# Expanding Access to Remdesivir via an Improved Pyrrolotriazine Synthesis: Supply Centered Synthesis

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Supplemental Information:

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General Remarks

**Instrumentation:** For all compounds, H and C NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer. Chemical shifts were measured relative to the residual solvent resonance for $^1$H and $^{13}$C NMR (CDCl$_3$ = 7.26 ppm for $^1$H and 77.2 ppm for $^{13}$C, DMSO-$d_6$ = 2.50 ppm for $^1$H and 39.2 ppm for $^{13}$C). Coupling constants $J$ are reported in hertz (Hz). The following abbreviations were used to designate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet, p, pentet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, double of triplet; ddt, doublet of doublet of triplet; m, multiplet; br, broad. Reactions were monitored by HPLC using the methods indicated. 2-Cyanopyrrole, N-amino-2-cyanopyrrole and triazine were monitored using identical HPLC methods (see Analytical Method Section for details). Glassware was oven-dried at 120 °C, assembled while hot, and cooled to ambient temperature under an inert atmosphere. Unless noted otherwise, reactions involving air sensitive reagents and/or requiring anhydrous conditions were performed under a nitrogen atmosphere.

**Reagents and solvents.** Reagents and solvents were purchased from Aldrich Chemical Company, Fisher Scientific, Alfa Aesar, Acros Organics, Oakwood, or TCI. Liquid reagents were purified by distillation when necessary. Unless otherwise noted, solid reagents were used without further purification. Methylene chloride (DCM) and dimethylformamide (DMF) taken from a solid-sorbant Solvent Dispensing System purchased from Pure Process Technologies or distilled as described in the literature.
Representative Procedures:

2-cyanopyrrole (Table S2, entry 10):

A 1 L three necked round bottom flask was equipped with a J-KEM internal temperature probe, overhead stirrer and nitrogen line. The flask was charged with anhydrous DMF (250 mL) and cooled to an internal temperature of 0 – 5 °C under an atmosphere of nitrogen. With stirring (300 RPM), phosphoryl trichloride (38.3 mL, 409.9 mmol, 1.1 eq.) was slowly charged while maintaining internal temperature below 15 °C. The reaction mixture was stirred at 20 °C for another 30 minutes. The reaction was cooled to 0 – 5 °C and pyrrole (25.0 g, 372.6 mmol, 1.0 eq.) was slowly charged while maintaining internal temperature below 15 °C. The reaction mixture was stirred at 20 °C for another 1h. The light brown solution was cooled to 0 – 5 °C and water (75 mL) was added while maintaining internal temperature below 15 °C. The reaction was stirred for 5 minutes at same temperature. Solid hydroxylamine hydrochloride (28.48 g, 409.9 mmol, 1.1 eq.), acetic anhydride (38.7 mL, 409.9 mmol, 1.1 eq.) and pyridine (150 mL, 1.863 mol, 5.0 eq.) were added sequentially, the internal temperature was not allowed to exceed 15 °C. After the addition was complete, the reaction mixture was heated to 90 °C (heating block) for overnight (16 hours). After complete consumption of the iminium chloride salt, as indicated by ¹H NMR, the reaction mixture was diluted with water (250 mL) and transferred to the separating funnel. The product was extracted from dark brown reaction mixture by using ethyl acetate (3 X 400 mL). The combined organic layer was washed with 1M HCl (250 mL) and brine (250 mL), dried over sodium sulfate and concentrated using rotary evaporation under reduced pressure. The desired 2-cyanopyrrole (34.7 g, 90% (adjusted with 89% purity by HPLC)) was distilled out from dark brown oil under vacuum at 100-140 °C. The obtained product was used for next (amination) step (52 g CSTR batch; in Batch Amination with NH₂Cl from CSTR section).

¹H NMR of intermediate iminium chloride salt (600 MHz, DMSO-d₆) δ 13.52 (br s, 1H), 9.03 (s, 1H), 7.49 – 7.50 (m, 1H), 7.17 – 7.17 (m, 1H), 6.53 – 6.54 (m, 1H) ppm.

¹H NMR of 2-cyanopyrrole (600 MHz, DMSO-d₆) δ 12.29 (br s, 1H), 7.13 – 7.15 (m, 1H), 6.89 – 6.91 (m, 1H), 6.21 – 6.23 (m, 1H) ppm. ¹³C NMR of 2-cyanopyrrole (150 MHz, DMSO-d₆) δ 125.1, 119.9, 115.4, 110.0, 100.0 ppm. Data match those previously reported.¹

Pyrrolotriazine (Table S6, Entry 4):

Monochloramine Synthesis (0.74M in MTBE):
NH₄Cl (24.6 g) in MTBE (330 mL) was cooled to -5 °C (internal temperature) in a 2L-round bottom flask, and concentrated NH₄OH (38.4 mL) was added. Sodium hypochlorite (10-15% solution, Sigma-Aldrich, 432 mL) was then added via addition funnel over 30 min. The mixture was stirred for 30 min, the layers were separated, and the organic layer was washed with brine (1 × 180 mL). The organic layer was dried over powdered CaCl₂ (15 g) in the freezer for at least 1 h and kept at the same temperature. Approximate concentration is 0.74 M.

A 0.5 L three necked round bottom flask was equipped with a J-KEM internal temperature probe and a stirring bar. A solution of 1H-pyrrole-2-carbonitrile (10.0 g, 105.3 mmol, 97% purity) in MTBE (100 mL) was added, cooled in an ice bath (internal temperature between 5-10 °C) and NaH (6.3 g, 158.0 mmol, 60% in mineral oil Sigma-Aldrich) was added in portions. The reaction was stirred for 30 min at room temperature. DMF (50 mL) was added and MTBE was distilled under vacuum at 20-30 °C (internal temperature). Chloramine in MTBE (170 mL, 125.8 mmol, 0.74 M solution) was added. The reaction was monitored by HPLC until conversion is >95% (less than 30 minutes). To the reaction mixture was added formamidine acetate (32.89 g, 315.9 mmol, 99% Chem-Impex). The mixture was heated while distilling MTBE at atmospheric pressure until internal temperature reaches 90-95 °C (heating block). The reaction was stirred at the same temperature for 16 hours. Assay ¹H NMR using 1,3,5-trimethoxybenzene as internal standard showed 75% assay yield to triazine. The mixture was concentrated through distillation under vacuum (40-45 mL of DMF was recovered) and 100 mL of water was added washing the vessel walls. The flask was placed in an ice-bath and the internal temperature was monitored until it reached ~5 °C. The mixture was filtered and washed with 20 mL of water. The brown solids were transferred to 250 mL round bottom flask, dried under vacuum and 100 mL of MTBE was added. The mixture was vigorously stirred until a uniform suspension is observed. The suspension was filtered giving the triazine as a brown powder which was dried under vacuum (8.53 g, 99.0% purity by HPLC, 60% yield).

¹H NMR (600 MHz, DMSO-d₆) δ 7.66-8.01 (m, 3H), 7.61 (s, 1H), 6.91 (s, 1H) 6.61 (s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 155.7, 148.1, 118.2, 114.2, 110.2, 101.5. Data match those previously reported.²

Alternative amination replacing DMF with diglyme: Synthesis of 1-amino-1H-pyrrole-2-carbonitrile (Table S7, Entry 3):

![](image)

To a solution of 1H-pyrrole-2-carbonitrile (400.0 mg, 4.34 mmol) in diglyme (4 mL) was added NaH (261.0 mg, 3.257 mmol, 60% in mineral oil Sigma-Aldrich), and the reaction was stirred for 30 min at room temperature. NH₄Cl (16 mL, ca. 0.56 M in MTBE) was added via syringe. After 1h (97% assay yield), the reaction mixture was passed through Celite, washed with ethyl acetate and solvent was removed under reduced pressure at 80 °C. The crude product was obtained as a brownish oil (603 mg, 65% purity, 84% yield). The mixture was subjected to column chromatography using ethyl acetate and hexanes as eluent solvents (5% → 25%). The pure fractions were concentrated to give the required product as a pale-yellow oil (220 mg, 98% purity, 47% yield).

¹H NMR (600 MHz, CDCl₃) δ 6.95 (s, 1H), 6.72 (d, J = 4.1 Hz, 1H), 6.08 (d, J = 3.6 Hz, 1H), 4.41 (br s, 2H) ppm. ¹³C NMR (150 MHz, CDCl₃) δ 128.1, 117.7, 113.4, 107.1, 105.4 ppm. Data match those previously reported.²
$^1$H NMR spectrum of 1-amino-1H-pyrrole-2-carbonitrile in CDCl$_3$ at 600 MHz:

$^{13}$C NMR spectrum of 1-amino-1H-pyrrole-2-carbonitrile in CDCl$_3$ at 151 MHz:
One-pot Synthesis of 2-Cyanopyrrole from Pyrrole

Table S1: Optimization for synthesis of 2-cyanopyrrole from pyrrole under acidic conditions.

| Entry | Scale (g) | Solvent (V) | ROH (V) | Temp. (°C) | AY (%) | IY (%) (Purity) (%) |
|-------|-----------|-------------|---------|------------|--------|---------------------|
| 1     | 0.5       | DMF (15)    | -       | 125        | 58     | -                   |
| 2     | 0.2       | DMF (10)    | -       | 90         | 25     | -                   |
| 3     | 0.2       | DMF (10)    | H₂O (3) | 90         | 78     | -                   |
| 4     | 0.2       | DMF (10)    | EtOH (3) | 90       | 84     | -                   |
| 5     | 0.25      | DMF (10)    | H₂O (3) | 80         | 74     | -                   |
| 6     | 0.25      | DMF (10)    | H₂O (5) | 80         | 61     | -                   |
| 7     | 0.25      | DMF (10)    | H₂O (10) | 80    | 51     | -                   |
| 8     | 0.25      | DMF (15)    | H₂O (3) | 80         | 74     | -                   |
| 9     | 0.25      | DMF (15)    | H₂O (5) | 80         | 94     | -                   |
| 10    | 0.25      | DMF (15)    | H₂O (10) | 80    | 47     | -                   |
| 11    | 25        | DMF (15)    | H₂O (5) | 80         | 93     | 72 (72)             |

The synthesis began with C-2 formylation of pyrrole and further its oxidation to nitrile. Both reactions are well known in literature, however, 2-formyl pyrrole is a low melting solid not easily distilled or recrystallized in good yield. Moreover, waste will be generated in the process of purifying the aldehyde, aldoxime or other intermediates. We wondered whether isolation of the aldehyde or aldoxime intermediate was necessary. Both reactions can be performed in same solvent, the iminium chloride salt could be used directly to form the nitrile in one-pot. Considering this, we began our efforts with formylation of pyrrole using POCl₃ (1.1 eq.) in DMF (15V) (Table S1, entry 1). The complete conversion of pyrrole to iminium chloride salt was observed in 1h, analyzed by ¹H NMR. According to the literature,¹ the oxidation of 2-formylpyrrole to 2-cyanopyrrole is feasible at higher temperature (125 °C). Keeping this in mind, the hydroxylamine hydrochloride (1.1 equiv.) was added to the reaction mixture and heated at 125 °C for 16 h (overnight). The reaction was dark and sluggish, however, gave 58% assay yield by ¹H NMR (mesitylene was used as internal standard). Further, the assay yield dropped to 25% by lowering the reaction temperature to 90 °C (Table S1, entry 2). The lower yields could be because of chemical incompatibility of hydroxylamine hydrochloride with residual POCl₃ and related species which would need to be quenched. Thus, water or ethanol was added to the reaction mixture prior to addition of hydroxylamine hydrochloride.
The HCl generated in the course of the quench could be used as catalyst for the dehydration of ald oxide. Surprisingly, the reactions gave >80% assay yield under these conditions (Table S1, entry 3 and 4).

**Exploring effect of amount of DMF and water on reaction:**

![Effect of amount of DMF and water](chart)

The effect of amount of solvents like, DMF and water on reaction was examined (Table S1, entry 5-10). The reaction with 15V of DMF and 5V of water provided 94% assay yield. Importantly, the results were reproduced at 25 g batch under similar reaction conditions (Table S1, entry 9). The reaction gave 72 % isolated yield (adjusted with 72% purity). However, the extraction of product was found difficult due to emulsion formation and was not feasible at large scale. This led us to further optimize our reaction conditions to make product isolation easy.

**Table S2:** Optimization for synthesis of 2-cyanopyrrole from pyrrole under basic conditions.

| Entry | Scale (g) | Solvent (V) | ROH (V) | Base (eq.) | Ac₂O (eq.) | Temp. (°C) | AY (%) | IY (%) |
|-------|-----------|-------------|---------|------------|------------|------------|--------|--------|
| 1     | 0.2       | DMF (10)    | H₂O (3) | Pyridine (5) | 1.2        | 90         | 92     | -      |
| 2     | 0.2       | DMF (10)    | EtOH (3) | Pyridine (5) | 1.2        | 90         | 93     | -      |
| 3     | 0.2       | MeCN (10)   | EtOH (3) | Pyridine (5) | 1.2        | 90         | 88     | -      |
| 4     | 0.2       | MeCN (10)   | EtOH (3) | Pyridine (3.5) | 1.2        | 90         | 88     | -      |
| 5     | 25        | MeCN (10)   | EtOH (3) | Pyridine (3.5) | 1.1        | 70         | -      | 81 (58) |
| 6     | 5         | MeCN (10)   | H₂O (3)  | Pyridine (3.5) | 1.2        | 70         | -      | 93 (80) |
| 7     | 25        | MeCN (10)   | H₂O (3)  | Pyridine (3.5) | 1.1        | 70         | -      | 76 (90) |
Next, the reactions were attempted under basic conditions by activating the \textit{in situ} formed oxime with acetic anhydride. Initially, the reactions were performed with pyridine (5 eq.) and acetic anhydride (1.2 equiv.) in DMF (Table 2S, entry 1 and 2). The reactions were smooth and provided excellent assay yields (>90%).

**Reaction screening for alternative solvents and amount of ethanol required for reaction:**

![Solvent screening vs quantity of EtOH](image1)

**Exploring solvent volumes and amount of pyridine:**

![DOE for amount of MeCN and pyridine](image2)
Further, the reaction was screened for an alternative solvents, concentration and reagents. In this regard, few experiments were performed on 0.2 g scale, and it was found that DMF and acetonitrile works well. Acetonitrile would be better compared to DMF, as it could be easily evaporated from reaction which will be helpful for isolation of the product. With this results in hand, the reaction was scaled up to 25 g under similar reaction conditions (Table S2, entry 5). The reaction gave 81% yield with 58% purity, however, triethylphosphate generated by reaction of EtOH with residual POCl₃ and its associated species was problematic for purification process. The addition of water instead of ethanol resolved this problem and reaction provided excellent yield and purity at 5 g and 25 g scale (Table S2, entry 6 and 7). In addition, inorganic bases, NaHCO₃ and NaOH, were tried instead of pyridine giving lower yields (Table S2, entry 8 and 9). We ended up with pyridine as best base for the reaction. Importantly, the reaction reproducibility was demonstrated at 100 g scale (Table S2, entry 11). The product was purified by vacuum distillation and used for next step.

HPLC Trace of Distilled 2-Cyanopyrrole:
As discussed in manuscript, in order to avoid dilute reaction conditions and increase throughput for \( N \)-amination of 2-cyanopyrrole, the solid aminating reagents like, \( O \)-(4-nitrobenzoyl)hydroxylamine (A) and \( O \)-(diphenylphosphinyl)hydroxylamine (B) were planned to use. At the outset, in 10 mL glass vials, the solution of 2-cyanopyrrole (100 mg) in DMF (2 mL) was treated with aminating reagents (A and B; 1.5 eq.; individually) in presence of NaH (1.5 eq.). However, both reactions were very thick (almost dry) and unable to stir with magnetic stir bar. Then, the reactions were attempted with excess amount of DMF (Table S3, entry 1 and 2) and heated at 80 °C overnight (16 h). The reactions showed >90% conversion (by HPLC method). The assay yields were derived by using \( ^1 \)H NMR (mesitylene was used as internal standard). The assay yields showed well agreement with conversion. However, the reactions were highly diluted at this stage. Then, reactions were examined in EasyMax instrument with overhead stirrer employing lower amount of solvents. The reactions were carried out on 500 mg scale with both aminating reagents (A and B; 1.5 eq.; individually) in presence of NaH (1.5 eq.).
B) in DMF (25V and 40V) under similar reaction conditions (Table 3S, entry 3 and 4). The results were excellent, showing ~90% conversion in both reactions.

**Exploring different solvents and bases with O-(4-nitrobenzoyl)hydroxylamine (A) reagent:**

![Solvent and base screening](image1)

**Exploring different solvents and bases with O-(diphenylphosphinyl)hydroxylamine (B) reagent:**

![Solvent and base screening](image2)

Next, we screened the effect of solvents (DMF and NMP) and bases (NaH, KOtBu and KOH) with both aminating reagents (A and B). NMP and KOtBu combination gave the best result. Both reagents showed 94% conversion and assay yields were in good agreement with conversion (Table 3S, entry 5 and 6). Further, these reactions conditions were taken up forward for large scale reaction. The reactions were examined to investigate the lowest solvent volume that could be used on EasyMax (Table 3S, entry 7-10). The reactions were carried out on 1.0 g scale with lowest 10V and 15V of solvent. The reaction with 15V of solvent gave good conversion and assay yield.

**Procedure for synthesis of N-amination of 2-cyanopyrrole:**

A 50 mL glass reactor was equipped in EasyMax instrument with an internal temperature probe, overhead stirrer and nitrogen line. The flask was charged with anhydrous NMP (15 mL) and cooled to an internal temperature of 0 – 5 °C under an atmosphere of nitrogen. With stirring (300 RPM), 2-cyanopyrrole (1.0 g, 10.86 mmol, 1.0 eq.) was charged. Then, solid KOtBu (1.34 g, 11.94 mmol, 1.1 eq.) was added to reaction mixture. The temperature was raised to 10 °C, reactions stirred for 10 minutes. To the reaction mixture
solid O-(diphenylphosphinyl)hydroxylamine (2.78 g, 11.94 mmol, 1.1 eq.) was charged in four portions over 10 minutes. The temperature of reaction was slowly increased to 80 °C and then reaction continued for overnight (16 hours). The consumption of the starting 2-cyanopyrrole was monitored with HPLC (95%) and assay yield (95%) was derived by using 1H NMR.
Investigation of NH₂Cl Extraction

3.00 g of NH₄Cl (57.2 mmol) was added to a 250 mL Erlenmeyer flask along with 4.70 mL of 14.5 M NH₄OH (68.1 mmol). The mixture was cooled to -5 °C and stirred on a magnetic hotplate. 72 mL of bleach (2.2 M, 158 mmol) was added via addition funnel over the course of 15 minutes. The concentration of the bleach was stated as 7.5% NaOCl (7.1% active chlorine) which translates to 15.8 wt% NaOCl and 2.2 M NaOCl as found by iodometric titration. Some of this apparent difference in concentration exists as a result of ambiguity in tradeterms of the bleaching industry. The same procedure was used with more concentrated bleach, but the portions of reagent were changed as follows: 4.08 g NH₄Cl, 6.39 mL NH₄OH, and 72 mL of 10.6% NaOCl were mixed. Care should be taken working with monochloramine. It is a reactive oxidant.

The mixture reacted for 10 minutes at -5 °C prior to sampling. The aqueous feed was then held at this temperature through course of the study. 8 mL aliquots were taken to explore extraction of NH₂Cl into organic solvent under various conditions. The aqueous chloramine was added to a 20 mL scintillation vial and vigorously shaken with organic solvent for 30 seconds. The biphasic mixture was allowed to separate and 1 mL of the organic phase was removed and titrated by the iodometric titration (See below). The results are listed in the table below.

Iodometric Titration: 6.20 g of Na₂S₂O₃·5(H₂O) was dissolved in 250 mL of water and set aside. 0.5 g of starch was dissolved in 50 mL of water by heating (hot plate) to 80 °C for 10 minutes and then set aside. 0.8 g of NaI was dissolved in 200 mL of water, and 10 mL of AcOH and 10 mL of the starch indicator were added. 20 mL of the iodide solution was transferred to a 50 mL Erlenmeyer flask with stir bar. 1.00 mL of NH₂Cl in organic solvent was added to the iodide solution which turned a purplish brown color. The oxidant solution was stirred rapidly on a magnetic hotplate. The thiosulphate solution was added dropwise until the solution became clear. The volume required to quench the oxidant was recorded and molarity of NH₂Cl was recorded.

Perhaps the difference between the reported and observed values can be explained as follows. A significant amount of volatile NH₂Cl could be lost as the literature procedure evaporates the organic layer prior to filtration. Also, the concentration of NaOCl was not stated. Strength of commercial bleach varies widely.

pH of reagents and reaction mixture (for batch conditions):
1) NH₄Cl (3.00 g) + NH₄OH (4.7 mL): 13.0 (NH₄Cl was not fully soluble)
2) Clorox (7.5% of NaOCl): 13.4
3) NH₄Cl (3.00 g) + NH₄OH (4.7 mL) + Clorox (7.5% of NaOCl, 72 mL): 13.5
4) NH₂Cl (extracted in MTBE): 11.5
5) Aqueous layer (after extraction of NH₂Cl): 14.0

pH of reagents and reaction mixture (for CSTR conditions):
1) NH₄Cl (3.00 g in 5 mL water): 6.0
2) NH₄Cl (3.00 g in 5 mL water) + NH₄OH (4.7 mL): 12.0
3) NH₄Cl (3.00 g in 5 mL water) + NH₄OH (4.7 mL) + Clorox (7.5% of NaOCl, 72 mL): 14.0
4) NH₂Cl (extracted in MTBE): 12.5
5) Aqueous layer (after extraction of NH₂Cl): 14.0

Note: pH measured with a pH meter
Table S4: Chloramine concentration from different solvents and bleach sources

| Entry | Solvent (Volume)                       | NaOCl (%) | NH$_2$Cl (M) |
|-------|---------------------------------------|-----------|--------------|
| 1     | Reported Literature Value             | unknown   | 0.09         |
| 2     | MTBE (11 mL)—(Repeat of Literature Procedure) | 7.5 (2.2 M) | 0.53         |
| 3     | MTBE (5.5 mL, 50% MTBE Charge)        | "         | 0.66         |
| 4     | MTBE (3.7 mL, 25% MTBE Charge)        | "         | 0.89         |
| 5     | MTBE (11 mL)                          | 10.6 (2.99 M) | 0.65         |
| 6     | MTBE (5.5 mL)                         | "         | 0.83         |
| 7     | MTBE (5.5 mL)—organic saturated with NaOAc | "         | 0.81         |
| 8     | MTBE (5.5 mL)—aqueous layer saturated with NaCl | "         | 0.84         |
| 9     | MTBE (3.7 mL)                         | "         | 0.90         |
| 10    | Et$_2$O (3.7 mL)                      | "         | 0.79         |
| 11    | 2-Methyl THF (3.7 mL)                 | "         | 0.80         |
| 12    | Dioxane (6 mL)                        | "         | 0.86         |
| 13    | CPME                                  | "         | 0.98         |
| 14    | DEGDBE (3.7 mL)                       | "         | 1.05         |
| 15    | THF (3.7 mL)                          | "         | 1.19         |
Continuous Production of Chloramine

A continuous stirred tank reactor (CSTR) was constructed from a 100 mL Schlenk flask with a liquid fill level set at 100 mL. Bleach, NH₄Cl/NH₄OH, and MTBE feeds were positioned below the liquid level surface, and the dip tubes removing reaction fluids were placed at the top of the fluid surface level, the 100 mL fill level volume. The CSTR was equipped with a large oval shaped stir bar, stirred at 800 rpm, and cooled to -5 °C. The total flow rate of fluids entering the CSTR was set at 10 mL/min to give a residence time of 10 min. The exit flow was set at 12 mL/min, faster than the entering flow to ensure a reactor volume of 100 mL/min. The exit stream flowed into the bottom of a gravity liquid-liquid settler made from a simple pressure-equalizing addition funnel (25 mL). The funnel was filled to a level of 25 mL, allowing the organic and aqueous layers to separate, and then the aqueous layer (bottom) was drained from the addition funnel, while the MTBE layer containing NH₂Cl was pumped off the top layer. Steady state was reached 30 minutes after the start of pumping (0.45 M NH₂Cl in MTBE).

The input feeds for the CSTR are as follows. 75.0 g of NH₄Cl (1.43 mol), 117.5 mL of concentrated NH₄OH and 125 mL of H₂O were combined and stirred until the mixture was homogeneous (Stream A). The total volume was 300 mL, and pumped at a rate of 0.619 mL/min (Stream A). Aqueous NaOCl was pumped at a rate of 3.71 mL/min (Stream B). Some ambiguity exists in the terminology describing concentration of NaOCl. Our bleach was 7.5% NaOCl (7.1% active chlorine, 15.8 wt% NaOCl, 2.2 M NaOCl as found by iodometric titration). MTBE was pumped at a rate of 5.67 mL/min (Stream C). The exit dip tubes were programmed to remove solution at a rate of 12 mL/min (2 pumps flowing at 5 and 7 mL/min). Peristaltic pumps from Vapourtec were used for fluid transport.
Batch Amination with NH₂Cl from CSTR

2-Cyanopyrrole (9.21 g, 10.0 mmol) was added to a 500 mL 3-neck round-bottom flask along with 100 mL of DMF. The solution was cooled to 0 °C with an ice-bath. 5.00 g of 60 wt% NaH in mineral oil was added slowly with stirring, keeping temperature below 35 °C. The round-bottom was connected to two condensers connected in series and cooled to -20 °C with a chiller. The condensers were configured to drain into a 250 mL receiving flask which was used to recycle the MTBE.

After the CSTR reached steady state, the stream of NH₂Cl in MTBE was connected to the basified pot of 2-cyanopyrrole. The NH₂Cl was added at a rate of 5.67 mL/min. It was added in 5 portions, where each fraction was flowed into the cyanopyrrole for 15 minutes. After every 15 minute addition, the chloramine feed was removed from the amination pot. The reaction vessel was set to 30 °C and placed under vacuum. The MTBE distilled and was collected in the receiving flask. After 14 minutes of evaporation, vacuum was turned off and the vessel was returned to atmospheric pressure. The MTBE was recycled and returned to the feedline used to extract NH₂Cl in the CSTR (Stream C).

After the fifth addition of chloramine, conversion was measured as 93%, and 360 mL of MTBE was recovered (84% recovery).
Continuous Amination in PFR with NH₂Cl from CSTR

A CSTR was made as described above in a 22 mL Scintillation vial with a fill volume of 20 mL. The total flow rate in was 2 mL/min, the exit was programmed at 3 mL/min, and the residence time was 10 min.

The input feeds for the CSTR are as follows. 15.0 g of NH₄Cl (286 mol), 23.5 mL of concentrated NH₄OH and 25 mL of H₂O were combined and stirred until the mixture was homogeneous (Stream A). The total volume was 60 mL, and pumped at a rate of 0.124 mL/min by syringe pump (Stream A). Aqueous NaOCl was pumped at a rate of 0.742 mL/min (Stream B). MTBE was pumped at a rate of 1.13 mL/min (Stream C). The exit dip tube was programmed to remove solution at a rate of 3 mL/min, and it transported the biphasic mixture to the gravity separator as described previously. Peristaltic pumps from Vapourtec were used for fluid transport.

The NH₂Cl in MTBE was pumped at a rate of 1.13 from the gravity separator, and mixed with a solution of 2-cyanopyrrole anion (0.609 M in DMF) via a T-Mixer (Idexx, 0.02 “ ID). The 2-cyanopyrrole mixture was flowed at 0.129 mL/min by syringe pump. The solution was made by dissolving 1.40 g of 2-cyanopyrrole (15.2 mmol, 1.00 equiv.), 1.22 g NaH (60 wt%, 30.4 mmol, 2.00 equiv.) in DMF to reach a total volume of 25 mL. The reaction mixture flowed into a PFR constructed from PFA tubing (0.06” ID, 5.04 mL, 4 min tᵣ). The reaction ran for 12 minutes before collecting sample for analysis. 89% conversion was observed.
**Batch Amination with Multicharge of Chloramine**

![Chemical structure](image)

**Multicharge of NH₂Cl in MTBE:**
- Add 1/4, then vacuum distillation of MTBE at 30 °C
- Repeat cycle for 3 more additions

**Typical multi-charge amination procedure:**

To a solution of 1H-pyrrole-2-carbonitrile (0.46 g, 5 mmol) in MTBE (5 mL) was added NaH (0.40 g, 10.0 mmol, 60% in mineral oil) in portions, and the reaction was stirred for 20 min at room temperature. DMF (5 mL) was added and MTBE was distilled under vacuum at 20-30 °C. NH₂Cl in MTBE (5 mL, 0.59M solution) was added via syringe. The mixture was let to stir between 20-30 °C for 5 min and HPLC analysis was carried out. MTBE was distilled under vacuum at 20-30 °C and NH₂Cl in MTBE (5 mL, 0.59M solution) was added via syringe. MTBE was distilled under vacuum at 20-30 °C and this cycle was repeated 2 more times. Methylethylketone was added as internal standard and the reaction was assayed by quantitative 1H NMR. This procedure was consistently repeated in 1, 5 and 30 mmol scale (Table S5).

**Table S5. Conversion and assay yield of chloramine multicharge reactions**

| Chloramine charge | Conversion (HPLC area %) | Assay Yield (NMR) |
|-------------------|--------------------------|-------------------|
|                   | 1 mmol (0.092 g)         | 5 mmol (0.460 g)  | 30 mmol (2.8 g)     |
| 1                 | 43                       | 38                | 43                 |
| 2                 | 70                       | 72                | 70                 |
| 3                 | 86                       | 92                | 89                 |
| 4                 | 98                       | 98                | 98                 |
| Assay Yield (NMR)| 87%                      | 91%               | 89%                |
Monochloramine (0.59M in MTBE) preparation procedure:

NH₄Cl (3.0 g) in MTBE (55 mL) was cooled to -5 °C (internal temperature), and concentrated NH₄OH (4.7 mL) was added. Commercial bleach (72 mL, Clorox ~7.5% NaOCl) was then added via addition funnel over 15 min. The mixture was stirred for 15 min, the layers were separated, and the organic layer was washed with brine (1 × 30 mL). The organic layer was dried over powdered CaCl₂ in the freezer for at least 1 h and kept at the same temperature. Approximate concentration is 0.59 M.
Amination and Triazine Formation in One-Pot

For scale-up to 10 g, a few changes were implemented: 1) In order to increase the throughput of the overall process, DMF was reduced from 10 V to 5 V in relation to 1H-pyrrole-2-carbonitrile with no impact to the reaction profile; 2) Based on our findings on the relation of NaH vs chloramine (see manuscript for details), NaH was reduced from 2 to 1.5 equiv.; 3) The number of chloramine charges were reduced from 4 to 3. This procedure was employed at in 2.8 g and 10 g scale furnishing the N-amino-2-cyanopyrrole product in 92% assay yield (Table S6, entries 1 and 2). The N-amino-2-cyanopyrrole product was cyclized by adding 3 equiv. of formamidine acetate and heating at 90-95 °C for 16 h. Assay 1H NMR using 1,3,5-trimethoxybenzene as internal standard showed 76% assay yield to triazine, which was isolated in 64% isolated yield after reducing the DMF volume and addition of water (78% purity). Preliminary purification studies showed that >97% pure triazine can be obtained by recrystallizing the crude solid from boiling water/EtOH (1:1), however, further studies showed that trituration with MTBE was more efficient for mass recovery in good purity.

Aiming at further increasing the throughput, more concentrated solutions of chloramine were investigated. Instead of using Clorox (~7.5% NaOCl), a sodium hypochlorite 10-15% solution from Sigma-Aldrich was used to prepare a ca. 0.74M chloramine solution in MTBE. By the addition of 170 mL (~1.2 equiv.) of this solution to deprotonated 1H-pyrrole-2-carbonitrile (10.0 g scale) in DMF gave 98% conversion to the N-amino-2-cyanopyrrole product (Table S6, Entry 4). It is important to mention that this batch employed a total of 22 volumes of solvent (5 V of DMF + 17 V of chloramine solution in MTBE), a reduction of approximately 5-fold compared to the Hynes Jr. procedure4 (20V of DMF + 80V of chloramine solution). The N-amino-2-cyanopyrrole solution was subjected to cyclization with formamidine acetate. The crude solid obtained from this batch was further purified by trituration with MTBE to give triazine in 60% isolated yield over the two steps.

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\text{NH}_2
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Table S6: Scale-up to 10g and evaluation of concentrated chloramine solution

| Entry | Scale (g) | Chloramine in MTBE charge (concentration) | Amination conversion (HPLC) | Cyclization AY (1H NMR) | Purity (HPLC) | IY yield | Comments |
|-------|-----------|------------------------------------------|---------------------------|------------------------|---------------|----------|----------|
| 1     | 2.8       | 3 charges (3 x 40mL) Concent.: 0.56M     | 97%                       | 74%                    | 82%           | 62%      | Analysis of the mother liquor showed ~2 g of triazine (~14% yield) |
| 2     | 10.0      | 3 charges (3 x 140mL) Concent.: 0.56M    | 98% (92% AY)              | 76%                    | 78%           | 64%      | Crude solid was resuspended/triturated in MTBE to give 98% purity triazine. |
| 3     | 10.0      | 2 charges (220 + 50 mL) Concent.: 0.68M  | 99%                       | NA                     | 98%           | 63%      | Crude solid was resuspended/triturated in MTBE to give 99% purity triazine. |
| 4     | 10.0      | 1 charge (170 mL) Concent.: 0.74M        | 98%                       | 75%                    | 99%           | 60%      | Crude solid was resuspended/triturated in MTBE to give 99% purity triazine. |

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\text{NH}_2
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HPLC trace of commercial Triazine (red) and M4ALL triazine (green):
We concluded our amination investigation by addressing the hazards surrounding use of NaH in conjunction with DMF. An interesting solution is the deprotonation of 1H-pyrrole-2-carbonitrile in MTBE, then solvent swap to DMF before adding the chloramine (Table S7, entry 2). This alternative was employed in the 10 g batches previously discussed. Alternative solvents and bases were also evaluated. Prior experience at M4ALL Institute⁴ suggested that NaH deprotonation can be successful in “glyme type” solvents. Diglyme and diethylene glycol dibutyl ether (DEDGBE) were explored furnishing high conversion to the desired N-aminated product (Table S7, Entries 3 and 4). THF and MTBE were also investigated, however, lower conversion was obtained (Table S7, entries 5 and 6). These results confirm the importance of the coordinating nature of the “glyme type” ethers which can be used as an alternative to DMF. Different bases like, NaHMDS, NaO\textsubscript{t}Bu and KO\textsubscript{t}Bu were examined, however the product is formed in reasonable conversion, the reaction profiles did not excel the use of NaH as base (Table S7, Entries 7-9).

**Typical amination screening procedure:**

To a solution of 1H-pyrrole-2-carbonitrile (1 mmol) in DMF or alternative solvent (1 mL) was added NaH or alternative base, and the reaction was stirred for 30 min at room temperature. NH\textsubscript{2}Cl (4 mL, ca. 0.56 M in MTBE) was added via syringe. The reaction conversion was monitored by HPLC after 1 hour.

**Table S7:** Evaluation of alternative bases and solvents for the amination reaction

| Entry | Solvent  | Base (equiv.) | Conversion (HPLC) | AY (NMR) | Comments                                                                 |
|-------|----------|---------------|-------------------|----------|--------------------------------------------------------------------------|
| 1     | DMF      | NaH (1.1 to 2)| >95%              | 89-95%   | See the manuscript for relation between NaH and Chloramine               |
| 2     | MTBE/DMF | NaH (2)       | >95%              | -        | Deprotonation was carried out in MTBE, then DMF was added and MTBE was distilled before adding chloramine solution |
| 3     | Diglyme  | NaH (1.5)     | >95%              | 97%      |                                                                         |
| 4     | Diethylene glycol dibutyl ether (DEGDBE) | NaH (2) | >95% | 92% |                                                                         |
| 5     | MTBE     | NaH (2)       | 45%               | -        |                                                                         |
| 6     | THF      | NaH (1.5)     | 26%               | -        |                                                                         |
| 7     | DMF      | NaHMDS (1.5)  | 73%               | -        |                                                                         |
| 8     | DMF      | NaO\textsubscript{t}Bu (1.5) | 71% | - | For a full screening of sodium and potassium tert-butoxide equivalents, see table S8 |
| 9     | DMF      | KO\textsubscript{t}Bu (1.5) | 81% | - |                                                                         |
Table S8: Evaluation of Sodium and Potassium tert-butoxide equivalents for the amination reaction

| Entry | Equiv. | Base     | Conversion (HPLC area %) |
|-------|--------|----------|--------------------------|
| 1     | 1      | KO' Bu   | 69                       |
| 2     |        | NaO' Bu  | 80                       |
| 3     | 1.2    | KO' Bu   | 85                       |
| 4     |        | NaO' Bu  | 80                       |
| 5     | 1.5    | KO' Bu   | 81                       |
| 6     |        | NaO' Bu  | 71                       |
| 7     | 2      | KO' Bu   | 78                       |
| 8     |        | NaO' Bu  | 66                       |
| 9     | 2.5    | KO' Bu   | 62                       |
| 10    |        | NaO' Bu  | 46                       |
| 11    | 3      | KO' Bu   | 31                       |
| 12    |        | NaO' Bu  | 19                       |
| 13    | 4      | KO' Bu   | 0                        |
| 14    |        | NaO' Bu  | 0                        |
NMR spectra

$^1$H NMR Spectra of Intermediate Iminium Chloride Salt in DMSO-$d_6$ at 600 MHz:
\( ^1H \) NMR Spectra 2-Cyanopyrrole in DMSO-\( d_6 \) at 600 MHz:

\[ \text{\includegraphics[width=\textwidth]{1H_NMR_spectra.png}} \]

\( ^{13}C \) NMR Spectra 2-Cyanopyrrole in DMSO-\( d_6 \) at 151 MHz:

\[ \text{\includegraphics[width=\textwidth]{13C_NMR_spectra.png}} \]
Typical $^1$H NMR spectrum of reaction mixture after amination step in DMSO-$d_6$ (600 MHz):

Typical $^1$H NMR spectrum of crude reaction mixture after cyclization step in DMSO-$d_6$ (600 MHz):
$^1$H NMR spectrum of triazine in DMSO-$d_6$ at 600 MHz:

$^{13}$C NMR spectrum of triazine in DMSO-$d_6$ at 151 MHz:
Analytical Method and HPLC chromatogram:

**Structures & IDs**

1. **1H-pyrrole**
   - Exact Mass: 67.04
   - Predicted pKa = 17.0

2. **1H-pyrrole-2-carbaldehyde**
   - Exact Mass: 95.04
   - Predicted pKa = 15.2

3. **2-cyanopyrrole**
   - Exact Mass: 92.04
   - Predicted pKa = 14.18

4. **1-amino-2-cyanopyrrole**
   - Exact Mass: 107.05
   - Predicted pKa = -4.62

5. **pyrrolo[2,1-f][1,2,4]triazin-4-amine**
   - Exact Mass: 134.06
   - Predicted pKa = 4.28

**Conditions**

- **Mobile Phase A:** 5 mM KH₂PO₄ in Water, pH ~5.0  
  (0.680g HPLC grade KH₂PO₄ in 1000 mL HPLC grade water)
- **Mobile Phase B:** Acetonitrile
- **Flow rate:** 1.5 mL/min
- **Injection volume:** 1 µL
- **Column:** Agilent Zorbax Eclipse C18 (4.6 x 100 mm; 3.5 µm)
- **Column temp:** 30 °C
- **Detector wavelength:** 230 nm (Pyrrole to Carbaldehyde), 260 nm (Carbaldehyde to Triazine)
- **Sample preparation:** Prepare samples at approximately 1.0 mg/mL in MeOH (preferred).

**Isocratic Table**

| Time (min) | %A | %B |
|-----------|----|----|
| 0         | 80 | 20 |
| 4.0       | 80 | 20 |

- Post-run equilibration: Off

**Retention Times**

| Compound                                    | Time (min) |
|--------------------------------------------|------------|
| Pyrrolo[2,1-f][1,2,4]triazin-4-amine        | 1.301 min  |
| 1H-Pyrrole-2-Carbaldehyde                  | 1.542 min  |
| 1-Amino-2-Cyanopyrrole                      | 1.894 min  |
| Pyrrole                                    | 2.052 min  |
| 2-Cyanopyrrole                             | 2.961 min  |
Representative Chromatogram(s) (attach additional chromatograms and spectra as needed)
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