Columbetdione, a New Cyclopentene Derivative from the Fruiting Bodies of *Tricholoma columbetta* (Basidiomycetes) − Structure and Synthesis[‡]

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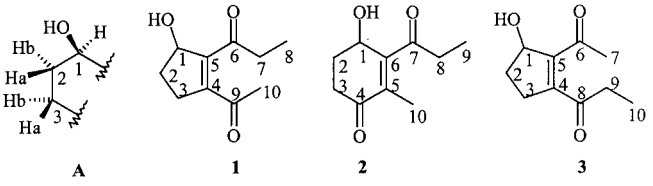
A chemical investigation of the fruiting bodies of *Tricholoma columbetta* (Agaricaceae, Basidiomycetes) led to the isolation of columbetdione, an unprecedented cyclopentene derivative. The structure was determined by spectroscopic methods and molecular modelling and unambiguously confirmed by total synthesis.

Introduction

Despite the wide occurrence of mushroom species of the genus *Tricholoma* (Basidiomycetes), a relatively limited number have been studied so far.[1−5] During our continuing work on the secondary metabolites of European Basidiomycetes as chemotaxonomic markers and potential pharmacological leads, we have completed the first chemical investigation of the fruiting bodies of *Tricholoma columbetta* (Fries) Quelet. This mushroom is common to Italy, where it can be collected in summer and in autumn amidst coniferous forests.[6] The fruiting bodies have no special smell or taste and are usually consumed as food. We were therefore interested in examining *T. columbetta* not only because several compounds with unusual chemical structures have been isolated from related species,[1−5] but also to exclude the presence of significant amounts of harmful compounds.

Results and Discussion

Young specimens (220 g) of *T. columbetta* that appeared undamaged were minced, frozen, and soaked in EtOAc at −5 °C a few hours after collection. After solvent evaporation, the residue was partitioned between hexane and MeOH/H_2O (9:1), and the alcoholic phase was concentrated and chromatographed on silica gel to give 4 mg of compound **1** as a pale yellow oil.



The hexane phase contained ubiquitous fungal metabolites,[1,2] such as glycerides and other fatty acid esters, and common sterols; they were not investigated further. The structure determination of compound **1** was based on NMR spectroscopic and MS data, which, considered together, suggested the elemental composition as $C_{10}H_{14}O_3$. HSQC and HMBC correlations (Figure 1) revealed three isolated proton spin systems, attributable to the moiety **A**, and to a methyl and an ethyl group, each linked to a different ketone carbonyl group (δ_C = 200.7 and 203.4 ppm, respectively). In addition, the ^{13}C NMR spectrum showed signals of two quaternary sp^2 olefinic carbon atoms (δ_C = 145.9 and 148.9 ppm), which allowed us to assemble the structure of the compound as a cyclopentenol derivative **1** or as a cyclohexenol diketone **2**.

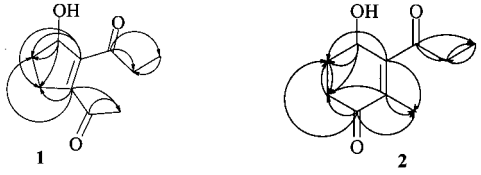


Figure 1. Pertinent HMBC correlations observed for compounds **1** and **2**

Both structures are compatible with a likely polyketide biosynthesis of the metabolite (vide infra). Structure **1** was, however, better supported by the C-H connectivity data inferred from an HMBC spectrum at 600 MHz (Figure 1); in particular, both the quaternary olefinic carbon atoms show correlations with both methylene protons of the ring. These

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correlations can take place through two and three bonds in the cyclopentene structure **1**, whereas they require two rarely observed four-bond connectivities in the cyclohexenol structure **2**. On the other hand, strong NOE interactions between the carbinol proton (signal at $\delta = 5.14$ ppm) and the ethyl group excluded structure **3**, which is a regioisomer of **1** with respect to the position of the side chains.

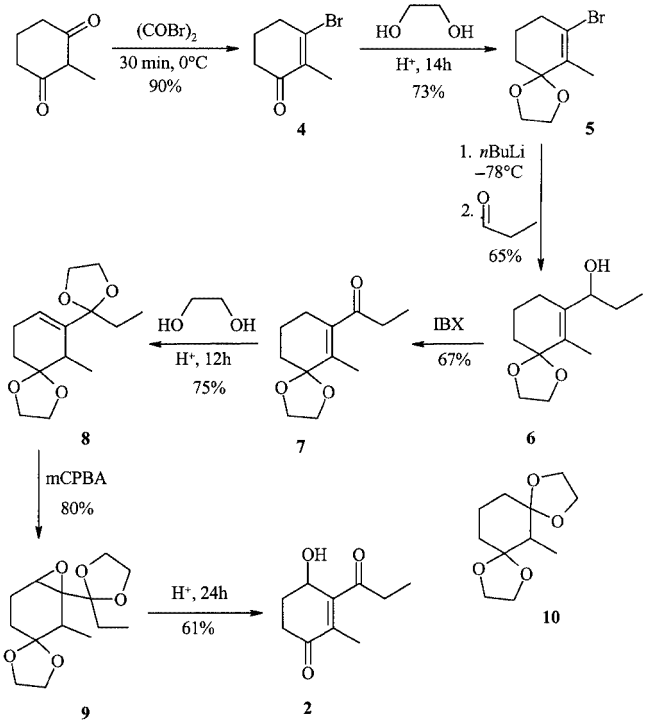
To confirm this structural assignment, a search of the conformational space of structures **1** and **2** was undertaken at the B3LYP/6-31G* level of calculation. In both cases two families of minimum energy conformations were located, each family being characterised by a particular geometry of the ring, while the various members of each family showed a different orientation of the side chains. The vicinal coupling constants of the ring protons of each family were then calculated along with the averaged values for each compound (Supporting Information, see the footnote on the first page of this article), which were compared with the pertinent experimental coupling constants of the compound isolated from *T. columbetta***.** The values calculated for the cyclopentene derivative **1** showed a better agreement, thus reinforcing our former assignment. By contrast, observed NOE effects were compatible with the interatomic distances measured for both structures **1** and **2** by molecular modelling.

In conclusion, both the spectroscopic data and the results of the molecular modelling support structure **1** better than **2**. On the other hand, both compounds are unknown in the chemical literature. Therefore, in order to assign unambiguously the structure of the metabolite isolated from *T. columbetta*, and to test the biological activities of **1** and **2**, we decided to embark on the total synthesis of the two structural isomers.

Total Synthesis of Compound 2

Compound **2** was synthesised according to Scheme 1, which is self-explanatory. Our synthetic strategy was based on the introduction of the propanoyl side-chain through the use of a β-bromo-α-methylcyclohexenone derivative as a β-acylvinyl anion equivalent[7] (step **5** → **6**), and on the installation of the allylic hydroxy group through an epoxide/allylic alcohol rearrangement (step **9** → **2**). The entire synthetic plan was completed uneventfully, except for the monoketalization of compound **4**[7] and the deprotection of bis(ketal) **9**. Both these transformations required careful optimisation of reaction conditions to reduce the formation of bis(ketal) **10** in the former reaction and aromatization of the γ-hydroxycyclohexenone **2** in the last step of the synthesis.

It is noteworthy that isomerization of the tetrasubstituted double bond did not occur in the ketalization of bromocyclohexenone **4**, as reported previously,[7] whereas double bond migration was observed in the ketalization of compound **7**. Eventually, ketone **2** was obtained in good overall yields and, as anticipated, the NMR spectra, consistent with the structure, were clearly different from those of the compound isolated from *T. columbetta*. Relevant HMBC

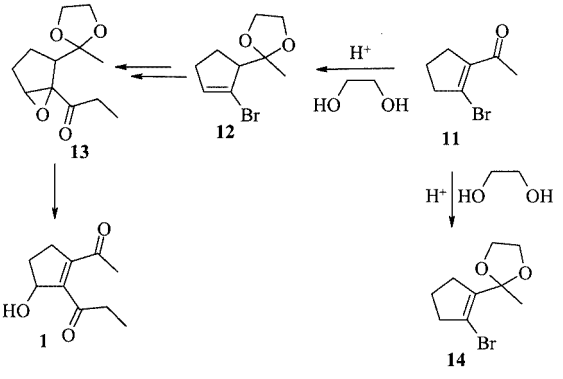


Scheme 1. Total synthesis of compound **2**

correlations observed for compound **2** are reported in Figure 1.

Total Synthesis of Compounds 1 and 3

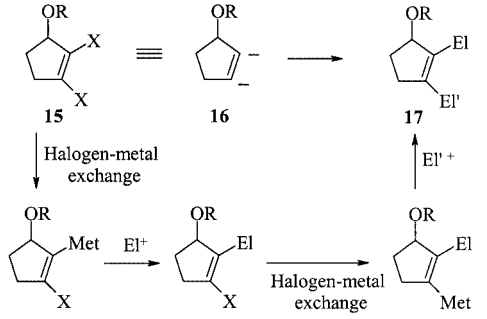
Inspired by the successful synthesis of cyclohexenone **2**, at first we envisioned a synthetic approach to compound **1** through an epoxide/allylic alcohol rearrangement (step **13**→**1** in Scheme 2). However, as observed above for the ketalization of vinyl bromide **4**,[7] ketalization of the known bromocyclopentenyl ketone **11**[8] occurred without concomitant double bond isomerization, furnishing acetal **14** instead of the required isomer **12**.



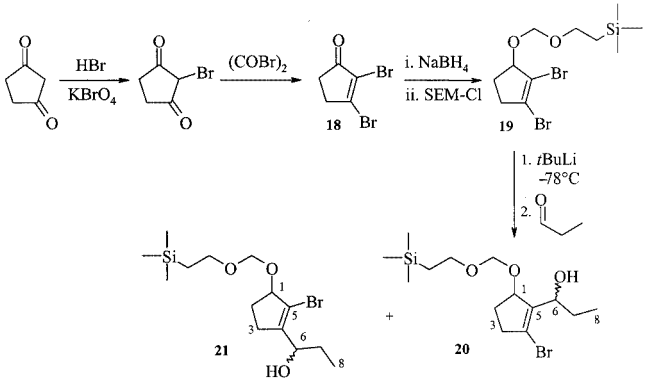
Scheme 2. Attempted synthesis of compound **1**

Consequently, we decided to explore a completely different route to alcohol **1** based on the development of a new methodology aimed at the direct introduction of two acyl chains onto a cyclopentenol double bond. We reasoned that

the protected 2,3-dihalocyclopentenol **15** could serve as a viable α,β-hydroxyvinyl dianion equivalent of cyclopentenols **16** if two consecutive regioselective halogen/metal exchanges, each followed by reaction of the corresponding organometallic species with an electrophile, could be realized. This methodology has a broad appeal since, in principle, the synthesis of several compounds of type **17** can thus become accessible from easily prepared **15** (X = Br or I) in two consecutive similar steps (Scheme 3). Moreover, to the best of our knowledge this chemistry is unprecedented in the literature, although Gassman has demonstrated the feasibility of a bromine/metal monoexchange reaction in a symmetrical 1,2-dihaloalkene, namely in 2,3-dibromobicyclo[2.2.1]hept-2-ene.[9] In this context, 2,3-dibromocyclopenten-1-ol (**15**: R = H; X = Br), obtained in three steps from 1,3-cyclopentanedione and protected as its SEM ether **19**, was treated with *tert*-butyllithium (2 equiv.) in THF at −78 °C, followed, after 5 min, by the addition of propionaldehyde (1 equiv.) at the same temperature (Scheme 4).



Scheme 3. Synthesis of 2,3-disubstituted cyclopentenols by using an α,β-hydroxyvinyl dianion synthon



Scheme 4. Synthesis of compounds **20** and **21**

GC analysis of the products revealed the presence of two adducts **20** and **21** (each as a mixture of diastereomeric alcohols) in a ratio of 1.5:1, which were isolated by column chromatography in 46% and 31% yield, respectively. The structures were firmly established by HMBC experiments (Figure 2). The low regioselectivity observed for the halogen/metal exchange in compound **19** was attributed to two opposed directing effects exerted by the SEM group, namely chelation of the lithium cation by the oxygen atoms, which should favour exchange of the bromine atom at C-2, and steric hindrance of the bulky protective group, which should direct the reaction towards the halogen on C-3. Although the effects of other protective groups on the regioselectivity of the reaction could be worthy of investigation, we decided to proceed with compounds **20** and **21** separately, thus having the opportunity to secure both the natural compound and its regioisomer **3** for comparison of the spectra and biological evaluation.

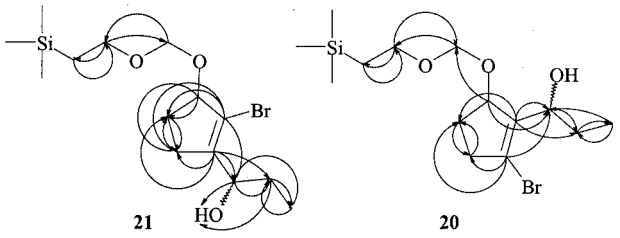
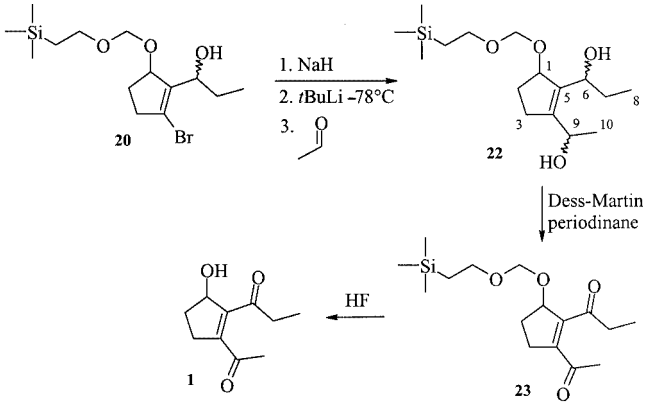


Figure 2. Pertinent HMBC correlations observed for compound **20** and **21**

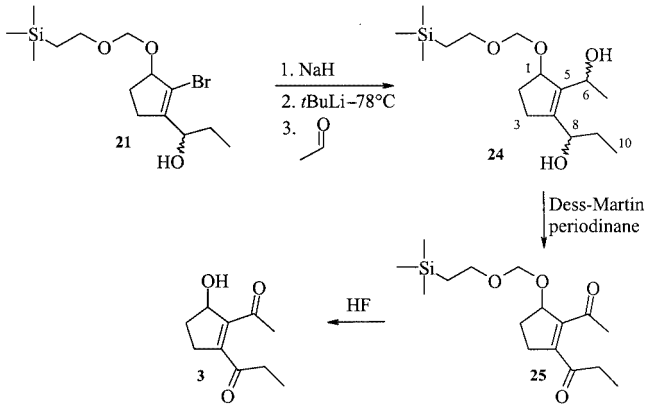
Scheme 5 depicts the synthetic pathway leading to compound **1**; an identical sequence of reactions was employed in the synthesis of diketone **3** starting from alcohol **21** (Scheme 6). Both the second bromine/metal exchange and the subsequent reaction of the anion with acetaldehyde gave satisfactorily high yields (71−73%) of the corresponding adduct (mixture of diastereomers at the two carbinol centres). The NMR spectra of compounds **1**−**3** are clearly different from each other; in addition, the spectra of **1** are superimposable with those of the metabolite isolated from *T. columbetta*.



Scheme 5. Final steps of the synthesis of compound **1**

Conclusions

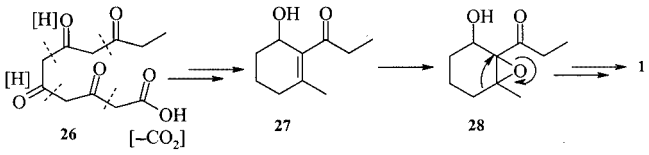
A new cyclopentene derivative was isolated from *T. columbetta*. The structure was established as **1** by spectroscopic data and definitely confirmed by total synthesis. The latter features a new methodology for installing two substituents on the olefinic carbon atoms of a 1,2-dibromocyclopentene ring through two consecutive bromine/lithium exchange reactions, followed by addition to an aldehyde.



Scheme 6. Final steps of the synthesis of compound **3**

Alhough compound **1** was obtained as the racemate, in principle this approach can afford enantiomerically enriched cyclopentenols of type **1** through asymmetric reduction of ketone **18**.

It is noteworthy that the structure of compound **1** seems to be unrelated to other fungal metabolites isolated from Basidiomycetes. Indeed, monocyclopentanoid natural products, such as the jasmonates and prostaglandins, are widely distributed in living organisms with the remarkable exception of Basidiomycetes.[1,2] Although no experimental evidence is available so far, the oxygenation pattern of structure **1** strongly suggests, as a biosynthetic route, an intramolecular aldol-like condensation of the pentaketide chain compound **26** formed by four acetate units and a propionate starter. However, intramolecular condensation of a polyketide precursor of this kind cannot afford a cyclopentene ring directly. Therefore, to account for the formation of compound **1**, we propose the initial formation of the six-membered ring compound **27**, followed by a ring contraction process, possibly involving the epoxide intermediate **28** (Scheme 7).



Scheme 7. Proposed biosynthesis of compound **1**

Interestingly, the hypothetical intermediate **27** has the same carbon skeleton as the cyclohexenone moiety of lascivol, isolated from *T. lascivum*,[10] and scalpturan, isolated from *T. scalpturatum*.[11] The possible biogenetic relationship of **27** with these *Tricholoma* metabolites enhances the plausibility of our biogenetic suggestion. Compounds **1**–**3** are devoid of antimicrobial activity and toxicity in the brine shrimp (*Artemia salina*) lethality assay. Compound **1** was named columbetdione. Interestingly, it is optically inactive at 589 nm, $[\alpha]_D^{20} = 0$ ($c = 0.2$, MeCN), whereas it shows a weak CD maximum at 246 nm ($\Delta\varepsilon = -0.6$).

Experimental Section

General: NMR spectra were recorded as $CDCl_3$ solutions at 25 °C, unless otherwise indicated, with a Bruker CXP 300 spectrometer. Chemical shifts are reported in δ units relative to $CHCl_3$ [δ_H = 7.26 ppm; δ_C (central line of t) = 77.0 ppm]; the abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, quint = quintuplet, m = multiplet, and br. = broad are used throughout. Coupling constants (*J*) are in Hz. The multiplicity (in parentheses) of each carbon signal was determined by DEPT experiments. Mass spectra (direct inlet system) were recorded at 70 eV (0.5 mA) with a Finnigan MAT 8222 instrument. IR spectra were obtained as thin films with a Perkin–Elmer FT-IR Paragon 100 PC spectrometer. Optical rotations were determined with a Perkin–Elmer 241 polarimeter at 20 °C and CD spectra with a Jasco J-715 spectropolarimeter, employing 1-cm optical path cuvettes. Analytical TLC was carried out on glass-backed plates, precoated with a 0.25 mm layer of silica gel, and viewing was effected with short-wavelength UV light (254 nm), with a 0.5% vanillin solution in H_2SO_4/EtOH (4:1), or with a 7% phosphomolybdic acid solution in EtOH, followed by heating. Liquid chromatography was accomplished with 60 Kieselgel (40–63 μm). All commercial reagent grade solvents were dried by standard techniques just before use.

Collection, Extraction, and Isolation: Fruiting bodies of *Tricholoma columbetta* (220 g) were collected in the vicinity of Savona (region Liguria) in October 1998. A few hours after the collection, the mushrooms were frozen and then extracted with EtOAc at −5 °C. The raw extract was dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure to give 0.7 g of crude extract, which was then partitioned between hexane and MeOH/H_2O (9:1). After dilution with brine, the hydroalcoholic phase was extracted with EtOAc and the organic layer was dried ($MgSO_4$) and the solvents were evaporated. Chromatographic separation of the residue (0.3 g) on silica gel with a CH_2Cl_2/EtOAc gradient mixture resulted in the isolation of columbetdione (**1**) (4 mg).

Columbetdione (1): Colourless oil, $[\alpha]_D^{20} = 0$ ($c = 0.2$, MeCN). CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 246 nm (−0.6). UV (MeCN): λ_{max} (log ε) = 240 (3.65) nm. IR: $\tilde{\nu}$ = 3432, 2944, 1688, 1622, 1411, 1360, 1244, 1128, 1052, 897, 803 cm^{-1}. ^1H NMR: δ = 1.11 (t, $J_{7\text{-}8} = J_{7'\text{-}8}$ = 7 Hz, 3 H, 8-H_3), 1.9 (dddd, $J_{2a\text{-}3b}$ = 5, $J_{2a\text{-}3a}$ = 9, $J_{2a\text{-}2b}$ = 18.5, $J_{2a\text{-}1}$ = 4.5 Hz, 1 H, 2-H[a]), 2.33 (s, 3 H, 10-H_3), 2.40 (d, $J_{OH\text{-}1}$ = 5.5 Hz, 1 H, OH), 2.41 (dddd, $J_{2b\text{-}3a}$ = 4.5, $J_{2b\text{-}3b}$ = 8.5, $J_{2b\text{-}1}$ = 7.5 Hz, 1 H, H-2[b]), 2.6 (dddd, $J_{3b\text{-}1}$ = 1.5, $J_{3a\text{-}3b}$ = 17.5 Hz, 1 H, 3-H[b]), 2.65 (m, $J_{7\text{-}7'}$ = 18.5 Hz, 2 H, 7-H and 7-H′), 2.86 (dddd, $J_{3a\text{-}1}$ = 2.5 Hz, 1 H, 3-H[a]), 5.15 (m, 1 H, 1-H) ppm. ^{13}C NMR: δ = 7.2 (q, C-8), 29.0 (q, C-10), 32.0 (t, C-3), 32.3 (t, C-2), 35.1 (t, C-7), 77.9 (d, C-1), 145.8 (s, C-5), 149.0 (s, C-4), 200.8 (s, C-6), 203.3 (s, C-9) ppm. EIMS: *m*/*z* (%) = 182 (10) [M^+], 164 (8), 153 (72), 126 (12), 111 (100), 97 (11), 83 (12), 57 (20), 43 (85). HRMS: calcd. for $C_{10}H_{14}O_3$ 182.0943; found 182.0939.

3-Bromo-2-methylcyclohex-2-en-1-one (4):[7] Oxalyl bromide (1.53 mL, 10.8 mmol) was added over 5 min to a solution of 2-methyl-1,3-cyclohexanedione (1.13 g, 9 mmol) and DMF (0.86 mL, 11.7 mmol) in CH_2Cl_2 (25 mL) at 0 °C under argon. The mixture was then warmed to 25 °C over 30 min, diluted with Et_2O (100 mL) and extracted with H_2O (40 mL). The organic layer was dried ($MgSO_4$), filtered and taken to dryness. Chromatographic separation of the residue on silica gel with hexane/EtOAc (4:1) as eluent gave the known 3-bromo-2-methylcyclohex-2-en-1-one[7] (1.53 g, 90% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2947, 1676, 1620, 1429, 1334, 1326, 1279, 1190, 1037, 967, 896 cm^{-1}. ^1H NMR: δ = 1.97 (t, *J* =

1.5 Hz, 3 H), 2.0−2.1 (m, 2 H), 2.49 (m, 2 H,), 2.92 (m, 2 H) ppm.

7-Bromo-6-methyl-1,4-dioxaspiro[4,5]dec-6-ene (5):[7] A solution of 3-bromo-2-methylcyclohex-2-en-1-one (**4**) (2 g, 10.6 mmol), ethylene glycol (1.31 g, 22.2 mmol), and *p*-toluenesulfonic acid monohydrate (45 mg) in dry benzene (300 mL) was refluxed for 14 h in a Dean−Stark apparatus. The reaction was quenched after conversion of about 80% of the starting ketone to prevent formation of diacetal **10**. Solid $CaCO_3$ (50 mg) was then added, followed by satd. aq. $NaHCO_3$ (20 mL). The organic layer was washed with brine, dried ($MgSO_4$), filtered and the solvents were evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (95:5) as eluent gave the known acetal **5**[7] (1.8 g, 73% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2945, 2876, 1290, 1175, 1144, 1100, 1071, 1050, 1018, 945, 925 cm^{-1}. ^1H NMR ([D$_6$]Me_2CO): δ = 1.75 (m, 7 H), 2.51 (m, 2 H), 4.00 (m, 4 H) ppm.

1-(6-Methyl-1,4-dioxaspiro[4,5]dec-6-en-7-yl)propan-1-ol (6): Butyllithium (1.6 M solution in hexane, 1.63 mL, 2.61 mmol) was added dropwise to bromoacetal **5** (0.541 g, 2.17 mmol) in dry THF (9 mL) at −78 °C under argon. After 15 min, propionaldehyde (0.159 g, 2.39 mmol) was added and stirring was continued at −78 °C for an additional hour. The mixture was then warmed to 0 °C, 4% aq. NH_4Cl (50 mL) added, and the mixture extracted with Et_2O (3 × 100 mL). The organic layer was washed with H_2O (60 mL), followed by brine (2 × 60 mL), dried (K_2CO_3), and the solvents were evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (7:3) as eluent gave acetal **6** (299.4 mg, 65% yield) as a colourless oil. IR: $\tilde{\nu}$ = 3385, 2932, 1584, 1458, 1258, 1071, 782 cm^{-1}. ^1H NMR: δ = 0.97 (t, *J* = 7.3 Hz, 3 H), 1.53−1.76 (m, 2 H), 1.76 (br. s, 3 H), 2.0 (quint, *J* = Hz7, 2 H), 2.24−2.60 (m, 4 H), 3.76 (br. s, 4 H), 4.65 (dd, *J* = 7.5 and 6.1 Hz, 1 H). $C_{12}H_{20}O_3$ (212.29): calcd. C 67.89, H 9.50; found C 67.79, H 9.56.

1-(6-Methyl-1,4-dioxaspiro[4,5]dec-6-en-7-yl)propan-1-one (7): 1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX)[12] (1.38 g, 5.1 mmol) was added to alcohol **6** (500 mg, 2.36 mmol) in dry DMSO (810 mL) at 25 °C. After 4 h, the reaction was quenched with satd. aq. $NaHCO_3$ (50 mL) and the mixture extracted with Et_2O (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and the solvents evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (7:3) as eluent gave ketone **7** (332 mg, 67% yield) as a pale yellow oil. IR: $\tilde{\nu}$ = 1686 cm^{-1}. ^1H NMR ([D$_6$]Me_2CO): δ = 1.02 (t, *J* = 7.2 Hz, 3 H), 1.65 (br. s, 3 H), 1.72 (m, 4 H), 2.17 (m, 2 H), 2.56 (q, *J* = 7.2 Hz, 2 H), 3.99 (s, 4 H) ppm. $C_{12}H_{18}O_3$ (210.27): calcd. C 68.54, H 8.63; found C 68.59, H 8.66.

7-(2-Ethyl-1,3-dioxolan-2-yl)-6-methyl-1,4-dioxaspiro[4,5]dec-7-ene (8): A solution of ketone **7** (172 mg, 0.82 mmol), ethylene glycol (512 mg, 8.2 mmol), and *p*-toluenesulfonic acid monohydrate (45 mg) in dry benzene (80 mL) was refluxed for 12 h in a Dean−Stark apparatus. The solution was cooled to 25 °C and extracted with satd. aq. $NaHCO_3$ (20 mL). The organic layer was washed with brine, dried (Na_2SO_4), filtered and the solvents were evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (4:1) as eluent gave diacetal **8** (156 mg, 75% yield) as a pale yellow oil. IR: $\tilde{\nu}$ = 2880, 1464, 1366, 1280, 1200, 1045, 947, 918, 864, 832 cm^{-1}. ^1H NMR ([D$_6$]Me_2CO): δ = 0.84 (t, *J* = 7.2 Hz, 3 H), 1.12 (d, *J* = 7.0 Hz, 3 H), 1.45−2.25 (m, 7 H), 3.70−4.0 (m, 8 H), 5.72 (t, *J* = 3.5 Hz, 1 H) ppm. ^{13}C NMR ([D$_6$]Me_2CO): δ = 7.6 (q), 18.1 (q), 23.9 (t), 25.8 (t), 30.7 (t), 37.9 (d), 64.2 (t), 64.2 (t), 64.4 (t), 64.6 (t), 110.8 (s), 110.9 (s), 122.5 (d), 140.5 (s) ppm. $C_{14}H_{22}O_4$ (254.32): calcd. C 66.12, H 8.72; found C 66.09, H 8.69.

1′-(2-Ethyl-1,3-dioxolan-2-yl)-2′-methylspiro[1,3-dioxolane-2,3′-[7]-oxabicyclo[4.1.0]heptane] (9): 3-Chloroperoxybenzoic acid (184 mg, 1.07 mmol) in dry CH_2Cl_2 (2 mL) was added to olefin **8** (227 mg, 0.89 mmol) in dry CH_2Cl_2 (4 mL) at 25 °C under argon, and stirring was continued for 5 h. The solution was diluted with Et_2O (30 mL) and washed with satd. aq $NaHCO_3$ (3 × 20 mL) followed by brine, dried (Na_2SO_4), filtered, and the solvents were evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (4:1) as eluent gave epoxide **9** (204 mg, 85% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2982, 2886, 1464, 1369, 1221, 1113, 1044, 951, 931, 805 cm^{-1}. ^1H NMR ([D$_6$]Me_2CO): δ = 0.88 (t, *J* = 7.3 Hz, 3 H), 1.06 (d, *J* = 7 Hz, 3 H), 1.14−2.26 (m, 7 H), 3.15 (br. s, 1 H), 3.75−4.10 (m, 8 H) ppm. ^{13}C NMR ([D$_6$]Me_2CO) of major stereoisomer: δ = 6.7 (q), 11.9 (q), 23.0 (t), 23.6 (t), 28.6 (t), 35.9 (d), 55.8 (d), 63.8 (t), 64.4 (s), 64.5 (t), 65.7 (t), 66.6 (t), 109.3 (s), 110.3(s) ppm. $C_{14}H_{22}O_5$ (270.32): calcd. C 62.20, H 8.20; found C 62.28, H 8.24.

4-Hydroxy-2-methyl-3-propionyl-2-cyclohexen-1-one (2): 1.2 M HCl (175 μL) was added to epoxide **9** (50 mg, 0.18 mmol) in MeCN (2 mL) and stirring was continued for 24 h at 25 °C. The solution was neutralised with satd. aq. $NaHCO_3$ and extracted with Et_2O (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (1:1) as eluent gave compound **2** (20 mg, 61% yield) as a white solid, m.p. 58−60 °C. IR (KBr): $\tilde{\nu}$ = 3440, 2940, 1668, 1513, 1449, 1409, 1379, 1330, 1308, 1249, 1186, 1147, 1108, 1042, 1017, 952, 919, 846, 700 cm^{-1}. ^1H NMR: δ = 1.19 (t, *J* = 7.3 Hz, 3 H, 9-H_3), 1.76 (br. s, 3 H, 10-H_3), 2.0−2.13 (m, 1 H, 2-H), 2.30−2.48 (m, 2 H, 3-H and 2-H′), 2.57−2.85 (m, 3 H, 8-H_2 and 3-H′), 4.76 (m, 1 H, 1-H) ppm. ^{13}C NMR: δ = 7.1 (q), 12.2 (q), 31.9 (t), 34.7 (t), 36.5 (t), 66.8 (d), 131.1 (s), 156.0 (s), 198.4 (s), 208.1 (s) ppm. EIMS: *m*/*z* (%) = 182 (63), 164 (100), 122 (13), 109 (10), 97 (20), 79 (30), 69 (10), 57 (20). $C_{10}H_{14}O_3$ (182.22): calcd. C 65.91, H 7.74; found C 65.88, H 7.71.

2-(2-Bromocyclopent-1-enyl)-2-methyl-1,3-dioxolane (14): A solution of the known ketone **11**[8] (100 mg, 0.53 mmol), ethylene glycol (70 mg, 1.13 mmol), and *p*-toluenesulfonic acid monohydrate (5 mg) in dry benzene (30 mL) was refluxed for 20 h in a Dean−Stark apparatus. The solution was cooled to 25 °C and extracted with satd. aq. $NaHCO_3$ (10 mL). The organic layer was washed with brine, dried (Na_2SO_4), filtered and the solvents were evaporated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (6:1) as eluent gave acetal **14** (112 mg, 91% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2955, 1638, 1371, 1195, 1042, 869 cm^{-1}. ^1H NMR: δ = 1.57 (s, 3 H, Me), 1.87−2.0 (m, 2 H), 2.40−2.50 (m, 2 H), 2.67−2.75 (m, 2 H), 3.87 (m, 2 H), 3.99 (m, 2 H) ppm. $C_9H_{13}BrO_2$ (233.10): calcd. C 46.37, H 5.62; found C 46.40, H 5.60.

2,3-Dibromocyclopent-2-enone (18): Oxalyl bromide (1.17 g, 5.4 mmol) was added over 5 min to freshly prepared 2-bromo-1,3-cyclopentanedione[13] (809 mg, 4.6 mmol) in CH_2Cl_2 (25 mL) and DMF (0.43 mL) at 0 °C under argon. The stirred solution was warmed to 25 °C over 30 min and partitioned between Et_2O (100 mL) and H_2O (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (4:1) as eluent gave ketone **18** (938 mg, 85% yield) as a white solid, m.p. 79−81 °C. IR (nujol): $\tilde{\nu}$ = 2923, 2854, 1707, 1575, 1459, 1377, 1241, 1214, 940, 843 cm^{-1}. ^1H NMR: δ = 2.75 (m, 2 H), 3.05 (m, 2 H) ppm. ^{13}C NMR: δ = 34.8 (t), 35.8 (t), 128.2 (s), 159.0 (s), 197.2 (s) ppm. EIMS: *m*/*z* (%) = 242 (50) [M^+, $^{81}Br_2$] (50), 240 (100) [M^+, $^{79+81}Br_2$], 238 (50) [M^+, $^{79}Br_2$], 161 (80), 133 (80), 119 (15), 79 (40), 51 (90), 42 (18).

$C_5H_4Br_2O$ (239.89): calcd. C 25.03, H 1.68; found C 24.98, H 1.65.

[2-{[(2,3-Dibromocyclopent-2-enyl)oxy]methoxy}ethyl]-trimethylsilane (19): $CeCl_3 \cdot 7H_2O$ (298 mg, 0.8 mmol) was added to ketone **18** (1.92 g, 8 mmol) in MeOH (25 mL). The solution was cooled to 0 °C and solid $NaBH_4$ (395 mg, 10.4 mmol) was added portionwise over 50 min. The stirred solution was warmed to 25 °C, diluted with H_2O (30 mL) and extracted with Et_2O (3 × 80 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to give 2,3-dibromocyclopent-2-enol **15** (R = H; X = Br) (1.90 g, 98% yield) which was used immediately in the following step. M.p. 43−44 °C. IR (nujol): $\tilde{\nu}$ = 3217, 2918, 1616, 1434, 1327, 1148, 1116, 1083, 1047, 966, 899, 839, 666 cm^{-1}. ^1H NMR: δ = 1.5−2.5 (m, 4 H), 4.06 (m, 1 H) ppm. ^{13}C NMR: δ = 31.7 (t), 37.2 (t), 78.3 (d), 125.9 (s), 126.8 (s) ppm. EIMS: *m*/*z* (%) = 244 (5) [M^+, $^{81}Br_2$], 242 (10) [M^+, $^{79-81}Br_2$], 240 (50) [M^+, $^{79}Br_2$], 225 (25), 163 (100), 82 (90), 63 (15), 53 (45), 43 (15). [2-(Trimethylsilyl)ethoxy]methyl chloride (1.04 g, 6.2 mmol) was added dropwise to a solution of 2,3-dibromocyclopent-2-enol (484 mg, 2 mmol) and *N*,*N*-diisopropylethylamine (1.27 g, 9.8 mmol) in dry CH_2Cl_2 (1 mL) at 25 °C under argon. The solution was stirred for 1 h at 25 °C, then diluted with H_2O (20 mL) and extracted with Et_2O (3 × 80 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (95:5) as eluent gave compound **19** (736 mg, 99% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2952, 2892, 1621, 1413, 1359, 1304, 1249, 1191, 1163, 1106, 1022, 938, 835, 758, 693 cm^{-1}. ^1H NMR: δ = 0.05 (s, 9 H, Me_3Si), 0.94−1.00 (m, 2 H, CH_2Si), 2.00−2.10 (m, 1 H), 2.37−2.48 (m, 1 H), 2.53−2.63 (m, 1 H), 2.76−2.87 (m, 1 H), 3.61−3.80 (m, 2 H, CH_2O), 4.66 (m, 1 H, CHOH), 4.80 (ABq, *J* = 7.1, 2 H, OCH_2O) ppm. $C_{11}H_{20}Br_2O_2Si$ (372.17): calcd. C 35.50, H 5.42; found C 35.51, H 5.43.

1-(2-Bromo-5-{[2-(trimethylsilyl)ethoxy]methoxy}cyclopent-1-en-1-yl)propan-1-ol (20) and 1-(2-Bromo-3-{[2-(trimethylsilyl)-ethoxy]methoxy}cyclopent-1-en-1-yl)propan-1-ol (21): *t*BuLi (1.7 M in pentane, 2.3 mL, 3.9 mmol) was added to a solution of compound **19** (699 mg, 1.88 mmol) in dry THF (9 mL) at −78 °C under argon. After stirring the mixture for 5 min, propionaldehyde (120 mg, 2.07 mmol) was added and stirring was continued for an additional hour at −78 °C. Satd. aq. NH_4Cl (10 mL) was carefully added and the mixture was extracted with Et_2O (3 × 20 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (95:5) as eluent gave compound **20** (205 mg, 31.5% yield) as a mixture of stereoisomers (undetermined stereochemistry) and compound **21** (299 mg, 46% yield) as a mixture of stereoisomers (undetermined stereochemistry). **20:** IR: $\tilde{\nu}$ = 3462, 2955, 1651, 1455, 1412, 1355, 1249, 1190, 1145, 1099, 1025, 939, 859, 836, 759, 694 cm^{-1}. ^1H NMR (major stereoisomer): δ = 0.04 (s, 9 H, Me_3Si), 0.87−1.0 (m, 5 H, CH_2Si and 8-H_3), 1.51−1.86 (m, 2 H, 7-H_2), 1.90−2.01 (m, 1 H), 2.28−2.40 (m, 1 H), 2.52−2.64 (m, 1 H), 2.75−2.89 (m, 1 H), 3.67−3.75 (m, 2 H, CH_2O), 4.48 (t, *J* = 7.1 Hz, 1 H, 1-H), 4.76 (ABq, *J* = 7, 2 H, OCH_2O), 4.88 (m, 1 H, 6-H) ppm. ^{13}C NMR (major stereoisomer): δ = −1.5 (3 overlapped q), 9.9 (q), 18.0 (t), 28.6 (t), 30.3 (t), 38.0 (t), 65.9 (t), 71.4 (d), 81.3 (d), 93.6 (t), 125.0 (s), 140.4 (s) ppm. $C_{14}H_{27}BrO_3Si$ (351.35): calcd. C 47.86, H 7.75; found C 47.91, H 7.79. **21:** Two stereoisomers in a ratio of 1:1 from NMR spectroscopic data. IR: $\tilde{\nu}$ = 3422, 2955, 2880, 1649, 1462, 1411, 1361, 1319, 1249, 1191, 1163, 1095, 1018, 920, 836, 758, 693 cm^{-1}. ^1H NMR: δ ([D$_6$]Me_2CO) = 0.04 (s, 9 H, Me_3Si), 0.87−1.0 (m, 5 H, CH_2Si and 8-H_3), 1.42−1.72 (m, 2 H, 7-H_2), 1.81−1.94 (m, 1 H), 2.20−2.36 (m, 1.5 H), 2.45 (br. t, *J* = 7.0, 1 H), 2.56−2.65 (m, 0.5 H), 3.60−3.81 (m, 2 H, CH_2O), 4.01 (d, *J* = 8.5, 0.5 H, OH), 4.03 (d, *J* = 8.3, 0.5 H, OH), 4.42−4.48 (m, 1 H, 1-H), 4.63−4.67 (m, 1 H, 6-H), 4.73 (ABq, 2 H, OCH_2O) ppm. ^{13}C NMR: δ ([D$_6$]Me_2CO) = −2.2 (3 overlapped q), 9.3 (q), 17.6 (t), 27.5 (t), 27.7 (t), 27.8 (t), 28.0 (t), 28.8 (t), 29.0 (t), 64.4 (t), 69.9 (d), 69.9 (d), 84.0 (d), 84.3 (d), 93.2 (t), 93.5 (t), 117.1 (s), 117.2 (s), 147.8 (s), 148.2 (s) ppm. $C_{14}H_{27}BrO_3Si$ (351.35): calcd. C 47.86, H 7.75; found C 47.81, H 7.71.

1-[2-(1-Hydroxyethyl)-5-{[2-(trimethylsilyl)ethoxy]methoxy}-cyclopent-1-en-1-yl]propan-1-ol (22): A solution of alcohol **20** (298 mg, 0.85 mmol) in dry THF (4.5 mL) was added at −78 °C to 35 mg of a prewashed (hexane) 60% paraffin dispersion of NaH at −78 °C under argon. The mixture was warmed to 0 °C for 5 min, then recooled to −78 °C and *t*BuLi (1.7 M in pentane, 1.1 mL, 1.87 mmol) was added, followed by acetaldehyde (41 mg, 0.93 mmol) after 5 min. After 1 h at −78 °C, the reaction was quenched with satd. aq. NH_4Cl (10 mL) and the mixture was extracted with Et_2O (3 × 30 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (3:2) as eluent gave compound **22** (196 mg, 73% yield) − a colourless oil − as a mixture of stereoisomers of undetermined stereochemistry. IR: $\tilde{\nu}$ = 3423, 2957, 1249, 1022, 834 cm^{-1}. ^1H NMR: δ = 0.04 (s, 9 H, Me_3Si), 0.80−0.98 (m, 5 H, CH_2Si and 8-H_3), 1.23 and 1.35 (2d, *J* = 7, 3H overall, 10-H_3), 1.49−1.87 (m, 3 H), 2.12−2.75 (m, 3 H), 3.57−3.75 (m, 2 H, CH_2O), 4.34−4.42 (m, 1 H, 1-H), 4.69−4.80 (m, 3 H, 9-H and OCH_2O), 4.93 (m, 1 H, 6-H) ppm. $C_{16}H_{32}O_4Si$ (316.51): calcd. C 60.72, H 10.19; found calcd. C 60.78; H, 10.15.

1-(2-Acetyl-5-{[2-(trimethylsilyl)ethoxy]methoxy}cyclopent-1-en-1-yl)propan-1-one (23): Dess−Martin periodinane[14] (678 mg, 1.6 mmol) was added to a solution of compound **22** (191 mg, 0.6 mmol) in dry CH_2Cl_2 (5 mL) at 25 °C. After 3 h, the reaction was quenched by the addition of satd. aq. $Na_2S_2O_3$ (10 mL) and $NaHCO_3$ (10 mL) and extracted with Et_2O (3 × 30 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to give compound **23** (177 mg, 94% yield) as a colourless oil. IR: $\tilde{\nu}$ = 2952, 2895, 1686, 1621, 1413, 1362, 1285, 1249, 1214, 1191, 1129, 1103, 1056, 1023, 940, 861, 837, 751, 693 cm^{-1}. ^1H NMR: δ = 0.04 (s, 9 H, Me_3Si), 0.92−0.98 (m, 2 H, CH_2Si), 1.13 (t, *J* = 7.2 Hz, 3 H, 8-H_3), 1.95−2.06 (m, 1 H), 2.31 (s, 3 H, 10-H_3), 2.31−2.42 (m, 1 H), 2.50−2.80 (m, 3 H), 2.80−2.92 (m, 1 H), 3.60−3.67 (m, 2 H, CH_2O), 4.74 (ABq, *J* = 7.1, 2 H, OCH_2O), 5.08 (m, 1 H, 1-H) ppm. $C_{16}H_{28}O_4Si$ (312.48): calcd. C 61.50, H 9.03; found C 61.58, H 9.07.

1-(2-Acetyl-5-hydroxycyclopent-1-enyl)propan-1-one (1): 40% aq. HF (0.5 mL) was added dropwise to a solution of compound **23** (89 mg, 0.28 mmol) in MeCN (2 mL) *contained in a polyethylene test tube.* After 24 h, an additional amount (0.5 mL) of HF was added and the mixture was left at 25 °C for further 24 h. The solution was taken to neutrality by the addition of satd. aq. $NaHCO_3$ and diluted with Et_2O (20 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Chromatographic separation of the residue on silica gel with hexane/EtOAc (3:2) as eluent gave compound **1** (45 mg, 88% yield) as a pale yellow oil. The IR, ^1H and ^{13}C NMR spectra, as well as the chromatographic properties of synthetic and natural samples of compound **1** are identical.

1-[2-(1-Hydroxyethyl)-3-{[2-(trimethylsilyl)ethoxy]methoxy}-cyclopent-1-en-1-yl]propan-1-ol (24): Compound **21** (298 mg, 0.9 mmol) was submitted to bromine/lithium exchange followed by reaction with acetaldehyde (Scheme 6) according to the same pro-

cedure described above for compound **20**. Diol **24** (201 mg, 71% overall yield) − a colourless oil − was obtained as two chromatographically separable mixtures (A and B) of diastereomers of undetermined stereochemistry. ^{1}H NMR ([D$_6$]Me$_2$CO) of mixture A: δ = 0.04 (s, 9 H, *Me*$_3$Si), 0.87 (t, *J* = 7 Hz, 3 H, 10-H$_3$), 0.87−0.97 (m, 2 H, C*H*$_2$Si), 1.32 (d, *J* = 6.7 Hz, 3 H,7-H$_3$), 1.45−1.70 (m, 2 H, 9-H$_2$), 1.70−1.82 (m, 1 H), 1.90−2.03 (m, 1 H), 2.35−2.40 (m, 2 H), 3.60−3.70 (m, 2 H, C*H*$_2$O), 3.82 (d, *J* = 5 Hz, 1 H, OH), 3.91 (d, *J* = 5.0 Hz, 1 H, O*H*), 4.44 (m, 1 H, 8-H), 4.70 (m, 3 H, C*H*O and OC*H*$_2$O), 4.86 (m, 1 H, C*H*O) ppm. IR: ṽ = 3390, 2957, 2895, 1249, 1026, 859, 835 cm^{-1}. ^{1}H NMR ([D$_6$]Me$_2$CO) of mixture B: δ = 0.04 (s, 9 H, *Me*$_3$Si), 0.82−0.99 (m, 5 H, 10-H$_3$ and C*H*$_2$Si), 1.29 and 1.32 (2 d, *J* = 6.7, 3H overall, 7-H$_3$), 1.40−1.80 (m, 3 H), 2.00−2.65 (m, 3 H), 3.58−3.76 (m, 2 H, C*H*$_2$O), 3.97, 4.00, 4.08 and 4.12 (4 d, *J* = 5.0, 2H overall, 2 O*H*), 4.39−4.51 (m, 1 H, C*H*O), 4.57−4.78 (m, 3 H, C*H*O and OC*H*$_2$O), 4.88−4.97 (m, 1 H, C*H*O) ppm. $C_{16}H_{32}O_4Si$ (316.51): calcd. C 60.72, H 10.19; found C 60.65, H 10.13.

1-[2-Acetyl-3-{[2-(trimethylsilyl)ethoxy]methoxy}cyclopent-1-en-1-yl]propan-1-one (25): Compound **24** (149 mg, 0.47 mmol) was treated with Dess−Martin periodinane[14] (Scheme 6) according to the same procedure described above for compound **22**. Diketone **25** (130mg, 89% yield) was obtained as a colourless oil. IR: ṽ = 2952, 1698, 1619, 1459, 1411, 1361, 1293, 1249, 1191, 1130, 1101, 1056, 1023, 939, 861, 837, 837, 759, 694 cm^{-1}. ^{1}H NMR: δ = 0.04 (s, 9 H, *Me*$_3$Si), 0.92−0.98 (m, 2 H, C*H*$_2$Si), 1.05 (t, *J* = 7.2 Hz, 3 H, 10-H$_3$), 1.95−2.10 (m, 1 H), 2.27 (s, 3 H, 7-H$_3$), 2.25−2.40 (m, 1 H), 2.54−2.63 (m, 2 H), 2.78−2.89 (m, 2 H), 3.65 (m, 2 H, OC*H*$_2$), 4.74 (ABq, *J* = 7, 2 H, OC*H*$_2$O), 5.07−5.12 (m, 1 H, 1-H) ppm. $C_{16}H_{28}O_4Si$ (312.48): calcd. C 61.50, H 9.03; found C 61.45, H 8.98.

1-(2-Acetyl-3-hydroxycyclopent-1-en-1-yl)propan-1-one (3): Compound **25** (89 mg, 0.28 mmol) was treated with 40% aq. HF (Scheme 6) according to the same procedure described above for compound **23**. Alcohol **3** (43 mg, 85% yield) was obtained as a colourless oil. IR: ṽ = 3422, 2977, 2940, 1683, 1618, 1499, 1458, 1455, 1409, 1362, 1215, 1128, 1049, 968, 874, 799 cm^{-1}. ^{1}H NMR ([D$_6$]Me$_2$CO): δ = 1.03 (t, *J* = 7.2 Hz, 3 H, 10-H$_3$), 1.75−1.93 (m, 1 H), 2.30 (s, 3 H, 7-H$_3$), 2.30−2.40 (m, 1 H), 2.45−2.62 (m, 3 H), 2.72−2.90 (m, 1 H), 4.45 (m, 1 H, O*H*), 5.12 (m, 1 H) ppm. ^{13}C NMR ([D$_6$]Me$_2$CO): δ = 6.6 (q), 28.5 (q), 31.7 (t), 33.1 (t), 33.8 (t), 76.9 (d), 145.6 (s), 149.9 (s), 198.5 (s), 203.6 (s) ppm. EIMS: *m*/*z* (%) = 182 (3) [M$^+$], 164 (8), 153 (28), 125 (22), 111 (7), 97 (16), 83 (57), 57 (12), 43 (100). $C_{10}H_{14}O_3$ (182.22): calcd. C 65.91, H 7.74; found C 65.72, H 7.75.

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