Synthesis of 2-Deoxybrassinosteroids Analogs with 24-nor, 22(S)-23-Dihydroxy-Type Side Chains from Hyodeoxycholic Acid

Rodrigo Carvajal 1, Cesar González 1, Andrés F. Olea 2,*, Mauricio Fuentealba 3 and Luis Espinoza 1, *

1 Departamento de Química, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2340000, Chile; rodrigo.carvajal@postgrado.usm.cl (R.C.); cesar.gonzalez@usm.cl (C.G.)
2 Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago 8910339, Chile
3 Laboratorio de Cristalografía, Pontificia Universidad Católica de Valparaíso, Avenida Universidad 330, Curauma, Valparaíso 2340000, Chile; mauricio.fuentealba@pucv.cl
* Correspondence: andres.olea@uautonoma.cl (A.F.O.); luis.espinozac@usm.cl (L.E.); Tel.: +56-32-2654225 (L.E.)

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Abstract: Natural brassinosteroids are widespread in the plant kingdom and it is known that they play an important role in regulating plant growth. In this study, two new brassinosteroid analogs with shorter side chains but keeping the diol function were synthesized. Thus, the synthesis of 2-deoxybrassinosteroids analogs of the 3α-hydroxy-24-nor, 22,23-dihydroxy-5α-cholestane side chain type is described. The starting material is a derivative from hyodeoxycholic acid (4), which was obtained with an overall yield of 59% following a previously reported five step route. The side chain of this intermediate was modified by oxidative decarboxylation to get a terminal olefin at the C22-C23 position (compound 20) and subsequent dihydroxylation of the olefin. The resulting epimeric mixture of 21a, 21b was separated and the absolute configuration at the C22 carbon for the main product 21a was elucidated by single crystal X-ray diffraction analysis of the benzoylated derivative 22. Finally, lactonization of 21a through a Baeyer-Villiger oxidation of triacetylated derivative 23, using CF3CO2H/CHCl3 as oxidant system, leads to lactones 24 and 25 in 35% and 14% yields, respectively. Deacetylation of these compounds leads to 2-deoxybrassinosteroids 18 and 19 in 86% and 81% yields. Full structural characterization of all synthesized compounds was achieved using their 1D, 2D NMR, and HRMS data.

Keywords: brassinosteroid analogs; hyodeoxycholic acid; 2-deoxybrassinosteroids; synthesis; short side chain

1. Introduction

Since the discovery of brassinolide (1), a polyhydroxysteroidal hormone that regulates plant growth and development, other brassinosteroids (BRs) have been found throughout all the plant kingdom and much effort has been dedicated to the synthesis of BR analogs. Most of this work has been focused on determining the structural requirements that these compounds should possess to elicit strong biological activity [1–3]. For example, in Figure 1 are shown the chemical structures of 1, castasterone (2) and typhasterol (3). The latter is a natural 2-deoxybrassinosteroid that may act as important biosynthetic precursors of more active brassinosteroids [4–7].
Natural occurring BRs show a variety of structural modifications in the A/B ring, but it seems that a vicinal 22R,23R diol structural functionality in the side chain is essential for high biological activity. In recent decades, many BR analogs with structural changes on the A/B rings and/or on the side chain (shorter side chains, different oxygenated functions, spirostanic, aromatic and cyclic substituents, methyl esters, carboxylic acids) have been synthesized [8–12]. Surprisingly, some BR analogs with drastic structural modifications in the side chain have also shown interesting activities as plant growth regulators. Thus, the structural requirement of a side chain with a cis diol, preferentially with R, R configurations, and a C-24 methyl or ethyl substituent, seems to be contradicted by an important number of BR analogs exhibiting strong biological activities. For example, in Figure 2 are shown a series of BR analogs with 24-nor-22,23-dihydroxy-type side chains, i.e., BRs analogs with shorter side chain as compared to naturally occurring BRs.

![Figure 1. Structure of brassinolide (1), castasterone (2) and typhasterol (3).](image)

![Figure 2. Synthetic 24-nor-22(S),23-dihydroxy analogs 5-16.](image)
and 8–9 have been synthesized from 4 following different synthetic routes [13,14]. In both cases, the modification in the side chain was achieved by decarboxylation and subsequent dihydroxylation of a terminal olefin. From these compounds only 8 and 9 were evaluated as potential neuroinflammation inhibitors [14]. On the other hand, compounds 10–13 were synthesized from deoxycholic acid, bearing oxygenated functions in ring C, with 24-nor-22(S),23-dihydroxy side chain and cis A/B ring fusion [15], whereas analogues 14–16 were obtained from deoxysclerol acid with 11-oxo-functionalized on C ring, 24-nor-22(S),23-dihydroxy and 22(S),23-diacetoxy [16]. Interestingly, compounds 10 and 13 have shown growth promoting activity in hypocotyl elongation and cotyledon expansion in a radish bioassay [17].

From the synthetic point of view, the tremendous effort dedicated to obtain a number of synthetic analogs has led to development of some convenient, effective and general methods of synthesis applicable to this compound class [18–31]. In this work, we describe the synthesis of new 2-deoxybrassinosteroid analogs bearing a shorter side chain but retaining the diol function, i.e., a 3α-hydroxy-24-nor-22,23-dihydroxy-5α-cholestane side chain type. The starting material is 17, a hyodeoxycholic acid derivative. Following this procedure two new 2-deoxybrassinosteroids analogs (compounds 18 and 19, Figure 3) have been prepared. The full structural characterization of these analogs is also given.

![Figure 3](image_url). New 2-deoxybrassinosteroids analogs (18 and 19) with 3α-hydroxy-24-nor-22,23-dihydroxy-5α-cholestane side chain type.

2. Results

The main goal of this work was to synthesize BR analogs where the main structural change was a reduction of the side alkyl chain length, as compared to brassinolide (1), but keeping the glycol function at the C22–C23 position. In the steroidal nucleus, the introduction of a glycol function at the C22–C23 position via dihydroxylation with OsO₄ requires the presence of a terminal double bond. In the case of hyodeoxycholic acid (4) this can be accomplished by oxidative decarboxylation using a Pb(OAc)₄/Cu(OAc)₂ system. This method has been proposed to obtain terminal double bonds from carboxylic acids [32], and has been used for the degradation of bile acid side chains [33], synthesis of BR analogs [14,15,17], and specifically for decarboxylation of the side chain of hyodeoxycholic acid (4) and derivatives [34,35]. Alternatively, the carboxylic degradation reaction may be carried out using PhI(OAc)₂/CuSO₄ system [13,34–45]. Hyodeoxycholic acid (4) is a common starting material because it is easily available, and it has been previously used to synthesize a number of BR analogs. Related to this work, we have recently reported the synthesis of compound 17 in a five step route with an overall yield of 59% [46]. This compound will be the intermediate for the synthesis of 18 and 19 (Scheme 1).

2.1. Synthesis of Brassinosteroids Analogs

Oxidative decarboxylation of the side chain of compound 17, with the Pb(OAc)₄/Cu(OAc)₂ system, leads to olefin 20 in 75% yield. Formation of 20 was confirmed by ¹H-NMR and ¹³C-NMR.

Dihydroxylation of alkene 20 with OsO₄ produces an epimeric mixture of 21a and 21b in 72% yield (Scheme 1). This is an expected outcome for this reaction in steroidal nucleus with a terminal
double bond at the C22–C23 position (24-nor-chol-22-ene), and the 22(S) alcohol is stereoselectively obtained [47]. Thus, epimeric mixtures have been obtained during the preparation of analogs 10, 11 and 12 (Figure 2) [15–17].

Integration areas of signals in the 1H-NMR spectrum of mixture 21a/21b, appearing at $\delta_H = 0.965$ and 0.928 ppm, respectively, and assigned to the H-21 methyl hydrogen (CH$_3$-C20), indicates that the major component on this mixture is the less polar glycol 21a in a ratio 7.5:1.0. Recrystallization of a mixture of 21a/21b (MeOH/Et$_2$O = 3/1) allowed for isolation of 21a in 64.0% yield.

The stereochemistry at the C22 carbon for compound 21a was assumed to be 22(S) based on previous results reported for similar hydroxylation reactions used to obtain analogues 10, 13 and 14 (Figure 2) [15–17]. In order to establish the absolute configuration at C22 carbon for compound 21a, the benzoylated derivative 22 (Scheme 1) was prepared. Treatment of 21a with PhCOCl/DMAP in CH$_2$Cl$_2$ and pyridine led to selective esterification of C23 as the only reaction product in 94.0% yield.

Finally, the molecular and crystaline structure of derivative 22 was determined using single crystal X-ray diffraction techniques. This structure crystallizes in the orthorhombic Sohncke space group $P2_12_12_1$. The ORTEP diagram appears in Figure 4, whereas X-ray data, bond distances and angles are given in Tables S1–S3, respectively, of the Supplementary Material.

The absolute configuration S for C22 of compound 22 cannot be reliably determined using only the Flack’s parameter value of $-0.2(3)$, calculated by using 1169 quotients of the type $|(I^+)- (I^-)|/|(I^+)+(I^-)|$ [48]. Nevertheless, the analysis of Bayesian statistics of Bijovet pairs is a much simpler and reliable method to determine the absolute configuration for molecules that contain atoms no heavier than oxygen [49]. The resulting values for the analysis of 2386 Bijovet pairs, Hooft’s parameter y: 0.0(2); P2(true): 1.00; P2(false): 1.743 $\times$ 10$^{-6}$; P3(true): 0.973; P3(false): 1.695 $\times$ 10$^{-6}$ and; P3(racemic twin): 0.027, have confirmed the absolute structure for compound 22. Additionally, considering that compound 22 was synthesized using enantiopure precursors, the configurations $R, S, S, S, R, S, R$ and $S$ have also been verified for atoms C3, C5, C8, C9, C10, C13, C14, C17 and C20, respectively.

A mild saponification reaction (K$_2$CO$_3$/MeOH, r.t.) of glycol 21a gave the brassinosteroid analog 9 in 97% yield (Scheme 2). This compound has been previously obtained by using a different synthetic route, and its structure was determined by $^1$H-, $^{13}$C-NMR spectroscopy, EIMS and HRMS spectrometry. However, as the assignment of NMR signals was not performed [14] both the $^1$H- and $^{13}$C-NMR spectra of this compound are given in the Supplementary Material.

![Scheme 1. Synthesis of compound 21a, followed by selective benzylation at the C-23 position to obtain the derivative 22, and synthesis of brassinosteroid analog 9. CC stands for Column Chromatography.](image-url)
Additionally, lactonization global yields are greater than those obtained when there are hydroxyl groups in the steroid structure [45]. For these reasons, Baeyer-Villiger oxidation of triacetylated steroids with oxygenated substituents at 2α and 3α positions occurs with regioselective control, favoring 7-oxalactone formation, when electron-withdrawing substituents (acetyl [13,35–37,43,50], benzoyl [36,50], tosyl [36,50], trifluoroacetyl [50] and acetonide [42] groups) are present in the C-3 position [50]. Also, the use of CF₃CO₂H as the oxidant agent has a marked effect upon the 6-oxa/7-oxa ratio, and can lead to preferential formation of the desired 7-oxa isomer [50]. Additionally, lactonization global yields are greater than those obtained when there are hydroxyl groups in the steroid structure [45]. For these reasons, Baeyer-Villiger oxidation of triacetylated

**Figure 4.** ORTEP diagram of derivative 22 showing full atom-numbering scheme. Thermal ellipsoids are drawn at the 50% probability level.

**Scheme 2.** Synthesis of triacetylated derivative 23 and its subsequent Baeyer-Villiger oxidation to obtain new 2-deoxybrassinosteroids 18 and 19.

In order to obtain 2-deoxybrassinosteroid analogs 18 and 19 a lactone group (B-homo-7-oxa and B-homo-6-oxa) must be introduced in the B ring of 21a (Scheme 2). It is known that Baeyer-Villiger oxidation of 5α-6-keto-steroids with oxygenated substituents at 2α–3α and 3α positions occurs with regioselective control, favoring 7-oxalactone formation, when electron-withdrawing substituents (acetyl [13,35–37,43,50], benzoyl [36,50], tosyl [36,50], trifluoroacetyl [50] and acetonide [42] groups) are present in the C-3 position [50]. Also, the use of CF₃CO₂H as the oxidant agent has a marked effect upon the 6-oxa/7-oxa ratio, and can lead to preferential formation of the desired 7-oxa isomer [50]. Additionally, lactonization global yields are greater than those obtained when there are hydroxyl groups in the steroid structure [45]. For these reasons, Baeyer-Villiger oxidation of triacetylated
derivative 23 instead of 21a is performed with CF$_3$CO$_2$H/CHCl$_3$ as the oxidant. Similar regioselectivity has been observed in the oxidation of a series of sterols with m-CPBA/NaHCO$_3$/CH$_2$Cl$_2$ system, but the rates of these reactions are very slow [50].

Standard acetylation (Ac$_2$O/DMAP) of compound 21a (Scheme 2) leads to triacetylated derivative 23 with 97% yield.

Baeyer-Villiger oxidation of 23 with CF$_3$CO$_2$H/CHCl$_3$ system produces lactones 24 and 25 with 35% and 14% yields, respectively. Finally, deacetylation reaction of lactones 24 and 25 under mild conditions (K$_2$CO$_3$/MeOH, at room temperature) produced the new analogs of 2-deoxy-brassinosteroids 18 and 19 with 86% and 81% yields, respectively.

2.2. Elucidation of Compound Structures

The full structure assignment of compounds 20, 21a, 9, 22, and 23 were carried out by analysis of spectroscopic data obtained from $^1$H-NMR, $^{13}$C-NMR, and HRMS of pure and isolated compounds.

In the $^1$H-NMR of compound 20 the protons H-22, H$_{23a}$-23 and H$_{cis}$-23 appear at $\delta_H = 5.66$ ppm (H-22), 4.93 ppm (H$_{23a}$-23) and 4.83 ppm (H$_{cis}$-23). In the $^{13}$C-NMR the carbons C22 and C23 appear at $\delta_C = 144.89$ and 111.84 ppm, respectively (Table 1). These data were consistent with those reported for a similar structure but with hydroxyl function at C-3$\alpha$ instead of acetyl group [14,43].

| C  | 20  | 21a | 22  | 9  |
|----|-----|-----|-----|----|
| 1  | 32.38 | 32.17 | 32.36 | 32.88 |
| 2  | 28.19 | 27.20 | 27.41 | 28.73 |
| 3  | 68.85 | 68.70 | 68.81 | 66.02 |
| 4  | 25.27 | 25.05 | 25.26 | 28.53 |
| 5  | 52.58 | 52.38 | 52.58 | 52.84 |
| 6  | 211.81 | 211.72 | 211.62 | 214.49 |
| 7  | 46.75 | 46.49 | 46.69 | 47.61 |
| 8  | 37.92 | 37.74 | 37.90 | 39.45 |
| 9  | 53.79 | 52.68 | 52.90 | 54.19 |
| 10 | 41.28 | 41.07 | 41.23 | 42.62 |
| 11 | 21.06 | 20.87 | 21.06 | 22.19 |
| 12 | 39.36 | 39.19 | 39.41 | 40.76 |
| 13 | 42.94 | 43.16 | 43.41 | 44.46 |
| 14 | 55.41 | 53.51 | 53.72 | 55.08 |
| 15 | 25.01 | 24.78 | 25.00 | 28.45 |
| 16 | 23.91 | 23.85 | 24.02 | 25.07 |
| 17 | 56.80 | 56.15 | 56.41 | 57.56 |
| 18 | 12.18 | 11.56 | 11.81 | 12.14 |
| 19 | 12.41 | 12.20 | 12.40 | 12.67 |
| 20 | 41.10 | 39.87 | 40.33 | 42.01 |
| 21 | 20.04 | 12.86 | 12.90 | 13.42 |
| 22 | 144.89 | 73.69 | 71.77 | 75.19 |
| 23 | 111.84 | 62.22 | 66.39 | 63.19 |
| CH$_3$CO | 170.26 | 170.15 | 170.27 |  |
| CH$_3$CO | 21.41 | 21.21 | 21.41 |  |
| CH$_3$CO | - | - | - |  |
| CH$_3$CO | - | - | - |  |
| CH$_3$CO | - | - | - |  |
| CH$_3$CO | - | - | - |  |
| CH$_3$CO | - | - | - |  |
| COAr | - | - | 167.01 |  |
| 1' | - | - | 129.86 |  |
| 2' | - | - | 129.63 |  |
| 3' | - | - | 128.45 |  |
| 4' | - | - | 133.22 |  |

* The $^{13}$C-NMR spectrum of compound 9 was recorded in MeOD solution.

In the $^1$H-NMR of 21a signals appearing at chemical shifts $\delta_H = 3.79$ ppm, 3.64 ppm, and 3.51 ppm are assigned to protons H-22, H-23a and H-23b, respectively. On the other hand, in the $^{13}$C-NMR spectrum, the signals at $\delta_C = 73.69$ and 62.22 ppm correspond to carbinolic carbons C22 and C23, respectively (see Table 1).
The $^1$H-NMR of triol 9 shows signals at $\delta_H = 4.04$–$4.03$, 3.71, 3.60, and 3.40 ppm, which are assigned to hydrogens H-3, H-22, H-23a and H-23b, respectively. In the $^{13}$C-NMR three carbinolic carbons (C22, C-3 and C23) were observed at $\delta_C = 75.19$, 66.02 and 63.19 ppm, (Table 1).

The structure of derivative 22 was established mainly by 1D and 2D NMR spectroscopy. In the $^1$H-NMR spectrum the aromatic protons appear at $\delta_H = 8.05$ (H$_{Ar}$-2'), 7.58 (H$_{Ar}$-4'), and 7.46 (H$_{Ar}$-3') ppm. Additionally, two low field shifted signals at $\delta_H = 4.50$ (dd, $J = 11.4$ and 1.7 Hz, 1H) and 4.20 (dd, $J = 11.3$ and 4.2 Hz, 1H) ppm correspond to H-23a and H-23b. In the $^{13}$C-NMR spectrum the signal at $\delta_C = 167.01$ ppm is assigned to carbonyl of aromatic ester, whereas the signals at $\delta_C = 129.86$, 129.63, 128.45 and 133.22 ppm (Table 1) are assigned to aromatic ring (each one of signals at $\delta_C = 129.63$ and 128.45 ppm correspond to two symmetrical carbons of the aromatic ring). In the 2D HMBC spectrum a heteronuclear correlation at $^3$J$_{H/C}$ between H-23a ($\delta_H = 4.50$ ppm) with carbonyl of aromatic ester ($\delta_C = 167.01$ ppm) was observed, confirming the presence of benzoyl ester at C23 position. The structure of compounds 18, 19, 24 and 25 were mainly elucidated by analysis of data obtained from $^1$H, $^{13}$C, $^{13}$C DEPT-135, 2D HSQC, 2D HMBC NMR, and HRMS measurements.

For compound 24, the position of the 7-oxa lactone function was established from the $^1$H-NMR spectrum where a signal observed at $\delta_H = 4.13$–$4.04$ ppm (m, 2H), was assigned to hydrogens H-7 and correlated by 2D $^1$H-$^{13}$C HSQC with the signal at $\delta_C = 70.31$ ppm (CH$_2$-7 from $^{13}$C and $^{13}$C DEPT-135 spectra, Table 2). Additionally, important heteronuclear correlations were obtained for hydrogens H-5$^\alpha$ and H-7 from a 2D $^1$H-$^{13}$C HMBC spectrum, i.e., (i) H-7 shows $^3$J$_{HC}$ correlations with the signal at $\delta_C = 175.97$ ppm (assigned to carbon C-6, C=O of lactone function, Table 2), and signals at $\delta_C = 58.31$ ppm (assigned to carbon C-9); and $^2$J$_{HC}$ correlation with signal appearing at $\delta_C = 39.42$ ppm (assigned to carbon C-8); (ii) H-5$^\alpha$ at $\delta_H = 3.03$ ppm shows $^3$J$_{HC}$ correlation with signals at $\delta_C = 14.54$, and 58.31 ppm, which were assigned to carbons CH$_3$-19 and C-9, respectively (Table 2); (iii) H-5$^\alpha$ exhibits $^2$J$_{HC}$ correlation with signals at $\delta_C = 29.7$, 36.1 and 176.0 ppm, assigned to carbons C-4, C-10 and C-6, respectively. These correlations are depicted in the 2D HMBC spectrum shown in Figure 5. These observations confirmed unequivocally the 7-oxalactone position for compound 24.

Table 2. $\delta$(ppm) $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) for compounds 18, 19, 23–25.

|     | C     | 23    | 24    | 25    | 18 * | 19 * |
|-----|-------|-------|-------|-------|------|------|
| 1   | 32.35 | 33.66 | 31.84 | 32.78 | 31.06|
| 2   | 27.01 | 26.95 | 26.65 | 24.84 | 25.25|
| 3   | 68.79 | 68.38 | 69.56 | 64.32 | 65.60|
| 4   | 25.24 | 29.74 | 32.95 | 32.50 | 35.39|
| 5   | 52.55 | 42.57 | 79.68 | 41.67 | 80.04|
| 6   | 211.49| 175.97| 174.64| 177.44| 176.08|
| 7   | 46.63 | 70.31 | 38.12 | 70.43 | 37.88|
| 8   | 37.85 | 39.42 | 38.31 | 39.54 | 39.89|
| 9   | 52.84 | 58.31 | 57.97 | 58.15 | 57.77|
| 10  | 41.18 | 36.14 | 39.55 | 36.17 | 39.64|
| 11  | 21.03 | 22.10 | 22.17 | 22.03 | 22.02|
| 12  | 39.38 | 39.53 | 39.69 | 40.03 | 39.48|
| 13  | 43.39 | 43.03 | 43.09 | 42.91 | 42.91|
| 14  | 53.67 | 52.80 | 55.13 | 52.70 | 54.94|
| 15  | 24.98 | 25.13 | 25.33 | 27.87 | 27.46|
| 16  | 23.94 | 24.83 | 24.83 | 27.20 | 26.76|
| 17  | 56.37 | 51.10 | 53.14 | 50.98 | 52.97|
| 18  | 11.81 | 11.57 | 11.59 | 11.41 | 11.32|
| 19  | 12.38 | 14.54 | 11.59 | 14.39 | 11.32|
| 20  | 38.32 | 38.32 | 34.78 | 39.33 | 34.77|
| 21  | 13.37 | 13.28 | 13.29 | 12.77 | 12.66|
| 22  | 73.97 | 73.80 | 73.80 | 73.57 | 73.50|
| 23  | 62.39 | 62.30 | 62.35 | 62.08 | 62.02|
Additionally, H-5 with signals appearing at δ = 57.97 ppm, assigned to carbons C-9. These correlations are shown in the 2D HMBC spectrum in Figure 6.

* The 13C-NMR spectrum of compound 18 and 19 were recorded in CDCl3/MeOD 2/1, solution.

Table 2. Cont.

|     | C   | 23  | 24  | 25  | 18 * | 19 * |
|-----|-----|-----|-----|-----|------|------|
| CH₃CO | 171.09 | 171.07 | 171.12 | -   | -    |
| CH₃CO | 21.39  | 21.36  | 21.32  | -   | -    |
| CH₃CO | 170.42 | 170.40 | 170.41 | -   | -    |
| CH₃CO | 21.23  | 21.21  | 21.24  | -   | -    |
| CH₃CO | 170.25 | 170.28 | 170.17 | -   | -    |
| CH₃CO | 20.86  | 20.85  | 20.88  | -   | -    |

A similar analysis was performed to determine the structure of 6-oxalactone 25. Thus, in the 1H-NMR spectrum a signal at δ = 4.46 ppm was assigned to H-5α, and correlated by 2D 1H-13C HSQC with the signal at δC = 79.68 ppm (CH with impair multiplicity from DEPT-135 spectrum). Additionally, H-5α shows 2J_HC correlation with signal at δC = 32.95 ppm that is assigned to carbon C-4; and 3J_HC correlation with signals at δC = 11.59, 57.94 and 174.64 ppm, which are assigned to carbons CH₃-19, C-9 and C-6, respectively (C=O, of lactone function, Table 2). On the other hand, the 1H-NMR signal at δH = 2.55–2.43 ppm, corresponding to H-7 (2H, m), shows 2J_HC correlation with signals appearing at δC = 38.31 and 174.64 ppm, which were assigned to carbons C-8 and C-6, respectively (Table 2); and 3J_HC correlation with signals at δC = 57.97 ppm, assigned to carbons C-9. These correlations are shown in the 2D HMBC spectrum in Figure 6.
(7-oxalactone) appears at higher field (δ = 4.60 ppm) (Figure 7). Similarly, H-7 in compound 19 is observed at downfield (δ = 2.53–2.43, m, 2H) (Figure 7).

Similar analyses were performed to determine the structure of compounds 18 and 19. In Figure 7 are shown parts of 1H-NMR spectra of compounds 18 and 19 where the major differences in chemical shift of protons H-5 and H-7 are observed for both molecules. For example, H-5 in compound 18 (7-oxalactone) appears at higher field (δ = 3.17 ppm) than in compound 19 (6-oxalactone) (δ = 4.60 ppm) (Figure 7). Similarly, H-7α and β in compound 18 are observed at downfield (δ = 4.08–4.07, m, 2H), while in 6-oxalactone 19 these H-atoms are displaced to high field (δ = 2.53–2.43, m, 2H) (Figure 7).
3. Materials and Methods

3.1. General Experimental Methods

All reagents were purchased from commercial suppliers, and used without further purification. Melting points were measured on a SMP3 apparatus (Stuart-Scientific, now Merck KGaA, Darmstadt, Germany) and are uncorrected. $^1$H-, $^{13}$C-, $^{13}$C DEPT-135, gs 2D HSQC and gs 2D HMBC NMR spectra were recorded in CDCl$_3$ or MeOD solutions, and are referenced to the residual peaks of CHCl$_3$ at $\delta = 7.26$ ppm and $\delta = 77.00$ ppm for $^1$H and $^{13}$C, respectively and CD$_3$OD at $\delta = 3.30$ ppm and $\delta = 49.00$ ppm for $^1$H and $^{13}$C, respectively, on an Avance 400 Digital NMR spectrometer (Bruker, Rheinstetten, Germany) operating at 400.1 MHz for $^1$H and 100.6 MHz for $^{13}$C. Chemical shifts are reported in ppm and coupling constants ($J$) are given in Hz, multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), triplet (t), quartet (q), multiplet (m). IR spectra were recorded as KBr disks in a FT-IR 6700 spectrometer (Nicolet, Thermo Scientific, San Jose, CA, USA) and frequencies are reported in cm$^{-1}$. High-resolution mass spectra (HRMS-ESI) were recorded in a Exactive Plus mass spectrometer (Thermo Scientific, Waltham, MA, USA). The analysis for the reaction products was performed with the following relevant parameters: heater temperature, 50 °C; sheath gas flow, 5 (arbitrary unit); sweep gas flow rate, 0 (arbitrary unit) and spray voltage, 3.0 kV at negative mode. The accurate mass measurements were performed at a resolving power: 140,000 FWHM at range m/z 300–500. Optical rotations were measured on a Model AA-5 polarimeter (Optical Activity, Ltd., NJ, USA) with a sodium lamp using a l = 0.1 dm cell and are reported as follows: $[\alpha]_D^\circ$ (c (g/100 mL), solvent). For analytical TLC, silica gel 60 in 0.25 mm layer was used and TLC spots were detected by heating after spraying with 25% H$_2$SO$_4$ in H$_2$O. Chromatographic separations were carried out by conventional column on silica gel 60 (230–400 mesh) using EtOAc-hexane gradients of increasing polarity. All organic extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure, below 40 °C.

3.2. X-ray Crystal Structure Determination

A suitable single crystal of compound 22 was mounted on a MiTeGen MicroMount (MiTeGen, Lansing, NY, USA) in a random orientation. Diffraction data was collected at 120 K on a D8 VENTURE diffractometer (Bruker, Rheinstetten, Germany) equipped with a bidimensional CMOS Photon100 detector, using graphite monochromated Cu-Kα radiation ($\lambda = 1.54178$ Å). The diffraction frames were integrated using the APEX2 package. The structure of 22 was solved using Olex2 [51], with the olex2.solve structure solution program using Charge Flipping [52] and refined with full-matrix least-square methods based on $F^2$ (SHELXL) [53]. Non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were included in their calculated positions, assigned fixed isotropic thermal parameters and constrained to ride on their parent atoms. A summary of the details about crystal data, collection parameters and refinement are documented in Supplementary Material, and additional crystallographic details are in the CIF files. CCDC 1583718 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk. ORTEP view was drawn using OLEX2 software [51].

3.3. Synthesis

$\alpha$-Acetoxy-24-nor-5$\alpha$-cholan-22-en-6-one (20). To a solution of 17 (4.00 g, 9.25 mmol) in dry benzene (150 mL) were added Cu(OAc)$_2$ (0.29 g, 1.60 mmol) and pyridine (1.5 mL). Then, under reflux, Pb(OAc)$_4$ (9.75 g, 22.0 mmol) was added in four portions at hourly intervals. After the addition was completed, the reaction was continued for 1 h. The end of reaction was verified by TLC, and then the mixture was filtered, and the solvent was evaporated under reduced pressure. The crude was re-dissolved in DCM (8 mL) and chromatographed on silica gel with PE/EtOAc mixtures of increasing
polarity (19.8:0.2 → 15.8:4.2). Compound 20 (2.67 g, 75% yield) was obtained as a colorless solid: m.p. 64.0–66.1 °C (hexane/Et2O = 1/1); [α]D 21 = −21.2° (c = 2.36, MeOH); 1H-NMR (CDCl3) δ 5.66 (ddd, J = 17.0; 10.2 and 8.4 Hz, 1H, H-22), 5.12 (m, 1H, H-3), 4.93 (dd, J = 17.0 and 1.8 Hz, 1H, Htrans-23), 4.83 (dd, J = 10.2 and 1.8 Hz, 1H, Hcis-23), 2.56 (dd, J = 12.1 and 3.2 Hz, 1H, H-5), 2.31 (dd, J = 13.1 and 4.5 Hz, 1H, H-7α), 2.04 (s, 3H, CH3CO), 1.04 (dd, J = 6.6 Hz, 3H, H-21), 0.748 (s, 3H, H-19), 0.694 (s, 3H, H-18); 13C-NMR (CDCl3) see Table 1; IR νmax: 3082 (CH=CH2); 2946; 2909; 2868 and 2849 (C-H), 1740 (C=O), 1708 (C=O), 1637 (C=O), 1263 (C-O), 1021 (C-O), 988 (CH=CH2), 926 (CH=CH2) cm−1. HRMS-ESI (positive mode): m/z calculated for C25H38O3: 386.2821 [M]+; found 387.2874 [M + H]+.

3α-Acetoxy-22(S), 23-dihydroxy-24-nor-5α-cholan-6-one (21a) and 3α-acetoxy-22(R), 23-dihydroxy-24-nor-5α-cholan-6-one (21b). To a solution of 20 (2.50 g, 6.47 mmol) in acetone (150 mL) was added NMO (0.45 g, 3.84 mmol). Then the mixture was homogenized by magnetic stirring and 2.0 mL of 4% OsO4 (0.210 mmol) was added dropwise with stirring for 36 h at room temperature. The end of the reaction was verified by TLC. Then the solvent was removed (up to 25 mL approximate volume) and water (25 mL) and Na2S2O4·5H2O (25 mL saturated solution) were added. The organic layer was extracted with EtOAc (2 × 30 mL), washed with water (2 × 50 mL), dried over Na2SO4, and filtered. The solvent was evaporated under reduced pressure. The crude was re-dissolved in DCM (10 mL) and chromatographed on silica gel with PE/EtOAc mixtures of increasing polarity (19.8:0.2 → 9.8:10.2).

A mixture of 21a/21b was 7.5/1.0 was obtained (1.97 g, 72% yield). Recrystallization of this mixture (MeOH/Et2O = 3/1) allows for compound 21a to be obtained as colorless solid (1.74 g, 64% yield): m.p. 250.1–253.8 °C; [α]D 21 = −7.41° (c = 5.40, CHCl3); 1H-NMR (CDCl3) δ 5.11 (m, 1H, H-3), 3.79 (dt, J = 9.6 and 3.6 Hz, 1H, H-22), 3.64 (ddd, J = 10.8 and 3.6 Hz, 1H, H-23a), 3.51 (ddd, J = 10.8 and 9.6 Hz, 1H, H-23b), 2.55 (dd, J = 12.1 and 3.2 Hz, 1H, H-5), 2.31 (dd, J = 13.1 and 4.5 Hz, 1H, H-7α), 2.03 (s, 3H, CH3CO), 0.954 (d, J = 6.9 Hz, 3H, H-21), 0.735 (s, 3H, H-19), 0.675 (s, 3H, H-18); 13C-NMR (CDCl3) see Table 1; IR νmax: 3519 (O-H), 2941 and 2885 (C-H), 1732 (C=O), 1708 (C=O), 1050 (C-O) cm−1; HRMS-ESI (negative mode): m/z calculated for C25H38O5: 420.2876 [M]+; found 419.2811 [M – H]+.

3α-Acetoxy-22(S)-hydroxy-24-nor-5α-cholan-6-oxa-23-benzoate (22). Compound 21a (0.5 g, 1.19 mmol) was dissolved in DCM (25 mL) and pyridine (1.0 mL). Later DMAP (0.5 mg) and PhCOCl (0.5 mL, 4.30 mmol) were added with slow stirring at room temperature. The end of the reaction was verified by TLC (2 h), solvent volume was reduced to about 10 mL, and then EtOAc (20 mL) were added. The organic layer was washed with 5% KHSO4 (2 × 5 mL) and water (2 × 10 mL), dried over Na2SO4 and filtered. The solvent was evaporated under reduced pressure. The crude was redissolved in DCM (5 mL) and chromatographed on silica gel with PE/EtOAc mixtures of increasing polarity (19.8:0.2 → 14.2:5.8). Compound 22 (0.59 g, 93.8% yield) was obtained as a colorless solid: m.p. 210.5–211.7 °C (MeOH/Et2O = 1/2); [α]D 22 = −17.1° (c = 1.75, CHCl3); 1H-NMR (CDCl3) δ 8.05 (d, J = 7.4 Hz, 2H, HAr-2’), 7.58 (t, J = 7.4 Hz, 1H, HAr-4’), 7.46 (t, J = 7.4 Hz, 2H, HAr-3’), 5.12 (m, 1H, H-3), 4.50 (dd, J = 11.4 and 1.7 Hz, 1H, H-23a), 4.20 (dd, J = 11.3 and 4.2 Hz, 1H, H-23b), 4.06 (m, 1H, H-22), 2.57 (dd, J = 12.0 and 2.9 Hz, 1H, H-5), 2.32 (dd, J = 13.1 and 4.5 Hz, 1H, H-7α), 2.04 (s, 3H, CH3CO), 1.06 (d, J = 6.9 Hz, 3H, H-21), 0.748 (s, 3H, H-19), 0.709 (s, 3H, H-18); 13C-NMR (CDCl3) see Table 1; IR νmax: 3502 (O-H), 2951, 2936, 2893 and 2870 (C-H), 1730 (C=O), 1715 (C=O), 1693 (C=O), 1601 (C=C Ar), 1276 (C-O), 1070 (C-O), 716 (C-H Ar) cm−1; HRMS-ESI (positive mode): m/z calculated for C32H44O6: 524.3138 [M]+; found 525.3186 [M + H]+.

3α,23-Trihydroxy-24-nor-5α-cholan-6-one (9). To a solution of 21a (0.5 g, 1.19 mmol) in MeOH (30 mL) was added K2CO3 (0.493 g, 3.57 mmol), then the suspension was stirred at room temperature for 3 h. The end of the reaction was verified by TLC. Then the solvent was removed to dryness and the residue acidified with 2% HCl (20 mL). The obtained solid was filtered and washed with 5% NaHCO3 (20 mL) and water (2 × 10 mL) and dried. Compound 9 (0.437 g, 97% yield) was obtained as a colorless solid: m.p. 227.0–229.1 °C (MeOH/Et2O = 3/1); [α]D 22 = −3.67° (c = 2.73, MeOH); 1H-NMR (CD2OD) δ 4.04–4.03 (m, 1H, H-3), 3.71 (dt, J = 8.9 and 3.2 Hz, 1H, H-22), 3.60 (dd, J = 11.3 and 2.7 Hz, 1H, H-23a),
3.40 (dd, J = 11.3 and 8.9 Hz, 1H, H-23b), 2.74 (t, J = 7.9 Hz, 1H, H-5), 2.21 (dd, J = 13.1 and 4.8 Hz, 1H, H-7α), 2.11 (t, J = 13.1 Hz, 1H, H-7x), 2.04 (dt, J = 12.2 and 2.2 Hz, 1H, H-12α), 0.943 (d, J = 6.9 Hz, 3H, H-21), 0.732 (s, 3H, H-19), 0.716 (s, 3H, H-18); 13C-NMR (CD2OD) see Table 1; IR νmax: 3387 (O-H), 2940, 2906 and 2871 (C-H), 1700 (C=O), 1246 (C-O), 1050 (C-O), 754 (CH) cm⁻¹; HRMS-ESI (positive mode): m/z calculated for C23H38O4: 378.2770 [M]+; found 379.2823 [M + H]+.

3α-22(S), 23-Triacetoxy-24-nor-5α-cholan-6-one (23). Compound 21a (2.0 g, 4.76 mmol) was dissolved in DCM (30 mL) and pyridine (3.0 mL). Later DMAP (5.0 mg) and Ac2O (1 mL, 10.6 mmol) were added to the solution and the reaction mixture was stirred at room temperature. The end of the reaction was verified by TLC (30 min), volume of solvent was reduced to about 5 mL and extracted with EtOAc (2 × 10 mL). The organic layer was washed with 5% KHSO4 (2 × 5 mL) and water (2 × 10 mL), dried over Na2SO4 and filtered. The solvent was evaporated under reduced pressure. The crude was redissolved in DCM (5 mL) and chromatographed on silica gel with PE/Et2O mixtures of increasing polarity (19.8:0.2 → 11.8:8.2). Compound 23 (2.33 g, 97% yield) was obtained as a colorless solid: m.p. 136.1–137.6 °C (Et2O/hexane); [α]D25 = +2.53° (c = 3.95, CHCl3); 1H-NMR (CDCl3) δ 5.10–5.09 (m, 2H, H-3 and H-22), 4.31 (dd, J = 11.9 and 1.9 Hz, 1H, H-23a), 3.99 (dd, J = 11.9 and 9.4 Hz, 1H, H-23b), 2.54 (dd, J = 12.1 and 3.1 Hz, 1H, H-5), 2.30 (dd, J = 13.1 and 4.4 Hz, 1H, H-7α), 2.06 (s, 3H, AcO), 2.04 (s, 3H, AcO), 0.973 (d, J = 7.0 Hz, 3H, H-21), 0.723 (s, 3H, H-19), 0.651 (s, 3H, H-18); 13C-NMR (CDCl3) (see Table 1); IR νmax: 2945, 2908 and 2871 (C-H), 1737 (C=O), 1711 (C=O), 1369 (CH3), 1242 (C-O), 1224 (C-O), 1051 (C-O), 757 (C-H) cm⁻¹; HRMS-ESI (positive mode): m/z calculated for C29H44O7: 530.3087 [M]+; found 505.3134 [M + H]+.

3α-22(S), 23-Triacetoxy-24-nor-B-homo-7-oxa-5α-cholan-6-one (24) and 3α-22(S), 23-triacetoxy-24-nor-B-homo-6-oxa-5α-cholan-6-one (25). Preparation of oxidant: 1.0 mL of H2O2 (30%), (9.77 mmol) was slowly dripped into a solution of (CF3CO2)2O (1.20 mL, 8.52 mmol) at 0 °C, diluted with CHCl3 (3 mL) and stirred for 30 min. The oxidant mixture (3.0 mL) was slowly added to the solution of compound 23 (1.00 g, 1.98 mmol in 10 mL of CHCl3) at 0 °C and slowly stirred in N2 atmosphere for 24 h. The end of reaction was verified by TLC, the mixture was filtered, then concentrated in a rotary evaporator to a volume of approximately 10 mL. Then Et2O (40 mL) was added and the organic layer was washed with saturated NaHCO3 solution (2 × 20 mL), water (2 × 15 mL), then dried over Na2SO4, and filtered. The solvent was evaporated and the crude was re-dissolved in DCM (5 mL) and chromatographed on silica gel with hexane/Et2O mixtures of increasing polarity (0.2:50.0 → 23.8:26.2). Three fractions were obtained. Fraction I (less polar): 0.363 g (35% yield), compound 24. Fraction II (medium polarity): 0.285 g, mixture of compounds 24 and 25. Fraction III (more polar): 0.146 g (14% yield) compound 25.

Compound 24 was obtained as a colorless solid: m.p. 90.3–91.8 °C (Hexane/Et2O = 2/1); [α]D19 = +50.9° (c = 4.13, CHCl3); 1H-NMR (CDCl3) δ 5.11–5.09 (m, 2H, H-3 and H-22), 4.32 (dd, J = 11.9 and 1.9 Hz, 1H, H-23a), 4.13–4.04 (m, 2H, H-7α and H-7x), 3.99 (dd, J = 11.9 and 9.4 Hz, 1H, H-23b), 3.03 (dd, J = 12.2 and 4.3 Hz, 1H, H-5), 2.08 (dddd, J = 16.2, 12.2 and 2.70 Hz, 1H, H-4α), 2.08 (s, 6H, AcO), 2.05 (s, 3H, AcO), 0.979 (d, J = 6.8 Hz, 3H, H-21), 0.897 (s, 3H, H-19), 0.701 (s, 3H, H-18); 13C-NMR (CDCl3) (see Table 2); IR νmax: 2965, 2946, 2909 and 2872 (C-H), 1735 (C=O), 1438 (C-H), 1368 (CH3), 1242 (C-O), 1182 (C-O), 1052 (C-O), 754 (C-H) cm⁻¹; HRMS-ESI (positive mode): m/z calculated for C29H44O8: 520.3036 [M]+; found 521.3085 [M + H]+.

Compound 25 was obtained as a colorless solid: m.p. 169.9–170.8 °C (Hexane/Et2O = 2/1); [α]D19 = +61.4° (c = 0.44, MeOH); 1H-NMR (CDCl3) δ 5.14–5.09 (m, 2H, H-3 and H-22), 4.46 (dd, J = 11.3 and 5.5 Hz, 1H, H-5), 4.32 (dd, J = 12.0 and 2.0 Hz, 1H, H-23a), 3.99 (dd, J = 12.0 and 9.4 Hz, 1H, H-23b), 2.55–2.43 (m, 2H, H-7α and H-7x), 2.07 (s, 6H, AcO), 2.05 (s, 3H, AcO), 0.971 (d, J = 6.9 Hz, 3H, H-21), 0.901 (s, 3H, H-19), 0.695 (s, 3H, H-18); 13C-NMR (CDCl3) see Table 2; IR νmax: 2965, 2949 and 2871 (C-H), 1743 (C=O), 1736 (C=O), 1725 (C=O), 1445 (C-H), 1368 (CH3), 1258 (C-O), 1239 (C-O), 1225 (C-O), 1044 (C-O), 1022 (C-O), 754 (C-H) cm⁻¹; HRMS-ESI (positive mode): m/z calculated for C29H44O8: 520.3036 [M]+; found 521.3082 [M + H]+.
3α-22(S), 23-Trihydroxy-24-nor-B-homo-7-oxa-5α-cholan-6-one (18). To a solution of 24 (0.15 g, 0.288 mmol) in MeOH (20 mL) was added K$_2$CO$_3$ (0.050 g, 0.307 mmol), then the suspension was stirred at room temperature for 3 h. The end of the reaction was verified by TLC. Then the solvent was removed to dryness and the residue acidified with 2% HCl (10 mL). The obtained solid was filtered and washed with 5% NaHCO$_3$ (20 mL) and water (2 × 10 mL) and dried. Compound 18 (0.098 g, 86% yield) was obtained as a colorless solid: m.p. 91.8–94.5 °C (MeOH/Et$_2$O = 3/1); [α]$_D^{19}$ = +54.2° (c = 2.95, MeOH); $^1$H-NMR (CDCl$_3$) δ 4.19–4.15 (m, H-3), 4.08–4.07 (m, 2H, H-7α and H-7α), 3.79 (dt, $J$ = 9.3 and 3.1 Hz, 1H, H-22), 3.64 (dd, $J$ = 11.0 and 2.8 Hz, 1H, H-23a), 3.52 (dd, $J$ = 11.0 and 9.4 Hz, 1H, H-23b), 3.17 (dd, $J$ = 12.3 and 4.4 Hz, 1H, H-5), 2.13 (ddd, $J$ = 13.7, 12.4 and 2.7 Hz, 1H, H-4α), 1.98 (dt, $J$ = 12.6 and 3.3 Hz, 1H, H-12α), 0.950 (d, $J$ = 6.9 Hz, 3H, H-21), 0.888 (s, 3H, H-19), 0.710 (s, 3H, H-18); $^{13}$C-NMR (CDCl$_3$/CD$_3$OD = 2/1) see Table 2; IR $\nu$ max: 3400 (O-H); 2959; 2941; 2902 and 2870 (C-H); 1709 (C=O); 1315 (C-H); 1249 (C-O); 1183 (C-O); 1065 (C-O); 1048 (C-O), 753 (C-H) cm$^{-1}$; HRMS-ESI (positive mode): $m/z$ calculated for C$_{25}$H$_{38}$O$_5$: 394.2719 [M]$^+$; found 395.2771 [M + H]$^+$. 

3α-22(S), 23-Trihydroxy-24-nor-B-homo-6-oxa-5α-cholan-6-one (19). Compound 19 was obtained from 25 by the same method described above. Compound 25 (0.05 g, 0.288 mmol), MeOH (20 mL), K$_2$CO$_3$ (0.050 g, 0.307 mmol). Compound 25 (0.092 g, 81% yield), colorless solid: m.p. 227.4–229.5 °C (MeOH/Et$_2$O = 3/1); [α]$_D^{19}$ = +27.1° (c = 1.48, MeOH); $^1$H-NMR (CDCl$_3$) δ 4.60 (dd, $J$ = 11.3 and 5.3 Hz, 1H, H-5), 4.23–4.21 (m, 1H, H-3), 3.79 (dt, $J$ = 9.4 and 3.1 Hz, 1H, H-22), 3.64 (dd, $J$ = 10.9 and 2.6 Hz, 1H, H-23a), 3.50 (dd, $J$ = 10.9 and 9.4 Hz, 1H, H-23b), 2.53–2.43 (m, 2H, H-7α and H-7α), 0.944 (d, $J$ = 6.9 Hz, 3H, H-21), 0.892 (s, 3H, H-19), 0.701 (s, 3H, H-18); $^{13}$C-NMR (CDCl$_3$/CD$_3$OD = 2/1) see Table 2; IR $\nu$ max: 3386 (O-H); 2942; 2889; 2869 and 2851 (C-H); 1710 (C=O); 1380 (C-O); 1278 (C-O); 1038 (C-O), 751 (C-H) cm$^{-1}$; HRMS-ESI (negative mode): $m/z$ calculated for C$_{23}$H$_{38}$O$_5$: 394.2719 [M]$^+$; found 393.2652 [M − H]$^−$. 

4. Conclusions

A new synthetic route has been used to obtain the known brassinosteroid analog 9 and new compounds 18, 19, 21a, 22–25. Compound 9 was obtained from 17 in a total yield of 46%, whereas new lactones analogues 18 and 19 were obtained from glycol 21a in 29% and 11% total yields. Additionally, using 1D, 2D NMR, and HRMS we have achieved full structural determination of all compounds shown in Schemes 1 and 2. The absolute stereochemistry at position C-22 was established a (S) by X-ray crystallography studies of the benzoylated derivative 22. This conclusion is in line with literature data reported for similar steroidal structures [14]. Finally, in order to establish a relationship between the side chain structure of BRs analogs and the promoting plant growth activity, additional changes on the side chain should be introduced.

Supplementary Materials: The following are available online, X-ray structure of compound 22 (CIF); Spectra $^1$H and $^{13}$C-NMR of compounds 9, 18–20, 21a, 23–25 (PDF); Spectra HRMS of compounds 9, 18–20, 21a, 23–25 (PDF).

Author Contributions: R.C. and C.G. carried out the synthesis, separation and purification of compounds. L.E. Project Administration, supervised the whole work, collaborated on the synthesis, structure determination by spectroscopic methods (1D, 2D NMR, HRMS and IR), and manuscript preparation. A.F.O. collaborated in the discussion and interpretation of the results, manuscript redaction and corrections. M.F. collaborated with X-ray crystallography studies.

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**Sample Availability:** Samples of the compounds 9, 17–20, 21a, 22–25 are available from the authors.