SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF SOME 6β-ACRYLAMIDO PENICILLINS

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(Received for publication August 5, 1992)

Syntheses are described for penicillins (**4b**～**4i**, **5a** and **5b**) which possess a 6β-(2-heteroaryl-3-substituted)-propenamido side-chain of fixed geometry. *In vitro* results for these compounds against a range of Gram-positive and Gram-negative bacteria showed in most cases good stability against both penicillinase and TEM-1 β-lactamase; analogues (**4b**～**4i**) bearing a 2-(2-aminothiazol-4-yl) unit showed the best intrinsic activity, the cyclohexyl compound (**4b**) being the most promising. The 1-acetoxyethyl ester (**6**) of **4b** was also prepared; in experimental animal studies the *in vivo* properties of this compound compared favourably with cefuroxime axetil and are reported together with selected *in vivo* data for the other compounds.

A continuing challenge to our research in these laboratories has been the design of semisynthetic penicillins that possess stability to bacterial β-lactamases. These enzymes have continued to proliferate, leading to the development of a variety of resistant strains of bacteria. Penicillins previously reported by us which addressed this problem may be divided into two groups:

A. Those bearing an appropriate 6α-substituent, as in temocillin[1] (BRL 17421) and BRL 36650[2]. Many other 6α-substituents were examined but proved to be less effective[3].

B. Those bearing a sterically hindered side-chain of fixed geometry, as in the alkoximino series typified by BRL 44154[4] (**1**).

The penicillins of group **A** exhibited high β-lactamase stability and broad-spectrum activity against Gram-negative bacteria, but lacked activity against important Gram-positive pathogens. On the other hand, those of group **B** combined adequate β-lactamase stability with good activity against Staphylococci and other Gram-positive species, including significant activity against methicillin-resistant strains[5], while showing restricted activity against many Gram-negative bacteria. Furthermore, we envisaged the possibility of an oral form of a group **B** penicillin through the use of a suitable pro-drug ester[6].

The important structural element of **1** for its activity and enzymic stability was the (*Z*)-alkoxyimino unit; hence we considered the possibility of finding equivalent or better biological properties through an isosteric oxime replacement. The most obvious replacement was a (*Z*)-olefinic bond, *viz.*, in a 6β-(*Z*)-(2,3-disubstituted)-acrylamido penicillin. Some penicillins of this type were already known, *e.g.* tetrasubstituted double bond analogues of type (**2**)[7], (3-heteroaryl)-acrylamido compounds such as (**3**)[8] and some 2-(2-aminothiazol-4-yl)-acrylamido penicillins of type (**4a**)[9] (including 3-alkyl and 3-heteroaryl

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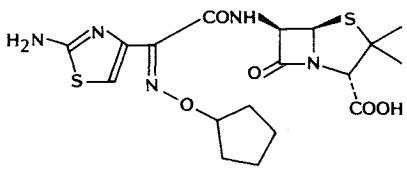
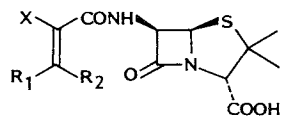
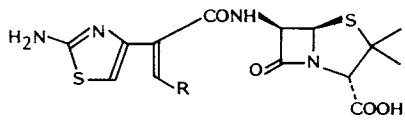
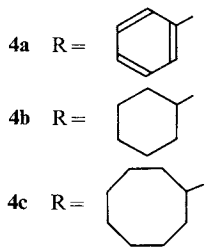


1



2 X=Ph or heteroaryl, $R_1=R_2$=alkyl
3 X=Heteroaryl, R_1=H, R_2=alkyl, aryl *etc.*



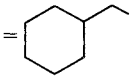


4a R=

4b R=

4c R=

4d R=



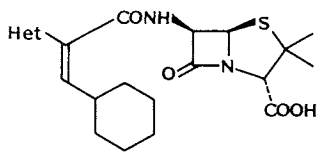
4e R=

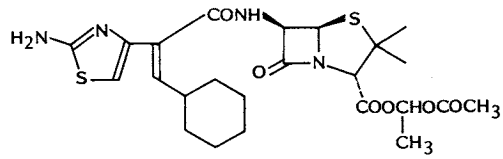
4f R=^{t}Bu

4g R=^{t}BuCH$_2$

4h R=

4i R=





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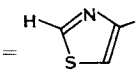
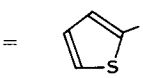


5a Het=



5b Het=

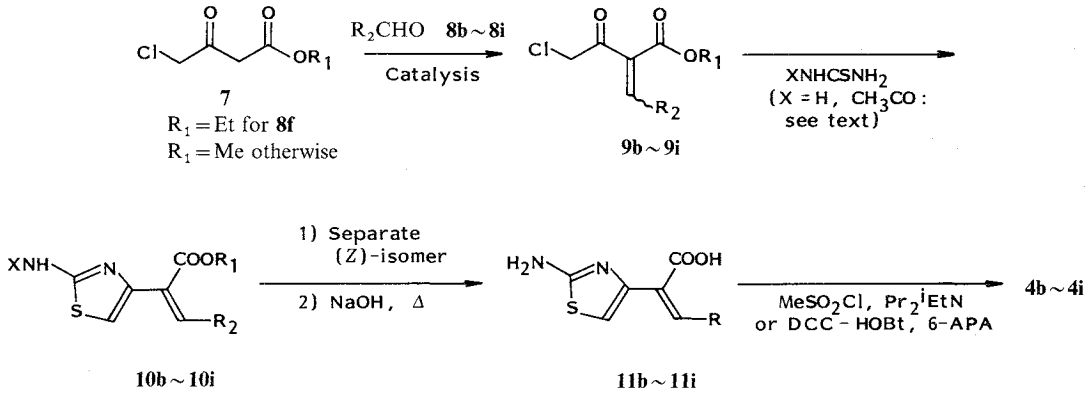
substituents as well as 3-aryl), through the main thrust of the latter series was in cephalosporins.

Our work in the oxime series based on **1** had shown that cycloalkyl- and *tert*-butyloxyimino substituents afforded highly β-lactamase stable penicillins[4] and it was natural to synthesise the corresponding acrylic analogues. We now report[10,11] on the synthesis and biological properties of a series of 6β-[2-(2-aminothiazol-4-yl)-3-substituted]-acrylamido penicillins (**4b**~**4i**) and the related heterocyclic analogues (**5a** and **5b**).

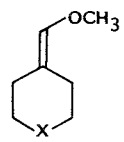
Chemistry

The synthetic route to penicillins (**4b**~**4i**) is summarised in Scheme 1. Condensation of the ethyl or methyl 4-chloro-3-oxobutanoate esters (**7**) with the appropriate aldehydes (**8b**~**8i**) was generally performed by mixing the neat liquids at low temperature with piperidine catalysis; sometimes Dean-Stark conditions proved superior. Synthesis of aldehydes (**8h** and **8i**) was achieved *via* the enol ethers (**12** and **13**) (for other aldehydes see Experimental section). The 4-tetrahydropyranyl analogue (**8h**) proved particularly troublesome, yields of **9h** being low and variable by either of the above procedures. A modification of

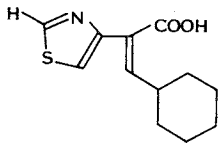
Scheme 1. Synthesis of penicillins (**4b**~**4i**).



6-APA = 6-aminopenicillanic acid. Subscripts **a**~**h** as defined for **4b**~**4i** above.



12 X=O
13 X=S



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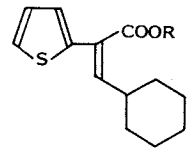


15 R=Et
16 R=H

the 'neat liquids' procedure, mixing in ethyl acetate at −10°C for 20 minutes, eventually proved best, the crude product after an aqueous work-up, then being taken through promptly to the next stage.

In general, the resulting acrylates (**9b**~**9i**) were sufficiently stable for chromatography and obtained in satisfactory yield (25~50%) but were best stored at −10°C and used fairly promptly. Mixtures of (*E*-) and (*Z*-) products resulted but this was not a problem as the desired (*Z*-) isomers were easily separated at the next stage.

In the case **9f**, R_2='Bu, cyclisation to the aminothiazole could be effected using thiourea; otherwise heating with *N*-acetylthiourea was necessary to avoid by-products of the types previously reported[12]. Readily separable mixtures of (*E*-) and (*Z*-) esters (**10b**~**10i**) resulted in 50~75% yield, all the desired *Z*-esters being crystalline solids. Saponification using excess sodium hydroxide in aqueous dioxan removed both ester and amide groups, affording amino acids (**11b**~**11i**) in 80% yield or better. A little (≤5%, NMR) (*Z*-) to (*E*-) isomerisation was observed in some hydrolyses, presumably formed by reversible Michael addition-elimination.

Finally, the coupling of side-chains (**11b**~**11i**) to 6-APA to give the desired penicillins (**4b**~**4i**) was achieved either by using a methanesulfonyl chloride mixed anhydride procedure or by using *N*,*N*'-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (DCC-HOBt); full details are given in the Experimental section.

The phenyl analogue (**4a**) was prepared for comparison by a published method[9]. The difference made by the 2-aminothiazole group is illustrated by comparison with the des-amino analogue (**5a**) and the 2-thienyl analogue (**5b**). To prepare the former compound, amino acid (**11b**) was deaminated by a known

procedure[13] followed by DCC - HOBt coupling of the resulting acid (**14**) to 6-APA giving **5a**. Condensation of ethyl 2-thiopheneacetate with aldehyde (**8b**) under Dean-Stark conditions gave workable amounts of the desired (*E*-) ester (**15**); sodium hydride gave very largely the (*Z*-) ester. Hydrolysis of **15** to give acid (**16**), followed by coupling to 6-APA, giving **5b**, were then routine.

The most interesting member of the series by virtue of its *in vitro* screen, was the cyclohexyl analogue (**4b**), BRL 48025, which was selected for conversion to a pro-drug ester. Reaction of **4b** with 1-acetoxyethyl bromide and triethylamine in DMF afforded the axetil ester (**6**), BRL 49753, the *in vivo* properties of which are discussed below.

Biological Evaluation

The *in vitro* activities of penicillins (**1**, **4b**~**4i**, **5a** and **5b**) (Table 1) were determined by serial dilution in agar against a range of clinically important aerobic bacteria. Analogues with the cyclic substituent linked directly to the acrylic system were active against *Staphylococcus aureus* and the respiratory pathogens

Table 1. MICs[a] (μg/ml) of 6β-acrylamido penicillins.

Organism	Derivative					
	4a	**4b**	**4c**	**4d**	**4e**	**4f**
Escherichia coli NCTC 10418	0.5	1.0	1.0	1.0	4.0	16
E. coli ESS	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.12
E. coli 1077*	16	8.0	16	>64	>64	>64
Proteus mirabilis C977	16	4.0	4.0	8.0	64	64
Haemophilus influenzae Q1	0.12	0.06	0.25	0.06	0.12	0.25
H. influenzae NEMC 1*	0.12	0.5	0.5	2.0	16	1.0
Moraxella catarrhalis Ravasio*	1.0	0.25	1.0	4.0	8.0	2.0
Staphylococcus aureus Oxford	1.0	0.25	0.5	0.5	0.5	4.0
S. aureus Russell*	2.0	1.0	0.5	1.0	2.0	4.0
S. aureus MB9*	4.0	2.0	1.0	4.0	32	8.0
S. aureus V573†	8.0	4.0	4.0	8.0	64	64
S. epidermidis PHLN 20	1.0	0.12	0.25	0.25	0.25	1.0
Streptococcus pneumoniae PU7†	8.0	1.0	2.0	4.0	1.0	16
S. pneumoniae 1761	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.12

Organism	Derivative					**1** (BRL 44154)
	4g	**4h**	**4i**	**5a**	**5b**	
Escherichia coli NCTC 10418	32	0.25	8.0	0.5	8.0	2.0
E. coli ESS	≤0.03	≤0.03	0.06	≤0.03	0.25	≤0.03
E. coli 1077*	>64	64	16	64	>64	32
Proteus mirabilis C977	32	2.0	4.0	8.0	>64	4.0
Haemophilus influenzae Q1	0.12	0.06	0.06	0.12	1.0	0.06
H. influenzae NEMC 1*	4.0	0.25	0.5	1.0	4.0	0.25
Moraxella catarrhalis Ravasio*	0.5	0.5	1.0	2.0	8.0	0.5
Staphylococcus aureus Oxford	0.12	0.5	0.5	0.5	1.0	0.25
S. aureus Russell*	0.5	1.0	0.5	2.0	1.0	0.25
S. aureus MB9*	2.0	4.0	1.0	8.0	4.0	0.5
S. aureus V573†	2.0	8.0	8.0	16	32	4.0
S. epidermidis PHLN 20	0.5	4.0	0.5	1.0	0.5	0.5
Streptococcus pneumoniae PU7†	1.0	8.0	1.0	4.0	2.0	2.0
S. pneumoniae 1761	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03

[a] MIC values were determined by serial dilution in Blood Agar Base (Oxoid) against an inoculum of 1×10^6 cfu.
* β-Lactamase producing strains.
† Intrinsically resistant strains.

Table 2. Comparative blood levels of **4b** by oral administration of **6** to the mouse (n=5) and the squirrel monkey (n=4).

Species	Compound	Route	Blood level				CD_{50}* (mg/kg)	
			Dose (mg/kg)	Cmax (μg/ml)	$T_{1/2}$ (minutes)	$AUC_{0\sim\infty}$** (μg·minutes/ml)	*S.a.*	*E.c.*
Mouse	**4b**	*S.c.*	50	24.2	13	666	14	12.4
		Oral	50	<0.01	—	—	>400	>200
	6	Oral	50	4.4	32	222	112	78
	5a	Oral	50	0.88	23	42.6	—	—
	5b	Oral	50	1.6	24	84.2	—	—
	Amoxycillin	Oral	50	4.9	38	308	—	50
	Cefuroxime axetil	Oral	50	8.6	25	403	—	—
						(μg·hours/ml)		
Squirrel monkey	**6**	Oral	25†	2.4	90.5	7.8		
	Cefuroxime axetil	Oral	25†	1.5	68.5	4.3		

S.a.; *Staphylococcus aureus* Smith p^-, *E.c.*; *Escherichia coli* $96R^-$.

† 25 mg/kg parent.

* Animals dosed at 1 hour and 5 hours after infection and the dose required (CD_{50}) to protect 50% of the animals calculated 4 days after infection.

** Calculated by the trapezoidal rule.

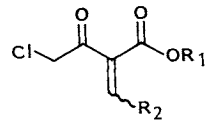
Haemophilus influenzae, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. These derivatives were generally more active than the *tert*-butyl analogue (**4f**) and activity was further affected by introduction of a methylene bridge. In the case of the cyclohexylmethyl analogue (**4e**) the additional methylene spacer reduced potency but for the *tert*-butyl example (**4g**) the reverse was true. *In vitro* studies also showed that the larger the ring the more stable the compounds was to staphylococcal β-lactamase *e.g.* using a concentrated β-lactamase preparation (cell free β-lactamase, substrate concentration 100 μg/ml, pH 7; 37°C) the half-life for analogue (**4d**) containing a five-membered ring was 7 minutes and 36 minutes for (**4b**) the six-membered ring analogue. When the ring was further enlarged to eight carbon atoms there was no further improvement in stability, **4c** having a similar half-life to **4b**.

Introduction of heteroatoms *viz.*, oxygen (**4h**) or sulfur (**4i**) into the ring led to a reduction in activity relative to **4b**. Replacement of the aminothiazolyl ring by thiazole as in **5a** or thienyl (**5b**) was also found to reduce potency. These same changes, however, had the reverse effect on oral absorption properties in mice with the thienyl analogue being the best absorbed compound and the aminothiazolyl analogue the worst (Table 2). The oral absorption of the aminothiazoly analogue (**4b**), however, could be improved by pro-drug esterification of the C-3 carboxylic acid. Administration of the acetoxyethyl ester (**6**) gave rise to high oral blood levels both in the mouse (Table 2) and in the squirrel monkey, where serum concentrations of **4b** were greater than those of cefuroxime administered as cefuroxime axetil, administered at the same dose level.

Experimental

MP's were determined on a Köfler hot stage and are uncorrected. IR spectra were recorded on KBr discs in a Perkin-Elmer 457 instrument unless otherwise stated. ^{1}H NMR spectra were recorded using a Bruker WM 250 instrument at 250 MHz unless otherwise stated, using an appropriate internal standard.

Table 3. ^{1}H NMR and IR spectral data of **9** (*E*/*Z* mixtures).



9

No.	Compound		^{1}H NMR, δ ($CDCl_3$), *inter alia*	Method of preparation (see text)	IR ($CHCl_3$) (cm^{-1})
	R_1	R_2			
9c	CH_3		3.80, 3.86 (3H, 2s), 4.37, 4.40 (2H, 2s), 6.97, 7.04 (1H, 2d, J=11 Hz)	A	1710, 1630, 1610[a]
9d	CH_3		3.82, 3.86 (3H, 2s), 4.40, 4.42 (2H, 2s), 7.02, 7.06 (1H, 2d, J=11 Hz)	A	1710, 1635
9e	CH_3		3.83, 3.87 (3H, 2s), 4.39, 4.42 (2H, 2s), 7.16, 7.21 (1H, 2t, J=8 Hz)	A	1710, 1635, 1610
9f	C_2H_5	^{t}Bu	1.20, 1.30 (3H, 2t), 4.25 (2H, 2q), 4.35, 4.37 (2H, 2s), 7.10 (1H, s)	B	—[b]
9g	CH_3	$(CH_3)_3CCH_2$	0.97, 0.98 (9H, 2s), 2.22, 2.38 (2H, 2d, J=8 Hz), 3.82, 3.86 (3H, 2s), 4.38, 4.40 (2H, 2s), 7.21, 7.26 (1H, 2t, J=8 Hz)	A	1710, 1630, 1615 (sh)[a]
9h	CH_3		3.82, 3.85 (3H, 2s), 4.40 (2H, s), 6.86, 6.95 (1H, 2d, J=10 Hz)	A	1710, 1620
9i	CH_3		3.82, 3.87 (3H, 2s), 4.38, 4.39 (2H, 2s), 6.84, 6.94 (1H, 2d, J=10 Hz)	A	1720, 1630

[a] Liquid film.
[b] Not isolated pure, fully characterised at next step.

Mass spectra were recorded using appropriate VG instruments in the electron impact (EI) or fast-atom bombardment (FAB) modes, as stated. Homogeneity of all products was assessed by TLC on Merck silica gel $60F_{254}$ plates, and by reverse-phase analytical HPLC on a Beckman Ultrasphere column where appropriate. Preparative chromatography was performed on Merck silica gel 7729 (finer than 230 mesh ASTM). Aldehydes **8b**, **8c** and **8f** were commercially available; **8d**[14], **8a**[15] and **8g**[16] were made by pyridinium chlorochromate oxidation of the corresponding primary alcohols in CH_2Cl_2. A sample of the penicillin **4a** was prepared using a published procedure[9]. Physico-chemical data for intermediates and products are summarised in Tables 3~6.

4-(Methoxymethylene)-4*H*-tetrahydropyran (**12**)

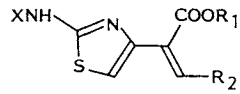
To a suspension of anhydrous (methoxymethyl)triphenylphosphonium chloride (5.47 g, 16.0 mM) in THF (15 ml) stirred under argon at 0°C was added a solution of 1 M lithium hexamethyldisilazide in THF (16 ml). After allowing to regain 20°C and stirring for 30 minutes the dark red solution was re-cooled to 0°C and 4*H*-tetrahydropyran-4-one (1.52 g, 15.2 mM) was added dropwise. The solution was again allowed to warm to 20°C and stirred for 16 hours, then diluted with Et_2O, washed twice with water, brine, dried ($MgSO_4$) and evaporated. Brief flash chromatography (eluant, EtOAc-*n*-hexane, 1:9) afforded product containing a little triphenylphosphine; by double Kugelrohr distillation (bp *ca.* 100°C/12 mm Hg) there was obtained the enol ether (**12**) as a colourless oil (1.07 g, 56%): IR ($CHCl_3$) cm^{-1} 1690; ^{1}H NMR (60 MHz, $CDCl_3$) 2.1, 2.4 (4H, 2t, 2×CCH_2), 3.65 (3H, s, CH_3O), 3.70 (4H, m, 2×OCH_2), 5.95 (1H, br s, olefinic H).

Anal Calcd for $C_7H_{12}O_2$: C 65.6, H 9.4.
Found: C 65.4, H 9.6.

4-(Methoxymethylene)-4*H*-tetrahydrothiapyran (**13**)

In an identical manner, reaction of 4*H*-tetrahydrothiapyran-4-one (4.5 g, 38.8 mM) afforded enol ether

Table 4. MP, ^{1}H NMR and IR spectral data of **10** (*Z*-isomers).



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No.	Compound X, R_1	Compound R_2	MP (°C)	^{1}H NMR, δ ($CDCl_3$), *inter alia*	IR (cm^{-1})
10c	CH_3CO, CH_3		141~143	2.20 (3H, s), 2.83 (1H, m), 3.86 (3H, s), 6.64 (1H, d, J=11 Hz), 6.92 (1H, s)	1719, 1655, 1560
10d	CH_3CO, CH_3		150~151	2.18 (3H, s), 2.98 (1H, m), 3.86 (3H, s), 6.64 (1H, d, J=10 Hz), 6.93 (1H, s)	1718, 1653, 1560
10e	CH_3CO, CH_3		141~142	2.20 (3H, s), 2.30 (2H, t, J=7.3 Hz), 3.85 (3H, s), 6.78 (1H, t, J=7.8 Hz), 6.94 (1H, s)	1710, 1535, 1440[a]
10f	H, C_2H_5	^{t}Bu	121~125	1.16 (9H, s), 1.36 (3H, t, J=7 Hz), 4.31 (2H, q, J=7 Hz), 6.24 (1H, s), 6.46 (1H, s)	1720, 1610, 1520[a]
10g	CH_3CO, CH_3	$(CH_3)_3CCH_2$	153.5~154.5	0.96 (9H, s), 2.23 (3H, s), 2.31 (2H, d, J=8 Hz), 3.86 (3H, s), 6.83 (1H, t, J=8 Hz), 6.92 (1H, s)	1727, 1655, 1553
10h	CH_3CO, CH_3		225~227	2.24 (3H, s), 2.90 (1H, m), 3.88 (3H, s), 6.59 (1H, d, J=10 Hz), 6.95 (1H, s)	1726, 1660, 1551
10i	CH_3CO, CH_3		181~186	2.25 (3H, s), 2.50~2.90 (5H, m), 3.88 (3H, s), 6.56 (1H, d, J=10 Hz), 6.96 (1H, s)	1700, 1580, 1540

Satisfactory microanalytical data (CHN, ±0.4%) and/or high resolution EI-MS molecular ion measurement were obtained for all the above esters.

[a] $CHCl_3$ solution.

13 (2.1 g, 38%) as a colourless oil: IR (film) cm^{-1} 1680.

4*H*-Tetrahydropyran-4-carboxaldehyde (**8h**)[17]

A solution of **12** (1.0 g, 7.81 mM) in THF (5 ml) and water (5 ml) was stirred at 20°C with 4-toluenesulfonic acid (1.48 g, 1 equiv). After 5 hours, reaction being complete by TLC, saturated aq $NaHCO_3$ (10 ml) was added and the product extracted with CH_2Cl_2 (4×), followed by drying ($MgSO_4$). Evaporation followed by Kugelrohr distillation gave the aldehyde (**8h**)[17] (0.73 g, 82%), as a colourless oil; IR (film) cm^{-1} 1725; ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.85 (4H, m, 2×CCH_2), 2.40 (1H, m, C*H*CHO), 3.20~4.20 (4H, m, 2×OCH_2), 9.81 (1H, br s, CHO).

4*H*-Tetrahydrothiapyran-4-carboxaldehyde (**8i**)[18]

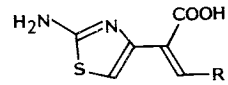
In an identical manner, hydrolysis of **13** (2.1 g, 14.6 mM) afforded aldehyde **8i**[18] (1.55 g, 82%) as a colourless oil; IR (film) cm^{-1} 1725; ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.50~1.90 (2H, m), 2.15~2.45 (2H, m), 2.55~2.85 (5H, m), 9.61 (1H, s).

Scheme 1 is illustrated by a fully detailed description of the synthesis of the leading compound of the series, **4b**. Spectroscopic and analytical data for the other penicillins (**4c**~**4i**, **5** and **6**) and intermediates are summarised in Tables as indicated; slight variations of procedure are also noted in the following text.

Methyl (*E*,*Z*)-4-Chloro-2-(cyclohexyl)methylidene-3-oxobutanoate (**9b**)

Method A: A mixture of cyclohexane carboxaldehyde (**8b**) (2.96 g, 26.4 mM) and methyl 4-chloro-3-oxobutanoate (**7**, R_1=Me) (3.61 g, 24 mM) was stirred at 0°C in the presence of piperidine (0.1 ml). After

Table 5. ^{1}H NMR and IR spectral data of **11**.



11

Compound No.	R	^{1}H NMR, δ [$(CD_3)_2SO$], *inter alia*	IR (cm^{-1})
11c		2.67 (1H, m), 6.37 (1H, d, J=10 Hz), 6.37 (1H, s), 7.04 (2H, br s)	1695, 1628, 1565, 1530
11d		2.90 (1H, m), 6.17 (1H, d, J=10 Hz), 6.40 (1H, s), 6.93 (2H, br s)	
11e		2.17 (2H, t, J=7.5 Hz), 6.39 (1H, s), 6.49 (1H, t, J=7.9 Hz), 7.03 (2H, br s)	1640, 1611, 1559, 1526
11f	^{t}Bu	1.11 (9H, s), 6.03 (1H, s), 6.24 (1H, s), 6.98 (2H, br s)	1610, 1565, 1525
11g	$(CH_3)_3CCH_2$	0.91 (9H, s), 2.18 (2H, d, J=8 Hz), 6.41 (1H, s, 5-H), 6.51 (1H, t, J=8 Hz), 7.21 (2H, br s)	1700 (sh), 1636, 1559, 1528
11h		2.78 (1H, m), 5.94 (1H, d, J=10 Hz), 6.45 (1H, s), 6.95 (2H, br s)	1630, 1569, 1530
11i		2.40~2.80 (5H, m), 6.26 (1H, d, J=10 Hz), 6.42 (1H, s), 7.06 (2H, br s)	1700 (sh), 1630, 1560, 1530

4 hours, when reaction appeared complete by TLC, the mixture was diluted with ethyl acetate (100 ml), washed with cold 1 M HCl (3×), water (2×), brine, dried ($MgSO_4$) and evaporated. Flash chromatography, eluting with 2~6% ethyl acetate-*n*-hexane, afforded on pooling and evaporation of appropriate fractions the chloroacrylates (**9b**) (2.11 g, 36%); IR (film) cm^{-1} 1715, 1630; ^{1}H NMR (90 MHz; $CDCl_3$) δ 0.90~2.80 (11H, M), 3.78, 3.84 (3H, 2s, OCH_3), 4.34, 4.37 (2H, 2s, $ClCH_2$), 6.86 and 6.92 (1H, 2d, J=9 Hz, olefinic H). Isomer ratio $Z:E \simeq 2:1$. (Found: M, 244.0858 by EI. $C_{12}H_{17}ClO_3$ requires M, 244.0866). For some aldehydes (especially **8f**) Method B was used: The aldehyde (nmM) and **7** (1.2 equiv) dissolved in benzene (5 ml) per mM aldehyde) was treated with piperidine and acetic acid (2% mol equiv each), then heated at reflux with a Dean-Stark water separator until reaction appeared complete by TLC (usually 2 hours). The solution was cooled, washed with water (3×) and brine, then worked up as in Method A to give the chloroacrylates. Spectral data for **9c~9i**, prepared by the method indicated in each case, are summarised in Table 1. See also the earlier comment on **9h**.

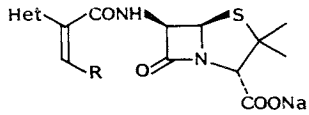
Ethyl *E*-[2-(2-Thienyl)-3-cyclohexyl]propenoate (**15**)

Similarly, condensation of ethyl 2-thiophenacetate (1.70 g, 10 mM) with aldehyde **8b** (1.12 g, 1 equiv) under Method B conditions, heating at reflux in toluene (20 ml) for 28 hours, afforded on work-up a crude product which was subjected to flash chromatography, eluting with 1~5% EtOAc-*n*-hexane. The first eluted product was the *E*-ester (**15**) (0.18 g, 7%), a colourless oil; ^{1}H NMR ($CDCl_3$) δ 1.05~1.40, 1.60~1.85 (10H, 2m, cyclohexyl H), 2.50~2.65 (1H, m, allylic H), 1.37 (3H, t, J=7 Hz, CH_3CH_2O), 4.34 (2H, q, J=7 Hz, CH_3CH_2O), 6.09 (1H, d, J=10 Hz, olefinic H), 6.95~7.05 (2H, m, aryl H), 7.18 (1H, dd, J=5 Hz, 1 Hz, 5-H). (Found: M, 264.1179 by EI. $C_{15}H_{20}O_2S$ requires M, 264.1184). Further elution afforded the corresponding *Z*-ester (0.07 g, 3%); ^{1}H NMR (60 MHz, $CDCl_3$), *inter alia*, 5.80 (1H, d, J=10 Hz, olefinic H), 6.50~7.00 (3H, m, aryl H).

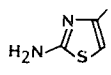
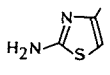
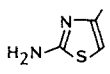
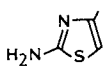
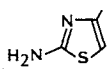
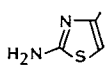
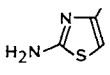
Methyl *Z*-[2-(2-Acetamidothiazol-4-yl)-3-cyclohexyl]propenoate (**10b**)

The isomeric chloroacrylates **9b** (1.15 g, 4.70 mM) and *N*-acetylthiourea (0.74 g, 1.3 equiv) were stirred and heated at 85°C in anhydrous DMF (5 ml) in the presence of 4 Å molecular sieves. After 2.5 hours the reaction (then complete by TLC) was cooled, diluted with EtOAc-Et_2O, 1:1 (30 ml) and washed

Table 6. ^{1}H NMR, MS and IR spectral data of **4**, **5a** and **5b**.



4, **5** and **6**

Compound No.	Het	R	^{1}H NMR, δ (D_2O), *inter alia*	FAB-MS (m/z)[a]	IR (cm^{-1})	Method of preparation (see text)
4c			1.55, 1.63 (6H, 2s), 2.53 (1H, m), 4.22 (1H, s), 5.65 (2H, ABq), 6.25 (1H, d, J=11 Hz), 6.42 (1H, s)[b]	501	1765, 1650 (sh), 1611, 1521	B
4d			1.49, 1.60 (6H, 2s), 2.61 (1H, m), 4.21 (1H, s), 5.58 (2H, ABq), 6.23 (1H, d, J=11 Hz), 6.43 (1H, s)	459	1763, 1659, 1604, 1527	B
4e			1.59, 1.65 (6H, 2s), 2.19 (2H, m), 4.24 (1H, s), 5.70 (2H, ABq), 6.43 (1H, t, J=8 Hz), 6.44 (1H, s)[b]	465	1764, 1655 (sh), 1609, 1525	B
4f		^{t}Bu	1.10 (9H, s), 1.49, 1.60 (6H, 2s), 4.20 (1H, s), 5.62 (2H, ABq), 6.28 (1H, s), 6.42 (1H, s)	447	1765, 1655 (sh), 1604, 1524	B
4g		$(CH_3)_3CCH_2$	1.48, 1.59 (6H, 2s), 2.12 (2H, m), 4.20 (1H, s), 5.58 (2H, ABq), 6.43 (1H, t, J=8 Hz), 6.45 (1H, s)	461	1764, 1655 (sh), 1609, 1530	B
4h			1.50, 1.59 (6H, 2s), 2.55 (1H, m), 4.22 (1H, s), 5.61 (2H, ABq), 6.12 (1H, d, J=10 Hz), 6.49 (1H, s)	453	1763, 1660 (sh), 1603, 1526	B
4i			1.45, 1.52 (6H, 2s), 3.82 (1H, s), 5.41 (2H, ABq), 6.02 (1H, d, J=10 Hz), 6.24 (1H, s)	—	1765, 1660 (sh), 1605, 1525	A
5a			1.51, 1.61 (6H, 2s), 2.34 (1H, m), 4.22 (1H, s), 5.63 (2H, ABq), 6.40 (1H, d, J=10.5 Hz), 7.42 (1H, d, J=2 Hz), 8.92 (1H, d, J=2 Hz)	458	1769, 1662, 1611, 1506	B
5b			1.47, 1.52 (6H, 2s), 2.33 (1H, m), 4.10 (1H, s), 5.58 (2H, ABq), 5.75 (1H, d, J=10 Hz), 6.85~7.22 (3H, m)	457	1769, 1660 (sh), 1609, 1498	B

[a] $(M+H)^+$.
[b] In $(CD_3)_2CO$-D_2O, 1:1.
[c] In $(CD_3)_2SO$.

thoroughly with water (6×) to remove *N*-acetylthiourea traces, then with brine, dried ($MgSO_4$) and evaporated. The resulting crude product (1.51 g) was subjected to flash chromatography, being applied in toluene and eluted with 20~40% EtOAc-*n*-hexane. First eluted was the desired *Z*-ester **10b** (X=MeCO) as a white solid (0.72 g, 56%), mp 146~149°C (from ethyl acetate-hexane): IR ($CHCl_3$) cm^{-1} 1710, 1560 (sh), 1535, 1510 (sh); ^{1}H NMR ($CDCl_3$) δ 1.24, 1.73 (10H, 2m, cyclohexyls), 2.18 (3H, s, CH_3CONH), 2.55 (1H, m, allylic H), 3.86 (3H, s, CH_3O), 6.55 (1H, d, J=10 Hz, olefinic H), 6.93 (1H, s, 5-H), 9.88 (1H, br s, D_2O exchangeable, NH).

Anal Calcd for $C_{15}H_{20}N_2O_3S$: C 58.4, H 6.5, N 9.1, S 10.4.
Found: C 58.5, H 6.5, N 9.3, S 10.3.

Further elution afforded the *E*-ester of **10b** which solidified less readily (0.23 g, 18%), mp 183~184°C (from ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$), δ *inter alia*, 6.90 (1H, s, 5-H), 7.05 (1H, d, J=10 Hz, olefinic H).

Anal Calcd for $C_{15}H_{20}N_2O_3S$: C 58.4, H 6.5, N 9.1, S 10.4.
Found: C 58.4, H 6.8, N 9.1, S 10.1.

Similarly prepared were **10a**~**10i**, with the exception that thiourea (1 equiv) in EtOH at 20°C for 16 hours was used in the preparation of **10f**; spectral data, Table 4.

Z-[2-(2-Aminothiazol-4-yl)-3-cyclohexyl]propenoic Acid (**11b**)

A solution of *Z*-ester **10b** (0.43 g, 1.38 mM) in purified dioxan (11 ml) was heated at 90~95°C with 1 M NaOH (7 ml, 5 equiv) for 5 hours, when hydrolysis appeared complete by TLC ($CHCl_3$ - MeOH - AcOH, 17:2:1). The solution was cooled, evaporated to dryness, redissolved in water and washed twice with EtOAc - Et_2O, 1:1. On acidification of the aqueous phase to pH 3.5 using 5 M HCl, with cooling, a dense white precipitate appeared which was filtered off, washed well with cold water and dried, giving the amino acid **11b** as an amorphous solid (0.30 g, 85%): IR cm^{-1} 1690 (sh), 1628, 1560, 1528; ^1H NMR [$(CD_3)_2SO$] δ 1.00~1.35, 1.55~1.80 (10H, 2m, cyclohexyl H), 2.45 (1H, m, allylic H), 6.29 (1H, d, J=10 Hz, olefinic H), 6.40 (1H, s, 5-H), 7.13 (2H, br s, D_2O exchangeable, NH_2), 12.85 (1H, vbrs, D_2O exchangeable, CO_2H); (Found; M, 252.0929 by EI. $C_{12}H_{16}N_2O_2S$ requires M, 252.0932). An analytical sample was obtained by reprecipitation from THF - *n*-hexene, mp 115~118°C.

Anal Calcd for $C_{12}H_{16}N_2O_2S \cdot 1H_2O$: C 53.3, H 6.7, N 10.35, S 11.85.
Found: C 53.7, H 6.7, N 10.15, S 11.7.

For acids **11d** and **11f**~**11i** it was necessary to acidify the aqueous solution to pH 2 and extract into EtOAc (+THF for **11h**); spectral data in Table 5.

Z-[2-(Thiazol-4-yl)-3-cyclohexyl]propenoic Acid (**14**)

A solution of acid **11b** (126 mg, 0.5 mM) in THF (20 ml) was added dropwise to a stirred solution of *tert*-amylnitrite (0.13 ml, 2equiv) in THF (3 ml) heated at 60°C[13]. After 1 hour the solution was cooled, concentrated to near dryness and redissolved in ethyl acetate followed by washing with water. The organic phase was extracted with satd aq $NaHCO_3$ (5×), then the combined aqueous extracts were acidified to pH2 using 2 M HCl and the product extracted into ethyl acetate. The organic extract was washed with water and brine, dried ($MgSO_4$) and evaporated to a reddish oil which was chromatographed, eluting with 0~10% MeOH in $CHCl_3$. Pooling and evaporation of appropriate fractions gave the acid **14** (22 mg, 19%) as a gum: ^1H NMR (90 MHz, $CDCl_3$) δ 0.90~1.40, 1.55~1.95 (10H, 2m, cyclohexyl H), 3.15 (1H, m, allylic H), 6.73 (1H, d, J=10 Hz, olefinic H), 7.43 (1H, d, J=2 Hz, 5-H), 8.77 (1H, d, J=2 Hz, 2-H); EI-MS m/z 237 (M^+); (Found: M, 237.0825. $C_{12}H_{15}NO_2S$ requires M, 237.0824).

E-[2-(Thien-2-yl)-3-cyclohexyl]propenoic Acid (**16**)

A solution of **15** (166 mg, 0.62 mM) in dioxan (2.5 ml) was treated with 1 M NaOH (2 ml) and heated at 70°C for 7 hours, when no ester was visible by TLC. Work-up was performed as for acids **11b**~**11i** (extraction variant) to afford **16** (156 mg, 94%) as a gum, homogeneous by TLC, sufficiently pure for further use: ^1H NMR [$(CD_3)_2CO$] δ 1.10~1.45, 1.60~1.85 (10H, 2m, cyclohexyl H), 2.70 (1H, m, allylic H), 6.10 (1H, d, J=9.9 Hz, olefinic H), 7.00, 7.11, and 7.34 (3H, 3dd, aryl H); EI-MS m/z 236 (M^+) (Found: M, 236.0881. $C_{13}H_{16}O_2S$ requires M, 236.0871).

6β-[*Z*-[2-(2-Aminothiazol-4-yl)-3-cyclohexyl]propenamido]penicillanic Acid (**4b**)

Method A: A solution of acid **11b** (12.17 g, 48.0 mM) in DMF (100 ml) was treated at 0°C with *N*,*N*-diisopropylethylamine (8.1 g, 62.8 mM). The solution as further cooled to −20°C and an solution of methanesulfonyl chloride (6.07 g, 53 mM) in DMF (20 ml) was added over 15 minutes. After another 5 minutes a solution of 6-APA (13.56 g, 62.7 mM) in water (20 ml) and triethylamine (12.2 g, 120 mM) was added in one portion. The mixture was stirred at 0°C for 30 minutes, diluted with water (200 ml) and adjusted to pH 7.5 (5 M HCl), then the clear solution was washed with EtOAc (2×200 ml). The aqueous phase was briefly concentrated to remove organic traces, then acidified to pH 2.5 using 5M HCl. After cooling in ice, the precipitate was filtered off, washed with water and dried to afford penicillin **4b** as a hygroscopic white solid (15.1 g, 70%): Spectral data on Na salt: IR cm^{-1} 1765, 1650 (sh), 1609, 1526; ^1H NMR (D_2O) δ 1.10~1.40, 1.55~1.80 (10H, m, cyclohexyl H), 1.51, 1.61 (6H, 2s, $(CH_3)_2C$), 2.24 (1H, m, allylic H), 4.23 (1H, s, 3-H), 5.60 (2H, ABq, J=4 Hz, 5-H+6-H), 6.15 (1H, d, J=10 Hz, olefinic H), 6.44 (1H, s, thiazole 5-H); FAB-MS m/z 495 $(M+Na)^+$, 473 $(M+H)^+$. The sodium salt was obtained

by dissolving **4b** in THF-EtOAc, adding water, adjusting the aqueous pH to 7 (satd $NaHCO_3$), then separating and freeze-drying the aqueous phase. This was the more convenient form for biological testing.

Method B: A solution of acid **11b** (100 mg, 0.40 mM) and 1-hydroxybenzotriazole (67 mg, 0.44 mM) in DMF (2 ml) was stirred at 0°C and *N*,*N'*-dicyclohexylcarbodiimide (90 mg, 0.44 mM) was added. The mixture was allowed to regain 20°C, stirred for a further 2 hours and filtered, then the filtrate was stirred with a solution of 6-APA (95 mg, 1.1 equiv) in water (2 ml) containing triethylamine (0.1 ml)†. Complete solution was obtained by addition of further DMF. After 2 hours the solution was again filtered, the precipitate washed with a little DMF and the combined filtrate and washings evaporated to near dryness. The residue was redissolved in water containing a little satd $NaHCO_3$ and washed twice with Et_2O-EtOAc, 1:1, then the aqueous phase was acidified to pH 2 (2 M HCl) and extracted with EtOAc (3×). The combined organic extracts were washed with water (4×), then water added and the pH adjusted to 7, with stirring, by addition of satd $NaHCO_3$. The aqueous phase was separated, combined with a further aqueous washing, and lyophilised to give crude product (135 mg). Chromatography was effected using either Diaion HP20SS resin, eluting with acetone-water mixtures, or silica gel, eluting with EtOAc-2-PrOH-water mixtures. The product (54 mg, 29%) had identical spectral data to material prepared by Method A.

Penicillins **4c**～**4i**, **5a** and **5b** were similarly prepared using Method A or B (as stated) and their spectral data are summarised in Table 6.

(1*R*,1*S*)-1-Acetoxyethyl 6β-[*Z*-[2-(2-Aminothiazol-4-yl)-3-cyclohexyl]propenamido]penicillanate (**6**)

A solution of **4b** (free acid; 3.0 g, 6.7 mM) in DMF (20 ml) was stirred under argon at 0°C and treated sequentially with triethylamine (1.86 ml, 2.0 equiv) and 1-bromoethyl acetate (2.26 g, 2.0 equiv). The solution was allowed to regain 20°C and stirred for 2 hours, when little further change was apparent by TLC, then it was evaporated to near dryness and diluted with ethyl acetate. This solution was then washed with 5% aqueous citric acid, brine, satd aq $NaHCO_3$, and brine (3×). Following drying ($MgSO_4$) and evaporation, the crude product (3.42 g) was chromatographed, being applied in CH_2Cl_2 and eluted with EtOAc-*n*-hexane mixtures from 1:2 to 2:1. Appropriate fractions were pooled and evaporated to give the ester **6** (2.30 g, 64%) as a foam; IR cm^{-1} 1765, 1656, 1612, and 1523; ^1H NMR ($(CD_3)_2CO$) δ 1.23 (3H, d, J=5 Hz, CH_3CH), 1.53, 1.64 (6H, 2s, $(CH_3)_2C$), 1.10～1.90 (10H, m, cyclohexyls; CH_3CO solvent-obscured); 2.59 (1H, m, allylic H), 4.40, 4.44 (1H, 2s, 3-H, isomers), 5.65～5.85 (2H, m, 5-H+6-H), 6.21 (1H, d, J=10 Hz, olefinic H), 6.36, 6.37 (1H, 2s, thiaz. 5-H), 6.41 (2H, br s, D_2O exchangeable, NH_2), 6.90 (1H, m, $CH_3CH(O)O$), 8.09 (1H, d, D_2O exchangeable, 6-NH); isomer ratio *ca.* 1:1; FAB-MS *m/z* 537 $(M+H)^+$.

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