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Identification of Novel Mammalian Squalene Synthase Inhibitors Using a Three-Dimensional Pharmacophore

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Abstract—Squalene synthase (E.C. 2.5.1.21) catalyses the reductive dimerisation of farnesyl diphosphate in a [1–4] head to head fashion to form squalene, and is the first committed step in cholesterol biosynthesis. Specific inhibitors of squalene synthase would inhibit cholesterol formation and allow production of other important compounds derived from the cholesterol biosynthetic pathway, namely the ubiquinones (co-enzyme Q_{10}), dolichol, and would also allow the isoprenylation process of ras by farnesyl-protein transferase. The construction of a hypothetical squalene synthase three-dimensional pharmacophore is presented. It serves as a template for the identification of several new potential classes of inhibitors. The synthesis, anti-microbial and mammalian pig liver squalene synthase activities of analogues based on the bicyclo[3.2.0]heptane and bicyclo[3.3.0]octane ring systems are reported. Analogues of the latter system are pro-drug type inhibitors and exhibit promising biological activity. © 2002 Published by Elsevier Science Ltd.

Introduction

Over the past 10 years inhibition of squalene synthase (E.C. 2.5.1.21) (SQS) has been the focus of many academic and industrial groups in the search for new cholesterol-lowering agents.[1] Efforts to find SQS inhibitors have been prompted as an alternative to the statin class of HMG–CoA reductase inhibitors as cholesterol-lowering agents. The latter class of inhibitors have the potential to cause depletion of other essential compounds, such as ubiquinones,[1] dolichols[2] and isoprenylated proteins[3] (ras proteins). Inhibition of SQS after farnesyl diphosphate (FPP) is considered advantageous, as it is the first step committed solely to the synthesis of cholesterol.

SQS is a 47 kDa membrane-associated enzyme which catalyses the remarkable reductive dimerisation of two molecules FPP in a [1–4] head-to-head fashion, via presqualene diphosphate (PSQPP) to form squalene (S) (Scheme 1).[1]

The SQS mediated reaction proceeds via two distinct steps. In the first step, two molecules of FPP react to form the stable cyclopropylcarbinyl diphosphate intermediate, PSQPP. Mutagenesis data identifying the residues that affect substrate binding and activity suggest that this step occurs at the end of a large central channel found in the middle of one face of the SQS domain.[5] The stable reaction intermediate (PSQPP) passes deeper into this central channel, where the second reaction step occurs and is sequestered from water. Here, PSQPP undergoes rearrangement, through a secondary cyclobutylcarbinyl diphosphate (CYPP) and then tertiary cyclopropylcarbinyl diphosphate (TPSQPP) and reduction with NADPH to form S.

An impressive array of structurally diverse inhibitors of SQS has been reported. Currently one of the most

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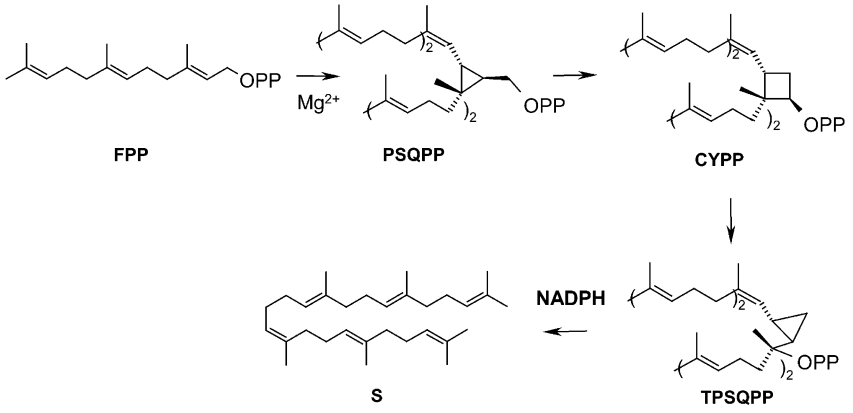


Scheme 1. The biosynthetic reaction mediated by SQS within cholesterol biosynthesis.

potent SQS inhibitors is Squalestatin S1[6] also known as Zaragozic acid A[7] (Fig. 1). S1 and its analogues exhibit fascinating biological activity and much work has focused on their total synthesis.[8] Unfortunately S1 is toxic in mammalian subjects, and work with the squalestatin class has been discontinued by Glaxo-Wellcome.[9]

Recently Pandit et al.[10] reported both the crystallisation of human SQS and co-crystal structure of SQS with various inhibitors (CP-424677, CP-458003 and CP-320473; Fig. 1) enzyme bound.[11–13] This remarkable goal represents 30 years of extensive efforts to obtain crystals of sufficient quality in order to allow X-ray determination. However, at the onset of this work no three-dimensional structure for SQS was available and hence we embarked on the construction of a pharmacophore that represents the inhibitors and intermediates (PSQPP, CYPP and TPSQPP) of SQS.

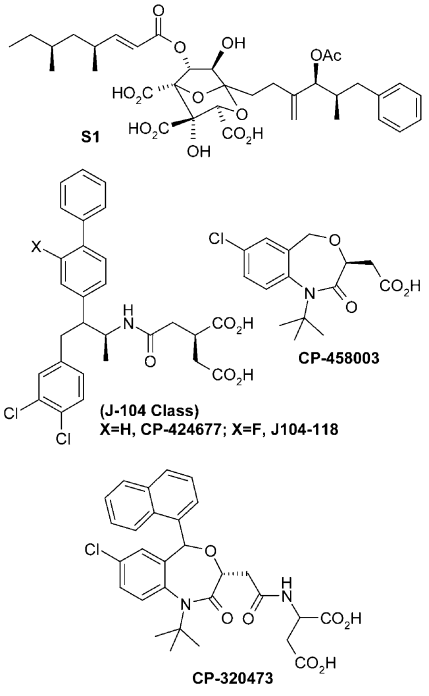


Figure 1. Squalestatin S1 (Zaragozic acid A).

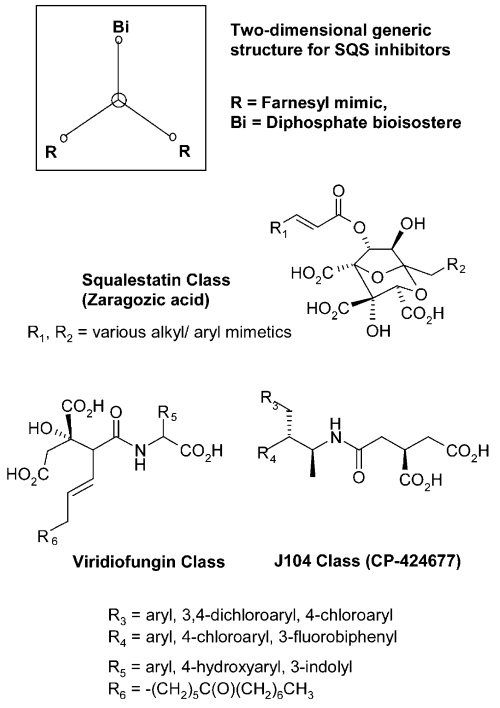
The X-ray structure shows that SQS is folded as a single domain and has a large channel in the centre of one face. The active sites of the two half-reactions are located in the central channel. This is lined on both sides by conserved aspartate and arginine residues. One end of this central channel is exposed to solvent (water), while the other end leads to a completely enclosed pocket surrounded by hydrophobic residues, which presumably accepts the hydrophobic farnesyl chain or mimetics thereof.

'The flap'—The central channel contains an important structural feature where one side is covered by a 'flap', residues 50 and 54, which connects the A and B helices. The side chain of Phe-54 forms one wall under the flap and is largely a hydrophobic cavity.[12] The X-ray co-crystal structures of SQS-inhibitor complexes show that bulky hydrophobic groups, for example naphthyl and biphenyl moieties, fill a cavity under this flap. Unfortunately the residues forming the flap and the side chain of Tyr-73 show large conformational differences in each enzyme–inhibitor complex.[12] Hydrophobic moieties are thought to stabilise a very flexible region. This property of the enzyme active site makes computational modelling with new structures difficult. Indeed, the inherent problems of hydrophobic binding due to rotation of the side chains Phe-54 and Tyr-73 and backbone rotations in the 'flap' residues illustrates the importance of rigidity when designing novel inhibitors based on the structures of the enzyme–inhibitor complexes. Moreover, it ought to be possible to take advantage of the information gained from the X-ray studies with the enzyme–inhibitors complexes, that is, by taking advantage of the 'flap' in order to design novel rigid bicyclic systems that include aromatic isosteres. Future computational molecular studies will address this issue.

Results—Pharmacophore for SQS

A pharmacophore is defined as the three-dimensional arrangement of the groups essential for recognition and activation.[14] In drug design, it also refers to a set of features that are common to both substrate/intermediates and inhibitors of a given receptor/enzyme.[15] Various methods exist for construction of the pharmacophore, such as the distance-geometry approach by

computational molecular modelling.[14,16] Using this approach, it is possible to gain information about the conformational properties of both substrate/intermediates and known inhibitors of SQS. The inherent conformational flexibility of the farnesyl chains increases the difficulty associated with modelling the substrate/intermediates of SQS, so one must focus attention on the known inhibitors of SQS to give a training set for the pharmacophore.



Scheme 2. Two-dimensional generic structure for SQS inhibitors.

SQS inhibitors share common structural features (Scheme 2): the presence of a hydrophilic di- or tri-acid core, which share electronic and steric similarities with the diphosphate head found in FPP and SQS intermediates; and hydrophobic-lipophilic groups which mimic the farnesyl moiety. A simple two-dimensional generic structure that represents the SQS inhibitors is presented in Scheme 2.

The initial generation of the pharmacophoric map using S1[6,7] and viridiofungin A[17] proved quite cumbersome, mainly due to the dissimilarity and flexibility found in the farnesyl mimetics. Other inhibitors chosen were the J-104[18] and 3-biarylquinuclidine[19] classes, which proved more useful. It was also possible to use modified S1 structures (MS1-A, MS1-B and MS1-C; Fig. 2).[20] Clearly, the chosen compounds are structurally distinct and were all selected as candidates in the training set for pharmacophore generation. As the phenyl and biphenyl moieties exhibit rigidity, molecular superposition of the unsaturated systems (π) and two oxygens (directly attached to carbon 'in bold' for the CP-, J-104 and S1 derivatives, and phosphorus for the biarylquinuclidines) was possible.[21]

In this model, the π-systems are described by a vector orthogonal to the plane of the π-system through its centre, and then measured with respect to the two oxygen atoms, that is forming several co-ordinates (Fig. 3, I). Four π-systems (π_1, π_2, π_3 and π_4; Fig. 2) were used in the model and the position of these with respect to the diphosphate head and di-acid bioisostere were determined.

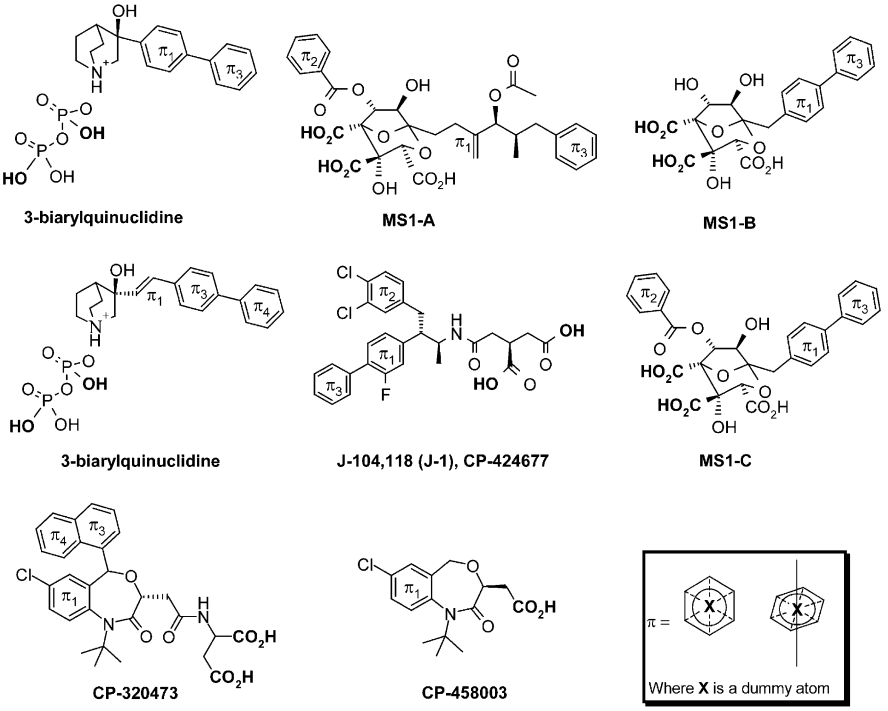


Figure 2. Structures used in the construction of the pharmacophore.

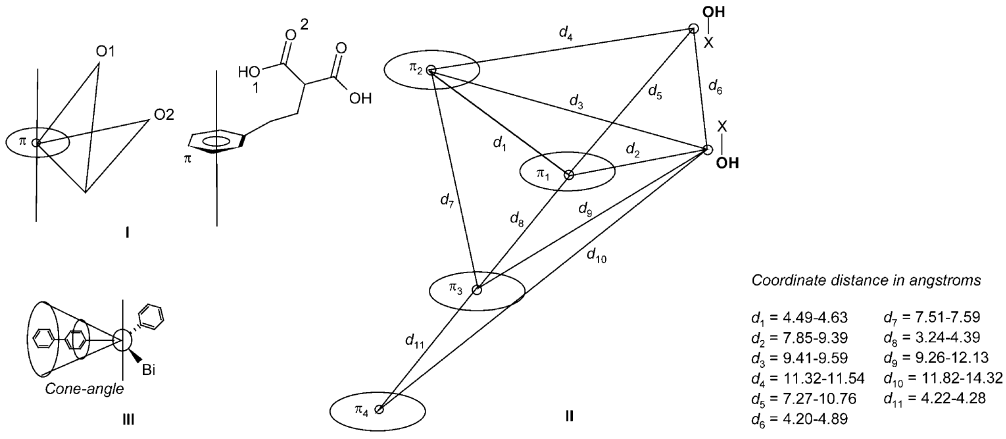


Figure 3. Proposed hypothetical pharmacophore for SQS.

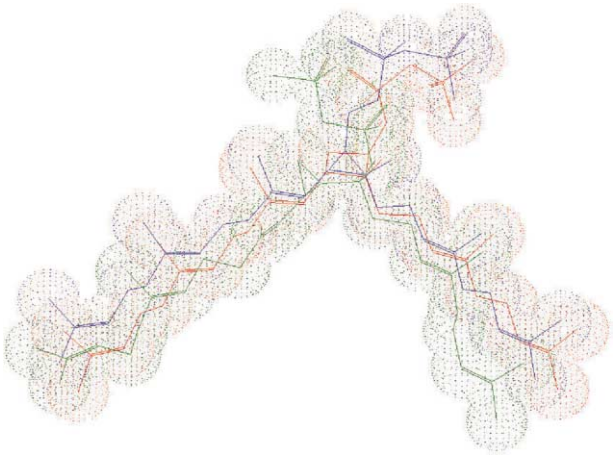


Figure 4. Overlays of the SQS intermediate structures.

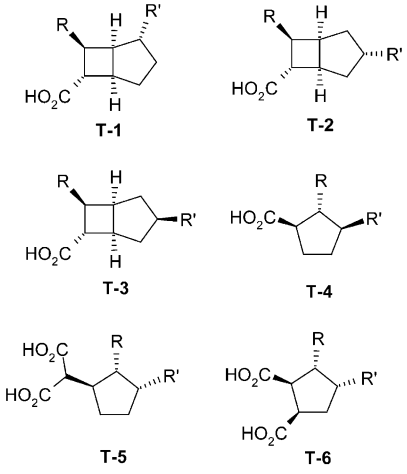


Figure 5. The proposed target structures.

This enabled the construction of a three-dimensional pharmacophore (Fig. 3, II). The molecular overlay of the SQS intermediates, PSQPP, CYPP and TPSQPP, simulated and overlaid using a best-fit parameter, fitted well into this model (Fig. 4).

The co-crystallised structures of CP-424677 (J-104), CP-458003 and CP-320473 inhibitors from human SQS,[12] also fit well into the pharmacophore.

Identification of new SQS inhibitors candidates

Rigid ring systems were introduced to increase the accuracy of the simulated structures.[14] The use of flexible structures might result in the introduction of error through unknown conformational properties.[14,21] Several cyclic and bicyclic structures were investigated, as the substituent orientation of these structures gives a small 'cone-angle' window (Fig. 3, III) and are considered more ideal pharmacophore candidates (Fig. 5).

The substituents of the target structures (T-1–T-6) were systematically varied so as to attain positional mimetics of the farnesyl chains and diphosphate head of the SQS intermediates. The best substituents were found to be phenyl and biphenyl moieties, which exhibit the highest rigidity with respect to the π-system.

The three-dimensional structures were minimized at the MM level until the lowest local energy minimum[22] (LEM) was attained. The pharmacophore π–π distances and limits are shown in Table 1. The target structures, T-1 through to T-6, demonstrate the desired orientation of the substituents. For the bicyclo[3.2.0]heptane series (T-1, T-2 and T-3) the bicyclic ring system exhibits a different conformation when R is changed from C2 to C3 (Fig. 6).[23] The *endo* envelope is preferred for T-1, whereas for T-2 the *exo* envelope is predominant. This difference demonstrated that a change in the orientation

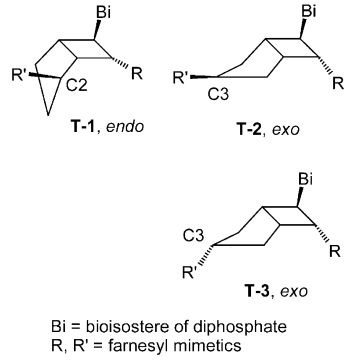


Figure 6. *Endo* and *exo* envelope conformations for T-1 and T-2.

Table 1. Distance coordinates for the proposed SQS pharmacophore

Distance co-ordinate[a]	T-1	T-2	T-3	T-4	T-5	T-6	Limits
d_1	6.41 (+1.78)	7.48 (+2.85)	6.79 (+2.16)	4.06 (−0.43)	3.83 (−0.66)	4.24 (−0.25)	4.49–4.63
d_2	4.42 (−3.43)	5.46 (−2.39)	6.29 (−1.56)	5.96 (−1.89)	5.38 (−2.47)	5.26 (−2.59)	7.85–9.39
d_3	8.67 (−0.74)	**9.41**	**9.52**	7.5 (−1.91)	6.83 (−2.58)	7.31 (−2.10)	9.41–9.59
d_4	9.40 (−1.92)	7.19 (−4.13)	8.87 (−2.45)	7.73 (−3.59)	7.34 (−3.98)	8.12 (3.20)	11.32–11.54
d_5	6.32 (−0.95)	**7.66**	**7.63**	6.23 (−1.04)	**7.27**	**7.42**	7.27–10.76
d_6	2.85 (−1.35)	**4.68**	2.75 (−1.45)	**4.20**	3.84 (−0.36)	3.35 (−0.85)	4.20–4.89
d_7	9.02 (+1.43)	10.94 (+3.35)	9.64 (+2.05)	7.12 (−0.39)	6.77 (−0.74)	7.48 (−0.03)	7.51–7.59
d_8	**4.38**	**4.27**	**4.31**	**4.37**	**4.26**	4.54 (+0.15)	3.24–4.39
d_9	7.98 (−1.28)	8.78 (−0.48)	**9.91**	9.22 (−0.04)	9.02 (−0.24)	**9.51**	9.26–12.13

The co-ordinate distances in bold lie between the calculated limits.
[a]Number in parentheses correspond to the difference in the calculated limits.

of R might also yield a potential structure and hence led to the simulation of T-3.

The cyclopentane structures (T-4, T-5 and T-6) exhibit good potential in this pharmacophore. Abbott Laboratories[24] recently published similar analogues to T-6, as potent in vitro inhibitors of SQS. This lends credibility to both the simulated pharmacophore and the other structures simulated from it.

The synthesis of analogues similar to the proposed targets, T-2, T-3 and T-4, have been the focus of our recent attention[25] and the preliminary biological activities, against a range of yeasts and bacteria and the enzyme SQS from mammalian pig liver, of several T-2, T-3 and T-4 analogues, are reported herein.

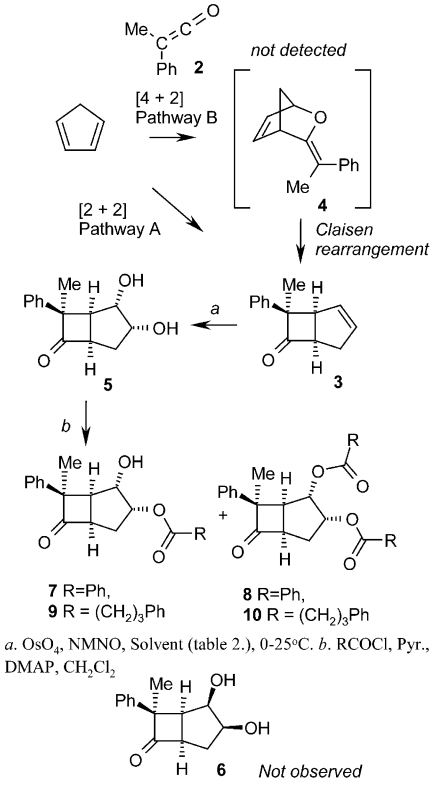
Synthesis of T-2 analogues

The bicyclo[3.2.0]heptane ring **3** was synthesised in 71% yield by [π2s + π2a] cycloaddition of phenyl(methyl)ketene **2** (generated in situ via dehydrochlorination of 2-phenyl-propanoyl chloride **1** with triethylamine) to [1,3]-cyclopentadiene (pathway A, Scheme 3).[26]

Recently, the mechanism of this reaction has received renewed interest.[27] The initial product is suggested to be a [π4s + π2s] cycloaddition product **4** which undergoes a [3,3]-sigmatropic rearrangement to the thermodynamically more stable expected [2 + 2] product **3** (pathway B, Scheme 3). However, we failed to detect this intermediate under various conditions (several reaction solvents and low temperatures).

The concave bicyclo[3.2.0]heptane ring enables regio and stereoselective functionalisation of the alkenic bond.[28,29] Using the method of Rahman,[29b] the *exo* diol **5** was synthesised by catalytic osmylation (OsO_4) of **3** in the presence of *N*-methyl-morpholine-*N*-oxide (NMNO) in a THF–^{t}BuOH–H_2O mixture (Table 2).

The *endo* diol **6** was not observed in this reaction, presumably due to the steric constraints imposed by the bicyclic ring, which blocks OsO_4 attack from the *endo* face.[29] Alteration of the solvent system led to an increase in the yield of **5** to 82%. C3-esterified derivatives were synthesised by simple treatment of **5** with the appropriate acyl chloride in the presence of pyridine and DMAP in CH_2Cl_2 (Table 3).



Scheme 3. Synthetic route to the T-2 analogues.

Table 2. Yields of the *exo* diol **5**

Solvent (ratio)[a]	Yield%
THF–^{t}BuOH–H_2O (8:5:3)	63
MeCN–THF–^{t}BuOH–H_2O (4:2:1:1)	82
MeCN–^{t}BuOH–H_2O (4:1:1)	74
$(CH_3)_2CO$–THF–^{t}BuOH–H_2O (4:2:1:1)	33

[a]All reactions were run on the same scale with 2 mol% of OsO_4 and 1 equiv NMNO in the specified solvent system.

Reaction of **5** with 1 equiv of the benzoyl chloride gave the C3 (**7**) mono-acylated and C2–C3 (**8**) di-acylated products, separable by flash chromatography in 46 and 23% yields, respectively. The *exo* configuration of the C2 substituent in both **7** and **8** were confirmed by fine ^1H coupling ($J_{1\text{-}2} = 1.8$ Hz) observed between the trans-coaxial H-1 and H-2 protons. The *cis* arrangement of both the C2 and C3 substituents were confirmed by small H-2 and H-3 coupling for **8** ($J_{2\text{-}3} = 4.0$ Hz). The

Table 3. Yields of two T-2 derivatives

Compd[a]	R	Yield[b] (%)
7 (**8**)	Ph	46 (23)
9 (**10**)	$Ph(CH_2)_3$	13 (44)

[a]Number in parentheses corresponds to the di-substituted derivative.
[b]Yields in parentheses refer to the C2–C3 di-substituted product.

selective C3 mono-acylation of **5** is particularly noteworthy, as both C2 and C3 alcohols are seemingly equally exposed. Molecular modelling[30] of **5** at the PM3 level of theory shows that the *exo* envelope is the lowest energy conformation in solution (Fig. 7).

It is clear that the C2 alcohol is slightly hindered by the cyclobutane ring. Reaction of **5** with 4-phenyl-butanoyl chloride under identical conditions gave predominantly the C2-C3 di-acylated product **10** in 44% and the C3 mono-acylated product **9** in 13% yield. The favorable formation of the di-acylated products is intriguing, although the origin of this effect may stem from differing conformations of diol **5** and the mono-acylated products **7** and **9**, where addition of the second acyl group is facilitated by introduction of the first group.

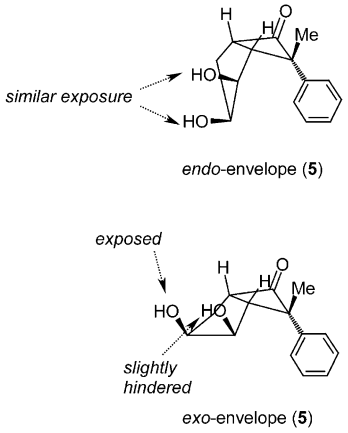


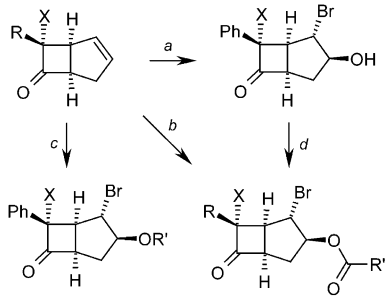
Figure 7. *Exo*-face selective osmylation products.

Synthesis of T-3 analogues

Previous work by Roberts et al. has shown that 7,7-disubstitutedbicyclo[3.2.0]alkenones undergo electrophilic bromination in a regioselective manner.[28,29a] It was rationalised that the introduction of farnesyl mimetics could be achieved using the electrophilic bromination reaction. The synthesis of T-3 analogues was accomplished with several 7,7-disubstituted bicyclo[3.2.0]hept-2-en-6-ones (**3**, **11**, **12** and **13**), using *N*-bromoacetamide (NBA) and the appropriate nucleophile (H_2O, ROH or RCO_2H) (Scheme 4). We found that *N*-bromosuccinimide (NBS) was a satisfactory source of electrophilic bromine when H_2O was used as the nucleophile. However, employment of other nucleophiles (ROH and RCO_2H) with NBS led to poor isolated yields of the required products.

The corresponding products and yields are given in Table 4. The electrophilic bromination of chlorobicycloheptenone **11** yielded two isomers **21** (9%) and **22** (37%). The minor isomer **21** was produced by addition of bromine from the more hindered face of the alkene, followed by nucleophilic attack from the *exo* face of the bicyclic ring (Scheme 5).[28]

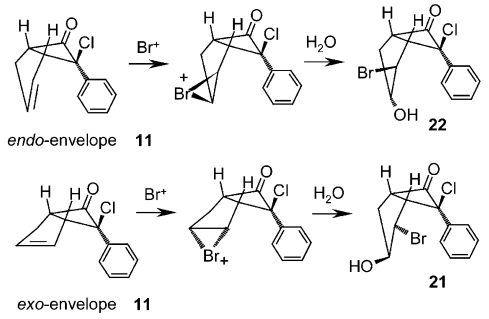
When other nucleophiles (MeOH, $MeCO_2H$ and $PhCO_2H$) were employed in the reaction of **11** with NBA, only the major *exo*-bromo stereoisomers (**23**, **24** and **25**) were observed.



Scheme 4. Synthesis of T-3 analogues. (a) NBA, $(CH_3)_2CO$–H_2O (3:1); (b) NBA, CH_2Cl_2, RCO_2H; (c) NBA, CH_2Cl_2, ROH; (d) RCOCl, Et_3N, DMAP.

Table 4. Synthesis of T-3 derivatives

Alkene	Nucleophile	Product	Compd	Yield %
R = Ph, X = Cl, **11**	H_2O	R = Ph, X = Cl, R_1 = H (C2 *endo*-Br)	**21**	9
		R = Ph, X = Cl, R_1 = H (C2 *exo*-Br)	**22**	37
R = Ph, X = Cl, **11**	MeOH	R = Ph, X = Cl, R_1 = Me	**23**	88
R = Ph, X = Cl, **11**	$MeCO_2H$	R = Ph, X = Cl, R_1 = COMe	**24**	77
R = Ph, X = Cl, **11**	$PhCO_2H$	R = Ph, X = Cl, R_1 = COPh	**25**	47
R = Ph, X = Cl, **11**	*p*-C_6H_5-$C_6H_4CO_2H$	R = Ph, X = Cl, R_1 = CO-C_6H_4-*p*-C_6H_5	**26**	0
R = Ph, X = H, **12**	$PhCO_2H$	R = Ph, X = Br, R_1 = COPh	**27**	62
R = Ph, X = Me, **3**	H_2O	R = Ph, X = Me, R_1 = H (C2 *exo*-Br only)	**14**	56
R = Ph, X = Me, **3**	MeOH	R = Ph, X = Me, R_1 = Me	**15**	82
R = Ph, X = Me, **3**	$MeCO_2H$	R = Ph, X = Me, R_1 = COMe	**16**	80
R = Ph, X = Me, **3**	$PhCO_2H$	R = Ph, X = Me, R_1 = COPh	**17**	63
R = Ph, X = Me, **3**	*p*-NO_2–$PhCO_2H$	R = Ph, X = Me, R_1 = CO(C_6H_4-*p*-NO_2)	**18**	0
R = Ph, X = Me, **3**	*p*-MeO–$PhCO_2H$	R = Ph, X = Me, R_1 = CO(C_6H_4-*p*-OMe)	**19**	0
R = Ph, X = Me, **3**	*p*-C_6H_5–$C_6H_4CO_2H$	R = Ph, X = Me, R_1 = CO-C_6H_4-*p*-C_6H_5	**20**	0
R = *p*-C_6H_5-C_6H_4, X = Me, **13**	$PhCO_2H$	R = *p*-C_6H_5-C_6H_4, X = Me, R_1 = COPh	**30**	58



Scheme 5. Major and minor stereoisomers formed from the *endo* and *exo* envelope conformations of the bicyclo[3.2.0]heptane ring.

The successful incorporation of the benzoyl ester (**25**) led reaction of **11** with *para*-phenylbenzoic acid, although no product (**26**) was formed in this reaction.

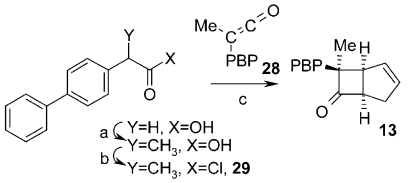
The use of other nucleophiles, such as *E*-geraniol, *E*,*E*-farnesol, benzyl alcohol, 4-phenyl-butanoic acid and decanoic acid failed to react.

The reaction of unhalogenated derivative **12** (X = H) with NBA in the presence of benzoic acid resulted in bromination of the C7 position, yielding the di-bromo derivative **27** in 62% yield. Roughly 2.5 equiv of NBA were required for this reaction to reach completion. Both bromination reactions proceed at roughly equivalent rates, as evidenced by the disappearance of the vinyl protons (H-2 and H-3) and the H-7 acidic proton by ^{1}H NMR.

Treatment of compound **3** with NBA in acetone and H_2O, yielded the *exo*-bromo stereoisomer **14** exclusively. This reaction was repeated several times, albeit producing none of the *endo*-bromo stereoisomer. This is the first case of a 7,7-disubstitutedbicyclo[3.2.0]hep-2-en-6-one affording one isomer exclusively under these conditions.[28,29]

The incorporation of methoxy **15**, acetoxy **16** and benzoxy **17** derivatives of **3** were relatively facile, with all reactions proceeding in good yield. Compound **3** failed to react with NBA in the presence of *p*-nitro-benzoic acid, *p*-methoxy-benzoic acid or *p*-phenylbenzoic acid. 7-*endo*-*p*-Biphenyl-7-*exo*-methylbicyclo[3.2.0]hept-2-en-6-one **13** was synthesised from the corresponding ketene **28** (generated in situ from **29** via dehydrochlorination with Et_3N) and [1,3]-cyclopentadiene (49% overall yield from commercially available *p*-biphenylacetic acid) (Scheme 6).

Compound **13** underwent selective reaction with NBA in the presence of benzoic acid to give **30** in good yield



Scheme 6. Synthesis of alkene **13**. (a) *n*-BuLi (2 equiv), THF, −78 °C, then CH_3l. (b) $(COCl)_2$, CHCl3, 0–25 °C. (c) Et_3N, 1,3-cyclopentadiene. (PBP = para-biphenyl).

(77%). We also found that it was possible to acylate the *endo* alcohol of **14** using benzoyl chloride under standard conditions to give **17** in near quantitative yield.

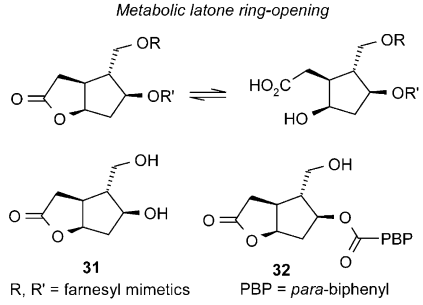
Synthesis of T-4 analogues

We envisaged that pro-drug type compounds based on the T-4 target might prove useful in vitro and in vivo inhibitors of SQS (Scheme 7).

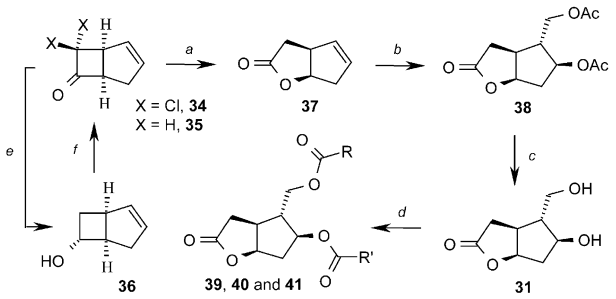
Functionalised bicyclo[3.3.0]octanes have been extensively developed as key useful intermediates in synthetic routes to the naturally occurring prostaglandins.[28,31] T-4 analogues can be easily approached using lactones **31** and **32**. The large scale synthesis of **31** was performed starting from dichloroacetyl chloride **33** (Scheme 8).[32]

Dehydrochlorination of **33** in situ using Et_3N in the presence of [1,3]-cyclopentadiene gave cycloadduct **34** in 90% yield. Dehalogenation of **34** using a zinc–copper couple in acetic acid gave bicyclo[3.2.0]hept-en-6-one **35** in 70% yield. Compound **35** could be resolved using fermenting Bakers yeast to give the *S*-alcohol **36** and *R*-ketone of **35**-(***R***).[33] Reoxidation of *S*-alcohol **36** with CrO_3–H_2SO_4/acetone yields *S*-ketone **35**-(***S***) in 32% overall yield from (±)-**35**. Baeyer–Villiger oxidation of **35** gave the lactone **37** in 75% yield.

The key-step in this series was Prins reaction of **37** with *para*-formaldehyde (monomerised with H_2SO_4 at 70 °C) in glacial acetic acid, followed by acylation of the crude mixture of products (di-acetate, and C4/C5 mono-acetates) yielding the di-acetate **38** in 63% yield. Hydrolysis of the acetates with sodium methoxide in methanol,



Scheme 7. Proposed metabolic lactone ring opening of the T-4 targets.

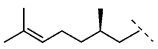
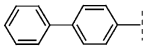
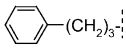
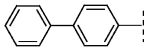


Scheme 8. Synthetic route toT-4 analogues. (a) MCPBA, $NaHCO_3$, CH_2Cl_2, 25 °C; (b) (i). $(CH_2O)_n$, H_2SO_4, AcOH, 80 °C; (ii). Ac_2O, DMAP, Pyr., CH_2Cl_2; (c) NaOMe, MeOH; (d) RCOCl, Pyr., DMAP, CH_2Cl_2, 25 °C; (e) Bakers Yeast, Sucrose, water—3 days; (f) Jones reagent.

and acidic work up afforded the known lactone **31** in 92% yield.[32]

The T-4 esterified compounds were attained by treatment of **31** or **32**[34] with the appropriate acyl chloride in a standard fashion (Table 5 and Scheme 8).

Table 5. Isolated yields of T-4 analogues

No.	R	R′	Yield%
39[a]			53
40[b]			58
41[b]			91

[a]Synthesised from compound **31**.
[b]Synthesised from compound **32**.

Antimicrobial activities

The test compounds were screened for anti-microbial activity.[35] Three yeasts (*Candida albicans*, *Shizosaccharomyces pombe* and *Saccharomyces cerevisiae*, Table 6) and three bacteria (*Botrytis cineria*, *Escherichia coli* and *Staphylococcus aureus*, Table 7) were chosen to evaluate the effectiveness of these compounds.

Each compound was evaluated at a concentration of 200 μg in triplicate, against two controls: squalestatin S1 (Table 8) and ethanol (inhibitor-free). It should be noted that all three yeasts are highly dependant on the steroid pathway and the latter species is more susceptible to SQS inhibitors.[36] This susceptibility was also confirmed in our own assay.

S1 exhibited no activity towards any of the bacteria screened. S1 is either unable to traverse the bacterial cell wall or the bacteria species screened are non-dependant on SQS and ultimately sterol biosynthesis.

The T-2 analogues (**5**, **7**, **8**, **9** and **10**) exhibited little activity against the three yeasts. The best inhibitor of this class was analogue **9**, expected on hydrophobic grounds to be an improved inhibitor. Interestingly, analogue **5** exhibits modest inhibitory activity towards *E. coli*.

The biological activities of the T-3 class were disappointing. Compounds **14**, **16**, **17** and **22** showed little or no activity against the yeasts. The best analogues were those that contained both a halogen at the C7 position and a benzoxy substituent at C3 (**25**, **27** and **30**). These were all modest inhibitors of *S. cerevisiae* and *S. pombe*.

On the whole, the T-3 class demonstrate little activity against any of the bacteria that were screened.

Table 6. Biological activities against yeasts

Compd	Inhibition zone (mm)[a,b]		
	C. albicans GDH2346	*S. cerevisiae* NCYC1467	*S. pombe*
5	0	0	5
7	8	8	7
8	0	8	0
9	0	8	11
10	0	6	8
14	0	0	7
15	—	—	—
16	7	7	0
17	0	0	7
21	—	—	—
22	0	7	5
23	—	—	—
24	7	10	9
25	7	12	11
27	0	10	11
30	11	10	12
31	14	—	—
32	21	11	12
39	12	7	10
40	18	19	19
41	9	16	13

[a]Diameter of inhibition zone.
[b]All discs contained 200 μg of the test compound. Student *t*-Test analysis demonstrates that all inhibition zones >5 mm show significant differences ($P<0.05$).

Table 7. Biological activities against bacteria

Compd	Inhibition zone (mm)[a,b]		
	B. subtilis	*E. coli*	*S. aureus*
5	8	13	6
7	7	8	7
8	—	—	—
9	7	9	0
10	8	5	0
14	—	—	—
15	0	0	0
16	—	—	—
17	0	0	7
21	0	0	0
22	0	0	0
23	—	—	—
24	—	—	—
25	0	0	0
27	0	0	0
30	7	0	5
32	0	8	0
39	0	12	—
40	0	15	—
41	0	15	—

[a]Diameter of inhibition zone.
[b]All discs contained 200 μg of the test compound. Student *t*-Test analysis demonstrates that all inhibition zones >5 mm show significant differences ($P<0.05$). For the controls, see the experimental section.

Table 8. Yeast biological activities of **S1**

Organism	Concentration (μg)[a]		
	200	20	2
Candida albicans	27	15	5
Saccharomyces cerevisiae	27	15	7
Shizosaccharomyces pombe	37	25	19

[a]Zones of inhibition (values are means of three experiments).

The T-4 analogues (**31**, **32**, **39**, **40** and **41**) exhibited the best activities against the yeasts screened. Compound **31**, without any hydrophobic substituents, is a good inhibitor of *C. albicans*; the yeast that is considered the 'most hardy' in the series with regards to SQS inhibitors—compare S1 (Table 8). The presence of a *p*-biphenyl moiety in **32** increases activity. Addition of a citranellyl side chain results in loss of activity, although this might be due to problems with cell transport and insufficient lactone hydrolysis in *C. albicans*. This is confirmed with analogue **41**, where only modest activity was observed. Compounds **32** and **39** are modest inhibitors of *S. cerevisiae* and *S. pombe*, which was surprising in light of the S1 results. Analogue **40** retained activity towards both *S. cerevisiae* and *S. pombe*, demonstrating the promise of this class. Analogue **41** also exhibits good activity towards *S. cerevisiae* and *S. pombe*. The inhibitory activities of the T-4 class against the bacteria *B. subtilis* and *S. aureus* were poor, although somewhat surprisingly good activities for analogues **39**, **40** and **41** were observed against *E. coli*. This final result was the most intriguing, as **39**, **40** and **41** are seemingly selective for *E. coli*.

Mammalian pig liver SQS (PL-SQS) activities

Several analogues that exhibited promising activity were evaluated for inhibition against PL-SQS using an adapted procedure as described by Higson et al.[37] S1 was used as a control and showed a 27.5% reduction in squalene formation at 10 μM. The relatively low activity is attributable to the crude liver cell extracts used in this assay, although we believe that the use of this type of enzyme preparation gives a clearer indication of the real activity of the compounds in vitro and also enables the T4-class to be 'activated' by hydrolysis of the lactone ring. The T-2 analogues all exhibited some inhibitory activity towards PL-SQS at 10 μM (Table 9).

Table 9. Pig liver SQS inhibition results

Compd[a]	Reduction in SQS Activity[b]	
S1 (ZA-A)	27.5	1
5	7.6	0.276
7	8.3	0.302
9	14.4	0.526
31	5.4	0.196
32	12.3	0.447
39	8.3	0.302
40	16.5	0.600
41	16.6	0.604

[a]All test compounds at 10 μM.
[b]Relative activity with S1 in bold.

For example, diol **5** caused a modest 7.6% reduction in squalene production. The presence of a benzoxy substituent in the *exo* position at C3 (**7**) marginally enhanced this activity (8.3%). Addition of a tether between the phenyl and the C3 position (**9**) resulted in a significant increase in activity (14.4%). The T-4 analogues exhibited activity that fits well with increasing the hydrophobicity via use of a *p*-biphenyl moiety and the fact that these compounds have a diphosphate isostere released on hydrolysis. We expected the basic 'core' analogue **31** to exhibit no activity, although a modest amount was observed (5.4%). The *p*-biphenyl analogue **32** was a surprise inhibitor, showing a reduction in squalene formation of 12.3%. The presence of a citranellyl chain (**40**) or an alkyl-phenyl tether (**41**) gave the best activities (16.5 and 16.6%, respectively) in the T-4 series.

Conclusion

We have designed a three-dimensional pharmacophore for SQS by simulation of the substrate/intermediates and known inhibitors of the enzyme. Utilization of the distance geometry approach provided by the pharmacophore has presented several novel targets for synthetic elaboration (T-1–T-6). The choice of the targets was based on rigidity and the desired orientation of the hydrophobic substituents. This was rather fortuitous in retrospect, although most appropriate when compared to the structural architecture provided by the X-ray co-crystal structures of SQS and inhibitors described recently by Pandit et al.[12] The synthesis of T-2 and T-3 analogues were approached with a view to identifying promising activity early without following a time-consuming path to these rather complex targets. The anti-microbial activities of these compounds were poor, although somewhat surprisingly, the T-2 analogues **5**, **7** and **9** exhibited activity against PL-SQS. We appreciate that these compounds have a large degree of hydrophobic character and this is most likely to be attributable to their poor anti-microbial activities. It seems plausible that within the mammalian cell, the cyclobutanones **5**, **7** and **9** might be converted to a more active form and future studies will address this.

The T-4 analogues exhibited the greatest activities against mammalian PL-SQS. It was pleasing to see that the presence of a *p*-biphenyl and citranellyl moiety or an alkyl-phenyl tether produced the best results. Future work will focus on the synthesis of the ring-opened forms of analogues **40** and **41,** and in order to evaluate whether hydrolysis is occurring within the cell, feeding studies with T-4 analogues will be conducted.

Experimental

Nuclear Magnetic Resonance (^{1}H and ^{13}C) spectra were recorded on a Jeol GSX270 (270 MHz) spectrometer. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. Coupling constants (*J* values) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). The relative proportion of solvents in mixed chromatography solvents refers to the volume/volume ratio. Triethylamine was dried over calcium hydride and distilled before use. CH_2Cl_2 was distilled from calcium hydride prior to use. All reactions were performed in an inert atmosphere created by a slight positive pressure of argon. GC spectra were recorded on a Finnigan 2000 series GC coupled to a Finnigan Trace MS, source Electron Impact (EI) 70 eV. 2-Phenylpropanoyl chloride, (+)-citranellyl carbonoyl chloride and

4-phenylbutanoyl chloride were synthesised from commercially available 2-phenyl-propanoic acid, (+)-citranellic acid and 4-phenyl-butyric acid, respectively. Benzoyl chloride and compound **32** were purchased from Lancaster Synthesis and used as received. 7-*exo*-Chloro-7-*endo*-phenylbicyclo[3.2.0]hept-2-en-6-one (**11**) and 7-*endo*-phenylbicyclo[3.2.0]hept-2-en-6-one (**12**) were synthesised using known literature procedures[29b] (±)-Bicyclo[3.2.0]hept-2-en-6-one (**35**) was prepared as previously described.[28] Synthesis of both enantiomers of bicyclo[3.2.0]hept-2-en-6-one [**35-(*R*)** and **35-(*S*)**] was performed on a 20 g scale.[33] The synthesis of compounds **37**, **38** and **31** were conducted on a 10 g scale as previously described.[32] High resolution mass spectra were run by the EPSRC high-resolution mass spectrometer facility at the University of Swansea (UK).

(±)-7-*endo*-Phenyl-7-*exo*-methylbicyclo[3.2.0]hept-2-en-6-one (3). 2-Phenylpropanoyl chloride **1** (3.01 g, 17.9 mmol) was added dropwise to a magnetically stirred solution of [1,3]-cyclopentadiene (2.6 g, 39.3 mmol, 2.2 equiv) and Et_3N (2.7 g, 26.8 mmol, 1.5 equiv) in $CHCl_3$ (100 mL) at 0 °C over 30 min. The mixture was allowed to warm to 25 °C and then stirred for 3 h. $CHCl_3$ was removed in vacuo to ca. 10 mL, then hexane (50 mL) was added and the white precipitate was filtered through a pad of Celite, which was washed with 3×10 mL portions of hexane. Concentration of the filtrate in vacuo afforded a dark coloured oil. Purification by flash chromatography using ether/hexane (1:6, *v*/*v*) afforded the known[38] *title compound* as a viscous lime green oil (2.52 g, 71.2%). ν_{max} (neat) cm^{-1} 3059, 2960, 2922, 1771, 1603, 1371, 1203, 702. δ ^1H ($CDCl_3$) 7.15–7.36 (5H, m, Ph-H), 5.55–5.70 (1H, m, C2-H), 5.47–5.52 (1H, m, C3-H), 3.99–4.06 (1H, m, C5-H), 3.55–3.59 (1H, m, C1-H), 2.63–2.73 (1H, m, C4-H *endo*), 2.41–2.54 (1H, m, C4-H *exo*), 1.67 (3H, s, C7(C1′)-H_3). δ ^{13}C ($CDCl_3$) 211.1 (C6), 140.7 (C1′ *ipso*), 133.2, 128.2 (2C), 127.4 (2C), 126.2, 126.1, 71.5 (C7), 57.2 (C5), 51.6 (C1), 34.2 (C4), 27.1 (C7(C1′)). LRMS (EI) *m*/*z* 198 (M^+) 6%, 183 (M^+-CH_3)$^+$, 170 (M^+-CO)$^+$, 155, 132 ($Ph(CH_3)CCO$)$^+$, 104 ($Ph(CH_3)C$)$^+$, 91, 77 (Ph)$^+$, 66 (C_5H_6)$^+$, 51 (100%).

(±)-2-*exo*-Hydroxy-3-*exo*-hydroxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0]hept-2-en-6-one (5). Solvent system A: Tetrahydrofuran (8 mL), *tert*-butyl alcohol (5 mL) and water (3 mL). To a stirred mixture of *N*-methylmorpholine-*N*-oxide (0.60 g, 5.11 mmol), osmium tetraoxide (25 mg, 0.1 mmol, 2 mol%) in solvent system A, was added compound **3** (1 g, 5.1 mmol) in small portions over a few min. The mixture was left to stir at room temperature under an atmosphere of argon and monitored by TLC. After 18 h the alkene was consumed completely (TLC). The mixture was diluted with CH_2Cl_2 (50 mL) and washed with 20% aqueous sodium sulphite solution (3×20 mL), 10% aqueous hydrochloric acid (2×20 mL) and finally water (2×20 mL). The aqueous phases were back extracted with CH_2Cl_2 (2×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil. Purification by flash chromatography using EtOAc/ hexane (1:1, *v*/*v*) gave the *title compound* as a white solid (0.73 g, 62.6%, R_f = 0.37, EtOAc, mp 98–99 °C). ν_{max} (KBr)/cm^{-1} 3349, 2957, 2868, 1770, 1602, 1493, 1445, 1292, 1241, 1058, 941, 862, 700, 534. δ ^1H ($CDCl_3$) 7.21–7.35 (5H, m, Ph-H), 3.64–3.82 (3H, m, C2-H, C3-H and C5-H), 2.87–2.89 (1H, d, *J* = 7.3, C1-H), 2.20–2.29 (2H, b, C2-OH and C3-OH), 2.12–2.19 (1H, m, C4-H *endo*), 1.84–1.96 (1H, m, C4-H *exo*), 1.62 (3H, s, C7(C1′-H_3)). δ ^{13}C 215.2 (C6), 139.7 (C7(C1′*ipso*), 128.7 (2C), 127.0 (2C), 125.9, 74.7 (C2), 73.9 (C3), 66.5 (C7), 57.0 (C5), 49.4 (C1), 32.8 (C4), 28.3 (C7(C1′)). LRMS (EI) *m*/*z* 232 (M^+), 215.2 (M^+-OH)$^+$, 198 (M^+-2OH)$^+$, 172, 158, 147 ($PhCH_3CCOCH$)$^+$ 100%, 132 ($PhCH_3CCO$)$^+$, 129, 115, 104.1 ($PhCCH_3$)$^+$, 99.1 ($C_5H_7O_2$)$^+$ 91, 77 (Ph)$^+$, 65 (C_5H_5)$^+$. Found: C, 72.65; H, 7.09 expected for $C_{14}H_{16}O_3$ C, 72.39; H, 6.94.

(±)-2-*exo*-Hydroxy-3-*exo*-benzoxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0]hept-2-en-6-one (7). Compound **5** (0.10 g, 0.44 mmol) was added to a stirred mixture of DMAP (5 mg, Cat.) in pyridine (2 mL). The mixture was stirred for 30 min and then benzoyl chloride (67 mg, 0.48 mmol, 1.1 equiv) in pyridine (0.5 mL) was added dropwise over 10 min. The mixture was placed under an atmosphere of argon and stirred for 18 h. The mixture was quenched with water (2 mL) and extracted with ether (3×5 mL). The combined organic extracts were washed with 10% aqueous hydrochloric acid (3×5 mL) and water (1×5 mL), dried ($MgSO_4$) and concentrated in vacuo to give an oil. Purification by flash chromatography using EtOAc/hexane (1:10, *v*/*v*) gave impure **8** as a colourless oil (32 mg, 16.3%, 23.4% based on recovered **5**, repeated chromatography failed to give **8** in higher purity) followed by **7** as a white solid (46 mg, 31.2%, 45.5% based on recovered **5**, mp 113–114 °C). Data for compound **7**: ν_{max} (KBr)/cm^{-1} 3555, 3026, 2967, 2872, 1949, 1769, 1743, 1602, 1585, 1444, 1103, 702. δ ^1H ($CDCl_3$) 7.94–7.97 (2H, d, *J* = 7.4, C3(C4′-H and C8′-H), 7.53–7.58 (1H, dd, *J* = 7.4 and 7.4, C3(C6′-H), 7.26–7.37 (7H, m, C3(C5′-H and C7′-H) and C7(Ph-H), 4.90–4.97 (1H, m, C3-H), 4.05 (1H, dd, *J* = 1.8 and 4.1 Hz, C2-H), 3.91–3.98 (1H, d, *J* = 8.1, C5-H), 2.99–3.02 (1H, dd, *J* = 8.1 and 1.8, C1-H), 2.40–2.47 (1H, m, C4-H *exo*), 2.16–2.24 (1H, m, C4-H *endo*), 2.02 (1H, s, C2-OH), 1.68 (3H, s, C7(C1′-H_3)). LRMS (EI) *m*/*z* 215 (M^+-OCOPh)$^+$ 21%, 196 ($M^+-OCOPh-OH$)$^+$, 132.0 ($PhCMeCO$)$^+$, 129.1, 122, 104.8 ($PhCMe$)$^+$ 100%, 91.0, 76.9 (Ph), 55, 51. Found: C, 74.80; H, 6.48, expected for $C_{21}H_{20}O_4$, C, 74.98; H, 5.99. Representative data for compound **8** (Selected): δ ^1H ($CDCl_3$) 8.02–8.07 (2H, m, C3(C4′-H and C8′-H)), 7.82–7.85 (2H, dd, *J* = 8.1 and 8.1, C2(C4′-H and C8′-H)), 7.26–7.62 (11H, m, C7(Ph-H), C3(C5′-H, C6′-H and C7′-H) and C2(C5′-H, C6′-H and C7′-H), 5.36–5.38 (1H, dd, *J* = 4.0 and 1.8, C2-H), 5.03–5.08 (1H, m, C3-H), 3.95–4.02 (1H, dd, *J* = 8.1 and 8.1, C5-H), 3.16–3.19 (1H, dd, *J* = 8.1 and 1.8, C1-H), 2.61–2.68 (1H, d, *J* = 19.4, C4-H *endo*), 2.27–2.36 (1H, m, C4-H *exo*), 1.70 (3H, s, C7(C1′-H_3).

(±)-2-*exo*-Hydroxy-3-*exo*-(3′-phenylpropyl)-carboxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0]hept-2-en-6-one (9) and (±)-2-*exo*-(3′-phenylpropyl)-carboxy-3-*exo*-(3′-phenylpropyl)-carboxy-7-*endo*-phenyl-7-*exo*-methyl bicyclo[3.2.0] hept-2-en-6-one (10). Following the procedure for compound **7**: Compound **5** (104 mg, 0.45 mmol) was reacted

with 4-phenyl butanoyl chloride (89.9 mg, 0.49 mmol) in pyridine (2 mL). Purification by flash chromatography using EtOAc/hexane (1:4, *v*/*v*) gave **10** as a colourless oil (87.7 mg, 37.2%, 43.9% based on recovered **5**) followed by **9** as a viscous yellow oil (19.2 mg, 11.3%, 13.4% based on recovered **5**). The last component eluted was **5** as a white solid (16 mg). Data for compound **9**: δ ^{1}H ($CDCl_3$) 7.13–7.42 (10H, m, Ph-H), 4.63–4.70 (1H, m, C3-H), 3.82–3.89 (2H, m, C2-H and C5-H), 2.89–2.92 (1H, dd, J = 1.8, 6.2, C1-H), 2.57–2.63 (2H, t, J = 7.4, C3 (C3′-H_2)), 2.23–2.40 (3H, m, C3 (C5′-H_2) and C4-H *exo*), 1.99–2.04 (1H, d, J = 12.1, C4-H *endo*), 1.89–1.98 (2H, m, C3(C4′-H_2)), 1.63 (3H, s, C7 (C1′-H_3)). δ ^{1}H–^{1}H COSY ($CDCl_3$) 4.63 (C3-H) and 3.82 (C2-H), 3.82 (C2-H and C5-H) and 2.89 (C1-H), 3.82 (C5-H) and 2.23 (C4-H *exo*), 4.88 (C3-H) and 2.23 (C4-H *exo*). Found: C, 75.74; H, 7.16, expected for $C_{21}H_{20}O_4$, C, 76.17; H, 6.92. Representative data for compound **10**: δ ^{1}H ($CDCl_3$) 7.07–7.41 (15H, m, C7 (Ph–H), C2 (C5′-Ph–H) and C3 (C5′-Ph–H)), 4.98–5.00 (1H, dd, J = 1.8, 4.1, C2-H), 4.70–4.78 (1H, m, C3-H), 3.79–3.86 (1H, d, J = 7.7, C5-H), 2.87–2.91 (1H, dd, J = 1.8, 7.7, C1-H), 2.62–2.65 (2H, t, J = 7.4, C2 (C3′-H_2)), 2.49–2.55 (2H, t, J = 7.5, C3 (C3′-H_2)), 2.27–2.38 (3H, m, C4-H *exo* and C2 (C4′-H_2)), 2.13–2.18 (2H, t, J = 7.5, C3 (C4′-H_2)), 1.75–2.02 (5H, m, C4-H *endo*, C2 (C5′-H_2) and C3 (C5′-H_3)), 1.63 (3H, s, C7 (C1′-H_3)). δ ^{13}C ($CDCl_3$) 211.1 (C6), 172.3 (C2 (C2′)), 171.9 (C3 (C2′)), 141.3 (C *ipso*), 138.7 (C *ipso*), 128.9, 128.4, 128.4, 128.35, 127.4 (2C), 126.1 (2C), 125.9, 125.7, 74.2 (C2), 73.3 (C3), 67.5 (4) (C7), 56.1 (C5), 47.5 (C1), 35.1 (C2 (C3′), 34.9 C3 (C3′), 33.6 (C2 (C4′), 33.3 (C4), 30.6 (C3 (C4′)), 28.2 (C2 (C4′)), 26.4 (C3 (C4′)), 26.2 (C8).

(±)-2-*endo*-Bromo-3-*exo*-hydroxy-7-*endo*-phenyl-7-*exo*-chlorobicyclo[3.2.0]hept-6-one (21) and (±)-2-*exo*-bromo-3-*endo*-hydroxy-7-*endo*-phenyl-7-*exo*-chlorobicyclo[3.2.0]-hept-6-one (22). To a stirred mixture containing **11** (2.46 g, 11.3 mmol) in acetone (30 mL) and water (10 mL) was added NBA (2.33 g, 16.9 mmol, 1.5 equiv) over 30 min. After 16 h compound **11** was completely consumed (TLC) and standard work up gave a yellow oil. Purification by flash chromatography using ether/hexane (1:4, v/v) gave the known[25] bromohydrin **22** as a fluffy white solid (1.33 g, 37.3%, mp 102–103 °C) followed by **21** as a white solid (0.33 g, 9.3%, mp 107–108 °C). Data for compound **21**: ν_{max} (KBr)/cm^{-1} 3552, 3079, 3022, 2961, 1786, 1487, 1256, 1145, 1029. δ ^{1}H ($CDCl_3$) 7.51–7.55 (2H, m, C7 (C2′-H and C6′-H)), 7.33–7.36 (3H, m, C7 (C3′-H, C4′-H and C5′-H)), 4.20–4.27 (2H, m, C2-H and C3-H), 4.02–4.08 (1H, m, C1-H), 3.66–3.72 (1H, m, C5-H), 2.51–2.59 (1H, m, C4-H *exo*), 2.16 (1H, s, C3-OH), 1.80 (1H, m, C4-H *endo*). LRMS (CI) *m*/*z* 334 (M ^{81}Br, $+NH_4$) 2%, 332 (M ^{79}Br, $+NH_4$) 1.5%, 314 (5%), 312 (5%), 260, 232, 216, 202 (100%), 185, 118, 108, 91. HRMS (CI): *m*/*z* exact mass calculated for $C_{13}H_{16}NO_2{}^{79}BrCl$ 332.0052; Found 332.0056. Data for compound **22**: ν_{max} (KBr)/cm^{-1} 3539, 3088, 3025, 2981, 2958, 1786, 1488, 1393, 1258, 1169, 1033, 990, 832, 700, 660, 629. δ ^{1}H ($CDCl_3$) 7.52–7.57 (2H, m, C7 (C2′-H and C6′-H)), 7.34–7.42 (3H, m, C7 (C3′-H, C4′-H and C5′-H)), 4.32–4.41 (2H, m, C2-H and C3-H), 4.05 (1H, m, C5-H), 3.93–3.95 (1H, dd, J = 1.0, 7.8, C1-H), 2.43–2.56 (1H, m, C4-H *exo*), 2.28–2.34 (1H, d, J = 14.8, C4-H *endo*), 1.62 (1H, s, C3-OH). δ ^{13}C ($CDCl_3$) 200.8 (C6), 136.9 (C7 (C1′ *ipso*)), 129.0, 128.9, 128.8, 127.6, 127.4, 80.9 (C3), 80.5 (C7), 59.8 (C2), 57.0 (C5), 53.1 (C1), 35.1 (C4). δ ^{1}H–^{1}H COSY ($CDCl_3$) 4.32 (C2-H and C3-H) and 2.43 (C4-H *exo*), 4.32 (C2-H and C3-H) and 4.05 (C1-H), 4.05 (C1-H) and 3.93 (C5-H). LRMS (CI) *m*/*z* 334 (M ^{81}Br, $+NH_4$) 7%, 332 (M ^{79}Br, $+NH_4$) 5.5%, 314 (2.5%), 312 (2%), 232, 218, 202 (100%), 185, 118, 108, 91, 55. HRMS (CI): *m*/*z* exact mass calculated for $C_{13}H_{16}NO_2{}^{79}BrCl$ 332.0052; found 332.0050.

(±)-2-*exo*-Bromo-3-*endo*-methoxy-7-*endo*-phenyl-7-*exo*-chlorobicyclo[3.2.0]hept-6-one (23). To a stirred mixture containing compound **11** (0.56 g, 2.56 mmol) in methanol (10 mL) was added NBA (0.53 g, 3.84 mmol, 1.5 equiv). Reaction time: 16 h (TLC) and standard work up gave an oil. Purification by flash chromatography using ether/hexane (1:4, v/v) gave the *title compound* as a colourless oil (0.74 g, 87.8%). ν_{max} (neat)/cm^{-1} 3064, 2989, 2931, 2829, 1794, 1600, 1581, 1450, 1088, 911, 732, 699, 658, 613. δ ^{1}H ($CDCl_3$) 7.26–7.60 (5H, m, Ph-H), 4.30 (1H, s, C3-H), 4.27–4.28 (1H, d, J = 4.8, C1-H), 3.90 (1H, s, C2-H), 3.86–3.89 (1H, d, J = 4.8, C5-H), 2.52 (3H, s, C3 (C2′-H_3)), 2.25–2.49 (2H, m, C4-H_2). δ ^{13}C ($CDCl_3$) 200.5 (C6), 136.9 (C7 (C1′ *ipso*)), 129.4, 129.4, 128.8, 128.1, 127.6, 89.3 (C3), 80.5 (C7), 59.9 (C1), 57.5 (C2), 55.9 (C5), 49.4 (C3 (C2′)), 33.1 (C4). Found: C, 50.52; H, 3.97, expected for $C_{14}H_{14}BrClO_2$ C, 51.01; H, 4.28.

(±)-2-*exo*-Bromo-3-*endo*-acetoxy-7-*endo*-phenyl-7-*exo*-chlorobicyclo[3.2.0]hept-6-one (24). To a stirred mixture containing compound **11** (1.0 g, 4.6 mmol) in acetic acid (10 mL) and CH_2Cl_2 (10 mL) was added NBA (0.94 g, 6.81 mmol, 1.5 equiv) over 30 min. Reaction time: 16 h (TLC). Purification by flash chromatography using EtOAc/hexane (1:9, v/v) gave the *title compound* as a light green crystalline solid (1.26 g, 77.3%, mp 38 °C, R_f = 0.12, EtOAc/hexane (1:9, v/v)). ν_{max} (neat)/cm^{-1} 3064, 2977, 2869, 1795, 1746, 1600, 1583, 1448, 1371, 1221, 1026, 910, 733, 699. δ ^{1}H ($CDCl_3$) 7.70–7.75 (2H, m, C7 (C2′-H and C6′-H)), 7.41–7.59 (3H, m, C7 (C3′-H, C4′-H and C5′-H)), 5.33–5.34 (1H, d, J = 4.0, C3-H), 4.53–4.59 (1H, dd, J = 4.4, 7.7, C5-H), 4.45 (1H, s, C2-H), 4.12–4.15 (1H, d, J = 7.7, C1-H), 2.66–2.77 (1H, m, C4-H *exo*), 2.44–2.50 (1H, d, J = 15.0, C4-H *endo*), 1.68 (3H, s, C3 (C3′-H_3)). δ ^{13}C ($CDCl_3$) 201.0 (C6), 168.8 (C3 (C2′)), 135.9 (C7 (C1′ *ipso*)), 129.3, 129.1, 128.9 (2C), 127.4, 82.2 (C3), 79.5 (C7), 60.6 (C5), 57.4 (C2), 49.7 (C1), 32.4 (C4), 20.5 (C3 (C3′)). δ ^{1}H–^{1}H COSY ($CDCl_3$) 5.33 (C3-H) and 2.66 (C4-H *exo*), 4.53 (C5-H) and 4.12 (C1-H), 2.66 (C4-H *exo*) and 2.44 (C4-H *endo*). LRMS (CI) *m*/*z* 376 (M ^{81}Br, $+NH_4$) 23%, 374 (M ^{79}Br, $+NH_4$) 17%, 342, 340, 298, 296, 260, 218 (100%), 200, 183, 155. HRMS (CI): *m*/*z* exact mass calculated for $C_{15}H_{18}NO_3{}^{79}BrCl$ 374.0158; Found 374.0154. Found: C, 50.23; H, 3.86; Br, 22.13, expected for $C_{15}H_{14}BrClO_3$ C, 50.38; H, 3.95; Br, 22.34.

(±)-2-*exo*-Bromo-3-*endo*-benzoxy-7-*endo*-phenyl-7-*exo*-chlorobicyclo[3.2.0]hept-6-one (25). To a stirred mixture

containing compound **11** (1.01 g, 4.6 mmol) and benzoic acid (4.47 g, 36.61 mmol, 8 equiv) in CH_2Cl_2 (30 mL) was added NBA (0.94 g, 6.8 mmol, 1.5 equiv) over 30 min. Reaction time: 16 h (TLC) and standard work up gave a yellow solid. Recrystallisation of the yellow solid from ether gave the *title compound* as white crystals (0.90 g, 47.1%, mp 148–149 °C). ν_{max} (KBr)/cm^{-1} 3070, 3030, 2996, 2949, 1948, 1852, 1781, 1722, 1599, 1493, 1261, 1098, 991, 826. δ ^{1}H ($CDCl_3$) 7.51–7.58 (3H, m, C3 (C4′-H), C3 (C6′-H) and C3 (C8′-H)), 7.33–7.44 (4H, m, C7 (C2′-H)), C7 (C6′-H), C3 (C5′-H and C7′-H)), 6.95–7.01 (1H, dd, J = 7.2 and 7.3, C7 (C4′-H)), 6.82–6.87 (2H, dd, J = 7.5, 7.5, C7 (C3′-H and C5′-H)), 5.31–5.32 (1H, d, J = 4.4, C3-H), 4.48–4.55 (2H, m, C5-H and C2-H), 3.02–3.07 (1H, m, C1-H), 2.64–2.74 (1H, m, C4-H *exo*), 2.50–2.55 (1H, d, J = 14.7, C4-H *endo*). δ ^{13}C ($CDCl_3$) 201.0 (C6), 165.1 (C3 (C2′)), 135.2 (C *ipso*), 134.6 (C *ipso*), 133.3, 129.9 (2C), 128.8 (2C), 128.6 (2C), 128.1 (2C), 127.3, 83.5 (C3), 79.8 (C7), 61.1 (C2), 57.3 (C5), 49.6 (C1), 32.1 (C4). Found: C, 57.33; H, 3.74, expected for $C_{20}H_{16}BrClO_3$ C, 57.24; H, 3.84.

(±)-2-*exo*-Bromo-3-*endo*-benzoxy-7-*endo*-phenyl-7-*exo*-bromobicyclo[3.2.0] hept-6-one (27). To a stirred mixture containing compound **12** (0.40 g, 2.2 mmol) and benzoic acid (1.59 g, 13.0 mmol, 6 equiv) in CH_2Cl_2 (20 mL) was added NBA (0.45 g, 3.3 mmol, 1.5 equiv) over 30 min. Further NBA (1 equiv) was added in one portion after 8 h. Reaction time: 16 h (TLC) and standard work up gave a yellow solid. Recrystallization of the yellow solid from ether gave the *title compound* as a white fluffy solid (0.52 g, 62.3%, mp 157–158 °C). ν_{max} (KBr)/cm^{-1} 3069, 3028, 2944, 1950, 1848, 1782, 1723, 1600, 1497, 1260, 998, 719. δ ^{1}H ($CDCl_3$) 7.54–7.66 (3H, m, C3 (C4′-H), C3 (C6′-H) and C3 (C8′-H)), 7.26–7.46 (4H, m, C7 (C2′-H), C7 (C6′-H), C7 (C3′-H) and C7 (C5′-H)), 6.94–6.99 (1H, dd, J = 7.5 and 7.5, C7 (C4′-H)), 6.81–6.87 (2H, dd, J = 7.5, 7.5, C3 (C5′-H) and C3 (C7′-H)), 5.34–5.35 (1H, d, J = 4.4, C3-H), 4.52–4.56 (2H, m, C5-H and C2-H), 2.98–3.02 (1H, m, C1-H), 2.66–2.77 (1H, m, C4-H *exo*), 2.51–2.56 (1H, d, J = 14.7, C4-H *endo*). δ ^{13}C ($CDCl_3$) 200.9 (C6), 165.4 (C3 (C2′)), 135.6 (C *ipso*), 133.6 (C *ipso*), 130.3, 128.9, 128.8, 128.7 (2C), 128.6 (2C), 128.3, 127.6, 127.5, 83.9 (C3), 71.7 (C7), 60.9 (C2), 57.9 (C5), 49.4 (C1), 31.9 (C4). Found: C, 51.24; H, 3.31; Br, 34.19 expected for $C_{20}H_{16}Br_2O_3$ C, 51.75; H, 3.47; Br, 34.43.

(±)-2-*exo*-Bromo-3-*endo*-hydroxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0] hept-6-one (14). To a stirred mixture containing compound **3** (0.52 g, 2.63 mmol) in acetone (15 mL) and water (5 mL) was added NBA (0.54 g, 3.95 mmol, 1.5 equiv) over 30 min. Reaction time: 16 h (TLC) and standard work up gave a white solid. Purification by flash chromatography using ether/hexane (1:3, *v*/*v*) gave the *title compound* as white crystals (0.43 g, 55.9%, mp 98–99 °C). ν_{max} (KBr)/cm^{-1} 3504, 3023, 2933, 2861, 1955, 1889, 1772, 1600, 1446, 1109, 1033, 948, 700. δ ^{1}H ($CDCl_3$) 7.18–7.40 (5H, m, Ph-H), 4.33–4.35 (1H, m, C3-H), 3.92–4.00 (2H, m, C2-H and C5-H), 3.31–3.34 (1H, d, J = 7.7, C1-H), 2.35–2.46 (1H, m, C4-H *exo*), 2.13–2.18 (1H, d, J = 14.3, C4-H *endo*), 1.63 (3H, s, C7 (C1′-H_3)), 1.33–1.35 (1H, s, C3-OH). LRMS (EI) m/z 294 (M^+ ^{79}Br) and 296 (M^+ ^{81}Br) 13%, 276 (M^+ ^{79}Br-H_2O)$^+$ and 278 (M^+ ^{81}Br−H_2O)$^+$, 215 (M^+−Br)$^+$, 197 (M^+−Br−H_2O)$^+$, 155, 132 (Ph(CH_3)CCO)$^+$, 104 (Ph(CH_3)C)$^+$, 91, 77 (Ph)$^+$, 55 (100%). LRMS (CI) m/z 314 (M ^{81}Br, +NH_4) 84%, 312 (M ^{79}Br, +NH_4) 85%, 296 (M^+ ^{81}Br) 10%, 294 (M^+ ^{79}Br) 10.5%, 232 (100%), 216, 197, 169, 160, 132, 55. HRMS (CI) m/z ($^+NH_4$ enhanced) exact mass calculated for $C_{14}H_{19}N^{79}O_2Br$ 312.0599; Found 312.0595. Found: C, 56.64; H, 5.37; Br, 26.72, expected for $C_{14}H_{15}BrO_2$, C, 56.97; H, 5.12; Br, 27.07.

(±)-2-*exo*-Bromo-3-*endo*-methoxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0] hept-6-one (15). To a stirred mixture containing compound **3** (206 mg, 1.04 mmol) in dry methanol (10 mL) was added NBA (0.196 g, 1.41 mmol, 1.4 equiv). Reaction time: 8 h (TLC) and standard work up gave an oil. Purification by flash chromatography using ether/hexane (1:4, v/v) gave the *title compound* as a colourless oil (0.26 g, 81.7%, mp 56–57 °C, R_f = 0.44, 3:7 ether/hexane *v*/*v*), which crystallised on standing. ν_{max} (KBr)/cm^{-1} 3525, 3061, 3019, 2923, 2829, 1951, 1879, 1772, 1600, 1493, 1250, 1089, 761, 695. δ ^{1}H ($CDCl_3$) 7.17–7.40 (5H, m, Ph–H), 4.36 (1H, s, C3-H), 3.91–3.97 (1H, m, C5-H), 3.86–3.88 (1H, d, J = 4.0, C2-H), 3.33–3.36 (1H, d, J = 7.7, C1-H), 2.49 (3H, s, C3 (C2′-H_3)), 2.33–2.42 (1H, m, C4-H *exo*), 2.21–2.26 (1H, d, J = 13.9, C4-H *endo*), 1.61 (3H, s, C7 (C1′-H_3)). δ ^{13}C ($CDCl_3$) 211.6 (C6), 141.7 (C *ipso*), 128.4 (2C), 126.2 (2C), 125.8, 89.8 (C3), 70.0 (C7), 58.0 (C2), 55.9 (C5), 53.6 (C1), 50.7 (C3 (C2′)), 32.8 (C4), 30.6 (C7 (C1′)). LRMS (EI) m/z 308.1 (M^+ ^{79}Br), 310.1 (M^+ ^{81}Br) 10%, 277.8 (M^+−OMe)$^+$, 229.1 (M^+−^{79}Br)$^+$, 197 (M^+−^{79}Br−OMe)$^+$, 131.8 (PhCMeCO)$^+$, 104.0 (PhCMe)$^+$, 91.0, 79.0 (^{79}Br), 77.0 (Ph)$^+$, 65 (C_5H_5)$^+$, 55 (100%). LRMS (CI) m/z 330 (M ^{81}Br, +NH_4) 100%, 328 (M ^{79}Br, +NH_4) 98%, 216, 197, 169, 132, 99. HRMS (CI) ($^+NH_4$ enhanced) m/z exact mass calculated for $C_{15}H_{21}N^{79}O_2Br$ 326.0755; Found 326.0758.

(±)-2-*exo*-Bromo-3-*endo*-acetoxy-7-*endo*-phenyl-7-*exo*-methylbicyclo[3.2.0]hept-6-one (16). To a stirred mixture containing compound **3** (640 mg, 3.2 mmol) in CH_2Cl_2 (10 mL) and acetic acid (3 mL) was added NBA (669 mg, 4.9 mmol, 1.5 equiv). Reaction time: 4 h (TLC) and standard work up gave a colourless oil. Purification by flash chromatography using ether/hexane (1:4, v/v) gave the *title compound* as a colourless oil (0.86 g, 79.2%). ν_{max} (neat)/cm^{-1} 3057, 3023, 2967, 1959, 1839, 1776, 1743, 1601, 1494, 1444, 1371, 1224, 1027, 764. δ ^{1}H ($CDCl_3$) 7.17–7.42 (5H, m, Ph–H), 5.18–5.20 (1H, d, J = 4.1, C3-H), 4.34 (1H, s, C2-H), 4.02–4.07 (1H, d, J = 8.0, C1-H), 3.42–3.50 (1H, m, C5-H), 2.47–2.57 (1H, m, C4-H *exo*), 2.02–2.08 (1H, d, J = 11.4, C4-H *endo*), 1.63 (3H, s, C7 (C1′-H_3)), 1.51 (3H, s, C3 (C3′-H_3)). δ ^{13}C ($CDCl_3$) 211.8 (C6), 169.2 (C3 (C1′)), 141.0 (C7 (C1′ *ipso*)), 128.6 (2C), 126.5 (2C), 125.5, 82.5 (C3), 69.8 (C7), 58.6 (C2), 53.6 (C5), 51.1 (C5), 31.8 (C4), 31.0 (C3 (C3′)), 20.4 (C7 (C1′)). LRMS (EI) m/z 198 (M^+−Br−OCOCH$_3$)$^+$ 8%, 182, 154.1, 141, 128, 115, 104 (PhCH$_3$C)$^+$, 91, 77 (Ph)$^+$, 65 (C_5H_5)$^+$, 55 (100%), 43 (COCH$_3$)$^+$. Found: C, 57.42; H, 5.45, expected for $C_{16}H_{17}BrO_3$ C, 56.99; H, 5.08.

(±)-2-*exo*-Bromo-3-*endo*-benzoxy-7-*endo*-phenyl-7-*exo* methylbicyclo[3.2.0]hept-6-one (17). To a stirred mixture containing compound **3** (606 mg, 3.06 mmol) and benzoic acid (2.24 g, 18.4 mmol, 6 equiv) in CH_2Cl_2 (30 mL) was added NBA (0.55 g, 3.98 mmol, 1.3 equiv). Reaction time: 24 h (TLC) and standard work up gave a white solid. Recrystallization from ether gave the *title compound* as white crystals (0.77 g, 62.7%, mp 147–148 °C). ν_{max} (KBr)/cm^{-1} 3059, 3030, 2931, 2898, 2867, 1952, 1842, 1773, 1720, 1602, 1492, 1320, 1178, 1096, 989, 887, 761, 706, 626. δ ^{1}H ($CDCl_3$) 6.76–7.61 (10H, br m, 2×Ph-H), 5.31–5.33 (1H, d, J=4.4, C3-H), 4.56 (1H, s, C2-H), 4.14–4.17 (1H, dd, J=4.2 and 7.7, C5-H), 3.45–3.48 (1H, d, J=7.7, C1-H), 2.60–2.70 (1H, m, C4-H *exo*), 2.41–2.46 (1H, d, J=14.7, C4-H *endo*), 1.62 (3H, s, C7 (C1′-H_3)). δ ^{13}C ($CDCl_3$) 211.8 (C6), 165.3 (C3 (C1′)), 139.8 (C *ipso*), 133.1 (C *ipso*), 130.1 (2C), 128.3 (2C), 127.9 (2C), 126.3 (2C), 125.6 (2C), 83.9 (C3), 70.1 (C7), 59.1 (C2), 53.5 (C5), 51.1 (C1), 31.8 (C4), 31.1 (C7 (C1′)). LRMS (EI) m/z 279.9 (M^+ ^{81}Br−PhCO)$^+$, 277.9 (M^+ ^{79}Br−PhCO)$^+$ 2%, 197 (M^+−^{79}Br−PhCO)$^+$, 132 ($PhCH_3CCO)^+$, 105 $(PhCO)^+$, 104 $(PhCH_3C)^+$, 91, 79.1 (^{79}Br)$^+$, 77 $(Ph)^+$, 65.1 $(C_5H_5)^+$, 55 (100%). LRMS (CI) m/z 418 (M ^{81}Br, +NH_4) 100%, 416 (M ^{79}Br, +NH_4) 98%, 338, 279, 277, 216, 199, 169, 132, 105. HRMS (CI): m/z exact mass calculated for $C_{21}H_{23}N^{79}BrO_3$ 416.0861; Found 416.0866. Found: C, 63.43; H, 5.24, expected for $C_{21}H_{19}BrO_3$ C, 63.17; H, 4.80.

2-*para*-Biphenylpropanoyl chloride (29). To a stirred solution of diisopropylamine (1.03 g, 10.3 mmol, 2.2 equiv) in dry THF (20 mL) at −84 °C was added *n*-butyl lithium (4 mL, 9.9 mmol, 2.1 equiv, 1.6 M solution in hexanes) over 20 min under an atmosphere of argon. The mixture was stirred for a further 30 min and then allowed to warm to −30 °C over 1 h. The colourless LDA solution was cooled to −84 °C and 2-*para*-biphenylpropanoic acid (1.0 g, 7.7 mmol, 1 equiv) in dry THF (5 mL) was added dropwise over 30 min. The mixture was allowed to stir for 2 h at −84 °C and then methyl iodide (0.66 g, 5.2 mmol, 1.1 equiv) in dry THF (2 mL) was added dropwise over 30 min. Stirring was continued for 2 h at −84 °C and then the mixture was allowed to warm to 25 °C over 3 h. The reaction was quenched with satd NH_4Cl (100 mL) and extracted with EtOAc (3×100 mL). The combined organic extracts were washed with satd brine (2×100 mL) and water (2×100 mL), dried ($MgSO_4$) and concentrated in vacuo to give a viscous brown oil. The oil was taken up in ether and hexane (1:1, 20 mL), and after standing for several hours the acid precipitated to give the *title compound* as a fine brown powder (0.81 g, 76.4%, mp 137–139 °C). ν_{max} (KBr)/cm^{-1} 3330, 2951, 1699, 1599, 1488, 1282, 1231, 1077, 1005, 953, 765, 722, 692. 2-*para*-Biphenylpropanoic acid (0.67 g, 2.9 mmol) in dry $CHCl_3$ (10 mL) was stirred at 0 °C for 30 min, then oxalyl chloride (0.75 g, 5.9 mmol, 2 equiv) in dry $CHCl_3$ (5 mL) was added dropwise over 15 min, followed by addition of 10 μL of *N*,*N*′-dimethylformamide (cat.) and stirred at 0 °C for 1 h and then allowed to warm to 25 °C and stirring continued overnight. Concentration in vacuo afforded a brown solid, which was taken up in ether (10 mL). The precipitate was filtered and the filtrate concentrated in vacuo to give the *title* compound as a light brown oil (0.80 g, 100%), which was used without further purification for the reaction with [1,3]-cyclopentadiene.

(±)-7-*endo*-*para*-Biphenyl-7-*exo*-methylbicyclo[3.2.0] hept-2-en-6-one (13). Following a similar procedure for the synthesis of compound **3**: 2-*para*-Biphenylpropanoyl chloride **29** (0.8 g, 3.3 mmol) was reacted with [1,3]-cyclopentadiene (0.86 g, 13 mmol, 4 equiv) and Et_3N (0.6 g, 5.9 mmol, 1.8 equiv) in $CHCl_3$ (30 mL). The usual work up gave a dark brown oil. Purification by flash chromatography using ether/hexane (1:6, v/v) gave the *title compound* (0.59 g, 65.2%, mp 35–36 °C) as light green crystals. δ ^{1}H ($CDCl_3$) 7.26–7.58 (9H, m, ar–H), 5.68–5.72 (1H, m, C2-H), 5.52–5.56 (1H, m, C3-H), 4.00–4.06 (1H, m, C5-H), 3.56–3.61 (1H, m, C1-H), 2.65–2.75 (1H, m, C4-H *endo*), 2.39–2.52 (1H, m, C4-H *exo*), 1.74 (3H, s, C7(C1′-H_3). δ ^{13}C ($CDCl_3$) 213.2 (C6), 140.9 (C7(C1′ *ipso*)), 139.8, 139.3, 133.4, 131.3, 128.7 (2C), 127.1 (2C), 126.9, 126.8, 126.6, 57.3 (C1), 51.6 (C5), 34.2 (C4), 26.9 (C7 (C1′)). LRMS (EI) m/z 274 (M^+) 6%, 208 (M^+−C_5H_6)$^+$, 165, 153 (C_6H_5−C_6H_4)$^+$, 151, 131, 115, 89, 66 $(C_5H_6)^+$ 100%, 51 $(C_4H_3)^+$. Found: C, 87.02; H, 6.76, expected for $C_{20}H_{18}O$; C, 86.92; H, 7.26.

(±)-2-*exo*-Bromo-3-*endo*-benzoxy-7-*endo*-*para*-biphenyl-7-*exo*-methylbicyclo[3.2.0]hept-6-one (20). To a stirred mixture containing compound **13** (128 mg, 0.47 mmol) and benzoic acid (0.46 g, 3.7 mmol, 8 equiv) in CH_2Cl_2 (30 mL) was added NBA (96.7 mg, 0.7 mmol, 1.5 equiv). Reaction time: 24 h (TLC) and standard work up gave an oil. Purification by flash chromatography using ether/hexane (1:4, v/v) gave the *title compound* as a viscous light green oil which crystallised on standing (172 mg, 77.0%, mp 65–66 °C). δ ^{1}H ($CDCl_3$) 7.47–7.60 (2H, m, Ph–H), 7.21–7.41 (8H, m, Ph–H), 6.98–7.02 (2H, m, Ph–H), 5.32–5.34 (1H, d, J=4.4, C3-H), 4.66 (1H, s, C2-H), 4.14–4.20 (1H, dd, J=4.4, 7.7, C5-H), 3.49–3.52 (1H, d, J=7.7, C1-H), 2.62–2.73 (1H, m, C4-H *exo*), 2.44–2.50 (1H, d, J=14.7, C4 *endo*), 1.66 (3H, s, C7 (C1′-H_3)). FAB LRMS m/z 476.2 (M^+ ^{81}Br) and 474.2 (M^+ ^{79}Br), 355.1 (M^+-$PhCO_2$)$^+$, 273.2, 245.2, 231.2, 208.2, 181.2, 153.1 (C_6H_5–C_6H_4)$^+$ 100%, 105.1. FAB HRMS: m/z exact mass calculated for $C_{27}H_{23}BrO_3Na$ 497.0728; found 497.0732. Found: C, 67.94; H, 4.89; Br, 16.65 expected for $C_{27}H_{23}BrO_3$ C, 68.22; H, 4.88; Br, 16.81.

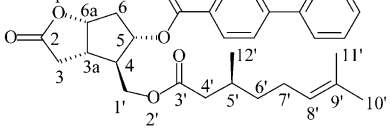
General procedure for the synthesis of the T-4 di-esters

To the alcohol **31** (100 mg, 0.28 mmol) in pyridine (5 mL) was added the acid chloride (0.31 mmol, 1.1 equiv) in CH_2Cl_2 (2 mL) over 15 min, followed by addition of DMAP (3.5 mg, 10 mol%). The mixture was stirred at 25 °C until all **31** was consumed (TLC). The mixture was diluted with water (5 mL) and extracted with EtOAc (3×10 mL). The combined extracts were washed successively with 5% aqueous hydrochloric acid (3×10 mL) and water (1×10 mL), dried ($MgSO_4$) and concentrated in vacuo to give the product as an oil or solid. Purification by flash chromatography gave the products as white solids or viscous oils. For alcohol **32**,

2.2 equiv of the acid chloride was used. All reactions were run on a 0.28–0.30 mmol scale.

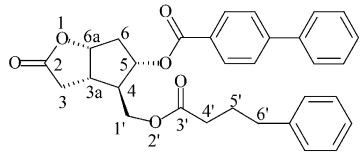
(3aα,4α,5β,6aα)-(−)-Hexahydro-4-(1′-phenylcarboxymethyl)-2-oxo-2*H*-cyclopenta[b]furan-5-yl-1-phenyl-5-carboxylate (39). Purification by flash chromatography using EtOAc–hexane (1:1, v/v) gave the *title compound* as a white solid, which was recrystallised from EtOAc and hexane (117 mg, 53.1%, mp 100–102 °C). δ ^{1}H ($CDCl_3$) 7.98–8.03 (4H, m, C4 (C5′-H and C9′-H) and C5 (C4′-H and C8′-H)), 7.53–7.62 (2H, m, C4 (C7′-H) and C5 (C6′-H)), 7.41–7.50 (4H, m, C4 (C6′-H and C8′-H) and C5 (C5′-H and C7′-H)), 5.45–5.50 (1H, m, C5-H), 5.11–5.16 (1H, dddd, J = 2.1, 2.2, 5.9, 5.9, C6a-H), 4.39–4.42 (2H, d, J = 7.0, C4 (C1′-H_2)), 2.88–3.02 (2H, m, C3a-H and C4-H), 2.61–2.70 (3H, m, C6-H *endo* and C3-H_2), 2.36–2.43 (1H, m, C6-H *exo*). δ ^{13}C ($CDCl_3$) 176.1 (C2), 166.5 (C5 (C2′), 165.9 (C4 (C3′), (1×C *ipso not observed*), 133.3 (C *ipso*), 129.6 (2C), 129.5 (2C), 128.5 (3C), 128.4, 83.9 (C5), 64.5 (C6a), 51.7 (C4 (C1′), 40.7 (C3a), 38.3 (C3), 35.7 (C6). FAB LRMS: m/z 380 (M^+), 303 (M^+-Ph)$^+$, 226 (M^+-2Ph)$^+$, 132 (Ph(Me)CCO)$^+$, 104 (Ph(Me)C)$^+$, 77 (Ph)$^+$. FAB HRMS: m/z exact mass calculated for $C_{22}H_{20}O_6$ 380.4011; found, 380.4016.

(3aα,4α,5β,6aα)-(−)-Hexahydro-4-(5′,9′-dimethyl-hept-8′-en-1′-carboxymethyl)-2-oxo-2*H*-cyclopenta[b]furan-5-yl-1,1′-biphenyl-5-carboxylate (40).



Purification by flash chromatography using EtOAc/hexane (1:1, v/v) gave the *title compound* as a light yellow viscous oil (83.1 mg, 58.1%). δ ^{1}H ($CDCl_3$) 7.36–8.08 (9H, br m, Ph–H), 5.39–5.41 (1H, m, C6a-H), 5.05–5.13 (2H, m, C5-H and C4 (C8′-H)), 4.14–4.16 (2H, d, J = 6.2, C4 (C1′-H_2)), 2.91–3.01 (1H, m, C4-H), 2.80–2.86 (1H, m, C3a-H), 2.31–2.64 (4H, m, C3-H_2 and C6-H_2), 1.97–2.21 (3H, m, C4 (C5′-H) and C4 (C4′-H_2)), 1.60 (3H, s, C4 (C10′-H_3)), 1.57 (3H, s, C4 (C11′-H_3)), 1.30–1.50 (4H, m, C4 (C6′-H_2) and C4 (C7′-H_2)), 0.93–1.01 (3H, d, J = 8.1, C4 (C12′-H_3)). Found: C, 73.18%; H, 7.15%, $C_{28}H_{24}O_6$ requires C, 73.79%; H, 7.19%.

(3aα,4α,5β,6aα)-(−)-Hexahydro-4-(3′-phenylpropyl carboxymethyl)-2-oxo-2*H*-cyclopenta[b]furan-5-yl-1,1′-biphenyl-5-carboxylate (41).



Purification by flash chromatography using EtOAc/hexane (3:2, v/v) gave the *title compound* as a white solid, which was recrystallised from EtOAc (28 mg, 90.8%. Mp 84–86 °C, R_f = 0.24, ethyl acetate/hexane, 3:2 v/v). δ ^{1}H ($CDCl_3$) 7.16–8.08 (14H, m, Ph–H), 5.35–5.40 (1H, m, C6a-H), 5.07–5.12 (1H, m, C5-H), 4.11–4.16 (2H, dd, J = 1.1, 7.7, C4 (C1′-H_2), 2.89–2.99 (1H, m, C4-H), 2.76–2.83 (1H, m, C3a-H), 2.61–2.68 (2H, m, C3-H_2), 2.33–2.51 (6H, m, C6-H_2, C4 (C4′-H_2) and C4 (C6′-H_2)), 1.91–2.05 (2H, m, C4 (C5′-H_2)). FAB LRMS: m/z 512.3 ($M+Na$)$^+$ 48%, 499.3 ($M+1$)$^+$ 17%, 353.2, 335.2 ($M-Ph(CH_2)_3CO_2$)$^+$, 198.1 ($C_6H_4C_6H_5CO_2+1$)$^+$, 196.1 ($C_6H_4C_6H_5CO_2-1$)$^+$, 181.1 ($C_6H_4C_6H_5CO$)$^+$ 100%, 165.1 ($C_6H_4C_6H_5C$)$^+$, 121.1 ($PhCO_2$)$^+$, 119.1. FAB HRMS: m/z exact mass calculated for $C_{31}H_{30}NaO_6$ 521.1940; found, 521.1940.

Antimicrobial procedures

The antimicrobial activity of each compound was assessed using an established standard disc-plate method.[35] Sabourands agar (Oxoid Products Ltd) was prepared according to the manufacturers instructions, then dispensed in 20 mL amounts into glass universal bottles and these were autoclaved at 121 °C and 15 lb for 15 min. The molten agar was poured into agar plates (Sterilin). Each strain was inoculated into 10 mL of Sabourands broth (prepared according to manufacturers instructions) and incubated at the appropriate temperatures (25 °C for *S. pombe* and *S. cerevisiae*, 37 °C for *C. albicans* for 12 h. After incubation the resultant broth culture was diluted in sterile saline to produce a 1/100 dilution. Aseptically, a 0.1 mL aliquot of this dilution was pipetted onto the agar plate and carefully spread around the agar surface. A sterile 4 mm paper disc was placed on the centre of agar plate and then the test compound (20 μL of a 10, 1, 0.1 or 0.01 mg/mL in absolute ethanol or water with final concentrations of 200, 20, 2, 0.2 μg, respectively) was inoculated onto the sterile paper disc. These plates were incubated at the appropriate temperatures 25 °C for *S. pombe* and *S. cerevisiae*, 37 °C for *C. albicans* for 24 h. To investigate the antimicrobial activity of the test compounds against bacteria, the previous procedure was used, however, nutrient agar (LAB M) and nutrient broth (LAB M) were the preferred growth media. Incubation of the bacterial species was conducted at 37 °C for 24 h. An antibiotic assay 'ring', which contains several known potent anti-bacterial agents (Novobiocin, Penicillin G, Streptomycin, Tetracyline, Chloroamphenicol, Erythromycin, Fusidic acid and Methicillin), was used as the control in these assays (individual discs contain 1 unit). Zones of inhibition (~5–8 mm) were observed for these compounds.

Pig liver SQS bioassay (HPLC-based)[37]

Pig liver was used as the mammalian source of SQS, transported at 0 °C and used within 24 h. Liver samples were perfused with HEPES buffer pH 7.5 to rinse the liver of excess blood whilst cooling the liver sample. The liver was minced and two volumes of pH 7.5HEPES lysis buffer (containing 1 mM PMSF, 3 mM DTT and 5.5 mM $MgCl_2$) were added. PMSF was included to decrease the rate of proteolysis in the samples. Metalloproteinase inhibitors, including EDTA, are metal chelators. Such compounds could not be used in this

analysis, since the SQS requires Mg^{2+} for its activity. Metalloproteinases remained active during these analyses. The minced sample was homogenised for 10 min, then divided into three aliquots and stored overnight at −80 °C. The frozen liver samples were slowly thawed, on ice, to limit any further protease activity. Stock solutions of KF, NADPH, NB-598 and test compounds were prepared. All enzyme inhibitors were dissolved in DMSO and added to enzyme preparations to give final concentrations of 3.2 nM (NB-598) and 10 μM (SQS test compounds and S1), respectively. Inhibition assays of each test compound were run in duplicate. Homogenate samples were divided into aliquots of 2 mL and placed into glass vials. The stock solutions of KF (KF is an inhibitor of phosphatase enzymes and added to limit farnesyl pyrophosphate degradation) and NADPH were added to give final concentrations of 11 and 1 mM, respectively. NB-598 is a potent squalene epoxidase inhibitor and was included so as to prevent squalene consumption during the assay. All potential inhibitors were compared to S1 and a control containing no inhibitor. Assay initiation was performed by addition of farnesyl pyrophosphate (5 μM). The SQS enzyme solutions were then shaken for 1 h at 37 °C. The enzyme reaction was stopped by addition of 10% KOH (1 mL) to each sample vial and thoroughly mixed by vortexing. Saponification: Failure to saponify liver samples resulted in low recoveries of squalene. The addition of a base dissolves rather than precipitates membrane proteins, thereby facilitating squalene release. Methanol (1 mL) was added to each sample to aid saponification, followed by heating to 60 °C for 90 min. Extraction of squalene and other non-polar metabolites: Heptane (15 mL) was added to the saponified liver homogenate sample (4 mL) and vortexed for 1 min, shaken for 5 min and then vortexed for a further 2 min. This ensured that the majority of squalene in the sample was transferred to the organic phase. Heptane removal and sample pretreatment for HPLC: Prior to HPLC analysis heptane was removed from each sample at 50 °C under a steady stream of nitrogen and then replaced with propan-2-ol (2 mL); a solvent previously used in our research for the dissolution of squalene.[37] The sample was then filtered to remove any particulate matter prior to HPLC analysis. A standard squalene sample was run by HPLC. Squalene (20 μM) on an ODS HPLC column at 30 °C had a retention time of 32 min. Duplicate extracted samples were analysed without SQS inhibitor (peak area; 57,977, ±2.85%). The S1 control inhibitor caused a reduction in the squalene concentration (peak area; 41,994, ±1.92%) equating to a 27.5% decrease in squalene. The inhibition results were compared directly to both control experiments.

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References and Notes

1. (a) For two excellent reviews in this area, see: Nadin, A.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1622. (b) Biller, S. A.; Neuenschwander, K.; Ponpipom, M. M.; Poulter, C. D. *Curr. Pharm. Des.* **1996**, *2*, 1.
2. Grunler, J. *Biochem. Biophys. Acta* **1994**, *12*, 259.
3. (a) Bhide, R. S.; Patel, D. V.; Patel, M. M.; Robinson, S. P.; Hunihan, L. W.; Gordon, E. M. *Biorg. Med. Chem. Lett.* **1994**, *4*, 2107. (b) Clarke, S. *Ann. Rev. Biochem.* **1992**, *61*, 355. (c) Schafer, W. R.; Rine, J. *Annu. Rev. Genet.* **1992**, *30*, 209. (d) Omer, C. A.; Gibbs, J. B. *Mol. Microbiol.* **1994**, *11*, 219. (e) Casey, P. J.; Seabra, M. C. *J. Biol. Chem.* **1996**, *271*, 5289.
4. (a) Biller, S. A.; Sofia, M. J.; Delange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Cioset, C. P., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8522. (b) Iwasawa, Y.; Hayashi, M.; Namoto, T.; Shibata, J.; Mitsuya, M.; Hirata, K.; Yonemota, M.; Kamai, T.; Miara, K.; Tomimoto, K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1989.
5. Gu, P.; Ishii, Y.; Spencer, T. A.; Shechter, I. *J. Biol. Chem.* **1998**, *273*, 12515.
6. Sidebottom, P. J.; Highcock, R. M.; Lane, S. J.; Procopiou, P. A.; Watson, N. S. *J. Biol. Chem.* **1992**, *45*, 648.
7. (a) Bergstrom, J. D.; Kurtz, M. M.; Rew, D. J.; Amend, A. M.; Karkas, J. D.; Bostedor, R. G.; Bansal, V. S.; Dufresne, C.; Van Middlesworth, F. L.; Hensens, O. D.; Liesch, J. M.; Zink, D. L.; Wilson, K. E.; Onishi, J.; Milligan, J. A.; Bills, G.; Kaplan, L.; Omstead, M. N.; Jenkins, R. G.; Huang, L.; Meinz, M. S.; Quinn, L.; Burg, R. W.; Kong, Y. L.; Mochales, S.; Mojena, M.; Martin, I.; Pelaez, F.; Diez, M. T.; Alberts, A. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 80. (b) Dufresne, C.; Wilson, K. E.; Zink, D. L.; Smith, T.; Bergstrom, J. D.; Kurtz, M.M; Rew, D.; Nallin-Omstead, M.; Jenkins, R. G.; Bartizal, K.; Trainor, C.; Bill, G.; Meinz, M.; Huang, L.; Onishi, J.; Milligan, J.; Mojena, M.; Pelaez, F. *Tetrahedron* **1992**, *48*, 10221.
8. (a) Carreira, E. M.; Du Bois, J. *J. Am. Chem. Soc.* **1994**, *116*, 10825. (b) Nicolaou, K. C.; Nadin, A.; Leresche, J. E.; Yue, E. W.; La Greca, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2190. (c) Evans, D. A.; Barrow, J. C.; Leighton, J. L.; Robichaud, A. J.; Sefkow, M. *J. Am. Chem. Soc.* **1994**, *116*, 12111.
9. Procopiou, P. Private communication, 1998.
10. Pandit, J.; Danley, D. E.; Schulte, G. K. *FASEB J.* **1999**, *13*, (Suppl. S), A1338.
11. (a) Pandit, J.; Thompson, J. F. Private communication. Pfizer USA Ltd., Groton (USA), Apr, 1999. (b) Harwood, H. J. Private communication. Pfizer Ltd., Groton (USA), Sept, 1999.
12. Pandit, J.; Danley, D. E.; Schulte, G. K.; Mazzalupo, S.; Pauly, T. A.; Hayward, C. M.; Hamanaka, E. S.; Thompson, J. F.; Harwood, J., Jr. *J. Biol. Chem.* **2000**, *275*, 30610.
13. Harwood, J., Jr.; Barbacci-Tobin, E. G.; Petras, S. F.; Lindsay, S.; Pellarin, L. D. *Biochem. Pharmacol.* **1997**, *53*, 839.
14. Leach, A. R. *Molecular Modelling, Principles and Applications*; Addison-Wesley-Longman: 1996; Chapter 10, p 543.
15. Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. *Computer-Assisted Drug Design*; ACS Symp. Series 112; American Chemical Society: Washington, DC, 1979, p 205.
16. (a) Byberg, J. R.; Jorgensen, F. S.; Klemmensen, P. D. *J. Comput. Aided Mol. Des.* **1987**, *1*, 181. (b) Gund, P. *Ann. Rep. Med. Chem.* **1979**, *14*, 299. (c) Humblet, C.; Marshall, G. R. *Ann. Rep. Med. Chem.* **1980**, *15*, 267.

17. (a) Harris, G. H.; Zink, D. L.; Jones, E. T.; Kong, Y. L. US Patent 5364948, 1994. (b) Mandela, S. M.; Thornton, R. A.; Frommer, B. R.; Dreikorn, S.; Kurtz, M. B. *J. Antibiot.* **1997**, *50*, 339.
18. Iwasawa, Y.; Shibata, J.; Mitsuya, M.; Masaki, H.; Hayashi, M.; Kanno, T.; Sawasaki, Y.; Hisaki, A.; Kamai, T.; Tomimoto, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 463.
19. Brown, G. R.; Clarke, D. S.; Foubister, A. J.; Freeman, S.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McCormick, J.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J. *J. Med. Chem.* **1998**, *41*, 2971.
20. The X-ray crystal structure of Squalestatin S1 was minimised at the PM3 level of theory. The core bicyclic structure was used as a template to which rigid farnesyl mimics—biphenyl and phenyl moieties could be easily attached. These structures were further minimised until the global energy minima was attained. Calculations were performed using MOPAC 6.0 and Quanta^{TM} on an Indy Silicon Graphics Machine.
21. Sheridan, R. P.; Nilakantan, R.; Dixon, J. S.; Venkataraghavan, R. *J. Med. Chem.* **1986**, *29*, 899.
22. It was cumbersome to determine the lowest energy minimum for these structures at the AM1 or PM3 level (MOPAC 6.0), due to the large amount of atoms involved in the semi-empirical calculations. Rigorous molecular mechanics (MM) calculations were performed several times, generating LEM structures. Calculations were performed using MOPAC 6.0 and Quanta^{TM} on an Indy Silicon Graphics workstation.
23. Mubarik Ali, S.; Crossland, N. M.; Lee, T. V.; Roberts, S. M.; Newton, R. F. *J. Chem. Soc., Perkin Trans. 1* **1979**, 122.
24. Brown, G. R.; Clarke, D. S.; Foubister, A. J.; Freeman, S.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McCormick, J.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J. *J. Med. Chem.* **1998**, *41*, 2971.
25. (a) Fairlamb, I. J. S. PhD thesis, Manchester Metropolitan University, UK, 2000. (b) Fairlamb, I. J. S.; Dickinson, J. M.; Cristea, I. M. *Tetrahedron Lett.* **2000**, *41*, 3739. (c) Fairlamb, I. J. S.; Dickinson, J. M.; Cristea, I. M. *Tetrahedron* **2001**, *7*, 2237.
26. Brady, W. T.; Roe, R., Jr. *J. Org. Chem.* **1970**, *35*, 1515.
27. (a) Yamabe, S.; Dai, T.; Minato, T.; Machiguchi, T.; Hasegawa, T. *J. Am. Chem. Soc.* **1996**, *118*, 6518. (b) Machiguchi, T.; Hasegawa, T.; Ishiwata, A.; Terashima, S.; Yamabe, S.; Minato, T. *J. Am. Chem. Soc.* **1999**, *121*, 4771.
28. Newton, R. F.; Roberts, S. M. *Tetrahedron* **1980**, *36*, 2163 and references cited therein.
29. (a) Rahman, S. S.; Wakefield, B. J.; Roberts, S. M.; Dowle, M. D. *Chem. Commun.* **1989**, 303. (b) Rahman, S. S. PhD Thesis, University of Salford, UK, 1988.
30. The initial minimised structures were attained at the MM level, followed by further minimisation at the PM3 level in an artificial box of solvent (CH_2Cl_2) at 25 °C. Several separate minimisations gave the *exo* envelope as the lowest energy conformation.
31. (a) Corey, E. J.; Weinshenker, N. M.; Schaaf, T. K.; Huger, W. *J. Am. Chem. Soc.* **1969**, *91*, 5675. (b) Corey, E. J.; Schaaf, T. K.; Huger, W.; Koelliker, U.; Weinshenker, N. M. J. *Amer. Chem. Soc.* **1970**, *92*, 397. (c) Corey, E. J.; Albocino, S. M.; Koelliker, U.; Schaaf, T. K.; Varma, R. K. *J. Am. Chem. Soc.* **1971**, *93*, 1491.
32. Tomoskozi, I.; Gruber, L. *Tetrahedron Lett.* **1976**, *50*, 4639.
33. Newton, R. F.; Hallett, P.; Paton, J.; Reynolds, D. P.; Young, S.; Green, S. M.; Roberts, S. M.; Wallace, T. W.; Dawson, M. J.; Lawrence, G. C.; Lilley, G.; Todd, W.; Noble, D. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2119.
34. Compound **32** is available commercially in racemic and enantiomerically pure form.
35. National Committee for Clinical Laboratory Standards, 1992. Reference method for broth dilution for anti-fungal susceptibility testing against yeasts. National Committee for Clinical Laboratory Standards, Villanova, PA, USA.
36. Dufresne, C.; Wilson, K. E.; Zink, D.; Smith, J.; Bergstrom, J. D.; Kurtz, M.; Rew, D.; Nallin, M.; Jenkins, R.; Bartizal, K.; Trainor, C.; Bills, G.; Meinz, M.; Huang, L.; Onishi, J.; Milligan, J.; Mojena, M.; Pelaez, F. *Tetrahedron* **1992**, *48*, 10221.
37. By adaption of the HPLC method previously reported: Grieveson, L. A.; Ono, T.; Sakakibara, J.; Derrick, J. P.; Dickinson, J. M.; McMahon, A.; Higson, S. P. J. *Anal. Biochem.* **1997**, *252*, 19.
38. Brady, W. T.; Smith, L. *J. Org. Chem.* **1971**, *36*, 1637 and references cited therein.