A Facile Construction of Withanolide Side Chains: Synthesis of Minabeolide-3

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A stereocontrolled construction of withanolide side chains, withanolide R-type **17**, and 27-deoxywithaferin A-type **21**, was accomplished from 20-carboxaldehyde **6**. The key features of the construction are based on the stereoselective synthesis of the furylcarbinol **12** and its transformation into pyranone derivatives. Synthesis of minabeolide-3 **28** was achieved from the key intermediate **18**.

Withanolides, a group of highly oxygenated ergostane type steroids possessing a δ-lactone in the side chain, have been isolated from the plants of the *Solanaceae* family[1] and also marine organisms.[2] Due to their novel structures and interesting biological activities,[3] such as antitumor and insect antifeedant properties, withanolides have been recognized as attractive synthetic targets.[1,4] As part of our continuing studies on the synthesis of physiologically active steroidal side chains using furan and butenolide as synthons,[5] we herein report a new method for the construction of the withanolide side chain bearing a (22*R*)-δ-lactone moiety and its application to the synthesis of minabeolide-3, which was isolated from a soft coral, *Minabea* sp., recently.[2] Although several syntheses of withanolides have been published to date,[4] many steps have been required to construct an unsaturated δ-lactone moiety in the synthesis of the withaferin A-type side chain.[4a,b] Our synthetic strategy of withanolide side chains is based on the stereoselective synthesis of the (22*S*)-furylcarbinol **2**, which could be easily transformed into the 27-deoxywithaferin A type **5** via the pyranone **3** and the withanolide R type **4** (Figure 1).

Results and Discussion

We began to explore the stereocontrolled synthesis of the (22*S*)-furylcarbinol **11** as a model compound (Scheme I). Our previous result[5e] indicated that nucleophilic addition of 2-lithiofuran **7** to the 20-carboxaldehyde **6** could provide mainly the (22*R*)-isomer **9**, a Cram product. For the synthesis of withanolide side chains, it was necessary to prepare the anti-Cram product **11**, and this was attempted using Yamamoto's method.[6] Unfortunately, addition of 2-lithiofuran[7] to the aldehyde **6**[8] in the presence of methylaluminum bis(2,4,6-tri-*tert*-butylphenoxide)[6] proceeded with the same selectivity as the previous result to give the isomers **9** and **11** in a ratio of 2:1, respectively. Therefore, conversion of **9** to **11**[9] was examined by oxidation/reduction sequence via the acyl furan **13**. Alcohol **9** was oxidized with pyridinium chlorochromate (PCC)[5e] to give the ketone **13**. Several reducing agents were surveyed for the reduction of **13**, and the results are shown in Table I. Interestingly, the reductions proceeded with moderate Cram selectivity to afford the isomer **11** predominantly in a ratio of 1.8–3.8:1, although reduction of 22-oxosteroids having saturated side chains provides mainly anti-Cram products.[10] This selectivity agrees with the previous observation in the reduction of 22-oxo-Δ^{23}-steroids with L-Selectride.[11]

Table I. Reduction of 13 with Reducing Agents

reducing agent	ratio of products 9:11	% yield
DIBAL	1:1.8	91.2
$Zn(BH_4)_2$	1:2.2	86.9
L-Selectride	1:3.2	88.8
$LiAlH_4$	1:3.6	91.1
$NaBH_4$	1:3.7	90.0
Red-Al	1:3.8	93.1

On the basis of these results, we prepared the key intermediate **12** for the construction of the withanolide side chains as follows. Addition of 2-lithio-3,4-dimethylfuran **8**, prepared by lithiation of 3,4-dimethylfuran,[12] to the aldehyde **6** produced the furylcarbinols **10** and **12** in a ratio of 3.2:1 (79.5%). Oxidation of the alcohol **10** with PCC employed conditions similar to the oxidation of **9**; however, it gave a mixture of products. Several oxidation conditions, such as dimethyl sulfoxide (DMSO)–oxalyl chloride[13] and manganese dioxide,[14] were investigated, and we found that **10** was successfully oxidized with use of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[15] in dioxane to afford the desired acyl furan **14** in 61.4% yield. Reduction of the ketone **14** with lithium aluminum hydride in tetrahydrofuran (THF) proceeded with high diastereoselectivity to

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(9) Mitsunobu inversion of **9** resulted in formation of a complex mixture probably due to the instability of the 2-furylcarbinol system (Mitsunobu, O. *Synthesis* **1981**, 1).

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(11) Takahashi, T.; Ootake, A.; Yamada, H.; Tsuji, J. *Tetrahedron Lett.* **1984**, *25*, 69. Midland, M. M.; Kwon, Y. C. *Ibid.* **1984**, *25*, 5981.

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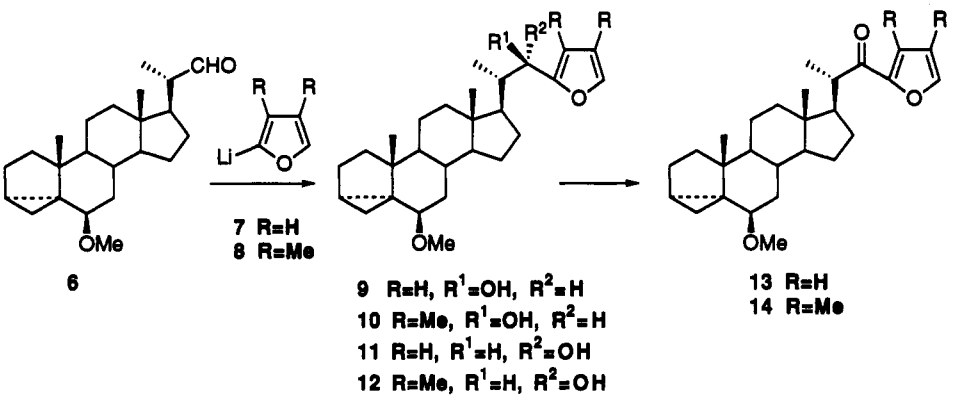
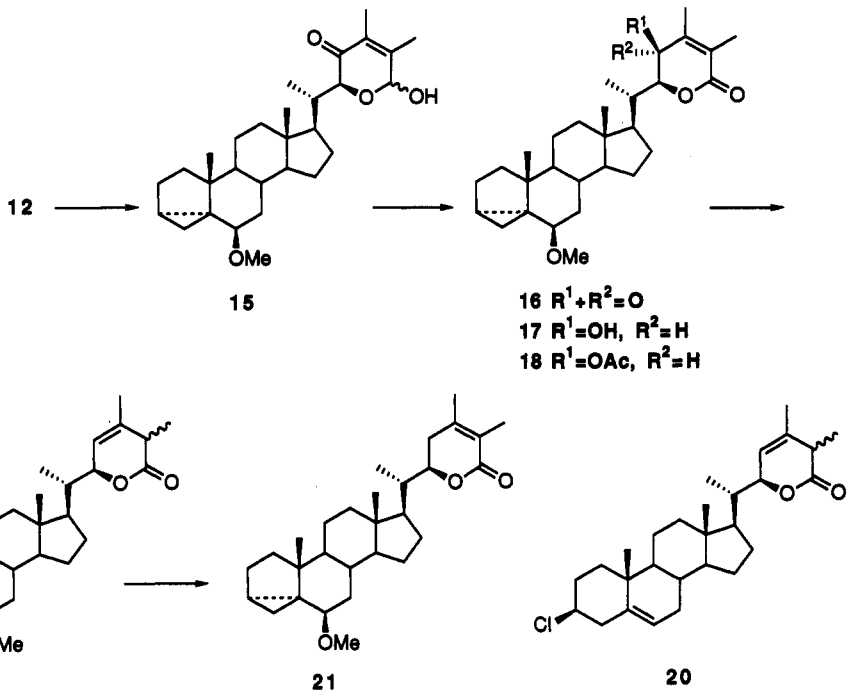
Scheme I



Scheme II



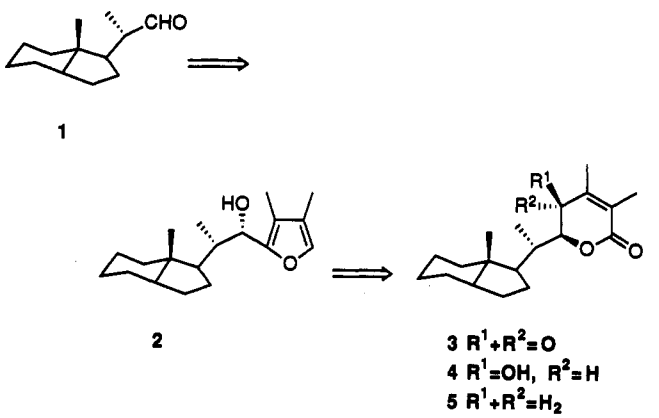


Figure 1.

furnish the desired (22*S*)-furylcarbinol **12** together with **10** in a ratio of 16:1.

With the requisite furylcarbinol **12** in hand, we embarked on the synthesis of the withanolide side chains as shown in Scheme II. Treatment of **12** with *N*-bromosuccinimide (NBS)[16] in aqueous THF brought about the ring enlargement to give the lactol **15** in 84.4% yield. Further oxidation of **15** with PCC provided the relatively unstable δ-lactone **16** which, without purification, was reduced with sodium borohydride to afford the alcohol **17**, the withanolide R-type side chain, as a sole product in 73.4% yield from **15**. The ^{1}H NMR spectrum of its acetate **18**, prepared by acetylation of the alcohol **17**, showed good accordance with that of withanolide R monoacetate.[17] The observed selectivity can be explained by assuming that the reduction of the ketone **16** would occur from the opposite side of steroid nucleus (Figure 2).

Figure 2.

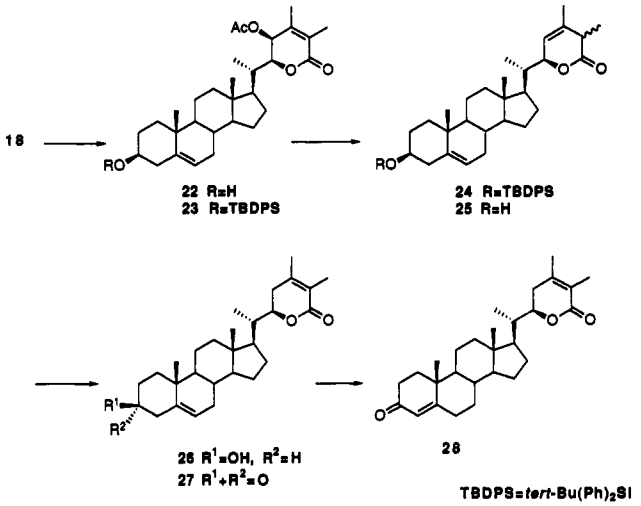
Reductive deacetoxylation of **18** with zinc–amalgam[18] in ethereal hydrogen chloride gave the olefin **19** in 20.8%

(16) Georgiadis, M. P.; Couladouros, E. A. *J. Org. Chem.* **1986**, *51*, 2725.

(17) Kirson, I.; Cohen, A.; Abraham, A. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2136.

(18) Corey, E. J.; Pyne, S. G.; Su, W.-g. *Tetrahedron Lett.* **1983**, *24*, 4883.

Scheme III



yield, together with the starting material (76.7%). Side reactions were observed under forced reaction conditions in which the cyclopropyl moiety of **18** was opened to produce the chloride **20** as a major product. Isomerization of the olefin **19** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[5b] furnished the α,β-unsaturated lactone **21**,[19] the 27-deoxywithaferin A side chain, whose spectroscopic data were identical with those reported.[4c]

Establishing a new method for the construction of the withanolide side chains, we next focused our attention on the synthesis of minabeolide-3 **28** from the lactone **18** (Scheme III). Due to the difficulty observed in the reductive deacetoxylation of **18**, ring opening of the cyclopropane in **18** was carried out prior to further transformation of the side-chain moiety. Acid treatment[20] of **18** with *p*-toluenesulfonic acid in aqueous dioxane afforded the olefinic alcohol **22** whose protection with *tert*-butylchlorodiphenylsilane[21] gave the silyl ether **23** quantitatively. Allyl acetate **23** was subjected to the reductive deacetoxylation to give the olefin **24** in 80% yield. Upon treatment with hydrogen fluoride,[22] **24** provided the alcohol **25** which was isomerized with DBU to the α,β-unsaturated lactone **26**. Finally, Swern oxidation of **26** gave the ketone **27** which was treated with oxalic acid in ethanol to afford minabeolide-3 **28** in 88.4% yield. The spectral data of the synthetic minabeolide-3 showed good agreement with those reported.[2]

Thus, we have developed a synthesis of the withanolide side chains by a facile method that could be applied to the synthesis of highly oxygenated withanolides.

Experimental Section

Melting points were measured with a Yanagimoto MP apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrophotometer. ^{1}H NMR spectra were obtained for solutions in $CDCl_3$ on a JEOL GSX-270 instrument, and chemical shifts are reported on the δ-scale from internal TMS. Mass spectra were measured with a JEOL JMS D-300 spectrometer. Optical rotations were taken with a JASCO DIP-360 polarimeter.

Addition of 2-Lithiofuran to the 20-Carboxaldehyde 6 with MAT. To a stirred solution of methylaluminum bis(2,4,6-tri-*tert*-butylphenoxide), prepared from 2,4,6-tri-*tert*-butylphenol (944 mg, 3.49 mmol) and Me_3Al (1.76 mL of a 0.99 M hexane solution, 1.74 mmol) in CH_2Cl_2 (12 mL) according to Yamamoto's method,[6] was added a solution of the aldehyde **6**[8] (200 mg, 0.58 mmol) in THF (3 mL) followed by 2-lithiofuran,[7] prepared from furan (0.17 mL, 2.32 mmol) and *n*-BuLi (1.13 mL of a 1.65 M hexane solution, 1.86 mmol) in THF (0.5 mL), at −78 °C. The reaction mixture was stirred for 2 h at room temperature, and aqueous saturated NH_4Cl solution was added. The product was extracted with CH_2Cl_2, and the organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (16:1, v/v) as eluent to afford the (22*R*)-alcohol **9** (110 mg, 45.5%) as colorless needles: mp 134–136 °C (hexane–AcOEt); IR ν_{max} 3360 cm^{-1}; ^{1}H NMR δ 0.77 (s, 3 H, 18-CH_3), 0.88 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 2.78 (t, J = 3.0 Hz, 1 H, 6-CH), 3.33 (s, 3 H, OCH_3), 4.86 (br s, 1 H, 22-CH), 6.21 (d, J = 3.1 Hz, 1 H, 24-CH), 6.33 (dd, J = 1.8 and 3.1 Hz, 1 H, 25-CH), 7.35 (d, J = 1.8 Hz, 1 H, 26-CH); HRMS calcd for $C_{27}H_{40}O_3$ (M$^+$) 412.2993, found (M$^+$) 412.2997. Anal. Calcd for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found C, 78.59; H, 9.92. The further elution gave the (22*S*)-alcohol **11** (53 mg, 21.9%) as colorless needles: mp 139–141 °C (hexane–AcOEt); IR ν_{max} 3350 cm^{-1}; ^{1}H NMR δ 0.76 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.02 (d, J = 6.1 Hz, 3 H, 21-CH_3), 2.76 (t, J = 2.7 Hz, 1 H, 6-CH), 3.32 (s, 3 H, OCH_3), 4.81 (d, J = 3.7 Hz, 1 H, 22-CH), 6.21 (d, J = 3.1 Hz, 1 H, 24-CH), 6.33 (dd, J = 1.8 and 3.1 Hz, 1 H, 25-CH), 7.35 (d, J = 1.8 Hz, 1 H, 26-CH); HRMS calcd for $C_{27}H_{40}O_3$ (M$^+$) 412.2993, found (M$^+$) 412.2975. Anal. Calcd for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found C, 78.46; H, 9.91.

(20*S*,23*Z*,25*Z*)-23,26-Epoxy-6β-methoxy-3α,5-cyclo-27-nor-5α-cholesta-23,25-dien-22-one (13). To a stirred suspension of PCC (56 mg, 0.26 mmol), Celite (60 mg), and anhydrous sodium acetate (22 mg, 0.26 mmol) in CH_2Cl_2 (0.2 mL) was added a solution of **9** in CH_2Cl_2 (0.4 mL) at room temperature. Stirring was continued for 1 h, and the reaction mixture was diluted with Et_2O (5 mL). The organic layer was decanted and evaporated to give a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (20:1, v/v) as eluent to afford the ketone **13** (25.3 mg, 70.9%) as a colorless gum: IR ν_{max} 1660 cm^{-1}; ^{1}H NMR δ 0.83 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 1.22 (d, J = 6.7 Hz, 3 H, 21-CH_3), 2.77 (t, J = 2.7 Hz, 1 H, 6-CH), 3.26 (dq, J = 6.7 and 10.4 Hz, 1 H, 20-CH), 3.32 (s, 3 H, OCH_3), 6.53 (dd, J = 1.8 and 3.7 Hz, 1 H, 25-CH), 7.20 (d, J = 3.7 Hz, 1 H, 26-CH), 7.60 (d, J = 1.8 Hz, 1 H, 24-CH); HRMS calcd for $C_{27}H_{38}O_3$ (M$^+$) 410.2821, found (M$^+$) 410.2822.

Reduction of the Ketone 13. General Procedure. To a solution of **13** (50 mg, 0.12 mmol) in appropriate solvent was added reducing agent at −78 °C under argon atmosphere. The reaction was warmed to room temperature, and general workup was performed. Purification of the crude product by column chromatography on silica gel using hexane–AcOEt (10:1, v/v) as eluent afforded a mixture of the isomeric alcohols **9** and **11**. Ratio of the products was estimated by the ^{1}H NMR spectrum of the mixture.

DIBAL Reduction of 13. Reduction of **13** with diisobutylaluminum hydride (DIBAL) (0.24 mL of a 1 M toluene solution, 0.24 mmol) in toluene (5 mL) was performed as above to afford a mixture of **9** and **11** (45.8 mg, 91.2%) in a ratio of 1:1.8.

$Zn(BH_4)_2$ Reduction of 13. Reduction of **13** with zinc borohydride [$Zn(BH_4)_2$] (2.73 mL of a 0.14M Et_2O solution, 0.38 mmol) in Et_2O (5 mL) was performed as above to afford a mixture of **9** and **11** (43.6 mg, 86.9%) in a ratio of 1:2.2.

L-Selectride Reduction of 13. Reduction of **13** with L-Selectide (0.24 mL of a 1 M THF solution, 0.24 mmol) in THF (5 mL) was performed as above to afford a mixture of **9** and **11** (44.6 mg, 88.8%) in a ratio of 1:3.2.

$LiAlH_4$ Reduction of 13. Reduction of **13** with lithium aluminum hydride ($LiAlH_4$) (7 mg, 0.18 mmol) in THF (2 mL) was performed as above to afford a mixture of **9** and **11** (45.8 mg, 91.1%) in a ratio of 1:3.6.

$NaBH_4$ Reduction of 13. Reduction of **13** with sodium borohydride ($NaBH_4$) (7 mg, 0.18 mmol) in MeOH (5 mL) was performed as above to afford a mixture of **9** and **11** (45.2 mg, 90.0%) in a ratio of 1:3.7.

Red-Al Reduction of 13. Reduction of **13** with Red-Al (0.04 mL of a 3.4 M toluene solution, 0.12 mmol) in toluene (2 mL) was

(19) Attempt to convert **17** into **21** employing Barton–McCombie's deoxygenation reaction was unsuccessful (Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574).

(20) Partridge, J. J.; Faber, S.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 764.

(21) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *55*, 2975.

(22) Newton, R. F.; Reynolds, D. P. *Tetrahedron Lett.* **1979**, 3981.

performed as above to afford a mixture of **9** and **11** (46.8 mg, 93.1%) in a ratio of 1:3.8.

Addition of 2-Lithio-3,4-dimethylfuran to the 20-Carboxaldehyde 6. To a stirred solution of 2-lithio-3,4-dimethylfuran, prepared from 3,4-dimethylfuran[12] (3.15 g, 32.8 mmol) and *n*-BuLi (18 mL of a 1.64 M hexane solution, 29.5 mmol) in THF (40 mL), was added a solution of the aldehyde **6** (5.63 g, 16.4 mmol) in THF (40 mL) at −78 °C. The reaction mixture was warmed to room temperature, and aqueous saturated NH_4Cl solution was added. Concentration of the solvent afforded an oil, which was extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (16:1, v/v) as eluent to afford the (22*R*)-alcohol **10** (4.38 g, 60.7%) as a colorless gum: $[\alpha]^{24}_D$ +32.2° (*c* 1.20, $CHCl_3$); IR ν_{max} 3370 cm^{-1}; ^1H NMR δ 0.73 (s, 3 H, 18-CH_3), 0.98 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.92 and 1.93 (2 × s, 2 × 3 H, $CH_3C{=}CCH_3$), 2.78 (t, J = 3.1 Hz, 1 H, 6-CH), 3.33 (s, 3 H, OCH_3), 4.83 (d, J = 2.4 Hz, 1 H, 22-CH), 7.08 (br s, 1 H, C=CH); HRMS calcd for $C_{29}H_{44}O_3$ (M^+) 440.3289, found (M^+) 440.3287. The further elution gave the (22*S*)-alcohol **12** (1.36 g, 18.8%) as colorless needles: $[\alpha]^{24}_D$ +43.6° (*c* 1.05, $CHCl_3$); mp 128–129 °C (hexane–AcOEt); IR ν_{max} 3450 cm^{-1}; ^1H NMR δ 0.77 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.13 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.92 (d, J = 1.8 Hz, 3 H, C=CCH_3), 1.96 (s, 3 H, C=CCH_3), 2.76 (t, J = 3.1 Hz, 1 H, 6-CH), 3.32 (s, 3 H, OCH_3), 4.72 (d, J = 4.3 Hz, 1 H, 22-CH), 7.09 (d, J = 1.8 Hz, 1 H, C=CH); HRMS calcd for $C_{29}H_{44}O_3$ (M^+) 440.3289, found (M^+) 440.3289.

Oxidation of the Alcohol 10. To a stirred suspension of DDQ (12.9 mg, 0.05 mmol) in dioxane (0.3 mL) was added a solution of **10** (20 mg, 0.045 mmol) in dioxane (0.5 mL) under argon at room temperature. Stirring was continued for 30 min, and the solvent was concentrated to give a residue. The residue was diluted with CH_2Cl_2, and the precipitate was filtered off. Evaporation of the filtrate afforded a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (20:1, v/v) as eluent to afford the ketone **14** (12.2 mg, 61.4%) as colorless plates: $[\alpha]^{21}_D$ +57.6° (*c* 1.47, $CHCl_3$); mp 161–161.5 °C (hexane–AcOEt); IR ν_{max} 1660 cm^{-1}; ^1H NMR δ 0.82 (s, 3 H, 18-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.17 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.97 (d, J = 1.2 Hz, 3 H, C=CCH_3), 2.30 (s, 3 H, C=CCH_3), 2.77 (t, J = 3.1 Hz, 1 H, 6-CH), 3.32 (s, 3 H, OCH_3), 3.41 (dq, J = 6.7 and 10.4 Hz, 1 H, 20-CH), 7.22 (br s, 1 H, C=CH); HRMS calcd for $C_{29}H_{42}O_3$ (M^+) 438.3132, found (M^+) 438.3130. Anal. Calcd for $C_{29}H_{42}O_3$: C, 79.40; H, 9.65. Found: C, 79.15; H, 9.83.

Reduction of the Ketone 14. To a stirred suspension of lithium aluminum hydride (768 mg, 20 mmol) in THF (20 mL) was added a solution of **14** (1.77 g, 4 mmol) in THF (40 mL) at −78 °C under argon atmosphere. The reaction was warmed to room temperature, and aqueous 1 M sodium hydroxide solution was added slowly. The precipitate was filtered off, and the filtrate was concentrated to give a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (10:1, v/v) as eluent, affording the isomeric alcohols **10** (104 mg, 5.8%) and **12** (1.67 g, 93.8%).

NBS Oxidation of the Alcohol 12. To a solution of **12** (1.15 g, 2.61 mmol) and anhydrous sodium acetate (235 mg, 2.87 mmol) in aqueous THF (15 mL, THF:H_2O = 4:1) was added portionwise *N*-bromosuccinimide (512 mg, 2.87 mmol) at 0 °C, and stirring was continued for 30 min. After addition of aqueous 10% KI solution and then aqueous saturated sodium thiosulfate solution to the reaction mixture, the product was extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (20:1, v/v) as eluent to afford the lactol **15** (1.0 g, 84.4%) as a colorless gum: IR ν_{max} 3380, 1670 cm^{-1}; ^1H NMR δ 0.73 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.02 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.77 and 1.96 (2 × br s, 2 × 3 H, $CH_3C{=}CCH_3$), 2.77 (t, J = 2.4 Hz, 1 H, 6-CH), 3.20 (br s, 1 H, OH), 3.32 (s, 3 H, OCH_3), 3.9–4.45 (m, 1 H, 22-CH), 5.44 (br s, 1 H, C*H*OH); HRMS calcd for $C_{29}H_{44}O_4$ (M^+) 456.3240, found (M^+) 456.3245. Anal. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found C, 76.52; H, 9.74.

(20*S*,22*S*,23*S*)-23-Hydroxy-6β-methoxy-3α,5-cyclo-5α-with-24-enolide (17). To a stirred suspension of PCC (1.39 g, 6.45 mmol), Celite (1 g), and anhydrous sodium acetate (530 mg, 6.45 mmol) in CH_2Cl_2 (5 mL) was added a solution of **15** (980 mg, 2.15 mmol) in CH_2Cl_2 (7 mL) at room temperature. Stirring was continued for 2 h, and the reaction mixture was diluted with Et_2O (60 mL). The organic layer was decanted and evaporated to give a residue. A small amount of the crude product was purified by column chromatography on silica gel using hexane–AcOEt (20:1, v/v) as eluent to afford the lactone **16** as a colorless gum: IR ν_{max} 1715, 1680 cm^{-1}; ^1H NMR δ 0.71 (s, 3 H, 18-CH_3), 0.96 (d, J = 6.7 Hz, 3 H, 21-CH_3), 0.99 (s, 3 H, 19-CH_3), 1.96 and 2.15 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.75 (t, J = 2.4 Hz, 1 H, 6-CH), 3.30 (s, 3 H, OCH_3), 4.85 (d, J = 2.5 Hz, 1 H, 22-CH); HRMS calcd for $C_{29}H_{42}O_4$ (M^+) 454.3082, found (M^+) 454.3077. The crude product obtained above was used without further purification due to the instability of **16**. To a solution of **16** in MeOH (8 mL) was added portionwise sodium borohydride (205 mg, 5.38 mmol) at 0 °C, and the reaction mixture was stirred for 30 min. Aqueous saturated NH_4Cl solution was added slowly, and the solvent was concentrated. The product was extracted with AcOEt, and the organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (4:1, v/v) as eluent to afford the title alcohol **17** (723 mg, 73.4% from **15**) as a colorless gum: $[\alpha]^{22}_D$ +71.2° (*c* 0.13, $CHCl_3$); IR ν_{max} 3400, 1700 cm^{-1}; ^1H NMR δ 0.78 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.21 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.90 and 2.04 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.78 (t, J = 2.4 Hz, 1 H, 6-CH), 3.33 (s, 3 H, OCH_3), 3.97 (br s, 1 H, 23-CH), 4.20 (dd, J = 1.8 and 6.1 Hz, 1 H, 22-CH); HRMS calcd for $C_{29}H_{44}O_4$ (M^+) 456.3240, found (M^+) 456.3243. Anal. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 75.78; H, 9.71.

(20*S*,22*S*,23*S*)-23-Acetoxy-6β-methoxy-3α,5-cyclo-5α-with-24-enolide (18). A mixture of the alcohol **17** (120 mg, 0.26 mmol), acetic anhydride (75 μL, 0.79 mmol), 4-(dimethylamino)pyridine (3.2 mg, 0.03 mmol), and pyridine (85 μL, 1.05 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C for 1 h. The reaction mixture was poured into water, and the product was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (7:1, v/v) as eluent to afford the acetate **18** (125 mg, 95.4%) as colorless plates: $[\alpha]^{23}_D$ +179.5° (*c* 2.72, $CHCl_3$); mp 194–195 °C (hexane–AcOEt); IR ν_{max} 1730, 1715 cm^{-1}; ^1H NMR δ 0.75 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.06 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.94 and 1.95 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.09 (s, 3 H, OAc), 2.77 (t, J = 2.4 Hz, 1 H, 6-CH), 3.32 (s, 3 H, OCH_3), 4.36 (dd, J = 1.8 and 5.5 Hz, 1 H, 22-CH), 5.47 (d, J = 1.8 Hz, 1 H, 23-CH); HRMS calcd for $C_{31}H_{46}O_5$ (M^+) 498.3344, found (M^+) 498.3339. Anal. Calcd for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30. Found: C, 74.84; H, 9.50. The ^1H NMR spectrum of **18** in pyridine-d_5 showed good accordance with that of withanolide R monoacetate:[15] ^1H NMR (pyridine-d_5) δ 0.66 (s, 3 H, 18-CH_3), 1.15 (s, 3 H, 19-CH_3), 1.19 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.92 and 1.96 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.16 (s, 3 H, OAc), 2.74 (br s, 1 H, 6-CH), 3.31 (s, 3 H, OCH_3), 4.56 (dd, J = 1.8 and 5.5 Hz, 1 H, 22-CH), 5.77 (br s, 1 H, 23-CH).

(20*S*,22*R*,25ξ)-6β-Methoxy-3α,5-cyclo-5α-with-23-enolide (19). To a suspension of zinc amalgam (79 mg, 1.2 mmol) and **18** (30 mg, 0.06 mmol) in Et_2O (2 mL) was added hydrogen chloride (47 μL of a 2.5 M Et_2O solution, 0.12 mmol) over 15 min at −15 °C under argon atmosphere, and the reaction was warmed to room temperature. The inorganic material was filtered off, and the organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (7:1, v/v) as eluent to afford the olefin **19** (5.5 mg, 20.8%) as a colorless gum: IR ν_{max} 1725 cm^{-1}; ^1H NMR δ 0.76 (s, 3 H, 18-CH_3), 0.86 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.41 (d, J = 7.3 Hz, 3 H, 27-CH_3), 1.59 (s, 3 H, 28-CH_3), 2.78 (t, J = 2.4 Hz, 1 H, 6-CH), 2.85–3.0 (m, 1 H, 25-CH), 3.33 (s, 3 H, OCH_3), 4.91 (br s, 1 H, 22-CH), 5.50 (br s, 1 H, 23-CH); HRMS calcd for $C_{29}H_{44}O_3$ (M^+) 440.3291, found (M^+) 440.3292. The further elution gave starting material **18** (23 mg, 76.7%).

(20*S*,22*R*,25ξ)-3β-Chlorowitha-5,23-dienolide (20). Reaction of **18** with hydrogen chloride (3.5 M Et_2O solution) was performed as above to afford the chloride **20** (95%) as a colorless gum: IR ν_{max} 1720 cm^{-1}; ^1H NMR δ 0.72 (s, 3 H, 18-CH_3), 0.86 (d, J = 6.7

Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.41 (d, J = 7.3 Hz, 3 H, 27-CH_3), 1.79 (s, 3 H, 28-CH_3), 2.85–3.0 (m, 2 H, 7-CH_2), 3.7–3.85 (m, 1 H, 3-CH), 4.91 (br s, 1 H, 22-CH), 5.37 (d, J = 5.5 Hz, 1 H, 6-CH), 5.51 (br s, 1 H, 23-CH); HRMS calcd for $C_{28}H_{41}O_2Cl$ (M^+) 444.2793, found (M^+) 444.2787. Anal. Calcd for $C_{28}H_{41}O_2Cl$: C, 75.56; H, 9.29. Found: C, 75.97; H, 9.65.

(20*S*,22*R*)-6β-Methoxy-3α,5-cyclo-5α-with-24-enolide (21). A mixture of the olefin **19** (5.5 mg, 0.01 mmol) and DBU (1 μL, 0.006 mmol) in THF (0.1 mL) was stirred at room temperature for 2 h. The reaction mixture was poured into water and the product was extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (7:1, v/v) as eluent to afford the title lactone **21** (5 mg, 90.9%) as a colorless gum: IR ν_{max} 1700 cm^{-1}; ^1H NMR δ 0.75 (s, 3 H, 18-CH_3), 1.01 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.88 and 1.94 (2 × s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.35–2.55 (m, 2 H, 23-CH_2), 2.77 (t, J = 3.1 Hz, 1 H, 6-CH), 3.32 (s, 3 H, OCH_3), 4.38 (dt, J = 3.7 and 13.4 Hz, 1 H, 22-CH); HRMS calcd for $C_{29}H_{44}O_3$ (M^+) 440.3291, found (M^+) 440.3293. The spectroscopic data of **21** were identical with those reported.[4c]

(20*S*,22*S*,23*S*)-23-Acetoxy-3β-hydroxywitha-5,24-dienolide (22). A mixture of **18** (50 mg, 0.1 mmol) and a catalytic amount of *p*-toluenesulfonic acid in aqueous dioxane (1 mL, dioxane:H_2O = 3:1) was heated at 80 °C for 1 h. The reaction mixture was poured into water, and the product was extracted with CH_2Cl_2. The organic layer was washed with aqueous saturated sodium bicarbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (2:1, v/v) as eluent afforded the alcohol **22** (48.5 mg, 99.8%) as a colorless amorphous: $[\alpha]^{21}_D$ +129.1° (*c* 0.32, $CHCl_3$); mp 251–252 °C (hexane–CH_2Cl_2); IR ν_{max} 3450, 1745, 1720 cm^{-1}; ^1H NMR δ 0.71 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.07 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.93 and 1.94 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.09 (s, 3 H, OAc), 3.45–3.6 (m, 1 H, 3-CH), 4.35 (dd, J = 2.4 and 5.5 Hz, 1 H, 22-CH), 5.34 (d, J = 5.5 Hz, 1 H, 6-CH), 5.46 (d, J = 2.4 Hz, 1 H, 23-CH); HRMS calcd for $C_{30}H_{44}O_5$ (M^+) 484.3187, found (M^+) 484.3192. Anal. Calcd for $C_{30}H_{44}O_5$: C, 74.34; H, 9.15. Found: C, 74.23; H, 9.36.

(20*S*,22*S*,23*S*)-23-Acetoxy-3β-[(*tert*-butyldiphenylsilyl)oxy]witha-5,24-dienolide (23). A mixture of **22** (49 mg, 0.1 mmol), *tert*-butylchlorodiphenylsilane (53 μL, 0.2 mmol), triethylamine (35 μL, 0.25 mmol), and 4-(dimethylamino)pyridine (1.3 mg, 0.01 mmol) in CH_2Cl_2 (1 mL) was refluxed for 6 h. The reaction mixture was poured into water, and the product was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (15:1, v/v) as eluent to afford **23** (72.5 mg, 99.2%) as a colorless gum: $[\alpha]^{23}_D$ +65.6° (*c* 0.38, $CHCl_3$); IR ν_{max} 1730, 1700 cm^{-1}; ^1H NMR δ 0.68 (s, 3 H, 18-CH_3), 0.98 (s, 3 H, 19-CH_3), 1.04 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.06 (s, 9 H, *t*-Bu), 1.92 and 1.93 (2 × br s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.06 (s, 3 H, OAc), 3.45–3.6 (m, 1 H, 3-CH), 4.33 (dd, J = 1.8 and 5.5 Hz, 1 H, 22-CH), 5.11 (d, J = 4.9 Hz, 1 H, 6-CH), 5.43 (d, J = 1.8 Hz, 1 H, 23-CH), 7.3–7.45 (m, 6 H, Ph), 7.65–7.7 (m, 4 H, Ph). Anal. Calcd for $C_{46}H_{62}O_5Si$: C, 76.41; H, 8.64. Found: C, 76.40; H, 8.76.

(20*S*,22*R*,25ξ)-3β-[(*tert*-Butyldiphenylsilyl)oxy]witha-5,23-dienolide (24). Reductive deacetoxylation of **23** was performed using the procedure described for **19** to afford the olefin **24** (80.0%) as a colorless gum: IR ν_{max} 1720 cm^{-1}; ^1H NMR δ 0.69 (s, 3 H, 18-CH_3), 0.83 and 0.84 (2 × d, 2 × J = 6.7 Hz, 3 H, 21-CH_3), 0.99 (s, 3 H, 19-CH_3), 1.06 (s, 9 H, *t*-Bu), 1.34 and 1.40 (2 × d, 2 × J = 7.3 Hz, 3 H, 27-CH_3), 1.77 (s, 3 H, 28-CH_3), 2.85–3.0 (m, 1 H, 25-CH), 3.45–3.6 (m, 1 H, 3-CH), 4.85–5.0 (m, 1 H, 22-CH), 5.12 (d, J = 4.9 Hz, 1 H, 6-CH), 5.4–5.5 (m, 1 H, 23-CH), 7.3–7.45 (m, 6 H, Ph), 7.65–7.7 (m, 4 H, Ph). Anal. Calcd for $C_{44}H_{60}O_3Si$: C, 79.47; H, 9.09. Found: C, 79.48; H, 9.28.

(20*S*,22*R*,25ξ)-3β-Hydroxywitha-5,23-dienolide (25). A mixture of **24** (60 mg, 0.09 mmol) and 50% HF (100 μL, 2.5 mmol) in MeCN (1 mL) was stirred for 4 h at room temperature. The reaction mixture was poured into water, and the product was extracted with $CHCl_3$. The organic layer was washed with aqueous saturated sodium bicarbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (3:1, v/v) as eluent, affording **25** (70 mg, 93.3%) as a colorless gum: IR ν_{max} 3350, 1720 cm^{-1}; ^1H NMR δ 0.72 (s, 3 H, 18-CH_3), 0.85 and 0.86 (2 × d, 2 × J = 6.7 Hz, 3 H, 21-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.35 and 1.41 (2 × d, 2 × J = 7.3 Hz, 3 H, 27-CH_3), 1.79 (s, 3 H, 28-CH_3), 2.2–2.35 (m, 1 H, 7-CH), 2.85–3.0 (m, 1 H, 25-CH), 3.45–3.6 (m, 1 H, 3-CH), 4.85–5.0 (m, 1 H, 22-CH), 5.35 (d, J = 4.9 Hz, 1 H, 6-CH), 5.45–5.55 (m, 1 H, 23-CH); HRMS calcd for $C_{28}H_{42}O_3$ (M^+) 426.3134, found (M^+) 426.3139. Anal. Calcd for $C_{28}H_{42}O_3$: C, 78.82; H, 9.92. Found: C, 78.52; H, 10.09.

(20*S*,22*R*)-3β-Hydroxywitha-5,24-dienolide (26). Isomerization of **25** was performed using the procedure described for **21** to afford the olefin **26** (98.4%) as a colorless plates: $[\alpha]^{24}_D$ +39.2° (*c* 0.28, $CHCl_3$); mp 222–223 °C (hexane–CH_2Cl_2); IR ν_{max} 3480, 1690 cm^{-1}; ^1H NMR δ 0.72 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.02 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.88 and 1.94 (2 × s, 2 × 3 H, 27-CH_3 and 28-CH_3), 3.45–3.6 (m, 1 H, 3-CH), 4.38 (dt, J = 3.7 and 13.4 Hz, 1 H, 22-CH), 5.35 (d, J = 4.9 Hz, 1 H, 6-CH); HRMS calcd for $C_{28}H_{42}O_3$ (M^+) 426.3134, found (M^+) 426.3137. Anal. Calcd for $C_{28}H_{42}O_3$: C, 78.82; H, 9.92. Found: C, 78.76; H, 10.09.

(20*S*,22*R*)-3-Oxowitha-5,24-dienolide (27). To a stirred solution of oxalyl chloride (15 μL, 0.17 mmol) in CH_2Cl_2 (0.2 mL) was added a solution of DMSO (14.5 μL, 0.21 mmol) in CH_2Cl_2 (0.2 mL) at −50 °C under argon atmosphere. After stirring for 15 min at the same temperature, the alcohol **26** (29 mg, 0.07 mmol) in CH_2Cl_2 (0.4 mL) was added and the reaction mixture was stirred for 30 min. Triethylamine (47 μL, 0.34 mmol) was added, and stirring was further continued for 15 min at the same temperature. After addition of aqueous saturated NH_4Cl solution, the product was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (4:1, v/v) as eluent to afford **27** (25.5 mg, 88.4%) as a colorless powder: $[\alpha]^{25}_D$ +63.0° (*c* 0.18, $CHCl_3$); IR ν_{max} 1690 cm^{-1}; ^1H NMR δ 0.75 (s, 3 H, 18-CH_3), 1.03 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.20 (s, 3 H, 19-CH_3), 1.89 and 1.94 (2 × s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.83 (dd, J = 2.5 and 16.5 Hz, 1 H, 4-C*H*H), 3.2–3.35 (m, 1 H, 4-CH*H*), 4.38 (dt, J = 3.7 and 13.4 Hz, 1 H, 22-CH), 5.3–5.4 (m, 1 H, 6-CH); HRMS calcd for $C_{28}H_{40}O_3$ (M^+) 424.2978, found (M^+) 424.2979.

Minabeolide-3 (28). A mixture of **27** (9 mg, 0.02 mmol) and oxalic acid (0.8 mg, 0.01 mmol) in EtOH (0.5 mL) was heated at 40 °C for 3 h. The reaction mixture was poured into water, and the product was extracted with AcOEt. The organic layer was washed with aqueous saturated sodium bicarbonate solution and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane–AcOEt (3:1, v/v) as eluent to afford **28** (9 mg, 100%) as colorless needles: $[\alpha]^{23}_D$ +131.6° (*c* 0.59, $CHCl_3$); mp 229–230 °C (CH_2Cl_2–AcOEt); IR ν_{max} 1695, 1660 cm^{-1}; ^1H NMR δ 0.75 (s, 3 H, 18-CH_3), 1.01 (d, J = 6.7 Hz, 3 H, 21-CH_3), 1.19 (s, 3 H, 19-CH_3), 1.89 and 1.94 (2 × s, 2 × 3 H, 27-CH_3 and 28-CH_3), 2.2–2.6 (m, 4 H, 6-CH_2 and 23-CH_2), 4.37 (dt, J = 3.7 and 13.4 Hz, 1 H, 22-CH), 5.73 (br s, 1 H, 4-CH); HRMS calcd for $C_{28}H_{40}O_3$ (M^+) 424.2978, found (M^+) 424.2981. The ^1H NMR spectrum of **28** was identical with that reported.[2]

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