FUNCTIONALIZATION OF THE DIHYDROTHIAZINE RING OF CEPHEM SULFONES

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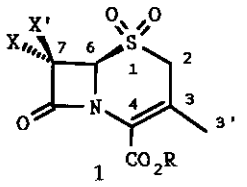
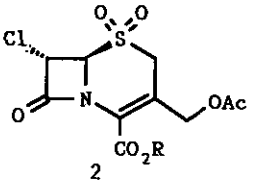
Abstract- Functionalization of the dihydrothiazine ring of cephem esters and 4-ketones was studied at the sulfone oxidation level. On the esters, alkylation and Michael addition gave 4β- and 2α,4β-substituted products. On the ketones, electrophilic insertion of carbon, halo, oxygen and sulfur substituents was achieved with acceptable selectivity at 2α. Nucleophilic sulfenylation 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 report[1] that esters of 7α-chloro- and 7α-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′ position[3] and derivatization of the C-4 carboxyl (esters,[4,5] amides,[5] thiolesters[6] 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-substituted[12] and 2,4-disubstituted[13] 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]



1

a: X= OMe, X'= H, R= Me
b: X= OMe, X'= H, R= *t*-Bu
c: X= Cl, X'= H, R= Me
d: X= Cl, X'= H, R= $CHPh_2$
e: X= H, X'= $PhOCH_2CONH$, R= $CHPh_2$



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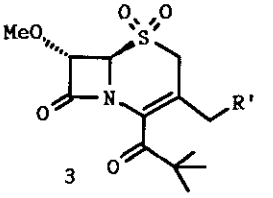
a: R= *t*-Bu
b: R= Bn
c: R= *p*-MB



3

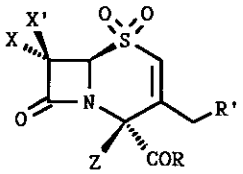
a: R'= H
b: R'= OAc
c: R'= Br

[*p-MB = 4-methoxybenzyl*]

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 4β-methylation on cephem sulfoxides,[16,17] one reported 4α-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 2α 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 4β. The divergence from the literature[14b] in this respect (run 4, 4β- instead of 2α-substitution) may depend on multiple differences in the sustrate 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 4β-alkylation, from the *S*-sulfoxides a mixture of 2α- 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 4β-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 (2α and 4β), 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.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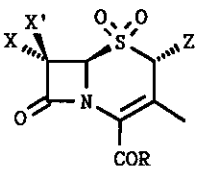
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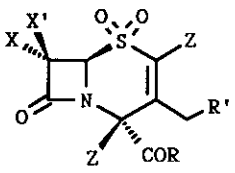
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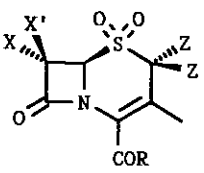
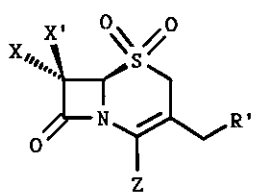

7

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

a : X= OMe, R= OMe, Z= Me
b : X= OMe, R= O*t*-Bu, Z= Me
c : X= OMe, R= O*t*-Bu, Z= CH_2CO_2t-Bu
d : X= Cl, R= $OCHPh_2$, Z= CH_2CH_2COMe
e : X= Cl, R= O*p*-MB, Z= CH_2CH_2COMe, R'= OAc
f : X= Cl, R= $OCHPh_2$, Z= CH_2CH_2CN
g : X= OMe, R= O*t*-Bu, Z= CH_2CH_2CN
h : X= H, X'= $PhOCH_2CONH$, R= $OCHPh_2$, Z= CH_2CH_2CN
h': X= H, X'= $PhOCH_2CONH$, R= OH, Z= CH_2CH_2CN
i : X= $PhOCH_2CONH$, R= $OCHPh_2$, Z= CH_2CH_2CN
i': X= $PhOCH_2CONH$, R= OH, Z= CH_2CH_2CN
j : X= Cl, R= OMe, Z= $CH{=}CHCO_2t$-Bu
j': X= Cl, R= OMe, Z= $CH{=}CHCO_2H$

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

k : X= OMe, R= *t*-Bu, Z= Me
l : X= OMe, R= *t*-Bu, Z= CH_2CO_2t-Bu
m : X= OMe, R= *t*-Bu, Z= CH_2CH_2COMe
n : X= OMe, R= *t*-Bu, Z= CH_2CH_2CN
o : X= OMe, R= *t*-Bu, Z= $CH{=}CHCO_2t$-Bu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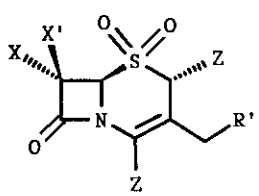
9

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

a: X= Cl, Z= CH_2CH_2COMe
b: X= Cl, Z= CH_2CH_2COMe, R'= OAc
c: X= Cl, Z= CH_2CH_2CN
d: X= OMe, Z= CH_2CH_2CN
e: X= H, X'= $PhOCH_2CONH$, Z= CH_2CH_2CN
f: X= $PhOCH_2CONH$, Z= CH_2CH_2CN

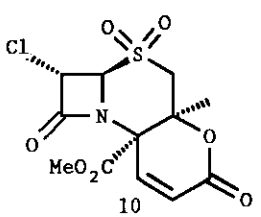
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Table 1. Alkylations and Michael additions on 1,1-dioxocephems

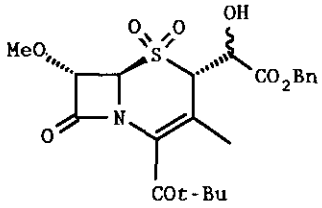
Run	Starting	Reagent	Conditions[a]	Products (% yield)[b] 4β	2,4β	2α	2,2
1	**1a**	MeI	i	**4a** (50)	--------	--------	--------
2	**1b**	MeI	i	**4b** (35)	--------	--------	--------
3	**3a**	MeI	i	**4k** (40)	--------	**5k**[c](15)	--------
4	**1b**	$BrCH_2CO_2t$-Bu	i	**4c** (20)	--------	--------	--------
5	**3a**	$BrCH_2CO_2t$-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**	$CH{\equiv}CCO_2t$-Bu	iv	**4j**[e](15)	**6j**[f](18)	--------	--------
14	**3a**	$CH{\equiv}CCO_2t$-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)* CH_2Cl_2, TEA catalysis, 4 h; *(v)* CH_2Cl_2, 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 2β-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 (4*E* and 4*Z*, 2:1); cumulative yield reported.
([f]) Isolated as a mixture of (2*E*,4*E*) and (2*E*,4*Z*) isomers (1:2).
([g]) Isolated as a single alkene isomer (*E*,*E*).

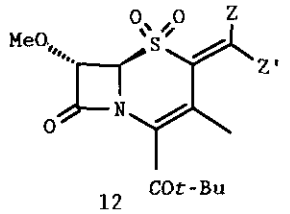
Hydrolysis of the ester group and decarboxylation[14c] was carried out on the 4-alkylation and alkenylation products (**4**, **6**) in order to obtain 3-cephem sulfones (**8**, **9**) carrying new types of substituents at *C*-4. In our experience this procedure is also an important tool for removing ambiguities in regioisomeric differentiation between disubstituted derivatives **6** and **7**, since in the latter compounds loss of CO_2 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 $NaHCO_3$ 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 ester[23] 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., CH_2Cl_2, -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 $CaCO_3$, H_2 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.



11

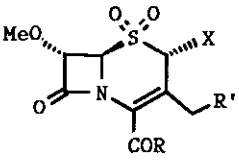


12

a: Z= H, Z'= CO_2Bn
b: Z= CO_2Bn, Z'= H
c: Z= Z'= H

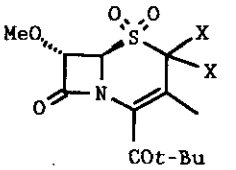
Halogenation at the C-2 position of cephem sulfones has no precedents, but the electrophilic bromination procedure (equimolar amounts of NBS and TEA, CH_2Cl_2, 30 min) recently developed by us[7] provided **13a** (92%) from the ketone (**3a**). This procedure has now been extended to the 3'-substituted 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, CCl_4-CH_2Cl_2 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.



a: R= *t*-Bu, R'= H, X= Br
b: R= *t*-Bu, R'= OAc, X= Br
c: R= *t*-Bu, R'= Br, X= Br
d: R= Ot-Bu, R'= H, X= Br
e: R= *t*-Bu, R'= H, X= I
f: R= *t*-Bu, R'= H, X= Cl

13



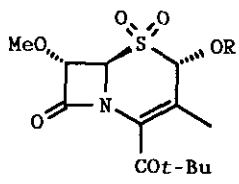
a: X= Cl
b: X= Br

14

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 accomodate a positive charge at C-2, as the sulfides do.[26] Displacement of α-halosulfones by a SN2 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, methatetical 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 sulfenyl bromide and the stabilized cephem carbanion; sulfenylation or reduction, respectively, might be determined by competition for this carbanion by a sulfenyl 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 sulfenylation 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-SO_2Ph and **3a**; **18f** (87:13 mixture with **19d**; 75%) from PyS-SPy and **1b**; **18g** (86:14 mixture with **20f**; 65%) from PhS-SO_2Ph and **1b**. Electrophilic acyloxylation was performed with benzoyl peroxide (DBN, MeCN, 30 min) on **3a**; in this case the desired 2α-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.



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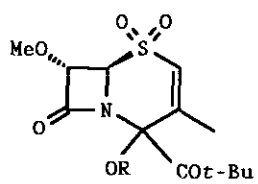
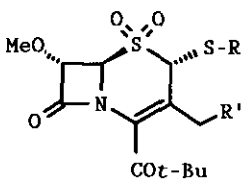
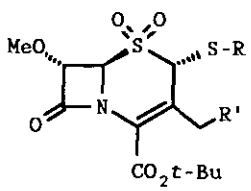


16

a: R= COMe
b: R= COPh

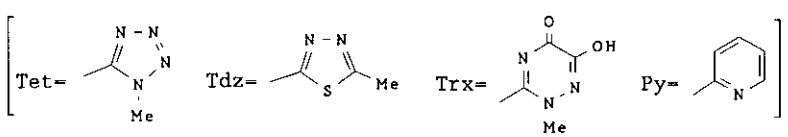


ketones 17



esters 18

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

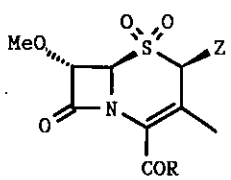


The characterization of C-2 epimeric compounds and 3-*exo* or Δ^2-cephem tautomers is a distinctive result of our structural studies. These compounds are reported in Table 2; spectral data for representative products are collectively presented in Table 5.

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

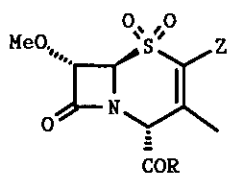
Run	Starting[a]	Conditions[b]	Products (isomeric ratio)[c] 2α	2β	Δ^2	3-*exo*
1	2b	i		2b (60)[d]	--------	21a (40)
2	3a	ii		3a (90)[d]	--------	21b (10)
3	5k	iii	5k (50)	19a (17)	20a (33)	--------
4	19a	iii	5k (50)	19a (17)	20a (33)	--------
5	5n,20b	---	5n (70)	--------	20b (30)	--------
6	17a,19b,20c	---	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 cephems.



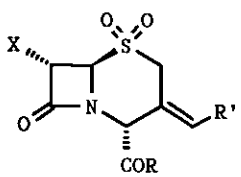
19

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= CH_2CH_2CN
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



21

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 (λ_{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 cabonyl 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-6α, as it would be expected if this group were α-oriented;[17,18] instead, NOEs were observed between H-6α 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.

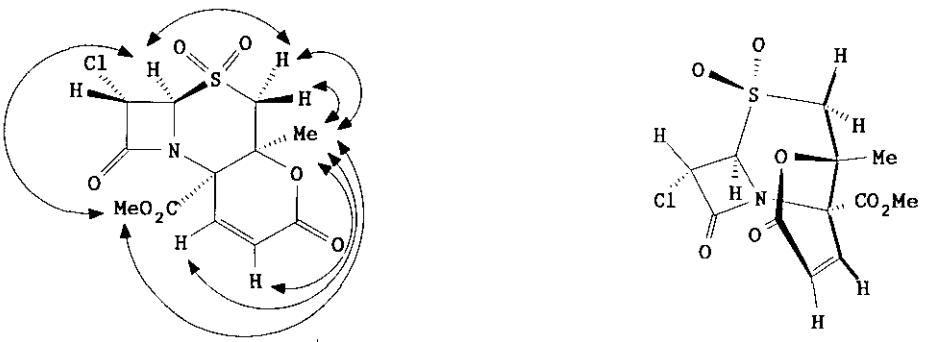


Figure 1. ^{1}H nmr analysis of tricyclic lactone (**10**). Arrows (left) indicate relevant NOEs; boldface bonds (right) indicate the "W" backbone responsible for the four-bond coupling (J= ca. 0.5 Hz) observed between H-2β and H-4'.

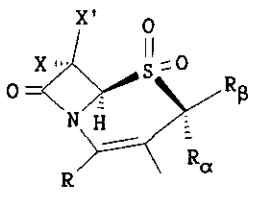
The 4-acyloxycephem (**16b**) poses an interesting question, since it was obtained as a single epimer by two different procedures. No resolutive NOE was observed on this compound; however, the absence of an NOE between H-6α and the *tert*-butyl group (contrarily to that observed on **4k**, see above) might be taken as a suggestion that benzoyloxylation occurred from the less hindered α-face. The 4α-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 selected[33] 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-6α and the C-2 proton characteristic of 2-unsubstituted cephem *S*-sulfoxides[34] and sulfones;[35] moreover, no correlation was found between these two protons by NOESY spectroscopy. These results would necessitate that the compounds are 2α-substituted, if the "open" half-chair conformation[34] 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 derivates (**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.

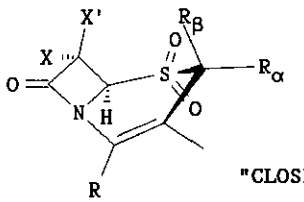
Table 3. Theoretical heats of formation[(a)] of cephem sulfone conformers predicted by MOPAC

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

[(a)] ΔH_f, kcal/mole



"OPEN"



"CLOSED"

While computational results (not considering stability contributions by the solvent) confirmed the accessibility of the closed conformation, this latter was not supported experimentally. Most resolutive was NOESY analysis of the isomeric 2-methylcephems (**5k**) and (**19a**); a strong NOE correlation existed between H-6 and 2-CH_3 in **5k**, and between H-6 and H-2 in **19a**, unequivocally proving that they are, respectively, 2α- and 2β-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 oucome of a "W" effect, a possibility proposed by De Angelis *et al.*[38] This evidence adds to others[35] 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-6α and H-2α in the open conformers). Within the 2-sulfenylated compounds, the minor isomers **19** were also characterized by an NOE and a ^4J between H-6 and H-2, supporting their attribution as 2β-substituted cephems 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 2α configuration and the open conformation, in analogy with **5k**. Remaining possibilities, 2α closed and 2β closed, are less likely. In fact, an NOE between H-2β and H-7β would be expected if they were 2α-substituted cephems in the closed conformation,[39] and a conformational preference for the closed conformation in 2β-substituted cephems 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 cephems 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 2α,3-dimethyl-7β-phenoxyacetamidocephem-4-carboxylate (1*S*)-oxide.[25] This compound, prepared by us as described, displayed a strong NOESY correlation for 2-CH_3 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-sulfenyl-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 ketones[33] 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 (4*Z*) isomer.
([d]) Mixture of (2*E*,4*E*) and (2*E*,4*Z*) isomers (1:2).
([e]) Equilibrates to a mixture of 2α- and 2β-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). ν_{max} 1787, 1735. λ_{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). ν_{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). ν_{max} 1800, 1735. λ_{max} <220.
4j	*E-isomer*: 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). ν_{max} 1800, 1745, 1710. *Z-isomer*: 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). ν_{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). ν_{max} 1780, 1705. λ_{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). ν_{max} 1770, 1695. λ_{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). ν_{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). ν_{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). ν_{max} ($CHCl_3$) 1795, 1748. λ_{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	*(2E,4Z)-isomer*: 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). ν_{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). ν_{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). ν_{max} 1795, 1700. λ_{max} 258.
7o	*(E,E)-isomer*: 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). ν_{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). ν_{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). ν_{max}($CHCl_3$) 1795. λ_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{max} 1780, 1698. λ_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{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). ν_{max} 1775, 1705.

([a]) Unless otherwise stated, nmr spectra (δ, ppm) were taken in $CDCl_3$ at 200 MHz, ir spectra (ν_{max}, cm^{-1}) in KBr, and uv spectra (λ_{max}, nm) in MeCN.

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