SHORT PAPER

J. Chem. Research (S), 2001, 498–499

Preparation of optically active ß-amino acids from microbial polyester polyhydroxyalkanoates

Sang Hyun Park[a,b], Seung Hwan Lee[a,b] and Sang Yup Lee[a]*

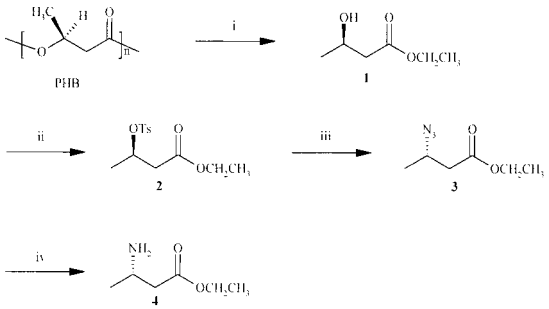
[a]*Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical Engineering and BioProcess Engineering Research Center, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea*
[b]*ChiroBio Inc., #2324 Undergraduate Building 2, 373-1 Kusong-dong, Yusong-gu, Taejon 305-301, Korea*

An efficient method for the preparation of optically active ethyl β-aminobutyrate from the biopolymer, poly-(*R*)-(-)-3-hydroxybutyrate (PHB) obtained from bacterial cells has been established using chemical transformations: simple recovery of PHB from bacterial cells followed by acidic alcoholysis, tosylation, nucleophilic substitution by azide, and an indium mediated reduction.

Keywords: optically active β-amino acids and esters, ethyl (*S*)-(+)-3-aminobutyrate, poly-(*R*)-(-)-3-hydroxybutyrate (PHB)

β-Amino acids, although less abundant than their α-analogues, are also present in peptides and other natural products, and in their free forms and derivatives show interesting pharmacological effects. A number of methods for synthesis and transformations leading to β-amino acids in diastereomerically and enantiomerically enriched forms have been reported.[1] The synthesis of modified peptides containing β-amino acids as key structural components has recently attracted attention in synthetic organic chemistry because of their usefulness as pharmaceutical agents. Peptides containing β-amino acids are generally more stable to enzymatic hydrolysis due to the inability of proteases and peptidases to cleave the amide bonds adjacent to the β-amino acid.[2–4] This has been demonstrated by somatostatin analogues containing β-amino acids.[5] Polyhydroxyalkanoates (PHAs) are a family of carbon, energy and/or reducing power storage polymers, which are accumulated intracellularly as distinct granules by numerous bacteria.[6] Interestingly, all the monomer units in PHAs are only in the (*R*)-(-)-configuration due to the stereospecificity of the biosynthetic enzymes.[7] More than 140 different kinds of enantiomerically pure (*R*)-hydroxycarboxylic acids have been shown to be incorporated into PHAs by employing various bacterial strains and culture conditions.[7,8] We have recently reported novel methods to efficiently produce various (*R*)-hydroxycarboxylic acids by *in vivo* depolymerisation.[9] We have also reported an efficient method for the preparation of alkyl (*R*)-(-)-3-hydroxybutyrate by acidic alcoholysis of poly-(*R*)-(-)-3-hydroxybutyrate (PHB).[10] In this paper, we report a novel efficient method for the synthesis of β-amino acids using the enantiopure monomers of PHAs as a chiral pool.

Chemical transformation of PHB resulted in alkyl (*R*)-(-)-3-hydroxybutyrates, which were further transformed into β-amino acids by simple chemical reactions. Using the same reaction condition described in our earlier work,[10] ethyl (*R*)-(-)-3-hydroxybutyrate (**1**) was prepared by acidic alcoholysis of PHB as shown in Scheme 1**.** Purified PHB was reacted with anhydrous ethyl alcohol in 1,2-dichloroethane in the presence of concentrated hydrochloric acid for 12 h, which resulted in **1a** in high yield. Compound **1a** exhibited a characteristic proton NMR signal at 3.15 ppm for the hydroxy hydrogen at carbon 3. In our experimental work, it was found that tosylation was sufficient to activate the hydroxy group of **1a** for nucleophilic substitution of azide. Tosylation of **1a** was carried out following the similar reaction procedure[11] to afford **2** in 71 % yield. The absence of the hydroxy hydrogen signal at 3.15 ppm indicated that the starting material was consumed. Compound **2** was reacted with sodium azide in water using hexadecyltributylphosphonium bromide as a phase transfer catalyst. This gave compound **3** in 76 % yield, whereas azidation was conducted in DMF or refluxing DMF in the previous reports. The IR spectrum of the azido ester showed a characteristic peak due to the presence of azido group at 2121 cm^{-1}. Reduction of **3** in tetrahydrofuran with indium in 1N HCl resulted in ethyl (*S*)-(+)-3-aminobutyrate (**4a**) in 61% yield as shown in Table 1. In view of increasing emphasis on environmentally benign and safe organic synthesis, it should be noted that we used mostly water as the reaction medium in the reaction of azido ester formation and indium mediated reduction.



Scheme 1 Reagents and conditions: i, EtOH, conc. HCl, $ClCH_2CH_2Cl$, reflux, 12h; ii, TsCl, pyridine, $CHCl_3$, 0°C,12h; iii, NaN_3, hexadecyltributylphosphonium bromide, H_2O, rt, 12h; iv, In, 1N HCl, THF, rt, 12h.

These methods were also applied to synthesise (*R*)-configurated β-amino acid. For example, **4b** could be made by using **1b** as a starting material (Table 1). Furthermore, these methods were successfully applied to prepare primary amines from the corresponding alcohols (Table 1).

In summary, treatment of ethyl (*R*)-(-)-3-hydroxybutyrate with *p*-toluenesulfonyl chloride and sodium azide allowed formation of the corresponding azido ester, which was then converted to the β-amino acid by indium mediated reduction. The overall reaction proceeded with inversion of configuration. This methodology is also expected to be applicable to the preparation of primary amines from the corresponding alcohols. This reaction reported herein expands the scope of synthetic transformations and offers a new and convenient

* To receive any correspondence. E-mail: leesy@mail.kaist.ac.kr
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

Table 1 Specific rotations of amino compounds (**4**)

Entry	**1**	**4**	Specific rotation
a	ethyl (*R*)-(-)-3-hydroxybutyrate	ethyl (*S*)-(+)-3-aminobutyrate	+8.0° (c 1, CH_3OH)
b	ethyl (*S*)-(+)-3-hydroxybutyrate	ethyl (*R*)-(-)-3-aminobutyrate	–7.5° (c 1, CH_3OH)
c	cyclohexanol	cyclohexylamine	Not applicable

method for the preparation of β-amino acids from diverse members of microbial polyester PHAs[7] as a chiral pool.

Experimental

Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck) and all chromatographic separations were monitored by TLC analyses, performing using glass plates precoated with 0.25-mm 230–400 mesh silica gel impregnated with a fluorescent indicators (254 nm). Optical rotations were measured on a JASCO DIP-181 Digital Polarimeter. IR spectra were recorded on a Bomen MB154 FTIR (KBr pellets or neat). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 500-MHz FTNMR spectrometer in $CDCl_3$, DMSO-d_6, or D_2O solution, and chemical shifts were recorded in ppm units using $SiMe_4$ as an internal standard. Mass spectra were measured on Varian MAT 371 Mass Spectrometer at 70 eV.

*Preparation of ethyl (R)-(-)-3-hydroxybutyrate (***1a***) by acidic alcoholysis of poly-(R)-(-)-3-hydroxybutyrate (PHB):* Ethyl (*R*)-(-)-3-hydroxybutyrate was prepared as previously described.[10] Purified PHB (1 g) was dissolved in a solution of 20 ml of 1,2-dichloroethane, 20 ml of anhydrous ethyl alcohol and 2 ml of concentrated hydrochloric acid. (Methyl benzoate was also added as an internal standard for the GC analysis.) This mixture was boiled for 12 h under reflux followed by washing with 20 ml each of half saturated sodium chloride aqueous solution, saturated sodium hydrogen carbonate aqueous solution and saturated sodium chloride aqueous solution. The aqueous layers were extracted twice with 20 ml of dichloromethane, and the extracts were added to the organic layer. The combined organic layer was dried over magnesium sulfate, filtered and concentrated using a rotary evaporator. Vacuum distillation gave 0.93 g of the title compound as a white oil from 1.00 g of PHB: $[\alpha]_D$ –44° (*c* 1, $CHCl_3$); IR (neat) 3448, 2979, 2936, 2909, 1735, 1719 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, *J = 6.3* Hz, 3H, $CH_3CH(OH)$), 1.26 (t, *J = 7.2* Hz, 3H, CH_3CH_2O), 2.39–2.51 (m, 2H, CH_2CO), 3.15 (s, OH), 4.15–4.23 (m, 3H, OCH and OCH_2); ^{13}C NMR ($CDCl_3$) δ 14.19, 22.47, 42.86, 60.67, 64.27, 173.16; CIMS, *m/z* 133 ([M+1]$^+$), 131, 128, 117, 115, 88, 87 (base), 85, 73, 71, 69, 60, 57.

Preparation of **2a** *(tosylation of ethyl (R)-(-)-3-hydroxybutyrate):* Ethyl *(R)*-(-)-3-hydroxybutyrate (0.65 ml, 5 mmol) was dissolved in chloroform (10ml) and cooled in an ice bath (0 °C). Pyridine (0.81 ml, 10 mmol) was then added, followed by the addition of *p*-toluenesulfonyl chloride (1.14 g, 6 mmol) in small portions with constant stirring. After 12 h, ether (20 ml) and water (10 ml) were added and the organic layer was washed successively with 1N HCl, 5 % $NaHCO_3$, and water and then dried (Na_2SO_4). The solvent was removed under reduced pressure and crude tosylate was column chromatographed (3 % ethyl ether : 97 % hexane) on a silica gel column to yield 1016 mg (71 %) of the title compound as a white oil: $[\alpha]_D$ +4.3° (*c* 1, $CHCl_3$); IR (neat) 2985, 1735, 1365, 1191 cm^{-1} ; ^{1}H NMR($CDCl_3$) δ 1.21 (t, *J = 7.1* Hz, 3H, OCH_2CH_3), 1.35 (d, *J = 6.3* Hz, 3H, $OCHCH_3$), 2.45 (s, 3H, $ArCH_3$), 2.46, 2.53 (dd, *J = 7* and *16* Hz, 1H, $COCH_2$), 2.71, 2.74 (dd, *J = 7* and *16* Hz, 1H, $COCH_2$), 3.99–4.11 (m, 1H, OCH_2), 4.98 (q, *J = 6.4* Hz, 1H, OCH), 7.34 (d, *J = 8.1 Hz*, 2H, ArH), 7.79 (d, *J = 8.1* Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.05, 20.92, 21.61, 41.53, 60.85, 75.91, 127.78, 130.02, 134.06, 144.75, 169.53; CIMS, *m/z* 287 ([M+1]$^+$), 286, 258, 241, 199, 198, 197,173 (base), 172, 156, 155, 131, 115, 114, 108, 107, 92, 91, 87, 86, 85, 73, 65.

*Ethyl (S)-(+)-3-azidobutyrate (***3a***):* Reaction mixture of tosylate (854 mg 2.98 mmol), hexadecyltributylphosphonium bromide (150 mg, 0.298 mmol) and sodium azide (387 mg, 5.96 mmol) in water (2.5ml) was vigorously stirred at room temperature. After 12 h, ether (20ml) was added and the organic layer was collected and dried over sodium sulfate. Evaporation under reduced pressure gave 355 mg (75.8 %) of the title compound as a light-yellow oil: $[\alpha]_D$ +41° (*c* 1, $CHCl_3$); IR (neat) 2982, 2936, 2122, 1377, 1295, 1254, 1185, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (q, *J = 7.0* Hz, 3H, OCH_2CH_3), 1,34 (d, *J = 6.5* Hz, 3H, $CH_3CH(N_3)$), 3.95–4.04 (m, 1H, CHN_3), 4.18 (q, *J = 7.0* Hz, 2H, OCH_2); ^{13}C NMR ($CDCl_3$) δ 14.17, 19.51, 41.17, 54.34, 60.87, 170.56; CIMS, *m/z* 157 (M^+), 155, 149, 131, 115, 91 (base), 88, 84, 73, 70, 69, 60, 57, 56, 55, 49.

*Ethyl (S)-(+)-3-aminobutyrate (***4a***):* To a solution of ethyl *(S)*-(+)-3-azidobutyrate(338 mg, 2.15 mmol) in tetrahydrofuran (1 ml), 1*N* HCl (3 ml), indium (370 mg, 3.23 mmol) were added. After 12 h, ether (20ml) was added, and aqueous layer was collected and evaporated under reduced pressure. Vacuum distillation gave 172 mg (60.9 %) of the title compound as a light-yellow oil: $[\alpha]_D$ +8.0° (*c* 1, CH_3OH); IR (neat) 3486, 3206, 1733, 1717, 1609, 1407 cm^{-1}; ^{1}H NMR(D_2O) δ 1.28 (t, *J = 7.0* Hz, 3H, OCH_2CH_3), 1,37 (d, *J = 6.5* Hz, 3H, $CH_3CH(NH_2)$), 2.78 (d, *J = 6.2* Hz, 2H, CH_2 C=O), 3.77–3.81 (m, 1H, $CH(NH_2)$), 4.22 (q, *J = 7.0* Hz, 2H, OCH_2); ^{13}C NMR(D_2O) δ 14.48, 18.90, 39.20, 45.58, 63.48, 173.46; CIMS, *m/z* 131 (M^+), 46(base).

1b, **2b**, **3b**, *and* **4b***:* Same as **1a**, **2a**, **3a** and **4a** except for the opposite signs of those specific rotations: $[\alpha]_D$ +46 ° (*c* 1, $CHCl_3$), –2 ° (*c* 1, $CHCl_3$), –35 ° (*c* 1, $CHCl_3$) and –7.5 ° (*c* 1, CH_3OH), respectively.

2c*:* IR (neat) 2950, 2863, 1599, 1457, 1355, 1188, 1175, 931, 873, 667, 571, 555 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 1.20–1.35 (m, 3H), 1.40–1.60 (m, 3H), 1.65–1.85 (m, 4H), 2.44 (s, 3H, $ArCH_3$), 4.46–4.56 (m, 1H, OCH_3), 7.32 (d, *J = 7.8* Hz, 2H, ArH), 7.79 (d, *J = 7.8* Hz, 2H, ArH); ^{13}C NMR($CDCl_3$) δ 21.62, 23.39, 24.86, 32.34, 81.69, 127.59, 129.74, 134.86, 144.33; CIMS, *m/z* 254 (M^+), 175, 174, 173, 172, 157, 156, 155, 139, 108, 107, 99, 92, 91, 90, 89, 83, 82 (base), 81, 79, 77, 68, 67, 65, 55, 54, 53.

3c*:* IR (neat) 3116, 2306, 2097 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 1.15–1.45 (m, 5H), 1.60–1.65 (m, 1H), 1.70–1.82 (m, 2H), 1.85–1.95 (m, 2H), 3.34 (s, 1H, N_3CH) ; ^{13}C NMR($CDCl_3$) δ 24.26, 25.30, 31.64, 59.94; CIMS, *m/z* 125 (M^+), 49 (base)

4c*:* IR (neat) 3355, 3277, 2924, 2852, 1603, 1449, 894, 842, 776 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 0.91–1.11 (m, 5H), δ 1.22–1.28 (m, 2H), 1.53 (d, *J = 12* Hz, 1H), 1.65 (d, *J = 13* Hz, 2H), 1.75 (d, *J = 11* Hz, 2H), 2.50–2.62 (m, 1H); ^{13}C NMR($CDCl_3$) δ 25.21, 25.75, 37.00, 50.53; CIMS, *m/z* 99 (M^+), 70, 56 (base), 49.

This work was supported by the First Presidential Young Investigator's Award to S. Y. Lee. S. H. Park was supported by the Brain Korea 21 Project from the Korean Ministry of Education.

Received 10 May 2001; accepted 16 August 2001
Paper 01/871

References

1 (a) E. Juaristi Ed. *Enatioselective Synthesis of β-Amino Acids*; John Wiley & Sons, Inc.: New York, 1996, pp. 1-491; (b) Cole, D. C. *Tetrahedron* 1994, **32**, 9517–9582.
2 (a) D. Seebach and J. Matthews *Chem. Commun.*, 1997, 2015–2022; (b) K. Gademann, M. Ernst, D. Hoyer, and D. Seebach *Angew. Chem. Int. Ed.* 1999, **38**, 1223–1226.
3 H. Yoshio, J. Katada, T. Harada, A. Tachiki, K. Iijima, Y. Takiguchi, M. Muramatsu, H. Miyazaki, T. Asari, T. Okazaki, Y. Sato, E. Yasuda, M. Yano, I. Uno, and I. Ojima *J. Med. Chem.* 1998, **41**, 2345–2360.
4 M. Werder, H. Hauser, A. Abele, and D. Seebach *Helv. Chim. Acta* 1999, **82**, 1774–1783.
5 K. Gademann, M. Ernst, D. Hoyer, and D. Seebach *Helv. Chim. Acta* 2000, **83**, 16-33.
6 S.Y. Lee *Trends Biotechnol*., 1996, **14**, 431–438.
7 S.Y. Lee *Biotechnol. Bioeng.*, 1996, **49**, 1–14.
8 (a) A. Stenbüchel and H.E. Valentin *FEMS Microb. Lett.* 1995, 128, 219-228; (b) D. Seebach, A. K. Beck, R. Breitschuh, and K. Job *Org. Synth.* 1992, **71**, 39–47; (c) D. Seebach and M. F. Zuger *Tetrahedron Lett.* 1985, **25**, 2747–2750.
9 S.Y. Lee, Y. Lee, and F. Wang *Biotechnol. Bioeng.* 1999, **65**, 363-368.
10 Y. Lee, S. H. Park, I.T. Lim, K. Han, and S.Y. Lee *Enzyme Microb. Tech.* 2000, **27**, 33–36.
11 W.H. Kruizinga, B. Strijtveen, and R. M. Kellog *J. Org. Chem.*, 1981, **46**, 4321.