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Studies on the chemoenzymatic synthesis of 3-phenyl-GABA and 4-phenyl-pyrrolid-2-one: the influence of donor of the alkoxy group on enantioselective esterification

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ABSTRACT

A new chemoenzymatic method for the synthesis of enantiomerically pure 3-phenyl-γ-aminobutyric acid **1** and 4-phenyl-pyrrolid-2-one **9** based on the enzymatic kinetic resolution of 3-phenyl-4-pentenoic acid **2** is described herein. Enzymatic resolution of the racemic substrate provided products with good enantioselectivity upon esterification. In these reactions, a new class of alkoxy group donor—orthoesters, acetals and ketals were used. The best results of the enzymatic kinetic resolution were obtained for triethyl orthoacetate in toluene solvent.

1. Introduction

3-PhenylGABA (phenibut) **1** is a lipophilic analogue of GABA (γ-aminobutyric acid), the main neurotransmitter of the central nervous system layout (CNS).[1] The disorders in the synthesis of GABA in an organism often lead to epilepsy, as well as other diseases such as Huntington's disease or Parkinson's disease[2] and other psychiatric disorders such as anxiety and pain. The direct application of GABA in treatment therapy is limited due to its hydrophilic nature. GABA has a low permeability through the blood–brain barrier.[3] Phenibut was discovered and introduced into clinical practice in Russia in the 1960s. The biological activity of this compound depends on its absolute configuration. The (*R*)-isomer is much more active than the (*S*)-isomer, and it is twice as potent as the racemate.[4]

Previously, enantiomerically pure phenibut **1** has been synthesized via chemical methods from α,β-unsaturated oxazolines **3** (Fig. 1).[5] There are several chemical routes in which asymmetric catalysts are used (with substrates **4**,[6] **5**[7] and **6**[8]).

There are also some examples of the enzymatic method of synthesis of enantiomerically pure 3-phenyl-GABA. Wang et al. described chemoenzymatic preparation of phenibut (85% ee) via hydrolysis of 3-phenylglutaronitrile **7** catalysed by *Rhodococcus* sp. AJ270 followed by Curtius rearrangement and consequent acidic hydrolysis.[9] Felluga et al. prepared compound **1** via enzymatic hydrolysis of racemic γ-nitroester **8** with α-chymotrypsin and subsequent reduction with Raney Nickel.[10]

3-Phenyl-4-pentenoic acid is a substrate for the synthesis of 4-phenyl-pyrrolid-2-one **9**. This class of compounds are selective inhibitors of cAMP phosphodiesterase. They are used in the therapy of ischaemic heart disease.[11] Compound **9** is a precursor for the synthesis of γ-aminobutyric acid (GABA) analogues, which are of great interest due to their importance in various nervous system functions

2. Results and discussion

We focused our attention on a novel irreversible method for the enzymatic esterification of carboxylic acids. We used orthoesters, acetals and ketals as new donors of alkoxy groups. The mechanism of this reaction is shown on Scheme 1. The racemic carboxylic acid **E** reacts with the hydroxyl group of enzyme **C** to form the acylenzyme intermediate **D** and a water molecule. In the next step the intermediate **D** reacts with alcohol **B** to give ester **A**. The alcohol **B** derives from the reaction between water and compound **F** to form compound **G**, which in the next step is transformed into compound **H** and alcohol **B**. The last step is irreversible.

Herein our aim was to develop enzymatic methods for the enantioselective synthesis of ester derivatives of 3-phenyl-4-pentenoic acid. In our previous study, we looked at the enzymatic esterification of 3-phenyl-4-pentenoic acid using triethyl orthoacetate as a donor of the alkoxy group.[12] The results obtained were very promising so we decided to expand upon this methodology using the other donors of alkoxy groups and enzymes.

At first, the racemic esters **11** and **13** were obtained in only 11% and 3% yields, respectively (Scheme 2).[13] In this reaction, a trialkyl orthoester was used. The addition of an orthoester shifts the equilibrium in favour of the ester side, due to the consumption of water formed through hydrolysis of the orthoester.

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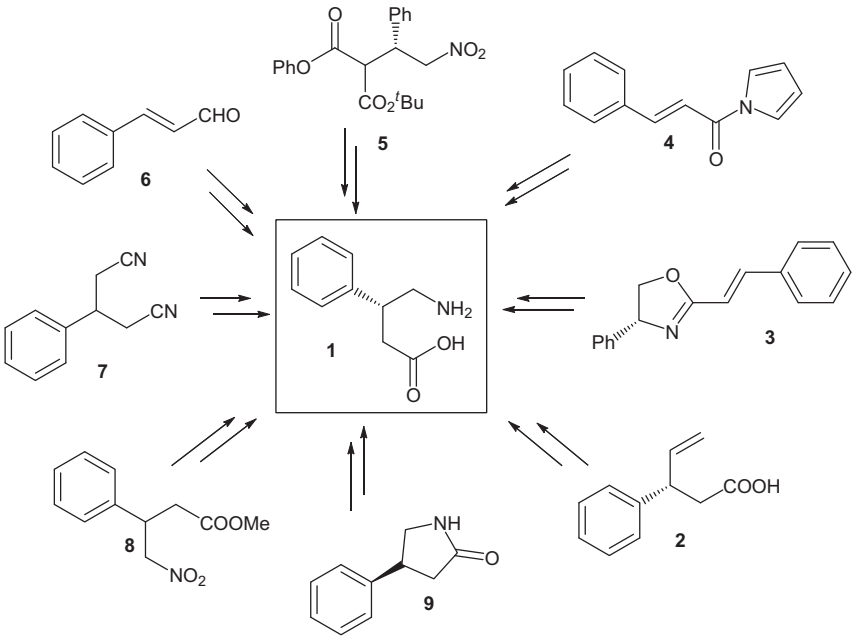
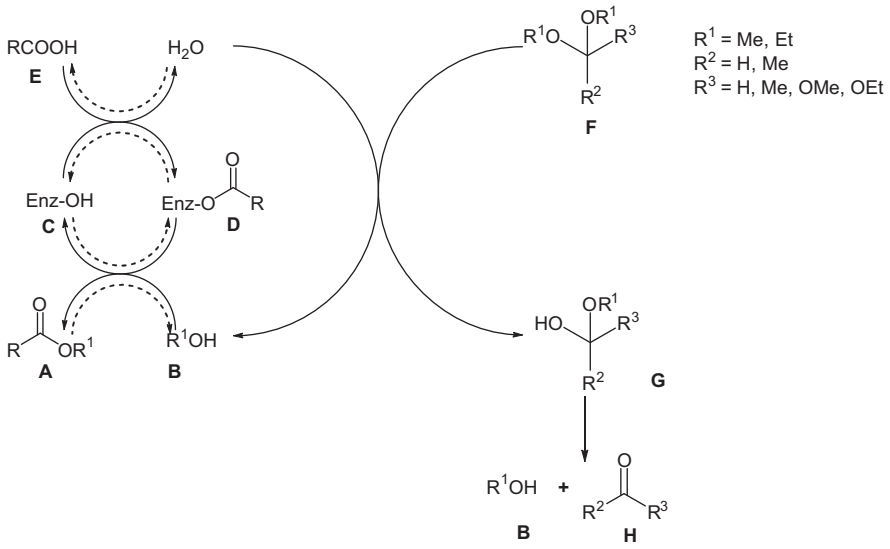
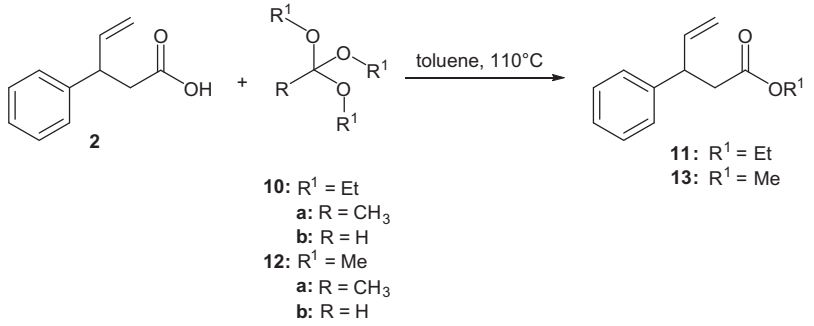


Figure 1. Substrates for the synthesis of 3-phenylGABA.



Scheme 1. The proposed mechanism for the enzymatic esterification.



Scheme 2. Synthesis of racemic esters of 3-phenyl-4-pentenoic acid.

2.1. Comparison of various solvents

Previously, we have found that the best biocatalyst for the enzymatic esterification was lipase from Wheat germ.[12] It is well documented that the solvent has a strong influence on biocatalytic reactions. In order to determine the best solvent for the kinetic resolution of 3-phenyl-4-pentenoic acid **2**, different solvents listed in Table 1, were used. These reactions, catalysed by lipase from Wheat germ, were conducted in an organic solvent at 40 °C for 24 h. Triethyl orthoacetate was used as a donor of the alkoxy group.

The experimental data show that the best enantioselectivity was obtained for toluene (Table 1, entry 2). Tetrahydrofuran, dimethylformamide and acetonitrile (Table 1, entries 5, 10 and 11) also exhibited good enantioselectivity but the substrate conversion was substantially lower.

2.2. Enantioselective synthesis of esters of 3-phenyl-4-pentenoic acid using various trialkyl orthoesters

Herein we have focused our attention on the synthesis of chiral non-racemic esters of 3-phenyl-4-pentenoic acid. We decided to test other orthoesters **10b**, **12a**, and **12b** as donors of an alkoxy group. In our studies commercially available lipases were used as biocatalysts as well as acetone powders [beef liver acetone powder (BLAP), turkey liver acetone powder (TLAP), chicken liver acetone powder (CLAP), deer liver acetone powder (JLAP), beef kidney acetone powder (BKAP) and goose liver acetone powder (GLAP)]. These reactions were conducted in toluene at 40 °C for 24–48 h (Scheme 3). The results obtained are shown in Table 2.

The results show that triethyl orthoesters **10** are better donors of the alkoxy group than trimethyl orthoesters **12**. In most cases triethyl orthoacetate **10a** exhibits very good enantioselectivity (Table 2, entries 3–11 and 13–15), while triethyl orthoformate **10b** gives a reasonable enantioselectivity for only one enzyme (Table 2, entry 18). However, the results obtained using trimethyl orthoacetate **12a** and trimethyl orthoformate **12b** as donors, reveal very low enantioselectivity despite a twofold time extension (Table 2, entries 22–29).

It is noteworthy that using trimethyl orthoformate as a donor of the methoxy group and GLAP as a biocatalyst (Table 2, entry 29), the absolute configuration of the product was opposite (*R*). It is interesting to note that simple change of the alkoxy group donor can reverse the stereochemical course of the enzymatic kinetic resolution.

Table 1
Results obtained in enzymatic esterification 3-phenyl-4-pentenoic acid in different solvents[a]

Entry	Solvent	Product	*c* (%)	ee[b] (%)	*E*[c]
1	—	(*S*)-**11**	30.5	69	7
2	Toluene	(*S*)-**11**	45	93	63
3	Hexane	(*S*)-**11**	19.5	52	4
4	Chloroform	(*S*)-**11**	27	3.7	1
5	THF	(*S*)-**11**	16	90	23
6	Dichloromethane	(*S*)-**11**	65	50	10
7	Ethyl ether	(*S*)-**11**	2.5	21	2
8	Dioxane	(*S*)-**11**	9.7	45	3
9	*tert*-Butyl-methyl ether	(*S*)-**11**	67	41	6
10	DMF	(*S*)-**11**	3.7	91	23
11	Acetonitrile	(*S*)-**11**	16	96	53
12	DMSO	(*S*)-**11**	38.5	14	1

[a] Conditions: **2** (1 mmol), **10a** (3 mmol), solvent (1.21 mL), lipase from Wheat germ (10 mg), 40 °C, 24 h.
[b] Determined by HPLC on a Daicel Chiracel OD-H column.
[c] Calculated according to Chen et al.,[14] using the equation: $E = (\ln[1 - c(1 + \text{eep})])/(\ln[1 - c(1 - \text{eep})])$.

2.3. Acetals and ketals as donors of alkoxy group

Based on the mechanism of enzyme catalysed reaction, we noted that another class of alkoxy group donors for enzymatic esterification reaction can be used. Therefore, in the next stage of our studies, we used acetals **14** and ketals **15** (Fig. 2). The reactions were conducted in toluene at 40 °C for 24–48 h. The results obtained are shown in Table 3.

The results show that the acetal donors of ethoxy group **14a** are better than methoxy **14b** and **15**, as in the case of orthoesters. In some cases, 1,1-diethoxyethane **14a** gave good enantioselectivity (Table 3, entries 4, 7 and 10). 1,1-Dimethoxyethane **14b** and 2,2-dimethoxypropane **15** exhibited lower enantioselectivity, however these values are acceptable for several enzymes (Table 3, entries 13, 14 and 16).

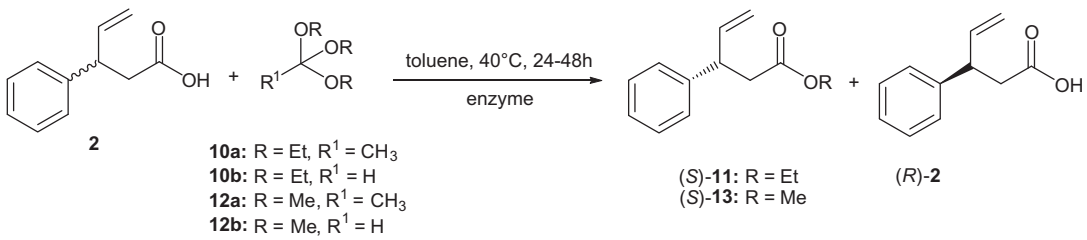
Comparing the results obtained for orthoesters and acetals we noticed that orthoesters are better donors of alkoxy group for the esterification of carboxylic acids than acetals.

2.4. Synthesis of 3-phenylGABA

The retrosynthetic analysis of 3-phenylGABA is shown in Figure 3.

3-PhenylGABA **A** can be obtained by functional group interconversion (FGI) from the corresponding azide **B** synthon obtained directly from the corresponding alcohol **C**. The hydroxyl group in alcohol **C** can be obtained via functional group interconversion of the double bond present in the 3-phenyl-4-pentenoic acid **2** structure.[15]

Stereodifferentiation was planned to take place during the enzymatic functionalization of acid **2**. For the synthesis of 3-phenylGABA, the obtained ethyl ester (*R*)-**11** was used (Scheme 4). Ozonolysis of the ester (*R*)-**11** double bond gives an aldehyde **16** (40% yield). Using sodium cyanoborohydride to reduce the carbonyl group did not lead to a desirable product. After many trials, we found that sodium cyanoborohydride reduces the aldehyde **16** efficiently and provides alcohol **17** (76% yield). In the next step, the reaction of alcohol **17** with methanesulfonic acid chloride led to the formation of compound **18** in 90% yield. Compound **18** was converted into azide **19** in 66% yield whose hydrogen/palladium reduction gave the respective amine **20** in 58% yield. The last stage

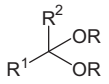


Scheme 3. Enzymatic kinetic resolution of compound **2** using different trialkyl orthoesters.

Table 2
Kinetic resolution of compound **2** using orthoesters[a]

Entry	Enzyme	Donor of the alkoxy group	Time (h)	Product	*c* (%)	% ee[b]	*E*[c]
1	—	**10a**	24	**11**	11	0	—
2	*Hog pancreas*	**10a**	24	(*S*)-**11**	50	0	—
3	*Penicilium roqueforti*	**10a**	24	(*S*)-**11**	52	90	776
4	*Aspergillus*	**10a**	24	(*S*)-**11**	23	92	34
5	*Wheat germ*	**10a**	24	(*S*)-**11**	45	93	63
6	*Pseudomonas cepacia*	**10a**	24	(*S*)-**11**	15.4	92	30
7	*Pseudomonas sp*	**10a**	24	(*S*)-**11**	37.5	93	50
8	*Mucor miehei*	**10a**	24	(*S*)-**11**	40.9	91	40
9	*Candida rugosa*	**10a**	24	(*S*)-**11**	30.1	90	29
10	*BLAP*	**10a**	24	(*S*)-**11**	24	90	26
11	*TLAP*	**10a**	24	(*S*)-**11**	54	82	41
12	*CLAP*	**10a**	24	**11**	9.3	—	—
13	*JLAP*	**10a**	24	(*S*)-**11**	52	83	32
14	*BKAP*	**10a**	24	(*S*)-**11**	25.7	93	37
15	*GLAP*	**10a**	24	(*S*)-**11**	25	97	82
16	—	**10b**	24	**11**	3	0	—
17	*Aspergillus*	**10b**	24	(*S*)-**11**	12.2	68	6
18	*Pseudomonas cepacia*	**10b**	24	(*S*)-**11**	5.9	89	19
19	*Wheat germ*	**10b**	24	(*S*)-**11**	43	71	10
20	*Mucor miehei*	**10b**	24	(*S*)-**11**	3.2	27	2
21	*Candida rugosa*	**10b**	24	(*S*)-**11**	6	44	3
22	—	**12a**	48	**2**	0	—	—
23	*Wheat germ*	**12a**	48	(*S*)-**13**	17	0.5	1
24	*Penicillium roqueforti*	**12a**	48	(*S*)-**13**	9	82	11
25	*GLAP*	**12a**	48	(*S*)-**13**	5.8	1.5	1
26	—	**12b**	48	**13**	8.9	0	—
27	*Wheat germ*	**12b**	48	**2**	0	—	—
28	*Penicillium roqueforti*	**12b**	48	(*S*)-**13**	9.2	82	11
29	*GLAP*	**12b**	48	(*R*)-**13**	18.5	16	1.4

[a] Conditions: **2** (1 mmol), **10/12** (3 mmol), toluene (1.21 mL), the enzyme (10 mg), 40 °C, 24–48 h.
[b] Determined by HPLC on a Daicel Chiracel OD-H column.
[c] Calculated according to Chen et al.,[14] using the equation: $E = (\ln[1 - c(1 + \text{eep})])/(\ln[1 - c(1 - \text{eep})])$.



14a: R = Et, $R^1 = CH_3$, R^2 = H
14b: R = Me, $R^1 = CH_3$, R^2 = H
15: R = Me, $R^1 = R^2 = CH_3$

Figure 2. Selected donors of the alkoxy group.

was the acidolysis of the ester group. In this reaction we obtained the desired product as hydrochloride **21** in 89% yield.

The assignment of the absolute configuration of the 3-phenylGABA stereogenic centre was based on the sign of specific rotation of hydrochloride **21**. The absolute configuration at carbon 3 was assigned as (*S*) by comparison of the specific rotation with the following literature value: $[\alpha]_D = -2.6$ (*c* 0.86, MeOH), [(*S*)-isomer].[7]

It is noteworthy that amine **20** was converted into (*S*)-(+)-4-phenyl-pyrrolid-2-one **9** with 60% yield. Compound **9** is an important substance for the study of the nervous system function.[11]

Table 3
Enzymatic kinetic resolution of **2** using acetals and ketals[a]

Entry	Enzyme	Donor of alkoxy group	Time (h)	Product	*c* (%)	% ee[b]	*E*[c]
1	—	**14a**	24	**11**	1	0	—
2	*Wheat germ*	**14a**	24	(*S*)-**11**	6.2	46	3
3	*P. roqueforti*	**14a**	24	(*R*)-**11**	6.7	33	2
4	*Aspergillus*	**14a**	24	(*S*)-**11**	1.5	>99	202
5	*Papaine*	**14a**	24	(*S*)-**11**	6.6	16	1
6	*Hog pancreas*	**14a**	24	(*S*)-**11**	23	30	2
7	*Rhizopus arrhizus*	**14a**	24	(*S*)-**11**	6.7	98	106
8	*Candida rugosa*	**14a**	24	(*S*)-**11**	25	12	1
9	*Mucor javanicus*	**14a**	24	(*S*)-**11**	5.8	18	2
10	*Rhizomucor miehei*	**14a**	24	(*S*)-**11**	4.4	98	104
11	*Novozym*	**14a**	24	**11**	2.5	0	—
12	*GLAP*	**14a**	24	**11**	2.8	0	—
13	*Wheat germ*	**14b**	48	(*S*)-**13**	2.6	88	16
14	*Penicillium roqueforti*	**14b**	48	(*S*)-**13**	1.3	90	19
15	*GLAP*	**14b**	48	(*S*)-**13**	1.6	58	4
16	*Wheat germ*	**15**	48	(*S*)-**13**	11.3	89	19
17	*Penicillium roqueforti*	**15**	48	(*R*)-**13**	1.1	57	4
18	*GLAP*	**15**	48	(*R*)-**13**	1.1	72	6

[a] Conditions: **2** (1 mmol), **14/15** (3 mmol), toluene (1.21 mL), the enzyme (10 mg), 40 °C, 24–48 h.
[b] Determined by HPLC on a Daicel Chiracel OD-H column.
[c] Calculated according to Chen et al.,[14] using the equation: $E = (\ln[1 - c(1 + \text{eep})])/(\ln[1 - c(1 - \text{eep})])$.

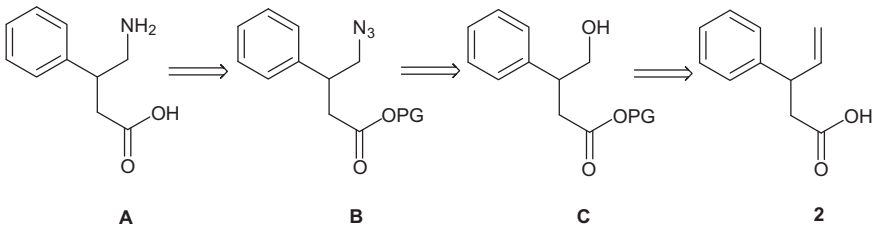
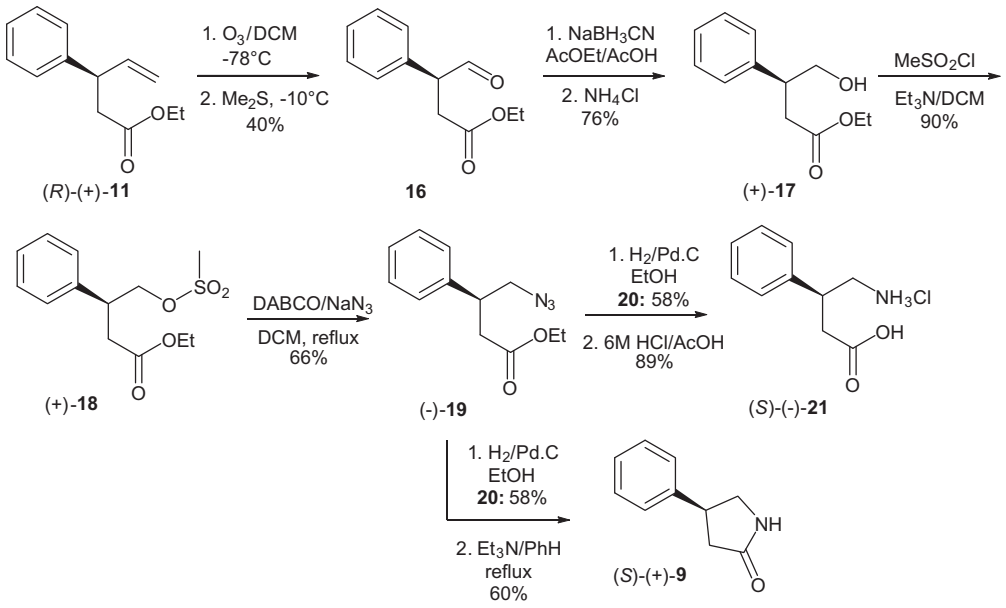


Figure 3. Retrosynthetic analysis of 3-phenylGABA.



Scheme 4. Formal synthesis of 3-phenylGABA.

3. Conclusion

We have studied the enzyme-catalysed synthesis of enantiomerically pure ester derivates of 3-phenyl-4-pentenoic acid. Our results demonstrate that several enzymes catalysed these reactions with excellent enantioselectivity, when we used triethyl orthoacetate as the donor of the alkoxy group. Trialkyl orthoesters were used previously in the kinetic resolution of racemic carboxylic acids, catalysed by enzymes, although this reactions required a few days and the enantiomeric excesses were lower.[16] We proved that other donors such acetals and ketals can be used in kinetic resolution of racemic 3-phenyl-4-pentenoic acid. In most cases, the proper choice of the enzyme and the alkoxy group donor is required to achieve excellent enantioselectivity.

The solvent effect in the reaction studied was investigated in an attempt to find suitable reaction conditions; the best result was obtained in toluene.

We have also provided a novel and convenient chemoenzymatic method for the preparation of biologically active compounds: 3-phenylGABA **1** and 4-phenyl-pyrrolid-2-one **9**. These two six-step syntheses have been developed starting from the readily available precursor (*R*) ethyl-3-phenyl-4-pentenoate **11**, through an efficient enzymatic kinetic resolution.

4. Experimental

4.1. General

The HPLC analyses were performed on a Chiracel OD-H column (4.6 mm × 250 mm, from Diacel Chemical Ind., Ltd) equipped with a pre-column (4 mm × 10 mm, 5 m) using an LC-6A Varian ProStar apparatus with UV Varian ProStar 330 detector and Chromatopac C-R6A analyzer. The elemental analyses were performed on CHN Perkin–Elmer 240 apparatus. All reactions were monitored by TLC on Merck silica gel Plates 60 F254. The lipases were purchased from Fluka. Novo sp 435A was purchased from Novo Industri A/S. The acetone powders were prepared in our laboratory.[17] All of the chemicals were obtained from commercial chemical sources. The solvents were of analytical grade.

4.2. Synthesis of 3-phenyl-4-pentenoic acid 2

3-Phenyl-4-pentenoic acid was synthesized according to the method described by Bermejo et al.[18] A mixture of cinnamyl alcohol (0.25 mol; 33.7 g), triethyl orthoacetate (0.25 mol; 46.1 mL) and hexanoic acid (1.5 mmol; 0.19 mL) was heated in an oil bath. The solution was placed in a flask with a Claisen head (for distilling off ethanol), condenser and thermometer. After 3 h, 0.1 mL of hexanoic acid was added. An additional portion (0.1 mL) of hexanoic acid was added at 3.5 and 4.5 h. After 6 h the temperature rose to 166 °C. After this time, 27 mL of ethanol were distilled off and TLC analysis indicated that no cinnamyl alcohol remained.

The mixture was cooled, and a solution of potassium hydroxide (0.35 mol; 19.7 g) in water (25 mL), and methanol (75 mL) were added. The mixture was heated at reflux for 1 h under nitrogen. After cooling, the solution was washed with ethyl ether and acidified with concentrated HCl to pH 1. The acidic solution was extracted with ethyl ether (3×) and the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure.

The crude acid was purified by crystallization from hexane (75% yield) to give a product that melted at 46 °C.[10] ^{1}H NMR (200 MHz, $CDCl_3$): 2.74 (dd, J = 15.0, 7.5 Hz, 1H), 2.78 (dd, J = 15.0, 7.8 Hz, 1H), 3.90 (q, J = 7.2 Hz, 1H), 5.00–5.18 (m, 2H), 5.90–6.10 (m, 1H), 7.19–7.40 (m, 5H); ^{13}C NMR (200 MHz, $CDCl_3$): 40.2, 45.5, 115.3, 127.1, 127.8, 128.9, 140.2, 142.2, 178.3.

4.3. Synthesis of racemic ethyl 3-phenyl-4-pentenoate 11

Compound **11** was synthesized as described by Gopalan.[13] To a solution of 3-phenyl-4-pentenoic acid (1 mmol) in toluene, the corresponding triethyl orthoester **10** (3 mmol) was added. The reaction mixture was heated at reflux (110 °C) for 24 h.

After cooling, 2 M HCl was added (1.1 mL). The organic layer was washed with saturated $NaHCO_3$ (1×) and brine (1×), and dried over anhydrous $MgSO_4$. The excess reagents and solvent were evaporated under vacuum.

The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate (99.5/0.5; v/v) as an eluent to afford the corresponding product **11** as a colourless oil; R_f = 0.8 (hexane/EtOAc, 8:2; v/v); HPLC analysis [hexane/*i*PrOH; 9:1; λ = 232 nm; 1.0 mL/min] $t_R(S)$ = 9.77 min; $t_R(R)$ = 11.03 min.

4.4. Enantioselectivity synthesis of ethyl 3-phenyl-4-pentenoate 11

To a solution of 3-phenyl-4-pentenoic acid (1 mmol) in toluene, the corresponding triethyl orthoester **10** or acetal **14** (3 mmol) and enzyme (10 mg) were added. The reaction mixture was stirred for 24 h at 40 °C.

After cooling, 2 M HCl was added (1.1 mL). The organic layer was washed saturated $NaHCO_3$ (1×) and brine (1×), and dried over anhydrous $MgSO_4$. The excess reagents and solvent were evaporated under vacuum.

The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate (99.5/0.5; v/v) as an eluent to afford the corresponding product **11** as a colourless oil; R_f = 0.8 (hexane/EtOAc, 8:2; v/v); HPLC analysis [hexane/*i*PrOH; 9:1; λ = 232 nm; 1.0 mL/min] $t_R(S)$ = 9.77 min; $t_R(R)$ = 11.03 min.

4.5. Synthesis of racemic methyl 3-phenyl-4-pentenoate 13

Compound **13** was synthesized as described by Gopalan.[13] To a solution of 3-phenyl-4-pentenoic acid (1 mmol) in toluene, the corresponding trimethyl orthoester **12** (3 mmol) was added. The reaction mixture was then heated at reflux (110 °C) for 48 h.

After cooling, 2 M HCl was added (1.1 mL). The organic layer was washed with saturated $NaHCO_3$ (1×) and brine (1×), and dried over anhydrous $MgSO_4$. The excess reagents and solvent were evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate (99.5/0.5; v/v) as an eluent to afford the corresponding product **13** as a colourless oil; R_f = 0.5 (hexane/EtOAc, 9:1; v/v); HPLC analysis [hexane/*i*PrOH; 9:1; λ = 232 nm; 1.0 mL/min] $t_R(S)$ = 4.70 min; $t_R(R)$ = 6.70 min.

4.6. Enantioselective synthesis of methyl 3-phenyl-4-pentenoate 13

To a solution of 3-phenyl-4-pentenoic acid (1 mmol) in toluene, the corresponding trimethyl orthoester **12** or acetal **14** or ketal **15** (3 mmol) and enzyme (10 mg) were added. The reaction mixture was then stirred for 48 h at 35–40 °C. After cooling, 2 M HCl was added (1.1 mL). The organic layer was washed with saturated $NaHCO_3$ (1×) and brine (1×), and dried over anhydrous $MgSO_4$. The excess reagents and solvent were evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate (99.5/0.5; v/v) as an eluent to afford the corresponding product **13** as a colourless oil; R_f = 0.5 (hexane/EtOAc, 9:1; v/v); HPLC analysis [hexane/*i*PrOH; 9:1; λ = 232 nm; 1.0 mL/min] $t_R(S)$ = 4.70 min; $t_R(R)$ = 6.70 min.

4.7. Enantioselective synthesis of ethyl 3-phenyl-4-pentenoate 11 in different solvents

To a solution of 3-phenyl-4-pentenoic acid (1 mmol) in an organic solvent, triethyl orthoacetate **10a** (3 mmol) and Lipase from Wheat germ (10 mg) were added. The reaction mixture was then stirred for 24 h at 40 °C. After cooling, 2 M HCl was added (1.1 mL). The organic layer was washed with saturated $NaHCO_3$ (1×) and brine (1×), and dried over anhydrous $MgSO_4$. The excess reagents and solvent were evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate (99.5/0.5; v/v) as an eluent to afford product **11** as a colourless oil; R_f = 0.8 (hexane/EtOAc, 8:2; v/v); HPLC analysis [hexane/*i*PrOH; 9:1; λ = 232 nm; 1.0 mL/min] $t_R(S)$ = 9.77 min; $t_R(R)$ = 11.03 min.

4.8. Synthesis of the ethyl ester of 3-phenyl-4-oxobutyric acid 16

A solution of the ethyl ester of (*R*)-(+)-3-phenyl-4-pentenoic acid (12.2 mmol) in dry dichloromethane was cooled to −78 °C. Next, this solution was saturated by ozone for 30 min in −78 °C. Next, air was blown for 5 min and dimethyl sulfide (1 mL) was added. After evaporating the excess solvent, the crude product was purified by silica gel flash chromatography using hexane/ethyl acetate as an eluent to afford product **16** as a colourless oil (40% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.21 (t, J = 7.12 Hz, 3H), 2.60 (dd, J = 7.0, 15.0 Hz, 1H), 3.18 (dd, J = 7.0, 15.0 Hz, 1H), 3.94–4.40 (m, 2H), 5.10 (dd, J = 7.0, 15.0 Hz, 1H), 7.10–7.58 (m, 5H), 9.60 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 34.9, 54.9, 61.1, 127.8, 128.3, 128.8, 129.0, 129.1, 129.5, 171.8, 198.8; ESI-MS HR, $[M+Na]^+$, calcd for $C_{12}H_{14}O_3Na$: 229.083, found (m/z) 229.082 (100%).

4.9. Synthesis of the ethyl ester of (+)-3-phenyl-4-hydroxybutyric acid 17

To a solution of acid **16** (3.0 mmol) in ethyl acetate/acetic acid (9/1, v/v, 5 mL) was added sodium cyanoborohydride (3 mmol) and stirred in room temperature for 20 min after which a saturated solution of ammonium hydrochloride was added. The reaction mixture was then extracted with ethyl acetate (3 × 5 mL). The organic layer was dried over anhydrous $MgSO_4$ and the excess solvent was evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate as an eluent to afford product **17** as a colourless oil (76% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22 (t, J = 7.2 Hz, J = 14.2 Hz, 3H), 2.71 (dd, J = 7.0, 15.0 Hz, 1H), 2.91 (dd, J = 7.0, 15.0 Hz, 1H), 3.33–3.47 (m, 1H), 3.73–3.89 (m, 2H), 4.12 (q, J = 7.0 Hz, 2H), 5.07 (1H), 7.22–7.44 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 21.0, 37.7, 44.7, 60.9, 67.0, 127.3, 128.0, 128.7, 128.9, 141.2, 173.0, 176.2; ESI-MS HR, $[M+Na]^+$, calcd for $C_{12}H_{16}O_3Na$: 231.099, found (m/z): 231.099; $[\alpha]_D^{25}$ = +4.8 (c 1.0, $CHCl_3$).

4.10. Synthesis of the ethyl ester of (+)-3-phenyl-4-methansulfonyloxybutyric acid 18

A solution of acid **17** (0.96 mmol, $[\alpha]_D$ = +4.8) and triethylamine (2.89 mmol) in dry dichloromethane was cooled to 0 °C and methanesulfonic acid chloride was added (2.74 mmol). The reac-

tion mixture was stirred at room temperature for 6 h, filtered and the excess solvent evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate as an eluent to afford the corresponding product **18** (90% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (t, J = 7.2 Hz, J = 14.2 Hz, 3H), 2.70–3.00 (m, 1H), 2.90 (s, 3H), 3.57–3.73 (m, 1H), 4.13 (q, J = 7.4 Hz, J = 7.0 Hz, 2H), 4.48–4.52 (m, 2H), 7.28–7.45 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 37.0, 37.5, 41.7, 61.0, 72.8, 128.0, 128.0, 129.1, 139.3, 171.5; ESI-MS HR, $[M+Na]^+$, calcd for $C_{13}H_{18}O_5NaS$: 309.076, found (m/z): 309.076; $[\alpha]_D^{25}$ = +1.05 (c 1.0, $CHCl_3$).

4.11. Synthesis of the ethyl ester of (-)-3-phenyl-4-azidobutyric acid 19

A solution of ester **18** (1.64 mmol), sodium azide (3.28 mmol), diazabicycloundecane (2.62 mmol), DMAP (10 mg) and crown ether B18C6 (10 mg) in dry dichloromethane (10 mL) was heated at reflux for 72 h. After filtration, the excess solvent was evaporated under vacuum. The crude product was purified by silica gel flash chromatography using hexane/ethyl acetate as an eluent to afford product **19** (66% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.18–1.30 (m, 3H), 2.64–3.12 (m, 2H), 3.40–3.90 (m, 4H), 4.06–4.24 (m, 1H), 7.24–7.47 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 38.1, 38.2, 49.0, 56.5, 127.7, 127.8, 127.9, 128.9, 129.0, 140.8, 140.9, 171.8; ESI-MS HR, $[M+Na]^+$, calcd for $C_{12}H_{15}N_3O_2Na$: 256.105, found (m/z): 256.104; $[\alpha]_D^{25}$ = −0.6 (c 1.0, $CHCl_3$).

4.12. Synthesis of the ethyl ester of 3-phenyl-4-aminobutyric acid 20

To a solution of ester **19** (0.38 mmol) in ethanol (5 mL) was added palladium on carbon. The reaction mixture was then stirred under hydrogen for 10 h, filtered and the excess solvent evaporated under vacuum. Product **20** was obtained in 58% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.16 (t, J = 7.2 Hz, 3H), 2.75 (dd, J = 5.5, 9.8 Hz, 1H), 2.84 (dd, J = 5.5, 16.1, 1H), 3.25 (q, 1H), 3.40 (m, 2H), 4.06 (q, J = 7.2 Hz, 2H), 7.37 (m, 3H), 7.43 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 22.1, 38.0, 38.9, 48.9, 126.6, 127.0, 127.7, 127.8, 128.1, 128.7, 128.9, 129.1, 140.5, 172.2; ESI-MS HR, $[M+Na]^+$, calcd for $C_{12}H_{18}NO_2$: 208.133, found (m/z): 208.132.

4.13. Synthesis of (*S*)-3-phenyl-4-aminobutyric acid hydrochloride (phenylGABA) 21

To a solution of ester **20** (0.58 mmol) in acetic acid (1 mL) was added hydrochloric acid (6 M, 0.1 mL). The reaction mixture was then stirred for 24 h at room temperature, after which the excess solvent was evaporated under vacuum. The crude product was purified by crystallization from benzene (89% yield). ^{1}H NMR (400 MHz, D_2O): δ 2.75 (dd, J = 5.5, 9.8 Hz, 1H), 2.84 (dd, J = 5.5, 16.1, 1H), 3.25 (m, 1H), 3.40 (m, 2H), 7.37 (m, 3H), 7.43 (m, 2H); ^{13}C NMR (50 MHz, D_2O): δ 38.5, 40.2, 44.1, 128.1, 128.6, 129.6, 138.6, 175.8—according to the literature;[10] ESI-MS HR, $[M]^+$, calcd for $C_{10}H_{14}NO_2$: 180.102, found (m/z): 180.102, $[\alpha]_D^{25}$ = −0.7 (c 1.0, H_2O).

4.14. Synthesis of the ethyl ester of (*S*)-(+)-4-phenyl-2-pyrollidone 9

Compound **20** (0.04 mmol) was suspended in toluene (3 mL) and triethylamine was dropped (0.04 mmol). The reaction mixture was then heated at reflux for 24 h, after which the excess solvent was evaporated under vacuum. The residue was washed with dichloromethane. The organic layer was washed with hydrochloric acid (10%, 3 × 5 mL) and water (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the excess solvent evaporated. The crude product was purified by crystallization from hexane/ethyl acetate (60% yield). ESI-MS HR, $[M]^+$, calcd for $C_{10}H_{11}NO$: 161.084, found (m/z): 161.085; mp 78 °C [lit. 76–77].[11]

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