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Synthesis of Optically Pure Mechanism-Based Inhibitors of γ-Aminobutyric Acid Aminotransferase (GABA-T) via Enzyme-Catalyzed Resolution

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Abstract: The kinetic resolution of γ-ethynyl- ,γ-allenyl- and γ-vinyl GABA was accomplished by penicillin acylase-catalyzed hydrolysis of their *N*-phenylacetyl derivatives. The procedure employs inexpensive commercially available immobilized enzyme.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter. When the concentration of GABA in the brain decreases below a threshold level, seizures and other neurological disorders occur.[1] The appropriate level of GABA at the synaptic cleft can be maintained by the irreversible inactivation of the enzyme GABA-T (EC 2.6.1.1 9), which is involved in the degradation of GABA.[2]

Several mechanism-based inhibitors of GABA (γ-ethynyl **1**, γ-allenyl **2** and γ-vinyl **3** GABA) have been designed and synthesized [2,3]. All these compounds have potential for therapeutic use and γ-vinyl GABA (vigabatrin) has already been approved in Europe as an effective drug for the treatment of epilepsy.[4]

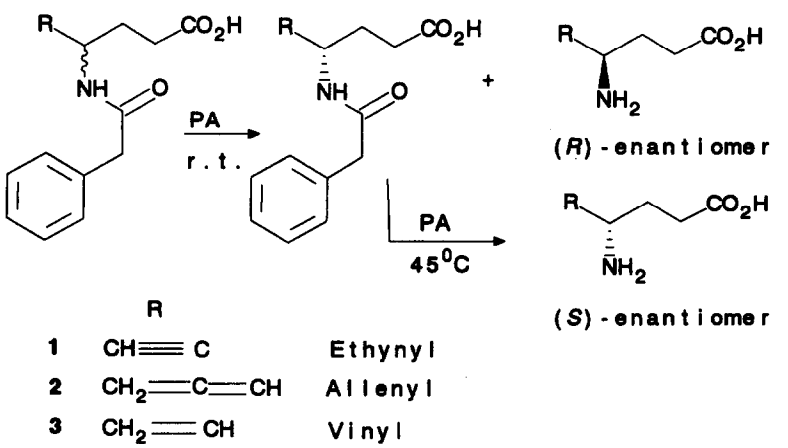
The biological activity of GABA-T inhibitors **1**, **2**, and **3** strongly depends on their absolute configuration. Thus, the biological activity of **2** and **3** resides in their *(S)*-enantiomer.[4,5] Conversely, *(R)*-**1** is more active as an anticonvulsant agent *in vivo* than its *(S)*-counterpart or the racemic compound.[6] So far, the enantiomers of **1**, **2** and **3** have been produced by asymmetric synthesis[5,7] or diastereomer crystallization.[8,9] These routes, however, are not suitable for large-scale synthesis , due to poor overall yield and/or lengthy synthetic sequences.

The use of enzymes as catalysts for stereoselective transformations[10] has recently gained in popularity, especially, in the synthesis of optically pure drugs.[11] Despite the ability of many enzymes to discriminate

between the enantiomers of a chiral compound, **1**, **2**, and **3** turned out to be difficult targets for enzyme-based resolution techniques. Enzymes, such as aminoacylases[12] and aminopeptidases,[13] that are used for the resolution of α-amino acids do not resolve γ-amino acids. Lipases do catalyze the enantioselective hydrolysis of the esters of *N*-acyl-**3**, but with modest stereoselectivity.[14] Resolution of these compounds by the newly developed technique with ω-amino acid transaminases,[15] may also present a serious problem since **1**, **2** and **3** are designed to irreversibly inhibit the very same group of enzymes.

Here, we report a simple procedure for the preparation of the enantiomers of **1**, **2** and **3** using penicillin acylase-catalyzed hydrolysis of the corresponding *N*-phenylacetyl derivatives. Penicillin acylase (PA) from *E. coli* (EC 3.5.1.11) is used in industry for the preparation of 6-aminopenicillanic acid from benzylpenicillin and can also be employed for the preparation of a variety of semi-synthetic ß-lactam antibiotics.[16] PA is highly selective to the phenylacetyl group and catalyzes its cleavage not only from penicillins, but also from other amines, peptides and alcohols.[17] The structure of the leaving group of the substrates minimally affects the rate constants of the PA-catalyzed hydrolytic reaction.[18] The enantioselectivity of PA was exploited in the preparation of amino acids,[19] aminoalkylphosphonic acids,[20] esters and alcohols,[21] although the hydrolysis of an ester bond normally results in products with modest optical purity. Recently, the high enantioselectivity of PA in the acylation reaction was demonstrated in the synthesis of a new carbacephalosporin, locarbef.[22]

We found that the combination of broad specificity of PA towards leaving groups in the substrate, with the potentially high enantioselectivity of the enzyme in the hydrolysis reactions is useful in the synthesis of optically pure GABA-T inhibitors **1**, **2** and **3**. The resolution procedure is outlined in the Scheme. In a typical experiment, PA immobilized on Eupergit C (0.7 g: Rohm Pharma), was suspended in a solution of *N*-phenylacetyl-**3** (1.0 g; 4 mmol) in 35 mL 0.1 M phosphate buffer pH 7.8. The mixture was stirred at room temperature for 5 h. The pH of the solution was adjusted to 2 with a 1 M HCl and the remaining substrate was extracted with CH_2Cl_2 (3 x 50 mL) to give *(S)*-*N*-phenylacetyl-**3** (organic layer) and *(R)*-**3** (aqueous layer). The layers were separated and the aqueous phase was chromatographed on Dowex 1 x 2-100 OH^-. *(R)*-**3** was eluted with 0.25 M acetic acid and lyophilized to afford 0.24 g (1.9 mmol; 47%) of *(R)*-**3**.



	R	
1	$CH{\equiv}C$	Ethynyl
2	$CH_2{=}C{=}CH$	Allenyl
3	$CH_2{=}CH$	Vinyl

R[a]	Relative reaction rate[b]	(*R*)-γ-amino acid[e] Yield (%)[c]; ee (%)[d]		(*S*)-γ-amino acid Yield (%)[c]; ee (%)[d]		E[f]
Ethynyl 1	100	48	>96[g]	41	83	>100
Allenyl 2	46	54	75	43	>98[g]	20
Vinyl 3	39	47	78	35	99	17

(a) *N*-phenylacetyl derivatives of 1, 2 and 3 were prepared under Schotten-Baumann conditions. The identities of the products have been confirmed by MS, ^{1}H NMR and microanalysis.

(b) [S] = 50 mM; pH 7.8; 25 °C.

(c) Isolated yields for lyophilized compounds.

(d) Enantiomeric excess for lyophilized compounds were determined by GC using Chirasil-Val column (Chrompack) according to the procedure of Wagner *et.al.*[23]

(e) The absolute configurations were assigned by direct comparison with authentic samples.

(f) The E values were calculated from the yields and the ee's obtained for the (*R*)-enantiomers.[24]

(g) The sensitivity limit of the method.

Since the deacylation of (*S*)-*N*-phenylacetyl-3 with 5 M HCl resulted in the formation of by-products, the same enzyme was used for the deacylation of (*S*)-*N*-phenylacetyl-3. To achieve an efficient hydrolysis, a larger amount of Eupergit-PA (1.5 g), higher reaction temperature (45°C), and longer reaction time (2 d) were employed. When the reaction was completed (HPLC), the Eupergit-PA was filtered off, the pH of the solution was adjusted to 2 with 1 M HCl, and phenylacetic acid was extracted with CH_2Cl_2 (3 x 50 mL).

The aqueous solution was purified as described for (*R*)-3 to give 0.18 g (1.4 mmol; 35%) of (*S*)-3. The enantiomers of 1 and 2 (0.1-0.5 g) were prepared by the same procedure.

One can see that this entire group of potent GABA-T inhibitors, which includes the pharmaceutically important (*S*)-enantiomers of 2 and 3, as well as both enantiomers of 1 can been synthesized in good yield and with high optical purity by the here described enzyme-catalyzed resolution technique. It should be stressed that this procedure employs an inexpensive, commercially available immobilized enzyme, which has already proven its excellent qualities in the reactions on a scale of more than 100 kg.

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