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SYNTHESIS OF PHENOXYACETYL-N-(HYDROXYDIOXOCYCLOBUTENYL)CYCLOSERINES

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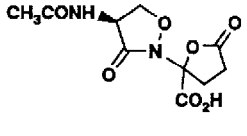
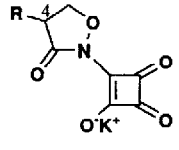
Abstract: The synthesis and antibacterial activity of a potential antibiotic, phenoxyacetyl-(L)-cycloserine activated by a cyclobutenedione moiety, is described.

β–Lactam antibiotics, such as penicillins and cephalosporins are known to interfere with the biosynthesis of the peptidoglycan layer of bacterial cell walls presumably by acylating certain enzymes (penicillin binding proteins) involved in the process [1] Traditional β-lactam antibiotics such as penicillins and the more recently discovered carbapenems[2] carry a strained bicyclic system, which results in an activated β-lactam amide bond with enhanced acylating power. The strained bicyclic ring system is thought to be essential for good antibacterial activity. The recent discovery of monocyclic lactams such as the monobactams[3] and the lactivicins[4] indicates that good antibacterial activity can also be attained with monocyclic lactams provided the lactam amide bond is activated by an electron withdrawing or an electronegative substituent. It has been suggested that activation of the β-lactam bond coupled with the presence of an anionic substituent in close proximity to the lactam bond[5] are important structural parameters necessary for useful antibacterial properties.

The new antibiotic, lactivicin (**1**) was of particular interest to us. Lactivicin, although not a β-lactam, has been shown to bind to penicillin binding proteins, thus indicating a similar mode of action to the β-lactam antibiotics.[4a] The cyclic amide of lactivicin is activated cumulatively by an electronegative oxygen bonded directly to the nitrogen and the accompanying lactone moiety.[4b] Recently, Baldwin and co-workers described the synthesis and antibacterial activity of cycloserine derivatives whose amide bond was activated by a group other than a lactone functionality.[6]



1

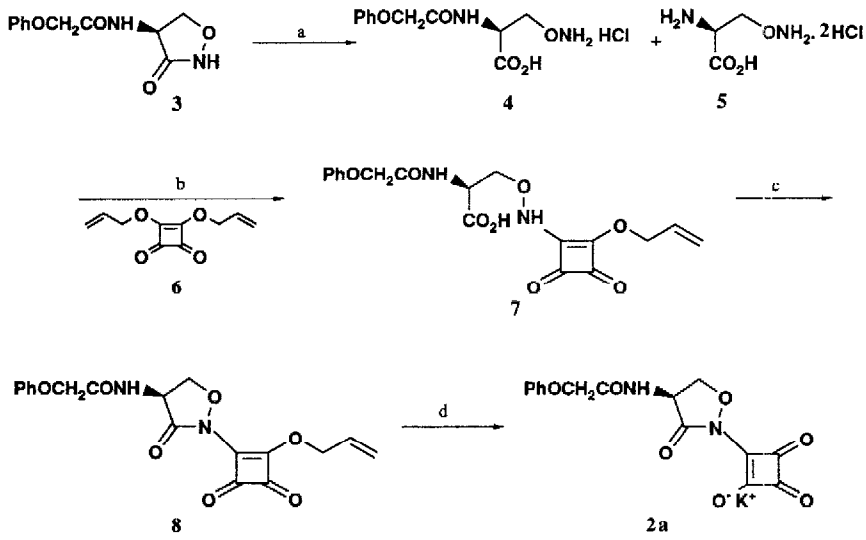


2a, R= 4β-(S)-NHCOCH$_2$OPh
2b, R= 4α-(R)-NHCOCH$_2$OPh

Here we report the synthesis of phenoxyacetyl-N-(hydroxydioxocyclobutenyl)cycloserines (**2**) The hydroxycyclobutenedione group in **2** is considered to activate the lactam amide by delocalization of the nitrogen lone pair. The hydroxycyclobutenedione moiety serves not only as an amide-activating functionality, but also as the source of the required[5] anionic center In particular, the (L)-isomer **2a** was thought to be a rational analog of lactivicin (**1**)

Phenoxyacetyl-(L)-cycloserine (**3**),[6] prepared by the method of Stammer,[7] was treated with 3N HCl in H_2O for 4 days at room temperature to afford phenoxyacetyl-(L)-O-aminoserine hydrochloride (**4**). The product was shown to be contaminated with O-aminoserine dihydrochloride (**5**) (see Scheme) as a result of further hydrolysis at the side chain amide.[8] The diallyl squarate (**6**)[9] was treated with the crude mixture containing **4** in the presence of Et_3N (2eq.) in DMF at room temperature. In this way O-(allyloxydioxocyclobutenyl)aminoserine **7**[10] was produced in 36% overall yield from **3**. Acylation using DCC as an activating agent proceeded smoothly, affording, after purification by silica gel[11] column chromatography in 51% yield, an acid sensitive cycloserine derivative **8**.[10] The allyl group was catalytically cleaved by palladium (0)[12] in the presence of N-methylaniline[13] to afford the target molecule **2a**[10] in 51% yield after purification on C-18 reverse phase silica [14]

SCHEME :



Reagents and Conditions:

a) 3N HCl/H_2O, rt, 4 days; b) Et_3N(2eq)/DMF, rt, 4-5 hrs,
c) Dicyclohexylcarbodiimide(DCC)/CH_2Cl_2, rt, 1 5 hrs, d) 1, $Pd(PPh_3)_4$/ CH_2Cl_2/N-methylaniline, rt, 3 hrs; 2, pH 7 0 potassium phosphate buffer

Repetition of the sequence described above, starting with (D)-cycloserine gave phenoxyacetyl-(D)-cycloserine analog **2b.**[14]

The (L)-cycloserine derivative **2a** exhibited moderate antibacterial activity against *Staph. aureus* A9537 (MIC, 32 μg/mL), *Strep. pneumoniae* A9585 (MIC, 4 μg/mL) and *Staph. epidermidis* A24548 (MIC, 32 μg/mL) As anticipated, (D)-cycloserine analog **2b** did not display any antibacterial activity.

The compounds **2** (both **2a** and **2b**) were found to be hydrolytically unstable, the chemical half-life being about 4 h in pH 7 buffer at 21°C, indicating the activating effect of the hydroxycyclobutenedione group.[15] The limited chemical stability is probably one of the factors associated with the relatively poor antibacterial activity of **2a.**

Acknowledgement: We would like to thank Dr. J. Fung-Tomc and her group for in vitro antibacterial assays and our Analytical Research Staff members for spectral and analytical measurements.

References and Notes:

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3. W H. Koster, C.M. Cimarusti and R.B. Sykes, in reference 1, Vol 3, pp 339-375.
4. a) Y. Nozaki, N. Katayama, H. Ono, S. Tsubotani, S. Harada, H. Okazaki, and Y. Nakao *Nature* **325**, 179 (1987).
b) S. Harada, S. Tsubotani, T. Hida, K. Koyama, M. Kondo, and H. Ono *Tetrahedron* **44**, 6589 (1988).
5 N C. Cohen *J. Med. Chem.* **26**, 259 (1983)
6 a) J.E. Baldwin, S.C. Ng and A.J. Pratt *Tetrahedron Letters* **28**, 4319 (1987).
b) J.E. Baldwin, C. Lowe and C.J. Schofield *Tetrahedron Letters* **31**, 2211 (1990).
c) See also a recent review article: J. E. Baldwin, G. P. Lynch and J. Pitlik *J. Antibiotics* **44**, 1(1991).
7. C.H Stammer, C.C. Kartha, N.C. Chaturvedi and J D Mckinney *J. Med. Chem.* **13**, 1013 (1970).
8. The other byproduct, phenoxyacetic acid was removed during the workup procedure
9. Prepared from squaric acid and allyl alcohol (U.S. Patent 4,092,146; May 30, 1978). For a review on squaric acid (dihydroxycyclobutenedione) see A H. Schmidt *Synthesis* 961 (1980).
10. Physical data: Compound **7**. yellowish foam; $[\alpha]^{20}_{D}$ -27.78° (c 0 16, CH_2Cl_2); IR (KBr): 3160 (br), 1800, 1720, 1650, 1600 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 4.19 (1H, dd, J=11, 4 Hz), 4 27 (1H, dd, J=11, 6 Hz), 4.53 (2H, s), 4.67 (1H, m), 5.07 (2H, d, J=5.5 Hz), 5 28 (1H, d, J=10.5 Hz), 5.39 (1H, d, J=17 Hz), 6.02 (1H, m), 6.97 (1H, t, J=7 Hz), 6.99 (2H, d, J=7 Hz), 7.30 (2H, t, J=7 Hz), 8 32 (1H, d, J=8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 52.37, 68.38, 74.78, 77.02, 116.51, 121.26, 123.04, 131.24, 134.07, 159.29, 169.63, 170.88, 172.19, 173 40, 186.67; MS (FAB/NOBA + Na): m/e 413 (M + Na); HRMS (FAB/NOBA): calcd for $C_{18}H_{19}N_2O_8$ (MH^+)

391.1141, found 391.1128; UV (CH_2Cl_2) λmax 270nm (ε = 2.22 x 10^4); Anal Calcd for $C_{18}H_{18}N_2O_8.1/2\ H_2O$: C, 54.14; H, 4.80; N, 7.02. Found: C, 53.99; H, 4.83; N, 7 01. Compound **8**: white solid, mp 104-106° C (i-PrOH); $[\alpha]^{20}_D$ -10.61° (c 0.13, CH_2Cl_2); IR (KBr): 1810, 1765, 1740, 1680, 1600 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ ppm: 4.59 (2H, s), 4.60 (1H, m), 4.89 (1H, t, J=8 5 Hz), 5.25 (1H, m), 5.27 (2H, d, J=5.5 Hz), 5.37 (1H, d, J=9.5 Hz), 5.42 (1H, d, J=17 Hz), 6.11 (1H, m), 6.97-7.02 (3H, m), 7.32 (2H, m), 8.32 (<1H, d, J=8 Hz); ^{13}C NMR (75 MHz, acetone-d_6) δ ppm: 50.73, 67.75, 72.67, 75.03, 115.55, 120.43, 122 47, 130.26, 132 60, 158.45, 165 84, 169.36, 180.23, 181.44, 187.75; MS (Isobutane-DCI): m/e 373 (MH^+); HRMS (FAB/NOBA) calcd for $C_{18}H_{17}N_2O_7$ (MH^+) 373.1036, found 373.1032; UV (CH_2Cl_2) λmax 226 (ε = 8.27 x 10^3), 260 (ε = 1.12 x 10^4), 312 nm (ε = 2.01 x 10^4); Anal. Calcd for $C_{18}H_{16}N_2O_7$ C, 58.07, H, 4 34; N, 7.53. Found: C, 57.72; H, 4.50; N, 7 33 Compound **2a**[14]: yellowish powder; $[\alpha]^{20}_D$ -45.25° (c 0.40, H_2O); IR (KBr): 1800, 1730 (sh), 1690, 1600 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ ppm 4 24 (1H, dd, J=10, 8 Hz), 4.50-4.55-4.56-4.61 (2H, Abq), 4.66 (1H, t, J=8 Hz), 5.08 (1H, m), 4.96 (3H, m), 7.30 (2H, m), 8.82 (1H, d, J=8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 51.71, 68.42, 72.58, 116.48, 123.06, 131.26, 159.24, 164 47, 168 69, 170 02, 187.24; MS (FAB/NOBA-Na) m/e 355 (M+H-K+Na); HRMS (FAB/NOBA) calcd for $C_{15}H_{12}N_2O_7K$ (MH^+) 371.0282, found 371.0278; UV (H_2O) λmax 252 (ε = 1.27 x 10^4), 322nm (ε = 1.56 x 10^4).

11. Silica gel 60 extra pure (E. Merck # 7754) was used since cycloserine derivative **8** decomposed on regular silica gel 60 (E. Merck # 7734).
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13. H. Mastalertz, M. Menard, V Vinet, J. Desiderio, J Fung-Tomc, R. Kessler, and Y. Tsai *J Med. Chem.* **31**, 1190 (1988).
14. The compounds **2a** and **2b** could not be obtained in pure form, due to the instability of these molecules in aqueous solution. Certain amounts of **2** decomposed during the lyophilization process.
15. The hydrolysis in pH 7 buffer gave a complex mixture of products. Formation of phenoxyacetylcycloserine was not detected during the hydrolysis, suggesting the initial cleavage may be at the lactam amide

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