

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

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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.<sup>1</sup> 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<sup>2</sup> 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.<sup>3</sup>

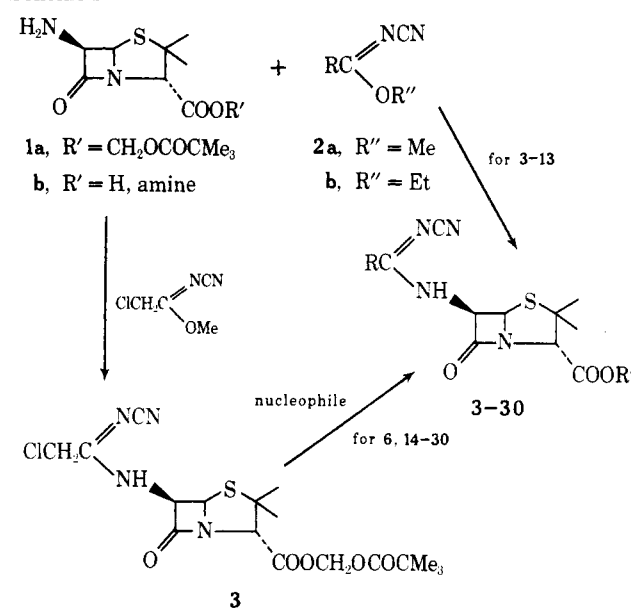
The strategy for the synthesis of the compounds of this study was based on the interaction of *N*-cyanoimides 2a,b with the amino group of 6-aminopenicillanic acid. It was appreciated that the unprotected 3-carboxyl group might cause adverse side reactions. A transient blocking could be achieved by the use of pivaloyloxymethyl esters. Acyloxymethyl esters have been reported to undergo facile enzymatic hydrolysis to the corresponding acids by non-specific serum esterases.<sup>1,4-6</sup> With the readily available pivaloyloxymethyl 6-aminopenicillanate (1a)<sup>4</sup> 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.<sup>4,7</sup>

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<sup>8</sup> or the imide<sup>9</sup> gave the *N*-cyanoimides 2a,b. Reaction of 2a,b with pivaloyloxymethyl 6-aminopenicillanate (1a)<sup>4</sup> constituted the basic route to the title esters, as outlined in Scheme I.

The 6-*N*'-cyano- $\alpha$ -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 quaternary compounds 23-30, whereas *N*-alkylation by the pivaloyloxymethyl ester of  $\alpha$ -chloromethylpenicillin

Scheme I



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.

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<]  $\rightleftharpoons$  RC(NHCN)=NCH<]. The nmr spectra of pivaloyloxymethyl esters in CDCl<sub>3</sub>, 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<sub>2</sub>O clearly demonstrated long-range coupling between the formyl H and 6-H, establishing the tautomeric structure HC(NHCN)=NCH<. Likewise, the diethylamine salt 11 in (CD<sub>3</sub>)<sub>2</sub>SO 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-  $\rightarrow$  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-

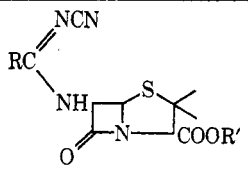
† This paper is dedicated to Professor Alfred Burger, with whom I had the pleasure to be associated at the Cobb Chemical Laboratory, University of Virginia, during 1962-1963.

Table I. *In Vitro* Antibacterial Activity<sup>a</sup> of 3-30 and Some Related Penicillins; IC<sub>50</sub> Concentration in µg/ml<sup>b</sup>

Compd	Staph. aureus		D. pneumoniae		Strept. pyogenes	L. monocytogenes	E. coli	K. pneumoniae	P. vulgaris	Sal. typhosa	Shig. dysenteriae
	LeoCJ	LeoCJ9 <sup>c</sup>	LeoCJ110 <sup>c</sup>	LeoEA <sup>d</sup>	NCTC 6175	NCTC 7973	LeoHA	LeoHE	NCTC 4175A	NCTC 5760	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 <sup>e</sup>											
6	2.0	>100	>100	0.50	0.40	4.0	16	13	13	13	16
8	0.63	>100	>100	0.13	0.13	0.40	80	13	>100	13	>100
9	0.063	16	50	0.02	0.016	0.20	8.0	6.3	1.3	10	10
Benzylpenicillin sodium salt	0.063	16	50	0.025	0.016	0.16	5.0	6.3	1.6	8.0	16
11	0.016	100	>100	0.01	0.008	0.10	25	2.5	0.40	2.0	5.0
10	0.05	63	50	0.01	0.01	0.16	16	20	1.3	32	10
	0.10	63	63	0.013	0.016	0.20	20	25	2.0	50	32
Pivaloyloxymethyl p-chlorobenzylpenicillanate <sup>f</sup>											
12	0.025	>100	>100	0.016	0.008	0.16	5.0	5.0	1.6	10.0	6.3
13	2.5	>100	>100	0.40	0.16	4.0	63	>100	40	>100	80
Pivaloyloxymethyl D-α-azidobenzylpenicillanate <sup>g</sup>											
13	0.05	>100	>100	0.01	0.005	0.13	4.0	4.0	0.50	16	13
3	0.80	80	>100	0.13	0.13	4.0	>100	>100	20	>100	>100
	0.40	>100	>100	0.10	0.05	0.63	63	13	13	10	16
Pivaloyloxymethyl α-chloromethylpenicillanate <sup>e</sup>											
14	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-azidomethylpenicillanate <sup>e</sup>											
7	0.32	>100	>100	0.025	0.04	0.40	16	6.3	13	5.0	13
16	4.0	>100	>100	0.50	0.40	1.6	>100	32	13	>100	32
17	13	>100	>100	1.3	1.3	4.0	>100	16	8.0	16	>100
18	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	40	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

<sup>a</sup>Pivaloyloxymethyl esters were pretreated with 20% mouse serum for 1.5 hr at 37° and pH 7.4. <sup>b</sup>IC<sub>50</sub> is the concentration causing 50% inhibition of growth after 20 hr of incubation at 36°. The IC<sub>50</sub>'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. <sup>c</sup>Penicillinase producing strain. <sup>d</sup>3% horse serum was added to the substrate. <sup>e</sup>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. <sup>f</sup>Mp 132-133°, prepared according to footnote e. <sup>g</sup>See ref 4.

Table II

								
Compd	R	R'	Mp, °C	Pro- cedure <sup>a</sup>	Yield, % <sup>b</sup>	Recrystn solvent(s)	Formula <sup>c</sup>	
3	ClCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	124 dec	A	69	Aq EtOH	C <sub>17</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>5</sub> S	
4	H	CH <sub>2</sub> OCOCMe <sub>3</sub>	159.5–160.5 dec	A	38	Aq EtOH	C <sub>16</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub> S	
5	H	H, Et <sub>2</sub> NH	129–133 dec	K	32	EtOH–Et <sub>2</sub> O	C <sub>14</sub> H <sub>23</sub> N <sub>5</sub> O <sub>5</sub> S · 1/9 H <sub>2</sub> O	
6	CH <sub>3</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	167.5–169 dec	B	4	Aq EtOH	C <sub>17</sub> H <sub>24</sub> N <sub>4</sub> O <sub>5</sub> S · 1/6 H <sub>2</sub> O	
				G	68			
7	MeOCOCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	144–148 dec	A	9	Aq EtOH	C <sub>19</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub> S	
8	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	163–164.5	B	26	Aq Me <sub>2</sub> CO	C <sub>23</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub> S	
9	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	H, Et <sub>2</sub> NH	119–120.5 dec	K	10	EtOH–Et <sub>2</sub> O	C <sub>21</sub> H <sub>29</sub> N <sub>5</sub> O <sub>5</sub> S · H <sub>2</sub> O	
10	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	147–150	B	16	Aq Me <sub>2</sub> CO	C <sub>23</sub> H <sub>27</sub> ClN <sub>4</sub> O <sub>5</sub> S	
11	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	H, Et <sub>2</sub> NH	126–128.5 dec	K	22	EtOH–Et <sub>2</sub> O	C <sub>21</sub> H <sub>28</sub> ClN <sub>5</sub> O <sub>5</sub> S	
12	C <sub>6</sub> H <sub>5</sub> CH(N <sub>3</sub> )	CH <sub>2</sub> OCOCMe <sub>3</sub>	148–149	A	13	Aq Me <sub>2</sub> CO	C <sub>23</sub> H <sub>27</sub> N <sub>7</sub> O <sub>5</sub> S	
13	C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	143–147	B	37	Aq Me <sub>2</sub> CO	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub> S	
14	ICH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	139–140.5 dec	C	88	Aq EtOH	C <sub>17</sub> H <sub>23</sub> IN <sub>4</sub> O <sub>5</sub> S <sup>d</sup>	
15	N <sub>3</sub> CH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	85–86	D	58	Aq EtOH	C <sub>17</sub> H <sub>23</sub> N <sub>7</sub> O <sub>5</sub> S	
16	NaS(O <sub>3</sub> )SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	126–140 dec	E	70	Me <sub>2</sub> CO–Et <sub>2</sub> O	C <sub>17</sub> H <sub>23</sub> N <sub>4</sub> NaO <sub>8</sub> S <sub>3</sub> · H <sub>2</sub> O	
17	4-MeC <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> CH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	158–159	F	39	Aq EtOH	C <sub>24</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
18	C <sub>6</sub> H <sub>5</sub> SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	144–146	H	45	Aq EtOH	C <sub>23</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
19	4- <i>t</i> -BuC <sub>6</sub> H <sub>4</sub> SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	148–150	H	66	Aq EtOH	C <sub>27</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
20	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	120–121.5	H	51	Aq Me <sub>2</sub> CO	C <sub>24</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
21	Me(CH <sub>2</sub> ) <sub>4</sub> SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	129–131	H	42	Aq Me <sub>2</sub> CO	C <sub>22</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
22	Me <sub>2</sub> CHCH(Me)SCH <sub>2</sub>	CH <sub>2</sub> OCOCMe <sub>3</sub>	130–132	H	47	Aq Me <sub>2</sub> CO	C <sub>22</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub> S <sub>2</sub>	
23	C <sub>5</sub> H <sub>5</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	206	J	30	Me <sub>2</sub> CO–Et <sub>2</sub> O	C <sub>22</sub> H <sub>28</sub> ClN <sub>5</sub> O <sub>5</sub> S	
24	4-Et-C <sub>5</sub> H <sub>4</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	151–152 dec	J	35	MeOH–Et <sub>2</sub> O	C <sub>24</sub> H <sub>32</sub> ClN <sub>5</sub> O <sub>5</sub> S · H <sub>2</sub> O	
25	4-(4'-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> )-C <sub>5</sub> H <sub>4</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	170–171 dec	J	24	MeOH–Et <sub>2</sub> O	C <sub>29</sub> H <sub>38</sub> ClN <sub>6</sub> O <sub>5</sub> S · H <sub>2</sub> O	
26	4-C <sub>6</sub> H <sub>5</sub> -C <sub>5</sub> H <sub>4</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	132.5–134 dec	J	31	MeOH–Et <sub>2</sub> O	C <sub>28</sub> H <sub>32</sub> ClN <sub>5</sub> O <sub>5</sub> S · H <sub>2</sub> O	
27	4-(4'-C <sub>5</sub> H <sub>4</sub> N)-C <sub>5</sub> H <sub>4</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	130–132 dec	J	42	MeOH–Et <sub>2</sub> O	C <sub>27</sub> H <sub>31</sub> ClN <sub>6</sub> O <sub>5</sub> S · H <sub>2</sub> O	
28	3-OH-C <sub>5</sub> H <sub>4</sub> N <sup>+</sup> CH <sub>2</sub> Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	176–177.5 dec	J	28	MeOH–Et <sub>2</sub> O	C <sub>22</sub> H <sub>28</sub> ClN <sub>5</sub> O <sub>6</sub> S <sup>e</sup>	
29	2-Isoquinolinium-methyl Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	169–170 dec	J	18	MeOH–Et <sub>2</sub> O	C <sub>26</sub> H <sub>30</sub> ClN <sub>5</sub> O <sub>5</sub> S	
30	1-Methylimidazolium-methyl Cl <sup>−</sup>	CH <sub>2</sub> OCOCMe <sub>3</sub>	180–181 dec	J	35	MeOH–Et <sub>2</sub> O	C <sub>21</sub> H <sub>29</sub> ClN <sub>6</sub> O <sub>5</sub> S	

<sup>a</sup>Capital letters refer to procedures in the Experimental Section. <sup>b</sup>No attempts were made to optimize yields. <sup>c</sup>Compounds were analyzed for C, H, N, S, and halogen. Unless otherwise stated analyses are within  $\pm 0.4\%$  of the theoretical values. <sup>d</sup>I: calcd, 24.31; found, 23.51. <sup>e</sup>C<sub>5</sub>H<sub>5</sub>N<sup>+</sup> or C<sub>5</sub>H<sub>4</sub>N<sup>+</sup> in the following are denoting *N*-pyridinium or substituted *N*-pyridinium. <sup>f</sup>Cl: calcd, 5.86; found, 6.47. <sup>g</sup>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<sub>50</sub> 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.<sup>1</sup>

Using *Sarcina lutea* as the test organism, it was found that the pivaloyloxymethyl esters of benzyl- and D-azidobenzylpenicillin<sup>4</sup> were 46 and 40% (respectively) hydrolyzed to the active penicillins by mouse serum. IC<sub>50</sub> 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 produc-

ing staphylococci (Table I). Their rate of  $\beta$ -lactam hydrolysis by *Bacillus cereus*  $\beta$ -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.<sup>10</sup> Gram-positive activity is even more pronounced for the  $\alpha$ -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<sup>−1</sup>, attributable to  $-\text{C}\equiv\text{N}$ ,<sup>11</sup> 1810–1740 cm<sup>−1</sup> ( $\beta$ -lactam

and ester carbonyls), and 1615–1560  $\text{cm}^{-1}$  ( $-\text{C}=\text{N}-$ ).<sup>11</sup> 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 ( $\text{R} = \text{H}$ ,  $\text{ClCH}_2$ ,  $\text{C}_6\text{H}_5$ ,  $\text{C}_6\text{H}_5\text{CH}_2$ ) was prepared by condensing the ortho ester with cyanamide in  $\text{Ac}_2\text{O}$ .<sup>8</sup> 2a ( $\text{R} = \text{MeOCOCH}_2$ ,  $\text{C}_6\text{H}_5$ ,  $\text{C}_6\text{H}_5\text{CH}_2$ , 4- $\text{ClC}_6\text{H}_4\text{CH}_2$ ) as well as 2b ( $\text{R} = \text{Me}$ ) was obtained by the reaction of the imidates with cyanamide in phosphate buffer.<sup>9</sup> Action of triethoxonium tetrafluoroborate<sup>12</sup> on D- $\alpha$ -azido- $\alpha$ -phenylacetamide (1 equiv) in  $\text{CH}_2\text{Cl}_2$  and direct conversion of the imide<sup>9</sup> furnished 2b [ $\text{R} = \text{C}_6\text{H}_5\text{CH}(\text{N}_3)$ ]. **Warning:** crude ethyl  $\alpha$ -azido- $\alpha$ -phenyl-N-cyanoacetimidate should not be evaporated exhaustively, but rather be reacted with 1a as a concentrated  $\text{Et}_2\text{O}$  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  $\text{Et}_2\text{O}$  (20 ml). The requisite 2a,b (9–13 mmol) was added at 0°, and stirring was maintained for 2 hr. *i*- $\text{Pr}_2\text{O}$  (20 ml) was added, and the mixture was kept overnight at 0°. The crystalline material was collected by filtration and washed with *i*- $\text{Pr}_2\text{O}$  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.  $\text{Et}_2\text{O}$  (150 ml) was added, and the solution was washed twice with cold 0.1 *N* HCl (100 ml), with 0.2 *N*  $\text{NaHCO}_3$ , and with pure  $\text{H}_2\text{O}$ , dried ( $\text{MgSO}_4$ ), 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  $\text{CH}_3\text{CN}$  (9 ml) was stirred at ambient temperature for 2 hr, while precipitation occurred (KCl). After standing at 0° (72 hr), evaporation, addition of  $\text{Et}_2\text{O}$  (30 ml) and  $\text{EtOAc}$  (20 ml), and washing with  $\text{H}_2\text{O}$  ( $2 \times 50$  ml) the organic solution was charcoaled and dried ( $\text{MgSO}_4$ ). Evaporation gave crude 14.

**Procedure D (Table II).** A solution of 3 (0.86 g, 2 mmol) and  $\text{NaN}_3$  (0.26 g, 4 mmol) in DMF (3 ml) was kept at 0° for 2 hr.  $\text{Et}_2\text{O}$  (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  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  (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  $\text{Et}_2\text{O}$ . The residue was dissolved in a 1:1 mixture of  $\text{EtOH}$ – $\text{Me}_2\text{CO}$  (12 ml), filtered, and evaporated, giving crude 16.

**Procedure F (Table II).** 4- $\text{MeC}_6\text{H}_4\text{SO}_2\text{Na}$  (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°.  $\text{Et}_2\text{O}$  (100 ml) was added, and after two washings with 0.2 *N*  $\text{NaHCO}_3$  (100 ml) the ethereal solution was dried ( $\text{Na}_2\text{SO}_4$ ) in the presence of charcoal. Evaporation yielded 17.

**Procedure G (Table II).** 3 (0.86 g, 2 mmol) in  $(\text{MeO})_3\text{P}$  (8 ml, technical grade), containing a trace of  $\text{CuCl}$ , was heated gradually to 80° over 20 min. After evaporation of  $(\text{MeO})_3\text{P}$ , a crude product was obtained. Recrystallization from aqueous  $\text{EtOH}$  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  $\text{KHCO}_3$  (0.2 g, 2 mmol) after 24 hr at 0° was stirred at 25° for 1–5 hr.  $\text{Et}_2\text{O}$  (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  $\text{Me}_2\text{CO}$  (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).  $\text{Et}_2\text{O}$  (30 ml) was then added (with 25 and 30  $\text{Et}_2\text{O}$  was added from the start, and stirring was maintained for 48 hr). The precipitate was collected and washed with  $\text{Et}_2\text{O}$ . 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  $\text{H}_2\text{O}$  (1.5 ml) at 0° was added  $\text{Et}_2\text{N}-\text{Pr}_2\text{N}$  (2.1 ml, 12 mmol). After 15 min 2a (10 mmol) was introduced dropwise, and the suspension was stirred at 0° for 6 hr.  $\text{NaHCO}_3$  (0.2 *N*, 75 ml) was then added, and two washings with  $\text{Et}_2\text{O}$  (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  $\text{Et}_2\text{O}$ – $\text{EtOAc}$  (100 ml). The combined extracts were washed with  $\text{H}_2\text{O}$  (75 ml) and dried ( $\text{MgSO}_4$ ).  $\text{Et}_2\text{NH}$  (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  $\text{Et}_2\text{O}$ . It was collected by filtration and washed with  $\text{Et}_2\text{O}$ .

**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  $\text{Et}_2\text{O}$ – $\text{EtOAc}$  (75 ml) gave a solution which on drying ( $\text{MgSO}_4$ ) and addition of  $\text{Et}_2\text{NH}$  (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 [ $\text{RC}(=\text{NCN})\text{NH}- \rightarrow \text{RC}(=\text{O})\text{NH}-$ ] 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– $\text{H}_2\text{O}$  (~4:1:5); 11,  $R_f = 0.60$ ; 4-chlorobenzylpenicillin,  $R_f = 0.51$  in a two-phase system of  $\text{EtOAc}$  and 0.1 *N* acetate buffer (pH 5.6).

**Assay of the Conversion of the Pivaloyloxymethyl Esters of Benzyl- and D- $\alpha$ -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- $\alpha$ -azidobenzylpenicillin K salt as reference compounds.

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