N-(FUNCTIONALIZED ALKYL) DERIVATIVES OF 6-AMINOPENICILLANIC ACID: A NEW SERIES OF SPECIFIC INHIBITORS OF β-LACTAMASE FROM *ENTEROBACTER CLOACAE* P99

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Eight of nine new *N*-alkylaminopenicillanic acids (**7a~c, e~j**), prepared *via* efficient direct monoalkylation reactions, were found to be specific inhibitors of cephalosporinase P99 with $IC_{50} \leq 4$ mg/liter, while representative corresponding *S*-oxidized derivatives were less active.

Since the disclosure of the β-lactamase inhibitor clavulanic acid[1], routine screening procedures have revealed the ability to protect susceptible β-lactam antibiotics against inactivation by β-lactamase in a number of semisynthetic, as well as natural, β-lactam-containing compounds. Prominent amongst these are penicillanic acid sulfone[2] (sulbactam) and 6β-bromopenicillanic acid[3] (BPA), whose structures lack the amino-nitrogen of 6-aminopenicillanic acid (6-APA), from which they are prepared, although other derivatives which retain this, and may therefore be more readily accessible, can also have interesting properties.[4,5] Described here are some new *N*-alkylaminopenicillanic acid derivatives of this type which are specific inhibitors of cephalosporinase P99. (The syntheses of some simple *N*-alkyl derivatives of 6-APA and thence of benzylpenicillin or phenoxymethylpenicillin as potential antibacterial agents have been previously reported[6,7]).

Chemistry

The general synthetic strategy used in the present work (Scheme 1) involves *N*-alkylating a carboxyl-protected derivative (**I**) of 6-APA to give an intermediate ester derivative (**III**) which could be readily purified prior to a clean deprotection step giving a title compound (**II**).

Thus, direct alkylation of allyl 6-aminopenicillanate (**1**)[8] with phenacyl bromide, bromoacetic esters, or iodoacetonitrile in DMF in the presence of $EtN(i\text{-}Pr)_2$ as proton acceptor gave selectively the expected *N*-monoalkylated products (**4**) in good yields (Table 1, Entries A~E). The alkyl groups introduced into these products (**4a~e**) have electron withdrawing substituents on the α-carbon and were chosen in the anticipation that they would decrease the nucleophilicity of the amino function and thus favor monoalkylation. (In fact, only traces of di-alkylated byproducts were detectable in the reaction mixtures under the conditions described). Benzylation (Table 1, Entry F) was less selective with respect to mono-

Scheme 1. General synthetic strategy.

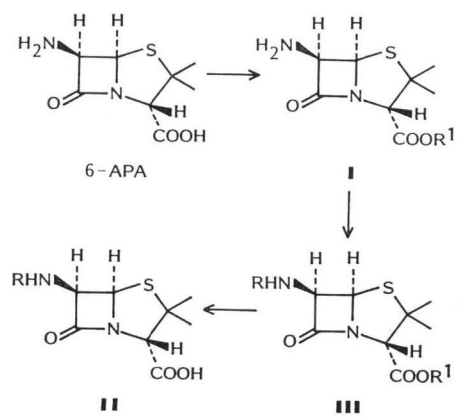


Table 1. *N*-Monoalkylations of esters of 6-APA, their β-sulfoxides, or sulfones.[a]

Entry	Product	Substrate[b]/ mmol	Reagent/mmol	Reaction time (hours)	Isolation procedure	Yield (%)	MP (°C)
A	**4a**·TsOH	**1** / 20	$PhCOCH_2Br$/21	1	A	87	126 (dec.)
B	**4b**	**1** / 10	$BrCH_2CO_2Et$/12.5	3	B (a)	89	oil[c]
C	**4c**·HCl	**1** / 10	$BrCH_2CO_2CH_2Ph$/12.7	3.5	B (b)+C	72	103~104.5
D	**4d**	**1** / 10	$BrCH_2CO_2CH_2CH=CH_2$/11	3	B (a)	88	oil[d]
E	**4e**	**1** / 20	ICH_2CN/21	4[e]	B (a)	74	oil[f]
F	**4f**·TsOH	**1** / 15	$PhCH_2Br$/16.5	3	B (b)+A	43	136~137.5
G	**4g**	**1** / 20	$CH_3COCH=CH_2$/40	64	B (a)	62	oil[g]
H	**4g**[h]·TsOH	**1**[h] / 15	$CH_3COCH=CH_2$/60	64	A	71	143~144
I	**5b**	**2**[i] / 2	$BrCH_2CO_2Et$/2.2	18	B (c)+D	53	98~99
J	**6a**[h]	**3**[h,j] / 10	$PhCOCH_2Br$/20	3	B (a)+D	41	107.5~108
K	**6g**[h]	**3**[h,j] / 10	$CH_3COCH=CH_2$/40	48	B (a)+D	33	86~87

[a] Substitution reactions in the presence of $EtN(i\text{-}Pr)_2$ in DMF at 50°C, according to General procedure (i); Addition reactions in CH_2Cl_2 at room temperature according to General Procedure (ii).

[b] Used as hydrotosylate.

[c] Characterized as hydrochloride, prisms, mp 102~104°C from EtOAc - isopropanolic HCl.

[d] Characterized as hydrochloride, mp 105~106.5°C from EtOAc - ethereal HCl.

[e] 1.5 hours at 50°C then 2.5 hours at 60°C. Corresponding reaction with $ClCH_2CN$ gives lower yield after longer reaction time.

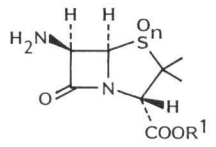
[f] Rf 0.36 (EtOAc - cyclohexane, 1:1). Although **4e** could not be characterized as a crystalline derivative, the corresponding ester **4e** ($R^1=CH_2Ph$), similarly prepared, gave a hydrotosylate (Isolation Procedure B (a)+A), mp 90~94°C (dec.), which was fully characterized.

[g] Characterized as hydrochloride, from EtOAc - ethereal HCl.

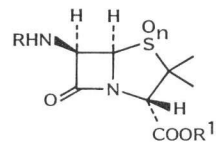
[h] Pivaloyloxymethyl ester ($R^1=CH_2O_2CBu^t$).

[i] Table 3, Entry A.

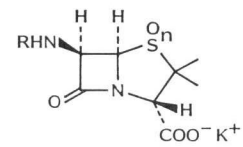
[j] **3** ($R^1=CH_2O_2CBu^t$) was prepared according to BARTH, W. E., U. S. Patent 4,260,598 (1981) and used as the *p*-TsOH addition salt, which we have found to have the curious property of crystallizing from EtOAc only in the presence of excess acid and then as a well-defined dihydrotosylate, monohydrate; needles, mp 102~105°C.



1 n=0
2 n=1 (β-sulfoxide)
3 n=2



4 n=0
5 n=1 (β-sulfoxide)
6 n=2



7 n=0
8 n=1 (β-sulfoxide)

a R=$PhCOCH_2$; **b** R=EtO_2CCH_2; **c** R=$PhCH_2O_2CCH_2$; **d** R=CH_2: $CHCH_2O_2CCH_2$;
e R=$NCCH_2$; **f** R=$PhCH_2$; **g** R=$CH_3COCH_2CH_2$; **h** R=$PhCH(OH)CH_2$;
i R=$CH_3CH(OH)CH_2CH_2$; **j** R=$K^+{}^-O_2CCH_2$
$R^1=CH_2CH$: CH_2, unless otherwise stated

versus di-alkylation but still of preparative value. 1,4-Additions of esters of 6-APA to methyl vinyl ketone in dichloromethane also gave mainly the desired monoalkylated derivatives (Table 1, Entries G, H). The alcohols (**4h**, **4i**) (as mixtures of diastereoisomers at the new chiral center in the side chain) were prepared by sodium borohydride reduction of the corresponding ketones (**4a**, **4g**).

Each of the allyl esters (**4a~i**), except **4d**, was readily converted to the corresponding pure crystalline potassium salt (**7**) for biological testing by catalytic transallylation with potassium 2-ethylhexanoate

Table 2. Potassium salts by catalytic deallylation.[a]

Entry	Product	Starting material	Scale (mmol)	Yield (%)	MP (°C(dec.))	IR (KBr)[b] (cm^{-1})
A	**7a**	**4a**·TsOH	1.0	75[c]	195~200	1773, 1670, 1600
B	**7b**	**4b**	1.0	88[c,d,e]	205~207	1745, 1600
C	**7c**	**4c**·HCl	1.0	85[f]	246~250	1768, 1730, 1600
D	**7e**	**4e**	5.3	83[d,f]	223~225	2250w[g], 1770, 1600
E	**7f**	**4f**·TsOH	1.9[h]	89[d]	198~207	1750, 1600
F	**7g**	**4g**	1.3	85[c,d]	194~200	1753, 1703, 1595
G	**7h**	**4h**	See Experimental			
H	**7i**	**4i**				
I	**7j**	**4d**	2.3[h]	77[i]	Amorphous	1752, 1600
J	**8a**	**5a**	1.0	85	Amorphous	1762, 1687, 1605
K	**8b**	**5b**	0.5	89[c,d]	170~172	1761, 1733, 1610
L	**8f**	**5f**	1.6	87	195~197	1755, 1600
M	**8g**	**5g**·TsOH	1.0	88[c,j]	179~180	1760, 1700, 1603

[a] Transallylation with potassium 2-ethylhexanoate in the presence of $Pd(PPh_3)_4$ in EtOAc, according to General Procedure (iii).
[b] Carbonyl absorptions.
[c] Hemihydrate.
[d] Rosettes.
[e] H_2O analysis: calcd. 2.58; found 3.02.
[f] Recrystallized from MeOH - Et_2O.
[g] CN absorption.
[h] Reaction run in acetone.
[i] After reprecipitation from MeOH - Et_2O.
[j] H_2O analysis: calcd. 2.58; found 3.26.

in the presence of $Pd(PPh_3)_4$[9] (Table 2, Entries A~H). The double deallylation of **4d** to give **7j** proceeded as anticipated, but only an amorphous product could be precipitated from various reaction media (Table 2, Entry I). This product, however, contained only trace amounts of monoallyl ester-salt (by NMR) and was tested as such.

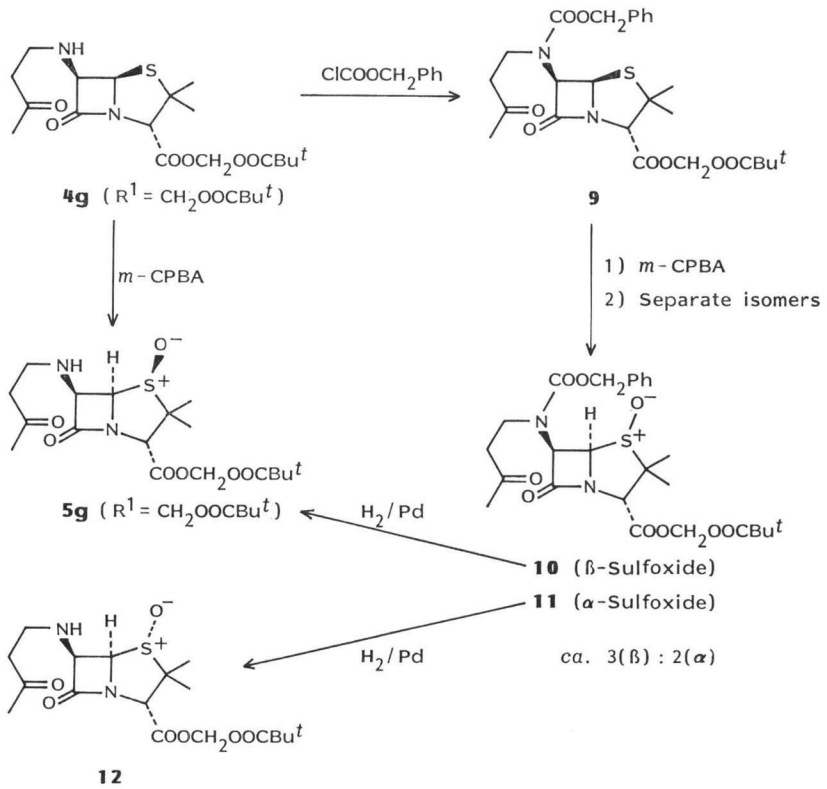
Representative sulfoxide and sulfone analogues of the new *N*-alkyl derivatives were prepared either *via* *N*-alkylation of an ester of 6-APA 1β-oxide (**2**) or 1,1-dioxide (**3**) (Table 1, Entries I~K) or, for sulfoxides, *via* *S*-oxidation of an *N*-alkylated 6-APA ester with *m*-chloroperbenzoic acid (*m*-CPBA) (Table 3, Entries B~E), a process which resulted in the exclusive formation of β-sulfoxides. (The corresponding reaction was used to prepare allyl 6β-aminopenicillanate β-sulfoxide (**2**) itself (Table 3, Entry A) as substrate for the *N*-alkylation reaction). The 1*S* stereochemistry of sulfoxides **2** and **5** is consistent with ^1H and ^{13}C NMR spectroscopic data ($CDCl_3$ solutions), for example the relatively large (~0.4 ppm) separation of the gem dimethyl singlets in the ^1H NMR, characteristic for a 6-APA ester β-sulfoxide derivative with not more than one substituent (alkyl or acyl) on the 6β-nitrogen (N. RASTRUP-ANDERSEN, personal communication). For the preparation of an example of an α-sulfoxide (Scheme 2), the oxidation step was performed after benzyloxycarbonylation of the amino group of the ester **4g** ($R^1=CH_2O_2CBu^t$), which eliminated the β-selectivity of reagent approach control in the reaction with *m*-CPBA associated with hydrogen-bonding to the 6β-NH group.[10] The product of hydrogenolysis of the major sulfoxide (**10**) was found to be identical to the single product obtained by direct oxidation of **4g** ($R^1=CH_2O_2CBu^t$) (Table 3, Entry E), and therefore the β-sulfoxide **5g** ($R^1=CH_2O_2CBu^t$), while the α-sulfoxide (**12**), produced in the isomeric series, was readily distinguishable (also from the corresponding sulfone

Table 3. β-Sulfoxide esters by *m*-CPBA oxidation.[a]

Entry	Product	Starting[b] material	Scale (mmol)	Isolation procedure	Yield (%)	MP (°C)
A	**2**·TsOH	**1**	5	A	88	144~148[c]
B	**5a**	**4a**	5	B (c)	82	83~84[d]
C	**5f**	**4f**	1.9	—[e]	93	oil[f]
D	**5g**·TsOH	**4g**[g]	6.3	A	60	120~125[c]
E	**5g**[h]	**4g**[h]	2	—[e]	97	oil[i]

[a] Reactions run in CH_2Cl_2 at 0°C according to General Procedure (iv).
[b] Used as hydrotosylate.
[c] With decomposition.
[d] Platelets from EtOAc - petroleum ether.
[e] Reaction product was chromatographically homogeneous.
[f] Rf 0.5 (EtOAc).
[g] Reaction run in EtOAc; **4g**·TsOH prepared *in situ*.
[h] Pivaloyloxymethyl ester ($R^1=CH_2O_2CBu^t$).
[i] Rf 0.14 (EtOAc); characterized as hydrotosylate, mp 126.5~127.5°C (dec.), platelets from EtOAc.

Scheme 2. Synthesis of α-sulfoxide (**12**).



(**6g**, $R^1=CH_2O_2CBu^t$)). (Compare, for example, the separations of the gem dimethyl signals in the ^1H and ^{13}C spectra of **5g** ($R^1=CH_2O_2CBu^t$) and **12** given in the Experimental). This and other features of the ^{13}C spectra are consistent with the present assignment of sulfoxide configuration by analogy with reference 11.) β-Sulfoxides which were prepared as allyl esters (**5**), were converted to the potassium salts (**8**) as above (Table 2, Entries J~M), but methodology for the preparation of allyl esters of the α-sul-

Table 4. Inhibition of *E. cloacae* P99 β-lactamase[a] activity by some *N*-alkyl 6-APA derivatives.

N-Alkyl group, R		IC_{50}[b] (mg/liter)		
		Sulfide **7** (**4**[c])	β-Sulfoxide **8** (**5**[c])	Sulfone (**6**[c])
EtO_2CCH_2	**b**	0.25	16	—
$CH_3CH(OH)CH_2CH_2$	**i**	0.32	—	—
$CH_3COCH_2CH_2$	**g**	0.40 (1.0)[d]	16 (63)[d,e]	(13)[f]
KO_2CCH_2	**j**	0.63	—	—
$NCCH_2$	**e**	1.6	—	—
$PhCOCH_2$	**a**	3.2	>100	(25)[f]
$PhCH_2O_2CCH_2$	**c**	4.0	—	—
$PhCH_2$	**f**	4.0	>100	—
$PhCH(OH)CH_2$	**h**	20	—	—

[a] Enzyme source was P99 grown in NIH medium, washed with pH 7 phosphate buffer, and adjusted to an activity of *ca.* 6U (1U=activity transforming 1 μmol substrate per minute).

[b] Concentrations of test compounds needed for 50% inhibition of enzyme activity on nitrocefin (100 μM), after a 30-minute preincubation of enzyme and test compound, measured using the method of C. H. O'CALLAGHAN *et al.*, Antimicrob. Agents Chemother. 1: 283~288, 1972. Values for reference compounds: BPA, 0.40; sulbactam, 1.6; clavulanic acid, 50 mg/liter. (Figures in parenthesis in the table refer to pivaloyloxymethyl esters tested immediately after hydrolysis by incubation with esterase from hog liver).

[c] Pivaloyloxymethyl ester ($R^1=CH_2O_2CBu^t$).

[d] Test compound was hydrotosylate.

[e] α-Sulfoxide (**12**), IC_{50} (mg/liter)=(50).

[f] The sulfones had comparable activity in a similar test against *S. aureus* enzyme (see text).

foxide and the sulfones (the syntheses of which as described all require a catalytic hydrogenation step) was not developed. Instead, these compounds were prepared as pivaloyloxymethyl esters,[12] which were enzymatically cleaved to the salts immediately prior to testing.

Microbiology

The test compounds were screened as inhibitors of β-lactamases from *Staphylococcus aureus* Leo strain CJ9, *Klebsiella aerogenes* 1082E, *Escherichia coli* W3110, and *Enterobacter cloacae* P99, but, with the exception of the sulfone derivatives, which were weak inhibitors of the *S. aureus* enzyme, activity (IC_{50}<100 mg/liter) was only observed against the *E. cloacae* cephalosporinase, with the sulfides being the most active series (Table 4). (Test compounds which were formal derivatives of benzylpenicillin were also prepared by including an *N*-phenylacetylation step in the syntheses of most of the test compounds described. These derivatives retained cephalosporinase inhibitory activity, but were invariably less active than the parent amines. No useful antibacterial activity was discovered in any of the new compounds (*cf.* references 6, 7)). Some of the compounds tested were more active inhibitors of the P99 enzyme than sulbactam or clavulanic acid and comparable in activity to BPA in our test system. β-Lactamase inhibitory activity was also indicated by synergistic effects in 1: 1 combinations with cephaloridine in preventing the growth of *E. cloacae*,

Table 5. Test compound concentrations (IC_{50}) needed to inhibit by 50% the growth of *E. cloacae* P99, determined using the agar incorporation technique.

Test compound	IC_{50} (mg/liter)
Cephaloridine (CER)	>180
7b	>250
CER+**7b**	63+63
7i	>160
CER+**7i**	25+25
7g	>180
CER+**7g**	30+30
BPA	180
CER+BPA	17+17

as shown in Table 5.

The type of enzymic specificity observed for the new *N*-alkylaminopenicillanic acids described here is reminiscent of that of cloxacillin,[18] which is a potent reversible inhibitor of the P99 β-lactamase. Monobactams also appear to share this specificity[14]. It seems likely that the particular *N*-alkyl derivatives investigated in this work are members of a general class of modified 6-APA's which, while lacking useful intrinsic antibacterial activity *per se*, may serve to protect β-lactam antibiotics against destruction by (type I) cephalosporinases.

Experimental

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer model 457 spectrophotometer. NMR spectra were measured on a Jeol FX 100 spectrometer using as internal reference HDO (δ=4.66 ppm) for D_2O solutions or otherwise Me_4Si (δ=0). All products gave IR and ^1H NMR spectra fully consistent with the assigned structures and, for crystalline products (needles, unless otherwise stated), C, H, N (S, Cl, H_2O) analyses correct within ±0.4% unless indicated otherwise. Yields for precipitated products refer to material recovered by filtration, washing with the reaction/(re)crystallization solvent and drying *in vacuo* at room temperature over KOH. Petroleum ether refers to the fraction bp <50°C. Organic solutions were dried over Na_2SO_4. Analytical TLC (to which Rf values refer) was performed on Merck plates pre-coated with silica gel 60 F_{254}.

General Procedure (i)

Substitutive *N*-Alkylation of Esters of 6-APA (Sulfoxide/Sulfone) (Table 1, Entries A~F, I, J): The specified alkyl halide was added to a solution of the substrate hydrotosylate and EtN(*i*-Pr)$_2$ (2.5 equiv., but 3.5 equiv. in the reaction with **3** (R^1=$CH_2O_2CBu^t$)·2TsOH) in DMF (*ca.* 3 ml/mmol substrate), and the mixture was stirred in the stoppered flask at 50°C for the specified time. After cooling, the clear reaction mixture was diluted with at least 5 volumes EtOAc and washed four times with H_2O and finally once with saturated NaCl solution. The EtOAc solution was dried and concentrated *in vacuo* to give an oil from which the specified product was isolated accordingly (see Isolation Procedures).

General Procedure (ii)

Alkylations with Methyl Vinyl Ketone (Table 1, Entries G, H, K): Redistilled methyl vinyl ketone was added to a solution of the substrate hydrotosylate and EtN(*i*-Pr)$_2$ (an amount equiv. to TsOH in substrate) in CH_2Cl_2 (*ca.* 3 ml/mmol substrate) and the mixture left at room temperature until TLC showed consumption of primary amine (2~3 days). The reaction solution was then concentrated *in vacuo*, taken up in EtOAc (*ca.* 10 ml/mmol substrate) and washed, dried, and concentrated as in General Procedure (i).

General Procedure (iii)

Potassium *N*-Alkylaminopenicillanates by Catalytic Deallylation of the Corresponding Ester (Table 2): A mixture of $Pd(PPh_3)_4$ (15 mg) and PPh_3 (10 mg) was added to stirred solution of the allyl ester (1 mmol) [free-base; recovered, if indicated, from the acid addition salt by suspending the salt in EtOAc (but CH_2Cl_2 for **5 g**) and shaking with excess 5% $NaHCO_3$ solution until the solid has dissolved. The organic layer was then separated, washed with saturated NaCl solution, dried, and concentrated to give the free base] and potassium 2-ethylhexanoate (1.25 mmol per allyl ester function, added as a 1 M solution in the reaction solvent) in 5 ml solvent (normally EtOAc). After stirring under an atmosphere of nitrogen for 0.5 hour, the precipitated salt was recovered by filtration. All the salts prepared by this method were hygroscopic, and several of the crystalline products analyzed as partial hydrates.

Selected NMR data (D_2O)

Sulfides (**7**): 5-H, δ 5.21~5.55; 6-H, δ 4.42~4.58; $J_{5,6}$ 3.8~4.0 Hz.

β-Sulfoxides (**8**): 5-H, δ 4.70~5.16; 6-H, δ 4.60~4.70; $J_{5,6}$ 4.1~4.5 Hz.

General Procedure (iv)

Preparation of β-Sulfoxide Esters by Oxidation with *m*-CPBA (Table 3): A 10% solution of *m*-CPBA (1 equiv.) in CH_2Cl_2 was added dropwise to a stirred suspension of the starting material hydrotosylate in CH_2Cl_2 (10 ml/mmol) at 0°C. The reaction mixture was diluted with more CH_2Cl_2 and extracted consecutively with 5% $NaHCO_3$ solution, 5% $NaHSO_3$ solution (which had been adjusted to pH 8 with Na_2CO_3), and saturated NaCl solution. The CH_2Cl_2 solution was dried and concentrated *in vacuo* to give the sulfoxide. For Entry D, the solvent was changed to CH_2Cl_2 prior to work-up.

Isolation Procedures

A: The product was redissolved in EtOAc and the salt precipitated from the stirred, ice-cooled solution by the addition of a slight excess of ~0.5 M *p*-TsOH·H_2O solution in EtOAc. B: The product was subjected to flash chromatography on silica gel, eluting with (a) EtOAc - petroleum ether, 1: 1, (b) EtOAc - petroleum ether, 1: 2, or (c) EtOAc. C: The product was redissolved in EtOAc (15 ml) and 2-PrOH (70 ml) and treated with HCl in Et_2O (3.7 M, 3 ml) with stirring at 0°C. D: The solid obtained after chromatography was recrystallized from Et_2O.

Pivaloyloxymethyl 6β-[*N*-Benzyloxycarbonyl-*N*-(3-oxobut-1-yl)amino]penicillanate (**9**)

Benzyl chloroformate (0.85 g, 5 mmol) was added to a vigorously stirred solution of **4g** ($R^1=CH_2O_2CBu^t$)·TsOH (2.29 g, 4 mmol) in a mixture of 60 ml each of EtOAc and 0.7 M aqueous potassium phosphate buffer (pH 7.0) at 0°C. After stirring for a further 10 minutes, dimethylamine hydrochloride (0.33 g) was added to react with any remaining acid chloride, before the EtOAc layer was separated, washed with water, saturated NaCl solution, and dried. Removal of the solvent *in vacuo* gave an oil which was purified by flash chromatography (100 g silica gel; EtOAc - petroleum ether, 1: 1 as eluant) to give **9** as a viscous oil (2.02 g, 94%): Rf 0.4; IR ($CHCl_3$) 1780, 1770 (sh), 1750 (sh), 1705 cm^{-1}; NMR ($CDCl_3$) δ 1.21 (9H, s, Bu^t), 1.49 (3H, s, 2α-CH_3), 1.66 (3H, s, 2β-CH_3), 2.10 (3H, s, $CH_3C=O$), 2.5~3.1 (2H, m, $CH_2C=O$), 3.4~4.1 (2H, m, CH_2N), 4.42 (1H, s, 3-H), 5.12 (2H, bs, 5-H+6-H), 5.41 (2H, bs, CH_2Ph), 5.74 and 5.86 (each 1H, d, J=5.5 Hz, OCH_2O), and 7.34 (5H, bs, Ph).

Pivaloyloxymethyl 6β-[*N*-Benzyloxycarbonyl-*N*-(3-oxobut-1-yl)amino]penicillanate 1β-Oxide (**10**) and 1α-Oxide (**11**)

General Procedure (iv) was followed for the *m*-CPBA oxidation using **9** (1.72 g, 3.2 mmol) as substrate. The crude product, which displayed two spots on TLC, was purified by flash chromatography (160 g silica gel; EtOAc - petroleum ether, 3: 1 as eluant) to give **10** as a white foam (0.99 g, 56%): Rf 0.57; IR ($CHCl_3$) 1791, 1750, 1710, 1686 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (12H, s, Bu^t+2α-CH_3), 1.64 (3H, s, 2β-CH_3), 2.07 and 2.16 (3H, 2 s from the two rotamers, $CH_3C=O$), 2.7~2.9 (2H, m, $CH_2C=O$), 3.73 (2H, t, J=7 Hz, CH_2N), 4.65 (1H, bs, 3-H), 5.0~5.2 (2H, m, 5-H+6-H), 5.0~5.3 (2H, bs, CH_2Ph), 5.72 and 5.95 (each 1H, d, J=5.5 Hz, OCH_2O), and 7.34 (5H, s, Ph), and **11** as a white foam (0.60 g, 34%): Rf 0.43; IR ($CHCl_3$) 1793, 1750, 1710, 1700 (sh) cm^{-1}; NMR ($CDCl_3$) 1.23 (12H, s, Bu^t+2α-CH_3), 1.68 (3H, bs, 2β-CH_3), 2.14 (3H, s, $CH_3C=O$), 2.6~3.0 (2H, m, $CH_2C=O$), 3.3~4.0 (2H, m, CH_2N), 4.5 (1H, bs, 3-H), 4.60 (1H, d, J=4.3 Hz, 6-H), 5.04 (1H, d, J=4.3 Hz, 5-H), 5.13 (2H, ABq, CH_2Ph), 5.75 and 5.91 (each 1H, d, J=5.4 Hz, OCH_2-O), and 7.36 (5H, s, Ph).

Pivaloyloxymethyl 6β-[(3-Oxobut-1-yl)amino]penicillanate 1β-Oxide (**5g**, $R^1=CH_2O_2CBu^t$) and 1α-Oxide (**12**) by Catalytic Hydrogenation

(a) A solution of **10** (0.48 g, 0.87 mmol) in EtOAc (10 ml) containing suspended 10% Pd-C (0.5 g) was hydrogenated at 1 atmosphere for 3 hours. The reaction mixture was filtered, concentrated *in vacuo*, and the product separated from unreacted **10** by preparative TLC (silica gel; EtOAc as eluant) to give **5g** ($R^1=CH_2O_2CBu^t$) as an oil (0.14 g, 38%): Rf 0.14; IR ($CHCl_3$) 1780 (br), 1755, 1710 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (12H, s, Bu^t+2α-CH_3), 1.66 (3H, s, 2β-CH_3), 2.17 (3H, s, $CH_3C=O$), 2.67 (2H, m, $CH_2C=O$), 2.86 (1H, bs, NH), 3.0 (2H, m, CH_2N), 4.56 (1H, d, J=4.6 Hz, 6-H), 4.59 (1H, s, 3-H), 4.94 (1H, d, J=4.6 Hz, 5-H), 5.71 and 5.95 (each 1H, d, J=5.4 Hz, OCH_2O); ^{13}C NMR ($CDCl_3$) 18.3 (2α-CH_3) and 19.0 (2β-CH_3). This product was identical to material obtained by direct oxidation of **4g** ($R^1=CH_2O_2CBu^t$) (Table 3, Entry E) (IR, NMR, TLC, and mp of hydrotosylate).

(b) **11** (0.32 g, 0.58 mmol) was hydrogenated as described in (a) to give a crude product which was

separated by flash chromatography (30 g silica gel; EtOAc as eluant) to return unreacted **11** (0.09 g) (Rf 0.53) and **12** as a colorless gum (0.08 g, 33%): Rf 0.26; IR ($CHCl_3$) 1775, 1750 (sh), 1707 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (9H, s, Bu^t), 1.34 (3H, s, 2α-CH_3), 1.61 (3H, s, 2β-CH_3), 2.17 (3H, s, CH_3C=O), 2.26 (1H, bs, NH), 2.70 (2H, t, J=6 Hz, CH_2C=O), 3.00 (2H, t, J=6 Hz, CH_2N), 4.33 (1H, s, 3-H), 4.58 (1H, d, J=3.8 Hz, 6-H), 4.66 (1H, d, J=3.8 Hz, 5-H), 5.75 and 5.91 (each 1H, d, J=5.5 Hz, OCH_2O); ^{13}C NMR ($CDCl_3$) δ 15.3 (2α-CH_3) and 23.8 (2β-CH_3).

Potassium 6β-[*N*-(2-Hydroxy-2-phenylethyl)amino]penicillanate (**7h**)

$EtN(i\text{-}Pr)_2$ (0.65 g, 5 mmol) was added to a suspension of **4a**·TsOH (2.73 g, 5 mmol) in CH_2Cl_2 (12 ml) at 0°C and the resulting solution diluted with EtOH (35 ml). $NaBH_4$ (0.34 g, 9 mmol) was added and stirring continued at 0°C for 10 minutes before the reaction mixture was partitioned between EtOAc (150 ml) and H_2O (100 ml). The EtOAc layer was separated, washed with H_2O (2×50 ml), saturated NaCl solution (50 ml), and dried. Removal of the solvent *in vacuo* gave an oil which was purified by flash chromatography (110 g silica gel; EtOAc - petroleum ether, 1: 1 as eluant) to give allyl 6β-[*N*-(2-hydroxy-2-phenylethyl)amino]penicillanate (**4h**) as an oil (1.53 g, 81%)*: a *ca.* 55: 45 mixture of diasteromers; Rf 0.31 (major component) and 0.34 (minor component); IR ($CHCl_3$) 1773, 1742 cm^{-1}; NMR ($CDCl_3$) δ 1.49 (3H, s, 2α-CH_3), 1.59 and 1.60 (3H, 2s, 2β-CH_3's), 2.8 (2H, bs, NH+OH), 2.7~3.1 (2H, m, CH_2N), 4.39 (1H, s, 3-H), 4.43 and 4.44 (1H, 2d, J=4 Hz, 6-H's), 4.65 (2H, m, OCH_2), 4.75 (1H, m, C*H*Ph), 5.2~5.5 (3H, m, CH_2=CH+5-H), 5.75~6.15 (1H, m, CH_2=C*H*), and 7.32 (5H, s, Ph). The entire product was submitted to the transallylation reaction with potassium 2-ethylhexanoate (5 mmol) in EtOAc using General Procedure (iii) to give **7h** (1.16 g, 77%, or 62% overall): rosettes, mp 222~224°C (dec.); IR (KBr) 1764, 1605 cm^{-1}; NMR (D_2O) δ 1.39 (6H, s, 2-(CH_3)$_2$), 2.8 (2H, m, CH_2N), 4.04 and 4.06 (1H, 2s, 3-H's), 4.42 (1H, d, J=3.8 Hz, 6-H), 4.7 (1H, m, PhC*H*), 5.34 and 5.37 (1H, 2d, J=3.8 Hz, 5-H's), and 7.33 (5H, s, Ph);

Anal. ($C_{16}H_{19}N_2O_4SK \cdot 0.25\ H_2O$): C, H, N, H_2O

Potassium 6β-[*N*-(3-Hydroxybut-1-yl)amino]penicillanate (**7i**)

$NaBH_4$ (0.25 g, 6.6 mmol) was added to a stirred and ice-cooled solution of **4g** (1.40 g, 4.3 mmol) prepared by dissolving the oil in EtOAc (6 ml) and diluting with EtOH (25 ml). After stirring at 0°C for 10 minutes, the reaction mixture was diluted with EtOAc (50 ml) and aqueous potassium phosphate buffer solution (1 M, pH 7, 20 ml) was added. The EtOAc layer was separated, washed with water, saturated NaCl solution, and dried. Removal of the solvent *in vacuo* gave the crude intermediate allyl 6β-[*N*-(3-hydroxybut-1-yl)amino]penicillanate (**2i**, R^1=$CH_2O_2CBu^t$). This was purified by flash chromatography (100 g silica gel; EtOAc as eluant) to give an oil (1.03 g), Rf 0.43, which was submitted to the catalytic transallylation reaction with potassium 2-ethylhexanoate (4 mmol) in EtOAc using General Procedure (iii) to give **7i** (0.67 g, 48% overall): rosettes, mp 217~221°C (dec.); IR (KBr) 1770, 1600 cm^{-1}; NMR (D_2O) δ 1.10 (3H, d, J=6.3 Hz, CH_3CH), 1.45 (3H, s, 2α-CH_3), 1.54 (3H, s, 2β-CH_3), 1.59 (2H, m, CH_2CH_2N), 2.64 (2H, m, CH_2N), 3.8 (1H, m, CH_3CH), 4.09 (1H, s, 3-H), 4.43 (1H, d, J=3.9 Hz, 6-H), and 5.42 (1H, d, J=3.9 Hz, 5-H);

Anal. ($C_{12}H_{19}N_2O_4SK$): C, H, N.

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* Although this intermediate could not be characterized as a crystalline derivative, the corresponding ester (**4h**, R^1=CH_2Ph) correspondingly prepared, gave a crystalline hydrotosylate (mp 132~134°C, from EtOAc) which gave a satisfactory analysis C, H, N, S.

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