European Journal of Medicinal Chemistry 216 (2021) 113270

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Balancing potency and basicity by incorporating fluoropyridine moieties: Discovery of a 1-amino-3,4-dihydro-2,6-naphthyridine BACE1 inhibitor that affords robust and sustained central Aβ reduction

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ARTICLE INFO

Article history:
Received 18 October 2020
Received in revised form
22 January 2021
Accepted 1 February 2021
Available online 7 February 2021

Keywords:
BACE1
β-secretase
Inhibitor
Naphthyridine
Basicity
X-ray analysis

ABSTRACT

β-Site amyloid precursor protein cleaving enzyme 1 (BACE1) has been pursued as a prime target for the treatment of Alzheimer's disease (AD). In this report, we describe the discovery of BACE1 inhibitors with a 1-amino-3,4-dihydro-2,6-naphthyridine scaffold. Leveraging known inhibitors **2a** and **2b**, we designed the naphthyridine-based compounds by removing a structurally labile moiety and incorporating pyridine rings, which showed increased biochemical and cellular potency, along with reduced basicity on the amidine moiety. Introduction of a fluorine atom on the pyridine culminated in compound **11** which had improved cellular activity as well as further reduced basicity and demonstrated a robust and sustained cerebrospinal fluid (CSF) Aβ reduction in dog. The crystal structure of compound **11** bound to BACE1 confirmed van der Waals interactions between the fluorine on the pyridine and Tyr71 in the flap.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia. The clinical symptoms of AD are progressive memory loss, learning impairment, and behavioral and psychiatric disturbances. Currently available treatments using acetylcholinesterase inhibitors and the *N*-methyl-D-aspartate receptor antagonist do not halt or even delay the disease progression and only provide temporary symptomatic effects, thus leaving an urgent unmet need to develop disease-modifying therapies [1].

The pathological hallmarks of AD patients are extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles consisting of aggregates of phosphorylated tau protein [1]. As Aβ levels become abnormal before the accumulation of tau proteins and Aβ42 oligomers, a toxic component of Aβ, inducing tau hyperphosphorylation, Aβ is thought to be a causative factor in the development of AD [2]. Amyloid precursor protein (APP) is processed sequentially by β-site amyloid precursor protein cleaving enzyme 1 (BACE1, also known as β-secretase) and γ-secretase, leading to the formation of Aβ peptides [3]. Because the clinical use of γ-secretase inhibitors was abandoned in the clinic due to the mechanism-based toxicities arising from inhibition of Notch processing, the inhibition of BACE1 remained as a promising therapeutic approach for the development of disease-modifying therapies for AD [4].

The large size of the catalytic site in BACE1 has hampered the search for BACE1 inhibitors. Initial investigations identified peptidomimetic inhibitors with high molecular weight and large polar surface area, which prevented both cellular and brain penetration

Abbreviations: Alzheimer's disease, AD; amyloid-β, Aβ; amyloid precursor protein, APP; area under the concentration-time curve, AUC; β-site amyloid precursor protein cleaving enzyme 1, BACE1; cerebrospinal fluid, CSF; N,N-dimethylformamide, DMF; efflux ratio, ER; 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate, HATU; homogeneous time-resolved fluorescence, HTRF.

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https://doi.org/10.1016/j.ejmech.2021.113270
0223-5234/

[4]. Identification of amidine-based inhibitors mitigated these issues and consequently led to the discovery of several clinical compounds, such as verubecestat (MK-8931) [5], elenbecestat (E−2609) [6], and atabecestat (JNJ-54861911) [7]. During the course of the research, controlling the basicity of the amidine moiety together with retaining the potency was key to the development of centrally active inhibitors, as they tend to show high basicity being associated with poor brain penetration due to recognition of the P-gp transporter [8]. Recently, we identified dihydro-1,3-oxazine **1** which led to significant Aβ reduction in vivo at a low dose through mitigation of the P-gp efflux resulting from moderate basicity ($pK_a = 7.2$). The fluorine at the 5-position in 1 reduced the basicity along with increasing the potency via stabilization of an active conformation (Fig. 1) [9]. Herein we report an alternative approach to controlling pK_a along with increasing potency, starting with fused inhibitors **2a** and **2b** [10a], which led to the identification of novel 1-amino-3,4-dihydro-2,6-naphthyridine BACE1 inhibitors [11]. Optimization efforts culminated in an orally efficacious compound **11**, which showed robust and sustained Aβ reduction in dog.

2. Results and discussion

We have reported benzene-fused amidines **2a** and **2b** with moderate to slightly high pK_a values of 7.7 and 7.2 in the patent literature [10a], which had comparable potency relative to non-substituted dihydro-1,3-oxazine **3** [9]. Because diversification of dihydro-1,3-oxazine head groups, as in **1** and **3**, was considered in our optimization strategy, compounds **2a** and **2b** served as promising scaffolds for the design of structurally differentiated inhibitors. The potential concern of racemization for **2** did occur when a racemic close analogue was subjected to chiral chromatography, and the enantiomers racemized again. Therefore, our effort started with removal of the labile *N*,*O*-acetal substructure and replacement of the phenyl moiety on the amidine with a variety of pyridine rings, in order to control basicity (Fig. 2).

Table 1 summarizes the key SAR for the newly designed compounds. Clearly, incorporation of pyridine rings impacted potency. Compound **4** with a nitrogen at the 1-position, with the atom shown in Table 1, exhibited modest biochemical and cellular potency, whereas compounds with nitrogen at other positions, such as compounds **5**, **6**, and **7**, were found to increase potency relative to **4** and the lead **2a** and **2b**. Of these, compounds **5** and **6** with nitrogen atoms at the 2- and 3-positions respectively, significantly improved BACE1 activity associated with excellent cellular potency. Introduction of the pyridine rings successfully reduced basicity for compounds **4**–**7** ($pK_a = 9.0$ to 9.8) when compared with the corresponding benzene analogue ($pK_a = 10.8$) reported by scientists at Roche [10b], although the pK_a values were still too high to gain brain penetration due to the potential of being P-gp substrates, according to our previous research and other reports [8,9].

Having identified promising pyridine-fused amidine scaffolds (1-amino-3,4-dihydro-2,6-naphthyridines), such as **5** and **6**, that showed cellular IC_{50} values of <2 nM, we optimized the scaffolds to reduce basicity. To this end, we examined incorporation of a fluorine atom on the pyridine ring. Compound **10** with a fluorine at the 1-positon decreased both enzymatic and cellular potency, whereas introduction of a fluorine at the 4-position yielded compounds with improved cellular potency (**8**, **9**, and **11**). As expected, further reduction of pK_a was realized by the incorporation of a fluorine on the pyridine; compounds **8** and **11** with a fluorine at the 4-position showed reduced pK_a values of 7.6 and 8.2, relative to those in non-substituted **5** and **6** ($pK_a = 9.5$ and 9.0, respectively), although we observed a difference in the extent to which the fluorine atom at the 4-position reduced basicity (**5** vs **8**; **6** vs **11**). Although incorporation of a fluorine at the 1-position achieved the lowest pK_a of 7.3, it was accompanied by loss in potency. As expected, the less basic profile translated into reduced P-gp efflux. Indeed, non-fluorine substituted **6** with a pK_a of 9.0 had a high P-gp efflux ratio (ER) of 37 in LLC-PK cells expressing the human MDR1 gene. Conversely, the corresponding fluorine analogue **11** with a pK_a of 8.2 displayed a reduced, but still significant P-gp ER of 13; compound **8** with a further reduced pK_a of 7.3 also exhibited the same P-gp ER value of 13. Unfortunately, all the less basic compounds of **8**, **10**, and **11** were found to be strong hERG inhibitors with percent inhibitions at 5 μM of 72%, 92%, and 88% in an automated patch clamp assay, which were comparable to those of non-substituted **5** and **6** (83% and 85%, respectively). As shown in a previous paper [12a], a pK_a reduction of less than **7** seems to be required to achieve preferable profiles for P-gp efflux and hERG inhibition, along with modifications at the amide substituent of the cyanopyridine group. Nevertheless, a representative compound of **11** was evaluated in vivo for the ability to reduce Aβ in the brain. Table 2.

Before measuring Aβ reduction in vivo, we profiled the pharmacokinetics properties for **11** in rat and dog, together with measuring metabolic stability. In rat, clearance was high (45 mL/min/kg) due to poor microsomal stability (22% remaining after 30 min). Combined with high tissue distribution (14 L/kg), this translated into a low maximum concentration (C_{max}) and area under the concentration-time curve (AUC). Reflecting high P-gp efflux, **11** exhibited a low total brain-to-plasma ratio (K_p) of 0.28. In dog, improved microsomal stability (93% remaining after 30 min) led to good PK profiles with a relatively high C_{max} and AUC value following oral administration (1 mg/kg), which prompted us to advance to PK/PD study. As shown in Table 3, compound **11** demonstrated robust and sustained reduction of total Aβ in the cerebrospinal fluid (CSF) by 49% and 47% at the 10 and 24 h time points, respectively, despite the low CSF-to-unbound plasma ratios of 0.070 and 0.083. The significant and sustained Aβ reduction was rationalized by the drug concentrations in the CSF at 10 and 24 h, where **11** exceeded the cellular IC_{50} value by 3.3- and 3.0-fold.

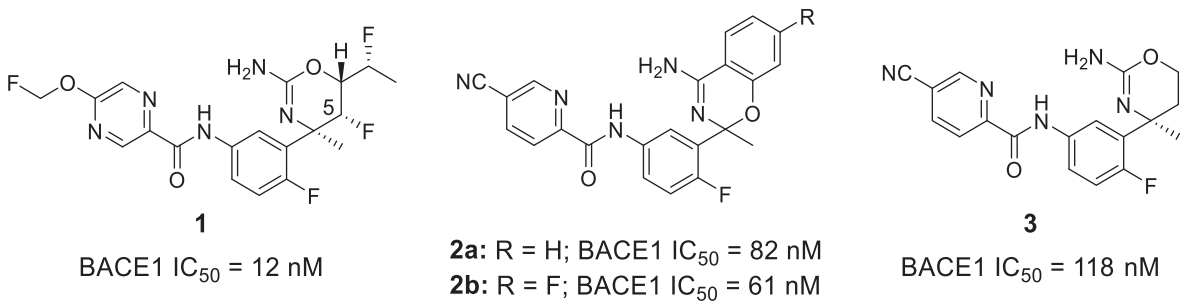


Fig. 1. Dihydro-1,3-oxazine BACE1 inhibitors **1** and **3** and lead compounds **2a** and **2b**

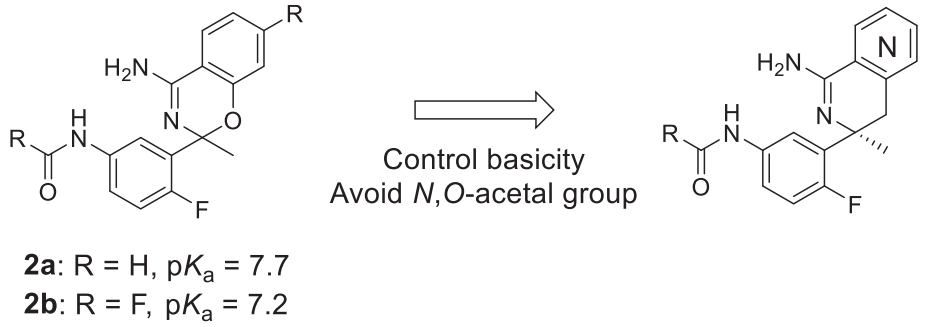
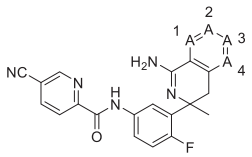


Fig. 2. Design of 1-amino-3,4-dihydro-2,6-naphthyridine BACE1 inhibitors.

Table 1
Exploration of pyridine-fused amidines.



compd		IC50 (nM)[a] BACE1[b]	Cellular Aβ[c]	pKa[d]
4		394	26	9.8
5		8.1	1.3	9.5
6		21	1.5	9.0
7		54	4.1	9.4
8		12	0.25	7.6
9		14	0.36	NT
10		118	11	7.3
11 (chiral, *S*)		9.1	0.41	8.2

[a] Values represent the mean values of at least two determinations.
[b] Biochemical homogeneous time-resolved fluorescence (HTRF)-based assay.
[c] IC_{50} determined by measuring the levels of secreted Aβ40 in human APP-transfected human neuroblastoma (SH-SY5Y) cells via an HTRF-based assay.
[d] pK_a determined by capillary electrophoresis. NT = not tested.

The X-ray cocrystal structure of **11** bound to BACE1 was solved at 2.3 Å resolution (PDB code 7D36 Fig. 3). The structure confirmed a binding mode similar to those seen for amidine-based BACE1 inhibitors [4,5a,12]. The amidine moiety displays hydrogen bond interactions with the catalytic aspartate dyad (Asp32 and Asp228). The amide N–H forms a hydrogen bond with the backbone carbonyl oxygen of Gly230. The quaternary center positions the fluorophenyl ring in an axial orientation to occupy the S1 pocket, and the nitrile substituent projects into the S3 pocket. Interestingly, the fluorine on the pyridine in **11** engages in van der Waals interactions with Tyr71 located in the flap. The corresponding hydrogen in **6** is likely to be involved in the same interaction, giving comparable activities to **11**. In contrast, the nitrogen atom at the same position on the pyridine in **7** may disrupt this favorable interaction, explaining why **7** showed reduced potency relative to the corresponding hydrogen or fluorine analogues, such as **6** and **11**. We also reasoned that the nitrogen and fluorine atoms at the 1-position (shown in Table 1) in **4** and **10**, respectively, would be proximal to the catalytic site and consequently cause electrostatically unfavorable interactions, resulting in reduced potency [13][[15]]. [10],

3. Chemistry

Synthesis of compound **4** started with commercially available acetophenone **12** (Scheme 1). The ketone moiety in **12** was converted to the corresponding boronate **14** via Wittig reaction and subsequent Miyaura borylation reaction [14]. Cross-coupling of **14** with 3-bromo-2-cyanopyridine gave compound **15**, which was then heated with ammonium chloride in the presence of trimethyl aluminum to afford **16**. Cyclization of the amidine **16** furnished a 1-amino-3,4-dihydro-2,6-naphthyridine scaffold followed by nitration of the fluorobenzene gave **17**. The amide moiety in **18** was successfully introduced through amidation with 5-cyanopicolinic acid, following Boc protection of **17** and subsequent reduction of the nitro group. Finally, deprotection of the Boc group in **18** under acidic condition furnished compound **4**.

The synthesis of compound **5** is shown in Scheme 2. Homologation of 2′-fluoroacetophenone **12** was achieved by nucleophilic addition of 3-cyano-4-methylpyridine and subsequent elimination of water to afford amide **19**, which was then dehydrated with trifluoroacetic anhydride to yield nitrile **20**. According to the methods described in Scheme 1, compound **5** was prepared from intermediate **20** as shown in Scheme 2. The synthesis of compounds **6** and **7** also followed the procedures described in Scheme 1 with some modifications to Suzuki cross-coupling conditions of boronate **14** (Schemes 3 and 4). The synthesis of compounds **8**–**11** started with Suzuki cross-coupling reaction of commercially available reagents **33**, **39**, and **45** with boronate **14** (Schemes 5–7). Intermediates **34**, **40**, and **46** were converted to the final compounds **8**–**11**, according to the procedures described in Scheme 1. Chiral separation of compound **9** using supercritical fluid chromatography (SFC) afforded the desired enantiomer **11**.

4. Conclusions

1-Amino-3,4-dihydro-2,6-naphthyridine BACE1 inhibitors were identified through incorporation of pyridine moieties and removal

Table 2
Pharmacokinetic profiles of **11** in rat and dog.

	LM (%)[a]	Serum f_u[b]	iv, 0.5 mg/kg, $n = 2$			po, 1 mg/kg, $n = 2$ (rat), 3 (dog)			
species			CL (mL/min/kg)[c]	Vd_{ss} (L/kg)[d]	B/P[e]	AUC (ng·h/mL)[f]	C_{max} (ng/mL)[g]	T_{max} (h)[h]	F (%)[i]
rat	22	0.15	45	14	0.28	122	9.0	4.0	33
beagle dog	93	0.14	NT	NT	NT	3890	78	4.3	NC

[a] % remaining in rat and dog liver microsomes after 30 min incubation.
[b] Fraction unbound in rat and dog serum.
[c] Total clearance.
[d] Volume of distribution at steady state.
[e] Total brain-to-plasma ratio (*Kp*).
[f] Area under the concentration-time curve.
[g] Maximum plasma concentration.
[h] Time at maximum concentration.
[i] Oral bioavailability. NT = not tested. NC = not calculated.

Table 3
Pharmacokinetic and pharmacodynamic profiles of **11** in dog.

	time after administration / po, 1 mg/kg, $n = 3$[a]			
	3 h	7 h	10 h	24 h
C_p (ng/ml)[b]	72	65	64	47
C_{CSF} (ng/ml)[c]	NT	NT	0.56	0.52
$C_{CSF}/C_{p,u}$[d]	NT	NT	0.070	0.083
CSF total Aβ reduction (%)	NT	NT	49	47
C_{CSF} /Cell IC_{50}	NT	NT	3.3	3.0

[a] Dosed to beagle dogs as a suspension in 0.5% MC.
[b] Plasma concentration.
[c] CSF concentration.
[d] CSF-to-unbound plasma ratio. NT = not tested.

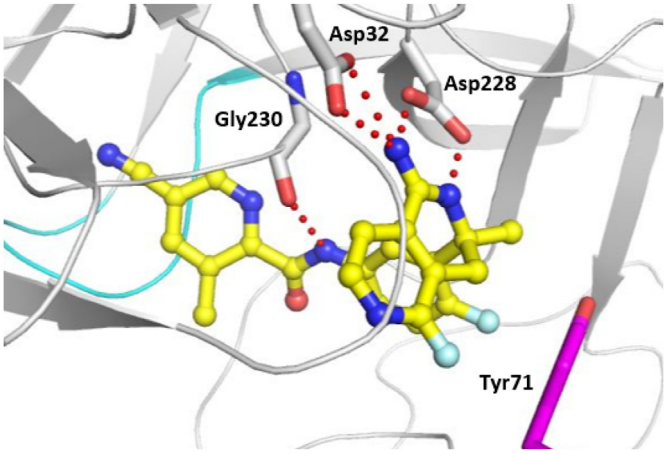


Fig. 3. Crystal structure of compound **11** complexed with BACE1.

of the *N,O*-acetal group in initial leads **2a** and **2b**. Further optimization by introducing a fluorine atom on the pyridine ring balanced basicity and cellular potency, leading to the discovery of compound **11** which showed a robust and sustained CSF Aβ reduction in dog, along with reduced P-gp efflux. The X-ray structure of compound **11** bound to BACE1 revealed the molecular interactions around the fluoropyridine moiety, which provide opportunities for further optimization of this analogue.

5. Experimental section

5.1. General chemistry

All commercial reagents and solvents were used without further purification. Flash column chromatography was conducted on an automated purification system using Yamazen or Fuji Silysia prepacked silica gel columns. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz Analytical LC/MS was carried out on a Shimadzu Shim-pack XR-ODS (C18, 2.2 μm, 3.0 × 50 mm, a linear gradient from 10% to 100% B over 3 min and then 100% B for 1 min (A = water + 0.1% formic acid, B = MeCN + 0.1% formic acid), flow rate 1.6 mL/min) using a Shimadzu UFLC system equipped with an LCMS-2020 mass spectrometer, LC-20AD binary gradient module, SPD-M20A photodiode array detector (detection at 254 nm), and SIL-20AC sample manager.

1-(1-Bromoprop-1-en-2-yl)-2-fluorobenzene (13)

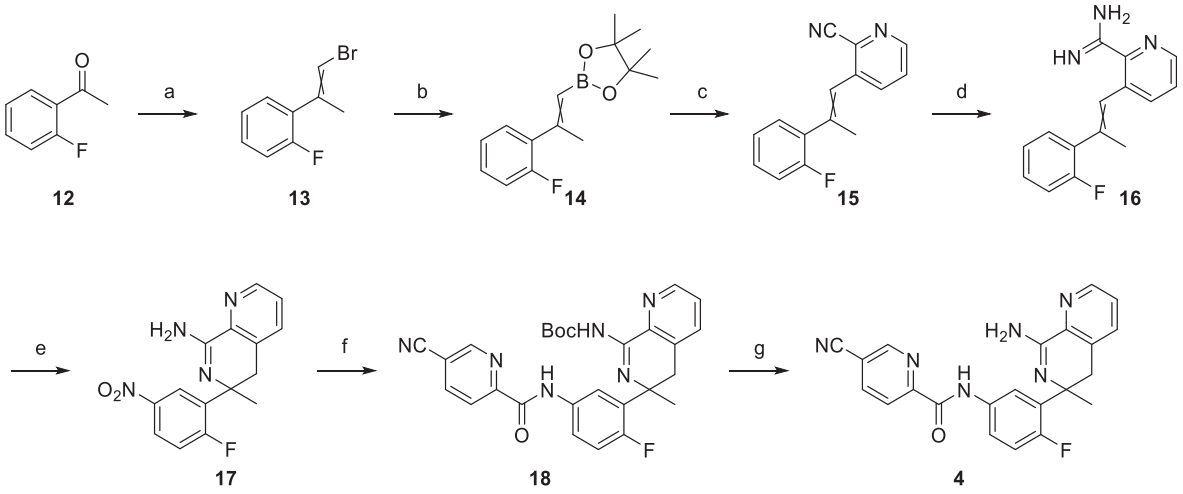
To a mixture of (bromomethyl)triphenylphosphonium bromide (7.58 g, 17.4 mmol) in THF (20 mL) was added dropwise *tert*-BuOK (1 M in THF; 17.4 mL, 17.4 mmol) at 0 °C. After the mixture was stirred at the same temperature for 10 min, 1-(2-fluorophenyl) ethanone (12, 1.79 mL, 14.5 mmol) was added dropwise to the mixture at 0 °C. The mixture was stirred at the same temperature for 90 min and then quenched with saturated aqueous NH_4Cl solution. The aqueous layer was separated and extracted with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; hexane) to give a 3:2 diastereomeric mixture of **13** (1.57 g, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.10 (d, J = 1.5 Hz, 3H, *major isomer*) and 2.19 (t, J = 1.4 Hz, 3H, *minor isomer*), 6.32 (q, J = 1.5 Hz, 1H, *major isomer*) and 6.39 (q, J = 1.4 Hz, 1H, *minor isomer*), 7.01–7.34 (4H, m).

2-(2-(2-Fluorophenyl)prop-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (14)

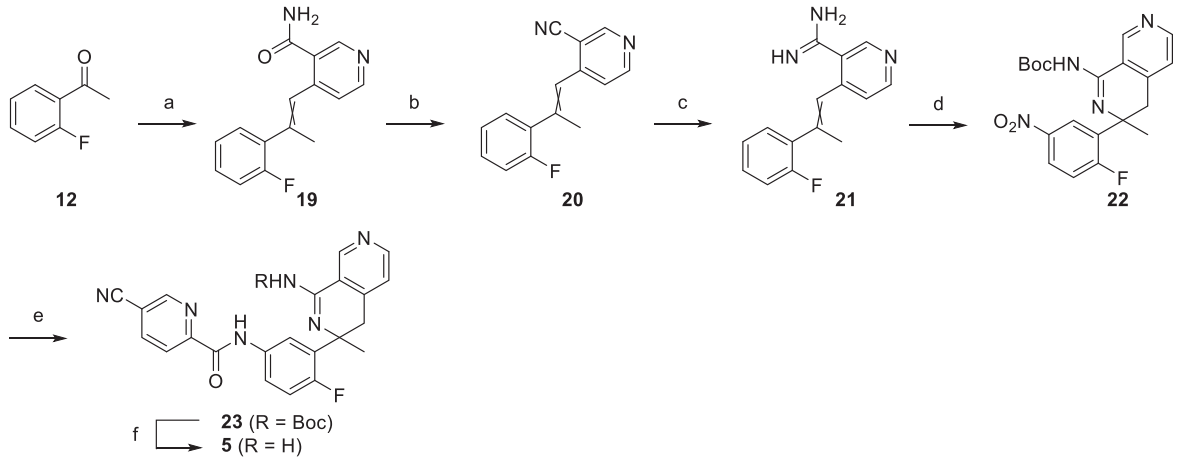
A mixture of **13** (2.24 g, 10.4 mmol), bis(4,4,5,5-tetramethyl-[1,3]dioxolan-2-yl)borane (3.97 g, 15.6 mmol), KOAc (3.07 g, 31.2 mmol), and $PdCl_2$(dppf)•CH_2Cl_2 (0.425 g, 0.521 mmol) in 1,4-dioxane (45 mL) was stirred at 65 °C for 18 h under N_2. The mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–10% EtOAc) to give a 3:2 diastereomeric mixture of **14** (2.15 g, 79%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 12H, *major isomer*), 1.31 (s, 12H, *minor isomer*), 2.18 (br s, 3H, *major isomer*), 2.37 (t, J = 1.2 Hz, 3H, *minor isomer*), 5.53 (s, 1H, *minor isomer*), 5.61 (q, J = 1.2 Hz, 1H, *major isomer*), 6.95–7.09 (2H, m), 7.14–7.30 (2H, m). MS-ESI (*m/z*): 263 $[M + H]^+$.

3-(2-(2-Fluorophenyl)prop-1-en-1-yl)picolinonitrile (15)

A mixture of **14** (223 mg, 0.852 mmol), 3-bromo-2-cyanopyridine (187 mg, 1.02 mmol), K_2CO_3 (2 M aqueous solution; 1.28 mL, 2.56 mmol), and $PdCl_2$(dppf)•CH_2Cl_2 (34.8 mg, 0.043 mmol) in THF (7 mL) was stirred at 70 °C for 9 h under N_2. The mixture was filtered through a Celite pad, and then the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4,



Scheme 1. Synthesis of Compound **4**. Reagents and conditions: (a) *t*-BuOK, bromomethyltriphenylphosphonium bromide, THF, 0 °C, 50%, dr = 3:2; (b) bis(4,4,5,5-tetramethyl-[1,3]-dioxolan-2-yl)borane, KOAc, $PdCl_2(dppf)•CH_2Cl_2$, 1,4-dioxane, 100 °C, 79%, dr = 3:2; (c) 3-bromo-2-cyanopyridine, K_2CO_3, $PdCl_2(dppf)•CH_2Cl_2$, THF-H_2O, 70 °C; (d) trimethylaluminum, NH_4Cl, toluene, 100 °C, 31% for 2 steps, dr = 3:2; (e) H_2SO_4, rt; then HNO_3, 0 °C, 75%; (f) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C; (iii) 5-cyanopicolinic acid hydrate, HATU, Et_3N, THF, rt, 71% for 3 steps; (g) formic acid, rt, 59%.



Scheme 2. Synthesis of Compound **5**. Reagents and conditions: (a) *t*-BuONa, 3-cyano-4-methylpyridine, DMF, rt, 66%, dr = 1:1; (b) trifluoroacetic anhydride, pyridine, 1,4-dioxane, rt; (c) trimethylaluminum, NH_4Cl, toluene, 100 °C, 74% for 2 steps, dr = 3:2; (d) (i) H_2SO_4, rt; then HNO_3, 0 °C; (ii) Boc_2O, THF, rt, 70% for 2 steps; (e) (i) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C; (ii) 5-cyano-2-pyridinecarboxylic acid, diphenyl phosphorochloridate, Et_3N, EtOAc, 0 °C, 77% for 2 steps; (f) TFA, CH_2Cl_2, rt, 67%.

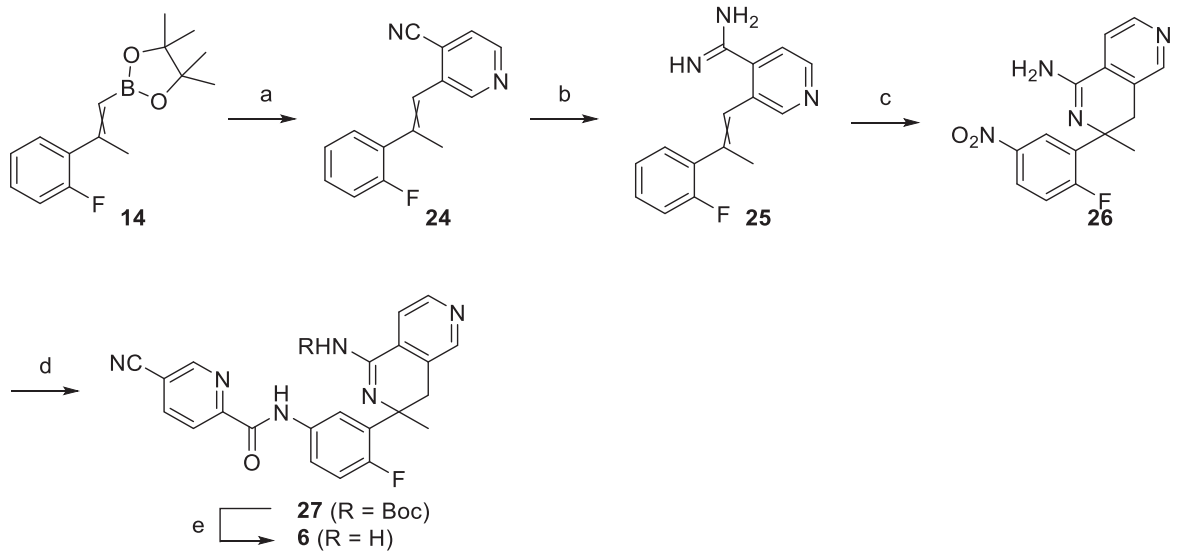
filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–10% EtOAc) to give a 1:1 diastereomeric mixture of **15** (207 mg, 3-bromo-2-cyanopyridine was contained) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (t, *J* = 1.2 Hz, 3H, *major isomer*) and 2.44 (d, *J* = 1.5 Hz, 3H, *minor isomer*), 6.75 (1H, br s), 6.97–7.56 (5H, m), 8.17 (s, *major isomer*) and 8.88 (s, *minor isomer*), 8.55 (d, *J* = 5.0 Hz, 1H, *major isomer*), 8.78 (d, *J* = 5.0 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 239 $[M + H]^+$.

3-(2-(2-Fluorophenyl)prop-1-en-1-yl)picolinimidamide (16)

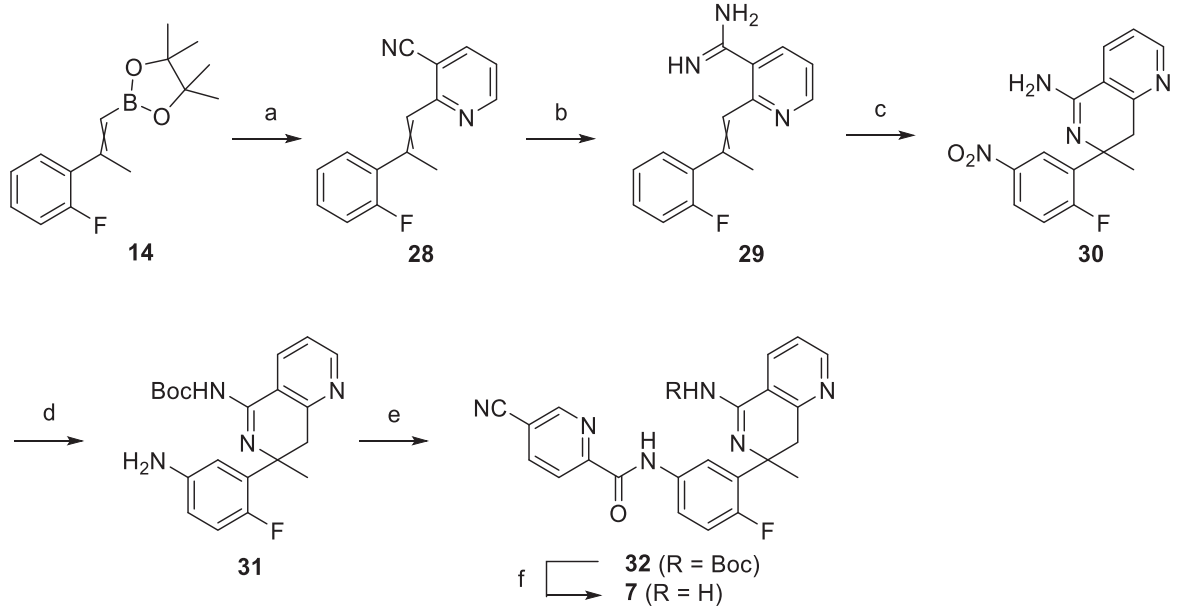
To a suspension of NH_4Cl (1.56 g, 29.2 mmol) in toluene (2 mL) was added dropwise trimethyl aluminum (14.6 mL, 29.2 mmol) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, **15** (199 mg, 0.833 mmol) in toluene (4 mL) was added. The mixture was stirred at 100 °C for 24 h. The reaction was quenched with potassium sodium tartrate and NaOH (2 M aqueous solution) at 0 °C. The mixture was stirred at rt for 1 h. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–10% MeOH) to give a 3:2 diastereomeric mixture of **16** (65.3 mg, 31% for 2 steps) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, *J* = 1.3 Hz, 3H, *minor isomer*), 2.26 (d, *J* = 1.5 Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 8.01 (s, 1H, *major isomer*), 8.71 (s, 1H, *minor isomer)*, 8.35 (d, *J* = 5.0 Hz, 1H, *major isomer*) and 8.58 (d, *J* = 4.9 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 256 $[M + H]^+$.

6-(2-Fluoro-5-nitrophenyl)-6-methyl-5,6-dihydro-1,7-naphthyridin-8-amine (17)

After a mixture of **16** (61.3 mg, 0.240 mmol) and conc. H_2SO_4 (0.50 mL, 9.00 mmol) was stirred at rt for 16 h, HNO_3 (0.021 mL, 0.480 mmol) was added at 0 °C, and the mixture was then stirred at 0 °C for 1 h. The reaction was quenched with NaOH (2 M aqueous solution) and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **17** (53.7 mg, 75%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (3H, s), 3.18 (1H, d,



Scheme 3. Synthesis of Compound **6**. Reagents and conditions: (a) 3-bromo-4-cyanopyridine, K_2CO_3, $PdCl_2(dppf)•CH_2Cl_2$, THF-H_2O, 70 °C, 84%, dr = 3:2; (b) trimethylaluminum, NH_4Cl, toluene, 100 °C, 97%; (c) conc. H_2SO_4, rt; then HNO_3, 0 °C, 86%; (c) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C; (d) 5-cyanopicolinic acid hydrate, HATU, Et_3N, THF, rt, 97% for 3 steps; (e) formic acid, rt, 52%.

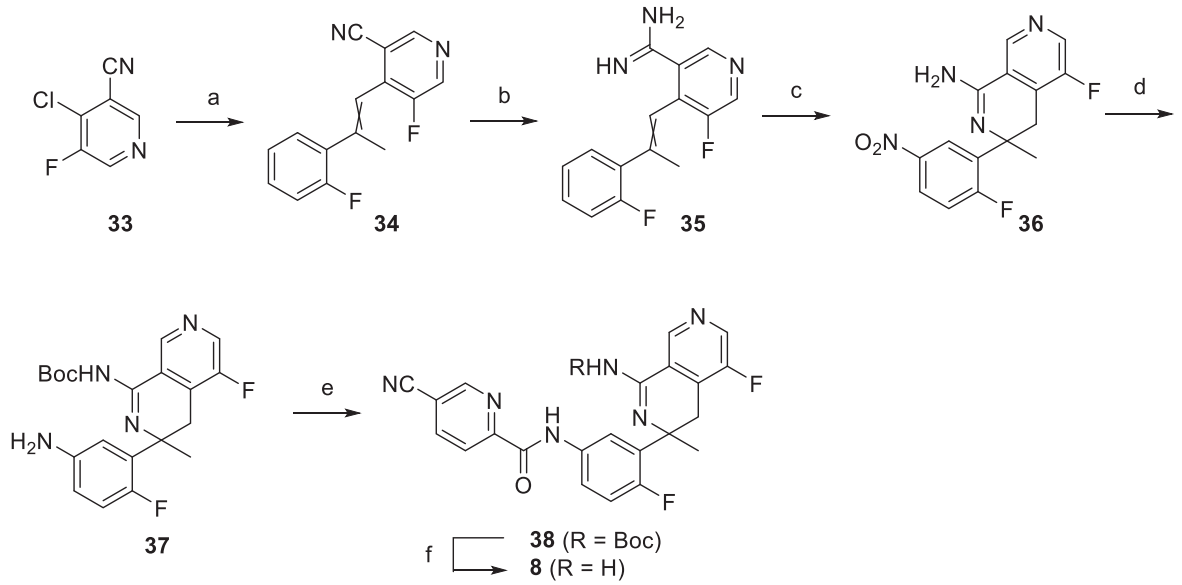


Scheme 4. Synthesis of Compound **7**. Reagents and conditions: (a) 2-chloro-3-cyanopyridine, K_2CO_3, $PdCl_2(dppf)•CH_2Cl_2$, 1,4-dioxane-H_2O, 100 °C, 84%, dr = 3:2; (b) trimethylaluminum, NH_4Cl, toluene, 100 °C, 97%, dr = 3:2; (c) conc. H_2SO_4, rt; then HNO_3, 0 °C, 81% for 2 steps; (d) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C, 70% for 2 steps; (e) 5-cyanopicolinic acid hydrate, HATU, Et_3N, THF, rt, 96%; (f) formic acid, rt, 84%.

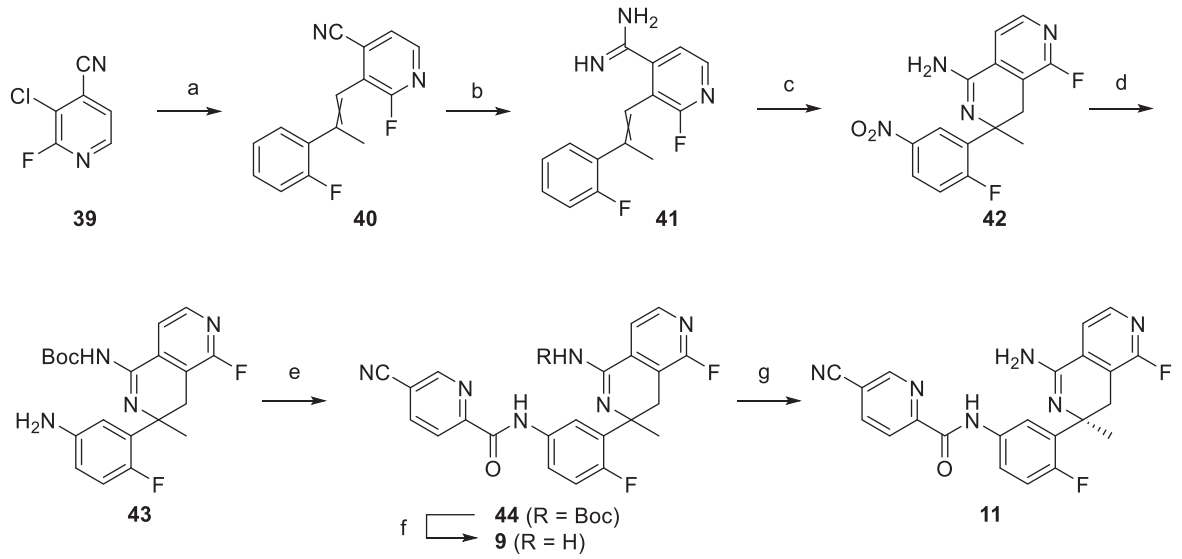
J = 15.9 Hz), 3.30 (1H, d, J = 15.9 Hz), 7.15 (1H, dd, J = 10.9, 8.9 Hz), 7.31 (1H, d, J = 5.0 Hz), 8.10 (1H, ddd, J = 8.9, 4.0, 3.0 Hz), 8.55 (1H, s), 8.63 (1H, d, J = 5.0 Hz), 8.68 (1H, dd, J = 7.0, 3.0 Hz). MS-ESI (m/z): 301 $[M + H]^+$.

***tert*-Butyl (6-(5-(5-cyanopicolinamido)-2-fluorophenyl)-6-methyl-5,6-dihydro-1,7-naphthyridin-8-yl)carbamate (18)**

After a mixture of **17** (54.9 mg, 0.183 mmol) and Boc_2O (0.042 mL, 0.183 mmol) in THF (0.83 mL) was stirred at rt for 15 h, MeOH (0.6 mL), H_2O (0.2 mL), Fe (57.2 mg, 2.90 mmol), and NH_4Cl (44.0 mg, 0.823 mmol) were added. The mixture was stirred at 70 °C for 90 min, and cooled to rt, and filtered through a Celite pad, and then the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to give the residue as a brown amorphous (64.5 mg). To the residue (64.5 mg) in THF (1.3 mL) were added 5-cyanopicolinic acid hydrate (35.0 mg, 0.211 mmol), HATU (87.0 mg, 0.228 mmol), and Et_3N (0.063 mL, 0.456 mmol) at 0 °C. The mixture was stirred at rt for 90 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column



Scheme 5. Synthesis of Compound **8**. Reagents and conditions: (a) **14**, K_2CO_3, $PdCl_2(dppf)•CH_2Cl_2$, THF-H_2O, 70 °C, 81%, dr = 3:2; (d) trimethylaluminum, NH_4Cl, toluene, 100 °C, 99%, dr = 3:2; (e) conc. H_2SO_4, rt; then HNO_3, 0 °C, 81%; (f) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C, 92% for 2 steps; (g) 5-cyano-2-pyridinecarboxylic acid, HATU, Et_3N, THF, rt, 96%; (h) formic acid, rt, 78%.



Scheme 6. Synthesis of Compounds **9** and **11**. Reagents and conditions: (a) **14**, K_2CO_3, $PdCl_2(dppf)•CH_2Cl_2$, 1,4-dioxane-H_2O, 100 °C, 32%, dr = 1:1; (b) trimethylaluminum, NH_4Cl, toluene, 100 °C, 39%; (c) conc. H_2SO_4, rt; then HNO_3, 0 °C, 81% for 2 steps; (d) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C, 70% for 2 steps; (e) 5-cyano-2-pyridinecarboxylic acid, HATU, Et_3N, THF, rt, 80%; (f) formic acid, rt or HCl in EtOAc, rt. (g) chiral separation by SFC, 29% for 2 steps.

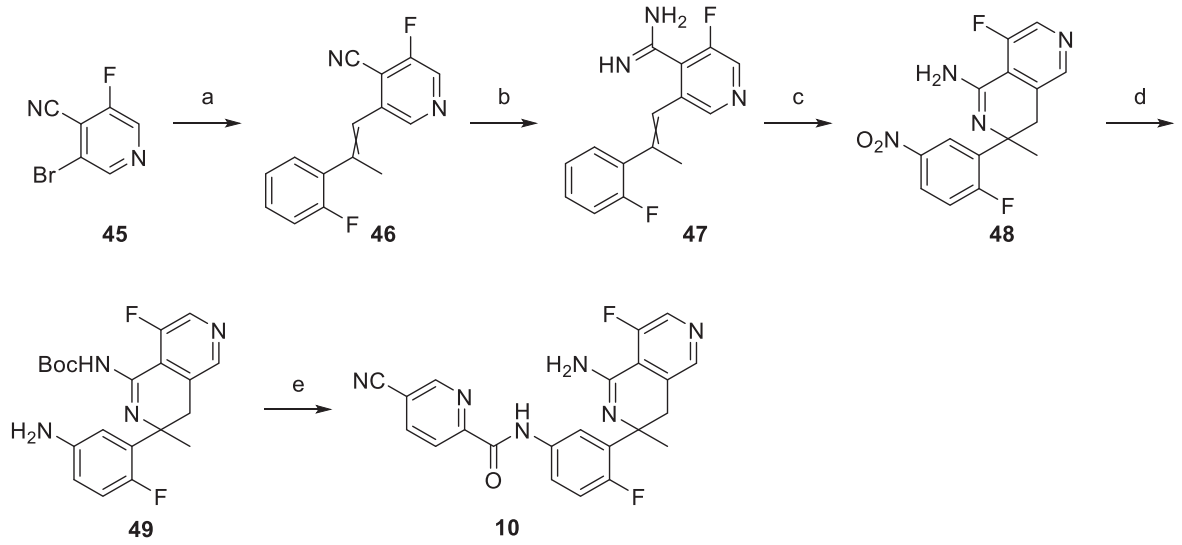
chromatography (silica gel; EtOAc/hexane, gradient: 50–75% EtOAc) to give **18** (62.5 mg, 71% for 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s), 1.89 (3H, s), 3.27 (1H, d, *J* = 15.9 Hz), 3.77 (1H, d, *J* = 15.9 Hz), 7.07 (1H, dd, *J* = 11.6, 8.9 Hz), 7.39 (1H, dd, *J* = 7.2, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, *J* = 5.0 Hz), 8.19 (1H, dd, *J* = 8.2, 2.0 Hz), 8.37 (1H, dd, *J* = 8.2, 0.8 Hz), 8.50 (1H, s), 8.54 (1H, d, *J* = 5.0 Hz), 8.88 (1H, dd, *J* = 2.0, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (*m/z*): 501 $[M + H]^+$.

N-(3-(8-Amino-6-methyl-5,6-dihydro-1,7-naphthyridin-6-yl)-4-fluorophenyl)-5-cyanopicolinamide (4)

A mixture of **18** (44.5 mg, 0.089 mmol) and formic acid (0.50 mL, 13.0 mmol) was stirred at rt for 14 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH). The crude product was recrystallized from hexane/EtOAc to give **4** (20.9 mg, 59%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.75 (3H, s), 3.27 (1H, d, *J* = 15.7 Hz), 3.63 (1H, d, *J* = 15.7 Hz), 7.08 (1H, dd, *J* = 12.2, 8.6 Hz), 7.39 (1H, dd, *J* = 7.6, 5.1 Hz), 7.67 (1H, d, *J* = 7.6 Hz), 7.72–7.78 (2H, m), 8.34 (1H, d, *J* = 8.1 Hz), 8.41–8.47 (2H, m), 9.04–9.05 (1H, m). MS-ESI (*m/z*): 401 $[M + H]^+$.

4-(2-(2-Fluorophenyl)prop-1-en-1-yl)nicotinamide (19)

To a mixture of *tert*-BuONa (4.49 g, 46.7 mmol) in DMF (18 mL) was added dropwise 3-cyano-4-methylpyridine (3.68 g, 31.2 mmol)



Scheme 7. Synthesis of Compound **10**. Reagents and conditions: (a) **14**, K_2CO_3, $PdCl_2(dppf)\bullet CH_2Cl_2$, THF-$H_2O$, 70 °C, 80%, dr = 3:2; (b) trimethylaluminum, NH_4Cl, toluene, 100 °C, 82%; (c) conc. H_2SO_4, rt; then HNO_3, 0 °C, 86%; (d) (i) Boc_2O, THF, rt; (ii) Fe, NH_4Cl, THF-MeOH-H_2O, 70 °C, 88% for 2 steps; (g) (i) 5-cyano-2-pyridinecarboxylic acid, HATU, Et_3N, THF, rt; (ii) formic acid, rt, 62% for 2 steps.

at 0 °C. After the mixture was stirred at 0 °C for 1 h, 1-(2-fluorophenyl)ethanone (4.03 mL, 32.7 mmol) was added dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 7 h and then rt for 15 h. The reaction was quenched with sat NH_4Cl at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH). The crude product was recrystallized from EtOAc/hexane to give a 1:1 diastereomeric mixture of **19** (5.27 g, 66%) as a brown gum. ^{1}H NMR (400 MHz, CDCl3) δ 2.19 (3H, t, J = 2.2 Hz, *major isomer*), 2.29 (3H, d, J = 1.5 Hz, *minor isomer*), 7.07–7.41 (6H, m), 8.27 (1H, *d*, J = 5.2 Hz, *minor isomer*), 8.70 (1H, d, J = 5.0 Hz, *major isomer*), 8.81 (1H, s, *minor isomer*), 9.04 (1H, s, *major isomer*). MS-ESI (*m/z*): 257 [M + H]+.

4-(2-(2-Fluorophenyl)prop-1-en-1-yl)nicotinimidamide (21)

To a solution of **19** (105 mg, 0.411 mmol) in 1,4-dioxane (1 mL) were added pyridine (0.073 mL, 0.904 mmol) and trifluoroacetic anhydride (0.064 mL, 0.452 mmol) at 0 °C. The mixture was stirred at rt for 3.5 h. The reaction was quenched with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with 10% citric acid, H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to give **20** as a brown oil (103 mg). To a mixture of NH_4Cl (279 mg, 5.21 mmol) in toluene (0.35 mL) was added dropwise trimethylaluminum (2.61 mL, 5.21 mmol) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, 20 (35.5 mg, 0.149 mmol) in toluene (0.7 mL) was added. The mixture was stirred at 100 °C for 27 h. The reaction was quenched with potassium sodium tartrate and NaOH (2 M aqueous solution) at 0 °C. The mixture was stirred at rt for 30 min. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–10% MeOH) to give a 3:2 diastereomeric mixture of **21** (28.1 mg, 74% for 2 steps) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, J = 1.3 Hz, 3H, *minor isomer*), 2.26 (d, J = 1.5 Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 8.01 (s, 1H, *major isomer*), 8.71 (s, 1H, *minor isomer)*, 8.35 (d, J = 5.0 Hz, 1H, *major isomer*), 8.58 (d, J = 4.9 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 256 $[M + H]^+$.

4-(2-(2-Fluorophenyl)prop-1-en-1-yl)nicotinonitrile (22)

After a mixture of **21** (25.5 mg, 0.100 mmol) and conc. H_2SO_4 (0.50 mL, 9.00 mmol) was stirred at rt for 19 h, HNO_3 (8.93 μL, 0.20 mmol) was added at 0 °C, and the mixture was then stirred at 0 °C for 1 h. The reaction was quenched with NaOH (2 M aqueous solution) and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to give the residue as a white solid (21.5 mg). After a mixture of the residue (21.3 mg, 0.071 mmol) and Boc_2O (0.016 mL, 0.071 mmol) in THF (0.4 mL) was stirred at rt for 17 h, the mixture was evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give **22** (28.2 mg, 70% for 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (t, J = 1.5 Hz, 3H, *minor isomer*), 2.45 (d, J = 1.5 Hz, 3H, *major isomer*), 6.23–6.26 (1H, m), 6.87–7.40 (5H, m), 8.15 (s, 1H, *major isomer*), 8.88 (s, 1H, *minor isomer)*, 8.35 (d, J = 5.0 Hz, 1H, *major isomer*), 8.58 (d, J = 5.0 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 401 $[M + H]^+$.

***tert*-Butyl (3-(5-(5-cyanopicolinamido)-2-fluorophenyl)-3-methyl-3,4-dihydro-2,7-naphthyridin-1-yl)carbamate (23)**

A mixture of **22** (34.0 mg, 0.085 mmol), Fe (26.6 mg, 0.476 mmol), and NH_4Cl (20.4 mg, 0.382 mmol) in THF (0.51 mL), MeOH (0.34 mL), and H_2O (0.14 mL) was stirred at 70 °C for 2 h. After cooling, the mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and aqueous saturated $NaHCO_3$. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to give a white solid (30.9 mg). To the residue in EtOAc (0.6 mL) were added 5-cyanopicolinic acid hydrate (15.0 mg, 0.090 mmol), diphenyl phosphorochloridate (0.020 mL, 0.098 mmol), and Et_3N (0.057 mL, 0.410 mmol) at 0 °C. The mixture was stirred at the same temperature for 90 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50–75% EtOAc) to give **23** (31.5 mg, 77% for 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63

(9H, s), 1.89 (3H, s), 3.27 (1H, d, $J = 15.9$ Hz), 3.77 (1H, d, $J = 15.9$ Hz), 7.07 (1H, dd, $J = 11.6$, 8.9 Hz), 7.39 (1H, dd, $J = 7.2$, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, $J = 5.0$ Hz), 8.19 (1H, dd, $J = 8.2$, 2.0 Hz), 8.37 (1H, dd, $J = 8.2$, 0.8 Hz), 8.50 (1H, s), 8.54 (1H, d, $J = 5.0$ Hz), 8.88 (1H, dd, $J = 2.0$, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (*m/z*): 501 $[M + H]^+$.

N-(3-(1-Amino-3-methyl-3,4-dihydro-2,7-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (5)

A mixture of **23** (31.2 mg, 0.062 mmol) and TFA (0.144 mL, 1.870 mmol) in CH_2Cl_2 (0.6 mL) was stirred at rt for 50 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH). The crude product was recrystallized from hexane/EtOAc/MeOH to give **5** (16.7 mg, 67%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (3H, s), 3.09 (1H, d, $J = 15.2$ Hz), 3.18 (1H, d, $J = 15.2$ Hz), 6.36 (2H, s), 7.11 (1H, dd, $J = 11.9$, 8.9 Hz), 7.29 (1H, d, $J = 4.6$ Hz), 7.68–7.71 (1H, m), 7.98–7.99 (1H, m), 8.26 (1H, d, $J = 8.1$ Hz), 8.47 (1H, d, $J = 4.6$ Hz), 8.57 (1H, dd, $J = 8.1$. 2.0 Hz), 8.82 (1H, s), 9.19 (1H, d, $J = 1.5$ Hz), 10.69 (1H, s). MS-ESI (*m/z*): 401 $[M + H]^+$.

3-(2-(2-Fluorophenyl)prop-1-en-1-yl)isonicotinonitrile (24)

A mixture of **14** (267 mg, 1.02 mmol), 3-bromo-4-cyanopyridine (205 mg, 1.12 mmol), K_2CO_3 (2 M aqueous solution; 1.53 mL, 3.06 mmol), and $PdCl_2$(dppf)•CH_2Cl_2 (41.6 mg, 0.051 mmol) in THF (8 mL) was stirred at 70 °C for 14 h under N_2. The resulting mixture was filtered through a Celite pad, and then the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 10–20% EtOAc) to give a 3:2 diastereomeric mixture of **24** (203 mg, 84%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (t, $J = 1.2$ Hz, 3H, *major isomer*) and 2.32 (d, $J = 1.5$ Hz, 3H, *minor isomer*), 6.75 (1H, br s), 6.97–7.56 (5H, m), 8.17 (s, *major isomer*) and 8.88 (s, *minor isomer*), 8.42 (d, $J = 5.0$ Hz, 1H, *major isomer*), 8.66 (d, $J = 5.0$ Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 239 $[M + H]^+$.

3-(2-(2-Fluorophenyl)prop-1-en-1-yl)isonicotinimidamide (25)

To a mixture of NH_4Cl (1.44 g, 27.0 mmol) in toluene (2 mL) was added dropwise trimethylaluminum (13.5 mL, 27.0 mmol) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, **24** (184 mg, 0.771 mmol) in toluene (4 mL) was added. The mixture was stirred at 100 °C for 26 h. The reaction was quenched with potassium sodium tartrate and NaOH (2 M aqueous solution) at 0 °C, which was then stirred at rt for 10 min. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give a 3:2 diastereomeric mixture of **25** (190 mg, 97%) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, $J = 1.3$ Hz, 3H, *minor isomer*), 2.26 (d, $J = 1.5$ Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 7.99 (s, 1H, *major isomer*), 8.33 (s, 1H, *minor isomer*), 8.45 (d, $J = 5.0$ Hz, 1H, *major isomer*), 8.99 (d, $J = 5.0$ Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 256 $[M + H]^+$.

3-(2-Fluoro-5-nitrophenyl)-3-methyl-3,4-dihydro-2,6-naphthyridin-1-amine (26)

After a mixture of **25** (179 mg, 0.699 mmol) and conc. H_2SO_4 (1.00 mL, 18.0 mmol) was stirred at rt for 15 h, to this mixture was added HNO_3 (0.062 mL, 1.40 mmol) at 0 °C, which was then stirred at the same temperature for 1 h. The reaction was quenched with NaOH (2 M aqueous solution) and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **26** (181 mg, 86%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (3H, s), 3.18 (1H, d, $J = 15.9$ Hz), 3.30 (1H, d, $J = 15.9$ Hz), 7.15 (1H, dd, $J = 10.9$, 8.9 Hz), 7.31 (1H, d, $J = 5.0$ Hz), 8.10 (1H, ddd, $J = 8.9$, 4.0, 3.0 Hz), 8.55 (1H, s), 8.63 (1H, d, $J = 5.0$ Hz), 8.68 (1H, dd, $J = 7.0$, 3.0 Hz). MS-ESI (*m/z*): 301 $[M + H]^+$.

***tert*-Butyl (3-(5-(5-cyanopicolinamido)-2-fluorophenyl)-3-methyl-3,4-dihydro-2,6-naphthyridin-1-yl)carbamate (27)**

After a mixture of **26** (156 mg, 0.519 mmol) and Boc_2O (0.120 mL, 0.519 mmol) in THF (2.3 mL) was stirred at rt for 15 h, MeOH (1.5 mL), H_2O (0.6 mL), Fe (162 mg, 2.90 mmol), and NH_4Cl (125 mg, 2.33 mmol) were added at rt. The mixture was stirred at 70 °C for 90 min and then filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give a residue as a yellow solid (228 mg). To the residue (68.8 mg) in THF (1.4 mL) were added 5-cyanopicolinic acid hydrate (37.0 mg, 0.223 mmol), HATU (92.0 mg, 0.241 mmol), and Et_3N (0.067 mL, 0.483 mmol) at 0 °C. The mixture was stirred at rt for 90 min and quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50–75% EtOAc) to give **27** (90.3 mg, 97% for 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (9H, s), 1.99 (3H, s), 3.26 (1H, d, $J = 152$ Hz), 3.78(1H, d, $J = 15.2$ Hz), 7.11 (1H, dd, $J = 11.6$, 8.9 Hz), 7.50 (1H, dd, $J = 7.2$, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, $J = 5.0$ Hz), 8.33 (1H, dd, $J = 8.2$, 2.0 Hz), 8.37 (1H, dd, $J = 8.5$, 1.0 Hz), 8.50 (1H, s), 8.54 (1H, d, $J = 5.0$ Hz), 8.88 (1H, dd, $J = 2.0$, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (*m/z*): 501 $[M + H]^+$.

N-(3-(1-Amino-3-methyl-3,4-dihydro-2,6-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (6)

A mixture of **27** (64.3 mg, 0.128 mmol) and formic acid (0.50 mL, 13.0 mmol) was stirred at rt for 14 h. The reaction was quenched with NaOH (2 M aqueous solution) and saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH). The crude product was recrystallized from hexane/EtOAc/MeOH to give **6** (26.5 mg, 52%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) d 1.75 (3H, s), 3.13 (1H, d, $J = 16.2$ Hz), 3.62 (1H, d, $J = 16.2$ Hz), 7.08 (1H, dd, $J = 11.9$, 8.9 Hz), 7.64–7.70 (2H, m), 7.77 (1H, dd, $J = 7.4$, 2.8 Hz), 8.34 (1H, dd, $J = 8.1$, 1.0 Hz), 8.42 (1H, dd, $J = 8.1$, 2.0 Hz), 8.46 (1H, s), 8.50 (1H, d, $J = 5.1$ Hz), 9.04 (1H, d, $J = 1.5$ Hz). MS-ESI (*m/z*): 401 $[M + H]^+$.

2-(2-(2-Fluorophenyl)prop-1-en-1-yl)nicotinonitrile (28)

A mixture of **14** (172 mg, 0.656 mmol), 2-chloro-3-cyanopyridine (109 mg, 0.787 mmol), K_2CO_3 (2 M aqueous solution; 0.984 mL, 1.97 mmol), and $PdCl_2$(dppf)•CH_2Cl_2 (26.8 mg, 0.033 mmol) in 1,4-dioxane (5 mL) was stirred at 100 °C for 4.5 h under N_2. The resulting mixture was filtered through a Celite pad. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over

Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–10% EtOAc) to give a 3:2 diastereomeric mixture of **28** (203 mg, 84%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (t, J = 1.2 Hz, 3H, *major isomer*) and 2.32 (d, J = 1.5 Hz, 3H, *minor isomer*), 6.75 (1H, br s), 6.97–7.56 (5H, m), 8.17 (s, *major isomer*) and 8.88 (s, *minor isomer*), 8.42 (d, J = 5.0 Hz, 1H, *major isomer*), 8.66 (d, J = 5.0 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 239 $[M + H]^+$.

2-(2-(2-Fluorophenyl)prop-1-en-1-yl)nicotinimidamide (29)

To a suspension of NH_4Cl (411 mg, 7.68 mmol) in toluene (0.5 mL) was added dropwise trimethylaluminum (3.84 mL, 7.68 mmol) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, 28 (52.3 mg, 0.220 mmol) in toluene (1 mL) was added. The mixture was stirred at 100 °C for 48 h. The reaction was quenched with potassium sodium tartrate and NaOH (2 M aqueous solution) at 0 °C. The mixture was stirred at rt for 1 h, and the aqueous layer was then separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–10% MeOH) to give a 3:2 diastereomeric mixture of **29** (21.7 mg, 39%) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, J = 1.3 Hz, 3H, *minor isomer*), 2.26 (d, J = 1.5 Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 8.01 (s, 1H, *major isomer*), 8.71 (s, 1H, *minor isomer)*, 8.35 (d, J = 5.0 Hz, 1H, *major isomer*) and 8.58 (d, J = 4.9 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 256 $[M + H]^+$.

7-(2-Fluoro-5-nitrophenyl)-7-methyl-7,8-dihydro-1,6-naphthyridin-5-amine (30)

After a mixture of **29** (179 mg, 0.699 mmol) and conc. H_2SO_4 (0.50 mL, 9.00 mmol) was stirred at rt for 14 h, HNO_3 (7.5 μL, 0.168 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction was quenched with NaOH (2 M solution) and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated to give **30** (181 mg) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s), 1.89 (3H, s), 3.27 (1H, d, J = 15.9 Hz), 3.77 (1H, d, J = 15.9 Hz), 7.07 (1H, dd, J = 11.6, 8.9 Hz), 7.39 (1H, dd, J = 7.2, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, J = 5.0 Hz), 8.19 (1H, dd, J = 8.2, 2.0 Hz), 8.37 (1H, dd, J = 8.2, 0.8 Hz), 8.50 (1H, s), 8.54 (1H, d, J = 5.0 Hz), 8.88 (1H, dd, J = 2.0, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (*m/z*): 301 $[M + H]^+$.

***tert*-Butyl (7-(5-amino-2-fluorophenyl)-7-methyl-7,8-dihydro-1,6-naphthyridin-5-yl)carbamate (31)**

After a mixture of **30** (20.5 mg, 0.068 mmol) and Boc_2O (0.016 mL, 0.068 mmol) in THF (0.6 mL) was stirred at rt for 13 h, MeOH (0.4 mL), H_2O (0.16 mL), Fe (21.4 mg, 0.382 mmol), and NH_4Cl (16.4 mg, 0.307 mmol) were added at rt. The mixture was stirred at 70 °C for 2 h and filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give a 3:2 diastereomeric mixture of **31** (21.7 mg, 39%) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, J = 1.3 Hz, 3H, *minor isomer*), 2.26 (d, J = 1.5 Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 8.01 (s, 1H, *major isomer*), 8.71 (s, 1H, *minor isomer)*, 8.35 (d, J = 5.0 Hz, 1H, *major isomer*) and 8.58 (d, J = 4.9 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 371 $[M + H]^+$.

***tert*-Butyl (7-(5-(5-cyanopicolinamido)-2-fluorophenyl)-7-methyl-7,8-dihydro-1,6-naphthyridin-5-yl)carbamate (32)**

To a solution of **31** (19.9 mg) in THF (0.6 mL) were added 5-cyanopicolinic acid hydrate (10.7 mg, 0.064 mmol), HATU (26.6 mg, 0.070 mmol), and Et_3N (0.019 mL, 0.140 mmol) at 0 °C. The mixture was stirred at rt for 1.5 h and then quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50–75% EtOAc) to give **32** (21.4 mg, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s), 1.89 (3H, s), 3.27 (1H, d, J = 15.9 Hz), 3.77 (1H, d, J = 15.9 Hz), 7.07 (1H, dd, J = 11.6, 8.9 Hz), 7.39 (1H, dd, J = 7.2, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, J = 5.0 Hz), 8.19 (1H, dd, J = 8.2, 2.0 Hz), 8.37 (1H, dd, J = 8.2, 0.8 Hz), 8.50 (1H, s), 8.54 (1H, d, J = 5.0 Hz), 8.88 (1H, dd, J = 2.0, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (*m/z*): 501 $[M + H]^+$.

N-(3-(5-Amino-7-methyl-7,8-dihydro-1,6-naphthyridin-7-yl)-4-fluorophenyl)-5-cyanopicolinamide (7)

A mixture of **32** (21.1 mg, 0.042 mmol) and formic acid (0.50 mL, 13.0 mmol) was stirred at rt for 14 h. The reaction was quenched with NaOH (2 M aqueous solution) and saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH). The crude product was recrystallized from hexane/CH_2Cl_2 to give **7** (12.3 mg, 73%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (3H, s), 3.22 (1H, d, J = 15.2 Hz), 6.33 (2H, brs), 7.12 (1H, dd, J = 11.9, 8.9 Hz), 7.31 (1H, dd, J = 7.6, 5.1 Hz), 7.67–7.71 (1H, m), 7.97–8.05 (2H, br m), 8.26 (1H, d, J = 8.1 Hz), 8.45 (1H, dd, J = 4.6, 1.5 Hz), 8.57 (1H, dd, J = 8.1, 2.0 Hz), 9.18–9.19 (1H, m), 10.69 (1H, s). MS-ESI (*m/z*): 401 $[M + H]^+$.

5-Fluoro-4-(2-(2-fluorophenyl)prop-1-en-1-yl)nicotinonitrile (34)

A mixture of **14** (223 mg, 0.852 mmol), 4-chloro-3-cyano-5-fluoropyridine (33, 209 mg, 1.33 mmol), K_2CO_3 (2 M aqueous solution; 2.00 mL, 4.00 mmol), and $PdCl_2(dppf)•CH_2Cl_2$ (54.4 mg, 0.067 mmol) in THF (11 mL) was stirred at 70 °C for 16 h under N_2. The resulting mixture was filtered through a Celite pad. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–10% EtOAc) to give a 3:2 diastereomeric mixture of **34** (277 mg, 81%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (t, J = 1.2 Hz, 3H, *major isomer*) and 2.32 (d, J = 1.5 Hz, 3H, *minor isomer*), 6.75 (1H, br s), 6.97–7.56 (5H, m), 8.17 (s, *major isomer*) and 8.88 (s, *minor isomer*), 8.42 (d, J = 5.0 Hz, 1H, *major isomer*), 8.66 (d, J = 5.0 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 257 $[M + H]^+$.

5-Fluoro-4-(2-(2-fluorophenyl)prop-1-en-1-yl)nicotinimidamide (35)

To a suspension of NH_4Cl (1.88 g, 35.1 mmol) in toluene (2.5 mL) was added dropwise trimethylaluminum (15.5 mL, 35.1 mmol) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, 34 (257 mg, 1.00 mmol) in toluene (5 mL) was added. The mixture was stirred at 100 °C for 21 h and then quenched with potassium sodium tartrate and NaOH (2 M solution) at 0 °C. The mixture was stirred at rt for 10 min. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–10% MeOH) to give a 3:2 diastereomeric mixture of **35** (270 mg, 99%) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (t, J = 1.3 Hz, 3H, *minor isomer*), 2.26 (d, J = 1.5 Hz, 3H, *major isomer*), 6.65–6.66 (1H, m), 6.87–7.40 (5H, m), 8.01 (s, 1H, *major isomer*), 8.71 (s, 1H, *minor isomer)*, 8.35 (d, J = 5.0 Hz, 1H, *major isomer*) and 8.58 (d, J = 4.9 Hz, 1H, *minor isomer*). MS-ESI (*m/z*): 274 $[M + H]^+$.

5-Fluoro-3-(2-fluoro-5-nitrophenyl)-3-methyl-3,4-dihydro-2,7-naphthyridin-1-amine (36)

After a mixture of **35** (234 mg, 0.857 mmol) and conc. H_2SO_4 (1.00 mL, 18.0 mmol) was stirred at rt for 13 h, HNO_3 (0.077 mL, 1.71 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1.5 h, then quenched with NaOH (2 M aqueous solution), and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–10% MeOH) to give **36** (220 mg, 81%) as a brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) d 1.53 (3H, s), 3.18 (1H, d, J = 15.9 Hz), 3.30 (1H, d, J = 15.9 Hz), 7.15 (1H, dd, J = 10.9, 8.9 Hz), 7.31 (1H, d, J = 5.0 Hz), 8.10 (1H, ddd, J = 8.9, 4.0, 3.0 Hz), 8.55 (1H, s), 8.63 (1H, d, J = 5.0 Hz), 8.68 (1H, dd, J = 7.0, 3.0 Hz). MS-ESI (m/z): 319 $[M + H]^+$.

***tert*-Butyl (3-(5-amino-2-fluorophenyl)-5-fluoro-3-methyl-3,4-dihydro-2,7-naphthyridin-1-yl)carbamate (37)**

After a mixture of **36** (174 mg, 0.549 mmol) and Boc_2O (0.127 mL, 0.549 mmol) in THF (2.6 mL) was stirred at rt for 16 h, MeOH (1.8 mL), H_2O (0.7 mL), Fe (172 mg, 3.07 mmol), and NH_4Cl (132 mg, 2.47 mmol) were added at rt. The mixture was stirred at 70 °C for 1.5 h and then filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 33–50% MeOH) to give **37** (196 mg, 92%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (3H, s), 3.18 (1H, d, J = 15.9 Hz), 3.30 (1H, d, J = 15.9 Hz), 7.15 (1H, dd, J = 10.9, 8.9 Hz), 7.31 (1H, d, J = 5.0 Hz), 8.10 (1H, ddd, J = 8.9, 4.0, 3.0 Hz), 8.55 (1H, s), 8.63 (1H, d, J = 5.0 Hz), 8.68 (1H, dd, J = 7.0, 3.0 Hz). MS-ESI (m/z): 389 $[M + H]^+$.

***tert*-Butyl (3-(5-(5-cyanopicolinamido)-2-fluorophenyl)-5-fluoro-3-methyl-3,4-dihydro-2,7-naphthyridin-1-yl)carbamate (38)**

To a solution of **37** (80.9 mg, 0.208 mmol) in THF (1.6 mL) were added 5-cyanopicolinic acid hydrate (41.5 mg, 0.250 mmol), HATU (103 mg, 0.271 mmol), and Et_3N (0.075 mL, 0.542 mmol) at 0 °C. The mixture was stirred at rt for 2 h and then quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 25–40% EtOAc) to give **38** (104 mg, 96%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (9H, s), 1.89 (3H, s), 3.27 (1H, d, J = 15.9 Hz), 3.77 (1H, d, J = 15.9 Hz), 7.07 (1H, dd, J = 11.6, 8.9 Hz), 7.39 (1H, dd, J = 7.2, 2.5 Hz), 7.73–7.79 (1H, m), 8.02 (1H, d, J = 5.0 Hz), 8.19 (1H, dd, J = 8.2, 2.0 Hz), 8.37 (1H, dd, J = 8.2, 0.8 Hz), 8.50 (1H, s), 8.54 (1H, d, J = 5.0 Hz), 8.88 (1H, dd, J = 2.0, 0.8 Hz), 9.70 (1H, s), 10.72 (1H, s). MS-ESI (m/z): 519 $[M + H]^+$.

N-(3-(1-Amino-5-fluoro-3-methyl-3,4-dihydro-2,7-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (8)

A mixture of **38** (65.0 mg, 0.125 mmol) and formic acid (0.65 mL, 17.0 mmol) was stirred at rt for 2.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc and then $CHCl_3$/MeOH. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–3% MeOH). The crude product was recrystallized from hexane/EtOAc/MeOH to give **8** (43.8 mg, 84%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.75 (3H, s), 3.02 (1H, d, J = 16.2 Hz), 3.78 (1H, d, J = 16.2 Hz), 7.08 (1H, dd, J = 11.7, 8.6 Hz), 7.64–7.68 (1H, m), 7.78 (1H, dd, J = 7.4, 2.8 Hz), 8.33 (1H, dd, J = 8.1, 1.0 Hz), 8.41 (1H, dd, J = 8.1, 2.0 Hz), 8.45 (1H, s), 9.03–9.04 (1H, m). MS-ESI (m/z): 419 $[M + H]^+$.

2-Fluoro-3-(2-(2-fluorophenyl)prop-1-en-1-yl)isonicotinonitrile (40)

A mixture of **14** (365 mg, 1.39 mmol), 2-chloro-3-fluorobenzonitrile (218 mg, 1.39 mmol), K_2CO_3 (2 M in H_2O; 2.09 mL, 4.18 mmol), and $PdCl_2$(dppf)•CH_2Cl_2 (56.9 mg, 0.070 mmol) in 1,4-dioxane (11 mL) was stirred at 100 °C for 18 h under N_2. The resulting mixture was filtered through a Celite pad, and the aqueous layer was then separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–10% EtOAc) to give a 1:1 diastereomeric mixture of **40** (174 mg, 49%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (d J = 1.3 Hz, 3H, *one isomer*) and 2.36 (d, J = 1.5 Hz, 3H, *the other isomer*), 6.52 (1H, s, *one isomer*), 6.56 (1H, s, *the other isomer*), 6.91–7.54 (5H, m), 8.12 (d, J = 4.9 Hz, 1H, *one isomer*), 8.28 (d, J = 5.0 Hz, 1H, *one isomer*), 8.31 (d, J = 5.0 Hz, 1H, *the other isomer*). MS-ESI (m/z): 257 $[M + H]^+$.

2-Fluoro-3-(2-(2-fluorophenyl)prop-1-en-1-yl)isonicotinimidamide (41)

To a suspension of NH_4Cl (1.16 g, 21.7 mmol) in toluene (1.6 mL) was added dropwise trimethylaluminum (10.8 mL, 21.7 mmol, 2 M in toluene) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, **40** (159 mg, 0.621 mmol) in toluene (3.2 mL) was added. The mixture was stirred at 100 °C for 23 h and then quenched with potassium sodium tartrate and 2 M aqueous NaOH solution at 0 °C. The mixture was stirred at rt for 50 min. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give a 3:2 diastereomeric mixture of **41** (98.3 mg, 58%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (t, J = 1.4 Hz, 3H, *minor isomer*), 2.26 (d, J = 0.9 Hz, 3H, *major isomer*), 6.47 (1H, s), 6.86–7.45 (5H, m), 8.03 (d, J = 5.0 Hz, 1H, *major isomer*), 8.23 (d, J = 5.0 Hz, 1H, *minor isomer*). MS-ESI (m/z): 274 $[M + H]^+$.

5-Fluoro-3-(2-fluoro-5-nitrophenyl)-3-methyl-3,4-dihydro-2,6-naphthyridin-1-amine (42)

After a mixture of **41** (82.9 mg, 0.303 mmol) and conc. H_2SO_4 (0.50 mL, 9.00 mmol) was stirred at rt for 15 h, HNO_3 (0.027 mL, 0.607 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1 h and then quenched with 2 M aqueous NaOH solution and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give **42** (71.6 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (3H, s), 3.11 (1H, d, J = 16.5 Hz), 3.37 (1H, d, J = 16.5 Hz), 4.93 (1H, brs), 7.18 (1H, dd, J = 10.8, 9.0 Hz), 8.13 (1H, dt, J = 8.8, 3.2 Hz), 8.23 (1H, d, J = 5.0 Hz), 8.70 (1H, d, J = 4.3 Hz). MS-ESI (m/z): 319 $[M + H]^+$.

***tert*-Butyl (3-(5-amino-2-fluorophenyl)-5-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-1-yl)carbamate (43)**

After a mixture of **42** (58.6 mg, 0.184 mmol) and Boc_2O (0.043 mL, 0.184 mmol) in THF (0.9 mL) was stirred at rt for 16 h, MeOH (0.6 mL), H_2O (0.2 mL), Fe (57.6 mg, 1.03 mmol), and NH_4Cl (44.3 mg, 0.829 mmol) were added at rt. The mixture was stirred at 70 °C for 1.5 h and filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and

extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 10–33% EtOAc) to give **43** (68.5 mg, 96%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (3H, s), 1.84 (3H, s), 3.04 (1H, d, J = 16.7 Hz), 3.50 (1H, brs), 3.84 (1H, d, J = 16.7 Hz), 6.32 (1H, dd, J = 6.8, 2.8 Hz), 6.46 (1H, td, J = 5.8, 3.0 Hz), 6.82 (1H, dd, J = 11.7, 8.6 Hz), 7.94 (2H, d, J = 5.6 Hz), 8.12 (2H, d, J = 5.6 Hz), 10.57 (1H, s). MS-ESI (m/z): 389 $[M + H]^+$.

***tert*-Butyl (3-(5-(5-cyanopicolinamido)-2-fluorophenyl)-5-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-1-yl)carbamate (44)**

To a solution of **43** (49.2 mg, 0.127 mmol) in THF (1.0 mL) at 0 °C were added 5-cyanopicolinic acid hydrate (25.3 mg, 0.152 mmol), HATU (62.6 mg, 0.165 mmol), and Et_3N (0.046 mL, 0.329 mmol). The mixture was stirred at rt for 1.5 h and then quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 25–40% EtOAc) to give **44** (65.7 mg, 100%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (9H, s), 1.91 (3H, s), 3.13 (1H, d, J = 16.8 Hz), 3.93 (1H, d, J = 16.8 Hz), 7.09 (1H, dd, J = 11.5, 8.9 Hz), 7.42 (1H, dd, J = 7.2, 2.6 Hz), 7.72–7.78 (1H, m), 7.96 (1H, d, J = 5.2 Hz), 8.12 (1H, d, J = 5.2 Hz), 8.19 (1H, dd, J = 8.2, 2.1 Hz), 8.36 (1H, d, J = 8.1 Hz), 8.88 (1H, d, J = 1.7 Hz), 9.71 (1H, s), 10.71 (1H, s). MS-ESI (m/z): 519 $[M + H]^+$.

N-(3-(1-Amino-5-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (9)

A mixture of **44** (51.2 mg, 0.099 mmol) and formic acid (0.50 mL, 13.0 mmol) was stirred at rt for 14 h. The reaction was quenched with NaOH (2 M aqueous solution) and saturated aqueous $NaHCO_3$ solution at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–3% MeOH). The crude product was recrystallized from hexane/EtOAc to give **9** (21.3 mg, 52%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) d 1.74 (3H, s), 3.01 (1H, d, J = 16.2 Hz), 3.66 (1H, d, J = 16.2 Hz), 7.09 (1H, dd, J = 11.9, 8.9 Hz), 7.64–7.67 (1H, m), 7.78 (1H, dd, J = 7.4, 2.8 Hz), 8.13 (1H, d, J = 5.1 Hz), 8.41 (1H, dd, J = 8.1, 2.0 Hz), 9.03–9.04 (1H, m). MS-ESI (m/z): 419 $[M + H]^+$.

(S)–N-(3-(1-Amino-8-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (11)

A mixture of **44** (2.00 g, 3.85 mmol) and HCl (EtOAc solution, 50 mL) was stirred at overnight. The reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by SFC to give **9** (445 mg, 29%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.72 (3H, s), 3.10 (1H, d, J = 16.1 Hz), 3.60 (1H, d, J = 16.1 Hz), 7.08 (1H, dd, J = 11.8, 8.8 Hz), 7.66–7.71 (1H, m), 7.80 (1H, dd, J = 7.4, 2.7 Hz), 8.33–8.36 (2H, m), 8.41–8.44 (2H, m), 9.04–9.05 (1H, m). MS-ESI (m/z): 419 $[M + H]^+$.

3-Fluoro-5-(2-(2-fluorophenyl)prop-1-en-1-yl)isonicotinonitrile (46)

A mixture of **14** (267 mg, 1.02 mmol), 3-bromo-5-fluoroisonicotinonitrile (45, 205 mg, 1.02 mmol), K_2CO_3 (2 M in H_2O; 1.53 mL, 3.06 mmol), and $PdCl_2(dppf)\bullet CH_2Cl_2$ (41.7 mg, 0.051 mmol) in THF (8 mL) was stirred at 70 °C for 24 h under N_2. The resulting mixture was filtered through a Celite pad, and the aqueous layer was then separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 0–15% EtOAc) to give a 3:2 diastereomeric mixture of **46** (208 mg, 80%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (t, J = 1.5 Hz, 3H, *minor isomer*), 2.34 (d, J = 1.5 Hz, 3H, *major isomer*), 6.71 (1H, s, 1H, *major*), 6.72 (1H, s, 1H, *minor*), 7.01–7.43 (5H, m), 7.98 (s, *major isomer*), 8.34 (s, *minor isomer*), 8.56 (s, *minor isomer*), 8.71 (s, 1H, *minor isomer*). MS-ESI (m/z): 257 $[M + H]^+$.

3-Fluoro-5-(2-(2-fluorophenyl)prop-1-en-1-yl)isonicotinimidamide (47)

To a suspension of NH_4Cl (1.50 g, 28.0 mmol) in toluene (2.0 mL) was added dropwise trimethylaluminum (14.0 mL, 28.0 mmol, 2 M in toluene) at 0 °C under N_2. After the mixture was stirred at rt for 1 h, **46** (205 mg, 0.800 mmol) in toluene (4.0 mL) was added. The mixture was stirred at 100 °C for 25 h and then quenched with potassium sodium tartrate and 2 M aqueous NaOH solution at 0 °C. The mixture was stirred at rt for 50 min. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; MeOH/$CHCl_3$, gradient: 0–5% MeOH) to give a 2:1 diastereomeric mixture of **47** (180 mg, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25–2.26 (s, 3H), 6.59 (1H, s), 6.90–7.39 (5H, m), 7.82 (s, 1H, *major isomer*), 8.23 (s, 1H, *major isomer*), 8.46 (s, 1H, *minor isomer*), 8.57 (s, 1H, *minor isomer*). MS-ESI (m/z): 274 $[M + H]^+$.

***tert*-Butyl (3-(5-amino-2-fluorophenyl)-8-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-1-yl)carbamate (48)**

After a mixture of **47** (155 mg, 0.568 mmol) and conc. H_2SO_4 (1.0 mL, 18.000 mmol) was stirred at rt for 13 h, HNO_3 (0.051 mL, 1.14 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 2.5 h and then quenched with 2 M aqueous NaOH solution and basified with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to give **48** (155 mg, 86%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (3H, s), 3.12 (1H, d, J = 15.8 Hz), 3.33 (1H, d, J = 15.8 Hz), 7.16 (1H, dd, J = 10.9, 8.9 Hz), 8.12 (1H, ddd, J = 8.9, 4.0, 3.0 Hz), 8.38 (1H, s), 8.48 (1H, d, J = 2.2 Hz), 8.74 (1H, dd, J = 6.9, 3.0 Hz). MS-ESI (m/z): 319 $[M + H]^+$.

***tert*-Butyl (3-(5-amino-2-fluorophenyl)-8-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-1-yl)carbamate (49)**

After a mixture of **48** (153 mg, 0.480 mmol) and Boc_2O (0.111 mL, 0.480 mmol) in THF (2.3 mL) was stirred at rt for 13 h, MeOH (1.5 mL), H_2O (0.6 mL), Fe (150 mg, 2.69 mmol), and NH_4Cl (115 mg, 2.16 mmol) were added at rt. The mixture was stirred at 70 °C for 1.5 h and filtered through a Celite pad, and the filtrate was evaporated. The residue was diluted with H_2O and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 10–33% EtOAc) to give **49** (164 mg, 88%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (9H, s), 1.81 (3H, s), 3.17 (1H, d, J = 15.7 Hz), 3.52 (2H, s), 3.74 (1H, d, J = 15.7 Hz), 6.41–6.54 (1H, m), 6.87 (1H, dd, J = 11.7, 8.6 Hz), 8.22 (1H, s), 8.39 (1H, d, J = 2.5 Hz), 10.68 (1H, s). MS-ESI (m/z): 389 $[M + H]^+$.

N-(3-(1-Amino-8-fluoro-3-methyl-3,4-dihydro-2,6-naphthyridin-3-yl)-4-fluorophenyl)-5-cyanopicolinamide (10)

To a solution of **49** (80.3 mg, 0.207 mmol) in THF (2.4 mL) at 0 °C were added 5-cyanopicolinic acid hydrate (41.2 mg, 0.248 mmol), HATU (102 mg, 0.269 mmol), and Et_3N (0.075 mL, 0.538 mmol). The mixture was stirred at rt for 2.5 h and then quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column

chromatography (silica gel; EtOAc/hexane, gradient: 33–67% EtOAc) to give a white solid (133 mg, impure).

A mixture of the solid (97.1 mg) and formic acid (0.50 mL, 13.0 mmol) was stirred at rt for 18 h. The reaction was quenched with NaOH (2 M aqueous solution) and saturated aqueous $NaHCO_3$ at 0 °C. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel; EtOAc/hexane, gradient: 50–80% EtOAc). The crude product was recrystallized from hexane/EtOAc/MeOH to give **10** (48.6 mg, 62%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.72 (3H, s), 3.10 (1H, d, J = 16.1 Hz), 3.60 (1H, d, J = 16.1 Hz), 7.08 (1H, dd, J = 11.8, 8.8 Hz), 7.66–7.71 (1H, m), 7.80 (1H, dd, J = 7.4, 2.7 Hz), 8.33–8.36 (2H, m), 8.41–8.44 (2H, m), 9.04–9.05 (1H, m). MS-ESI (m/z): 419 $[M + H]^+$.

5.2. Biochemical BACE1 Assay

The biochemical BACE1 IC_{50} values were measured by HTRF assay using an APP-derived peptide as reported previously.

5.3. Cellular Aβ Assay

The cellular Aβ IC_{50} values were determined by measuring Aβ40 using HTRF assay in SH-SY5Y cells expressing human APP as reported previously.

5.4. In Vitro ADMET Assays

Metabolic stability in microsomes, P-gp efflux ratio, solubility, protein binding, and hERG inhibitory activity were determined according to procedures reported previously.

5.5. pKa assays

pKa values were determined using capillary electrophoresis method according to procedures reported previously.[9,12a]

5.6. In vivo experiments

All the procedures for the animal studies were in accordance with regulations and established guidelines and were approved by the Shionogi Animal Care and Use Committee.

5.6.1. In vivo pharmacokinetics study

The test compound was dissolved in 20% HPBCD, and the details were reported previously.

5.6.2. Dog PK/PD study

The test compound was suspended in 0.5% MC and was orally administered to beagle dogs (n = 3) at 1 mg/kg dose. The total Aβ levels were measured using an ELISA method; the details were reported previously.

5.7. X-ray crystallographic study

The X-ray structure for **11** was solved according to the method described previously.[15] X-ray data collection and refinement statistics for compound **11** are described the below table.

PDB code	7D36
Wavelength (Å)	1.5418
Resolution range (Å)	50.0–2.30 (2.34–2.30)
Space group	P 6122
Unit cell	102.531 102.531 170.115 90.0 90.0120.0
Total reflections	24 100
Unique reflections	24 100
Completeness (%)	99.5 (99.3)
Redundancy	8.3 (8.4)
Mean I/sigma(I)	24.5 (3.7)
Wilson B-factor	46.2
R-merge	9.5 (52.0)
Reflections used in the refinement	21 623
R-work	0.213
R-free	0.265
Number of non-hydrogen atoms	
macromolecules	2902
ligands	32
RMS (bonds)	0.011
RMS (angles)	1.363
Ramachandran favored (%)	97.6
Ramachandran allowed (%)	2.40
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0
Average B-factor	
macromolecules	45.2
Ligands	56.1
Solvent	45.3

Accession codes

The PDB accession code for the X-ray structure of **11** bound to human BACE1 is 7D36.

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.

Acknowledgements

We thank Maki Hattori, Masanori Nakae, and Maito Douma for the biological assays and Eriko Matsuoka for the X-ray analysis. We gratefully thank the members of the Janssen-Shionogi research collaboration for the support of this program. We gratefully thank Dr. Harrie J.M. Gijsen for his help with reviewing the manuscript. We are grateful to Judy Noguchi for proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113270.

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