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Synthesis of purine modified 2′-*C*-methyl nucleosides as potential anti-HCV agents

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ABSTRACT

Based on the anti-hepatitis C activity of 2′-*C*-methyl-adenosine and 2′-*C*-methyl-guanosine, a series of new modified purine 2′-*C*-methyl nucleosides was prepared as potential anti-hepatitis C virus agents. Herein, we report the synthesis of both 6-modified and 2-modified purine 2′-*C*-methyl-nucleosides along with their anti-HCV replication activity and cytotoxicity in different cells.

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Hepatitis C virus (HCV) infects more than 200 million individuals worldwide according to the World Health Organization. In addition, an estimated 3–4 million people contract HCV each year. Long-term infection can lead to chronic liver disease, such as cirrhosis of the liver or hepatocellular carcinoma. Until recently the standard of care therapy employed interferon-α (IFN) often in combination with the nucleoside analog ribavirin. The impact on standard of care by approval of the two HCV protease inhibitors Incivek and Victrelis remains unclear as both drugs require response-guided therapy regimens that can shorten the duration of IFN therapy in infected persons with an early viral response from 48 weeks to as few as 24 weeks. In addition, serious side effects and limited efficacy emphasize the urgent need for improved therapeutic agents.[1] Moreover, there is no established vaccine for HCV. As a result, there is an urgent need for safe and effective therapeutic agents that combat HCV infection and that have high genetic barrier to resistance.

Among the most successful preclinical and clinical nucleosides with potent HCV NS5B polymerase inhibition are 2′-*C*-methyl analogs.[2] 2′-*C*-Methyl adenosine, **1** and 2′-*C*-methyl guanosine, **2** (Fig. 1) display selective in vitro anti-HCV activity as non-obligatory chain terminators toward RNA elongation. However, in vivo these analogs display poor bioavailability due to deamination of the adenine base and purine nucleoside phosphorylase (PNP) promoted glycosidic bond cleavage in the case of **1** and poor cellular uptake and/or inefficient phosphorylation for **2**.[3]

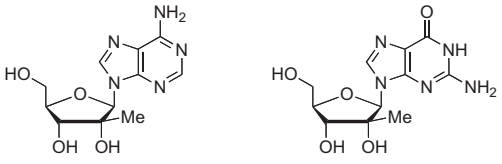

Figure 1. Important 2′-*C*-methyl purine analogs.

Base-modification of nucleoside analogs has been successful in the development of potent antiviral agents.[4] Within the 2′-*C*-methyl nucleoside series, the 7-deaza modification of the adenosine analog results in a significant improvement of in vivo anti-HCV activity.[5] In this study, novel purine modified 2′-*C*-methyl nucleoside analogs were prepared which explored the steric, electronic, and hydrogen bonding effects on anti-HCV activity as measured by a cell-based replication assay.

The synthesis of 2′-*C*-methyl-2-amino-6-methyl purine analogs is outlined in Scheme 1. Compound **3**, as a single isomer, was readily prepared in three sugar modification steps[5] from commercially

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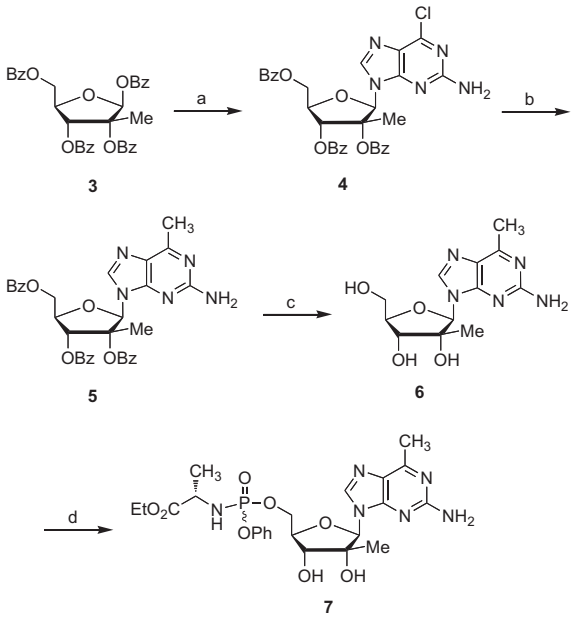


Scheme 1. Reagents and conditions: (a) 2-amino-6-chloropurine, DBU, TMSOTf, CH_3CN, 80 °C, 5 h, 92%; (b) $Pd(Ph_3P)_4$, $Al(CH_3)_3$, THF, 75 °C, 8 h, 54%; (c) NH_3/CH_3OH, rt, 2 days, 62%; (d) *t*-BuMgCl, phenyl ethoxyalaninyl phosphorochloridate, THF, rt, 18 h, 9%.

available starting materials. Condensation of **3** with 2-amino-6-chloropurine in the presence of DBU and TMSOTf at 80 °C for 5 h gave the 6-chloro nucleoside **4** as the pure β-anomer in 92% yield. A modification to the standard Vorbrüggen conditions involving the addition of TMSOTf at −78 °C improved the reaction yield from around 60%[3a] to over 90%. Cross-coupling reactions have been used successfully for the introduction of carbon-linked substituents into purine moieties.[6] Hence compound **4** was allowed to react with trimethylaluminum under $Pd(PPh_3)_4$ catalyzed conditions in THF to give the corresponding 6-methyl-purine derivative **5** in 54% yield. Subsequently, the benzoyl groups were removed to afford the 6-methyl purine nucleoside analog **6**.[7] Monophosphate prodrug, (aryloxy)phosporamidate **7**, was prepared by following Uchiyama's procedure in the presence of *tert*-butyl magnesium chloride in THF.[8,9]

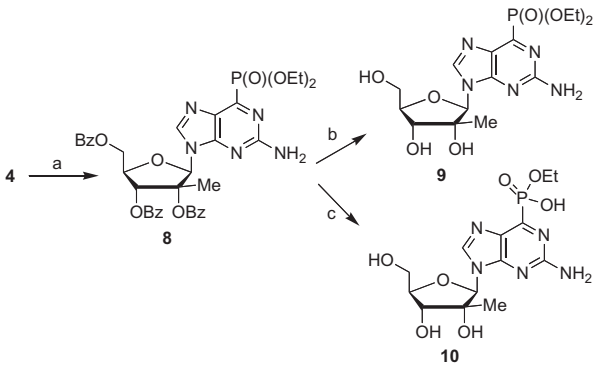
The 6-phosphonate substituted purine is an attractive target because the phosphonate group may mimic the hydrogen bonding characteristics of a 6-amino or 6-hydroxyl group of endogenous nucleoside bases and thus may result in nucleoside analogs that are inhibitors of adenosine deaminase (ADA).[10] Application of the Arbuzov reaction to 6-chloro purine **4** by treatment with triethyl phosphite afforded 6-phosphonate **8** in 78% yield (Scheme 2).[11] Removal of the benzoyl groups with saturated NH_3 in ethanol or NaOEt in ethanol provided **9**[12] and **10** respectively.

A series of 1,2,4-triazolo[5,1-*i*]adenine derivatives were identified as potent adenosine A_{2a} receptor antagonists.[13] Application of these interesting base modifications to a 2′-Me sugar nucleoside provides potential HCV inhibitors. Among many synthetic approaches for preparation of these purine base modifications,[14] an efficient cyclization method utilizing *N,O*-bis-(trimethylsilyl)acetamide (BSA) to undergo a dehydrative rearrangement was chosen as shown in Scheme 3.

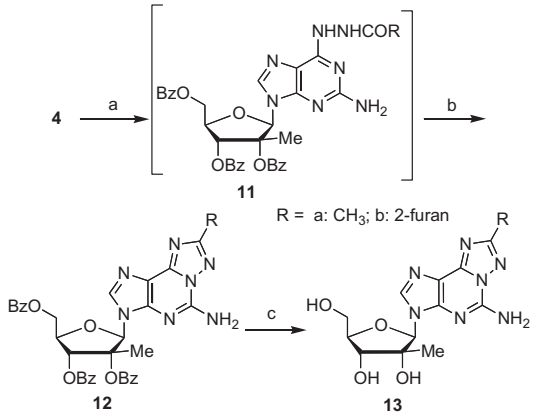
The ipso displacement of 6-chloro group of compound **4** by acetohydrazide or 2-furoic hydrazide afforded compounds **11a** and **11b**, which are used for the next step without purification. Heating with BSA for 5 h furnished the desired tricycles **12** via a dehydrative rearrangement process.[13] After removal of the benzoyl protecting groups, **13a** and **13b** were isolated in 50% and 90% yield, respectively.

The 6-H nucleoside **15** was isolated in 21% yield during the preparation of **12a** (Fig. 2). Formation of **15** can be explained by a base promoted Wolff–Kishner reduction.[15] This postulate is supported by the detection of the 6-hydrazo nucleoside **14** by LC/MS during the preparation of **11a**. During the BSA promoted dehydrative rearrangement reaction, intermediate **14** was converted to **15** while the intermediate **11a** proceeded to **12a**. In a confirmation reaction, 6 equivalents of hydrazine were allowed to react with compound **4** at 110 °C for 2 days. The reduced nucleoside **15** was cleanly formed without removal of the benzoyl groups.

Previous studies have found 2-position modified purines with potent antiviral activity and/or a reduction of toxicity.[16] The 2-hydrazine-, 2-azido- and 2-triazole-purine nucleosides were targeted as they potentially offer a variety of steric, electronic, and hydrogen bonding interactions which may enhance recognition by HCV NS5B polymerase. Treatment of the 2,6-dichloro purine nucleoside **16** with methanolic ammonia removed the benzoyl protecting groups with concomitant amination and methoxylation to afford 6-aminopurine **17** and 6-methoxypurine **18**, respectively (Scheme 4). Nucleophilic substitution of the 2-chloro group of **17** with hydrazine hydrate gave the 2-hydrazine substituted purine nucleoside **19**.[17] Treatment of **19** with sodium nitrite in acetic acid provided a 63% yield of the 2′-azidopurine **20**. Based on analysis of the ^1H NMR, compound **20** exists in both an azido and an N1 tetrazole tautomeric form.[18] 1,3-Dipolar cycloaddition of azide **20** with ethynyltrimethylsilane through a Cu(I)-catalyzed 1,3-cycloaddition reaction generated the triazole analogs **21**[19] and compound **22**.[20] Compound **21** is formed by desilylation during the cycloaddition reaction. Compound **21** may also be prepared from **22** by treatment with



Scheme 2. Reagents and conditions: (a) $P(OEt)_3$, 130 °C, 18 h; 78%; (b) $NH_3/EtOH$, rt, 4 days, 44%; (c) NaOEt/EtOH, rt, 2 days, then 50 °C for 30 min, 19%.



Scheme 3. Reagents and conditions: (a) $H_2NNHCOR$, 110 °C, 2 days; (b) BSA, 130 °C, 5 h, **12a**: 39%; **12b**: 72%; (c) NaOMe/MeOH, rt, 18 h, **13a**: 50%; **13b**: 90%.

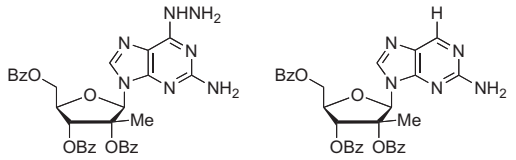
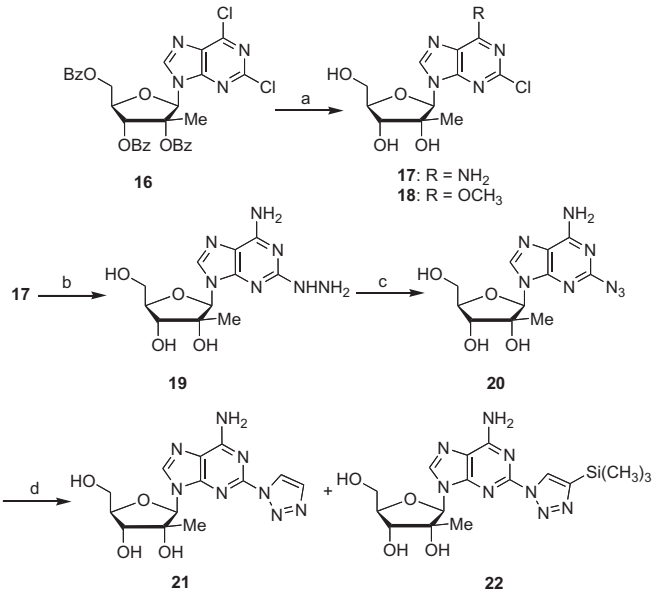
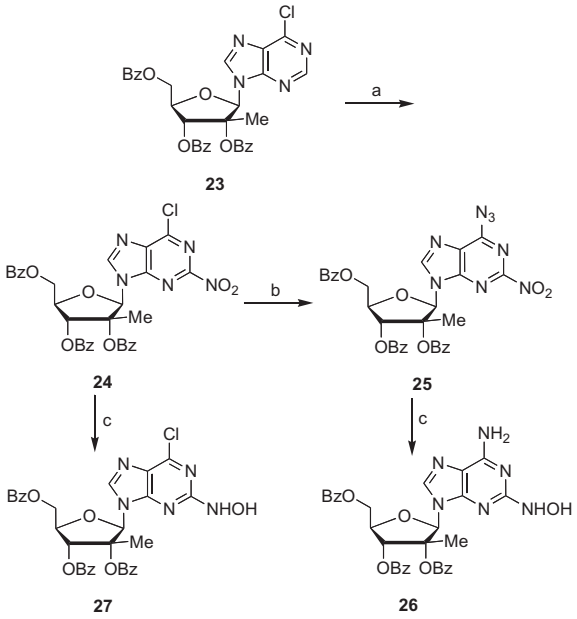


Figure 2. Side products of **12a** formation.



Scheme 4. Reagents and conditions: (a) NH_3/CH_3OH, THF, rt, 3 days, **17**: 46%, **18**: 21%; (b) hydrazine, EtOH, rt, 16 h, 92%; (c) $NaNO_2$, HOAc, rt, 1 h, 78%; (e) sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, TMSCCH, *t*-BuOH/H_2O (v/v, 1:1), rt, 18 h, **21**: 12%, **22**: 30%.



Scheme 5. Reagents and conditions: (a) TBAN, TFAA, CH_2Cl_2, 85 °C, 3 h, 79%; (b) NaN_3, DMF, −18 °C, 1 h, then 0 °C, 2 h, 99%; (c) H_2 (1 atm), Pd/C, EtOAc, EtOH, 45–50 °C, **26**: 1.5 h, 43%; **27**: 3 h, 41%.

aqueous HF in THF and formation of **21** during the cycloaddition step may be suppressed by addition of BSA to the reaction mixture.[21]

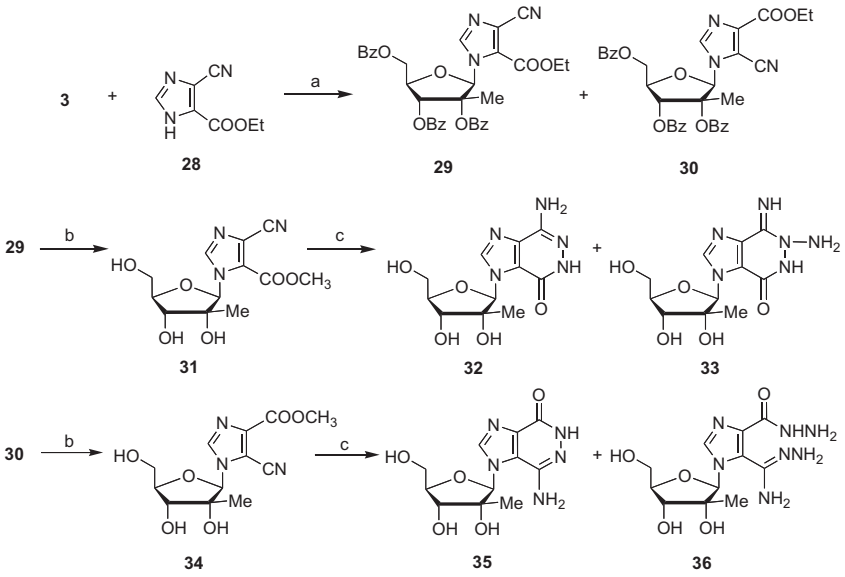
The 2-hydroxyamino substitution was selected for evaluation due to its structural and electronic similarity to hydrazine. As shown in Scheme 5 the 6-chloropurine **23** was nitrated with tetrabutylammonium nitrate (TBAN) and trifluoroacetic anhydride (TFAA) to afford the 2-nitro analog **24** in 79% yield.[22] Nucleophilic substitution with sodium azide produced compound **25** in 99% yield. Side reactions and lower yields occurred when more than 1 equiv of sodium azide was utilized in this reaction. Careful hydrogenation under mild heating afforded 2-hydroxyaminopurines **26** and **27** in modest yields.[23] However, removal of the benzoyl groups of **26** or **27** with NH_3/CH_3OH or sodium methoxide under a variety of conditions failed to give the desired compounds, but instead lead to complex mixtures.

Based on the biological activity of the 4-amino-1*H*-imidazo[4,5-*d*]pyridazin-7(6*H*)-one ring system as a purine isostere,[24] 4-amino-1-(2-methyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*]pyriazin-7-(6*H*)-one, **32** and 7-amino-1-(2-methyl-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*d*]pyridazin-4-(5*H*)-one, **35** were synthesized (Scheme 6). The Vorbrüggen glycosylation reaction of **4** with ethyl 5(4)-cyano-1*H*-imidazole 4(5)carboxylate **28** gave two regioisomeric nucleosides **29** and **30** in 64% and 36%, respectively. The regioisomeric structures were assigned based on analysis of their ^1H NMR data and comparison to their ribofuranosyl analogs.[25] Compounds **29** and **30** were independently treated with excess *t*-butylamine in ethanol to obtain the deprotected and transesterified **31** and **34** in 91% and 78% yield, respectively. The two compounds were separately reacted with hydrazine, followed by heating with sodium ethoxide in anhydrous ethanol at reflux to yield the respective target nucleoside analogs **32** and **35** in 71% and 62% yield. The unexpected bis-adduct products **33** and **36** were also obtained albeit in low yields of 5% and 12%, respectively.

All of the modified purine 2′-*C*-methyl nucleosides were evaluated for inhibition of HCV RNA replication at 10 μM in Huh7 cells using a subgenomic HCV replicon system.[26] Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and ribosomal RNA (rRNA).[27] To determine the spectrum of activity of the compounds, anti-HIV activity was evaluated versus HIV-1_{LAI} in primary human peripheral blood mononuclear (PBM) cells and AZT was used as a positive standard. Cytotoxicity was determined in PBM, human lymphoblastoid CEM, and African Green monkey Vero cells.[28] A subset of the compounds were tested for their ability to inhibit and/or act as substrates of adenosine deaminase.[29] The antiviral and cytotoxicity results are summarized in Table 1.

In general, nucleosides with the 6-position modifications of the purine base did not display any marked anti-HCV activity (up to 10 μM) or toxicity to Huh7 PBM, CEM, or Vero cell lines. The lack of anti-HCV activity or cytotoxicity of these nucleosides may be due to inefficient uptake and/or their inability to be intracellularly metabolized to the corresponding nucleoside triphosphates. However, the monophosphate prodrug **7**, which would bypass the initial phosphorylation step, also did not display any inhibition of HCV replicon RNA replication.

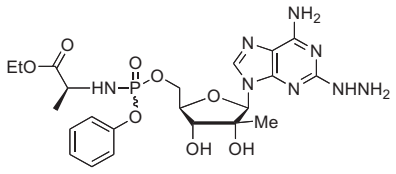
Modification in the 2-position of the purine base proved to be somewhat interesting. The substituted purines 2-Cl, 6-NH_2, **17**, 2-Cl, 6-OMe, **18**, 2-azido, 6-NH_2, **20**, 2-(1*H*-triazole), 6-NH_2, **21**, 2-(4-(trimethylsilyl)-1*H*-triazole), 6-NH_2, **22** along with the purine isosteres **32**–**36** all displayed no antiviral activity or cytotoxicity. In contrast, hydrazine **19** possessed anti-HCV activity (85% inhibition at 10 μM; EC_{50} = 6 μM) with no observed cytotoxicity which encouraged us to prepare its (2*S*)-ethyl(phenoxy)phosphorylamino)-propanoate prodrug (Fig. 3). This phosphoramidate prodrug was more active versus HCV replication with an EC_{50} of 0.9 μM. However stability studies of **19** in pH 7.4 phosphate buffer at 23 °C showed complete conversion of **19** to a complex mixture of



Scheme 6. Reagents and conditions: (a) DBU, TMSOTf, CH_3CN, 0 °C, 30 min, 50 °C, overnight, **29**: 64%, **30**: 36%; (b) *t*-butylamine, MeOH, rt, 2 days, for **31**: 91%, for **34**: 78%; (c) (i) hydrazine monohydrate, MeOH, 0 °C; (ii) NaOEt (cat), EtOH, reflux, **32**: 71%, **33**: 5%, **35**: 62%, **36**: 12%.

Table 1
Anti-HCV activity, anti-HIV activity, and cytotoxicity of synthesized compounds in cellular assays

Compd	Anti-HCV activity:% inhib @ 10 μM in Huh7 cells		Anti-HIV activity (μM)		Cytotoxicity in: CC_{50} (μM)		
	HCV	rRNA	EC_{50}	EC_{90}	PBM	CEM	Vero
6	0	0	70	>100	>100	13	>100
7	0	0	68	>100	>100	>100	>100
9	19	15	>100	>100	>100	>100	>100
10	19	15	>100	>100	>100	>100	>100
13a	0	0	>100	>100	>100	>100	>100
13b	0	0	>100	>100	>100	>100	>100
17	0	5.8	>100	>100	>100	>100	>100
18	14	9.8	>100	>100	>100	>100	>100
19	85	7.0	>100	>100	>100	>100	>100
20	5.2	0	>100	>100	>100	>100	>100
21	0	0	>100	>100	>100	>100	>100
22	0	0	>100	>100	>100	>100	>100
26	66	76	8.7	42	>100	27	42
27	100	100	17	65	43	7.3	>100
32	0	0	>100	>100	>100	>100	>100
33	0	0	>100	>100	>100	>100	>100
35	0	0	>100	>100	>100	>100	>100
36	0	9.0	>100	>100	>100	>100	>100



Phosphoramidate of **19**

Figure 3. (2*S*)-Ethyl(phenoxy)phosphorylamino)-propanoate prodrug.

products after 3 days. One of decomposition products was identified as 2′-*C*-methyladenosine, a known inhibitor of HCV NS5B polymerase.[30]

The two 2-hydroxyamino nucleosides **26** and **27** displayed broad toxicity against most cell lines tested. Compounds **9**, **10**, **13b**, **21**, **32**, and **35** were tested for their ability to inhibit the ADA catalyzed conversion of adenosine to inosine. These compounds were chosen due to their structural similarity to known ADA inhibitors.[10,13] Only compound **21** was found effective with an average IC_{50} = 3.7 ± 0.4 μM. Compounds **9**, **10**, **21**, and **32** were evaluated as potential substrates of adenosine deaminase; however, none underwent a nitrogen to oxygen transformation.

In conclusion, a variety of purine-modified 2′-*C*-methyl nucleosides were synthesized and evaluated as potential anti-HCV agents. Among these synthesized nucleoside analogs, none displayed potent and selective anti-HCV or anti-HIV activity. Interestingly, some of these purine analogs warrant further study as potential inhibitors of adenosine deaminase.[31]

Acknowledgments

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7. *Spectral data for compound* ***6***: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.46 (s, 1H), 5.98 (s, 1H), 4.17 (d, J = 9.2 Hz, 1H), 4.00 (m, 1H), 3.97 (d, J = 2.0 Hz, 1H), 3.81 (dd, J = 2.8 Hz, J = 12.4 Hz, 1H), 2.56 (s, 3H), 0.92 (s, 3H). LC/MS (m/z), calcd for $C_{12}H_{18}N_5O_4{\cdot}(M^+{+}H)$: 296.13; found, 296.19.
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12. *Spectral data for compound* ***9***: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.63 (s, 1H), 6.04 (s, 1H), 4.27 (m, 4H), 4.18 (d, J = 9.2 Hz, 1H), 3.97 (m, 2H), 3.81 (dd, J = 3.2 Hz, J = 12.8 Hz, 1H), 1.33 (t, J = 7.2 Hz, 6H), 0.94 (s, 3H). ^{13}C NMR (CD_3OD, 400 MHz): δ 158.97, 152.61, 149.80, 147.58, 141.32, 89.56, 81.47, 77.54, 70.59, 62.46, 58.15, 17.45, 13.91. LC/MS (m/z), calcd for $C_{15}H_{25}N_5O_7P$ (M^++H), 418.14; found, 418.33.
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19. *Spectral data for compound* ***21***: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.79 (d, J = 1.2 Hz, 1H), 8.59 (s, 1H), 7.87 (d, J = 1.2 Hz, 1H), 6.16 (s, 1H), 4.18 (d, J = 9.2 Hz, 1H), 4.00 (m, 2H), 3.86 (dd, J = 3.2 Hz, J = 12.8 Hz, 1H), 0.96 (s, 3H). ^{13}C NMR (CD_3OD, 400 MHz): δ 156.96, 149.80, 149.56, 140.32, 133.57, 123.63, 118.39, 91.87, 83.21, 79.03, 72.28, 59.85, 19.07. LC/MS (m/z), calcd for $C_{13}H_{17}N_8O_4$ (M^++H), 349.13; found, 349.16.
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