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A simple synthesis of cytotoxic endoperoxide lactones

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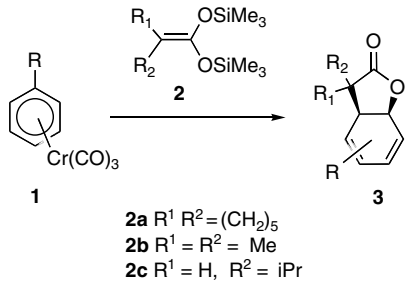
Abstract—A series of dihydrobenzofuran-2-one **3** have been submitted to various dienophiles: whereas maleic anhydride led only to the adducts **4a**,**b**, singlet oxygen gave the expected new endoperoxide lactones **5a**–**d**, **6** and **7a**,**b**, three of them showing interesting cytotoxicity towards various cancer cell lines.

The one-pot, double nucleophilic addition of bis (TMS) ketene acetals **2** to a carbon–carbon double bond of a series of arene chromium tricarbonyl complexes **1** (R = H, *i*Pr, *t*Bu, OPh, $SiMe_3$) induced successively by *t*BuOK and I_2, led in satisfactory yields (from 24% to 71%) to the bi- and tri-cyclic dienes **3**[1a–c] (Scheme 1).

Most of these compounds are however unstable due to the presence of the easy-to-cleave lactone function. They undergo indeed a facile rearomatization to the corresponding arylacetic acids in the presence of weak acids, precluding their purification even by silica gel chromatography. We thought that a way to stabilize these lactones would be to carry out 4 + 2 cycloadditions involving either olefines or oxygen.[2,3] If successful, such transformations would not only allow for the preservation of the lactone function, but also give access in the case of oxygen to a potentially important class of compounds, endoperoxide lactones. Indeed, whereas peroxide-containing substrates already show for some of them important cytotoxic properties,[4] peroxide-containing lactones are among the most active substrates towards *Plasmodium falciparum* malaria parasite,[5–9] but are now also recognized as potent cytotoxic substrates,[10] inhibitors of the mitochondrial respiratory chain,[11] for their activity against fungal pathogens,[12] AIDS opportunistic parasitic infections,[13] and their antiviral properties.[14]



Scheme 1.

In this letter, we disclose a series of transformations which demonstrate that such reactions can indeed lead to stable cycloadducts, and that moreover, the new polycyclic peroxide lactones revealed interesting cytotoxic properties.

The interaction of the dienes **3** (R = H, $R_1R_2 = C_5H_{10}$ or R_1R_2 = Me) with maleic anhydride however did not lead to the expected adducts: whereas no reaction took place at room temperature, decomposition of the dienes into the corresponding acids was observed in refluxing diethyl ether (see Scheme 2).

Only the trimethylsilyl-substituted dienes **3a** ($R_1R_2 = C_5H_{10}$) and **3b** (R_1R_2 = Me) slowly led, over five days at room temperature, to the adducts **4a** (10%) and **4b**

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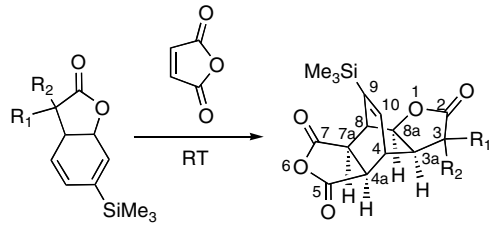
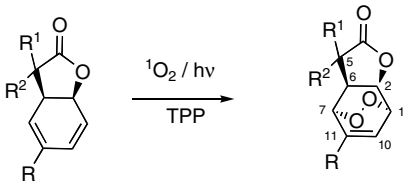


3a **4a**
3b **4b**

Scheme 2.



3c R = OPh R^1 R^2=$(CH_2)_5$ **5a**
3d R = H R^1 R^2=$(CH_2)_5$ **5b**
3e R = *i*Pr R^1 R^2=$(CH_2)_5$ **5c**
3f R = *t*Bu R^1 R^2=$(CH_2)_5$ **5d**

Scheme 3.

(23%) which were characterized both by mass and NMR spectroscopies.[15] The ^{13}C NMR spectrum of **4b** confirmed the presence of the anhydride and of the lactone functions with signals at, respectively, δ 171.3, 171.15 and 180.2 ppm. The ^1H NMR spectrum disclosed signals for a vinylic proton at δ 6.57 ppm (d, H_{10}, $J = 5.8$ Hz), the typical signal at δ 4.86 ppm for the proton geminated to the oxygen of the lactone (dd, H_{8a}, $J = 7.4$ and 3.6 Hz). The geometry of this adduct was assessed by an X-ray crystal structure determination, an *endo* addition of the double bond to the diene having taken place (Fig. 1).[16]

However, singlet oxygen appeared to be a more gratifying reagent since all of the dienes led to rather stable endoperoxides. Thus, irradiation of dichloromethane solutions of the diene **3c** in the presence of catalytic amounts of tetraphenylporphyrin (TPP) and oxygen led after 6 h to a new, more polar crystalline compound which could be purified by silica gel chromatography (58% yield, mp 96–98 °C). Its mass spectrum was in agreement with **5a**, the addition product of molecular oxygen to **3c** (see Scheme 3).

The NMR data of this adduct were in full agreement with structure **5a**.[17] The ^1H NMR spectrum disclosed, besides signals for the aromatic protons, low-field signals for the four protons H_1, H_2, H_7 and H_{10} whereas the ^{13}C NMR spectra confirmed first the presence of a lactone (δ 180.2 ppm), of the double bond of an enol ether with signals at δ 156.9 (C_{11}) and 97.1 (C_{10}), and of three signals for carbons bearing an oxygen atom, at δ 73.1 (C_7), 72.0 (C_1), 70.2 (C_2).

Similarly, the difficult-to-separate 6:5 mixture of the two enolethers **3g** and **3h** was oxidized under the same conditions (see Scheme 4). Fortunate enough, after 1 h of irradiation a complete disappearance of the less polar isomer **3g** was observed leading almost to a single, more polar compound which was easily purified by silicagel chromatography to give a white solid **6** (68% with respect to the starting dienol ether), mp 138 °C, **3h** being recovered. The NMR spectra of **6** confirmed the presence of a disubstituted double bond with signals at δ 6.74 (H_{11}, dd, J 9.2 and 5.2 Hz) and 6.47 (H_{10}, d, $J = 9.2$ Hz) ppm, of signals for two methine groups bearing oxygen at δ 4.80 (H_2, d, $J = 7.8$ Hz) and 4.66 (H_7, m), the carbon C_1 giving a signal at δ 102.56 confirming thus the presence of two oxygen atoms on the same carbon.[18] The selective addition of oxygen to **3g** rather than to **3h** can be inferred to be due to both electronic and steric factors.[19,20]

Lactones **3d–f** (R = H, *i*Pr, *t*Bu, $R^1R^2 = C_5H_{10}$) led similarly to the lactone peroxides **5b–d** in respectively 95%, 32% and 13% yields. Both their NMR data and their elemental analyses or mass spectra were in agreement with the proposed structures.[17]

Finally, the trimethylsilyl-bearing derivatives **3a** and **3b** were also submitted to this transformation: in both cases, satisfactory yields of the expected peroxylactones **7a** (40%) and **7b** (38%) were obtained (see Scheme 5). For example, the physical data of the crystalline peroxylactone **7b**, mp 154 °C were in agreement with the suggested structure, the lactone function giving a signal at

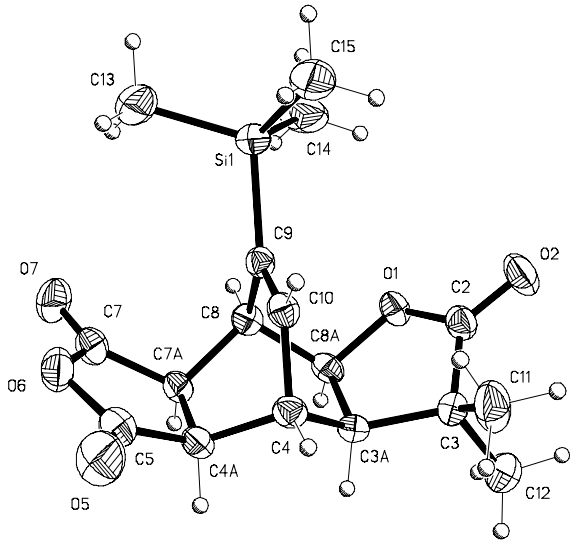
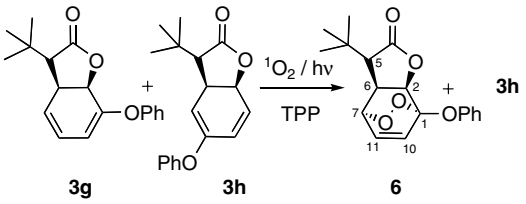
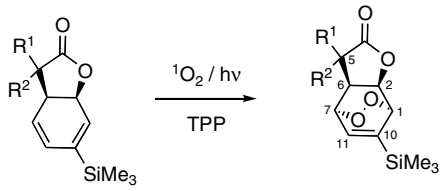


Figure 1. X-ray structure of the adduct **4b**.

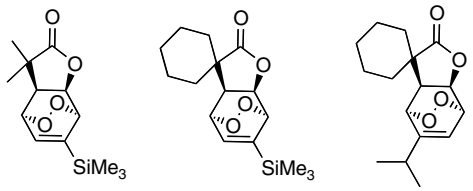


3g **3h** **6**

Scheme 4.



Scheme 5.



Scheme 6.

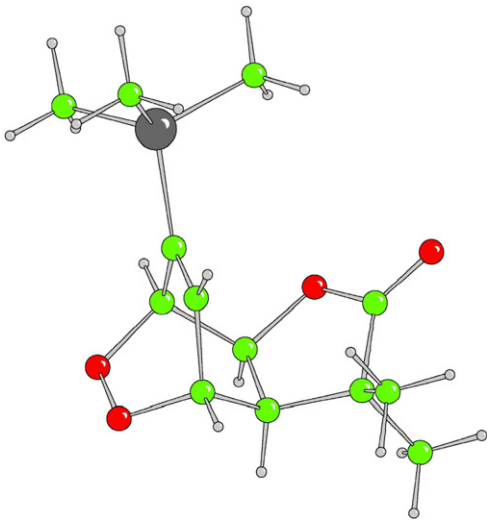


Figure 2. X-ray structure of the oxygen adduct **7b**.

δ 180.2 ppm, and the trisubstituted double bond giving two signals, respectively, at δ 146.2 (C_{10}) and 138.1 ppm (C_{11}), the proton H_{11} appearing as a doublet at δ 6.75 ppm.[21] Crystals suitable for an X-ray structure determination were grown from mixtures of hexane and dichloromethane.[22] The structure of this compound is displayed in Figure 2 and confirms the 4 + 2 addition of oxygen to the diene **3b**, the peroxo bridge being *cis* with respect to the hydrogens at the ring junction. Having in hand a series of new peroxylactones, we submitted them to the classical cell growth inhibition tests to evaluate their possible cytotoxicity. Compounds **7a**, **7b** and **5c** were found by far the most active against HCT15 (colon cancer), MCF-7 (breast cancer), K-562 CML (leukaemia), U-251 (central nervous system), PC-3 (prostate cancer), and SKLU-1 (lung cancer) tumor cell lines (Table 1) and their IC_{50} values were compared to those of known cytotoxic compounds doxorubicin and *cis*-platin.[23]

Thus, only three compounds bearing as a substituent of the diene either a trimethylsilyl or an isopropyl group showed activities better or comparable to those of *cis*-platin, whereas compounds bearing substituents such as *t*Butyl or phenoxy were devoid of significant activity (see Scheme 6).

In conclusion, we have established that lactone endoperoxides can be prepared in a few steps from aromatic derivatives, their cytotoxicity being highly dependent on the nature of the substituent on the remaining double bond.

Acknowledgements

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Table 1.

Cell lines	U251	PC3	K562	HTC15	MCF7	SKLU1
IC_{50} (μM)						
7b	4.7	5.5	4.6	3.4	6.0	6.9
7a	12.8	9.4	3.4	5.8	5.4	4.6
5c	11.45	3.55	9.46	2.08	24.49	15.89
Doxorubicin	0.09	0.32	0.28	0.23	0.10	0.28
Cis-platin	9.48	15.9	13.52	13.48	25.8	nd

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15. Compound **4b**: white solid, mp 160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (d, 1H, H_{10}); 4.86 (dd, 1H, H_{8a}); 3.81 (s, 1H, H_8); 3.28 (m, 1H, H_4); 3.16 (ddd, 2H, $H_{7a,4a}$); 2.20 (d, 1H, H_{3a}); 1.23 (s, 3H, CH_3); 1, 22 (s, 3H, CH_3); 0.04 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.2 (C_2); 171.35, 171.15 ($C_{7,5}$); 147.4 (C_9); 139.0 (C_{10}); 75.65 (C_{8a}); 49.13 (C_{3a}); 44.46 (C_{4a}); 41.01 (C_3); 40.67 (C_{7a}); 38.92 (C_8); 34.11 (C_4); 29.38, 20.40 ($2CH_3$), −2.66 ($Si(CH_3)_3$). MS, calcd 334.44; found 334.44.
16. Crystal data for **4b**: CCDC 607822 can be obtained free of charge from the Cambridge Data Centre via www.ccdc.ac.uk/data.report/cif.
17. Lactone peroxide **5a**: white solid, mp 96–98 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (m, 5H, ArH); 4.99–4.89 (m, 3H, $H_{1,10,2}$); 4.78 (br s, 1H, H_7); 2.97 (m, 1H, H_6); 1.85–1.12 (m, 10H, C_5H_{10}); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.2 (C_4), 156.9 (C_{11}); 153.0 (C_{iPh}); 130.1 (C_{mPh}); 125.8 (C_{oPh}); 120.7 (C_{pPh}), 97.1 (C_{10}), 73.1 (C_7); 72.0 (C_1); 70.2 (C_2); 44.9 (C_5); 43.6 (C_6); 35.5; 26.9; 24.9; 22.3; 21.8. HRMS calcd for $C_{19}H_{20}O_5$, 329.1389; found; 329.1382. Lactone peroxide **5b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.80 (τ, 1H, H_{11}, $J = 7.9$ Hz); 6.60–6.56 (m, 1H, H_{10}); 5.10 (m, 2H, H_1 and H_2); 4.79 (dd, 1H, H_7, $J = 7.0$, 3.0 Hz); 3.05 (dd, 1H, H_6, $J = 7.0$, 3.0 Hz), 1.76–1.28 (cyclohexyl); Lactone peroxide **5c**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.18 (d, 1H, H_{10}, $J = 6$ Hz); 5.00–4.97 (m, 2H, $H_{1,7}$); 4.65 (m, 1H, H_2); 3.00 (q, 1H, H_6, $J = 3.4$, 6.9 Hz); 1.64–1.12 (m, 17H, $H_{i\text{Pr, cyclohexyl}}$); ^{13}C NMR ($CDCl_3$, 75.43 MHz) δ 180.23 (C_4); 151.21 (C_{11}); 120.58 (C_{10}); 74.28 (C_2); 71.30 (C_7); 70.23 (C_1); 44.80 (C_6); 44.37 (C_5); 35.70, 31.97, 27.92, 25.10, 23.13, 22.38, 21.94, 19.24 ($C_{12,\ 13,\ \text{cyclohexyl}}$). MS: calcd for $C_{16}H_{22}O_4$, 278; found, 278.
18. Lactone peroxide **6**: white solid, mp 138 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.20 (m, 5H, ArH); 6.74 (dd, 1H, H_{11}, $J = 5.2$, 9.2 Hz); 6.47 (d, 1H, H_{10}, $J = 9.2$ Hz); 4.80 (d, 1H, H_2, $J = 7.8$ Hz); 4.66 (m, 1H, H_7), 3.27 (dt, 1H, H_6, $J = 7.8$, 4.2, 4.2 Hz); 1.83 (d, 1H, H_5, $J = 4$ Hz); 1.07 (s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 175.82 (C_4); 153.14 (ArC–O), 133.31; 131.22; 124.85 (ArC); 131.22 (C_{11}); 121.36 (C_{10}); 102.56 (C_1); 75.82 (C_7); 74.46 (C_2); 52.90 (C_5); 41.10 (C_6); 34.22 ($C(CH_3)_3$); 27.25 $(CH_3)_3$. Anal. for $C_{18}H_{20}O_5$, 0.25 H_2O; calcd, C, 67.39; H, 6.37; found, C, 67.42; H, 6.52.
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21. Lactone peroxide **7b**: white solid, mp 154 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.75 (m, 1H, H_{11}); 4.94–4.89 (m, 2H, H_1, H_2); 4.39–4.36 (m, 1H, H_7); 2.65 (m, 1H, H_6); 1.15 (s, 3H, CH_3); 0.98 (s, 3H, CH_3); 0.00 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.2 (C_4); 146.2 (C_{10}); 138.1 (C_{11}); 72.6 (C_1); 70.6 (C_7); 70.00 (C_2); 46.2 (C_6); 40.1 (C_5); 28.7 (CH_3); 19.0 (CH_3); −2.6 ($Si(CCH_3)_3$); HRMS, calcd for $C_{13}H_{21}O_4Si$, 269.1209; found, 269.1207.
22. Crystal structure of lactone peroxide **7b**: CCDC 278208 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge crystallographic Data Centre via www.ccdc.ac.uk/data.report/cif.
23. Colon cancer (HCT-15), breast cancer (MCF-7), leukaemia (K-562 CML), central nervous system (U-2521 Glio), prostate cancer (PC-3) and lung cancer (SKLU-1) cell lines were supplied by National Cancer Institute (NCI), USA and by Instituto de Cancerologia de la Ciudad de México, respectively. The cytotoxicity towards the tumor cells of the test compounds was determined by using the protein-binding dye sulforhodamine in microculture assay to measure cell growth.[24]
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