Evolution of the Synthesis of Remdesivir. Classical Approaches and Most Recent Advances

Didier F. Vargas, Enrique L. Larghi,* and Teodoro S. Kaufman*

ABSTRACT: The broad-spectrum antiviral Remdesivir, a monophosphate nucleoside analogue prodrug (ProTide), was repurposed. In May 2020, it received emergency approval by the FDA, being the first drug approved to fight the new coronavirus (COVID-19) disease which targets the virus directly. The main synthetic strategies toward Remdesivir, and their relevant modifications, are presented and discussed, to provide a panoramic view of the state-of-the-art and the more important advances in this field. Recent progress, proposed improvements, and uses of novel technologies for the synthetic sequence are also detailed.

1. INTRODUCTION

In 2012, Cho et al. discovered that C-nucleotide mimics containing 1′-substituted 4-aza-7,9-dideazaadenosine are analogues of ATP with potent activity against RNA viruses. The concomitant outbreak of the Ebola epidemics of 2014 prompted the development of new specific antivirals.

The incorporation of phosphate groups into drugs is problematic, due to their high metabolic lability and charged nature, which hinder membrane crossing. Therefore, they are synthesized as prodrugs, such as phosphoramidates, where metabolically labile protecting groups replace the acidic oxygens of the phosphate. This results in compounds which are uncharged, more lipophilic, and less prone to phosphoesterase-mediated hydrolysis; they can be converted into the bioactive forms inside the cells.

Remdesivir (1, Scheme 1), a phosphoramidate prodrug nucleotide (ProTide), is a result of this approach. Developed by Gilead Sciences Inc. as GS-5734 since 2009 to fight the hepatitis C virus, the drug was later claimed as active against the Ebola virus and others, including those causing SARS and MERS, as well as against the Hendra, Junin, Lassa, Nipah, Marburg, Zika, and respiratory syncytial viruses.

In vitro, 1 blocks virus infection at low micromolar concentration with high selectivity index. These bioactivity data and the preexistence of safety and clinical data eased FDA approval of 1 as a repurposed drug for treatment of severe COVID-19 cases, being the first approved treatment that directly targets the virus.
The pharmaceutical use of 1 instead of compound 3 improves the potency and efficacy of the treatment. The ProTide 1 can enter the cells (passive diffusion or via the nucleoside transporter), while the permeabilities of 3 and its monophosphate derivative GS-704277 (3a) are much lower. Inside the cells, 1 undergoes an esterase-mediated in vivo bioactivation, ultimately leading to the monophosphate 3a. Phosphate loss under the action of phosphatase or nucleosidase gives the adenosine analogue GS-441524 (3), which has shown in vivo efficacy in a veterinary setting. In the cells, the monophosphate 3a (a species which avoids the rate-limiting first phosphorylation step) can also be in equilibrium with the nucleoside 3.

Then, a kinase-mediated phosphorylation yields the active form, the triphosphate 4 (GS-443902). The latter confuses the RNA-dependent viral RNA polymerase, decreasing viral RNA production; when incorporated into viral RNA, it compromises H2O2,0 RNA-dependent viral RNA polymerase,6 decreasing viral RNA among the target tissues.

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UTP uptake in the parenteral dosage form (Veklury), which contains sodium EtOH, re

NH2OH, Ac2O, Py, H2O, 90°CHT, 10 h (85%) or 1. I2, Py, EtOAc, 10°C, 5 h (95%) or ICl, DMF −25 °C → −10 °C, 3 h (90%) or 1. I2, Py, EtOAc, 10 °C, 1 h; 2. H2O2, 20 °C, 10 h (85%).

2. SYNTHESIS OF THE KEY FRAGMENTS OF REMDESIVIR

Retrosynthetically, 1 can be disconnected into an activated phosphoramidate (2) and an adenosine mimic (3), which in turn can be further retrosynthetically disassembled to unveil an activated pyrrolo[2,1-f][1,2,4]triazine-4-amine (6) along with a suitably protected d-ribo-1,4-lactone precursor (5) and a cyanide source (7).

2.1. Pyrrolo[2,1-f][1,2,4]triazine-4-amine Core. Klein et al. (Scheme 2) provided the first concise and efficient approach9 to this long known heterocycle (12).7 2-Formylpyrrole (8) was treated with hydroxylamine-O-sulfonic acid (HOSA) in aqueous KOH to give 1-amino-2-pyrrrolonitrile (10) in 43% yield, along with 37% yield of 2-pyrrrolonitrile (11). Next, 10 was cyclodepsamidated with formamide acetate in refluxing ethanol under mild basic conditions (K2CO3), furnishing 12 in 66% yield.

However, for the synthesis of Remdesivir, 12 was prepared from 2,5-dimethoxytetrahydrofuran (15) and tert-butyl carbonate in dioxane at 90°C under HCl catalysis to give 16 (59% yield).10 This was exposed to chlorosulfonyl isocyanate in MeCN, resulting in 67% yield of nitrile 17 after reaction with DMF, which converted the N-chlorosulfonyl amide intermediate into a nitrile moiety.

Acid-mediated deprotection of 17 with HCl in dioxane gave an improved yield of 10 (85%),11 which in turn delivered 12 in 81% yield in three crops, when the cyclodepsamidation with formamide acetate was performed using potassium phosphate as base (31% overall yield from 15).

Finally, the heterocycle was selectively brominated with 1,3-dibromo-5,5-dimethylhydantoin (14) in DMF to give 13 in 90% yield.12 This heterocycle was employed for the first-generation synthesis of Remdesivir. Other Remdesivir generations and their modifications13 used 18, obtained in 95% yield by iodination of 12 with N-iodosuccinimide (NIS).14 Alternative procedures toward 18 employed ICl (90% yield)15 and molecular iodine (85% yield)16 as iodinating reagents.

Remdesivir sparked renewed interest in the synthesis of 12. In an improved alternative (39% overall yield),17 the reaction of 8 and HOSA with NaHCO3 afforded 11 (92% yield), and 10 was obtained in 71% yield by N-amination with NaH and CINH2. However, the approach of Sneed et al.18 is the best one. It is based on a one-pot oxidative Vilsmier cascade from pyrrole (9) to afford 10 through the intermediacy of 8 and further cyclocondensation of 10 to 12 with formamide acetate (59% overall yield).

2.2. d-Ribonolactone Moiety. The d-ribo-1,4-lactone (19) derivative 22 used for the first-generation synthesis of Remdesivir was obtained employing the Albright–Goldman oxidation (Ac2O, DMSO) of lactol 21 (96% yield),19 which in turn can be accessed in high yield from d-ribose (20). For the second-generation synthesis, lactone 22 was prepared (~95% yield), using a TEMPO-catalyzed NaClO oxidation (Scheme 3).20

Scheme 2

\[ \text{Scheme 2} \]

Reagents and conditions: (a) 1. MeOH, H2SO4, rt (79%); 2. BnBr, NaH, DMF, rt; 3. AcOH, H2O, 100 °C (95%); (b) DMSO, Ac2O, rt, 48 h (96%) or NaClO, KBr, K2HPO4, TEMPO, MTBE-H2O (~ 4:1), 1 °C (~95%); (c) CCl3C(NH)OBn, TfOH, dioxane, 0 °C, 3 h (80%); (d) TBDMSCl, imidazole, DMF, 65 °C, 5 h (96%); (e) SOBr2, Py, CH2Cl2, rt, 1 h (93%).
Oxidants such as pyridinium chlorochromate (PCC)\textsuperscript{21} have also been used.\textsuperscript{22} Given its wide interest, additional syntheses of 22 have been reported, including one which proceeds through the debenzylic lactonization of 23 with SOBr\textsubscript{2}.\textsuperscript{23} On the other hand, compound 24, used for the third-generation synthesis of Remdesivir, was prepared by conventional silylation of 19.\textsuperscript{24}

2.3. Phosphoramidate Fragment. Three generations of the key phosphoramidate fragment have been reported. The product of the first generation (26) was obtained in two steps and 83% yield by reaction of alanine 2-ethylbutylester hydrochloride (25) with phenyl dichlorophosphate in CH\textsubscript{2}Cl\textsubscript{2} at −78 °C (Scheme 4).

The second-generation phosphoramidate (28) was accessed by in situ exposure of 26 to 4-nitrophenol. This furnished 27 in 80% yield, which was fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.

A third-generation synthesis of phosphoramidates based on a dynamic kinetic resolution approach was first disclosed in a 2016 patent by Gilead, without informing yields.\textsuperscript{14,25} The method involved preparation of 26 in i-PrOAc at −20 °C and its in situ conversion into (R\textsubscript{p}/S\textsubscript{p})-27 by reaction with 4-nitrophenol under Et\textsubscript{3}N promotion.

The reaction was quenched and subjected to acid and base washings; next, the organic phase was concentrated and then fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.

Scheme 4

The reaction was quenched and subjected to acid and base washings; next, the organic phase was concentrated and then fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.

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3. SYNTHETIC ROUTES TOWARD REMDESIVIR

Gilead devised three main generations of the same strategy toward Remdesivir. They were partially reviewed in contributions with different scopes.\textsuperscript{26,27} The approval of the drug for COVID-19 treatment resulted in additional synthetic contributions since the pandemic’s outbreak.

3.1. First Generation. A New Antiviral Prodrug. The first synthetic route toward Remdesivir was disclosed in 2012 (Scheme 5).\textsuperscript{28} There, 13 was subjected to a temporary N,N-bis-silylation for N-amino protection. Next, metal–halogen exchange in THF at −78 °C and reaction with 22 afforded lactol 31, as a mixture of anomers. Use of n-BuLi and TMSCl gave a meagre 25% yield of 31.\textsuperscript{11}

Therefore, NaH and ClSi(Me)\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}(Me)\textsubscript{2}SiCl were employed for the N-amino protection step, and then n-BuLi executed the metal–halogen exchange.\textsuperscript{1,29,30} The modification notably improved the efficiency (up to 60% yield) of this poorly reliable C-glycosylation step. Qin et al. demonstrated that replacing NaH with the reagent couple n-BuLi/i-Pr\textsubscript{2}O, THF, 0 °C (21%); (e) chiral HPLC.

The reaction was quenched and subjected to acid and base washings; next, the organic phase was concentrated and then fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.

Scheme 5

The reaction was quenched and subjected to acid and base washings; next, the organic phase was concentrated and then fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.

The reaction was quenched and subjected to acid and base washings; next, the organic phase was concentrated and then fractionally crystallized from di-isopropyl ether to give (S\textsubscript{p},S\textsubscript{p})-28 in 39% yield, as proven by single-crystal X-ray diffraction.
The transformation gave 21% yield of an ~1:1 mixture of diastereomers (34), which was resolved by chiral HPLC to afford Remdesivir (1). Overall, the synthetic sequence was straightforward but required several HPLC separations (including chiral HPLC in the final step) and proceeded in low overall yield (0.6–1.5% from 13 and 22).

3.2. Second Generation. The Optically Pure Phosphoramidite Alternative. In 2016, a multigram synthesis of the drug was disclosed (Scheme 6), and a 2017 patent included additional details. This new generation synthesis comprised several improvements, including a TEMPO-catalyzed NaClO oxidation to access 22 and a C-glycosylation which was executed with iodide 18 and Grignard reagents. This combination proved to be superior to that of the first-generation synthesis (based on 13 and n-BuLi, NaH, or n-BuLi/-PrNH2). In a modification of this reaction, the LaCl3:2LiCl complex was added to generate a less basic carbanion intermediate, which avoids deprotonation of lactone 22.

![Scheme 6](https://doi.org/10.1021/acsomega.1c03082

Reagents and conditions: (a) TMSCI, PhMgCl, i-PrMgCl:LiCl, THF, −20 °C, 1 h (42%); (b) TMSCl, TMSOTf, TIOH, CH2Cl2, −78 °C, 2 h (85%); (c) 1. BCl3, CH2Cl2, −40 °C, 2 h; 2. Et3N, MeOH, −78 °C → rt (86%); (d) 2,2-DMP, H2SO4, Me2CO, rt, 0.5 h; 45 °C, 0.5 h (99%); (e) MgCl2, DIPEA, MeCN, 50 °C, 0.5 h (70%); (f) 12 N HCl, THF (1:5), rt, 5 h (69%).

The efficiency of this 1,2-addition was improved, and intermediate 31 was obtained in 42% yield, consistently, with less costly reagents and under milder conditions (−20 °C), ensuring scalability. Other lanthanide salts (CeCl3, NdCl3, YCl3) seem to have been tested, without disclosing product yields. However, NdCl3 was preferred for process scale up (68% yield of 31 from 282 kg of 22).

Another modification was the addition of an acid (TIOH, TFA) to the original reagent couple TMSCl/TMSOTf, to carry out the required stereoselective cyanation. This change improved the access to 32 (85% yield; βα > 95:5). The exhaustive debenzylation step was refined, being performed at −40 °C to give triol 33 in 86% yield.

As proven by Gilead scientists, mild coupling of 28 and 35 did not affect the stereochemical integrity of the phosphorus center, suggesting that coupling of 28 and 33 could have the same outcome. However, in order to overcome the poor yields of the coupling stage between 26 and 33, the vicinal diol moiety of the latter was protected. Hence, 33 was exposed to 2,2-dimethoxypropane/H2SO4, giving the acetonide 35 in 99% yield; interestingly, 35 was synthesized earlier in undisclosed yield, under di-p-nitrophenylphosphoric acid catalysis.

Then, 35 was reacted with the second-generation phosphoramidite S0−28, furnishing 36 (70% yield). The process is a nucleophilic substitution, where MgCl2 activates the phosphorus center and DIPEA promotes the coupling, with configurational inversion at the P-center. Final deprotection of 36 with concentrated HCl in THF gave optically pure Remdesivir (1) in 69% yield. Under the rather mild conditions developed for the coupling, the product exhibited stereochemical integrity of its stereogenic center.

Interestingly, the Gilead 2016 patent also disclosed a t-BuMgCl-mediated coupling between unprotected compound 33 and the (R0/S0)-diastereomeric mixture of 27 (Scheme 4), which gave (R0/S0)-1 in 43% yield. The resulting diastereomers could barely be separated by gradient reverse-phase HPLC (Kinetex C-18 column); however, they were cleanly separated by chiral HPLC (Lux Cellulose-2 column, eluting with MeCN:MeOH (95:5, v/v)).

Therefore, the three-step coupling sequence (48% overall yield) for the diastereoselective synthesis of Remdesivir compared favorably with the original coupling-chiral HPLC approach (12.5% overall yield) and the t-BuMgCl-mediated coupling (~21.5% overall yield). Diol protection and use of the enantiopure phosphoramido derivative (S0)-28 were key to this notable improvement.

The whole sequence proceeded in only six linear steps from known lactone 22 and iodide 18 and in 14.7% overall yield. This approach avoided costly, bottleneck HPLC separations and enabled preparation of over 200 g of Remdesivir, to support preclinical efficacy and toxicity studies. The preparation of the radiolabeled analogue ([14C]GS-5734 (58.0 mCi/mmol) was carried out with the same strategy, using [14C]TMSCl.

Kappe et al. developed a small-scale flow chemistry approach of the C-glycosylation step toward 31, which afforded the product in a stable 47% yield, with a total residence time of <1 min and a starting material throughput of 51.8 mmol/h (~690 mg/day). They optimized the reaction stoichiometries to suppress generation of identified impurities arising from onward reaction of the intermediate. The procedure did not provide a significantly higher yield with regard to the original batch conditions (40%), but it offered a marked improvement in processability.

In addition, a manufacturing scale (~200 kg/run) continuous flow chemistry approach for the cyanation of 31 at −30 °C was reported. The process afforded 32 in 78% yield and 99.9% purity, at a rate of 6 mol/h, comparing favorably with a batch process (71%, 99.2% purity). It also provided improved control over the reaction conditions and increased diastereoselectivity [d.r. (βα) = 96:4].

Use of large quantities of cyanide derivatives (added in excess) required special conditions for plant design, including detailed written instructions and cyanide detectors as well as...
suitable personal training and protective measures. Further, use of cold basic reaction quenching conditions (KOH, H₂O, −10 °C) prevented the generation of HCN gas, whereas the waste streams from the process were treated with bleach until the cyanide levels were below the limit of cyanide test strip detection.⁶⁰

Shen et al.⁴⁸ recently reported a simplified and efficient C-glycosylation strategy which avoids the formation of over-addition byproducts (Scheme 7). It involves a Weinreb amidation of 22, followed by in situ temporary silylation of 37, addition of the Grignard reagent derived from 18 (MeMgBr was used instead of PhMgCl), and final cyclization to afford 31 in 65% overall yield at a kilogram scale.

In the absence of additives, it has been shown that the coupling between 26 and the diol 33 gave an ~1:1 mixture of diastereomers. However, efficient organocatalytic asymmetric P-phosphoramidation alternatives to the fragment coupling stage were recently reported by the groups of Zhang⁶⁹ and Hung.⁶⁸ Their approach involved dynamic kinetic asymmetric transformations (DyKATS) (Scheme 8) with chiral imidazole derivatives (38 and 39) as organocatalysts, mimicking the mechanism of the enzyme glucose-6-phosphatase.⁶⁸

Scheme 7

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\[ \text{Reagents and conditions: (a) MeONH}_2\cdot\text{HCl, i-PrMgCl, THF, 0 °C; (b) TMSCl, MeMgBr, i-PrMgCl-LiCl, THF, −10 °C → rt (65% overall).} \]
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Scheme 8

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\[ \text{Reagents and conditions: (a) 1. i-PrMgCl, TMSCl, 0 °C; 2. i-PrMgCl, −15 °C; 3. LaCl}_3\cdot\text{2LiCl, −15 °C; (b) 1. TMSCN, TMSOTf, TIOH, CH}_2\text{Cl}_2, −30 °C, 15 min; 2. Et}_3\text{N, 0 °C (β:α = 3.8:1); (c) 28, MgCl}_2\cdot\text{-Pr}_2\text{NEt, THF, 50 °C, 21 h or 30, t-BuMgCl, THF, −10 °C → 5 °C, 16 h; (d) 12 N HCl, 20 °C, 72 h or TBAF orKF or Py-HF.} \]
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In the procedure devised by Zhang et al., catalyst 38 (10 mol %) was employed under 2,6-lutidine promotion, to diasteroselectively afford the protected intermediate 36 (Scheme 8) after 96% conversion (d.r. = 22:1:1). The improved coupling−deprotection−crystallization sequence of Hung et al. gave 1 in 70% yield (d.r. = 99:3:0.7) under the assistance of 39, which was recovered (83% yield) for reuse.

The solid-state characteristics of Remdesivir were ascertained employing ssNMR, X-ray powder diffraction, calorimetric (DSC, TGA), and dynamic vapor sorption (DVS) methods. Four polymorphic forms of the drug were differentiated, and a maleate salt was prepared.⁴¹

3.3. Third Generation. The Silyl Ether Approach. The sequence was first reported in 2016 without disclosing the yields of any step.⁴¹ There, the silyl lactone 24 was condensed with iodide 18, using the previously developed Grignard reagents strategy (Scheme 9), and the LaCl₃·2LiCl complex was added to improve access to 40 and avoid side products. In turn, compound 40 is subjected to cyanation with the TMSCN/TMSOTf reagent system at −30 °C, with the addition of TFA. This selectively affords the monodeprotected nitrile after quenching with Et₃N; however, the product 41 was isolated as a mixture of diastereomers (β:α = 3.8:1), which required a preparative HPLC separation for purification.

Nitrile 41 was further coupled in THF with the enantiomerically pure phosphoramido 28 under MgCl₂/i-Pr₂NEt promotion to furnish 42. In an alternative setup, the use of the phosphoramide 30 carrying a pentafluorophenyl leaving group was also proposed,⁴² but the coupling was performed with t-BuMgCl in THF at −10 to 5 °C. Incompatibility between the nitro group in 28 with the Grignard reagent may have forced the use of 30. Acid- (HCl) or fluoride- (TBAF, KF, or Py-HF) mediated desilylation finally provided Remdesivir (1). Since no reaction yields were given, efficiency comparisons with previous syntheses are not possible. However, this route has some clear advantages, such as the selective monodeprotection of the primary silyl ether, which greatly simplifies the last steps (debenzylation, protection, coupling, and deprotection), enabling direct coupling of 41 with the phosphoramido moiety. Another advantage is the milder (room temperature) final deprotection step.
Both the cyanation−monodesilylation step aided by TFA and the use of the pentafluorophenyl derivative 30 for the coupling stage are interesting and novel developments. On the other side, however, the need for preparative HPLC for accessing enantiomerically pure intermediate 41 is a disadvantage.

4. PREPARATION OF (R)-REMDESIVIR

The S<sub>p</sub>-isomer Remdesivir was originally selected for development due to its greater selectivity and better therapeutic window. However, the R<sub>p</sub>-isomer is also active. The enzymes carboxylate esterase 1 (CES1) and cathepsin A (CatA), which have variable expression levels among individual patients, have been identified as the major contributors of the initial activation step inside the cells.

CatA is more widespread and has preference for hydrolysis of (S<sub>p</sub>)-Remdesivir, whereas CES1 has a more limited tissue distribution but a strong preference for hydrolysis of the (R<sub>p</sub>)-isomer. The new coronavirus affects primarily the lungs, rich in CatA; however, since it attacks multiple tissues, the synthesis of (R<sub>p</sub>)-Remdesivir (Scheme 10) becomes relevant.

This prompted Raushel et al. to prepare the key intermediate 43 through a chemoenzymatic strategy, employing the In1W variant of the phosphotriesterase from <i>P. diminuta</i> (In1W-PTE). The product was obtained satisfactorily (97% yield with regards to 27, ee > 95%), as assessed by 31P NMR. Other strategies toward this end have been reviewed. This group finally achieved the synthesis of R<sub>p</sub>-Remdesivir, using the t-BuMgCl-mediated coupling conditions (38% yield).

5. SUMMARY AND OUTLOOK

This review highlighted how synthetic efforts have improved the synthesis of Remdesivir in terms of cost, yield, and scalability. It also revealed not only how synthetic organic chemistry and its interaction with other disciplines can give specific responses to nowadays urgent needs but also how difficult it still is for science to come out with state-of-the-art suitable pharmaceutical solutions with regard to small organic molecules, even under the pandemic’s pressure. Any specific drug against coronavirus should target the virus and its respiratory and cardiovascular effects. While discovery of this magic bullet is underway, the lessons learned during this pandemic will enhance our preparedness for the next time, when urgency will knock the door again.

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Notes

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