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A Straightforward Synthesis of Proclavaminic Acid, a Biosynthetic Precursor of Clavulanic Acid

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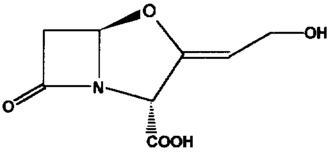
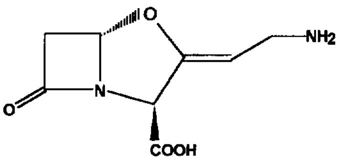
Abstract: A stereocontrolled synthesis of (2*S*,3*R*)-5-amino-3-hydroxy-2-(2-oxoazetin-1-yl) pentanoic acid, proclavaminic acid **3**, has been achieved via the homochiral 4,5-disubstituted oxazolidin-2-one **4**. Copyright © 1996 Elsevier Science Ltd

Clavulanic acid **1**, a medicinally important bicyclic β-lactam produced by *Streptomyces clavuligerus*,[1] is a potent inhibitor of many bacterial β-lactamases[2] present in Gram-positive and Gram-negative organisms and usually used clinically in formulation with amoxycillin to treat infections caused by β-lactamase producing bacteria. Recently, great efforts have been made to clarify the biosynthetic pathway of clavulanic acid. These studies have demonstrated[3] that the biosynthesis of clavulanic acid proceeds *via* clavaminic acid **2**, which is in turn produced from (2*S*,3*R*)-5-amino-3-hydroxy-2-(2-oxoazetin-1-yl)-pentanoic acid, proclavaminic acid **3**, by the enzyme clavulanic acid synthase (CAS), a 2-oxoglutarate-linked oxygenase. The proclavaminic acid is presumably obtained[4] by the action of a hydrolase present in the *S. clavuligerus*, named proclavaminic acid amidino hydrolase, on the corresponding N-guanidino proclavaminic acid. The last biosynthetic intermediate is produced[4] by the action of CAS on N^2-(2-carboxyethyl)-L-arginine *via* the initial formation of the β-lactam ring, followed by hydroxylation.



1



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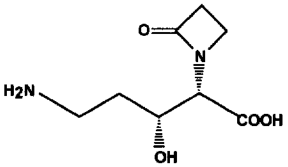
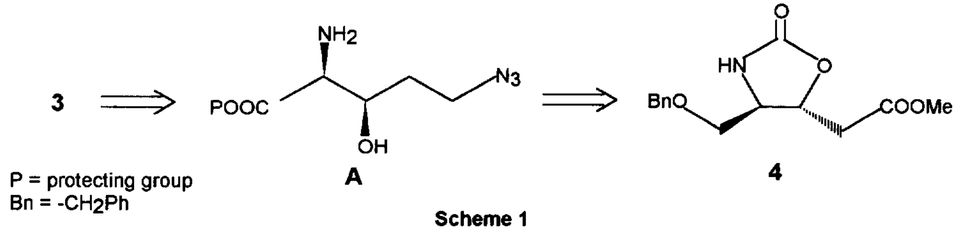


3

Chemical synthesis of this important β-hydroxy-α-amino acid has been reported by Baggaley *et al.* [5] with the aim of assigning its absolute configuration. The synthesis was based on an aldol reaction between a suitable 3-azidopropionaldehyde or 3-(benzyloxycarbonylamino)propionaldehyde with a Schiff's base of glycine ester to enantiomers of the *threo*-amino alcohols were resolved by the use of the acylase [EC 3.5.1.11] from *Escherichia coli*. The (2*S*,3*R*)-isomer was transformed into proclavaminic acid and then bioconverted into clavulanic acid by the action of CAS. A second approach[6] was based on a [3+2] cycloaddition of a suitable *N*-benzyl nitrone with protected *N*-Cbz-L-vinyl glycine benzyl ester to give the corresponding *erythro* and *threo* isoxazolidines in a 2:3 ratio. The major isoxazolidine was converted into (2*S*,3*R*) proclavaminic acid.

Unfortunately the required protected vinyl-glycine was difficult to prepare in optically active form following the Hanessian procedure, as reported by Townsend *et al.*,[6] due to racemization which occurs during the decarboxylative oxidation.

As part of ongoing studies[7] directed to the synthesis of biologically important β-hydroxy-α-amino acids, we wish to report a straightforward synthesis of the title compound **3** by the use of the homochiral 4,5-disubstituted oxazolidin-2-one **4**, through the intermediate **A** as shown in Scheme 1. The azido group was used as a protecting group for the 5-amine function during the construction of the β-lactam ring.



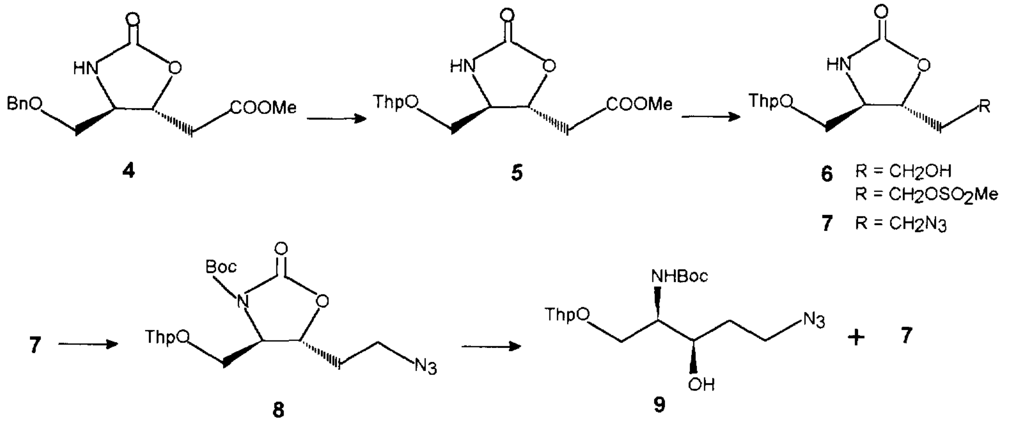
Scheme 1

The chiral heterocycle **4** has been reported by us[7] as a convenient intermediate for the synthesis of (2*S*,3*R*)-3-hydroxy-ornithine, *threo*-3-hydroxy-glutamic acid, and (2*S*,3*R*)-3-hydroxy-proline. Compound **4** is easily prepared[7a] *via* highly stereoselective iodocyclocarbamation of methyl (2*Z*,4*R*)-5-benzyloxy-4-[(benzyloxycarbonyl)-amino]-2-pentenoate, readily available from L-serine,[8] followed by tributyltin hydride reduction.

In the event, according to the synthetic pathway shown in scheme 2, the benzyl ether in **4** was rapidly converted, in 95% yield, into the tetrahydropyranyl ether **5** by catalytic hydrogenolysis followed by reprotection,[9] in order to have a protecting group compatible with the presence of the azide function in the successive intermediates.

Treatment of **5** with $LiAlH_4$ at -10 °C allowed the clean reduction, in 87% yield, of the ester function to give the crystalline alcohol **6**, which was in turn converted into the azide **7** (95% yield) through the corresponding mesylate. With this intermediate in hand, the synthetic plan foresaw the opening of the oxazolidin-2-one ring followed by the construction of the azetidinone ring.

Treatment of 7 with Boc_2O in the presence of catalytic DMAP gave in almost quantitative yield the N-Boc derivative **8**. Although it has been reported[10a] that treatment of N-Boc oxazolidin-2-ones with Cs_2CO_3 in MeOH proceeds with good regioselective cleavage of the ring, in our hands the reaction of **8** under the above reaction conditions gave only a mixture of **9** and 7, in almost 1:1 ratio, after 16 h. The lack of regioselectivity for this reaction seems to be general,[11] when it is applied to 4-alkyl substituted N-Boc oxazolidin-2-ones compared to the good regioselectivity observed[10,11] for the substrates where an acyl group is present in the 4-position.



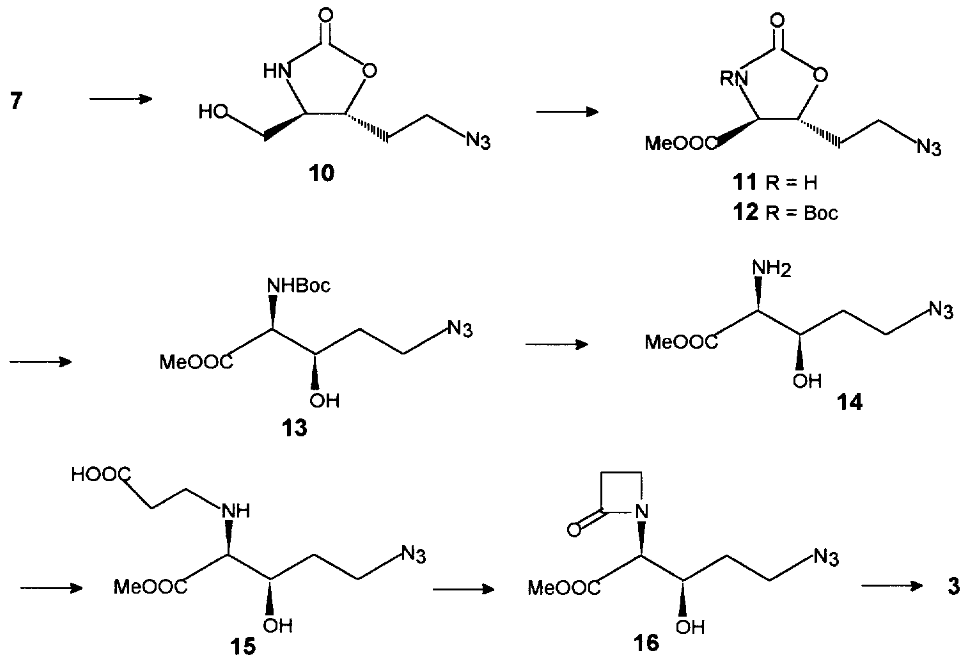
Scheme 2

To overcome this problem the tetrahydropyranyl ether in compound 7 was quantitatively converted[9] into the free alcohol **10** followed by oxidation under Jones conditions.[12] The acid was isolated as the methyl ester **11** in 72% yield from 7. Notably a two step oxidation (TEMPO, NaClO/NaBr[13] and 1M $KMnO_4$/5% NaH_2PO_4[14]) of **10**, followed by esterification, gave lower yields (60%) of **11**.

The last compound was then converted into the N-Boc derivative **12**, according to the conditions described above, and treated with Cs_2CO_3 (0.25 eq.) in MeOH at room temperature.

Ring opening took place cleanly after 4h, to give the N-Boc amino alcohol **13** in 80% yield based on **11**. The next step was the removal of the Boc-protecting group, which was realized under neutral conditions, by a slight modification of Ohfune's procedure[15] to give directly the required amino-alcohol **14** in 84% yield (Scheme 3). Alternative procedures, based on the use of either acidic conditions or of catechol boron halides,[16] gave a complex mixture of products, due probably to competitive β-elimination.

The free amino alcohol was treated[5] with an excess of acrylic acid in hot CH_3CN to obtain the β-amino acid **15** in 76% yield. The β-lactam formation was then studied. The presence of the azido group prevents the use of triphenylphosphine based condensing reagents to avoid competitive reactions through the formation of



Scheme 3

an imino phosphorane.[17] Nevertheless, after some attempts, the use of the Mukayama reagent,[18] 2-chloro *N*-methyl pyridinium iodide, in hot acetonitrile gave the expected 2-azetidinon-1-yl derivative **16** in 65% yield.

The title compound was finally obtained from **16** by preferential ester cleavage with LiI in anhydrous pyridine,[19] followed by catalytic hydrogenolysis to give **3**, which showed analytical data in agreement with those already reported.[5,6]

In conclusion, we have described a straightforward synthesis of proclavaminic acid **3** by the use of the homochiral 4,5-disubstituted oxazolidin-2-one **4**; the present method proves to be a suitable alternative to the known procedures. Further applications of the above 4,5-disubstituted oxazolidin-2-ones for the synthesis of threonine analogues are currently under investigation and will be published in due course.

Experimental

Melting points were determined in open capillaries using a Büchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5DX FT-IR spectrophotometer. ^{1}H- and ^{13}C NMR spectra were run on a Varian Gemini 300 spectrometer at 300 and 75 MHz respectively, in $CDCl_3$, unless otherwise reported. Chemical shifts (δ scale) are relative to TMS as internal reference. Compounds **5-9** were a 1:1 mixture of diastereoisomers. The proton chemical-shift in ^{1}H NMR spectra were assigned by HETCOR correlations with carbon signals, but the multiplicities were not determined. Optical rotations were determinated on a Perkin

Elmer 243 polarimeter at 21 °C. All solvents were dried[20] prior to use. Thin layer chromatography was performed on Merck silica gel 60 F_{254} glass plates.

Methyl (4*R*,5R)-4-(tetrahydropyranyloxy)methyl-2-oxo-5-oxazolidineacetate 5. A solution of **4** (2.00 g, 7.17 mmol) in absolute EtOH (70 ml) was hydrogenated over 10% Pd/C at 1 atm for 18 h. After that time the solution was diluted with EtOH (50 ml), filtered over Celite and concentrated under reduced pressure. To a solution of the above hydroxy ester in dry CH_2Cl_2 (50 ml) containing pyridinium *p*-toluensolfonate (20 mg, 0.08 mmol) was added 3,4-dihydro-2H-pyran (1.06 ml, 11.6 mmol) and the mixture was stirred at room temperature for 4 h, then the solution was diluted with CH_2Cl_2 and washed with brine (2 × 100 ml). The organic layer was dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by silica gel chromatography with $CHCl_3$/MeOH (98:2) as eluent to give **5** (1.87 g, 95%) as a colourless oil. $[\alpha]_D$ = + 44.50 (c = 0.044, $CHCl_3$); IR (neat) ν: 3312 cm^{-1}, 2951,1745, 1426, 1384, 1128, 1032. ^1H NMR (1:1 mixture of diastereoisomers) δ: 6.23, 6.13 (1H each, s, NH), 4.71, 4.69 (1H each, m, CHO), 4.61 (2H, m, ΣJ = 11.0 Hz, OCHO, Thp), 3.85 (1H, dd, J = 9.0 and 5.0 Hz, $C\underline{H}_AH_BO$), 3.78, 3.54 (2H each, m, CH_2O, Thp), 3.77, 3.53 (1H each, m, CHN), 3.73 (3H × 2, s, $COOCH_3$), 3.45 (1H, dd, J = 9.0 and 6.0 Hz, $CH_A\underline{H}_BO$); 2.87, 2.76 (2H each, dd, J = 15.0 and 6.0 Hz, CH_2CO), 1.8-1.4 (2H × 3, m, 3 × CH_2,Thp). ^{13}C NMR δ: 169.69 (s, $\underline{C}OOCH_3$), 158.38 (s, CONH), 99.41, 99.22 (d each, OCHO, Thp), 74.91, 74.84 (d each, OCHO), 69.40, 68.90 (t each, CH_2O), 62.51, 62.40 (t each, CH_2O, Thp), 57.18, 56.97 (d each, CHN), 52.04 (t, $COO\underline{C}H_3$), 38.96 (t, $\underline{C}H_2CO$), 30.34, 30.24, 25.16, 25.12, 19.27, 19.21 (t each, 3 × CH_2, Thp). Anal. Calcd. for $C_{12}H_{19}NO_6$: C, 52.74%; H, 7.01; N, 5.13. Found: C, 52.39%; H, 6.81; N, 5.17.

(4*R*,5*R*)-4-(Tetrahydropyranyloxy)methyl-5-(2-hydroxyethyl)-2-oxo-oxazolidine 6. To a solution of **5** (1.82 g, 6.66 mmol) in dry THF (50 ml) at -10 °C (ice + NaCl) a solution of $LiAlH_4$ (1M in THF, 7.32 ml) was added dropwise over 15 min with stirring under N_2. The reaction mixture was stirred at room temperature for 2 h followed by the addition of EtOAc (10 ml) and H_2O (2 ml). The mixture was stirred for 15 min, diluted with EtOAc (50 ml), filtered through a pad of Celite and dried over Na_2SO_4. The organic solution was filtered, concentrated under reduced pressure and purified by silica gel chromatography using $CHCl_3$/MeOH (95:5) as eluent to give **6** (1.43 g, 87%) as a white solid. M.p. 85-6 °C (EtOAc/pentane). $[\alpha]_D$ = +73.68 (c = 0.05, MeOH). IR(KBr) ν: 3318 cm^{-1}. 2942, 1724, 1470. ^1H NMR (1:1 mixture of diastereoisomers) δ: 5.58, 5.40 (1H each, brs, NH), 4.59 (2H, m, ΣJ = 12.0 Hz, OCHO, Thp), 4.50 (2H, m, ΣJ = 28.0 Hz, CHO), 3.85 (4H, t, J = 6.0 Hz, $C\underline{H}_2OH$), 3.83, 3.45 (2H each, m, CH_2O), 3.80, 3.55 (2H each, m, CH_2O, Thp), 3.75 (2H, m, CHN), 2.04, 1.95 (2H each, m, $C\underline{H}_2CH_2OH$), 1.8-1.4 (12H, m, 3 × CH_2, Thp). ^{13}C NMR δ: 158.36 (s, CONH), 99.76, 99.54 (d each, OCHO, Thp), 77.31, 77.20 (d each, CHO), 69.96, 69.36 (t each, CH_2O), 62.86 (t, CH_2O, Thp), 58.59, 58.51 (t each, CH_2OH), 57.53, 57.21 (d each, CHN), 37.34, 37.23 (t each, $\underline{C}H_2CH_2OH$), 30.45, 30.36, 25.16, 25.12, 19.51 (t each, 3 × CH_2, Thp). Anal. Calcd. for $C_{11}H_{19}NO_5$: C, 53.87%; H, 7.81; N, 5.71. Found: C, 53.94%; H, 7.78; N, 5.69.

(4*R*, 5*R*)-4-(Tetrahydropyranyloxy)methyl-5-(2-azidoethyl)-2-oxo-oxazolidine 7. A solution of alcohol **6** (1.4 g, 5.71 mmol) in dry CH_2Cl_2 (30 ml) was cooled at 0°C and treated with Et_3N (1.2 ml, 8.6 mmol) and methanesulfonyl chloride (0.531 ml, 6.85 mmol). After stirrring at 0 °C for 30 min, the reaction was quenched with H_2O (10 ml) and concentrated under reduced pressure. The aqueous residue was extracted with EtOAc (3 × 100 ml), and the combined organic layers were washed with H_2O (100 ml), brine (100 ml), dried over

Na_2SO_4, filtered and concentrated under reduced pressure. The crude sulfonate was dissolved in dry DMF (35 ml) and treated with excess of NaN_3 (2.23 g, 34.3 mmol). The resultant suspension was stirred for 16 h at 65 °C, diluted with EtOAc (100 ml) and filtered through Celite. The organic solution was washed with 10% $NaHCO_3$ (150 ml), H_2O (100 ml), brine (100 ml), and dried over Na_2SO_4. The solution was filtered, concentrated under reduced pressureand purified by silica gel chromatography using EtOAc/hexane (55:45) as eluent to give the compound **7** (1.47 g, 95%) as a colourless oil. $[\alpha]_D = +67.23$ (c = 0.03, $CHCl_3$). IR (KBr) ν: 3279 cm^{-1}, 2943, 2098, 1753, 1376, 1256, 1128, 1032. ^1H NMR (1:1 mixture of diastereoisomers) δ: 5.71, 5.56 (1H each, brs, NH), 4.60 (2H, m, $\Sigma J = 12.0$ Hz, OCHO, Thp), 4.41 (2H, dt, $J = 2 \times 10.0$ and 5.0 Hz, CHO), 3.81, 3.55 (2H each, m, CH_2O, Thp), 3.75 (2H, m, $C\underline{H}_AH_BO$), 3.73 (2H, m, CHN), 3.54 (2H × 2, m, CH_2N_3), 3.43 (2H, dd, $J = 10.0$ and 7.0 Hz, $CH_A\underline{H}_BO$), 2.03, 1.93 (2H each, m, $C\underline{H}_2CH_2N_3$), 1.76, 1.55 (2H, 4H, m, 3 × CH_2, Thp). ^{13}C NMR δ: 158.17 (s, CONH), 99.72, 99.39 (d each, OCHO, Thp), 76.32, 76.21 (d each, CHO), 69.69, 69.02 (t each, CH_2O), 62.81, 62.67 (t each, CH_2O, Thp), 57.42, 57.15 (d each, CHN), 47.05 (t, CH_2N_3), 34.24, 34.18 (t each, $\underline{C}H_2CH_2N_3$), 30.41, 30.30, 25.17, 25.11, 19.45, 19.36 (t each, 3 × CH_2, Thp). Anal. Calcd. for $C_{11}H_{18}N_4O_4$: C, 48.88%; H, 6.71; N, 20.73. Found: C, 49.01%; H, 6.91; N, 20.77.

(4*R*,5*R*)-3-(*tert*-Butoxycarbonyl)-4-(tetrahydropyranyl)methyl-5-(2-azidoethyl)-2-oxo-oxazolidine 8 . To a well stirred solution of the compound **7** (0.45 g, 1.66 mmol) in dry THF (22 ml) was added Et_3N (0.278 ml, 1.99 mmol), Boc_2O (0.474 g, 2.17 mmol) and DMAP (0.041 g, 0.33 mmol). The solution was stirred overnight at room temperature and concentrated under reduced pressure. The residue was diluted with $CHCl_3$ (100 ml), washed with 5% $NaHCO_3$ (50 ml), brine (100 ml) and dried over Na_2SO_4. The organic solution was filtered, concentrated under reduced pressure followed by purification over silica gel with EtOAc/hexane (1:1) as eluent to give the N-Boc derivative **8** (558 mg, 95%) as a solid. M.p. 59-60 °C (Et_2O/hexane). $[\alpha]_D = +9.2$ (c = 0.1, $CHCl_3$). IR (KBr) ν: 2935 cm^{-1}, 2106, 1802, 1713, 1376, 1286, 1196, 1073. ^1H NMR (1:1 mixture of diastereoisomers) δ: 4.67 (1H, t, $J = 3.0$ Hz, OCHO, Thp), 4.59 (1H, dd, $J = 5.0$ and 3.0 Hz, OCH, Thp), 4.57 (2H, m, CHO), 4.07 (1H, dt, $J = 6.0$ and 2 × 3.0 Hz, CHN), 4.04 (1H, dt, $J = 5.5$ and 2 × 3.0 Hz, CHN), 4.00 (1H, dd, $J = 10.0$ and 3.5 Hz, $C\underline{H}_AH_BO$), 3.75 (1H, m, $C\underline{H}_CH_DO$, Thp), 3.74 (2H × 2, m, 2 × CH_2O, Thp), 3.6 (1H, m, $CH_A\underline{H}_BO$), 3.45 (1H, m, $CH_C\underline{H}_DO$,Thp), 3.40 (2H × 2, m, CH_2N_3), 1.99, 1.89 (2H each, m, CH_2), 1.56 (2H × 2, m, 2 × CH_2, Thp), 1.55 [9H × 2, s, 2 × $C(CH_3)_3$], 1.54 (2H × 4, m, 4 × CH_2, Thp). ^{13}C NMR δ: 151.51, 151.45 (s each, NCOO), 149.18 [s, $N\underline{C}OOC(CH_3)_3$], 99.75, 98.28 (d each, OCHO, Thp), 84.21, 84.18 [s each, $O\underline{C}(CH_3)_3$], 73.55, 73.48 (d each, CHO), 66.27, 66.59 (t each, CH_2O), 62.65, 61.86 (t each, CH_2O, Thp), 59.25, 59.09 (d each, CHN), 46.78, 46.73 (t each, CH_2N_3), 34.33, 34.21 (t each, CH_2), 30.30, 30.17 (t each, $-CH_2$, Thp), 27.94 [q, $C(\underline{C}H_3)_3$], 25.22, 25.19 (t each, $-CH_2$, Thp), 19.33, 18.73 (t each, $-CH_2$, Thp). Anal. Calcd. for $C_{16}H_{26}N_4O_6$: C, 51.88%; H, 7.08; N, 15.13. Found: C, 51.64%; H, 7.16; N, 15.15.

(2*R*,3*R*)-5-Azido-3-hydroxy-2-*tert*-butoxycarbonylamino-1-tetrahydropyranyloxypentane 9 . To a well stirred solution of **8** (0.5 g, 1.35 mmol) in dry MeOH (20 ml) was added Cs_2CO_3 (88 mg, 0.27 mmol) in one portion at room temperature. The mixture was stirred for 16 h at the same temperature, neutralized with citric acid and concentrated under reduced pressure. The residue was purified over silica gel chromatography using EtOAc/hexane (65:35) as eluent to give **9** (183 mg, 40%) and **7** (128 mg, 35%). $[\alpha]_D = -8.6$ (c = 0.1, $CHCl_3$). IR (neat) ν: 3427 cm^{-1}, 2943, 2098, 1712, 1515, 1171, 1032. ^1H NMR (1:1 mixture of diastereoisomers) δ: 5.24, 5.19 (1H each, brd, $J = 9.0$ Hz, NH), 4.57 (1H, brt, $J = 3.0$ Hz, OCH, Thp), 4.55 (1H, dd, $J = 5.0$ and

2.0 Hz, OCH, Thp), 4.09, 4.05 (1H each, m, CHO), 3.90, 3.85 (1H each, m, $C\underline{H}_AH_BO$), 3.86 (2H, m, -CH_2O, Thp), 3.65 (2H, m $CH_A\underline{H}_BO$), 3.60 (2H, m, CHN), 3.54 (2H, m, -CH_2O, Thp), 3.48 (2H , brt, J = 7.0 Hz, CH_2N_3), 1.78 (2H, m, $C\underline{H}_EH_F$), 1.75 (2H × 2, m, -CH_2O × 2, Thp), 1.72 (2H, m, $CH_E\underline{H}_F$), 1.50 (2H × 2, m, -CH_2 × 2, Thp), 1.45 (2H × 2, m, -CH_2 × 2, Thp), 1.45, 1.44 [9H each, s, $C(CH_3)_3$]. ^{13}C NMR δ: 156.03 (s, CONH), 99.89, 99.61 (1H each, OCH, Thp), 79.61, 79.53 [s each, $O\underline{C}(CH_3)_3$], 70.45, 69.31 (t each, CH_2O), 70.32, 68.89 (d each, CHO), 63.48, 62.75 (t each, CH_2O, Thp), 52.96, 52.84 (d each, CHN), 48.24, 48.12 (t each, CH_2N_3), 33.05, 32.70 (t each, CH_2), 30.63, 30.29 (t each, CH_2, Thp), 28.30 [q, $C(\underline{C}H_3)_3$], 25.06 (t, CH_2, Thp), 20.05, 19.59 (t each, CH_2, Thp). Anal. Calcd. for $C_{15}H_{28}N_4O_5$: C, 52.31%; H, 8.19; N, 16.27. Found: C, 52.44%; H, 8.29; N, 16.39.

(4*R*,5*R*)-4-(Hydroxymethyl)-5-(2-azidoethyl)-2-oxo-oxazolidine 10 . A solution of the THP ether **7** (1.40 g, 5.18 mmol) and pyridinium toluene-4-sulfonate (0,131 g, 0.520 mmol) in EtOH (42 ml) was stirred at 55 °C for 3h. After that time the solvent was evaporated under reduced pressure and the residue was purified over silica gel chromatography with $CHCl_3$/MeOH (93:7) as eluent to give **10** (0.956 g, 99%) as a solid. m.p. 55-56 °C (EtOAc/pentane). $[\alpha]_D$ = +39.66 (c = 0.04 MeOH). IR (KBr) ν: 3270 cm^{-1}, 2959, 2098, 1753, 1470, 1245, 1071. ^1H NMR δ: 4.37 (1H, ddd, J = 9.0, 5.5 and 4.5 Hz, CHO), 3.73 (1H, dd, J = 11.0 and 3.0 Hz, $C\underline{H}_AH_BO$), 3.66 (1H, dt, J = 2 × 5.5 and 3.0 Hz, CHN), 3.60 (1H, dd, J = 11.0 and 5.5 Hz, $CH_A\underline{H}_BO$), 3.51, 3.49 (1H each, ddd, J = 12.5, 7.5 and 6.0 Hz, CH_2N_3), 1.98 (1H, ddt, J = 14.0, 9.0 and 2 × 6.0 Hz, $C\underline{H}_CH_D$), 1.89 (1H, ddt, J = 14.0, 2 × 7.5 and 4.5 Hz, $CH_C\underline{H}_D$). ^{13}C NMR δ: 158.62 (s, CO), 75.65 (d, CHO), 63.42 (t, CH_2O), 59.78 (d, CHN), 47.15 (t, CH_2N_3), 34.04 (t, CH_2). Anal. Calcd. for $C_6H_{10}N_4O_3$: C, 38.71%; H, 5.41; N, 30.09. Found: C, 38.88%; H, 5.53; N, 30.14.

(4*S*,5*R*)-4-(Carboxymethyl)-5-(2-azidoethyl)-2-oxo-oxazolidine 11 . The Jones oxidation[12] was performed. A solution of hydroxy azide **10** (0.8 g, 4.3 mmol) in acetone (90 ml) was added dropwise at -5 °C to a solution of Jones reagent (13.6 ml) in acetone (37 ml). The Jones reagent was a solution of chromic trioxide (26.5 g), conc. sulfuric acid (23 ml) and water (to a total vol. of 100 ml). After 4 h at -5 °C, 2-propanol was added dropwise until the medium turned blue, water was added, and extraction with EtOAc (3 × 150 ml) was performed. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. The resulting crude acid was dissolved in EtOAc (10 ml), cooled at 0 °C and treated with an etheral solution of CH_2N_2, followed by stirring for 45 min at room temperature. Excess of CH_2N_2 was destroyed by addition of CH_3COOH (5M in CH_2Cl_2, 1 ml). After evaporation, the crude mixture was purified by silica gel chromatography using EtOAc/hexane (6:4) as eluent to give **11** (641 mg, 72%) as an oil. $[\alpha]_D$ = +68.90 (c = 0.02, $CHCl_3$). IR (neat) ν: 3305 cm^{-1}, 2104, 1769, 1405, 1222. ^1H NMR δ: 6.47 (1H, brs, NH), 4.75 (1H, dt, J = 7.5 and 2 × 5.0 Hz, CHO), 4.13 (1H, d, J = 5.0 Hz, CHN), 3.84 (3H, s, $COOCH_3$), 3.60 (1H, dt, J = 12.0 and 2 × 6.0 Hz, $C\underline{H}_AH_BN_3$), 3.54 (1H, ddd, J = 12.0, 7.0 and 5.0 Hz, $CH_A\underline{H}_BN_3$), 2.10 (1H, ddt, J = 14.0, 7.0 and 2 × 6.0 Hz, $C\underline{H}_CH_D$), 2.04 (1H, ddt, J = 14.0, 2 × 6.0 and 5 Hz, $CH_C\underline{H}_D$). ^{13}C NMR δ: 169.99 (s, $\underline{C}OOCH_3$), 157.95 (s, CONH), 76.14 (d, CHO), 58.60 (d, CHN), 53.30 (q, $COO\underline{C}H_3$), 46.93 (t, CH_2N_3), 34.59 (t, CH_2). Anal. Calcd. for $C_7H_{10}N_4O_4$: C, 39.26%; H, 4.71; N, 26.16. Found: C, 39.51%; H, 4.68; N, 26.09.

(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-4-(carboxymethyl)-5-(2-azidoethyl)-2-oxo-oxazolidine 12 . To a well stirred solution of the azido ester **11** (0.631 g, 2.95 mmol) in dry THF (30 ml) was added Et_3N (0.526 ml, 3.77 mmol), Boc_2O (0.895 g, 4.1 mmol) and DMAP (0.077 g, 0.61 mmol). The reaction was performed as for compound **8** to give **12** (0.927 g, 94%) as an oil. $[\alpha]_D$ = +11.86 (c = 0.11, $CHCl_3$). IR (neat) ν: 2970 cm^{-1}, 2100, 1829, 1757, 1376, 1336, 1164, 1072. ^1H NMR δ: 4.51 (1H, dt, *J* = 8.0 and 2 × 5.0 Hz, CHO), 4.45 (1H, d, *J* = 5.0 Hz, CHN), 3.84 (3H, s, $COOCH_3$), 3.59 (1H, dt, *J* = 11.0 and 2 × 6.0 Hz, $C\underline{H}_AH_BN_3$), 3.55 (1H, dt, *J* = 11.0 and 5.5 Hz, $CH_A\underline{H}_BN_3$), 2.06 (1H, ddt, *J* = 14.5, 7.5 and 5.5 Hz, $C\underline{H}_CH_D$), 2.01(1H, m, $CH_C\underline{H}_D$), 1.52 (9H, s, $C(CH_3)_3$). ^{13}C NMR δ: 168.64 (s, $\underline{C}OOCH_3$), 150.26 (s, CONH), 148.38 [s, $\underline{C}OOC(CH_3)_3$], 84.88 [s, $COO\underline{C}(CH_3)_3$], 72.58 (d, CHO), 60.94 (d, CHN), 53.19 (q, $COO\underline{C}H_3$), 46.40 (t, CH_2N_3), 34.47 (t, CH_2), 27.72 (q, CH_3). Anal. Calcd. for $C_{12}H_{18}N_4O_6$: C, 45.86%; H, 5.77; N, 17.83. Found: C, 45.73%; H, 5.61; N, 17.68.

Methyl (2*S*, 3*R*)-5-azido-3-hydroxy-2-*tert*-butoxycarbonylamino pentanoate 13 . To a stirred solution of **12** (0.9 g, 2.86 mmol) in dry methanol (43 ml), Cs_2CO_3 (0.233 g, 0.715 mmol) was added at room temperature. The mixture was stirred at the same temperature for 4h, neutralized with citric acid and concentrated under reduced pressure. The residue was dissolved in EtOAc (100 ml), washed with H_2O, brine and dried over Na_2SO_4. The residue was purified by silica gel chromatography using EtOAc/hexane (60:40) as eluent to give **13** (0.696 g, 83%) as a solid. M.p. 75-6 °C (EtOAc/pentane). $[\alpha]_D$ = +12.73 (c = 0.07, MeOH). IR (KBr) ν: 3451 cm^{-1}, 2945, 2106, 1745, 1696. ^1H NMR δ: 5.35 (1H, d, *J* = 9.0 Hz, NH), 4.32 (1H, dd, *J* = 9.0 and 2.0 Hz, CHN), 4.27 (1H, ddt, *J* = 2 × 6.5, 5.5 and 2.0 Hz, CHO), 3.79 (3H, s, $COOCH_3$), 3.53, 3.50 (1H, dt, *J* = 12.0 and 2 × 6.5 Hz, CH_2N_3), 2.54 (1H, d, *J* = 5.5 Hz, OH), 1.78 (2H, q, *J* = 6.5 Hz, CH_2), 1.46 [9H, s, $C(CH_3)_3$]. ^{13}C NMR δ: 171.59 (s, $\underline{C}OOCH_3$), 156.03 (s, CONH), 80.36 [s, $O\underline{C}(CH_3)_3$], 69.59 (d, CHO), 57.69 (d, CHN), 52.69 (q, OCH_3), 48.29 (t, CH_2N_3), 32.60 (t, CH_2), 28.23 (q, CH_3). Anal. Calcd. for $C_{11}H_{20}N_4O_5$: C, 45.83%; H, 6.99; N, 19.43. Found: C, 46.09%; H, 6.81; N, 19.38.

Methyl (2*S*,3*R*)-5-azido-3-hydroxy-2-amino pentanoate 14 . To a stirred solution of **13** (0.5 g, 1.74 mmol) in dry CH_2Cl_2 (5.0 ml) containing 2,6 lutidine (0.828 ml, 6.96 mmol) was added dropwise at room temperature *tert*-butyldimetyl silyl trifluoromethane sulfonate (1.2 ml, 5.25 mmol). The reaction mixture was stirred for 20 min at the same temperature, followed by the addition of a satd. solution of NH_4Cl (2 ml). The mixture was extracted with Et_2O (3 × 10 ml) and the combined organic extracts were washed with brine (20 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue containing the silyl carbamate was dissolved in dry THF (3.5 ml) and treated with a solution of tetrabutylammonium fluoride (1M in THF, 3.48 ml). The reaction was stirred for 1h at room temperature, then quenched with solid NH_4Cl and concentrated under reduced pressure. The residue was carefully purified on silica gel using $CHCl_3$/MeOH (90:10 → 80:20) as eluent to give the amino alcohol **14** (275 mg, 84%) as a solid. M.p. 66-7 °C (EtOAc/pentane). $[\alpha]_D$ = +5.3 (c = 0.1 $CHCl_3$); IR (KBr) ν: 3450 cm^{-1}, 2920, 2106, 1745, 1696, 1460, 1360, 1176. ^1H NMR δ: 3.89 (1H, dt, *J* = 9.0 and 2 x 4.5 Hz, CHO), 3.77 (3H, s, OCH_3), 3.52 (1H, dt, *J* = 12.5 and 2 × 6.0 Hz, $C\underline{H}_AH_BN_3$), 3.48 (1H, dt, *J* = 12.5 and 2 × 7.5 Hz, $CH_A\underline{H}_BN_3$), 3.38 (1H, d, *J* = 4.5 Hz, CHN), 1.81 (1H, ddt, *J* = 14.0, 9.0, and 2 × 6.0 Hz, $C\underline{H}_CH_D$), 1.79 (1H, ddt, *J* = 14.0, 2 × 7.5 and 4.5 Hz, $CH_C\underline{H}_D$). ^{13}C NMR δ: 171.46 (s, CO), 69.26 (d, CHO), 58.33 (d, CHN), 52.39 (q, OCH_3), 48.24 (t, CH_2N_3), 33.13 (t, CH_2). Anal. Calcd. for $C_6H_{12}N_4O_3$: C, 38.30%; H, 6.43; N, 29.77. Found: C, 38.34%; H, 6.45; N, 29.70.

Methyl (2*S*,3*R*)-2-(2-carboxyethyl)-5-azido-3-hydroxypentanoate 15 . To a solution of the amino alcohol **14** (0.15 g, 0.8 mmol) in dry CH_3CN (10 ml) was added acrylic acid (0.556 ml, 8.0 mmol) and the solution was stirred at 45°C for 24h. After this time the solvent and excess of acrylic acid was removed under vacuum and the residue purified by silica gel chromatography with $CH_3COOH/CHCl_3/MeOH$ (5%/75%/20%) as eluent to give the β-amino acid **15** (0.158 g, 76%) as a solid. M.p. 77-8 °C (EtOAc/hexane). $[\alpha]_D = +4.3$ (c = 0.1, H_2O). IR (KBr) ν: 3308 cm^{-1}, 2945, 2102, 1720, 1541, 1252. ^1H NMR (D_2O) δ: 4.39 (1H, brq, $J = 6.0$ Hz, NH), 4.32 (1H, q, $J = 6.0$ Hz, CHO), 4.12 (1H, t, $J = 6.0$ Hz, CHN), 3.82 (3H, s, OCH_3), 3.47, 3.25 (1H each, m, CH_2N_3), 3.37 (1H, dt, $J = 13.0$ and 2 × 6.0 Hz, $C\underline{H}_AH_BN$), 3.13 (1H, dt, $J = 13.0$ and 2 × 7.0 Hz, $CH_A\underline{H}_BN$), 2.61, 2.55 (1H each, m, CH_2CO), 2.15, 1.95 (1H each, m, CH_2). ^{13}C NMR (D_2O) δ: 175.95 (s, COOH), 170.71 (s, $\underline{C}OOCH_3$), 70.16 (d, CHO), 56.78 (d, CHN), 52.66 (q, OCH_3), 47.36 (t, CH_2N_3), 37.87 (t, $\underline{C}H_2CO$), 34.02 (t, CH_2N), 33.44 (t, CH_2). Anal. Calcd. for $C_9H_{16}N_4O_5$: C, 41.54%; H, 6.20; N, 21.53. Found: C, 41.44%; H, 6.24; N, 21.65.

Methyl (2*S*,3*R*)-5-azido-3-hydroxy-2-(2-oxoazetidine-1-yl)pentanoate 16 . To an acetonitrile suspension (26 ml) of the β-amino acid **15** (0.135 g, 0.52 mmol) and 2-chloro-1-methylpyridinium iodide (0.166 g, 0.65 mmol) was added Et_3N (0.182 ml, 1.3 mmol). The mixture was stirred for 24 h at 60 °C, followed by evaporation of the solvent. The crude residue was purified by silica gel chromatography to give the compound **16** (82 mg, 65%) as a slight yellow oil. $[\alpha]_D = +23.89$ (c = 0.05, $CHCl_3$). IR (neat) ν: 3409cm^{-1}, 2942, 2104. 1733, 1552, 1358. ^1H NMR δ: 4.89 (1H, dt, $J = 2 \times 9.5$ and 3.0 Hz, CHO), 4.43 (1H, d, $J = 9.5$ Hz, CHN), 3.86 (1H, ddd, $J = 15.0$, 9.0 and 6.0 Hz, $C\underline{H}_AH_BN$), 3.79 (3H, s, OCH_3), 3.77 (1H, ddd, J=15.0, 9.5, and 6.5 Hz, $CH_A\underline{H}_BN$), 3.59, 3.55(1H each, m, CH_2N_3), 3.09 (1H, ddd, $J = 15.0$, 9.5 and 6.0 Hz, $C\underline{H}_CH_DCO$), 2.99 (1H, ddd, $J = 15.0$, 9.0 and 6.5 Hz, $CH_C\underline{H}_DCO$), 2.12 (1H,ddd, $J = 14.5$, 12.0, 7.5 and 3.0 Hz, $C\underline{H}_EH_F$), 1.99 (1H, ddt, $J = 14.5$, 2 × 9.0 and 4.0 Hz, $CH_E\underline{H}_F$). ^{13}C NMR δ: 169.94 (s, $\underline{C}OOCH_3$), 169.18 (s, CON), 72.75 (d, CHO), 57.55 (d, CHN), 52.98 (q, OCH_3), 46.95 (t, CH_2N_3), 38.09 (t, CH_2CO), 33.90 (t, CH_2N), 32.16 (t, CH_2). Anal. Calcd. for $C_9H_{14}N_4O_4$: C, 4.63%; H, 5.83; N, 23.13. Found: C, 44.79%; H, 5.91; N, 23.02.

(2*S*,3*R*)-5-Amino-3-hydroxy-2-(2-oxoazetidin-1-yl)pentanoic acid 3 . To a solution of **16** (0.08 g, 0.33 mmol) in anhydrous pyridine (1.4 ml) was added LiI (0.131 g, 0.99 mmol) and the mixture was stirred at 85 °C for 4 h under N_2. The yellow solution was cooled, diluted with EtOAc (10 ml) and poured into 10 ml of cooled (-10 °C) 10% aq. HCl. The aqueous phase was extracted with EtOAc (2 × 10 ml); the combined organic phase was washed with cold water and dried over Na_2SO_4, followed by removal of the solvent under reduced pressure. The crude acid was dissolved in a mixture of $EtOH/H_2O$ (6:3) and hydrogenated (1 atm) over 5% Pd/C catalyst at room temperature for 3 h. The mixture was then filtered through a pad of Celite and the filtrate was processed according to the ref.[6], to afford **3** (43 mg, 65%) as a solid. M.p. 128-132 °C. $[\alpha]_D = +7.1$ (c = 0.04, H_2O) [Lit.[5]: m.p. 127-130 °C; $[\alpha]_D = +7.3$ (c = 1.3, H_2O), Lit.[6]: m.p. 130-135 °C; $[\alpha]_D = +7.8$ (c = 1.0, H_2O). ^1H- and ^{13}C NMR as reported.[5,6]

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References

1. Brown, A. G.; Corbett, D. F.; Goodacre, J.; Harbridge, J. B.; Howarth, T. T.; Ponsford, R. J.; Stirling, I.; King, T.J. *J. Chem. Perkin Trans. 1* **1984**, 635.

2. Brown, A. G.; Butterworth, D.; Cole, M.; Hanscomb, G.; Hood, J. D.; Reading, C.; Rolinson, G. N. *J. Antibiot.* **1976**, *29*, 668.
3. Elson, S.W.; Baggaley, K. H.; Gillett, J.; Holland, S.; Nicholson, N. H.; Sime, J. T.; Woroniecki, S. R. *J. Chem. Soc. Chem. Commun.* **1987**, 1736, 1739.
4. Baldwin, J. E.; Lloyd, M. D.; Wha-Son, B.; Schofield, C. J.; Elson, S. W.; Baggaley, K. H.; Nicholson, N. H.; *J. Chem. Soc. Chem. Commun.* **1993**, 500. Elson, S. W.; Baggaley, K. H.; Davison, M.; Fulston, M.; Nicholson, N. H.; Risbridger, G. D.; Tyler, J. W. *J. Chem. Soc. Chem. Commun.* **1993**, 1212.
5. Baggaley, K. H.; Sime, J. T.; Nicholson, N. H.; Elson, S. W.; Gillett, J.; Holland, S.; Woroniecki, S. R. *J. Chem. Soc. Chem. Commun.* **1987**, 1738. Baggaley, K. H.; Nicholson, N. H.; Sime, J. T. ibid. 1988, 567; Baggaley, K. H.; Elson, S. W.; Nicholson, N. H.; Sime, J. T. *J. Chem. Soc. Perkin Trans 1* **1990**, 1513, 1520.
6. Krol, W. J.; Mao, S.; Steele, D. L.; Townsend, C. A. *J. Org. Chem.* **1991**, *56*, 728.
7. (a) Di Giovanni, M. C.; Misiti, D.; Zappia, G.; Delle Monache, G. *Tetrahedron* 1993; 49; 11321; (b) Dell'Uomo, N.; Di Giovanni, M. C.; Misiti, D.; Zappia, G.; Delle Monache, G. *Liebigs Ann. Chem.* **1994**, 641; (c) Dell'Uomo, N.; Di Giovanni, M. C.; Misiti, D.; Zappia, G.; Delle Monache, G. *Tetrahedron: Asymmetry* **1996**, *7*, 181.
8. Delle Monache, G.; Di Giovanni, M. C.; Maggio, F.; Misiti, D.; Zappia, G. *Synthesis* **1995**, 1155.
9. Miyashita, N.; Yoshikoshi, A.; Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772.
10. (a) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**; *28*; 4185; (b) Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**; *36*; 6595.
11. Di Giovanni, M. C.; Misiti, D.; Zappia, G.; Delle Monache, G. Unpublished results.
12. Bowden, K.; Heilbron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc. A* **1946**, 39.
13. Leanna, M. R.; Sowin, T. J.; Morton, H. E. *Tetrahedron Lett.* **1992**, *33*, 5029.
14. Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, *27*, 4537.
15. Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870.
16. Boeckman, Jr., R. K.; Potenza, J. C. *Tetrahedron Lett.* **1985**, 26, 1411.
17. See in *The Chemistry of the Azido Group*, S. Patai Ed., J. Wiley: London, 1971.
18. Huang, H.; Iwasawa, N.; Mukaiyama, T. *Chem. Lett.* **1984**, 1465.
19. Kamiya, T.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron* **1979**, *35*, 323. Bose, A. K.; Manhas, M. S.; Kapur, J. C.; Sharma, S. D.; Amin, S. G. *J. Med. Chem.* **1974**; *17*; 541 and references therein.
20. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1988.

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