Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3885–3888
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological activity of 22-oxa CD-ring modified analogues of 1α,25-dihydroxyvitamin D_3: *cis*-perhydrindane CE-ring analogues

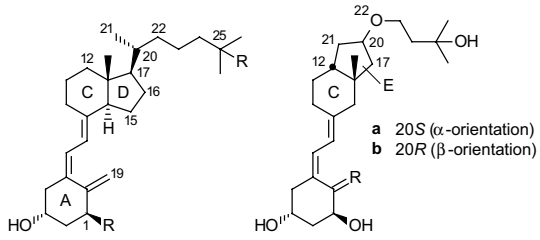
Samuël Demin,[a] Dirk Van Haver,[a] Maurits Vandewalle,[a] Pierre J. De Clercq,[a,*] Roger Bouillon[b] and Annemieke Verstuyf[b]

[a]*Department of Organic Chemistry, Ghent University, Krijgslaan 281 S4, B-9000 Gent, Belgium*
[b]*Laboratory of Experimental Medicine and Endocrinology, Catholic University of Leuven, Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium*

Received 6 April 2004; accepted 25 May 2004
Available online 17 June 2004

Abstract—The synthesis and biological activity of novel CD-ring modified analogues of 22-oxa-1α,25-dihydroxyvitamin D_3, lacking the D-ring and featuring a connection between C-12 and C-21 (*cis*-perhydrindane CE-ring analogues), is described. The synthesis of the CE-ring system follows Meyers' methodology for the preparation of enantiomerically pure hydrinden-2-ones. The analogues show a complete lack of binding affinity for the vitamin D receptor (pig nVDR) and of antiproliferative activity (MCF-7 cells), as compared to calcitriol.

In the context of the search for therapeutically useful structural analogues of 1α,25-dihydroxyvitamin D_3 (**2**, calcitriol),[1] the hormonally active metabolite of vitamin D_3 (**1**; cholecalciferol), based on the dissociation of the calcemic and other (prodifferentiating, antiproliferative, immunoregulating) activities,[2] in this and the accompanying paper we wish to describe the synthesis and biological activity of two different series of CD-ring modified analogues of calcitriol (**2**), which are also characterised by the presence of a 22-oxa side chain and of a C-20 epimerisable position, and by the possible deletion of C-19. In this work the central CD-ring system consists of a *cis*-fused perhydrindane CE-ring system such as present in **3** and **4** instead of the natural *trans*-fused perhydrindane CD-ring skeleton. Next to this important structural modification, **3** and **4** also feature: (i) a 22-oxa side chain, which is known to have led in the natural hormone case to the first analogue (OCT) in which a dissociation of activities has been observed;[3] (ii) a stereoisomeric position at C-20, reminiscent of 20-*epi* analogues, which in the natural hormone case (MC1288) has led to superagonistic activity;[4] (iii) the possibility of C-19 deletion, which in general is accompanied by a reduction in calcemic activity.[5]



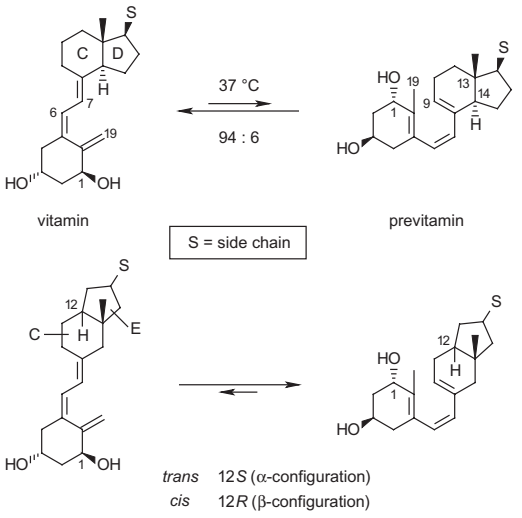
1 R = H (cholecalciferol)
2 R = OH (calcitriol)
3 R = CH_2
4 R = H,H

The implementation of this modified CD-ring system, featuring the deletion of C-15 and of C-16, and a connection between C-12 and C-21 (CE-ring system), is in line with previous work of our laboratories that has focused on systematic structural changes in the central hydrophobic part of the molecule.[6]

Of particular importance in the case of perhydrindane CE-ring analogues is the vitamin–previtamin equilibration issue (Scheme 1). The process is analogous to the

* Corresponding author. Tel.: +32-9-2644463; fax: +32-9-2644998; e-mail: pierre.declercq@UGent.be

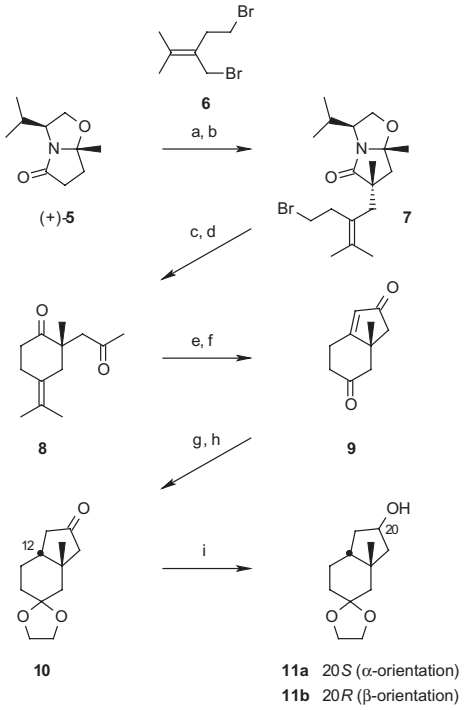
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.05.067



Scheme 1. The vitamin–previtamin equilibrium.

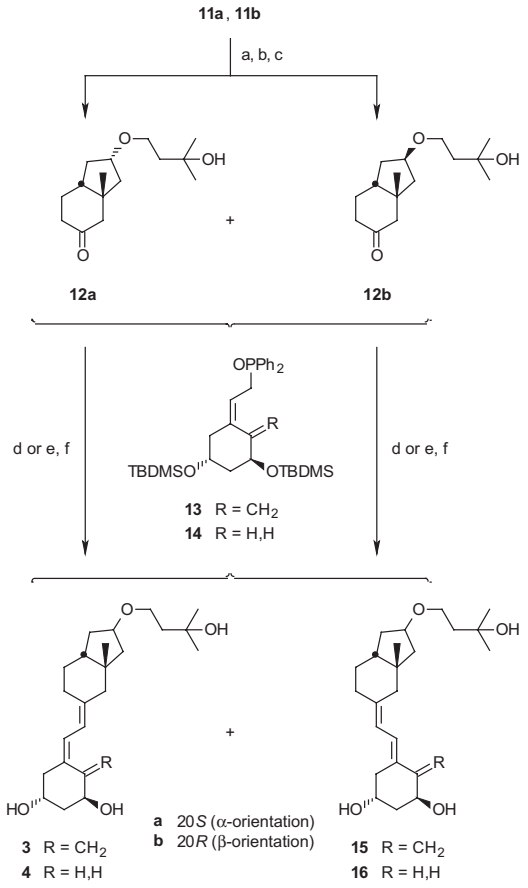
well known isomerisation that occurs during the bioconversion of 7-dehydrocholesterol into vitamin D_3.[7] It implies the reversible [1,7]-sigmatropic H-shift between positions C-19 and C-9. In the natural series, involving a *trans*-fused perhydrindane CD-ring system, this equilibrium is in favour of the vitamin form, notwithstanding the more substituted nature of the triene system in the previtamin form.[8] It has been shown that this originates from conformational aspects related to the substitution pattern of the C-ring, in particular to the presence of a *trans*-fusion at C-13–C-14.[9] In the case of the 14-*epi* derivative, that is, the epimeric *cis*-fused CD-ring system, the equilibrium is largely shifted towards the previtamin form (5:95 at 80 °C).[10] For the perhydrindane CE-ring analogues under investigation, having the ring fusion at C-12–C-13, molecular mechanics calculations indicate the vitamin–previtamin equilibrium mixture, in the case of a *cis*-fusion as well as a *trans*-fusion, to consist of almost exclusively the previtamin (>99%), so that problems to isolate the pure vitamin could be expected. The choice of a *cis*-fused perhydrindane system (**3**), as compared to the isomeric *trans*-fused system, is dictated by the expectation that a more mobile central part would be able to better adjust to the central region of the vitamin D receptor cavity.[11] Moreover, since the vitamin–previtamin equilibration issue becomes irrelevant in the absence of C-19, the corresponding 19-nor analogues **4** were also prepared.[12] From a synthetic point of view, both the natural (**3**) and the 19-nor series (**4**) are accessible from a common intermediate, if the Wittig–Horner reaction, as introduced by Lythgoe and co-workers,[13] is used for attachment of the A-ring.[14]

Central in the synthesis of analogues **3** and **4** stand the epimeric alcohols **11a** and **11b**. Their enantioselective synthesis follows a straightforward sequence based on Meyers' methodology for the preparation of enantiomerically pure hydrinden-2-ones (Scheme 2).[15] Starting from the chiral nonracemic bicyclic lactam **5**,[16] alkylation with methyl iodide (LDA; 96% yield), followed by



Scheme 2. Reagents and conditions: (a) (i) LDA, THF, −78 °C; (ii) MeI, −78 °C, 2 h (96%); (b) (i) LDA, DMPU, THF, −78 °C; (ii) **6**, −78 °C; 3 h (84%); (c) *t*-BuLi, KH, THF, −78 °C, 45 min; (d) $Bu_4NH_2PO_4$, EtOH, 75 °C, 12 h (68% from **7**); (e) KOH, EtOH, rt, 16 h (89%); (f) O_3, CH_2Cl_2–MeOH, −78 °C (73%); (g) $HO(CH_2)_2OH$, TsOH, toluene, reflux, 1.5 h (92%); (h) H_2, Pd/C, EtOAc, rt, 3 h (100%); (i) Li, liq NH_3, −78 °C, 1 h (95%).

alkylation with the known dibromide **6** (LDA; 84% yield),[17] led to a mixture of **7** and its epimer (not shown; ratio *endo*/*exo* 7:3), which were separated by column chromatography. The structural assignment of both isomers follows from ^{1}H NMR NOE measurements involving the two quaternary Me groups. On the basis of the enantiomeric purity of lactam **5**, which was determined to be virtually complete,[18] it may be safely assumed that the enantiomeric purity of **7** and subsequent intermediates is also very high (>99%). Further conversion of **7** follows Meyers' procedure (*t*-BuLi, KH; followed by buffered acid hydrolysis) affording diketone **8** in 68% yield. Subsequent intramolecular aldol condensation (KOH, EtOH; 89% yield), followed by selective ozonisation (O_3, Me_2S, CH_2Cl_2–MeOH), led to enone **9** (73% yield). After selective protection of the saturated carbonyl in **9** ($HO(CH_2)_2OH$, toluene; 92% yield), catalytic hydrogenation of the conjugated double bond (H_2, Pd/C, EtOAc) led to the *cis*-fused bicyclic ketone **10** in quantitative yield. The stereochemistry at C-12 was assigned on the basis of ^{1}H NMR NOE difference experiments involving H-12 and the angular methyl group. Finally, dissolved metal reduction of **10** (Li, liq NH_3) gave a mixture of alcohols **11a** and **11b** (ratio 1:1; 95% yield), which were separated by HPLC for analytical purposes. Again ^{1}H NMR NOE measurements involving H-20 and the angular methyl group allowed for the structural assignment of both isomers.



Scheme 3. Reagents and conditions: (a) NaH, $BrCH_2CH{=}C(CH_3)_2$, *n*-Bu_4NI, DMF, 0 °C → rt, 24 h (78%); (b) $Hg(OAc)_2$, THF–H_2O; $NaBH_4$, NaOH, rt, 1.5 h (80%); (c) TsOH, Me_2CO, H_2O, rt, 16 h (80%); (d) **13**, *n*-BuLi, THF, −78 °C; (e) **14**, *n*-BuLi, THF, −78 °C; (f) TBAF, THF, rt (**a**: 84% from **12**; **b**: 67% from **12**).

In the final sequence to analogues **3** and **4**, the mixture of **11a** and **11b** was converted to **12a** and **12b** (Scheme 3). The latter were separated by column chromatography. The sequence involved alkylation of the alkoxides (NaH) with 4-bromo-2-methyl-2-butene in the presence of tetrabutylammonium iodide (DMF; 78% yield), followed by mercuric acetate promoted water addition (80% yield) and acetal deprotection (80% yield). Again the stereochemistry of separated **12a** and **12b** was established through ^{1}H NMR spectral analysis as for **11a** and **11b**.

Reaction of **12a** and **12b** with phosphine oxide **13**,[19] followed by silyl ether deprotection, led to (*E*)- and (*Z*)-mixtures of **3a** and **15a** (ratio 6:4), and of **3b** and **15b** (ratio 92:8), respectively, which we were not able to separate by chromatography. Furthermore, upon repeated attempts at purification, more complex mixtures were obtained, presumably due to vitamin–previtamin equilibration. Hence, the coupling of **12a** and of **12b** was further examined with phosphine oxide **14**,[20] which afforded, after deprotection, mixtures of the corresponding 19-nor (*E*)- and (*Z*)-derivatives **4a** and **16a** (ratio 8:2), and of **4b** and **16b** (ratio 6:4), respectively. Also in this case the mixtures could not be separated.

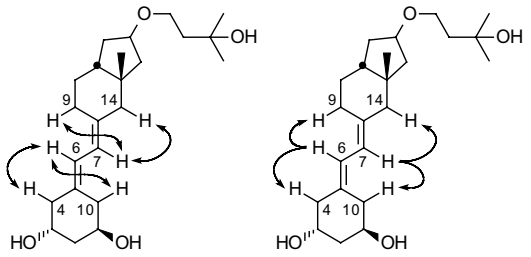


Figure 1. ^{1}H NMR COSY (left) and NOE studies (right) of **4**.

The determination of the relative (*E*/*Z*)-ratio's and the structural assignment of the double-bond geometry in **4**, in both the **a** and the **b** series, were performed via ^{1}H NMR spectral analysis of the mixtures (Fig. 1). Satisfactory analytical and physical data for all described intermediates were obtained.[21]

Analogues **4a** and **4b** were subjected to biological evaluation, despite the presence of a large fraction of their corresponding (*Z*)-isomer. The results show a complete lack of binding affinity for the vitamin D receptor (pig nVDR) and of antiproliferative activity (MCF-7 cells), as compared to calcitriol (**2**). In the context of SAR studies, however, the present result is interesting, since a few C-12 substituted analogues have recently been described, one of which (12β-Me-calcitriol) showing superagonistic activity.[22] In our case presumably the position of the side chain on the five-membered E-ring (C-20) does not allow for an optimal fit in the receptor cavity. Future studies will be directed towards the synthesis of analogues with a 22-oxa side chain located at C-17.

Acknowledgements

Financial assistance of the FWO-Vlaanderen (projects G.0150.02 and G.0036.00) is gratefully acknowledged. S.D. thanks the IWT for a scholarship.

References and notes

1. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200–257.
2. (a) *Vitamin D*; Feldman, D., Glorieux, F. H., Eds.; Academic Press: San Diego, USA, 1997; 1285 pp; (b) *Proceedings of the Eleventh Workshop on Vitamin D*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; University of California, Riverside, USA, 2000; pp 1–992, and the previous 10 volumes in this series.
3. Nishii, Y.; Okano, T. *Steroids* **2001**, *66*, 137–146, and references cited therein.
4. Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. *Biochem. Pharmacol.* **1991**, *42*, 1569–1575.
5. Perlman, K. L.; Sicinski, R. R.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1990**, *31*, 1823–1824.
6. (a) Bouillon, R.; De Clercq, P.; Pirson, P.; Vandewalle, M. *Novel Structural Analogues of Vitamin D*. Patent PCT/EP 93,202037,3, 1993; *Chem. Abstr.* **1996**, *122*, 314933m;

(b) Chen, Y.-J.; Gao, L.-J.; Murad, I.; Verstuyf, A.; Verlinden, L.; Verboven, C.; Bouillon, R.; Viterbo, D.; Milanesio, M.; Van Haver, D.; Vandewalle, M.; De Clercq, P. J. *Org. Biomol. Chem.* **2003**, *1*, 257–267, and references cited therein.

7. Velluz, L.; Amiard, G.; Goffinet, B. *Bull. Soc. Chim. Fr.* **1955**, *22*, 1341–1348.
8. Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958–6966.
9. (a) Havinga, E. *Experientia* **1973**, *29*, 1181–1316; (b) Schlatmann, J. L. M. A.; Pot, J.; Havinga, E. *Rec. Trav. Chim.* **1964**, *83*, 1173–1184.
10. Maynard, D. F.; Trankle, W. G.; Norman, A. W.; Okamura, W. H. *J. Med. Chem.* **1994**, *37*, 2387–2393.
11. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholtz, B.; Moras, D. *Mol. Cell* **2000**, *5*, 173–179.
12. Bouillon, R.; Sarandeses, L. A.; Allewaert, K.; Zhao, J.; Mascareñas, J. L.; Mouriño, A.; Van Baelen, H.; Vrielynck, S.; De Clercq, P.; Vandewalle, M. *J. Bone Miner. Res.* **1993**, *8*, 1009–1015.
13. Kociensky, P. J.; Lythgoe, B.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1045–1050.
14. (a) Zhu, G.-D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877–1952; (b) Posner, G. H.; Kahraman, M. *Eur. J. Org. Chem.* **2000**, 3889–3895.
15. Snyder, L.; Meyers, A. I. *J. Org. Chem.* **1993**, *58*, 7507–7515.
16. (a) Harre, M.; Meyers, A. I.; Garland, R. *J. Am. Chem. Soc.* **1984**, *106*, 1146–1148; (b) Meyers, A. I.; Romo, D.; Midura, W. *Tetrahedron* **1990**, *46*, 4951–4994.
17. Posner, G. H.; Hamill, T. G. *J. Org. Chem.* **1988**, *53*, 6031–6035.
18. Racemic **5** was synthesised; both enantiomers were shown to be fully separated by chiral GC: 0.25 μm SUPELCO β-DEX 120 column, 30 m × 0.25 mm, 120 °C.
19. Baggiolini, E. G.; Iacobelli, J. A.; Hennesy, B. M.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 2945–2948.
20. Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1991**, *32*, 7663–7666.
21. ^{1}H NMR (500 MHz, $CDCl_3$) spectral data, for **7**: δ 4.18 (dd, $J = 8.8$, 8.8 Hz, 1H), 3.77 (dd, $J = 7.9$, 7.9 Hz, 1H), 3.59–3.54 (m, 1H), 3.39–3.34 (m, 1H), 3.28–3.22 (m, 1H), 2.39 (s, 2H), 2.68–2.62 (m, 1H), 2.29–2.23 (m, 1H), 2.24 (d, $J = 13.5$ Hz, 1H), 1.88 (d, $J = 13.3$ Hz, 1H), 1.71 (s, 3H), 1.66–1.63 (m, 1H), 1.63 (s, 3H), 1.50 (s, 3H), 1.29 (s, 3H), 1.04 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H) ppm. For **8**: δ 2.98 (d, $J = 17.7$ Hz, 1H), 2.69–2.63 (m, 2H), 2.56–2.47 (m, 3H), 2.44–2.38 (m, 1H), 2.34 (d, $J = 14.1$ Hz, 1H), 2.10 (s, 3H), 1.71 (s, 3H), 1.70 (s, 3H), 1.03 (s, 3H) ppm. For **9**: δ 6.02 (d, $J = 1.7$ Hz, 1H), 3.06 (ddd, $J = 14.6$, 7.5, 1.4 Hz, 1H), 2.83 (dddd, $J = 14.6$, 13.1, 6.7, 1.7 Hz, 1H), 2.67–2.61 (m, 2H), 2.52–2.44 (m, 2H), 2.40 (d, $J = 1.7$ Hz, 2H), 1.25 (s, 3H) ppm. For **10**: δ 3.93–3.90 (m, 4H), 2.73 (d, $J = 18.4$ Hz, 1H), 2.47 (dd, $J = 18.8$, 7.7 Hz, 1H), 2.04 (dd, $J = 18.8$, 4.2 Hz, 1H), 1.95–1.93 (m, 1H), 1.87 (d, $J = 18.4$ Hz, 1H), 1.85–1.80 (m, 1H), 1.71–1.66 (m, 2H), 1.59–1.48 (m, 3H), 1.12 (s, 3H) ppm. For **11a**: δ 4.15–4.11 (m, 1H), 3.54–3.48 (m, 4H), 2.10 (d, $J = 14.0$ Hz, 1H), 1.99 (td, $J = 13.7$, 7.9 Hz, 1H), 1.88 (dd, $J = 13.6$, 3.9 Hz, 1H), 1.82–1.75 (m, 2H), 1.65–1.62 (m, 1H), 1.60–1.51 (m, 4H), 1.38–1.35 (m, 1H), 1.21 (br s, 1H), 1.03 (s, 3H) ppm. For **11b**: δ 4.08 (p, $J = 3.5$ Hz, 1H), 3.50–3.47 (m, 4H), 2.14 (dd, $J = 13.7$, 7.5 Hz, 1H), 1.79–1.72 (m, 3H), 1.53 (d, $J = 13.5$ Hz, 1H), 1.65–1.46 (m, 3H), 1.45 (dd, $J = 14.0$, 1.7 Hz, 1H), 1.44–1.38 (m, 1H), 1.31 (dd, $J = 13.7$, 3.9 Hz, 1H), 1.26 (s, 3H) ppm. For **12a**: δ 4.02–3.97 (m, 1H), 3.61 (t, $J = 5.9$ Hz, 2H), 3.24 (s, 1H), 2.61 (d, $J = 14.2$ Hz, 1H), 2.36 (ddd, $J = 16.0$, 8.8, 5.1 Hz, 1H), 2.28 (dt, $J = 14.0$, 7.0 Hz, 1H), 2.17–2.09 (m, 1H), 2.09 (d, $J = 14.2$ Hz, 1H), 1.98–1.93 (m, 1H), 1.86–1.69 (m, 6H), 1.63 (dd, $J = 13.7$, 4.4 Hz, 1H), 1.21 (s, 6H), 0.98 (s, 3H) ppm. For **12b**: δ 3.99 (m, 1H), 3.63 (t, $J = 5.9$ Hz, 2H), 3.40 (s, 1H), 2.34–2.30 (m, 1H), 2.28 (d, $J = 14.3$ Hz, 1H), 2.21–2.17 (m, 1H), 2.12 (d, $J = 14.1$ Hz, 1H), 2.07–1.99 (m, 3H), 1.91–1.85 (m, 1H), 1.84 (dd, $J = 13.9$, 6.3 Hz, 1H), 1.78–1.69 (m, 3H), 1.64 (dd, $J = 14.3$, 3.7 Hz, 1H), 1.24 (s, 6H), 1.11 (s, 3H) ppm. For *E*/*Z*-mixture **4a**/**16a** (ratio 8:2): δ 6.20 (d, $J = 11.2$ Hz, 1H of *E*-isomer), 6.17 (d, $J = 11.4$ Hz, 1H of *Z*-isomer), 6.10 (d, $J = 11.4$ Hz, 1H of *Z*-isomer), 5.96 (d, $J = 11.2$ Hz, 1H of *E*-isomer), 4.11–4.06 (m, 2H), 4.00–3.96 (m, 1H), 3.65–3.57 (m, 2H), 2.66 (dd, $J = 13.2$, 3.8 Hz, 1H of *E*-isomer), 2.59 (dd, $J = 13.2$, 3.4 Hz, 1H of *Z*-isomer), 2.50–2.46 (m, 1H), 2.36–2.10 (m, 4H), 1.93–1.82 (m, 2H), 1.73 (t, $J = 5.8$ Hz, 2H), 1.74–1.50 (m, 10H), 1.26 (s, 6H of *Z*-isomer), 1.22 (s, 6H of *E*-isomer), 0.93 (s, 3H of *Z*-isomer), 0.92 (s, 3H of *E*-isomer) ppm. For *E*/*Z*-mixture **4b**/**16b** (ratio 6:4): δ 6.20 (d, $J = 11.4$ Hz, 1H of *E*-isomer), 6.18 (d, $J = 11.5$ Hz, 1H of *Z*-isomer), 6.10 (d, $J = 11.5$ Hz, 1H of *Z*-isomer), 5.96 (d, $J = 11.4$ Hz, 1H of *E*-isomer), 4.11–4.05 (m, 2H), 4.00–3.96 (m, 1H), 3.61 (t, $J = 5.8$ Hz, 2H), 2.66 (dd, $J = 13.3$, 3.7 Hz, 1H of *E*-isomer), 2.58 (dd, $J = 13.5$, 3.6 Hz, 1H of *Z*-isomer), 2.51–2.47 (m, 1H), 2.37–2.11 (m, 4H), 2.05 (d, $J = 13.5$ Hz, 1H of *E*-isomer), 1.86 (d, $J = 14.5$ Hz, 1H of *E*-isomer), 2.07–1.81 (m, 7H), 1.74 (t, $J = 5.8$ Hz, 2H), 1.70–1.62 (m, 1H), 1.48–1.36 (m, 3H), 1.23 (m, 6H), 1.02 (s, 3H of *Z*-isomer), 1.01 (s, 3H of *E*-isomer) ppm.
22. Gonzalez-Avion, X. C.; Mouriño, A. *Org. Lett.* **2003**, *5*, 2291–2293.