Synthesis of steroidal [4,6-*b*,*c*]-benzothiazepines, a new class of aromatase inhibitor

Herbert L. Holland, Sudalaiyandi Kumaresan, and Gingipalli Lakshmaiah

Abstract: Several steroidal [4,6-*b*,*c*]benzothiazepines have been prepared via base-catalyzed Michael addition of 2-aminothiophenol to 3β,17β-diacetoxyandrost-4-en-6-one. The product of this reaction has the 4β,5α stereochemistry, but cyclizes to a benzothiazepine with the steroidal 4β,5β configuration, confirmed by X-ray crystallographic analysis. 2-Aminothiophenol reacts with 3β,17β-diacetoxyandrost-4-en-6-one under acidic conditions to give a steroidal 6-spiro-benzothiazole. An androst-4-ene-3,17-dione-based [4,6]benzothiazepine has been shown to be a moderate competitive inhibitor of the human placental aromatase enzyme with $IC_{50} = 42.3$ μM.

Key words: steroid, aromatase, benzothiazepine.

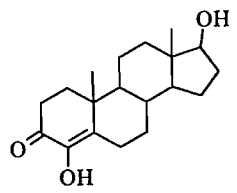
Résumé : On a préparé plusieurs [4,6-*b*,*c*]benzothiazépines stéroïdales par le biais d'une addition du 2-aminothiophénol sur la 3β,17β-diacétoxyandrost-4-én-6-one. Le produit possède une stéréochimie 4β,5α, mais il se cyclise en une benzothiazépine de configuration stéroïdale 4β,5β qui a été confirmée par diffraction des rayons X. Dans des conditions acides, le 2-aminothiophénol réagit avec la 3β,17β-diacétoxyandrost-4-én-6-one pour donner un 6-spiro-benzothiazole. Il a été démontré qu'une [4,6]benzothiazépine basée sur une androst-4-én-3,17-dione est un inhibiteur modérément compétitif de l'enzyme aromatase du placenta humain; $IC_{50} = 42,3$ μM.

Mots clés : stéroïde, aromatase, benzothiazépine.

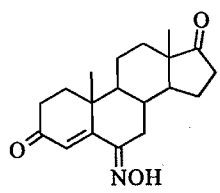
[Traduit par la rédaction]

The enzyme aromatase (estrogen synthetase), responsible for the production of the estrogen hormones in human females, is currently of interest in view of its role as a possible regulator in the etiology of breast cancer (1). Of the many steroids that have been examined for their potential as aromatase inhibitors (1), two of the most potent are the 4-enol **1** (2) and the 6-oxime **2** (3, 4). These observations, together with a demonstration of the importance of the Δ^4-3-keto unit and substitution at C-4 and C-6 in the development of efficient steroidal aromatase inhibitors (4, 5), suggested that a steroid such as **3**, carrying the [4,6]benzothiazepine unit, might function as an efficient aromatase inhibitor.

The reactions between 2-aminothiophenol and 3β-acetoxy-Δ^4-6-ketosteroids with the stigmastane (6) and cholestane (7) skeletons (Scheme 1) have been reported to produce the corresponding 4α-substituted-5α-steroids, **4**. We have applied this reaction to the synthesis of **3** as outlined in Scheme 2, starting from 3β,17β-diacetoxyandrost-4-en-6-one (**5**), whose preparation we have previously described (3). When performed under acid-catalyzed conditions as described (6), addition of



1



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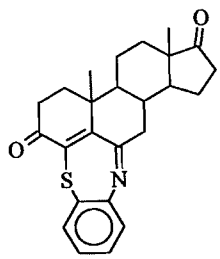

3

2-aminothiophenol to the ketone **5** gave only the spiro(benzothiazole) steroid **6** in 68% isolated yield. We were not able to isolate the expected product, benzothiazepine **8**, from this reaction. The structure of **6** follows from spectral data reported in the Experimental section and in Table 1, the significant nmr resonances being those associated with carbons 4–6. The configuration of **6** at C-6 has not been determined.

Reaction between ketone **5** and 2-aminothiophenol occurred to give the Michael addition product, 5α steroid **7**, in 87% yield

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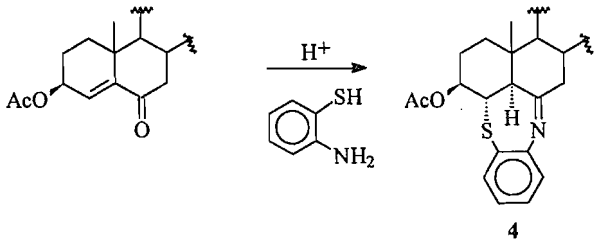
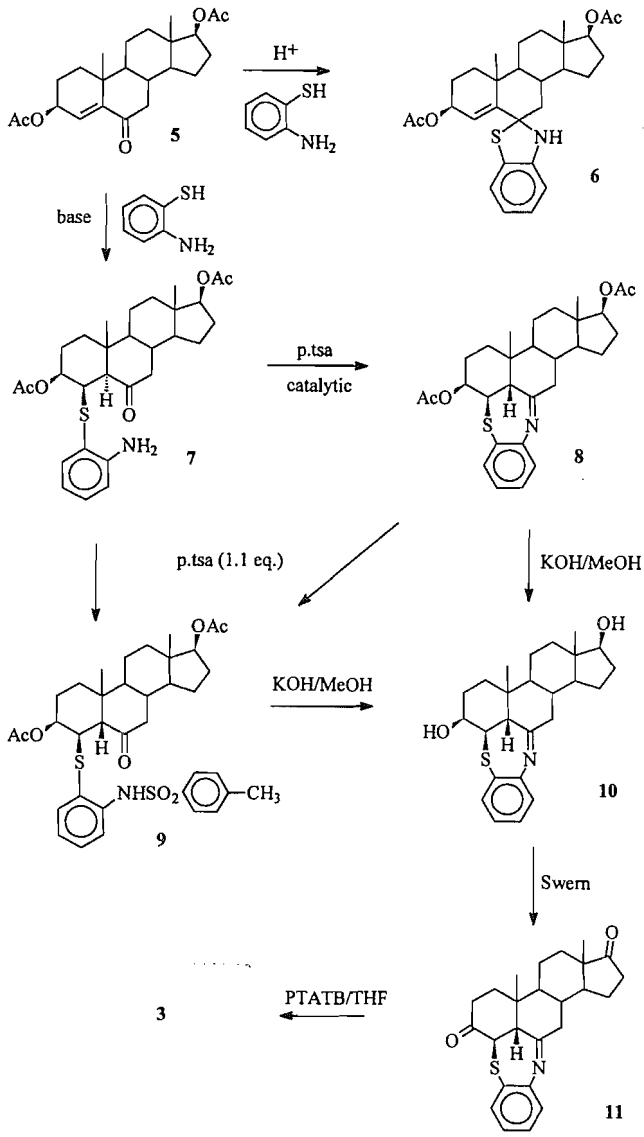
Scheme 1.



Scheme 2.



when carried out using basic catalysis. The stereochemistry of **7** at C-4, together with that of the AB ring junction, was confirmed as follows. That **7** is a 5α steroid follows clearly from its ^{13}C nmr spectrum (Table 1), with shifts for C-9 and C-19 being diagnostic of this stereochemistry (8, 9). The 4β configuration is then indicated by the ^{13}C resonance position of C-2, which shows a γ-*gauche* upfield shift of −3.5 ppm from the position of C-2 in corresponding C-4-unsubstituted steroids (9); the C-19 resonance position (3.6 ppm downfield from that of corresponding C-4-unsubstituted steroids (9)); and the appearance of the C-4 hydrogen resonance at δ 4.06 ppm as narrow doublet of doublets (J = 3.8 and 3.2 Hz), indicating the presence of only axial–equatorial couplings to hydrogens at C-3 and C-5. Michael addition to **5** from the axial (4β) side is predictable on stereoelectronic grounds (10) and is in accord with literature precedent (11): the 4α stereochemistry assigned earlier to similar products was based on "expectation" and is not substantiated by spectral data (7).

The addition product **7** is converted in high yield (91%) to the benzothiazepine **8** on treatment with a catalytic amount of *p*-toluenesulfonic (p.tsa) acid in refluxing benzene. It is clear from nmr analysis that **8**, and the subsequently derived intermediates **9–11**, possess the 5β configuration. This conclusion follows from the ^{13}C nmr data presented in Table 1, particularly the characteristic shifts for C-9 and C-19 (9), and the observation that the C-4 hydrogen resonance now appears at δ 4.01 ppm as a doublet of doublets with J = 12.9 and 2.5 Hz (in **8**, for example) caused by diaxial, and axial–equatorial couplings to hydrogens at C-5 and C-3, respectively. Proton nmr resonances at C-3, -4, and -5 were assigned in compounds **7–11** by ^1H–^1H COSY experiments, and the associated carbon resonances then assigned by SFORD spectra. In view of its unusual origin, this stereochemical analysis was confirmed by X-ray structure determination of the hydrolysis product, diol **10**, which is presented in Fig. 1.[2]

The formation of benzothiazepine **8** with 4β, 5β stereochemistry is at variance with the results of Mushfiq et al. depicted in Scheme 1 (6, 7), but their reported proton nmr data (6), in particular the assignment of the C-4 hydrogen at δ 2.8 ppm, do not support either their proposed structure or its 4α,5α stereochemistry. Condensation of **7** to a product with 5β stereochemistry is apparently thermodynamically driven. MM2 energy calculations on minimized structures using the Alchemy© or Hyperchem© modelling programmes indicate that **8** is more stable than the corresponding 5α isomer by 15.0 kcal/mol, the difference being associated entirely with angle and torsion strain. This assumption is strengthened by the isolation of the 5β sulfonamide **9** on treatment of **7** with larger amounts of p.tsa; this latter product was also obtained from **8** on further acid treatment, suggesting that formation of **9** from **7** proceeds via the benzothiazepine **8**. A possible route for the conversion of **8** to **9** in the presence of a larger amount of p.tsa may involve the acid-catalyzed addition of p.tsa across the C=N bond, followed by ring opening and O to N migration of the *p*-toluenesulfonyl group.

The diol **10**, obtained by hydrolysis of **8** or from cyclization of **9** under basic conditions, was converted in 90% yield by low-temperature Swern oxidation to the dione **11**. The latter then afforded benzothiazepine **3** on treatment with phenyl trimethyl ammonium tribromide. The target steroid **3** had an IC_{50} value of 42.3 μM when tested against human placental aromatase using 2.5 μM testosterone as substrate,[3] making it a moderately effective inhibitor of this enzyme. In spite of the importance of substitution at C-6 in the design of effective aromatase inhibitor (4), the recent suggestion (5) that the

[2] This analysis was carried out by Professor Ling-Kang Liu of the Institute of Chemistry, Academia Sinica, Taiwan.

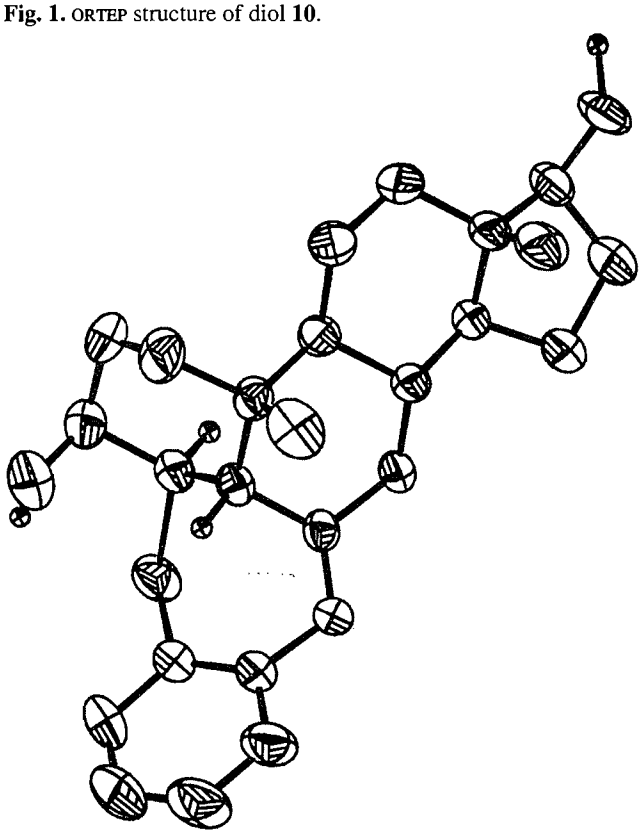
[3] This assay was carried out by Drs. V.C.O. Njar and P. Dorfmüller, Department of Pharmaceutical Chemistry, Universität des Saarlandes, Germany.

Table 1. The ^{13}C nmr resonance positions of **3** and **5–11**.[a]

Carbon	3	5	6	7	8	9	10	11
1	36.7*	34.8	37.8	37.2	28.6	28.0	27.2	34.6*
2	33.8	23.2*	23.4*	23.2*	23.3*	22.9*	26.0	35.7*
3	192.6	69.2	70.6	75.8	72.7	72.4	70.2	204.5
4	135.3	129.0	108.9	43.8	57.6	49.9	61.6	63.6
5	159.0	147.8	144.4	60.2	48.2	56.7	48.4	53.0
6	169.9	201.6	78.8	207.7	178.3	196.2	179.2	174.5
7	45.0	45.7	48.5	46.1	39.4	35.7	39.7	37.6
8	34.1	34.0	31.6	35.6	35.1	35.2	35.2	35.3
9	52.8	51.1	53.5	54.0	41.0	39.9	41.0	43.7
10	43.0	38.3	37.4	40.4	37.8	39.4	39.0	38.7
11	20.7	20.3	20.3	20.3	20.7	20.6	20.7	20.5
12	31.1	36.4	36.5	36.3	36.7	36.2	36.5	31.3
13	47.7	42.7	42.6	42.6	42.9	43.0	43.2	47.7
14	51.3	51.1	49.8	51.1	51.3	50.2	51.6	51.6
15	21.7	24.1*	24.2*	23.2*	24.3*	24.8*	23.2	21.6
16	35.7*	27.4	27.4	27.4	27.6	27.4	30.6	35.7
17	219.1	82.2	82.4	82.2	82.4	81.9	81.6	219.4
18	13.7	12.0	12.1	12.0	12.1	12.0	11.1	13.7
19	17.8	19.6	21.3	16.6	24.3	24.5	24.2	22.7

[a]Chemical shifts marked with an asterisk may be interchanged in vertical columns.

Fig. 1. ORTEP structure of diol **10**.



enzyme's active site possesses limited accessible volume in the C-6 region may account for the lack of high inhibitory activity of benzothiazepine **3**.

Experimental

Apparatus, materials, and methods

Melting points were determined on a Kofler heating stage. The nmr spectra were recorded at 200 MHz (routine ^1H) or 50 MHz (routine ^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ or TMS as internal standard. Proton signals were assigned from ^1H–^1H COSY data and carbon signals from a combination of JMOD and SFORD spectra. Mass spectra were obtained with a Kratos 1S instrument operating in EI mode. Thin-layer chromatography was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh. Microanalyses were performed by Guelph Chemical Laboratories, Guelph, Ontario.

X-ray structure determination was carried out with a 0.64 × 0.39 × 0.51 mm crystal on a Nonius diffractometer using the θ/2θ scan mode. Crystal data: $C_{25}H_{33}NO_2S$; FW 411.60; orthorhombic $P2_12_12_1$, a = 12.1438(13), b = 13.2605(19), c = 13.9445(12) Å, V = 2245.5(4) Å^3, Z = 4, ρ_c 1.218 g cm^{-3}, μ = 0.16 mm^{-1} (20°C, MoKα_1, λ = 0.70930 Å), $2\theta_{max}$ = 44.9°; 1688 unique reflections were obtained and the last least-squares cycle was calculated with 62 atoms, 263 parameters, and 1460 observed reflection ($I > 2\sigma_I$) out of 1688 total reflections; R = 0.036, R_w = 0.037. Details of the X-ray measurements and tables of data have been deposited as supplementary material.[4]

2-[6-Spiro-(3β,17β-diacetoxyandrost-4-enyl)]-4,5-benzo-2[*H*]-thiazole, 6

A mixture of androst-4-ene-3β,17β-diol-6-one diacetate (**5**)

[4] Tables of bond lengths and angles, torsion angles, atomic parameters and thermal parameters, together with ORTEP structures, may be purchased from: The Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, Canada K1A 0R6. Tables of bond lengths and angles, atomic parameters, and the ORTEP structures have also been deposited with the Cambridge Crystallographic Data Centre, and can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, U.K.

(3) (1.164 g, 0.3 mmol), 2-aminothiophenol (0.414 g, 3.3 mmol), dry methanol (8 mL), and 2 drops of concentrated HCl was refluxed under an argon atmosphere for 90 min. The mixture was then cooled in ice, and the precipitated solid collected by filtration and washed with cold methanol (2 mL). The resulting colourless solid (1.01 g) was crystallized from methanol–chloroform to yield **6**, mp 178–179°C; ^{1}H nmr included signals at δ: 0.85 (3H, s, 18-H), 1.27 (3H, s, 19-H), 2.03 (6H, s, OAc), 2.51 (1H, dd, J = 2.5, 12 Hz, 7-H), 3.88 (1H, s, exchanges D_2O, NH), 4.57 (1H, t, J = 8.8 Hz, 17-H), 5.34 (1H, d of t, J = 2.5, 9 Hz, 3-H), 6.15 (1H, s, 4-H), and 6.57–6.96 (4H, m, Ar-H) ppm; ms *m/z*(%): 495 (M^+, 7), 435(100), 420(42), 346(5), 241(27). Anal. calcd. for $C_{29}H_{37}NO_4S$: C 70.27, H 7.52, N 2.83, S 6.47%; found: C 70.04, H 7.92, N 3.11, S 6.44%.

3β,17β-Diacetoxy-4β-(2-aminothiophenyl)-5α-androstan-6-one, 7

Androst-4-ene-3β,17β-diol-6-one diacetate (**5**) (3) (0.582 g, 1.5 mmol) was added to a solution of 2-aminothiophenol (0.207 g, 1.66 mmol) in dry methanol in which a catalytic amount of sodium had been dissolved. The resulting mixture was refluxed under argon for 90 min, cooled in ice, and the precipitated solid was collected by filtration, washed with cold methanol, and crystallized from methanol–chloroform to give **7** (0.675 g, 87%), mp 190°C (sharp); ^{1}H nmr included signals at δ: 0.80 (3H, s, 18-H), 1.07 (3H, s, 19-H), 1.51 (3H, s, OAc), 2.05 (3H, s, OAc), 4.05 (1H, dd, J = 3.8, 3.2 Hz, 4-H), 4.6–4.8 (2H, m, 3- and 17-H), 5.30 (2H, s, exchanges D_2O, NH_2), 6.60 (2H, m, Ar-H), 7.04 (1H, t, Ar-H), and 7.54 (1H, d, Ar-H) ppm; ms *m/z*(%) 513(M^+, 2.5), 435(3.5), 388(7.5), 346(100), 328(17), 313(57). Anal. calcd. for $C_{29}H_{39}NO_5S$: C 67.81, H 7.65, N 2.73, S 6.24%; found: C 67.42, H 7.78, N 3.24, S 6.52%.

2′3′-Dihydro-3β,17β-diacetoxy-5β-androstano[4β,6-*b*,*c*]-1′,5′-benzothiazepine, 8

A solution of **7** (0.400 g, 0.78 mmol) and a catalytic amount of *p*-toluene sulfonic acid in dry benzene (30 mL) was refluxed for 3 h using a Dean–Stark trap. The solution was then cooled, ether (50 mL) added, and the solution was washed with water, followed by saturated aqueous sodium bicarbonate, water, and then dried over magnesium sulfate. Evaporation afforded a yellow solid that was crystallized from benzene–hexane to give 0.350 g (91%) of colourless **8**, mp 270°C (sharp); ^{1}H nmr included signals at δ: 0.81 and 0.83 (each 3H, s, 18- and 19-H), 2.0 and 2.05 (each 3H, s, OAc), 4.02 (1H, dd, J = 2.5, 12.9 Hz, 4-H), 4.65 (1H, t, J = 8.5 Hz, 17-H), 5.1 (1H, br s, 3-H), 7.0–7.4 (4H, m, Ar-H) ppm; ms *m/z*(%): 495(M^+, 100), 435(7.5), 215(43). Anal. calcd. for $C_{29}H_{37}NO_4S$: C 70.27, H 7.52, N 2.83, S 6.47%; found: C 69.74, H 7.83, N 2.97, S 6.48%.

3β,17β-Diacetoxy-4β-(2-[*p*-tolylsulfonylamino]thiophenyl)-5β-androstan-6-one, 9

(a) From 3β,17β-diacetoxy-4β-(2-aminothiophenyl)-5α-androstan-6-one (7)

A mixture of the adduct **7** (0.400 g, 0.78 mmol), *p*-toluenesulfonic acid monohydrate (0.163 g, 0.86 mmol), and benzene (25 mL) was refluxed in a Dean–Stark apparatus for 2.5 h. Chloroform (20 mL) was then added, and the resulting solution was washed with water, aqueous sodium bicarbonate, dried, and evaporated to give 0.300 g (58%) of **9**, crystallized from chloroform – petroleum ether, mp 243°C (sharp); ^{1}H nmr included signals at δ: 0.77 and 0.87 (each 3H, s, 18- and 19-H), 1.94 and 2.06 (each 3H, s, OAc), 2.36 (3H, s, Ar-CH_3), 3.70 (1H, dd, J = 2.6, 12.6 Hz, 5-H), 4.35 (1H, dd, J = 2.6, 12.6 Hz, 4-H), 4.56 (1H, t, J = 8.8 Hz, 17-H), 5.10 (1H, br s, 3-H), and 7.16–7.96 (8H, m, Ar-H) ppm; ms *m/z*(%): 495 (M − p.tsa, 100), 453(3.5), 435(10). Anal. calcd. for $C_{36}H_{45}NO_7S_2$: C 64.74, H 6.79, N 2.10, S 9.60%; found: C 64.63, H 6.99, N 2.19, S 9.76%.

*(b) From 2′,3′-dihydro-3β,17β-diacetoxy-5β-androstano[4β,6-*b,c*]-1′,5′-benzothiazepine (8)*

A mixture of the benzothiazepine **8** (0.040 g, 0.06 mmol), *p*-toluenesulfonic acid monohydrate (0.017 g, 0.09 mmol), and dry benzene was refluxed for 2.5 h. Chloroform (10 mL) was then added, and the solution was washed with water, aqueous sodium bicarbonate, dried, and evaporated to give **9** (0.040 g), identical in all respects with the sample prepared in (*a*).

2′,3′-Dihydro-3β,17β-dihydroxy-5β-androstano[4β,6-*b*,*c*]-1′,5′-benzothiazepine, 10

*(a) From 2′,3′-dihydro-3β,17β-diacetoxy-5β-androstano[4β,6-*b,c*]-1′,5′-benzothiazepine (8)*

A solution of the diacetate **8** (0.100 g, 0.15 mmol) in dry methanol (3 mL) containing KOH (0.100 g) was stirred at room temperature for 24 h. The solvent was removed by evaporation, ice-water (5 mL) was added to the residue, and the mixture stirred for 10 min. The resulting solid was collected by filtration and dried to yield 0.076 g (92%) of **10**, crystallized from methanol–chloroform, mp 262–264°C; ^{1}H nmr included signals at δ 0.77 and 0.80 (each 3H, s, 18- and 19-H), 3.70 (1H, t, 17-H), 3.86 (1H, br s, 3-H), 4.07 (1H, dd, J = 2.5, 12.6 Hz, 4-H), and 7.03–7.55 (4H, m, Ar-H) ppm; ms *m/z*(%): 411 (M^+, 100), 258(16), 162(15), 149(23). Anal. calcd. for $C_{25}H_{33}NO_2S$: C 72.95, H 8.08, N 3.40%; found: C 72.84, H 7.94, N 3.44%.

(b) From 3β,17β-Diacetoxy-4β-(2-[p-tolylsulfonyl-amino]thiophenyl)-5β-androstan-6-one (9)

Treatment of **9** (1.00 g) by the procedure described in (*a*) resulted in the formation of 0.59 g (96%) of **10**, identical in all respects with the sample described above.

2′,3′-Dihydro-3,17-diketo-5β-androstano[4β,6-*b*,*c*]-1′,5′-benzothiazepine, 11

A solution of dimethyl sulfoxide (18 mg) in dichloromethane (0.5 mL) was added dropwise at −65°C under an argon atmosphere to a solution of oxalyl chloride (13.5 mg) in dichloromethane (0.30 mL), and the resulting mixture stirred for 10 min at that temperature. A suspension of the diol **10** (20 mg, 0.05 mmol) in dichloromethane was then added over a period of 10 min; the mixture was stirred for 1 h at −65°C and then quenched by the addition of triethylamine (49.2 mg). The solution was allowed to reach room temperature, water (2 mL) was added, and the mixture was then extracted with dichloromethane. The extract was washed with water followed by saturated NaCl, dried, and evaporated to give a residue that on chromatography (hexane–acetone, eluting with 12% acetone

in hexane) gave the title product (18 mg, 90%), mp 231–233°C; ^{1}H nmr included signals at δ: 0.87 and 0.95 (each 3H, s, 18- and 19-H), 4.45 (1H, d, J = 14 Hz, 4-H), and 7.0–7.62 (4H, m, Ar-H) ppm; ms *m/z*(%): 407 (M^+, 34), 373(36), 358(52), 299(35), 243(35), 215(100). Anal. calcd. for $C_{25}H_{29}NO_2S$: C 73.67, H 7.17, N 3.44%; found: C 73.78, H 7.23, N 3.44%.

3,17-Diketo-androsteno[4,6-*b*,*c*]-1′,5′-benzothiazepine, 3

A solution of phenyl trimethyl ammonium tribromide (73 mg, 0.194 mmol) in dry THF (1.5 mL) was added at −2°C to a stirred solution of the diketone **11** (80 mg, 0.196 mmol) in dry THF over a period of 10 min, and the mixture stirred at this temperature for 2 h. The mixture was then extracted into dichloromethane, and the extract was washed with water followed by saturated NaCl, dried, and evaporated. The residue was chromatographed using hexane–acetone, the product (45 mg, 57%) being eluted with 10% acetone – hexane, mp 242–244°C; ^{1}H nmr included signals at δ: 0.95 (6H, s, 18- and 19-H) and 7.03–7.45 (4H, m, Ar-H) ppm; ms *m/z*(%): 405 (M^+, 10), 373(68), 358(100), 344(12), 316(16). Anal. calcd. for $C_{25}H_{27}NO_2S$: C 74.04, H 6.71, N 3.45%; found: C 74.03, H 6.64, N 3.43%.

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