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Improved Procedures for Gram-Scale Synthesis of Galeterone 3β-Imidazole and Galeterone 3β-Pyridine Methoxylate, Potent Androgen Receptor/Mnk Degrading Agents

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Keywords: C3-imidazole/pyridyl methoxylate synthesis, androgen receptor/Mnk degrading agents, aminosteroids, anticancer agents.

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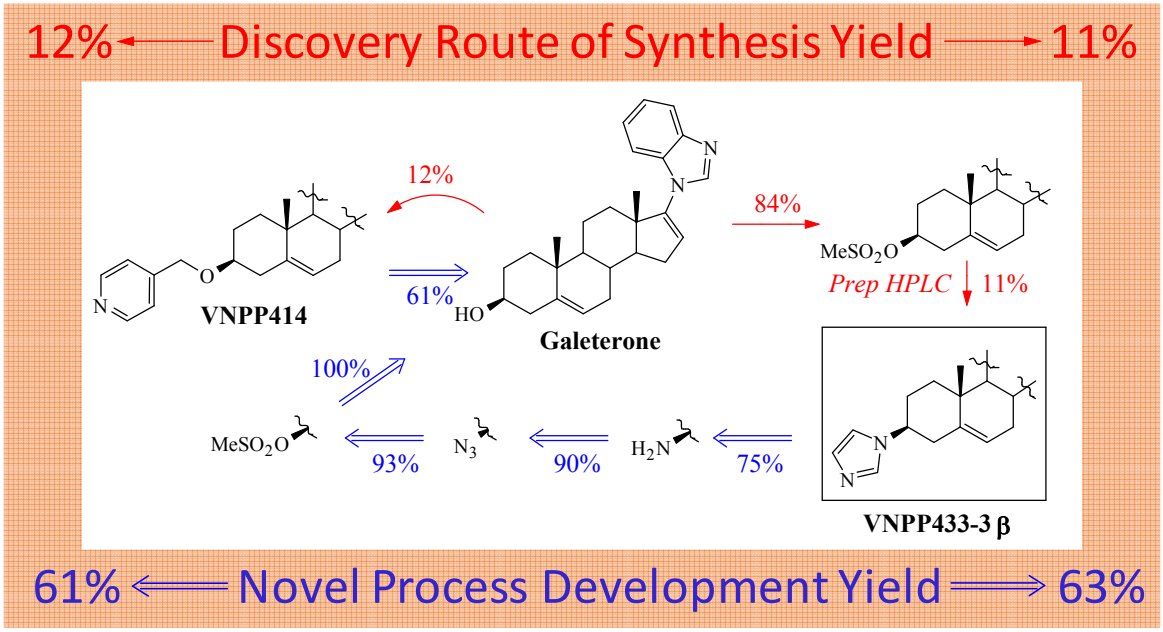
12% ← Discovery Route of Synthesis Yield → 11%
12%
61%
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VNPP414
Galeterone
MeSO2O
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75%
VNPP433-3 β
61% ⟸ Novel Process Development Yield ⟹ 63%

ABSTRACT

Galeterone (**1**) and its C-3 analogs are of substantial interest because of their multi-target anticancer activities, including AR and Mnk degrading activities. Here, we describe improved and efficient procedures for the gram-scale synthesis of 3β-(1*H*-imidazole-1-yl)-17-(1*H*-benzimidazole-1-yl)-androsta-5,16-diene (galeterone 3β-imidazole, **2**) and 3β-(pyridine-4-ylmethoxy)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (galeterone 3β-pyridine methoxylate, **3**). Whereas compound **2** was synthesized in 63% overall yield from galeterone (**1**) over four steps, *via* key intermediate, 3β-azido galeterone (**8**); compound **3** was synthesized in 61% overall yield from **1** in one step. This article also reports on the facile synthesis of other potential AR/Mnk degrading agents (ARDAs/MNKDAs), including galeterone 3α-imidazole (**5**) and galeterone 3β-amine (**10**), both in excellent overall yields. Notably, except for the one-step synthesis of compound **3** which required purification by flash column chromatography, none of the intermediates and target compound **2** required extensive chromatographic purifications or multiple crystallizations.

INTRODUCTION

In the course of studies to develop potent androgen receptor degrading agents (ARDAs),[1] using our phase 3 clinical candidate, galeterone (**1**) (**Figure 1**)[2, 3] as lead, to modulate AR signaling in prostate cancer models,[1] we discovered that these novel ARDAs also effectively target oncogenic eukaryotic protein translation, via modulation of Mnk-eIF4E axis. Since we determined that these agents also suppress oncogenic peIF4E via degradation of Mnk1 and 2, they are also referred to as Mnk degrading agents (MNKDAs). We note that these targets have been implicated in the development, progression, metastasis and drug resistance of a variety of cancers, including prostate[4-8] and pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC).[9-12] These studies enabled us to synthesize and identify 3*β*-(1*H*-imidazole-1-yl)-17-(1*H*-benzimidazole-1-yl)-androsta-5,16-diene (galeterone 3*β*-imidazole, **2**) (**Figure 1**) and 3*β*-(pyridine-4-ylmethoxy)-17-(1H-benzimidazol-1-yl)androsta-5,16-diene (galeterone 3*β*-pyridine methoxylate, **3**) (**Figure 1**) as promising new leads with superior anti-prostate cancer activities compared to galeterone (1).[13, 14] However, these two promising lead compounds, **2** and **3**, were obtained in very low discouraging overall yields of 11 and 12%, respectively.

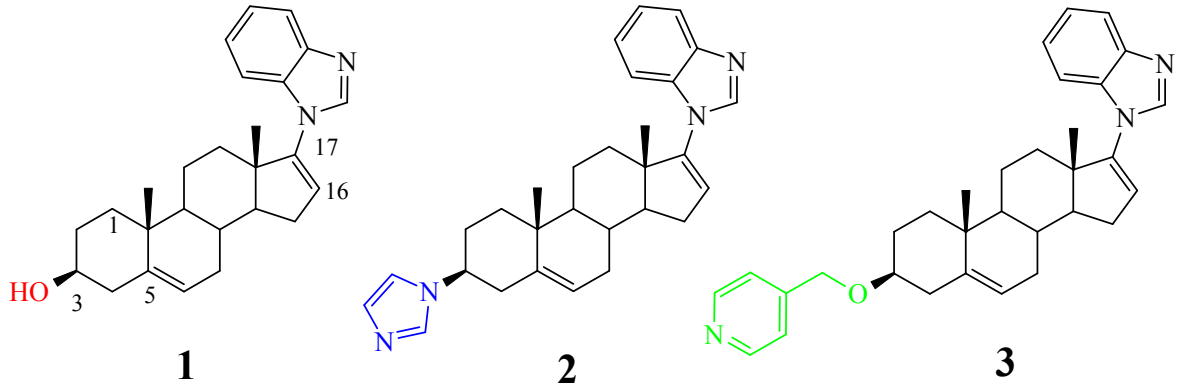
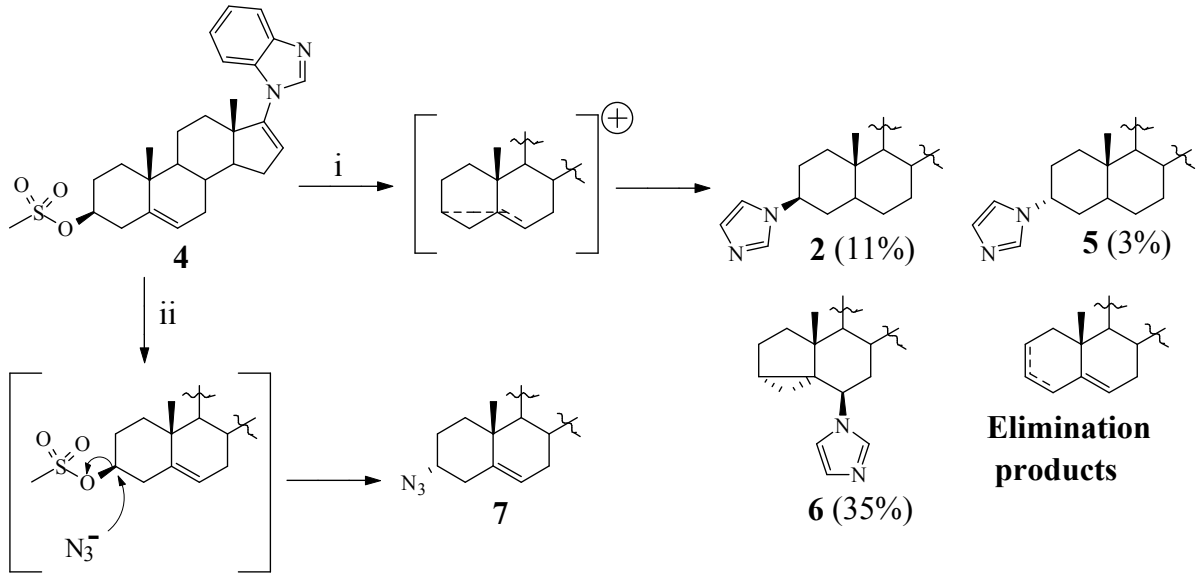


Figure 1: Structure of galeterone (1) and new analogs 2 and 3.

Our strategy to improved and efficient procedures for the gram-scale synthesis of compounds **2** and **3**, was based on critical analyses of our prior synthetic procedures, including the by-products that were obtained. First, the treatment of 3*β*-mesyl galeterone (**4**) with imidazole in refluxing toluene afforded the desired compound **2** (11%), stereo 3α-isomer (**5**, 3%), positional 6*β*-isomer of 3α, 5α-cycloandrostane (**6**, 35%) and uncharacterized elimination products (**Scheme 1**).[13, 14] We note that these three products were isolated following tedious flash column chromatography and preparative HPLC procedures. Similar products were also obtained when the reaction was conducted in pyridine at 85 ^{o}C.



Scheme 1: Influence of nucleophiles on reaction of Δ^5 steroids[a]

[a]***Reagents and conditions:*** (i) imidazole, $PhCH_3$, reflux, 12 h or pyridine/DMF, 85 ^{o}C; (ii) DMF, NaN_3, 15-crown-5, 80 ^{o}C, 18 h.

Based on literature precedence,[15-19] the basis for the formation of stereo/positional isomers in our previous reported synthetic method for **2** is depicted in **Scheme 1**.[13, 14] Formation of the 6*β*-substituted imidazole (**6**) as major product is probably a result of ionization of mesylate prior to the attack of nucleophile at C3 position (S_N1 mechanism), which further forms

homoallylic hybrid carbonium ion intermediate due to the participation of C5 double bond as previously reported by other investigators.[15-19] Attack of nucleophile on hybrid carbonium ion has been demonstrated to be faster at C6 position than at C3 due to the difference in their reactivity.[20] However, strong nucleophile, such as, azide predominantly follows S_N2 mechanism to yield stereo inverted C3-azide (**7**).[21] From these observations, it is clear that the nature of nucleophile has influence on mechanism of S_N reaction of Δ^5 steroids. Weak nucleophile such as imidazole predominantly follow S_N1 mechanism while reactive nucleophiles follow S_N2 mechanism. Therefore, direct installation of imidazole unit at C3 position in the presence of C5 double bond may be impractical. Thus, synthesis of imidazole ring starting from azide by functional group modification provides an attractive route as described below.

Second, for the synthesis of the pyridyl methoxylate (**3**), the strategy was to improve on our reported procedure of Williamson's etherification following treatment of galeterone (**1**) with 4-(bromomethyl)pyridine hydrobromide in the presence of sodium hydride as base in DMF at 65 ^{o}C.[13, 14] Specifically, we explored using different solvents and salt effects. This strategy proved successful as described below.

RESULTS AND DISCUSSION

Synthesis of galeterone 3*β*-imidazole (2): We first envisioned and designed a new synthetic route that would enable the tethering of the imidazole moiety to C3 (3*α*/*β*-isomers) of galeterone (**12/1**) as depicted in **Figure 2A**. Retro-synthetically, the 3*β*-imidazole ring can be constructed from 3*β*-amine (**10**) by applying Heinrich Debus cyclization method,[22, 23] where the 3*β*-amine (**10**) would be obtained from selective reduction of key intermediate, 3*β*-azide (**8**), without affecting the double bonds in rings B and D.[24] Next, we envisioned two possible routes for the

synthesis of 3β-azide (**8**). The first method involves conversion of galeterone (**1**) to the 3β-mesylate (**4**) followed by treatment with trimethylsilyl azide ($TMSN_3$) and boron trifluroride etherate (BF_3•OEt_2) in dichroromethane (DCM). This procedure is termed, Lewis acid mediated *i*-steroid/ retro-*i*-steroid rearrangement method (**Figure 2B**).[24, 25] Alternatively, **8** can also be obtained from epi-galeterone (**12**) either by Mitsunobu method of using hydrogen azide or via treatment of α-mesyl derivative (**13)** with NaN_3 (**Figure 2B**). Both methods involving epi-galaterone (**12**) are known to produce the azide as major product, with inversion of configuration.[21, 26]

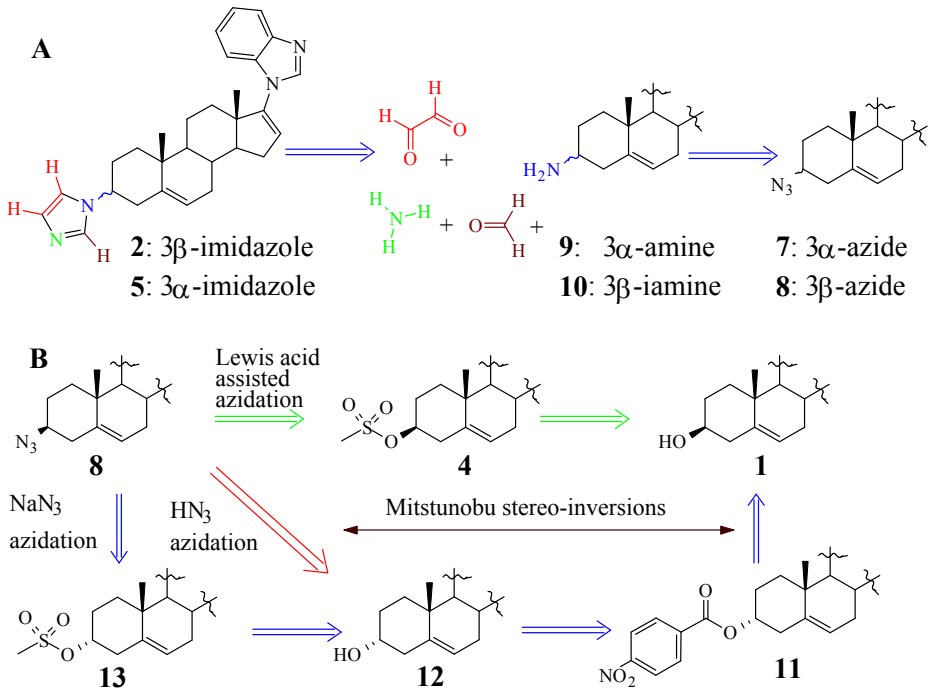


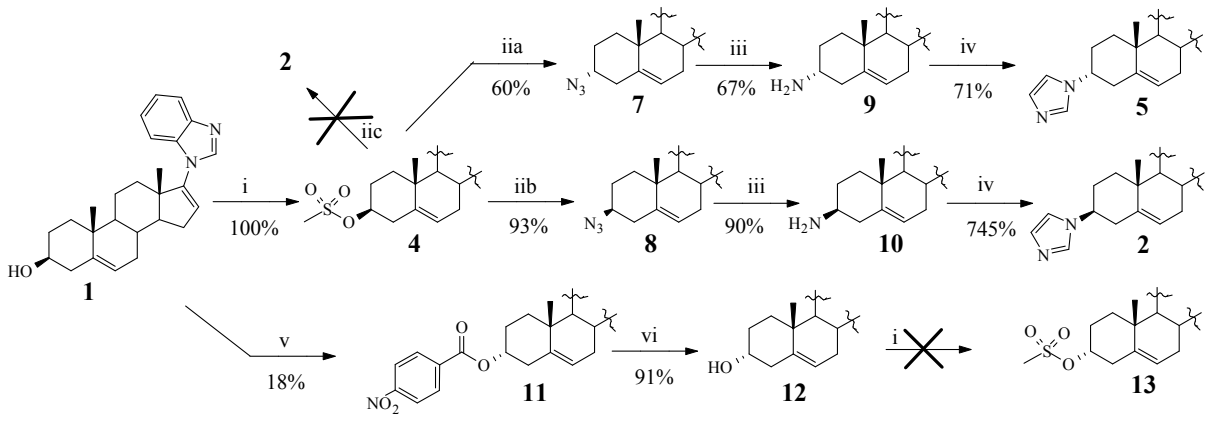
Figure 2A & B: Retrosynthetic procedure for synthesis of compounds 2 and 5 and key intermediate 8.

The Heinrich Debus method for the synthesis of imidazole ring from primary amines is widely reported in the literature.[22, 27, 28] However, its applicability to aminosteroids has not been reported.

Before initiating any efforts towards the stereospecific synthesis of our key intermediate, 3β-azide (**8**), we considered it prudent to first evaluated the synthetic feasibility of imidazole ring from amine at C3 position of steroid using easily accessible 3α-azide (**7**), previously reported by our group.[21] In addition, the resultant galeterone 3α-imidazole (**5**), a possible metabolite of galeterone 3β-imidazole (**2**) would be valuable during pharmacokinetics (PK) and other *in vivo* studies.

Accordingly, our experimentation began with the mesylation of galaterone (**Scheme 2**). Although we have reported the milligram scale synthesis of 3β-mesyl galeterone (**4**), in 84% yield, using pyridine as solvent and base, its application in gram scale yield intractable mixture of products.[1] Therefore, we modified the procedure, by replacing DCM as solvent and using triethyl amine (TEA) as base.[24] This modification gave **4**, in 100% yield after simple solvent removal, water wash of residue solids, filtration and drying under vacuum. The conversion of **4** to 3α-azide (**7**) was achieved in relatively shorter time (18 h) in comparison to our previous method (48 h, 60% yield),[21] by conducting the reaction in the presence of catalytic amount of 15-crown-5 (yield 60%).[29] For the reduction of 3α-azide **7** to 3α-amine **9**, a reported $LiAlH_4$ in ether method a gave good yield (60 %).[24] Considering the requirement of anhydrous condition and difficulties in handling pyrophoric reagent in scale-up, we looked for a nonhazardous reduction method. Application of Staudinger method of azide reduction, using PPh_3 in THF/MeOH/water (4:4:1) at 60 °C smoothly converted **7** into 3α-amine, **9** within 10 h.[30] The product, in 67% yield, was isolated by simple acid-base workup. The cyclization of **9** into 3α-imidazole (**5**) was achieved by reacting with aq. ammonia, formaldehyde and glyoxal at 70 °C for 5 h.[27] As in the case of amine, the imidazole product was also isolated by acid base workup and further purified by passing through a plug of silica using 1-5% metanol in ethyl acetate. The pure product

obtained was reprocessed with acid-base to obtain solvent free product in 71% yield. This route of synthesis gave 28% overall yield of galeterone 3α-imidazole (**5**) starting from **4** in three steps (β-mesyl $\rightarrow$ α-N_3 $\rightarrow$ α-NH_2 $\rightarrow$ α-imidazole). By these test reactions using 3α-azide (**7**), we not only establish the viability of our intended synthetic strategy, but also established reaction conditions and purification procedures which were extended to the synthesis of the desired galeterone 3β-imidazole (**2**).



Scheme 2: Stereo-elective synthesis of galeterone 3β-imidazole (2) and galetrone 3α-imidazole (5)[a].

[a]***Reagents and conditions****:* (i) anhydrous DCM, TEA, 4 ºC, Mesyl chloride, 30 min, ~22 ºC, 16 h; (iia) anhydrous DMF, NaN_3, 15-Crown-5, 80 ºC, 18 h; (iib) anhydrous DCM, $TMSN_3$, $BF_3.OEt_2$, ~22 ºC, 5 h, 2M aq. NaOH, ~22 ºC, 6 h; (iic) anhydrous DCM, TMS-imidazole, $BF_3.OEt_2$, ~22 ºC, 12-48 h, 2M aq. NaOH, ~22 ºC, 5-20 h; (iii) PPh_3, THF/MeOH/water (4:4:1), 60 ºC, 3-10 h; (iv) NH_3 (aq. 25%), dist. H_2O, MeOH, glyoxal trimer dihydrate, HCHO (aq. 36%), 70 ºC, 5 h;(v) p-nitrobenzoicacid, PPh_3, THF, 4 ºC, DEAD (40% in Toluene), ~22 ºC, 12 h. vi) THF/MeOH (2:1), aq. NaOH (1.0 N), ~22 ºC 2.5 h.

Having demonstrated the working synthetic strategy, we directed our efforts towards the synthesis of key intermediate 3β-azide (**8**). Recently, Sun *et al.* reported a practical synthesis for the conversion of cholest-5-en-3β-ol methanesulfonate to 3β-amino-5-cholestene with retention of configuration in 93% yield).[24] The method involves use of $TMSN_3$ in the presence of Lewis acid which led to *C3 steroidal azidation with complete stereo-retention*.

Thus, we attempted this method on 3β-mesylate (**4**) using the reported ratio of reagents and also doubled the quantity of reagents with longer duration of reaction time. Neither of these reaction conditions indicated formation of the expected 3β-azido product, **8**. We therefore pursued the epi-galaterone (**12**) route for the synthesis of 3β-azido (**8**) (**Scheme 2**). The desired *epi*-galaterone (**12**) was obtained from galeterone (**1)** by Mitsunobu stereo-inversion method.[31] This involves the formation of nitrobenzoicacid 3α-epiester (**11**) and its hydrolysis with aq. NaOH to obtain *epi*-galaterone (**12**). This stereo-inversion method required cumbersome purification procedure due to the polar nature of substrate, product, and by-products, such as, PPh_3O, diethyl 1,2-hydrazinedicarboxylate of reagents PPh_3, DEAD. As a result, it provided only 17% yield of **12** from galeterone (**1**).

For the conversion of *epi*-galaterone (**12**) to desire 3β-azide (**8**) we only attempted mesylation route (**Figure 2B, Scheme 2**) to avoid hazardous nature of hydrogen azide in Mitsunobu method. Unfortunately, our attempts to synthesize 3α-mesyl (**13**) resulted in elimination products along with intractable adducts.

This unexpected difficulty in functional group modifications inspired us to revisit the *i*-steroid and retro-*i*-steroid rearrangement method for steroidal 3β-azide synthesis. As stated earlier, Sun *et al* reported that, the method worked efficiently on 3β-cholesteryl mesylate while using 1 equivalent of $TMSN_3$ and 2 equivalents $BF_3.OEt_3$ in DCM.[24] They also stated that, no reaction was observed when solvents bearing heteroatoms that function as Lewis base were used.[24] Based on these data, we reasoned that the failure in our earlier efforts to synthesize 3β-azide (**8**) from 3β-mesylate (**4**) could be due to presence of heteroatoms in the substrate, **4** (i.e.,benzimidazole hetero-nitrogen atoms). Thus, we hypothesized that the amount of BF_3•OEt_2

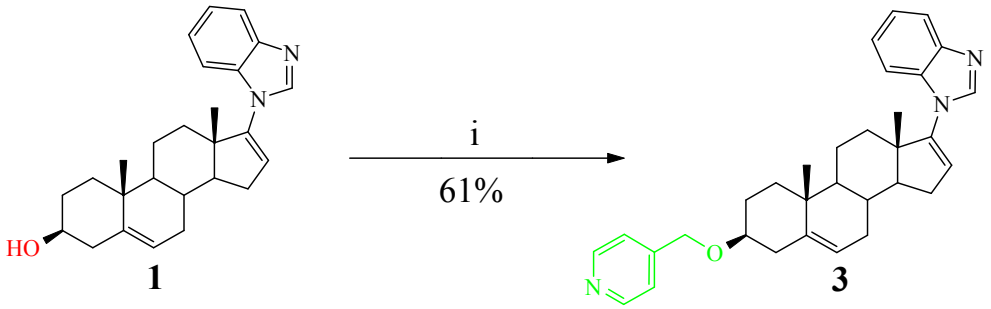
used in the earlier reactions could be consumed by nitrogen atoms of benzimidazole ring of compound **4**. To evaluate this possibility, we setup a reaction using 10 equivalents of BF_3•OEt_2 which indicated very little progress in the reaction as evidenced by TLC and was incomplete even after longer duration of reaction time (48 h). To determine the optimum conditions required for this azidation reaction, we evaluated various ratio of $TMSN_3/BF_3.OEt_3$. We eventually found that the best yield (93%), was obtained *when 15 and 27 equivalents of $TMSN_3$ and $BF_3.OEt_2$, respectively, were used for 1 equivalent of **4** in anhydrous DCM at ~22 ºC for 5 h*. Pure Lewis acid salt free 3β-azido product (**8)** was isolated by filtration after neutralizing the reaction mixture (exothermic) as well as breaking the complex of product with Lewis acid with aqueous NaOH for 6 h.

Finally, reduction of 3β-azide (**8**) to 3β-amine (**10**, 90%) followed by its cyclization, as described about for 3α-amine (7) gave the desired 3β-imidazole (**2)** in 75% yield. Surprisingly, both Staudinger azide reduction and Heinrich Debus method of imidazole synthesis gave better yield in shorter time in the case of β-epimer in comparison to α-epimer. This may be due to easy accessibility of equatorial functions in comparison to axial on steroid scaffold.[32] Functional group modification (β-mesyl $\rightarrow$ β-N_3 $\rightarrow$ β-NH_2 $\rightarrow$ β-imidazole) proceeded rapidly and in high yield (overall yield 63%) *with complete retention of configuration*. The whole process was repeated twice to confirm the yields with standard deviation of ~2%.

We also evaluated the possibility of extension of this *i*-steroid and reto-*i*-steroid method with TMS-imidazole which could provide the desired compound **2** from **4** in one step (**Scheme 2**). Unfortunately, the method was unsuccessful even with various ratios of TMS-imidazole and BF_3•OEt_3; as it gave small amount elimination product and majority of unreacted **4**.

Synthesis of galeterone 3*β*-pyridine methoxylate (3): To improve on our reported low yield synthesis of compound **3**,[13, 14] we initially attempted the synthesis following the procedure reported by Jilka *et al*.[33] where galeterone (**1**) in THF was treated with sodium hydride (NaH) at 0 °C and stirred at room temperature for 10 minutes followed by addition of 4-(bromomethyl)pyridine hydrobromide solution [**14**; THF/DMF (1:1), TEA]. The product was isolated in low yields of 24% although it was slightly higher than our previously 12% yield reported in our recent reports.[13, 14] In an effort to improve the product yield, DMF and DMSO were investigated as alternative reaction solvents to THF/DMF mixture, as well as addition of phase transfer catalyst (tetrabutylmmonium iodide), but insignificant product formation improvement was observed by TLC analysis and isolated product yields.

Salts are known to affect the transition state of S_N2 reactions positively.[34] In attempt to investigate salt effect in our substrate reaction, we incorporated lithium carbonate (Li_2CO_3) which concurrently eliminated neutralization of **14** with TEA. The procedure that we developed is depicted in **Scheme 3**.



Scheme 3: Synthesis of galeterone 3*β*-pyridine methoxylate (3)[a]

[a]***Reagents and conditions:*** (i) NaH, Li_2CO_3, DMF/THF, 0 °C, 4-(bromomethyl)pyridine hydrobromide (**14**), rt, 18 h.

Briefly, galeterone (**1**) in THF was treated with NaH followed by addition of DMF after one minute. Li_2CO_3 followed by reagent **13** were then added to give the product in good yields (61-64%) and unreacted compound **1** was recovered. Several reactions conditions were explored in an attempt to drive the Li_2CO_3 reaction to completion without success. Although we obtained an excellent yield of the desired compound **3**, it is unclear at this time why the reaction could not be driven to completion.

Overall, several important observations were noted. (1) The reaction done with DMF as the solvent produced a green/blue reaction mixture color and no product formed irrespective of the reaction duration while THF revealed low product formation as analyzed by TLC. (2) The appropriate ratio of DMF to THF should not be > 1:2 a ratio of 1:1 gave no product. (3) The optimized DMF to THF ratio of 1:16 was applicable to both milligram (≥ 250 mg) and gram (1-5 g) scales reactions. (4) DMSO and THF (1:2) solvent mixture showed no product formation. (5) Cesium carbonate salt gave 50% of product. (6) The color of the reaction mixture after addition of all reactants should remain colorless for about one hour and gradually change to dark red after 18 h. (7) The developed reaction procedure was conducted at 50 °C as an attempt to optimize the yield but the isolated yield was 45% which was significantly lower compared to 61% as reported at room temperature. In summary, the order of the reagent/solvent addition as well as temperature control during addition was found to be crucial to attaining the desired product.

CONCLUSION

In conclusion, we have developed efficient four-step synthesis of galeterone 3β-imidazole (**2**) and a one-step synthesis of galeterone 3β-pyridine methoxylate (**3**) in 63 and 61% overall yields, respectively, both from galeterone (**1**). We also developed an interesting stereospecific synthesis of galeterone 3α-imidazole (**5**), which could be useful in pharmacokinetics studies of

compound **2**. In addition, the penultimate precursor of compound **2**, 3*β*-amino galeterone (**10**), obtained in 84% overall yield from galeterone (**1**) is also proving to be a potent ARDA/MNKDA. These new and improved procedure for the synthesis of compound **2** do not require column chromatography or multiple crystallizations and both procedures for target compounds **2** and **3** are amendable to commercial productions. The efficient production of these new and improved lead ARDAs/MNKDAs will undoubtedly facilitate our ongoing anti-tumor efficacy studies.

EXPERIMENTAL SECTION

General: Galeterone used in this process development was provided by Tokai Pharmaceutical Inc. (TPI). All other reagents were obtained from Sigma-Aldrich or Acros and were used without further purification. Room temperature means ~22 ºC. Reactions were conducted using oven dried glassware under a positive pressure of argon. Anhydrous tetrahydrofuran (THF, 99.8%, sigma), triethylamine (TEA, 99.5%, sigma), anhydrous *N,N*-dimethylformamide (DMF, 99.8%, Acros), anhydrous Dimethyl sulfoxide (DMSO, 99.8%, sigma) were used as supplied. Reactions were monitored by analytical thin-layer chromatography on Silica plate TLC aluminum baked plates coated with 200 μm silica gel, indicator F254 and Flash Column Chromatography (FCC) was performed using silica gel (230-400 mesh, 60 Å). Melting points were recorded on Fisher-Johns melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker *Ascend 400* spectrometer, and chemical shifts δ are expressed in ppm relative to TMS as internal reference (^{1}H and ^{13}C). 'H and C NMR data's analyzed and reports were generated by using ACD/NMR Processor Academic Edition. High-resolution mass spectrometry was obtained on Bruker 12T APEX-Qe FTICR-MS instrument by positive ion ESI mode by Isaiah Ruhl, Interim

Facility Director, College of Sciences Major instrumentation cluster, Old Dominion University, Norfolk, VA. Purity of intermediates and final compound were determined by HPLC method.

Purity check of compounds used for biological activity (HPLC Chromatograms). The purity of compounds determined by reverse phase on LC system of Waters Acquity Preparative HPLC 2535 Quaternary Gradient Module coupled with a Waters 2489 UV/visible photodiode array detector operated at 254 nm using Novapak C18 4 µl, 3.9 X 150 mm column as the stationary phase at room temperature. **Mobile phase-A** comprised of Water/MeOH/CH_3CN (20: 50:30, v/v/v + 1 mL of TEA) and maintained isocratically at the flow rate of 2.5 mL/min for mesyl (**4**), imidazoles derivatives (**2** and **5**) and epi-galeterone (**12**) compound. Where purity of azide (**7**-**8**) and amines (**9**-**10**) were determined by applying gradient method of using above **Mobile phase-A** and **Mobile Phase-B** contained methanol with flow rate of1.5 mL/min (see chromatograms for detail in SI). Similarly, a gradient method applied for compound **3** by using **Mobile phase-C** comprised of 10 mM ammonium acetate (NH_4OAc) buffer solution and **Mobile phase-D** comprised of Water/MeOH/CH_3CN (35: 35:30, v/v/v + 20 µL of TEA + 77 mg of NH_4OAc) with flow rate of 0.8 mL/min. Purity of all compounds are > 96.5%.

3*β*-(1*H*-imidazol-1-yl)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (**2**). A mixture of amine **10** (6.50 g, 16.8 mmol), ammonia (25% aq., 3.90 mL, 48.3 mmol), distilled water (5 mL) and MeOH (120 mL) at ~22 ºC was added glyoxal trimer dihydrate (5.50 g, 26.2 mmol) and formaldehyde (37% aq., 2.14 mL, 29.3 mmol) simultaneously. The reaction mixture was immediately taken to 70 ºC (pre heated oil bath) and stirred for 2 h, and then additional half more quantity of ammonia and formaldehyde added and continued. When **10** was consumed as evidenced by TLC (~ 3 h), reaction mixture evaporated under vacuum at 60 ºC, reconstituted

with 120 mL of DCM and extracted with 1N HCl (60 mL x 2). Aqueous phase collected washed with DCM and neutralized with 1N aq. NaOH and resulting precipitate is extracted with ethyl acetate (60 mL x 2). Biphasic solution passed through a plug of celite, organic phase collected, washed with water and evaporated to obtain yellowish red sticky crude product. Sticky mass absorbed on silica (1.5 w/w) and passed through a short bed of silica using 1-5% methanol in ethyl acetate to obtain a cream solid **2**. Product is reprocessed with acid-base and collected by filtration to obtain solvent free **2** (5.50 g, 75%), mp 180-184 ºC; R_f = 0.21 (DCM/MeOH/TEA, 10/0.5/0.025); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (s, 3 H, 18-CH_3), 1.14 (s, 3 H, 19-CH_3), 3.87 - 4.00 (m, 1 H, 3α-H), 5.50 (d, *J*=5.14 Hz, 1 H, 6-H), 5.96 - 6.03 (m, 1 H, 16-H), 7.01 (s, 1 H, Ar'-4-H), 7.10 (s, 1 H, Ar'-5-H), 7.27 - 7.35 (m, 2 H, 6-Hs), 7.46 - 7.54 (m, 1 H, Ar-7-H), 7.67 (br. s., 1 H, Ar'-2-H), 7.79 - 7.86 (m, 1 H, Ar-4-H), 7.97 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.1, 143.2, 141.6, 140.0, 135.2, 134.5, 129.0, 124.0, 123.4, 122.5, 122.2, 120.2, 116.8, 111.1, 57.4, 55.8, 50.5, 47.2, 40.5, 37.8, 36.9, 34.8, 31.0, 30.2, 30.2, 29.9, 20.6, 19.3, 16.0; HPLC: t_R 2.26 min 97.58%; HRMS calcd 339.2856 ($C_{26}H_{34}N_4H^+$), found 339.3856.

3*β*-(pyridine-4-ylmethoxy)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (3): Sodium hydride {3.00 g, 125 mmol, 10 eq. (5.00 g of 60% NaH in oil)} was added to a solution of galeterone (**1**, 5.00 g, 12.9 mmol, 1 eq.) in THF (80 mL) at 0 °C under argon atmosphere. A white precipitate formed and after stirring for one minute, DMF (5.0 mL) was added and the reaction mixture was allowed to stir for 10 minutes at room temperature. The reaction mixture was placed at 0 °C and lithium carbonate (5.00 g, 67.7 mmol, 5 eq.) was added, followed immediately by 4-(bromomethyl)pyridine hydrobromide (**13**, 10.0 g, 39.5 mmol, 3 eq.) and the reaction mixture was allowed to stir at 0 °C for 30 minutes. The reaction mixture was removed from 0 °C and stirring continued at ambient temperature for 18 h under argon atmosphere. The

color of the reaction mixture changed from clear to dark red during the 18 h period. After 18 h, the reaction mixture was placed at 0 °C, water (30 mL) was added to quench unreacted NaH, and the mixture was stirred for 10 minute. Volatile (THF) were removed in vacuo and water (70 mL) was added to the residue aqueous phase. The aqueous phase was extracted with ethyl acetate (EtOAc) (150 mL x 3). Ethyl acetate extract was washed with brine (80 mL x 2), dried with anhydrous Na_2SO_4 and concentrated in vacuo to give a dark red crude product. Purification by flash chromatography using 3% MeOH/EtOAc as eluent afforded compound **3** as a white solid (3.78 g, 7.88 mmol, 61%), mp 177-179 °C. R_f = 0.31 (5% MeOH/EtOAc). ^{1}H NMR (400 MHz, CDCl3) δ 1.02 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 3.35 - 3.21 (m, 1 H, 3α-H), 4.59 (s, 2 H, 2″-CH_2), 5.42 (d, J = 5.0 Hz, 1 H, 6-H), 5.96 - 6.00 (m, 1 H, 16-H), 7.27-7.31 (m, 4 H, aromatic and pyridinyl -Hs), 7.48-7.50 (m, 1 H, aromatic-H), 7.80-7.83 (m, 1 H, aromatic-H), 7.96 (s, 1 H, 2′-H), 8.57 (d, J = 5.4 Hz, 2 H, pyridinyl-Hs). ^{13}C NMR (100 MHz, CDCl3) δ 19.3, 20.6, 28.3, 30.2, 30.3, 31.1, 34.8, 37.0, 37.1, 39.0, 47.2, 50.5, 55.8, 68.3, 79.1, 111.1, 120.2, 121.2, 121.7, 122.4, 123.3, 124.0, 134.5, 141.0, 141.6, 143.2, 147.1, 148.2, 149.8. HPLC: t_R 3.721 min 100%; HRMS calcd 502.2828 ($C_{32}H_{37}N_3O2Na^+$, found 502.2834.

3*β*-Mesyloxy-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (4). To a solution of galeterone (12.0 g, 30.9 mmol) in anhydrous DCM (100 mL) at 4 ºC was added TEA (6.46 mL, 46.4 mmol), followed by the addition of a solution of mesyl chloride (2.87 mL, 37.1 mmol) in anhydrous DCM (30 mL) drop wise. The reaction was continued at 4 ºC for 30 min and then stirred at ~22 ºC for total of 16 h. The reaction mixture was concentrated in *vacuo*, residue solid treated with water, filtered and dried to afford **4** (14.4 g, 100%) as a white solid, mp 172-174 ºC; R_f = 0.4 (DCM/ EtOH/TEA, 10:0.25:0.025); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.08 (s, 3 H, 19-CH_3), 3.02 (s, 3 H, Mesyl-CH_3), 4.49 - 4.61 (m, 1 H, 3*α*-H), 5.49 (d, *J*=4.89 Hz, 1 H,

6-H), 5.99 (m, 1 H, 16-H), 7.28 - 7.34 (m, 2 H, Ar-5, 6-Hs), 7.47 - 7.52 (m, 1 H, Ar-7-H), 7.79 - 7.85 (m, 1 H, Ar-4-H), 7.95 (s, 1 H, Ar-2-H); 147.1, 143.2, 141.6, 139.1, 134.5, 124.0, 123.4, 123.1, 122.4, 120.2, 111.1, 81.6, 55.7, 50.2, 47.2, 39.1, 38.8, 36.7, 36.6, 34.7, 31.0, 30.2, 28.8, 20.6, 19.1, 16.0; HPLC: t_R 1.62 min 97.69%; HRMS calcd 955.4472 $(C_{27}H_{34}N_2O_4S)_2Na^+$ (note: dimer formation), found 955.4468.

3*α*-(1*H*-imidazol-1-yl)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (**5**). A mixture of amine **9** (0.20 g, 0.52 mmol), ammonia (25% aq., 0.12 mL, 1.76 mmol), distilled water (0.15 mL) and MeOH (7.5 mL) at ~22 ºC was added glyoxal trimer dihydrate (0.17 g, 0.81 mmol) and formaldehyde (37% aq., 0.07 mL, 0.85 mmol) simultaneously. The reaction mixture immediately taken to 70 ºC (pre heated oil bath) and stirred for 3 h before the addition of one more portion of glyoxal trimer dihydrate (0.16g), formaldehyde (0.067 mL) and ammonia (0.12 mL), and continued stirring at 70 ºC for 2 h. When **9** was consumed as evidenced by TLC, reaction mixture allowed to cool, filtered through celite, residue washed with MeOH (5 mL), and combined methanolic solutions evaporated. Resulting sticky crude product was reconstituted with DCM (20 mL) and washed with water, then extracted with 1N HCl solution (10 mL x 2). Combined acid extracts were washed with DCM (7.5 mL x 2), and then aqueous layer basified to neutralization with saturated $NaHCO_3$ solution to obtain yellow solid. Colored product was dissolved in DCM, absorbed on silica (1.5 eq. w/w) and passed through a short bed of silica using 1-5% methanol in ethyl acetate to obtain a cream solid, which reprocessed with acid-basification to obtain solvent free **5** (0.16 g, 71%), mp 207-209 ºC; R_f = 0.2 (DCM/MeOH/ TEA, 10/0.5/0.025); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.14 (s, 3 H, 19-CH_3), 4.41 (br. s., 1 H, 3*β*-H), 5.56 (d, *J*=3.18 Hz, 1 H, 6-H), 5.94 - 6.06 (m, 1 H, 16-H), 7.03 (d, *J*=3.67 Hz, 2 H, Ar'-4, 5-Hs), 7.28 - 7.37 (m, 2 H, Ar-5, 6-Hs), 7.45 - 7.54 (m, 1 H, Ar-7-H), 7.73 (s, 1

H, Ar'-2-H), 7.77 - 7.86 (m, 1 H, Ar-4-H), 7.95 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.0, 143.2, 141.6, 139.1, 136.7, 134.5, 128.5, 124.1, 123.4, 123.3, 122.4, 120.2, 118.6, 111.1, 55.7, 52.9, 50.1, 47.2, 37.2, 35.9, 34.7, 32.2, 31.1, 30.2, 30.1, 28.3, 20.2, 19.3, 16.0; HPLC: t_R 1.83 min 98.83%; HRMS calcd 439.2856 ($C_{26}H_{34}N_4H^+$), found 439.3856.

3α-Azido-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**7**). NaN_3 (0.42 g, 6.43 mmol) was added to a stirred solution of mesylate **4** (1.00 g, 2.14 mmol) and 15-crown-5 (0.005 g, 0.210 mmol) in anhydrous DMF (7.5 mL). The mixture was heated at 80 ºC for 18 h. After cooling, the reaction mixture was poured into ice water mixture, stirred for 30 min, filtered and dried to obtain 0.86g of crude product. Pure product was obtained after crystallization from ethyl acetate and petroleum ether (0.53 g, 60%), mp 164-165 ºC; R_f = 0.21 (petroleum ether : ethyl acetate, 2:1) ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 3 H, 18-CH_3), 1.06 (s, 3 H, 19-CH_3), 3.91 (t, *J*=2.81 Hz, 1 H, 3β-H), 5.46 (d, *J*=5.14 Hz, 1 H, 6-H), 5.93 - 6.01 (m, 1 H, 16-H), 7.27 - 7.34 (m, 2 H, Ar-5, 6-Hs), 7.46 - 7.52 (m, 1 H, Ar-7-H), 7.78 - 7.84 (m, 1 H, Ar-4-H), 7.97 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.1, 143.2, 141.6, 138.5, 134.5, 124.1, 123.3, 122.5, 122.4, 120.2, 111.1, 58.1, 55.7, 50.2, 47.2, 37.3, 36.0, 34.8, 33.5, 31.0, 30.2, 30.2, 26.0, 20.3, 18.9, 16.0; HPLC: t_R 7.02 min 98.16%; HRMS calcd 414.2652 ($C_{26}H_{31}N_5H^+$), found 414.2652.

3β-Azido-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**8**). To a solution of mesylate **4** (13.0 g, 27.8 mmol) in anhydrous DCM (100 mL) was added $TMSN_3$ (55.5 mL, 418 mmol), followed by $BF_3.OEt_2$ (71.2 mL, 753 mmol). The reaction was stirred at ~22 ºC for 5 h. When starting material was completely consumed as evidenced by TLC (petroleum ether : ethyl acetate, 2:1; R_f = 0.44 for product and Lewis acid complex), reaction was slowly poured into RB flask on ice bath containing aqueous 2M NaOH (750 ml) and stirred at ~22 ºC for 6 h. When

product is completely free of Lewis acid salt as evidenced by TLC, DCM from aqueous suspension was evaporated. The resulting precipitate containing product and inorganic salts were filtered and washed with water. The dry crude product then stirred with 75 mL chloroform and filtered, residue washed with chloroform (75 mL). Combined filtrates evaporated to obtain pure product **8** (10.7 g, 93%), mp 136-137 ºC; R_f = 0.21 (petroleum ether : ethyl acetate, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 3 H, 18-CH_3), 1.08 (s, 3 H, 19-CH_3), 3.19 - 3.32 (m, 1 H, 3α-H), 5.48 (d, *J*=5.38 Hz, 1 H, 6-H), 6.01 (dd, *J*=3.18, 1.71 Hz, 1 H, 16-H), 7.29 - 7.37 (m, 2 H, Ar-5, 6-Hs), 7.47 - 7.56 (m, 1 H, Ar-7-H), 7.81 - 7.88 (m, 1 H, Ar-4-H), 7.98 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.1, 143.2, 141.6, 140.2, 134.5, 124.0, 123.3, 122.4, 121.8, 120.2, 111.1, 61.0, 55.8, 50.4, 47.2, 38.1, 37.4, 36.8, 34.8, 31.0, 30.2, 27.8, 20.5, 19.2, 16.0; HPLC: t_R 7.86 min 100%; HRMS calcd 414.2652 ($C_{26}H_{31}N_5H^+$), found 414.2653.

3α-Amino-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**9**). A solution of compound **6a** (0.40 g, 0.97 mmol) and PPh_3 (1.00 g, 3.97 mmol) in THF/MeOH/water (4/4/1 mL) was stirred at 60 ºC for 10 h. Solvents evaporated under *vacuo,* residue reconstituted with DCM (25 mL) and extracted with 1N HCl (20 mL x 2). Combined aqueous extracts washed with DCM, then aqueous layer neutralized with 1N NaOH solution. Resulting solid filtered, washed with water and dried under *vacuo* to obtain pure **9** (0.25 g, 67%), mp 104-106 ºC; Rf = 0.2 (DCM:MeOH:TEA,10:1:0.05); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 3.31 (br. s., 1 H, 3β-H), 5.47 (d, *J*=4.40 Hz, 1 H, 6-H), 5.93 (br. s., 1 H, 16-H), 7.26 - 7.31 (m, 2 H, Ar-5, 6-Hs), 7.45 - 7.51 (m, 1 H, Ar-7-H), 7.76 - 7.83 (m, 1 H, Ar-4-H), 7.95 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.1, 143.2, 141.5, 138.4, 134.5, 123.8, 123.4, 123.3, 122.4, 120.1, 111.1, 55.7, 50.4, 47.2, 47.1, 38.8, 37.5, 34.8, 32.8, 31.1, 30.3, 30.2, 28.2, 18.8, 16.0; HPLC: t_R 8.18 min 96.72%; HRMS calcd 388.2747 ($C_{26}H_{33}N_3H^+$), found 388.2747.

3β-Amino-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**10**). A solution of compound **8** (9.00 g, 21.8 mmol) and PPh_3 (22.9 g, 87.1 mmol) in THF/MeOH/water (145/145/36 mL) was stirred at 60 ºC for 3 h. Solvents evaporated under *vacuo,* residue reconstituted with DCM (200 mL). To this solution under stirring added 1N HCl (100 mL) and stirred for 5 min to obtain precipitate which collected by filtration (filtrate discarded). Solid product on Buchner funnel made slurry with DCM (20 mL), filtered and suck dried. The second filtrate collected and separately processed for second crop. The above solid product suspended in water (100 mL) and neutralized with aq. NaOH solution (1.25 g in 25 mL). The product filtered, washed with water (50 mL x 3), suck dried and further dried under oven at 45-50 ºC (7.36 g, 87%). For the second crop, second filtrate containing DCM and aqueous phase was treated with fresh 1N HCl (10 mL). The precipitate collected by filtration, solids treated with aq. NaOH solution, filtered and washed with water, and suck dried (0.28g, 3%). Altogether 90% yield. mp 155-156 ºC; Rf = 0.2 (DCM:MeOH:TEA,10:1:0.05); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-C H_3), 1.05 (s, 3 H, 19-CH_3), 2.58 - 2.69 (m, 1 H, 3α-H), 5.38 (d, *J*=4.89 Hz, 1 H, 6-H), 5.98 (dd, *J*=2.93, 1.71 Hz, 1 H, 16-H), 7.47 - 7.52 (m, 1 H, 7-H), 7.78 - 7.85 (m, 1 H, Ar-4-H), 7.96 (s, 1 H, , Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.2, 143.2, 142.1, 141.6, 134.5, 124.0, 123.3, 122.4, 120.1, 120.0, 111.1, 55.9, 51.9, 50.6, 47.2, 43.2, 38.0, 36.7, 34.9, 32.4, 31.0, 30.3, 30.3, 20.6, 19.3, 16.0; HPLC: t_R 8.26 min 97.47%; HRMS calcd 388.2747 ($C_{26}H_{33}N_3H^+$), found 388.2747.

3α-(*p*-Nitrophenylcarbonyloxy)-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**11**). To a two neck flask was added glaterone (**1**, 2.00 g, 5.15 mmol), 0.95 g (5.66 mmol) of *p*-nitrobenzoicacid, 1.50 g (5.66 mmol) of PPh_3 and 30 mL anhydrous THF. The mixture was stirred until all solids dissolved and then cooled to 4 ºC in an ice-water bath. A solution of 40% DEAD 2.46 mL (5.66 mmol) in anhydrous toluene was added drop wise, allowed to attain room

temperature, stirred for 12 h. The reaction mixture concentrated in *vacuo* and the resulting sticky residue was suspended in 5 mL of ethyl acetate, stirred, cooled and filtered. Mother liquor collected, stirred vigorously and 30 mL of petroleum ether added slowly. Resulting sticky suspension filtered, washed with 10% ethyl acetate in petroleum ether (25 mL) and dried under *vacuo*. The sticky solid made slurry on Buckner funnel with ether (7.5 mL x 2), filtered and dried under *vacuo*. Free solids (0.8 g) obtained was a mixture of product **11**, traces of tripheylphosphinoxide and diethyl 1,2-hydrazinedicarboxylate. The crude product dissolved in hot IPA, allowed to cool to room temperature, resulting precipitate filtered and dried under *vacuo* to obtain a white solid (0.50 g, 18%) of pure **11**, mp 193-194 ºC; R_f = 0.4 (3% acetone in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 3 H, 18-CH_3), 1.13 (s, 3 H, 19-CH_3), 5.31 (br. s., 1 H, 3*β*-H), 5.39 (d, *J*=4.89 Hz, 1 H, 6-H), 6.00 (br. s., 1 H, 16-H), 7.28 - 7.34 (m, 2 H, Ar-5, 6-Hs), 7.48 - 7.52 (m, 1 H, Ar-7-H), 7.80 - 7.85 (m, 1 H, Ar-4-H), 7.97 (s, 1 H, Ar-2-H), 8.17 (m, *J*=8.80 Hz, 2 H, Ar'-2, 6-Hs), 8.30 (m, *J*=8.80 Hz, 2 H, Ar'-3, 5-Hs); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 150.4, 147.1, 141.6, 138.6, 136.4, 130.5, 124.1, 123.5, 123.4, 122.5, 122.0, 120.2, 111.1, 72.3, 55.8, 50.7, 47.2, 37.3, 36.4, 34.8, 33.9, 31.1, 30.3, 30.2, 26.2, 25.3, 20.4, 18.8, 16.0.

3α-Hydroxy-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (**12**). Ester **11** (0.50 g, 0.93 mmol) was dissolved in THF/MeOH solvent mixture (2:1, 7.5 mL), and the resulting solution was treated with 1N aq. NaOH solution (1.25 mL). The mixture was stirred at ~22 ºC for 2.5 h and then solvents evaporated under *vacuo* at ~40 ºC. The residue solid treated with water, filtered, washed with water and dried to afford **12** (0.33 g, 91%) as a white solid, mp 203 ºC; R_f = 0.34 (DCM/ MEOH/TEA, 10:0.5:0.025); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 4.05 (br. s., 1 H, 3β-H), 5.47 (d, *J*=4.89 Hz, 1 H, 6-H), 5.99 (br. s., 1 H, 16-H), 7.28 - 7.38 (m, 2 H, Ar-5, 6-Hs), 7.50 (d, *J*=5.38 Hz, 1 H, Ar-7-H), 7.77 - 7.88 (m, 1 H,

Ar-4-H), 7.97 (s, 1 H, Ar-2-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.2, 143.2, 141.6, 139.1, 134.5, 124.1, 123.3, 123.1; 122.4, 120.2, 111.1, 67.0, 55.8, 50.6, 47.2, 39.8, 37.5, 34.8, 33.1, 31.1, 30.3, 30.2, 28.8, 20.3, 18.6, 16.0; HPLC: t_R 1.89 min 99.28%; HRMS calcd 429.2587 ($C_{26}H_{32}N_2OH^+$), found 429.2587.

ASSOCIATED CONTENT

Supporting Information

The Supporting is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.5b00394.

Full characterization (^{1}H, ^{13}C NMR and HRMS) for all compounds (intermediate and final products) and HPLC chromatograms for final compounds (PDF).

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Notes

Vincent C. O. Njar is the lead inventor of galeterone and new analogs, patents and technologies thereof owned by the University of Maryland, Baltimore, and licensed to Tokai Pharmaceuticals, Inc. Vincent Njar and Puranik Purushottamachar are co-inventors of all compounds described in this report. A patent application to protect these novel improved synthetic procedures has been filed, and the authors of this manuscript are co-inventors of the patent application.

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