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Synthesis Toward and Stereochemical Assignment of Clathsterol: Exploring Diverse Strategies to Polyoxygenated Sterols

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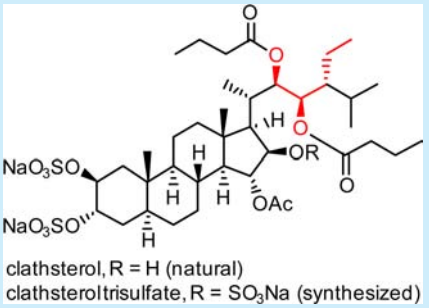
Supporting Information

ABSTRACT: Herein we describe a synthesis of the trisulfate derivative of clathsterol (**1**), a marine sterol endowed with impressive structural features and moderate inhibitory activity against HIV-1 reverse transcriptase. By synthesizing two possible isomers of the side chain, the stereochemistry of **1** is assigned. In creating chiral side chains from steroidal lactone, our strategies, including an addition/reduction procedure to give C22*R*−OH, an epoxide-opening reaction, and a [3.3]-rearrangement to induce the generation of C24*S*-Et and C24*R*-Et respectively, are highly flexible and complementary to each other.



Polyoxygenated steroids are widely isolated from marine and terrestrial organisms and possess a variety of pharmaceutically attractive bioactivities.[1] Their diverse and complex structures have attracted extensive and fruitful synthetic endeavors.[2] For targets with similar structural features, developing robust and flexible synthetic strategies are encouraged. Herein we report our efforts on the synthesis of clathsterol (**1**, Figure 1), the strategies of which might be applicable to many natural sterols.

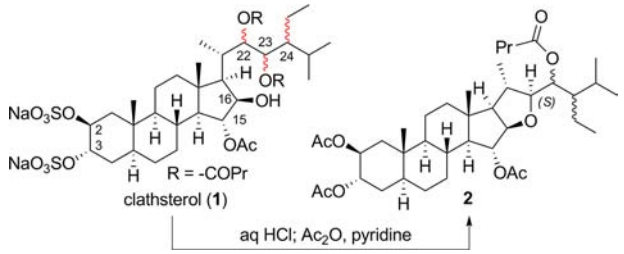


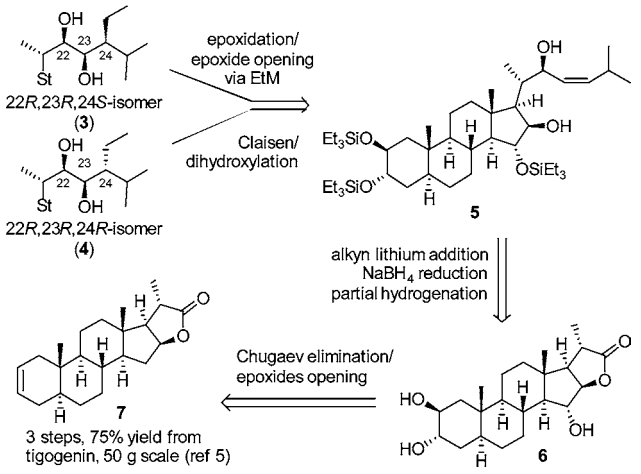
Figure 1. Structures of clathsterol.

Clathsterol (**1**) is a sterol sulfate isolated by Kashman and coworkers from the Red Sea sponge *Clathria* sp. in 2001.[3] It inhibits human deficiency virus type 1 (HIV-1) reverse transcriptase at a concentration of 10 μM. Kashman assigned the structure of **1** mainly by interpretation of spectral data of **1** and its derivative **2**, but left three chiral centers (C22, C23, and C24) unassigned due to the conformational lability of the side chain, thus arising eight possible isomers. Possessing three sets of vicinal diols that present differentially as 15α-acetate, 22,23-dibutylate, and 2β,3α-disulfate salt, along with its unassigned side-chain configuration, **1** was a challenging and intriguing target for us.

We narrowed the eight isomers of **1** down to 22*R*,23*R*,24*S*-**3** (*syn*, *syn*) and 22*R*,23*R*,24*R*-**4** (*syn*, *anti*) (Scheme 2) and set them as targets for two reasons. First, in natural sterols, most of the 22,23-diols, if any, are *cis*- and 22*R*,23*R*-configured.[4] Second, the configuration of C22 in **2** was assigned as *S*, we believed that the configuration of C22 in **1** was inverted during the ring-closing reaction although certain unambiguity of the mechanism might exist.

Our retrosynthetic analysis is depicted in Scheme 1. We considered allylic alcohol **5** as a common precursor. The *syn*,*syn*-unit of **3** was to be constructed through an epoxidation/epoxide-opening process, while the *syn*,*anti*-unit of **4** was to be established through a Claisen-type rearrangement followed by a dihydroxylation. Alcohol **5** could be stereoselectively prepared by an

Scheme 1. Retrosynthetic Analysis



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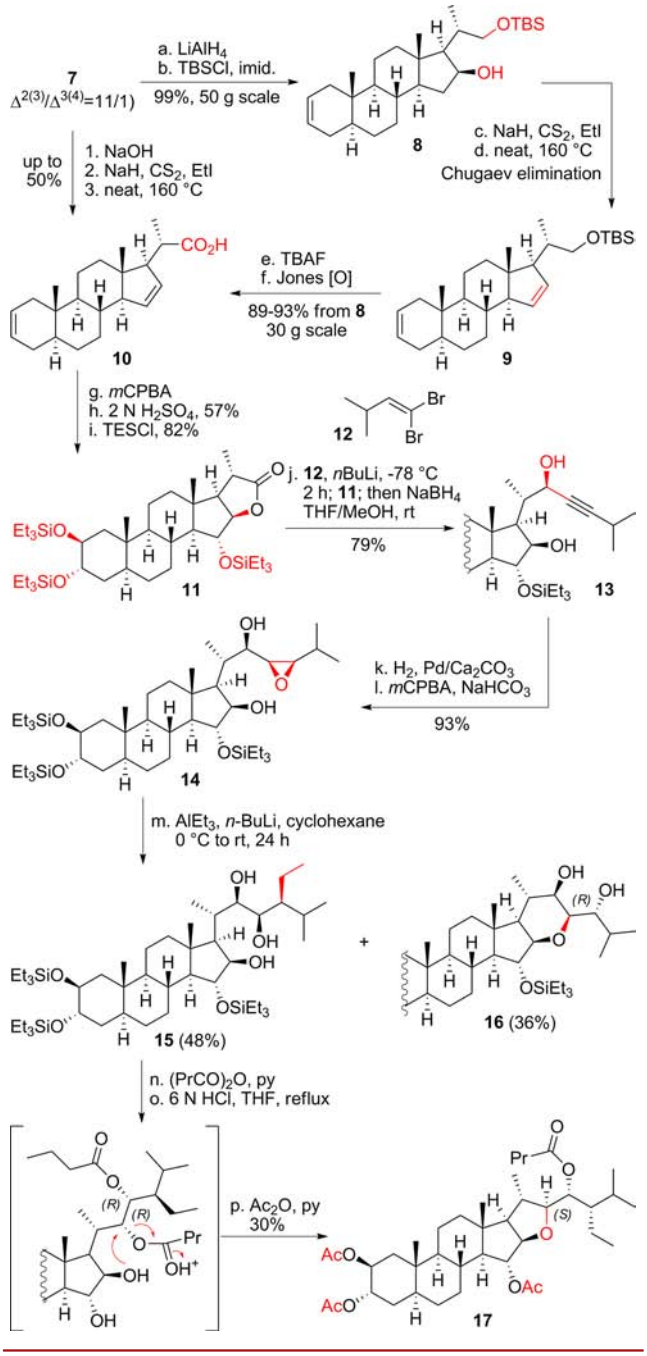
Scheme 2. Synthesis of 22,23,24-*syn,syn* Isomer



addition of isopentynyl lithium to lactone **6** followed by $NaBH_4$ reduction and Rosenmund reduction. Two *trans*-diol units in lactone **6** were to be introduced via epoxide-opening reactions of a diepoxide derived from lactone **7**. To gain more isomers for the situation of clathsterol (**1**) being neither **3** nor **4**, we could change the configuration of C22 in **5** from *R* to *S*, C23−C24 double bond from *cis* to *trans*, and diol-forming processes on steroidal core from the epoxide-opening reaction to dihydroxylation.

We have reported a concise synthesis of **6**.[5] Therein lactone **7** was prepared from tigogenin in three steps and on the 50 g scale with an overall yield of 75%,[6] and diene acid **10** was prepared through a three-step procedure featuring a Chugaev elimination of xanthate derived from 16-hydroxyl-22-carboxylate dianion. However, the xanthate was obtained in unstable yields (30−50%) and was difficult to purify. We needed a robust method to support further exploration. Therefore, **7** was reduced with $LiAlH_4$ and the primary C22-OH of the resultant diol was selectively protected as TBS ether to give **8**. The exposed secondary C16-OH in **8** was then converted into xanthate, which underwent Chugaev elimination to install the required C15−C16 double bond. Desilylation and Jones oxidation provided acid **10** in 89−93% yield from **8** on the 30 g scale. With this six-step procedure we accumulated **10** more rapidly (only one purification was needed).

Both the C2−C3 and the C15−C16 double bonds in **10** were epoxidized with *m*CPBA, and the diepoxide was treated with 2 N H_2SO_4 to open the epoxides intra- and intermolecularly, giving lactone **11** after protecting all the hydroxyl groups in **6** as triethylsilyl (TES) ethers. Then **11** was treated with isopentynyl lithium and the resultant hemiketal was reduced with $NaBH_4$ to afford diol **13** in 79% yield on the multigram scale as a single isomer. Although the mechanism of the excellent stereocontrol observed herein was not fully understood, this lactone addition/reduction process provides a rapid access to C22*R*-configured sterols,[7] which are difficult to obtain with high stereoselectivity from 22-aldehydes.[8] Partial hydrogenation of the triple bond of **13** to *cis*-double bond through Rosenmund reduction gave **5**, and a highly stereoselective epoxidation of **5** directed by its C22*R*−OH[9] provided **14** in 93% yield.

To install the correct configuration of the side chain of brassinolide, Mori and co-workers developed an effective procedure for opening a similar epoxide with $AlMe_3$.[8b] Rather smoothly a similar reaction proceeded by treating **14** with a premixed solution of 10 equiv of Et_3Al and 0.6 equiv of *n*-BuLi in cyclohexane at ambient temperature for 24 h, providing the desired product **15** in 48% yield, along with **16**, the intramolecular epoxide opening product, in 36% yield. Clearly, under the activation of Lewis acid $AlEt_3$, the epoxide in **14** was attacked by the adjacent C16-OH, hence generating **16**.

Before optimizing this epoxide-opening reaction, two butyrate esters at C22 and C23 were installed by treating **15** with butyrate anhydride in pyridine for 2 days in 84% yield. We found that both the C22−H and the C23−H displayed as single peaks in ^1H NMR, which were different from those of natural products (d for C22−H and dd for C23−H). Further verification was needed. Acid hydrolysis of the butyrate, as reported by Kashman and co-workers, followed by acetylation of the crude product with Ac_2O in pyridine provided triacetate **17** in 35% yield. The 2D NOESY analysis assigned the configuration of C22 in **17** to be *S* (cross peaks of C22−H and C16−H/C21−Me), which suggested that the ring-closing reaction is a simple S_N2 process without neighbor group participation involved, and thus, C22 of clathsterol is *R*-configured. However, although the ^1H NMR data of **17** highly resemble those of **2**, the ^{13}C NMR signals are apparently different at C18−C29 area (see Supporting Information for a detailed list). The correct configuration of **1** is not 22*R*,23*R*,24*S* (*syn*, *syn*). Focus was then turned to the 22*R*,23*R*,24*R*-isomer **4**.

To synthesize the *syn*,*anti*-product, we planned to transfer the stereochemistry from C22 to C24 through a Claisen-type rearrangement (Scheme 3).[8f,g,10] As in **14**, the unprotected C16−OH participated in the reaction again. Heating **5** with excess triethyl orthoacetate in the presence a catalytic amount of propionic acid, the typical Johnson−Claisen rearrangement conditions, produced trace or no desired product **18**. An intramolecular substitution occurred, forming a THF ring. We found that it was propionic acid that caused the side reaction. Refluxing a solution of **5** in triethyl orthoacetate without

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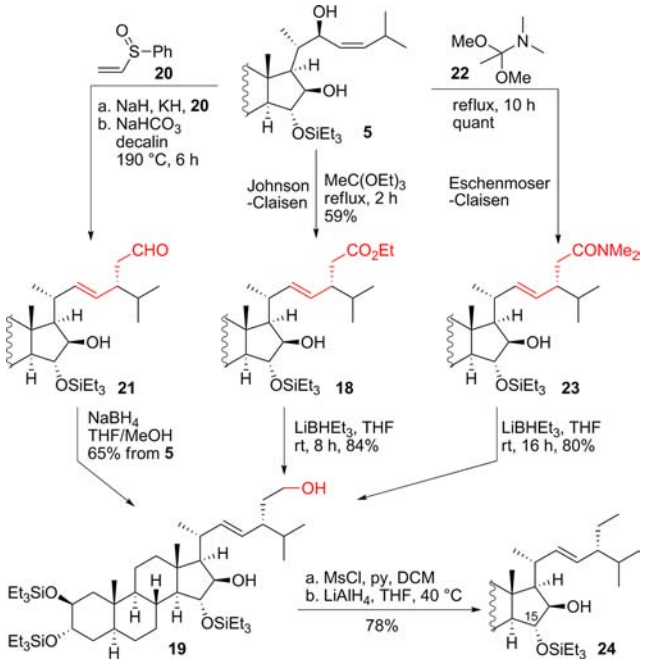
Scheme 3. Synthesis of 19 by Claisen-Type Rearrangements

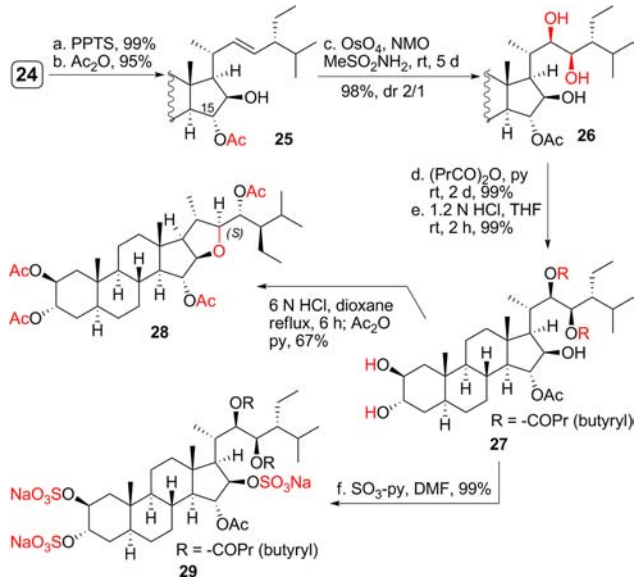


propionic acid for 2 h successfully delivered **18** in good yield, which was reduced with $LiBHEt_3$ to give **19**. Likewise, other [3.3]-rearrangements that could proceed under neutral conditions also gave good results. Employing a modified Claisen rearrangement of allylic vinyl ethers,[11] **19** was prepared in 65% overall yield through a sequence involving a NaH/KH promoted Michael-type addition of **5** to aryl vinyl sulfoxide **20** at room temperature, a concurrent thermal elimination and [3.3]-rearrangement, and a $NaBH_4$ reduction of the resulting aldehyde **21**. Allylic alcohol **5** also underwent Eschenmoser−Claisen rearrangement[12] to furnish amide **23**, which was easily reduced to **19** with $LiBHEt_3$. Finally, the C29−OH of **19** was removed through mesylation and reduction with $LiAlH_4$, giving **24** in excellent yield.

The C15−OH of **24** was selectively exposed by removing the TES group with a catalytic amount of PPTS in MeOH/DCM and acetylated with Ac_2O to provide **25** in excellent yield (Scheme 4). Dihydroxylation and esterification of the hydroxyl groups were then investigated. Dihydroxylation of **25** gave the products in excellent yield and moderate selectivity (2/1 by ^1H NMR), favoring the desired **26**.[13,14] The newly generated C22-OH and C23-OH in **26** were acylated with butyrate anhydride in pyridine and the TES ethers at ring A were removed with HCl, giving **27**. The NMR data of D ring and side chain of **27** were in good agreement with those of **1** since they have the same side chain, which was remote from the variation on the steroidal skeletons. To further verify the structure of **1**, we also tried to convert **27** to **2**. Acid hydrolysis of **27** (6 N HCl, dioxane, reflux, 6 h) unexpectedly removed its C23-butylate, giving not **2** but **28** after treating with Ac_2O in pyridine. The 2D NOESY analysis assigned the configuration of C22 in **28** to be *S*, thus verifing the stereochemistry of **26**. Pleasingly, we found that the NMR data of **28** were in good agreement with those of **2**. We assigned the correct configuration of **1** as 22*R*,23*R*,24*R* (*syn*,*anti*).

Then we reached the endgame stage of this synthesis. The C16−OH is usually unreactive toward many reagents due to the steric hindrance. Therefore, we considered the selective sulfation of **27** to be feasible but found it quite challenging. Reaction with

Scheme 4. Synthesis of 29



SO_3·py in DMF at 90 °C (24 h, or microwave, 5 min) and treatment with $NaHCO_3$ (ion exchange) directly formed the trisulfate sodium salt **29** in high yield. Lowering temperature and employing other sulfation reagents[15] gave complex reaction mixture, suggesting that the reactivities of three hydroxyl groups toward these sulfation reagents are too close to be effectively differentiated, thus giving mixtures of random sulfated products or fully sulfated product. Because these sulfate sodium salts were unable to isolate from a mixture unless the reaction was clean, we did not obtain a sample pure enough for collecting satisfactory spectral data of clathsterol (**1**) although it was generated as minor product in some cases. Selective sulfation method for substitutes with many hydroxyl groups, such as carbohydrates, whose sulfate derivatives often have biological activities, is valuable and needs further investigation.

In summary, based on a flexible synthetic strategy we assigned the clathsterol (**1**) as 22*R*,23*R*,24*R* by comparing the NMR data of the synthetic samples with those of natural samples. The synthesis of trisulfate sodium salt **29** was accomplished in 21 steps and 11% overall yield starting from **7**. Noteworthy transformations include a convenient and scalable nine-step procedure for lactone **11**, an addition of alkynyl lithium to lactone followed by $NaBH_4$ reduction to stereoselectively give C22*R*−OH, a selective epoxidation/epoxide-opening procedure to install the 22*R*,23*R*,24*S*-configured side chain and Claisen-type rearrangements under nonacidic conditions to transfer the stereochemistry from C22 to C24. Our endeavors allow quick access to C22*R*−OH compounds from steroidal lactone and C23*R*-, C24*R*/*S*-stereochemistry from C22*R*−OH and therefore are valuable for establishing chiral side chains that are frequently associated with the unusual structural features of a great number of natural sterols.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01029.

Experimental details, spectral data, and ^1H and ^{13}C NMR spectra of all the new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839−1895. (b) Sarma, N. S.; Krishna, M. S.; Pasha, S. G.; Rao, T. S. P.; Venkateswarlu, Y.; Parameswaran, P. S. *Chem. Rev.* **2009**, *109*, 2803−2828. (c) Lee, S.; LaCour, T. G.; Fuchs, P. L. *Chem. Rev.* **2009**, *109*, 2275−2314. (d) Li, J.; Risinger, A. L.; Peng, J.; Chen, Z.; Hu, L.; Mooberry, S. L. *J. Am. Chem. Soc.* **2011**, *133*, 19064−19067. (e) Qi, J.; Ojika, M.; Sakagami, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1961−1966. (f) Chen, B.-W.; Chang, S.-M.; Huang, C.-Y.; Su, J.-H.; Wen, Z.-H.; Wu, Y.-C.; Sheu, J.-H. *Org. Biomol. Chem.* **2011**, *9*, 3272−3278. (g) Li, Y.-M.; Wang, L.-H.; Li, S.-L.; Chen, X.-Y.; Shen, Y.-M.; Zhang, Z.-K.; He, H.-P.; Xu, W.-B.; Shu, Y.-L.; Liang, G.-D.; Fang, R.-X.; Hao, X.-J. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 8083−8088. (h) Adam, G.; Schmidt, J.; Schneider, B. Brassinosteroids. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Tamm, C., Eds.; Springer: Vienna, 1999; Vol. *78*, pp 1−46. (i) Jiang, Q.-W.; Chen, M.-W.; Cheng, K.-J.; Yu, P.-Z.; Wei, X.; Shi, Z. *Med. Res. Rev.* **2016**, *36*, 119−143.

(2) (a) Lee, S.; LaCour, T. G.; Fuchs, P. L. *Chem. Rev.* **2009**, *109*, 2275−2314. (b) Renata, H.; Zhou, Q.; Baran, P. S. *Science* **2013**, *339*, 59−63. (c) Mukai, K.; Urabe, D.; Kasuya, S.; Aoki, N.; Inoue, M. *Angew. Chem., Int. Ed.* **2013**, *52*, 5300−5304. (d) Jiang, B.; Shi, H.-P.; Tian, W.-S.; Zhou, W.-S. *Tetrahedron* **2008**, *64*, 469−476. (e) Heretsch, P.; Tzagkaroulaki, L.; Giannis, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 3418−3427. (f) Tang, Y.-P.; Li, N.-G.; Duan, J.-A.; Tao, W.-W. *Chem. Rev.* **2013**, *113*, 5480−5514.

(3) Rudi, A.; Yosief, T.; Loya, S.; Hizi, A.; Schleyer, M.; Kashman, Y. *J. Nat. Prod.* **2001**, *64*, 1451−1453.

(4) (a) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C. *Nature* **1979**, *281*, 216−217. (b) Yokota, T.; Arima, M.; Takahashi, N. *Tetrahedron Lett.* **1982**, *23*, 1275−1278. (c) Baba, J.; Yokota, T.; Takahashi, N. *Agric. Biol. Chem.* **1983**, *47*, 659−661. (d) Abe, H.; Morishita, T.; Uchiyama, M.; Takatsuto, S.; Ikekawa, N. *Agric. Biol. Chem.* **1984**, *48*, 2171−2172. (e) Yokota, T.; Baba, J.; Koba, S.; Takahashi, N. *Agric. Biol. Chem.* **1984**, *48*, 2529−2534. (f) Kim, S. K.; Yokota, T.; Takahashi, N. *Agric. Biol. Chem.* **1987**, *51*, 2303−2305. (g) Schmidt, J.; Yokota, T.; Spengler, B.; Adam, G. *Phytochemistry* **1993**, *34*, 391−392. (h) Abe, H.; Takatsuto, S.; Nakayama, M.; Yokota, T. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 176−178. (i) Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly, M. *J. Org. Chem.* **1999**, *64*, 6706−6709.

(5) Cong, R.-G.; Zhang, Y.-H.; Tian, W.-S. *Tetrahedron Lett.* **2010**, *51*, 3890−3892.

(6) Lactone **7** was mixed with inseparable C3−C4 isomer, which was separated after the epoxide opening stage. For clarity, the inseparable side products were not mentioned in main text.

(7) Various organolithium reagents have been examined and all exhibited excellent 22*R* stereoselectivity. The structures of some products were confirmed by X-ray analysis. Wang, T. The addition reaction of steroidal lactone and its application to synthesis of some natural products. PhD thesis, Shanghai Institute of Organic Chemistry, China, 2004.

(8) (a) Zhou, W.-S.; Tian, W.-S. *Tetrahedron* **1987**, *43*, 3705−3712. (b) Mori, K.; Sakakibara, M.; Okada, K. *Tetrahedron* **1984**, *40*, 1767−1781. (c) Matsuya, Y.; Yamakawa, Y.-I.; Tohda, C.; Teshigawara, K.; Yamada, M.; Nemoto, H. *Org. Lett.* **2009**, *11*, 3970−3973. (d) Fung, S.; Siddall, J. B. *J. Am. Chem. Soc.* **1980**, *102*, 6580−6581. (e) Prestwich, G. D.; Phirwa, S. *Tetrahedron Lett.* **1983**, *24*, 2461−2464. (f) Wiersig, J. R.; Waespe-Sarcevic, N.; Djerassi, C. *J. Org. Chem.* **1979**, *44*, 3374−3382. (g) Hirano, Y.; Djerassi, C. *J. Org. Chem.* **1982**, *47*, 2420−2426.

(9) Back, T. G.; Blazecka, P. G.; Krishna, M. V. *Can. J. Chem.* **1993**, *71*, 156−163.

(10) (a) Giroux, S.; Corey, E. J. *Org. Lett.* **2008**, *10*, 801−802. (b) Khripach, V. A.; Zhabinskii, V. N.; Konstantinova, O. V.; Khripach, N. B.; Antonchick, A. P.; Schneider, B. *Steroids* **2002**, *67*, 597−603. (c) Watanabe, T.; Noguchi, T.; Yokota, T.; Shibata, K.; Koshino, H.; Seto, H.; Kim, S.-K.; Takatsuto, S. *Phytochemistry* **2001**, *58*, 343−349.

(11) (a) Mandai, T.; Ueda, M.; Hasegawa, S.-I.; Kawada, M.; Tsuji, J.; Saito, S. *Tetrahedron Lett.* **1990**, *31*, 4041−4044. (b) Mandai, T.; Matsumoto, S.; Kohama, M.; Kawada, M.; Tsuji, J.; Saito, S.; Moriwake, T. *J. Org. Chem.* **1990**, *55*, 5671−5673.

(12) (a) Wick, A. E.; Felix, D.; Steen, K.; Eschenmoser, A. *Helv. Chim. Acta* **1964**, *47*, 2425−2429. (b) Loh, T.-P.; Hu, Q.-Y. *Org. Lett.* **2000**, *3*, 279−281.

(13) (a) Thompson, M. J.; Meudt, W. J.; Mandava, N. B.; Dutky, S. R.; Lusby, W. R.; Spaulding, D. W. *Steroids* **1982**, *39*, 89−105. (b) Thompson, M. J.; Mandava, N. B.; Meudt, W. J.; Lusby, W. R.; Spaulding, D. W. *Steroids* **1981**, *38*, 567−580. (c) Sun, L.-Q.; Zhou, W.-S.; Pan, X.-F. *Tetrahedron: Asymmetry* **1991**, *2*, 973−976. (d) Brosa, C.; Peracaula, R.; Puig, R.; Ventura, M. *Tetrahedron Lett.* **1992**, *33*, 7057−7060. (e) Anastasia, M.; Ciuffreda, P.; Fiecchi, A. *J. Chem. Soc., Perkin Trans. 1* **1983**, 379−382. (f) Anastasia, M.; Ciuffreda, P.; Del Puppo, M.; Fiecchi, A. *J. Chem. Soc., Perkin Trans. 1* **1983**, 383−386.

(14) Close examination of the NMRs of the reported sterols revealed that the chemical shifts of 22*R*,23*R*-diols are between 3.5 and 3.8 ppm, while those of 22*S*,23*S*-diols are between 3.3 and 3.5 ppm. The dihydroxylation of **24** gave two products. The less polar one has peaks at 3.33 (dd) and 3.52 (m) ppm, while the more polar one is at 3.55 (dd) and 3.77 (m) ppm. According to the above observation, we assigned the former as 22*S*,23*S*- and the latter as 22*R*,23*R* (**26**). On a similar substrate, we also observed the *R*/*S* selectivity was enhanced with $(DHQD)_2PHAL$ and reversed with $(DHQ)_2PHAL$ (see Supporting Information).

(15) (a) Kakiyama, G.; Muto, A.; Shimada, M.; Mano, N.; Goto, J.; Hofmann, A. F.; Iida, T. *Steroids* **2009**, *74*, 766−772. (b) Gunnarsson, G. T.; Riaz, M.; Adams, J.; Desai, U. R. *Bioorg. Med. Chem.* **2005**, *13*, 1783−1789. (c) Santos, G. A. G.; Murray, A. P.; Pujol, C. A.; Damonte, E. B.; Maier, M. S. *Steroids* **2003**, *68*, 125−132. (d) Sepe, V.; Ummarino, R.; D'Auria, M. V.; Renga, B.; Fiorucci, S.; Zampella, A. *Eur. J. Org. Chem.* **2012**, *2012*, 5187−5194. (e) Huibers, M.; Manuzi, Á.; Rutjes, F. P. J. T.; van Delft, F. L. *J. Org. Chem.* **2006**, *71*, 7473−7476.

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