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Fit-for-purpose synthesis of dual leucine zipper kinase (DLK) inhibitor GNE-834

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ABSTRACT

A practical fit-for-purpose synthesis of dual leucine zipper kinase (DLK) inhibitor **GNE-834 (1)** was developed. The key C—C bond was constructed via a Suzuki–Miyaura cross-coupling of iodopyrazole **2** and pyridine boronic ester **3** to afford ketone **12**. Subsequent selective reductive amination of ketone **12** with morpholine followed by a resolution via tosylate salt formation provided **GNE-834 (1)** in 39% overall yield from iodide **2** with 98.9 A% HPLC purity.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's Disease, is a rare but devastating progressive neurodegenerative disease which results in the wasting away of muscle and loss of movement [1]. There are an estimated 15,000 people in the United States living with ALS [2]. Currently, there is no known effective cure for ALS and the average survival from onset to death is two to four years with deteriorating quality of life [3]. The cause of ALS is currently unknown [2]. In brains of patients with ALS, an elevated dual leucine zipper kinase (DLK) pathway activity was observed and DLK was reported to regulate c-Jun *N*-terminal kinases (JNK) which induces brain-cell death. It was hypothesized that DLK inhibition could be a potential approach to treat neuronal degeneration disease such as ALS [4].

GNE-834 (1) is a small molecule DLK inhibitor that was discovered and developed for the potential treatment of ALS (Fig. 1) [5]. We describe herein a fit-for-purpose synthesis of **GNE-834 (1)** which enabled the fast delivery of materials for pre-clinical studies. Retrosynthetically, we envisioned that a Suzuki–Miyaura cross-coupling [6] between iodopyrazole **2** and pyridine boronic ester **3** could construct the key C—C bond between the pyrazole and pyridine moieties; the morpholine group could be installed via a diastereoselective reductive amination; and the iodopyrazole **2** could be derived from further functionalization of ketone **4** (Scheme 1).

Results and discussions

We commenced our synthesis with protection of 3-cyclopentenol **5** with *tert*-butyldiphenylchlorosilane (TBDPSCl) followed by $Rh_2(OAc)_4$ catalyzed cyclopropanation to give ester **6** [7]. Conversion of ester **6** to ketone **4** proceeded smoothly via Weinreb amide [8] formation followed by MeMgBr addition in 59% overall yield from 3-cyclopentenol **5** over 5 steps. Claisen condensation between ketone **4** and oxalic acid ethyl ester followed by pyrazole formation with 1-isopropylhydrazine delivered the desired bicyclopyrazole **7** in 67% yield over 2 steps. Saponification of ester **7** and Curtius rearrangement [9] of the resulting acid gave Cbz protected pyrazole amine **8** in 70% yield over 2 steps. Cbz deprotection and Sandmeyer [10] reaction then afforded the desired pyrazole iodide **9** in 47% yield. Final TBDPS deprotection and Dess–Martin periodinane oxidation [11] furnished the iodoketone as a mixture of 2:1 exo:endo isomers [12]. Separation of the undesired *endo*-isomer from the desired *exo*-isomer **2** by silica gel column chromatography provided **2** in 60% yield (Scheme 2).

From commercially available 2-amino-3-trifluoromethylpyridine **10**, an *N*-bromosuccinimide (NBS) bromination afforded the desired bromopyridine **11** in 99% yield.

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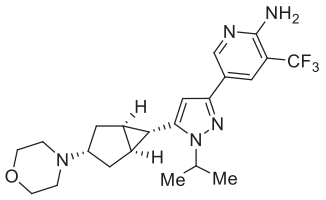
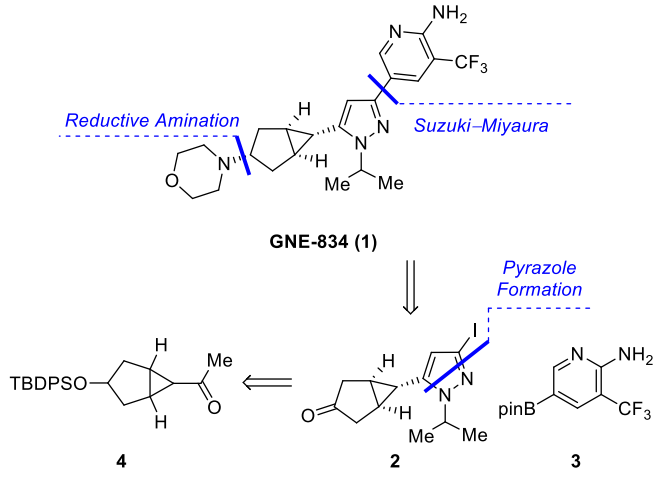


GNE-834 (1)

Fig. 1. GNE-834 (1), a DLK inhibitor.

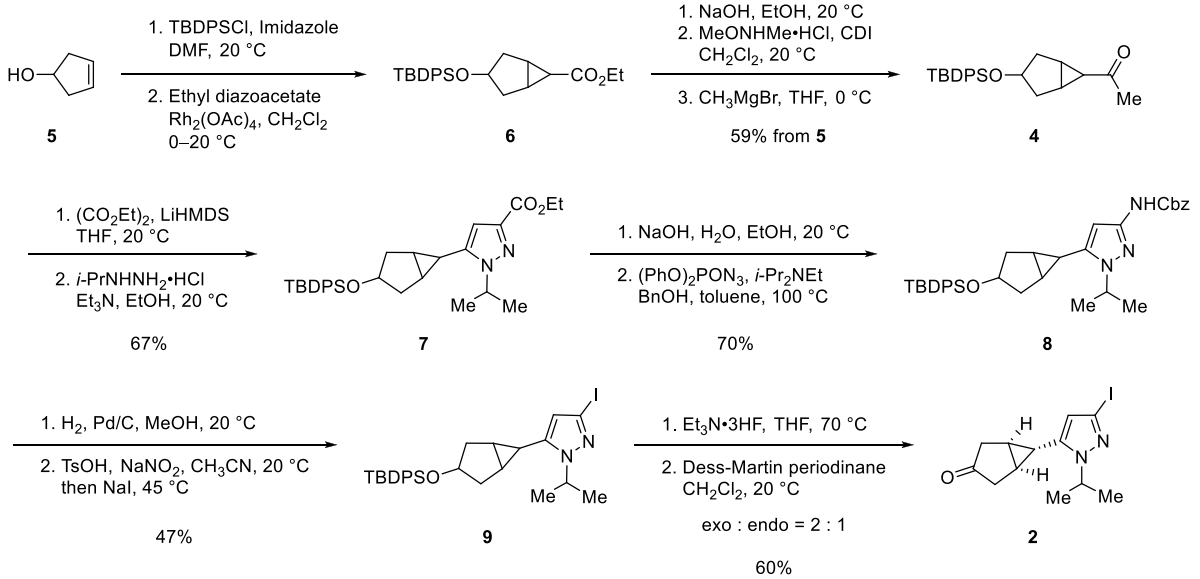


Scheme 1. Retrosynthetic analysis of **GNE-834 (1)**.

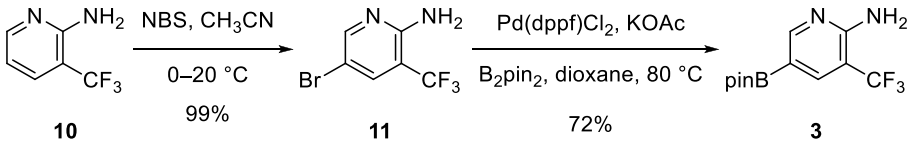
Bromopyridine **11** was then converted to the corresponding boronic ester **3** with bispinacolatodiboron (B_2pin_2) in 72% yield (Scheme 3) [13].

With both starting materials in hand, we started to examine the Suzuki–Miyaura coupling of iodopyrazole **2** and aminopyridine boronic ester **3**. The major challenge we needed to address for this transformation was to suppress the impurity resulting from aldol self-condensation of iodoketone **2** under basic conditions. Indeed, when a strong base such as NaO*t*-Bu was employed in the Suzuki–Miyaura coupling in the presence of 5 mol% $Pd(dppf)Cl_2$ in dioxane/H_2O (5:1) at 90 °C, the reaction gave complete conversion but with a significant level (43 A% by HPLC analysis) of aldol condensation side product (Table 1, entry 1). Phosphate base resulted in a slow reaction (Table 1, entry 2). K_2CO_3 afforded 93% conversion and 70 A% HPLC of the desired product **12** (Table 1, entry 3). Besides dppf, other ligands including A-[ca]Phos, XPhos, *t*-BuXPhos, and P(*t*-Bu)$_3$ were also studied [14] (Table 1, entries 4–7). However, none of them gave better conversion and reaction profile than $Pd(dppf)Cl_2$. Since 1,4-dioxane is an environmentally undesirable solvent, other solvents were explored. Compared to 1,4-dioxane (Table 1, entry 10), THF and ethanol gave lower conversion and inferior assay yield (Table 1, entries 8 and 11). DMF led to complete conversion (Table 1, entry 9), but similar to NaO*t*-Bu, large amounts of aldol condensation side product resulted in lower assay yield. Using *i*-PrOH as reaction solvent minimized the formation of the aldol condensation side product and afforded 100% conversion in high assay yield of 88% (Table 1, entry 12). By increasing the reaction temperature from 70 °C to 85 °C, the reaction time was also reduced to 3 h from 24 h with better reaction profile (Table 1, entry 13). The reaction also tolerated lower catalyst loading (1 mol %) (Table 1, entry 14) and lower boronic ester **3** (110 mol%) (Table 1, entry 15). Thus, the optimized reaction conditions employed 1 mol % $Pd(dppf)Cl_2$, and 110 mol% boronic ester **3** in *i*-PrOH/H_2O at 85 °C for 3 h, under which the reaction afforded complete conversion and 96% assay yield. Isolation of ketone **12** by direct crystallization from *i*-PrOH/H_2O mixture was achieved by adding H_2O (10 mL/g) directly into the reaction mixture, generating the coupling product in 93% isolated yield and 99.6 A% purity by HPLC.

We then focused our effort on the formation of **GNE-834** (**1**) by a reductive amination approach. We started our optimization of the reductive amination of **12** and morpholine employing 10 equiv of AcOH and a variety of reducing agents in toluene (Table 2). Generally, borohydride and borane reagents afforded better conversion than other reducing agents such as LiAlH(O*t*-Bu)$_3$ (Table 2, entries 1–5). The reaction performed the best in terms of diastereoselectivity when $NaBH_3CN$ was employed as the reducing agent (Table 2, entry 6). Other aromatic solvents performed similarly to toluene (Table 2, entries 7–9). Lewis Acids such as Ti(O*i*-Pr)$_4$ [15] and $ZrCp_2Cl_2$ were also studied (Table 2, entries 10–11). Similar to

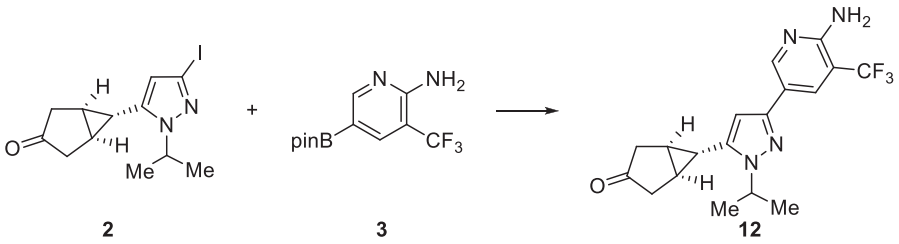


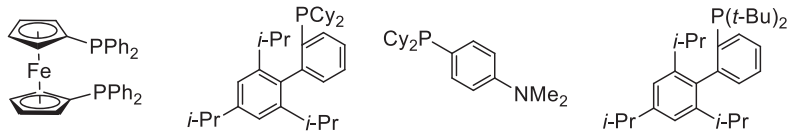
Scheme 2. Synthesis of iodoketone **2.**



Scheme 3. Synthesis of Boronic Ester **3.**

Table 1
Suzuki reaction optimization.





entry[a]	base	catalyst	solvent	time	temp (°C)	conv (%)[b]	A% of **12**[b]	assay yield (%)[b]
1	NaO*t*-Bu	Pd(dppf)Cl_2	dioxane/H_2O	3 h	90	100	57	–
2	K_3PO_4	Pd(dppf)Cl_2	dioxane/H_2O	24 h	90	83	57	–
3	K_2CO_3	Pd(dppf)Cl_2	dioxane/H_2O	24 h	90	93	70	–
4	K_2CO_3	XPhos Pd G3	dioxane/H_2O	24 h	90	63	38	–
5	K_2CO_3	(A-caPhos)$_2$ $PdCl_2$	dioxane/H_2O	24 h	90	78	56	–
6	K_2CO_3	*t*-BuXPhos Pd G1	dioxane/H_2O	24 h	90	86	50	–
7	K_2CO_3	P(*t*-Bu)$_3$Pd G2	dioxane/H_2O	24 h	90	65	60	–
8	K_2CO_3	Pd(dppf)Cl_2	THF/H_2O	24 h	70	79	67	77
9	K_2CO_3	Pd(dppf)Cl_2	DMF/H_2O	24 h	70	100	70	61
10	K_2CO_3	Pd(dppf)Cl_2	dioxane/H_2O	24 h	70	89	76	85
11	K_2CO_3	Pd(dppf)Cl_2	EtOH/H_2O	24 h	70	86	57	56
12	K_2CO_3	Pd(dppf)Cl_2	*i*-PrOH/H_2O	24 h	70	100	82	88
13	K_2CO_3	Pd(dppf)Cl_2	*i*-PrOH/H_2O	3 h	85	100	94	89
14[c]	K_2CO_3	Pd(dppf)Cl_2[c]	*i*-PrOH/H_2O	3 h	85	100	94	88
15[d]	K_2CO_3	Pd(dppf)Cl_2	*i*-PrOH/H_2O	3 h	85	100	96	93[e]

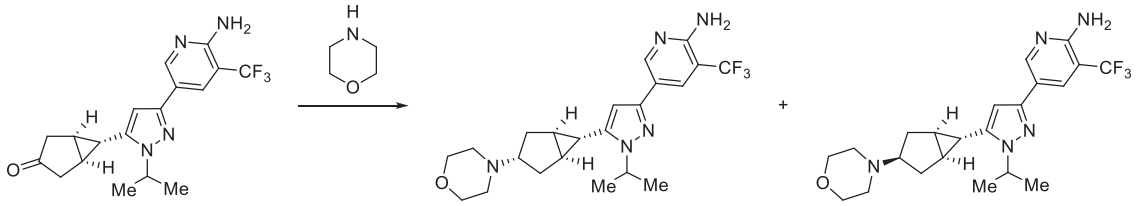
[a] Reaction conditions: **2** (100 mg, 0.303 mmol), **3** (105 mg, 0.363 mmol, 120 mol%), base (200 mol%), and catalyst (5 mol%) in different organic solvent (15 mL/g) and H_2O (3 mL/g).
[b] Determined by quantitative HPLC analysis of the crude reaction mixture
[c] cPd(dppf)Cl_2 (2.2 mg, 0.0030 mmol, 1 mol%)
[d] Reaction condition: **2** (466 g, 1.41 mol), **3** (447 g, 1.55 mol, 110 mol%), K_2CO_3 (390 g, 2.82 mol, 200 mol%), and Pd(dppf)Cl_2 (10.3 g, 0.0141 mol, 1 mol%) in *i*-PrOH (10 mL/g) and H_2O (2 mL/g)
[e] Isolated yield

AcOH, both gave complete conversion and similar diastereoselectivity. Since both diastereomers **1** and **13** have low solubility in toluene, direct precipitation was achieved by adding water and aqueous Na_2CO_3 solution into the reaction mixture to provide **1** and **13** (dr = 67:33) mixture in > 99% yield. Further purification by silica gel column chromatography produced **1** in 58% yield.

Next, we set out to investigate the separation of the diastereomers by crystallization to eliminate chromatographic purification. We first examined the solubility of the diastereomers **1** and **13** at 25 ℃ in a number of organic solvents. The initial study indicated that both diastereomers have similarly low solubility in common organic solvents (<30 mg/mL). Therefore, the separation of the diastereomers by direct crystallization was deemed impractical [16]. We then prepared the corresponding tosylate salt of both diastereomers **1** and **13**. The solubility of the tosylate salts was examined in multiple solvents and we were delighted to see that by using MeOH as the solvent, a significant solubility difference (40–50 mg/mL) between the tosylate salts could be achieved at elevated temperatures (Fig. 2). Generally, the solubility of **1**•TsOH is low and relatively temperature independent. However, the solubility of **13**•TsOH is highly dependent on temperature. This led us to envision a crystallization by tosylate salt formation in MeOH at high temperature so that **1**•TsOH could be obtained and the undesired **13**•TsOH purged in the mother liquor after hot filtration.

Different temperatures were then evaluated for the resolution by tosylate salt formation (Table 3). Starting from 67:33 mixture

Table 2
Reductive amination condition optimization.



entry[a]	reducing agent	acid	solvent	**1** : **13**[b]	conv (%)[b]	A% of **1**[b]
1	$NaBH_4$	AcOH	toluene	53:47	92	48
2	2-Picoline•borane	AcOH	toluene	48:52	100	48
3	5-Ethyl-2-picoline•borane	AcOH	toluene	44:56	100	44
4	$NaBH(OAc)_3$	AcOH	toluene	38:62	79	30
5	$LiAlH(Ot\text{-}Bu)_3$	AcOH	toluene	–	<5	–
6	$NaBH_3CN$	AcOH	toluene	67:33	100	67
7	$NaBH_3CN$	AcOH	xylene	58:42	100	58
8	$NaBH_3CN$	AcOH	mesitylene	62:38	100	62
9	$NaBH_3CN$	AcOH	$CF_3C_6H_5$	55:45	100	55
10	$NaBH_3CN$	$Ti(Oi\text{-}Pr)_4$	toluene	66:34	100	66
11	$NaBH_3CN$	$ZrCp_2Cl_2$[c]	toluene	65:35	100	65

[a] Reaction conditions: **12** (50.0 mg, 0.137 mmol), reducing agent (0.206 mmol, 150 mol%), morpholine (0.119 mL, 1.37 mmol, 1000 mol%), and acid (1.37 mmol, 1000 mol%) in different organic solvent (15 mL/g) at 80 °C for 3 h.
[b] Determined by HPLC analysis of the crude reaction mixture.
[c] 200 mol%.

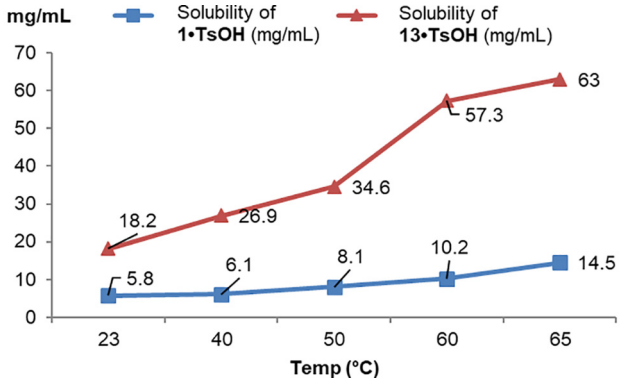
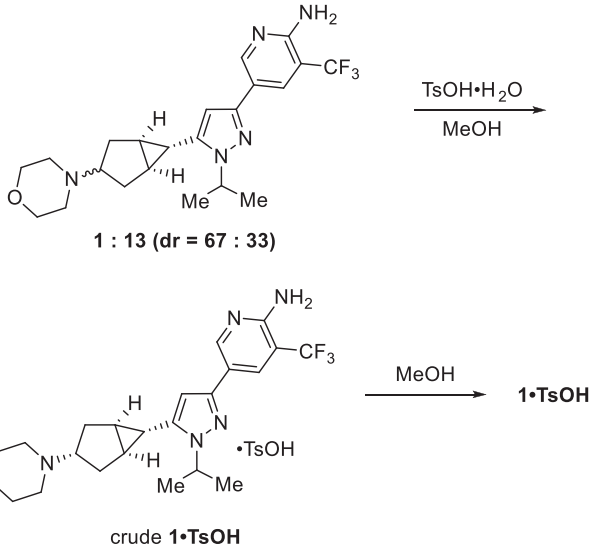


Fig. 2. Solubility of **1•TsOH** and **13•TsOH** in MeOH.

of **1** and **13** freebase, we were able to increase the dr of the isolated tosylate salt to 94:6 at 50 °C (Table 3, entry 1). Additional methanol slurry at 50 °C was able to increase the dr to 99:1. However, hot filtration at higher temperatures resulted in marginally lower overall yield (Table 3, entries 2 and 3). Conducting resolution and hot filtration at lower temperature 45 °C afforded a slightly lower diastereoselectivity (Table 3, entry 4). To balance the recovery and purging of **13**, 50 °C was pursued further. This process was first scaled up to 3 g (Table 3, entry 5), consistent dr (99.4:0.6) and recovery (53%) was obtained. When the reaction was scaled up to 59 g, a lower dr (94:6) was observed after the tosylate salt formation. The subsequent MeOH slurry did not upgrade the purity as effectively as in the small scale (Table 3, entry 5), presumably due to the presence of trapped freebase diastereomer **13** due to insufficient charge of TsOH•H_2O. An additional MeOH slurry of crude **1**•TsOH in the presence of 5 mol% TsOH•H_2O was thus performed to further improve the diastereoselectivity and lead to comparable dr (98.9:1.1) and yield (50%) (Table 3, entry 6).

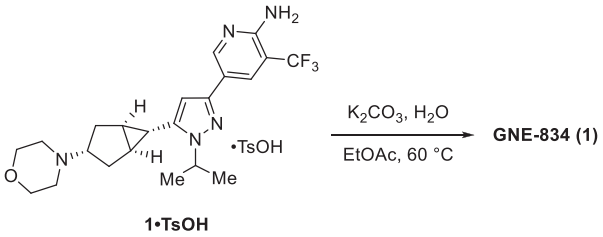
Table 3
Crystallization of tosylate salt at different temperatures.



entry[a]	temp (°C)	dr[b] of crude **1**•TsOH	dr[b] of **1**•TsOH	yield (%)[c]
1	50	94.0:6.0	99.2:0.8	55
2	55	96.5:3.5	99.4:0.6	53
3	60	93.6:6.4	99.2:0.8	52
4	45	92.6:7.4	98.8:1.2	56
5[d]	50	97.6:2.4	99.4:0.6	53
6[e]	50	94.0:6.0	98.9:1.1	50

[a] Reactions conditions: **1** and **13** mixture (300 mg, 0.689 mmol), with TsOH·H_2O (131 mg, 0.689 mmol, 100 mol%) in MeOH (5 mL/g) at different temperature.
[b] Determined by HPLC analysis of the isolated tosylate salt.
[c] Isolated yield from **1** and **13** mixture (dr = 67:33).
[d] 2.7 g scale.
[e] 59.4 g scale, one additional slurry with 5 mol% TsOH•H_2O in MeOH was used.

By treating **1•TsOH** with a biphasic mixture of K_2CO_3 aqueous solution and EtOAc, the freebase was effectively liberated into EtOAc layer. Subsequent azeotropic distillation of the EtOAc layer followed by crystallization afforded **1**. This process was carried out at 25 g scale to give **GNE-834** (**1**) in 87% yield and 99.6 A% HPLC purity.



1•TsOH

Conclusion

In conclusion, a fit-for-purpose synthesis of DLK inhibitor **GNE-834** (**1**) was developed. An efficient Suzuki–Miyaura coupling was developed to construct the key C—C bond between the pyrazole and the pyridine moieties in high yield. Despite the modest diastereoselectivity (67:33) observed in the reductive amination, **GNE-834** (**1**) was obtained in excellent diastereoselectivity (99:1) by resolution using tosylate salt formation without chromatographic purification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152430.

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