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The synthesis of 2-oxyalkyl-cyclohex-2-enones, related to the bioactive natural products COTC and antheminone A, which possess anti-tumour properties

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ABSTRACT

The syntheses of five novel 2-oxyalkyl-cyclohex-2-enones, structurally related to the natural products COTC and antheminone A, are described. The target structures were selected in order to probe the influence of several key structural parameters on in vitro anti-cancer bioactivity. The results of a cytotoxicity bioassay of the compounds against non-small-cell lung cancer cell lines A549 and H460 are reported. The biological data provides useful information, which will help guide the future design of compounds in this class with enhanced anti-cancer activity.

1. Introduction

The 2-oxyalkyl-cyclohex-2-enone moiety is a common structural feature in many natural products, including keto-carbasugars such as 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC, **1**)[1] and gabosine E (**2**),[2] as well as a variety of terpenoids including antheminone A (**3**)[3] and phorbasin B (**4**)[4] (Fig. 1). Of these, (**1**) and (**3**) have been shown to display notable toxicity towards a range of different cancer cell lines[3,5]—a finding that has stimulated much interest from both chemists and biologists in compounds of this type.

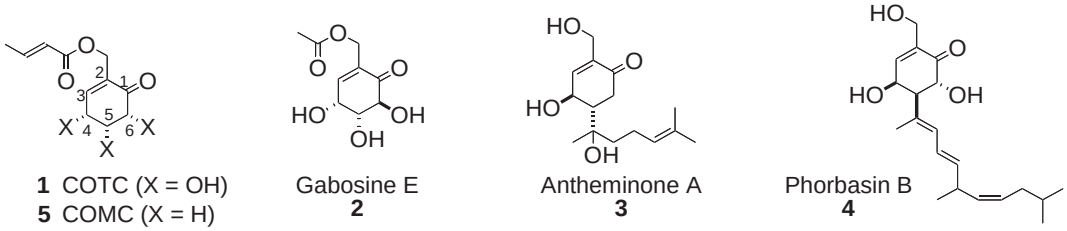

Fig. 1.

Extensive investigations into the anti-tumour properties of COTC, and its non-hydroxylated analogue, 2-crotonyloxymethyl-cyclohex-2-enone (COMC, **5**), by Creighton, Ganem and others have delineated a plausible general mechanism for anti-cancer activity of the cyclohexenones which is depicted for (**1**) in Scheme 1.[6,7] According to this mechanism, conjugate addition of glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) to the enone moiety of (**1**) gives enol (**6**), which undergoes expulsion of crotonic acid to generate a glutathionylated exocyclic enone (**7**); the formation of enol (**6**) is facile but the rate of reaction in cells is substantially increased by enzymatic catalysis via glutathione transferase (GST).[6] Alkylation of intracellular proteins and/or nucleic acids by (**7**) is believed to then lead to cell death.

Other processes may also contribute to the anti-cancer activity of the cyclohexenones. Thus, GSH conjugate (**9**), derived by trapping the exocyclic enone (**7**) with GSH, has been shown to be a competitive inhibitor of human glyoxalase 1 (Glo1):[7] this enzyme (inhibitors of which have previously been demonstrated to have anti-cancer properties) is vital for cell survival as part of a detoxification system for cytotoxic 2-oxoaldehydes such as methylglyoxal.[8] Furthermore, general depletion of intracellular GSH resulting from formation of (**9**), or indeed the bis-GSH adduct (**10**), may also contribute to anti-cancer activity due to a resulting

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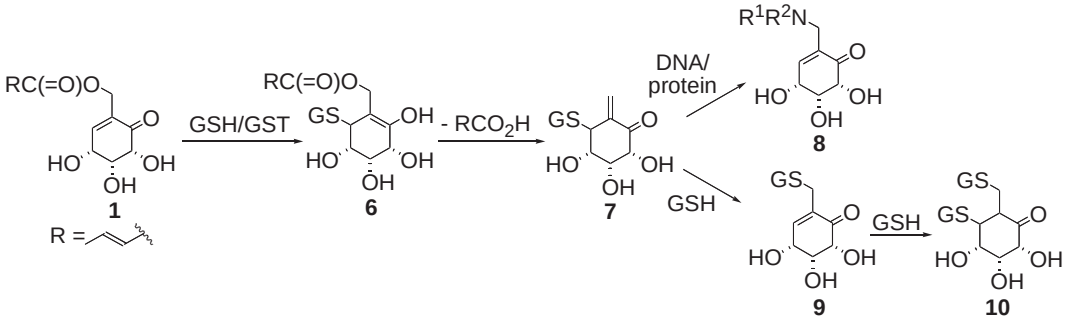


Scheme 1.

increase in concentration of 2-oxoaldehydes or other toxic species. A consequence of these mechanisms of action is the probability that cells, which require high levels of GSH/GST compared with normal cells (e.g., many inherently drug-resistant tumour cells) may exhibit enhanced sensitivity towards compounds containing the 2-oxyalkyl-cyclohex-2-enone moiety.

Prompted by the findings described above, one focus of our current research concerns the preparation of analogues of compounds (**1**–**4**) having generic structure (**11**) wherein there are five loci for diversification: the acyl substituent R^1, the side-chain branching substituent R^2 and the C4, C5 and C6 substituents on the cyclohexenone core (X, Y and Z).[9–11] In this paper, we provide full details of the syntheses of five novel 2-oxyalkyl-cyclohex-2-enones (**12**–**16**) (Fig. 2) as well as the results of their bioassay against GSH-rich lung cancer cell lines, A549 and H460.

itself. We felt that a key intermediate in the synthesis of (**12**) would be the hydroxyl-protected derivative of (4*S*)-4-hydroxycyclohex-2-enone (**17**), which could be converted to the target compound via a three-step reaction sequence of Morita–Baylis–Hillman reaction,[12] esterification and deprotection (Scheme 2).

A large-scale synthesis of TBS-ether (**17**) from (−)-quinic acid (**20**) has been described by Danishefsky and co-workers during the course of which isopropylidene protection was employed for the vicinal *cis*-diol moiety of (**20**).[13] In our hands, although this synthetic route was successful, it proved to be a little capricious with regard to reproducibility of yields as well as ease of isolation of some intermediates (e.g., the isopropylidene analogue of triol (**22**)). We opted, therefore, to modify the route to (**17**) and to utilise cyclohexylidene protection for the *cis*-diol moiety in (**20**) (Scheme 3).

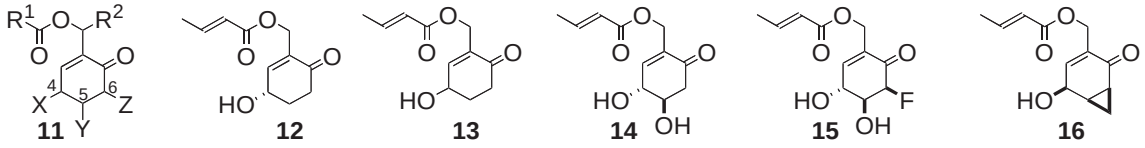
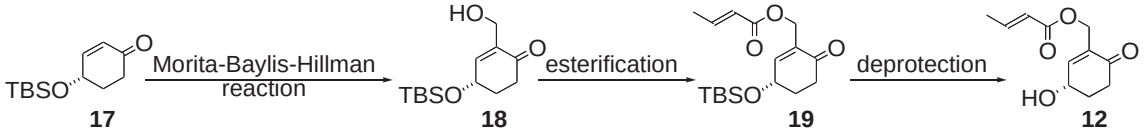


Fig. 2.



Scheme 2.

The selection of the target molecules was initiated by an early observation made by Douglas and co-workers that the non-hydroxylated synthetic compound COMC (**5**) was generally more toxic towards cancer cell lines than the tri-hydroxylated natural product COTC (**1**).[5] Thus, compounds (**12**), (**13**) and (**14**) were chosen in order to probe the importance of the extent of hydroxylation, as well as absolute configuration, on anti-cancer activity. Compound (**15**) was selected in order to examine the biological effect of incorporation of an activating and lipophilic fluorine atom adjacent to the carbonyl group and compound (**16**) was chosen in order to discover whether incorporation of a second electrophilic site into the structures (α,β-cyclopropyl ketones are pseudo-Michael acceptors) would result in enhanced biological potency.

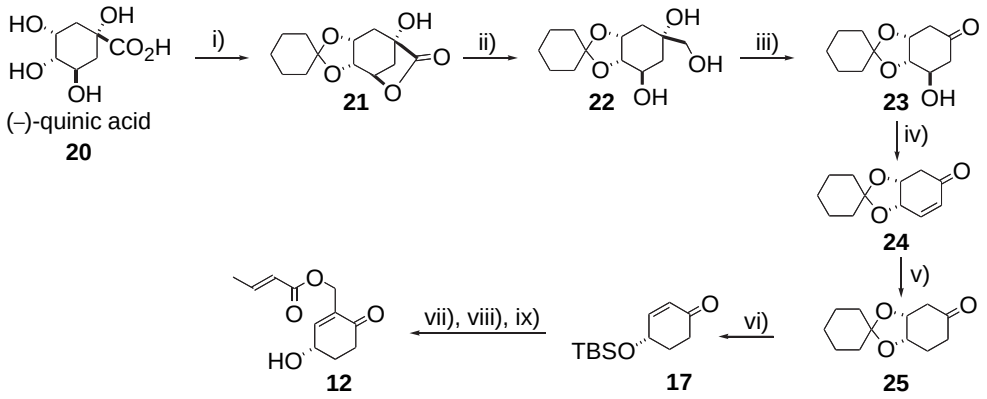
2. Results and discussion

2.1. Mono-hydroxylated analogues of COTC (12) and (13)

The first objective of our research programme was the preparation of enantiomerically pure mono-hydroxylated analogue (**12**), which possesses the same absolute configuration at C4 as COTC

Using an adaptation of a published procedure, cyclohexylidene quinide (**21**) was prepared in excellent yield from (**20**).[14] Reductive ring-opening of the γ-lactone moiety in (**21**) proceeded smoothly under mild conditions ($NaBH_4$) and subsequent oxidative cleavage of the vicinal diol moiety in (**22**) gave β-hydroxyketone (**23**) in acceptable overall yield from (**21**).[15] In a similar manner to the work reported by Danishefsky, dehydration of (**23**) to give α,β-enone (**24**) could be achieved using methanesulfonyl chloride and Et_3N. This reaction, however, was sluggish even at elevated temperature and the transformation was better accomplished at room temperature via an intermediate triflate and in the presence of pyridine. Catalytic hydrogenation of (**24**) proceeded with good chemoselectivity to give cyclohexanone (**25**) and subsequent base-mediated elimination of the cyclohexylidene protecting group with concomitant O-silylation gave (**17**), which was identical in all respects to the material reported by Danishefsky and co-workers.[13]

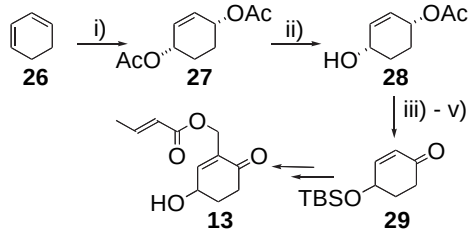
The Morita–Baylis–Hillman reaction represents a powerful procedure for the introduction of a hydroxyalkyl group at the α-position of α,β-unsaturated carbonyl compounds however, with cyclic enone substrates, the reaction can be quite unpredictable with regard to reaction time as well as isolated yield.[12] We were



Scheme 3. Reagents: (i) cyclohexanone, DMF/C_6H_6 (1:1), Amberlite IR 120 (H)®, reflux (Dean and Stark), 5 h, 92%; (ii) $NaBH_4$, CH_3OH, 0 °C to rt, 24 h; (iii) $NaIO_4$, H_2O, CH_3OH, 0 °C, 1.5 h, 58% from **21**; (iv) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0 °C to rt, 12 h, 82%; (v) H_2, 10% Pd on C, EtOAc, rt, 17 h, 92%; (vi) DBU, TBSCl, C_6H_6, reflux, 6 h, 80%; (vii) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 52%; (viii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 68%; (ix) TFA/H_2O (7:1), 0 °C, 1 h, 98%.

pleased, therefore, to discover that treatment of (**17**) with aqueous formaldehyde and a catalytic amount of DMAP for 24 h furnished hydroxymethyl compound (**18**) in an acceptable yield (52%).[16] Finally, crotonylation of the primary hydroxyl group of (**18**) and acid-mediated removal of the silyl protecting group both proceeded smoothly to give target compound (**12**), the enantiomeric purity of which was confirmed by chiral GC analysis using an essentially racemic sample for comparison (vide infra).

The synthesis of analogue (**13**) was achieved in a similar fashion to (**12**) via racemic silyl-ether (**29**), which in turn was prepared in five synthetic operations from commercially available 1,3-cyclohexadiene (**26**). Thus, using the excellent protocol described by Bäckvall, palladium-catalysed *cis*-1,4-diacetoxylation of (**26**) provided the *meso*-diacetate (**27**).[17] In accordance with the observations of Kazlauskas and co-workers,[18] enzymatic de-symmetrisation of (**27**) using electric eel cholinesterase[19] then gave essentially racemic mono-acetate (**28**) (synthetic (**28**): $[\alpha]_D^{21}$ −1.8 (*c* 2.21, CH_2Cl_2); (−)-(**28**): $[\alpha]_D$ −100.0 (*c* 1.3, CH_2Cl_2)). Subsequent silylation of the free hydroxyl of (**28**) with TBSOTf followed by methanolysis of the allylic acetate and oxidation using the Ley–Griffith reagent (TPAP)[20] gave enone (**29**) in acceptable yield. Conversion of (**29**) to mono-hydroxylated analogue (**13**) was then accomplished using an identical three-step sequence to that described for the preparation of (**12**) (Scheme 4).



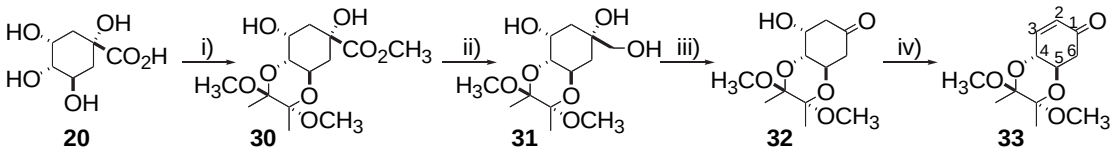
Scheme 4. Reagents: (i) MnO_2, LiCl, *p*-benzoquinone, $Pd(OAc)_2$, $LiOAc{\cdot}2H_2O$, AcOH, rt, 3 d, 69%; (ii) electric eel cholinesterase, NaN_3, phosphate buffer (pH 6.85), 20 °C, 30 h, 63%; (iii) TBSOTf, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 82%; (iv) K_2CO_3, CH_3OH, rt, 3 h, 88%; (v) TPAP, NMO, 4 Å mol. sieves, CH_2Cl_2, rt, 7 h, 65%.

2.2. Dihydroxylated analogues of COTC (14) and (15)

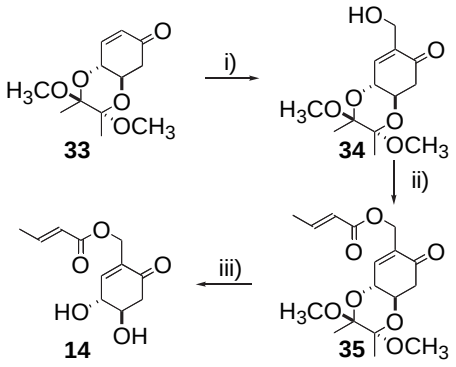
The key intermediate in our approach to dihydroxylated analogue (**14**) was butane-diacetal (BDA) protected dihydroxycyclohexenone (**33**), the cyclohexane-diacetal (CDA) protected variant of which was first described in 1996 by Brückner and Gebauer.[21] Using a variation of the synthetic sequence reported by these authors, (**33**) was prepared in four steps from (−)-quinic acid (**20**) in an overall yield of 79% (Scheme 5). Accordingly, using a modification of the procedure described by Frost and co-workers, BDA-protected methyl quinate (**30**) was prepared from (**20**) in almost quantitative yield.[22,23] Reduction of the α-hydroxy ester moiety of (**30**) was initially carried out using a large excess of $NaBH_4$ in aqueous methanol, however mass-recovery of the highly polar and partially water-soluble triol (**31**) using this procedure was quite variable. A more reliable procedure was developed, therefore, whereby treatment of (**30**) with 2 equiv of $LiBH_4$ in THF resulted in a good recovery of crude triol (**31**) after 90 min and subsequent oxidative cleavage, using the silica-supported $NaIO_4$ reagent described by Shing and Zhong,[24] gave β-hydroxyketone (**32**) in excellent yield over two steps from (**30**). Dehydration of (**32**) to give enone (**33**) was then accomplished, via an intermediate mesylate, using similar conditions to those described by the original authors.[21]

Similarly to the mono-hydroxylated series (vide supra), treatment of (**33**) with formaldehyde in the presence of a catalytic amount of DMAP, under the 'aqueous' conditions described by Rezgui and El Gaied,[16] furnished hydroxymethyl compound (**34**) in excellent yield (Scheme 6). Alternative conditions for this reaction, including the use of DABCO as a nucleophilic catalyst[25] and the use of surfactant additives,[26] were less successful with regard to both the isolated yield of (**34**) as well as reproducibility. Crotonylation of the free hydroxyl of (**34**) and subsequent acid-mediated removal of the BDA protecting group both proceeded smoothly to give the dihydroxylated target compound (**14**), which was purified by reverse phase HPLC.

Unambiguous confirmation of the structure of the product of the Morita–Baylis–Hillman reaction was gained by X-ray analysis of a crystal of (**34**) obtained from petroleum ether/ethyl acetate (Fig. 3).



Scheme 5. Reagents and conditions: (i) butan-2,3-dione, $(CH_3O)_3CH$, camphorsulfonic acid, CH_3OH, Δ, 12 h, 98%; (ii) $LiBH_4$, THF, rt, 1.5 h; (iii) $NaIO_4$ on silica gel, CH_2Cl_2, 1.5 h, 82% over two steps from **30**; (iv) Et_3N, CH_3SO_2Cl, CH_2Cl_2, 3 h, 98%.



Scheme 6. (i) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 80%; (ii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 67%; (iii) TFA/H_2O (7:1), 0 °C, 30 min, then HPLC, 75%.

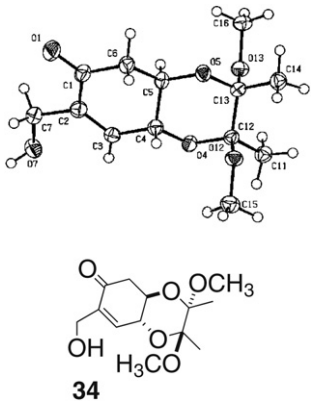


Fig. 3. Crystal structure of (**34**) with ellipsoids at 50% probability.

Incorporation of a highly electronegative and lipophilic fluorine atom into natural products can have a profound and often beneficial effect on their bioactivity. We were interested, therefore, to discover whether incorporation of a fluorine atom adjacent to the ketone carbonyl of dihydroxylated analogue (**14**) would have a positive influence on anti-cancer activity. It was envisaged that the synthesis of the target α-fluoro ketone (**15**) may be achievable via quenching an enolate derived from enone (**33**) with an electrophilic source of fluorine. In this regard, we were particularly encouraged by results communicated by O'Brien and co-workers who reported a procedure for introduction of a bromine atom at C6 of (**33**), via the intermediate generation of trimethylsilylenol-ether (**36**).[27] After much experimental investigation, it was gratifying to find that, using a modification of these workers' procedure, enol-ether (**36**) could be quenched with the 'F^+-source', SelectFluor®,[28] to give a single fluorine-containing compound (**37**) in acceptable yield (Scheme 7). Morita–Baylis–Hillman reaction of (**37**) with formaldehyde then proceeded in excellent yield to give allylic alcohol (**38**), which was converted in the usual manner to fluorinated COTC analogue (**15**).

The facial selectivity of the reaction of (**36**) with SelectFluor®, to give axially fluorinated compound (**37**), was confirmed by the use of 2D heteronuclear Overhauser effect spectroscopy (HOESY) of the hydroxymethylated compound (**38**). Figure 4 compares the normal proton spectrum (top) of (**38**) for the region around δ 4.8 ppm with a cross-section (bottom) through the F_2 dimension of a 2D gradient HOESY spectrum at the fluorine chemical shift. As expected a strong NOE is seen to the geminal proton C(6)*H* (53 Hz doublet, one half of which overlaps the vicinal proton resonance C(4)*H* at δ 4.84 ppm), and a weaker transannular NOE to C(4)*H*.

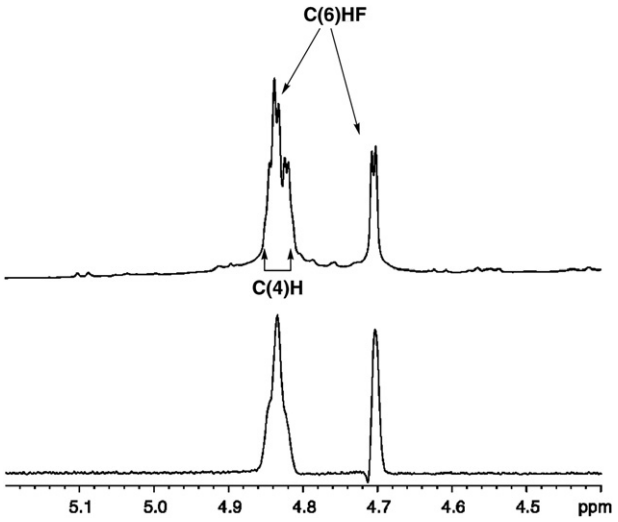
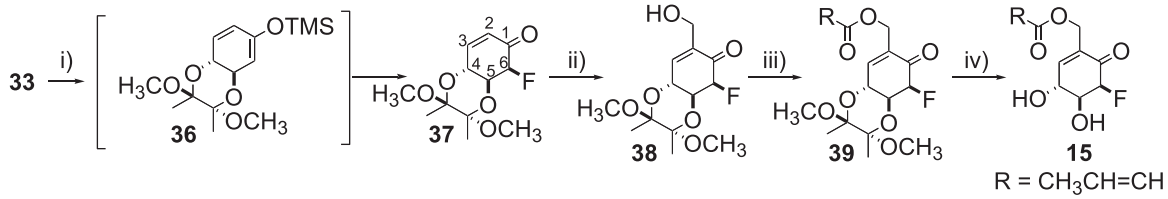


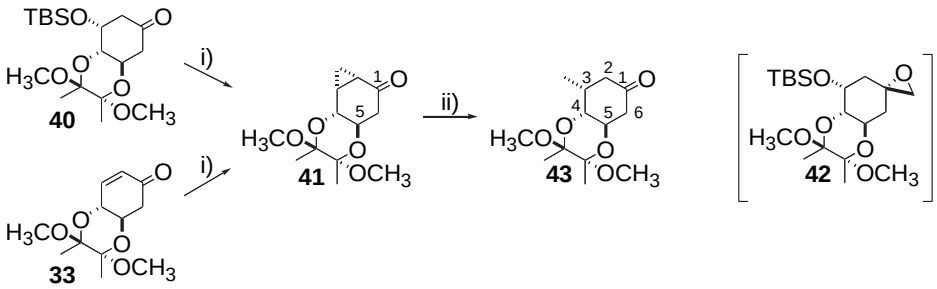
Fig. 4.

2.3. α,β-Cyclopropyl ketone (16)

It is well documented that α,β-cyclopropyl ketones can react as pseudo-Michael acceptors towards a range of different nucleophiles and this observation prompted our interest in the preparation of COTC analogue (**16**). This compound possesses both a conjugated ketone and an α,β-cyclopropyl ketone moiety and might, therefore, be expected to display enhanced GSH/GST-mediated anti-cancer activity. As a prequel to the synthesis of compound (**16**), a pertinent observation was made during earlier investigations into the reactivity of β-silyloxy ketone (**40**) towards a variety of nucleophiles: thus, exposure of the latter compound to dimethylsulfoxonium methylide (Corey's ylid)[29] resulted in face-selective formation of cyclopropane (**41**) as the only isolable product (rather than the spiro-epoxide (**42**)) (Scheme 8). The formation of (**41**) can be rationalised as arising from base-mediated elimination of *tert*-butyldimethylsilanoxide to generate enone (**33**), followed by stereoelectronically favoured 'axial attack' at C3 by the ylid. In accord with this suggestion, treatment of enone (**33**) with a slight excess of Corey's ylid furnished cyclopropane (**41**) in very good yield.



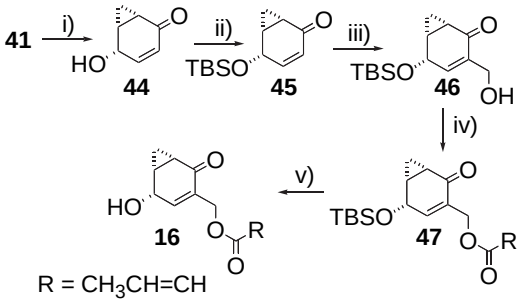
Scheme 7. Reagents and conditions: (i) KHMDS (0.75 M in toluene), TMSCl, THF, −78 °C to 0 °C, 1 h then SelectFluor®, CH_3CN, 0 °C, 30 min, 57%; (ii) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 94%; (iii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 39%; (iv) TFA/H_2O (7:1), rt, 3 h, then HPLC, 95%.



Scheme 8. Reagents and conditions: (i) trimethylsulfoxonium iodide, NaH, DMSO, rt, 1.5 h, 56% from **40**, 87% from **33**; (ii) SmI_2, THF, DMPU, rt, 15 min, 54%.

Initially, the stereochemical assignment of (**41**) was based on the observation of a significant NOE from the axially located hydrogen at C5 (δ 3.87 ppm) to the cyclopropyl methylene *endo* hydrogen (δ 1.48 ppm). Further evidence was gained from spectroscopic analysis of β-methylcyclohexanone (**43**), the product of reductive ring-opening of α,β-cyclopropyl ketone (**41**):[30] a significant NOE between the axially located hydrogen at C5 and the methyl hydrogens together with a small vicinal coupling constant between C(4)*H* and C(3)*H* of 4.6 Hz are both in accord with an axial location for the C3 substituent in this compound.

Conversion of α,β-cyclopropyl ketone (**41**) to the target analogue (**16**) was accomplished in five synthetic operations. Thus, prolonged exposure of (**41**) to aqueous acidic conditions resulted in sequential removal of the BDA protecting group and elimination of a molecule of water to generate γ-hydroxy-enone (**44**) (Scheme 9). Protection of the allylic hydroxyl of (**44**) then proceeded smoothly to give TBS-ether (**45**) and subsequent incorporation of a hydroxymethyl group at C2 was best accomplished, albeit in very disappointing yield, via an imidazole-mediated Morita–Baylis–Hillman reaction with formaldehyde.[31] Conversion of (**46**) to target structure (**16**) was then accomplished in the usual manner.



Scheme 9. Reagents and conditions: (i) TFA/H_2O (7:1), rt, 12 h, then K_2CO_3, H_2O, CH_3OH, rt, 30 min, 99%; (ii) TBSCl, DMAP (cat.), Et_3N, CH_2Cl_2, rt, 6 d, 78%; (iii) imidazole (cat.), H_2CO, THF, Na_2CO_3 (aq), 40 °C, 7 d, 23%; (iv) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 24 h, 48%; (v) TFA/H_2O (7:1), 0 °C, 1 h, 94%.

2.4. Toxicity of COTC analogues (12–16) towards cancer cell lines

The results of toxicity bioassays of the five novel analogues of COTC against two lung cancer cell lines (A549 and H460) are shown in Table 1 together with the corresponding data for COMC (**5**), which was prepared according to a two-step procedure described by Ganem and co-workers.[6] These cell lines were chosen because they showed the highest level of GSH from among a panel of human tumour cell lines used in chemosensitivity testing.[32] Intracellular GSH concentrations are 16.9 and 22.3 mM, respectively. Furthermore, the A549 cells show high levels of cytosolic πGST. The cytotoxicity assays were carried out by exposing cells to varying concentrations of each compound for 4 days. The number of surviving cells was then determined by the use of the MTT assay.[33] Values of IC_{50} are the drug concentrations required to reduce cell number by 50% relative to untreated, control cells.

Table 1
IC_{50} values (μM) of compounds (**12**–**16**) towards lung cancer cell lines A549 and H460. Experiments were repeated twice and data within individual experiments were derived from four separate observations: average values are given in the table

		Compound					
		COMC (**5**)	(**12**)	(**13**)	(**14**)	(**15**)	(**16**)
Cell line	A549	54.5	16.7	23.6	147.4	163.7	18.1
	H460	40.4	10.9	10.5	158.0	>200	20.4

The bioassay results allow several conclusions to be reached regarding the structural features that influence the toxicity of this class of compounds towards the two lung cancer cell lines:

- incorporation of a single hydroxyl group at C4 results in a significant improvement in potency over the non-hydroxylated compound, COMC (**5**) (data for (**12**), (**13**) and (**16**) vs data for (**5**));
- the absolute configuration at C4 has little influence on potency (data for (**12**) vs data for (**13**));
- incorporation of a second hydroxyl group at C5 results in a significant erosion of potency (data for (**14**) and (**15**) vs data for (**5**), (**12**), (**13**) and (**16**));
- incorporation of a lipophilic and activating fluorine atom adjacent to the carbonyl group has no beneficial influence on bioactivity (data for (**15**) vs data for (**14**));
- incorporation of an additional electrophilic site into the COTC analogues has no beneficial influence on bioactivity (data for (**16**) vs data for (**12**) and (**13**));

The enhanced potency of the mono-hydroxylated analogues compared to COMC (**5**) prompted us to investigate the toxicity of compounds (**12**) and (**16**) towards a small panel of human cancer cell lines (Table 2).

The data presented here clearly demonstrate that both mono-hydroxylated compounds display significant toxicity towards all of the cell lines investigated with the α,β-cyclopropyl ketone (**16**) being between two and five times less potent than (**12**).

3. Conclusion

In conclusion, five novel 2-oxyalkyl-cyclohex-2-enones (**12**–**16**), which are structurally related to the natural products COTC (**1**) and antheminone A (**3**), have been prepared. The target compounds were selected in order to probe the influence of several key structural parameters on the cytoxicity of this structural class towards non-small-cell lung cancer cell lines. Four of the compounds (**12** and **14**–**16**) were enantiomerically pure and were synthesised from the 'chiral pool' material (−)-quinic acid. The remaining compound (**13**), a racemate, was synthesised from cyclohexa-1,3-diene.

Cytotoxicity bioassay of the new compounds against non-small-cell lung cancer cell lines, A549 and H460, was carried out and the results of this allow several general conclusions to be made:

Table 2
IC_{50} values (μM) of compounds (**12**) and (**16**) towards an array of human cancer cell lines. Experiments were repeated three times and data within individual experiments were derived from four separate observations: average values are given in the table

		Cell line					
		MDA231 (breast)	T47D (breast)	HCT116 (colon)	HT29 (colon)	RT112 (bladder)	HT1080 (fibrosarcoma)
Compound	(**12**)	5.4±2.7	10.0±0.9	6.4±2.4	8.3±3.0	3.7±0.5	4.5±0.4
	(**16**)	18.8±5.0	20.1±2.3	12.4±2.2	12.2±4.4	14.2±2.2	24.2±5.9

- analogues possessing a *single* free hydroxyl group (e.g., (**12**), (**13**) and (**16**)) display the greatest anti-cancer activity
- the absolute configuration at C4 has little influence on bioactivity
- an additional electrophilic moiety (α,β-cyclopropyl ketone) does not enhance biological potency

The synthetic chemistry described herein, coupled with the results of cytotoxicity bioassay, will provide useful information to guide the future design and synthesis of novel compounds of this class with enhanced anti-cancer activity.

4. Experimental

4.1. General

Solvents were dried and distilled before use. Chromatography was performed over Merck silica gel 60 (40–63 μm). IR spectra were recorded on a Perkin–Elmer 881 spectrometer or an AT1-Mattson Genesis Series FTIR spectrometer or a JASCO FT/IR-4100 spectrometer. ^{1}H, ^{13}C and ^{19}F spectra were recorded on a Varian Inova 400 MHz spectrometer, a Varian Inova 300 MHz spectrometer or a Bruker AMX 500 MHz spectrometer. Chemical shifts are referenced to the residual solvent peak. Mass spectra were recorded on Micromass Trio 2000 quadrupole (EI/CI, low resolution), Thermo Finnigan MAT 95 XP (EI/CI, high resolution), Micromass Platform (electrospray) spectrometers. Melting points were recorded using a Sanyo Gallenkamp MPD350 heater and are uncorrected.

4.2. Preparation of mono-hydroxylated compound (12)

*4.2.1. (1S,3R,4R,5R)-3-O,4-O-Cyclohexylidene-7-oxo-6-oxabicyclo [3.2.1]octan-1,3,4-triol (**21**)*[14]. A mixture of (−)-quinic acid (40.0 g, 208 mmol), cyclohexanone (140 mL, 1.35 mol), DMF (170 mL) and benzene (160 mL) was heated under reflux in a flask fitted with a Dean and Stark trap and condenser until no more water was collected. After cooling to room temperature, Amberlite® resin IR 120 (H) (35 g) was added. The suspension was heated at reflux for a further 5 h (until no more water was collected) and then allowed to cool to room temperature. The resin was removed by filtration and the excess solvent removed in vacuo. Toluene (150 mL) was added and the mixture washed with a saturated aqueous solution of sodium bicarbonate (2×100 mL), followed by water (2×100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give the crude product as a waxy solid. Petroleum ether (200 mL) was added and the mixture was cooled in ice for 1 h. The solid was collected by filtration, washed with cold petroleum ether and dried under vacuum to afford the title compound as colourless crystals (49.1 g, 92%); mp 140.8–141.3 °C [lit.[14] mp 139–141 °C]; $[\alpha]_D^{22}$ −36.0 (*c* 0.60, CH_2Cl_2) [lit.[14] $[\alpha]_D^{22}$ −33.0 (*c* 1.05, $CHCl_3$)]; ν_{max} (film)/cm^{-1} 3434br (O–H), 2930s (C–H), 1795s (C=O); δ_H (300 MHz; $CDCl_3$) 1.40–1.80 (10H, m, 5×CH_2 of cyclohexane), 2.21 (1H, dd, *J* 14.5, 3.0, one of C(2)H_2), 2.28–2.45 (2H, m, one of C(2)H_2 and one of C(6)H_2), 2.76 (1H, br d, *J* 11.5, one of C(6)H_2), 4.38 (1H, ddd, *J* 6.5, 2.5, 1.0, C(4)*H*), 4.48–4.56 (1H, m, C(3)*H*), 4.80 (1H, dd, *J* 6.0, 2.5, C(5)*H*); δ_C (75 MHz; $CDCl_3$) 23.8, 24.2, 25.3, 33.9, 37.1 (5×CH_2 of cyclohexane), 34.7 (*C*(6)H_2), 38.8 (*C*(2)H_2), 71.3 (*C*(5)H), 71.9 (*C*(4)H), 72.1 (*C*(3)H), 76.3 (*C*(1)), 110.9 (acetal *C*), 178.9 (*C*=O); *m*/*z* (CI/NH_3) 272 ($[M+NH_4]^+$, 100%), 255 ($[M+H]^+$, 30), 211 (10); (Found: 255.1213. $C_{13}H_{19}O_5$ ($[M+H]^+$) requires 255.1227).

*4.2.2. 3-O,4-O-Cyclohexylidene-(3R,4S,5R)-trihydroxycyclohexan-1-one (**23**).* The lactone quinide (**21**) (0.87 g, 3.43 mmol) was stirred in methanol (60 mL) at 0 °C and sodium borohydride (1.29 g, 34.3 mmol) was carefully added portionwise. Once effervescence had ceased, the reaction mixture was stirred for a further 24 h at room temperature when it was quenched by the addition of a saturated aqueous solution of ammonium chloride (40 mL). Organic material was extracted into ethyl acetate (3×40 ml) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the crude triol (**22**) as a colourless solid (0.88 g). Methanol (80 mL) was added to the solid and the resulting dispersion was stirred at 0 °C. A saturated aqueous solution of sodium periodate (19.6 mL) was added to the dispersion and stirring was continued at 0 °C for 1.5 h. The reaction was quenched by the addition of brine (20 mL) and organic material was then extracted into ethyl acetate (2×20 mL) and diethyl ether (2×20 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a clear oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 1:1) furnished the title compound as a colourless solid (0.45 g, 58% from (**21**)); R_f 0.12 (petroleum ether/diethyl ether, 1:1); mp 94–95 °C [lit.[15] mp 97–98 °C]; $[\alpha]_D^{20}$ +98.2 (*c* 0.88, CH_2Cl_2) [lit.[15] $[\alpha]_D$ +100.3 (*c* 0.44, CH_3OH)]; ν_{max} (film)/cm^{-1} 3419br (O–H), 2935w (C–H), 1700s (C=O); δ_H (300 MHz; $CDCl_3$) 1.39–1.62 (10H, m, 5×CH_2 of cyclohexane), 2.44 (1H, ddd, *J* 18.0, 3.0, 2.0, C(6)H_α), 2.63–2.71 (2H, m, C(2)H_β and C(6)H_β), 2.81 (1H, dd, *J* 18.0, 3.0, C(2)H_α), 3.17 (1H, br s, O*H*), 4.22–4.24 (1H, m, C(5)*H*), 4.32 (1H, dt, *J* 9.0, 3.0, C(4)*H*), 4.70 (1H, dt, *J* 9.0, 3.0, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 23.7, 24.1, 25.4, 33.5 and 36.5 (5×CH_2 of cyclohexane), 30.6 (*C*(6)H_2), 40.5 (*C*(2)H_2), 68.3 (*C*(5)H), 71.9 (*C*(4)H), 74.8 (*C*(3)H), 109.7 (acetal *C*), 209.5 (*C*(1)O); *m*/*z* (CI/NH_3) 244 ($[M+NH_4]^+$, 100%), 227 ($[M+H]^+$, 40); (Found: 227.1277. $C_{12}H_{19}O_4$ ($[M+H]^+$) requires 227.1278).

*4.2.3. (4R,5S)-4-O,5-O-Cyclohexylidene-4,5-dihydroxycyclohex-2-en-1-one (**24**).* A solution of β-hydroxyketone (**23**) (1.00 g, 4.43 mmol) in dichloromethane (22 mL) was stirred at −10 °C under an atmosphere of N_2. Pyridine (0.43 mL, 5.31 mmol) and trifluoromethanesulfonic anhydride (0.87 mL, 5.31 mmol) were added dropwise to the stirred solution, which was then allowed to warm to room temperature. After 3 h, a further portion of pyridine (0.43 mL, 5.31 mmol) was added and the reaction mixture was stirred overnight. The reaction was quenched by the careful addition of a saturated aqueous solution of sodium bicarbonate (10 mL) and the organic phase was collected. The aqueous phase was extracted with a further portion of dichloromethane (10 mL) and the combined organic extracts were washed sequentially with a saturated aqueous solution of sodium bicarbonate (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as an orange oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 2:1) provided enone (**24**) as a colourless solid (0.75 g, 82%); R_f 0.20 (petroleum ether/diethyl ether, 2:1); mp 50–51 °C [lit.[34] mp 55–58 °C]; $[\alpha]_D^{20}$ +113 (*c* 1.81, CH_2Cl_2) [lit.[34] $[\alpha]_D$ +135 (*c* 1.0, $CHCl_3$)]; ν_{max} (film)/

cm^{-1} 3143m (C–H), 1659s (C=O); δ_H (300 MHz; $CDCl_3$) 1.41–1.68 (10H, m, 5×CH_2 of cyclohexane), 2.71 (1H, dd, *J* 17.7, 3.9, one of C(6)H_2), 2.98 (1H, dd, *J* 17.7, 2.7, one of C(6)H_2), 4.68–4.77 (2H, m, C(4)*H* and C(5)*H*), 6.04 (1H, dt, *J* 10.5, 1.2, C(2)*H*), 6.68 (1H, ddd, *J* 10.5, 2.7, 2.1, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 24.0, 24.1, 25.2, 36.5 and 37.7 (5×CH_2 of cyclohexane), 39.1 (*C*(6)H_2), 70.9 (*C*(4)H or *C*(5)H), 72.1 (*C*(4)H or *C*(5)H), 110.8 (acetal *C*), 128.9 (*C*(2)H), 146.5 (*C*(3)H), 195.9 (*C*(1)O); *m*/*z* (CI/NH_3) 226 ($[M+NH_4]^+$, 50%), 209 ($[M+H]^+$, 100); (Found: 208.1099. $C_{12}H_{16}O_3$ (M^+) requires 208.1094).

*4.2.4. (3R,4S)-3-O,4-O-Cyclohexylidene-3,4-dihydroxycyclohexanone (**25**).* A solution of the enone (**24**) (352 mg, 1.69 mmol) in ethyl acetate (13 mL) was stirred at room temperature and palladium on carbon (10%, 35 mg) was added. The reaction vessel was flushed several times with hydrogen and the reaction mixture was then stirred under an atmosphere of hydrogen for 17 h. After this time, the reaction mixture was filtered through a pad of Celite®, eluting with ethyl acetate, and concentration of organic solvents in vacuo then yielded the crude product as a yellow oil. Purification by flash column chromatography (SiO_2; diethyl ether/petroleum ether, 1:1) furnished the title compound as a colourless solid (329 mg, 92%); R_f 0.22 (diethyl ether/petroleum ether, 7:3); mp 84.6–85.7 °C [lit.[34] mp 86–87 °C]; ν_{max} (film)/cm^{-1} 3169s (C–H), 2070w (C–H), 1649s (C=O); δ_H (300 MHz; $CDCl_3$) 1.40–1.65 (10H, m, 5×CH_2 of cyclohexane), 1.88 (1H, ~tdd, *J* 15.0, 6.0, 3.0, C(5)H_α), 2.09–2.17 (1H, m, C(5)H_β), 2.22–2.30 (1H, m, one of C(6)H_2), 2.47 (1H, ddd, *J* 15.0, 6.0, 3.0, one of C(2)H_2), 2.54 (1H, ddd, *J* 12.0, 6.0, 3.0, one of C(6)H_2), 2.71 (1H, dd, *J* 15.0, 3.0, one of C(2)H_2), 4.56–4.60 (1H, m, C(4)*H*), 4.65–4.69 (1H, m, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 23.8, 24.2, 25.5, 25.9 and 33.5 (5×CH_2 of cyclohexane), 33.8 (*C*(6)H_2), 36.0 (*C*(5)H_2), 42.3 (*C*(2)H_2), 71.3 (*C*(4)H), 72.6 (*C*(3)H), 108.6 (acetal *C*), 210.3 (*C*(1)O); *m*/*z* (CI/NH_3) 211 ($[M+H]^+$, 100%), 210 (M^+, 20); (Found: 210.1253. $C_{12}H_{18}O_3$ (M^+) requires 210.1256).

*4.2.5. (4S)-4-O-tert-Butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**17**)[13].* *tert*-Butyldimethylsilyl chloride (0.30 g, 1.99 mmol) and DBU (0.28 mL, 1.86 mmol) were added at room temperature to a solution of ketone (**25**) (0.35 g, 1.66 mmol) in benzene (14 mL). The reaction mixture was stirred at room temperature for 10 min and then heated under reflux for 3 h. After cooling to room temperature, a second portion of DBU (70 μL, 0.46 mmol) was added and heating under reflux was continued for a further 3 h. The reaction mixture was then allowed to cool to room temperature and diethyl ether (15 mL) was added. The resulting organic phase was washed sequentially with water (10 mL), 1 M aqueous HCl (10 mL), saturated aqueous sodium bicarbonate solution (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 9:1) furnished the title compound as a colourless oil (0.30 g, 80%); R_f 0.22 (petroleum ether:diethyl ether, 9:1); $[\alpha]_D^{20}$ −111.6 (*c* 0.81, CH_2Cl_2) [lit.[13] $[\alpha]_D^{20}$ −115.9 (*c* 1.06, $CHCl_3$); ν_{max} (film)/cm^{-1} 2940w (C–H), 2920s (C–H), 1675s (C=O); δ_H (300 MHz; $CDCl_3$) 0.15 (3H, s, SiCH_3), 0.16 (3H, s, SiCH_3), 0.95 (9H, s, C(CH_3)$_3$), 2.03 (1H, tdd, *J* 12.9, 9.2, 4.5, C(5)H_α), 2.24 (1H, dqd, *J* 12.9, 4.5, 1.8, C(5)H_β), 2.38 (1H, ddd, *J* 16.8, 12.9, 4.5, C(6)H_β), 2.61 (1H, br dt, *J* 16.8, 4.5, C(6)H_α), 4.56 (1H, ddt, *J* 9.2, 4.5, 1.8, C(4)*H*), 5.96 (1H, d, *J* 10.2, C(2)*H*), 6.86 (1H, dt, *J* 10.2, 1.8, C(3)*H*); δ_C (75 MHz; $CDCl_3$) −4.9 (SiCH_3), −4.3 (SiCH_3), 26.0 (C(CH_3)$_3$), 33.2 (*C*(5)H_2 or *C*(6)H_2), 35.8 (*C*(5)H_2 or *C*(6)H_2), 67.3 (*C*(4)H), 128.9 (*C*(2)H), 154.2 (*C*(3)H), 199.2 (*C*(1)O); *m*/*z* (CI/NH_3) 244 ($[M+NH_4]^+$, 100%), 227 ($[M+H]^+$, 20); (Found: 244.1736. $C_{12}H_{26}NO_2Si$ ($[M+NH_4]^+$) requires 244.1727).

*4.2.6. (4S)-2-Hydroxymethyl-4-O-tert-butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**18**).* The enone (**17**) (140 mg, 0.62 mmol) was stirred in a mixture of tetrahydrofuran/water (1:1) (8 mL) at room temperature. Formaldehyde (37% aqueous solution) (115 μL, 1.55 mmol) and a crystal of 4-dimethylaminopyridine were added and the reaction mixture was stirred at 40 °C for 24 h. The reaction mixture was then acidified with 1.5 M aqueous hydrochloric acid and extracted with dichloromethane (2×10 mL). The combined organic extracts were washed sequentially with brine (2×10 mL) and saturated aqueous sodium bicarbonate solution (2×10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash chromatography (SiO_2; petroleum ether/diethyl ether, 1:1) yielded the title compound as a colourless oil (82 mg, 52%); R_f 0.18 (petroleum ether/diethyl ether, 3:1); $[\alpha]_D^{20}$ −50.5 (*c* 1.14, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3425br (O–H), 2932m (C–H), 1675s (C=O); δ_H (300 MHz; $CDCl_3$) 0.16 (3H, s, SiCH_3), 0.17 (3H, s, SiCH_3), 0.95 (9H, s, C(CH_3)$_3$), 1.96–2.69 (4H, m, C(5)H_2 and C(6)H_2), 4.23 (1H, d, *J* 13.5, CH_aH$_b$OH), 4.35 (1H, d, *J* 13.5, CH$_a$H$_b$OH), 4.56–4.63 (1H, m, C(4)*H*), 6.79–6.82 (1H, m, C(3)*H*); δ_C (75 MHz; $CDCl_3$) −4.5 (SiCH_3), −4.4 (SiCH_3), 26.0 (C(CH_3)$_3$), 33.3 (*C*(5)H_2 or *C*(6)H_2), 36.1 (*C*(5)H_2 or *C*(6)H_2), 61.7 (CH_2OH), 67.3 (*C*(4)H), 137.1 (*C*(2)), 151.1 (*C*(3)H), 200.0 (*C*(1)O); *m*/*z* (CI/NH_3) 257 ($[M+H]^+$, 10%); (Found: 257.1572. $C_{13}H_{25}O_3Si$ ($[M+H]^+$) requires 257.1567).

*4.2.7. (4S)-2-((E)-Crotonyloxymethyl)-4-O-tert-butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**19**).* A solution of hydroxymethyl compound (**18**) (81.9 mg, 0.32 mmol) in dichloromethane (5 mL) was stirred at room temperature under an atmosphere of N_2. Crotonic anhydride (104 μL, 0.70 mmol), a crystal of 4-dimethylaminopyridine and pyridine (0.23 mL, 2.84 mmol) were added and the reaction mixture was left to stir for 24 h. The reaction was quenched by the addition of a saturated aqueous solution of sodium bicarbonate (5 mL) and organic material was extracted into dichloromethane (2×10 mL). The combined organic extracts were then washed with saturated aqueous sodium bicarbonate solution (10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash chromatography (SiO_2; petroleum ether/diethyl ether, 6:1) furnished the title compound as a colourless oil (70 mg, 68%); R_f 0.13 (petroleum ether/diethyl ether, 6:1); $[\alpha]_D^{20}$ −45.9 (*c* 0.75, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2955m (C–H), 1725m (C=O, ester), 1650s (C=O, enone); δ_H (300 MHz; $CDCl_3$) 0.15 (3H, s, SiCH_3), 0.16 (3H, s, SiCH_3), 0.95 (9H, s, C(CH_3)$_3$), 1.92 (3H, dd, *J* 6.9, 1.8, CH_3CH=CH), 1.98–2.70 (4H, m, C(5)H_2 and C(6)H_2), 4.57–4.64 (1H, m, C(4)*H*), 4.79 (1H, d, *J* 13.8, CH_aH$_b$OC(=O)), 4.87 (1H, d, *J* 13.8, CH$_a$H$_b$OC(=O)), 5.91 (1H, dq, *J* 15.3, 1.8, CH$_3$CH=CH), 6.83–6.84 (1H, m, C(3)*H*), 7.05 (1H, dq, *J* 15.3, 6.9, CH$_3$C*H*=CH); δ_C (75 MHz; $CDCl_3$) −4.4 (SiCH_3), −4.3 (SiCH_3), 18.3 (*C*H$_3$CH=CH), 26.0 (C(CH_3)$_3$), 33.2 (*C*(5)H_2 or *C*(6)H_2), 35.9 (*C*(5)H_2 or *C*(6)H_2), 60.7 (CH_2OC(=O)), 67.4 (*C*(4)H), 122.5 (CH$_3$CH=CH), 133.8 (*C*(2)), 145.6 (CH$_3$*C*H=CH), 150.9 (*C*(3)H), 166.3 (CH$_2$O*C*(=O)), 197.5 (*C*(1)O); *m*/*z* (CI/NH_3) 342 ($[M+NH_4]^+$, 40%), 325 ($[M+H]^+$, 60%); (Found: 325.1826. $C_{17}H_{29}O_4Si$ ($[M+H]^+$) requires 325.1830).

*4.2.8. (4S)-2-((E)-Crotonyloxymethyl)-4-hydroxycyclohex-2-en-1-one-4-ol (**12**).* Silyl-ether (**19**) (36 mg, 0.11 mmol) was stirred in a mixture of TFA and H_2O (7:1, 0.8 mL) at 0 °C for 1 h, after which time the solvents were removed in vacuo to give the crude product as a yellow oil. Purification by flash chromatography (SiO_2; petroleum ether/diethyl ether, 3:1) provided the title compound as a colourless oil (23 mg, 98%); R_f 0.17 (petroleum ether/diethyl ether, 3:1); $[\alpha]_D^{20}$ −23.0 (*c* 0.45, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3434br (O–H), 2361w (C–H), 1716s (C=O, ester), 1658s (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.93 (3H, dd, *J* 6.0, 1.5, CH_3CH=CH), 1.98–2.73 (4H, m, C(5)H_2 and C(6)H_2), 4.62–4.70 (1H, m, C(4)*H*), 4.78–4.92 (2H, m, CH_2OC(=O)), 5.91 (1H, dq, *J* 15.0, 1.5, CH$_3$CH=CH), 6.92–6.93 (1H, m, C(3)*H*), 7.06 (1H, dq, *J* 15.0, 6.0, CH$_3$C*H*=CH); δ_C (75 MHz; $CDCl_3$) 18.4 (*C*H$_3$CH=CH), 32.5 (*C*(5)H_2 or *C*(6)H_2), 35.7 (*C*(5)H_2 or *C*(6)H_2), 60.7 (*C*H$_2$OC(=O)), 66.7 (*C*(4)H), 122.3 (CH$_3$CH=CH), 134.5 (*C*(2)), 146.4 (*C*(3)H), 149.2 (CH$_3$*C*H=CH), 166.6 (CH$_2$O*C*(=O)), 197.7 (*C*(1)

O); *m/z* (CI/NH_3) 228 ($[M+NH_4]^+$, 50%), 211 ($[M+H]^+$, 100%); (Found: 228.1238. $C_{11}H_{18}NO_4$ ($[M+NH_4]^+$) requires 228.1230).

4.3. Preparation of racemic enone (29)

*4.3.1. meso-3,6-Di-O-acetyl-cyclohex-1-ene-3,6-diol (**27**)*[17]. Manganese(IV) oxide (2.72 g, 31.3 mmol) was added to a stirred solution of lithium chloride (207 mg, 4.9 mmol), *p*-benzoquinone (640 mg, 5.9 mmol), palladium(II) acetate (280 mg, 1.25 mmol) and lithium acetate dihydrate (8.60 g, 84.3 mmol) in acetic acid (40 mL). 1,3-Cyclohexadiene (2.4 mL, 25.0 mmol) in pentane (50 mL) was then added to the reaction mixture and the resulting solution was stirred at room temperature for 3 days. Brine (50 mL) was added to dilute the mixture, which was extracted sequentially with pentane (3×100 mL) and pentane/diethyl ether (1:1 mixture, 3×80 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as an orange oil. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 9:1) furnished the title compound as a yellow oil (3.42 g, 69%); R_f 0.18 (petroleum ether/ethyl acetate, 9:1); ν_{max} (film)/cm^{-1} 2953s (C–H), 1736s (C=O); δ_H (300 MHz; $CDCl_3$) 1.73–1.88 (4H, m, C(4)H_2 and C(5)H_2), 1.97 (6H, s, 2×CH_3), 5.13 (2H, br s, C(3)*H* and C(6)*H*), 5.80 (2H, s, C(1)*H* and C(2)*H*); δ_C (75 MHz; $CDCl_3$) 21.3 (2×*C*H_3), 25.0 (*C*(4)H_2 and *C*(5)H_2), 67.4 (*C*(3)H and *C*(6)H), 130.4 (*C*(1)H and *C*(2)H), 170.5 (2×*C*=O); *m/z* (CI/NH_3) 216 ($[M+NH_4]^+$, 90%), 199 ($[M+H]^+$, 10), 139 (100); (Found: 216.1226. $C_{10}H_{18}NO_4$ ($[M+NH_4]^+$) requires 216.1230).

*4.3.2. (3S,6R/3R,6S)-3-O-Acetyl-cyclohex-1-ene-3,6-diol (**28**)*[19]. The *meso*-diacetate (**27**) (300 mg, 1.51 mmol) was stirred in aqueous phosphate buffer (0.58 M, pH=6.85, 50 mL) together with sodium azide (2 mg, 0.03 mmol) and electric eel cholinesterase (1 mg, 292 units) at 20 °C for 30 h. Organic material was then extracted into a mixture of ethyl acetate and diethyl ether (1:1, 3×20 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a red oil. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 3:1) furnished the title compound as a clear oil (149 mg, 63%); R_f 0.11 (petroleum ether/ethyl acetate, 3:1); ν_{max} (film)/cm^{-1} 3402br (O–H), 2950s (C–H), 1735s (C=O); δ_H (300 MHz; $CDCl_3$) 1.70–1.98 (4H, m, C(4)H_2 and C(5)H_2), 2.04 (3H, s, OC(=O)CH_3), 2.60 (1H, br, O*H*), 4.18–4.20 (1H, m, C(6)*H*), 5.15–5.18 (1H, m, C(3)*H*), 5.77 (1H, br dd, *J* 10.2, 3.6, C(1)*H*), 5.95 (1H, br dd, *J* 10.2, 2.9, C(2)*H*); δ_C (75 MHz; $CDCl_3$) 21.5 (OC(=O)CH_3), 25.3 (*C*(4)H_2 and *C*(5)H_2, signals coincident), 65.5 (*C*(6)H), 67.6 (*C*(3)H), 127.8 (*C*(1)H), 135.3 (*C*(2)H), 171.1 (*C*=O); *m/z* (CI/NH_3) 174 ($[M+NH_4]^+$, 100%), 139 (100); (Found: 174.1136. $C_8H_{16}NO_3$ ($[M+NH_4]^+$) requires 174.1125).

4.3.3. (3S,6R/3R,6S)-3-O-Acetyl-6-O-tert-butyldimethylsilyl-cyclohex-1-ene-3,6-diol. A stirred solution of allylic acetate (**28**) (200 mg, 1.28 mmol) in dichloromethane (10 mL) was cooled to 0 °C. A crystal of 4-dimethylaminopyridine was added, followed by triethylamine (0.27 mL, 1.92 mmol) and a solution of TBDMS–OTf (0.32 mL, 1.41 mmol) in dichloromethane (10 mL). The reaction mixture was stirred for 1 h at 0 °C when it was quenched by the careful addition of water (20 mL). The organic phase was collected and combined with two further dichloromethane extracts (2×20 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 15:1) provided the title compound as a colourless oil (284 mg, 82%); R_f 0.26 (petroleum ether/diethyl ether, 15:1); δ_H (300 MHz; $CDCl_3$) 0.01 (6H, s, (Si(CH_3)$_2$)), 0.92 (9H, s, C(CH_3)$_3$), 1.72–1.97 (4H, m, C(4)H_2 and C(5)H_2), 2.07 (3H, s, OC(=O)CH_3), 4.16–4.22 (1H, m, C(6)*H*), 5.15–5.19 (1H, m, C(3)*H*), 5.75–5.77 (1H, m, C(1)*H* or C(2)*H*), 5.89–5.91 (1H, m, C(1)*H* or C(2)*H*); δ_C (75 MHz; $CDCl_3$) −4.4 (SiCH_3), −4.3 (SiCH_3), 18.4 (*C*(CH_3)$_3$) 21.6 (OC(=O)CH_3), 25.6 (*C*(4)H_2 or *C*(5)H_2), 26.1 (C(CH_3)$_3$), 28.7 (*C*(4)H_2 or *C*(5)H_2), 66.6 (*C*(6)H), 67.3 (*C*(3)H), 126.5 (*C*(1)H or *C*(2)H), 136.7 (*C*(1)H or *C*(2)H), 170.6 (*C*=O); *m/z* (CI/NH_3) 288 ($[M+NH_4]^+$, 10%), 271 ($[M+H]^+$, 5); (Found: 288.1997. $C_{14}H_{30}NO_3Si$ ($[M+NH_4]^+$) requires 288.1998).

4.3.4. (3S,6R/3R,6S)-6-O-tert-Butyldimethylsilyl-cyclohex-1-ene-3,6-diol. Potassium carbonate (150 mg, 1.05 mmol) was added portionwise, at room temperature, to a stirred solution of racemic 3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-cyclohex-1-ene-3,6-diol (284 mg, 1.05 mmol) in methanol (10 mL) and stirring was continued for 3 h. The reaction was quenched by the addition of water (10 mL) and organic components were extracted into ethyl acetate (2×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a pale yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 3:1) provided the title compound as a colourless oil (210 mg, 88%); R_f 0.11 (petroleum ether/diethyl ether, 3:1); ν_{max} (film)/cm^{-1} 3400br (O–H), 2953w (C–H), 2856w (C–H); δ_H (300 MHz; $CDCl_3$) 0.01 (6H, s, Si(CH_3)$_2$), 0.92 (9H, s, C(CH_3)$_3$), 1.67–1.88 (4H, m, C(4)H_2 and C(5)H_2), 4.09–4.18 (2H, m, C(3)*H* and C(6)*H*), 5.75–5.84 (2H, m, C(1)*H* and C(2)*H*); δ_C (75 MHz; $CDCl_3$) −4.4 (SiCH_3), −4.3 (SiCH_3), 26.1 (*C*(4)H_2 or *C*(5)H_2), 28.5 (*C*(4)H_2 or *C*(5)H_2), 28.7 (C(CH_3)$_3$), 65.1 (*C*(3)H or *C*(6)H), 66.6 (*C*(3)H or *C*(6)H), 130.8 (*C*(1)H or *C*(2)H), 134.5 (*C*(1)H or *C*(2)H); *m/z* (ESI$^+$) 251 ($[M+Na]^+$, 100%), 229 ($[M+H]^+$, 10).

*4.3.5. (4S/R)-4-O-tert-Butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**29**).* A solution of racemic 6-*O*-*tert*-butyldimethylsilyl-cyclohex-1-ene-3,6-diol (200 mg, 0.88 mmol) in dichloromethane (15 mL) was stirred at room temperature. Molecular sieves (1.5 g), 4-methylmorpholine *N*-oxide (130 mg, 0.97 mmol) and TPAP (15.5 mg, 0.04 mmol) were added and stirring was continued at room temperature for 7 h. The reaction mixture was filtered through a pad of silica, eluting successively with dichloromethane (20 mL) and ethyl acetate (20 mL) and the eluate was concentrated in vacuo to yield the crude product as a brown oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 5:1) furnished the title compound as a pale yellow oil (130 mg, 65%); analytical data as for compound (**17**) (vide supra).

4.4. Preparation of dihydroxylated compound (14)

*4.4.1. (2′S,3′S)-Methyl-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-quinate (**30**).* Prepared as described previously.[35]

*4.4.2. (2′S,3′S,3R,4R,5R)-4-O,5-O-(2′,3′-Dimethoxybutane-2′,3′-diyl)-cyclohexan-1-one-3,4,5-triol (**32**).* Lithium borohydride (0.68 g, 31.2 mmol) was stirred in freshly distilled THF (30 mL) at room temperature under an atmosphere of N_2 for 15 min. A solution of BDA-protected methyl quinate (**30**) (5.0 g, 15.6 mmol) in THF (30 mL) was added at 0 °C and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was then poured into a saturated aqueous solution of ammonium chloride (60 mL). Water and THF were removed in vacuo to yield a colourless solid to which ethyl acetate (200 mL) was added. The resulting mixture was filtered to remove the suspended solid, which was washed repeatedly with ethyl acetate (3×40 mL). The combined washings were dried ($MgSO_4$) and concentrated in vacuo to yield the crude triol (**31**) as a colourless solid (4.48 g).

Sodium periodate (8.20 g, 38.3 mmol) was dissolved in water (30 mL) with gentle warming. To the resulting solution, silica gel (20.5 g) was carefully added with vigorous stirring to produce silica-supported sodium periodate as a free flowing powder. A solution of the crude triol (**31**) in dichloromethane (60 mL) was added to

a vigorously stirred suspension of the silica-supported sodium periodate reagent in dichloromethane (120 mL). The heterogeneous reaction mixture was stirred at room temperature for 1.5 h then filtered and the silica-residue washed repeatedly with dichloromethane (3×50 mL). The filtrate was dried ($MgSO_4$) and concentrated in vacuo to yield the title compound as a pale yellow solid (3.34 g, 82%); R_f 0.26 (ethyl acetate/40–60 petroleum ether, 1:1); mp 167.9–169.7 °C [lit.[36] mp 163–165 °C]; $[\alpha]_D^{24}$ +147 (*c* 1.0, CH_2Cl_2) [lit.[36] $[\alpha]_D^{20}$ +159.8 (*c* 0.59, CH_2Cl_2)]; ν_{max} (film)/cm^{-1} 3442br (O–H), 2991w (C–H), 2948w (C–H), 2991w (C–H), 2834w (C–H), 1721s (C=O); δ_H (400 MHz; $CDCl_3$) 1.32 (3H, s, butyl CH_3), 1.35 (3H, s, butyl CH_3), 2.40 (1H, s, O*H*), 2.47–2.54 (2H, m, one of C(2)H_2 and one of C(6)H_2), 2.64–2.70 (2H, m, one of C(2)H_2 and one of C(6)H_2), 3.24 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 3.90 (1H, dd, *J* 10.1, 2.5, C(4)*H*), 4.25–4.32 (2H, m, C(3)*H* and C(5)*H*); δ_C (100 MHz; $CDCl_3$) 17.6 (butyl *C*H_3), 17.7 (butyl *C*H_3), 44.7 (*C*(2)H_2 or *C*(6)H_2), 46.1 (*C*(2)H_2 or *C*(6)H_2), 48.0 (O*C*H_3), 48.1 (O*C*H_3), 63.2 (*C*(3)H or *C*(5)H), 67.7 (*C*(3)H or *C*(5)H), 72.2 (*C*(4)H), 99.2 (acetal *C*), 100.3 (acetal *C*), 205.5 (*C*(1)O); *m*/*z* (CI/NH_3) 278 ($[M+NH_4]^+$, 60%), 246 (90), 229 (60), 214 (50); (Found: 278.1606. $C_{12}H_{24}NO_6$ ($[M+NH_4]^+$) requires 278.1598).

*4.4.3. (2′S,3′S,4R,5R)-4-O,5-O-(2′,3′-Dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**33**).* A solution of hydroxyketone (**32**) (1.0 g, 3.8 mmol) in dichloromethane (20 mL) was stirred under an atmosphere of N_2 at 0 °C and methanesulfonyl chloride (0.36 mL, 4.61 mmol), followed by triethylamine (1.55 mL, 11.2 mmol) were carefully added. The reaction mixture was allowed to warm to room temperature and it was then stirred at that temperature for a further period of 3 h. The reaction was quenched by the addition of water (20 mL). The organic phase was collected and combined with two subsequent dichloromethane extracts (2×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the title compound as a slightly off-white solid (0.91 g, 98%); (Found: C, 59.61; H, 7.47. $C_{12}H_{18}O_5$ requires C, 59.49; H, 7.49%); mp 181.8–183.0 °C [lit.[36] mp 182–184 °C]; $[\alpha]_D^{22}$ +68.8 (*c* 1.2, CH_2Cl_2) [lit.[36] $[\alpha]_D^{20}$ +64.4 (*c* 0.39, CH_2Cl_2)]; ν_{max} (film)/cm^{-1} 3054w (C–H), 2854s (C–H), 1685 (m, C=O); δ_H (300 MHz; $CDCl_3$) 1.38 (3H, s, butyl CH_3), 1.40 (3H, s, butyl CH_3), 2.52 (1H, dd, *J* 16.5, 13.2, C(6)H_β), 2.80 (1H, dd, *J* 16.5, 4.8, C(6)H_α), 3.30 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 4.08 (1H, ddd, 13.2, 9.3, 4.8, C(5)*H*), 4.55 (1H, dt, *J* 9.3, 1.8, C(4)*H*), 6.05 (1H, dd, *J* 10.2, 1.8, C(2)*H*), 6.92 (1H, dd, *J* 10.2, 1.8, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 17.9 (butyl *C*H_3), 18.0 (butyl *C*H_3), 42.3 (*C*(6)H_2), 48.4 (O*C*H_3), 48.5 (O*C*H_3), 68.4 (*C*(5)H), 69.5 (*C*(4)H), 100.0 (acetal *C*), 101.1 (acetal *C*), 130.4 (*C*(3)H), 148.8 (*C*(2)H), 197.5 (*C*(1)O); *m*/*z* (CI/NH_3) 260 ($[M+NH_4]^+$, 10%), 228 (90); (Found: 260.1491. $C_{12}H_{22}NO_5$ ($[M+NH_4]^+$) requires 260.1492).

*4.4.4. (2′S,3′S,4R,5R)-2-Hydroxymethyl-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**34**).* A solution of the enone (**33**) (300 mg, 1.24 mmol) was stirred in a mixture of tetrahydrofuran and water (1:1, 10 mL) at room temperature. Formaldehyde (37%) (0.19 mL, 2.49 mmol) and a crystal of 4-dimethylaminopyridine (15 mg, 0.12 mmol) were added and the reaction mixture was warmed and stirred at 40 °C for 24 h. The reaction mixture was then acidified with 1.5 M aqueous hydrochloric acid and extracted with dichloromethane (3×10 mL). The combined organic extracts were washed sequentially with brine (2×10 mL) and a saturated aqueous solution of sodium bicarbonate (2×10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow solid. Purification by flash chromatography (SiO_2; petroleum ether/ethyl acetate, 1:1) furnished the title compound as a colourless solid (270 mg, 80%). Found: C, 57.24; H, 7.51. $C_{13}H_{20}O_6$ requires C, 57.34; H, 7.40%; R_f 0.36 (petroleum ether/ethyl acetate, 1:1); mp 179.8–181.2 °C; $[\alpha]_D^{22}$ +34.5 (*c* 0.9, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3435br (O–H), 3054m (C–H), 1677m (C=O); δ_H (300 MHz; $CDCl_3$) 1.38 (3H, s, butyl CH_3), 1.40 (3H, s, butyl CH_3), 2.58 (1H, dd, *J* 16.5, 15.0, C(6)H_β), 2.80 (1H, dd, *J* 16.5, 4.8, C(6)H_α), 3.30 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 4.09 (1H, ddd, *J* 15.0, 9.3, 4.8, C(5)*H*), 4.25 (1H, d, *J* 14.4, C$H_a$$H_b$OH), 4.36 (1H, d, *J* 14.4, C$H_a$$H_b$OH), 4.58 (1H, dd, *J* 9.3, 1.8, C(4)*H*), 6.88 (1H, d, *J* 1.8, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 17.9 (butyl *C*H_3), 18.0 (butyl *C*H_3), 42.4 (*C*(6)H_2), 48.4 (O*C*H_3), 48.5 (O*C*H_3), 61.1 (*C*H_2OH), 68.3 (*C*(5)H), 69.3 (*C*(4)H), 100.0 (acetal *C*), 101.1 (acetal *C*), 139.3 (*C*(2)), 144.7 (*C*(3)H), 197.5 (*C*(1)O); *m*/*z* (CI/NH_3) 290 ($[M+NH_4]^+$, 40%), 273 (10), 258 (90); (Found: 290.1597. $C_{13}H_{24}NO_6$ ($[M+NH_4]^+$) requires 290.1598).

*4.4.5. (2′S,3′S,4R,5R)-2-((E)-Crotonyloxymethyl)-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**35**).* A solution of hydroxymethyl compound (**34**) (175 mg, 0.73 mmol) in dichloromethane (20 mL) was stirred under an atmosphere of N_2 at room temperature. Crotonic anhydride (0.24 mL, 1.60 mmol), a crystal of 4-dimethylaminopyridine and pyridine (0.52 mL, 6.45 mmol) were added sequentially and the reaction mixture was then left to stir for 1.5 h at room temperature. The reaction was quenched by the addition of a saturated aqueous solution of sodium bicarbonate (20 mL) and organic material was then extracted into dichloromethane (2×20 mL). The combined organic extracts were washed with saturated sodium bicarbonate solution (10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow solid. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 5:1) yielded the title compound as a colourless solid (165 mg, 67%); R_f 0.22 (petroleum ether/ethyl acetate, 5:1); mp 76.2–76.8 °C; $[\alpha]_D^{22}$ +72.3 (*c* 0.6, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2854m (C–H), 1720m (C=O, ester), 1685w (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.38 (3H, s, butyl CH_3), 1.40 (3H, s, butyl CH_3), 1.95 (3H, dd, *J* 6.9, 1.8, CH_3CH=CH), 2.58 (1H, dd, *J* 16.5, 13.5, C(6)H_β), 2.82 (1H, dd, *J* 16.5, 4.8, C(6)H_α), 3.30 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 4.01 (1H, ddd, *J* 13.5, 9.3, 4.8, C(5)*H*), 4.58 (1H, dd, *J* 9.3, 1.5, C(4)*H*), 4.76 (1H, d, *J* 14.4, C$H_a$$H_b$OC=O), 4.81 (1H, d, *J* 14.4, C$H_a$$H_b$OC=O), 5.90 (1H, dq, *J* 15.6, 1.8, CH_3CH=CH), 6.84 (1H, d, *J* 1.5, C(3)*H*), 7.05 (1H, dq, *J* 15.6, 6.9, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) 17.9 (butyl *C*H_3), 18.0 (butyl *C*H_3), 18.3 (CH_3CH=CH), 42.3 (*C*(6)H_2), 48.4 (O*C*H_3), 48.5 (O*C*H_3), 60.3 (*C*H_2OC=O), 68.2 (*C*(5)H), 69.4 (*C*(4)H), 99.9 (acetal *C*), 101.1 (acetal *C*), 109.9 (CH_3CH=CH), 122.4 (CH_3CH=CH), 135.8 (*C*(2)), 145.1 (*C*(3)H), 166.1 (CH_2OC=O), 195.4 (*C*(1)O); *m*/*z* (CI/NH_3) 358 ($[M+NH_4]^+$, 70%), 341 (60), 326 (40); (Found: 358.1866. $C_{17}H_{28}NO_7$ ($[M+NH_4]^+$) requires 358.1860).

*4.4.6. (4R,5R)-2((E)-Crotonyloxymethyl)-4,5-(dihydroxy)-cyclohex-2-en-1-one (**14**).* Removal of the BDA protecting group from (**35**) was accomplished using trifluoroacetic acid in the same manner as deprotection of silyl-ether (**19**). Purification by reverse phase HPLC eluting with acetonitrile/water (20:80) afforded the title compound as a pale yellow oil (75%); $[\alpha]_D^{18}$ +11.7 (*c* 2.5, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3402br (O–H), 2963w (C–H), 2362w (C–H), 1719m (C=O, ester), 1678m (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.94 (3H, dd, *J* 6.9, 1.8, CH_3CH=CH), 2.52 (1H, dd, *J* 16.5, 12.3, C(6)H_β), 2.92 (1H, dd, *J* 16.5, 4.8, C(6)H_α), 4.01 (1H, ddd, *J* 12.3, 8.4, 4.8, C(5)*H*), 4.58 (1H, dd, *J* 8.4, 2.4, C(4)*H*), 4.80–4.90 (2H, m, CH_2OC=O), 5.90 (1H, dq, *J* 15.6, 1.8, CH_3CH=CH), 6.84 (1H, d, *J* 2.4, C(3)*H*), 7.05 (1H, dq, *J* 15.6, 6.9, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) 18.4 (CH_3CH=CH), 44.8 (*C*(6)H_2), 60.3 (*C*H_2OC=O), 72.9 (*C*(5)H), 73.3 (*C*(4)H), 122.2 (CH_3CH=CH), 135.2 (*C*(2)), 146.4 (*C*(3)H), 146.8 (CH_3CH=CH), 166.4 (CH_2OC=O), 195.8 (*C*(1)O); *m*/*z* (CI/NH_3): 244 ($[M+NH_4]^+$, 90%), 227 ($[M+H]^+$, 20); (Found: 244.1181. $C_{11}H_{18}NO_5$ ($[M+NH_4]^+$) requires 244.1179).

4.5. Preparation of fluorinated compound (15)

*4.5.1. (2′S,3′S,4R,5S,6S)-6-Fluoro-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**37**).* A solution of the enone (**33**) (200 mg, 0.83 mmol) in tetrahydrofuran (5 mL) was added, via cannula and under an atmosphere of N_2, to a stirred

solution of potasium bis(trimethylsilyl)amide (3.3 mL of a 0.75 M solution in toluene, 2.48 mmol) in tetrahydrofuran (5 mL) at −78 °C in a two-neck round bottom flask attached to a high vacuum Büchi rotary evaporator (no vacuum applied). The resulting solution was stirred for 1 h at −78 °C. Freshly distilled trimethylsilyl chloride (0.52 mL, 4.13 mmol) was added dropwise and the reaction mixture was stirred for 30 min at −78 °C, and then at 0 °C for a further 30 min. After being allowed to warm to room temperature the solvents were removed in vacuo and the crude trimethylsilylenol-ether (**36**) was stored under vacuum. A solution of SelectFluor® (0.59 g, 1.65 mmol) in acetonitrile (10 mL) was then added via syringe to the crude enol-ether at 0 °C and the resulting solution was stirred at 0 °C for 30 min. The reaction mixture was quenched by the addition of a saturated aqueous solution of ammonium chloride (20 mL), ethyl acetate (20 mL) was added and the organic phase was collected and combined with two subsequent ethyl acetate extracts (2×20 mL). The combined organic extracts were washed with brine (2×10 mL), dried ($MgSO_4$) and concentrated in vacuo to give the crude product as a yellow solid. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 9:1) yielded the title compound as a colourless solid (123 mg, 57%); R_f 0.28 (petroleum ether/ethyl acetate, 9:1); mp 170–171 °C; $[\alpha]_D^{22}$ −45.0 (*c* 1.2, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3003w (C–H), 2948s (C–H), 1688s (C=O); δ_H (300 MHz; $CDCl_3$) 1.38 (3H, s butyl C*H*$_3$), 1.40 (3H, s, butyl C*H*$_3$), 3.38 (3H, s, OC*H*$_3$), 3.40 (3H, s, OC*H*$_3$), 4.05 (1H, ddd, *J* 35.1, 8.7, 2.4, C(5)*H*), 4.84 (1H, ~dt, *J* 51.9, 2.4, C(6)*H*F), 4.93 (1H, ~dq slightly obscured, *J* 8.7, 1.8, C(4)*H*), 6.12 (1H, dq, *J* 10.2, 1.8, C(2)*H*), 7.03 (1H, dd, *J* 10.2, 1.8, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 17.8 (butyl *C*H$_3$), 17.9 (butyl *C*H$_3$), 48.5 (OCH$_3$), 48.6 (OCH$_3$), 64.2 (d, *J* 7.2, *C*(4)H), 71.4 (d, *J* 18.2, *C*(5)H), 89.9 (d, *J* 182.3, *C*(6)HF), 91.1 (acetal *C*), 100.8 (acetal *C*), 127.9 (*C*(2)H), 150.3 (*C*(3)H), 190.7 (d, *J* 16.9, *C*(1)O); δ_F (282 MHz; $CDCl_3$) −20.0 (dd, *J* 51.9, 35.1, C(6)H*F*); *m*/*z* (CI/NH_3) 278 ($[M+NH_4]^+$, 80%), 246 (60), 214 (40); (Found: 278.1393. $C_{12}H_{21}FNO_5$ ($[M+NH_4]^+$) requires 278.1398).

*4.5.2. (2′S,3′S,4R,5S,6S)-6-Fluoro-2-hydroxymethyl-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**38**).* Morita–Baylis–Hillman reaction of enone (**37**) (123 mg, 0.47 mmol) was carried out in a similar manner to that described for (**33**) (vide infra). Purification by flash chromatography (SiO_2; petroleum ether/ethyl acetate, 3:1) provided the hydroxymethyl compound (**38**) as a colourless oil (94%); R_f 0.16 (petroleum ether/ethyl acetate 3:1); $[\alpha]_D^{20}$ −53.4 (*c* 0.75, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3435br (O–H), 2361w (C–H), 1636w (C=O); δ_H (300 MHz; $CDCl_3$) 1.40 (3H, s, butyl C*H*$_3$), 1.42 (3H, s, butyl C*H*$_3$), 1.60 (1H, br, O*H*), 3.38 (3H, s, OC*H*$_3$), 3.40 (3H, s, OC*H*$_3$), 4.04 (1H, ddd, *J* 35.1, 8.1, 2.1, C(5)*H*), 4.37 (2H, s, C*H*$_2$OH), 4.88 (1H, dd, *J* 52.2, 2.1, C(6)*H*F), 4.94 (1H, dt slightly obscured, *J* 8.1, 1.8, C(4)*H*), 7.04 (1H, q, *J* 1.8, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 17.4 (butyl *C*H$_3$), 17.9 (butyl *C*H$_3$), 48.5(OCH$_3$), 48.6 (OCH$_3$), 60.5 (*C*H$_2$OH), 63.9 (d, *J* 7.2, *C*(4)H), 71.1 (d, *J* 18.1, *C*(5)H), 90.0 (d, *J* 181.4, *C*(6)HF), 100.8 (acetal *C*), 101.1 (acetal *C*), 137.5 (*C*(2)), 145.7 (*C*(3)H), 190.7 (d, *J* 16.9, (*C*(1)O)); δ_F (282 MHz; $CDCl_3$) −29.0 (dd, *J* 52.2, 35.1, C(6)H*F*); *m*/*z* (CI/NH_3) 308 ($[M+NH_4]^+$, 100%), 290 (20), 276 (80); (Found: 308.1501. $C_{13}H_{23}FNO_6$ ($[M+NH_4]^+$) requires 308.1504).

*4.5.3. (2′S,3′S,4R,5S,6S)-2((E)-Crotonyloxymethyl)-6-fluoro-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-4,5-dihydroxycyclohex-2-en-1-one (**39**).* Crotonylation of hydroxmethyl compound (**38**) (125 mg, 0.43 mmol) was carried out in a similar manner to that used for (**34**). Purification by flash chromatography (SiO_2; petroleum ether/ethyl acetate, 9:1) provided the title compound as a colourless oil (39%); R_f 0.18 (petroleum ether/ethyl acetate, 9:1); $[\alpha]_D^{22}$ −2.8 (*c* 1.1, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2952m (C–H), 1726s (C=O, ester), 1695s (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.39 (3H, s, butyl C*H*$_3$), 1.41 (3H, s, butyl C*H*$_3$), 1.93 (3H, dd, *J* 9.0, 3.0, C*H*$_3$CH=CH), 3.35 (3H, s, OC*H*$_3$), 3.38 (3H, s, OC*H*$_3$), 4.04 (1H, ddd, *J* 33.0, 9.0, 3.0, C(5)*H*), 4.87–4.88 (2H, m, C*H*$_2$OC=O), 4.90 (1H, dd, *J* 51.0, 3.0, C(6)*H*F), 4.94 (1H, dq, *J* 9.0, 3.0, C(4)*H*), 5.91 (1H, dq, *J* 15.0, 3.0, CH$_3$CH=CH), 7.00 (1H, q, *J* 3.0, C(3)*H*), 7.08 (1H, dq slightly obscured, *J* 15.0, 9.0, CH$_3$C*H*=CH); δ_C (75 MHz; $CDCl_3$) 17.7 (butyl *C*H$_3$), 19.9 (butyl *C*H$_3$), 18.4 (*C*H$_3$CH=CH), 48.6 (2×OCH$_3$, coincident), 59.9 (*C*H$_2$OC=O), 64.0 (d, *J* 7.4, *C*(4)H), 71.1 (d, *J* 17.7, *C*(5)H), 89.9 (d, *J* 182.0, *C*(6)HF), 100.8 (acetal *C*), 101.2 (acetal *C*), 122.2 (CH$_3$CH=CH), 133.9 (*C*(2)), 146.2 (*C*(3)H or CH$_3$*C*H=CH), 146.6 (*C*(3)H or CH$_3$CH=CH), 165.9 (CH$_2$O*C*=O), 189.2 (d, *J* 17.1, (*C*(1)O)); δ_F (282 MHz; $CDCl_3$) −29.5 (dd, *J* 51.0, 33.0, C(6)H*F*); *m*/*z* (CI/NH_3) 376 ($[M+NH_4]^+$, 50%), 359 ($[M+H]^+$, 10), 344 (20); (Found: 376.1773. $C_{17}H_{27}FNO_7$ ($[M+NH_4]^+$) requires 376.1766).

*4.5.4. (4R,5S,6S)-(2(E)-Crotonyloxymethyl)-6-fluoro-4,5-(dihydroxy)-cyclohex-2-en-1-one (**15**).* Removal of the BDA protecting group from (**39**) (43 mg, 0.12 mmol) was accomplished using trifluoroacetic acid in the same manner as for deprotection of silyl-ether (**19**). Purification by reverse phase HPLC eluting with acetonitrile/water (20:80) afforded the title compound as a clear oil (95%); $[\alpha]_D^{18}$ −4.4 (*c* 1.36, H_2O); ν_{max} (film)/cm^{-1} 3402br (O–H), 2900w (C–H), 1708s (C=O, ester), 1680s (C=O, enone); δ_H (400 MHz; $CDCl_3$) 1.74 (1H, br, O*H*), 1.91 (3H, d, *J* 6.8, C*H*$_3$CH=CH), 3.10 (1H, br, O*H*), 4.30 (1H, ~d, *J* 18.4, C(5)*H*), 4.67 (1H, br s, C(4)*H*), 4.86 (1H, d, *J* 14.8, C*H*$_a$H$_b$OC=O), 4.90 (1H, d, *J* 14.8, CH$_a$*H*$_b$OC=O), 5.22 (1H, ~d, *J* 50.0, C(6)*H*F), 5.89 (1H, d, *J* 14.0, CH$_3$CH=CH), 6.86 (1H, br s, C(3)*H*), 6.99–7.09 (1H, m, CH$_3$C*H*=CH); δ_C (100 MHz; $CDCl_3$) 18.1 (*C*H$_3$CH=CH), 59.7 (*C*H$_2$OC=O), 68.1 (d, *J* 7.6, *C*(4)H), 74.1 (d, *J* 17.5, *C*(5)H), 90.7 (d, *J* 182.8, *C*(6)HF), 121.8 (CH$_3$CH=CH), 134.0 (*C*(2)), 143.0 (*C*(3)H), 146.2 (CH$_3$CH=CH), 165.9 (CH$_2$OC=O), 190.7 (*C*(1)O); δ_F (376 MHz; $CDCl_3$) −206.8 (dd, *J* 50.0, 18.4, C(6)H*F*); *m*/*z* (CI/NH_3): 262 ($[M+NH_4]^+$, 100%), 245 ($[M+H]^+$, 30); (Found: 262.1077. $C_{11}H_{17}FNO_5$ ($[M+NH_4]^+$) requires 262.1085).

4.6. Preparation of α,β-cyclopropyl ketone (16)

*4.6.1. (2′S,3′S,2S,3R,4S,5R)-2,3-Cyclopropyl-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-cyclohexan-1-one-4,5-diol (**41**).* A solution of the enone (**33**) (1.50 g, 6.19 mmol) in a 1:1 mixture of DMSO and THF (75 mL) was added carefully at room temperature to neat sodium hydride (0.19 g, 7.9 mmol) and trimethylsulfonium iodide (1.71 g, 8.38 mmol). The resulting orange slurry was stirred under a nitrogen atmosphere, becoming homogeneous within 5 min. Stirring was continued for 1 h when THF was removed in vacuo and the residue was quenched by the careful addition of brine (50 mL). The organic components were extracted into dichloromethane (3×50 mL) and the combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash chromatography (SiO_2; petroleum ether/diethyl ether, 3:2) afforded the title compound as a colourless solid (1.38 g, 87%); R_f 0.21 (petroleum ether/diethyl ether, 3:2); mp 160–161 °C; $[\alpha]_D^{22}$ +123.1 (*c* 1.0, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2950w (C–H), 1685s (C=O); δ_H (300 MHz; $CDCl_3$) 1.15–1.25 (1H, m, cyclopropyl C*H*$_{exo}$), 1.30 (3H, s, butyl C*H*$_3$), 1.37 (3H, s, butyl C*H*$_3$), 1.43–1.52 (1H, m, cyclopropyl C*H*$_{endo}$), 1.75–1.86 (1H, m, C(3)*H*), 1.93–2.01 (1H, m, C(2)*H*), 2.30 (1H, dd, *J* 18.3, 12.0, C(6)*H*$_\beta$), 2.59 (1H, dd, *J* 18.3, 6.2, C(6)*H*$_\alpha$), 3.25 (3H, s, OC*H*$_3$), 3.31 (3H, s, OC*H*$_3$), 3.89 (1H, ddd, *J* 12.0, 9.8, 6.2, C(5)*H*), 4.19 (1H, dd, *J* 9.8, 3.7, C(4)*H*); δ_C (75 MHz; $CDCl_3$) 9.9 (cyclopropyl *C*H$_2$), 17.9 (butyl, *C*H$_3$), 18.1 (butyl, *C*H$_3$), 18.9 (*C*(3)H), 26.9 (*C*(2)H), 41.8 (*C*(6)H$_2$), 48.3 (2×OCH$_3$ coincident), 61.8 (*C*(5)H), 67.6 (*C*(4)H), 99.7 (acetal *C*), 100.3 (acetal *C*), 205.5 (*C*(1)O); *m*/*z* (CI/NH_3) 274 ($[M+NH_4]^+$, 20%), 225 (100); (Found: 274.1647. $C_{13}H_{24}NO_5$ ($[M+NH_4]^+$) requires 274.1649).

*4.6.2. (2′S,3′S,3R,4S,5R)-3-Methyl-4-O,5-O-(2′,3′-dimethoxybutane-2′,3′-diyl)-cyclohexan-1-one-4,5-diol (**43**).* To a stirred solution of

cyclopropyl ketone (**41**) (0.5 g, 1.95 mmol) in a mixture of anhydrous DMPU (4.5 mL) and dry THF (35 mL) (degassed with N_2 for an hour prior to use), was added dropwise a 0.1 M solution of samarium iodide in THF (39.0 mL, 3.90 mmol) at room temperature under a nitrogen atmosphere. After ~15 min the reaction mixture underwent a colour-change from blue to yellow and a saturated aqueous solution of sodium hydrogen carbonate solution (30 mL) was added. The organic components were extracted into diethyl ether (3×30 mL), dried ($MgSO_4$) and volatile solvents then removed in vacuo to give the crude product as a viscous orange oil. Purification by flash chromatography (SiO_2; petroleum ether/diethyl ether, 1:1) afforded the title compound as a colourless oil (0.27 g, 54%); R_f 0.35 (ethyl acetate/40–60 petroleum ether, 1:7); mp 79.3–79.9 °C; $[\alpha]_D^{29}$ +151.2 (*c* 1.0, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2987w (C–H), 2957m (C–H), 2904w (C–H), 2831w (C–H), 1720s (C=O); δ_H (400 MHz; C_6D_6) 0.91 (3H, d, *J* 7.5, C(3)HCH_3), 1.30 (3H, s, butyl CH_3), 1.33 (3H, s, butyl CH_3), 1.89–2.06 (3H, m, C(2)H_2 and C(3)*H*), 2.30 (1H, ~t, *J* 13.1, C(6)H_β), 2.63 (1H, ddd, *J* 13.1, 5.5, 2.5, C(6)H_α), 2.93 (3H, s, OCH_3), 3.11 (3H, s, OCH_3), 3.83 (1H, dd, *J* 10.0, 4.5, C(4)*H*), 3.98 (1H, ddd, *J* 13.1, 10.0, 5.5, C(5)*H*); δ_C (100 MHz; C_6D_6) 13.9 (C(3)HCH_3), 18.3 (butyl CH_3), 18.4 (butyl CH_3), 31.8 (*C*(3)H), 45.7 (*C*(6)H_2), 47.0 (*C*(2)H_2), 47.9 (OCH_3), 48.0 (OCH_3), 64.8 (*C*(5)H), 72.1 (*C*(4)H), 99.6 (acetal *C*), 100.4 (acetal *C*), 205.8 (*C*(1)O); *m*/*z* (EI) 227 ([M−OCH_3]$^+$, 8%), 127 (12), 110 (65), 101 (45), 68 (100); (Found: 227.1272. $C_{12}H_{19}O_4$ ([M−OCH_3]$^+$) requires 227.1278).

*4.6.3. (4S,5R,6S)-5,6-Cyclopropyl-cyclohex-2-en-1-one-4-ol (**44**).* The α,β-cyclopropane (**41**) (0.24 g, 0.94 mmol) was stirred in a mixture of TFA and H_2O (7:1, 4 mL) at room temperature overnight. The reaction mixture was then concentrated in vacuo to give an oily residue, which was re-dissolved in methanol (20 mL) and the resulting solution was cooled to 0 °C. Potassium carbonate (0.13 g, 0.94 mmol) was added in one portion and the dispersion was stirred for 30 min at 0 °C. Water (20 mL) was added and organic components were extracted into ethyl acetate (3×10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as an orange oil. Purification by flash column chromatography (SiO_2; petroleum ether/ethyl acetate, 1:1) furnished the title compound as a viscous oil (0.12 g, 99%); $[\alpha]_D^{22}$ −130.2 (*c* 1.3, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3499br (O–H), 2351s (C–H), 1660s (C=O); δ_H (300 MHz; $CDCl_3$) 1.13–1.18 (1H, m, one of cyclopropyl CH_2), 1.26–1.33 (1H, m, one of cyclopropyl CH_2), 1.98–2.13 (2H, m, C(5)*H* and C(6)*H*), 4.96 (1H, ~dt, *J* 6.0, 3.0, C(4)*H*), 5.77 (1H, ~dt, *J* 12.0, 3.0, C(2)*H*), 6.40 (1H, ~dt, *J* 12.0, 3.0, C(3)*H*); δ_C (75 MHz; $CDCl_3$) 11.6 (cyclopropyl CH_2), 18.9 (*C*(5)H or *C*(6)H), 23.9 (*C*(5)H or *C*(6)H), 64.7 (*C*(4)H), 125.4 (*C*(2)H), 146.8 (*C*(3)H), 197.5 (*C*(1)O); *m*/*z* (CI/NH_3) 142 ([M+NH_4]$^+$, 100%), 125 ([M+H]$^+$, 100).

*4.6.4. (4S,5R,6S)-5,6-Cyclopropyl-4-O-tert-butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**45**).* A solution of the allylic alcohol (**44**) (0.96 g, 0.77 mmol) in dichloromethane (8 mL) was stirred at 0 °C under a nitrogen atmosphere. Triethylamine (0.80 mL) and a crystal of 4-dimethylaminopyridine were added followed by TBDMS–Cl (0.14 g, 0.92 mmol) and the resulting solution was stirred at room temperature for 96 h. The reaction was quenched by the addition of water (8 mL) and organic components were extracted into dichloromethane (3×10 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether/diethyl ether, 5:1) afforded the title compound as a colourless oil (0.14 g, 78%); R_f 0.30 (petroleum ether:diethyl ether, 5:1); $[\alpha]_D^{22}$ −50.5 (*c* 1.1, CH_2Cl_2); ν_{max} (film)/cm^{-1} 2351w (C–H), 1680s (C=O); δ_H (300 MHz; $CDCl_3$) 0.16 (6H, s, Si(CH_3)$_2$), 0.94 (9H, s, C(CH_3)$_3$), 1.13–1.28 (2H, m, cyclopropyl CH_2), 1.81–1.96 (2H, m, C(5)*H* and C(6)*H*), 4.91–4.95 (1H, m, C(4)*H*), 5.68 (1H, br d, *J* 10.4, C(2)*H*), 6.24 (1H, br d, *J* 10.4, C(3)*H*); δ_C (75 MHz; $CDCl_3$) −4.6 (SiCH_3), −4.2 (SiCH_3), 11.8 (cyclopropyl CH_2), 19.1 (*C*(5)H or *C*(6)H), 23.9 (*C*(5)H or *C*(6)H), 26.9 (C(CH_3)$_3$), 65.4 (*C*(4)H), 125.1 (*C*(2)H), 147.3 (*C*(3)H), 197.2 (*C*(1)O); *m*/*z* (CI/NH_3) 256 ([M+NH_4]$^+$, 40%), 239 ([M+H]$^+$, 100); (Found: 238.1380. $C_{13}H_{22}NO_2Si$ (M^+) requires 238.1384).

*4.6.5. (4S,5R,6S)-5,6-Cyclopropyl-2-hydroxymethyl-4-O-tert-butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**46**).* Morita–Baylis–Hillman reaction of enone (**45**) (123 mg, 0.47 mmol) using imidazole as nucleophilic catalyst was carried out in a similar manner to that described for the preparation of (**34**) to give hydroxymethyl compound (**46**) as a colourless oil (23%); ν_{max} (film)/cm^{-1} 3053s (C–H), 1667s (C=O); δ_H (300 MHz; $CDCl_3$) 0.18 (6H, s, Si(CH_3)$_2$), 0.97 (9H, s, C(CH_3)$_3$), 1.15–1.30 (2H, m, cyclopropyl CH_2), 1.85–2.00 (2H, m, C(5)*H* and C(6)*H*), 2.53 (1H, dd, *J* 7.3, 5.5, CH_2O*H*), 4.15 (1H, dd, *J* 13.3, 7.3, CH_aH$_b$OH), 4.30 (1H, dd, *J* 13.3, 5.5, CH$_a$H$_b$OH), 4.95–5.00 (1H, m, C(4)*H*), 6.19–6.23 (1H, m, C(3)*H*); δ_C (75 MHz; $CDCl_3$) −4.5 (SiCH_3), −4.2 (SiCH_3), 12.2 (cyclopropyl CH_2), 18.5 (*C*(CH_3)$_3$), 19.0 (*C*(5)H or *C*(6)H), 24.4 (*C*(5)H or *C*(6)H), 26.1 (C(CH_3)$_3$), 62.1 (CH_2OH), 65.3 (*C*(4)H), 133.4 (*C*(2)), 143.1 (*C*(3)H), 199.0 (*C*(1)O); *m*/*z* (CI/NH_3) 286 ([M+NH_4]$^+$, 35%), 269 ([M+H]$^+$, 100), 137 (30); (Found: 268.1496. $C_{14}H_{24}O_3Si$ (M^+) requires 268.1489).

*4.6.6. (4S,5R,6S)-2-Crotonyloxymethyl-5,6-cyclopropyl-4-O-tert-butyldimethylsilyl-cyclohex-2-en-1-one-4-ol (**47**).* Crotonylation of hydroxmethyl compound (**46**) was carried out in a similar manner to that used for the preparation of (**35**) to give crotonate ester (**47**) as a colourless oil (48%); ν_{max} (film)/cm^{-1} 2951s (C–H), 2857m (C–H), 1726s (ester C=O), 1674s (enone C=O); δ_H (300 MHz; $CDCl_3$) 0.19 (6H, s, (SiCH_3)$_2$), 0.97 (9H, s, C(CH_3)$_3$), 1.15–1.29 (2H, m, cyclopropyl CH_2), 1.92 (3H, dd, *J* 6.9, 1.5, CH_3CH=CH), 1.90–2.02 (2H, m, C(5)*H* and C(6)*H*), 4.76–4.79 (2H, m, CH_2OH), 4.95–4.99 (1H, m, C(4)*H*), 5.90 (1H, br d, *J* 15.6, CH_3CH=CH), 6.23–6.26 (1H, m, C(3)*H*), 7.03 (1H, dq, *J* 15.6, 6.9, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) −4.5 (SiCH_3), −4.2 (SiCH_3), 11.9 (cyclopropyl CH_2), 18.3 (*C*(5)H or *C*(6)H), 18.5 (*C*(CH_3)$_3$), 19.0 (CH_3CH=CH), 24.3 (*C*(5)H or *C*(6)H), 26.1 (C(CH_3)$_3$), 60.9 (CH_2OC=O), 65.4 (*C*(4)H), 122.5 (CH_3CH=CH), 130.1 (*C*(2)), 144.0 (*C*(3)H), 145.6 (CH_3CH=CH), 166.2 (CH_2O*C*=O), 195.9 (*C*(1)O); *m*/*z* (CI/NH_3) 354 ([M+NH_4]$^+$, 8%), 337 ([M+H]$^+$, 100), 311 (35), 251 (40), 205 (32); (Found: 337.1835. $C_{18}H_{29}O_4Si$ ([M+H]$^+$) requires 337.1830).

*4.6.7. (4S,5R,6S)-2-Crotonyloxymethyl-5,6-cyclopropyl-cyclohex-2-en-1-one-4-ol (**16**).* Removal of the silyl protecting group in (**47**) was accomplished in the same manner as deprotection of silyl-ether (**19**) to give the title compound as a colourless oil (94%); $[\alpha]_D^{20}$ −84.2 (*c* 0.57, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3424 (br, OH), 1713 (s, ester C=O), 1666 (s, enone C=O); δ_H (300 MHz; $CDCl_3$) 1.11–1.34 (2H, m, cyclopropyl CH_2), 1.93 (3H, dd, *J* 6.0, 3.0, CH_3CH=CH), 2.07–2.12 (2H, m, C(5)*H* and C(6)*H*), 2.60 (1H, br, O*H*), 4.72–4.87 (2H, m, CH_2OC=O), 4.96–4.98 (1H, m, C(4)*H*), 5.90 (1H, dq, *J* 15.0, 3.0, CH_3CH=CH), 6.35–6.36 (1H, m, C(3)*H*), 7.05 (1H, dq, *J* 15.0, 6.0, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) 11.3 (cyclopropyl CH_2), 18.4 (CH_3CH=CH), 18.9 (*C*(5)H or *C*(6)H), 24.3 (*C*(5)H or *C*(6)H), 60.7 (CH_2OC=O), 64.9 (*C*(4)H), 122.3 (CH_3CH=CH), 130.7 (*C*(2)), 141.9 (*C*(3)H), 146.1 (CH_3*C*H=CH), 166.3 (CH_2O*C*=O), 195.6 (*C*(1)O); *m*/*z* (CI/NH_3) 240 ([M+NH_4]$^+$, 15%), 223 ([M+H]$^+$, 100); (Found: 223.0969. $C_{12}H_{15}O_4$ ([M+H]$^+$) requires 223.0965).

4.7. X-ray crystallographic analysis of (34)

The selected crystal was an extremely thin, easily distorted blade, which was mounted along a glass fibre for support. All measurements were carried out on an Enraf-Nonius Kappa CCD diffractometer employing graphite monochromated, Mo Kα radiation, λ=0.71073 Å. Crystal data is as follows.

$C_{13}H_{20}O_6$ M_r=272.29, D_x=1.377 Mg m^{-3}, Monoclinic $P2_1$, *a*=6.8277(7), *b*=6.9460(7), *c*=14.0151(14) Å, β=98.995(5)°,

V=656.49(11) Å^3, Z=2, F_{000}=292, μ=0.11 mm^{-1}, T=100(2) K, crystal dimensions 0.3×0.8×0.03 mm.

The structure was solved by direct methods using SHELXS and subjected to full-matrix least-squares refinement using SHELXL.[37] The final R values were $R[F^2>2\sigma(F^2)]$=0.063 and $wR(F^2)$=0.152 for 1520 unique reflections. All non-hydrogen atoms were subjected to anisotropic refinement and hydrogen atoms were constrained to chemically reasonable positions.

Crystallographic data (excluding structure factors) for (**34**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 764787. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.8. Cytotoxicity bioassay of COTC analogues

General procedures for culturing human tumour cells and assays for toxicity have been described previously.[38] Briefly, all cell lines were maintained in exponential growth phase in RPMI 1640 medium supplemented with 10% foetal bovine serum and 2 mM glutamine. Exponentially growing cells were harvested and seeded into 96-well microtitre plates at an appropriate density that would allow exponential growth for 4 days. The COTC analogues were dissolved in DMSO and appropriate concentrations added to each well of the microtitric plates so that the final concentration of DMSO in the media was always ≤1%. Cells were then incubated at 37 °C (5% CO_2, 100% humidity) for 4 days. Cytotoxicity was then determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5;diphenyltetrazolium bromide) assay.[39] MTT (50 μL at 2 mg/mL) was aliquoted into the wells and the cells incubated for a further 4 h. Medium and unconverted MTT was removed, formazan crystals were solubilised with DMSO and optical densities determined at 540 nm using a Titertek plate reader. Values of IC_{50}, the concentration of drugs required to reduce optical density to 50% of control, were used as the measure of cellular sensitivity to a given agent.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.08.072.

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