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Synthesis of [28-^{13}C]-24-methylenecholesterol using 1-*tert*-butyl-1*H*-tetrazol-5-yl [^{13}C]-methyl sulfone to methylenate an isopropyl ketone intermediate

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Dedicated to Professor Samir Z. Zard on the occasion of his retirement and in recognition of his seminal contributions to the theory and practice of synthetic organic chemistry.

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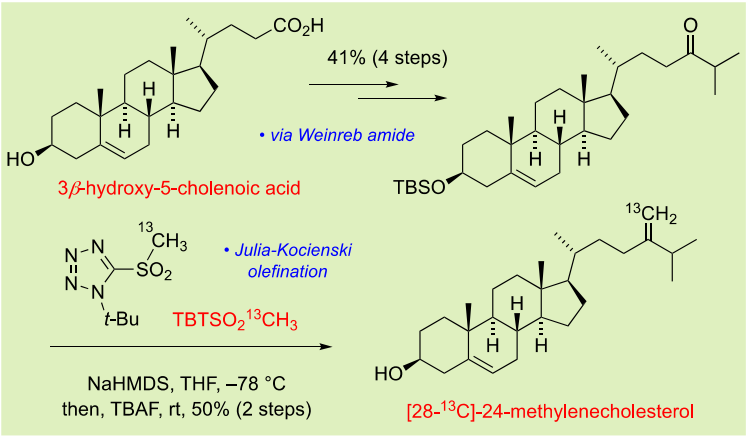
Abstract

[28-^{13}C]-24-Methylenecholesterol, a labelled form of a phytosterol found in pollen that is a critical micronutrient for honey bees (*Apis mellifera*, L.), was prepared in six steps and 20% overall yield from 3β-hydroxy-5-cholenoic acid. The acid was converted to the corresponding isopropyl ketone via its TBS ether protected Weinreb amide derivative and the carbonyl group was subsequently methylenated (in 51% yield) by a Julia-Kocienski olefination using 1-*tert*-butyl-1*H*-tetrazol-5-yl [^{13}C]-methyl sulfone to introduce the ^{13}C-labelled methylene carbon-atom.



Keywords: Julia-Kocienski olefination, isotopologue, methylenation, sterol

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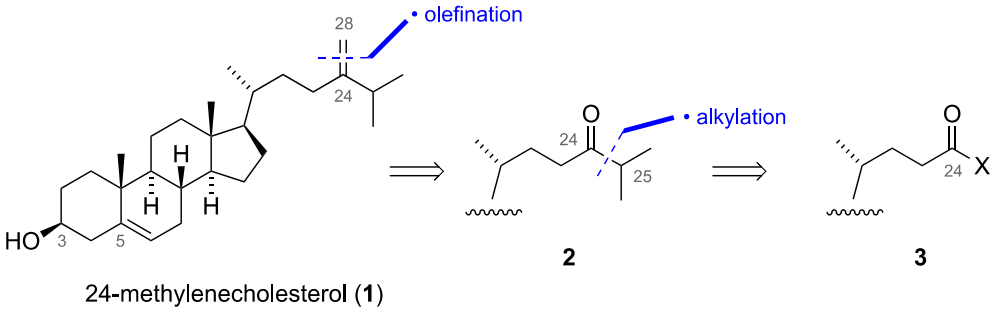
Introduction

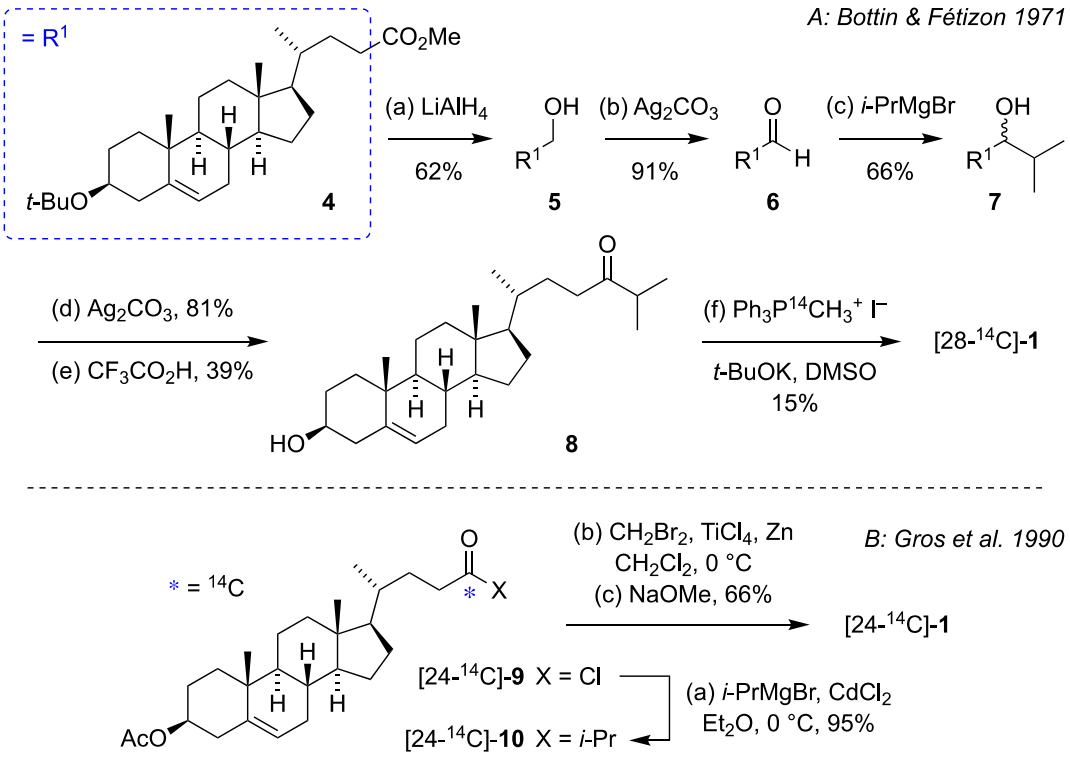
Phytosterols are critically important not only to the biological function of the plants that produce them, but also to the many organisms that assimilate them due to a plant-based diet.[1] For example, the physiology of insects, which are incapable of biosynthesizing sterols for themselves, is often dependent on the consumption of dietary phytosterols which are either used directly or as the starting materials for other needed functional molecules. In insects, sterols play vital structural roles in cell membranes and also in important biochemical pathways, for example, as the precursors to moulting hormones.[2,3,4] In this regard, 24-methylenecholesterol (**1**), a phytosterol found in plant pollen but first identified as ostreasterol following its isolation from molluscs such as oysters,[5] is an exemplar and it has been discovered that this compound is an essential micronutrient for the honey bee (*Apis mellifera* Linnaeus, 1758).[6,7] 24-Methylenecholesterol (**1**) makes up approximately 50% of the total sterols found in bee pupae[8] and a selective transfer of this sterol from nursing adult honey bees to the young larvae occurs via brood food produced by the nurses.[9,10] Research on this micronutrient has revealed its importance for honey bee colony health and individual bee physiology. When honey bee colonies were fed an artificial diet supplemented with sterol **1** , brood production as well as overall longevity were enhanced.[6] In recent laboratory cage studies, it was found that honey bees fed with an artificial diet fortified with 24-methylenecholesterol exhibited better survival rates, consumed more diet, and attained higher head protein and abdominal lipid contents than honey bees that did not receive the sterol.[11,12,13] In apiculture, honey bee colonies are often supplemented with artificial protein diets when pollen is scarce in the landscape; however, these diets lack critical phytosterols, especially 24-methylenecholesterol, that honey bees need (pollen is the only natural source of phytosterols for all bee species).[14] Thus, it is hypothesized that the apiculture industry could benefit by using specially formulated honey bee diets that include the provision of 24-methylenecholesterol.[11]



Scheme 1. 24-Methylenecholesterol (**1**) and a retrosynthetic overview of the traditional synthetic strategy used to access it from a cholenoic acid derivative (**3**, X = nucleofugal atom or residue).

24-Methylenecholesterol is not a naturally abundant sterol and so access to this valuable material has generally been made possible by its semi-synthesis from more plentiful cholesterol-related congeners. The most typical strategy adopted involves conversion of a suitably decorated 3β-hydroxy-5-cholenoic acid derivative (**3**) into a 24-ketocholesterol derivative (**2**) followed by methylenation of the ketone to install the C28 atom (Scheme 1). Interestingly, the inaugural synthetic preparation of **1** took this kind of approach[15] and the methylenation step, which was achieved using methylenetriphenylphosphorane ($Ph_3P{=}CH_2$), represented an early application of what was at the time, the newly introduced Wittig reaction.[16] A majority of later elaborations of **1** have followed this lead and likewise used the venerable Wittig reaction for the methylenation step; for example, Bottin and Fétizon secured the [28-^{14}C]-isotopologue of **1** by direct olefination of 24-ketocholesterol (**8**) using

the ylide generated *in situ* from [^{14}C]-methyltriphenylphosphonium iodide (Scheme 2).[17] Ketone **8** was itself obtained from the *tert*-butyl ether of methyl 3β-hydroxyl-5-cholenoate (**4**) by a serviceable albeit over long sequence of steps involving redundant redox level changes. The synthesis of a 24-methylenecholesterol isotopologue by Gros and co-workers avoids this pitfall by direct conversion of a carboxyl-level functional group at C24, found within acid chloride [24-^{14}C]-**9** (obtained in four steps from 3-oxopregn-4-ene-20-carbaldehyde), into the requisite isopropyl ketone using a stoichiometric organocadmium reagent.[18] Subsequent methylenation was achieved in this case with a good yield under the Lombardo conditions[19] and afforded [24-^{14}C]-24-methylenecholesterol (**1**) following saponification of the C3 acetoxy group. In a combination of the aforementioned illustrated approaches, Whiting et al. realized a synthesis of [28-^{14}C]-**1** via a 24-ketocholesterol derivative generated using *i*-Pr_2Cd and again a Wittig reaction with $Ph_3P{=}^{14}CH_2$ to install the labelled methylene group.[20]



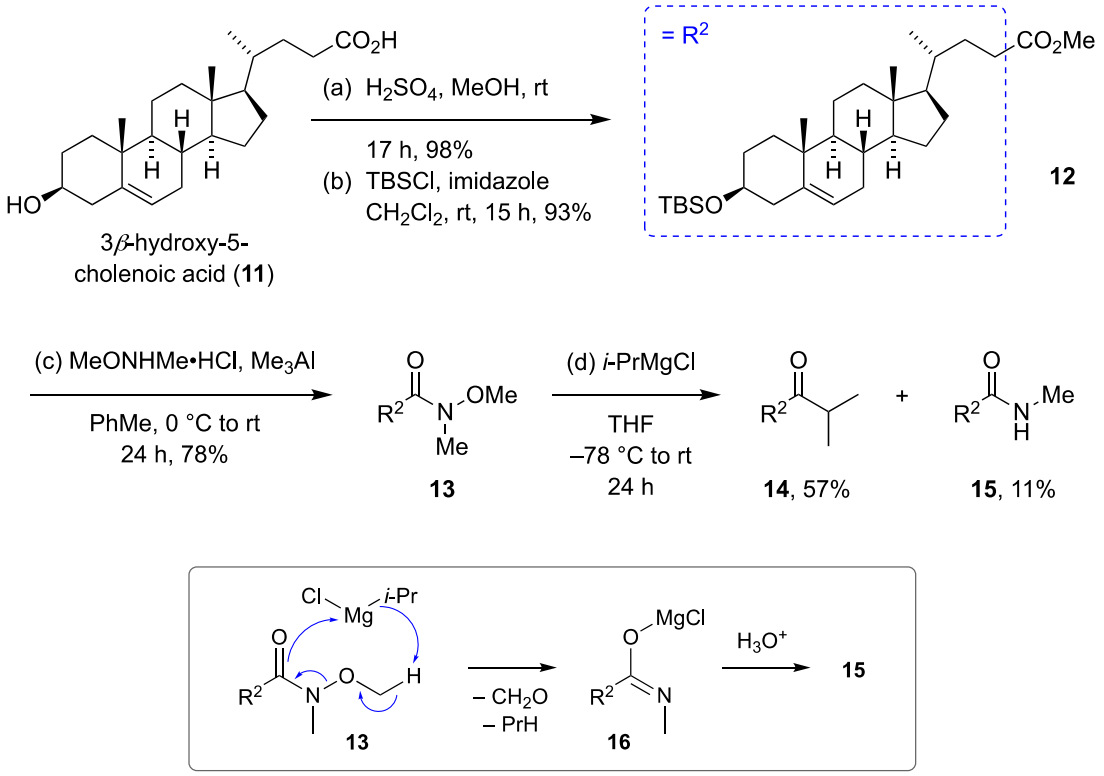
Scheme 2. Two representative syntheses of 24-methylenecholesterol isotopologues that utilize a strategy based on semi-synthesis from a cholenoic acid derivative.[17,18]

For the purposes of a planned study to determine the effectiveness of 24-methylenecholesterol as a honey bee dietary supplement and examining its effects on honey bee physiology and colony growth under semi-field conditions, a reliable source of a safe and traceable form of this micronutrient, chosen to be the stable isotopologue [28-^{13}C]-24-methylenecholesterol (**1**), was required. Accordingly, and as described below, a convenient and robust synthesis of [28-^{13}C]-**1** capable of meeting the supply needs of the field study was developed via a route that minimizes redox level changes and that utilizes a Julia-Kocienski olefination[21] to install the stable isotope labelled methylene moiety at C28.

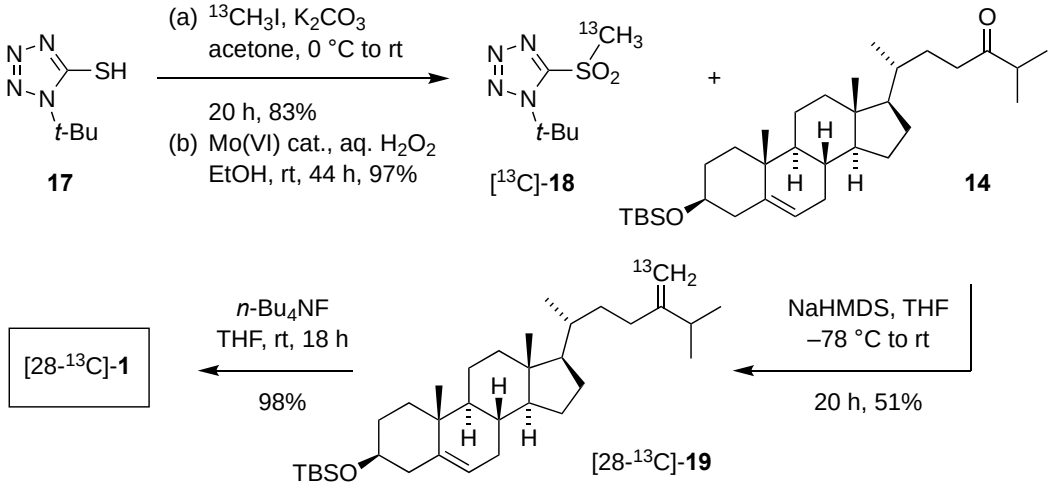
Results and Discussion

To facilitate the efficient conversion of a cholenic acid derivative to an isopropyl ketone intermediate required for methylenation, and wishing to avoid use of the kind of organocadmium reagent seen above (Scheme 2B), we elected to pursue a Weinreb amide-based approach.[22] An appropriate Weinreb amide for this purpose (**13**) was prepared in three steps and 71% overall yield from inexpensive 3β-hydroxy-5-cholenoic acid (**11**) by Fisher esterification, protection of the carbinol with a *tert*-butyldimethylsilyl (TBS) group, and then amidation of the resulting ester **12** with the aluminum reagent generated *in situ* from *N,O*-dimethylhydroxylamine hydrochloride and trimethylaluminum (Scheme 3). Addition of isopropylmagnesium chloride to Weinreb amide **13** gave the desired ketone **14** in a reasonable yield (57%) but this product was accompanied by a significant quantity of the demethoxylated form of the starting material, secondary amide **15** (11%). The reduction of the N–O bond in Weinreb amides is a side-reaction that may be encountered when attempting to substitute the N(Me)OMe nucleofuge for strongly basic nucleophiles.[23,24] When originally observed,[25] it was noted that this process occurs alongside hydroxymethylation of the nucleophile and the proposed mechanism involves E2 elimination, with loss of formaldehyde, from the hydroxamate (see **13** to **16**, insert Scheme 3).[25,26] More recently, this type of dealkoxylation process has been deliberated exploited in synthetically useful transition metal-catalyzed[27] and organophotocatalytic[28] transformations. In view of the yield limiting side-reaction encountered from amide **13**, the conversion of ester **12** to ketone **14** was also evaluated by a route analogous to that involved above in the transformation of **4** into **8** [steps (a) to (d), Scheme 2A]. Superior yields were obtained for individual steps using more modern redox reagents than those employed by Bottin and Fétizon[17] (i.e., DIBAL-H in place of $LiAlH_4$, Dess-Martin periodinane in place of Ag_2CO_3), but the overall yield for this four step sequence (48%) was comparable to the more convenient two step sequence via Weinreb amide **12** [i.e., steps (c) and (d), 44% overall, Scheme 3] and so the shorter route came to be regarded as the preferred approach for production purposes.

With ketone **14** in hand its methylenation was examined; in this task, we elected to explore the Julia-Kocienski olefination, a process not previously used in this context (Scheme 4). When sterically unencumbered (i.e., small alkyl groups attached at sulfur), the activated sulfones typically used for the Julia-Kocienski olefination [e.g., benzothia-2-yl (BT) and 1-phenyl-1*H*-tetrazol-5-yl (PT) sulfones] have a tendency to self-condense upon metalation, wherein one sulfone anion attacks another by ipso substitution.[21] Kocienski and coworkers introduced 1-*tert*-butyl-1*H*-tetrazol-5-yl (TBT) sulfones as bulkier alternatives that are more resistant to such side-reactions,[29] and Aïssa subsequently demonstrated that the methylated congener of this system, 1-*tert*-butyl-1*H*-tetrazol-5-yl methyl sulfone ($TBTSO_2CH_3$, **18**), is well suited to perform methylenation reactions.[30] Successful conversions of modestly hindered ketones to 1,1-disubstituted alkenes using $TBTSO_2CH_3$ (**18**) are known,[31] and other specialized activated sulfones (e.g., 1-methylbenzimidazol-2-yl sulfones) have since been introduced for the same purpose.[32] Thus, to complete the synthesis of [28-^{13}C]-**1**, we prepared the requisite ^{13}C-labelled isotopologue of Aïssa's reagent, 1-*tert*-butyl-1*H*-tetrazol-5-yl [^{13}C]methyl sulfone ($TBTSO_2{}^{13}CH_3$, [^{13}C]-**18**), and deployed it for the methylenation of isopropyl ketone **14** (Scheme 4). As illustrated, under Aïssa's standard type A reaction conditions (sulfone and carbonyl compound premixed in THF solvent at –78 °C prior to addition of NaHMDS base),[30] the methylenation reaction proceeded uneventfully and generated the TBS ether of [28-^{13}C]-**1** (**19**) in good yield (51%). By contrast, Aïssa's alternative type B reaction conditions (Cs_2CO_3, DMF-THF, reflux) resulted in significant decomposition. To conclude the synthesis, silyl ether deprotection of [28-^{13}C]-**19** using TBAF gave [28-^{13}C]-24-methylenecholesterol (**1**) material in pure form which displayed the expected NMR spectral signatures and other characteristics concordant with its structure and in agreement with literature reports.[33]



Scheme 3. Conversion of 3β-hydroxy-5-cholenoic acid (**11**) to a 24-ketocholesterol derivative (**14**) via Weinreb amide **13** and observation of demethoxylation side-product (**15**) formation during addition of isopropylmagnesium chloride. Insert = putative mechanism for demethoxylation.



Scheme 4. Preparation of labelled methylenation reagent [^{13}C]-**18** and its use in the Julia-Kocienski olefination of 24-ketocholesterol derivative **14** to provide the target molecule, [28-^{13}C]-24-methylenecholesterol (**1**).

Conclusions

In summary, a six step synthesis of [28-^{13}C]-24-methylenecholesterol (**1**) was achieved in 20% overall yield from inexpensive 3β-hydroxy-5-cholenoic acid (**11**) by a route incorporating generation of an isopropyl ketone (a 24-

ketocholesterol derivative) from the corresponding Weinreb amide (**13**) and installation of the ^{13}C-atom at C28 by a Julia-Kocienski olefination. The findings serve to highlight a detrimental demethoxylation side-reaction that may complicate ketone synthesis when adding strong sterically hindered nucleophiles to Weinreb amides, and document the first utilization of an isotopologue of Aïssa's sulfone [$TBTSO_2{}^{13}CH_3$, [^{13}C]-**18**] for the introduction of a labelled methylene moiety. Sulfone **18** has received comparatively little attention since its introduction over 15 years ago[30] but the ease of its synthesis, coupled with the good operational characteristics shown herein, and elsewhere,[31] bode well for the prospect of its wider application in future endeavors.

Experimental Section

General. All reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of Ar. Anhydrous solvents were obtained from a commercially available solvent purification system (SPS) employing activated Al_2O_3 drying columns. Preparative chromatographic separations were performed on silica gel 60 (35-75 μm) and reactions followed by TLC analysis using silica gel 60 plates (2-25 μm) with fluorescent indicator (254 nm) and visualized with UV or phosphomolybdic acid stain. All commercially available reagents were used as received unless otherwise noted. Infra-red (IR) spectra were recorded in Fourier transform mode using an ATR probe for solids and oils were supported between NaCl plates. ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the field strength specified and from the indicated deuterated solvents in standard 5 mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows: $CDCl_3$ δ_H ($CHCl_3$) = 7.26 ppm, δ_C = 77.2 ppm. Multiplicities in the ^{1}H NMR spectra are described as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Numbers in parentheses following carbon atom chemical shifts refer to the number of attached hydrogen atoms as revealed by the DEPT spectral editing technique. Low (MS) and high resolution (HRMS) mass spectra were obtained using either electrospray ionization (ES+) or atmospheric pressure chemical ionization (APCI+). Ion mass/charge (*m/z*) ratios are reported as values in atomic mass units.

Methyl 3β-[(*tert*-butyldimethyl)silyloxy]-5-cholenate (12): [Esterification] A stirred solution of 3β-hydroxychol-5-en-24-oic acid (**11**, 1.00 g, 2.67 mmol, 1 eq) in MeOH (20 mL) at rt was treated dropwise with conc. H_2SO_4 (1.00 mL). The resulting mixture was stirred for 17 h and then diluted with EtOAc (150 mL) and sat. aq. $NaHCO_3$ (20 mL) added cautiously in a separatory funnel. The layers were separated and the organic phase washed with further portions of sat. aq. $NaHCO_3$ (3x20 mL) until effervescence ceased. The combined mildly basic aqueous phases were extracted with EtOAc (50 mL) and the combined organic phases were washed with H_2O (50 mL), brine (30 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to afford the desired methyl cholenate ester (1.015 g, 2.61 mmol, 98%) as a colorless waxy solid. The ^{1}H NMR spectrum for the material so obtained was in complete agreement with such a spectrum collected from a commercial sample of methyl 3β-hydroxy-5-cholenate and the ester was therefore used without further purification. [Silyl Ether Formation] A stirred solution of the methyl ester (1.015 g, 2.61 mmol, 1 eq) and imidazole (351 mg, 5.15 mmol, 2 eq) in anhydrous CH_2Cl_2 (17 mL) at 0 °C under Ar was treated with tert-butylchlorodimethylsilane (482 mg, 3.20 mmol, 1.2 eq). The reaction mixture was allowed to warm to rt while being stirred for 15 h. After this time, sat. aq. $NaHCO_3$ (10 mL) was added and the layers were shaken and separated. The aqueous phase was extracted with CH_2Cl_2 (2x10 mL) and the combined organic phases were washed with H_2O (20 mL) and brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO2, eluting with 10% EtOAc in hexanes) to afford silyl ether **12** (1.22 g, 2.43 mmol, 93%) as a colorless solid: IR (neat) 2930, 1744, 1638,

1472, 1251, 1101, 837, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.33-5.30 (1H, m), 3.66 (3H, s), 3.48 (1H, tt, *J* = 11.0, 4.6 Hz), 2.35 (1H, ddd, *J* = 15.5, 10.3, 5.4 Hz), 2.28-2.13 (3H, m), 2.02-1.67 (6H, m), 1.63-1.38 (9H, m), 1.37-1.23 (3H, m), 1.20-1.05 (3H, m), 0.99 (3H, s), 0.92 (3H, d, *J* = 6.4 Hz), 0.89 (9H, s), 0.67 (3H, s), 0.05 (6H, s) ppm; MS (ES+) *m/z* 503 $(M+H)^+$; HRMS (ES+) *m/z* 503.3921 (calcd. $C_{31}H_{55}O_3Si$: 503.3920).

***N*-Methoxy-*N*-methyl 3β-[(*tert*-butyldimethyl)silyloxy]-5-cholenamide (13):** A stirred suspension of *N,O*-dimethylhydroxylamine hydrochloride (273 mg, 2.80 mmol, 3.5 eq) in PhMe (2 mL) at rt under Ar, was treated dropwise with trimethylaluminum (1.40 mL, 2.0 M in PhMe, 2.80 mmol, 3.5 eq). Gas evolution was observed during the addition process and the suspension became an homogenous solution once the addition was complete. After stirring for 30 min, the solution of aluminum amide reagent so-made was cooled to 0 °C and a solution of methyl ester **12** (352 mg, 0.700 mmol, 1 eq) in PhMe (2 mL+1 mL washing rinse) was added dropwise during 1 min. The reaction mixture was allowed to warm to rt and stirred for a total duration of 24 h. After this time (and completion of the transformation was confirmed by TLC analysis), sat. aq. sodium potassium tartrate (Rochelle's salt, 5 mL) was added and the biphasic mixture stirred vigorously for 2 h. The mixture was partitioned between EtOAc (15 mL) and H_2O (10 mL) and the layers separated. The aqueous phase was extracted with EtOAc (2x5 mL) and the combined organic phases washed with brine (5 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 25% EtOAc in hexanes) to afford Weinreb amide **13** (290 mg, 0.545 mmol, 78%) as a colorless solid: mp = 139-142 °C; $[\alpha]_D^{20}$ = –25.6 ($CHCl_3$, c = 0.39); IR (neat) 2934, 1648, 1460, 1382, 1255, 1087, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.33-5.28 (1H, m), 3.69 (3H, s), 3.48 (1H, tt, *J* = 10.9, 5.0 Hz), 3.17 (3H, s), 2.49-2.12 (5H, m), 2.06-1.65 (7H, m), 1.64-1.26 (8H, m), 1.21-1.02 (5H, m), 1.00 (3H, s), 0.95 (3H, d, *J* = 6.5 Hz), 0.89 (9H, s), 0.68 (3H, s), 0.05 (6H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.5 (0), 141.7 (0), 121.4 (1), 72.8 (1), 61.4 (3), 56.9 (1), 56.0 (1), 50.3 (1), 44.8 (3), 43.0 (2), 42.5 (0), 39.9 (2), 37.5 (2), 36.7 (0), 35.8 (1), 32.2 (2), 32.08 (2), 32.05 (1), 30.9 (2), 29.0 (2), 28.3 (2), 26.1 (3C, 3), 24.5 (2), 21.2 (2), 19.6 (3), 18.7 (3), 18.5 (0), 12.1 (3), –4.4 (2C, 3) ppm; HRMS (ES+) *m/z* 532.4193 (calcd. $C_{32}H_{58}NO_3Si$: 532.4186).

Reaction of Weinreb amide 13 with isopropylmagnesium chloride: A stirred solution of Weinreb amide **13** (2.40 g, 4.52 mmol) in anhydrous THF (40 mL) at –78 °C under Ar was treated with a solution of isopropylmagnesium chloride (13.6 mL, 2.0 M in THF, 27.2 mmol, 6 eq) down the cold flask side wall during 3 min. The solution was allowed to warm slowly to rt and stirred for a total duration of 24 h. After this time, sat. aq. sodium potassium tartrate (Rochelle's salt, 25 mL) was added and the mixture stirred vigorously for 2 h. The reaction mixture was then partitioned between EtOAc (50 mL) and H_2O (100 mL) and the layers separated. The aqueous phase was extracted with EtOAc (2x30 mL) and the combined organic phases washed with brine (20 mL), dried (Na_2SO_4), and then concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 20% EtOAc in hexanes) to afford in order of elution, the desired ketone **14** (1.33 g, 2.58 mmol, 57%) and the by-product secondary amide **15** (243 mg, 0.484 mmol, 11%) both as colorless solids.

Data for *O*-[(*tert*-butyldimethyl)silyl]-24-ketocholesterol (**14**): mp = 133-135 °C; $[\alpha]_D^{20}$ = –21.6 ($CHCl_3$, c = 0.26); IR (neat) 2930, 1714, 1471, 1381, 1255, 1096, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.33-5.30 (1H, m), 3.48 (1H, tt, *J* = 10.9, 6.4 Hz), 2.60 (1H, septet, *J* = 6.9 Hz), 2.46 (1H, ddd, *J* = 16.6, 10.0, 5.3 Hz), 2.38 (1H, dd, *J* = 10.0, 6.1 Hz), 2.30-2.13 (3H, m), 2.03-1.92 (3H, m), 1.90-1.66 (6H, m), 1.64-1.30 (11H, m), 1.09 (6H, d, *J* = 6.9 Hz), 1.00 (3H, s), 0.92 (3H, d, *J* = 6.5 Hz), 0.89 (9H, s), 0.67 (3H, s), 0.05 (6H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 215.8 (0), 141.7 (0), 121.3 (1), 72.8 (1), 56.9 (1), 56.0 (1), 50.3 (1), 43.0 (2), 42.5 (0), 41.0 (1), 39.9 (2), 37.5 (2), 37.4 (2), 36.7 (0), 35.6 (1), 32.2 (2), 32.1 (2), 32.0 (1), 30.0 (2), 28.3 (2), 26.1 (3C, 3), 24.4 (2), 21.2 (2), 19.6 (3), 18.7 (3), 18.6 (3), 18.50 (3), 18.48 (0), 12.0 (3), –4.4 (2C, 3) ppm; HRMS (ES+) *m/z* 515.4274 (calcd. $C_{33}H_{59}O_2Si$: 515.4284).

Data for *N*-methyl 3β-[(*tert*-butyldimethyl)silyloxy]-5-cholenamide (**15**): mp 217-219 °C; $[\alpha]_D^{20}$ = –29.6 ($CHCl_3$, c = 1.02); IR (neat) 3282, 2933, 1632, 1566, 1460, 1253, 1084, 838, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.44-5.36 (1H, br s), 5.32-5.30 (1H, m), 3.48 (1H, tt, *J* = 10.7, 5.0 Hz), 2.81 (3H, d, *J* = 4.9 Hz), 2.30-2.10 (3H, m), 2.10-1.90 (3H, m), 1.90-1.60 (4H, m), 1.60-1.00 (15H, m), 0.99 (3H, s), 0.93 (3H, d, *J* = 6.6 Hz), 0.89 (9H, s), 0.67 (3H, s), 0.06 (6H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 174.4 (0), 141.7 (0), 121.3 (1), 72.8 (1), 56.9 (1), 56.0 (1), 50.3 (1), 43.0 (2), 42.5 (0), 39.9 (2), 37.5 (2), 36.7 (0), 35.7 (1), 33.8 (2), 32.2 (2), 32.07 (2), 32.04 (1), 32.02 (2), 28.4 (2), 26.5 (3), 26.1 (3C, 3), 24.4 (2), 21.2 (2), 19.6 (3), 18.6 (3), 18.5 (0), 12.0 (3), –4.4 (2C, 3) ppm; HRMS (ES+) *m/z* 502.4075 (calcd. $C_{31}H_{56}NO_2Si$: 502.4080).

1-*tert*-Butyl-1*H*-tetrazol-5-yl [^{13}C]-methyl sulfone (18): [Alkylation Step] A stirred suspension of TBTSH (**17**, 1.44 g, 9.10 mmol, 1.3 eq, prepared from *t*-BuNCS and NaN_3 as described in ref. 29) and K_2CO_3 (2.51 g, 18.2 mmol, 2.6 eq) in acetone (25 mL) at 0 °C was treated dropwise with neat [^{13}C]-iodomethane (1.00 g, 7.00 mmol, 1 eq; from a freshly opened ampule). The resulting yellow mixture was allowed to warm to rt and stirred vigorously for 20 h. After this time, the mixture was concentrated *in vacuo* and the residue partitioned between EtOAc (20 mL) and H_2O (20 mL). The aqueous phase was extracted with EtOAc (10 mL) and the combined organic phases washed with brine (5 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford the thioether (1.01 g, 5.84 mmol, 83%) as a colorless solid which was used directly in the oxidation step without purification. [Oxidation Step] 30% aq. H_2O_2 (2.40 mL, d = 1.11, 2.65 g, eff. 0.79 g H_2O_2, 23.4 mmol, 4 eq) was added dropwise to a stirred suspension of thioether (1.01 g, 5.84 mmol, 1 eq) and ammonium molybdate catalyst (0.72 g, 5.84 mmol, 10 mol %) in EtOH (20 mL) at 0 °C. The reaction mixture was allowed to warm to rt and after 20 h, TLC analysis revealed that that oxidation beyond the sulfoxide was not completed. Accordingly, a second portion of 30% aq. H_2O_2 (1.20 mL, 2 eq) was added and vigorous stirring at rt continued for a further 24 h. After this time, the reaction mixture was concentrated *in vacuo* and the residue partitioned between EtOAc (30 mL), H_2O (20 mL) and brine (10 mL). The aqueous phase was extracted with EtOAc (2x10 mL) and the combined organic phases were washed with H_2O (20 mL), brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 25% EtOAc in hexanes) to afford sulfone [^{13}C]-**18** (1.16 g, 5.66 mmol, 97%) as a colorless solid: mp 81-83 °C; IR (neat) 3000, 2922, 1337, 1168, 1960, 751, 562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.65 (3H, d, J^1_{CH} = 141 Hz), 1.85 (9H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$): δ 154.7 (0, d, *J* = 10.4 Hz), 65.6 (0), 44.8 (3, ^{13}C enriched atom), 29.8 (3C, 3) ppm. HRMS (ES+) *m/z* 206.0788 (calcd. $^{12}C_5{}^{13}CH_{13}N_4O_2S$: 206.0793).

[28-^{13}C]-24-Methylenecholesterol *tert*-butyldimethylsilyl ether (19): A stirred solution of ketone **14** (336 mg, 0.653 mmol) and TBT sulfone [^{13}C]-**18** (268 mg, 1.31 mmol, 2 eq) in anhydrous THF (5 mL) at –78 °C under Ar was treated dropwise with NaHMDS (1.37 mL, 1.00 M in THF, 1.37 mmol, 2 eq) during 2 min. The resulting yellow solution was stirred for 20 h while being allowed to warm slowly to rt. After this time, sat. aq. NH_4Cl (5 mL) was added and the mixture partitioned between EtOAc (15 mL) and H_2O (10 mL). The aqueous phase was extracted with EtOAc (5 mL) and the combined organic phases were washed with brine (5 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 2-5% EtOAc in hexanes) to afford TBS ether [28-^{13}C]-**19** (171 mg, 0.333 mmol, 51%) as a colorless waxy solid: mp 110-115 °C; $[\alpha]_D^{20}$ = –23.5 ($CHCl_3$, c = 0.09); IR (neat) 2931, 1620, 1463, 1254, 1095, 836, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.33-5.31 (1H, m), 4.71 (1H, d, J^1_{CH} = 154.1 Hz), 4.65 (1H, dm, J^1_{CH} = 154.1 Hz), 3.48 (1H, tt, *J* = 11.0, 4.9 Hz), 2.32-1.67 (10H, m), 1.64-1.36 (9H, m), 1.35-1.06 (7H, m), 1.03 (3H, d, *J* = 6.8 Hz), 1.02 (3H, d, *J* = 6.9 Hz), 1.00 (3H, s), 0.95 (3H, d, *J* = 6.5 Hz), 0.89 (9H, s), 0.68 (3H, s), 0.07 (6H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$): δ 157.1 (0, d, *J* = 71.8 Hz), 141.7 (0), 121.4 (1), 106.1 (2, ^{13}C enriched atom), 72.8 (1), 57.0 (1), 56.1 (1), 50.3 (1), 43.0 (0), 42.5 (2), 40.0 (2), 37.6 (2), 36.8 (0), 35.9 (1), 34.8 (2), 33.9 (1), 32.3 (2), 32.10 (1), 32.06 (2), 31.1 (2), 28.4 (2), 26.1 (3C, 3), 24.5 (2), 22.2 (3, d, *J* = 3.0 Hz), 22.1 (3, d, *J* = 3.1 Hz), 21.2 (2), 19.6 (3), 18.9 (3), 18.5 (0), 12.0 (3), –4.4 (2C, 3) ppm; HRMS (APCI+) *m/z* 512.4360 (calcd. $^{12}C_{33}{}^{13}CH_{59}OSi$: 512.4369).

[28-^{13}C]-24-Methylenecholesterol (1): A stirred solution of the TBS ether [28-^{13}C]-**19** (394 mg, 0.768 mmol) in anhydrous THF (10 mL) at rt under Ar was treated with a solution of tetra-*n*-butylammonium fluoride (3.84 mL, 1.0 M in THF, 3.84 mmol, 5 eq). The resulting reaction mixture was stirred for 18 h and then partitioned between EtOAc (25 mL) and a mixture of H_2O (20 mL) and brine (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (2x5 mL). The combined organic phases were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 25% EtOAc in hexanes) to afford [28-^{13}C]-**1** (301 mg, 0.753 mmol, 98%) as a colorless solid: mp = 136-138 °C; $[\alpha]_D^{20}$ = –34.3 ($CHCl_3$, c = 0.18); IR (neat) 3413 (br s), 2939, 1638, 1465, 1380, 1048, 800, 668 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 5.35 (1H, dt, *J* = 5.3, 2.0 Hz), 4.71 (1H, d, J^1_{CH} = 153.7 Hz), 4.65 (1H, dm, J^1_{CH} = 154.2 Hz), 3.52 (1H, tt, *J* = 11.2, 4.4 Hz), 2.30 (1H, ddd, *J* = 13.1, 5.0, 2.3 Hz), 2.26-2.20 (2H, m), 2.09 (1H, ddt, *J* = 15.2, 10.9, 4.9 Hz), 2.01 (1H, dt, *J* = 12.6, 4.0 Hz), 1.97 (1H, dtd, *J* = 17.5, 5.2, 2.7 Hz), 1.91-1.81 (4H, m), 1.60-1.39 (8H, m), 1.30-1.25 (1H, m), 1.19-1.04 (6H, m), 1.05-0.90 (2H, m), 1.03 (3H, d, *J* = 6.8 Hz), 1.02 (3H, d, *J* = 6.9 Hz), 1.01 (3H, s), 0.95 (3H, d, *J* = 6.6 Hz), 0.68 (3H, s) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 157.0 (0, d, *J* = 71.6 Hz), 140.6 (0), 121.9 (1), 106.1 (2, ^{13}C enriched atom), 72.0 (1), 56.9 (1), 56.1 (1), 50.2 (1), 42.51 (0), 42.45 (2), 39.9 (2), 37.4 (2), 36.7 (0), 35.9 (1), 34.8 (2), 33.9 (1), 32.1 (2), 32.1 (1, d, *J* = 2.2 Hz), 31.8 (2), 31.1 (2), 28.4 (2), 24.5 (2), 22.2 (3, d, *J* = 2.9 Hz), 22.1 (3, d, *J* = 3.1 Hz), 21.2 (2), 19.6 (3), 18.9 (3), 12.0 (3) ppm; HRMS (APCI+) *m/z* 398.3489 (calcd. $^{12}C_{27}{}^{13}CH_{45}O$: 398.3504). ^{1}H and ^{13}C NMR spectral data are in agreement with those previously reported for 24-methylenecholesterol (**1**) with the expected differences to account for the enrichment at the C28 methylene position by a ^{13}C atom.[33]

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Supplementary Material

^{1}H and ^{13}C NMR spectra.

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