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Efficient Chemoenzymatic Synthesis of (*S*)- and (*R*)-5-(1-Aminoethyl)-2-(cyclohexylmethoxy)benzamide: Key Intermediate for Src-SH2 Inhibitor

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Abstract—A facile chemoenzymatic synthesis of both the *S* and *R* forms of 5-(1-aminoethyl)-2-(cyclohexylmethoxy)benzamide a key intermediate of non-peptidic Src SH2 inhibitors is described. Both the enantiomers were synthesized in high optical purity (>99% ee) by reduction followed by lipase-mediated acylation of the precursor **6** in one-pot. Immobilized *Pseudomonas cepacia* lipase offered high degree of enantioselectivity with spontaneity.

Src-homology-2 (SH2) domain and their associated catalytic or non-catalytic protein constitute critical signal transduction target for drug discovery, which is both challenging and promising with respect to the design of novel inhibitors. SH2 domain are found in the regulation of number of cellular processes, including growth, mitogenesis, motility, metabolism, immune response and gene transcription.[1] Of particular interest are inhibitors of the SH2 domain of the tyrosine kinase $pp60^{c\text{-}Src}$, which has been implicated as a potential target[2] for the therapeutic intervention for both osteoporosis[3] and breast cancer.[4] There has been interest to develop completely non-peptidic Src-SH2 inhibitors based on phosphotyrosine (pTyr) portion of tetrapeptide **1** (Fig. 1), a high affinity ligand for the Src-family SH2 domain. Recently a non-peptidic, monocharged compound has been designed which displays good binding affinity for the p561ck SH2 domain and good cell permeation properties.[5] There are some reports[6–8] that have examined pTyr replacement attached to a novel non-peptidic 1-aminoethyl substituted benzamide for the Glu-Glu-Ile-NH_2 within 'tail' portion of **1**. This modified compound **2** exhibits improved binding affinity over **1**, whereas compound **3**, which possesses same 'tail' but attached to a 4-carboxyphenyl phosphate 'head' group, shows comparable binding affinity as **1**.[9–11] The crystallographic study[8] shows that two key polar contacts are attained in the binding of these peptides with SH2 domain. First, the NH of the amide attached to phosphophenyl group is a hydrogen bond donor to His 58 (CO) of the Src-SH2 domain, thus mimicking the interaction observed with P+1[12] NH in the 11-mer.[13] Second, and more interestingly, the carboxamide substituted on phenyl in the linker template displaces water molecules found in the 11-mer X ray structure and binds directly to Lys 60 (NH) of Src-SH2 domain.

We have been interested in the development of non-peptidic Src-SH2 inhibitors and to investigate an efficient chemoenzymatic enantioselective methodology employing lipases. In this context, the starting materials for the key intermediate **5** of non-peptidic Src SH2 inhibitors of type **2**–**4** that contains acetophenone moiety provides the required secondary alcohol upon reduction, which was resolved by lipase-mediated acylation in the same pot. The synthesis of non-peptidic Src-SH2 inhibitor, reported in the literature for this compound has either employed racemic amine **5** or one of the enantiomerically enriched *S* amine **5b**.

In continuation of our efforts towards the synthesis of biologically important chiral compounds employing enzymes,[14,15] it is considered of interest to enzymatically resolve the precursors of this amine **5**, which would allow the preparation of both the *R* and *S* enantiomers. The chiral synthesis reported in the literature for this key intermediate involves reduction of methyl 5-acetyl-2-(cyclohexylmethoxy)benzoate **6** using (+)DIP-chloride

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at −13 °C for 2.5 days to afford the corresponding alcohol in 94% ee, which leads to the required amine **5b** in 88% ee. Whereas, another protocol employs the chiral 1-(4-methoxy-phenyl)ethylamine as the precursor, which yielded the required amine **5b** in 94% ee.[9] However, no efforts have been made in the literature to prepare both the *R* and *S* forms of the amine **5**, which could be of significance to unravel the correlation between stereoselectivity and biological activity of these inhibitors.

In an attempt to exploit the synthesis of the key intermediate for SH2 inhibitor, herein we report for the first time, a new chemoenzyamtic method that produces the chiral amine **5** with high degree of selectivity. Methyl 5-acetyl-2-(cyclohexylmethoxy)benzoate **6** was reduced using sodium borohydride and moist neutral alumina in hexane followed by lipase-mediated resolution in the same pot to afford the corresponding alcohol **7** and acetate **8** in high enantiomeric purity (Scheme 1).

One mmol of **6** was reduced by sodium borohydride with moist neutral alumina[16] in hexane at 40 °C and subjected for lipase-mediated resolution process (1 equiv w/w) at room temparature. It was observed that immobilized lipase; PS-C 'Amano' II and PS-D 'Amano' I have shown high degree of selectivity both in alcohol **7** and acetate **8**. Lipase from porcine pancrease (PPL) and wheat germ source were inactive towards this one-pot acetylation. Further there was no significant conversion employing lipase from *Candida antarctica*, *Candida cylindracea*, *Candida rugosa* and *Mucor miehei* (lipozyme). Lipase from Amano-PS and *Ps. fluorescence* has shown effective conversion in 42 h with high enantioselectivity in acetate **8**. Amongst all, the immobilized lipase PS-C 'Amano' II afforded good conversion and high degree of selectivity in shorter duration (Table 1).

In a typical representative procedure for lipase-mediated acylation, to a solution of the **6** (1 mmol) in 10 mL of hexane was added moist neutral alumina (1.0 g) and $NaBH_4$ (2 mmol). The resulting reaction mixture was stirred at 40 °C for 3 h and monitored for the completion of the reduction by TLC. At the end of the reaction was added lipase PS-C 'Amano' II (1 equiv w/w) and isopropenyl acetate (0.65 mL). The reaction was stirred at room temperature for 5–6 h till the reaction reached to 50% conversion. The reaction was filtered through Celite, diluted with EtOAc and washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give an oily residue, which was purified by silica gel column chromatography to obtain enantiomerically pure acetate and alcohol.[17] Products, thus obtained were analyzed by chiral HPLC and compared with corresponding racemic products.[18]

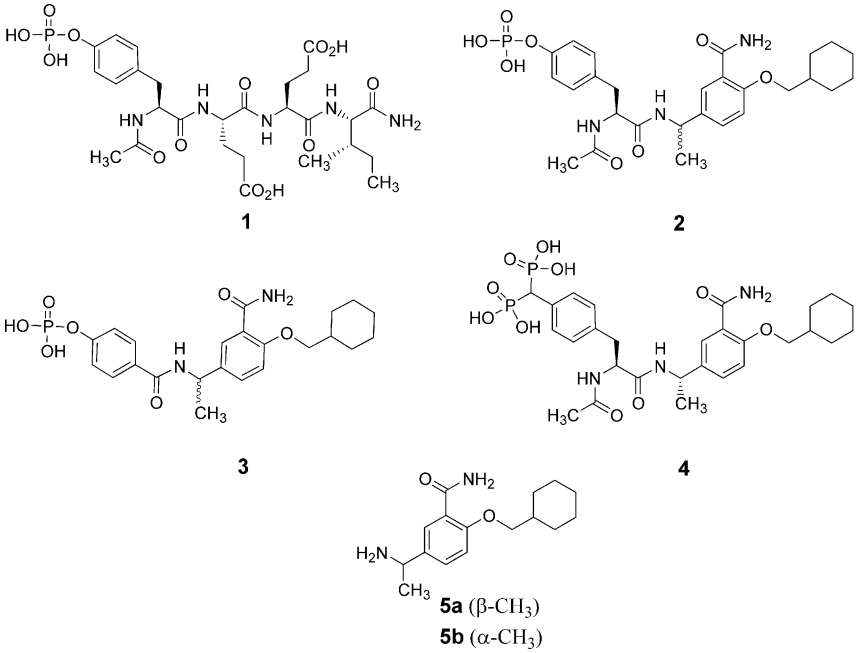
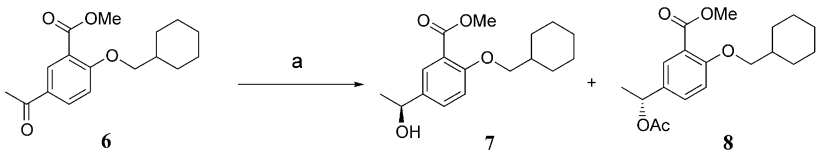
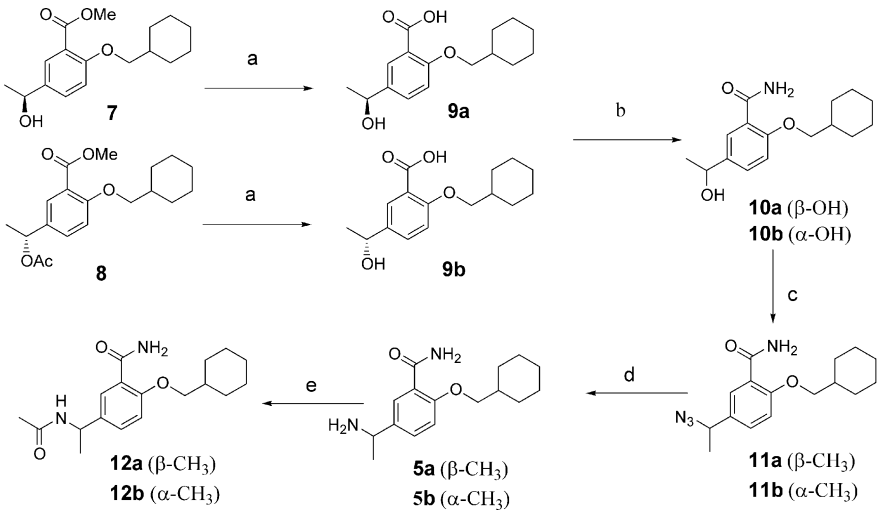


Figure 1.



Scheme 1. Reagents and conditions: (a) $NaBH_4$, moist Al_2O_3 (neutral), hexane, lipase PS-C 'Amano' II, isopropenyl acetate, 50% (**7**), 47% (**8**).



Scheme 2. Reagents and conditions: (a) K_2CO_3, MeOH, 98%; (b) HOBt, EDC, NMM, NH_4OH, THF, 91%; (c) DPPA, DBU, toluene, 71%; (d) 10% Pd/C, H_2, EtOAc, 88%; (e) CH_3COCl, Et_3N, CH_2Cl_2, 99%.

Table 1. In-situ transesterification employing different lipases

Lipase	Time (h)[a]	Conversion (%)[b]	ee (%)[c]	
			7	8
PS 'Amano'	42	46	84	>99
PS-C 'Amano' II	6	50	>99	>99
PS-D 'Amano' I	12	50	>99	>99
Ps. fluorescence	42	40	65	>99

[a]Time taken for transesterification at which the ratio of 7/8 remains constant.
[b]Calculated by; % conversion $= \frac{ee_7}{ee_7+ee_8} \times 100$.
[c]Determined by chiral HPLC.

Chiral alcohol **7** and acetate **8** were further transformed to the highly enantiopure amine **5a** and **5b** by employing the modified procedures of Luke and Holt (Scheme 2).[9] The alcohol **7** and acetate **8** were hydrolyzed independently by potassium carbonate in methanol to their corresponding benzoic acids **9a** and **9b**, which were further converted to their carboxamides **10a** and **10b**. Substitution of secondary hydroxy group by an azide was carried out employing diphenyl phosphorylazide, that usually involves the change of configuration at the chiral center, but in this case interestingly no racemization was observed. Finally, the azide was reduced to the desired (*R*) and (*S*) 5-(1-aminoethyl)-2-(cyclohexylmethoxy)benzamide (**5**) which was isolated in high enantiomeric excess (**5a** ee >99%; **5b** ee >99%).[19] The enantioselectivity was determined by chiral HPLC studies through their acetyl derivative (**12**).[20]

In summary, one-pot protocol was developed for the $NaBH_4/Al_2O_3$ reduction accompanied by in-situ lipase-mediated transesterification, for the synthesis of both *R* and *S* forms of 5-(1-aminoethyl)-2-(cyclohexylmethoxy)-benzamide, an important intermediate required for the preparation of structurally modified non-peptidic Src-SH2 inhibitors. Both the enantiomers were synthesized in high optical purity for the first time and could assist researchers to design and synthesize novel analogues in the desired stereochemical forms.

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12. Nomenclature for binding sites designates P for pTyr site. Residues N-terminal to P are P-1, P-2 and so on; residue C-terminal to P are P+1, P+2, and so on.

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16. Neutral alumina (S. d. fine chemicals, 10.0 g) was dried in an oven at 500 °C for 6 h; distilled water (1.1 mL) was added portionwise while shaking at every min, to afford free-flowing moist neutral alumina.
17. Spectral data for compound **7**: $[\alpha]_D^{25}$ −30.77 (*c* 1.0, $CHCl_3$); IR (KBr) 3424, 2928, 1712 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.1–1.4 (5H, m), 1.5 (3H, d, $J=6.11$ Hz), 1.6–1.9 (6H, m), 3.8 (2H, d, $J=4.90$ Hz), 3.9 (3H, s), 4.8 (1H, q, $J=6.11$ Hz), 6.9 (1H, d, $J=8.55$ Hz), 7.4 (1H, dd, $J=8.55$, 2.45 Hz), 7.7 (1H, d, $J=2.45$ Hz), MS (EI) *m*/*z* 292 (M^+). Spectral data for compound **8**: $[\alpha]_D^{25}$ +85.45 (*c* 1.1, $CHCl_3$); IR (KBr) 2928, 1728 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.1–1.3 (5H, m), 1.5 (3H, d, $J=7.33$ Hz), 1.7–1.9 (6H, m), 2.0 (3H, s), 3.8 (2H, d, $J=6.11$ Hz), 3.9 (3H, s), 5.8 (1H, q, $J=7.33$ Hz), 6.9 (1H, d, $J=8.56$ Hz), 7.4 (1H, dd, $J=8.56$, 2.45 Hz), 7.8 (1H, d, $J=2.45$ Hz); MS (EI) *m*/*z* 334 (M^+).
18. Enantioselectivity of **7** and **8** was determined by chiral HPLC (Chiracel OD column, Daicel) employing hexane/isopropanol = 90/10 (v/v) as mobile phase, 0.5 mL/min flow and monitored at 254 nm wavelength.
19. Spectral data for compound **5a** (white solid): IR (KBr) 1653, 2923, 3184, 3492 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.0–1.4 (5H, m), 1.5 (3H, d, $J=6.59$ Hz), 1.7–2.0 (6H, m), 3.9 (2H, d, $J=5.86$ Hz), 4.1 (1H, q, $J=6.59$ Hz), 5.8 (1H, br s), 6.9 (1H, d, $J=8.80$ Hz), 7.4 (1H, dd, $J=8.79$, 2.20 Hz), 7.8 (1H, br s), 8.1 (1H, d, $J=2.20$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 167.36, 156.29, 140.18, 130.38, 129.64, 120.56, 112.49, 74.69, 50.44, 37.49, 29.96, 26.24, 25.57, 25.53; MS (FAB) *m*/*z* 277 ($M^+ + 1$); HRMS data: calcd for $C_{16}H_{25}N_2O_2$ 277.191603, found 277.192082; mp 123.4 °C; $[\alpha]_D^{25}$ +23.7 (*c* 0.8, $CHCl_3$). Spectral data for compound **5b** (white solid): IR (KBr) 1653, 2923, 3169, 3415 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.0–1.4 (5H, m), 1.5 (3H, d, $J=6.59$ Hz), 1.7–2.0 (6H, m), 3.9 (2H, d, $J=5.86$ Hz), 4.1 (1H, q, $J=6.59$ Hz), 5.8 (1H, br s), 6.9 (1H, d, $J=8.79$ Hz), 7.4 (1H, dd, $J=8.79$, 2.20 Hz), 7.8 (1H, br s), 8.1 (1H, d, $J=2.20$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 167.33, 156.30, 139.79, 130.44, 129.63, 120.54, 112.48, 74.66, 50.39, 37.43, 29.90, 26.19, 25.52, 25.14; MS (FAB) *m*/*z* 277 ($M^+ + 1$); HRMS data: calcd for $C_{16}H_{25}N_2O_2$ 277.191603, found 277.191445; mp 123.8 °C; $[\alpha]_D^{25}$ −23.3 (*c* 1.2, $CHCl_3$).
20. Enantioselectivity of the amine **5a** and **5b** was determined through their acetates **12a** and **12b** by chiral HPLC (Chiracel, OJ-H column, Daicel) employing hexane/isopropanol/diethylamine = 90/10/0.1 (v/v/v) as mobile phase, 0.5 mL/min flow and monitored at 254 nm wavelength.