

Full Paper

Development of a Gram-Scale Synthesis of PBRM, an Irreversible Inhibitor of 17 β -Hydroxysteroid Dehydrogenase Type 1

René Maltais, and Donald Poirier

Org. Process Res. Dev., **Just Accepted Manuscript** • DOI: 10.1021/acs.oprd.8b00402 • Publication Date (Web): 19 Sep 2019Downloaded from pubs.acs.org on September 19, 2019

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

1
2
3
4 *Organic Process Research and Development (second revised version)*
5
6
7
8

9 **Development of a Gram-Scale Synthesis of PBRM, an Irreversible Inhibitor of 17beta-**
10 **Hydroxysteroid Dehydrogenase Type 1**
11
12

13
14
15 René Maltais[†] and Donald Poirier^{*†,‡}
16
17
18
19
20
21
22
23

24 [†] Laboratory of Medicinal Chemistry, Endocrinology and Nephrology Unit, CHU de Québec –
25 Research Center, Québec, QC, Canada
26

27 [‡] Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, QC, Canada
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44 (*) Corresponding Author:

45 Donald Poirier

46 Laboratory of Medicinal Chemistry

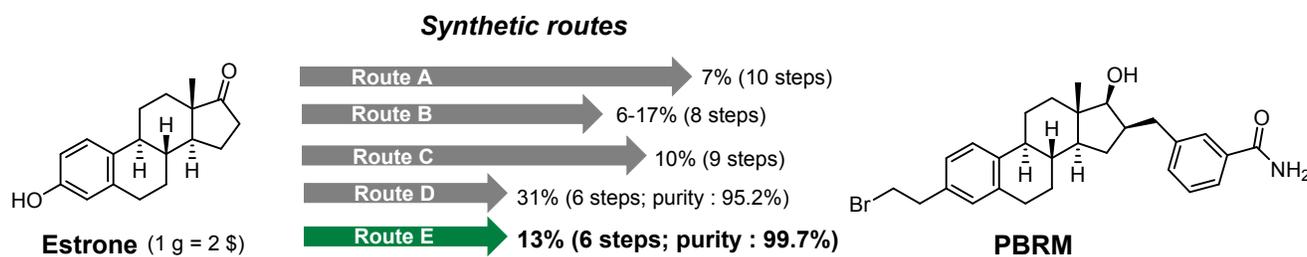
47 CHU de Québec – Research Center (CHUL, T4-42)

48 2705 Laurier Boulevard

49 Québec, QC, G1V 4G2, Canada

50 Tel.: 1-418-654-2296; Fax: 1-418-654-2298; E-mail: donald.poirier@crchul.ulaval.ca
51
52
53
54
55
56
57
58
59
60

Table of Contents:



ABSTRACT

Efforts toward the development of a reliable gram scale synthesis of PBRM, a potent and selective steroidal covalent inhibitor of 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1), are described. Among the three synthetic routes (C-E) developed herein, route E is the most efficient one with only 6 chemical steps from commercially available estrone, and an overall yield of 13% leading to PBRM with a high HPLC grade purity (99.7%) after recrystallization. Important improvements have been achieved in this sequence from previous reported routes (A and B). Notably, we used a palladium catalyzed Suzuki-Miyaura cross-coupling reaction to rapidly install the requested C3 chain on estrone. Also, catalytic hydrogenation of the C16-enone was shortened by half using Pearlman's catalyst. Finally, we used a selective bromination through deoxygenation of alcohol at the last step of the sequence to provide PBRM without dehydration of its carboxamide functionality, a persistent problem observed in other routes. Crystals of PBRM were also obtained from recrystallization in acetonitrile and submitted to x-ray analysis, which confirmed the PBRM structure. This work now makes it possible to start a proof-of-principle in a non-human primate model for the treatment of endometriosis, while supporting its future pharmacological development.

KEYWORDS: Covalent inhibitor, 17beta-hydroxysteroid dehydrogenase type 1, steroid, multigram synthesis

1. INTRODUCTION

PBRM, formally named as 3-[[[(16 β ,17 β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-trien-16-yl]methyl]benzamide (Figure 1), is the first selective covalent inhibitor of 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1).¹ Since this enzyme is involved in the last step of estrogen biosynthesis by converting the weak estrogen estrone (E1) into the highly potent estrogen estradiol (E2), the use of a 17 β -HSD1 inhibitor for the treatment of estrogen-dependent diseases (EDDs) has been identified as a promising therapeutic approach.² Clinical validation of this therapeutic target remains to be done for the treatment of EDDs including breast cancer,³ endometrial cancer,⁴ and endometriosis.⁵ PBRM has been identified as a promising lead candidate with desirable properties such as strong inhibitory activity on 17 β -HSD1 (*in vitro* and *in vivo*), good oral bioavailability, and low toxicity profile in rodent.⁶ A successful proof-of-principle study on a xenograft model with estrogen-sensitive human breast cancer (T-47D cells) in nude mice demonstrated the potential of such a selective covalent inhibitor to treat EDDs.⁷ As the next step of its therapeutic validation, we are now interested in studying the potency of PBRM for the treatment of endometriosis.⁸ Importantly, PBRM is an irreversible inhibitor that requires the presence of a histidine (His) residue for an alkylation on 17 β -HSD1.⁹ Considering the presence of a glycine rather than His at key position 221 in rodent, this animal model is inadequate for efficiency studies. Fortunately, monkeys represent a valuable model to evaluate PBRM efficiency, since they possess the same His as humans.¹⁰ Moreover, PBRM has been shown to be a potent inhibitor of 17 β -HSD1 in ovarian tissue from monkeys with a comparable level of inhibition as those measured in human ovarian tissues.^{9b} Thus, in order to validate the potency of PBRM in a relevant non-human primate endometriosis model such as the baboon,¹¹ the elaboration of a synthetic route allowing the preparation of a gram-scale quantity was absolutely necessary.

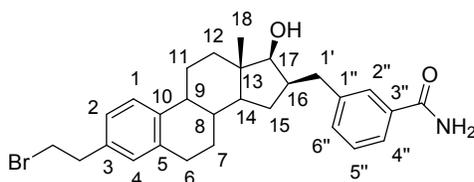
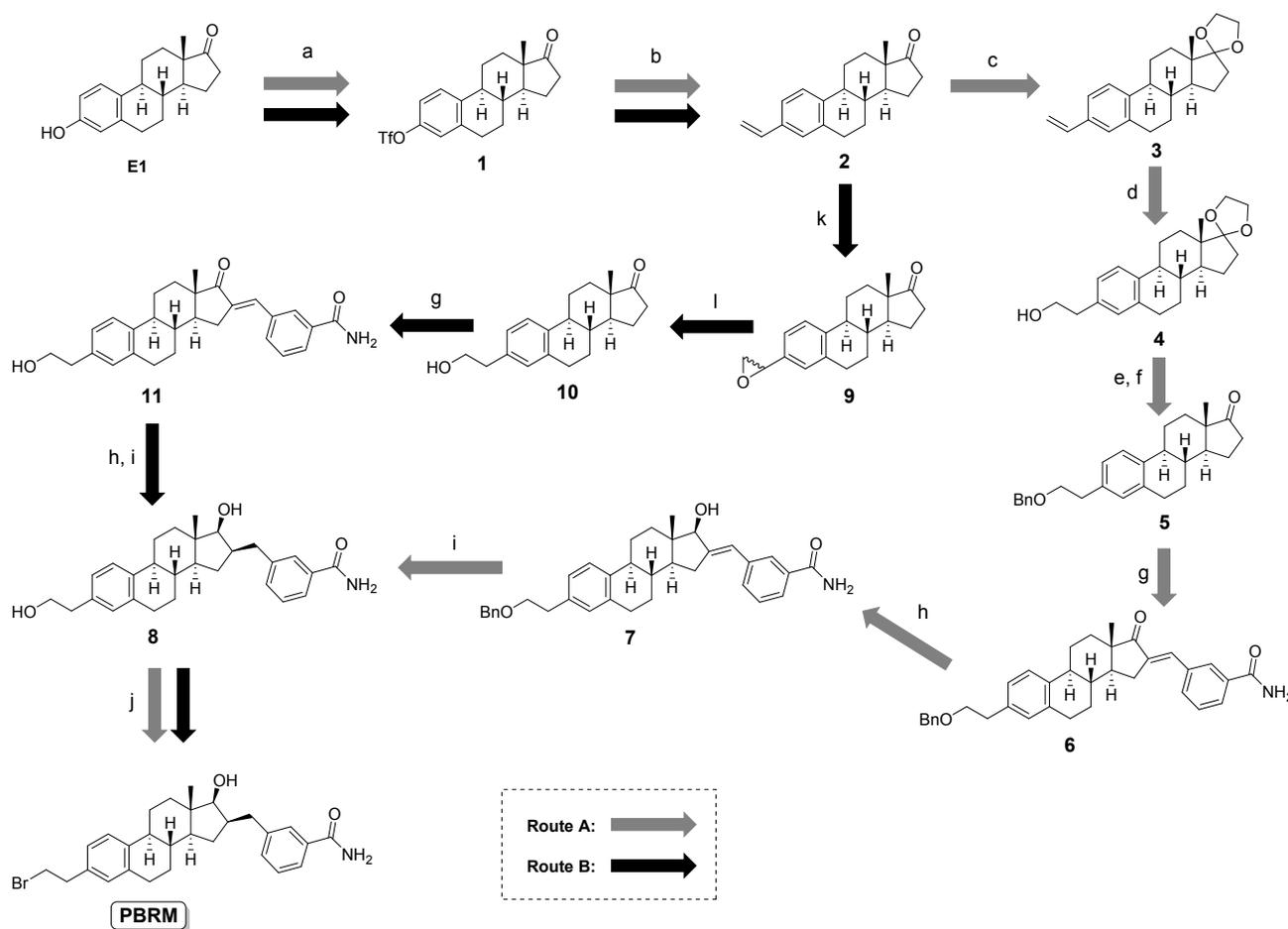


Figure 1. Carbon numbering and molecular structure of PBRM, a steroid derivative inhibiting 17 β -HSD1.

Two chemical syntheses of PBRM have been initially reported (Scheme 1, routes A and B).¹ The first one (route A), developed in a medicinal chemistry context, leads to milligram quantities and

1
2 allowed the *in vitro* assessment of PBRM inhibitory activities. This initial route consisted in 10 steps
3 from commercially available E1 and resulted in an overall yield of 7% (Table 1).^{1a} To obtain a few
4 grams of PBRM and to proceed to an *in vivo* proof-of-principle on a breast cancer xenograft model in
5 mice,⁷ this first synthesis was later optimized and shortened to 8 steps (route B, scale-up 1) with an
6 overall yield of 17%.^{1b} However, major limitations were encountered with these two routes, including
7 variable yields, incomplete and/or unselective bromination, as well as laborious final product
8 purification. These problems forced us to redesign the synthesis to provide an optimal route. We
9 present herein, the optimization efforts that led to a shorter synthesis of 6 steps that provided the
10 required quantity of PBRM. This work represents a critical step to start our proof-of-principle study in
11 baboons, and consequently to promote the use of PBRM as a new drug to treat endometriosis and other
12 EDDs.
13
14
15
16
17
18
19
20
21



52 **Scheme 1. Synthesis of PBRM via route A (10 steps) and route B (8 steps) from E1.** *Reagents and*
53 *conditions:* (a) Trifluoromethanesulfonic anhydride, TEA, DCM, rt; (b) $\text{CH}_2=\text{CHSnBu}_3$, $\text{Pd}(\text{PPh}_3)_4$,
54 LiCl , THF, rt (route A) or Potassium vinyltrifluoroborate, PdCl_2 , Cs_2CO_3 , PPh_3 , THF/ H_2O (9:1), 80 °C
55 (route B); (c) Ethylene glycol, p-TSA, Dean-Stark, toluene, reflux; (d) *i*) $\text{BH}_3\text{-(CH}_3)_2\text{S}$, THF, -78 °C-rt;
56 *ii*) NaHCO_3 , H_2O_2 , rt, 3 h; (e) NaH, benzylbromide, DMF, 0 °C-rt, 18 h; (f) HCl, MeOH, rt, 5 h; (g) 3-
57
58
59
60

1
2 Formyl-benzamide, KOH, EtOH, reflux, 0.5 h; (h) NaBH₄, MeOH, DCM, rt, 1 h; (i) H₂, 10% Pd/C,
3 MeOH, rt, 36 h; (j) PPh₃, CBr₄, DCM, 0 °C to rt; (k) Oxone, NaHCO₃, acetone/ACN (1:2), rt; (l) Pd/C
4 (10%), ammonium formate, MeOH, 70 °C.
5
6
7

8 2. RESULTS AND DISCUSSION 9

10 11 *Limitations of synthetic route B and attempts for optimization* 12

13
14 The major issue encountered with the last reaction step strongly limited the conversion of route
15 B into a reliable scale-up synthesis. The selective bromination of the primary alcohol **8** into PBRM
16 gave unreproducible yields varying from 30-73%, but most of the time below 50%. Different aspects of
17 this reaction were found to be problematic. First, the solubility of alcohol **8** in dichloromethane (DCM),
18 the usual solvent used for the Appel reaction,¹² was very limited and forced us to increase the number
19 of equivalents of PPh₃ and CBr₄ to drive the reaction to completion. Under this large reagent excess,
20 the carboxamide functionality on the 16β-side chain was found vulnerable to dehydration leading to the
21 corresponding nitrile. Second, 17β-OH was also found to react in those conditions and to form the
22 corresponding secondary bromide as a minor side product (10-15%). As a consequence of reagent
23 excess, PBRM was difficult to isolate from the triphenylphosphine oxide formed as a by-product. Their
24 very similar mobility on silica gel led to multiple laborious flash chromatography purifications.
25 Therefore, we explored different bromination methods as alternatives to the Appel reaction. The first
26 method tested was based on the use of (chloro-phenylthio-methylene)dimethylammonium chloride
27 (CPMA) in the presence of tetrabutylammonium bromide (TBAB), which has been reported for the
28 selective bromination of primary alcohol.¹³ Interestingly, CPMA provided a good yield (70%) for
29 halogenation of **8** selectively as opposed to the secondary 17-beta alcohol. Unfortunately, the presence
30 of a chlorination compound, found in a ratio 1:3 versus the bromide (PBRM), disqualified this reaction
31 as both compounds were found to be inseparable by chromatography. Varying the TBAB equivalent,
32 the temperature and the concentration did not alter this ratio. Two other bromination assays, using N-
33 bromosuccinimide and triphenylphosphine in DMF¹⁴ or tetraethylammonium halide and Et₂NSF₂BF₄
34 (XtalFluor-E) in DCM,¹⁵ also gave unsatisfactory results by leading to carboxamide dehydration.
35 Overall, route B from batch 1 and 2, was found to be more straightforward than route A, but provided
36 variable overall yields (6-17%, Table 1). The direct bromination of the primary alcohol in presence of
37 the carboxamide remains the major limitation to its translation to a reliable and scalable synthesis of
38 PBRM and resulted in variable yields.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Comparison of the five synthetic route efficiencies for the synthesis of PBRM.

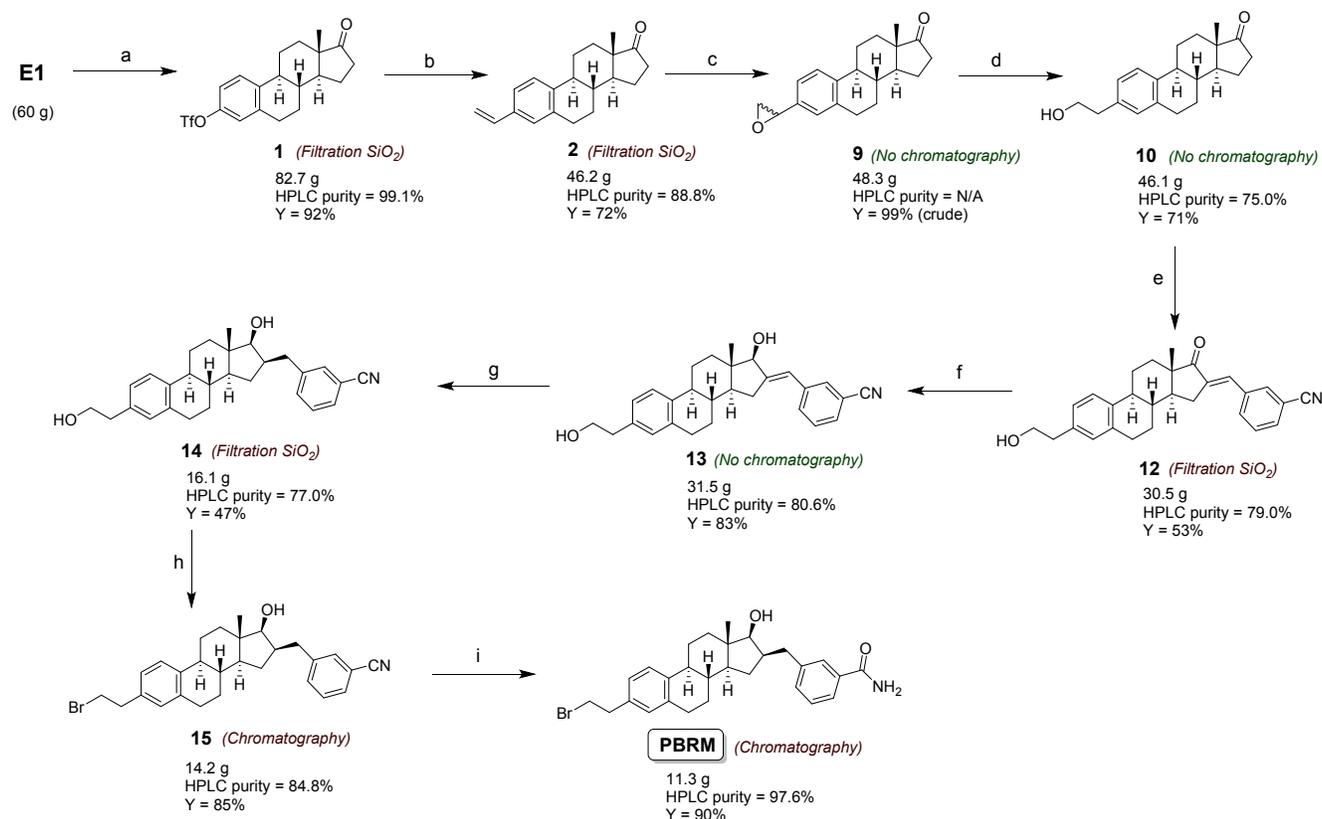
Synthetic route	Number of steps	Starting material (g) (Estrone)	Final product (g) (PBRM)	Overall yield (%)	HPLC purity (%)
A ^{1a}	10	1.6	0.2	7	98.5
B ^{1b} (batch 1)	8	8.6	2.5	17	98.5
B (batch 2)	8	33.6	3.7	6	96.7
C	9	60.0	11.3	10	97.6
D	6	38.0	23.5	31	95.2
E	6	30.0	9.2 (6.9*)	17 (13*)	95.1 (99.7*)

* Obtained after recrystallization from 9.2 g of PBRM in ACN.

Synthetic Route C

Synthesis of PBRM via route B was successful on a relatively small scale (< 5 g), but with important limitations related to the last synthetic step (bromination). Consequently, we redesigned the synthetic sequence, seeking to avoid the bromination in the presence of the carboxamide functionality (Scheme 2). Thus, we preserved the first four steps of route B and replaced the carboxamide by a nitrile group, which would be transformed back to primary amide at the last step of the sequence using mild hydration conditions.¹⁶ Prior starting the sequence, we first tried to optimize the reactions needed for the synthesis of alcohol **10**, with a particular interest for the opening of epoxide **9** (Scheme 1). In fact, we observed variable yields (31-71%) for the epoxide opening in the scale-up process necessary to obtain a larger quantity of **10**. The formation of the corresponding methyl-ether (20-25%), coming from the competition of methanol over hydrogen for the nucleophilic attack, was found to be in large part responsible for this significant lower yield. To avoid this side reaction, we tried to replace the methanol by less nucleophilic hindered alcohols such as isopropanol and *tert*-butyl-alcohol, but without significant improvement. Change in reaction temperature or reagent concentration was also found ineffective to avoid this by-product formation. Nevertheless, we obtained a sufficient quantity of alcohol **10** (46 g) to engage next step of the sequence. Indeed, alcohol **10** underwent aldolization with 3-CN-benzaldehyde using NaOH solution in EtOH at room temperature instead of KOH solution in refluxing EtOH as reported in routes A and B. This strategy limited the hydrolysis of nitrile to carboxamide and provided an acceptable yield (> 53%) of **12**. The subsequent reduction of the enone to allylic alcohol **13** proceeded in high yield as previously reported for routes A and B. The

1
2 stereoselective catalytic hydrogenation of the allylic alcohol **13** to 16 β -benzyl-CN derivative **14** was
3
4 however a potential issue because the nitrile group is known to be sensitive to catalytic hydrogenation,
5 thus forming the corresponding primary amine via an imine intermediate.¹⁷ In fact, the nitrile function
6 was partially hydrogenated during the reaction time (96 h) necessary to complete the double bond
7 reduction of the 17 β -allylic alcohol **13**. As result, a moderated yield (47%) of the saturated alcohol **14**
8 was obtained. The amine by-product was however easily removed by a simple filtration on silica gel.
9 Unexpectedly, the hydrogenation of the allylic alcohol was not completely stereoselective and gave a
10 85:15 mixture of 16 β /16 α benzyl-CN side-chain, as determined by NMR using the representative 18-
11 CH₃ and 17-CH signals. The key ¹H and ¹³C NMR signals for each of the four possible diastereomers
12 at C16/C17-stereogenic centers were previously identified by us.¹⁸ Fortunately, this 16 α -benzyl-CN
13 isomer was efficiently removed by chromatography in subsequent steps of purification, and no trace of
14 this isomer was found in the final PBRM product. The subsequent bromination of alcohol **14** gave, as
15 expected, a good yield (85%) of compound **15**. Finally, the conversion of the nitrile to corresponding
16 carboxamide by a palladium-dichloride catalyzed hydration with acetamide¹⁶ provided a satisfactory
17 90% of PBRM in a pure form after flash chromatography. A quantity of 11.3 g of PBRM was thus
18 generated in a high purity level (97.6%) using route C (Table 1).
19
20
21
22
23
24
25
26
27
28
29
30



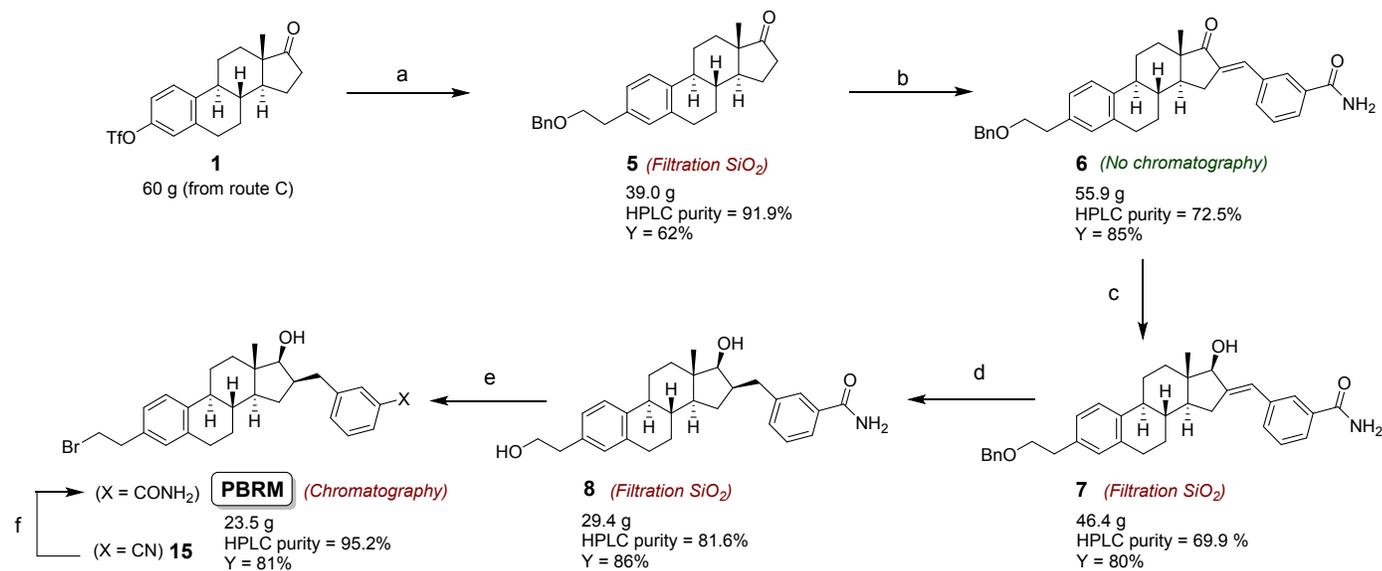
Scheme 2. Gram scale synthesis of PBRM via route C (9 steps) from E1. *Reagents and conditions:* (a) Trifluoromethanesulfonic anhydride, TEA, DCM, rt; b) Potassium vinyltrifluoroborate, PdCl₂, Cs₂CO₃, PPh₃, THF/H₂O (9:1), 80 °C; (c) Oxone, NaHCO₃, acetone/ACN (1:2); (d) Pd/C (10%), ammonium formate, MeOH, 70 °C (e) 3-CN-benzaldehyde, NaOH, EtOH, rt; (f) NaBH₄, MeOH, DCM, rt, 3 h; (g) H₂, 10% Pd/C, EtOH, rt, 96 h; (h) PPh₃, CBr₄, DCM, 0 °C to rt; (i) PdCl₂, acetamide, THF/H₂O, rt, 24 h. (*) Yields for this reaction varied from 37 to 71%, based on different assays..

Synthetic Route D

The variable yields for the formation and opening of epoxide **9** and the moderate yields for the aldolization and catalytic hydrogenation steps lead us to doubt that route E could be an efficient synthetic strategy for scale-up. For this reason, we had considered using a straightforward method to install the bromoethyl side chain directly at the C3 position of the E1 scaffold. At first sight, the use of a Suzuki-Miyaura palladium catalyzed cross-coupling reaction,¹⁹ between estrone-triflate (**1**) and alkoxyethylboron coupling partner, represented an attractive option.²⁰ Unfortunately, the synthetic methods available for the preparation of the alkoxyethylboron compounds were quite limited at that time and were reported only on very small scales.²¹ As a possible alternative, Fleury-Brégeot *et al.*²² reported the successful synthesis of different potassium alkoxyethyltrifluoroborates via a copper-catalyzed borylation. This method would simplify the synthesis protocol and solve stability issues encountered with alkoxyethylboron derivatives.²² Therefore we revisited the initial sequence based on a Suzuki-Miyaura reaction and elaborated a new synthetic strategy (route D, Scheme 3).

As validation step, we first performed the Suzuki-Miyaura coupling with estrone-triflate (**1**) on a small scale. Since Fleury-Brégeot *et al.*,²² reported coupling only with aryl halides, we used the conditions published by Molander *et al.*,²³ who reported different examples of cross-coupling reactions of potassium alkyltrifluoroborate with aryl triflates. We obtained a similar coupling yield (67%) as those published (66-75%) with the exception that a small amount (15%) of the aryl-triflate reductive product (3-deoxy-E1). Inseparable at this stage, the mixture was found to be separable later in the sequence of reactions by chromatography. We then proceeded to this coupling reaction on a larger quantity with estrone-triflate (30 g) and potassium (2-benzyloxyethyl)trifluoroborate (21 g) with comparable yield to small scale assay. The 2-benzyloxyethyl)trifluoroborate reagent being successfully prepared using the published procedure.²² With a multigram quantity of **5** in hands, we then followed the previously reported sequence of reactions^{1b} to 1) introduce the 16 β -benzylcarboxamide chain via an aldol condensation to provide **6**, 2) reduce the enone to allylic alcohol **7**, and 3) hydrogenate the double

bond to benzylcarboxamide derivative **8**. This sequence of reactions had the advantage to remove the benzyl ether protecting group during the palladium catalytic hydrogenation of the allylic alcohol **7**. On this larger scale, we however observed that the reaction time to complete the reduction of the allylic alcohol was found to be much longer than observed in smaller scale (from 1 to 9 days).



Scheme 3. Gram scale synthesis of PBRM via route D (6 steps) from E1. *Reagents and conditions:* (a) Potassium (2-benzyloxyethyl)trifluoroborate, PPh₃, PdCl₂, Cs₂CO₃, THF/H₂O (9:1), 90 °C; (b) 3-Carboxamide-benzaldehyde, NaOH (10%), EtOH/DCM (9:1); (c) NaBH₄, MeOH, DCM, rt, 1 h; (d) H₂, 10% Pd/C, EtOH, rt, 9 days; (e) PPh₃, CBr₄, THF, 0 °C to rt; (f) PdCl₂, acetamide, THF/H₂O, rt, 24 h.

Even though intermediate **8** was obtained in good overall yield, we had not resolved the problem regarding the last bromination step. The very low solubility of diol **8** in DCM was a possible cause of this reactivity problem, because of incomplete and slow conversion of the alcohol was observed. Consequently, we investigated the tolerance of the Appel reaction for other solvents than DCM. Among aprotic solvents that allow complete dissolution of **8** at 0 °C, THF was selected as an alternative to DCM. To our knowledge, no examples of Appel's reaction were reported using THF. We performed the bromination of **8** using conventional conditions, exchanging DCM for anhydrous THF. Interestingly, we obtained PBRM in good and reproducible yields with a rapid and complete conversion of the starting alcohol. However, as observed with the classic Appel reaction conditions,²⁴ the formation of the by-product **15** (35%) coming from carboxamide dehydration was also obtained. Fortunately, this nitrile side product was easily separated from PBRM by flash chromatography and

1
2 successfully recycled to PBRM using the palladium-catalyzed hydration conditions previously used in
3 route C.¹⁶ During this last flash chromatography, the 3-deoxy-E1 by-product present during all the
4 sequence starting from the Suzuki-Miyaura reaction was easily removed.
5
6

7 Another challenge for obtaining PBRM was the removal of PPh₃O by-product formed during
8 the Appel reaction. Solvent systems usually used in normal-phase flash chromatography (ex:
9 EtOAc/hexanes; DCM/MeOH, acetone/hexanes, etc.) lead to laborious separation of PBRM from
10 PPh₃O. These two compounds have a similar mobility on SiO₂ and were only separated following
11 successive flash chromatographic steps on normal phase with a high ratio of silica gel:product (100:1)
12 and this separation can be only viable in a relatively small scale (< 3 g of PBRM).^{1a} In an ultimate
13 attempt, we investigated the capacity of unusual solvent systems to provide a clean separation and to
14 simplify the final purification process of PBRM. As result, a ternary solvent system
15 (diethylether/DCM/MeOH: 48/48/4) provided an efficient separation of PPh₃O from PBRM in a single
16 chromatography step, being efficient even on a larger scale chromatographic column (> 10 g of
17 PBRM). A potential alternative to avoid flash chromatography would have be to remove PPh₃O by
18 precipitation with zinc chloride,²⁵ but this interesting new methodology was found only partially
19 effective in our case.
20
21
22
23
24
25
26
27
28

29 Analysis of the final PBRM indicated the presence of a small amount (4.6%) of the
30 corresponding chloride analog. This compound, also present from route C, but in a smaller proportion
31 (1%), could not be removed either by flash chromatography or by crystallization. However, the
32 presence of chloride derivative is not a problem at this stage of the proof-of-principle in monkeys,
33 because its pharmacology is closely related to PBRM, showing lower inhibition of 17β-HSD1 than
34 PBRM but without estrogenic properties.^{1a} Even if we did not yet identify the exact source of this side
35 product, we suspected an halogen exchange with the PdCl₂ used during the recycling step of the
36 benzonitrile derivative **15** to PBRM, or it could also be the result of a contamination of the CBr₄
37 reagent with chloride impurities.
38
39
40
41
42
43
44
45
46

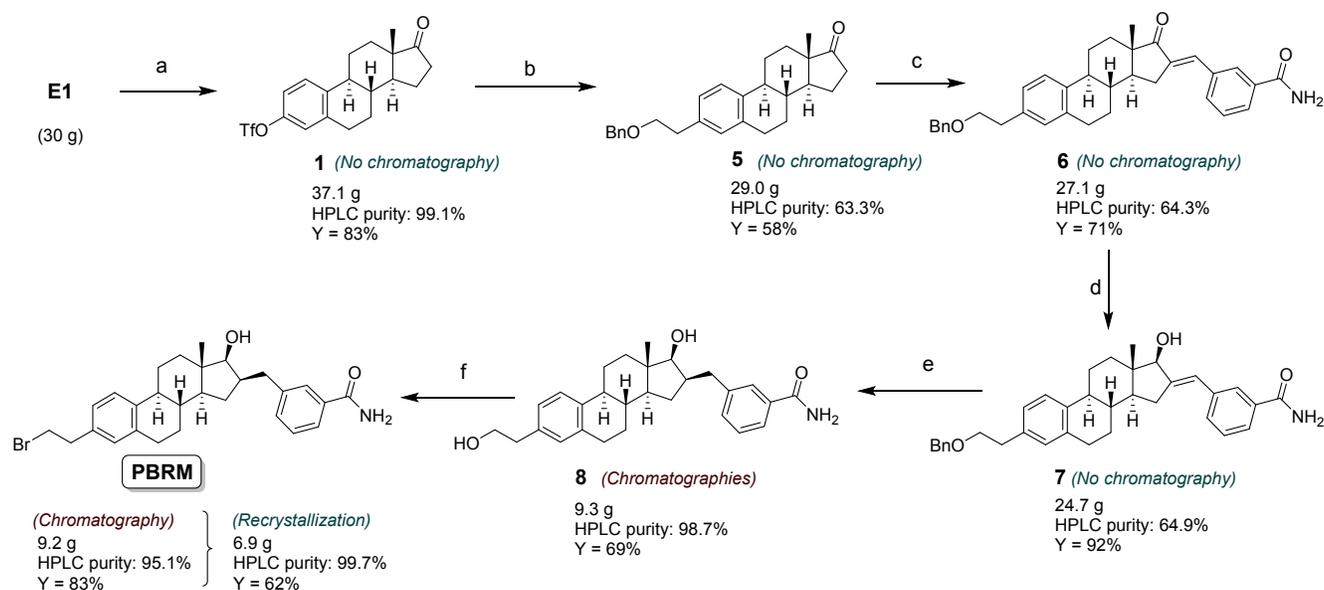
47 *Synthetic Route E*

48
49 Route D has considerably improved the first syntheses of PBRM, but some problems remain,
50 such as: 1) the number of silica gel filtrations (5 for 6 steps) needed during the sequence of reactions, 2)
51 the long time needed for the catalytic hydrogenation reaction (9 days), and 3) the unselective final
52 bromination of primary alcohol vs the carboxamide and 17β-OH functions. We thus engaged additional
53 efforts toward optimization (route E, Scheme 4) to address these weaknesses.
54
55
56
57
58
59
60

1
2 At the first step, the purification of estrone-triflate (**1**) was performed by trituration with
3 hexanes to remove trimethylamine salt residues. This procedure gave a comparative high-grade purity
4 (HPLC purity = 99.1%) to route D, but the yield of the reaction was slightly lower (83% vs 95%). The
5 condition of the next reaction, based on a palladium catalyzed Suzuki-Miyaura cross-coupling, was
6 unchanged from route D, but the SiO₂ filtration was replaced by a trituration of crude compound **5** with
7 toluene, which removed a large amount of the E1 impurity, known to be almost insoluble in that
8 solvent.²⁶ Efforts to recrystallize this crude compound **5** (HPLC purity = 63.3%) were unsuccessful
9 because this hydrophobic steroid derivative (cLog P = 5.8) was an amorphous solid and found to be
10 soluble in almost all organic solvents, greatly limiting the possibility of recrystallization. Compound **5**
11 was used as such for the next aldolization reaction, where a smaller amount of 3-formyl carboxamide
12 was used (1.1 vs 2.0 eq in scale-up 1) to limit the quantity of 3-formyl-carboxamide remaining.
13 Purification of **6** (HPLC purity = 64.3%) by recrystallization was also found to be unsuccessful,
14 considering the large solubility of this intermediate in organic solvents. Crude compound **6** was then
15 submitted to a stereoselective reduction of the C17-carbonyl with NaBH₄ at -40 °C rather than at room
16 temperature, to maximize the selectivity formation of 17β-OH. ¹H NMR analysis of the resulting
17 alcohol **7** showed no trace of 17α-OH reduction product by NMR analysis.¹⁸ Once again,
18 recrystallization attempts of crude compound **7** (HPLC purity = 64.9%) were ineffective, presumably
19 due to its hydrophobic nature (cLog P = 6.6). Thus, at this stage, all purification attempts from
20 intermediates **5-7** by recrystallization have failed, probably related to high hydrophobicity, high
21 solubility in organic solvent and the amorphous solid state of the compounds.
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 The next objective was to reduce the time necessary for catalytic hydrogenation, which required
37 over a week to be completed. We thus changed palladium 10% on charcoal (Pd/C) for palladium
38 hydroxide (20%) on charcoal (Pearlman's catalyst),²⁷ a catalyst reported as a useful alternative when
39 classical Pd/C conditions are inefficient.²⁸ The new conditions were beneficial and shortened the
40 reaction time by more than an half (4 days instead of 9). At this stage, the more polar nature of
41 compound **8** (cLog P = 4.1) offered a better opportunity for recrystallization of the crude mixture than
42 previous intermediates (only 72.0% of **8** by HPLC, with 8.3% of 3-OH and 6.8% of 3-H analogs).
43 Among the solvents screened for recrystallization (ACN, MeOH, EtOH, *i*-PrOH, 1-butanol, THF,
44 EtOAc, AcOH, DCE, toluene, and acetone), ACN and acetone offered the best properties. Indeed, the
45 compound **8** mixture was almost insoluble at room temperature in those two solvents, and completely
46 soluble around their refluxing temperature. Unfortunately, despite variation of solution concentration
47 (1-5%), duration and temperature of recrystallization, we never observed the formation of crystals.
48
49
50
51
52
53
54
55
56
57
58
59
60

Consequently, we proceeded to the bromination of **8**, thus generating crude PBRM, and to see if separation of the impurities could be possible. Using the Appel reaction conditions of route D, we observed that it was impossible to separate the 3-H analog by-product from PBRM in all eluent systems tried. Also, recrystallization assays of this mixture of PBRM and 3-H analog in ACN led to crystals, but co-crystallization with 3-H impurity was obtained (88% PBRM and 6.0% of 3-H analog). Therefore, two chromatographies using different systems (1: DCM/MeOH and 2: EtOAc 100%) were needed to remove the two major impurities (3-OH and 3-H analogs) and obtain **8** in a pure form (HPLC purity = 98.7%). These two chromatographic steps were however harmful on the yield compared to route D (69 vs 86%).



Scheme 4. Gram scale synthesis of PBRM via route E (6 steps) from E1. Reagents and conditions: (a) Trifluoromethanesulfonic anhydride, TEA, DCM, rt; (b) Potassium (2-benzyloxyethyl)trifluoroborate, PPh₃, PdCl₂, Cs₂CO₃, THF/H₂O (9:1), 90 °C; (c) 3-Carboxamide-benzaldehyde, NaOH (10%), EtOH/DCM (9:1); (d) NaBH₄, MeOH, DCM, -40 °C rt, 1 h; (e) H₂, 10% Pd(OH)₂/C, EtOH, rt, 4 days; (f) PPh₃, TBAI, 1,2-dibromoethane, 60°C.

Finally, to fix the unselective bromination reaction of primary alcohol **8** over carboxamide and 17β-OH functionalities, we investigated several other conditions not yet tried. Our attention was first focused on the use of trichlorotriazine (TCT), which in addition to NaBr in DMF, was reported for the bromination of alcohols.²⁹ However, since a residual chloride derivative (20%) was observed in the reported methodology, we adapted the protocol by running the reaction in anhydrous THF in the presence of LiBr. The high solubility of this salt in THF increased the equivalents of bromine ions in

1
2 solution *vs* the use of NaBr. As a result, we obtained PBRM with only a slight contamination by the
3 chloride analog (3%), but in a moderate yield (35%) because we observed 40% of the nitrile by-product
4 resulting from carboxamide dehydration. Thus, this reaction led to the same problem observed with
5 other methodologies used previously, but without presence of PPh₃O. Another promising reaction we
6 tried was the visible-light-mediated photocatalytic alcohol activation with Ru(bpy)₃Cl₂, which converts
7 alcohols to the corresponding bromides using CBr₄ in DMF.³⁰ Unfortunately, the elimination product
8 (nitrile) was also observed (35%) in addition to PBRM. In an ultimate attempt, we tried the reaction
9 developed by Chen *et al.*³¹ and reporting a bromination from deoxygenation of alcohols using
10 tetrabutylammonium iodide and triphenylphosphine in heated dibromoethane. For the first time in all
11 our bromination assays, we were delighted to see no trace of nitrile by-product, thus leading to a good
12 yield of PBRM (83%) after purification by chromatography (HPLC purity = 95.1%). Even if PPh₃O
13 was formed during the reaction, this method eliminates the need for a supplementary step to recycle the
14 nitrile by-product into PBRM and also eliminate the formation of the inseparable chloride analog
15 observed in route D.
16
17
18
19
20
21
22
23
24
25
26
27

28 *Recrystallization of PBRM*

29
30 Considering the advantages of recrystallization in the development of a drug candidate,
31 especially regarding chemistry, manufacturing and control (CMC).³² we were highly interested in
32 obtaining PBRM in a crystal form. Identification of a valuable solvent of recrystallization was thus
33 explored by screening the solubility of PBRM in a range of organic solvents, including DCM, DMF,
34 EtOAc, ACN, diethylether, methanol, toluene, and acetone. Among all these, two solvents (acetone and
35 ACN) stood out and allowed to recrystallize PBRM with a lower recrystallization yield (42%) from
36 acetone and with a good recrystallization yield (75%) from ACN. In route E, these later conditions
37 were used to recrystallize PBRM in 74% yield with a high purity of 99.7%. X-ray analysis of crystals
38 (Fig. 2 and Supporting Information) confirmed the PBRM molecular structure.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

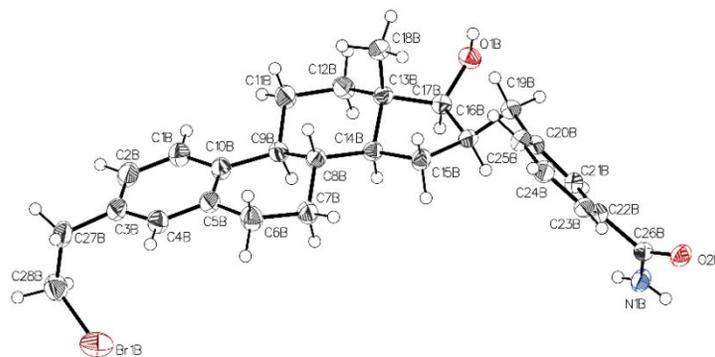


Figure 2. Molecular structure of PBRM obtained from X-ray analysis.

3. CONCLUSIONS

The development of an efficient chemical route that affords a gram quantity of the selective covalent 17 β -HSD1 inhibitor PBRM has been successfully achieved. In fact, the redesign of synthetic routes A and B previously reported for the preparation of PBRM on small scales led to new routes C, D and E for gram scale purposes. Route E emerged as the most convenient one taking into account the synthesis length (6 steps), the overall yield of 13%, and the purity level of final compound obtained from recrystallization (HPLC purity = 99.7%). This new synthesis represents a significant improvement compared to the ten steps required in the initial route A and the overall yield of 7%. The success of route E was based on two strategic modifications of the initial synthetic sequence. First, a Suzuki-Miyaura reaction was used as a key step to rapidly install the C3 side chain on the estrane scaffold. Second, the use of a recent methodology for bromination through deoxygenation of alcohol using a PPh₃/BrCH₂CH₂Br/TBAI reagent system provided selectivity over carboxamide functionality. Overall, the multigram quantity of PBRM obtained from novel synthetic routes C-E will now enable to start a proof-of-principle study on primates to assess its therapeutic potential to treat endometriosis. At the same time, the obtainment of a more reliable synthesis of PBRM supports its further pharmacological development toward the treatment of estrogen-dependent diseases.

4. EXPERIMENTAL SECTION

General

Reagents and solvents

Estrone was purchased from Zhejiang Xianju Pharmaceuticals Co. (Xiangju, Zhejiang, China). Triphenylphosphine, oxone and sodium borohydride were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Palladium 10% on charcoal was purchased from either BeanTown Chemicals (Hudson, NH, USA) or Acros Organic (Belgium Town, WI, USA). Trifluoroacetic anhydride and acetamide were purchased from Oakwood Chemicals (Estill, SC, USA). Carbon tetrabromide was purchased from either TCI (Portland, OR, USA) or Oakwood Chemicals. Cesium carbonate was purchased from Chem-Impex (Wood Dale, IL, USA). Ammonium formate and triethylamine was acquired from Alfa Aesar (Tewksbury, MA, USA). Palladium dichloride was obtained from Aurum Pharmatech Inc (Franklin Park, NJ, USA). Anhydrous acetonitrile (ACN), dichloromethane (DCM), diethyl ether, dimethylformamide (DMF), isopropanol, and tetrahydrofuran (THF) were obtained from Sigma-Aldrich, but the usual solvents ethyl acetate (EtOAc), dichloromethane (DCM), hexanes, methanol (MeOH), and ethanol (EtOH), were purchased from Fisher Scientific (Montréal, QC, Canada).

Purification

Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20-mm silica gel 60 F254 plates (E. Merck; Darmstadt, Germany) and with 230-400 mesh ASTM silica gel 60 (Silicycle, Québec, QC, Canada), respectively. HPLC purities were determined with a Shimadzu apparatus using a Shimadzu SPD-M20A photodiode array detector, an Alltima HP C18 reversed-phase column (250 mm x 4.6 mm, 5 μ m), and a solvent gradient of MeOH:H₂O. Semi-preparative HPLC purifications were performed to separate chloride analog from PBRM using an Alltima HP C18 (250 mm x 10 mm; 5 μ m) with a solvent gradient from 70:30 MeOH/H₂O to 100% MeOH on 60 min at a flow rate of 10 mL/min. The wavelength of the UV detector was selected at maximal compound absorbance.

Characterization

Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ¹H and 100.6 MHz for ¹³C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) were expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm) or acetone (2.05 and 28.9 ppm) for ¹H and ¹³C NMR, respectively. Infrared (IR) spectra were recorded on a MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands are reported in cm⁻¹. Low-

1
2 resolution mass spectra (LRMS) were recorded on a Shimadzu apparatus (Kyoto, Japan) equipped with
3 a turbo ion-spray source. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the
4 Chemistry Department of Université Laval (Québec, Qc, Canada). Melting points were determined on a
5 Gallenkamp melting point apparatus (England). The chemical names of the steroid derivatives were
6 generated with ACD/Laboratories (chemist version) software (Toronto, ON, Canada).
7
8
9

10 11 12 13 **Synthetic Routes A and B**

14
15
16 The experimental details and compound characterization (NMR, IR, MS) for the synthesis of
17 PBRM according to routes A and B were previously reported.¹
18
19
20
21

22 **Synthetic Route C**

23 *17-oxoestra-1,3,5(10)-trien-3-yl trifluoromethanesulfonate (1)*

24
25 In a three-neck round-bottom flask (3 L) under an argon atmosphere were added estrone (0.22
26 mol, 60.0 g), DCM (1.5 L) and TEA (0.79 mol, 108 mL). The solution was cooled to 0 °C and
27 trifluoromethanesulfonic anhydride (0.35 mmol, 49.1 mL) was slowly added over a period of 15 min.
28 The solution was stirred at 0 °C for 1 h and then at room temperature overnight. The resulting solution
29 was poured into water, washed with brine, dried over sodium sulfate, filtered and evaporated under
30 reduced pressure. The crude compound (105 g) was purified using a 2 L filter loaded with 1.5 L of
31 silica gel which was beforehand solvated with EtOAc/hexanes (1/9). The compound was solubilized in
32 DCM (150 mL) and gently deposited on the top of silica gel in order to be adsorbed. At this point,
33 portions of eluent (20 x 450 mL (EtOAc/hexanes: 1/9) were passed through silica gel and harvested
34 using vacuum Erlenmeyer (500 mL). The fractions 6-26, containing the desired compound as identified
35 by molybdate indicator on TLC plate (R_f = 0.49; EtOAc/hexanes: 3/7), were combined and evaporated
36 under reduced pressure to give estrone-triflate (**1**) (82.7 g, 92%). HPLC purity = 99.1%. NMR data
37 agree with those reported in literature.³³
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 *3-ethenylestra-1,3,5(10)-trien-17-one (2)*

53
54 In a three-neck round-bottom flask (2 L) under an argon atmosphere were added estrone-triflate
55 (**1**) (0.103 mol, 41.4 g), THF (225 mL) and H₂O (25 mL). The solution was then degassed for 15 min
56
57
58
59
60

1
2 with bubbling argon. Potassium vinyltrifluoroborate (0.123 mol, 16.5 g), cesium carbonate (0.31 mol,
3 100.5 g), triphenylphosphine (6.2 mmol, 1.62 g) and palladium dichloride (2.1 mmol, 0.365 g) were
4 added under a stream of argon. The solution was degassed with bubbling argon again for 15 min under
5 vigorous stirring. The heterogeneous mixture was gradually heated to 80 °C and then stirred for 4 days.
6 The resulting black solution was filtered over a celite pad (1 L) and washed with EtOAc (1 L). The
7 organic phase was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced
8 pressure. The crude compound (65.5 g) was purified using a 2 L filter loaded with 1.5 L of silica gel
9 which was previously solvated with EtOAc/hexanes (3/7). The compound was solubilized in DCM
10 (150 mL) and gently deposited on the top of the silica gel to be adsorbed. At this point, portions of
11 eluent (10 x 450 mL of EtOAc/hexanes: 3/7) were passed through silica gel and harvested using
12 vacuum Erlenmeyers (500 mL). The fractions 2-11, containing the desired compound as identified by
13 molybdate indicator on TLC plate ($R_f = 0.45$; EtOAc/hexanes: 3/7), were combined and evaporated
14 under reduced pressure to give **2** (23.1 g). This reaction was repeated a second time using the same
15 conditions to give a total quantity of 46.2 g (Yield = 72%). HPLC purity = 88.2%. NMR data agree
16 with those reported in literature.^{1b}
17
18
19
20
21
22
23
24
25
26
27
28
29

30 *3-(oxiran-2-yl)estra-1,3,5(10)-trien-17-one (9)*

31
32 In a 5 L round-bottom flask, the 3-vinyl-deoxyestrone (**2**) (0.082 mol, 23.1 g) was dissolved
33 using a mixture of acetone (500 mL) and ACN (1 L), followed by the addition of an aqueous solution
34 of NaHCO₃ (10%) (1 L). Oxone (0.50 mol, 76.0 g) was then added by small portions over 5 min and
35 the solution was vigorously stirred for an additional 40 min. The resulting solution was poured into
36 water (1 L) and extracted with EtOAc (1 L). The organic layer was then washed with brine, dried over
37 sodium sulfate, filtered and evaporated under reduced pressure. The reaction was repeated a second
38 time with another 23.1 g portion of compound **2** to give a total quantity of 48.3 g of crude oxirane **9**
39 (Yield = 99%). This compound was used for the next step without further purification. NMR data agree
40 with those reported in literature.^{1b}
41
42
43
44
45
46
47
48

49 *3-(2-hydroxyethyl)estra-1,3,5(10)-trien-17-one (10)*

50
51 In a 2 L flask, dried beforehand under an argon atmosphere, was added ammonium formate
52 (0.81 mol, 50.8 g) and palladium on charcoal (10%) (24.0 g). Isopropanol (550 mL) was added to those
53 solids and the resulting suspension was heated 5 min at 40 °C under an argon atmosphere. The crude
54 oxirane **9** (24.0 g) was dissolved in isopropanol (350 mL) and the solution was rapidly added using an
55
56
57
58
59
60

1
2 addition funnel and stirred at 60 °C for 1 h. The solution was allowed to return at room temperature and
3 then filtered over celite (1 L). The celite pad was washed with a mixture of DCM/MeOH (4 L) and the
4 organic solvent was evaporated under reduced pressure. The crude mixture was triturated with DCM,
5 filtered and evaporated under reduced pressure to give 23.1 g of crude ethyl alcohol derivative **10**. This
6 compound was used for the next step without further purification. The reaction was repeated a second
7 time with another portion (24.0 g) of compound **9** to give a total quantity of 46.1 g of crude alcohol **10**
8 (Yield = 71%). HPLC purity = 75.0%. NMR data agree with those reported in literature.^{1b}
9
10
11
12
13
14
15

16 *3-{(E)-[(16E)-3-(2-hydroxyethyl)-17-oxoestra-1,3,5(10)-16-ylidene]methyl}benzonitrile (12)*
17

18 To a solution of crude alcohol **10** (23.0 g) in EtOH (595 mL) were added 3-cyano-benzaldehyde
19 (0.268 mol, 35.2 g) and an aqueous solution of NaOH (10%) (105 mL). The solution was stirred for 15
20 min at room temperature and then poured into water. The resulting solution was neutralized to pH 7
21 with an aqueous solution of HCl (10%) and then extracted two times with EtOAc. The organic layer
22 was washed with brine, dried with sodium sulfate, filtered and evaporated under reduced pressure. The
23 reaction was repeated a second time with another portion (23.0 g) of alcohol **10**. The combined crude
24 compound of both assays (118 g) was purified using a 2 L filter loaded with 1.5 L of silica gel which
25 was beforehand solvated with EtOAc/hexanes (3/7). The compound was solubilized in DCM (150 mL)
26 and gently deposited on the top of silica gel in order to be absorbed. At this point, portions of different
27 eluents (10 x 450 mL of EtOAc/hexanes (3/7)), 10 x 450 mL of EtOAc/hexanes (4/6) and 5 x 450 mL
28 of EtOAc/hexanes (1/1)), were passed through silica gel and harvested using vacuum Erlenmeyers (500
29 mL). The fractions 10-22, containing the desired compound (R_f = 0.26; EtOAc/Hexanes (1/1)) as
30 identified by molybdate indicator on TLC plate, were combined and evaporated under reduced pressure
31 to give compound **12** (30.5 g, 53%). IR (NaCl film): 3420 (OH), 2230 (CN), 1720 (C=O), 1628 (C=C).
32 ¹H NMR (CDCl₃): 1.01 (s, 18-CH₃), 1.23-2.61 (unassigned CH and CH₂), 2.83 (t, J = 6.5 Hz,
33 CH₂CH₂OH), 2.95 (m, 6-CH₂ and 1H of 15-CH₂), 3.87 (broad t, J = 6.0 Hz, CH₂CH₂OH), 7.00 (s,
34 4-CH), 7.04 (d, J = 8.0 Hz, 2-CH), 7.26 (d, J = 7.6 Hz, 1-CH), 7.41 (s, 1'-CH), 7.54 (t, J = 7.8 Hz, 5''-
35 CH), 7.64 (d, J = 7.8 Hz, 6''-CH), 7.74 (d, J = 7.9 Hz, 4''-CH), 7.83 (s, 2''-CH). ¹³C NMR (CDCl₃):
36 14.4, 25.7, 26.9, 29.1, 29.3, 31.6, 37.8, 38.7, 44.3, 47.9, 48.5, 63.6, 113.1, 118.4, 125.6, 126.5,
37 129.6, 129.7, 130.4, 132.1, 132.9, 134.4, 136.0, 136.5, 136.9, 137.7, 138.5, 208.7. LRMS for
38 C₂₈H₃₀NO₂ [M + H]⁺ 412.2 m/z. HPLC purity = 79.0%.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

3-{(E)-[(16E,17β)-17-hydroxy-3-(2-hydroxyethyl)estra-1,3,5(10)-16ylidene]methyl}benzonitrile (13)

To a solution of compound **12** (0.074 mol, 30.5 g) in a mixture of MeOH/DCM (9/1) (1.3 L) at 0 °C was added NaBH₄ (0.25 mol, 9.5 g) by small portions over 15 min. The solution was stirred for 1 h at 0 °C before returning to room temperature and being stirred for an additional 2 h. The resulting solution was concentrated to a volume of approximately 500 mL and then poured into water to be extracted four times with EtOAc (4 x 500 mL). The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to give crude compound **13** (31.5 g, 83%), which was used for the next step without further purification. IR (NaCl film): 3387 (OH), 2230 (CN). ¹H NMR (CDCl₃): 0.74 (s, 18-CH₃), 1.35-2.50 (unassigned CH and CH₂), 2.75 (dd, J₁ = 5.7 Hz, J₂ = 16.9 Hz, 1H of 15-CH₂), 2.82 (t, J = 6.5 Hz, CH₂CH₂OH), 2.92 (m, 6-CH₂), 3.86 (broad q, J = 6.2 Hz, CH₂CH₂OH), 4.17 (d, J = 8.7 Hz, OH), 4.76 (d, J = 4.2 Hz, OH), 6.54 (d, J = 2.4 Hz, 1'-CH), 6.99 (s, 4-CH), 7.03 (d, J = 8.0 Hz, 2-CH), 7.27 (d, J = 6.1 Hz, 1-CH), 7.46 (m, 5''-CH and 6''-CH), 7.59 (d, J = 7.6 Hz, 4''-CH), 7.69 (s, 2''-CH). ¹³C NMR (CDCl₃): 11.0, 26.0, 27.4, 29.4, 30.6, 36.3, 38.0, 38.6, 43.3, 44.3, 47.6, 63.6, 84.9, 112.5, 119.0, 121.3, 125.5, 126.4, 129.2, 129.7 (2C), 131.2, 132.6, 135.8, 136.7, 138.1, 139.0, 149.0. LRMS for C₂₈H₃₀NO [M - H₂O + H]⁺ 396.3 m/z. HPLC purity = 80.6%.

34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3-{[(16β,17β)-17-hydroxy-3-(2-hydroxyethyl)estra-1,3,5(10)-16-yl]methyl}benzonitrile (14)

To a solution of crude compound **13** (10.5 g) in anhydrous EtOH (500 mL) in a three-neck round-bottom flask (2 L), was added palladium on charcoal 10% (2.1 g) at room temperature under an argon atmosphere. The solution was submitted to vacuum and purged with hydrogen for 2 min. This operation was repeated three times. The three necks of the round-bottom flask were connected with hydrogen balloons which were periodically refilled, as needed. The solution was stirred for 96 h under a hydrogen atmosphere, until no trace of the olefin signal (δ = 6.58 ppm, 1'-CH) was present in ¹H NMR spectrum. The resulting solution was filtered over a celite pad (500 mL), washed with MeOH (1 L), and evaporated under reduced pressure to give 10.1 g of crude compound **14**. In fact, this reaction was performed in parallel (3 x 10.1 g of **13**) in three 2 L three-neck round-bottom flasks to increase the contact surface, maximize the hydrogen saturation of the solutions and thus accelerate the rate of the reaction. The combined crude compound (30.3 g) was purified using a 2 L filter loaded with silica gel (1 L), which was solvated beforehand with EtOAc/hexanes (1/1). The compound was solubilized in DCM (150 mL) and gently deposited on the top of the silica gel to be adsorbed. Portions of eluent (6 x 450 mL of EtOAc/hexanes (1/1)) were then passed through silica gel and harvested using vacuum

1
2 Erlenmeyers (500 mL). The fractions 2-7, containing the desired compound ($R_f = 0.25$; EtOAc/hexanes
3 (1/1)) as identified by molybdate indicator on a TLC plate, were combined and evaporated under
4 reduced pressure to give compound **14** (17.0 g). At this point, we proceeded to a NMR analysis and
5 observed that compound **14** contained a small proportion of 17-ketone (15%), which was surprisingly
6 formed during the reaction, and the isomer 16 α -benzyl-CN (15%). We thus submitted the crude
7 mixture to a NaBH₄ reduction in MeOH to regenerate the 17 β -alcohol from 17-ketone by-product. The
8 usual work-up for NaBH₄ reduction, as reported previously for the synthesis of **13**, was performed and
9 lead to crude compound **14** (16.1 g, 47%). IR (NaCl film): 3410 (OH), 2230 (CN). ¹H NMR (CDCl₃):
10 0.87 (s, 18-CH₃), 1.05-2.54 (unassigned CH and CH₂), 2.84 (m, CH₂CH₂OH and 6-CH₂), 3.15 (d,
11 $J = 8.8$ Hz, 1'-CH₂), 3.85 (m, CH₂CH₂OH and OH), 6.95 (s, 4-CH), 7.01 (dd, $J_1 = 1.6$ Hz, J_2
12 = 8.0 Hz, 2-CH), 7.24 (d, $J = 7.9$ Hz, 1-CH), 7.39 (t, $J = 7.6$ Hz, 5"-CH), 7.47 (m, 4"-CH and 6"-CH),
13 7.52 (s, 2"-CH). ¹³C NMR (CDCl₃): 12.5, 25.9, 27.3, 29.3, 32.0, 37.2, 37.5, 37.9, 38.5, 41.5, 44.2,
14 44.3, 48.6, 63.5, 81.8, 112.1, 119.0, 125.4, 126.2, 128.9, 129.4, 129.5, 132.2, 133.4, 135.6, 136.7,
15 138.3, 143.7. LRMS for C₂₈H₃₂NO [M - H₂O + H]⁺ 398.0 m/z. HPLC purity = 77.0%.

26
27
28
29 **3-{[(16 β ,17 β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-16-yl]methyl}benzonitrile (**15**)**

30
31 To a solution of crude compound **14** (16.1 g) in anhydrous THF (750 mL) at 0 °C was added
32 triphenylphosphine (0.078 mol, 20.4 g) and carbon tetrabromide (0.78 mol, 25.8 g). The solution was
33 stirred at 0 °C for 5 min and then allowed to return to room temperature and stirred for 70 min. The
34 resulting solution was poured into water, extracted with EtOAc, washed with brine, dried over sodium
35 sulfate, filtered and evaporated under reduced pressure to give 54.4 g of crude material. Purification by
36 flash chromatography using EtOAc/hexanes (2:8) as eluent give compound **15** (14.2 g, 85%). IR (NaCl
37 film): 3479 (OH), 2230 (CN). ¹H NMR (CDCl₃): 0.87 (s, 18-CH₃), 1.03-2.57 (unassigned CH and
38 CH₂), 2.84 (m, 6-CH₂), 3.10 (t, $J = 7.8$ Hz, CH₂CH₂Br), 3.15 (dd, $J_1 = 3.3$ Hz, $J_2 = 12.2$
39 Hz, 1'-CH₂), 3.55 (t, $J = 7.8$ Hz, CH₂CH₂Br), 3.87 (d, $J = 9.3$ Hz, OH), 6.93 (s, 4-CH),
40 6.99 (d, $J = 8.0$ Hz, 2-CH), 7.24 (d, $J = 8.1$ Hz, 1-CH), 7.38 (t, $J = 7.6$ Hz, 5"-CH), 7.48 (m, 4"-CH
41 and 6"-CH), 7.52 (s, 2"-CH). ¹³C NMR (CDCl₃): 12.6, 26.0, 27.3, 29.4, 32.1, 32.9, 37.3, 37.6, 38.0,
42 39.0, 41.6, 44.3, 44.4, 48.7, 81.9, 112.3, 119.1, 125.6, 125.9, 129.0, 129.2, 129.5, 132.3, 133.4, 136.2,
43 136.9, 138.9, 143.7. LRMS for C₂₈H₃₃BrNO [M + H]⁺ 476.2 and 478.2 m/z. HPLC purity = 84.8%.

44
45
46
47
48
49
50
51
52
53
54
55
56 **3-{[(16 β ,17 β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-trien-16-yl]methyl}benzamide (**PBRM**)**

To a solution of compound **15** (0.03 mol, 14.2 g) in a THF/H₂O (3:1) mixture (150 mL) was added acetamide (0.24 mol, 13.9 g) and PdCl₂ (2.92 mmol, 518 mg) under an argon atmosphere. The solution was stirred at room temperature for 24 h and then poured into water and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to give 16.4 g of crude material. Purification by flash chromatography using EtOAc/hexanes (8/2) gave 11.3 g (90%) of PBRM. NMR data agree with those reported in literature.^{1a} HPLC purity = 97.6%.

Synthetic Route D

Potassium (2-benzyloxyethyl)trifluoroborate

In a 500 mL flask was successively added bis(pinacolato)biboron (88.5 g, 0.35 mol), lithium methoxide (17.7 g, 0.47 mol), triphenylphosphine polymer-bound loaded at 1.5 mmol/g (20.0 g, 30 mmol), and CuI (4.42 g, 23 mmol). The flask was purged with argon, followed by the addition of degassed anhydrous DMF (1.2 L) and benzyl 2-bromoethyl ether (50 g, 0.23 mol). The solution was bubbled with argon for an additional 10 min, and then vigorously stirred at room temperature for 20 h under an argon atmosphere. The resulting solution was diluted with DCM, filtered over celite, and evaporated under reduced pressure. The crude mixture was diluted with diethyl ether and washed with water. The organic layer was washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. The resulting crude mixture was treated with a solution of potassium hydrogen fluoride in THF/H₂O (1000/200 mL) and stirred overnight at room temperature. Evaporation of THF/H₂O under reduced pressure gave a crude mixture that was triturated with diethyl ether. The resulting white solid was washed with diethyl ether and dried under reduced pressure to give 44.8 g (80%) of potassium (2-benzyloxyethyl)trifluoroborate. NMR data agree with those reported in literature.²²

3-[2-(benzyloxy)ethyl]estra-1,3,5(10)-trien-17-one (5)

In a Schlenk tube (300 mL) was added estrone-triflate (**1**) (0.075 mol, 30.0 g), cesium carbonate (0.224 mol, 72.9 g), PdCl₂ (1.5 mmol, 265 mg), triphenylphosphine (4.5 mmol, 1.17 g) and potassium (2-benzyloxyethyl)trifluoroborate (0.09 mol, 21.7 g), which was prepared beforehand following the Fleury-Brégeot methodology.²³ To the solids, under an argon atmosphere was added 100 mL of a

1
2 degassed solution of THF/H₂O (9/1) and the solution was heated at 90 °C and vigorously stirred for 24
3 h. The solution was cooled, poured into water, and extracted with EtOAc. The organic phase was
4 washed with brine, dried with sodium sulfate, filtered and evaporated under reduced pressure. The
5 reaction was repeated a second time from 30 g of **1**. The crude material from both reactions were
6 combined (60.5 g) and purified using a 2 L filter loaded with silica gel (1.5 L), which was beforehand
7 solvated with EtOAc/hexanes (1/9). The compound was solubilized in DCM (150 mL) and gently
8 deposited on the top of silica gel in order to be adsorbed. At this point, portions of eluents (15 x 450
9 mL of EtOAc/hexanes: 1/9) have been passed through silica gel and harvested using vacuum
10 Erlenmeyers (500 mL). The fractions 4-15, containing the desired compound (R_f = 0.50;
11 EtOAc/hexanes (1:9)) as identified by molybdate indicator on TLC plate, were combined and
12 evaporated under reduced pressure to give compound **5** (39.0 g, 67%), including 15% of 3-deoxy-
13 estrone, which was inseparable at this stage. HPLC purity = 91.9%. NMR data of both compounds
14 agree with those reported in literature.^{1a, 33}

25
26
27 *3-{(E)-[(16E)-3-[2-(benzyloxy)ethyl]-17-oxoestra-1,3,5(10)-trien-16-ylidene]methyl}benzamide (6)*

28
29 To a solution of compound **5** (0.098 mol, 38.0 g) in a mixture of EtOH/DCM (9/1) (1 L) was
30 added 3-formylbenzamide (0.195 mol, 29.2 g) and an aqueous solution of NaOH (10%) (150 mL). The
31 solution was stirred at room temperature for 5 h. The resulting solution was then poured into water (2
32 L) and neutralized at pH 7 with an aqueous solution of HCl (10%) before being extracted with EtOAc
33 (5 x 500 mL). The combined organic phases were washed with brine, dried over sodium sulfate,
34 filtered and evaporated under reduced pressure to give 55.9 g of crude compound **6** (Yield = 85%).
35 This compound was used for the next step without further purification. HPLC purity = 72.5%.

36
37
38
39
40
41
42
43 *3-{(E)-[(16E,17β)-3-[2-(benzyloxy)ethyl]-17-hydroxyestra-1,3,5(10)-trien-16*
44 *ylidene]methyl}benzamide (7)*

45
46
47 To a solution of crude compound **6** (55.9 g) in a mixture of MeOH/DCM (9/1) (1 L) was added
48 NaBH₄ (0.45 mol, 16.9 g) at 0 °C. The solution was stirred for 30 min at 0 °C and allowed to return to
49 room temperature and stirred for an additional 2 h. The resulting solution was concentrated under
50 reduced pressure to a volume of about 500 mL and then poured into water (2 L). The aqueous solution
51 was extracted with EtOAc (3 x 400 mL), washed with brine, dried over sodium sulfate, filtered and
52 evaporated under reduced pressure to give 56.0 g of crude compound. The crude material was purified

1
2 using a 2 L filter loaded with silica gel (1.5 L), which was solvated beforehand with EtOAc/hexanes
3 (7/3). The compound was solubilized in DCM (200 mL) and gently deposited on the top of the silica
4 gel to be adsorbed. At this point, portions of eluent (30 x 450 mL of EtOAc/hexanes (7/3)) have been
5 passed through silica gel and harvested using vacuum Erlenmeyers (500 mL). The fractions 8-27,
6 containing the desired compound ($R_f = 0.17$; EtOAc/hexanes (7/3)) as identified by molybdate
7 indicator on TLC plate, were combined and evaporated under reduced pressure to give compound **7**
8 (46.4 g, 80%). The corresponding 3-deoxy-estrone derivative (15%) was present, but inseparable at this
9 stage. IR (NaCl film): 3366 and 3209 (OH and NH₂), 1666 (C=O, amide). ¹H NMR (CDCl₃): 0.74 (s,
10 18-CH₃), 1.35-2.45 (unassigned CH and CH₂), 2.79 (dd, $J_1 = 5.8$ Hz, $J_2 = 17.0$ Hz, 1H of
11 15-CH₂), 2.88 (broad t, $J = 7.2$ Hz, CH₂CH₂O and 6-CH₂), 3.69 (t, $J = 7.2$ Hz, CH₂CH₂OBn),
12 4.15 (s, 17 α -CH), 4.54 (s, OCH₂Ph), 5.80-6.20 (2 broad s, CONH₂), 6.59 (d, $J = 2.0$ Hz,
13 1'-CH), 6.97 (s, 4-CH), 7.02 (d, $J = 7.9$ Hz, 2-CH), 7.23 (d, $J = 8.0$ Hz, 1-CH), 7.32 (m, OCH₂Ph),
14 7.42 (t, $J = 7.7$ Hz, 5"-CH), 7.56 (d, $J = 7.8$ Hz, 6"-CH), 7.61 (d, $J = 7.7$ Hz, 4"-CH), 7.87 (s, 2"-CH).
15 ¹³C NMR (CDCl₃): 11.1, 26.1, 27.5, 29.4, 30.6, 35.8, 36.3, 38.1, 43.3, 44.4, 47.7, 71.3, 72.9, 84.9,
16 122.3, 124.9, 125.3, 126.3, 127.4, 127.5, 127.6 (2C), 128.3 (3C), 128.6, 129.5, 131.5, 133.5, 136.2,
17 136.4, 137.9, 138.4, 147.6, 169.6. LRMS for C₃₅H₄₀NO₃ [M + H]⁺ 522.3 m/z. HPLC purity = 69.9%.

31
32
33 *3-{[(16 β ,17 β)-17-hydroxy-3-(2-hydroxyethyl)estra-1,3,5(10)-trien-16-yl]methyl}benzamide (**8**)*

34
35 In a 2 L three-neck round-bottom flask, compound **7** (15.4 g) obtained above was dissolved in
36 anhydrous EtOH (1 L) and palladium on charcoal 10% (3.2 g) was added under an argon atmosphere.
37 The solution was submitted to vacuum and then purged with hydrogen for 2 min. This operation was
38 repeated three times. The three necks of the round-bottom flask were connected with hydrogen
39 balloons which were periodically refilled, as needed. The solution was then stirred for 9 days under
40 hydrogen atmosphere, until no trace of the olefin signal ($\delta = 6.58$ ppm, 1'-CH) was present in ¹H NMR
41 spectrum. The resulting solution was filtered over a celite pad (500 mL), washed with MeOH (1 L), and
42 evaporated under reduced pressure to give crude compound **8**. In fact, this reaction was performed in
43 parallel (3 x 15.4 g of **7**) in three 2 L three-neck round-bottom flasks to increase the contact surface,
44 maximize the hydrogen saturation of the solutions, and thus accelerate reaction rate. Crude compound
45 from the three reactions were combined. At this point, we proceeded to a NMR analysis and observed
46 that crude compound **8** contained a small proportion of 17-ketone (15%). We thus submitted the crude
47 compound to a NaBH₄ reduction in MeOH in order to regenerate the 17 β -alcohol from 17-ketone side
48
49
50
51
52
53
54
55
56
57

1
2 product. The usual work-up for NaBH₄ reduction was performed as reported previously for the
3 synthesis of **13**. The crude material (40.6 g) was then divided in two portions (20.3 g) and purified by
4 flash chromatography using EtOAc/hexanes (8/2) to give a total of 29.4 g (86%) of compound **8**,
5 including 7% of the 16 α -benzylbenzamide isomer which was found inseparable at this stage. NMR data
6 agree with those reported in literature.^{1a} HPLC purity = 81.6%.
7
8
9

10 11 12 13 *3-}{[(16 β ,17 β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-16-yl]methyl}benzamide (PBRM)*

14
15 To a solution of compound **8** (0.012 mol, 5.0 g) obtained above in anhydrous THF (50 mL) at 0
16 °C was added triphenylphosphine (0.023 mol, 6.06 g) and carbon tetrabromide (0.023 mol, 7.68 g). The
17 solution was stirred at 0 °C for 10 min, and then allowed to return to room temperature and stirred for 1
18 h. The resulting mixture was poured into water and extracted three times with EtOAc. The organic
19 phase was washed with brine, dried over sodium sulfate, filtered, and evaporated under reduced
20 pressure to give 15.6 g of crude material. Purification by flash chromatography using diethyl
21 ether/DCM/MeOH (48/48/4) as eluent provided PBRM (2.5 g) and a less polar mixed fraction (3.0 g)
22 containing in part the nitrile derivative **15**, a by-product coming from dehydration of carboxamide, and
23 CBr₄/CHBr₃ by-product. To regenerate PBRM from **15**, this mixed fraction (3.0 g) was submitted
24 overnight to hydration conditions using PdCl₂ (0.92 mmol, 160 mg) and acetamide (74.4 mmol, 4.4 g)
25 in a mixture of THF/H₂O (3/1) (50 mL). Purification by flash chromatography using
26 diethylether/DCM/MeOH (48:48:4) as eluent provided an additional 1.4 g of PBRM, which was
27 combined with the 2.5 g obtained previously to give a total of 3.9 g (68%) of PBRM. The entire
28 process was repeated with two larger portions of compound **8** (12.25 g) and gave reproducible yields
29 (9.5 g and 9.8 g) of PBRM. NMR data agree with those reported in literature.^{1a} The PBRM portions
30 originating from the bromination assays performed (3.9 + 9.5 + 9.8 = 23.5 g (81%)) were combined,
31 showing an HPLC purity of 95.2 %. An impurity representing 4.6% was identified as (3-}{[(16 β ,17 β)-3-
32 (2-chloroethyl)-17-hydroxyestra-1,3,5(10)-16-yl]methyl}benzamide after isolation from a semi-
33 preparative HPLC chromatography on a small portion (200 mg) of the final PBRM batch. NMR data of
34 this chloro derivative agree with those reported in literature.^{1b}
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 **Synthetic Route E**

53 54 *17-oxoestra-1,3,5(10)-trien-3-yl trifluoromethanesulfonate (1)*

1
2 In a three-neck round-bottom flask (3 L) under an argon atmosphere were added estrone (0.11
3 mol, 30.0 g), DCM (750 mL) and TEA (0.40 mol, 54 mL). The solution was cooled to 0 °C and
4 trifluoromethanesulfonic anhydride (0.18 mmol, 24.6 mL) was slowly added over a period of 15 min.
5 The solution was stirred at 0 °C for 1 h and then at room temperature overnight. The resulting solution
6 was poured into water, washed with brine, dried over sodium sulfate, filtered and evaporated under
7 reduced pressure. The crude compound (51 g) was purified by successive washing with hexanes (5 x 1
8 L). The combined solutions were evaporated under reduced pressure to give estrone-triflate (**1**) (37.1 g,
9 83%). NMR data were identical to those reported for **1** in route C.
10
11
12
13
14
15
16
17

18 *3-[2-(benzyloxy)ethyl]estra-1,3,5(10)-trien-17-one (5)*
19

20
21 In a Schlenk tube (300 mL) was added estrone-triflate (**1**) (0.091 mol, 37.0 g), cesium carbonate
22 (0.28 mol, 89.9 g), PdCl₂ (1.8 mmol, 326 mg), triphenylphosphine (7.4 mmol, 1.92 g) and potassium
23 (2-benzyloxyethyl)trifluoroborate (0.11 mol, 29.0 g), which was prepared beforehand following the
24 Fleury-Brégeot methodology.²² To the solids, under an argon atmosphere was added 120 mL of a
25 degassed solution of THF/H₂O (9/1) and the solution was heated at 90 °C and vigorously stirred for 24
26 h. The solution was cooled, poured into water, and extracted with EtOAc. The organic phase was
27 washed with brine, dried with sodium sulfate, filtered and evaporated under reduced pressure to give
28 29.0 g (52%). HPLC purity = 63.3%. This compound was used for the next step without further
29 purification. NMR data were consistent to those reported for compound **5** in route D.
30
31
32
33
34
35
36
37
38

39 *3-{(E)-[(16E)-3-[2-(benzyloxy)ethyl]-17-oxoestra-1,3,5(10)-trien-16-ylidene]methyl}benzamide (6)*
40

41 To a solution of compound **5** (74.6 mmol, 29.0 g) in a mixture of EtOH/DCM (85/15) (595 mL)
42 was added 3-formylbenzamide (82 mmol, 12.3 g) and an aqueous solution of NaOH (10%) (105 mL).
43 The solution was stirred at room temperature for 5 h. The resulting solution was then poured into water
44 (2 L) and neutralized at pH 7 with an aqueous solution of HCl (10%) before being extracted with
45 EtOAc (5 x 500 mL). The combined organic phases were washed with brine, dried over sodium sulfate,
46 filtered and evaporated under reduced pressure to give 27.1 g of crude compound **6** (Yield = 71%).
47 This compound was used for the next step without further purification. HPLC purity = 64.9%. NMR
48 data were consistent to those reported for compound **6** in route D.
49
50
51
52
53
54
55
56
57
58
59
60

1
2 *3-{(E)-[(16E,17β)-3-[2-(benzyloxy)ethyl]-17-hydroxyestra-1,3,5(10)-trien-16*
3 *ylidene]methyl}benzamide (7)*
4

5
6 To a solution of crude compound **6** (27.1 g) in a mixture of MeOH/DCM (9/1) (600 mL) at -40
7 °C was added NaBH₄ (104 mmol, 3.95 g) at 0 °C. The solution was stirred at -40 °C for 2 h. The
8 resulting solution was concentrated under reduced pressure to a volume of about 500 mL and then
9 poured into water (2 L). The aqueous solution was extracted with EtOAc (3 x 400 mL), washed with
10 brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to give 24.7 g of crude
11 compound. This compound was used for the next step without further purification. HPLC purity =
12 69.9%. NMR data were identical to those reported for compound **7** in route D.
13
14
15
16
17
18
19
20

21 *3-{[(16β,17β)-17-hydroxy-3-(2-hydroxyethyl)estra-1,3,5(10)-trien-16-yl]methyl}benzamide (8)*
22

23 In a 2 L three-neck round-bottom flask, compound **7** (24.7 g) obtained above was dissolved in
24 anhydrous EtOH (500 mL) and palladium hydroxide (20% on charcoal) (3.0 g) was added under an
25 argon atmosphere. The solution was submitted to vacuum and then purged with hydrogen for 2 min.
26 This operation was repeated three times. The three necks of the round-bottom flask were connected
27 with hydrogen balloons which were periodically refilled, as needed. The solution was then stirred for 4
28 days under hydrogen atmosphere, until no trace of the olefin signal ($\delta = 6.58$ ppm, 1'-CH) was present
29 in ¹H NMR spectrum. The resulting solution was filtered over a celite pad (250 mL), washed with
30 MeOH (500 L), and evaporated under reduced pressure to give crude compound **8**. At this point, we
31 proceeded to a NMR analysis and observed that crude compound **8** contained a small proportion of 17-
32 ketone (15%). We thus submitted the crude compound to a NaBH₄ reduction in MeOH in order to
33 regenerate the 17β-alcohol from 17-ketone side product. The usual work-up for NaBH₄ reduction was
34 performed as reported previously for the synthesis of **13**. The crude material (20.2 g) purified by flash
35 chromatography using DCM/MeOH (9/1) to give a total of 13.4 g of compound **8**, but still containing
36 3-OH by-product (3-{[(16β,17β)-3,17-dihydroxyestra-1,3,5(10)-trien-16-yl]methyl}benzamide). A
37 second flash chromatography using EtOAc provide 9.3 g of pure compound **8**. NMR data were
38 consistent to those reported for compound **8** in route D. HPLC purity = 98.7%.
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 *3-{[(16β,17β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-16-yl]methyl}benzamide (PBRM)*
54
55
56
57
58
59
60

To compound **8** (21.4 mmol, 9.3 g) in a 500 mL flask was added triphenylphosphine (38.5 mmol, 10.1 g), tetrabutylammonium iodide (38.5 mmol, 14.2 g) and 1,2-dibromoethane (250 mL). The solution was stirred at 60 °C for 1 h. The resulting solution was evaporated under reduced pressure. Purification by flash chromatography using diethyl ether/DCM/MeOH (49:49:2) provided PBRM (8.8 g, 83%) showing an HPLC purity of 95.1%. Recrystallization from acetonitrile provided 6.9 g (74% of recrystallization yield) of PBRM with a high HPLC purity (99.7%). The impurity (0.26%) corresponding to PBRM-O, oxidized form at position C17, and identified by LC-MS from previous data.⁶ NMR data were consistent to those reported for PBRM in previous routes.

PBRM recrystallization assays and X-ray analysis

Recrystallization in acetonitrile

To 200 mg of PBRM at room temperature was added 10 mL of ACN. The suspension was heated at reflux for 5 min and cooled to room temperature. The solution was left to rest at room temperature for 45 min and cooled at 4 °C overnight. Crystals were filtered, washed with cold ACN and dried over vacuum to give 151 mg of PBRM (75% of recovery). M.p. = 183-184°C. ¹H NMR was identical to the starting PBRM.

Recrystallization in acetone

To 100 mg of PBRM at room temperature was added 5 mL of acetone. The suspension was heated at reflux for 5 min and cooled to room temperature. The solution was left to rest at room temperature for 3 days and cooled at 4 °C for a week. Crystals were filtered, washed with cold acetone, and dried over vacuum to give 42 mg of PBRM (42% of recovery). M.p. = 183-184°C. ¹H NMR was identical to the starting PBRM.

X-ray analysis

Two PBRM crystals were analyzed by the X-ray Diffraction Laboratory (Department of Chemistry, Université de Montréal, Montréal, QC, Canada) and details of x-ray analysis and data were provided in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

1) ¹H NMR spectra of PBRM from routes B (batch 2), C, D, and E. 2) HPLC chromatograms of PBRM from routes B (batch 2), C, D, and E. 3) X-ray analysis of PBRM from route D. This material is available free of charge via internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

Phone: 1-418-654-2296. Fax: 1-418-654-2298. E-Mail: Donald.poirier@crchul.ulaval.ca

ORCID

Donald Poirier: 0000-0002-7751-3184

René Maltais: 0000-0002-0394-1653

Author Contributions

RM performed the laboratory work and participated in the writing of the manuscript; DP contributed to the writing. Both have approved the final version of the manuscript.

Funding

We thank the Canadian Institutes of Health Research (CIHR) for an operating grant.

Notes

The authors declare the following competing financial interest (s): RM and DP have ownership interest in a patent application.

ACKNOWLEDGMENTS

We are grateful to Mr. Thierry Maris (Université de Montréal, Montréal, QC, Canada) for x-ray analysis, and to Mrs. Micheline Harvey for careful reading of this manuscript.

REFERENCES

- (1) (a) Maltais, R.; Ayan, D.; Poirier, D., Crucial role of 3-bromoethyl in removing the estrogenic activity of 17 β -HSD1 inhibitor 16 β -(*m*-carbamoylbenzyl)estradiol. *ACS Med. Chem. Lett.* **2011**, *2*,

- 678-681; (b) Maltais, R.; Ayan, D.; Trottier, A.; Barbeau, X.; Lague, P.; Bouchard, J. E.; Poirier, D., Discovery of a non-estrogenic irreversible inhibitor of 17 β -hydroxysteroid dehydrogenase type 1 from 3-substituted-16 β -(*m*-carbamoylebenzyl)-estradiol derivatives. *J. Med. Chem.* **2014**, *57*, 204-222
- (2) (a) Labrie, F.; Luu-The, V.; Lin, S.-X.; Labrie, C.; Simard, J.; Breton, R.; Bélanger, A., The key role of 17 β -hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* **1997**, *62*, 148-158; (b) Luu-The, V., Analysis and characteristics of multiple types of human 17 β -hydroxysteroid dehydrogenase. *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 143-151; (c) Purohit, A.; Tutill, H. J.; Day, J. M.; Chander, S. K.; Lawrence, H. R.; Allan, G. M.; Fischer, D. S.; Vicker, N.; Newman, S. P.; Potter, B. V. L.; Reed, M. J., The regulation and inhibition of 17 β -hydroxysteroid dehydrogenase in breast cancer. *Mol. Cell. Endocrinol.* **2006**, *248*, 199-203; (d) Poirier, D., Contribution to the development of inhibitors of 17 β -hydroxysteroid dehydrogenase types 1 and 7: key tools for studying and treating estrogen-dependent diseases. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 83-94; (e) He, W.; Gauri, M.; Li, T.; Wang, R.; Lin, S. X., Current knowledge of the multifunctional 17 β -hydroxysteroid dehydrogenase type 1 (HSD17B1). *Gene* **2016**, *588*, 54-61.
- (3) Husen, B.; Huhtinen, K.; Poutanen, M.; Kangas, L.; Messinger, J.; Thole, H., Evaluation of inhibitors for 17 β -hydroxysteroid dehydrogenase type 1 in vivo in immunodeficient mice inoculated with MCF-7 cells stably expressing the recombinant human enzyme. *Mol. Cell. Endocrinol.* **2006**, *248*, 109-113.
- (4) (a) Cornel, K. M.; Krakstad, C.; Delvoux, B.; Xanthoulea, S.; Jori, B.; Bongers, M. Y.; Konings, G. F.; Kooreman, L. F.; Kruitwagen, R. F.; Salvesen, H. B.; Romano, A., High mRNA levels of 17 β -hydroxysteroid dehydrogenase type 1 correlate with poor prognosis in endometrial cancer. *Mol. Cell. Endocrinol.* **2017**, *442*, 51-57; (b) Sinreih, M.; Knific, T.; Anko, M.; Hevir, N.; Vouk, K.; Jerin, A.; Frkovic Grazio, S.; Rizner, T. L., The significance of the sulfatase pathway for local estrogen formation in endometrial cancer. *Front. Pharmacol.* **2017**, *8*, 368.
- (5) (a) Messinger, J.; Husen, B.; Koskimies, P.; Hirvelä, L.; Kallio, L.; Saarenketo, P.; Thole, H., Estrone C15 derivatives-a new class of 17 β -hydroxysteroid dehydrogenase type 1 inhibitors. *Mol. Cell. Endocrinol.* **2009**, *301*, 216-224; (b) Arnold, C.; Einspanier, A., Medical treatment improves social behavior in a primate endometriosis model (*Callithrix jacchus*). *J. Med. Primatol.* **2013**, *42*, 112-119.
- (6) Maltais, R.; Trottier, A.; Roy, J.; Ayan, D.; Bertrand, N.; Poirier, D., Pharmacokinetic profile of PBRM in rodents, a first selective covalent inhibitor of 17 β -HSD1 for breast cancer and endometriosis treatments. *J. Steroid Biochem. Mol. Biol.* **2018**, *178*, 167-176.
- (7) Ayan, D.; Maltais, R.; Roy, J.; Poirier, D., A new nonestrogenic steroidal inhibitor of 17 β -hydroxysteroid dehydrogenase type I blocks the estrogen-dependent breast cancer tumor growth induced by estrone. *Mol. Cancer Ther.* **2012**, *11*, 2096-2104.
- (8) Simoens, S.; Dunselman, G.; Dirksen, C.; Hummelshoj, L.; Bokor, A.; Brandes, I.; Brodsky, V.; Canis, M.; Colombo, G. L.; DeLeire, T.; Falcone, T.; Graham, B.; Halis, G.; Horne, A.; Kanj, O.; Kjer, J. J.; Kristensen, J.; Lebovic, D.; Mueller, M.; Viganò, P.; Wullschleger, M.; D'Hooghe, T., The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Hum. Reprod.* **2012**, *27*, 1292-1299.
- (9) (a) Trottier, A.; Maltais, R.; Ayan, D.; Barbeau, X.; Roy, J.; Perreault, M.; Poulin, R.; Lague, P.; Poirier, D., Insight into the mode of action and selectivity of PBRM, a covalent steroidal inhibitor of 17 β -hydroxysteroid dehydrogenase type 1. *Biochem. Pharmacol.* **2017**, *144*, 149-161; (b) Li, T.; Maltais, R.; Poirier, D.; Lin, S. X., Combined biophysical chemistry reveals a new covalent inhibitor with a low-reactivity alkyl halide. *J. Phys. Chem. Lett.* **2018**, 5275-5280.
- (10) D'Hooghe, T. M.; Nyachio, A.; Chai, D. C.; Kyama, C. M.; Spiessens, C.; Mwenda, J. M., Reproductive research in non-human primates at Institute of Primate Research in Nairobi, Kenya

- (WHO Collaborating Center): a platform for the development of clinical infertility services? *ESHRE Monographs* **2008**, *2008*, 102-107.
- (11) Braundmeier, A. G.; Fazleabas, A. T., The non-human primate model of endometriosis: research and implications for fecundity. *Mol. Hum. Reprod.* **2009**, *15*, 577-586.
- (12) Appel, R., Tertiary phosphane/tetrachloromethane, a versatile reagent for chlorination, dehydration, and P-N linkage. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 801-811.
- (13) Gomez, L.; Gellibert, F.; Wagner, A.; Mioskowski, C., (Chloro-phenylthio-methylene)dimethylammonium chloride (CPMA) an efficient reagent for selective chlorination and bromination of primary alcohols. *Tetrahedron Lett.* **2000**, *41*, 6049-6052.
- (14) Ponpipom, M. M.; Hanessian, S., Preparative and exploratory carbohydrate chemistry. Method for the selective bromination of primary alcohol groups. *Carbohyd. Res.* **1971**, *18*, 342-344.
- (15) Pouliot, M.-F.; Mahe, O.; Hamel, J.-D.; Desroches, J.; Paquin, J.-F., Halogenation of primary alcohols using a tetraethylammonium halide/[Et₂NSF₂]BF₄ combination. *Org. Lett.* **2012**, *14*, 5428-5431.
- (16) Maffioli, S. I.; Marzorati, E.; Marazzi, A., Mild and reversible dehydration of primary amides with PdCl₂ in aqueous acetonitrile. *Org. Lett.* **2005**, *7*, 5237-5239.
- (17) Chakraborty, S.; Berke, H., Homogeneous hydrogenation of nitriles catalyzed by molybdenum and tungsten amides. *ACS Catal.* **2014**, *4*, 2191-2194.
- (18) Dionne, P.; Tchédam Ngatcha, B.; Poirier, D. D-ring allyl derivatives of 17β- and 17α-estradiols: Chemical synthesis and ¹³C NMR data. *Steroids* **1997**, *62*, 674-681.
- (19) Lennox, A. J.; Lloyd-Jones, G. C., Selection of boron reagents for Suzuki-Miyaura coupling. *Chem Soc. Rev.* **2014**, *43*, 412-443.
- (20) Molander, G. A.; Ito, T., Cross-coupling reactions of potassium alkyltrifluoroborates with aryl and 1-alkenyl trifluoromethanesulfonates. *Org. Lett.* **2001**, *3*, 393-396.
- (21)(a) Lawrence, J. D.; Takahashi, M.; Bae, C.; Hartwig, J. F., Regiospecific functionalization of methyl C-H bonds of alkyl groups in reagents with heteroatom functionality. *J. Am. Chem. Soc.* **2004**, *126*, 15334-15335; (b) Murphy, J. M.; Lawrence, J. D.; Kawamura, K.; Incarvito, C.; Hartwig, J. F., Ruthenium-catalyzed regiospecific borylation of methyl C-H bonds. *J. Am. Chem. Soc.* **2006**, *128*, 13684-13685.
- (22) Fleury-Bregeot, N.; Presset, M.; Beaumard, F.; Colombel, V.; Oehlich, D.; Rombouts, F.; Molander, G. A., Suzuki-Miyaura Cross-coupling of potassium alkoxyethyltrifluoroborates: Access to aryl/heteroarylethoxy motifs. *J. Org. Chem.* **2012**, *77*, 10399-10408.
- (23) Molander, G. A.; Yun, C.-S.; Ribagorda, M.; Biolatto, B., B-Alkyl Suzuki-Miyaura cross-coupling reactions with air-stable potassium alkyltrifluoroborates. *J. Org. Chem.* **2003**, *68*, 5534-5539.
- (24) Shipilovskikh, S. A.; Vaganov, V. Y.; Denisova, E. I.; Rubtsov, A. E.; Malkov, A. V., Dehydration of amides to nitriles under conditions of a catalytic Appel reaction. *Org. Lett.* **2018**, *20*, 728-731.
- (25) Batesky, D. C.; Goldfogel, M. J.; Weix, D. J., Removal of triphenylphosphine oxide by precipitation with zinc chloride in polar solvents. *J. Org. Chem.* **2017**, *82*, 9931-9936.
- (26) Ruchelman, M.W., Solubility studies of estrone in organic solvents using gas-liquid chromatography. *Analytical Biochem.* **1967**, *19*, 98-108.
- (27) Pearlman, W.M., Noble metal hydroxides on carbon nonpyrophoric dry catalysts. *Tetrahedron Lett.* **1967**, *17*, 1663-1664.
- (28) Misra, R.N.; Brown, B.R.; Han, W.C.; Harris, D.N.; Hedberg, A.; Webb, M.L.; Hall, S.E. Interphenylene 7-oxabicyclo[2.2.1]heptane thromboxane A₂(TxA₂) antagonists. Semicarbazone .omega.-chains *J. Med. Chem.* **1991**, *34*, 2882-2891.
- (29) De Luca, L.; Giacomelli, G.; Porcheddu, A. An efficient route to alkyl chlorides from alcohols using the complet TCT/DMF. *Org. Lett.* **2002**, *4*, 553-555.

- 1
2 (30) Dai, C.D.; Narayaman, J.M.R.; Stephenson, C.R.J., Visible-light-mediated conversion of alcohols
3 to halides. *Nat. Chem.* **2011**, *3*, 140-145.
4 (31) Chen, J., Lin, J-H., Xiao, J-C., Halogenation through deoxygenation of alcohols and aldehydes.
5 *Org. Lett.* **2018**, *20*, 3061-3064.
6 (32) Javadzadeh, Y.; Hamedeyazdan, S.; Asnaashari, S. In *Recrystallization of drugs: significance on*
7 *pharmaceutical processing*, InTech: **2012**; pp 425-446.
8 (33) Tremblay, M. R.; Boivin, R. P.; Luu-The, V.; Poirier, D., Inhibitors of type 1 17 β -hydroxysteroid
9 dehydrogenase with reduced estrogenic activity: Modifications of the positions 3 and 6 of
10 estradiol. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 153-163.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60