Development of a Large-Scale Cyanation Process Using Continuous Flow Chemistry En Route to the Synthesis of Remdesivir

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ABSTRACT: The implementation of cyanation chemistry at manufacturing scales using batch equipment can be challenging because of the hazardous nature of the reagents employed and the tight control of reaction parameters, including cryogenic temperatures, that help to afford acceptable selectivity and conversion for the desired reaction. Application of continuous flow chemistry offers a means to mitigate the risk associated with handling large amounts of hazardous reagents and to better control the reaction parameters. A case study describing the cyanation of a glycoside using continuous flow chemistry toward the synthesis of the drug candidate remdesivir is presented.

KEYWORDS: continuous flow chemistry, cyanation, remdesivir, COVID-19

INTRODUCTION

In response to the 2014 outbreak of Ebola virus disease in Libera, Guinea, and Sierra Leone, Gilead Sciences, Inc., in collaboration with the Centers for Disease Control and Prevention (CDC) and the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), identified an adenosine nucleotide analogue, remdesivir, that demonstrated strong antiviral activity against multiple strains of the Ebola virus in vitro and in animal models (Scheme 1). Remdesivir has also demonstrated broad-spectrum antiviral activity against coronaviruses, including SARS-CoV and MERS-CoV, in primary human cell culture models and in vivo models of viral pathogenesis. More recently, remdesivir has been found to exhibit in vitro inhibition of SARS-CoV-2, the novel 2019 coronavirus responsible for the outbreak of COVID-19. On the basis of this observed activity toward coronaviruses, remdesivir has been applied, on compassionate use grounds, in response to the outbreak of COVID-19 infection, and clinical trials have been initiated.

The synthesis of remdesivir has been reported previously and proceeds through a six-step sequence (Scheme 1) that includes the installation of a cyano group through the stereoselective addition of cyanide to the 1'-position of riboside 1. In the initial process, this transformation required cryogenic conditions (−78 °C) and had the potential to liberate significant quantities of hydrogen cyanide. Preclinical and clinical evaluation of remdesivir would necessitate the production of multikilogram quantities of compound 2, requiring the establishment of a safe, robust process for its synthesis. Herein we report the development of continuous flow conditions for the preparation of 2.

RESULTS AND DISCUSSION

Batch-Mode Preparation of Compound 2. Two manufacturing processes for the preparation of compound 2 were developed: (i) a batch cyanation process for preclinical and early-stage clinical remdesivir demands and (ii) a cyanation process using continuous flow for advanced-phase clinical trials and commercial manufacturing.

As previously reported, the cyanation reaction was performed through combination of compound 1 (Scheme 1) in dichloromethane (DCM) with trifluoromethanesulfonic acid (TfOH), trimethylsilyl trifluoromethanesulfonate (TMSOTf), and trimethylsilyl cyanide (TMSCN) while the reaction temperature was maintained at −78 °C. These reaction conditions afford a clean reaction profile with good diastereoselectivity (Table 1, entry 1). The first challenge to be addressed was the requirement of cryogenic reaction temperatures (Scheme 2). When the reaction was conducted at −15 °C, rapid and significant decomposition of the starting material was observed along with reduced diastereoselectivity for the transformation (Table 1, entry 2). Alternative Brønsted acids were evaluated, and trifluoroacetic acid (TFA) was found to be a promising lead (Table 1, entries 3−7), providing good solution purity of compound 2 and acceptable diastereoselectivity. Decreasing the reaction temperature to −30 °C was found to increase the solution purity as a result of decreased degradation of reactive intermediates while providing a modest...
improvement in the diastereoselectivity (Table 1, entry 5 vs entry 8). The order of addition was also found to impact the diastereoselectivity. For example, adding TMSCN prior to the addition of TFA and TMSOTf significantly eroded the diastereoselectivity of the reaction (Table 1, entry 5 vs entry 9). Reagent equivalents were investigated and observed to play a role in the reaction performance, as increasing the quantities of both TMSOTf and TMSCN to 6 equiv increased the selectivity of the reaction, affording a high diastereoselectivity of 93:7 (Table 1, entries 10−12).

With the optimized conditions in hand, a larger-scale experiment was performed (Table 1, entry 13). During the scale-up experiment, TMSOTf and TMSCN were each added over approximately 30 min to maintain the reaction at the target temperature of −30 °C. The 30 min addition time resulted in significant decomposition of the reaction mixture and a lower diastereoselectivity (Table 1, entry 13). This finding was addressed by first combining the starting material 1 in DCM with TFA at −30 °C and then adding a mixture of TMSOTf and TMSCN in DCM that had been precooled to −30 °C. This new order of addition prevented the starting material from decomposing while maintaining acceptable diastereoselectivity (Table 1, entry 14).

After viable conditions for the formation of compound 2 were identified, attention was directed toward isolation of the product. On the basis of a number of laboratory trials in solvent screening (over 40 single solvents or solvent combinations were investigated), it was determined that compound 2 exhibited favorable solid-state properties and could be selectively crystallized from either a mixed solvent system (e.g., ethyl acetate and heptane) or a single solvent (e.g., 2-propanol or toluene). Following additional development studies, including hazard assessments and safety studies, the batch cyanation process was successfully executed for the preparation of compound 2.

### Table 1. Optimization of Cyanation Batch-Mode Reaction Conditions

| entry | input mass of 1 (mg) | Brønsted acid | equiv of acid | equiv of TMSOTf | equiv of TMSCN | reaction temperature (°C) | solution purity of 2 (%) | in-process content of 3 (%) | d.r. (2:3) |
|-------|---------------------|---------------|--------------|----------------|----------------|--------------------------|--------------------------|--------------------------|-----------|
| 1     | 100                  | TfOH          | 2            | 2              | 3              | −78                      | 90.4                     | 3.6                      | 96:4      |
| 2     | 100                  | TfOH          | 2            | 2              | 3              | −15                      | 32.3                     | 13.3                     | 71:29     |
| 3     | 100                  | TsOH          | 2            | 4              | 4              | −15                      | 41.0                     | 5.8                      | 88:14     |
| 4     | 100                  | MsOH          | 2            | 4              | 4              | −15                      | 16.4                     | 7.8                      | 68:32     |
| 5     | 100                  | TFA           | 2            | 4              | 4              | −15                      | 54.1                     | 9.5                      | 85:15     |
| 6     | 100                  | HCO₂H         | 2            | 3              | 3              | −15                      | 11.1                     | 5.7                      | 66:34     |
| 7     | 100                  | AcOH          | 2            | 3              | 3              | −15                      | 2.4                      | 1.4                      | 64:36     |
| 8     | 200                  | TFA           | 3            | 3              | 3              | −30                      | 75.7                     | 8.7                      | 90:10     |
| 9     | 200                  | TFA           | 2            | 3              | 3              | −15                      | 34.8                     | 23.0                     | 60:40     |
| 10    | 200                  | TFA           | 1            | 1              | 3              | −30                      | 48.6                     | 17.4                     | 74:26     |
| 11    | 200                  | TFA           | 1            | 3              | 3              | −30                      | 84.0                     | 8.4                      | 91:9      |
| 12    | 200                  | TFA           | 3            | 6              | 6              | −30                      | 88.6                     | 6.8                      | 93:7      |
| 13    | 66                   | TFA           | 3            | 6              | 6              | −30                      | 69.2                     | 8.0                      | 90:10     |
| 14    | 200                  | TFA           | 3            | 6              | 6              | −30                      | 83.2                     | 14.7                     | 85:15     |
| 15    | 200                  | TFA           | 2            | 4              | 6              | −30                      | 78.3                     | 20.4                     | 79:21     |

"Reaction conditions: Compound 1 in DCM was cooled to the indicated temperature, and the indicated Brønsted acid, TMSOTf, and TMSCN were added sequentially. "Purity was determined by LC analysis. "Reaction conditions: Compound 1 in DCM was cooled to the indicated temperature, and TMSCN was added, followed by TFA and TMSOTf. "The additions of TMSOTf and TMSCN were performed over about 30 min each. "Reaction conditions: TFA was added to a solution of compound 1 in DCM at −30 °C, and a solution of TMSCN and TMSOTf in DCM precooled to −30 °C was subsequently added."
early deliveries of remdesivir (Table 2). This enabled acceleration of the program at a time when there was an urgent need for remdesivir for clinical evaluation due to the escalating Ebola virus epidemic. For the planned large-scale batch size, two key challenges needed to be addressed: (i) improvement of the safety measures because of the increased operational hazards associated with the larger quantities of acidic reaction mixtures containing cyanide and (ii) the increased operational time required to combine large quantities of compound 1/TFA with TMSOTf and TMSCN, which could negatively impact the diastereoselectivity and yield.

Feasibility Studies for the Preparation of Compound 2 via Continuous Flow Chemistry. On the basis of the above challenges associated with further increasing the batch size, alternative processing modes were evaluated. Isolated literature reports discuss the use of continuous flow chemistry to perform C-glycosylations and O-glycosylations and the use of cyanide in flow chemistry directly or via a cyanide precursor. This mode of processing offers several unique advantages to traditional batch processing, including enabling large-scale chemistry to be performed with reduced reaction volumes and improved temperature control and mixing efficiency. Application of these advantages of continuous flow chemistry could in principle address the challenges associated with the batch cyanation process to prepare compound 2. For instance, smaller quantities of the hazardous reaction mixture would be present during the reaction and could be rapidly quenched once the reaction stream exited the flow reactor, thereby mitigating a portion of the safety risks associated with the process. The continuous flow chemistry manufacturing mode would also enable processing facilities to perform the process at about −30 °C with relatively inexpensive cooling units. An appropriately designed flow reactor would improve mixing and cooling compared with batch-mode chemistry, both of which could impact the process (Table 1, entries 13 and 14). Lastly, the process was believed to be viable for continuous flow chemistry because all of the reaction components formed clear solutions (upon dissolution in DCM) and the reaction mixture was not observed to become heterogeneous throughout the course of the reaction.

The development of the continuous flow chemistry process was first investigated on a Vapourtec R-series system consisting of four HPLC pumps and 3/16″ PFA tubing. In the initial configuration, shown in Figure 1, compound 1 was sequentially combined with TFA, followed by TMSOTf and then TMSCN. On the basis of the short reaction times observed during the development of the batch-mode process, the residence times were initially set at 0.5, 0.4, and 1.8 min for the reactions of compound 1 with TFA, TMSOTf, and TMSCN, respectively. These initial conditions resulted in high conversion with good diastereoselectivity (Table 3, entry 1). It appeared that the Brønsted acid (TFA) was not necessary at small scale for the continuous flow chemistry process (Table 3, entry 2). The results at larger scale (discussed later) suggested that the presence of TFA was important for process robustness. Evaluation of the residence times indicated that 0.5 min was optimal for the reaction with TMSOTf and 2 min was sufficient for the reaction with TMSCN (Table 3, entry 2 vs entries 3 and 4). Decreasing the equivalents of reagents was found to negatively impact the reaction performance, with a decrease in both conversion and diastereoselectivity under the flow chemistry conditions, regardless of whether TFA was present (Table 3, entries 9–11).

After acceptable continuous flow conditions were established, an in-house model system was assembled, consisting of three diaphragm metering pumps and 3/16″ PFA tubing. The more powerful diaphragm pumps (compared to the Vapourtec HPLC pump system) allowed a significant increase in throughput (ca. 317 g/h consumption of compound 1). With this system, the process was tested at 100 g input of compound 1 and afforded high diastereoselectivity (94:6) and a solution purity of compound 2 of 93.5% (Table 3, entry 12). Subsequent isolation of the quenched reaction stream afforded

![Figure 1. Schematic drawing of continuous flow chemistry setup.](https://dx.doi.org/10.1021/acs.oprd.0c00172)
compound 2 in 84% yield with 99.8% purity. This larger-scale test demonstrated the viability of the continuous flow process and enabled the shift from batch mode to continuous flow for future preparation of compound 2.

**Kilo-Lab-Scale Preparation of Compound 2 via Continuous Flow Chemistry.** Following the successful 100 g demonstration with the in-house model continuous flow chemistry system (Table 3, entry 12), an improved system was designed for the preparation of 20 kg of compound 2. The following criteria were considered necessary during the design of this continuous flow reactor: improved safety for larger-scale manufacturing, compatibility of the reagents and product with the materials of construction of the reactor system, heating/cooling requirements, mixing requirements, and increased throughput relative to the lab-scale system. A stainless steel (SS) reactor was designed and fabricated (Figure 2a), which improved the durability of the reactor and helped prevent potential loss of containment. SS was selected as the material of construction because it was found to be compatible with the individual reaction components or mixtures thereof (elemental impurities due to leaching were not detected in the reaction streams by ICP-MS). With regard to the cooling requirements for the continuous flow chemistry system, the heat of reactions was found to be $-133 \text{ kJ/mol}$ for the reaction of compound 1 with TMSOTf and $-68 \text{ kJ/mol}$ for the reaction of this stream with TMSCN. These exotherms would give adiabatic temperature rises of 9 and 4 K, respectively, which could be easily controlled by bath cooling when the SS reactor was used. Quenching of the reaction mixture was accomplished in a semibatch mode by constant delivery of the reaction mixture into a caustic aqueous mixture. A complete system that was assembled on the basis of the established conditions is shown in Figure 2b.

With the SS continuous flow system, an experimental run was performed with a 100 g input of compound 1 (without the use of TFA) to confirm the system performance. The results obtained were within expectations, although there was a slight increase in the residual amount of starting material (2.5%) along with a decrease in diastereoselectivity (91:9) (Table 4, entry 1). At the time, these lower-than-expected results were attributed to diffusion at the beginning and end of the experiment, as a significant amount of non-steady-state reaction mixture was collected for this smaller trial run using the SS reactor system. With the operation of the SS system confirmed, the process was piloted at a 2.75 kg scale (compound 1 input) using the preferred conditions identified in Table 3, entry 12 with 0 equiv of TFA. However, more pronounced challenges were encountered. Incomplete conversion of the starting material and a significant reduction in diastereoselectivity were observed (Table 4, entry 2). Investigation into equipment setup and execution with the previously used systems (small-scale experiments with the Vapourtec system and 100 g experiments with either the in-house $1/16"$ PFA reactor system or the SS reactor system) and the 2.75 kg pilot run with the SS reactor system when TFA was not added to the reaction mixture (Table 3, entries 11 and 12, and Table 4, entry 1 vs Table 4 entry 2), suggested that the reactor configuration and materials of construction were not the cause of the change in reaction performance.

An alternative explanation for the discrepancy between the small-scale experiments and the 2.75 kg pilot run was sought. It was hypothesized that residual water could have generated trace amounts of TIOH through hydrolysis of TMSOTf. The generated TIOH could then act as a catalyst, driving both the selectivity and the conversion of the transformation. This effect would likely have a larger impact on smaller-scale reactions (i.e., 1 g or even 100 g) with decreasing impact as the scale increased (i.e., 2.75 kg). To test this hypothesis, TIOH was introduced into the TMSOTf/DCM stock solution of a 150 g reaction using the SS reactor system, and it was found that the conversion and diastereoselectivity were fully restored (Table 4, entry 3). The use of TFA in the reaction mixture was assessed further, and experiments at the 150 g scale were conducted with the SS reactor system (Table 4, entries 4–6). It was found that 0.5 molar equiv of TFA afforded acceptable conversion and selectivity during the process (Table 4, entry

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**Table 3. Development of Continuous Flow Chemistry Conditions**

| entry | equiv of TFA/TMSOTf/TMSCN | reaction loop 1 | reaction loop 2 | reaction loop 3 | in-process content of 1 (%) | solution purity of 2 (%) | in-process content of 3 (%) | d.r. (2:3) |
|-------|--------------------------|----------------|----------------|----------------|---------------------------|--------------------------|---------------------------|----------|
| 1     | 3/6/6                    | 0.5            | 0.4            | 1.8            | 0.5                       | 89.3                     | 6.3                       | 93:7     |
| 2     | 0/6/6                    | −              | 0.5            | 2.0            | 0.6                       | 93.4                     | 5.6                       | 94:6     |
| 3     | 0/6/6                    | −              | 0.5            | 3.9            | 1.3                       | 92.1                     | 6.0                       | 94:6     |
| 4     | 0/6/6                    | −              | 2.5            | 2.0            | 2.1                       | 91.6                     | 5.8                       | 94:6     |
| 5     | 0/6/6                    | −              | 2.4            | 3.7            | 1.7                       | 90.5                     | 7.1                       | 93:7     |
| 6     | 0/6/6                    | −              | 0.5            | 2.0            | 4.0                       | 81.5                     | 9.0                       | 90:10    |
| 7     | 0/6/6                    | −              | 0.4            | 1.8            | 3.8                       | 87.4                     | 7.8                       | 92:8     |
| 8     | 0/6/6                    | −              | 2.1            | 1.8            | 0.3                       | 77.8                     | 6.4                       | 92:8     |
| 9     | 1/3/3                    | 0.8            | 0.7            | 2.9            | 3.7                       | 81.9                     | 13.1                      | 86:14    |
| 10    | 0/3/3                    | −              | 0.7            | 2.9            | 4.7                       | 69.1                     | 15.3                      | 82:18    |
| 11    | 2/2/2                    | 0.5            | 0.4            | 1.9            | 5.7                       | 74.4                     | 10.8                      | 87:13    |
| 12    | 0/6/6                    | −              | 0.5            | 2.0            | 0.7                       | 93.5                     | 5.6                       | 94:6     |

Reactions were performed at 1 g input of compound 1 on a Vapourtec R-series flow chemistry system using $1/16"$ o.d. PFA tubing. Reaction components were combined according to Figure 1. Residence times were achieved through increasing the volume of the appropriate reaction loop while maintaining the flow rates constant. Reaction temperatures were maintained at about $-40^\circ C$ through immersion of the flow reaction loops in an isopropyl alcohol (IPA)/dry ice bath. Only steady-state reaction streams were used to analyze in-process reaction progress.

Purity was determined by LC analysis of an aliquot of the quenched reaction mixture. The experiment was performed at 100 g input of compound 1 on an in-house-built system consisting of three diaphragm pumps and $1/16"$ o.d. PFA tubing. The quenched reaction stream was isolated via crystallization to afford compound 2 in 84% yield with 99.8% purity by LC.
To accommodate potential fluctuations in the flow rates of the three-pump system and increase the robustness of the process, 1.0 equiv of TFA was selected for subsequent runs. During implementation of this change, the TFA/TMSOTf feed line experienced increased back pressure and ultimately plugged. After additional experimentation to evaluate the source of the plugging, it was discovered that TFA (melting point of \(-15.4^\circ C\)) had limited solubility in the TMSOTf/DCM mixture at \(-40^\circ C\) and likely crystallized during precooling of the feed line. To circumvent this issue, the TFA/TMSOTf/DCM feed precooling loop was removed. Additionally, the reaction temperature was adjusted from \(-40^\circ C\) to \(-30^\circ C\) to decrease the risk of TFA crystallization from the TFA/TMSOTf/DCM feed solution. These changes did not have a substantial impact on the performance of the reaction, as the solution purity of compound 2 and the diastereoselectivity of the reaction were similar despite the increase in reaction temperature and removal of the TFA/TMSOTf/DCM precooling loop (Table 4, entries 5 and 6).

Under these newly established conditions, the subsequent five runs with a 2.75 kg input of compound 1 using the SS reactor system provided high solution purity and diastereoselectivity (Table 5, entries 2–6). The quenched effluents from the multiple runs were combined, and 13.0 kg of compound 2 was isolated in 66% yield with 99.7% purity.

### Table 4. Performance of the Stainless Steel Continuous Flow Reactor System

| entry | input of 1 | reaction temperature (°C) | equiv of TFA/TMSOTf/TMSCN | in-process content of 1 (%) | solution purity of 2 (%) | in-process content of 3 (%) | d.r. (2:3) |
|-------|------------|---------------------------|---------------------------|-----------------------------|-------------------------|-----------------------------|-------------|
| 1     | 100 g      | -40                       | 0/6/6                     | 2.4                         | 87.7                    | 8.8                         | 91:9        |
| 2     | 2.75 kg    | -40                       | 0/6/6                     | 14.1                        | 63.6                    | 18.4                        | 78:22       |
| 3     | 150 g      | -40                       | 0.5/3/3                   | 1.4                         | 86.8                    | 7.7                         | 92:8        |
| 4     | 150 g      | -40                       | 0.1/6/6                   | 3.9                         | 77.3                    | 13.3                        | 85:15       |
| 5     | 150 g      | -30                       | 0.5/6/6                   | 1.2                         | 86.4                    | 7.1                         | 92:8        |
| 6     | 150 g      | -30                       | 1/6/6                     | 0.9                         | 88.7                    | 7.9                         | 92:8        |

Reactions were performed using the SS continuous flow reactor system displayed in Figure 2 with residence times of 0.5 min for the reaction of compound 1 with TFA/TMSOTf and 2.0 min for the reaction with TMSCN. The reaction temperatures were maintained at about \(-30^\circ C\) through immersion of the flow reaction loops in an IPA/dry ice bath. Purity was determined by LC analysis of an aliquot of the quenched reaction mixture.

### Table 5. In-Process Results for the Stainless Steel Continuous Flow Reactor Runs

| entry | equiv of TFA/TMSOTf/TMSCN | scale (kg) | in-process content of 1 (%) | solution purity of 2 (%) | in-process content of 3 (%) | d.r. (2:3) |
|-------|----------------------------|------------|-----------------------------|-------------------------|-----------------------------|-------------|
| 1     | 0/6/6                      | 2.75       | 14.1                        | 63.6                    | 18.4                        | 78:22       |
| 2     | 1/6/6                      | 2.76       | 3.3                         | 88.3                    | 5.0                         | 95:5        |
| 3     | 1/6/6                      | 2.75       | 2.4                         | 91.3                    | 4.7                         | 95:5        |
| 4     | 1/6/6                      | 2.75       | 0.4                         | 93.0                    | 5.6                         | 94:6        |
| 5     | 1/6/6                      | 2.75       | 2.1                         | 90.8                    | 5.7                         | 94:6        |
| 6     | 1/6/6                      | 2.76       | 0.7                         | 92.6                    | 5.6                         | 94:6        |

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### Plant-Manufacturing-Scale Preparation of Compound 2 via Continuous Flow Chemistry

The results from the gram-scale to kilogram-scale runs using the various continuous flow reactor systems, along with the process parameters applied to each system, served as the basis for the design of the plant continuous flow reactor system. The following major factors were used to define the plant system:

1. First, the plant system needed to be well-contained in order to ensure safe handling of the cyanide-containing reaction mixture.
2. Second, the process parameters for each system were critical for achieving the desired purity and diastereoselectivity.
3. Third, the scalability of the reactor system was a key factor in determining the feasibility of the plant-scale implementation.

## Figure 2.
Kilo-lab flow chemistry equipment: (a) flow reactor; (b) kilo-lab flow reactor assembly.
mixture. To address this concern, the flow reactor and ancillary equipment were constructed from stainless steel and underwent rigorous pressure testing prior to use.

Second, the plant system needed to have the capacity to produce hundreds of kilograms of compound 2 in a reasonable time frame. Table 6 presents a comparison of process parameters for the different systems. The interaction among the residence time, reactor volume, and throughput (consumption of compound 1) can be clearly observed. The results shown in this table suggest that an SS tube reactor with a volume of 2.5 L would achieve a throughput of approximately 2 kg/h, enabling processing of 200 kg of compound 1 in about 4 days. This duration was deemed acceptable for the manufacturing requirements. In order to consistently achieve the desired flow rates of the three feeds, three diaphragm pumps constructed of SS and PTFE and equipped with back-pressure regulators were selected as the feed-delivery devices and were calibrated prior to use.

Lastly, the plant system needed to have sufficient cooling capacity and efficiency. The cooling capacity would be required both to adjust the reagent solutions to about −30 °C prior to reaction and to rapidly scavenge heat generated during the reaction in order to ensure that high-quality product streams were produced. These factors were mitigated through use of the SS construction of a tube-in-shell reactor system, enabling rapid heat transfer, along with use of a high-capacity heating/cooling unit.

Following the fabrication and installation of the plant-scale continuous flow reactor system (Figure 3), a pilot run was performed with an 86 kg input of compound 1 to confirm the operability of the plant system for manufacturing of compound 2. As observable in Figure 4, after a brief induction period

Table 6. Key Parameters for Continuous Flow Reactor Systems

| entry | system description | material of construction | reaction loop 1 | reaction loop 2 | reaction loop 1 | reaction loop 2 | throughput of compound 1 |
|-------|--------------------|--------------------------|-----------------|-----------------|-----------------|-----------------|-------------------------|
| 1     | Vapourtec R-series | PFA                      | 0.5             | 2.0             | 2 mL           | 10 mL          | 9 g/h                   |
| 2     | in-house PFA reactor system | PFA               | 0.5             | 2.0             | 59 mL          | 300 mL        | 317 g/h                 |
| 3     | kilo-lab SS reactor system | stainless steel   | 0.5             | 2.0             | 60 mL          | 300 mL        | 317 g/h                 |
| 4     | plant-scale reactor system | stainless steel | 0.5             | 2.0             | 0.5 L          | 2.0 L         | 1.98 kg/h               |

Figure 3. Schematic drawing of the plant-manufacturing-scale continuous flow reactor.

Figure 4. Flow chemistry reaction profile for the 86 kg pilot run. The reaction profile was obtained through sampling and LC analysis of aliquots of the quenched reaction mixture.
whereby steady-state operation of the system was obtained, a consistent reaction profile was observed throughout the duration of the flow chemistry run. Numerical results demonstrated that the plant-scale system afforded similar reaction diastereoselectivity and solution purity of compound 2 compared to the kilo-lab SS system (Table 7, entry 1 vs Table 5, entries 2–6). The quenched reaction mixture was carried through an aqueous workup in the same fashion as for the kilo-lab batch (see Experimental Details) to afford a stock solution of compound 2 in DCM. Following solvent exchange into toluene, the product was crystallized and filtered to obtain 68 kg of compound 2 (Table 7, entry 1). This delivery enabled the preparation of remdesivir to support clinical evaluation of the drug candidate. Two additional deliveries were subsequently performed using the same plant-scale continuous flow reactor system, at 280 and 250 kg inputs of 1, to provide 204 and 198 kg of compound 2, respectively (Table 7, entries 2 and 3).

**CONCLUSION**

The development of a batch cyanation process to prepare compound 2 and enable the development of the clinical candidate remdesivir was described. Translation of the batch-mode chemistry into a continuous flow process was accomplished, providing improved control over the reaction conditions and increased diastereoselectivity. Four unique continuous flow chemistry reaction systems were evaluated, including two systems that were utilized for large-scale manufacture of compound 2. The preparation of about 500 kg of compound 2 through continuous flow chemistry was reported, enabling the preparation and clinical evaluation of remdesivir for the treatment of Ebola virus and coronavirus diseases.

**EXPERIMENTAL DETAILS**

**General Information.** Commercially available solvents and reagents were used as received without further purification. LC data were collected on a Waters Acuity or Waters Acuity H-class UPLC instrument with detection by UV. UPLC conditions were as follows: Acuity UPLC BEH C18, 130 Å, 1.7 μm, 2.1 mm × 100 mm; 75–5% gradient of 20 mM ammonium acetate at pH 9.5 in water and 100% acetonitrile; flow rate of 0.4 mL/min; acquisition time of 12 min; UV detection at 245 nm. Purity was calculated through percent area normalization.

**Preparation of Compound 1.** Anhydrous neodymium-(III) chloride (1.0 equiv), tetra-n-butylammonium chloride (1.0 equiv), and tetrahydrofuran (THF, 9 volumes) were combined, and the resulting mixture was concentrated to about 4.5 volumes under ambient pressure at about 66 °C. THF (4.5 volumes) was charged, and the concentration was repeated. The mixture was cooled to about 22 °C, and 2,3,5-tri-O-benzyl-d-ribo-1,4-lactone (5) (282 kg, 1.0 equiv) was charged. After about 30 min, the mixture was cooled to about −20 °C and held at that temperature. In a separate reaction vessel, 7-iodopyrrolo[2,1-f][1,2,4]triazin-4-ylamine (1.10 equiv) was added slowly, and after about 30 min, the mixture was cooled to about −10 °C. Phenylmagnesium chloride (2 M in THF, 2.17 equiv) was added slowly (Scheme 3), and after about 30 min, the reaction mixture was cooled to about −20 °C. Isopropylmagnesium chloride (2 M in THF, 1.13 equiv) was added slowly, and after about 2 h, the Grignard reaction mixture was transferred into the lactone/NaCl/N-BuNCl/THF mixture. The resulting mixture was agitated at about −20 °C, and after about 8 h, a solution of acetic acid (1 volume) and water (4 volumes) was added. The resulting mixture was warmed to about 22 °C, and isopropyl acetate (i-PrOAc) was added. The layers were separated, and the organic layer was washed sequentially with aqueous potassium bicarbonate and aqueous sodium chloride. The organic layer was concentrated under vacuum to about 4.5 volumes, and i-PrOAc was charged. The organic layer was washed with water twice and concentrated to about 4.5 volumes under vacuum. i-PrOAc was charged, and the concentration was repeated. The mixture was filtered, and the filtrate was concentrated under vacuum to about 3 volumes. Methyl tert-butyl ether (MTBE) was charged, and the mixture was adjusted to about 22 °C. Seed crystals of compound 1 were charged, followed by n-heptane, and the mixture was cooled to about 0 °C. The solids were isolated by filtration and rinsed forward with an MTBE/n-heptane mixture. The resulting solids were dried under vacuum to afford compound 1 in 69% yield with 100% purity. The analytical data were consistent with data previously reported in the literature.

**Preparation of Compound 2.** Safety Notice. The chemistry described below was conducted using TMSCN,
which may generate HCN upon exposure to water or protic solvents or in the presence of acids. HCN is a highly toxic, colorless, flammable liquid that boils at 25.6 °C. The SDS should be consulted, and in consultation with appropriate environmental, health, and safety personnel, adequate precautions should be taken. Additional precautions may include items such as the following:

- written documentation outlining handling procedures for reagents, stock solutions, process streams, waste, and loss of containment or exposure
- higher level of personal protective equipment use
- cyanide detectors
- trained response personnel with access to cyanide antidote

Batch Process to Prepare Compound 2. To a solution of compound 1 (23 kg, 1.0 equiv) in DCM (10 volumes) precooled to about −40 °C was charged trifluoroacetic acid (3.0 equiv) followed by a solution of TMSOTf (6.0 equiv) and TMSCN (6.0 equiv) in DCM (5 volumes) precooled to about −30 °C, while the internal temperature was maintained below about −25 °C. The reaction mixture was agitated at about −30 °C for no less than 10 min and quenched into a precooled (about −10 °C) solution of KOH (35.8 equiv) in water (14 volumes). The biphasic mixture was warmed to ambient temperature. The organic layer was separated and washed three times with aqueous sodium chloride. The organic phase was concentrated under vacuum to about 4 volumes, diluted with DCM (8 volumes), and concentrated under vacuum to about 6 volumes. The mixture was filtered, and the filtrate was concentrated to about 4 volumes. The concentrate was diluted with toluene (19 volumes) and then concentrated under vacuum to about 14 volumes at about 50 °C. The mixture was heated to about 90 °C, cooled to about 55 °C, and seeded with compound 2. The mixture was agitated at about 55 °C for about 1 h and cooled to about 0 °C over about 6 h. The solids were isolated by filtration, and the filter cake was washed with toluene (3 volumes). The solids were dried under vacuum at about 50 °C to afford compound 2 in 71% yield with 99.2% purity.

Continuous Flow Chemistry Process to Generate Compound 2. Stock solutions of compound 1 (250 kg, 1.0 equiv) in DCM (15.0 volumes) (feed 1), TMSOTf (6.0 equiv) and TFA (1.0 equiv) in DCM (4.4 volumes) (feed 2), and TMSCN (6.0 equiv) in DCM (4.5 volumes) (feed 3) were prepared in separate reactors or feed vessels. Feed 1 was pumped at a flow rate of approximately 207 mL/min through a precooling loop at about −30 °C, and Feed 2 was pumped at a flow rate of approximately 504 mL/min through a precooling loop at about −30 °C. The effluent was then combined with feed 3 (pumping at approximately 189 mL/min through a precooling loop at about −30 °C) in reaction loop #2 at about −30 °C for about 2 min. The effluent of the combined feeds was collected directly into a vessel containing a solution of KOH (19.7 equiv) in water (8 volumes) at about −10 °C. The mixture was adjusted to about 22 °C, and then 2-propanol was added. The layers were separated, and the organic layer was washed with aqueous sodium chloride twice and concentrated. The resulting solution was filtered. Toluene was charged to the filtrate, and the mixture was concentrated. The mixture was heated to about 55 °C and then cooled to about 0 °C. The resulting slurry was filtered, rinsed with toluene, and dried at about 60 °C to afford compound 2 in 78% yield with 99.9% purity. Analytical data were consistent with data previously reported in the literature. Waste streams from the cyanosis process (both laboratory- and plant-scale) were monitored for cyanide content and were treated with bleach until the cyanide levels were below the limit of detection by cyanide test strips.

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**Notes**

The authors declare the following competing financial interest(s): The authors are employees of Gilead Alberta ULC, Edmonton, Alberta, Canada and Gilead Sciences, Inc., Foster City, California, USA.

**Acknowledgments**

The authors thank Anna Chiu, Dragos Vizitiu, Jeff Ng, Kevin Allan, Mark Scott, Richard Yu, and Zhongxin Zhou for thoughtful discussions and feedback during the preparation of this article.

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TFA was selected over TfOH because of its less corrosive nature and lower cost.

Alternatively, 7-bromopyrrolo[2,1-f][1,2,4]triazin-4-ylamine can be used in place of 7-iodopyrrolo[2,1-f][1,2,4]triazin-4-amine under the same reaction conditions.