Tetrahedron Vol. 44, No. 23, pp. 7283 to 7292, 1988
Printed in Great Britain.

0040-4020/88 $3.00+.00

X=Y-ZH SYSTEMS AS POTENTIAL 1,3-DIPOLES. PART 22.[1] CYCLOADDITION REACTIONS OF PYRIDOXAL IMINES. RELEVANCE TO α-AMINO ACID RACEMASES AND TRANSAMINASES.[2]

Ronald Grigg*, Sunit Thianpatanagul and (in part) James Kemp

(Chemistry Department, Queen's University, Belfast BT9 5AG, Northern Ireland)

(*Received in UK* 21 *September* 1988)

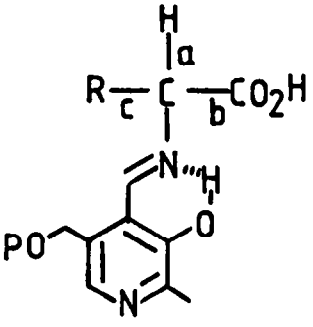
Abstract. Pyridoxal imines of α-amino acid esters and related amines undergo cycloaddition to N-phenylmaleimide on heating in acetonitrile, toluene or xylene. The cycloadditions proceed in good yield, are stereospecific, and involve an endo-transition state. The reactive intermediates are postulated to be NH azomethine ylides produced stereospecifically from the imines by prototropy.

Our general concept of 1,2-prototropy in X=Y-ZH systems[3], generating 1,3-dipoles $X=\overset{+}{Y}(H)-\overset{-}{Z}$ under thermal activation, was subsequently illustrated in a range of applications to hydrazones[4], oximes[5] and imines.[1,6] Imines, in which the ZH proton is labilised by a range of electron withdrawing groups, proved especially valuable precursors of azomethine ylides.[1,7] The success of this new concept in generating azomethine ylides led us to consider the possible relevance of such prototropy in biochemical processes effected by pyridoxal enzymes.[2,8,9] In this context labilisation of the ZH proton by ester or carboxylic acid substituents are the important cases.[9-11] We have subsequently reported full details of our work that is relevant to pyridoxal-dependant decarboxylases[9,12] and we now report full details of the reactions of pyridoxal imines of α-amino acid esters relevant to pyridoxal-dependant racemases and transaminases.

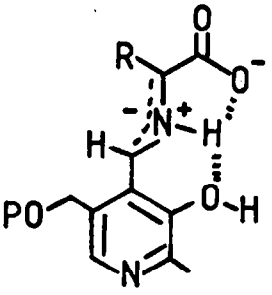
Pyridoxal phosphate-dependant enzymes effect the transamination, racemisation, α, β- and β, γ-elimination, and decarboxylation of α-amino acids *in vivo*. In these processes the key intermediate is the α-amino acid-pyridoxal imine (1).[13] Imine formation activates the aza-allylic bonds a-c in (1) to cleavage due to the facility with which the protonated pyridyl ring can delocalise a negative charge. Stereoelectronic effects dictate that the breaking bond, a, b or c, in (1), be aligned with the pyridyl azomethine π-system.[14] The rich chemistry of the enzyme bound imine derives from this activation moderated by the steric environment of the enzyme.

Transaminases and racemases activate bond a, the C-H bond, in (1) to cleavage. Removal of this proton in conjunction with hydrogen bonding involving the imine nitrogen atom, the ortho-phenolic group, and the carboxylic acid moiety, or other suitable hydrogen bonding sources at the enzyme active site would result in (2) or a related hydrogen-bonded species. Such species are azomethine ylides but until our preliminary work[2] were not recognised as such. Although it is widely believed that protonation of the pyridyl nitrogen atom plays an integral

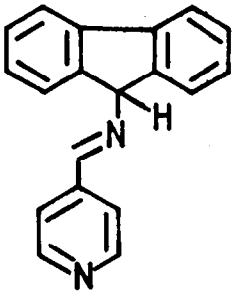
part in the activation of the aza-allylic bonds, our experience of 1,2-prototropy



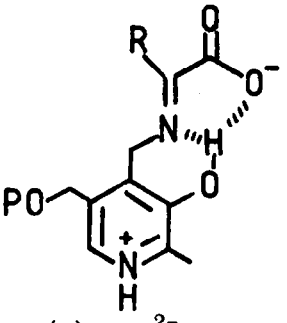
(1) $P=PO_3^{2-}$



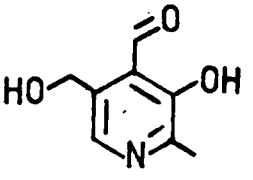
(2)



(3)



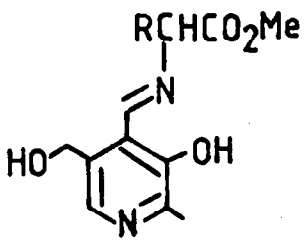
(4) $P=PO_3^{2-}$



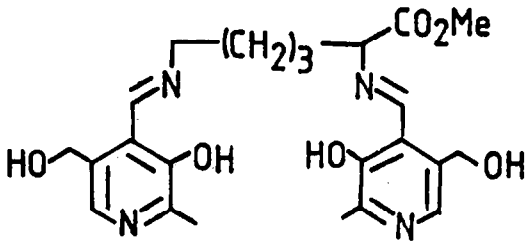
(5)

in imines of α-amino acids and their esters suggests the unprotonated ring is also capable of providing sufficient activation.[1,6,10,15] Note also that base catalysed 1,3-prototropic rearrangement of (3) at pH 7.4 does not involve prior protonation of either the azomethine or pyridine ring nitrogen atom.[16] On the other hand, hydrogen bonding between the imine nitrogen atom, and the carboxylate and phenolate oxygen atoms, will strongly activate bonds a and c in (1) to cleavage. The lability of bond a in (1) has been demonstrated in numerous in vitro studies. The prototropic shift is the slow step in aldimine(1)-ketamine(4) tautomerism and this step is accelerated by general acid-base catalysis (e.g. imidazole, imidazoline buffers).[18] Snell[19] demonstrated that exclusive transamination occurs at low pH whilst at higher pH racemisation is much more rapid than transamination. The pH maximum for transamination corresponds to the pK_a of pyridinium nitrogen, whereas the optimum pH for racemisation is one at which the pyridine nitrogen atom is not protonated. We have demonstrated related Bronsted and Lewis acid catalysis of azomethine ylide formation from aryl imines of α-amino acids and their esters and commented on similarities between the acid-base chemistry of such compounds and carbonyl compounds bearing α-CH groups.[6,10]

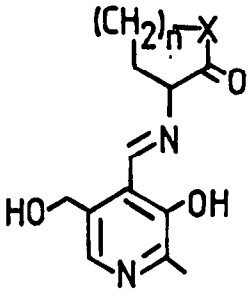
Our suggestion[2] that the intermediate (2), or a related species, involved in transamination and racemisation of α-amino acids in vivo is an azomethine ylide, was tested by experiments designed to trap the intermediate 1,3-dipole. Thus a range of imines (6)-(10) derived from pyridoxal (5) and α-amino acid esters and related compounds was prepared (Table 1).



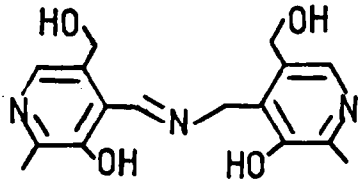
(6)



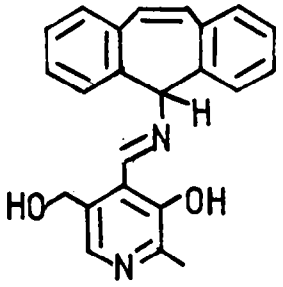
(7)



(8) a. X=S, n=1
b. X=NH, n=3



(9)



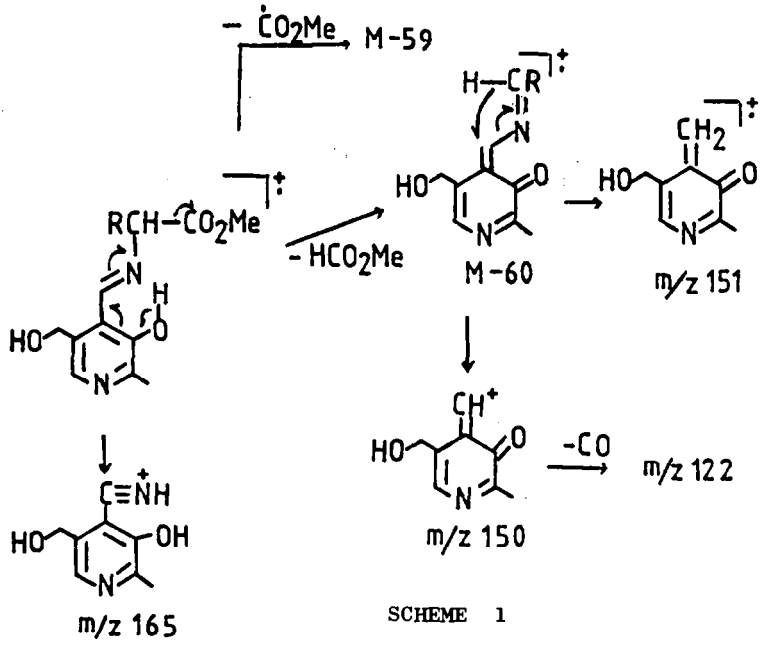
(10)

Table 1. Yields and ^1H n.m.r. Data (δ, $CDCl_3$) for Pyridoxal Imines (6)-(10)

Imine	Yield (%)	CH=N	6-H	CH_2O	CH-N	OMe	2-Me
6, R=H	30	8.91	7.94	4.74	4.51	3.80	2.49
6, R=Me[a]	43	8.97	7.83	4.73	4.33	3.70	2.47
6, R=CHPr^i	70	8.85	7.60	4.70	4.15	3.75	2.45
6, R=CH_2OH[b]	61	9.45	8.30	5.05	4.65	3.7	2.65
6, R=CH_2Ph	57	9.4	7.7	4.5	4.35	3.8	2.45
6, R=p-HOC_6H_4[a]	84	8.72	7.94	4.69	4.44	3.77	2.46
6, R=3-indolylmethyl	77	8.12	7.45	4.22	4.22	3.73	2.35
6, R=Ph	70	8.9	7.75	4.75	5.25	3.75	2.5
7[c]	68	8.84	7.58	4.64	4.05	3.77	2.32
8a	34	8.95	7.84	4.80	4.2	-	2.49
8b[d]	36	8.83	7.84	4.62	5.34	-	2.37
9[b,e]	80	9.45	8.2	5.1	4.95	-	2.6
10	38	8.95	7.9	4.65	5.7	-	2.4

a. N.m.r. determined in acetone-d_6; b. N.m.r. determined in pyridine-d_5;
c. Signals for the second pyridoxal ring occur at δ 8.79 (CH=N), 7.52 (6-H), and 2.29 (2-Me); d. N.m.r. determined in DMSO-d_6; e. Signals for the second pyridoxal ring occur at δ 8.5 (6-H), 5.3 (CH_2O), and 2.75 (2-Me).

Pyridoxal reacts with lysine methyl ester to give a mixture of the bis-imine (7) and mono-imine. Use of an excess of pyridoxal gives (7) as the sole product. Most of the pyridoxal imines exhibit a molecular ion in their mass spectra and the general fragmentation pattern of imines (6) is shown in Scheme 1.

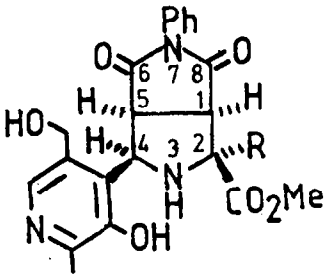


SCHEME 1

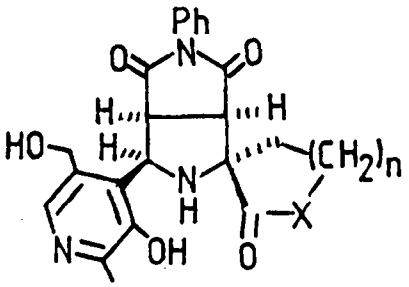
Stereospecific cycloaddition of the pyridoxal imines, (6) and (8)-(10), to N-phenylmaleimide (NPM) occurs on heating in acetonitrile, toluene or xylene to give a single stereoisomer, (11)-(14) repsectively, in each case (Table 2). These cycloadditions were completed before our studies on Bronsted and Lewis acid catalysis of such processes[15] and our subsequent development of the room temperature metal ion-triethylamine catalysed cycloadditions.[6] However, the reaction of (6,R=Ph) with NPM in acetic anhydride containing 5% acetic acid (12h,25°C) gave (75%) a 1.1:1 mixture of (1,R=Ph) and (15) indicating that these room temperature catalytic methods should be generally applicable to the imines described in this paper.

Table 2. Cycloadducts derived from the cycloaddition of imines (6) and (8-10) with NPM

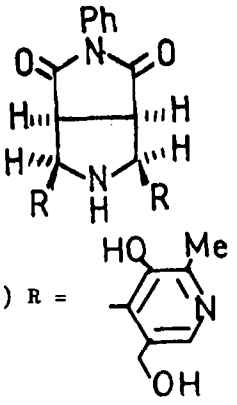
Imine	Solvent	Reaction time (h)	Temp.(°C)	Product	Yield(%)
6, R=H	xylene	6	140	11,R=H	66
6, R=Me	xylene	8	140	11,R=Me	86
6, R=CHPr^i	xylene	7	140	11,R=CHBr^i	63
6, R=CH_2OH	MeCN	12	80	11,R=CH_2OH	75
6, R=CH_2Ph	toluene	12	110	11,R=CH_2Ph	84
6, R=p-HOC_6H_4	xylene	7	140	11,R=p-HOC_6H_4	72
6, R=3-indolylmethyl	xylene	12	140	11,R=3-methylindolyl	58
6, R=Ph	xylene	3.5	140	11,R=Ph	79
8a	xylene	29	130	12a	64
8b	MeCN	60	80	12b	71
9	MeCN	20	80	13	72
10	MeCN	24	80	14	44



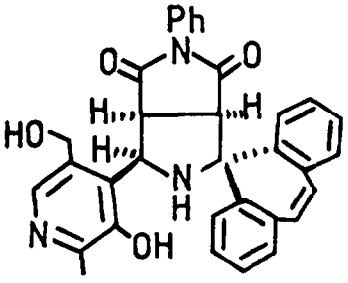
(11)



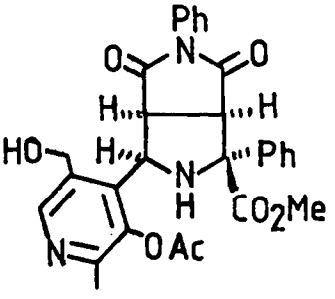
(12) a. X=S, n=1
b. X=NH, n=3



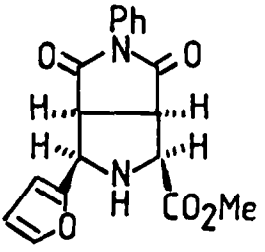
(13) R =



(14)



(15)

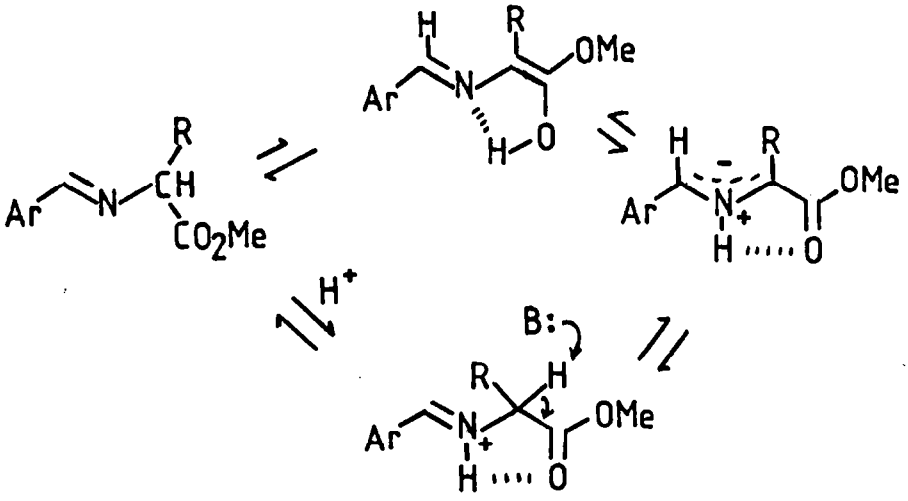


(16)

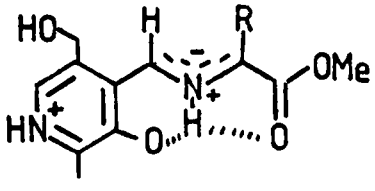
The stereochemistry of the cycloadducts (11)-(15) is based on interpretation of their ^{1}H n.m.r. spectra (Table 3), by spectral comparisons with a wide range of other NPM cycloadducts[10], and on a single crystal X-ray structure of a related cycloadduct (16).[20] Thus a comparison of coupling constants ($J_{4,5}$ 8.8-10.5Hz, $J_{1,5}$ 7.1-8.5Hz)(Table 3) with those of analogous cycloadducts[10] establishes the all-cis arrangement of the 1-,4- and 5-H atoms. Cycloadducts (11) and (12) thus arise from a dipole with configuration (17) undergoing cycloadditon via an endo-transition state. Previous extensive studies have shown that this dipole configuration is generated under kinetic control.[10,21] This is ascribed to either (i) delivery of the proton to the nitrogen atom via the ester enolate, with intramolecular hydrogen-bonding helping to maintain the configuration (a bridging water molecule may be involved in this hydrogen-bonding) or (ii) prior protonation of the imine followed by deprotonation by an external base (Scheme 2). Imine (9) gives rise to a single cycloadduct (13) whose stereochemistry identifies (18), or an analogously configured dipole, as its precursor. Both (17) and (18) offer multiple opportunities for prototropy and we have no evidence of the precise location of the labile protons. Indeed, it is likely that several prototropic species are in equilibrium in each case. Protonated Ruhemann's purple (19) is a related case for which a single crystal X-ray structure has been determined.[22] However, it is clear that the second pyridoxal group in (9) can assist dipole formation both as a proton source, and as a base, and that its hydrogen bonding capabilities favour (18) or a similar species as the kinetic dipole. The presence of an ortho-hydroxy group in the imines (6)-(10) does not in itself promote dipole formation. Indeed, studies of salicylidene imines show the ortho-hydroxy group dramatically inhibits dipole formation.[15] This inhibition is ascribed to the

well documented[23] strong intramolecular hydrogen bond in such imines, or their existence in the N-protonated form[24], rendering the imino-nitrogen atom unavailable for functioning as a base (Scheme 2). In the case of pyridoxal imines the pyridine nitrogen atom can assume this function.

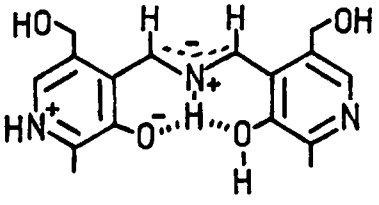
Another noteworthy feature of the cycloaddition of pyridoxal imines (5) and (8)-(10) to NPM is that the reactions proceed in good to excellent yield despite the array of potentially interferring nucleophilic substituents on the pyridoxal. The presence of the 3-hydroxypyridine moiety also creates a potential site for an



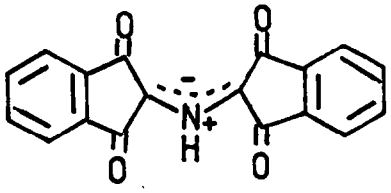
SCHEME 2



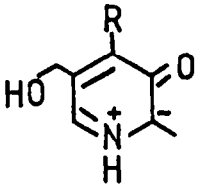
(17)



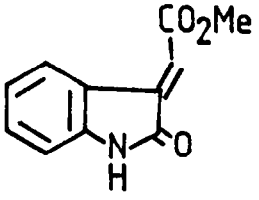
(18)



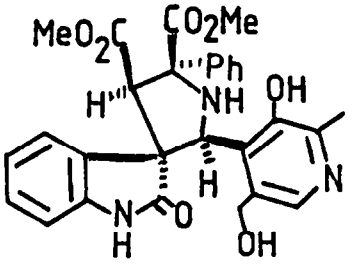
(19)



(20)



(21)



(22)

alternative 1,3-dipolar cycloaddition involving the pyridine ring[25], i.e. (20). We do not detect any products arising from such processes. The cycloaddition across the aldimine system is clearly favoured over cycloaddition to the incipient pyridium betaine with its attendant loss of aromaticity.

Table 3. Chemical shifts (δ) and coupling constants (pyridine-d_5) for cycloadducts (11) - (15)

Cycloadduct	4-H[a]	5-H	1-H	$J_{1,5}$(Hz)	$J_{4,5}$(Hz)
11, R=H[b]	5.73	4.41	4.26	8.1	9.2
11, R=Me	5.96	4.58	3.96	8.2	9.7
11, R=CHPr^i	5.88	4.61	3.97	8.1	9.6
11, R=CH_2OH	5.98	4.62	4.02	8.5	9.9
11, R=CH_2Ph[c]	5.35	4.2	3.7	8.0	9.9
11, R=p-HOC_6H_4	6.2	4.6	4.17	8.0	10.0
11, R=3-indolylmethyl	6.21	4.77	4.39	8.1	9.9
11, R=Ph	5.56	4.56	4.91	7.7	9.9
12a	5.90	4.66	4.12	8.1	8.8
12b	5.90	4.50	4.40	8.5	9.9
13[d]	5.66	4.34	-	-	6.2
14	5.54	3.69	4.33	7.3	10.5
15	5.24	4.47	5.04	7.8	9.8

a. For numbering scheme see formula (11); b. 2-H occurs at δ 4.64 ($J_{1,2}$ 7.7Hz); c. In $CDCl_3$; d. The molecule is symmetrical, 2-H≡4-H and 1-H≡5-H.

One other dipolarophile (21) was briefly investigated with imine (6, R=Ph) and found to react slowly (xylene, 130°C, 5d) to give (22)(49%).

The trapping, via cycloadduct formation, of a thermally generated intermediate arising from pyridoxal imines by prototropic processes constitutes, we believe, good evidence for formulating the intermediate as an N-protonated azomethine ylide (17) or (18) and, by analogy, implicates related species in the biochemical processes mediated by racemases and transaminases.

Experimental. General experimental details were as previously noted.[15] Petroleum ether refers to the fraction with b.p. 40-60°C.

General Method for the Preparation of Pyridoxal Imines. The amino acid methyl ester hydrochloride (1mol) and pyridoxal hydrochloride (1mol) were mixed and dissolved in 1N potassium hydroxide (2mol) with stirring, giving a bright yellow solution together with an insoluble yellow oil. The mixture was stirred at room temperature for 30 min. and then extracted with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure to leave a yellow oil which crystallised on standing. The imines were further recrystallised from an appropriate solvent as noted below. Yields together with most of the ^1H n.m.r. data are collected in Table 1.

N-Pyridoxylideneglycine methyl ester (6, R=H). Yellow prisms from methanol, m.p. 112-114°C (Found: C, 54.75; H, 6.05; N, 10.95. $C_{11}H_{14}N_2O_4.0.5$ MeOH requires C, 54.35; H, 6.35; N, 11.00%); $\nu_{max.}$ 3100, 1745 and 1635 cm^{-1}; m/z(%) 238 (M^+, 29), 179(37), 165(31), 150(41) and 149(100); δ 4.51 (s,2H,CH_2CO_2Me).

N-Pyridoxylidenealanine methyl ester (6, R=Me). Yellow rods from acetone-petroleum ether, m.p. 118-120°C (Found: C, 57.25; H, 6.30; N, 10.95. $C_{12}H_{16}N_2O_4$ requires C, 57.15; H, 6.40; N, 11.10%); $\nu_{max.}$ 3205, 1745 and 1625 cm^{-1}; m/z(%) 252 (M^+,48), 193(42), 165(100), 150(67), and 149(74); δ (acetone-d_6), 4.33 (q,1H,CHMe), and 1.5 (d,3H,CHMe).

N-Pyridoxylideneleucine methyl ester (6, R=CHPr^i). Obtained as a yellow viscous oil which decomposed on attempted distillation and hydrolysed on attempted preparative t.l.c. (Found: C, 54.75; H, 7.75. $C_{15}H_{22}N_2O_4.2H_2O$ requires C, 54.55; H, 7.95%); $\nu_{max.}$(film) 1740 and 1625 cm^{-1}; m/z(%) 294 (M^+,59),

235(5), 212(17), 165(21), 149(12) and 86(100); δ 1.6 (m,1H,CHMe$_2$) and 1.92 (d,6H,CHMe$_2$).

N-Pyridoxylideneserine methyl ester (6, R=CH$_2$OH). Yellow rods from methanol, m.p. 136°C (Found: C, 53.50; H, 5.90; N, 10.20. $C_{12}H_{16}N_2O_4$ requires C, 53.70; H, 6.00; N, 10.45%); ν_{max} 3350, 3100, 1735 and 1620 cm^{-1}; m/z(%) 268 (M$^+$,28), 238(7), 209(8), 165(60), 151(10), 150(65) and 149(100); δ (pyridine-d$_5$) 4.65 (m,1H,CHCH$_2$OH) and 4.35 (m,2H,CHCH$_2$OH).

N-Pyridoxylidenephenylalanine methyl ester (6, R=CH$_2$Ph). Obtained as a yellow powder from ether-petroleum ether, m.p. 52-54°C (Found: C, 65.35; H, 6.55; N, 8.20. $C_{18}H_{20}N_2O_4$ requires C, 65.85; H, 6.15; N, 8.55%); ν_{max} (nujol) 1740 and 1625 cm^{-1}; m/z(%) 328 (M$^+$,4), 269(1), 237(2), 120(53) and 88(100); δ 6.7 (dd,2H,ArCH$_2$).

Pyridoxylidenetyrosine methyl ester (6, R=p-HOC$_6$H$_4$). Yellow rods from acetone-petroleum ether, m.p. 140-142°C (Found: C, 62.90; H, 5.75; N, 7.80. $C_{18}H_{20}N_2O_5$ requires C, 62.80; H, 5.85; N, 8.15%); ν_{max} 3200, 1745 and 1635 cm^{-1}; m/z(%) 344 (M$^+$,1), 237(1), 165(2), 136(26), 108(32), 107(100), 91(17) and 77(22); δ (acetone-d$_6$) 3.29 and 3.16 (2xq, 2x1H, ArCH$_2$).

N-Pyridoxylidenetryptophan methyl ester (6, R=3-methylindolyl). Obtained as a yellow froth which resisted attempts at crystallisation (Found: C, 59.20; H, 5.85; N, 10.30. $C_{20}H_{21}N_3O_4.2H_2O$ requires C, 59.55; H, 6.25; N, 10.40%); ν_{max} 3400, 1730 and 1625 cm^{-1}; m/z(%) 367 (M$^+$,1), 218(33), 159(19), 131(57) and 130(100); δ 9.13 (s,1H, indole NH) and 3.41 (m,2H,CHCH$_2$).

N-Pyridoxylidenephenylglycine methyl ester (6, R=Ph). Yellow needles from methanol, m.p. 131-134°C (Found: C, 62.80; H, 5.80; N, 8.35. $C_{17}H_{18}N_2O_4.0.5H_2O$ requires C, 63.15; H, 5.90; N, 8.65%); ν_{max} 3210, 1735 and 1620 cm^{-1}.

N,N'-Di(pyridoxylidene)lysine methyl ester (7). Obtained as a yellow froth which resisted attempted crystallisation. (Found: C, 53.90; H, 7.00; N, 10.95. $C_{23}H_{30}N_4O_6.3H_2O$ requires C, 53.90; H, 7.10; N, 10.95%); ν_{max} 3200, 1735 and 1625 cm^{-1}; m/z(%) 399 (M-CO$_2$Me,1) and 151(100); δ 2.17-1.18 (m,6H,3xCH$_2$).

N-Pyridoxylidene-3-aminotetrahydrothiophene-2-one (8a). Yellow rods from acetonitrile, m.p. 140-142°C (Found: C, 53.95; H, 5.30; N, 10.55. $C_{12}H_{14}N_2O_3S$ requires C, 54.15; H, 5.30; N, 10.50%); ν_{max} 3210, 1685 and 1615 cm^{-1}; m/z(%) 266 (M$^+$,3), 150(4), 83(100) and 47(CH$_2$=SH,16); δ 4.20 (q,1H,CHCOS), 3.4 (m,2H,CH$_2$S) and 2.5 and 2.6 (2xm, 2x2H, ring CH$_2$).

N-Pyridoxylidene-ε-caprolactam (8b). Prepared by the general method but with the 1N potassium hydroxide solution (1mol) being added to a solution of pyridoxal hydrochloride and ε-caprolactam in ethanol. The product crystallised from the reaction mixture as yellow rods, m.p. 224-226°C (decomp.) (Found: C, 60.65; H, 6.90; N, 15.15. $C_{14}H_{19}N_3O_3$ requires C, 60.10; H, 6.75; N, 14.90%); ν_{max} 3360, 3160, 1665 and 1625 cm^{-1}; m/z(%) 277 (M$^+$,7), 165(9) and 149(100); δ (DMSO-d$_6$) 5.34 (t,1H,CHCON), 3.14 (m,2H,CH$_2$N) and 2.0-1.4 (m,6H,3xCH$_2$).

N-Pyridoxylidenepyridoxylamine (9). Prepared by the general method from pyridoxal hydrochloride and pyridoxylamine dihydrochloride but using 1N potassium hydroxide (3mol). The product crystallised from the reaction mixture as yellow needles, m.p. 235°C (decomp.)(Found: C, 60.65; H, 5.80; N, 13.10. $C_{16}H_{19}N_3O_4$ requires C, 60.55; H, 6.05; N, 13.25%); ν_{max} 3350, 3150 and 1625 cm^{-1}; m/z(%) 317 (M$^+$,1), 152(10), 151(89) and 149(100).

N-Pyridoxylidene-5-amino-5H-dibenzo[a,d]cycloheptene (10). Prepared by the general method from pyridoxal hydrochloride and 5-amino-5H-dibenzo[a,d]cycloheptene but with addition of 1N potassium hydroxide (1mol). The product crystallised from methanol as yellow rods, m.p. 203-205°C(decomp.)(Found: C, 73.45; H, 5.90; N, 7.25. $C_{23}H_{20}N_2O_2.H_2O$ requires C, 73.80; H, 5.90; N, 7.50%); ν_{max} 1622 cm^{-1}; m/z(%) 356 (M$^+$,1), 207(100), 191(94) and 165(15).

General Procedure for the Cycloaddition of Pyridoxal Imines and NPM. A solution of the pyridoxal imine (10mmol) and NPM (10mmol) in boiling xylene (140°C), toluene (110°C) or acetonitrile (80°C)(60ml) was heated for the time noted in Table 2. The solvent was then removed under reduced pressure and the residue crystallised from methanol. Yields are noted in Table 2 and chemical shifts and coupling constants of the pyrrolidine ring protons are collected in Table 3.

Methyl 4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=H). Colourless rods, m.p. 227-230°C (decomp.)(Found: C, 60.95; H, 5.50; N, 10.25. $C_{21}H_{21}N_3O_6$ requires C, 61.30; H, 5.15; N, 10.20%); ν_{max} 3290, 1735 and 1705; m/z(%) 411 (M$^+$,3), 238(12), 173(93) and 149(100); δ (pyridine-d$_5$) 8.83 (s,1H,PyH), 7.42 (m,5H,ArH), 4.98 (q,2H,CH$_2$O), 3.84 (s,3H,OMe) and 2.6 (s,3H,PyMe).

Methyl 2-methyl-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=Me). Colourless rods, m.p. 232-234°C (decomp.)(Found: C, 61.90; H, 5.45; N, 9.85. $C_{22}H_{23}N_3O_6$ requires C, 62.15; H, 5.45; N, 9.90%); ν_{max} 3300, 1735 and 1705 cm^{-1}; m/z(%) 424 (M$^+$,72) 366(12) 252(20), 175(100) and 173(29); δ (pyridine-d$_5$) 8.26 (s,1H,PyH), 7.39 (m,5H,ArH), 5.03 (q,2H,CH$_2$O), 3.83 (s,3H,OMe), 2.67 (s,3H,PyMe) and 1.9 (s,3H,Me).

Methyl 2-sec-butyl-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=CHPr^i). Colourless rods, m.p. 248-250°C (decomp.)(Found: C, 63.90; H, 6.30; N, 8.90. $C_{25}H_{29}N_3O_6$ requires C, 64.20; H, 6.25; N, 9.00%); ν_{max} 3320, 1730 and 1710 cm^{-1}; m/z(%) 467 (M$^+$,100), 408(16), 390(50), 294(18) and 173(32); δ

(pyridine-d_5) 8.27 (s,1H,PyH), 7.40 (m,5H,ArH), 5.5 (br s, 1H,NH), 5.06 (q,2H,CH_2O), 3.93 (s,3H,OMe), 2.60 (s,3H,PyMe), 2.14 (m,3H,$\underline{CH_2CH}Me$) and 0.99 (2xd, 2x3H, $CHMe_2$).

Methyl 2-hydroxymethyl-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=CH_2OH). Colourless rods, m.p. 232-234°C (decomp.)(Found: C, 59.50; H, 5.15; N, 9.80. $C_{22}H_{23}N_3O_7$ requires C, 59.85; H, 5.25; N, 9.50%); ν_{max} 3500,3300,1730 and 1705 cm^{-1}; δ 8.28 (s,1H,PyH), 7.41 (m,5H,ArH), 4.92 (q,2H,$PyCH_2O$), 4.72 and 4.30 (2xd, 2H, CH_2O), 3.78 (s,3H,OMe) and 2.69 (s,3H,PyMe).

Methyl 2-benzyl-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=CH_2Ph). Colourless prisms from methylene chloride-ether, m.p. 180-183°C (Found: C, 67.25; H, 5.30; N, 8.15. $C_{28}H_{27}N_3O_6$ requires C, 67.05; H, 5.45; N, 8.40%); ν_{max} (nujol) 3315 and 1720 cm^{-1}; δ 7.85 (s,1H,PyH), 7.25 (m,10H,ArH), 4.7 (s,2H,CH_2O), 3.90 (s,3H,OMe), 3.0 and 2.8 (2xd, 2x1H, $ArCH_2$) and 7.6 (s,3H,PyMe).

Methyl 2-(4'-hydroxyphenyl)-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=p-HOC_6H_4). Colourless rods, m.p. 242-244°C (decomp.)(Found: C, 64.75; H, 5.45; N, 8.00. $C_{28}H_{27}N_3O_7$ requires C, 65.00; H, 5.25; N, 8.10%); ν_{max} 3420, 3300, 1750 and 1705 cm^{-1}; m/z(%) 517 (M^+,1), 457(2), 410(8), 344(6), 173(96) and 107(100); δ (pyridine-d_5) 8.23 (s,1H,PyH), 7.25 (m,5H,ArH), 5.1 (s,2H,CH_2O), 3.93 (s,3H,OMe), 3.87 and 3.37 (2xd, 2x1H, $ArCH_2$) and 2.57 (s,3H,PyMe).

Methyl 2-(3'-indolylmethyl)-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=3-indolylmethyl) Colourless rods, m.p. 254-256°C (decomp.)(Found: C, 65.00; H, 5.30; N, 10.10. $C_{30}H_{28}N_4O_6$.MeOH requires C, 65.00; H, 5.65; N, 9.80%); ν_{max} 3300, 1725 and 1705 cm^{-}; m/z(%) 368(100) and 367(21); δ (pyridine-d_5) 8.24 (s,1H,PyH), 8.22-7.27 (m,10H,ArH), 5.1 (q,2H,CH_2O), 4.11 and 3.36 (2xd, 2x1H, indolyl-CH_2), 3.92 (s,3H,OMe) and 2.5 (s,3H,PyMe).

Methyl 2,7-diphenyl-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (11, R=Ph). Colourless rods, m.p. 275-276°C (decomp.)(Found: C, 66.35; H, 5.20; N, 8.50. $C_{27}H_{25}N_3O_6$ requires C, 66.50; H, 5.15; N, 8.60%); ν_{max} 3300, 1720 and 1705 cm^{-1}; m/z(%) 487 (M^+,27), 428(10), 410(33), 314(14) and 173(100); δ (pyridine-d_5) 8.21 (s,1H,PyH), 8.08-7.21 (m,10H,ArH), 4.77 (q,2H,CH_2O), 3.83 (s,3H,OMe) and 2.62 (s,3H,PyMe).

2,2-Spiro(3',3'-tetrahydro-2-oxo-thienyl)-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (12a). Colourless needles, m.p. 257-258°C (decomp.)(Found: C, 59.95; H, 4.75; N, 9.45. $C_{22}H_{21}N_3O_5S$ requires C, 60.15; H, 4.80; N, 9.55%); ν_{max} 3385, 3110 and 1710 cm^{-1}; m/z(%) 439 (M^+,2), 173(43) and 149(100); δ (pyridine-d_5) 8.22 (s,1H,PyH), 7.44 (m,5H,ArH), 4.99 (s,2H,CH_2O), 3.65 and 3.35 (2xm, 2x1H, CH_2S), 2.95 and 2.44 (2xm, 2x1H, $\underline{CH_2}CH_2S$) and 2.61 (s,3H,PyMe).

2,2-Spiro(3',3'-hexahydro-2-oxo-azepinyl)-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (12b). Colourless needles, m.p. 277-279°C (decomp.)(Found: C, 59.25; H, 5.90; N, 11.25. $C_{24}H_{26}N_4O_5.2H_2O$ requires C, 59.25; H, 6.20; N, 11.5%); ν_{max} 3480, 3250 and 1710 cm^{-1}; δ (pyridine-d_5) 8.27 (s,1H,PyH), 7.42 (m,5H,ArH), 5.06 (s,2H,CH_2O), 3.8 and 3.4 (2xm, 2x1H, CH_2N), 2.64 (s,3H,PyMe) and 2.4-1.4 (m,6H,3xCH_2).

2,4-Bis(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (13). Colourless needles from acetonitrile, m.p. 225-227°C (decomp.)(Found: C, 57.15; H, 5.40; N, 10.05. $C_{26}H_{26}N_4O_6.3H_2O$ requires C, 57.34; H, 5.90; N, 10.30%); ν_{max} 3200 and 1705 cm^{-1}; m/z(%) 322(3), 149(19), 93(100) and 77(26); δ (pyridine-d_5) 8.36 (s,2H,PyH), 7.36 (m,5H,ArH), 5.06 (s,4H,2xCH_2O) and 2.69 (s,6H,2xPyMe).

2,2-Spiro(5',5'-dibenzo[a,d]cycloheptyl)-4-(3'-hydroxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14). Yellow prisms, m.p. 275-276°C (decomp.)(Found: C, 70.40; H, 5.80; N, 7.05. $C_{33}H_{27}N_3O_4.2H_2O$ requires C, 70.05; H, 5.50; N, 7.45%); ν_{max} 3275 and 1705 cm^{-1}; m/z(%) 529 (M^+,2), 356(6) and 191(100); δ (pyridine-d_5) 7.93 (s,1H,PyH), 7.40 (m,13H,ArH), 6.96 (2xd, 2x1H, CH=CH), 4.6 (q,2H,CH_2O) and 2.46 (s,3H,PyMe).

Methyl 2,7-diphenyl-4-(3'-acetoxy-5'-hydroxymethyl-2'-methyl-4'-pyridyl)-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (15). A solution of methyl N-pyridoxylidenephenylglycinate (314mg, 1mmol) and NPM (180mg, 1mmol) in acetic anhydride containing 5% acetic acid (3ml) was kept at room temperature for 12h. A pale yellow solid began to precipitate after ca.20min. The solid (200mg, 41%) was removed by filtration and found to be identical with (11, R=Ph)(above). The filtrate was evaporated to dryness and partitioned between ethyl acetate and 5% aqueous sodium carbonate solution. The organic layer was separated, washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was triturated with ether to afford a colourless solid which, on crystallisation from methanol, afforded the product (180mg, 34%) as colourless needles, m.p. 244-246°C (decomp.)(Found: C, 65.45; H, 5.20; N, 8.15. $C_{29}H_{27}N_3O_7$ requires C, 65.75; H, 5.15; N, 7.95%); ν_{max} 3300, 1730 and 1710 cm^{-1}; m/z(%) 529 (M^+,19); δ (pyridine-d_5) 8.3 (s,1H,PyH), 8.10-7.3 (m,10H,ArH), 6.5 (br s, 1H,NH), 5.24 (d,1H, J 9.8Hz, 4-H), 5.04 (d,1H, J 7.8Hz, 1-H), 4.99 (s,2H,CH_2O), 4.47 (dd,1H,5-H), 3.85 (s,3H,OMe), 2.59 (s,3H,PyMe) and 1.87 (s,3H,OCOMe).

Cycloaddition of methyl N-pyridoxylidenephenylglycinate and methyl 3-isatylidene acetic acid. A solution of methyl N-pyridoxylidenephenylglycinate (100mg, 0.32mmol) and methyl 3-isatylidene acetic acid (70mg, 0.40mmol) in dry xylene (5ml) was heated at 130°C for 5d. The solvent was then removed under reduced pressure and the residue triturated with benzene. The resulting solid was crystallised from methanol to afford the cycloadduct (22)(80mg,49%), as colourless needles, m.p. 212°C (decomp.) (Found: C, 65.00; H, 5.25; N, 8.10. $C_{28}H_{27}N_3O_7$ requires C, 65.00; H, 5.40; N, 8.10%); ν_{max} 3300, 1730 and 1610 cm^{-1}; m/z(%) 314(10), 255(5), 203(56) and 165(40); δ(pyridine-d_5) 8.12 (s,1H,PyH), 8.1-6.6 (m,9H,ArH), 6.16 (d,1H,5-H, J 5.1Hz, coupled to NH), 5.06 (s,1H,3-H), 4.97 (br s,1H,NH), 4.55 (q,2H,CH_2O), 3.9 and 3.2 (2xs, 2x3H, OMe) and 2.8 (s,3H,PyMe).

We thank Queen's University for support.

References

1. Part 21. R. Grigg, G. Donegan, D. Kennedy, H.Q.N. Gunaratne, J.F. Malone, V. Sridharan & S. Thianpatanagul, Tetrahedron, in press.
2. Preliminary communication: R. Grigg & J. Kemp, Tetrahedron Letters, 1978, 2823.
3. R. Grigg, J. Kemp & N. Thompson, Tetrahedron Letters, 1978, 2827.
4. R. Grigg, M. Dowling, M.W. Jordan, J. Kemp, V. Sridharan & S. Thianpatanagul, Tetrahedron, 1987, 43, 5873.
5. R. Grigg & S. Thianpatanagul, J.Chem.Soc.,Perkin Trans.1, 1984, 653; see also P. Armstrong, R. Grigg & W.J. Warnock, J.Chem.Soc.,Chem.Commun., 1987, 1325; P. Armstrong, R. Grigg, S. Surendrakumar & W.J. Warnock, ibid, 1987, 1327; R. Grigg, M.R.J. Dorrity, F. Heaney, J.F. Malone, S. Rajviroongit, V. Sridharan & S. Surendrakumar, Tetrahedron Letters, 1988, 29, 4323.
6. R. Grigg, Chem.Soc.Rev., 1987, 16, 89; D.A. Barr, R. Grigg, H.Q.N. Gunaratne, J. Kemp, P. McMeekin & V. Sridharan, Tetrahedron, 1988, 44, 557.
7. M. Joucla & J. Hamelin, Tetrahedron Letters, 1978, 2885; O. Tsuge, K. Ueno, S. Kanemesa & K. Yovozu, Bull.Chem.Soc.Jpn., 1986, 59, 1809.
8. P. Armstrong, D.T. Elmore, R. Grigg & C.H. Williams, Biochem.Soc.Trans., 1986, 404.
9. M.F. Aly, R. Grigg, S. Thianpatanagul & V. Sridharan, J.Chem.Soc., Perkin Trans. 1, 1988, 949.
10. K. Amornraksa, R. Grigg, H.Q.N. Gunaratne, J. Kemp & V. Sridharan, J. Chem. Soc., Perkin Trans.1, 1987, 2285.
11. M. Joucla, J. Mortier & J. Hamelin, Tetrahedron Letters, 1985, 26, 2775; A. Mkairi & J. Hamelin, ibid, 1987, 28, 1397; O. Tsuge, S. Kanemasa, M. Ohe, K. Yorozu, S. Tekenaka & K. Ueno, Bull.Chem.Soc.Jpn., 1987, 60, 4067; idem, Chem.Letters, 1986, 1271.
12. R. Grigg, S. Surendrakumar, S. Thianpatanagul & D. Vipond, J. Chem. Soc., Perkin Trans.1, 1988, 2693; R. Grigg, J. Idle, P. McMeekin, S. Surendrakumar & D. Vipond, Ibid, 1988, 2703; H. Ardill, R. Grigg, V. Sridharan & S. Surendrakumar, Tetrahedron, 1988, 44, 4953; H. Ardill, R. Grigg, V. Sridharan & J.F. Malone, J.Chem.Soc.,Chem.Commun., 1987, 1296.
13. E.E. Snell, P.M. Fasella, A. Braunstein & A.R. Fanelli, eds., "Chemical and Biological Aspects of Pyridoxal Catalysis", McMillan Co., New York, 1963; J.C. Vederas & H.G. Floss, Acc.Chem.Res., 1980, 13, 455.
14. H.C. Dunathan, Proc.Natl.Acad.Sci, U.S.A., 1966, 55, 712; J.R. Fischer & E.H. Abbot, J.Am.Chem.Soc., 1979, 101, 2781.
15. R. Grigg, H.Q.N. Gunaratne & V. Sridharan, Tetrahedron, 1987, 43, 5887; R. Grigg, H.Q.N. Gunaratne & J. Kemp, J.Chem.Soc.,Perkin Trans.1, 1984, 41.
16. N.M. Vernon & R.A. More O'Ferrall, Bull.Soc.Chim.Belg., 1982, 91, 403.
17. A.E. Martell, Adv.Enzymol., 1982, 53, 163.
18. C. Walsh, "Enzymatic Reaction Mechanisms", Freeman, San Francisco, 1979,, pp. 777 et seq.
19. J. Olivard, D.E. Metzler & E.E. Snell, J. Biol. Chem., 1952, 198, 353.
20. R. Grigg, J. Kemp, G. Sheldrick & J. Trotter, J.Chem.Soc.,Chem.Commun., 1978, 109.
21. R. Grigg, M.W. Jordan, J.F. Malone & P. Armstrong, Tetrahedron, 1985, 41, 3547.
22. R. Grigg, J.F. Malone, T. Mongkolaussavaratana & S. Thianpatanagul, J. Chem. Soc., Chem. Commun., 1986, 421.
23. J.W. Ledbetter, J.Phys.Chem., 1967, 71, 2351; 1968, 72, 4111; 1982, 86, 2449.
24. G.O. Dudek & R.H. Holm, J.Am.Chem.Soc., 1961, 83, 2099, 3914; D. Heinert & A.E. Martell, ibid, 1962, 84, 3257; G.O. Dudek & E.P. Dudek, ibid, 1966, 88, 2047; idem, Tetrahedron, 1967, 23, 3245.
25. N. Dennis, A.R. Katritzky & Y. Takeuchi, Angew. Chem. Internat. Edn. Engl., 1976, 15, 1.