Pergamon

SCIENCE DIRECT®

TETRAHEDRON

Tetrahedron 59 (2003) 1349–1357

Synthesis of perfragilin A, B and some analogues

Vitor F. Ferreira,[a] Aeri Park,[b] Francis J. Schmitz[b,*] and Fred A. Valeriote[c]

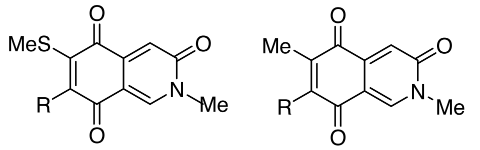
[a]*Departamento de Química Orgânica, Universidade Federal Fluminense, Niterói, Rio de Janeiro CEP 24020-150, Brazil*
[b]*Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, OK, 73019-0370, USA*
[c]*Josephine Ford Cancer Center, One Ford Place, 1-D, Detroit, MI 48202, USA*

Received 30 September 2002; revised 3 December 2002; accepted 3 December 2002

Abstract—Synthesis of the cytotoxic isoquinoline quinone perfragilin A, an improved synthesis of perfragilin B and preparation of some analogues of both these compounds are described. Cytotoxicity evaluation of a number of the products is reported. The regioselectivity in Diels–Alder reactions of differently substituted benzoquinones with 2-aza-1,3-bis(*t*-butyldimethylsilyloxy)-1,3-butadiene is described. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Perfragilin A (**1**) and B (**2**) belong to a small class of marine metabolites having an isoquinoline quinone skeleton. These compounds were isolated by our group from the bryozoan *Membranipora perfragilis*[1,2] and their structures fully assigned based on NMR and X-ray data.[3] The isoquinoline quinone skeleton is also found in a few other marine metabolites such as mimosamycin (**3**) isolated from several sponges, e.g. *Reniera* sp[4,5], *Xestopongia caycedoi*[6] and cribrostatin 2 (**4**) isolated from a *Cribrochalina* sp[7] sponge. Perfragilin A and B were toxic to murine leukemia cells (P388), with perfragilin B being the more potent, ED_{50} 0.8 and 0.07 μg/mL, respectively. Perfragilin B (**1**) showed some selectivity for renal and breast cancer cells in the NCI human tumor cell panel.[8]



R = NH_2, Perfragilin A (**1**) R = OMe, Mimosamycin (**3**)
R = SMe, Perfragilin B (**2**) R OEt, Cribostatin (**4**)

Sometime ago we described briefly a total synthesis for perfragilin B (**2**) in 3.6% overall yield from benzoquinone.[9] A key step in constructing the isoquinoline quinone skeleton was a hetero Diels–Alder reaction as had previously been used in the syntheses of amphimedine[10] and mimosamycin.[11,12] In order to obtain more perfragilin B (**2**) for biological evaluation, we undertook a study to improve yields of individual steps and possibly shorten the synthesis of this compound, to prepare some analogues, and to synthesize the related perfragilin A (**1**).

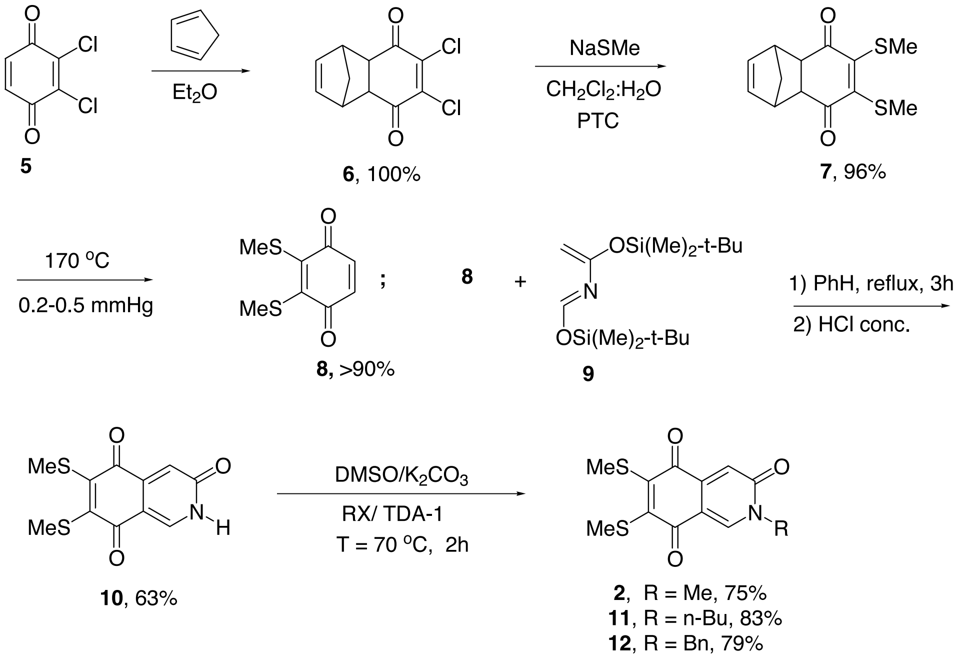
2. Results and discussion

The synthesis of perfragilin B (**2**) following the general route described earlier is outlined in Scheme 1. 2,3-Dichloro-1,4-benzoquinone (**5**) was prepared from 1,4-benzoquinone via the procedure described by Norris.[13] Reproducibility and substantial improvement in yield in this synthesis were achieved by using a modified procedure for preparing 5,6-dichlorocyclohexen-2-ene-1,4-dione.[14] Reaction of **5** with cyclopentadiene produced **6** in quantitative yield. Replacement of the chlorines by thiomethoxy groups was achieved in our earlier work and by others[15] in moderate to good yields (maximum reported 80%) by carefully controlling the pH of reaction mixture. In our current work we found that under phase transfer catalysis conditions, **6** reacted with sodium thiomethoxide very rapidly and the desired product **7** was obtained in quantitative yield. Not only is the reaction rapid under these conditions, but the product is not exposed to protic, basic (or buffered) conditions which facilitate enolization of **7** to its hydroquinone form, thereby lowering the yield. The retro Diels–Alder reaction of **7** carried out at high temperature and low pressure yielded 2,3-di-(thiomethoxy)-1,4-benzoquinone (**8**) which, without purification, was reacted with 2-aza-1,3-bis-(*t*-butyldimethylsilyloxy)-1,3-butadiene (**9**)[16] in refluxing benzene. The hot reaction mixture was quenched with concentrated HCl to produce the isoquinoline quinone **10** in 63% yield from **7**. *N*-Methylation of **10** was previously carried out in DMF promoted by tris-(3,6-dioxaoctyl)-amine (TDA-1)[17] as phase transfer catalyst. This procedure gave good yields in the synthesis of

Keywords: synthesis; cytotoxicity; antitumor; Diels–Alder; regioselectivity; quinones; isoquinoline.

* Corresponding author. Tel.: +1-405-325-5581; fax: +1-405-325-6111; e-mail: fjschmitz@ou.edu

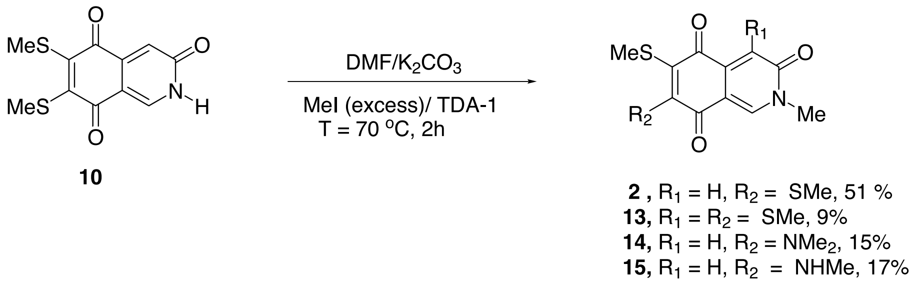
PII: S0040-4020(02)01591-0



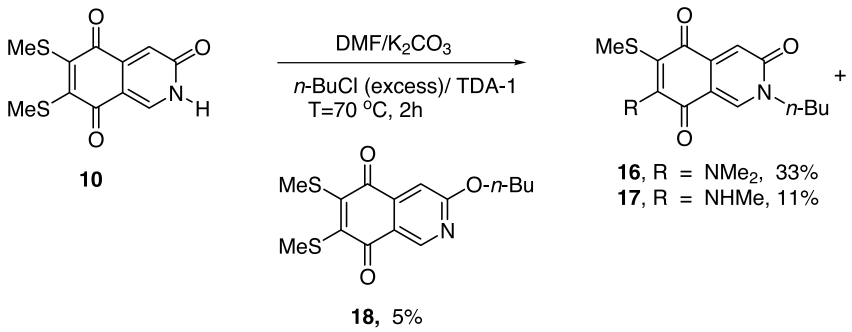
Scheme 1.

mimomycin,[11] but only moderate yield in the synthesis of perfragilin B (**2**).[9] In the current work we found that using DMSO as solvent resulted in conversion of **10** to **2** in 75% yield. The *N*-butyl (**11**) and *N*-benzyl (**12**) analogues of perfragilin B (**2**), respectively, were obtained in 83 and 79% yield from **10** using DMSO/K_2CO_3/TDA-1 and butyl iodide and benzyl chloride, respectively. Use of *n*-butyl chloride in DMF gave rise to different products, see Scheme 2 below.

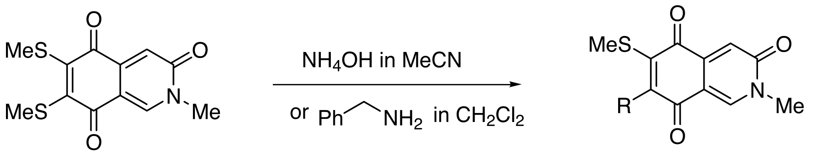
A detailed analysis of the reaction mixture from methylation of **10** in DMF was carried out in hopes of determining the cause of the low yield of the desired product. In addition to **2** (51% yield), the side products **13–15** were isolated in 9, 17, and 15%, respectively (Scheme 3). The latter two unexpected products revealed operation of a highly regioselective Michael reaction that could be taken advantage of to synthesize perfragilin A (**1**). Product **13** was identified by high resolution mass and NMR data (loss of the δ 7.08 ppm singlet characteristic of H-4 and presence of an additional *S*-methyl singlet δ H/C 2.66:18.0). Products **14** and **15** likewise were identified by mass (high resolution only for **15**) and NMR data [loss of one SMe signal and replacement by signals for $-NMe_2$ (δ 3.26) and $-NHMe$ (δ 3.48)], respectively. The assigned regiochemistry of the



Scheme 2.



Scheme 3.



2

1, R = NH_2, 22%
19, R = NHBn, 29%

Scheme 4.

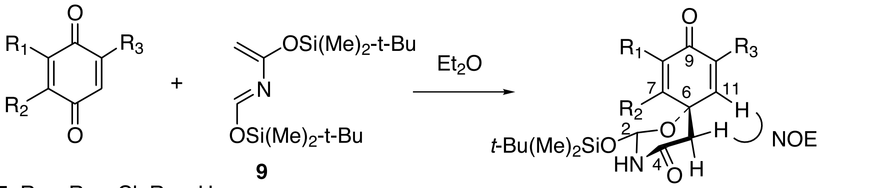
quinone substituents was not proven by spectroscopic methods, but can be rationalized on electronic grounds, and was confirmed by the synthesis of perfragilin A (**1**), see below. The C-5 carbonyl should be more electrophilic than C-8 since the former is cross-conjugated with two other α,β-unsaturated carbonyl systems while the latter is part of a conjugated enamino ketone system. Hence Michael addition at C-7 should be preferred. Decomposition of DMF by base and heat[18] is assumed to generate the methyl- and dimethylamine needed to form these products. Dimethylformamide had been carefully purified[18] and NMR analysis of the solvent confirmed its purity. The origin of **13** is more obscure, but may involve Michael addition at C-4 by thiomethoxide (released in formation of **14/15**) followed by air oxidation to give back the conjugated system of **13**.

Reaction of **10** with *n*-butyl chloride in DMF with K_2CO_3/TDA-1 gave no detectable amounts of the desired product **11**, but instead products **16–18** were isolated in 33, 11, and 5% yield, respectively, see Scheme 3. The structures of **16** and **17** were established by mass spectral analysis and comparison of their NMR data with that of **14** and **15**. The structure of **18** was deduced by comparison of its proton NMR data with that of **11**. The lower field shifts of the signals H-1, H-4, and the CH_2O of the butyl ether argue for the O-alkylated structure for **18**.

The serendipitous synthesis of **14–17** indicated that perfragilin A (**1**) and other substituted amino analogues could be synthesized from perfragilin B (**2**) by selective displacement of the C-7 thiomethoxy group with ammonia or other amines. Indeed, reaction of **2** with aqueous ammonia in acetonitrile yielded **1** in 22% yield along with many other side products which were not individually separated. Synthetic and natural **1** exhibited the same TLC behavior and identical proton NMR spectra. Preparation of **1** by this route confirms the regiochemistry of products **14–17**. The *N*-benzyl analogue of perfragilin A, **19**, was prepared in 29% yield by reaction of **2** with benzylamine in CH_2Cl_2 (Scheme 4). Selective and facile displacement of the thiomethoxy group suggests that **2** may a biogenetic precursor of **1**.

All the synthetic compounds gave sharp, clean ^1H NMR spectra. However all the compounds containing a 7-NH_2 or 7-NHR group gave very weak (broad) ^{13}C NMR signals for the –SMe group and many of the signals for C's -5 to 9 were also weak or not observed. It is possible that this is due to the presence of a radical in this conjugated compound as has been reported in some other cases.[19]

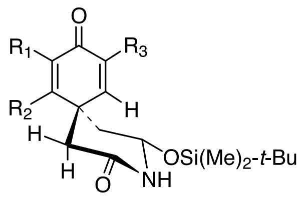
The synthesis of perfragilin B (**2**) could perhaps be shortened if quinone **5** would undergo a Diels–Alder reaction directly with azadiene **9** to give an isoquinoline quinone analogous to **10**, but with chlorines in place of thiomethoxy groups. Methylation of such an intermediate followed by displacement of the chlorines with appropriate nucleophiles would give compounds in the perfragilin series. Reaction of **5** and **9** in refluxing ether gave a low yield of a spiro product **22** in 26% yield (Scheme 5). The ^1H NMR spectrum of **22** showed two doublets at δ 7.16 and 6.31 confirming that the unsubstituted carbon–carbon double bond of the quinone **5** had not formed a Diels–Alder adduct with the diene. Also there was a doublet at δ 6.23 which was coupled to a broad signal δ 6.46 which was considered to be due to an amide proton. Two geminal



5, $R_1 = R_2$ = Cl, R_3 = H
20, R_1 = H, $R_2 = R_3$ = Cl
21, R_1 = H, $R_2 = R_3$ = SMe

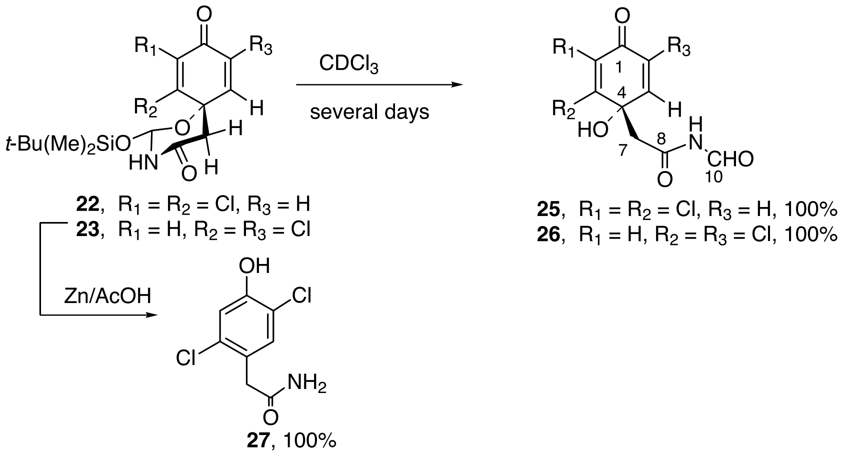
9

22, $R_1 = R_2$ = Cl, R_3 = H, 26%
23, R_1 = H, $R_2 = R_3$ = Cl, 44%
24a, R_1 = H, $R_2 = R_3$ = SMe, 4%



24b, R_1 = H, $R_2 = R_3$ = SMe, 16%

Scheme 5.



Scheme 6.

proton signals at δ 2.56 and 3.09 and the *t*-BuMe$_2$Si group resonances were also present. Furthermore, one of the ^{13}C NMR carbonyl carbon resonances of the starting quinone was lacking, but there were signals for an amide carbonyl (δ 165.7) and an ortho-amide carbon C-2 (δ 95.3). This led to the conclusion that one of the carbonyl carbons of **5** had reacted with the diene **9** to yield the spiro compound **22**. A similar spiro compound has already been reported in connection with the synthesis of amphimedine using the azadiene **9**.[10]

The ^{13}C NMR spectrum and the mass data of *m/z* 378 were in good agreement with structure **22**. The stereochemistry at C-6 was established from observing an NOE between one of the H-5 protons (δ 2.56) and H-11. No NOE was observed between H-2 and H-11 as would be expected from the diastereoisomer with the opposite configuration at C-6, see Scheme 5.

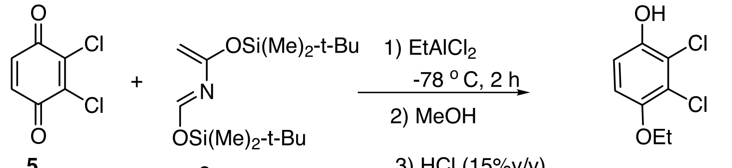
Adduct **22** hydrolyzed to **25** during storage in an NMR tube (CDCl$_3$) for a few days at 4°C, presumably due to the presence of a trace of water (Scheme 6). The ^{1}H NMR spectrum of **25** showed an additional signal for a hydroxyl proton at δ 3.75 and signals for the formamide group at δ 8.6 and 9.5, but lacked the proton resonance at δ 6.23 due to H-2 of **22**. The ^{13}C NMR spectrum of **25** showed three carbonyl resonances (δ 160.9, 168.9, 175.9) and lacked the ortho-amide carbon resonance present in **22**. Mass spectral data [263, M$^+$, 265 (M+2)$^+$] was also in good agreement with the structure **25**. It is not known whether **22** (and **23**, **24**, see below) is formed by a concerted or two-step process. Calculations currently in progress indicate that it is a two-step process with initial addition of the quinone oxygen to the imino moiety of **9**.[20]

Two other quinones were subjected to the Diels–Alder reaction with the azadiene **9** to see if they also reacted preferentially with the carbonyl group. Reaction of 2,5-dichlorobenzoquinone (**20**) with **9** in diethyl ether at room temperature produced the spiro compound **23** in 44% yield and none of the "normal" Diels–Alder product (Scheme 5). The structure of **23** was deduced from NMR data following arguments parallel to those applied for **22**. High resolution mass data confirmed the formula. The stereochemistry was again assigned on the basis of an NOE effect observed between H-5 and H-11 and lack of NOE between H-2/H-11. Compound **23** hydrolyzed to **26** upon storage in chloroform for a few days. Reduction of **23** with Zn/AcOH afforded the phenol derivative **27**. Ultrasonication-promoted[21] reaction of **20** and **9** in ether yielded spiro adduct **23** in 28% yield, but none of the desired Diels–Alder product. A *t*-butyldimethylsilyl triflate catalyzed reaction[22] of **20** and **9** in ether gave **23** in 3% yield and 75% of the starting material was recovered.

When 2,5-dichloroquinone **20** was reacted with the diene **9** in chloroform at refluxing temperature, 5% of the adduct **23** was obtained and 77% unreacted quinone was recovered.

Dienophile 2,5-dimethylthioquinone **21** did not react with **9** under normal refluxing conditions. However, when **21** and **9** were reacted in 5 M LiClO$_4$/ether[23,24] at room temperature, the diastreoisomers **24a** and **24b** were obtained in a 1:4 ratio. The ^{1}H NMR spectra of **24a** and **24b** showed similar resonances except that the signals for the C-5 diastereoisomeric protons were about 0.5 ppm apart in the spectrum of **24a** whereas they were 1.0 ppm apart in **24b**. The structures of these diastereoisomers were deduced from ^{1}H, ^{13}C NMR spectra and NOE analysis following arguments similar to those invoked for **22**. The molecular formula, $C_{17}H_{28}NO_4S_2Si$, was confirmed for **24b** from high resolution FAB MS analysis. For the minor diastereoisomer, **24a**, the olefinic proton resonance at C-11 showed NOE with one of the protons at C-5 (δ 2.96) and the methylthio group at δ 2.20, while the methylthio group at δ 2.36 showed NOE with the H-2 proton and the olefinic proton at H-8. This is only consistent with the isomer **24a**. The signal for the olefinic proton at C-11 in **24b** showed an NOE with H-2 and one of the proton signals at C-5 (δ 3.28), while both C-5 protons showed an NOE with one of the methylthio groups (δ 2.36). This is only consistent with structure **24b** in the conformation shown.

In order to promote the desired reaction between **5** and **9** Lewis acid catalysis was tried. When ethylaluminum dichloride was used to catalyze the reaction in CH$_2$Cl$_2$ at −78°C (Scheme 7), the *O*-ethylated hydroquinol **28** was obtained in 75–85% yield. This reaction and related ones are reported elsewhere.[25–27]



Scheme 7.

3. Biological evaluation

Natural perfragilin B was found to be cytotoxic to P-388 murine leukemia cells (ED_{50} 0.07 mg/mL). Synthetic perfragilin B was tested against the NCI 60-cell line tumor panel[8] where the average molar $\log_{10} GI_{50}$ for five leukemia cell lines was −6.24, and the mean-graph midpoint $\log GI_{50}$ against all cell lines was −5.34. Some selectivity against renal and breast cell lines was observed in the overall panel evaluation. Pefragilin B (**2**) was evaluated further in NCI's in vivo hollow fiber assay against 12 cell lines but was found not to meet NCI's criteria for further in vivo testing. Perfragilin B was also found to be inactive in an in vitro anti-HIV drug screen. Perfragilin B (**2**) and synthetic compounds **11**–**13**, **16**, and **19** were screened in the Corbett–Valeriote soft agar diffusion assay[28] which evaluates compounds for differential cytotoxicity between human leukemia and various human and murine solid tumors. None of the compounds showed solid tumor selectivity. Compound **16** was slightly more cytotoxic to two leukemia cell lines (murine L1210 and human CCRF-CEM) than perfragilin B (**2**); zone sizes in the primary assay were as follows: [μg/disk, L1210/CCRF-CEM] **2**: 16.5, 450/200; **16**: 18, 650/500. The activity of **16** is interesting from a structure–activity relationship point of view in that perfragilin A (**1**), which has a similar substitution pattern but in which the aromatic amino group is not substituted, is much less active in vitro than perfragilin B (**2**) (see opening paragraph).

4. Conclusions

In summary, an improved synthesis of perfragilin B (45% overall from **5**) was developed along with the synthesis of two *N*-alkylated analogues **11** and **12**. Perfragilin A (**1**) and various of its amino alkylated analogues were prepared by regioselective displacement of one thiomethoxy group in perfragilin B (**2**). Perfragilin B was found to be inactive in vivo anticancer testing (hollow fiber assay) although it is quite cytotoxic. The synthetic analogues of perfragilin A and B were in general less cytotoxic than **2**, except for **16** which was slightly more cytotoxic.

5. Experimental

5.1. General

High resolution fast atom bombardment mass spectra (HRFABMS) were recorded in a 3-NBA matrix in the positive ion mode on a VG ZAB-E mass spectrometer. Low resolution electron-impact mass spectra (12 eV) were measured on a Hewlett Packard 5985 instrument. NMR experiments were performed on Varian XL-300, VXR-400 and VXR-500 instruments; signals are reported in parts per million (δ), referenced to the solvent used. All NMR pulse sequences were run using standard Varian software version 4.3. IR spectra were recorded on a Bio-Rad 3240-spc FT spectrophotometer. Freshly purified samples were used for measurement of physical constants and spectral data. The reaction mixtures were separated using preparative TLC plates coated with silica gel using a Chromatotron model 7924 (Harrison Research Co.). Freshly purified samples were used for measurement of physical constants and spectral data.

5.1.1. 4,5-Dichlorotricyclo[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-dione (6). 2,3-Dichloro-1,4-benzoquinone (**5**) was prepared from 1,4-benzoquinone according to the procedure of Norris[13] except that the intermediate 5,6-dichlorocyclohexen-2-ene-1,4-dione was prepared by the following procedure.[14] To a stirred solution of benzoquinone (600 mg, 5.56 mmol) in dry ether (14 mL) was slowly added (3 mL/h) a solution of sulfuryl dichloride (1.0 mL, 13.24 mmol) in ether (6 mL) which had previously been treated with triethylamine (0.08 mL, 0.28 mmol, see below).[14] After the addition, the mixture was stirred for an additional 30 min and the solvent was evaporated on a rotary evaporator first using aspirator pressure and then a high vacuum to produce a slightly yellow solid in quantitative yield. This product was used directly for preparing **6**.

To a solution of 2,3-dichloro-1,4-benzoquinone (**5**, 1.0 mmol) dissolved in ether (5 mL) was added at room temperature 0.2 mL of freshly distilled cyclopentadiene. The solution was stirred for 5 h and then solvent removed under reduced pressure first using a water aspirator, then under high vacuum for 2 h. The product **6** was analyzed by ^{1}H NMR and this indicated the presence only the *endo* adduct in quantitative yield. Light yellow solid mp 104–106°C (lit.[15] 109–110°C); ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 1H, *J*=8.8 Hz, H-11), 1.59 (d, 1H, *J*=8.8 Hz, H-11), 3.42 (dd, 2H, *J*=1.8 Hz, H-2, H-7), 3.61 (m, 2H, H-1, H-8), 6.10 (t, 2H, *J*=1.8 Hz, H-9, H-10); ^{13}C NMR ($CDCl_3$) δ 48.6 (C-2, C-7), 48.9 (C-1, C-8), 49.5 (C-11), 135.4 (C-9, C-10), 147.3 (C-4, C-5), 188.8 (C-3, C-6) ppm; LREIMS obs *m/z* 241.9 (100%), 243.9 (60.6%).

5.1.2. 4,5-Dithiomethoxytricyclo[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-dione (7). A solution of 4,5-dichlorotricyclo-[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-dione (**6**, 341 mg, 1.40 mmol) in 20 mL of dichloromethane was placed into a separatory funnel. To this mixture was added at once a solution of sodium thiomethoxide (197 mg, 2.81 mmol) and tetrabutylammonium hydrogen sulfate (20 mg, 0.06 mmol) in 20 mL of water. The mixture was shaken for 2 min and the phases separated. The organic phase was washed with water (8 mL), dried over anhydrous sodium sulfate, and the solvent evaporated under reduced pressure producing **7** as a yellow solid (361 mg, 97%). Mp(crude) 110–112°C (lit.[15]113–115°C); ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 1H, J=8.8 Hz, H-11), 1.61 (d, 1H, J=8.8 Hz, H-11), 2.46 (s, 3H, Me), 3.33 (dd, 2H, J=1.8 Hz, H-2, H-7), 3.45 (m, 2H, H-1, H-8), 6.08 (t, 2H, J=1.8 Hz, H-9, H-10) ppm; ^{13}C NMR ($CDCl_3$) δ 16.8 (Me), 46.8 (C-2, C-7), 48.2 (C-1, C-8), 50.5 (C-11), 136.1 (C-9, C-10), 150.2 (C-4, C-5), 191.4 (C-3, C-6) ppm.

5.1.3. 2,3-Di-(thiomethoxy)-1,4-benzoquinone (8). Compound **7** (60 mg, 0.22 mmol) was ground to a fine powder and placed in the terminal round bottom flask (10 mL) of a triple bulb (10 mL ea.) Kugelrohr short-path distillation tube. This glassware assembly was connected to a vacuum pump (0.5 mmHg) and placed into a Kugelrohr oven preheated to 165–170°C with one bulb remaining outside for cooling with dry ice. After 3–4 min a red material distilled to the edge of the first bulb. The distillation bulbs were removed from the oven and left to cool under vacuum. The reaction product (49 mg crude yield) was removed from the first bulb by dissolving it in dichloromethane. ^{1}H NMR analysis indicated that the mixture was composed of **8** and the corresponding hydroquinone of **7**[15] in a ratio of 9:1; ^{1}H NMR ($CDCl_3$) δ 2.63 (s, 6H, SMe), 6.72 (s, 2H, H_{olefin}).

5.1.4. *N*-Demethyl-perfragilin B (10)[9] [2,3,5,8-tetrahydro-6,7-di-(thiomethoxy)-3,5,8-trioxoisoquinoline]. A solution of **8** (15 mg, 0.075 mmol) and azadiene **9** (90 mg, 0.225 mmol) in dry benzene (1.5 mL) was refluxed for 3 h under nitrogen. To this hot mixture was added 5 drops of concentrated hydrochloric acid and heating continued for 15 more minutes. After cooling, the solvent was evaporated under reduced pressure (water aspirator) then under high vacuum for 3 h. The residue was chromatographed on a silica gel column and eluted initially with CH_2Cl_2 (150 mL) then with CH_2Cl_2/MeOH (95:5). The desired product **10** (12.6 mg, 63%) is orange and moved on the column only when the latter solvent mixture was introduced. Orange solid; mp 208–209°C; IR (film) 1683 (s), 1652 (s), 1630 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.69 (s, 3H, SMe), 2.76 (s, 3H, SMe), 5.92 (s, 1H, N–H), 7.12 (s, 1H, H-4), 8.21 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.1 (SMe), 18.7 (SMe), 113.3 (C-9), 117.9 (C-4), 138.7 (C-1), 141.2 (C-10), 146.9 (C-6), 151.2 (C-9), 157.2 (C-3), 175.1 (C-8), 176.3 (C-5); LREIMS 12 eV) m/z (relative intensity) 267 (M^+, 27), 252 (100); HRFABMS obs. m/z 268.0102 ($[M+1]^+$ (calcd for $C_{11}H_9NO_3S_2$, 268.0102).

5.2. General procedure for obtaining 2, 12 and 13 in DMSO

To a solution of **10** (0.1 mmol) in 1 mL of DMSO was added the appropriate alkylating agent (20–40 equiv.), anhydrous potassium carbonate (0.3 mmol) and 1–2 drops of TDA-1.[17] The mixture was stirred at room temperature under nitrogen for 3 h, poured into (15 mL) water and then extracted with dichloromethane (5×15 mL). The combined organic phase was washed with brine (3×15 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was chromatographed over a silica gel column and eluted with CH_2Cl_2/MeOH (95:5).

5.2.1. Perfragilin B (2)[9] [2,3,5,8-tetrahydro-6,7-di-(thiomethoxy)-2-methyl-3,5,8-trioxoisoquinoline]. Obtained in 75% yield (13.9 mg from 17 mg of **10**) using methyl iodide as the alkylating agent, mp 120–121°C; IR (film) ν_{max} 1679 (m), 1635 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.68 (s, 3H, SMe), 2.74 (s, 3H, SMe), 3.65 (s, 3H, N–Me), 7.08 (s, 1H, H-4), 8.24 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.1 (SMe), 18.7 (SMe), 38.5 (N–Me), 111.9 (C-9), 117.4 (C-4), 139.7 (C-10), 142.4 (C-1), 147.3 (C-6), 150.7 (C-7), 162.5 (C-3), 175.6 (C-8), 176.6 (C-5).

5.2.2. *N*-Demethyl-*N*-*n*-butyl perfragilin B (11) [2,3,5,8-tetrahydro-6,7-di-(thiomethoxy)-2-*n*-butyl-3,5,8-trioxoisoquinoline]. Obtained in 83% yield (10.6 mg from 10 mg of **10**) using *n*-butyl iodide as the alkylating agent. IR (film) ν_{max} 1694 (m), 1654 (m), 1636 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J=7 Hz, Me), 1.37 (t, 2H, J=7 Hz, CH_2), 1.75 (p, 2H, J=7 Hz, CH_2), 2.67 (s, 3H, SMe), 2.74 (s, 3H, SMe), 4.01 (t, 2H, J=7 Hz, CH_2–N), 7.06 (s, 1H, H-4), 8.24 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 13.5 (Me), 18.0 (SMe), 18.6 (SMe), 19.7 (CH_2), 31.1 (CH_2), 50.5 (N–CH_2), 111.7 (C-9), 117.7 (C-4), 139.2 (C-10), 141.6 (C-1), 147.2 (C-6), 150.6 (C-7), 162.0 (C-3), 175.3 (C-5), 176.6 (C-8); LREIMS (12 eV) m/z (relative intensity) 325 (M^+, 41), 308 (100); HRFABMS obs. m/z 326,0884 ($[M+3]^+$ (calcd for $C_{15}H_{20}NO_3S_2$, 326.0884).[29]

5.2.3. *N*-Demethyl-*N*-benzyl perfragilin B (12) [2,3,5,8-tetrahydro-6,7-di-(thiomethoxy)-2-benzyl-3,5,8-trioxoisoquinoline]. Obtained in 79% yield (6.3 mg from 6 mg of **10**) yield using benzyl chloride as the alkylating agent. IR (film) ν_{max} 1694 (m), 1654 (m), 1636 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H, SMe), 2.71 (s, 3H, SMe), 5.19 (s, 2H, N–CH_2), 7.12 (s, 1H, H-4), 8.23 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.1 (SMe), 18.7 (SMe), 53.0 (N–CH_2), 112.2 (C-9), 118.0 (C-4), 128.5 (Ph), 128.8 (Ph), 129.2 (Ph), 134.7 (Ph), 139.4 (C-10), 141.5 (C-1), 147.2 (C-6), 150.8 (C-7), 162.1 (C-3), 175.2 (C-5), 176.5 (C-8); HRFABMS obs. m/z 360.0665 ($[M+3]^+$ (calcd for $C_{18}H_{18}NO_3S_2$, 360.0728).[29]

5.3. Reaction of 10 with methyl iodide in DMF

To a solution of **10** (17 mg, 0.064 mmol) in 1.5 mL of DMF was added methyl iodide (12 equiv., 0.05 mL), anhydrous potassium carbonate (18 mmol) and TDA-1 (2 drops). The mixture was stirred at 70°C under nitrogen for 2 h, poured into 15 mL of water and then extracted with ethyl acetate (5×10 mL). The combined organic phase was washed with brine (3×15 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed over silica gel and eluted with CH_2Cl_2/MeOH (95:5). Perfragilin B (**2**) was obtained in 51% yield along with **13**–**15**.

5.3.1. 2,3,5,8-Tetrahydro-4,6,7-tri-(thiomethoxy)-2-methyl-3,5,8-trioxoisoquinoline (13). Obtained in 7% (1.5 mg) yield. IR (film) ν_{max} 1684 (s), 1652 (s), 1630 (s), 1558 (s) (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H, Me), 2.66 (s, 3H, Me), 2.68 (s, 3H, Me), 3.63 (s, 3H, N–Me), 8.10 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.0 (SMe), 18.1 (SMe), 18.2 (SMe), 39.0 (N–Me), 112.6 (C-9), 135.4 (C-10), 138.2 (C-1), 145.7 (C-4), 146.6 (C-6), 150.9 (C-7), 160.8 (C-3), 175.3 (C-8), 177.4 (C-5); LRFABMS *m/z* (relative intensity) 328 [$(M+1)^+$, 15]; HRFABMS obs. *m/z* 328.0145 ($[M+1]^+$ (calcd for $C_{13}H_{14}NO_3S_3$, 328.0136).

5.3.2. 2,3,5,8-Tetrahydro-7-dimethylamino-6-thiomethoxy-2-methyl-3,5,8-trioxoisoquinoline (14). Obtained in 15% (2.5 mg) yield. IR (film) ν_{max} 3233 (bm), 1684 (s), 1645 (s), 1559 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24 (s, 3H, SMe), 3.26 (s, 6H, NMe_2), 3.63 (s, 3H, NMe), 7.07 (s, 1H, H-4), 8.17 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.1 (weak, SMe), 38.4 (N–Me), 44.8 (NMe_2), 111.8 (C-9), 116.0 (C-4), 116.6 (C-6, very weak), 139.7 (C-10), 142.2 (C-1), (C-7, not obs.), 163.0 (C-3), (C-8, not obs.), 179.6 (C-5); LREI (12 eV) *m/z* 279.9 [M^+] (88%), 263.0 $[M-15]^+$ (100%).

5.3.3. 2,3,5,8-Tetrahydro-7-methylamino-6-thiomethoxy-2-methyl-3,5,8-trioxoisoquinoline (15). Obtained in 17% (3 mg) yield. IR (film) ν_{max} 1684 (m), 1652 (s), 1600 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, SMe), 3.48 (d, 3H, J=6 Hz, NHMe), 3.64 (s, 3H, NMe), 6.90 (bs, 1H, NH), 7.16 (s, 1H, H-4), 8.27 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.5 (very weak, SMe), 33.7 (NH–Me), 38.4 (NMe), 116.6 (C-4), (C-9, not obs.), 131.3 (C-6), 140.2 (C-10), 142.5 (C-1), (C-7, not obs.), 163.0 (C-3), 176.9 (C-5), (C-8, not obs.); LRFABMS *m/z* (relative intensity) 264 (M^+, 100), 249 (56), 231 (29); HRFABMS obs. *m/z* 265.0643 ($[M+1]^+$ (calcd for $C_{12}H_{13}N_2O_3S$, 265.0647).

5.4. Reaction of 10 with *n*-butyl chloride in DMF

To a solution of **10** (13 mg, 0.05 mmol) in 1.5 mL of DMF was added *n*-butyl chloride (0.05 mL), anhydrous potassium carbonate (15 mg, 0.15 mmol) and TDA-1 (2 drops). The mixture was stirred at 70°C under nitrogen for 5 h, poured into 20 mL of water and then extracted with ethyl acetate (4×15 mL). The combined organic phase was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed on a silica gel TLC preparative plate (500 mm) and eluted with CH_2Cl_2/MeOH (95:5).

5.4.1. 2,3,5,8-Tetrahydro-7-dimethylamino-6-thiomethoxy-2-*n*-butyl-3,5,8-trioxoisoquinoline (16). Obtained in 33% yield (5.2 mg) yield. IR (film) ν_{max}1684 (m), 1654 (m), 1559 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3H, J=7 Hz, Me), 1.38 (sextet, 2H, J=7 Hz, CH_2), 1.71 (quint., 2H, J=7 Hz, CH_2), 2.21 (s, 3H, SMe), 3.24 (s, 6H, NMe_2), 3.98 (t, 2H, J=7 Hz, CH_2–N), 7.04 (s, 1H, H-4), 8.12 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 13.5 (Me), 18.1 (SMe), 19.8 (CH_2), 31.1 (CH_2), 44.7 (NMe_2), 50.4 (N–CH_2), 111.7 (C-9), 116.3 (C-4), 123.6 (C-6), 139.3 (C-10), 141.5 (C-1), 155.5 (C-7), 162.5 (C-3), 178.7 (C-8), 179.2 (C-5); LRFABMS *m/z* (relative intensity) 321 [$(M+1)^+$, 100), 307 (82); HRFABMS obs. *m/z* 321.1286 ($[M+1]^+$ (calcd for $C_{16}H_{21}N_2O_3S_2$, 321.1273).

5.4.2. 2,3,5,8-Tetrahydro-7-methylamino-6-thiomethoxy-2-*n*-butyl-3,5,8-trioxoisoquinoline (17). Obtained in 11% yield (1.2 mg) yield. IR (film) ν_{max} 3230 (bm), 1684 (s), 1652 (s), 1558 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, J=7 Hz, 3H, Me), 1.36 (sextet, J=7 Hz, 2H, CH_2), 1.73 (quint., J=7 Hz, 2H, CH_2), 2.29 (s, 3H, SMe), 3.47 (d, J=6 Hz, 6H, NHMe), 3.99 (t, J=7 Hz, CH_2–N), 6.76 (bs, 1H, NH), 7.13 (s, 1H, H-4), 8.22 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 13.6 (Me), (SMe, not obs.), 19.8 (CH_2), 31.2 (CH_2), 33.7 (NHMe), 50.5 (N–CH_2), (C-9, not obs.), 117.0 (C-4), (C-7, not obs.), 139.9 (C-10), 142.0 (C-1), 153.0 (very weak, C-7), 162.6 (C-3), 177.0 (very weak, C-5), 180.1 (very weak, C-8); LRFABMS *m/z* (relative intensity) 307 [$(M+1)^+$, 100); HRFABMS obs. *m/z* 307.1111 ($[M+1]^+$ (Calcd for $C_{15}H_{19}N_2O_3S_2$, 307.1116).

5.4.3. 5,8-Dihydro-6,7-di-(thiomethoxy)-3-butoxy-5,8-dioxoisoquinoline (18). Obtained in 5% yield. IR (film) ν_{max} 1735 (bm), 1664 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, 3H, J=7 Hz, Me), 1.46 (h, 2H, J=7 Hz, CH_2), 1.76 (p, 2H, J=7 Hz, CH_2), 2.67 (s, 3H, SMe),), 2.78 (s, 3H, SMe), 4.41 (t, 2H, J=7 Hz, CH_2–N), 7.20 (s, 1H, H-4), 8.85 (s, 1H, H-1); LREIMS *m/z* (relative intensity) 323 (M^+, 64), 308 (100), 251 (17); HRFABMS obs. *m/z* 326.0897 $(M+3)^+$ (Calcd for $C_{15}H_{19}NO_3S_2$ 326.0885).[29]

5.4.4. Perfragilin A (1) [2,3,5,8-tetrahydro-7-amino-6-thiomethoxy-2-methyl-3,5,8-trioxoisoquinoline]. To a solution of perfragilin B (**2**) (8 mg, 0.03 mmol) in acetonitrile (1.5 mL) was added a solution of concentrated NH_4OH (0.1 mL) and the mixture stirred at room temperature for 1 h. The reaction mixtures was initially concentrated in a rotary evaporator and then the residue dried under high vacuum overnight. Chromatography on a silica gel preparative TLC plate (500 mm) using CH_2Cl_2/MeOH (95:5) as eluent afforded $\mathbf{1_a}$ as a yellow solid, 22% (1.7 mg) yield. ^1H NMR ($CDCl_3$) δ 2.68 (s, 3H, SMe), 3.64 (s, 3H, N–Me), 7.17 (s, 1H, H-4), 8.29 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 16.7 (SMe), 38.4 (N–Me), 110.5 (C-6), 113.1 (C-9), 117.2 (C-4), 140.2 (C-10), 142.5 (C-1), 152.1 (C-7), 162.9 (C-3), 175.2 (C-8), 176.9 (C-5); LRFABMS *m/z* 251 (16%); HRFABMS obs. *m/z* 251.0496 $[M+3]^+$ (calcd for $C_{11}H_{10}N_2O_3S$, 251.0490).[29]

5.4.5. *N*-Benzyl perfragilin A (19) [2,3,5,8-tetrahydro-7-benzylamino-6-thiomethoxy-2-methyl-3,5,8-trioxoisoquinoline]. To a solution of perfragilin B (**2**) (15 mg, 0.05 mmol) in CH_2Cl_2 (5 mL) was added benzylamine (0.05 mL) and the mixture stirred at room temperature for 2 h. The deep red reaction mixture was initially concentrated in a rotary evaporator, then worked up in the same manner as for **1** to give **19** as a yellow glass in 29% (5 mg) yield. IR (film) ν_{max} 3309 (bm), 1684 (s), 1652 (s), 1558 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H, SMe), 3.65 (s, 3H, NMe), 6.95 (bs, 1H, N–H), 7.15 (s, 1H, H-4), 8.27 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 18.2 (very weak, SMe), 38.4 (NCH_3), 49.8 (CH_2), (C-9, not obs.), 116.7 (C-4), 125.6 (C-6), 127.5 (C-H, *o* or *m*-Ph), 127.9 (C-H, *p*-Ph), 129.0 (C-H, *o* or *m*-Ph), 137.8 (C, Ph), 140.1 (C-10), 142.9 (C-1), 157.2 (C-7, very weak), 162.9 (C-3), 176.8 (C-5), (C-8, not obs.); LREIMS *m/z* (relative intensity) 340 (M^+, 100); HRFABMS obs. *m/z* 341.0947 $[M+1]^+$ (calcd for $C_{18}H_{17}N_2O_3S$, 341.0960).

5.4.6. 1-Oxa-3-aza-2-(*t*-butyldimethylsilyloxy)-7,8-dichloro-4,9-dioxospiro[5,5]undeca-7,10-diene (22). A solution of **5** (24 mg, 0.14 mmol) and **9** (71 mg, 0.19 mmol) in anhydrous diethyl ether (1 mL) was refluxed under a nitrogen atmosphere for 3.5 h. After cooling to rt the reaction was quenched by addition of methanol and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel flash column using dichloromethane/acetone (95:5) to give **22** as yellow solid (13.5 mg, 26% yield); mp 109–110°C; IR (film) ν_{max} 3290 (br), 1685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.13 (s, 3H, Si–Me), 0.16 (s, 3H, Si–Me), 0.89 (s, 9H, Si–*t*-Bu), 2.56 (d, 1H, *J*=16 Hz, H-5′), 3.09 (d, 1H, *J*=16 Hz, H-5), 6.23 (d, 1H, *J*=2 Hz, H-2), 6.31 (d, 1H, *J*=10 Hz, H-10), 6.46 (br, 1H, NH), 7.16 (d, 1H, *J*=10 Hz, H-11); ^{13}C NMR ($CDCl_3$) δ −5.2 (Si–Me), −4.5 (Si–Me), 17.7 (Si–*t*-Bu), 25.4 (Si–*t*-Bu), 39.1 (C-5), 73.7 (C-6), 95.3 (C-2), 125.3 (C-10), 133.2 (C-8), 147.5 (C-11), 149.8 (C-7), 165.7 (C-4), 175.9 (C-9); LRFABMS *m/z* 378 (relative intensity) [(M+H)$^+$, 64), 380 [(M+H+2)$^+$, 42].

5.4.7. 1-Oxa-3-aza-2-(*t*-butyldimethylsilyloxy)-7,10-dichloro-4,9-dioxospiro[5,5]undeca-7,10-diene (23). A solution of **20** (50 mg, 0.28 mmol) and **9** (135 mg, 0.34 mmol) in anhydrous diethyl ether (1 mL) was refluxed under a nitrogen atmosphere for 3 h. After cooling to rt the reaction was quenched by addition of methanol and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel flash column using dichloromethane/acetone (95:5) to give **23** as yellow solid (48 mg, 44% yield); mp 119–120°C; IR (film) ν_{max} 3240 (br), 1682 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 3H, Si–Me), 0.14 (s, 3H, Si–Me), 0.88 (s, 9H, Si–*t*-Bu), 2.59 (d, 1H, *J*=16 Hz, H-5′), 3.04 (d, 1H, *J*=16 Hz, H-5), 6.18 (d, 1H, *J*=2 Hz, H-2), 6.52 (d, 1H, *J*=10 Hz, H-8), 7.06 (br, 1H, NH), 7.39 (d, 1H, *J*=10 Hz, H-11); ^{13}C NMR ($CDCl_3$) δ −5.2 (Si–Me), −4.5 (Si–Me), 17.7 (Si–*t*-Bu), 25.4 (Si–*t*-Bu), 38.5 (C-5), 73.5 (C-6), 95.2 (C-2), 127.8 (C-8), 131.5 (C-10), 143.1 (C-11), 154.5 (C-7), 165.7 (C-4), 176.0 (C-9); HRFABMS obs. *m/z* 378.0667 [M+1, 49]$^+$ (calcd for $C_{15}H_{22}NO_4SiCl_2$, 378.0695), 380.0609 [(M+H+2)$^+$, 38].

5.4.8. 1-Oxa-3-aza-2-(*t*-butyldimethylsilyloxy)-7,10-di-(methylthio)-4,9-dioxospiro[5,5]undeca-7,10-diene (24a and 24b). A solution of **21** (60 mg, 0.30 mmol) and **9** (225 mg, 0.56 mmol) in 0.5 M LiClO4/ether was stirred at rt for 48 h. The reaction was quenched by addition of 45 mL of cold water and extracted with chloroform (3×45 mL). The combined chloroform extracts were evaporated under reduced pressure and the residue chromatographed on a silica a gel flash column using a gradient from dichloromethane to acetone. After repetitive chromatography **24a** and **24b** were obtained as yellow solids in 4% and 16% (20.9 mg) yield, respectively; **24a**; mp 173–174°C; IR (film) ν_{max} 3235 (br), 1651, 1567, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 3H, Si–Me), 0.15 (s, 3H, Si–Me), 0.89 (s, 9H, Si–*t*-Bu), 2.20 (s, S–Me at C-10), 2.36 (s, 3H, S–Me at C-7), 2.56 (d, 1H, *J*=16 Hz, H-5′), 2.96 (d, 1H, *J*=16 Hz, H-5), 6.02 (s, 1H, H-8), 6.18 (d, *J*=2 Hz, 1H, H-2), 6.45 (br, 1H, NH), 6.60 (s, 1H, H-11); ^{13}C NMR ($CDCl_3$) δ −5.1 (Si–Me), −4.3 (Si–Me), 13.2 (S–Me at C-10), 14.3 (S–Me at C-7), 17.8 (Si–*t*-Bu), 25.5 (Si–*t*-Bu), 43.5 (C-5), 74.4 (C-6), 95.2 (C-2), 118.8 (C-8), 134.7 (C-11), 138.7 (C-10), 166.1 (C-7), 166.7 (C-4), 178.2 (C-9).

24b: mp 171–172°C; IR (film) ν_{max} 3217 (br), 1692, 1643, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.18 (s, 3H, Si–Me), 0.19 (s, 3H, Si–Me), 0.90 (s, 9H, Si–*t*-Bu), 2.19 (s, S–Me at C-10), 2.36 (s, 3H, S–Me at C-7), 2.36 (d, 1H, *J*=16 Hz, H-5′), 3.28 (d, 1H, *J*=16 Hz, H-5), 6.04 (s, 1H, H-8), 6.12 (d, 1H, *J*=2 Hz, H-2), 6.31 (s, 1H, H-11), 6.45 (br, 1H, NH); ^{13}C NMR ($CDCl_3$) δ −5.2 (Si–Me), −4.5 (Si–Me), 13.4 (S–Me at C-10), 14.5 (S–Me at C-7), 17.9 (Si–*t*-Bu), 25.5 (Si–*t*-Bu), 43.7 (C-5), 75.1 (C-6), 94.3 (C-2), 118.8 (C-8), 131.3 (C-11), 140.3 (C-10), 166.7 (C-7), 167.3 (C-4), 177.9 (C-9); HRFABMS *m/z* 402.1265 [M+1]$^+$ (calcd for $C_{17}H_{28}NO_4SiS_2$, 402.1229).

5.5. General procedure for obtaining 26 and 27

When the compounds **22** (2 mg) and **23** were stored in $CDCl_3$ at 4°C for several days they hydrolyzed to **26** and **27**, respectively, in quantitative yields.

5.5.1. 2,3-Dichloro-4-*N*-formylacetamido-4-hydroxy-cyclohexadienone (25). Yellow solid; mp 112–113°C; IR (film) ν_{max} 3305 (br), 1743, 1683, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.65 (d, 1H, *J*=16 Hz, H-7′), 3.24 (d, 1H, *J*=16 Hz, H-7), 6.39 (d, 1H, *J*=10 Hz, H-6), 7.09 (d, 1H, *J*=10 Hz, H-5), 8.60 (br, 1H, NH), 9.05 (d, 1H, *J*=10 Hz, H-10); ^{13}C NMR ($CDCl_3$) δ 45.5 (C-7), 71.7 (C-4), 127.0 (C-6), 133.3 (C-2), 146.5 (C-5), 150.6 (C-3), 160.9 (C-10), 168.9 (C-8), 175.9 (C-1); LREIMS *m/z* 263 (M$^+$, 64), 265 (M+2)$^+$.

5.5.2. 2,5-Dichloro-4-*N*-formylacetamido-4-hydroxy-cyclohexadienone (26). Yellow solid. IR (film) ν_{max} 3400–3250, 1743, 1682 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 2.70 (d, 1H, *J*=15 Hz, H-7′), 2.90 (d, 1H, *J*=15 Hz, H-7), 6.38 (s, 1H, H-6), 7.12 (s, 1H, H-5), 8.90 (s, 1H, H-10); LREIMS *m/z* 263 (M$^+$, 64), 265 (M+2)$^+$.

5.5.3. 2′,5′-Dichloro-4-hydroxyphenylacetamide (27). A solution of **23** (24 mg, 0.063 mmol) in ether was refluxed with zinc dust (15 mg) and acetic acid (0.5 mL) overnight. The resulting mixture was partitioned between chloroform and water. The combined chloroform layers afforded **27** as white solid in a quantitative yield after removal of solvent; mp 109–110°C; IR (film) ν_{max} 3350, 1668 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 3.67 (s, 2H, H-2), 6.95 (s, 1H, H-3′), 7.20 (s, 1H, H-6′), 9.06 (br s, OH); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 39.7 (C-2), 117.3 (C-3′ or 6′), 119.6 (C-3′ or 6′), 123.0 (C-1′), 132.4 (C-2′ or 5′), 133.4 (C-2′ or 5′), 153.2 (C-4′), 163.4 (C-1); Low Resolution Thermospray MS *m/z* 220 (M+H)$^+$, 222 (M+H+2) $^+$, 237 (M+NH_4)$^+$, 239 [(M+NH_4+2)$^+$.

Acknowledgements

A Fellowship from CAPES (Brazil) to V. F. F. is gratefully acknowledged. This work was supported in part by Department of Commerce, NOAA Sea Grant Project NA66RGO172.

References

1. Schmitz, F. J.; De Guzman, F. S.; Choi, Y.-H.; Hossain, M. B.;

Rizvi, S. K.; van der Helm, D. *Pure Appl. Chem.* **1990**, *62*, 1393–1396.
2. Choi, Y.-H.; Park, A.; Schmitz, F. J.; van Altena, I. J. *Nat. Prod.* **1993**, *56*, 1431–1433.
3. Rizvi, S. K.; Hossain, M. B.; van der Helm, D. *Acta Crystallogr., Sect. C* **1993**, *49*, 151–154.
4. Frincke, J. M.; Faulkner, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 265–269.
5. McIntyre, D. E.; Faulkner, D. J.; van Engen, D.; Clardy, J. *Tetrahedron Lett.* **1979**, 4163–4166.
6. McKee, T. C.; Ireland, C. M. *J. Nat. Prod.* **1987**, *50*, 754–756.
7. Pettit, G. R.; Collins, J. C.; Herald, D. L. D.; Boyd, M. R.; Schmidt, J. M.; Hooper, N. A.; Tackett, L. P. *Can. J. Chem.* **1992**, *70*, 1170–1175.
8. Boyd, M. R.; Paull, K. D. *Drug Development Res.* **1995**, *34*, 91–108.
9. Schmitz, F. J.; Park, A. *Tetrahedron Lett.* **1993**, *34*, 3983–3984.
10. Echavarren, A. M.; Stille, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 4049–4053.
11. McKillop, A.; Brown, S. P. *Synth. Commun.* **1987**, *17*, 657–667.
12. Fukumi, H.; Kurihara, H.; Hata, T.; Tamura, C.; Mishima, H.; Kubo, A.; Arai, T. *Tetrahedron Lett.* **1977**, 3825–3828.
13. Norris, R. K.; Sternhell, S. *Aust. J. Chem.* **1973**, *26*, 333–343.
14. Ferreira, V. F.; Schmitz, F. *J. Org. Prep. Proced. Int.* **1998**, *30*, 115–116.
15. Wladislaw, B.; Marzorati, L.; Di Vita, C. *Synthesis* **1983**, 464–466.
16. Sainte, F.; Serckx-Poncin, B.; Hesbain-Frisque, A.-M.; Ghosez, L. *J. Am. Chem. Soc.* **1982**, *104*, 1428–1430.
17. Soula, G. *J. Org. Chem.* **1985**, *50*, 3717–3721.
18. Armarego, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*; Butterworth-Heinemann: Oxford, 1992; pp 192–193.
19. Gould, S. J.; Melville, C. K. *Tetrahedron Lett.* **1997**, *38*, 1473–1476, In the case of **14** an instrument problem contributed to the weakness of the spectrum. Compound **14** decomposed by the time a second spectral analysis was attempted.
20. Personal communication from Dr V. F. Ferreira.
21. Lee, J.; Mei, H. S.; Snyder, J. K. *J. Org. Chem.* **1990**, *55*, 5013–5016.
22. Lamg-Schelkens, H.; Giomi, D.; Ghosez, L. *Tetrahedron Lett.* **1989**, *30*, 5887–5890.
23. Braun, R.; Sauer, *J. Chem. Ber.* **1986**, *119*, 1269–1274.
24. Grieco, P. A. In *Organic Chemistry: Its Language and Its State of Art*; Kisakürek, M. V., Ed.; VHCA/VCA: Basel/Weinheim, 1993; pp 133–146.
25. Florjanczyk, Z.; Kuran, W.; Pasynkiewicz, S.; Kwas, G. *J. Organomet. Chem.* **1976**, *112*, 21–28.
26. Florjanczyk, Z.; Szymanska-Zachara, E. *J. Organomet. Chem.* **1983**, *259*, 127–137.
27. Ferreira, V. F.; Schmitz, F. J. *J. Organomet. Chem.* **1998**, *571*, 1–6.
28. (a) Valeriote, F.; Corbett, T.; LoRusso, P.; Moore, R. E.; Scheuer, P. J.; Patterson, G.; Paul, V.; Grindey, G.; Bonjouklian, R.; Pearce, H.; Suffness, M. *I. J. Pharmacog.* **1995**, *33*, 59–66. (b) Valeriote, F.; Corbett, T.; Edelstein, M.; Baker, L. *Cancer Invest.* **1996**, *14*, 124–141. (c) Valeriote, F.; Grieshaber, C. K.; Media, J.; Pietraszkiewicz, H.; Hoffmann, J.; Pan, M.; McLaughlin, S. *J. Exp. Therap. Oncol.* **2002**, *2*, 228–236.
29. Quinones often show prominent M+2 ions in EIMS and M+3 ions in their FABMS corresponding to their reduced hydroquinol forms. See: Fu, X.; Hossain, M. B.; Schmitz, F. J.; van der Helm, D. *J. Org. Chem.* **1997**, *62*, 3810–3819, and references cited.