A concise synthesis of (*S*)-ESBA, the first selective KATII inhibitor and their analogs

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Abstract: A straightforward preparation of racemic and enantiomerically highly enriched *N*-substituted 4-(ethylX)benzoylalanine (X – S, SO, SO_2) will be presented. This process involves the tandem of crystallization-induced asymmetric transformation with conjugate addition of ammonia and chiral *N*-nucleophiles to the corresponding aroylacrylic acids. Further transformations of *N*-substituted oxoamino acids to the 4-(ethylsulfanyl)-, 4-(ethylsulfenyl)- and 4-(ethylsulfonyl)benzoylalanine in racemic and enantiomerically enhanced from *via* periodate oxidation are also described. The targeted amino acid (*S*)-4-(ethylsulfonyl)benzoylalanine (*S*)-ESBA is the first known selective kynurenine aminotransferase (KAT II) inhibitor.

Keywords: crystallization-induced asymmetric transformation, periodate degradation, ESBA, amino acid, kynurenine

1. Introduction

Kynurenine aminotransferase (KAT) is a pyridoxal 5´-phosphate (PLP)-dependent enzyme catalyzing the irreversible transamination of L-kynurenine (KYN) to produce kynurenic acid (KYNA). KYN is the central metabolite in the kynurenine pathway (KP), the main catabolic process of tryptophan in most living organisms [1]. At least two isoforms (KAT I and KAT II) are present in the mammalian brain and have been characterized by biochemical and genetical methods [2].

From the known enzyme inhibitors of KP, substituted aroylalanines – structural analogues of kynurenine, represent the intensively studied derivatives. Among them, *m*-nitrobenzoylalanine (*m*-NBA) and 3,4-dichlorobenzoylalanine (FCE28833) possess kynurenine 3-monooxygenase (3-KMO) inhibition activity. (4*R*)-5-Bromodihydro-L-kynurenine was found to be one of the most potent inhibitors of kynureninase [3]. The straightforward synthesis of several haloaroylalanines we described in our previous paper [4]. Another important medicinal target of such type of aroylalanines represents 4-ethylsulfonylbenzoylalanine (ESBA) which show kynurenine aminotransferease II (KAT II) inhibition activity [5].

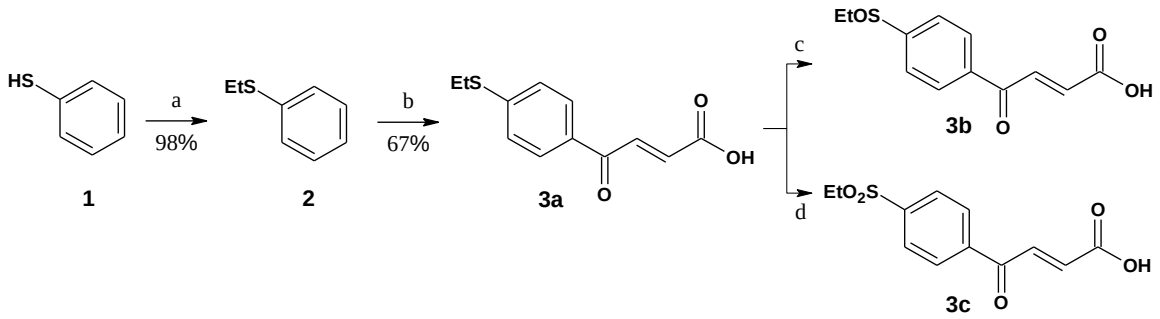
Our synthetical approach for preparation of (*S*)-ESBA is based on the crystallization-induced asymmetric transformation (CIAT). CIAT is a useful methodology for the control of

the stereochemical outcome of diverse chemical reactions. There are number of intriguing applications where CIAT has been used to obtain enantiomerically and diastereomerically pure molecules simply by crystallization of one of two equilibrating isomers. Recoveries can approach 100 % based on the mixture regardless of the equilibrium constant in solution. The CIAT approach has been used for control of the absolute configuration of stereogenic carbons or other heteroelements [6].

2. Results and discussion

The starting substituted (*E*)-4-aryl-4-oxo-2-butenoic acids 3 were prepared *via* the Friedel–Craft's acylation of substituted benzenes **2** with maleic anhydride [7]. Ethylsulfanyl benzene was prepared from thiophenol **1** and etylbromide by nucleophilic substitution (Scheme 1).

Scheme 1. Synthesis of aroylacrylic acid **3a-c**



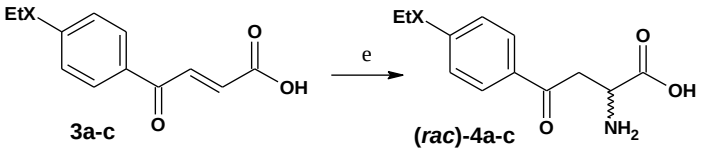
(a) Ethylbromide, NaOH, TBABr, H_2O, toluene; (b) $AlCl_3$, DCM, maleic anhydride; (c) 1 eq. H_2O_2 in CH_3COOH, 70°C; (d) 2 equiv. H_2O_2 in CH_3COOH, 70°C

Table 1. Results of preparation of aroylacrylic acid **3a-c**

Entry	X	yield [%]	m.p. [°C]
3a	S	67	120-124
3b	S=O	70	140-143
3c	O=S=O	98	148-150

Sulfide oxidation is one of the standard approaches to sulfones, where a stoichiometric or larger amount of common oxidants such as $KMnO_4$, MCPBA and magnesium monoperoxyphthalate (MMPP) is often required[8, 9]. However, sulfides could be efficiently oxidized with hydrogen peroxide in presence of acetic acid. The oxidation to the corresponding sulphoxide which produces 4-(ethylsulfenyl)benzoylacrylic acid was acoomplished with one equivalent of reagent in very short time. The excess of hydrogen peroxide caused consecutive oxidation into second level and 4-(ethylsulfonyl)benzoylacrylic acid was obtained in 98 % yield as light yellow crystals (Scheme 1). Next, we continued with racemic aroylalanines (*rac*)-**4a-c** synthesis *via* the conjugate addition of aqueous ammonia to substituted Michael acceptors **3a-c.** The results are summarized in Scheme 2.

Scheme 2. Preparation of racemic amino acids **4a-c**

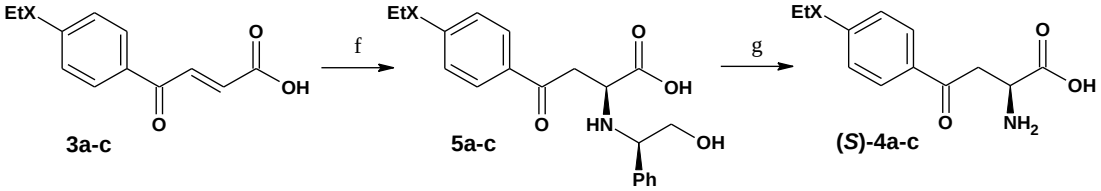


Entry	X	yield [%]	m.p. [°C]
4a	S		212-215
4b	S=O		185-187
4c	O=S=O		209-213

e) aqueous ammonia (12-15 %)

The synthesis of enantiomerically pure aroylalanines 5a-c was realized by the addition of an chiral, enantiomerically pure *N*-nucleophile to the aroylacrylic acids **3a-c** in tandem with crystallization-induced asymmetric transformation, which is intensively studied in our laboratory[4, 6, 10-15]. Identically to all previously described examples, at the very beginning the asymmetric transformation produced a mixtures of both diastereomers in approximately equal proportion (Figure 1, 2). However the situation changed with the time when the conditions for effective equilibrium between both diastereoisomers in solution are fulfilled. The speed of diastereoisomer alteration is largely depending on the substrate solubility. For example for the less soluble **5a** the high degree of asymmetric transformation was obtained after 34 hours of stirring. The compound **5c** with more polar sulfonyl group the equilibrium was obtained within 3 hours (Figure 2). After the corresponding time (HPLC control) the filtration of the reaction suspension allowed to obtain practically one of two possible diastereoisomers in high yield and with excellent diastereomeric purity (Table 2).

Scheme 3. Preparation of adducts **5** and optically pure amino acids **4**



(f) (*R*)-(−)-2-Phenylglycinol, MeOH; (g) $NaIO_4$, H_2O, NH_3

Table 2. Preparation of N-substituted adducts **5** and targeted amino acids **4**

Entry	X	yield [%]	d.r./ e.r.	Configuration	$[\alpha]_D^{20}$	m.p. [°C]
5a	S	90	98:2	(2*S*,1′*R*)		182-184
5b	S=O	80	98:2	(2*S*,1′*R*)	+ 53.176	166-168
5c	O=S=O	84	98:2	(2*S*,1′*R*)	+ 40.309	191-193
4a	S			(2*S*)		212-215
4b	S=O			(2*S*)		186-188
4c	O=S=O	35	>97:3	(2*S*)		211-213

Finally, oxidative degradation of vicinal aminoalcohols with sodium periodate was realized on prepared adducts **5a-c**. The neutral oxidative conditions, which we used in our laboratory previously, didn`t work. [13] Considering lower solubility of oxoamino acids **5a-c** we have used the slightly alkaline condition by addition of aqueous ammonia. The desired free amino acids (*S*)-**4a-c** were obtained in poor to acceptable yields (Table 2). The lower yields were

caused by higher solubility and low stability of prepared amino acids (mainly for **4b,c)** (Scheme 3).

Figure 1. The stereochemical development of addition of (*R*)-Phenylglycinol to acid **3b**; (■) (2*S*,1´*R*)-diastereomer, (□) (2*R*,1´*R*)-diastereomer

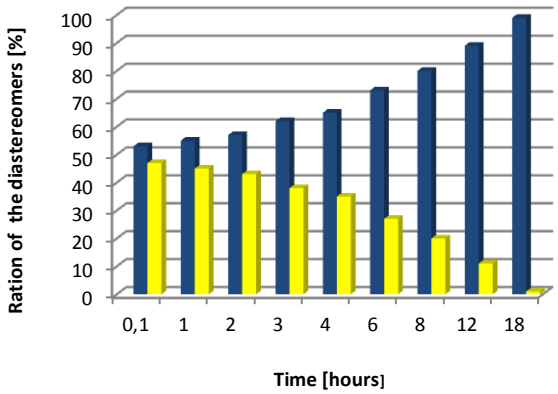
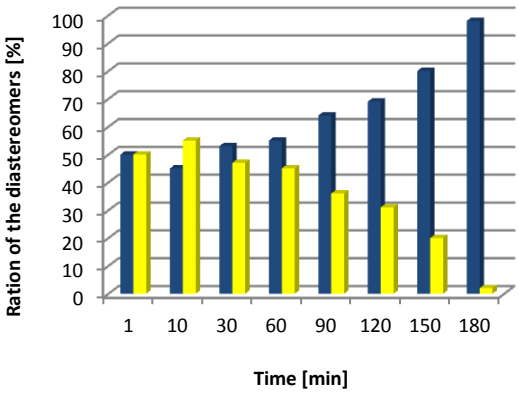


Figure 2. The stereochemical development of addition of (*R*)-Phenylglycinol to acid **3c**; (■) (2*S*,1´*R*)-diastereomer, (□) (2*R*,1´*R*)-diastereomer



3. Conclusions

We have developed an easy a straightforward synthesis of 4-(ethylX)benzoyl alanines (X= S. SO, SO_2) in both racemic and also enantiomerically highly enriched forms using tandem *aza*-Michael addition and crystallization-induced asymmetric transformation of accessible 4-(ethylX)bezoylacrylic acids. The synthetic pathway represents an short alternative for the preparation of (*S*)-ESBA known as a first KAT II inhibitor.

4. Experimental section

General

All reagents were used as received without further purification unless otherwise specified. (*R*)-(−)-Phenylglycine (98% ee) was obtained from ACROS Organics which was used for the preparation of (*R*)-(−)-Phenylglycinol (98% ee). Optical rotations ware measured with P-1020 polarimeter from JASCO company and with digital polarimeter POLAR L-µP (IBZ Messtechnik) at a wavelength of sodium line D (λ = 589nm). Specific rotations are given in units of 10^{-1} deg.cm^{-2}.g^{-1} and concentrations are given in g.cm^{-3}. Melting point were obtained using a Koffler hot plate and are uncorrected.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR-300 spectrometer (299,94MHz for ^{1}H NMR or 75,43MHz for ^{13}C NMR). Chemical shift (δ)is quoted in parts per million and is referenced to the tetramethylsilane (TMS) as in internal standard (δ_{TMS}= 0.00 ppm). Coupling constant (*J*) is recorded in Hertz. The following abbreviations were used through to characterize signal multiplicities: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad).

HPLC experiments were performed on VARIAN Pro Star chromatographic system (pump, UV detector, autosampler). The following columns and eluents were used for HPLC experiments: C18 5μm reverse phase column Phenomenex Luna C18 250 x 4,6 mm or Phenomenex Luna Phenyl-Hexyl 250 x 4,6 mm or XTerra® RP18 250 x 4,6 mm. Eluent was a mixture of acetonitile/water/ triethylamine 200:500:15 or 250:750:15, pH adjusted to 3,5 or 3,2 by H_3PO_4. For determination of enantiomeric purity we used CROWNPAK CR(+) 150 x 4 mm, as a eluent was used solution of $HClO_4$ adjusted to pH=2. The mobile phase was pumped through the system at 0,5 – 1,5 mL.min^{-1} at room temperature.

*Ethylphenyl sulfide (**2**)*

Towards the prepared heterogenous mixture water (80 mL) and toluene (120 mL) was added *tetra*-butylamoniumu bromide (8 g, 0.027 mol), NaOH (8.8 g, 0.22 mol) and ethylbromide (24 g, 0.22 mol). Reaction mixture was intensive stirred at r.t. Then thiophenol (22 g, 0.2 mol) was added stepwise. After 3 hours stirring the organic layer was separated and washed with 2x50 mL 10% NaOH and 2x50 mL H_2O and dried over Na_2SO_4. Toluene was distilled off and residuum was distilled by reduced pressure (20 mm Hg, 100°C). We obtained colorless oil (27 g, 98%, b.p. 200-203°C)

^{1}H NMR ($CDCl_3$), δ: 7.37 – 7.27 (m, 4H, H-2´,3´,5´,6´), 7.21 – 7.16 (m, 1H, H-4´), 2.96 (q, 2H, *J*=7.3, H-1), 1.33 (t, 3H, *J*=7.3, H-2),
^{13}C NMR (CDCl3), δ: 129.0, 128.8, 125.8 (C-Ar), 27.6 (C-1), 14.4 (C-2).

*(2E)-4-[4-(ethylsulfanyl)phenyl]-4-oxobut-2-enoic acid (**3a**)*

$AlCl_3$ (50.5 g, 0.38 mol) was suspended in dichloromethane (300 mL) and then was slowly added maleic anhydride (18.7 g, 0.19 mol). The reaction mixtures was stirred about 30 min and ethylphenyl sulfide (24 g, 0.17 mol) was added dropwise. The reaction mixture was stirred next 3 hours. Crude reaction mixture was poured into mixtures of ice and conc. HCl (1:4). Organic layer was separated and water was extracted with dichloromethane (2x30 mL). Organic layers was combined, dichloromethane was evaporated out. Crude aroylacrylic acid was crystallized from toluene (27 g, 67%, m.p. 120-124°C)

^{1}H NMR (CD_3OD), δ: 7.99 (d, 1H, *J*=15.5,H-3), 7.93 (d, 2H, *J*=8.5, H-Ar), 7.35 (d, 1H, *J*=8.5, H-Ar), 6.90 (d, 1H, *J*=15.5, H-2), 3.06 (q, 2H, *J*=7.4,H-1´), 1.41 (t, 3H, *J*=7.4,H-2´),
^{13}C NMR (CD_3OD), δ: 187.8 (C-4), 169.9 (C-1), 146.7, 138.3 (C-Ar), 132.8 (C-3), 130.9 (C-2), 129.3, 126.2 (C-Ar), 25.7 (C-1´), 13.8 (C-2´).

*(2E)-4-[4-(ethylsulfonyl)phenyl]-4-oxobut-2-enoic acid (**3c**)*

Acrylic acid 3a (9.93 g, 42 mmol) was dissolved in acetic acid (200 mL) and 30% hydrogen peroxide was added (9.5 mL, 92.4 mmol). The suspension was stirred by 70°C 20 min and then solvent was evaporated. Crude acid 3c was crystallized from ethyl acetate and hexane. The light yellow crystals (11.0 g, 98%, m.p. 148-150°C) were obtained.

^{1}H NMR (CD_3OD), δ: 8.24 (d, 2H, *J*=8.5, H-Ar), 8.09 (d, 2H, *J*=8.4, H-Ar), 7.93 (d, 1H, *J*=15.6, H-3), 6.83 (d, 1H, *J*=15.6, H-2), 3.28 (q, 2H, *J*=7.4, H-1´), 1.23 (t, 3H, *J*=7.4, H-2´), ^{13}C NMR (CD_3OD), δ: 190.4 (C-4), 168.1 (C-1), 144.1, 142.0 (C-Ar), 137.1 (C-3), 134.9 (C-2), 130.7, 129.9 (C-Ar), 51.0 (C-1´), 7.5 (C-2´).

*(2E)-4-[4-(ethylsulfinyl)phenyl]-4-oxobut-2-enoic acid (**3b**)*

Acrylic acid 3a (1.0 g, 4.23 mmol) was dissolved in acetic acid (20 mL) and 30% hydrogen peroxide was added (0.432 mL, 4.23 mmol). The suspension was stirred by 70°C 20 min and then solvent was evaporated. Crude acid **3b** was crystallized from ethyl acetate. We obtained yellow crystals (0.75 g, 70%, m.p. 140-143°C).

^{1}H NMR (CD_3OD), δ: 8.23 (d, 2H, *J*=8.6, H-Ar), 7.96 (d, 1H, *J*=15.6, H-3),7.85 (d, 2H, *J*=8.6Hz, H-Ar), 6.83 (d, 1H, *J*=15.6, H-2), 3.14 (qd, 1H, *J*=7.4, *J*=14.7, H-1´), 2.91 (qd, 1H, *J*=7.3, *J*=14.6, H-1´), 1.21 (t, 1H, *J*=7.4, H-2´)
^{13}C NMR (CD_3OD), δ: 190.4 (C-4), 168.2 (C-1), 149.5, 140.2 (C-Ar), 137.2 (C-3), 134.6 (C-2), 130.6, 126.0 (C-Ar), 50.6 (C-1´), 6.0 (C-2´)

*A typical procedure for the preparation of racemic amino acid **(rac)-4a** is as follows:*

Aroylacrylic acids **3a-c** was suspended in water and 10% solution of aqueous ammonia was added. The reaction mixture was stirred 20 min. The solution was evaporated off, residuum was suspended in MeOH and filtered off. Racemic amino acids were obtained as white solid crystals.

*A typical procedure for the preparation of adducts **(2S,1´R)-5a-c** is as follows:*

(*R*)-Phenyglycinol (1.2 equiv) was added to a stirred suspension of requisite acid **3a-c** in MeOH. The resulting mixture was stirred for 3 - 34 hours at room temperature, d.r. being monitored by HPLC. Precipitated crystalline solid was filtered off, washed with a small amount of MeOH and ether, and dried under reduced pressure.

*A typical procedure for the preparation of pure free amino acids **(2S)-4a-c** is as follows:*

The starting adducts 5a-c was suspended in water and aqueous solution of $NaIO_4$ (1 equiv.) was added. Weakly solubility of adducts was improved by addition of aqueous ammonia (1 equiv.). The reaction mixtures was stirred about 5 min and then it was extracted with ether. Water layer was evaporated and in this way we obtained mixtures of free amino acid and salts of iodate. The compound (2*S*)-4a-c was separated by addition of acetonitrile in which the amino acids was dissolved in contrast to the salts.

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