Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1255–1272

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Inhibitory Action on HMG-CoA Synthase of Racemic and Optically Active Oxetan-2-ones (β-Lactones)

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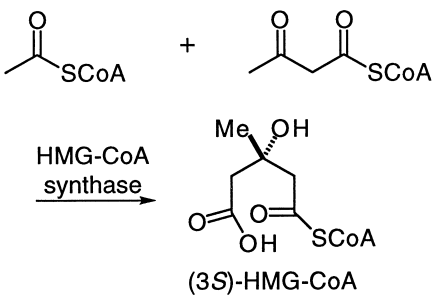
Received 25 March 1998; accepted 8 June 1998

Abstract—A homologous series of both C3-unsubstituted and C3-methyl substituted oxetan-2-ones (β-lactones) was investigated as potential inhibitors of yeast 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase. Several reported methods for racemic β-lactone synthesis were studied for preparation of the target series. In addition, a novel aluminum-based Lewis acid obtained by combination of Et_2AlCl with (1*R*,2*R*)-2-[(diphenyl)hydroxymethyl] cyclohexan-1-ol was studied for the asymmetric [2 + 2] cycloaddition of aldehydes and trimethylsilylketene. This Lewis acid exhibited good reactivity but variable enantioselectivity (22-85% ee). In in vitro assays using both native and recombinant HMG-CoA synthase from *Saccharomyces cerevisiae*, oxetan-2-ones mono-substituted at C4 with linear alkyl chains gave IC_{50}s that decreased monotonically with chain length up to 10 carbons and then rose rapidly for longer chains. The *trans* isomers of 3-methyl-4-alkyl-oxetan-2-ones showed a similar trend but had 1.3- to 5.6-fold lower IC_{50}s. The results imply a substantial hydrophobic pocket in this enzyme that interacts with both C-3 and C-4 substituents of oxetan-2-one inhibitors.

Introduction

The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase[1] catalyzes the formal aldol condensation of 1 mol of acetyl-coenzyme A (AcCoA) and 1 mol of acetoacetyl-coenzyme A (AcAcCoA). The reaction product, (3*S*)-HMG-CoA, is the universal precursor of terpenes and steroids,[2] being converted by the action of HMG-CoA reductase to mevalonate and thence via isopentenyl diphosphate and dimethylallyl diphosphate through to geranyl (GPP), farnesyl (FPP), or other diphosphates.[2,3] GPP and FPP act as progenitors for mono- and sesquiterpenes, respectively, while in the sterol sequence, FPP is converted via squalene and lanosterol into cholesterol and other sterols.[3] Inhibition of the many enzymes along this biosynthetic pathway thus offers the potential to regulate sterol biosynthesis; indeed the commercial statin drugs such as lovastatin and fluvastatin, which inhibit HMG-CoA reductase,[4] have proved to be highly effective at reducing sterol levels in humans.[5] Inhibitors of this pathway can also act as potent antimicrobial agents, especially for cells such as fungi which possess active sterol biosynthetic machinery.[6] HMG-CoA synthase is also involved in ketogenesis.[1a,6]



Key words: β-Lactones; HMG-CoA synthase; inhibition; asymmetric synthesis; chiral Lewis acid.
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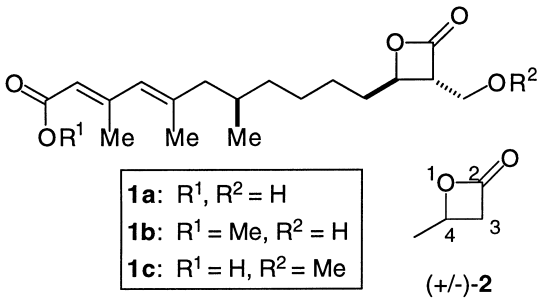
0968-0896/98/$—see front matter
PII: S0968-0896(98)00114-X

HMG-CoA synthase has been isolated from a range of species, including yeast,[7] and the livers of ox,[8] chicken[9] and man.[10] The human[10b] and avian liver[9] enzymes have been cloned and purified to homogeneity;[10] however no cloning work has been undertaken on the yeast enzyme. Detailed kinetic studies have been performed on the synthase from a range of these species to determine the sequence of events during catalysis of this condensation reaction; the enzyme exhibits BiBi ping-pong kinetics with AcCoA binding first to form an acetyl-enzyme covalent intermediate. The latter is isolable,[9] and the point of attachment has been shown to be a conserved cysteine residue (Cys-129 in both man[10a] and avian liver[9]). Binding of AcAc-CoA, via an interaction with His-264 (human enzyme residue),[11] then occurs; binding to free enzyme results in an abortive complex, accounting for the observed substrate inhibition, while binding to the acetyl–enzyme intermediate leads to aldol condensation and hence to a CoA-HMG-enzyme covalent intermediate.[12] Hydrolysis of the latter generates HMG-CoA and free synthase ready for another catalytic cycle.

As well as the statins, there exists an array of other natural products which act as inhibitors of the sterol pathway. However, aside from inhibition by one of the substrates (AcAcCoA) and other primary metabolic CoA esters such as succinyl-CoA,[13] the sole known metabolite which is specifically active against HMG-CoA synthase is the antibiotic F-244 (**1a**),[14] also known as L-659,699[14b,15] and 1233A.[16] This β-lactone, which has been isolated from *Scopulariopsis, Fusarium*, and *Cephalosporium* sp.,[14–16] along with a range of synthetic analogues which possess similar structures,[17] has been shown to be an irreversible inactivator of the enzyme in in vitro assays,[10a,14,15,17,18] as well as to be a potent inhibitor in in vivo systems.[14,15,17,19] The observation that both the methyl ester **1b** and *O*-methyl ether **1c** of F-244[14a,15b,17d,18b] exhibited little if any reduction in inhibition indicates that neither the carboxy nor hydroxy groups of F-244 participate in strong hydrogen-bond donor interactions with the enzyme active site, and therefore suggests that hydrophobic interactions are important in determining the affinity of F-244 and analogs for the enzyme. Thus a variety of non-polar substituents have been attached to the oxetan-2-one (β-lactone) C4-carbon, including 2-arylethyl[17b,c,f] and decyl.[17g] However, a systematic investigation of the effect of the length of alkyl groups attached at this site has not been conducted to the best of our knowledge.

In an earlier report, we showed that β-butyrolactone (**2**), a simple β-lactone analogue of F-244, was an irreversible inhibitor of the synthase, with an inactivation rate constant (k_{inact}) similar to F-244, but with much weaker binding affinity: K_I was ca. 10^5-fold higher than that for



F-244.[20] This result established that the lactone ring is the only structural component required for irreversible inhibition, and suggests that the ring substituents play a role solely in guiding the inhibitor into the enzyme active site. In order to explore the role of substituents at C3 and C4 of the oxetan-2-one ring, we thus prepared a series of β-lactones bearing either hydrogen or methyl at C-3 and alkyl chains of increasing length at C4, as well as several β-lactones containing variations on these substitution patterns. The biological activity of these simple F-244 analogs in in vitro assays with HMG-CoA synthase is described herein.

A range of methods for preparing racemic β-lactones has been reported and we investigated several of these routes to prepare our target series of compounds. This enabled a comparative study of these methods. The most efficient routes are those which involve a single-pot transformation such as the [2+2] cycloaddition of ketenes and aldehydes,[21] aldol-lactonizations,[22] and most recently the tandem Mukaiyama aldol-lactonization (TMAL) reaction.[23]

We were also interested in determining if the absolute stereochemistry of simple 4-alkyloxetan-2-ones would have a bearing on the inhibition of HMG-CoA synthase.[17a] The [2+2] cycloaddition of ketenes and aldehydes is one of the most concise routes to β-lactones. The first example of this reaction was reported by Staudinger in 1911,[21a] however more recently, Zaitseva showed that trimethylsilyl ketenes can participate in this reaction with Lewis acid catalysis.[21b] Recent studies with trimethylsilylketene and aldehydes have shown that high diastereoselectivity can be obtained with bulky, achiral Lewis acids.[24] In addition, the recent use of chiral Lewis acids has allowed access to optically active β-lactones.[25] However, a general chiral Lewis acid that provides high reactivity and enantioselectivity with a broad range of aldehyde substrates has yet to be found. We have also been studying this cycloaddition reaction as a concise entry into optically active β-lactones. In this regard, we have recently reported that the $TiCl_2$-TADDOL catalyst exhibits low to good enantioselectivity (9–80% ee) in this reaction.[26] Herein, we disclose a novel aluminum

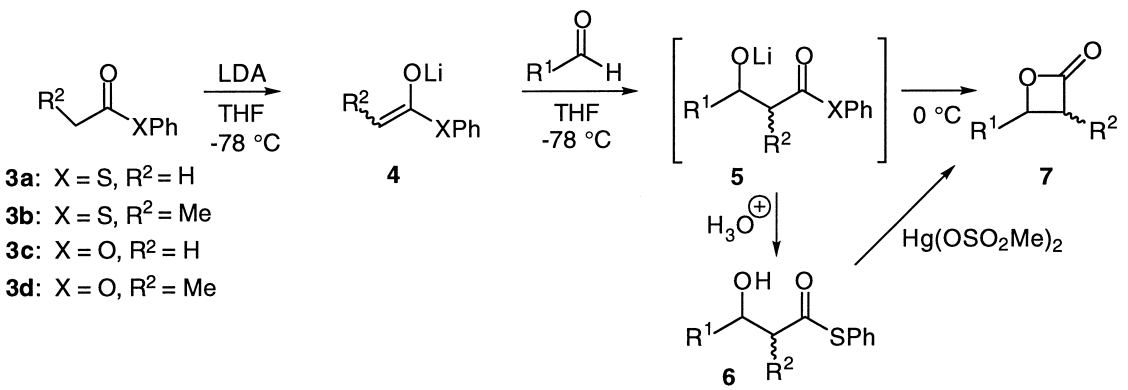
Lewis acid for this cycloaddition. While this Lewis acid provides the highest enantioselectivities reported to date for α-unbranched, aliphatic aldehydes, it exhibits enantioselectivity which appears to be substrate dependent.

Results

Preparation of oxetan-2-ones (β-lactones). Several of the reported methods for the preparation of racemic β-lactones were investigated. The desired β-lactones were prepared by the following methods: (1) the one-pot aldol-lactonization procedure reported initially by Danheiser[22a] and subsequently modified by Schick[22b] (Scheme 1, Table 1); (2) an aldol followed by a separate cyclization of the hydroxy acid derivative by the methods of Wemple[27] and Masamune[28] (Scheme 1, Table 1); (3) the tandem Mukaiyama-aldol lactonization (Scheme 2, Table 2); or (4) the catalyzed [2+2] cycloaddition of aldehydes and ketene using both $BF_3{\cdot}OEt_2$[21] (Scheme 3, Table 3) and a new, chiral aluminum-based Lewis acid (Scheme 5, Table 4).

A chiral promoter based on the chiral diol, (1*R*,2*R*)-2-[(diphenyl)hydroxymethyl] cyclohexan-1-ol (**13**),[29] was studied for the [2+2] cycloaddition of trimethylsilylketene and aldehydes. Diol **13** was readily prepared in two steps from β-ketoester **11** employing the modified Noyori reduction conditions reported by Taber in 67% yield and 64% ee.[30] Addition of excess phenyl magnesium bromide to the *trans*-β-hydroxyester **12** afforded the diol **13** in 72% yield. After a single recrystallization, the diol **13** could be enriched to 99% ee. Treatment of this diol, which was sparingly soluble in toluene with a slight deficiency of Et_2AlCl, resulted in a homogeneous solution of the presumed Lewis acid **14**. The structure of the Lewis acid has not been determined and the representation provided is meant only to show the presumed Lewis acid composition (Scheme 4).

Use of 20 mol of this promoter led to a variety of optically active α-silyl-β-lactones **10** with enantioselectivities ranging from 22–85%. In some cases, the β-lactones were more readily isolated after desilylation which gave the monosubstituted β-lactones **7** (Table 4).

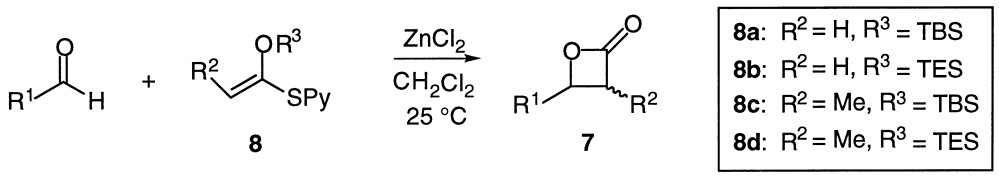


Scheme 1.

Table 1. Synthesis of racemic β-lactones via one-pot or two-step aldol-lactonizations

Entry	β-Lactone	R^1	R^2	Ester	*trans*/*cis* Ratio (**7**)	Method[a]	% Yield
1	**7a**	C_7H_{15}	Me	**3b**	>19:1[b]	A	32
2	**7a**	C_7H_{15}	Me	**3d**	>19:1[b]	B	39
3	**7b**	C_8H_{17}	H	**3a**	—	C	56[c]
4	**7c**	C_9H_{19}	H	**3a**	—	C	61[c]
5	**7d**	C_9H_{19}	Me	**3b**	>19:1[b]	A	37
6	**7d**	C_9H_{19}	Me	**3d**	>19:1[b]	B	35
7	**7d**	C_9H_{19}	Me	**3b**	1.2:1	C	66[c]

[a]Method A: Danheiser one-pot aldol-lactonization (ref 22a) but the aldehyde was added at −100 °C. Method B: Schick one-pot aldol-lactonization (ref 22b). Method C: Two-step procedure involving aldol reaction according to the method of Wemple (ref 27) and subsequent lactonization by the method of Masamune (ref 28).
[b]*trans*/*cis* Ratio determined by 200 MHz ^{1}H NMR on the purified β-lactones.
[c]Yields are for the two-step aldol-lactonization sequence.

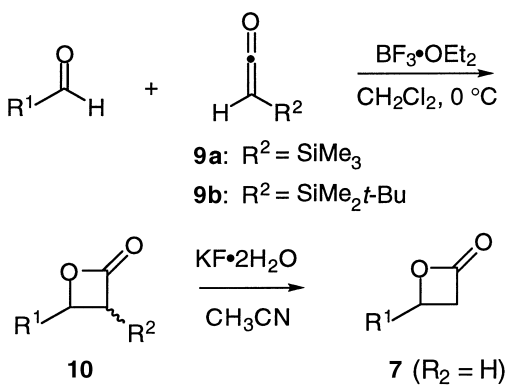


Scheme 2.

Table 2. Synthesis of racemic β-lactones via the tandem Mukaiyama aldol-lactonization (TMAL) reaction

Entry	β-Lactone	R^1	R^2	Ketene acetal	*trans*/*cis* Ratio (7)[a]	% Yield
1	**7e**[b]	$Ph(CH_2)_2$	H	**8b**	—	66
2	**7f**[b]	*t*-Bu	H	**8a**	—	42
3	**7g**[b]	C_7H_{15}	H	**8b**	—	65
4	**7c**	C_9H_{19}	H	**8b**	—	59
5	**7h**	C_4H_9	Me	**8c**	> 19/1	34
6	**7i**	C_5H_{11}	Me	**8c**	> 19/1	36
7	**7j**	C_6H_{13}	Me	**8c**	> 19/1	39
8	**7a**[b]	C_7H_{15}	Me	**8d**	39/1	60
9	**7k**	C_8H_{17}	Me	**8c**	> 19/1	35
10	**7d**	C_9H_{19}	Me	**8c**	> 19/1	35
11	**7l**	$C_{10}H_{21}$	Me	**8c**	> 19/1	38
12	**7m**	$C_{11}H_{23}$	Me	**8c**	> 19/1	41
13	**7n**	$C_{13}H_{27}$	Me	**8c**	> 19/1	40
14	**7o**	$C_{17}H_{35}$	Me	**8c**	> 19/1	32
15	**7p**[b]	*p*-NO_2Ph	Me	**8c**	< 1/19	36
16	**7q**[b]	$TBSO(CH_2)_4$	Me	**8c**	> 19/1	47
17	**7r**[b]	*c*-C_6H_{11}	Me	**8c**	> 19/1	16

[a]Ratios estimated or determined by ^{1}H NMR (200 MHz) or by GC, respectively.
[b]These β-lactones have been described previously (refs. 23b and c).



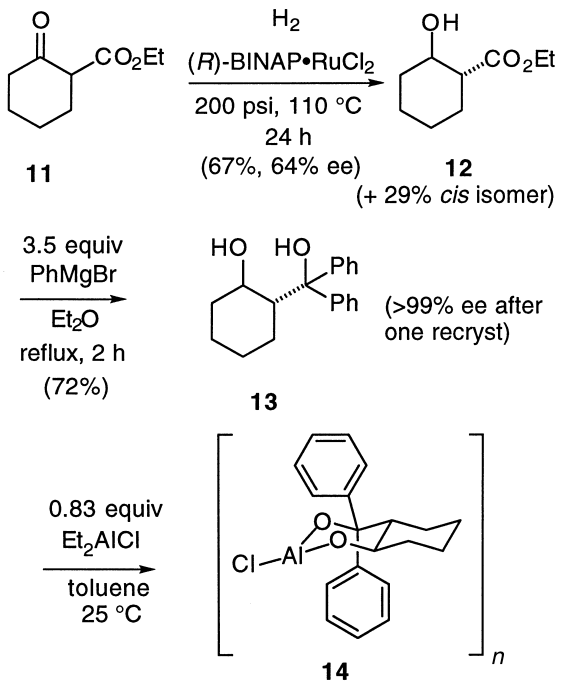
Scheme 3.

Table 3. Synthesis of racemic β-lactones via [2 + 2] cycloadditions of silylketenes and aldehydes

Entry	β-Lactone	Silylketene	R^1	% Yield (**7**)[a]
1	**7s**	**9a**	C_4H_9	74
2	**10t**	**9b**	C_5H_{11}	94[b]
3	**7u**	**9a**	C_6H_{13}	74
4	**7g**	**9a**	C_7H_{15}	80
5	**7b**	**9a**	C_8H_{17}	78
6	**7c**	**9a**	C_9H_{19}	84
7	**7v**	**9a**	$C_{10}H_{21}$	82
8	**7w**	**9a**	$C_{11}H_{23}$	79

[a]Yield is for the two steps of [2 + 2] cycloaddition and desilylation.
[b]Yield is for α-silyl-β-lactone **10t** as a mixture of *trans*/*cis* diastereomers (1/4.7).

Isolation of HMG-CoA synthase. Native HMG-CoA synthase was isolated from yeast as previously described,[20] and purified through the hydroxylapatite step to a specific activity of 0.13 μmol min^{-1} mg^{-1} (units/mg). For the recombinant synthase, oligonucleotide primers complementary to the *Saccharomyces cerevisiae* putative HMG-CoA synthase sequence obtained from GenBank (accession no. Z50178), bearing *Hind*III and *Nde*I restriction endonuclease sites at the 3′ and 5′ termini, respectively, were prepared. These primers were then used to amplify the putative HMG-CoA synthase gene from a genomic *S. cerevisiae* DNA preparation by PCR. The resulting product (1.5 kb) was digested with *Hind*III and *Nde*I, purifed and cloned into the



Scheme 4.

corresponding restriction sites on the plasmid pET-22b. After transforming *E. coli* BL21 (DE3) cells with this plasmid, plasmid DNA from three clones was prepared. The entire 1500 bases of interest were sequenced using primers for the T7 promoter and terminator sequences present in the plasmid, and an additional primer which was complementary to a central region of the gene. The results show that in all three clones there are three silent mutations relative to the original database sequence, as well as one non-silent mutation (nt 223 from T to C) corresponding to codon 75 being changed from serine to proline. Induction of expression of HMG-CoA synthase [MW 47.5 kD] in one of these clones was achieved by IPTG as assessed by SDS–PAGE of total cell protein. After cell lysis, the crude cell-free extract possessed a specific activity of synthase of 0.04 units/mg, compared to uninduced and insert-free controls which were inactive.

The recombinant enzyme was partially purified by a sequence of ammonium sulfate precipitation, anion exchange (Q-Sepharose) and size exclusion (S-200) chromatography to a final specific activity of 0.85 units/mg protein (Table 5).

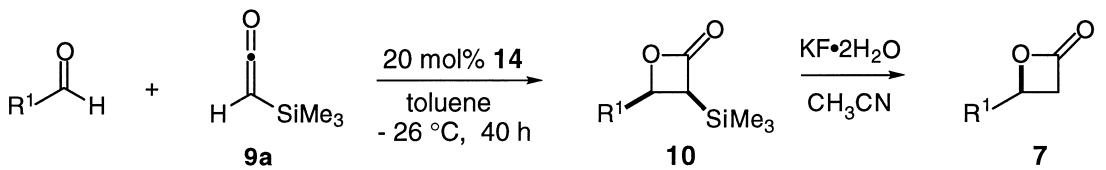
IC_{50} values for β-lactones. Initially, we screened the β-lactones **7a–d, 7g–o** and **7s–w** for inhibitory activity against HMG-CoA synthase using an in vitro assay against either native or recombinant enzyme. While IC_{50} is a poor measure of activity for irreversible inhibitors, this value does provide a comparison between inactivators determined with the same sample under identical conditions, as was the case in this study. Since IC_{50} is much more readily obtained, compared to the more fundamental constants of inhibition, k_{inact} and K_I, it was practical to obtain this value for all the inhibitors. All assays were performed by measuring the percentage of enzyme inhibited, relative to an inhibitor-free control, during five minutes of preincubation of inhibitor with enzyme, followed by addition of adequate substrates to prevent further inactivation of the enzyme, as demonstrated by linear substrate consumption during the assay period (Fig. 1). The IC_{50} values were then obtained by plotting % inhibition versus concentration of inhibitor for a series of assays performed at inhibitor concentrations above, below and around the estimated IC_{50}, followed by extrapolation of the IC_{50} value (Fig. 2). For F-244, as well as several of the synthetic β-lactones studied, the IC_{50} values were determined repetitively in several independent trials, and statistical analysis gave estimates of errors associated with these determinations. The results are shown in Table 6.

Several other synthetic β-lactones were also investigated for inhibitory efficacy against the synthase. The results for IC_{50} in these cases are shown in Table 7.

Discussion

Synthetic studies

Synthesis of racemic β-lactones. Danheiser and Nowick reported the first one-pot condensation of enolates with aldehydes to give variously substituted β-lactones.[22a] They employed thiophenyl ester enolates (**4a** or **4b**) and the presumed, initially formed aldolates **5** spontaneously lactonized on warming (Scheme 1). However,



Scheme 5.

Table 4. Synthesis of optically active β-lactones via [2 + 2] cycloadditions of trimethylsilyl-ketene and aldehydes

Entry	β-Lactone	R^1	*cis*/*trans* (10)	% Yield (7)[a]	% ee	Config. (C4)
1	**7x**[b]	*c*-C_6H_{11}	> 99:1[c]	83[d]	84[e]	S[f]
2	**7s**	*n*-Bu	> 99:1[c]	86[d]	85[e]	R[f]
3	**7y**[b]	*t*-Bu	—	no rxn	—	—
4	**7z**	Ph	> 99:1[c]	82[d]	28[e]	ND
5	**7aa**	$PhCH_2$	> 19:1[g]	45[d]	75[h]	R[i]
6	**7e**[b]	$PhCH_2CH_2$	> 19:1[g]	60	36[h]	S[f]
7	**7bb**	$TSBO(CH_2)_5$	> 19:1[g]	55	46[j]	ND
8	**7cc**	$CH_2=CH(CH_2)_7$	> 19:1[g]	71	22[j]	ND
9	**7dd**	$(CH_3CH_2)_2CH$	> 19:1[g]	46	56[j]	ND

[a]Yield of **7** for two steps (not including recovered aldehyde).
[b]These β-lactones have been described previously (refs 23b–c and 26).
[c]Determined on the crude reaction mixtures by GC.
[d]Yield of **10**.
[e]% ee's are for **10** and were determined by chiral GC(TBS-cyclodextrin, ref 31).
[f]Absolute configuration were assigned after alcoholysis of **7** to the corresponding known, β-hydroxy esters and comparison of optical rotation data.
[g]Determined on the crude reaction mixtures by ^1H NMR (200 or 300 MHz).
[h]% ee's are for **7** and were determined by chiral HPLC (Chiralcel OD).
[i]Absolute configuration was assigned by comparison of optical rotation data to the known β-lactone (ref 32).
[j]% ee's of **7** determined by chiral GC (TBS-cyclodextrin).

Table 5. Purification of recombinant yeast HMG-CoA synthase

Step	Vol. (mL)	Total protein (mg)	Specific activity (units/mg)[a]	Yield (%)	Fold purification
Cell-free Extract	20	30	0.04	100	1
$(NH_4)_2SO_4$ (25–40%)	5	50	0.10	104	2.5
Q-Sepharose	10	3	0.5	63	12
S-200	10	1	0.85	35	21

[a]One unit = 1 μmol/min.

condensation of the enolate derived from phenyl thioacetate with hexanal, octanal or decanal failed to give any α-unsubstituted-β-lactones in our hands. In an analogous one-step lactonization, Schick and co-workers[22b] used the enolates of phenyl esters (**4c** or **4d**). However, this method also failed to provide the expected β-lactone **7t** by condensation of the enolate of phenyl acetate with hexanal. In contrast, addition of either octanal or decanal (−100 °C) to either the phenyl or thiophenyl propionate (**3b** or **3d**) derived enolates (**4**) gave lactones **7a** and **7d** in yields of 32–39% (Table 1, entries 1–2 and 5–6) with a *trans*/*cis* ratio of > 19/1 (200 MHz ^1H NMR). The lower reaction temperature was crucial to obtain the desired β-lactones. Thus, both methods provide good selectivity but low yields in the preparation of 3,4-disubstituted oxetan-2-ones, and appear to be unsuitable for the preparation of C4-monosubstituted-β-lactones.

An alternative approach to C-4-monosubstituted oxetan-2-ones involves isolation of the aldol adduct **6**, followed by a separate lactonization (Scheme 1). Wemple

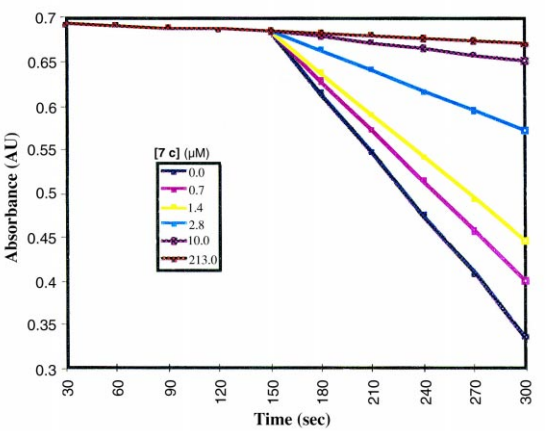


Figure 1. Inhibition of HMG-CoA Synthase by β-Lactone **7c**. Enzyme was incubated with the concentrations of **7c** shown for 5 min, then acetoacetyl-CoA (AcAcCoA) was added (time 0). Absorbance of AcAcCoA at 303 nm was monitored for 150 s (providing a control for AcAcCoA thiolase), then acetyl-CoA was added, and the reaction was monitored until time 300 s.

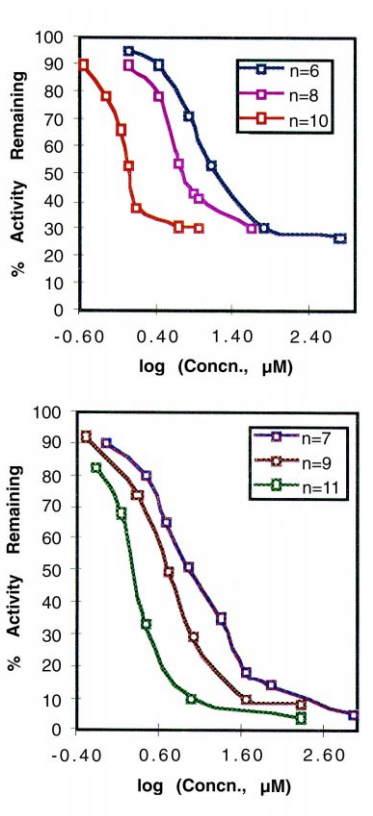


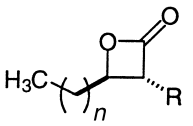
Figure 2. Representative inhibition curves for HMG-CoA synthase. Data from Figure 1 and similar plots for β-lactones **7b, 7g, 7u, 7v,** and **7w** are re-plotted to show percent inhibition as a function of β-lactone concentration. IC_{50} values were determined by extrapolation.

condensed the enolate of phenyl thioacetate with aldehydes at low temperature, then quenched the resulting aldol alkoxide **5** to give β-hydroxythiophenylesters **6**.[27] Masamune cyclized β-hydroxythiophenylesters using $Hg(O_3SCH_3)_2$ to give various substituted β-lactones.[28] This sequential route furnished racemic 4-octyl-(**7b**) and 4-nonyl-oxetan-2-one (**7c**) from thiophenyl acetate and nonanal, or decanal in two steps in 56 and 61% overall yield, respectively (entries 3 and 4, Table 1). However, the corresponding condensation of phenyl thiopropionate with decanal followed by lactonization gave the lactone **7d** in a ca. 1/1.2 ratio of cis/trans diastereomers and 66% overall yield. Thus, while this method provides a route to both C3, C4-disubstituted as well as C4-monsubstituted-β-lactones, undesirable features include the use of mercury salts, the low diastereoselectivity with the propionate enolate, and the fact that it is a two step process. We next investigated the use of the 2-thiopyridylketene acetals **8** for the single pot preparation of β-lactones, according to a method first reported by Hirai and further developed in our laboratories (Scheme 2, Table 2).[23] Condensation of the TBS(*tert*-butyldimethylsilyl)ketene acetal **8c** with a variety of aldehydes using $ZnCl_2$ as a promoter, afforded the corresponding 3-methyl-4-alkyloxetan-2-ones in yields of 32–47% with high diastereoselectivities (*trans*/*cis*, >19/1). As previously described,[23b,c] this method could also be used to access C4-monsubstituted-β-lactones unavailable by other one-pot aldol-lactonization methods described above. We have also previously reported that higher yields of β-lactones using propionate and especially acetate derived thiopyridylketene acetals could be achieved by employing the triethylsilyl (TES) ketene acetals **8b** and **8d**. This was further demonstrated in this study (i.e. entries 1, 3–4 and 8, Table 2).[23c]

The Lewis acid catalyzed [2+2] cycloaddition of trimethylsilylketene and aldehydes first reported by Zaitseva[27] was also investigated. Trimethylsilylketene[33] was prepared and its [2+2] cycloaddition with a range of saturated aldehydes afforded monosubstituted β-lactones **7b–c, 7g, 7s–7w** in good yields (74–84%, Table 3) after a subsequent desilylation step of the α-silyl-β-lactones **10**. This two step process provides a concise route to racemic, C4-monosubstituted β-lactones. Limitations to this method are the somewhat tedious preparation of ethoxyacetylene[34] required for the synthesis of trimethylsilylketene and the fact that it is a two step process. In summary, for *trans*-3-methyl-4-alkyloxetan-2-ones, the methods of Danheiser and Schick are comparable in both yield and diastereoselectivity, removing the requirement for diastereomer separation inherent in the method due to Wemple. The TMAL reaction provides a concise, and highly diastereoselective route to both C4-monosubstituted and C3, C4-disubstituted-β-lactones. In most cases, these methods are complementary to one another since for example, the single-pot aldol-lactonizations proceed efficiently with ketone substrates but less so with aldehyde substrates. In contrast, the TMAL reaction provides good yields with aldehyde substrates including the use of acetic acid derived ketene acetals leading to C4-monosubstituted β-lactones. In some cases, comparable diastereoselectivity but higher yields can be obtained via the TMAL reaction in comparison to the methods of Danheiser and Schick (cf. entry 1 and 2, Table 1 and entry 8, Table 2). In addition the one step TMAL reaction gives comparable yields to the two-step aldol-lactonization (Wemple/Masamune) for the synthesis of C4-monosubstituted β-lactones (cf. entry 4, Table 1 vs entry 4, Table 2). However, the [2+2] cycloaddition of aldehydes and ketenes provides the highest overall yields for C4-monosubstituted β-lactones but is a two step procedure.

Synthesis of optically active β-lactones. In the design stages of the aluminum complex **14**, we envisioned a tetrahedral complex (**A** or **B**) formed upon complexation of

Table 6. IC_{50} values for inhibition of HMG-CoA synthase with β-lactones of varying chain length[a]



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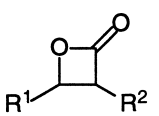
n	R = H				R = Me			
	Entry	β-Lactone	Enz[b]	IC_{50} (μM)[c]	Entry	β-Lactone	Enz[b]	IC_{50} (μM)[c]
—	1	**15**	N	500 (5)	13	—	—	—
0	2	**2**	N,R	2,000 (1) ± 40[d]	14	—	—	—
3	3	**7s**	R	200 (4); 150 (4)[e]	15	**7h**	R	88 (5)
4	4	**7t**	—	—	16	**7i**	R	26 (1)
5	5	**7u**	N	13 (1)	17	**7j**	R	10 (1)
6	6	**7g**	N	9.0 (8)	18	**7a**	N	1.6 (1)
7	7	**7b**	N	6.3 (5)	19	**7k**	R	1.3 (1)
8	8	**7c**	N,R	2.0 (2) ± 0.17[f]	20	**7d**	N	1.0 (1) ± 0.28[d]
9	9	**7v**	N,R	1.4 (2) ± 0.08[g]	21	**7l**	R	0.28 (1)
10	10	**7w**	R	2.0 (1)	22	**7m**	R	2.7 (1)
12	11	—	—	—	23	**7n**	R	280 (8)
16	12	—	—	—	24	**7o**	R	280 (8)

[a]Determined for 5 min inactivation.
[b]Enz = enzyme source, N = native, R = recombinant.
[c]Values in parentheses are errors in the last decimal place from a single inhibition plot; errors from statistical analysis of several repeat experiments are shown as ± one standard deviation.
[d]Average and standard deviation (±) from five determinations.
[e]First value is for racemic, second for β-lactone (4*R*)-**7s** (85% ee).
[f]Average and standard deviation (±) from six determinations.
[g]As for note f, from four determinations.

Table 7. IC_{50} values for inactivation of HMG-CoA synthase by β-lactones with varying substitution[a]



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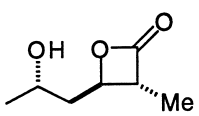

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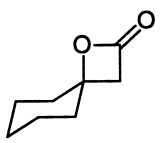

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Entry	β-Lactone	R_1	R_2	Config.	IC_{50} (μM)[b]
1	**10t**	C_5H_{11}	$SiMe_2t$-Bu	*RS-trans*/*cis*[c]	> 2,000
2	**7x**[d]	*c*-C_6H_{11}	H	*RS*	350 (5)
3	**7x**[d]	*c*-C_6H_{11}	H	4-*S*	184 (4)
4	**7e**[d]	$Ph(CH_2)_2$	H	*RS*	1800 (50)
5	**7bb**[d]	$Ph(CH_2)_2$	Me	*RS-trans*	1500 (50)
6	**7p**[d]	*p*-NO_2Ph	Me	*RS-cis*	> > 2,000
7	**16**[d]	$CH_2CH(OH)CH_3$	Me	3*R*,4*R*,2′*S*	283 (15)
8	**17**[d]	$-(CH_2)_5-$	H	achiral	2300 (50)[e]
9	(F-244) **1**	—	—	4-*R-trans*	0.0100 ± 0.0006[f]

[a]Determined for 5 min inactivation.
[b]Values in parentheses are errors in the last decimal place from a single inhibition plot; errors from statistical analysis of several repeat experiments are shown as ± one standard deviation.
[c]This was a 4.7/1 ratio of *cis*/*trans* β-lactones.
[d]These β-lactones have been described previously, see refs 22a, 23c, and d.
[e]Reversible inhibitor.
[f]Average and standard deviation (±) from six determinations.

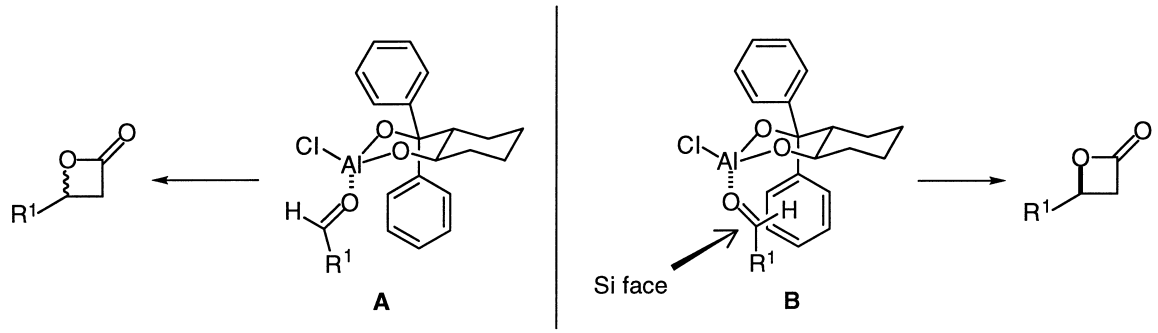

Figure 3. Proposed conformations for complexation of aldehydes with monomeric Lewis acid **14**.

the (monomeric) Lewis acid with an aldehyde (Fig. 3). In this situation, assuming the chlorine adopts a pseudoequatorial position upon complexation, the aldehyde would be in a pseudoaxial position. Two conformations are likely, **A** and **B**, and it was not clear at the outset which would predominate. These conformations avoid 1,3-diaxial type interactions between the formyl hydrogen or *R* group of the aldehyde and the axial phenyl group. However, it appeared that conformation **A** would provide low facial selectivity while conformation **B** may lead to high facial selectivity since the pseudoaxial phenyl effectively blocks the *Re* face of the aldehyde. The possibility of formyl C-H–O hydrogen bonding to oxygen, recently proposed by Corey to explain a number of enantioselective transformations,[35] found in conformer **B** was also considered as a possible control element to favor conformation **B**. However, analysis of the complex by ^{27}Al NMR indicates that the Lewis acid **14** in toluene-d_8 may exist as two species, one of which is a dimer, making a rationalization of the results more difficult.[36]

The Lewis acid **14** derived from diol **11** and Et_2AlCl led to good reactivity but variability in the enantioselectivity for the [2+2] cycloaddition of trimethylsilyl ketene and various aldehydes (Table 4). Interestingly, the same facial selectivity is observed with most aldehydes for which the absolute configurations have been determined (entries 1, 2 and 5 but not entry 6, Table 4) and is consistent with attack on the *Si* face of the aldehyde on conformer **B**. It should be noted that the difference in absolute configuration for the β-lactones derived from cyclohexanecarboxaldehyde and *n*-butanal (cf. entry 1 and 2, Table 4) is due to a change in substituent priority rather than a change in facial selectivity of the aldehyde. While these are the highest enantioselectivities reported to date for α-unbranched, aliphatic aldehydes (i.e. entry 2, Table 4) in this [2+2] cycloaddition, the reactivity and generality must obviously be improved before this becomes a practical procedure for the preparation of optically active β-lactones.

Inhibition studies

Although the native HMG-CoA synthase from yeast is available, as we have described previously,[20] difficulties in the purification of this enzyme to homogeneity, coupled with the availability of the sequence of the yeast genome, led us to investigate a genetic cloning approach. Cloning of the putative *S. cerevisiae* HMG-CoA synthase gene, which has ca. 40% sequence similarity to the known human sequence, into an *E. coli* host under control of the T7 promoter, followed by induction gives HMG-CoA synthase of defined sequence, which is fully catalytically competent and resembles the native enzyme in many respects. The putative role of this gene in yeast is thus confirmed. The mutation of Ser-75 to Pro-75 which was detected in this study has little effect on the competency of the protein. The IC_{50} values for several of the β-lactone inhibitors (**2, 7s, 7c**, and **7v**) were determined with both recombinant and native enzyme preparations, and in each case the values obtained were found to be essentially identical. The recombinant and native HMG-CoA synthases behave similarly during purification; however the higher initial specific activity in the recombinant case allows for a purer preparation overall (ca. 25% pure based on titration with F-244). The IC_{50} for both β-lactone 2 and F-244 (**1**) was determined at various stages of purification

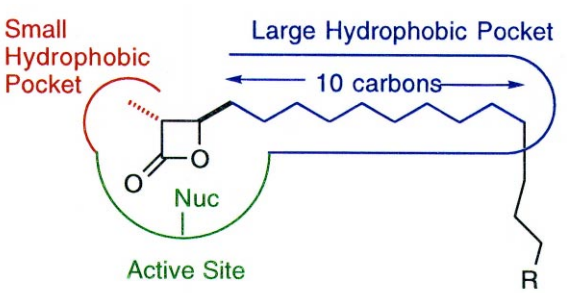

Figure 4. Proposed HMG-CoA synthase binding/active site for β-lactone inhibitors.

of both enzyme preparations, and was found to be invariant with enzyme purity, indicating that there are no interferences with inhibition through processes such as protein–protein interactions, and allowing further studies of the inhibition process with the partially purified material. An overall 21-fold purification of the recombinant synthase was obtained in three steps; completion of the purification is currently in progress.

Although the amino acid sequence for yeast synthase is substantially different than that for human enzyme, the results of this study confirm that the yeast synthase is a valid model for the human protein. Thus, the IC_{50} value for F-244 was found to be essentially identical to values reported previously.

Two sets of β-lactones bearing either hydrogen or methyl at C-3 and alkyl chains from one to 18 carbons at C-4 were evaluated for inhibitory efficacy. The results show a roughly monotonic decrease in IC_{50} with C-4 chain length until an optimum length of 10 carbons is reached. Thereafter, the IC_{50} rises sharply again. The results are most simply interpreted in terms of a substantial hydrophobic binding pocket on the enzyme; increasing alkyl chain length gives rise to tighter binding, and hence to reduced IC_{50} (Fig. 4). Alkyl chains of greater than 10 carbons 'overflow' out of the pocket, perhaps into a hydrophilic region or into the aqueous environment, making this arrangement disfavoured. Comparison of the C3-methyl series with the C4-monosubstituted β-lactones as inhibitors shows a consistent improved affinity for the enzyme when the 3-methyl group is present, by a factor of between 1.3 and 5.6. This result implies that a substituent at C-3 can also provide a substantial hydrophobic interaction with the enzyme. Although this study was performed largely with racemic lactones, due to ease of synthesis, lactone **7a** in enantiomerically enriched form was investigated, to establish that the inhibitors were acting enantioselectively. Comparison of the results with racemic and chiral materials shows that the enantiomer corresponding in configuration to F-244 is most effective, suggesting that the compound is binding in the synthase active site in a similar manner to F-244. It is interesting to note from Fig. 2 that β-lactones in the odd *n* series abolished synthase activity completely, but that even *n* β-lactones appeared to permit some residual enzyme activity even at high concentrations. The source of this effect is currently unclear, and investigations of this phenomenon are ongoing. The acute loss of activity for very long chains, as well as the discrimination for one enantiomer of **7a** are both inconsistent with inhibitory activity depending on aggregation of the hydrophobic β-lactones. Thus, the critical micellar concentration of long-chain β-lactones is expected to decrease with increasing chain length to 18 carbons.

These results are consistent with data from a range of previous studies that have shown, for example, that F-244 methyl ester, F-244 *O*-methyl ether and several oxetan-2-ones possessing phenalkyl groups at C4 exhibit little loss of potency compared to the parent antibiotic. However, the present set of data rigorously establish the approximate size requirements for the hydrophobic cavity at the active site of HMG-CoA synthase. Thus, Nature has selected the optimal chain length in the natural inhibitor F-244 (**1**).

The results with several lactones which are reported in Table 7 demonstrate that the enzyme is not highly tolerant of major changes in structure of the inhibitor at either C3 or C4. Thus, replacement of a C3-methyl by *tert*-butyldimethylsilyl gives an extremely poor inhibitor (cf. entry 16, Table 6 with entry 1, Table 7). Likewise, a *p*-nitrophenyl substituent at C4 resulted in only weak activity. The results with β-lactones **7e** and **7bb** show that a phenethyl substituent at C4 gives poor activity against yeast HMG-CoA synthase. This result is in contrast to those of Hashizume with rat liver enzyme.[17b] This observation suggests a difference between the two proteins for these inhibitors, whereas F-244 shows similar activity with both enzymes. That such a difference could be exploited in species selective inhibition of the synthase is under active investigation. In contrast, 2′-hydroxypropyl or cyclohexyl substituents at C4 give inhibitory activities which approximate those of the straight-chain compounds which possess the same chain length (cf. entry 2 and 7, Table 7 with entry 3, Table 6). In the case of the cyclohexyl substituent, enantiomerically enriched material exhibited an IC_{50} of approximately one-half that of the racemic compound (entries 2 and 3, Table 7), again showing the binding to be enantioselective.

Conclusion

For the synthesis of racemic β-lactones from aldehydes, the aldol-lactonization methods of Danheiser and Schick are comparable in terms of yields and selectivity for the preparation of C3, C4-disubstituted β-lactones. However, these methods do not allow access to C4-monosubstituted-β-lactones. The TMAL reaction provides a single step route to C4-monsubstituted-β-lactones using readily available thiopyridyl ketene acetals, however the two step procedure involving [2 + 2] cycloaddition of aldehydes and silylketenes provides higher overall yields. In addition, the TMAL reaction provides good yields and high diastereoselectivity for the synthesis of C3,C4-disubstituted-β-lactones. A new, aluminum chiral Lewis acid has been applied to the [2 + 2] cycloaddition of aldehydes and trimethylsilylketene and provides good reactivity but

variable enantioselectivity dependent on the substrate structure. Both 4-alkyl- and 3-methyl-4-alkyl-oxetan-2-ones exhibit a logical trend in inhibition of HMG-CoA synthase with potencies which are dependent on the length of the C4 substituent; a chain of 10–11 carbons gives maximal inhibition. The results can be explained by hydrophobic interactions with the synthase active site.

Experimental

General synthetic procedures

All reactions were performed in flame-dried glassware under a positive pressure of nitrogen and magnetically stirred unless otherwise indicated. Toluene was distilled from sodium immediately prior to use. Methylene chloride was distilled from CaH_2 immediately prior to use. Tetrahydrofuran and diethyl ether were distilled from sodium-benzophenone ketyl radical immediately prior to use. Acetonitrile was heated at reflux overnight with P_2O_5, distilled, and kept under argon over activated 3 Å molecular sieves. Commercially available aldehydes were purchased from Aldrich Chemical Co. and other aldehydes were prepared from the corresponding alcohols by oxidation. All aldehydes were distilled (Kugelrohr distillation) or purified by flash chromatography (silica gel) immediately prior to use. Commercial-grade reagents (Aldrich) and solvents (Caledon) were used without further purification except as indicated below. Flash column chromatography was accomplished using Merck 60 (230–400 mesh) silica gel or Baxter S/P Silica Gel 60 Å (230–400 mesh ASTM). Thin layer chromatography was performed using Merck 60 F-254 plates, and β-lactones were visualized through treatment with a solution of phosphomolybdic acid in 10% sulfuric acid (5 g/100 mL).

Mass spectra were obtained on a VG analytical 70S high-resolution, double focusing, sectored (EB) mass spectrometer at the Center for Chemical Characterization and Analysis (Texas A&M) or on a VG Analytical ZAB-E mass spectrometer (McMaster). Enantiomeric excess was determined by GC (Hewlett–Packard 5880A gas chromatograph) analysis using a TBS-β-cyclodextrin column[31] or HPLC (RAININ SD-200 with DYNAMAX UV-C detector) analysis using a Chiralcel OD column. IR spectra were recorded on a Nicolet Impact 410DSP (Texas A&M) or on a BIO-RAD FTS-40 FT-IR spectrometer (McMaster). ^{1}H NMR spectra were recorded on a Varian VXR-300 (300 MHz) or 200E spectrometer (200 MHz) (Texas A&M) or Bruker AC-200, AC-300, or DRX-500 spectrometers (McMaster) and chemical shifts are reported in ppm using tetramethylsilane (δ 0.0) or $CHCl_3$ (δ 7.26) as internal reference. ^{13}C NMR spectra were recorded on a Varian VXR-300 (75 MHz) or XL 200E (50 MHz) and chemical shifts are reported in ppm using $CDCl_3$ (δ 77.0) as internal reference. ^{27}Al NMR spectra were recorded on a Varian XL 200 (52 MHz) in toluene-d_8 and chemical shifts are reported in ppm relative to $Al(acac)_2$ (δ 0.0) as external reference.

Methods A,[22a] B,[22b] and C[22,28] (Table 1) were performed according to the published procedures except that in method A, the aldehyde was added at −100 °C. The characterization of β-lactones prepared by these methods is described below.

General procedure for the [2 + 2] cycloaddition of aldehydes and trimethylsilyl ketene and desilylation as described for 4-nonyl-oxetan-2-one (7c). A solution of decanal (0.403 g, 2.62 mmol) and trimethylsilylketene **9a**[34] (0.334 g, 2.91 mmol, 1.1 equiv) dissolved in dichloromethane (5 mL) was cooled to 0 °C. While stirring, boron trifluoride etherate (0.4 mL of a 0.1 M solution in dichloromethane) was added dropwise until IR indicated loss of trimethylsilylketene starting material (ca. 50 min). The reaction was quenched with two drops of water and the solution was filtered through a short plug of anhydrous sodium sulfate. After solvent removal on the rotary evaporator, the crude oil was dissolved in 7 mL of acetonitrile. To this solution, finely crushed $KF{\cdot}2H_2O$ (0.496 g, 5.23 mmol, 2.0 equiv) was added and the mixture was vigorously stirred for 20 min. The resulting mixture was filtered through florisil with ether washing and the solvent was removed on a rotary evaporator. The crude oil was purified via flash chromatography (EtOAc/hexanes, 10/90) to give 432 mg of lactone **7c** (84%) as a colorless oil. IR (thin film) 2928, 1831 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.49 (m, 1H), 3.51 (dd, J = 16.2, 5.7 Hz, 1H), 3.05 (dd, J = 16.2, 4.3 Hz, 1H), 1.76 (m, 2H), 1.28 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 71.5, 42.8, 34.6, 29.4 (2C), 29.2, 24.8, 22.6, 14.1.

4-Butyloxetan-2-one (7s). The [2 + 2] cycloaddition of pentanal (0.197 g, 1.74 mmol) with trimethylsilylketene **9a** (0.215 g, 1.82 mmol) and then desilylation with potassium fluoride dihydrate (0.322 g, 3.41 mmol) was performed according to the general procedure. Work-up followed by purification afforded 187 mg (74%) of product **7s** as a colorless oil. IR (thin film) 2931, 1828 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.50 (m, 1H), 3.50 (dd, J = 16.2, 5.7 Hz, 1H), 3.04 (dd, J = 16.2, 4.3 Hz, 1H), 1.86 (m, 2H), 1.30 (m, 8H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 71.2, 42.8, 34.6, 31.5, 28.7, 24.7, 22.4, 13.9.

4-Hexyloxetan-2-one (7u). The [2 + 2] cycloaddition of heptanal (0.197 g, 1.74 mmol) with trimethylsilylketene

9a (0.215 g, 1.82 mmol) and then desilylation with potassium fluoride dihydrate (0.322 g, 3.41 mmol) was performed according to the general procedure. Work-up followed by purification afforded 187 mg (74%) of product **7u** as a colorless oil. IR (thin film) 2931, 1828 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.50 (m, 1H), 3.50 (dd, $J=16.2$, 5.7 Hz, 1H), 3.04 (dd, $J=16.2$, 4.3 Hz, 1H), 1.86 (m, 2H), 1.30 (m, 8H), 0.88 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 71.2, 42.8, 34.6, 31.5, 28.7, 24.7, 22.4, 13.9.

4-Heptyloxetan-2-one (7g). The [2 + 2] cycloaddition of octanal (0.217 g, 1.63 mmol) with trimethylsilylketene **9a** (0.200 g, 1.73 mmol) and then desilylation with potassium fluoride dihydrate (0.306 g, 3.24 mmol) was performed according to the general procedure. Work-up followed by purification afforded 214 mg (80%) of product **7g** as a colorless oil. IR (thin film) 2930, 1830 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.50 (m, 1H), 3.50 (dd, $J=16.2$, 5.7 Hz, 1H), 3.04 (dd, $J=16.2$, 4.3 Hz, 1H), 1.80 (m, 2H), 1.27 (m, 10H), 0.88 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 71.4, 42.8, 34.6, 31.5, 28.9, 24.8, 22.4, 14.0.

4-Octyloxetan-2-one (7b). The [2 + 2] cycloaddition of nonanal (0.319 g, 2.26 mmol) with trimethylsilylketene **9a** (0.287 g, 2.52 mmol) and then desilylation with potassium fluoride dihydrate (0.413 g, 4.46 mmol) was performed according to the general procedure. Work-up followed by purification afforded 317 mg (78%) of product **7b** as a colorless oil. IR (thin film) 2928, 1831 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.48 (m, 1H), 3.49 (dd, $J=16.2$, 5.7 Hz, 1H), 3.04 (dd, $J=16.2$, 4.3 Hz, 1H), 1.79 (m, 2H), 1.26 (m, 10H), 0.86 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.3, 71.2, 42.8, 34.6, 31.7, 31.5, 29.3, 29.1, 24.8, 22.6, 14.0.

4-Decyloxetan-2-one (7v). The [2 + 2] cycloaddition of undecanal (0.640 g, 3.69 mmol) with trimethylsilylketene **9a** (0.475 g, 4.14 mmol) and then desilylation with potassium fluoride dihydrate (0.708 g, 7.43 mmol) was performed according to the general procedure. Work-up followed by purification afforded 651 mg (82%) of product **7v** as a colorless oil. IR (thin film) 2926, 1830 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.50 (m, 1H), 3.50 (dd, $J=16.2$, 5.7 Hz, 1H), 3.05 (dd, $J=16.2$, 4.3 Hz, 1H), 1.79 (m, 2H), 1.26 (m, 16H), 0.88 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) 168.3, 71.3, 42.9, 34.7, 31.9, 29.4 (3C), 29.3, 29.1, 24.9, 22.6, 14.1.

4-Undecyloxetan-2-one (7w). The [2 + 2] cycloaddition of dodecanal (0.433 g, 2.32 mmol) with trimethylsilylketene **9a** (0.285 g, 2.49 mmol) and then desilylation with potassium fluoride dihydrate (0.428 g, 4.63 mmol) was performed according to the general procedure. Work-up followed by purification afforded 413 mg (79%) of product **7w** as a colorless oil. IR (thin film) 2926, 1831 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.50 (m, 1H), 3.50 (dd, $J=16.2$, 5.7 Hz, 1H), 3.05 (dd, $J=16.2$, 4.3 Hz, 1H), 1.79 (m, 2H), 1.25 (m, 18H), 0.88 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 71.3, 42.9, 34.7, 31.9, 29.6, 29.4 (3C), 29.3, 29.2, 24.9, 22.7, 14.1.

***cis*/*trans*-4-Butyl-3-*tert*-butyldimethylsilyloxetan-2-one (10t).** The [2 + 2] cycloaddition of hexanal (0.242 g, 2.4 mmol) with *tert*-butyldimethylsilyl ketene (0.390 g, 2.5 mmol) was performed according to the general procedure with the exception that the desilylation was not performed. Purification (EtOAc/hexanes, 10/90) gave 0.561 g (94%) of β-lactone **10t** (*cis*/*trans*, 4.7/1, 200 MHz ^1H NMR). IR (thin film) 2932, 1806 cm^{-1}; *cis*-**10t**: ^1H NMR (200 MHz, $CDCl_3$) δ 4.61 (m, 1H), 3.58 (d, $J=6.3$ Hz, 1H), 1.76 (m, 2H), 1.31 (m, 6H), 0.91 (m, 12H), 0.16 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 74.9, 43.8, 34.8, 32.0, 26.8, 23.2, 17.3, 14.2, −7.1, −7.3; *trans*-**10t**: ^1H NMR (200 MHz, $CDCl_3$) δ 4.36 (m, 1H), 3.58 (d, $J=4.1$ Hz, 1H), 1.76 (m, 2H), 1.31 (m, 6H), 0.91 (m, 12H), 0.16 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 73.7, 46.2, 36.3, 32.2, 26.8, 23.2, 17.3, 14.2, −7.1, −7.3.

The tandem Mukaiyama aldol-lactonization (TMAL) reaction was used to prepare the following β-lactones and was performed as described previously.[23c]

4-Heptyl-3-methyloxetan-2-one (7a). The reaction of octanal (0.216 g, 1.63 mmol) with ketene acetal **8c** (0.504 g, 1.78 mmol) was performed using 0.533 g (2.35 mmol) zinc chloride in 10 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7a** (108 mg, 39%) as a clear oil. IR (thin film) 2930, 1825 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.14 (td, $J=6.7$, 4.0 Hz, 1H), 3.20 (qd, $J=7.5$, 4.0 Hz, 1H), 1.77 (m, 2H), 1.35 (d, $J=7.5$ Hz, 3H), 1.26 (m, 10H), 0.87 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.5, 50.6, 34.0, 31.5, 31.3, 29.0, 24.6, 22.4, 13.8, 12.4.

4-Nonyl-3-methyloxetan-2-one (7d). The reaction of decanal (0.104 g, 0.647 mmol) with ketene acetal **8c** (0.203 g, 0.712 mmol) was performed using 0.203 g (0.887 mmol) zinc chloride in 5 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7d** (46 mg, 35%) as a clear oil. IR (thin film) 2928, 1826 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.16 (td, $J=6.7$, 4.0 Hz, 1H), 3.20 (qd, $J=7.5$, 4.0 Hz, 1H), 1.79 (m, 2H), 1.37 (d, $J=7.5$ Hz, 3H), 1.26 (m, 14H), 0.87 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 79.6, 50.8, 34.2, 31.9, 31.6, 29.5 (2C), 29.3, 25.0, 22.7, 14.5, 12.6.

4-Butyl-3-methyloxetan-2-one (7h). The reaction of pentanal (0.125 g, 1.33 mmol) with ketene acetal **8c** (0.416 g, 1.46 mmol) was performed using 0.429 g (1.85 mmol) zinc chloride in 7 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7h** (58 mg, 34%) as a clear oil. IR (thin film) 2936, 1824 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.14 (td, $J=6.7$, 4.0 Hz, 1H), 3.18 (qd, $J=7.5$, 4.0 Hz, 1H), 1.78 (m, 2H), 1.32 (d, $J=7.5$ Hz, 3H), 1.24 (m, 4H), 0.89 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.5, 50.6, 33.7, 26.9, 22.2, 13.7, 12.4.

4-Pentyl-3-methyloxetan-2-one (7i). The reaction of hexanal (0.163 g, 1.62 mmol) with ketene acetal **8c** (0.509 g, 1.78 mmol) was performed using 0.479 g (2.12 mmol) zinc chloride in 10 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7i** (83 mg, 36%) as a clear oil. IR (thin film) 2933, 1825 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.15 (dt, $J=6.7$, 4.0 Hz, 1H), 3.20 (dq, $J=7.5$, 4.0 Hz, 1H), 1.79 (m, 2H), 1.36 (d, $J=7.5$ Hz, 3H), 1.24 (m, 6H), 0.89 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.5, 50.6, 34.0, 31.3, 24.6, 22.4, 13.8, 12.4.

4-Hexyl-3-methyloxetan-2-one (7j). The reaction of heptanal (0.183 g, 1.55 mmol) with ketene acetal **8c** (0.488 g, 1.71 mmol) was performed using 0.489 g (2.15 mmol) zinc chloride in 10 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7j** (94 mg, 39%) as a clear oil. IR (thin film) 2932, 1827 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.15 (td, $J=6.7$, 4.0 Hz, 1H), 3.20 (qd, $J=7.5$, 4.0 Hz, 1H), 1.78 (m, 2H), 1.36 (d, $J=7.5$ Hz, 3H), 1.27 (m, 8H), 0.87 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 76.4, 50.6, 34.1, 31.5, 28.8, 24.9, 22.4, 13.9, 12.4.

4-Octyl-3-methyloxetan-2-one (7k). The reaction of nonanal (0.169 g, 1.19 mmol) with ketene acetal **8c** (0.34 g, 1.21 mmol) was performed using 0.364 g (1.62 mmol) zinc chloride in 5 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7k** (110 mg, 35%) as a clear oil. IR (thin film) 2929, 1826 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.14 (td, $J=6.7$, 4.0 Hz, 1H), 3.19 (qd, $J=7.5$, 4.0 Hz, 1H), 1.76 (m, 2H), 1.35 (d, $J=7.5$ Hz, 3H), 1.24 (m, 12H), 0.85 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.4, 50.6, 34.0, 31.7, 29.3, 29.1, 29.0, 24.9, 22.5, 14.0, 12.4.

4-Decyl-3-methyloxetan-2-one (7l). The reaction of undecanal (0.188 g, 1.04 mmol) with ketene acetal **8c** (0.328 g, 1.14 mmol) was performed using 0.336 g (1.50 mmol) zinc chloride in 5 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7l** (83 mg, 38%) as a clear oil. IR (thin film) 2928, 1827 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.15 (td, $J=6.7$, 4.0 Hz, 1H), 3.19 (qd, $J=7.5$, 4.0 Hz, 1H), 1.79 (m, 2H), 1.34 (d, $J=7.5$ Hz, 3H), 1.24 (m, 16H), 0.86 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.5, 50.6, 34.1, 31.8, 29.5 (2C), 29.4, 29.2, 29.1, 24.9, 22.6, 14.0, 12.4.

4-Undecyl-3-methyloxetan-2-one (7m). The reaction of dodecanal (0.291 g, 1.64 mmol) with ketene acetal **8c** (0.508 g, 1.80 mmol, 1.1 equiv) was performed using 0.649 g (2.93 mmol, 1.6 equiv) zinc chloride in 7 mL dichloromethane according to the general procedure. Work-up followed by purification gave 157 mg of β-lactone **7m** (41%) as a clear oil. IR (thin film) 2929, 1826 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.16 (dt, $J=6.7$, 4.0 Hz, 1H), 3.20 (dq, $J=7.6$, 4.0 Hz, 1H), 1.77 (m, 2H), 1.37 (d, $J=7.6$ Hz, 3H), 1.25 (m, 18H), 0.87 (t, $J=6.7$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 79.6, 50.7, 34.1, 31.9, 29.6 (3C), 29.4, 29.3, 29.2, 24.9, 22.7, 14.1, 12.5.

4-Tridecyl-3-methyloxetan-2-one (7n). The reaction of tetradecanal (0.270 g, 1.29 mmol) with ketene acetal **8c** (0.406 g, 1.43 mmol) was performed using 0.396 g (1.74 mmol) zinc chloride in 8 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7n** (131 mg, 40%) as a clear oil. IR (thin film) 2923, 1819 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.14 (td, $J=6.7$, 4.0 Hz, 1H), 3.19 (qd, $J=7.5$, 4.0 Hz, 1H), 1.75 (m, 2H), 1.41 (d, $J=7.5$ Hz, 3H), 1.23 (m, 22H), 0.86 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.5, 50.6, 34.1, 31.9, 29.6, 29.4, 29.2, 24.9, 22.6, 14.0, 12.5.

4-Heptadecyl-3-methyloxetan-2-one (7o). The reaction of octadecanal (0.179 g, 0.679 mmol) with ketene acetal **8c** (0.217 g, 0.747 mmol) was performed using 0.231 g (1.02 mmol) zinc chloride in 5 mL dichloromethane according to the general procedure. Work-up followed by purification afforded **7o** (67 mg, 32%) as a clear oil. IR (thin film) 2924, 1818 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.16 (td, $J=6.7$, 4.0 Hz, 1H), 3.21 (qd, $J=7.5$, 4.0 Hz, 1H), 1.75 (m, 2H), 1.38 (d, $J=7.5$ Hz, 3H), 1.25 (m, 30H), 0.90 (t, $J=6.9$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.0, 79.6, 50.7, 34.1, 31.9, 31.6, 29.7, 29.4, 29.2, 24.9, 22.7, 14.1, 12.5.

Preparation of (1*R*,2*R*)-2-ethoxycarbonyl cyclohexan-1-ol (12). This β-hydroxy ester was prepared according to known procedures[30] with a few modifications. To a dried metal Parr reactor were added ethyl 2-cyclohexanonecarboxylate (10.98 g, 64.5 mmol), CH_2Cl_2 (50 mL), Ru-(*R*)-BINAP catalyst (4 mL; from 40 mg of (*R*)-BINAP) which was prepared by the method of Taber,[30a] and Dowex-50 resin (700 mg; washed with water, methanol, diethyl ether, and methanol then dried). Hydrogenation was carried out at 200 psi H_2 and 110 °C

for 24 h. After cooling, the reaction mixture was filtered through celite and concentrated in vacuo. Flash chromatography (EtOAc/Hexanes, 10/90) gave a mixture of *cis*-β-hydroxy ester (3.2 g, 29%) and *trans*-β-hydroxy ester **12** (7.4 g, 67%) as pale-yellow oils. Spectral data for the *trans*-β-hydroxy ester **12** matched that previously reported:[30c] 64% ee; R_f 0.19 (EtOAc/hexanes, 20/80); $[\alpha]_D^{22}$ −43.1° (*c* 0.58, ether); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.18 (q, J = 7.1 Hz, 2H), 3.77 (ddd, J = 4.4, 10.2, 10.2 Hz, 1H), 2.77 (bs, 1H), 2.24 (ddd, J = 3.7, 10.1, 12.0 Hz, 1H), 2.04 (m, 2H), 1.75 (m, 2H), 1.30 (m, 4H), 1.25 (t, J = 7.1 Hz, 3H).

Preparation of chiral diol ligand ((1*R*,2*R*)-2-[(diphenyl)-hydroxymethyl] cyclohexan-1-ol) 13. To a solution of *trans*-β-hydroxy ester **12** (1.0 g, 5.8 mmol) in anhydrous diethyl ether (60 mL) was added phenylmagnesium bromide (20.3 mL of 1.0 M in THF, 20.3 mmol) slowly and gradually at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was heated to reflux for 2 h. The mixture was cooled to ambient temperature and quenched with saturated aqueous NH_4Cl solution. The resulting precipitates were redissolved by the addition of aqueous 2 N HCl and the mixture was extracted with ether (3×50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (EtOAc/hexanes, 10/90) gave 1.17 g (72%) of diol as a pale-yellow solid. Optically pure diol (>99.9% ee) was obtained as white crystals after a single recrystallization from $CHCl_3$. Spectral data for this compound matched that previously reported:[29] >99.9% ee; R_f 0.37 (EtOAc/hexanes, 20/80); mp 176.5–177.5 °C (lit. 177–178 °C); $[\alpha]_D^{22}$ +8.9° (*c* 1.46, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.25–7.55 (m, 10H), 3.80 (bs, 1H), 3.32 (ddd, J = 4.6, 9.9, 9.9 Hz, 1H), 2.50 (ddd, J = 2.7, 9.8, 12.2 Hz, 1H), 0.85–1.95 (m, 8H).

General procedure for the asymmetric [2 + 2] cycloaddition reaction as described for *cis*-4-cyclohexyl-3-(trimethylsilyl)oxetan-2-one (10x). To a solution of chiral diol **13**, (60.4 mg, 0.214 mmol) in toluene (6 mL) was added Et_2AlCl (0.10 mL of 1.8 M in toluene, 0.178 mmol) at 0 °C slowly. After stirring for 5 h at 0 °C, the reaction mixture was cooled to −78 °C then cyclohexanecarboxaldehyde (108 mL, 0.892 mmol) was added. After stirring for 15 min at −78 °C, a solution of trimethylsilylketene **9a**[34] (153 mg, 1.34 mmol) in toluene (2 mL) was added and the mixture was further stirred for 30 min at −78 °C. The resulting reaction mixture was kept in a freezer (−26 °C) for 40 h. After addition of pH 7 buffer (1 mL) at −26 °C, the reaction mixture was warmed to room temperature and filtered through celite. The solution was diluted with ethyl acetate (10 mL), washed with brine, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography (EtOAc/hexanes, 5/95) gave 167 mg (83%) of α-silyl-β-lactone **10x** as a colorless oil. Spectral data for this compound matched that previously reported:[37] 84% ee; R_f 0.35 (EtOAc/hexanes, 10/90); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (dd, J = 5.9, 10.5 Hz, 1H), 3.29 (d, J = 5.9 Hz, 1H), 0.83–2.09 (m, 11H), 0.25 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 78.0, 45.9, 40.6, 28.9, 28.6, 25.9, 25.1, 25.0, −0.85; IR (thin film) 2935, 2855, 1799, 1453, 1252, 1127 cm^{-1}.

***cis*-4-Butyl-3-(trimethylsilyl)oxetan-2-one (10s).** This α-silyl-β-lactone was obtained from valeraldehyde (95 μL, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition. Flash chromatography (EtOAc/hexanes, 5/95) gave 153 mg (86%) of α-silyl-β-lactone **10s** as a colorless oil. Spectral data for this compound matched that previously reported:[26] 85% ee; R_f 0.36 (EtOAc/hexanes, 10/90); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (m, 1H), 3.33 (d, J = 6.0 Hz, 1H), 1.32–1.88 (m, 6H), 0.93 (t, J = 7.2 Hz, 3H), 0.22 (s, 9H); IR (thin film) 2958, 2933, 2875, 1800, 1492, 1260, 1123 cm^{-1}.

***cis*-4-Phenyl-3-(trimethylsilyl)oxetan-2-one (10z).** This α-silyl-β-lactone was obtained from benzaldehyde (91 μL, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition. Flash chromatography (EtOAc/hexanes, 5/95) gave 161 mg (82%) of α-silyl-β-lactone **10z** as a colorless oil. Spectral data for this compound matched that previously reported:[37] 28% ee; R_f 0.44 (EtOAc:hexanes, 10/90); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.46 (m, 5H), 5.71 (d, J = 6.3 Hz, 1H), 3.72 (d, J = 6.3 Hz, 1H), −0.13 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 136.8, 128.5, 128.3, 125.4, 72.6, 49.7, −1.86; IR (thin film) 3067, 3036, 2955, 2901, 1811, 1453, 1255, 1110 cm^{-1}.

***cis*-4-Benzyl-3-(trimethylsilyl)oxetan-2-one (10aa).** This α-silyl-β-lactone was obtained from phenylacetaldehyde (104 μL, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition. Flash chromatography (EtOAc/hexanes, 5/95) gave 94 mg (45%) of α-silyl-β-lactone **10aa** as a colorless oil: 75% ee; R_f 0.33 (EtOAc/hexanes, 10/90); $[\alpha]_D^{22}$ +96.1° (*c* 1.02, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24–7.36 (m, 5H), 4.83 (ddd, J = 3.3, 6.3, 10.2 Hz, 1H), 3.44 (d, J = 6.6 Hz, 1H), 3.15 (dd, J = 10.2, 14.7 Hz, 1H), 3.05

(dd, $J = 3.3$, 14.7 Hz, 1H), 0.29 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 136.7, 128.7, 128.6, 127.0, 74.0, 46.5, 39.7, −1.1; IR (KBr) 1785 cm^{-1}; HRMS calcd for $C_{13}H_{18}O_2Si$ [M + Na]: 257.0974. Found: 257.0984.

(*S*)-4-Cyclohexyloxetan-2-one (7x). The α-silyl-β-lactone **10x** (150 mg, 0.66 mmol) was desilylated according to the general procedure in CH_3CN (4 mL) using $KF{\cdot}2H_2O$ (125 mg, 1.33 mmol) at ambient temperature. Flash chromatography (5→10% ethyl acetate in hexanes) afforded 92 mg (91%) of β-lactone **7x** as a colorless oil, which exhibited spectral data which matched that previously reported:[23c] R_f 0.22 (EtOAc/hexanes, 10/90); 84% ee; $[\alpha]_D^{22}$ +15.9° (*c* 1.67, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.20 (ddd, $J = 4.2$, 5.7, 8.4 Hz, 1H), 3.43 (dd, $J = 5.7$, 16.2 Hz, 1H), 3.12 (dd, $J = 4.2$, 16.2 Hz, 1H), 1.55–2.00 (m, 5H), 1.0–1.37 (m, 6H).

(*R*)-4-Butyloxetan-2-one (7s). This β-lactone was obtained from α-silyl-β-lactone **10s** (100 mg, 0.50 mmol) and $KF{\cdot}2H_2O$ (94 mg, 1.0 mmol) according to the general procedure for desilylation. Flash chromatography (EtOAc/hexanes, 10/90) gave 58 mg (91%) of β-lactone **7s** as a colorless oil: 85% ee; R_f 0.23 (EtOAc/hexanes, 10/90); $[\alpha]_D^{22}$ +24.7 (*c* 1.75, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.51 (m, 1H), 3.51 (dd, $J = 6.0$, 16.3 Hz, 1H), 3.06 (dd, $J = 4.2$, 16.3 Hz, 1H), 1.70–1.90 (m, 2H), 1.36–1.50 (m, 4H), 0.93 (t, $J = 7.2$ Hz, 3H); IR (thin film) 2957, 2930, 2860, 1828, 1125 cm^{-1}.

(*R*)-4-Benzyloxetan-2-one (7aa). This β-lactone was obtained from α-silyl-β-lactone **10aa** (85 mg, 0.36 mmol) and $KF{\cdot}2H_2O$ (68 mg, 0.73 mmol) according to the general procedure for desilylation. Flash chromatography (10/90, EtOAc/hexanes) gave 53 mg (91%) of β-lactone **7aa** as a colorless oil. Spectral data for this compound matched that previously reported:[32] R_f 0.44 (1/3, EtOAc/hexanes); $[\alpha]_D^{22}$ +6.5 (*c* 4.77, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.21–7.35 (m, 5H), 4.74 (dddd, $J = 4.2$, 5.7, 6.3, 6.3 Hz, 1H), 3.48 (dd, $J = 5.7$, 16.5 Hz, 1H), 3.22 (dd, $J = 6.3$, 14.4 Hz, 1H), 3.15 (dd, $J = 4.2$, 16.5 Hz, 1H), 3.06 (dd, $J = 6.3$, 14.4 Hz, 1H).

4-(2-Phenylethyl)oxetan-2-one (7e). This β-lactone was obtained from hydrocinnamalaldehyde (117 μL, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition followed by desilylation according to the general procedure. Flash chromatography (EtOAc/hexanes, 10/90) gave 94 mg (60% yield for two sequences) of β-lactone **7e** as a colorless oil. Spectral data for this compound matched that previously reported:[23c] 36% ee; R_f 0.27 (EtOAc/hexanes, 1/5); ^1H NMR (300 MHz, $CDCl_3$) δ 7.20–7.36 (m, 5H), 4.45–4.55 (m, 1H), 3.48 (dd, $J = 5.7$, 16.5 Hz, 1H), 3.03 (dd, $J = 4.5$, 16.5 Hz, 1H), 2.65–2.88 (m, 2H), 2.01–2.25 (m, 2H).

4-(5-*tert*-Butyldimethylsiloxypentyl)oxetan-2-one (7bb). This β-lactone was obtained from (6-*tert*-butyldimethylsiloxy)hexanal (205 mg, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition followed by desilylation according to the general procedure. Flash chromatography (EtOAc/hexanes, 10/90) gave 113 mg (55% yield for two sequences) of β-lactone **7bb** as a colorless oil: 46% ee; R_f 0.47 (EtOAc/hexanes, 1/5); ^1H NMR (300 MHz, $CDCl_3$) δ 4.48–4.55 (m, 1H), 3.61 (t, $J = 6.6$ Hz, 2H), 3.52 (dd, $J = 6.0$, 16.5 Hz, 1H), 3.07 (dd, $J = 4.2$, 16.5 Hz, 1H), 1.70–1.92 (m, 2H), 1.35–1.60 (m, 6H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 71.2, 62.8, 42.8, 34.6, 32.4, 25.9, 25.4, 24.6, 18.3, −5.4; IR (thin film) 1829 cm^{-1}; HRMS calcd for $C_{14}H_{28}O_3$ [M + H]: 273.1886. Found: 273.1890.

4-(8-Nonenyl)oxetan-2-one (7cc). This β-lactone was obtained from 9-decenal (138 mg, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition followed by desilylation according to the general procedure. Flash chromatography (EtOAc/hexanes, 10/90) gave 124 mg (71% yield for two sequences) of β-lactone **7cc** as a colorless oil: 22% ee; R_f 0.32 (EtOAc/hexanes, 10/90); ^1H NMR (300 MHz, $CDCl_3$) δ 5.81 (ddt, $J = 6.6$, 10.5, 17.1 Hz, 1H), 4.91–5.03 (m, 2H), 4.47–4.55 (m, 1H), 3.52 (dd, $J = 5.7$, 16.2 Hz, 1H), 3.06 (dd, $J = 4.5$, 16.2 Hz, 1H), 2.00–2.08 (m, 2H), 1.68–1.92 (m, 2H), 1.30–1.50 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 138.8, 114.1, 71.2, 42.7, 34.5, 33.6, 29.1, 28.9, 28.7, 28.6, 24.7; IR (thin film) 1829 cm^{-1}; HRMS calcd for $C_{12}H_{20}O_2$: 219.1361. Found: 219.1359.

4-(1-Ethylpropyl)oxetan-2-one (7dd). This β-lactone was obtained from 2-ethylbutyraldehyde (110 μL, 0.892 mmol) and trimethylsilylketene **9a** (153 mg, 1.34 mmol) using Lewis acid **14** prepared from Et_2AlCl (0.1 mL of 1.8 M in toluene, 0.178 mmol) and chiral diol **13** (60.4 mg, 0.214 mmol) according to the general procedure for asymmetric [2 + 2] cycloaddition followed by desilylation using $KF{\cdot}2H_2O$ according to the general procedure. Flash chromatography (EtOAc/hexanes, 10/90) gave 58 mg (46% yield for two sequences) of β-lactone **7dd** as a colorless oil: 56% ee; R_f 0.46 (EtOAc/hexanes, 20/80);

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.36 (ddd, J = 4.6, 5.6, 8.4 Hz, 1H), 3.47 (dd, J = 6.0, 16.2 Hz, 1H), 3.11 (dd, J = 4.4, 16.2 Hz, 1H), 1.20–1.70 (m, 5H), 0.91 (t, J = 7.2 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 73.7, 44.5, 41.5, 21.4, 20.8, 10.9, 10.2; IR (thin film) 2971, 2936, 2871, 1835 cm^{-1}; HRMS calcd for $C_8H_{14}O_2$ [M$^+$]: 142.0994. Found: 142.0982.

Ethyl-(*S*)-3-hydroxy-3-cyclohexylpropanoate. This β-hydroxy ester was prepared by the reaction of β-lactone **7x** (26 mg, 0.17 mmol) with NaOEt (14 mg, 0.20 mmol, 1.2 equiv) in EtOH-THF at ambient temperature.[38] General work-up followed by purification by flash chromatography (EtOAc/hexanes, 20/80) gave 29 mg (86%) of β-hydroxy ethyl ester. Spectral data for this compound matched that previously reported:[39] 63% ee; R_f 0.30 (EtOAc/hexanes, 1/6); $[\alpha]_D^{22}$ −15.2 (*c* 0.66, $CHCl_3$); lit. $[\alpha]_D^{22}$ +27.8 (*c* 0.66, $CHCl_3$) for *R*-enantiomer;[38] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.18 (q, J = 7.2 Hz, 2H), 3.74–3.81 (m, 1H), 2.88 (d, J = 4.2 Hz, 1H), 2.52 (dd, J = 2.7, 16.5 Hz, 1H), 2.41 (dd, J = 9.3, 16.5 Hz, 1H), 1.60–1.90 (m, 5H), 1.28 (t, J = 7.2 Hz, 3H), 0.98–1.42 (m, 6H).

Methyl-(*R*)-3-hydroxyheptanoate. This β-hydroxy ester was prepared by the reaction of β-lactone **7a** (45 mg, 0.35 mmol, 85% ee) with NaOMe (22 mg, 0.42 mmol, 1.2 equiv) in MeOH-THF at ambient temperature.[38] General work-up followed by purification by flash chromatography (EtOAc/hexanes, 20/80) gave 47 mg (85%) of β-hydroxy methyl ester. Spectral data for this compound matched that previously reported:[30b] 85% ee; R_f 0.25 (EtOAc/Hexanes, 20/80); $[\alpha]_D^{22}$ −1.67 (*c* 2.58, EtOH); lit. $[\alpha]_D^{22}$ +1.85 (*c* 2.58, EtOH) for *S*-enantiomer; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.01 (m, 1H), 3.71 (s, 3H), 2.87 (bs, 1H), 2.30–2.60 (m, 2H), 1.20–1.60 (m, 6H), 0.91 (t, J = 7.3 Hz, 3H); IR (thin film) 3455, 2927, 1730 cm^{-1}.

General biochemical procedures. Biochemical reagents were purchased from Sigma and chemical reagents from Aldrich. HMG-CoA synthase assays, protein determinations and other biochemical procedures were carried out as described previously.[20] Cloning of the HMG-CoA synthase gene of *S. cerevisiae* into *E. coli* to give strain Y-HMGS was performed according to standard, established procedures.[40]

Cell growth and lysis. The *E. coli* strain Y-HMGS was grown overnight on LB-amp plates at 37 °C. A single colony was then used to inoculate LB-amp liquid medium (50 mL) which was grown overnight at 37 °C with shaking (250 rpm). LB-amp liquid culture (1 L) was then inoculated with the overnight culture (7 mL) and grown to OD_{600} = 0.6 (37 °C, 250 rpm). Solid IPTG (95 mg) was then added and incubation was continued for a further 2.5 h. The cells were collected by centrifugation (8000 *g*, 5 min), washed with 0.85% NaCl, and resuspended in lysis buffer (50 mM HEPES, pH 8.0, 50 mM NaCl, 10 mM EDTA, 15 mL). The cells were twice passed through the French Press (20,000 psi) and cellular debris was removed by centrifugation (8000 *g*, 5 min).

Ammonium sulphate precipitation. To cell-free extract (20 mL), cooled in an ice bath, was gradually added finely crushed ammonium sulphate with stirring to 25% saturation. Stirring was continued for 20 min at 40 °C. The precipitate was removed by centrifugation (10,000 *g*, 10 min). Additional ammonium sulphate was then added to 45% saturation, and the precipitate was collected by centrifugation as above. The pellet was resuspended in 25 mM HEPES (pH 8.2), and dialyzed for 3 h two times against 1L of the same buffer.

Q-Sepharose chromatography. The dialyzed solution was applied onto a 50 mL Q-Sepharose FPLC column, pre-equilibrated with loading buffer (25 mM HEPES, pH 8.2). After washing with one column volume of loading buffer (10 min), the column was eluted with a continuous salt gradient (0–0.5 M NaCl, 5 mL/min, over 60 min). Fractions (5 mL) were collected and monitored for HMG-CoA synthase activity. Maximum enzyme activity was found in fractions 32–35, which were pooled and concentrated to 0.5 mL in a Gelman concentrating centrifuge tube (MW cut-off = 30 kD, 10000 *g*, 60 min).

S-200 chromatography. The resulting solution was loaded onto a S-200 column (600 mL) which had been pre-equilibrated with loading buffer (25 mM HEPES, pH 8.2, 50 mM NaCl). Elution (same buffer, 0.3 mL/min) gave fractions (6 mL). Active fractions (30–34) were pooled and concentrated as above.

Acknowledgements

Financial support for this work from the Natural Sciences and Engineering Research Council of Canada through grants to both P.H. and G.D.W., the Medical Research Council of Canada (GDW) and the National Science Foundation (Grant CHE-9624532 to D.R.) is gratefully acknowledged. We thank Dr. Lloyd Sumner and Dr. Barbara Wolf of the Texas A&M Center for Characterization for mass spectral analyses obtained on instruments acquired by generous funding from the NSF (CHE-8705697) and the TAMU Board of Regents Research Program.

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