  signals associated to the (23*S*)-23-hydroxymethylene-16 $\beta$ ,22:22,25-diepoxy side chain of compound **12**, (see Figure 4 for key H-H correlations and supplementary information file for annotated spectra).

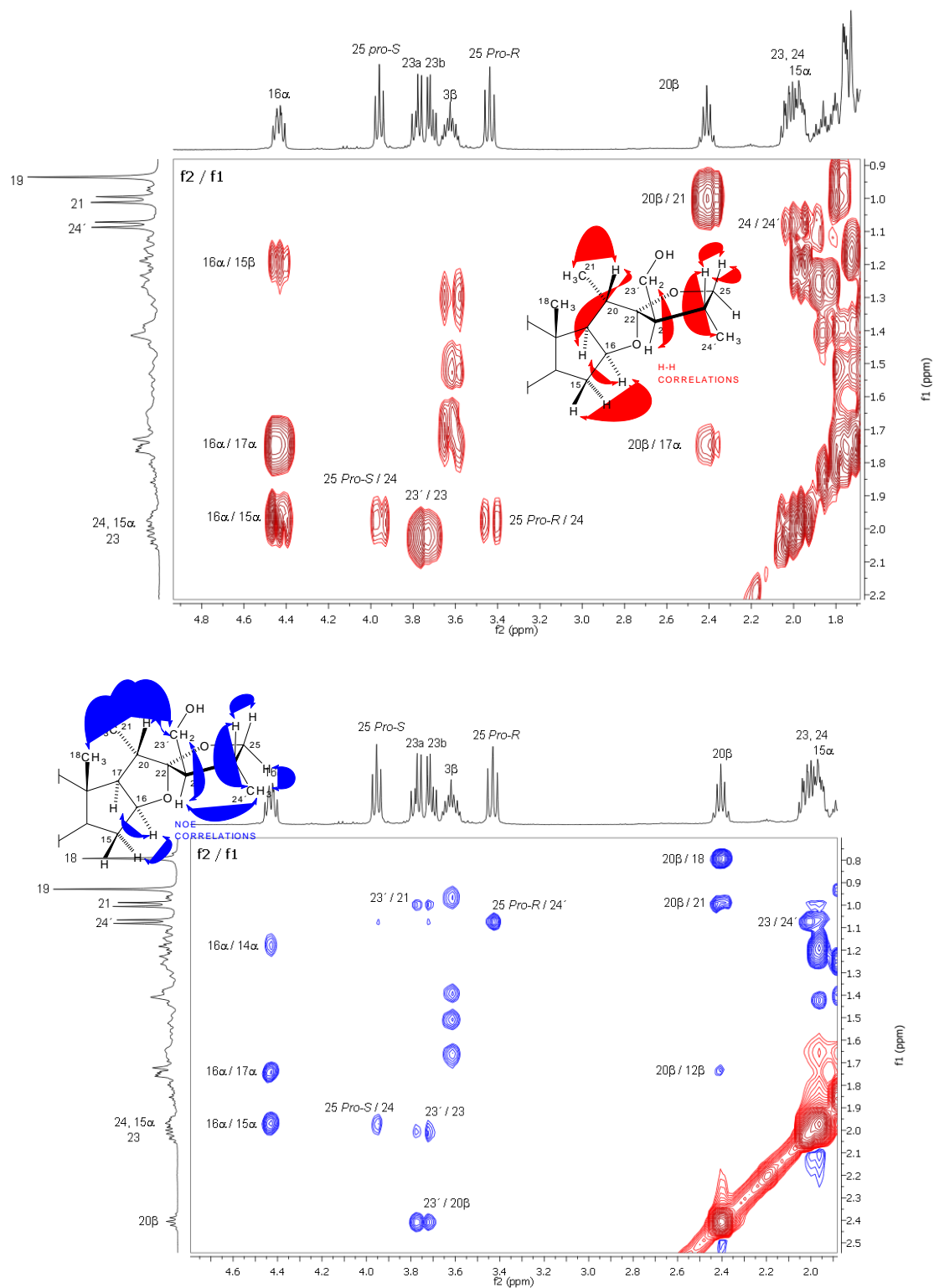
**Table 2.** Main hetero- and homonuclear correlations of the side chain of compound **12**

H	COSY	NOESY	HMBC H-C	
	H-H	H-H	2 bonds	3 bonds
20 $\beta$	17 $\alpha$ , 21	12 $\beta$ , 18, 21	17, 21, 22	13, 23,
21	20 $\beta$	20 $\beta$ , 23'a, 23'b	20	17, 22
23 ** 24	23'a, 23'b	23'a, 23'b, 24'	24	-
23' a	23	21, 23, 20 $\beta$	23	22
23' b	23	21, 23, 20 $\beta$	23	22, 24
24 ** 23	25 <i>Pro-R</i> , 25 <i>Pro-S</i>	25 <i>Pro-S</i>	23, 25	-
24'	24	23, 25 <i>Pro-R</i>	24	23, 25
25 <i>Pro-R</i>	24	24'	24	24'
25 <i>Pro-S</i>	24	24	24	22, 23

\*\*overlapped

While the comparison between the coupling constants of the protons in the side chain of the spirostane derivative **9** with those of the furospirostanes **10** to **12** indicates that the conversion of 6-membered F-ring into a 5-membered ring results in the expected and evident conformational changes, the coincidence of the coupling constants of H-23 *pro-R* in compounds **11** and **12** suggests that the conversion of the carboxymethyl moiety of **11** into the exocyclic hydroxymethylene of **12** does not induce significant conformational changes in the side chain.

**$^{13}\text{C}$  NMR discussion.** In addition to the extensive collection of the  $^{13}\text{C}$  chemical shifts of around 400 steroids built up by Blunt and Stothers,<sup>8a</sup> Agrawal published an excellent review which comprises the assignment of the  $^{13}\text{C}$  chemical shifts of 66 steroid sapogenins bearing the 16 $\beta$ ,22:22,26-diepoxy in the side chain.<sup>8b</sup> Some of our previous works on this subject have also provided rules for the systematization of the effects exerted by different functional groups in NMR signals of the 16 $\beta$ ,22:22,26-diepoxy side chain.<sup>8c-e</sup> Consequently, the discussion here will be focused in the main NMR characteristic associated with the 5-membered 16 $\beta$ ,22:22,25-diepoxy side chain result of the F-ring contraction (see crystallographic studies).



**Figure 4.** Selected H-H and NOE correlations in the side chain of compound 12.

**Table 3.**  $^1\text{H}$  chemical shifts of the compounds **9** to **12**

H	<b>9</b> *	H	<b>10</b>	<b>11</b>	<b>12</b>
1 $\alpha$	1.80	1 $\alpha$	1.79	1.78	1.78
1 $\beta$	1.02	1 $\beta$	0.96	1.01	0.97
2 $\alpha$	1.38	2 $\alpha$	1.29	1.37	1.30
2 $\beta$	1.67	2 $\beta$	1.66	1.66	1.66
3 $\beta$	4.71 (m)	3 $\beta$	3.62 (m)	4.69 (m)	3.62 (ddd, $J = 15.7$ , 10.9, 4.7)
4 $\alpha$	1.80	4 $\alpha$	1.73	1.79	1.74
4 $\beta$	1.53	4 $\beta$	1.51	1.52	1.51
5 $\beta$	1.44	5 $\beta$	1.40	1.43	1.39
6 $\alpha$	1.23	6 $\alpha$	1.25	1.23	1.53
6 $\beta$	1.85	6 $\beta$	1.85	1.84	1.84
7 $\alpha$	1.07	7 $\alpha$	1.09	1.07	1.10
7 $\beta$	1.38	7 $\beta$	1.43	1.37	1.40
8 $\beta$	1.57	8 $\beta$	1.57	1.57	1.57
9 $\alpha$	1.40	9 $\alpha$	1.40	1.39	1.41
11 $\alpha$	1.38	11 $\alpha$	1.39	1.70	1.40
11 $\beta$	1.23	11 $\beta$	1.21	1.21	1.24
12 $\alpha$	1.13	12 $\alpha$	1.12	1.12	1.17
12 $\beta$	1.74	12 $\beta$	1.71	1.69	1.73
14 $\alpha$	1.15	14 $\alpha$	1.13	1.14	1.17
15 $\alpha$	1.98	15 $\alpha$	1.98	1.95	1.99
15 $\beta$	1.27	15 $\beta$	1.26	1.23	1.22
16 $\alpha$	4.59 (td, $J =$ 7.6, 6.2)	16 $\alpha$	4.42 (td, $J =$ 7.7, 6.0)	4.40 (td, $J =$ 7.7, 6.0)	4.43 (m)
17 $\alpha$	1.74	17 $\alpha$	1.71	1.69	1.74
18 (CH <sub>3</sub> )	0.74 (s)	18 (CH <sub>3</sub> )	0.75 (s)	0.74 (s)	0.79 (s)
19 (CH <sub>3</sub> )	0.93 (s)	19 (CH <sub>3</sub> )	0.92 (s)	0.92 (s)	0.93 (s)
20 $\beta$	2.86 (quin, $J =$ 6.9)	20 $\beta$	2.11 (quin, $J =$ 6.8)	2.10 (quin, $J =$ 6.8)	2.41 (quin, $J = 6.8$ )
21 (CH <sub>3</sub> )	0.92 (d, $J = 7.0$ )	21 (CH <sub>3</sub> )	0.93 (d, $J = 7.4$ )	0.92 (d, $J = 6.9$ )	1.00 (d, $J = 6.9$ )
23	-	23	2.84 (d, $J = 9.4$ )	2.82 (d, $J = 9.3$ )	1.99 (m)
23' a	-	23' a	-	-	3.78 (dd, $J = 10.9$ , 7.1)
23' b	-	23' b	-	-	3.71 (dd, $J = 10.9$ , 5.5)



**Table 3.** Continued

H	<b>9</b> *	H	<b>10</b>	<b>11</b>	<b>12</b>
24 <i>Pro-S</i> (ax.)	2.42 (m)	-	-	-	-
24 <i>Pro-R</i> (eq.)	2.47 (dd, <i>J</i> = 10.7, 10.7)	-	-	-	-
25	2.27 (m)	24	2.65 (m)	2.64 (m)	1.99 (m)
26 <i>Pro-S</i> (eq.)	3.57 (ddd, <i>J</i> = 11.2, 4.6, 1.4)	25 <i>pro-S</i>	4.03 (dd, <i>J</i> = 7.7, 7.7)	4.01 (dd, <i>J</i> = 7.7, 7.7)	3.95 (d, <i>J</i> = 7.6, 7.6)
26 <i>Pro-R</i> (ax.)	3.77 (dd, <i>J</i> = 11.3, 11.3)	25 <i>pro-R</i>	3.47 (dd, <i>J</i> = 10.1, 8.1)	3.46 (dd, <i>J</i> = 10.1, 8.1)	3.43 (dd, <i>J</i> = 9.3, 8.2)
27 (CH <sub>3</sub> )	0.92 (d, <i>J</i> = 6.6)	24' (CH <sub>3</sub> )	1.03 (d, <i>J</i> = 6.6)	1.01 (d, <i>J</i> = 6.6)	1.07 (d, <i>J</i> = 6.3)
	2.01 (s)	CH <sub>3</sub> COO-3	-	2.00 (s)	-
		CH <sub>3</sub> O	3.72 (s)	3.71 (s)	

\*Signals of compound **9**<sup>8e</sup> are included for reference

### <sup>13</sup>C NMR characteristics of the 16β,22:22,25-diepoxy side chain.

The conversion of the F-ring of **9** into the 5-membered ring of **10** produces the downfield shift of C-25 (formerly C-26 in **9**) that now resonates at between 71.8 and 72.5 ppm as a result of the lost of the 1,3 shielding interactions present in the 6-membered F-ring of compound **9**. In addition, the 16β,22:22,25-diepoxy side chains of compounds **10** to **12** are characterized by the presence of a deshielded signal corresponding to the spiroketal carbon C-22 between 119 and 120 ppm (See Table 4).

In particular the <sup>13</sup>C spectra of compounds **10** and **11** are characterized by the presence of the signal corresponding to C-23 around 59.7 ppm and the those corresponding to the exocyclic carboxymethyl group attached to C-23 around 172 ppm (C=O) and 51.8 (OCH<sub>3</sub>). The F-ring contraction also produces a slight upfield shift on C-24' (formerly C-27 in compound **9**) as well as slight downfield shifts on C-20, C-21 and C-24 (formerly C-25 in compound **9**).

As expected, the reduction of the exocyclic carboxylic ester moiety to produce an exocyclic hydroxy methylene results on the upfield shifts of the signals of C-23 and C-23'. Provided that the reduction of the C-23' carboxyl ester moiety attached to C-23 does not produce significant conformational changes in the side chain (*vide supra*), the slight upfield and downfield shifts observed in other C nuclei of the side chain should be attributed to field changes associated to the conversion of the magnetically anisotropic carboxymethyl moiety into a hydroxymethylene group.

## Crystallographic studies

Table 4.  $^{13}\text{C}$  chemical shifts of compounds 9 to 12

<b>C</b>	<b>9*</b>	<b>C</b>	<b>10**</b>	<b>11**</b>	<b>12**</b>
<b>1</b>	35.0	<b>1</b>	35.3	35.0	35.3
<b>2</b>	26.6	<b>2</b>	30.5	26.5	30.5
<b>3</b>	74.2	<b>3</b>	71.7	74.2	71.8
<b>4</b>	32.2	<b>4</b>	36.4	32.2	36.4
<b>5</b>	41.8	<b>5</b>	42.0	41.8	42.0
<b>6</b>	26.9	<b>6</b>	27.1	26.9	27.1
<b>7</b>	26.5	<b>7</b>	26.6	26.5	26.7
<b>8</b>	35.4	<b>8</b>	35.5	35.5	35.5
<b>9</b>	40.5	<b>9</b>	40.5	40.4	40.5
<b>10</b>	34.7	<b>10</b>	34.7	34.7	34.7
<b>11</b>	20.5	<b>11</b>	20.5	20.5	20.6
<b>12</b>	40.0	<b>12</b>	40.0	39.9	40.1
<b>13</b>	41.1	<b>13</b>	41.1	41.1	41.2
<b>14</b>	56.4	<b>14</b>	56.2	56.2	56.2
<b>15</b>	31.7	<b>15</b>	31.8	31.8	31.9
<b>16</b>	83.4	<b>16</b>	81.4 (-2.0)	81.4 (0)	81.0 (-0.4)
<b>17</b>	61.8	<b>17</b>	62.7 (+0.9)	62.7 (0)	62.9 (+0.2)
<b>18</b>	16.1	<b>18</b>	16.0 (-0.1)	16.0 (0)	16.4 (+0.4)
<b>19</b>	23.3	<b>19</b>	23.3	23.3 (0)	23.4 (+0.1)
<b>20</b>	34.7	<b>20</b>	37.4 (+2.7)	37.4 (0)	35.8 (-1.6)
<b>21</b>	14.4	<b>21</b>	15.5 (+1.1)	15.5 (0)	16.2 (+0.7)
<b>22</b>	109.8	<b>22</b>	119.3 (+9.5)	119.3 (0)	120.2 (+0.9)
<b>23</b>	201.8	<b>23</b>	59.7 (-142.1)	59.6 (-0.1)	55.7 (-3.9)
<b>24</b>	45.2	<b>23'</b>	172.5	172.4 (-0.1)	62.5 (-109.9)
<b>25</b>	35.8	<b>24</b>	37.5 (+1.7)	37.5 (0)	36.9 (-0.6)
<b>26</b>	65.6	<b>25</b>	72.5 (+6.9)	72.5 (0)	72.2 (-0.3)
<b>27</b>	17.1	<b>24'</b>	15.4 (-1.7)	15.4 (0)	16.3 (+0.9)
<b>CH3COO-3</b>	170.5	<b>CH3COO-3</b>	-	170.5	-
<b>CH3COO-3</b>	21.4	<b>CH3COO-3</b>	-	21.4	-
		<b>CH3O-23'</b>	51.8	51.8 (0)	-

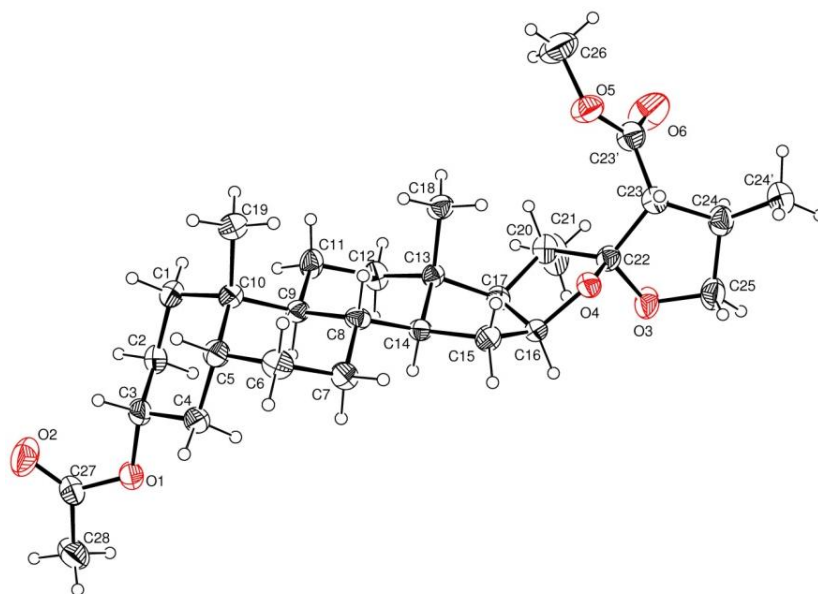
\*Signals of compound **9**<sup>8c</sup> are included for reference. \*\* $\Delta$  ppm associated to the changes in the side chain are given in parentheses and were calculated using the synthetic precursor as reference.

The absolute configuration of the studied compounds was considered and corroborated as that from the starting steroid sapogenin.<sup>8c</sup> Studied compounds **11** and **12** contain the steroid ABCD fused ring framework with the axial methyl groups attached to C-10 and C-13 cis A/B, trans B/C, and trans C/D rings junctions, they also bear 16 $\beta$ ,22:22,25-diepoxy moiety characteristic of the furospirostane side chain. Puckering parameters were calculated as described by Cremer and Pople.<sup>9a</sup>

In compound **11** the equatorial acetoxy group bonded to C-3 is arranged in such a way that the carbonyl O atom nearly eclipses the axial H atom attached to C-3 and does not disturb ring A, which present a chair conformation with a puckering amplitude (Q) = 0.555(2) Å,  $\theta$  = 178.0(2) °,  $\varphi$  = 242(5) °, if the calculation starts from C-1 to C-10 and proceeds in a counterclockwise direction. Ring B assumes a conformation very similar to a chair form with a puckering amplitude Puckering Amplitude (Q) = 0.578(2) Å,  $\theta$  = 3.2(2) °,  $\varphi$  = 226(4) °, if the calculation starts from C-5 to C-10 and proceeds in a counterclockwise direction. Ring C assumes a conformation close to a chair with a puckering amplitude (Q) = 0.569(2) Å,  $\theta$  = 9.6(2) °,  $\varphi$  = 254.9(14) °, if the calculation starts from C-8 to C-14 and proceeds in a clockwise direction. Ring D can be described as cyclopentane twisted on C-13-C-14 with puckering parameters of Q(2) = 0.445(2) Å,  $\varphi$  (2) = 202.3(3) °, if the calculation starts from C-13 to C-17 and proceeds in a clockwise direction. Ring E display an envelope O(4)-exo with absolute L configuration with puckering parameters Q(2) = 0.350(2) Å,  $\varphi$  (2) = 186.2(4) °, if the calculation follow this order; O(4), C-22, C-20, C-17, C-16. The tetrahydrofuran ring E is cis-fused to the cyclopentane ring D. Finally the F-ring, which bisects the normal plane of the steroid skeleton, shows a C-25-endo envelope conformation with puckering parameters Q(2) = 0.369(3) Å,  $\varphi$  (2) = 146.3(4)°, if the calculation follow this order: O(3), C-22, C-23, C-24, C-25, (See Table 5). The crystal structure of compound **11** is shown in Figure 5, with the ellipsoid drawn at the 50% of probability.<sup>9b</sup>

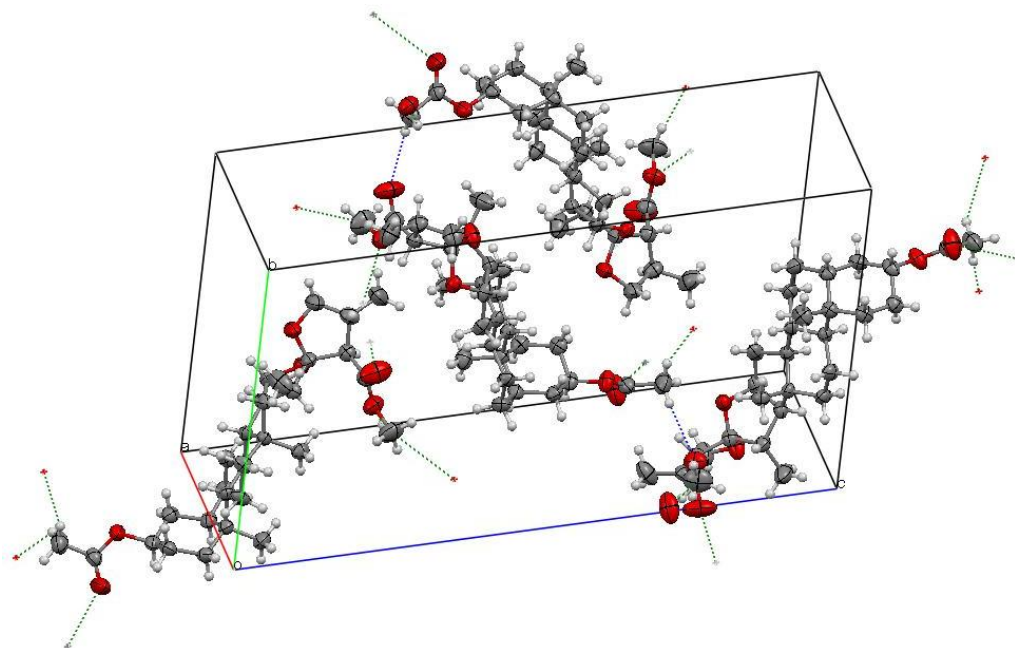
**Table 5.** Calculated puckering parameters for compound **11**

Ring	Q / Å	$\theta$ / °	$\varphi$ / °	Order and direction of the calculation
A	0.555(2)	178.0(2)	242(5)	From C(1) to C(10), counterclockwise
B	0.578(2)	3.2(2)	226(4)	From C(5) to C(10), counterclockwise
C	0.569(2)	9.6(2)	255(1)	From C(8) to C(14), clockwise
<b>Ring</b>	<b>Q(2) / Å</b>	<b><math>\varphi</math> / °</b>		
D	0.445(2)	202.3(3)	-	From C(13) to C(17), counterclockwise
E	0.350(2)	186.2(4)	-	In this order; O(4), C(22), C(20), C(17), C(16)
F	0.369(3)	146.3(4)	-	In this order: O(3), C(22), C(23), C(24), C(25)



**Figure 5.** Crystal structure of compound **11**.

The molecules in the unit cell of **11** show non-classic hydrogen bonds and they are linked via one hydrogen bond in the same cell C(24)-H(24)···O(6) (2.50 H...O, 2.887(4) C O) with other to the next cell, C(28)-H(28B)···O(6) (2.54 H O, 3.439(3) C O). The packing of the crystal is assumed to be dictated by van der Waals interactions and intermolecular CH---O hydrogen bonds. In figure 6 the main contacts of this molecule are shown.

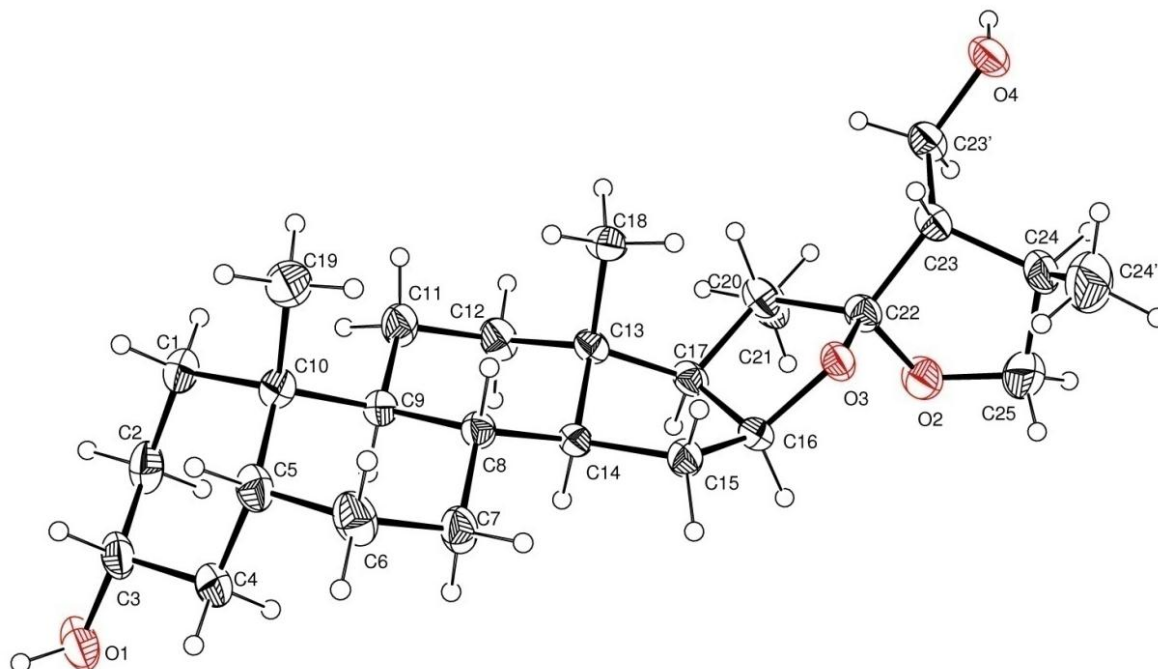


**Figure 6.** View of the unit cell and the main contacts of **11**.

As expected, in compound **12**, the presence of the smaller equatorial hydroxyl substituent attached to C-3 does not disturb the chair conformation of ring A, which shows a puckering amplitude ( $Q$ ) = 0.560(2) Å,  $\theta$  = 175.0(2) °,  $\varphi$  = 282(2) °, if the calculation starts from C-1 to C-10 and proceeds in the counterclockwise direction. Ring B assumes a conformation very similar to a chair form with a puckering amplitude Puckering Amplitude ( $Q$ ) = 0.573(2) Å,  $\theta$  = 4.5(2) °,  $\varphi$  = 227(2) °, if the calculation starts from C-5 to C-10 and proceeds in a counterclockwise direction. Ring C assumes a conformation close to a chair with a puckering amplitude ( $Q$ ) = 0.5550(19) Å,  $\theta$  = 10.7(2) °,  $\varphi$  = 247.5(11) °, if the calculation starts from C-8 to C-14 and proceeds in a clockwise direction. Ring D can be described as cyclopentane twisted on C-13-C-14 with a conformation very similar to a half chair with puckering parameters of  $Q(2)$  = 0.4503(19) Å,  $\varphi(2)$  = 199.1(2) °, if the calculation starts from C-13 to C-17 and proceeds in a clockwise direction. Ring E display an envelope O(3)-exo with absolute L configuration with puckering parameters  $Q(2)$  = 0.3624(17) Å,  $\varphi(2)$  = 183.4(3) °, if the calculation follow this order; O(3), C-22, C-20, C-17, C-16. The tetrahydrofuran ring E is cis-fused to the cyclopentane ring D. Finally the F-ring, which is perpendicular to the normal plane of the skeleton, shows an C-22-endo envelope conformation with puckering parameters  $Q(2)$  = 0.336(2) Å,  $\varphi(2)$  = 212.5(4) °, if the calculation follow this order: O(2), C-22, C-23, C-24, C-25, (See Table 6). The crystal structure of compound **12** is shown in Figure 7, with the ellipsoid drawn at the 50% of probability.<sup>9b</sup>

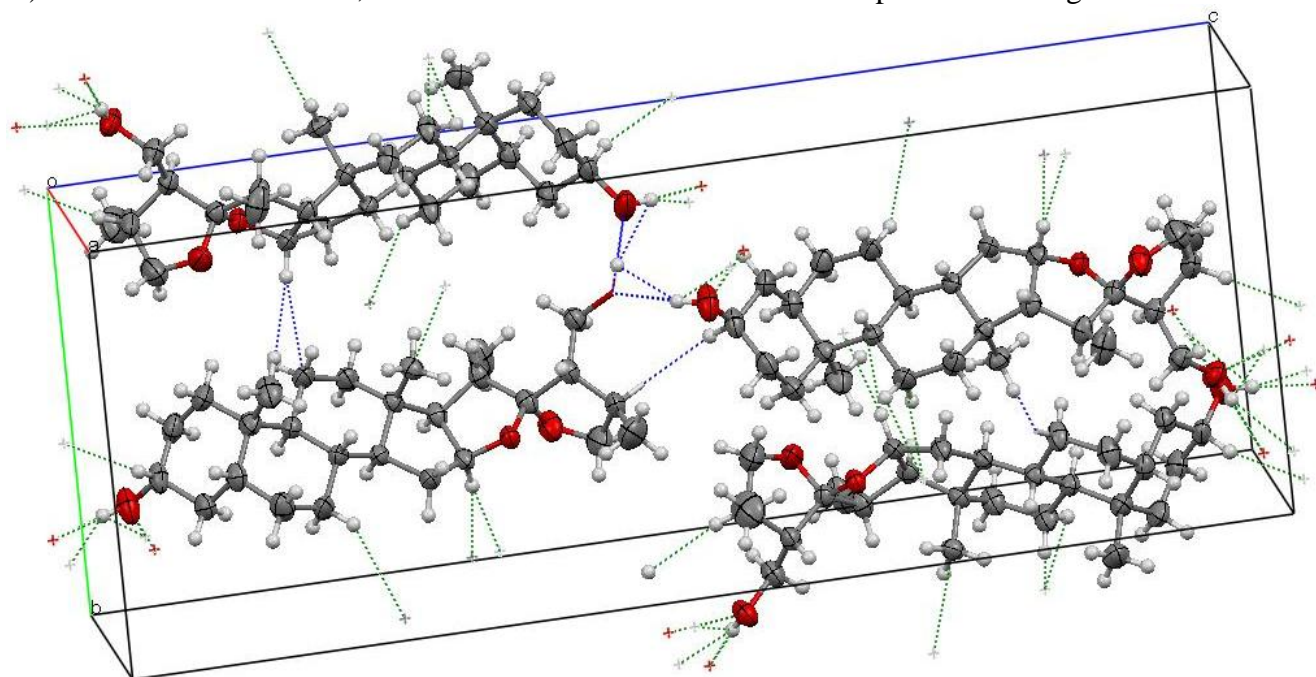
**Table 6.** Calculated puckering parameters for compound **12**

Ring	$Q / \text{Å}$	$\theta / ^\circ$	$\varphi / ^\circ$	Order and direction of the calculation
A	0.560(2)	175.0(2)	282(2)	From C(1) to C(10), counterclockwise
B	0.573(2)	4.5(2)	227(2)	From C(5) to C(10), counterclockwise
C	0.555(2)	10.7(2)	248(1)	From C(8) to C(14), clockwise
Ring	$Q(2) / \text{Å}$	$\varphi / ^\circ$		
D	0.450(2)	199.1(2)	-	From C(13) to C(17), counterclockwise
E	0.362(2)	183.4(3)	-	In this order: O(3), C(22), C(20), C(17), C(16)
F	0.336(2)	212.5(4)	-	In this order: O(2), C(22), C(23), C(24), C(25)



**Figure 7.** Crystal structure of compound **12**.

The molecules in the unit cell of **12** are connected head to tail through these hydrogen bonds: O(1)-H(10). O(4) (1.93(4) H O, 2.777(2) O O) and O(4)-H(40). O(1) (1.82(4) H O, 2.704(2) O O) in consecutive unit cells, the main contacts for this molecule are presented in Figure 8.



**Figure 8.** View of the main contacts of **12**.

In order to establish quantitative differences between the studied compounds **11** and **12**, a least-squares overlay analysis of the structures is performed. Table 7 shows the results of least-squares overlay analysis obtained with the Mercury program.<sup>9c</sup> From the obtained data it is clear that, as expected, the modifications introduced in the side chain do not produce significant conformational changes in the steroid framework, at least qualitatively.

**Table 7.** Least squares overlay analysis of the rings

RINGS	RMS of 12 vs 11
<b>A</b>	0.0186
<b>B</b>	0.0088
<b>C</b>	0.0108
<b>D</b>	0.0150
<b>E</b>	0.0115
<b>F</b>	0.1770

Interestingly, when F-rings of **11** and **12** are compared, it becomes evident that the envelope configuration shifts from C(25) in **11** to C(22) in **12**, making the overlay between these two rings very bad and indicating evident differences in the conformations adopted by the side chains of **11** and **12**. Table 8 shows the puckering descriptors as defined by Cremer and Pople<sup>9a</sup> and calculated using the PLATON program.<sup>9d-e</sup> The minor differences observed amongst rings A to D across all compounds may be attributed to the different intermolecular interactions that are, of course, influenced by the changes introduced in the side chain.

**Table 8.** Puckering descriptors obtained using the PLATON program

	<b>11</b>	<b>12</b>
<b>Ring A</b>	C-form	Very Similar to a C-form
<b>Ring B</b>	Very Similar to a C-form	Very Similar to a C-form
<b>Ring C</b>	Close to a C-form	Close to a C-form
<b>Ring D</b>	Twisted on C(13)-C(14)	Twisted on C(13)-C(14)
<b>Ring E</b>	Envelope O(4)-exo	Envelope O(3)-exo
<b>Ring F</b>	Envelope <b>C(25)-endo</b>	Envelope <b>C(22)-endo</b>

While the quite similar coupling constants of F-ring protons of compounds **11** and **12** (see Table 3) indicate that, in solution, both side chains adopt very similar conformations, the important *-and somehow unexpected-* conformational changes observed between both side chains in the solid state, should be associated with the different crystal packing of compounds **11** and **12**, (see Figures 6 and 8).

## Experimental Section

**General.** Reactions were monitored by TLC on Alugram® SIL G/UV254 plates from Macherey-Nagel. Chromatographic plates were sprayed with a 1% solution of vanillin in 50% HClO<sub>4</sub> and heated until color developed. Melting points were measured on a Melt-Temp II equipment. Mass spectra were registered in a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in CDCl<sub>3</sub> solution in a Varian Inova 400 spectrometer using the solvent signal 7.26 ppm for <sup>1</sup>H and 77.00 ppm for <sup>13</sup>C as references. NMR signals assignment was made using a combination of 2D homonuclear and heteronuclear correlation techniques, which included <sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>1</sup>H Nuclear Overhauser effect (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC). All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer and processed using Mestrenova (<http://mestrelab.com>). Copies of the spectra can be found in the supplementary information file.

**(22*S*,23*R*,24*R*)-16β, 22:22, 25-diepoxy-23-carboxymethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate (11).** Acetic anhydride 0.8 mL was added to a solution of **10**<sup>If</sup> in pyridine (8 mL), the mixture was stirred overnight, poured into a mixture of ice and 50 mL of 10% HCl. The mixture was extracted with ethyl acetate (2x40 mL) and the organic layer was washed with 10% HCl solution (2x25 mL), 5% CuSO<sub>4</sub> solution (3x25 mL), water (2x30 mL), dried (anh. Na<sub>2</sub>SO<sub>4</sub>) and evaporated to afford the acetylated product **11** (1.06 g, 2.11 mmol, 75%). m.p. 172-174 °C from acetone/hexane. See tables 3 and 4 for <sup>1</sup>H and <sup>13</sup>C NMR signals. MS (EI, 70 eV): 503 MH<sup>+</sup>, 471, 469. Elem. Anal. Calc. for C<sub>27</sub>H<sub>44</sub>O<sub>4</sub>: C, 71.68, H, 9.22%. Found: C, 71.61, H 9.31%.

**(22*S*,23*S*,24*R*)-16β,22:22,25-diepoxy-23-hydroxymethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol (12).** A solution of the rearranged product **10**<sup>If</sup> (643 mg, 1.39 mmol) in THF (15 ml) was slowly added to a refluxing suspension of LiAlH<sub>4</sub> (643 mg, 1.39 mmol) in THF (25 mL) under nitrogen and the mixture was refluxed for 2 hours. Ethyl acetate (30 mL) was carefully added to destroy the excess of LiAlH<sub>4</sub> (caution !!) followed by water (30 mL) and the mixture was extracted with ethyl acetate (2x50 mL). The organic layer was washed with water (5x30 mL) dried (anh. Na<sub>2</sub>SO<sub>4</sub>) and evaporated to afford the reduced compound **12**, (512.5 mg, 1.18 mmol, 85%). m.p. 177-178 °C from ethyl acetate/hexane. See tables 3 and 4 for <sup>1</sup>H and <sup>13</sup>C NMR signals. MS (EI, 70e V): 433 MH<sup>+</sup>, 415, 397, 385, 367, 285, 255, 155, 137, 125. HRMS-EI 433.3321 (MH<sup>+</sup> C<sub>27</sub>H<sub>45</sub>O<sub>4</sub> requires 433.3318)

Suitable crystals for X-Ray diffraction studies were obtained from the analytical samples as follows: crystals of **11** from a 10/1 mixture of hexane/ethyl acetate by slow evaporation; and crystals of **12** from an acetone solution left at 4° C for several days. Compounds **11** and **12** crystallized as orthorhombic systems with space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>. X-Ray diffraction measurements were performed at 293 (2) K on an Oxford Diffraction Atlas (Gemini) diffractometer with Mo K<sub>α</sub> radiation λ = 0.71073 Å. Data collection routine and data reduction were carried out with CrysAlisPro, Oxford Diffraction Ltd. Oxford Diffraction.<sup>10a</sup> The structures of both molecules



were solved using SIR2004<sup>10b</sup> and refined using SHELXL-97.<sup>10c</sup> All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were found in the difference Fourier maps, placed at geometrically calculated positions and refined using the riding model. The obtained bond lengths and angles of each compound are normal and are available from the electronic supporting information (CIF file) for the structure. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary material numbers CCDC 773328 (**11**) and CCDC 773330 (**12**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. E-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk).

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