The Chemistry of Pseudomonic Acid Part 14

Synthesis and *In Vivo* Biological Activity of Heterocyclyl Substituted Oxazole Derivatives

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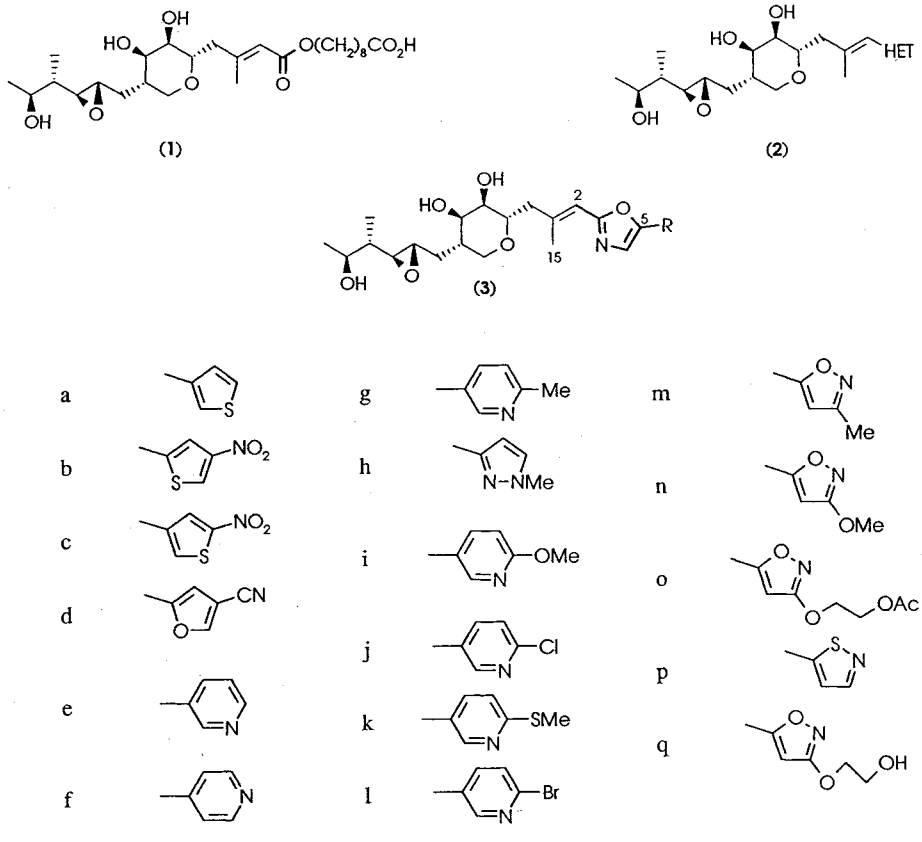
Semisynthetic analogues of pseudomonic acid A have been prepared containing a heterocyclyl substituted oxazole. Derivatives in which the heterocycle was thiophene, furan, pyridine, or isoxazole showed good antibacterial potency and were further evaluated *in vivo*. Both pharmacokinetic parameters and oral activity against an experimental intraperitoneal sepsis were superior to results obtained from previously described pseudomonic acid A derivatives.

We have previously described the semisynthetic derivatives[1] of the naturally occurring antibiotic pseudomonic acid A† (**1**), including a series of heterocyclic derivatives (**2**), in which the metabolically labile ester group has been replaced by various heterocycles[2].

Amongst these derivatives the greatest biological activity was found to reside in compounds in which the heterocycle was a 2,5-disubstituted oxazole (**3**), and we

Fig. 1. Structures of the pseudomonic acid derivatives.



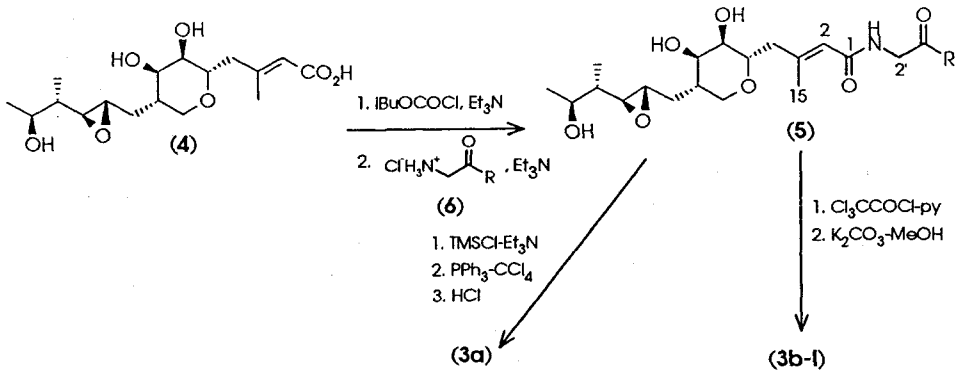
* Substituents a~q remain the same in all the structures used in this paper.

† The approved generic name for pseudomonic acid A is mupirocin.

have previously reported the synthesis and *in vitro* antibacterial activity of such compounds when the 5-substituent R is alkyl and aryl[3].

We have now found that derivatives in which R is a heterocyclic group have improved antibacterial activity compared to the previously reported derivatives. This paper describes the synthesis and *in vitro* and *in vivo* biological properties of the more active of these

Scheme 1. Synthesis of oxazoles (**3a**~**l**).



Scheme 2. Synthesis of α-aminoketones (**6**).

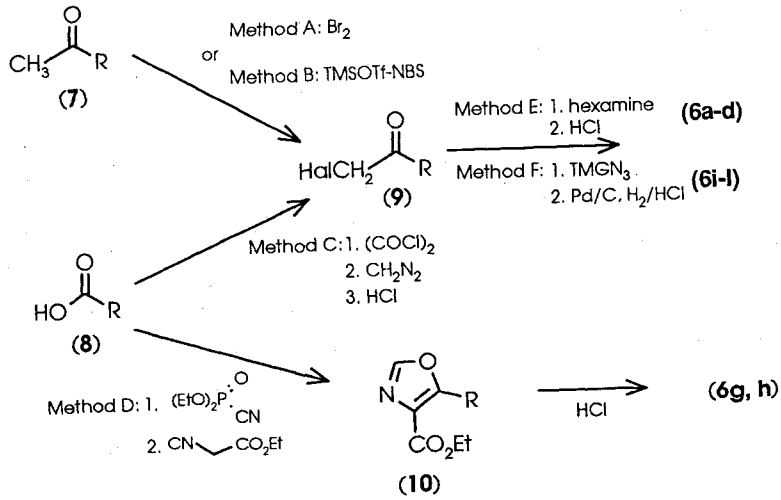


Table 1. Synthesis and properties of the α-aminoketones (**6**).

(6)	Method(s)	Yield(s) %	^{1}H NMR (ppm)			
			Solvent	CH_2	CH_3	Aromatic
a	A, E	85, 75	CD_3OD	4.52	—	7.60, 8.55
b	A, E	86, 74	$(CD_3)_2SO$	4.65	—	8.80, 9.30
c	C[a], E	75, 88	D_2O	4.61	—	8.46, 8.68
d	B, E	23, 96	D_2O	—	—	7.70, 8.50
e	[b]	92	D_2O	4.75	—	8.22, 8.95, 9.15, 9.33
f	[b]	87	D_2O	4.90	—	8.30, 8.90
g	D	46	D_2O	4.84	2.90	8.15, 9.02, 9.32
h	D	84	D_2O	4.52	3.95	6.90, 7.69
i	C, F	31, 85	$(CD_3)_2SO$	4.56	3.95	7.02, 8.25, 8.90
j	C, F	96, 45	$(CD_3)_2SO$	4.63	—	7.78, 8.41, 9.04
k	C, F	70, 40	D_2O	4.67	2.67	7.66, 8.38, 8.97
l	C[c], F	26, 50	D_2O	4.65	—	7.82, 8.22, 8.87

[a] HBr was used in place of HCl.
[b] The method of Ref. 6 was used but prolonged heating was found necessary in the final hydrolysis.
c $(COBr)_2$/HBr was used instead of $(COCl)_2$/HCl.

compounds, (**3a**～**q**).

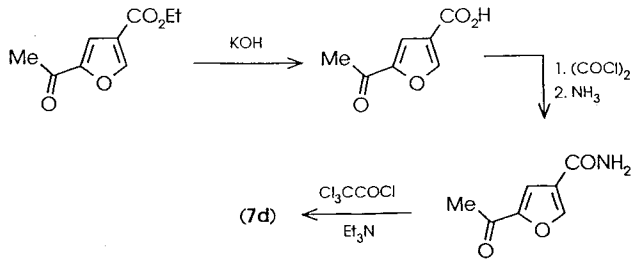
Chemistry

Two general methods were used for the preparation of the heterocyclyl oxazoles, both starting from monic acid A[4] (**4**). The first, shown in Scheme 1, uses the previously described dehydrative cyclisation of keto-amides (**5**) prepared from the corresponding α-amino-ketones (**6**)[2].

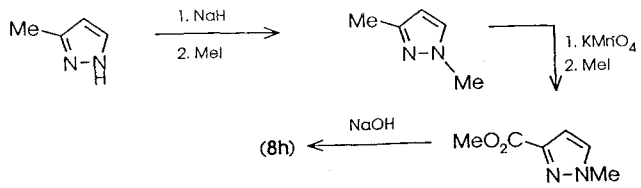
The methods used to prepare the appropriate α-aminoketones are shown in Scheme 2 and Table 1.

The Delepine synthesis (acidic hydrolysis of a hexa-methylenetetraammonium salt)[5] was satisfactory for the preparation of the thiophene and furan derivatives (**6a**～**d**). However this method could not be used for the more electron-deficient pyridyl and pyrazolyl derivatives (**6e**～**l**), and a variety of methods were developed for these compounds. The unsubstituted derivatives (**6e,f**) were prepared according to a literature method using the Neber rearrangement[6], and the methyl pyridine and methylpyrazole (**6g, h**) *via* the corresponding oxazole[7] (**10**). Neither of these two methods was successful for the functionalised pyridines (**6i**～**l**), and in these cases the use of very mild conditions *via* hydrogenation of an azidomethyl ketone was required.

Scheme 3. Synthesis of ketone (**7d**).



Scheme 4. Synthesis of acid (**8h**).

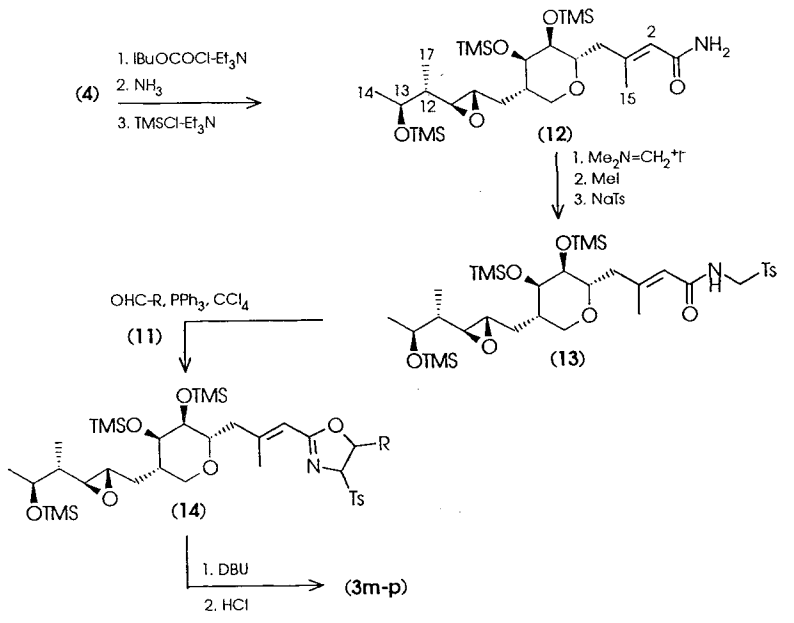


The appropriate starting methyl ketones (**7**) and acids (**8**) were available either commercially or by literature procedures (see Experimental) with the exception of; (**7d**) prepared as shown in Scheme 3, (**8h**) as in Scheme 4, and (**8i**), prepared by hydrolysis of the corresponding ester.

All the above conditions proved unsuitable for the preparation of the even more electron-deficient isoxazolyl and isothiazolyl aminomethyl ketones (**6m**～**p**) and the use of aminoketone intermediates was therefore abandoned. An alternative oxazole synthesis shown in Scheme 5 was therefore developed using a cycloaddition strategy.

Tosylmethyl amide intermediate (**13**) was prepared in 6 steps from (**4**), and then dehydration in the presence of electron-deficient aldehydes R-CHO (**11**) gave oxazolines (**14**). Subsequent base treatment and deprotection liberated the oxazoles (**3m**～**p**)[8]. Conversely this route is unsuitable for electron-rich aldehydes, when ring

Scheme 5. Synthesis of oxazoles (**3m**～**p**).



opening of the oxazoline takes place in preference to elimination of *p*-toluenesulphinic acid.

Aldehydes (**11m~o**) were prepared as shown in Schemes 6 and 7.

Oxazole (**3q**) was prepared from (**3o**) by hydrolysis (K_2CO_3 - MeOH).

Results and Discussion

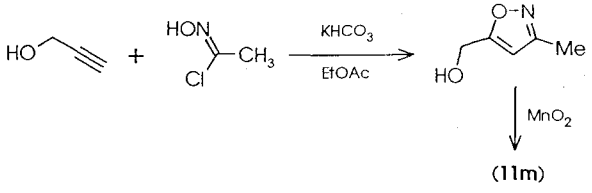
The presently described series of derivatives were prepared as a continuation of the series of alkyl and aryl oxazoles mentioned previously. Heterocycles incorporating a single heteroatom were investigated first, and three series: thiophenes, furans, and pyridines, were found to confer good activity. In order to investigate whether heterocycles containing two heteroatoms would be more active isoxazoles, isothiazoles, and pyrazoles were then prepared.

All the oxazoles had the same spectrum of antibacterial activity as pseudomonic acid A. The degree of potency was remarkably high and present in several of the series of heterocyclic oxazoles (Table 2), the level of activity being close to that of pseudomonic acid A and equal or superior to that of the aryl and alkyl oxazoles reported previously[3].

In both the aryl and heterocyclic series, potent activity was associated with compounds containing a nitro group: *p*-nitrophenyl in the case of aryl derivatives; 4-nitro-2-thienyl (**3b**) and 2-nitro-4-thienyl (**3c**) in the heterocyclic series.

An advantage for the majority of the heterocyclic oxazoles was lower human serum binding than that of

Scheme 6. Synthesis of aldehyde (**11m**).



Scheme 7. Synthesis of aldehydes (**11n, o**).

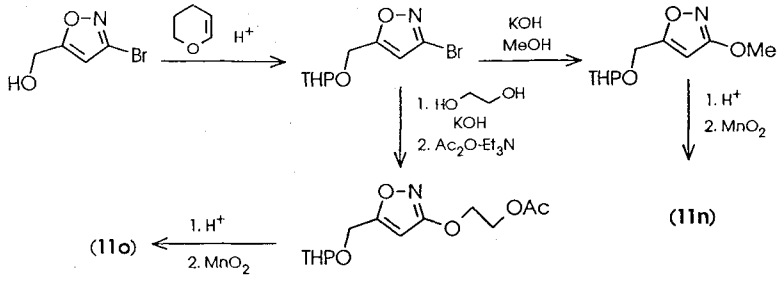


Table 2. Antibacterial activity of the oxazoles (**3**).

	Antibacterial activity (MIC $\mu g\,ml^{-1}$)*						
	Escherichia coli DC0	*Staphylococcus aureus* NCTC 6571	*Staphylococcus aureus* Smith	*Streptococcus pyogenes* CN10	*Streptococcus pneumoniae* 1629	*Haemophilus influenzae* WY21	*Moraxella catarrhalis* 1502
1	>128	0.13	0.25	0.13	0.13	0.06	0.13
3a	128	1	1	1	0.50	0.06	4
3b	128	0.25	0.06	0.25	0.25	0.50	0.50
3c	128	0.25	0.25	0.25	0.25	0.06	0.25
3d	>128	0.50	1	0.50	0.13	0.13	0.25
3e	>128	1	2	0.25	1	0.25	4
3f	>128	1	—	0.25	1	0.50	4
3g	>128	1	1	0.50	0.25	0.50	—
2h	>128	2	4	4	0.25	—	8
3i	>128	0.50	1	0.50	1	0.25	1
3j	>128	1	2	0.50	0.25	0.13	1
3k	>128	1	1	1	0.25	1	1
3l	>128	2	1	1	0.25	0.25	1
3m	128	1	1	0.50	0.13	0.13	1
3n	>128	1	1	1	0.50	0.50	1
3p	>128	8	2	2	—	0.13	8
3q	>128	2	2	0.25	0.25	0.25	0.50

* Standard agar dilution procedure using: medium, blood agar base plus 5% chocolated horse blood: inoculum, $10^5 \sim 10^6$ cfu per 1 μl spot: incubation, 18 hours at 37°C: end-point (MIC), lowest concentration preventing visible growth.

pseudomonic acid A (>95%) (Table 3). Only thienyl derivatives (**3a**~**c**) and the 2-methylthio-5-pyridyl analogue (**3k**) were more than 90% bound, and of these only (**3c**) was more highly bound than pseudomonic acid A.

The high and consistent level of antibacterial activity and favourable serum binding exhibited by the furyl, thienyl, pyridyl, and isoxazolyl oxazoles made the use of *in vitro* criteria alone inadequate for selection of candidate compounds for progression to detailed biological investigation. The increased level of metabolic stability conferred by replacement of the metabolically labile ester linkage allowed compounds to be compared on the basis of murine pharmacokinetic parameters (Table 3) and efficacy in an experimental infection model (Table 4). In terms of the area under the blood concentration/time curve (AUC), only the unsubstituted 3-thienyl oxazole (**3a**) was clearly inferior to the other derivatives. The remaining compounds all gave substantial blood levels by both oral and parenteral routes. A selection of these compounds with a range of blood levels and antibacterial potency was therefore evaluated by oral administration to mice with experimental intraperitoneal sepsis caused by *Staph. aureus* Smith (MIC range 0.25~2 $\mu g\,ml^{-1}$). The results obtained are shown in Table 4 and were remarkably similar for all the compounds tested indicating that high potency with lower blood levels and *vice versa* were equally effective combinations. Nitrothiophene (**3c**) was clearly the least effective derivative: the poor performance of this compound could be attributed to dosing problems associated with its very poor aqueous solubility.

The level of activity generally shown in the infection model was slightly poorer than that of the anti-staphylococcal penicillin, flucloxacillin, but equal or superior to erythromycin base. None of the compounds approached the *in vivo* potency of amoxycillin.

The heterocyclic oxazoles were thus demonstrated to be active *in vivo*, but the curative dose in this infection model was large in view of their potent *in vitro* activity and good blood levels.

Further infection studies to clarify this anomaly and to select one or more of the heterocyclic oxazoles for further progression are in hand and will be reported subsequently.

Table 3. Serum binding and blood levels of the oxazoles (**3**).

	Human[a] serum binding % bound	Mouse blood level[b] AUC ($\mu g\,ml^{-1}$ minute)	
		po	sc
1	>95	0	32
3a	93	71	184
3b	94	348	1165
3c	97	283	511[c]
3d	87	1113	2360
3e	76	520	560
3f	89	366	1327
3g	75	599	1161
3h	—	—	—
3i	90	873	1716
3j	85	529	1147
3k	93	557	678
3l	90	641	1255
3m	81	661	921
3n	76	608	1147
3p	—	—	—
3q	—	191	622

[a] By ultrafiltration (Amicon microfree partition apparatus) using sterile pooled human serum; initial compound concentration 40 $\mu g\,ml^{-1}$.
[b] Dose = 50 $mg\,kg^{-1}$.
[c] Dose = 25 $mg\,kg^{-1}$, aqueous solubility poor.

Table 4. Oral activity of selected derivatives against a systemic staphylococcal infection in mice.*

(**3**)	CD_{50} ($mg\,kg^{-1}$)	MIC ($\mu g\,ml^{-1}$) *S. aureus* Smith
b	84	0.06
c	220	0.25
d	84	1
e	96	2
i	74	1
j	82	2
l	>66	1
m	96	1
Amoxycillin	0.2~0.6	0.25
Flucloxacillin	46~66	0.25
Erythromycin	90~132	0.25
Ciprofloxacin	8~10	0.12

* OLAC MFI mice were infected intraperitoneally with 2~9 × 10^6 cfu of *Staph. aureus* Smith contained in 0.5 ml of 3% hog gastric mucin and 1% carboxymethyl cellulose. Compounds were administered orally as a solution or suspension in 1% hydroxypropylmethyl cellulose at 1 and 5 hours post infection. The CD_{50} was calculated on the second day post infection as the total dose required to protect 50% of the mice from death.

Experimental

^1H NMR data were recorded at 250 MHz on a Bruker AC-250F spectrometer and are expressed relative to tetramethylsilane as an internal standard, infrared data on a Perkin-Elmer PE983 machine, ultraviolet data on a Beckman DU68, and mass spectra on a VG-ZAB spectrometer. The silica used for TLC and column chromatography was Merck type 60.

α-Aminoketone Hydrochlorides (**6a**~**l**)

Prepared by the general methods described below, as shown in Scheme 2 and Table 1. The appropriate starting methyl ketones and carboxylic acids were either: com-

mercially available, (**7a**), (**7c**), (**7e**), (**7f**), (**8g**), and (**8j**); or were prepared as described previously, (**7b**)[9], (**8k**)[10], and (**8l**)[11]; or were prepared as described below.

2-Acetyl-4-cyanofuran (**7d**)

Ethyl 2-acetyl-4-furoate[12] (5 g, 27 mmol) in ethanol (35 ml) was treated with aqueous potassium hydroxide (16 ml, 2.5 M, 40 mmol) for 15 minutes at 60°C. The mixture was cooled, acidified, and extracted four times with ethyl acetate. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated to give the crude acid (3.0 g, 70%) which was suspended in dichloromethane (60 ml) and treated with oxalyl chloride (1.7 ml, 20 mmol). After 0.5 hour at 20°C, evaporation gave the crude acid chloride which was dissolved in dry THF (120 ml). Ammonia (g) was bubbled through the solution for 0.5 hour at 5°C, and then ethyl acetate and water were added. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give the primary amide (2.2 g, 73%).

The primary amide (2.2 g, 14 mmol) was suspended in dichloromethane (70 ml) at 5°C and triethylamine (4.0 ml, 30 mmol) and trichloroacetyl chloride (1.8 ml, 16 mmol) were added. After 0.5 hour at 5°C, the solution was washed with 1 M aqueous sodium hydroxide, 1 M hydrochloric acid, and brine, dried ($MgSO_4$), and evaporated. Chromatography of the residue eluting with ethyl acetate/hexane mixtures gave the title compound 1.3 g (68%); mp 92~93°C; IR ν_{max} ($CHCl_3$) cm^{-1} 2240 and 1685; ^1H NMR ($CDCl_3$) δ 2.52 (3H, s), 7.42 (1H, s), and 8.20 (1H, s); MS *m/z* 135 (M^+) (Found: M^+ 135.0323, $C_7H_5NO_2$ requires 135.0320).

1-Methylpyrazole-3-carboxylic Acid (**8h**)

3-Methylpyrazole (12.0 g, 150 mmol) was dissolved in dry THF (120 ml) at 0°C and sodium hydride (4.8 g × 75% in oil, 150 mmol) added in portions. After 0.5 hour iodomethane (13.7 ml, 220 mmol) was added, and after 3 hours at 20°C diethyl ether (120 ml) was added and the mixture was filtered and evaporated. The evaporation residue was dissolved in aqueous potassium hydroxide (75 ml, 2 M, 150 mmol) and potassium permanganate (23.7 g, 150 mmol) added. After 2 hours at 100°C, the solution was filtered (Kieselguhr) and evaporated to give a crude sample of the title compound potassium salt.

This was purifed by chromatography as its methyl ester: the potassium salt was stirred with DMF (100 ml) and iodomethane (17.4 ml, 280 mmol) for 48 hours at 20°C: diethyl ether (200 ml) was then added and the solution filtered and evaporated. The evaporation residue was purified by chromatography, eluting with ethyl acetate/hexane mixtures to give pure methyl ester, 0.9 g (5%); ^1H NMR ($CDCl_3$) δ 3.89 (3H, s), 3.96 (3H, s), 6.78 (1H, d), and 7.38 (1H, d).

The free acid was liberated as a mixture with sodium chloride by stirring with THF (10 ml)/aqueous sodium hydroxide (7 ml, 1 M, 7 mmol) for 2 hours at 50°C then cooling, washing with ethyl acetate, acidifying, and evaporating the aqueous solution.

2-Methoxypyridine-5-carboxylic Acid (**8i**)

A mixture of ethyl 2-methoxy-pyridine-5-carboxylate[13] (0.45 g, 2.5 mmol), THF (10 ml), and aqueous sodium hydroxide (16 ml, 0.25 M, 4.0 mmol) was stirred for 5 hours at 20°C, and then treated with hydrochloric acid (4 ml, 1.0 M, 4.0 mmol) and evaporated. The residue was partitioned between water and 4-methylpentan-2-one. Evaporation of the organic layer gave the title compound 0.29 g, (75%); ^1H NMR [$(CD_3)_2CO$] δ 3.96 (3H, s), 6.82 (1H, d), 8.19 (1H, dd), and 8.80 (1H, d).

General Methods for the Preparation of (**6**)

Method A: the appropriate acetyl heterocycle (**7**) (10 mmol) was dissolved in anhydrous diethyl ether (20 ml) containing aluminium chloride (50 mg). Bromine (10 mmol) was then added dropwise. When the solution became colourless it was washed with water and evaporated *in vacuo* to give the bromoketone (**9**).

Method B: the appropriate acetyl heterocycle (**7**) (10 mmol) in dichloromethane (40 ml) at 5°C was treated with triethylamine (15 mmol) and trimethylsilyl trifluor omethanesulphonate (15 mmol). After 30 minutes at 20°C N-bromosuccinimide (12 mmol) was added, and after another 45 minutes the solution was washed with water and evaporated *in vacuo* to give the bromoketone (**9**).

Method C: a suspension of the heterocyclyl carboxylic acid (**8**) (10 mmol) in dichloromethane (50 ml) was treated with oxalyl chloride (12 mmol), and stirred at 20°C until reaction was complete by IR analysis. The solution was then added to excess diazomethane in ether solution. After 2 hours at 20°C the resulting solution was treated with gaseous hydrogen chloride for 10 minutes (alternatively 9 M hydrobromic acid was used). Evaporation of the organic fraction gave the bromo- or chloroketone (**9**).

Method D: to a solution of the appropriate heterocyclyl carboxylic acid (**8**) (10 mmol) in DMF (15 ml) at −15°C were added diethyl cyanophosphonate (11 mmol) and triethylamine (10 mmol). After 20 minutes a solution containing ethyl isocyanoacetate (9 mmol) and triethylamine (25 mmol) in DMF (10 ml) was added dropwise. After 2 hours at −15°C saturated aqueous sodium hydrogen carbonate was added and extraction with ethyl acetate then gave the intermediate oxazole (**10**). This was dissolved in 5 M hydrochloric acid and the solution heated at 100°C for 5 hours and then evaporated to give (**6**).

Method E: The appropriate haloacetyl heterocycle (**9**) (10 mmol) and hexamethylenetetramine (10 mmol) were dissolved in dichloromethane (50 ml). When precipitation of the salt was complete it was collected by filtration, dried, and added to a mixture of ethanol (10 ml) and 11 M hydrochloric acid (5 ml). After 18 hours at 20°C the aminoketone hydrochloride was collected by filtration and recrystallised from an appropriate alcohol to remove ammonium chloride.

Method F: a mixture of the appropriate haloketone

Table 5. Properties of keto amides (**5**).

(5)	Yield (%)	IR (cm^{-1}) (neat)	^1H NMR (ppm)					^{13}C NMR (ppm)			
			Solvent	H-2	CH_3-15	CH_2-2′	Heteroaryl	Solvent	C-1	C-2	C-2′
a	15	1680, 1660, 1630	CD_3OD	5.89	2.18	4.63	7.51, 7.59, 8.42	CD_3OD	169.8	120.8	119.2
b	46	1690, 1660, 1630	CD_3OD	5.87	2.15	4.66	8.48, 8.88	CD_3OD	169.9	120.4	189.5
c	59	1700, 1660, 1630	CD_3OD	5.88	2.16	4.61	8.39, 8.66	CD_3OD	169.8	120.5	190.3
d	26	2240, 1720, 1660, 1630	CD_3OD	5.87	2.15	4.53	7.71, 8.54	CD_3OD	168.2	118.8	183.7
e	25	1690, 1655, 1635	$CDCl_3$	5.89	2.21	4.82	7.48, 8.28, 8.84, 9.22	$CDCl_3$	167.5	119.4	194.1
f*	8	1810, 1655, 1635	$CDCl_3$	—	2.20	—	7.79, 8.85	—	—	—	—
g	38	1690, 1660, 1640	$CDCl_3$	5.83	2.20	4.79	7.31, 8.16, 9.09	$CDCl_3$	167.8	119.6	193.8
h	45	1700, 1660, 1630	CD_3OD	5.89	2.16	4.67	6.79, 7.67	CD_3OD	169.9	120.8	191.7
i	58	—	$CDCl_3$	5.83	2.21	4.75	6.82, 8.14, 8.83	$CDCl_3$	167.4	119.6	192.7
j	21	1700, 1660	$CDCl_3$	5.82	2.20	4.78	7.50, 8.23, 8.99	—	—	—	—
k	25	1695, 1660, 1630	CD_3OD	5.90	2.17	4.69	7.39, 8.13, 9.00	CD_3OD	169.8	120.7	195.0
l	30	—	CD_3OD	5.88	2.17	4.68	7.78, 8.23, 8.96	CD_3OD	169.8	120.4	195.0

* Impure.

Table 6. Properties of the oxazoles (**3**).

(3)	Yield (%)	MP	CHN	M^+ (EI)	UV (EtOH)		IR (cm^{-1}) (neat)
					λ_{max} (nm)	ε_m	
a	40	—	—	449	300	19,800	1650, 1610, 1540
b	41	—	—	494	325	25,500	1650, 1600, 1530
c	58	133～4°	C, H, N	494	296	35,000	1650, 1520
d	44	—	—	458	—	—	2250, 1650
e	65	—	—	444	305	24,200	1650, 1525
f	32	—	—	444	312	23,500	1650, 1610
g	67	—	—	458	302	24,400	1650
h	67	—	—	447	292	22,500	1650
i	82	—	—	474	302	25,900	1670, 1620
j	52	—	—	478	—	—	—
k	53	—	—	490	328	29,900	1650
l	40	—	—	522	313	25,100	—
m	25	100～3°	C, H, N	448	299	25,000	1630
n	32	104～7°	—	464	298	19,600	1660
o	60	—	—	537	298	27,700	1640
p	7	—	—	450	316	18,400	1650, 1520
q	96	—	—	494	300	25,400	1650

(3)	^1H NMR (ppm)					^{13}C NMR (ppm)		
	Solvent	H-2	CH_3-15	Oxazole-H	Heteroaryl	Solvent	C-2	Oxazole-C
a	$CDCl_3$	6.26	2.30	7.18	7.29, 7.37, 7.60	$CDCl_3$	113.2	129.4, 146.9, 160.6
b	$CDCl_3$	6.24	2.29	7.18	7.95, 8.20	CD_3OD	112.6	125.3, 144.4, 161.0
c	CD_3OD	6.25	2.29	7.50	7.99, 8.31	CD_3OD	113.3	124.8, 146.2, 162.8
d	CD_3OD	6.25	2.29	7.48	7.03, 8.38	CD_3OD	113.0	125.7, 146.5, 163.2
e	$(CD_3)_2CO$	6.30	2.33	7.45	7.36, 7.90, 8.53, 8.90	$CDCl_3$	112.8	124.1, 146.8, 161.6
f	$CDCl_3$	6.30	2.34	7.56	7.49, 8.62	$CDCl_3$	112.8	126.5, 147.1, 162.6
g	$CDCl_3$	6.29	2.32	7.39	7.21, 7.79, 8.79	$CDCl_3$	112.9	123.3, 147.2, 161.6
h	CD_3OD	6.23	2.28	7.36	6.56, 7.66	CD_3OD	113.4	123.5, 146.3, 162.3
i	$CDCl_3$	6.26	2.30	7.27	6.82, 7.81, 8.46	$CDCl_3$	113.0	121.8, 147.6, 161.1
j	$CDCl_3$	6.29	2.32	7.44	7.85, 8.67, 8.85	$CDCl_3$	113.0	124.8, 148.9, 162.4
k	CD_3OD	6.27	2.30	7.57	7.33, 7.90, 8.72	CD_3OD	113.5	124.1, 149.8, 163.0
l	CD_3OD	6.28	2.31	7.66	7.71, 8.71, 8.00	CD_3OD	113.3	125.3, 147.5, 163.7
m	$CDCl_3$	6.29	2.31	7.52	6.29	$CDCl_3$	112.5	127.3, 149.1, 162.3
n	$CDCl_3$	6.29	2.14	7.47	6.12	$CDCl_3$	111.7	126.3, 149.2, 162.1
o	$CDCl_3$	6.30	2.30	7.30	6.10	—	—	—
p	CD_3OD	6.25	2.30	7.60	7.58, 8.45	—	—	—
q	$CDCl_3$	6.30	2.30	7.50	6.10	—	—	—

(**9**) (10 mmol), N,N,N′,N′-tetramethylguanidinium azide (11 mmol), and dichloromethane (40 ml) was stirred for 1.5 hours at 20°C then evaporated *in vacuo*. Chromatography (silica gel, dichloromethane - methanol mixtures) gave the azidoketone. The azidoketone (10 mmol) was dissolved in a mixture of THF (30 ml) and 1 M hydrochloric acid (25 ml) and then hydrogenated over 10% palladium on charcoal (50 mg) for 5 minutes. Filtration and evaporation gave the aminoketone hydrochloride (**6**).

Preparation of Oxazoles (**3a**～**l**) *via* Amides (**5a**～**l**)

The methods previously described were used as shown in Scheme 1[2]. The properties of the compounds prepared are shown in Tables 5 and 6.

Aldehydes (**11m**～**p**)

Aldehyde (**11p**) was commercially available. Aldehydes (**11m**～**o**) were prepared as follows:

3-Methylisoxazole-5-carboxyaldehyde (**11m**)

A solution of acetohydroxamoyl chloride[14] (16 g, 0.17 mmol) in ether (50 ml) was added over 0.5 hour to a well stirred mixture of potassium hydrogen carbonate (44 g, 0.45 mmol), prop-2-yn-1-ol (18 ml, 0.30 mmol), ethyl acetate (600 ml), and water (6 ml). After 18 hours at 20°C the mixture was washed with water, dried ($MgSO_4$), and evaporated to give 3-methylisoxazole-5-methanol as a yellow oil, 11.2 g (60%).

A mixture of this alcohol (11.2 g, 0.10 mmol), active manganese dioxide (17 g), and benzene (100 ml) was refluxed under a Dean and Stark trap for 3 hours, then filtered and evaporated. Purification of the residue by chromatography eluting with methanol - dichloromethane mixtures gave the title compound, 6.6 g (59%); ^{1}H NMR ($CDCl_3$) δ 2.47 (3H, s), 6.98 (1H, s), and 10.12 (1H, s).

3-Methoxyisoxazole-5-carboxaldehyde (**11n**)

A mixture of 3-bromoisoxazole-5-methanol[15] (33 g, 0.19 mmol), 2,3-dihydropyran (22 ml, 0.25 mmol), diethyl ether (500 ml), and *p*-toluenesulphonic acid (0.01 g) was stirred for 2 hours at 20°C then washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated to give the THP ether, 46 g (95%). This was added to a solution of potassium hydroxide (87 g, 1.50 mmol) in methanol (500 ml). After 2 hours at reflux the solution was cooled and water added. Extraction with diethyl ether, drying ($MgSO_4$), and evaporation gave 3-methoxyisoxazole-5-methanol THP ether as a yellow oil, 25 g (60%).

This was stirred with methanol (500 ml) and Amberlyst IR-120 (acid form) (10 g) for 18 hours at 20°C. The solution was then filtered and evaporated to give an oil, which was purified by chromatography eluting with diethyl ether - hexane mixtures to give the alcohol 9.0 g (60%). Oxidation using the method described for (**11m**) gave the title compound, 5.4 g (60%), ^{1}H NMR ($CDCl_3$) δ 4.02 (3H, s), 6.56 (1H, s), 9.80 (1H, s).

3-(2-Acetoxyethoxy)isoxazole-5-carboxaldehyde (**11o**)

A mixture of 3-bromoisoxazole-5-methanol THP ether prepared as described in the previous example (1.9 g, 7.2 mmol), potassium hydroxide (21 g, 72 mmol), and ethylene glycol (65 ml) was heated at 80°C for 4 hours then cooled. Hydrochloric acid (7.2 ml, 5 M, 36 mmol) was added and the solution then extracted three times with diethyl ether. The combined extracts were dried ($MgSO_4$) and evaporated and the residue purified by chromatography eluting with diethyl ether to give 3-(2-hydroxyethoxy)isoxazole-5-methanol 0.8 g (45%).

To a solution of this material in dichloromethane (20 ml) at 0°C were added triethylamine (1.9 ml, 13 mmol) and acetic anhydride (0.95 ml, 10 mmol). After 3 hours at 20°C the solution was washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography eluting with ethyl acetate - hexane mixtures to give 3-(2-acetoxyethyl)isoxazole-5-methanol, 1.2 g (94%).

Deprotection and oxidation using the method described for (**11n**) gave the title compound 0.38 g (45% overall); ^{1}H NMR ($CDCl_3$) δ 2.10 (3H, s), 4.4～4.6 (4H, m) (4H, m), 6.65 (1H, s) and 9.80 (1H, s).

Preparation of Oxazoles (**3m**～**p**) by Cycloaddition

Monic Acid Amide, Tris(trimethylsilyl ether) (**12**)

Monic acid A[4] (17 g, 50 mmol) was dissolved in dry THF (100 ml) at 0°C and triethylamine (7.0 ml, 50 mmol) isobutyl chloroformate (6.5 ml, 50 mmol) were added. After 0.5 hour at 0°C the solution was filtered and ammonia (g) was passed through the filtrate for 0.75 hour. The mixture was again filtered and then evaporated. The residue was dissolved in THF (100 ml), and triethylamine (19 ml, 140 mmol), N,N-dimethylaminopyridine (20 mg), and chlorotrimethylsilane (18 ml, 140 mmol) were added. After 1.5 hours at 20°C the solution was filtered and methanol (50 ml) added. After 0.5 hour the solution was evaporated, the residue taken up in diethyl ether (100 ml), and the resulting solution filtered and evaporated to give the title compound as an oil, 9.0 g (38%); IR ν_{max} (neat) cm^{-1} 3440, 3360, 3200, 1675, 1640 and 1615; ^{1}H NMR ($CDCl_3$) δ 0.11 (3H, s, TMS), 0.14 (6H, s, 2 × TMS), 0.91 (3H, d, CH_3-17, $J_{12,17}$ = 7.1 Hz), 1.22 (3H, d, CH_3-14, $J_{13,14}$ = 6.3 Hz), 2.18 (3H, s, CH_3-15), 5.52 (2H, br s, NH_2) and 5.70 (1H, s, H-2).

Monic Acid, N-*p*-Toluenesulphonylmethyl Amide, Tris(trimethylsilyl ether) (**13**)

Primary amide (**12**) (9.0 g, 18 mmol) was dissolved in THF (50 ml) and N,N-dimethylmethyleneammonium iodide (4.0 g, 20 mmol) was added. After 1.5 hours at 20°C, ethyl acetate was added and the solution washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated to give the dimethylaminomethyl amide.

This was dissolved in THF (50 ml) and iodomethane (1.2 ml, 38 mmol) was added. After 1 hour at 20°C the solution was evaporated to give the crude quaternary ammonium salt, which was dissolved in DMF (100 ml). Triethylamine (6.3 ml, 45 mmol) and sodium *p*-toluenesulphinate monohydrate (5.9 g, 27 mmol) were added. After 18 hours at 20°C ethyl acetate was added and the solution was washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography eluting with methanol-dichloromethane mixtures to give the title compound as an oil, 8.1 g (85%); ^1H NMR ($CDCl_3$) δ 0.12 (9H, s, TMS), 1.94 (3H, d, CH_3-14, $J_{13,14}=7.0$ Hz), 1.29 (3H, d, CH_3-17, $J_{12,17}=6.3$ Hz), 1.95 (3H, s, CH_3-15), 2.42 (3H, s, aryl-Me), 4.70 (2H, d, CH_2SO_2, $J=6.8$ Hz), 5.56 (1H, s, H-2), 6.12 (1H, t, NH, $J=6.8$ Hz), and 7.52 (4H, ABq, aryl).

General Method for the Preparation of Oxazoles (**3m**~**p**) *via* Aldehydes (**11m**~**p**)

Carbon tetrachloride (1.3 ml, 14 mmol) was added to a mixture of amide (**13**) (1.0 g, 1.4 mmol), the appropriate aldehyde (**11**) (2.8 mmol), triphenylphosphine (1.1 g, 4.2 mmol), triethylamine (0.8 ml, 5.6 mmol) and acetonitrile (10 ml). After 2 hours at 20°C ethyl acetate was added and the solution washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated to give crude oxazoline (**14**).

This was dissolved in acetonitrile (10 ml) and DBU (0.22 ml, 1.5 mmol) was added. After 2 hours at 20°C ethyl acetate was added and the solution washed with aqueous ammonium chloride, dried ($MgSO_4$), and evaporated. The residue was dissolved in a mixture of THF (30 ml) and hydrochloric acid (6 ml, 0.4 M, 2.4 mmol). After 2 minutes at 20°C aqueous sodium hydrogen carbonate and ethyl acetate were added. The organic layer was dried ($MgSO_4$) and evaporated, and the residue purified by chromatography, eluting with methanol-dichloromethane mixtures to give oxazoles (**3m**~**p**), whose properties are shown in Table 6.

2-(Normonyl)-5-[(3-2-hydroxyethyl)isoxazol-5-yl]-oxazole (**3q**)

A mixture of acetate (**3o**) (40 mg, 0.075 mmol), potassium carbonate (16 mg, 0.113 mmol) and methanol (5 ml) was stirred for 1 hour at 0°C, then ethyl acetate was added and the solution washed with aqueous sodium chloride. The organic layer was dried ($MgSO_4$) and evaporated, and the residue purified by chromatography, eluting with methanol-dichloromethane mixtures to give (**3q**) with the properties shown in Table 6.

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