Design and Synthesis of Estrarubicin: a Novel Class of Estrogen-Anthracenedione Hybrids

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Received April 27, 1998

Keywords: Cycloadditions / Quinones / Hormones / Antitumor agents / Hybrid molecules

The synthesis of estrarubicin, a member of a new class of estrogen-anthraquinone hybrids, has been accomplished in an 8-step sequence starting from estrone. The octacyclic carbon skeleton has been elaborated by a Diels-Alder reaction using diene **5** and known epoxy-tetrone **6** as precursors. The cycloaddition reaction, performed in 5 M $LiClO_4$ in diethyl ether, revealed notable diastereofacial selectivity of the diene, leading mainly to cycloadduct **8**. Elaboration of the dihydroxyanthraquinone moiety and highly stereoselective epoxidation of the $\Delta^{17(20)}$ bond yielded estrarubicin **2**.

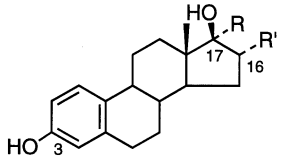
Introduction

The anthraquinone subunit is contained in a broad range of biologically important natural products[1]. In anthracycline-based anticancer agents[2] it exhibits strong antineoplastic activity due to its DNA strand cleavage ability[3]. As a consequence, great effort has been devoted both to the synthesis of the natural products themselves and to the development of new analogs[4].

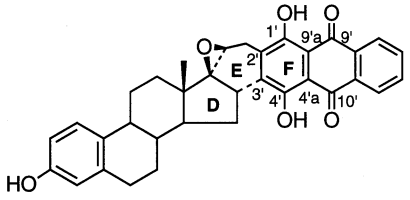
Recently, attempts have been made to increase the organ and/or tissue specificity of anticancer agents by linking them to various steroidal hormones[5]. In particular, the utilization of estrogens as vectors for cytotoxic agents has long been established[6]. These agents are designed and synthesized to facilitate the transport and accumulation of the cytotoxic moieties inside the cell via the estrogen receptor (ER), which is present in high concentration in breast cancer, prostatic carcinoma, ovarian adenocarcinoma, and endometrial carcinoma[5]. Recent examples of cytotoxic agents linked to estradiol include estramustine phosphate[7], a 17β-nitrosourea derivative[8], the estramycines[9], and the tricarbonyl(cyclopentadienylestradiol)rhenium[10].

Previous work has shown that the hormonal vehicle should have a high affinity to the ER. In order to obtain a good association of modified estrogens with the nuclear receptor, it is essential to preserve the C-3 phenolic group and the β-oriented C-17 oxygenated moiety. Both these moieties are present in estradiol (**1**) and are intimately involved in the recognition process[11]. However, modifications can be made to the 17α[10] (−R) and 16α[12] (−R′) positions with maintenance of a high level of molecular recognition.

In this paper we report the synthesis of hybrid **2**, named estrarubicin, the first member of a new family of artificial estrogens. In compound **2** the antitumor pharmacophore dihydroxyanthracenedione has been stereoselectively linked at the 16α,17α positions to the steroidal D ring.



1, R = R' = H

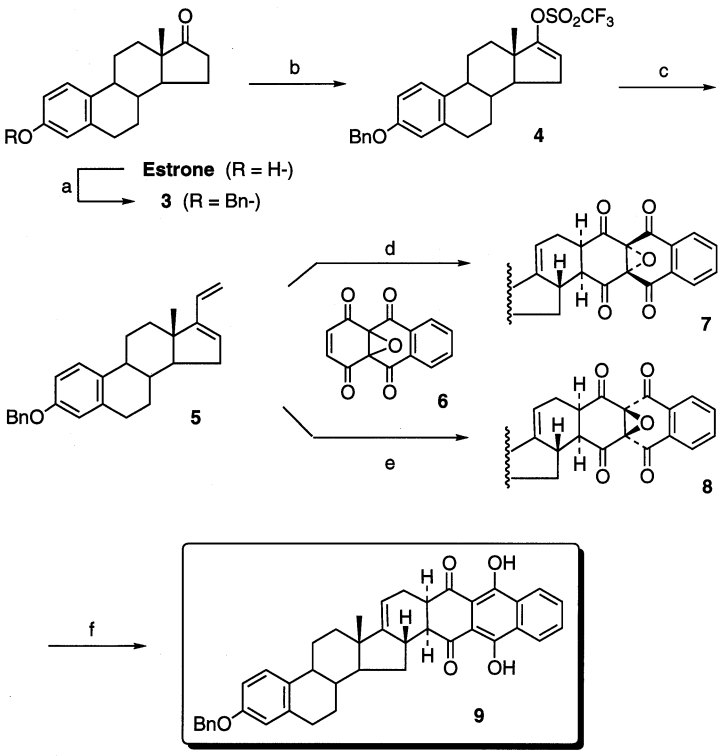


2

The synthetic strategy envisioned for connecting the two molecular portions took advantage of our recently developed route to cytotoxic steroid-anthraquinone hybrids[13]. We have extended the versatility of this strategy, maintaining a high level of stereoselectivity in the Diels-Alder cycloaddition step.

  1434−193X/98/0909−1965 $ 17.50+.50/0

Scheme 1. Reagents and conditions: (a) 3.0 equiv. of K_2CO_3, 3.0 equiv. of BnBr, CH_2Cl_2/MeOH (2:1), reflux, 12 h, 80%; (b) 2.0 equiv. of $LiN[Si(CH_3)]_2$, 1.6 equiv. of $PhN(SO_2CF_3)_2$, THF, 2 h, −78 °C to 25 °C, 95%; (c) 4.5 equiv. of LiCl, 0.03 equiv. of $Pd(PPh_3)_4$, 1.1 equiv. of $Bu_3SnCH{=}CH_2$, THF, reflux, 2 h, 79%; (d) 1.1 equiv. of **6**, toluene, 60 °C to reflux, 3 h, 29−42%; (e) 1.1 equiv. of **6**, 5 M $LiClO_4$ in Et_2O/CH_2Cl_2 (2:1), 25 °C, 18 h; (f) 10 equiv. of Zn, CH_3COOH, 18 h

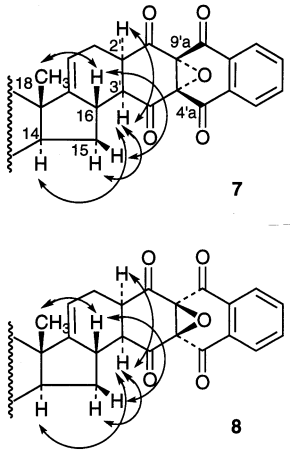


Results and Discussion

The first steps toward the synthesis of compound **2** are depicted in Scheme 1. Estrone (**3**) was benzylated and transformed into the 17-enol trifluoromethanesulfonate **4** with lithium bis(trimethylsilyl)amide and *N*-phenyltrifluoromethanesulfonimide, according to the McMurry procedure[14]. Enol triflate **4** was then treated with tributyl(vinyl)tin in the presence of lithium chloride and tetrakis(triphenylphosphanyl)palladium(0)[15] to give **5** in 79% yield. With diene **5** in our hands we were ready for the Diels-Alder reaction with the easily prepared dienophile 4a,9a-epoxy-4a,9a-dihydroanthracene-1,4,9,10-tetrone (**6**)[16], which is a versatile synthon in anthracycline synthesis. The oxirane **6** was thus treated with compound **5** in toluene. The reaction was performed at different temperatures (60 °C to reflux) and concentrations of the reactants (0.03−0.15 M). When the reactions were complete (TLC analysis) a white precipitate was isolated from the complex mixture of stereoisomers cooled at 0 °C (**7**, 29−42% yield, m. p. 225−228 °C).

Full ^{1}H- and ^{13}C-NMR chemical shift assignments of the octacyclic derivative **7** were carried out using COSY-DQF[17] and HSQC[18] experiments. The stereochemistry at the C-16, C-2′, and C-3′ stereogenic centers was deduced on the basis of the connectivities observed in NOE and ROESY[19] experiments (Figure 1). ROESY crosspeaks between the signals at δ = 0.85 (18-CH_3), 2.66 (16-H), and 1.95 (15β-H) defined (*S*) stereochemistry at C-16. Strong correlations were also detected between the signals at δ = 3.41 (2′-H), 3.02 (3′-H), 1.63 (15α-H), and 1.25 (14-H), suggesting an *anti-cis* D−E−F ring junction (2′*R*,3′*S*).

Figure 1. Selected ROESY interactions for adducts **7** and **8**



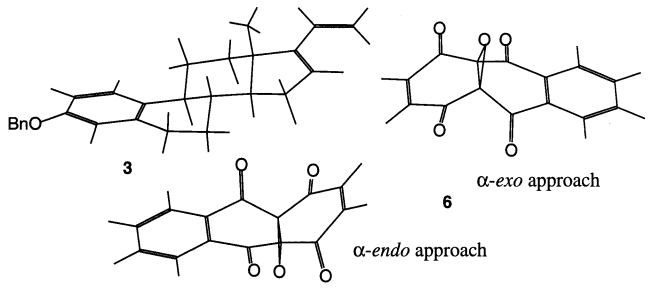
In order to improve the stereoselectivity of the cycloaddition we performed the Diels-Alder reaction in a non-conventional way using 5 M lithium perchlorate in diethyl ether[20]. Under these conditions the reaction proceeded smoothly and gave, in almost quantitative yield, adduct **8** which appeared to be different from **7** (^{1}H-NMR analysis). COSY-DQF[17], DEPT, HSQC[18] experiments allowed all of the ^{1}H- and ^{13}C-NMR resonances of **8** to be assigned. The stereochemistry of the D−E−F ring junctions was

then clarified by an ROESY[19] spectrum (Figure 1). This showed correlations between the signals at δ = 0.84 (18-CH_3), 2.83 (16-H), and 1.68 (15β-H) and the signals at δ = 3.10 (2′-H), 2.46 (3′-H), 1.88 (15α-H), and 1.23 (14-H) suggesting that this adduct has also *anti-cis* D−E−F ring junctions like **7**.

Therefore the only difference between **7** and **8** should reside in the stereochemistry of the epoxide ring. Accordingly, the reductive deoxygenation of the oxirane group of both **7** and **8**, with zinc in acetic acid, produced the same compound **9** (Scheme 1). The comparison of the chemical shift values of C-2′ and C-3′ protons in the ^{1}H-NMR spectra of **7** and **8** allowed a tentative assignment of the relative stereochemistry of the oxirane ring in the two compounds. In fact the higher chemical shift values of the C-2′ and C-3′ protons in compound **7** (δ = 3.41 and 3.02)[21] was attributed to a *syn* deshielding effect of the epoxide oxygen atom on these protons. In adduct **8** the same protons resonate at higher field (δ = 3.10 and 2.46).

According to this evidence adducts **7** and **8** are formed through the least hindered *exo* transition state[22], which is usually not favoured in Diels-Alder reactions, the dienophile approaching from the sterically favourable α side of the steroid skeleton[22] (Figure 2). The only difference between the two α-*exo* transition states lies in the spatial position of the oxirane rings.

Figure 2. Possible approaches of the dienophile from the steroidal α side



Scheme 2. Reagents and conditions: (a) 1.2 equiv. of $Pb(OAc)_4$, CH_3COOH, 25°C, 3 h; (b) 0.40 equiv. of Et_3N, 25°C, 6 h, 30−32% from **5**, four steps; (c) 2 equiv. of *m*-CPBA, CH_2Cl_2, 0°C, 3 h, 60%; (d) Pd/C, 5 h, 52%

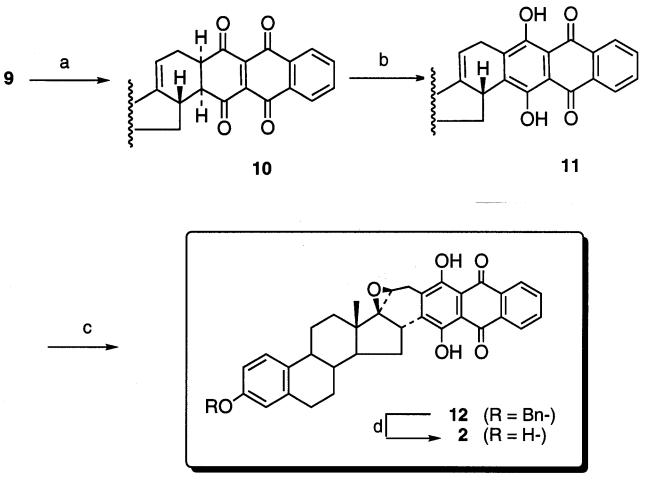
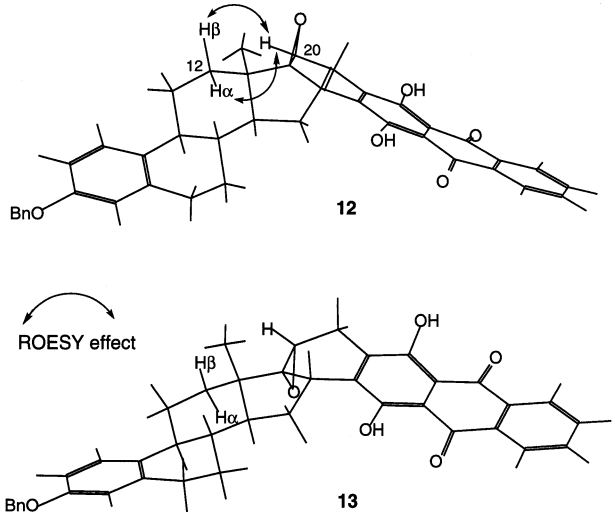


Figure 3. Most stable conformation of **12** and **13** and selected ROESY contacts for **12**



Treatment of **9** with lead tetraacetate in acetic acid (Scheme 2) furnished the tetrone **10**[23], which in the presence of triethylamine tautomerized into the dihydroxyanthracenedione derivative **11**[16].

Furthermore, stereoselective *m*-choroperbenzoic acid oxidation of the $\Delta^{17(20)}$ bond introduced the epoxide functionality.

The stereochemical assignment of **12** was achieved through the unambiguous rationalization of ^{1}H- and ^{13}C-NMR resonances using a combination of COSY-DQF[17], HSQC[18], HMBC[24], and ROESY[19] techniques. In particular the ROESY spectrum showed two key crosspeaks: a very strong correlation, between the signals at δ = 3.70 (20-H) and 1.42 (12α-H_{ax}) and a weaker correlation, between the signals at δ = 3.70 (20-H) and 1.73 (12β-H_{eq}). This means that the position of the 20-H and 12α-H are on the same side, supporting a β stereochemistry for the epoxide ring (20*S*). This conclusion was also corroborated by molecular modeling of the two possible epoxides **12** and **13** (Figure 3).

Conformational minimization, using the MM2 force field[25], showed that in the predicted most stable conformation of **12** the distance between 20-H and 12α-H is 2.36 Å, while the distance between 20-H and 12β-H is 2.64 Å. In the predicted most stable conformation for **13** the distance between 20-H and 12β-H is 2.73, while the distance between 20-H and 12α-H is 3.28 Å.

Finally, oxirane **12** was debenzylated with Pd/C to give target compound **2** (52% yield).

In summary, we have described a stereoselective synthesis of a potentially useful anthraquinone-estrogen hybrid. The availability of the starting materials and the convergent approach stategy provide a simple access to the preparation of other members of this new class of compounds.

The results of the biological testing with **2** will be reported in due course.

This work has been supported by *MURST* and *CNR* (Rome, Italy). The mass spectra were obtained from the *Servizio di Spettrometria di Massa del CNR e dell' Università di Napoli*; the staff is gratefully acknowledged.

Experimental Section

General Techniques: All reactions were carried out under a dry argon using freshly distilled solvents unless otherwise noted. Tetrahydrofuran was distilled from sodium and benzophenone. Toluene and dichloromethane were distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) before use. When necessary, compounds were dried by azeotropic removal of water with toluene under reduced pressure. Commercial reagents were purchased from Aldrich and used without further purification. − Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel plates (0.25 mm) and visualized using UV light, spraying with H_2SO_4/$Ce(SO_4)_2$ solution and drying. Reaction temperatures were measured externally. Flash chromatography was performed on Merck silica gel (60, particle size: 0.040−0.063 mm). Yields refer to chromatographically and spectroscopically (^{1}H NMR) pure materials. − NMR spectra were recorded in $CDCl_3$ solutions with Bruker AM-250, DRX 400, and DRX 600 spectrometers at ambient temperature. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ_H = 7.26; $^{13}CDCl_3$: δ_C = 77.0). − Optical rotations were recorded in $CHCl_3$ solutions with a JASCO DIP-1000 polarimeter. − Mass spectra (E.I., 70 eV) were recorded with a VG TRIO 2000 mass spectrometer. High-resolution mass spectra were recorded with a VG 70-250SE (70 eV, source temp. 200°C, resolution 5000 m/Δm, 10% valley). − UV/Vis spectra were recorded in dioxane with a Beckman DU 600 spectrometer. − Melting points were recorded with a digital Electrothermal 9100.

Benzyl Ether **3**: To a solution of estrone (5.0 g, 18.5 mmol) in CH_2Cl_2/MeOH (39 ml; 2:1 v/v ratio) at room temp., K_2CO_3 (7.67 g, 55.5 mmol) and benzyl bromide (6.60 ml, 55.5 mmol) were added. The resulting mixture was refluxed overnight. Filtration and removal of the solvent in vacuo afforded a residue which was crystallized ($CHCl_3$/MeOH) to give **3** (5.29 g, 80% yield) as a white solid, m.p.: 130−131°C; R_f = 0.56 (silica gel, 20% ethyl acetate in petroleum ether); $[\alpha]_D$ = +112.4 (c = 2.0, $CHCl_3$). − ^{1}H NMR (250 MHz): δ = 0.93 (3 H, s, 18-CH_3), 2.91 (2 H, m, 6-H), 5.05 (2 H, s, $C_6H_5CH_2$), 6.76 (1 H, d, J = 3.0 Hz, 4-H), 6.81 (1 H, dd, J = 8.5, 3.0 Hz, 2-H), 7.23 (1 H, d, J = 8.5 Hz, 1-H), 7.33−7.46 (5 H, m, C_6H_5). − ^{13}C NMR (62.5 MHz): δ = 13.8, 21.5, 25.8, 26.4, 29.6, 31.5, 35.8, 38.2, 43.9, 47.9, 50.3, 69.8, 112.3, 114.8, 126.3, 127.4 (× 2), 127.8, 128.5 (× 2), 132.2, 137.1, 137.7, 156.8, 211.9. − EI MS; m/z: 360 [M^+].

Vinyl Triflate **4**: To a solution of lithium bis(trimethylsilyl)amide (29.4 ml, 1.0 M in THF, 29.4 mmol) at −78°C, **3** (5.29 g, 14.7 mmol) in dry THF (50.0 ml) was added. After 1 h, *N*-phenyltrifluoromethanesulfonimide (8.40 g, 23.5 mmol) was added to the resulting enolate. After 20 min, the reaction mixture was slowly warmed to room temp. and stirred for 1 h. The reaction was quenched with water, the mixture concentrated in vacuo to remove the excess of THF, and extracted with diethyl ether. The organic layer was washed with a saturated solution of NH_4Cl, then with brine and finally dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was crystallized ($CHCl_3$/MeOH) to give **4** (6.87 g, 95% yield) as a white solid, m.p. 74−75°C. − R_f = 0.66 (silica gel, 10% ethyl acetate in petroleum ether); $[\alpha]_D$ = +52.6 (c = 2.5, $CHCl_3$). − ^{1}H NMR (250 MHz): δ = 1.03 (3 H, s, 18-CH_3), 2.91 (2 H, m, 6-H), 5.06 (2 H, s, $C_6H_5CH_2$), 5.64 (1 H, dd, J = 1.5, 0.8 Hz, 16-H6), 6.76 (1 H, d, J = 3.0 Hz, 4-H), 6.81 (1 H, dd, J = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, J = 8.5 Hz, 1-H), 7.26−7.45 (5 H, m, C_6H_5). − ^{13}C NMR (62.5 MHz): δ = 15.3, 25.8, 26.7, 28.3, 29.4, 32.7, 36.6, 44.2, 45.0, 53.5, 70.0, 112.3, 114.5, 114.9, 126.0, 127.4 (× 2), 127.9, 128.5 (× 2), 132.4, 137.2, 137.7, 156.8, 159.3. − EI MS; m/z: 492 [M^+].

Diene **5**: To a solution of **4** (2.63 g, 5.34 mmol) in dry THF (10 ml), $Pd(PPh_3)_4$ (0.185 g, 0.16 mmol), LiCl (1.020 g, 24.0 mmol), and tributyl(vinyl)tin (1.86 g, 5.88 mmol) were added. The reaction mixture was stirred at reflux for 2 h and then quenched with water (10 ml), concentrated in vacuo to remove the excess of THF and extracted with diethyl ether. The organic phase was washed with a 10% aqueous solution of NH_4OH, brine and then dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was flash-chromatographed (silica gel, 15% ethyl acetate in petroleum ether) to give **5** (1.56 g, 79% yield) as a white solid, m.p. 89−91°C; R_f = 0.78 (silica gel, 10% ethyl acetate in petroleum ether); $[\alpha]_D$= +23.1 (c = 1.6, $CHCl_3$). − ^{1}H NMR (250 MHz): δ = 0.95 (3 H, s, 18-CH_3), 2.91 (2 H, m, 6-H), 5.01 (1 H, d, J = 11.3 Hz, 21-H), 5.05 (2 H, s, $C_6H_5CH_2$), 5.38 (1 H, d, J = 17.9 Hz, H′-21), 5.77 (1 H, br. s, 16-H), 6.35 (1 H, dd, J = 17.9, 11.3 Hz, 20-H), 6.75 (1 H, d, J = 3.0 Hz, 4-H), 6.81 (1 H, dd, J = 8.5, 3.0 Hz, 2-H), 7.22 (1 H, d, J = 8.5 Hz, 1-H), 7.33−7.47 (5 H, m, C_6H_5). − ^{13}C NMR (62.5 MHz): δ = 16.0, 26.6, 27.7, 29.7, 31.0, 35.4, 37.1, 44.2, 46.4, 56.5, 69.9, 112.2, 112.9, 114.8, 126.0, 127.4 (× 2), 127.9, 128.5 (× 2), 129.3, 132.4, 133.2, 137.1, 138.0, 153.3, 156.9. − UV/Vis: λ_{max} (ε) = 237.2 (15400), 246 (sh, 9400), 256−280 (3300−3400), 286 (sh, 2900). − EI MS; m/z: 370 [M^+].

Diels-Alder Adducts **7** *and* **8**

Adduct **7**: Diene **5** (0.200 g, 0.540 mmol) and dienophile **6** (0.136 g, 0.540 mmol) were dissolved in anhydrous toluene and heated at different temperatures (60°C to reflux) until the reaction was complete (1−5 h, TLC analysis). Concentration under reduced pressure to a smaller volume gave a white suspension which was cooled at 0°C for 6 h and filtered to give adduct **7** (29−42% yield) as a white solid. The best yield of **7** (42%) was obtained refluxing **5** (0.200 g, 0.540 mmol) and **6** (0.136 g, 0.540 mmol) in toluene (20 ml) for 3 h; m.p. 225−228°C; R_f = 0.48 (silica gel, 25% ethyl acetate in petroleum ether); $[\alpha]_D$ = −3.3 (c = 0.50, $CHCl_3$). − ^{1}H NMR (600 MHz): δ = 0.85 (3 H, s, 18-CH_3), 1.25 (1 H, m, 14-H), 1.63 (1 H, m, 15α-H), 1.95 (1 H, m, 15β-H), 2.25 (1 H, m, 21β-H), 2.35 (2 H, m, 21α-H and 11-H), 2.66 (1 H, br. dd, J = 9.9, 9.9 Hz, 16-H), 2.86 (2 H, m, 6-H), 3.02 (1 H, dd, J = 11.2, 11.2 Hz, 3′-H), 3.41 (1 H, m, 2′-H), 5.03 (2 H, s, $C_6H_5CH_2$), 5.43 (1 H, m, 20-H), 6.72 (1 H, d, J = 3.0 Hz, 4-H), 6.81 (1 H, dd, J = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, J = 8.5 Hz, 1-H), 7.33−7.45 (5 H, m, C_6H_5), 7.83 (2 H, m, 6′-H and 7′-H), 8.15 (2 H, m, 5′-H and 8′-H). − ^{13}C NMR (150 MHz): δ = 18.2 (C-18), 22.0 (C-21), 26.3 (C-11), 27.5 (C-7), 29.8 (C-6), 30.7 (C-15), 33.9 (C-16), 35.3 (C-12), 38.2 (C-8), 43.8 (C-2′), 44.0 (C-13), 44.2 (C-9), 51.9 (C-14), 52.2 (C-3′), 63.1, 64.0 (C-4′a, C-9′a), 69.9 ($C_6H_5CH_2$), 110.4 (C-20), 112.3 (C-2), 114.8 (C-4), 126.3 (C-1), 127.4 (× 2, *Ph*CH_2), 127.8 (× 3, C-5′, C-8′, *Ph*CH_2), 128.5 (× 2, *Ph*CH_2), 131.0, 131.1 (C-8′a, C-10′a), 132.7 (C-10), 135.4 (× 2, C-6′ and C-7′), 137.3 (*Ph*CH_2), 138.0 (C-5), 154.1 (C-17), 156.7 (C-3), 182.3, 183.4 (C-9′, C-10′), 196.6, 197.5 (C-1′, C-4′). − UV/Vis: λ_{max} (ε) = 237 (25000), 259−282 (12600−10400), 300−321 (6500−5200). − EI MS; m/z: 624 [M^+].

Adduct **8**. Diene **5** (0.300 g, 0.810 mmol) and dienophile **6** (0.225 g, 0.892 mmol) were suspended in a mixture of 5 M $LiClO_4$ in diethyl ether/CH_2Cl_2 (2:1, 27 ml). After 16 h, the reaction was quenched with water and the mixture extracted with CH_2Cl_2. The lower phase was washed with brine and dried (Na_2SO_4). Concentration under reduced pressure gave adduct **8** as a brown-orange

oil (purity: 95%,^{1}H-NMR analysis); R_f = 0.37 (silica gel, 25% ethyl acetate in petroleum ether); $[\alpha]_D$ = +1.7 (*c* = 0.5, $CHCl_3$). − ^{1}H NMR (600 MHz): δ = 0.84 (3 H, s, C*H*$_3$-18), 1.23 (1 H, m, 14-H), 1.68 (1 H, m, 15β-H), 1.88 (1 H, dd, *J* = 11.1, 7.8 Hz, 15α-H), 2.36 (2 H, m, 11-H and 21-H), 2.46 (1 H, dd, *J* = 11.6, 8.4 Hz, 3′-H), 2.65 (1 H, br. dd, *J* = 16.7, 6.1 Hz, H′-21), 2.83 (3 H, m, H$_2$-6 and 16-H), 3.10 (1 H, dd, *J* = 8.4, 8.4 Hz, 2′-H), 5.03 (2 H, s, $C_6H_5CH_2$), 5.45 (1 H, m, 20-H), 6.72 (1 H, d, *J* = 3.0 Hz, 4-H), 6.78 (1 H, dd, *J* = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, *J* = 8.5 Hz, 1-H), 7.33−7.45 (5 H, m, C_6H_5), 7.77 (2 H, m, 6′-H and 7′-H), 8.01 (2 H, m, 5′-H and 8′-H). − ^{13}C NMR (150 MHz): δ = 18.2 (C-18), 23.9 (C-21), 26.2 (C-11), 27.4 (C-7), 28.2 (C-15), 29.7 (C-6), 35.3 (C-12), 36.3 (C-16), 38.2 (C-8), 44.2 (C-9), 44.5 (C-13), 47.3 (C-2′), 51.5 (C-14), 52.2 (C-3′), 62.4, 64.5 (C-4′a, C-9′a), 69.9 ($C_6H_5CH_2$), 110.8 (C-20), 112.3 (C-2), 114.8 (C-4), 126.2 (C-1), 127.4 (× 2, *Ph*CH_2), 127.6, 127.8 (C-5′, C-8′), 127.8 (*Ph*CH_2), 128.5 (× 2, *Ph*CH_2), 130.8, 131.1 (C-8′a, C-10′a), 132.7 (C-10), 135.3 (× 2, C-6′ and C-7′), 137.2 (*Ph*CH_2), 138.0 (C-5), 154.9 (C-17), 156.7 (C-3), 183.2, 184.0 (C-9′, C-10′), 196.0, 197.0 (C-1′, C-4′). − UV/Vis: λ_{max} (ε) = 236 (17200), 256−282 (6500−7600), 287 (sh, 5200). − EI MS; *m*/*z*: 624 [M$^+$].

Dione **9**: To a stirred solution of **7** or **8** (0.226 g, 0.363 mmol) in acetic acid (6 ml) at room temp., powdered zinc (0.237 g, 3.63 mmol) was added. After 18 h, the mixture was partitioned between $CHCl_3$ and water. The organic layer was washed several times with a diluted $NaHCO_3$ solution, dried (Na_2SO_4), and filtered. Concentration in vacuo gave **9** as a yellow oil; R_f = 0.75 (silica gel, 20% ethyl acetate in petroleum ether); $[\alpha]_D$ = +73.3 (*c* = 0.5, $CHCl_3$). − ^{1}H NMR (250 MHz): δ = 0.75 (3 H, s, 18-C*H*$_3$), 2.70 (2 H, br. s), 2.84 (2 H, m), 3.25 (1 H, br. d, *J* = 15.8 Hz), 3.46 (1 H, br. d, *J* = 6.8), 5.03 (2 H, s, $C_6H_5CH_2$), 5. 32 (1 H, br. s, 20-H), 6.70 (1 H, d, *J* = 3.0 Hz, 4-H), 6.76 (1 H, dd, *J* = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, *J* = 8.5 Hz, 1-H), 7.33−7.45 (5 H, m, C_6H_5), 7.77 (2 H, m, 6′-H and 7′-H), 8.48 (2 H, m, 5′-H and 8′-H), 13.44 (1 H, s, O*H*), 13.62 (1 H, s, O*H*). − ^{13}C NMR (62.5 MHz): δ = 17.2, 21.4, 26.2, 27.4, 27.5, 29.7, 35.0, 37.4, 38.6, 44.2, 44.5, 44.7, 51.5, 51.8, 69.9, 106.0, 107.0, 110.1, 112.3, 114.8, 124.7 (× 2), 127.4 (× 2), 127.8, 128.5 (× 2), 129.6 (× 2), 130.2 (× 2), 130.5, 132.8, 137.3, 138.0, 151.1, 154.5, 155.8, 156.7, 202.0, 204.3. − UV/Vis: λ_{max} (ε) = 238 (27400), 256 (23500), 278 (23300), 286 (21400), 339 (13100), 417 (12500). − EI MS; *m*/*z*: 610 [M$^+$].

Dione **11**: To a stirred solution of **9** (0.210 g, 0.345 mmol) in acetic acid (5 ml) at room temp., $Pb(OAc)_4$ (0.184 g, 0.414 mmol) was added. After 3 h, the mixture was partitioned between $CHCl_3$ and water. The organic layer was washed with a diluted $NaHCO_3$ solution, dried (Na_2SO_4) and filtered. Concentration in vacuo yielded **10** as brown solid (R_f = 0.46, silica gel, 20% ethyl acetate in petroleum ether); which was used in the next step without purification. To a stirred solution of **10** (1.0 g, 1.64 mmol) in $CHCl_3$ (15 ml) at room temp., triethylamine (0.09 ml, 0.66 mmol) was added. After 3 h, the mixture was partitioned between $CHCl_3$ and dilute hydrochloric acid. The organic layer was washed with water, dried (Na_2SO_4), filtered and concentrated in vacuo. The tautomerization also occurred spontaneously on smaller aliquots in chloroform solutions requiring prolonged times. The residue was flash-chromatographed (silica gel, 35% petroleum ether in toluene + 0.1% CH_3COOH) to give **11** as a brown solid (30−32% yield from **5**, four steps); R_f = 0.70 (silica gel, 20% ethyl acetate in petroleum ether). − ^{1}H NMR (250 MHz): δ = 1.03 (3 H, s, 18-C*H*$_3$), 2.19 (1 H, m, 15-H), 2.35 (1 H, m, H′-15), 2.84 (2 H, m, 6-H), 3.15 (1 H, br. dd, *J* = 22.6, 10.9 Hz, 21-H), 3.58 (1 H, br. dt, *J* = 22.6, 6.2 Hz, H′-21), 3.82 (1 H, m, 16-H), 5.03 (2 H, s, $C_6H_5CH_2$), 5.53 (1 H, br. s, 20-H), 6.72 (1 H, d, *J* = 3.0 Hz, 4-H), 6.81 (1 H, dd, *J* = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, *J* = 8.5 Hz, 1-H), 7.33−7.45 (5 H, m, C_6H_5), 7.77 (2 H, m, 6′-H and 7′-H), 8.35 (2 H, m, 5′-H and 8′-H), 13.59 (1 H, s, O*H*), 13.73 (1 H, s, O*H*). − UV/Vis: λ_{max} (ε) = 258 (70000), 401 (8800), 424 (11200), 487 (19800), 519 (13500). − EI MS; *m*/*z*: 608 [M$^+$].

Epoxide **12**: To a stirred solution of **11** (0.051 g, 0.084 mmol) in CH_2Cl_2 (0.6 ml) at 0°C, *m*-CPBA (0.029 g, 0.168 mmol) was added. After 3 h, the mixture was partitioned between CH_2Cl_2 and a Na_2SO_3 (5% w/v, aqueous solution). The organic layer was washed with water, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue (0.048 g) was flash-chromatographed (silica gel, 50−60% chloroform in petroleum ether) to give **12** as a red solid (60% yield); R_f = 0.44 (silica gel, 20% ethyl acetate in petroleum ether); $[\alpha]_D$ = −86.7 (*c* = 0.5, $CHCl_3$). − ^{1}H NMR (600 MHz): δ = 1.16 (3 H, s, 18-C*H*$_3$), 1.42 (1 H, ddd, *J* = 13.9, 13.9, 4.2 Hz, 12α-H), 1.56 (1 H, m, 14-H), 1.73 (1 H, br. d, *J* = 13.9 Hz, 12β-H), 1.91 (1 H, m, 15α-H), 2.48 (1 H, m, 15β-H), 2.84 (2 H, m, 6-H), 3.15 (1 H, dd, *J* = 21.9, 3.3 Hz, 21-H), 3.40 (1 H, dd, *J* = 21.9, 2.7 Hz, 21-H′), 3.70 (1 H, d, *J* = 3.3, 20-H), 3.82 (1 H, br. d, *J* = 11.3 Hz, 16-H), 5.03 (2 H, s, $C_6H_5CH_2$), 6.72 (1 H, d, *J* = 3.0 Hz, 4-H), 6.81 (1 H, dd, *J* = 8.5, 3.0 Hz, 2-H), 7.20 (1 H, d, *J* = 8.5 Hz, 1-H), 7.33−7.45 (5 H, m, C_6H_5), 7.80 (2 H, m, 6′-H and 7′-H), 8.37 (2 H, m, 5′-H and 8′-H), 13.54 (1 H, s, O*H*), 13.59 (1 H, s, O*H*). − ^{13}C NMR (125 MHz): δ = 14.1 (C-18), 24.9 (C-15), 25.7 (C-21), 26.8 (C-11), 29.7 (C-7), 31.3 (C-6), 32.8 (C-12), 35.0 (C-16), 38.9 (C-8), 40.4 (C-13), 43.9 (C-9), 49.7 (C-14), 52.5 (C-20), 69.9 ($C_6H_5CH_2$), 71.2 (C-17), 109.6, 110.1 (C-4′a, C-9′a), 112.3 (C-2), 114.8 (C-4), 126.2 (C-1), 126.8 (× 2, C-5′ and C-8′), 127.4 (× 2, *Ph*CH_2), 127.8 (*Ph*CH_2), 128.5 (× 2, *Ph*CH_2), 132.4 (C-10), 132.6 (C-2′), 133.5 (× 2, C-10′a and C-8′a), 134.2 (× 2, C-6′ and C-7′), 137.8 (C-5), 138.6 (C-3′), 156.7 (C-4′), 156.8 (C-3), 157.5 (C-1′), 186.3, 186.4 (C-9′, C-10′). − UV/Vis: λ_{max} (ε) = 255 (26600), 285 (9800), 401 (3200), 423 (4000), 484 (5100), 516 (3300). − EI MS; *m*/*z*: 624 [M$^+$].

Estrarubicin (**2**): To a solution of **12** (0.036 g, 0.058 mmol) in ethanol/dioxane (2:1) (1.5 ml) at room temp., Pd/C (0.018 g) was added. The flask was evacuated and flushed with hydrogen three times. The reaction mixture was then stirred vigorously. After 2 h, the palladium was filtered off and the mixture was concentrated in vacuo. The residue (0.036 g) was flash-chromatographed (silica gel, 80−90% chloroform in petroleum ether) to give **12** (0.010 g) and a red solid (0.012 g, 52% yield based on recovered starting material); R_f = 0.65 (silica gel, 35% ethyl acetate in petroleum ether); $[\alpha]_D$ = +179.8 (*c* = 0.3, $CHCl_3$). − ^{1}H NMR (600 MHz): δ = 1.15 (3 H, s, 18-C*H*$_3$), 1.91 (1 H, m, 15α-H), 2.48 (1 H, m, 15β′-H), 2.84 (2 H, m, 6-H), 3.15 (1 H, dd, *J* = 21.9, 3.3 Hz, 21-H), 3.40 (1 H, dd, *J* = 21.9, 2.7 Hz, 21′-H), 3.70 (1 H, d, *J* = 3.3, 20-H), 3.82 (1 H, br. d, *J* = 11.3 Hz, 16-H), 6.55 (1 H, d, *J* = 3.0 Hz, 4-H), 6.62 (1 H, dd, *J* = 8.5, 3.0 Hz, 2-H), 7.14 (1 H, d, *J* = 8.5 Hz, 1-H), 7.80 (2 H, m, 6′-H and 7′-H), 8.32 (2 H, m, 5′-H and 8′-H), 13.52 (1 H, s, O*H*), 13.62 (1 H, s, O*H*). − ^{13}C NMR (150 MHz): δ = 14.1 (C-18), 25.0 (C-15), 25.8 (C-21), 26.8 (C-11), 29.7 (C-7), 31.3 (C-6), 32.8 (C-12), 35.1 (C-16), 39.0 (C-8), 40.4 (C-13), 43.9 (C-9), 49.7 (C-14), 52.6 (C-20), 71.3 (C-17), 109.7, 110.1 (C-4′a, C-9′a), 112.8 (C-2), 115.3 (C-4), 126.4 (C-1), 126.9 (× 2, C-5′ and C-8′), 132.2 (C-10), 132.6 (C-2′), 133.6 (× 2, C-10′a and C-8′a), 134.2 (× 2, C-6′ and C-7′), 138.1 (C-5), 138.6 (C-3′), 153.5 (C-3), 156.8 (C-4′), 157.6 (C-1′), 186.4, 186.5 (C-9′, C-10′). − UV/Vis: λ_{max} (ε) = 256 (35000), 277 (sh, 1900), 400 (5400), 419 (6100), 484 (5500), 516 (3600). − HR EIMS; *m*/*z*: 534.2048 [M$^+$]; calcd. for $C_{34}H_{30}O_6$: 534.2042.

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