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Beyond enzymatic promiscuity: Asymmetric induction by L-proline on lipase catalyzed synthesis of polyfunctionalized 4*H*-pyrans

Pranjal P Bora[a], Manisha Bihani[a] and Ghanashyam Bez[a]*

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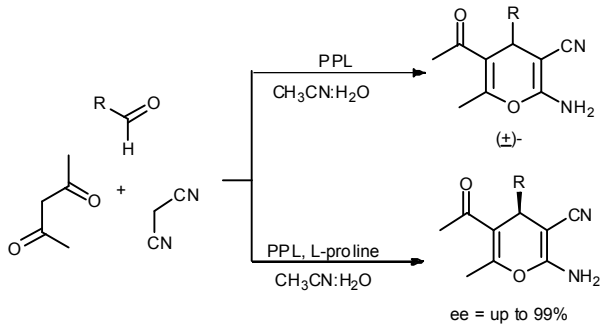
We report herein the synthesis of polysubstituted 4*H*-pyrans catalysed by PPL via a three-component reaction of aldehyde, malononitrile and acetyl acetone. The catalytic activity of PPL with other lipases and its reactivity profile in different reaction medium was thoroughly studied. Although the change in enzyme reaction specificity induced by metal substitutions and amino acids is well known, non-covalent introduction of an amino acid into enzymatic promiscuous reaction to achieve stereoselectivity through co-operative catalysis is not explored yet. In an interesting observation, we have noted that addition of L-proline to PPL catalysed reaction can induce stereoselectivity in the formation of polysubstituted 4*H*-pyrans while such MCRs catalyzed by PPL alone give only racemic products.

Recently synthesis of pyrans, one of the most privileged heterocyclic scaffolds, have found a lot of attention due to their simple structural complexity and important biological activities, such as antimicrobial,[1,2] antiviral,[3,4] anticonvulsant,[5] cytotoxic,[6] and antigenotoxic,[7] etc. Among the pyrans, polyfunctionalized 4*H*-pyran is a major constituent of many natural products[8-10] and displays a wide array of biological activities such as antitumor, antibacterial, antiviral, spasmolytic, and antianaphylactic.[11-14] Compounds with 4*H*-pyran core have also shown applications in the treatment of Alzheimer, Schizophrenia, and Mycolonous diseases.[15] Nevertheless, heterocyclic compounds bearing 4*H*-pyran units have played increasing roles in synthetic strategies to promising compounds in the field of medicinal chemistry.[16-18]

Given the importance of polyfunctionalized *4H*-pyrans, many synthetic strategies have been reported to achieve the target by adopting simple reaction strategies. Commonly used approach for the said synthesis include two-step or three-component reaction catalyzed by organic bases,[19-20] ionic liquids,[21-22] hexadecyltrimethyl ammonium bromide,[23] Mg/La mixed metal oxides,[24] Cu(II) oxymetasilicate,[25] rubidinium fluoride,[26] Pd/C[27] and nanocrystalline ZnO,[28] etc. Recently, we have also reported a completely green protocol for the synthesis of polyfunctionalized 4*H*-pyran derivatives using Amberlyst A21, a basic resin bound catalyst.[29]

Recently, enzyme catalyzed reaction have found profound applications in carbon-carbon bond forming reactions. The fact that enzymes works under extremely mild conditions, near neutral pH, both in aqueous and organic medium and are recyclable, use of enzyme in place of conventional acid and base catalyst have found tremendous applications. In a bid to use environmentally benign reaction conditions, Mane *et al*.[30] reported baker yeast catalyzed one-pot reaction of aldehydes, malononitrile and ethylacetoacetate or acetylacetone in dimethylacetamide at room temperature approach to achieve polyfunctionalized 4*H*-pyrans in moderate to good yields. Although baker's yeast is known to catalyze many stereoselective reactions,[31-33] the authors did not report any such observation. The fact that there are scant reports on stereoselective synthesis of polyfunctionalized 4*H*-pyrans, we wanted to explore the catalytic effect of the lipase from *Procine pancreas* (PPL) on the synthesis of polyfunctionalized 4*H*-pyrans and its selectivity. The role of solvents and additional amino acids in the lipase catalyzed reaction was also explored in inducing stereoselectivity of this otherwise promiscuous catalysis (Scheme 1).



Scheme 1 Synthesis of 4*H*-pyrans

RESULTS & DISCUSSION

In our quest to develop a lipase catalyzed stereoselective MCR protocol, we selected the reaction of 4-fluorobenzaldehyde, malononitrile and acetylacetone to synthesize 4*H*-pyran as our pilot reaction. Therefore, a mixture of 4-fluorobenzaldehyde (0.124 g, 1 mmol), malononitrile (0.066 g, 1 mmol) and acetylacetone (0.125 g, 1.25 mmol) in 10 mL ethanol was stirred at room temperature in the presence of PPL (lipase from *Procine pancreas*). The reaction led to formation of the desired product and gave excellent yield of the desired polyfunctionalized pyran derivative within 36 h. The structure of the product was confirmed from its IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopic data along with melting point and elemental

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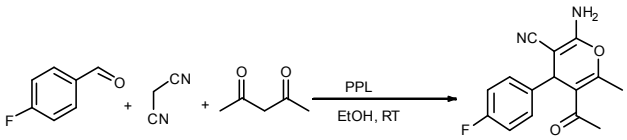
analysis. However, to our dismay, the HPLC analysis of the product employing chiralcel AD-H column with 12% isopropanol in hexane at flow rate = 0.5 mL/min showed two peaks of equeal intensity at retention time t_R = 18.9 min and t_R = 23.3 min to confirm only the formation of a racemic mixture. The fact that other than in promiscuous catalysis, where the oxyanion hole of the catalyst is responsible for catalysis, lipase catalyzed reactions are known to be substrate specific. Therefore, we decided to carry out the reaction of 4-fluorobenzaldehyde, malononitrile and acetylacetone with other lipases such as lipase from *Pseudomonas cepacia*, Amano lipase AK from *Pseudomonas fluorescens*, lipase from *Penicilum camemberti* and lipase from *Aspergillus niger* (ANL) (Table 1). As it can be seen form the Table 1, stereoselectivity was not observed with these lipases in spite of showing moderate to good reactivities. This observation led us to believe that the reaction was catalyzed by the non-specific oxyanion hole rather than the enzyme active site.

Table 1 : Screening of lipases[a]



1

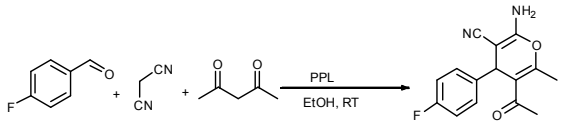
Entry	Lipase	Time/h	%Yield[b,c]
1	Lipase Amano AK	48	34
2	Lipase from *Porcine pancreas* (PPL)	36	85
3	Lipase from *Aspergillus niger* (ANL)	48	77
4	Lipase from *Penicilum camemberti*	48	40
5	Lipase from *Pseudomonas cepacia*	48	65

[a]Reaction conditions: 4-Fluoro benzaldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with lipase (30 mg) in ethanol (10 mL) at 30 °C. [b]Yield of the pure product, purified by recrystallization from ethanol. [c]Enantiomeric ratio (e. r.) was determined by chiral HPLC analysis.

The fact that the enantioselectivity of hydrolases dramatically changes with change in solvent, the potential of 'medium engineering' for modifying enzyme selectivity is well established.[34] Since the lipase from *Procine panacrease* (PPL) was found to the best in terms of both yield and reaction time, we screened out various solvent systems (Table 2) in order to explore plausible effect on stereoselectivity and reactivity. Best yield was observed in case of 1: 1 water-acetonitrile mixture, but enantioselectivity was not observed at all.

Since PPL catalyzed synthesis of the stated polyfunctionalized 4*H*-pyran worked under extremely benign reaction conditions, we decided to complete a detail study on enzyme loading and substrate scope. Henceforth, the pilot reaction was carried out with different catalyst loadings and 25 mg of PPL against 1 mmol of the starting aldehyde was found to be optimum in terms of both yield and reaction time (See Table 1S in the Supporting information).

Table 2 : Screening of lipases[a]



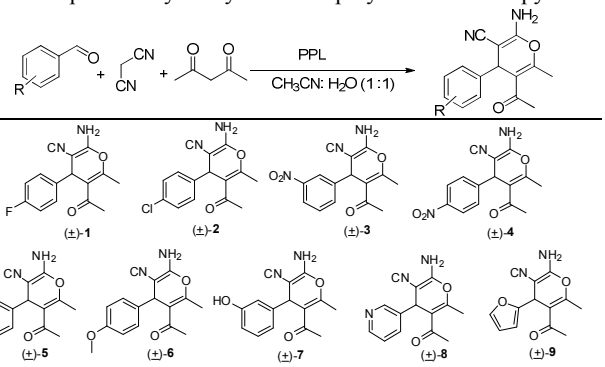
1

Entry	Solvent	%Yield[b]
1	Water	52
2	Ethanol	85
3	*t*-BuOH	70
4	DMSO	70
5	Acetonitrile	58
6	**Water-acetonitrile (1: 1)**	**88**
7	$MeOCH_2CH_2OMe$	82
8	Cycloheaxne	<10
9	Hexane	<10

[a]Reaction conditions: 4-Fluoro benzaldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with lipase (30 mg) in ethanol (10 mL) at 30 °C. [b]Yield of the pure product, purified by recrystallization from ethanol.

Since the lipase catalyzed synthesis of polyfunctionalized 4*H*-pyrans have not been explored yet, we wanted expand the catalytic promisicuity of PPL for other aldehydes. Various aromatic aldehydes with both electron-donating (+M) (entries 6-7, Table 3) and electron withdrawing substituents (-M) (entries 1-4, Table 3) in the phenyl ring were subjected to the reaction with acetyl acetone and malononitrile under optimized reaction conditions to achieve corresponding 4*H*-pyrans in excellent yields. Of course, the aldehydes with electron-donating (+M) substituent on the phenyl ring took longer reaction time.

Table 3 Lipase catalyzed synthesis of polyfunctionalized pyran[a]



[a]Reaction conditions: Aldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with PPL (25 mg) in (1: 1) acetonitrile water mixture (10 mL) at 30 °C. [b]Yield of the pure product, purified by recrystallization from ethanol. [c]No enantiomeric excess (%ee) was observed upon analysis by chiral HPLC with OD-H and AD-H column.

The first and only asymmetric synthesis of polyfunctionalized 4*H*-pyrans via Michael was demonstrated by Gonzalaz *et al.* starting from a chiral aldehyde which controlled the stereochemistry of the product in a two-step mechanism.[35] Given

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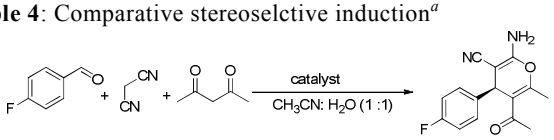
the scant attention paid to the stereoselective synthesis of polyfunctionalized 4*H*-pyrans and the lack of multicomponent protocol to achieve this objective, there lies two-fold challenge for synthesis of asymmetric polysubstituted 4*H*-pyrans. Firstly to devise a lipase catalyzed asymmetric MCR via C-C bond forming reaction and then improvisation of stereoselective induction in enzymatic 'oxyanion hole' assisted promiscuous catalysis by solvent engineering, catalyst engineering, or both. No such attempts have been reported in the literature.

Incidentally, the change in enzyme reaction specificity induced by metal substitutions is well known. Kaiser and Lawrence[36] induced promiscuous oxidase activities in several hydrolytic enzymes[37,38] by introduction of copper ions. Likewise carbonic anhydrase are known to catalyse styrene epoxidation[39] upon replacement of the native Zn^{2+} by Mn^{2+}, while rhodium-substituted carbonic anhydrase acts as a hydrogen-utilizing reductase.[40] Even incorporation of selenocysteine into the active sites of subtilisin,[41] glyceraldehyde-3-phosphate dehydrogenase,[42] and GST[43] endowed these enzymes with novel peroxidase activities. Such alteration of reaction specificity by enzymatic infidelity in the presence of metal ions or amino acids led us to believe that non-covalent introduction of an amino acid into enzymatic promiscuous reaction may lead to formation of stereochemically different product. We presumed that if the hydrogen bonding of the oxyanion hole in promicuous catalysis is disturbed by addition of external amino acid, the non-specific enzyme-substrate complex might get affected to change either the reaction kinetics or the product. To test our assumption, we carried out the pilot reaction (1 mmol level) in 1: 1 acetonitrile-water mixture in the presence of 15 mol% L-proline and 20 mg of PPL at room temperature. We were pleased to observed that the reaction completed in relatively shorter reaction time (28 h) and gave excellent yield (94%). The HPLC analysis of the compound employing chiralcel AD-H column with isopropanol and hexane (88: 12) as mobile phase at flow rate of 0.5 mL/min provided an exciting result: the reaction was enantioselective and gave 46 %ee. In spite of having many reported lietrature that showed the formation of racemic mixture in L-proline catalyzed multicomponent reactions,[44-48] we carried out the pilot reaction in acetonitrile-water medium in the presence of 15 mol% L-proline as catalyst. The reaction led to completion in 48 h to give the desired polyfunctionalized 4*H*-pyran derivative with 56% isolated yield. Intetrestingly, the HPLC analysis of the product employing chiralcel AD-H column showed 34% enantiomeric excess. Above observations (Table 4) revealed that stereoselctive induction can be achieved from L-proline catalyzed multicomponent reaction and the combination of lipase and L-proline can cooperatively catalyze such reaction to increase enantioselectivity, in this case by 12%. In a bid to determine the appropiate combination of PPL and L-proline, we carried out the pilot reaction with various combination of PPL and L-proline (see Table 2S in the supporting information) and the mixture of 30 mg PPL and 15 mol% L-proline worked best for the reaction at 1 mmol level, both in terms of yield and enantioselectivity. To the best of our knowledge, this kind of co-operative bio-organocatalysis has not been reported in the literature.

Table 4: Comparative stereoselctive induction[a]

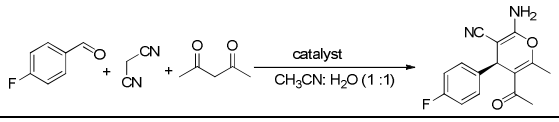


Entry	Catalyst	Time/h	% yield[b]	% ee[c]
1	PPL	36	88	0
2	L-proline	48	56	34
3	PPL, L-proline	28	90	46

[a]Reaction conditions: 4-Fluoro benzaldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with lipase (30 mg) in ethanol (10 mL) at 30 °C. [b]Yield of the pure product, purified by recrystallization from ethanol. [c]Enantiomeric ratio (e. r.) was determined by chiral HPLC analysis.

The fact that cooperative catalysis was observed in case L-proline with PPL, we sought to study whether other amino acids also can cooperatively induce stereoslectivity with PPL for the pilot reaction. To this effect, we screened some amino acids at our disposal and the results are summerized in the Table 5. Among the amino tested, L-serine addition showed noticable stereochemical induction while L-Cysteine very low enantioselectivity. L-Alanine and L-Valline did not show any stereoselective induction at all. The observation that some amino acids other than L-proline also catalyzed in tendem with PPL to obviate stereoselective 4*H*-pyrans led us to believe that the stereoselective induction might not be due to formation of specific enzyme-amino acid complexation, but some non-specific interactions of the enzyme with amino acids in general to form stetreoselective 'oxyanion hole'.

Table 5: Effect of other amino acid on stereoselctive induction[a]



Entry	Catalysts	Time/h	% yield	% ee
1	L-Serine	72	30	0
2	L-Serine, PPL	36	91	8%
3	L-Cysteine	120	-	0
4	L-Cysteine, PPL	36	84	<3%
5	L-Alanine, PPL	36	87	0
6	L-Valline	36	88	0

[a]Reaction conditions: 4-Fluoro benzaldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with lipase (30 mg) and amino acid (15 mol%) in 1:1 acetonitrile-water (10 mL) at 30 °C. [b]Yield of the pure product, purified by recrystallization from ethanol. [c]Enantiomeric ratio (e. r.) was determined by chiral HPLC analysis.

After acheiving the elusive enantioselectivity, we were excited to study the scope of our protocol for other aldehydes as well (Table 6). The reaction of 4-chlorobenzaldehyde, malononitrile and acetylacetone under optimized condition

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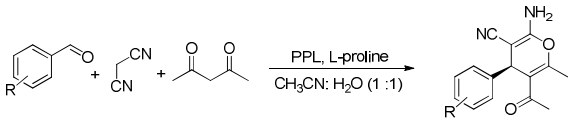
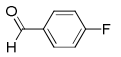
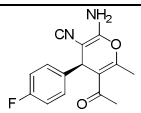
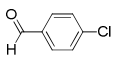
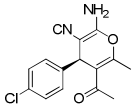
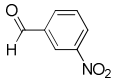
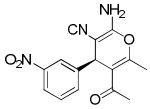
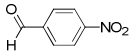
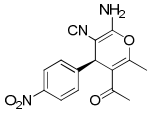
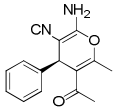
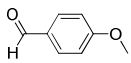
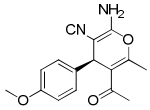
resulted in formation of the corresponding pyran derivative **2** in excellent yield with 79% ee. Similarly, the reaction of benzaldehyde, malononitrile and acetylacetone under our reaction conditions also gave excellent yield of the product with an excellent optical purity (enantiomeric excess of more than 99%) as confirmed from the HPLC analysis. Ironically, the reaction of nitro-substituted benzaldehydes (entry 3-4, Table 6) failed to induce any enantioselectivity in the resulting pyran in spite of giving excellent yields. Further studies on the role of amino acids other than L-proline are in progress.

Table 6: Enantioselective synthesis of polyfunctionalized 4*H*-pyran[a]



[a]

Entry	Aldehyde	Product[b]	Time/h	% yield[c]	er[d]
1		 **1**	28	94	73: 27
2		 **2**	28	92	89: 11
3		 **3**	32	95	50:50
4		 **4**	24	93	50:50
5		 **5**	30	86	99.6: 0.4
6		 **6**	48	91	79: 21

[a]Reaction conditions: 4-Fluoro benzaldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol) were stirred with lipase (30 mg) in ethanol (10 mL) at 30 °C. [b]Absolute configuration is not determined [c]Yield of the pure product, purified by recrystallization from ethanol. [d]Enantiomeric ratio (e. r.) was determined by chiral HPLC analysis.

EXPERIMENTAL

General Information

All reagents were commercially available and used without further purification. All products were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis. The IR spectra were recorded on a Perkin Elmer spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AC-400 using DMSO-d_6 as solvent and TMS as internal standard, unless otherwise stated. HPLC analyses were performed on an Waters M515 series equipped with a chiral column (Chiralcel AD-H and Chiralcel OD-H), using mixtures of *n*-hexane/isopropyl alcohol (IPA) as mobile phase at 25 °C. For column chromatography, we employed Merck silica gel 60-120 mesh.

Materials

Lipase from Pseudomonas cepacia (Lot# E3993333/1V, Pcode: 101026286, 46 U/mg), lipase from Pseudomonas fluorescens (Lot# BCBB6276, Pcode: 100923033, 40 U/mg), lipase from A. niger, ANL (Lot# 0001448380, Pcode: 101007887, 236 U/g), lipase from Penicillium camemberti (Lot# BCBB7618, Pcode: 100994489, 50.6 U/mg) Amano Lipase AK from P. fluorescens (Batch # 07919JE), and lipase from procine pancreas, PPL (Lot# BCBC7768, Pcode: 100973041, 16.5 U/mg) were purchased from Sigma–Aldrich.

General procedure for PPL catalyzed synthesis of polyfunctionalized 4H-pyrans (Method A): To an ethanolic solution of aldehyde (1 mmol), malononitrile (1 mmol) and acetylacetone (1.25 mmol), 25 mg PPL was added and stirred at 30°C for the specified time. After complete consumption of the starting materials the reaction mixture was diluted with 30 mL of ethanol to dissolve the solid precipitate The lipase was removed by centrifugation and the solution was concentrated in rotavapor and kept in refrigerator for crystallization.

General procedure for enantioselective synthesis of polyfunctionalized 4H-pyrans (Method B): To a mixture of aldehyde (1 mmol) and malononitrile (66 mg, 1 mmol) in 5 ml of 1: 1 acetonitrile-water mixture, 17 mg L-proline (15 mol%) and 20 mg PPL were added and stirred for 5 min. Then acetylacetone (125 mg, 1.25 mmol) was added and allowed to stir for the specified time at room temperature, the starting materials got converted into the desired product and precipitated as solid. After completion, as evident from TLC, the acetonitrile was removed in vacuum and extracted with ethyl acetate (15 ml × 3). The solvent was removed and purified by recrystallization from ethanol to achieve the pure product.

Spectral data of the products

5-Acetyl-2-amino-6-methyl-4-(4-fluorophenyl)-4H-pyran-3-carbonitrile, ***1***[30]: White solid; Yield: 90%; IR (KBr): 1658, 2190, 3356 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.78 (s, 3H), 1.92 (s, 3H), 4.04 (s, 1H), 6.64 (s, 2H), 6.92-7.06 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.3, 29.7, 38.5, 61.7, 114.5, 115.6, 116, 116.1, 118.8, 128.9, 129.2, 138.7, 139.1, 157.7, 197.5; MS (ES+): *m/z* 273 (M + H)+, 295 (M + Na)+; Elemental analysis for

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$C_{15}H_{13}FN_2O_2$:Calculated C 66.17, H 4.81, N 10.29 %; Found C 66.09, H 4.69, N 10.34 %.

5-Acetyl-2-amino-6-methyl-4-(4-chlorophenyl)-4H-pyran-3-carbonitrile, ***2***[29]: Pale yellow solid; Yield: 91%; IR (KBr): 1669, 2193, 3337 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 3H), 1.99 (s, 3H), 4.25 (s, 1H), 6.70 (s, 2H), 7.02 (d, J = 7.6 Hz, 2H), 7.12 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.5, 29.8, 38.0, 57.2, 114.7, 119.6, 128.6, 129, 129, 129.9, 131.5, 143.5, 155, 158.2, 198.1; MS (ES+): *m/z* 311 (M + Na)+; Elemental analysis for $C_{15}H_{13}ClN_2O_2$: Calculated C 62.40, H 4.54, N 9.70 %; Found C 62.38, H 4.50, N 9.66 %.

5-Acetyl-2-amino-6-methyl-4-(3-nitrophenyl)-4H-pyran-3-carbonitrile, ***3***[28]: Yellow crystalline solid; Yield: 95%; IR (KBr): 1532, 1675, 2192, 3396 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.89 (s, 3H), 2.06 (s, 3H), 4.47 (s, 1H), 6.83 (s, 2H), 7.42-7.45 (m, 2H), 7.87-7.90 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.7, 30.1, 38.2, 56.7, 114.5, 119.4, 121.4, 122, 130.4, 133.9, 146.9, 147.9, 156, 158.5, 197.8; MS (ES+): *m/z* 300 (M + H)+; Elemental analysis for $C_{15}H_{13}N_3O_4$: Calculated C 60.20, H 4.38, N 14.04%. Found C 60.15, H 4.40, N 14.01%.

5-Acetyl-2-amino-6-methyl-4-(4-nitrophenyl)-4H-pyran-3-carbonitrile, ***4***[29]: Yellowish solid; Yield: 94%; IR (KBr): 1524, 1677, 2189, 3349 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.90 (s, 3H), 2.08 (s, 3H), 4.42 (s, 1H), 6.85 (s, 2H), 7.23 (d, J = 8 Hz, 2H), 8.00 (J = 8 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.7, 29.7, 38.5, 57.6, 113.9, 119, 123.4, 127.7, 146.2, 150, 155.8, 156.9, 196.9; MS (ES+): 322 (M + Na)+; Elemental analysis for $C_{15}H_{13}N_3O_4$: Calculated C 60.20, H 4.38, N 14.04%; Found C 60.17, H 4.34, N 13.98 %.

5-Acetyl-2-amino-6-methyl-4-phenyl-4H-pyran-3-carbonitrile, ***5***[28]: Yellowish solid; Yield: 88%; IR (KBr): 1669, 2197, 3189, 3380 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.97 (s, 3H), 2.10 (s, 3H), 4.31 (s, 1H), 6.81 (s, 2H), 6.90-7.14 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.2, 29.1, 38.2, 56.8, 115.3, 121.3, 127.4, 127.6, 128.7, 128.9, 129.0, 140.2, 155.3, 158.5, 198.2; MS (ES+): *m/z* 255 (M + H)+; Elemental analysis for $C_{15}H_{14}N_2O_2$: Calculated C 70.85, H 5.55, N 11.02%; Found C 70.87, H 5.49, N 10.95%.

5-Acetyl-2-amino-6-methyl-4-(4-methoxyphenyl)-4H-pyran-3-carbonitrile, ***6***[28]: Yellowish solid; Yield: 81%; IR (KBr): 1254, 1672, 2188, 3370 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.91 (s, 3H), 2.12 (s, 3H), 3.56 (s, 3H) 4.34 (s, 1H), 6.74 (s, 2H), 7.06 (d, J = 7.6 Hz, 2H), 7.23 (J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.4, 29.5, 38.7, 55.4, 56.9, 116.4, 121.3, 122.5, 128.5, 129.2, 150.4, 158.1, 159.8, 197.8; MS (ES+): *m/z* 285 (M + H)+; Elemental analysis for $C_{16}H_{16}N_2O_3$: Calculated C 67.59, H 5.67, N 9.85%. Found C 67.51, H 5.54, N 9.72%.

5-Acetyl-2-amino-6-methyl-4-(3-hydroxyphenyl)-4H-pyran3-carbonitrile, ***7***[29]: Light yellow crystal; Yield: 86%; IR (KBr): 1664, 2190, 3368 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 3H), 1.99 (m, 3H), 4.12 (s, 1H), 6.31-6.39 (m, 3H), 6.62 (s, 2H) 6.87 (t, J = 7.6 Hz, 1H), 9.20 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.3, 29.6, 38.6, 57.8, 113.7, 114.1, 114.8, 117.7, 119.8, 129.0, 145.8, 154.5, 157.6, 158.1, 198.4; MS (ES+): *m/z* 271 (M +H)+, 293 (M + Na)+; Elemental analysis for $C_{15}H_{14}N_2O_3$: Calculated C 66.66, H 5.22, N 10.36%. Found C 66.61, H 5.18, N 10.32%.

5-Acetyl-2-amino-6-methyl-4-(pridin-3-yl)-4H-pyran-3-carbonitrile, ***8***[29]: Brown solid; Yield: 88%; IR (KBr): 972, 1069, 1217, 1261, 1367, 1431, 1688, 2189, 2942, 3150, 3366, 3572 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.93 (s, 3H), 2.10 (s, 3H), 4.35 (s, 1H), 6.38 (2H), 7.17-7.20 (m, 1H), 7.38 (d, J = 8 Hz, 1H), 8.24-8.28 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.6, 29.9, 36.2, 56.8, 114.4, 119.5, 124.0, 134.7, 139.9, 148.1, 148.3, 155.8, 158.4, 197.8; MS (ES^+): *m/z* 278 (M + Na)$^+$; Elemental analysis for $C_{14}H_{13}N_3O_2$: Calculated C 65.87, H 5.13, N 16.46%; Found C 65.82, H 5.09, N 16.39%.

5-Acetyl-2-amino-4-(furan-2-yl)-6-methyl-4H-pyran-3-carbonitrile, ***9***[29]: Light brownish crystal; Yield: 85%; IR (KBr): 1663, 2198, 3454 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.17 (s, 3H), 2.18 (s, 3H), 4.61 (s, 1H), 6.14 (d, J = 2.8 Hz, 1H), 6.34-6.36 (m, 1H), 7. 00 (s, 2H), 7.53 (d, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.4, 29.5, 38.5, 54.3, 105.6, 110.5, 113.0, 119.6, 142.4, 155.7, 155.8, 159.3, 197.7; MS (ES+): *m/z* 245 (M + H)+, 267 (M + Na)+; Elemental analysis for $C_{13}H_{12}N_2O_3$: Calculated C, 63.93; H, 4.95; N, 11.47%; Found C 63.89, H 4.88, N 11.42%.

HPLC data of the products obtained via Method B

5-Acetyl-2-amino-6-methyl-4-(4-fluorophenyl)-4H-pyran-3-carbonitrile, ***1***: Enantiomeric ratio: 73: 27; HPLC analysis: Chiralcel AD-H; isopropanol/hexane (12: 88); flow rate = 0.5 ml/ min.; λ = 230 nm; t_R = 17.4 (minor), t_R = 21.9 (major).

5-Acetyl-2-amino-6-methyl-4-(4-chlorophenyl)-4H-pyran-3-carbonitrile, ***2***: Enantimeric ratio: 89.3: 10.7; HPLC analysis: Chiralcel AD-H; isopropanol/hexane (12: 88); flow rate = 0.5 ml/ min.; λ = 230 nm; t_R = 18.1 (minor), t_R = 24.4 (major).

5-acetyl-2-amino-6-methyl-4-phenyl-4H-pyran-3-carbonitrile, ***5***: Yellowish solid; Yield: 86%; Enantiomeric ratio: 99.6: 0.4; HPLC analysis: Chiralcel AD-H; isopropanol/hexane (12: 88); flow rate = 0.5 ml/ min.; λ = 230 nm; t_R = 18.4 (minor), t_R = 22.2 (major).

5-Acetyl-2-amino-6-methyl-4-(4-methoxyphenyl)-4H-pyran-3-carbonitrile, ***6***: Yellowish solid; Yield: 91%; Enantimeric ratio: 79: 21; HPLC analysis: Chiralcel AD-H; isopropanol/hexane (12: 88); flow rate = 0.5 ml/ min.; λ = 230 nm; t_R = 21.8 (minor), t_R = 33.9 (major).

CONCLUSION

In conclusion, a novel and efficient synthesis of polyfunctionalized 4*H*-pyrans by PPL catalysed multicomponent reaction of aldehyde, malononitrile and acetylacetone is developed. Excellent yields, use of lipase as catalyst and ethanol as solvent, and no column chromatography involving

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environmentally incompatible organic solvents are some of the highlights of our protocol. For the first time, we have reported that PPL catalyzed promiscuous synthesis of racemic 4*H*-pyrans can be made stereoselective by addition of external L-proline which works co-operatively with PPL to obviate optically active 4*H*-pyrans.

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Notes and references

[a] *Department of Chemistry, North Eastern Hill University, Shillong-793022; Tel: 91 364 272 2624; E-mail: bez@nehu.ac.in*

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