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SYNTHESIS OF N-BUTANOYL L-HOMOSERINE LACTONE A PHARMACEUTICALLY IMPORTANT QUORUM SENSING DRUG

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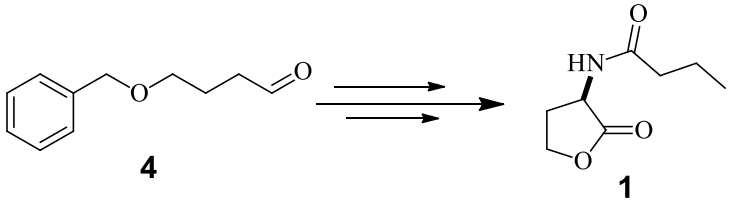
Abstract :

The different varieties of N-acyl homoserine lactones (N-AHLs) produced by different bacteria and are differ in their length of the acylated side chain of amino ***lactone****. These are small molecules as a part of quorum sensing system of bacteria and are used by bacteria during their virulence pathogenic involvement. Herein, we would like introduce a new synthetic approach for the synthesis of (R)-isomer of N-acylated homoserine lactone via L-proline catalyzed asymmetric α-amination reaction. Enantioselective synthesis of N-butanoyl-(R)-homoserine (1) lactone from 4-benzyloxy butyraldehyde (4) via L-proline catalyzed asymmetric α-amination of aldehyde. Synthesis of this target molecule is straightforward and completed in five steps with overall yield of 32% and 92% ee.*



(R)-3-aminodihydrofuran-2(3H)-one

Keywords: *α-Amination, Pinnick oxidation, Swern oxidation, Hydrogenolysis.*

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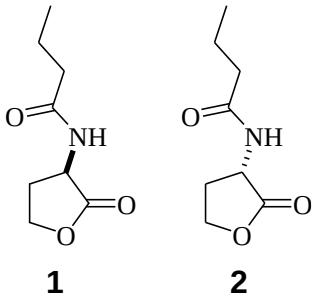
INTRODUCTION:

The bacteria which are producing N-butanoyl L-homoserine lactone are pathogen that infects immuno compromised patients and is found in lungs of infections of cystic fibrosis patients. These bacteria's are mainly relies on N-butanoyl L-homoserine lactone for their quorum sensing system. It is a method of communication between bacteria that enables the group based behavior on population density. The N-butanoyl-L-homoserine lactone **2** (BHL) is a small diffusible signaling molecule involved in controlling the gene expression and cellular metabolism [1]. Its D-Form of homoserine lactone **7** is very useful for the synthesis of important drug molecule which behaves as an antibiotics [2] antifungal peptides, syringotoxin-B, syringostatin-A, serine protease inhibitors and phenacyl homoserine lactones which is used as an anticancer compound [3]. There are only few methods are available in the literature on asymmetric synthesis of N-acyl L-homoserine lactone and most of them are concerns with synthesis of the (*S*)-isomer homo serine lactone **2**. These methods reported in the literature includes resolution by penicillin G acylase immobilized on Eupergit C [4] cationic Aza-Cope rearrangements [5] photo induced radical additions of alcohols, ether to chiral 4-methyleneoxazolidin-5-one and used antimicrobial activity [6,7]. However, there is lack of report on synthesis of the (*R*)-isomer of homoserine lactone **1**. In this context, we are interested to provide a new simple and efficient approach towards synthesis of N-acyl-(*R*)-homoserine lactone based on L-proline catalyzed asymmetric α-amination reaction [8].



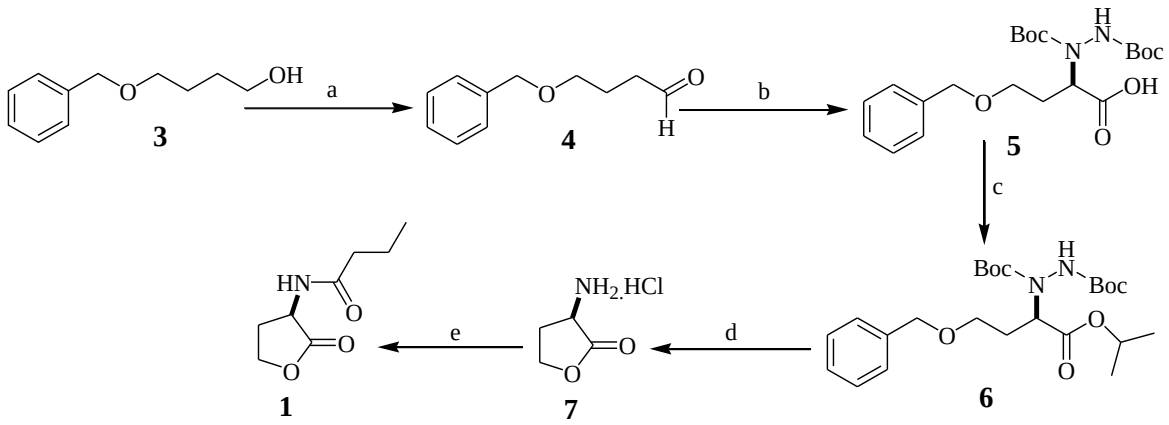
1 **2**

Fig.1: N-butanoyl homoserine lactone (BHL)

Recently, use of L-proline as organocatalyst is extensively explored in the field of asymmetric synthesis. Since L-proline is a naturally occurring α-amino acid; it is cheap and easily obtained in its high optical purity; therefore, its role as a chiral catalyst for introduction of the chirality becoming the powerful tool. As a result, L-proline catalyzed α-amination of aldehydes could be easily performed in the presence ditertbutylazodicarboxylate and obtained the chiral α-amino aldehyde in a good yield with excellent enantioselectivity. We employed this as a key reaction during the synthesis of N-butanoyl (*R*)-homoserine lactone and reporting here for the first time.

RESULT AND DISCUSSION:

Our synthetic strategy for N-butanoyl D-homoserine lactone is as given in the **Scheme 1** and it is based on use of commercially available 1,4-butane diol as a starting material. It was subjected to monobenzalytion for protection of one of the two hydroxyl group with help of benzyl bromide in the presence of sodium hydride in anhydrous DMF [9]. The unprotect hydroxyl was then oxidized to corresponding aldehyde **4** under the Swern oxidation reaction at -78 ºC [10]. The IR spectrum of aldehyde shows appearance of characteristic band at 1722 cm^{-1}, which corresponding to carbonyl group of aldehyde. Furthermore, asymmetric *α*-amination of the aldehyde **4** was carried out using ditertbutylazodicarboxylate (DBAD) in the presence of L-proline in dry CH_3CN at 0ºC [8]. Without isolating the chiral *α*-amino aldehyde it was transformed to *α*-amino acid **5** under the Pinnick oxidation reaction using $NaClO_2$ in the presence of NaH_2PO_4 in t-BuOH [11]. This *α*-amino acid **5 i**s a key intermediate to N-butanoyl D-homoserine lactone; therefore, it was isolated and purified by column chromatophy over 60-120 mesh size silica. The yield of the pure (*R*)-α-amino acid **5** was 90% and its melting point was 90ºC. The measurement of the specific optical rotation of the acid **5** was done in chloroform and it was found to be $[\alpha]_D^{25}$= -5.8º (*c*=1). This rotation corresponds to optical purity of 92% ee of (*R*)-*α*-amino acid **5** and it was confirmed by CHIRAL HPLC analysis over LUX-Cellulose-1 chiral column.



***Scheme 1**: a) $COCl_2$, DMSO, Et_3N, H_2O, DCM,-78 °C, 71%; b) i) DBAD, L-Proline, CH_3CN, 0 °C; ii) $NaClO_2$, NaH_2PO_4, H_2O, t-BuOH, 90%; c) i) NaOH, THF, H_2O; ii) Isopropyl iodide, Bu_4NF, THF, 92%; d) Raney Nickel, H_2, EtOH, reflux, HCl, EtOH, 68%; e) Butanoyl chloride, Na_2CO_3, CH_2Cl_2:H_2O, 86%.*

The ^{1}H NMR spectrum of acid **5** was difficult to characterize in the presence of intense peak due to Boc protection which masked the splitting pattern of the other proton signals. The ES-Mass spectrospcopic analysis showed the presence of peak at 447.2936. It is as a result of sodium cationic molecular peak [M^++Na] of (*R*)-α-amino acid **5**. This acid **5** was then converted to its isopropyl ester **6** by alkylation of the carboxylate anion with isopropyl iodide. This reaction was performed through generation of the sodium salt of acid with alkali under the biphasic solvent system THF: H_2O in the presence of phase transfer catalyst t-butyl ammonium fluoride [12]. The Boc deprotection with subsequent cleavage of N-N bond followed by debenzylation was done to make the free amino functionality as well hydroxyl; it was then cyclized to form the lactone under the influence of Raney nickel catalyzed reduction using H_2 gas at 60 psi pressure in ethanol [13]. This ethanolic reaction mixture was filtrated through celite and concentrated on rotary evaporator under the reduced pressure. The residual solution thus obtained was treated with dil. HCl to give yellow colored solid of hydrochloride salt **7** of (*R*)-*α*-amino lactone. It was characterized by ^{1}H-NMR it is indicated that there is presence of triplet at δ 4.52–4.55 with J = 9.4 Hz. This chemical shift is assigned to *α*-proton of the lactone who is involved in coupling to neighboring β-C$\underline{H_2}$ protons. The signal at δ 2.29-2.44 and 2.67-2.76 is multiplet it is as a result of coupling of the β-C$\underline{H_2}$ protons with *α*-proton and two γ-protons of the lactone. The other signal appearing at δ 4.35-4.49 is also multiplet and it is assigned to γ-protons of the lactone. This multiplet is resulting from the coupling of two *β*-protons. These are the protons are diastereotopic since they are adjacent to the chiral center of (*R*)-*α*-amino lactone therefore instead of triplet it resulting the multiplet. The hydrochloride salt **7** was also characterized by ^{13}C-NMR spectroscopy it was observed that single at δ. 48.61 Hz is due to *α*-carbon of the lactone while other two signals appearing at δ 26.80 and δ 67.47 Hz are corresponding to the β and γ carbon of the (*R*)-*α*-amino lactone. The other characteristic peak appearing at δ 175.48 Hz is assigned to the carbonyl carbon (*R*)-*α*-amino lactone. Thus it confirmed the structure of lactone **7**. Finally, the N-acylation was successfully accomplished by treating (R)-*α*-amino butyrolactone hydrochloride salt with butyryl chloride in the presence of Na_2CO_3 as base to give N-butanoyl (*R*)-homoserine lactone **1** which was confirmed by its IR, ^{1}H NMR, and Mass spectroscopic analysis [14].

CONCLUSION:

In summary, we demonstrated enantioselective synthesis of N-butynoyl (*R*)-homoserine lactone by simple and efficient manner. The naturally occurring L-proline was used as catalyst for *α*-amination and ditertbutylazodicarboxylate used as a source for nitrogen during the amination. Our synthesis of N-butynoyl (*R*)-homoserine lactone (**1**) completed in five steps starting from monobenzyl protected 1,4-butane diol. The overall yield of the final product was 32 % and optical purity was found to be 92% ee.

Supporting information

Full experimental detail as ^{1}H and ^{13}C NMR spectra are available via ‘‘Supplementary Content’’

ACKNOWLEDGEMENT:

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SUPPLEMENTARY INFORMATION

Synthesis of N-butanoyl L-homoserine lactone a pharmaceutically important quorum sensing drug

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Experimental

Solvents were purified and dried by standard procedures prior to use. Monitoring of the reactions was carried out using TLC, [TLC Silica gel 60 F254 (Merk)] and visualization with UV light (254 and 365 nm). Other techniques to identify the TLC were use of I_2 and solution of anisaldehyde in ethanol as developing reagents. IR spectra were recorded on Shimadzu FTIR 8400 instrument as a thin film or KBr pellets and the IR frequency expressed in terms of cm^{-1}. The ^1H NMR and ^{13}C NMR spectrum were recorded in $CDCl_3$ on Bruker AC-200 NMR and Varian Mercury 300 MHz NMR spectrometers. Optical rotations were obtained on Jasco P-1020 digital Polarimeter. Elemental analysis was carried out with C, H, N-analyzer Thermoelectron Corporation Model EA-1112 series. GC-MS analyses were carried out using Shimadzu GCMS-QP5050A spectrometer and Phenomenex Chiral HPLC analysis on Lux 5u Cellulose -1 (250 x 4.60 mm) column.

4-(benzyloxy)butanoyl (4).

A solution of oxalyl chloride (3.02 mL, 33 mmol) was taken in a 250 mL RB and 50 ml dry DCM was added to it with the help of a syringe. The resulting solution was cooled to -78 ^oC under argon atmosphere. A solution of DMSO (4.7 ml, 66 mmol) in 5 mL DCM was added at a rate such that the reaction temperature remained below -65 ^oC. After stirring for 5 min, a solution of the alcohol **3** (6 g, 33 mmol) in DCM (5 mL) was added dropwise into the RB and the resulting solution was stirred for 15 min. Next, dry NEt_3 (23 mL, 166 mmol) was added slowly into the reaction using syringe. After stirring the solution for an additional 10 min at -70 ^oC, the cooling bath was removed and the reaction was warmed for 45 min. The reaction mixture was allowed to reach room temperature and 50 ml water was added to it and stirring continued for 15 min. It was then transferred to a separating funnel and washed by HCl (50 mL), saturated $NaHCO_3$ (100 mL) and brine (50 mL). The organic layer was combined, dried over Na_2SO_4 and evaporate under reduced pressure to get desired aldehyde **4** (4.2g, 71%), IR (KBr) ν_{max}/cm^{-1} 2922, 2854, 1722, 1554, 1452, 1097, 740,700; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.93 (2H, quin., J = 6.8 Hz, CH_2), 2.55 (2H, t, J = 6.0 Hz, CH_2), 3.51 (t, J = 6.0 Hz, 2H, CH_2), 4.47 (2H, s, CH_2), 7.32 (6H, m, Ar-H), 9.75 (1H, s, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 202.15, 138.12, 128.24, 127.46, 72.76, 68.97, 40.76, 22.38, GC-Mass: **178**, 160, 150, 131, 116, 107, 91, 79, 65, 41.

(*R*)-4-benzyloxy)-2-(1,2-bis(tert-butoxycarbonyl)hydrainyl)butanoic acid (5).

i) In a 50 ml one neck RB placed in ice bath, the aldehyde **4** (0.3g, 1.68 mmol) was taken in 5ml acetonitrile followed by addition of DBAD (Di tert-butyl azo dicarboxylate) (0.388g, 1.68 mmol) and L-Proline (0.038g, 0.168 mmol).The reaction mixture was stirred at 0 ^oC for two hours and then warmed to 20oC for 1h. After the mixture became colorless, it was cooled to 0 ^oC.

ii) To the above reaction mixture at 0 ^oC, 10 mL of t-BuOH was added, followed by addition of $NaClO_2$ (1.21g, 13.48 mmol) and $NaHPO_4$ (1.58g, 10.11 mmol) in H_2O over 5 min. The reaction was stirred for an additiona l 5 min. The aqueous layer was separated and extracted with 2x10 ml EtOAc. The combined organic extracts was washed with 30 ml of 1M $Na_2S_2O_4$ and 15 ml of brine, dried and evaporated to get oily product. The product was purified by flash chromatography on silica eluting with 90% EtOAc: MeOH. The collected fractions were evaporated on rotary evaporator to afford the acid in quantitative yield **5** (0.643g, 90%), mp = 97 ^oC, $[\alpha]_D^{25}$ = -5.8^o (c 1, $CHCl_3$); IR (KBr) ν_{max}/cm^{-1} 3456, 3296, 2926, 1718, 1458, 1371, 1153, 740; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.48 (18H, s, Boc), 1.96 (1H, m, CH_2), 2.13 (1H, m, CH_2), 3.52 (1H, t, J = 6.0 Hz, CH_2), 3.61 (1H, broad s), 4.49 (2H, s, CH_2), 6.91 (1H, broad s), 6.91 (1H, broad s), 7.33 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 154.52, 154.07, 137.87, 128.36, 127.68, 96.09, 83.30, 73.03, 66.74, 59.47, 28.08, 27.99; Found LC-MS: 447.2936 (M^+ + Na); required 447.2107; enantiomeric purity: 92% ee determined by HPLC analysis (Chiral LUX-Cellulose-1 chiral column, MeOH:H_2O 75:25, 0.5 mL/min, 272 nm). Retention time: t_{minor} = 20.27 and t_{major} = 19.15 min.

(*R*)-di-tert-butyl1-(4-(benzyloxy)-1-isopropoxy-1-oxobutan-2-yl)hydrazine-1,2-dicarboxylate (6).

To a solution of compound **5** (200mg, 0.471 mmol) in THF/H_2O (6 mL, 1:1) was added at room temperature a aqueous solution of sodium hydroxide (0.019g, 0.471mmol). The mixture was stirred at room temperature for 24 h and concentrated in vacuo. The resultant white solid was suspended in toluene and concentrated to remove traces of water to give sodium carboxylate salt. THF (10 mL), tetrabutylammonium fluoride (109mg, 0.536mmol) and a

solution of Isopropyl iodide (0.082g, 0.447mmol) were added. After stirring at room temperature for 32 h, the reaction mixture was cooled and concentrated. The residue was partitioned between ethyl acetate (25 mL) and 0.5 M aqueous sodium bisulfate (10 mL). The organic phase was washed with portions of saturated aqueous sodium bicarbonate (1x10 mL) and brine (1x10 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was chromatographed on silica gel with a mixture of petroleum ether and ethyl acetate as eluant to give ester **6** as an oil (202mg, 92%), $[\alpha]_D^{25}$ = -5° (c 1, $CHCl_3$); IR (Neat) ν_{max}/cm^{-1} 3450, 2960, 1545, 1660, 1350, 1120; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.19 (6H, m, CH_3), 1.39 (18H, m, CH_3), 1.80 (1H, m, CH), 2.09 (1H, m, CH), 3.61 (2H, m, CH_2), 4.47 (2H, s, CH_2), 4.94 (2H, m, CH_2), 6.40 (1H, m), 7.23 (5H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 171.35, 155.51, 154.93, 138.64, 128.22, 127.68, 127.35, 96.14, 73.09, 68.89, 66.68, 59.06, 29.33, 28.18, 28.12, 21.80. Anal. calcd. for $C_{24}H_{38}N_2O_7$: C, 61.78; H, 8.21; N, 6.00; found: C, 61.75; H, 8.24; N, 6.04;

(*R*)-3-aminodihydrofuran-2(3H)-one (7).

A solution of compound **6** in absolute Ethanol was added excess Raney Ni and was stirred under H_2 atmosphere (60psi). After reaction was complete the catalyst was removed on celite through filtration of reaction mixture and filtrate concentrated in vacuo. The residue was stirred by the addition of dil. HCl in Ethanol for 8 h. The reaction mixture was dried by evaporating the solvent under reduced pressure to get corresponding product **7** (0.040gm, 68%), mp = 216-218 °C; $[\alpha]_D^{25}$= +25° (c 1, H_2O); IR (KBr) ν_{max}/cm^{-1} 3450, 2965, 1772, 1530, 1270, 1015; ^1H NMR (300 MHz, D_2O) δ (ppm) 2.29-2.44 (1H, m, CH_2), 2.67-2.76 (1H, m, CH_2), 4.31-4.49 (2H, m, CH_2O), 4.52-4.55 (1H, t, *J* = 9.4Hz, CH); ^{13}C NMR (75 MHz, D_2O) δ (ppm) 174.48(CO), 67.479(CH_2), 48.61(CH), 26.80(CH_2); GC-Mass: **101**, 85, 60, 41.

(*R*)-N-(2-oxotetrahydrofuran-3-yl)butyramide (1).

In a 50 mL RB was charged with butanoyl chloride (0.03 mL, 0.291 mmol), (R)-(α)-amino-γ-butyro lactone hydrochloride **7** (0.040g, 0.291mmol), CH_2Cl_2 (5 mL), water (5 mL) and Na_2CO_3 (0.123 g, 1.16 mmol). The title compound was obtained as a white solid **1 (** 0.042 mg, 86%). mp = 112-114 °C, $[\alpha]_D^{25}$ = +14.5° (c 1, $CHCl_3$), lit[14].+16 (c = 1, $CHCl_3$ for 99% for its antipode); IR (KBr) ν_{max}/cm^{-1} 3307, 2959, 2931, 2871, 1774, 164; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.93-0.97 (3H, t, *J* = 7.5 Hz, CH_3), 1.62-1.74 (2H, app. sextet, *J* =7.5 Hz, CH_2), 2.11-2.27 (3H, m, CH_2, CH_2), 2.80-2.87 (1H, m, CH_2), 4.20-4.29 (1H, ddd, *J* = 11.5, 9.5, 6.0 Hz, CH_2), 4.33-4.54 (1H, app. td, *J* = 9.0, 1.0 Hz, CH_2), 4.45 (1H, ddd, *J* = 11.5, 8.5, 6.0 Hz, CH), 6.32 (1H, s, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.0, 174.0, 66.5, 49.6, 38.4, 30.9, 19.3, 14.1; GC-Mass: **171**, 164, 140, 139, 123, 109, 96, 83, 69, 41.