SYNTHESIS OF POTASSIUM 3S-PHENYLACETAMIDO-1-(1-CARBOXY-METHYLTETRAZOL-5-YL)-AZETIDIN-2-ONE AND POTASSIUM 3S-PHENYLACETAMIDO-1-(2-CARBOXYMETHYL-TETRAZOL-5-YL)-AZETIDIN-2-ONE

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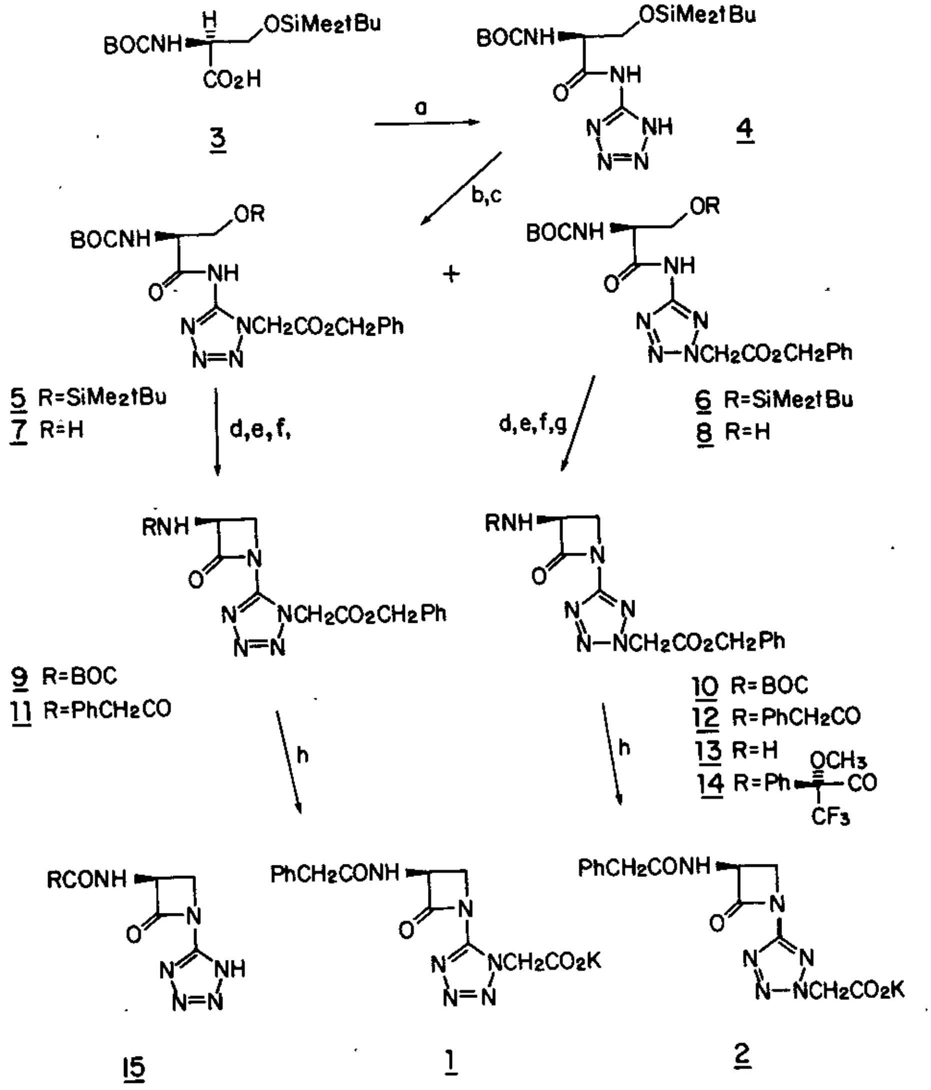
Abstract - The synthesis and some biological properties of the title compounds, beta-lactam antibiotics, are described.

Recent discoveries in the field of beta-lactam antibiotics show that the geometric relationship between the N1-C2 amide bond and the anionic binding site is likely to be an important determinant of antimicrobial potency.[1] We sought to probe the limiting distance between these moieties by the synthesis of **1** and **2**, wherein a tetrazole ring serves as a rigid spacer between the azetidin-2-one and carboxyl function. In addition to providing geometric definition to this relationship, the tetrazole function serves to withdraw electron density from the amide bond by induction, another effect usually correlated with bioactivity.

As in our previous report[2] on N-(5-tetrazolyl)azetidin-2-ones, N-Boc-L-serine was protected as its tert-butyldimethylsilyl ether **3**[3] and coupled with 5-aminotetrazole to give **4**. Alkylation with benzyl bromoacetate and triethylamine in acetonitrile afforded a 3.5/1 mixture of isomers. Different bases and solvents did not dramatically alter this ratio. Although the isomers could not be conclusively identified at this stage, literature precedent holds that 5-carbon-substituted tetrazoles alkylate predominantly at the 2-position and that 1- and 2-substituted tetrazole isomers exhibit consistent spectral and physical differences.[4] The assignment of **5** and **6** was ultimately verified by a crystallographic study (vide infra). After separation by chromatography, **5** and **6** were desilylated by hydrogen fluoride in acetonitrile.[5] Cyclization of **7** and **8** was achieved with diisopropylazodicarboxylate/triphenylphosphine[6] to yield azetidin-2-ones **9** and **10**. Deblocking with trifluoroacetic acid and acylation of the evaporated salts with phenylacetyl chloride afforded **11** and **12**. Hydrogenolysis with palladium in the presence of potassium phosphate buffer gave **1** and **2**.

To verify that the stereochemical integrity of intermediates was retained throughout the synthesis, **10** was deblocked to the free amine **13** and acylated with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(+)MTPACl][7] to give **14**. Examination of the 200 MHz pmr spectrum showed **14** to be homogeneous with a single NH doublet at 7.46 δ (J = 7 Hz) and a single methoxyl quartet[8] at 3.42 δ (J = 1.2 Hz). Therefore, any racemization taking place is undetectable by pmr. To firmly establish the identity of the tetrazolyl isomers, the structure of **10** was confirmed by X-ray crystallography.[9]



BOC = tert-butyloxycarbonyl

a) DCC, l-hydroxybenzotriazole, 5-amino tetrazole, DMF; 41% or iBuOCOCl, N-methylmorpholine, THF, one hour −15°, then 5-amino tetrazole, N-methylmorpholine; 68%

b) $BrCH_2CO_2CH_2Ph$, Et_3N, DMF; 16% 5 and 59% 6

c) 48% HF, CH_3CN; 85% 7, 98% 8

d) $iPrO_2CN = NCO_2iPr$, Ph_3P, THF; 59% 9, 57% 10

e) CF_3CO_2H, CH_2Cl_2

f) $PhCH_2COCl$, pyr, CH_2Cl_2; 36% 11 from 9, 83% 12 from 10

g) (+)MTPACl, pyr, CH_2Cl_2; 95% 14 from 13

h) H_2, 10% Pd/C, THF, EtOH, pH 7 potassium phosphate buffer; 30% 1, 47% 2

A 116-microgram sample of **1** on a 6-mm paper disc imparted a 14 mm zone of inhibition in a culture of *Staph. aureus*. Likewise, 59 micrograms of **2** gave a 21 mm zone. However, both compounds were inactive (>128 μg/ml 10^4 colony forming units) against a variety of more clinically representative bacteria, by a tube-dilution, minimum inhibitory concentration assay.[10] This result stands in marked contrast to the significant antimicrobial activity of the N-tetrazolylazetidin-2-ones **15**.[2] As the inductive effect upon the amide bond in **1** and **2** should be greater than that induced in **15** by the anionic tetrazole, we conclude that the distance[11] between the electrophilic center and the anionic function is too large (5.08 Å) for efficient recognition by target enzyme binding sites.[12]

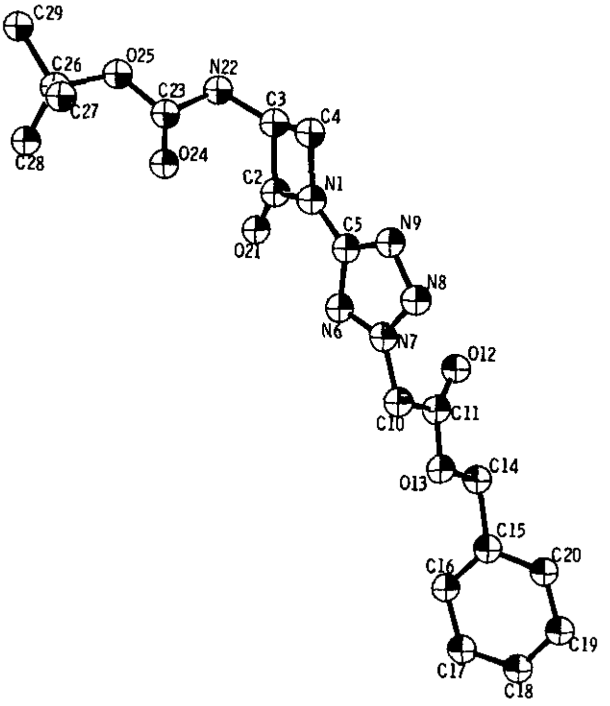


Figure 1. A computer-generated drawing of 10 derived from X-ray coordinates with hydrogens omitted for clarity.

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3) Selected physical data: **4**: mp 152-55°C $[\alpha]_D^{25}$ = +2.8° (c = 1, CH_3OH); IR (CH_3CN): 3300, 1720, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.19 (1 H, d, J = 7), 4.56 (1 H, m), 4.28 (1 H, dd, J = 3, 10.5), 3.97 (1 H, dd, J = 4, 10.5), 1.43 (9 H, s), 0.80 (9 H, s), 0.04 (6 H, s). **5**: IR ($CHCl_3$): 3410, 1755, 1715, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (5 H, m), 5.38 (2 H, s), 5.26 (2 H, s), 4.40 (1 H, m), 4.10 (1 H, dd, J = 4, 10), 3.80 (1 H, dd, J = 6, 10), 1.46

(9 H, s), 0.88 (9 H, s), 0.06 (6 H, s). **6**: IR ($CHCl_3$): 3405, 1760, 1715, 1555 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40 (5 H, m), 5.46 (2 H, s), 5.24 (2 H, s), 4.40 (1 H, m), 4.18 (1 H, dd, J = 4, 10), 3.76 (1 H, dd, J = 7, 10), 1.50 (9 H, s), 0.88 (9 H, s), 0.10 (6 H, s). **8**: ^{1}H NMR ($CDCl_3$) δ 9.82 (1 H, br s), 7.40 (5 H, s), 5.77 (1 H, d, J = 7), 5.43 (2 H, s), 5.28 (2 H, s), 4.44 (1 H, m), 4.29 (1 H, dd, J = 3, 11.5), 3.79 (1 H, dd, J = 4.5, 11.5), 1.49 (9 H, s). **9**: IR ($CHCl_3$): 1790, 1760, 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36 (5 H, m), 5.70 (1 H, d, J = 17.5), 5.48 (1 H, d, J = 17.5), 5.24 (1 H, d, J = 12), 5.16 (1 H, d, J = 12), 4.79 (1 H, br s), 4.1 (2 H, m), 1.46 (9 H, s). **10**: mp 188-90°: IR ($CHCl_3$): 3460, 1790, 1765, 1720, 1560 cm^{-1}; ^{1}H ($CDCl_3$) δ 7.41, (5 H, br s), 5.41 (2 H, s), 5.27 (2 H, s), 5.06 (1 H, m), 4.22 (1 H, m), 4.02 (1 H, dd, J = 3.5, 6), 1.46 (9 H, s); MS: 347, 329. **11**: IR ($CHCl_3$): 1795, 1770, 1685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.34 (10 H, m), 5.98 (1 H, d, J = 6.5), 5.72 (1 H, d, J = 17.5), 5.48 (1 H, d, J = 17.5), 5.28 (1 H, d, J = 12), 5.14 (1 H, d, J = 12), 4.74 (1 H, ddd, J = 3.5, 6.5, 7.0), 4.12 (1 H, dd, J = 6.5, 6.5), 4.04 (1 H, dd, J = 3.5, 7), 3.66 (2 H, s). **12**: mp 135° $[\alpha]_D^{25}$ = +36.8° (c = 1, $CHCl_3$); IR ($CHCl_3$): 3940, 1790, 1765, 1680, 1560, 1560 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40-7.30 (10 H, m), 6.08 (1 H, d, J = 7), 5.40 (2 H, s), 5.23 (2 H, s), 5.09 (1 H, ddd, J = 3, 6, 7), 4.18 (1 H, dd, J = 6, 6), 3.95 (1 H, dd, J = 3, 6), 3.67 (2 H, s). **14**: IR (CH_2Cl_2): 3400, 1795, 1760, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.64 (1 H, d, J = 7), 7.5-7.3 (10 H, m), 5.41 (2 H, s), 5.27 (1 H, m), 5.23 (2 H, s), 4.26 (1 H, dd, J = 6, 6), 3.94 (1 H, dd, J = 3, 6), 3.42 (3 H, q, J = 1.2). **1**: IR (KBr): 1785 cm^{-1}; ^{1}H NMR (D_2O) δ 7.40 (5 H, m), 5.35 (1 H, d, J = 16), 5.20 (1 H, d, J = 16), 5.15 (1 H, m), 4.24 (1 H, dd, J = 5.5, 6.5), 4.08 (1 H, dd, J = 3.5, 5.5), 3.72 (2 H, s). **2**: IR (KBr): 3400 br, 1775, 1700 broad cm^{-1}; ^{1}H NMR (D_2O) δ 7.36 (5 H, m), 5.27 (2 H, s), 5.08 (1 H, dd, J = 3, 5.5), 4.20 (1 H, dd, J = 3, 5.5, 5.5), 3.99 (1 H, dd, J = 3, 5.5), 3.68 (2 H, s).

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8) Acylation of aniline with (+)-MTPACl gave the amide, mp 73°C, showing a PMR quartet (1:2:2:1), centered at 3.54 δ, J = 1.5 Hz ($CDCl_3$). This five-bond coupling to fluorine has been noted before: B. L. Hirschbein and G. M. Whitesides, J. Am. Chem. Soc. **104**, 4458 (1982).

9) Suitable crystals for X-ray analysis of **10** formed from methylene chloride/hexane mixtures. The symmetry space group was $P2_12_12_1$ with a = 9.324(1)Å, b = 9.438(1)Å, and c = 22.742(2)Å for Z = 4. An automatic four-circle diffractometer equipped with Cu radiation (λ = 1.5418Å) was used to measure 1601 unique reflections with $2\theta \leq 114°$. Of these 1373 were observed ($I \geq 3\sigma I$) and corrected for Lorentz and polarization effects. A multi-solution tangent formula approach [the following library of crystallographic programs was used: MULTAN 80, Univ. of York, York, Eng. (1980); Structure Determination Package V17.0, Enraf-Nonius

Co., Delft, Holland (1981); ORTEP-II, Oak Ridge National Laboratory, Oak Ridge, Tenn. (1970)] with tangent formula recycling [J. Karle, Acta Cryst. **B24**, 182 (1968)] was used to find initial positions for all non-hydrogen atoms. Full matrix least squares and difference Fourier methods were used to find refined positions. The function minimized was $\Sigma\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ to give an unweighted residual index of .053. Tables containing the final fractional coordinates, temperature parameters, bond distances and bond angles, have been deposited in the Cambridge Crystallographic Database.

10) E. coli, Sal. typhimurium, Ent. cloacae, Ent. aerogenes, K. pneumoniae, Prot. vulgaris, Prot. morganii, Prot. mirabilis, Ps. aeruginosa, Ser. marcesans.

11) Calculated by rotation of N7-C10 in **10** to minimize the C11-021 distance. This value is beyond the range of active compounds (<4.1 Å) cited by Cohen.[1]

12) It must be cautioned that any observed activity of **1** or **2** may not be due to the same mechansims of action shared by "classical" beta-lactam antibiotics, i.e., the penicillin-binding proteins and cell-wall synthesis inhibition D. J. Waxman and J. L. Strominger, Annual Rev. Biochem. **52**, 825 (1983).

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