ORALLY ACTIVE 2-(ALKYLOXYCARBONYL)-2-ALKYLIDENEETHYL ESTERS OF CEPHALOSPORINS

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2-(Alkyloxycarbonyl)-2-alkylideneethyl esters of various aminothiazole-oxyimino cephalosporins have been synthesized and studied. They are useful alternatives to the currently existing orally active esters. Among the new esters synthesized, the 3'-azidomethyl cephem ester Ro 41-3399 (**7k**) presented an oral bioavailability superior to the corresponding pivaloyloxymethyl ester (**9**) in a rat model and was selected as a candidate for further evaluation.

In contrast to the large number of parenteral cephalosporins in clinical use, the number of orally active cephalosporins is small[1] and there is a need both to broaden their antibacterial spectrum and to increase their potency. This imbalance is due in part to the fact that the structural features required for good absorption from the intestinal tract are different from those needed for the desired antibacterial activity[2,3]. Some clinically useful broad-spectrum cephalosporins are satisfactorily absorbed as the free acid[1], while others, *e.g.* cefetamet (**4**), a so-called third-generation cephalosporin in the late stages of clinical development[4], must be transformed into hydrolyzable esters such as the pivaloyloxymethyl ester **8** for use as orally active agents.

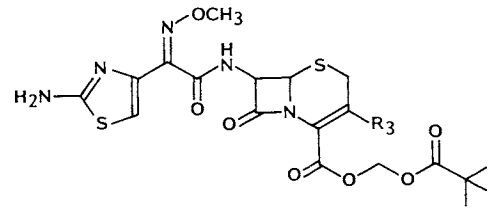
Ro 40-6890 (**5**) is a third-generation cephalosporin with a broad spectrum of activity against both Gram-positive and Gram-negative bacteria[5]. The compound is not well absorbed after oral administration, and, although the pivaloyloxymethyl ester **9** is better absorbed, improved absorption would be desirable. A great deal of effort has been expended to identify new types of cephalosporin esters[6] that improve oral absorption. In this report we present the results with novel 2-(alkyloxycarbonyl)-2-alkylideneethyl esters of various cephalosporins. The esters of several aminothiazole-oxyimino cephalosporins lead to increased plasma levels of the cephalosporin acids in animal models.

Results and Discussion

Chemistry

The observation in our laboratory the acidic hydroxyl groups (*e.g.* those of oximes) protected with α-bromomethyl acrylates are easily liberated under various mild conditions led to the idea that this moiety could be useful for making novel cephalosporin ester prodrugs. The processes for the

Fig. 1. Chemical structures of pivaloyloxymethyl esters of cephalosporins.



8 $R_3 = CH_3$ (cefetamet pivoxyl, Globocef)
9 $R_3 = CH_2N_3$

Dedicated to Prof. J.-P. FLEURY, on the occasion of his 65th anniversary.

Table 1. Yield and characterization of the esters **6** and **7**.

Ester No.	R_1	R_2	R_3	Yield (%)	Formula	Analysis (%) Calcd/Found			IR (cm^{-1})
						C	H	N	
6a	Me	Et	Me	58	$C_{21}H_{25}N_5O_7S_2$	48.17	4.81	13.28	1776
						47.90	4.97	13.26	
6b	Me	*iso*-Butyl	Me	17	$C_{23}H_{29}N_5O_7S_2$		NA		1780
6c	Me	Cyclohexyl	Me	79	$C_{25}H_{31}N_5O_7S_2$	51.98	5.41	12.12	1781
						51.95	5.96	12.04	
6d	Me	Cyclohexyl-methyl	Me	52	$C_{26}H_{33}N_5O_7S_2$	52.78	5.62	11.84	1778
						52.73	5.74	11.86	
6e	Me	Ethoxy-ethoxy-methyl	Me	42	$C_{23}H_{29}N_5O_8S_2$	48.67	5.15	12.34	1779
						48.37	5.41	12.57	
6f	Me	2,3-Dimethoxy propyl	Me	48	$C_{24}H_{31}N_5O_9S_2$	48.23	5.23	11.72	1780
						48.00	5.40	11.40	
6g	Me	2-Ethyl-hexyl	Me	60	$C_{27}H_{37}N_5O_7S_2$		NA		1778
6h	Me	*tert*-Butyl	Me	49	$C_{23}H_{29}N_5O_7S_2$	50.08	5.30	12.70	1780
						49.89	5.39	12.60	
6i	Me	*iso*-Propyl	Me	27	$C_{22}H_{27}N_5O_7S_2$	49.15	5.06	13.03	1780
						50.14	5.66	13.22	
6j	Et	Et	Me	32	$C_{22}H_{27}N_5O_7S_2$	49.15	5.06	13.03	1779
						49.21	5.08	13.09	
6k	Et	*iso*-Butyl	Me	69	$C_{24}H_{31}N_5O_7S_2$	50.96	5.52	12.38	1780
						50.64	5.78	12.30	
6l	Et	2-(Tetrahydro-pyranyl)methyl	Me	41	$C_{26}H_{33}N_5O_8S_2$		NA		1781
6m	Et	4-(Tetrahydro-pyranyl)	Me	52	$C_{25}H_{31}N_5O_8S_2$	50.58	5.26	11.80	1781
						50.54	5.52	11.60	
6n	Phenyl	Et	Me	56	$C_{26}H_{27}N_5O_7S_2$	53.32	4.65	11.96	1780
						53.17	4.83	11.60	
7a	Me	Et	CH_2N_3	85	$C_{21}H_{24}N_8O_7S_2$	44.67	4.28	19.85	1786
						44.83	4.31	19.58	
7b	Me	*iso*-Butyl	CH_2N_3	20	$C_{23}H_{28}N_8O_7S_2$	46.61	4.76	18.91	1786
						46.65	4.92	18.57	
7d	Me	Cyclohexyl-methyl	CH_2N_3	51	$C_{26}H_{32}N_8O_7S_2 \cdot HCl$	46.67	4.97	16.75	1790
						46.13	5.17	16.40	
7e	Me	Ethoxy-ethoxy-methyl	CH_2N_3	66	$C_{23}H_{28}N_8O_7S_2$	45.39	4.64	18.41	1785
						45.45	4.71	18.05	
7k	Et	*iso*-Butyl	CH_2N_3	51	$C_{24}H_{30}N_8O_7S_2$	47.52	4.98	18.47	1786
						47.49	4.99	18.19	

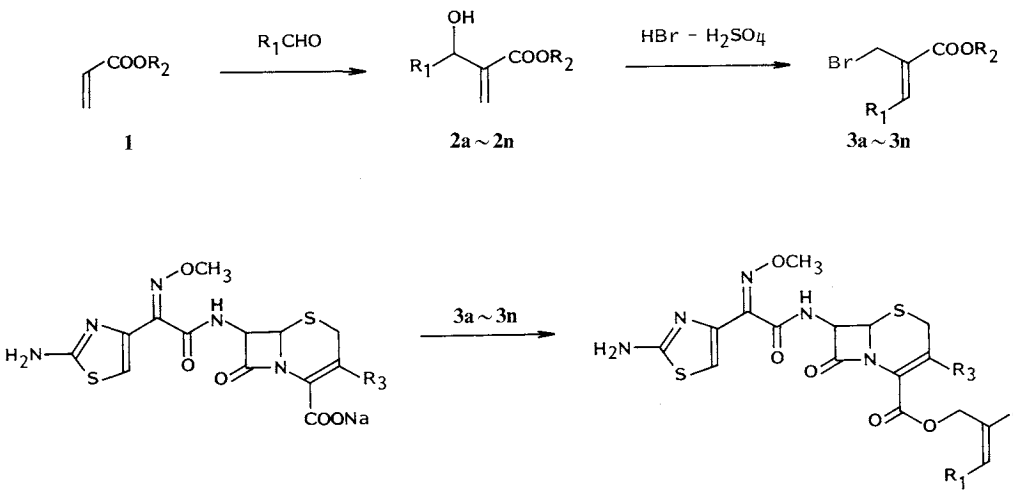
NA: Not available.

preparation of the esters **6a**~**6n** and **7a**, **7b**, **7d**, **7e**, **7k** listed in Table 1 are outlined in Scheme 1. The alkyl α-bromomethyl alken-2-oates **3a**~**3n** necessary for the preparation of the esters were obtained by base catalyzed (DABCO[7]) addition of aldehydes to the acrylates **1** followed by allylic transposition (HBr-H_2SO_4[8] or NBS-dimethyl sulfide[9]) of the intermediate allylic alcohols **2a**~**2n**. Treatment of the sodium salts of cephalosporins **4** and **5** with the bromides **3a**~**3n** in DMF at 0°C afforded the corresponding esters **6** and **7**, which were purified by crystallization.

Oral Absorption of Cephalosporin Esters

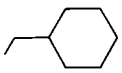
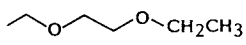
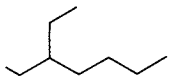
A mouse model employing suspensions of cephalosporin esters in 2% aqueous Tween 80 was used as an initial screen to identify novel esters yielding significant blood levels of free cephalosporins. We followed a two part strategy: a) Identification of novel groups, suitable to derivatize the carboxylic acid of the

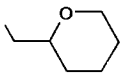
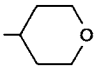
Scheme I. Synthesis of the new cephalosporin esters.



4 $R_3=CH_3$
5 $R_3=CH_2N_3$

6a ~ 6n $R_3=CH_3$
7a, 7b, 7d, 7e, 7k $R_3=CH_2N_3$

a $R_1=CH_3$ $R_2=C_2H_5$
b $R_1=CH_3$ $R_2=$ *iso*-Butyl
c $R_1=CH_3$ $R_2=$ Cyclohexyl
d $R_1=CH_3$ $R_2=$ 
e $R_1=CH_3$ $R_2=$ 
f $R_1=CH_3$ $R_2=$ 
g $R_1=CH_3$ $R_2=$ 

h $R_1=CH_3$ $R_2=$ *tert*-Butyl
i $R_1=CH_3$ $R_2=$ *iso*-Propyl
j $R_1=C_2H_5$ $R_2=C_2H_5$
k $R_1=C_2H_5$ $R_2=$ *iso*-Butyl
l $R_1=C_2H_5$ $R_2=$ 
m $R_1=C_2H_5$ $R_2=$ 
n $R_1=$ Phenyl $R_2=C_2H_5$

model cephalosporin **4**, which enhanced the oral absorption and b) test the best of these groups using 3′-azidomethyl cephalosporin (**5**), which has a broader antibacterial spectrum than 3′-methyl cephalosporin (**4**). The area under the curve (AUC) values of the liberated cephalosporins were used to provide an estimation of the absorption of the new esters in the mouse model (Table 2). Because the AUC values of iv injected **4** were not available, we were not able to calculate the absolute bioavailabilities of the new esters. This was however performed in the rat with selected compounds showing high AUC in mice (see below Table 3). A comparison of the data for po administration of the pivaloyloxymethyl (PIV) ester **8** and sc administration of the corresponding acid **4** (AUC = 24 μg·hour/ml) suggests that the new esters (*e.g.* **6a**, **6e**, **6j ~ 6l**) are absorbed as well as the PIV ester in this mouse model.

All modifications of the parent 2-(alkyloxycarbonyl)-2-alkylideneethyl structure either greatly reduced, or totally eliminated, the oral activity (*e.g.* 3-(alkyloxycarbonyl)-2-alkylideneethyl, saturation of the double bond). The replacement of the carboxylate by other electron withdrawing groups (*e.g.* ketone, sulfone, nitrile) led to poorer oral activity (data not shown). Within the parent structure, there was a tendency for small R_1 groups (*e.g.* methyl, ethyl) to enhance absorption relative to a phenyl group (*e.g.* **6m**). However,

Table 2. AUC in mouse, stability and physico-chemical properties of esters **6** and **7**.

Ester No.	AUC[a] (μg·hour/ml)	$t_{1/2}$ pH 8 (hours)	$t_{1/2}$ pH 0 (hours)	Solubility (μg/ml)	$\log_{10}$ [P]
6a	15.6	8	>20	129	1.72
6b	11.4	6	>20	38	2.44
6c	8.1	>24	>24	36	2.19
6d	12.2	>12	>20	6	3.03
6e	17.6	3.7	>20	658	1.07
6f	13.0	2.4	>20	>880	0.88
6g	14.8	>12	>20	3	2.82
6h	11.0	>12	1	160	2.27
6i	14.3	12	>24	—	1.80
6j	15.5	8	>20	101	2.13
6k	16.9	8.4	>24	51	2.19
6l	15.7	8	>24	175	2.31
6m	5.3	>12	>24	420	1.69
6n	9.8	>8	>24	17	2.60
7a	14.1	2.9	>24	134	2.12
7b	8.4	2.9	>24	39	2.59
7d	22.2	<5	>24	37	1.67
7e	12.6	2.8	>24	257	1.80
7k	19.9	3.5	>24	16	2.51
8	16.2	2.0	>6	377	1.68
9	20.7	0.38	9.5	133	1.90

[a] Calculated for a dose equivalent to 20 mg/kg of cephalosporins **4** and **5**.

aside from the necessity of the 2-(alkyloxycarbonyl)-2-alkylideneethyl ester structure, the substituent changes in the ester (*e.g.* ethyl, iso-butyl, cyclohexylmethyl, ethoxy-ethoxymethyl, 2-(tetrahydropyranyl)methyl) did not lead to major changes in observed AUCs. In many cases, the values are identical within the experimental error. Therefore we conclude that, in contrast to the results in rats (see Table 3), the mouse model used here (*i.e.* with a Tween 80 suspension) does not have the selectivity to discriminate between the wide range of esters tested. The physico-chemical properties of the cephalosporin esters **6a**~**6n**, **7a**, **7b**, **7d**, **7e**, **7k**, **8** and **9** indicated that there are no clear correlations between the other measured parameters (Table 2), when taken individually, and the observed AUC values. Whether this means that there is a complex relation between the AUC values and several of the parameters, or that the parameters have no relationship to the AUC values, remains to be determined.

Table 3. AUC and bioavailability (F) of some selected esters **7** in the rat.

Ester No.	R_1	R_2	AUC[a] (μg·hour/ml)	F in % (n=)
7a	Me	Et	17.4	45 (1)
7b	Me	*iso*-Butyl	13.1	34 (3)
7d	Me	Cyclohexyl-methyl	14.6	38 (3)
7e	Me	Ethoxy-ethoxy-methyl	13.4	35 (2)
7k	Et	*iso*-Butyl	22.8	61 (4)
9		(PIV ester)	17.3	45 (3)

[a] Calculated for a dose equivalent to 20 mg/kg of 3′-azidocephem **5**.

One possible cause for low AUC values of the esters (relative to iv administered cephalosporins) is the *in vivo* isomerization of the Δ-3 double bond to the Δ-2 position, which gives a biologically inactive cephalosporin isomer. This isomerization has been identified as a problem with other cephalosporin esters[10]. By synthesizing the Δ-2 isomer of **7k** and testing it in the mouse model, it was determined that the AUC

of the liberated Δ-2 isomer of the cephalosporin **5** was 40% of that obtained with the Δ-3 isomer. After po administration of the latter, the HPLC data (data not shown) demonstrate that the serum levels of the Δ-2 isomer of **5** were less than 5% of the level of the Δ-3 isomer (limit of sensitivity). Therefore isomerization of the cephalosporin ester does not appear to be responsible for the low bioavailability in this mouse model.

Because of the inability of the model using Tween 80 suspensions to discriminate between various esters of **5**, a rat model in which the esters were administered as suspensions of micronized material in water was used. The results (Table 3) showed that **7k** was significantly better absorbed than any of the other esters tested.

Current studies of the biochemical pathway of the ester cleavage are in progress and will be reported elsewhere.

Conclusion

A novel class of orally active cephalosporin esters has been identified. Among the esters studied, the 2-isobutoxycarbonyl-2-propylidene ethyl ester of the 3'-azido cephem **5** (**7k**, Ro 41-3399) was selected as a candidate for further testing.

Experimental

The ^{1}H NMR spectra were recorded with a Bruker AC 250 (250 MHz) spectrometer. Chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The IR spectra were recorded on a Nicolet FTIR spectrometer as KBr pellets. The IR, NMR data of all compounds were consistent with the assigned structures. Elemental analyses of carbon, hydrogen and nitrogen were within 0.4% of the theoretical values. All organic phases were dried over anhydrous $MgSO_4$ and concentrated on a Büchi rotatory evaporator at aspiratory pressure. Chromatography was done using the medium-pressure flash method using Merck Silica gel 60 (230~400 mesh ASTM). The PIV esters **8** and **9** were prepared according to literature[11].

Preparation of the Alcohols **2a**~**2n**

To a mixture of **1** (6.44 mol) and 1,4-diazabicyclo[2.2.2]octane (0.64 mol) was added dropwise the desired aldehyde (9.68 mol). After stirring at room temperature for two weeks, the reaction mixture was diluted with ethyl acetate, washed with water, dried and concentrated under reduced pressure. The residue was distilled under vacuum to afford a colorless mobile oil.

Preparation of the Bromides **3a**~**3n**

The alcohol **2** (0.46 mol) was cooled to 0°C and treated dropwise with 45% hydrobromic acid (144 ml, 1.16 mol). After a few minutes, conc sulfuric acid (132 ml, 2.41 mol) was added dropwise and the reaction mixture was allowed to reach room temperature. After stirring at room temperature overnight, the resulting brown reaction mixture was transferred into a separatory funnel, the lower layer was discarded and the upper layer was extracted twice with ether. The combined ether extracts were sequentially washed with saturated sodium bicarbonate and water before treatment with charcoal, drying and concentration. As the bromides **3** are thermally unstable, they were purified by chromatography (*n*-hexane-EtOAc, 9:1 (v/v)) and characterized by their NMR spectra.

3k: ^{1}H NMR ($CDCl_3$) δ 6.97 (1H, t, J=8 Hz, =CH), 4.23 (2H, s, CH_2Br), 3.98 (2H, d, J=4.8 Hz, OCH_2), 2.32 (2H, q, J=7.6 Hz, CH_2CH_3), 2.01 (1H, m, $CHCH_3$), 1.13 (3H, t, J=7.6 Hz, CH_2CH_3), 0.98 (6H, d, J=7 Hz, $(CH_3)_2$). IR (film) cm^{-1} 1717 (C=O).

Anal Calcd for $C_{10}H_{17}BrO_2$: C 48.21, H 6.88.
Found: C 48.54, H 6.97.

Preparation of the Esters **6a**~**6n** and **7a**, **7b**, **7d**, **7e**, **7k**

A solution of the cephalosporin **4** or **5** (0.25 mol) in abs DMF (2 liters) was cooled to 0°C and treated

dropwise with a solution of the bromide **3** (0.3 mol) in abs DMF (100 ml). The resulting solution was stirred for 24 hours at room temperature then diluted with toluene (2 liters) and washed with water (4 × 1 liter). The organic layer was treated with charcoal, dried, filtered and concentrated to about 500 ml. The resulting solution was added dropwise to vigorously stirred *n*-hexane (2.5 liters). The resulting crystals were collected by filtration.

Preparation of the Hydrochloride of Compound **7k**

The cephalosporin (**7k**, 117 g, 0.215 mol) dissolved in abs ethanol (800 ml) was treated dropwise at 5°C with 4.9 N hydrochloric acid in *iso*-propanol (0.43 mol). The solution was further stirred at this temperature for 1 hour then treated dropwise with *n*-hexane (1.2 liters) and stirred overnight. The resulting crystals were collected by filtration and washed with ethanol - *n*-hexane (2 : 3) (v/v) and *n*-hexane. The yield after drying was 130 g (66%).

Area Under the Curve Measurements

For the mouse model, AUC determination of the liberated cephalosporins (**4** and **5**) were made using pooled plasma from three mice at specific time points (from 0 to 45 minutes) after oral administration (0.5 ml, 20 mg/kg) of the ester in Tween 80 (4%). Blood samples were removed, prepared and analyzed by HPLC using the method of WYSS and BUCHELI[12], substituting trifluoroacetic acid for perchloric acid in the mobile phase.

For the rat model, jugular-catheterized animals were dosed either through the jugular vein (**5**, 20 mg/kg) or *via* gastric intubation (**7a**, **7b**, **7d**, **7e**, **7k**, 20 mg of the ester/kg). Blood samples (0.3 ml) were taken before treatment and over a period of 8 hours *via* the catheter. Plasma analysis[12] of **5** was performed by HPLC using a Spherisorb ODS-1 column. Bioavailability (F) was calculated by AUC of **5** following oral administration of **7a**, **7b**, **7d**, **7e**, **7k** divided by AUC of **5** after iv administration, after correcting for the differing molecular weights of the esters.

Stability in Aqueous Solution

Stability of the cephalosporin esters was measured both in phosphate buffer[13] (pH 8) and in 1.0 N HCl (pH 0) containing 1% acetonitrile at 37° by HPLC analysis of the parent esters using a Synchropak C_{18} column (4 × 250 mm) equilibrated in phosphate buffer (50 mM, pH 7) containing varying amounts of acetonitrile (from 15 to 35%). The half-life ($t_{1/2}$) of the esters was calculated from the data by fitting to a simple exponential decay.

Solubility in Water

The solubility in phosphate buffer (10 mM, pH 7) was measured by spectrophotometry at 240 nm. Addition of 100 μl of an acetonitrile solution of the ester to 1.0 ml of buffer, followed by *in vacuo* evaporation of the acetonitrile and centrifugation led to a saturated solution of the ester. An aliquot of this solution (50 ~ 500 μl) was removed, diluted to 1.0 ml of 50% aq acetonitrile, and the absorbance was measured. The concentration was determined from the absorbance values of known concentrations of the ester measured in 50% aq acetonitrile.

Measurement of Partition Coefficient ($\log_{10}$ [P])

Octanol solutions of the ester (1.0 ml, 0.5 mg/ml) were extracted with phosphate buffer (1.0 ml). The concentration of ester in the respective phases was determined spectrophotometrically at 240 nm.

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