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Article

Structural Analysis and Antitumor Activity of Androstane D-Seco-mesyloxy Derivatives

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Neste artigo, a influência dos compostos esteróides nas culturas de células tumorais foi estudada: o crescimento de células, a indução de apoptose e/ou alterações do ciclo celular. Esta é uma forma comum na descoberta de compostos terapêuticos potenciais para o tratamento de pessoas que sofrem de doenças dependentes de hormônios. Os tipos de terapêutica para o tratamento de diferentes tipos de câncer são os mais importantes devido à elevada taxa de mortalidade relacionada com esta classe de doenças. O trabalho apresenta a síntese de dois estereoisómeros 16,17-secoandrostane mesilados e seus precursores 17-hidroxi, e o estudo da sua atividade antiproliferativa, influência sobre o ciclo celular e indução de apoptose, assim como a análise cristalográfica destes compostos.

The study of the influence of steroidal compounds on tumor cell cultures, cell growth, induction of apoptosis and/or cell cycle changes, is a common way of discovering potential therapeutics for treating people suffering from hormone-dependent problems and diseases. Because of the very high mortality rate associated with this class of disease, therapeutics for treating different types of cancers are among the most important. This work presents the synthesis of two stereoisomeric 16,17-secoandrostane mesyloxy derivatives and their 17-hydroxy precursors. Compounds were structurally analyzed by X-ray crystallography, and their antiproliferative activity, influence on the cell cycle and potential to induce apoptosis were investigated.

Keywords: 16,17-seco mesyloxy androstane derivatives, X-ray crystallography, tumor cell cytotoxicity, apoptosis, cell cycle

Introduction

Androgens play an important role in the development of diseases such as benign prostate hyperplasia and prostate cancer.[1,2] On the other hand, estrogen-dependent breast cancer is the most common cancer among women and continues to be a major cause of cancer-related deaths.[3] The efficacy of adjuvant endocrine therapy in hormone receptor-positive early breast cancer is constantly examined.[4] Since many estrogen and androgen derivatives exhibit anti-hormone and/or cytotoxic activity, a large number of steroidal derivatives have been synthesized from appropriate estrogen or androgen precursors in order to obtain potential therapeutics for the treatment of steroid-dependent cancers.[5-15]

It is known that many sulfur-containing compounds display biological potency.[16-19] In addition, some steroidal compounds induce apoptosis or cause changes in the cell cycle of tumor cells.[20-22] In light of the above, as a continuation of our ongoing efforts on D-seco steroid synthesis as potential anticancer agents, in the present study, we report the structural analysis of recently (**5**)[23] and newly (**6**) synthesized stereoisomeric D-seco-mesyloxy derivatives, the study of their antiproliferative activity against tumor cell lines and their impact on the cell cycle and apoptosis of tumor cells.

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Experimental

Chemical synthesis

General

Melting points were measured on a Nagema Rapido melting point microscope. Infrared (IR) spectra were recorded on a Nexus 670 FTIR spectrometer. ^{1}H (250 MHz) and ^{13}C (62.5 MHz) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 250 apparatus using tetramethylsilane as an internal standard. High-resolution mass spectroscopy (HRMS) data (TOF) were recorded on a 6210 time-of-flight LC/MS Agilent Technologies (ESI$^+$) instrument. Organic solutions were dried with Na_2SO_4. Column chromatography (CC) was performed on silica gel 60 (0.04-0.063 μm, Merck).

3β-Acetoxy-(17*S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**) and 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**)

A mixture of compounds **3** and **4** served as starting material for the synthesis of mesylates **5** and **6**. Compounds **5** and **6** were separated by flash chromatography (petroleum ether-ethyl acetate 3:1; 2:1; 1.5:1) giving pure compound **5** (0.4938 g, 78%, white crystals, mp 158-160 °C, after crystallization from ethyl acetate/hexane) and **6** (0.0579 g, 9%, white crystals, mp 160-161 °C, after crystallization from ethyl acetate/hexane).

Compound **5**: IR ν/cm^{-1} 3019, 2943, 2243, 1728, 1247, 1173, 896, 755; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.041 (s, 3H, H19); 1.050 (s, 3H, H18); 1.412 (d, 3H, *J* 6.25 Hz, H20); 2.046 (s, 3H, Ac); 3.054 (s, 3H, Ms); 4.600 (m, 1H, H3); 4.782 (q, 1H, *J* 6.41 Hz, H17); 5.391 (d, 1H, *J* 4.54 Hz, H6); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 15.07 (CH_2); 15.39 (C-19); 17.18 (C-18); 19.11 (C-20); 19.76 (CH_2); 21.39 (CH_3, Ac); 27.54 (CH_2); 31.00 (CH_2); 31.88 (CH_2); 31.91 (CH); 36.57 (CH_2); 36.76 (q); 37.65 (CH_2); 39.19 (CH_3, Ms); 40.53 (q); 42.09 (CH); 48.61 (CH); 73.56 (C-3); 82.83 (C-17); 119.04 (C-16); 121.30 (C-6); 139.26 (C-5); 170.53 (Ac); HRMS calculated $(M + NH_4)^+$: 455.25742; measured: 455.25771.

Compound **6**: IR, ν/(cm^{-1}) 2941, 2243, 1727, 1241, 1172, 901, 800; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.045 (s, 3H, H19); 1.142 (s, 3H, H18); 1.410 (d, 3H, *J* 6.50 Hz, H20); 2.047 (s, 3H, Ac); 3.051 (s, 3H, Ms); 4.600 (m, 1H, H3); 4.798 (q, 1H, *J* 6.50 Hz, H17); 5.380 (d, 1H, *J* 4.54 Hz, H6); ^{13}C NMR (62.5 MHz, $CDCl_3$), δ 15.38 (C-19); 15.52 (CH_2); 17.47 (C-18); 19.09 (C-20); 19.27 (CH_2); 21.36 (CH_3, Ac); 27.51 (CH_2); 30.39 (CH_2); 31.82 (CH_2); 32.93 (CH); 36.57 (CH_2); 36.84 (q); 37.65 (CH_2); 39.02 (CH_3, Ms); 40.52 (q); 44.23 (CH); 48.93 (CH); 73.50 (C-3); 85.43 (C-17); 118.68 (C-16); 120.99 (C-6); 139.55 (C-5); 170.50 (Ac); HRMS calculated $(M + NH_4)^+$: 455.25742; measured: 455.25753.

Biological tests _ *In vitro* antitumor assay

Cell lines and cell culture

Six human tumor cell lines (ER+ breast adenocarcinoma, MCF-7; ER-breast adenocarcinoma, MDA-MB-231; prostate cancer, PC-3; cervical carcinoma, HeLa; myelogenous leukemia, K-562; colon adenocarcinoma, HT-29) and one human non-tumor cell line (normal fetal lung fibroblasts MRC-5) were used in this study. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose (MCF-7; MDA-MB-231; PC-3; HeLa; HT-29; MRC-5) or RPMI 1640 medium (K562). Medium was supplemented with 10% of fetal calf serum (FCS, Sigma) and antibiotics: 105 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37 °C in a 100% humidity atmosphere supplemented with 5% of CO_2. Only viable cells were used in the assays. Cell viability was determined by the trypan blue dye exclusion assay.

Antiproliferative activity

Steroidal compounds **2** and **4**-**6** were evaluated for antiproliferative activity using the tetrazolium colorimetric MTT assay, after treating tumor cells with the study compounds for 48 h. The assay is based on conversion of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan by mitochondrial dehydrogenases in viable cells.[24] Doxorubicin (Dox) was used as a positive control. Exponentially growing cells were harvested, counted by the trypan blue exclusion test, and plated onto 96 well microplates (Costar) at an optimal seeding density of 10^4 (K-562) or 5×10^3 (other cell lines) cells *per* well, to assure a logarithmic growth rate throughout the assay period. Viable cells were plated in a volume of 90 μL *per* well, and pre-incubated in complete medium at 37 °C for 24 h, to allow cell stabilization prior to the addition of test substances. Tested compounds were added in volume of 10 μL *per* well of appropriate concentration to all wells except controls in order to achieve the required final concentration in the growth medium (10^{-8} to 10^{-4} mol L^{-1}). Microplates were then incubated for 48 h. Wells containing cells without tested compounds were used as control.

After 48 h incubation, 10 μL of MTT solution were added to each well. MTT was dissolved in the medium at 5 mg mL^{-1} and filtered sterilized to remove a small amount of insoluble residue present in some batches of MTT. Acidified 2-propanol (100 mL of 0.04 mol L^{-1} HCl in 2-propanol)

was added to each well and mixed thoroughly to dissolve the dark blue crystals. When all crystals were dissolved, plates were read on a spectrophotometer microplate reader (Multiscan MCC340, LabSystems) at 540/690 nm. Wells without cells containing complete medium and MTT only were used as a blank. Antiproliferative activity was expressed as the IC_{50} (50% inhibitory concentration).

Data analysis

Two independent experiments were conducted in quadruplicate for each concentration of tested compound; mean values and standard deviations (SD) were calculated for each experiment. IC_{50} defines the dose of compound that inhibits cell growth by 50%. The IC_{50} value of each tested compound was determined by median effect analysis.[25]

Morphological analysis of apoptosis and flow cytometric cell cycle analysis

The MDA-MB-231 human breast adenocarcinoma (ER-) cell line was used to examine induction of apoptosis by the steroidal test compounds. Apoptosis was monitored by observing morphological changes in cells using light microscopy. The same cell line was used for flow cytometry cell cycle analysis.

Cell culture and cell treatment

Cells were grown in the same conditions as for the MTT test. Each culture of both control and experimental groups was seeded in duplicate. Flasks with seeded cells were left at 37 °C, in an atmosphere comprising 5% of CO_2, during the following 24 h to allow cells to adhere to the flask surface. After 24 h, test steroid compounds (**4**-**6** and the reference formestane) were added to experimental cultures in 1 mL volume in order to achieve the appropriate final concentrations (equal to their IC_{50} concentrations, which were estimated earlier by cytotoxicity assays; equitoxic doses). Cells were then again incubated in total darkness at 37 °C in a 100% humidity atmosphere with 5% CO_2. Total incubation time with steroid compounds was 48 h, allowing cells to undergo two mitotic divisions, the same as in the control sample.

Cell harvesting

Cell harvesting and slide preparation were conducted under aseptic conditions, following a modified cytogenetic preparation procedure for micronuclei testing.[26] Each culture was harvested and processed separately. Cell suspension preparations involved hypotonic treatment in order to achieve adequate cell spreading and high-quality cell preparation for scoring. Hypotonic treatment, fixation and centrifugation were modified to preserve the cell cytoplasm. The cell cytoplasm was retained to enable reliable detection and identification of all morphological features related to apoptosis.

After 48 h of treatment, cells were separated from culture flask walls by trypsinization (0.5% solution of trypsin). One part was used for slide preparation, and the other for cell cycle analysis.

Slide preparation for morphological analysis

Cells were gently centrifuged (1200 rpm) for 5 min and the supernatant culture medium was removed. Cells were then hypotonically treated with 7.0 mL of cold (4 °C) 0.075 mol L^{-1} KCl and centrifuged immediately at 1200 rpm for 8 min. The supernatant was removed and replaced with 5 mL of fixative, consisting of a mixture containing methanol and acetic acid (3:1) with 1% formaldehyde. The fixative was added while agitating the cells in order to prevent clump formation. The cells were then centrifuged again at 1100 rpm for 8 min, washed twice with fixative formaldehyde and gently resuspended. The final suspension was dropped onto clean glass slides and allowed to dry. Specimen staining (after 24 h) was performed using 2% Giemsa stain (Merck) in potassium phosphate buffer (pH 7.3).

Analysis and scoring criteria

Prepared material was observed and analyzed by light microscopy (Olympus BX51). All specimens, including controls, were independently coded before microscopic analysis and analyzed with no prior information regarding the origin of the material. Cells were counted and at least 1000 cells were scored for each specimen. Scored features included normal cells and all forms of induced morphological changes that can be attributed to apoptosis.[27]

Image capturing and data processing

Images were captured with a 12 megapixel digital camera (Canon 350D) attached to a computer. Data were processed with Microsoft Excel.

Flow cytometric cell cycle analysis

The cell cycle was investigated using a flow cytometer, by analyzing the DNA content of ethanol-fixed MDA-MB-231

cells stained with propidium iodide (PI). For this purpose, a Becton Dickinson (BD) Immunocytometry System[28] was used to analyze cell distributions.

After trypsinization, cell fixation (70% ethanol 30 min on ice) and centrifugation, a solution of ribonuclease A (RNase A, 100 units mL^{-1}) and propidium iodide (400 mg mL^{-1}) were added to the cell pellet. Cell suspensions were incubated in the dark at room temperature for 30 min. After incubation, and prior to analysis by flow cytometry, each FACSCalibur BD sample was filtered through a 35 µm grid. The cell excitation wavelength was 488-514 nm, and the fluorescence emission of PI was approximately 610 nm. The FL2 parameter of BD FACSCalibur was used for one parametric analysis.

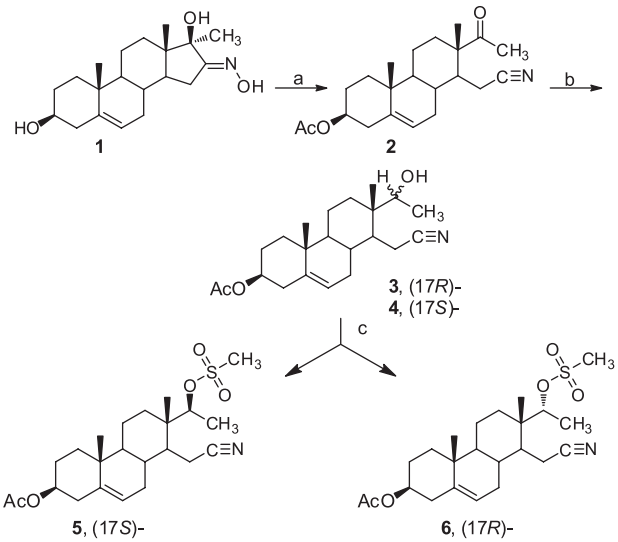
Structure determination

Diffraction data for compounds **5** and **6** were collected using an Oxford Diffraction Gemini S four-circle goniometer equipped with a Sapphire CCD detector. The crystal to detector distance was 45.0 mm and graphite monochromated Mo K_α (λ = 0.71073 Å) radiation was used for the experiment. Data were reduced using the CrysAlis PRO program.[29] A semi-empirical absorption-correction, based upon the intensities of equivalent reflections, was applied, and the data were corrected for Lorentz, polarization and background effects. The structure was solved by direct methods using the Sir97 program,[30,31] and refined by full-matrix least-squares procedures on F^2 using SHELXL-97 programs,[32] as implemented in the WinGX program suite.[33] Non-H atoms were refined anisotropically. Hydrogen atoms were treated by a mixture of independent and constrained refinement. The figures representing molecular structure were made using the ORTEP-3[34] and PLATON[35] programs.

Results and Discussion

Hydroxy oxime **1** by Beckmann fragmentation with acetic anhydride afforded D-seco derivative **2**, which was reduced, giving a mixture of epimer alcohols **3** and **4**. Mesylation of **3** and **4** resulted in D-seco mesyloxy derivatives **5** and **6** (Scheme 1).[36,23]

The obtained mesyloxy derivatives **5** and **6** were successfully separated by column chromatography, and the crystallization afforded crystals suitable for X-ray structural analysis. X-ray structural analysis revealed that the major product, compound **5**, has a (17*S*)-configuration, while the side-product **6** has a (17*R*)-configuration. Based on these results, it can indirectly be concluded that the reduction of D-seco ketone **2** with sodium boron hydride afforded a mixture of stereoisomeric alcohols **3** and **4**, which could not be separated chromatographically, but from which the main product **4**, with a (*17S*)-configuration, was isolated by crystallization (hexane/acetone, 3:1).[36]



Scheme 1. a: Ac_2O, Py; b: $NaBH_4$, EtOH, CH_2Cl_2; c: MsCl, Py.

Biological tests

Antiproliferative activity

The primary tests for biological and anti-proliferative activities of compounds **2** and **4**-**6** were *in vitro* cytotoxicity assays against six human cancer cell lines: estrogen receptor negative (ER-) breast adenocarcinoma (MDA-MB-231); estrogen receptor positive (ER+) breast adenocarcinoma (MCF-7); prostate cancer (PC-3); human cervical carcinoma (HeLa); myelogenous leukemia (K-562); colon adenocarcinoma (HT-29); and normal fetal lung fibroblast (MRC-5) cells (Table 1 and Figure 1). Results were compared with the non-selective cytotoxic drug doxorubicin.

D-seco 17-oxo derivative **2** exhibited weak antiproliferative activity only against human cervical carcinoma cells (HeLa). However, its C-17 hydroxy derivative **4** showed strong antiproliferative activity against MDA-MB-231 breast cancer cells and moderate activity against HeLa cells, with no inhibition potency displayed against other cell lines. The introduction of a C-17 mesyloxy function significantly increased the antiproliferative activity of both epimers **5** and **6** against the majority of the studied tumor cells, compared to precursors **2** and **4**. Namely, mesyloxy derivatives **5** and **6** displayed the strongest antiproliferative activity against MDA-MB-231 breast cancer cells. In addition, compounds **5** and **6** displayed respectively 16- and 8-fold greater antiproliferative activity

Table 1. The results of *in vitro* MTT cytotoxicity tests of synthesized steroidal derivatives **2**, **4**, **5** and **6** and doxorubicin against a panel of six human cancer cell lines and one normal human cell line, expressed as IC_{50} values. Doxorubicin served as a reference cytoxicity compound

Compound	Cell lines and growth inhibitory concentrations, IC_{50} / (μmol L^{-1}) after 72 h culture in the presence of either test steroid derivatives **2**, **4**-**6** and control						
	MCF-7	MDA-MB 231	PC-3	HeLa	K562	HT-29	MRC-5
2	> 100	> 100	> 100	44.68	> 100	> 100	> 100
4	> 100	14.23	> 100	50.99	99.78	> 100	> 100
5	> 100	6.58	5.97	17.51	> 100	21.10	> 100
6	62.53	8.93	12.23	14.82	86.32	38.27	> 100
Dox	0.75	0.12	95.61	1.17	0.36	0.32	0.12

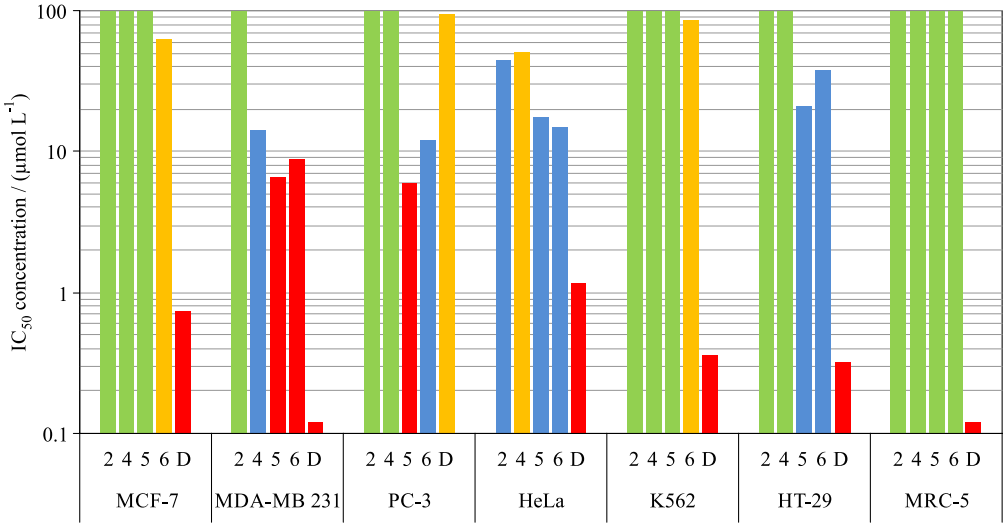


Figure 1. Cytotoxicity of synthesized steroidal derivatives **2**, **4**-**6** and doxorubicin against six human cancer cell lines and one normal human cell line, expressed as IC_{50} values.

against PC-3 cells than Dox, and also strongly inhibited HeLa and HT-29 tumor cell growth. (*17R*)-Stereoisomer **6** was a slightly less potent antiproliferative agent against the same cell cultures than its (17*S*)-stereoisomer **5**.

Doxorubicin displayed significant cytotoxicity against nearly all cancer cell types, but was also highly toxic to healthy MRC-5 (control) cells, possibly explaining the severe side effects associated with Dox chemotherapy, including cardiotoxicity and nephrotoxicity.[37] In contrast, the new steroidal compounds investigated in the present study were selectively cytotoxic being non-toxic to healthy MRC-5 cells.

Effects of steroidal derivatives **4**-**6** and formestane on the cell cycle

Since compounds **4**-**6** expressed significant antiproliferative potential, especially against estrogen receptor negative MDA-MB-231 breast cancer cells, the following experiments in our research were aimed at identifying a preliminary mechanism for their action. Particular interest was focused on their effect on the cell cycle and their potential to induce apoptosis. We performed cell cycle analysis and studied the morphological changes in MDA-MB-231 cells treated with equitoxic doses (IC_{50} concentrations) of the tested compounds over a 48 h time period.

Figure 2 shows the effect of the tested compounds on treated cells, while the percentages of cells in G1/M, S, G2/M and subG1 phases of the cell cycle were calculated and presented in Table 2. Formestane was used as a reference compound.

The treatment of MDA-MB-231 cells for 48 h with each of the newly synthesized compounds resulted in almost the same number of cells in G1/M1 phase, while the treatment with formestane resulted in a slight decrease, compared to control. The cell population in the synthetic (S) phase in cultures treated with compounds **4** and **5** was very similar to the control sample, and somewhat lower in the sample treated with compound **6**. Formestane slightly increased

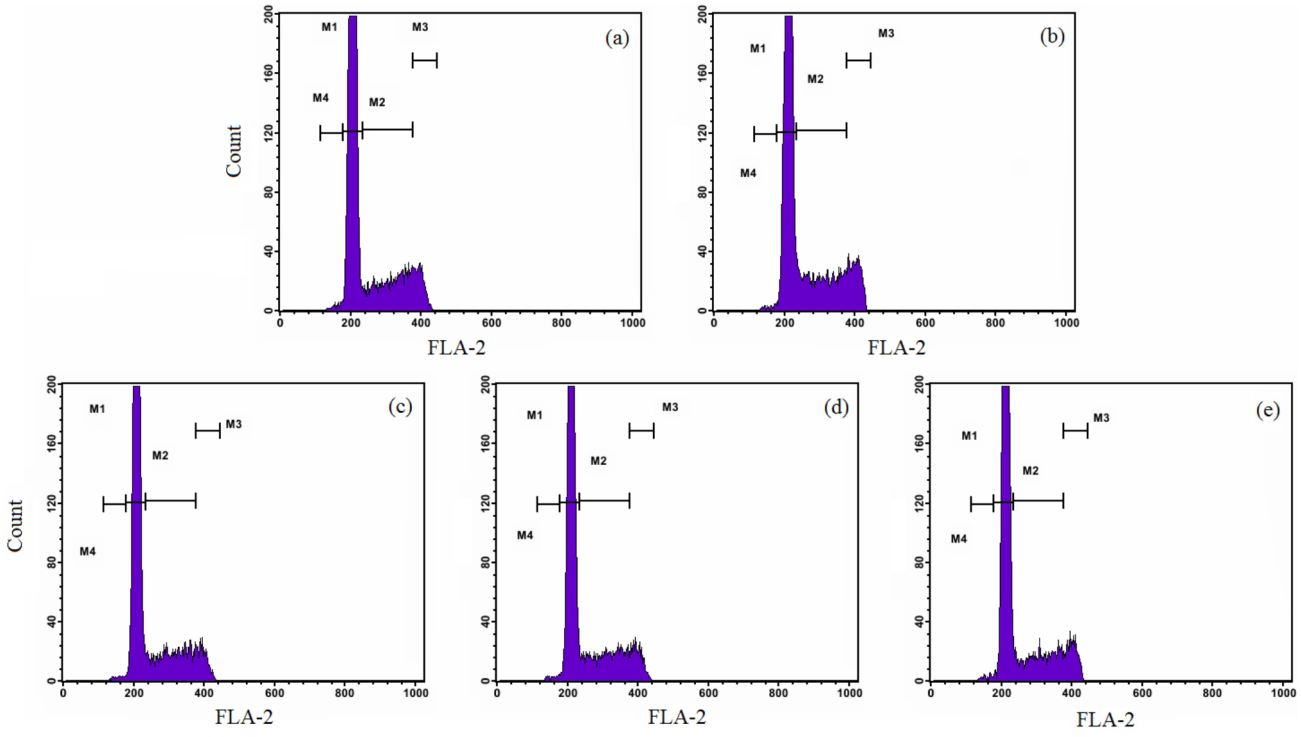


Figure 2. The effects of the investigated steroidal derivatives on the cell cycle of MDA-MB-231 cells. Cells were collected, stained with propidium iodide and analyzed by flow cytometry after treatment with equitoxic doses over 48 h: (a) untreated control cells; (b) formestane; (c), (d) and (e) compounds **4**, **5** and **6**, respectively. Marks M1, M2, M3 and M4 on the histograms correspond to G1/M, S, G2/M and subG1 phases of cell cycle.

Table 2. Distribution of MDA-MB-231 cells between cell cycle phases in control and treated samples

Sample	Distribution of cells between cell cycle phases / %			
	G1/M	S	G2/M	subG1
Control	78.30	15.75	5.62	0.58
4	77.15	16.95	5.77	0.46
5	77.12	16.44	6.20	0.47
6	77.77	14.83	7.32	0.42
Formestane	68.42	21.55	9.95	0.47

the number of cells in S-phase. Concerning G2/M phase, compounds **4** and **5** exerted similar effects compared to the control sample, while compound **6** and formestane showed a modest increase. The number of hypodiploid (subG1) cells, which are generally regarded as an apoptotic population, was very low after 48 h incubation, and practically the same as the control sample (Table 3).

Induction of apoptotic cellular morphology by steroidal derivatives

Analysis of the morphology of MDA-MB-231 cells, which were treated for 48 h with equitoxic doses of compounds **4**-**6** and formestane (as a reference compound), was performed by visual observations using a light microscope. No significant difference was found concerning the quality of specimens between control and treated cells by light microscopy. The quality and clearness of the treated specimens were comparable to those of the control, regardless of the test compound.

It was found that the number of cells with apoptotic morphology increased after treatment with investigated steroidal derivatives. Compounds **4** and **5** slightly increased the number of cells featuring apoptotic cellular morphology (less than 4%, compared to control samples). Compound **6** modestly affected this ratio (9.5%), while formestane increased the number of cells with apoptotic morphology more than the other tested compounds (12.5%).

Apoptotic cells were identified by a series of typical morphological changes, and morphology still constitutes an important experimental proof of the underlying processes. To study the mechanism(s) behind the antiproliferative activity displayed by these novel seco-androstane derivatives, compounds **4**-**6** were selected and light microscopy was used to determine whether they induce apoptosis in MDA-MB-231 cells at the single cell level. As a reference molecule, the steroid formestane were used. Treated and untreated MDA-MB-231 cells were stained with Giemsa. Figure 3 shows the morphology of MDA-MB-231 cells following exposure to equitoxic doses

of the tested compounds (**4**-**6** and formestane) for 48 h, as well as untreated cells. The percentage of apoptotic cells, as estimated by visual observation of cell morphology, is given in Table 3. Numerous morphological changes indicative of apoptosis were detected: plasma membrane blebbing, cellular shrinkage, chromatin condensation and nuclear degradation (Figure 4). Most of the control cells appeared normal with round and homogeneous nuclei (Figure 3a).

Table 3. Percentage of apoptotic cells estimated by visual observation of the cell morphology of MDA-MB-231 cells after treatment for 48 h with equitoxic doses of steroidal derivatives **4**-**6** and formestane

Sample	Control	Formestane	**4**	**5**	**6**
Number of cells with normal morphology	1083	1035	1001	1027	1006
Number of cells with apoptotic morphology	126	308	162	171	247
The total number of cells examined	1209	1343	1163	1198	1253
Apoptotic cell / %	10.42	22.93	13.93	14.27	19.71

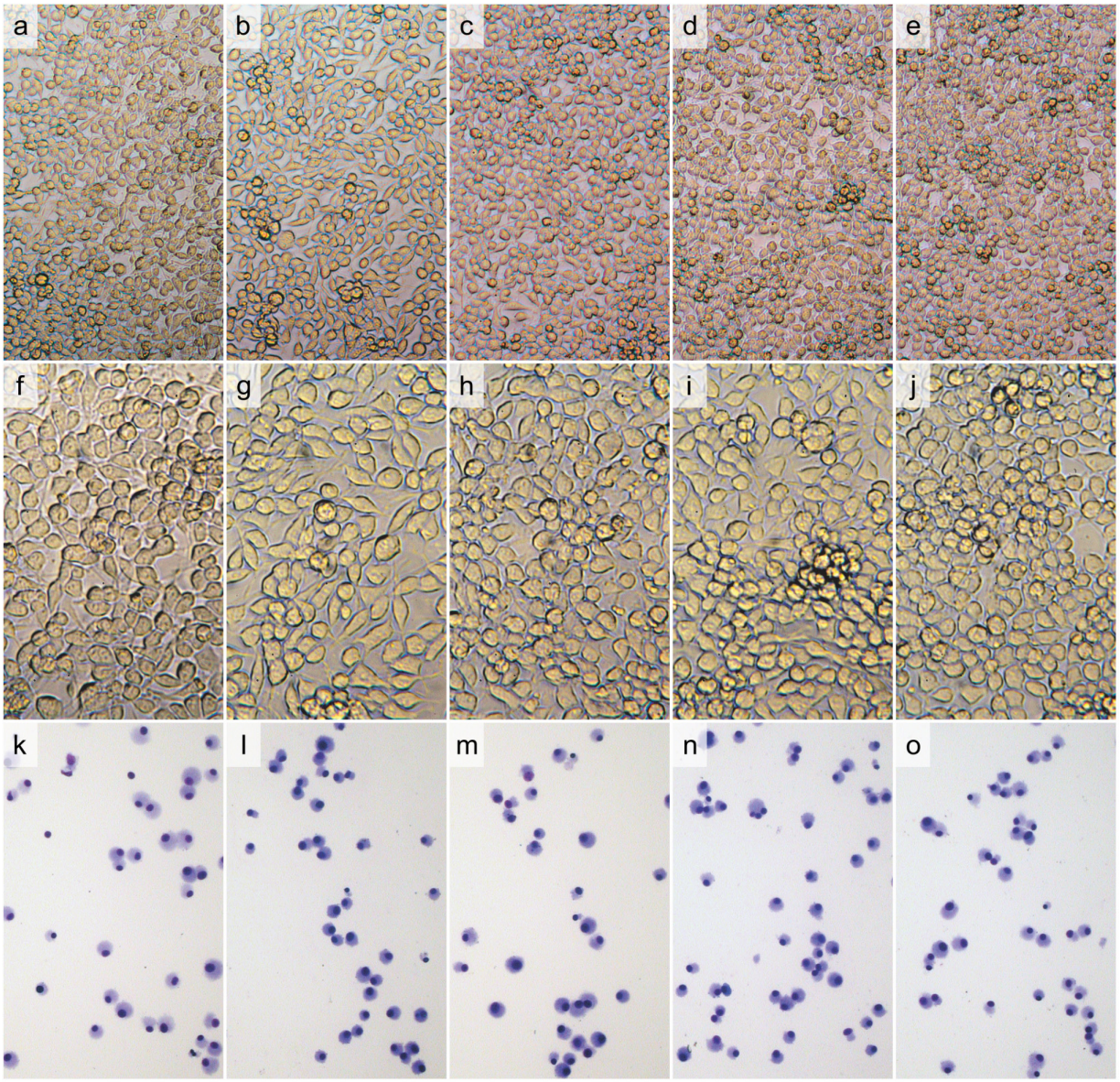


Figure 3. Morphology of MDA-MB-231 cells treated with no substance (a, f, k; control), with formestane (b, g, l), compound **4** (c, h, m), **5** (d, i, n) or **6** (e, j, o) for 48 h. Images in the 1st and 2nd rows were photographed with a Canon 350D digital camera attached to a Reichart BioStar inverted microscope at 10 × 10 and 20 × 10 magnification, respectively; images in the 3rd row were photographed with an Olympus Camedia 3040 digital camera attached to an Olympus BX51 microscope at 10 × 15 magnification, after staining with giemsa.

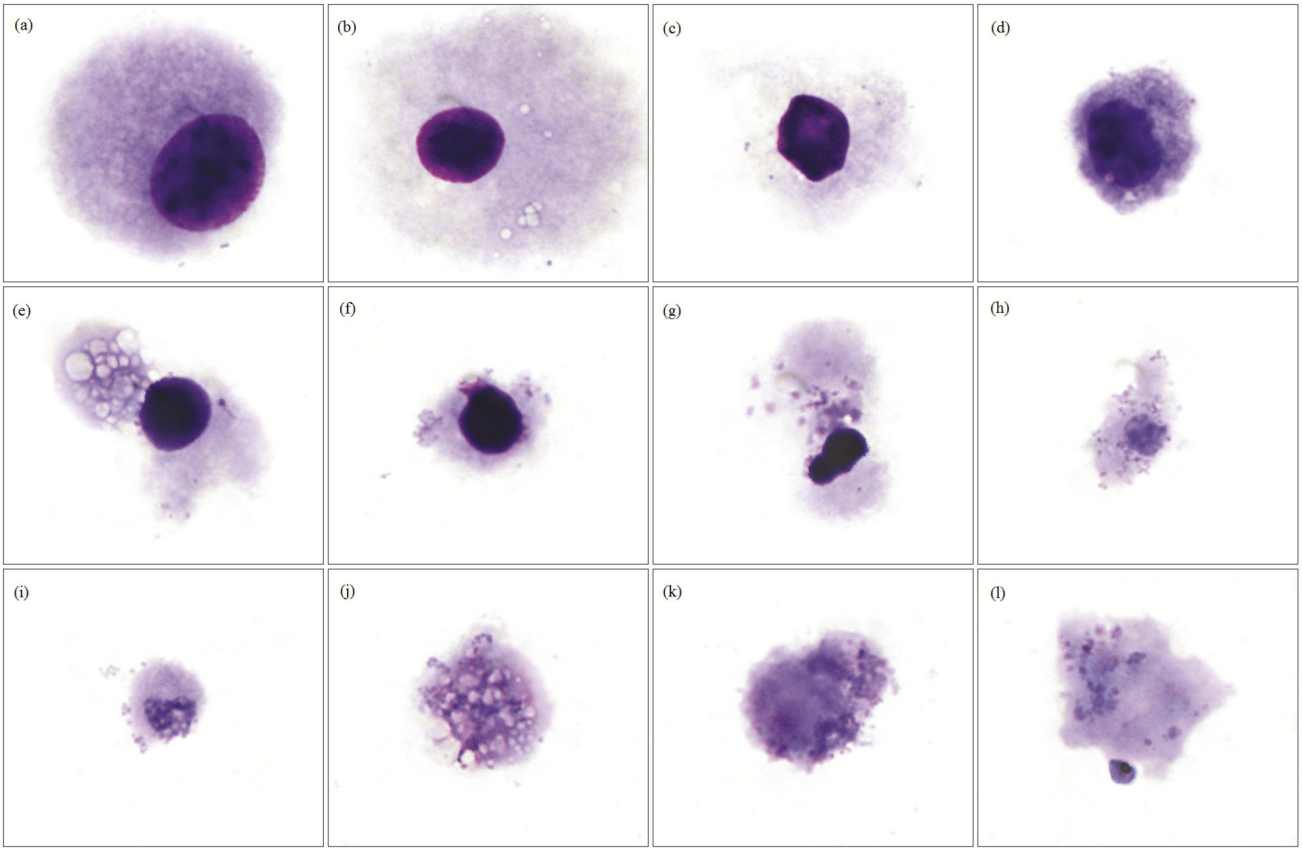


Figure 4. Cells in different stages of apoptosis in treated cultures are easily distinguishable. Most cells had normal morphology (a). Nuclear condensation is evident in cells (dark, condensed and rounded nuclei), as well as vacuolated cytoplasm (b-e). Degradation of nuclei and cytoplasm is also present (f-k). Membrane blebbing and apoptotic bodies are also evident.

After treatment with test compounds, alterations in the size, shape and structure of treated cell nuclei were detected in slightly larger number. Chromatin condensation, cell shrinkage and nuclear fragmentation, as well as the formation of apoptotic bodies, were observed. In addition to these morphological changes, treated cells also showed signs of impairment of the plasma membrane and cell disintegration (Figure 3).

In order to study the origin of the differences between the biological activities of the two stereoisomeric mesyloxy derivatives **5** and **6**, monocrystals of these two compounds were prepared, and their crystal structures by X-ray crystallography were analyzed. Comparing the structural features of compound **5** and **6**, it is possible to notice that the bond lengths and bond angles, especially in the D-seco region, do not differ significantly (Table S11 in the Supplementary Information (SI) section). On the other hand, differences between conformations in the D-seco region were confirmed, as can be seen by inspection of selected torsion angles (Table S12 in the SI section).

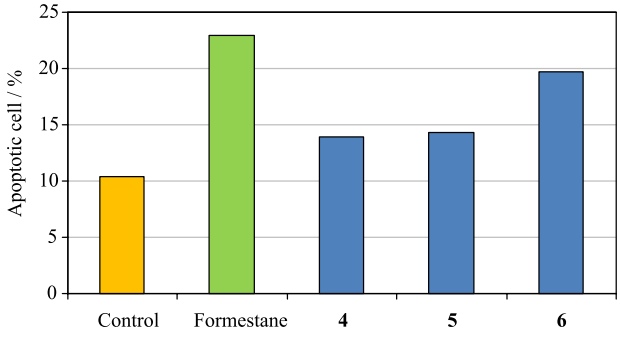


Figure 5. Percentage of apoptotic cells, as estimated by visual observation of the morphology of MDA-MB-231 cells treated for 48 h with equitoxic doses of steroidal derivatives **4**-**6** and formestane.

A perspective view of the molecular structure of compounds **5** and **6** is shown in Figure 6. Selected bond lengths and bond angles are given in Table S11 (in the SI section). Torsion angles are given in Table S12 (in the SI section).

A structural alignment of the X-ray crystal structures of the two stereoisomeric mesyloxy derivatives **5** and **6** is shown in Figure 7. It can be seen that compound **5** has a (17*S*)-configuration, while **6** has a (17*R*)-configuration. Bond lengths and bond angles in the D-seco region do not differ significantly (Table S11), in agreement with the slight differences in their observed cytotoxic and pro-apoptotic potency. The differences between the conformations in the

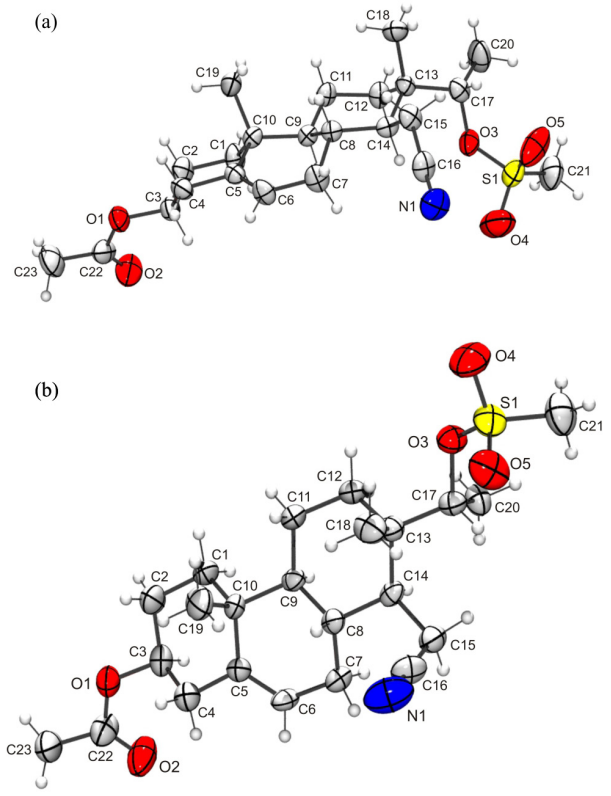


Figure 6. ORTEP drawings of the molecular structures of compound **5** (a) and **6** (b). Non-H atoms are labelled. Displacement ellipsoids are shown at 50% probability level and H atoms are drawn as spheres of arbitrary radii.

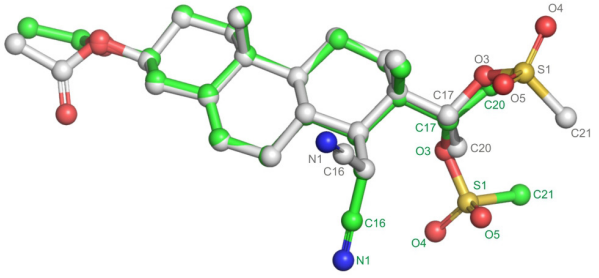


Figure 7. Superimposed fit of the molecular structures of compound **5** (C atoms in green) and compound **6** (C atoms in gray).

D-seco region are connected with the differences in the values of selected torsion angles (Table S12 in the SI section).

Conclusions

Starting from a 16,17-seco alcohol, two stereoisomeric mesyloxy derivatives were obtained (**5** and **6**). Both mesylates expressed strong antiproliferative activity against steroid receptor-independent tumor cell lines: breast adenocarcinoma (MDA-MB-231), prostate cancer (PC-3) and cervical carcinoma (HeLa), with no cytotoxicity against healthy cells (MRC-5). Their precursor, seco alcohol **4**, strongly inhibited the growth of ER-breast adenocarcinoma cell line (MDA-MB-231). These three compounds did not influence the cell cycle of MDA-MB-231 cells, but slightly induced apoptosis in these cells. Slight differences in their cytotoxic and proapoptotic potency could be correlated with their molecular structures, i.e., with differences between their conformations in the D-seco region. Comparing cytotoxicity of D-seco ketone **2**, (17*S*)-secocyanoalcohol **4** and both (17*S*) and (17*R*) D-seco mesyloxy derivatives **5** and **6**, respectively, it may be concluded that compounds **4**, **5** and **6** were much more effective in reducing tumor cell culture growth. It can be presumed that this difference arises from the differences in distances of oxygen atom at C-17 from steroidal skeleton: in molecule **2**, in which hybridization of C-17 is sp^2, it is less than in other compounds, in which hybridization of C-17 is sp^3. On the other hand, antiproliferative effects of these three compounds, as well as their influences on the tumor cell cycle and inducing of apoptosis, are quite similar with each other, even though apoptosis induced by compound **6** is very slightly higher expressed.

Based on these results, it is propose that compounds **4**, **5** and **6** could be used to develop more potent inhibitors of breast and prostate cancer growth, with reduced cytotoxicity toward normal, non-cancerous cells. Synthesis of new steroidal compounds with mesyloxy function, but modified steroidal moiety, is being planned.

The time- and dose-dependencies of the processes by which the tested steroidal compounds influence cell cycle progression (if at all) remain to be clarified. Western-blot analysis would help determine the exact pathways of the compound mode of action. In order to estimate if these compounds induce apoptosis in treated cells, translocation of phosphatidylserine at the cell surface could be examined using an annexine binding assay.

Supplementary Information

Data concerning the IR, ^{1}H, ^{13}C NMR, crystallographic and mass characterizations of the mesyloxy derivatives **5** and **6** are available free of charge at http://jbcs.sbq.org.br, or as a pdf file. CCDC 943744 and 943745 contains the supplementary crystallographic data for this work.

Acknowledgments

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Supplementary Information

Structural Analysis and Antitumor Activity of Androstane D-Seco-mesyloxy Derivatives

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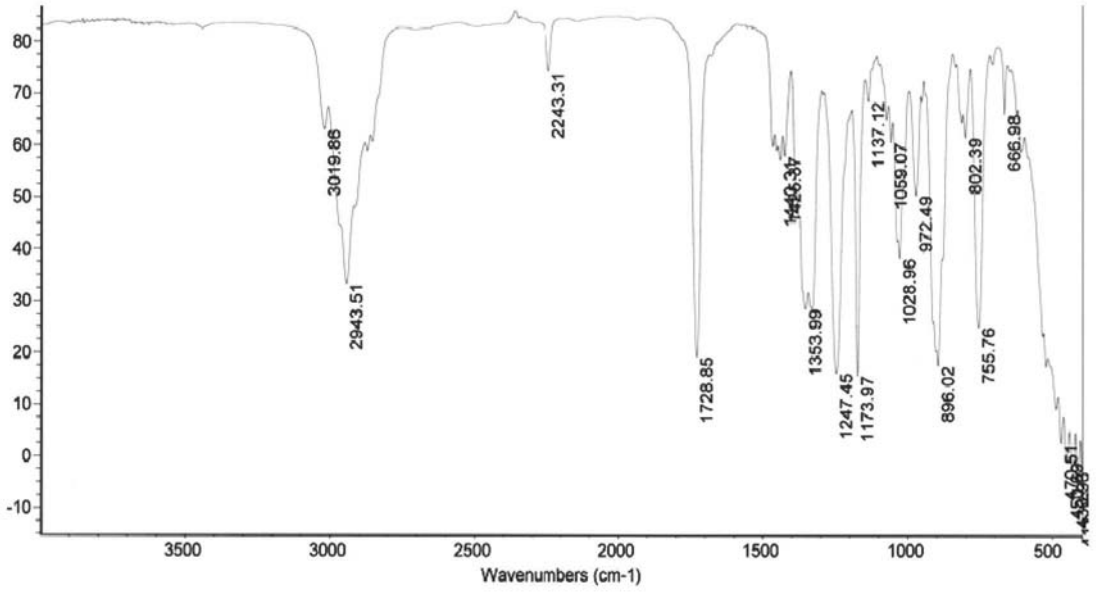

Figure S1. IR spectrum of 3β-acetoxy-(*17S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**).

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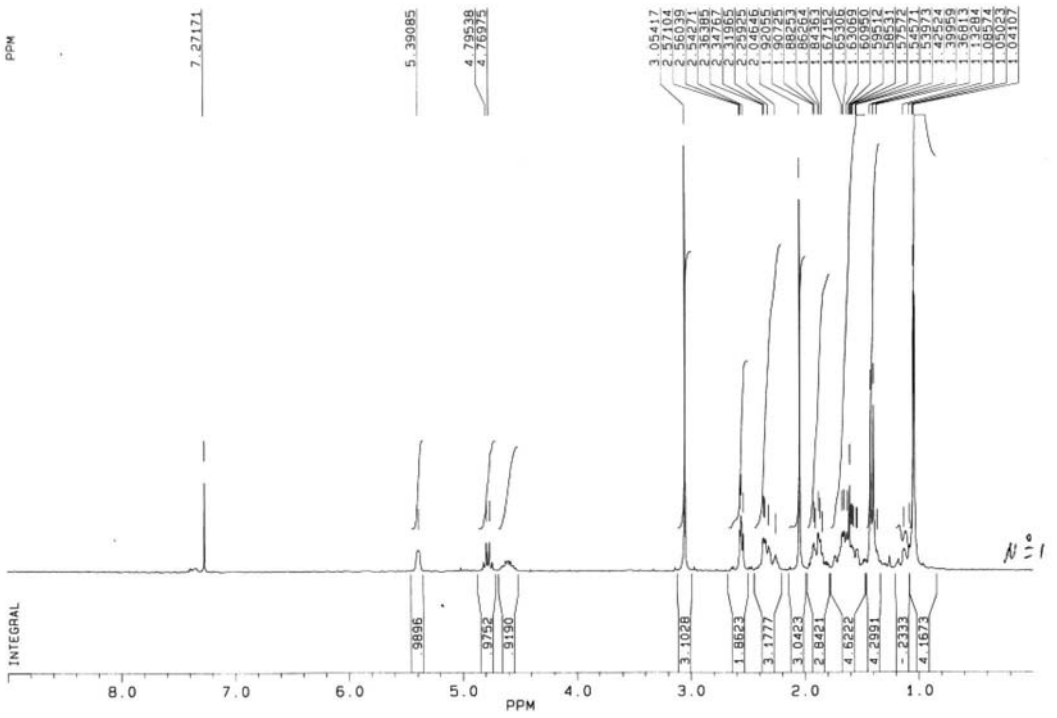


Figure S2. ^{1}H NMR spectrum (250 MHz, $CDCl_3$) of 3β-acetoxy-(*17S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**).

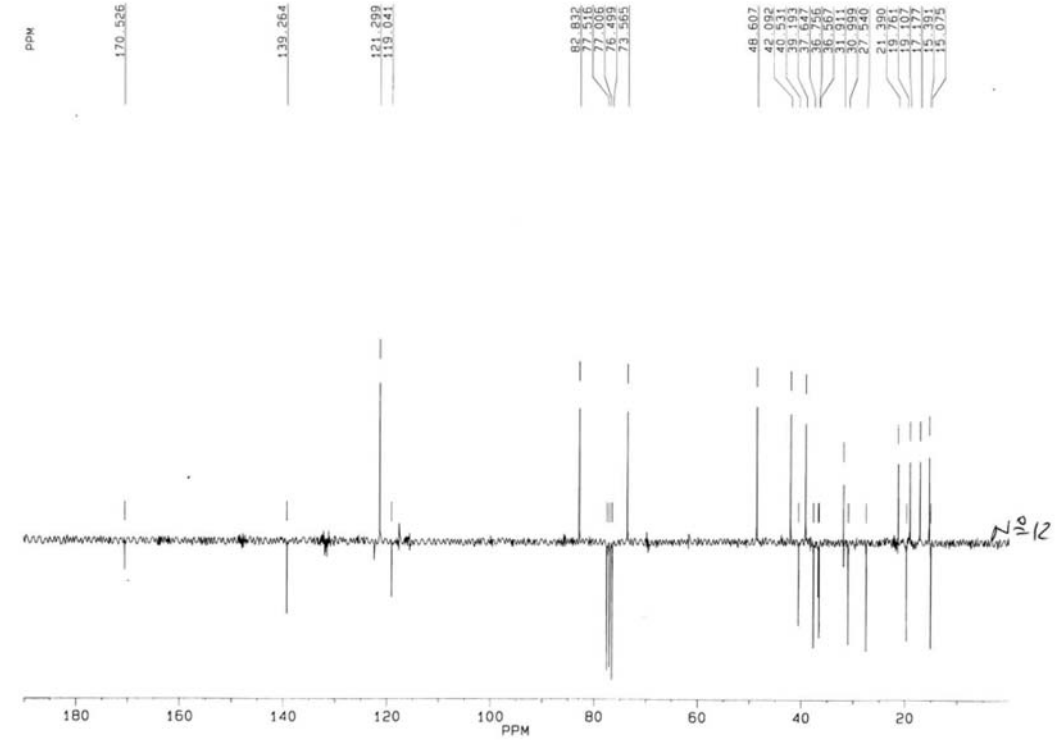


Figure S3. ^{13}C NMR spectrum (62.5 MHz, $CDCl_3$) of 3β-acetoxy-(*17S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**).

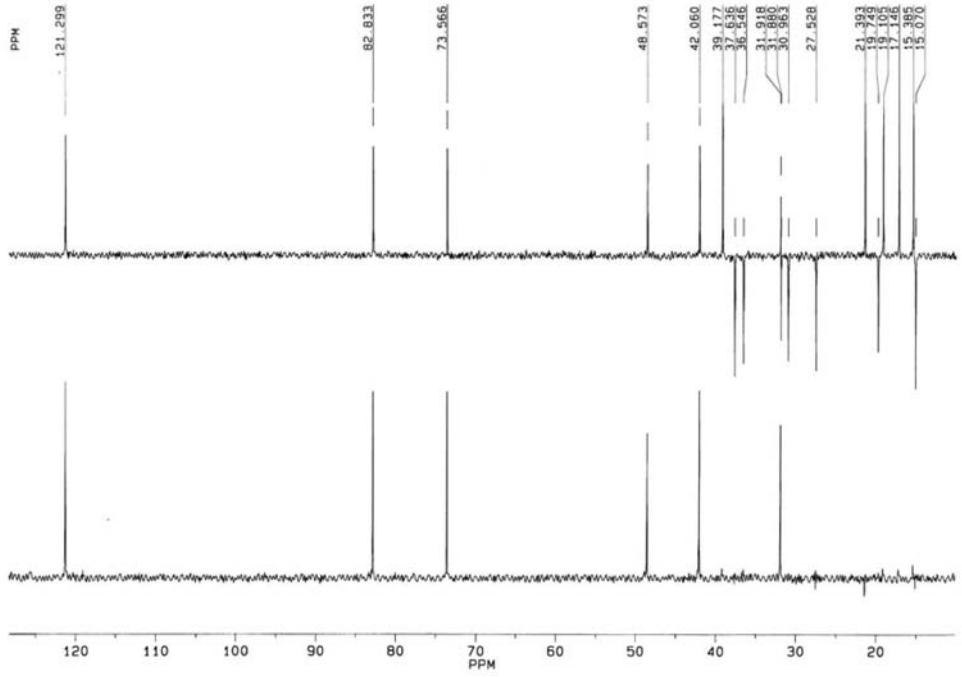
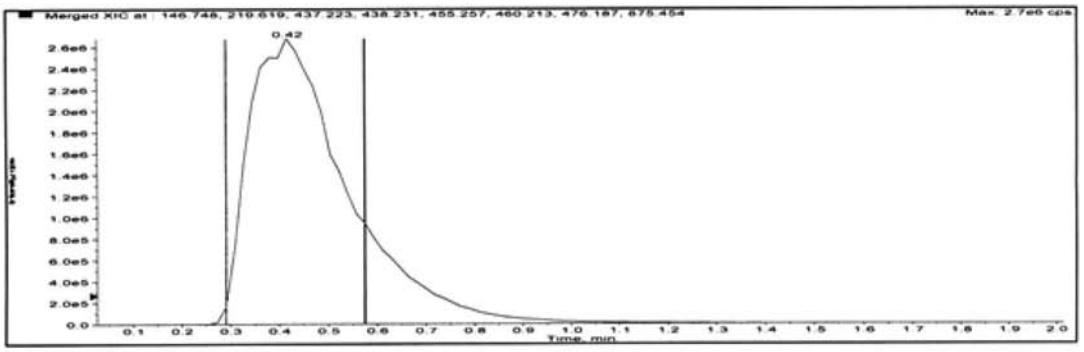


Figure S4. DEPT 135 and DEPT 90 spectrum (62.5 MHz, $CDCl_3$) of 3β-acetoxy-(*17S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**).



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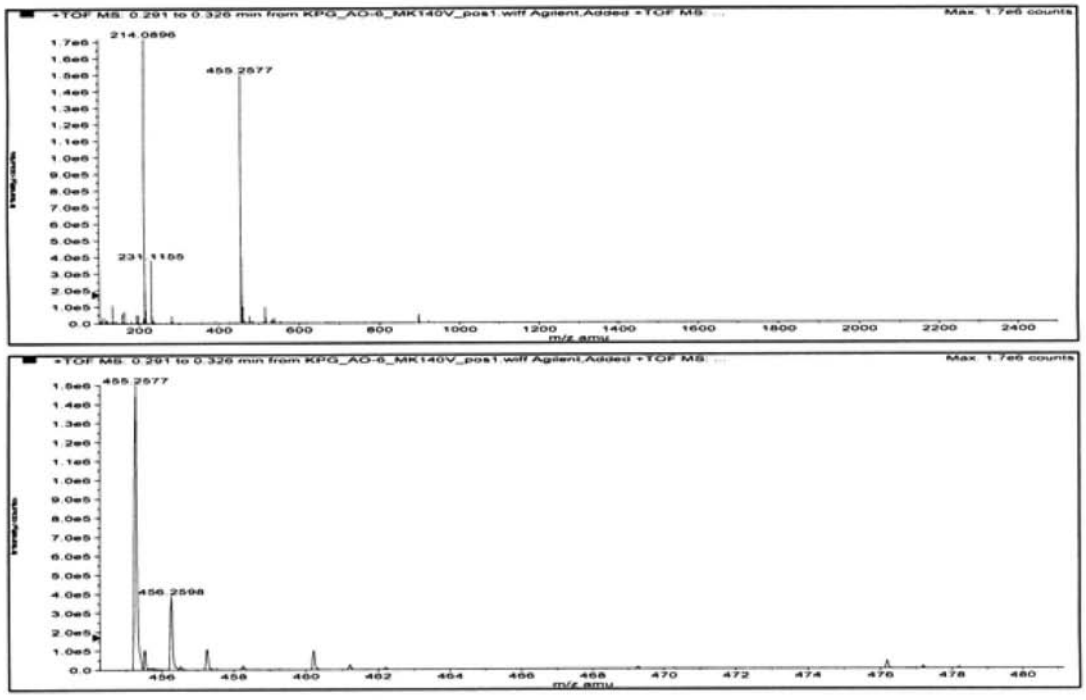


Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C23H35NO5S	--	437.22359	0.42	3.75659 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+NH4]+	1506511.36	455.25742	455.25771	0.28895	0.63	--
[M+Na]+	101743.68	460.21282	460.21217	-0.64697	-1.41	--
[M+K]+	41807.72	476.18675	476.18622	-0.52749	-1.11	--

Figure S5. HRMS (ESI$^+$/TOF) spectrum of 3β-acetoxy-(*17S*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**5**).

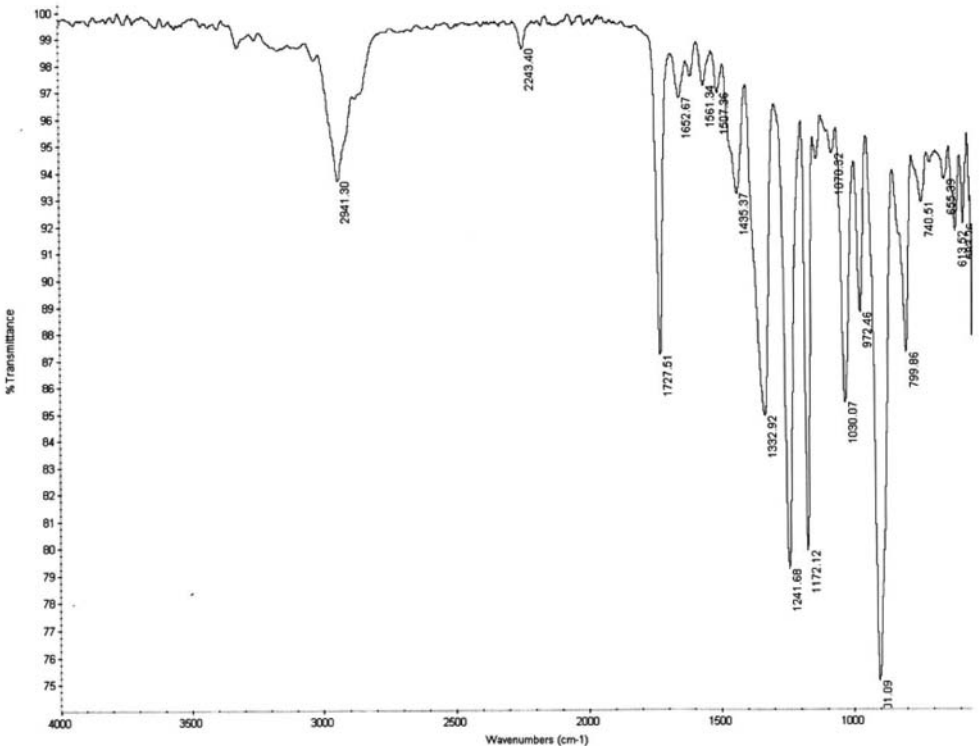


Figure S6. IR spectrum of 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**).

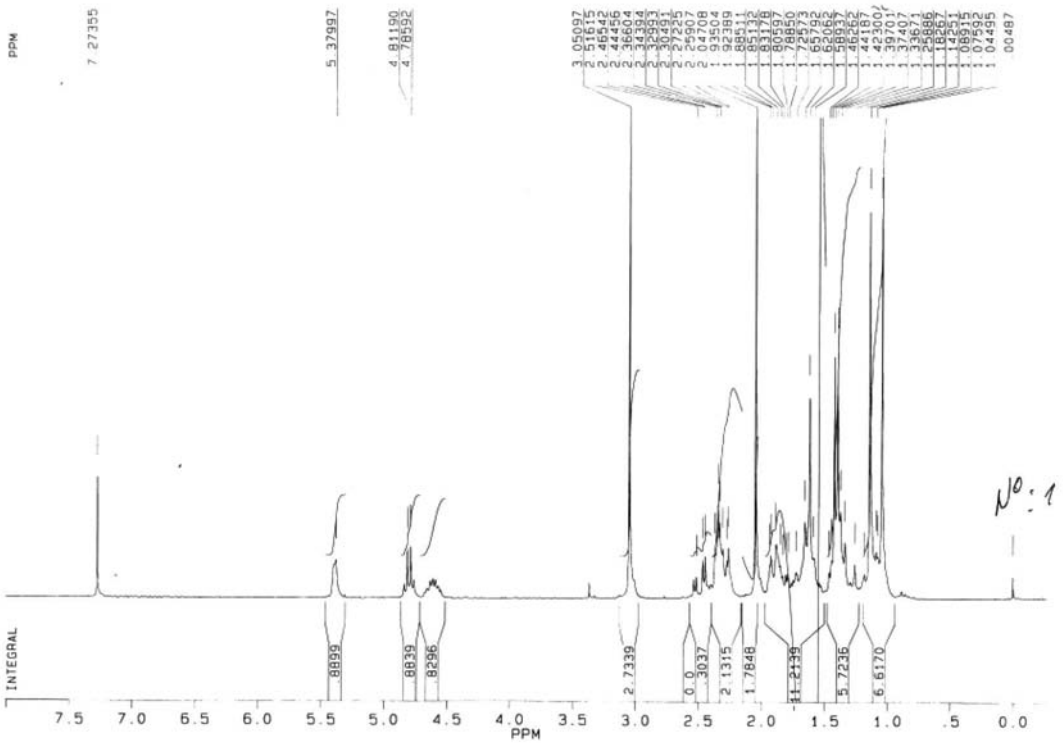


Figure S7. ^{1}H NMR spectrum (250 MHz, $CDCl_3$) of 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**).

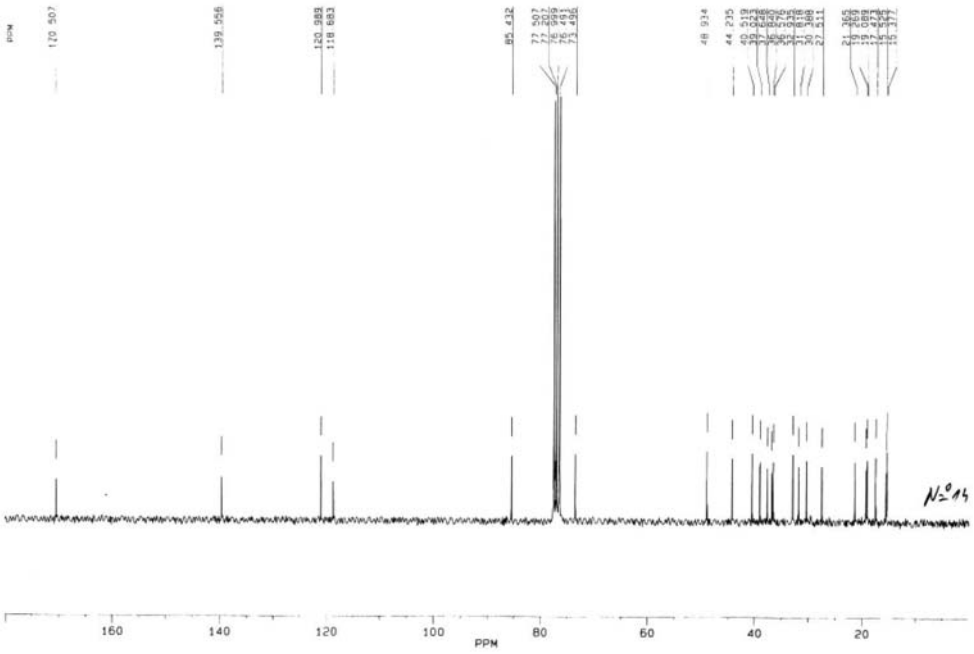


Figure S8. ^{13}C NMR spectrum (62.5 MHz, $CDCl_3$) of 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**).

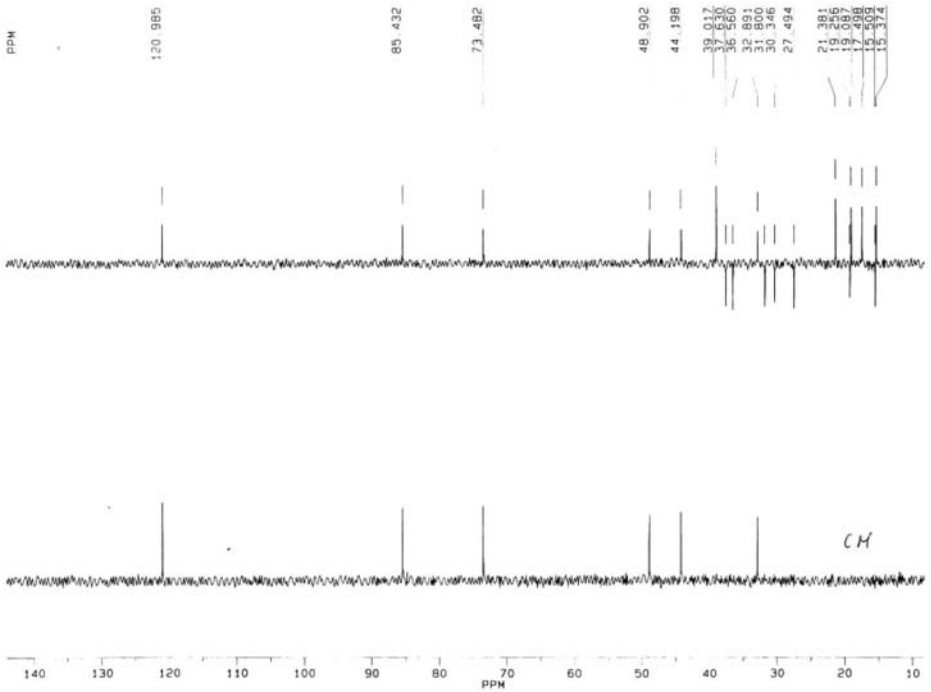
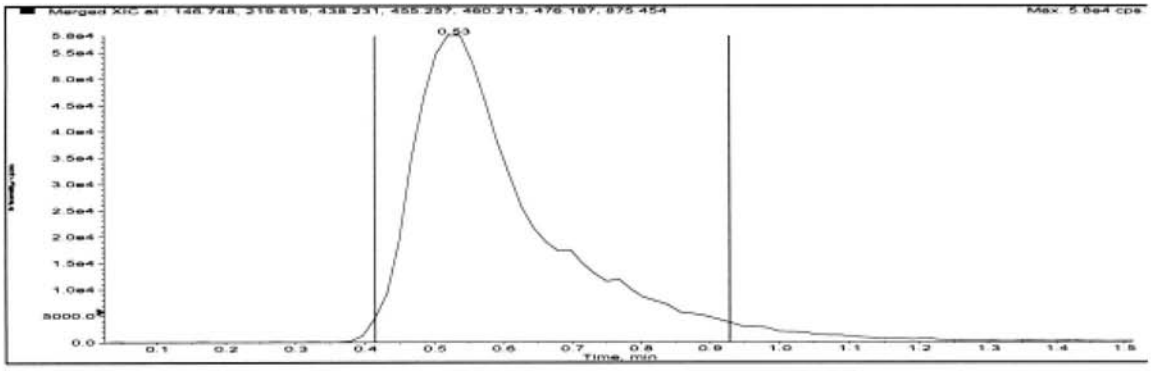


Figure S9. DEPT 135 and DEPT 90 spectrum (62.5 MHz, $CDCl_3$) of 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**).



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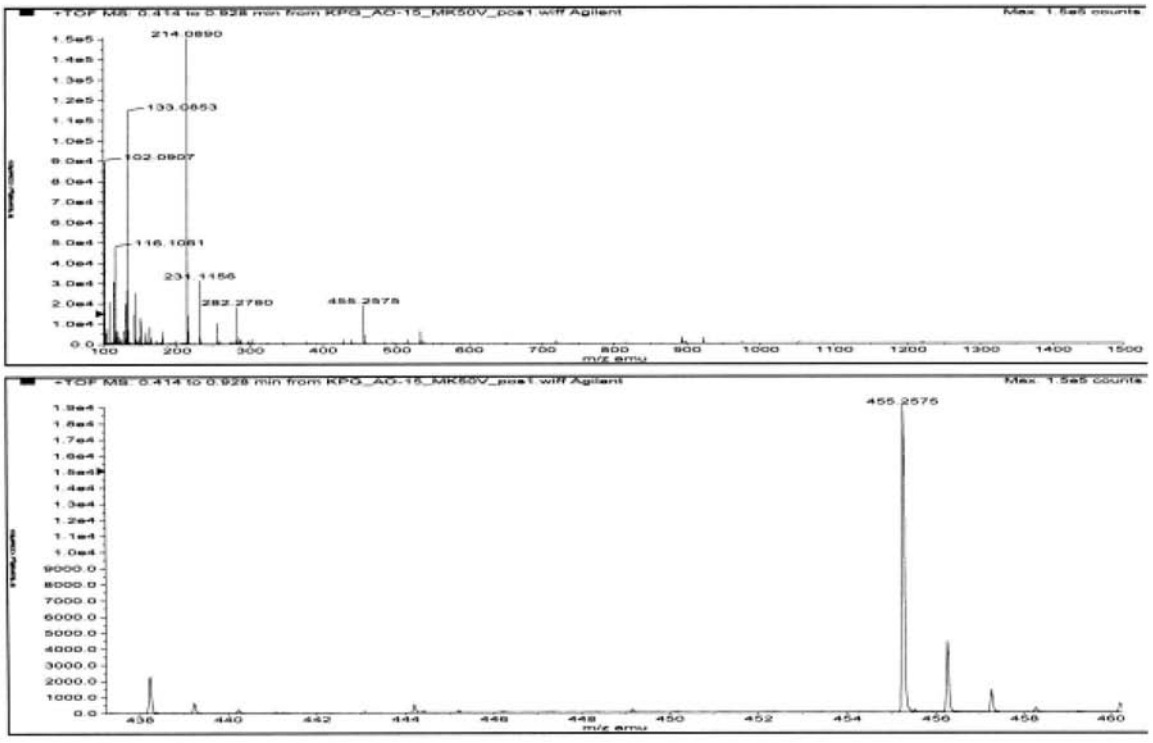

Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C23H35NO5S	--	437.22359	0.53	7.22937 E5	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	2416.57	438.23087	438.23088	0.01272	0.03	--
[M+NH4]+	19680.11	455.25742	455.25753	0.10898	0.24	--

Figure S10. HRMS (ESI$^+$/TOF) spectrum of 3β-acetoxy-(17*R*)-mesyloxy-17-methyl-16,17-secoandrost-5-ene-16-nitrile (**6**).

Table S1. Crystal data and structure refinement for compound **5**

Identification code	Compound **5**	
Empirical formula	$C_{23}H_{35}NO_5S$	
Formula weight	437.58	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 8.539(5) Å	α = 90°
	b = 9.190(5) Å	β = 90°
	c = 30.024(5) Å	γ = 90°
Volume	2356.1(19) Å^3	
Z	4	
Density (calculated)	1.234 mg m^{-3}	
Absorption coefficient	0.170 mm^{-1}	
F(000)	944	
Crystal size	0.569 × 0.328 × 0.082 mm^3	
Theta range for data collection	3.14 to 25.00°	
Index ranges	$-10 \leq h \leq 7$, $-9 \leq k \leq 10$, $-31 \leq l \leq 35$	
Reflections collected	6767	
Independent reflections	3816 [R(int) = 0.0261]	
Completeness to theta = 25.00°	99.7%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.91093	
Refinement method	full-matrix least-squares on F^2	
Data / restraints / parameters	3816 / 0 / 279	
Goodness-of-fit on F^2	1.122	
Final R indices [I > 2sigma(I)]	R1 = 0.0581, wR2 = 0.1217	
R indices (all data)	R1 = 0.0680, wR2 = 0.1267	
Absolute structure parameter	–0.05(12)	
Largest diff. peak and hole	0.198 and –0.238 e Å^{-3}	

Table S2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for compound **5**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

	x	y	z	U(eq)
S(1)	–3506(1)	8472(1)	7900(1)	51(1)
O(1)	237(3)	2182(2)	10881(1)	48(1)
O(3)	–2358(3)	8724(3)	8297(1)	47(1)
C(10)	731(4)	5245(3)	9791(1)	32(1)
C(13)	210(4)	8079(3)	8582(1)	33(1)
O(2)	–1903(4)	2824(4)	11263(1)	75(1)
O(5)	–2624(4)	8233(4)	7505(1)	83(1)
O(4)	–4565(4)	7386(3)	8035(1)	92(1)
N(1)	–1793(5)	3929(4)	7773(1)	63(1)
C(14)	–285(4)	6451(3)	8558(1)	32(1)
C(6)	–121(5)	3121(3)	9335(1)	42(1)
C(11)	571(4)	7760(3)	9417(1)	37(1)
C(9)	29(4)	6171(3)	9408(1)	30(1)
C(8)	337(4)	5511(3)	8945(1)	30(1)
C(5)	384(4)	3633(3)	9720(1)	32(1)
C(4)	666(5)	2647(3)	10113(1)	41(1)
C(1)	–67(5)	5699(3)	10232(1)	46(1)
C(15)	199(5)	5769(3)	8103(1)	42(1)
C(17)	–672(5)	8979(3)	8230(1)	39(1)
C(12)	–184(5)	8641(3)	9048(1)	40(1)
C(16)	–936(5)	4725(4)	7922(1)	44(1)
C(22)	–747(5)	2095(4)	11228(1)	49(1)
C(7)	–386(5)	3986(3)	8923(1)	45(1)
C(2)	287(5)	4721(3)	10631(1)	46(1)
C(3)	–189(4)	3175(3)	10522(1)	42(1)
C(18)	1965(4)	8257(4)	8485(1)	50(1)
C(21)	–4537(6)	10104(4)	7860(2)	71(1)
C(19)	2504(4)	5465(4)	9831(1)	50(1)
C(23)	–215(6)	981(5)	11556(1)	73(1)
C(20)	–394(6)	10611(4)	8242(1)	69(1)

Table S3. Bond lengths (Å) and angles (degree) for compound **5**

S(1)-O(4)	1.406(3)	C(18)-H(18C)	0.9600	C(7)-C(8)-C(9)	109.3(2)
S(1)-O(5)	1.422(3)	C(21)-H(21A)	0.9600	C(7)-C(8)-C(14)	110.0(3)
S(1)-O(3)	1.561(3)	C(21)-H(21B)	0.9600	C(9)-C(8)-C(14)	113.5(2)
S(1)-C(21)	1.743(4)	C(21)-H(21C)	0.9600	C(7)-C(8)-H(8)	108.0
O(1)-C(22)	1.342(4)	C(19)-H(19A)	0.9600	C(9)-C(8)-H(8)	108.0
O(1)-C(3)	1.457(4)	C(19)-H(19B)	0.9600	C(14)-C(8)-H(8)	108.0
O(3)-C(17)	1.473(4)	C(19)-H(19C)	0.9600	C(6)-C(5)-C(4)	121.5(3)
C(10)-C(5)	1.525(4)	C(23)-H(23A)	0.9600	C(6)-C(5)-C(10)	122.1(3)
C(10)-C(19)	1.533(5)	C(23)-H(23B)	0.9600	C(4)-C(5)-C(10)	116.3(3)
C(10)-C(1)	1.546(4)	C(23)-H(23C)	0.9600	C(5)-C(4)-C(3)	111.5(3)
C(10)-C(9)	1.552(4)	C(20)-H(20A)	0.9600	C(5)-C(4)-H(4A)	109.3
C(13)-C(12)	1.530(4)	C(20)-H(20B)	0.9600	C(3)-C(4)-H(4A)	109.3
C(13)-C(18)	1.535(5)	C(20)-H(20C)	0.9600	C(5)-C(4)-H(4B)	109.3
C(13)-C(17)	1.538(5)	O(4)-S(1)-O(5)	118.1(2)	C(3)-C(4)-H(4B)	109.3
C(13)-C(14)	1.556(4)	O(4)-S(1)-O(3)	106.82(19)	H(4A)-C(4)-H(4B)	108.0
O(2)-C(22)	1.197(5)	O(5)-S(1)-O(3)	109.09(16)	C(2)-C(1)-C(10)	115.2(3)
N(1)-C(16)	1.127(4)	O(4)-S(1)-C(21)	107.8(2)	C(2)-C(1)-H(1A)	108.5
C(14)-C(8)	1.541(4)	O(5)-S(1)-C(21)	110.1(2)	C(10)-C(1)-H(1A)	108.5
C(14)-C(15)	1.560(4)	O(3)-S(1)-C(21)	103.96(19)	C(2)-C(1)-H(1B)	108.5
C(14)-H(14)	0.9800	C(22)-O(1)-C(3)	117.1(3)	C(10)-C(1)-H(1B)	108.5
C(6)-C(5)	1.320(4)	C(17)-O(3)-S(1)	122.3(2)	H(1A)-C(1)-H(1B)	107.5
C(6)-C(7)	1.488(4)	C(5)-C(10)-C(19)	109.3(3)	C(16)-C(15)-C(14)	114.4(3)
C(6)-H(6)	0.96(3)	C(5)-C(10)-C(1)	107.2(3)	C(16)-C(15)-H(15A)	108.7
C(11)-C(12)	1.515(4)	C(19)-C(10)-C(1)	109.4(3)	C(14)-C(15)-H(15A)	108.7
C(11)-C(9)	1.532(4)	C(5)-C(10)-C(9)	110.8(2)	C(16)-C(15)-H(15B)	108.7
C(11)-H(11A)	0.9700	C(19)-C(10)-C(9)	111.5(3)	C(14)-C(15)-H(15B)	108.7
C(11)-H(11B)	0.9700	C(1)-C(10)-C(9)	108.5(2)	H(15A)-C(15)-H(15B)	107.6
C(9)-C(8)	1.540(4)	C(12)-C(13)-C(18)	110.6(3)	O(3)-C(17)-C(20)	107.9(3)
C(9)-H(9)	0.9800	C(12)-C(13)-C(17)	109.8(3)	O(3)-C(17)-C(13)	107.4(3)
C(8)-C(7)	1.533(4)	C(18)-C(13)-C(17)	106.9(3)	C(20)-C(17)-C(13)	116.1(3)
C(8)-H(8)	0.9800	C(12)-C(13)-C(14)	107.8(2)	O(3)-C(17)-H(17)	106.0(19)
C(5)-C(4)	1.507(4)	C(18)-C(13)-C(14)	111.0(3)	C(20)-C(17)-H(17)	109.2(17)
C(4)-C(3)	1.509(5)	C(17)-C(13)-C(14)	110.7(3)	C(13)-C(17)-H(17)	109.7(17)
C(4)-H(4A)	0.9700	C(8)-C(14)-C(13)	114.3(2)	C(11)-C(12)-C(13)	113.3(3)
C(4)-H(4B)	0.9700	C(8)-C(14)-C(15)	110.0(3)	C(11)-C(12)-H(12A)	108.9
C(1)-C(2)	1.529(4)	C(13)-C(14)-C(15)	110.7(2)	C(13)-C(12)-H(12A)	108.9
C(1)-H(1A)	0.9700	C(8)-C(14)-H(14)	107.1	C(11)-C(12)-H(12B)	108.9
C(1)-H(1B)	0.9700	C(13)-C(14)-H(14)	107.1	C(13)-C(12)-H(12B)	108.9
C(15) -C(16)	1.468(5)	C(15)-C(14)-H(14)	107.1	H(12A)-C(12)-H(12B)	107.7
C(15)-H(15A)	0.9700	C(5)-C(6)-C(7)	126.0(3)	N(1)-C(16)-C(15)	178.3(4)
C(15)-H(15B)	0.9700	C(5)-C(6)-H(6)	117.3(17)	O(2)-C(22)-O(1)	123.5(3)
C(17)-C(20)	1.519(5)	C(7)-C(6)-H(6)	116.7(18)	O(2)-C(22)-C(23)	125.2(4)
C(17)-H(17)	0.94(3)	C(12)-C(11)-C(9)	111.6(3)	O(1)-C(22)-C(23)	111.3(4)
C(12)-H(12A)	0.9700	C(12)-C(11)-H(11A)	109.3	C(6)-C(7)-C(8)	113.0(3)
C(12)-H(12B)	0.9700	C(9)-C(11)-H(11A)	109.3	C(6)-C(7)-H(7A)	109.0
C(22)-C(23)	1.493(5)	C(12)-C(11)-H(11B)	109.3	C(8)-C(7)-H(7A)	109.0
C(7)-H(7A)	0.9700	C(9)-C(11)-H(11B)	109.3	C(6)-C(7)-H(7B)	109.0
C(7)-H(7B)	0.9700	H(11A)-C(11)-H(11B)	108.0	C(8)-C(7)-H(7B)	109.0
C(2)-C(3)	1.513(5)	C(11)-C(9)-C(8)	109.9(2)	H(7A)-C(7)-H(7B)	107.8
C(2)-H(2A)	0.9700	C(11)-C(9)-C(10)	113.2(2)	C(3)-C(2)-C(1)	109.2(3)
C(2)-H(2B)	0.9700	C(8)-C(9)-C(10)	112.8(2)	C(3)-C(2)-H(2A)	109.8
C(3)-H(3)	0.9800	C(11)-C(9)-H(9)	106.9	C(1)-C(2)-H(2A)	109.8
C(18)-H(18A)	0.9600	C(8)-C(9)-H(9)	106.9	C(3)-C(2)-H(2B)	109.8
C(18)-H(18B)	0.9600	C(10)-C(9)-H(9)	106.9	C(1)-C(2)-H(2B)	109.8

Table S3. continuation

H(2A)-C(2)-H(2B)	108.3	S(1)-C(21)-H(21A)	109.5	C(22)-C(23)-H(23B)	109.5
O(1)-C(3)-C(4)	106.2(3)	S(1)-C(21)-H(21B)	109.5	H(23A)-C(23)-H(23B)	109.5
O(1)-C(3)-C(2)	111.2(3)	H(21A)-C(21)-H(21B)	109.5	C(22)-C(23)-H(23C)	109.5
C(4)-C(3)-C(2)	110.4(3)	S(1)-C(21)-H(21C)	109.5	H(23A)-C(23)-H(23C)	109.5
O(1)-C(3)-H(3)	109.7	H(21A)-C(21)-H(21C)	109.5	H(23B)-C(23)-H(23C)	109.5
C(4)-C(3)-H(3)	109.7	H(21B)-C(21)-H(21C)	109.5	C(17)-C(20)-H(20A)	109.5
C(2)-C(3)-H(3)	109.7	C(10)-C(19)-H(19A)	109.5	C(17)-C(20)-H(20B)	109.5
C(13)-C(18)-H(18A)	109.5	C(10)-C(19)-H(19B)	109.5	H(20A)-C(20)-H(20B)	109.5
C(13)-C(18)-H(18B)	109.5	H(19A)-C(19)-H(19B)	109.5	C(17)-C(20)-H(20C)	109.5
H(18A)-C(18)-H(18B)	109.5	C(10)-C(19)-H(19C)	109.5	H(20A)-C(20)-H(20C)	109.5
C(13)-C(18)-H(18C)	109.5	H(19A)-C(19)-H(19C)	109.5	H(20B)-C(20)-H(20C)	109.5
H(18A)-C(18)-H(18C)	109.5	H(19B)-C(19)-H(19C)	109.5		
H(18B)-C(18)-H(18C)	109.5	C(22)-C(23)-H(23A)	109.5		

Table S4. Anisotropic displacement parameters ($Å^2 \times 10^3$) for compound **5**. The anisotropic displacement factor exponent takes the form: $2\pi^2 [h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	47(1)	51(1)	54(1)	0(1)	−12(1)	7(1)
O(1)	47(2)	58(1)	40(1)	13(1)	4(1)	4(1)
O(3)	42(2)	63(2)	34(1)	5(1)	1(1)	9(1)
C(10)	30(2)	32(2)	35(2)	–5(1)	–2(2)	3(1)
C(13)	35(2)	32(2)	31(2)	−1(1)	2(2)	−2(2)
O(2)	58(2)	112(2)	55(2)	15(2)	19(2)	19(2)
O(5)	68(2)	134(3)	47(2)	−32(2)	−13(2)	24(2)
O(4)	66(2)	68(2)	144(3)	11(2)	−17(2)	−14(2)
N(1)	76(3)	67(2)	46(2)	−18(2)	–7(2)	−10(2)
C(14)	31(2)	35(2)	31(2)	–9(1)	3(2)	0(2)
C(6)	56(2)	24(2)	45(2)	–2(1)	1(2)	−1(2)
C(11)	44(2)	34(2)	32(2)	–6(1)	–6(2)	2(2)
C(9)	31(2)	28(1)	32(2)	–5(1)	−1(2)	3(1)
C(8)	32(2)	27(1)	32(2)	–5(1)	2(2)	1(2)
C(5)	30(2)	31(2)	35(2)	0(1)	5(2)	2(2)
C(4)	40(2)	36(2)	46(2)	5(1)	5(2)	−3(2)
C(1)	66(3)	40(2)	31(2)	–3(1)	−3(2)	6(2)
C(15)	51(2)	44(2)	30(2)	–6(1)	4(2)	−2(2)
C(17)	49(2)	38(2)	30(2)	–3(1)	3(2)	−3(2)
C(12)	54(2)	31(2)	34(2)	–8(1)	–6(2)	5(2)
C(16)	62(3)	41(2)	29(2)	−10(2)	−3(2)	4(2)
C(22)	48(2)	67(2)	32(2)	0(2)	−2(2)	−11(2)
C(7)	64(3)	33(2)	37(2)	−10(1)	–4(2)	–4(2)
C(2)	55(3)	55(2)	28(2)	–4(1)	0(2)	1(2)
C(3)	39(2)	49(2)	37(2)	9(2)	2(2)	4(2)
C(18)	43(2)	52(2)	55(2)	2(2)	−3(2)	−16(2)
C(21)	65(3)	72(3)	77(3)	13(2)	−12(3)	26(2)
C(19)	46(3)	46(2)	58(2)	12(2)	−15(2)	–4(2)
C(23)	83(4)	86(3)	49(2)	23(2)	11(3)	1(3)
C(20)	98(4)	40(2)	70(3)	14(2)	−24(3)	–8(2)

Table S5. Torsion angles (degree) for compound **5**

O(4)-S(1)-O(3)-C(17)	140.7(3)	C(15)-C(14)-C(8)-C(7)	–61.9(4)	C(14)-C(13)-C(17)-O(3)	54.5(3)
O(5)-S(1)-O(3)-C(17)	12.0(3)	C(13)-C(14)-C(8)-C(9)	49.9(4)	C(12)-C(13)-C(17)-C(20)	56.4(4)
C(21)-S(1)-O(3)-C(17)	−105.4(3)	C(15)-C(14)-C(8)-C(9)	175.3(3)	C(18)-C(13)-C(17)-C(20)	–63.7(4)
C(12)-C(13)-C(14)-C(8)	–50.7(4)	C(7)-C(6)-C(5)-C(4)	−177.8(3)	C(14)-C(13)-C(17)-C(20)	175.3(3)
C(18)-C(13)-C(14)-C(8)	70.6(3)	C(7)-C(6)-C(5)-C(10)	1.5(6)	C(9)-C(11)-C(12)-C(13)	–60.8(4)
C(17)-C(13)-C(14)-C(8)	−170.8(3)	C(19)-C(10)-C(5)-C(6)	−109.2(4)	C(18)-C(13)-C(12)-C(11)	–65.3(4)
C(12)-C(13)-C(14)-C(15)	−175.7(3)	C(1)-C(10)-C(5)-C(6)	132.3(3)	C(17)-C(13)-C(12)-C(11)	176.9(3)
C(18)-C(13)-C(14)-C(15)	–54.4(4)	C(9)-C(10)-C(5)-C(6)	14.1(5)	C(14)-C(13)-C(12)-C(11)	56.3(4)
C(17)-C(13)-C(14)-C(15)	64.2(4)	C(19)-C(10)-C(5)-C(4)	70.2(4)	C(14)-C(15)-C(16)-N(1)	158(14)
C(12)-C(11)-C(9)-C(8)	55.0(4)	C(1)-C(10)-C(5)-C(4)	–48.3(4)	C(3)-O(1)-C(22)-O(2)	–3.0(5)
C(12)-C(11)-C(9)-C(10)	−177.9(3)	C(9)-C(10)-C(5)-C(4)	−166.5(3)	C(3)-O(1)-C(22)-C(23)	176.8(3)
C(5)-C(10)-C(9)-C(11)	−170.2(3)	C(6)-C(5)-C(4)-C(3)	−127.0(4)	C(5)-C(6)-C(7)-C(8)	13.3(6)
C(19)-C(10)-C(9)-C(11)	–48.2(4)	C(10)-C(5)-C(4)-C(3)	53.6(4)	C(9)-C(8)-C(7)-C(6)	–42.2(4)
C(1)-C(10)-C(9)-C(11)	72.4(3)	C(5)-C(10)-C(1)-C(2)	50.5(4)	C(14)-C(8)-C(7)-C(6)	−167.5(3)
C(5)-C(10)-C(9)-C(8)	–44.7(4)	C(19)-C(10)-C(1)-C(2)	–67.9(4)	C(10)-C(1)-C(2)-C(3)	–57.4(5)
C(19)-C(10)-C(9)-C(8)	77.3(3)	C(9)-C(10)-C(1)-C(2)	170.2(3)	C(22)-O(1)-C(3)-C(4)	−157.4(3)
C(1)-C(10)-C(9)-C(8)	−162.1(3)	C(8)-C(14)-C(15)-C(16)	89.9(4)	C(22)-O(1)-C(3)-C(2)	82.5(4)
C(11)-C(9)-C(8)-C(7)	−173.3(3)	C(13)-C(14)-C(15)-C(16)	−142.7(3)	C(5)-C(4)-C(3)-O(1)	−177.3(3)
C(10)-C(9)-C(8)-C(7)	59.5(4)	S(1)-O(3)-C(17)-C(20)	97.0(3)	C(5)-C(4)-C(3)-C(2)	–56.7(4)
C(11)-C(9)-C(8)-C(14)	–50.1(4)	S(1)-O(3)-C(17)-C(13)	−137.2(2)	C(1)-C(2)-C(3)-O(1)	175.6(3)
C(10)-C(9)-C(8)-C(14)	−177.3(3)	C(12)-C(13)-C(17)-O(3)	–64.4(3)	C(1)-C(2)-C(3)-C(4)	58.1(4)
C(13)-C(14)-C(8)-C(7)	172.7(3)	C(18)-C(13)-C(17)-O(3)	175.6(3)		

Table S6. Crystal data and structure refinement for compound **6**

Identification code	Compound **6**	
Empirical formula	$C_{23}H_{35}NO_5S$	
Formula weight	437.58	
Temperature	293(2) K	
Wavelength	0.71069 Å	
Crystal system	monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 10.644(5) Å	α = 90°
	b = 9.823(5) Å	β = 94.995(5)°
	c = 11.068(5) Å	γ = 90°
Volume	1152.8(10) Å^3	
Z	2	
Density (calculated)	1.261 mg m^{-3}	
Absorption coefficient	0.174 mm^{1}	
F(000)	472	
Crystal size	0.532 × 0.178 × 0.079 mm^3	
Theta range for data collection	3.28 to 25.00°	
Index ranges	8 ≤ h ≤ 12, 11 ≤ k ≤ 11, 13 ≤ l ≤ 12	
Reflections collected	4336	
Independent reflections	3448 [R(int) = 0.0249]	
Completeness to theta = 25.00°	99.7%	
Absorption correction	semiempirical from equivalents	
Max. and min. transmission	1.00000 and 0.74065	
Refinement method	full matrix least squares on F^2	
Data / restraints / parameters	3448 / 1 / 275	
Goodnessoffit on *F2*	1.058	
Final R indices [I > 2sigma(I)]	R1 = 0.0535, wR2 = 0.1017	
R indices (all data)	R1 = 0.0678, wR2 = 0.1108	
Absolute structure parameter	0.01(10)	
Largest diff. peak and hole	0.169 and 0.200 e Å^3	

Table S7. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for compound **6**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

	x	y	z	U(eq)
S(1)	10266(1)	7848(1)	5579(1)	52(1)
O(3)	9606(2)	8391(2)	6687(2)	47(1)
O(1)	849(3)	8888(3)	11208(2)	55(1)
O(5)	10936(3)	6673(3)	6002(3)	77(1)
C(9)	5251(3)	9044(3)	8542(3)	35(1)
C(8)	5071(3)	9556(3)	7227(3)	34(1)
O(4)	9383(3)	7722(3)	4561(3)	72(1)
C(13)	7373(3)	8856(3)	6900(3)	37(1)
C(11)	6300(3)	7986(4)	8712(4)	46(1)
C(14)	6322(3)	9957(3)	6723(3)	34(1)
C(10)	3991(3)	8562(3)	9016(3)	37(1)
C(5)	2955(3)	9594(3)	8692(3)	38(1)
C(20)	9115(3)	10708(3)	7172(3)	47(1)
C(3)	1986(4)	9309(4)	10662(3)	49(1)
O(2)	-72(3)	10907(3)	10828(3)	88(1)
C(7)	4168(3)	10776(3)	7159(3)	41(1)
C(12)	7520(4)	8504(4)	8252(3)	46(1)
C(6)	3074(4)	10579(4)	7900(3)	42(1)
C(16)	5236(4)	9660(4)	4607(4)	51(1)
C(17)	8612(3)	9450(3)	6500(3)	41(1)
C(4)	1753(4)	9426(4)	9309(3)	50(1)
C(18)	7026(4)	7583(3)	6134(4)	53(1)
C(15)	6083(4)	10494(4)	5402(3)	45(1)
C(1)	4171(4)	8469(4)	10412(3)	51(1)
C(2)	2955(4)	8212(4)	11009(4)	56(1)
C(21)	11347(4)	9112(5)	5278(4)	72(1)
C(22)	−110(4)	9763(4)	11217(4)	60(1)
C(23)	−1217(4)	9131(5)	11716(4)	73(1)
C(19)	3580(4)	7164(3)	8483(4)	57(1)
N(1)	4557(4)	9017(4)	4005(3)	85(1)

Table S8. Bond lengths (Å) and angles (degree) for compound **6**

S(1)-O(4)	1.409(3)	C(1)-H(1B)	0.9700	C(5)-C(10)-C(9)	110.3(3)
S(1)-O(5)	1.415(3)	C(2)-H(2A)	0.9700	C(19)-C(10)-C(9)	111.4(3)
S(1)-O(3)	1.559(3)	C(2)-H(2B)	0.9700	C(1)-C(10)-C(9)	108.7(3)
S(1)-C(21)	1.744(4)	C(21)-H(21A)	0.9600	C(6)-C(5)-C(4)	121.0(3)
O(3)-C(17)	1.486(4)	C(21)-H(21B)	0.9600	C(6)-C(5)-C(10)	122.5(3)
O(1)-C(22)	1.336(5)	C(21)-H(21C)	0.9600	C(4)-C(5)-C(10)	116.5(3)
O(1)-C(3)	1.459(4)	C(22)-C(23)	1.481(6)	C(17)-C(20)-H(20A)	109.5
C(9)-C(11)	1.526(5)	C(23)-H(23A)	0.9600	C(17)-C(20)-H(20B)	109.5
C(9)-C(8)	1.535(4)	C(23)-H(23B)	0.9600	H(20A)-C(20)-H(20B)	109.5
C(9)-C(10)	1.556(5)	C(23)-H(23C)	0.9600	C(17)-C(20)-H(20C)	109.5
C(9)-H(9)	0.9800	C(19)-H(19A)	0.9600	H(20A)-C(20)-H(20C)	109.5
C(8)-C(7)	1.534(5)	C(19)-H(19B)	0.9600	H(20B)-C(20)-H(20C)	109.5
C(8)-C(14)	1.538(5)	C(19)-H(19C)	0.9600	O(1)-C(3)-C(4)	111.3(3)
C(8)-H(8)	0.9800	O(4)-S(1)-O(5)	118.3(2)	O(1)-C(3)-C(2)	105.3(3)
C(13)-C(12)	1.530(5)	O(4)-S(1)-O(3)	110.11(16)	C(4)-C(3)-C(2)	110.9(3)
C(13)-C(18)	1.538(5)	O(5)-S(1)-O(3)	105.52(17)	O(1)-C(3)-H(3)	109.8
C(13)-C(17)	1.542(5)	O(4)-S(1)-C(21)	108.4(2)	C(4)-C(3)-H(3)	109.8
C(13)-C(14)	1.556(5)	O(5)-S(1)-C(21)	108.8(2)	C(2)-C(3)-H(3)	109.8
C(11)-C(12)	1.523(5)	O(3)-S(1)-C(21)	104.88(18)	C(6)-C(7)-C(8)	112.8(3)
C(11)-H(11A)	0.9700	C(17)-O(3)-S(1)	119.6(2)	C(6)-C(7)-H(7A)	109.0
C(11)-H(11B)	0.9700	C(22)-O(1)-C(3)	118.9(3)	C(8)-C(7)-H(7A)	109.0
C(14)-C(15)	1.555(4)	C(11)-C(9)-C(8)	111.7(3)	C(6)-C(7)-H(7B)	109.0
C(14)-H(14)	0.9800	C(11)-C(9)-C(10)	113.2(3)	C(8)-C(7)-H(7B)	109.0
C(10)-C(5)	1.518(5)	C(8)-C(9)-C(10)	112.3(3)	H(7A)-C(7)-H(7B)	107.8
C(10)-C(19)	1.542(5)	C(11)-C(9)-H(9)	106.3	C(11)-C(12)-C(13)	112.6(3)
C(10)-C(1)	1.542(5)	C(8)-C(9)-H(9)	106.3	C(11)-C(12)-H(12A)	109.1
C(5)-C(6)	1.320(5)	C(10)-C(9)-H(9)	106.3	C(13)-C(12)-H(12A)	109.1
C(5)-C(4)	1.511(5)	C(7)-C(8)-C(9)	109.1(3)	C(11)-C(12)-H(12B)	109.1
C(20)-C(17)	1.516(5)	C(7)-C(8)-C(14)	109.9(3)	C(13)-C(12)-H(12B)	109.1
C(20)-H(20A)	0.9600	C(9)-C(8)-C(14)	112.8(3)	H(12A)-C(12)-H(12B)	107.8
C(20)-H(20B)	0.9600	C(7)-C(8)-H(8)	108.3	C(5)-C(6)-C(7)	125.7(3)
C(20)-H(20C)	0.9600	C(9)-C(8)-H(8)	108.3	C(5)-C(6)-H(6)	118(2)
C(3)-C(4)	1.501(5)	C(14)-C(8)-H(8)	108.3	C(7)-C(6)-H(6)	116(2)
C(3)-C(2)	1.517(5)	C(12)-C(13)-C(18)	110.8(3)	N(1)-C(16)-C(15)	178.4(5)
C(3)-H(3)	0.9800	C(12)-C(13)-C(17)	110.5(3)	O(3)-C(17)-C(20)	106.6(3)
O(2)-C(22)	1.205(5)	C(18)-C(13)-C(17)	108.6(3)	O(3)-C(17)-C(13)	108.2(3)
C(7)-C(6)	1.493(5)	C(12)-C(13)-C(14)	107.0(3)	C(20)-C(17)-C(13)	116.2(3)
C(7)-H(7A)	0.9700	C(18)-C(13)-C(14)	111.1(3)	O(3)-C(17)-H(17)	108.6
C(7)-H(7B)	0.9700	C(17)-C(13)-C(14)	108.9(3)	C(20)-C(17)-H(17)	108.6
C(12)-H(12A)	0.9700	C(12)-C(11)-C(9)	111.6(3)	C(13)-C(17)-H(17)	108.6
C(12)-H(12B)	0.9700	C(12)-C(11)-H(11A)	109.3	C(3)-C(4)-C(5)	112.7(3)
C(6)-H(6)	0.89(3)	C(9)-C(11)-H(11A)	109.3	C(3)-C(4)-H(4A)	109.0
C(16)-N(1)	1.132(5)	C(12)-C(11)-H(11B)	109.3	C(5)-C(4)-H(4A)	109.0
C(16)-C(15)	1.456(6)	C(9)-C(11)-H(11B)	109.3	C(3)-C(4)-H(4B)	109.0
C(17)-H(17)	0.9800	H(11A)-C(11)-H(11B)	108.0	C(5)-C(4)-H(4B)	109.0
C(4)-H(4A)	0.9700	C(8)-C(14)-C(15)	110.6(3)	H(4A)-C(4)-H(4B)	107.8
C(4)-H(4B)	0.9700	C(8)-C(14)-C(13)	114.3(2)	C(13)-C(18)-H(18A)	109.5
C(18)-H(18A)	0.9600	C(15)-C(14)-C(13)	114.3(3)	C(13)-C(18)-H(18B)	109.5
C(18)-H(18B)	0.9600	C(8)-C(14)-H(14)	105.6	H(18A)-C(18)-H(18B)	109.5
C(18)-H(18C)	0.9600	C(15)-C(14)-H(14)	105.6	C(13)-C(18)-H(18C)	109.5
C(15)-H(15A)	0.9700	C(13)-C(14)-H(14)	105.6	H(18A)-C(18)-H(18C)	109.5
C(15)-H(15B)	0.9700	C(5)-C(10)-C(19)	109.2(3)	H(18B)-C(18)-H(18C)	109.5
C(1)-C(2)	1.525(5)	C(5)-C(10)-C(1)	107.5(3)	C(16)-C(15)-C(14)	114.8(3)
C(1)-H(1A)	0.9700	C(19)-C(10)-C(1)	109.7(3)	C(16)-C(15)-H(15A)	108.6

Table S8. continuation

C(14)-C(15)-H(15A)	108.6	C(3)-C(2)-H(2B)	109.5	C(22)-C(23)-H(23B)	109.5
C(16)-C(15)-H(15B)	108.6	C(1)-C(2)-H(2B)	109.5	H(23A)-C(23)-H(23B)	109.5
C(14)-C(15)-H(15B)	108.6	H(2A)-C(2)-H(2B)	108.1	C(22)-C(23)-H(23C)	109.5
H(15A)-C(15)-H(15B)	107.5	S(1)-C(21)-H(21A)	109.5	H(23A)-C(23)-H(23C)	109.5
C(2)-C(1)-C(10)	114.0(3)	S(1)-C(21)-H(21B)	109.5	H(23B)-C(23)-H(23C)	109.5
C(2)-C(1)-H(1A)	108.7	H(21A)-C(21)-H(21B)	109.5	C(10)-C(19)-H(19A)	109.5
C(10)-C(1)-H(1A)	108.7	S(1)-C(21)-H(21C)	109.5	C(10)-C(19)-H(19B)	109.5
C(2)-C(1)-H(1B)	108.7	H(21A)-C(21)-H(21C)	109.5	H(19A)-C(19)-H(19B)	109.5
C(10)-C(1)-H(1B)	108.7	H(21B)-C(21)-H(21C)	109.5	C(10)-C(19)-H(19C)	109.5
H(1A)-C(1)-H(1B)	107.6	O(2)-C(22)-O(1)	123.2(4)	H(19A)-C(19)-H(19C)	109.5
C(3)-C(2)-C(1)	110.9(3)	O(2)-C(22)-C(23)	125.3(4)	H(19B)-C(19)-H(19C)	109.5
C(3)-C(2)-H(2A)	109.5	O(1)-C(22)-C(23)	111.5(3)		
C(1)-C(2)-H(2A)	109.5	C(22)-C(23)-H(23A)	109.5		

Table S9. Anisotropic displacement parameters ($Å^2 \times 10^3$) for compound **6**. The anisotropic displacement factor exponent takes the form: $2\pi\ 2[h^2\ a^{*2}\ U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	53(1)	50(1)	54(1)	–8(1)	11(1)	9(1)
O(3)	48(2)	48(1)	45(2)	–1(1)	5(1)	9(1)
O(1)	52(2)	53(2)	61(2)	11(1)	16(2)	–4(1)
O(5)	91(3)	54(2)	88(2)	7(2)	16(2)	30(2)
C(9)	35(2)	27(2)	41(2)	4(2)	–6(2)	–2(2)
C(8)	36(2)	27(2)	39(2)	–1(2)	–3(2)	–5(2)
O(4)	68(2)	97(2)	52(2)	–19(2)	5(2)	8(2)
C(13)	36(2)	34(2)	41(2)	1(2)	0(2)	1(2)
C(11)	40(2)	44(2)	54(2)	19(2)	3(2)	5(2)
C(14)	42(2)	27(2)	32(2)	1(2)	–3(2)	–3(2)
C(10)	36(2)	33(2)	43(2)	11(2)	2(2)	–4(2)
C(5)	34(2)	42(2)	38(2)	4(2)	1(2)	2(2)
C(20)	44(2)	43(2)	54(2)	–5(2)	11(2)	–4(2)
C(3)	49(2)	47(2)	51(2)	5(2)	12(2)	–9(2)
O(2)	97(3)	56(2)	118(3)	23(2)	50(2)	19(2)
C(7)	49(2)	37(2)	36(2)	6(2)	1(2)	6(2)
C(12)	37(2)	48(2)	51(2)	14(2)	–1(2)	7(2)
C(6)	42(2)	39(2)	44(2)	7(2)	0(2)	15(2)
C(16)	60(3)	60(3)	31(2)	–6(2)	–6(2)	10(2)
C(17)	41(2)	36(2)	44(2)	2(2)	0(2)	6(2)
C(4)	45(2)	55(2)	50(2)	6(2)	5(2)	–1(2)
C(18)	48(2)	37(2)	73(3)	–9(2)	1(2)	3(2)
C(15)	47(2)	44(2)	43(2)	6(2)	5(2)	7(2)
C(1)	42(2)	62(3)	48(2)	20(2)	–1(2)	3(2)
C(2)	51(3)	73(3)	45(2)	17(2)	7(2)	–2(2)
C(21)	72(3)	72(3)	75(3)	–7(3)	31(3)	–3(3)
C(22)	72(3)	52(3)	58(3)	5(2)	17(2)	4(2)
C(23)	56(3)	82(3)	83(3)	27(3)	21(2)	7(3)
C(19)	57(3)	38(2)	77(3)	2(2)	9(2)	–5(2)
N(1)	109(4)	78(3)	61(3)	–13(2)	–26(2)	19(3)

Table S10. Torsion angles (degree) for compound **6**

O(4)-S(1)-O(3)-C(17)	−38.6(3)	C(8)-C(9)-C(10)-C(19)	75.3(3)	C(12)-C(13)-C(17)-O(3)	62.3(4)
O(5)-S(1)-O(3)-C(17)	−167.4(3)	C(11)-C(9)-C(10)-C(1)	68.7(4)	C(18)-C(13)-C(17)-O(3)	–59.4(4)
C(21)-S(1)-O(3)-C(17)	77.8(3)	C(8)-C(9)-C(10)-C(1)	−163.6(3)	C(14)-C(13)-C(17)-O(3)	179.4(3)
C(11)-C(9)-C(8)-C(7)	−170.7(3)	C(19)-C(10)-C(5)-C(6)	−109.2(4)	C(12)-C(13)-C(17)-C(20)	–57.5(4)
C(10)-C(9)-C(8)-C(7)	60.9(3)	C(1)-C(10)-C(5)-C(6)	131.8(4)	C(18)-C(13)-C(17)-C(20)	−179.2(3)
C(11)-C(9)-C(8)-C(14)	–48.2(4)	C(9)-C(10)-C(5)-C(6)	13.5(5)	C(14)-C(13)-C(17)-C(20)	59.7(4)
C(10)-C(9)-C(8)-C(14)	−176.6(3)	C(19)-C(10)-C(5)-C(4)	69.7(4)	O(1)-C(3)-C(4)-C(5)	−169.0(3)
C(8)-C(9)-C(11)-C(12)	52.8(4)	C(1)-C(10)-C(5)-C(4)	–49.3(4)	C(2)-C(3)-C(4)-C(5)	–52.1(4)
C(10)-C(9)-C(11)-C(12)	−179.2(3)	C(9)-C(10)-C(5)-C(4)	−167.6(3)	C(6)-C(5)-C(4)-C(3)	−129.5(4)
C(7)-C(8)-C(14)-C(15)	–56.8(4)	C(22)-O(1)-C(3)-C(4)	–70.1(5)	C(10)-C(5)-C(4)-C(3)	51.6(4)
C(9)-C(8)-C(14)-C(15)	−178.8(3)	C(22)-O(1)-C(3)-C(2)	169.7(3)	N(1)-C(16)-C(15)-C(14)	63(16)
C(7)-C(8)-C(14)-C(13)	172.5(3)	C(9)-C(8)-C(7)-C(6)	–41.6(4)	C(8)-C(14)-C(15)-C(16)	–46.8(4)
C(9)-C(8)-C(14)-C(13)	50.4(4)	C(14)-C(8)-C(7)-C(6)	−165.8(3)	C(13)-C(14)-C(15)-C(16)	83.9(4)
C(12)-C(13)-C(14)-C(8)	–53.8(4)	C(9)-C(11)-C(12)-C(13)	–60.2(4)	C(5)-C(10)-C(1)-C(2)	52.0(4)
C(18)-C(13)-C(14)-C(8)	67.2(4)	C(18)-C(13)-C(12)-C(11)	–62.8(4)	C(19)-C(10)-C(1)-C(2)	–66.6(4)
C(17)-C(13)-C(14)-C(8)	−173.2(3)	C(17)-C(13)-C(12)-C(11)	176.8(3)	C(9)-C(10)-C(1)-C(2)	171.4(3)
C(12)-C(13)-C(14)-C(15)	177.2(3)	C(14)-C(13)-C(12)-C(11)	58.4(4)	O(1)-C(3)-C(2)-C(1)	175.6(3)
C(18)-C(13)-C(14)-C(15)	–61.7(4)	C(4)-C(5)-C(6)-C(7)	−174.5(4)	C(4)-C(3)-C(2)-C(1)	55.1(4)
C(17)-C(13)-C(14)-C(15)	57.9(4)	C(10)-C(5)-C(6)-C(7)	4.3(6)	C(10)-C(1)-C(2)-C(3)	–57.2(4)
C(11)-C(9)-C(10)-C(5)	−173.7(3)	C(8)-C(7)-C(6)-C(5)	10.5(5)	C(3)-O(1)-C(22)-O(2)	–2.3(6)
C(8)-C(9)-C(10)-C(5)	–46.1(4)	S(1)-O(3)-C(17)-C(20)	−115.6(3)	C(3)-O(1)-C(22)-C(23)	175.6(3)
C(11)-C(9)-C(10)-C(19)	–52.3(4)	S(1)-O(3)-C(17)-C(13)	118.8(3)		

Table S11. Selected bond lengths and bond angles for compounds **5** and **6**

Bond	Compound **5**	Compound **6**	Angle / degree	Compound **5**	Compound **6**
	Bond length / Å			Bond angle / degree	
S1-O4	1.406 (3)	1.409 (3)	O4-S1-O5	118.1 (2)	118.3 (2)
S1-O5	1.422 (3)	1.415 (3)	O4-S1-O3	106.82 (19)	110.11 (16)
S1-O3	1.561 (3)	1.559 (3)	O5-S1-O3	109.09 (16)	105.52 (17)
S1-C21	1.743 (4)	1.744 (4)	O4-S1-C21	107.8 (2)	108.4 (2)
O3-C17	1.473 (4)	1.486 (4)	O5-S1-C21	110.1 (2)	108.8 (2)
C13-C18	1.535 (5)	1.538 (5)	O3-S1-C21	103.96 (19)	104.88 (18)
C13-C17	1.538 (5)	1.542 (5)	C17-O3-S1	122.3 (2)	119.6 (2)
C17-C20	1.519 (5)	1.516 (5)	O3-C17-C20	107.9 (3)	106.6 (3)
C13-C12	1.530 (4)	1.530 (5)	C12-C13-C17	109.8 (3)	110.5 (3)
C13-C18	1.535 (5)	1.538 (5)	C18-C13-C17	106.9 (3)	108.6 (3)
C13-C14	1.556 (4)	1.556 (5)	C17-C13-C14	110.7 (3)	108.9 (3)

Table S12. Selected torsion angles for compounds **5** and **6**

	Compound **5**	Compound **6**
	Torsion angle / degree	
O4-S1-O3-C17	140.7 (3)	38.6 (3)
O5-S1-O3-C17	12.0 (3)	167.4 (3)
C21-S1-O3-C17	105.4 (3)	77.8 (3)
S1-O3-C17-C13	137.2 (2)	118.8 (3)
S1-O3-C17-C20	97.0 (3)	115.6 (3)
C12-C13-C17-C20	56.4 (4)	57.5 (4)
C18-C13-C17-C20	63.7 (4)	179.2 (3)
C14-C13-C17-C20	175.3 (3)	59.7 (4)
C12-C13-C17-O3	64.4 (3)	62.3 (4)
C18-C13-C17-O3	175.6 (3)	59.4 (4)
C14-C13-C17-O3	54.5 (3)	179.4 (3)
C8-C14-C15-C16	89.9 (4)	46.8 (4)
C13-C14-C15-C16	142.7 (3)	83.9 (4)
C14-C15-C16-N1	158 (14)	63 (16)