STRUCTURE-ACTIVITY RELATIONSHIPS IN 1,2,3-TRIAZOL-1-YL DERIVATIVES OF CLAVULANIC ACID

ROBERT C. MEARMAN, CHRISTOPHER E. NEWALL and ALAN P. TONGE*

Chemical Research Department, Glaxo Group Research Ltd.,
Greenford, Middlesex UB6 OHE, England

(Received for publication March 6, 1984)

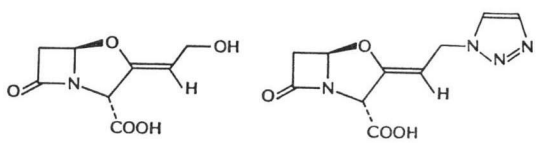
The synthesis of a series of analogues of clavulanic acid, possessing a substituted 1,2,3-triazole ring in place of the exocyclic hydroxyl group, is described. Quantitative structure-activity relationships in this series are discussed.

Clavulanic acid (**1**) is a moderately-active broad-spectrum antibiotic and potent β-lactamase inhibitor which is used as a potentiator of the antibacterial activity of β-lactamase sensitive antibiotics[1]. As part of an extensive program[2] directed at the synthesis of novel derivatives of **1** we have prepared a series of 1,2,3-triazol-1-yl analogues (**2**, **5**, **9a**~**i**).

Chemistry

The 1,2,3-triazole esters (**4**, **6**, **7a**~**i**, **8a**, **8h**) were prepared by the 1,3-dipolar cycloaddition reaction of acetylenes[3] to the azido ester (**3**) (Scheme 1). The rate of reaction was found to be a function of the nature of the acetylene substituent, many reactions being sluggish and requiring several days to yield even moderate amounts of product. Thus, whereas diethyl acetylenedicarboxylate reacted with the azide (**3**) at room temperature to give the 4,5-disubstituted product (**4**) in good yield, acetylenes with only a single electron-withdrawing substituent were found to require warming at 40~45°C to initiate reaction and those with no activating substituent (propargyl alcohol, phenylacetylene and acetylene itself) needed heating at 70~75°C. Yields at these higher temperatures were generally poor due to competing side reactions and the thermal instability of the azido ester (**3**).

Fig. 1.

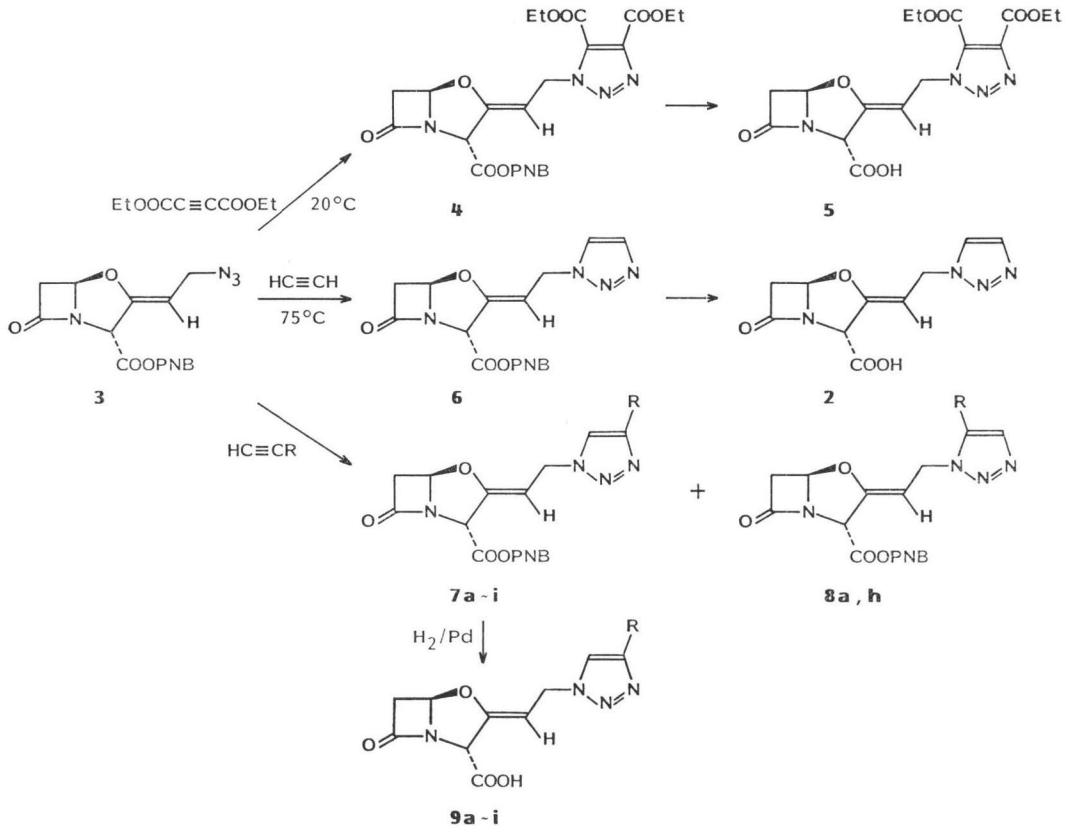


1 **2**

The protecting 4-nitrobenzyl group of the triazole esters was removed by hydrogenolysis over palladium on carbon in ethyl acetate solution. For the poorly-soluble esters (**7e** and **7f**), 1,4-dioxane was used as a co-solvent. The products were isolated by precipitation of their sodium or potassium salts following treatment of the reaction solution with the corresponding salts of 2-ethylhexanoic acid.

Structural assignments for the 4- and 5-monsubstituted triazole isomers were based on a comparison of their spectroscopic properties with those of the unsubstituted ester (**6**) and the 4,5-disubstituted analogue (**4**). The proton magnetic resonance (^{1}H NMR) spectrum of the parent triazole (**6**) in $CDCl_3$ solution showed the resonance of the prochiral methylene protons adjacent to the triazole ring as a singlet at δ 5.08, while the analogous group in the 4,5-disubstituted compound (**4**) appeared as a multiplet at δ 5.28, the proximity of the 5-substituent making apparent the non-equivalence of the two protons, which are shifted downfield relative to the 5-unsubstituted derivatives. Spectroscopic data for the deprotected salts are given in Table 1.

Scheme 1. Preparation of 1,2,3-triazole derivatives of clavulanic acid.
PNB=4-Nitrobenzyl



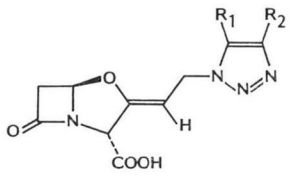
7, 8, 9a	R=$-COOCH_2CH_3$	7, 9e	R=$-CONH_2$
7, 9b	R=$-COOCH_3$	7, 9f	R=$-CONHCH_3$
7c	R=-COOPNB	7, 9g	R=-CN
9c	R=-COOH	7, 8, 9h	R=$-CH_2OH$
7, 8, 9d	R=$-COCH_3$	7, 9i	R=$-C_6H_5$

Biological Activity

The antibacterial and β-lactamase inhibitory properties of the triazole series are given in Table 2 and show some improvement upon clavulanic acid (**1**). The 4-substituted derivatives prepared were chosen, within the limits of synthetic accessibility, to give as broad a range of substituent properties as possible, in order that quantitative structure-activity relationships (QSAR) in this series could be studied with minimal cross-correlation between different substituent parameters[4]. Regression analyses were performed on the antibacterial activity of 4-monosubstituted derivatives against substituent parameters representing steric, electronic and lipophilic properties. Values for substituent lipophilicity π and substituent electronic parameters were taken from published tables[4] and the steric parameter V, representing VAN DER WAALS volume, was calculated by the method of MORIGUCHI *et al.*[5].

The antibacterial activity was expressed as $\log_2 A$, the mean logarithm to the base two of MIC values (in μmol/ml) (Table 3); for the purpose of calculating $\log_2 A$, MIC's of >250 μg/ml were assigned values of 500 μg/ml. The analysis was applied to those organisms having similar cell-wall membranes[6] and was therefore limited to activity against ten members of the *Enterobacteriaceae* against which all

Table 1. Spectroscopic data.



	R_1	R_2	^1H NMR (D_2O) chemical shifts (δ)					IR (Nujol) ν_{max} (cm^{-1})	
			Triazole	H-5	H-3	CH_2N	H-6	β-Lactam	CO_2^-
5	COOEt	COOEt	—	5.72	4.93	5.30 (m)	3.02, 3.58	1792	1620
2	H	H	7.82, 8.00	5.82	5.02	5.15 (s)	3.12, 3.61	1785	1620
9a	H	COOEt	8.53	5.81	5.05	5.16 (s)	3.13, 3.61	1792	1628
9b	H	COOMe	8.48	5.77	5.00	5.10 (s)	3.12, 3.60	1785	1620
9c	H	COONa	8.24	5.82	5.06	5.15 (s)	3.15, 3.62	1788	1620
9d	H	$COCH_3$	8.56	5.82	5.05	5.16 (s)	3.13, 3.61	1790	1626
9e	H	$CONH_2$	8.44	5.83	5.06	5.16 (s)	3.16, 3.64	1782	1620
9f	H	$CONHCH_3$	8.37	5.83	5.06	5.15 (s)	3.13, 3.64	1782	1615
9g	H	CN	8.58	5.78	5.01	5.09 (s)	3.12, 3.61	1790	1620
9h	H	CH_2OH	7.92	5.78	5.02	5.09 (s)	3.12, 3.59	1790	1620
9i	H	C_6H_5	8.00	5.80	5.00	5.00 (s)	3.08, 3.62	1790	1620

compounds had been tested (Table 2). A significant parabolic relationship was observed between the biological activity A and substituent lipophilicity π:

$$\log_2 A = 0.68\pi^2 + 1.05\pi - 4.76 \quad (1)$$
$$n = 10 \qquad r^2 = 0.92$$

Regression of the activity A against V^2 also showed good correlation, but as π and V show cross-correlation[5] this was not to be unexpected. None of the electronic parameters investigated (σ, F and R)[4] were found to contribute towards biological activity.

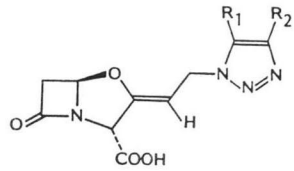
The parabolic form of Equation 1 is typical of QSAR studies modelling transport across biological membranes[7] and suggests that the antimicrobial activity of this series of compounds is related to their ability to permeate passively across cell membranes. However, other processes dependent upon the hydrophilic-lipophilic properties of the substrate, such as interaction with a receptor[8], cannot be excluded. The lack of activity of the 4,5-diester (**5**) against most Gram-negative organisms appears to be attributable to poor penetration of the cell membrane, since activity against the cell wall mutant DC2 is similar to the 4-monosubstituted compounds studied (Table 2).

Experimental

MICs were determined by the agar dilution method in Casitone agar. Serial dilutions of freshly prepared antibiotic solutions were added to the agar to give concentrations in the range 250 to 0.1 micrograms per milliliter. The MIC, in micrograms per milliliter, was read after 18-hour incubation at 37°C as the lowest concentration that inhibited visible growth. β-Lactamase enzyme preparations were made as previously described[9] and study of β-lactamase inhibition performed by the spectrophotometric monitoring[9] of hydrolysis of a standard (ampicillin) in the presence and absence of clavulanic acid derivative.

NMR spectra were recorded at 100 MHz using Varian HA-100 and Jeol-MH 100 spectrometers. IR spectra were taken on a Perkin Elmer 257 spectrophotometer. Preparative TLC was carried out

Table 2. Antibacterial and β-lactamase



	R_1	R_2	*Staphylococcus aureus*		S.f.** 850	*Escherichia coli*			S.t.** 804*
			663	853		1193* (TEM$^+$)	DC0 (DC2)	851*	
1	Clavulanic acid		4	4	>250	16	16	16	16
2	H	H	2	2	NT	8	NT	4	16
5	COOEt	COOEt	1	1	>250	>250	>250 (8)	>250	>250
9a	H	COOEt	<0.1	<0.1	>250	8	4 (4)	4	8
9b	H	$COOCH_3$	0.5	0.5	>250	4	NT	4	16
9c	H	COOH	8	8	>250	8	8 (8)	8	8
9d	H	$COCH_3$	<0.1	<0.1	>250	8	4 (1)	4	8
9e	H	$CONH_2$	0.5	0.5	>250	4	4 (4)	4	8
9f	H	$CONHCH_3$	0.5	1	>250	8	8	8	16
9g	H	CN	0.2	<0.1	>250	4	NT	2	8
9h	H	CH_2OH	1	1	NT	8	NT	8	4
9i	H	C_6H_5	0.2	0.2	62	250	NT	250	>250

NT: Not tested.

Figures in parenthesis indicate MIC against the cell wall mutant strain DC2[12].

† With respect to inhibition of hydrolysis of ampicillin by isolated penicillinases.

on Merck pre-coated F-254 silica gel plates. Hydrogenations were carried out at 1 atmosphere pressure, the hydrogenation mixture being shaken vigorously for several minutes on a mechanical shaker until completion of reaction. Petroleum ether refers to the fraction boiling at 40~60°C. The following examples comprise a representative selection of preparative procedures and further examples may be found in the patent literature[10].

Sodium (3*R*,5*R*,*Z*)-2-[2-(1,2,3-Triazol-1-yl)ethylidene]clavam-3-carboxylate (**2**)

Acetylene gas (dried by passage through conc H_2SO_4) was passed through a solution of 4-nitrobenzyl (3*R*,5*R*,*Z*)-2-(2-azidoethylidene)clavam-3-carboxylate[11] (3.0 g) in dioxane (50 ml) at 70°C for 1 hour. The reaction mixture was sealed and heated at 70~75°C for 65 hours. The mixture was then concentrated and passed down a column of silica gel (50 g) using ethyl acetate - petroleum ether (3: 1) eluent to give 0.88 g (25%) of the ester (**6**); mp 121~123°C (EtOH); IR ($CHBr_3$) 1800, 1750, 1520 and 1342 cm^{-1}; NMR ($CDCl_3$) δ 7.70 and 7.53 (s, triazole protons), 5.80 (1H, d, J=2 Hz, 5-H), 5.28 (2H, s, benzyl protons), 5.08 (2H, s, CH_2N), 3.60 (dd, J=3 and 16 Hz, 6α-H) and 3.14 (d, J=16 Hz, 6β-H).

Anal Calcd for $C_{17}H_{15}N_5O_6$: C 53.0, H 3.9, N 18.2.
Found: C 52.8, H 4.0, N 18.0.

A solution of the ester (**6**) (0.24 g) in ethyl acetate (25 ml) was hydrogenated over palladium on carbon (10%, 0.35 g) until uptake of hydrogen ceased. The mixture was filtered through diatomaceous earth and the filtrate treated with a solution of sodium 2-ethylhexanoate (0.08 g) in ethyl acetate (3 ml). The mixture was concentrated and the deposited solid filtered off to yield 0.09 g of the title compound; IR (Nujol) 1785 and 1620 cm^{-1}; NMR (D_2O) δ 8.00 and 7.82 (s, triazole protons), 5.82 (1H, d, J=3 Hz, 5-H), 5.15 (2H, s, CH_2N), 5.02 (1H, s, 3-H), 3.61 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.12 (1H, d, J=17 Hz, 6β-H).

Anal Calcd for $C_{10}H_9N_4O_4Na \cdot 1.5H_2O$: C 40.15, H 4.05, N 18.7.
Found: C 40.45, H 4.25, N 17.95.

inhibitory activities (μg/ml).

Enterobacter cloacae		*Klebsiella aerogenes*		P.mi.**	P.mo.**	S.m.**	*Pseudomonas aeruginosa*		*Haemophilus influenzae*		I_{50}†	
1051* (P99+)	1521* (P99−)	1082* (K1+)	1522* (K1−)	451*	1606*	1324*	1571	150	1184	1788	TEM III	K1
16	16	31	31	16	31	31	62	125	31	31	1.35	0.62
8	8	31	8	4	16	8	8	NT	16	16	0.21	0.55
>250	>250	>250	>250	>250	>250	>250	>250	>250	125	31	0.21	0.8
16	16	62	8	8	8	125	>250	>250	62	16	0.07	0.35
16	16	125	16	8	16	62	62	>250	62	62	NT	NT
16	8	62	16	8	8	31	62	>250	31	16	0.11	0.2
8	8	62	8	8	8	62	>250	>250	62	31	0.18	0.16
4	4	62	8	8	8	8	62	>250	31	16	0.13	0.34
16	16	62	16	16	16	31	250	>250	31	16	0.36	0.7
4	4	31	8	4	4	31	31	NT	16	31	0.10	0.39
4	4	31	8	8	8	16	16	>250	31	31	NT	NT
>250	>250	>250	250	250	250	>250	>250	>250	16	31	NT	NT

* Strains used in calculation of $\log_2$ A.

** S.f. *Streptococcus faecalis*, S.t. *Salmonella typhi*, P.mi. *Proteus mirabilis*, P.mo. *Proteus morganii*, S.m. *Serratia marcescens*.

Table 3. Substituent parameters for QSAR analysis.

Compound	$\log_2$ A (μmol/ml)	π	V(A^3)
2	−4.9	0	1.2
9a	−4.6	0.51	57.7
9b	−4.4	−0.01	42.3
9c	−4.75	−0.32	25.6
9d	−4.8	−0.55	34.2
9e	−5.4	−1.49	28.7
9f	−4.25	−1.27	44.9
9g	−5.5	−0.57	19.0
9h	−5.25	−1.03	23.5
9i	0.05	1.96	70.7

Sodium (3*R*,5*R*,*Z*)-2-[2-(4,5-Diethoxycarbonyl-1,2,3-triazol-1-yl)ethylidene]clavam-3-carboxylate (**5**)

A solution of diethyl acetylenedicarboxylate (0.25 g) and the azido ester (**3**) (0.54 g) in tetrahydrofuran (3 ml) was allowed to stand at room temperature for 3 days. The mixture was chromatographed on preparative silica gel plates, using ethyl acetate - petroleum ether (1: 1) eluent to give 0.38 g of the ester (**4**); IR ($CHBr_3$) 1804, 1750, 1730, 1530 and 1348 cm^{-1}; NMR ($CDCl_3$) 5.73 (1H, d, J=2 Hz, H-5), 5.28 (2H, m, CH_2N), 5.25 (2H, s, benzyl protons), 5.12 (1H, s, 3-H), 4.95 (1H, t, J=7 Hz, =CH–), 3.53 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.13 (1H, d, J=17 Hz, 6β-H).

Anal Calcd for $C_{28}H_{28}N_5O_{10}$: C 52.2, H 4.4, N 13.2.
Found: C 52.0, H 4.5, N 13.6.

A solution of the ester (**4**) (0.59 g) in ethyl acetate (50 ml) was hydrogenated over palladium on carbon (10%, 0.45 g) until uptake of hydrogen ceased. The mixture was filtered through diatomaceous earth and a solution of sodium 2-ethylhexanoate (0.116 g) in ethyl acetate (10 ml) added to the filtrate, which was concentrated to a small volume and diluted with ether (50 ml). The deposited solid was filtered off and dried, yielding 0.33 g of the sodium salt (**5**); IR (Nujol) 1792, 1730, 1700 and 1620 cm^{-1}; UV (H_2O) 223 nm (ε 13,600); NMR (D_2O) δ 5.72 (1H, d, J=2 Hz, 5-H), 5.30 (2H, m, CH_2N), 5.00 (1H, t, J=13 Hz, =CH–), 4.93 (s, 3-H), 4.44 (4H, m, OCH_2CH_3), 3.58 (1H, dd, J=2 and 17 Hz, 6α-H), 3.02 (1H, d, J=17 Hz, 6β-H) and 1.35 (6H, m, OCH_2CH_3).

Anal Calcd for $C_{16}H_{17}N_4O_8Na \cdot H_2O$: C 44.2, H 4.4, N 12.9.
Found: C 44.4, H 4.6, N 12.6.

4-Nitrobenzyl (3*R*,5*R*,*Z*)-2-[2-(4- and 5-Ethoxycarbonyl-1,2,3-triazol-1-yl)ethylidene]clavam-3-carboxylate (**7a** and **8a**)

A solution of ethyl propiolate (0.75 ml) and the azido ester (**3**) (2.0 g) in benzene (10 ml) was warmed on an oil bath at 40°C for 3 days. The mixture was then chromatographed on silica gel plates using ethyl acetate - petroleum ether (2: 1) eluent to give 0.20 g (8%) of the 5-ethoxycarbonyl ester (**8a**); mp 142~144°C (EtOH - EtOAc, 1: 1); IR ($CHBr_3$) 1800, 1752, 1725, 1704, 1552 and 1344 cm^{-1}; NMR ($CDCl_3$) δ 8.13 (1H, s, triazole proton), 5.78 (1H, d, J=2 Hz, H-5), 5.42 (2H, m, CH_2N), 5.27 (2H, s, benzyl protons), 5.14 (1H, s, 3-H), 5.00 (1H, t, J=7 Hz, =CH–), 4.37 (2H, q, J=7 Hz, OCH_2CH_3), 3.56 (1H, dd, J=2 and 17 Hz, 6α-H), 3.17 (1H, d, J=17 Hz, 6β-H) and 1.38 (3H, t, J=7 Hz, OCH_2CH_3).

Anal Calcd for $C_{20}H_{19}N_5O_8$: C 52.5, H 4.2, N 15.3.
Found: C 52.3, H 4.2, N 15.35.

0.90 g (35%) of the 4-ethoxycarbonyl ester (**7a**); IR ($CHBr_3$) 1802, 1750, 1716, 1522 and 1348 cm^{-1}; NMR ($CDCl_3$) δ 8.05 (1H, s, triazole proton), 5.79 (1H, d, J=2 Hz, 5-H), 5.28 (2H, s, benzyl protons), 5.21 (1H, s, H-3), 5.16 (s, CH_2N), 4.41 (2H, q, J=7 Hz, OCH_2CH_3), 3.59 (1H, dd, J=2 and 17 Hz, 6α-H), 3.13 (1H, d, J=17 Hz, 6β-H) and 1.39 (3H, t, J=7 Hz, OCH_2CH_3).

Anal Calcd for $C_{20}H_{19}N_5O_8$: C 52.5, H 4.2, N 15.3.
Found: C 52.4, H 4.25, N 14.9.

Sodium (3*R*,5*R*,*Z*)-2-[2-(4-Carbamoyl-1,2,3-triazol-1-yl)ethylidene]clavam-3-carboxylate (**9e**)

A solution of propiolamide (1.4 g) and the azido ester (**3**) (7.0 g) in tetrahydrofuran (35 ml) was warmed at 38°C for one week. The deposited crystals were filtered off, yielding 1.75 g (21%) of the 4-carbamoyl ester (**7e**); mp 185~188°C; IR (Nujol) 1794, 1750, 1694, 1634, 1524 and 1348 cm^{-1}; NMR (DMSO-d_6) δ 8.52 (1H, s, triazole proton), 8.25 and 7.63 (4H, m, phenyl protons), 7.80 and 7.47 (2H, m, $CONH_2$), 5.87 (1H, d, J=2 Hz, 5-H), 5.60 (1H, s, 3-H), 5.38 (2H, s, benzyl protons), 5.13 (2H, s, CH_2N), 3.78 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.23 (1H, d, J=17 Hz, 6β-H).

Anal Calcd for $C_{18}H_{16}N_6O_7$: C 50.45, H 3.75, N 19.6.
Found: C 50.5, H 3.75, N 19.8.

A solution of the ester (**7e**) (0.75 g) in 1,4-dioxane - ethyl acetate (2: 1, 30 ml) (ester dissolved by warming) was added to a suspension of palladium on carbon (10%, 1.0 g) in dioxane - ethyl acetate (2: 1, 30 ml) and the mixture shaken on a hydrogenator until uptake of hydrogen ceased. The mixture was filtered through diatomaceous earth and a solution of sodium 2-ethylhexanoate (0.20 g) in ethyl acetate (5 ml) added to the filtrate. The deposited solid was filtered off, washed with ethyl acetate and dried to yield 0.32 g of the sodium salt (**9e**); IR (Nujol) 1782, 1660 and 1620 cm^{-1}; NMR (D_2O) δ 8.44 (1H, s, triazole proton), 5.83 (1H, d, J=2 Hz, H-5), 5.17 (2H, s, CH_2N) and 5.06 (1H, s, 3-H).

Anal Calcd for $C_{11}H_{10}N_5O_5Na \cdot 2H_2O$: C 37.6, H 4.0, N 19.9.
Found: C 37.5, H 3.5, N 18.9.

Potassium (3*R*,5*R*,*Z*)-2-[2-(4-Hydroxymethyl-1,2,3-triazol-1-yl)ethylidene]clavam-3-carboxylate (**9h**)

A solution of propargyl alcohol (1.7 g) and the azido ester (**3**) (5.0 g) in dioxane (25 ml) was heated at 70°C for 115 hours. The mixture was concentrated and chromatographed on a column of silica gel (150 g) using ethyl acetate - tetrahydrofuran (2: 1) eluent to give 0.52 g (9%) of the 5-hydroxymethyl ester (**8h**); IR ($CHBr_3$) 3600, 1810, 1760, 1705 and 1615 cm^{-1}; NMR ($CDCl_3$) δ 7.50 (1H, s, triazole proton), 5.78 (1H, d, J=2 Hz, H-5), 5.28 (2H, s, benzyl protons), 4.8~5.05 (3H, m, H-3 and CH_2N), 4.70 (2H, s, CH_2OH), 3.55 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.14 (1H, d, J=17 Hz, 6β-H). Further elution yielded 0.46 g (8%) of the 4-hydroxymethyl ester (**7h**); IR ($CHBr_3$) 3600, 1808, 1760, 1704, 1528 and 1340 cm^{-1}; NMR ($CDCl_3$) δ 7.54 (1H, s, triazole proton), 5.80 (1H, d, J=2 Hz, 5-H), 5.29 (2H, s, benzyl protons), 5.03 (2H, s, CH_2N), 4.76 (2H, s, CH_2OH), 3.60 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.15 (1H, d, J=17 Hz, 6β-H).

A solution of the ester (**7h**) (0.45 g) in ethyl acetate (25 ml) was hydrogenated over palladium on carbon (10%, 0.50 g) until uptake of hydrogen ceased. The mixture was filtered through diatomaceous earth and the filtrate treated with a solution of potassium 2-ethylhexanoate (0.10 g) in ethyl acetate. The

mixture was then concentrated to *ca.* 5 ml and diluted with ether (5 ml). The precipitated solid was filtered off and dried to give 0.12 g (34%) of the potassium salt (**9h**); IR (Nujol) 1790, 1700 and 1620 cm^{-1}; NMR (D_2O) δ 7.92 (1H, s, triazole proton), 5.78 (1H, d, J=2 Hz, 5-H), 5.09 (2H, s, CH_2N), 5.02 (1H, s, 3-H), 3.59 (1H, dd, J=2 and 17 Hz, 6α-H) and 3.12 (1H, d, J=17 Hz, 6β-H).

Anal Calcd for $C_{11}H_{11}N_4O_5K \cdot 2H_2O$: C 37.4, H 4.3, N 15.8.
Found: C 37.9, H 4.7, N 15.2.

Potassium (3*R*,5*R*,*Z*)-2-[2-(4-Phenyl-1,2,3-triazol-1-yl)ethylidene]clavam-3-carboxylate (**9i**)

Phenylacetylene (3.2 ml) was added to a solution of the azido ester (**3**) (5.0 g) in dioxane (25 ml) and the mixture heated at 70°C for 65 hours. The reaction mixture was concentrated and passed down a column of silica gel (150 g) using ethyl acetate - petroleum ether (3: 2) eluent to give 0.41 g (6%) of the 4-phenyl ester (**7i**); IR ($CHBr_3$) 1800 and 1760 cm^{-1}; NMR ($CDCl_3$) δ 8.2, 7.9~7.1 (10H, m, aromatic protons), 5.82 (1H, d, J=2 Hz, 5-H), 5.29 (2H, s, benzyl protons), 5.22 (1H, s, 3-H), 5.10 (2H, s, CH_2N), 3.62 (1H, dd, J=2 and 16 Hz, 6α-H) and 3.16 (1H, d, J=16 Hz, 6β-H).

A solution of the ester (0.38 g) in ethyl acetate (20 ml) was hydrogenated over palladium on carbon (10%, 0.50 g) until uptake of hydrogen ceased. The mixture was filtered through diatomaceous earth and a solution of potassium 2-ethylhexanoate (0.10 g) in ethyl acetate added to the filtrate. The filtrate was concentrated to *ca.* 5 ml, then diluted with ether (10 ml). The precipitated solid was filtered off and dried, yielding 0.13 g (43%) of the potassium salt (**9i**); IR (Nujol) 1790 and 1720 cm^{-1}; UV (pH 6 buffer) 241.5 nm (ε 13,700); NMR (D_2O) δ 8.00 (1H, s, triazole proton), 7.8~7.2 (5H, m, phenyl protons), 5.80 (1H, d, J=2 Hz, 5-H), 5.00 (3H, s, 3-H and CH_2N), 3.08 and 3.62 (2H, ABq, J=17 Hz, 6α-H and 6β-H). The NMR spectrum showed the presence of *ca.* 20% of an *E*-isomer of the phenyltriazole.*

Anal Calcd for $C_{16}H_{13}N_4O_4K \cdot 2H_2O$: C 47.9, H 4.3, N 14.0.
Found: C 47.6, H 4.3, N 13.5.

References

1) Hunter, P. A.; K. Coleman, J. Fischer & P. Taylor: *In vitro* synergistic properties of clavulanic acid with ampicillin, amoxycillin and ticarcillin. J. Antimicrob. Chemother. 6: 455~470, 1980
2) Newall, C. E.: Clavams, penems and ethylidene penams derived from clavulanic acid. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.* Gregory, G. I., pp. 151~169, Royal Soc. Chem., London, 1981
3) Gilchrist, T. L. & G. E. Gymer: 1,2,3-Triazoles. *In* Advances in Heterocyclic Chemistry. *Eds.* Katritzky, A. R. & A. J. Boulton, Vol. 16, pp. 33~85, Academic Press, New York, 1974
4) Hansch, C. & A. Leo: Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley-Interscience, New York, 1979
5) Moriguchi, I.; Y. Kanada & K. Komatsu: Van der Waals volume and related parameters for hydrophobicity in structure-activity studies. Chem. Pharm. Bull. 24: 1799~1806, 1976
6) Sykes, R. B. & N. H. Georgopapadakou: Bacterial resistance to β-lactam antibiotics: An overview. *In* β-Lactam Antibiotics — Mode of Action, New Developments and Future Prospects. *Eds.* Salton, M. & G. D. Shockman, pp. 199~214, Academic Press, New York, 1981
7) Blankley, C. J.: A review of QSAR methodology. *In* Quantitative Structure-Activity Relationships of Drugs. *Ed.* Topliss, J. G., pp. 1~21, Academic Press, New York, 1983
8) Hansch, C.; R. Li, J. M. Blaney & R. Langridge: Comparisons of the inhibition of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase by 2,4-diamino-5-(substituted-benzyl)pyrimidines: Quantitative structure-activity relationships, X-ray crystallography, and computer-graphics in structure-activity analysis. J. Med. Chem. 25: 777~784, 1982
9) O'Callaghan, C. H.; P. W. Muggleton & G. W. Ross: Effects of β-lactamase from Gram-negative organisms on cephalosporins and penicillins. Antimicrob. Agents Chemother. -1968: 57~63, 1969
10) Newall, C. E. & A. P. Tonge: Clavulanic acid derivatives. U.K. Patent Appl. 2,006,769, 1979 (Chem. Abstr. 92: 22497u)
11) Cherry, P. C.; C. E. Newall & A. P. Tonge: Clavulanic acid derivatives. Ger. Offen. 2,747,599, 1978 (Chem. Abstr. 89: 59890f)
12) Richmond, M. H.; D. C. Clark & S. Wotton: Indirect method for assessing the penetration of beta-lactamase-nonsusceptible penicillins and cephalosporins in *Escherichia coli* strains. Antimicrob. Agents Chemother. 10: 215~218, 1976

* *E*-Isomers of clavulanic acid derivatives are considerably less active biologically than the corresponding *Z*-isomers.