SCIENTIFIC LETTER N°14

Edito

Dans le contexte particulier que nous vivons actuellement, Jacques LEBRETON a souhaité vous faire partager le potentiel de certains analogues nucléosidiques pour le traitement des infections humaines au Coronavirus.
Au cours des dernières années, notre équipe de chimistes a travaillé sur plusieurs projets dans le domaines des nucléosides et à travers ces travaux, notre équipe a acquis une expertise dans cette chimie si particulière.

Notre prochain Webinaire intitulé « Nucléosides : les diamants sont éternels » qu'animera Jacques LEBRETON le 18 Juin prochain abordera sous toutes ces facettes la chimie des Nucléosides.
De plus tous les mois, nous maintenons ce partage avec vous à travers nos Posts #TheMinuteOfChemistry sur la page LinkedIn d'AtlanChim Pharma.

Alors qu'AtlanChim Pharma continue de gagner en reconnaissance auprès de ses clients, l'innovation n'est pas notre seul objectif. Nous savons l'importance d'établir une relation de confiance avec vous, nos clients. Nous intégrons quotidiennement les valeurs qui sont les nôtres à savoir la Transparence, la Communication et l'Orientation Client. C'est pourquoi nos chimistes sont toujours à votre écoute pour répondre à vos besoins en synthèse à façon, du marquage à froid ou bien encore la synthèse d'impuretés et faire de votre projet un succès.

Enfin, nous tenons à remercier nos clients et nos collaborateurs pour leur compréhension et leur soutien alors que nous mettions en place les nouveaux protocoles pour répondre aux contraintes sanitaires actuelles.

Bonne lecture,

L'équipe AtlanChim Pharma

Editorial

In this specific context, Jacques LEBRETON wanted to share with you the potential of some promising nucleoside analogues for the treatment of human Coronaviruses infections. During the last years, our team of chemists has worked on several projects dealing with Nucleosides. Throughout theses works, they acquired a significant expertise in this chemistry.

During our next Webinar untitled « Nucleosides : Diamonds are Forever » , scheduled on the 18th of June, Jacques LEBRETON will address Nucleoside chemistry in all its aspects. Every month we are still sharing with you our knowledge through our posts #TheMinuteOfChemistry on our LinkedIn page .

As AtlanChim Pharma continues to gain recognition among its customers, Innovation is not our unique focus. We know how important it is to establish a relationship of trust with you, our clients. We integrate into our daily activities our values that are Transparency, Communication and Customer Orientation. This is why our chemists are at your full disposal to study your requests on custom synthesis, isotope labelling or identification and synthesis of impurities in order to make your project a success.

Finally, we would like to thank our customers and collaborators for their understanding and support while we were putting in practice the new protocol to meet current health protocol.

Enjoy your reading

AtlanChim Pharma's Team

Nucleoside analogues for the treatment of coronavirus infections: an overview of their chemistry.*

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** In memory of Prof. Chris McGuigan*

In the last 20 years, three human Coronaviruses (CoV) have emerged, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV, now named SARS-CoV-1) first identified in China, (November 2002), Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV) isolated from a lung sample of an adult patient in Saudi Arabia (April 2012) and SARS-CoV-2 (initially named 2019-nCoV) identified in December 2019 in the city of Wuhan, China[1]. This latest disease caused by SARS-CoV-2 is named Coronavirus Diseases-2019 (Covid-19). Due to the current dramatic and unexpected pandemic, research on effective treatments for Covid-19 must be greatly accelerated to find safe and effective therapeutic agents that will also need to be able to prevent the emergence of resistance. It is also now clear and accepted that new coronaviruses may emerge in the future. Unfortunately so far, there haven't been yet any approved drugs or vaccines for human CoV infections, although there are many clinical trials going on.

In this context, nucleoside analogues are a unique class of antiviral agents which inhibit viral replication through a variety of mechanisms mimicking naturally occurring nucleosides.[2,3] In this regard, several recent studies have highlighted the potential of nucleoside analogues for the treatment of human CoV infections.[4,5] This series of promising nucleoside analogues with broad-spectrum activity against human CoV infections are Ribavirin **1**, Mizoribine **2**, Gemcitabine **3**, β-D-*N*[4]-hydroxycytidine **4**, Remdesivir **5**, BCX4430 **6** and Acyclovir analogue **7** as outlined in Figure 1.

[1] G. Kroemer *et al*., *Cell Stress* **2020**, *4*, 66-75 (doi: 10.15698/cst2020.04.216) and cited literature.
[2] M. K. Yates, K. L. Seley-Radtke, *Antiviral Res*. **2019**, *162*, 5-21.
[3] K. L. Seley-Radtke, M. K. Yates, *Antiviral Res*. **2018**, *154*, 66-86.
[4] A. A. Elfiky, *Life Sciences* **2020**, *253*, 117592 (https://doi.org/10.1016/j.lfs.2020.117592).
[5] A. J. Pruijssers, M. R. Denison, *Curr. Opin. Virol*. **2019**, *35*, 57-62

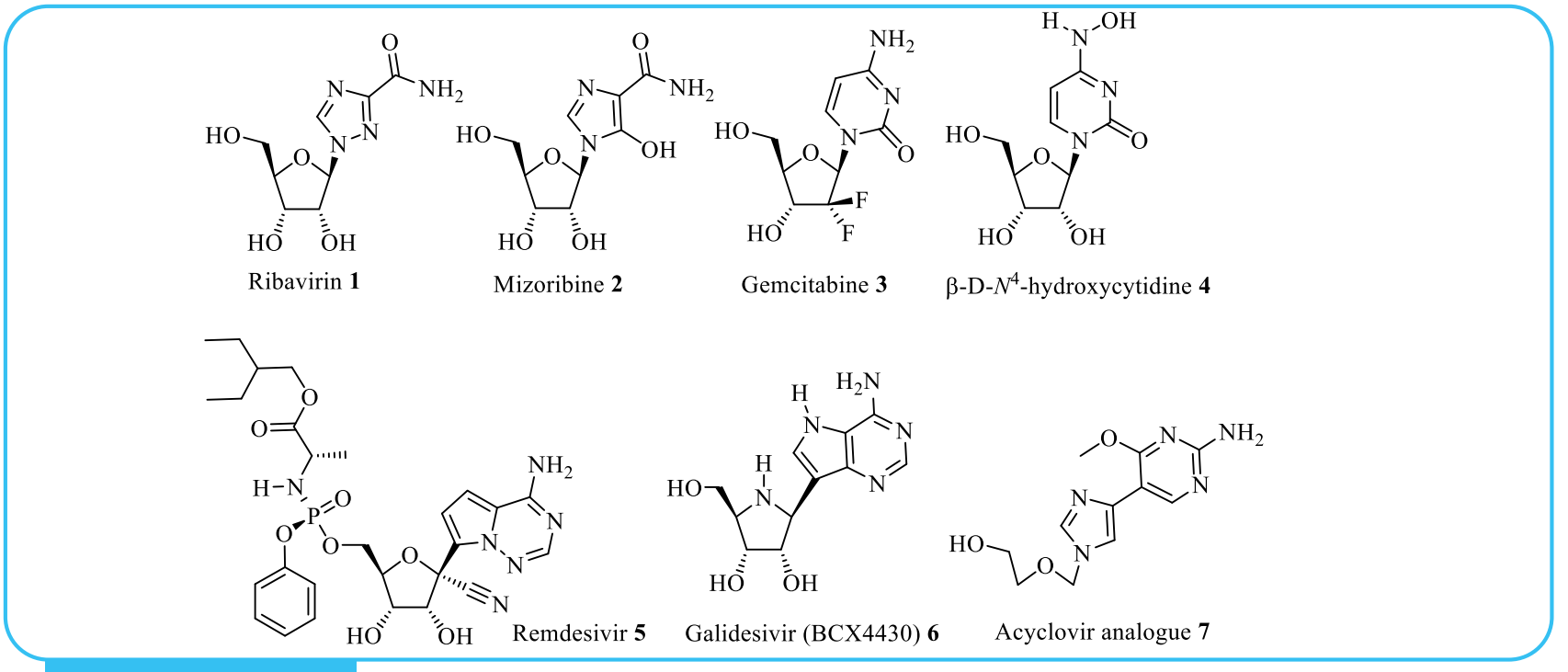


Figure 1

Figure 1. Structures of promising bioactive nucleoside analogues in the context of human CoV infections.

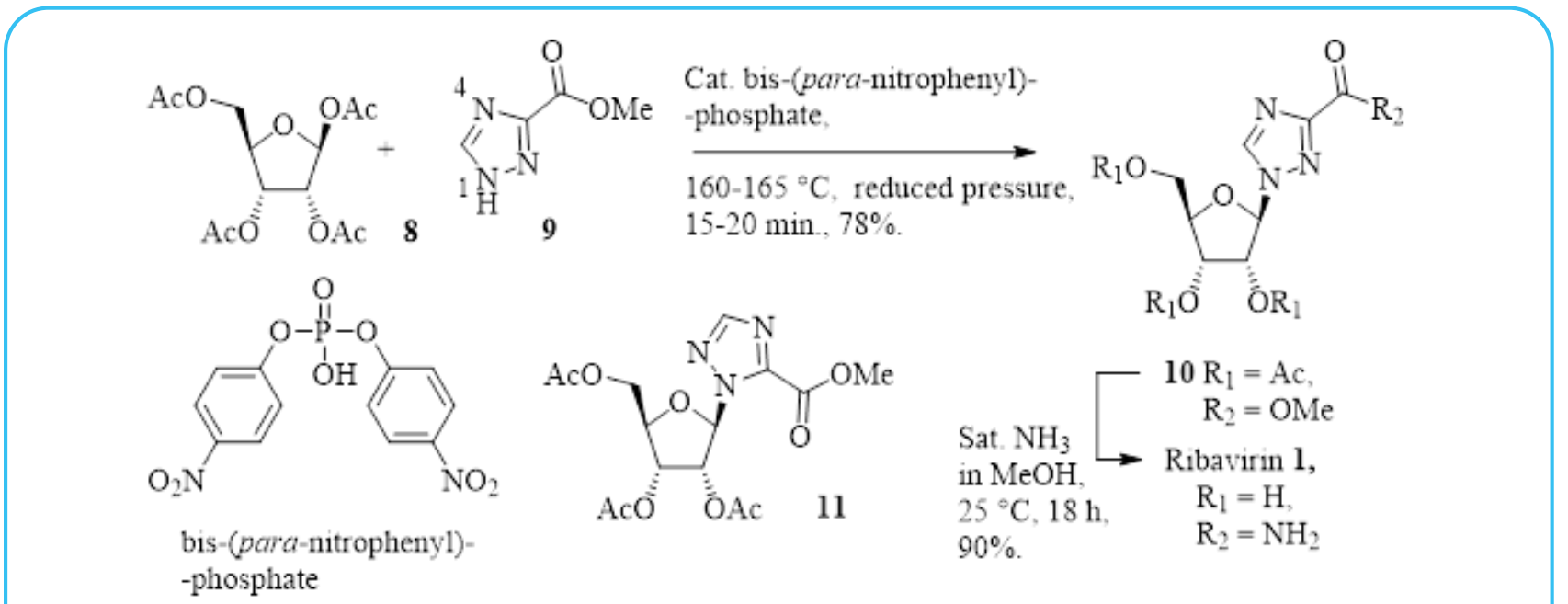
In the recent decades, nucleoside chemistry has occupied a pivotal role in innovative drug discovery and continues to attract attention. The chemistry presented in this new AtlanChim Pharma Letter fully illustrates the quest to identify the best nucleobase and/or ribose modifications as well as the most efficient choice for the prodrug strategy, in the context of treatment of human CoV infections.

*Ribavirin **1***

Ribavirin **1** (see Figure 1) is an important synthetic antiviral drug first synthetized in 1972 by Witkowski and coll.[6] Ribavirin **1** is used for treatment of influenza, severe Respiratory Syncytial virus (RSV) infection, some viral hemorrhagic fevers and in combination with interferon-α, for Hepatitis C Virus (HCV) therapy.[7,8] Ribavirin 1 shows some efficacy *in vitro*, on the three human Coronaviruses, SARS-CoV-1, MERS-CoV and Covid-19. Unfortunately, Ribavirin **1** does not provide clinical benefit to humans with coronavirus infections.[9,10]

[6] J. T. Witkowski, R. K. Robins, R.W. Sidwell, L. N. Simon, *J. Med. Chem.* **1972**, *15*, 1150-1154.
[7] V. Loustaud-Ratti, M. Debette-Gratien, J. Jacques, S. Alain, P. Marquet, D. Sautereau, A. Rousseau, P. Carrier, *World J. Hepatol*. **2016**, *8*, 123-130.
[8] For an excellent review concerning the biological activities of nucleoside analogues possessing five-membered azaheterocyclic bases, see: J. Zeidler, D. Baraniak, T. Ostrowsk, *Eur. J. Med. Chem*. **2015**, *97*, 409-418.
[9] J. S. Khalili, H. Zhu, N. S. A. Mak, Y. Yan, Y. Zhu, *J. Med. Virol.* **2020**, https://doi.org/10.1002/jmv.25798.
[10] M. Saijo, S. Morikawa, S. Fukushi, T. Mizutani, H. Hasegawa, N. Nagata, N. Iwata, I. Kurane, *Antiviral Res.* **2005**, *66*, 159-163.

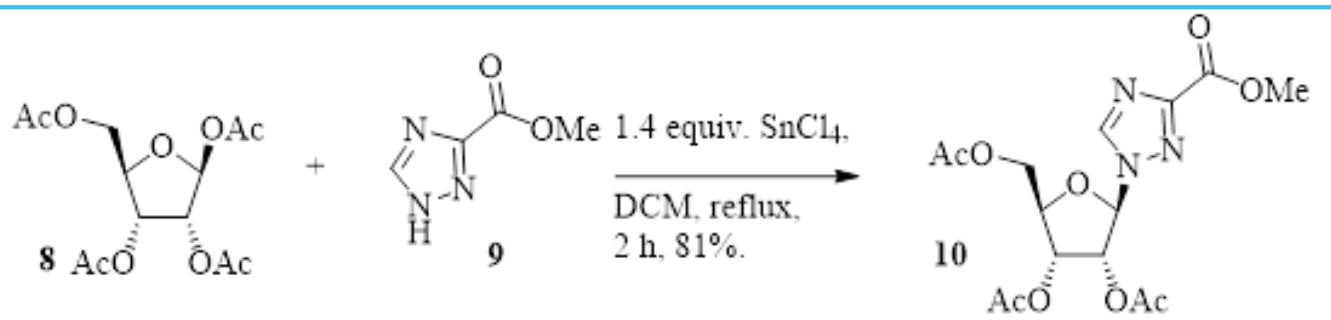
Most of synthetic approaches for preparing Ribavirin **1** reported in the literature are based on the coupling reaction under various conditions between the 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose **8** with methyl 1,2,4-triazole-3-carboxylate **9** as depicted in Scheme 1. In their pioneering work, Witkowski and coll.[6] obtained Ribavirin **1** by fusion of equimolar mixture of 3-carbomethoxytriazole **9** and tetraacetylribose **8** under *vacuum* at 160-165 °C, in the presence of bis-(*para*-nitrophenyl)phosphate. Under these rather drastic conditions, absence of solvent, Ribavirin precursor **10** was isolated in a remarkable yield of about 80% after crystallization. It should be pointed out that this reaction is highly regioselective, the minor regioisomer **11** formed by glycosylation at the N-4 atom of the triazole moiety was isolated in only 8% yield by column chromatography of the filtrate from crystallization of Ribavirin precursor **10**. Nevertheless, these extreme and critical process conditions are not easily transposable to the industrial scale. At this point, to complete the synthesis, methyl ester **10** was treated with methanolic ammonia solution providing concomitant cleavage of the acetate groups and amide formation leading to Ribavirin **1** in 90% yield.



SCHEME 1

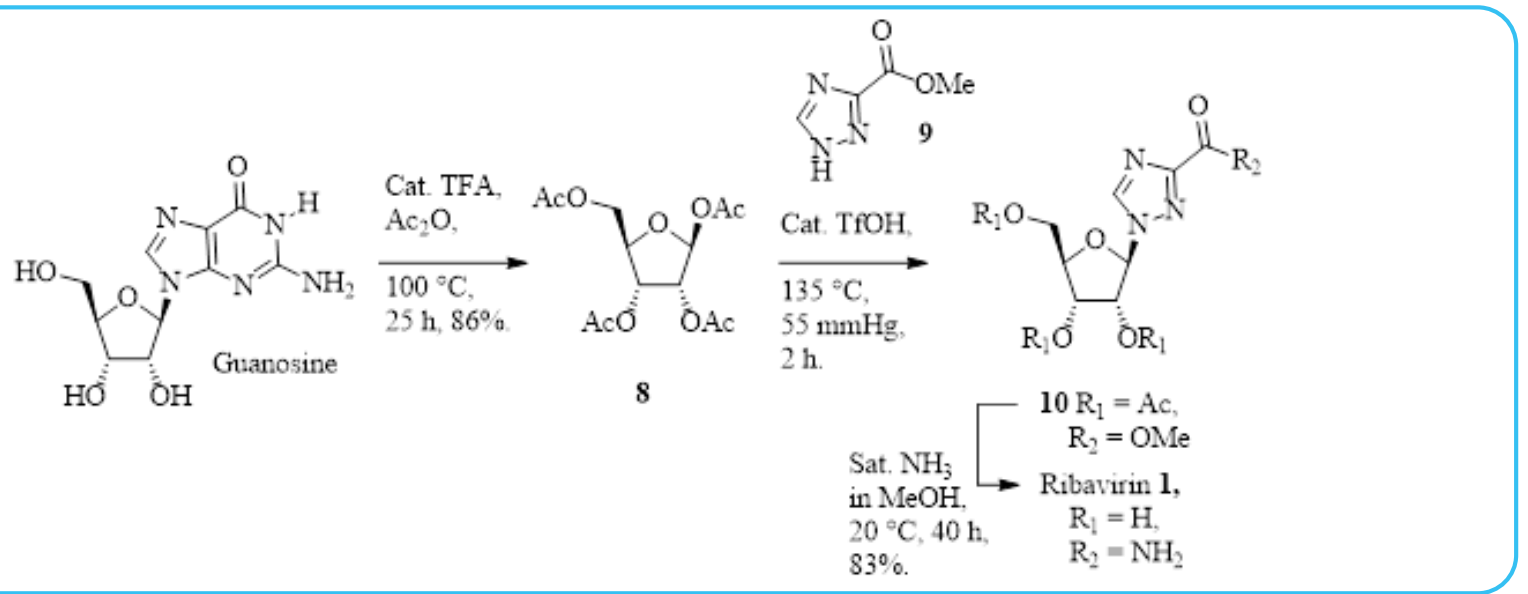
In 2007, Banfi and coll. patented a large-scale synthesis of Ribavirin **1**, in which the glycosylation between tetraacetylribose **8** and triazole **9** was carried on in refluxing DCM in the presence of 1.4 equivalents of tin tetrachloride giving the desired intermediate **10** in 81% yield (392 g scale) as outlined in Scheme **2**.[11]

[11] A. Banfi, B. Dall'Oro, M. Frigerio, A. Mancini, US Patent 7285659, **2007**.



SCHEME 2

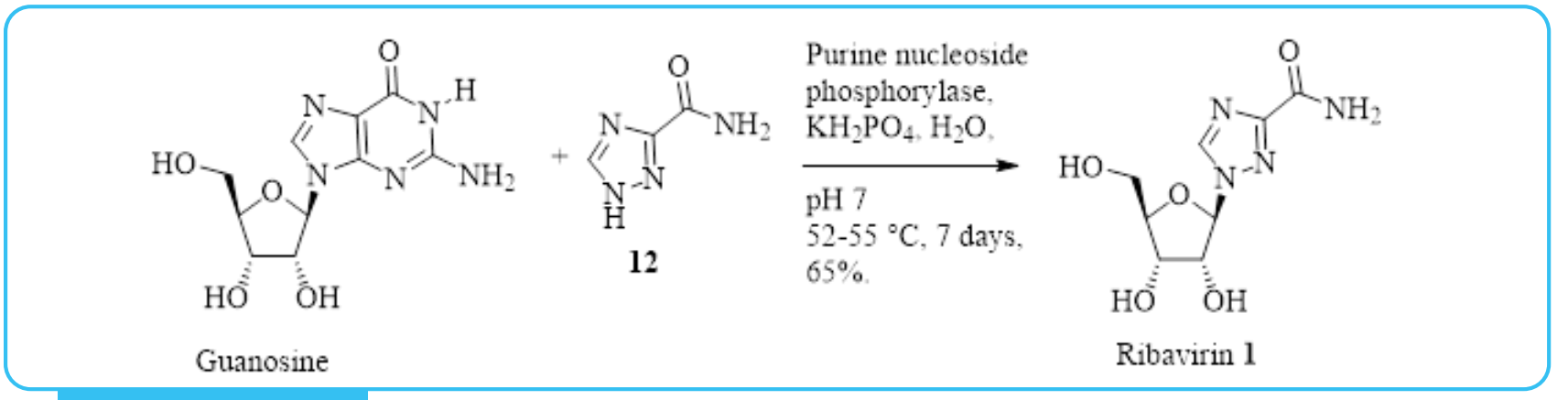
More recently, Tan and coll. published an economic route to Ribavirin **1** from Guanosine as cheap nucleoside starting materials.[12] Thus, the treatment of Guanosine (90 € *per* mole)[13] in acetic anhydride and TFA as catalyst at 100 °C provided the desired tetraacylated sugar **8** (70 € *per* mole)[13] in 86% yield (40 g scale) as presented in Scheme 3. Next, the glycosylation between this tetraacetylribose **8** and triazole **9** by fusion under *vacuum* (about 55 mmHg) at 135 °C for two hours led to intermediate **10** which was directly treated without purification with methanolic ammonia solution, as previously described, to afford Ribavirin **1** isolated in 71% yield (53 g scale) based on Guanosine, after crystallization.



SCHEME 3

It is noteworthy that various chemoenzymatic syntheses of Ribavirin **1** have been reported in the literature. In recent investigations, from 1,2,4-triazole-3-carboxamide **12** and Guanosine as ribose donor, a catalyzed transglycosylation reaction using a purine nucleoside phosphorylase (PNP) (isolated from *Escherichia coli* BL21(DE3)/pERPUPHHO1 strain) provided Ribavirin **1** in 65% yield (11 g scale), after crystallization (see Scheme 4).[14]

[12] Y. S. Li, J. J. Zhang, L. Q. Mei, C. X. Tan, *Org*. *Prep. Proced*. *Int*. **2012**, *44*, 387-391.
[13] Comparative prices of these chemicals from the same supplier (May 2020).
[14] V. Sakharov, S. Baykov, I. Konstantinova, R. Esipov, M. Dorogov, *Int. J. Chem. Eng*. **2015**, Article ID 734851, http://dx.doi.org/10.1155/2015/734851.



SCHEME 4

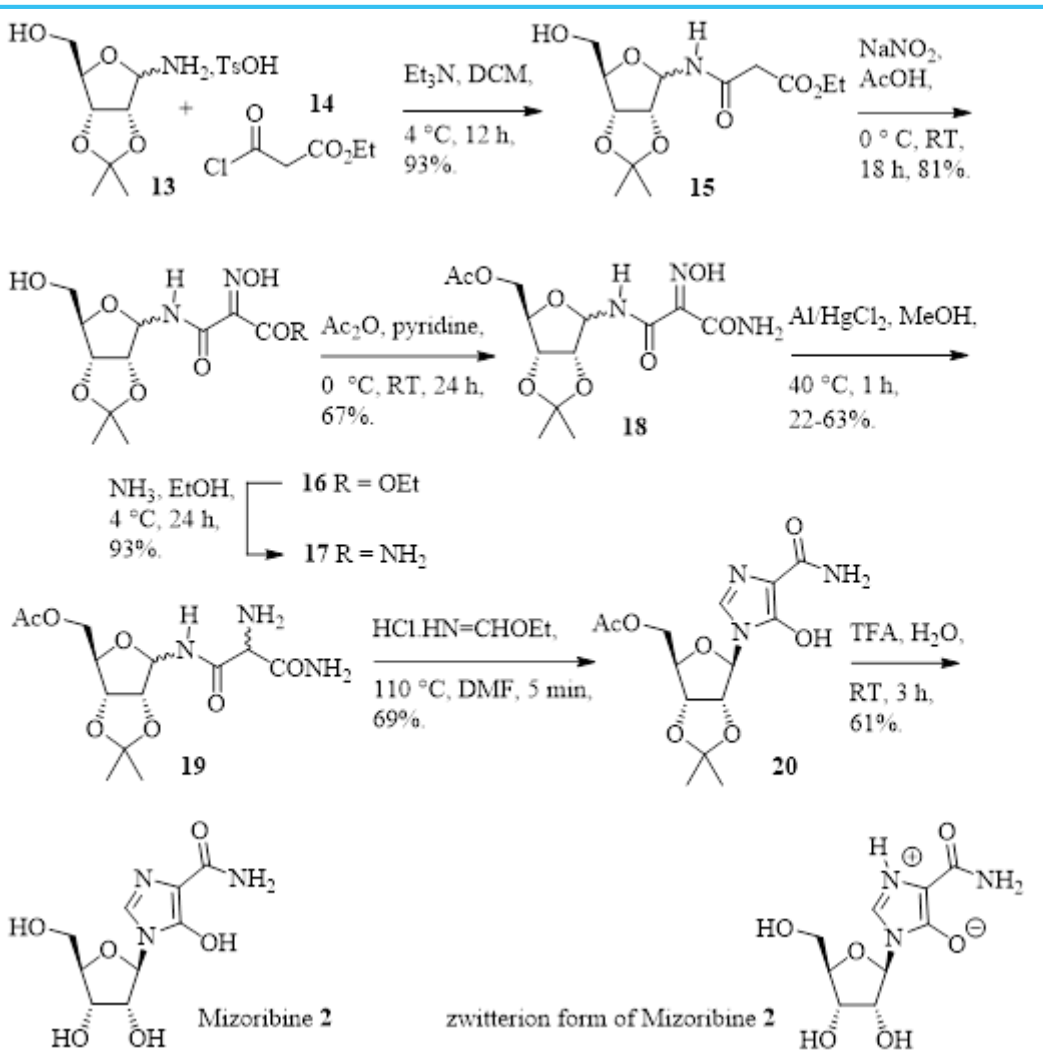
*Mizoribine **2***

Mizoribine **2** (see Figure 1, trade name Bredinin™ registered by Asahi Kasei Pharma Corporation) is an antiviral imidazole nucleoside (structurally similar to Ribavirin **1**)[8] originally isolated from *Eupenicillium brefeldianum* M-2166, an ascomycetes harvested from the soil of Hachijo Island (Japan) in 1971.[15] In the beginning of the 80's in Japan, Mizoribine **2** has found clinical use as an immunosuppressant for post-transplant patients to prevent from rejection in renal transplantation.[16,17] More recently, it has been used in association with other immunosuppressants, such as cyclosporine or tacrolimus, for transplantation.[18]

A recent synthesis of Mizoribine **2** published by Boa, Mackenzie and coll. from an acyclic precursor, which implied the construction of the 5-hydroxyimidazole base, is shown in Scheme 5.[19] The 2,3-*O*-isopropylidene-β-D-ribofuranosylamine[20] as the crystalline *para*-toluenesulfonate salt **13** was acylated with ethyl malonyl chloride **14** in the presence of triethylamine in dichloromethane to provide an inseparable anomeric mixture of amides **15** which was then subjected to aqueous sodium nitrite solution in acetic acid giving the corresponding anomeric mixture of oximes **16** in 75% for the two steps. At this point, various attempts to reduce the oxime intermediate **16** were unsuccessful. In order to circumvent this problem, treatment of this later oxime **16** with ethanolic ammonia solution provided the β-D-ribofuranosyl malondiamide derivative **17** which was then reacted with acetic anhydride in pyridine to afford the corresponding 5'-*O*-acetyl derivative **18** in 62% yield for the two steps. The acetylation of the 5' hydroxyl group in **17** greatly facilitated the chromatographic purification of later products in this sequence. Reduction of this new oxime **18** performed with an aluminium–mercury amalgam yielded to the desired 2-amino-*N*-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl) malondiamide **19** as a complex mixture of anomers and diastereoisomers.

[15] K. Mizuno, M. Tsujino, M. Takada, M. Hayashi, K. Atsumi, *J. Antibio*t. **1974**, *27*, 775-782.
[16] Y. Kawasaki, *Clin. Dev. Immunol*. **2009**, doi:10.1155/2009/681482.
[17] Y. Ichikawa, H. Ihara, S. Takahara, *Transplantation* **1984**, *38*, 262-267.
[18] T. Nakatani *et al*., *Int. J. Urol.* **2018**, *25*, 141-145, doi: 10.1111/iju.13476.
[19] R. W. Humble, D. F. Middleton, J. Banoub, D. F. Ewing, A. N. Boa, G. Mackenzie, *Tetrahedron Letters* **2011**, *52*, 6223-6227.
[20] R. S. Tipson, *J. Org. Chem*. **1961**, *26*, 2462-2464.

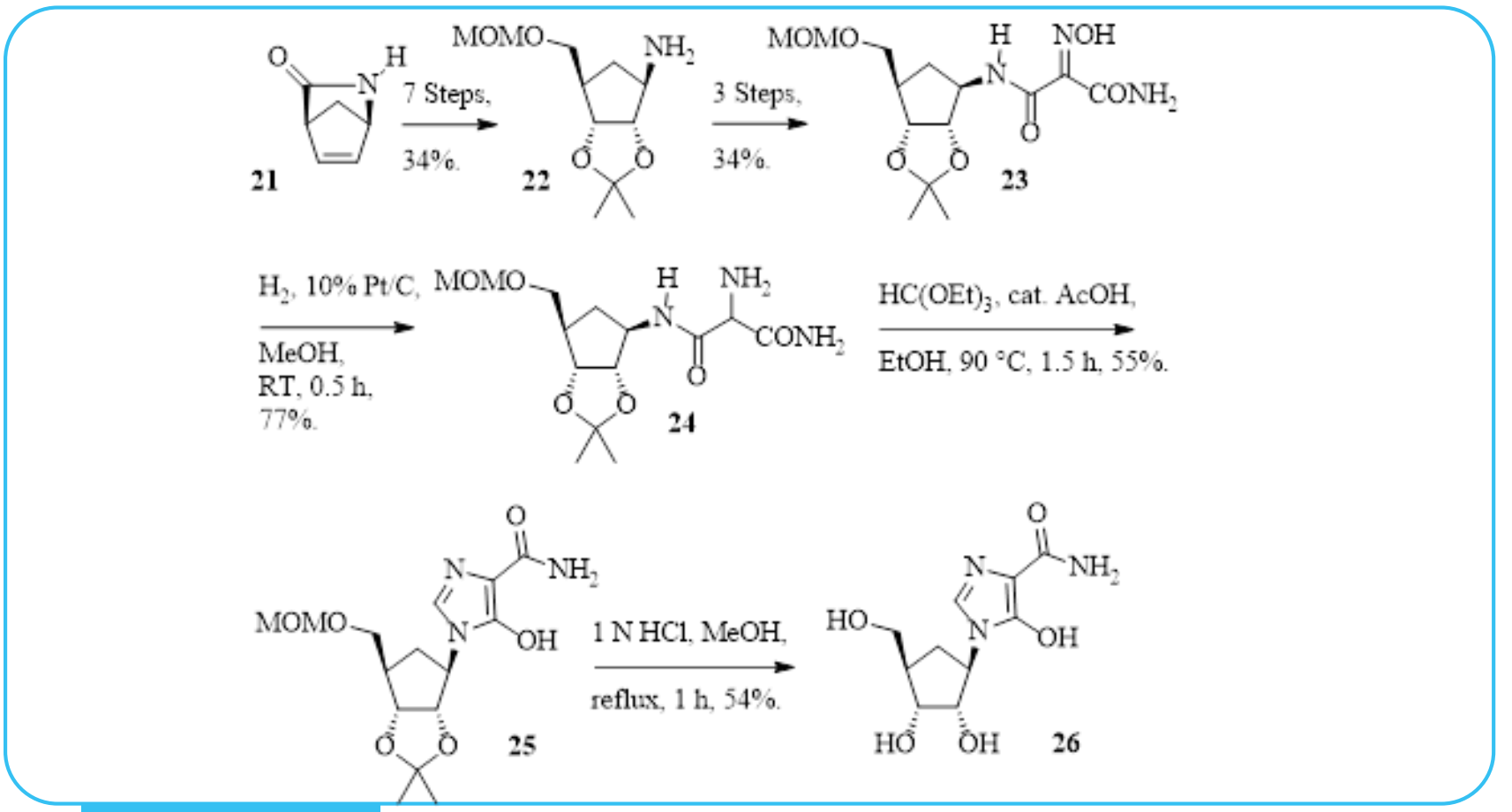
Concerning this reduction step, it is of interest that on small scales the desired 2-amino-(β-D-ribofuranosyl) malonamide **19** was isolated in about 20% yield, whereas on larger scales yields up to 60% could be obtained. Exposure of 2-amino-(β-D-ribofuranosyl) malonamide **19** to one equivalent of freshly prepared ethyl formimidate hydrochloride in DMF at 110 °C for five minutes allowed for the construction of the 5-hydroxyimidazole moiety to provide **20** in 69% yield. Surprisingly, under the standard conditions with triethyl orthoformate in DMF to build this heterocycle (*vide supra*), only by-products were formed. At the final step, the previous intermediate **20** was treated with aqueous TFA and after separation of the two anomers by chromatography on silica gel, Mizoribine **2** was isolated in 61% yield. It is important to mention that Mizoribine **2** is isolated in its zwitterion form (see Scheme **5**).



SCHEME 5

In 2013, Nair and Zhang used this previous synthetic route from the commercially available chiral bicyclic lactam (1*R*,4*S*)-2-azabicyclo[2.2.1] hept-5-en-3-one **21** (1500 € *per* mole) to prepare the carbocyclic analogue **26** of Mizoribine **2**, as briefly outlined in Scheme **6**.[21]

It should be pointed out that this chiral starting material **21** (also known as (*R*)-(-)-Vince lactam), prepared from racemic mixture using kinetic enzymatic resolution, is an extremely versatile synthon for the preparation of carbocyclic nucleoside analogues.[22] Hydrogenation of oxime **23** in the presence of catalytic amount of 10% platinum on carbon afforded the desired amine **24** in 77% yield. At this point, as previously discussed, the construction of imidazole ring was tricky in this series, and inefficient using orthoformate or formimidate reagents in DMF. However, cyclization to the 5-hydroxyimidazole ring occurred efficiently when amino malonamide carba-ribose **24** was heated at 90 °C with triethyl orthoformate in the presence of catalytic amounts of acetic acid in ethanol to give, after purification by HPLC, the protected carbocyclic Mizoribine **25** in 55% yield. Removal of protecting groups in acidic medium led to carbocyclic Mizoribine **26** in 54% yield, after HPLC purification.



SCHEME 6

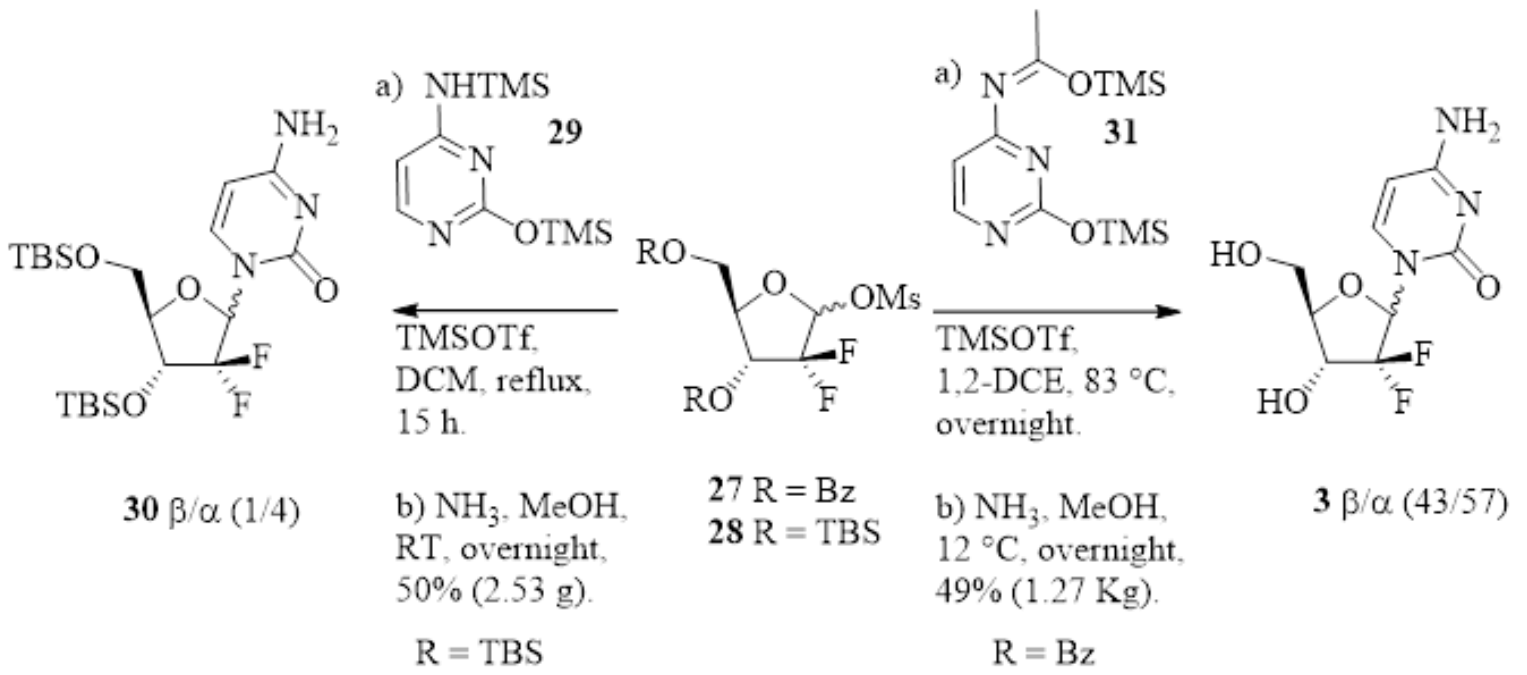
*Gemcitabine **3***

Gemcitabine **3** (see Figure 1, 2'-deoxy-2',2'-difluorocytidine, trade name Gemzar®) is a chemotherapy drug used to treat various types of cancer.[23] Gemcitabine **3** was patented in 1983 by Eli Lilly Company, approved for medical use in 1995 and its generic versions were introduced in Europe in 2009 and one year later in the US.

[21] V. Nair, F. Zhang, *Molecules* **2013**, *18*, 11576-11585.
[22] For a recent example, see: S. Gao, S. Zhu, R. Huang, Y. Lu, G. Zheng, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 3878-3881.
[23] For an excellent review on synthesis of Gemcitabine, see: K. Brown, M. Dixey, A. Weymouth-Wilson, B. Linclau, *Carbohyd. Research* **2014**, *387*, 59-73 and cited literature.

In 2014, a study reported that Gemcitabine **3** inhibits both MERS-CoV and SARS-CoV with micromolar EC_{50} (1.2 µM and 4.9 µM, respectively) and low toxicity.[24]

The preparation of Gemcitabine **3** from protected difluororibofuranosyl methanesulfonate derivatives such as **27** or **28** by condensation of persilylated cytosine base derivatives **29**[25] or **31**[26] illustrated the crucial problem in this field to form only the requisite β-configuration at the anomeric position (see Scheme 7). Under the Vorbrüggen protocol in these two examples, nucleobase introduction carried out by displacement of the mesylate leaving group by nucleophilic persilylated pyrimidine derivatives led preferentially to the undesired α-anomers. Due to its importance as an anticancer agent, this key glycosylation step in the preparation of Gemcitabine **3** has been the subject of intense investigations to increase the yield and the stereoselectivity. Different difluorosugar donors with various anomeric leaving groups as well as hydroxyl protections have been evaluated, leading only to moderate improvements. In this context, the development of selective crystallization procedures to provide anomerically pure Gemcitabine **3** from the previous mixtures is a key issue.



SCHEME 7

Scheme 7

An effective and elegant large-scale synthesis of Gemcitabine **3** was carried out without chromatography, using a *para*-phenylbenzoyl protective group (PhBz-) in order to enhance the crystallinity of the intermediates and thereby facilitate fractional crystallization of the α-ribofuranosyl bromide **35** out of the anomeric mixture, as reported in Scheme 8.[27]

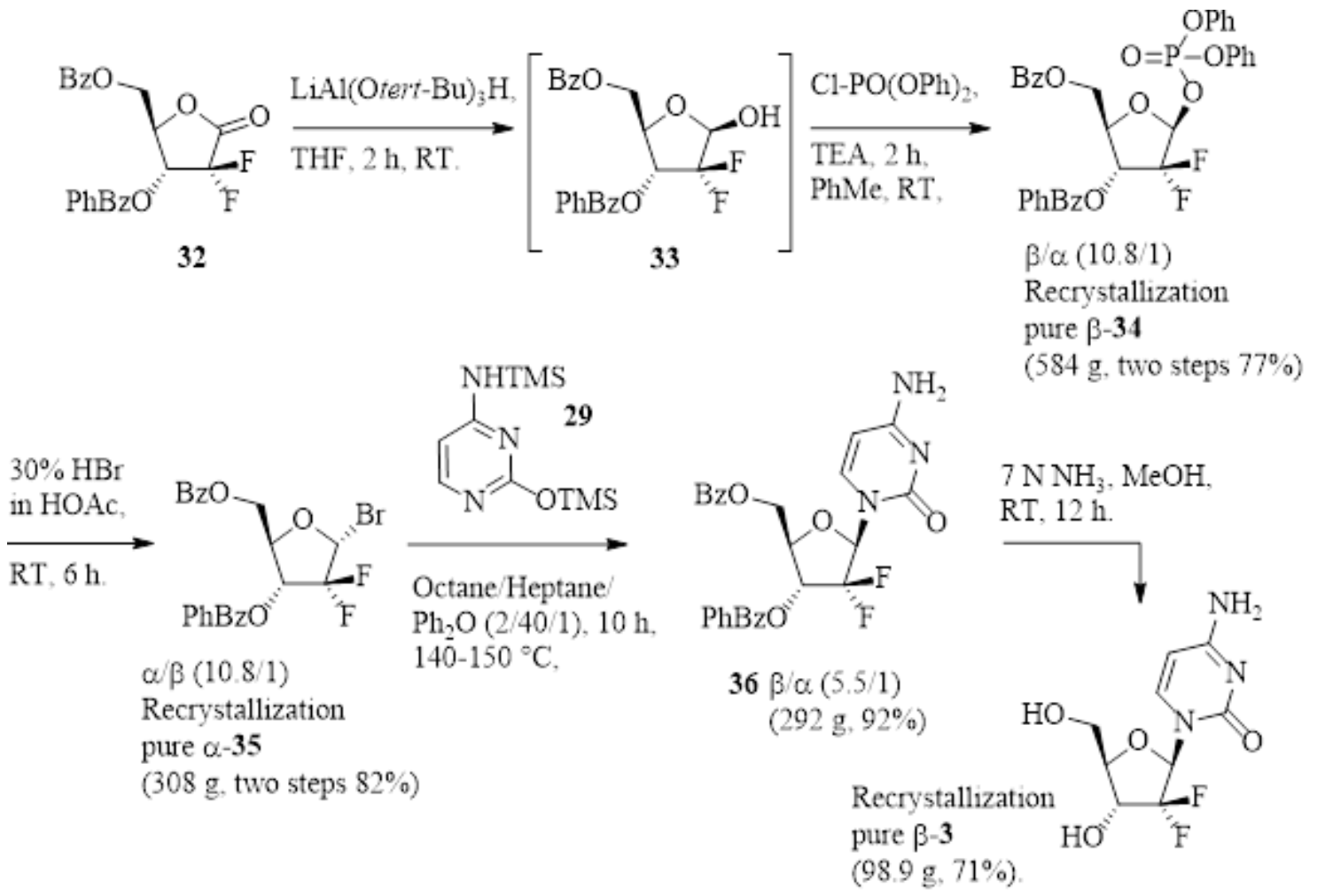
[24] M. B. Frieman *et al*., *Antimicrob. Agents Chemother*. **2014**, *58*, 4885-4893.
[25] L. W. Hertel, J. S. Kroin, J. W. Misner, J. M. Tustin, *J. Org. Chem*. **1988**, *53*, 2406-2409.
[26] T. S. Chou, P. C. Heath, L. E. Patterson, L. M. Poteet, R. E. Lakin, A. H. Hunt, *Synthesis* **1992**, 565-570.

The protected 2',2'-difluoro-2'-deoxyribose sugar **32** was prepared following a well-known seven-step sequence, starting with D-mannose, involving a Reformatsky reaction with the D-glyceraldehyde intermediate and ethyl bromodifluroacetate (not shown).[28] Reduction of the lactone **32** was achieved in the presence of $LiAl(O\textit{tert}\text{-}Bu)_3H$. The resulting lactol **33** was converted into the corresponding diphenylphosphate intermediate **34** as a 1/10.8 α/β anomeric mixture which after recrystallization enhanced this ratio to >2/98. Next, pure β -diphenylphosphate intermediate **34** was treated with 30% HBr in acetic acid to provide a mixture of solid ribofuranosyl bromides **35** as a 10.8/1 α/β anomeric mixture which, one again, after recrystallization enhanced this ratio to >99.7/0.3. This three-step sequence permitted the large-scale synthesis (308 g) of the required anomerically α-pure bromide **35** in about 63% overall yield from the lactone **32**. Concerning the key glycolysation step after some experimentations, it was noticed that removal of TMSBr formed *in situ* during the reaction by continuous distillation of heptane, as a carrier with dropwise simultaneous addition of this solvent, was crucial. In this condition, the protected gemcitabine **36** was isolated in 92% yield as a 5.5/1 β/α anomeric mixture which was treated without further purification with ammonia in methanol giving after recrystallization the pure β-Gemcitabine **3** (containing less than 0.02% of its α-anomer based on HPLC analysis) in 65% overall yield (98.9 g) from the α-pure bromide **35**. A control experiment was done without distilling off TMSBr during this glycosylation step, providing the protected gemcitabine **36** as a 1/1 anomeric mixture. It was assumed by the authors that TMSBr formed in the reaction mixture, promoted anomerization of the anomerically α-pure bromide **35** bromide via a S_N1-type reaction involving an oxocarbenium ion intermediate and/or a S_N2-type reaction.[29]

[27] Y.-K. Chang, J. Lee, G.-S. Park, M. Lee, C. Hyun Park, H. Kyong Kim, G. Lee, B.-Y. Lee, J. Y. Baek, K. S. Kim, *Tetrahedron* **2010**, *66*, 5687-5691.

[28] For preparation of this protected 2',2'-difluoro-2'-deoxyribose sugar **32**, see Z. Chen, T. C. Ku, K. L. Seley-Radtke, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 4274-4276 and cited literature.

[29] For recent investigations concerning this *N*-glycosylation, see: T. Liu, J. Tang, J. Liang, Y. Chen, X. Wang, J. Shen, D. Zhao, B. Xiong, J.-D. Cen, Y.-L. Chen, *Tetrahedron* **2019**, *75*, 1203-1213.



SCHEME 8

*β-D-N^4-Hydroxycytidine **4***

Recent publications pointed out that β-D-N^4-Hydroxycytidine **4** (see Figure 1) was more potent than Ribavirin **1** to treat alphavirus infections such as Chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV).[30,31] This year, a set of investigations highlighted the antiviral potency of the orally bioavailable EIDD-2801 **37** (see Figure 2), an analogue of β-D-N^4-Hydroxycytidine **4**, against human CoVs including COVID-19 developed by researchers from the University of North Carolina at Chapel Hill (UNC-Chapel Hill, USA).[32]

[30] M. Ehteshami, S. Tao, K. Zandi, H.-M. Hsiao, Y. Jiang, E. Hammond, F. Amblard, O. O Russell, A. Merits, R. F. Schinazi, *Antimicrob. Agents Chemother.* **2017**, *61*, e02395-16. https://doi.org/10,1128/AAC.02395-16.

[31] N. Urakova, V. Kuznetsova, D. K. Crossman, A. Sokratian, D. B. Guthrie, A. A. Kolykhalov, M. A. Lockwood, M. G. Natchus, M. R. Crowley, G. R. Painter, E. I. Frolova, I. Frolov, *J. Virol*. **2018**, *92*, e01965-17. https://doi.org/10.1128/JVI.01965-17.

[32] T. P. Sheahan *et al*., *Sci. Transl. Med*. **2020**, *12*, eabb5883, http://stm.sciencemag.org/content/12/541/eabb5883.

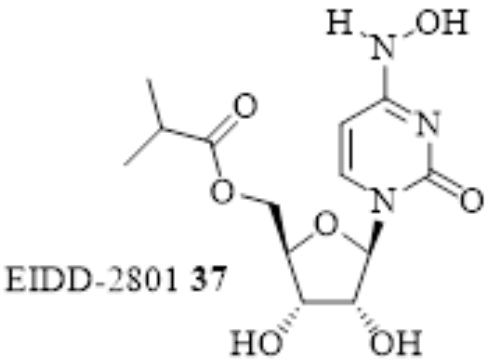
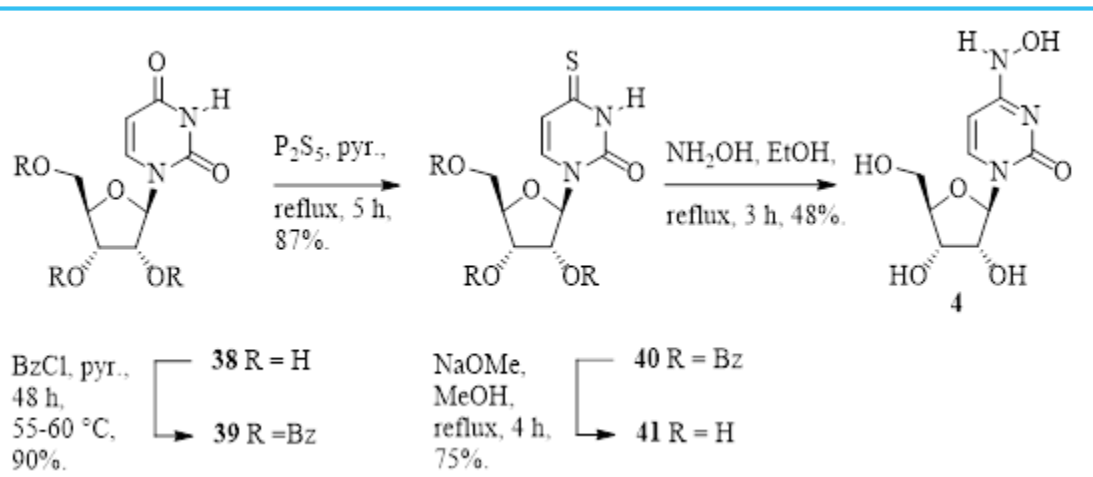


Figure 2

Figure 2. Structure of the orally bioavailable EIDD-2801 **37**.

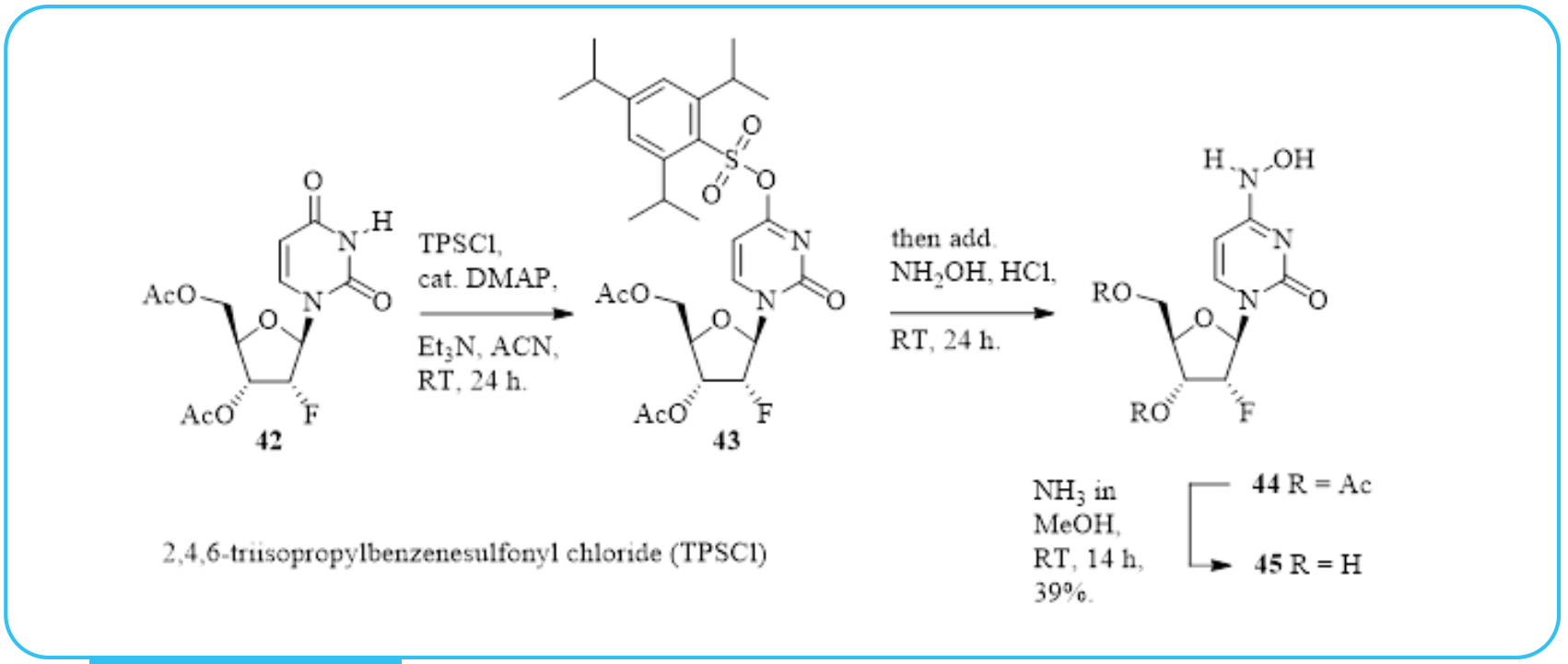
One of the oldest synthesis of β-D-N^4-Hydroxycytidine **4** is outlined in Scheme 9.[33] Starting from Uridine **38**, treatment with benzoyl chloride afforded the 2,3,5-tri-*O*-benzoyl-uridine **39** which was treated with phosphorus pentasulfide to give the protected 4-thiouridine **40** in 78% overall yield for the two steps. After cleavage of the benzoyl groups upon treatment with sodium methoxide in methanol, the resulting intermediate **41** was refluxed in ethanolic solution of hydroxylamine (a weaker base compared to ammonia) providing β-D-N^4-Hydroxycytidine **4** in a modest overall yield for this sequence.



SCHEME 9

In more recent investigations, the 4-position was activated as a sulfonate followed by treatment with hydroxylamine, as exemplified in the synthesis of β-D-N^4-hydroxycytidine fluoro nucleoside analogue **45** (see Scheme 10).[34] Treatment of compound **42** with 2,4,6-triisopropylbenzenesulfonylchloride (TPSCl) in the presence of Et_3N afforded the desired sulfonate **43**. At the final stage from this later intermediate **43**, nucleophilic substitution with hydroxylamine hydrochloride followed by deprotection with ammonia in methanol afforded β-D-N^4-Hydroxycytidine fluoro nucleoside **45** in 39% overall yield for this three-step sequence.

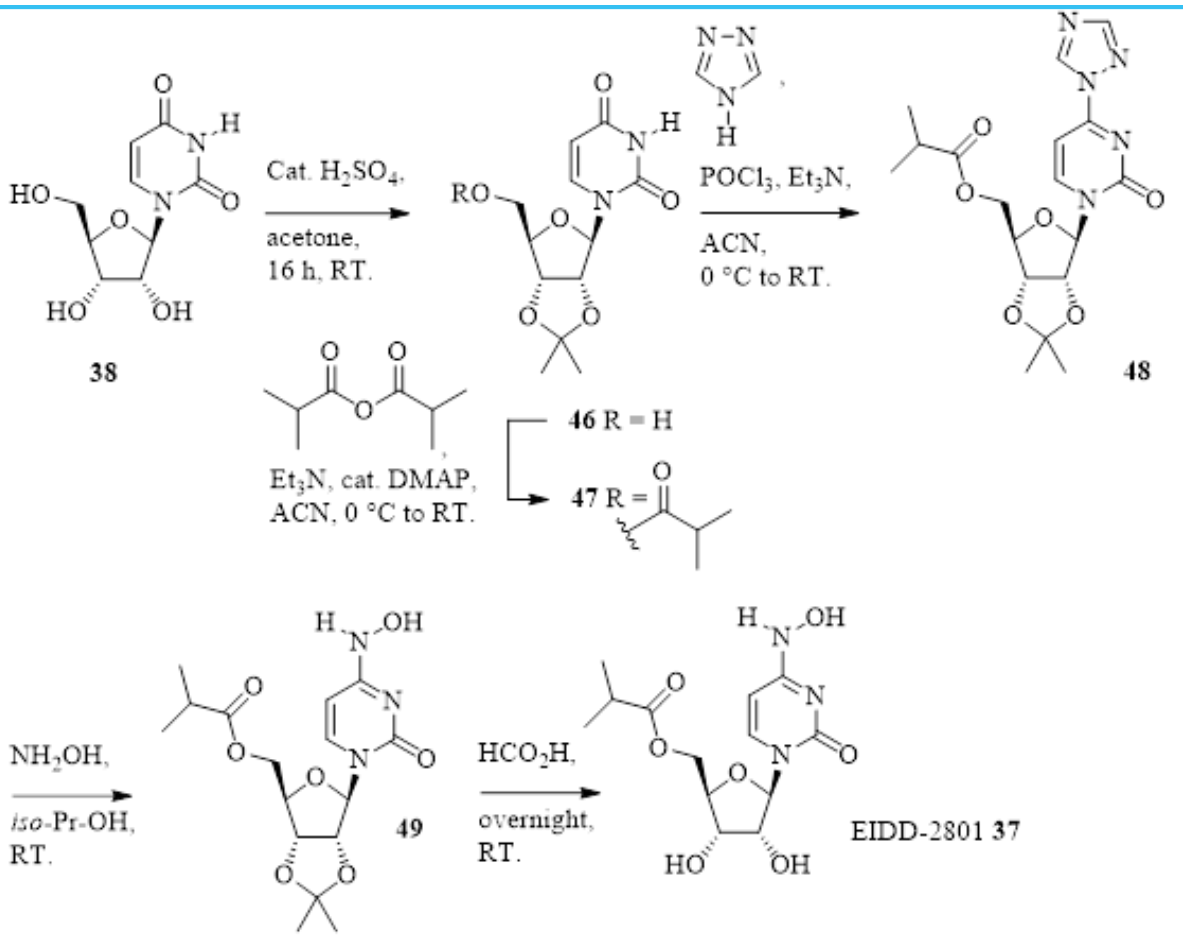
[33] J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, G. B. Brown, *J. Am. Chem. Soc.* **1958**, *81*, 178-187.
[34] J. Shi *et al*., *Bioorg. Med. Chem*. **2005**, *13*, 1641-1652.



SCHEME 10

The preparation of the orally bioavailable EIDD-2801 **37**, is described in a patent, and briefly presented in Scheme 11.[35] The 2',3'-*O*-isopropylidene uridine derivative **46** (prepared from Uridine **38** by treatment in acetone containing a catalytic amount of H_2SO_4) was acetylated with *iso*-propanoic anhydride to afford **47** which was treated with 1,2,4-triazole, triethylamine, and phosphorous oxychloride in acetonitrile to provide the triazolyl derivative **48**. Then from this later compound **48**, upon reaction with hydroxylamine followed by cleavage of the 2',3'-*O*-isopropylidene protecting group of **49** gave the targeted EIDD-2801 **37**.

[35] G. R. Painter, G. R. Bluemling, M. G. Natchus, D. Guthrie, WO 2019113462 A1 20190613, **2019**.



SCHEME 11

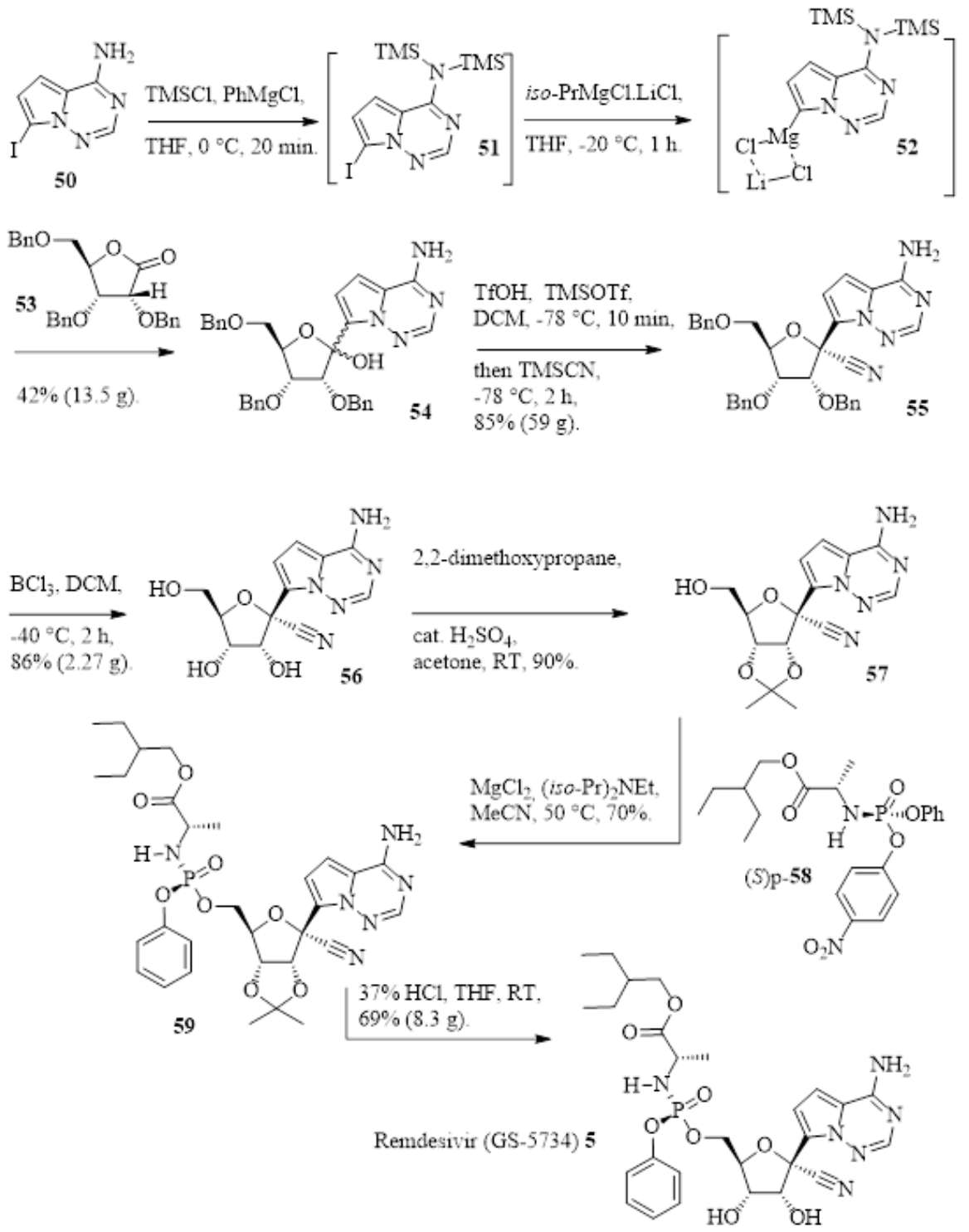
Remdesivir (GS-5734) ***5***

Remdesivir (GS-5734) **5** (see Figure 1) is a monophosphoramidate prodrug of an adenosine *C*-analogue which was developed by Gilead Sciences for the treatment for Ebola Hemorrhagic Virus.[36] A very recent study of potential Covid-19 treatments showed that the median time to recovery was 11 days for patients receiving Remdesivir **5** *versus* 15 days for those receiving placebo.[37,38,39,40] A scalable preparation of Remdesivir **5** is described in Scheme 12.[41] To avoid the use of cryogenic temperatures for the key glycosylation step, 4-aza-7-deaza-9-iodo-adenine **50** (1.000 € *per* mole) was temporally protected as the *N,N*-bis-silylated amine **51** which was submitted to a magnesium-halogen exchange using *iso*-PrMgCl·LiCl complex to afford the organometallic species **52**. Then, nucleophilic addition of the lithium-magnesium heterocycle complex **52** in solution to tribenzyl lactone **53** gave the 1'-hydroxy-1'-*C*-nucleoside **54** as an anomeric mixture, in a modest yield of 42%. This later hemiketal **54** was reacted with TMSCN, TMSOTf, and TfOH at -78 °C to provide the cyano derivative **55** in 85% yield in >95/5 anomeric ratio.

[36] S. Bavai *et al., Nature* **2016**, *514*, 47-53 and cited literature.
[37] H.C. Lane *et al.*, *N. Engl. J. Med*. **2020**, DOI: 10.1056/NEJMoa2007764.
[38] R. T. Eastman, J. S. Roth, K. R. Brimacombe, A. Simeonov, M. Shen, S. Patnaik, M. D. Hall, *ACS Cent. Sci*. **2020**, https://dx.doi.org/10.1021/acscentsci.0c00489.
[39] A. Shannon, N. T.-T. Le, B. Selisko, C. Eydoux, K. Alvarez, J.-C. Guillemot, E. Decroly, O. Peersen, F. Ferron, B. Canard, *Antiviral Res*. **2020**, *178*, 104793, https://doi.org/10.1016/j.antiviral.2020.104793.
[40] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, *Cell Research* **2020**, *30*, 269-271 and cited literature.
[41] R. L. Mackman *et al.*, *J. Med. Chem*. **2017**, *60*, 1648−1661.

It was also mentioned by the authors that the addition of TfOH was crucial to promote the high yield and high selectivity favoring the desired β-anomer. In the same conditions, an experiment carried out without TfOH led to the cyano derivative **55** in 65% yield in 85/15 anomeric ratio. Also, concerning this transformation, it was reported in the literature that at -78 °C chelation of the silicon atom of the trimethylsilyl cyanide to oxygen electron lone pair of the 2'-benzyl ether results in the delivery of the cyanide from the α-face preferentially.[42] From this later compound **55**, for an optimal introduction of *para*-nitrophenolate 2-ethylbutyl-L-alaninate prodrug precursor **58** at C5', subsequent debenzylation using BCl_3 followed by acetonide protection of the 2′,3′-hydroxyl moieties of the resulting triol **56** with 2,2-dimethoxypropane in the presence of H_2SO_4 furnished the desired primary alcohol **57** in 90% overall yield. At the final stage, the pure single (*S*)p diastereomer of the *para*-nitrophenolate prodrug precursor **58** in the presence of $MgCl_2$ and Hünig's base was reacted with primary alcohol **57** to furnish intermediate **59** which was engaged in a deprotection of the acetonide with concentrated HCl in THF giving the Remdesivir (GS-5734) **5** in about 50% overall yield for this two-step sequence.

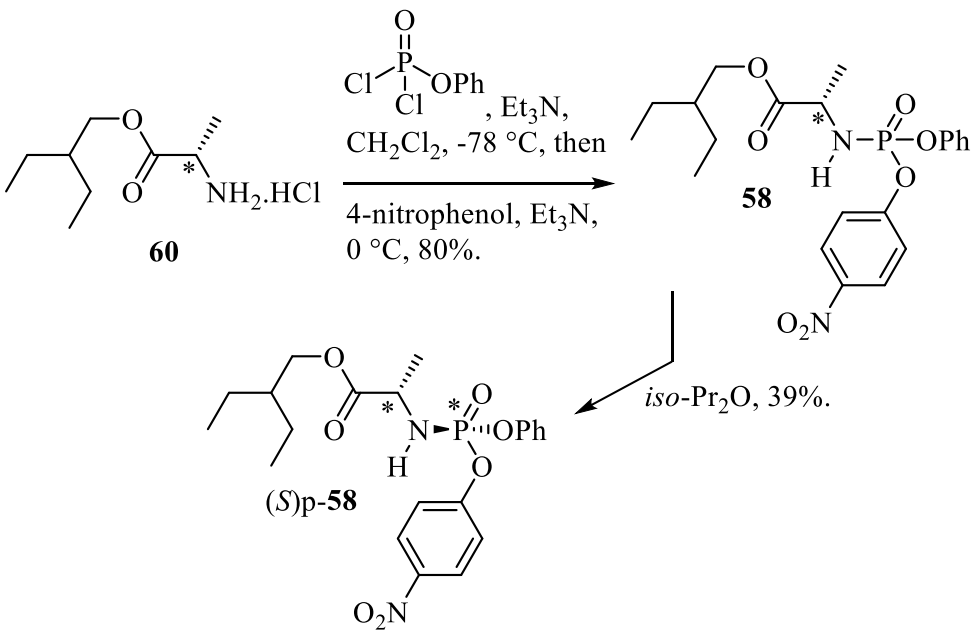
[42] S E. Metobo, J. Xu, O. L. Saunders, T. Butler, E. Aktoudianakis, A. Cho, C. U. Kim, *Tetrahedron Letters* **2012**, *53*, 484-486.



SCHEME 12

The pure, single (*S*)p diastereomer of the *para*-nitrophenolate prodrug precursor (*S*)p-**58** was prepared as presented in Scheme 13.[41,43] The treatment of 2-ethylbutyl-L-alanine **60** with $OP(OPh)Cl_2$, followed by addition of 4-nitrophenol, allowed the formation of **58** as a diastereomeric mixture at phosphorus atom in 80% yield. From this previous mixture, the pure (*S*)p diastereomeric prodrug precursor (*S*)p-**58** was obtained in 39% yield after resolution through crystallization in di-*iso*-propyl ether.

[43] Y. Mehellou, H. S Rattan, J. Balzarini, *J. Med. Chem.* **2018**, *61*, 2211-2226.



SCHEME 13

Galidesivir (BCX4430) ***6****.*

Galidesivir (BCX4430) **6** (see Figure 1) a viral RNA-dependent RNA polymerase inhibitor exhibits broad-spectrum antiviral activity.[44,45] Galidesivir (BCX4430) **6** has recently been reported to be effective against coronavirus infections including SARS-CoV-2.[46] The preparation of Galidesivir (BCX4430) **6** started from the protected 1,4-dideoxy-1,4-imino-D-ribitol **61**[47] as outlined in Scheme 14.[48] Treatment of this later iminoribitol derivative **61** with *N*-chlorosuccinimide (NCS) provided the *N*-chlorinated intermediate which by reaction with a strong base (Lithium tetramethylpiperidide (LiTMP)) gave the corresponding imine **62**. This resulting intermediate **62** was condensed, without further purification, with the lithiated acetonitrile at low temperature to lead after purification to the desired cyanomethyl *C*-glycoside derivative **63** in 36% overall yield, from the protected 1,4-dideoxy-1,4-imino-D-ribitol **61**. After protection of amine **62**, as a *N*-Boc derivative **64** under standard conditions, treatment with *tert*-butoxy-bis(*N*,*N*-dimethylamino)methane (Bredereck's reagent) in DMF at 70 °C gave the enamine **65**, which was subjected, without any purification, to mild acid hydrolysis to afford the enol **66** in 61% overall yield for the three steps. Exposure of this enol **66** to ethyl glycinate hydrochloride furnished the enamine **67** which was subsequently treated with an excess of benzylchloroformate and DBU leading to the pyrrole **68** with N-1 protected as the carbamate. Following this two-step sequence, the *N*-protected pyrrole **68** was isolated in 65% overall yield. At the final stage, hydrogenolysis of **68** readily afforded pyrrole **69**, which was subsequently treated with formamidine acetate to generate the deazapurine aza-C-glycoside **70**. Deprotection with TFA provided the target Galidesivir (BCX4430) **6** in 74% overall yield from **69**.

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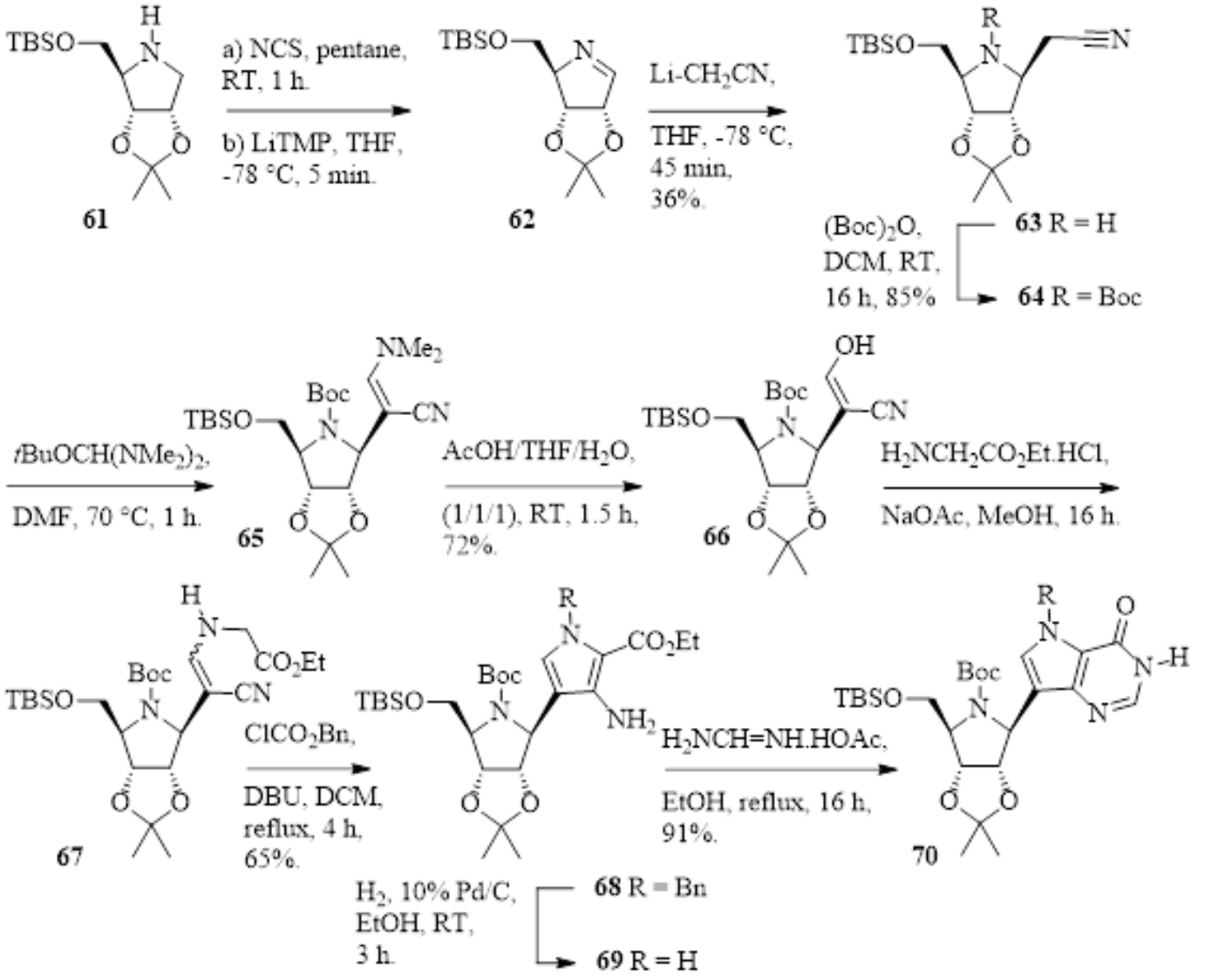
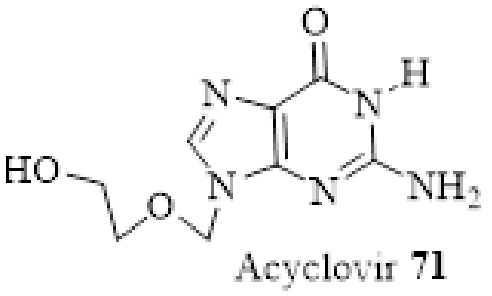
[44] W. P. Sheridan, *J. Infect. Public Heal.* **2016**, *9*, 220-226.
[45] S. Bavai *et al*., *Nature* **2016**, *514*, 47-53 and cited literature.
[46] W.-F. Zhang, P. Stephen, J.-F. Thériault, R. Wang, S.-X. Lin, *J. Phys. Chem. Lett*. **2020**, *11*, 4430−4435.
[47] For a preparation of protected 1,4-dideoxy-1,4-imino-D-ribitol **61**, see: A. Gillig, S. R. Majjigapu, B. Sordat, P. Vogel, *Helv. Chim. Acta* **2012**, *95*, 34-42, and cited literature.
[48] G. B. Evans, R. H. Furneaux, G. J. Gainsford, V. L. Schramm, P. C. Tyler, *Tetrahedron* **2000**, *56*, 3053-3062.


a) NCS, pentane, RT, 1 h.
b) LiTMP, THF, -78 °C, 5 min.
Li-CH2CN,
THF, -78 °C, 45 min, 36%.
(Boc)2O, DCM, RT, 16 h, 85%
63 R = H
64 R = Boc
tBuOCH(NMe2)2,
DMF, 70 °C, 1 h.
AcOH/THF/H2O, (1/1/1), RT, 1.5 h, 72%.
H2NCH2CO2Et.HCl, NaOAc, MeOH, 16 h.
ClCO2Bn, DBU, DCM, reflux, 4 h, 65%.
H2, 10% Pd/C, EtOH, RT, 3 h.
68 R = Bn
69 R = H
H2NCH=NH.HOAc, EtOH, reflux, 16 h, 91%.
TFA, RT, overnight, 81%
Galidesivir (BCX4430) 6

SCHEME 14

Acyclovir analogue 7

Acyclovir **71** (see Figure 3) is one of the most important antiviral drugs, primarily used for the treatment of herpes simplex virus infections. This flexible nucleoside analogue (called ‘‘Fleximers” or “Flex-analogues”) is mimicking guanosine.



Acyclovir **71**

Figure 3

Figure 3. Structure of Acyclovir **71**.

In 2015, Seley-Radtke and coll. published the synthesis and evaluation of a series of Acyclovir analogues with anti-coronavirus activity, such as the acyclic nucleoside analogue **7** (see Scheme 15).[50] It should be pointed out that this contribution reported the first nucleoside analogues to exhibit significant activity against MERS-CoV.[51] Surprisingly, this compound **7** displayed any antiviral activity towards SARS-CoV. It is also worth noticing that Acyclovir **71**, the parental compound, has no significant activity against MERS-CoV and SARS-CoV. Using modified Vorbrüggen conditions, coupling 2-acetoxyethyl acetoxymethylether **73** with diiodoimidazole **72** gave the intermediate **74** in modest yield (see Scheme 15). Then, this later compound **74** after standard protection group manipulations provided **76** which was submitted to selective deiodination with EtMgBr to afford the key intermediate **77**[52] in 40% yield for this three-step sequence. At the final stage, organotin pyrimidine **78** (prepared from commercially available 2-amino-5-chloro-6-methoxy pyrimidine according to literature)[53] was engaged with the iodo derivative **77** in a palladium-catalyzed cross-coupling to furnish the protected acyclic nucleoside analogue **79** in 42% yield. At this point, the hydrogenolysis of benzyl ether protecting group in compound **79**, in the presence of 2-amino-6-methoxy pyrimidine moiety, was problematic: ammonium formate in EtOH under reflux in presence of Pd/C gave the targeted acyclic nucleoside analogue **7** in 22% yield, as well as recovered starting material **79** (52%).

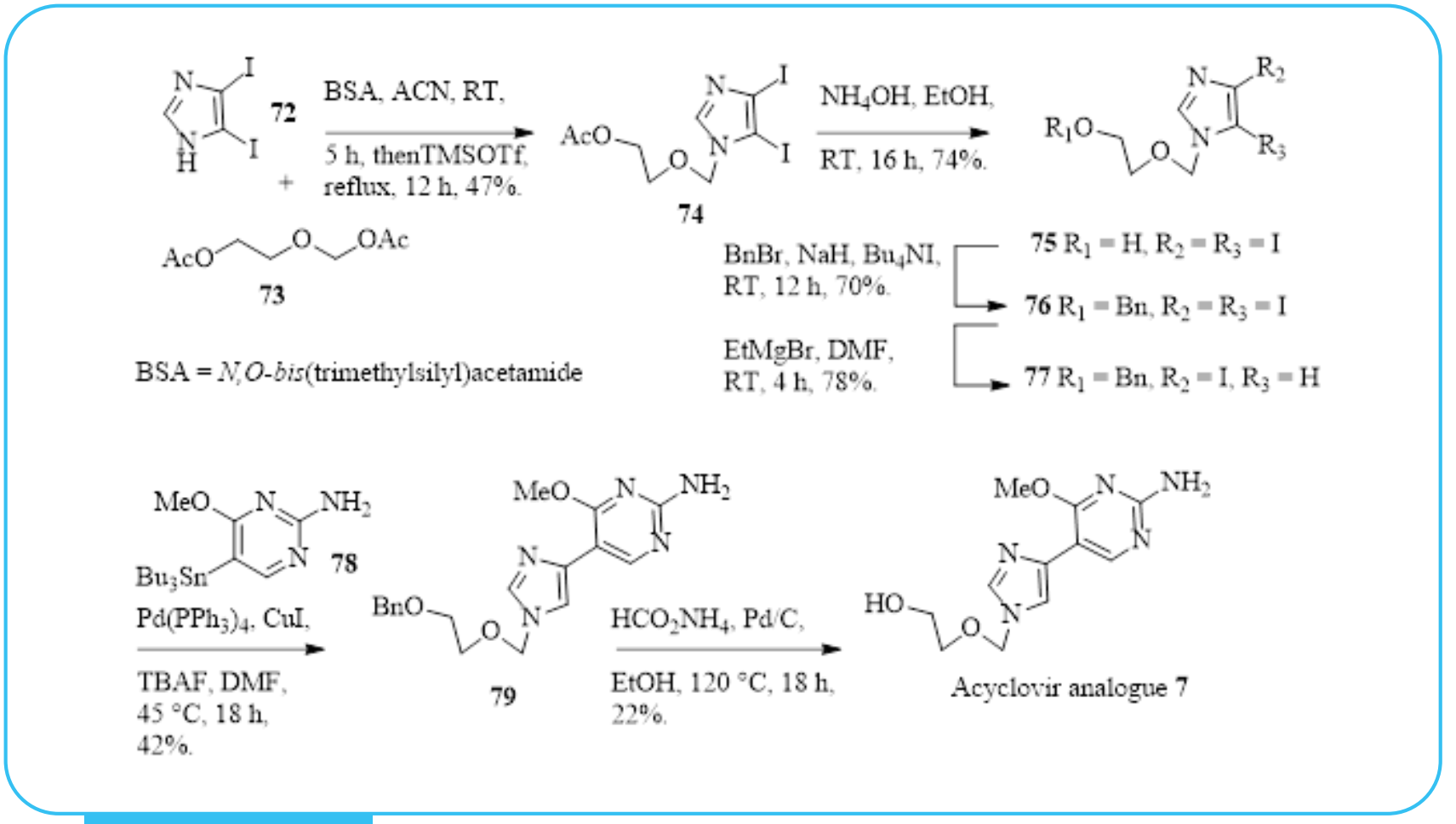
[49] H. Gao, A. K. Mitra, *Synthesis* **2000**, 329-351.

[50] H. L. Peters, D. Jochmans, A. H. de Wilde, C. C. Posthuma, E. J. Snijder, J. Neyts, K. L. Seley-Radtke, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 2923-2926.

[51] For an excellent review on this field, see: M. K. Yates, P. Chatterjee, M. Flint, Y. Arefeayne, D. Makuc, J. Plavec, C. F. Spiropoulou, K. L. Seley-Radtke, *Molecules* **2019**, *24*, 3184; doi:10.3390/molecules24173184.

[52] For recent improvements concerning the preparation of this intermediate, see: M. K. Yates, M. R. Raje, P. Chatterjee, C. F. Spiropoulou, S. Bavari, M. Flint, V. Soloveva, K. L. Seley-Radtke, *Bioorg. Med. Chem. Lett*. **2017**, *27*, 2800-2802.

[53] A. Matsuda *et al*., *J. Am. Chem. Soc.* **2003**, *125*, 9970-9982.



SCHEME 15

Conclusion

There is an urgent need to develop new, effective and safer drugs for the treatment of coronavirus infections, including Covid-19. In this regard, nucleosides have recently shown great potential which, in turn, has resurrected interest in this class of molecules. In this context, there are great opportunities for chemists to develop new and original structures, as well as to improve current routes to reach promising nucleosides under clinical trials.

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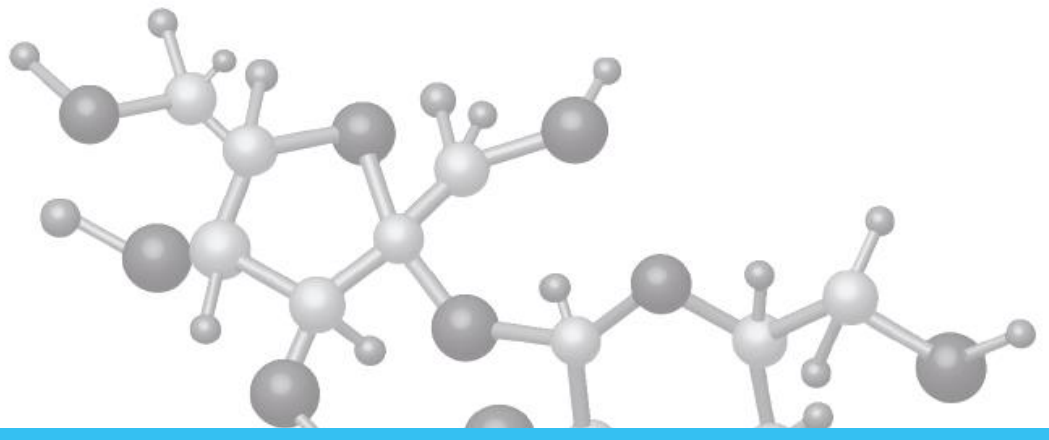

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