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Article

Klein's Remdesivir-Nucleobase Synthesis Revisited: Chemoselective Cyanation of Pyrrol-2-carboxaldehyde

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4-Aminopyrrolo[2,1-*f*][1,2,4]triazine is a fundamental raw material in the synthesis of remdesivir, which demand has increased due to the tests and potential repositioning of this drug against Coronavirus disease 2019 (COVID-19). Here, three chemical steps route for the preparation of remdesivir's nucleobase is described. Particularly, a highly chemoselective cyanation of Klein's route and successful application of monochloramine prepared from commercial bleach as an *N*-amination reagent are presented.

Keywords: remdesivir's nucleobase, triazine, monochloramine, Klein's route

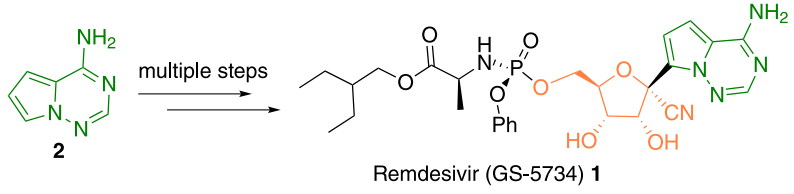
Introduction

In view of the urgent need for the availability of the remdesivir **1** imposed by the Coronavirus disease 2019 (COVID-19) pandemic and the synthetic challenges of its production, the scientific community has been dedicated to seek improvements to the complex process of obtaining this broad-spectrum *C*-nucleoside antiviral (Scheme 1).[1] In this context, the preparation of unnatural nucleobase 4-aminopyrrolo[2,1-*f*][1,2,4]triazine **2** (Scheme 1), an essential unit of **1**, is still a challenging task, since only a limited number of synthetic routes is available (Scheme 2). In addition, pyrrolotriazine is a starting material that is early involved in the total synthesis of remdesivir, participating in stages that generally occur with moderate yield.

The oldest route for the preparation of **2** was described in 1994[2] and allows access to the pyrrolotriazine base in two steps from the pyrrole-2-carboxaldehyde **3** in 28% overall yield (Scheme 2). In this approach, the first synthetic step has poor chemoselectivity, providing the reactive intermediate 1-aminopyrrol-2-carbonitrile **4** in only 43% yield, as a ca. 1:1 mixture with the presence of the non-reactive intermediate, pyrrole-2-carbonitrile **5** (obtained in 37% yield (Scheme 3)). On the other hand, the pyrrolotriazine formation step was particularly simple, in terms of operational (total reaction time to access the base was short (ca. 8 h) and led to reasonable yield).

The 2007 Bayer's route,[3] which is currently commercially employed in remdesivir's production, is a linear sequence of four steps that uses 2,5-dimethoxytetrahydrofuran **6** and *tert*-butyl carbazate **7** and produced the nitrogenous base in an overall yield of 31% (Scheme 2). According to Snead and co-workers,[3] the use of protecting groups and derivatized starting materials contribute to mass inefficiency, and adds to the step-count, decreasing overall yield of the Bayer route.

Recently, Snead and co-workers[3] described a synthetic proposal of recognized efficiency when compared to Bayer's commercial route (Scheme 2). Beginning from pyrrole **8**, the access to the nitrogenous base involves, sequentially, a cyanation step, an amination step followed



Scheme 1. Remdesivir and pyrrolotriazine chemical structures.

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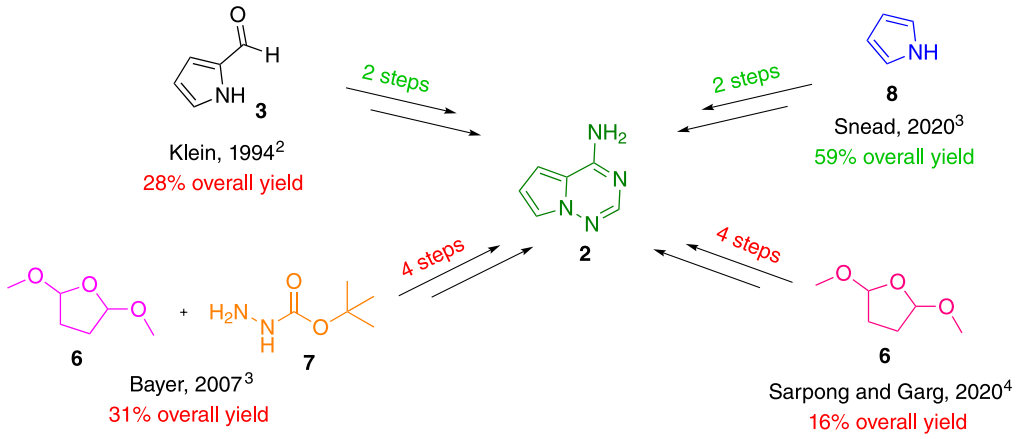
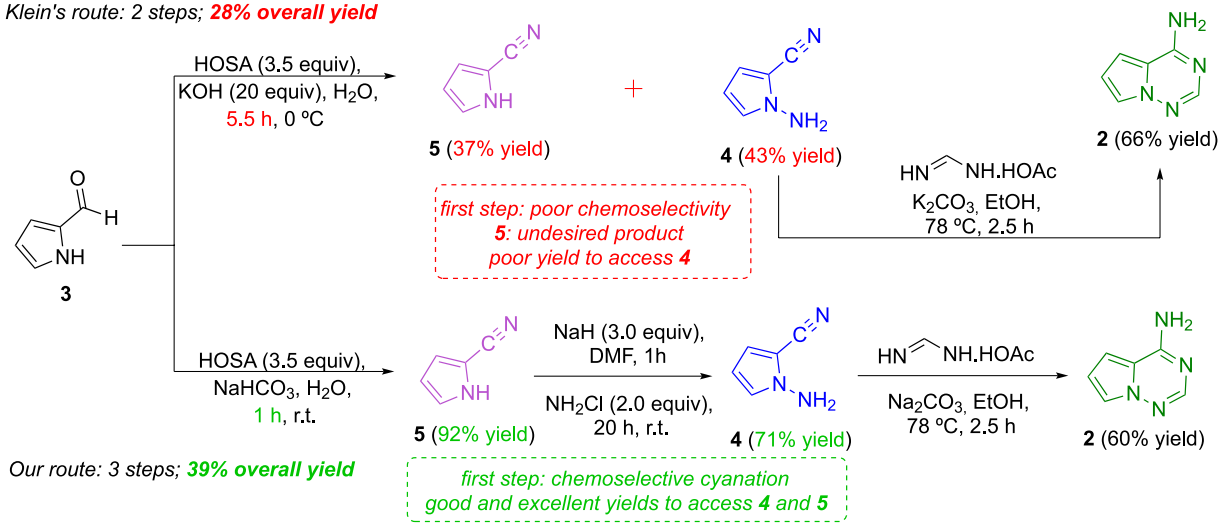


Scheme 2. Available literature[2-4] routes to achieve **2**.



Scheme 3. Klein's remdesivir nitrogen base synthesis, and our approach.

by the formation of **2**. The overall reported yield is 59%; the synthesis involves modern approaches such as one pot and flow experiments and has already been described in a gram scale synthesis.

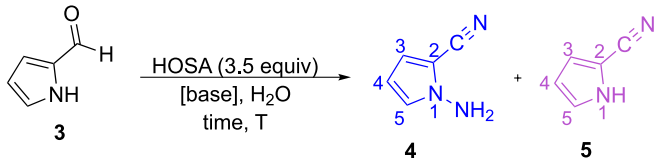
During the development of our work, a fourth synthetic approach was reported in the literature by a collaboration between the Sarpong's and Garg's research groups.[4] Their strategy has a cyanoamidine cyclization as a key step and provides the nucleobase in 16% overall yield (Scheme 2). Like Bayer's route, this synthesis requires 2,5-dimethoxytetrahydrofuran compound as the starting material, and a linear sequence of four steps.

Due to that, we were encouraged to revisit the available routes, particularly the one described by Klein.[2] As a result, a route of three linear steps from pyrrole-2-carboxaldehyde **3** that provides the desired nitrogen base in an overall yield up to 39% is described (Scheme 3). Our approach solves the problem of poor chemoselectivity in Klein's route, and applies commercial bleach for the generation of the *N*-amination agent monochloramine (NH_2Cl).

Results and Discussion

We carried out our initial experiments investigating the experimental details of the step of *N*-amination, and conversion of the pyrrole-2-carboxaldehyde **3** to nitrile derivative **5** using hydroxylamine-*O*-sulfonic acid (HOSA), as described by Klein.[2] As a result, we obtained a mixture of the **4** and **5** in poor yields, in both tested scales (Table 1, entries 1 and 2). Monitoring of the reaction by thin-layer chromatography (TLC), and subsequent analysis of the crude ^{1}H nuclear magnetic resonance (NMR) mixture suggested the presence of a mixture of the two reaction intermediates, probably in the oxime-*O*-sulfonate forms. Alternatively, the addition of an excess of base into this aqueous phase did not promote the formation of the desired products. The reaction monitoring by TLC also indicated that in the first hour only the conversion of **3** to **5** had occurred. Thus, we evaluated the influence of KOH concentration/equivalent and the reaction temperature to improve the reaction efficacy and selectivity for obtaining the intermediate **5** (entry 3). This outcome

Table 1. Optimization and control studies for the first step in the synthesis of **2**



3 **4** **5**

entry	Amount of **3** / mmol	Concentration of base[a] / (mol L^{-1})	time[b] / h	T[c] / °C	Yield (**4** + **5**) / %
1	1.5	5.32 KOH	3.5	r.t.	13 + 15[d]
2	10.5	5.32 KOH	3.5	r.t.	13 + 18[d]
3	1.5	0.532 KOH	0.5	0	0 + 80[e]
4	1.5	0.532 KOH	2.5	0	0 + 85[e]
5	1.5	0.532 KOH	18.0	0	0 + 92[e]
6	1.5	$NaHCO_3$ until pH ca. 6-7	1.0	–	0 + 80[e]
7	10.5	$NaHCO_3$ until pH ca. 6-7	1.0	–	0 + 92[e]

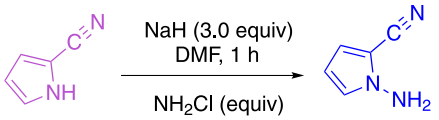
[a]20.0 equiv of KOH; [b]reaction time after base addition; [c]temperature after base addition; [d]isolated yield after purification through flash chromatography; [e]yield after liquid-liquid extraction. r.t.: room temperature.

suggested that the use of 0.53 mol L^{-1} of aqueous KOH solution (instead of 5.32 mol L^{-1}) is sufficient to promote the selective formation of **5** in up to 92% yield (entries 3-5). Furthermore, the intermediate **5** was satisfactory obtained in 80% yield in only 30 min (entry 3). To comprehend that the performance of the KOH was restricted to neutralize the cyanation reaction, we investigated the effects of replacing the base by $NaHCO_3$. To our delight, the selective preparation of **5** has been achieved in only 1 h of reaction at room temperature, followed by neutralization with $NaHCO_3$.

In addition to the reached chemoselectivity, the chromatographic column purification step was eliminated from the process. The pyrrole-2-carbonitrile **5** was obtained in its pure form after only liquid-liquid extraction. The product was isolated in 80% yield on 1.5 mmol scale (entry 6). Scale up of this cyanation step to 10.5 mmol of the aldehyde provided the desired product in up to 92% yield (entry 7).

Inspired by the successful cyanation step, we further explored the *N*-amination with monochloramine, which has been shown to be superior for the electrophilic amination of heterocycles when compared to other NH_2^+ transfer reagents, like HOSA.[5] Monochloramine can be easily prepared[5] from inexpensive precursors (NH_4Cl, NH_4OH, NaOCl),[3] which is more evident with the use of commercial bleach ($NaOCl_{(aq)}$, 2.5-3.5 m/m). With minor modification of the literature[6] method (replacing EtOAc by CH_2Cl_2 in liquid-liquid extraction) an increase on literature reported yield (from 54 to 71%) was achieved, considering different scale experiments (Table 2, entries 1 and 2). Moreover, the recovered starting material can be reused. We also evaluated the effect of the concentration/equivalent amount of the solution of NH_2Cl in diethyl ether for the efficiency of the transformation (entries 3-4). The results suggested that increasing the equivalents and maintaining the molar concentration the reactivity was not influenced (entry 3). Nevertheless, the use of a higher concentration of ethereal solution of NH_2Cl promoted a subtle adverse effect on the *N*-amination process (entry 4).

Table 2. Optimization and control studies for the second step: synthesis of **4**



5 **4**

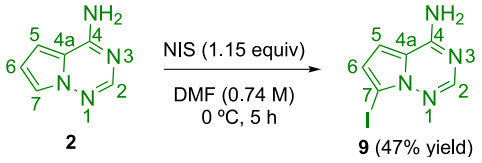
entry	Amount of **5** / mmol	[NH_2Cl] (equiv) / (mol L^{-1})	time / h	Conversion[a] / %	Yield[b] / %
1	0.33	0.15 (2.0)	5.5	ca. 70	NA
2	9.72	0.15 (2.0)	21.0	> 70	71
3	9.12	0.15 (5.0)	21.0	> 70	NA
4	8.76	0.90 (2.0)	18.0	ca. 50	NA

[a]Conversion calculated from the ^{1}H NMR analysis of crude reaction mixture; [b]isolated yield after purification through flash chromatography. NA: not available.

For the preparation of pyrrolotriazine, we followed the satisfactory Klein's methodology with minor modifications, thus obtaining similar yields (60%) in both reactions scales (1.82 and 6.89 mmol of **4**) (Scheme 3). It is worth mentioning that Snead's approach requires a longer reaction time (16 h) *versus* 2.5 h of Klein's route to reach comparable yield (60% *versus* 66%).

Finally, the halogenation of **2** seems to be particularly important for the remdesivir manufacturing, and the

halogenated nucleobase **9** has been required in recent literature reports.[1] As an application, the intermediate **2** was reacted with *N*-iodosuccinimide (NIS) in dimethylformamide (DMF) to provide the corresponding 7-iodopyrrolo[2,1-*f*][1,2,4]triazin-4-amine **9** in 47% yield (Scheme 4).[1]



2 **9** (47% yield)

Scheme 4. Synthesis of **9**.

Conclusions

In summary, a synthetic route to access 4-aminopyrrolo[2,1-*f*][1,2,4]triazine **2** is described. Our approach involves inexpensive and commercially available starting materials allowing to cover the high remdesivir's nucleobase demand. Particularly, after exclusion of hydroxide treatment, a chemoselective cyanation process was achieved, leading exclusively to **5**. Moreover, commercial bleach was successfully applied in the preparation of monochloramine giving **4**. Together, these experimental changes on Klein's route gave the corresponding remdesivir nucleobase in an overall yield up to 39%. Finally, regioselective halogenation of the nucleobase gave the iodopyrrolotriazine derivative **9** in 47% yield.

Experimental

Thin-layer chromatography (TLC) was performed on TLC plates (silica gel 60 F_{254}) and visualized by a UV lamp. Column chromatography was performed using 230-400 mesh silica gel. Commercial bleach was purchased from local supermarket ($NaOCl_{aq}$, 2.5-3.5 m/m, Classic®, Juiz de Fora, MG, Brazil). All the other reagents and solvents were purchased from Sigma-Aldrich (Saint Louis, MO, USA), Merck (Darmstadt, Germany), Vetec Química (Duque de Caxias, RJ, Brazil), LabSynth (Diadema, SP, Brazil), and were used without further purification.

The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on Bruker Avance III 500 MHz spectrometer (Bruker do Brasil, Atibaia, SP, Brazil). Chemical shifts for ^{1}H and ^{13}C NMR were reported as δ (parts *per* million) relative to residual signals of the solvent ($CDCl_3$ or dimethyl sulfoxide (DMSO-d_6)). Chemical shifts are reported employing the following peak abbreviation pattern: br, broad; s, singlet; d, doublet; dd, double doublet and m, multiplet. Melting points were recorded on a melting point apparatus (Microquímica MQAPF-301, Palhoça, SC, Brazil).

1-Aminopyrrol-2-carbonitrile (**4**) and pyrrole-2-carbonitrile (**5**)

The mixture was prepared according to the literature method.[2] These compounds were obtained as pale-yellow oils (0.142 g, 1.32 mmol, 13% of **4**; 0.175 g, 1.90 mmol, 18% of **5**) from 10.5 mmol of **3**.

Pyrrole-2-carbonitrile (**5**)

Caution: as a general rule, *O*-sulfonylhydroxylamines like HOSA are thermally labile.[7] Pyrrole-2-carboxaldehyde **3** (10.5 mmol, 1.0 equiv) was dissolved in 32.0 mL of water and then hydroxylamine-*O*-sulfonic acid (36.78 mmol, 3.5 equiv) was added. The mixture was stirred at room temperature for 1 h, when no starting material was detected by TLC analysis. The yellow-colored solution was cooled to 0 °C (ice bath) and treated by the $NaHCO_3$ portion additions until pH ca. 6-7. Then, the reaction was extracted several times with CH_2Cl_2. The organic phases were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting material **5** was obtained in pure form as an oil and did not require further purification (0.895 g, 9.72 mmol, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (br s, 1H), 6.96-6.95 (m, 1H), 6.88-6.87 (m, 1H), 6.26 (dd, 1H, *J* 3.6, 1.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 124.0, 120.3, 115.0, 110.0, 100.4. Spectral data is in accordance with that described in the literature[8] for **5**.

1-Aminopyrrol-2-carbonitrile (**4**) using NH_2Cl

Caution: chloramine is not sufficiently stable for storage and can be disproportionated to $NHCl_2$ and NCl_3 (a shock-sensitive compound).[3,9] To a solution of pyrrole-2-carbonitrile **5** (9.72 mmol, 1.0 equiv) in DMF (5.0 mL), NaH (29.12 mmol, 3.0 equiv, 60% in mineral oil) was added in small portions and the mixture was stirred for 1 h at room temperature. Then, the freshly prepared solution of NH_2Cl (19.44 mmol, 2.0 equiv) in diethyl ether (97.5 mL)[5] was added and the resulting mixture was stirred at room temperature and monitored by TLC and ^{1}H NMR analysis over a period of 20 h. Then, the yellow precipitate was filtered, and the solvent was evaporated. The resulting crude material was diluted with cold water and extracted several times with CH_2Cl_2. Then, it was dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified through flash chromatography on silica gel (hexanes/EtOAc = 90:10 to 80:20) to give the product **4** as an oil (0.739 g, 6.90 mmol, 71% of **4**); Rf = 0.8 (hexanes/EtOAc = 50:50) and the starting material **5** could

be recovered. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93-6.91 (m, 1H), 6.69 (dd, 1H, *J* 4.3, 1.7 Hz), 6.07 (dd, 1H, *J* 4.3, 3.1 Hz), 5.01 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.2, 118.3, 113.2, 107.4, 105.8. Spectral data is in accordance with that described in the literature[2] for **4**.

4-Aminopyrrolo[2,1-*f*][1,2,4]triazine (**2**)

The nitrogenous base **2** was prepared according to the literature method,[2] using Na_2CO_3 instead of K_2CO_3 given our unavailability of the latter and obtained as a brown solid (0.554 g, 4.13 mmol, 60%). From 1.32 mmol of **4**, the product was obtained in the same yield (0.142 g, 1.06 mmol, 60%). mp > 240 °C (mp literature[2] 236-239 °C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.69 (br s, 2H), 7.59 (dd, 1H, *J* 2.5, 1.5 Hz), 6.85 (dd, 1H, *J* 4.3, 1.5 Hz), 6.59 (dd, 1H, *J* 4.3, 2.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.6, 147.9, 118.1, 114.4, 110.1, 101.2. Spectral data is in accordance with that described in the literature[2] for **2**.

7-Iodopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**9**)

The iodinated derivative **9** was prepared according to the literature method[1] and obtained as a beige solid (0.046 g, 0.18 mmol, 47%, uncorrected) from 0.38 mmol of **2**; mp 210-211 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.91 (s, 1H), 7.80 (br s, 2H), 6.99 (d, 1H, *J* 4.4 Hz), 6.83 (d, 1H, *J* 4.4 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.2, 148.6, 118.3, 117.6, 104.0, 71.3. Spectral data is in accordance with that described in the literature[1] for **9**.

Supplementary Information

Supplementary information (copies of NMR spectra) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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