

Communication

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Improvement of the C-glycosylation Step for the Synthesis of Remdesivir

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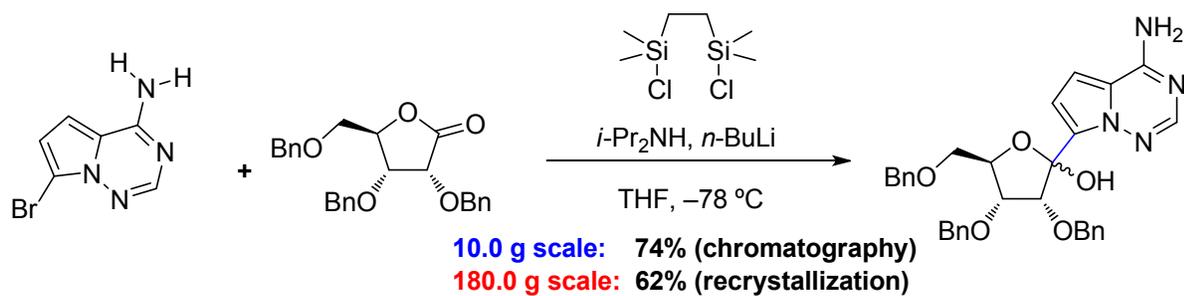
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For Table of Contents Only



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2
3 ABSTRACT: The bulk supply of the antiviral *C*-nucleoside analogue remdesivir is largely
4 hampered by a low-yielding *C*-glycosylation step in which the base is coupled to the pentose
5 unit. Here we disclose a significantly improved methodology for this critical transformation. By
6 utilizing diisopropylamine as a cost-effective additive, the addition reaction furnishes an optimal
7 yield of 75% of the desired ribofuranoside adduct, representing the highest yield obtained thus
8 far for this key step. The method proved suitable for hectogram scale synthesis without column
9 chromatographic operations.
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21 KEYWORDS: Diisopropylamine, *C*-glycosylation, Remdesivir, COVID-19
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INTRODUCTION

The global outbreak of Corona Virus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a severe threat to human health.¹⁻⁵ Compared to its relatives (e.g., SARS-CoV and MERS-CoV), the new coronavirus exhibits a more significant person-to-person transmission tendency. Most importantly, no drugs or vaccines have thus far been approved to treat infections of SARS-CoV-2, which seems to render this pandemic uncontrollable, with the number of infected cases worldwide exceeding ten million. Under such circumstances, several existing antiviral drugs and drug candidates are actively evaluated for their therapeutic potential for this disease.^{6,7,8} Among them, remdesivir (GS-5734, **1**; Figure 1) represents one of the most promising compounds.^{8,9,10}

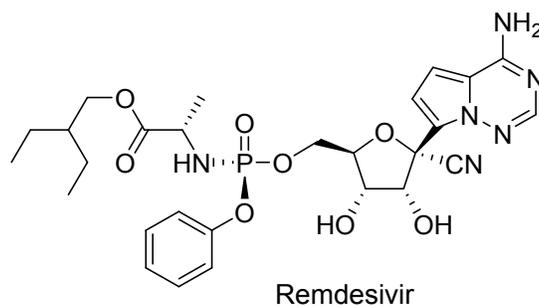


Figure 1. Structure of remdesivir.

Remdesivir is an investigational small-molecule drug originally developed to treat Ebola virus infections and later found to exhibit broad-spectrum activity against various RNA viruses.¹¹⁻¹⁷ In particular, a recent study (published on February 4th, 2020) clearly demonstrated that remdesivir was effective against SARS-CoV-2 in vitro with an EC₅₀ of 0.77 μ M in Vero E6 cells.¹⁷ Of note, approximately one week prior to this report, Holshue et al disclosed that the first COVID-19 patient in the USA had gradually recovered after receiving a compassionate

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3 administration of remdesivir.¹⁸ Subsequently, a number of clinical trials have been launched
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5 worldwide to evaluate the efficacy and safety of remdesivir,¹⁹ and other compassionate use of
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7 this molecule has also been initiated,²⁰ in the hope that it would benefit the treatment of the
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9 epidemic COVID-19. Although the earliest clinical trials conducted in China did not yield
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11 positive results in terms of reducing the death rate of severely affected patients due to
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13 insufficient data,²¹ preliminary data from a clinical trial led by the National Institute of Allergy
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15 and Infectious Diseases (NIAID) suggest a promising therapeutic effect of remdesivir.²² On May
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17 1st 2020, the Food and Drug Administration (FDA) issued an Emergency Use Authorization
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19 (EUA) for remdesivir to treat COVID-19 patients.²² Once further evidence supports the broad
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21 clinical use of remdesivir, the bulk supply of the active pharmaceutical ingredients required for
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23 the production of remdesivir would be an urgent concern.²³
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30 Structurally, remdesivir consists of three fragments: an adenine analogue (base), a pentose
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32 unit, and a phosphoramidate side chain. A key challenge in the preparation of this *C*-nucleoside
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34 analogue is the coupling of the ribose and base moieties. Previously, two-generation syntheses of
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36 remdesivir, including three different methods for accessing ribofuranoside **4**, have been
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38 developed by chemists at Gilead Sciences (**2** + **3** to **4**; Figure 2).^{11,12,24-27} Temporary silyl
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40 protection of the free primary amine group in the base fragment **2** was required in the addition
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42 reaction between **2** and **3**. Two silyl reagents (i.e., trimethylchlorosilane and 1,1,4,4-tetramethyl-
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44 1,4-dichloro disilylethylene (**5**)) were employed to generate the corresponding protected
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46 intermediates **6** and **7**,²⁸ respectively. In the first generation synthesis, subjection of bromide **2a**
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48 and ribolactone **3** to the conditions of TMSCl and *n*-BuLi at -78°C furnished the desired *C*-
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50 glycosylation product **4** in a low yield of 25% (method A; Figure 2).^{24,25} Alternatively, the use of
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52 disilane **5** and NaH in this transformation could deliver **4** in a reported yield of 60% on 4.4 g
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3 scale (method B; Figure 2).^{12,26,27} The authors of the original studies added that “*the efficiency of*
4 *both conditions (in the first generation synthesis) was suboptimal as the yields were capricious*
5 *and highly dependent on the cryogenic temperatures and the rate of *n*-BuLi addition required for*
6 *the transformation*”.¹² In the second generation synthesis (method C; Figure 3), iodide **2b** was
7 employed as the source of the base unit, with a combination of TMSCl, PhMgCl, and *i*-
8 PrMgCl·LiCl reagents, which resulted in a 40% yield of **4**.^{11,25,26} We attempted to reproduce
9 these documented procedures on a decagram scale^{11,25,26} which resulted in the isolation of **4** with
10 yields in the range of 21–42% (see Table S1 in the Supporting Information). Evidently, none of
11 the three above approaches efficiently addressed this problematic synthetic step, and the
12 aforementioned techniques are not suitable for scale-up preparation of remdesivir. Until very
13 recently, during the course of our manuscript preparation, the Gilead scientists reported an
14 optimization of method C for the critical *C*-glycosylation step, in which NdCl₃ and *n*-Bu₄NCl
15 were employed to facilitate the addition of lactone **3** with TMS-protected amine **2b** (Method D;
16 Figure 2).²⁹ The 282 kg scale-up synthesis of compound **4** with 69% yield proved the reliability
17 of this method. In this report, we describe an alternative improvement for the *C*-glycosylation
18 step, which renders the synthesis of remdesivir simple and efficient, with the aim of meeting the
19 demand for its large-scale synthesis.
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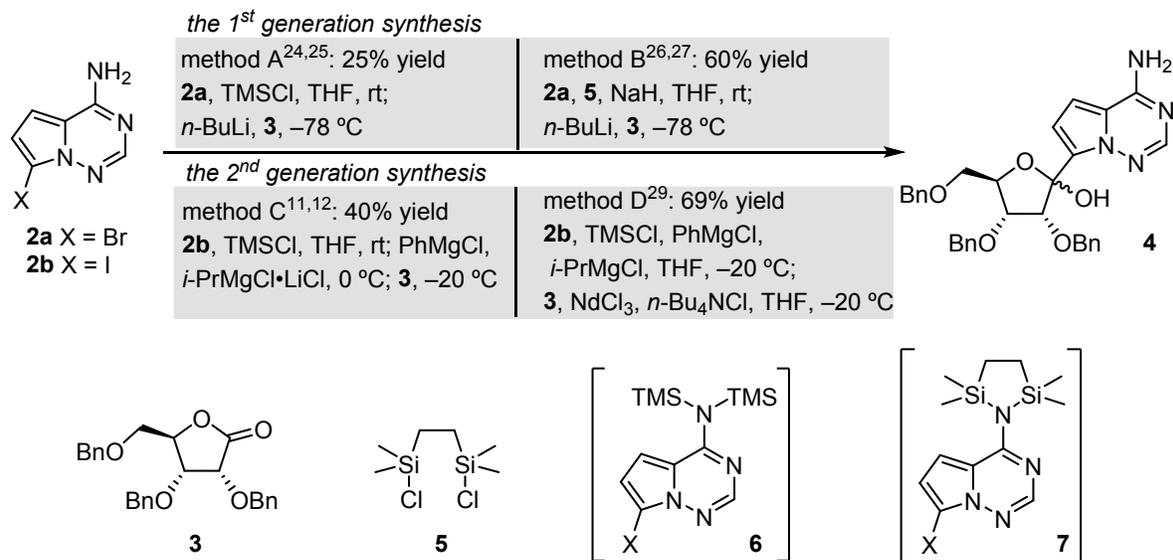


Figure 2. Reported methods for accessing a key intermediate (**4**) in the remdesivir synthesis.

RESULTS AND DISCUSSION

Efficient protection of the free amine group in **2** would be essential for the successful addition reaction of base **2** to lactone **3**. We envisioned that the addition of a secondary amine might be helpful for improving the reaction efficiency (Figure 3). First, the secondary amine could serve to activate disilane **5** through the formation of a disilyl substituted cyclic quaternary amine salt **8**,³⁰ thereby promoting the exchange of the silyl groups between the inactive primary amine in **2** and the secondary amine to form cyclic disilazane **7**. Second, a stabilized lithium intermediate **9** could be formed in the presence of the secondary amine after lithium-halogen exchange with **7**, which would subsequently facilitate the addition of the base motif to lactone **3** to yield the desired ribofuranoside **4**.

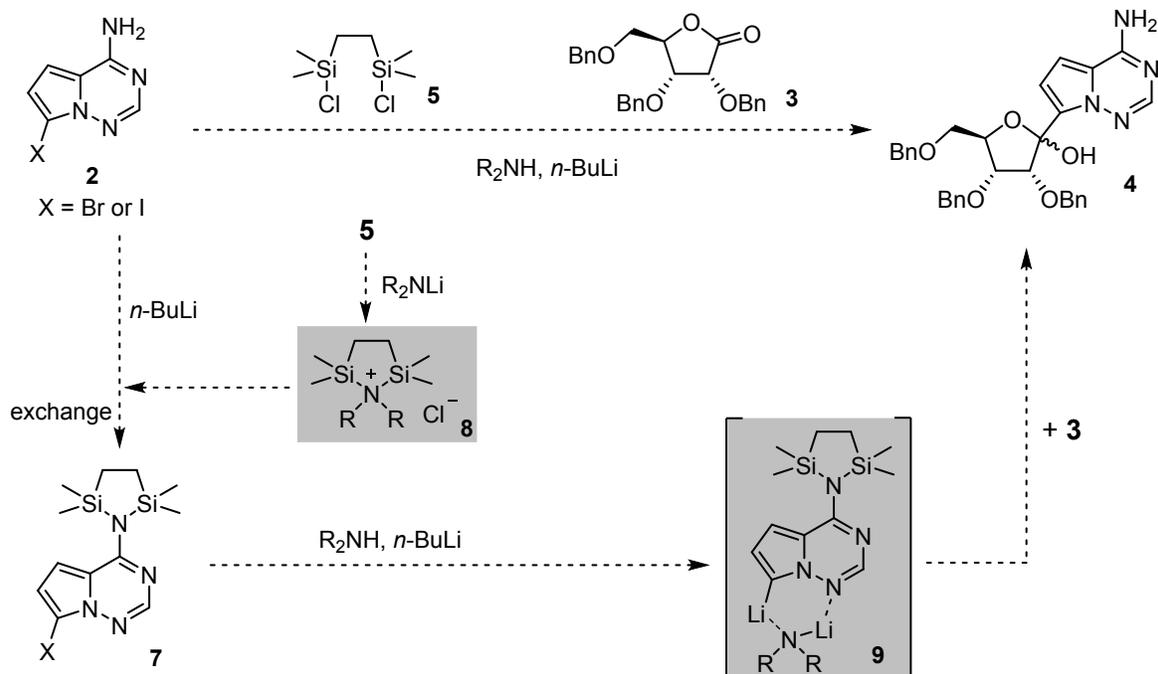
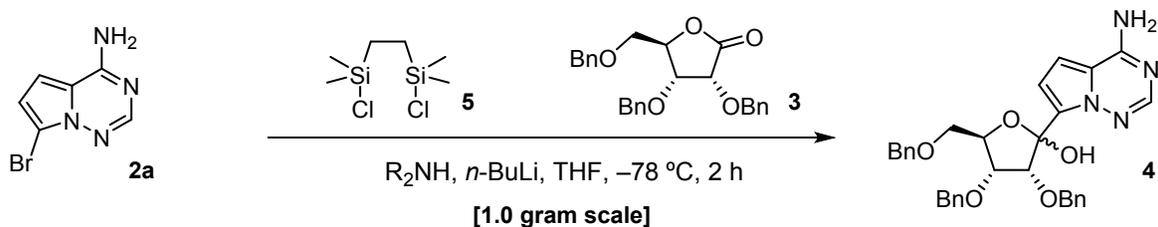


Figure 3. Working hypothesis for an improved synthesis of **4**.

With the above working hypothesis in mind, we initiated our studies by performing the reactions with bromide **2a** (1.0 gram scale) and lactone **3** employing disilane **5** in the presence of $n\text{-BuLi}$ with various secondary amines (Table 1). When secondary amines bearing two linear alkyl groups (such as diethylamine, dipropylamine, and dibutylamine) were used, the addition reactions proceeded to afford **4** in moderate yields (41–54%, entries 1–3). To our delight, bulkier alkyl substituents in the secondary amine were beneficial for improving the reaction efficiency. As a result, conducting the reaction in the presence of diisopropylamine led to the generation of **4** in 74% yield (entry 4). Notably, similar results were obtained with other bulky secondary amines: diisobutylamine (71%, entry 5), dicyclohexylamine (70%, entry 6), and 2,2,6,6-tetramethylpiperidine (74%, entry 7). For the purposes of comparison, the use of tertiary amines was likewise evaluated. For instance, we performed the addition reaction of **2a** and **3** under the

identical conditions of entry 4, except for the replacement of diisopropylamine with diisopropylethylamine, which resulted in a lower yield (49%, entry 8) of product **4**.

Table 1. Effect of Organic Amines in the Addition Reaction^a



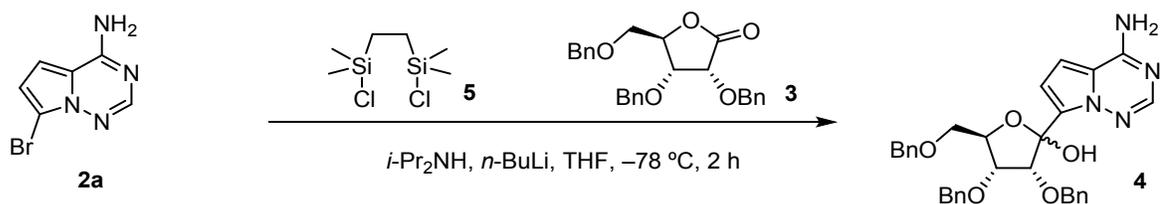
Entry	R ₂ NH	Yield (%)	Entry	R ₂ NH	Yield (%)
1		41	5		71
2		45	6		70
3		54	7		74
4		74	8		49

^a Reactions were conducted on 1.0 gram scale of **2a** with 1.1 equiv. of **5**, 1.1 equiv. of R₂NH, 4.3 equiv. of *n*-BuLi, and 2.0 equiv. of **3** at 0.08 mol/L concentration unless otherwise stated. Yields were determined according to the isolated materials through column chromatography.

Based on the above preliminary results, we then carried out scale-up experiments employing diisopropylamine as an additive (Table 2). A 10 g-scale addition of **2a** to lactone **3**

was performed according to the conditions described in Table 1, leading to the generation of adduct **4** in 75% yield (entry 1). To further improve the practicality of the addition process, we sought to reduce the amount of solvent and increase the reaction concentration. Specifically, a 74% yield was observed when conducting the reaction at the concentration of 0.20 mol/L (entry 2), comparable to that of 0.08 mol/L (entry 1), while the reaction at 0.25 mol/L led to a slightly diminished yield (70%, entry 3). Moreover, effect of the equivalents of lactone **3** was investigated. Lowering the loading of **3** delivered inferior results (i.e., 1.8 equiv., 62%, entry 4; 1.6 equiv., 59%, entry 5) compared to that obtained with 2.0 equiv. of **3**. With the optimized reaction conditions (entry 2), a 180 g-scale experiment was conducted, which occurred smoothly to provide **4** with 62% yield and 99.3% purity after recrystallization without the need of column chromatographic operations (entry 6).

Table 2. Further optimization studies and scale-up synthesis^a



Entry	Scale of 2a	Equiv. of 3	Concentration (mol/L)	Yield (%)
1	10.0 g	2.0	0.08	75 ^b
2	10.0 g	2.0	0.20	74 ^b
3	10.0 g	2.0	0.25	70 ^b

4	10.0 g	1.8	0.20	62 ^b
5	10.0 g	1.6	0.20	59 ^b
6	180.0 g	2.0	0.20	62 ^c

^a Reactions were conducted with 1.1 equiv. of **5**, 1.1 equiv. of *i*-Pr₂NH, and 4.3 equiv. of *n*-BuLi unless otherwise stated. ^b Yields were calculated based on the materials isolated through column chromatography. ^c The yield was obtained after recrystallization.

CONCLUSION

In summary, the crucial *C*-glycosylation step that ordinarily limits the overall efficiency of remdesivir synthesis has been significantly improved by the use of a cost-effective secondary amine, diisopropylamine. Thus, subjecting the base unit **2a** and ribolactone **3** to the optimal reaction conditions delivered up to 75% yield of the anticipated ribofuranoside **4**, which, to the best of our knowledge, represents the highest yield obtained for this critical transformation thus far. Furthermore, the procedure is amenable to hectogram scale synthesis without column chromatographic operations, and as such would be highly advantageous for the large-scale manufacture of the antiviral, remdesivir.

EXPERIMENTAL DETAILS

General Information. All reagents were purchased from commercial suppliers and were used without further purification. The product of the decagram scale synthesis was purified by flash column chromatography on silica gel (200 – 300 meshes) from the Anhui Liangchen Silicon Material Company in China. ¹H NMR and ¹³C NMR spectra were recorded on Varian INOVA-400/54, Agilent DD2-600/54 and calibrated by using deuterated dimethyl sulfoxide (DMSO-d₆,

¹H NMR: δ = 2.50, ¹³C NMR: δ = 39.5) unless otherwise noted. High-resolution mass spectra (HRMS) were recorded on Agilent LC-MSD TOF ESI mass spectrometers.

General Procedure for Synthesis of Compound 4 by Using Different Amines. To an oven-dried round-bottom flask equipped with a stir bar was added 7-bromopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**2a**, 1.00 g, 4.694 mmol, 1.0 equiv.). The flask was evacuated and backfilled with argon for three times. Under the protection of argon, anhydrous THF (40 mL) was added and the mixture was stirred at room temperature for 5 min before a solution of 1,2-bis(chlorodimethylsilyl) ethane (**5**, 1.111 g, 5.163 mmol, 1.1 equiv.) in anhydrous tetrahydrofuran (5 mL) was added. The resulting mixture was stirred for 10 min. Then amine (5.163 mmol, 1.1 equiv.) was added. After stirring at room temperature for 5 min, the resulting mixture was cooled to -78 °C over 20 min. At this point, *n*-BuLi (2.5 M in *n*-hexane, 8.1 mL, 20.18 mmol, 4.3 equiv.) was added dropwise over 30 min through a syringe to the mixture and the reaction was allowed to stir at -78 °C for additional 15 min. Next, a solution of lactone **3** (3.930 g, 9.388 mmol, 2.0 equiv.) in anhydrous THF (5 mL) was added dropwise over 10 min under the argon atmosphere through a syringe. After the reaction was stirred at -78 °C for 2 h, it was allowed to warm slowly to 0 to 10 °C. The reaction was quenched by adding 1 M citric acid (50 mL) and stirred vigorously for 15 min. Then the layers were separated and the aqueous layer was extracted with EtOAc (3 \times 30 mL). The organic layers were combined and washed sequentially with water (1 \times 50 mL), saturated aqueous NaHCO₃ (1 \times 50 mL) and brine (1 \times 50 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (50% EtOAc in petroleum ether to 100% EtOAc to 10% MeOH in EtOAc) to give **4** as white foam.

Decagram Scale Synthesis of Compound 4 Under the Optimized Reaction Conditions. An oven-dried three-neck round-bottom flask equipped with a stir bar and an additional funnel was charged with 7-bromopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**2a**, 10.0 g, 46.94 mmol, 1.0 equiv.). The above flask was evacuated and backfilled with argon for three times. Under the protection of argon, anhydrous THF (80 mL) was added and the mixture was stirred at room temperature for 5 min before a solution of 1,2-bis(chlorodimethylsilyl) ethane (**5**, 11.1 g, 51.63 mmol, 1.1 equiv.) in anhydrous tetrahydrofuran (20 mL) was added. The resulting mixture was stirred for 10 min. Then diisopropylamine (7.3 mL, 51.63 mmol, 1.1 equiv.) was added. After stirring at room temperature for 5 min, the resulting mixture was cooled to $-78\text{ }^{\circ}\text{C}$ over 20 min. At this point, *n*-BuLi (2.5 M in *n*-hexane, 81 mL, 201.8 mmol, 4.3 equiv.) was added dropwise over 45 min through the additional funnel to the mixture. *Note: the dropwise addition of n-BuLi is essential for this exothermic step. The addition time depends on the volume of n-BuLi, during which the reaction temperature should be kept below $-78\text{ }^{\circ}\text{C}$. In the decagram scale case, a 45 min period could guarantee the complete formation of lithium intermediate.* After the additional funnel was rinsed by anhydrous THF, a solution of lactone **3** (39.3 g, 93.88 mmol, 2.0 equiv.) in anhydrous THF (50 mL) was added dropwise over 20 min under the argon atmosphere, and the reaction was allowed to stir at $-78\text{ }^{\circ}\text{C}$ for 2 h. Then the cold bath was removed and the mixture was slowly warmed to 0 to $10\text{ }^{\circ}\text{C}$. The reaction was quenched by adding 1 M citric acid (200 mL) and stirred vigorously for 15 min. Then the layers were separated and the aqueous layer was extracted with EtOAc ($3 \times 200\text{ mL}$). The organic layers were combined and washed sequentially with water ($1 \times 250\text{ mL}$), saturated aqueous NaHCO_3 ($1 \times 250\text{ mL}$) and brine ($1 \times 250\text{ mL}$). The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (50% EtOAc in petroleum ether

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3 to 100% EtOAc to 10% MeOH in EtOAc) to give the mixture of isomers **4** (19.2 g, 74%) as
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5 white foam. The analytical data were consistent with data previously reported in the literature.¹¹

6
7 **¹H-NMR (400 MHz, DMSO-*d*₆)**: δ 8.10 (br s, 2H), 8.02 (s, 1H), 7.37 – 7.23 (m, 11H), 7.17 –
8
9 7.12 (m, 3H), 7.03 – 7.01 (m, 2H), 6.98 (d, *J* = 4.0 Hz, 1H), 5.41 (d, *J* = 4.0 Hz, 1H), 5.10 (d, *J* =
10
11 8.0 Hz, 1H), 4.61 – 4.56 (m, 2H), 4.54 – 4.47 (m, 4H), 4.08 – 3.99 (m, 1H), 3.98 – 3.95 (m, 1H),
12
13 3.71 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.50 (dd, *J* = 8.0, 4.0 Hz, 1H); **¹³C-NMR (100 MHz, DMSO-*d*₆)**:
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15 δ 187.97, 155.86, 148.98, 138.63, 138.45, 138.42, 138.15, 128.63, 128.25, 128.22, 128.16,
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17 128.15, 127.83, 127.77, 127.73, 127.64, 127.61, 127.55, 127.49, 127.46, 127.42, 127.28, 127.22,
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19 127.11, 127.04, 118.57, 117.52, 103.12, 102.27, 81.89, 80.85, 72.48, 72.31, 71.70, 71.40, 69.37;
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24 **HRMS (ESI)**: *m/z* calcd. for C₃₂H₃₂N₄NaO₅ [M + Na]⁺ 575.2265; found 575.2260.
25
26

27 **Hectogram Scale Synthesis of Compound 4 Under the Optimized Reaction Conditions.**

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29 An oven-dried 20 L reactor was charged with 7-bromopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**2a**,
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31 180.0 g, 0.845 mol, 1.0 equiv.) and anhydrous THF (1.44 L) under an atmosphere of nitrogen at
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33 20 °C. The mixture was stirred for 10 min before a solution of 1,2-bis(chlorodimethylsilyl)
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35 ethane (**5**, 200.0 g, 0.929 mol, 1.1 equiv.) in anhydrous tetrahydrofuran (360 mL) was added at
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37 the same temperature. The resulting mixture was stirred for 15 min. Then diisopropylamine (94.1
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39 g, 131 mL, 0.929 mol, 1.1 equiv.) was added. After stirring for 10 min, the reaction was cooled
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41 to –85 to –78 °C. At this point, *n*-BuLi (**4**, 2.5 M in *n*-hexane, 1.45 L, 3.634 mol, 4.3 equiv.) was
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43 added dropwise over 4 h while maintaining the reaction temperature below –78 °C during the
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45 exothermic addition, and the resulting mixture was allowed to stir at –85 to –78 °C for 30 min.
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49 Next, a solution of lactone **3** (707.2 g, 1.69 mol, 2.0 equiv.) in anhydrous THF (900 mL) was
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51 added dropwise over 3 h while maintaining the temperature between –85 to –78 °C. After the
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53 reaction was stirred at the same temperature for another 2 h, it was allowed to warm slowly to 0
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3 to 10 °C. The reaction was quenched by the addition of 1 M citric acid (3.6 L) and the
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5 temperature was maintained below 25 °C while vigorously stirring for 10 min. Then the layers
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7 were separated and the aqueous layer was extracted with EtOAc (3 × 3.6 L). The organic layers
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9 were combined and washed sequentially with water (1 × 3.6 L), saturated aqueous NaHCO₃ (1 ×
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11 3.6 L) and brine (1 × 3.6 L). The organic phase was then concentrated in vacuo at 30 °C to
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13 remove the majority of organic solvents. The residual solvents were removed by adding *tert*-
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15 butyl methyl ether (3.6 L) and evaporated at 30 °C under reduced pressure. The mixture was
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17 finally concentrated in vacuo to a total batch volume of 540 mL, whereupon *n*-heptane (3600
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19 mL) was added. The resulting mixture was heated to 50 °C and stirred at this temperature for 30
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21 min. Then it was allowed to cool to 0 °C. The resulting pale yellow solid was collected by
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23 filtration. The filter cake was rinsed with *n*-heptane (540 mL) and dried under vacuum at 30 °C
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25 to afford pale yellow solid of product **4** (291.5 g, 62%). Purity: 99.3% (Flow rate: 1.0 mL/min;
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27 Column: Waters XTERRA MS C18 Column, 4.6 × 150 mm, 3.5 μm; Temperature: 45 °C;
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29 Mobile phase A: water with 0.1% (v/v) trifluoroacetic acid; Mobile phase B: acetonitrile with
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31 0.1% (v/v) trifluoroacetic acid; Gradient elution: mobile phase A/B = 95:5 (0-5 min) to mobile
32
33 phase A/B = 5:95 (5-32 min) to mobile phase A/B = 95:5 (32-40 min), *t_R* = 19.272 min).
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44 ASSOCIATED CONTENT

45 46 47 **Supporting Information.**

48 The Supporting Information is available free of charge at DOI:

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52 NMR spectra of compounds; (PDF)
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Notes

The authors declare no competing financial interest.

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