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Review

Convergent synthesis of double point modified analogs of 1α,25-dihydroxyvitamin D_2 for biological evaluation

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ABSTRACT

There is a long lasting controversy over the biological activity of vitamin D_2 as compared to vitamin D_3 in terms of maintaining of calcium homeostasis and raising the level of circulating 25-OH-D. To shed more light on this relationship we synthesized 1α,25-dihydroxyvitamin D_2, by a novel convergent strategy, to compare this compound directly with the activity of 1α,25-dihydroxyvitamin D_3. The same synthetic strategy also provided a series of (5*E*,7*E*) geometric isomers of the natural 1α,25-dihydroxyvitamin D_2 as well as a series of double point modified analogs of its (24*R*)-epimer, including C-22 hydroxy derivatives. The structure of the new analogs was determined by ^{1}H and ^{13}C NMR as well as by mass spectrometry. The influence of (5*E*,7*E*) modification, alone or in combination with additional modifications in the side chain, on the activity profile and metabolic deactivation of analogs of 1α,25-dihydroxyvitamin D_2 still remains unknown. (5*E*,7*E*) modification in the structure of new analogs of 1α,25-dihydroxyvitamin D_2 is expected to give analogs with no influence on calcium level, as was previously obtained for the analogs of 1α,25-dihydroxyvitamin D_3. Investigation of the affinities for the vitamin D receptor and cell differentiation, transcriptional and calcium activities of the most active form of vitamin D_2 and of (5*E*,7*E*) analogs, compared to 1α,25-dihydroxyvitamin D_3, is underway in the collaborating laboratories.

Contents

1. Introduction

Nuclear receptors, both steroid and *seco*-steroid [1], as ligand-activated transcription factors [2] are very sensitive to changes in the ligand structure. Even a subtle modification of the structure of the ligand results in an altered affinity for the receptor and subsequently alteration of biological function [1]. Exogenous vitamin D_2 differs from the endogenous vitamin D_3 by the additional saturation at C-22 and methyl at C-24. It is, therefore, very intriguing that the active form of vitamin D_2, 1α,25-dihydroxyergocalciferol [1α,25-dihydroxyvitamin D_2, 1α,25$(OH)_2D_2$,Fig. 1] shows very similar activity [3] as one of the hormonal forms of vitamin D_3, 1α,25-dihydroxycholecalciferol [1α,25-dihydroxyvitamin D_3, 1α,25$(OH)_2D_3$]. 1α,25-dihydroxyvitamin D_2 was first isolated from an *in vitro* rat and chick kidney mitochondrial system using a tritiated analog of vitamin D_2 [4].

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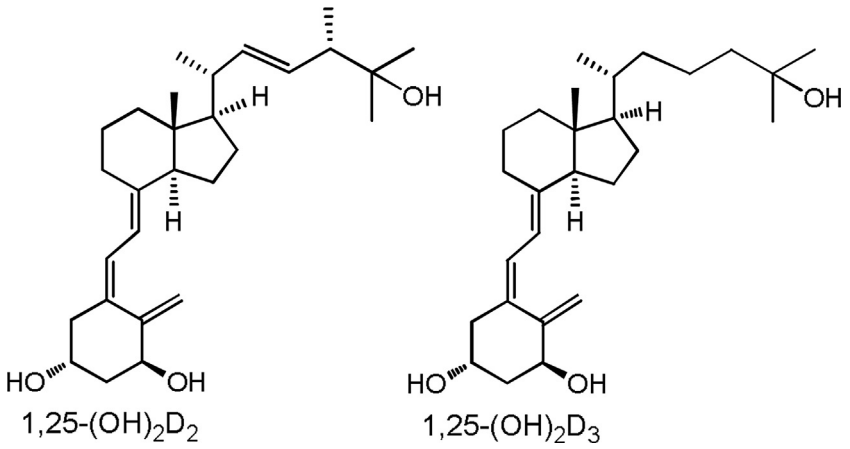

Fig. 1. Chemical structures of 1α,25-dihydroxyvitamin D_2 and 1α,25-dihydroxyvitamin D_3.

The antirachitic activity of 1α,25$(OH)_2D_2$ in the rat was equal to that of 1α,25$(OH)_2D_3$. Quite surprisingly, the same kind of activity of 1α,25$(OH)_2D_2$ in the chick was only 10–20% of the activity of 1α,25$(OH)_2D_3$ [5]. The initial bioassay measuring the potency of plain vitamin D_2 and vitamin D_3 in humans indicated their equal potency, while later studies showed higher potency of vitamin D_3 [6] as compared to vitamin D_2 in elevating or sustaining of serum 25-OH-D.

The synthetic precursor of 1α,25$(OH)_2D_2$, 1α-hydroxyvitamin D_2 (1α-OH-D_2) was less toxic than 1α-OH-D_3 but equally active in bone mineralization in the rat [7]. Deactivating side-chain hydroxylation and C24-C25 cleavage by CYP24A1 is thought to be responsible for different function of 1α,25-$(OH)_2D_2$ as compared the one of 1α,25-$(OH)_2D_3$ [3]. Although 1α,25$(OH)_2D_2$ has been already obtained by several syntheses [8–11] the direct comparison of the activity profile of this compound with 1α,25-$(OH)_2D_3$ is far from being well documented. To enable such a systematic comparison we have developed a convenient and scalable convergent synthesis of 1α,25$(OH)_2D_2$ that provides the compound in quantity. Introducing of (5*E*,7*E*) modification into the structure of 1α,25$(OH)_2D_3$ resulted previously in an non-hypercalcemic analog [12]. We have also found out that this modification resulted in an increased potency of the analog [13] in inhibiting Lewis lung carcinoma tumor growth with no influence on calcium level. To explore this finding further we have now synthesized a series of (5*E*,7*E*) analogs [8,10] of 1α,25-$(OH)_2D_2$, including (5*E*,7*E*) geometric analog of the natural 1α,25-$(OH)_2D_2$ (PRI-1731, Fig. 2) as well as the double point modified analogs [14] including

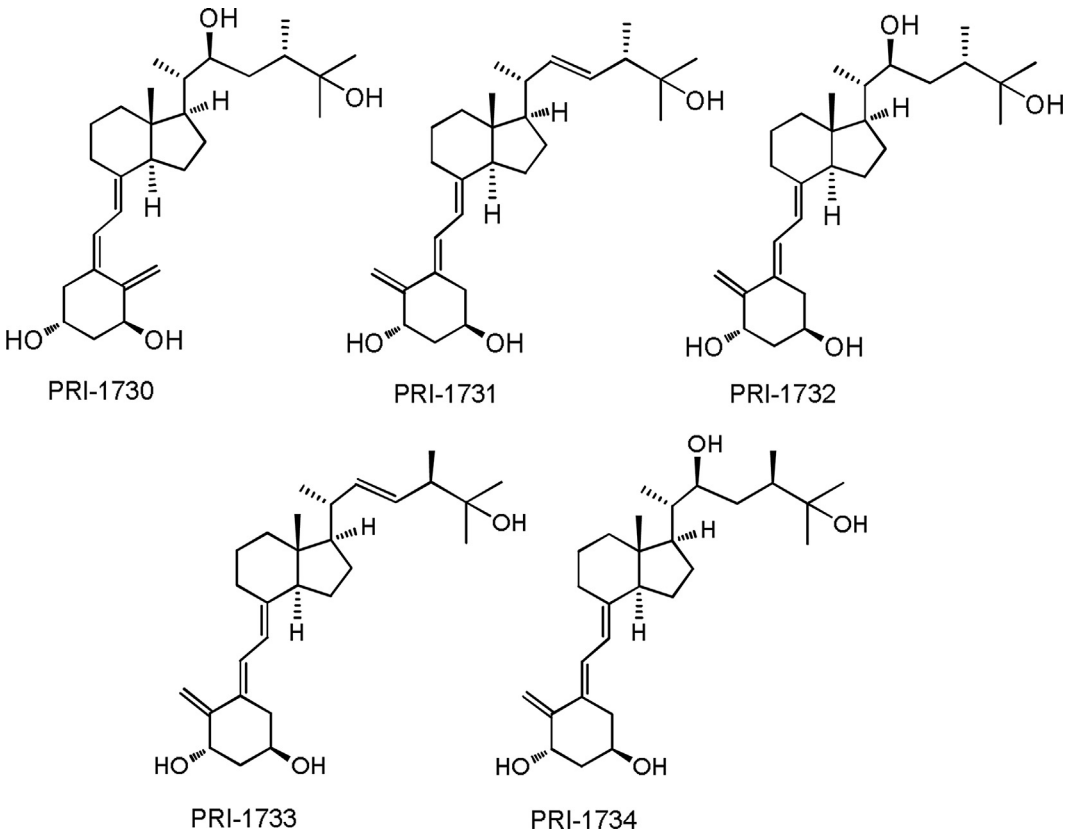

Fig. 2. Chemical structures of double point modified analogs of 1α,25-dihydroxyvitamin D_2.

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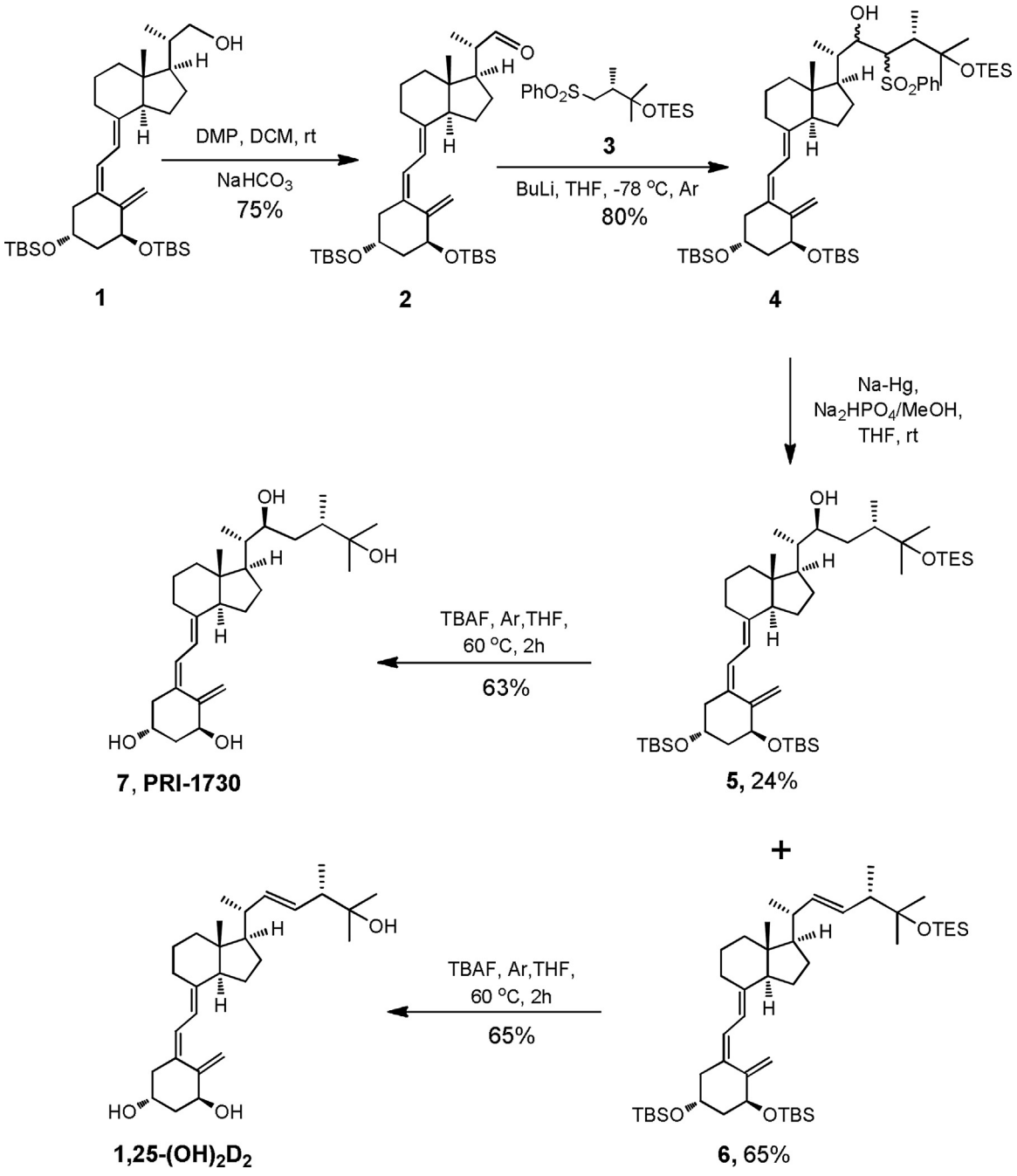
(24*R*)-epimer of 1α,25-$(OH)_2D_2$ (PRI-1733). C-22Hydroxy analogs, PRI-1730, PRI-1732 and PRI-1734, were also isolated and identified, as synthetic byproducts, to test the impact of the more hydrophilic side-chain on the activity.

2. Results and discussion

2.1. Synthesis of 1α,25-dihydroxyvitamin D_2 and its (22S)-hydroxy analog

We synthesized 1α,25-$(OH)_2D_2$ (Scheme 1) by a short convergent synthesis [15] from advanced intermediates, C-22 aldehyde **2**, as vitamin D synthon [16], and chiral phenylsulfone **3** [10], as a side-chain fragment, in 25% total yield. Vitamin D C-22 alcohols, bearing the labile triene system, were oxidized to the respective C-22 aldehydes by a classical Swern oxidation or by modified Swern oxidations [17,18]. TPAP was also used for this purpose [19]. For the oxidation of alcohol **1** we have first used Dess-Martin periodinane (DMP). Alcohol **1** was obtained in this laboratory [16] from vitamin D_2 by a multistep procedure. Sulfone **3** was obtained from (*R*)-3-hydroxy-2-methylpropionate [10]. Julia olefination of aldehyde **2** with sulfone **3** gave a diastereomeric mixture of hydroxysulfones **4**. Dehydroxy-desulfonylation of this mixture with 20% sodium amalgam gave olefin **6**. Desulfonylation product, C-22 alcohol **5** was also obtained in 9% of total yield, as a single diastereomer, as showed by ^{1}H NMR (individual 18-CH_3 singlet) [20]. The tentative (22*S*) absolute configuration at C-22 in **5** is to be confirmed by chiroptical methods or by X-ray crystallography. Fluoride anion-promoted desilylation of alcohol **5** and olefin **6** gave final analog **7** (PRI-1730) and 1α,25$(OH)_2D_2$, respectively.



Scheme 1. Synthesis of 1α,25-dihydroxyvitamin D_2 and its (22*S*)-hydroxy C-22 saturated analog PRI-1730.

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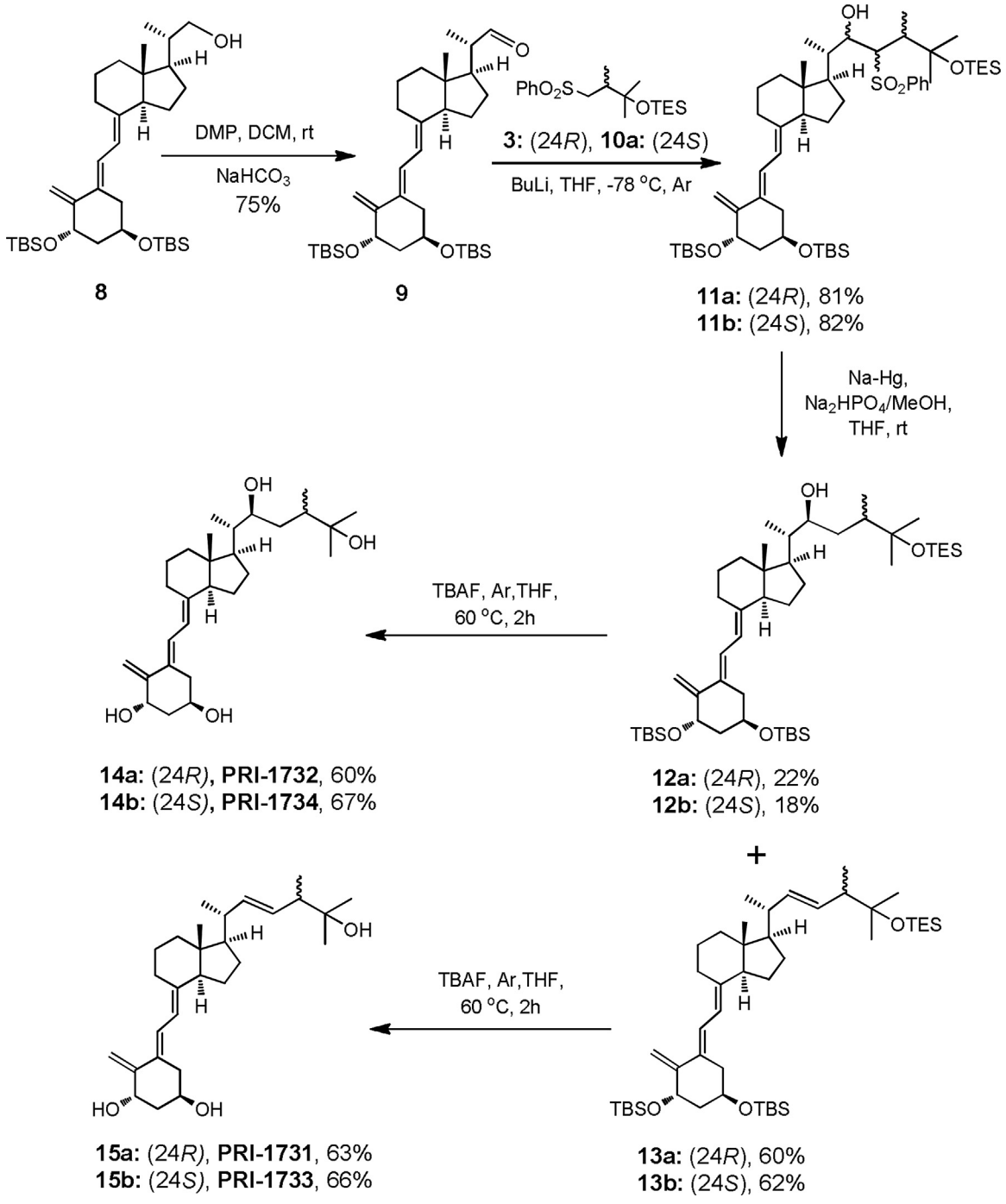
2.2. Synthesis of (5E,7E) analogs of 1α,25-dihydroxyvitamin D_2 and their (22S)-hydroxy derivatives

In a similar way as described above and starting from the previously obtained (5*E*,7*E*) alcohol **8** (Scheme 2) we have synthesized a series of (5*E*,7*E*) analogs **14a**, **14b**, **15a** and **15b** (PRI-1732, PRI-1734, PRI-1731 and PRI-1733) in 8, 7, 23 and 25% of total yield, respectively. The yields of the C-22 alcohols at the desulfonylation step were also around 20% (22 and 18% for **14a** and **14b**, respectively).

The binding affinity for vitamin D receptor as well as cell differentiation, transcriptional and calcium activity of the most active form of vitamin D_2 and of the analogs (PRI-1730, PRI-1731, PRI-1732, PRI-1733 and PRI-1734), compared to 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_2, is underway in the collaborating laboratories [13,14].

3. Conclusion

A series of geometric isomers of the natural 1α,25-dihydroxyvitamin D_2 as well as a series of double point modified analogs of its (24*R*)-epimer, including C-22 hydroxy derivatives, might be conveniently obtained by the common convergent strategy starting from the C-22 vitamin D alcohol or its (5*E*,7*E*) geometric isomer. (5*E*,7*E*) Modification is expected to give new analogs of 1α,25-dihydroxyvitamin D_2 with no influence on calcium level, as



Scheme 2. Synthesis of (5*E*,7*E*) analogs of 1α,25-dihydroxyvitamin D_2.

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was previously obtained for the analogs of 1α,25-dihydroxyvitamin D_3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2015.08.022.

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[20] ^{1}H data of new analogs (600MHz, CD3OD, 25.8 °C,TMS): **6**, (PRI-1730): ^{1}H NMR, δ = 6.326 (d, J = 10.8Hz, 1H, 6-H), 6.096 (d, J = 10.8Hz, 1H, 7-H), 5.291 (s, 1H, 19E-H), 4.903 (s, 1H, 19Z-H), 4.535 (t, J = 6.0, 1H, 1-H), 4.127 (q, J = 4.2Hz, 1H, 3-H), 3.725 (d, J = 10.2, 1H, 22-H), 1.152, 1.131 (s, s, 3H, 3H, 26-CH_3, 27-CH_3), 0.944 (d, J = 7.2Hz, 3H, 21-CH_3), 0.922 (d, J = 6.6Hz, 3H, 28-CH_3), 0.580 (s, 3H, 18-CH_3). **14a**, (PRI-1732): ^{1}H NMR, δ = 6.530 (d, J = 10.8 Hz, 1H, 6-H), 5.921 (d, J = 10.8 Hz, 1H, 7-H), 5.040 (s, 1H, 19E-H), 4.939 (s, 1H, 19Z-H), 4.417 (t, J = 3.6, 1H, 3-H), 4.116 (q, J = 4.2 Hz, 1H, 1-H), 3.740 (d, J = 10.2, 1H, 22-H), 1.155, 1.134 (s, s, 3H, 3H, 26-CH_3, 27-CH_3), 0.951 (d, J = 6.6 Hz, 3H, 21-CH_3), 0.927 (d, J = 6.6 Hz, 3H, 28-CH_3), 0.598 (s, 3H, 18-CH_3). **14b**, (PRI-1734): ^{1}H NMR, δ = 6.530 (d, J = 11.4 Hz, 1H, 6-H), 5.920 (d, J = 11.4 Hz, 1H, 7-H), 5.040 (s, 1H, 19E-H), 4.939 (s, 1H, 19Z-H), 4.418 (t, J = 2.4, 1H, 3-H), 4.109 (q, J = 3.6 Hz, 1H, 1-H), 3.740 (d, J = 7.2, 1H, 22-H), 1.147, 1.136 (s, s, 3H, 3H, 26-CH_3, 27-CH_3), 0.952 (d, J = 7.2 Hz, 3H, 21-CH_3), 0.928 (d, J = 6.6 Hz, 3H, 28-CH_3), 0.604 (s, 3H, 18-CH_3). **15a**, (PRI-1731): ^{1}H NMR, δ = 6.525 (d, J = 11.4 Hz, 1H, 6-H), 5.906 (d, J = 11.4 Hz, 1H, 7-H), 5.355 (q, J = 8.4 Hz, 1H, 22-H), 5.281 (q, J = 8.4 Hz, 1H, 23-H), 5.038 (s, 1H, 19E-H), 4.938 (s, 1H, 19Z-H), 4.410 (t, J = 3.6, 1H, 3-H), 4.113 (q, J = 3.6 Hz, 1H, 1-H), 1.140, 1.102 (s, s, 3H, 3H, 26-CH_3, 27-CH_3), 1.045 (d, J = 6.6 Hz, 3H, 21-CH_3), 0.998 (d, J = 6.6 Hz, 3H, 28-CH_3), 0.601 (s, 3H, 18-CH_3). **15b**, (PRI-1733): ^{1}H NMR, δ = 6.524 (d, J = 11.4 Hz, 1H, 6-H), 5.904 (d, J = 11.4 Hz, 1H, 7-H), 5.320 (q, J = 8.4 Hz, 1H, 22-H), 5.274 (q, J = 8.4 Hz, 1H, 23-H), 5.038 (s, 1H, 19E-H), 4.938 (s, 1H, 19Z-H), 4.415 (t, J = 3.6, 1H, 3-H), 4.108 (q, J = 3.6 Hz, 1H, C-1), 1.148, 1.104 (s, s, 3H, 3H, 26-CH_3, 27-CH_3), 1.036 (d, J = 6.6 Hz, 3H, 21-CH_3), 0.989 (d, J = 6.6 Hz, 3H, 28-CH_3), 0.602 (s, 3H, 18-CH_3)..