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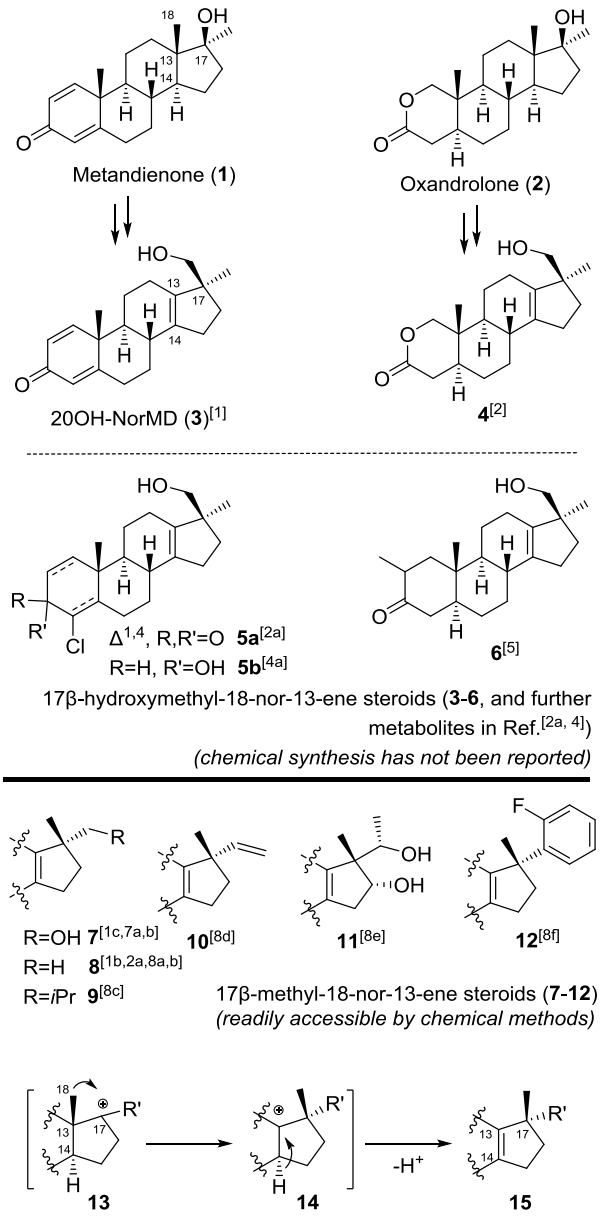
C-H Acetoxylation Based Chemical Synthesis of 17β-Hydroxymethyl-17α-methyl-18-norandrost-13-ene Steroids

Alaksiej L. Hurski,*[a] Maryia V. Barysevich,[a] Tatsiana S. Dalidovich,[a] Marharyta V. Iskryk,[a] Nastassia U. Kolasava,[a] Vladimir N. Zhabinskii,[a] and Vladimir A. Khripach[a]

Abstract: Palladium-catalyzed C-H acetoxylation has been proposed as a key transformation in the first chemical synthesis of steroids bearing a unique 17β-hydroxymethyl-17α-methyl-18-nor-13-ene D-fragment. This C-H functionalization step was crucial for inverting the configuration at the quaternary stereocenter of a readily available synthetic intermediate. The developed approach was applied to prepare metandienone metabolite needed as a reference substance in anti-doping analysis to control the abuse of this androgenic anabolic steroid.

17β-Hydroxymethyl-17α-methyl-18-norandrost-13-enes 20OH-NorMD (**3**)[1] and **4**[2] are human metabolites of the 17-methylated androgenic anabolic steroids (AAS) metandienone (**1**) and oxandrolone (**2**) banned in sports (Scheme 1). The use of **3** and **4** as reference substances in anti-doping analysis resulted in an enormous increase of adverse analytical findings of the parent drugs due to the considerable extension of the detection window.[3] The metabolite **3** is detectable for up to 19 days after metandienone (**1**) abuse whereas the detection period of its other metabolites is only 4-6 days.[1a] It is expected that long-term metabolites of dehydrochloromethyltestosterone (DHCMT)[2a, 4] (including **5a**[2a] and **5b**[4a]), oxymetholone **6**[5] and metabolites of other frequently abused 17-methylated AAS[2a] also bear analogous D-fragment. But their full structural elucidation still remains an unresolved issue. These metabolites cannot be isolated in quantities sufficient for NMR analysis, and the only way to prove their structures is preparation of synthetic samples for comparison purposes.[6]

Structure of the D-ring in steroids **3**-**6** is unique due to the configuration of its quaternary C17 stereocenter which in combination with an endocyclic C13-C14 double bond makes this fragment a challenging target for chemical synthesis. This fact is particularly impressive, since epimeic at C17 D-fragment **7**[1c, 7] as well as other 18-nor-13-ene steroids **8**-**12**[1b, 2a, 8] bearing a non-hydroxylated 17β-methyl group are readily available *via* a stereospecific Wagner-Meerwein shift of 18-methyl group in the cationic intermediates **13** (Scheme 1). The only known routes to steroids **16** have been based on a biotechnological oxidation of the rearranged intermediates **8** (Scheme 2).[1b, 2] Application of these methods allowed to prepare metabolites **3**[1b] and **4**[2b] in quantities sufficient for their structural elucidation. Inspired by challenging structure of the compounds **3-6** and analogous metabolites[2a, 4] along with their importance for anti-doping analysis, we have undertaken studies on the fully chemical synthesis of the 17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene steroids.



Scheme 1. Androgenic anabolic steroids, their metabolites and 17β-methyl-18-nor-13-ene steroids.

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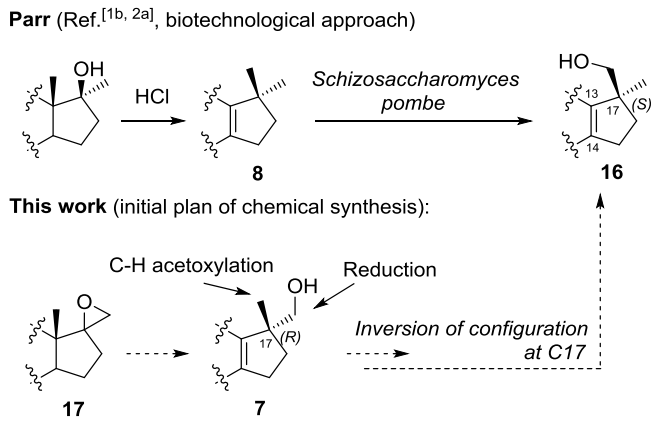
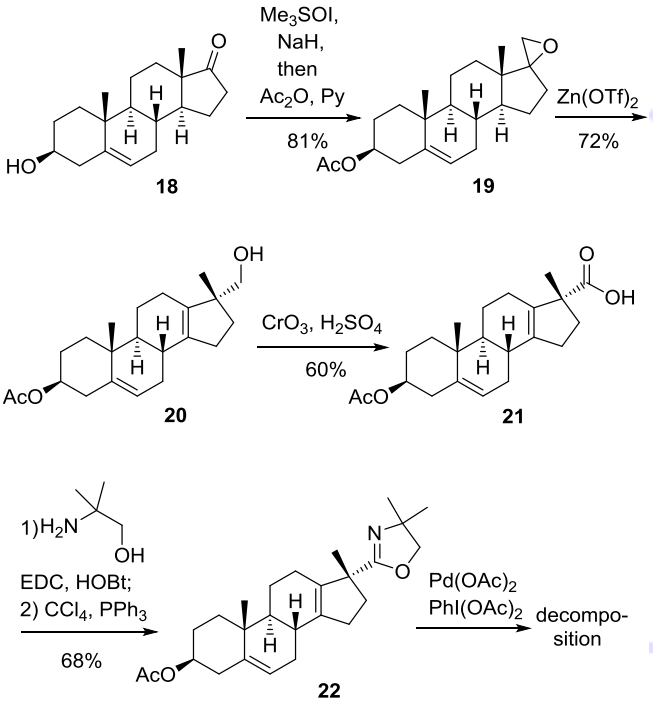


Scheme 2. Approaches to the construction of the steroidal fragment **16**.

While searching for a suitable starting material, our attention was focused on availability of steroids with D-fragment **15**. Synthesis of the target steroids **16** in living organisms is based on steroselective C-H oxidation of 17β-methyl group in 17,17-dimethyl intermediate **8** (Scheme 2).[1-2] We speculated that chemical C-H activation methods[9] could be applied for oxidation of 17β-methyl substituent as well. To perform this functionalization selectively, the appropriate directing group is necessary. In this respect, steroids with epimeric D-fragment **7** are suitable intermediates for our synthesis as their hydroxyl group could be transformed to any directing ligand. Finally, our synthetic plan included two stages (Scheme 2). At first stage, carbon backbone of the target fragment was thought to be built through a Lewis acid catalyzed Wagner-Meerwein rearrangement of a readily available epoxide **17** to alcohol **7**.[7] At the second stage of the synthesis we planned to perform C-H acetoxylation[10] of 17β-methyl group and a complete reduction of functionalities at 17α-substituent. The result of the latter set of transformations is inversion of configuration at the quaternary C17 stereocenter of intermediate **7**.

The synthesis started from androstenolone (**18**), which reacted with dimethyloxosulfonium methylide[11] to give, after acetylation, a 2:3 mixture of isomeric spirooxiranes **19** (Scheme 3). The Wagner-Meerwein rearrangement of the intermediate **19** was promoted by a catalytic amount of $Zn(OTf)_2$ at room temperature to afford alcohol **20** that bears the necessary carbon backbone with a C13-C14 double bond. To carry out the implied Pd-catalyzed C–H oxidation, the 17α-hydroxymethyl group of compound **20** had to be converted into an appropriate directing ligand.

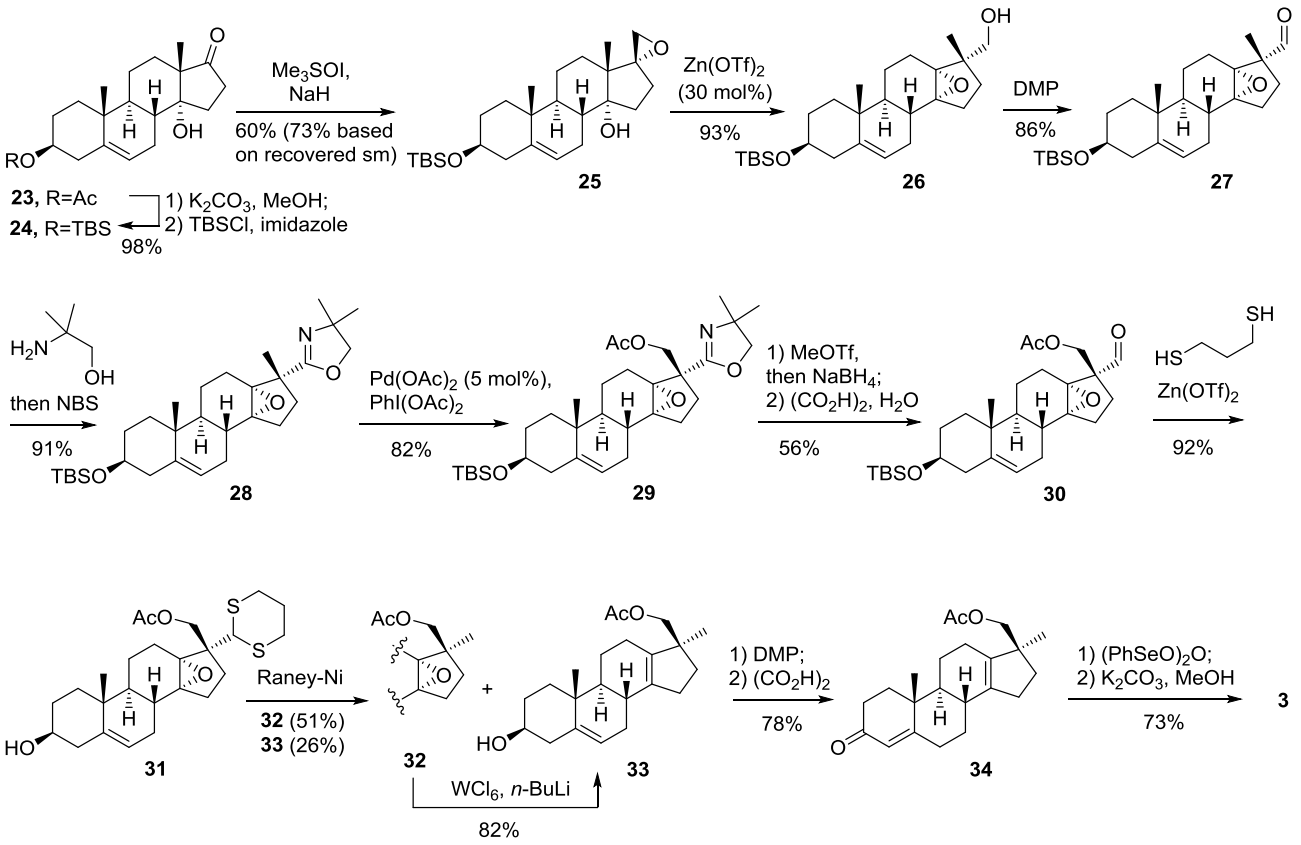
Existing directing groups can be simply introduced into the molecules but their reduction to alkane could be problematic as only amides, oxime derivatives and heterocycles can direct palladium catalyzed acetoxylation of C^{sp3}-H bonds.[10] Our attention was drawn to the oxazoline group[12] which can be smoothly prepared from aldehydes or acids and after the acetoxylation could be easily converted into aldehydes or alcohols suitable for further deoxygenation.[13] The oxidation of **20** with Jones reagent yielded the acid **21** that was amidated with 2-amino-2-methylpropanol. Reaction of the resulting amide with Ph_3P and CCl_4 led to oxazoline ring closure.[14] However, heating of the oxazoline **22** with iodobenzene diacetate[10a] in the presence of a catalytic amount of palladium diacetate led to the destruction of the starting material. We assumed that the decomposition of **22** could be caused by the reactions of intermediate palladium-oxazoline complex with a proximate C13-C14 alkenyl fragment. Another C5-C6 double bond in the substrate was expected to be stable under the reaction conditions.[15]



Scheme 3. Initial efforts to oxidize the 17β-methyl group.

To test this hypothesis, synthesis of steroidal oxazoline **28** bearing a C13-C14 epoxide as the protecting group for double bond was undertaken staring from 14α-hydoxyandrostenolone acetate (**23**).[16] The acetyl group in **23** was replaced by a tert-butyldimethylsilyl protecting group and the resulting compound **24** was treated with dimethyloxosulfonium methylide to afford the spirooxirane **25** as a single isomer (Scheme 4). Its Wagner-Meervein rearrangement is expected to proceed via the formation of a carbocation similar to **14** which undergoes the nucleophilic attack of the adjacent hydroxyl group to form oxirane ring.[17] This transformation was effected in a yield of 81% using zinc triflate as the catalyst under ambient conditions. Alternatively, it is possible to synthesize the compound **26** from commercial androstenolone (**18**) in four steps and in 44% overall yield (see SI). Conversion of the hydroxymethyl group of intermediate **26** to the required directing oxazoline group was achieved in two efficient synthetic steps. Oxidation of **26** by Dess-Martin periodinane gave aldehyde **27** that was subjected to one-pot reaction sequence involving formation of intermediate

oxazolidine and its oxidation with NBS[18] to afford oxazoline **28**. It should be noted that application of the latter transformation in the synthesis of compound **22** was not possible as the C13-C14 alkenyl fragment reacted with NBS.



Scheme 4. Chemical synthesis of the metabolite **3**.

Having in hands steroid **28** with oxazoline directing group and a protected double bond, we moved on to explore the Pd-catalyzed C–H acetoxylation. The reaction of **28** with $PhI(OAc)_2$ in the presence of catalytic amounts of $Pd(OAc)_2$ at 100°C proceeded smoothly over 45 min, providing product **29** in a good 82% yield. The next task to be performed was the conversion of the oxazoline heterocycle into a methyl group. It was desirable to use the synthetic routes that are compatible with the acetyl protecting group in **29**. Quaternization of **29** with methyl triflate followed by treatment with sodium borohydride and hydrolysis of the resulting aminal with oxalic acid[13a] gave the aldehyde **30** in 56% overall yield. Deoxygenation of the aldehyde carbonyl group was effected via the dithiane intermediate **31**. Zinc triflate catalyzed thioacetalization was accompanied by loss of the silyl protecting group. Desulfurization of dithiane **31** under the action of Raney nickel was unexpectedly accompanied by deoxygenation providing a mixture of epoxide **32** and the target 13-ene steroid **33**. The deprotection of Δ^{13}-double bond by Raney nickel was desirable, but neither increasing the amount of the reagent nor lengthening the reaction time could drive the transformation to completion. Ultimately, epoxide **32** was successfully transformed into **33** with WCl_6 and *n*-BuLi in the presence of LiI.[19]

Compound **33** possesses the required features of ring D and 3β-hydroxy-Δ^5-fragment allowing functionalization of this part of the molecule by conventional steroid chemistry methods.[20] The scope of the developed approach was evaluated through the synthesis of metandienone metabolite **3**. Alcohol **33** was oxidized by Dess-Martin reagent to yield the corresponding Δ^5-3-ketone, which was isomerized under acidic conditions[21] to give conjugated Δ^4-3-ketone **34** in 78% yield. The application of the Oppenauer method to the oxidation of **33** proved to be less efficient, resulting in the desired product in only moderate yield (58%). Transformation of Δ^4-3-ketone to $\Delta^{1,4}$-3-ketone was performed in 81% yield using benzeneseleninic anhydride as an oxidant.[22] Employment of a more common oxidant DDQ[20, 23] led to the dienone in a yield of only 10%. Removal of the acetate protecting group at the last step of the synthesis furnished metabolite **3** whose 1D and 2D NMR spectra were consistent with the assigned structure. [1b, c][24]

In conclusion, we have developed the first fully chemical route to the construction of a key fragment of long-term metabolites of AAS. These 17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene steroids are needed as synthetic reference substances for effective anti-doping programs to ensure fair sport competitions.[3] Palladium catalyzed C-H acetoxylation of 17β-

methyl group was successfully used for the inversion of configuration at a quaternary stereocenter of the readily available epimeric synthetic intermediate. Although this transformation required 2 additional steps for the formation of the directing oxazoline group and 4 steps were necessary to reduce the directing group to methyl, the developed approach allowed the preparation of the challenging compound **3** in an acceptable 8.5% overall yield.

Acknowledgements

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Keywords: C-H activation • steroids • rearrangement • oxidation • protecting groups

[1] (a) W. Schänzer, H. Geyer, G. Fußholler, N. Halatcheva, M. Kohler, M.-K. Parr, S. Guddat, A. Thomas, M. Thevis, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2252-2258; (b) A. Zöllner, M. K. Parr, C.-A. Drägan, S. Dräs, N. Schlörer, F. T. Peters, H. H. Maurer, W. Schänzer, M. Bureik, *J. Biol. Chem.* **2010** *391*, 119-127; (c) M. K. Parr, A. Zöllner, G. Fussholler, G. Opfermann, N. Schlörer, M. Zorio, M. Bureik, W. Schänzer, *Toxicol. Lett.* **2012**, *213*, 381-391.

[2] For detection of 17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene human metabolites of 17-methylated AAS (oxandrolone, DHCMT, methyl-1-testosterone and methyltestosteron), and for formation of steroids with analogous D-ring by biotransfornation of 17,17-dimethyl-18-norandrost-13-enes, see: (a) M. K. Parr, G. Fußhöller, M. Gütschow, C. Hess., W. Schänzer, in *Recent advances in doping analysis (18)*, Sportverlag Strauß, Cologne, **2010**, 64-73; for biotechnological synthesis of **4** and its full characterization, see: (b) Guddat, G. Fußhöller, S. Beuck, A. Thomas, H. Geyer, A. Rydevik, U. Bondesson, M. Hedeland, A. Lagojda, W. Schänzer, M. Thevis, *Anal. Bioanal. Chem.* **2013** *405*, 8285-8294.

[3] H. Geyer, W. Schänzer, M. Thevis, *Br. J. Sports Med.* **2014**, *48*, 820-826.

[4] For detection of **5a**, **5b** and further DHCMT metabolites with similar D-ring, see: (a) T. Sobolevsky, G. Rodchenkov, *J. Steroid Biochem. Mol. Biol.* **2012**, *128*, 121-127; (b) M. Fernández-Álvarez, J. Lu, D. Cuervo, Y. Xu, J. Muñoz-Guerra, R. Aguilera, in *Recent advances in doping analysis (22)*, Sportverlag Strauß, Cologne, **2014**, 182-187.

[5] T. Sobolevsky, G. Rodchenkov, *Drug Test. Anal.* **2012**, *4*, 682-691.

[6] W. Abushareeda, A. Fragkaki, A. Vonaparti, Y. Angelis, M. Tsivou, K. Saad, S. Kraiem, E. Lyris, M. Alsayrafi, C. Georgakopoulos, *Bioanalysis* **2014**, *6*, 881-896.

[7] (a) D. N. Kirk, M. A. Wilson, *J. Chem. Soc. C* **1971**, 414-424; (b) J. R. Bull, L. M. Steer, *Tetrahedron* **1990**, *46*, 5389-5400.

[8] (a) D. M. P. E. Caspi, *Can. J. Chem.* **1963**, *41*, 2294-2298; (b) W. Schänzer, G. Opfermann, M. Donike, *Steroids* **1992**, *57*, 537-550; (c) D. G. Loughhead, *J. Org. Chem.* **1985**, *50*, 3931-3934; (d) G. R. Pettit, R. F. Mendonça, J. C. Knight, R. K. Pettit, *J. Nat. Prod.* **2011**, *74*, 1922-1930; (e) C.-X. Huang, Y. Shi, J.-R. Lin, R.-H. Jin, W.-S. Tian, *Tetrahedron Lett.* **2011**, *52*, 4123-4125; (f) G. Deniau, K. T. Sillar, D. O'Hagan, *J. Fluorine Chem.* **2008**, *129*, 881-887.

[9] (a) J. F. Hartwig, *J. Am. Chem. Soc.* **2016**, *138*, 2-24; (b) T. Newhouse, P. S. Baran, *Angew. Chem.* **2011**, *123*, 3422-3435; *Angew. Chem. Int. Ed.* **2011**, *50*, 3362-3374; (c) D. Y. K. Chen, S. W. Youn, *Chem. Eur. J.* **2012**, *18*, 9452-9474; (d) Y. Qiu, S. Gao, *Natural Product Reports* **2016**, *33*, 562-581.

[10] (a) T. W. Lyons, M. S. Sanford, *Chem. Rev.* **2010**, *110*, 1147-1169; (b) X. Yang, G. Shan, L. Wang, Y. Rao, *Tetrahedron Lett.* **2016**, *57*, 819-836.

[11] G. Drefahl, K. Ponsold, H. Schick, *Chem. Ber.* **1964**, *97*, 3529-3535.

[12] R. Giri, J. Liang, J. G. Lei, J. J. Li, D. H. Wang, X. Chen, I. C. Naggar, C. Guo, B. M. Foxman, J. Q. Yu, *Angew. Chem.* **2005**, *117*, 7586-7590; *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 7420-7424.

[13] (a) T. G. Gant, A. I. Meyers, *Tetrahedron* **1994**, *50*, 2297-2360; (b) G. S. K. Wong, W. Wu, in *Oxazoles: Synthesis, Reactions, and Spectroscopy*, John Wiley & Sons, Inc., **2004**, pp. 331-528.

[14] H. Vorbrüggen, K. Krolikiewicz, *Tetrahedron Lett.* **1981**, *22*, 4471-4474.

[15] For examples of palladium-catalyzed C-H functionalyzation of substrates with alkenyl fragments, see: (a) M. Wasa, J.-Q. Yu, *J. Am. Chem. Soc.* **2008**, *130*, 14058-14059; (b) S.-Y. Zhang, G. He, Y. Zhao, K. Wright, W. A. Nack, G. Chen, *J. Am. Chem. Soc.* **2012**, *134*, 7313-7316; (c) G. Shan, X. Yang, Y. Zong, Y. Rao, *Angew. Chem.* **2013**, *125*, 13851-13855; *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 13606-13610; (d) W.-H. Rao, B.-B. Zhan, K. Chen, P.-X. Ling, Z.-Z. Zhang, B.-F. Shi, *Org. Lett.* **2015**, *17*, 3552-3555.

[16] A. F. S. André, H. B. MacPhillamy, J. A. Nelson, A. C. Shabica, C. R. Scholz, *J. Am. Chem. Soc.* **1952**, *74*, 5506-5511.

[17] (a) M. Tanabe, D. F. Crowe, *J. Org. Chem.* **1963**, *28*, 3197-3199; (b) V. A. Khripach, V. N. Zhabinskii, A. I. Kotyatkina, G. P. Fando, A. S. Lyakhov, A. A. Govorova, M. Groen, J. van der Louw, A. de Groot, *Mendeleev Commun.* **2001**, 144-145.

[18] K. Schwekendiek, F. Glorius, *Synthesis* **2006**, *2006*, 2996-3002.

[19] K. B. Sharpless, M. A. Umbreit, M. T. Nieh, T. C. Flood, *J. Am. Chem. Soc.* **1972**, *94*, 6538-6540.

[20] J. Fried, J. A. Edwards, *Organic Reactions in Steroid Chemistry*, Van Nostrand Reinhold Comp., New York, **1972**.

[21] L. F. Fieser, *J. Am. Chem. Soc.* **1953**, *75*, 5421-5422.

[22] D. H. R. Barton, D. J. Lester, S. V. Ley, *J. Chem. Soc., Perkin Trans. 1* **1980**, 2209-2212.

[23] K. Chen, C. Liu, L. Deng, G. Xu, *Steroids* **2010**, *75*, 513-516.

[24] ^{13}C NMR spectral data of the synthesized compound were in a good agreement with those reported for 20OH-NorMD (**3**) obtained by biotechnological approach[1b, c] while small chemical shift differences were observed in the ^{1}H NMR spectra (see SI).

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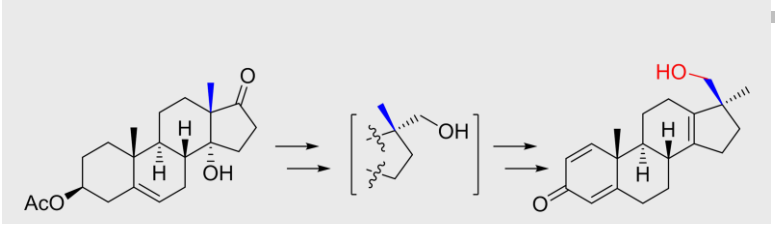

Palladium-catalyzed C-H acetoxylation was used in the first chemical synthesis of 17β-hydroxymethyl-17α-methyl-18-nor-13-ene steroids for inversion of configuration at quaternary stereocenter of the readily available synthetic intermediate.

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