Synthesis and Biological Activity of 25-Methoxy-, 25-Fluoro-, and 25-Azabrassinolide and 25-Fluorocastasterone: Surprising Effects of Heteroatom Substituents at C-25

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The CuCN-catalyzed addition of 2-propenylmagnesium bromide to (*threo*-2*R*,3*S*,5α,22*R*,23*R*,24*S*)-23,24-epoxy-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestan-22-ol (**11a**) afforded the corresponding Δ^{25}-22,23-diol **12**. This was converted into 25-methoxybrassinolide (**7**) by protection as the 22,23-acetonide **13**, oxymercuration in methanol, Baeyer−Villiger oxidation, and deprotection. Similarly, the addition of pyridinium poly(hydrogen fluoride) to **13** and deprotection afforded 25-fluorocastasterone (**8**), which was converted into 25-fluorobrassinolide (**9**) by Baeyer−Villiger oxidation. Treatment of *threo*-epoxide **11a** with Me_2NMgBr, followed by Baeyer−Villiger oxidation of the corresponding tetraacetate and saponification, provided 25-azabrassinolide (**10**). Epoxide **11a** is therefore a versatile intermediate for the synthesis of side-chain analogues of brassinolide (**1**). 25-Methoxybrassinolide (**7**) displayed strong activity in the rice leaf lamina inclination bioassay, which was significantly enhanced by the simultaneous application of an auxin, indole-3-acetic acid (IAA). Thus, the presence of a 25-methoxy substituent, like that of the previously reported 25-hydroxy group in the 24-epibrassinolide series, yields a molecule with potent biological activity. On the other hand, **8**−**10** showed no bioactivity with or without IAA. This suggests that either the 25-fluoro and 25-aza substituents interfere with binding to a putative brassinosteroid receptor or that they prevent the in vivo enzymatic oxidation at C-25 that is required for bioactivity.

Brassinolide (**1**) is a powerful steroidal plant growth regulator that was discovered in 1979 by Grove and Mandava and co-workers[1] and displays activity at doses as low as 1 ng per individual plant in some species. This compound, as well as related brassinosteroids that were discovered subsequently, have therefore attracted considerable attention[2] to their synthesis,[3] biosynthesis and metabolism,[4] bioactivity and field applications,[5] and molecular biology.[6] Investigations of structure−activity relationships[5a,d,7] in brassinosteroids have revealed that the (2α,3α)- and (22*R*,23*R*)-vicinal diol moieties are required for optimum bioactivity, although O-methylation of the side-chain hydroxyl groups of **1** affords a product that retains significant biological activity.[8] The B-ring lactone moiety of **1** tolerates considerable variation in its structure, but the complete absence of a polar functional group results in the loss of all activity.[9] Castasterone (**2**), the B-ring ketone analogue of **1**, is also intrinsically bioactive in some plant species, although it serves as the biosynthetic precursor of **1** in others.[4b,10] The 5α-configuration is generally required, although some 5*β*-analogues have recently been reported to show substantial activity.[7a] Several variations of the side chain segment C-24 to C-28 have also been reported. Some of these, such as 24-epibrassinolide (**3**) and 28-homobrassinolide (**4**), possess substantial bioactivity and have been widely employed in field trials because of their greater synthetic accessibility compared to **1**.[3b,11]

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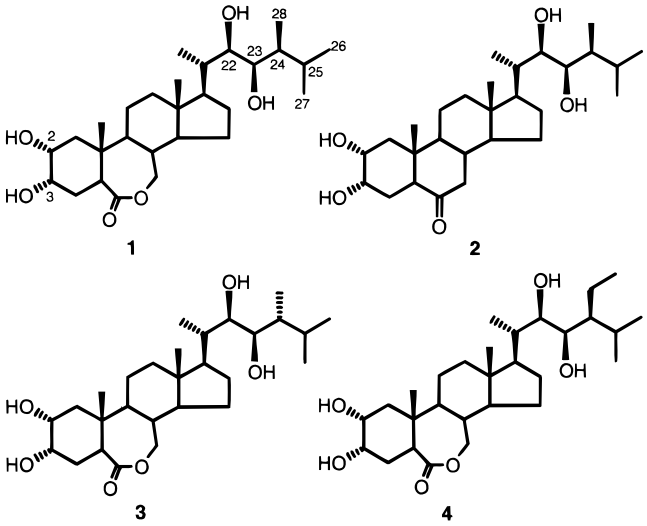
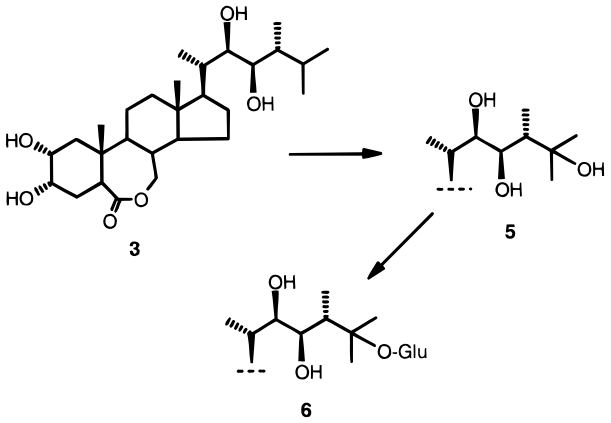


It was recently reported by Adam et al.[4a] that plants metabolize and ultimately deactivate brassinosteroids such as **3** by, inter alia, hydroxylation at C-25, followed by glucosylation at that and other hydroxylated sites (Scheme 1). Whereas the 25-hydroxy metabolite **5** displays increased biological activity compared to **3**, subsequent glucosylations render the products (e.g., **6**) relatively inactive. Therefore, it is of interest to prepare novel brassinosteroid analogues where this metabolic pathway is blocked by appropriate structural modifications. Such compounds could have longer lasting activity and, therefore, be less expensive in field applications, if they prevent or retard metabolic deactivation in plants while still retaining the growth-promoting properties of active brassinosteroids. We now report the preparation and bioactivity of several novel brassinosteroids designed for this purpose, including 25-methoxybrassinolide (**7**), 25-fluorocastasterone (**8**), 25-fluorobrassinolide (**9**), and 25-azabrassinolide (**10**). Our premise was that **8**−**10** would be unable to undergo the initial enzymatic hydroxylation at C-25 because the site is blocked by the heteroatom, whereas the methyl ether moiety of **7** would preclude subsequent glucosylation of the C-25 hydroxyl group. The strong bioactivity retained after O-methylation of the C-22 and C-23 hydroxyl groups of brassinolide[8] suggested to us that the 25-methoxy analogue **7** might be similarly active. Moreover, fluorination at C-25 should result in a relatively small perturbation to the castasterone and brassinolide structures because of the small size of the fluorine atom, again leading us to expect high bioactivity for the fluorinated analogues **8** and **9**. 25-Azabrassinolide **10** was included in our investigation because it was of interest to observe whether the dimethylamino group of **10** would act as an isostere of the isopropyl moiety of **1** while blocking the hydroxylation pathway.

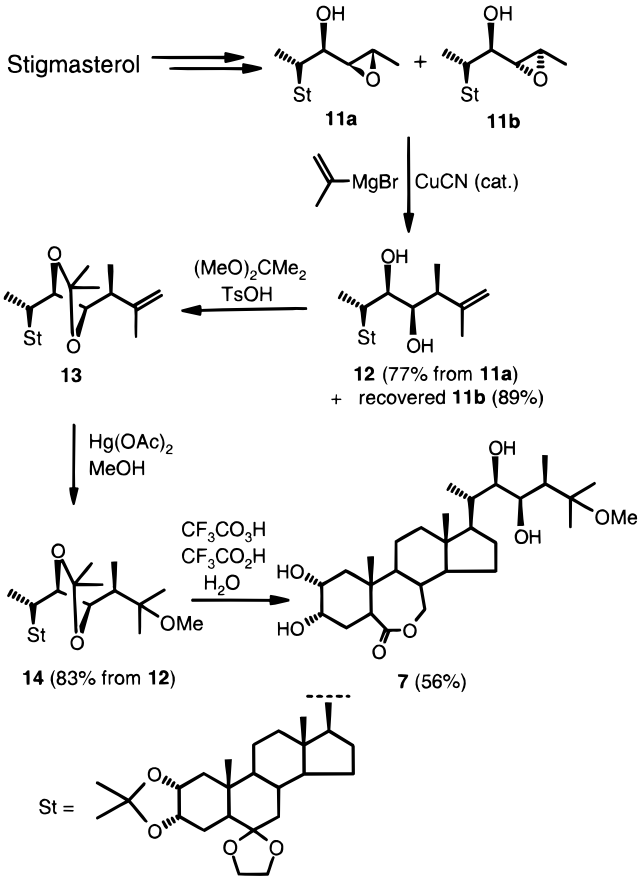
Scheme 1



Results and Discussion

The synthesis of compounds **7**−**10** is shown in Schemes 2−4. The mixture of epoxides **11a** and **11b** is readily available from stigmasterol and was employed in our recent synthesis of **1**[12] and related compounds.[8,9,13] In general, the *threo*-epoxide **11a** reacts considerably faster with Grignard reagents than does the erythro isomer **11b**, thereby permitting a facile separation of the diol produced from **11a** from the less polar unreacted **11b**. The reaction of the mixture of **11a** and **11b** with 2-propenylmagnesium bromide in the presence of catalytic CuCN thus afforded **12** and recovered **11b**. Protection of the side chain diol moiety to produce **13**,[14] followed by oxymercuration in methanol and a one-pot deprotection and Baeyer−Villiger oxidation,[12,15] afforded the 25-methoxy derivative **7** (Scheme 2). The reaction of **13** with pyridinium poly(hydrogen fluoride,[16] produced 25-fluo-

Scheme 2



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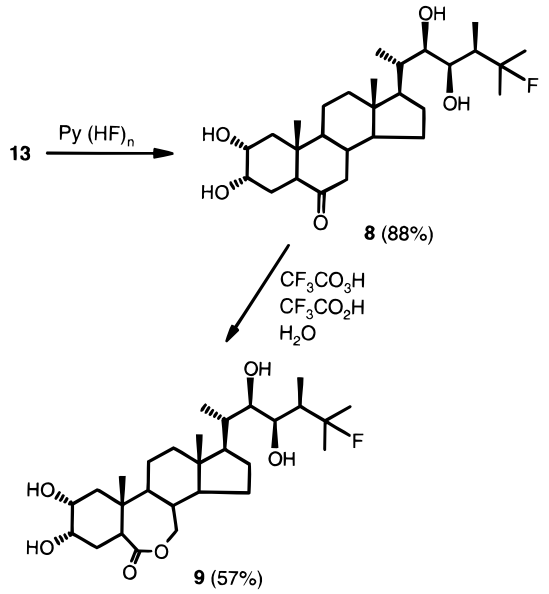
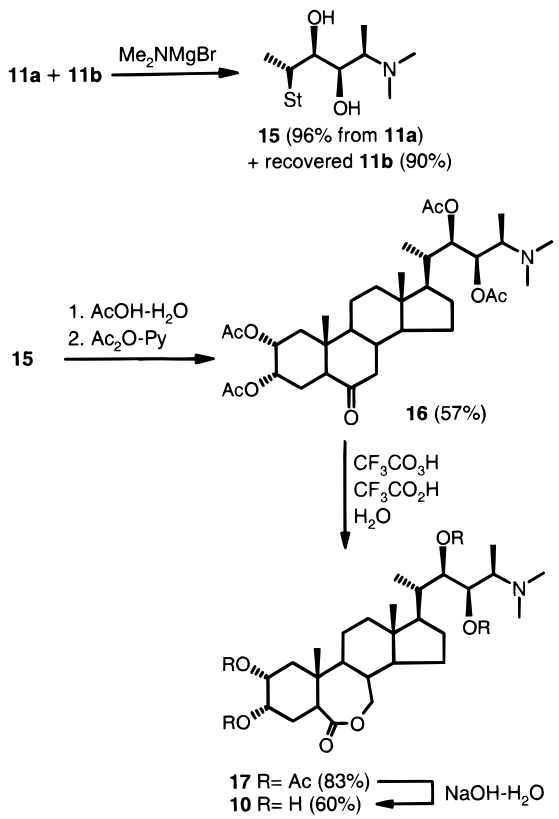
(14) The side-chain diol moiety of **12** was protected as the acetonide **13** before the oxymercuration reaction was carried out because of the ease with which intramolecular alkoxymercuration proceeds when a five-membered or six-membered cyclic ether is formed: Larock, R. C. *Solvomercuration/Demercuration Reactions in Organic Synthesis*; Springer-Verlag: Berlin, 1986; p 276.

Scheme 3



rocastasterone (**8**), which was converted into 25-fluorobrassinolide (**9**) in the usual manner (Scheme 3). The mixture of epoxides **11a** and **11b** was also treated with Me_2NMgBr to produce the 25-aza derivative **15**. Baeyer−Villiger oxidation of the corresponding tetraacetate analogue **16**, followed by saponification of **17**, provided 25-azabrassinolide (**10**) (Scheme 4). The *threo*-epoxide **11a** thus serves as a versatile intermediate for novel brassinosteroid analogues with altered side chains.

Scheme 4



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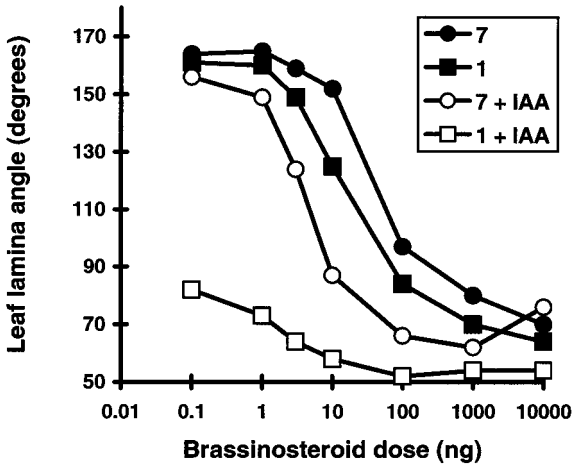

Figure 1. Rice leaf lamina inclination assay of 25-methoxybrassinolide (**7**) vs Brassinolide (**1**) with and without IAA.

The brassinolide analogues **7**, **9**, and **10**, as well as 25-fluorocastasterone (**8**), were then subjected to a suitable bioassay. Several bioassays have been developed to measure brassinosteroid activity,[5a,17] but the rice leaf lamina inclination assay[18] is particularly sensitive, convenient, and reproducible and was, therefore, chosen for the present work. The leaf lamina angle in rice seedlings decreases from a control value of ca. 160−170° upon exposure to active brassinosteroids, and the angle can be conveniently plotted vs the logarithm of the brassinosteroid dose in nanograms. The results for compound **7** are shown in Figure 1, using brassinolide (**1**) as a standard for comparison. Auxins are known to synergize the effects of brassinosteroids,[5d,8,9,18] and the application of **1** with an auxin can yield a 10- to 30-fold increase in bioactivity relative to **1** alone. The present bioassay was therefore also conducted by the simultaneous application of an optimal amount (1000 ng) of indole-3-acetic acid (IAA) with the brassinosteroid.

Figure 1 reveals that the bioactivity of the 25-methoxy analogue **7** resembles that of brassinolide (**1**) across a wide dosage range, although **1** is 2- to 10-fold more potent, depending on the dose. When **7** was administered together with IAA, a synergistic effect was observed, resulting in 10- to 100-fold greater bioactivity at all but the highest dosage level[19] than produced by **1** alone. However, the combination of **1** with IAA produced the greatest synergistic effect. Thus, the introduction of a methoxy group at C-25 of brassinolide has only a slightly adverse effect upon its activity. Since it will be recalled that glucosylation at this position in the closely related 24-epi series comprises a principal deactivation pathway in plants, compound **7** is therefore a suitable candidate for further studies of enhanced persistence in field applications.

In contrast to our expectations, and to the strong bioactivity of **7**, the 25-fluoro analogues **8** and **9**, as well as 25-azabrassinolide (**10**), proved completely inactive at all doses studied. Moreover, coapplication with IAA also failed to produce any significant activity. It is possible

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(19) We have previously observed that the leaf lamina bending is partly reversed (i.e., the leaf lamina angle increases from its minimum value) when very high doses of certain other brassinosteroids are similarly applied, especially in the presence of IAA (see ref 9). The reason for this behavior is not known at this time.

that the introduction of fluorine or nitrogen heteroatoms at C-25 of brassinolide disrupt its recognition by a putative receptor in plant cells by, for instance, changing the conformation of the side chain (e.g., through intramolecular hydrogen bonding). An intriguing alternative explanation for the lack of bioactivity of **8**−**10** is that oxidation at C-25 of brassinosteroids to the corresponding 25-hydroxy derivatives is a prerequisite for biological activity[20] and that such activity is lost when this transformation is blocked. This in turn implies that brassinolide may not be the actual plant hormone, but is merely the precursor of an active 25-hydroxy metabolite.

Experimental Section

NMR spectra were obtained in deuteriochloroform unless otherwise noted, with residual chloroform used as the internal standard for ^{1}H and ^{13}C spectra. ^{19}F NMR spectra were recorded with hexafluorobenzene (δ −162.9 ppm) as the internal standard and are reported relative to $CFCl_3$ (δ 0.0 ppm). Flash chromatography was performed using Merck silica gel, 230−400 mesh. The mixture of epoxides **11a** and **11b** was prepared according to our previously reported procedure.[12] 2-Propenylmagnesium bromide was prepared in THF solution and was titrated prior to use with 1 M *sec*-butyl alcohol in xylene, using 1,10-phenanthroline as the indicator.[21] The rice leaf lamina assay was conducted by the same protocol as described earlier.[8,18] Typically, 36 rice plants were used for each data point in Figure 1, except at the highest doses of 1000 and 10000 ng, where 24 plants were used.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-6,6-(Ethylenedioxy)-2,3-(isopropylidenedioxy)-25-ergostene-22,23-diol (12). A solution of 2-propenylmagnesium bromide (10 mmol) in THF was added to a stirred suspension of CuCN (44 mg, 0.50 mmol) in 10 mL of THF at −78 °C. After 1 h, the mixture of epoxides **11a** and **11b** (500 mg, 0.991 mmol, 1.5:1) was added dropwise in 10 mL of THF. The reaction mixture was stirred for 1 h at −78 °C and 3.5 h at 0 °C, followed by the addition of 20% aqueous NH_4Cl solution, and extraction three times with ether. The organic extracts were washed twice with $NaHCO_3$ solution and once with NaCl solution, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography (elution with 45−60% ether−hexanes) afforded 89% of recovered *erythro*-epoxy alcohol **11b** and 250 mg (77%; based on the amount of *threo*-epoxide **11a** in the original mixture of epoxides) of the 22,23-diol **12** as a colorless oil: IR (KBr) 3442, 1640, 1228, 1055 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.93 (br s, 1 H), 4.81 (br s, 1 H), 4.29 (m, 1 H), 4.09 (m, 1 H), 3.92 (m, 3 H), 3.76 (m, 1 H), 3.60 (br s, 2 H), 1.78 (s, 3 H), 1.47 (s, 3 H), 1.32 (s, 3 H), 1.01 (d, J = 7.0 Hz, 3 H), 0.96 (d, J = 6.0 Hz, 3 H), 0.83 (s, 3 H), 0.68 (s, 3 H); ^{13}C NMR (100 MHz) δ 147.9, 111.6, 109.7, 107.5, 73.7, 73.0, 72.9, 72.8, 65.5, 64.1, 55.8, 52.9, 52.4, 45.5, 42.7, 42.3, 41.8, 41.0, 39.7, 38.0, 37.5, 32.9, 28.6, 27.7, 26.5, 24.0, 22.1, 22.0, 20.8, 13.4, 12.2, 11.9, 11.7; mass spectrum *m*/*z* (relative intensity) 546 (M^+, 10), 335 (100); exact mass calcd for $C_{33}H_{54}O_6$ 546.3920, found 546.3947.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-6,6-(Ethylenedioxy)-2,3,22,23-bis(isopropylidenedioxy)-25-ergostene-22,23-diol (13). 2,2-Dimethoxypropane (0.41 mL, 3.3 mmol), followed by *p*-toluenesulfonic acid (8.4 mg, 0.04 mmol), was added to a solution of compound **12** (183 mg, 0.335 mmol) in dichloromethane, at room temperature. After 20 min, 15 mg of K_2CO_3 was added, and the solution was allowed to stir for another 5 min. The mixture was poured into water and extracted three times with dichloromethane, and the organic extracts were washed with $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography (elution with 10% ethyl acetate−hexane) provided a quantitative yield of **13** as a colorless oil: IR (KBr) 1229, 1053 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.79 (br s, 1 H), 4.76 (br s, 1 H), 4.29 (m, 1 H), 4.11 (m, 1 H), 3.92 (m, 4 H), 3.68 (m, 2 H), 1.66 (s, 3 H), 1.48 (s, 3 H), 1.38 (s, 3 H), 1.36 (s, 3 H), 1.33 (s, 3 H), 1.13 (d, J = 6.9 Hz, 3 H), 0.94 (d, J = 5.8 Hz, 3 H), 0.83 (s, 3 H), 0.62 (s, 3 H). This compound was used in subsequent reactions without further purification.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-25-Methoxy-6,6-(ethylenedioxy)-2,3;22,23-bis(isopropylidenedioxy)ergostane-22,23-diol (14). Compound **13** (100 mg, 0.170 mmol) in 1 mL of methanol was added to a vigorously stirred suspension of mercuric acetate (109 mg, 0.341 mmol) in 1 mL of methanol. The mixture was allowed to stir for 2 h at room temperature, followed by the addition of 2 mL of 3 M NaOH solution. After 5 min, 2 mL of 0.5 M $NaBH_4$ in 3 M NaOH was added, and the mixture was stirred for an additional 2 h at room temperature. It was then filtered through Celite, and the filtrate was extracted three times with diethyl ether. The organic extracts were washed with $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography over silica gel (elution with 10% ethyl acetate−hexanes) afforded compound **14** (88 mg, 83%) as a colorless oil: IR (KBr) 1232, 1151, 1054 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.29 (m, 1 H), 4.02 (m, 5 H), 3.72 (m, 2 H), 3.16 (s, 3 H), 1.48 (s, 3 H), 1.33 (br s, 9 H), 1.13 (s, 6 H), 0.98 (d, J = 6.1 Hz, 3 H), 0.90 (d, J = 7.0 Hz, 3 H), 0.83 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (50 MHz) δ 109.7, 107.5, 80.0, 75.0, 72.9, 72.8, 65.5, 64.1, 55.7, 53.4, 52.9, 48.6, 45.5, 42.7, 42.4, 41.0, 40.3, 39.5, 38.0, 35.1, 32.9, 28.6, 27.9, 27.3, 27.2, 26.5, 24.0, 22.8, 22.0, 20.7, 13.3, 12.5, 11.8, 8.7; mass spectrum *m*/*z* (relative intensity) 618 (M^+, 0.2), 73 (100); exact mass calcd for $C_{37}H_{62}O_7$ 618.4496, found 618.4496.

25-Methoxybrassinolide (7). Aqueous hydrogen peroxide (0.19 mL of a 30% solution, ca. 2 mmol) was slowly added to trifluoroacetic anhydride (1.54 mL, 10.9 mmol) at 0 °C, and the mixture was stirred for 30 min. In a separate vessel, trifluoroacetic acid (1.3 mL) was added to a solution of **14** (87 mg, 0.14 mmol) in 5 mL of chloroform. The latter solution was stirred at room temperature for 40 min and was then added slowly to the pregenerated trifluoroperoxyacetic acid solution at 0 °C, followed by warming to room temperature and stirring for an additional 1.5 h. The mixture was diluted with chloroform, washed twice with 10 mL of water and twice with 10 mL of 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography (elution with 5−10% methanol−chloroform) provided 52 mg (72%) of a mixture of **7** and its 6-oxa-7-oxo regioisomer in a ratio of 8:1 (NMR integration). Recrystallization from methanol afforded 40 mg (56%) of pure **7**: mp 228−230 °C; IR (KBr) 3481, 1707, 1132, 1066 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.03 (m, 4 H) 3.73 (m, 1 H), 3.56 (br d, J = 7.0 Hz, 1 H), 3.23 (s, 3 H), 3.13 (dd, J = 11.9, 4.5 Hz, 1 H), 1.33 (s, 3 H), 1.22 (s, 3 H), 0.96 (d, J = 6.9 Hz, 3 H), 0.93 (s, 3 H), 0.92 (d, J = 6.3 Hz, 3 H), 0.71 (s, 3 H); mass spectrum *m*/*z* (relative intensity) 442 (17), 40 (100). Anal. Calcd for $C_{29}H_{50}O_7$: C, 68.20; H, 9.87. Found: C, 68.14; H, 9.88.

25-Fluorocastasterone (8). Alkene **13** (175 mg, 0.298 mmol) in 5 mL of THF was slowly added to 11 mL of a solution of 70% HF−pyridine in a plastic vessel under nitrogen at 0 °C. The mixture was allowed to stir for 1 h, followed by extraction three times with chloroform. The combined organic extracts were washed twice with water and twice with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to dryness. The white solid was chromatographed over silica gel (elution with 10% methanol−chloroform) to furnish 125 mg (88%) of compound **8**: mp 318−320 °C; IR (KBr) 3483, 1693, 1226 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.06 (m, 1 H), 3.97 (br d, J = 8.7 Hz, 1 H), 3.77 (m, 1 H), 3.59 (br d, J = 8.5 Hz, 1 H), 2.70 (dd, J = 12.5, 3.4 Hz, 1 H), 1.36 (d, J = 9.3 Hz, 3 H), 1.25 (d, J = 7.0 Hz, 3 H), 0.96 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 7.1 Hz, 3 H), 0.77 (s, 3 H), 0.69 (s, 3 H); ^{19}F NMR (400 MHz,

(20) It will be recalled that the 25-hydroxy analogue **5** has greater activity than its biosynthetic precursor **3** in the 24-epi series (see ref 4a). This too is consistent with, but does not prove, the hypothesis that hydroxylation at C-25 is required to activate brassinosteroids. It is also interesting that O-methylation of the 25-hydroxyl group (i.e., as in **7**) has little adverse effect upon the bioactivity, as was also observed previously with the corresponding 22,23-di-O-methylated analogue (ref 8).

(21) Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165.

$CDCl_3-C_6F_6$) δ −142.0 (m, 1 F). The poor solubility and low volatility of **8** necessitated its conversion into the corresponding tetraacetate (treatment with acetic anhydride and DMAP in pyridine for 10 h at room temperature) for further spectroscopic characterization: ^{13}C NMR (50 MHz) 210.3, 170.4, 170.2, 170.1, 169.9, 97.8, 75.2, 71.1, 69.0, 68.1, 56.5, 53.6, 52.3, 51.7, 46.3, 42.7, 42.6, 42.4, 42.3, 39.2, 37.5, 37.0, 28.1, 25.0, 24.8, 24.4, 24.2, 23.7, 21.1, 21.0, 20.9, 20.7, 13.5, 12.7, 11.8, 8.7, 8.6; mass spectrum *m*/*z* (relative intensity) 631 (M^+ − F, 0.1), 630 (M^+ − HF, 0.05), 590 (M^+ − AcOH, 5), 387 (100); exact mass calcd for $C_{36}H_{55}O_9$ (M^+ − F) 631.3846, found 631.3875.

25-Fluorobrassinolide (9). A solution of compound **8** (88 mg, 0.18 mmol) in 5 mL of chloroform was added slowly to pregenerated trifluoroperoxyacetic acid solution (as in the preparation of **7**) at 0 °C, followed by warming to room temperature and stirring for an additional 2 h. The mixture was diluted with chloroform, washed twice with 2 mL of water and twice with 2 mL of 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$), and concentrated under vacuum. The resulting white solid was chromatographed over silica gel (elution with 5−10% methanol−chloroform) to provide 66 mg (73%) of a mixture of **9** and its 6-oxa-7-oxo regioisomer in a ratio of 8:1 (NMR integration). Recrystallization from methanol−dichloromethane solution afforded 52 mg (57%) of pure **9**: mp 339−341 °C; IR (KBr) 3469, 1695, 1232 cm^{-1}; ^{1}H NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (br s, 1 H), 3.97 (br d, J = 8.4 Hz, 1 H), 3.72 (m, 1 H), 3.58 (br d, J = 9.0 Hz, 1 H), 3.13 (dd, J = 12.2, 4.4 Hz, 1 H), 1.35 (d, J = 9.2 Hz, 3 H), 1.32 (d, J = 13.2 Hz, 3 H), 0.95 (d, J = 6.7 Hz, 3 H), 0.93 (s, 3 H), 0.86 (d, J = 7.1 Hz, 3 H), 0.72 (s, 3 H); ^{19}F NMR (400 MHz, $CDCl_3-C_6F_6$) δ −141.1 (m). Product **9** was converted into the corresponding tetraacetate (treatment with acetic anhydride and DMAP in pyridine for 10 h at room temperature) for further spectroscopic characterization: ^{13}C NMR (100 MHz) 174.9, 170.4, 170.1, 170.0, 169.9, 97.8, 75.1, 71.1, 70.3, 68.9, 67.9, 58.3, 52.3, 51.3, 42.6, 42.5, 42.0, 39.4, 39.1, 38.8, 38.3, 37.1, 29.3, 28.0, 25.1, 24.9, 24.6, 24.4, 24.1, 22.2, 21.1, 21.0, 20.7, 15.4, 12.6, 11.6, 8.7, 8.6; mass spectrum *m*/*z* (relative intensity) 605 (1), 586 (M^+ − HF and AcOH, 13), 43 (100); exact mass calcd for $C_{34}H_{50}O_8$ (M^+ − HF and AcOH) 586.3506, found 586.3471.

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-25-Aza-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)ergostane-22,23-diol (15). A 2.0 M solution of dimethylamine in THF (3.0 mL, 6.0 mmol) was added slowly to a 2.0 M hexylmagnesium bromide solution (2.4 mL, 4.8 mmol), and the mixture was allowed to stir at 35−40 °C for 1 h. The mixture of epoxides **11a** and **11b** (600 mg, 1.19 mmol; 1.5:1) in THF was added to the reaction mixture at room temperature, and the solution was again warmed to 35−40 °C for 1 h. The solvent was evaporated, and the crude mixture was chromatographed over silica gel (elution with 67% ethyl acetate, 28% methanol ,and 5% triethylamine) to give 90% of recovered *erythro*-epoxy alcohol **11b** and a white solid residue. The residue was triturated with K_2CO_3 solution and extracted several times with ether, and the combined ether layers were dried ($MgSO_4$) and concentrated under vacuum to afford 375 mg of compound **15** (96%, based on *threo*-epoxide **11a**) as a colorless oil: IR (KBr) 3451, 1229 cm^{-1}; ^{1}H NMR (200 MHz) δ 4.29 (m, 1 H), 4.09 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 2 H), 3.58 (m, 1 H), 2.38 (m, 1 H), 2.36 (s, 6 H), 1.48 (s, 3 H), 1.33 (s, 3 H), 1.03 (d, J = 6.0 Hz, 3 H), 0.98 (d, J = 6.0 Hz, 3 H), 0.84 (s, 3 H), 0.68 (s, 3 H); ^{13}C NMR (100 MHz) δ 109.6, 107.4, 73.0, 72.8, 72.7, 71.6, 65.4, 64.0, 61.1, 55.7, 52.8, 52.2, 45.4, 42.6, 42.3, 42.2, 40.9, 39.6, 37.9, 37.8, 32.8, 28.5, 27.7, 26.5, 24.0, 21.9, 20.7, 13.3, 12.3, 11.8, 9.8; mass spectrum *m*/*z* (relative intensity) 549 (M^+, 1), 72 (100); exact mass calcd for $C_{32}H_{55}NO_6$ 549.4029, found 549.4043.

25-Azacastasterone Tetraacetate (16). Compound **15** (375 mg, 0.682 mmol) was treated with 5 mL of 80% aqueous acetic acid for 30 min. The mixture was concentrated under vacuum, and the residue was basified with 10% NaOH solution, followed by extraction five times with chloroform. The combined organic extracts were dried ($MgSO_4$) and evaporated under vacuum to give a white powder. This was acetylated with acetic anhydride (0.84 mL, 8.9 mmol) and DMAP (82 mg, 0.67 mmol) in pyridine for 5 h at room temperature. The mixture was poured into ice-cold 10% HCl solution, followed by extraction three times with chloroform. The combined organic layers were washed twice with saturated $NaHCO_3$ solution and once with brine, dried ($MgSO_4$), and evaporated to dryness. The crude product was chromatographed over silica gel (elution with 50% ethyl acetate−hexanes) to afford 247 mg (57%) of the tetraacetate **16** as a colorless oil: ^{1}H NMR (200 MHz) δ 5.39 (m, 1 H), 5.20 (m, 2 H), 4.96 (m, 1 H), 2.60 (m, 1 H), 2.24 (s, 6 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.03 (d, J = 6.7 Hz, 3 H), 1.01 (d, J = 6.7 Hz, 3 H), 0.83 (s, 3 H), 0.68 (s, 3 H). This compound was used directly in the next step.

25-Azabrassinolide Tetraacetate (17). A solution of compound **16** (245 mg, 0.387 mmol) in 10 mL of chloroform was added slowly to the pregenerated trifluoroperoxyacetic acid solution (as in the preparation of **7**) at 0 °C, followed by stirring at room temperature for an additional 2 h. The mixture was diluted with chloroform and washed twice with saturated $NaHCO_3$ solution, twice with water, and twice with 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$), and concentrated under vacuum. The product was chromatographed over silica gel (elution with 0−2% methanol−chloroform) to provide 209 mg (83%) of a mixture of **17** and its 6-oxa-7-oxo regioisomer in the ratio of 9:1 (NMR integration) as a colorless oil: IR (KBr) 1739, 1244, 1042, 1026 cm^{-1}; ^{1}H NMR (400 MHz) δ 5.37 (br s, 1 H), 5.20 (m, 1 H), 5.12 (br d, J = 7.7 Hz, 1 H), 4.89 (m, 1 H), 4.08 (m, 2 H), 3.00 (dd, J = 12.3, 4.4 Hz, 1 H), 2.65 (m, 1 H), 2.26 (s, 6 H), 2.11 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 2.00 (s, 3 H), 1.02 (two overlapping d, J = 6.8 Hz, 6 H), 0.99 (s, 3 H), 0.72 (s, 3 H); ^{13}C NMR (100 MHz) δ 175.0, 170.5, 170.3, 170.2, 169.9, 75.2, 72.5, 70.4, 68.9, 67.9, 59.4, 58.3, 52.7, 51.3, 42.5, 42.0, 41.1, 39.4, 39.1, 38.8, 38.4, 37.6, 29.3, 27.9, 24.7, 22.2, 21.1, 21.0, 20.8, 15.4, 13.3, 11.5, 9.2; mass spectrum *m*/*z* (relative intensity) 649 (M^+, <0.1), 634 (M^+ − CH_3, 4), 72 (100); exact mass calcd for $C_{35}H_{55}NO_{10}$ 649.3826, found 649.3772.

25-Azabrassinolide (10). The tetraacetate **17** (209 mg, 0.322 mmol) was refluxed in 30 mL of NaOH solution (prepared by dissolving 1.17 g of NaOH in 3 mL of water and 45 mL of methanol) for 2.5 h. THF (30 mL), followed by 11 mL of 6 N HCl solution, was added to the reaction mixture at room temperature, and stirring was continued for 2.5 h. The solvent was removed under vacuum, and the residue was basified with 10% NaOH solution. The mixture was extracted five times with 10% methanol−chloroform. The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness. The resulting white powder was rinsed with 20 mL of chloroform and dried under high vacuum to afford 121 mg (78%) of a mixture of **10** and its 6-oxa-6-oxo regioisomer in the ratio of 9:1 (NMR integration). Recrystallization from methanol−dichloromethane solution afforded 92 mg (60%) of pure **10**: mp 273−275 °C; IR (KBr) 3458, 1691, 1263, 1066 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3-CD_3OD$) δ 3.89 (m, 2 H), 3.82 (br s, 1 H), 3.61 (dd, J = 7.3, 2.6 Hz, 1 H), 3.49 (dd, J = 7.7, 2.6 Hz, 1 H), 3.35 (d, J = 7.2 Hz, 1 H), 3.01 (dd, J = 12.2, 4.5 Hz, 1 H), 2.29 (m, 1 H), 2.20 (s, 6 H), 0.89 (d, J = 6.7 Hz, 3 H), 0.81 (d, J = 6.6 Hz, 3 H), 0.78 (s, 3 H), 0.60 (s, 3 H); mass spectrum *m*/*z* (relative intensity) 481 (M^+, 1), 72 (100). Anal. Calcd for $C_{27}H_{47}NO_6$: C, 67.33; H, 9.84; N, 2.91. Found: C, 67.43; H, 10.05; N, 2.95.

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Supporting Information Available: The ^{1}H and ^{13}C NMR spectra of compounds **12**, **14**, **15**, and **17**, the ^{1}H NMR spectra of compounds **7**−**10**, and the ^{13}C NMR spectra of the tetraacetates of compounds **8** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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