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Bioorganic & Medicinal Chemistry Letters 14 (2004) 3517–3520
Bioorganic & Medicinal Chemistry Letters

Synthesis of new 2′-β-*C*-methyl related triciribine analogues as anti-HCV agents

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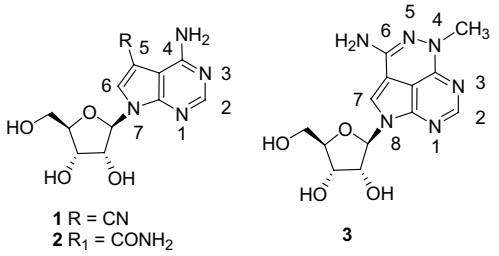
Received 16 February 2004; accepted 19 April 2004

Abstract—Ten new β-D-ribofuranosyl and 2′-β-*C*-methyl-β-D-ribofuranosyl triciribine derivatives **4**–**13** with various *N4* and 6-*N* substituents on the tricyclic ring were synthesized from the corresponding toyocamycin and new 2′-β-*C*-methyl toyocamycin derivatives. The inhibitory studies of these compounds in the HCV replicon assay reveal that some of them possess interesting anti-HCV properties with low cytotoxicity.

Hepatitis C virus (HCV) infection is an important public health problem worldwide and is recognized as the major cause of non-A, non-B hepatitis. HCV is an enveloped positive-sense single-stranded RNA virus belonging to the *Flaviviridae* family.[1] HCV is a major human pathogen, and infects an estimated 170 million people worldwide. The virus establishes chronic infection in up to 80% of infected individuals and persists for decades with a substantial risk of developing liver cirrhosis and hepatocellular carcinoma (HCC).[2] There is no vaccine available against HCV. Monotherapy using interferon-α (IFN-α) has 8–10% sustained virological response (SVR).[3] Combination therapy of IFN-α-2b, or pegylated IFN, and ribavirin, a nucleoside analogue, improves the overall SVR to 41–55%.[4] However, combination therapy shows a low response in patients with HCV genotypes 4–11, and a majority of patients infected with HCV genotype 1, the predominant genotype in the western world, are not responsive to the current treatments.[5] A large number of HCV patients are still waiting for novel direct anti-HCV agents. Clearly, more efficacious therapies are urgently needed to combat this important viral disease.

Toyocamycin **1** and sangivamycin **2** are 7-deazaadenosine nucleoside analogues that show interesting biological properties.[6] 6-Amino-4-methyl-8-(β-D-ribofuranosyl)-(4*H*,8*H*)-pyrrolo[4,3,2-*d*]pyrimido[4,5-*c*]pyridazine (triciribine, TCN, **3**) (Fig. 1), a cyclic sangivamycin analogue, exhibits anti-cancer and anti-viral activity.[7] Triciribine 5′-monophosphate (TCN-P), having higher water solubility, has been extensively studied as a prodrug of TCN and advanced to phase II clinical studies as a potential anti-neoplastic agent.[8] Acyclic[9] and various deoxy[10] modified triciribine analogues have been reported without obvious anti-viral and anti-cancer activity. However, a couple of 6-*N*-acylated triciribine analogues showed improved cell permeability with anti-viral activity but higher cytotoxicity.[11] We anticipated that a 2′-alkyl modification would reduce cytotoxicity and improve both pharmacological and biological activities based on the fact that 2′-β-*C*-methyl-β-D-ribonucleosides have a high population of the anti-3′-endo ($^3T_2$) 'northern' sugar conformation.[12] It has been



1 R = CN
2 R_1 = $CONH_2$

3

Figure 1. Structure of toyocamycin 1, sangivamycin 2, and triciribine 3.

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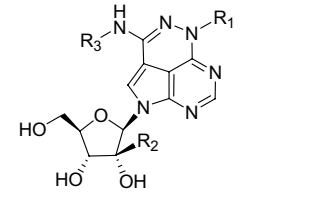
doi:10.1016/j.bmcl.2004.04.067

demonstrated that 2′-β-*C*-methyl derivatives are efficient chain terminators to inhibit HCV genome replication.[13]

In order to study direct anti-hepatitis C activity of triciribine derivatives, we designed and synthesized β-D-ribofuranosyl triciribine analogues **4**–**6** with different *N4* substitutions (Fig. 2). We further designed and synthesized 2′-β-*C*-methyl-β-D-ribofuranosyl triciribine derivatives **7**–**13** with *N4* and 6-*N*substitutions. New triciribine derivatives **4**–**13** were studied for their ability to inhibit hepatitis C viral RNA replication in Huh-7 replicon cell culture.[14]

We first explored β-D-ribofuranosyl triciribine derivatives **4**–**6** (Scheme 1). The tri-*O*-benzoyl-protected pyrrolopyrimidine nucleoside derivative **14**, synthesized by mimicking the literature procedure,[15] was reacted with different N-substituted hydrazines at room temperature (Scheme 1). The resulted 4-hydrazino derivatives were cyclized with the cyano group at the 5-position of the pyrrolopyrimidine ring at the refluxing temperature to provide the corresponding tri-*O*-benzoylated triciribine derivatives **15**–**17** in 70–90% yields. Deprotection of **15**–**17** with ammonia solution in methanol generated *N4*-substituted triciribine analogues **4**–**6** in high yields.

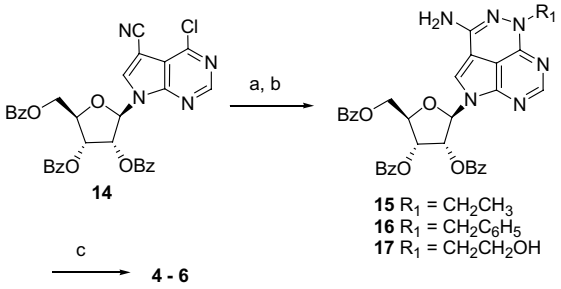


$R_2 = R_3 = H$ **4** $R_1 = CH_2CH_3$; **5** $R_1 = CH_2C_6H_5$; **6** $R_1 = CH_2CH_2OH$

$R_2 = CH_3$, $R_3 = H$ **7** $R_1 = CH_3$; **8** $R_1 = CH_2CH_3$; **9** $R_1 = CH_2CH_2CH_3$; **10** $R_1 = CH_2CH_2CH_2CH_3$; **11** $R_1 = CH_2CH_2OH$

$R_2 = CH_3$, $R_3 = CH_3CO$ **12** $R_1 = CH_3$; **13** $R_1 = CH_2CH_2OH$

Figure 2. New β-D-ribofuranosyl and 2′-β-*C*-methyl-β-D-ribofuranosyl triciribine derivatives **4**–**13**.



14

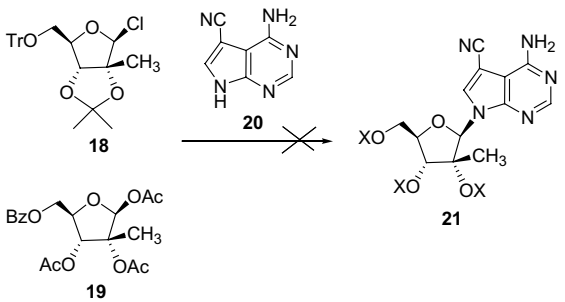
15 $R_1 = CH_2CH_3$; **16** $R_1 = CH_2C_6H_5$; **17** $R_1 = CH_2CH_2OH$

4 - 6

Scheme 1. Synthesis of β-D-ribofuranosyl triciribines **4**–**6**. Reagents and conditions: (a) R_1NHNH_2, $CHCl_3$, EtOH, 25 °C; (b) EtOH, HCl, reflux; (c) NH_3, MeOH, rt 18 h.

There has been increasing interest in *C*-methyl-ribonucleosides, such as the reported synthesis of 3′-*C* and 5′-*C*-methylsangivamycins, as well as 4′-*C* and 5′-*C* β-methyltoyocamycins in searching for anti-viral and anticancer agents.[12,16] However, there exists no efficient and practical route for the synthesis of the corresponding 2′-β-*C*-methyl analogues. Murai and co-workers[12] reported the glycosylation of chloro-ribose **18** with a 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine derivative resulting in a mixture of α- and β-anomers and the 1-glycosylated isomers. To obtain the key intermediate, we investigated the coupling of the 2′-*C*-β-methyl ribose derivative **19** with 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine derivative **20**. Different protected ribose sugars **18**[12] and **19** were reacted with **20**, and the couplings were explored in different combinations under various conditions, for example, NaH, HMDS/TMSCl/TMSOTf, and BSA/TMSOTs (Scheme 2). It was anticipated that by modifying either the protecting groups of ribofuranose, or the base substituents, we might be able to better understand the steric and electrophilic requirements necessary to facilitate the desired coupling. However, these approaches proved to be low yielding, and the resulting mixtures of isomers for the synthesis of compounds **21** (X = acetyl or XX = dimethylketal) were difficult to be separated. We then utilized the 6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**22**)[16] and the tetra-*O*-benzoyl-protected 2′-β-*C*-methyl-β-D-ribofuranose **23**[17] for glycosylation studies (Scheme 3). The bromo base **22** was first silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and then coupled with **23**, mediated by TMSOTf, at 65 °C for 18 h. The desired β-coupled product **24** was obtained in 50–70% yields as the only observed anomer. Apparently, the coupling reaction favors the use of a ribose containing a 2′-α-benzoyl protecting group to assist in the cleavage of the labile 1′-ester, in order to generate sufficiently electrophilic species and to guide the 1′-β-glycosylation. Although one additional reaction step is needed to prepare the desired intermediate **21**, the high-yielding and efficient glycosylation overcame many of the hurdles for the coupling between other ribose and heterocyclic bases. This proved to be an excellent way for the synthesis of difficult pyrrolopyrimidine nucleoside derivatives.

The bromo derivative **24** was hydrogenated over 10% Pd/C to afford the corresponding debrominated benzoyl protected 2′-β-*C*-methyl toyocamycin **21** in 90–97% yields. The 4-amino group was then oxidized by treat-



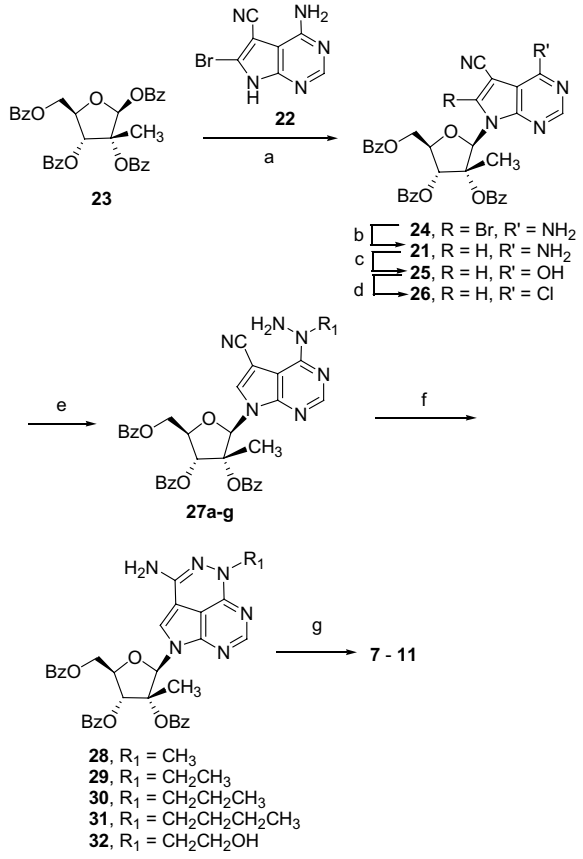
18

19

20

21

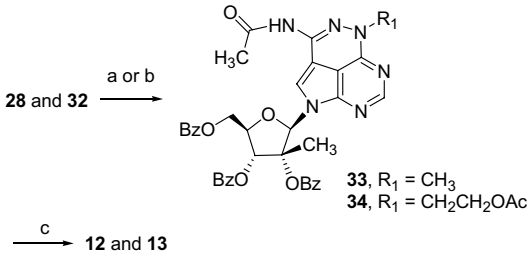
Scheme 2. Attempted synthesis of nucleosides **21**.



Scheme 3. Synthesis of triciribines **7**–**11**. Reagents and conditions: (a) BSA (2 equiv), CH_2ClCH_2Cl, 65 °C, 18 h; (b) H_2 (30 psi), Pd/C, Et_3N, dioxane; (c) $NaNO_2$, AcOH, H_2O, 80 °C, 2 h; (d) $POCl_3$, reflux, 1 h; (e) $RNHNH_2$, $CHCl_3$, EtOH, 25 °C; (f) EtOH, HCl, reflux; (g) NH_3, MeOH, 18 h.

ment with sodium nitrite in a 4:1 mixture of acetic acid and water to afford **25** in 90% yield. Compound **25** was successfully converted to its corresponding 4-chloro derivative **26** in 90% yield by refluxing in $POCl_3$. After treatment of **26** with methyl hydrazine at room temperature for 4 h, the intramolecular nucleophilic cyclization of the amino and cyano groups of the resultant intermediate **27a** afforded the corresponding triciribine derivative **28** by refluxing in ethanol and a catalytic amount of hydrochloric acid. The benzoyl groups of **28** were then removed by treatment with ammonia solution in methanol to afford the desired 2′-β-*C*-methyl triciribine **7** in 78% overall yield from **26**. Utilizing this strategy, we were also able to synthesize other N4 substituted TCN analogues **8**–**11**, by the reaction of **26** with the corresponding substituted hydrazines in place of methyl hydrazine. The resulting intermediates **27b**–**g** were cyclized to give the corresponding protected triciribine derivatives **29**–**32**, which were deprotected to give 2′-β-*C*-methyl-β-D-ribofuranosyl triciribines **8**–**11**.

In order to improve cell permeability and oral bioavailability of the triciribine derivatives, we further synthesized the 6-*N*-acetylated triciribine analogues **12** and **13** (Scheme 4). Tri-benzoyl-protected triciribines **28** and **32** were acetylated with acetic anhydride or acetyl chloride in pyridine. The resulting tri-ester **33** and tetra-

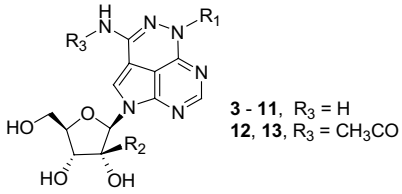


Scheme 4. Synthesis of triciribines **12** and **13**. Reagents and conditions: (a) Ac_2O, pyridine; (b) CH_3COCl, pyridine, DAMP; (c) NH_3, MeOH, 25 °C, 18 h.

ester **34** were deprotected at room temperature with ammonia solution in methanol. The ester-based protecting groups of **33** and **34** were selectively removed without affecting the amide group affording the desired 2′-β-*C*-methyl-β-D-ribofuranosyl triciribine amides **12** and **13**.[18]

The new triciribine analogues **4**–**13**, synthesized herein, were assayed for their ability to inhibit hepatitis C viral RNA replication in Huh-7 cells.[14] The inhibitory potency of these compounds in HCV replicon assay is expressed as EC_{50}. The MTS[19] assay was utilized to test the associated cytotoxicity (CC_{50}). Table 1 lists the HCV replicon activity and cytotoxicity of compounds **3**–**13**. Compound **4** exhibits the highest potency with an EC_{50} of 1.0 μM. Compounds **6**–**8** show modest inhibitory activity. Compounds **4**, **7**, and **8** with smaller substituents at the *N*4 position exhibit higher potency than the related compounds **5**, **9**, and **10** with bigger substituents. The 6-*N* acetylation, compound **12** and **13**, decreases potency comparing to the un-acetylated derivative **7**.

Table 1. Inhibitory potency and cytotoxicity of **3**–**13** in HCV replicon assay



No.	R_2	R_1	EC_{50}[a]/CC_{50}[b] (μM)
3	H	CH_3	2/>300
4	H	CH_2CH_3	1/150
5	H	$CH_2C_6H_5$	125/>300
6	H	CH_2CH_2OH	20/>300
7	CH_3	CH_3	18/>300
8	CH_3	CH_2CH_3	15/>200
9	CH_3	$CH_2CH_2CH_3$	100/>300
10	CH_3	$CH_2CH_2CH_2CH_3$	100/>300
11	CH_3	CH_2CH_2OH	200/>300
12	CH_3	CH_3	150/300
13	CH_3	CH_2CH_2OH	100/200

[a] Compounds were incubated in cell culture for 72 h prior to determination of the relative amount of HCV replicon RNA using a luciferase reporter assay.

[b] Compound cytotoxicity was determined by MTS assay on parallel samples.

Most compounds show low cytotoxicity with an exception of **4**, which nonetheless still shows a reasonably high selectivity index (CC_{50}/EC_{50}).

In summary, *N*4 substituted β-D-ribofuranosyl triciribines **4**–**6** were synthesized. We have established an efficient coupling of a 2′-β-*C*-methyl-β-D-ribofuranose with 4-amino-7-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine.

Employing this strategy, new *N*4 and 6-*N* substituted 2′-β-*C*-methyl-β-D-ribofuranosyl triciribines **7**–**13** were synthesized as potential anti-HCV agents. Some of them were found to exhibit anti-viral activity in HCV replicon cell culture with minimal cytotoxicity. These results should promote the synthetic studies of other 2′-β-*C*-methyl nucleosides, such as the biologically active toyocamycin and sangivamycin, as potential therapeutics against human diseases.

Acknowledgements

The authors thank Jeffrey Abt and Jenny Song for the synthesis of **25**.

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