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Synthesis of Menarandroside A from Dehydroepiandrosterone

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Abstract

Menarandroside A, which bears a 12α-hydroxypregnenolone steroid backbone, was isolated from the plant, *Cynanchum menarandrense*. Treatment of extracts from this plant containing menarandroside A against secretin tumor cell line (STC-1) intestinal cells, resulted in an increased secretion of glucagon-like peptide 1 (GLP-1), a peptide that plays a role in the regulation of blood sugar levels. Increase in GLP-1 is beneficial for the treatment of type 2 diabetes. We disclose the synthesis of menarandroside A from dehydroepiandrosterone (DHEA). Key features of this synthesis include: (i) Wittig reaction of the C17-ketone of a 12-oxygenated DHEA derivative to introduce the C17-acetyl moiety, and (ii) the stereoselective reduction of a C12-keto intermediate bearing an sp^2-center at C17 to yield the C12α-hydroxy group. In addition, an oxidation of a methyl enol ether derivative to an α-hydroxy methyl ester using tetrapropylammonium perruthenate (TPAP) and N-methyl-morpholine-N-oxide (NMO) was discovered.

1. Introduction

Menarandroside A (**1**), a 12α-hydroxypregnenolone derivative, has been identified from plant extracts of *Cynanchum menarandrense*, which stimulated the secretion of glucagon-like peptide 1 (GLP-1) in secretin tumor cell line (STC-1) intestinal cells (Figure 1, **1**).[1] In patients with type 2 diabetes, GLP-1 is lowered.[2] Therefore, accessing the natural product and its derivatives could help identify possible treatments for diabetes and other diseases.[3] Here, we report a synthesis of menarandroside A (**1**) from dehydroepiandrosterone (DHEA).

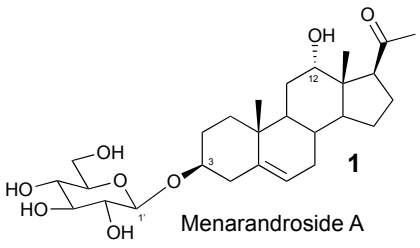


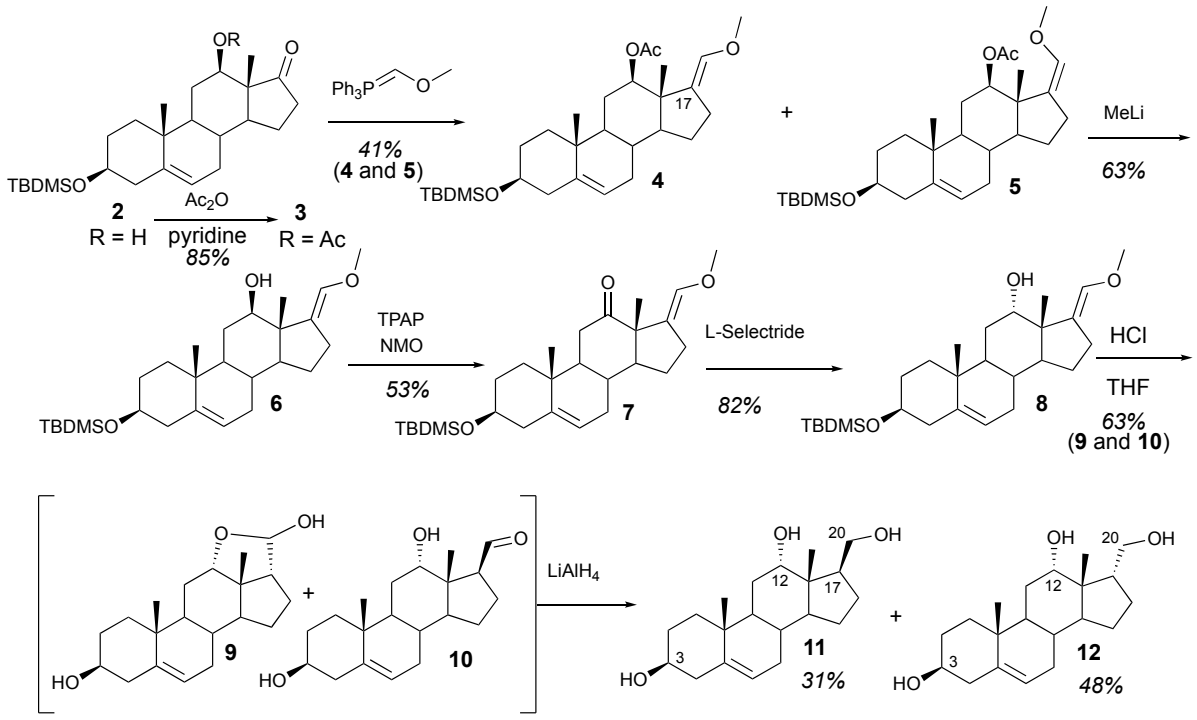
Figure 1. Structure of menarandroside A (**1**).

2. Results and Discussion

Synthesis of 12α-Hydroxypregnenolone from Dehydroepiandrosterone (DHEA)

The 3-TBDMS ether of 12β-hydroxy DHEA (**2**) was synthesized from DHEA according to our previous studies.[4,5] The 12β-hydroxy group of **2** was protected as acetate **3** with Ac_2O and pyridine (Scheme 1). The C17-ketone of **3** was treated with the ylide, methoxymethylidene-triphenylphosphorane, to extend the side chain of the D ring to yield methyl enol ether **4**. This Wittig reaction yielded a mixture of E- and Z- isomers (**4** and **5**) in a 3 to 1 ratio, which co-eluted by column chromatography. The resulting acetate at C12 was cleaved with methyl lithium to yield

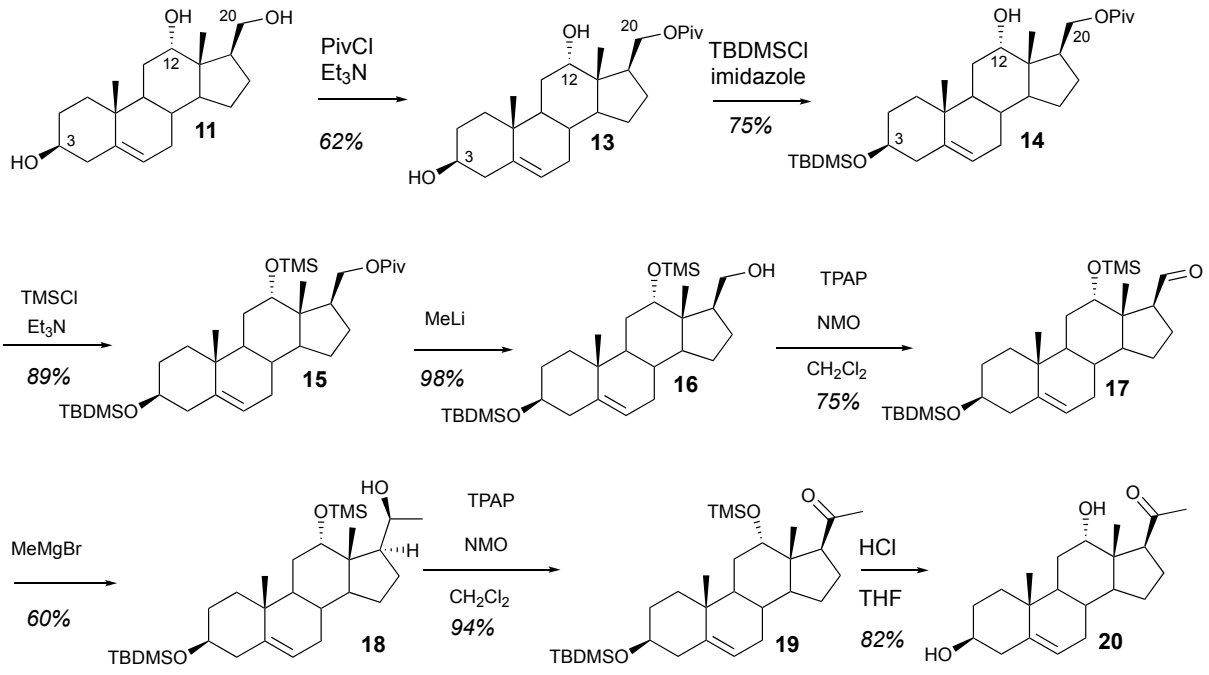
the 12β-hydroxy group (compound **6**). At this stage, the E-isomer of the methyl enol ether product was separable from the Z- isomer by column chromatography, and the E-isomer was carried forward in subsequent steps. The alcohol at the C12-position of **6** was oxidized under Ley-Griffith conditions to afford the C12-ketone (**7**). When the Z-isomer bearing the 12-hydroxy group was oxidized under the Ley Griffith condition, the methyl enol ether would hydrolyze to afford the aldehyde. Subsequent reduction of ketone **7** with L-Selectride gave the desired C12α-hydroxy product (**8**). Methyl enol intermediate **8** was refluxed with HCl in THF/H_2O for 12 hours to yield an inseparable mixture of the lactol and aldehyde in 60% yield (**9** and **10**, 1:1, this ratio was determined by comparing the integrals of the acetal proton and the aldehyde proton at δ 5.16 and δ 9.79, respectively). In addition to the lactol/aldehyde (**9**/**10**) mixture, a cyclic methyl acetal was isolated (7%, see SI Part 1), which presumably could be further hydrolyzed to **9**/**10** after longer treatment with HCl in THF/H_2O. The mixture of lactol **9**/aldehyde **8** was subsequently reduced with $LiAlH_4$ to afford the two triol diastereomers, **11** and **12,** in 31% and 48% yield, respectively. These diastereomers (**11** and **12**) were separable by column chromatography. Although triol **11** with the desired C17β-stereochemistry was obtained in a low yield (31%) relative to triol **12** (48% yield), a synthetic route was developed to convert the C17α-stereocenter of triol **12** to the desired C17β- through epimerization of a C17-aldehyde intermediate under basic conditions (see SI Part 7, Scheme S7-2, **S7-9** to **17**).



Scheme 1. Synthesis of triol intermediate **11**.

The primary alcohol of **11** was selectively protected with pivaloyl chloride as the pivaloyl ester **13** (Scheme 2). The resulting diol (**13**) was regioselectively protected at C3 with TBDMSCl and imidazole to yield alcohol **14**. Alcohol **14** was protected as TMS ether **15** with TMSCl in the presence of triethylamine. Pivalate ester **15** was deprotected with methyl lithium to yield the primary alcohol (**16**). Alcohol **16** was oxidized under Ley-Griffith conditions to give the aldehyde **17**, which was treated with MeMgBr to yield carbinol **18**. The C20-stereocenter of the major diastereomer of the Grignard adduct (**18**) was assigned as the S stereochemistry from the comparison of the ^{1}H NMR spectrum of a previously synthesized steroid derivative.[6] The C18-methyl group of the major diastereomer of the Grignard adduct had a chemical shift of δ 0.68 ppm (labeled as **18** in the experimental section) while the minor diastereomer had a chemical shift of δ 0.75 ppm (labeled as **18b**) – these chemical shifts match the ones from our previous analysis.[6] The

resulting carbinol (**18**) was oxidized under Ley Griffith conditions to yield desired ketone **19**. The cleavage of the TMS and TBDMS protecting groups with HCl in THF afforded 12α-hydroxypregnenolone **20**.

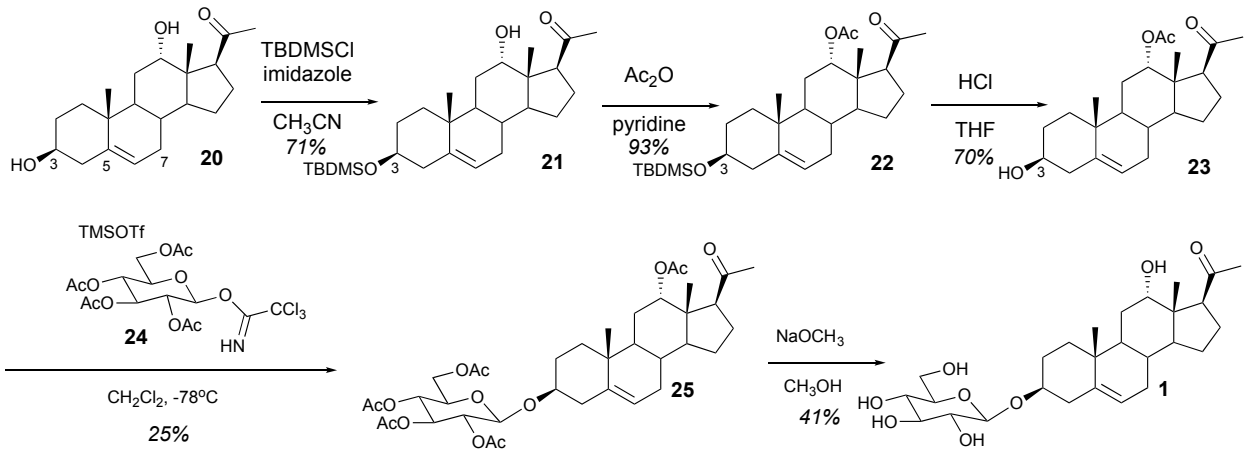


Scheme 2. Synthesis of 12α-hydroxypregnenolone (**20**) from triol **11**.

Synthesis of Menarandroside A from 12α-Hydroxypregnenolone – Sugar Coupling at C3

In order to access menarandroside A (**1**) from 12α-hydroxypregnenolone (**20**), the C12α-hydroxy group was protected as the acetate to set the stage for coupling the sugar group at C3 (Scheme 3, **20** to **23**). First, 12α-hydroxypregnenolone (**20**) was protected as the C3-TBDMS ether (**21**) with TBDMSCl and imidazole. The resulting TBDMS ether (**21**) was protected with Ac_2O in pyridine to yield the C12-acetate (**22**). The silyl group was selectively deprotected with HCl in THF/H_2O to yield 12α-acetoxypregnenolone (**23**). The free alcohol at C3 was subsequently coupled with

trichloroacetimidate **24** to yield the polyacetylated steroid (**25**). Although other options to form the glycosidic bond have been previously reported[7] (also see SI Part 8), the desired stereochemistry at the anomeric carbon was achieved by using the trichloroacetimidate coupling partner in the presence of TMS triflate at -78 °C.[8] Polyacetate **25** was deprotected with $NaOCH_3$ and CH_3OH to yield menarandroside A (**1**).

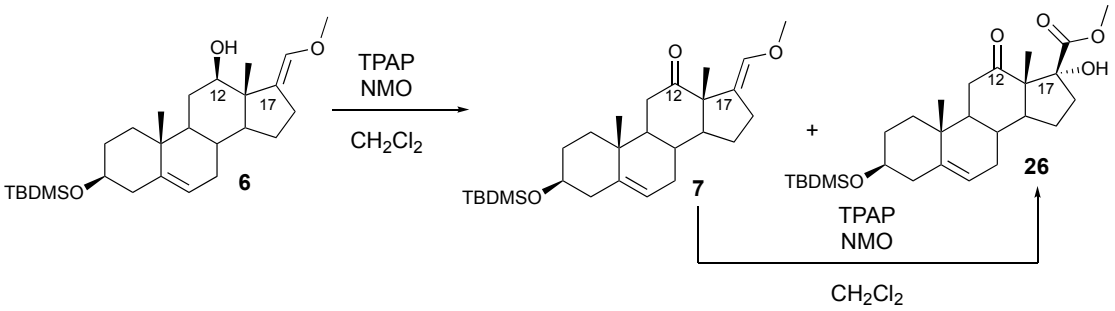


Scheme 3. Synthesis of menarandroside A (**1**) from 12α-hydroxypregnenolone (**20**).

A New Oxidation of the Methyl Enol Ether Functional Group under Ley-Griffith Conditions

The identification of the Ley-Griffith conditions to oxidize methyl enol ether **6** to 12-ketone **7** was not a trivial task (Scheme 1, **6** to **7**). In preliminary attempts to oxidize the C12 hydroxy position of **6** (i.e. Dess-Martin periodinane, Oppenauer oxidation, pyridinium chlorochromate and Swern), the methyl enol ether functional group at C17 was labile. In the end, the mild Ley-Griffith oxidation conditions consistently yielded the desired C12-ketone (Scheme 4, **6** to **7**) while keeping the methyl enol ether group at C17 intact. Interestingly, the methyl ester (**26**) was also obtained

during these conditions. The crystal structure of the methyl ester was obtained (Figure 2, **26**). In order to understand the formation of the undesired methyl ester product (Scheme 4, **6** to **26**), various conditions were performed, which involved adjusting the stoichiometry of the oxidant, N-methylmorpholine N-oxide (NMO) (summarized in Table 1). Adding excess NMO (20 mol equivalents) resulted in the formation of the methyl ester (**26**). All oxidations at C12 (Scheme 3) required the presence of TPAP (i.e. when no TPAP was present, no reaction was observed).



Scheme 4. Successful oxidation at C12 of 12β-hydroxy intermediate (**6**) to the C12-ketone (**7**) using the Ley-Griffith oxidation conditions. Table showing optimization conditions to understand methyl ester (**26**) formation.

Table 1. Summary of conditions to form methyl ester **26** from **6** or **7**.

Entry	Starting Material	TPAP	NMO	time	Ketone **7**	Methyl Ester **26**
1	**7**	0.05 eq	20 eq	60 h	56%[a]	44%[a]
2	**7**	0.05 eq	20 eq	18 h	59%[a]	41%[a]
3	**6**	0.10 eq	20 eq	36 h	28%[b]	78%[b]
4	**7**	0.05 eq	5 eq	60 h	71%[b]	29%[b]
5	**7**	0.05 eq	10 eq	60 h	49%[b]	51%[b]
6	**7**	0.05 eq	10 eq	18 h	66%[b]	34%[b]
7	**7**	0.05 eq	5 eq	60 h	76%[b]	24%[b]
8	**7**	0.1 eq	1.5 eq	0.5 h	71%[c]	15%[c]

[a]: percentage of ketone and methyl ester determined from the crude reaction mixture of the ^{1}H NMR spectrum and integrating the singlets at δ 3.6 ppm and 3.8 ppm respectively. [b]: percentage of ketone and methyl ester determined from the crude reaction mixture of the ^{1}H NMR spectrum and integrating the multiplets at δ 6.4 ppm (vinyl proton) and 4.7 ppm (C17 O-H proton) respectively. [c]: isolated yields.

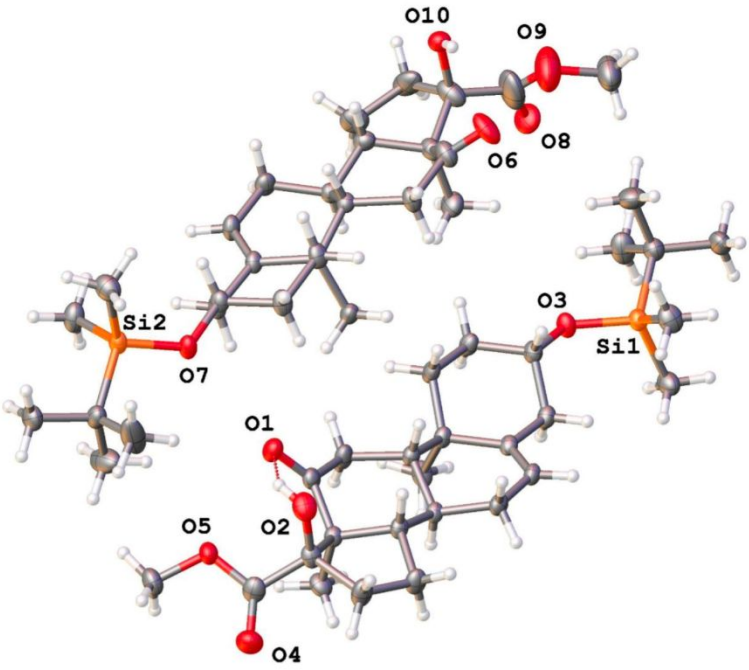


Figure 2. Crystal structure of methyl ester **26**.

3. Conclusion

In conclusion, menarandroside A (**1**) was synthesized from DHEA. Key transformations of this synthesis include: (i) a mild Ley-Griffith oxidation of the 12β-hydroxy intermediate to retain the methoxy vinyl substituent at C17 (Scheme 1, **6** to **7**), (ii) a stereoselective reduction of a C12-ketone bearing a methoxy vinyl substituent at the C17-position to yield the desired 12α-hydroxy product (Scheme 1, **7** to **8**), and (iii) the coupling of the sugar at C3 (Scheme 3, **23** to **25**).

4. Materials and Methods

Bruker instruments (500 MHz: TopSpin 3.5pl6 on Windows 7/11.7 Tesla/54 mm bore/Prodigy CryoProbe/5 mm probe with PFG $^{1}H/^{19}F$, and 300 MHz: TopSpin 3.5pl6 software on Windows 7,

7.05 Tesla, 54 mm bore) were used to record the NMR spectra of all synthesized compounds. LTQ Orbitrap XL connected to a Waters Acuity UPLC was used to obtain high resolution mass spectra of all synthesized compounds (XCalibur Software). IR spectra were taken on a Nicolet iS50 FT-IR spectrometer (Thermo Fischer Scientific, Waltham, MA). Melting points were taken on a Global Medical and Lab Solutions instrument (India). Optical rotations were measured on a JASCO P-1010 polarimeter (Easton, MD) instrument. NMR solvents for $CDCl_3$ and CD_3OD were referenced to δ 7.26 ppm and δ 3.31 ppm for the proton NMR spectra and δ 77.16 ppm and δ 49.00 ppm for the ^{13}C NMR spectra, respectively.[9]

Chemical Syntheses:

See Supporting Information (SI) file for the syntheses of compounds in the main text.

X-Ray Crystallography for Methyl Ester 26:

Single crystals of $C_{54}H_{88.25}O_{9.25}Si_2$ (methyl ester **26**) were prepared by slow evaporation of an acetone solution. A suitable colorless plate-like crystal with dimensions of 0.133 mm × 0.069 mm × 0.035 mm, was mounted in paratone oil onto a nylon loop. All data were collected at 100.0(1) K, using a XtaLAB Synergy/ Dualflex, HyPix fitted with CuK_α radiation (λ = 1.54184 Å). Data collection and unit cell refinement were performed using *CrysAlisPro* software.[10] The total number of data were measured in the range 4.93° < 2θ < 153.2°, using ω scans. Data processing and absorption correction, giving minimum and maximum transmission factors (0.698, 1.000) were accomplished with *CrysAlisPro*[10] and *SCALE3 ABSPACK*[11], respectively. The structure, using Olex2[12], was solved with the ShelXT[13] structure solution program using direct methods and refined (on F^2) with the ShelXL[14] refinement package using full-matrix, least-squares techniques. All non-

hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atom positions were determined by geometry and refined by a riding model. All oxygen bound hydrogen atom positions, on compound **26** were placed on a calculated position. The crystal structure of compound **26** contains two molecules in the asymmetric unit. The molecule with the silicon atom, Si2, is a 75/ 25% mixture of compound **26** and the starting material of compound **6**, respectively. Structure of the crystal is shown in Figure 2.

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