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THE DETERMINATION OF ENANTIOMERIC EXCESS OF THE PRODUCTS OF BAKER'S YEAST-MEDIATED REDUCTION OF δ–ALKOXYCARBONYL–β–KETO ESTERS, *VIA* THE DERIVATION TO ALEXAKIS' ALKOXYDIAZAPHOSPHORIDINE

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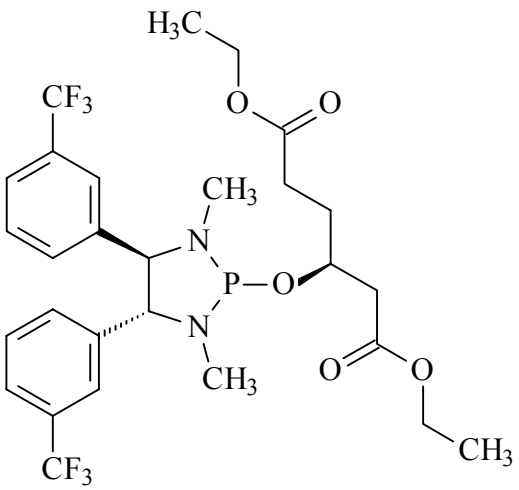
Abstract – Reduction of 5–alkoxycarbonyl–3–oxopentanoates (**1a–1j**) with baker's yeast turned to be enantioselective, and the *e.es* of the products (**2a–2j**) were determined by the NMR measurement of the corresponding Alexakis' alkoxydiazaphosphoridines. Stereochemistry was established with X–Ray crystallography.

Advantages of enzymatic catalysis in synthetic organic chemistry have recently been recognized and reported. Baker's yeast[1-3] is of particular interest: it is inexpensive, versatile and its growth does not require the assistance of a microbiologist. Many of functional groups can be reduced by baker's yeast. Among these groups, carbonyl group is the most interesting from the synthetic point of view.[1]

Although biocatalyzed enantioselective reduction of δ–alkoxycarbonyl–β–keto esters has been extensively explored,[4–6] the knowledge of requirements for baker's yeast and its specific activity is still inadequate. Also, lactonization of the bioreduction products leads to a butyrolactone derivatives which may serve as a very useful intermediates for the synthesis of various natural products.[7,8] We therefore decided to undertake a more systematic study on the reduction of δ–alkoxycarbonyl–β–keto esters with baker's yeast.

The present paper describes the results of the reduction of 5–alkoxycarbonyl–3–oxopentanoates (**1a**–**1j**) by the raw baker's yeast. It has already been shown[9,10] that the bioreduction of the commercially available methyl 5-methoxycarbonyl-3-oxopentanoate (**1a**) proceeded in 19% chemical yield with 48% ee. The product of this reaction–alcohol (**2a**) appears to be an attractive chiral starting material, so we decided to investigate the possibility to improve the outcome of this process. The ee of alcohols (**2a–2j**) was

determinated by Alexakis method,[11] based on the reaction of the hydroxy compound with PCl_3 and the appropriate enantiomerically pure diamine. For example, when (*1R,2R*)–*N,N'*–dimethyl–1,2–bis(3–trifluoromethylphenyl)–1,2–ethylenediamine was used for the reaction with racemic ethyl 5-ethoxycarbonyl-3–hydroxypentanoate, we observed a good separation of both phosphorous absorption signals in ^{31}P NMR spectrum of **3** (Figure 1, (a)). The relative proportion of both signals was considerably changed when the same procedure was applied to alcohol (**2b**) derived from bioreduction (Figure 1, (b)). This observation prompted us to use this method for establishing the enantiomeric composition of other enantioenriched alcohols. It is worth to notice that NMR spectral technique proved to be very useful in such determinations.[12]



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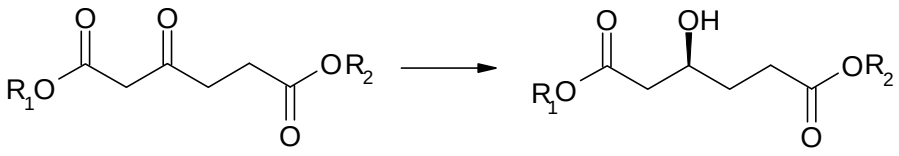
Each baker's yeast reduction of a δ–alkoxycarbonyl–β–keto esters (**1a**–**1j**) gave chiral non–racemic δ–alkoxycarbonyl–β–hydroxy esters (**2a**–**2j**) having the same sign of the optical rotation and very similar chemical shifts in ^{31}P NMR spectra (Table 2) which strongly suggested the same absolute stereochemistry on the stereogenic center (most probably *S*).

When diethyl 3-oxohexanedioate (**1b**) was applied, the product (**2b**) was obtained in 60% chemical yield with 90% ee. Also, the time of this reaction was considerably shorter than in the previous case (Table 1). These findings indicated that the size of the alkoxy group may affect the stereochemical outcome of this reaction. Earlier studies on the effect of the size of ester moieties in 5-alkoxycarbonyl-3–oxopentanoate in the reduction with baker's yeast, provided very interesting results.[9] To extend the study, new compounds (**1e**–**1j**) had to be prepared. Such derivatives are usually difficult to access *via* classical methods.[13] However, we found that a careful regioselective transesterification of the commercial methyl ester (**1a**) could be used for this purpose. Both methyl 5–ethoxycarbonyl–3–oxopentanoate (**1c**) and ethyl 5–

methoxycarbonyl–3–oxopentanoate (**1d**) were examined in order to check if only one or both ester moieties are important for the reaction course.

The baker's yeast reduction of the diesters (**1c**) and (**1d**) showed that the reaction time depended on the nature of the both ester groups and was much shorter for esters bigger than 5-ethoxycarbonyl.



1a–1j **2a–2j**

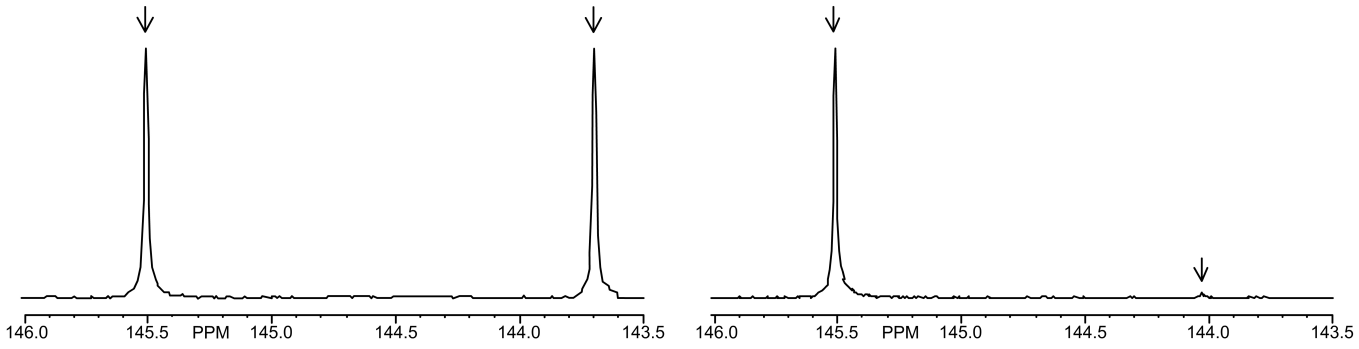
a $R_1 = R_2 = Me$
b $R_1 = R_2 = Et$
c $R_1 = Me$, $R_2 = Et$
d $R_1 = Et$, $R_2 = Me$
e $R_1 = R_2 = Pr$
f $R_1 = Me$, $R_2 = Pr$
g $R_1 = Pr$, $R_2 = Me$
h $R_1 = R_2 = Bu$
i $R_1 = Me$, $R_2 = Bu$
j $R_1 = Bu$, $R_2 = Me$

Scheme 1.

Table 1. Raw baker's yeast reduction

Substrate	Product	Reaction time [h]	Yield [%]	$[\alpha]_D$ (temp) [°C]	ee [%]
1a	**2a**	92	45	+7.7 (20)	68
1b	**2b**	26	60	+9.5 (22)	90
1c	**2c**	96	40	+6.5 (22)	66
1d	**2d**	29	45	+9.8 (22)	94
1e	**2e**	26	62	+8.5 (22)	96
1f	**2f**	26	34	+7.9 (27)	82
1g	**2g**	25	53	+8.9 (22)	96
1h	**2h**	26	44	+9.2 (22)	96
1i	**2i**	24	52	+5.8 (27)	72
1j	**2j**	29	56	+7.8 (27)	96

We also found that the methyl group R_1 strongly diminishes the optical yield (Table 1). Other alkyls seem to improve the output considerably–even up to 96% ee and 56% isolated yield–in the case of n–butyl (compound (**1j**)).



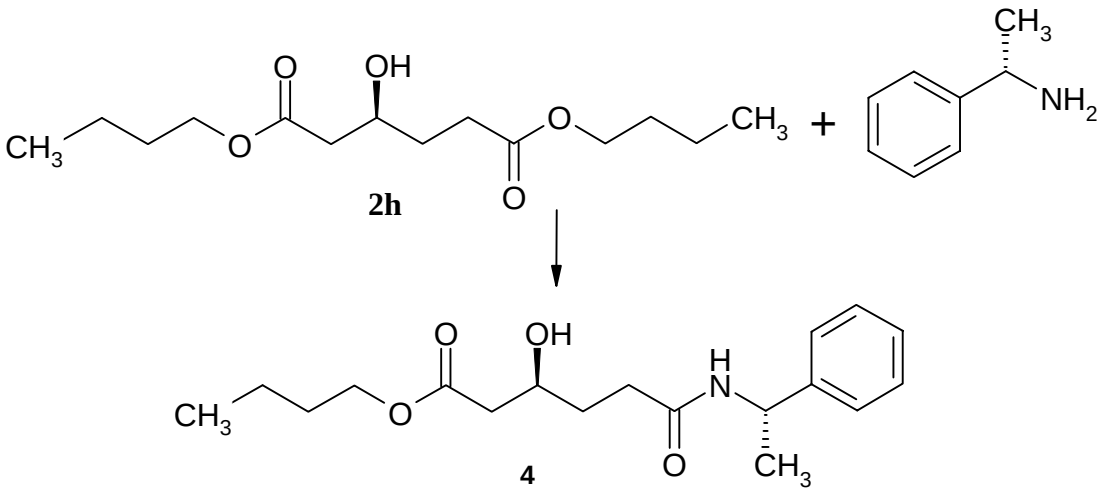
a b

Figure 1. ^{31}P NMR spectrum of ethyl 5-ethoxycarbonyl-3–hydroxypentanoate (**2b**): (a) racemic; (b) chiral non-racemic; in $CDCl_3$

Table 2. ^{31}P NMR chemical shifts of compounds type (**3**) obtained from alkohols (**2a – 2j**)

Alkohol	^{13}P NMR δ (ppm)(%)	
2a	145.601(84)	144.516(16)
2b	145.561(95)	144.195(5)
2c	145.072(83)	144.091(17)
2d	145.337(97)	143.847(3)
2e	145.562(98)	144.069(2)
2f	145.466(91)	144.516(9)
2g	145.703(98)	144.063(2)
2h	145.517(98)	144.035(2)
2i	145.454(86)	144.510(14)
2j	145.669(98)	144.030(2)

In order to establish unambiguously the stereochemistry at C–3 we subjected compound (**2h**) to the reaction with the enantiomerically pure *S*–(-)–α–methylbenzylamine to form butyl (3*S*)-3-hydroxy-6-oxo-6-{[(1*S*)-1-phenylethyl]amino}hexanoate (**4**) (Scheme 2). The single crystal of compound (**4**) was then subjected to an X–Ray crystallography measurement that finally allowed to assign *S* configuration on C–3 chiral center (Figure 2).



Scheme 2.

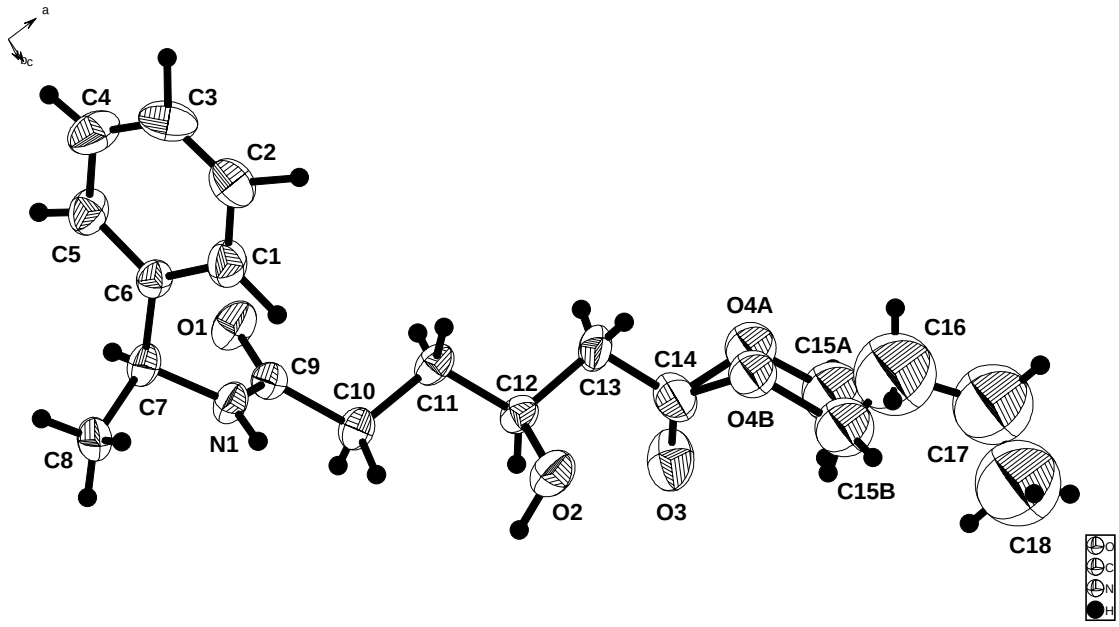


Figure 2. ORTEP diagram for compound (**4**).

In conclusion, we can say that the yeast-catalyzed reduction of δ–alkoxycarbonyl–β–keto esters proceeds in good to high enantioselectivity, judging from the NMR measurement of the corresponding Alexakis' heterocyclic derivatives, with acceptable chemical yield.

EXPERIMENTAL

^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded with Varian UNITY Plus spectrometer at 500, 125,87 and 80,95 MHz respectively. Specific rotations were measured with Perkin–Elmer 241 polarimeter. Merck's 60 F254 plates and Merck's 60 (230–400 mesh) silica columns were used for

chromatography. The commercial raw baker's yeast (Polmos, Poland) was used. X–Ray measurement for compound (**4**) was performed on Kuma KM4CCD κ-axis diffractometer with graphite-monochromated MoKα radiation. The crystal was positioned at 65 mm from the KM4CCD camera. The number of 796 frames was measured at 1.6^{o} intervals with a counting time of 35 sec. The data were corrected for Lorentz and polarization effects. No absorption correction was applied. Data reduction and analysis were carried out with the Kuma Diffraction (Wrocław, Poland) programs.

The structure was solved by direct method[14] and refined using SHELXL software.[15] The refinement was based on F^2 for all reflections except those with very negative F^2. Weighted R factors wR and all goodness-of-fit S values are based on F^2. Conventional R factors are based on F with F set to zero for negative F^2. The $F_0^2 > 2s(F_0^2)$ criterion was used only for calculating R factors and is not relevant to the choice of reflections for the refinement. The R factors based on F^2 are about twice as large as those based on F. All hydrogen atoms were located from a differential map and refined isotropically.

Nutrition: The addition of sugar to a yeast–water suspension has occasionally been found to be of minor importance for asymmetric reduction of ketones.[16] In the present experiments dimethyl 3–oxohexanedioate (**1a**) was found to disappear progressively from yeast–water suspension in 92 h, neither the substrate nor the product could be found. With glucose used as nutrient, dimethyl 3–hydroxyhexanedioate (**2a**) was isolated in 45% yield.

Concentration of baker's yeast cells: To shorten the time for the reduction of **1a**, attempts were made to increase the concentration of yeast cells. Unfortunately after the concentration had been raised to 40 g/L, the reaction time remained unaffected whereas the chemical and the optical yields dropped. The concentrate given below may be regarded as optimized.

Determination of enantiomeric composition of compounds (2a–2j): Into a dry NMR tube were placed the (*1R,2R*)–*N,N'*–dimethyl–1,2–bis(–3–trifluoromethylphenyl)–1,2–ethylenediamine (0.15 mmol, 56.45 mg) and $CDCl_3$ (800 μL). The tube was shaken until complete dissolution. The base (0.75 mmol, 91 mg, *N,N*–diethylaniline) was added with a microsyringe, followed by slow addition of PCl_3 (12.8 μL, 0.15 mmol) also with a microsyringe. The NMR tube was shaken and an exothermic reaction took place. Samples of compounds (**2a–2j**) were added in 0.3 mL of dry $CDCl_3$ and the ^{31}P NMR spectra was recorded accordingly and in each case the enantiomeric composition was deduced from the integration of signals at approximately 143.7 and 145.5.[11]

General procedure for the baker's yeast reduction of compounds (1a–1j): A sample of 1 mmol of δ–alkoxycarbonyl–β–keto ester was added to an aqueous baker's yeast–glucose mixture (150 mL) with continuous stirring at 35°C. A TLC analysis of worked–up aliquots (*vide infra*) was used to follow the reactions. After the substrate had almost disappeared (see Table 1), the reaction mixture was extracted with chloroform (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and

then the solvent was removed at reduced pressure. Pure products (**2a**–**2j**) were obtained after column chromatography on silica using cyclohexane–ethyl acetate (10%, 15%, 20% and 50% of ethyl acetate) as eluent. The yields, reaction time, $[\alpha]_D$ and ee are listed in Table 1.

Methyl (*S*)-5-methoxycarbonyl–3–hydroxypentanoate (2a): 1. Baker's yeast (6 g); glucose (20 g,another 10 g added after 44 h); water (75 mL); **1a** (1 mmol); 92 h; yield of **2a** 45%; oil; $[\alpha]_D^{20}$ +7.7° (c 1.0 in $CHCl_3$).

2. Baker's yeast (12 g); glucose (40 g, another 10 g added after 44 h); water (75 mL); **1a** (1 mmol); 92 h; yield of **2a** 21%; oil; $[\alpha]_D^{20}$ +6.6° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.47 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.68 (s, 3H, C(6)–O–C$\underline{H}_3$), 3.72 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.05 (q, 1H, *J* = 6.1 Hz, C(3)$\underline{H}$), 4.50 (br s, 1H, O$\underline{H}$); ^{13}C NMR ($CDCl_3$, δ, ppm) 30.20 (C5), 31.70 (C4), 41.80 (C2), 51.70 (C1–O–$\underline{C}$), and (C6–O–$\underline{C}$), 67.30 (C3), 172.70 (C6), 174.20 (C1).

Ethyl (*S*)-5-ethoxycarbonyl–3–hydroxypentaoate (2b): Baker's yeast (5.25 g); glucose (17.5 g); water (260 mL); **1b** (3.5 mmol); 26 h; yield of **2b** 60%; oil; $[\alpha]_D^{22}$ +9.5° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 1.25 (t, 3H, *J* = 7.0 Hz, C(6)–O–CH_2–C$\underline{H}_3$), 1.27 (t, 3H, *J* = 7.0 Hz, C(1)–O–CH_2–C$\underline{H}_3$), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.48 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.15 (br s, 1H, O$\underline{H}$), 4.05 (m, 1H, C(3)$\underline{H}$), 4.13 (q, 2H, *J* = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_3), 4.20 (q, 2H, *J* = 7.0 Hz, C(1)–O–C$\underline{H}_2$–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 14.20 (2 × O–C–$\underline{C}$), 30.50 (C5), 31.50 (C4), 41.60 (C2), 60.50 (C6–O–$\underline{C}$), 60.70 (C1–O–$\underline{C}$), 67.20 (C3), 172.50 (C6), 173.70 (C1).

Methyl (*S*)–5–ethoxycarbonyl–3–hydroxypentanoate (2c): Baker's yeast (3 g); glucose (10 g, another 10 g added after 50 h); water (150 mL); **1c** (2 mmol); 96 h; yield of **2c** 40%; oil; $[\alpha]_D^{22}$ +6.5° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 1.26 (t, 3H, *J* = 7.0 Hz, C(6)–O–CH_2–C$\underline{H}_3$), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.48 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.17 (br s, 1H, O$\underline{H}$), 3.72 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.05 (m, 1H, C(3)$\underline{H}$), 4.14 (q, 2H, *J* = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 14.20 (O–C–$\underline{C}$), 30.50 (C5), 31.40 (C4), 41.30 (C2), 51.80 (C1–O–$\underline{C}$), 60.50 (C6–O–$\underline{C}$), 67.20 (C3), 173.00 (C6), 173.70 (C1).

Ethyl (*S*)-5-metoxycarbonyl–3–hydroxypentaoate (2d): Baker's yeast (3.6 g); glucose (11.2 g); water (180 mL); **1d** (2.4 mmol); 29 h; yield of **2d** 45%; oil; $[\alpha]_D^{22}$ +9.8° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 1.28 (t, 3H, *J* = 7.0 Hz, C(1)–O–CH_2–C$\underline{H}_3$), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.48 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.18 (br s, 1H, O$\underline{H}$), 3.68 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.05 (m, 1H, C(3)$\underline{H}$), 4.18 (q, 2H, *J* = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 14.20 (O–C–$\underline{C}$), 30.20 (C5), 31.50 (C4), 41.60 (C2), 51.70 (C6–O–$\underline{C}$), 60.70 (C1–O–$\underline{C}$), 67.20 (C3), 172.60 (C6), 174.10 (C1).

Propyl (*S*)-5-propoxycarbonyl–3–hydroxypentanoate (2e): Baker's yeast (6.0 g); glucose (20 g); water (150 mL); **1e** (2 mmol); 26 h; yield of **2e** 62%; oil; $[\alpha]_D^{22}$ +8.5° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ,

ppm) 0.94 (t, 6H, J = 7.0 Hz, 2 × –O–CH_2–CH_2–C$\underline{H}_3$), 1.65 (m, 4H, 2 × –O–CH_2–C$\underline{H}_2$–CH_3), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.50 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.15 (br s, 1H, O$\underline{H}$), 4.05 (m, 1H, C(3)$\underline{H}$), 4.04 (t, 2H, J = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_2–CH_3), 4.08 (t, 2H, J = 7.0 Hz, C(1)–O–C$\underline{H}_2$–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 10.40 (2 × –O–C–C–$\underline{C}$), 21.90 (C6–O–C–$\underline{C}$–C), 22.00 (C1–O–C–$\underline{C}$–C), 30.40 (C5), 31.30 (C4), 41.30 (C2), 66.10 (C6–O–$\underline{C}$), 66.40 (C1–O–$\underline{C}$), 67.20 (C3), 172.90 (C6), 173.80 (C1).

Methyl (*S*)-5-propoxycarbonyl–3–hydroxypentanoate (2f): Baker's yeast (6 g); glucose (20 g); water (150 mL); **1f** (2 mmol); 26 h; yield of **2f** 34%; oil; $[\alpha]_D^{27}$ +7.9° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 0.94 (t, 3H, J = 7.0 Hz, C(6)–O–CH_2–CH_2–C$\underline{H}_3$), 1.65 (sextet, 2H, J = 7.0 Hz, C(6)–O–CH_2–C$\underline{H}_2$–CH_3), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.50 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.14 (br s, 1H, O$\underline{H}$), 3.72 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.04 (superposition of m, 1H, C(3)$\underline{H}$ and t, 2H, J = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 10.40 (C6–O–C–C–$\underline{C}$), 22.00 (C6–O–C–$\underline{C}$–C), 30.40 (C5), 31.30 (C4), 41.30 (C2), 51.80 (C1–O–$\underline{C}$), 66.20 (C6–O–$\underline{C}$), 67.20 (C3), 173.10 (C6), 173.80 (C1).

Propyl (*S*)-5-methoxycarbonyl–3–hydroxypentanoate (2g): Baker's yeast (6 g); glucose (20 g);water (150 mL); **1g** (2 mmol); 25 h; yield of **2g** 53%; oil; $[\alpha]_D^{22}$ +8.9° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 0.95 (t, 3H, J = 7.0 Hz, C(1)–O–CH_2–CH_2–C$\underline{H}_3$), 1.66 (sextet, 2H, J = 7.0 Hz, C(1)–O–CH_2–C$\underline{H}_2$–CH_3), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.50 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.16 (br s, 1H, O$\underline{H}$), 3.68 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.04 (m, 1H, C(3)$\underline{H}$), 4.08 (t, 2H, J = 7.0 Hz, C(1)–O–C$\underline{H}_2$–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 10.40 (C1–O–C–C–$\underline{C}$), 21.90(C1–O–C–$\underline{C}$–C), 30.20 (C5) 31.30 (C4), 41.20 (C2), 51.70 (C6–O–$\underline{C}$), 66.40 (C1–O–$\underline{C}$), 67.10 (C3), 172.80 (C6), 174.10 (C1).

Butyl (*S*)-5-butoxycarbonyl–3–hydroxypentanoate (2h): Baker's yeast (6 g); glucose (20 g); water (150 mL); **1h** (2 mmol); 26 h; yield of **2h** 44%; oil; $[\alpha]_D^{22}$ +9.2^o (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 0.93 (t, 3H, J = 7.0 Hz, (C(6)–O–CH_2–CH_2–CH_2–C$\underline{H}_3$), 0.94 (t, 3H, J = 7.0 Hz, C(1)–O–CH_2–CH_2–CH_2–C$\underline{H}_3$), 1.38 (m, 4H, 2 × –O–CH_2–CH_2–C$\underline{H}_2$–CH_3), 1.61 (m, 4H, 2 × –O–CH_2–C$\underline{H}_2$–CH_2–CH_3), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.50 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.15 (d, 1H, J = 3.5 Hz, O$\underline{H}$), 4.04 (m, 1H, C(3)$\underline{H}$), 4.08 (t, 2H, J = 7.0 Hz, C(6)–O–C$\underline{H}_2$–CH_2–CH_2–CH_3), 4.12 (t, 2H, J = 7.0 Hz, C(1)–O–C$\underline{H}_2$–CH_2–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 13.70 (2 × –O–C–C–C–$\underline{C}$), 19.10 (2 × –O–C–C–$\underline{C}$–C), 30.40 (C5), 30.60 (2 × –O–C–$\underline{C}$–C–C), 31.30 (C4), 41.20 (C2), 64.40 (C6–O–$\underline{C}$), 64.70 (C1–O–$\underline{C}$), 67.20 (C3), 172.90 (C6), 173.80 (C1).

Methyl (*S*)-5-butoxycarbonyl–3–hydroxypentanoate (2i): Baker's yeast (6 g); glucose (20 g); water (150 mL); **1i** (2 mmol); 24 h; yield of **2i** 52%; oil; $[\alpha]_D^{27}$ +5.8° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 0.93 (t, 3H, J = 7.0 Hz, C(6)–O–CH_2–CH_2–CH_2–C$\underline{H}_3$), 1.37 (m, 2H, C(6)–O–CH_2–CH_2–C$\underline{H}_2$–CH_3), 1.61 (m, 2H, C(6)–O–CH_2–C$\underline{H}_2$–CH_2–CH_3), 1.80 (m, 2H, C(4)$\underline{H}_2$), 2.50 (m, 4H, C(2)$\underline{H}_2$ and C(5)$\underline{H}_2$), 3.20 (br s, 1H, O$\underline{H}$), 3.72 (s, 3H, C(1)–O–C$\underline{H}_3$), 4.08 (superposition of m, 1H, C(3)$\underline{H}$ and t, 2H, J = 7.0

Hz, C(6)–O–C$\underline{H_2}$–CH_2–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 13.70 (C6–O–C–C–C–$\underline{C}$), 19.10 (C6–O–C–C–$\underline{C}$–C), 30.40 (C5), 30.60 (C6–O–C–$\underline{C}$–C–C), 31.30 (C4), 41.10 (C2), 51.80 (C1–O–$\underline{C}$), 64.50 (C6–O–$\underline{C}$), 67.20 (C3), 173.10 (C6), 173.80 (C1).

Butyl (*S*)-5-methoxycarbonyl–3–hydroxypentanoate (2j): Baker's yeast (6 g); glucose (20 g); water (150 mL); **1j** (2 mmol); 29 h; yield of **2j** 56%; oil; $[\alpha]_D^{27}$ +7.8° (c 1.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm) 0.94 (t, 3H, *J* = 7.0 Hz, C(1)–O–CH_2–CH_2–CH_2–C$\underline{H_3}$), 1.38 (m, 2H, C(1)–O–CH_2–CH_2–C$\underline{H_2}$–CH_3), 1.62 (m, 2H, C(6)–O–CH_2–C$\underline{H_2}$–CH_2–CH_3), 1.80 (m, 2H, C(4)$\underline{H_2}$), 2.50 (m, 4H, C(2)$\underline{H_2}$ and C(5)$\underline{H_2}$), 3.16 (br s, 1H, O$\underline{H}$), 3.68 (s, 3H, C(6)–O–C$\underline{H_3}$), 4.03 (m, 1H, C(3)$\underline{H}$), 4.12 (t, 2H, *J* = 7.0 Hz, C(1)–O–C$\underline{H_2}$–CH_2–CH_2–CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm) 13.70 (C1–O–C–C–C–$\underline{C}$), 19.10 (C1–O–C–C–$\underline{C}$–C), 30.20 (C5), 30.60 (C1–O–C–$\underline{C}$–C–C), 31.30 (C4), 41.30 (C2), 51.70 (C6–O–$\underline{C}$), 64.70 (C1–O–$\underline{C}$), 67.10 (C3), 172.80 (C6), 174.10 (C1).

Butyl (3*S*)-3-hydroxy-6-oxo-6-{[(1*S*)-1-phenylethyl]amino}hexanoate (4): A sample of 555 mg, 0.5 mmol of compound **2h** and of 343 mg, 0.7 mmol of *S*–(-)–α–methylbenzylamine in 10 mL dry toluene was refluxed at on the argon atmosphere for 20 h. The mixture was then washed with two portions 6% hydrochloric acid and three time with water. The solvent was evaporated under reduced pressure, and the product was isolated by column chromatography (silica 200 mesh, eluent: $CHCl_3$–MeOH, 200:1); yield of 278 mg (43%); $[\alpha]_D^{20}$ -53.8° (*c* 1.0 in $CHCl_3$), mp 43–46°C. ^{1}H NMR ($CDCl_3$, δ, ppm) 0.93 (t, 3H, *J* = 7.0 Hz, C(6)–O–CH_2–CH_2–CH_2–C$\underline{H_3}$), 1.38 (m, 2H, C(6)–O–CH_2–CH_2–C$\underline{H_2}$–CH_3), 1.61 (m, 2H, C(6)–O–CH_2–C$\underline{H_2}$–CH_2–CH_3), 1.82 (m, 2H, C(4)$\underline{H_2}$), 2.50 (m, 4H, C(2)$\underline{H_2}$ and C(5)$\underline{H_2}$), 3.08 (br s, 1H, O$\underline{H}$), 4.03 (m, 1H, C(3)$\underline{H}$), 4.10 (t, 2H, *J* = 7.0 Hz, C(6)–O–C$\underline{H_2}$–CH_2–CH_2–CH_3), 6.15 (d, 1H, J =14.0 Hz, N–H), 5.10 (m, 1H, N–C$\underline{H_3}$), 1.48 (d, 3H, *J* = 3.5 Hz, N–CH–C$\underline{H_3}$); ^{13}C NMR ($CDCl_3$, δ, ppm) 13.70 (C1–O–C–C–C–$\underline{C}$), 19.10 (C1–O–C–C–$\underline{C}$–C), 21.70 (N–C–$\underline{C}$), 30.05 (C4), 31.70 (C1–O–C–$\underline{C}$–C–C), 32.80 (C5), 41.40 (C2), 48.80 (N–$\underline{C}$–C), 64.60 (C1–O–$\underline{C}$–C–C–C), 67.50 (C3), 172.10, 172.80 (C=O), benzyl 126.10, 127.30, 128.6,0 143.10.

A single crystal for an X–Ray study was obtained by slow evaporation of ether–hexane solution.

$C_{18}H_{27}NO_4$, M=321.41, space group P2(1), a=11.838(2) Å, b=5.0432 Å, c=15.685 Å, V=929.0(3) Å^3, Z=2, D_c=1.149 g cm^3, F(000)=348 μ, (MoK\α)=0.71073 cm^{-1}.

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