Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 427–431

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of 21,21-Difluoro-3β-hydroxy-20-methylpregna-5,20-diene and 5,16,20-Triene as Potential Inhibitors of Steroid $C_{17(20)}$ Lyase

Philip M. Weintraub,[a,*] Amy K. Holland,[b] Cynthia A. Gates,[c] William R. Moore,[d] Robert J. Resvick,[†,a] Philippe Bey[e] and Norton P. Peet[f,*]

[a]*Aventis Pharmaceuticals; Rte 202-206, PO Box 6800, Bridgewater, NJ 08807-0800, USA*
[b]*Vankel, 13000 Weston Parkway, Cary, NC 27513, USA*
[c]*Vertex Pharmaceuticals Inc., 130 Waverly St., Cambridge, MA 02139-4242, USA*
[d]*Locus Discoveries, Inc., 512 Township Line Rd, Blue Bell, PA 19422, USA*
[e]*ArQule, 19 Presidential Way, Woburn, MA 01801-5140, USA*
[f]*Aurigene Discovery Technologies, 8-A Preston Court, Bedford, MA 01730, USA*

Received 22 May 2002; accepted 29 August 2002

Abstract—Novel 21,21-difluorovinyl steroids, designed as difluorinated $C_{20(21)}$ enol mimics of pregnenolone, were targeted as potential mechanism-based inhibitors of $C_{17(20)}$ lyase, a crucial enzyme in the biosynthesis of testosterone. Addition of (difluoromethyl)diphenylphosphine oxide reagent to 17-acetyl steroids was the approach chosen for the construction of these compounds. Of particular interest were the abnormal Wittig products which formed during attempted preparation of the triene **9**. The target difluoroolefin **3** was found to be a moderately potent, time-dependent inhibitor of the enzyme.

Introduction

Prostate cancer is a leading cause of cancer-related deaths among men.[1] Treatment is primarily aimed at blocking the synthesis and action of androgens. Androgen deprivation can be achieved surgically or through the use of LHRH analogues in combination with antiandrogens. A key enzyme in the biosynthetic pathway for the synthesis of androgens is $C_{17(20)}$ lyase.[2,3] This enzyme converts the C_{21}-progestins to C_{19}-androgens by cleavage of the two-carbon acetyl side chain.

A variety of $C_{17(20)}$ lyase inhibitors of different mechanistic types and structural classes has been described.[4] Our laboratory has been interested in developing mechanism-based inhibitors of this enzyme and we have prepared a number of C_{17}-cyclopropylamines,[5] C_{17}-cyclopropyl ethers,[6] and C_{17}-heterocyclic compounds[7] for this purpose as well as combined $C_{17(20)}$ lyase/5α-reductase inhibitors.[8] We recently reported fluoro-olefins which were $C_{17(20)}$ enol mimics of pregnenolone, which displayed potent, time-dependent inhibition of $C_{17(20)}$ lyase.[9] This report is an extension of our work on enol mimics as potential $C_{17(20)}$ lyase inhibitors.

The fluoro-olefins which we designed as $C_{17(20)}$ enol mimics are compounds **1** and **2**[9] as shown in Figure 1.

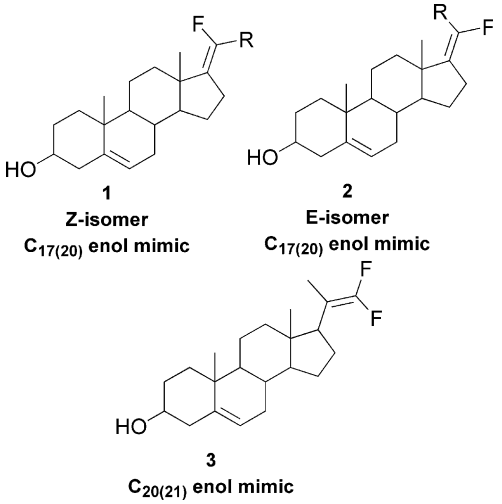


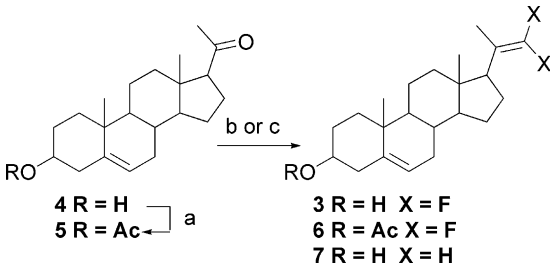
Figure 1. $C_{17(20)}$ and $C_{20(21)}$ fluoroolefin enol mimics.

*Corresponding authors. Tel.: +1-908-231-2572, e-mail: philip.weintraub@aventis.com (P. M. Weintraub); Tel.: +1-978-804-8099, e-mail: norton_p@aurigene.com (N. P. Peet).
†Deceased 28 April 1998.

0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00434-0

Table 1. Inhibition of cymologous monkey testicular $C_{17(20)}$ lyase with fluoroolefin enol mimics of pregnenolone and related compounds[16]

Compd	Enol mimic	Concentration (μM)	Preincubation (min)	Inhibition (%)
1	C17(20)	10	0	78
		10	40	94
		1	0	49
		1	40	72
2	C17(20)	10	0	88
		10	40	94
		1	0	54
		1	40	60
3	C20(21)	1	0	10
		1	40	40
		0.1	0	11.5
		0.1	40	8
6	C20(21)	1	0	7
		1	40	12
		0.1	0	5
		0.1	40	5
7	C20(21)	1	0	70
		1	40	60
9	C20(21)	1	0	64
		1	40	62
14	C17(20)	1	0	100
		1	40	85



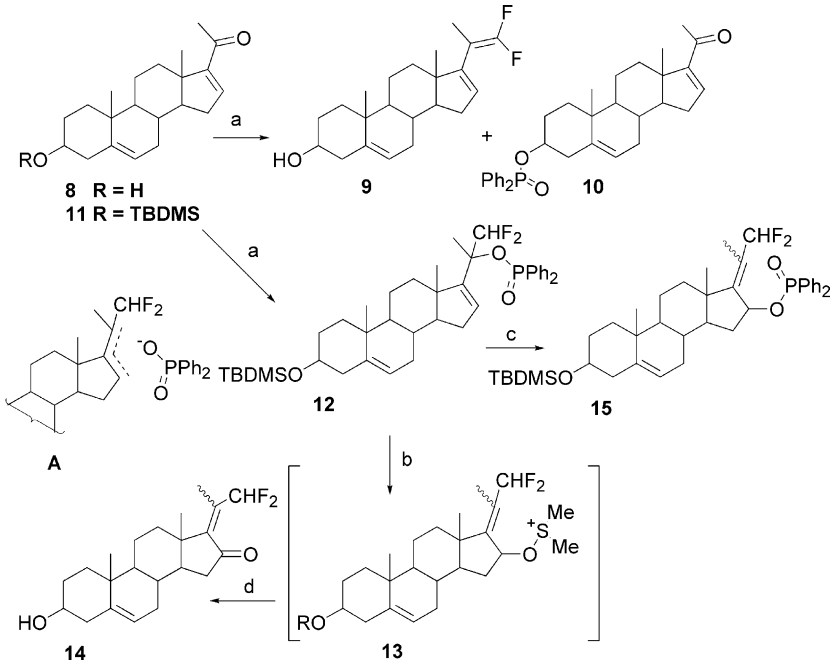
Scheme 1. Reagents: (a) Ac_2O; (b) $Ph_2P(O)CHF_2$; (c) Ph_3P+CH_3Br-.

These compounds are potent inhibitors of cynomolgous monkey testicular $C_{17(20)}$ lyase (Table 1). From the preincubation experiments, the *Z*-isomer **1** is clearly demonstrating time-dependent behavior. Thus, we wanted to explore the activity of novel difluoroolefin **3**, which we felt would provide a somewhat analogous mimic of the $C_{20(21)}$ enol of pregnenolone.

Starting with pregnenolone (**4**), we prepared pregnenolone acetate (**5**) and treated this ketone with (difluoromethyl)diphenylphosphine oxide[10] as shown in Scheme 1. A mixture of products was obtained which included the desired difluorolefin **3** (20%). Also produced in 14% yield was **6**, the acetate of **3**, along with pregnenolone (30%) and pregnenolone acetate (7%). As a reference compound, we also prepared the simple, nonfluorinated vinyl compound **7**[11] as also shown in Scheme 1.

Since compound **3** did display inhibition of the target enzyme (Table 1), although modest, we decided to prepare the Δ^{16} analogues as we have previously found that this additional double bond imparted enhanced inhibitory activity to a series of C_{17}-heterocyclic compounds.[7,12] As shown in Scheme 2, diene **8** was treated with (difluoromethyl)diphenyphosphine oxide to provide a separable mixture of the desired 21,21-difluoro-3β-hydroxy-20-methylpregna-5,16,20-triene (**9**) and phosphonate **10**. Compound **10** is probably produced by addition of the C_3-alcoholate of **8** to the difluoromethyl phosphinate with subsequent elimination of the relatively stable difluoromethyl anion. Similar anamolous reaction products have been observed in the difluoromethenylation of ketones.[13,14]

In an attempt to circumvent the production of the undesired phosphinate ester **10**, a side reaction which was consuming starting material **8**, we chose to protect the alcohol. Treatment of **8** with TBDMS-Cl gave silyl



Scheme 2. Reagents: (a) $Ph_2P(O)CHF_2$; (b) TBDMS-Cl; (c) DMSO, 160–170 °C, 4 h; (d) 3-bromotoluene, reflux, 3 h.

ether **11** which was subsequently treated with (difluoromethyl)-diphenylphosphine oxide. The presence of the silyl ether changed the course of the reaction, such that diphenylphonic ester **12** was isolated. Recognizing that compound **12** could represent an intermediate on the path to the desired difluoroolefin via a potential thermal elimination of diphosphosphonic acid, we attempted to effect this elimination at elevated temperatures. Thermal gravimetric analysis (TGA) showed no weight loss to 210 °C at which temperature a pronounced loss occurred.

Thermolysis of **12** in dimethylsulfoxide at 160–170 °C for 4 h gave a new product which was isolated and identified by spectral analysis as α,β-unsaturated ketone **14**. It seems likely that **14** emanated from sulfonium ion **13** by oxidative 1,2-elimination of a proton, analogous to the Swern oxidation. Several possible routes to **13** can be envisioned. Direct S_N2' displacement of diphenylphosphinic acid by DMSO can give **13**. Alternatively, E_1 or E_2 elimination of $Ph_2PO_2^-$ with subsequent trapping of the resulting carbonium ion **A** by dimethyl sulfoxide can give **13**. One can also propose a [3,3]-sigmatropic rearrangement of **12** to give **15** which then could suffer E_1 elimination of $Ph_2PO_2^-$ in the polar DMSO and subsequent trapping by solvent. The possible intermediancy of **15** is suggested by the thermolysis of **12**, in 3-bromotoluene for 3 hr at reflux temperature from which compound **14** was isolated.[15]

Results and Discussion

The novel compounds prepared in this study were evaluated for their ability to inhibit cynomolgous monkey testicular $C_{17(20)}$ lyase at two concentrations, with and without preincubation of enzyme with the potential inhibitor. These results are presented in Table 1. Also included in Table 1 are the two $C_{17(20)}$ fluoroolefin enol mimics (*Z*-isomer **1** and *E*-isomer **2**) which were previously prepared,[9] for comparison.

The target molecule of this study, difluorolefin $C_{20(21)}$ enol mimic **3**, was a time-dependent inhibitor of the monkey enzyme at the 1 μM concentration, although not quite so potent as the $C_{17(20)}$ enol mimics **1** and **2**. Compound **3** was the only new compound in Table 1 to display time-dependency, although three additional compounds **7**, **8** and **14** were potent inhibitors of the enzyme at both test concentrations.

Compound **6**, the *O*-acetate of inhibitor **3**, was essentially inactive, which underscores the necessity of the free hydroxyl group at the 3-position for active site affinity. Interestingly, compound **7**, the dihydrogen analogue of inhibitor **3**, was a potent inhibitor but did not display time-dependency. This suggests that the fluoro groups do provide a special contribution to enzyme inhibition.

Compound **9**, the 16,17-unsaturated version of inhibitor **3**, was a potent inhibitor of the monkey enzyme but was not time-dependent. Perhaps the conjugation imposes a geometry which is suitable for enzyme affinity but does not facilitate a time-dependent process.

And lastly, the conformationally rigid enone **14** displayed potent enzyme inhibition but no time-dependency. Since compound **14** is a Michael acceptor, the potential exists for it to interact with an active site nucleophile. However, if this interaction is operative, it is a fast process.

Conclusion

In summary, we have extended our studies on fluoroolefin pregnenolone enol mimetics from the $C_{17(20)}$ to the $C_{20(21)}$ mimetics. Indeed, our target $C_{20(21)}$ pregnenolone enol mimetic, difluoroolefin **3**, was synthesized and shown to be a moderately potent, time-dependent inhibitor of cymologous monkey testicular $C_{17(20)}$ lyase.

Experimental

General

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC analyses were performed with Merck DC-F254 silica gel plates, with visualization by UV light and alkaline permanganate. Flash chromatography was performed with Merck silica gel 60 (0.040–0.063 μm). NMR spectra were recorded on Unity 400 or Gemini-300 spectrometers in $CDCl_3$, unless otherwise stated. ^1H and ^{13}C NMR signals are reported in ppm from tetramethylsilane (s, d, t, m and br for singlet, doublet, triplet, multiplet and broad, respectively) and coupling constants are reported in hertz (Hz). Infrared spectra were recorded on a Perkin–Elmer Model 1800 or Mattson Galaxy 5020 FT-IR spectrophotometer. Mass spectral data were collected at 70 eV on a Finnigan MAT 4600, Mat TSQ-700 or VG Analytical Ltd ZAB2-SE mass spectrometer and computerized for HRMS. Combustion analyses were performed using a Perkin–Elmer Model 2400 elemental analyzer and fell within ±0.4% of calculated values. The organic extracts were dried over magnesium sulfate or sodium sulfate prior to evaporation in vacuum on a rotary evaporator. Anhydrous solvents and starting materials were purchased from Aldrich Chemical Co. and used as obtained with the following exception: dehydroepiandrosterone was purchased from Proquina.

21,21-Difluoro-20-methylpregna-5,20-dien-3β-ol (3) and 21,21-difluoro-20-methylpregna-5,20-dien-3β-ol Acetate (6). To a solution of lithium diisopropyl amide (4.00 mmol) in THF (50 mL) cooled to −78 °C was added a solution of (difluoromethyl)diphenylphosphine oxide (1.01 g, 4.00 mmol) in THF (2 mL). After 50 min, a solution of pregnenolone acetate (1.43 g, 4.00 mmol) in THF (15 mL) was added dropwise. The reaction was warmed to room temperature and heated at reflux for 90 min. The cooled reaction was quenched by pouring into water. The products were extracted into methylene chloride and purified by flash chromatography eluting with hexane −20% ethyl acetate (33×50 mL) and hexane–40% ethyl acetate (8×100 mL).

The first material to elute from the column was concentrated to give 215 mg (13.7%) of **6**, mp 124–130 (aq MeOH): IR ν 1734, 1250 cm^{-1}; MS (FAB) *m/e* 415 (85%, M + Na), 333 (100%, M + 1-AcOH); ^1H NMR δ 32;($CDCl_3$) 0.65 (3H, s), 1.05 (s), 1.56–1.61 (m, C_{21}–Me), 4.53–4.68 (1H, m, C_3–H), 5.34–5.42 (1H, m, C_6–H); F NMR δ 32;($CDCl_3$)-92.65 (d, $J = 55$ Hz), −93.35 (dq, $J = 3.3$, 55 Hz). Anal. calcd for $C_{24}H_{34}F_2O_2$: C, 73.44; H, 8.73. Found: C, 73.86; H, 9.19.

The next material to elute from the column was concentrated to give 100 mg (7.0%) of recovered starting material.

The third material to elute from the column was concentrated to give 281 mg (20.0%) of **3**, mp 150–151 °C (EtOAc–hexane): IR ν 3428, 1734 cm^{-1}; MS (CI) *m/e* 351 (8%, M + 1), 350 (18%, M^+), 349 (25%, M-1), 333 (100%, M + 1-H_2O), 313 (15%, 333-HF); (EI) *m/e* 350 (100%, M^+); 332 (45%, M–H_2O), 335 (12%, M–CH_3), 317 (99%, 335-H_2O), 213 (95%); ^1H NMR δ(CDCl3) 0.64 (3H, s), 1.02 (s), 1.54 (t, C_{21}–Me), 3.46–3.58 (1H, m, C_3–H), 5.33–5.38 (1H, m, C_6–H); F NMR δ ($CDCl_3$)−92.60 (d, $J = 2.7$ Hz), −93.32 (dq, $J = 56$ Hz). Anal. calcd for $C_{22}H_{32}F_2O$: C, 75.39; H, 9.20. Found: C, 74.70; H, 9.03.

The last material to elute from the column was concentrated to give 382 mg (30.2%) of pregnenolone.

20-Methylpregna-5,20-dien-3β-ol (7). Mp 125–126 °C [CH_2Cl_2-hexane; reported mp 134–135 °C (MeOH)[11]]; IR ν 3400 cm^{-1}; MS (CI) *m/e* 315 (15%, M + 1), 314 (27%, M^+), 297 (100%, M + 1–H_2O); ^1H NMR δ ($CDCl_3$) 0.60 (3H, s), 1.02 (s), 1.77 (s, C_{21}–Me), 3.28–3.38 (1H, m, C_3–H), 4.72 + 4.86 (2H, s + s, C_{21}-CH_2), 5.34–5.37 (1H, m, C_6–H). Anal. calcd for $C_{22}H_{34}O$: C, 84.02; H, 10.90. Found: C, 83.71; H, 10.81.

21,21-Difluoro-20-methylpregna-5,16,20-trien-3β-ol (9) and 3β-(diphenyl-phosphoryloxy)-pregn-5-en-20-one (10). To a solution of $Ph_2P(O)CHF_2$ (2.05 g, 8.13 mmol) in THF (8 mL) at −78 °C was added a 1.5 M LDA/THF solution (22.3 mL, 33.4 mmol). After 1 h, the steroid **8** (5.0 g, 15.9 mmol) in THF (50 mL) was added. The reaction was stirred at −78 °C for 1.5 h, warmed to room temperature, then heated under reflux for 2 h. The reaction was quenched with H_2O, extracted into CH_2Cl_2 and the products separated by flash chromatography (SiO_2, CH_2Cl_2–2% MeOH). Product containing fractions were pooled, concentrated and rechromatographed (hexane–35% EtOAc) The least polar product fractions were combined, concentrated and the residue crystallized (CH_2Cl_2–hexane) to give **9** (54 mg, 0.95%); mp 106 °C (EtOAc–hexane); UV λ 223 nm (lg ε = 3.769); MS (CI) *m/e* 349 (18%, M + 1), 348 (30%, M^+), 347 (28%, M-1), 331 (10%, M + 1–H_2O); ^1H NMR δ ($CDCl_3$) 0.91 (3H, s), 1.04 (3H, s), 1.79 (t, C_{21}−Me), 3.47–3.60 (1H, m, C_3–H), 5.34–5.41 (1H, m, C_6–H), 5.63–5.67 (1H, m, C_{16}–H); ^{19}F NMR δ −88.58 (d, $J = 45$ Hz), −93.34 (dd, $J = 2.5$, 46 Hz). Anal. calcd for $C_{22}H_{30}F_2O$: C, 75.83; H, 8.68. Found: C, 75.22; H, 9.09.

Fractions of the most polar material from the column were combined and concentrated to a white, crystalline solid, **10** (241 mg, 2.9%); ^1H NMR δ ($CDCl_3$) 0.81 (3H, s), 1.05 (s), 2.26 (3H, s, C_{21}–Me), 4.15–4.33 (1H, m, C_3–H), 5.28–5.36 (1H, m, C_6–H), 6.71 (1H, t, C_{16}-H), 7.41–7.57 (6H, m), 7.78–7.90 (4H, m).

3β-(Dimethyl *tert*-butylsilyloxy)-pregna-5,16-dien-20-one (11). To a solution of alcohol **8** (5.0 g, 15.9 mmol) in pyridine (30 mL) was added *tert*-butyldimethysilyl chloride (2.7 g, 18.2 mmol) and dimethylaminopyridine (0.213 g). After 2.5 h, additional TBDMS-Cl (1.35 g, 9.1 mmol) and DMAP (106 mg) were added and stirring was continued for 3 days. The reaction mixture was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O, twice with 1 N HCl, with brine, dried and concentrated. Purification by flash chromatography (SiO_2, hexane–15% EtOAc) gave **11** (6.03 g, 80.5%), mp 150–152 °C.

21,21-Difluoro-3β-(dimethyl *tert*-butylsilyloxy)-3β-(diphenylphosphoryloxy)-20-methylpregna-5,16-diene (12). To a stirred solution of 1.5 M LDA/THF (9.5 mL, 14.2 mmol) at −78 °C was added a solution of $Ph_2P(O)CHF_2$ (3.55 g, 14.0 mmol) in THF (7 mL). After 1 h, the enone **11** (6.03 g, 14.1 mmol) in THF (45 mL) was added and the reaction mixture was allowed to warm slowly to room temperature. It was then heated at reflux for 2 h and stirred overnight at room temperature. The reaction was quenched with H_2O and extracted with CH_2Cl_2. Concentration of the organic layer and purification by flash chromatography and crystallization (Et_2O) gave **12**, (692 mg, 19.7%): IR ν 1439, 1231, 1090, 982 cm^{-1}; MS (CI) *m/e* 681 (10%, M + 1), 679 (21%, M-1), 549 (28%, M + 1-TBDMS-OH), 219 (100%, Ph_2PO_2H + H); ^1H NMR δ ($CDCl_3$) 0.07 (6H, s, 2×Si-CH_3), 0.89 (9H, s, *tert*-Bu-CH_3), 0.95 (s), 0.99 (s), 1.69 (d, C_{20}–Me), 3.42–3.54 (1H, m, C_3–H), 5.30–5.35 (1H, m, C_6–H), 5.86–5.90 (1H, m, C_{16}–H), 6.22 (1H, t, CHF2), 7.38–7.55 (7H, m), 7.74–7.89 (4H, m); F NMR δ ($CDCl_3$)−128.89 (dd, $J = 5$, 129 Hz); P NMR Δ−80.01. Anal. calcd for $C_{40}H_{55}F_2O_3PS$: C, 70.55; H, 8.14. Found: C, 70.37; H, 8.18.

21,21-Difluoro-3β-(dimethyl-^{t}butylsilyloxy)-20-methylpregna-5,17(20)-dien-16-one (14). Material recovered from the crystalization filtrate of phosphoryl diene **12** was suspended in DMSO and heated at 160–170 °C for 4 h. The solids dissolved and the solution gradually turned a blood-red color. The cooled solution was poured into H_2O and extracted with Et_2O. After drying and concentrating, the resulting residue was flash chromatographed (SiO_2, hexane–25% EtOAc). Product containing fractions were pooled, concentrated and crystallized from CH_2Cl_2–hexane to give **14** (102 mg) mp 139–140 °C; IR ν 3422, 3229, 1721, 1642 cm^{-1}; MS (CI) *m/e* 365 (67%, M + 1), 347 (55%, M + 1-H_2O), 345 (100%, M + 1-HF), 327 (20%, M + 1-HF-H_2O); ^1H NMR δ ($CDCl_3$) 1.04 (3H, s), 1.10 + 1.11 (3H, s + s, 2:1), 1.94 + 2.16 (s + s, C_{20}–Me, 2:1), 3.49–3.62 (1H, m, C_3–H), 5.33–5.40 (1H, m, C_6–H), 6.63 (0.33H, t, CHF_2), 7.54 (0.67H, d, CHF2); F NMR δ ($CDCl_3$)−116.62 (dd, $J = 55$, 322 Hz), −119.47 (dd, $J = 55$, 322 Hz), −120.26 (dd, $J = 56$, 324 Hz), −122.08 (dd, $J = 56$, 324 Hz). Anal. calcd for $C_{40}H_{55}F_2O_3PS$: C, 70.55; H, 8.14. Found: C, 70.37; H, 8.18.

References and Notes

1. De Coster, R.; Wouters, W.; Bruynseels, J. *J. Steroid Biochem. Molec. Biol.* **1996**, *56*, 133.
2. Chung, B.-C.; Picado-Leonard, J.; Haniu, M.; Bienkowski, M.; Hall, P. F.; Shively, J. F.; Miller, W. L. *Proc. Nat. Acad. Sci. U.S.A.* **1987**, *84*, 407.
3. Bradshaw, K. D.; Waterman, M. R.; Couch, R. T.; Simpson, E. R.; Zuber, M. X. *Molec. Endocr.* **1987**, *1*, 348.
4. Njar, V. C. O.; Brodie, A. M. H. *Current Pharm. Des.* **1999**, *5*, 163.
5. Angelastro, M. R.; Laughlin, M. E.; Schatzman, G. L.; Bey, P.; Blohm, T. R. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1571.
6. Angelastro, M. R.; Marquart, A. L.; Weintraub, P. M.; Gates, C. A.; Laughlin, M. E.; Blohm, T. R.; Peet, N. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 97.
7. Burkhart, J. P.; Gates, C. A.; Laughlin, M. E.; Resvick, R. J.; Peet, N. P. *J. Bioorg. Med. Chem.* **1996**, *4*, 1411.
8. Weintraub, P. M.; Gates, C. A.; Angelastro, M. R.; Curran, T. P.; Johnston, J. O. US Patent 5,486,511 **1996**, *Chem. Abstr.* **1995**, *122*, 214328.
9. Burkhart, J. P.; Weintraub, P. M.; Gates, C. A.; Resvick, R. J.; Vaz, R. J.; Friedrich, D.; Angelastro, M. R.; Bey, P.; Peet, N. P. *Bioorg. Med. Chem.* **2002**, *10*, 929.
10. Edwards, M. L.; Stemerick, D. M.; Jarvi, E. T.; Matthews, D. P.; McCarthy, J. R. *Tetrahedron Lett.* **1990**, *31*, 5571.
11. Carlson, R. M. K.; Popov, S.; Massey, I.; Delseth, C.; Ayanoglu, E.; Varkony, T. H.; Djerassi, C. *Bioorg. Chem.* **1978**, *7*, 453.
12. Also finding similar results were Jarmon, M., Barrie, S. E., Llera, J.M. *J. Med. Chem.* **1998**, *41*, 5375.
13. Phillion, D. P.; Cleary, D. G. *J. Org. Chem.* **1992**, *57*, 2763.
14. Piettre, S. R.; Cabanas, L. *Tetrahedron Lett.* **1996**, *37*, 5881.
15. Thermolysis of **15** in DMSO could not be performed due to lack of material.
16. For experimental details, see ref 8.