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Synthesis of Fluorinated Brassinosteroids Based on Alkene Cross-Metathesis and Preliminary Biological Assessment

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Three types of brassinosteroid analogues with perfluoroalkylated side chains were synthesized by using alkene cross-metathesis of a brassinosteroid derivative bearing a terminal alkene moiety with different (perfluoroalkyl)propenes. The presence of the double bonds in the cross-metathesis products allowed a facile one-step double dihydroxylation to provide intermediates that after Baeyer−Villiger oxidation afforded the target compounds. Biological activity of the prepared analogues was tested in $GABA_A$ receptor, cytotoxic, and brassinolide activity, which reached in some cases the same range as their nonfluorinated analogues.

Introduction

Fluorine-containing compounds have been an ever increasing target of the pharmaceutical and specialty chemicals industry.[1−3] This stems from the fact that the presence of fluorine or fluorinated functional groups with their unique properties gives the target molecules special (desirable) properties (e.g., metabolic stability, increased lipophilicity, etc.).[4] Brassinosteroids are an important class of plant hormone with many potential applications in agrochemistry due to their ability to stimulate growth of plants under undesirable conditions.[5,6] In addition, some exerted unexpected antiviral and cancerostatic activity.[7−10] However, they are easily inactivated, among other reactions, by the conversion to more hydroxylated derivatives.[11] The site of such a hydroxylation has been found in the side chain, e.g., in position 26. Several papers describe metabolic accumulation of C-26-, C-28-hydroxylated, or further oxidized products. Because the metabolic stability of the C−F bond is very high, fluorine-containing brassinosteroids would have practical significance because of a good stability of products. This suggestion was confirmed by synthesis and metabolic stability studies of their monofluoroderivatives.[12] Similar results were obtained for brassinosteroids bearing perfluoroalkylated ester side chains instead of the classical sterol one. They were found to possess the activity comparable to natural brassinosteroids.[13]

Despite considerable advances in perfluoroalkylation methodology,[14−16] the search for new more synthetically flexible procedures is highly desirable. One such attractive process is highly selective alkene cross-metathesis under mild reaction conditions[17] that has been utilized in the synthesis of a number of fluorinated compounds.[18−27] Recently, we have also reported that the perfluoroalkylation could be achieved by cross-metathesis with perfluoroalkylpropenes[28] that showed higher reactivity, selectivity, and efficiency in comparison with other methods.

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In this regard, we aimed at two goals: the synthesis of a modified molecule and the development of a suitable synthetic methodology. As for the former, the synthesis of brassinosteroids bearing fluorinated side chains such as **1a**−**1c** (Figure 1) was expected to afford a new type of brassinosteroid derivatives resistant to the above-mentioned undesirable side reaction with increased metabolic stability,[4,12,13] and perhaps better biological activity. As for the latter, to demonstrate that cross-metathesis of terminal alkenes with perfluoroalkylpropenes is a convenient method for synthesis of brassinosteroids bearing perfluoroalkylated side chains.

Results and Discussion

Synthesis. The underlying strategy was based on the following assumption: because the brassinolide side chain contains a 1,2-diol moiety, it could be conveniently prepared by dihydroxylation of a suitable unsaturated intermediate, which in turn could be prepared by cross-metathesis of a terminal alkene with the appropriately substituted perfluoroalkylpropene (Scheme 1).

The starting substrate **3** was prepared by standard synthetic methodology in six steps from commercially available carboxylic acid **2** (Scheme 2).[29] Ester **3** was then reduced by $LiAlH_4$ to alcohol **4** (88%), which was oxidized by using Dess−Martin reagent to aldehyde **5** (76%),[30] whose Wittig olefination afforded alkene **6** (93%).[31] Deprotection of its carbonyl group under acidic conditions afforded the required alkene **7** in good 96% isolated yield. With the alkene **7** on hand, cross-metatheses with perfluorohexyl- **8a**, perfluoropropyl- **8b**, and perfluoroisopropylpropene **8c** (2-fold molar excess) were carried out. On the basis of previous results, the reaction was catalyzed by Hoveyda−Grubbs second-generation catalyst[32,33] (10 mol %) in refluxing dichloromethane for 4 h.[28] In all cases the cross-metathesis proceeded smoothly to give the corresponding perfluoroalkylated products **9a**−**9c** in good 67, 71, and 59% isolated yields, respectively. According to NMR analysis, compounds **9a** and **9b** were obtained as pure trans double-bond isomers; only in the case of **9c** a 19/1 trans/cis mixture was

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obtained. The structure of *trans*-**9c** was unequivocally confirmed by a single crystal X-ray analysis.

Because compounds **9a**−**9c** possess two double bonds within the molecule, simultaneous dihydroxylation was attempted. The hydroxylation of the double bonds was carried out by catalytic amount of OsO_4 (15 mol %) and excess *N*-methyl morpholine *N*-oxide (3.5 fold excess).[13] Initial hydroxylation of **9a** for 2 h led only to a 1/1.5 mixture of **10a** and **11a** in 50% isolated yield. The previous findings clearly demonstrated that dihydroxylation takes place preferentially on the more electron-rich double bond in the cyclohexane ring. To achieve full conversion, the hydroxylation time was prolonged to 16 h. Under these conditions, **9a**−**9c** were fully converted to tetraols **11a**−**11c** that were isolated as single diastereoisomers in good isolated yields of 68, 50, and 46%, respectively. The observed dihydroxylation diastereoselectivity could be explained as follows: in case of the cyclohexene ring, an oxidizing agent is approaching the double bond from the less hindered side, i.e., from the bottom side of the molecule, and in the case of the side chain double bond it is controlled by the presence of the center of chirality on C-20. Although the formation of other possible diastereoisomers can not be excluded, they were not detected in the reaction mixture.

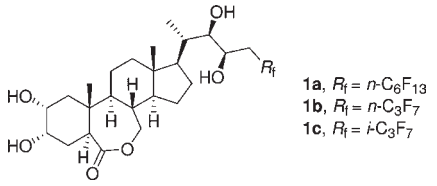
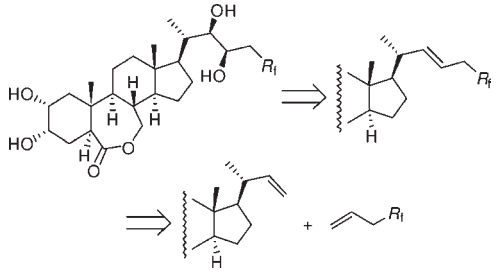


Figure 1. Brassinosteroids **1a**−**1c** with perfluoroalkylated side chain.

Scheme 1. Construction of Perfluoroalkylated 28-Norbrassinosteroid Side Chain



Finally, the synthesis was completed by Baeyer−Villiger oxidation of **11a**−**11c** by trifluoroperacetic acid (prepared by mixing trifluoroacetic anhydride and hydrogen peroxide in dichloromethane) under ambient conditions. In each case, the oxidation afforded a mixture of two regioisomeric lactones in 4:1 ratio in favor of the desired regioisomers **1a**−**1c** with natural configuration of diol moiety in the side chain. The desired brassinosteroids **1a**−**1c** were isolated by preparative HPLC in 62, 70, and 61% yields, respectively.

Biology. Because the brassinosteroids are known to possess various biological activities, the newly attained compounds, **1a**−**1c** and **11a**−**11c**, constituted ideal substrates for testing in various assays because of their new structural and previously unexplored feature, a perfluoroalkylated side chain.

$GABA_A$ Receptors Activity. Initially, the binding of the compounds **1a**−**1c** to $GABA_A$ receptors was tested in vitro using membranes isolated from the brains of male Wistar rats. The specific steroid binding was detected by the decrease in the binding of 2 nM [^{35}S]-*tert*-butylbicyclo-[2.2.2] phosphorothionate (TBPS) after application of the tested compounds incubated for 1 h at 37 °C. The results (see Table 1) could be summarized as follows: the heptafluoro derivative **1c** compares favorably to natural hormone allopregnanolone **12** and its higher metabolic stability (with respect to potential hydroxylation of the side chain)[4,12,13] should more than compensate for its slightly lower GABA-like activity. The results are in agreement with structural similarity of **1c** and 28-norbrassinolide **13** (Figure 2).[13] Compound **1a**, which does not contain the steroidal *i*-octyl side chain, is active at a higher concentration only and compound **1b** is inactive.

Scheme 2. Synthesis of Brassinosteroids with Perfluoroalkylated Side Chains

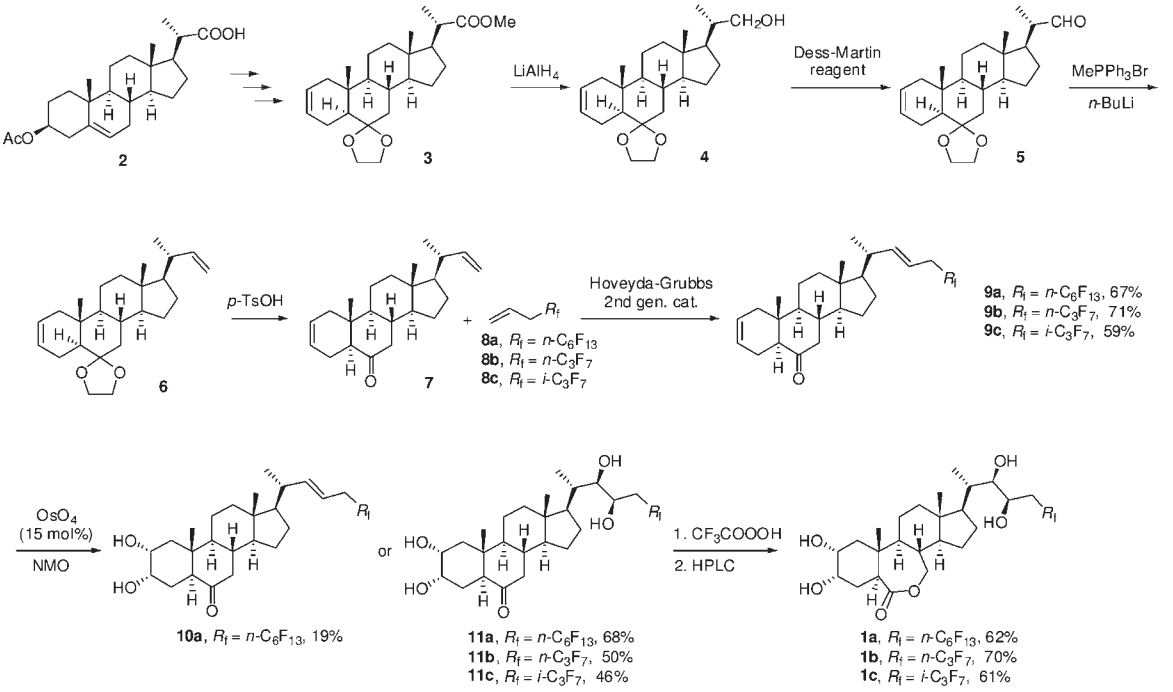


Table 1. Modulatory Effect on $GABA_A$ Receptors

compd	[^{35}S]-TBPS (%)[a]	I_{max}(%)[b]	IC_{50} (nM)[c]
allopregnanolone **12**	56.2 ± 6.0*	79.0	80
1a	47.2 ± 15.1*	57.9	900
1b	95.1 ± 14.8	[d]	[d]
1c	56.6 ± 14.6*	59.4	100

[a] The values of samples containing tested compounds at 100 nM concentrations were related to these of control samples with the buffer and expressed in %. Data are presented as means ± SD (obtained from triplicates or quadruplicates). To estimate statistical significance, nonparametric Kruskal−Wallis test for a global comparison ($\chi^2(4) = 13.95$, $p = 0.0074$) and Mann−Whitney−Wilcoxon test for pairwise comparisons (it was calculated with respect to the control samples, $*p < 0.050$). [b] The maximal suppression of the binding. [c] the steroid concentration producing a half-maximal inhibition were estimated using 1 nM TO 10 μM concentrations (in duplicates) of compounds. [d] Not determined.

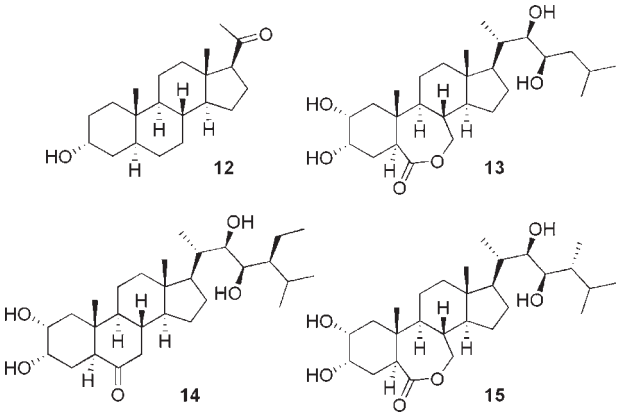


Figure 2. Structures of allopregnanolone **12**, 28-norbrassinolide **13**, 28-homocastasterone **14**, and 24-epibrassinolide **15**.

Anticancer Activity. Brassinosteroids are also known to exhibit anticancer activity.[7,8,10] The cytotoxic activity of **1a**−**1c** was determined by comparing human normal (fibroblast BJ) and cancer cell lines (T-lymphoblastic leukemia CEM and breast carcinoma MCF 7). These were exposed to six serial 4-fold dilutions of each drug for 72 h, the proportions of surviving cells were then estimated, and IC_{50} values were calculated (28-homocastasterone was used as a positive control). Unfortunately, only **11b** exhibited slight activity against CEM cell line (IC_{50} = 35.3 μM) (see Table 2). The other tested compounds such as **1a**−**1c**, **11a**, and **11c** had extremely weak or no detectable activity (IC_{50} > 50 μM). However, it is important to emphasize that tested compounds are not toxic toward normal human cells at all.

Brassinolide-Type Activity. Last but not least, brassinolide activity was measured by the bean second-internode bioassay.[13,34] The length of the second internodes was measured 5 days after the application of tested compounds in lanoline and the difference in length between treated and control plants provided a measure of activity (see Table 3). It was found that compound **1b** exhibited expressive swelling of the second bean internode at concentration 10^{-7} mol·L^{-1}. Moreover, compound **1c** exhibited surprising activity at lower and higher concentrations differing by 5 orders (10^{-7} and 10^{-12} mol·L^{-1}, +15.9 and +10.7 mm, respectively).

Conclusion

The synthetic route based on the cross-metathesis between terminal alkenes and perfluoroalkylpropenes constitutes an easy access to a new type of brassinosteroids with perfluoroalkylated side chains. The flexibility of our approach permits, in principle, introduction of any kind of side chains not only on the brassinosteroid but also other frameworks and provides a powerful tool for an analogue development and for an elucidation of biological functions.

The preliminary biological testing showed that some of the prepared brassinosteroids with fluorinated side chain exhibit the $GABA_A$ activity comparable with the endogeneous neurosteroid allopregnanolone. On the other hand, the anticancer activity was insignificant. Furthermore, the results of the brassinolide activity of the prepared perfluoroalkylated compounds were in the range of previously tested nonfluorinated analogues. These studies thus open not only new possibilities for the synthesis new functionalized brassinosteroid analogues but also could serve as a general strategy for the preparation of other classes of compounds bearing perfluorinated side chain.

Table 2. Cytotoxic Activity (IC_{50}) of Brassinosteroids Determined by Calcein-AM Assays[a]

compd	CEM[b] (μM)	MCF 7[c] (μM)
28-homocastasterone **14**	13 ± 2.8	> 50
1a	> 50	> 50
1b	> 50	> 50
1c	> 50	> 50
11a	> 50	> 50
11b	35.3 ± 1.6	48.2 ± 0.6
11c	> 50	> 50

[a] The IC_{50} values are expressed as mean ± SD values of three independent experiments performed in triplicate. [b] T-lymphoblastic leukemia cell line CEM. [c] Breast carcinoma cell lines MCF-7.

Table 3. Activity in the Bean Second-Internode Bioassay

compd	PSI[a]	SD
24-epibrassinolide **15**	32.3	±5.7
1a	3.1	±1.1
1b	11.0	±3.7
1c	0.9	±0.3
11a	14.1	±4.1
11b	9.6	±3.1
11c	11.6	±4.9

[a] PSI: Difference of prolongation of the Second Internode SD (mm) at concentration 10^{-10} mol·L^{-1} to control.

Experimental Section

General. All solvents were used as obtained unless otherwise noted. THF was distilled from sodium and benzophenone. Perfluoroalkylated propenes **8a**, **8b**, and **8c** (spectral characteristics were in agreement with the previously reported data)[35−37] were prepared according to the previously reported procedure.[38] All other reagents were obtained from commercial sources. The NMR spectra were measured on Bruker AVANCE 500 and 600 instruments (^{1}H at 500 or 600 MHz, ^{13}C at 125.7 or 150.9 MHz, and ^{19}F NMR at 470.3 MHz) as solutions in $CDCl_3$ at 27 °C unless otherwise noted. Chemical shifts are given in δ scale (^{1}H NMR spectra were referenced to TMS as an internal standard, ^{13}C NMR spectra to $CDCl_3$ at δ 77.0, and ^{19}F NMR to C_6F_6 δ −163.0), coupling constants *J* are given in Hz. Melting points (uncorrected) were determined using a Kofler apparatus. Infrared spectra were recorded as $CHCl_3$ solutions or as KBr tablets and are reported in wave numbers (cm^{-1}). The HPLC system used consisted of a high pressure pump (model 361, Gilson), Rheodyne valve, preparative column (10 mm × 250 mm) with silica gel filling (Biosher PSI 200 7micro-m, Labio), preparative ELSD detector (Gilson) connected with PC (software Trilution

LC, Gilson), and automatic fraction collector (model 346, Gilson). Purity of the prepared compounds was determined by a combination of ^{1}H NMR and by HLPC techniques and was >95%.

General Procedure for Cross-Metathesis of 7 with (Perfluoroalkyl)propenes 8. To a mixture of a terminal alkene **7** (1 mmol) and (perfluoroalkyl)propenes **8a**−**8c** (2 mmol) in CH_2Cl_2 (8 mL) under Ar atmosphere was added Hoveyda−Grubbs second-generation catalyst (63 mg, 0.1 mmol) and it was stirred at 42 °C for 4 h. Evaporation of the volatiles under reduced pressure followed by column chromatography on silica gel afforded expected compounds.

(20*S*)-20-(4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,9′-Tridecafluorononen-1′-en-1′-yl)-5α-pregn-2-en-6-one (9a). The reaction was carried out with **7** (250 mg, 0.77 mmol) and **8a** (551 mg, 1.53 mmol) according to the general procedure. Column chromatography on silica gel (50/1 hexane/EtOAc) and crystallization (MeOH) yielded 338 mg (67%) of the title compound **9a** as white needles: mp 101−102 °C (MeOH); $[\alpha]_D^{20}$ +6.4 (*c* 0.22, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.70 (s, 3H), 0.72 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 2.75 (m, 2H), 5.32 (dt, *J* = 15.2, 7.2 Hz, 1H), 5.55 (ddt, *J* = 15.3, 8.8, 1.3 Hz, 1H), 5.57 (m, 1H), 5.69 (m, 1H). HR-MS (EI) calcd. for $C_{30}H_{35}OF_{13}$ [M^+] 658.2480, found 658.2485. R_f (20/1 hexane/EtOAc) = 0.24.

(20*S*)-20-(4′,4′,5′,5′,6′,6′,6′-Heptafluorohex-1′-en-1′-yl)-5α-pregn-2-en-6-one (9b). The reaction was carried out with **7** (360 mg, 1.1 mmol) and **8b** (463 mg, 2.2 mmol) according to the general procedure. Column chromatography on silica gel (50/1 hexane/EtOAc) and crystallization (MeOH) yielded 397 mg (71%) of the title compound **9b** as white crystals: mp 117−119 °C (MeOH); $[\alpha]_D^{20}$ +12.9 (*c* 0.20, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.70 (s, 3H), 0.72 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 2.75 (m, 2H), 5.32 (dt, *J* = 15.3, 7.1 Hz, 1H), 5.55 (ddt, *J* = 15.3, 8.8, 1.2 Hz, 1H), 5.57 (m, 1H), 5.69 (m, 1H). HR-MS (ESI) calcd. for $C_{27}H_{36}OF_7$ [M^+ + 1] 509.2649, found 509.2650. R_f (20/1 hexane/EtOAc) = 0.24.

22-(*E*)-(20*S*)-25,26,26,26,27,27,27-Heptafluoro-cholesta-2,22-dien-6-one (9c). The reaction was carried out with **7** (340 mg, 1.04 mmol) and **8c** (440 mg, 2.0 mmol) according to the general procedure. Column chromatography on silica gel (50/1 hexane/EtOAc) and crystallization (MeOH) yielded 315 mg (59%) of the title compound **9c** as white crystals: mp 125−126 °C (MeOH); $[\alpha]_D^{20}$ +19.5 (*c* 0.17, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.69 (s, 3H), 0.71 (s, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 2.77 (bdd, *J* = 20.0, 7.0 Hz, 2H), 5.30 (bdt, *J* = 15.1, 7.0 Hz, 1H), 5.52 (bdd, *J* = 15.1, 8.8 Hz, 1H), 5.57 (m, 1H), 5.69 (m, 1H). HR-MS (ESI) calcd for $C_{27}H_{36}OF_7$ [M^+ + 1] 509.2649, found 509.2649. R_f (20/1 hexane/EtOAc) = 0.24.

General Procedure for Dihydroxylation.[13] A solution of OsO_4 (13 mg, 0.05 mmol) in 2-methyl-propan-2-ol (0.12 mL) was added to a solution of olefin **9** (0.35 mmol) in acetone (8 mL) and tetrahydrofuran (8 mL). Next, *N*-methylmorpholine *N*-oxide (140 mg, 1.2 mmol) in water (0.2 mL) was added. The mixture was stirred under Ar atmosphere for 16 h at room temperature. A solution of sodium sulfite (5 mL, 10%) was then added and the mixture was stirred for 30 min, poured into water, and extracted with chloroform. Column chromatography on silica gel (1/2 hexane/EtOAc) and crystallization (heptane/acetone) yielded compounds **11a**−**11c** as a white crystals.

(20*S*,1′*R*,2′*R*)-2α,3α,1′,2′-Tetrahydroxy-20-(4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,9′-tridecafluorononen-1′-yl)-5α-pregnan-6-one (11a). The reaction was carried out with **9a** (115 mg, 0.17 mmol). Column chromatography on silica gel (1/2 hexane/EtOAc) afforded 86 mg (68%) of the title compound **11a** as white crystals: mp 220−221 °C (acetone/heptane); $[\alpha]_D^{20}$ −1.9 (*c* 0.11, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 0.70 (s, 3H), 0.76 (s, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 2.48 (m, 1H), 2.69 (ddd, *J* = 12.6, 3.4, 0.8 Hz, 1H), 3.52 (dd, *J* = 4.6, 1.7 Hz, 1H), 3.77 (ddd, *J* = 11.8, 4.8, 3.2 Hz, 1H), 4.06 (q, *J* = 3.3 Hz, 1H), 4.30 (ddd, *J* = 8.0, 3.8, 1.7 Hz, 1H). HR-MS (ESI) calcd for $C_{30}H_{39}O_5F_{13}Na$ [M^+ + Na] 749.2482, found 749.2476. R_f (2/3 toluene/EtOAc) = 0.23.

(20*S*,1′*R*,2′*R*)-2α,3α,1′,2′-Tetrahydroxy-20-(4′,4′,5′,5′,6′,6′,6′-heptafluorohex-1′-yl)-5α-pregnan-6-one (11b). The reaction was carried out with **9b** (190 mg, 0.37 mmol). Column chromatography on silica gel (1/2 hexane/EtOAc) afforded 107 mg (50%) of the title compound **11b** as white crystals: mp 176−178 °C (acetone/heptane); $[\alpha]_D^{20}$ −23.9 (*c* 0.13, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3OD) δ 0.75 (s, 3H), 0.77 (s, 3H), 1.10 (d, *J* = 6.6 Hz, 3H), 2.40 (m, 2H), 2.74 (bdd, *J* = 11.5, 3.4 Hz, 1H), 3.44 (dd, *J* = 4.9, 1.5 Hz, 1H), 3.66 (ddd, *J* = 11.8, 4.8, 3.0 Hz, 1H), 3.95 (bq, *J* = 3.0, 1H), 4.22 (ddd, *J* = 7.8, 3.9, 1.5 Hz, 1H). HR-MS (ESI) calcd for $C_{27}H_{40}O_5F_7$ [M^+ + 1] 577.2758, found 577.2759. R_f (20/1 toluene/EtOAc) = 0.23.

(20*S*,22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-25,26,26,26,27,27,27-heptafluoro-cholestan-6-one (11c). The reaction was carried out with **9c** (190 mg, 0.37 mmol). Column chromatography on silica gel (1/2 hexane/EtOAc) afforded 98 mg (46%) of the title compound **11c** as white crystals and 11 mg (5%) of the compound *cis*-**10c** as a colorless oil. **11c**: mp 153−154 °C (acetone/heptane); $[\alpha]_D^{20}$ −12.0 (*c* 0.16, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3OD) δ 0.74 (s, 3H), 0.77 (s, 3H), 1.09 (d, *J* = 6.6 Hz, 3H), 2.42 (m, 2H), 2.74 (ddd, *J* = 12.5, 3.4, 1.0 Hz, 1H), 3.41 (dd, *J* = 5.0, 1.5 Hz, 1H), 3.66 (ddd, *J* = 11.8, 4.8, 3.0 Hz, 1H), 3.95 (bq, *J* = 3.0 Hz, 1H), 4.16 (bd, *J* = 7.8 Hz, 1H). HR-MS (ESI) calcd. for $C_{27}H_{40}O_5F_7$ [M^+ + 1] 577.2758, found 577.2759. R_f (20/1 toluene/EtOAc) = 0.23.

General Procedure for Baeyer−Villiger Oxidation.[39] A solution of trifluoroperoxyacetic acid in dichloromethane (20 mL), prepared from trifluoroacetic anhydride (3.23 g, 8.24 mmol) and 30% hydrogen peroxide (0.5 mL, 4.8 mmol), was added to a solution of ketone **11** (2 mmol) in dichloromethane (16 mL) and stirred for 4 h. Then the reaction mixture was poured into a 10% $KHCO_3$ solution (200 mL), extracted with $CHCl_3$ (3 × 150 mL), the combined organic extracts washed with water (200 mL), and dried over anhydrous $MgSO_4$. Evaporation of the volatiles followed by column chromatography on silica gel (6/1 EtOAc/hexane) afforded 4/1 mixture of regioisomeric lactones **11**/**11′**. Further preparative HPLC (6/1 EtOAc/hexane) yielded target compounds.

(20*S*,1′*R*,2′*R*)-2α,3α,1′,2′-Tetrahydroxy-7-oxa-7a-homo-20-(4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,9′-tridecafluorononen-1′-yl)-5α-preg-nan-6-one (1a). The reaction was carried out with **11a** (95 mg, 0.13 mmol) and trifluoroperoxyacetic acid (2 mL). Column chromatography followed by HPLC afforded 60 mg (62%) of the title compound **1a** as a colorless oil: $[\alpha]_D^{20}$ +7.5 (*c* 0.11, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.73 (s, 3H), 0.92 (s, 3H), 1.05 (d, *J* = 7.0 Hz, 3H), 2.48 (m, 1H), 3.12 (dd, *J* = 12.3, 4.5 Hz, 1H), 3.51 (dd, *J* = 4.7, 1.6 Hz, 1H), 3.72 (ddd, *J* = 12.1, 4.7, 2.8 Hz,1H), 4.03 (bq, *J* = 3.0 Hz, 1H), 4.08 (m, 2H), 4.28 (ddd, *J* = 7.9, 3.9, 1.6 Hz, 1H). HR-MS (ESI) calcd for $C_{30}H_{38}O_6F_{13}$ [M^+ − 1] 741.2466, found 741.2446. R_f (6/1 EtOAc/hexane) = 0.16.

(20*S*,1′*R*,2′*R*)-2α,3α,1′,2′-Tetrahydroxy-7-oxa-7a-homo-20-(4′,4′,5′,5′,6′,6′,6′-heptafluorohex-1′-yl)-5α-pregnan-6-one (1b). The reaction was carried out with **11b** (70 mg, 0.12 mmol) and trifluoroperoxyacetic acid (2 mL). Column chromatography followed by HPLC afforded 51 mg (70%) of the title compound **1b** as a colorless oil: $[\alpha]_D^{20}$ +7.0 (*c* 0.14, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.73 (s, 3H), 0.92 (s, 3H), 1.05 (d, *J* = 7.0 Hz, 3H), 2.48 (m, 1H), 3.12 (dd, *J* = 12.3, 4.5 Hz, 1H), 3.51 (dd, *J* = 4.7, 1.6 Hz, 1H), 3.72 (ddd, *J* = 12.1, 4.7, 2.8 Hz, 1H), 4.03 (bq, *J* = 3.0 Hz, 1H), 4.08 (m, 2H), 4.28 (ddd, *J* = 7.9, 3.9, 1.6 Hz, 1H). HR-MS (ESI) calcd for $C_{27}H_{38}O_6F_7$ [M^+ − 1] 591.2562, found 591.2550. R_f (6/1 EtOAc/hexane) = 0.16.

(20*S*,22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-7-oxa-7a-homo-25,26,26,26,27,27,27-heptafluoro-cholestan-6-one (1c). The reaction was carried out with **11c** (85 mg, 0.15 mmol) and trifluoroperoxyacetic acid (2 mL). Column chromatography followed by HPLC afforded 54 mg (62%) of the title compound **1c** as a colorless oil: $[\alpha]_D^{20}$ +29.4 (*c* 0.07, MeOH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.73 (s, 3H), 0.92 (s, 3H), 1.04 (d, *J* = 7.0 Hz, 3H), 2.75 (m, 1H), 3.12 (dd, *J* = 12.3, 4.5 Hz, 1H), 3.46 (dd, *J* = 4.7, 1.7 Hz, 1H), 3.72 (ddd, *J* = 12.1, 4.7, 2.7 Hz, 1H), 4.03 (bq, *J* = 3.0 Hz, 1H), 4.09 (m, 2H), 4.21

(m, 1H). HR-MS (ESI) calcd for $C_{27}H_{38}O_6F_7$ [$M^+ - 1$] 591.2562, found 591.2547. R_f (6/1 EtOAc/hexane) = 0.16.

Biological Evaluation. $GABA_A$ receptor binding assay,[40,41] calcein-AM cytotoxicity assay,[10] and the Bean second-internode bioassay[13,34] were carried out according to previously reported methods (for details, see Supporting Information).

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Supporting Information Available: All experimental procedures, detailed compound characterization data, biological evaluation methods, copies of spectra, and crystallographic information file. This material is available free of charge via the Internet at http://pubs.acs.org.

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