Dioxidosqualenes: Characterization and Activity as Inhibitors of 2,3-Oxidosqualene–Lanosterol Cyclase[1]

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The preparation and characterization of dioxidosqualenes **4–10** is reported. Treatment of the appropriate epoxysqualene **1**, **2**, or **3** with NBS followed by chromatographic purification afforded the corresponding epoxybromohydrins **11–14** as diastereomeric mixtures, with the exception of compound **11**, which could be separated into the respective racemates **11a** and **11b**. Further dehydrobromination with NaH in THF led to the respective dioxidosqualenes **4–8** in good conversion yields. Dioxides **9** and **10** were isolated from the crude reaction mixture of the treatment of epoxide **2** with dimethyldioxirane. Characterization of compounds **4–10** was carried out by combining ^1H and ^{13}C NMR spectral means with positive GC–MS–CI analysis. The GC–MS–CI analysis included the identification of the carbonyl compounds resulting from the cleavage of dioxido derivatives **4–10** with periodic acid. Finally, data on the activity of dioxidosqualenes as oxidosqualene–lanosterol cyclase (OSLC) inhibitors in rat liver microsomes are also presented. In this respect, 2,3:18,19-dioxidosqualene (**7**) was found to be the best inhibitor within the compounds assayed (IC_{50} = 0.11 μM), although dioxides **4**, **5**, and **9** also exhibited a potent inhibitory activity (IC_{50} = 21.3, 13.0, and 9.3 μM, respectively). The fact that these compounds could be potentially generated in an organism constitutes a remarkable difference relative to other OSLC inhibitors described to date.

Introduction

(3*S*)-2,3-Oxidosqualene (**1**) undergoes a fascinating cyclization reaction to give lanosterol in a process that constitutes the main biosynthetic entry to the sterol skeleton in mammals and fungi.[2] This cyclization is mediated by 2,3-oxidosqualene–lanosterol cyclase (OSLC, EC 5.4.99.7).[3] Although the mechanism of this multistep transformation has been the subject of intense debate, it seems that the membrane-associated enzyme binds substrate **1** folded into a chair–boat–chair conformation and then mediates the sequential formation of four carbon–carbon bonds and backbone rearrangements leading to the lanosterol molecule.[4] On the other hand, the ability of OSLC to bind and cyclize a variety of substrates other than epoxide **1** has been used for the large-scale production of sterol analogs[5] or for studies directed to the elucidation of the cyclization mechanism.[4b,6]

Inhibition of OSLC has constituted an attractive approach for the development of potential hypocholesteremic agents, since this enzymatic step is beyond the synthesis of other important terpenoid biomolecules such as dolichol and ubiquinones. In this respect, OSLC inhibitors described so far include substrate mimics, product mimics, transition-state analogs, and irreversible inactivators.[4a,7] Some of these inhibitors are structurally related to squalene or to its 2,3-epoxy derivative **1**. A general strategy followed for obtaining potent mechanism-based enzyme inactivators has been the introduction of structural variations in the vicinity of the different trisubstituted carbon–carbon double bonds present in the squalene skeleton, with a particular emphasis on the C-18,C-19 region.[7,8] In this context, it could be anticipated that a second epoxide moiety replacing a carbon–carbon double bond in the natural substrate **1** could interfere in the cyclization process thus originating an inhibition effect. This possibility was attractive in the sense that dioxidosqualenes could be present as endogenous compounds resulting from potential overoxidation of squalene under certain physiological conditions. However, there has not been a systematic study on the activity of dioxidosqualenes as OSLC modulators. In fact, from the different dioxidosqualenes, only the 2,3:22,23-dioxidosqualene (**8**) had been previously described.[9] This compound exhibited a moderate angiotoxic activity in rabbits,[9b] and recently its formation from **1** with partially purified pig liver squalene epoxidase and its activity as inhibitor of crude and purified pig liver OSLC (IC_{50} = 16 μM) has been also reported.[10]

With these antecedents, the present paper reports the preparation and characterization of dioxidosqualenes **4–10** (Scheme I). Results on the biological activity of these compounds as rat liver microsomal OSLC inhibitors are also discussed.[11]

(1) Abbreviations used: OSLC, oxidosqualene–lanosterol cyclase; DMD, 3,3-dimethyldioxirane; MTBE, methyl *tert*-butyl ether.

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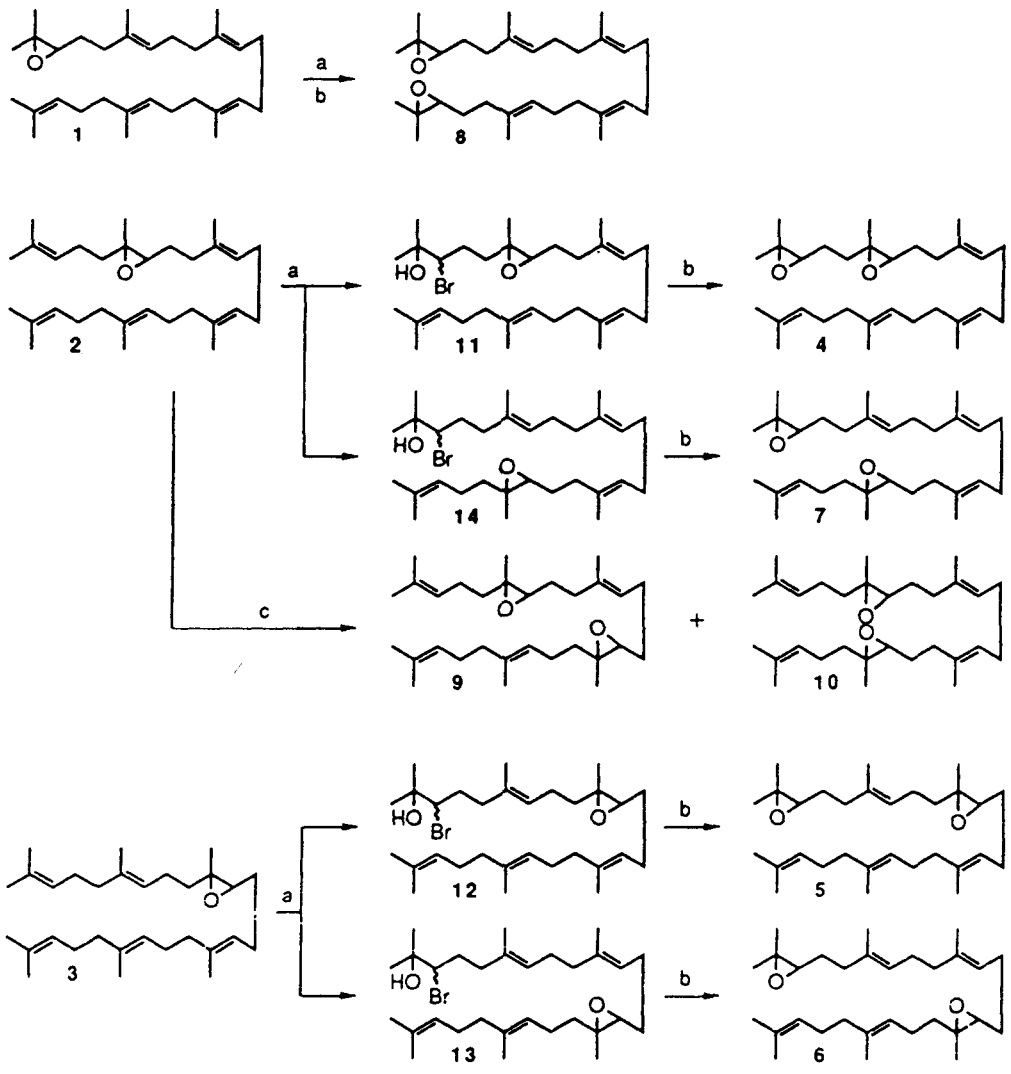
0022-3263/93/1958-3991$04.00/0

Scheme I[a]



[a] Reaction conditions: (a) NBS/THF/H_2O; (b) NaH/THF; (c) DMD/acetone/CH_2Cl_2. For details on purification procedures, see Experimental Section.

Results and Discussion

Preparation of Dioxidosqualenes. The procedures used for the preparation of the dioxidosqualenes **4–10** are shown in Scheme I. Since we were interested in carrying out preliminary screening assays on the activity of these compounds as OSLC inhibitors, our aim was the isolation of the different dioxidosqualenes as diastereomeric mixtures, and no procedures for their stereoselective formation were initially contemplated. Preparation of dioxides **4–8** was carried out by treatment of oxidosqualenes **1**, **2**, or **3**,[8] as appropriate, with NBS in THF/H_2O to give the corresponding epoxybromohydrins **11–14**, followed by dehydrobromination with NaH in THF. The observed attack of NBS predominantly at the terminal C=C bonds of the corresponding epoxysqualene skeleton under the conditions assayed and the better purification of the crude mixtures achieved at the epoxybromohydrin stage favored the election of this synthetic route in comparison with that involving the direct epoxidation of compounds **1–3** with peroxy acids or dimethyldioxirane (DMD, results not shown). Epoxybromohydrins **11** and **14** were obtained from epoxide **2**. In this case, the diastereomeric mixture **11** could be separated by chromatographic means to give epoxybromohydrins **11a** (less polar in TLC) and **11b**, although their relative stereochemistry was not determined. On the other hand, treatment of epoxide **3** with NBS led to a mixture of epoxybromohydrins from which compounds **12** and **13** could be isolated as diastereomeric mixtures. Conversion of epoxybromohydrins **11–14** into the corresponding dioxido derivatives was achieved with good conversion yields, and compounds **4–7** were purified by chromatography on silica gel. In the case of dioxides **4a** or **4b**, this purification was carried out with silica gel impregnated with triethylamine to minimize decomposition of the compounds. As it has been shown elsewhere, dioxido compounds formally derived from vicinal double bonds of terpenoid structures are rather unstable.[12]

Internal dioxido derivatives **9** and **10** were isolated from the treatment of epoxide **2** with DMD. This reaction afforded a mixture of polyoxidosqualenes from which dioxides **4**, **7**, **9**, and **10** and unreacted **2** were the major components. A first chromatographic separation afforded pure compounds **2**, **4** (the most polar dioxide), and **10** (the less polar dioxide), whereas a further chromatography on silica gel impregnated with silver nitrate was required for the separation of dioxides **7** and **9**.

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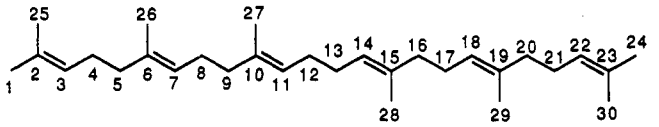
Table I. ^{1}H NMR Chemical Shifts in ppm Downfield from TMS of Selected Signals Corresponding to Oxidosqualenes 1–3 and Dioxidosqualenes 4–10[a]



	1	2	3	4a	4b	5	6	7	8	9	10
H(1)	1.30 (s)	1.68 (s)	1.68 (s)	1.31 (s)	1.31 (s)	1.30 (s)	1.30 (s)	1.30 (s)	1.30 (s)	1.68 (s)	1.68 (s)
H(3)	2.71 (t)[b]	5.19 (m)	5.10 (m)	2.73 (m)	2.73 (m)	2.70 (t)	2.71 (t)	2.71 (t)	2.71 (t)	5.20 (m)	5.18 (m)
H(7)	5.12 (m)	2.70 (t)	5.16 (m)	2.70 (t)	2.72 (t)	5.11 (m)	5.13 (m)	5.15 (m)	5.13 (m)	2.70 (t)	2.71 (t)
H(11)	5.12 (m)	5.10 (m)	2.73 (t)	5.11 (m)	5.12 (m)	2.73 (t)	5.13 (m)	5.15 (m)	5.13 (m)	5.08 (m)	5.08 (m)
H(14)	5.12 (m)	5.10 (m)	5.10 (m)	5.11 (m)	5.12 (m)	5.11 (m)	2.73 (t)	5.15 (m)	5.13 (m)	2.73 (t)	5.08 (m)
H(18)	5.12 (m)	5.10 (m)	5.10 (m)	5.11 (m)	5.12 (m)	5.11 (m)	5.13 (m)	2.71 (t)	5.13 (m)	5.08 (m)	2.71 (t)
H(22)	5.12 (m)	5.10 (m)	5.10 (m)	5.11 (m)	5.12 (m)	5.11 (m)	5.13 (m)	5.15 (m)	2.71 (t)	5.08 (m)	5.08 (m)
H(24)	1.68 (s)	1.68 (s)	1.68 (s)	1.68 (s)	1.68 (s)	1.68 (s)	1.68 (s)	1.68 (s)	1.30 (s)	1.68 (s)	1.68 (s)
H(25)	1.26 (s)	1.60 (s)	1.60 (s)	1.26 (s)	1.27 (s)	1.26 (s)	1.26 (s)	1.26 (s)	1.26 (s)	1.60 (s)	1.61 (s)
H(26)	1.62 (s)	1.25 (s)	1.60 (s)	1.28 (s)	1.27 (s)	1.62 (s)	1.62 (s)	1.62 (s)	1.62 (s)	1.25 (s)	1.25 (s)
H(27)	1.60 (s)	1.62 (s)	1.26 (s)	1.62 (s)	1.62 (s)	1.26 (s)	1.62 (s)	1.60 (s)	1.60 (s)	1.64 (s)	1.61 (s)
H(28)	1.60 (s)	1.60 (s)	1.62 (s)	1.60 (s)	1.60 (s)	1.62 (s)	1.26 (s)	1.62 (s)	1.60 (s)	1.25 (s)	1.61 (s)
H(29)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.25 (s)	1.62 (s)	1.60 (s)	1.25 (s)
H(30)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.60 (s)	1.26 (s)	1.60 (s)	1.61 (s)

[a] The methylene protons α to double bond appeared as a complex signal (1.8–2.3). The methylene protons α to epoxide appeared as a complex signal (1.2–1.7). [b] The coupling constants for the hydrogen atom linked to the epoxide ranged from 6.0 to 6.5 Hz.

Characterization of Dioxidosqualenes. Structural identification of the different dioxido derivatives 4–10 was carried out by a combination of ^{1}H and ^{13}C NMR and GC–MS-CI methods. For the interpretation of NMR spectra, information related to epoxides 1–3 obtained in this laboratory and elsewhere[13] was used.

^{1}H NMR Spectra. Table I shows the diagnostic ^{1}H NMR signals of compounds 4–10. The presence of two epoxides in these molecules was inferred from the relative integrations of peaks assigned to the olefin protons, the hydrogen atoms of the epoxide rings, and the CH_3 groups bound to the epoxide rings. On the other hand, analysis of the chemical shifts of the epoxide protons, the CH_3 groups linked to the epoxide and to the olefin moieties in squalene and in monoepoxides 1–3 permitted the unequivocal assignation of these signals for each oxidosqualene. With these data, the different dioxidosqualenes 4–10, with the exception of 5 and 6, could be then identified.

Thus, the resonances from CH_3 groups permitted us to distinguish among dioxido derivatives with an oxirane ring in a terminal position (4–8) and the others (9 and 10). In addition, dioxide 8 could be differentiated from compounds 4–7. On the other hand, chemical shifts of hydrogen atoms from oxirane rings permitted the differentiation of dioxides 5, 6, and 9 from 7, 8, and 10, which would allow the distinction of 7 from 5 and 6 and of 9 from 10. Finally, the complexity of the absorption due to the oxirane hydrogen atom was characteristic in dioxides 4. COSY experiments performed with dioxides 4a and 4b, which showed only one coupling pattern among CH_2 protons α to epoxide with those α to a double bond, supported the identification of these diastereomers.

^{13}C NMR Spectra. Table II shows the ^{13}C NMR signals of dioxidosqualenes 4–10. In this case, the comparison of the spectra of squalene and monoepoxides 1–3 revealed the importance of the β-effect exerted by an oxirane ring on a CH_2 moiety. The presence of this effect, which accounts for a 3–3.5 ppm upfield shift in all cases, was used to confirm the identification of the different dioxidosqualenes. Thus, compounds bearing the epoxide ring at the third terpene unit (i.e., 5, 6, and 9) showed a β-effect on the characteristic CH_2 group from the tail-to-tail linkage region, together with the shift corresponding to the β-CH_2 present at the other side of the oxirane. When the epoxide was at the second terpene unit, the observed shifts corresponded to CH_2 groups linked to a CH= moiety and to a quaternary carbon atom. As expected, this latter shift was the only one observed for CH_2 groups in β to terminal epoxides. Concerning the CH_3 groups, the *cis*-CH_3 linked to internal oxirane rings appeared at 16.5–16.6 ppm, whereas those linked to C-2 appeared at 18.7 ppm. This latter signal was absent in dioxido derivatives 9 and 10. Finally, compounds 5 and 6 could only be differentiated by the higher complexity exhibited by the spectra of 5, which was attributed to the proximity of the oxirane moieties in this dioxide.

GC–MS-CI Analysis. The structural information obtained from the GC–MS-CI of the different dioxidosqualenes 4–10 was confirmed by the identification of the carbonyl compounds resulting from the cleavage of these dioxides with periodic acid. This reaction, which had been previously used for the cases of epoxides 2 and 3 in a preparative scale,[8,14] was performed on a microscale on compounds 4–10 to give the corresponding carbonyl derivatives 15–25 (Scheme II). Table III shows the characteristic fragment ions obtained from the GC–MS-CI analysis of these derivatives. Aldehydes resulting from the treatment of epoxides 1, 2, or 3 with periodic acid were also analyzed for comparison purposes.

Thus, the GC–MS-CI spectrum of epoxide 1 revealed the presence of a ion at m/z 153 (data not shown); this peak is characteristic of terpene units bearing a terminal oxirane.[15] On the other hand, cleavage of epoxide 1 led to the formation of aldehyde 15, while 2 afforded ketone 16 and aldehyde 17 and 3 gave geranylacetone (18) and aldehyde 19. Ketones 16 and 18 were identified by comparison with authentic standards, while aldehydes 15,

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(14) Xiao, X.-Y.; Prestwich, G. D. *Tetrahedron Lett.* **1991**, *32*, 6843–6846.

(15) This peak corresponds to a fragmentation at the allylic C8–C9 bond (see: Liedtke, R. J.; Djerassi, C. *J. Org. Chem.* **1972**, *37*, 2111–2119). For the presence of this peak in oxidosqualene derivatives, see: Katayama, M.; Marumo, S. *Tetrahedron Lett.* **1976**, *16*, 1293–1294.

Table II. ^{13}C NMR Chemical Shifts in ppm Downfield from TMS of Oxidosqualenes 1–3 and Dioxidosqualenes 4–10[a]

	1	2	3	4a	4b	5	6	7	8	9	10
C(1)	24.9	25.7	25.7	24.8	24.8	24.9	24.9	24.9	24.9	25.7	25.7
C(2)	58.3	131.7	131.3	58.4	58.4	58.3	58.2	58.3	58.3	131.8	131.8
C(3)	64.2	123.7	124.3	64.0	63.9	64.1	64.2	64.2	64.2	123.7	123.7
C(4)	27.4	23.8	26.6	24.7	24.6	27.4	27.5	27.5	27.4	23.9	23.9
C(5)	36.3	38.8	39.7	35.6	35.3	36.3	36.3	36.3	36.3	38.8	38.8
C(6)	133.9	60.7	135.9	60.4	60.3	134.6	134.1	134.0	133.9	60.8	60.8
C(7)	124.9	63.3	123.5	63.5	63.0	124.2*	124.8	124.9	124.8	63.3	63.3
C(8)	26.6	27.3	23.7	27.2	27.2	23.7	26.6	26.7	26.6	27.3	27.3
C(9)	39.6	36.3	38.9	36.3	36.3	38.8	39.6	39.7	39.6	36.3	36.3
C(10)	134.9	134.1	60.8	134.1	134.1	60.8	135.5	135.1	134.9	135.0	134.3
C(11)	124.2	124.8	63.3	125.0	125.0	63.3*	123.4	124.2	124.3	123.8*	124.7
C(12)	28.2	28.1	29.0	28.2	28.2	28.9	24.9	28.3	28.2	24.9	28.2
C(13)	28.2	28.2	24.8	28.3	28.3	24.9	29.0	28.2	28.2	28.9	28.2
C(14)	124.2	124.1	123.2	124.1	124.1	123.2	63.3	124.8	124.3	63.3	124.7
C(15)	134.9	134.8	135.4	134.9	134.9	135.9	60.7	134.2	134.9	60.8	134.3
C(16)	39.7	39.7	39.6	39.7	39.7	39.7	38.9	36.3	39.6	38.8	36.3
C(17)	26.6	26.6	26.6	26.6	26.6	26.6	23.7	27.3	26.6	23.8	27.3
C(18)	124.3	124.2	124.1	124.2	124.2	124.1	123.6	63.4	124.8	123.5	63.3
C(19)	135.1	135.2	135.0	135.3	135.3	135.0	135.8	60.8	133.9	135.5	60.8
C(20)	39.7	39.7	39.7	39.7	39.7	39.7	39.7	38.8	36.3	39.7	38.8
C(21)	26.7	26.7	26.7	26.7	26.7	26.7	26.7	23.9	27.4	26.7	23.9
C(22)	124.4	124.4	124.2	124.4	124.4	124.3	124.3	123.7	64.2	124.2	123.7
C(23)	131.2	131.8	131.2	131.3	131.3	131.3	131.3	131.8	58.3	131.4	131.8
C(24)	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	24.9	25.7	25.7
C(25)	18.7	17.6	17.6	18.7	18.6	18.7	18.7	18.7	18.7	17.6	17.6
C(26)	16.0	16.5	16.0	16.4	16.7	16.0	16.0	16.0	16.0	16.5	16.5
C(27)	16.0	16.0	16.6	16.0	16.0	16.6*	16.0	16.0	16.0	16.0*	16.0
C(28)	16.0	16.0	16.0	16.0	16.0	16.0	16.6	16.0	16.0	16.6	16.0
C(29)	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.5	16.0	16.0	16.5
C(30)	17.7	17.6	17.6	17.7	17.7	17.7	17.6	17.6	18.7	17.7	17.6

[a] An asterisk denotes the presence of a second peak at the same chemical shift which corresponds to the other diastereomer.

17, and 19 exhibited fragmentation patterns in the MS-CI spectra consistent with their structure (Table III).

Concerning the GC–MS-CI spectra of dioxidosqualenes 4–10, those dioxides bearing a terminal oxirane, i.e., compounds 5–8, showed the ion at m/z 153 (which was the base peak for dioxide 8). The other dioxido derivative with a terminal oxirane ring, i.e., compound 4, showed a different behavior. Although both diastereomers suffered extended decomposition in the chromatographic system giving rise to several peaks, all these peaks showed molecular ions at m/z 443 and a characteristic base peak at m/z 155. The instability of dioxide 4 led to unsuccessful results in the reaction with periodic acid.

Periodic acid cleavage of dioxido derivatives 5–10 led to reaction products which were in agreement with the structure of parent compounds. Thus, dioxido derivative 5 was the only one to give a ketoaldehyde (i.e., 20). This compound showed a characteristic base peak at m/z 93. This ion was only present, also as base peak, in the fragmentation pattern of 24, a dialdehyde originated from the cleavage of dioxide 9, the other compound with alternate terpene units bearing oxirane rings.

Dioxides 6, 7, and 8 afforded dialdehydes 21, 22,[16] 23,[17] respectively, as carbonyl products originated from the fragment of the parent dioxide comprised between the two oxirane rings. Identification of ketones 18 and 16 completed the structural assignation for parent dioxides 6 and 7, respectively. Finally, the presence of dioxide 10 was confirmed by the identification of dialdehyde 25 and ketone 16 as cleavage products.

Inhibition of OSLC Activity. OSLC assays with oxido and dioxido derivatives 2–10 were carried out by using rat liver microsomes, and the amount of lanosterol formed was quantified by GC. Results obtained on the inhibition of OSLC activity are shown in Table IV. Although for the case of dioxides these assays were performed with the diastereomeric mixtures, some conclusions could be drawn. As shown, dioxide 7 was the most potent inhibitor of the compounds tested (IC_{50} = 0.11 μM), being 800-fold more active than epoxide 2. This activity is comparable to that reported for the most potent OSLC inhibitors described so far.[7] From the kinetic data obtained it was shown that inhibition elicited by compound 7 was essentially noncompetitive.[11] In addition to the potency of the inhibitory effect elicited by dioxido derivative 7, results obtained from the activity of this dioxide deserve further comments. First, it can be expected that the inhibitory activity observed would be due, at least, to diastereomers with the (3*S*) configuration of the OSLC natural substrate, although a stereochemical requirement at C-18,C-19 could also be necessary. Therefore, the activity of the stereoisomer responsible for the inhibition effect would be even higher, and work along this line is now under investigation. On the other hand, the occurrence of epoxide 2 in nature[18] raises the possibility of formation of dioxide 7 under certain physiological conditions, which could give a particular importance to the activity herein reported for this compound. This aspect is also under current research in our laboratory.

Compounds 5 and 9, the two dioxido derivatives with the oxirane rings in alternate terpene units, exhibited an OSLC inhibitory activity which is also within the order of that found for some of the most potent inhibitors reported in the literature.[8,19] It is worth noting that epoxide 3, which could be considered as a putative precursor of dioxides 5 and 9, showed a poor inhibitory activity under our assay conditions. By contrast, when two terpene units were intercalated between those bearing the oxirane rings,

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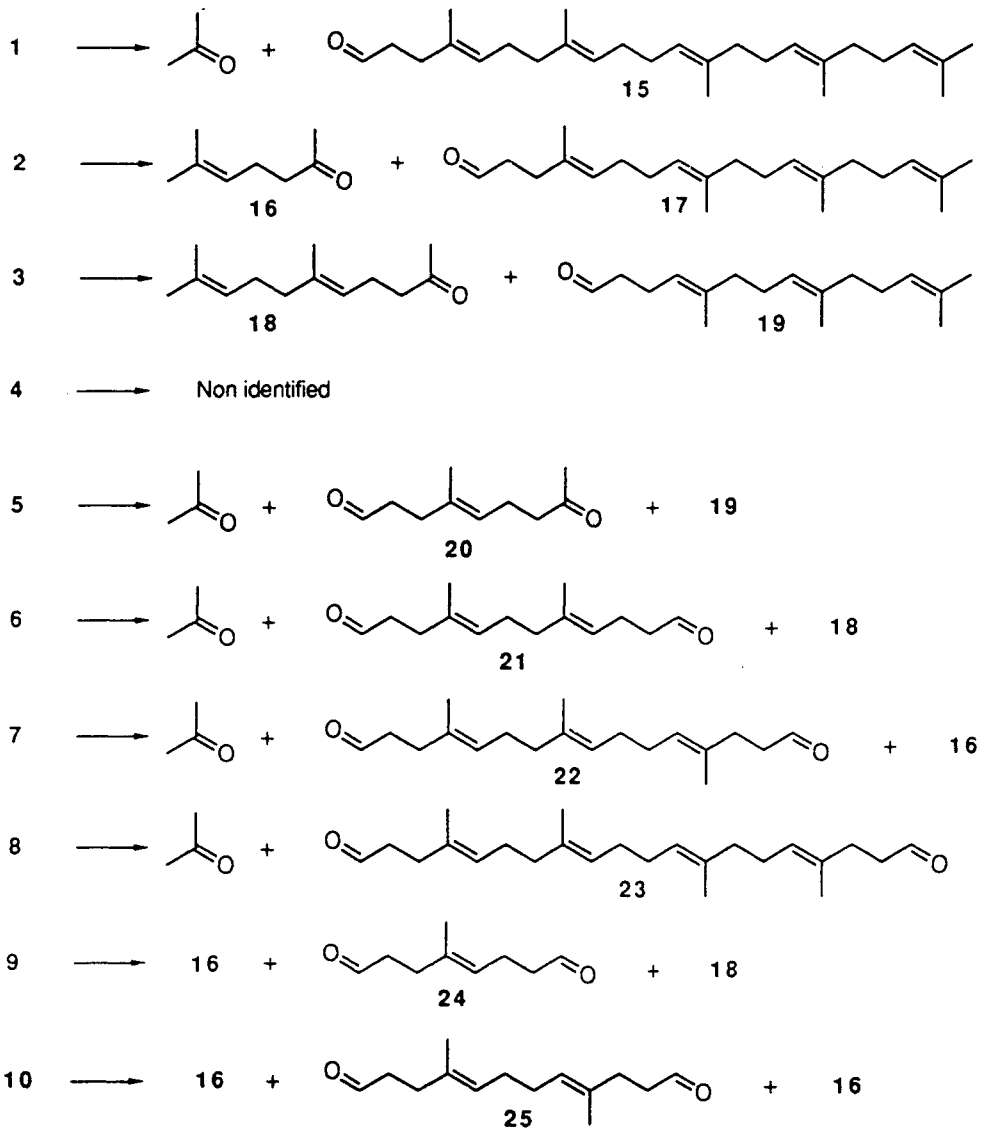
Scheme II[a]



[a] Reaction conditions: H_5IO_6 in diethyl ether at 20 °C.

Table III. Characteristic Fragment Ions Obtained in the GC–MS-CI (Positive Mode, CH_4) Analyses of the Carbonyl Compounds Formed from the Treatment of Oxido- and Dioxidosqualenes 1–10 with H_5IO_6 (cf. Scheme II)

compd	m/z
15	413 (M + 29), 385 (M + 1), 383 (M − 1), 367, 137[a]
16[b]	127 (M + 1), 109, 56[a]
17	345 (M + 29), 317 (M + 1), 315 (M − 1), 299, 137[a]
18[b]	195 (M + 1), 193 (M − 1), 177[a]
19	249 (M + 1), 247 (M − 1), 231, 137[a]
20	197 (M + 29), 169 (M + 1), 151, 93[a]
21	223 (M + 1), 205, 187, 111[a]
22	319 (M + 29), 291 (M + 1), 273, 255, 111[a]
23	359 (M + 1), 341, 111[a]
24	137, 93[a]
25	223 (M + 1), 205, 187, 111, 107[a]

[a] Base peak. [b] The fragmentation pattern was coincident with that showed by authentic standards.

i.e., dioxides **6** and **10**, the compounds were inactive as OSLC inhibitors. Finally, compound **4** exhibited a moderate OSLC inhibitory activity. In this case, biological assays were also carried out with the mixture of four diastereomers, and it was observed that dioxide **4**, in spite of the instability shown during the purification procedures,

Table IV. Inhibition of Oxidosqualene–Lanosterol Cyclase (OSLC) Elicited by Racemic Oxido- and Dioxidosqualenes in Rat Liver Microsomes[a]

compd	IC_{50} (μM)	compd	IC_{50} (μM)
2	83.5[b]	7	0.11[b]
3	220	8	142[b]
4	21.3	9	9.3
5	13.0	10	c
6	c		

[a] For assay details and concentrations of inhibitors used, see Experimental Section. [b] Values taken from ref 11. [c] No dose-effect response was obtained within the concentration range assayed with this compound (1–40 μM).

remained unaltered in the presence of the buffer solution used for the inhibition assay.

In summary, dioxidosqualenes **4**, **5**, **9**, and particularly **7** have shown an inhibitory activity within the range of the best OSLC inhibitors reported to date, and these compounds are of additional interest in that, in contrast

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to the later inhibitors, they can be potentially generated by the organism itself.[10,20]

Experimental Section

Apparatus. All NMR spectra were performed in neutralized $CDCl_3$ solutions, and chemical shifts are given in ppm downfield from tetramethylsilane. The gas chromatography–mass spectrometry analyses with positive chemical ionization (GC–MS-CI) were performed with a Hewlett-Packard 5890 chromatograph coupled to a Finnigan MAT Model Incos XL mass spectrometer, using methane as ionization gas and a 30-m HP-5 bonded-phase capillary column (0.25-mm i.d.). Elemental analyses were performed with a Carlo Erba 1108 instrument (Microanalysis Service, CID).

Compounds. All squalene derivatives synthesized were isolated as diastereomeric mixtures with the exception of epoxybromohydrins **11a** and **11b** and dioxido derivatives **4a** and **4b**, which were obtained as a racemic mixture. For the sake of clarity, those absorptions observed in the ^{13}C NMR spectra attributed to the same carbon atom of different diastereomers are marked with an asterisk. Unless otherwise stated, organic solutions obtained from workup of crude reaction mixtures were dried over $MgSO_4$. 2,3-Oxidosqualene (**1**)[9a] was prepared by treatment of squalene with NBS in THF/H_2O[21] followed by reaction of the purified bromohydrin with NaH in THF. 6,7-Oxidosqualene (**2**) and 10,11-oxidosqualene (**3**) were prepared by epoxidation of squalene with *m*-CPBA as described elsewhere.[8] Similar results were obtained when the epoxidation was carried out with DMD in acetone/CH_2Cl_2. In both cases, separation of epoxides from epoxide **1** was achieved by preparative TLC (12:1 hexane/MTBE, three elutions). Then, compounds **2** and **3** were separated by flash chromatography on silica gel impregnated with 10% $AgNO_3$ (5:1 toluene/EtOAc).

Preparation of Intermediate Bromohydrins 11–14.[22] A solution of epoxide **2** (90 mg, 0.2 mmol) in a 5:1 THF/H_2O mixture (6 mL) maintained at 0 °C was treated with a solution of NBS (1 mol equiv) in THF (0.5 mL) and the mixture was stirred for 2 h at the same temperature. Removal of THF under vacuum led a residue which was extracted with hexane, dried, and evaporated. Flash chromatography on silica gel (95:5 hexane/MTBE) of the residue obtained after solvent removal gave epoxybromohydrin **14** (15 mg, 14%), epoxybromohydrin **11a** (6 mg, 6%), and epoxybromohydrin **11b** (6 mg, 6%), together with 35 mg of unreacted epoxide **2**. **3-Bromo-18,19-epoxy-2-hydroxy-2,3,18,19-tetrahydrosqualene (14)**: 5.24–5.04 (4 H), 3.98 (dd, 1 H, J_1 = 11.5, J_2 = 2 Hz, CHBr), 2.71 (t, 1 H, J = 6 Hz), 2.40–1.90 (16 H), 1.68 (br s, 3 H, J_1 = 1 Hz), 1.62 (br s, 3 H), 1.60 (br s, 9 H), 1.90–1.18 (4 H), 1.35 (s, 3 H), 1.33 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR δ 135.0 (C), 134.2 (C), 133.0 (C), 131.8 (C), 126.0 (CH), 124.8 (CH), 124.3 (CH), 123.7 (CH), 72.4 (C), 71.0 (CH), 63.4 (CH), 60.8 (C), 39.6 (CH_2), 38.8 (CH_2), 38.2 (CH_2), 36.3 (CH_2), 32.1 (CH_2), 28.2 (CH_2), 28.2 (CH_2), 27.3 (CH_2), 26.6 (CH_3, CH_2), 25.8 (CH_3), 25.7 (CH_3), 23.9 (CH_2), 17.7 (CH_3), 16.5 (CH_3), 16.0 (CH_3), 16.0 (CH_3), 15.8 (CH_3). **3-Bromo-6,7-epoxy-2-hydroxy-2,3,6,7-tetrahydrosqualene (11a**, less polar mixture): ^1H NMR δ 5.26–5.04 (4 H), 3.98 (dd, 1 H, J_1 = 11 Hz, J_2 = 2 Hz, CHBr) 2.78 (t, 1 H, J = 6 Hz), 2.40–1.90 (16 H), 1.68 (br s, 3 H), 1.62 (br s, 3 H), 1.60 (br s, 9 H), 1.90–1.10 (4 H), 1.36 (s, 3 H), 1.34 (s, 3 H), 1.27 (s, 3 H); ^{13}C NMR δ 135.3 (C), 134.9 (C), 134.0 (C), 131.3 (C), 125.0 (CH), 124.4 (CH), 124.2 (CH), 124.1 (CH), 72.6 (C), 70.6 (CH), 62.7 (CH), 59.9 (C), 39.7 (CH_2), 37.1 (CH_2), 36.3 (CH_2), 29.3 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 27.2 (CH_2), 26.7 (CH_2), 26.7 (CH_3), 26.6 (CH_2), 26.0 (CH_3), 25.7 (CH_3), 17.7 (CH_3), 17.1 (CH_3), 16.1 (CH_3), 16.0 (CH_3), 16.0 (CH_3). **3-Bromo-6,7-epoxy-2-hydroxy-2,3,6,7-tetrahydrosqualene (11b,** more polar mixture): ^1H NMR δ 5.28–5.04 (4 H), 4.00 (dd, 1 H, J_1 = 11 Hz, J_2 = 2 Hz, CHBr), 2.73 (t, 1 H, J = 6 Hz), 2.40–1.90 (16 H), 1.68 (br s, 3 H), 1.62 (br s, 3 H), 1.60 (br s, 9 H), 1.90–1.10 (4 H), 1.37 (s, 3 H), 1.35 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR δ 135.3 (C), 134.9 (C), 134.0 (C), 131.3 (C), 125.0 (CH), 124.4 (CH), 124.2 (CH), 124.1 (CH), 72.6 (C), 71.3 (CH), 63.7 (CH), 60.4 (C), 39.7 (CH_2), 38.0 (CH_2), 36.3 (CH_2), 29.6 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 27.2 (CH_2), 26.9 (CH_2), 26.7 (CH_3), 26.6 (CH_2), 25.9 (CH_3), 25.7 (CH_3), 17.7 (CH_3), 16.4 (CH_3), 16.1 (CH_3), 16.0 (CH_3), 16.0 (CH_3).

The same general procedure was carried out with epoxide **3** (87 mg, 0.2 mmol). Flash chromatography of the crude reaction mixture afforded unreacted epoxide **3** (30 mg), epoxybromohydrin **12** (17 mg, 16%), and epoxybromohydrin **13** (17 mg, 16%). **3-Bromo-10,11-epoxy-2-hydroxy-2,3,10,11-tetrahydrosqualene (12)**: ^1H NMR δ 5.28–5.04 (4 H), 3.98 (1 H, CHBr) 2.73 (t, 1 H, J = 6.5 Hz), 2.40–1.90 (16 H), 1.68 (s, 3 H), 1.63 (s, 3 H), 1.60 (br s, 9 H), 1.90–1.19 (4 H), 1.34 (s, 6 H), 1.26 (s, 3 H); ^{13}C NMR δ 136.0 (C), 135.0 (C), 133.6 (C), 131.3 (C), 125.3 (CH), 125.2 (CH), 124.3 (CH), 124.1 (CH), 123.2 (CH), 72.5 (C), 70.6 (CH), 70.4 (CH), 63.3 (CH), 63.2 (CH), 60.8 (C), 39.7 (CH_2), 38.7 (CH_2), 38.0 (CH_2), 32.0 (CH_2), 32.0 (CH_2), 28.9 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 26.3 (CH_3), 26.3 (CH_3), 26.1 (CH_3), 26.0 (CH_3), 25.7 (CH_3), 24.9 (CH_2), 23.7 (CH_2), 23.7 (CH_2), 17.7 (CH_3), 16.5 (CH_3), 16.4 (CH_3), 16.0 (CH_3), 16.0 (CH_3), 15.9 (CH_3), 15.9 (CH_3). **3-Bromo-14,15-epoxy-2-hydroxy-2,3,14,15-tetrahydrosqualene (13)**: ^1H NMR δ 5.26–5.04 (4 H), 3.98 (dd, 1 H, J_1 = 11.5 Hz, J_2 = 2 Hz, CHBr) 2.73 (t, 1H, J = 6.5 Hz), 2.40–1.90 (16 H), 1.68 (br s, 3 H), 1.62 (br s, 3 H), 1.60 (br s, 9 H), 1.90–1.19 (4 H), 1.35 (s, 3 H), 1.33 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR δ 135.7 (C), 135.6 (C), 133.2 (C), 131.4 (C), 125.9 (CH), 124.3 (CH), 123.6 (CH), 123.5 (CH), 72.4 (C), 70.8 (CH), 63.3 (CH), 60.8 (C), 39.7 (CH_2), 39.6 (CH_2), 38.9 (CH_2), 38.2 (CH_2), 32.2 (CH_2), 29.0 (CH_2), 26.7 (CH_2), 26.6 (CH_2), 26.6 (CH_2), 25.9 (CH_3), 25.7 (CH_3), 24.9 (CH_3), 23.8 (CH_2), 17.7 (CH_3), 16.6 (CH_3), 16.0 (CH_3), 16.0 (CH_3), 15.9 (CH_3).

Preparation of Squalene Dioxides 4–8. General Procedure. A solution of the respective epoxybromohydrin in THF was added to a suspension of NaH (1.2 molar equiv) in the same solvent, and the mixture was stirred at 20 °C under nitrogen until the reaction was completed (TLC monitoring). The crude reaction mixture was filtered through Celite 545, and the filtrate was concentrated under vacuum. Unless stated otherwise, the residue obtained was purified by flash chromatography on silica gel eluting with 95:5 hexane/MTBE to give the corresponding dioxide.

2,3:6,7-Dioxidosqualene (4a). Starting from 6 mg of epoxybromohydrin **11a**, this compound was isolated as an oil (4 mg, 80%) after purification with column chromatography on silica gel deactivated with 2.5% triethylamine: MS (CI, CH_4) *m/z* 443 (M + 1), 425 (base peak, M − 18 + 1), 191, 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.26; H, 11.37.

2,3:6,7-Dioxidosqualene (4b). Starting from 6 mg of epoxybromohydrin **11b**, this compound was isolated as an oil (4 mg, 80%) after purification with column chromatography on silica gel deactivated with 2.5% triethylamine: MS (CI, CH_4) *m/z* 443 (M + 1), 425 (base peak, M − 18 + 1), 191, 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.54; H, 11.49.

2,3:10,11-Dioxidosqualene (5). Starting from 15 mg of epoxybromohydrin **12**, this compound was isolated as an oil (10 mg, 78% yield): MS (CI, CH_4) *m/z* 443 (M + 1), 425 (base peak, M − 18 + 1), 191, 153, 137. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.22; H, 11.40.

2,3:14,15-Dioxidosqualene (6). Starting from 15 mg of epoxybromohydrin **13**, this compound was isolated as an oil (10 mg, 78% yield): MS (CI, CH_4) *m/z* 443 (M + 1), 425 (base peak, M − 18 + 1), 191, 153, 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.35; H, 11.31.

2,3:18,19-Dioxidosqualene (7).[11] Starting from 14 mg of epoxybromohydrin **14**, this compound was isolated as an oil (10 mg, 84% yield): MS (CI, CH_4) *m/z* 443 (M + 1), 425 (base peak, M − 18 + 1), 191, 153, 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.06; H, 11.31.

2,3:22,23-Dioxidosqualene (8).[23] This compound was prepared from the corresponding pure bisbromohydrin by using NaH

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(21) Nadeau, R. G.; Hanzlik, R. P. In *Methods in Enzymology*; Clayton, R. B., Ed.; Academic Press: New York, 1969; Vol. 15, pp 346–351.

(22) The epoxybromohydrins herein described are compounds which showed a moderate unstability. For that reason, they were characterized only by their ^1H and ^{13}C NMR spectra and rapidly converted into the respective dioxido derivatives.

(23) Heintz, R.; Schaefer, P. C.; Benveniste, P. *Chem. Commun.* **1970**, 946–947.

in THF for the dehydrobromination: MS (CI, CH_4) m/z 443 (M + 1), 425 (M − 18 + 1), 191, 153 (base peak), 135, 127.

Preparation of Dioxides 9 and 10. A solution of epoxide **2** (200 mg, 0.47 mmol) in CH_2Cl_2 (5 mL) was treated with 1 mol equiv of DMD (a 80 mM solution in acetone[12]) for 1 h at 0 °C. The residue obtained after elimination under vacuum of solvents and excess reagent was purified by preparative TLC (12:1 hexane/MTBE, five elutions) to give unreacted **2** (80 mg), dioxide **10** (15 mg, 8% yield), dioxide **4** (8 mg, 6% yield), and a mixture of dioxides **7** and **9** (42 mg). Flash chromatography on silica gel impregnated with $AgNO_3$ (see above) permitted the separation of this mixture to afford dioxide **7** (18 mg, 9% yield) and dioxide **9** (17 mg, 8% yield). Yield of pure dioxide **9** was not improved by treatment of epoxide **3** with DMD or *m*-CPBA. **6,7:14,15-Dioxidosqualene (9)**: MS (CI, CH_4) m/z 443 (M + 1), 425 (base peak, M − 18 + 1), 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.43; H, 11.43. **6,7:18,19-Dioxidosqualene (10)**: MS (CI, CH_4) m/z 443 (M + 1), 425 (base peak, M − 18 + 1), 137, 135. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.37; H, 11.38. Found: C, 81.50; H, 11.44.

Reaction of Oxido Derivatives 1–10 with Periodic Acid. Following the general procedure described by Ceruti et al.,[8] 0.4 mol equiv of the corresponding oxide or dioxide were added to a suspension of periodic acid in diethyl ether (2 mL), and the mixture was stirred at 20 °C until reaction was completed (1 h, GC monitoring). The crude reaction mixture was washed with $NaHCO_3$ saturated solution and dried. The residue obtained after solvent elimination was redissolved in isooctane and injected onto the GC–MS-CI system. In those cases where volatile products could be present, the dried solution was injected directly onto the GC–MS system.

Assay Method for OSLC. Isopropyl alcohol solutions of the substrate and inhibitors were added to the test tubes (the alcohol contents did not exceed 1% of the overall test mixture), followed by addition of 0.1 M phosphate buffer (pH 7.4), EDTA (final concentration 0.1 mM), Tween-80 (final concentration 0.15% w/v), 100 μL of rat liver microsomal suspension, and 200 μl of cytosolic fraction. Microsomes (from Sprague-Dawley males) were prepared as described elsewhere.[19b] For determinations of IC_{50} values final concentration of substrate (*R,S*)-2,3-oxidosqualene (**1**) was 40 μM and OSLC activity was 3.1 ± 0.4 nmol/h/mg protein (N = 8). The mixture (final volume 1 mL) was flushed with nitrogen and incubated anaerobically for 60 min at 37 °C. The enzymatic reaction was quenched by addition of 1 mL of 6% KOH in methanol and incubated for 60 min at 37 °C. Then, 24,25-dihydrolanosterol was added as internal standard, and the mixture was extracted three times with an equivalent volume of hexane. The combined extracts were evaporated to dryness, redissolved in a 10:1 hexane/MTBE mixture (3 × 40 μL), loaded onto a column fitted with silica gel (0.75 g, 40–60 μm), and eluted with the same solvent mixture. After discarding the first 7 mL, lanosterol and internal standard were collected in a 6-mL fraction. The eluates were evaporated to dryness, redissolved in hexane, and injected onto a GC system. Conditions of analysis were: SPB-5 capillary column (15 m, 0.32-mm i.d.), gradient temperature from 240 °C (1 min) up to 300 °C at 2 °C/min. Under these conditions, retention times for 24,25-dihydrolanosterol and lanosterol were 13.86 and 14.72 min, respectively. Incubations were performed by duplicate, and a minimum of two experiments per point were carried out. The IC_{50} values for each inhibitor were estimated by interpolation from the respective plot of percent inhibition vs log [*I*]. The plots were generated by using five different concentrations of inhibitor; the ranges of concentrations used were 10–200 μM (for **2** and **8**), 80–400 μM (for **3**), 1–40 μM (for **4**, **5** and **9**), and 0.01–1 μM (for **7**).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **4a**, **4b**, and **5–10** (24 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.