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Synthesis and antihormonal properties of novel 11β-benzoxazole-substituted steroids

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ABSTRACT

Early studies led to the identification of 11β-aryl-4′,5′-dihydrospiro[estra-4,9-diene-17β,4′-oxazole] analogs with potent and more selective antiprogestational activity compared to antiglucocorticoid activity than mifepristone. In the present study, we replaced the 4′-dimethylaminophenyl group of mifepristone with the benzoxazol group to give **5a–d**. We also prepared the 17β-formamido analogs **6a,b** using a new synthetic strategy via the intermediate epoxide **21**. These compounds were evaluated for their antagonist hormonal properties using the T47D cell-based alkaline phosphatase assay and the A549 cell-based functional assay. Compound **5c** showed potent antagonist activity at GR with better selectivity for GR versus PR than mifepristone and is a promising lead for further development.

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Nuclear receptors play crucial roles in development, homeostasis and other biological processes in mammals.[1] A subset of these receptors binds specific steroids and related ligands to mediate the effects by influencing transcription of target genes.[2] One such receptor, the glucocorticoid receptor (GR), acts primarily via binding to cortisol in humans and is crucially involved in the normal development and maintenance of basal and stress-related homeostasis.[3] Potent and selective GR antagonists have been suggested for the possible treatment of depression,[4] diabetes,[5] glaucoma,[6] hypertension[7] and Cushing's disease.[8] The well-known steroidal GR antagonist, mifepristone (RU 486, **1**, Fig. 1), was introduced by the Roussel-Uclaf group in 1980s.[9] A serious drawback of mifepristone is its abortifacient effects due to its potent progesterone receptor (PR) antagonism.[10] Since the discovery of mifepristone, hundreds of analogs have been synthesized to optimize the potency and selectivity for GR or PR.[11] Nonetheless, mifepristone remains the most used drug to date for the study of biology involving GR.

Steroid receptors are closely related in the structure and their mechanism of action.[12] Crystal structures of mifepristone bound to GR and PR have recently been solved.[13,14] Superposition of mifepristone bound to GR and PR ligand binding domains (LBD) revealed its different binding interactions in GR and PR, indicating that modification of the steroidal structure would have a profound effect on the affinity and activity for the corresponding receptors.[14] SAR studies from our laboratory as well as others have shown that combinations of specific substitutions at the 11- and 17-positions have a marked influence on the antihormonal properties at GR and PR.[11,15–24] For example, replacement of the *N*,*N*-dimethylamino group of mifepristone with a diethoxyphosponyl group led to a potent and selective GR antagonist **2**.[22] Interestingly, substitution of the methyl on the propynyl group with dialkyl or dialkoxyphosphonyl groups resulted in selective PR antagonists.[21] Some time ago, we found that various nitro[25] and amido (**3**)[26] substituents on the 17β-position could also generate potent antihormonal effects, in some cases, with dissociated activities at GR versus PR. We also showed that replacement of the 17β-hydroxyl and 17α-propynyl groups with a substituted 17,17-spiro-oxazole group (**4**) led to potent antagonism at PR with considerably reduced activity at GR.[24] Oxazoles have been suggested as bioisosteres for the carboxylic ester group.[27] We envisioned that oxazole could also serve as a bioisostere in steroids for the *N*,*N*-dimethylamino group in mifepristone or the diethoxyphosponyl group in **2**. In this Letter, we describe the synthesis of several 11β-benzoxazole-substituted 17β-hydroxy- or 17β-formamido-steroids, and report their antagonist activities at GR and PR.

Retrosynthetic analysis suggested that dieneketal **10** was a suitable starting material for making the target compounds with the major question being the order of introduction of the substituents to give intermediate **8** or **9** (Scheme 1). The synthesis of 11β-benzoxazole-substituted 17β-hydroxy-steroids **5a–d** is presented in Scheme 2. Regioselective 5,10-epoxidation of commercial dieneketal **10** using hexafluoroacetone hydroperoxide, generated in situ from hexafluoroacetone trihydrate and hydrogen peroxide, afforded

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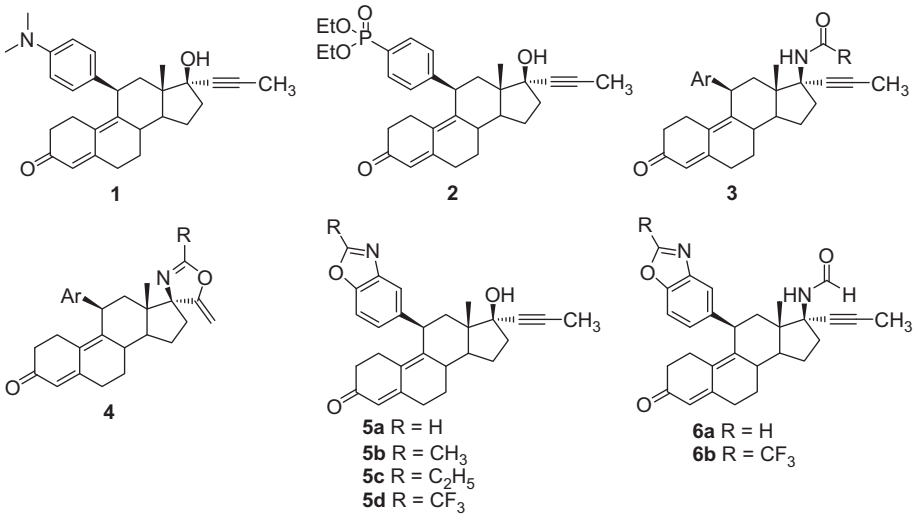
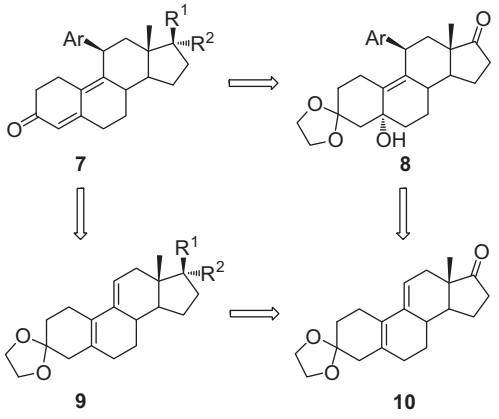
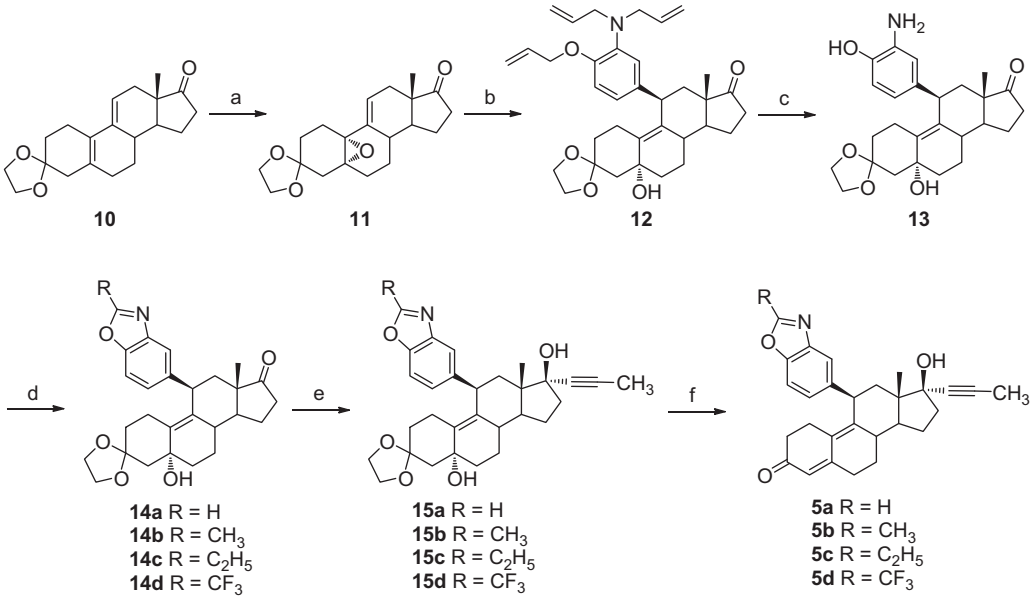


Figure 1. Structures of mifepristone (**1**) and 11β-arylsteroids.

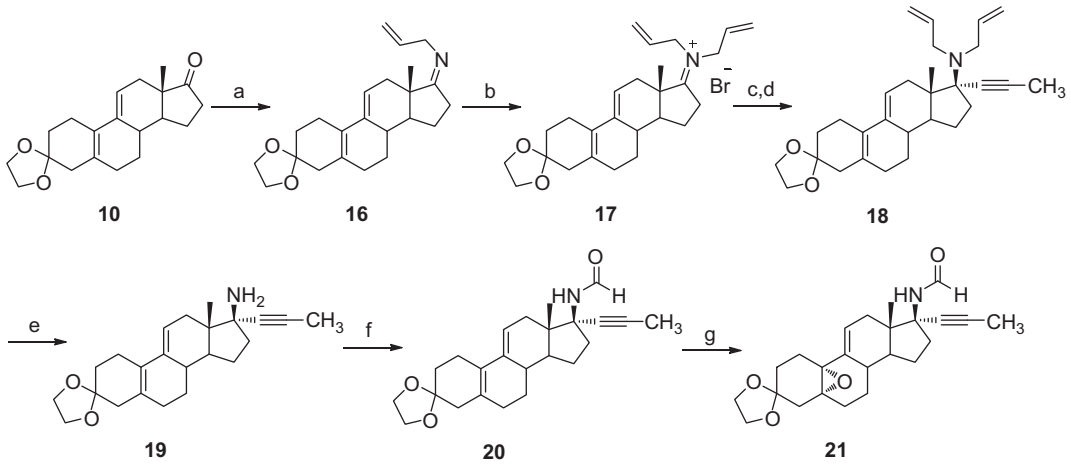


Scheme 1. Retrosynthesis of 11β-aryl 17,17-disubstituted steroids.

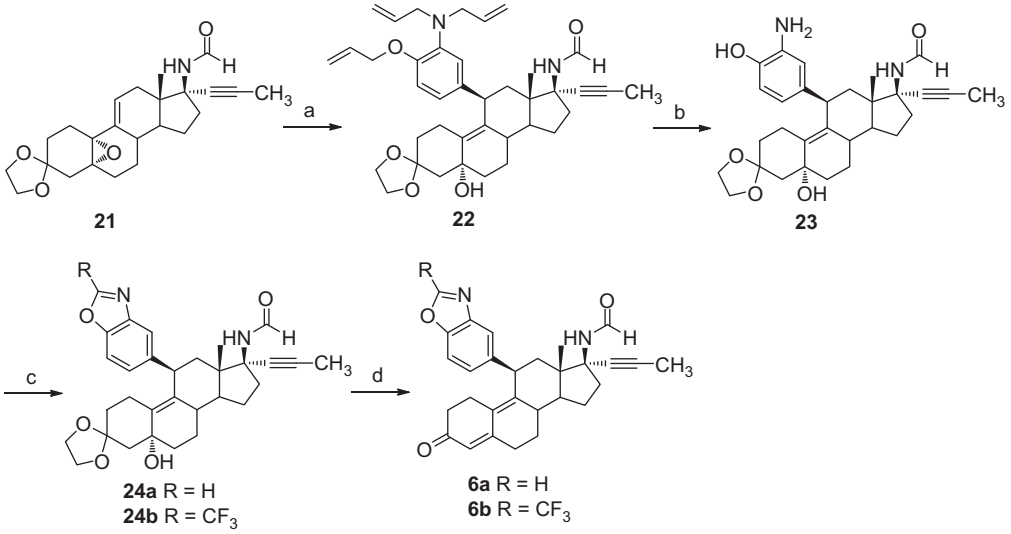
epoxide **11** in 53% yield.[25] Original attempts to generate the Grignard or lithiate reagent from the corresponding 5-bromobenzoxazoles followed by addition to epoxide **11** were not successful. It was therefore necessary to introduce the oxazole moiety at a later stage in the synthesis. Thus, treatment of diallyamino-2-allyloxy-5-bromobenzene, prepared from 2-amino-4-bromophenol with ally bromide and potassium hydroxide, with magnesium in tetrahydrofuran (THF) afforded the required Grignard reagent. Subsequent addition of the Grignard reagent to a solution of **11** in THF in the presence of CuI gave **12** in 85% yield.[28] Deprotection of the allyl groups was achieved in 69% yield by using *N*,*N*′-dimethylbarbituric acid and catalytic $Pd(PPh_3)_4$ to give aminophenol **13**.[29] Ring formation with *s*-triazine[30] or an appropriate ortho ester[31] provided oxazoles **14a–c** in 57–73% yields. The trifluoromethyl analog **14d** was synthesized in 67% yield using freshly prepared 2,2,2-trifluoroacetimidic acid methyl ester.[32,33] Addition of 1-propynylmagnesium bromide followed by deketalization and dehydration with trifluoroacetic acid



Scheme 2. Reagents and conditions: (a) CF_3COCF_3, Na_2HPO_4, H_2O_2, 0 °C, 53%; (b) diallylamino-2-allyloxy-5-bromobenzene/Mg, THF, CuI, 0 °C, 85%; (c) $Pd(PPh_3)_4$, *N*,*N*′-dimethylbarbituric acid, CH_2Cl_2, 40 °C, 69%; (d) **14a**: *s*-triazine, toluene, reflux, 57%; **14b**: triethyl orthoacetate, DMF, 100 °C, 73%; **14c**: triethyl orthopropionate, DMF, 100 °C, 69%; **14d**: 2,2,2-trifluoroacetimidic acid methyl ester, $CHCl_3$, rt, then toluene, reflux, 67%; (e) 1-propynylmagnesium bromide, THF, rt, 80–87%; (f) TFA, CH_2Cl_2, H_2O, 0 °C, 70–83%.



Scheme 3. Reagents and conditions: (a) allylamine, *p*-TsOH, benzene, reflux; (b) allyl bromide, CH_2Cl_2, rt; (c) KCN, MeOH, rt; (d) 1-propynylmagnesium bromide, THF, rt, 25% over 4 steps; (e) $Pd(PPh_3)_4$, *N*,*N*′-dimethylbarbituric acid, CH_2Cl_2, 35 °C, 84%; (f) cyanomethyl formate, CH_2Cl_2, rt, 75%; (g) CF_3COCF_3, Na_2HPO_4, H_2O_2, 0 °C, 90%.



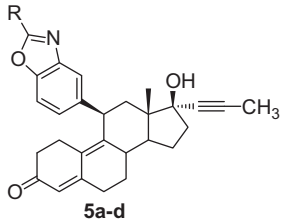
Scheme 4. Reagents and conditions: (a) diallylamino-2-allyloxy-5-bromobenzene/Mg, THF, CuI, 0 °C, 71%; (b) $Pd(PPh_3)_4$, *N*,*N*′-dimethylbarbituric acid, CH_2Cl_2, 40 °C, 77%; (c) **24a**: *s*-triazine, toluene, reflux, 79%; **24b**: 2,2,2-trifluoroacetimidic acid methyl ester, $CHCl_3$, rt, then toluene, reflux, 77%; (d) TFA, CH_2Cl_2, H_2O, 0 °C, 92–98%.

(TFA) furnished the title compounds **5a–d** in 56–72% yields over two steps.

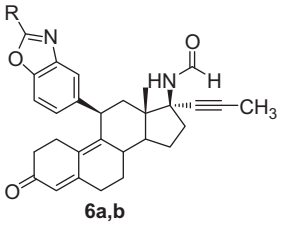
The synthesis of 17β-formamido analogs **6a,b** was investigated next. Attempts to convert the 17-keto group of intermediate **12** or **14** to the 17α-propynyl-17β-formamido[26] functionalities were not successful. Therefore, an alternative synthetic strategy, outlined in Schemes 3 and 4, was pursued, in which the 17-substituents were introduced first. Reaction of dieneketal **10** with allylamine followed by excess allyl bromide afforded the iminium salt **17** (Scheme 3). Addition of potassium cyanide to the iminium moiety followed by substitution with 1-propynylmagesium bromide under Bruylants reaction conditions[34] provided the 17β-diallylamino-17α-propynyl derivative **18** in 25% yield over four steps. Pd(0)-catalyzed deprotection of the ally groups afforded amine **19** in 84% yield. Formylation of **19** with cyanomethyl formate[35] followed by epoxidation gave the key epoxide **21** in 67% yield. Following a similar procedure analogous to that for preparing **5a–d**, CuI-catalyzed addition of the Grignard reagent, generated from diallyamino-2-allyloxy-5-bromobenzene, to **21** afforded the Grignard adduct **22** in 71% yield (Scheme 4). Ally groups deprotection using *N*,*N*′-dimethylbarbituric acid and catalytic $Pd(PPh_3)_4$ gave aminophenol **23** in 77% yield. Oxazole ring formation with *s*-triazine or 2,2,2-trifluoroacetimidic acid methyl ester gave **24a,b** in 79% and 77% yield, respectively. Finally, deketalization and dehydration with TFA provided 17β-formamido analogs **6a,b** in 92–98% yields. All synthesized steroids **5a–d** and **6a,b** were ≥98% pure as determined by HPLC analyses. The ^1H NMR and HRMS analyses of the compounds were in agreement with the assigned structures.[36]

Compounds **5a–d** and **6a,b** were evaluated for GR antagonist activity based on the ability to inhibit dexamethasone-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549.[37] Their PR antagonist activity was tested on the ability to block progesterone induction of alkaline phosphatase activity in the human breast cancer cell line T47D.[37] The IC_{50} values of the tested compounds were determined using regression analysis of the luminescent data and are listed in Table 1. The ratio of the T47D IC_{50} to the A549 IC_{50} was also calculated as a measure of the separation of GR and PR antagonism. The commercial drug mifepristone was tested as a control. Compounds **5a–d** are potent GR antagonists with similar or better selectivity, though less potent, than mifepristone. The best selectivity (fivefold) in differentiating GR

Table 1
Antihormonal properties of 11β-benzoxazole-substituted steroids



5a-d



6a,b

Compound	R	A549 (GR) and T47D (PR) activity		
		A549 IC_{50} (nM)	T47D IC_{50} (nM)	Ratio (PR/GR)
Mifepristone[a]		1.6	1.4	0.9
5a	H	45	101	2.2
5b	CH_3	23	35	1.5
5c	C_2H_5	13	65	5.0
5d	CF_3	61	85	1.4
6a	H	60	41	0.7
6b	CF_3	142	46	0.3

[a] Data taken from Ref. 37.

from PR was realized when R = Et (**5c**). Compound **5c** is also the most potent compound in the series at GR with an IC_{50} value of 13 nM. It is interesting to note that the 17β-amido analogs **6a,b** are slightly more selective than mifepristone toward PR.

In summary, a series of novel 11β-benzoxazole-substituted 17β-hydroxy- or 17β-formamido-steroids were synthesized and evaluated for their antihormonal properties at GR and PR. Although all the compounds in this series were less potent than mifepristone, representative compound demonstrated a better selectivity for GR versus PR. Further modification of **5c** is underway to gain additional SAR information for potent and selective GR antagonist.

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References and notes

1. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schütz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. *Cell* **1995**, *83*, 835.
2. Egea, P. F.; Klaholz, B. P.; Moras, D. *FEBS Lett.* **2000**, *476*, 62.
3. DeRijik, R. H.; Sternberg, E. M.; deKloet, E. R. *Curr. Opin. Endocrinol. Diabetes* **1997**, *4*, 185.
4. Brown, E. S. *Ann. N.Y. Acad. Sci.* **2009**, *1179*, 41.
5. Gettys, T. W.; Watson, P. M.; Taylor, I. L.; Collins, S. *Int. J. Obes. Relat. Metab. Disord.* **1997**, *21*, 865.
6. Southren, A. L.; Wandel, T.; Gordon, G. G.; Weinstein, B. I. *J. Ocul. Pharmacol.* **1994**, *10*, 385.
7. Nussinovitch, U.; de Carvalho, J. F.; Pereira, R. M.; Shoenfeld, Y. *Curr. Pharm. Des.* **2010**, *16*, 3574.
8. Chu, J. W.; Matthias, D. F.; Belanoff, J.; Schatzberg, A.; Hoffman, A. R.; Feldman, D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3568.
9. Teutsch, G.; Philibert, D. *Hum. Reprod.* **1994**, *9*, 12.
10. Im, A.; Appleman, L. *J. Expert Opin. Pharmacother.* **2010**, *11*, 481.
11. Clark, R. D. *Curr. Top. Med. Chem.* **2008**, *8*, 813. and references therein.
12. Carson-Jurica, M. A.; Schrader, W. T.; O'Malley, B. W. *Endocr. Rev.* **1990**, *11*, 201.
13. Kauppi, B.; Jakob, C.; Färnegårdh, M.; Yang, J.; Ahola, H.; Alarcon, M.; Calles, K.; Engström, O.; Harlan, J.; Muchmore, S.; Ramqvist, A. K.; Thorell, S.; Ohman, L.; Greer, J.; Gustafsson, J. A.; Carlstedt-Duke, J.; Carlquist, M. *J. Biol. Chem.* **2003**, *278*, 22748.
14. Raaijmakers, H. C.; Versteegh, J. E.; Uitdehaag, J. C. *J. Biol. Chem.* **2009**, *284*, 19572.
15. Wehle, H.; Moll, J.; Cato, A. C. B. *Steroids* **1995**, *60*, 368.
16. Gebhard, R.; Van der Voort, H.; Schuts, W.; Schoonen, W. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2229.
17. Wagner, B. L.; Pollio, G.; Giangrande, P.; Webster, J. C.; Breslin, M.; Mais, D. E.; Cook, C. E.; Vedeckis, W. V.; Cidlowski, J. A.; McDonnell, D. P. *Endocrinology* **1999**, *140*, 1449.
18. Fuhrmann, U.; Hess-Stumpp, H.; Cleve, A.; Neef, G.; Schwede, W.; Hoffmann, J.; Fritzemeier, K. H.; Chwalisz, K. *J. Med. Chem.* **2000**, *43*, 5010.
19. Cook, C. E.; Raje, P.; Lee, D. Y. –W.; Kepler, J. A. *Org. Lett.* **2001**, *3*, 101.
20. Jiang, W. Q.; Allan, G.; Fiordeliso, J. J.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P. F.; Gunnet, J.; Demarest, K.; Lundeen, S.; Sui, Z. H. *Bioorg. Med. Chem.* **2006**, *14*, 6726.
21. Jiang, W. Q.; Allan, G.; Chen, X.; Fiordeliso, J. J.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P. F.; Gunnet, J.; Demarest, K.; Lundeen, S.; Sui, Z. H. *Steroids* **2006**, *71*, 949.
22. Jiang, W. Q.; Fiordeliso, J. J.; Allan, G.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P. F.; Gunnet, J.; Demarest, K.; Lundeen, S.; Sui, Z. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1471.
23. Jin, C.; Burgess, J. P.; Kepler, J. A.; Cook, C. E. *Org. Lett.* **2007**, *9*, 1887.
24. Jin, C.; Manikumar, G.; Kepler, J. A.; Cook, C. E.; Allan, G. F.; Kiddoe, M.; Bhattacharjee, S.; Linton, O.; Lundeen, S. G.; Sui, Z. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5754.
25. Cook, C. E.; Kepler, J. A.; Shetty, R. S.; Bartley, G. S.; Lee, D. Y. -W. U.S. Patent 5,962,444, 1999.
26. Cook, C. E.; Kepler, J. A.; Bartley, G. S. U.S. Patent 6,262,042, 2001.
27. Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23.
28. Teutsch, G.; Klich, M.; Bouchoux, F.; Cerede, E.; Philibert, D. *Steroids* **1994**, *59*, 22.
29. Garro-Helion, F.; Merzouk, A.; GuibB', F. *J. Org. Chem.* **1993**, *58*, 6109.
30. Grundmann, C.; Kreutzberger, A. *J. Am. Chem. Soc.* **1955**, *77*, 6559.
31. Meyers, A. I.; Yamamoto, Y. *Tetrahedron* **1984**, *40*, 2309.
32. Brown, H. C.; Wetzel, C. R. *J. Org. Chem.* **1965**, *30*, 3724.
33. Braz, G. I.; Myasnikova, G. V.; Yakubovich, A. Y.; Bazov, V. P.; Kardash, I. E.; Pravednikov, A. N. *Chem. Heterocycl. Compd.* **1967**, *3*, 158.
34. Beaufort-Droal, V.; Pereira, E.; Thery, V.; Aitcen, D. J. *Tetrahedron* **2006**, *62*, 11948.
35. Deutsch, J.; Niclas, H.-J. *Synth. Commun.* **1993**, *23*, 1561.
36. The stereochemistry of **5a**–**d** and **6a**,**b** was assigned based on comparison of their NMR spectra with those of reported similar compounds (References 23,26,28).
37. Kang, F. A.; Allan, G.; Guan, J. H.; Nareshkumar, J.; Linton, O.; Tannenbaum, P.; Xu, J.; Zhu, P. F.; Gunnet, J.; Chen, X.; Demarest, K.; Lundeen, S.; Sui, Z. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 907.