Toward a Biorelevant Structure of Protein Kinase C Bound Modulators: Design, Synthesis, and Evaluation of Labeled Bryostatin Analogs for Analysis with REDOR NMR Spectroscopy

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Supporting Information

Table of Contents

General Methods

Unless otherwise noted, all reactions were run under a nitrogen atmosphere in flame-dried glassware. Reactions were stirred using Teflon-coated magnetic stirrer bars. Reactions were monitored using thin layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates with fluorescent indicator from Merck. Plates were visualized by treatment with UV, acidic *p*-anisaldehyde stain, or $KMnO_4$ stain with gentle heating. Products were purified by column chromatography using the solvent systems indicated. Silica gel 60, 230-400 mesh, was purchased from Fisher Scientific.

When necessary, solvents and reagents were purified before use. Tetrahydrofuran (THF), diethyl ether (ether), benzene, toluene (PhMe), and dichloromethane were passed through an alumina drying column (Solv-Tek Inc. or Innovative Technologies) using nitrogen pressure. Anhydrous dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetone, acetonitrile (MeCN), and methanol (MeOH) were obtained from Sigma-Aldrich. Ethyl acetate (EtOAc), petroleum ether, pentane, hexanes, MeOH, ether, dichloromethane, MeCN, PhMe, and THF were obtained from Fischer Scientific. Powdered 4Å molecular sieves (< 5 micron) were purchased from Aldrich and stored/activated as indicated. Amine bases (NEt_3, pyridine, diisopropylamine, diisopropylethylamine [Hünig's base]) were distilled over CaH_2 under nitrogen. Sodium borodeuteride was purchased from Cambridge Isotope Laboratories, and acetic acid-2-^{13}C was obtained from Isotec. All other reagents were purchased from commercial suppliers (Aldrich, Acros) and were either used as received without additional purification or were purified using standard methods. Preparative HPLC was carried out using an MeCN:H_2O gradient using a Shimadzu Prominence system equipped with a Restek 18 column (5 μm, 21 x 250 mm). NMR spectra were measured on a Varian INOVA 500 (^{1}H at 500 MHz, ^{13}C at 125 MHz), a Varian 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz), or a Varian INOVA 600 MHz (^{1}H at 500 MHz, ^{13}C at 150 MHz) magnetic resonance spectrometer, as noted. ^{1}H chemical shifts are reported relative to the residual solvent peak (chloroform = 7.26 ppm; benzene = 7.16 ppm)[1] as follows: chemical shift (δ), (multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, hept. = heptet, b = broad, *app* = apparent), integration, coupling constant(s) in Hz, proton ID [when available, designated by carbon number]). Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. Proton assignments were made via 2D spectroscopy (COSY, HSQC, and/or HMBC) or analogy. ^{13}C chemical shifts are reported relative to the residual deuterated solvent ^{13}C signals ($CDCl_3$ = 77.16 ppm, C_6D_6 = 128.06 ppm).[1] Infrared spectra were recorded on a Perkin-Elmer 1600 Series Fourier Transform spectrometer (FTIR) and are reported in wavenumbers (cm^{-1}). Optical rotation data were obtained using a JASCO P-2000 Polarimeter are reported as $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\boldsymbol{T}}$ (*c* = grams/100 mL), where D indicates the sodium D line (589 nm) and T indicates temperature (all optical rotation values were obtained at ambient temperature, ca. 22-25 °C). Unless otherwise indicated, optical rotations are the average (± standard deviation) of 10 individual measurements. Optical rotations were not recorded for isomeric mixtures. High resolution mass spectra were obtained at the Vincent Coates Mass Spectrometry Laboratory, Stanford, CA 94305.

[1] Gottlieb, H.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

Computational Methods

This section discusses the details of the modeling calculations for bryostatin 1 (**1**) and analogs **3** and **4** as a way to illustrate the development of the modeling method. An analogous method was applied to B-ring dioxane analog **5**.

Modeling and visualization was performed on a Dell Precision 390 workstation running the Ubuntu Linux distribution (10.0 LTS release) with Intel Core 2 Duo CPUs. The primary modeling programs were the Schrödinger Suite (Macromodel v. 9.5/Maestro GUI v. 8.0) for model building, simple minimizations, and conformer searching, PyMol (Molecular Graphics System, v. 1.3) for visualization, MOPAC or Gaussian for refined geometry minimization calculations and the CSD for crystallographic data retrieval.

Initial conformational search efforts targeted the bryostatin scaffold in an attempt to generate distances for all potential labelling strategies. This method was later refined due to the potential of ^{19}F labels to alter conformational preferences. These initial efforts are included as they help illustrate project development. These first conformational searches were restricted to the bryostatin core structure in which the C20 octadienoate sidechain was converted to a simplified C20 acetate sidechain (Figure S1). Conformational sampling of this structure allowed for determination of conformer families associated with the bryostatin 1 macrocyclic core. Removal of several degrees of freedom associated with the conversion of the C20 sidechain to an acetate substituent allowed for a more efficient search focusing upon conformational variables associated with the core as opposed to those resulting from variation of the alkyl sidechain. Following the first conformational search, the resulting structures were then fed once again into a new search to confirm that conformational sampling had been sufficient. As no additional conformers were obtained in the second conformational search, it was concluded that sampling had been sufficient.

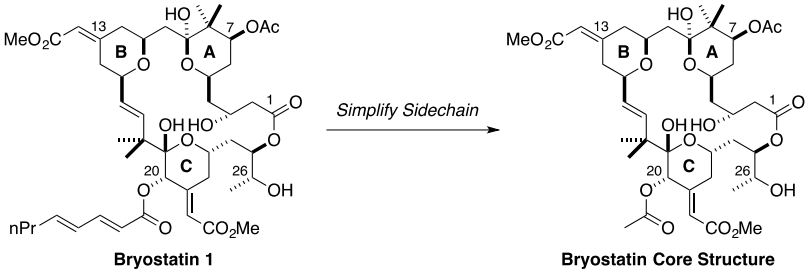


Figure S1. Bryostatin 1 core used for conformational searches.

Two key assumptions were made during the course of this conformational search effort. First, it was assumed that the simplification of the octadienoate sidechain to an acetate would have a minor effect upon the conformation of the macrocyclic core (see ref. 28 in text). Secondly, it was assumed that the conformations generated using this simplified bryostatin core structure would apply to both bryostatin 1 as well as designed isotopically-labeled analogs. However, subsequent conformational searches of the designed analogs would verify whether this assumption would be the case (*vide infra*). While two of the three isotopic labels chosen would not change the overall conformation of the bryostatin scaffold (i.e. ^{2}H and ^{13}C), the incorporation of a ^{19}F label may result in variation of the conformation of the molecule.

The first conformational search was performed using the following parameters:

Bryostatin 1 core conformational search:
Run #1:
Potential tab
Force field: MMFFs
Solvent: $CHCl_3$

Mini tab
PRCG minimization steps: 2000 gradient steps were found to be sufficient to minimize the vast majority of conformers while not wasting undue computational time on unreasonable conformers

CSearch Tab
Automatic setup used with "Torsion Sampling Options": Extended
Maximum number of steps: 170,000

Atoms used for comparison are shown in Figure S2. All carbons, oxygens, and hydroxyl hydrogens were utilized for comparison of the conformers.

Result: 10,033 unique bryostatin conformers were found.

Data reduction
Conformers within 5 kcal/mol above the global energy minimum structure were extracted and numbered 241 conformers. These conformers were then utilized to seed the second conformational search.

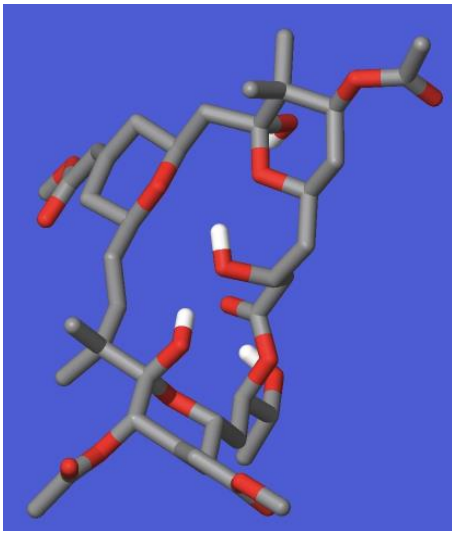


Figure S2. Atoms utilized for comparison of conformers obtained in the conformer search of the bryostatin 1 core structure

The second conformational search was performed using the following parameters:

Run #2:
Potential tab
Force field: MMFFs
Solvent: $CHCl_3$

Mini tab
PRCG minimization steps: 2000 gradient steps

CSearch Tab
Automatic setup used with "Torsion Sampling Options": Extended
Maximun number of steps: 100,000

Atoms used for comparison are the same as in run #1 (Figure S2).

Result: 10,033 unique bryostatin conformers were found.

Data reduction

Conformers within 5 kcal/mol above the global energy minimum structure were extracted and numbered 240 conformers. As the obtained number of conformers was not significantly different from the first run, which produced 241 conformers, the search was judged to be complete. Further reduction of the data was then performed.

The 240 conformers from the second conformer search were reduced via a redundant conformer elimination procedure (mmod_confelim), in which the core ring structure excluding side chains and all hydrogens were used for reduction. This procedure reduced the 240 conformers to 87 conformers. These 87 conformers were then clustered to produce conformer families using the XCluster utility. Atoms used for comparison were the same as those used in the redundant conformer elimination procedure. XCluster reduced the set of conformers to 29 conformer families. A lead structure was then extracted for each conformer family to yield 29 core structures.

Bryostatin 1 full structure search:
Potential tab
Force field: MMFFs
Solvent: $CHCl_3$

Mini tab
PRCG minimization steps: 2000 gradient steps

CSearch Tab
Only C20 sidechain atoms were selected for torsion sampling. All other atoms were allowed to relax.

Maximum number of steps: 100,000

The 29 conformer families generated from the core-based search above were seeded into this run. The atoms used for comparison between the resulting structures are shown in Figure S3.

Result: 2,183 unique bryostatin conformers were found.

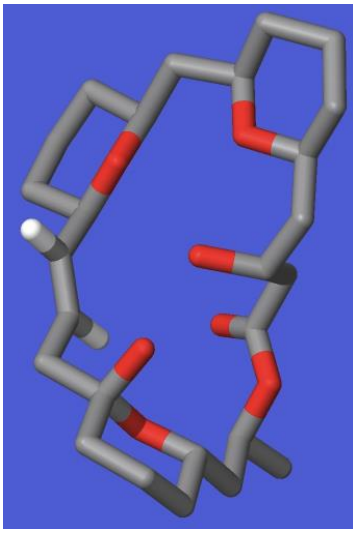


Figure S3. Atoms utilized for comparison of conformers obtained in the conformer search of the full bryostatin 1 molecular structure.

Data reduction

Conformers within 5 kcal/mol above the global energy minimum structure were extracted and numbered 55 conformers. A redundant conformer elimination procedure was deemed unnecessary, and so the 55 conformers were then clustered to produce conformer families using the XCluster utility. This procedure reduced the 55 conformers to 26 conformer families. A lead structure was then extracted for each conformer family to yield 26 structures, representing the possible conformational classes available to bryostatin 1. These structures were then utilized to determine a suitable isotopic labeling strategy that would allow for experimental differentiation of the various proposed conformer families.

Distance table for Bryostatin 1 conformers

Following generation of each of the 26 conformer structures of bryostatin 1 detailed above, distance tables were generated based upon the incorporation of isotopic labels at various positions on the bryostatin 1 scaffold. An example of a distance table shown below that led to the design of **3** and **4** is provided below (Table S1). The numbering refers to the standard numbering scheme for the bryostatin scaffold. The term C13F corresponds to the distance from a hypothetical ^{19}F isotopic label replacement of the hydrogen on the sp^2 carbon of the C13 methyl enoate substituent. C20 corresponds to the distance from a hypothetical ^{13}C isotopic label on the carbonyl of the octadienoate ester. C26D corresponds to the distance from a hypothetic ^{2}H isotopic label incorporated at the C26 position. C7 corresponds to the distance from a hypothetical ^{13}C isotopic label on the carbonyl carbon of the C7 acetate substituent. The energy (MMFF94s) of each conformer above the global energy minimum is also provided for the 26 conformer structures.

Table S1. Intramolecular distance data for selected distances in the 26 low energy conformer structures of bryostatin 1. Distances given in Å.

Energy (kcal/mol)	Distance C7-C13F	Distance C13F-C26D	Distance C20-C13F	Distance C20-C26D	Distance C7-C26D	Distance C7-C20
0	11.95	10.09	12.17	8.72	12.84	15.28
0.22	11.63	11.27	12.23	8.63	6.46	13.75
1.25	11.73	12.75	8.32	7.82	8.91	9.94
1.90	12.25	12.16	10.53	8.71	12.60	15.66
2.49	11.74	11.35	12.28	8.68	6.53	13.77
2.58	11.95	9.33	12.16	8.06	14.14	15.38
3.20	11.63	9.83	12.23	7.00	11.42	12.28
3.21	11.92	6.17	12.09	7.17	12.64	13.40
3.31	11.64	11.00	12.27	8.59	6.58	13.56
3.34	11.76	10.31	12.22	8.66	13.15	14.97
3.41	11.76	11.20	12.02	8.66	6.45	14.27
3.53	11.79	5.87	11.95	6.96	12.45	12.45
3.55	10.92	13.42	8.01	7.88	11.98	9.31
3.55	10.83	11.18	12.36	7.76	13.58	13.95
3.89	10.95	8.44	12.35	7.95	13.53	14.01
4.17	11.93	12.74	8.57	7.97	10.34	9.69
4.40	12.23	11.62	9.64	8.65	13.02	15.30
4.48	11.69	9.57	12.10	7.00	12.38	12.03
4.48	12.22	11.56	10.60	8.00	13.96	15.80
4.52	10.63	9.80	12.21	8.71	12.74	17.27
4.57	11.69	12.18	12.39	6.95	8.18	12.51
4.63	11.93	6.25	12.17	7.25	12.70	14.21
4.68	11.12	10.41	12.26	8.61	13.79	16.38
4.80	11.75	8.67	12.14	7.98	14.24	15.17
4.95	11.66	9.87	12.02	6.94	11.83	13.33
4.96	11.72	10.15	12.32	6.98	11.86	13.27
Min.	**10.63**	**5.87**	**8.01**	**6.94**	**6.45**	**9.31**
Max	**12.25**	**13.42**	**12.39**	**8.72**	**14.24**	**17.27**
Max Δ	**1.62**	**7.55**	**4.39**	**1.78**	**7.79**	**7.96**

Of note, the labels presented above are only a sampling of those considered. Figure S4 shows all the positions that were considered to be synthetically accessible (keeping in mind that the later one can install an isotopic label, the more one can rely on previous prepared lab stocks and does not have to worry about incurring large costs associated with using significant amounts of labeled reagents). The combination of these various potential labels, if sticking to the caveats detailed in the main text, yields 24 potential labeling strategies that were to be evaluated.

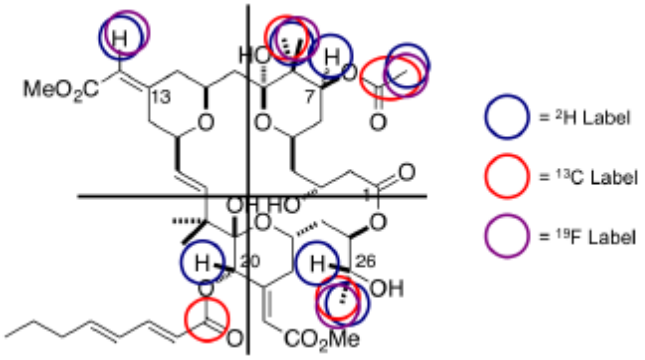


Figure S4. Identification of sites on the bryostatin 1 scaffold that are amenable to the incorporation of NMR-active ^{2}H, ^{13}C or ^{19}F labels. Note: both the *E*- and *Z*-isomers at C13 were considered

Analog 3 and 4 Conformational Search:

Following identification of the 24 proposed isotopic labeling strategies, a conformer search was performed on each potential analog. This would verify that the labeling strategy would not drastically alter the ability to differentiate between proposed binding conformers (the 26 conformer families detailed above). In the case of analogs **3** and **4**, a ^{19}F isotopic label was incorporated on the C13 exocyclic methyl enoate. Since the two analogs differ only in the placement of ^{13}C and ^{2}H labels, analog **3** was used as a conformational guide for both (isotopic substitution is not treated by the modeling program and was thus expected to have no effect on the search results). This was seeded with the 29 unique bryostatin core conformers described above. A similar strategy was applied to the other potential labeled analogs.

Potential tab
Force field: MMFFs
Solvent: $CHCl_3$

Mini tab
PRCG minimization steps: 2000 gradient steps

CSearch Tab
Only C20 sidechain atoms were selected for torsion sampling. All other atoms were allowed to relax.
Maximun number of steps: 100,000

Atoms used for comparison are shown in Figure S3.

Result: 1,708 unique bryostatin conformers were found.

Data reduction

Conformers within 5 kcal/mol above the global energy minimum structure were extracted and numbered 49 conformers. A redundant conformer elimination procedure was deemed unnecessary, and so the 49 conformers were then clustered to produce conformer families using the XCluster utility. This procedure reduced the 49 conformers to 23 conformer families. A lead structure was then extracted for

each conformer family to yield 23 structures. These structures have been utilized in preliminary docking studies and await further refinement from REDOR solid-state NMR experimental results.

*Distance table for analog **3** and **4** conformers*

Following generation of each of the 23 conformer structures detailed above, distance tables were generated based upon the incorporation of isotopic labels at the indicated positions on the analog scaffold (Table S2). The numbering refers to the standard numbering scheme for the bryostatin scaffold. The term C13F corresponds to the distance from a ^{19}F isotopic label replacement of the hydrogen on the sp^2 carbon of the C13 methyl enoate substituent. C20 corresponds to the distance from a ^{13}C isotopic label on the carbonyl of the octadienoate ester. C26D corresponds to the distance from a ^{2}H isotopic label incorporated at the C26 position. C7 corresponds to the distance from a ^{13}C isotopic label on the methyl carbon of the C7 acetate substituent. The energy (MMFF94s) of each conformer above the global energy minimum is also provided for the 23 conformer structures.

Table S2. Intramolecular distance data for selected distances in the 23 low energy conformer structures of analogs **3** and **4**. Distances given in Å.

Energy (kcal/mol)	Distance C7-C13F	Distance C13F-C26D	Distance C20-C13F	Distance C20-C26D	Distance C7-C26D	Distance C7-C20
0	11.90	10.13	12.43	8.70	12.86	15.27
0.29	11.61	11.38	12.49	8.62	6.46	13.75
1.55	11.70	12.88	8.44	7.82	8.74	9.92
2.12	12.25	12.36	10.69	8.68	12.61	15.68
2.42	11.72	11.47	12.55	8.65	6.53	13.75
2.78	11.91	9.44	12.40	8.04	14.15	15.38
3.36	11.76	11.33	12.29	8.65	6.45	14.26
3.38	11.91	6.38	12.35	7.14	12.62	13.37
3.38	11.70	10.41	12.48	8.64	13.17	14.96
3.49	11.60	10.03	12.47	6.97	11.43	12.24
3.70	11.62	11.14	12.54	8.58	6.57	13.57
3.93	10.75	11.27	12.62	7.74	13.56	13.97
4.23	10.91	8.59	12.61	7.94	13.52	14.02
4.29	11.77	6.16	12.20	6.95	12.48	12.43
4.37	11.74	12.45	9.00	7.96	9.87	9.18
4.37	10.88	13.56	8.21	7.87	11.84	8.92
4.50	12.26	1.78	9.61	8.63	13.01	15.34
4.60	10.52	9.79	12.43	8.69	12.73	17.26
4.71	11.67	9.76	12.33	6.97	12.38	12.00
4.78	11.92	6.50	12.44	7.23	12.70	14.17
4.79	12.22	11.77	10.77	7.98	13.96	15.82
4.85	11.68	12.34	12.66	6.90	8.09	12.43
4.88	11.04	10.50	12.51	8.60	13.78	16.37
Min.	**10.52**	**6.16**	**8.21**	**6.90**	**6.45**	**8.92**
Max	**12.26**	**13.56**	**12.66**	**8.70**	**14.15**	**17.26**
Max Δ	**1.75**	**7.41**	**4.44**	**1.81**	**7.70**	**8.35**

Analog 5 Conformational Search:
Due to the significantly different nature of the B-ring dioxane analog core structure, it was deemed appropriate to re-run the core conformation search for each analog of this class separately. In each case, the 23 input conformers were seeded based on modified versions of the analog **3** conformers found in order to ensure conformational coverage included at least those conformers already identified. In each case, parameter sets were identical to those used above.

***In Silico* Docking Studies:**
The above efforts all focus on the ligand only to avoid biasing the system. It is certainly possible to add in peptide component, and this has been done both in our lab in 2004 and later by others (see ref. 18 in the main text). The most recent ligand-peptide structures from our lab are shown below, displaying two possible orientations.

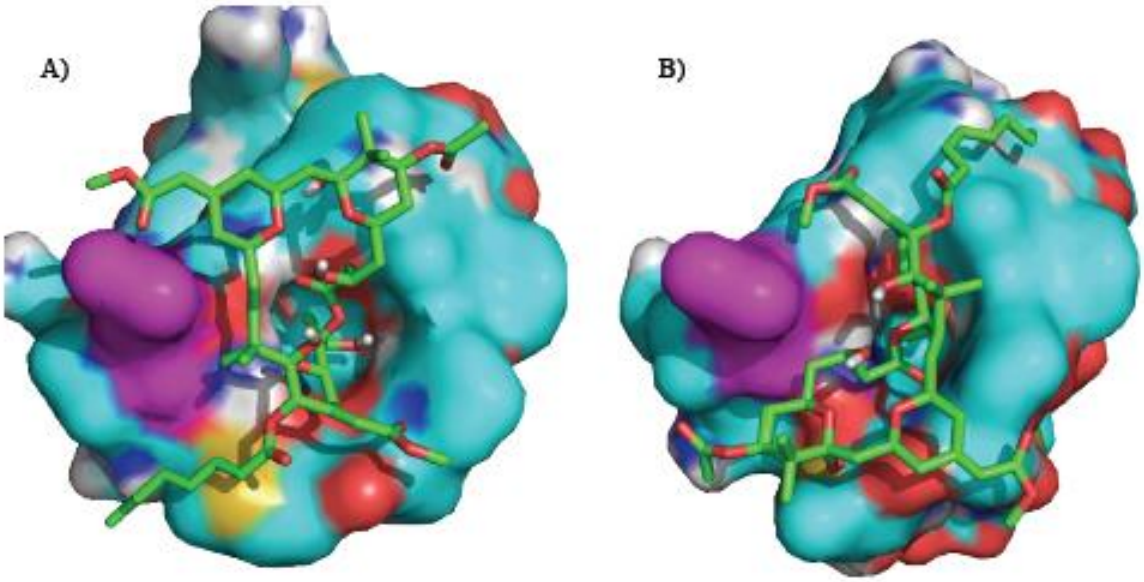


Figure S5. Docking models of bryostatin 1 to PKCδ C1b domain generated with Autodock

Modern molecular dynamics simulations are capable of incorporating the membrane component (something we are actively working on), but as is detailed in the text, all of these studies are theoretical. Obtaining experimental information on these tertiary complexes will allow us to constrain the various *in silico* models, and thus begin to remove some of the speculation associated with interpreting our current structural understanding of the PKC-ligand-membrane interaction.

For ease of comparison, all proton assignments are given by carbon number as it corresponds to the bryostatin 1 scaffold (see Figure S5). For instance, the carbons of the C20 octanoyl chain are both designated as C39-C46, even though analog **5** only contains 38 total carbons.

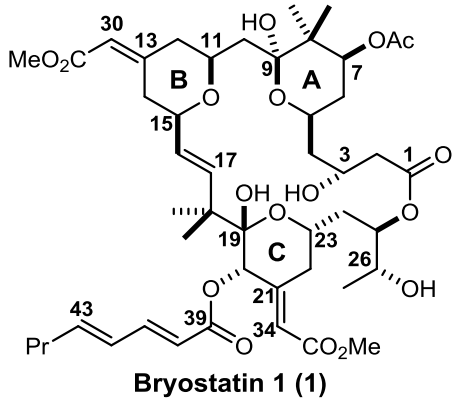
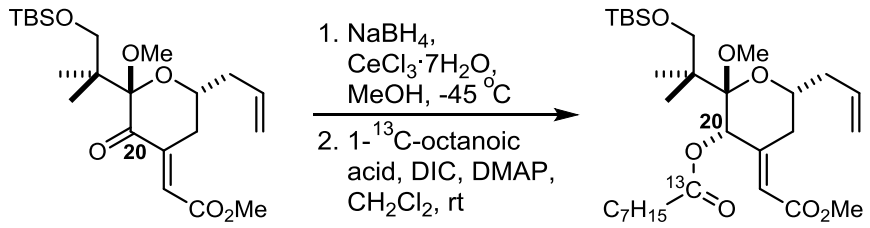


Figure S5. Bryostatin 1 carbon numbering



8 ***76% over 2 steps*** **9**

Procedure for ester 9

A solution of enone **8** (166 mg, 0.39 mmol) was prepared in MeOH (8.8 mL) in a 25 mL round bottom flask, affording a bright yellow solution. $CeCl_3 \cdot 7H_2O$ (148 mg, 0.40 mmol) was added and the resulting solution was stirred at ambient temperature until all of the solids dissolved. After ~10 min, the reaction was cooled to -40 °C in a MeCN/CO_2 bath. After 5 min, $NaBH_4$ (30.0 mg, 0.79 mmol) was added in one portion. Vigorous bubbling was observed and the solution became progressively clear over the next 5 minutes, at which point the reaction was quenched into saturated aqueous NH_4Cl (15 mL) and diluted with water (15 mL), brine (20 mL) and Et_2O (50 mL). The layers were separated, and the organic layer was extracted with Et_2O (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting light yellow oil contained a single C20 epimer (dr > 20:1) and was further purified via flash chromatographythrough a plug of silica gel (5→8% ethyl acetate:pentane, 1.5% increments) to afford a pale yellow oil that was used directly in the next step.

The crude material from the previous step was dissolved in CH_2Cl_2 (4.1 mL) in a 25 mL round bottom flask at ambient temperature. DMAP (61.9 mg, 0.51 mmol) was added followed by 1-^{13}C-octanoic acid (81.0 µL, 0.51 mmol), then DIC (78.5 µL, 0.51 mmol). The reaction was stirred overnight, producing a turbid, tan solution. After 18 hours, the reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was washed with saturated NH_4Cl (50 mL) then brine (50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification via silica gel column chromatography (2→3.5→5→8% ethyl acetate:pentane) afforded ester **9** (166.2 mg, 76.3% over two steps) as a pale, yellow oil.

*Characterization Data for ester **9**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 6.20 (d, 1H, *J* = 0.2 Hz, C34), 5.96 (d, 1H, *J* = 3.6 Hz, C20), 5.81 (ddt, 1H, *J* = 16.3, 10.9, 7.1 Hz, C25), 5.03 (s, 1H, C26), 5.01 (dd, 1H, *J* = 7.8, 2.0 Hz, C26), 3.85 (*app* q, 2H, *J* = 10.1 Hz, C17), 3.69 (tdd, 1H, *J* = 9.3, 4.8, 2.3 Hz, C23), 3.49 (d, 1H, *J* = 16.5 Hz, C22), 3.36 (s, 3H, CO_2Me), 3.16 (s, 3H, C19-OMe), 2.64 (dd, 1H, *J* = 16.1, 12.1 Hz, C22), 2.23-2.05 (m, 4H, C24, C40), 1.61-1.51 (m, 2H, C41), 1.31 (s, 3H, C18-Me), 1.27 (s, 3H, C18-Me), 1.23 (t, 2H, *J* = 6.8 Hz, C42), 1.20-1.13 (m, 6H, C43-C45), 1.00 (s, 9H, TBS), 0.87 (t, 3H, *J* = 7.2 Hz, C46), 0.10 (s, 3H, TBS), 0.10 (s, 3H, TBS) ppm

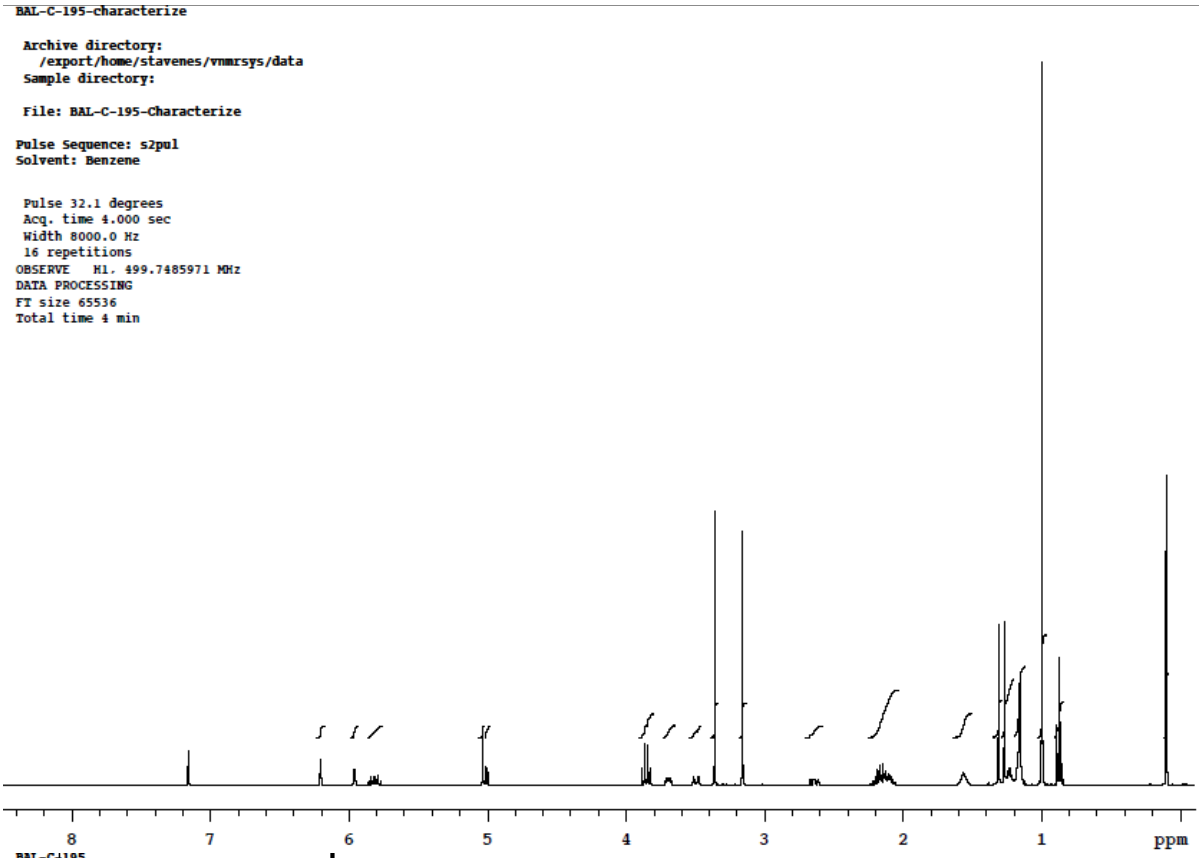
^{13}C NMR (C_6D_6, 125 MHz): δ = 171.5 (^{13}C label), 166.3, 154.3, 134.1, 117.8, 116.0, 103.2, 71.6, 70.9, 67.9, 50.7, 50.4, 47.7, 40.1, 34.7, 34.2, 33.5, 32.0, 29.3, 29.3, 29.2, 26.2, 25.0, 22.9, 21.0, 20.9, 18.6, 14.2, -5.3 ppm

IR (thin film): 3079, 2930, 2858, 1722, 1668, 1471, 1436, 1390, 1361, 1256, 1224, 1192, 1157, 1083, 1006, 939, 916, 837, 775, 726, 669 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{29}\{^{13}C\}H_{54}NaO_7Si^+$: 578.3565, Found: 578.3556

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22.7\ ^\circ C}}$ = -13.4 ± 0.3° (*c* = 0.76, CH_2Cl_2)

$\mathbf{R}_f$ = 0.40 (10% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV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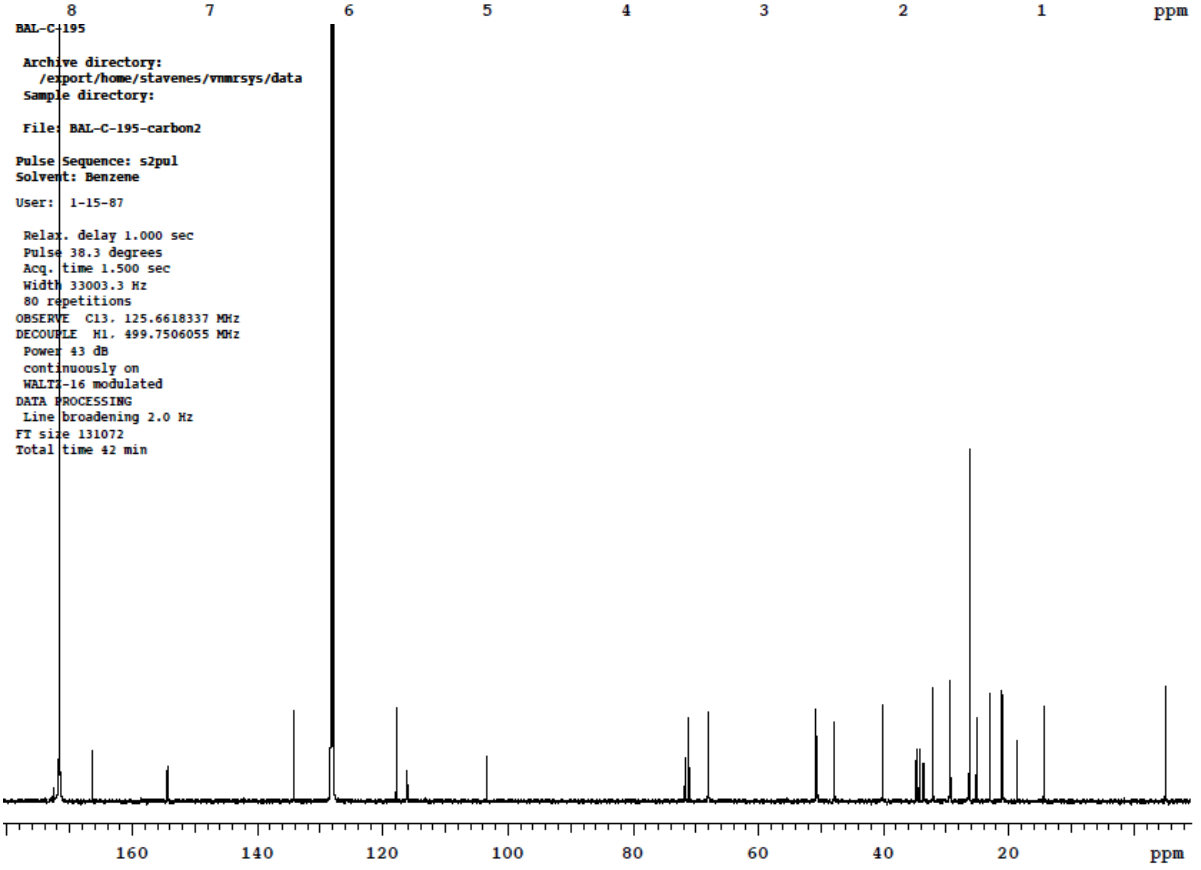
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80 repetitions
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DECOUPLE H1, 499.7506055 MHz
Power 43 dB
continuously on
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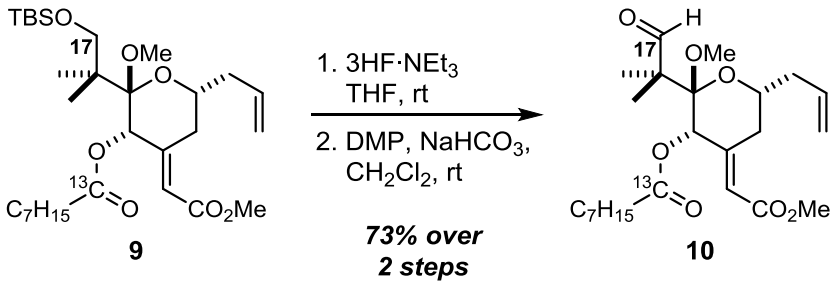


Procedure for C17 aldehyde 10

Ester **9** (166 mg, 0.299 mmol), was dissolved in THF (2.9 mL) in a 15 mL polypropylene vial under nitrogen gas. $3HF{\cdot}Et_3N$ (0.51 mL) was added via syringe over 3 min. The resulting solution was stirred vigorously at room temperature for 40 h. An additional 0.125 mL $3HF{\cdot}Et_3N$ was added. After an additional 5 h, the reaction was quenched into saturated aqueous $NaHCO_3$ (50 mL) and then extracted with Et_2O (4 x 50 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a crude residue which was purified via silica gel column chromatography (15→17.5→20% ethyl acetate:pentane) to provide a clear, colorless oil which was used directly in the next step.

The crude oil from the previous step was dissolved in CH_2Cl_2 (12.3 mL) in a 50 mL round bottom flask. $NaHCO_3$ (88 mg, 1.05 mmol) was then added in one portion, followed by DMP (223 mg, 0.525 mmol) in one portion, and the resulting white solution was stirred at ambient temperature. After 24 h, the reaction was still not complete, and an additional 88 mg $NaHCO_3$ and 223 mg DMP was added. After an additional 4 h, the reaction was quenched with 1:1 saturated aqueous $Na_2S_2O_3$:saturated aqueous $NaHCO_3$ (24 mL) and stirred vigorously for 15 min until the organic layer was clear. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a crude residue which was purified via silica gel column chromatography (3→4.5→6→8% ethyl acetate:pentane) to provide aldehyde **10** (95.3 mg, 72.7% over 2 steps) as a pale, yellow oil.

*Characterization Data for C17 aldehyde **10**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 9.93 (s, 1H, C17), 6.26 (d, 1H, *J* = 1.9 Hz, C34), 5.77 (ddt, 1H, *J* = 17.1, 10.2, 7.0 Hz, C25), 5.49 (d, 1H, *J* = 3.8 Hz, C20), 5.04-4.98 (m, 2H, C26), 3.91 (dd, 1H, *J* = 14.8, 2.3 Hz, C22), 3.59 (dddd, 1H, *J* = 11.7, 7.2, 4.5, 2.6 Hz, C23) 3.29 (s, 3H, CO_2Me), 2.98 (s, 3H, C19-OMe), 2.33-2.21 (m, 2H, C22, C24), 2.18-2.04 (m, 3H, C24, C40), 1.58-1.44 (m, 2H, C41), 1.25-1.17 (m, 2H, C42), 1.17-1.09 (m, 9H, C18-Me, C42-C44), 0.99 (s, 3H, C18-Me), 0.86 (t, 3H, *J* = 7.2 Hz, C46) ppm

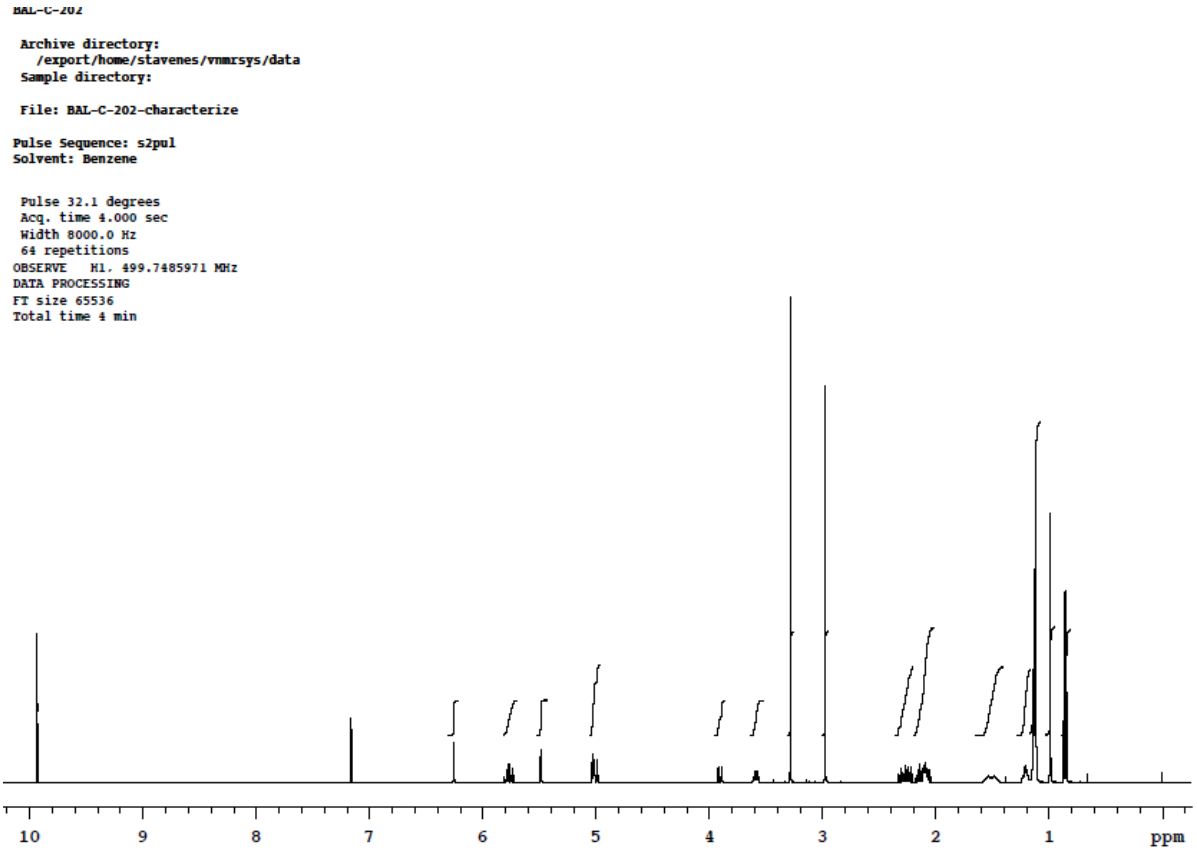
^{13}C NMR (C_6D_6, 125 MHz): δ = 201.1, 171.5 (^{13}C label), 166.2, 151.2, 133.9, 119.9, 117.9, 102.6, 71.9, 71.6, 54.1, 50.9 ($^1J_{CC}$ = 26.5 Hz), 40.1, 34.3, 33.8, 31.9, 31.1, 29.3, 29.2 ($^2J_{CC}$ = 3.7 Hz), 24.7, 22.9, 19.0, 16.7, 14.3 ppm

IR (thin film): 2929, 2856, 1722, 1667, 1436, 1394, 1362, 1260, 1223, 1181, 1160, 1131, 1103, 1048, 993, 917, 880 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{23}\{^{13}C\}H_{38}NaO_7^+$: 462.2543, Found: 462.2533

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22.2\ ^\circ C}}$ = -3.1 ± 0.2° (*c* = 0.88, CH_2Cl_2)

$\mathbf{R}_f$ = 0.35 (10% EtOAc in pentane), one black spot, *p*-anisaldehyde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Width 8000.0 Hz
64 repetitions
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Total time 4 min
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8
7
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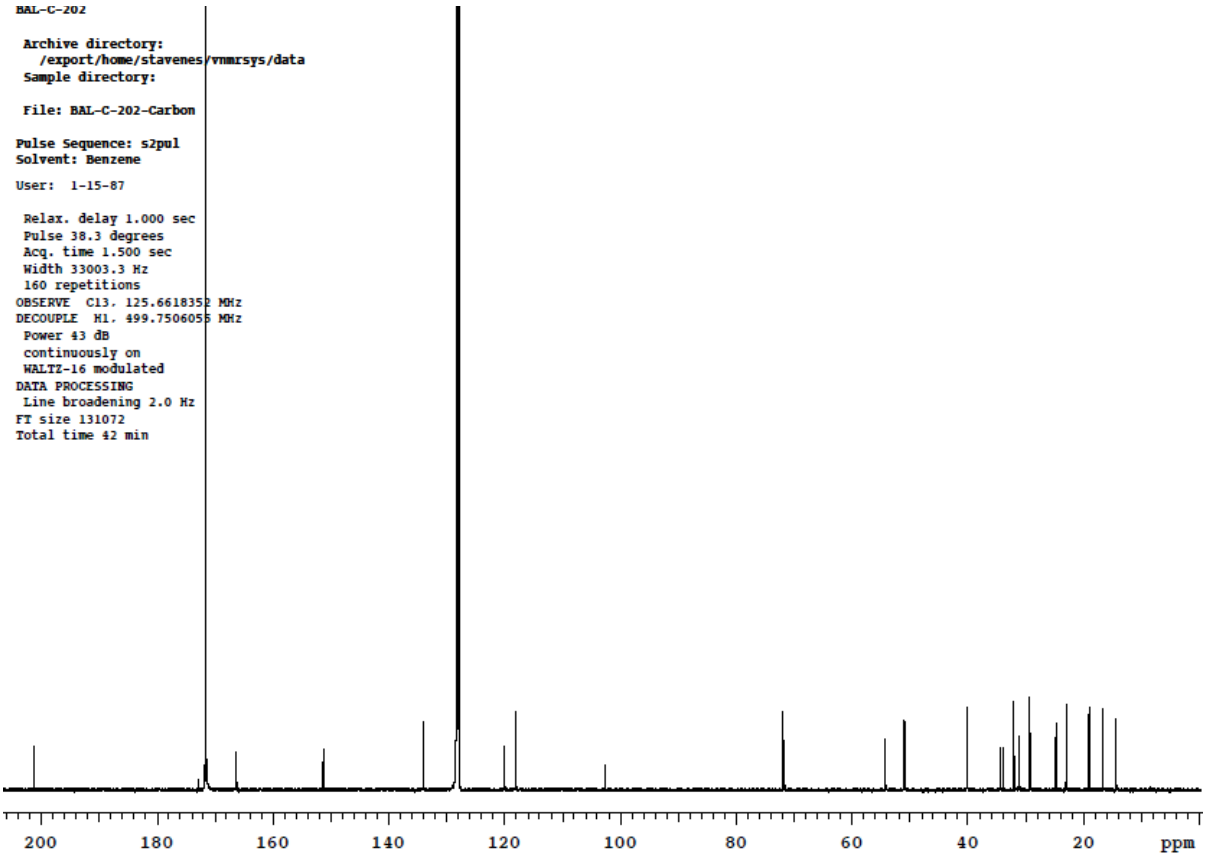
ppm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User: 1-15-87
Relax. delay 1.000 sec
Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
160 repetitions
OBSERVE C13, 125.6618352 MHz
DECOUPLE H1, 499.7506055 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
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FT size 131072
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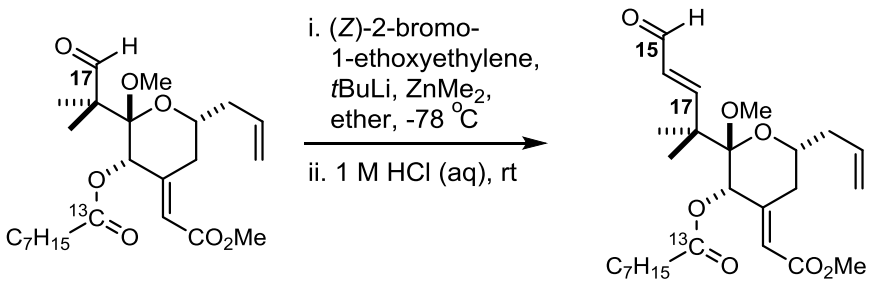


10 ***80%*** **11**

Procedure for C15 enal 11

(*Z*)-1-bromo-2-ethoxyethane (134 µL, 1.26 mmol) was added to a 25 mL flask containing Et_2O (3.2 mL) under nitrogen gas and cooled to -78 °C. *t*BuLi (1.68 mL, 1.48 M in pentane, 2.51 mmol) was added dropwise via syringe over 1 min, and the resulting solution was stirred at -78 °C for 30 min. Me_2Zn (1.07 mL, 1.2 M in toluene, 1.29 mmol) was then added dropwise via syringe over 2 min, and the solution was stirred at -78 °C for an additional 30 min. **10** (137.8 mg, 0.314 mmol) was then added dropwise as a solution in Et_2O (2.0 mL with 2 x 0.6 mL washes) and stirred at -78 °C for 3 h. TLC indicated consumption of starting material. 1 M HCl (5.4 mL) was then added, and the reaction was allowed to warm to ambient temperature. The resulting solution was stirred vigorously at rt for 41 h, at which point the reaction was quenched into saturated aqueous $NaHCO_3$ (20 mL) and diluted with water (20 mL) and EtOAc (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 50 mL), dried over Na_2SO_4, filtered and concentrated. Purification via silica gel column chromatography (5→11% ethyl acetate:pentane, 2% increments) afforded enal **11** (116.2 mg, 79.6%) as a pale, yellow oil.

*Characterization Data for C15 enal **11**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 9.49 (d, 1H, *J* = 7.6 Hz, C15), 7.12 (m, 1H, C17), 6.08 (s, 1H, C34), 5.91 (dd, 1H, *J* = 16.1, 7.6 Hz, C16), 5.72 (ddt, 1H, *J* = 16.9, 10.3, 6.9 Hz, C25), 5.64 (d, 1H, *J* = 3.9 Hz, C20), 5.00 (d, 1H, *J* = 0.6 Hz, C26), 4.98-4.95 (m, 1H, C26), 3.57-3.51 (m, 2H, C22, C23), 3.29 (s, 3H, CO_2Me), 2.99 (s, 3H, C19-OMe), 2.44 (dd, 1H, *J* = 15.2, 12.6 Hz, C22), 2.13-1.86 (m, 4H, C24, C40), 1.47-1.38 (m, 2H, C40), 1.23-1.16 (m, 2H, C41), 1.14-1.10 (m, 6H, C42-C45), 0.99 (s, 3H, C18-Me), 0.93 (s, 3H, C18-Me), 0.84 (t, 3H, *J* = 7.1 Hz, C46) ppm

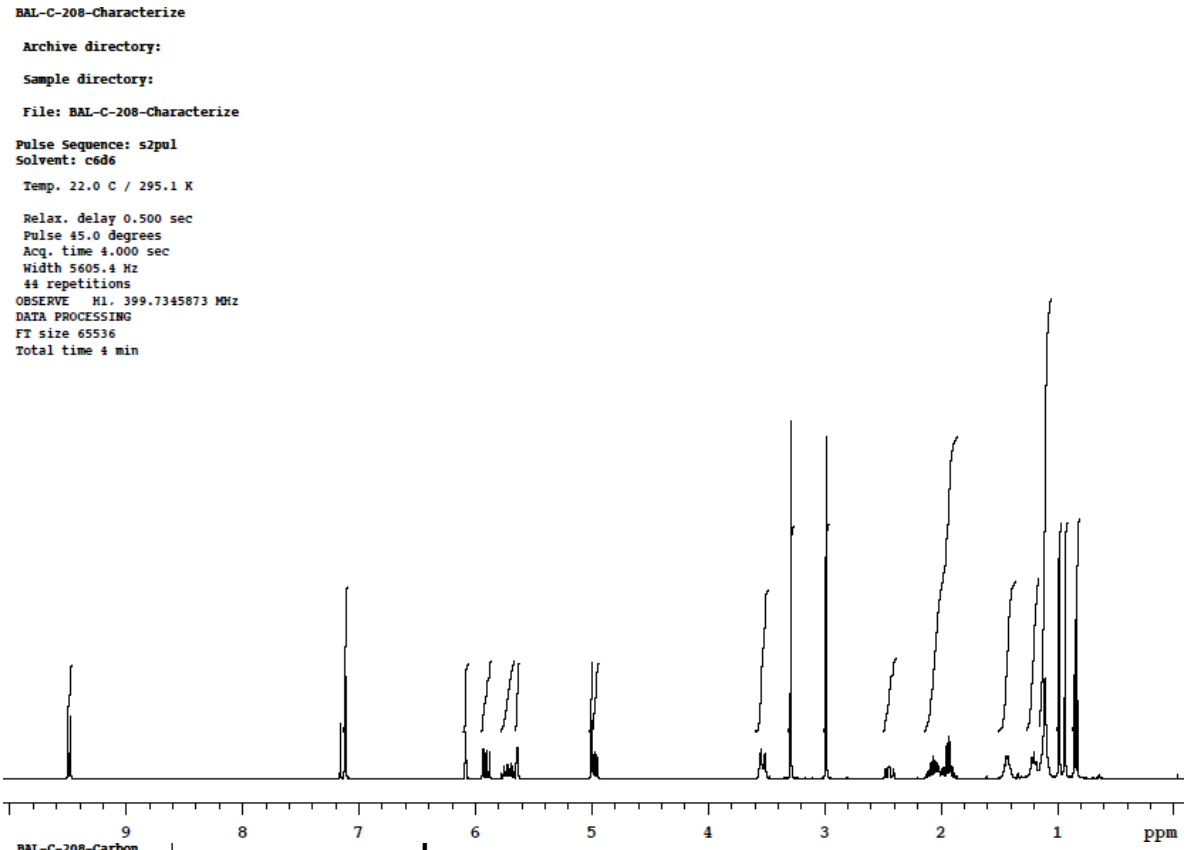
^{13}C NMR (C_6D_6, 125 MHz): δ = 193.2, 171.4 (^{13}C label), 166.2, 165.3, 152.7, 133.8, 127.5, 118.0, 117.3, 102.8, 71.6, 70.9 ($^2J_{CC}$ = 2.6 Hz), 50.7, 47.4, 40.0, 34.7, 34.1, 32.6, 32.0, 29.2, 29.2, 24.9, 23.3, 22.9, 22.1, 14.3 ppm

IR (thin film): 2930, 2858, 2720, 1720, 1690, 1628, 1460, 1436, 1382, 1362, 1258, 1226, 1179, 1140, 1104, 1044, 916 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{25}\{^{13}C\}H_{40}NaO_7^+$: 488.2700, Found: 488.2698

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22.0\ °C}}$ = -31.7 ± 0.6° (*c* = 0.97, CH_2Cl_2)

$\mathbf{R}_f$ = 0.20 (10% EtOAc in pentane), one black spot, *p*-anisaldehyde +UV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8
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6
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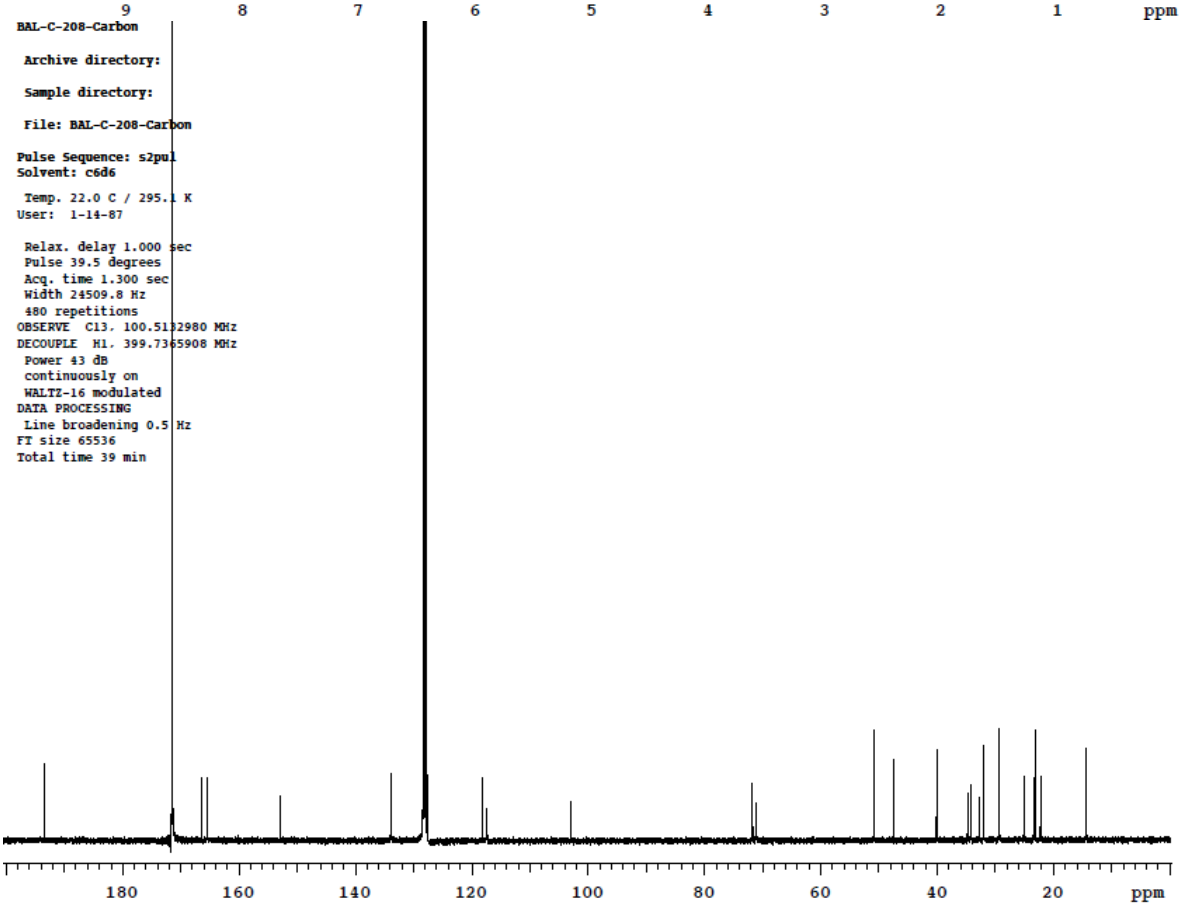
ppm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User: 1-14-87
Relax. delay 1.000 sec
Pulse 39.5 degrees
Acq. time 1.300 sec
Width 24509.8 Hz
480 repetitions
OBSERVE C13, 100.5132980 MHz
DECOUPLE H1, 399.7365908 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
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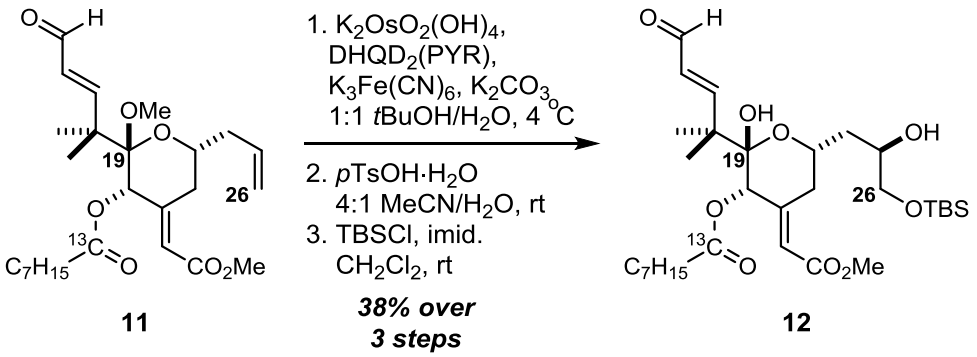
ppm



Procedure for C26 silyl ether 12

In a 25 mL round bottom flask was added $(DHQD)_2PYR$ (24.0 mg), $K_2OsO_2(OH)_4$ (4.0 mg), $K_3Fe(CN)_6$ (1.13 g), K_2CO_3 (2.68 g), *t*BuOH (13.5 mL) and H_2O (13.5 mL). The resulting biphasic mixture was stirred vigorously at ambient temperature for 2 h prior to use. Enal **11** (226 mg, 0.485 mmol) was cooled in a small vial in an ice bath, and then 7.3 mL of the biphasic catalyst solution (0.006 mol% osmium) was added via syringe in one portion. The reaction mixture was stirred at 0 °C for 10 min and then moved to the 4 °C cold room. After 72 h, the reaction was diluted with water (40 mL) and EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (4 x 50 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and then concentrated *in vacuo* to afford a crude residue which was purified via silica gel column chromatography (15→90→100% ethyl acetate:pentane →5→10% MeOH:$CHCl_3$) to provide 181 mg of the two C25 epimers (75%, dr = 2.8:1 β:α) as clear, colorless oil which was used directly in the next step.

The colorless oil from the previous step was dissolved in a 50 mL round bottom flask in MeCN (27.6 mL) and water (6.9 mL). *p*TsOH·H_2O (691 mg, 3.63 mmol) was added in one portion, and the resulting solution was stirred at ambient temperature. After 41 h, the reaction was diluted with saturated aqueous $NaHCO_3$ (100 mL) and EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (100 mL) then dried over Na_2SO_4, filtered, and then concentrated *in vacuo* to afford a crude residue, still containing both C25 epimers, that was used directly in the next step.

The oil from the previous step and imidazole (111 mg, 1.64 mmol) were dissolved in CH_2Cl_2 (36.3 mL) in a 100 mL round bottom flask. TBSCl (545 μL, 1 M in CH_2Cl_2, 0.545 mmol) was added in one portion via syringe. The reaction was stirred at ambient temperature. After 4 hr, the reaction was quenched into saturated aqueous NH_4Cl (50 mL), diluted with water (50 mL) and extracted with EtOAc (4 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification via silica gel column chromatography (20→25→27→29→32→35% ethyl acetate:pentane) afforded the pale, yellow oil of southern fragment **12** (109.7 mg, 37.7% over three steps) as a single epimer at C25.

*Characterization Data for C26 silyl ether **12**:*

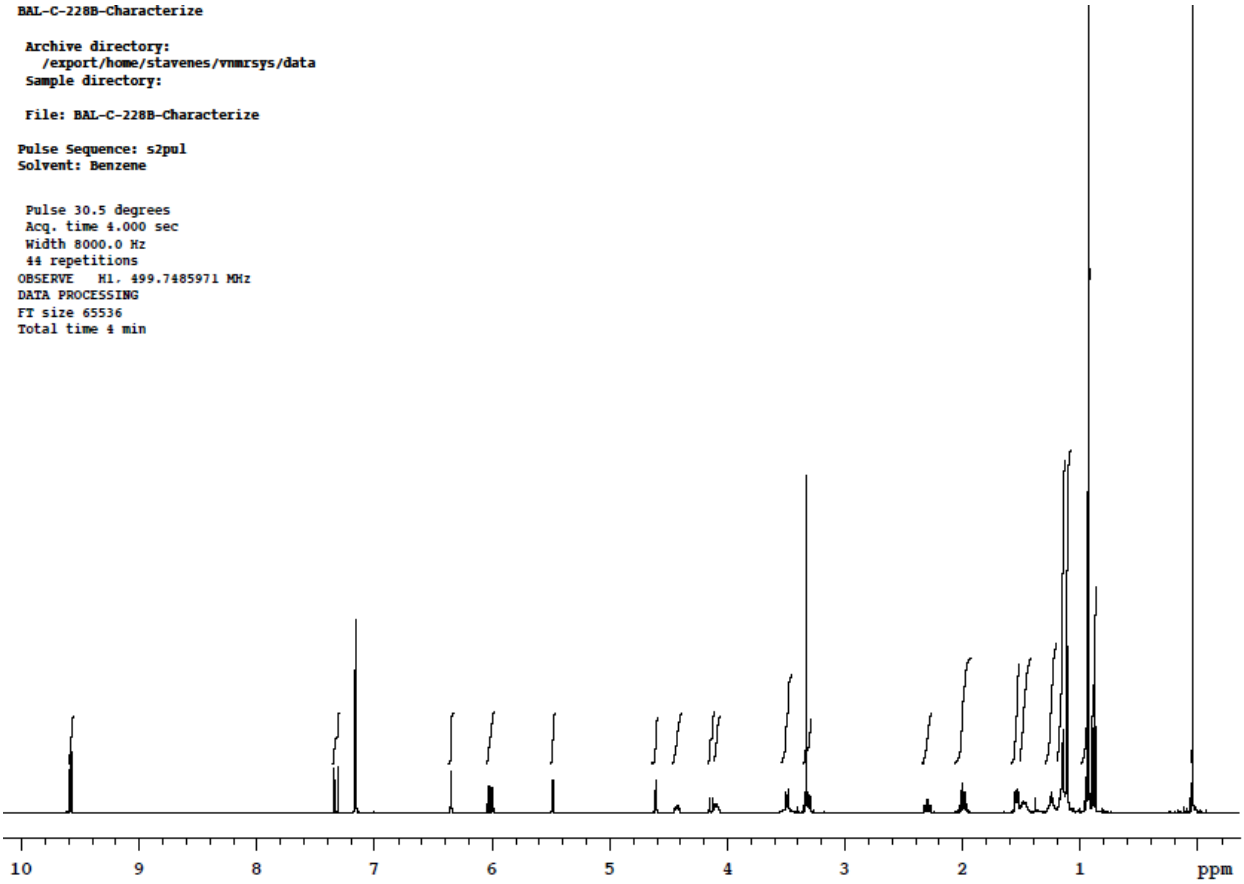
^{1}H NMR (C_6D_6, 500 MHz): δ = 9.58 (d, 1H, *J* = 7.6 Hz, C15), 7.32 (d, 1H, *J* = 16.1 Hz, C17), 6.35 (d, 1H, *J* = 1.8 Hz, C34), 6.01 (dd, 1H, *J* = 16.1, 7.6 Hz, C16), 5.48 (d, 1H, *J* = 3.7 Hz, C20), 4.61 (s, 1H, C19-OH), 4.43 (dqt, 1H, *J* = 9.3, 4.5, 2.8 Hz, C23), 4.14 (dd, 1H, *J* = 13.9, 2.2 Hz, C22), 4.09 (td, 1H, *J* = 6.4, 3.8 Hz, C25), 3.49 (m, 2H, C26, C25-OH), 3.33 (s, 3H, CO_2Me), 3.30 (dd, 1H, *J* = 6.8, 3.4 Hz, C26), 2.30 (ddd, 1H, *J* = 13.8, 11.7, 1.9 Hz, C22), 2.06-1.94 (m, 2H, C40), 1.55-1.53 (m, 2H, C24), 1.53-1.43 (m, 2H, C41), 1.24 (dt, 2H, *J* = 13.7, 6.5 Hz, C42), 1.18-1.15 (m, 6H, C43-C45), 1.12 (s, 3H, C18-Me), 1.11 (s, 3H, C18-Me), 0.93 (s, 9H, TBS), 0.88 (t, 3H, *J* = 7.2 Hz, C46), 0.04 (s, 6H, TBS) ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 193.3, 171.4 (^{13}C label), 166.3, 164.8, 150.9, 127.9,[2] 121.1, 100.3, 73.1, 68.5, 67.8, 67.1, 50.9, 45.9, 39.2, 34.9, 34.4, 32.0, 31.7, 29.2, 29.2, 26.1, 24.8, 23.0, 22.9, 20.3, 18.6, 14.3, -5.2 ppm

IR (thin film): 3392, 2930, 2858, 1693, 1471, 1436, 1384, 1257, 1104, 986, 939, 889, 838, 779, 456 cm^{-1}

[2] The solvent peak covers this resonance, but it is predicted to be at this shift based on analogy to preceding intermediates.

HRMS (ES+, *m/z)* calculated for $C_{30}\{^{13}C\}H_{54}NaO_9Si^+$: 622.3463, Found: 622.3467
$[\alpha]_D^{24.1\,°C}$ = -31.3 ± 0.3° (*c* = 0.80, CH_2Cl_2)
R_f = 0.35 (50% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV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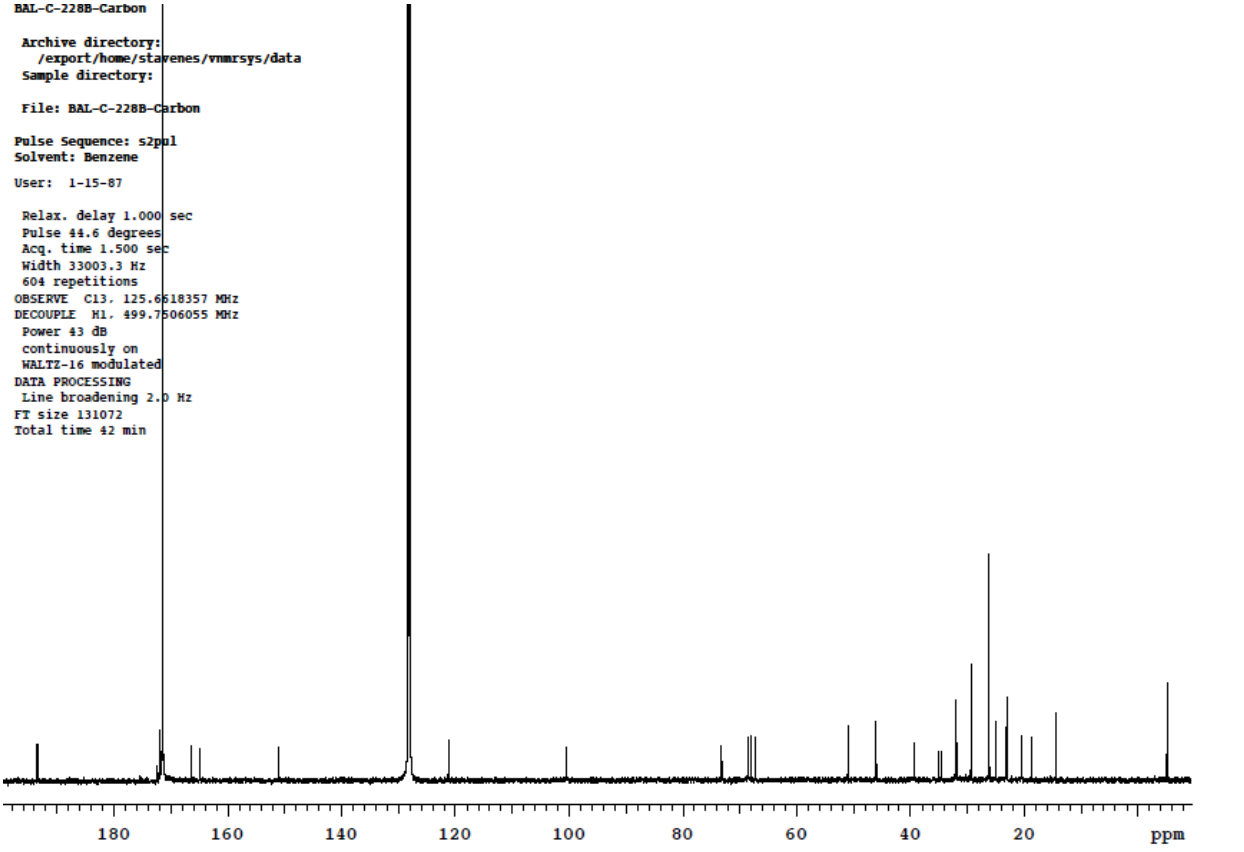
ppm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Pulse 44.6 degrees
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DECOUPLE H1, 499.7506055 MHz
Power 43 dB
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WALTZ-16 modulated
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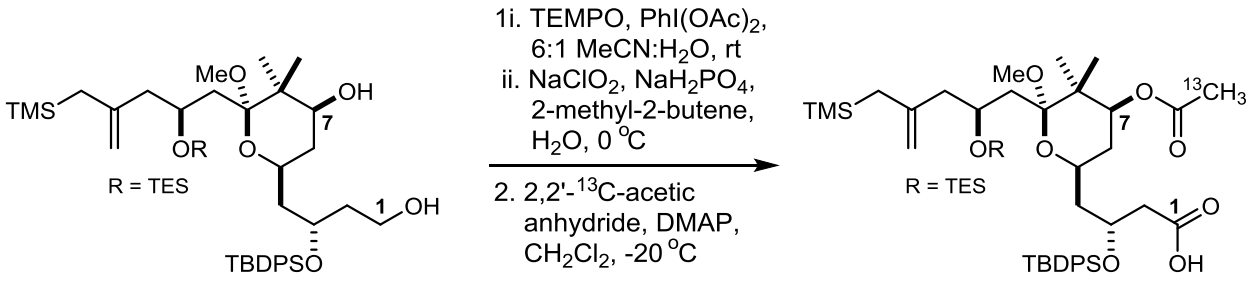


13 ***56% over 2 steps*** **14**

Procedure for C1 carboxylic acid 14

Diol **13** (44.0 mg, 0.057 mmol) was dissolved in MeCN (3.0 mL) under ambient temperature. Water (0.5 mL) was added, followed by TEMPO (2.7 mg, 0.017 mmol), then $PhI(OAc)_2$ (56.9 mg, 0.177 mmol). The resulting tan-brown solution was stirred at room temperature for 1 h and then cooled to 0 °C. Water (0.9 mL) was then added, followed by 2-methyl-2-butene (1.3 mL), NaH_2PO_4 (69.1 mg, 0.576 mmol), and $NaClO_2$ (31.9 mg, 0.353 mmol). The resulting solution was then stirred at 4 °C for 3.5 h and then quenched with saturated aqueous $Na_2S_2O_3$ (6 mL) and diluted with brine (3 mL). The aqueous layer was then extracted with Et_2O (4 x 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting pale brown crude material was used directly in the next step.

The crude material from the previous step was dissolved in CH_2Cl_2 (1.0 mL) in a small vial under N_2 and cooled to -30 °C. DMAP (82.9 mg, 0.678 mmol) was added in one portion followed by 2,2'-^{13}C acetic anhydride (53.9 µL, 0.570 mmol). The resulting solution was stirred at -30 °C for 2 h, at which time sat. aq. $NaHCO_3$ (1 mL) and H_2O (1 mL) was added. The resulting biphasic mixture was stirred vigorously at ambient temperature for 20 h. The reaction was then diluted with saturated aqueous NH_4Cl (20 mL) and then extracted with Et_2O (4 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification via silica gel column chromatography (12→14→16→18% ethyl acetate:pentane) afforded northern fragment **14** (26.4 mg, 55.9% over two steps) as a clear, colorless oil.

*Characterization Data for C1 carboxylic acid **14**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 7.86-7.77 (m, 4H, TBDPS), 7.30-7.23 (m, 6H, TBDPS), 5.41 (dd, 1H, *J* = 4.8, 11.8 Hz, C7), 4.79 (br s, 1H, C14), 4.69 (br s, 1H, C14), 4.45-4.30 (m, 2H, C3, C11), 3.48-3.31 (m, 1H, C5), 3.02 (s, 3H, C9-OMe), 2.70 (dd, 1H, *J* = 4.1, 15.1 Hz, C2), 2.60 (dd, 1H, *J* = 7.2, 15.1 Hz, C2), 2.35 (dd, 1H, *J* = 5.1, 13.0 Hz, C12), 2.26 (dd, 1H, *J* = 8.0, 13.0 Hz, C12), 2.12 (dd, 1H, *J* = 3.4, 15.9 Hz, C10), 2.07-1.93 (m, 2H, C4, C10), 1.77-1.69 (m, 1H, C4), 1.71 (d, 3H, $^1J_{CH}$ = 128.9 Hz,[3] C7-OAc), 1.60 (d, 2H, *J* = 4.0 Hz, C30), 1.54-1.48 (m, 1H, C6), 1.19 (s, 9H, TBDPS), 1.16 (s, 3H, C8-Me), 1.15 (s, 3H, C8-Me), 1.09 (t, *J* = 8.0 Hz, 9H, TES), 1.08 (m, 1H, C6), 0.74 (q, *J* = 7.7 Hz, 6H, TES), 0.10 (s, 9H, TMS) ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 176.2, 169.6 (d, *J* = 59.9 Hz), 144.7, 136.3, 136.3, 134.3, 133.7, 130.2, 130.1, 128.3, 128.1, 128.1, 127.9, 110.9, 104.6, 95.9, 73.8, 69.4, 68.7, 66.2, 48.9, 48.3, 43.9, 42.8, 42.3,40.1, 30.0, 27.5, 27.2, 21.1, 20.7 (^{13}C label), 20.5, 19.5, 17.5, 7.4, 6.0, -1.3 ppm

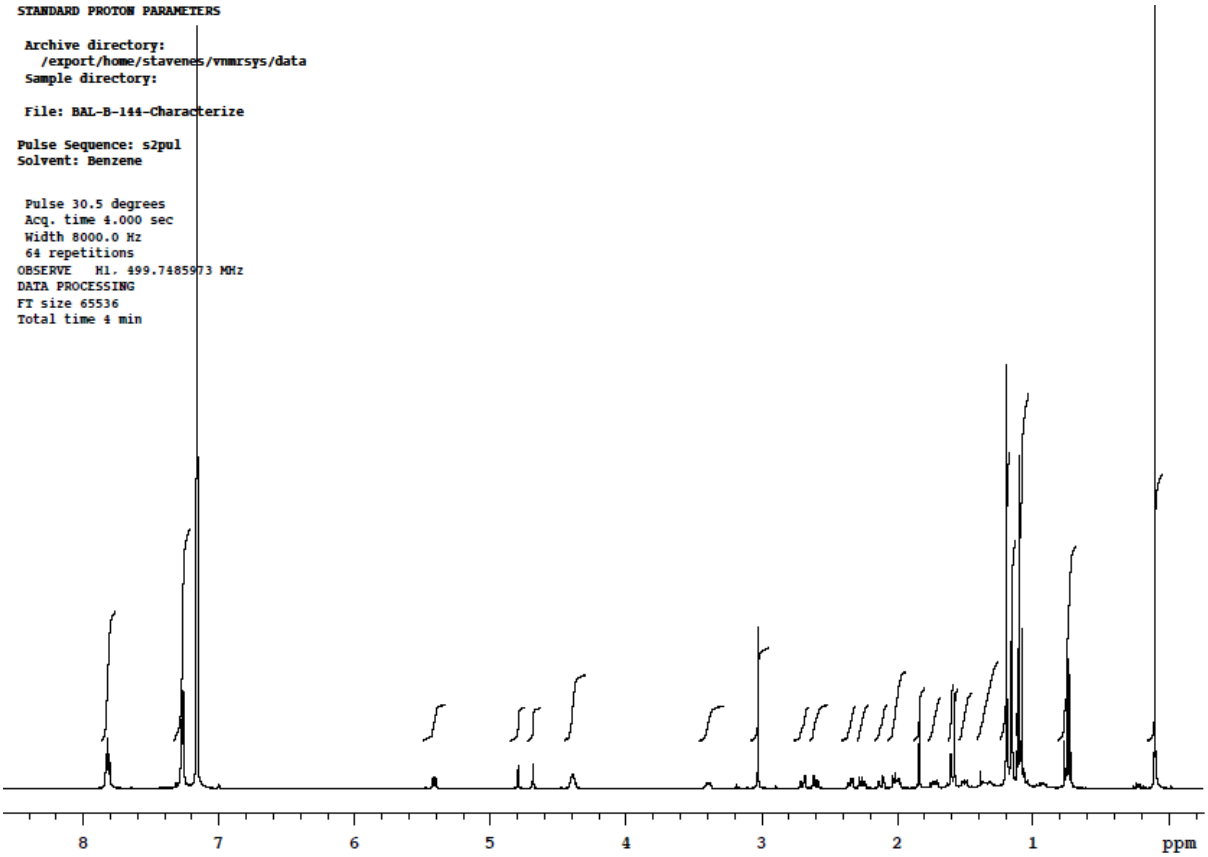
IR (thin film): 3071, 2953, 1738, 1731, 1713, 1427, 1391, 1359, 1245, 1185, 1143, 1111, 1037, 1016, 854, 822, 739, 702 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{44}\{^{13}C\}H_{74}NaO_8Si_3^+$: 850.4617, Found: 850.4640

$[\alpha]_D^{24.3\,°C} = 29.9 \pm 1.9°$ (*c* = 0.21, CH_2Cl_2)

R_f = 0.20 (25% EtOAc in pentane), one purple spot, *p*-anisaldehyde

[3] Large coupling constant due to ^{13}C-induced splitting.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7
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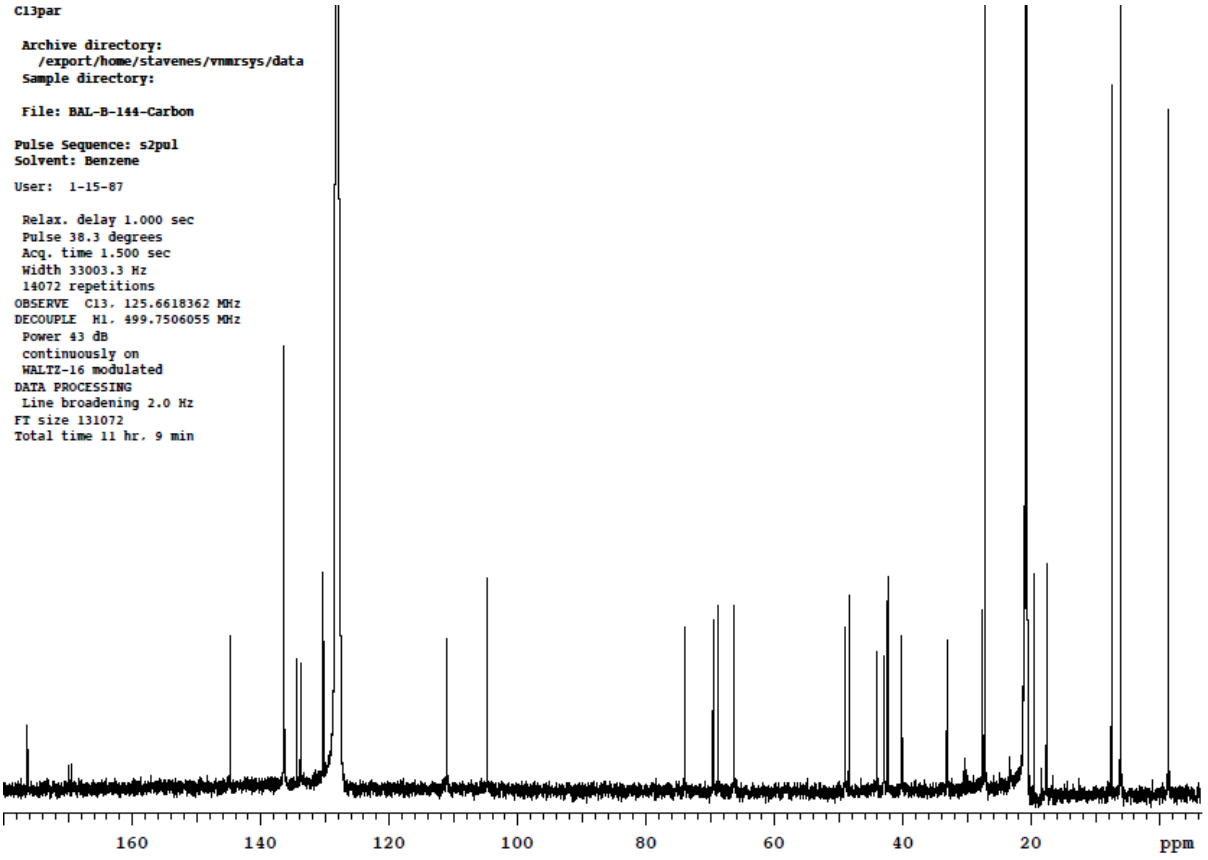
ppm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Power 43 dB
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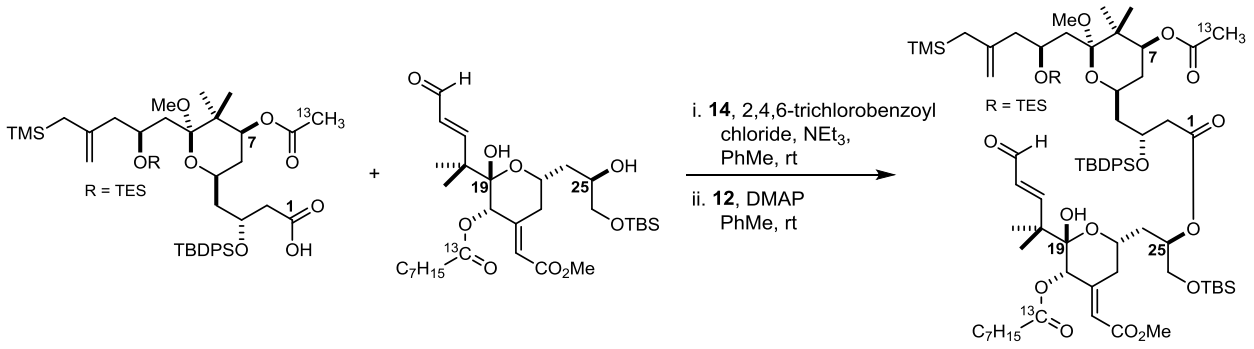
60
40
20
ppm



14 **12** ***78%*** **16**

Procedure for C1 ester 16

C1 carboxylic acid northern fragment **14** (95.1 mg, 0.115 mmol) was dissolved in toluene (3.6 mL) in a small oven-dried vial equipped under N_2. To this solution was added triethylamine (73.6 μL, 0.528 mmol), then 2,4,6-trichlorobenzoyl chloride (19.0 μL, 0.122 mmol). The resulting solution was stirred for 4.5 hrs at ambient temperature, and the formation of salts was observed. A toluene solution of southern fragment **12** (85.9 mg, 0.144 mmol) and DMAP (37.9 mg, 0.310 mmol) was then added in toluene (3.0 mL) via cannula (with 2 x 1.2 mL washes to ensure material transfer). The resulting solution was stirred for 75 min, at which time it was loaded directly onto a slurry-packed silica gel column. Purification by column chromatography (7→9→11→13→15% ethyl acetate:pentane) provided desired ester **16** (126.5 mg, 78.0%) as a pale yellow oil.

*Characterization Data for C1 ester **16**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 9.72 (d, 1H, *J* = 7.6 Hz, C15), 7.83-7.81 (m, 4H, TBDPS), 7.47 (d, 1H, *J* = 16.1 Hz, C17), 7.32-7.26 (m, 6H, TBDPS), 6.31 (d, 1H, *J* = 1.8 Hz, C34), 6.04 (dd, 1H, *J* = 16.1, 7.6 Hz, C16), 5.51 (m, 2H, C20, C25), 5.35 (dd, 1H, *J* = 11.8, 4.8 Hz, C7), 4.81 (bs, 1H, C14), 4.71 (bs, 1H, C14), 4.43-4.35 (m, 2H, C3, C11), 4.21-4.11 (m, 2H, C22, C23), 3.65 (dd, 1H, *J* = 10.7, 5.6 Hz, C26), 3.60 (dd, 1H, *J* = 10.8, 5.6 Hz, C22), 3.54-3.48 (m, 1H, C5), 3.39 (s, 1H, C19-OH), 3.28 (s, 3H, CO_2Me), 3.08 (s, 3H, C9-OMe), 2.67-2.57 (m, 2H, C2), 2.38-2.23 (m, 3H, C22, C12, C12), 2.10-1.96 (m, 6H, C4, C10, C10, C40, C40, C24), 1.76-1.64 (m, 2H, C4, C24), 1.70 (d, 3H, $^1J_{CH}$ = 129.0 Hz,[4] C7-OAc), 1.62 (d, 2H, *J* = 3.3 Hz, C30), 1.59-1.54 (m, 1H, C6), 1.51-1.43 (m, 1H, C41), 1.23-1.17 (m, 14H, Me, Me, C42-C45), 1.17-1.13 (m, 15H, Me, Me, TBDPS), 1.10 (t, 9H, *J* = 8.0 Hz, TES), 1.08 (m, 1H, C6), 0.97 (s, 9H, TBS), 0.89 (t, 3H, *J* = 7.2 Hz, C46), 0.77-0.72 (q, 6H, *J* = 8.0 Hz, TES), 0.11-0.10 (m, 9H, TMS), 0.09 (m, 6H, TBS) ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 193.2, 175.1, 173.9, 172.3, 171.6, 171.4 (C20 octanoyl ^{13}C label), 170.1 ($^1J_{CC}$ = 42.6 Hz), 166.1, 164.8, 150.9, 144.7, 136.36, 136.23, 134.3, 133.9, 130.20, 130.12, 121.3, 111.0, 104.6, 100.0, 74.2, 73.0, 73.0, 71.8, 68.7, 68.6, 66.6, 65.8, 65.3, 50.8, 49.0, 48.4, 45.9, 43.6, 42.4, 42.3, 40.1, 37.9, 34.9, 34.4, 32.9, 32.0, 31.5, 29.2, 29.2, 27.6, 27.2, 26.0, 24.8, 24.8, 22.9, 21.1, 20.8 (C7 acetoxy ^{13}C label), 20.6, 19.5, 18.5, 17.5, 14.3, 7.4, 6.0, -1.3, -5.1, -5.1 ppm

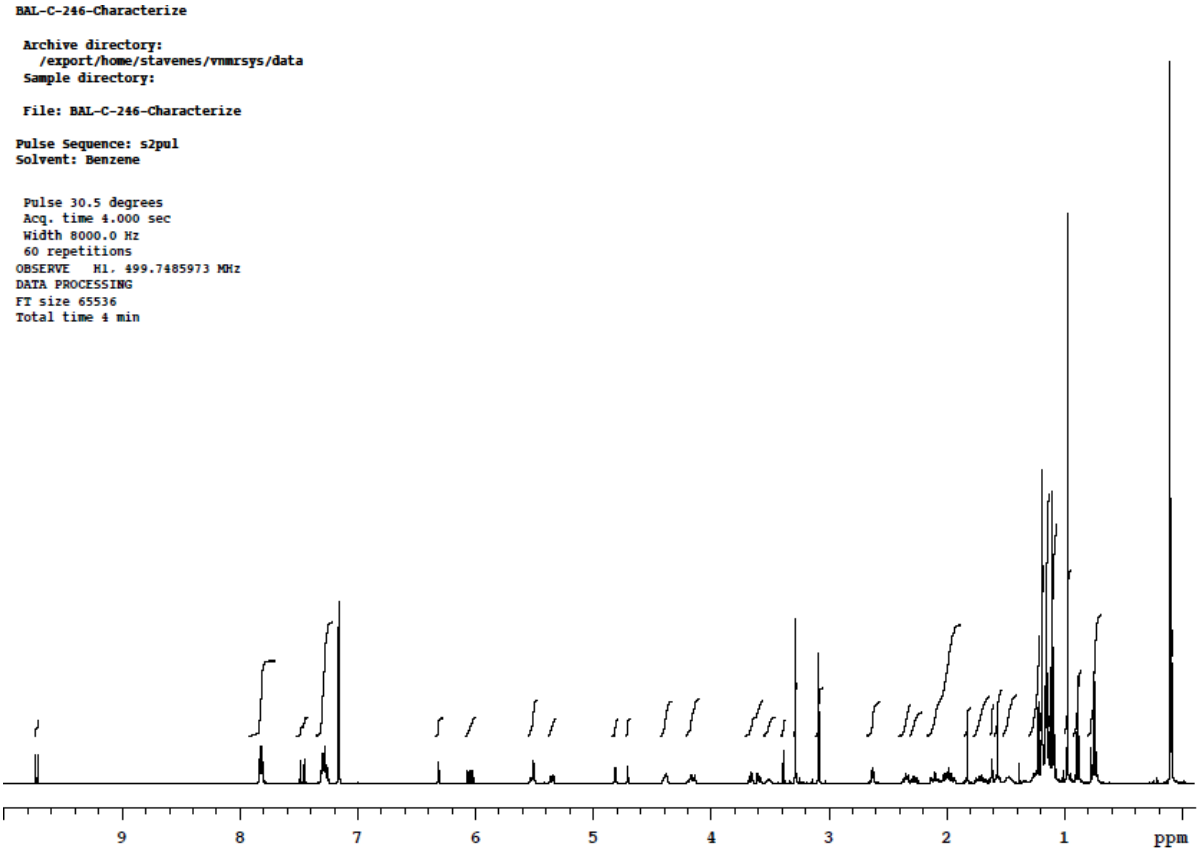
IR (thin film): 3487, 3072, 2954, 2858, 1725, 1692, 1631, 1472, 1429, 1389, 1360, 1248, 1111, 839, 778, 740, 703, 612 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{74}\{^{13}C_2\}H_{128}NaO_{16}Si_4^+$: 1431.8082, Found: 1431.8114

$[\alpha]_D^{22.6\,°C}$ = -14.6 ± 0.2° (*c* = 1.1, CH_2Cl_2)

R_f = 0.60 (25% EtOAc in pentane), one purple spot, *p*-anisaldehyde + UV

[4] Large coupling constant due to ^{13}C-induced splitting.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6
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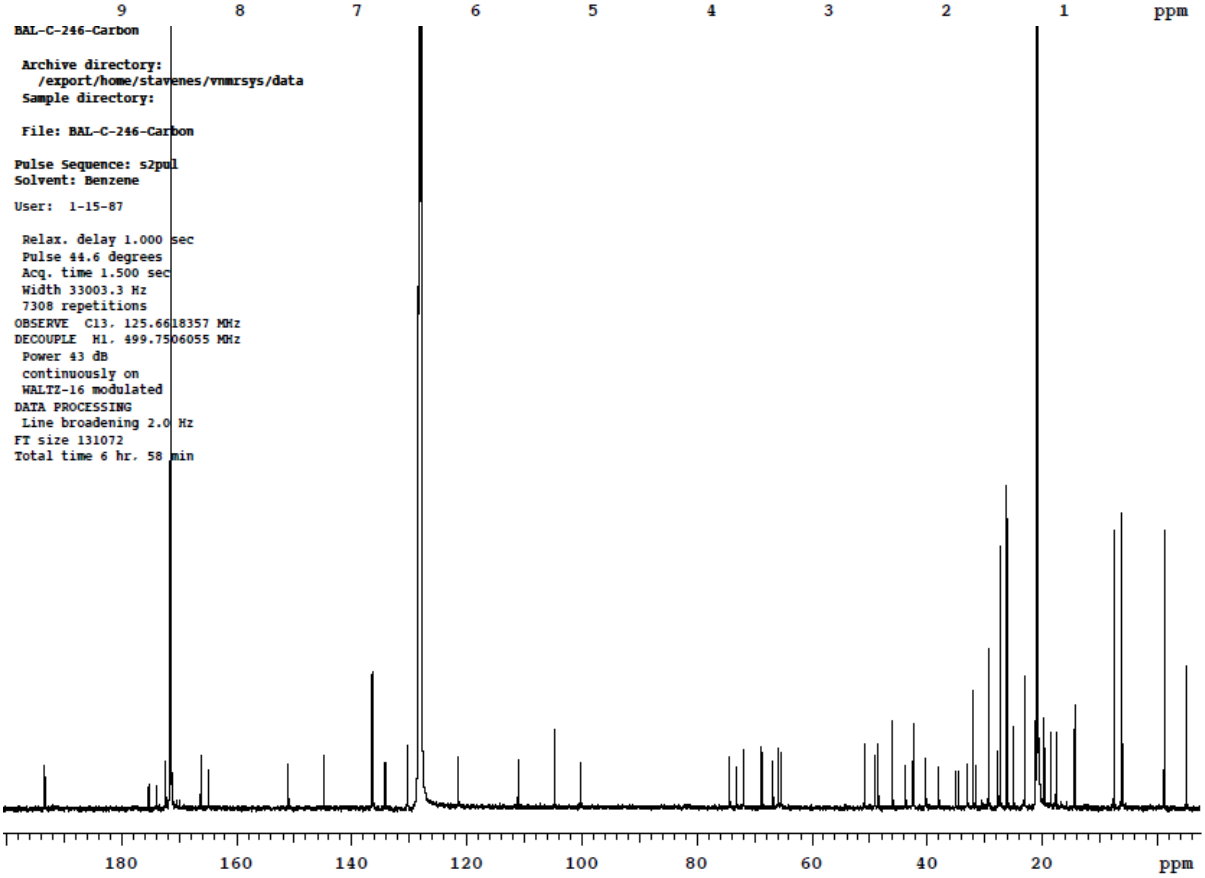
4
3
2
1
ppm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Power 43 dB
continuously on
WALTZ-16 modulated
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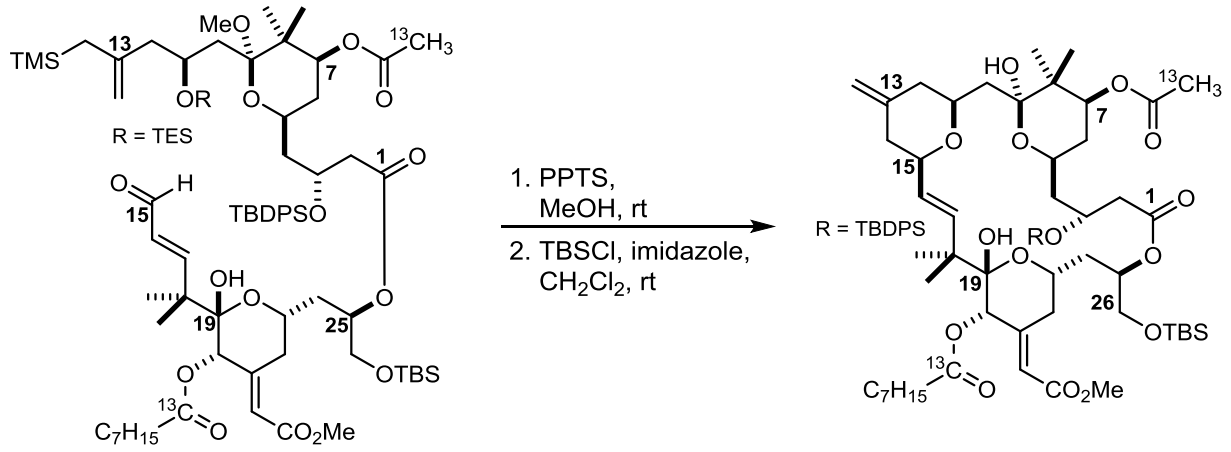


16 ***57% over 2 steps*** **18**

Procedure for C13 exocyclic olefin 18

Neat ester **16** (126.5 mg, 0.090 mmol) was dissolved in a solution of pyridinium *p*-toluenesulfonate (PPTS) in anhydrous MeOH (4 mM, 4.49 mL, 0.018 mmol). The reaction was stirred under ambient for 20 hrs, at which time the solution was diluted with Et_2O (40 mL), H_2O (15 mL) and brine (15 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (4 x 40 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated to afford a crude residue that was used directly in the next step.

The crude material from the previous step was dissolved in CH_2Cl_2 (9.1 mL). Imidazole (91.6 mg, 1.35 mmol) was added, followed by TBSCl (1.0 M in CH_2Cl_2, 449 μL, 0.449 mmol). The colorless reaction mixture was stirred at rt for 2 hrs. The reaction became slightly cloudy during the course of the reaction. The reaction was diluted with sat. aq. NH_4Cl (25 mL) and extracted with Et_2O (4 x 40 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified via silica gel chromatography (7→16% ethyl acetate:pentane, 3% increments) to afford 61.1 mg C13 olefin **18** (56.5% over two steps) as a clear, colorless oil.

*Characterization Data for C13 exocyclic olefin **18**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 7.74 (dd, 2H, *J* = 8.0, 1.3 Hz, TBDPS), 7.71-7.69 (m, 2H, TBDPS), 7.29-7.21 (m, 6H, TBDPS), 6.37 (d, 1H, *J* = 16.0 Hz, C17), 6.32 (d, 1H, *J* = 1.9 Hz, C34), 5.82 (dd, 1H, *J* = 16.1, 6.9 Hz, C16), 5.62-5.58 (m, 2H, C7, C20), 5.58-5.54 (m, 1H, C25), 4.83-4.78 (m, 3H, C3, C30, C30), 4.32-4.27 (m, 1H, C15), 4.23-4.17 (m, 2H, C22, C23), 4.04 (*app* t, 1H, *J* = 9.9 Hz, C5), 3.69 (*app* t, 1H, *J* = 10.3 Hz, C11), 3.32 (m, 2H, C26), 3.14 (s, 3H, CO_2Me), 2.96 (s, 1H, C19-OH), 2.87 (dd, 1H, *J* = 16.9, 3.7 Hz, C2), 2.72 (s, 3H, C9-OMe), 2.39 (dd, 1H, *J* = 16.9, 10.1 Hz, C2), 2.32 (*app* t, 1H, *J* = 12.6 Hz, C22), 2.22 (d, 1H, *J* = 12.2 Hz, C14), 2.15-2.05 (m, 4H, C10, C40, C40, C14), 2.00 (d, 1H, *J* = 12.2 Hz, C12), 1.89 (*app* t, 1H, *J* = 12.2 Hz, C24), 1.70 (d, 3H, $^1J_{CH}$ = 128.3Hz,[5] C7-OAc), 1.71-1.61 (m, 4H, C6, C24, C24, C4), 1.56-1.48 (m, 3H, C41, C41, C10), 1.43-1.30 (m, 2H, C6, C4), 1.24-1.18 (m, 5H, Me, C45), 1.18-1.16 (m, 3H, Me), 1.16-1.08 (m, 18H, Me, TBDPS, C42-C44), 1.08-1.06 (m, 3H, Me), 0.90-0.87 (m, 9H, TBS), 0.86 (t, 3H, *J* = 7.4 Hz, C46), -0.05 (s, 3H, TBS), -0.08 (s 3H, TBS) ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 174.3, 172.0, 171.5 (C20 octanoyl ^{13}C label), 170.3, 169.7 ($^1J_{CC}$ = 59.9 Hz), 166.3, 151.1, 145.3, 136.1, 136.0, 135.3, 135.1, 134.8, 134.4, 130.0, 128.3, 128.1, 121.0, 108.7, 103.3, 98.7, 78.6, 74.5, 74.0, 73.3, 70.1, 67.5, 65.6, 65.4, 64.7, 50.6, 47.9, 45.1, 45.0, 43.2, 42.1, 41.7, 40.9, 39.7, 37.4, 34.9, 34.4, 33.9, 31.9, 29.2, 29.2, 29.2, 27.2, 26.0, 25.0, 24.3, 22.9, 20.8 (C7 acetate ^{13}C label), 20.7, 20.6, 19.4, 18.4, 17.7, 14.2, -5.3, -5.3 ppm

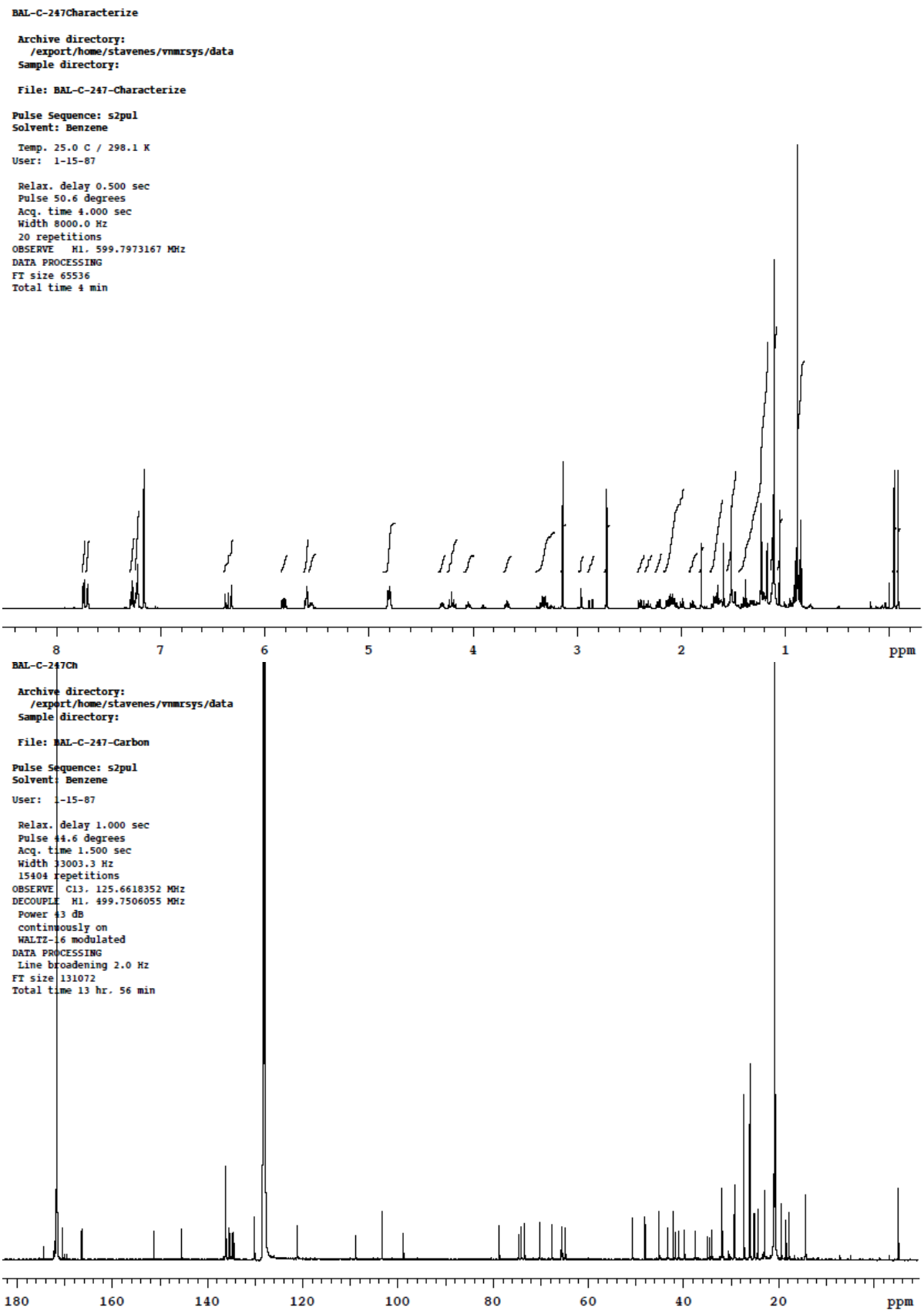
IR (thin film): 3510, 3071, 2930, 2857, 1735, 1664, 1473, 1428, 1388, 1364, 1317, 1246, 1164, 1143, 1104, 1020, 1004, 985, 886, 838, 777, 740, 704, 623, 611 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{65}\{^{13}C_2\}H_{102}NaO_{15}Si_2^+$: 1227.6717, Found: 1227.6688

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23.1\,°C}} = 13.5 \pm 0.3°$ (*c* = 0.71, CH_2Cl_2)

R_f = 0.35 (30% EtOAc in pentane), one black spot, *p*-anisaldehyde

[5] Large coupling constant due to ^{13}C-induced splitting.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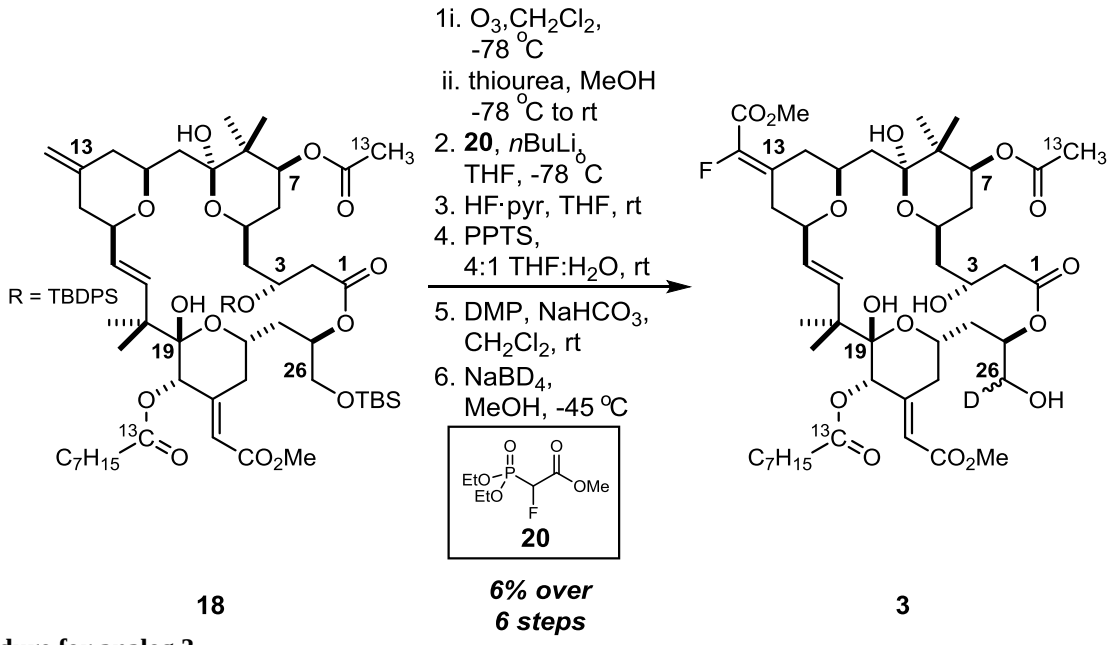


Procedure for analog 3

Approximately 50 mL dry CH_2Cl_2 in a dry flask under N_2 was cooled to -78 ºC before bubbling through ozone for ~5 min at 2 LPM (generated at 70 V). The resulting blue solution was assumed to be ~0.025 M O_3 in CH_2Cl_2. Olefin **18** (21.4 mg, 0.018 mmol) was dissolved in 815 µL dry CH_2Cl_2 under nitrogen and cooled to -78 ºC. The ozone solution (1.86 mL, ~0.047 mmol) was added in one portion via syringe. The reaction mixture was allowed to stir 5 minutes at -78 ºC, at which point starting material had been consumed. Methanol (2.68 mL) and thiourea (25.5 mg, 0.35 mmol) were added in one portion each before removing the cold bath and stirring at room temp for 24 hrs. The reaction was concentrated under a stream of nitrogen, and the crude residue was purified via flash chromatography over silica (12→16% ethyl acetate:hexanes, 2% increments), providing 17.7 mg of the C13 ketone. A similar method was applied to an additional 39.7 mg (0.033 mmol) of olefin **18**. Unfortunately, storage of this sample under vacuum even for <10 hrs led to significant decomposition. The two batches were re-purified and immediately moved into the next step.

Phosphonoacetate **20** (54.8 mg, 0.24 mmol) was dissolved in dry THF (1.4 mL) in a dry vial under an inert atmosphere and cooled to -78 ºC. *n*Butyl lithium (2.12 M in hexanes, 105 µL, 0.22 mmol) was added dropwise over 30 seconds. The mixture was allowed to stir 1.5 hrs at -78 ºC. The C13 ketone (29 mg, ~0.024 mmol) was dissolved in 700 µL dry THF in a separate dry vial under N_2 before cannulating into the HWE reagent solution. The transfer was quantified with two 350 µl portions of dry THF. The reaction mixture was stirred 1.5 hrs at -78 ºC before quenching with 3 mL sat. NH_4Cl and diluting with 5 mL ether. The phases were separated, and the aqueous phase was extracted three times with 5 mL portions of ether. The combined organic phases were dried over anhydrous Na_2SO_4, filtered to remove solids, and concentrated under vacuum. Flash chromatography over silica (12→16% ethyl acetate:hexanes, 2% increments) afforded the HWE product as a mixture of C13 isomers as a clear, colorless oil (24.5 mg)

The resulting C13 enoate ester mixture (24.5 mg, ~19 µmol) was dissolved in 7.6 mL dry THF in a Falcon tube flushed with nitrogen. HF·pyridine (1.9 mL) was added dropwise over a minute. The reaction mixture was stirred for 52 hrs to achieve full conversion. The reaction was quenched by pouring into 50 mL sat. $NaHCO_3$ and diluted with 50 mL ethyl acetate. The phases were separated, and the aqueous phase was extracted with two 50 mL portions of ethyl acetate. The combined organic phases were washed with 50 mL of 0.2 M HCl. The acidic aqueous phase was then extracted with two 50 mL portions of ethyl acetate. All of the organic phases were then combined, washed with 50 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated *in vacuo*. The crude orange solid (still a mixture of C13 isomers) was moved on without further purification.

The crude product from above was dissolved in 2.4 mL dry THF and 600 µL H_2O in a dry vial under a nitrogen atmosphere. PPTS (38 mg, 0.15 mmol) was added in one portion. The reaction mixture was stirred 36 hrs at room temp. Water (5 mL) and EtOAc (5 mL) were added, and the phases were separated. The aqueous phase was extracted four times with 5 mL portions of ethyl acetate. The combined organic phases were washed with 5 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated under vacuum. The crude residue was purified via flash chromatography over silica (30→80% ethyl acetate:pentane, 10% increments). The *des*-^{2}H version of labeled analog **3** (**21**) was obtained as a white solid (10.7 mg, 61.2%) along with 4.8 mg of what appears to be the *des*-TBS starting material. This byproduct was re-exposed to the above conditions (HF·pyr, THF then PPTS, THF:H_2O) to provide an additional 1.8 mg of the desired intermediate (12.5 mg total, 71.5% over two steps through the two passes). Both C13 diastereomers were still present at this point.

des-^{2}H labeled intermediate **21** (8.9 mg, 9.7µmol) was dissolved in 1.5 mL dry CH_2Cl_2 in a dry vial under an atmosphere of nitrogen. $NaHCO_3$ (4.0 mg, 49 µmol) and DMP (10.3 mg, 24 µmol) were added in one portion each. The reaction mixture was stirred 18 hrs at room temp before quenching with 1 mL sat. $Na_2S_2O_3$. This mixture was stirred vigorously for ~5 min until it was no longer cloudy. The biphasic mixture was further diluted with 500 µL sat. $NaHCO_3$, 500 µL brine, and 2 mL ethyl acetate. The phases were separated, and the aqueous phase was extracted with four 2 mL portions of ethyl acetate. The combined organic phases were washed with 2 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated under vacuum. The white solid (8.4 mg of the two C13 isomers) was moved on without further purification.

The crude C26 aldehyde was dissolved in 1.5 mL dry MeOH in a dry vial under N_2 then cooled to -45 ºC (MeCN/CO_2). $NaBD_4$ (4.1 mg, 98 µmol) was added in one portion. The reaction mixture was stirred 45 min at -45 ºC at which point the starting material was consumed by TLC analysis. The reaction was quenched with 1 mL sat. NH_4Cl then diluted with 1 mL H_2O and 2 mL ethyl acetate. The phases were separated, and the aqueous phase was extracted with 2 mL portions of ethyl acetate four times. The phases were separated, and the aqueous phase was extracted with four 2 mL portions of ethyl acetate. The combined organic phases were washed with 2 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated under vacuum. The crude residue was purified with flash chromatography over a silica pipet column (50→60% EtOAc:pentane). The desired product was further purified with reverse phase HPLC (70→100% MeCN:H_2O, 30 min run, product loaded with 6:3:1 mix of MeOH to MeCN to DMSO). The labeled analog **3** was obtained as a white solid (2.1 mg, 6.4% over six steps as a 1:1 mix of C26 epimers). The HPLC trace revealed the C13-C30 *Z*:*E* ratio of the final product to be 7.7:1 (assuming equivalent UV activity at 254 nm). Both were isolated as single entities, but there was not a sufficient amount of the *E* isomer to fully characterize.

*Characterization Data for analog **3**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 6.47 (s, 1H, C34), 6.18 (d, 1H, *J* = 15.9 Hz, C17), 5.71 (*app* d, 1H, *J* = 3.7 Hz, C20), 5.63 (s, 1H, C19-OH), 5.56-5.48 (m, 2H, C16, C25), 5.43 (dd, 1H, *J* = 11.7, 4.8 Hz, C7), 4.53 (d, 1H, *J* = 11.4 Hz, C3-OH), 4.48-4.39 (m, 2H, C15, C23), 4.28 (d, 1H, *J* = 13.4 Hz, C22), 4.18-4.07 (m, 3H, C3, C5, C11), 3.75-3.64 (m, 1.5H, C12, C26), 3.51-3.47 (m, 0.5H, C26), 3.39 (s, 3H, B-ring CO_2Me), 3.22 (s, 3H, C-ring CO_2Me), 2.90 (d, 1H, *J* = 13.4 Hz, C14), 2.71 (t, 1H, *J* = 12.0 Hz, C2), 2.66 (br s, 1H, C26-OH), 2.38 (d, 1H, *J* = 13.0 Hz, C22), 2.34 (t, 1H, *J* = Hz, C2), 2.23-2.10 (m, 3H, C10, C40), 1.98-1.88 (m, 1H, C12), 1.70 (d, 3H, $^1J_{CH}$ = 129.5 Hz, $^{13}CH_3$), 1.80-1.68 (m, 3H, C10, C14, C24), 1.63 (*app* t, 1H, *J* = Hz, C24), 1.63-1.53 (m, 2H, C41), 1.56-1.48 (m, 1H, C4), 1.46 (s, 3H, C18-Me), 1.47-1.41 (m, 1H, C6), 1.27 (s, 3H, C18-Me), 1.25-1.19 (m, 1H, C6), 1.25-1.11 (m, 8H, C42-C45), 0.99-0.94 (m, 1H, C4), 0.93 (s, 3H, C8-Me), 0.92 (s, 3H, C8-Me), 0.88 (t, 3H, *J* = 7.2 Hz, C46) ppm

^{13}C NMR (C_6D_6, 150 MHz): δ = 185.2, 172.8, 171.8 (C20 octanoyl ^{13}C label), 166.6, 162.0, 152.4, 142.3, 139.7, 131.4, 129.6, 120.4, 101.9, 99.6, 78.7, 74.8, 73.0, 71.5, 71.1, 68.8, 65.6, 65.1, 65.0, 51.4, 50.5, 42.3, 42.2, 39.5, 35.1, 34.7, 33.6, 33.5, 31.8, 31.7, 29.1,[6] 25.1, 22.7, 20.7, 20.6 (C7 acetoxy ^{13}C label), 19.5, 16.7, 14.0 ppm[7]

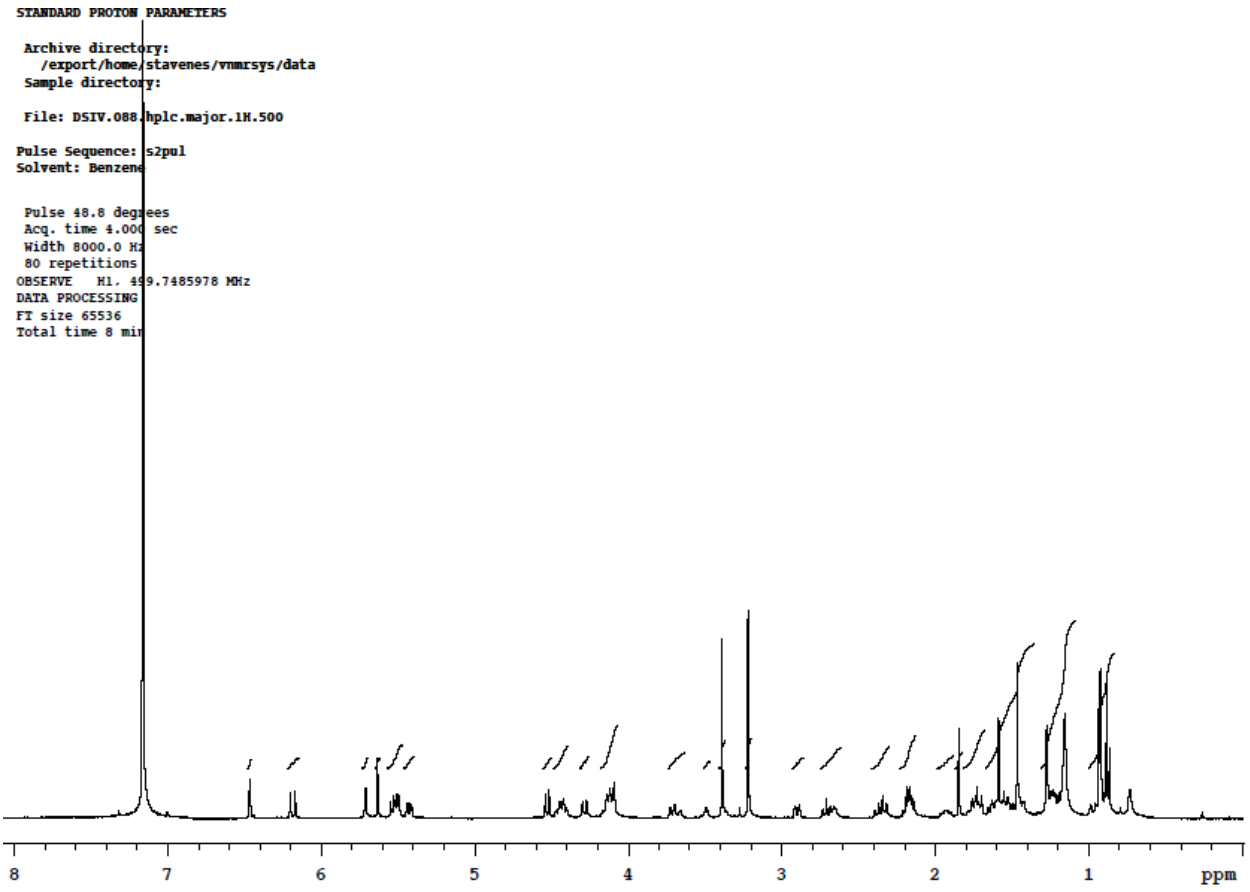
IR (thin film): 3464, 3343, 2928, 2848, 1725, 1437, 1362, 1312, 1245, 1156, 1098, 1079, 1003, 984, 736, 680 cm^{-1}

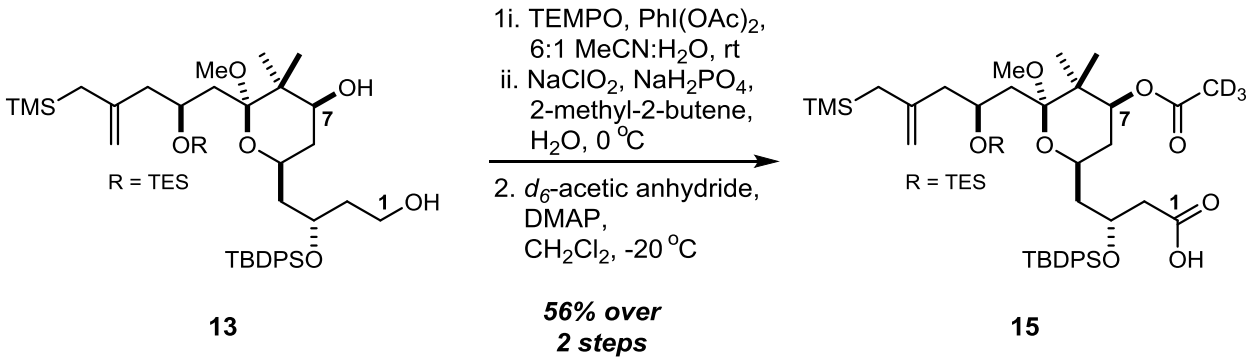
HRMS (ES+, *m/z)* calculated for $C_{44}\{^{13}C_2\}H_{68}DFNaO_{17}^+$: 938.4541, Found: 938.4565

[6] Suspected to be two unresolved peaks based on analogy to preceding intermediates.

[7] ^{13}C resonances obtained from HMBC and HSQC NMR data.

$[\alpha]_D^{23.3\,°C}$ = -19.7 ± 2.0° (*c* = 0.17, CH_2Cl_2)
R_f = 0.40 (60% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV





Procedure for C1 carboxylic acid 15

Diol **13** (27.1 mg, 0.0351 mmol) was dissolved in MeCN (1.9 mL) under ambient temperature. Water (0.3 mL) was added, followed by TEMPO (1.6 mg, 0.0105 mmol), then $PhI(OAc)_2$ (35.0 mg, 0.109 mmol). The resulting tan-brown solution was stirred at room temperature for 1 h and then cooled to 0 °C. Water (0.55 mL) was then added, followed by 2-methyl-2-butene (0.85 mL), NaH_2PO_4 (42.6 mg, 0.355 mmol), and $NaClO_2$ (19.7 mg, 0.218 mmol). The resulting solution was then stirred at 4 °C for 1.5 h and then quenched with saturated aqueous $Na_2S_2O_3$ (2 mL) and diluted with brine (1 mL). The aqueous layer was then extracted with Et_2O (4 x 4 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting crude material was used directly in the next step.

The crude material from the previous step was dissolved in CH_2Cl_2 (0.5 mL) in a small vial under N_2 and cooled to 0 °C. DMAP (5.1 mg, 0.418 mmol) was added in one portion followed by d_6-acetic anhydride (33.2 µL, 0.351 mmol). The solution was stirred on ice for 40 min and then diluted with saturated aqueous $NaHCO_3$ (0.5 mL) and water (0.5 mL). The reaction was warmed to ambient temperature and stirred vigorously overnight (16 h). The reaction was then diluted with saturated aqueous NH_4Cl (3 mL) and then extracted with Et_2O (3 x 6 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification via silica gel column chromatography (12→18% EtOAc:pentane, 2% increments) afforded northern fragment **15** (16.2 mg, 55.5% over two steps) as a clear, colorless oil.

*Characterization Data for C1 carboxylic acid **15**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 7.82 (t, 4H, *J* = 6.3 Hz, TBDPS), 7.29-7.24 (m, 6H, TBDPS), 5.41 (dd, 1H, *J* = 11.7, 4.7 Hz, C7), 4.79 (bs, 1H, C14), 4.69 (bs, 1H, C14), 4.39 (m, 2H, C3, C11), 3.41-3.39 (m, 1H, C5), 3.03 (s, 3H, C9-OMe), 2.70 (dd, 1H, *J* = 15.0, 3.8 Hz, C2), 2.62-2.58 (m, 1H, C2), 2.34 (dd, 1H, *J* = 13.1, 4.9 Hz, C12), 2.26 (dd, 1H, *J* = 12.6, 7.6 Hz, C12), 2.11 (dd, 1H, *J* = 15.8, 2.8 Hz, C10), 2.01 (m, 2H, C4, C10), 1.74-1.70 (m, 1H, C4), 1.63-1.58 (m, 2H, C30), 1.52-1.50 (m, 1H, C6), 1.38-1.36 (m, 1H, C6), 1.19 (s, 9H, TBDPS), 1.16 (s, 3H, C8-Me), 1.15 (s, 3H, C8-Me), 1.10 (t, 9H, *J* = 6.7 Hz, TES), 0.74 (q, 6H, *J* = 7.9 Hz, TES), 0.10 (s, 9H, TMS) ppm

^{2}H NMR (C_6D_6, 600 MHz): δ = 1.63 ppm.

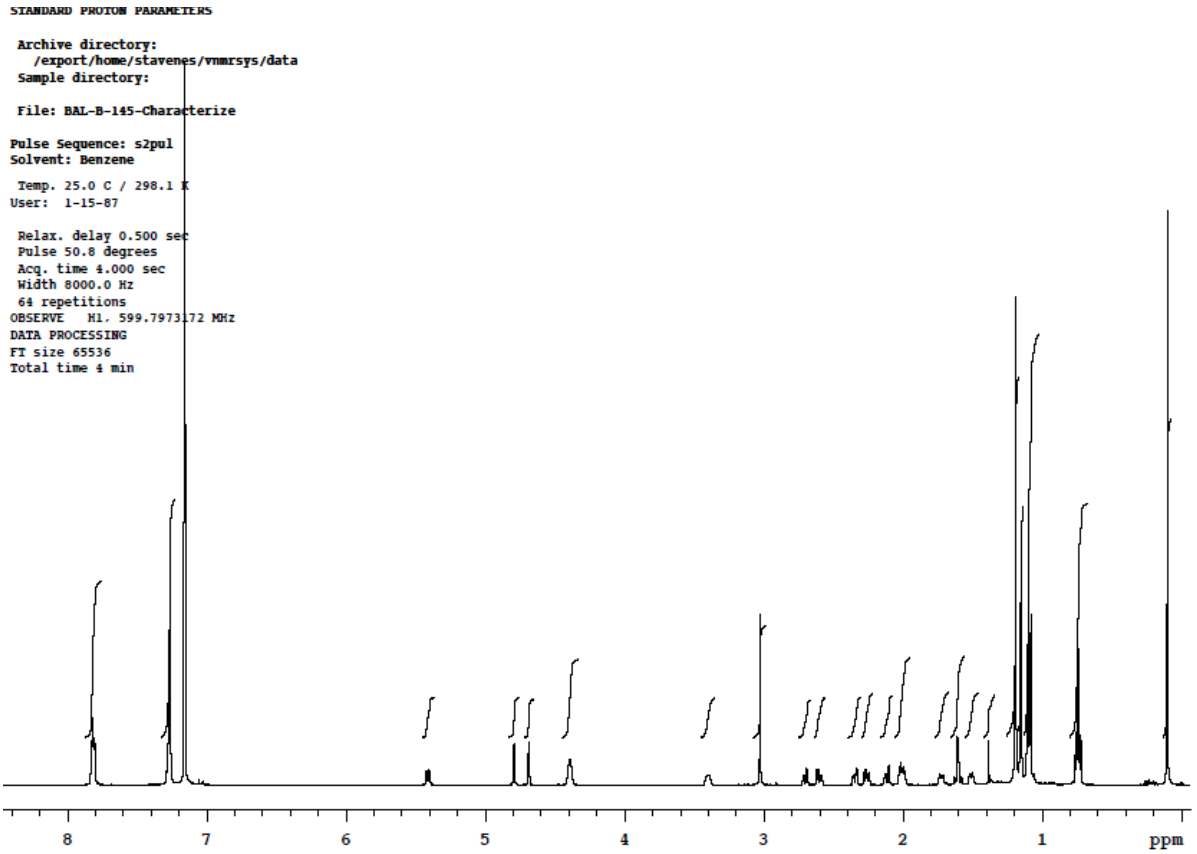
^{13}C NMR (C_6D_6, 125 MHz): δ = 176.7, 169.7, 144.7, 136.3, 136.3, 134.3, 133,7, 130.2, 130.1, 128.3, 128.1, 128.1, 127.9, 110.9, 104.6, 73.8, 69.4, 68.7, 66.2, 48.9, 48.3, 43.9, 42.8, 42.3, 40.1, 33.0, 27.5, 27.2, 21.1, 19.5, 17.5, 7.4, 6.0, -1.3 ppm

IR (thin film): 3072, 2954, 1738, 1713, 1427, 1250, 1185, 1111, 1075, 1005, 854, 739, 702 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{45}H_{71}D_3NaO_8Si_3^+$: 852.4772, Found: 852.4758

$[\alpha]_D^{23.2\,°C} = 18.1 \pm 1.2°$ (*c* = 0.74, CH_2Cl_2)

R_f = 0.20 (25% EtOAc in pentane), one purple spot, *p*-anisaldehyde + UV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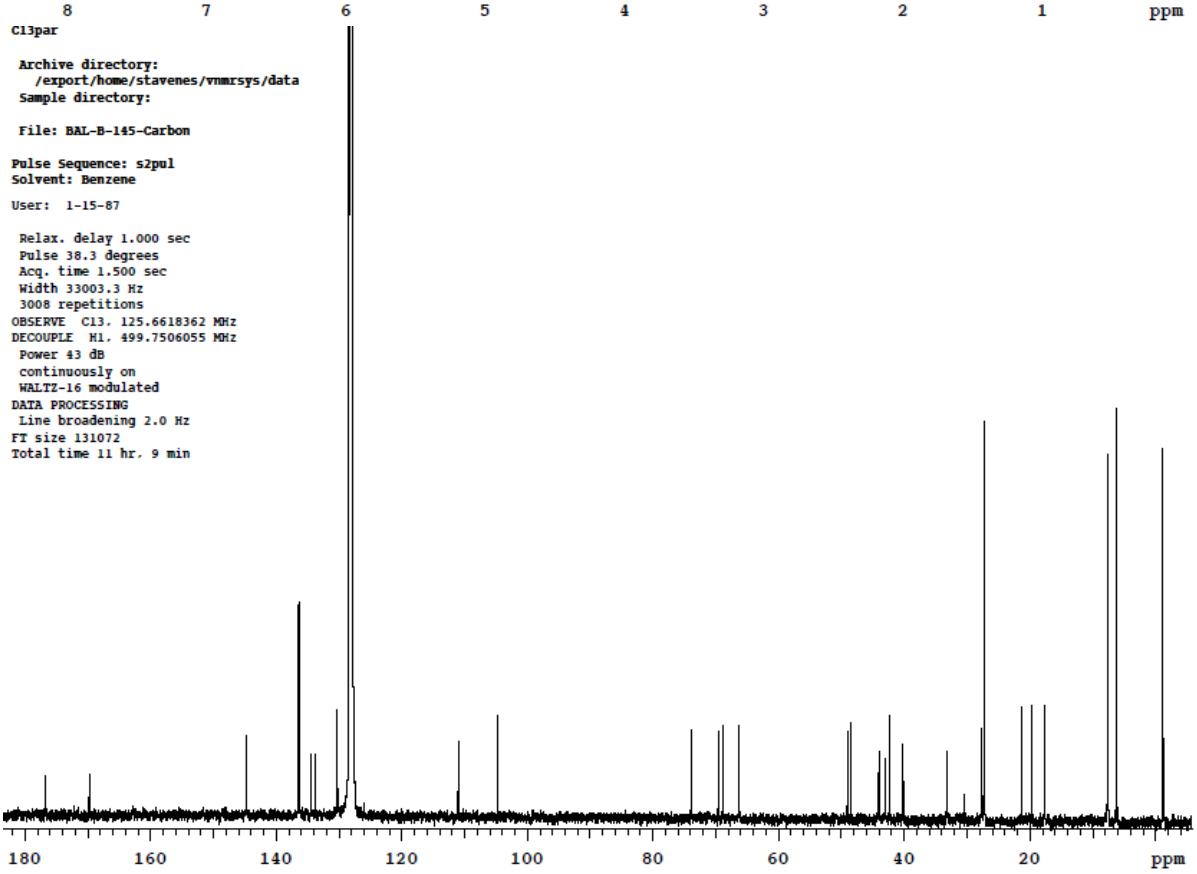
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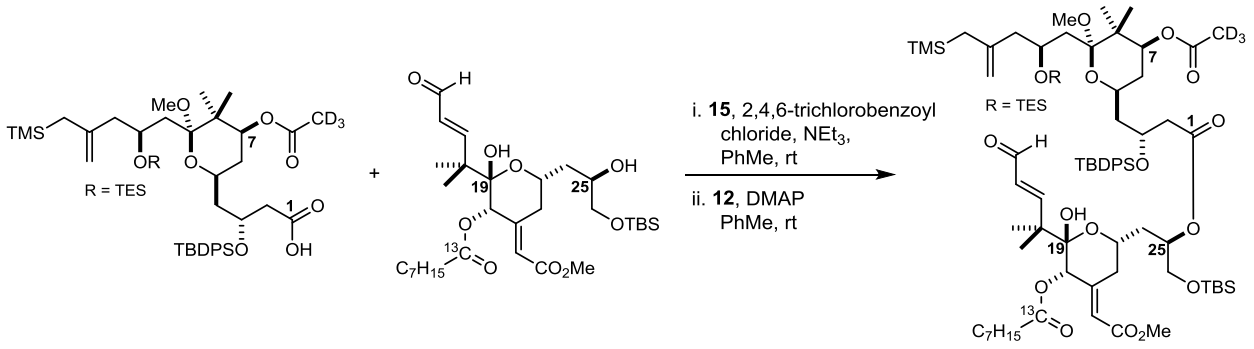


15 **12** ***78%*** **17**

Procedure for C1 ester 17

C1 carboxylic acid northern fragment **15** (81.0 mg, 0.0975 mmol) was dissolved in toluene (3.05 mL) in a small oven-dried vial equipped with under N_2. To this solution was added triethylamine (62.5 μL, 0.449 mmol), then 2,4,6-trichlorobenzoyl chloride (16.2 μL, 0.103 mmol). The resulting solution was stirred for 3.5 hrs at ambient temperature, and the formation of salts was observed. A toluene solution of southern fragment **12** (73.0 mg, 0.122 mmol) and DMAP (32.2 mg, 0.263 mmol) was then added in toluene (2.0 mL) via cannula (with 2 x 1.3 mL washes to ensure material transfer). The resulting solution was stirred for 45 min, at which time it was loaded directly onto a slurry-packed silica gel column. Purification by column chromatography (7→15% EtOAc to pentane, 2% increments) provided desired ester **17** (106.9 mg, 77.6%) as a pale yellow.

*Characterization Data for C1 ester **17**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 9.71 (d, 1H, *J* = 7.6 Hz, C15), 7.83-7.80 (m, 4H, TBDPS), 7.46 (d, 1H, *J* = 16.1 Hz, C17), 7.31-7.25 (m, 6H, TBDPS), 6.30 (d, 1H, *J* = 1.8 Hz, C34), 6.03 (dd, 1H, *J* = 16.1, 7.6 Hz, C16), 5.49 (d, 2H, *J* = 3.7 Hz, C20, C25), 5.34 (dd, 1H, *J* = 11.5, 4.7 Hz, C7), 4.80 (bs, 1H, C14), 4.70 (bs, 1H, C14), 4.38 (s, 2H, C3, C11), 4.14 (m, 2H, C22, C23), 3.66 (dd, 1H, *J* = 10.6, 5.5 Hz, C26), 3.59 (dd, 1H, *J* = 10.7, 5.5 Hz, C26), 3.54-3.47 (m, 1H, C5), 3.42 (d, 1H, *J* = 2.3 Hz, C19-OH), 3.28 (s, 3H, CO_2Me) 3.08 (s, 3H, C9-OMe), 2.64-2.57 (m, 2H, C2), 2.41-2.20 (m, 3H, C22, C12, C12), 2.09-1.95 (m, 6H, C4, C10, C10, C40, C40, C24), 1.76-1.64 (m, 2H, C4, C24), 1.61 (d, 2H, *J* = 2.9 Hz, C30), 1.58-1.54 (m, 1H, C6), 1.52-1.40 (m, 3H, C41), 1.26-1.20 (m, 9H, C6, C42-C45), 1.20-1.17 (m, 6H, Me, Me), 1.14 (m, 15H, Me, Me, TBDPS), 1.10 (t, 9H, *J* = 7.9 Hz, 9H, TES), 0.97 (s, 9H, TBS), 0.89 (td, 3H, *J* = 7.1, 4.1 Hz, C46), 0.77-0.72 (q, 6H, *J* = 8.0 Hz, TES), 0.10 (s, 9H, TMS), 0.09 (s, 6H, TBS) ppm

^{2}H NMR (C_6D_6, 600 MHz): δ = 1.64 ppm

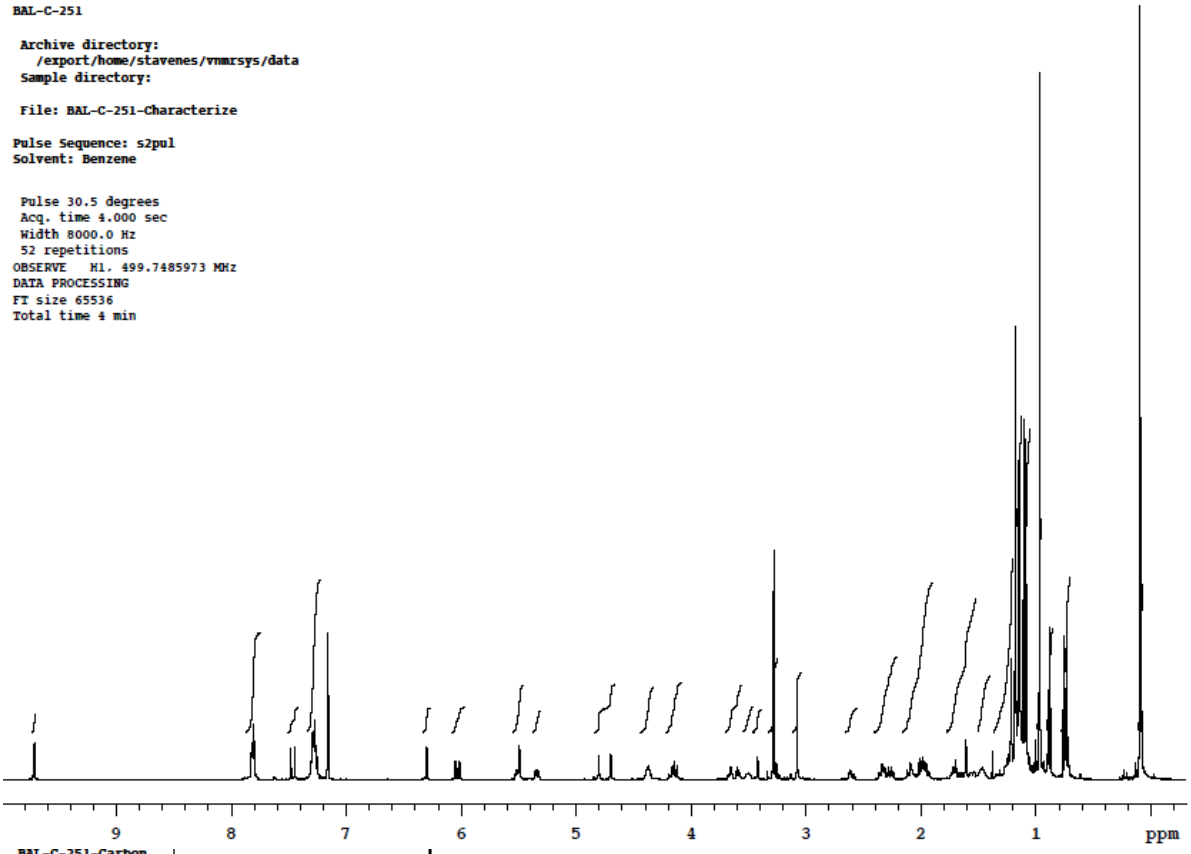
^{13}C NMR (C_6D_6, 125 MHz): δ = 193.3, 175.1, 173.9, 171.3 (^{13}C label), 171.1, 166.1, 164.8, 150.9, 144.7, 136.4, 136.2, 134.2, 133.8, 130.2, 130.1, 128.3, 128.1, 127.9, 121.3, 111.0, 104.6, 100.0, 74.2, 73.0, 71.8, 68.7, 66.6, 65.7, 65.3, 50.8, 48.9, 48.4, 45.9, 43.6, 42.4, 42.2, 40.1, 37.9, 34.9, 34.4, 32.9, 31.9, 31.5, 29.2, 29.2, 27.6, 27.2, 26.0, 24.8, 23.0, 22.9, 21.1, 20.3, 19.5, 18.5, 17.5, 14.3, 7.4, 6.0, -1.3, -5.1 ppm

IR (thin film): 3484, 3071, 2953, 2857, 1724, 1630, 1463, 1428, 1388, 1361, 1251, 1143, 1111, 1006, 983, 838, 778, 740, 726, 702 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{75}\{^{13}C_2\}H_{123}D_3NaO_{16}Si_4^+$: 1433.8237, Found: 1433.8273

$[\alpha]_D^{23.4\,°C}$ = -15.1 ± 0.4° (*c* = 1.0, CH_2Cl_2)

R_f = 0.60 (25% EtOAc in pentane), one purple spot, *p*-anisaldehyde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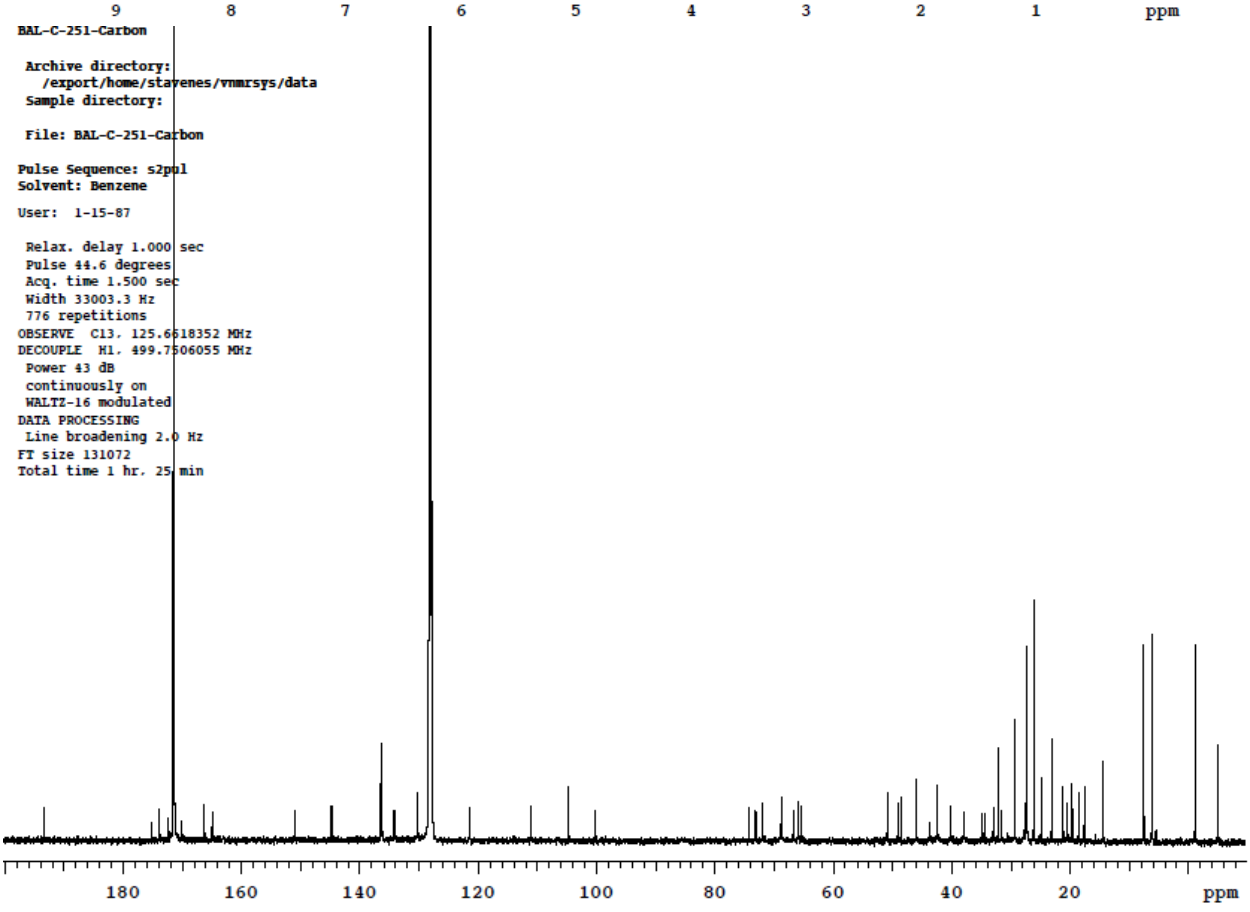
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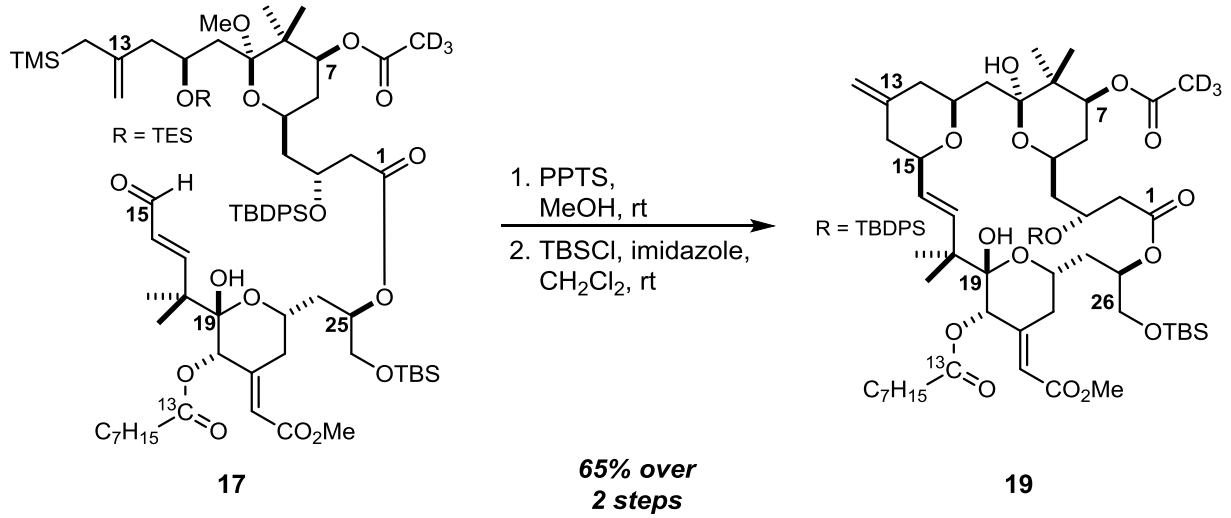

17 ***65% over 2 steps*** **19**

Procedure for C13 exocyclic olefin 19

Neat ester **17** (106.9 mg, 0.0757 mmol) was dissolved in a solution of pyridinium *p*-toluenesulfonate (PPTS) in anhydrous MeOH (4 mM, 3.78 mL, 0.0151 mmol). The reaction was stirred under ambient atmosphere for 21 h, at which time the solution was diluted with Et_2O (40 mL), H_2O (15 mL) and brine (15 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (4 x 40 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated to afford a crude residue that was used directly in the next step.

The crude material from the previous step was dissolved in CH_2Cl_2 (7.65 mL). Imidazole (77.3 mg, 1.14 mmol) was added, followed by TBSCl (1.0 M in CH_2Cl_2, 378 μL, 0.378 mmol). The colorless reaction mixture was stirred at rt for 2 h. The reaction became slightly cloudy during the course of the reaction. The reaction was diluted with sat. aq. NH_4Cl (25 mL) and extracted with Et_2O (4 x 40 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified via silica gel chromatography (7→10→13→16% EtOAc:pentane) to afford 59.5 mg olefin **19** (65.1% over two steps) as a clear, colorless oil.

*Characterization Data for C13 exocyclic olefin **19**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 7.73 (d, 2H, *J* = 3.2 Hz, TBDPS), 7.72-7.68 (m, 2H, TBDPS), 7.29-7.20 (m, 6H, TBDPS), 6.37 (d, 1H, *J* = 16.0 Hz, C17), 6.32 (d, 1H, *J* = 1.8 Hz, C34), 5.82 (dd, 1H, *J* = 15.9, 7.0 Hz, C16), 5.61 (m, 2H, C7, C20), 5.57-5.51 (m, 1H, C25), 4.82-4.75 (m, 3H, C3, C30, C30), 4.29 (ddd, 1H, *J* = 10.6, 7.5, 2.5 Hz, C15), 4.26-4.15 (m, 2H, C22, C23), 4.04 (*app* t, 1H, *J* = 10.4 Hz, C5), 3.68-3.64 (m, 1H, C11), 3.38-3.21 (m, 2H, C26), 3.13 (s, CO_2Me), 2.96 (s, 1H, C19-OH), 2.89-2.84 (m, 1H, C2), 2.71 (s, 3H, C9-OMe), 2.41-2.28 (m, 2H, C2, C22), 2.24-2.18 (m, 1H, C14), 2.17-2.02 (m, 4H, C10, C40, C40, C14), 2.02-1.95 (m, 1H, C12), 1.91-1.85 (m, 1H, C12), 1.71-1.57 (m, 4H, C4, C24, C24, C6), 1.57-1.44 (m, 3H, C41, C41, C10), 1.41-1.28 (m, 2H, C6, C4), 1.24 (s, 3H, Me), 1.22-1.16 (m, 2H, C45), 1.17 (s, 3H, Me), 1.14-1.08 (m, 18H, Me, Me, TBDPS, C42-C44), 1.06 (3H, Me), 0.88 (s, 9H, TBS), 0.86 (t, 3H, *J* = 7.3 Hz, C46), -0.05 (s, 3H, TBS), -0.9 (s, 3H, TBS) ppm

^{2}H NMR (C_6D_6, 600 MHz): δ = 1.64 ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 174.3, 171.8, 171.5 (^{13}C label), 170.3, 169.8, 166.3, 151.1, 145.3, 136.1, 135.3, 135.2, 134.8, 134.4, 130.0, 130.0, 128.3, 128.1, 121.0, 108.7, 103.3, 98.7, 78.5, 74.5, 74.0, 73.3, 70.1, 67.5, 67.5, 65.9, 65.6, 65.4, 64.7, 50.6, 47.9, 45.1, 45.0, 42.1, 41.7, 40.9, 39.7, 37.4, 34.9, 34.4, 33.9, 31.9, 31.7, 29.2, 29.2, 27.2, 6.0, 25.0, 24.3, 22.9, 20.7, 20.5, 19.4, 18.4, 17.7, 14.3, -5.3, -5.3 ppm

IR (thin film): 3510, 3071, 2931, 2857, 1735, 1473, 1465, 1430, 1388, 1364, 1254, 1161, 1143, 1105, 1003, 983, 914, 886, 838, 777, 740, 704, 663, 622, 613 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{66}\{^{13}C\}H_{99}D_3NaO_{15}Si_2^+$: 1229.6871, Found: 1229.6863

$[\alpha]_D^{22.3\,°C} = 11.7 \pm 0.2°$ (*c* = 1.1, CH_2Cl_2)

R_f = 0.40 (30% EtOAc in pentane), one purple spot, *p*-anisaldehyde + UV

STANDARD PROTON PARAMETERS

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Sample directory:

File: BAL-C-252-Characterize

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Solvent: Benzene

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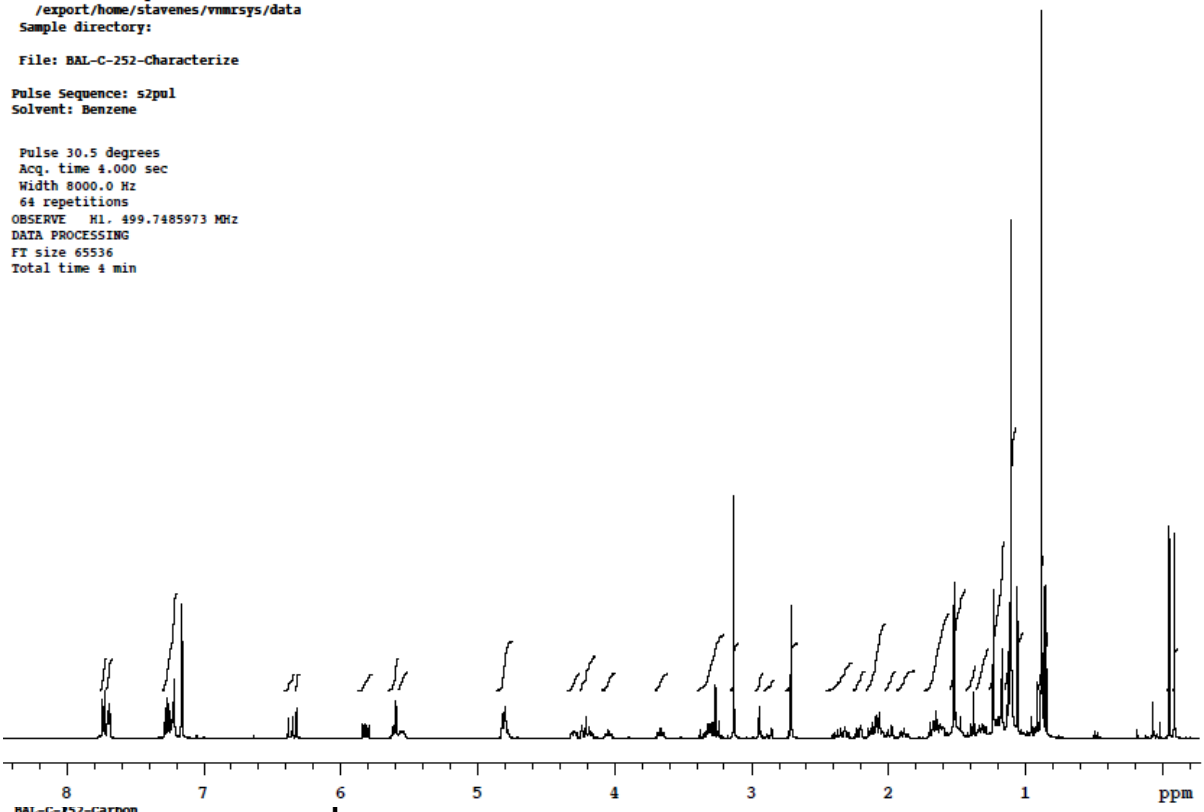

BAL-C-252-Carbon

Archive directory:
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Sample directory:

File: BAL-C-252-Carbon

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Solvent: Benzene
User: 1-15-87

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OBSERVE C13, 125.6618352 MHz
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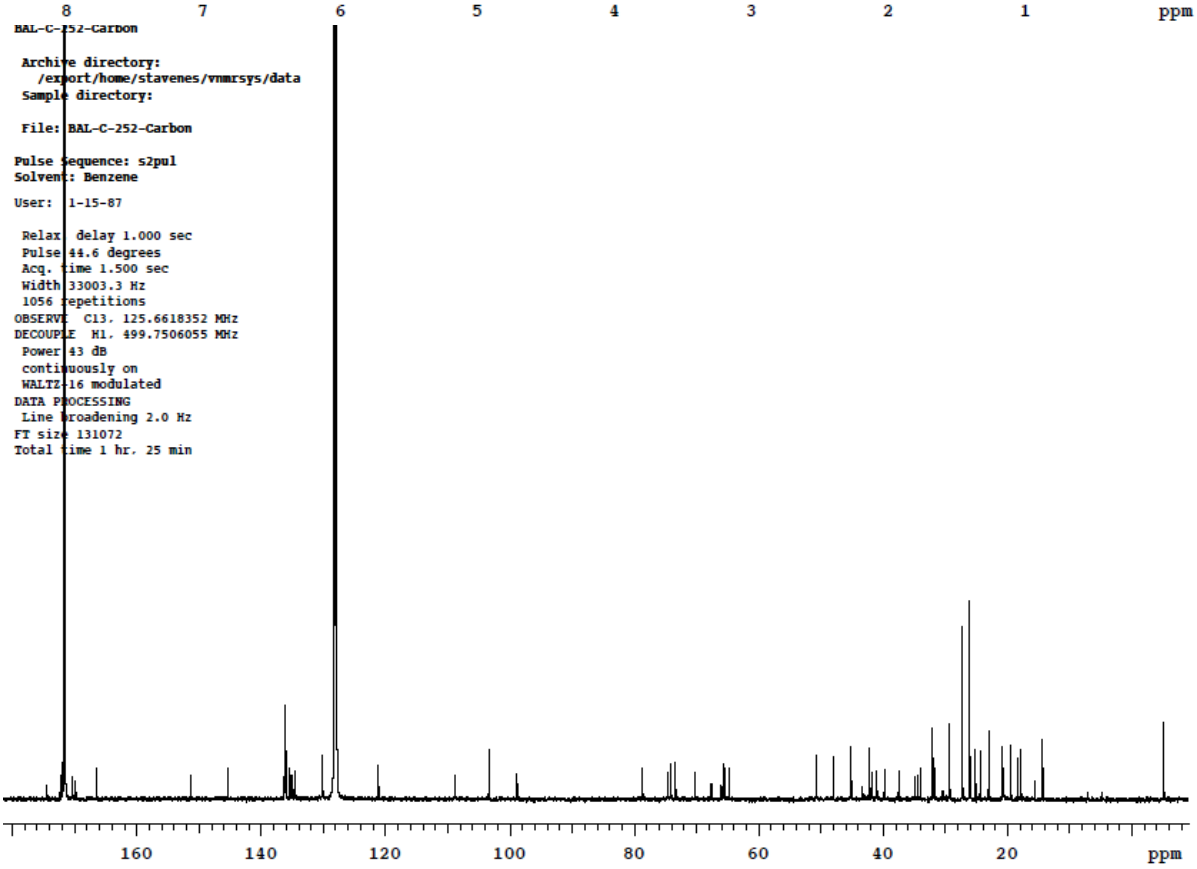

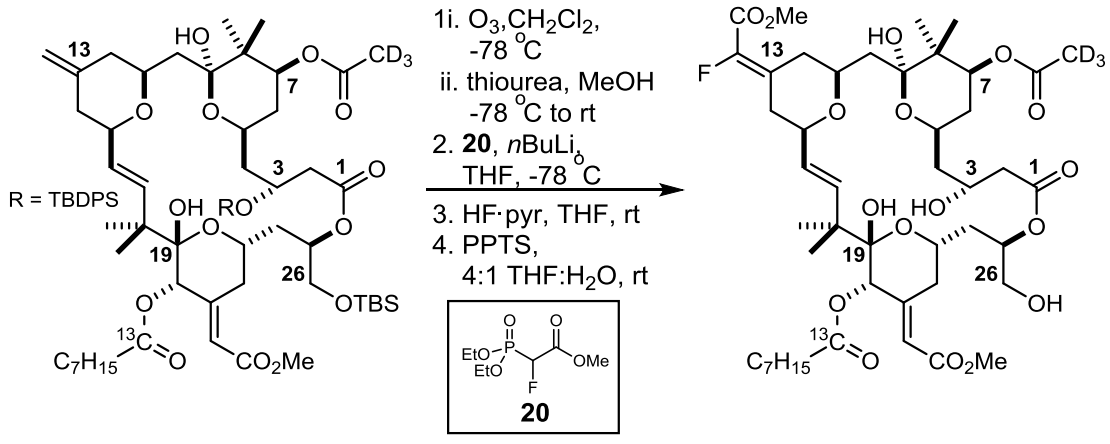

Procedure for analog 4

Approximately 50 mL dry CH_2Cl_2 in a dry flask under N_2 was cooled to -78 ºC before bubbling through ozone for ~3 min at 2.5 LPM (generated at 70 V). The resulting blue solution was assumed to be ~0.025 M O_3 in CH_2Cl_2. Olefin **19** (33.0 mg, 0.027 mmol) was dissolved in 1.0 mL dry CH_2Cl_2 under nitrogen and cooled to -78 ºC. The ozone solution (1.6 mL, ~0.041 mmol) was added in one portion via syringe. The reaction mixture was allowed to stir 5 minutes at -78 ºC, at which point starting material had been consumed as visualized by TLC. Thiourea (39 mg, 0.54 mmol) then dry methanol (2.6 mL) were added in one portion each before removing the cold bath and stirring at room temp for 18 hrs. The reaction was concentrated under a stream of nitrogen, and the crude residue was purified via flash chromatography over silica (5→20% ethyl acetate:pentane, 5% increments, residue loaded with PhMe), providing 22.5 mg of the C13 ketone.

Phosphonoacetate **20** (42 mg, 0.19 mmol) was dissolved in dry THF (900 µL) in a dry vial under an inert atmosphere and cooled to -78 ºC. *n*Butyl lithium (1.6 M in hexanes, 110 µL, 0.18 mmol) was added dropwise down the side of the vial over 30 seconds. The mixture was allowed to stir 1.5 hrs at -78 ºC. The C13 ketone (~0.019 mmol) was dissolved in 600 µL dry THF in a separate dry vial under N_2 before transferring into the HWE reagent solution down the side of the vial via syringe over 1 min. The transfer was quantified with two 200 µl portions of dry THF. The reaction mixture was stirred 1.5 hrs at -78 ºC before quenching with 2 mL sat. NH_4Cl and diluting with 1 mL ether. The phases were separated, and the aqueous phase was extracted four times with 2 mL portions of ether. The combined organic phases were washed with 2 mL brine, dried over anhydrous Na_2SO_4, filtered to remove solids, and concentrated under vacuum. Flash chromatography over silica (4→16% ethyl acetate:hexanes, 4% increments, crude residue loaded with PhMe) afforded the HWE product as a white solid (21.4 mg). Both the *E*- and the *Z*-isomers at C13 were isolated together, with their R_f values being different by only ~0.05. ^{19}F NMR of the mixture revealed two peaks at -130.5 and -130.7 ppm in a 1:5.5 ratio (later determined to be the *E*- and *Z*-isomers respectively).

The mix of enoate esters (~17 µmol) was dissolved in 5.6 mL dry, degassed THF in a Falcon tube flushed with nitrogen. HF·pyridine (1.25 mL) was added dropwise over a minute. The reaction mixture was stirred for 3 days to achieve full conversion. The reaction was quenched by pouring into 10 mL sat. $NaHCO_3$ and diluted with 10 mL ethyl acetate. The phases were separated, and the aqueous phase was extracted with three 10 mL portions of ethyl acetate. The combined organic phases were washed with 10 mL of 0.2 M HCl. The acidic aqueous phase was then extracted with two 10 mL portions of ethyl acetate. All of the organic phases were then combined, washed with 10 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated *in vacuo*. The crude solid (still a mixture of C13 diastereomers) was moved on without further purification.

The crude product from above was dissolved in 2.0 mL dry, degassed THF, followed by the addition of 0.5 mL water and 34 mg PPTS (0.13 mmol). The reaction mixture was stirred 24 hrs at room temp under an Ar atmosphere.

The reaction mixture was diluted with 2 mL water and 3 mL ether, then the phases were separated. The aqueous phase was extracted four times with 3 mL portions of ether. The combined organic phases were washed with 2 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated under vacuum. The crude residue was purified via pipet-sized flash chromatography over silica (30→70% ethyl acetate:pentane, 20% increments). The desired product was further purified with reverse phase HPLC (70→100% MeCN:H_2O, 30 min run, product loaded with 450 μL 2:1 MeOH:MeCN). This method was not sufficient to fully separate the C13 diastereomers, so the mixed fractions were exposed to a second HPLC run. A small portion of the desired *E*-isomer was still mixed with the *Z*-isomer, but this amount was considered negligible. Approximately 2 mg of mixture were obtained that was mostly *Z*-isomer. The pure labeled analog **4** was obtained as a white solid (5.8 mg, 23.2% over four steps).[8]

*Characterization Data for analog **4**:*
^{1}H NMR ($CDCl_3$, 600 MHz): δ = 5.98 (d, *J* = 1.9 Hz, 1H, C34), 5.77 (d, 1H, *J* = 15.8 Hz, C17), 5.35-5.31 (m, 1H, C25), 5.31 (dd, 2H, *J* = 15.9, 8.4 Hz, C16), 5.16 (dd, 1H, *J* = 11.8, 4.8 Hz, C7), 5.15 (s, 1H, C19-OH), 5.13 (*app* d, 1H, *J* = 3.4 Hz, C20), 4.25 (d, 1H, *J* = 12.3 Hz, C5), 4.16 (br s, 1H, C3), 4.06-4.00 (m, 2H, C15, C23), 3.84 (dd, 1H, *J* = 7.4, 3.1 Hz, C26), 3.83 (s, 3H, B-ring CO_2Me), 3.83-3.78 (m, 1H, C11), 3.69 (dd, 1H, *J* = 13.9, 2.1 Hz, C22), 3.68 (s, 3H, C-ring CO_2Me), 3.63 (dd, 1H, *J* = 12.2, 5.8 Hz, C26), 3.43 (d, 1H, *J* = 14.4 Hz, C12), 2.70 (d, 1H, *J* = 14.7 Hz, C14), 2.54-2.44 (m, 1H, C2), 2.35-2.22 (m, 3H, C2, C40), 2.11-1.96 (m, 5H, C10, C22, C4, C24, C12), 1.85-1.71 (m, 4H, C14, C24, C6, C12), 1.65-1.56 (m, 3H, C41, C4), 1.46 (*app* quart., 1H, *J* = 12.2 Hz, C6), 1.30-1.23 (m, 8H, C42-C45), 1.16 (s, 3H, C18-Me), 1.01 (s, 3H, C18-Me), 1.00 (s, 3H, C8-Me), 0.95 (s, 3H, C8-Me), 0.87 (t, 3H, *J* = 7.1 Hz, C46) ppm
^{13}C NMR ($CDCl_3$, 125 MHz): δ = 172.7,[9] 172.4 (^{13}C label), 171.2, 162.0 (J^2_{CF} = 34.4 Hz), 151.7, 141.9 (J^1_{CF} = 251.3 Hz), 139.6, 130.9 (J^2_{CF} = 14.1 Hz), 120.0, 101.9, 99.0, 78.6, 74.4, 73.0, 71.7, 71.0, 68.6, 65.9, 65.5, 64.8, 52.3, 51.3, 45.0, 42.4,[10] 42.1, 41.1, 39.8, 36.0, 35.0, 34.8, 34.5, 33.5, 31.8, 31.1, 29.1 (J^1_{CC} = 3.7 Hz), 29.0, 24.8, 24.7, 22.7, 21.2,[11] 19.8, 17.0, 14.2 ppm
IR (thin film): 3464, 3343, 2931, 1725, 1437, 1364, 1313, 1255, 1158, 1078, 984, 734 cm^{-1}
HRMS (ES+, *m/z)* calculated for $C_{45}\{^{13}C\}H_{66}D_3FNaO_{17}^+$: 939.4633, Found: 939.4650
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23.6\,^{\circ}C}} =$ -7.7 ± 0.5° (*c* = 0.60, CH_2Cl_2)
$\mathbf{R}_f$ = 0.40 (60% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV

[8] Final mass of compound determined by quantitative ^{1}H NMR with dimethyl terephthalate as the external standard and benzene as the internal standard.
[9] This resonance is suspected to be the C7-*d_3*-OAc carbonyl carbon, thus its broadened and misshapen appearance could arise from ^{2}H-induced splitting.
[10] This resonance is suspected to be the C7-*d_3*-OAc -CD_3 carbon, and thus is broad with low signal-to-noise.
[11] Suspected to be two unresolved peaks based on analogous scaffolds.

STANDARD PROTON PARAMETERS

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Sample directory:

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DATA PROCESSING
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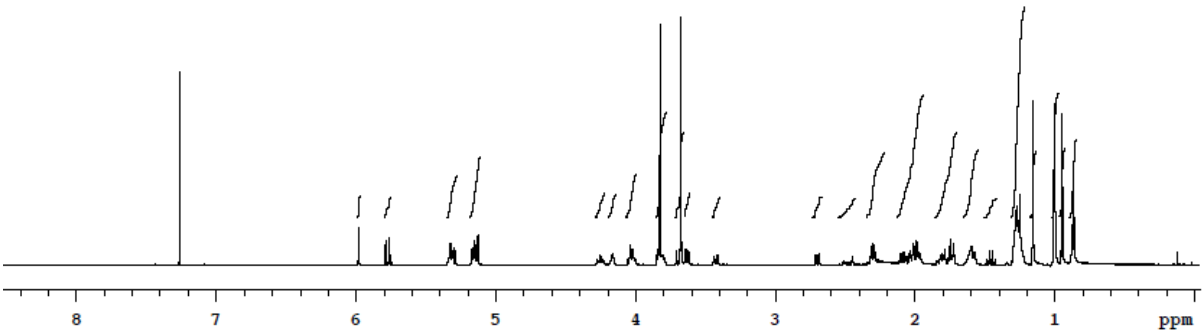

C13par

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User: 1-15-87

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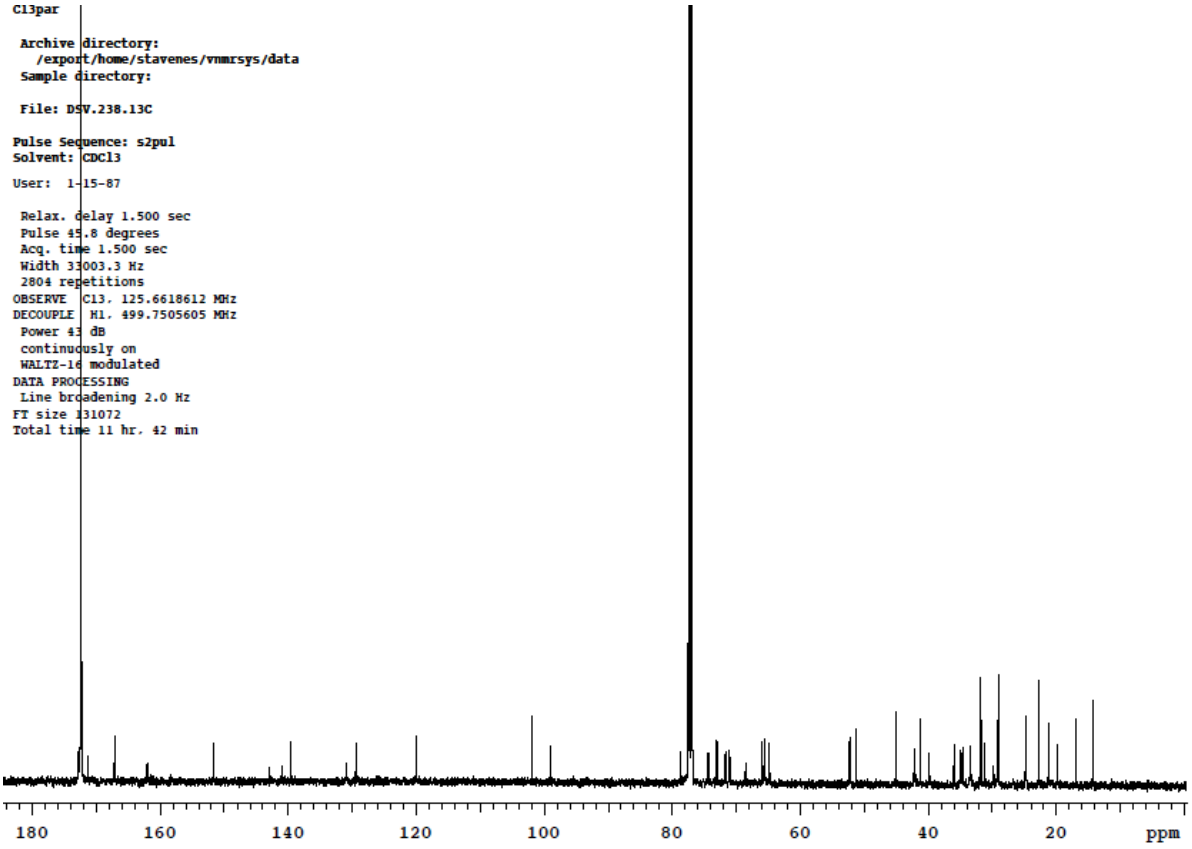

^{19}F NMR of diastereomeric mixture of HWE products:

```
Std Fluorine experiment

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 Sample directory:

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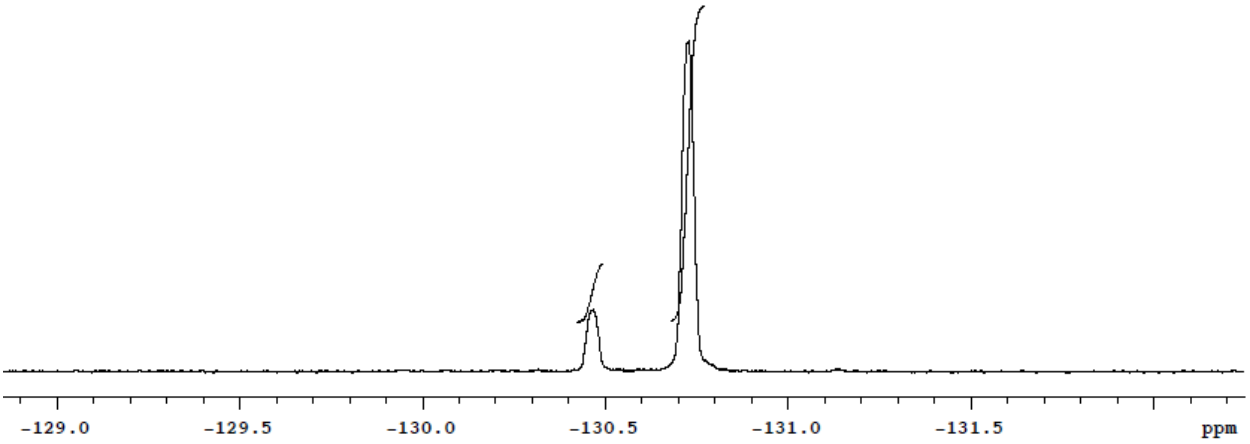


Procedure for exploring HWE diastereoselectivity in late-stage C13 olefination

As noted in the text, Horner-Wadsworth-Emmons olefination of the C13 ketone led to unexpectedly high diastereoselectivities. To try to understand this observation, a small screen of conditions was run on the C13 ketone intermediate **S1** (obtained through ozonolysis of **19**, *vide infra*).

Generation of ylide solutions: Phosphonoacetate **20** was dissolved in dry THF to generate a 0.5 M stock solution. From this stock, two 100 μL aliquots (0.05 mmol) were transferred into separate vials under inert atmosphere. One aliquot was diluted with 400 μL dry THF and cooled to -78 ºC before adding 20 μL *n*-BuLi (2.5 M in hexanes, 0.05 mmol) down the side of the vial via syringe to generate the lithium-based ylide. The second aliquot was diluted with 350 μL dry THF and cooled to -78 ºC before adding 50 μL NaHMDS (1.0 M in THF, 0.05 mmol) down the side of the vial via syringe to generate the sodium-based ylide. Both solutions were stirred 1.5 hrs at -78 ºC before use and were assumed to be 0.1 M with respect to the ylide.

Four separate vials, each containing 2.3 mg of C13 ketone **S1** (1.9 μmol) under an atmosphere of nitrogen, were diluted with 380 μL dry THF. Two vials were cooled to -78 ºC, two were cooled to -10 ºC (brine/ice). One vial at each temperature received the 0.1 M Li-derived ylide solution, likewise for the 0.1 M Na-based ylide solution. Each ylide stock (95 μL, 9.5 μmol) was added via syringe down the side of the vial over the course of 15 seconds to the solutions of **S1**. These reactions were stirred 1.5 hrs at their respective temperatures, each being complete as visualized by TLC with the exception of the Li-based olefination at -78 ºC which required 2.5 additional equivalents of ylide and an additional hr of reaction time. All reactions were quenched and purified as follows. The reaction mixture was quenched with 1 mL of sat. NH_4Cl and diluted with 1 mL ether. The phases were separated, and the aqueous phase was extracted four times with 1 mL portions of ether. The combined organic phases were washed with 1 mL brine, dried over anhydrous sodium sulfate, filtered to remove solids, and concentrated under vacuum. The crude residue was further purified via pipet-scale flash chromatography over silica (10→20% ethyl acetate:pentane), although both the *E*- and *Z*-isomers at C13 were intentionally collected together despite having ~0.05 difference in R_f value by TLC. While it was difficult to cleanly obtain diastereomeric ratios from ^{1}H NMR, ^{19}F NMR of the mixtures showed only two peaks that reliably fell at -130.5 and -130.7 ppm,[12] assumed to be the C13 α-fluoro enoate ester diastereomers. Integration of these peaks provided the ratios seen below (Figure S5).

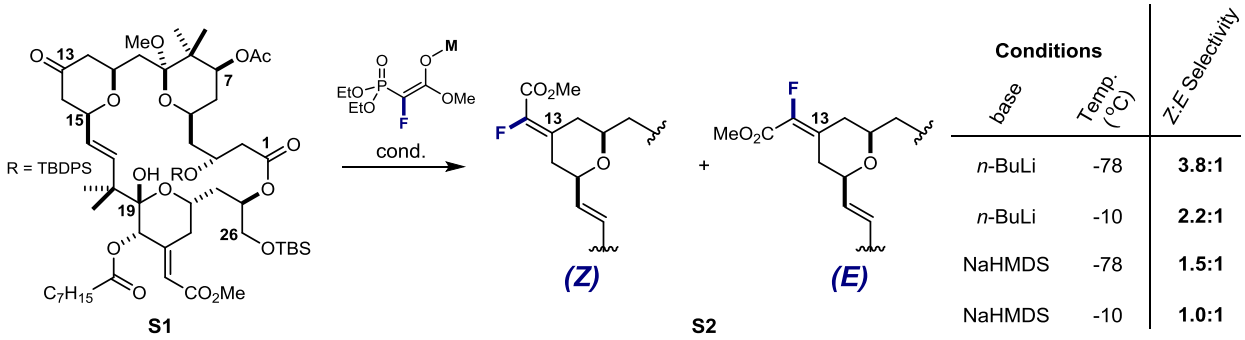


Figure S6. Olefination selectivities as a function of temperature and counterion.

^{1}H and COSY NMR data was used to determine which isomer was favored, specifically focusing on the location of the C12 and C14 resonances. 4-Alkylidenyl tetrahydropyran moieties show distinct anisotropic interactions between the olefin substituents and the equatorial hydrogens in the 3- and/or 5-positions. As seen in the bryostatin C-ring, methyl enoate esters will cause the C22 hydrogens to be split nearly 2 ppm from each other, with the equatorial resonance being significantly downfield (e.g. 3.69 and ~2.08 ppm for **4**). An α-fluoro enoate ester has previously been demonstrated[13] to display similar effects, with the methyl ester side maintaining a comparable ~3.5 ppm shift at the equatorial position, but the fluoro-substitute side displays a smaller anisotropic effect, only shifting the equatorial resonance up to ~2.8 ppm. Gratifyingly, all olefinated bryostatin-based products described in this report matched these expected shifts, and the *E* vs. *Z* geometry could be assigned based on whether C12 or C14 had a proton that was observed around 3.5 ppm. The shifts for the olefinated product detailed above (**S2**) are shown in Figure S7.

[12] Only the products of the Li ylide olefination at -10 ºC were different, being observed at -130.6 and -130.9 ppm.
[13] Abraham, R. *J. Chem. Soc. Perkins Trans. II* **1987**, 977-985.

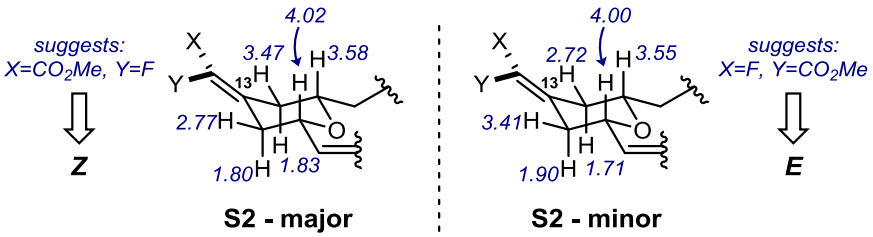


Figure S7. Distinguishing resonances allowing for determination of C13 olefin geometry. Proton assignments made with ^{1}H and COSY NMR.

As was detailed in the text, the selectivities observed in this small screen of conditions suggest that the diastereoselectivity is kinetically controlled and that the lithium counterion is playing a functional role that sodium cannot effectively mimic. While this information does not necessarily explain the selectivity, it does fall in line with the posited directing effect from the C9-OMe, presumably through a Li salt bridge (though other explanations are possible). Supporting the involvement of C9 functionality, prior efforts within the group[14] had demonstrated that different geometries at C9 could, in fact, lead to minor diastereoselective control at C13 (see Figure S7). This control was minimal and not easily predicted, thus chiral phosphonoacetates were ultimately employed to control the olefin geometry in our total synthesis of bryostatin 9.[15,16] While the reactions seen below are only two additional examples, it does seem to point to a role being played by C9, and given that these olefinations were run with the least selective conditions (Na counterion, on ice), it is possible that an even larger effect could have been observed on these substrates.

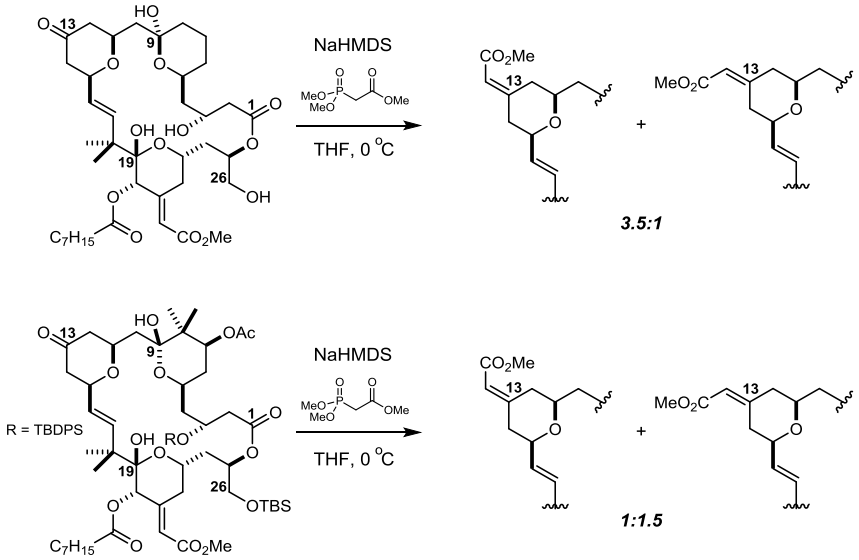


Figure S8. Prior observations potentially supporting the role of C9 functionality in controlling C13 olefin geometry

Also worth noting, the olefination run en route to **3** and **4** appeared to give higher selectivities than the analogous conditions in Figure S5. It is assumed that this discrepancy is the result of more effective cooling in the larger scale reactions. For instance, **4** was prepared on ~10-fold larger scale, allowing for slower addition times and more reliable cooling of the ylide solution during delivery into the reaction mixture. While the ylide solution remained cold up until being taken into the syringe, the small volume likely warmed up relatively quickly, and perhaps did not completely re-cool to -78 °C before reaching the reaction mixture. The data in Figure S6 suggests that even transient heating of the reaction mixture could abrogate selectivity, and this likely led to slightly lower

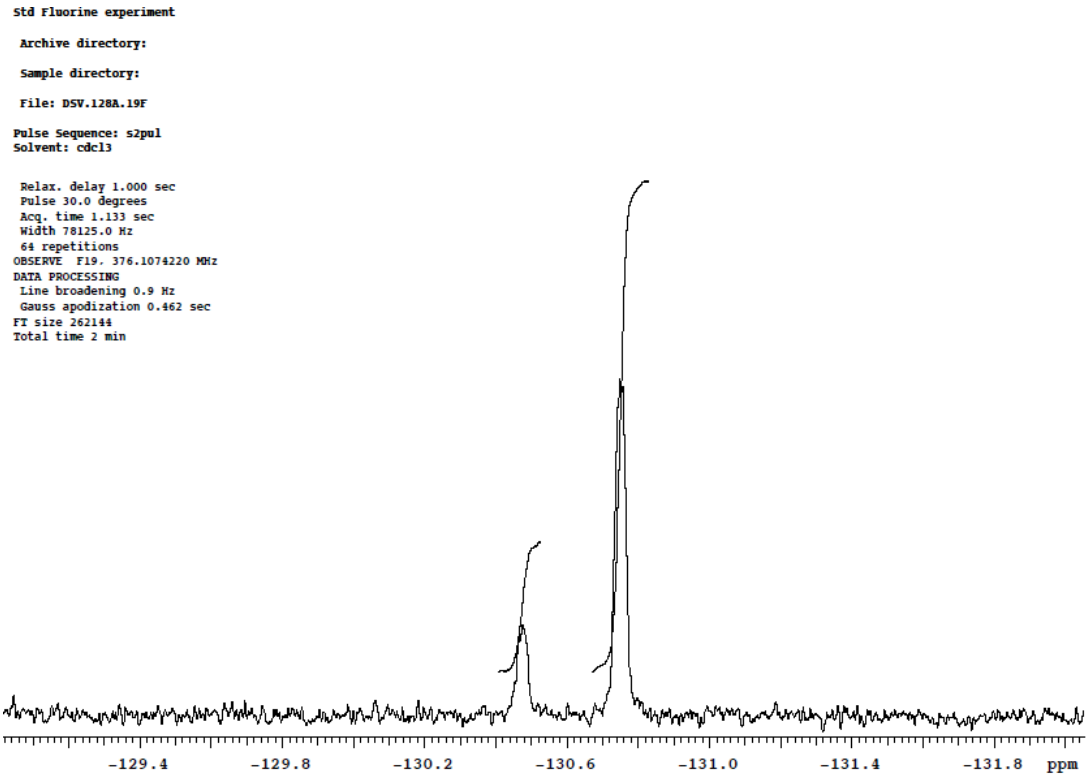
[14] Thesis of Adam Schrier, **2011**. Stanford University, Department of Chemistry. Wender Group.

[15] Wender, P.; Schrier, A. *J. Am. Chem. Soc.* **2011**, *133*, 9228-9231.

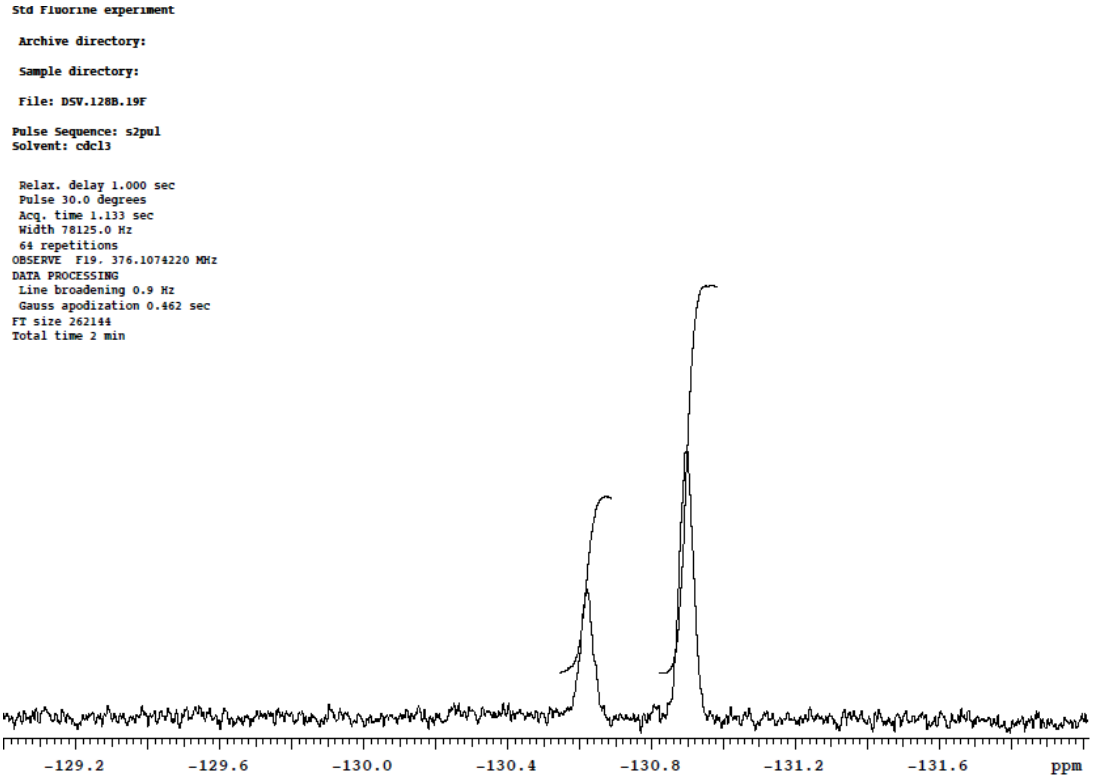
[16] Minimal diastereoselective control was also observed by other groups attempting HWE olefinations of various C13 ketone intermediates en route to bryostatins. See references 39 and 40 in the main text for more detail.

selectivities in the small scale screen than would have been observed had the conditions been run on the same scale as the analog syntheses.

Li ylide, -78 ºC:



Li ylide, -10 ºC:



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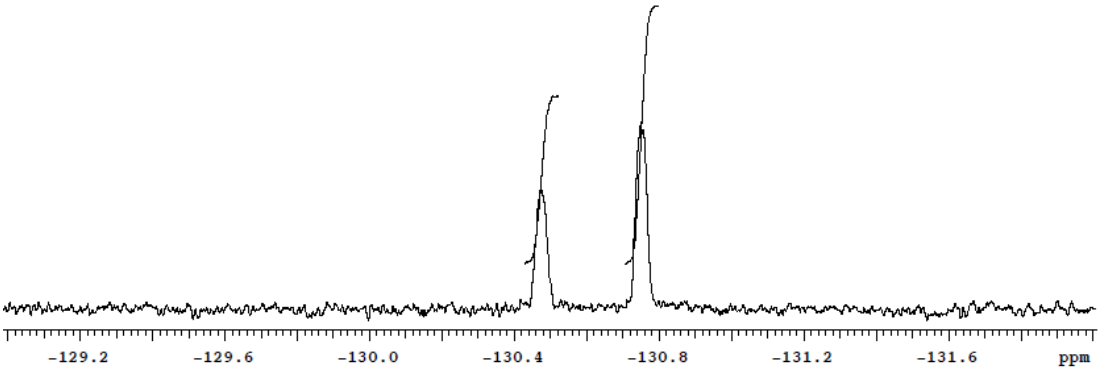

Na ylide, -10 ºC:

Std Fluorine experiment

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DATA PROCESSING
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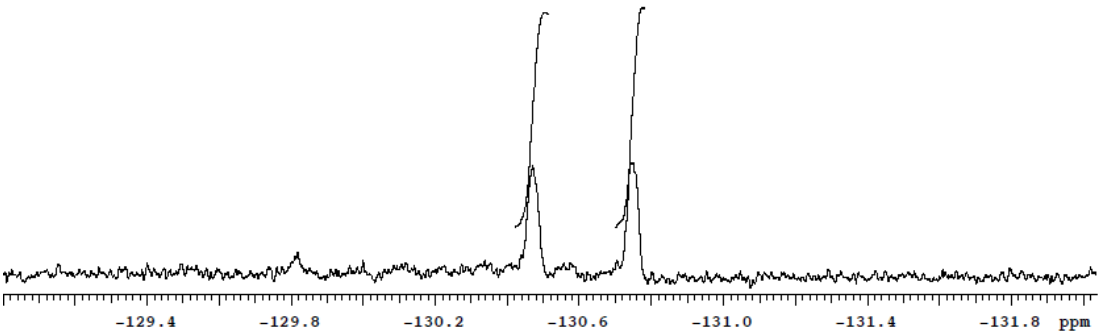
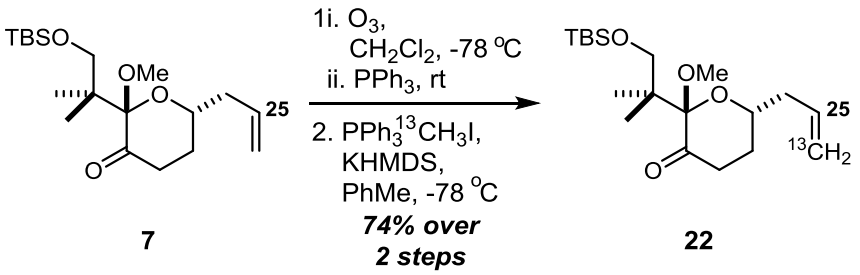




7 **22**

Procedure for labeled olefin 22

An oven dried flask was charged with stir bar and ketone **7** (107 mg, 0.30 mmol). Solvent (DCM, 17 mL) was added and the flask cooled to -78 °C in a dry ice/acetone bath. Ozone was introduced into the cold stirring solution via bubbling through the solution (via glass pipette at a rate of ~2 L/min) until the solution turned blue (~30 seconds). The stream of ozone was immediately replaced with a stream of nitrogen bubbles until no blue color remained (~2 minutes). Triphenylphosphine (221 mg, 0.84 mmol) was then added to the cold solution and the flask was allowed to come to room temperature with stirring. TLC analysis indicated the completion of the reaction at 2.5 hrs. The reaction solution was concentrated to a minimum solvent volume (ca 1 mL) and was loaded directly onto a pre-packed silica column. This was followed by chromatography (25→45% Ethyl acetate / Pet. Ether) to isolate the intermediate aldehyde spot (108 mg, 0.30 mmol, quant. yield). This intermediate aldehyde was used directly in the next step.[17]

An oven-dried flask was charged with stirbar and oven-dried triphenylphosphonium iodide[18] (^{13}C-labeled, 121 mg, 0.298 mmol). The flask was flushed with N_2 followed by addition of 1.5 mL of toluene. The mixture was cooled to 0 °C in an ice bath followed by the addition of KHMDS (597 µL, 0.298 mmol, 0.5 M in toluene, fresh bottle) dropwise over 2 min. The resulting yellow solution of ylide was allowed to stir at 0°C for 20 min before warming to rt for 10. This solution was then added via syringe (dropwise, 5 min) to a stirring solution of the intermediate aldehyde (107 mg, 0.298 mmol, 1.0 eq) at -78 °C in toluene (1.5 mL). The resulting deep red solution was allowed to stir at -78 °C for 1.5 h. The cold bath was removed and the reaction was allowed to warm to rt and stir for 30 minutes before re-cooling and quenching at -78 °C with 1 mL saturated $NH_4Cl_{(aq)}$. The solution was warmed to rt with stirring before dilution with H_2O and Et_2O (5 mL each). The phases were separated, and the aqueous layer was extracted with three 5 mL portions of ether. The combined organic phases were dried over $MgSO_4$, filtered to remove solids, and concentrated under vacuum. The crude residue was purified via column chromatography over silica (2%→4% ethyl acetate:petroleum ether) to provide the ^{13}C-labeled olefin **22** as a clear, colorless oil (78.4 mg, 74% over two steps).

*Characterization Data for labeled olefin **22**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.90 (ddt, 1H, *J* = 17.1, 10.0, 7.1 Hz, C25), 5.30-5.24 (m, 1H, C26), 4.99-4.93 (m, 1H, C26), 3.95-3.90 (m, 1H, C23), 3.68 (d, 1H, *J* = 9.9 Hz, C17), 3.32 (d, 1H, *J* = 9.9 Hz, C17), 3.26 (s, 3H, CO_2Me), 2.65 (ddd, 1H, *J* = 18.1, 6.8, 3.6 Hz, C21), 2.43-2.29 (m, 3H, C21, C22), 1.96-1.87 (m, 2H, C24), 1.02 (s, 3H, C18-Me), 0.92 (s, 3H, C18-Me), 0.86 (s, 9H, TBS), 0.01 (s, 3H, TBS), 0.01 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 207.1, 134.1 ($^1J_{CC}$ = 69.6 Hz), 117.8 (^{13}C label), 103.5, 73.2 ($^2J_{CC}$ = 3.5 Hz), 68.4, 52.0, 45.9, 40.4, 37.7, 28.4, 26.1, 20.2, 20.0, 18.6, -5.4, -5.4 ppm

IR (thin film): 2953, 2929, 2857, 1725, 1621, 1472, 1392, 1361, 1253, 1188, 1087, 1051, 1004, 963, 938, 907, 834, 775, 734, 668 cm^{-1}

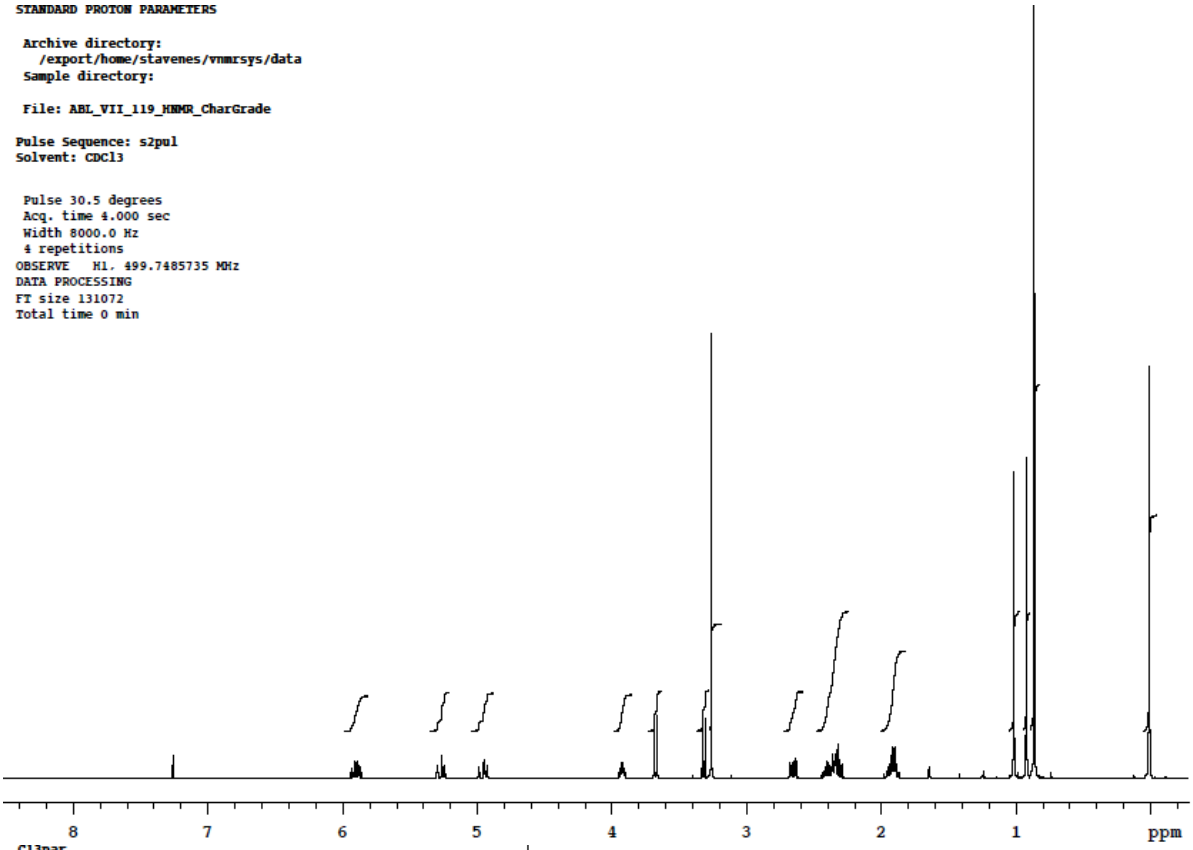
HRMS (ES+, *m/z)* calculated for $C_{18}\{^{13}C\}H_{36}O_4SiNa^+$: 380.2314, Found: 380.2307

$[\alpha]_D^{24.6\,°C} = 4.1 \pm 0.1°$ (*c* = 3.8, CH_2Cl_2)

R_f = 0.50 (10% EtOAc in petroleum ether), one black spot, *p*-anisaldehyde

[17] The C25 aldehyde has previously been characterized: Wender, P.; Schrier, A. *J. Am. Chem. Soc.* **2011**, *133*, 9228.

[18] The labeled triphenylphosphonium iodide was dried in a high-vacuum oven at 80 ºC overnight prior to use as described here: Kawasaki, T.; Matsumura, Y.; Tsutsumi, T.; Suzuki, K.; Ito, M.; Soai, K. "Asymmetric autocatalysis triggered by carbon isotope (13C/12C) chirality." *Science* **2009**, *324*, 492-495.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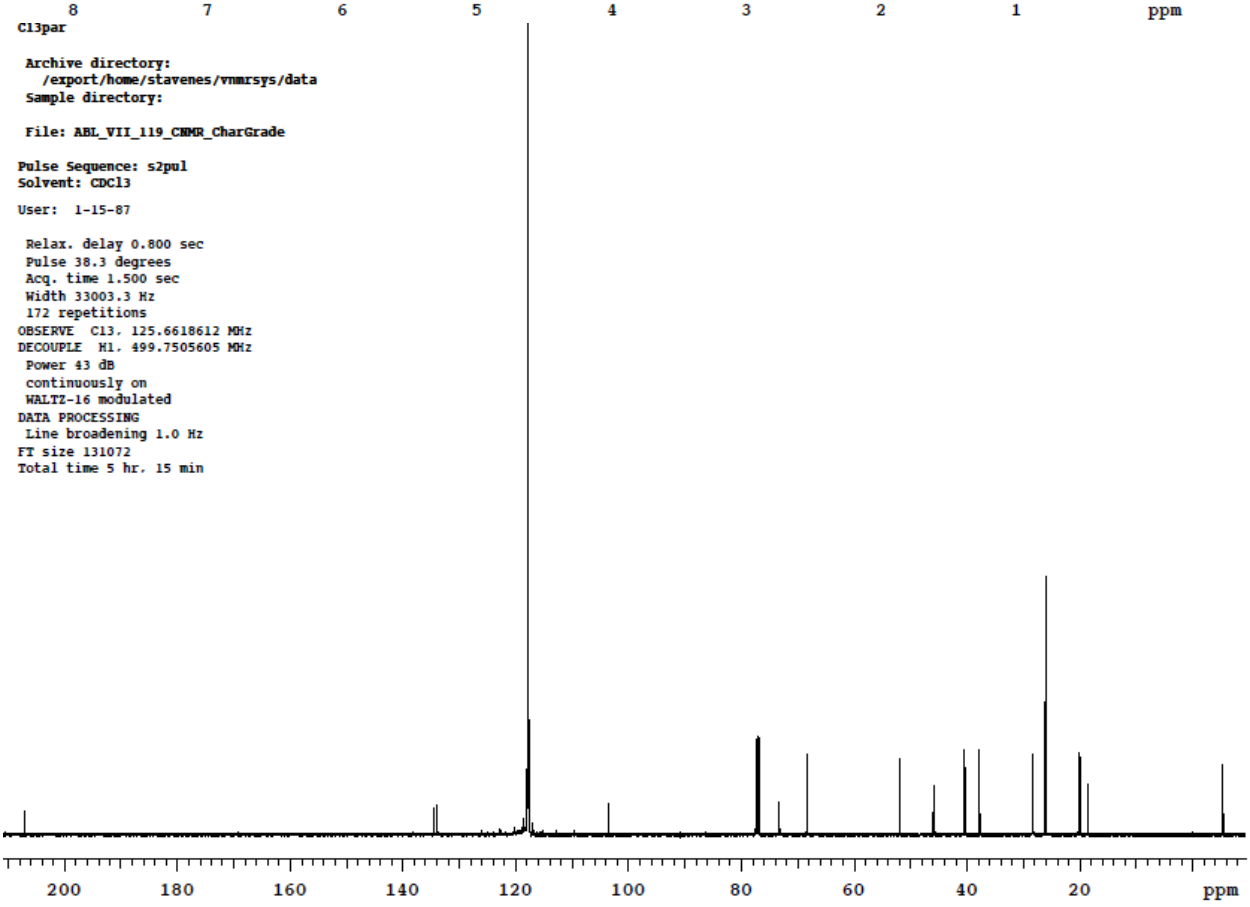
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Solvent: CDCl3
User: 1-15-87
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Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
172 repetitions
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DECOUPLE H1, 499.7505605 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
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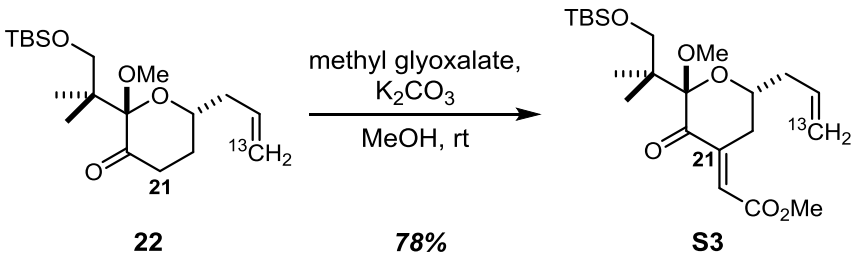


22 ***78%*** **S3**

Procedure for C21 methyl enoate ester S3

An oven-dried vial was charged with stir bar, precursor **22** (34 mg, 0.095 mmol), MeOH (0.9 mL), and methyl glyoxylate (240 µL of a 2.0M solution in THF, 0.475 mmol). To this stirring mixture was added K_2CO_3 (72 mg, 0.523 mmol). The resulting mixture was stirred at rt for 40 min and then quenched with saturated NH_4Cl (3 mL) and Et_2O/Pentane (1:1 mixture, 3 mL). The organic layer was collected and the aqueous layer was extracted with Et_2O/Pentane (1:1 mixture, 4 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to an orange oil which was purified by column chromatography (silica gel, 5→6% EtOAc:pet. ether) to yield the product as a bright yellow oil (31.6 mg, 0.074 mmol, 78% yield).

*Characterization Data for C21 methyl enoate ester **S3**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 6.93 (dd, 1H, *J* = 3.3, 1.5 Hz, C34), 5.86-5.77 (m, 1H, C25), 5.19-5.14 (m, 1H, C26), 4.87-4.83 (m, 1H, C26), 3.89 (d, 1H, *J* = 9.9 Hz, C17), 3.66-3.60 (m, 1H, C23), 3.53 (d, 1H, *J* = 9.9 Hz, C17), 3.48 (dt, 1H, *J* = 17.7, 1.7 Hz, C22), 3.33 (s, 3H, CO_2Me), 3.11 (s, 3H, C19-OMe), 2.78 (ddd, 1H, *J* = 17.9, 12.4, 3.3 Hz, C22), 2.15-2.05 (m, 2H, C24), 1.28 (s, 3H, C18-Me), 1.14 (s, 3H, C18-Me), 0.92 (s, 9H, TBS), 0.02 (s, 3H, TBS), 0.01 (s, 3H, TBS) ppm

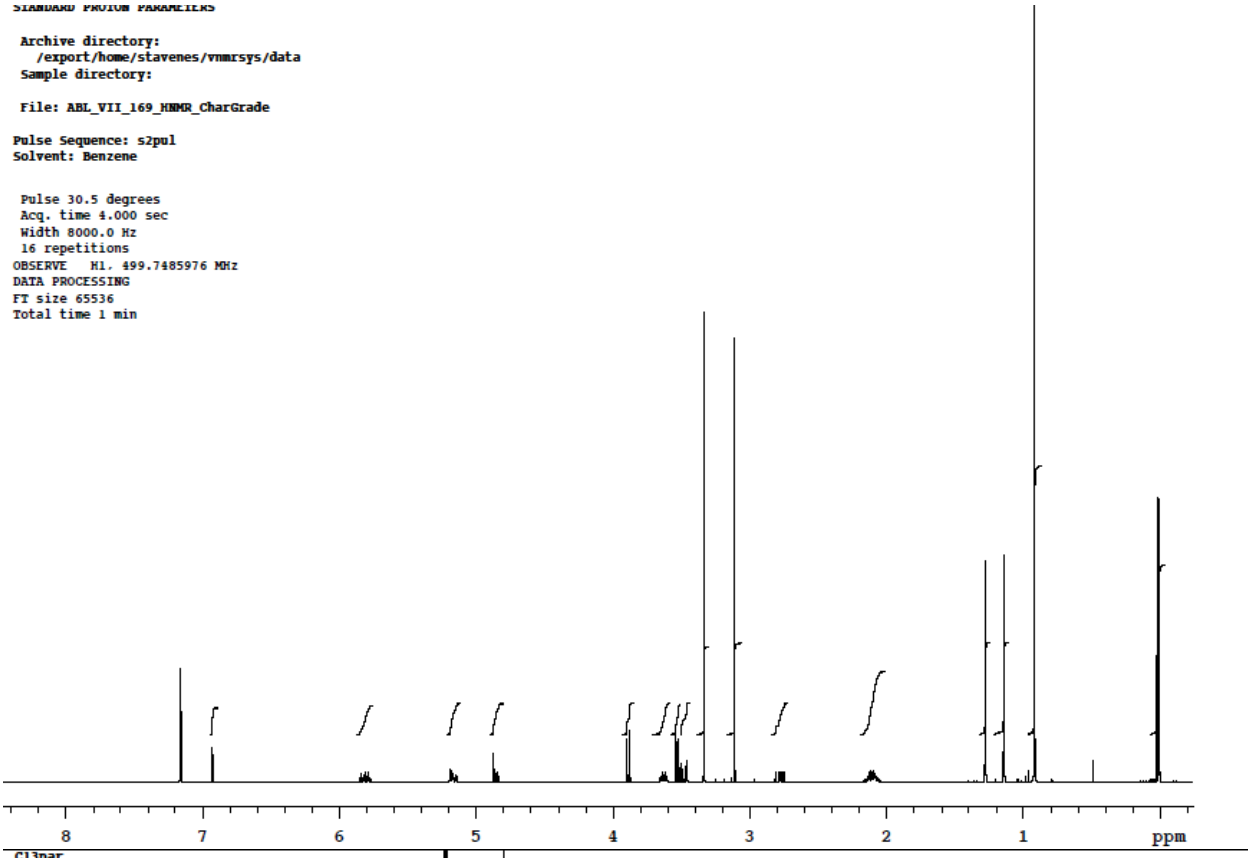
^{13}C NMR (C_6D_6, 125 MHz): δ = 195.6, 166.3, 148.3, 133.8 ($^1J_{CC}$ = 69.9 Hz), 122.5, 118.0 (^{13}C label), 104.6, 72.7 ($^2J_{CC}$ = 3.3 Hz), 68.6, 52.1, 51.3, 46.8, 40.0, 34.7, 26.2, 20.4, 20.0, 18.8, -5.4, -5.5 ppm

IR (thin film): 3067, 2953, 2929, 2857, 1726, 1709, 1633, 1471, 1435, 1392, 1358, 1253, 1203, 1178, 1127, 1089, 1061, 1005, 937, 909, 836, 778, 667 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{21}\{^{13}C\}H_{38}O_6Si^+$: 450.2369, Found: 450.2364

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24.6\,°C}}$ = -66.3 ± 0.1° (*c* = 1.55, CH_2Cl_2)

$\mathbf{R}_f$ = 0.50 (10% EtOAc in petroleum ether), one black spot, *p*-anisaldehyde + UV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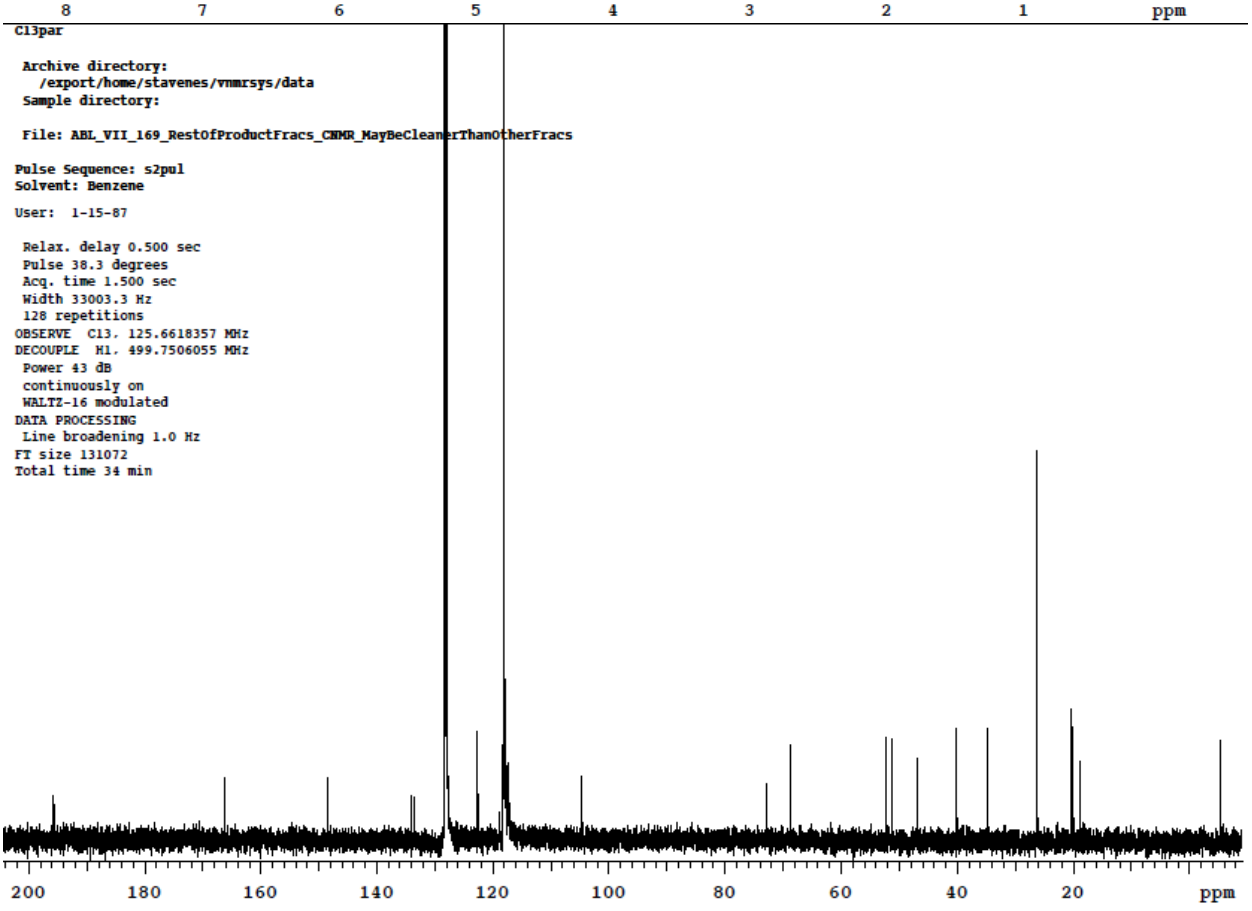
ppm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Solvent: Benzene
User: 1-15-87
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Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
128 repetitions
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DECOUPLE H1, 499.7506055 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
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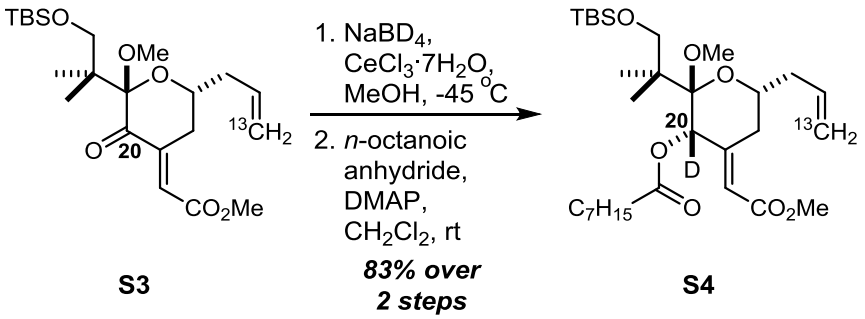


S3 ***83% over 2 steps*** **S4**

Procedure for C17 silyl ether S4

An oven-dried vial was charged with stir bar, precursor **S3** (31 mg, 0.072 mmol), MeOH (1.6 mL), and $CeCl_3 \cdot 7H_2O$ (28 mg, 0.074 mmol). The mixture was stirred until all solids were dissolved and then cooled to -40 °C (dry ice/MeCN bath) before addition of $NaBD_4$ (6.2 mg, 0.148 mmol) in one portion. The yellow color of the initial solution dissipated over ~5 minutes. The reaction was stopped after 18 minutes by addition of NH_4Cl (2 mL) after TLC indicated consumption of starting material. The reaction was diluted with H_2O (1 mL), and 1:1 Et_2O:pentane. The organic layer was collected and the aqueous layer was extracted with Et_2O/Pentane (1:1 mixture, 4 x 5 mL). The combined organic layers were dried over $MgSO_4$ and filtered to provide the crude product as a single C20 epimer (dr > 20:1), which was used in the next step without further purification.

The crude alcohol intermediate prepared above was dissolved in CH_2Cl_2 (0.75 mL). 4-Dimethylaminopyridine (31 mg, 0.252 mmol) was added. The mixture was stirred until the solids dissolved, followed by addition of octanoic anhydride (43 µL, 0.144 mmol). The reaction was allowed to stir for 3 hrs at which time the reaction was complete by TLC. The reaction mixture was loaded directly on a packed silica column and was purified by column chromatography (silica gel, gradient 5-8% Et_2O/petroleum ether) to yield intermediate **S4** (33.2 mg, 0.060 mmol, 83% over 2 steps) as a pale yellow oil.

*Characterization Data for C17 silyl ether **S4**:*

^{1}H NMR (C_6D_6, 500 MHz): δ = 6.25 (dd, 1H, *J* = 2.2, 1.5 Hz, C34), 5.87-5.79 (m, 1H, C25), 5.20-5.16 (m, 1H, C26), 4.89-4.85 (m, 1H, C26), 3.90 (d, 1H, *J* = 9.3 Hz, C17), 3.87 (d, 1H, *J* = 9.3 Hz, C17), 3.74-3.68 (m, 1H, C23), 3.55-3.51 (m, 1H, C22), 3.35 (s, 3H, CO_2Me), 3.17 (s, 3H, C19-OMe), 2.70-2.64 (m, 1H, C22), 2.23-2.10 (m, 4H, C24, C40), 1.60-1.56 (m, 2H, C41), 1.34 (s, 3H, C18-Me), 1.30 (s, 3H, C18-Me), 1.27-1.21 (m, 2H, C42), 1.20-1.15 (m, 6H, C43-C45), 1.01 (s, 9H, TBS), 0.88 (t, 3H, *J* = 7.2 Hz, C46), 0.12 (s, 6H, TBS) ppm

^{2}H NMR (C_6D_6, 77 MHz): δ = 6.64 ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 171.5, 166.3, 154.2, 134.1 ($^1J_{CC}$ = 69.9 Hz), 117.8 (^{13}C label), 116.9, 103.2, 71.0 ($^2J_{CC}$ = 3.3 Hz), 67.9, 50.7, 50.5, 47.7, 40.2, 34.5, 33.5, 32.0, 29.35, 29.29, 26.3, 25.1, 23.0, 21.0, 21.0, 18.7, 14.3, -5.2, -5.2 ppm[19]

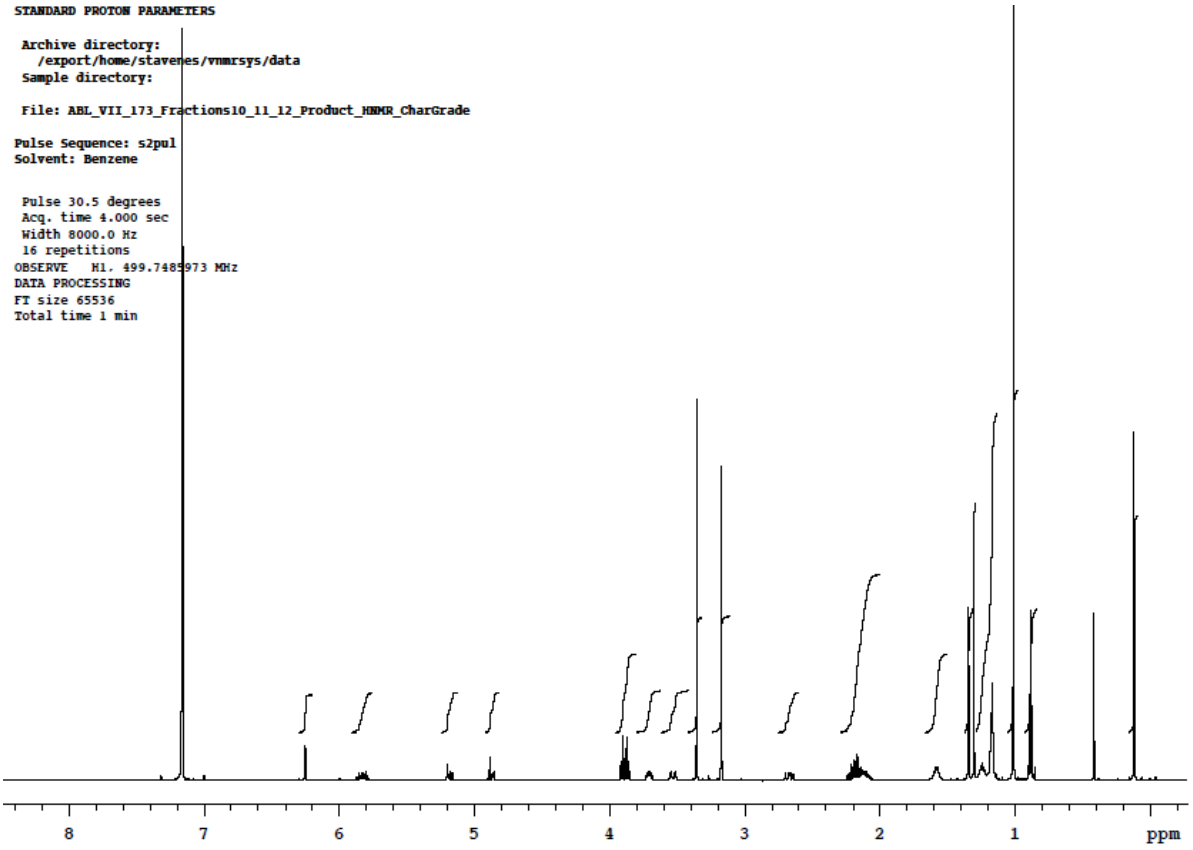
IR (thin film): 2923, 2855, 1746, 1722, 1666, 1470, 1435, 1359, 1252, 1167, 1081, 908, 836, 774, 666 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{29}\{^{13}C\}H_{53}DO_7SiNa^+$: 579.3633, Found: 579.3645

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24.6\ ^\circ C}} = -6.8 \pm 0.6°$ (*c* = 0.88, CH_2Cl_2)

$\mathbf{R}_f$ = 0.60 (10% EtOAc in petroleum ether), one black spot, *p*-anisaldehyde + UV

[19] No ^{13}C signal corresponding to C20 could be detected, likely due to the ^{2}H-induced splitting.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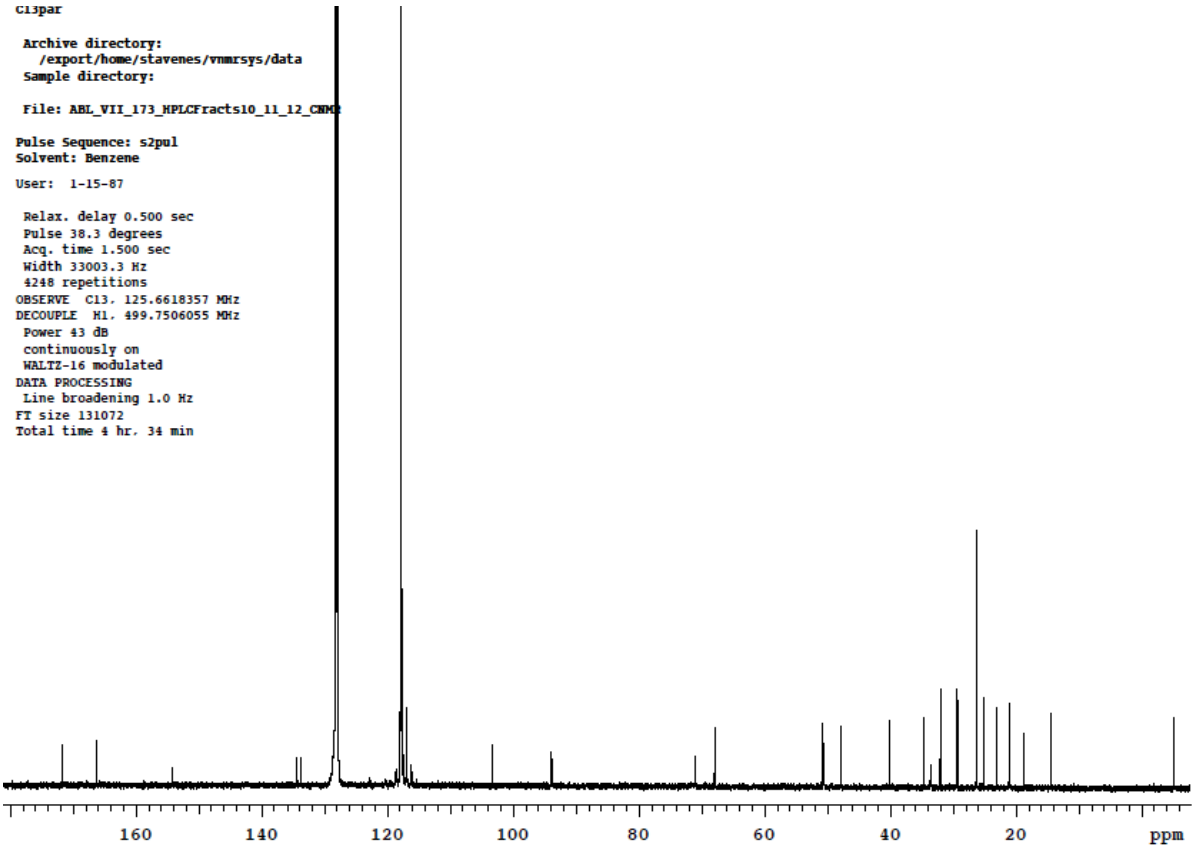
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Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
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DECOUPLE H1, 499.7506055 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
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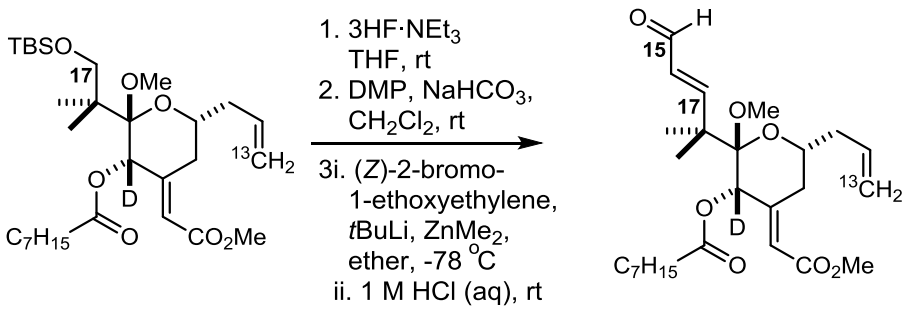

S4 **S5**

Procedure for C15 enal S5

To a solution of TBS ether **S4** (65 mg, 0.117 mmol) in THF (1.05 mL) at rt was added 3HF·Et_3N (190 µL). The reaction mixture was stirred for 26 h and then diluted with diethyl ether (6 mL). The organic phase was washed with saturated $NaHCO_3$ (2 x 3 mL), dried over $MgSO_4$, and eluted on a silica column in a gradient of 0→30% EtOAc to petroleum ether. The residue obtained after evaporation of solvent (43.4 mg) was carried forward without further purification.

To a solution of the intermediate alcohol in CH_2Cl_2 (2.0 mL), at rt, was added Dess-Martin Periodinane (104 mg, 0.245 mmol) was added. The reaction mixture was stirred for 30 min before TLC indicated that the reaction was not yet complete. Treatment with an additional 104 mg of the periodinane drove the reaction to completion after 30 additional minutes at rt. The reaction was quenched with 3 mL of a sat. solution of $Na_2S_2O_3$ and diluted with 3 mL Et_2O. The mixture was stirred vigorously until the organic layer was no longer cloudy. The quenched reaction mixture was then poured into a separatory funnel. The aqueous layer was extracted with Et_2O (4 x 3 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated. Purification via column chromatography (0-6% EtOAc/Pet. Ether) yielded the intermediate aldehyde **23** (32.4 mg) as a yellow oil.

(*Z*)-1-bromo-2-ethoxyethylene (37 µL, 0.294 mmol) was added to an oven-dried flask containing diethyl ether (1.5 mL). The solution was cooled to –78°C, and *t*-BuLi (350 µL, 0.559 mmol, 1.6 M in pentane) was added, dropwise. The reaction was stirred, at –78°C, for 30 min. Me_2Zn (250 µL, 0.298 mmol, 1.2 M in toluene) was added, dropwise, and the reaction was stirred, at –78°C, for 30 min. A solution of the intermediate aldehyde **23** (0.32 mg, 0.073 mmol dissolved in 0.5 mL Et_2O) was added dropwise, via syringe, with 2x0.5 mL rinses, and the reaction was stirred for 2 hrs at –78°C. The reaction was quenched at -78° C with a 1.0 M solution of HCl (2.2 mL) and allowed to warm to rt. The mixture was stirred vigorously for 18 h and was diluted with Et_2O (2 mL) and H_2O (2 mL). The separated aqueous phase was extracted with Et_2O (3 x 4 mL) and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification via HPLC (75-100% MeCN/H_2O, C18 column) gave pure C15 enal **S5** (24.6 mg, 45% yield over 3 steps) as a colorless oil.

*Characterization Data for C15 enal **S5**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.56 (d, 1H, *J* = 7.8 Hz, C15), 7.34 (d, 1H, *J* = 16.1 Hz, C17), 5.98-5.89 (m, 3H, C15, C25), 5.39-5.29 (m, 1H, C26), 5.06-4.98 (m, 1H, C26), 3.90-3.84 (m, 1H, C23), 3.70 (s, 3H, CO_2Me), 3.55 (dd, 1H, *J* = 15.8, 2.2 Hz, C22), 3.36 (s, 3H, C19-OMe), 2.48-2.40 (m, 2H, C40), 2.36-2.30 (m, 1H, C22), 2.20-2.05 (m, 2H, C24), 1.56-1.50 (m, 2H, C41), 1.30-1.20 (m, 8H, C42-C45), 1.19 (s, 3H, C18-Me), 1.15 (s, 3H, C18-Me), 0.87 (t, 3H, *J* = 7.0 Hz, C46) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 194.9, 171.9, 167.3, 166.6, 151.6, 133.6 ($^1J_{CC}$ = 69.7 Hz), 126.8, 118.4 (^{13}C label), 118.3, 102.7, 72.1, 72.0, 51.5, 51.4, 47.5, 40.2, 34.6, 31.8, 31.5, 29.1, 29.0, 24.7, 24.0, 22.7, 21.8, 14.2 ppm

IR (thin film): 2919, 2855, 2721, 1745, 1722, 1693, 1463, 1435, 1360, 1222, 1168, 1114, 1077, 974, 911, 789, 726 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{25}\{^{13}C\}H_{39}DO_7Na^+$: 489.2768, Found: 489.2765

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24.6\,^{\circ}C}}$ = -30.8 ± 0.1° (*c* = 1.25, CH_2Cl_2)

$\mathbf{R}_f$ = 0.45 (20% EtOAc in petroleum ether), one black spot, *p*-anisaldehyde + UV

STANDARD PROTON PARAMETERS

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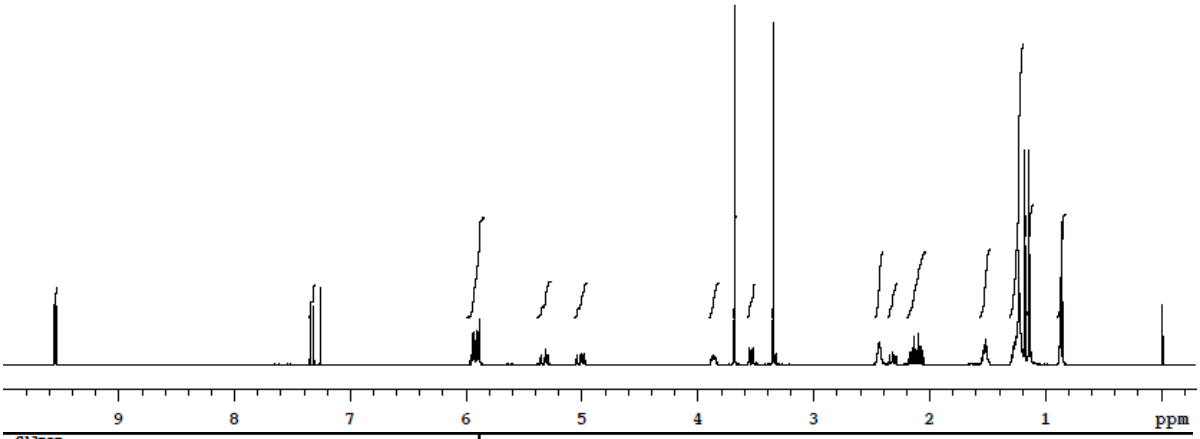

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User: 1-15-87

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Width 33003.3 Hz
148 repetitions
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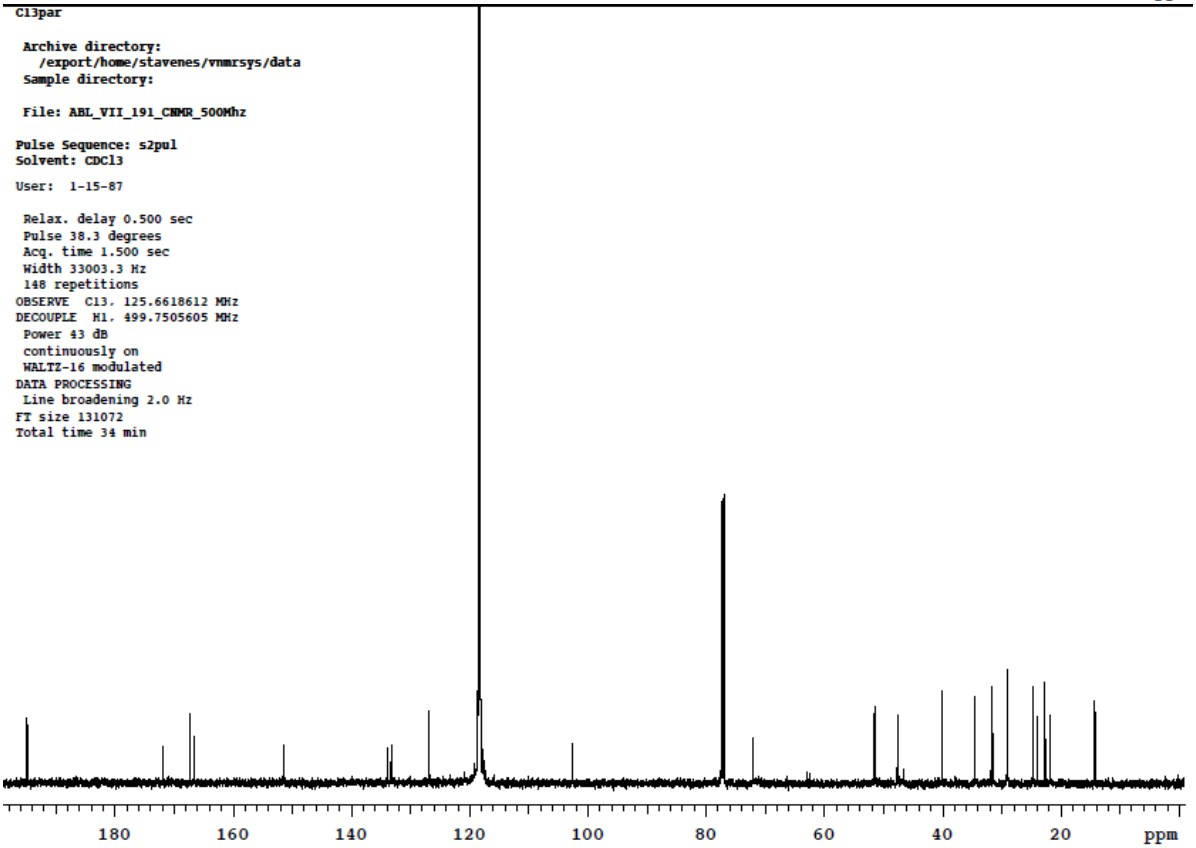
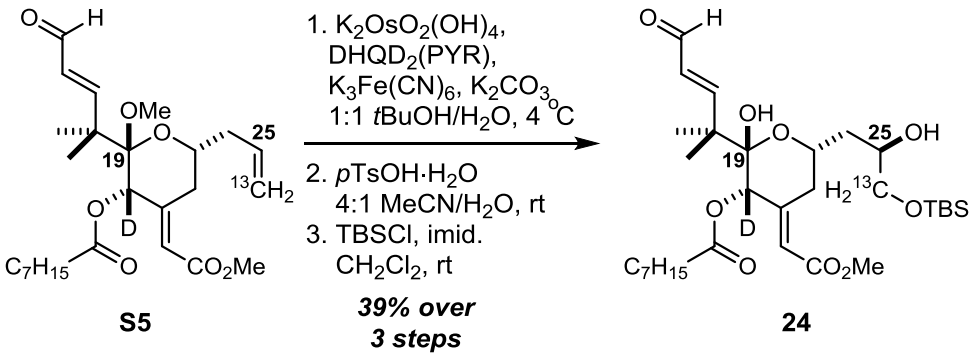




Procedure for C25 β-alcohol 24

An oven dried round-bottomed flask with stir bar was charged with $K_2OsO_2(OH)_4$ (2.9 mg, 0.0079 mmol), $(DHQD)_2Pyr$ (18 mg, 0.020 mmol), $K_3Fe(CN)_6$ (2.01 g, 6.11 mmol), and K_2CO_3 (845 mg, 6.11 mmol). The vessel was flushed with N_2 before addition of H_2O (10.1 mL) and *t*BuOH (10.1 mL). The resulting mixture was allowed to stir vigorously for 2 hrs at rt. A separate vial containing the C25-C26 olefin **S5** (58 mg, 0.124 mmol) was charged with a stir bar, flushed with N_2, and cooled to 0 °C in an ice bath prior to the addition of the dihydroxylation mixture prepared above (1.92 mL, 0.75 μmol [Os]) in a single portion via syringe. The resulting mixture was allowed to stir at ~4 °C (cold room) for 20 h. The mixture was then diluted with H_2O (25 mL) prior to extraction of the resulting biphasic mixture with four 100 mL EtOAc aliquots. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under vacuum prior to column chromatography (50→100% EtOAc:petroleum ether, 10% increments) to yield 46.9 mg of the intermediate diol. A reproduction of this procedure on 69 mg of **S5** yielded 52 mg of material. Both batches of diol were obtained as mixtures of C25 epimers with each dr being 2.5:1 β:α.

A vial containing the intermediate from step 1 (46.0 mg, ~ 0.092 mmol), and stirbar was charged with H_2O (1.8 mL) and MeCN (7.4 mL) followed by *p*TsOH·H_2O (175 mg, 0.919 mmol). The reaction was allowed to stir for 40 hrs before quenching with 10 mL sat. $NaHCO_3$ (aq). The resulting biphasic mixture was extracted with four 10 mL portions of EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to yield 42.9 mg of the C19 hemiketal, which was used directly in the next step. A repeat of this procedure on 52 mg of intermediate from step 1 yielded 51.2 mg. Both crude products were still mixtures of C25 epimers.

Crude product from step 2 (51.2 mg, ~0.105 mmol) was dissolved in dry CH_2Cl_2 (1.0 mL) in a dry vial under inert atmosphere. Imidazole (21.5 mg, 0.32 mmol) was then added and allowed to stir 3 min at rt to fully dissolve. A 1 M solution of TBSCl in dry CH_2Cl_2 (116 μL, 116 mmol) was added via syringe in one portion. The reaction mixture was allowed to stir 15 min at rt (complete by TLC), before being quenched with 10 mL of sat. NH_4Cl (aq) and diluted with Et_2O (10 mL). The layers were separated and the aqueous phase was extracted with three portions of Et_2O (10 mL each). The combined organic phases were dried over Na_2SO_4 and filtered prior to concentration *in vacuo*. Crude product was purified via column chromatography (60→80% ether:petroleum ether, 5% increments). This procedure was repeated for the second portion of crude C19 hemiketal. The product (**24**) was isolated as a single C25 epimer as a colorless oil (62.8 mg from both batches, 38.6% over 3 steps).

*Characterization Data for C25 β-alcohol **24**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.56 (d, 1H, *J* = 8.0 Hz, C15), 7.33 (d, 1H, *J* = 16.1 Hz, C17), 6.02 (*app.* d, 1H, *J* = 1.8 Hz, C34), 5.95 (dd, 1H, *J* = 16.1, 7.8 Hz, C16), 4.21-4.14 (m, 1H, C23), 4.08 (br s, 1H, C25-OH), 4.00-3.94 (m, 1H, C25), 3.70 (ddd, 1H, *J* = 141.7, 9.9, 3.5 Hz, C26), 3.69 (s, 3H, CO_2Me), 3.71-3.66 (m, 1H, C22), 3.48 (ddd, 1H, *J* = 140.9, 10.2, 5.8 Hz, C26), 2.13-1.98 (m, 3H, C22, C40), 1.74-1.62 (m, 2H, C24), 1.48 (*app.* quint., 2H, *J* = 7.3 Hz, C41), 1.31-1.16 (m, 8H, C42-C45), 1.13 (s, 3H, C18-Me), 1.12 (s, 3H, C18-Me), 0.91 (s, 9H, TBS), 0.86 (t, 3H, *J* = 7.2 Hz, C46), 0.09 (s, 6H, TBS) ppm[20]

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 194.7, 171.8, 166.5, 166.3, 150.2, 127.7, 120.8, 99.8, 72.4,[21] 68.0, 67.4 (^{13}C label), 66.7, 51.4, 45.9, 39.0, 34.6, 31.7, 31.2, 29.0, 29.0 26.1, 24.6, 23.2, 22.7, 20.1, 18.6, 14.2, -5.2 ppm

IR (thin film): 3422, 2952, 2929, 2856, 1720, 1689, 1466, 1436, 1379, 1252, 1169, 1060, 972, 837, 778 cm^{-1}

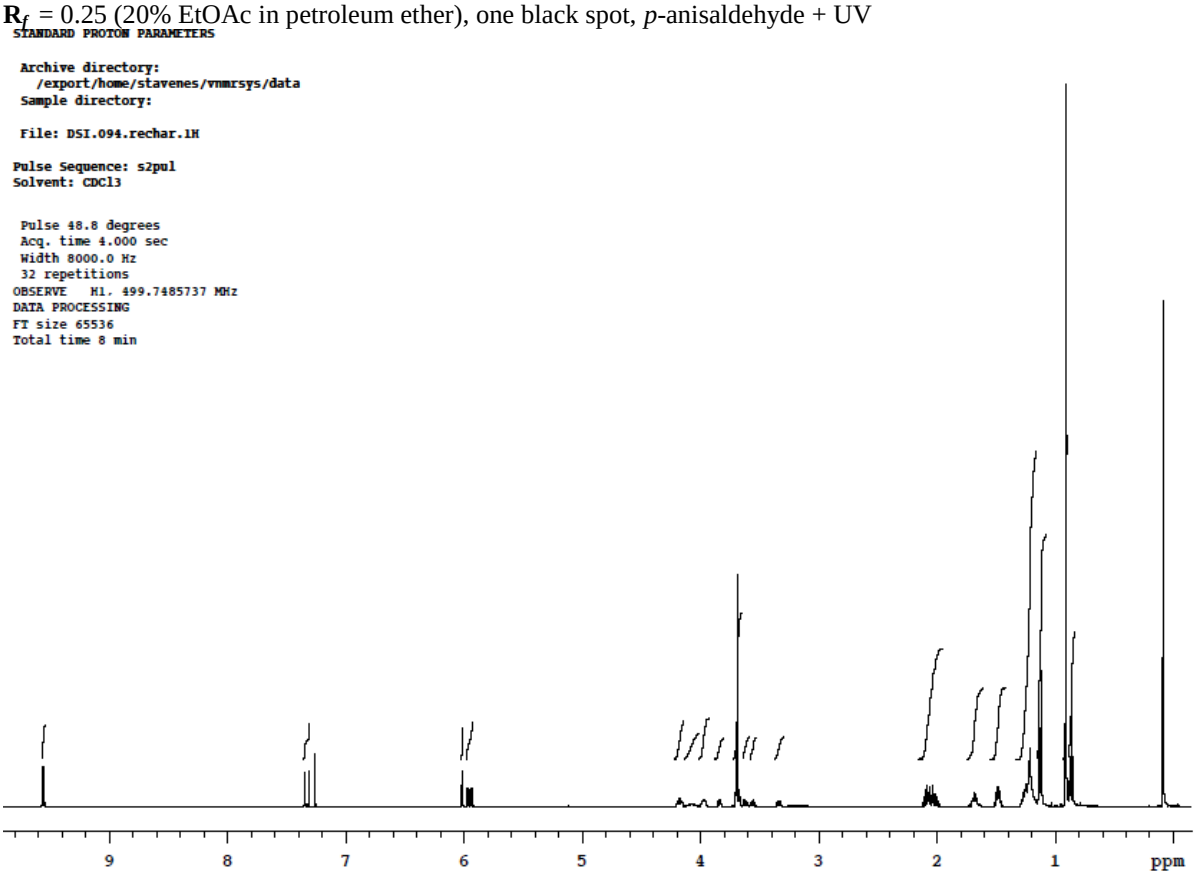
HRMS (ES+, *m/z)* calculated for $C_{30}\{^{13}C\}H_{53}D\ NaO_9Si^+$: 623.3526, Found: 623.3522

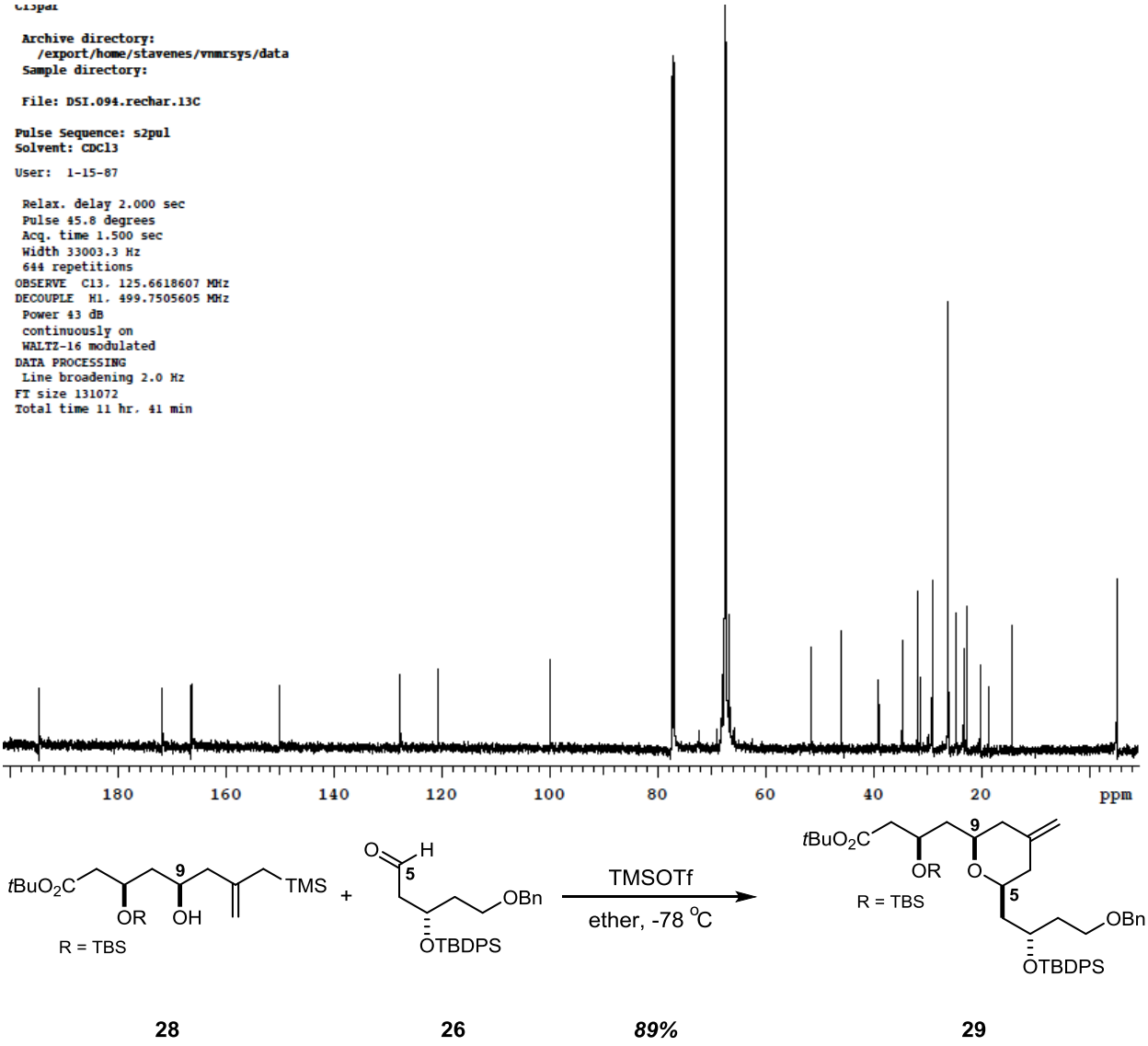
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24.6\ °C}} = -21.7 \pm 0.3°$ (*c* = 1.1, CH_2Cl_2)

[20] Large *J* couplings (>100 Hz) the result of the ^{13}C label at C26.

[21] The low signal-to-noise of this resonance is suspected to be due to its proximity to the ^{2}H label (tentatively assigned as C20 based on analogy to southern fragment **12**).

$\mathbf{R_f}$ = 0.25 (20% EtOAc in petroleum ether), one black spot, *p*-anisaldehyde + UV





28 **26** ***89%*** **29**

Procedure for C7 olefin 29

Aldehyde **26** (38.2 mg, 0.085 mmol) was dissolved in Et_2O (1.1 mL) and added to alcohol **28** (18.4 mg, 0.043 mmol). The solution was cooled in a CO_2/acetone bath and freshly distilled TMSOTf (11.6 µL, 0.064 mmol) was added via syringe over 30 seconds. The reaction was allowed to stir for 30 minutes and was then quenched with 1 M NaOH (500 µL) and stirred for 15 minutes at ambient temperature. The solution was diluted with water (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (5 mL), which was then extracted with EtOAc (5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification by silica gel column chromatography (3→7% EtOAc:pentane, 2% increments) afforded 30.0 mg (89.3% yield) of C7 olefin **29** as a clear oil.

*Characterization Data for C7 olefin **29**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.80-7.60 (m, 4H, TBDPS), 7.52-7.13 (m, 11H, TBDPS, OBn), 4.66 (s, 1H, C14), 4.63 (s, 1H, C14), 4.37 (s, 2H, C*H*$_2$Ph), 4.21 (quint, 1H, *J* = 6.1 Hz, C11), 4.11 (quint, 1H, *J* = 5.9 Hz, C3), 3.49 (m, 2H, C1), 3.15-3.38 (m, 2H, C5, C9), 2.37 (m, 2H, C12), 2.23 (d, 1H, *J* = 12.9 Hz, C6 or C8), 1.95 (d, 1H, *J* = 12.9 Hz, C6 or C8), 1.91-1.68 (m, 6H, C2, C4, C6, C8, C10), 1.67-1.58 (m, 2H, C4, C10), 1.47 (s, 9H, *t*Bu), 1.05 (s, 9H, TBDPS), 0.89 (s, 9H, TBS), 0.10 (s, 3H, TBS), 0.07 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 170.8, 144.7, 138.7, 136.0, 136.0, 134.6, 134.4, 129.6, 128.4, 127.7, 127.7, 127.5, 108.4, 80.4, 75.4, 75.0, 72.8, 68.8, 67.0, 66.4, 44.6, 43.8, 43.7, 41.1, 40.6, 37.7, 28.3, 27.2, 26.0, 19.6, 18.1, -4.5, -4.6 ppm
IR (thin film): 3070, 2930, 2890, 2856, 1731, 1651, 1589, 1472, 1462, 1427, 1390, 1316, 1253, 1153, 1110, 1043, 1005, 954, 889, 836, 776, 736, 701, 611 cm^{-1}
HRMS (ES+, *m/z)* calculated for $C_{47}H_{70}O_6Si_2Na^+$: 809.4603, Found: 809.4606
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22.6\ ^{\circ}C}} = 68.9 \pm 0.2°$ (*c* = 0.58, CH_2Cl_2)
$\mathbf{R}_f$ = 0.35 (5% EtOAc in pet. ether), one purple spot, *p*-anisaldehyde + UV

BAL-A-194

Archive directory:
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Sample directory:

File: BAL-A-194

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Solvent: CDCl3

Pulse 48.0 degrees
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Width 8000.0 Hz
32 repetitions
OBSERVE H1, 499.7485607 MHz
DATA PROCESSING
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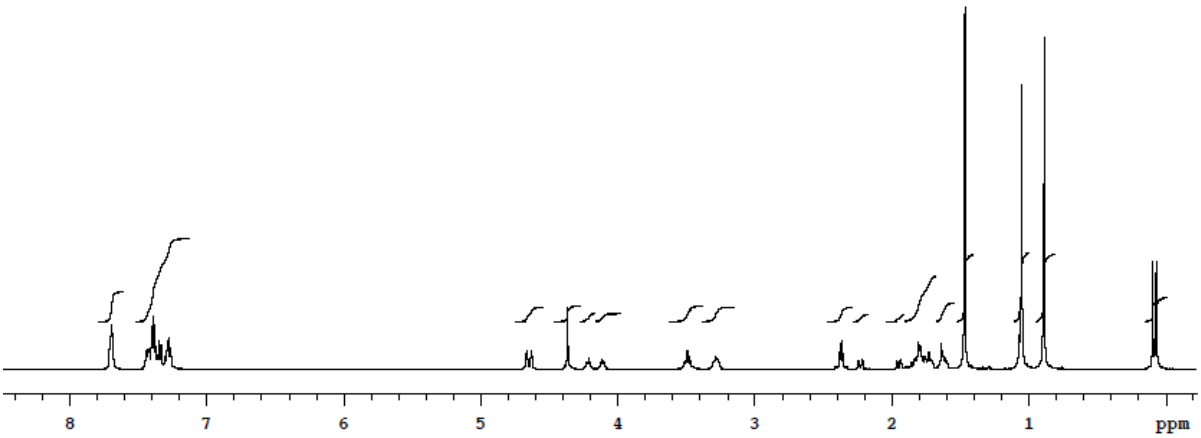
Total time 2 min



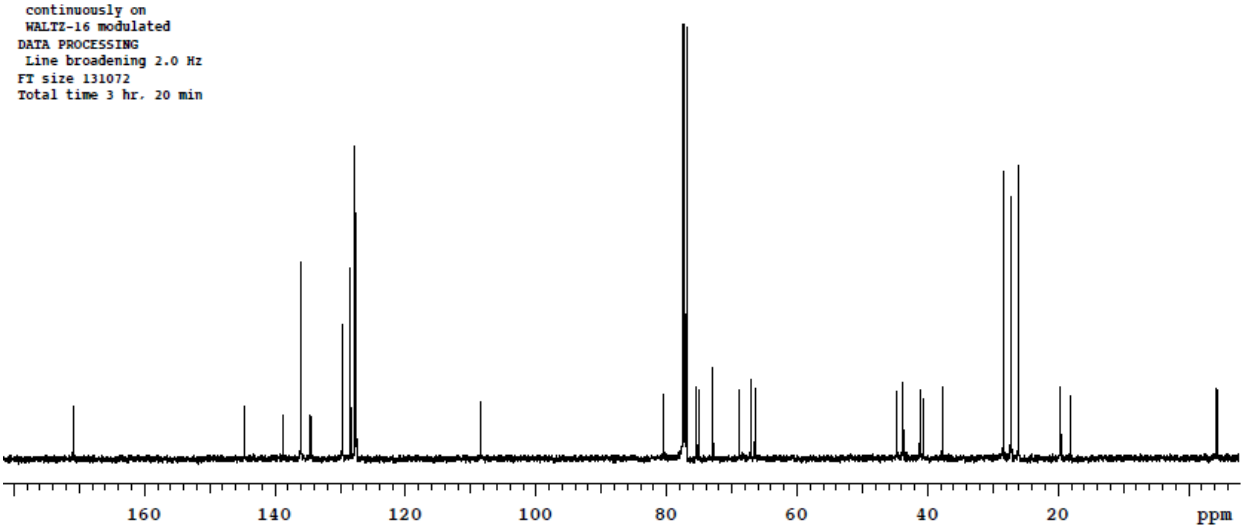
C13par

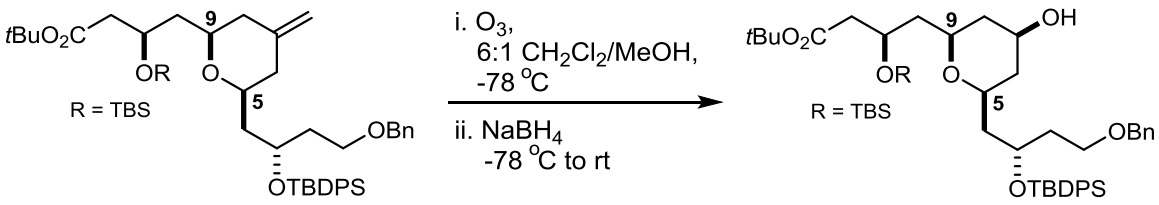
Archive directory:
/export/home/stavenes/vnmrsys/data
Sample directory:

File: BAL-A-194-Carbon

Pulse Sequence: s2pul
Solvent: CDCl3
User: 1-15-87

Relax. delay 1.500 sec
Pulse 37.8 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
400 repetitions
OBSERVE C13, 125.6618627 MHz
DECOUPLE H1, 499.7505605 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 3 hr, 20 min





29 ***98%*** **30**

Procedure for C7 β-alcohol 30

Pyran **29** (13.2 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (5.8 mL) and MeOH (1 mL) and cooled to –78 °C. O_3 was bubbled through the reaction until the solution turned blue (60 s). The reaction was purged with O_2 until the solution became colorless, then $NaBH_4$ (6.4 mg, 0.17 mmol) was added in one portion and the reaction was transferred to an ice bath and allowed to stir for 3.5 h as the ice melted. Sat. aq. NH_4Cl (8 mL) was added to the reaction and the solution extracted with CH_2Cl_2 (3 x 8 mL). The combined orgranic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Flash chromatography (13→19% ethyl acetate:pentane, 2% increments) afforded 13.0 mg of C7 β-alcohol **30** (97.8% yield) as a colorless oil.

*Characterization Data for C7 β-alcohol **30**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.76-7.59 (m, 4H, TBDPS), 7.50-7.13 (m, 11H, TBDPS, OBn), 4.38-4.30 (s, 2H, C*H*$_2$Ph), 4.17 (quint, 1H, *J* = 6.0 Hz, C11), 4.10 (t, 1H, *J* = 6.0 Hz, C3), 3.55 (m, 1H, C7), 3.46 (m, 2H, C1), 3.34-3.14 (m, 2H, C5, C9), 2.33 (t, 2H, *J* = 6.0 Hz, C12), 1.93 (d, 1H, *J* = 6.0 Hz, C6), 1.84-1.50 (m, 8H, C2, C4, C7-OH, C8, C10), 1.44 (s, 9H, *t*Bu), 1.03 (s, 9H, TBS), 1.05-0.88 (m, 2H, C6, C8), 0.86 (s, 9H, TBDPS), 0.06 (s, 3H, TBS), 0.03 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 170.7, 138.6, 136.0, 136.0, 134.5, 134.4, 129.7, 129.6, 128.4, 127.7, 127.7, 127.7, 127.5, 80.4, 72.9, 72.3, 72.0, 68.8, 68.1, 66.9, 66.4, 44.4, 43.7, 43.3, 41.6, 40.9, 37.8, 28.3, 27.2, 26.0, 19.6, 18.1, -4.4, -4.5 ppm

IR (thin film): 3431, 3070, 2930, 2888, 2857, 1731, 1472, 1462, 1427, 1390, 1367, 1316, 1256, 1154, 1110, 1075, 1006, 836, 776, 738, 702, 666, 611 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{46}H_{70}O_7Si_2Na^+$: 813.4552, Found: 813.4539

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22.9\ ^\circ C}} = 5.2 \pm 0.1°$ (*c* = 1.27, CH_2Cl_2)

$\mathbf{R}_f$ = 0.30 (20% EtOAc in pet. ether), one dark green spot, *p*-anisaldehyde

Std proton

Archive directory:

Sample directory:

File: BAL-A-199

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Solvent: cdcl3

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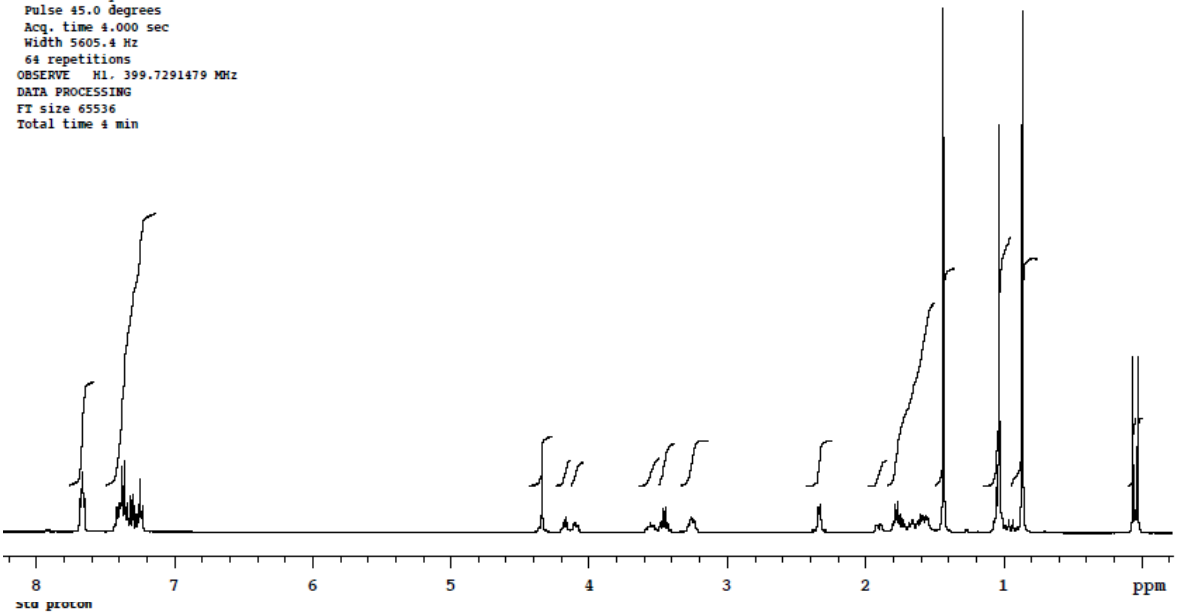
Total time 4 min



Std proton

Archive directory:

Sample directory:

File: BAL-A-199-Carbon

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Solvent: cdcl3

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User: 1-14-87

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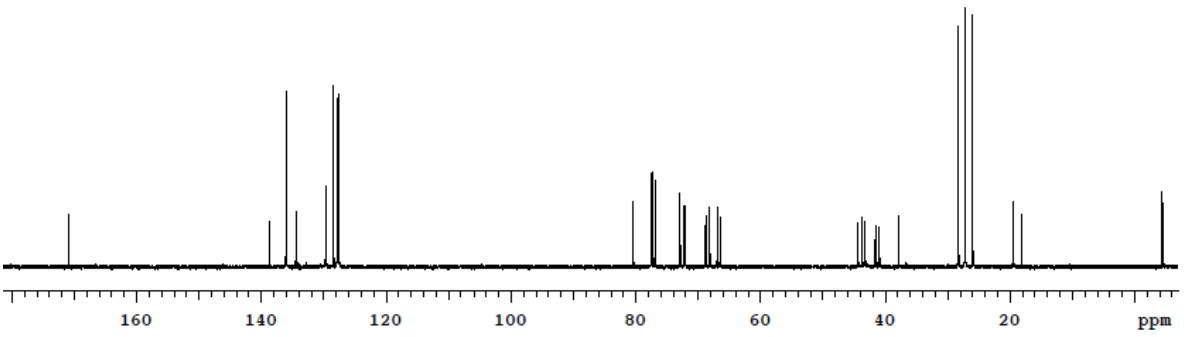

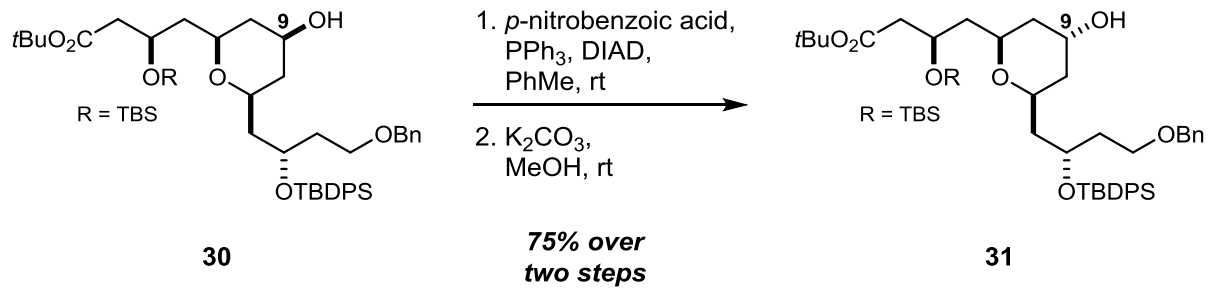

30 ***75% over two steps*** **31**

Procedure for C7 α-alcohol 31

Alcohol **30** (40 mg, 0.051 mmol) was dissolved in toluene (1.50 mL). Triphenylphosphine (60.2 mg, 0.230 mmol) was added in one portion, followed by *p*-nitrobenzoic acid (43 mg, 0.255 mmol) in one portion, followed by DIAD (40 μL, 0.204 mmol) via syringe over 5 s. The reaction was allowed to stir at rt for 1.5 hrs, then diluted with Et_2O (4 mL) and washed with $NaHCO_3$ (2 mL), H_2O (2 mL), and brine (2 mL). The combined aqueous layers were extracted with Et_2O (2 x 2 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was filtered through a short silica plug (25% EtOAc / pet. ether) and concentrated *in vacuo*. The residue was then dissolved in MeOH (3.00 mL), K_2CO_3 (92 mg) was added in one portion, and the reaction was allowed to stir at rt for 4 h. The reaction was diluted with H_2O (5 mL) and extracted with Et_2O (3 x 8 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (25% EtOAc:pet. ether) afforded 30 mg (75.0%) of the desired C7 α-alcohol **31** as a clear, colorless oil.

*Characterization Data for C7 α-alcohol **31**:*

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.70-7.65 (m, 4H, TBDPS), 7.42-7.23 (m, 11H, TBDPS, OBn), 4.35 (s, 2H, C*H*$_2$Bn), 4.16 (*app* quint., 1H, *J* = 6.0 Hz, C11), 4.10-4.04 (m, 2H, C3, C7), 3.71-3.61 (m, 2H, C5, C9), 3.51-3.44 (m, 2H, C1), 2.31 (d, 2H, *J* = 6.0 Hz, C12), 1.79 (*app* dd, 1H, *J* = 12.8, 6.5 Hz, C2), 1.71-1.46 (m, 7H, C2, C4, C7-OH, C10), 1.43 (s, 9H, *t*Bu), 1.36-1.17 (m, 4H, C6, C8), 1.03 (s, 9H, TBDPS), 0.85 (s, 9H, TBS), 0.05 (s, 3H, TBS), 0.03 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.9, 138.7, 136.1, 136.0, 134.7, 134.4, 129.7, 129.6, 128.4, 127.7, 127.7, 127.6, 127.5 80.2, 72.9, 68.8, 68.4, 68.2, 67.1, 66.4, 64.7, 44.6, 43.8, 43.5, 39.0, 38.3, 37.8, 28.3, 27.2, 26.0, 19.6, 18.1, -4.3, -4.6 ppm

IR (thin film): 3452, 2924, 2855, 1730, 1471, 1427, 1367, 1255, 1155, 1109, 1072, 835 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{46}H_{70}NaO_7Si_2^+$: 813.4540, Found: 813.4539

$[\alpha]_D^{25.5\,°C} = 3.5 \pm 0.3°$ (*c* = 0.60, CH_2Cl_2)

R_f = 0.40 (20% EtOAc in pet. ether), one dark green spot, *p*-anisaldehyde + UV

STANDARD 1H OBSERVE

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Sample directory:

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Solvent: CDCl3

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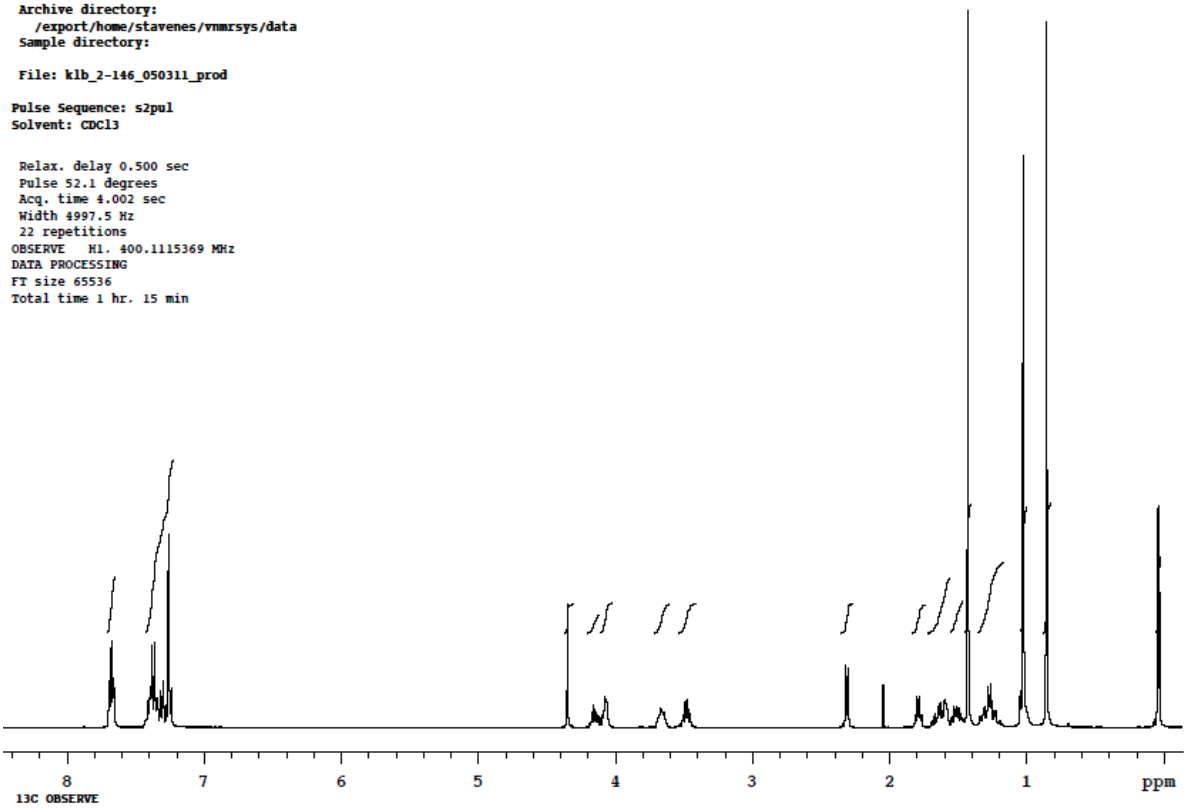

13C OBSERVE

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Sample directory:

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Solvent: CDCl3

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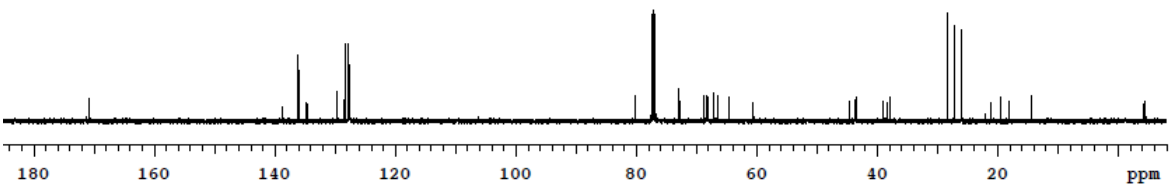

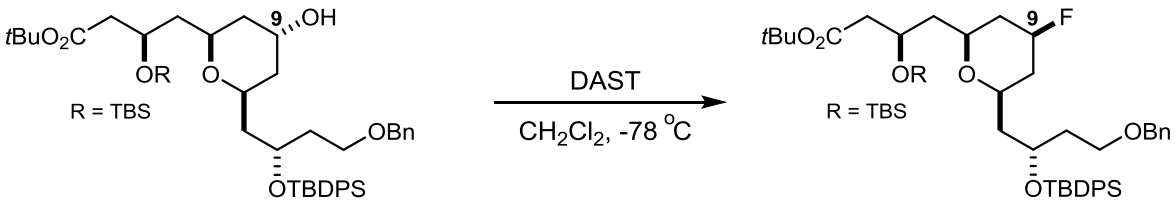

31 ***50%*** **27**

Procedure for C7 fluoride 27

Alcohol **31** (5.0 mg, 0.0063 mmol) was dissolved in CH_2Cl_2 (0.200 mL) in an over dried round bottom flask under atmosphere of nitrogen. The reaction was cooled to -78 °C with dry ice/acetone bath. Diethylammonium sulfur trifluoride (1.0 uL, 0.0076 mmol) was added dropwise via syringe to reaction. After stirring at -78 °C for 20 min, the reaction was warmed to 0 °C with an ice bath. After 20 additional min, the reaction was quenched with H_2O (0.50 mL) and extracted with EtOAc (3 x 1 mL), dried over a small amount of $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was filtered through a short silica plug (25% EtOAc / pet. ether) and concentrated *in vacuo*. The residue was then dissolved in t-BuOH (0.50 mL), H_2O (0.50 mL), $K_2OsO_4 \cdot 2H_2O$ (4.0 mg), $K_3Fe(CN)_6$ (80 mg), K_2CO_3 (40 mg), and the reaction was allowed to stir at rt for 15 h. The reaction was diluted with H_2O (2 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (5% EtOAc / pentane) afforded 2.5 mg (49.9%) of the desired C7-fluoride (**27**) as a clear oil.

*Characterization Data for C7 fluoride **27**:*

^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.67-7.62 (m, 4H, TBDPS), 7.44-7.21 (m, 11H, TBDPS, Ph), 4.48-4.32 (m, 1H, C7), 4.37-4.31 (m, 2H, CH_2Bn), 4.15 (t, 1H, *J* = 5.8 Hz, C11), 4.08 (t, 1H, *J* = 5.1 Hz, C3), 3.45 (*app* td, 1H, *J* = 13.6, 6.8 Hz, C1), 3.25-3.18 (m, 2H, C5, C9), 2.36-2.26 (m, 2H, C12), 2.06-1.99 (m, 1H, C6), 1.81-1.67 (m, 3H, C2, C8), 1.63-1.49 (m, 4H, C4, C10), 1.43 (s, 9H, *t*Bu), 1.27-1.11 (m, 2H, C6, C8), 1.02 (s, 9H, TBDPS), 0.85 (s, 9H, TBS), 0.05 (s, 3H, TBS), 0.02 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.7, 138.7, 136.0, 134.5, 134.4, 129.7, 128.4, 127.7, 127.6,[22] 89.4 ($^1J_{CF}$ = 175.5 Hz), 80.5, 72.9, 71.8 ($^3J_{CF}$ = 11.3 Hz), 71.5 ($^3J_{CF}$ = 11.3 Hz), 68.7, 66.9, 66.3, 44.3, 43.7, 43.1, 38.8 ($^2J_{CF}$ = 17.2 Hz), 38.2 ($^2J_{CF}$ = 17.2 Hz), 37.8, 28.3, 27.2, 26.0, 19.6, 18.1, -4.4, -4.5 ppm

^{19}F NMR ($CDCl_3$, 500 MHz): -170.4 ppm

IR (thin film): 2956, 2929, 2856, 1731, 1472, 1428, 1367, 1255, 1159, 1106, 1005, 836 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{46}H_{69}O_6FSi_2Na^+$: 815.4509, Found: 815.4519

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23.4\ °C}} = 8.8 \pm 0.3°$ (*c* = 0.66, CH_2Cl_2)

$\mathbf{R}_f$ = 0.35 (5% EtOAc in pet. ether), one black spot, *p*-anisaldehyde + UV

[22] Aromatic resonances from the two phenyl groups on the TBDPS could not be resolved, leading to only 8 unique peaks in the aromatic region (as opposed to 12 in the starting material).

STANDARD PROTON PARAMETERS

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Sample directory:

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User: 1-15-87

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Acq. time 4.000 sec
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DATA PROCESSING
Resol. enhancement -0.0 Hz
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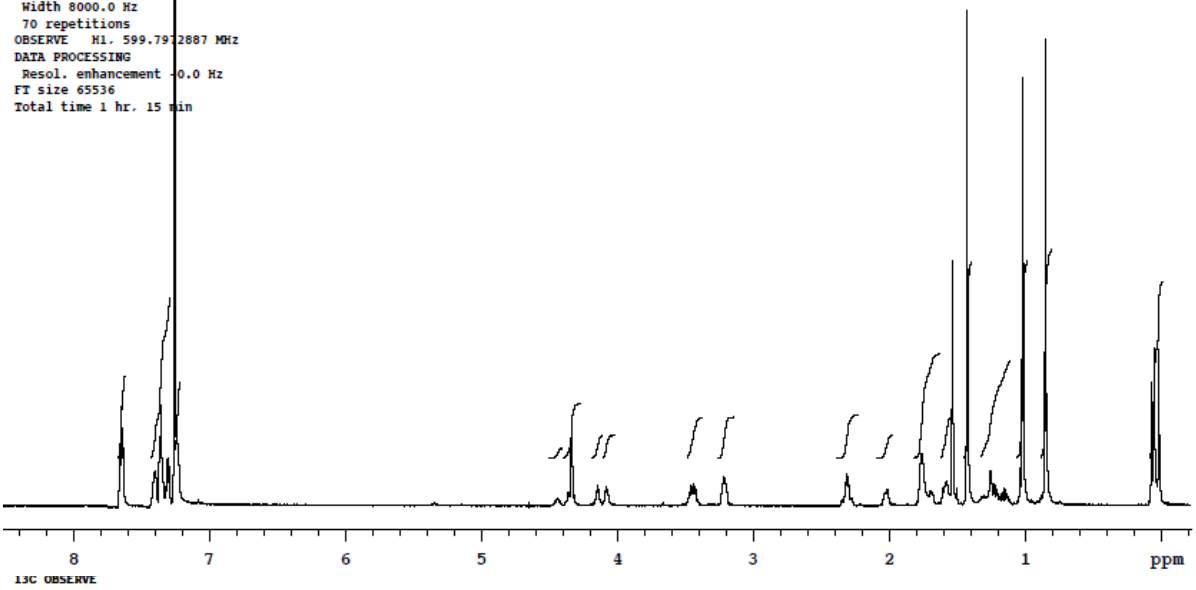

13C OBSERVE

Archive directory:
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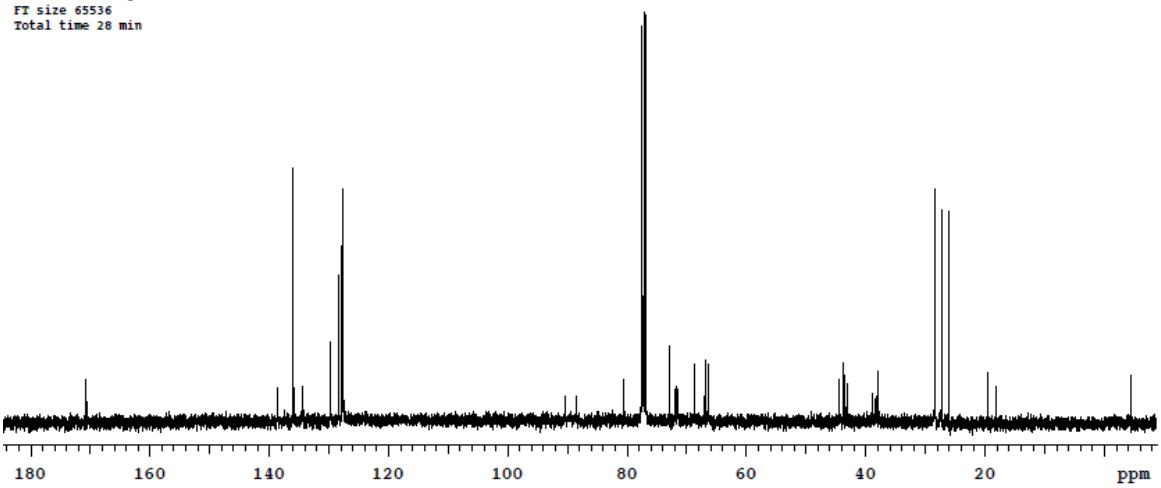
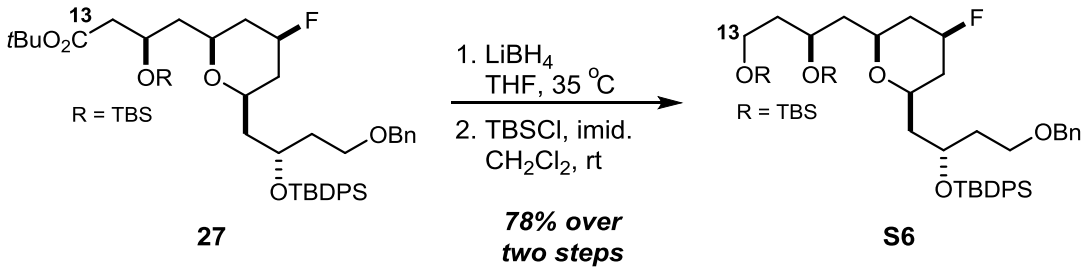
Sample directory:

File: klb_C_2-147_050711_prod

Pulse Sequence: s2pul
Solvent: CDCl3

Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
534 repetitions
OBSERVE C13, 100.6081116 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 28 min





27 **S6**

Procedure for C13 silyl ether S6

C13 ester **27** (17.1 mg, 0.022 mmol) was dissolved in THF (1.0 mL) and $LiBH_4$ (2.0 M in THF, 500 µL, 0.102 mmol) was added via syringe over 10 s and the reaction was allowed to stir at 35 °C for 24 h. The reaction was quenched with sat. aq. NH_4Cl (1 mL) and the reaction allowed to stir vigorously at rt for 15 min until bubbling ceased. The solution was diluted with H_2O (1 mL) and extracted with EtOAc (3 x 2 mL), dried over a small amount of $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified via flash chromatograph (10%→25% EtOAc:pet. ether). The resultant C13 alcohol was moved on without further purification.

The residue was dissolved in CH_2Cl_2 (1.00 mL). Imidazole (3.5 mg, 0.053 mmol) was added in one portion, followed by TBSCl (5.0 mg, 0.0.31 mmol). The reaction was allowed to stir for 15 min at rt and then quenched with sat. aq. NH_4Cl (1.00 mL). The solution was diluted with H_2O (1 mL) and extracted with Et_2O (3 x 3 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (5% EtOAc:pet. ether) afforded 14.0 mg of C13 silyl ether **S6** (77.5% yield) as a clear, colorless oil.

*Characterization Data for C13 silyl ether **S6**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.65 (t, 4H, *J* = 8.0 Hz, TBDPS), 7.41-7.23 (m, 11H, TBDPS), 4.50-4.31 (m, 1H), 4.37-4.30 (m, 2H, C*H*$_2$Bn), 4.10-4.04 (m, 1H, C3), 3.91 (t, 1H, *J* = 5.7 Hz, C11), 3.61 (t, 2H, *J* = 6.8 Hz, C13), 3.50-3.39 (m, 2H, C1), 3.27-3.20 (m, 2H, C5, C9), 2.08-1.98 (m, 1H, C6), 1.81-1.47 (m, 9H, C2, C4, C8, C10, C12), 1.28-1.10 (m, 2H, C6, C8), 1.02 (s, 9H, TBDPS), 0.88 (s, 9H, TBS), 0.86 (s, 9H, TBS), 0.07 (s, 3H, TBS), 0.04 (s, 3H, TBS), 0.03 (s, 3H, TBS), 0.00 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.6, 136.0,[23] 134.5, 134.4, 129.7,[1] 129.7, 128.4, 127.7, 127.7,[1] 127.6, 89.5 ($^1J_{CF}$ = 174.7 Hz), 72.9, 71.8, 71.7, 68.7, 66.9, 66.4, 59.7, 44.4, 43.1, 40.4, 38.9,[24] 38.3,[2] 27.2, 26.1, 26.0, 19.6, 18.4, 18.2, -4.3, -5.2 ppm

IR (thin film): 2956, 2855, 1661, 1578, 1537, 1471, 1427, 1361, 1256, 1110, 836 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{48}H_{77}O_5FSi_3Na^+$: 859.4955, Found: 859.4963

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23.0\,°C}} = 5.4 \pm 0.4°$ (*c* = 0.33, CH_2Cl_2)

$\mathbf{R}_f$ = 0.45 (5% EtOAc in pet. ether), one black spot, *p*-anisaldehyde + UV

[23] Believed to be two unresolved resonances based on analogy to preceding intermediates.

[24] Low resolution on these peaks believed to be due to C-F coupling (tentatively assigned as C6 and C8).

STANDARD PROTON PARAMETERS

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Sample directory:

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Solvent: CDCl3

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Width 8000.0 Hz
14 repetitions
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DATA PROCESSING
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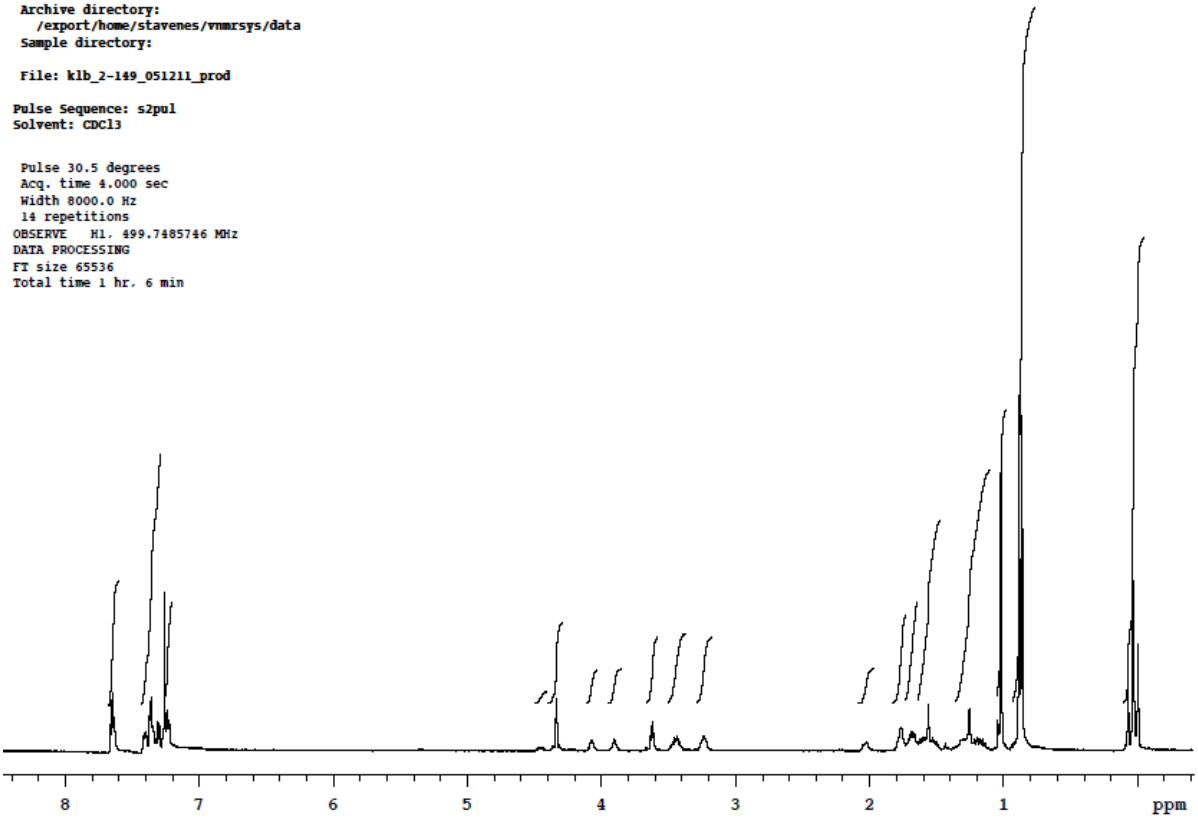

C13par

Archive directory:
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Sample directory:

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Solvent: CDCl3

User: 1-15-87

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Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
1224 repetitions
OBSERVE C13, 125.6618607 MHz
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Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
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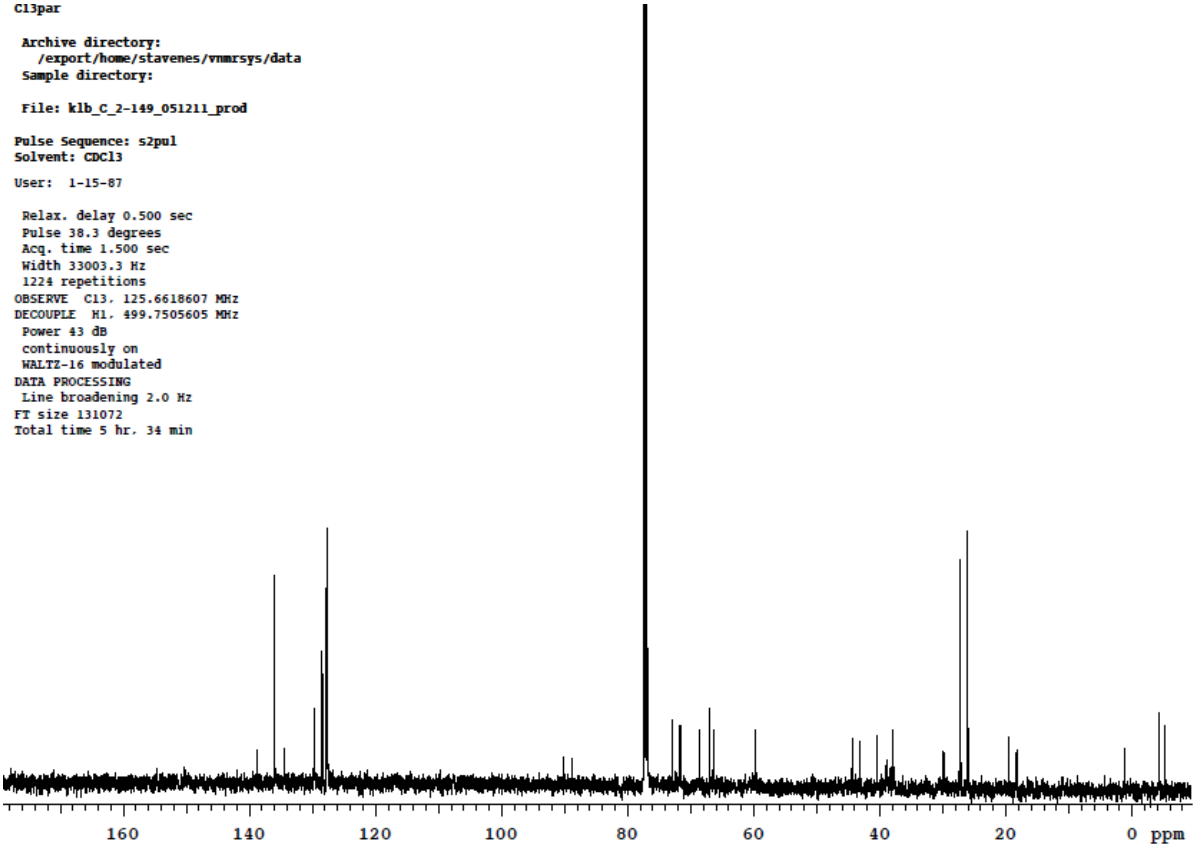
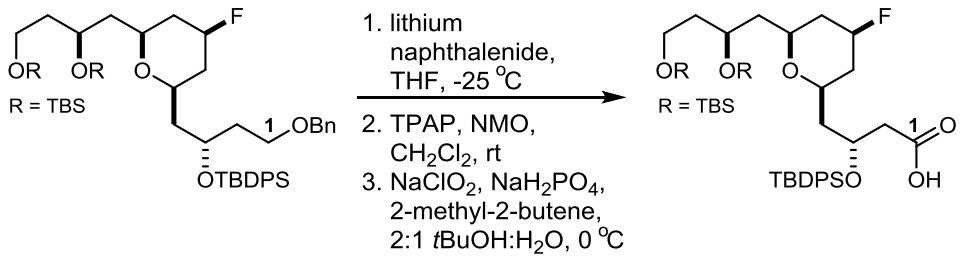




S6 ***74% over three steps*** **32**

Procedure for C1 carboxylic acid 32

A solution of C1 benzyl ether **S6** (20.7 mg, 0.025 mmol) in THF (2.0 mL) was cooled in a CO_2/MeCN bath (external temperature: -25 ºC). The lithium naphthalenide reagent (ca. 1.00 M in THF, 500 µL, 0.50 mmol) was added dropwise over 10 min. After complete addition of the lithium naphthalenide reagent, the opaque green mixture was allowed to stir for 20 min, at which time TLC demonstrated complete consumption of starting material. The reaction was quenched with saturated aqueous NH_4Cl (2 mL), effecting the immediate disappearance of color. The biphasic mixture was diluted with H_2O (2 mL) and Et_2O (5 mL). The organic and aqueous layers were partitioned, and the aqueous layer was extracted with Et_2O (2 x 5 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated to afford a residue that was purified via silica gel chromatography (15% EtOAc:pet. ether) to afford C1 alcohol 15.1 mg (~82%) as a clear, colorless oil. R_f = 0.25 (10% EtOAc in pet. ether), *p*-anisaldehyde + UV.

The crude C1 alcohol was dissolved in 1.0 mL dry CH_2Cl_2 in a dry vial under nitrogen. Powdered 4Å molecular sieves (100 mg) were added. *N*-methylmorpholine *N*-oxide (NMO) (7.1 mg, 0.061 mmol) was added as a solution in CH_2Cl_2 (0.15 mL). Tetrapropylammonium perruthenate (TPAP) (0.7 mg, 0.002 mmol) was added as a solution in CH_2Cl_2 (0.15 mL). The resulting green mixture was allowed to stir at ambient temperature for 15 min, at which time TLC demonstrated complete consumption of starting material. At 15 min, the reaction was directly loaded onto a plug of silica and eluted with 20% ethyl acetate in pet. ether, affording C1 aldehyde as a colorless oil.

The aforementioned oil was dissolved in *t*BuOH (1.5 mL) under ambient atmosphere. Water (0.75 mL) was added, followed by 2-methyl-2-butene (125 µL, 1.18 mmol). The resulting cloudy mixture was stirred vigorously and cooled in an ice water bath. To this suspension was added NaH_2PO_4 (24.2 mg, 0.20 mmol) followed by $NaClO_2$ (11.0 mg, 0.12 mmol). The mixture was stirred for 25 min, at which time it was quenched with 1:1 saturated aqueous NaCl : saturated aqueous $Na_2S_2O_3$ (2 mL). The resulting mixture was diluted with water (2 mL) and Et_2O (5 mL). The layers were partitioned, and the aqueous layer was extracted with Et_2O (3 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to afford an oil that was purified via silica gel chromatography (20% EtOAc:petroleum ether) to afford 14.0 mg (74.4% over three steps) carboxylic acid **32** as a viscous, colorless oil.

*Characterization Data for C1 carboxylic acid **32**:*

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.68-7.64 (m, 4H, TBDPS), 7.45-7.35 (m, 6H, TBDPS), 4.48-4.31 (m, 1H, C7), 4.30 (quint, 1H, *J* = 5.9 Hz, C3), 3.89 (quint, 1H, *J* = 6.1 Hz, C11), 3.62 (t, 2H, *J* = 6.7 Hz, C13), 3.21 (*app* td, 1H, *J* = 11.3, 6.2 Hz, C5), 3.12 (t, 1H, *J* = 9.9 Hz, C9), 2.52 (d, 2H, *J* = 5.9 Hz, C2), 2.03-1.97 (m, 1H, C6), 1.79-1.60 (m, 6H, C4, C8, C10, C12), 1.55-1.47 (m, 1H, C10), 1.25-1.16 (m, 2H, C6, C8), 1.03 (s, 9H, TBDPS), 0.88 (s, 9H, TBS), 0.86 (s, 9H, TBS), 0.04 (s, 3H, TBS), 0.04 (s, 3H, TBS), 0.03 (s, 3H, TBS), 0.01 (s, 3H, TBS) ppm

^{13}C NMR ($CDCl_3$, 125 MHz): δ = 175.5, 136.0, 136.0, 133.9, 133.6, 130.0, 129.9, 127.9, 127.8, 89.2 ($^1J_{CF}$ = 175.8 Hz), 71.9, 71.8, 71.7, 68.4, 66.3, 59.9, 44.1, 43.1, 43.0, 40.3, 38.9 ($^2J_{CF}$ = 16.5 Hz), 38.2 ($^2J_{CF}$ = 16.5 Hz), 27.1, 26.1, 26.0, 19.5, 18.4, 18.2, -4.4, -4.4, -5.2 ppm

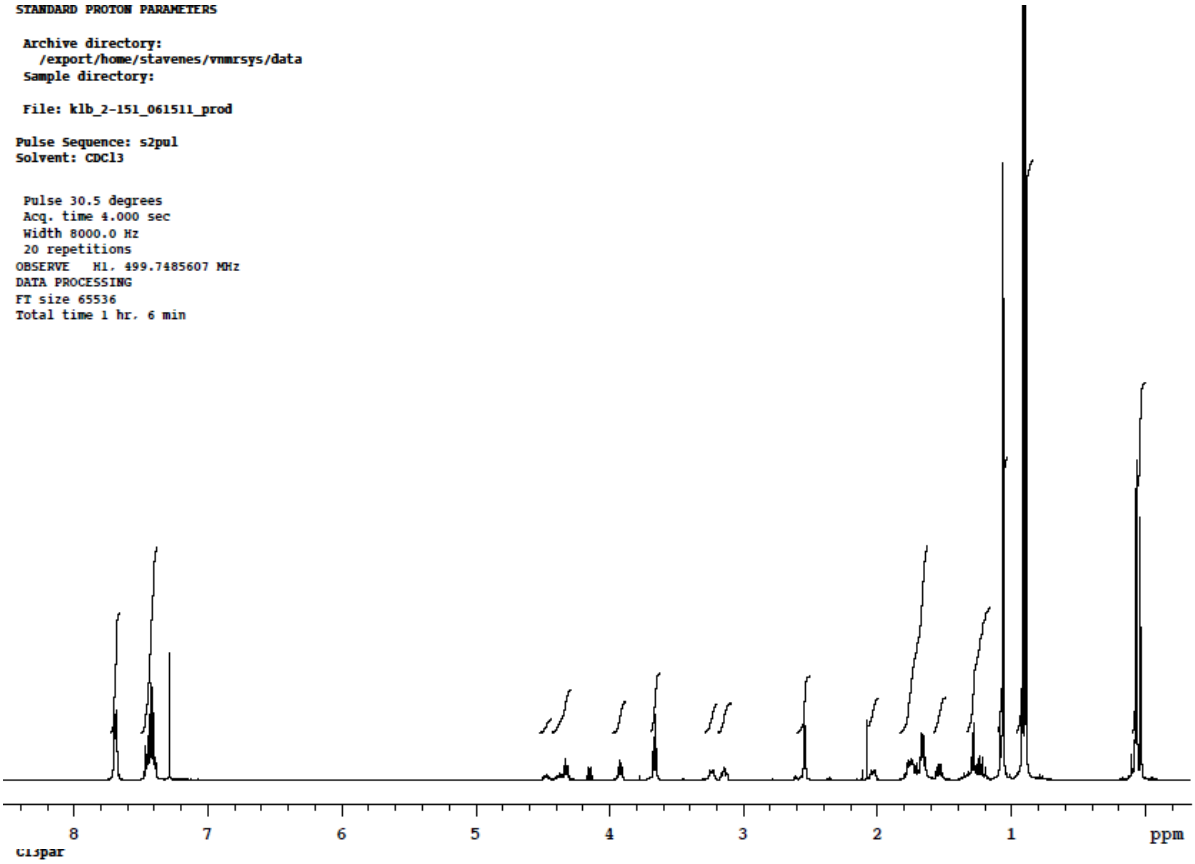
^{19}F-NMR ($CDCl_3$, 500 MHz): δ = -170.4 ppm

IR (thin film): 2929, 2855, 1731, 1472, 1427, 1367, 1254, 1154, 1106, 836 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{41}H_{69}FNaO_6Si_3^+$: 783.4278, Found: 783.4281

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23.2\ ^\circ C}} = 0.3 \pm 0.7°$ (*c* = 0.14, CH_2Cl_2)

R_f = 0.35 (15% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV


STANDARD PROTON PARAMETERS
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Sample directory:
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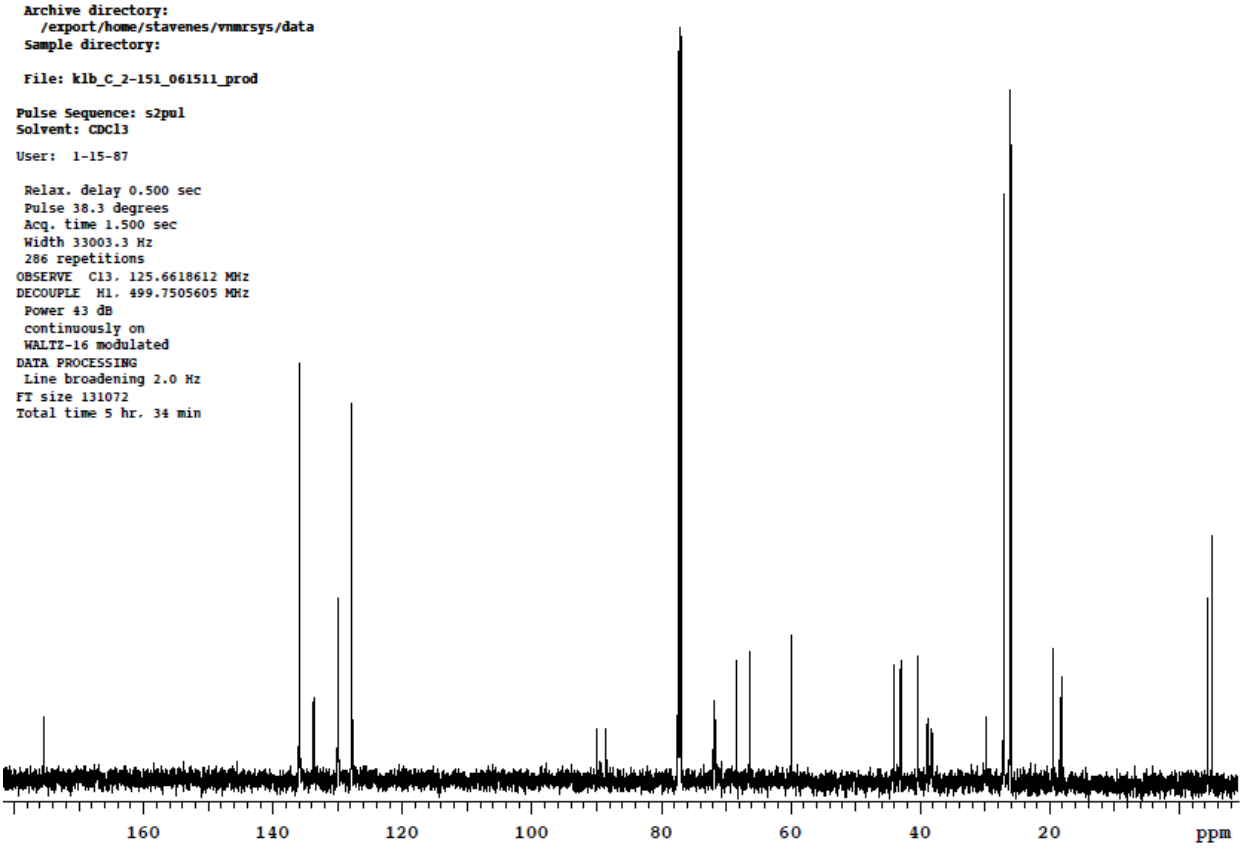
Solvent: CDCl3
Pulse 30.5 degrees
Acq. time 4.000 sec
Width 8000.0 Hz
20 repetitions
OBSERVE H1, 499.7485607 MHz
DATA PROCESSING
FT size 65536
Total time 1 hr, 6 min
8
7
6
5
4
3
2
1
ppm


C13par
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Sample directory:
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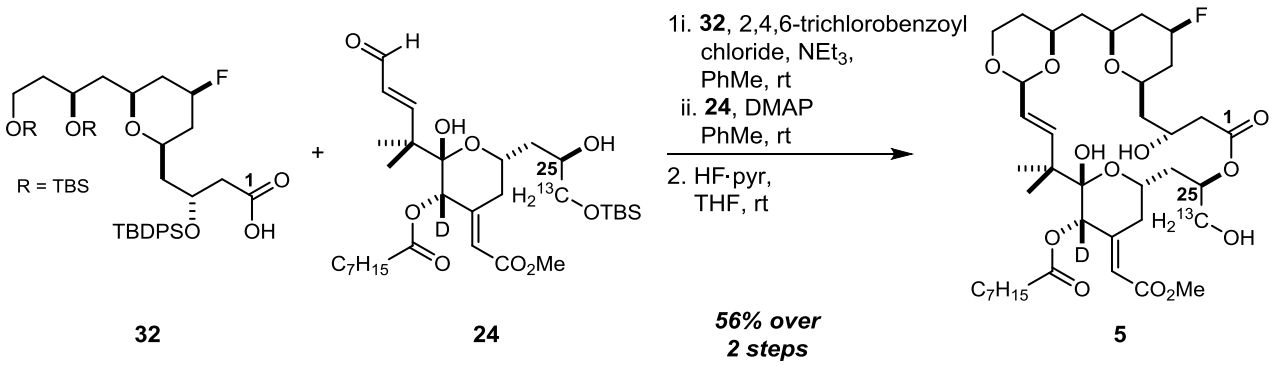
Solvent: CDCl3
User: 1-15-87
Relax. delay 0.500 sec
Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
286 repetitions
OBSERVE C13, 125.6618612 MHz
DECOUPLE H1, 499.7505605 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 5 hr, 34 min
160
140
120
100
80
60
40
20
ppm



32 **24** **5**

Procedure for labeled analog 5

Northern fragment **32** (400 µL from a 2.0 mg/100 µL PhMe stock solution, 0.011 mmol) was transferred into a dry, N_2-flushed vial via syringe followed by two 100 µL rinses of PhMe. Freshly distilled NEt_3 (7.3 µL, 0.053 mmol, 5 eq) was added, followed by acid chloride (2.5 µL, 0.016 mmol, 1.5 eq) in one portion each. The reaction was then stirred at rt under inert atmosphere for 4 h. Salts accumulate slowly during this time, generating a cloudy reaction mixture. After 4 h, southern fragment **24** (250 µL from a 35.1 mg/924 µL PhMe stock solution, 0.016 mmol, 1.5 eq) was added, followed by 4-dimethylaminopyridine (DMAP, 250 µL from a 26.4 mg/1.29 mL PhMe stock solution, 0.042 mmol, 4 eq) via syringe in one portion each. The reaction mixture became cloudier upon addition. After stirring for an additional 30 min at rt, the reaction mixture was loaded directly onto a silica gel column and purified via column chromatography (5→15% EtOAc:Pet. Ether). The intermediate product was obtained as an oil and carried directly onto the next step.

The crude product above was dissolved in THF (3.20 mL) under N_2 in a polypropylene tube. The solution was cooled to -78 °C (dry ice/acetone) before addition of HF·pyridine (750 µL, 28.9 mmol) dropwise over 2 min. The cooling bath was removed, and the reaction was allowed to stir at rt for 40 h. The reaction was quenched by pipetting directly (in aliquots to avoid violent bubbling) into a 75 mL solution of sat. $NaHCO_{3(aq)}$. The organic and aqueous layers were separated, and the aqueous phase was extracted 5x25 mL EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a residue. This residue was subjected to column chromatography (60→100% Et_2O:Pet. Ether) with 5% solvent strength increments to give pure analog **5** as a white residue (4.4 mg, 56.2% over 2 steps).

*Characterization Data for labeled analog **5**:*

^{1}H NMR (C_6D_6, 600 MHz): δ = 6.43-6.40 (m, 2H, C34, C17), 5.82 (dd, 1H, *J* = 16.0, 7.4 Hz, C16), 5.59 (s, 1H, C19-OH), 5.53-5.49 (m, 1H, C25), 5.38 (d, 1H, *J* = 7.4 Hz, C15), 4.48-4.42 (m, 2H, C23, C3-OH), 4.33-4.26 (m, 1H, C22), 4.15-4.02 (m, 2H, C3, C7), 3.90 (dd, 1H, *J* = 11.2, 4.3 Hz, C13), 3.79-3.55 (m, 2H, C26, C13), 3.54-3.32 (m, 2H, C26, C11), 3.21 (s, 3H, CO_2Me), 2.73-2.66 (m, 1H, C5), 2.58-2.49 (m, 1H, C9), 2.38-2.32 (m, 1H, C22), 2.26-2.05 (m, 4H, C2, C40), 1.79-1.71 (m, 1H, C24), 1.65-1.60 (m, 1H, C10), 1.59-1.48 (m, 5H, C41, C24, C12, C8), 1.47 (s, 3H, C18-Me), 1.41-1.30 (m, 4H, C44, C6, C4), 1.25 (s, 3H, C18-Me), 1.24-1.19 (m, 2H, C45), 1.17-1.01 (m, 6H, C42, C43, C8, C6), 0.86 (t, 3H, *J* = 7.3 Hz, C46), 0.76-0.69 (m, 3H, C12, C10, C4) ppm

^{13}C NMR (C_6D_6, 125 MHz): δ = 172.2, 171.6, 166.8, 152.4, 142.5, 126.8, 120.6, 103.1, 99.7, 88.0 ($^1J_{CF}$ = 177.5 Hz), 75.8 ($^3J_{CF}$ = 10.9 Hz), 75.6, 74.5,[25] 72.7 ($^3J_{CF}$ = 11.0 Hz), 72.0 ($^1J_{CC}$ = 40.3 Hz), 68.6, 66.1, 65.8 (^{13}C label), 65.2 ($^2J_{CC}$ = 3.2 Hz), 50.6, 45.6, 42.5, 42.5, 39.2, 38.6 ($^2J_{CF}$ = 25.2 Hz), 38.4 ($^2J_{CF}$ = 25.5 Hz), 36.2, 34.8, 32.6, 32.0, 31.8, 29.3, 29.3, 25.1, 24.9, 23.0, 19.7, 14.3 ppm

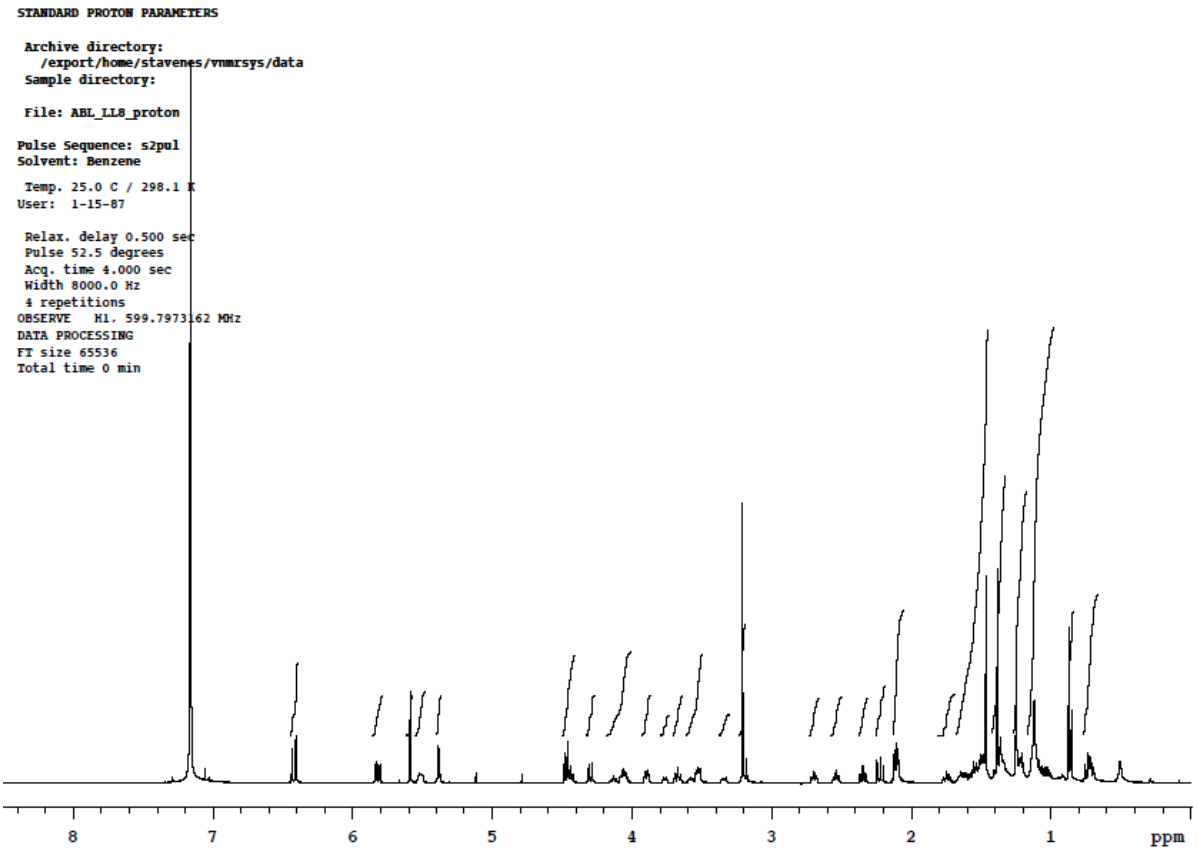
^{19}F-NMR ($CDCl_3$, 500 MHz): δ = -171.4 ppm

IR (thin film): 2929, 2855, 1731, 1472, 1427, 1367, 1254, 1154, 1106, 836 cm^{-1}

HRMS (ES+, *m/z)* calculated for $C_{37}\{^{13}C\}H_{58}DFNaO_{13}^+$: 767.3928, Found: 767.3915

R_f = 0.25 (80% EtOAc in pentane), one black spot, *p*-anisaldehyde + UV

[25] This resonance (corresponding to C20) was only visible via an HMBC experiment due to ^{2}H splitting.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User: 1-15-87
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Pulse 52.5 degrees
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Width 8000.0 Hz
4 repetitions
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Total time 0 min
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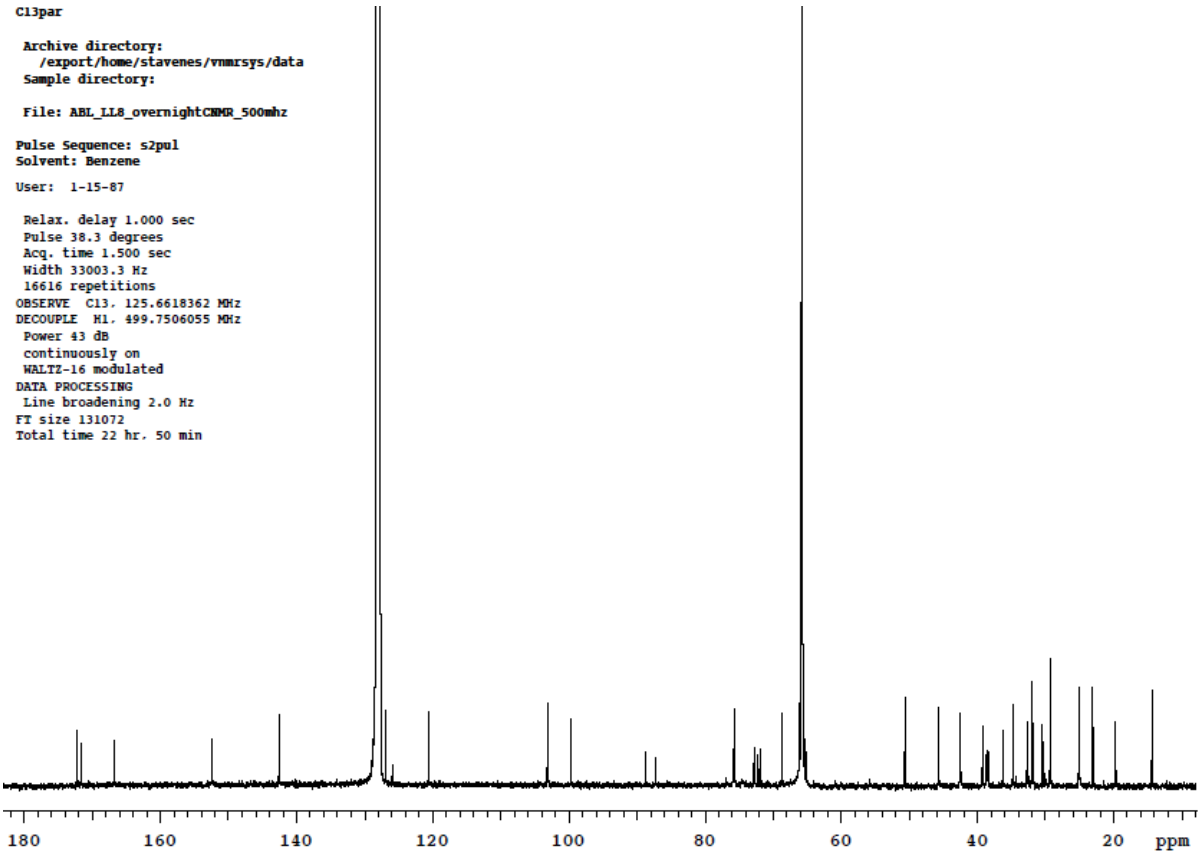
7
6
5
4
3
2
1
ppm


C13par
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User: 1-15-87
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Pulse 38.3 degrees
Acq. time 1.500 sec
Width 33003.3 Hz
16616 repetitions
OBSERVE C13, 125.6618362 MHz
DECOUPLE H1, 499.7506055 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 22 hr, 50 min
180
160
140
120
100
80
60
40
20
ppm