

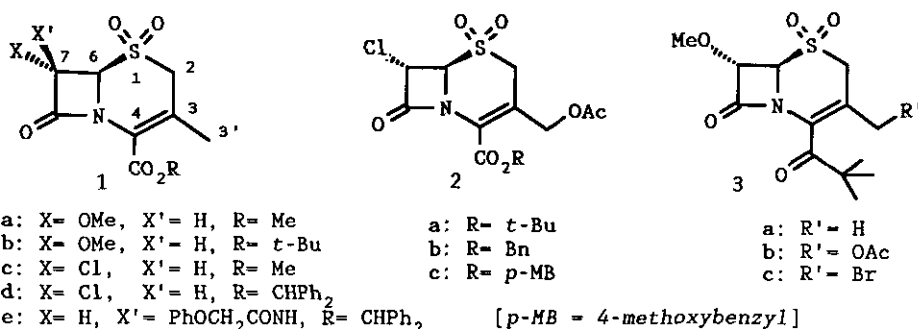
FUNCTIONALIZATION OF THE DIHYDROTHIAZINE RING OF CEPHEM SULFONES

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Abstract- Functionalization of the dihydrothiazine ring of cephem esters and 4-ketones was studied at the sulfone oxidation level. On the esters, alkylation and Michael addition gave 4 β - and 2 α ,4 β -substituted products. On the ketones, electrophilic insertion of carbon, halo, oxygen and sulfur substituents was achieved with acceptable selectivity at 2 α . Nucleophilic sulfonylation and acyloxylation was successful on 2-bromocephem ketones, themselves electrophilic bromination products. Noticeable events included shift of the 3-cephem double bond and epimerization at C-2. Configurations and ring conformation were evinced by nmr studies. Several compounds were potent inhibitors of human leukocyte elastase.

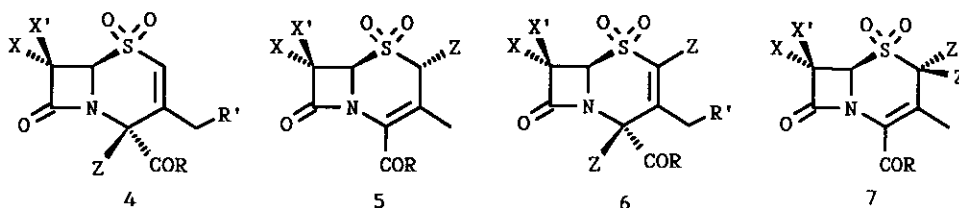
The recent report¹ that esters of 7 α -chloro- and 7 α -methoxycephem sulfones are good inhibitors of human leukocyte elastase (HLE), a serine proteinase involved in the connective-tissue destruction associated with several pathologies,² has provided a fresh impetus to the chemical modification of cephems. Conventional functionalization at the C-3' position³ and derivatization of the C-4 carboxyl (esters,^{4,5} amides,⁵ thioesters⁶ and ketones^{7,8}) was extensively investigated. We conjectured that the activity of cephem sulfones as HLE-inhibitors might be retained, and perhaps increased, by a more substantial alteration of their dihydrothiazine ring. Indeed, insertion of methyl, methoxy and phenylthiomethyl substituents at the C-2 position of 1,1-dioxocephem *tert*-butyl esters produced a relevant increase in their inhibitory potency.⁹ This finding was independently confirmed in our laboratories after the synthesis and biochemical evaluation of original 2-substituted,^{7,8,10} 2,2-disubstituted,¹¹ 4-substituted¹² and 2,4-disubstituted¹³ 1,1-dioxocephems. Though the majority of modified cephem sulfones reported in the literature were obtained by a chemistry performed at the sulfide or sulfoxide oxidation level,^{9,14,15} our synthetic strategy was characterized by the chemical manipulation of the preformed 1,1-dioxocephem skeleton. Here we wish to give a comprehensive account of our progressing studies on the direct functionalization of the dihydrothiazine ring of cephem sulfones, which include insertion of carbon, halo, oxygen and sulfur substituents on different 1,1-dioxocephem substrates whose carboxyl group had been previously derivatized as esters (1a-e, 2a-c)⁶ or as the *tert*-butyl ketone (3a-c).⁷



Reactions with carbon electrophiles (alkylations and Michael additions) were examined first. The C-2 position of the 1,1-dioxocephems (1-3) benefits from an electronic environment (α -sulfonyl group and vinylogous ester or ketone moiety) promoting deprotonation under mild conditions, but several possibilities exist for the outcome of electrophilic substitution at the resulting delocalized anionic species. Previously reported alkylations were carried out with methyl iodide or bromoacetic esters. With MeI, two papers reported 4 β -methylation on cephem sulfoxides,^{16,17} one reported 4 α -methylation on a single cephem sulfide,¹⁸ while methylation of cephem sulfones was not described. With bromoacetic esters, reaction of several cephem sulfoxides^{14b,19} and a single cephem sulfone^{14b} was stated to occur at 2 α exclusively. Our results (Table 1) indicate that ketone (3a) yields 2-substituted products, especially with *tert*-butyl bromoacetate, but the esters (1a, 1b) undergo alkylation at 4 β . The divergence from the literature^{14b} in this respect (run 4, 4 β - instead of 2 α -substitution) may depend on multiple differences in the substrate and in the reaction conditions.

Addition of Michael acceptors (methyl vinyl ketone and acrylonitrile) to cephem derivatives was extensively studied by Bremner *et al.* On esters of cephem sulfides^{14a,14c,20,21} and *R*-sulfoxides^{14a} this reaction resulted in 4 β -alkylation, from the *S*-sulfoxides a mixture of 2 α - and 2,2-disubstituted products was reported,^{14a} and the sulfone (1e) was stated to provide the 2,2-disubstituted derivative (7h).^{14c} In spite of the identity in reaction conditions, 2,2-disubstitution was observed by us only with the ketone (3a) (Table 1, runs 8, 12), while with the esters 4 β -substitution was usually the main outcome (runs 6, 7, 9, 10). To check the possible influence of the C-7 substituent (α -oriented Cl or MeO vs. β -oriented acylamino groups) on regioselectivity, the addition of acrylonitrile to ester (1e) was repeated as described^{14c} (run 11). In our hands, this reaction was complicated by base-assisted epimerization at C-7; isolated products were the 2,4-adducts (6h, 6i), contaminated by minor amounts of mono-adducts (2 α and 4 β), but the reported 2,2-adduct (7h) was neither isolated nor detected in the crude reaction mixture.

Finally, addition of *tert*-butyl propiolate to ester (1c) and ketone (3a) was briefly investigated (runs 13, 14). Trends observed in the addition of alkenes were reproduced, complicated by *E,Z* isomerism: the ester underwent prevailing reaction at C-4, while the ketone reacted at the 2-position almost exclusively.

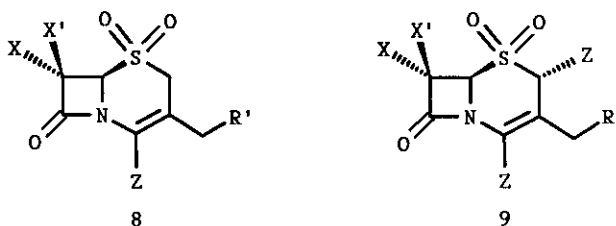


4-carboxy derivatives (R' and $X' = H$ unless otherwise stated):

- a : $X = \text{OMe}$, $R = \text{OMe}$, $Z = \text{Me}$
 b : $X = \text{OMe}$, $R = \text{Ot-Bu}$, $Z = \text{Me}$
 c : $X = \text{OMe}$, $R = \text{Ot-Bu}$, $Z = \text{CH}_2\text{CO}_2t\text{-Bu}$
 d : $X = \text{Cl}$, $R = \text{OCHPh}_2$, $Z = \text{CH}_2\text{CH}_2\text{COMe}$
 e : $X = \text{Cl}$, $R = \text{Op-MB}$, $Z = \text{CH}_2\text{CH}_2\text{COMe}$, $R' = \text{OAc}$
 f : $X = \text{Cl}$, $R = \text{OCHPh}_2$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 g : $X = \text{OMe}$, $R = \text{Ot-Bu}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 h : $X = \text{H}$, $X' = \text{PhOCH}_2\text{CONH}$, $R = \text{OCHPh}_2$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 h' : $X = \text{H}$, $X' = \text{PhOCH}_2\text{CONH}$, $R = \text{OH}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 i : $X = \text{PhOCH}_2\text{CONH}$, $R = \text{OCHPh}_2$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 i' : $X = \text{PhOCH}_2\text{CONH}$, $R = \text{OH}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 j : $X = \text{Cl}$, $R = \text{OMe}$, $Z = \text{CH=CHCO}_2t\text{-Bu}$
 j' : $X = \text{Cl}$, $R = \text{OMe}$, $Z = \text{CH=CHCO}_2\text{H}$

4-keto derivatives (R' and $X' = H$):

- k : $X = \text{OMe}$, $R = t\text{-Bu}$, $Z = \text{Me}$
 l : $X = \text{OMe}$, $R = t\text{-Bu}$, $Z = \text{CH}_2\text{CO}_2t\text{-Bu}$
 m : $X = \text{OMe}$, $R = t\text{-Bu}$, $Z = \text{CH}_2\text{CH}_2\text{COMe}$
 n : $X = \text{OMe}$, $R = t\text{-Bu}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 o : $X = \text{OMe}$, $R = t\text{-Bu}$, $Z = \text{CH=CHCO}_2t\text{-Bu}$



4-decarboxy derivatives (R' and $X' = H$ unless otherwise stated):

- a : $X = \text{Cl}$, $Z = \text{CH}_2\text{CH}_2\text{COMe}$
 b : $X = \text{Cl}$, $Z = \text{CH}_2\text{CH}_2\text{COMe}$, $R' = \text{OAc}$
 c : $X = \text{Cl}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 d : $X = \text{OMe}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 e : $X = \text{H}$, $X' = \text{PhOCH}_2\text{CONH}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$
 f : $X = \text{PhOCH}_2\text{CONH}$, $Z = \text{CH}_2\text{CH}_2\text{CN}$

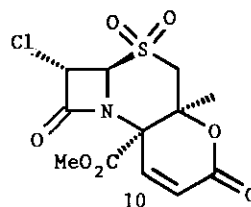


Table 1. Alkylations and Michael additions on 1,1-dioxocephems

Run	Starting	Reagent	Conditions ^(a)	4 β	Products (% yield) ^(b)		
					2,4 β	2 α	2,2
1	1a	MeI	i	4a (50)	-----	-----	-----
2	1b	MeI	i	4b (35)	-----	-----	-----
3	3a	MeI	i	4k (40)	-----	5k ^(c) (15)	-----
4	1b	BrCH ₂ CO ₂ t-Bu	i	4c (20)	-----	-----	-----
5	3a	BrCH ₂ CO ₂ t-Bu	i	-----	-----	5l (45)	7l (10)
6	1d	CH ₂ =CHCOMe	ii	4d (61)	-----	-----	-----
7	2c	CH ₂ =CHCOMe	ii	4e (52)	6e (24)	-----	-----
8	3a	CH ₂ =CHCOMe	ii	-----	-----	5m (45)	7m (20)
9	1d	CH ₂ =CHCN	ii	4f (37)	6f (22)	-----	-----
10	1b	CH ₂ =CHCN	ii	4g (35)	6g (44)	-----	-----
11	1e	CH ₂ =CHCN	ii	4h, 4i (5)	6h, 6i (35)	5i (6)	-----
12	3a	CH ₂ =CHCN	iii	-----	-----	5n ^(d) (20)	7n (55)
13	1c	CH $\equiv$ CCO ₂ t-Bu	iv	4j ^(e) (15)	6j ^(f) (18)	-----	-----
14	3a	CH $\equiv$ CCO ₂ t-Bu	v	-----	-----	-----	7o ^(g) (20)

(^a) (i) DMF, t-BuOK, -20°C, 1 h; (ii) neat, TEA catalysis, 1-40 h; (iii) neat, TEA catalysis, 6 days (70% conversion by tlc); (iv) CH₂Cl₂, TEA catalysis, 4 h; (v) CH₂Cl₂, TEA catalysis, 48 h (50% conversion by tlc).

(^b) After isolation by flash chromatography. Cumulative yields are reported for the C-7 isomers in run 11 (4h and 4i, 3:2; 6h and 6i, 3:2). Yields in runs 12 and 14 are based on reacted substrate.

(^c) Minor amounts of the 2 β -epimer (19a) and Δ^2 -cephem tautomer (20a) were also isolated; see Table 2.

(^d) This product was obtained as an inseparable mixture of Δ^3 - and Δ^2 -cephem isomers (5n and 20b, ca. 2.3:1); see Table 2.

(^e) Isolated as two separate alkene isomers (4E and 4Z, 2:1); cumulative yield reported.

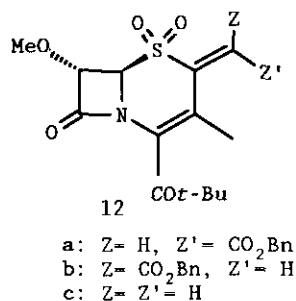
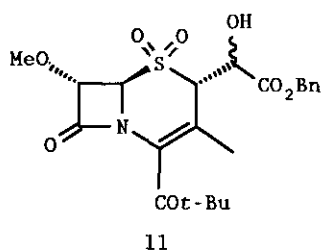
(^f) Isolated as a mixture of (2E,4E) and (2E,4Z) isomers (1:2).

(^g) Isolated as a single alkene isomer (E,E).

Hydrolysis of the ester group and decarboxylation^{14c} was carried out on the 4-alkylation and alkenylation products (4, 6) in order to obtain 3-cephem sulfones (8, 9) carrying new types of substituents at C-4. In our experience this procedure is also an important tool for removing ambiguities in regioisomeric differentiation between disubstituted derivatives 6 and 7, since in the latter compounds loss of CO₂ from the carboxylate salts cannot be assisted by extensive delocalization of the negative charge. Exposure to TFA (20 mol equiv., anisole), followed by stirring in a biphasic EtOAc/aqueous NaHCO₃ system, gave 8a from 4d, 8b from 4e, 9b from 6e, 9c from 6f, 9d from 6g, and 9e from 6h (45-75% estimated yields).²² The controversial products of acrylonitrile addition to 1e gave a mixture of compounds, wherefrom 5i', 8e, 8f, 9e and 9f were isolated; compound (7h') which would arise from 7h was not detected. Hydrolysis of the *tert*-butyl propiolate adducts gave

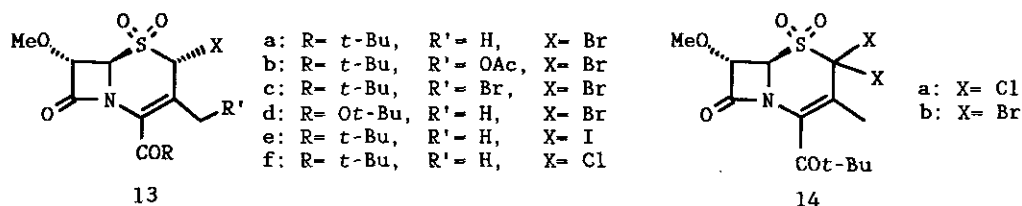
the corresponding acids; 4j' (*E*-isomer) was obtained from the *E*-isomer of 4j, while the *Z*-isomer spontaneously cyclized to the tricyclic lactone (10; 50% overall). Formation of the latter compound is a unique example of addition to the activated double bond of Δ^2 -cephem sulfones; addition of external nucleophiles is expected to be easy but reversible.

The reaction of cephem sulfones with aldehyde derivatives was investigated next. Activated aldehydes (pyruvic aldehyde, trifluoroacetaldehyde) were reported to add at the C-2 and C-4 position of a 3'-ylidenecephem sulfide ester²³ with poor selectivity. We found that benzyl glyoxylate (1 mol equiv. of reagent and TEA, 4Å molecular sieves, benzene) regioselectively reacts with the cephem sulfone (3a) to provide a mixture of epimeric alcohols (11; 2.5:1 ratio, 65%). The major epimer afforded the acrylates (12a) (63%) and (12b) (21%) when exposed to mesyl chloride (2 mol equiv.) and TEA (4 eq., CH₂Cl₂, -30°C to room temperature). Formaldehyde under Mannich conditions is known to provide 2-methylenecephem derivatives.²⁴ In the case of the 1,1-dioxocephem ketone (3a), we found that conversion to the 2-methylenecephem product (12c) can be accomplished in virtually quantitative yield by the use of *N,N*-dimethylmethyleneimmonium chloride (*t*-BuOH/dioxane 1:2, reflux temperature, 8 h). Hydrogenation^{9,25} of 12c provided the opportunity, in alternative to direct alkylation of 3a, to prepare the 2-methyl derivative (5k). However, the main hydrogenation product (5% Pd on CaCO₃, H₂ 4 atm., EtOAc/EtOH) was the β -epimer (19a) (3:1 with 5k), owing to preferential attack of the catalyst to the unhindered α -face of the cephem molecule.



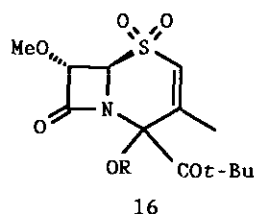
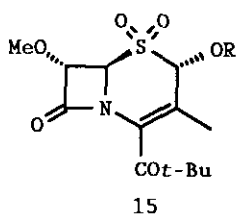
Halogenation at the C-2 position of cephem sulfones has no precedents, but the electrophilic bromination procedure (equimolar amounts of NBS and TEA, CH₂Cl₂, 30 min) recently developed by us⁷ provided 13a (92%) from the ketone (3a). This procedure has now been extended to the 3'-substituted derivatives (13b from 3b and 13c from 3c; 75-80%), and to all of the esters in somewhat lower yields (e.g., 13d from 1b, 65%). Further, we have found that bromination at C-2, sequential to bromination at C-3, occurs also under forcing radical conditions; by this way (NBS 2 mol equiv., AIBN catalysis, CCl₄-CH₂Cl₂ 5:1, reflux temperature, 6 h) the useful 2,3'-dibromo derivative (13c) was obtained in one step from

3a in 75% yield. Electrophilic iodination and chlorination of 3a were also attempted. With I_2 /TEA the 2-iodo derivative (13e) was detected by nmr but decomposed upon chromatography. With NCS/TEA, the *gem*-dichloro compound (14a) was isolated (35%) along with the product of mono-substitution (13f) (20%) and unreacted 3a (35%). By contrast, the dibromo analog (14b) was formed in trace amounts in the reaction of 3a with an equimolar amount of NBS.

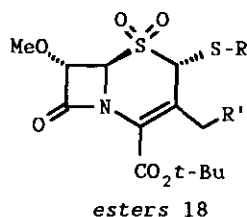
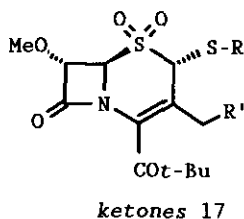


Two strategies were envisaged for the insertion of sulfur and oxygen substituents on the dihydrothiazine ring of cephem sulfones. The first strategy encompassed bromination at C-2, as above described, and reaction with the appropriate nucleophile. Cephem sulfones were not expected to easily accommodate a positive charge at C-2, as the sulfides do.²⁶ Displacement of α -halosulfones by a S_N2 mechanism is expected to be retarded by a combination of polar and steric effects;²⁷ in this situation attack of the nucleophile at the halogen atom may be preferred. In fact, as found independently by Botta *et al.*,¹⁵ displacement of the bromine atom with alcohols, alkyl thiols and triphenylphosphine failed; the reduced precursors (3a from 13a and 1b from 13d) were isolated instead. However, methathetical reaction of 13a with silver acetate and silver benzoate gave the corresponding 2-acyloxy derivatives (15a) (60%) and (15b) (68%), accompanied by minor amounts (ca. 10%) of the 4-regioisomers (16a), (16b). Moreover, displacement of the bromoketone (13a) to the 2-heterothiocephems (17a-c) was observed in good to excellent yields (65-90%) with the heterocyclic thiols commonly employed for derivatization at the 3'-position of classical cephalosporins, either as the sodium salts or in the presence of TEA (MeCN or DMF, 30 min). Further to this finding,⁷ we have observed that smooth displacement also occurs on the 3'-acetoxy derivative (13b) and on the bis(2,3')-bromide (13c) to provide, respectively, 17d (90%) and 17e (85%). On the latter substrate the reaction is sequential, substitution at C-3' occurring first, so that regioselective introduction of two different heterocyclylthio groups may be achieved if desired. Debromination instead of substitution was observed in the reaction of the 2-bromoketone (13a) with 2-mercaptopyridine (PySH) and thiophenol (PhSH), and on the 2-bromoester (13d) with all of the heterocyclic thiols. A possible interpretation for this mechanistic dichotomy was formulated after the observation that reduction of 13a to 3a was maximized (95%) by using 2 mol equiv. each of PhSH and TEA, and under these conditions phenyl disulfide was generated in almost quantitative yield. Thus, what appears as a nucleophilic substitution at the 2-carbon atom of the 2-bromocephems might be a reduction and

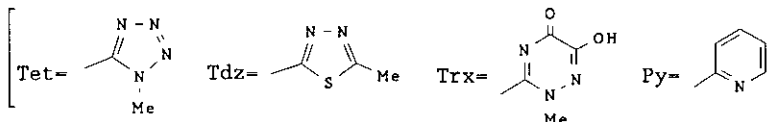
electrophilic substitution sequence.²⁷ Attack of the thiolate to the bromine atom would result in formation of sulphenyl bromide and the stabilized cephem carbanion; sulphenylation or reduction, respectively, might be determined by competition for this carbanion by a sulphenyl species and the proton source. The second strategy for the preparation of 2-thio- and 2-acyloxycephem sulfones was devised on this ground. Direct electrophilic sulphenylation of ketone (3a) and ester (1b) was achieved by reaction of bis(2-pyridyl) disulfide or *S*-phenyl benzenethiosulfonate (1 mol equiv. DBN, MeCN). Yields were good, though the products were often obtained as mixtures of interconverting isomers, as discussed below (Table 2): 17f (90:6:4 mixture with 19c and 20d; 73%) from PyS-SPy and 3a; 17g (65%) and 20e (30%) from PhS-SO₂Ph and 3a; 18f (87:13 mixture with 19d; 75%) from PyS-SPy and 1b; 18g (86:14 mixture with 20f; 65%) from PhS-SO₂Ph and 1b. Electrophilic acyloxylation was performed with benzoyl peroxide (DBN, MeCN, 30 min) on 3a; in this case the desired 2 α -benzoyloxycephem (15b; 35%) was accompanied by a regioisomer, (16b) (12%). The two compounds were identical to samples isolated from reaction of the bromide (13a) with silver benzoate.



a: R= COMe
b: R= CPh



a: R= Tet, R'= H
b: R= Tdz, R'= H
c: R= Trx, R'= H
d: R= Tet, R'= OAc
e: R= Tet, R'= S-Tet
f: R= Py, R'= H
g: R= Ph, R'= H



The characterization of C-2 epimeric compounds and 3-exo or Δ^2 -cephem tautomers is a distinctive result of our structural studies. These compounds are reported in Table 2; spectral data for representative products are collectively presented in Table 5.

Table 2. Equilibrations observed in 1,1-dioxocephems derivatives

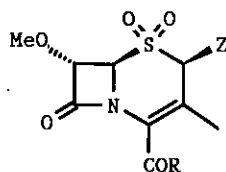
Run	Starting ^(a)	Conditions ^(b)	Products (isomeric ratio) ^(c)			
			2 α	2 β	Δ^2	3-exo
1	2b	i		2b (60) ^(d)	-----	21a (40)
2	3a	ii		3a (90) ^(d)	-----	21b (10)
3	5k	iii	5k (50)	19a (17)	20a (33)	-----
4	19a	iii	5k (50)	19a (17)	20a (33)	-----
5	5n, 20b	---	5n (70)	-----	20b (30)	-----
6	17a, 19b, 20c	---	17a (93)	19b (2)	20c (5)	-----
7	17f, 19c, 20d	iii	17f (87)	19c (6)	20d (7)	-----
8	17g	iii	17g (65)	-----	20e (35)	-----
9	18f, 19d	iii	18f (85)	19d (15)	-----	-----

(^a) Mixtures indicate that the pertinent compounds were obtained as such under their preparation conditions by flash-chromatography over silica. Relative ratios were: runs 5 and 6, as indicated (no treatment); run 7, 90:6:4; run 9, 87:13.

(^b) (i) TEA in acetone, 2 h; (ii) TEA in CHCl_3 , 2 days; (iii) TEA in CDCl_3 , 2 h; (---) no treatment.

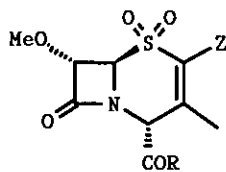
(^c) Approximate ratio by nmr integration (400 MHz) in CDCl_3 solution. Isolation (flash-chromatography over silica; gradient elution with *n*-hexane/ethyl acetate mixtures) was carried out after run 1 (21a, 38%), run 2 (21b, 5%), and run 3 (19a, 13%; 20a, 24%). The compounds of run 4 coelute. The 2-sulfenyl derivatives (runs 5-9) undergo equilibration over silica. Other products were characterized by nmr in the initial and final isomeric mixtures.

(^d) 2-Unsubstituted cepheps.



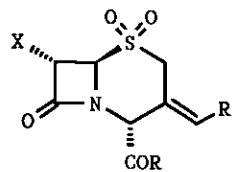
19

a: R = *t*-Bu, Z = Me
b: R = *t*-Bu, Z = S-Tet
c: R = *t*-Bu, Z = S-Py
d: R = *Ot*-Bu, Z = S-Py



20

a: R = *t*-Bu, Z = Me
b: R = *t*-Bu, Z = $\text{CH}_2\text{CH}_2\text{CN}$
c: R = *t*-Bu, Z = S-Tet
d: R = *t*-Bu, Z = S-Py
e: R = *t*-Bu, Z = S-Ph
f: R = *Ot*-Bu, Z = S-Ph



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a: X = Cl, R = OBn, R' = O/
b: X = OMe, R = *t*-Bu, R' = H

The 3-*endo*/3-*exo* double bond isomerism (Table 2, runs 1 and 2) was unexpected; previously, only the reverse isomerization process was reported.^{16,28} Remarkably, on the 2-substituted derivatives another type of equilibration was observed, Δ^3 -/ Δ^2 -isomerism (runs 3-8). The relative stability of the Δ^2 -structure in 1,1-dioxocephem esters and ketones was unexpected as well;²⁹ to our knowledge 20a-20e are the first Δ^2 -cephem sulfones possessing a hydrogen atom at C-4 reported to date. The equilibration between C-2 epimers was anticipated by the literature existing on esters of 2-substituted cephem sulfoxides^{25,30} and, for the ketones, by our previous studies on the enolization process.³¹ Characterization of minor isomeric products (19) was precious for configurational and conformational studies (see below).

Structural determination of other compounds in the present study deserves mention. Differentiation between Δ^3 -cephem sulfones and protomeric or regioisomeric Δ^2 -cephem structures (5 and 19 vs. 20; 5 and 19 vs. 4; 7 vs. 6; 15 vs. 16) was made by uv, ir and nmr spectroscopy. Structures differing for the presence of a vinylic proton at C-2 (4 and 16) were immediately recognized by nmr (quartet at 6.2-6.5 ppm, $J = 1.2$ -1.4 Hz). Otherwise, uv spectroscopy was exquisitely diagnostic, since the cephem chromophore characteristic of the Δ^3 -isomers ($\lambda_{\max}$ 253-265 nm; ϵ 7,000-8,200) was found to be absent in Δ^2 -cephem sulfones, and ir spectroscopy (conjugated vs. unconjugated carbonyl stretchings) offered in many instances another first-sight indication. The α -orientation of the 4-carbonyl or 4-carboxyl group in the 4-alkylation and alkenylation products (4, 6) is anticipated on mechanistic grounds (repulsion between the α -oriented lone pair of N and that of C-4 in the carbanion intermediate).^{14a,30b} Consistently, NOESY analysis of ester (4b) and ketone (4k) did not show proximity of the 4-methyl group with H-6 α , as it would be expected if this group were α -oriented;^{17,18} instead, NOEs were observed between H-6 α and the *tert*-butyl groups. Consistency is observed also in the structure of lactone (10), whose nmr analysis suggests the configuration (3*S*,4*R*) and the conformation shown in Figure 1.

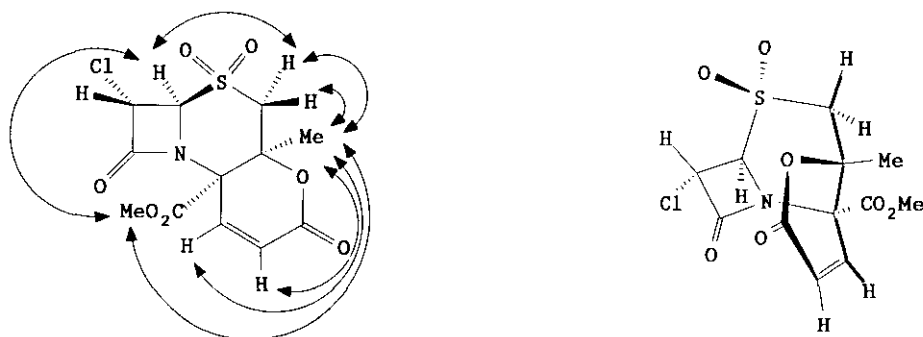


Figure 1. ^1H nmr analysis of tricyclic lactone (10). Arrows (left) indicate relevant NOEs; boldface bonds (right) indicate the "W" backbone responsible for the four-bond coupling ($J = \text{ca. } 0.5 \text{ Hz}$) observed between H-2 β and H-4'.

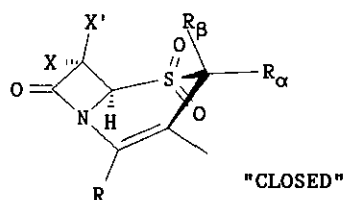
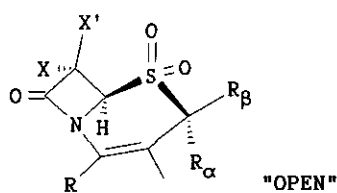
The 4-acyloxycephem (16b) poses an interesting question, since it was obtained as a single epimer by two different procedures. No resolutive NOE was observed on this compound; however, the absence of an NOE between H-6 α and the *tert*-butyl group (contrarily to that observed on 4k, see above) might be taken as a suggestion that benzoyloxylation occurred from the less hindered α -face. The 4 α -stereochemistry of Δ^2 - and 3-exo-cephem tautomers (20 and 21) was assigned by analogy with literature reports.^{16,32} The alkene geometry of the *tert*-butyl propiolate adducts (4j, 4j', 6j, 6o) was evident from the coupling constants of the contiguous vinylic protons. When this evidence was lacking (trisubstituted olefins 12a, 12b and 21a), NOESY analysis was resolutive. In particular, NOEs were observed between the 2-vinyl proton and 3-Me in 12b, and between the 3-vinyl proton and H-4 in 21a, which established the (*Z*)-alkene configuration of these compounds.

Configurational and conformational assignment of the selected³³ 2-substituted compounds was derived as follows. The major or exclusive isomers within this class (5, 9, 11, 13, 15, 17 and 18) lacked the long-range coupling ($J = 1.2$ -1.7 Hz) between H-6 α and the C-2 proton characteristic of 2-unsubstituted cephem *S*-sulfoxides³⁴ and sulfones;³⁵ moreover, no correlation was found between these two protons by NOESY spectroscopy. These results would necessitate that the compounds are 2 α -substituted, if the "open" half-chair conformation³⁴ for the dihydrothiazine ring is taken for granted (left-hand structure shown in Table 3). However, conformational analysis of cephem sulfones was never addressed previously, and the possibility exists for a conformational change. Calculations were made on the two 2-methyl derivatives (5k, 19a) and on their parent 3a using the semiempirical molecular orbital program MOPAC;³⁶ geometry optimizations were run with MNDO, MINDO/3 and PM3 methods. Interestingly (Table 3), the theoretical heats of formation obtained by MNDO would suggest that the closed conformers are favoured over the open ones; on 3a and 19a the open conformations were even unattainable.³⁷ By the other methods, conformational preferences were small and incongruent, except for 19a, whose closed conformation was favoured by ca. 1 Kcal/mole.

Table 3. Theoretical heats of formation^(a) of cephem sulfone conformers predicted by MOPAC

	MNDO		MINDO/3		PM3	
	closed	open	closed	open	closed	open
3a ($R_\alpha = R_\beta = H$)	-19.16	-----	-136.62	-135.66	-151.39	-151.54
5k ($R_\alpha = Me$)	-18.47	-18.10	-139.65	-137.94	-151.32	-152.98
19a ($R_\beta = Me$)	-20.13	-----	-139.58	-138.53	-152.49	-151.52

(a) ΔH_f , kcal/mole



While computational results (not considering stability contributions by the solvent) confirmed the accessibility of the closed conformation, this latter was not supported experimentally. Most resolute was NOESY analysis of the isomeric 2-methylcephems (5k) and (19a); a strong NOE correlation existed between H-6 and 2-CH₃ in 5k, and between H-6 and H-2 in 19a, unequivocally proving that they are, respectively, 2 α - and 2 β -methyl epimers in the open conformation. The open conformation of 19a established by NOESY spectroscopy necessitates that the four-bond coupling between H-6 and H-2 ($J = 1.5$ Hz) observed in this compound and in 2-unsubstituted cephem sulfones is not the outcome of a "W" effect, a possibility proposed by De Angelis *et al.*³⁸ This evidence adds to others³⁵ suggesting that this coupling should be related to a σ - π orbital overlap in the system formed by a central S=O bond and two axial protons (H-6 α and H-2 α in the open conformers). Within the 2-sulphenylated compounds, the minor isomers 19 were also characterized by an NOE and a 4J between H-6 and H-2, supporting their attribution as 2 β -substituted cepheps in the open conformation. Conversely, the major or unique 2-substituted isomers (5l-n, 9b-e, 11, 13a-k, 15a, 15b, 17a-g, 18f, 18g), lacking the $^4J_{2,6}$ and an NOE between H-2 and H-6, were tentatively assigned the 2 α configuration and the open conformation, in analogy with 5k. Remaining possibilities, 2 α closed and 2 β closed, are less likely. In fact, an NOE between H-2 β and H-7 β would be expected if they were 2 α -substituted cepheps in the closed conformation,³⁹ and a conformational preference for the closed conformation in 2 β -substituted cepheps is unexpected on steric grounds (large axial substituent protruding in the concave face of the molecule).

After the present study, the four literature reports^{25,38,40} on cepheps in the closed conformation should be critically considered. The attribution made by De Angelis *et al.*³⁸ was disputed by us previously.³⁵ The other compound pertinent to our work is trichloroethyl 2 α ,3-dimethyl-7 β -phenoxyacetamidocephem-4-carboxylate (1S)-oxide.²⁵ This compound, prepared by us as described, displayed a strong NOESY correlation for 2-CH₃ and H-6, whereas H-2 and H-6 were correlated in its C-2 epimer. Thus, both 2-methylcephem S-sulfoxide epimers are "open" cephem conformers; epimerization at C-2, contrarily to what proposed,²⁵ is not accompanied by a conformational change.

Kinetic parameters of HLE inhibition displayed by a few representative structures are presented in Table 4. The second order rate constant, k_{on} , combines information on both enzyme recognition and inhibitor reactivity. The steady state inhibition constant, $K_i(ss)$, contains further information on early reactivation of enzymatic activity and is reported as an empirical index of relative *in vitro* potency of the inhibitors. The excellent activity (high k_{on} and low $K_i(ss)$ values) of the 2-acyloxy- and 2-sulphenyl-1,1-dioxocephem ketones (15 and 17) can be appreciated. The ester derivatives were less active (18f vs. 17f), and the 2-methyl compound (19a) was a comparatively poor inhibitor. High potency, however, was inherent to some highly modified structures, e.g. the 2,4-disubstituted- Δ^2 -cephem sulfone (6j). Full accounts on the structure-activity relationships uncovered in the class of cephem ketones³³ and on the *in vivo* activity of selected compounds in animal models will be reported in due time.

Table 4. Kinetic parameters^(a) for HLE inhibition displayed by selected functionalized cephem sulfones and precursors (1a, 3b)

Compound	$k_{on} \times 10^3 [M^{-1}sec^{-1}]$	$K_i(ss) [nM]$
1b	nd ^(b)	5000
3a	0.09	1300
4j ^(c)	7.2	64
6j ^(d)	70	5.2
8b	0.21	nd
12c	nd	390
15b	1500	≤ 1
17a	100	8.3
17d	200	6.0
17f	82	10
18f	4.7	180
19a ^(e)	2.0	2400

(^a) See ref. 6 for experimental details.

(^b) nd: Not determined.

(^c) Isolated (4Z) isomer.

(^d) Mixture of (2E,4E) and (2E,4Z) isomers (1:2).

(^e) Equilibrates to a mixture of 2 α - and 2 β -epimers under the testing conditions.

This exploratory study was aimed at showing that direct insertion of carbon, halo, oxygen, and sulfur substituents on the dihydrothiazine ring of cephem sulfones is possible and convenient for the rapid screening of new HLE inhibitors. Care was devoted to the structural characterization of as many products as possible, rather than to the optimization of experimental conditions. We hope that our results may constitute a useful basis for future works aimed at improving or redressing the regio- and stereoselectivity, according to the individual chemical and biochemical targets.

ACKNOWLEDGEMENTS

We thank G. Franceschi for encouragement and stimulating discussions, V. Rizzo (Analytical Chemistry) for biochemical data, M. D'Anello (Industrial Development) for a generous supply of ketone (3a), and IMI (Istituto Mobiliare Italiano) for financial support.

Table 5. Distinctive spectral data for selected compounds ^(a)

4b	δ 1.48 (9H,s); 1.88 (3H,s); 1.91 (3H,d,J=1.4 Hz); 3.54 (3H,s); 4.67 (1H,d,J=1.3 Hz); 5.09 (1H,d,J=1.3 Hz); 6.25 (1H,q,J=1.4 Hz). $\nu_{\max}$ 1787, 1735. $\lambda_{\max}$ <220.
4d	δ 1.72 (3H,d,J≤1.1 Hz); 2.14 (3H,s); 2.2-3.0 (4H,m); 4.76 (1H,d,J=1.1 Hz); 5.26 (1H,d,J=1.1 Hz); 6.35 (1H,q,J≤1.1 Hz); 6.93 (1H,s); 7.2-7.5 (10H,m). $\nu_{\max}$ 1800, 1750, 1712.
4g	δ 1.51 (9H,s); 1.95 (3H,d,J=1.4 Hz); 2.0-3.2 (3H,m); 3.0-3.2 (1H,m); 3.57 (3H,s); 4.67 (1H,d,J=1.4 Hz); 5.10 (1H,d,J=1.4 Hz); 6.48 (1H,q,J=1.4 Hz). $\nu_{\max}$ 1800, 1735. $\lambda_{\max}$ <220.
4j	<i>E-isomer</i> : δ 1.26 (9H,s); 1.90 (3H,d,J=1.4 Hz); 3.92 (3H,s); 4.84 (1H,d,J=1.6 Hz); 5.29 (1H,d,J=1.6 Hz); 6.09 (1H,d,J=16 Hz); 6.38 (1H,q,J=1.4 Hz); 7.26 (1H,d,J=16 Hz). $\nu_{\max}$ 1800, 1745, 1710. <i>Z-isomer</i> : δ 1.46 (9H,s); 2.02 (3H,d,J=1.4 Hz); 3.82 (3H,s); 5.05 (1H,d,J=1.4 Hz); 5.25 (1H,d,J=1.4 Hz); 5.95 (1H,d,J=11.7 Hz); 6.28 (1H,d,J=11.7 Hz); 6.33 (1H,q,J=1.4 Hz). $\nu_{\max}$ 1805, 1750, 1720.
4k	δ 1.24 (9H,s); 1.77 (3H,d,J=1.4 Hz); 1.90 (3H,s); 3.53 (3H,s); 4.59 (1H,d,J=1.2 Hz); 5.04 (1H,d,J=1.2 Hz); 6.32 (1H,q,J=1.4 Hz). $\nu_{\max}$ 1780, 1705. $\lambda_{\max}$ <220.
5k	δ 1.24 (9H,s); 1.64 (3H,d,J=7.3 Hz); 1.71 (3H,s); 3.29 (1H,q,J=7.3 Hz); 3.53 (3H,s); 4.69 (1H,d,J=1.7 Hz); 5.16 (1H,d,J=1.7 Hz). $\nu_{\max}$ 1770, 1695. $\lambda_{\max}$ 256 (ϵ =7200).
5l	δ 1.23 (9H,s); 1.45 (9H,s); 1.66 (3H,s); 2.87 (1H,dd,J=6.1,18.0 Hz); 3.11 (1H,dd,J=3.5,18.0 Hz); 3.53 (3H,s); 3.54 (1H,m); 5.11 (1H,d,J=1.7 Hz); 5.15 (1H,d,J=1.7 Hz). $\nu_{\max}$ (CHCl ₃) 1795, 1730, 1700.
6f	δ 1.79 (3H,s); 2.3-3.3 (8H,m); 4.78 (1H,d,J=1.4 Hz); 5.29 (1H,d,J=1.4 Hz); 6.94 (1H,s); 7.2-7.5 (10H,m). $\nu_{\max}$ 1800, 1745.
6g	δ 1.51 (9H,s); 2.00 (3H,s); 2.2-3.2 (8H,m); 3.56 (3H,s); 4.70 (1H,d,J=1.1 Hz); 5.17 (1H,d,J=1.1 Hz). $\nu_{\max}$ (CHCl ₃) 1795, 1748. $\lambda_{\max}$ <220.
6h	δ 1.84 (3H,s); 2.4-3.4 (8H,m); 4.55 (2H,s); 4.76 (1H,d,J=4.8 Hz); 5.91 (1H,dd,J=4.8, 10.6 Hz); 6.95 (1H,s); 6.8-7.5 (15H,m); 7.99 (1H,d,J=10.6 Hz).
6i	δ 1.75 (3H,s); 2.4-3.4 (8H,m); 4.35 (1H,d,J=15.3 Hz); 4.47 (1H,d,J=15.3 Hz); 5.05 (1H,d,J=1.7 Hz); 5.12 (1H,dd,J=1.7,7.2 Hz); 6.8-7.4 (17H,m).
6j	(<i>2E,4Z</i>)- <i>isomer</i> : δ 1.44 (9H,s); 1.49 (9H,s); 1.86 (3H,s); 3.85 (3H,s); 5.25 (1H,d,J=1.5 Hz); 5.41 (1H,d,J=1.5 Hz); 6.00 (1H,d,J=12.5 Hz); 6.19 (1H,d,J=16.2 Hz); 6.33 (1H,d,J=12.5 Hz); 6.76 (1H,d,J=16.2 Hz). $\nu_{\max}$ 1810, 1720(br).
7m	δ 1.23 (9H,s); 1.56 (3H,s); 2.17 (3H,s); 2.21 (3H,s); 2.1-2.7 (7H,m); 2.9-3.1 (1H,m); 3.56 (3H,s); 4.73 (1H,d,J=1.7 Hz); 5.18 (1H,d,J=1.7 Hz). $\nu_{\max}$ 1800, 1720, 1705 (sh).
7n	δ 1.25 (9H,s); 1.69 (3H,s); 2.1-3.1 (8H,m); 3.57 (3H,s); 4.56 (1H,d,J=1.4 Hz); 5.23 (1H,d,J=1.4 Hz). $\nu_{\max}$ 1795, 1700. $\lambda_{\max}$ 258.
7o	(<i>E,E</i>)- <i>isomer</i> : δ 1.30 (9H,s); 1.49 (9H,s); 1.50 (3H,s); 1.52 (9H,s); 3.53 (3H,s); 4.81 (1H,d,J=1.8 Hz); 5.14 (1H,d,J=1.8 Hz); 5.96 (1H,d,J=15.8 Hz); 6.17 (1H,d,J=16.0 Hz); 6.83 (1H,d,J=16.0 Hz); 7.08 (1H,d,J=15.8 Hz). $\nu_{\max}$ 1805, 1725.
8a	δ 2.03 (3H,s); 2.17(3H,s); 2.6-3.2(4H,m); 3.41 (1H,d,J=18.0 Hz); 3.88 (1H,br-d,J=18.0 Hz); 4.67 (1H,m); 5.25 (1H,d,J=1.9 Hz). $\nu_{\max}$ 1790, 1715.
9d	δ 1.93 (3H,s); 2.1-2.4 (2H,m); 2.6-2.9 (5H,m); 3.0-3.2 (1H,m); 3.25 (1H,m); 3.57 (3H,s); 4.68 and 5.15 (1H,d,J=1.8 Hz). $\nu_{\max}$ (CHCl ₃) 1795. $\lambda_{\max}$ 253 (ϵ =7650).
9e	δ 1.96 (3H,s); 2.1-2.3 (2H,m); 2.6-2.9 (5H,m); 3.0-3.2 (1H,m); 3.25 (1H,m); 4.58 (2H,s); 4.85 (1H,d,J=4.8 Hz); 6.19 (1H,dd,J=4.8,10.8 Hz); 6.9-7.4 (5H,m); 7.93 (1H,d,J=10.8 Hz). $\nu_{\max}$ 1790, 1700.
9f	1.95 (3H,s); 2.2-2.3 (2H,m); 2.6-2.9 (5H,m); 3.0-3.1 (1H,m); 3.31 (1H,m); 4.57 (2H,s); 5.10 (2H,m); 6.8-7.4 (6H,m). $\nu_{\max}$ 1785, 1690.
10	δ [400 MHz] 1.45 (3H,s); 3.48 (1H,dd,J=15.2 and <1 Hz); 3.74 (1H,d,J=15.2 Hz); 3.98 (3H,s); 5.18 (1H,d,J=1.5 Hz); 5.30 (1H,d,J=1.5 Hz); 6.40 (1H,d,J=10.0 Hz); 7.24 (1H,dd,J=10.0 and <1 Hz). $\nu_{\max}$ 1798, 1742.

Table 5. Continued

11	[major isomer] δ 1.21 (9H,s); 1.28 (9H,s); 3.35 (1H,dd,J=0.9,4.0 Hz); 3.51 (3H,s); 3.70 (1H,br s); 4.95 (1H,dd,J=1.3,3.8 Hz); 5.02 (1H,d,J=1.9 Hz); 5.13 (1H,dd,J=1.9 Hz); 5.21 (1H,d,J=11.7 Hz); 5.30 (1H,d,J=11.7 Hz). $\nu_{\max}$ 1785, 1750, 1700.
12a	δ 1.24 (9H,s); 1.67 (3H,s); 3.54 (3H,s); 4.92 (1H,d,J=2.0 Hz); 5.23 (1H,d,J=2.0 Hz); 5.25 (2H,s); 7.05 (1H,s); 7.36 (5H,m). $\nu_{\max}$ (CHCl ₃) 1795, 1730, 1700.
12c	δ 1.26 (9H,s); 1.81 (3H,s); 3.55 (3H,s); 4.77 (1H,d,J=1.8 Hz); 5.23 (1H,d,J=1.8 Hz); 5.91 (1H,d,J=2.0 Hz); 6.50 (1H,d,J=2.0 Hz). $\nu_{\max}$ 1780, 1698. $\lambda_{\max}$ 226 (ϵ =7450), 300 (ϵ =10,300).
13a	δ 1.26 (9H,s); 1.82 (3H,s); 3.57 (3H,s); 4.90 (1H,s); 5.17 (1H,d,J=2.0 Hz); 5.32 (1H,d,J=2.0 Hz). $\nu_{\max}$ 1800, 1705.
13c	δ 1.28 (9H,s); 3.57 (3H,s); 3.76 (1H,d,J=11.7 Hz); 4.07 (1H,d,J=11.7 Hz); 5.17 (1H,d,J=2.1 Hz); 5.35 (1H,d,J=2.1 Hz); 5.45 (1H,s). $\nu_{\max}$ 1805, 1705.
13d	δ 1.55 (9H,s); 2.08 (3H,s); 3.58 (3H,s); 4.92 (1H,s); 5.14 (1H,d,J=1.8 Hz); 5.25 (1H,d,J=1.8 Hz). $\nu_{\max}$ 1805, 1720.
14a	δ 1.28 (9H,s); 1.93 (3H,s); 3.60 (3H,s); 5.29 (1H,d,J=1.8 Hz); 5.44 (1H,d,J=1.8 Hz). $\nu_{\max}$ 1810, 1705.
15b	δ 1.30 (9H,s); 1.76 (3H,s); 3.57 (3H,s); 4.88 (1H,d,J=1.8 Hz); 5.20 (1H,d,J=1.8 Hz); 5.92 (1H,s); 7.4-7.8 (5H,m). $\nu_{\max}$ 1795, 1755, 1700.
16b	δ [400 MHz] 1.54 (3H,s); 1.80 (3H,d,J=1.5 Hz); 3.53 (3H,s); 5.05 (1H,d,J=1.2 Hz); 5.12 (1H,d,J=1.2 Hz); 6.49 (1H,q,J=1.2 Hz); 7.4-8.2 (5H,m). $\nu_{\max}$ (CHCl ₃) 1810, 1735, 1710.
17a	δ 1.24 (9H,s); 1.92 (3H,s); 3.54 (3H,s); 4.08 (3H,s); 4.98 (1H,s); 5.10 (1H,d,J=1.9 Hz); 5.17 (1H,d,J=1.9 Hz). $\nu_{\max}$ 1800, 1705.
17b	δ 1.20 (9H,s); 1.89 (3H,s); 2.78 (3H,s); 3.53 (3H,s); 5.17 (1H,d,J=1.8 Hz); 5.20 (1H,s); 5.24 (1H,d,J=1.8 Hz). $\nu_{\max}$ (CHCl ₃) 1800, 1700.
17e	δ 1.27 (9H,s); 3.53 (3H,s); 3.68 (1H,d,J=14.2 Hz); 3.94 (3H,s); 4.11 (3H,s); 4.34 (1H,d,J=14.2 Hz); 5.10 (1H,d,J=1.9 Hz); 5.20 (1H,d,J=1.9 Hz); 5.55 (1H,s). $\nu_{\max}$ 1800 1700.
17f	δ 1.29 (9H,s); 3.54 (3H,s); 3.86 (1H,d,J=14.6 Hz); 3.92 (1H,d,J=18.0 Hz); 3.98 (1H,d,J=14.6 Hz); 4.09 (1H,dd,J=1.2,18.0 Hz); 4.67 (1H,m); 5.15 (1H,d,J=1.7 Hz); 7.15 (1H,m); 7.27 (1H,m); 7.62 (1H,m); 8.49 (1H,m). $\nu_{\max}$ 1780, 1695.
18f	δ [400 MHz] 1.56 (9H,s); 2.14 (3H,s); 3.55 (3H,s); 4.97 (1H,d,J=1.5 Hz); 5.13 (1H,d,J=1.5 Hz); 6.20 (1H,s); 7.16 (1H,m); 7.27 (1H,m); 7.62 (1H,m); 8.51 (1H,m). $\nu_{\max}$ (CHCl ₃) 1800, 1730.
18g	δ 1.54 (9H,s); 2.23 (3H,s); 3.50 (3H,s); 4.19 (1H,s); 4.70 (1H,d,J=1.7 Hz); 5.10 (1H,d,J=1.7 Hz). $\nu_{\max}$ (CHCl ₃) 1800, 1732.
19a	δ 1.24 (9H,s); 1.54 (3H,d,J=7.3 Hz); 1.63 (3H,d,J=1.0 Hz); 3.55 (3H,s); 3.82 (1H,m); 4.64 (1H,dd,J=1.5,1.6 Hz); 5.17 (1H,d,J=1.6 Hz). $\nu_{\max}$ 1790, 1698.
20a	δ 1.26 (9H,s); 1.65 (3H,s); 2.07 (3H,d,J=0.9 Hz); 3.49 (3H,s); 4.59 (1H,s); 5.20 (1H,s); 5.30 (1H,br s). $\nu_{\max}$ (CHCl ₃) 1795, 1715.
20e	δ 1.28 (9H,s); 1.93 (3H,s); 3.50 (3H,s); 4.74 (1H,s); 5.25 (1H,s); 5.53 (1H,s); 7.2-7.7 (5H,m).
21a	δ [400 MHz] 2.22 (3H,s); 3.71 (1H,dd,J=1.5,14.0 Hz); 4.34 (1H,d,J=14.0 Hz); 5.04 (1H,s); 5.06 (1H,d,J=1.5 Hz); 5.20 and 5.26 (2H,ABq,J=12.2 Hz); 5.25 (1H,d,J=1.5 Hz); 7.3-7.4 (5H,m); 7.64 (1H,d,J=1.5 Hz). $\nu_{\max}$ (CHCl ₃) 1800, 1740.
21b	δ 1.24 (9H,s); 3.52 (3H,s); 3.70 (1H,d,J=14.2 Hz); 4.30 (1H,dt,J=1.4,14.2 Hz); 4.94 (1H,d,J=1.1 Hz); 5.04 (1H,d,J=1.1 Hz); 5.41 (2H,m); 5.54 (1H,s). $\nu_{\max}$ 1775, 1705.

(^a) Unless otherwise stated, nmr spectra (δ , ppm) were taken in CDCl₃ at 200 MHz, ir spectra ($\nu_{\max}$, cm⁻¹) in KBr, and uv spectra ($\lambda_{\max}$, nm) in MeCN.

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Received, 21st December, 1992