Synthesis of Pivaloyloxymethyl 6-N'-Cyanoamidinopenicillanates and the Antibacterial Activity of the Corresponding Acids

Hans Jørgen Petersen†

Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark. *Received May 31, 1973*

A series of pivaloyloxymethyl esters of 6-N'-cyanoamidinopenicillanic acids was prepared in order to study the effect on antibacterial activity of substituting the 6-carboxamido residue of penicillins by the potentially bioisosteric cyanoamidino group. For testing the pivaloyloxymethyl esters were enzymatically hydrolyzed to the corresponding acids which showed *in vitro* antibacterial properties generally restricted to activity against gram-positive organisms. The 6-N'-cyanoamidinopenicillanic acids exhibited activity at comparable levels to structurally analogous penicillins.

Recently it was shown that the substitution of an amidino function for the 6-carboxamido residue of penicillins can produce potent antibacterials. In particular the activity peak of the antibacterial spectra of N'-disubstituted 6-formamidinopenicillanic acids was markedly displaced toward the gram-negative end.[1] Our attention was focused on the N-cyanoamidino group [RC(=NCN)NH-] as an alternative type of 6-substituent of penicillanic acid, since it might have the quality of furnishing penicillin bioisosteres. The cyanoimino fraction could constitute the equivalent of carbonyl oxygen in the broad definition of bioisosterism[2] in combination with the retention of the structural elements of the 6-amidinopenicillanic acids. Related 6-N'-hydroxyamidinopenicillanic acids have been reported to be active against gram-positive bacteria.[3]

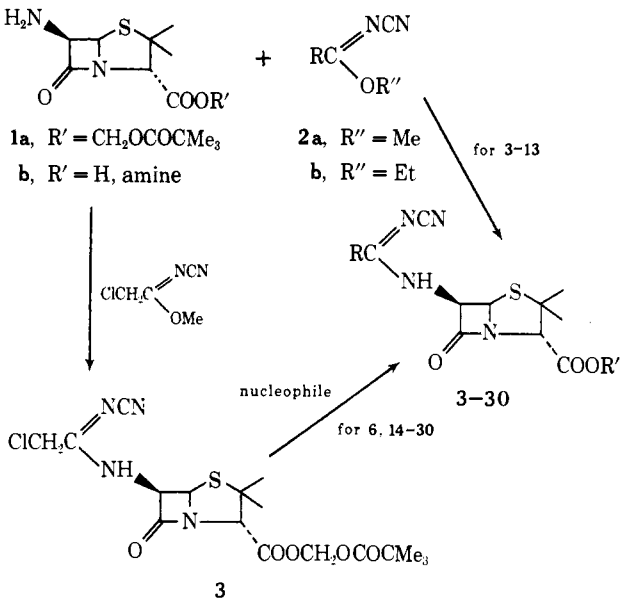
The strategy for the synthesis of the compounds of this study was based on the interaction of N-cyanoimidates **2a,b** with the amino group of 6-aminopenicillanic acid. It was appreciated that the unprotected 3-carboxyl group might cause adverse side reactions. A transient blocking could be achieved by the use of pivaloyloxymethyl esters. Acyloxymethyl esters have been reported to undergo facile enzymatic hydrolysis to the corresponding acids by nonspecific serum esterases.[1,4-6] With the readily available pivaloyloxymethyl 6-aminopenicillanate (**1a**)[4] as a convenient starting material the title compounds would be suitable for an *in vitro* screening program after serum hydrolysis to 6-N'-cyanoamidinopenicillanic acids. Furthermore, the esters were expected to be useful for *in vivo* absorption studies. Improved oral absorption of pivaloyloxymethyl esters of other substituted 6-aminopenicillanic acids has been demonstrated.[4,7]

Some results of the *in vitro* antibacterial testing after mouse serum hydrolysis were compared with those of structurally analogous penicillins under identical conditions. A few pivaloyloxymethyl 6-N'-cyanoamidinopenicillanates, after serum ester hydrolysis, were matched with the direct results for the corresponding acids, eventually obtained as diethylamine salts.

Chemistry. Cyanamide with the requisite orthoester[8] or the imidate[9] gave the N-cyanoimidates **2a,b**. Reaction of **2a,b** with pivaloyloxymethyl 6-aminopenicillanate (**1a**)[4] constituted the basic route to the title esters, as outlined in Scheme I.

The 6-N'-cyano-α-chloroacetamidinopenicillanic ester **3** was transformed into compounds **6** and **14-30** by nucleophilic displacement. Nucleophilic reagents included iodide, azide, thiosulfate, *p*-toluenesulfinate, and thiols, giving **14-22**. Tertiary N-heterocycles, bearing no ortho substituents, reacted with **3** satisfactorily, giving the quarternary compounds **23-30**, whereas N-alkylation by the pivaloyloxymethyl ester of α-chloromethylpenicillin under like conditions did not occur. **3** with trimethyl phosphite gave **6** by a Michaelis-Arbusov type of reaction. **6** was also obtained in lower yield from **1a** and **2b**.

Scheme I



Compounds **5**, **9**, and **11** could be prepared by the action of **2a** on a tertiary amine salt of 6-aminopenicillanic acid. The products were isolated as crystalline diethylamine salts. Attempts to apply this method to the preparation of other penicillanic acids, including the free acid of **3**, were unsuccessful.

The compounds **3-30** could in principle exist in tautomeric forms [RC(=NCN)NHCH< ⇌ RC(NHCN)=NCH<]. The nmr spectra of pivaloyloxymethyl esters in $CDCl_3$, excluding **23-30**, which had to be run in H- and D-exchanging solvents, showed coupling between the two protons of the 6-(NHCH<) group, thus supporting the tautomeric equivalence with penicillins as proposed (Scheme I). Conversely, the diethylamine salt **5** in D_2O clearly demonstrated long-range coupling between the formyl H and 6-H, establishing the tautomeric structure *H*C(NHCN)=NC*H*<. Likewise, the diethylamine salt **11** in $(CD_3)_2SO$ gave no indication of 6-(NHCH<) coupling. Clearly these observations of tautomers in esters and salts would not justify any conclusions as to the relative tautomer concentrations for the derived N'-cyanoamidinopenicillanic acids under antibacterial screening conditions, after enzymatic ester hydrolysis.

The possibility that the recorded antibacterial activities of compounds **3-30** are due to their hydrolysis to penicillins (that is R-C(=NCN)-NH- → R-C(=O)-NH-) was excluded by their chemical stability and the recognition that enzymatic hydrolysis of the pivaloyloxymethyl esters **4** and **10** yielded compounds which were chromatographi-

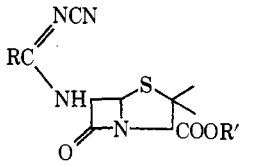
† This paper is dedicated to Professor Alfred Burger, with whom I had the pleasure to be associated at the Cobb Chemical Laboratory, University of Virginia, during 1962-1963.

Table I. *In Vitro* Antibacterial Activity[a] of **3–30** and Some Related Penicillins; IC_{50} Concentration in μg/ml[b]

Compd	*Staph. aureus* LeoCJ	*Staph. aureus* LeoCJ9[c]	*Staph. aureus* LeoCJ110[c]	*D. pneumoniae* LeoEA[d]	*Strept. pyogenes* NCTC 6175	*L. monocytogenes* NCTC 7973	*E. coli* LeoHA	*K. pneumoniae* LeoHE	*P. vulgaris* NCTC 4175A	*Sal. typhosa* NCTC 5760	*Shig. dysenteriae* NCTC 8217
5	3.2	50	50	0.40	0.13	1.6	13	5.0	10	4.0	5.0
4	2.5	80	63	0.80	0.16	1.6	13	6.3	8.0	5.0	10
Pivaloyloxymethyl 6-formamidopenicillanate[e]	2.0	>100	>100	0.50	0.40	4.0	16	13	13	13	16
6	0.63	>100	>100	0.13	0.13	0.40	80	13	>100	13	>100
8	0.063	>100	>100	0.02	0.016	0.20	8.0	6.3	1.3	10	10
9	0.063	16	50	0.025	0.016	0.16	5.0	6.3	1.6	8.0	16
Benzylpenicillin sodium salt	0.016	100	>100	0.01	0.008	0.10	25	2.5	0.40	2.0	5.0
11	0.05	63	50	0.01	0.01	0.16	16	20	1.3	32	10
10	0.10	63	63	0.013	0.016	0.20	20	25	2.0	50	32
Pivaloyloxymethyl *p*-chlorobenzylpenicillinate[f]	0.025	>100	>100	0.016	0.008	0.16	5.0	5.0	1.6	10.0	6.3
12	2.5	>100	>100	0.40	0.16	4.0	63	>100	40	>100	80
Pivaloyloxymethyl D-α-azidobenzylpenicillinate[g]	0.05	>100	>100	0.01	0.005	0.13	4.0	4.0	0.50	16	13
13	0.80	80	>100	0.13	0.13	4.0	>100	>100	20	>100	>100
3	0.40	>100	>100	0.10	0.05	0.63	63	13	13	10	16
Pivaloyloxymethyl α-chloromethylpenicillinate[e]	0.80	>100	>100	0.13	0.10	0.50	13	13	10	10	13
14	4.0	80	50	0.40	0.40	1.3	40	13	4.0	40	40
15	0.40	63	>100	0.13	0.04	0.40	32	8.0	4.0	5.0	10
Pivaloyloxymethyl α-azidomethylpenicillinate[e]	0.32	>100	>100	0.025	0.04	0.40	16	6.3	13	5.0	13
7	4.0	>100	>100	0.50	0.40	1.6	>100	32	13	>100	32
16	13	>100	>100	1.3	1.3	4.0	>100	16	8.0	16	>100
17	4.0	>100	>100	0.40	0.25	5.0	>100	>100	40	>100	>100
18	0.40	>100	>100	0.032	0.016	0.50	13	16	1.6	40	20
19	0.063	>100	>100	0.016	0.013	0.40	100	>100	40	>100	>100
20	0.16	>100	>100	0.05	0.04	0.40	>100	>100	5.0	>100	>100
21	0.40	>100	>100	0.05	0.032	0.40	>100	80	4.0	>100	>100
22	0.40	>100	>100	0.05	0.04	1.3	>100	50	13	>100	>100
23	0.50	>100	>100	0.08	0.05	0.80	20	13	>100	8.0	16
24	0.32	>100	>100	0.05	0.02	0.40	16	5.0	13	5.0	10
25	0.32	25	>100	0.025	0.04	0.50	25	13	13	10	13
26	0.10	32	>100	0.016	0.013	0.40	8.0	13	20	8.0	8.0
27	0.16	40	>100	0.05	0.04	1.3	>100	16	40	16	25
28	1.3	20	>100	0.13	0.13	1.3	25	13	25	13	16
29	0.13	>100	>100	0.013	0.013	0.40	6.3	4.0	13	2.5	5.0
30	0.40	>100	>100	0.04	0.04	0.50	40	5.0	40	10	16

[a] Pivaloyloxymethyl esters were pretreated with 20% mouse serum for 1.5 hr at 37° and pH 7.4. [b] IC_{50} is the concentration causing 50% inhibition of growth after 20 hr of incubation at 36°. The IC_{50}'s were determined by serial dilution in a medium containing 0.5% yeast extract (Difco), 1.5% casein hydrolysate (pancreatic), 0.1% dextrose, 0.25% sodium chloride, and 0.005% L-cystine in distilled water of pH 7.1 after autoclaving. [c] Penicillinase producing strain. [d] 3% horse serum was added to the substrate. [e] The compound was obtained as an oil by coupling **1a** with the appropriate carboxylic acid, using dicyclohexylcarbodiimide and standard work-up. The purity was checked by ir and tlc. [f] Mp 132–133°; prepared according to footnote *e*. [g] See ref 4.

Table II



Compd	R	R′	Mp, °C	Procedure[a]	Yield, %[b]	Recrystn solvent(s)	Formula[c]
3	$ClCH_2$	$CH_2OCOCMe_3$	124 dec	A	69	Aq EtOH	$C_{17}H_{23}ClN_4O_5S$
4	H	$CH_2OCOCMe_3$	159.5–160.5 dec	A	38	Aq EtOH	$C_{16}H_{22}N_4O_5S$
5	H	H, Et_2NH	129–133 dec	K	32	EtOH–Et_2O	$C_{14}H_{23}N_5O_3S \cdot {}^1/_9H_2O$
6	CH_3	$CH_2OCOCMe_3$	167.5–169 dec	B	4	Aq EtOH	$C_{17}H_{24}N_4O_5S \cdot {}^1/_6H_2O$
				G	68		
7	$MeOCOCH_2$	$CH_2OCOCMe_3$	144–148 dec	A	9	Aq EtOH	$C_{19}H_{26}N_4O_7S$
8	$C_6H_5CH_2$	$CH_2OCOCMe_3$	163–164.5	B	26	Aq Me_2CO	$C_{23}H_{28}N_4O_5S$
9	$C_6H_5CH_2$	H, Et_2NH	119–120.5 dec	K	10	EtOH–Et_2O	$C_{21}H_{29}N_5O_3S \cdot H_2O$
10	4-$ClC_6H_4CH_2$	$CH_2OCOCMe_3$	147–150	B	16	Aq Me_2CO	$C_{23}H_{27}ClN_4O_5S$
11	4-$ClC_6H_4CH_2$	H, Et_2NH	126–128.5 dec	K	22	EtOH–Et_2O	$C_{21}H_{28}ClN_5O_3S$
12	$C_6H_5CH(N_3)$	$CH_2OCOCMe_3$	148–149	A	13	Aq Me_2CO	$C_{23}H_{27}N_7O_5S$
13	C_6H_5	$CH_2OCOCMe_3$	143–147	B	37	Aq Me_2CO	$C_{22}H_{26}N_4O_5S$
14	ICH_2	$CH_2OCOCMe_3$	139–140.5 dec	C	88	Aq EtOH	$C_{17}H_{23}IN_4O_5S$[d]
15	N_3CH_2	$CH_2OCOCMe_3$	85–86	D	58	Aq EtOH	$C_{17}H_{23}N_7O_5S$
16	$NaS(O_3)SCH_2$	$CH_2OCOCMe_3$	126–140 dec	E	70	Me_2CO–Et_2O	$C_{17}H_{23}N_4NaO_8S_3 \cdot H_2O$
17	4-$MeC_6H_4SO_2CH_2$	$CH_2OCOCMe_3$	158–159	F	39	Aq EtOH	$C_{24}H_{30}N_4O_7S_2$
18	$C_6H_5SCH_2$	$CH_2OCOCMe_3$	144–146	H	45	Aq EtOH	$C_{23}H_{26}N_4O_5S_2$
19	4-*t*-$BuC_6H_4SCH_2$	$CH_2OCOCMe_3$	148–150	H	66	Aq EtOH	$C_{27}H_{36}N_4O_5S_2$
20	$C_6H_5CH_2SCH_2$	$CH_2OCOCMe_3$	120–121.5	H	51	Aq Me_2CO	$C_{24}H_{30}N_4O_5S_2$
21	$Me(CH_2)_4SCH_2$	$CH_2OCOCMe_3$	129–131	H	42	Aq Me_2CO	$C_{22}H_{34}N_4O_5S_2$
22	$Me_2CHCH(Me)SCH_2$	$CH_2OCOCMe_3$	130–132	H	47	Aq Me_2CO	$C_{22}H_{34}N_4O_5S_2$
23	$C_5H_5N^+CH_2Cl^-$[e]	$CH_2OCOCMe_3$	206	J	30	Me_2CO–Et_2O	$C_{22}H_{28}ClN_5O_5S$
24	4-Et-$C_5H_4N^+CH_2Cl^-$	$CH_2OCOCMe_3$	151–152 dec	J	35	MeOH–Et_2O	$C_{24}H_{32}ClN_5O_5S \cdot H_2O$
25	4-(4′-NO_2-$C_6H_4CH_2$)-$C_5H_4N^+CH_2Cl^-$	$CH_2OCOCMe_3$	170–171 dec	J	24	MeOH–Et_2O	$C_{29}H_{33}ClN_6O_7S \cdot H_2O$
26	4-C_6H_5-$C_5H_4N^+CH_2Cl^-$	$CH_2OCOCMe_3$	132.5–134 dec	J	31	MeOH–Et_2O	$C_{28}H_{32}ClN_5O_5S \cdot H_2O$
27	4-(4′-C_5H_4N)-$C_5H_4N^+$-CH_2Cl^-	$CH_2OCOCMe_3$	130–132 dec	J	42	MeOH–Et_2O	$C_{27}H_{31}ClN_6O_5S \cdot H_2O$[f]
28	3-OH-$C_5H_4N^+CH_2Cl^-$	$CH_2OCOCMe_3$	176–177.5 dec	J	28	MeOH–Et_2O	$C_{22}H_{28}ClN_5O_6S$[g]
29	2-Isoquinolinium-methyl Cl^-	$CH_2OCOCMe_3$	169–170 dec	J	18	MeOH–Et_2O	$C_{26}H_{30}ClN_5O_5S$
30	1-Methylimidazolinium-methyl Cl^-	$CH_2OCOCMe_3$	180–181 dec	J	35	MeOH–Et_2O	$C_{21}H_{29}ClN_6O_5S$

[a]Capital letters refer to procedures in the Experimental Section. [b]No attempts were made to optimize yields. [c]Compounds were analyzed for C, H, N, S, and halogen. Unless otherwise stated analyses are within ±0.4% of the theoretical values. [d]I: calcd, 24.31; found, 23.51. [e]$C_5H_5N^+$ or $C_5H_4N^+$ in the following are denoting *N*-pyridinium or substituted *N*-pyridinium. [f]Cl: calcd, 5.86; found, 6.47. [g]C: calcd, 50.23; found, 49.22.

cally distinguishable from the corresponding penicillins. Hydrolytic results with **4** and **10** and the corresponding diethylamine salts **5** and **11** are detailed in the Experimental Section.

Antibacterial Activity. The results of the *in vitro* antibacterial screening after mouse serum ester hydrolysis are compiled in Table I. For **3**, **4**, **10**, **12**, and **15** the IC_{50} figures are matched with those of the respective penicillin pivaloyloxymethyl esters under the same conditions. The diethylamine salts **5**, **9**, and **11** were tested without enzymatic pretreatment. **9** and **11** are compared with benzylpenicillin sodium salt. Except for the findings with **12** no significant departure is seen in terms of activity against specific organisms or displacement of the activity spectrum, as experienced with the 6-formamidinopenicillanic acids.[1]

Using *Sarcina lutea* as the test organism, it was found that the pivaloyloxymethyl esters of benzyl- and D-α-azidobenzylpenicillin[4] were 46 and 40% (respectively) hydrolyzed to the active penicillins by mouse serum. IC_{50} values for the esters **4**, **8**, and **10** and the corresponding salts **5**, **9**, and **11** seem to indicate the same or a higher order of recovery of active penicillanic acids from the esters.

9 and **11** show penicillin G-like activity. They appear to be very weakly active against two penicillinase producing staphylococci (Table I). Their rate of β-lactam hydrolysis by *Bacillus cereus* β-lactamase at pH 7.4 and 37° was evaluated as half of that found for benzylpenicillin, using iodometric assay.

The quaternary derivatives **23–30** exhibit gram-positive activity, but they are rather inactive against gram-negative species. In this respect they may be compared with the quaternary (1-methyl-3-pyridyl)methylpenicillin rather than with the pyridylmethylpenicillins.[10] Gram-positive activity is even more pronounced for the α-thiomethyl analogs **18–22**.

The members of this new class of 6-substituted penicillanic acids generally possess levels of gram-positive antibacterial activity similar to the activities of structurally analogous penicillins with which they have been compared. This lends support to the concept of the cyanoamidino group functioning as a carboxamido bioisostere.

Experimental Section

Melting points were uncorrected; they were taken in open capillaries using a Tottoli apparatus (N. Büchi, Flawil, Switzerland). Elemental analyses were performed by G. Cornali and W. Egger of these laboratories and are given as stated in Table II, footnote *c*. Ir spectra (KBr) were recorded with a Perkin-Elmer PE 457 spectrometer. In accordance with the assigned structure for **3–30** (Scheme I) pivaloyloxymethyl esters had absorptions at 2200–2180 cm^{-1}, attributable to $-C{\equiv}N$,[11] 1810–1740 cm^{-1} (β-lactam

and ester carbonyls), and 1615–1560 cm^{-1} (–C=N–).[11] Nmr spectra were run on a Varian A-60A spectrometer. The data were consistent with structures **3–30** (Scheme I) or amidino tautomers.

N-Cyanoimidates (**2a,b, Scheme I**). **2a** (**R** = **H**, $ClCH_2$, C_6H_5, $C_6H_5CH_2$) was prepared by condensing the ortho ester with cyanamide in Ac_2O.[8] **2a** (**R** = **MeOCOCH**$_2$, C_6H_5, $C_6H_5CH_2$, 4-$ClC_6H_4CH_2$) as well as **2b** (**R** = **Me**) was obtained by the reaction of the imidates with cyanamide in phosphate buffer.[9] Action of triethoxonium tetrafluoroborate[12] on D-α-azido-α-phenylacetamide (1 equiv) in CH_2Cl_2 and direct conversion of the imidate[9] furnished **2b** [**R** = $C_6H_5CH(N_3)$]. *Warning:* crude ethyl α-azido-α-phenyl-*N*-cyanoacetimidate should not be evaporated exhaustively, but rather be reacted with **1a** as a concentrated Et_2O solution, since violent spontaneous decomposition of a small dried sample has occurred once.

Pivaloyloxymethyl 6-*N'*-Cyanoamidinopenicillanates. Procedure A (3, 4, 7, and 12, Table II). 1a (3.3 g, 10 mmol) was dissolved in Et_2O (20 ml). The requisite **2a,b** (9–13 mmol) was added at 0°, and stirring was maintained for 2 hr. *i*-Pr_2O (20 ml) was added, and the mixture was kept overnight at 0°. The crystalline material was collected by filtration and washed with *i*-Pr_2O to yield essentially pure compounds.

Procedure B (6, 8, 10, and 13, Table II). 1a (6.6 g, 20 mmol) and **2a,b** (20 mmol) were dissolved in DMF (20 ml). A catalytic amount of 1-methylimidazole was added, and the solution was kept at 0° for 48 hr. Et_2O (150 ml) was added, and the solution was washed twice with cold 0.1 *N* HCl (100 ml), with 0.2 *N* $NaHCO_3$, and with pure H_2O, dried ($MgSO_4$), and evaporated. After trituration of the residue with petroleum ether the crude product was collected.

Procedure C (Table II). A solution of **3** (0.86 g, 2 mmol) and KI (0.50 g, 3 mmol) in CH_3CN (9 ml) was stirred at ambient temperature for 2 hr, while precipitation occurred (KCl). After standing at 0° (72 hr), evaporation, addition of Et_2O (30 ml) and EtOAc (20 ml), and washing with H_2O (2 × 50 ml) the organic solution was charcoaled and dried ($MgSO_4$). Evaporation gave crude **14**.

Procedure D (Table II). A solution of **3** (0.86 g, 2 mmol) and NaN_3 (0.26 g, 4 mmol) in DMF (3 ml) was kept at 0° for 2 hr. Et_2O (100 ml) was added, and work-up according to procedure C yielded solid **15**.

Procedure E (Table II). To **3** (0.86 g, 2 mmol) in ice-cooled DMF (10 ml) was added $Na_2S_2O_3 \cdot 5H_2O$ (0.45 g, 1.8 mmol). The mixture was kept (3 hr) at room temperature. After high vacuum removal of DMF, the residue was triturated repeatedly with Et_2O. The residue was dissolved in a 1:1 mixture of EtOH–Me_2CO (12 ml), filtered, and evaporated, giving crude **16**.

Procedure F (Table II). 4-$MeC_6H_4SO_2Na$ (0.71 g, 4 mmol) was dissolved in DMF (10 ml) at 0°, and **3** (0.86 g, 2 mmol) was added. After 4 hr at 0° the mixture was heated briefly to 25°. Et_2O (100 ml) was added, and after two washings with 0.2 *N* $NaHCO_3$ (100 ml) the ethereal solution was dried (Na_2SO_4) in the presence of charcoal. Evaporation yielded **17**.

Procedure G (Table II). **3** (0.86 g, 2 mmol) in $(MeO)_3P$ (8 ml, technical grade), containing a trace of CuCl, was heated gradually to 80° over 20 min. After evaporation of $(MeO)_3P$, a crude product was obtained. Recrystallization from aqueous EtOH afforded a compound which was identical with **6**, prepared by procedure B.

Procedure H (18–22, Table II). **3** (0.86 g, 2 mmol) in DMF (6 ml) with the appropriate thiol (2 mmol) and solid $KHCO_3$ (0.2 g, 2 mmol) after 24 hr at 0° was stirred at 25° for 1–5 hr. Et_2O (100 ml) was then introduced, and work-up was carried out according to procedure F.

Procedure J (23–30, Table II). **3** (0.86 g, 2 mmol) in Me_2CO (5 ml) was reacted with the appropriate N-heterocycle (2.4 mmol), while stirring at 25° for 72 hr (24 hr with **27** and **28**). Et_2O (30 ml) was then added (with **25** and **30** Et_2O was added from the start, and stirring was maintained for 48 hr). The precipitate was collected and washed with Et_2O. For 4,4'-bipyridine 6 equiv were required to warrant a fair yield of **27**.

6-*N'*-Cyanoamidinopenicillanic Acids, Diethylamine Salts. Procedure K (5, 9, and 11, Table II). To a stirred suspension of 6-aminopenicillanic acid (3.24 g, 15 mmol) in DMF (15 ml) and H_2O (1.5 ml) at 0° was added Et-*i*-Pr_2N (2.1 ml, 12 mmol). After 15 min **2a** (10 mmol) was introduced dropwise, and the suspension was stirred at 0° for 6 hr. $NaHCO_3$ (0.2 *N*, 75 ml) was then added, and two washings with Et_2O (75 ml) were carried out. The cooled aqueous solution was gently acidified (pH 2.5) with 4 *N* HCl and extracted twice with a 4:1 mixture of Et_2O–EtOAc (100 ml). The combined extracts were washed with H_2O (75 ml) and dried ($MgSO_4$). Et_2NH (0.5 ml, 5 mmol) was added to the stirred solution at 0°. The amorphous precipitate was crystallized by scratching, decanting, and repeated trituration with Et_2O. It was collected by filtration and washed with Et_2O.

Attempted Hydrolysis of the Diethylamine Salts 5 and 11. The salt (1 mmol) was dissolved in 0.07 *M* phosphate buffer (75 ml) of pH 7.4 and kept at 37° for 3 hr. Acidification (pH 2.5) and extraction with a 1:1 mixture of Et_2O–EtOAc (75 ml) gave a solution which on drying ($MgSO_4$) and addition of Et_2NH (0.1 ml, 1 mmol) deposited starting material only, as checked by ir and tlc.

Study of the Enzymatic Conversion of the Pivaloyloxymethyl Esters 4 and 10. The ester (20 mg) was treated with 20% mouse serum in phosphate buffer (5 ml, pH 7.4) for 2 hr at 37°. The pivaloyloxymethyl esters of 6-formamidopenicillanic acid and 4-chlorobenzylpenicillin were subjected to the same treatment, and together with **5** and **11** they were run as controls for hydrolyzed **4** and **10** on paper bioautograms with *Sarcina lutea* as the test organism. Hydrolyzed **4** and **10** showed only the presence of inhibitory zones with R_f values identical with those of the corresponding salts **5** and **11**, indicating that hydrolysis [RC(=NCN)NH– → RC(=O)NH–] had not occurred. Comparative R_f values: **5**, R_f = 0.65; 6-formamidopenicillanic acid, R_f = 0.55 in the system *n*-BuOH–AcOH–H_2O (~4:1:5); **11**, R_f = 0.60; 4-chlorobenzylpenicillin, R_f = 0.51 in a two-phase system of EtOAc and 0.1 *N* acetate buffer (pH 5.6).

Assay of the Conversion of the Pivaloyloxymethyl Esters of Benzyl- and D-α-Azidobenzylpenicillin into Active Penicillins. The esters (20 mg) were treated for 2 hr with 20% mouse serum in phosphate buffer (5 ml) (pH 7.4) at 37°. Penicillin concentrations were assessed by the agar diffusion technique (*Sarcina lutea* as test organism) using benzylpenicillin Na salt and D-α-azidobenzylpenicillin K salt as reference compounds.

Acknowledgments. The author wishes to thank Mr. F. Lund of these laboratories and Professor W. D. Ollis, University of Sheffield, England, for their interest and valuable discussions, Mr. N. Rastrup Andersen for providing the nmr data, and Mr. L. Tybring for the antibacterial screening. The technical assistance by Mr. J. R. Niemelä is also gratefully acknowledged.

References

(1) F. Lund and L. Tybring, *Nature (London), New Biol.*, **236**, 135 (1972).
(2) A. Burger, "Medicinal Chemistry," Part 1, 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 72.
(3) J. C. Godfrey, U. S. Patent 3,322,781 (1967).
(4) W. von Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Mørch, H. J. Petersen, K. Roholt, L. Tybring, and W. O. Godtfredsen, *J. Med. Chem.*, **13**, 607 (1970).
(5) A. B. A. Jansen and T. J. Russell, *J. Chem. Soc.*, 2127 (1965).
(6) H. P. K. Agersborg, A. Batchelor, G. W. Cambridge, and A. W. Rule, *Brit. J. Pharmacol.*, **26**, 649 (1966).
(7) F. Lund, British Patent 1,293,590 (1972).
(8) K. R. Huffman and F. C. Schaeffer, *J. Org. Chem.*, **28**, 1816 (1963).
(9) W. Lwowski, *Synthesis*, 263 (1971).
(10) R. J. Stedman, A. C. Swift, L. S. Miller, M. M. Dolan, and J. R. E. Hoover, *J. Med. Chem.*, **10**, 363 (1967).
(11) K. R. Huffman and F. C. Schaeffer, *J. Org. Chem.*, **28**, 1812 (1963).
(12) H. Meerwein, W. Florian, N. Schön, and G. Stopp, *Justus Liebigs Ann. Chem.*, **641**, 1 (1961).