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Synthesis and Cytotoxic Activity of Tetracenomycin D and of Saintopin Analogues

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Abstract—Regiospecific synthesis of title compounds is based either on cycloaddition of ketene acetals derived from Hagemann's ester or of homophthalic anhydrides. Thus, tetracenomycin D and 3,8-di-*O*-methyl saintopin have been prepared in few steps. New derivatives of 10-deoxysaintopin have been also obtained. Evaluation of their cytotoxicity against L1210 leukemia cells are reported.

Introduction

A large number of naturally occuring polycyclic quinones are known, and some of them such as the anthracyclines are largely used in cancer chemotherapy.[1] In addition to the latter which are tetrahydro derivatives, fully aromatized naphthacenequinones have been obtained from anthracycline producing strains or from other organisms. Among these, saintopin **1**,[2] saintopin E **2**,[3] UCE 1022 **3**[4] and UCE 6 **4**,[5] isolated from Actinomycete fermentation broths, have been shown to be cytotoxic, **4** being equipotent to camptothecin against several cancer cell lines.[5] Saintopin is a dual inducer of topoisomerase I- and topisomerase II-mediated DNA cleavage[6] while UCE 6 and UCE 1022 are specific inducers of topoisomerase I. All these antibiotics seem to act by trapping the reversible topoisomerase I/DNA cleavable complex.[7] Interaction of saintopin with DNA has been studied by means of surface-enhanced Raman scattering spectroscopy[8] and it has been shown that only partial intercalation (A and B rings) occurs and that the peripheral ring D hydroxyl groups are buried in DNA. Taking into account the interesting pharmacological profile of the recently introduced camptothecin derivatives Irinotecan and Topotecan,[9] it may be of interest to search for other specific topo I inhibitors based on the tetracyclic core of saintopin and UCE-6. In this respect, it is worthy to note that the closely related tetracenomycins A_2 **5**, B_1 **6**, B_2 **7**, B_3 **8**, D (or D_1) **9** and D_3 **10** (Fig. 1) have been isolated from *Streptomyces glaucescens* TÜ 49[10] and *Streptomyces olivaceus* TÜ 2353[11] but no cytotoxic activity was reported for these compounds. To our knowledge, only one total synthesis of tetracenomycins A_2, B_1, B_2 and D has been described in 1992 by Cameron[12] based on two Diels–Alder cycloadditions and a regiospecific deoxygenation in eight steps from chloronaphthazarin.

We now describe a new synthesis of tetracenomycin D and preparation of saintopin analogues and a preliminary evaluation of their cytotoxic activity.

Results

Chemistry

The first approach to these tetracyclic targets was based on our previous synthesis of 11-deoxy anthracyclinones starting from Hagemann's ester **11**.[13] It was anticipated that easy aromatization of intermediate 9,10-dihydro tetracyclic derivatives should afford naphthacenequinones in few steps. Thus, the known ketene acetal **12**[13]

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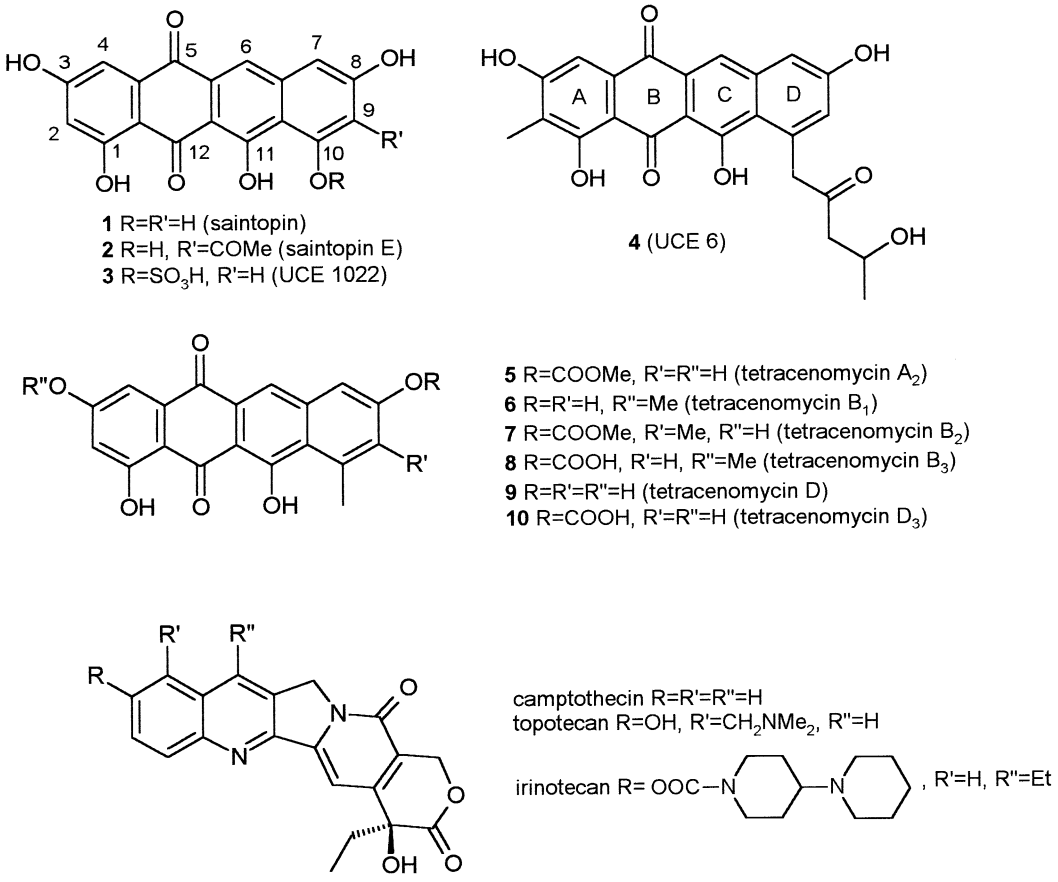


Figure 1.

was condensed with quinone **13** (prepared from 2,5-dichloro benzoquinone and 3-methoxy-1-ethoxy-1-trimethylsilyloxy-buta-1,3-diene)[14] to give a mixture of **14a** and of naphthacenequinone **15** (a trace of **14b** was also noticed). This mixture was treated with DDQ[15] to afford **15** which upon deprotection with BBr_3 gave 10-deoxy saintopin **16** (28%) as a dark red solid. The same reaction sequence was then applied to a synthesis of the corresponding 3-*O*-methyl derivative **20** (as in tetracenomycin B_1). Since selective deprotection of the ethoxy group failed,[16] replacement of the latter by a silyl ether protecting group was then considered. **17** was obtained by the method of Mander[17] (TBSOTf, Et_3N, 90%) from **11** without noticeable formation of the more stable endocyclic dienol silyl ether. After conversion to ketene acetal **18**, condensation with quinone **13** gave the fully aromatized tetracycle **19** (albeit isolated in a low 8% yield) together with a mixture of this material with the expected 9,10-dihydro derivative, which could not be aromatized to **19** upon treatment with DDQ or Pd/C. Fluoride induced desilylation of **19** afforded 3-*O*-methyl-10-deoxy saintopin **20**.

Taking into account potential functionalization of Hagemann's ester, the preparation of analogues bearing side chains at C-7 or C-10 was then investigated. First, a 3-hydroxypropyl side chain at C-7 was chosen by analogy with the UCE-6 side chain (which is indeed located at C-10). The sodium enolate of **11** (NaH, DMF) was treated with 1 equiv of the TPS ether of 3-bromo-1-propanol to give **21** in 29% isolated yield. As shown before, only formation of silyl enol ether **22** was detected (TBSOTf, Et_3N, CH_2Cl_2, 86%). **22** was then converted to **23** (LDA, THF, TMSCl, 71%) and condensed with **13** to give the fully aromatized derivative **24** (29%) and, after deprotection, **25** (91%) (Fig. 2).

Then, application of the above methodology to the synthesis of tetracenomycin D was attempted. The required 6-methyl derivative **26** was prepared as reported from acetaldehyde and ethyl acetoacetate.[18] After conversion to enol ether **27**, the sensitive ketene acetal **28** was prepared as usual (LDA-TMSCl) and condensed with **13**. There was obtained a disappointing low 13% yield of tetracycle **29** which could not be aromatized to 3,8-di-*O*-methyl tetracenomycin D **30** either in presence of Pd/C (diglyme at reflux) or DDQ.

The rather unexpected failure to aromatize **29** led us to use the base catalyzed condensation of a homophthalic anhydride with a quinone, a strategy first introduced by Tamura[19] for tetracyclic compounds synthesis. Enol ether **27** was converted to **31** (90%) upon heating with Pd/C in diglyme (trace amounts of 3,5-dimethyl anisole were also isolated). Homophthalic anhydride **32**[19] was then prepared in three steps: ethoxycarbonylation to **33**,[20] hydrolysis to **34** and dehydration (Fig. 3). Although the last two steps resulted in high yields, the first one turned out to be difficult. After an extensive study of reaction conditions, including the corresponding acid, **33** was obtained in 30% yield (42% based on recovered starting material). Upon deprotonation with

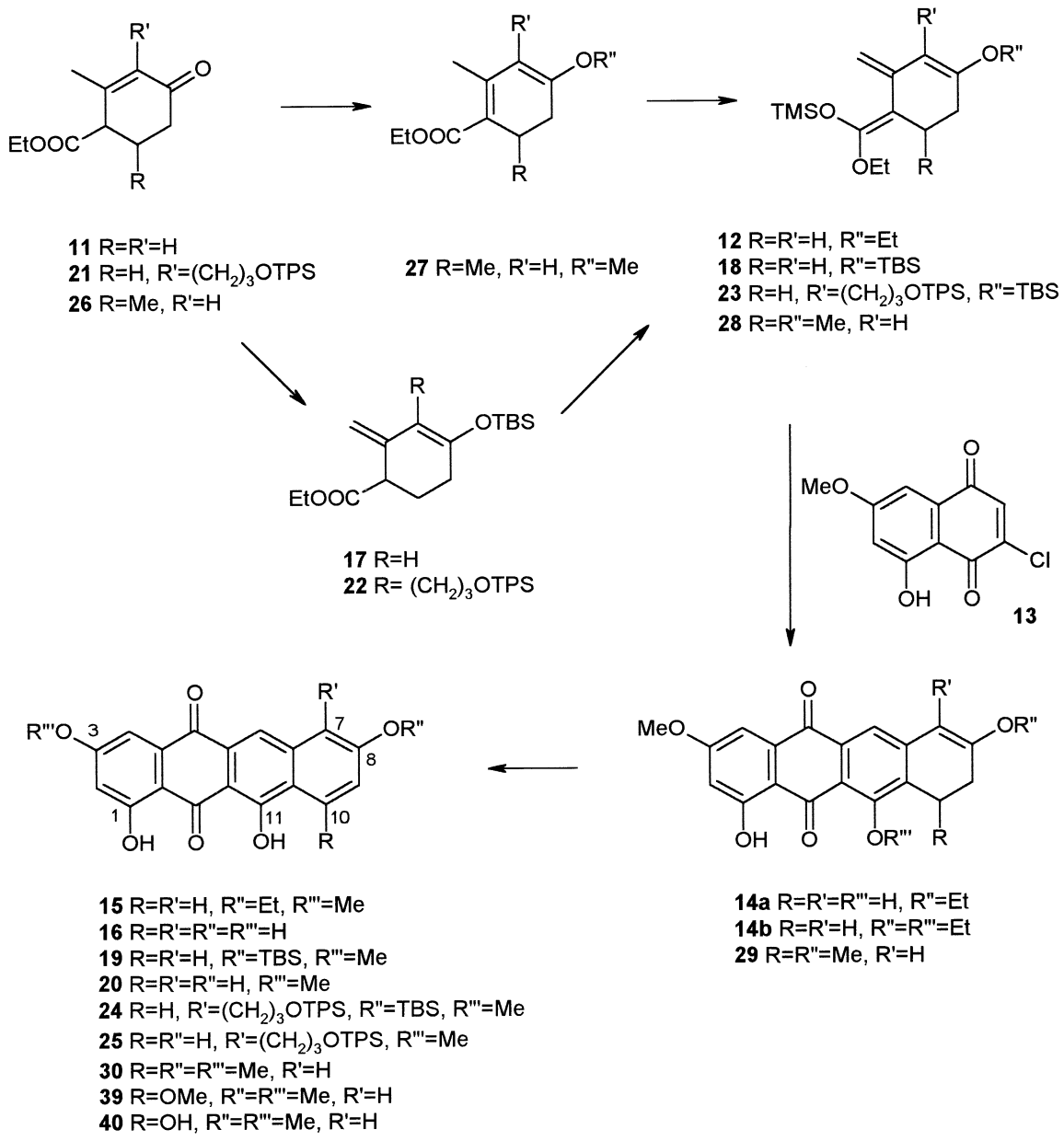
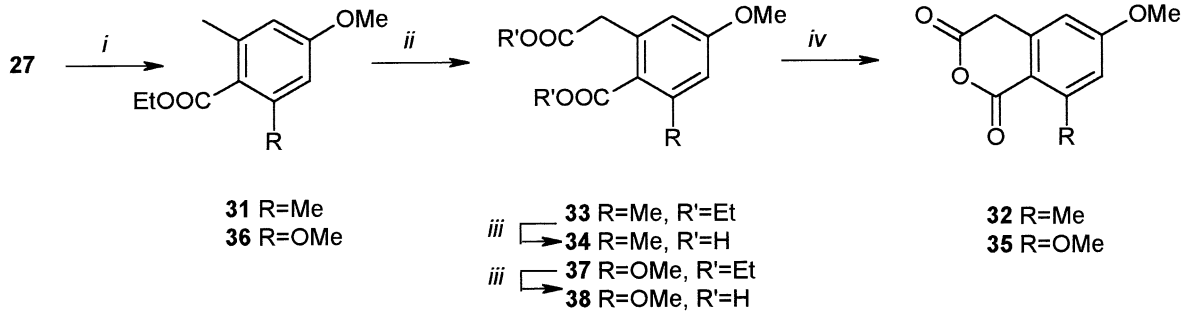


Figure 2.



i: Pd/C, diglyme, reflux, 90% for **31**; *ii*: LDA, THF-hexane, $(EtO)_2CO$, -78°C, 30%;
iii: NaOH, EtOH-water, 82% for **34**; *iv*: AcCl, acetone, 20°C, quant. for **32**.

Figure 3.

Table 1. Cytotoxicity and cell cycle effects of the tetracyclic compounds

Compound	Cytotoxicity IC_{50} L1210 (μM)	Percent of L1210 cells in the cell cycle phases[a]				
		G_1	S	G_2M	> G_2M	Conc.μM
9	22.1	6	7	83	3	50
15	> 10	NE[b]	—	—	—	—
16	2.8	7	9	75	8	5
20	14.7	34	25	39	1	100
25	40.3	NE[b]	—	—	—	—
30	13.3	41	28	24	1	20
39	13.9	41	28	24	1	20
40	2.3	63	15	21	1	5

[a]Percent of untreated control L1210 cells in the phases of the cell cycle: 41% (G_1), 28% (S), 24% (G_2M), 1% (8N). NE, not evaluated.

LDA, **32** was condensed with **13** to afford **30** (60%). Final demethylation with excess BBr_3 at −78 °C gave tetracenomycin D **9** (43%) as a dark red unsoluble solid. ^{1}H NMR data and UV spectra of this material were identical to those reported by Zeek[10] and by Cameron.[12]

Application of this route to a synthesis of saintopin was then attempted. The known homophthalic anhydride **35**[21] was prepared from ethyl 2,4-dimethoxy-6-methyl benzoate **36** (Fig. 3): ethoxycarbonylation to **37** (34%), hydrolysis to **38** (98%) and dehydration (quant). Condensation of **35** with quinone **13** afforded 3,8,10-tri-*O*-methyl saintopin **39** (72%). Attempted perdemethylation with excess BBr_3 of this material afforded an untractable complex mixture of dark red solids. This result is not surprising in view of the recently reported instability of saintopin which easily affords artefacts upon extraction from the culture broth.[22]However 3,8-di-*O*-methyl saintopin **40** resulting from easy demethylation of the chelated ether at C-10 could be isolated if the reaction is rapidly stopped after addition of BBr_3.

Cytotoxic activity

Tetracyclic derivatives were tested in vitro against L1210 leukemia and for their effect on the L1210 cell cycle.[23] Results are reported in Table 1.

These data show that 10-deoxy saintopin **16** and 3,8-di-*O*-methyl saintopin **40** are the most active compounds prepared in this study.

Etherification at C-3 or C-8 or introduction of a side chain at C-10 (tetracenomycin D) or C-7 (**25**) resulted in lower cytotoxicity. However, introduction of a hydroxyl at C-10 (**40** vs **15**) enhanced cytotoxicity. Finally, it is interesting to note that **16** and **40** have different effects on the cell cycle: 75% of the cells in the G_2M phase for **16** compared with 24% for control and 63% of the cells in the G_1 phase for **40** compared with 41% for control.

Conclusion

We have shown that substituted naphthacenequinones related to tetracenomycin D and saintopin can be obtained in few steps by cycloaddition of silyl ketene acetals or homophthalic anhydrides with a naphthoquinone. Following these synthetic schemes, and taking into account the cytotoxicity data observed above, further modifications are underway to increase the potency of these quinones.

Experimental

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a 300 MHz spectrometer, using $CDCl_3$ as solvent with TMS as internal standard. Assignment of ^{1}H NMR and ^{13}C NMR spectra were achieved using DEPT (Multiplicity by DEPT: s = C, d = CH, t = CH_2, q = CH_3). High resolution MS were performed by the 'Service Central de Microanalyse' (CNRS, Lyon). All reactions were run under an inert atmosphere. THF was dried over and distilled from sodium/benzophenone and CH_2Cl_2 was distilled from P_2O_5. Organic extract mixtures were dried over anhydrous Na_2SO_4 and filtered and the solvent was then removed under reduced pressure. All separations were done under flash chromatography (MPLC) conditions on silica gel (25–40 μm) completed, if necessary, by preparative thin-layer chromatography (TLC) performed on silica gel plates (60 GF_{254}).

9,10-Dihydro-8-ethoxy-1,11-dihydroxy-3-methoxy-naphthacene-5,12-dione (14a) and 9,10-dihydro-8,11-diethoxy-1-hydroxy-3-methoxy-naphthacene-5,12-dione (14b). To a solution of **13** (270 mg, 1.16 mmol) in anhydrous THF (23 mL) maintained at 0 °C was added ketene acetal **12** (359 mg, 1.1 equiv) in THF (10 mL). After stirring at rt for 48 h, hydrolysis with 10% HCl was followed by extraction with CH_2Cl_2. Chromatography over silica gel using CH_2Cl_2 as eluent afforded **14a** and **15** (142 mg, 39%) together with a mixture of 14a and 14b (30 mg, 8%). Preparative TLC afforded almost pure 14a and 14b contaminated by ca. 20% of **14a**.

14a. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (t, 3H, J = 7 Hz, CH_3), 2.48 (t, 2H, J = 8.6 Hz, H-9), 3.01 (t, 2H, J = 8.6 Hz, H-10), 3.93 (s, 3H, OCH_3), 3.99 (q, 2H, J = 7 Hz, OCH_2), 5.61 (s, 1H, H-7), 6.67 (d, 1H, J = 2.5 Hz, H-2), 7.35 (d, 1H, J = 2.5 Hz, H-4), 7.42 (s, 1H, H-6), 12.46 (s, 1H, OH) and 12.49 (s, H, OH).

14b. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (t, 3H, J = 7 Hz, CH_3), 1.51 (t, 3H, J = 7 Hz, CH_3), 2.43 (t, 2H,

$J=8.3$ Hz, H-9), 3.06 (t, 2H, $J=8.3$ Hz, H-10), 3.91 (s, 3H, OCH_3), 4.02 (q, 2H, $J=7$ Hz, OCH_2), 5.66 (s, 1H, H-7), 6.68 (d, 1H, $J=2.5$ Hz, H-2), 7.28 (d, 1H, $J=2.5$ Hz, H-4), 7.63 (s, 1H, H-6), 13.46 (s, 1H, OH).

8-Ethoxy-1,11-dihydroxy-3-methoxy-naphthacene-5,12-dione (15). To a solution of the above mixture of **14a**,**b** and **15** (171 mg) in toluene (13 mL) was added DDQ (106 mg, 0.467 mmol). After stirring at reflux for 6 h, toluene was removed in vacuo and the crude reaction mixture was extracted with CH_2Cl_2. Recristallization from CH_2Cl_2 afforded **15** (170 mg, 95%). Mp 264 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (t, 3H, $J=7$ Hz, CH_3), 3.95 (s, 3H, OCH_3), 4.21 (q, 2H, $J=7$ Hz, OCH_2), 6.72 (d, 1H, $J=2.5$ Hz, H-2), 7.25 (s, 1H, H-7), 7.32 (d, 1H, $J=9$ Hz, H-9), 7.43 (d, 1H, $J=2.5$ Hz, H-4), 8.17 (s, 1H, H-6), 8.39 (d, 1H, $J=9$ Hz, H-10), 12.56 (s, 1H, OH) and 13.83 (s, 1H, OH).MS (EI) m/z 364 (100), 336 (73). HRMS (EI) m/z calcd ($C_{21}H_{16}O_6$): 364.0946, obsd : 364.0927.

1,3,8,11-Tetrahydroxy-naphthacene-5,12-dione (10-deoxy saintopin) (16). BBr_3 (0.1 mL, 1.09 mmol) was added to a well stirred suspension of **15** (80 mg, 0.22 mmol) in CH_2Cl_2 (1.5 mL) maintained at −78 °C under nitrogen. After stirring for 16 h at rt, the crude reaction mixture was poured into iced water and extracted with CH_2Cl_2 and the unsoluble fraction was filtered. A dark red solid was obtained which was dried and triturated with ether to give **16** (22 mg, 28%). Mp > 250 °C (dec). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.58 (s, 1H, H-2), 7.14 (s, 1H, H-7), 7.26 (d, 1H, $J=9$ Hz, H-9), 7.36 (s, 1H, H-4), 8.01 (s, 1H, H-6), 8.21 (d, 1H, $J=9$ Hz, H-10), 10.75 (s, 1H, OH), 11.30 (s, 1H, OH), 12.29 (s, 1H, OH) and 13.78 (s, 1H, OH). HRMS (EI) m/z calcd ($C_{18}H_{10}O_6$): 322.0477, obsd: 322.0463.

Ethyl 2-Methylene-4-*tert*butyldimethylsilyloxy-cyclohexa-3-ene carboxylate (17). To a solution of Hagemann's ester **11** (250 mg, 1.37 mmol) in CH_2Cl_2, was added Et_3N (0.28 mL, 2.06 mmol, 1.5 equiv) and *tert*-butyldimethylsilyl triflate (0.41 mL, 1.78 mmol, 1.3 equiv). After stirring for 40 min at rt, dilution with CH_2Cl_2 and extraction as usual, there was obtained a crude oil which was rapidly filtered over florisil (eluent: EtOAc/pet ether 2.5:97.5). The unstable oily enol ether **17** (367 mg, 90%) was rapidly used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.15 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 1.26 (t, 3H, $J=7$ Hz, CH_3), 1.85–2.3 (m, 4H, H-5 and H-6), 3.24 (t, 1H, $J=5$ Hz, H-1), 4.17 (q, 2H, $J=7$ Hz, OCH_2), 4.65 (broad s, 1H, C=CH), 4.76 (broad s, 1H, C=CH), 5.50 (s, 1H, H-3).

1-(1-Ethoxy-1-trimethylsilyloxy-methylene)-2-methylene-4-*tert*butyldimethylsilyloxy-cyclohex-3-ene (18). To a cooled (−78 °C) solution of LDA prepared from diisopropylamine (0.38 mL, 2.73 mmol) and 1.6 N nBuLi in hexane (1.7 mL, 2.72 mmol) in THF (1.5 mL), under N_2, was added a solution of **17** (367 mg, 1.24 mmol) in THF (0.3 mL). After stirring for 1 h at −78 °C, TMSCl (0.36 mL, 2.85 mmol) was added and the mixture was stirred overnight at rt. After removal of excess TMSCl and solvents under vacuo, the residue was diluted with anhydrous ether and filtered. After evaporation ketene acetal **18** (440 mg, 96%) was obtained as a pale yellow oil which was rapidly used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06–0.23 (m, 15H, $Si(CH_3)_2$ and $Si(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 1.25 (t, 3H, $J=7$ Hz, CH_3), 2.13 (t, 2H, $J=6.5$ Hz, H-5), 2.43 (t, 2H, $J=6.5$ Hz, H-6), 3.85 (q, 2H, $J=7$ Hz, OCH_2), 4.79 (broad s, 1H, C=CH), 5.04 (broad s, 1H, C=CH), 5.41 (s, 1H, H-3).

8-*tert*Butyldimethylsilyloxy-1,11-dihydroxy-3-methoxy-naphthacene-5,12-dione (19). Same procedure as for **14a,b**. After chromatography **19** (6%) was obtained together with a mixture of the corresponding 9,10-dihydro (11-OH and 11-OEt) derivatives (35%). Attempted aromatization of the latter with DDQ failed. **19**: mp 200–210 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 6H, $Si(CH_3)_2$), 1.03 (s, 9H, $Si(CH_3)_3$), 3.95 (s, 3H, CH_3), 6.71 (d, 1H, $J=2.5$ Hz, H-2), 7.22 (dd, 1H, $J=2.3$ and 9 Hz, H-9), 7.30 (d, 1H, $J=8.3$ Hz, H-7), 7.43 (d, 1H, $J=2.5$ Hz, H-4), 8.14 (d, 1H, $J=2.3$ Hz, H-6), 8.39 (d, 1H, $J=9$ Hz, H-10), 12.55 (s, 1H, OH) and 13.86 (s, 1H, OH); MS (EI) m/z 450 (77), 393 (100), 197 (44), 73 (32), 57 (34). HRMS (EI) m/z calcd ($C_{25}H_{26}O_6Si$): 450.1498, obsd: 450.1503.

1,8,11-Trihydroxy-3-methoxy-naphthacene-5,12-dione (3-*O*-methyl 10-deoxy saintopin) (20). Desilylation of **19** (27 mg, 0.06 mmol) to **20** was carried out by treatment with Bu_4NF (31 mg, 0.12 mmol) in THF (0.25 mL). Extraction as usual with CH_2Cl_2 afforded **20** (14 mg, 69%) as a red solid after precipitation from EtOAc/Pet ether: mp 190 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H, CH_3), 6.68 (d, 1H, $J=2.5$ Hz, H-2), 7.05–7.35 (m, 2H, H-7 and H-9), 7.36 (d, 1H, $J=2.5$ Hz, H-4), 7.92 (s, 1H, H-6), 8.20 (d, 1H, $J=9$ Hz, H-10). MS (EI) m/z 336 (25), 142 (90), 100 (100), 58 (70); HRMS (EI) m/z calcd ($C_{19}H_{12}O_6$): 336.0633, obsd: 336.0637.

Ethyl 2-methyl-3-(3-*tert*butyldiphenylsilyloxypropyl)-cyclohex-2-en-4-one carboxylate (21). To a cooled (0 °C) solution of Hagemann's ester **11** (250 mg, 1.37 mmol) in DMF (7.5 mL) was slowly added a 60% suspension of NaH (55 mg, 1.375 mmol). After stirring for 20 min, 3-bromo-1-*tert*butyldiphenylsilyloxypropane (52 mg, 1.38 mmol) was added and the mixture was stirred for 24 h at rt. After hydrolysis with 1 N HCl and extraction as usual with ether, flash chromatography over silica gel (eluent EtOAc/Pet ether) afforded **21** as an oil (189 mg, 29%). ^{1}H NMR δ 0.15 (s, 6H, $Si(CH_3)_2$), 1.05 (s, 9H, $C(CH_3)_3$), 1.26 (t, 3H, $J=7$ Hz, CH_3), 1.57 (m, 2H, CH_2), 1.99 (s, 3H, $C=CCH_3$), 2.22-255 (m, 6H, H-5, H-6 and $C=CCH_2$), 3.26 (t, 1H, $J=5$ Hz, H-1), 3.67 (t, 2H, $J=6$ Hz, CH_2OSi), 4.18 (q, 2H, $J=7$ Hz, OCH_2), 7.38 (m, 6H, H-arom), 7.67 (m, 4H, H-arom); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2 (CH_3), 19.1, 34.7, 47.6 (C-4), 61.15 (OCH_2), 63.55 (COSi), 127.6, 129.5, 133.95, 135.45 (C-arom), 137.3 (C-2), 150.1 (C-3), 172.3 (COO), 197.3 (CO); MS (FAB) m/z 479 (43, MH^+), 422 (33), 421 (100), 401 (29), 223 (59). HRMS (FAB) m/z calcd (MH^+, $C_{29}H_{39}O_4Si$): 479.2617, obsd: 479.2634.

Ethyl 2-methylene-4-*tert*butyldimethylsilyloxy-3-(3-*tert*-butyldiphenylsilyloxypropyl)-cyclo-hex-3-ene carboxylate

(22). Same procedure as for **17,** starting from **21**. Compound **22** was isolated as a colorless oil (86%) which was rapidly used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $C(CH_3)_3$), 1.04 (s, 9H, $C(CH_3)_3$), 1.24 (t, 3H, $J=7$ Hz, CH_3), 1.55–2.35 (m, 8H, H-5, H-6, CH_2, $C=CCH_2$), 3.26 (t, 1H, $J=5$ Hz, H-1), 3.64 (t, 2H, $J=6$ Hz, CH_2OSi), 4.16 (q, 2H, $J=7$ Hz, OCH_2), 4.71 (broad s, 1H, C=CH), 4.98 (broad s, 1H, C=CH), 7.38 (m, 6H, H-arom), 7.67 (m, 4H, H-arom).

1-(1-Ethoxy-1-trimethylsilyloxy-methylene)-2-methylene-3-(3-*tert*butyldiphenylsilyloxypropyl)-4-*tert*butyldimethylsilyloxy-cyclohex-3-ene (23). Same procedure as for **19**, starting from **22**. Compound **23** was isolated as a pale yellow oil (71%) which was rapidly used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05–0.25 (m, 15H, $Si(CH_3)_2$ and $Si(CH_3)_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.06 (s, 9H, $C(CH_3)_3$), 1.24 (t, 3H, $J=7$ Hz, CH_3), 1.80 (m, 2H, CH_2), 2.21 and 2.39 (2m, 6H, $C=CCH_2$, H-5, H-6), 3.69 (t, 2H, $J=6$ Hz, CH_2OSi), 3.87 (q, 2H, $J=7$ Hz, OCH_2), 5.13 (broad s, 2H, $C=CH_2$), 7.39 (m, 6H, H-arom), 7.70 (m, 4H, H-arom).

8-*tert*Butyldimethylsilyloxy-7-(3-*tert*butyldiphenylsilyloxypropyl)-1,11-dihydroxy-3-methoxy-naphthacene-5,12-dione (24). Same procedure as for **14a,b,** starting from **23**. Compound **24** (29%) was obtained as a red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.28 (s, 6H, $Si(CH_3)_2$), 0.99 (s, 9H, $C(CH_3)_3$), 1.08 (s, 9H, $C(CH_3)_3$), 1.89 (m, 2H, CH_2), 3.13 (t, 2H, $J=8$ Hz, CH_2Ar), 3.80 (t, 2H, $J=6.5$ Hz, CH_2OSi), 3.94 (s, 3H, OCH_3), 6.69 (d, 1H, $J=2.5$ Hz, H-2), 7.16 (d, 1H, $J=9$ Hz, H-9), 7.36 (m, 6H, H-arom), 7.43 (d, 1H, $J=2.5$ Hz, H-4), 7.69 (m, 4H, H-arom), 8.26 (d, 1H, $J=9$ Hz, H-10), 8.43 (s, 1H, H-6), 12.55 (s, 1H, OH), 13.80 (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −3.9 ($Si(CH_3)_2$), 18.3 and 19.25 ($\underline{C}(CH_3)_3$), 22.5 (CH_2), 25.8 and 27.0 ($C(\underline{C}H_3)_3$), 33.35 ($\underline{A}rCH_2$), 55.95 (OCH_3), 64.15 ($CH_2O\underline{S}i$), 106.6 d, 107.05 s, 107.45 d, 111.1 s, 118.25 d, 121.9 d, 122.8 s, 124.1 d, 127.5 d, 128.3 s, 129.5 d, 129.8 s, 133.9 s, 135.6 d, 136.2 s, 136.7 s, 155.7 s, 163.4 s, 164.95 s, 166.1 s, 170.1 s 181.6 and 189.6 (CO). MS (EI) *m*/*z* 746 (12), 731 (17), 717 (28), 703 (86), 689 (100); HRMS (EI) *m*/*z* calcd ($C_{44}H_{50}O_7Si_2$): 746.3095, obsd: 746.3081.

1,8,11-Trihydroxy-7-(3-hydroxypropyl)-3-methoxy-naphthacene-5,12-dione (25). A solution of **24** (150 mg, 0.2 mmol) and nBu$_4$NF (190 mg, 0.73 mmol) in THF (1.2 mL) was stirred for 5 h at rt, poured over satd NH_4Cl and extracted with CH_2Cl_2. The insoluble fraction was dissolved in CH_2Cl_2/MeOH and **25** was precipitated with pet ether as a dark red solid (72 mg, 91%). Mp 190 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66 (m, 2H, CH_2), 3.01 (m, 2H, $ArCH_2$), 3.50 (t, 2H, $J=6.5$ Hz, CH_2O), 3.93 (s, 3H, CH_3), 6.86 (d, 1H, $J=2.2$ Hz, H-2), 7.21 (d, 1H, $J=2.2$ Hz, H-4), 7.36 (d, 1H, $J=9$ Hz, H-9), 8.18 (d, 1H, $J=9$ Hz, H-10), 8.25 (s, 1H, H-6). MS (EI) *m*/*z* 394 (24), 350 (43), 142 (100), 100 (59), 57 (31); HRMS (EI) *m*/*z* calcd ($C_{22}H_{18}O_7$): 394.1052, obsd: 394.1058.

Ethyl 2,6-dimethyl-4-methoxy-cyclohexa-1,3-diene carboxylate (27). To a solution of **26**[18] (20 g, 102 mmol), in anhydrous acetone, was added K_2CO_3 (21.1 g, 154 mmol) then dimethyl sulfate (12.6 mL, 133 mmol). After refluxing for 48 h, the mixture was cooled, filtered and concentrated under reduced pressure. Separation by flash chromatography (EtOAc/pet ether, 5:95) afforded **27** (13.7 g, 64%) and starting material **26** (3.6 g, 18%). **27**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (d, 3H, $J=6.6$ Hz, CH_3), 1.30 (t, 3H, $J=7.3$ Hz, CH_3), 1.94 (broad d, 1H, $J=16$ Hz, H-5a), 2.20 (s, 3H, CH_3), 2.64 (dd, 1H, $J=16$ and 8 Hz, H-5b), 2.92 (m, 1H, H-6), 3.66 (s, 3H, OCH_3), 4.18 (q, 2H, J = 7.3 Hz), 4.94 (d, 1H, $J=1.4$ Hz, H-3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3 q, 17.8 q, 21.8 q, 29.3 d, 34.7 t, 54.8 q, 59.3 t, 98.8 d, 119.6 s, 144.7 s, 162.0 s, 168.0 s. HRMS (EI) *m*/*z* calcd ($C_{12}H_{18}O_3$): 210.1255, obsd: 210,1237.

1-(1-Ethoxy-1-trimethylsilyloxy-methylene)-4-methoxy-6-methyl-2-methylene-cyclohex-3-ene (28). Same procedure as for **18,** starting from **27**. Compound **28** was obtained (quant) as a pale yellow oil which was rapidly used in the next step. **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.2 (s, 9H, $Si(CH_3)_3$), 1.00 (d, 3H, $J=7$ Hz, CH_3), 1.26 (t, 3H, $J=7$ Hz, CH_3), 1.87 (broad d, 1H, $J=17$ Hz, H-5a), 2.50 (broad dd, 1H, $J=17$ and 7 Hz, H-5b), 3.14 (m, 1H, H-6), 3.59 (s, 3H, OCH_3), 3.87 (q, 2H, $J=7$ Hz, OCH_2), 4.92 (d, 1H, $J=2$ Hz, =CH), 5.10 (d, 1H, $J=2$ Hz; =CH), 5.27 (s, 1H, =CH).

4.151,11-Dihydroxy-3,8-dimethoxy-10-methyl-9,10-dihydronaphthacene-5,12-dione (29). Same procedure as for 14a,b starting from **28**. Compound **29** was isolated (13%) as a red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (d, 3H, $J=7$ Hz, CH_3), 2.18 (broad d, 1H, $J=16$ Hz, H-9a), 2.80 (broad dd, 1H, $J=16$ and 7 Hz, H-9b), 3.56 (m, 1H, H-10), 3.78 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.62 (s, 1H, H-7), 6.65 (d, 1H, $J=2$ Hz, H-2), 7.32 (d, 1H, $J=2$ Hz, H-4), 7.43 (s, 1H, H-6), 12.43 (s, 1H, OH) and 12.47 (s, 1H, OH). HRMS (EI) *m*/*z* calcd ($C_{21}H_{16}O_6$): 364.0947, obsd: 364.0961.

Ethyl 4-methoxy-2,6-dimethyl benzoate (31). A mixture of 10% Pd/C (100 mg) and **27** (500 mg, 2.37 mmol) was heated at 220 °C for 14 h. After dilution with EtOAc and filtration, flash chromatography (eluent EtOAc/pet ether 5:95) afforded **31** as a colorless oil (446 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, 3H, $J=7$ Hz, CH_3), 2.32 (s, 6H, CH_3), 3.76 (s, 3H, OCH_3), 4.35 (q, 2H, $J=7$ Hz, OCH_2), 6.56 (broad s, 2H, H-3, H-5); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2 ($OCH_2\underline{C}H_3$), 20.2 (2 CH_3), 55.1 (OCH_3), 60.6 (OCH_2), 113.2 (C-3, C-5), 126.6 (C-1), 137.2 (C-2, C-6), 160.0 (C-4), 169.7 (COO). MS (EI) *m*/*z* 208 (M), 163 (100); HRMS (EI) *m*/*z* calcd ($C_{12}H_{16}O_3$): 208.1099, obsd: 208.1095.

4.17Ethyl 2-Ethoxycarbonylmethyl-4-methoxy-6-methyl benzoate (33). To a cooled (−78 °C) solution of **31** (1 g, 4.26 mmol) in anhydrous THF (4 mL) was added a 1.5 M solution of LDA in hexane–THF (1:2 v/v, 3.52 mL, 5.28 mmol), and, after 5 min, diethyl carbonate (2.38 mL, 19.2 mmol). Stirring was continued under N_2 for 1 h and the reaction mixture was then poured over satd NH_4Cl. After extraction with CH_2Cl_2 as usual, flash chromatography (eluent EtOAc/pet ether 5:95 to 10:90) afforded diester **33** as a colorless oil (386 mg,

30%), together with ethyl 2-diethoxycarbonylmethyl-4-methoxy-6-methyl benzoate (105 mg, 8%) and starting material **31** (278 mg, 28%). 33: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, 3H, $J=7$ Hz, CH_3), 1.36 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.71 (s, 2H, $ArCH_2$), 3.80 (s, 3H, OCH_3), 4.14 (q, 2H, $J=7$ Hz, OCH_2), 4.35 (q, 2H, $J=7$ Hz, OCH_2), 6.64 (broad s, 1H, H-arom), 6.66 (broad s, 1H, H-arom); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0 (2 C, $OCH_2\underline{C}H_3$), 21.0 ($ArCH_3$), 40.0 ($ArCH_2$), 55.0 (OCH_3), 60.6 (2C, OCH_2), 114.1 (C-3, C-5), 125.5 (C-1), 134.6 and 139.0 (C-2, C-6), 160.0 (C-4), 168.6 and 170.9 (COO). MS (EI) m/z 280 (M, 25), 234 (55), 206 (55), 179 (100), 162 (58); HRMS (EI) m/z calcd ($C_{15}H_{20}O_5$): 280.1310, obsd: 280.1313.

2-Carboxymethyl-4-methoxy-6-methyl benzoic acid (34). A solution of **33** (200 mg, 0.71 mmol), NaOH (1 g) in ethanol (5 mL) and water (1 mL) was heated under reflux for 16 h. After extraction with CH_2Cl_2, diacid 34 was obtained as a white solid (130 mg, 82%). ^{1}H NMR (300 MHz, acetone d_6) δ 2.42 (s, 3H, CH_3), 3.80 (broad s, 5H, OCH_3 and $ArCH_2$), 6.76 (broad s, 2H, H-3 and H-5), 10 (broad s, 2H, COOH); ^{13}C NMR (75.5 MHz, acetone-d_6) δ 21.5 ($ArCH_3$), 40.3 ($ArCH_2$), 55.7 (OCH_3), 115.4 and 115.5 (C-3, C-5), 127.0 (C-1), 136.5 (C-2), 139.7 (C-6), 161.2 (C-4), 170.5 and 172.6 (COO). MS (EI) m/z 224 (M, 13), 180 (50), 178 (55), 162 (88), 149 (80), 91 (100); HRMS (EI) m/z calcd ($C_{11}H_{12}O_5$): 224.0685, obsd: 224.0675.

6-Methoxy-8-methyl-isochroman-1,3-dione (32). A solution of acetyl chloride (84 mg, 1.07 mmol) in anhydrous acetone (0.1 mL) was added to a diacid **34** (60 mg, 0.27 mmol) in acetone (0.5 mL). After stirring for 2 h at rt, concentration under reduced pressure led to anhydride **32** (quant) as a white solid. ^{1}H NMR (300 MHz, acetone-d_6) δ 2.63 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.22 (s, 2H, Ar–CH_2), 6.90 (d, 1H, $J=2$ Hz, H-arom), 6.92 (d, 1H, $J=2$ Hz, H-arom). MS (EI) m/z 206 (M, 30), 162 (100), 149 (30), 134 (31), 119 (24), 91 (60); HRMS (EI) m/z calcd ($C_{11}H_{10}O_4$): 206.0579, obsd: 206.0587.

1,11-Dihydroxy-3,8-dimethoxy-10-methyl-naphthacene-5,12-dione (30). A solution of **32** (75 mg, 0.36 mmol) in anhydrous THF (6 mL) was treated with 60% NaH (17.5 mg, 0.44 mmol) at 0 °C. After stirring for 5 min at 0 °C, a solution of **13** (86 mg, 0.36 mmol) in anhydrous THF (4.5 mL) was added and the resulting mixture was stirred for 24 h at rt, quenched with satd NH_4Cl and then with 1 N HCl. After extraction as usual with CH_2Cl_2, and evaporation, **30** was obtained by precipitation from CH_2Cl_2 by addition of pet ether as a red solid (80 mg, 60%). Mp 240 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96 (s, 3H, CH_3), 3.94 (s, 6H, OCH_3), 6.71 (d, 1H, $J=2$ Hz, H-2), 7.01 (d, 1H, $J=2$ Hz, H-9), 7.09 (d, 1H, $J=2$ Hz, H-7), 7.40 (d, 1H, $J=2$ Hz, H-4), 8.10 (s, 1H, H-6), 12.50 (s, 1H, OH), 14.60 (s, 1H, OH). MS (EI) m/z 364 (M, 100), 350 (7), 321 (12), 303 (8); HRMS (EI) m/z calcd ($C_{21}H_{18}O_6$): 364.0947, obsd: 364.0961.

Tetracenomycin D (1,3,8,11-tetrahydroxy-10-methyl-naphthacene-5,12-dione) (9). To a cooled (−78 °C) suspension of **30** (25 mg, 0.069 mmol) in CH_2Cl_2 (0.8 mL) was added BBr_3 (33 μL, 0.34 mmol). After stirring for 48 h at rt followed by addition of water (10 mL), the insoluble fraction was filtered and purified by preparative TLC (eluent CH_2Cl_2/MeOH, 95:5) to afford tetracenomycin D (10 mg, 43%) as a dark red solid. ^{1}H NMR (300 MHz, methanol-d_4) δ 2.78 (s, 3H, CH_3), 6.35 (d, 1H, $J=2$ Hz, H-2), 6.82 (d, 1H, $J=2$ Hz, H-9), 6.91 (d, 1H, $J=2$ Hz, H-7), 7.03 (d, 1H, $J=2$ Hz, H-4), 7.72 (s, 1H, H-6); UV (methanol) λ (ε) 219 (22,400), 240 (28,800), 276 (26,200), 292 (20,200), 307 (19,100), 338 (15,100), 493 (12,700). MS (EI) m/z 336 (M, 100).

1,11-Dihydroxy-3,8,10-trimethoxy-naphthacene-5,12-dione (39). Same procedure as for **30**, starting from **13** and **35**[21] (prepared as above from **36** in three steps). Compound **39** was isolated as a dark red solid (72%). Mp 284–285 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.63 (d, 1H, $J=2$ Hz, H-9), 6.71 (d, 1H, $J=2.5$ Hz, H-2), 6.87 (d, 1H, $J=2$ Hz, H-10), 7.40 (d, 1H, $J=2.5$ Hz, H-4), 8.06 (s, 1H, H-6), 12.50 (s, 1H, OH), 14.80 (s, 1H, OH). HRMS (EI) m/z calcd ($C_{21}H_{16}O_7$): 380.0896, obsd: 380.0887.

4.233,8-Di-*O*-methyl saintopin (1,10,11-trihydroxy-3,8-dimethoxy-naphthacene-5,12-dione (40). Same procedure as for tetracenomycin D (5 min, 0 °C), starting from **39**. Compound **40** was obtained as a dark red solid (undeterminated yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 3.93 (s, 6H, 2 OCH_3), 6.80 (d, 1H, $J=2$ Hz), 6.82 (d, 1H, $J=2$ Hz), 7.21 (d, 1H, $J=2$ Hz), 7.33 (d, 1H, $J=2$ Hz), 8.10 (s, 1H), 12.50 (s, 1H, OH). HRMS (EI) m/z calcd ($C_{20}H_{14}O_7$): 366.0739, obsd: 366.0732.

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