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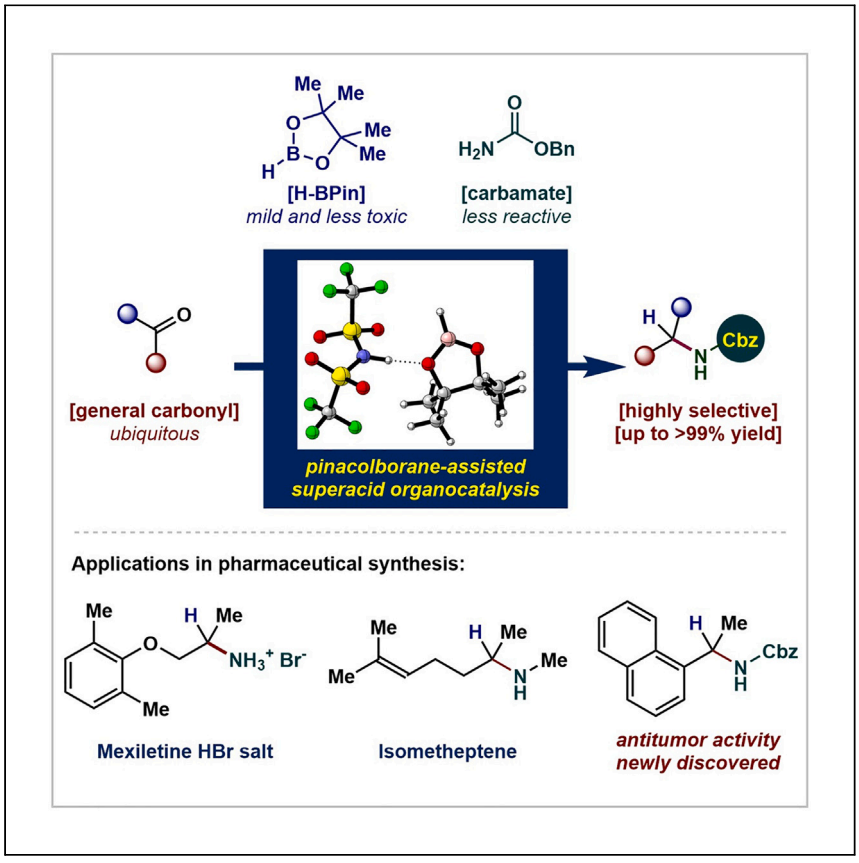

Kim et al. demonstrate the direct synthesis of *N*-benzyloxycarbonyl alkyl amines using a superacid organocatalyst. Selective reductive amination provides access to a range of scaffolds, including products demonstrating potent cytotoxicity *in vitro*.

Woo Hee Kim, Seon Bin Song, Da Eun Lee, ..., Ki Hyun Kim, Joonsuk Huh, Han Yong Bae

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hybae@skku.edu (H.Y.B.)

Highlights

Pinacolborane-assisted superacid catalysis toward alkyl *N*-Cbz amines using carbonyls

Robust catalysis achieves a general substrate scope with reliable scalability

The formation of protonated pinacolborane enables reductive amination with carbamates

Cytotoxicity against human cancer cell lines is demonstrated

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Pinacolborane-assisted superacid organocatalysis enabled direct access to cytotoxic alkyl *N*-Cbz amines

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SUMMARY

α-Secondary alkyl amines are structural motifs frequently encountered in a wide variety of natural products and pharmaceuticals. The *N*-benzyloxycarbonyl (Cbz) compound is a widely used precursor, acknowledged for its efficacy in implementing a masked amine strategy to access a privileged moiety. Although reductive amination is conducted as a crucial portion of the pharmaceutical industry, direct catalytic access to alkyl Cbz-amine is still rare due to the low reactivity of carbamate. Here, we show a superacid organocatalyst enabled direct access to bioactive Cbz-protected α-secondary alkyl amines using general ketones as the starting material. Through the highly selective and robust catalytic process, a wide substrate scope including drug precursor scaffolds in preparative scalability (up to >99% yield) with practical pharmaceutical syntheses is achieved. The obtained *N*-Cbz products are found to possess strong cytotoxicities in *in vitro* bioactivity evaluations, indicating their potential as promising candidates for new anticancer drug discovery.

INTRODUCTION

α-Secondary alkyl amines are essential chemical structures frequently encountered in various naturally occurring products, pharmaceuticals, and peptides.[1,2] The synthesis of intricate molecules incorporating such a privileged moiety requires implementing a masked amine strategy, recognized as a highly advantageous process for efficiently constructing the desired target molecules. In particular, since the pioneering discovery by Bergmann and Zervas,[3] the benzyloxycarbonyl (Cbz) group has become one of the most widely utilized amine-protecting tools due to its redox/acid-base tolerance and mild deprotection properties.[4] As conventional approaches to synthesizing such *N*-Cbz-protected alkyl amines, (1) a simple protection process with benzyl chloroformate of free-alkyl (primary) amine and (2) an addition reaction of nucleophilic reagent to *N*-Cbz-aldimine[5] (Mannich-type reaction) are used to introduce additional aryl/alkyl substituents. Multistep syntheses of precursors starting from raw materials were mandatory (Figure 1A).

An ideal method to further simplify the desired preparation process is to envision the reductive amination of raw materials such as ketones, for which a wider variety of substituents are readily available.[6–11] The study by Roughley et al. revealed that 23% of carbon-heteroatom bond-forming reactions in the pharmaceutical industry are carried out via reductive amination.[2] Catalytic processes to access alkyl-/aryl-substituted or free amines

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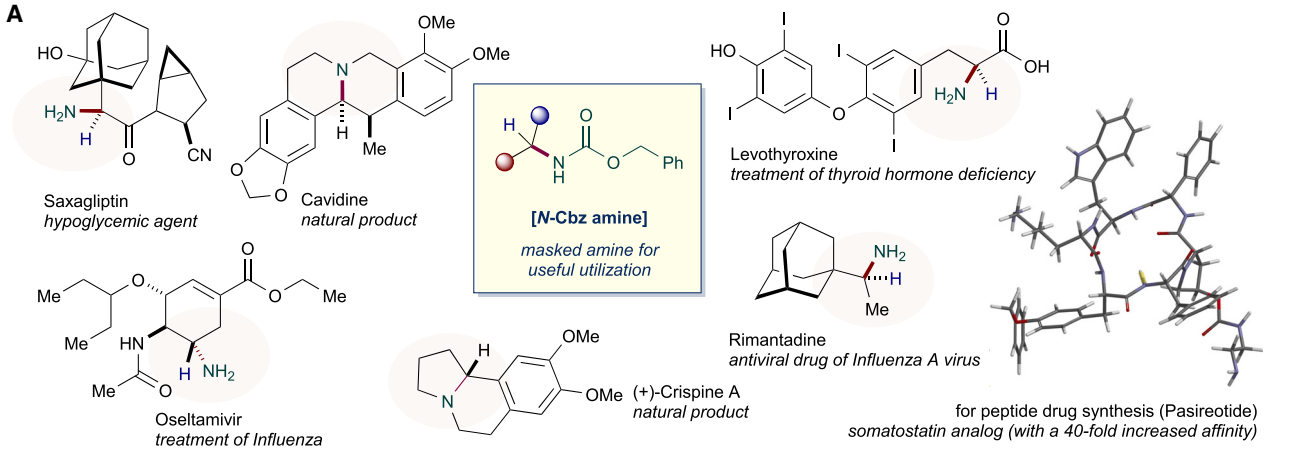
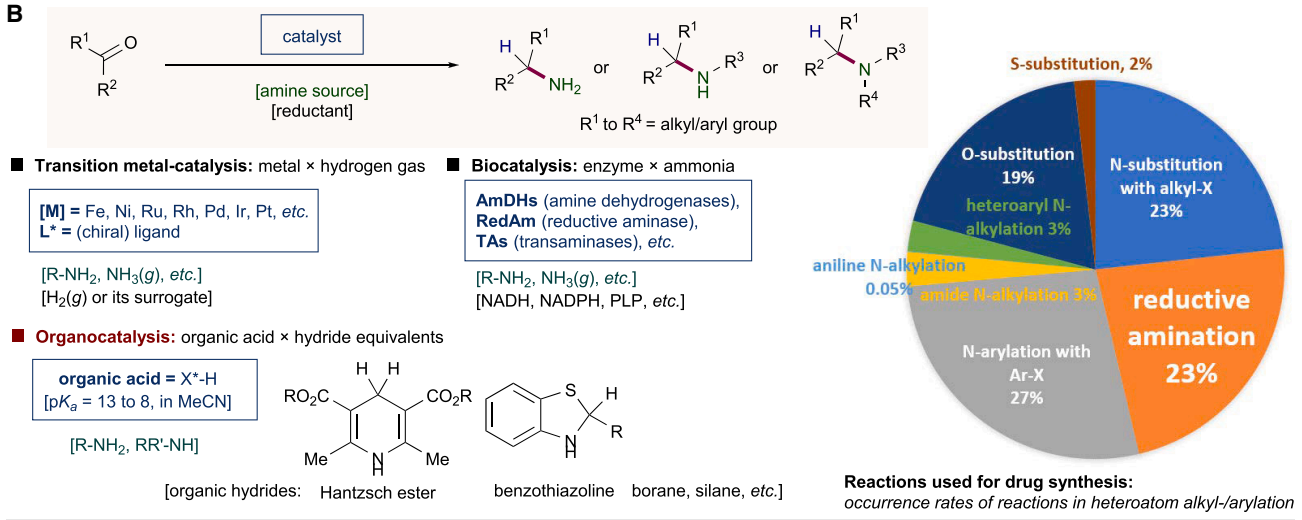

A
Saxagliptin
hypoglycemic agent
Cavidine
natural product
[N-Cbz amine]
masked amine for
useful utilization
Levothyroxine
treatment of thyroid hormone deficiency
Oseltamivir
treatment of Influenza
(+)-Crispine A
natural product
Rimantadine
antiviral drug of Influenza A virus
for peptide drug synthesis (Pasireotide)
somatostatin analog (with a 40-fold increased affinity)


B
catalyst
[amine source]
[reductant]
R1 to R4 = alkyl/aryl group
Transition metal-catalysis: metal × hydrogen gas
[M] = Fe, Ni, Ru, Rh, Pd, Ir, Pt, etc.
L* = (chiral) ligand
[R-NH2, NH3(g), etc.]
[H2(g) or its surrogate]
Biocatalysis: enzyme × ammonia
AmDHs (amine dehydrogenases),
RedAm (reductive aminase),
TAs (transaminases), etc.
[R-NH2, NH3(g), etc.]
[NADH, NADPH, PLP, etc.]
Organocatalysis: organic acid × hydride equivalents
organic acid = X*-H
[pKa = 13 to 8, in MeCN]
[R-NH2, RR'-NH]
[organic hydrides: Hantzsch ester
benzothiazoline
borane, silane, etc.]
S-substitution, 2%
O-substitution 19%
N-substitution with alkyl-X 23%
heteroaryl N-alkylation 3%
aniline N-alkylation 0.05%
amide N-alkylation 3%
reductive amination 23%
N-arylation with Ar-X 27%
Reactions used for drug synthesis:
occurrence rates of reactions in heteroatom alkyl-/arylation

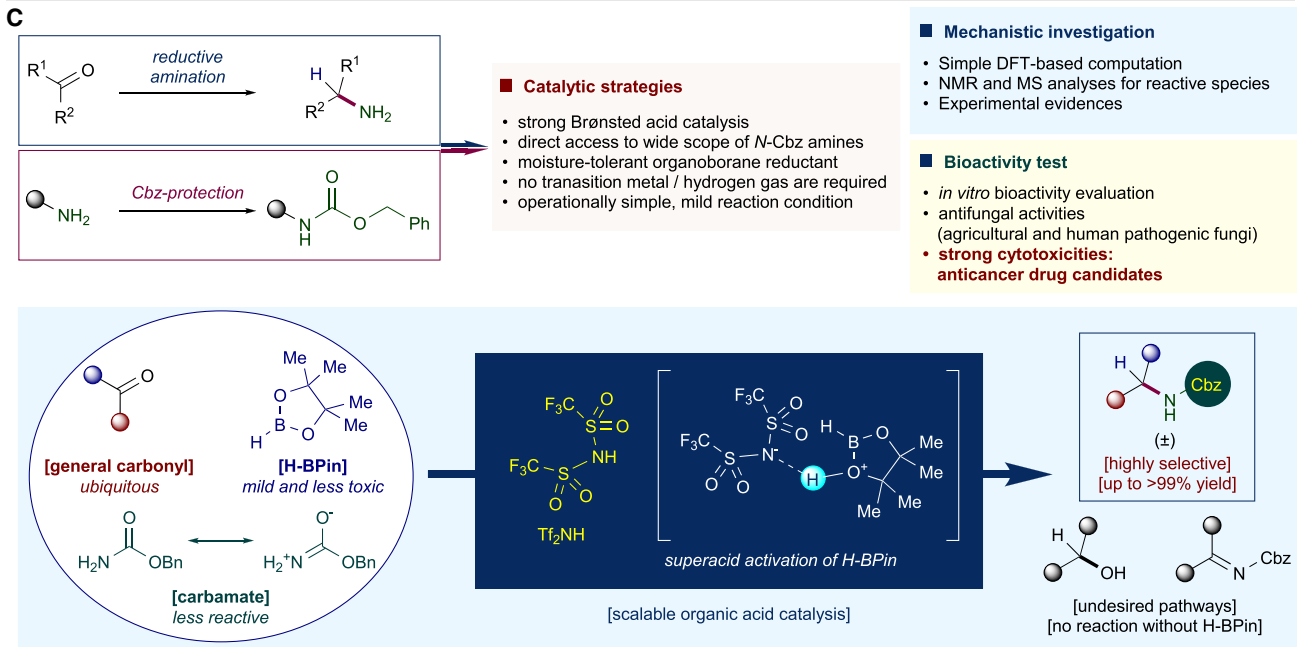

C
reductive amination
Cbz-protection
Catalytic strategies
strong Brønsted acid catalysis
direct access to wide scope of N-Cbz amines
moisture-tolerant organoborane reductant
no tranasition metal / hydrogen gas are required
operationally simple, mild reaction condition
Mechanistic investigation
Simple DFT-based computation
NMR and MS analyses for reactive species
Experimental evidences
Bioactivity test
in vitro bioactivity evaluation
antifungal activities
(agricultural and human pathogenic fungi)
strong cytotoxicities:
anticancer drug candidates
[general carbonyl]
ubiquitous
[H-BPin]
mild and less toxic
[carbamate]
less reactive
Tf2NH
superacid activation of H-BPin
[scalable organic acid catalysis]
(±)
[highly selective]
[up to >99% yield]
[undesired pathways]
[no reaction without H-BPin]

Figure 1. Overview and importance of reductive amination to access α-secondary alkyl amines and approaches that appeared in this study
(A) Chemical structures of α-secondary-alkyl-amine-incorporated pharmaceuticals and natural products.
(B) Established representative catalytic reductive aminations and their key features.
(C) This work: direct catalytic access to *N*-Cbz alkyl amine. Cbz, benzyloxycarbonyl; PLP, pyridoxal 5′-phosphate; NAD^+, nicotinamide adenine dinucleotide; $NADP^+$, nicotinamide adenine dinucleotide phosphate.

are well established using transition metals, biocatalysts, and organocatalysts[1,5] (Figure 1B). We investigated whether it would be possible to develop a process for matters that remain elusive in chemical catalysis on these terms: (1) the use of a less toxic organoborane reductant[12]; (2) an option for a user-friendly method, that is, one that can be performed under transition-metal-free, moisture-tolerant, or high-pressure H_2-free conditions[13]; (3) a practical process capable of preparative-scale *N*-Cbz amine synthesis; and (4) obtained products further possessing practical bioactivity or not.

The hydrogenation of preformed ketone-derived imines (ketimines)[14] is a widely applied process in the synthesis of α-secondary alkyl amines. Methods employing various metal catalysts with borane reductants have been reported.[15] However, most studies are limited to the use of *N*-alkyl- or *N*-aryl-protected imines (with Cbz-ketimine unknown), and transition-metal-free examples are quite rare.[16] External Lewis acid catalysts are mainly required as promoters for reduction with organoborane (e.g., pinacolborane = H–BPin). In a seminal example, in 2012, the Crudden group showed that cooperative boron Lewis acid-amine Lewis base pairs can be utilized in the hydroboration of preformed *N*-alkyl-protected ketimines.[17] Later, in 2017, the Oestereich and Melen groups reported that tris[3,5-bis-(trifluoromethyl)phenyl]borane Lewis acid catalyzed a similar reaction using *N*-aryl-/*N*-alkyl-/*N*-tosyl-protected ketimines.[18] When focusing on three-component reductive amination with carbonyl as a raw starting material, Gao et al. reported a tris(pentafluorophenyl)borane-catalyzed approach using aldehydes.[19] This method requires a highly reactive pyrrolidine (secondary amine) as the aminating reagent. Utilizing ketones as raw materials in this category of organoboron chemistry is exceptionally challenging because ketones are readily converted to alcohols (hydroboration with H–BPin) due to the intensive uncatalyzed reaction, as reported by Hreczycho et al.[20] The catalytic reductive amination of ketones to obtain Cbz-alkyl amines via hydroboration is yet underdeveloped. Though a silane-mediated rhenium(VII) oxide (Re_2O_7) catalysis approach is known,[21] rhenium is a very rare, expensive transition metal. Its toxicity guideline is unknown; therefore, an efficient and sustainable process via scalable catalysis is demanded.[22]

Herein, we report a new direct organocatalytic access to Cbz-protected α-secondary alkyl amines using ketones as the starting material. A general substrate scope with preparative scalability is achieved due to the highly chemoselective and efficient transition-metal-free catalytic process (up to >99% yield). Experimental, analytical, and computational approaches support the *in situ* activation of pinacolborane efficiently promoting the desired reductive amination. *In vitro* bioactivity evaluations reveal the cytotoxicity of *N*-Cbz amines and that they can be potential candidates for new anticancer drugs (Figure 1C).

RESULTS AND DISCUSSION

Establishment of reductive amination

Our main objective was to develop a reductive amination reaction via the catalytic hydroboration of ketimines generated *in situ* from general ketone substrates (Figures 2 and 3). To achieve this goal, we selected 2-heptanone (**1**) as the unbiased model substrate, benzyl carbamate (**2a**) as the aminating reagent, and pinacolborane (H–BPin,

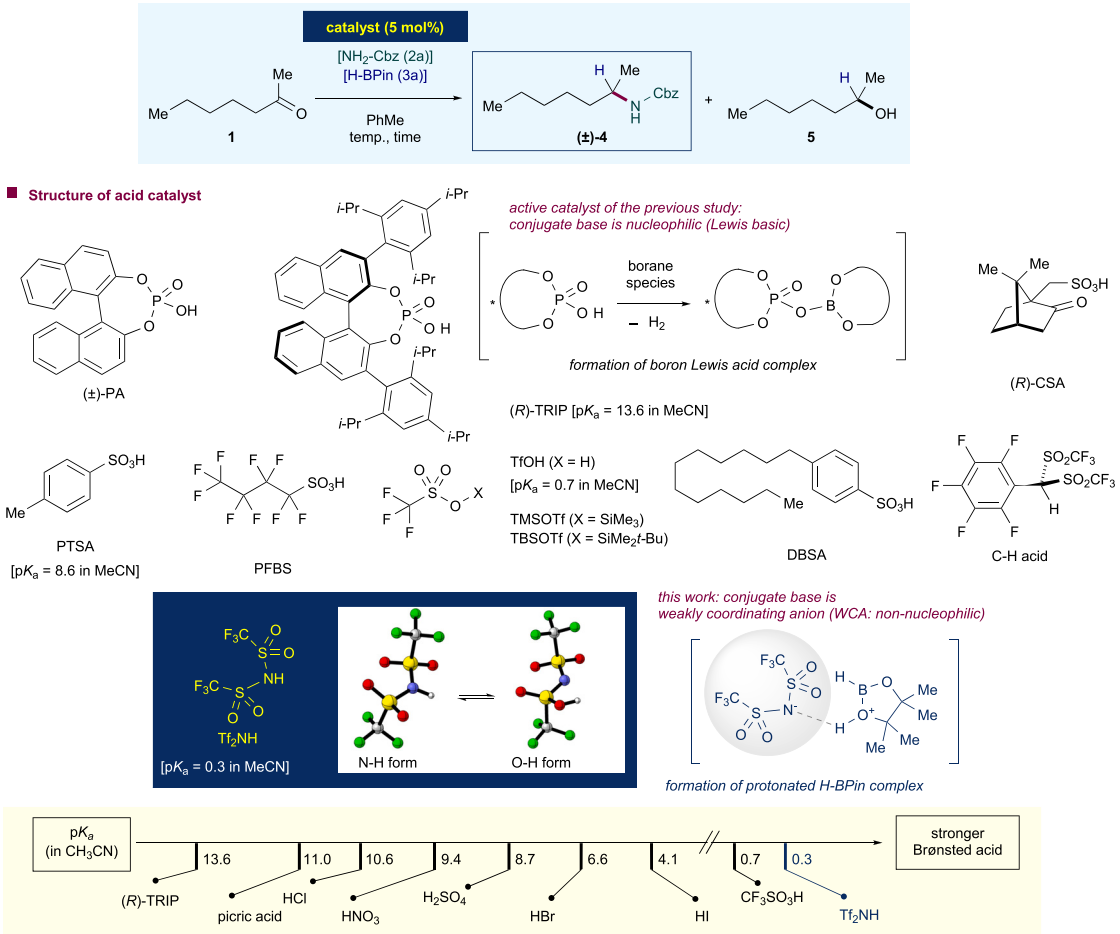
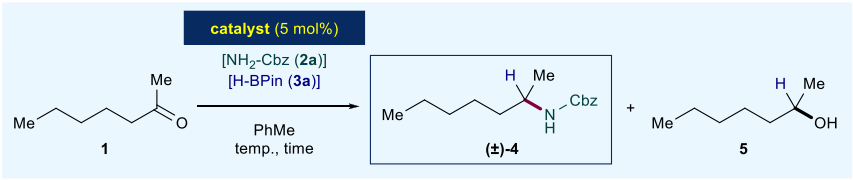


Figure 2. Structure of screened catalysts

Chemical structures of Brønsted acid organocatalysts used in this study and their reported pK_a values in CH_3CN.

3a) as the organoborane reductant (Figure 3). No reaction was detected without any catalyst in PhMe at 24°C (entry 1). Initially, we employed a typical metallic Lewis acid[23] for catalysis. Metallic salts such as $Fe(OTf)_3$, $Sc(OTf)_3$, Ag(OTf), $Cu(OTf)_2$, $Bi(OTf)_3$, and $Bi(OAc)_3$ were not effective in the reaction, and only a small amount of reductive amination product **4** was obtained (0%–10% yield, entries 2–7). The undesired hydroboration pathway from ketone **1** to alcohol **5** was also problematic (21% yield, entry 6). We wondered whether employing purely organic Brønsted acids[24] such as BINOL-derived phosphoric acid (PA) or its 3,3′-bis(2,4,6-triisopropylphenyl) substituent (*R*)-TRIP (pK_a = 13.6 in MeCN[25]), camphorsulfonic acid (CSA), *p*-toluenesulfonic acid (PTSA; pK_a = 8.6 in MeCN[26]), and 4-dodecylbenzenesulfonic acid (DBSA) could solve the chemoselectivity issue. Significantly improved results, except for (*R*)-TRIP (no reaction observed, entry 9), albeit still with low yields of product **4**, were obtained (19%–33%), with a partial decomposition of the side product **5** (entries 8, 10, and 11).

Antilla and co-workers previously reported that chiral PA catalyst in combination with boranes (catecholborane or pinacolborane) assembles the Lewis acid complex through hydrogen gas evolution. In their studies, electron-rich aniline-type aminating sources worked well due to their high nucleophilicity.[27–29] Inspired by these discoveries, we hypothesized that higher acidic organocatalysts can provide different performances in the case of electron-deficient aminating reagents.



Entry	Catalyst	T (°C)	t (h)	Yield of **4** (%)	Yield of **5** (%)
1	*No catalyst*	25	24	0	0
2	$Fe(OTf)_3$	25	24	0	not detected
3	$Sc(OTf)_3$	25	24	0	0
4	Ag(OTf)	25	24	10	0
5	$Cu(OTf)_2$	25	24	8	9
6	$Bi(OTf)_3$	25	24	10	21
7	$Bi(OAc)_3$	25	24	0	0
8	(±)-PA	25	24	26	8
9	(*R*)-TRIP	30	24	0	0
10	(*R*)-CSA	25	24	19	not detected
11	PTSA	25	24	33	not detected
12	DBSA	25	24	28	not detected
13	PFBS	25	24	70	not detected
14	TfOH	25	24	76	21
15	TfOH	60	24	64	26
16	TfOH	0	72	61	39
17	TMSOTf	25	24	76	21
18	TMSOTf	0	72	62	38
19	TBSOTf	25	24	70	24
20	TBSOTf	0	72	63	36
21	C-H acid	30	24	74	26
22	Tf_2NH	25	24	85	15
23[a]	Tf_2NH	25	24	80	20
24[b]	Tf_2NH	25	24	84	16
25	Tf_2NH	30	24	91	9

Figure 3. Catalyst screening

Reaction condition: reactions were performed with **1** (0.2 mmol), benzyl carbamate **2a** (1.2 equiv, 0.24 mmol), pinacolborane **3a** (2.0 equiv, 0.4 mmol), and acid catalyst (5 mol %, 0.01 mmol) in solvent (1 mL). Yield (%) was determined by ^{1}H NMR integration (internal standard: 1,3,5-trimethoxybenzene).

[a]1.3 equiv pinacolborane **3a**.

[b]2.5 equiv pinacolborane **3a**.

Interestingly, in the cases of significantly more acidic Brønsted acids such as perfluorobutanesulfonic acid (PFBS) and triflic acid (TfOH; pK_a = 0.7 in MeCN[30]), noticeably higher yields were obtained (70% and 76% yields of **4**; entries 13 and 14, respectively). Lowered (0°C) or elevated (60°C) temperatures were not helpful for achieving better chemoselectivity (**4**/**5** = 61%:39%, 0°C, entry 16; **4**/**5** = 64%:26%, 60°C, entry 15). The silylium Lewis acids of triflates trimethylsilyl trifluoromethanesulfonate (TMSOTf) or *tert*-butyldimethylsilyltrifluoromethanesulfonate (TBSOTf) exhibited similar levels of reactivity and chemoselectivity (entries 17–20). A strong C–H acid (pentafluorophenylbis(triflyl)methane)[31] showed better results (**4** = 74%; **5** = 26%, entry 21). To our surprise, a super Brønsted acid catalyst[32,33] such as triflimide[34,35] (Tf_2NH; pK_a = 0.3 in MeCN[30]), where its conjugate base is a non-nucleophilic weakly coordinating anion (WCA),[36] showed among the best results in terms of reactivity and selectivity (up to 91% yield of **4**, entries 22–25).

In-depth optimization of reaction parameters

The reaction parameters were then investigated in detail. Reductive amination is highly dependent on solvent and temperature. After screening several solvents, we determined that the PhMe solvent (polarity index = 2.4) at 30°C was the optimum condition for the selective synthesis of the product (91% yield). Relatively more polar solvents such as dichloromethane (polarity index = 3.1) and acetonitrile (polarity index = 5.8) were also recommended solvents, though slightly lower yields were obtained (75% and 74% yields, respectively) (Figures 4A and 4B). To further investigate the aminating reagent, we studied several candidate categories, beginning with the carbamate types. 9-Fluorenylmethyl carbamate (**2b**) resulted in its corresponding product, **6**, at a moderate yield (54%); however, no reaction occurred with *tert*-butyl carbamate (**2c**). Hydrazides such as **2d** (benzhydrazide) and **2e** (4-methoxybenzhydrazide) provided low yields of products **7** (34%) and **8** (30%), respectively. Other amines such as acetamide (**2f**), diphenyl phosphoramidate (**2h**), and Ellman's sulfinamide[37] (**2i**) did not proceed to the product. Benzamide (**2g**) afforded 12% of the desired product **9**. Electron-rich *p*-anisidine (**2j**) gave the desired product **10** in 89% yield, where (*R*)-TRIP also efficiently worked (Figure 4C).

The selection of a suitable reductant is a key factor in the success of the reaction. When catecholborane (H–BCat, **3b**) was used, a relatively low efficiency was observed (53% yield), where combination with (*R*)-TRIP gave 22% yield. In the ^{11}B nuclear magnetic resonance (NMR) analyses (^{1}H coupled), a significant boron–hydrogen coupling was observed in the borane combinations of Tf_2NH (both H–BPin and H–BCat); however, no signal was detected in the case of (*R*)-TRIP: this fact supports that the boron Lewis acid complex is formed through H_2 evolution[27–29] (Figure S1; supplemental experimental procedures). Other boranes such as 2,3-dihydro-1*H*-naphtho[1,8-de][1,3,2]diazaborinine (**3c**) were inactive in catalysis. A representative silicon reductant,[38] triethylsilane (**3d**), was not effective (n.d. [not detected]). Hantzsch ester (**3e**), which is a widely utilized hydride donor[39] mainly used with preformed *N*-aryl/-alkyl ketimines in the presence of chiral PA or hydrogen-bonding donor organocatalysts,[5] was also inactive under the reaction conditions (Figure 4D).

Generality, late-stage modification, scale-up synthesis, and synthetic application

A wide variety of ketone substrates were investigated under optimized conditions. First, we used a series of alkyl-alkyl ketones. As shown in Figure 5A, from short- (CH_3) to long-chain (n-C_8H_{17}) alkyl-group-incorporated ketones are smoothly converted into the desired products in good to quantitative yields (up to >99% yield, compounds **4**, **11**–**19**, and **22**). Product **20** (70% yield) can be used as a synthetic precursor for the lisdexamfetamine (treatment of attention-deficit/hyperactivity disorder). Five-, six-, and twelve-membered cycloalkane-substituted ketones were also highly active (67%–99% yield, **27**, **26**, and **28**, respectively). In addition, terminal alkenyl, cyclopropyl, chloroalkyl, internal alkenyl, and aryl ether functionalized groups were tolerated in the reactions (63%–80% yield, **21**, **22**, **23**, **24**, and **25**, respectively). Other aryl-alkyl ketones were also smoothly converted to the desired products in moderate to good yields (**29**–**41**). Product **38** (71% yield) also can be transformed into a synthetic precursor for cinacalcet (treatment for hyperparathyroidism) (Figure 5B). Other classes of carbonyl, including alkyl-/aryl-/alkenyl-group-bearing aldehydes, reacted under similar catalytic conditions to produce *N*-Cbz α-primary amines (up to 95% yield, **42**–**47**; Figure 5C).

Complex bioactive molecules such as nabumetone (a nonsteroidal anti-inflammatory drug), androstenolone (an endogenous steroid hormone), and Hedione (a fragrance),

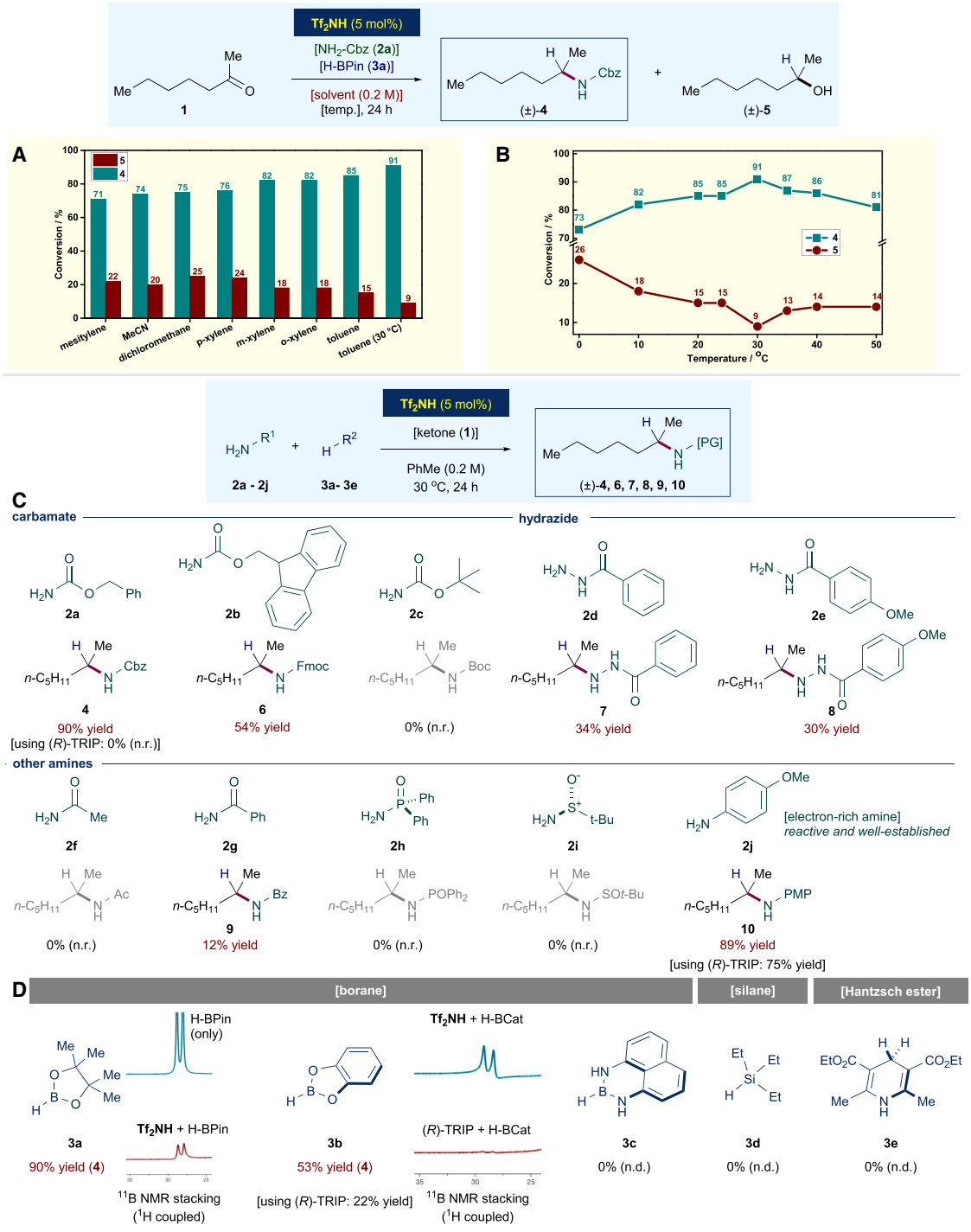


Figure 4. Optimization of reaction parameters

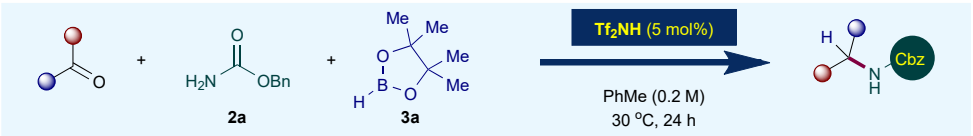
(A) Effect of solvent (at 24°C).

(B) Effect of temperature (in PhMe).

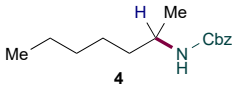
(C) Amine screening.

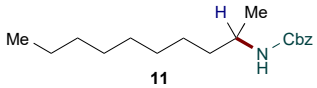
(D) Reductant screening.

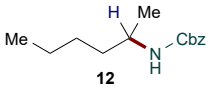
Reaction condition: reactions were performed with **1** (0.2 mmol), amine (1.2 equiv, 0.24 mmol), reductant (2.0 equiv, 0.4 mmol), and acid catalyst Tf_2NH (5 mol %, 0.01 mmol) in solvent (1 mL). Conversion (A and B) was determined by ^1H NMR integration (internal standard: 1,3,5-trimethoxybenzene). The yield (C and D) was determined after purification by column chromatography.

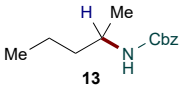


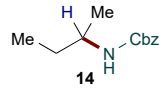
A Alkyl-alkyl ketone

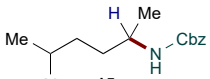

4
90% yield
[8.8 mmol scale]

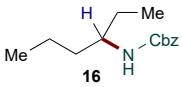

11
>99% yield

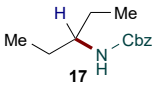

12
80% yield

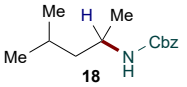

13
85% yield

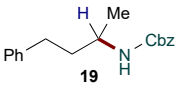

14
81% yield

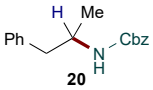

15
80% yield

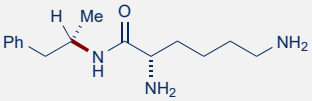

16
73% yield

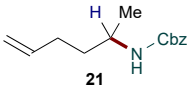

17
62% yield

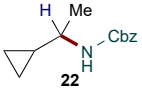

18
89% yield

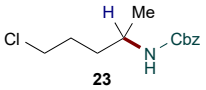

19
70% yield

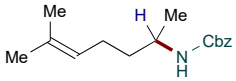

20
70% yield
[using 10 mol% cat.]

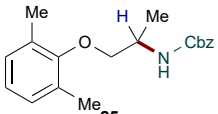

Lisdexamfetamine *treatment of ADHD*

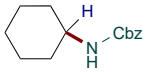

21
79% yield

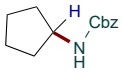

22
61% yield

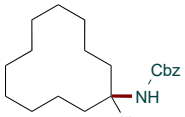

23
80% yield


24
66% yield
[4.0 mmol scale]


25
63% yield
[0.4 mmol scale]

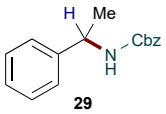

26
67% yield

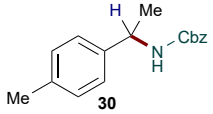

27
>99% yield

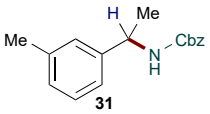

28
73% yield
[4.0 mmol scale]

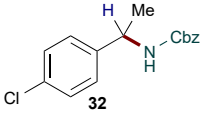

[0.93 gram]

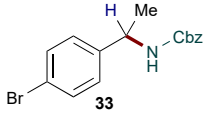
B Aryl-alkyl ketone

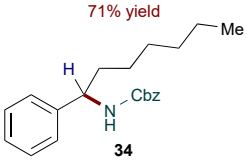

29
71% yield

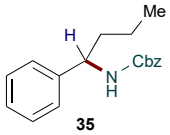

30
72% yield

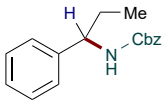

31
74% yield

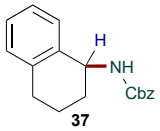

32
52% yield

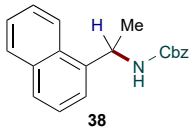

33
56% yield

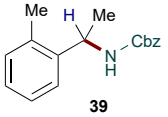

34
60% yield

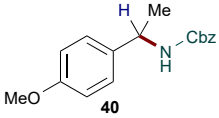

35
56% yield

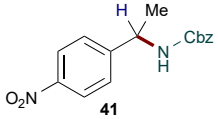

36
53% yield

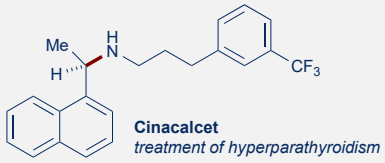

37
65% yield


38
71% yield

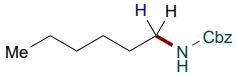

39
76% yield

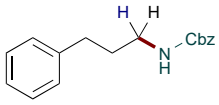

40
76% yield

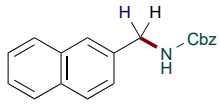

41
58% yield

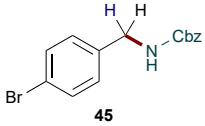

Cinacalcet
treatment of hyperparathyroidism

C Aldehyde


42
73% yield (0°C)


43
80% yiel (0 °C)


44
95% yield


45
91% yield

46
64% yield

47
99% yield

Figure 5. Substrate scope
(A) Alkyl-alkyl ketone.
(B) Aryl-alkyl ketone.
(C) Aldehyde.
Reaction condition: reactions were performed with ketone (0.20 mmol), benzyl carbamate **2a** (0.24 mmol, 1.2 equiv), pinacolborane **3a** (0.4 mmol, 2 equiv), and Tf_2NH catalyst (0.01 mmol, 5.0 mol %) in PhMe (1 mL). The yield was determined after purification by column chromatography.

which incorporate ketone moieties, were converted to *N*-Cbz amines in high yields (86%–92% yields, **48**–**50**). This fact supports the idea that our method can be further harnessed in the late-stage modification of functional organic molecules (Figure 6A). Preparative-scale syntheses were also conducted; 1.0 g of each of the starting ketones was converted to the desired *N*-Cbz amine with good to excellent yields (**4** = 1.97, **23** = 1.80, **27** = 2.60, and **19** = 1.33 g; Figure 6B). Finally, the synthetic application of reductive amination was investigated. Direct cyclization of product **23** afforded a pyrrolidine scaffold (78% yield, **51**), an essential *N*-heterocyclic building block in pharmaceutical compounds. A simple deprotection of compound **25** provided the HBr salt form of mexiletine (**52**, treatment of abnormal heart rhythms) in 96% yield. Methylation of the compound **24** provided product **53** (>99% yield), and subsequent Cbz deprotection resulted in isometheptene **54** (treatment of migraines and headaches) in >99% yield (Figure 6C).

Evaluation of antitumor and antifungal bioactivities of alkyl *N*-Cbz amines

To investigate further the biochemical applicability of the obtained *N*-Cbz products, we implemented *in vitro* bioactivity evaluations for antifungal activities against (1) agricultural pathogenic fungi and (2) human pathogenic fungi and for cytotoxicity against (3) human cancer cell lines (Figure 7). The relevant evaluation results are summarized and indicated as the size of the colored square (for the experimental details, see section 8 in the supplemental experimental procedures). The structures of tested compounds in this study are summarized (**KG-1** to **KG-16**).

At first, compounds **KG-1**, **KG-2**, and **KG-5** exhibited antifungal activities against *Rhizoctonia solani* (KACC 48921), with MIC_{50} (MIC, minimum inhibitory concentrations) values of 50, 25, and 100 μg/mL, respectively. Compounds **KG-4**, **KG-5**, and **KG-6** showed MIC_{50} values of 100 μg/mL against *Fusarium asiaticum* (KACC 46429), while *Fusarium solani* (KACC 44891) was the most chemically insensitive species in the tested compounds (Table S5). The results of antifungal activity against agricultural pathogenic fungi showed that compound **KG-2** had the lowest MIC_{50} (25 μg/mL), implying that it can protect vegetables and crops from infection by the fungal pathogen *R. solani* (for full evaluation data, see Table S5).

Secondly, compound **KG-11** exhibited meaningful antifungal activity against *Trichophyton rubrum* and *T. mentagrophytes*, which are the two most representative dermatophytes that cause tinea pedis[40]; MICs of **KG-11** against *T. rubrum* and *T. mentagrophytes* were 25 and 25 μg/mL, respectively. In addition, the MICs of **KG-1** and **KG-2** against *T. mentagrophytes* and the MICs of **KG-7** and **KG-15** against *T. rubrum* were all 50 μg/mL, suggesting that the compounds showed moderate antifungal activity. However, weak antifungal activity for the other compounds was detected against human pathogenic fungi (for full evaluation data, see Table S6).

Most importantly, compound **KG-4** showed significant cytotoxicity against all tested cancer cell lines, namely A549, SK-OV-3, SK-MEL-2, and HCT-15, with IC_{50} values of 3.83, 5.04, 3.38, and 4.78 μM, respectively. Compounds **KG-1**, **KG-3**, **KG-5**, **KG-6**, **KG-9**, and **KG-16** had different levels of cytotoxicity against some of the cell lines. Notably, among the cytotoxic molecules, compound **KG-4** showed higher antitumor

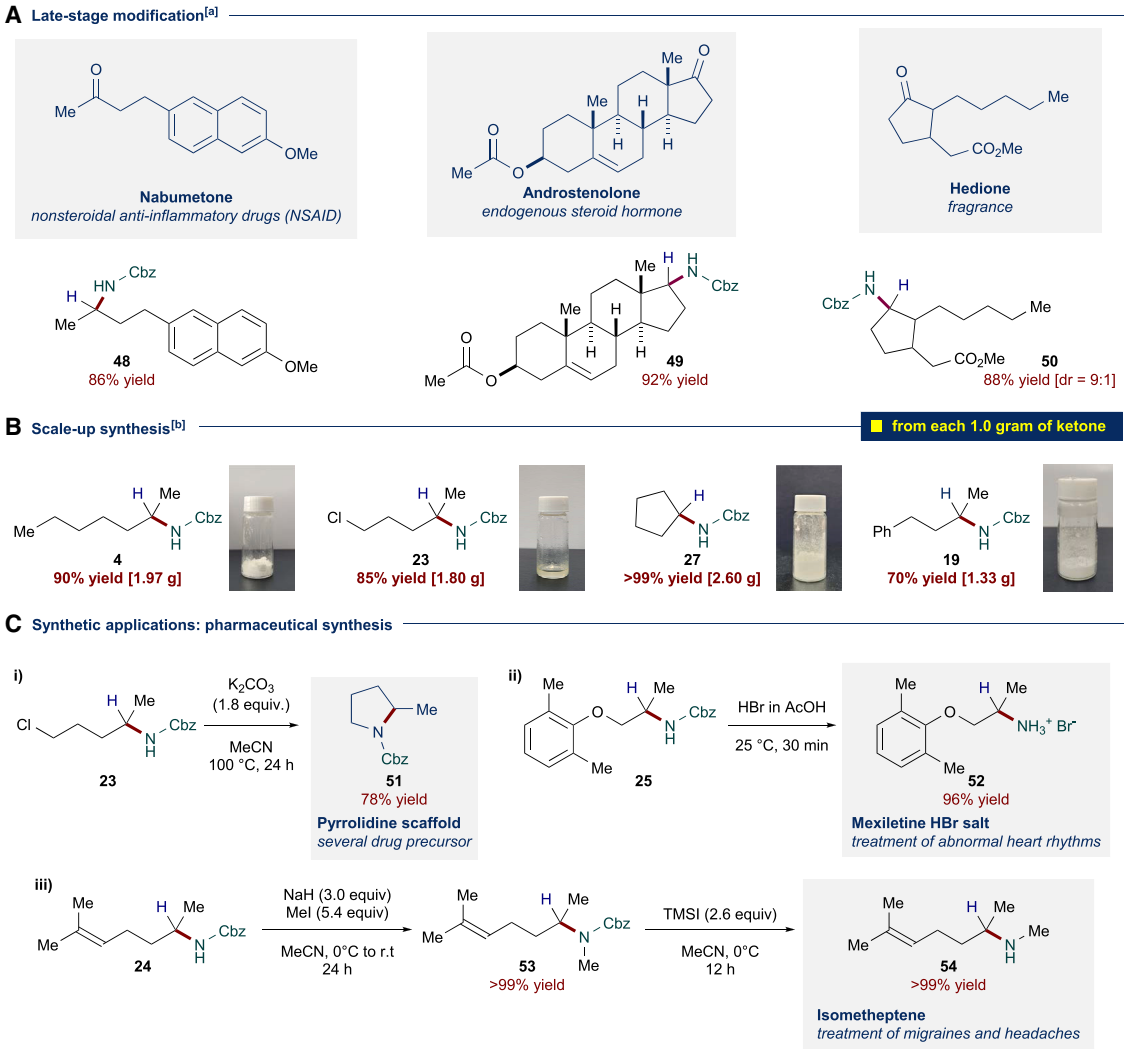


Figure 6. Synthetic utilities

(A) Late-stage modification.

(B) Scale-up synthesis.

(C) Synthetic applications: pharmaceutical synthesis.

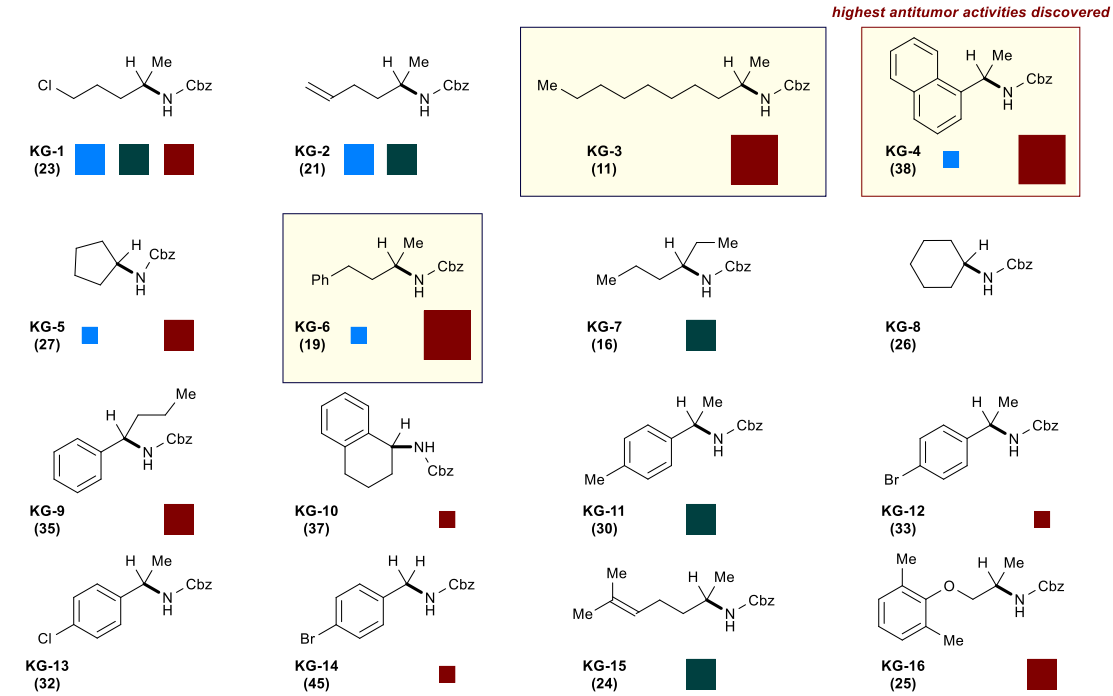
[a]Reaction condition: reactions were performed with ketone (0.20 mmol), benzyl carbamate **2a** (0.24 mmol, 1.2 equiv), pinacolborane **3a** (0.4 mmol, 2 equiv), and Tf_2NH catalyst (0.01 mmol, 5.0 mol %) in PhMe (1 mL). The yield was determined after purification by column chromatography.

[b]Scale-up syntheses were conducted using ketone (1.00 g), benzyl carbamate **2a** (1.2 equiv), pinacolborane **3a** (2 equiv), Tf_2NH (5 mol %), and PhMe (0.2 M).

activities than those of compounds with alkyls or other aromatics (**KG-1**, **KG-3**, **KG-5**, **KG-6**, **KG-9**, **KG-10**, **KG-12**, **KG-14**, and **KG-16**), suggesting that the sterically demanded 1-naphthyl moiety might influence the enhanced cytotoxicity of this kind of compound. To the best of our knowledge, this result is the first report of the cytotoxic bioactivities of *N*-Cbz amine molecules.

Mechanistic investigation: Experimental, analytical, and computational studies

Experimental, analytical, and computational studies were conducted to elucidate the detailed reaction mechanism. Initially, control experiments were implemented by varying the established standard conditions. In the absence of benzyl carbamate (**2a**), the hydroboration of ketone afforded 38% yield of the alcohol compound **5**



Compound / tumor cell line	A549[a]	SK-OV-3[a]	SK-MEL-2[a]	HCT-15[a]
KG-1	13.64±1.74	> 30.0	21.30±2.30	23.93±0.94
KG-2	22.26±2.66	> 30.0	> 30.0	> 30.0
KG-3	7.20±0.40	7.58±0.44	6.38±0.03	5.82±0.44
KG-4	3.83±0.34	5.04±0.32	3.38±0.30	4.78±0.25
KG-5	16.59±2.87	22.27±2.73	17.88±3.59	21.07±3.31
KG-6	9.56±0.73	14.13±1.76	6.71±0.55	12.11±0.92
KG-7	> 30.0	> 30.0	> 30.0	> 30.0
KG-8	> 30.0	> 30.0	> 30.0	> 30.0
KG-9	18.37±2.45	17.30±2.54	16.52±1.28	16.98±1.74
KG-10	> 30.0	> 30.0	20.53±1.27	> 30.0
KG-11	> 30.0	> 30.0	> 30.0	> 30.0
KG-12	> 30.0	> 30.0	20.74±1.24	> 30.0
KG-13	> 30.0	> 30.0	> 30.0	> 30.0
KG-14	> 30.0	25.46±2.59	22.78±2.25	> 30.0
KG-15	> 30.0	> 30.0	> 30.0	> 30.0
KG-16	> 30.0	> 30.0	22.95±2.10	> 30.0
Etoposide[b]	1.19±0.16	1.97±0.17	1.27±0.08	1.11±0.17

Figure 7. *In vitro* bioactivity evaluations: Potential drug discovery

[a]IC_{50} value of compounds against each tumor cell line, defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*. The data are presented as the mean ± SEM of at least three distinct experiments.

[b]Etoposide served as positive control.

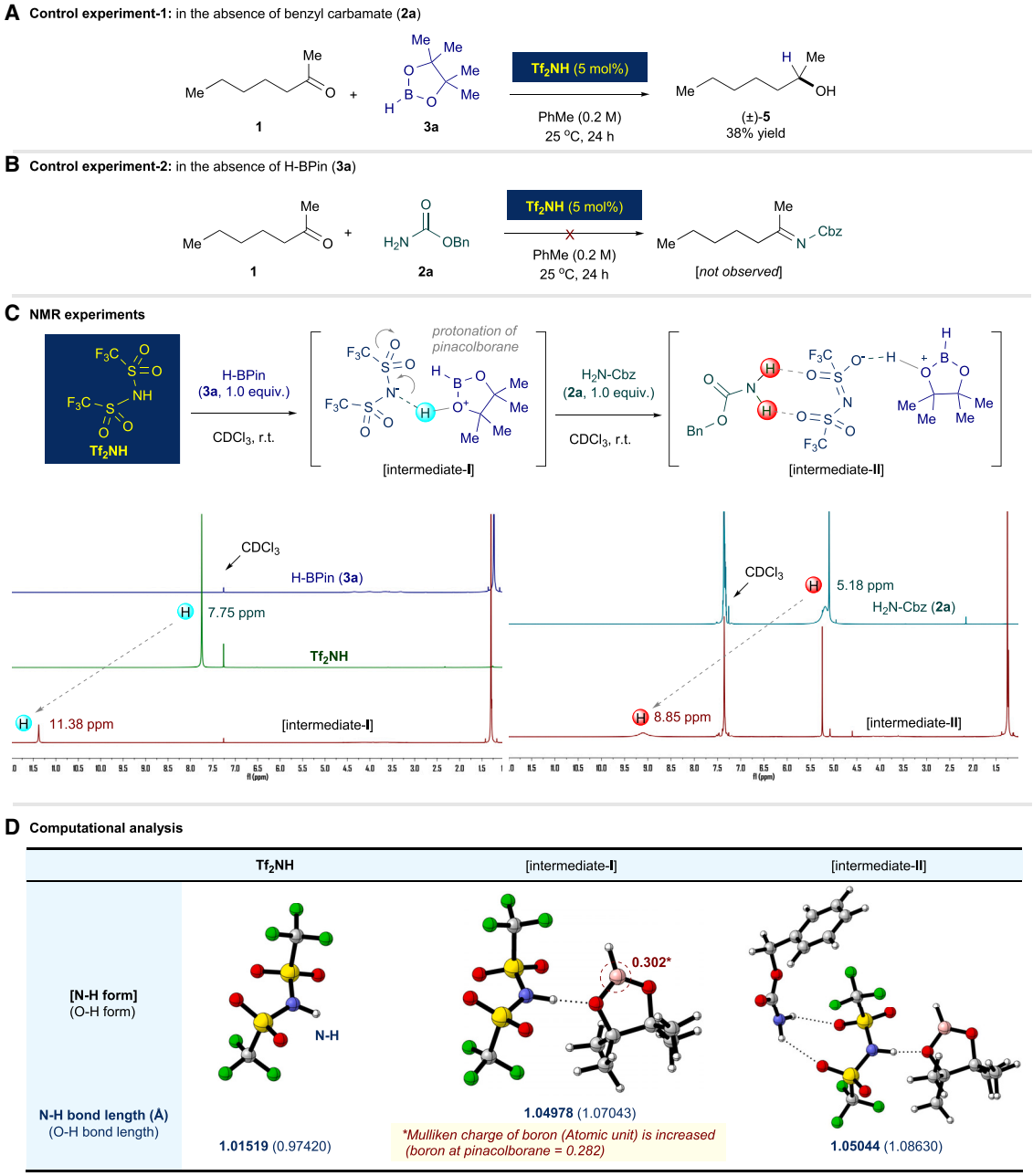


Figure 8. Mechanistic investigation

(A) Control experiment 1: in the absence of benzyl carbamate (**2a**).

(B) Control experiment 2: in the absence of H-BPin (**3a**).

(C) NMR experiments.

(D) Computational analysis.

(Figure 8A). Without compound **3a**, the *N*-Cbz ketimine, a plausible product precursor, was not observed, which may be due to the low nucleophilicity of amine **2a** under single-catalyst activation. Therefore, it is likely that compound **3a** operates not only as a reductant but also as a co-catalyst (Figure 8B).

We then performed ^{1}H NMR spectroscopy analysis to observe the catalytically active species (Figure 8C). When H–BPin (**3a**) was added to the $CDCl_3$ solution of Tf_2NH (1:1 = mol:mol) at room temperature (r.t.), a remarkable downfield shift of the proton peak at the acid catalyst (δ = 7.75 to 11.38 ppm, a singlet) was observed: this fact indicates that protonated H-BPin complex (intermediate-**I**) is generated. Subsequently, benzyl carbamate (**2a**, 1.0 equiv) was added to the mixture, a proton peak at the acid catalyst (δ = 11.38 ppm) disappeared, and a significant downfield shift of the NH_2 proton at benzyl carbamate (**2a**) was observed (δ = 5.18 to 8.85 ppm, a broad singlet). This interaction perhaps indicates the formation of the plausible ternary complex intermediate-**II**.

To support experimental and analytical observations, a systematic computational study to elucidate catalytically active species was conducted (Figure 8D). Density functional theory (DFT)-based optimization of the Tf_2NH with H–BPin (**3a**) structures led to the formation of a complex in which the proton at Tf_2NH (N–H form) is bound to the oxygen atom of compound **3a** by hydrogen-bonding interaction. Due to Tf_2NH being able to exist as a tautomerized O–H form in solution,[41] both N–H and O–H models were investigated. The length of the N–H/O–H bond stretched from 1.01519/0.97420 (single acid) to 1.04978/1.07043 Å [intermediate-**I**] respectively, which very well matches with the ^{1}H NMR observation. Based on the charge analysis, an increased positive value of 0.302 for the [intermediate-**I**] was observed where 0.282 for simple H-BPin due to the decrease of the electronic charge density of the boron center. As a result, the increased negative value of oxygen electron density makes higher Lewis acidity (of the boron center, increased by protonation). Further addition of benzyl carbamate **2a** provided a ternary hydrogen-bonding complex, and the N–H/O–H bond stretched to 1.05044/1.08630 Å [intermediate-**II**] (where $N–H^1$ and $N–H^2$ [1.00393 and 1.00456 Å, respectively] at compound **2a** stretched to 1.00627 and 1.00706 [N–H form] and 1.00613 and 1.00791 Å [O–H form] at [intermediate-**II**]; see section 7 in the supplemental experimental procedures).

Based on our preliminary experimental, analytical, and computational studies, we suggest a plausible catalytic cycle for the reductive amination as displayed in Figure 9. We additionally observed that the ^{19}F NMR peak of Tf_2NH is slightly shifted[42] when [intermediate-**I**] is formed via protonation. This result may be due to the electron delocalization of the weakly coordinating triflimidate (δ_F = −74.96 to −75.44 ppm; see Figure S3 for spectra and detailed discussion). The carbonyl group of starting ketone **1** is activated by the Lewis acidic boron center on [intermediate-**II**], enabling the formation of a covalent carbon–nitrogen bond with *N*-Cbz amine source, which affords to compound **A** (detected by *in situ* high-resolution mass spectrometry [HR-MS] supercritical fluid chromatography quadrupole-time-of-flight [SFC-QTOF] analysis; $[C_{21}H_{34}BNO_5 + Na]+$, *m/z* 414.2432 [expected] and 414.2434 [detected]; Figure S6). This fact supports that it is likely to facilitate the transient formation of reactive N-Cbz ketimine, which is inaccessible without **3a** (Figure 8B).

According to the crude ^{11}B NMR (^{1}H-decoupled) analysis, boric acid pinacol ester (HO–Bpin) was observed in the reaction mixture (δ_B = 21.64 ppm).[28] Perhaps the release of the compound is a driving force of the imine formation. Further acid-catalyzed reduction of imine via hydroboration with another equivalent of H–BPin afforded the desired product **4** and bispinacolatobiborate (PinB–O–Bpin), which could be detected by ^{11}B NMR (δ_B = 22.67 ppm)[28] and HR-MS SFC-QTOF ($[C_{12}H_{24}B_2O_5 + H]^+$, *m/z* 271.1883 [expected] and 271.1878 [detected]) analyses (see Figures S4 and S5 for detailed data).

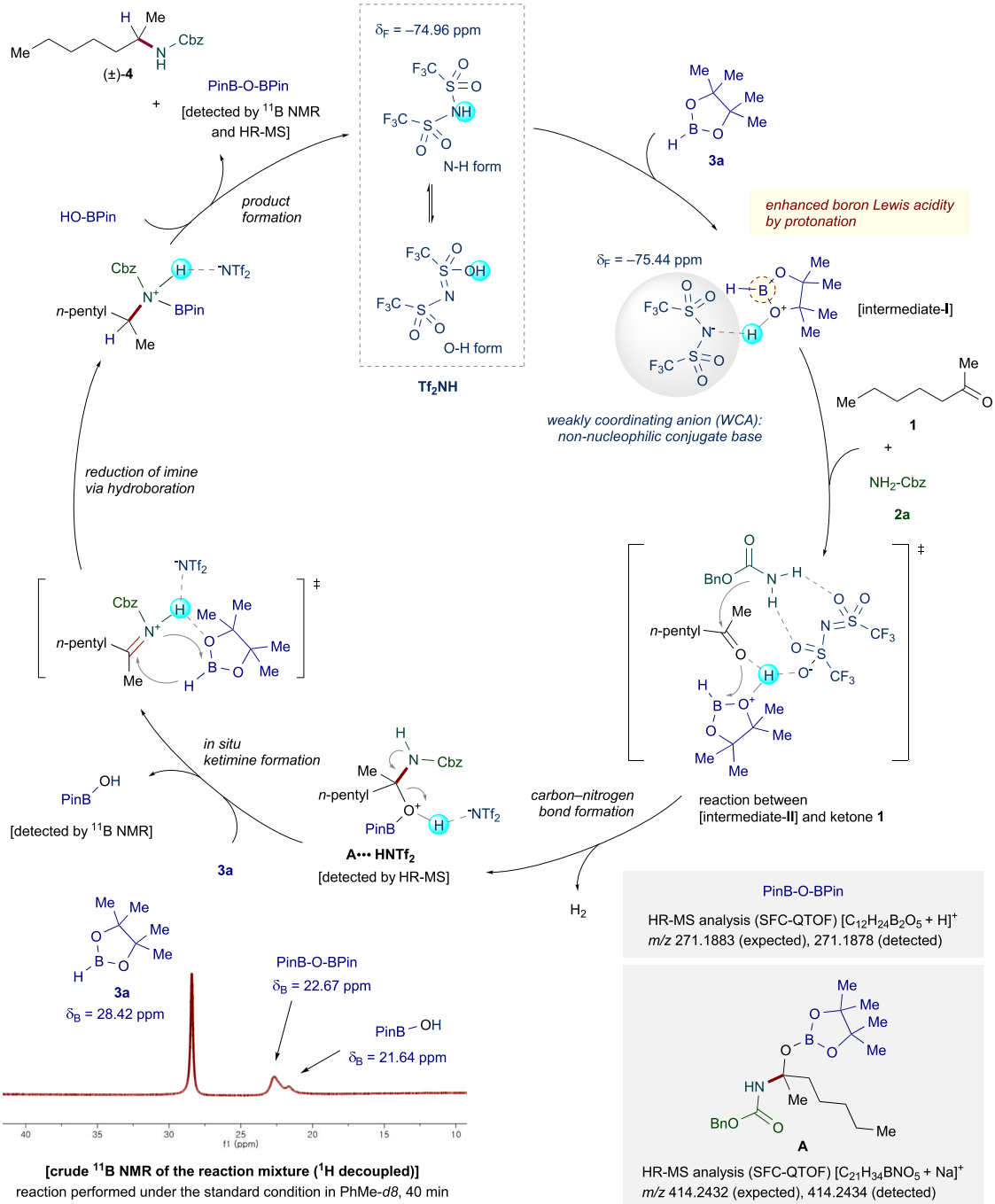


Figure 9. Proposed catalytic cycle

The carbonyl group of ketone **1** is activated by the Lewis acidic boron center in intermediate **II**, forming a key covalent C–N bond.

In conclusion, we developed a pinacolborane-assisted organic superacid catalysis for direct access to alkyl *N*-Cbz amines using ketones and aldehydes as the starting material. The robust catalytic efficiency (up to >99% yield) achieved a general substrate scope with reliable preparative scalability. Experimental, analytical, and computational approaches support the *in situ* formation of a protonated complex of pinacolborane, unlike the previously reported boron Lewis acid complex through H_2 evolution, which efficiently initiates the challenging reductive amination with electron-deficient carbamates. More

importantly, bioactivity evaluations for cytotoxicity against human cancer cell lines were described for the first time. This study can be a starting point for the development of potential anticancer small-molecule drugs. We anticipate that our study might be beneficial for efficient access to further bioactive target-oriented alkyl amines for drug discovery.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Han Yong Bae (hybae@skku.edu).

Materials availability

Chemicals were purchased from commercial vendors (e.g., Aldrich, Alfa Aesar, Combi-Blocks, TCI) and used as received unless otherwise stated. Anhydrous solvents, NMR solvents, and additional organic solvents were purchased from commercial vendors (e.g., Aldrich, Alfa Aesar, CIL, Merck, Wako) and used without further distillation or purification.

Data and code availability

All data generated and analyzed during this study are included in this article and its supplemental information files.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrp.2024.101786.

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AUTHOR CONTRIBUTIONS

W.H.K., P.G., and H.Y.B. conducted the catalytic reaction development. S.B.S., Y.K.C., S.H., and Y.O. conducted DFT calculations and wrote the paper. D.E.L., S.C., W.H.J., S.U.C., K.H.K., and J.H. conducted the biological experiments and wrote the paper. H.Y.B. designed the overall experiments, wrote the paper, and oversaw the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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