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Chemical and biological effects of substitution of the 2-position of ring-expanded ('fat') nucleosides containing the imidazo[4,5-*e*][1,3]diazepine-4,8-dione ring system: The role of electronic and steric factors on glycosidic bond stability and anti-HCV activity

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Abstract—The attempted removal of the aralkyl group of 2-bromo-1-*p*-methoxybenzyl-6-octylimidazo[4,5-*e*][1,3]diazepine (**ZP-33**) with trifluoroacetic acid resulted in replacement of the bromo group with a carbonyl at position-2 in addition to the desired deprotection at the 1-position. 2′-Deoxynucleosides of 2-bromo-substituted-imidazole-4,5-diesters (**ZP-35** and **ZP-103**) were synthesized by direct glycosylation of the corresponding heterocycles. The attempted ring-closure of the above nucleosides resulted in deglycosylation to form the starting heterocycles. The 2-phenyl derivatives of the above nucleosides (**ZP-45** and **ZP-73**) were successfully prepared by Suzuki coupling with the appropriate phenylboronic acids, but the attempted ring-closure with guanidines to form the desired 5,7-fused ring-expanded nucleosides (RENs) resulted once again in the formation of the corresponding heterocyclic aglycons (**ZP-64** and **ZP-75**). The first successful 2-substituted REN (**ZP-110**) was synthesized by replacing the 2-deoxyribose sugar moiety with a ribosyl group and replacing the bromo group with a *p*-methoxyphenyl substituent at the 2-position. A number of imidazole riboside diester precursors containing a substituted phenyl group at the 2-position were synthesized in order to prepare analogues of **ZP-110**. The substituents on the phenyl ring included a variety of electron-donating or electron-withdrawing groups operating through inductive and/or resonance effects. However, the final ring-closure of the diesters with guanidines in order to prepare RENs was successful only in a limited number of cases, including the ones containing a *p*-fluorophenyl (**ZP-121**), a *m*-methoxyphenyl (**ZP-122**), or an unsubstituted phenyl (**NZ-53**) at the 2-position. Deglycosylation and incomplete ring-closure of the intermediates were the major problems encountered with most other cases. The stability of glycosidic bonds was found to be dependent on several factors including, but not limited to, the electron-donating inductive effect of the 2-phenyl substituents and the temperature of the reaction medium. The three target RENs **ZP-110**, **ZP-121**, and **ZP-122** were screened for in vitro anti-HCV activity, employing an HCV RNA replicon assay. While **ZP-121** was inactive, the other two compounds showed only weak anti-HCV activity.

1. Introduction

We have recently reported[1,2] the in vitro inhibitory activity of a number of ring-expanded nucleosides against NTPases/helicases of a family of *Flaviviridae*, which includes, but not limited to, the hepatitis C virus (HCV)[1] and the West Nile virus (WNV)[1,2] Both WNV[3–5] and HCV[6–11] are among the current list of deadly viruses eliciting global response on human health. Helicases are capable of unwinding duplex RNA and DNA structures by disrupting the hydrogen bonds that keep the two strands together.[12,13] This unwinding activity is essential for the virus replication, and requires the hydrolysis of a molecule of nucleoside-5′-triphosphate (NTP), which is catalyzed by the viral NTPase.[1,2] While most of the active anti-Flaviviridae compounds were nucleoside

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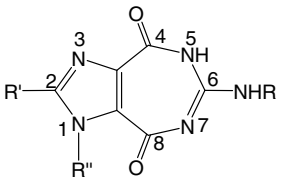
analogues bearing a ribosyl or 2-deoxyribosyl sugar moiety at the 1-position of the heterocycle, there were also a few active compounds that simply possessed an aralkyl moiety in place of a sugar. This latter observation evoked our interest in exploring the structure–activity relationships (SAR) in various heterocyclic aglycons of the above nucleosides with an aralkyl moiety substituting for the sugar.[14] This initiative led us to the mapping of a general structure **I** bearing a few key features required for anti-HCV activity, including a bulky substituent such as a bromo or phenyl group at position-2, an aralkyl substituent at position-1, and an aminoalkyl chain at position-6.[14] The observed enhancement of antiviral activity of heterocyclic aglycons by bulky substituents at the 2-position raised another issue about the significance of substituting this position in the original ring-expanded nucleoside analogues (RENs), most of which contained no substituents at position-2. So, the present work is an attempt to introduce various hydrophobic and hydrophilic substituents at position-2 of the title RENs as well as to explore the effects of such substituents on the observed anti-HCV activity.

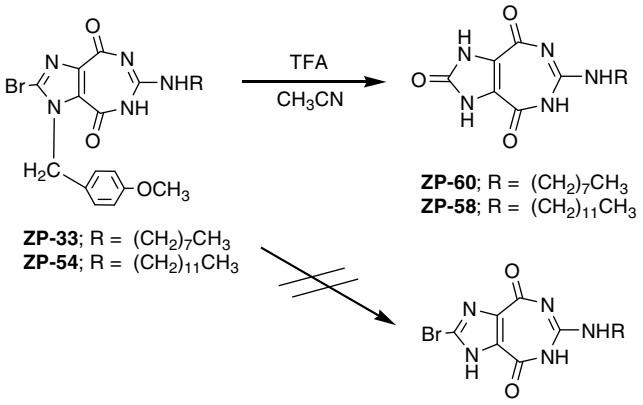


I

2. Results and discussion

The compound with the most promising anti-HCV activity in the heterocyclic series with the structural skeleton **I** was the 2-bromo-1-*p*-methoxybenzyl-6-octyl derivative (**ZP-33**). Therefore, the most convenient and facile method to prepare the corresponding nucleoside analogue appeared to be the deprotection of the aralkyl group of **ZP-33** with a reagent such as trifluoroacetic acid or by catalytic hydrogenation, followed by glycosylation. However, while catalytic hydrogenation of **ZP-33** did not yield the desired compound, the treatment with trifluoroacetic acid in acetonitrile, on the other hand, resulted in the formation of **ZP-60** (see Scheme 1), containing a carbonyl group in place of the bromo substituent at the 2-position, as evidenced by analytical and spectroscopic data. An analogous result was obtained with the N^6-dodecyl analogue **ZP-54**, which formed the corresponding carbonyl compound **ZP-58**.

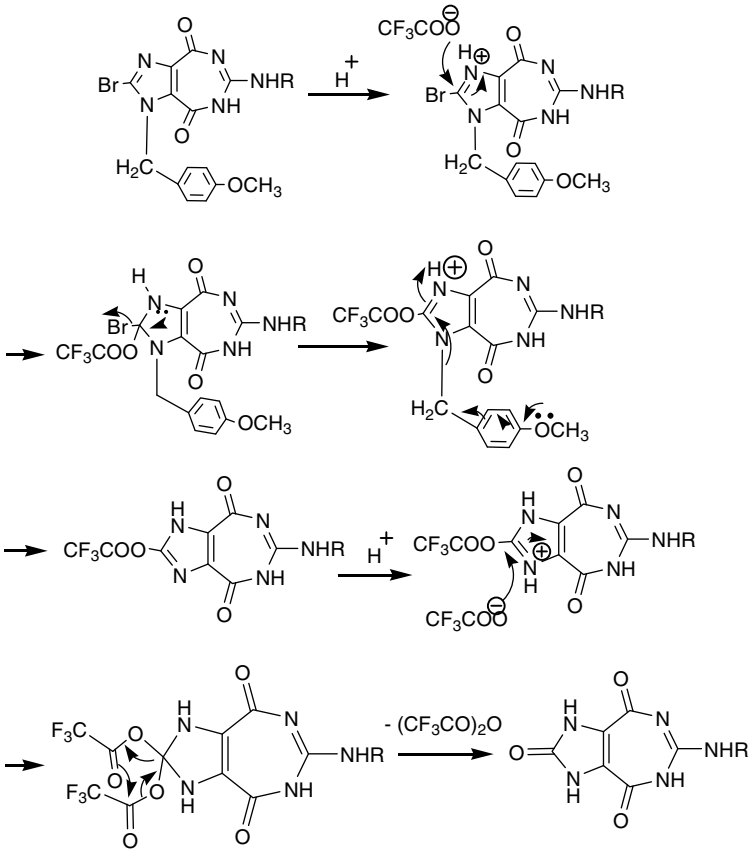
Suspecting the presence of adventitious water in the reaction medium, we repeated the reaction with an extensively dried reagent as well as the solvent, but obtained the same result as before, suggesting that the reaction mechanism for the formation of **ZP-60** and **ZP-58** from their respective precursors **ZP-33** and **ZP-54** does not involve water and that the carbonyl oxygen in the products must be coming from the trifluoroacetic acid employed in the reaction. A tentative mechanism for the product formation is outlined in Scheme 2.



Scheme 1.

In view of the above result, we resorted to an alternative approach to synthesize the desired nucleoside analogues. The strategy involved glycosylation of an appropriate 2-bromoimidazole precursor before condensation with guanidine. Thus, 2-bromoimidazole-4,5-dicarboxylic acid esters, **ZP-32** and **ZP-72**,[14] were glycosylated using standard procedures[2] to obtain the respective nucleoside analogues **ZP-35** and **ZP-103**, along with the corresponding α anomers as minor products in miniscule amounts (Scheme 3). However, all attempts at ring-closure of **ZP-35** and **ZP-103** with substituted guanidines to prepare the target nucleoside analogues resulted in intractable mixtures of products as suggested by their TLC and ^{1}NMR spectral analyses. In order to simplify and facilitate the product analysis of the complex reaction mixture that also contained the anticipated secondary products arising from the removal of the toluoyl protecting groups with the excess guanidine used, we decided to carry out the sugar deprotection with a hindered base before condensation with guanidine. Surprisingly, the treatment of **ZP-35** and **ZP-103** with *t*-butylamine in MeOH resulted in deglycosylation to form the starting heterocycles **ZP-32** and **ZP-72**, respectively, even at room temperature. In view of the expected greater nucleophilicity of guanidines as compared with *t*-butylamine, it is safe to conclude that the original ring-closure reactions of **ZP-35** and **ZP-103** with excess guanidines must have also resulted in the deglycosylated products.

The observed deglycosylation was tentatively attributed to the electron-withdrawing inductive effect of the bromo group. As our SAR model **I** described earlier calls for a hydrophobic group at position-2, we decided to replace the bromo group with a phenyl substituent, which should be less electron-withdrawing, but more bulky and hydrophobic than the bromo group. To that end, **ZP-35** was converted into two 2-phenyl (**ZP-45**) and 2-*p*-methoxyphenyl (**ZP-73**) analogues via Suzuki coupling[15] with the appropriate phenylboronic acids (Scheme 4). Compound **ZP-45** indeed underwent smooth sugar deprotection with *t*-butylamine in methanol to form the expected nucleoside **ZP-52**. Unfortunately, however, the attempted condensations of a substituted guanidine with either the sugar unprotected (**ZP-52**) or the sugar-protected (**ZP-45** and **ZP-73**)

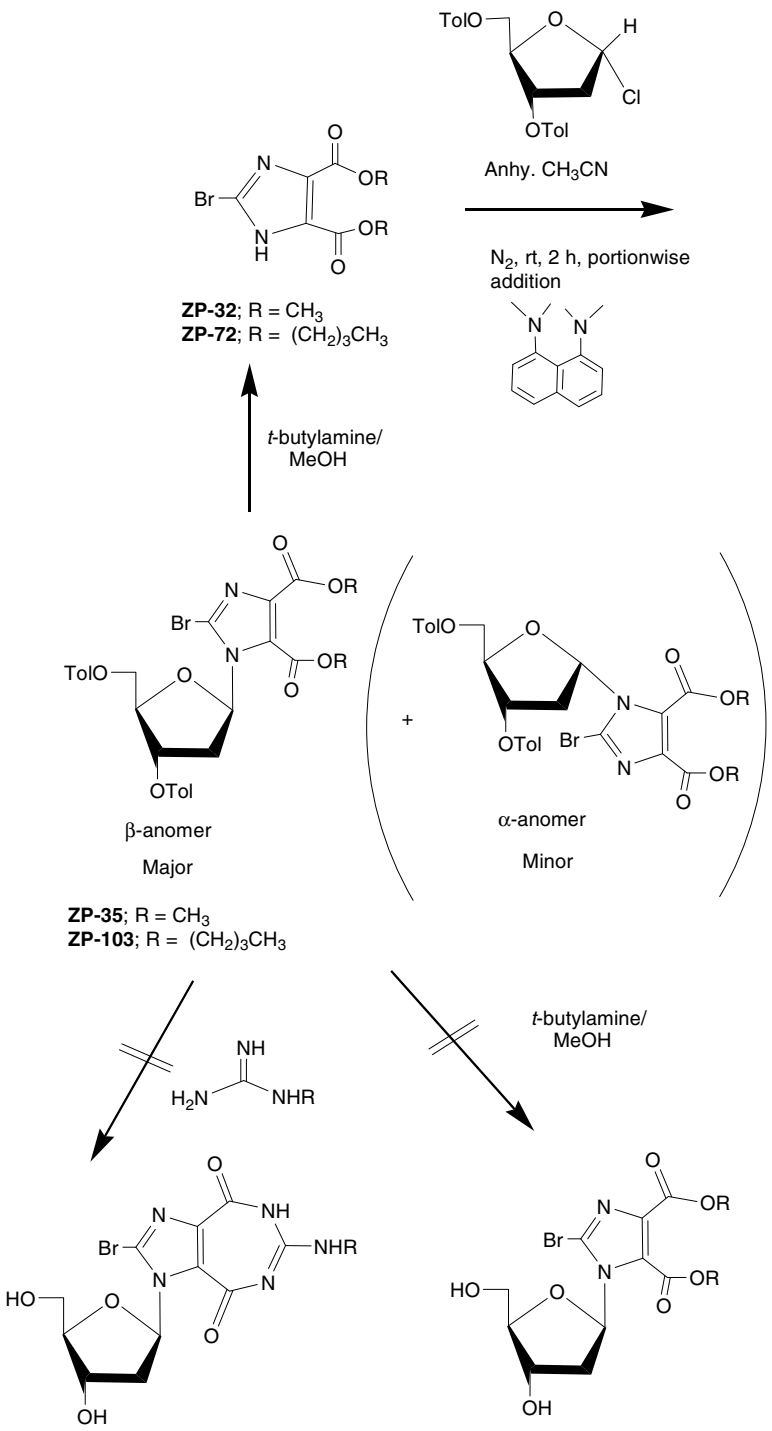


Scheme 2.

nucleosides only produced the corresponding deglycosylated products **ZP-64**, **ZP-75**, and **ZP-109**. Apparently, the glycosidic bonds in the expected nucleoside products are still too weak to withstand the experimental reaction conditions employed for ring-closures of nucleoside precursors **ZP-45** and **ZP-73** with guanidine.

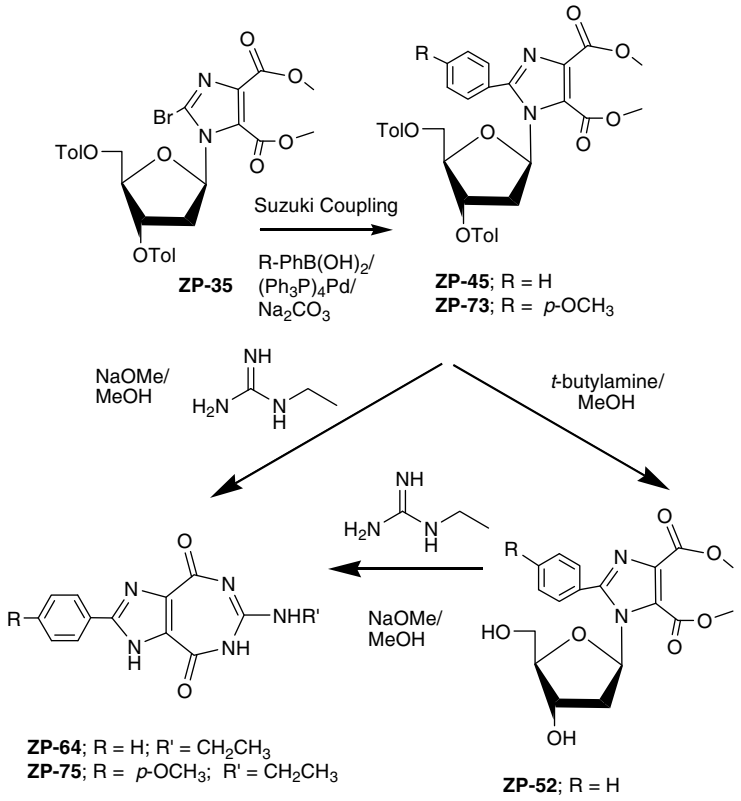
As it is well known that glycosidic bonds in 2′-deoxynucleosides/-tides (DNA) are nearly two orders of magnitude less stable than their ribose counterparts (RNA),[16] our best hope for preparing the target 2-substituted 5:7-fused nucleoside analogues was to replace their 2′-deoxyribose moiety with a ribosyl sugar at position-1. Besides, since the target *Flaviviridae* are RNA viruses, synthesis of ribose nucleoside analogues as potential inhibitors would only be logical, notwithstanding the potent in vitro anti-HCV/WNV activities of a few 2′-deoxyribose analogues of RENs.[1,2] To this end, we synthesized the required diester precursors in the ribose series, namely **ZP-115** and **ZP-77**, by standard Vorbrüggen ribosylation[17–19] of **ZP-32** and **ZP-72**, respectively (Scheme 5). Whereas an attempted condensation reaction of **ZP-115** with *N*-octylguanidine resulted in the formation of the deglycosylated, ring-closed heterocycle (**ZP-16**), the conversion of the 2-bromo group of **ZP-77** into the corresponding *p*-methoxyphenyl derivative (**ZP-108**) via Suzuki coupling[15] proceeded with no problem. The ring-closure of **ZP-108** with guanidine provided the desired **ZP-110**, representing our first successful synthesis of a 2-substituted ring-expanded nucleoside. The compound was completely characterized by ^{1}H and ^{13}C NMR analyses, coupled with mass spectral and microanalytical data.

Encouraged by the above result, we set out to synthesize several other ribonucleosides containing the title 5:7-fused heterocyclic system with a variety of substituted phenyl and other substituents at the 2-position. The necessary diester nucleoside precursors for guanidine ring-closures were successfully prepared, as before, from **ZP-115** or **ZP-77**, using Suzuki coupling.[15] These precursors include (Scheme 6) 2-phenyl (**ZP-78**), 2-*p*-F-phenyl (**ZP-118**), 2-*m*-methoxyphenyl (**ZP-119**), 2-*p*-phenylphenyl (**ZP-123**), 2-*p*-*tert*-butylphenyl (**ZP-124**), 2-*m*-nitrophenyl (**ZP-129**), 2-*p*-trifluoromethylphenyl (**ZP-132** and **ZP-175**), and 2-thienyl (**ZP-128**) derivatives. However, the attempted ring-closures of these precursors with guanidine or substituted guanidines were successful only in a limited number of cases, in addition to the mentioned *p*-methoxyphenyl (**ZP-110**), which included *p*-fluorophenyl (**ZP-121**), *m*-methoxyphenyl (**ZP-122**), and unsubstituted phenyl (**NZ-53**) derivatives. While the reaction of **ZP-108** with guanidine yielded the cleanest product (**ZP-110**), all other reactions were accompanied with one or more side-products, which often included the ring-closed or ring-open heterocyclic aglycons and/or ring-open nucleosides as outlined in Scheme 7 for the reactions of **ZP-136** and **ZP-119** with *N*-methylguanidine and guanidine, respectively. While individual components in the reaction mixture of products formed from the reaction of **ZP-136** with *N*-methylguanidine were identified mainly through



Scheme 3.

high resolution mass spectral data, those produced from the reaction of **ZP-119** with guanidine were isolated and the products were fully characterized by NMR as well as elemental microanalytical and/or high resolution mass spectral data. Our experience with these reactions suggests that several factors, including the type of substituents attached to the phenyl ring, the temperature of the reaction medium, the molar ratio of reactants used, the reaction time, as well as the reaction work-up procedure, affect the type, number, and yield of the products formed. Especially important are the contributions of electron-donating substituents at the *p*-position of the phenyl ring, through resonance and inductive effects, which seem to enhance the stability of the glycosidic bond toward hydrolytic and nucleophilic cleavage. Another important factor is the temperature of the reaction medium, the higher temperature leading to deglycosylation, while the ice-cold temperature yields un-ring-closed intermediates. The excess base facilitates the final ring-closure, but it also adversely affects the stability of the glycosidic bond, especially if the base is also a good nucleophile.



Scheme 4.

Finally, the three ring-expanded nucleosides **ZP-110**, **ZP-121**, and **ZP-122** were screened for anti-HCV activity in a primary HCV RNA replicon assay[20,21] through contractual arrangements with the National Institute of Allergy and Infectious Diseases (NIAID) at its Antimicrobial Acquisition and Coordinating Facility (AACF) in Birmingham, Alabama. The results are collected in Table 1. Out of the three compounds tested, the one with *p*-F substituent is completely inactive, while the other two with a methoxy substituent in the *m*- and *p*-position, are only weakly active, with the *meta* preferred to *para* position.

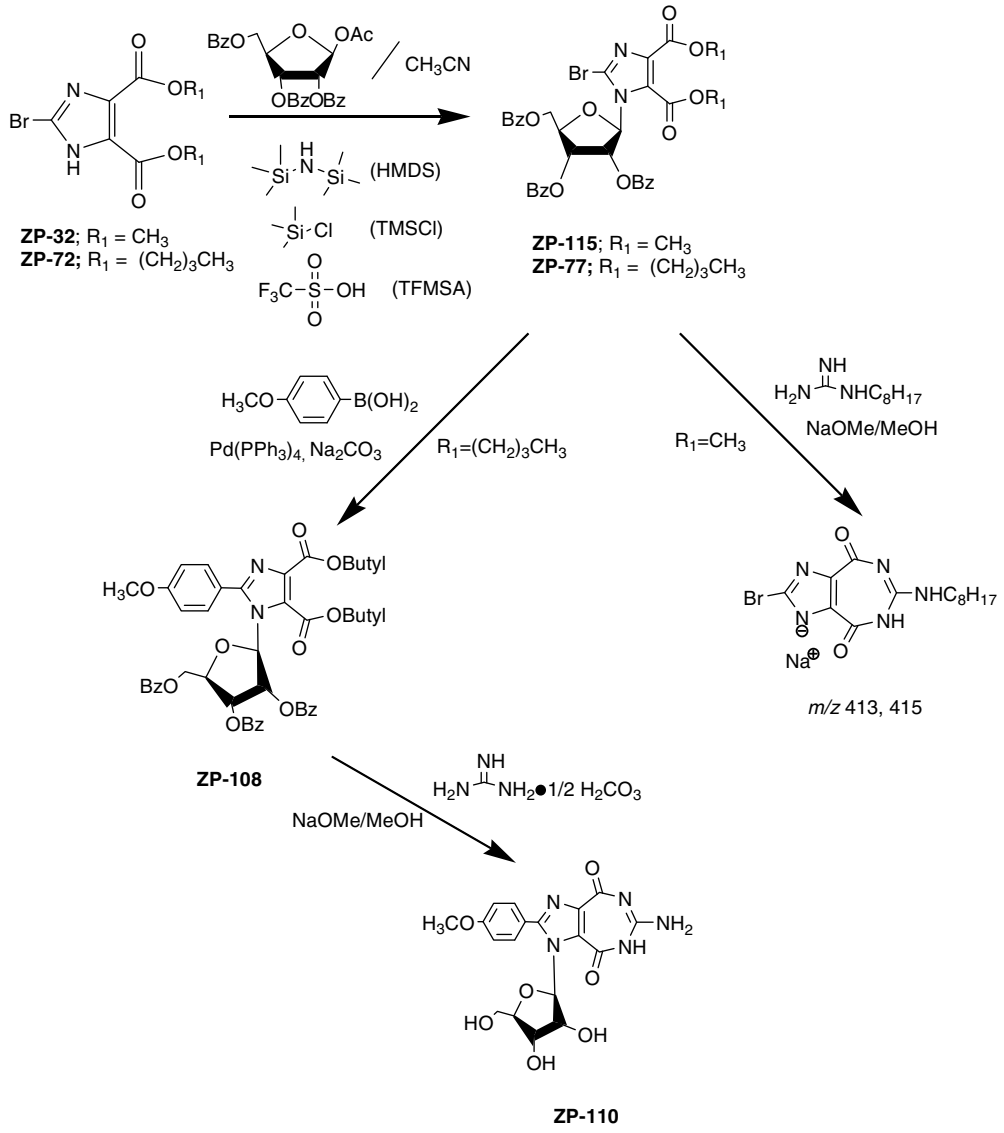
3. Conclusion

Substitution of position-2 of ring-expanded nucleosides containing the title imidazo[4,5-*e*][1,3]diazepine-4,8-dione ring system turned out to be challenging. The attachment of the bromo or the phenyl group at this position leads to an unstable and fragile glycosidic bond, often producing the corresponding aglycons either as sole or by-products. Deglycosylation is especially more pronounced in 2′-deoxyribonucleosides as compared to their ribose counterparts. Exchange of the bromo group with phenyl considerably enhances the glycosidic bond stability. The stability is further improved with an electron-donating group on the phenyl ring, in particular, when located at a position capable of imparting resonance effect (e.g., *para* vs *meta*). Another key factor that governs the outcome of the final ring-closure reaction is the temperature of the medium, with elevated temperatures leading to deglycosylation, while ice-cold temperatures resulted in ring-open intermediates. Two of the three target compounds screened showed only marginal activity against HCV in vitro. The structure–activity relationships (SAR) on the tested compounds as well as on the whole class of 2-substituted RENs with the title ring system still remain inconclusive for the following reasons: (a) the number of compounds tested are far too limited to draw any meaningful inference, and (b) none of the three tested compounds possesses the long alkyl chain at position-6, which may still be required for activity as has been the case with all of the RENs that have so far exhibited potent activity against *Flaviviridae*. Nonetheless, the outlined research is a good exercise for exploring the problems involved in substitution of the 2-position of nucleosides bearing the target 5:7-fused ring system, while also providing directions for future rational designs of 2-substituents and sugar moieties that do not adversely affect the glycosidic bond stability of target RENs.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a General Electric QE-300 NMR spectrometer or on an Oxford AS400 NMR spectrometer operating at 300/400 MHz for ^{1}H and 75/100 MHz for ^{13}C. The Chemical shift data are reported with reference to Me_4Si (internal standard) for ^{1}H and ^{13}C NMR spectra. The data are reported in

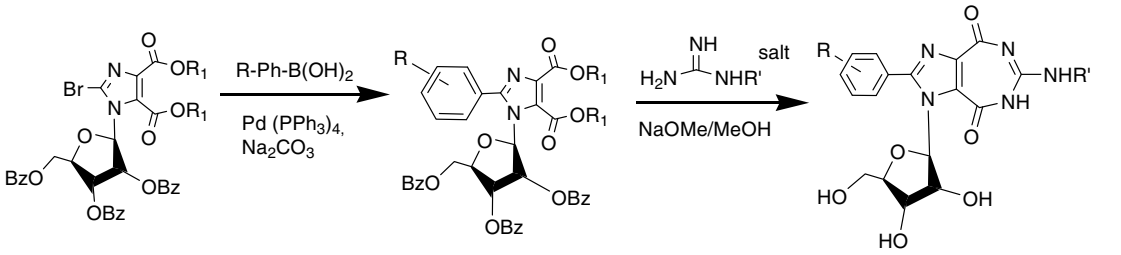


Scheme 5.

the following format: Chemical shift, multiplicity (s, singlet; d, doublet; dt, double triplet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad; coupling constants, integration, and assignment). Elemental Microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. The mass spectra were recorded at the Mass Spectral Facility, Department of Biochemistry, Michigan State University, or Mass Spectrometry Analysis Laboratory, Department of Chemistry and Biochemistry, University of Maryland, College Park or Mass Spectrometry Facility, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County. Thin layer chromatography was performed on Merck Kieselgel 60 GF_{254} plates (0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Dry solvents were prepared as follows: methanol was distilled from calcium hydride and was stored over molecular sieves (type 3 Å); methylene chloride was distilled from calcium hydride and was stored over molecular sieves (type 3 Å); dimethylformamide was dried over calcium oxide and then distilled under reduced pressure from calcium hydride, and was subsequently stored over molecular sieves (type 3 Å); acetonitrile was distilled from calcium hydride and was stored over molecular sieves (type 3 Å). Starting materials were purchased from Aldrich Chemical Co., Acros or Lancaster. All solvents are of reagent grade and were purchased from VWR Scientific or Fisher Scientific. All yields reported are for dry compounds that require no further purification for use in other reactions.

4.2. 6-*N*-Dodecylamino-1,3,4,5-tetrahydroimidazo[4,5-*e*]-[1,3]diazepine-2,4,8-trione (ZP-58)

To a suspension of **ZP-54** (0.275 g, 0.5 mmol) in 30 mL of dry acetonitrile, anhyd TFA (3 mL) was added slowly. The clear solution was stirred at 50 °C for 72 h. The reaction mixture was filtered to give white solid. Yield: 0.15 g (70%). $R_f = 0.75$ (chloroform/methanol/ammonium hydroxide, 2:1:0.3), mp >220 °C, ^{1}H NMR (DMSO-d_6): δ 11.2, 6.82 (2br, NH, exchangeable



ZP-115; $R_1 = CH_3$
ZP-77; $R_1 = (CH_2)_3CH_3$

ZP-78; R = H, $R_1 = (CH_2)_3CH_3$
ZP-108; R = *p*-OCH_3, $R_1 = (CH_2)_3CH_3$
ZP-118; R = *p*-F, $R_1 = (CH_2)_3CH_3$
ZP-119; R = *m*-OCH_3, $R_1 = (CH_2)_3CH_3$
ZP-123; R = *p*-Ph, $R_1 = CH_3$
ZP-124; R = *p*-*tert*-butyl, $R_1 = CH_3$
ZP-128; 2-Thienyl, $R_1 = CH_3$
ZP-129; R = *m*-NO_2, $R_1 = CH_3$
ZP-132; R = *p*-CF_3, $R_1 = CH_3$
ZP-136; R = *p*-CH_3, $R_1 = CH_3$
ZP-175; R = *p*-CF_3, $R_1 = (CH_2)_3CH_3$

ZP-110; R = *p*-OCH_3; R' = H
ZP-121; R = *p*-F; R' = H
ZP-122; R = *m*-OCH_3; R' = H
NZ-53; R = H; R' = CH_3

Scheme 6.

with D_2O), 5.08 (br, 2H, D_2O exchangeable), 3.16 (m, 2H, NCH_2), 1.19, 1.42 (2m, 20H, $C_{10}H_{20}$), 0.83 (t, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 202.6, 183.1, 166.4, 158, 123.1, 99.7, 81.3, 31.1, 29, 28.8, 28.5, 22, 13.7. MS (FAB) *m*/*z* 364.2; HRMS (FAB) calcd for $C_{18}H_{29}N_5O_3$ (MH^+) *m*/*z* 364.2349, found, 364.2364.

4.3. 6-*N*-Octylamino-1,3,4,5-tetrahydroimidazo[4,5-*e*]-[1,3]diazepine-2,4,8-trione (ZP-60)

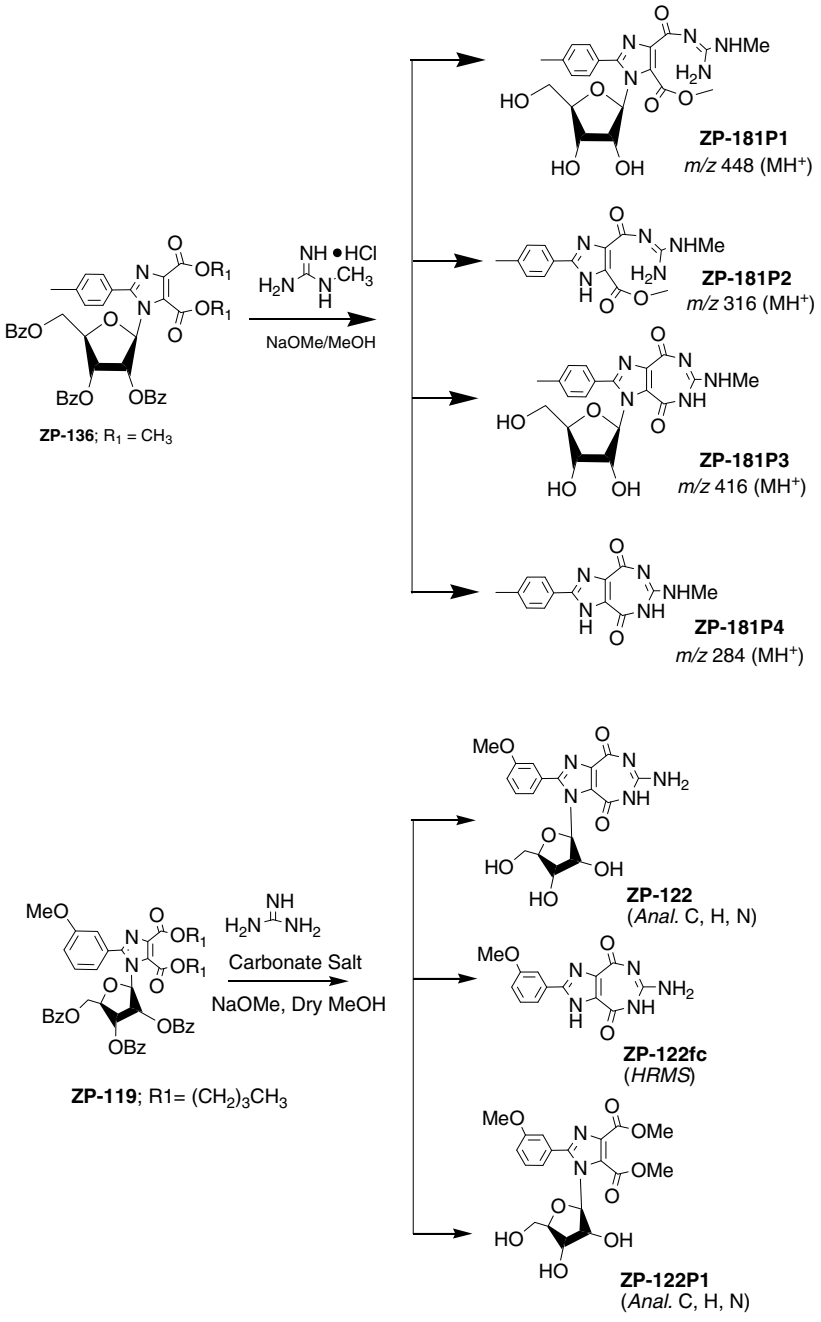
ZP-33 (0.17 g, 0.35 mmol) was suspended in 20 mL of dry CH_3CN. TFA (2 mL) was added to give a clear solution. The solution was heated at 45 °C for 48 h. Some white solid precipitate out, which was collected by filtration and dried. Yield: 15 mg (13%), mp 215 °C (dec), $R_f = 0.47$ (chloroform/methanol/ammonium hydroxide, 2:1:0.3). ^1H NMR (DMSO-d_6): δ 11.25 (br, 1H, NH, D_2O exchangeable), 6.92 (br, 1 H, NH, D_2O exchangeable), 4.80 (br, 2H, D_2O exchangeable), 3.20 (m, 2H, NCH_2), 1.47, 1.26 (2m, 12H, $(CH_2)_6$), 0.85 (t, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 223.8, 184.7, 163.7, 142.7, 133.0, 87.2, 38.2, 28.3, 26.0, 21.7, 13.5. MS (FAB) calcd for $C_{14}H_{21}N_5O_3$ (MH^+) *m*/*z* 308.2, found, 308.1.

4.4. 2-Bromo-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-erythro pentofuranosyl)imidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-35)

To a mixture of **ZP-32**[14] (0.65 g, 2.48 mmol) and proton sponge (0.53 g, 2.48 mmol), 20 mL dry acetonitrile was added. Then **ZP-3** (1-α-chloro-2-deoxy-D-ribofuranosyl-3,5-bis(*p*-toluate), chlorosugar)[2] (1.00 g, 2.48 mmol) was added portion-wise over a period of 2 h under nitrogen. The reaction mixture was kept stirring for another 3 h after addition. After filtration, the reaction mixture was evaporated to give a syrup. The syrup was dissolved in 10 mL chloroform and adsorbed onto flash silica gel. Flash chromatography was performed using chloroform/ ethanol (100:1). The appropriate fractions ($R_f = 0.14$ in the same solvent system used for elution) were combined and evaporated to give 1.39 g white foam. (91%). ^1H NMR (DMSO-d_6): δ 7.1–7.9 (4m, 8H, $2C_6H_4$), 6.42 (dd, $J_1 = 6.2$ Hz, $J_2 = 2.6$ Hz, 1H, 1′-H), 5.45 (m, 1H, 3′-H), 4.6–4.7 (m, 1H, 4′-H), 4.35–4.4 (m, 2H, 5′,5″-H), 3.71, 3.73 (2s, 6H, OCH_3), 2.6–2.8 (2m, 2H, 2′,2″-H), 2.28, 2.32 (2s, 6H, 2 PhMe). Anal. ($C_{28}H_{27}BrN_2O_9 \cdot 2\ H_2O$) C, H, N.

4.5. 2-Bromo-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β/α-D-erythropentofuranosyl)-4,5- imidazoledicarboxylic Acid Dibutyl Ester (ZP-103)

1-α-Chloro-2-Deoxy-D-ribofuranosyl-bis(*p*-toluate) (**ZP-3**) (505 mg, 1.25 mmol) was slowly added portion-wise into a solution of **ZP-72** (434 mg, 1.25 mmol) and sodium hydride (50 mg, 1.25 mmol) in anhyd acetonitrile (20 mL) in a period of 2 h. The reaction mixture was stirred overnight. It was evaporated and purified by flash chromatography eluting with chloroform. Appropriate fractions were collected and evaporated to give two products, one as a clear syrup (major product, β anomer) and the other as a purple syrup (minor product, α anomer). The physicochemical data of the two products are as follows: *The* β *anomer:* Yield: 51%, ^1H NMR ($CDCl_3$) δ 7.93 (d, $J = 7.6$ Hz, 4H, Ar–H), 7.28, 7.20 (2d, $J = 8.0$ Hz, 8.4, 4H, Ar–H), 6.38 (dd, $J_1 = 8.44$ Hz, $J_2 = 6.04$ Hz, 1H, 1′-H), 5.57, (m, 1H, 3′-H), 4.70 (m, 1H, 4′-H), 4.53 (m, 2H, 5′,5″-H), 4.3 (m, 4H, OCH_2 of butyl), 3.08 (m, 1H, 2′-H), 2.63 (ddd, $J_1 = 14.2$ Hz, $J_2 = 5.96$ Hz, $J_3 = 2.28$ Hz, 1H, 2″-H), 2.42, 2.39 (2s, 6H, $2PhCH_3$), 1.69, 1.35 (2m, 8H, $(CH_2)_2$ of butyl), 0.94, 0.88 (2 t, $J = 7.3$ Hz, 6H, $2CH_3$). NOE: no effect between 1′-H and 4′-H. ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.07, 165.58, 160.88, 160.56, 144.37, 143.88, 129.66, 129.64, 129.15, 129.0, 126.59, 126.19, 87.46, 82.21, 77.35, 77.03, 76.71, 73.79, 66.66, 65.15, 63.49, 38.07, 30.56, 30.03, 21.54, 18.91, 13.59, 13.51. Anal. ($C_{34}H_{39}BrN_2O_9 \cdot 1.5H_2O$) C, H, N. The α anomer: Yield (trace), ^1H NMR ($CDCl_3$) δ 7.93, 7.82, 7.25, 7.21 (4d, $J = 7.8$-8.0 Hz, 8H, Ar–H), 6.34 (t, $J = 5.2$ Hz, 1H, 1′-H), 5.56, (m, 1H, 3′-H), 4.83 (q,



Scheme 7.

Table 1. In vitro Anti-HCV Activity in a primary (single dose) HCV RNA replicon assay of three ring-expanded nucleosides containing the target imidazo[4,5-*e*][1,3]diazepine-4,8-dione ring system

Compound ID	Substituent at the 2-position	Antiviral Activity % Inhibition[a]	Cytotoxicity % Cell Control[b]
ZP-110	*p*-OMe-Ph	9.5	109.8
ZP-121	*p*-F-Ph	0	108.6
ZP-122	*m*-OMe-Ph	31.8	97.2
Alpha-IFN (Ref.)	NA	96.1	119.6

[a] Antiviral activity is assessed with an HCV RNA replicon containing stable luciferase (LUC) reporter. The extent of LUC expression, which is directly proportional to HCV RNA levels, is used as an indirect measure of HCV replication.[20]

[b] Compound cytotoxicity is assessed as the percent viable cells relative to the untreated cell controls.

J = 4.0 Hz, 1H, 4′-H), 4.55 (m, 2H, 5′,5″-H), 4.29 (m, 4H, OCH_2 of Butyl), 3.13, 2.87 (2m, 2H, 2′,2″-H), 2.42, 2.39 (2s, 6H, $2PhCH_3$), 1.66, 1.35 (2m, 8H, $(CH_2)_2$ of Butyl), 0.92, 0.89 (2 t, J = 7.2 Hz, 6H, $2CH_3$). NOE: effect between 1′-H and 4′-H. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.31, 160.99, 160.73, 144.34,

132.8, 129.9, 129.7, 129.32, 129.16, 127.2, 126.2, 118.11, 90.0, 83,81, 77.55, 77.43, 77.23, 76.92, 73.96, 67.1, 65.31, 64.1, 39.77, 30.73, 30.26, 21.73, 19.05, 13.75. Anal. ($C_{34}H_{39}BrN_2O_9$) C, H, N.

4.6. 1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-erythro-pentofuranosyl)-2-phenylimidazo-4,5-dicarboxylic Acid Dimethyl Ester (ZP-45)

A 25 mL round-bottomed flask was charged with 1.56 g of **ZP-35** (1.7 mmol), 0.22 g (1.8 mmol) of phenylboronic acid, 0.57 g of sodium carbonate (5.4 mmol dissolved in 2.7 mL water), 5.2 mL benzene, and 0.52 mL ethanol. The flask was covered with aluminum foil and a trace amount of tetrakis(triphenylphosphino) palladium was added. The reaction was heated to reflux for 60 h and the solvent was evaporated. The residue was dissolved in 100 mL of dichloromethane and washed by water (3×50 mL). The extract was dried over anhyd Na_2SO_4, filtered, and evaporated to give a brown foam, which was purified by column chromatography, eluting with chloroform/methanol (60:1). The appropriate fraction ($R_f = 0.18$ (chloroform/methanol, 60:1)) was pooled and evaporated to give some white foam, which was dried in vacuo overnight. Yield: 0.75 g (71.4%). ^{1}H NMR (DMSO-d_6) δ 7.75, 7.48, 7.25 (3m, 15H, Ar–H), 6.66 (t, $J = 6.6$ Hz, 1H, 1′-H), 5.3 (m, 1H, 3′-H), 4.56–4.28 (2m, 3H, 4′,5′,5″-H), 3.75, 3.7 (2s, 6H, OCH_3), 2.68-3.08 (m, 2H, 2′,2″-H), 2.32 (s, 6H, 2 PhCH3). Anal. ($C_{34}H_{32}N_2O_9$) C, H, N.

4.7. 1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-erythro-pentofuranosyl)-2-(*p*-methoxylphenyl)imidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-73)

Reaction conditions and work-up are the same as in **ZP-45** using *p*-methoxy-phenylboronic acid instead. Yield: 64%, $R_f = 0.29$ (chloroform/methanol, 60:1), purified by silica gel flash chromatography, eluting with chloroform/methanol (60:1). ^{1}H NMR (DMSO-d_6) δ 7.81, 7.62, 7.51, 7,29, 7.04 (5m, 12H, Ar–H), 6.64 (t, $J = 3.7$ Hz, 1H, 1′-H), 5.39 (m, 1H, 3′-H), 4.57, 4.41 (2m, 3H, 4′,5′,5″-H), 3.81, 3.80, 3.77 (3s, 9H, OCH_3), 3.48, 3.41 (2m, 2H, 2′,2″-H), 2.39 (s, 6H, phenyl-CH_3). Anal. ($C_{32}H_{32}N_2O_{10}\cdot 0.5\ H_2O$) C, H, N.

4.8. General method for ring-closure condensation reactions with substituted guanidine

Hydrochloride, carbonate, sulfate/hemisulfate, or nitrate salt of a substituted guanidine (4 mmol) was suspended in anhydrous methanol (6.0 mL) and cooled to 0 °C. A solution of sodium methoxide (25 wt%, 2.1 mL, 9.2 mmol) was added. The resulting mixture was stirred in an ice bath for 30 min. The precipitated sodium chloride was removed by filtration, and the filtrate was poured into a methanolic solution (20 mL) of ring-closure precursor (1 mmol). The mixture was stirred at room temperature for 16–72 h, and was monitored by frequent TLC analysis to check for the completion of reaction. The reaction mixture was filtered if necessary, and the clear solution was evaporated and purified by flash chromatography on a silica gel column. The appropriate fractions were combined and evaporated to obtain the product. The latter was recrystallized from an appropriate solvent if necessary. The necessary ring-closure precursor, the spectral and analytical data of the products, along with solvent of recrystallization and/or solvent of elution for chromatography, are collected as below.

4.8.1. 6-*N*-Ethylamino-7,8-dihydro-2-phenyl-1*H*-imidazo-[4,5-*e*][1,3]diazepine-4,8-dione (ZP-64). Ring-closure precursor: **ZP-45** condensed with ethylguanidine sulfate. Yield: 12.9%, purified by silica gel flash chromatography, eluting with chloroform/methanol (60:1, 30:1; 10:1; 5:1; 1:1) and methanol, mp >220 °C. $R_f = 0.75$ (chloroform/methanol/ammonium hydroxide, 2:1:0.3). ^{1}H NMR (DMSO-d_6) δ 13.78, 10.49, 7.04 (3br, 3H, NH, exchangeable with D_2O), 8.18, 7.48 (2m, 5H, Ar–H), 3.34 (m, 2H, $NHCH_2$), 1.12 (t, $J = 6.6$ Hz, 3H, CH_3). Anal. ($C_{14}H_{13}N_5O_2$) C, H, N.

4.8.2. 6-*N*-Ethylamino-7,8-dihydro-2-*p*-methoxyphenyl-1*H*-imidazo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-75). Ring-closure precursor: **ZP-73**, condensed with ethylguanidine sulfate, isolated by silica gel flash chromatography, eluting with a gradient of chloroform/methanol (30:1 to 10:1). Yield: 4%, ^{1}H NMR (DMSO-d_6) δ 13.59, 10.47, 4.58 (3br, 3H, NH, exchangeable with D_2O), 8.16, 7.04, (2m, 4H, Ar–H), 3.82 (s, 3H, OCH_3), 3.15 (q, $J = 7.3$ Hz, 2H, $NHCH_2$), 1.18 (t, 3H, CH_3).

4.8.3. 7,8-Dihydro-2-*p*-methoxyphenyl-6-*N*-methylamino-1*H*-imidazo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-109). Ring-closure precursor: **ZP-73**, condensed with methylguanidine hydrochloride. Yield: 5%, purified by silica gel flash chromatography, eluting with mixtures of chloroform/methanol (60:1, 30:1; 10:1; 5:1; 1:1) and methanol. $R_f = 0.52$ (chloroform/methanol/ammonium hydroxide, 2:1:0.3). Yield: ^{1}H NMR (DMSO-d_6) δ 13.58, 10.62, 6.95 (3br, 3H, NH, exchangeable with D_2O), 8.15, 7.06 (2m, 5H, Ar–H), 2.81 (s, 3H, OCH_3), 2.80 (s, 3H, $NHCH_3$). Anal. ($C_{14}H_{13}N_5O_3\cdot 0.75\ H_2O$) C, H, N.

4.8.4. 1-(2-Deoxy-β-D-erythropentofuranosyl)-2-phenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-52). *t*-Butylamine (1.5 mL) was added to a solution of **ZP-45** (0.22 g, 0.36 mmol) in 10 mL methanol. The reaction mixture was stirred at room temperature for 20 h and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography, eluting with chloroform/methanol (30:1). The appropriate fractions were pooled and evaporated to give a white foam. Yield: 103 mg, (76.3%), ^{1}H NMR (DMSO-d_6) δ 7.59–7.36 (m, 5H, Ar–H), 5.95 (t, $J = 7.3$ Hz, 1H, 1′-H), 4.64, 5.24 (2m, 2H, OH, exchangeable with D_2O), 4.11 (m, 1H, 3′-H), 3.75–3.84 (2s, 6H, OCH_3), 3.15–3.48 (m, 3H, 4′,5′,5″-H), 2.12–2.34 (m, 2H, 2′,2″-H). Anal. ($C_{18}H_{20}N_2O_7\cdot 0.5\ H_2O$) C, H, N.

4.8.5. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-bromoimidazole-4,5-dicarboxylic Acid Dibutyl Ester (ZP-77). To a mixture of **ZP-72** (1.74 g, 5 mmol) and 1-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.55 g, 5 mmol),

25 mL acetonitrile was added and the solution was placed in an ice bath. HMDS (1,1,1,3,3,3-hexamethyldisilazane) (3.55 mL, 16.5 mmol), TMSCl (trimethylsilyl chloride) (2.29 mL, 18 mmol), and TFMSA (trifluoromethanesulfonic acid) (1.63 mL, 18 mmol) were added successively into the solution with caution. The reaction was completed in 1.5 hrs. The solvent and extra reactant were removed by evaporation, and the residue was dissolved in 40 mL $CHCl_3$. The solution was washed with a saturated solution of $NaHCO_3$ (2×) and water (2×) successively. The organic layer was adsorbed onto flash silica gel and applied to a flash chromatography column made in $CHCl_3$. The column was eluted with a mixture of chloroform/methanol, 100:1. The appropriate fractions were collected, and evaporated to obtain two products, the second of which corresponded to **ZP-77** as determined by its ^{1}H NMR. $R_f = 0.21$ (chloroform). Yield: 25%, ^{1}H NMR (DMSO-d_6): δ 8.11-7.27 (5m, 15H, 3phenyl), 6.48 (m, 1H, 1′-H), 6.02 (m, 1H, 2′-H), 5.76 (m, 1H, 3′-H), 4.90, 4.74, 4.56 (3m, 3H, 4′,5′,5″-H), 4.18 (m, 4H, $2OCH_2$), 1.61, 1.51, 1.33, 1.22 (4m, 8H, $4CH_2$), 0.89, 0.77 (2 t, $J = 7.0$ Hz, 6H, $2CH_3$). Anal. ($C_{39}H_{39}BrN_{12}O_{11}$) C, H, N, Br.

4.8.6. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-bromoimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-115). Experimental and work-up are the same as in **ZP-77** using **ZP-32** as reactant. Yield: 30%, ^{1}H NMR (DMSO-d_6): δ 8.07, 7.92, 7.56, 7.42, 7.36 (5m, 15H, 3 phenyl), 6.40 (d, $J = 6$ Hz, 1H, 1′-H), 6.17 (t, $J = 6$ Hz, 1H, 2′-H), 5.87 (t, $J = 6$ Hz, 1H, 3′-H), 5.81, 4.67 (2m, 3H, 4′,5′,5″-H), 3.90 (t, $J = 3$ Hz, 6H, $2CH_3$). MS (FAB) *m*/*z* 728.9, 730.9 (MH^+).

4.8.7. 2-Bromo-7,8-dihydro-6-*N*-octylamino-1*H*-imidazo[4,5-*e*][1,3]diazepine-4,8-dione Sodium Salt (ZP-16). Ring-closure precursor: **ZP-115**, condensed with octylguanidine hemisulfate, isolated by silica gel flash chromatography eluting with chloroform/methanol (60:1, 30:1; 10:1; 5:1; 1:1) and methanol. ^{1}H NMR (DMSO-d_6) δ 3.14, 1.43, 1.26, 0.85 (NH-C_8H_{17}); MS (FAB) 416, 418 (MH^+).

4.8.8. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-methoxyphenylimidazole-4,5-dicarboxylic Acid Dibutyl Ester (ZP-108). Reaction conditions and work-up are the same as in **ZP-73** except using **ZP-77** as the reactant. Yield: 74.8%, $R_f = 0.1$ (chloroform), purified by silica gel flash chromatography, eluting with chloroform. ^{1}H NMR ($CDCl_3$) δ 8.06–7.37 (6m, 19H, Ar–H), 6.90 (m, 1H, 1′-H), 6.22 (m, 1H, 2′-H), 5.87 (m, 1H, 3′-H), 4.62-4.32 (2m, 3H, 4′,5′,5″-H), 3.82 (s, 3H, OCH_3), 1.71, 1.41 (2m, 8H, $(CH_2)_2$ of 2 butyl), 0.94 (m, 6H, CH_3 of Butyl). Anal. ($C_{46}H_{46}N_2O_{12}\cdot 2H_2O$) C, H, N.

4.8.9. 6-*N*-Amino-7,8-dihydro-2-*p*-methoxyphenyl-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-110). Ring-closure precursor: **ZP-108** condensed with guanidine carbonate (see General procedure for ring-closure reactions described above), isolated by silica gel flash chromatography, eluting with chloroform/methanol (20:1, 5:1, 1:1) and methanol. The appropriate fractions were collected and evaporated. Yield: 49%, $R_f = 0.38$ (chloroform/methanol/ammonium hydroxide, 2:1:0.3). ^{1}H NMR (DMSO-d_6) δ 10.70, 7.6 (2br, 2H, NH, exchangeable with D_2O), 7.70, 7.09 (2m, 4H, Ar–H), 6.49 (br, 1H, NH, exchangeable with D_2O), 5.81(d, $J = 5.5$ Hz, 1H, 1′-H), 5.36, 4.99 (2d, $J = 5.9$ Hz, 2H, 2′-OH, 3′-OH, exchangeable with D_2O), 4.75 (t, $J = 6.1$ Hz, 1H, 5′-OH, exchangeable with D_2O), 4.47 (t, $J = 6.0$ Hz, 1H, 2′-H), 3.83 (s, 3H, OCH_3), 3.75, 3.63, 3.54, 3.47 (4m, 4H, 3′,4′,5′,5″-H). ^{13}C NMR (300 MHz, DMSO) δ 160.8, 148.7, 131.6, 121.4, 116.6, 113.9, 91.4, 85.1, 71.8, 69.1, 61.5, 55.3. Anal. ($C_{18}H_{19}N_5O_7\cdot 0.25H_2O$) C, H, N. MS *m*/*z* 440.2, 418.2, 286.2, 156.6; HRMS (FAB) calcd for $C_{18}H_{20}N_5O_7$ (MH^+) *m*/*z* 418.1363, found, 418.1349.

4.9. General method for suzuki coupling reactions with substituted boronic acids

A 25 mL round-bottomed flask was charged with 1 mol of **ZP-77** or **ZP-115**, 1 mmol of substituted boronic acid, 0.32 g of sodium carbonate (3 mmol dissolved in 1.5 mL water), 3 mL benzene, and 0.3 mL ethanol. The flask was covered with aluminum foil and a trace amount of tetrakis(triphenylphosphino)palladium was added. The reaction mixture was heated to reflux for 48–60 h and the solvent was evaporated. The residue was dissolved in 100 mL of dichloromethane or chloroform and washed with water (3 × 50 mL). The extract was dried over anhyd Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography or by recrystallization from an appropriate solvent. The spectral and analytical data, along with solvent of recrystallization and/or solvent of elution for chromatography, are collected as below.

4.9.1. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-phenylimidazole-4,5-dicarboxylic Acid Dibutyl Ester (ZP-78). Reactant: **ZP-77**, coupling with phenylboronic acid, eluting with chloroform/methanol, 100:1. Yield: 0.92 g (53.5%), $R_f = 0.14$ (chloroform), purified by silica gel flash chromatography, eluting with chloroform. ^{1}H NMR ($CDCl_3$): δ 8.14–7.18 (4m, 20H, 4 Ar–H), 6.31, 6.24, 5.85, 4.72, 4.55, (5m, 6H, 1′,2′,3′,4′,5′,5″-H), 4.32 (m, 4H, OCH_2), 1.71, 1.42 (2m, 8H, CH_2 of butyl group), 0.95 (2t, 6H, CH_3 of butyl group). Anal. ($C_{45}H_{44}N_2O_{11}\cdot 0.5H_2O$) C, H, N.

4.9.2. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-fluorophenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-118). Reactant: **ZP-115**, coupling with *p*-fluorophenylboronic acid, triturating with MeOH. Yield: 30%, ^{1}H NMR ($CDCl_3$) δ 8.04, 7.80, 7.57, 7.40, 7.28, 7.26, 7.07 (7m, 19H, Ar–H), 6.30, 6.14, 5.81, 4.70, 4.61, 4.54 (6m, 6H, 1′,2′,3′,4′,5′,5″-H), 3.96, 3.88 (2s, 6H, 2 OMe). Anal. ($C_{39}H_{31}FN_2O_{11}$) C, H, N.

4.9.3. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*m*-methoxyphenylimidazole-4,5-dicarboxylic Acid Dibutyl Ester (ZP-119). Reactant: **ZP-77**, coupling with *m*-methoxyphenylboronic acid, triturating with MeOH. Yield: 36%, ^{1}H NMR ($CDCl_3$) δ 8.05, 7.84, 7.54, 7.40, 7.33, 7.16, 7.02 (7m, 19H, Ar–H), 6.27, 5.85, 4.72, 4.58 (4m, 6H, 1′,2′,3′,4′,5′,5″-H), 4.32 (m, 4H, CH_2 of 2 Butyl),

1.71, 1.42 (2m, 8H, $(CH_2)_2$ of 2 Butyl), 0.95 (m, 6H, CH_3 of Butyl). Anal. ($C_{46}H_{46}N_2O_{12}\cdot 0.5\ H_2O$) C, H, N.

4.9.4. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-phenylphenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-123). Reactant: **ZP-115**, coupling with *p*-phenylphenylboronic acid, triturating with MeOH. Yield: 44.7%, ^{1}H NMR ($CDCl_3$) δ 8.55, 7.81, 7.67, 7.58, 7.47, 7.36 (6m, 24H, Ar–H), 6.35 (t, $J = 6$ Hz, 1H, 1′-H), 6.28 (d, $J = 3$ Hz, 1H, 2′-H), 5.87 (d, $J = 6.6$ Hz, 1H, 3′-H), 4.73, 4.63, 4.56 (3m, 3H, 4′,5′,5″-H), 3.97, 3.94 (2s, 6H, OCH_3). ^{13}C NMR (100 MHz, DMSO) δ 163.9, 163.7, 149.6, 142.3, 138.9, 132.5, 132.2, 129.6, 128.8, 127.9, 127.4, 127.3, 88.6, 78.9, 75.6, 73.1, 69.0, 52.2, 51.3. Anal. ($C_{45}H_{36}N_2O_{11}\cdot 3.5\ H_2O$) C, H, N.

4.9.5. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-*t*-butylphenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-124). Reactant: **ZP-115**, coupling with *p*-*t*-butylphenylenebisboronic acid, eluting with chloroform/MeOH, 100:1. Yield: 77.9%, ^{1}H NMR ($CDCl_3$) δ 8.03, 7.82, 7.50, 7.39 (4m, 19H, Ar–H), 6.33 (t, $J = 6$ Hz, 1H, 1′-H), 5.85 (d, $J = 6$ Hz, 1H, 2′-H), 5.23 (dd, $J_1 = J_2 = 3$ Hz, 1H, 3′-H), 4.74 – 4.48 (m, 3H, 4′,5′,5″-H), 3.97, 3.92 (2s, 6H, OCH_3), 1.31 (s, 9H, 3 CH_3). Anal. ($C_{43}H_{40}N_2O_{11}\cdot 2\ H_2O$) C, H, N.

4.9.6. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thienylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-128). Reactant: **ZP-115**, coupling with thienylboronic acid, eluting with chloroform. Yield: 38%, ^{1}H NMR ($CDCl_3$) δ 8.05, 7.82, 7.55, 7.39, 7.26 (5m, 18H, Ar–H), 6.34 (d, $J = 5.5$ Hz, 1H, 1′-H), 6.27 (t, $J = 6.4$ Hz, 1H, 2′-H), 5.82 (t, $J = 6.9$ Hz, 1H, 3′-H), 4.71, 4.62, 4.58 (3m, 3H, 4′,5′,5″-H), 3.96, 3.92 (2s, 6H, OCH_3). Anal. ($C_{37}H_{30}N_2O_{11}S\cdot 2\ H_2O$) C, H, N.

4.9.7. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*m*-nitrophenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-129). Reactant: **ZP-115**, coupling with 3-nitrophenylboronic acid, eluting with chloroform. Yield: 83%, ^{1}H NMR ($CDCl_3$) δ 8.12, 7.97, 7.52, 7.48, 7.40 (5m, 19H, Ar–H), 6.46 (d, $J = 6$ Hz, 1H, 1′-H), 6.23 (t, $J = 6.3$ Hz, 1H, 2′-H), 5.93 (m, 1H, 3′-H), 4.85, 4.74, 4.60 (m, 3H, 4′,5′,5″-H), 3.97, 3.96 (2s, 6H, OCH_3). Anal. ($C_{39}H_{31}N_3O_{13}\cdot 1.5\ H_2O$) C, H, N.

4.9.8. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-trifluoromethylphenylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-132). Reactant: **ZP-115**, coupling with *p*-trifluoromethylphenylboronic acid, product was recrystallized from MeOH. Yield: 23.8%, ^{1}H NMR ($CDCl_3$) δ 8.05, 7.80, 7.73, 7.64, 7.55, 7.41, 7.33, 7.29 (8m, 19H, Ar–H), 6.30 (t, $J = 7.3$ Hz, 1H, 1′-H), 6.12 (d, $J = 6$ Hz, 1H, 2′-H), 5.85 (t, $J = 6$ Hz, 1H, 3′-H), 4.72, 4.62, 4.59 (3m, 3H, 4′H,5′,5″-H), 3.98, 3.94 (2s, 6H, OCH_3). Anal. ($C_{40}F_3H_{31}N_2O_{11}\cdot 0.5\ H_2O$) C, H, N.

4.9.9. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-tolylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-136). Reactant: **ZP-115**, coupling with tolylboronic acid, eluting with chloroform. Yield: 47.5%, ^{1}H NMR ($CDCl_3$) δ 7.94, 7.73, 7.51, 7.36, 7.11 (5m, 19H, Ar–H), 6.01 (d, 1H, 1′-H), 5.61 (m, 1H, 2′-H), 5.33 (m, 1H, 3′-H), 4.85, 4.53 (2m, 3H, 4′,5′,5″-H), 3.94, 3.85 (2s, 6H, OCH_3), 2.27 (s, 3H, CH_3). Anal. ($C_{40}H_{34}N_2O_{11}\cdot 2.5\ H_2O$) C, H, N.

4.9.10. 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-*p*-trifluoromethylphenylimidazole-4,5-dicarboxylic Acid Dibutyl Ester (ZP-175). Reactant: **ZP-77**, coupling with *p*-trifluoromethylphenylboronic acid, product was recrystallized from MeOH. Yield: 40%, ^{1}H NMR ($CDCl_3$) δ 8.05, 7.90, 7.53, 7.40, 7.38 (5m, 19H, Ar–H), 6.41 (d, $J = 7.3$ Hz, 1H, 1′-H), 6.17 (t, $J = 6$ Hz, 1H, 2′-H), 5.87 (t, $J = 6$, 1H, 3′-H), 4.80, 4.58 (2m, 3H, 4′,5′,5″-H), 4.27 (m, 4H, 2 OCH_2), 1.71, 1.39 (m, 8H, 4 CH_2), 0.93 (2 t, $J = 7.3$ Hz, 6H, CH_3). Anal. ($C_{46}H_{43}F_3N_2O_{11}\cdot 3.5\ H_2O$) C, H, N.

4.10. General method for ring-closure condensation reactions with guanidine

Carbonate/Hydrochloride salt of guanidine (4 mmol) was suspended in anhydrous methanol (6.0 mL) and cooled to 0 °C. A solution of sodium methoxide (25 wt%, 2.1 mL, 9.2 mmol) was added. The resulting mixture was stirred in an ice bath for 30 min. The precipitated sodium salt was removed by filtration, and the filtrate was poured into a methanolic solution (20 mL) of ring-closure precursor (1 mmol). The mixture was stirred at room temperature for 16–72 h, and was monitored by frequent TLC analysis to check for the completion of reaction. The reaction mixture was filtered if necessary, and the clear solution was evaporated and purified by flash chromatography on a silica gel column, eluting with a mixture of chloroform/methanol (20:1, 5:1, 1:1) and methanol, successively. The appropriate fractions were combined and evaporated to obtain the product. The latter was recrystallized from an appropriate solvent if necessary. The spectral and analytical data, along with solvent of recrystallization and/or solvent of elution for chromatography are collected as below.

4.10.1. 6-*N*-Amino-7,8-dihydro-2-*p*-fluorophenyl-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-121). Ring-closure precursor: **ZP-118**, condensed with guanidine carbonate, isolated by silica gel flash chromatography (for details, see General Procedure above). The appropriate fractions were collected and evaporated to dryness. The residue was recrystallized from ethyl acetate. Yield: 50 mg (10%), $R_f = 0.24$ (chloroform/methanol/ammonium hydroxide = 2:1:0.3). ^{1}H NMR (DMSO-d_6) δ 10.81, 8.06 (2br, 2H, NH, exchangeable with D_2O), 7.82, 7.38 (2m, 4H, Ar–H), 6.86 (br, 1H, NH, exchangeable with D_2O), 5.85 (d, $J = 6$ Hz, 1H, 1′-H), 5.35, 4.99 (2d, $J = 5.2$ Hz, 2H, 2′-OH, 3′-OH, exchangeable with D_2O), 4.75 (d, $J = 5.6$ Hz, 1H, 5′-OH, exchangeable with D_2O), 4.47 (t, $J = 6.0$ Hz, 1H, 2′-H), 3.7, 3.65, 3.51, 3.45 (4m, 4H, 3′,4′,5′,5″-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.47, 162.15, 151.61, 149.21, 132.73, 132.64, 125.96, 115.68, 115.46, 91.09, 85.16, 71.99, 68.99, 61.45, 48.48. Anal. ($C_{17}H_{16}FN_5O_6\cdot 2H_2O$) C, H, N. MS (ESI) *m*/*z* 274.08,

306.11, 406.13; HRMS (ESI) calcd for $C_{17}H_{17}FN_5O_6$ (MH^+) *m/z* 406.11629, found, 406.11693.

4.10.2. 6-*N*-Amino-7,8-dihydro-2-*m*-methoxyphenyl-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-122). Ring-closure precursor: **ZP-119**, condensed with guanidine carbonate, isolated by silica gel flash chromatography (for details, see General Procedure above). The appropriate fractions were collected and evaporated to obtain a solid. Yield: 15%, ^{1}H NMR (DMSO-d_6) δ 10.72, 6.65 (2br, 1H, NH, exchangeable with D_2O), 7.45, 7.32, 7.12 (3m, 4H, Ar–H), 5.81(d, J = 5.6 Hz, 1H, 1′-H), 5.37, 4.97 (2d, J = 6 Hz, 2H, 2′-OH, 3′-OH, exchangeable with D_2O), 4.7 (t, J = 6 Hz, 1H, 5′-OH, exchangeable with D_2O), 4.54 (m, 1H, 2′-H), 3.82 (s, 3H, OCH_3), 3.76, 3.63, 3.5, 3.4 (4m, 4H, 3′,4′,5′,5″-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.08, 149.05, 130.44, 129.81, 122.55, 116.47, 115.24, 91.5, 85.13, 72.16, 69.59, 61.78, 55.37. Anal. ($C_{18}H_{19}N_5O_7 \cdot 0.5\ H_2O$) C, H, N.

4.10.3. 6-*N*-Amino-7,8-dihydro-2-*m*-methoxyphenylimida-zo[4,5-*e*][1,3]diazepine-4,8-dione (ZP-122fc). ^{1}H NMR (DMSO-d_6) δ 7.70 (s, 1H, Ar–H), 7.69 (d, J = 6.8 Hz, 1H, Ar–H), 7.22 (t, J = 8 Hz,1H, Ar–H), 6.76 (d, J = 8.4 Hz, 1H, Ar–H), 3.80 (s, 3H, OMe). MS *m/z* 286.08; HRMS (ES) calcd for $C_{13}H_{12}N_5O_3$ (MH^+) *m/z* 286.09401, found, 286.09339.

4.10.4. 2-(*m*-Methoxyphenyl)-1-β-D-ribofuranosylimidazole-4,5-dicarboxylic Acid Dimethyl Ester (ZP-122P1). ^{1}H NMR (DMSO-d_6) δ 7.442, 7.326, 7.127 (3m, 4H, Ar–H), 5.746 (d, 1H, J = 6 Hz, 1′-H), 5.647, 5.157 (2d, 2H, J = 6.8 Hz, J = 6 Hz, 2′,3′-OH, exchangeable with D_2O), 4.832 (t, 1H, J = 6 Hz, 5′-H, exchangeable with D_2O), 4.491 (q, 1H, J = 6.4 Hz, 2′-H), 3.849, 3.822, 3.797 (3s, 9H, OCH_3), 3.673, 3.441, 3.374 (3m, 4H, 3′,4′,5′,5″-H). Anal. ($C_{19}H_{22}N_2O_9 \cdot 1.5\ H_2O$) C, H, N.

4.10.5. 7,8-Dihydro-6-*N*-methylamino-2-phenyl-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine-4,8-dione (NZ-53). Ring-closure precursor: **ZP-78**, condensed with methylguanidine, isolated by silica gel flash chromatography, eluting with mixtures of chloroform/methanol (20:1, 5:1, 1:1) and methanol. The appropriate fractions were collected and evaporated to obtain a solid. Yield: 53%, ^{1}H NMR (DMSO-d_6) δ 10.77, 7.1 (br, 2H, NH, exchangeable with D_2O), 7.72, 7.5 (m, 5H, Ar–H), 5.82(d, J = 5.7 Hz, 1H, 1′-H), 5.33, 4.99 (2d, J = 6 Hz, 2H, 2′-OH, 3′-OH, exchangeable with D_2O), 4.77 (t, J = 6 Hz, 1H, 5′-OH, exchangeable with D_2O), 4.35 (m, 1H, 2′-H), 3.64, 3.47 (2m, 4H, 3′,4′,5′,5″-H), 2.75 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162, 159, 147, 130.2, 130, 129.5, 128, 91, 85, 72, 70, 62, 29. Anal. ($C_{18}H_{19}N_5O_6 \cdot 3.5\ H_2O$) C, H, N. MS *m/z* 402.0, 424.0; HRMS (ES) calcd for $C_{18}H_{20}N_6O_5$ (MH^+) *m/z* 402.1414, found, 402.1398.

4.10.6. Reaction of ZP-136 with *N*-methylguanidine to produce mixture of products (ZP-181). ZP-136 was condensed with *N*-methylguanidine using the general procedure described above. The products were isolated by silica gel flash chromatography, eluting with mixtures of chloroform/methanol (20:1, 5:1, 1:1) and methanol. The fractions eluted with methanol were collected and evaporated to obtain a yellow solid. The ^{1}H NMR data suggested that it is a mixture. MS (FAB) of the reaction mixture exhibited the following ions: *m/z* 284.1, 316.1, 416.2, and 448.2. HRMS (ES) calcd for $C_{19}H_{22}N_5O_6$ and $C_{20}H_{26}N_5O_7$ (MH^+): *m/z* 416.15701 and 448.18322, found, 416.15923, 448.18029.

4.11. Primary HCV RNA replicon assay

The cell line Huh7 ET (luc-ubi-neo/ET), which contains an HCV RNA replicon with a stable luciferase (LUC) reporter, was used for the assay.**T**he HCV RNA replicon ET contains the 5′ NTR (IRES) of HCV (5′) which drives the production of a firefly luciferase (Luc), ubiquitin (Ubiq), and neomycin phosphotransferase (Neo) fusion protein. Ubiquitin cleavage releases the LUC and Neo genes. The EMCV IRES element (E-I) controls the translation of the HCV structural proteins NS3-NS5. The NS3 protein cleaves the HCV polyprotein to release the mature NS3, NS4A, NS4B, NS5A, and NS5B proteins that are required for HCV replication. At the 3′ end of the replicon is the 3′ NTR of HCV. The LUC reporter is used as an indirect measure of HCV replication. The activity of the LUC reporter is directly proportional to HCV RNA levels and positive control antiviral compounds behave comparably using either LUC or RNA endpoints.

In the primary assay, the effect of RENs was examined in triplicate at a single high-test concentration of 20 μM on HCV RNA-derived LUC activity and cytotoxicity. Human interferon alpha-2b is included in each run as a positive control compound. Subconfluent cultures of the ET line are plated out into 96-well plates that are dedicated for the analysis of cell numbers (cytotoxicity) or antiviral activity and the next day drugs are added to the appropriate wells. Cells are processed 72 hr later when the cells are still subconfluent. Compound cytotoxicity is assessed as the percent viable cells relative to the untreated cell controls. The results are collected in Table 1 in the text.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.04.043.

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