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The Synthesis and Evaluation of 3-Substituted-7-(alkylidene)cephalosporin Sulfones as β-Lactamase Inhibitors

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Abstract—A series of 3-substituted-7-(alkylidene)cephaloporin sulfones were prepared and evaluated as inhibitors of representative class A and class C serine β-lactamase. Appropriate substituents resulted in a 1000-fold improvement in the inhibition of the class A enzymes and a simultaneous 20-fold improvement in the inhibition of class C. These new compounds have achieved the goal of creating broad scale inhibitors in the cephalosporin series. © 2000 Elsevier Science Ltd. All rights reserved.

As discussed in the previous article,[1] we have developed a program directed toward generating efficient inhibitors of β-lactamases, enzymes capable of hydolyzing penicillin and cephalosporin antibiotics. β-Lactamases represent the most common form of antibiotic resistance.[2] The 255 currently identified β-lactamases[3] have been separated into four classes, A through D.[4] Classes A, C, and D are serine enzymes, while class B are zinc metalloenzymes. Current commercial inhibitors target only the class A enzymes.

Known β-lactamase inhibitors, such as the penam sulfones (sulbactam and tazobactam) and the clavams (clavulanic acid), are of the bicyclo[3.2.0]heptane ring system. Surprisingly, relatively few cephalosporin-derived inhibitors of β-lactamases have been documented. We have been exploring modifications of our previously disclosed inhibitors[5] with the goal of expanding their ability to inhibit both class A and class C enzymes. In the previous article[1] we prepared selected C-2 modifications of a cephalosporin-derived inhibitor. This study resulted in the discovery of a highly potent inhibitor of the class C β-lactamase derived from *Enterobacter cloacae* P99. We now explore the effect of substitution at the C-3′ position of the cephalosporin.

Synthesis

Two procedures were employed in the preparation of these materials. In the first, cephalosporanate **1**[6] (exclusively the 7*Z*-isomer as depicted) was equilibrated with the corresponding Δ-2,3 isomer, **2**, then the acetate was carefully hydrolyzed to produce alcohol **3**. The prior equilibration of the Δ-3,4 to the Δ-2,3 isomer is necessary to avoid formation of the lactone under these conditions. This material was then oxidized with pyridinium dichromate to produce aldehyde **4**. Reaction of **4** with a series of Wittig reagents resulted in the production of dienes **5a**–**5f** (>9:1 = *E*:*Z*), which were subsequently oxidized with mCPBA. This oxidation also resulted in concurrent isomerization of the double bond to the desired 3,4-position. Removal of the protecting group and treatment with bicarbonate then permitted isolation of the carboxylate salts **7**. Deprotection of **6f** resulted in the production of two compounds, **7f** and **7g**.

Condensation of the aldehyde **4** with nitromethane produced exclusively the *E*-isomer of nitroalkene **8**, which was transformed into **7h**. Reaction of **4** with hydroxylamine produced oxime **9**, which was converted to nitrile **10**, oxidized and deprotected to produce carboxylate **11**.

During the course of this work, we developed an alternative synthesis that avoided the troublesome separation of **1** and **2**. In this alternate scheme, 7-ACA was first hydrolyzed to the corresponding 3′-alcohol, then the amino group, carboxylic acid, and the 3′-alcohol were sequentially protected to generate **13**. Deprotection of the amine, conversion to 7-diazocephalosporanate and oxidation using our previously identified reaction conditions produced ketone **15**. Conversion to either the 7-(2′-pyridyl) or to the 7-(2′-thiazolidinyl)methylidene-cephalosporanate (exclusively the *Z*-isomer shown), followed by removal of the alloc protecting group produced alcohol **17**, which was sensitive to lactonization

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upon treatment with acids (including silica gel). Oxidation, as before, produced aldehydes **18a** and **18b**.

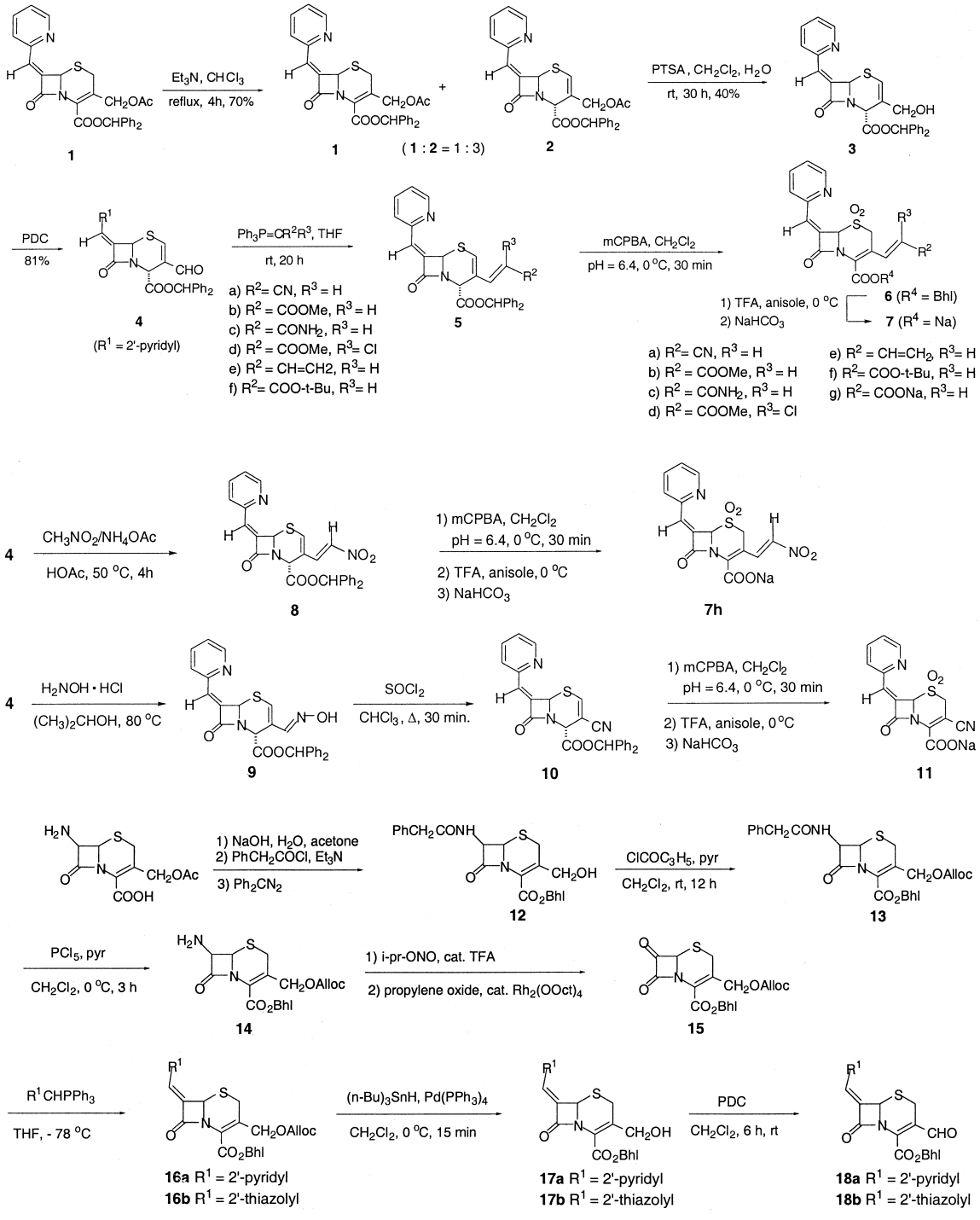
Like **4**, aldehydes **18a** and **18b** reacted with Wittig reagents to produce dienes such as **19** and **20**; **19** could either be selectively oxidized to the sulfone and converted to **7i**, or could be oxidized to the pyridine-*N*-oxide and deprotected to produce **7j**; **20** was oxidizied and deprotected to produce **21**.

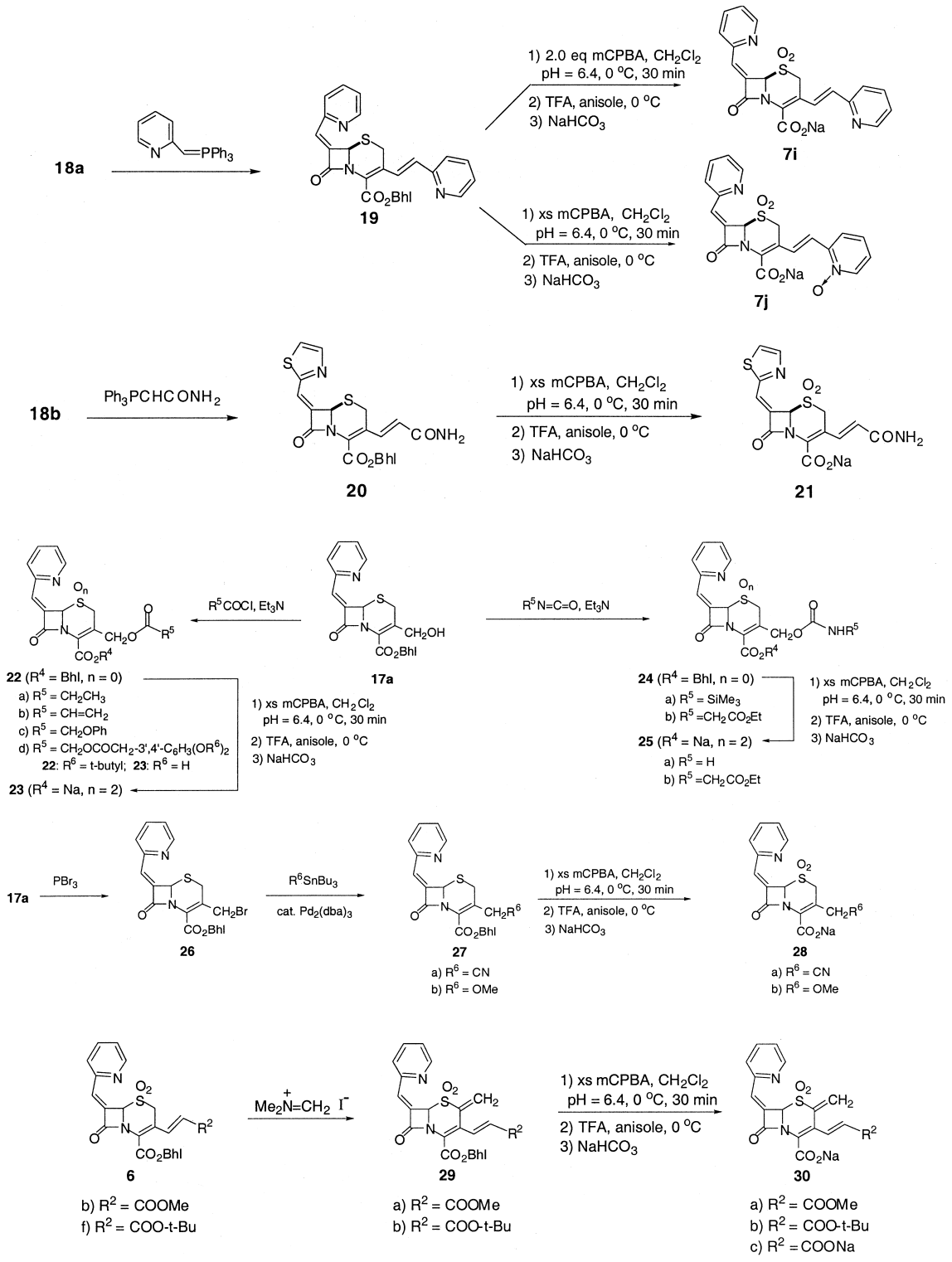
As shown below, intermediate alcohol **17a** was either directly acylated (using the appropriate acid chloride or isocyanate) to prepare a number of *O*-acyl derivatives or was converted to the corresponding bromide which could then be coupled with suitable organostannanes.

Lastly, as shown below, three 2-methylidene analogues of these new inhibitors were prepared by reaction with Eschenmoser's Salt.

Results and Discussion

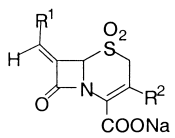
In Table 1 is displayed the activity of these compounds as inhibitors of the class C β-lactamase derived from *Enterobacter cloacae* strain P99, the class A β-lactamase





TEM-1, the class A PC1 β-lactamase, which is derived from *Staphylococcus aureus*, and the GC1 extended spectrum class C β-lactamase.[7] Especially in comparison with the parent compound, **31**, it is clear that several of the 3-vinyl-substituted cephems displayed outstanding activity versus both class C and class A lactamases. This is especially true of substituents which incorporate a vinylogous electron-withdrawing group (i.e. R^2 = HC= CH–EWG). Note that neither the electron withdrawing group itself (**11**) nor unsaturation (**7e**) produced a comparable effect. That the electron withdrawing group is necessary is further supported by the lower activity of **7g** (R^2 = COONa). However, the strongly electron withdrawing groups of **7b**, **7c** and **7h** do not follow the order predicted based on their electronegativity (**7h** >**7a** >**7b** >**7c**), but instead exhibit biological activity in the order **7a** >**7c** >**7b** >**7h**. This would imply that a recognition factor is also involved. Furthermore, it should be

Table 1. Inhibition of representative serine β-lactamases[8]



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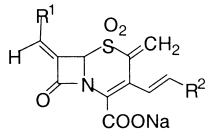


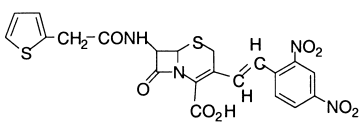
II

Compd	Type	R^1	R^2	IC_{50} (μM)			
				P99	TEM-1	PC1	GC1
Tazo				49.8	0.32	2.8	3.4
7a	I	2′-py.	*E*–CH=CH–CN	0.01	0.014	0.72	0.012
7b	I	2′-py.	*E*–CH=$CHCO_2Me$	0.20	0.02	0.30	0.30
7c	I	2′-py.	*E*–CH=$CHCONH_2$	0.026	0.09	0.10	0.01
7d	I	2′-py.	*Z*–CH=$CClCO_2Me$	0.90	0.07	1.4	0.18
7e	I	2′-py.	*E*–CH=CH–CH=CH_2	24	68	75	NT
7f	I	2′-py.	*E*–CH=$CHCO_2Bu^t$	1.48	NT	240	NT
7g	I	2′-py.	*E*–CH=$CHCO_2Na$	0.31	2.5	31	NT
7h	I	2′-py.	*E*–CH=$CHNO_2$	0.02	0.07	0.20	0.10
7i	I	2′-py.	*E*–CH=CH–2″-py	0.18	0.20	4.3	NT
7j	I	2′-py.	*E*–CH=CH–2″-py-*N*-ox	0.60	0.006	8.6	0.10
11	I	2′-py.	CN	0.029	2.34	280	NT
21	I	2′-thzl	*E*–CH=$CHCONH_2$	0.29	0.90	154	NT
23a	I	2′-py.	CH_2–O–$COCH_2CH_3$	NT	311	15.4	NT
23b	I	2′-py.	CH_2–O–COCH=CH_2	0.10	NT	110	0.9
23c	I	2′-py.	CH_2–O–$COCH_2OPh$	0.10	0.80	100	0.13
23d	I	2′-py.	CH_2–O–$COCH_2$–$C_6H_3(OH)_2$	3.6	17.4	67	NT
25a	I	2′-py.	CH_2–O–CO–NH_2	2.3	10.5	7.9	0.26
25b	I	2′-py.	CH_2–O–CO–$NHCH_2CO_2Et$	0.40	260	33.4	45.0
28a	I	2′-py.	CH_2–CN	0.90	5.6	8.9	NT
28b	I	2′-py.	CH_2–OCH_3	0.70	0.70	28.0	1.2
30a	II	2′-py.	*E*–CH=CH–CO_2Me	0.03	2.9	6.0	0.06
30b	II	2′-py.	*E*–CH=CH–CO_2Bu^t	440	NT	NT	150
30c	II	2′-py.	*E*–CH=CH–CO_2Na	6.60	2.5	NT	NT
31[1]	I	2′-py.	CH_2–O–$COCH_3$	0.50	0.30	2.6	NT

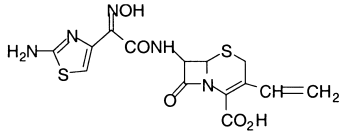
noted that the sterically bulky *tert*-butoxycarbonyl group loses much activity, relative to the methyl ester (compare **7f** and **7b**). R^2 groups incorporating a potential leaving group (**23a**–**d** and **25a,b**) and electronegative substituents attached to an sp^3-hybridized CH_2 (**28a,b**) are uniformly less active. Incorporation of an exocyclic methylidene at C-2 decreased inhibition of the class A enzymes (compare **7b** and **30a**), in agreement with our previous results.[1] It is likely that the presence of the 2-methylidene results in a nonplanar conformation of the 3′-alkylidene, interfering with conjugation, and reducing its ability to withdraw electron density.

Historically, the class A β-lactamases have been regarded as penicillinases, since they prefer penicillins as substrates, while the class C enzymes are cephalosporinases. More recently, however, Frère has demonstrated that selected penicillins can be good substrates for the class C enzymes[9] while many cepalosporins are excellent substrates of the class A β-lactamases.[10] For example, the chromogenic substrate nitrocefin, which contains a 3′-vinyl substituent, is an excellent substrate of both class A and class C enzymes. β-Lactamase inhibitors must mimic substrates in their first two interactions with the enzyme: recognition and acylation of the active site serine. Thus, we believed a cephalosporin-derived inhibitor, in particular a 3′-vinylcephem, was potentially capable of targeting both classes of enzymes. Other 3′-vinylcephalosporins, such as cefdinir, are known to be orally active, broad spectrum antibiotics.[11]

In summary, we have prepared the first cephalosporin-derived β-lactamase inhibitors to effectively inactivate both class A and class C β-lactamases. Crystallographic investigations are underway to determine the nature of the inhibited enzyme.



Nitrocefin



Cefdinir

Acknowledgements

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8. Four minutes incubation of a solution of inhibitor and enzyme (0.1–1 μM in enzyme, depending on the β-lactamase), followed by transfer of an aliquot into a dilute solution of the substrate nitrocefin. Hydrolysis is monitored spectrophotometrically at 480 nm for 1 min (rate was constant). Error is ±10%, based on multiple experiments. NT = not tested.
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