Supporting Information

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S1. Fractions of reaction

Pairwise estimation. In a given trial, the fraction of reaction of each sample aliquot was determined from the HPLC peak integrations observed during isolation of the residual substrate. If x_i and y_i are the peak integrations for the residual substrate and product formed, respectively, observed in the i-th sample each divided by the peak integration of pMAP, then a pairwise estimate of the i-th fraction of reaction can be calculated from the ratio

$$\frac{1 - x_i/x_j}{1 - (x_i/x_j)/(y_i/y_j)}, \quad i \neq j. \qquad [11]$$

If n reaction aliquots are obtained, then $n-1$ such pairwise estimates are obtained for each fraction of reaction, and the median value is taken as the overall best point estimate. In the present study, $n = 6$ reaction aliquots were taken in each experimental trial.

There are three reasons this approach was used to determine the fractions of reaction. First, it does not rely on the extinction coefficients of the substrate and product, which are imprecisely known, at the HPLC detector wavelength. Second, it does not require standardization of all peak integrations versus any one peak integration. Finally, the standard deviation of the pairwise estimates of any one fraction of reaction offers a measure of its uncertainty (see below) (1).

Error in variables. As described above, pairwise estimation of the fractions of reaction using Eq. (11) provides a measure of their uncertainty. If this uncertainty is large with respect to the range of values measured, it causes an error in variables problem and can bias the inferences. The standard deviations of the pairwise fractions of reaction had a median value of 0.06 and an interquartile range of 0.04 to 0.10. However, no meaningful differences were observed between regressions of individual trials ignoring error in variables and those accounting for this variability by introducing additional Gaussian parameters for each fraction of reaction (44). This robustness against error in variables is likely due to the roughly linear form of $R(f; {}^{D}k, \rho)$ with slope close to 0 when ${}^{D}k \approx 1$. Therefore, the hierarchical Bayesian analysis detailed in the *Methods* ignores error in variables in order to minimize model complexity.

S2. Processing of mass spectrometry data

In any given processed spectrum, a number of signals could be observed corresponding to different ion adducts of the residual substrate. Of these complexes, the $[M + \mathrm{Na}]^+$ ion of the unlabeled and deuterated substrate were consistently observed in all spectra with good intensity at 425.25 and 426.25 m/z, respectively. For this reason, the $[M + \mathrm{Na}]^+$ ion species forms the basis of the following analysis. However, consideration of other ion species generally gave similar results when it was possible to observe them at a consistently reasonable intensity.

MS peaks corresponding to the $[M + \mathrm{Na}]^+$ ions for the monoisotopic substrate and the $M + 1$ polyisotopic substrate were analyzed in pairs by fitting a sum of Gaussian lineshapes to the averaged intensities $I(m)$ at each acquired mass to charge ratio m according to

$$I(m; \beta_1, \beta_2, \mu_1, \mu_2, \sigma) = \sum_{i=1}^{2} \beta_i \exp\left(\frac{-(m - \mu_i)^2}{2\sigma^2}\right). \qquad [12]$$

In doing so, five parameters were fit using the standard Gauss-Newton nonlinear least squares regression algorithm. The parameters μ_1 and μ_2 represent the peak center m/z values for the M and $M+1$ peaks and were allowed to float independently of one another. The parameters β_1 and β_2 represent the corresponding maximum peak intensities and were allowed to float independently of one another. The parameter σ denotes the scale of each Gaussian lineshape and was constrained to be the same for both peaks. An example of a fitted pair of peaks is shown in Fig. S1.

The ratio of two integrated Gaussian lineshapes having the same scale parameter (i.e., σ) is equal to the ratio of the peak maxima. Therefore, the MS signal intensity ratio R_M ($M + 1$ intensity divided by M intensity) was determined from the ratio

$$R_M = \frac{\hat{\beta}_2}{\hat{\beta}_1}, \qquad [13]$$

where $\hat{\beta}_1$ and $\hat{\beta}_2$ are the fitted values of β_1 and β_2 from Eq. (12). The MS intensity ratio R_M for a sample is not equivalent to the ratio of mole fractions for the labeled versus unlabeled isotopologs in the sample. This is because the $M + 1$ peak corresponds to a mixture of isotopologs. It has previously been shown (15, 19, 45) that the observed enrichment ratio r is related to the MS intensity ratio according to

$$r = R_M - \alpha, \qquad [14]$$

where α is a correction term that accounts for natural abundance labeling in the sample. The value 0.2650 was used to correct all MS signal intensity ratios to enrichment ratios and is derived in Sec. S5. It should be emphasized that the correction term α is itself susceptible to kinetic isotope effects and changes with the fraction of reaction. The correction term may also vary between the preparations of different isotopologs. Section S5 also provides a quantitative analysis of these potential sources of bias and demonstrates that they will not be meaningful within the overall uncertainty of the present measurements.

S3. Sensitivity analysis

Sensitivity of the posterior distribution of ${}^{D}k$ to the choice of prior was assessed by simulating the 68% and 95% highest posterior density (HPD) intervals for ${}^{C12D}k_{non}$ and ${}^{C7D}k_{non}$ when the scale (i.e., standard deviation) of $p(\log {}^{D}k)$ was decreased and increased by ca. 50% (see *Methods*). When the scale is reduced from 0.18 to 0.10, the combined prior probability of large KIEs (i.e., greater than 20%) becomes less

than 10%; however, the posterior HPD intervals are essentially unaffected as shown in Tbl. S1. When the scale is increased from 0.18 to 0.27, the prior probability of large KIEs (i.e., between approximately 20 and 40%) increases to 50%, and the prior probability of extreme values (i.e., greater than 40%) becomes 20%. In this case, the simulated 68% interval is again effectively unchanged though there is some broadening of the 95% HPD intervals due to enchanced tailing. Therefore, the influence of the prior only becomes apparent when it is relaxed to the point that it admits extreme values for ${}^{D}k$ with significant probability. This implies that the prior described by Eq. (3) is indeed representative of prior knowledge regarding α-secondary deuterium kinetic isotope effects and that inferences are appropriately informed by the data.

S4. Total samples and outliers

Summary parameters for the simulated posterior marginal distributions of all parameters for each nonenzymatic KIE analyzed are shown in Tbl. S2. The corresponding results for the enzymatic KIEs are shown in Tbl. S3.

Nonenzymatic. A total of 108 samples were collected over all the nonenzymatic KIEs (18 per KIE). One sample was fouled and unusable, and one sample associated with a ${}^{C12D}k_{non}$ measurement was excluded as an outlier due to clear deviation from the overall trend of the data (see C12D-trial-3 in Fig. S3). Thus, a total of 106 samples were used in the analysis of the nonenzymatic KIEs with at least 17 samples over 3 trials per KIE.

Enzymatic. A total of 114 samples were collected over all the enzymatic KIEs (18 per KIE and 24 for ${}^{C7D}k_{enz}$). Two samples each from the ${}^{C7D}k_{enz}$ and ${}^{C2D}k_{enz}$ sample sets that were collected and analyzed in the same batch demonstrated clear deviations from the overall trend in the same direction (see C7D-trial-1 and C2D-trial-1 in Fig. S4). This suggested that they had been fouled during sample preparation and were therefore excluded from the analysis. One sample from the ${}^{C14D}k_{enz}$ (C14D-trial-3) and a third sample from the ${}^{C2D}k_{enz}$ (C2D-trial-2) collections were also identified as deviating significantly from the overall trend in the data and their corresponding mass spectra showed additional overlapping peaks inconsistent with the other spectra in the set. Therefore, they were also excluded from the analysis. Thus a total of at least 17 samples over at least 3 trials were collected for each KIE other than ${}^{C2D}k_{enz}$, which had 15 samples over 3 trials.

S5. The correction term α

The parameter α is used to correct the MS signal intenstity ratios to isotopolog enrichment ratios according to Eq. (14). As indicated in Sec. S2, this parameter is susceptible to isotope effects and may also vary between synthetic preparations of the istopologs. In the following sections, we derive the value of α used in the present studies, evaluate how α can *change* over the course of an experiment and analyze the consequences of using an *inaccurate* value of α.

Derivation. In the measurement of any given kinetic isotope effect, the centers of an ion observed by MS can be divided into the single position of interest (i.e., the KIE *target site*) and all remaining positions (i.e., the *nontarget sites*). The following analysis adapted from that of Anderson and coworkers (19) assumes that *the presence of an isotope at any given site is independent of the presence of an isotope at any other site.* In other words, each position in the mixture of isotopologs is assumed to be independently enriched. Let $P_i({}^{1}\mathrm{H})$ denote the probability of observing protium at the i-th hydrogenic site in the sodiated ion adduct of **4**. Similarly define $P_j({}^{12}\mathrm{C})$ and $P_k({}^{16}\mathrm{O})$ for the carbogenic and oxygenic sites, respectively.*

If χ_H and χ_D are the mole fractions of the unlabeled and labeled isotopologs in the sample, then the integrated MS signal intensity β_1 of the monoisotopic ion will then be proportional to the probability of finding the monoisotopic ion species according to

$$\beta_1 \propto \chi_H \prod_{i=1}^{n_H-1} P_i({}^{1}\mathrm{H}) \prod_{j=1}^{n_C} P_j({}^{12}\mathrm{C}) \prod_{k=1}^{n_O} P_k({}^{16}\mathrm{O}), \qquad [15]$$

where n_H, n_C and n_O are the total number of hydrogenic, carbogenic and oxygenic sites in the ion and the n_H-th hydrogenic site is considered the target site, i.e.,

$$\chi_H = P_{n_H}({}^{1}\mathrm{H}), \qquad [16a]$$

$$\chi_D = P_{n_H}({}^{2}\mathrm{H}). \qquad [16b]$$

Likewise, the integrated signal intensity of the polyisotopic species at ca. 1 m/z unit greater will be given by

$$\begin{aligned}\beta_2 \propto \chi_D &\prod_{i=1}^{n_H-1} P_i({}^{1}\mathrm{H}) \prod_{j=1}^{n_C} P_j({}^{12}\mathrm{C}) \prod_{k=1}^{n_O} P_k({}^{16}\mathrm{O}) + \\ \chi_H &\prod_{j=1}^{n_C} P_j({}^{12}\mathrm{C}) \prod_{k=1}^{n_O} P_k({}^{16}\mathrm{O}) \sum_{m=1}^{n_H-1} \left(P_m({}^{2}\mathrm{H}) \prod_{i\neq m}^{n_H-1} P_i({}^{1}\mathrm{H}) \right) + \\ \chi_H &\prod_{i=1}^{n_H-1} P_i({}^{1}\mathrm{H}) \prod_{k=1}^{n_O} P_k({}^{16}\mathrm{O}) \sum_{m=1}^{n_C} \left(P_m({}^{13}\mathrm{C}) \prod_{j\neq m}^{n_C} P_j({}^{12}\mathrm{C}) \right) + \\ \chi_H &\prod_{i=1}^{n_H-1} P_i({}^{1}\mathrm{H}) \prod_{j=1}^{n_C} P_j({}^{12}\mathrm{C}) \sum_{m=1}^{n_O} \left(P_m({}^{17}\mathrm{O}) \prod_{k\neq m}^{n_O} P_k({}^{16}\mathrm{O}) \right). \end{aligned} \qquad [17]$$

Since the constants of proportionality relating the observed signal intensities with the corresponding probabilities should be the same in any given MS spectrum, we have $R_M = \beta_2/\beta_1$, which implies

$$R_M = \frac{\chi_D}{\chi_H} + \sum_{i=1}^{n_H-1} \frac{P_i({}^{2}\mathrm{H})}{P_i({}^{1}\mathrm{H})} + \sum_{j=1}^{n_C} \frac{P_j({}^{13}\mathrm{C})}{P_j({}^{12}\mathrm{C})} + \sum_{k=1}^{n_O} \frac{P_k({}^{17}\mathrm{O})}{P_k({}^{16}\mathrm{O})}. \qquad [18]$$

We can simplify the notation considerably by defining the *nontarget enrichment ratios* as

$$R_i^H := P_i({}^{2}\mathrm{H})/P_i({}^{1}\mathrm{H}), \qquad [19a]$$

$$R_j^C := P_j({}^{13}\mathrm{C})/P_j({}^{12}\mathrm{C}), \qquad [19b]$$

$$R_k^O := P_k({}^{17}\mathrm{O})/P_k({}^{16}\mathrm{O}). \qquad [19c]$$

Since $r = \chi_D/\chi_H$, we can write the following much more compact expression for R_M

$$R_M = r + \sum_{i=1}^{n_H-1} R_i^H + \sum_{j=1}^{n_C} R_j^C + \sum_{k=1}^{n_O} R_k^O. \qquad [20]$$

*Contributions from sodium in the $[M+\mathrm{Na}]^+$ ion can be shown to be negligible and are ignored for the sake of brevity.

Therefore, if we define α as

$$\alpha := \sum_{i=1}^{n_H-1} R_i^H + \sum_{j=1}^{n_C} R_j^C + \sum_{k=1}^{n_O} R_k^O, \quad [21]$$

we obtain

$$R_M = r + \alpha, \quad [22]$$

from which Eq. (14) follows immediately.

Equation (21) can also be used to obtain an estimate of the correction term. Assuming that the nontarget enrichment ratios at each site are similar for like isotopes, we can write

$$\alpha \approx (n_H - 1)R^H + n_C R^C + n_O R^O, \quad [23]$$

and using the standard natural abundance ratios (46)

$$R^H = P(^2\mathrm{H})/P(^1\mathrm{H}) = 1.15 \times 10^{-4}, \quad [24a]$$
$$R^C = P(^{13}\mathrm{C})/P(^{12}\mathrm{C}) = 1.08 \times 10^{-2}, \quad [24b]$$
$$R^O = P(^{17}\mathrm{O})/P(^{16}\mathrm{O}) = 3.81 \times 10^{-4}, \quad [24c]$$

we then have the following approximation for α in the case of the sodium ion adduct of **4**:

$$\begin{aligned}\alpha &\approx (34-1) \times 1.15 \times 10^{-4} \\ &\quad + 24 \times 1.08 \times 10^{-2} \\ &\quad + 5 \times 3.81 \times 10^{-4}, \\ &= 0.2650.\end{aligned}$$

This value of α was used consistently in the calculation of all enrichments.

KIEs at nontarget sites. The compounds prepared in the present study actually represent a population of isotopologs due to natural abundance labeling at each nontarget site. The presence of different isotopes at the nontarget sites will lead to additional fractionation of the residual starting material due to possible isotope effects associated with those sites. This potential confound manifests itself as an isotope effect on the correction term α which may therefore change as a function of the fraction of reaction. To assess the extent to which this may bias measurements of the enrichment r, we seek an expression for the change in α as a function of the fraction of reaction.

Equation (21) implies that we can write α at any fraction of reaction f as a sum of the enrichment ratios at each nontarget site. If N is the total number of nontarget sites, i.e.,

$$N = n_H + n_C + n_O - 1,$$

then let ${}^X\mathbf{k}$ be the column-vector of kinetic isotope effects at all nontarget sites, $\mathbf{R}_0$ be the column-vector of *initial* enrichment ratios at all nontarget sites (see Eq. (19)) and define the vector-valued function $\mathbf{g}(f; {}^X\mathbf{k})$ parameterized in terms of ${}^X\mathbf{k}$ as

$$\mathbf{g}(f; {}^X\mathbf{k}) := \begin{bmatrix} (1-f)^{1/{}^X k_1 - 1} \\ (1-f)^{1/{}^X k_2 - 1} \\ \vdots \\ (1-f)^{1/{}^X k_N - 1} \end{bmatrix}. \quad [25]$$

Since isotopologs will fractionate independently of one another, Eq. (21) implies that α can be expressed as a function of the fraction of reaction f parameterized in terms of $\mathbf{R}_0$ and ${}^X\mathbf{k}$ using the vector product

$$\alpha(f; \mathbf{R}_0, {}^X\mathbf{k}) = \mathbf{R}_0^\intercal \mathbf{g}(f; {}^X\mathbf{k}), \quad 0 \le f < 1, \quad [26]$$

since the KIEs at each site are close to unity. Here, $^\intercal$ denotes vector/matrix transposition.

The roughly unit KIEs also imply that $\mathbf{g}$ can be approximated using a first-order, multivariate Taylor expansion about the vector of unit isotope effects such that

$$\alpha(f; \mathbf{R}_0, {}^X\mathbf{k}) \approx \mathbf{R}_0^\intercal \left(\mathbf{1} + (\mathbf{1} - {}^X\mathbf{k}) \log(1-f)\right), \quad [27]$$

where $\mathbf{1}$ is the N-vector $[1, 1, \ldots, 1]^\intercal$. Since $\alpha(0; \mathbf{R}_0, {}^X\mathbf{k})$ is equal to the vector product $\mathbf{R}_0^\intercal \mathbf{1}$, we can express the net *change* in α at fraction of reaction f as

$$\Delta\alpha(f; \mathbf{R}_0, {}^X\mathbf{k}) \approx \mathbf{R}_0^\intercal (\mathbf{1} - {}^X\mathbf{k}) \log(1-f). \quad [28]$$

Note that if all the nontarget KIEs are unity (i.e., ${}^X\mathbf{k} = \mathbf{1}$), then $\Delta\alpha = 0$, whereas if they are all normal or all inverse, then $\Delta\alpha$ is an increasing or decreasing function of f, respectively, on $[0, 1)$ as expected. Having developed a general form for $\Delta\alpha$, we can use it to analyze the bias for the present experiments.

Since only those sites undergoing rehybridization are likely to exhibit relatively large KIEs (i.e., $> 1\%$), we can approximate an upper limit of the vector product $\mathbf{R}_0^\intercal(\mathbf{1} - {}^X\mathbf{k})$ by assigning 5% KIEs to the hydrogenic sites at C4, C7, C11 and C14 and 2% KIEs to the corresponding carbogenic sites all in the same direction,

$$\begin{aligned}|\mathbf{R}_0^\intercal(\mathbf{1} - {}^X\mathbf{k})| &\approx 4 \times 0.05 \times R^H + 4 \times 0.02 \times R^C, \\ &\approx 0.001.\end{aligned}$$

Furthermore, the value of this result is expected to decrease as either a mixture of inverse and normal effects is considered or one of the sites of rehybridization is a target site.

The fitting results in Tbls. S2 and S3 imply that the most credible values of the standard deviation σ_w about the regression during fitting each enrichment curve (i.e., $R(f)$ vs. f) are at least 0.004. Table S4 lists values of $\Delta\alpha(f; \mathbf{R}_0, {}^X\mathbf{k})$ versus the fraction of reaction f when $\mathbf{R}_0^\intercal(\mathbf{1} - {}^X\mathbf{k}) = \pm 0.001$ and shows that any bias incurred in the observed values of r due to changes in α on account of nontarget site KIEs is well within the uncertainty about the regression. This implies that treatment of α as a constant is a reasonable approximation.

Accuracy. To assess the severity of bias introduced due to *inaccuracies* in the choice of a constant value of α, the simplified relative rate expression (1) can be solved for ${}^Dk$ as a function of f, α, R_M and R_{M0} (i.e., the MS intensity ratio at 0% reaction) according to

$${}^Dk(f, \alpha, R_{M0}, R_M) = \frac{\log(1-f)}{\log[(1-f)(R_M - \alpha)/(R_{M0} - \alpha)]}, \quad [29]$$

where $0 < f < 1$. The bias in ${}^Dk$ specifically due to inaccuracy in the choice of α is approximated by the differential

$$d^Dk = \delta\alpha \frac{\partial {}^Dk}{\partial \alpha}, \quad [30]$$

where $\delta\alpha$ is the discrepancy between the true value of the correction term (treated as a constant) and the value α used in the analysis (i.e., 0.2650). The partial derivative of ${}^Dk$

with respect to α holds f, R_M and R_{M0} constant and is to be evaluated at f, α, R_M and R_{M0}. This derivative is given by

$$\frac{\partial {}^D k}{\partial \alpha} = \frac{{}^D k^2}{\log(1-f)} \left(\frac{1}{R_M - \alpha} - \frac{1}{R_{M0} - \alpha} \right),$$

where the arguments of ${}^D k$ have been suppressed for brevity. Defining r_0 and r_f as

$$r_0 := R_{M0} - \alpha, \quad [31a]$$

$$r_f := R_M - \alpha, \quad [31b]$$

we can then express the relative bias in the measurement of ${}^D k$ due to $\delta\alpha$ as

$$\frac{d {}^D k}{{}^D k} = \frac{\delta\alpha \, {}^D k}{\log(1-f)} \left(\frac{1}{r_f} - \frac{1}{r_0} \right). \quad [32]$$

Using the approximation,

$$r_f \approx r_0 (1-f)^{1/{}^D k - 1},$$

and expanding $d {}^D k / {}^D k$ as a first-order Taylor series about ${}^D k = 1$, we get

$$\frac{d {}^D k}{{}^D k} \approx \delta\alpha ({}^D k - 1)/r_0. \quad [33]$$

Therefore, if $r_0 > 0.35$ (see ρ_i in Tbls. S2 & S3) and the KIEs are roughly 5%, then a 15% inaccuracy in α (i.e., $|\delta\alpha| \leq 0.04$) translates to an ca. 0.5% bias in ${}^D k$, which is well within the uncertainty of the present KIE estimates. Since point estimates of α based on MS of natural abundance material suggested that α was accurate to 10%, the measurements were considered robust to inaccuracies in this correction term.

S6. Enzymatic vs. nonenzymatic KIEs

In the absence of the SpnF enzyme, the measured KIE (i.e., ${}^D k$) corresponds to the isotope effect on the apparent first order rate constant k_{non} for nonenzymatic cyclization. Likewise, competitive measurements of enzymatic reactions furnish isotope effects on the specificity constant (14)

$$k_{enz} := (V/K)/e_0, \quad [34]$$

where e_0 denotes the total enzyme used in the experiment. Thus, ${}^D k$ would be replaced by

$${}^D k_{enz} := {}^D (V/K), \quad [35]$$

for reactions run with enzyme. In the case of SpnF, however, the nonenzymatic reaction competes with the enzymatic reaction introducing a bias to the observed KIE that needs to be appraised.

In the experiments with SpnF present, the labeled and unlabeled substrate (i.e., reactant) will be consumed according to the rate equations

$$\left. \frac{ds_H}{dt} \right|_t = -\left(k^H_{non} + k^H_{enz} e(t) \right) s_H(t), \quad [36a]$$

$$\left. \frac{ds_D}{dt} \right|_t = -\left(k^D_{non} + k^D_{enz} e(t) \right) s_D(t), \quad [36b]$$

where $s_H(t)$ and $s_D(t)$ are the concentrations of the unlabeled and labeled substrate at time t, respectively, and $e(t)$ is the concentration of the free enzyme at time t. These differential equations imply

$$\frac{ds_H/dt|_t}{ds_D/dt|_t} = \frac{k^H_{non} + k^H_{enz} e(t)}{k^D_{non} + k^D_{enz} e(t)} \cdot \frac{s_H(t)}{s_D(t)}. \quad [37]$$

Therefore, whenever the enzymatic or nonenzymatic reaction can be neglected, the ratio

$$\frac{k^H_{non} + k^H_{enz} e(t)}{k^D_{non} + k^D_{enz} e(t)}$$

reduces to the expected constant, and Eq. (1) can be derived (20). However, in the experiments with SpnF present, this ratio is time-dependent, which precludes a facile correction by which ${}^D k_{enz}$ can be extracted from the apparent KIE.

An alternative approach is to identify conditions where the majority of reaction flux is via enzyme-catalyzed cyclization such that the bias introduced to the observed isotope effect is negligible given the precision of the estimates. Under the present conditions, the combined initial substrate concentrations were approximately 1.5 mM with measurements generally taken to less than 90% reaction. Consequently, a total substrate range of ca. 1.5–0.15 mM is reflected in the experiments, and the enzyme remains approximately 90–60% saturated throughout the experiment given its Michaelis constant of approximately 120 μM (1). Therefore, it may be possible to approximate the fractional concentration of free enzyme ϕ as roughly constant. When this assumption is reasonable,[†] the apparent KIE will be approximated by

$${}^D k_{app} \approx \frac{k^H_{non} + \phi e_0 k^H_{enz}}{k^D_{non} + \phi e_0 k^D_{enz}}, \quad [38]$$

where e_0 is the total enzyme concentration. Therefore, our goal is to find an expression for the difference between the apparent and enzymatic KIEs, i.e.,

$$\Delta {}^D k := {}^D k_{app} - {}^D k_{enz}, \quad [39]$$

that suggests conditions where this difference is minimized in so far as the experiment remains feasible.

Combining Eq. (38) and Eq. (39) we get

$$\Delta {}^D k \approx \frac{k^H_{non} ({}^D k_{non} - {}^D k_{enz})}{k^H_{non} + \phi e_0 k^H_{enz} ({}^D k_{non}/{}^D k_{enz})}. \quad [40]$$

Since the isotope effects are both close to unity and ${}^D k_{non}/{}^D k_{enz} \approx 1$, the denominator term in this expression approximates the apparent first order rate constant for the consumption of substrate when enzyme is present. We can therefore rewrite Eq. (40) in terms of half-lives, which are experimentally more convenient. Doing so yields

$$\Delta {}^D k = \frac{t_{1/2}}{t^{non}_{1/2}} ({}^D k_{non} - {}^D k_{enz}), \quad [41]$$

where $t^{non}_{1/2} \approx 24$ min is the half-life in the absence of enzyme (1), and $t_{1/2}$ is the half-life when enzyme is present.

Assuming the difference between ${}^D k_{non}$ and ${}^D k_{enz}$ is unlikely to be greater than 0.05, a bias of $\Delta {}^D k \approx 0.005$ (i.e., 0.5% error) suggests using a half-life of $t_{1/2} \approx 2.5$ min, which is experimentally feasible. Therefore, if the reaction reaches roughly 80% completion within 6 min, the relative flux through the enzymatic reaction will be sufficient to ensure that the bias from the nonenzymatic reaction is within the uncertainty of the measurement.

[†] Numerical analysis of the differential equations in Eq. (36) indicated that this was indeed the case.

S7. Computed KIEs

The published coordinates of the optimized reactant and transition state structures for the gas-phase model of Hess & Smentek (13) were used as inputs for *Gaussian03W* frequency calculations with the same basis set and level of theory (B3LYP/6-31G(d)) (47). The resulting Hessian matrix in Cartesian coordinates was mass-weighted and diagonalized using scripts written in *Python* with the *NumPy/SciPy* packages (48, 49) in order to obtain the frequencies for the normal modes of vibration. The vibrational frequencies were scaled by 0.9614 (50) and used to calculate the reduced partition functions at 300 K for the reactant and transition states from which the KIEs were obtained as previously described (51, 52).

S8. Synthesis of isotopologs

Overall synthetic schemes are shown in Figs. S5, S6 and S7.

Fragment A. Fragment A was prepared according to a previously reported procedure(2).

(S)-5-Hydroxy-N-methoxy-N-methylheptanamide (12). (−)-(1*S*,2*R*)-*N*,*N*-dibutylnorephedrine (DBNE, 0.273 mL, 0.984 mmol) was added to a mixture of aldehyde **11** (2.61 g, 16.4 mmol) in 50 mL anhydrous hexanes at room temperature, and the reaction was stirred at room temperature for 30 min (53). The reaction was then cooled to 0 °C and diethyl zinc (1.1 M in toluene, 37 mL, 41 mmol) was added. After stirring for 24 h, the reaction was quenched by the addition of a saturated ammonium chloride solution (30 mL). The mixture was extracted with CH_2Cl_2 (30 mL ×5), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate: 1/3) to afford alcohol **12** (2.05 g, 10.8 mmol, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.65 (s, 3H, OMe), 3.48 (ddd, J = 12, 7.9, 4.5 Hz, 1H, 5-H), 3.15 (s, 3H, NMe), 2.43 (br t, J = 5.5 Hz, 2H, 2-H), 1.77–1.67 (m, 2H, 3-H), 1.53–1.37 (m, 4H, 4-H and 6-H), 0.91 (t, J = 7.5 Hz, 3H, 7-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7, 72.6, 61.2, 36.5, 32.2, 31.6, 30.1, 20.3, 9.90. HRMS (CI, positive) m/z for $C_9H_{20}NO_3$ $[M+H]^+$: calc. 190.1443, found 190.1447.

To determine the absolute stereochemistry as well as the enantiomeric purity of the product, the Mosher method (54) based on ^{19}F-NMR analysis of the diastereomeric MTPA ester derivatives of alcohol **12** was applied. ***(S)*-MTPA-ester of 12:** To a clear solution of alcohol **12** (0.05 g, 0.26 mmol) in 4 mL anhydrous CH_2Cl_2 at room temperature was added dry pyridine (0.065 mL, 0.81 mmol) followed by (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid chloride ((*R*)-(−)-MTPA-Cl, 0.095 mL, 0.49 mmol). After stirring for 2 h, the reaction mixture was quenched by the addition of water (1 mL). The aqueous layer was extracted with CH_2Cl_2 (3 mL ×3), and the combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (hexanes/ethyl acetate: 1/1) to afford the (*S*)-MTPA-ester (0.086 g, 0.22 mmol). In an entirely analogous fashion, the (*R*)-MTPA-ester was prepared using (*S*)-(+)-MTPA-Cl. ***(R)*-MTPA-ester of 12:** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54–7.52 (m, 2H, Ph), 7.39–7.36 (m, 3H, Ph), 5.07–5.03 (m, 1H, 5-H), 3.63 (s, 3H, OMe), 3.54–3.53 (m, 3H, OMe from Mosher), 3.14 (s, 3H, NMe), 2.44–2.30 (m, 2H, 2-H), 1.71–1.51 (m, 6H, 3-H + 4-H + 6-H), 0.91 (t, J = 7.5 Hz, 3H, 7-H). ^{19}F NMR ($CDCl_3$, 470 MHz) δ −71.72 ppm (integration: 907.52), −71.76 (integration: 77.68). ***(S)*-MTPA-ester of 12:** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54–7.52 (m, 2H, Ph), 7.39–7.36 (m, 3H, Ph), 5.07–5.02 (m, 1H, 5-H), 3.64 (s, 3H, OMe), 3.54–3.53 (m, 3H, OMe from Mosher), 3.15 (s, 3H, NMe), 2.48–2.34 (m, 2H, 2-H), 1.72–1.57 (m, 6H, 3-H + 4-H + 6-H), 0.79 (t, J = 7.5 Hz, 3H, 7-H). ^{19}F NMR ($CDCl_3$, 470 MHz) δ −71.72 ppm (integration: 84.77), −71.76 (integration: 941.45).

(S)-N-Methoxy-5-(4-methoxybenzyloxy)-N-methylheptanamide (13). *p*-Methoxybenzyl chloride (17.2 mL, 126.8 mmol) was added to a clear solution of alcohol **12** (20 g, 105.7 mmol) in anhydrous DMF (210 mL) at 0 °C. NaH (5.07 g, 126.8 mmol) was slowly added over 20 min, and the reaction mixture was stirred overnight, while the temperature was allowed to rise to room temperature. The reaction mixture was cooled to 0 °C again and quenched by adding water (20 mL) and concentrated under reduced pressure. Brine (300 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (150 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated and purified by flash column chromatography (EtOAc/hexanes: 1/2) to afford benzyl ether **13** (23.9 g, 77.3 mmol, 73.4%). ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 7.25 (d, 2H, J = 8.9 Hz, PhH of PMB), 6.84 (d, 2H, J = 8.9 Hz, PhH of PMB), 4.44 (d, 1H, J = 11.1 Hz, CH_2 of PMB), 4.40 (d, 1H, J = 11.1 Hz, CH_2 of PMB), 3.77 (s, 3H, OCH_3 of PMB), 3.63 (s, 3H, OCH_3), 3.34–3.29 (m, 1H, 5-H), 3.15 (s, 3H, NCH_3), 2.40 (t, 2H, J = 7.4 Hz, 2-H), 2.14–1.60 (m, 2H, 3-H), 1.58–1.51 (m, 4H, 4-H + 6-H), 0.89 (t, 3H, J = 7.4 Hz, 7-H). ^{13}C NMR ($CDCl_3$, 125 MHz) d (ppm) 174.60, 159.03, 131.23, 129.28, 113.72, 79.65, 70.45, 61.16, 55.26, 33.11, 31.94, 26.27, 20.64, 9.49. HRMS (CI, positive) m/z for $C_{17}H_{28}NO_4$ $[M+H]^+$: calc. 310.2018, found 310.2021.

(S)-5-(4-Methoxybenzyloxy)heptanal (14). DIBAL-H (1.0 M in CH_2Cl_2, 110 mL, 110 mmol) was added drop-wise to a solution of benzyl ether **13** (16.9 g, 54.6 mmol) in anhydrous CH_2Cl_2 (100 mL) at −78 °C over 2 h, and the reaction mixture was stirred at −78 °C for 2 h, at which time methanol (60 mL) was added followed by a saturated solution of Rochelle's salt (90 mL). The reaction mixture was stirred for an additional 30 min at room temperature. The reaction mixture was filtered on a Celite pad and washed with CH_2Cl_2 (50 mL), and the organic layer was separated. The aqueous layer was partitioned with EtOAc (100 mL ×2), and the combined organic layers were dried over anhydrous sodium sulfate, concentrated and purified by flash column chromatography (EtOAc/hexanes: 1/9, then 1/5) to afford the aldehyde **14** (8.95 g, 35.8 mmol, 65.5%).

(2R,3S,7S)-1-((R)-4′-Benzyl-2′-oxazolidin-3′-yl)-3-hydroxy-2-methyl-7-(4-methoxybenzyloxy)nonan-1-one (16). Dibutylboron triflate (1.0 M) in CH_2Cl_2 (30.12 mL, 30.12 mmol) was added drop-wise to a clear solution of oxazolidinone **15** (5.81 g, 24.9 mmol) in anhydrous CH_2Cl_2 (100 mL) at 0 °C over 15 min and triethylamine (4.78 mL, 34.27 mmol) was added slowly over 10 min before the reaction mixture was cooled down to −78 °C (55). Aldehyde **14** (5.2 g, 20.8 mmol) (see above) in CH_2Cl_2 (20 mL) was added drop-wise over 40 min at −78 °C with continuous stirring. The reaction mixture was then warmed to 0 °C and stirred overnight. A pH 7 tetrasodium

ethylenediaminetetraacetate buffer solution (21 mL) was added to the mixture at 0 °C to quench the reaction. MeOH (42 mL) was added to the solution followed by a 30% solution of hydrogen peroxide (21 mL). After 1 h, the reaction mixture was poured into brine (150 mL), the organic layer was separated, and the aqueous layer was partitioned with CH_2Cl_2 (100 mL ×2). The combined organic layers were dried over sodium sulfate, concentrated and purified by flash column chromatography (EtOAc/hexanes = 1/4) to afford the *syn*-aldol product **16** (6.07 g, 12.6 mmol, 60.4%). ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 7.35–7.20 (m, 5H, Ph of Bn), 7.26 (m, 2H, Ph of PMB), 6.85 (dt, 2H, J = 8.7, 2.9, 2.1 Hz, Ph of PMB), 4.72–4.68 (m, 1H, CH of oxazolidinone), 4.44 (dd, 2H, J = 14.5, 11.2 Hz, CH_2 of PMB), 4.24–4.18 (m, 2H, CH_2 of Bn), 3.95–3.92 (m, 1H, 3-H), 3.79 (s, 3H, OCH_3 of PMB), 3.77–3.72 (m, 1H, 7-H), 3.33–3.29 (m, 1H, 3-H), 3.26 (dd, 1H, J = 13.4, 3.4 Hz, CH_2 of oxazolidinone), 2.84 (br, 1H, OH), 2.79 (dd, 1H, J = 13.5, 9.5 Hz CH_2 of oxazolidinone), 1.60–1.36 (m, 8H, 4-H + 5-H + 6-H + 8-H), 1.25 (d, 3H, J = 7.0 Hz, 2-CH_3), 0.91 (t, 3H, J = 7.4 Hz, 9-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 177.53, 159.02, 152.98, 135.03, 131.26, 129.41, 129.29, 128.97, 127.43, 113.72, 79.79, 79.61, 71.46, 71.40, 70.50, 70.42, 66.15, 55.27, 55.09, 42.17, 37.81, 33.98, 33.92, 33.37, 33.24, 26.29, 21.86, 10.05, 9.54. HRMS (ESI, positive) m/z for $C_{28}H_{37}NO_6Na$ $[M + Na]^+$: calc. 506.2513, found 506.2514.

(2R,3S,7S)-1-((R)-4′-Benzyl-2′-oxazolidin-3′-yl)-3-(tert-butyldimethylsilyloxy)-2-methyl-7-(4-methoxybenzyloxy)nonan-1-one (17). 2,6-Lutidine (9.58 mL, 82.7 mmol) was added to a solution of the *syn*-aldol adduct **16** in anhydrous CH_2Cl_2 (100 mL) at −78 °C followed by drop-wise addition of *t*-butyldimethylsilyl trifluoromethanesulfonate (9.12 mL, 39.70 mmol). The reaction mixture was stirred for 6 h allowing the temperature to rise to room temperature. The reaction mixture was then poured into a saturated aqueous solution of sodium bicarbonate (100 mL), partitioned with CH_2Cl_2 (100 mL ×2), dried over sodium sulfate, concentrated and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford the silyl ether **17** (17.4 g, 29.1 mmol, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.35–7.20 (m, 5H, Ph of Bn), 7.26 (d, 2H, J = 8.4 Hz, Ph of PMB), 6.85 (dt, 2H, J = 8.7, 2.9, 2.1 Hz, Ph of PMB), 4.55–4.59 (m, 1H, CH of oxazolidinone), 4.43 (dd, 2H, J = 19.3, 11.1 Hz, CH_2 of PMB), 4.13 (dd, 1H, J = 9.0, 2.2 Hz, CH_2 of Bn), 4.09 (dd, 1H, J = 8.9, 7.3 Hz, CH_2 of Bn), 4.01 (q, 1H, J = 5.5 Hz, 3-H), 3.77 (s, 3H, OCH_3 of PMB), 3.30–3.25 (m, 2H, CH_2 of oxazolidinone + 7-H), 2.76 (dd, 1H, J = 13.3, 9.6 Hz, CH_2 of oxazolidinone), 1.55–1.30 (m, 8H, 4-H + 5-H + 6-H + 8-H), 1.21 (d, 3H, J = 6.9 Hz, 2-CH_3), 0.90 (t, 3H, J = 7.4 Hz, 9-H), 0.884 (s, 9H, CH_3 of TBS), 0.038 (s, 3H, CH_3 of TBS), −0.00 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 175.17, 158.94, 152.92, 135.32, 131.19, 129.37, 129.21, 129.12, 128.83, 127.22, 113.63, 113.62, 79.72, 79.70, 72.80, 70.42, 70.40, 65.86, 55.67, 55.65, 55.13, 42.75, 37.51, 35.73, 33.76, 26.27, 26.24, 25.76, 21.00, 17.96, 11.70, 11.62, 9.43, −4.16, −4.88. HRMS (ESI, positive) m/z for $C_{34}H_{51}NO_6SiNa$ $[M + Na]^+$: calc. 620.3378, found 620.3377.

(4R,5S,6S,10S)-6-(tert-Butyldimethylsilyloxy)-5-methyl-10-(4-methoxybenzyloxy)dodec-1-en-4-ol (20). A 2 M solution of $LiBH_4$ in THF (69 mL, 137.5 mmol) was added drop-wise to a solution of the silyl ether **17** (17.4 g, 29.1 mmol) in anhydrous THF (350 mL) and MeOH (3.53 mL, 87.3 mmol) over 1 h. The reaction mixture was stirred at 0 °C for 30 min, and warmed to room temperature with stirring for 3 h. The reaction mixture was then cooled to 0 °C again and quenched by adding a 15% aqueous solution of sodium hydroxide (200 mL) over 30 min and stirred for one additional hour. The reaction mixture was extracted with EtOAc (100 mL ×3), and the combined organic layers were dried with sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/4) to afford the corresponding alcohol compound **18** (7.4 g, 17.4 mmol, 59.9%).

Activated molecular sieves (4 Å, powder, 5.0 g) was added to a solution of the primary alcohol **18** (7.4 g, 17.4 mmol) in anhydrous CH_2Cl_2 (150 mL) at room temperature, and the reaction mixture was stirred for 2 h at room temperature before adding *N*-methylmorpholine-*N*-oxide (4.08 g, 34.8 mmol) and tetrapropylammonium perruthenate (TPAP) (306 mg, 0.87 mmol) stepwise. The reaction mixture was then stirred for another 1 h at room temperature. A 10% aqueous solution of sodium sulfite (50 mL) was then added, and the mixture was filtered on a Celite pad with CH_2Cl_2 (50 mL) washing. The layers were allowed to separate, and the aqueous layer was partitioned with CH_2Cl_2 (50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexanes: 1/4) to afford the aldehyde compound **19** (6 g, 14.2 mmol, 81.5%).

To a solution of (+)-diisopinocampheylchloroborane (1.6 M solution in THF, 12.1 mL, 19.31 mmol) and allylmagnesium bromide (1.0 M solution in THF, 18.46 mL, 18.46 mmol) in anhydrous THF at −78 °C was added the aldehyde compound **19** (6 g, 14.20 mmol) in anhydrous THF slowly and stirred for 4 h. MeOH (42.6 mL), 1 N NaOH (42.6 mL) and hydrogen peroxide (14.2 mL) were added sequentially at 0 °C to quench the reaction. The organic layer was separated and extracted with EtOAc (100 mL ×2). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by flash column chromatography (EtOAc/hexanes: 1/7) to afford the allylic alcohol **20** (6.16 g, 13.25 mmol, 93.3%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.23 (d, 2H, J = 8.7 Hz, Ph of PMB), 6.85 (d, 2H, J = 8.7 Hz, Ph of PMB), 5.83–5.76 (m, 1H, 2-H), 5.11–5.05 (m, 2H, 1-H), 4.44 (d, 1H, J = 11.2 Hz, CH_2 of PMB), 4.37 (d, 1H, J = 11.2 Hz, CH_2 of PMB), 3.81–3.76 (m, 5H, OCH_3 of PMB + 4-H + 6-H), 3.29–3.25 (m, 1H, 10-H), 2.68 (br s, 1H, OH), 2.29–2.17 (m, 2H, 3-H), 1.60–1.40 (m, 7H, 5-H + 7-H + 9-H + 11-H), 1.33–1.17 (m, 2H, 8-H), 0.89 (t, 3H, J = 7.4 Hz. 12-H), 0.88–0.86 (m, 12H, 5-CH_3 + CH_3 of TBS), 0.068 (s, 3H, CH_3 of TBS), 0.061 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 159.09, 135.49, 131.16, 129.26, 117.15, 113.74, 79.61, 77.31, 74.15, 70.48, 55.26, 39.83, 39.20, 34.90, 33.60, 26.28, 25.95, 25.87, 21.40, 18.13, 18.01, 9.53, 5.89, −3.62, −4.53. HRMS (CI, negative) m/z for $C_{27}H_{47}O_4Si$ $[M - H]^-$: calc. 463.3244, found 463.3248.

(3R,4R,5S,9S)-3,5-Bis(tert-butyldimethylsilyloxy)-4-methyl-9-(4-methoxybenzyloxy)undecan-1-ol (22). *t*-Butyldimethylsilyl trifluoromethanesulfonate (4.57 mL, 19.88 mmol) was added drop-wise to a solution of compound **20** (6.16 g, 13.25 mmol) and 2,6-lutidine (3.84 mL, 33.1 mmol) in anhydrous CH_2Cl_2 (100 mL) over 20 min at −78 °C. The reaction mixture was

stirred at −78 °C for 1 h, at which time the reaction was removed from the dry ice bath and stirred for an additional 2 h at room temperature. The reaction mixture was then poured into a saturated aqueous solution of sodium bicarbonate (100 mL), extracted with CH_2Cl_2 (100 mL ×2), dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography (EtOAc/hexanes: 1/15) to afford silyl ether compound **21** (7 g, 12.09 mmol, 91.2%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.28–7.25 (m, 2H, Ph of PMB), 6.87 (dt, 2H, J = 8.7, 2.8, 2.1 Hz, Ph of PMB), 5.84–5.76 (m, 1H, 2-H), 5.05–5.00 (m, 2H, 1-H), 4.43 (dd, 2H, J = 18.6, 11.2 Hz, CH_2 of PMB), 3.81–3.77 (m, 4H, OCH_3 of PMB + 4-H), 3.72–3.68 (m, 1H, 6-H), 3.31–3.26 (m, 1H, 10-H), 2.31–2.28 (m, 2H, 3-H), 1.65–1.40 (m, 9H, 5-H + 7-H + 8-H + 9-H + 11-H), 0.94–0.88 (m, 24H, CH_3 of TBS + 12-H + 5-CH_3), 0.05–0.03 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 159.04, 135.04, 131.28, 129.24, 116.76, 113.71, 79.89, 72.66, 72.20, 70.50, 55.27, 40.55, 39.64, 35.04, 33.95, 26.31, 25.96, 20.95, 18.15, 9.50, 9.38, −3.80, −4.46. HRMS (ESI, positive) m/z for $C_{33}H_{62}O_4Si_2Na$ $[M + Na]^+$: calc. 601.4079, found 601.4078.

To a solution of the silyl ether compound **21** (7 g, 12.09 mmol) in THF (48 mL), acetone (48 mL) and pH 7 phosphate buffer (48 mL) at room temperature was added *N*-methylmorpholine oxide (NMO, 2.13 g, 18.14 mmol) followed by osmium tetroxide (0.154 g, 0.60 mmol), and the reaction was stirred at room temperature overnight. The reaction was poured into a 10% solution of sodium thiosulfate ($Na_2S_2O_3$, 50 mL), extracted with ethyl acetate (50 mL ×3), washed with brine (40 mL), dried over anhydrous sodium sulfate, concentrated under reduced pressure and used without further purification. Sodium periodate (10.34 g, 48.36 mmol) was then added to a clear solution of the crude oil in THF (150 mL) and pH 7 phosphate buffer (50 mL) at room temperature, and the reaction was stirred for 3 h. Next, the reaction was poured into a saturated solution of sodium bicarbonate (50 mL), extracted with ethyl acetate (50 mL ×3), washed with brine (40 mL), dried over anhydrous sodium sulfate, concentrated under reduced pressure and used without further purification. To a clear solution of the crude aldehyde in ethyl alcohol (70 mL) at room temperature was added sodium borohydride (732 mg, 19.34 mmol), and the reaction was stirred for 1 h, at which time the reaction was poured into a saturated solution of ammonium chloride (50 mL), extracted with ethyl acetate (50 mL ×3), washed with brine (40 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/19) to afford the primary alcohol **22** (6.58 g, 11.29 mmol, 93.4% over 3 steps). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.26–7.23 (m, 2H, Ph of PMB), 6.86–6.84 (m, 2H, Ph of PMB), 4.44 (dd, 1H, J = 11.2, 5.0 Hz, CH_2 of PMB), 4.39 (dd, 1H, J = 11.2, 6.6 Hz, CH_2 of PMB), 3.88–3.85 (m, 1H, 3-H), 3.80–3.75 (m, 4H, 1-H + OCH_3 of PMB), 3.70–3.63 (m, 2H, 1-H + 5-H), 3.29–3.25 (m, 1H, 9-H), 2.28 (br s, 1H, OH), 1.88–1.71 (m, 3H, 2-H and 4-H), 1.56–1.21 (m, 8H, 10-H + 8-H + 7-H + 6-H), 0.90–0.85 (m, 24H, CH_3 of TBS, 11-H, 4-CH_3), 0.077 (s, 3H, CH_3 of TBS), 0.039 (s, 3H, CH_3 of TBS), 0.016 (s, 3H, CH_3 of TBS), 0.005 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 159.07, 131.20, 129.30, 113.74, 79.84, 72.65, 72.40, 70.54, 70.39, 59.79, 55.27, 40.32, 35.55, 35.43, 33.93, 26.29, 25.94, 21.20, 18.13, 9.79, 9.47, −3.68, −4.13, −4.49. HRMS (ESI, positive) m/z for $C_{32}H_{62}O_5Si_2Na$ $[M + Na]^+$: calc. 605.4026, found 605.4026.

(3R,4R,5S,9S)-3,5-Bis(tert-butyldimethylsilyloxy)-9-(4-methoxybenzyloxy)-4-methylundecyl-5-sulfonyl-1-phenyl-1H-tetrazole (24). 1-Phenyl-1*H*-tetrazole-5-thiol (3.017 g, 16.93 mmol), triphenyl phosphine (4.45 g, 16.93 mmol), and diisopropyl azodicarboxylate (3.36 mL, 16.93 mmol) were sequentially added to a solution of alcohol **22** (6.58 g, 11.29 mmol) in anhydrous THF (30 mL) at 0 °C. The resulting yellow suspension was stirred at 0 °C for 1 h and then warmed to room temperature over the course of an hour. The reaction mixture was concentrated under reduced pressure and subjected to flash column chromatography (EtOAc/hexanes = 1/9) to afford thioether compound **23** (7.15 g, 9.62 mmol, 85.2%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.56–7.52 (m, 5H, Ph of Ph), 7.26–7.23 (m, 2H, Ph of PMB), 6.86–6.83 (m, 2H, Ph of PMB), 4.42 (dd, 2H, J = 18.3, 11.1 Hz, CH_2 of PMB), 3.86–3.76 (m, 5H, OCH_3 of PMB + 3-H + 5-H), 3.40–3.29 (m, 3H, 1-H and 9-H), 2.20–1.95 (m, 2H, 2-H), 1.70–1.65 (m, 1H, 4-H), 1.59–1.26 (m, 8H, 6-H + 7-H + 8-H + 10-H), 0.91–0.86 (m, 24H, CH_3 of TBS + 11-H + 4-CH_3), 0.05–0.02 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 159.00, 154.29, 133.79, 131.25, 129.98, 129.72, 129.21, 123.78, 113.69, 79.75, 72.41, 72.14, 70.45, 55.23, 35.42, 33.85, 28.91, 26.27, 25.90, 21.20, 18.10, 9.76, 9.50, −3.66, −4.10, −4.38, −4.41. HRMS (ESI, positive) m/z for $C_{39}H_{67}N_4O_4Si_2S$ $[M + H]^+$: calc. 743.4416, found 743.4415.

To a solution of the thioether **23** (6.5 g, 8.75 mmol) in ethyl alcohol (50 mL) at 0 °C was added the premixed oxidant (ammonium molydate; 2.7 g, 30% H_2O_2; 10.72 mL), and the reaction was stirred at 0 °C for 24 h, at which time the mixture was poured into water (30 mL), extracted with EtOAc (50 mL ×3), washed with brine (50 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/49) to afford the sulfone **24** (6 g, 7.74 mmol, 88.5%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.71–7.69 (m, 2H, Ph of Ph), 7.62–7.57 (m, 3H, Ph of Ph), 7.26 (d, 2H, J = 9.4 Hz, Ph of PMB), 6.85 (dd, 2H, J = 8.8, 2.1 Hz, Ph of PMB), 4.46–4.39 (m, 2H, CH_2 of PMB), 3.90–3.74 (m, 7H, OCH_3 of PMB + 3-H + 5-H + 1-H), 3.31–3.29 (m, 1H, 9-H), 2.31–2.13 (m, 2H, 2-H), 1.61–1.24 (m, 9H, 4-H + 6-H + 7-H + 8-H + 10-H), 0.91–0.85 (m, 24H, CH_3 of TBS + 12-H + 4-CH_3), 0.086 (s, 3H, CH_3 of TBS), 0.067 (s, 3H, CH_3 of TBS), 0.053 (s, 3H, CH_3 of TBS), 0.019 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.03, 153.48, 133.09, 131.37, 131.20, 129.68, 129.23, 125.00, 113.72, 79.63, 71.68, 71.53, 70.49, 55.24, 52.11, 40.69, 35.45, 33.71, 26.34, 25.90, 21.43, 18.09, 18.05, 9.62, 9.49, −3.57, −4.27, −4.48. HRMS (ESI, positive) m/z for $C_{39}H_{66}N_4O_6Si_2SNa$ $[M + Na]^+$: calc. 797.4134, found 797.4143.

Fragment B.

(R)-1-(1′,3′-Dithian-2-yl)-3-(4″-methoxybenzyloxy)propan-2-ol (29). Known compound **28** was prepared from D-mannitol. D-mannitol (109.3 g, 0.60 mol) and *p*-toluenesulfonic acid (0.6 g, 3.2 mmol) were stirred in a solution of 2,2-dimethoxypropane (180 mL, 1.50 mol) and DMSO (180 mL) at room temperature for 2 d. Aqueous 3% sodium bicarbonate (360 mL) was slowly added to the reaction mixture, and the aqueous fraction

was partitioned with ethyl acetate (1 L ×3). The combined organic fractions were washed with water (300 mL ×3), dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was refluxed in hexane solution (1.8 L) for 1 h and then allowed to cool to 0 °C for 18 h. The white precipitate was filtered through a glass filter and air-dried to afford a white solid (**25**, 99.1 g, 63%).

Lead(IV) acetate (200.0 g, 0.92 mol) was slowly added portion-wise to a solution of (1*S*,2*S*)-1,2-bis((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (120.2 g, 0.46 mol) in THF (600 mL) at 0 °C while maintaining the temperature below 10 °C for 2 h. The reaction mixture was then stirred at 0 °C for 30 min and at room temperature for 30 min, followed by filtration through a Celite pad and washing with THF. Sodium borohydride (35.2 g, 0.90 mol) in aqueous 4% sodium hydroxide was slowly added to the resulting filtrate at 0 °C over 3 h while maintaining the temperature below 10 °C. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1.5 h. The reaction mixture was quenched by the addition of solid ammonium chloride to adjust the pH to 8.0. The reaction mixture was then filtered through Celite, and the organic fraction was separated. The aqueous fraction was saturated with sodium chloride and partitioned with ethyl acetate (300 mL ×3). The combined organic layers were washed with aqueous 5% sodium hydroxide in a saturated sodium chloride solution (1 L), dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was vacuum-distilled at 90 °C to afford **26** (91.9 g, 76%).

Sodium hydride (33.2 g, 0.83 mol) was added portion-wise to a solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (91.5 g, 0.69 mol) in anhydrous DMF (1 L) at 0 °C over 30 min. After stirring for 30 min at 0 °C, PMBCl (124.1 g, 0.79 mol) was added to the reaction mixture over 30 min. The reaction mixture was allowed to warm to room temperature with vigorous stirring over 18 h. After quenching the reaction mixture in an ice-bath (1 L), the aqueous layer was partitioned with ethyl acetate (500 mL ×3). The combined organic layers were washed with brine (500 mL), dried over sodium sulfate and concentrated under reduced pressure to afford the crude residue. The crude residue (174 g, 0.69 mol) was stirred in methanol (1.75 L) with (±)-camphorsulfonic acid (8.0 g, 34.6 mmol) at room temperature for 2 d. The reaction mixture was concentrated under reduced pressure and mixed with water (1 L) and ethyl acetate (1 L). The organic layer was partitioned with ethyl acetate (500 mL ×2), and washed out with brine (500 mL). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/1) to afford **27** (132.0 g, 90%).

p-Toluenesulfonyl chloride (148.0 g, 0.78 mol) was added to a solution of the diol **27** (132.0 g, 0.62 mol), triethylamine (121.4 mL, 0.87 mol) and di-*n*-butyltin oxide (7.7 g, 31.1 mmol) in CH_2Cl_2 (1.5 L) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature with stirring for 18 h. The reaction mixture was quenched with dilute hydrochloric acid solution (0.1 N, 400 mL), and partitioned with CH_2Cl_2 (200 mL ×2). The combined organic layers were washed with brine (500 mL), dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was directly used for the epoxidation without further purification. Sodium hydride (29.9 g, 0.75 mol) was added to a solution of the residue in THF (1.3 L) at 0 °C over 30 min under a nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by the addition of a saturated solution of ammonium chloride (500 mL) at 0 °C, and the aqueous fraction was partitioned with ethyl acetate (500 mL ×2). The combined organic layers were washed with brine (300 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/3) to afford **28** (83 g, 69%).

n-Butyl lithium (2.5 M solution in hexanes, 4.95 mL, 12.4 mmol) was added to a solution of 1,3-dithiane (1.44 g, 11.9 mmol) in tetrahydrofuran (10 mL) at −30 °C, and the mixture was stirred for 2 h while the temperature was maintained below −10 °C. After warming to 0 °C, the epoxide **28** (1.66 g, 8.53 mmol) was added to the solution. After 2 h of stirring, the reaction was quenched by adding a half-saturated ammonium chloride solution (20 mL) at room temperature, and the resulting mixture was partitioned with ethyl acetate (30 mL ×3). The combined organic extracts were washed with brine (50 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 2/5) to afford the secondary alcohol **29** (2.51 g, 7.98 mmol, 94%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27–7.23 (m, 2H, Ph of PMB), 6.90–6.86 (m, 2H, Ph of PMB), 4.48 (d, $J = 1.7$ Hz, 2H, CH_2 of PMB), 4.25 (dd, $J = 9.6$, 4.9 Hz, 1H, 2-H), 4.13–4.09 (m, 1H, 2′-H), 3.80 (s, 3H, OCH_3 of PMB), 3.48 (dd, $J = 9.6$, 3.4 Hz, 1H, 3-H), 3.34 (dd, $J = 9.6$, 6.9 Hz, 1H, 3-H), 2.94–2.79 (m, 4H, 3′-H, 5′-H), 2.50 (d, $J = 4.1$ Hz, 1H, OH), 2.14–1.78 (m, 4H, 1-H and 4′-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 129.8, 129.3, 113.8, 73.6, 73.0, 67.1, 55.2, 43.7, 38.9, 30.3, 30.0, 25.9. HRMS (CI, positive) m/z for $C_{15}H_{22}NaO_3S_2$ $[M+Na]^+$: calc. 337.0904, found 337.0903.

(R)-(1-(1′,3′-Dithian-2′-yl)-3-(4′′-methoxybenzyloxy)propan-2-yloxy)(tert-butyl)dimethylsilane (30). The secondary alcohol **29** (110 g, 345.0 mmol) was dissolved in a solution of imidazole (66.6 g, 0.98 mol) in DMF (1.1 L) at room temperature. TBDMSCl (76.0 g, 0.49 mol) was then added to the reaction mixture with vigorous stirring. After 18 h of stirring at ambient temperature, the reaction mixture was quenched with a saturated solution of ammonium chloride (500 mL), and the aqueous fraction was partitioned with ethyl acetate (500 mL ×4). The combined organic fractions were washed with brine (400 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/6) to afford **30** (110 g, 259.0 mmol, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.26 (m, 2H, Ph of PMB), 6.92–6.88 (m, 2H, Ph of PMB), 4.47 (s, 2H, CH_2 of PMB), 4.17–4.09 (m, 2H, 2-H and 2′-H), 3.83 (s, 3H, OCH_3 of PMB), 3.34–3.31 (m, 2H, 3-H), 2.89–2.75 (m, 4H, 4′′-H and 6′′′-H), 2.15–1.62 (m, 4H, 1-H and 5′′-H), 0.91 (s, 9H, CH_3 of TBS), 0.13 (s, 3H, CH_3 of TBS), 0.09 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.1, 130.32, 129.2, 113.7, 74.3, 72.9, 67.9, 55.3, 43.6, 40.3, 30.5, 29.9, 26.0, 25.9, 18.1, −4.4, −4.8. HRMS (CI, positive) m/z for $C_{21}H_{37}O_3S_2Si$ $[M+H]^+$, calc. 429.1955, found 429.1948.

(R)-3-(tert-Butyldimethylsilyloxy)-4-(4′-methoxybenzyloxy)butanal (31). Iodomethane (72.6 mL, 1.17 mol) and calcium carbonate (70.0 g, 0.70 mol) were added to a solution of dithiane

30 (50.0 g, 0.12 mol) in acetonitrile (700 mL) and water (175 mL), and the mixture was refluxed for 8 h. After cooling and filtering over a Celite pad, the filtrate was concentrated under reduced pressure to 200 mL to remove the acetonitrile and partitioned with ethyl acetate (300 mL ×4). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford aldehyde **31** (31.0 g, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 9.72 (t, $J = 2.4$ Hz, 1H, 1-H), 7.17 (m, 2H, Ph of PMB), 6.82 (m, 2H, Ph of PMB), 4.39 (s, 2H, CH_2 of PMB), 4.28 (tt, $J = 6.4$, 5.1 Hz, 1H, 3-H), 3.75 (s, 3H, OCH_3 of PMB), 3.41 (dd, $J = 9.5$, 5.1 Hz, 1H, 4-H), 3.30 (dd, $J = 9.5$, 6.4 Hz, 1H, 4-H), 2.58 (ddd, $J = 15.9$, 5.1, 2.1 Hz, 1H, 2-H), 2.50 (ddd, $J = 15.9$, 6.7, 2.7 Hz, 1H, 2-H), 0.80 (s, 9H, CH_3 of TBS), 0.00 (s, 6H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 201.5, 159.2, 123.0, 119.3, 113.8, 73.7, 73.0, 67.3, 55.3, 49.0, 25.7, 18.0, −4.5, −5.0. HRMS (CI, positive) m/z for $C_{18}H_{31}O_4Si$ $[M + H]^+$: calc. 339.1986, found 339.1984.

(2R,4R)-1-(4-Methoxybenzyloxy)-2,4-bis(tert-butyldimethylsilyloxy) hept-6-ene (33). (+)-Ipc_2B(allyl) was prepared by mixing (+)-diisopinocampheylchloroborane (77.8 mL, 1.6 M, 0.13 mol) and allyl magnesium bromide (119.1 mL, 1.0 M, 0.12 mol) in anhydrous THF (274 mL) at 0 °C for 1 h. After cooling to −78 °C, the aldehyde **31** (31.0 g, 0.09 mol) in THF (92 mL) was added to the reaction mixture over 1 h, which was stirred at −78 °C for 2 h and allowed to warm to room temperature over 1 h with stirring. When the aldehyde was no longer detectable, the reaction mixture was quenched by the addition of methanol (274 mL), aqueous 1 N sodium hydroxide (274 mL) and hydrogen peroxide (92 mL) at 0 °C. This reaction mixture was stirred at 0 °C for 3 h. After filtering through a paper filter, the filtrate was partitioned with ethyl acetate (300 mL ×4), and the combined organic layers were washed with brine (500 mL) and dried over sodium sulfate. After concentration under reduced pressure, the residue was subjected to flash column chromatography (EtOAc/hexanes: 1/9) to afford **32** (35.2 g, 91%).

t-Butyldimethylsilyl trifluoromethanesulfonate (21 mL, 0.09 mol) was added over 20 min to a solution of **32** (29.0 g, 76.2 mmol) and 2,6-lutidine (21.3 mL, 0.18 mol) in CH_2Cl_2 (381 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 2 h and allowed to warm to 0 °C over 18 h. The reaction mixture was quenched by the addition of a saturated solution of ammonium chloride (200 mL), and the aqueous fraction was partitioned with CH_2Cl_2 (200 mL ×4). The combined organic layers were washed with brine (200 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatograph (EtOAc/hexanes: 1/19) to afford **33** (34.1 g, 68.9 mmol, 98%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.26 (d, $J = 8.7$ Hz, 2H, Ph of PMB), 6.89 (d, $J = 8.5$ Hz, 2H, Ph of PMB), 5.81 (m, 1H, 6-H), 5.05 (app d, $J = 14$ Hz, 2H, 7-H), 4.46 (s, 2H, CH_2 of PMB), 3.97–3.83 (m, 2H, 2-H and 4-H), 3.80 (s, 3H, OCH3 of PMB), 3.38 (d, $J = 4.9$ Hz, 2H, 1-H), 2.34–2.12 (m, 2H, 5-H), 1.78–1.58 (m, 2H, 3-H), 0.91 (s, 9H, CH_3 of TBS), 0.90 (s, 9H, CH_3 of TBS), 0.08 (s, 3H, CH_3 of TBS), 0.07 (s, 3H, CH_3 of TBS), 0.05 (s, 3H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 159.0, 135.1, 130.5, 129.2, 116.9, 113.6, 74.6, 72.8, 69.1, 68.8, 55.2, 42.1, 41.6, 25.9, 25.9, 18.1, 18.0, −4.23, −4.41, −4.54, −4.82. HRMS (CI, positive) m/z for $C_{27}H_{49}O_4Si_2$ $[M - H]^+$: calc. 493.3169, found 493.3149.

(3S,5R)-3,5-Bis(tert-butyldimethylsilyloxy)-6-(4-methoxybenzyloxy) hexanal (34). Allyl compound **33** (10.0 g, 0.02 mol) was mixed with water and a solution of sodium (meta)periodate (13.0 g, 0.06 mol), osmium(VIII) oxide (0.12 g, 0.48 mmol), and 2,6-lutidine (3.5 mL, 0.03 mol) in 1,4-dioxane (61 mL) at 0 °C for 18 h. The reaction mixture was quenched by the addition of 1 N sodium thiosulfate (40 mL) and stirred for 1 h. The reaction

partitioned with CH_2Cl_2 (100 mL ×3). The combined organic layers were washed with brine (150 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford aldehyde **34** (9.8 g, 19.73 mmol, 98.6%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.77 (dd, $J = 3.2$, 2.1 Hz, 1H, 1-H), 7.22 (d, $J = 8.8$ Hz, 2H, Ph of PMB), 6.85 (d, $J = 8.6$ Hz, 2H, Ph of PMB), 4.42 (s, 2H, CH_2 of PMB), 4.37–4.32 (m, 1H, 3-H), 3.87–3.82 (m, 1H, 5-H), 3.79 (s, 3H, OCH_3 of PMB), 3.37–3.30 (m, 2H, 6-H), 2.58–2.43 (m, 2H, 2-H), 1.75 (t, $J = 6.8$ Hz, 2H, 4-H), 0.85 (s, 9H, CH_3 of TBS), 0.84 (s, 9H, CH_3 of TBS), 0.03–0.02 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.2, 159.1, 130.25, 129.2, 113.7, 74.3, 72.9, 68.7, 65.5, 55.2, 50.4, 42.7, 25.8, 25.7, 18.1, 17.9, −4.23, −4.41, −4.82, −4.87. HRMS (CI, positive) m/z for $C_{26}H_{47}O_5Si_2$ $[M - H]^+$: calc. 495.2962, found 495.2970.

(2R,4R,E)-1-(4-Methoxybenzyloxy)-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-ene (35). A solution of the aldehyde **34** (8.95 g, 18 mmol) and chromium(II) chloride (11.1 g, 91 mmol) in anhydrous THF (217 mL) was stirred at 0 °C for 30 min. Next, a solution of iodoform (14.3 g, 36 mmol) in anhydrous THF (36 mL) was added to the mixture at 0 °C over 15 min under a nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 2 d. The reaction mixture was then added to water (150 mL), filtered through a paper filter and washed with ethyl acetate (300 mL ×2). The aqueous fraction was partitioned with ethyl acetate (300 mL ×3). The combined organic layers were washed with brine (300 mL), dried over sodium sulfate and concentrated under reduced pressure in a bath maintained below 30 °C. The resulting residue was purified twice using flash column chromatography (hexanes only remove to iodoform → EtOAc/hexanes: 3/97) to afford the vinyl iodide **35** (9.4 g, 15.1 mmol, 84.1%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23 (d, $J = 8.7$ Hz, 2H, Ph of PMB), 6.86 (d, $J = 8.7$ Hz, 2H, Ph of PMB), 6.53–6.43 (m, 1H, 6-H), 5.99 (d, $J = 14$ Hz, 1H, 7-H), 4.42 (s, 2H, CH_2 of PMB), 3.87–3.81 (m, 2H, 2-H and 4-H), 3.79 (s, 3H, OCH_3 of PMB), 3.37–3.27 (m, 2H, 1-H), 2.28–2.20 (m, 1H, 5-H), 2.14–2.04 (m, 1H, 5-H), 1.72–1.54 (m, 2H, 3-H), 0.86 (s, 9H, CH_3 of TBS), 0.85 (s, 9H, CH_3 of TBS), 0.03–0.00 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.0, 143.2, 130.4, 129.2, 113.7, 74.5, 72.9, 68.9, 68.0, 55.3, 43.4, 42.3, 25.9, 25.8, 18.1, 18.0, −4.20, −4.48, −4.56, −4.79. HRMS (CI, positive) m/z for $C_{27}H_{48}IO_4Si_2$ $[M - H]^+$: calc. 619.2136, found 619.2136.

(2R,4R,E)-2,4-Bis(tert-butyldimethylsilyloxy)-7-iodohept-6-enal (37). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 1.36 g, 5.99 mmol) was added to a solution of the PMB ether **35** (3.10 g, 4.99 mmol) in CH_2Cl_2/MeOH: 10/1 (150 mL) at 0 °C. After 12 h of stirring, the solution was treated with a saturated solution of sodium bicarbonate (100 mL). The mixture was then partitioned with CH_2Cl_2 (50 mL

×3), and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 1/19) to afford the primary alcohol **36** (2.35 g, 4.69 mmol, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.46 (dt, J = 14.4, 7.5 Hz, 1H, 6-H), 6.03 (dt, J = 14.4, 1.2 Hz, 1H, 7-H), 3.89–3.76 (m, 2H, 2-H and 4-H), 3.58–3.51 (m, 1H, 1-H), 3.48–3.40 (m, 1H, 1-H), 2.30–2.12 (m, 2H, 3-H), 1.73–1.58 (m, 2H, 5-H), 0.87 (s, 9H, CH_3 of TBS), 0.86 (s, 9H, CH_3 of TBS), 0.06–0.03 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.6, 77.0, 69.8, 68.2, 66.1, 43.7, 41.3, 25.79, 25.77, 18.0, 17.9, −4.36, −4.55, −4.64. HRMS (CI, positive) m/z for $C_{19}H_{42}IO_3Si_2$ $[M+H]^+$: calc. 501.1717, found 501.1713.

Dess-Martin periodinane (890 mg, 2.10 mmol) was added to a solution of the resulting alcohol **36** (1.00 g, 2.00 mmol) in CH_2Cl_2 (25 mL) at 0 °C. After stirring at room temperature for 1 h, the solution was diluted with ethyl ether (50 mL). The resulting mixture was then washed with a saturated solution of sodium bicarbonate (50 mL), a saturated solution of sodium thisulfate (50 mL) and brine (50 mL). The organic phase was then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 1/19) to afford **37** (fragment B) (891 mg, 1.79 mmol, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.57 (d, J = 1.4 Hz, 1H, 1-H), 6.46 (dt, J = 14.4, 7.8 Hz, 1H, 6-H), 6.05 (dt, J = 14.4, 1.3 Hz, 1H, 7-H), 4.05 (td, J = 6.1, 1.4 Hz, 1H, 2-H), 3.98–3.93 (m, 1H, 4-H), 2.29–2.16 (m, 2H, 5-H), 1.83–1.75 (m, 2H, 3-H), 0.90 (s, 9H, CH_3 of TBS), 0.85 (s, 9H, CH_3 of TBS), 0.07–0.04 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.4, 142.3, 77.1, 74.7, 66.8, 43.5, 40.2, 25.8, 25.7, 18.1, 17.9, −4.36, −4.51, −4.64, −4.95. HRMS (CI, positive) m/z for $C_{19}H_{40}IO_3Si_2$ $[M+H]^+$: calc. 499.1561, found 499.1572.

Fragment C. Fragment C was prepared according to the previously reported procedure (2).

(E)-3-(Tributylstannyl)prop-2-en-1-ol (38). The stereoselective addition of tin to propargylic alcohol was conducted following a known procedure (56). Propargylic alcohol (1.50 g, 26.7 mmol) was mixed with tributyltin hydride (9.21 mL, 34.7 mmol), to which was added 2, 2′-azobis(2-methylpropionitrile) (AIBN, 43.8 mg, 0.267 mmol) at room temperature. The reaction was gradually heated to 80 °C over 1 h and allowed to react overnight under reflux. After completion of the reaction was confirmed by TLC analysis, the crude product was directly subjected to flash column chromatography (hexanes) to afford the vinyl tin species **38** (4.55 g, 13.1 mmol, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.24–6.11 (m, 2H, 1-H and 2-H), 4.15 (br d, J = 3.1 Hz, 2H, 3-H), 1.58–1.43 (m, 6H, Bu_3Sn), 1.40–1.26 (m, 6H, Bu_3Sn), 0.98–0.80 (m, 15H, Bu_3Sn). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.0, 128.3, 66.4, 29.1, 27.3, 13.7, 9.4.

Ethyl (2E,4E)-5-(tributylstannyl)penta-2,4-dienoate (40). Activated manganese oxide (3.85 g, 44.3 mmol) was added to a solution of the alcohol **38** (1.54 g, 4.43 mmol) in acetone (50 mL) at room temperature. After stirring overnight, the reaction mixture was filtered over a pad of Celite to remove the manganese oxide. The filtrate was concentrated under reduced pressure, and the crude residue was briefly purified by flash column chromatography (hexanes only) to afford the aldehyde **39** (1.29 g, 3.75 mmol, 85%). The resulting aldehyde **39** was immediately used for the Horner-Wadsworth-Emmons reaction in the next step. To a solution of triethyl phosphonoacetate (1.08 mL, 5.45 mmol) in tetrahydrofuran (15 mL) at 0 °C was slowly added sodium hydride (60% in mineral oil, 327 mg, 5.45 mmol). The aldehyde **39** (1.26 g, 3.64 mmol) was then added to the resulting suspension. After stirring at 0 °C for 4 h, the reaction was quenched by the addition of a saturated solution of ammonium chloride (15 mL), and the mixture was partitioned with ethyl acetate (20 mL ×3). The combined organic extracts were washed with brine (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 1/39) to afford **40** (fragment C) (1.10 g, 2.65 mmol, 74%) for the Stille cross coupling. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18 (dd, J = 15.4, 10.2 Hz, 1H, 4-H), 6.81 (d, J = 18.8 Hz, 1H, 2-H), 6.64 (dd, J = 18.8, 10.2 Hz, 1H, 3-H), 5.79 (d, J = 15.4 Hz, 1H, 5-H), 4.20 (q, J = 7.1 Hz, 1H, CH_3CH_2OCO), 1.56–1.41 (m, 6H, Bu_3Sn), 1.30–1.26 (m, 9H, Bu_3Sn), 0.95–0.78 (m, 15H, Bu_3Sn, CH_3CH_2OCO). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.4, 147.2, 146.3, 144.2, 119.9, 60.2, 29.0, 27.2, 13.7, 9.6. HRMS (CI, positive) m/z for $C_{19}H_{37}O_2Sn$ $[M+H]^+$: calc. 417.1816, found 417.1821.

(1E,4R,6R,7E,10R,11R,12S,16S)-1-Iodo-4,6,10,12-tetrakis(tert-butyldimethylsilyloxy)-11-methyl-16-(4-methoxybenzyloxy)octadeca-1,7-diene (41). Potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 5.76 mL, 2.88 mmol) was added drop-wise to a solution of fragment A (**24**) (1.49 g, 1.92 mmol) in THF (19 mL) at −78 °C over 10 min, and the reaction was kept stirring at −78 °C for 1 h, at which time fragment B (**37**) (1.05 g, 2.11 mmol) in THF (10 mL) was added to the resulting yellow solution at −78 °C over 30 min. After 4 h, the temperature was slowly raised to room temperature over 1 h, at which time the mixture was poured into a saturated solution of sodium bicarbonate (20 mL), extracted with EtOAc (20 mL ×3), dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/49) to afford the vinyl iodide **41** (1.61 g, 1.54 mmol, 80.0%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.26 (t, 2H, J = 4.4 Hz, PhH of PMB), 6.86 (dt, 2H, J = 8.7, 2.8 Hz, PhH of PMB), 6.53–6.47 (m, 1H, 2-H), 6.09–5.98 (1H, J = 34.4, 14.4, 1.3 Hz, 1-H), 5.57–5.51 (m, 1H, 8-H), 5.43–5.38 (dd, 1H, J = 15.4, 6.8 Hz, 7-H), 4.43 (s, 2H, CH_2 of PMB), 4.12–4.08 (m, 1H, 6-H), 3.84–3.80 (m, 1H, 4-H), 3.80 (s, 3H, OCH_3 of PMB), 3.75 (q, 1H, J = 5.6 Hz, 10-H), 3.67 (q, 1H, J = 5.6 Hz, 12-H), 3.29 (quint, 1H, J = 5.7 Hz, 16-H), 2.31–2.21 (m, 3H, 3-H and 9-H), 2.15–2.08 (m, 1H, 3-H), 1.74–1.69 (m, 1H, 5-H), 1.62–1.25 (m, 10H, 5-H + 11-H + 13-H + 14-H + 15-H + 17-H), 0.93–0.84 (m, 42H, 11-CH_3 + 18-H + CH_3 of TBS), 0.10–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 143.31, 135.54, 129.17, 126.81, 113.71, 79.79, 72.90, 72.25, 70.74, 70.45, 68.22, 55.26, 46.03, 43.47, 41.26, 37.87, 35.40, 33.91, 26.25, 25.99, 25.90, 25.85, 21.30, 18.18, 18.14, 18.12, 18.01, 9.52, −4.24, −4.35, −4.42, −4.49, −4.61, −4.71. HRMS (ESI, positive) m/z for $C_{51}H_{99}IO_6Si_4Na$ $[M+Na]^+$: calc. 1069.5461, found 1069.5460.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl 9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-16-methyl-21-(4-methoxybenzyloxy)tri-

cosa-2,4,6,12-tetraenoate (42). Tris-(dibenzylideneacetone) dipalladium (71 mg, 0.077 mmol) and triphenylarsine (71 mg, 0.23 mmol) were added to a solution of the vinyl iodide **41** (1.61 g, 1.54 mmol) and fragment C (**40**) (0.96 g, 2.31 mmol) in anhydrous dimethylformamide (30 mL) at room temperature, and the reaction mixture was stirred for 24 h at room temperature. EtOAc (150 mL) was then added, and the reaction mixture was washed with H_2O (50 mL ×4). The organic layer was dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to afford **42** (1.23 g, 1.18 mmol, 76.4%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.32–7.25 (m, 3H, 3-H and PhH of PMB), 6.87–6.85 (d, 2H, J = 8.6 Hz, PhH of PMB), 6.54–6.49 (m, 1H, 5-H), 6.23–6.18 (m, 1H, 4-H), 6.15–6.10 (m, 1H, 6-H), 5.94–5.88 (m, 1H, 7-H), 5.86–5.83 (d, 1H, J = 15.4 Hz, 2-H), 5.57–5.51 (m, 1H, 13-H), 5.43–5.38 (m, 1H, 7-H), 4.43 (s, 2H, CH_2 of PMB), 4.20 (q, 2H, J = 7.1 Hz, CH_2CH_3 of OEt), 4.15–4.11 (m, 1H, 11-H), 3.86–3.82 (m, 1H, 9-H), 3.79 (s, 3H, OCH_3 of PMB), 3.76–3.75 (m, 1H, 15-H), 3.69–3.68 (m, 1H, 17-H), 3.30–3.28 (m, 1H, 21-H), 2.39–2.36 (m, 1H, 8-H), 2.27–2.17 (m, 3H, 14-H and 8-H), 1.74–1.69 (m, 1H, 10-H), 1.63–1.26 (m, 13H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H + CH_2CH_3 of OEt), 0.93–0.84 (m, 42H, 16-CH_3, 23-H + CH_3 of TBS), 0.04–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.17, 159.02, 144.72, 140.89, 136.54, 135.62, 132.03, 129.18, 128.13, 126.76, 120.27, 113.71, 79.79, 72.89, 72.27, 70.81, 70.46, 68.95, 60.19, 55.25, 46.16, 41.26, 40.76, 37.89, 35.42, 33.91, 26.26, 25.99, 25.98, 25.91, 25.86, 21.30, 18.18, 18.14, 18.04, 14.32, 9.52, −3.72, −3.93, −4.26, −4.31, −4.37, −4.51, −4.72. HRMS (ESI, positive) m/z for $C_{58}H_{108}O_8Si_4Na$ $[M+Na]^+$: calc. 1067.7019, found: 1067.7005.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (44). A solution of 0.5 N lithium hydroxide (8 mL) was added to a solution of **42** (0.31 g, 0.30 mmol) in THF (8 mL) and MeOH (8 mL) at room temperature, and the mixture was stirred under reflux for 3 h before the volatile solvents were evaporated under reduced pressure. The pH of the aqueous solution was adjusted to approximately 6, and the mixture was partitioned with EtOAc (20 mL ×3). The organic extracts were pooled, washed with brine (20 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/4) to afford the carboxylic acid **43** (0.24 g, 0.236 mmol, 79.6%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.37 (dd, 1H, J = 15.1, 11.5 Hz, 3-H), 7.27–7.25 (m, 2H, PhH of PMB), 6.85 (dt, 2H, J = 8.7, 2.8, 2.1 Hz, PhH of PMB), 6.56 (dd, 1H, J = 14.8, 10.9 Hz, 5-H), 6.23 (dd, 1H, J = 14.8, 11.6 Hz, 4-H), 6.17–6.12 (m, 1H, 6-H), 5.98–5.92 (m, 1H, 7-H), 5.86 (dd, 1H, J = 15.1, 10.3 Hz, 2-H), 5.57–5.51 (m, 1H, 13-H), 5.40 (dd, 1H, J = 15.4, 6.8 Hz, 7-H), 4.43 (s, 2H, CH_2 of PMB), 4.13 (q, 1H, J = 6.2 Hz, 11-H), 3.85–3.83 (m, 1H, 9-H), 3.79 (s, 3H, OCH_3 of PMB), 3.75 (q, 1H, J = 5.6 Hz, 15-H), 3.70–3.67 (m, 1H, 17-H), 3.30–3.27 (m, 1H, 21-H), 2.41–2.37 (m, 1H, 8-H), 2.27–2.17 (m, 3H, 14-H and 8-H), 1.75–1.26 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.90–0.84 (m, 42H, 16-CH_3 + 23-H + CH_3 of TBS), 0.04–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.03, 147.05, 142.06, 135.60, 131.94, 129.19, 127.86, 126.80, 118.65, 113.72, 79.80, 72.90, 72.27, 70.82, 70.47, 68.92, 55.26, 46.17, 41.27, 40.77, 37.89, 35.42, 33.92, 26.26, 25.98, 25.91, 25.86, 21.31, 18.18, 18.15, 18.05, 9.51, −3.72, −3.75, −3.91, −4.25, −4.31, −4.37, −4.50, −4.71. HRMS (ESI, negative) m/z for $C_{56}H_{103}O_8Si_4$ $[M-H]^-$: calc. 1015.6736, found 1015.6731.

2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 107 mg, 0.47 mmol) was added to a solution of **43** (0.24 g, 0.236 mmol) from the previous step in CH_2Cl_2 (21.6 mL) and pH 7 phosphate buffer (2.4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 6 h, at which time a saturated solution of sodium bicarbonate (6 mL) was added. The two layers were separated, and the aqueous layer was partitioned with EtOAc (40 mL ×3). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes = 1/4, then 1/1) to afford the *seco*-acid **44** (0.16 g, 0.178 mmol, 75.4%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.36 (dd, J = 15.3, 11.3 Hz, 1H, 3-H), 6.55 (dd, J = 14.8, 10.5 Hz, 1H, 5-H), 6.22 (dd, J = 14.8, 11.3 Hz, 1H, 4-H), 6.14 (dd, J = 15.3, 10.5 Hz, 1H, 6-H), 5.94 (dt, J = 15.3, 7.4 Hz, 1H, 7-H), 5.82 (d, J = 15.1 Hz, 1H, 2-H), 5.54 (dt, J = 15.5, 7.2 Hz, 1H, 13-H), 5.39 (dd, J = 15.5, 6.8 Hz, 1H, 12-H), 4.12 (br q, J = 12.6, 7.1 Hz, 1H, 11-H), 3.81 (br quint, J = 17.0, 11.8, 6.6 Hz, 1H, 9-H), 3.73 (q, J = 11.0, 5.5 Hz, 1H, 15-H), 3.67 (q, J = 10.5, 5.5 Hz, 1H, 17-H), 3.51–3.45 (m, 1H, 21-H), 2.39–2.34 (m, 1H, 8-H), 2.24–2.18 (m, 3H, 8-H and 14-H), 1.70 (ddd, J = 13.5, 7.7, 5.5 Hz, 1H, 10-H), 1.61–1.18 (m, 10H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.92 (t, J = 7.5 Hz, 3H, 23-H), 0.87–0.83 (m, 36H, CH_3 of TBS), 0.83 (d, J = 7 Hz, 3H, 16-Me), 0.04–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 171.3, 147.0, 142, 0, 137.4, 135.8, 132.0, 127.9, 126.9, 120.0, 73.1, 72.6, 72.2, 70.9, 69.0, 60.2, 46.2, 40.8, 40.6, 37.7, 37.4, 35.1, 30.1, 25.95, 25.93, 25.87, 21.3, 18.2, 18.16, 18.13, 18.0, 9.9, 9.3, −3.77, −3.82, −3.99, −4.28, −4.37, −4.38, −4.59, −4.71. HRMS (CI, negative) m/z for $C_{48}H_{96}O_7Si_4$ $[M]^-$: calc. 896.6233, found 896.6230.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-macrolactone (45). A solution of *N*,*N*-diisopropylethylamine (0.92 mL, 0.4 M in THF, 0.37 mmol) was mixed with the *seco*-acid **44** (164 mg, 0.18 mmol) in THF (36 mL). A solution of 2,4,6-trichlorobenzoyl chloride (0.50 mL, 0.4 M, 0.20 mmol) was added to the mixture at room temperature. The reaction mixture was stirred at room temperature for 3 h and concentrated under reduced pressure to afford the crude anhydride intermediate. A solution of the anhydride in toluene (20 mL) was added to a solution of *N*,*N*-dimethylaminopyridine (DMAP; 67 mg, 0.55 mmol) in toluene (30 mL) using a syringe pump over 3 h. At the end of the addition, the syringe was rinsed with additional toluene (2 mL). After stirring for 18 h, the mixture was quenched with a saturated solution of sodium bicarbonate (20 mL), and the aqueous fraction was partitioned with ethyl acetate (40 mL ×3). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to afford the macrolactone **45** (141 mg, 0.16 mmol, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.24 (dd, J = 15.3, 11.3 Hz, 1H, 3-H), 6.47 (dd, J = 14.8, 10.5 Hz, 1H, 5-H), 6.21 (dd, J = 14.8, 11.3 Hz, 1H, 4-H), 6.11 (dd, J = 15.3, 10.5 Hz, 1H, 6-H), 5.79

(d, J = 15.1 Hz, 1H, 2-H), 5.77 (dt, J = 15.3, 7.4 Hz, 1H, 7-H), 5.38 (dt, J = 15.5, 7.2 Hz, 1H, 13-H), 5.27 (dd, J = 15.5, 6.8 Hz, 1H, 12-H), 4.85 (m, 1H, 21-H), 4.01 (br dd, J = 13.4, 6.7 Hz, 1H, 11-H), 3.75 (br ddd, J = 13.0, 9.0, 5.0 Hz, 1H, 9-H), 3.67 (br dd, J = 9.0, 5.5 Hz, 1H, 15-H), 3.57 (br dd, J = 10.0, 6.0 Hz, 1H, 17-H), 2.46–2.42 (m, 1H, 8-H), 2.24–2.18 (m, 2H, 14-H and 8-H), 2.12–2.08 (m, 1H, 14-H), 1.42–1.17 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91 (t, J = 7.5 Hz, 3H, 23-H), 0.874 (s, 9H, CH_3 of TBS), 0.897 (s, 9H, CH_3 of TBS), 0.85 (s, 9H, CH_3 of TBS), 0.93 (s, 9H, CH_3 of TBS), 0.74 (d, 3H, J = 7.0 Hz, 16-CH_3), 0.04–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.0, 144.8, 140.9, 136.0, 135.2, 132.0, 128.0, 127.0, 120.8, 75.2, 73.2, 72.1, 71.1, 69.1, 46.6, 42.3, 42.1, 38.4, 34.4, 33.4, 29.7, 27.8, 26.04, 25.99, 25.91, 25.8, 21.1, 18.18, 18.14, 18.08, 18.02, 10.2, 9.9, −3.47, −3.87, −3.97, −4.33, −4.41, −4.53, −4.62. HRMS (CI, negative) m/z for $C_{48}H_{94}O_6Si_4$ $[M]^-$: calc. 878.6128, found 878.6128.

Monomacrolactone (2). A 1 M solution of *tetra-n*-butylammonium fluoride in THF (10 mL) was added to a solution of the protected macrolactone **45** (31.2 mg, 35.4 μmol) in THF (1 mL) at 0 °C. The reaction was stirred for 4 d while the temperature was maintained at 4 °C. After completion of the reaction was confirmed by TLC analysis, the reaction was quenched by careful addition of a saturated solution of sodium bicarbonate at 0 °C. The mixture was then extracted with chloroform (20 mL ×3), and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (CH_2Cl_2/CH_3OH: 93/7) to afford macrolactone **2** (9.6 mg, 22 μmol) in 64% yield. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.25 (dd, J = 15.2, 11.3 Hz, 1H, 3-H), 6.70 (dd, J = 14.9, 10.9 Hz, 1H, 5-H), 6.35 (dd, J = 14.9, 11.3 Hz, 1H, 4-H), 6.18 (dd, J = 15.2, 10.9 Hz, 1H, 6-H), 5.89 (ddd, J = 15.2, 10.3, 5.4 Hz, 1H, 7-H), 5.85 (d, J = 15.2 Hz, 1H, 2-H), 5.29 (app dd, J = 15.4, 7.2 Hz, 1H, 12-H), 5.18 (ddd, J = 15.4, 7.6, 5.9 Hz, 1H, 13-H), 4.75 (quint, J = 6.2 Hz, 1H, 21-H), 4.64 (br s, 1H, OH), 4.51 (br s, 1H, OH), 4.38 (br m, 2H, OH), 3.81–3.74 (m, 1H, 11-H), 3.72–3.66 (m, 1H, 9-H), 3.52–3.48 (m, 1H, 15-H), 3.47–3.43 (m, 1H, 17-H), 2.52–2.50 (m, 1H, 8-H), 2.08–1.86 (m, 3H, 8-H and 14-H), 1.60–1.45 (m, 5H, 10-H + 19-H + 22-H), 1.40–1.21 (m, 5H, 10-H + 18-H + 20-H), 1.19–1.54 (m, 1H, 16-H), 0.84 (t, J = 7.3 Hz, 3H, 23-H), 0.67 (d, J = 7.1 Hz, 3H, 16-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.9, 144.7, 141.5, 137.0, 136.2, 131.7, 127.8, 126.0, 120.2, 74.7, 74.4, 73.3, 69.4, 66.9, 54.9, 45.8, 42.8, 38.4, 33.8, 32.8, 27.3, 21.3, 9.72, 6.06. HRMS (CI, negative) m/z for $C_{24}H_{38}O_6$ $[M]^-$: calc. 422.2668, found 422.2664.

SpnM natural substrate (3). SpnJ (7.0 mL of 50 μM in 50 mM Tris·HCl buffer, pH 8) was added to the solution of macrolactone **2** (20.7 mg, 4 mM in DMSO) in Tris·HCl buffer (4.03 mL, 50 mM Tris·HCl buffer, pH 8.0) at 30 °C to initiate the enzymatic reaction (total volume 12.25 mL, 10% v/v of DMSO). The reaction was monitored by HPLC using a 4 × 250 mm Econosil C_{18} column (Alltech). The detector was set at 254 nm, and the flow rate was 1 mL/min using the following gradient: start at 30% acetonitrile, linearly increase to 45% over 30 min, linearly increase to 80% over 3 min and decrease linearly back to 30% over 3 min. The reaction was completed after 2.5 h incubation, and the reaction mixture was directly filtered through a YM-10 filter by centrifugation at 4000 rpm for 40 min. The filtrate was purified by semi-preparative HPLC using a 10 × 250 mm Econosil C_{18} column (Alltech). The detector was set at 254 nm, and the solution was eluted from the column at 4 mL/min using the following gradient: start at 30% acetonitrile, linearly increase to 45% over 30 min, linearly increase to 80% over 3 min and decrease linearly back to 30% over 3 min. The collected fractions were pooled, partitioned with EtOAc (50 mL ×3), dried over sodium sulfate and concentrated under reduced pressure to afford **3** (19.0 mg, 0.045 mmol, 91.3%) ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.24 (dd, J = 15.1, 11.2 Hz, 1H, 3-H), 6.70 (dd, J = 14.8, 10.7 Hz, 1H, 5-H), 6.34 (dd, J = 14.8, 11.2 Hz, 1H, 4-H), 6.19 (dd, J = 15.0, 10.9 Hz, 1H, 6-H), 5.86 (app q, J = 15.1 Hz, 1H, 7-H), 5.85 (d, J = 15.1 Hz, 1H, 2-H), 5.42 (ddd, J = 15.5, 7.4, 5.8 Hz, 1H, 13-H), 5.29 (app dd, J = 15.5, 6.9 Hz, 1H, 12-H), 4.78–4.69 (m, 1H, 21-H), 4.67 (d, J = 4.7 Hz, 1H, OH), 4.61 (d, J = 4.8 Hz, 1H, OH), 4.50 (d, J = 5.9 Hz, 1H, OH), 3.86 (app quint, J = 6.1 Hz, 1H, 11-H), 3.62–3.54 (m, 2H, 9-H and 17-H), 3.14 (dd, J = 18.3, 7.6 Hz, 1H, 14-H), 3.03 (dd, J = 18.3, 6.9 Hz, 1H, 14-H), 2.48–2.36 (m, 2H, 8-H and 16-H), 2.11 (dt, J = 13.1, 8.3 Hz, 1H, 8-H), 1.61–1.16 (m, 10H, 10-H + 18-H + 19-H + 20-H + 22-H), 0.89 (d, J–7.1 Hz, 3H, 16-CH_3), 0.83 (t, J = 7.3 Hz, 3H, 23-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 210.7, 166.0, 144.8, 141.5, 137.2, 136.6, 132.3, 127.9, 121.7, 120.3, 75.3, 71.3, 69.0, 67.2, 51.0, 45.0, 44.8, 41.6, 34.0, 32.3, 26.9, 22.0, 11.0, 9.71. HRMS (CI, negative) m/z for $C_{24}H_{36}O_6$ $[M]^-$: calc. 420.2512, found 420.2510.

C4D isotopolog.

(E)-2-Deuterio-3-(tributylstannyl)prop-2-en-1-ol (46). Propargyl alcohol (0.94 mL, 16.30 mmol) was mixed with tributyltin deuteride (5 g, 17.12 mmol), to which was added 2, 2′-azobis(2-methylpropionitrile) (AIBN, 27 mg, 0.16 mmol) at room temperature. The reaction was gradually heated up to 100 °C over 1 h and maintained at that temperature overnight. After completion of the reaction was confirmed by TLC analysis, the crude was directly subjected to flash column chromatography (hexanes only) to afford vinyl tin species **46** (2.04 g, 5.86 mmol, 39.5%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 6.27–6.13 (m, 1H, 3-H), 4.18–4.16 (m, 2H, 1-CH_2), 1.57–1.47 (m, 6H, Bu_3Sn), 1.41–1.27 (m, 6H, Bu_3Sn), 1.02–0.82 (m, 15H, Bu_3Sn). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 146.86, 146.67, 146.48, 128.20, 66.32, 29.06, 27.27, 13.68, 9.44.

Ethyl (2E,4E)-4-deuterio-5-(tributylstannyl)penta-2,4-dienoate (48). Activated manganese oxide (5.10 g, 58.6 mmol) was added to a solution of alcohol **46** (2.04 g, 5.86 mmol) in acetone (50 mL) at room temperature. After stirring overnight, the reaction mixture was filtered over a pad of Celite to remove the manganese oxide. The filtrate was then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexanes only) to afford the aldehyde **47** (1.2 g, 3.47 mmol, 59.2%). The resulting aldehyde was immediately used for the subsequent Hornor-Wadsworth-Emmons reaction. Sodium hydride (0.208 g, 5.20 mmol, 60% in mineral oil) was first slowly added to a solution of triethyl phosphonoacetate (1.03 mL, 5.20 mmol) in anhydrous THF (20 mL) at 0 °C. Aldehyde **47** was then added to the resulting suspension. After 4 h, the reaction was quenched by the addition of a saturated solution of ammonium chloride (15 mL), and the

mixture was partitioned with EtOAc (20 mL ×3). The combined organic layers were washed with brine (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexanes: 1/39) to afford **48** (0.9 g, 2.16 mmol, 62.3%) for the Stille coupling. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.19 (d, 1H, J = 15.4 Hz, 3-H), 6.80 (s, 1H, 5-H), 5.82 (d, 1H, J = 15.4 Hz, 2-H), 4.20 (q, 2H, J = 7.1 Hz, $\mathbf{CH}_2$-CH_3), 1.55–1.47 (m, 6H, Bu_3Sn), 1.35–1.27 (m, 9H, Bu_3Sn+CH_2−$\mathbf{CH}_3$), 0.96–0.87 (m, 15H, Bu_3Sn). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.41, 147.01, 146.25, 144.08, 119.88, 60.26, 29.03, 27.22, 14.30, 13.66, 9.63. HRMS (CI, positive) m/z for $C_{19}H_{36}DO_2Sn$ $[M+H]^+$: calc. 418.1882, found 418.1882.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl-4-deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-16-methyl-21-(4-methoxybenzyloxy)tricosa-2,4,6,12-tetraenoate (49). Compound **49** was prepared following the same procedure as compound **42** using compound **48** instead of **40** with a yield of 78%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.31–7.25 (m, 3H, Ph of PMB + 3-H), 6.87–6.84 (m, 2H, Ph of PMB), 6.52 (d, 1H, J = 10.8 Hz, 5-H), 6.17–6.10 (m, 1H, 6-H), 5.94–5.89 (m, 1H, 7-H), 5.86 (d, 1H, J = 15.3 Hz, 2-H), 5.57–5.51 (m, 1H, 13-H), 5.41 (dd, 1H, J = 15.4, 6.8 Hz, 12-H), 4.43 (s, 2H, CH_2 of PMB), 4.20 (q, 2H, J = 7.1 Hz, $CH_3\mathbf{CH}_2CO(O)$), 4.15–4.11 (q, 1H, J = 6.6 Hz, 11-H), 3.86–3.81 (m, 1H, 9-H), 3.79 (s, 3H, OCH_3 of PMB), 3.77–3.74 (m, 1H, 15-H), 3.70–3.67 (m, 1H, 17-H), 3.30–3.27 (m, 1H, 21-H), 2.40–2.35 (m, 1H, 8-H), 2.27–2.20 (m, 3H, 8-H and 14-H), 1.75–1.69 (m, 1H, 10-H), 1.62–1.25 (m, 13H, 10-H + 16-H +18-H +19-H +20-H + 22-H + $\mathbf{CH}_3CH_2CO(O)$), 0.92–0.84 (m, 42H, CH_3 of TBS + 16-CH_3 + 23-H), 0.04–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.15, 159.01, 144.64, 140.78, 136.49, 135.61, 131.99, 131.29, 129.17, 126.75, 120.23, 113.70, 79.78, 72.88, 72.25, 70.80, 70.45, 68.94, 60.17, 55.24, 46.15, 41.23, 40.75, 37.87, 35.41, 33.90, 25.98, 25.90, 25.86, 21.29, 18.16, 18.13, 18.03, 14.31, 9.50, −3.74, −3.76, −3.94, −4.27, −4.32, −4.38, −4.52, −4.73. HRMS (ESI, positive) m/z for $C_{58}H_{107}DO_8Si_4Na$ $[M + Na]^+$: calc. 1068.7076, found 1068.7065.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-4-Deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (50). Compound **50** was prepared following the same procedure as compound **44** using compound **49** instead of **42** with a yield of 43% over two steps. ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.38 (d, J = 15.1 Hz, 1H, 3-CH), 6.56 (d, J = 11 Hz, 1H, 5-H), 6.15 (dd, 1H, J = 14.8, 11.0 Hz, 6-H), 5.96–5.95 (m, 1H, 7-H), 5.87 (d, 1H, J = 15.1 Hz, 2-H), 5.59–5.52 (m, 1H, 13-H), 5.42 (dd, 1H, J = 15.4, 6.7 Hz, 12-H), 4.14 (q, 1H, J = 6.6 Hz, 11-H), 3.84 (q, 1H, J = 5.6 Hz, 9-H), 3.75 (q, 1H, J = 5.3 Hz, 15-H), 3.69 (q, 1H, J = 5.1 Hz, 17-H), 3.51–3.48 (m, 1H, 21-H) 2.40–2.38 (m, 1H, 8-H), 2.27–2.22 (m, 3H, 8-H + 14-H), 1.75–1.26 (m, 11H, 10-H + 16-H +18-H +19-H + 20-H + 22-H), 0.95–0.84 (m, 42H, CH_3 of TBS + 16-CH_3 + 23-H), 0.04–0.02 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 171.93, 146.90, 141.89, 137.30, 135.64, 132.00, 127.58, 126.87, 126.68, 119.23, 73.12, 73.09, 72.77, 72.64, 72.18, 70.79, 68.99, 46.14, 40.90, 40.81, 40.63, 37.80, 37.67, 37.41, 35.05, 30.09, 29.69, 27.84, 27.03, 21.32, 21.26, 18.19, 18.15, 18.12, 18.04, 16.49, 9.87, 9.31, −3.78, −3.82, −3.96, −4.00, −4.28, −4.31, −4.32, −4.38, −4.60, −4.71. HRMS (CI, negative) m/z for $C_{48}H_{94}DO_7Si_4$ $[M - H]^-$: calc. 896.6223, found 896.6215.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-4-deuterio-macrolactone (51). Compound **51** was prepared following the same procedure as compound **45** with a yield of 72%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.24 (d, J = 15.0 Hz, 1H, 3-H), 6.45 (d, J = 11.0 Hz, 1H, 5-H), 6.11 (dd, 1H, J = 15.5, 11.0 Hz, 6-H), 5.80 (d, 1H, J = 15.1 Hz, 2-H), 5.78 (dt, J = 15.5, 8.0 Hz, 1H, 7-H), 5.42–5.22 (m, 2H, 12-H and 13-H), 4.90–4.80 (m, 1H, 21-H), 4.01 (q, J = 6.6 Hz, 1H, 11-H), 3.80–3.72 (m, 1H, 9-H), 3.71–3.64 (m, 1H, 15-H), 3.61–3.52 (m, 1H, 17-H), 2.50–2.06 (m, 4H, 8-H and 14-H), 1.78–1.16 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91–0.80 (m, 39H, CH_3 of TBS + 23-H), 0.75 (d, J = 7.0 Hz, 3H, 16-CH_3), 0.08–0.06 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 166.9, 144.7, 140.8, 136.0, 135.2, 131.9, 128.0, 127.1, 120.8, 75.2, 73.2, 71.1, 69.1, 46.6, 42.3, 42.1, 38.4, 34.4, 33.4, 27.9, 26.1, 26.0, 25.9, 25.8, 21.1, 18.19, 18.15, 18.12, 18.04, 10.2, 9.9, −3.5, −3.8, −4.0, −4.3, −4.4, −4.5, −4.51, −4.6, −4.62, −4.70, −4.71. HRMS (ESI, positive) m/z for $C_{48}H_{93}DO_6Si_4Na$ $[M + Na]^+$: calc. 902.6082, found 902.6071.

Monomacrolactone (52). Compound **52** was prepared following the same procedure as compound **2** with a yield of 61%. HRMS (ESI, positive) m/z for $C_{24}H_{37}DO_6Na$ $[M + Na]^+$: calc. 446.2623, found 446.2625.

[C4-^{2}H]-SpnM substrate (53). Compound **53** was prepared following the same procedure as compound **3** with a yield of 90.3%. HRMS (ESI, positive) m/z for $C_{24}H_{35}DO_6Na$ $[M+Na]^+$: calc. 444.2467, found 444.2461.

C7D isotopolog.

(3S,5R)-3,5-Bis(tert-butyldimethylsilyloxy)-6-(4-methoxybenzyloxy) hexan-1,1-dideuterio-1-ol (55). *N*-Iodosuccinimide (NIS, 11.3 g, 0.05 mol) and potassium carbonate (6.9 g, 0.05 mol) were added to a solution of aldehyde **34** (9.94 g, 0.02 mol) in anhydrous MeOH (100 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 h and quenched by the addition of a saturated solution of sodium thiosulfate (200 mL). The mixture was partitioned with CH_2Cl_2 (150 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford the methyl ester **54** (7.63 g, 14.5 mmol, 72.4%). Lithium aluminum deuteride (144 mg, 3.42 mmol) was slowly added to a solution of compound **54** (3 g, 5.7 mmol) in anhydrous diethylether (40 mL) at −78 °C, and the reaction mixture was stirred at −78 °C for 6 h. Rochelle's salt solution (10 mL) was slowly added in order to quench the reaction. The organic layer was separated and extracted with EtOAc (100 mL ×3), and the combined organic layers were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9, then 1/4) to afford alcohol **55** (1.2 g, 2.39 mmol, 42.0%). Some of starting material was also recovered (1.5 g, 2.9 mmol, 51%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.22 (dt, 2H, J = 8.7, 2.8, 2.1 Hz, PhH of PMB), 6.85 (dt, 2H, J = 8.7, 2.8, 2.1 Hz, PhH of PMB), 4.42 (s, 2-H, CH_2 of PMB), 4.10–4.04 (m, 1H,

5-H), 3.83–3.79 (m, 1H, 3-H), 3.77 (s, 3H, OCH_3 of PMB), 3.36–3.29 (m, 2H, 6-H), 1.85–1.76 (m, 2H, 2-H), 1.71–1.59 (m, 2H, 4-H), 0.87 (s, 9H, CH_3 of TBS), 0.86 (s, 9H, CH_3 of TBS), 0.06 (d, 6H, J = 7.0 Hz, CH_3 of TBS), 0.02 (s, 6H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.09, 130.30, 129.15, 113.65, 74.66, 72.89, 68.93, 55.14, 51.31, 41.78, 37.15, 25.80, 25.77, 18.02, 17.84, −4.21, −4.49, −4.83, −4.90. HRMS (ESI, positive) m/z for $C_{26}H_{48}D_2O_5Si_2Na$ $[M+Na]^+$: calc. 523.3215, found 523.3206.

(2R,4R,E)-2,4-Bis(tert-butyldimethylsilyloxy)-7-iodohept-6-deuterio-6-en-1-ol (58). Dess-Martin periodinate (3.19 g, 7.52 mmol) was added to a solution of compound **55** (3.14 g, 6.26 mmol) in anhydrous CH_2Cl_2 (150 mL), and the reaction mixture was stirred for 2 h. The reaction mixture was washed sequentially with saturated sodium bicarbonate solution (70 mL), a solution of sodium thiosulfate (50 mL) and brine (80 mL). The organic layer was dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/19) to give the aldehyde **56** (2.9 g, 5.83 mmol, 93.2%). The aldehyde was not stable enough for spectroscopic analysis, so it was used for the next step directly. A solution of the aldehyde **56** (2.9 g, 5.83 mmol) and iodoform (4.6 g, 11.66 mmol) in THF (20 mL) was added to a mixture of $CrCl_2$ (3.58 g, 29.13 mmol) in THF (70 mL) and protected from light for 70 min at 0 °C. The reaction mixture was then stirred for 14 h at 4 °C and stirred for additional 2 h at room temperature. The reaction mixture was diluted with EtOAc (200 mL) and washed with brine (250 mL) and water (200 mL). The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/39) to give the vinyl iodide compound **57** (1.89 g, 3.04 mmol, 52.1%). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 0.828 g, 3.65 mmol) was added to the product **57** in CH_2Cl_2 (300 mL) and H_2O (30 mL). The reaction mixture was stirred for 3 h at room temperature and washed with a saturated solution of sodium bicarbonate (150 mL). The organic layer was dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/19) to afford the hydroxyl compound **58** (1.16 g, 2.31 mmol, 75.9%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 6.05 (s, 1H, 1-H), 3.90–3.81 (m, 2H, 4-H and 6-H), 3.60–3.57 (m, 1H, 7-H), 3.49–3.44 (m, 1H, 7-H), 2.29–2.25 (m, 1H, 3-H), 2.21–2.16 (m, 1H, 3-H), 1.74–1.63 (m, 2H, 5-H), 0.90 (s, 9H, CH_3 of TBS), 0.89 (s, 9H, CH_3 of TBS), 0.09 (s, 6H, CH_3 of TBS), 0.06 (d, 6H, J = 4.4 Hz, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 142.47, 142.27, 142.08, 69.81, 68.26, 66.19, 43.58, 41.36, 25.82, 18.06, 17.98, −4.34, −4.50, −4.61. HRMS (ESI, positive) m/z for $C_{19}H_{41}DIO_3Si_2$ $[M+H]^+$: calc. 502.1771, found 502.1774.

(2R,4R,E)-2,4-Bis(tert-butyldimethylsilyloxy)-7-iodohept-6-deuterio-6-en-1-al (59). Compound **59** was prepared following the same procedure as compound **37** with a yield of 83.4%.

(1E,4R,6R,7E,10R,11R,12S,16S)-1-Iodo-2-deuterio-4,6,10,12-tetrakis(tert-butyldimethylsilyloxy)-11-methyl-16-(4-methoxybenzyloxy)octadeca-1,7-diene (60). Potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 3 mL, 1.5 mmol) was added drop-wise over 10 min to a solution of compound **24** (0.5 g, 1.0 mmol) in anhydrous THF (20 mL) at −78 °C, and the reaction mixture was stirred for 1 h, at which time compound **59** (fragment B) (0.853 g, 1.1 mmol) was added to the solution at −78 °C. After 4 h, the temperature was slowly raised to room temperature over 1 h, and then the reaction mixture was poured into a saturated solution of sodium bicarbonate (10 mL). The resulting mixture was partitioned with EtOAc (20 mL ×3), washed with brine (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to give species **60** (0.57 g, 0.54 mmol, 54.4%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.27–7.25 (m, 2H, Ph of PMB), 6.87–6.85 (m, 2H, Ph of PMB), 5.99 (s, 1H, 1-H), 5.57–5.51 (m, 1H, 8-H), 5.41 (dd, 1H, J = 15.4, 6.8 Hz, 7-H), 4.43 (s, 2H, CH_2 of PMB), 4.12–4.08 (m, 1H, 6-H), 3.84–3.81 (m, 1H, 4-H), 3.80 (s, 3H, OCH_3 of PMB), 3.77–3.74 (m, 1H, 10-H), 3.70–3.65 (m, 1H, 12-H), 3.32–3.27 (m, 1H, 16-H) 2.31–2.21 (m, 3H, 3-H and 9-H), 2.15–2.08 (m, 1H, 3-H), 1.74–1.69 (m, 1H, 5-H), 1.62–1.25 (m, 10H, 5-H + 11-H + 13-H + 14-H + 15-H + 17-H), 0.93–0.84 (m, 42H, CH_3 of TBS + 11-CH_3 + 18-H), 0.10–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.01, 143.31, 143.33, 135.54, 131.31, 129.17, 126.81, 113.71. 79.79, 76.45, 74.78, 72.90, 72.25, 70.74, 70.45, 68.22, 55.26, 46.03, 43.37, 41.26, 37.84, 35.40, 33.91, 25.99, 25.90, 25.85, 21.30, 18.18, 18.14, 18.12, 18.01, 9.52, −3.72, −3.90, −4.24, −4.35, −4.42, −4.49, −4.61, −4.71. HRMS (ESI, negative) m/z for $C_{51}H_{98}DIO_6Si_4Cl$ $[M+Cl]^-$: calc. 1082.5320, found 1082.5324.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl-7-deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-16-methyl-21-(4-methoxybenzyloxy)tricosa-2,4,6,12-tetraenoate (61). Compound **61** was prepared following the same procedure as compound **42** using compound **40** with a yield of 71%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.31–7.25 (m, 3H, Ph of PMB + 3-H), 6.87–6.84 (m, 2H, Ph of PMB), 6.52 (d, 1H, J = 10.8 Hz, 5-H), 6.23–6.18 (m, 1H, 4-H), 6.17–6.10 (m, 1H, 6-H), 5.86 (d, 1H, J = 15.3 Hz, 2-H), 5.57–5.51 (m, 1H, 13-H), 5.41 (dd, 1H, J = 15.4, 6.8 Hz, 12-H), 4.43 (s, 2H, CH_2 of PMB), 4.20 (q, 2H, J = 7.1 Hz, $CH_3\mathbf{CH}_2CO(O)$), 4.13 (q, 1H, J = 6.6 Hz, 11-H), 3.86–3.81 (m, 1H, 9-H), 3.79 (s, 3H, OCH_3 of PMB), 3.77–3.74 (m, 1H, 15-H), 3.70–3.67 (m, 1H, 17-H), 3.30–3.27 (m, 1H, 21-H) 2.40–2.35 (m, 1H, 8-H), 2.27–2.20 (m, 3H, 8-H and 14-H), 1.75–1.69 (m, 1H, 10-H), 1.62–1.25 (m, 13H, 10-H + 16-H +18-H + 19-H +20-H + 22-H + $\mathbf{CH}_3CH_2CO(O)$), 0.92–0.84 (m, 42H, CH_3 of TBS + 16-CH_3 + 23-H), 0.04–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.17, 159.02, 144.72, 140.89, 136.54, 135.62, 132.03, 131.31, 129.18, 128.13, 120.23, 113.70, 79.79, 72.89, 72.25, 70.80, 70.46, 68.94, 60.17, 55.24, 46.15, 41.23, 40.75, 37.87, 35.41, 33.90, 25.98, 25.90, 25.86, 21.29, 18.16, 18.13, 18.03, 14.31, 9.50, −3.74, −3.76, −3.94, −4.27, −4.32, −4.38, −4.52, −4.73. HRMS (ESI, positive) m/z for $C_{58}H_{107}DO_8Si_4Na$ $[M+Na]^+$: calc. 1068.7076, found 1068.7065.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-7-Deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (62). Compound **62** was prepared following the same procedure as compound **44** using compound **61** instead of **42** with a yield of 60% over two steps. HRMS (CI, negative) m/z for $C_{48}H_{94}DO_7Si_4$ $[M-H]^-$: calc. 896.6223, found 896.6215.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-7-deuterio-macrolactone (63). Compound **63** was prepared following the same procedure as compound **45** with a yield of 56.8%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.30–7.24 (m, 1H, 3-H), 6.48 (dd, J = 15.0, 10.5 Hz, 1H, 5-H), 6.25–6.20 (m, 1H, 4-H), 6.13 (d, 1H, J = 10.5 Hz, 6-H), 5.82 (d, 1H, J = 15.0 Hz, 2-H), 5.43–5.27 (m, 2H, 12-H and 13-H), 4.90–4.80 (m, 1H, 21-H), 4.05–4.00 (m, 1H, 11-H), 3.80–3.72 (m, 1H, 9-H), 3.71–3.64 (m, 1H, 15-H), 3.61–3.52 (m, 1H, 17-H), 2.46 (d, J = 10.0 Hz, 1H, 8-H), 2.37–2.20 (m, 2H, 8-H and 14-H), 2.14–2.04 (m, 1H, 14-H), 1.78–1.20 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.90–0.82 (m, 39H, CH_3 of TBS and 23-H), 0.75 (d, J = 7.0 Hz, 3H, 16-CH_3), 0.08–0.06 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 166.9, 144.8, 140.9, 135.2, 131.9, 128.0, 127.1, 120.8, 75.2, 73.2, 72.1, 71.1, 69.1, 46.6, 42.4, 42.0, 38.4, 34.4, 33.4, 27.9, 27.8, 26.1, 26.0, 25.9, 25.8, 21.1, 18.25, 18.11, 18.08, 18.01, 10.2, 9.9, −3.47, −3.86, −3.96, −4.32, −4.39, −4.52, −4.516, −4.62. HRMS (ESI, positive) m/z for $C_{48}H_{93}DO_6Si_4Na$ $[M + Na]^+$: calc. 902.6082, found 902.6082.

Monomacrolactone (64). Compound **64** was prepared following the same procedure as compound **2** with a yield of 65%. HRMS (ESI, positive) m/z for $C_{24}H_{37}DO_6Na$ $[M + Na]^+$: calc. 446.2623, found 446.2625.

[C7-^{2}H]-SpnM substrate (65). Compound **65** was prepared following the same procedure as compound **3** with a yield of 91%. HRMS (ESI, positive) m/z for $C_{24}H_{35}DO_6Na$ $[M+Na]^+$: calc. 444.2465, found 444.2467.

C11D isotopolog.

3-(4-Methoxybenzyloxy)propane-1,2-diol (66). Glycerol (92.0 g, 1.0 mol) was mixed with 2,2-dimethoxypropane (136 mL, 1.1 mol) in DMSO (200 mL) containing a catalytic amount of *p*-toluenesulfonic acid (1.9 g, 10 mmol) at room temperature for 18 h. Aqueous 3% sodium bicarbonate (360 mL) was slowly added to the reaction mixture, and the aqueous fraction was partitioned with ethyl acetate (1 L ×3). The combined organic fractions were washed with water (300 mL ×3), dried over sodium sulfate and concentrated under reduced pressure. The residue was vacuum-distilled at 70–80 °C to afford the intermediate (boiling point 188 °C at 760 torr; 129 g, 98%).

Sodium hydride (60% in mineral oil; 39.2 g, 1.18 mol) was added portion-wise over 30 min to a solution of the above intermediate (129 g, 0.98 mol) in DMF (0.98 L) at 0 °C with mechanical stirring. After stirring for an additional 30 min, *p*-methoxybenzyl chloride (freshly prepared) from the reaction of *p*-methoxybenzyl alcohol (154.7 g, 1.12 mol) and thionyl chloride ($SOCl_2$; 233.0 g, 1.96 mol) in diethyl ether (1.12 L) was added to the reaction mixture at 0 °C over 1 h. The reaction mixture was allowed to warm to room temperature with vigorous stirring for 18 h. The reaction mixture was quenched by the addition of water (0.98 L) at 0 °C over 30 min, the mixture was partitioned with ethyl acetate (500 mL ×4). The combined organic layers were washed with brine (500 mL), dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was concentrated further under high vacuum to produce a quantitative amount of an intermediate, which was used directly in the next step without further purification.

The above intermediate was directly dissolved in a solution of aqueous 1.0 M hydrochloric acid (250 mL) and methanol (375 mL) at room temperature and stirred at room temperature for 18 h. After removal of the methanol under reduced pressure, the mixture was partitioned by the addition of water (400 mL) and ethyl acetate (250 mL ×4). The combined organic layers were washed with brine (250 mL), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/1) to afford the vicinal diol **66** (152 g, 0.72 mol, 73%).

2-Deuterio-3-(4-methoxybenzyloxy)propane-1,2-diol (67). *tert*-Butyl (chloro)diphenylsilane (80.0 g, 0.29 mol) was added drop-wise over 30 min to a solution of diol compound **66** (62.0 g, 0.29 mol) and imidazole (39.5 g, 0.58 mol) in anhydrous CH_2Cl_2 (580 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h and quenched by the addition of a saturated solution of ammonium chloride (500 mL). The mixture was partitioned with CH_2Cl_2 (300 mL ×2) and washed with brine (500 mL) and water (500 mL). The organic layer was dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford an intermediate (125 g, 0.28 mol, 96%).

Dess-Martin periodinane (103.5 g, 0.24 mol) was added portion-wise to a solution of the above compound (100.0 g, 0.22 mol) in anhydrous CH_2Cl_2 (500 mL) at 0 °C over 30 min with vigorous stirring. The reaction mixture was allowed to warm to room temperature over 4 h with stirring. An aqueous solution of 10% sodium thiosulfate (500 mL) was added to the reaction mixture and stirred for additional 30 min. The mixture was partitioned with CH_2Cl_2 (200 mL ×3), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford a ketone intermediate (95 g, 0.21 mol, 94%).

Lithium aluminum deuteride (10.6 g, 254 mmol) was added to a solution of the keto compound (95.0 g, 0.21 mol) in anhydrous THF (1.05 L) at −78 °C over 30 min. The reaction mixture was kept stirring at −78 °C for 2 h and allowed to warm to room temperature over 18 h with stirring. The mixture was quenched by the careful addition of 10% sodium hydroxide (420 mL) and a saturated solution of sodium potassium tartrate (420 mL). The reaction mixture was then stirred for one additional hour, filtered through filter paper and washed with ethyl acetate (200 mL ×3). The mixture was partitioned with ethyl acetate (300 mL ×3), dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/1 then EtOAc only) to afford the diol **67** (26.0 g, 0.12 mol, 58%).

1-Deuterio-2-((4-methoxybenzyloxy)methyl)oxirane (68). Triethylamine (23.8 mL, 0.171 mol) and dibutyltin oxide (1.52 g, 0.006 mol) were added to a solution of the diol **67** (26 g, 0.122 mol) in anhydrous CH_2Cl_2 (150 mL) at 0 °C. The suspension was stirred at 0 °C for 10 min followed by portion-wise addition of tosyl chloride (24.4 g, 0.128 mol) over 20 min. The reaction mixture was then stirred at 0 °C for 30 min and at room temperature for an additional 18 h. The reaction mixture was quenched by the addition of water (50 mL) and 1 N HCl (50 mL), and the mixture was partitioned with CH_2Cl_2 (100 mL ×2). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford a tosylated intermediate.

Sodium hydride (5.9 g, 0.148 mol) was added portion-wise to a solution of the intermediate in THF (150 mL) at 0 °C over 20 min. The resulting white suspension was stirred at room temperature for 18 h. The reaction mixture was quenched by the addition of a saturated solution of ammonium chloride (100 mL) at 0 °C. The mixture was partitioned with EtOAc (150 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/6) to afford the epoxide **68** (16.87 g, 70.9%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25–7.20 (m, 2H, Ph of PMB), 6.86–6.81 (m, 2H, Ph of PMB), 4.49 (d, $J = 11.4$ Hz, 1H, CH_2 of PMB), 4.44 (d, $J = 11.4$ Hz, 1H, CH_2 of PMB), 3.75 (s, 3H, OCH_3), 3.69 (d, $J = 11.6$ Hz, 1H, 3-H), 3.36 (d, $J = 11.6$ Hz, 1H, 3-H), 2.73 (d, $J = 5.2$ Hz, 1H, 1-H), 2.55 (d, $J = 5.2$ Hz, 1H, 1-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.2, 129.8, 129.3, 113.7, 72.8, 70.3, 55.1, 50.4 (t, $J = 26.8$ Hz), 44.1. HRMS (CI, positive) m/z for $C_{11}H_{14}DO_3$ $[M + H]^+$: calc. 196.1006, found 196.1003.

1-(1,3-Dithian-2-yl)-2-deuterio-3-(4-methoxybenzyloxy)propan-2-yl-oxy-tert-butyldimethylsilane (70). *n*-BuLi (51.8 mL, 0.130 mol) was added drop-wise to a solution of dithiane (14.5 g, 0.121 mol) in THF (150 mL) cooled to −78 °C over 30 min. The resulting brownish solution was stirred at 0 °C for another 1 h, and a solution of the epoxide **68** (16.87 g, 0.086 mol) in THF (50 mL) was added drop-wise over 45 min. The reaction was stirred at 0 °C for 2.5 h. The mixture was quenched by the addition of saturated ammonium chloride (100 mL) at 0 °C. After the removal of THF under reduced pressure, the residue was partitioned with CH_2Cl_2 (200 mL ×3). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to afford the secondary alcohol **69**.

The crude alcohol **69** from the previous step was mixed with imidazole (12.9 g, 0.190 mol) in anhydrous *N*,*N*-dimethylformamide (150 mL).*tert*-Butyldimethylsilyl chloride (14.3 g, 0.095 mol) was then added to the mixture over 30 min at 0 °C. The reaction mixture was then stirred at room temperature for 24 h before concentration under reduced pressure and partitioning of the residue with CH_2Cl_2 (250 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford compound **70** (36.59 g, 0.085 mol, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.26–7.20 (m, 2H, Ph of PMB), 6.88–6.81 (m, 2H, Ph of PMB), 4.42 (s, 2H, CH_2 of PMB), 4.10 (dd, $J = 9.6$, 4.8 Hz, 1H, 4-H), 3.78 (s, 3H, OCH_3 of PMB), 3.38 (d, $J = 9.8$ Hz, 1H, 1-H), 3.31 (d, $J = 9.8$ Hz, 1H, 1-H), 2.90–2.69 (m, 4H, 2′-H and 4′-H), 2.12–2.02 (m, 1H, 3′-H), 1.94 (dd, $J = 14.2$, 9.6 Hz, 1H, 3-H), 1.92–1.79 (m, 2H, 3-H and 3′H), 0.87 (s, 9H, TBS), 0.08 (s, 3H, TBS), 0.04 (s, 3H, TBS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 159.1, 130.3, 129.2, 113.7, 74.2, 72.9, 67.5 (t, $J = 21.2$ Hz, C-3), 55.2, 43.6, 40.2, 30.4, 29.8, 26.0, 25.9, 18.1, −4.4, −4.9. HRMS (ESI, positive) m/z for $C_{21}H_{35}DO_3S_2SiNa$ $[M+Na]^+$: calc. 452.1830, found 452.1838.

3-(tert-Butyldimethylsilyloxy)-3-deuterio-4-(4-methoxybenzyloxy)butanal (71). A mixture of the substituted dithiane **70** (37.13 g, 86.4 mmol), calcium carbonate (51.9 g, 518.5 mmol), iodomethane (53.8 mL, 864 mmol) in acetonitrile/water co-solvent (575 mL, $v/v = 4/1$) was refluxed for 12 h. Calcium carbonate was filtered off through a Celite pad, and the filtrate was concentrated under reduced pressure to remove acetonitrile. The crude residue was partitioned with CH_2Cl_2 (200 mL ×2). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/6) to afford the aldehyde **71** (21.73 g, 64.1 mmol, 74.2%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.77 (dd, $J = 2.8$, 2.4 Hz, 1H, 1-H), 7.24–7.18 (m, 2H, Ph of PMB), 6.89–6.83 (m, 2H, Ph of PMB), 4.43 (s, 2H, CH_2 of PMB), 3.79 (s, 3H, OCH_3 of PMB), 3.45 (d, $J = 9.6$ Hz, 1H, 4-H), 3.34 (d, $J = 9.6$ Hz, 1H, 4-H), 2.62 (dd, $J = 15.6$, 2.4 Hz, 1H, 2-H), 2.54 (dd, $J = 15.6$, 2.8 Hz, 1H, 2-H), 0.84 (s, 9H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.5, 159.2, 130.0, 129.3, 113.8, 73.6, 73.0, 67.0 (t, $J = 21.5$ Hz), 55.2, 48.8, 25.7, 18.0, 4.5, 5.0. HRMS (ESI): m/z for $C_{18}H_{29}DO_4SiNa$ $[M + Na]^+$: calc. 362.18683, found 362.18680.

(4R)-6-(tert-Butyldimethylsilyloxy)-6-deuterio-7-(4-methoxybenzyloxy)hept-1-en-4-ol (72). A solution of allylmagnesium bromide (1 M in ethyl ether, 89 mL, 89 mmol) was added drop-wise to a solution of (+)-diisopinocampeylchloroborane (1.6 M in THF, 56 mL, 89.6 mmol) in THF at 0 °C over 1.5 h. After stirring at 0 °C for 30 min, a solution of the aldehyde **71** (21.73 g, 64 mmol) in THF (50 mL) was added drop-wise to the mixture at −78 °C over 45 min. The mixture was stirred at −78 °C for 1.5 h and then quenched by the slow addition of methanol (125 mL), sodium hydroxide (1 N, 125 mL) and 30% hydrogen peroxide (125 mL). The mixture was then filtered on a Celite pad and partitioned with CH_2Cl_2 (200 mL ×2). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/19 then 1/9) to afford the allyl alcohol **72** (21.4 g, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.24–7.18 (m, 2H, Ph of PMB), 6.90–6.80 (m, 2H, Ph of PMB), 5.88–5.72 (m, 1H, 6-H), 5.13–5.03 (m, 2H, 7-H), 4.46 (d, $J = 11.6$ Hz, 1H, CH_2 of PMB), 4.43 (s, 1H, CH_2 of PMB), 3.92–3.78 (m, 1H, 4-H), 3.79 (s, 3H, OCH_3 of PMB), 3.43 (d, $J = 9.6$ Hz, 1H, 1-H), 3.39 (d, $J = 9.6$ Hz, 1H, 1-H), 3.22 (br s, 1H, OH), 3.13 (br s, 1H, OH), 2.21 (br t, $J = 6.4$ Hz, 2H, 5-H), 1.73 (ddd, $J = 20.0$, 14.4, 2.4 Hz, 1H, 3-H), 1.58 (ddd, $J = 16.0$, 14.4, 8.8 Hz, 1H, 3-H), 0.85 (s, 9H, CH_3 of TBS), 0.06 (s, 3H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 159.2, 134.5, 130.05, 129.35, 129.33, 117.4, 113.7, 74.7, 73.0, 69.1, 67.7, 55.2, 42.4, 40.9, 25.8, 18.0, −4.2, −4.6, −4.8, −5.0. HRMS (ESI, positive) m/z for $C_{21}H_{35}DO_4SiNa$ $[M + Na]^+$: calc. 404.23378, found 404.23305.

(2R,4R,E)-1-(4-Methoxybenzyloxy)-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-ene (75). Compound (**73**) was prepared following the same procedure as compound (**33**) with a yield of 98%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.25–7.21 (m, 2H, Ph of PMB), 6.87–6.83 (m, 2H, Ph of PMB), 5.85–5.72 (m, 1H, 6-H), 5.05–4.98 (m, 2H, 7-H), 4.44 (d, $J = 11.8$ Hz, 1H, CH_2 of PMB), 4.41 (d, $J = 11.8$ Hz, 1H, CH_2 of PMB), 3.90–3.80 (m, 1H, 4-H), 3.79 (s, 3H, OCH_3 of PMB), 3.37–3.30 (m, 2H, 1-H), 2.30–2.09 (m, 2H, 5-H), 1.69 (dd, $J = 13.8$, 6.8 Hz, 1H, 3-H), 1.62–1.55 (m, 1H, 3-H), 0.86–0.84 (m, 18H, CH_3 of TBS), 0.05–0.00 (m, 12 H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 159.1, 135.1, 130.6, 129.2, 116.9, 113.7,

75.1, 72.9, 69.5, 68.9 (t, J = 20.3 Hz, C-2), 55.3, 42.7, 42.0, 26.05, 25.90, 18.26, 18.14, −3.9, −4.0, −4.2, −4.2.

To a solution of the olefin **73** (27.1 g, 54.6 mmol) in 1,4-dioxane/water co-solvent (200 mL, v/v = 3/1) cooled to 0 °C was added 2,6-lutidine (9.5 mL, 81.8 mmol), osmium tetroxide (250 mg, 0.98 mmol) and sodium periodate (35.1 g, 164 mmol). The suspension was stirred at 0 °C for 30 min and then at room temperature for 12 h. The pale yellow reaction mixture was quenched by the addition of a solution of sodium thiosulfate (1 N, 100 mL). The reaction mixture was filtered on a Celite pad, and the filtrate was partitioned with CH_2Cl_2 (100 mL ×2). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49, then 1/9) to afford the aldehyde **74** (20.2 g, 40.6 mmol, 74%). HRMS (ESI, postive): m/z for $C_{26}H_{47}DO_5Si_2Na$ $[M+Na]^+$: calc. 520.2995, found 520.2984.

A solution of iodoform (32 g, 81.3 mmol) in THF (80 mL) was added to a solution of the aldehyde **74** (20.2 g, 40.6 mmol) and $CrCl_2$ (24.9 g, 203 mmol) in THF (400 mL) at 0 °C. The resulting black solution was stirred at 0 °C for 4 d. The reaction was quenched by the addition of water (300 mL) at 0 °C, and the mixture was stirred at room temperature for another 1 h. After the removal of the THF under reduced pressure, the remaining solution was partitioned with CH_2Cl_2 (300 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49 then 1/19) to give the vinyl iodide **75** (18.6 g, 29.9 mmol, 73.7%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26–7.19 (m, 2H, Ph of PMB), 6.89–6.83 (m, 2H, Ph of PMB), 6.48 (dt, J = 14.4, 7.2 Hz, 1H, 6-H), 6.00 (dt, J = 14.4, 1.2 Hz, 1H, 7-H), 4.48–4.36 (m, 2H, CH_2 of PMB), 4.00–3.76 (m, 1H, 4-H), 3.79 (s, 3H, OCH_3 of PMB), 3.37–3.27 (m, 2H, 1-H), 2.38–2.02 (m, 2H, 5-H), 1.72–1.51 (m, 2H, 3-H), 0.89 (br s, 9H, CH_3 of TBS), 0.84 (br s, 9H, CH_3 of TBS), 0.08–0.00 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) 159.1, 159.1, 143.2, 143.0, 130.4, 130.3, 129.2, 129.2, 113.7, 113.6, 76.6, 76.6, 74.5, 74.4, 72.9, 72.8, 68.7, 68.0, 55.2, 44.4, 43.4, 42.9, 42.2, 25.9, 25.9, 25.8, 25.8, 18.2, 18.1, 18.0, 18.0, −4.0, −4.2, −4.22, −4.4, −4.5, −4.51, −4.6, −4.8. HRMS (ESI, positive): m/z for $C_{27}H_{48}DIO_4Si_2Na$ $[M+Na]^+$: calc. 644.2169, found 644.2168.

(2R,4R,E)-2-Deuterio-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-en-1-ol (76). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 8.15 g, 35.9 mmol) was added portion-wise to a solution of PMB ether **75** (18.6 g, 29.9 mmol) in CH_2Cl_2/MeOH/phosphate buffer co-solvent (108 mL, v/v = 10/1/1) at 0 °C over 30 min. After 6 h of stirring at 0 °C, the solution was treated with a saturated solution of sodium bicarbonate (100 mL). The mixture was then partitioned with CH_2Cl_2 (200 mL ×3), and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 1/12) to afford the primary alcohol **76** (15.0 g, 39%). Two diastereomers at C-2 could be separated at this step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.48 (dt, J = 14.6, 7.4 Hz, 1H, 6-H), 6.04 (dt, J = 14.6, 1.2 Hz, 1H, 7-H), 3.80 (br quint, J = 6.0 Hz, 1H, 4-H), 3.55 (dd, J = 11.2, 4.8 Hz, 1H, 1-H), 3.44 (dd, J = 11.2, 7.8 Hz, 1H, 1-H), 2.30–2.12 (m, 3H, 5-H and OH), 1.68 (dd, J = 14.0, 6.8 Hz, 1H, 3-H), 1.62 (dd, J = 14.0, 5.2 Hz, 1H, 3-H), 0.88 (s, 9H, CH_3 of TBS), 0.87 (s, 9H, CH_3 of TBS), 0.06 (s, 6H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS), 0.03 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.6, 77.1, 69.3 (t, J = 21.0 Hz), 68.2, 66.1, 43.7, 41.2, 25.8, 25.8, 18.0, 18.0, −4.4, −4.5, −4.51, −4.6. HRMS (ESI, positive): m/z for $C_{19}H_{40}DIO_3Si_2Na$ $[M+Na]^+$: calc. 524.1594, found 524.1601.

Diastereomer of 76. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.48 (dt, J = 14.4, 7.2 Hz, 1H, 6-H), 6.03 (dt, J = 14.6, 1.2 Hz, 1H, 7-H), 3.76 (br quint, J = 6.0 Hz, 1H, 4-H), 3.55 (br d, J = 10.4 Hz, 1H, 1-H), 3.42 (br d, J = 10.4 Hz, 1H, 1-H), 2.35–2.12 (m, 2H, 5-H), 1.93 (br s, 1H, OH), 1.70–1.57 (m, 2H, 5-H), 0.88 (s, 9H, CH_3 of TBS), 0.86 (s, 9H, CH_3 of TBS), 0.07 (s, 6H, CH_3 of TBS), 0.04 (s, 6H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.6, 77.0, 70.4 (t, J = 21.2 Hz), 68.8, 66.6, 44.1, 41.6, 25.8, 25.8, 18.0, 18.0, −4.2, −4.29, −4.3, −4.4.

(2R,4R,E)-2-Deuterio-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-en-1-al (77). Compound **77** was prepared following the same procedure as compound **37** with a yield of 96%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 9.56 (s, 1H, 7-H), 6.45 (dt, J = 14.4, 7.6 Hz, 1H, 2-H), 6.03 (dt, J = 14.4, 1.2 Hz, 1H, 1-H), 3.94 (quint, J = 6.0 Hz, 1H, 4-H), 2.30–2.12 (m, 2H, 3-H), 1.83–1.72 (m, 2H, 5-H), 0.89 (s, 9H, CH_3 of TBS), 0.85 (s, 9H, CH_3 of TBS), 0.06 (s, 3H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS), 0.03 (s, 6H, CH_3 of TBS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 203.5, 142.3, 77.1, 74.3 (t, J = 21.3 Hz, C-6), 66.7, 43.5, 40.1, 25.80, 25.72, 18.07, 18.01, −4.36, −4.51, −4.63, −4.93. HRMS (ESI, positive) m/z for $C_{19}H_{39}DIO_3Si_2$ $[M+H]^+$: calc. 500.1618, found 500.1609.

(1E,4R,6R,7E,10R,11R,12S,16S)-6-Deuterio-4,6,10,12-tetrakis(tert-butyldimethylsilyloxy)-11-methyl-16-(4-methoxybenzyloxy)octadeca-1,7-diene (78). Potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 5.2 mL, 2.6 mmol) was added drop-wise over 30 min to a solution of compound **24** (2.0 g, 2.58 mmol) in anhydrous THF (50 mL) at −78 °C, and the reaction mixture was stirred for 1 h, at which time a solution of compound **77** (fragment B) (1.63 g, 3.26 mmol) in THF (10 mL) was added to the mixture at −78 °C over 1 h. After 4 h, the temperature was slowly raised to room temperature over 1 h, and then the reaction mixture was poured into a saturated solution of sodium bicarbonate (10 mL). The resulting mixture was partitioned with EtOAc (20 mL ×3), washed with brine (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to give **78** (2.1 g, 2.00 mmol, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28–7.21 (m, 2H, Ph of PMB), 6.87–6.81 (m, 2H, Ph of PMB), 6.47 (dt, J = 14.6, 7.4 Hz, 1H, 2-H), 5.98 (br d, J = 14.6 Hz, 1H, 1-H), 5.59–5.43 (m, 1H, 8-H), 5.38 (br d, J = 15.6 Hz, 1H, 7-H), 4.41 (s, 2H, CH_2 of PMB), 3.78 (s, 3H, OCH_3 of PMB), 3.86–3.70 (m, 2H, 4-H and 10-H), 3.70–3.56 (m, 1H, 12-H), 3.27 (quint, J = 5.6 Hz, 1H, 16-H), 2.32–2.18 (m, 3H, 9-H and 3-H), 2.18–2.02 (m, 1H, 3-H), 1.75–1.64 (m, 1H, 11-H), 1.64–1.18 (m, 10H, 5-H + 13-H + 14-H + 15-H + 17-H), 0.96–0.74 (m, 42H, CH_3 of TBS + 11-CH_3 + 18-H), 0.04–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.0, 143.3, 135.4, 131.2, 129.2, 126.8, 113.7, 79.8, 72.9, 72.2, 70.4, 68.2, 55.2, 45.9, 43.5, 41.2, 37.8, 35.4, 33.9, 26.2, 26.0, 26.0, 25.9, 25.8, 25.8, 21.3, 18.2, 18.1, 18.1, 18.0, −3.6, −3.7, −3.7, −3.9, −4.0, −4.3, −4.4, −4.5, −4.7.

HRMS (ESI, positive): m/z calc. for $C_{51}H_{98}DIO_6Si_4Na$ $[M + Na]^+$: 1070.5518, found 1070.5546.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl-11-deuterio-9,11,15, 17-tetrakis(tert-butyldimethylsilyloxy)-16-methyl-21-(4-methoxybenzyloxy)tricosa-2,4,6,12-tetraenoate (79). Compound **79** was prepared following the same procedure as compound **42** using compound **40** with a yield of 57%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (dd, $J = 15.2$, 11.2 Hz, 1H, 3-H), 7.28–7.21 (m, 2H, Ph of PMB), 6.87–6.81 (m, 2H, Ph of PMB), 6.50 (dd, $J = 14.6$, 11.0 Hz, 1H, 5-H), 6.18 (dd, $J = 14.6$, 11.2 Hz, 1H, 4-H), 6.11 (dd, $J = 15.0$, 11.0 Hz, 1H, 6-H), 6.00–5.78 (m, 1H, 7-H), 5.82 (d, $J = 15.2$ Hz, 1H, 2-H), 5.58–5.44 (m, 1H, 13-H), 5.38 (br d, $J = 15.6$ Hz, 1H, 12-H), 4.40 (s, 2H, CH_2 of PMB), 4.18 (q, $J = 7.2$ Hz, 2H, CH_2 of C(=O)OEt), 3.77 (s, 3H, OCH_3 of PMB), 3.88–3.70 (m, 2H, 9-H and 15-H), 3.70–3.56 (m, 1H, 17-H), 3.27 (quint, $J = 5.6$ Hz, 1H, 21-H), 2.54–2.12 (m, 4H, 8-H and 14-H), 1.78–1.20 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 1.27 (t, $J = 7.2$ Hz, 3H, CH_3 of C(=O)OEt), 0.94–0.78 (m, 42H, CH_3 of TBS + 16-CH_3 + 23-H), 0.08–0.04 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 159.0, 144.7, 140.9, 136.5, 135.5, 132.0, 131.2, 129.2, 128.1, 126.8, 120.2, 113.7, 79.8, 72.2, 70.4, 68.9, 60.2, 55.2, 46.0, 41.2, 40.8, 35.4, 33.9, 26.2, 26.0, 26.0, 25.9, 25.8, 25.8, 21.3, 18.2, 18.1, 18.0, 14.3, 9.5, −3.7, −3.8, −3.9, −4.3, −4.4, −4.5, −4.7. HRMS (ESI, positve): m/z for $C_{58}H_{107}DO_8Si_4Na$ $[M + Na]^+$: calc. 1068.7076, found 1068.7078.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-11-Deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6, 12-tetraenoic acid (80). Compound **80** was prepared following the same procedure as compound **44** using compound **79** instead of **42** with a yield of 51% for two steps. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (dd, $J = 15.3$, 11.5 Hz, 1H, 3-H), 6.55 (dd, $J = 15.0$, 10.8 Hz, 1H, 5-H), 6.22 (dd, $J = 15.0$, 11.5 Hz, 1H, 4-H), 6.14 (dd, $J = 15.0$, 10.8 Hz, 1H, 6-H), 5.94 (dt, $J = 15.0$, 7.5 Hz, 1H, 7-H), 5.83 (d, $J = 15.3$ Hz, 1H, 2-H), 5.55 (dt, $J = 15.0$, 7.5 Hz, 1H, 13-H), 5.40 (br d, $J = 15.0$ Hz, 1H, 12-H), 3.81 (quint, 1H, $J = 6.0$ Hz, 9-H), 3.73 (q, 1H, $J = 5.5$ Hz, 15-H), 3.67 (q, 1H, $J = 5.5$ Hz, 17-H), 3.54–3.44 (m, 1H, 21-H), 2.48–2.32 (m, 1H, 8-H), 2.32–2.16 (m, 3H, 8-H and 14-H), 1.70 (dd, $J = 13.5$, 6.0 Hz, 1H, 10-H), 1.65–1.16 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H + OH), 0.92 (t, $J = 7.5$ Hz, 3H, 23-H), 0.90–0.84 (m, 36H, CH_3 of TBS), 0.83 (d, $J = 7.0$ Hz, 3H, 16-CH_3), 0.07–0.02 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 147.0, 142.0, 137.4, 135.6, 132.0, 127.9, 126.9, 119.0, 73.1, 72.6, 72.2, 69.0, 46.0, 40.8, 40.6, 37.7, 37.4, 35.1, 30.1, 26.0, 26.0, 25.9, 25.9, 21.3, 18.2, 18.2, 18.1, 18.1, 9.9, 9.3, −3.8, −3.82, −4.0, −4.3, −4.4, −4.6, −4.7. HRMS (ESI, positive): m/z for $C_{48}H_{95}DO_7Si_4Na$ $[M + Na]^+$: calc. 920.61880, found 920.61790.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-11-deuterio-macrolactone (81). Compound **81** was prepared following the same procedure as compound **45** with a yield of 72%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24 (dd, $J = 15.0$, 11.0 Hz, 1H, 3-H), 6.47 (dd, $J = 15.0$, 10.5 Hz, 1H, 5-H), 6.21 (dd, $J = 15.0$, 11.0 Hz, 1H, 4-H), 6.12 (dd, $J = 15.5$, 10.5 Hz, 1H, 6-H), 5.80 (d, $J = 15.0$ Hz, 1H, 2-H), 5.78 (dt, $J = 15.0$, 7.5 Hz, 1H, 7-H), 5.42–5.22 (m, 2H, 12-H and 13-H), 4.90–4.80 (m, 1H, 21-H), 3.80–3.72 (m, 1H, 9-H), 3.71–3.64 (m, 1H, 15-H), 3.61–3.52 (m, 1H, 17-H), 2.50–2.06 (m, 4H, 8-H and 14-H), 1.78–1.16 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91 (t, $J = 7.5$ Hz, 3H, 23-H), 0.90–0.80 (m, 36H, CH_3 of TBS), 0.75 (d, $J = 7.0$ Hz, 3H, 16-CH_3), 0.08–0.02 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.9, 144.7, 140.9, 136.0, 135.2, 132.0, 128.0, 127.1, 120.8, 75.2, 73.2, 69.1, 46.5, 42.3, 42.1, 38.3, 34.4, 33.4, 27.8, 26.0, 26.0, 26.0, 25.9, 25.8, 25.8, 21.1, 18.2, 18.2, 18.1, 18.1, 18.1, 18.0, 18.0, 10.2, 9.8, −3.5, −3.8, −4.0, −4.3, −4.4, −4.48, −4.5, −4.6, −4.62, −4.7. HRMS (ESI, positive): m/z for $C_{48}H_{93}DO_6Si_4$ $[M + Na]^+$: calc. 902.60820, found 902.60860.

Monomacrolactone (82). Compound **82** was prepared following the same procedure as compound **2** with a yield of 65%. HRMS (ESI, positive) m/z for $C_{24}H_{37}DO_6Na$ $[M + Na]^+$: calc. 446.2623, found 446.2625.

[C11-^{2}H]-SpnM substrate (83). Compound **83** was prepared following the same procedure as compound **3** with a yield of 92%. HRMS (ESI, positive) m/z for $C_{24}H_{35}DO_6Na$ $[M+Na]^+$: calc. 444.2465, found 444.2467.

C12D isotopolog.

(2R,4R,E)-Methyl 2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-enoate (84). *N*-Iodosuccinimide (NIS; 2.3 g, 10 mmol) and potassium carbonate (1.4 g, 10 mmol) were added sequentially to a solution of the aldehyde **37** (2.0 g, 4 mmol) in anhydrous methanol (20 mL) at room temperature and covered with aluminum foil. The reaction mixture was stirred at room temperature for 18 h. The reaction was then quenched by the addition of a saturated solution of sodium thiosulfate (20 mL), and the mixture was partitioned with CH_2Cl_2 (50 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/19) to afford the methyl ester **84** (1.5 g, 2.89 mmol, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 6.50 (ddd, 1H, $J = 7.1$, 8.0, 14.5 Hz, 6-H), 6.05 (d, 1H, $J = 14.5$ Hz, 7-H), 4.28 (dd, $J = 5.6$, 7.1 Hz, 2-H), 3.90–3.85 (m, 1H, 4-H), 3.71 (s, 3H, OCH_3), 2.34–2.29 (m, 1H, 5-H), 2.23–2.17 (m, 1H, 5-H), 1.91–1.80 (m, 2H, 3-H), 0.90 (s, 9H, CH_3 of TBS), 0.88 (s, 9H, CH_3 of TBS), 0.073 (s, 3H, CH_3 of TBS), 0.052 (s, 3H, CH_3 of TBS), 0.047 (s, 3H, CH_3 of TBS), 0.045 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 173.77, 142.83, 137.46, 69.38, 67.82, 51.78, 43.33, 42.20, 25.81, 25.70, 18.19, 17.99, −4.55, −4.70, −4.70, −5.28. HRMS (ESI, positive) m/z for $C_{20}H_{41}IO_4Si_2Na$ $[M + Na]^+$: calc. 551.1480, found 551.1484.

(2R,4R,E)-1,1-Dideuterio-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-en-1-ol (85). Lithium aluminum deuteride ($LiAlD_4$, 265 mg, 5.6 mol) was added portion-wise to a solution of the ester **84** (2.0 g, 3.8 mmol) in anhydrous THF (37 mL) at −78 °C. The reaction mixture was kept stirring at −78 °C for 2 h and subsequently quenched by the addition of a saturated solution of ammonium chloride (37 mL). The mixture was then filtered on a Celite pad and washed with ethyl acetate (30 mL). The filtrate was then partitioned with ethyl acetate (50 mL ×3). The combined organic layers were dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/9) to afford the primary alcohol **85** (0.84 g, 1.67 mmol, 44%) as well as residual starting material (0.92 g, 46%).

^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 6.46 (ddd, 1H, J = 7.1, 8.0, 14.5 Hz, 6-H), 6.04 (d, 1H, J = 14.7 Hz, 7-H), 3.85 (dd, J = 5.7, 7.6 Hz, 2-H), 3.83–3.79 (m, 1H, 4-H), 2.27–2.22 (m, 1H, 5-H), 2.20–2.15 (m, 1H, 5-H), 1.71–1.61 (m, 2H, 3-H), 0.88 (s, 9H, CH_3 of TBS), 0.87 (s, 9H, CH_3 of TBS), 0.066 (s, 3H, CH_3 of TBS), 0.062 (s, 3H, CH_3 of TBS), 0.041 (s, 3H, CH_3 of TBS), 0.032 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 173.77, 142.60, 137.30, 69.66, 68.30, 65.44 (quint, J = 20.4 Hz, C-1), 43.68, 41.29, 25.81, 25.78, 18.04, 17.96, −4.35, −4.46, −4.52, −4.63. HRMS (ESI, positive) m/z for $C_{19}H_{39}D_2IO_3Si_2Na$ $[M+Na]^+$: calc. 525.1657, found 525.1657.

(2R,4R,E)-1-Deuterio-2,4-bis(tert-butyldimethylsilyloxy)-7-iodohept-6-enal (86). Compound (**86**) was prepared following the same procedure as compound (**37**) with a yield of 85%.

(1E,4R,6R,7E,10R,11R,12S,16S)-7-Deuterio-4,6,10,12-tetrakis(tert-butyldimethylsilyloxy)-11-methyl-16-(4-methoxybenzyloxy)octadeca-1,7-diene (87). Potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 5.2 mL, 2.6 mmol) was added drop-wise over 30 min to a solution of compound **24** (2.0 g, 2.58 mmol) in anhydrous THF (50 mL) at −78 °C, and the reaction mixture was stirred for 1 h, at which time a solution of compound **86** (fragment B) (1.55 g, 3.10 mmol) in THF (10 mL) was added to the mixture at −78 °C over 1 h. After 4 h, the temperature was slowly raised to room temperature over 1 h. The reaction mixture was then poured into a saturated solution of sodium bicarbonate (10 mL). The resulting mixture was partitioned with EtOAc (20 mL ×3), washed with brine (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to give **87** (2.28 g, 2.17 mmol, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.27 (d, 2H, J = 8.8 Hz, Ph of PMB), 6.87 (d, 2H, J = 8.5 Hz, Ph of PMB), 6.54–6.48 (m, 1H, 2-H), 6.01 (d, 1H, J = 14.4 Hz, 1-H), 5.54 (t, 1H, J = 7.1 Hz, 8-H), 4.44 (s, 2H, CH_2 of PMB), 4.11 (t, 1H, J = 5.9 Hz, 6-H), 3.84–3.81 (m, 4H, 4-H + OCH_3 of PMB), 3.79–3.75 (m, 1H, 10-H), 3.72–3.69 (m, 1H, 12-H), 3.31–3.28 (m, 1H, 16-H), 2.32–2.24 (m, 3H, 3-H + 9-H), 2.16–2.10 (m, 1H, 3-H), 1.77–1.70 (m, 1H, 11-H), 1.65–1.27 (m, 10H, 5-H + 13-H + 14-H + 15-H + 17-H), 0.94–0.86 (m, 42H, CH_3 of TBS + 11-CH_3 + 18-H), 0.075–0.022 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.01, 143.30, 135.17 (t, J = 19.1 Hz, C-7), 131.30, 129.17, 126.69, 113.71, 79.78, 76.45, 72.89, 72.24, 70.65, 70.45, 68.21, 55.25, 46.01, 43.46, 41.25, 37.82, 35.39, 33.90, 26.34, 26.25, 25.99, 25.90, 25.85, 21.28, 18.17, 18.14, 18.11, 18.01, 9.51, −3.73, −3.91, −4.25, −4.35, −4.43, −4.50, −4.62, −4.72. HRMS (ESI, positive) m/z for $C_{51}H_{98}DIO_6Si_4Na$ $[M+Na]^+$: calc. 1070.5518, found 1070.5506.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl-12-deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-16-methyl-21-(4-methoxybenzyloxy)tricosa-2,4,6,12-tetraenoate (88). Compound **88** was prepared following the same procedure as compound **42** using compound **40** with a yield of 81%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.40 (dd, 1H, J = 14.1, 11.3 Hz, 3-H), 7.24 (d, 2H, J = 8.6 Hz, Ph of PMB), 6.84 (d, 2H, J = 8.7 Hz, Ph of PMB), 6.50 (dd, 1H, J = 14.5, 10.8 Hz, 5-H), 6.18 (dd, 1H, J = 14.8, 11.5 Hz, 4-H), 6.11 (dd, 1H, J = 15.3, 11.1 Hz, 6-H), 5.91–5.84 (m, 1H, 7-H), 5.82 (d, 1H, J = 15.3 Hz, 2-H), 5.51 (dd, 1H, J = 7.2, 6.8 Hz, 13-H), 4.44 (s, 2H, CH_2 of PMB), 4.18 (q, 2H, J = 7.1 Hz, CH_2 of C(=O)OEt), 4.13–4.10 (m, 1H, 21-H), 3.84–3.80 (m, 1H, 11-H), 3.77 (s, 3H, OCH_3 of PMB), 3.75–3.72 (m, 1H, 9-H), 3.68–3.65 (m, 1H, 15-H), 3.28–3.26 (m, 1H, 17-H), 2.38–2.20 (m, 4H, 8-H and 14-H), 1.72–1.21 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.95–0.82 (m, 45H, 23-H + CH_3 of C(=O)OEt + CH_3 of TBS + 16-CH_3), 0.03–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.16, 159.02, 147.15, 144.71, 140.89, 140.27, 136.97, 136.53, 135.21 (t, J = 19.5 Hz, 12-C), 132.02, 131.30, 129.17, 129.16, 128.13, 126.65, 120.27, 114.31, 113.71, 79.78, 72.89, 72.26, 70.72, 70.46, 70.43, 68.95, 60.17, 55.24, 46.13, 41.24, 40.75, 37.84, 37.77, 35.41, 33.91, 29.14, 27.84, 26.83, 26.62, 25.98, 25.90, 25.86, 21.29, 18.17, 18.13, 18.08, 18.05, 18.03, 17.52, 16.42, 14.31, 13.58, 13.55, 9.51, 9.50, −3.73, −3.76, −3.94, −4.26, −4.38, −4.52, −4.72. HRMS (ESI, positive) m/z for $C_{58}H_{107}DO_8Si_4Na$ $[M+Na]^+$: calc. 1068.7076, found 1068.7075.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-12-Deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (89). Compound **89** was prepared following the same procedure as compound **44** using compound **88** instead of **42** with a yield of 40% for two steps. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.38 (dd, 1H, J = 14.9, 11.2 Hz, 3-H), 6.57 (dd, 1H, J = 14.6, 10.4 Hz, 5-H), 6.24 (dd, 1H, J = 14.8, 11.5 Hz, 4-H), 6.16 (dd, 1H, J = 15.1, 11.1 Hz, 6-H), 5.99–5.93 (m, 1H, 7-H), 5.85 (d, 1H, J = 15.2 Hz, 2-H), 5.55 (dd, 1H, J = 7.1, 7.0 Hz, 13-H), 4.14–4.11 (m, 1H, 21-H), 3.86–3.81 (m, 1H, 11-H), 3.76–3.73 (m, 1H, 9-H), 3.71–3.68 (m, 1H, 15-H), 3.26–3.18 (m, 1H, 17-H), 2.42–2.33 (m, 1H, 8-H), 2.27–2.22 (m, 3H, 8-H and 14-H), 1.75–1.09 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.94 (t, 3H, J = 7.4 Hz, 23-H), 0.887–0.881 (m, 36H, CH_3 of TBS), 0.94 (d, 3H, J = 6.8 Hz, 16-CH_3), 0.045–0.017 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 171.37, 147.04, 142.05, 137.37, 135.15 (t, J = 19.5 Hz, C-12), 131.99, 129.25, 128.62, 127.90, 126.77, 119.06, 113.71, 73.08, 72.63, 72.18, 70.77, 68.97, 46.12, 40.81, 40.64, 37.63, 37.42, 35.05, 30.11, 27.84, 26.83, 25.95, 25.93, 25.86, 21.32, 18.19, 18.15, 18.12, 18.05, 17.52, 13.58, 9.87, 9.31, −3.78, −3.82, −4.00, −4.29, −4.35, −4.38, −4.59, −4.71. HRMS (ESI, positive) m/z for $C_{48}H_{95}DO_7Si_4Na$ $[M+Na]^+$: calc. 920.6188, found 920.6178.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-12-deuterio-macrolactone (90). Compound **90** was prepared following the same procedure as compound **45** with a yield of 62%. HRMS (ESI, positive) m/z for $C_{48}H_{93}DO_6Si_4Na$ $[M+Na]^+$: calc. 902.60820, found 902.60860.

Monomacrolactone (91). Compound **91** was prepared following the same procedure as compound **2** with a yield of 22%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.25 (dd, 1H, J = 14.8, 10.7 Hz, 3-H), 6.70 (dd, 1H, J = 14.9, 11.1 Hz, 5-H), 6.35 (dd, 1H, J = 14.8, 11.0 Hz, 4-H), 6.18 (dd, 1H, J = 15.0, 10.6 Hz, 6-H), 5.92–5.88 (m, 1H, 7-H), 5.85 (d, 1H, J = 15.6 Hz, 2-H), 5.18 (dd, 1H, J = 7.4, 6.6 Hz, 13-H), 4.76–4.73 (m, 1H, 21-H), 3.79–3.75 (m, 1H, 11-H), 3.71–3.64 (m, 1H, 9-H), 3.57–3.51 (m, 1H, 15-H), 3.49–3.43 (m, 1H, 17-H), 2.48–2.09 (m, 4H, 8-H and 14-H), 1.73–1.22 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.88–0.81 (m, 6H, 23-H + 16-CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.62 (1-C=O), 145.47 (C-3), 142.22 (C-5), 137.68 (C-7), 132.39 (C-6), 128.47 (C-4), 126.57 (C-13), 120.92 (C-2), 75.46, 75.12, 74.07, 70.07, 67.64, 60.44,

46.52, 43.51, 34.57, 27.98, 22.02, 10.43, 6.78. HRMS (ESI, positive) m/z for $C_{24}H_{37}DO_6Na$ $[M + Na]^+$: calc. 446.2623, found 446.2626.

[C12-^{2}H]-SpnM substrate (92). Compound **92** was prepared following the same procedure as compound **3** with a yield of 90%. HRMS (ESI, positive) m/z for $C_{24}H_{35}DO_6Na$ $[M+Na]^+$: calc. 444.2465, found 444.2467.

C2D isotopolog.

2-Bromo-2,2-dideuterioacetic acid (93). Preparation of **93** followed a previously reported procedure (57). Trifluoroacetic anhydride (14.12 mL, 100 mmol) was carefully added drop-wise to glacial acetic acid-d_3 (2.42 mL, 42.3 mmol) at 0 °C under a nitrogen atmosphere over 20 min. The mixture was stirred for 1 h, bromine (2.2 mL, 43 mmol) was added drop-wise to the mixture at 0 °C, and the mixture was stirred for 18 h. The mixture was quenched by the careful addition of distilled water (2.4 mL, 133 mmol) at 0 °C. The solution was distilled using a distillation setup to remove most of the trifluoroacetic acid with an oil bath heated to 120 °C, and the trace residual TFA was blown away by an air stream to afford solid **93** (4.89 g, 34.7 mmol, 81.4%). HRMS (CI, positive) m/z for $C_2H_2D_2BrO_2$ $[M + H]^+$: calc. 140.9520, found 140.9520.

Methyl (2E,4E)-5-(tributylstannyl)penta-2-deuterio-2,4-dienoate (94). Oxalyl chloride (3.57 mL, 41.6 mmol) was added drop-wise to a solution of compound **93** (4.89 g, 34.7 mmol) in anhydrous ethyl ether (70 mL) at 0 °C. The mixture was then warmed to room temperature over 30 min. After stirring for 2 h at room temperature, the mixture was concentrated under reduced pressure. Anhydrous methanol (14.03 mL, 347 mmol) was then carefully added to the residue at 0 °C, and the mixture was stirred for 12 h before dilution by the addition of ethyl ether (50 mL) and a saturated solution of ammonium chloride (10 mL). The mixture was then partitioned with ethyl ether (20 mL ×3). The combined organic layers were then dried over sodium sulfate and concentrated under reduced pressure. The residue was used directly in the next step. HRMS (CI, positive) m/z for $C_3H_4D_2BrO_2$ $[M + H]^+$: calc. 154.9677, found 154.9672.

The crude methyl ester (2.4 g, 15.5 mmol) and triethylphosphite (1.83 mL, 15.5 mmol) were mixed at room temperature, and the mixture was stirred at 90 °C for 12 h before being concentrated under reduced pressure with quantitative yield.

To a solution of methyl 2-(diethoxyphosphoryl)-2,2-dideuterioacetate (2.95 g, 16.02 mmol) in tetrahydrofuran (20 mL) at 0 °C was slowly added sodium hydride (60% in mineral oil, 640 mg, 16.02 mmol). The aldehyde **39** (1.84 g, 5.34 mmol) was then added to the resulting suspension. After stirring at 0 °C for 4 h, the reaction was quenched by the addition of a saturated solution of ammonium chloride (15 mL), and the mixture was partitioned with ethyl acetate (20 mL ×3). The combined organic extracts were washed with brine (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (EtOAc/hexanes: 1/39) to afford **94** (fragment C) (1.82 g, 4.50 mmol, 84.3%) for the Stille cross coupling. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.16 (dt, J = 11.9, 1.6 Hz, 1H, 3-H), 6.79 (d, J = 18.6 Hz, 1H, 5-H), 6.64 (dd, J = 18.6, 10.2 Hz, 1H, 4-H), 3.72 (s, 3H, C(=O)OCH_3), 1.61–1.45 (m, 6H, Bu_3Sn), 1.32–1.26 (m, 9H, Bu_3Sn), 0.98–0.86 (m, 15H, Bu_3Sn, $\mathbf{CH}_3CH_2OCO$). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 167.8, 147.5, 146.6, 144.1, 51.5, 29.0, 27.2, 13.6, 9.6. HRMS (ESI, positive) m/z for $C_{18}H_{33}DO_2SnNa$ $[M + Na]^+$: calc. 426.1539, found 426.1547.

(3S,7S,8R,9R,13R,15R,11E,17E)-7,9,13,15-Tetrakis(tert-butyldimethylsilyloxy)-18-iodo-8-methyloctadeca-11,17-dien-3-ol (95). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 286 mg, 1.26 mmol) was added to a solution of compound **41** (1.1 g, 1.05 mmol) in CH_2Cl_2 (10 mL) and methanol (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 6 h, at which time a saturated solution of sodium bicarbonate (6 mL) was added. The two layers were separated, and the aqueous layer was partitioned with EtOAc (40 mL ×3). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/10) to afford the secondary alcohol **95** (0.83 g, 0.89 mmol, 85.3%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 6.51–6.46 (m, 1H, 17-H), 6.00 (d, 1H, J = 14.4 Hz, 18-H), 5.56–5.50 (m, 1H, 11-H), 5.41–5.37 (m, 1H, 12-H), 4.11–4.07 (m, 1H, 13-H), 3.81–3.76 (m, 1H, 15-H), 3.73 (q, 1H, J = 5.4 Hz, 9-H), 3.68 (q, 1H, J = 5.4 Hz, 7-H), 3.50–3.46 (m, 1H, 3-H), 2.29–2.19 (m, 3H, 10-H and 16-H), 2.13–2.09 (m, 1H, 16-H), 1.72–1.67 (m, 1H, 14-H), 1.60–1.21 (m, 10H, 2-H + 4-H + 5-H + 6-H + 8-H + 14-H), 0.93–0.82 (m, 42H, 8-CH_3 + 1-H + CH_3 of TBS), 0.03–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 143.3, 135.6, 126.9, 126.7, 73.1, 72.6, 70.8, 68.3, 46.03, 43.52, 40.62, 37.66, 37.43, 35.04, 30.14, 25.97, 25.95, 25.91, 25.86, 21.31, 18.16, 18.16, 18.13, 18.02, 9.89, 9.32, −3.77, −3.81, −3.97, −4.32, −4.36, −4.43, −4.59, −4.72. HRMS (ESI, positive) m/z for $C_{43}H_{91}IO_5Si_4Na$ $[M + Na]^+$: calc. 949.4880, found 949.4878.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Methyl 2-deuterio-9,11,15,17-tetrakis(tertbutyldimethylsilyloxy)-16-methyl-21-hydroxy-tricosa-2,4,6,12-tetraenoate (96). Tris(dibenzylideneacetone) dipalladium (12 mg, 0.01 mmol) and triphenylarsine (12 mg, 0.04 mmol) were added to a solution of vinyl iodide **95** (250 mg, 0.27 mmol) and compound **94** (fragment C) (114 mg, 0.28 mmol) in anhydrous dimethylformamide (10 mL) at room temperature, and the reaction mixture was stirred for 24 h at room temperature. EtOAc (50 mL) was then added, and the reaction mixture was washed with H_2O (20 mL ×4). The organic layer was dried over sodium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to afford **96** (158 mg, 0.17 mmol, 64.1%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.28 (d, 1H, J = 11.5 Hz, 3-H), 6.51 (dd, 1H, J = 14.9, 10.8 Hz, 5-H), 6.20 (dd, 1H, J = 14.9, 11.3 Hz, 4-H), 6.12 (dd, 1H, J = 15.1, 10.8 Hz, 6-H), 5.93–5.89 (m, 1H, 7-H), 5.57–5.51 (m, 1H, 13-H), 5.42–5.37 (m, 1H, 12-H), 4.13–4.10 (m, 1H, 11-H), 3.82–3.78 (m, 1H, 9-H), 3.74–3.71 (m, 4H, C(=O)OCH_3 and 15-H), 3.68–3.66 (m, 1H, 17-H), 3.50–3.46 (m, 1H, 21-H), 2.38–2.34 (m, 1H, 8-H), 2.25–2.19 (m, 3H, 14-H and 8-H), 1.72–1.67 (m, 1H, 10-H), 1.62–1.20 (m, 10H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.92 (t, 3H, J = 7.5 Hz, 23-H), 0.87–0.85 (m, 36H, CH_3 of TBS), 0.83 (d, 3H, J = 6.9 Hz, 16-CH_3), 0.03–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 167.6, 144.9, 141.1, 136.6, 135.6, 132.0, 128.1, 126.9, 114.3, 73.04, 72.61, 72.17, 70.85, 68.98, 60.19, 51.44, 46.13, 40.80, 40.60, 37.43,

35.05, 30.13, 25.94, 25.92, 25.86, 21.32, 18.19, 18.15, 18.12, 18.05, 9.88, 9.30, −3.78, −3.82, −3.96, −4.00, −4.29, −4.34, −4.38, −4.39, −4.60, −4.72. HRMS (ESI, positive) m/z for $C_{49}H_{97}DO_7Si_4Na$ $[M + Na]^+$: calc. 934.6345, found: 934.6341.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-2-Deuterio-9,11,15,17-tetrakis(tert-butyldimethylsilyloxy)-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (97). A 0.5 N solution of lithium hydroxide (9 mL) was added to a solution of **96** (393 mg, 0.43 mmol) in THF (9 mL) and MeOH (9 mL) at room temperature, and the mixture was stirred under reflux for 3 h before the volatile solvents were evaporated under reduced pressure. The pH of the aqueous solution was adjusted to approximately 6, and the mixture was partitioned with EtOAc (20 mL ×3). The organic extracts were pooled, washed with brine (20 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/4) to afford the carboxylic acid **97** (251 mg, 0.28 mmol, 65.0%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.35 (d, 1H, J = 11.5 Hz, 3-H), 6.55 (dd, 1H, J = 14.9, 10.7 Hz, 5-H), 6.22 (dd, 1H, J = 15.0, 11.4 Hz, 4-H), 6.14 (dd, 1H, J = 14.8, 10.6 Hz, 6-H), 5.96–5.91 (m, 1H, 7-H), 5.57–5.51 (m, 1H, 13-H), 5.42–5.38 (m, 1H, 7-H), 4.14–4.08 (m, 1H, 11-H), 3.83–3.79 (m, 1H, 9-H), 3.73 (q, 1H, J = 5.6 Hz, 15-H), 3.68 (q, 1H, J = 5.1 Hz, 17-H), 3.50–3.46 (m, 1H, 21-H), 2.40–2.35 (m, 1H, 8-H), 2.25–2.20 (m, 3H, 14-H and 8-H), 1.72–1.19 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.92 (t, 3H, J = 7.5 Hz, 23-H), 0.87–0.86 (m, 36H, CH_3 of TBS), 0.83 (d, 3H, J = 6.8 Hz, 16-CH_3), 0.02–(−0.003) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 148.4, 146.9, 142.0, 137.4, 135.6, 132.0, 127.8, 126.9, 73.06, 72.61, 72.17, 70.85, 68.96, 46.14, 40.81, 40.62, 37.67, 37.43, 35.05, 30.12, 25.95, 25.93, 25.87, 21.33, 18.19, 18.16, 18.12, 18.05, 9.89, 9.31, −3.77, −3.82, −3.99, −4.28, −4.38, −4.57, −4.59, −4.71. HRMS (ESI, positive) m/z for $C_{48}H_{95}DO_7Si_4Na$ $[M + Na]^+$: calc. 920.6188, found 920.6175.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-2-deuterio-macrolactone (98). Compound **98** was prepared following the same procedure as compound **45** with a yield of 91%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.23 (d, J = 10.5 Hz, 1H, 3-H), 6.46 (dd, J = 14.9, 10.7 Hz, 1H, 5-H), 6.21 (dd, J = 14.9, 11.2 Hz, 1H, 4-H), 6.11 (dd, J = 15.2, 10.8 Hz, 1H, 6-H), 5.81–5.75 (m, 1H, 7-H), 5.41–5.32 (m, 1H, 13-H), 5.27 (dd, J = 15.3, 6.9 Hz, 1H, 12-H), 4.87–4.83 (m, 1H, 21-H), 4.01 (q, J = 6.7 Hz, 1H, 11-H), 3.77–3.73 (m, 1H, 9-H), 3.69–3.66 (m, 1H, 15-H), 3.59–3.55 (m, 1H, 17-H), 2.46–2.43 (m, 1H, 8-H), 2.33–2.08 (m, 3H, 14-H and 8-H), 2.02–1.18 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91 (t, J = 7.5 Hz, 3H, 23-H), 0.873 (s, 9H, CH_3 of TBS), 0.866 (s, 9H, CH_3 of TBS), 0.853 (s, 9H, CH_3 of TBS), 0.827 (s, 9H, CH_3 of TBS), 0.74 (d, 3H, J = 6.9 Hz, 16-CH_3), 0.06–(−0.03) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.9, 144.6, 140.9, 136.0, 135.2, 132.0, 128.0, 127.1, 75.2, 73.2, 72.04, 71.08, 69.12, 46.58, 42.35, 42.12, 38.36, 34.38, 33.41, 31.58, 27.81, 26.03, 25.99, 25.90, 25.88, 25.87, 25.86, 25.85, 25.84, 25.82, 21.11, 10.20, 9.85, −3.48, −3.88, −3.98, −4.34, −4.41, −4.54, −4.55, −4.63. HRMS (ESI, positive): m/z for $C_{48}H_{93}DO_6Si_4Na$ $[M + Na]^+$: calc. 902.60820, found 902.6075.

Monomacrolactone (99). Compound **99** was prepared following the same procedure as compound **2** with a yield of 28.8%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.20 (d, 1H, J = 11.2 Hz, 3-H), 6.52 (dd, 1H, J = 14.9, 10.9 Hz, 5-H), 6.26 (dd, 1H, J = 15.0, 11.2 Hz, 4-H), 6.16 (dd, 1H, J = 15.2, 10.8 Hz, 6-H), 5.83–5.78 (m, 1H, 7-H), 5.55–5.49 (m, 1H, 13-H), 5.43 (dt, 1H, J = 15.7, 6.7 Hz, 12-H), 4.93–4.87 (m, 1H, 21-H), 4.13 (td, 1H, J = 8.8, 3.4 Hz, 11-H), 3.96–3.92 (m, 1H, 9-H), 3.78 (td, 1H, J = 7.8, 1.8 Hz, 15-H), 3.73–3.69 (m, 1H, 17-H), 2.61–2.58 (m, 1H, 8-H), 2.47–2.13 (m, 3H, 8-H and 14-H), 1.68–1.23 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91–0.87 (m, 3H, 23-H), 0.75 (dd, 3H, J = 14.1, 7.1 Hz, 16-CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.4, 144.5, 140.5, 135.2, 133.3, 128.5, 128.1, 127.9, 76.38, 76.03, 73.19, 72.53, 70.25, 43.34, 41.46, 40.20, 38.24, 38.17, 34.69, 32.60, 29.69, 27.59, 22.24, 9.77, 4.13. HRMS (ESI, positive) m/z for $C_{24}H_{37}DO_6Na$ $[M + Na]^+$: calc. 446.2623, found 446.2625.

[C2-^{2}H]-SpnM substrate (100). Compound **100** was prepared following the same procedure as compound **3** with a yield of 92%. HRMS (ESI, positive) m/z for $C_{24}H_{35}DO_6Na$ $[M+Na]^+$: calc. 444.2465, found 444.2467.

C14D isotopolog.

(4R,5S,6S,10S)-4,6-Bis(tert-butyldimethylsilyloxy)-5-methyl-10-(4-methoxybenzyloxy)-1,1,3,3-tetradeuterio-dodec-1-ene (101). To a solution of (+)-diisopinocampheylchloroborane (1.6 M solution in THF, 4.02 ml, 6.43 mmol) and allylmagnesium bromide-d_4 (ca. 1.0 M solution in anhydrous THF, 6.15 mL, 6.15 mmol) at −78 °C was added aldehyde compound **19** (2 g, 4.73 mmol) in anhydrous THF slowly and stirred for 4 h. MeOH (14.2 ml), 1 N NaOH (14.2 mL) and hydrogen peroxide (4.7 mL) were added sequentially at 0 °C to quench the reaction. The organic layer was separated and extracted with EtOAc (100 mL ×2). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by flash column chromatography (EtOAc/hexanes: 1/7) to afford the crude allyic alcohol. *t*-Butyldimethylsilyl trifluoromethanesulfonate (4.57 mL, 7.10 mmol) was added drop-wise to a solution of the crude allylic alcohol and 2,6-lutidine (1.37 mL, 11.83 mmol) in anhydrous CH_2Cl_2 (50 mL) over 10 min at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, at which time the dry ice bath was removed, and the reaction mixture was stirred for an additional 2 h at room temperature. The reaction mixture was then poured into a saturated solution of aqueous sodium bicarbonate (100 mL), extracted with CH_2Cl_2 (50 mL ×2), dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography (EtOAc/hexanes: 1/15) to afford the silyl ether compound **101** (1.56 g, 2.68 mmol, 56.7% over two steps).

(3R,4R,5S,9S)-3,5-Bis(tert-butyldimethylsilyloxy)-9-(4-methoxybenzyloxy)-4-methyl-2,2-dideuterio-undecan-1-ol (102). *N*-Methylmorpholine oxide (NMO, 0.47 g, 4.02 mmol) followed by osmium tetroxide (34 mg, 0.13 mmol) was added to a solution of silyl ether compound **101** (1.56 g, 2.68 mmol) in THF (11 mL), acetone (11 mL) and pH 7 phosphate buffer (11 mL) at room temperature, and the reaction was stirred at room temperature overnight. The reaction was poured into a 10% solution of sodium thiosulfate ($Na_2S_2O_3$, 15 mL), extracted with ethyl acetate (20 mL ×3), washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated under

reduced pressure and used without further purification. Sodium periodate (2.29 g, 10.72 mmol) was then added to a clear solution of the crude oil in THF (30 mL) and pH 7 phosphate buffer (15 mL) at room temperature, and the reaction was stirred for 3 h. Next, the reaction was poured into a saturated solution of sodium bicarbonate (30 mL), extracted with ethyl acetate (20 mL ×3), washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated under reduced pressure and used without further purification. Sodium borohydride (162 mg, 4.29 mmol) was added to a clear solution of the crude aldehyde in ethyl alcohol (30 mL) at room temperature, and the reaction was stirred for 1 h, at which time the reaction was poured into a saturated solution of ammonium chloride (20 mL), extracted with ethyl acetate (20 mL ×3), washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/19) to afford the primary alcohol **102** (1.46 g, 2.50 mmol, 93.1% over 3 steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.26–7.23 (m, 2H, Ph of PMB), 6.86–6.84 (m, 2H, Ph of PMB), 4.44 (dd, 1H, J = 11.2, 5.0 Hz, CH_2 of PMB), 4.38 (dd, 1H, J = 11.2, 6.6 Hz, CH_2 of PMB), 3.88–3.85 (m, 1H, 3-H), 3.80–3.75 (m, 4H, 1-H + OCH_3 of PMB), 3.70–3.63 (m, 2H, 1-H + 5-H), 3.29–3.25 (m, 1H, 9-H), 2.28 (br s, 1H, OH), 1.88–1.71 (m, 1H, 4-H), 1.56–1.21 (m, 8H, 10-H + 8-H + 7-H + 6-H), 0.90–0.85 (m, 24H, CH_3 of TBS, 11-H, 4-CH_3), 0.077 (s, 3H, CH_3 of TBS), 0.039 (s, 3H, CH_3 of TBS), 0.016 (s, 3H, CH_3 of TBS), 0.005 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 159.07, 131.20, 129.30, 113.74, 79.84, 72.65, 72.40, 70.54, 70.39, 59.79, 55.27, 40.32, 35.55, 35.43, 33.93, 26.29, 25.94, 21.20, 18.13, 9.79, 9.47, −3.68, −4.13, −4.49. HRMS (ESI, positive) m/z for $C_{32}H_{60}D_2O_5Si_2Na$ $[M + Na]^+$: calc. 607.4153, found 607.4146.

(3R,4R,5S,9S)-2,2-Dideuterio-3,5-bis(tert-butyldimethylsilyloxy)-9-(4-methoxybenzyloxy)-4-methylundecyl-5-sulfonyl-1-phenyl-1H-tetrazole (104). 1-Phenyl-1H-tetrazole-5-thiol (669 mg, 3.75 mmol), triphenyl phosphine (985 mg, 3.75 mmol), and diisopropyl azodicarboxylate (0.74 mL, 3.75 mmol) were sequentially added to a solution of alcohol **102** (1.46 g, 2.50 mmol) in anhydrous THF (10 mL) at 0 °C. The resulting yellow suspension was stirred at 0 °C for 1 h and then warmed to room temperature over the course of an hour. The reaction mixture was then concentrated under reduced pressure and subjected to flash column chromatography (EtOAc/hexanes: 1/9) to afford thioether compound **103** (1.50 g, 2.01 mmol, 80.2%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.56–7.52 (m, 5H, Ph of Ph), 7.26–7.23 (m, 2H, Ph of PMB), 6.86–6.83 (m, 2H, Ph of PMB), 4.42 (dd, 2H, J = 18.3, 11.1 Hz, CH_2 of PMB), 3.86–3.76 (m, 5H, OCH_3 of PMB + 3-H + 5-H), 3.40–3.29 (m, 3H, 1-H and 9-H), 1.70–1.65 (m, 1H, 4-H), 1.59–1.26 (m, 8H, 6-H + 7-H + 8-H + 10-H), 0.91–0.86 (m, 24H, CH_3 of TBS + 11-H + 4-CH_3), 0.05–0.02 (m, 12H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.00, 154.29, 133.79, 131.25, 129.98, 129.72, 129.21, 123.78, 113.69, 79.75, 72.41, 72.14, 70.45, 55.23, 35.42, 33.85, 28.91, 26.27, 25.90, 21.20, 18.10, 9.76, 9.50, −3.66, −4.10, −4.38, −4.41. HRMS (ESI, positive) m/z for $C_{39}H_{65}D_2N_4O_4Si_2S$ $[M + H]^+$: calc. 745.4529, found 745.4530.

A premixed solution of ammonium molydate oxidant (620 mg, 30% H_2O_2; 2.46 ml) was added to a solution of thioether **103** (1.50 g, 2.01 mmol) in ethyl alcohol (10 mL) at 0 °C, and the reaction was stirred at 0 °C for 24 h. The mixture was then poured into water (10 mL), extracted with EtOAc (20 mL ×3), washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/hexanes: 1/49) to afford the sulfone **104** (1.30 g, 1.67 mmol, 83.0%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.71–7.69 (m, 2H, Ph of Ph), 7.62–7.57 (m, 3H, Ph of Ph), 7.26 (d, 2H, J = 9.4 Hz, Ph of PMB), 6.85 (dd, 2H, J = 8.8, 2.1 Hz, Ph of PMB), 4.46–4.39 (m, 2H, CH_2 of PMB), 3.90–3.74 (m, 7H, OCH_3 of PMB + 3-H + 5-H + 1-H), 3.31–3.29 (m, 1H, 9-H), 1.61–1.24 (m, 9H, 4-H + 6-H + 7-H + 8-H + 10-H), 0.91–0.85 (m, 24H, CH_3 of TBS + 12-H + 4-CH_3), 0.086 (s, 3H, CH_3 of TBS), 0.067 (s, 3H, CH_3 of TBS), 0.053 (s, 3H, CH_3 of TBS), 0.019 (s, 3H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 159.03, 153.48, 133.09, 131.37, 131.20, 129.68, 129.23, 125.00, 113.72, 79.63, 71.68, 71.53, 70.49, 55.24, 52.11, 40.69, 35.45, 33.71, 26.34, 25.90, 21.43, 18.09, 18.05, 9.62, 9.49, −3.57, −4.27, −4.48. HRMS (ESI, positive) m/z for $C_{39}H_{64}D_2N_4O_6Si_2SNa$ $[M + Na]^+$: calc. 799.4265, found 799.4273.

(1E,4R,6R,7E,10R,11R,12S,16S)-1-Iodo-9,9-dideuterio-4,6,10,12-tetrakis(tert-butyldimethylsilyloxy)-11-methyl-16-(4-methoxybenzyloxy) octadeca-1,7-diene (105). Potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 5.01 mL, 2.51 mmol) was added drop-wise over 10 min to a solution of compound **104** (1.30 g, 1.67 mmol) in anhydrous THF (20 mL) at −78 °C, and the reaction mixture was stirred for 1 h. Compound **37** (fragment B) (1.42 g, 1.84 mmol) was then added to the solution at −78 °C. After 4 h, the temperature was slowly raised to room temperature over 1 h, and the reaction mixture was poured into a saturated solution of sodium bicarbonate (10 mL). The resulting mixture was partitioned with EtOAc (20 mL ×3), washed with brine (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (EtOAc/hexanes: 1/49) to give compound **105** (883 mg, 0.84 mmol, 50.4%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.27–7.25 (m, 2H, Ph of PMB), 6.87–6.85 (m, 2H, Ph of PMB), 6.47 (dt, 1H, J = 14.6, 7.4 Hz, 2-H), 5.99 (d, 1H, J = 14.6 Hz , 1-H), 5.54 (d, 1H, J = 15.4 Hz, 8-H), 5.41 (dt, 1H, J = 15.4, 6.8 Hz, 7-H), 4.43 (s, 2H, CH_2 of PMB), 4.12–4.08 (m, 1H, 6-H), 3.84–3.81 (m, 1H, 4-H), 3.80 (s, 3H, OCH_3 of PMB), 3.77–3.74 (m, 1H, 10-H), 3.70–3.65 (m, 1H, 12-H), 3.32–3.27 (m, 1H, 16-H) 2.31–2.21 (m, 1H, 3-H), 2.15–2.08 (m, 1H, 3-H), 1.74–1.69 (m, 1H, 5-H), 1.62–1.25 (m, 10H, 5-H + 11-H +13-H +14-H +15-H + 17-H), 0.93–0.84 (m, 42H, CH_3 of TBS + 11-CH_3 + 18-H), 0.10–0.01 (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 159.01, 143.31, 143.33, 135.54, 131.31, 129.17, 126.81, 113.71. 79.79, 76.45, 74.78, 72.90, 72.25, 70.74, 70.45, 68.22, 55.26, 46.03, 43.37, 41.26, 37.84, 35.40, 33.91, 25.99, 25.90, 25.85, 21.30, 18.18, 18.14, 18.12, 18.01, 9.52, −3.72, −3.90, −4.24, −4.35, −4.42, −4.49, −4.61, −4.71. HRMS (ESI, positive) m/z for $C_{51}H_{97}D_2IO_6Si_4Na$ $[M + Na]^+$: calc. 1071.5581, found 1071.5575.

(3S,7S,8R,9R,13R,15R,11E,17E)-7,9,13,15-Tetrakis(tert-butyldimethylsilyloxy)-10,10-dideuterio-18-iodo-8-methyloctadeca-11,17-dien-3-ol (106). Compound **106** was prepared following the same procedure as compound **95** with a yield of 79.3%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.51–6.46 (m, 1H, 17-H), 6.00 (d,

1H, J = 14.4 Hz, 18-H), 5.53 (d, 1H, J = 15.3 Hz, 11-H), 5.41–5.37 (m, 1H, 12-H), 4.11–4.07 (m, 1H, 13-H), 3.81–3.76 (m, 1H, 15-H), 3.74–3.72 (m, 1H, 9-H), 3.70–3.67 (q, 1H, 7-H), 3.50–3.46 (m, 1H, 3-H), 2.29–2.19 (m, 1H, 16-H), 2.13–2.09 (m, 1H, 16-H), 1.72–1.67 (m, 1H, 14-H), 1.60–1.21 (m, 10H, 2-H + 4-H + 5-H + 6-H + 8-H + 14-H), 0.93–0.82 (m, 42H, 8-CH_3 + 1-H + CH_3 of TBS), 0.03–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 143.3, 135.6, 126.9, 126.7, 73.1, 72.6, 70.8, 68.3, 46.03, 43.52, 40.62, 37.66, 37.43, 35.04, 30.14, 25.97, 25.95, 25.91, 25.86, 21.31, 18.16, 18.16, 18.13, 18.02, 9.89, 9.32, −3.77, −3.81, −3.97, −4.32, −4.36, −4.43, −4.59, −4.72.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-Ethyl-9,11,15,17-tetrakis (tert-butyldimethylsilyloxy)-14,14-dideuterio-16-methyl-21-hydroxy-tricosa-2,4,6,12-tetraenoate (107). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.28 (dd, 1H, J = 15.4, 11.5 Hz, 3-H), 6.51 (dd, 1H, J = 14.9, 10.8 Hz, 5-H), 6.20 (dd, 1H, J = 14.9, 11.3 Hz, 4-H), 6.12 (dd, 1H, J = 15.1, 10.8 Hz, 6-H), 5.93–5.89 (m, 1H, 7-H), 5.86 (d, 1H, J = 15.4 Hz, 2-H), 5.54 (d, 1H, J = 15.3 Hz, 13-H), 5.42–5.37 (m, 1H, 12-H), 4.20 (q, 2H, J = 7.1 Hz, $\mathbf{CH}_2CH_3$ of OEt) 4.13–4.10 (m, 1H, 11-H), 3.82–3.78 (m, 1H, 9-H), 3.74–3.71 (m, 1H, 15-H), 3.68–3.66 (m, 1H, 17-H), 3.50–3.46 (m, 1H, 21-H), 2.38–2.34 (m, 1H, 8-H), 2.25–2.19 (m, 1H, 8-H), 1.72–1.67 (m, 1H, 10-H), 1.62–1.20 (m, 13H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H + $CH_2\mathbf{CH}_3$ of OEt), 0.92 (t, 3H, J = 7.5 Hz, 23-H), 0.87–0.85 (m, 36H, CH_3 of TBS), 0.83 (d, 3H, J = 6.9 Hz, 16-CH3), 0.03–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.6, 144.9, 141.1, 136.6, 135.6, 132.0, 128.1, 126.9, 114.3, 73.04, 72.61, 72.17, 70.85, 68.98, 60.19, 51.44, 46.13, 40.80, 40.60, 37.43, 35.05, 30.13, 25.94, 25.92, 25.86, 21.32, 18.19, 18.15, 18.12, 18.05, 9.88, 9.30, −3.78, −3.82, −3.96, −4.00, −4.29, −4.34, −4.38, −4.39, −4.60, −4.72. HRMS (ESI, positive) m/z for $C_{50}H_{98}D_2O_7Si_4Na$ $[M+Na]^+$: calc. 949.6569, found 949.6565.

(2E,4E,6E,9R,11R,12E,15R,16R,17S,21S)-9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-14,14-dideuterio-21-hydroxy-16-methyltricosa-2,4,6,12-tetraenoic acid (108). Compound **108** was prepared following the same procedure as compound **97** with a yield of 80.4%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.36 (dd, J = 15.3, 11.3 Hz, 1H, 3-H), 6.55 (dd, J = 14.8, 10.5 Hz, 1H, 5-H), 6.22 (dd, J = 14.8, 11.3 Hz, 1H, 4-H), 6.14 (dd, J = 15.3, 10.5 Hz, 1H, 6-H), 5.94 (dt, J = 15.3, 7.4 Hz, 1H, 7-H), 5.82 (d, J = 15.1 Hz, 1H, 2-H), 5.54 (d, J = 15.5 Hz, 1H, 13-H), 5.39 (dd, J = 15.5, 6.8 Hz, 1H, 12-H), 4.14–4.08 (m, 1H, 11-H), 3.83–3.79 (m, 1H, 9-H), 3.75–3.71 (m, 1H, 15-H), 3.69–3.66 (m, 1H, 17-H), 3.50–3.46 (m, 1H, 21-H), 2.39–2.34 (m, 1H, 8-H), 2.24–2.18 (m, 1H, 8-H), 1.70–1.18 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.92 (t, J = 7.5 Hz, 3H, 23-H), 0.87–0.83 (m, 36H, CH_3 of TBS), 0.83 (d, J = 7 Hz, 3H, 16-Me), 0.04–(−0.01) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 171.3, 147.0, 142, 0, 137.4, 135.8, 132.0, 127.9, 126.9, 120.0, 73.1, 72.6, 72.2, 70.9, 69.0, 60.2, 46.2, 40.8, 40.6, 37.7, 37.4, 35.1, 30.1, 25.95, 25.93, 25.87, 21.3, 18.2, 18.16, 18.13, 18.0, 9.9, 9.3, −3.77, −3.82, −3.99, −4.28, −4.37, −4.38, −4.59, −4.71. HRMS (ESI, positive) m/z for $C_{48}H_{94}D_2O_7Si_4Na$ $[M+Na]^+$: calc. 921.6251, found 921.6236.

9,11,15,17-Tetrakis(tert-butyldimethylsilyloxy)-14,14-dideuterio-macrolactone (109). Compound **109** was prepared following the same procedure as compound **45** with a yield of 72.0%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.23 (dd, 1H, J = 15.3, 11.2 Hz, 3-H), 6.46 (dd, 1H, J = 14.9, 10.7 Hz, 5-H), 6.21 (dd, 1H, J = 14.9, 11.2 Hz, 4-H), 6.11 (dd,1H, J = 15.2, 10.8 Hz, 6-H), 5.81 (d, 1H, J = 15.3, 2-H), 5.80–5.75 (m, 1H, 7-H), 5.36 (d, 1H, J = 15.3 Hz, 13-H), 5.27 (dd, 1H, J = 15.3, 6.9 Hz, 12-H), 4.87–4.83 (m, 1H, 21-H), 4.01 (q, J = 6.7 Hz, 1H, 11-H), 3.77–3.73 (m, 1H, 9-H), 3.69–3.66 (m, 1H, 15-H), 3.59–3.55 (m, 1H, 17-H), 2.46–2.43 (m, 1H, 8-H), 2.33–2.08 (m, 1H, 8-H), 2.02–1.18 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91 (t, J = 7.5 Hz, 3H, 23-H), 0.873 (s, 9H, CH_3 of TBS), 0.866 (s, 9H, CH_3 of TBS), 0.853 (s, 9H, CH_3 of TBS), 0.827 (s, 9H, CH_3 of TBS), 0.74 (d, 3H, J = 6.9 Hz, 16-CH_3), 0.06–(−0.03) (m, 24H, CH_3 of TBS). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.9, 144.6, 140.9, 136.0, 135.2, 132.0, 128.0, 127.1, 75.2, 73.2, 72.04, 71.08, 69.12, 46.58, 42.35, 42.12, 38.36, 34.38, 33.41, 31.58, 27.81, 26.03, 25.99, 25.90, 25.88, 25.87, 25.86, 25.85, 25.84, 25.82, 21.11, 10.20, 9.85, −3.48, −3.88, −3.98, −4.34, −4.41, −4.54, −4.55, −4.63. HRMS (ESI, positive): m/z for $C_{48}H_{92}D_2O_6Si_4Na$ $[M+Na]^+$: calc. 903.6139, found 903.6134.

Monomacrolactone (110). Compound **110** was prepared following the same procedure as compound **2** with a yield of 59%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.20 (dd, 1H, J = 15.2, 11.2 Hz, 3-H), 6.52 (dd, 1H, J = 14.9, 10.9 Hz, 5-H), 6.26 (dd, 1H, J = 15.0, 11.2 Hz, 4-H), 6.16 (dd, 1H, J = 15.2, 10.8 Hz, 6-H), 5.85 (d, 1H, J = 15.2 Hz, 2-H), 5.83–5.78 (m, 1H, 7-H), 5.52 (d, 1H, J = 15.5 Hz, 13-H), 5.43 (dt, 1H, J = 15.5, 6.7 Hz, 12-H), 4.93–4.87 (m, 1H, 21-H), 4.13 (td, 1H, J = 8.8, 3.4 Hz, 11-H), 3.96–3.92 (m, 1H, 9-H), 3.78 (td, 1H, J = 7.8, 1.8 Hz, 15-H), 3.73–3.69 (m, 1H, 17-H), 2.61–2.58 (m, 1H, 8-H), 2.47–2.13 (m, 1H, 8-H), 1.68–1.23 (m, 11H, 10-H + 16-H + 18-H + 19-H + 20-H + 22-H), 0.91–0.87 (m, 3H, 23-H), 0.75 (dd, 3H, J = 14.1, 7.1 Hz, 16-CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.4, 144.5, 140.5, 135.2, 133.3, 128.5, 128.1, 127.9, 76.38, 76.03, 73.19, 72.53, 70.25, 43.34, 41.46, 40.20, 38.24, 38.17, 34.69, 32.60, 29.69, 27.59, 22.24, 9.77, 4.13. HRMS (ESI, positive) m/z for $C_{24}H_{36}D_2O_6Na$ $[M+Na]^+$: calc. 447.2679, found 447.2682.

[C14-$^2H_2$]-SpnM substrate (111). Compound **111** was prepared following the same procedure as compound **3** with a yield of 91.3%. HRMS (ESI, positive) m/z for $C_{24}H_{34}D_2O_6Na$ $[M+Na]^+$: calc. 445.2522, found 445.2524.

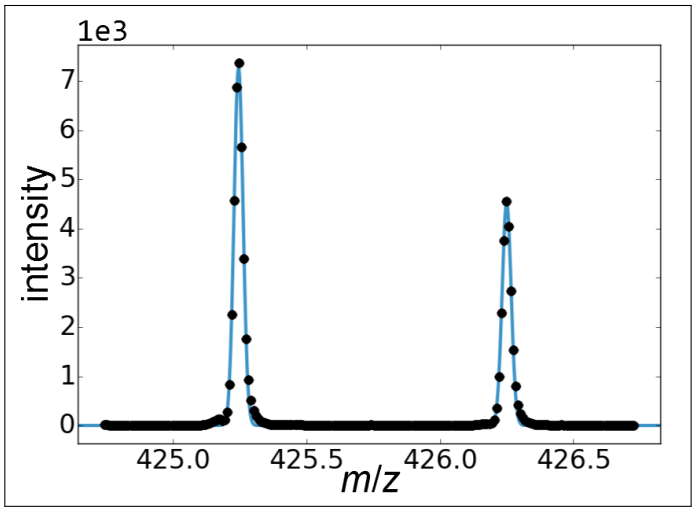

Fig. S1. Example mass spectrum used to determine a single observed MS peak intensity ratio R_M (see $\mathrm{Eq.}\ (12)$). Signals correspond to the M and $M+1$ peaks for the sodiated ion of **4**. Black circles denote the MS intensity upon averaging over the roughly 175 acquisitions during the 3 min direct infusion described in the text. The blue line corresponds to the best constrained fit of $\mathrm{Eq.}\ (12)$ to the data as described in Sec. S2.

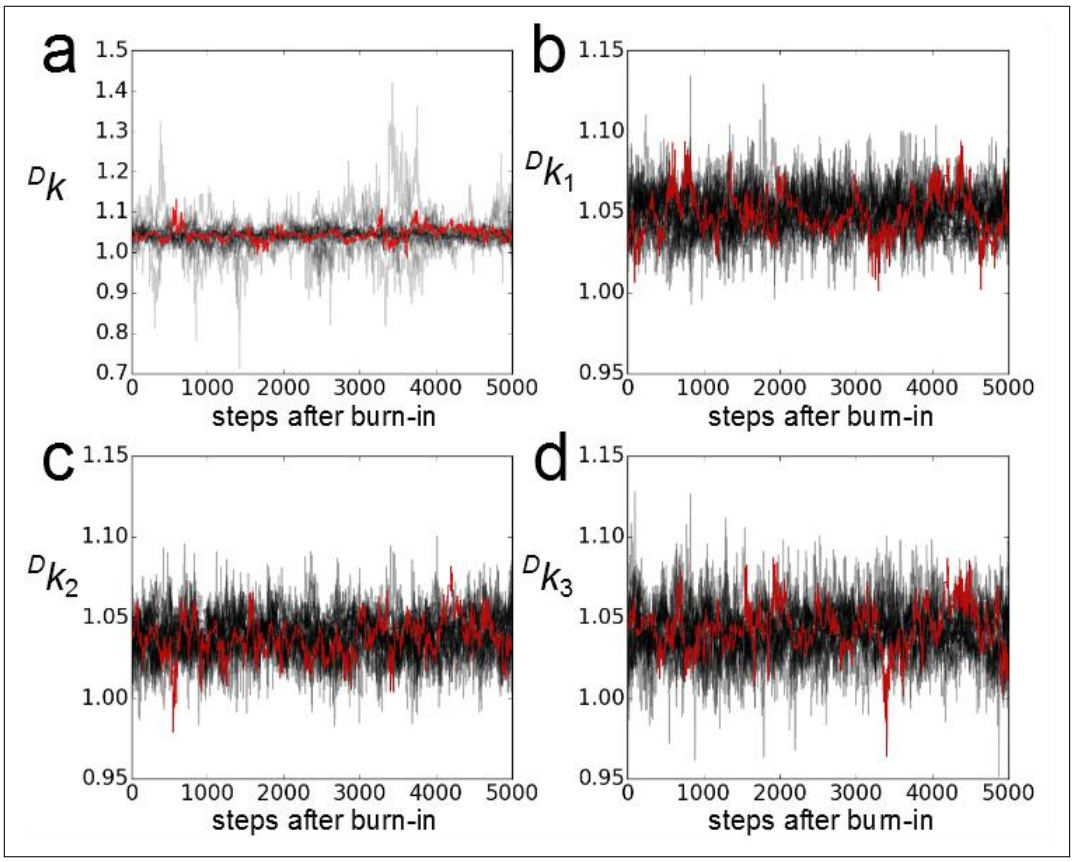

Fig. S2. Plots of 25 randomly selected walker trajectories (Markov chains) obtained during MCMC sampling of the posterior joint distribution for the C12D nonenzymatic KIE. Only the ^Dk (a), $^Dk_1$ (b), $^Dk_2$ (c), $^Dk_3$ (d) coordinates of the walker positions in the 9-dimensional parameter space are shown for brevity; however, the values of the coordinates corresponding to the remaining nuisance parameters (i.e., σ_b, σ_w, ρ_1, ρ_2 and ρ_3) demonstrated similar behavior. The 25 chains are overlaid with partial transparency; however, a single chain has been colored solid red to aid in visualization. Lack of drift in the chains is consistent with equilibration.

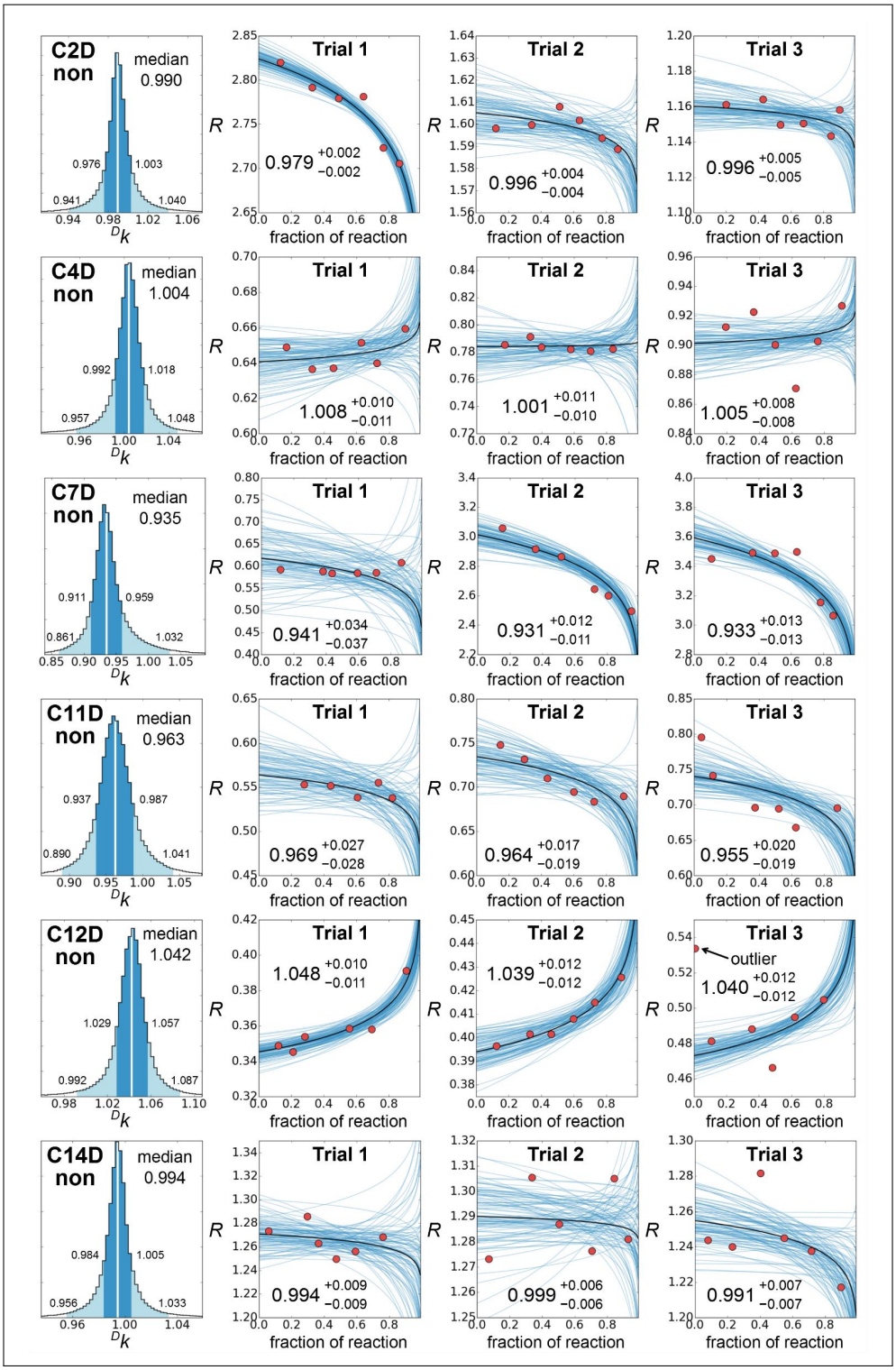

Fig. S3. Summary of fitting results for each site specific nonenzymatic KIE. The leftmost column shows the simulated posterior marginal distribution for the overall KIE parameter (^Dk) as a histogram. The dark blue region represents the 68% highest posterior density (HPD) interval, whereas the dark and light blue regions together represent the 95% HPD interval. The histogram is annotated with the lower and upper limits of these intervals. The median is denoted by a vertical white line. The three rightmost columns plot (red circles) the observed enrichments versus fraction of reaction for each of the three separate experimental trials. Data points excluded as outliers are annotated as such (see Sec. S4 for exclusion criteria). The blue lines represent 100 realizations of the function (1) based on random draws of $(^D\mathbf{k}, \boldsymbol{\rho})$ vector pairs from the simulated posterior joint distribution. In other words, a single draw from the posterior joint distribution of all parameters provides the three values of ^Dk_i and the three values of ρ_i used to construct a blue curve in each plot. The black curves represent the function (1) parameterized in terms of the median values of ^Dk_i and ρ_i marginalized over all other parameters and thus treated as the overall best point estimates. Each plot is labeled with this median value of ^Dk_i and its 68% HPD interval.

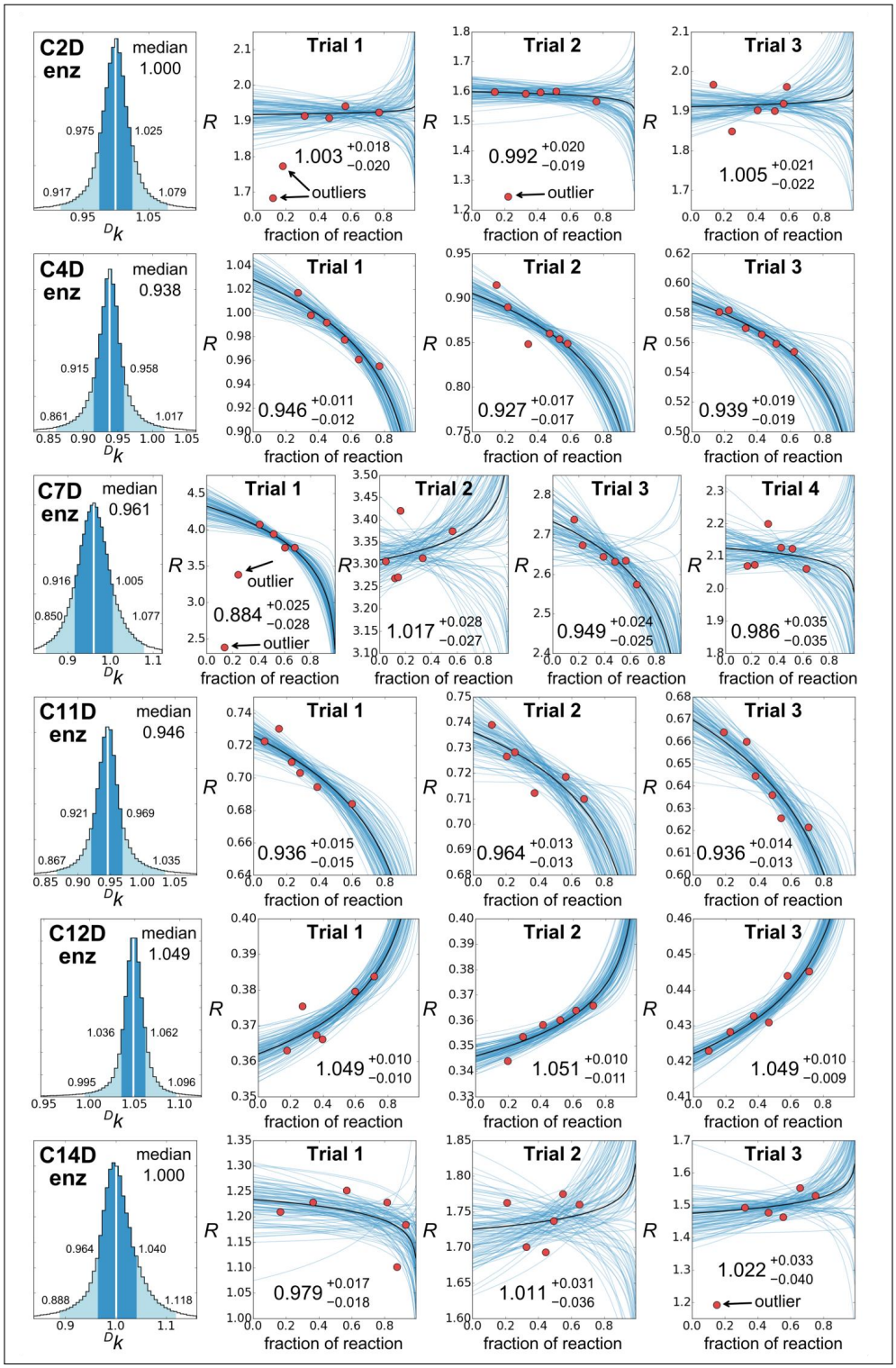

Fig. S4. Summary of fitting results for each site specific enzymatic KIE. The leftmost column shows the simulated posterior marginal distribution for the overall KIE parameter (^{D}k) as a histogram. The dark blue region represents the 68% highest posterior density (HPD) interval, whereas the dark and light blue regions together represent the 95% HPD interval. The histogram is annotated with the lower and upper limits of these intervals. The median is denoted by a vertical white line. The three to four rightmost columns plot (red circles) the observed enrichments versus fraction of reaction for each of the three separate experimental trials. Data points excluded as outliers are annotated as such (see Sec. S4 for exclusion criteria). The blue lines represent 100 realizations of the function (1) based on random draws of $(^{D}\mathbf{k}, \boldsymbol{\rho})$ vector pairs from the simulated posterior joint distribution. In other words, a single draw from the posterior joint distribution of all parameters provides the three (or four) values of $^{D}k_i$ and the three (or four) values of ρ_i used to construct a blue curve in each plot. The black curves represent the function (1) parameterized in terms of the median values of $^{D}k_i$ and ρ_i marginalized over all other parameters and thus treated as the overall best point estimates. Each plot is labeled with this median value of $^{D}k_i$ and its 68% HPD interval.

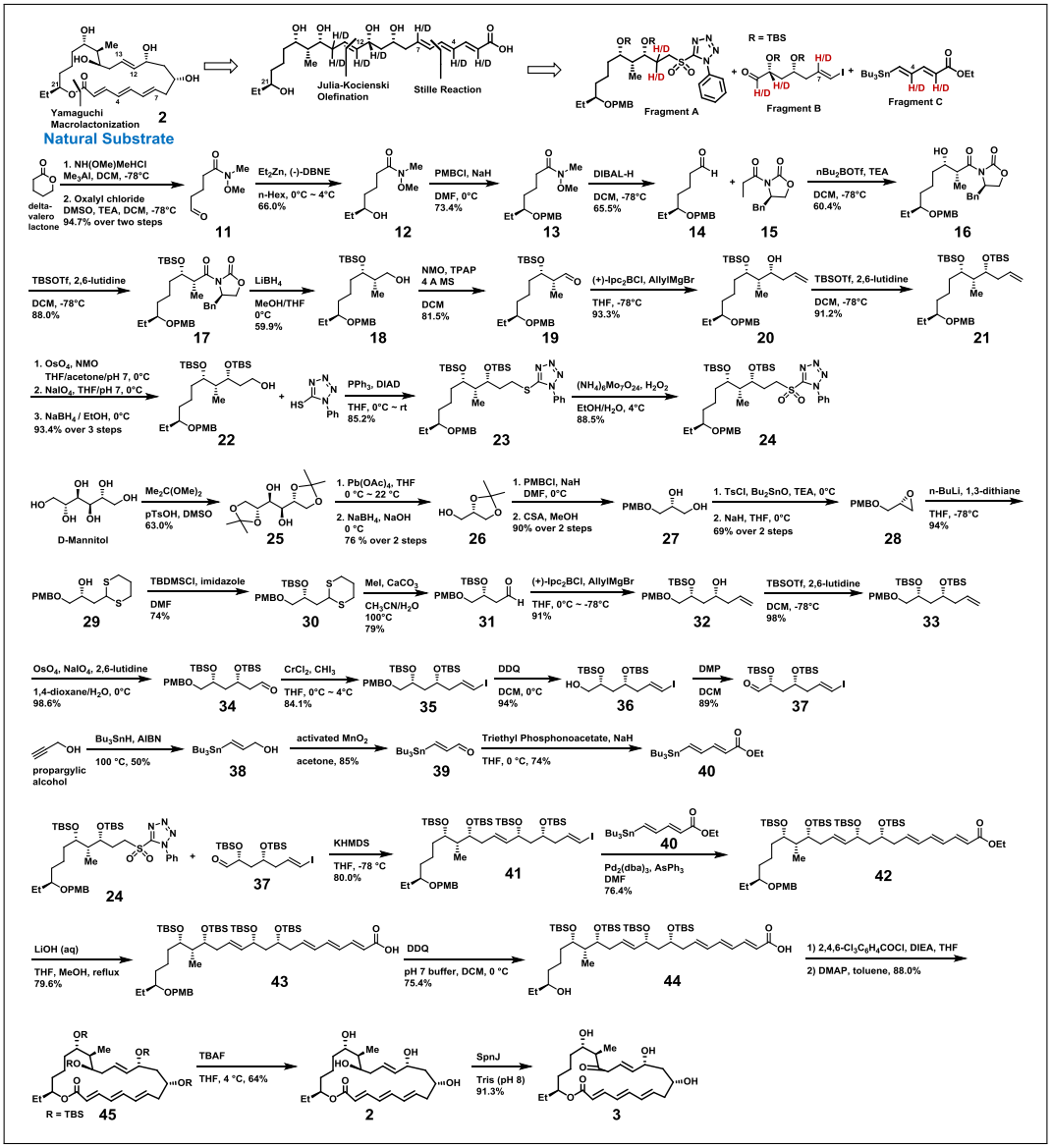

Fig. S5. Synthesis of the SpnM substrate (**3**) with natural abundance labeling.

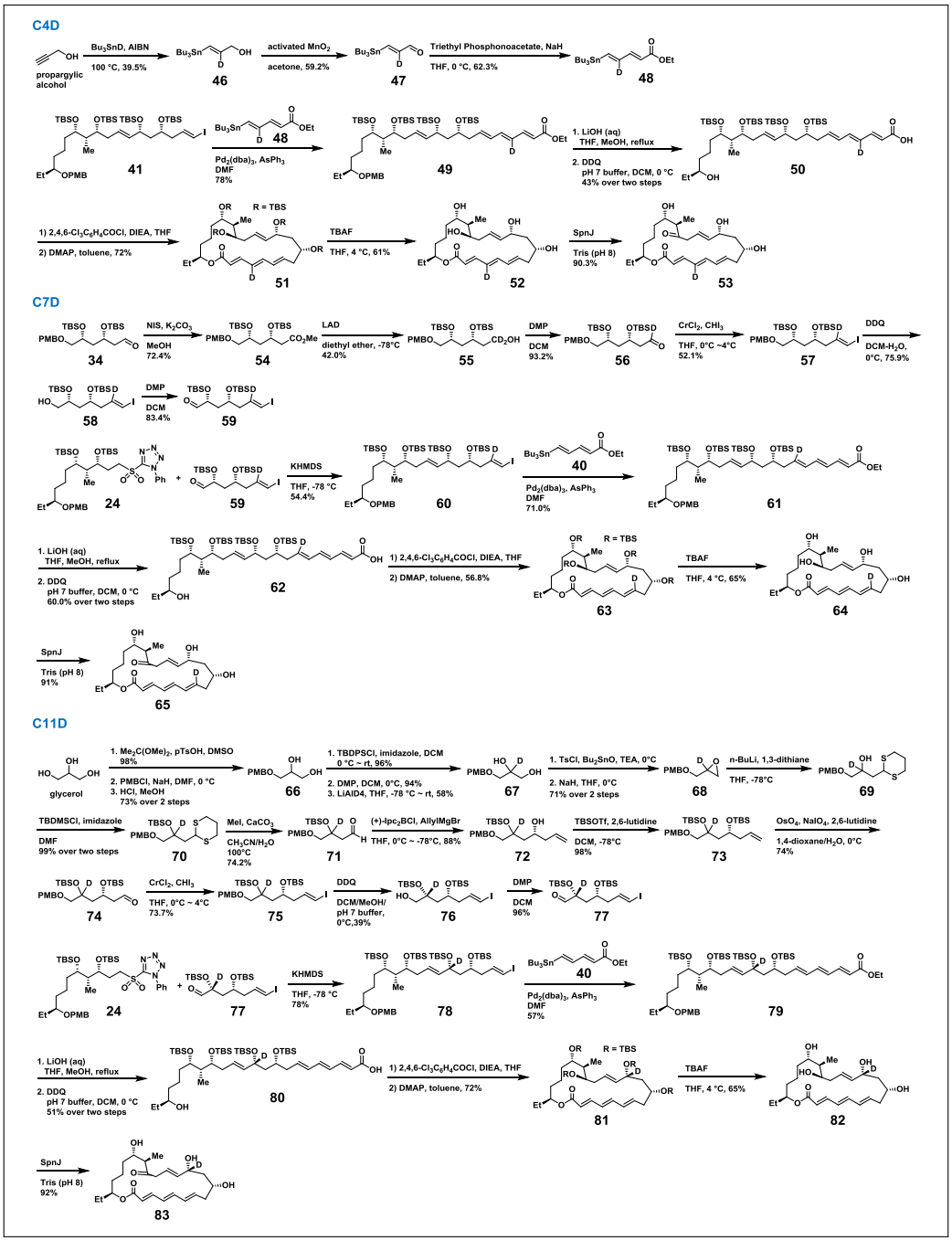

Fig. S6. Synthesis of the C4D (**53**), C7D (**65**) and C11D (**83**) enriched SpnM substrate isotopologs.

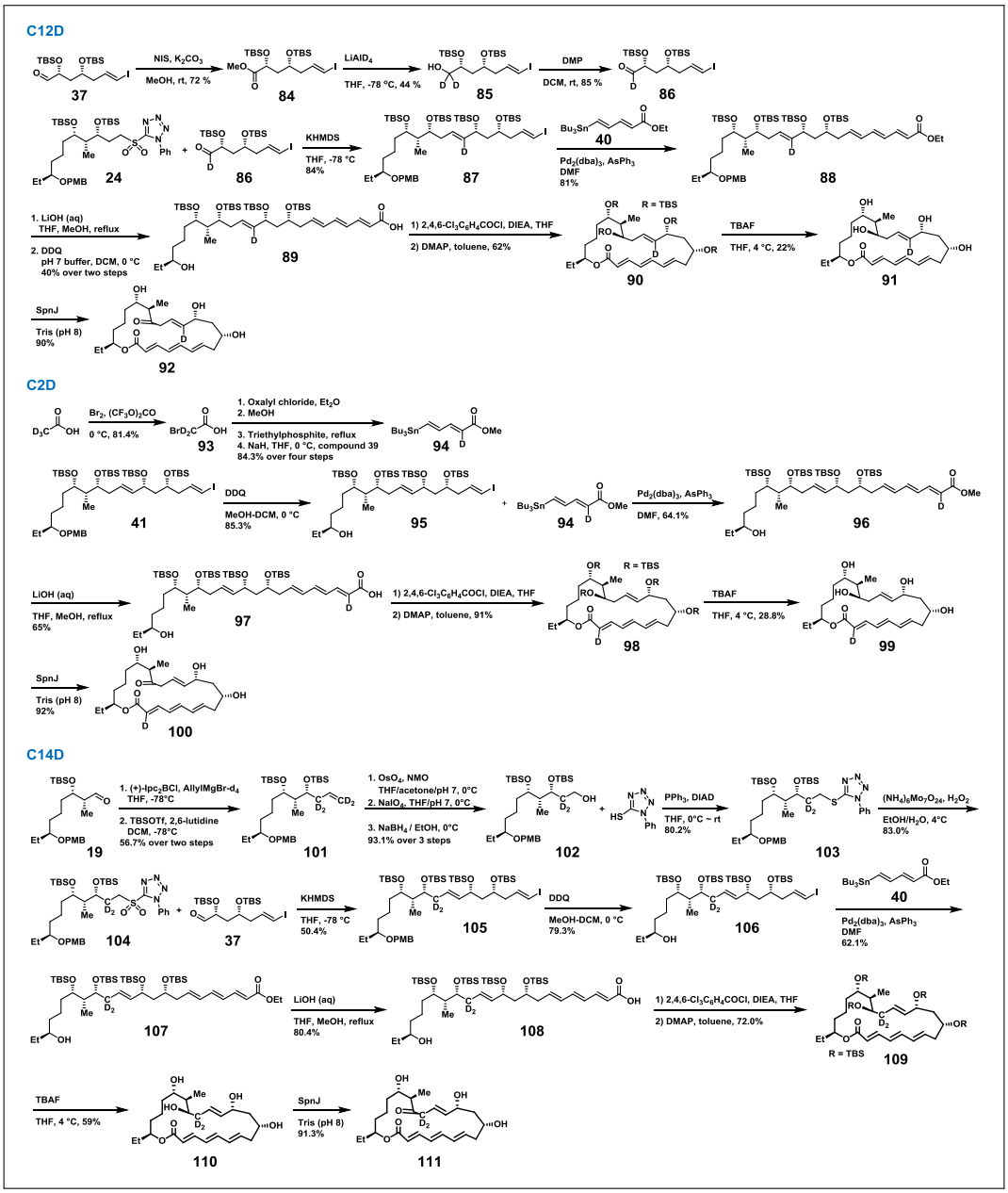

Fig. S7. Synthesis of the C12D (**92**), C2D (**100**) and C14D (**111**) enriched SpnM substrate isotopologs.

Table S1. Sensitivity of simulated highest posterior density (HPD) intervals for fitted KIEs (i.e., ^{D}k) to different scales in the prior distribution $p(\log {}^{D}k)$. The median values of the simulated posterior marginal distributions for $^{C7D}k_{non}$ and $^{C12D}k_{non}$ are shown with the negative and positive deflections defining the simulated 68% and 95% HPD intervals. The scale of 0.18 was used in the actual study.

C7D Nonenzymatic			
scale	0.10	0.18	0.27
68% HPD int.	$0.938^{+0.024}_{-0.026}$	$0.935^{+0.023}_{-0.024}$	$0.935^{+0.028}_{-0.028}$
95% HPD int.	$0.938^{+0.101}_{-0.061}$	$0.935^{+0.097}_{-0.074}$	$0.935^{+0.128}_{-0.101}$
C12D Nonenzymatic			
scale	0.10	0.18	0.27
68% HPD int.	$1.041^{+0.015}_{-0.013}$	$1.042^{+0.015}_{-0.014}$	$1.042^{+0.017}_{-0.014}$
95% HPD int.	$1.041^{+0.043}_{-0.053}$	$1.042^{+0.044}_{-0.050}$	$1.042^{+0.062}_{-0.071}$

Table S2. Summary statistics for the simulated posterior marginal distributions of each parameter in the fitted hierarchical Bayesian model for each nonenzymatic kinetic isotope effect. The median value, 68% and 95% highest posterior density (HPD) intervals of the marginal distributions are shown. Values are listed arbitrarily to 3 decimal places. The maximum potential scale reduction factor (PSRF) as well as the minimum effective sample size (ESS) among all parameters are also provided for each fit.

	C2D Nonenzymatic ESS $> 14.5 \times 10^3$, PSRF < 1.04			C14D Nonenzymatic ESS $> 12.5 \times 10^3$, PSRF < 1.04		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
^{D}k	0.990	$[0.976, 1.003]$	$[0.941, 1.040]$	0.994	$[0.984, 1.005]$	$[0.956, 1.033]$
$^{D}k_1$	0.979	$[0.977, 0.981]$	$[0.974, 0.984]$	0.994	$[0.985, 1.003]$	$[0.973, 1.014]$
$^{D}k_2$	0.996	$[0.991, 1.000]$	$[0.987, 1.004]$	0.999	$[0.992, 1.004]$	$[0.987, 1.011]$
$^{D}k_3$	0.996	$[0.991, 1.000]$	$[0.986, 1.006]$	0.991	$[0.984, 0.998]$	$[0.976, 1.004]$
ρ_1	2.824	$[2.816, 2.832]$	$[2.807, 2.840]$	1.271	$[1.260, 1.281]$	$[1.249, 1.293]$
ρ_2	1.605	$[1.597, 1.612]$	$[1.590, 1.621]$	1.290	$[1.279, 1.301]$	$[1.266, 1.314]$
ρ_3	1.160	$[1.153, 1.168]$	$[1.145, 1.176]$	1.255	$[1.244, 1.265]$	$[1.233, 1.277]$
σ_b	0.019	$[0.006, 0.035]$	$[0.000, 0.100]$	0.012	$[0.000, 0.020]$	$[0.000, 0.078]$
σ_w	0.010	$[0.008, 0.012]$	$[0.006, 0.015]$	0.017	$[0.013, 0.020]$	$[0.011, 0.026]$
	C4D Nonenzymatic ESS $> 13.2 \times 10^3$, PSRF < 1.05			C12D Nonenzymatic ESS $> 13.3 \times 10^3$, PSRF < 1.04		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
^{D}k	1.004	$[0.992, 1.018]$	$[0.957, 1.048]$	1.042	$[1.029, 1.057]$	$[0.992, 1.087]$
$^{D}k_1$	1.008	$[0.997, 1.018]$	$[0.987, 1.032]$	1.048	$[1.037, 1.059]$	$[1.027, 1.072]$
$^{D}k_2$	1.001	$[0.990, 1.012]$	$[0.976, 1.023]$	1.039	$[1.029, 1.049]$	$[1.016, 1.060]$
$^{D}k_3$	1.005	$[0.997, 1.013]$	$[0.988, 1.023]$	1.040	$[1.029, 1.052]$	$[1.014, 1.065]$
ρ_1	0.641	$[0.632, 0.650]$	$[0.622, 0.659]$	0.345	$[0.341, 0.350]$	$[0.336, 0.354]$
ρ_2	0.784	$[0.774, 0.794]$	$[0.765, 0.804]$	0.394	$[0.389, 0.399]$	$[0.384, 0.404]$
ρ_3	0.901	$[0.892, 0.910]$	$[0.881, 0.921]$	0.473	$[0.468, 0.478]$	$[0.462, 0.485]$
σ_b	0.013	$[0.000, 0.024]$	$[0.000, 0.087]$	0.016	$[0.000, 0.026]$	$[0.000, 0.094]$
σ_w	0.014	$[0.011, 0.017]$	$[0.009, 0.022]$	0.008	$[0.006, 0.009]$	$[0.005, 0.012]$
	C7D Nonenzymatic ESS $> 12.0 \times 10^3$, PSRF < 1.04			C11D Nonenzymatic ESS $> 13.0 \times 10^3$, PSRF < 1.03		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
^{D}k	0.935	$[0.911, 0.959]$	$[0.861, 1.032]$	0.963	$[0.937, 0.987]$	$[0.890, 1.041]$
$^{D}k_1$	0.941	$[0.903, 0.975]$	$[0.860, 1.077]$	0.969	$[0.940, 0.996]$	$[0.911, 1.045]$
$^{D}k_2$	0.931	$[0.920, 0.943]$	$[0.907, 0.956]$	0.964	$[0.945, 0.981]$	$[0.926, 1.004]$
$^{D}k_3$	0.933	$[0.920, 0.945]$	$[0.906, 0.960]$	0.955	$[0.936, 0.975]$	$[0.914, 0.996]$
ρ_1	0.618	$[0.573, 0.666]$	$[0.517, 0.711]$	0.564	$[0.543, 0.586]$	$[0.516, 0.609]$
ρ_2	3.013	$[2.954, 3.071]$	$[2.887, 3.136]$	0.735	$[0.717, 0.753]$	$[0.696, 0.772]$
ρ_3	3.592	$[3.530, 3.654]$	$[3.462, 3.720]$	0.740	$[0.724, 0.756]$	$[0.706, 0.775]$
σ_b	0.028	$[0.000, 0.051]$	$[0.000, 0.161]$	0.027	$[0.000, 0.044]$	$[0.000, 0.137]$
σ_w	0.085	$[0.065, 0.099]$	$[0.056, 0.128]$	0.028	$[0.022, 0.033]$	$[0.018, 0.043]$

Table S3. Summary statistics for the simulated posterior marginal distributions of each parameter in the fitted hierarchical Bayesian model for each enzymatic kinetic isotope effect. The median value, 68% and 95% highest posterior density (HPD) intervals of the marginal distributions are shown. Values are listed arbitrarily to 3 decimal places. The maximum potential scale reduction factor (PSRF) as well as the minimum effective sample size (ESS) among all parameters are also provided for each fit.

	C2D Enzymatic ESS $> 13.1 \times 10^3$, PSRF < 1.05			C14D Enzymatic ESS $> 14.0 \times 10^3$, PSRF < 1.04		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
${}^D k$	1.000	$[0.975, 1.025]$	$[0.917, 1.079]$	1.000	$[0.964, 1.040]$	$[0.888, 1.118]$
${}^D k_1$	1.003	$[0.983, 1.020]$	$[0.963, 1.046]$	0.979	$[0.962, 0.996]$	$[0.946, 1.014]$
${}^D k_2$	0.992	$[0.973, 1.012]$	$[0.949, 1.037]$	1.011	$[0.975, 1.042]$	$[0.947, 1.090]$
${}^D k_3$	1.005	$[0.983, 1.026]$	$[0.960, 1.057]$	1.022	$[0.981, 1.055]$	$[0.955, 1.101]$
ρ_1	1.918	$[1.881, 1.952]$	$[1.841, 1.994]$	1.234	$[1.201, 1.266]$	$[1.162, 1.302]$
ρ_2	1.598	$[1.572, 1.624]$	$[1.543, 1.656]$	1.725	$[1.687, 1.765]$	$[1.638, 1.806]$
ρ_3	1.911	$[1.883, 1.939]$	$[1.851, 1.970]$	1.475	$[1.429, 1.524]$	$[1.376, 1.570]$
σ_b	0.026	$[0.000, 0.048]$	$[0.000, 0.165]$	0.052	$[0.000, 0.083]$	$[0.000, 0.238]$
σ_w	0.035	$[0.026, 0.042]$	$[0.022, 0.056]$	0.044	$[0.033, 0.052]$	$[0.028, 0.068]$
	C4D Enzymatic ESS $> 13.3 \times 10^3$, PSRF < 1.04			C12D Enzymatic ESS $> 13.1 \times 10^3$, PSRF < 1.06		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
${}^D k$	0.938	$[0.915, 0.958]$	$[0.861, 1.017]$	1.049	$[1.036, 1.062]$	$[0.995, 1.096]$
${}^D k_1$	0.946	$[0.934, 0.957]$	$[0.924, 0.969]$	1.049	$[1.039, 1.060]$	$[1.027, 1.073]$
${}^D k_2$	0.927	$[0.910, 0.944]$	$[0.891, 0.959]$	1.051	$[1.040, 1.061]$	$[1.028, 1.075]$
${}^D k_3$	0.939	$[0.920, 0.958]$	$[0.894, 0.986]$	1.049	$[1.039, 1.058]$	$[1.029, 1.069]$
ρ_1	1.028	$[1.017, 1.039]$	$[1.006, 1.051]$	0.362	$[0.359, 0.365]$	$[0.356, 0.368]$
ρ_2	0.905	$[0.895, 0.915]$	$[0.885, 0.927]$	0.346	$[0.343, 0.349]$	$[0.340, 0.352]$
ρ_3	0.588	$[0.579, 0.596]$	$[0.569, 0.606]$	0.422	$[0.419, 0.425]$	$[0.416, 0.428]$
σ_b	0.027	$[0.000, 0.042]$	$[0.000, 0.162]$	0.012	$[0.000, 0.024]$	$[0.000, 0.104]$
σ_w	0.012	$[0.009, 0.014]$	$[0.007, 0.018]$	0.004	$[0.003, 0.005]$	$[0.003, 0.006]$
	C7D Enzymatic ESS $> 16.3 \times 10^3$, PSRF < 1.05			C11D Enzymatic ESS $> 11.8 \times 10^3$, PSRF < 1.03		
	Median	68% HPD Int.	95% HPD Int.	Median	68% HPD Int.	95% HPD Int.
${}^D k$	0.961	$[0.916, 1.005]$	$[0.850, 1.077]$	0.946	$[0.921, 0.969]$	$[0.867, 1.035]$
${}^D k_1$	0.884	$[0.856, 0.909]$	$[0.832, 0.947]$	0.936	$[0.921, 0.950]$	$[0.905, 0.966]$
${}^D k_2$	1.017	$[0.990, 1.045]$	$[0.959, 1.073]$	0.964	$[0.951, 0.977]$	$[0.937, 0.990]$
${}^D k_3$	0.949	$[0.924, 0.973]$	$[0.899, 1.004]$	0.936	$[0.923, 0.951]$	$[0.907, 0.966]$
${}^D k_4$	0.986	$[0.951, 1.021]$	$[0.916, 1.062]$			
ρ_1	4.323	$[4.202, 4.450]$	$[4.058, 4.577]$	0.726	$[0.720, 0.731]$	$[0.714, 0.737]$
ρ_2	3.309	$[3.275, 3.343]$	$[3.239, 3.382]$	0.736	$[0.729, 0.743]$	$[0.724, 0.750]$
ρ_3	2.732	$[2.683, 2.780]$	$[2.629, 2.833]$	0.670	$[0.661, 0.677]$	$[0.654, 0.685]$
ρ_4	2.124	$[2.078, 2.171]$	$[2.029, 2.220]$			
σ_b	0.083	$[0.037, 0.123]$	$[0.001, 0.232]$	0.033	$[0.000, 0.049]$	$[0.000, 0.167]$
σ_w	0.056	$[0.043, 0.065]$	$[0.037, 0.085]$	0.008	$[0.006, 0.010]$	$[0.005, 0.013]$

Table S4. Absolute values of $\Delta\alpha$ versus fraction of reaction f when $\mathbf{R}_0^\intercal(\mathbf{1} - {}^X\mathbf{k})$ is equal to ± 0.001.

f	0.15	0.30	0.50	0.70	0.85
$\lvert\Delta\alpha\rvert$	0.00016	0.00036	0.00069	0.0012	0.0019

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