ORGANIC LETTERS

2004
Vol. 6, No. 18
3127−3130

Synthesis of Simplified Hybrid Inhibitors of Type 1 17*β*-Hydroxysteroid Dehydrogenase via Cross-Metathesis and Sonogashira Coupling Reactions

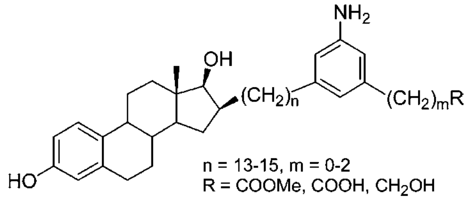
Marie Bérubé and Donald Poirier*

Medicinal Chemistry Division, Oncology and Molecular Endocrinology Research Center and Université Laval, Centre Hospitalier Universitaire de Québec (CHUQ), Pavillon CHUL, 2705 Laurier Boulevard, Quebec, Qc, G1V 4G2, Canada

donald.poirier@crchul.ulaval.ca

Received June 21, 2004

ABSTRACT



2

The inhibitor of type 1 17*β*-hydroxysteroid dehydrogenase EM-1745 (1) exhibits affinity for both the substrate (estrone or estradiol) and the cofactor (NAD(P)H) binding domains. However, to increase its bioavailability, this compound needs to be simplified. The efficient and convergent synthesis of simplified substrate/cofactor hybrid inhibitors (compounds 2) involving a cross-metathesis and a Sonogashira coupling reaction as key steps is described. Compounds 2a–c were also tested as enzyme inhibitors and compared to EM-1745.

Estrogens are known to be an important factor in the development of estrogen-dependent breast cancer.[1] A complementary approach to the treatment of this type of cancer with an antiestrogen[2] is to lower the level of estradiol by inhibiting one of the enzymes involved in its biosynthesis.[3] Among these enzymes, type 1 17*β*-hydroxysteroid dehydrogenase (17*β*-HSD) catalyzes the last step in the biosynthesis of estradiol.[4] During this enzymatic process, the nicotinamide moiety of the cofactor NADPH transfers a hydride to the α-face of the steroid carbon-17 to reduce estrone to the most potent estrogen estradiol.[5] Furthermore, the reductive activity of type 1 17*β*-HSD is more important in breast tumors than in normal breast tissue.[6]

The design of type 1 17*β*-HSD inhibitors has been attempted during the past decades, but without achieving progress significant enough to justify their use in the treatment of estrogen-dependent breast cancer.[7] Recently, a new class of inhibitors was developed based on the three-dimensional structures of the apoenzyme[8] and of a binary

---

(1) (a) von Angerer, E. *The Estrogen Receptor as a Target for Rational Drug Design*; R.G. Landes Co.: Austin, TX, 1995. (b) Dickson, R. B.; Lippman, M. E. *Endocr. Rev.* **1987**, *8*, 29−43. (c) Poulin, R.; Labrie, F. *Cancer Res.* **1986**, *46*, 4933−4937.

(2) Macgregor, J. I.; Jordan, V. C. *Pharmacol. Rev.* **1998**, *50*, 151−196.

(3) (a) Labrie, F.; Luu-The, V.; Lin, S. X.; Simard, J.; Labrie, C.; El-Alfy, M.; Pelletier, G.; Bélanger, A. *J. Mol. Endocrinol.* **2000**, *25*, 1−16. (b) Smith, H. J.; Nicholls, P. J.; Simons, C.; Le Lain, R. *Exp. Opin. Ther. Patents* **2001**, *11*, 789−824.

(4) (a) Luu-The, V.; Zhang, Y.; Poirier, D.; Labrie, F. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 581−587. (b) Luu-The, V. *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 143−151.

(5) Breton, R.; Housset, D.; Mazza, C.; Fontecilla-Camps, J. C. *Structure* **1996**, *4*, 905−915.

(6) McNeil, J. M.; Reed, M. J.; Beranek, P. A.; Bonney, R. C.; Ghilchik, M. W.; Robinson, D. J.; James, V. H. T. *Int. J. Cancer* **1986**, *38*, 193−196.

(7) For review on type 1 17*β*-HSD inhibitors, see: (a) Poirier, D. *Curr. Med. Chem.* **2003**, *10*, 1241−1253. (b) Penning, T. M. *Endocr. Relat. Cancer* **1996**, *3*, 41−56.

10.1021/ol048820u CCC: $27.50

complex (the enzyme and estradiol)[9] and on results of a structure/activity relationship (SAR) study.[10] This new category of substrate/cofactor hybrid inhibitor was designed to interact with both the substrate (estrone or estradiol) and the cofactor (NAD(P)H) enzyme-binding domains.[11] An eight-methylene spacer between the estradiol and the adenosine moiety of the cofactor gave the best in vitro inhibitor of type 1 17β-HSD (EM-1745, **1**, Figure 1) with an IC_{50} of 52 nM and a K_i value of 3.0 nM.[11a] Furthermore, the complex EM-1745/type 1 17β-HSD was crystallized and X-ray analysis confirmed that compound **1** interacts with both binding domains.[11a] However, this inhibitor needs to be simplified, especially on the adenosine moiety and on the ester link, to improve its in vivo stability and activity. Compounds **2** were proposed as simplified hybrid inhibitors of type 1 17β-HSD on the basis of main interactions between **1** and the enzyme (Figure 1). It was assumed that estradiol core of **2** would form strong hydrogen bonds with the amino acids His221, Ser142, and Tyr155, while the amino and R groups of the adenosine mimic would interact with Ser11 and Asp65. Furthermore, it is known that the cofactor NADPH has a better affinity for the enzyme than NADH.[12] The structural difference between these two entities is the presence of a phosphate group (NADPH) on the ribose. So, a phosphate derivative, or a carboxylic acid as a bioisostere,[13] added on aniline could interact well with the cofactor-binding domain. Other functionalities (ester, bromide, alcohol) will also be added to the aniline to extend the SAR study, and the alkyl spacers ($n = 13-15$, $m = 0-2$) will be optimized. We report herein a convergent and efficient route to prepare inhibitors **2a−c** using olefin cross-metathesis and Sonogashira coupling reactions as key steps. Preliminary biological evaluation is also reported.

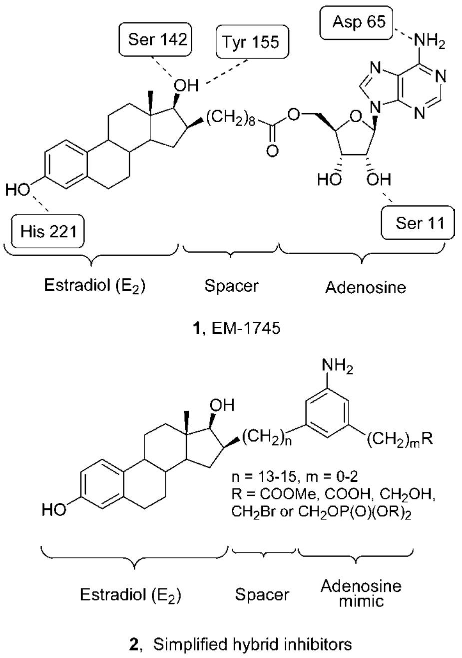
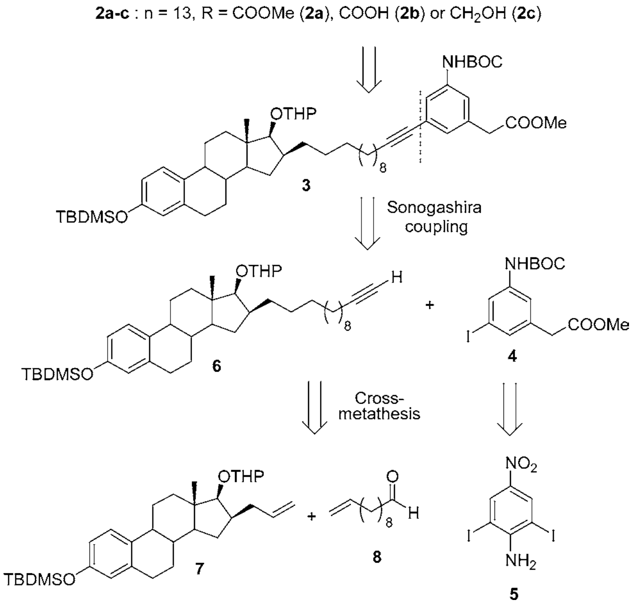


Figure 1. Type 1 17β-HSD hybrid inhibitor **1** (EM-1745) showing main interactions with this enzyme, as well as proposed simplified hybrid inhibitors **2**.

Scheme 1. Retrosynthetic Analysis of Compounds **2a−c**



The retrosynthetic strategy selected to synthesize inhibitors **2** is outlined in Scheme 1. To limit the number of steps in the preparation of compounds **2a−c**, introduction of molecular diversity (R = COOMe, COOH, CH_2OH) and removal of protective groups (TBDMS, THP, and BOC) would be done in the last stages. Because our synthetic plan is based on a convergent strategy, compound **3** would be obtained by a Sonogashira coupling reaction between the highly functionalized aryl iodide **4**, generated from **5**, and the alkyne **6**. A cross-metathesis between the suitably protected allylestradiol **7** and the olefin aldehyde **8** would be used to link the steroid and the alkyl spacer to give **6**. Thus, any alkyl spacers' length can be attached to the estradiol derivative **7** by using the appropriate olefin aldehyde.

Aniline **4** was prepared from the commercially available 2,6-diiodo-4-nitroaniline (**5**) (Scheme 2). Diazotization of **5**

---

(8) (a) Ghosh, D.; Pletnev, V. Z.; Zhu, D. W.; Wawrzak, Z.; Duax, W. L.; Pangborn, W.; Labrie, F.; Lin, S. X. *Structure* **1995**, *3*, 503−513. (b) Lin, S. X.; Zhu, D. W.; Azzi, A.; Campbell, R. L.; Breton, R.; Labrie, F.; Ghosh, D.; Pletnev, V.; Duax, W. L.; Pangborn, W. *J. Endocrinol.* **1996**, *150*, S13−20.

(9) Azzi, A.; Rhese, P. H.; Zhu, D. W.; Campbell, R.; Labrie, F.; Lin, S. X. *Nat. Struct. Biol.* **1996**, *3*, 665−668.

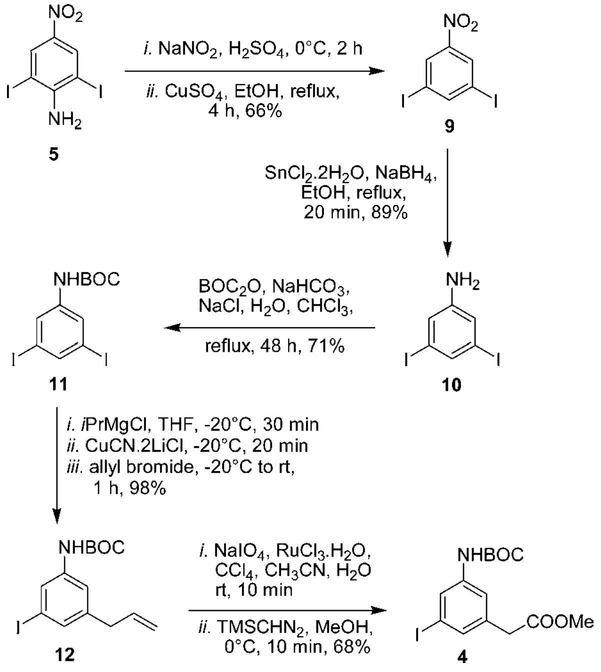
(10) (a) Tremblay, M. R.; Poirier, D. *J. Steroid Biochem. Mol. Biol.* **1998**, *66*, 179−191. (b) Tremblay, M. R.; Lin, S. X.; Poirier, D. *Steroids* **2001**, *66*, 821−831.

(11) (a) Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S. X. *FASEB J.* **2002**, *16*, 1829−1831. (b) Poirier, D.; Boivin, R. P.; Bérubé, M.; Lin, S. X. *Synth. Commun.* **2003**, *33*, 3183−3192.

(12) Huang, Y. W.; Pineau, I.; Chang, H. J.; Azzi, A.; Bellemare, V.; Laberge, S.; Lin, S. X. *Mol. Endocrinol.* **2001**, *15*, 2010−2020.

(13) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, CA, 1992; pp 19−23.

Scheme 2. Synthesis of Aniline **4**

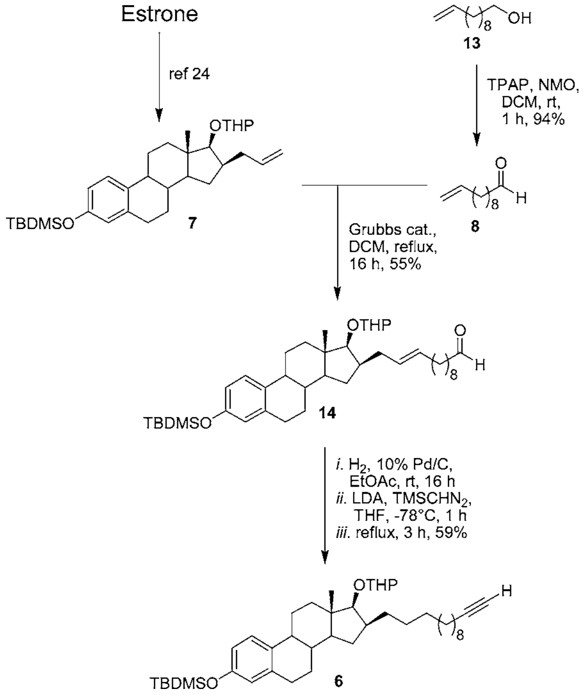


followed by reduction with copper sulfate in refluxing ethanol afforded **9** as described by Brink et al.[14] Nitro compound **9** was smoothly reduced with $NaBH_4$ and $SnCl_2$[15] to provide aniline **10** in good yield and purity without purification. Protection of aniline **10** with a BOC group was more difficult than expected. Classic protective conditions (BOC_2O, Et_3N in DCM or DMF, or BOC_2O in refluxing THF) did not allow completion of the reaction even after a long time. However, a biphasic system (BOC_2O, $NaHCO_3$, NaCl, H_2O, and $CHCl_3$)[16] gave a complete reaction, affording the protected aniline **11**. Desymmetrization of **11** was accomplished using a metal halogen exchange methodology. Adapting the chemistry of Knochel,[17] metalation of **11** using *iso*-propylmagnesium chloride was explored as a means for generating the arylmagnesium. When exactly 2 equiv of metalating agent were used, we found that only one I−Mg exchange took place. Thus, treatment of **11** with 2 equiv of *iso*-propylmagnesium chloride at −20 °C for 30 min afforded the formation of the carbamate anion and the I−Mg exchange, giving the corresponding Grignard reagent in >95% yield (NMR analysis of aliquots of the reaction mixture after quenching with water). This Grignard reagent was quenched with allyl bromide in the presence of CuCN·2LiCl[18] to give **12** in excellent yield (98%). Finally, oxidative cleavage[19] of allyl **12** with $RuCl_3$ and $NaIO_4$ followed by esterification with trimethylsilyldiazomethane[20] produced ester **4**.

For the attachment of the spacer to the steroid, several strategies were considered. For example, α-alkylation of steroidal 17-ketone, using LiHMDS as a base and a bromoalkyl chain, could be one way of joining the alkyl spacer to the TBDMS-estrone. However, this strategy was used when synthesizing **1** and only a low alkylation yield was obtained, which is explained by the less reactive 17-ketone of estrone. In fact, α-alkylation of 17-ketosteroid can only be obtained in good yield with an activated electrophile such as allyl bromide[21,10] or with an unactivated electrophile when the ketone is activated.[22] This later methodology was not selected because it is not a convergent strategy and its three-step sequence would have added too many steps to the synthesis of alkyne **6**.

We developed instead a convergent and improved strategy in which various alkyne lengths can be obtained in three steps (Scheme 3). This new approach is based on a cross-metathesis[23] between allyl-estradiol **7**[24] and aldehyde **8**, obtained from oxidation of alcohol **13** with TPAP, which

Scheme 3. Synthesis of Alkyne **6**



---

[14] Brink, G. J. T.; Vis, J. M.; Arends, I. W. C. E.; Sheldon, R. A. *Tetrahedron* **2002**, *58*, 3977−3983.

[15] (a) Satoh, T.; Mitsuo, N.; Nishiki, M.; Inoue, Y.; Ooi, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1443−1445. (b) Halterman, R. L.; McEvoy, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 980−985.

[16] Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730−732.

[17] (a) Boymond, L.; Rottländer, M.; Cahiez, G.; Knochel, P. *Angew. Chem., Int. Ed.* **1998**, *37*, 1701−1703. (b) Abarbri, M.; Thibonnet, J.; Bérillon, L.; Dehmel, F.; Rottländer, M.; Knochel, P. *J. Org. Chem.* **2000**, *65*, 4618−4634. (c) Rottländer, M.; Boymond, L.; Bérillon, L.; Leprêtre, A.; Varchi, G.; Avolio, S.; Laaziri, H.; Quéguiner, G.; Ricci, A.; Cahiez, G.; Knochel, P. *Chem. Eur. J.* **2000**, *6*, 767−770. (d) Herrinton, P. M.; Owen, C. E.; Gage, J. R. *Org. Process Res. Dev.* **2001**, *5*, 80−83. (e) Knochel, P.; Dohle, W.; Gommermann, N.; Kneisel, F. F.; Kopp, F.; Korn, T.; Sapountzis, I.; Anh Vu, V. *Angew. Chem., Int. Ed.* **2003**, *42*, 4302−4320.

[18] Knochel, P.; Yeh, M. C. P.; Berk, S. C.; Talbert, J. *J. Org. Chem.* **1988**, *53*, 2390−2392.

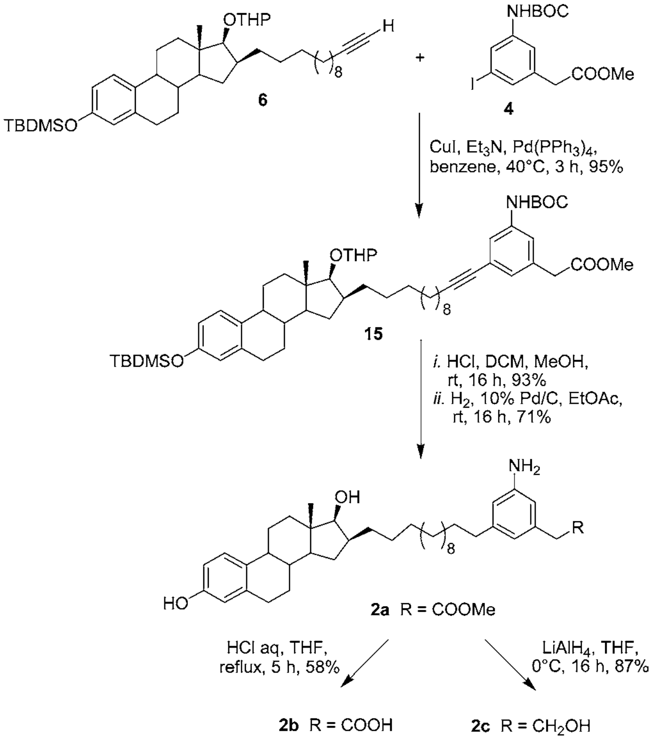
[19] Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936−3938.

[20] Gomez, A. M.; Lopez, J. C.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 3859−3870.

led to the product **14** in an acceptable but not optimized 55% yield. Hydrogenation of alkene **14** with palladium, followed by treatment with LDA, trimethylsilyl-diazomethane, and heat provided alkyne **6** in 59% yield for two steps.[25]

The next step is the Sonogashira coupling[26] between alkyne **6** and aryl iodide **4** (Scheme 4). Under optimized conditions $(Pd(PPh_3)_4$, CuI, Et_3N, benzene),[27] product **15** was obtained in excellent yield (95%). Finally, after cleavage of all three protective groups in acidic solution and hydrogenation of the alkyne, ester **2a** was successfully obtained. From ester **2a**, acid **2b** and alcohol **2c** were obtained after a hydrolysis and a hydride reduction treatment, respectively.

Scheme 4. Final Coupling and Molecular Diversities



Compounds **2a**−**c** were evaluated for their ability to inhibit the in vitro transformation of estrone into estradiol by type 1 17β-HSD according to an established procedure.[10b] Preliminary results revealed that **2b** (90% inhibition) was a more potent inhibitor than **2a** and **2c** (32 and 16% inhibition, respectively) at 1 μM. When compared to EM-1745 (**1**), the inhibitory potency of **2b** was approximately the same (90% versus 95% for **1**) at 1 μM and slightly weaker at 0.1 μM (56% versus 90% for **1)**. The good inhibition obtained for **2b** could be explained by the fact that the carboxylic acid is a bioisostere of the phosphate of NADPH and probably interacts with the same amino acids in the cofactor-binding domain.

In conclusion, we developed an efficient strategy for the preparation of simplified hybrid inhibitors **2a**−**c** in six steps from known intermediate **7** and with an overall yield of 20%. Halogen−metal exchange and olefin metathesis and Sonogashira coupling reactions are key steps in this synthesis. This methodology has been applied to the synthesis of analogues of compounds **2** (spacers of 14 and 15 methylenes, aryl diversity) with similar overall yields (data not shown). This strategy could also be used for the preparation of any bisubstrate inhibitors joined by a methylene spacer. For this purpose, the steroidal nucleus could be replaced by a phenolic ring, extending our methodology to other families of therapeutic agents.

Acknowledgment. We thank the Canadian Institutes of Health Research (CIHR) for financial support and scholarship (M.B.). We also thank Ms. Sylvie Méthot for careful reading of the manuscript and Drs. René Maltais and Shankar M. Singh for helpful suggestions.

Supporting Information Available: Experimental procedure and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048820U

---

(21) Fevig, T. L.; Katzenellenbogen, J. A. *J. Org. Chem.* **1987**, *52*, 247−251.

(22) (a) Tremblay, M. R.; Auger, S.; Poirier, D. *Synth. Commun.* **1995**, *25*, 2483−2495. (b) Tremblay, M. R.; Auger, S.; Poirier, D. *Bioorg. Med. Chem.* **1995**, *5*, 505−523.

(23) For recent reviews in this area, see: (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413−4450. (b) Connon, S. J.; Blechert, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1900−1923.

(24) Tremblay, M. R.; Poirier, D. *J. Comb. Chem.* **2000**, *2*, 41−65.

(25) Miwa, K.; Aoyama, T.; Shioiri, T. *Synlett* **1994**, 107−108.

(26) Sonogashira, K. *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon Press: Oxford, 1991; Vol. 3, Chapter 2.4, pp 521−549.

(27) Nicolaou, K. C.; Dai, W. M.; Hong, Y. P.; Tsay, S. C.; Baldridge, K. K.; Siegel, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 7944−7953.