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Tetrahedron Letters 45 (2004) 2657–2661
Tetrahedron Letters

Synthesis of C4-linked imidazole ribonucleoside phosphoramidite with pivaloyloxymethyl (POM) group

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Received 25 November 2003; revised 13 January 2004; accepted 23 January 2004

Abstract—A novel C4-linked imidazole ribonucleoside phosphoramidite was designed and successfully synthesized starting from tribenzylribofuranosylimidazole. This phosphoramidite product enables incorporation of the imidazole moiety into an RNA sequence and hence allows study of its role in the general acid and base catalysis of ribozymes. Pivaloyloxymethyl (POM) was first introduced as an N-protecting group for the imidazole ribonucleoside that can be readily removed under mild basic condition.

The study of ribozyme is important because it allows insight into how a simple RNA molecule can catalyze a reaction with a low repertoire of chemical groups compared to protein. The VS ribozyme,[1] encoded in the RNA transcribed from the Varkud satellite DNA of *Neurospora* mitochondria, catalyzes the site-specific cleavage of a phosphodiester linkage to generate products containing 2′,3′-cyclic phosphate and 5′-OH termini. Recently, in an extensive mutagenesis study, we have identified a critical nucleotide (A756) that is very likely a key residue in the active site of the VS ribozyme.[2] Further experiments by replacing adenosine with various analogues[3] indicate that the scissile phosphate of the substrate is in close proximity to A756 and, therefore, A756 may play a role in general acid and base catalysis. Unlike HDV ribozyme where cleavage activity of the uracil-substituted mutant of the antigenomic ribozyme αC76U can be restored by free imidazole in a buffer,[4] the secondary structure of the VS ribozyme may prevent free imidazole from entering the active site and thereby may terminate its function. Other potential places such as G8 and A39 of the Hairpin ribozyme, where mutagenesis and chemical substitution indicate they are critical for catalysis,[5] may also participate in general acid and base catalysis. Given that imidazole with a pK_a of 7.1 is both a good donor and acceptor of proton, the synthesis of an imidazole C-nucleoside will allow incorporation at the desired position to probe general acid and base catalysis. We herein describe the synthesis of novel C4-linked imidazole ribonucleoside phosphoramidite **1**, which enables the insertion of an imidazole base into RNA sequence (Fig. 1). In this

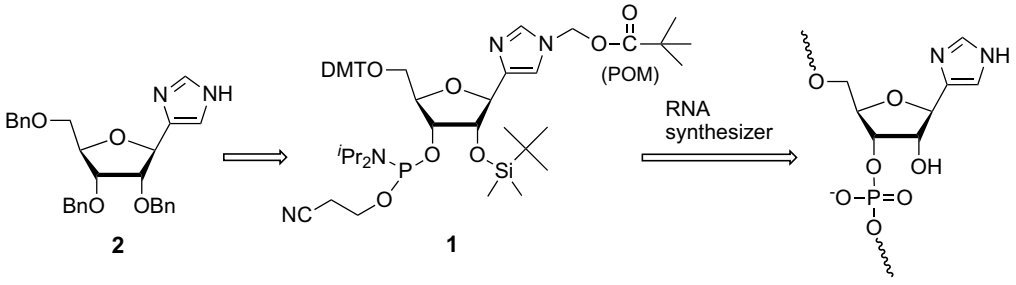

Figure 1.

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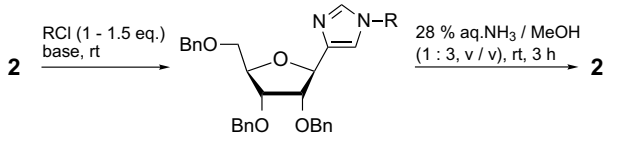
0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.01.132

synthetic study, pivaloyloxymethyl (POM) was used as a unique N-protecting group for the imidazole ribonucleoside.

We previously reported an efficient and stereoselective synthesis of 4(5)-(β-D-ribofuranosyl)imidazole (**3a**), its N-ethoxy carbonylated compound (**3b**), and 4(5)-2,3,5-tri-*O*-benzyl-β-D-ribofuranosylimidazole (**2**), which was provided from 2,3,5-tri-*O*-benzyl-D-ribose in three steps in 87% overall yield (Scheme 3).[6] We first aimed at a synthesis of the ribonucleoside phosphoramidite from the unsubstituted imidazole **3a** or **3b** protected imidazole-N by an ethoxycarbonyl group, which could be easily removed by aqueous ammonia (Table 1, entry 1). However, the dimethoxytritylation of **3a** or **3b** failed in spite of many trials due to instability of 5′-*O*-dimethoxytrityl (DMT) products (Scheme 1). We next sought a more suitable protecting group of imidazole-N, which might not only contribute to the stability of the synthetic intermediates, but also should be compatible with RNA deprotection condition at the end of the synthesis by the phosphoramidite approach.[7] It is further required to be tolerated under the debenzylation-condition of N-protected C-nucleosides **4**.

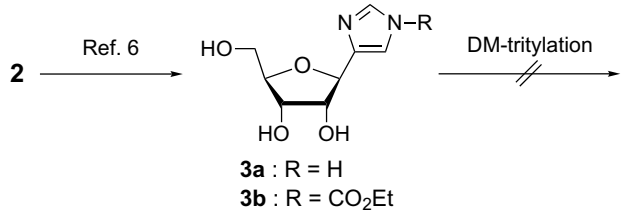
Table 1



4b - e

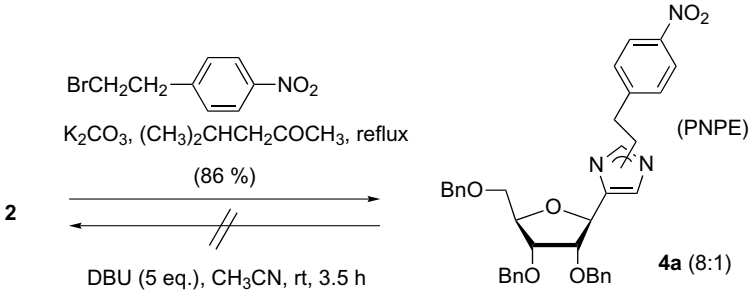
Entry	Reaction condition (equiv, h)	**4** (%)	**2** (%)
1	EtOCOCl (1.0), pyridine, cat DMAP, 1.5	**4b**,[a] 96	96
2	Cl_3CCH_2OCOCl (1.1), DMAP, benzene, 4	**4c**,[b] 89	89
3	*p*-$CH_3OC_6H_4COCl$ (1.0), pyridine, 1.5	**4d**,[b] 74	73
4	$(CH_3)_3CCOOCH_2Cl$ (1.5), NaH, THF, 5	**4e**,[c] 91	92

[a] Ref. 6.
[b] Ref. 13.
[c] See text.



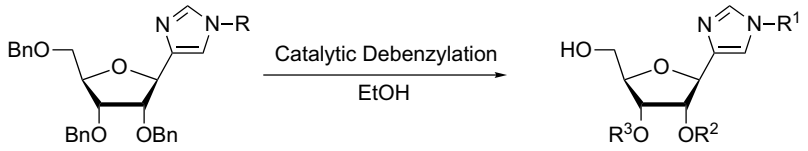
Scheme 1.

On the other hand, 2,2,2-trichloroethoxycarbonyl (Troc),[10] *p*-anisoyl (An),[11] and the POM groups[12] were easily introduced into the imidazole of **2** via the corresponding chlorides to give **4c–e** (Table 1) as single isomers.[9] The location of the protecting groups was tentatively assigned to be nitrogen because N-acylation of the imidazole ring occurs regioselectively on the less-hindered τ-nitrogen.[13] They could be removed by aqueous ammonia–MeOH (1:3, v/v) at rt after 3 h in high yields.[14] We next evaluated **4c–e** under catalytic debenzylation as shown in Table 2. Hydrogenolysis of **4c** or **4d** over Pd–C cleaved Troc or An groups as well as benzyl groups to give a nonprotected imidazole C-nucleoside **3a** (R_1, R_2, R_3 = H, quant) (Table 2, entries 1 and 2). Interestingly, **4e** maintained the POM group under the reduction conditions to give partial debenzylated products **5** and **6** (Table 2, entry 3). Further, treatment of **4e** with $Pd(OH)_2$–C/cyclohexene in refluxing ethanol produced N-POM-imidazole C-nucleoside **7**[15] in quantitative yield. The POM group of **7** was determined to be at the τ nitrogen by a convenient NMR method using ^1H–^{15}N HMBC, which was very recently reported by Zaramella et al.[16]: a ^1H (C1′)/^{15}N (π) cross-peak could clearly be seen in ^1H–^{15}N HMBC signals [δ



Scheme 2.

Table 2



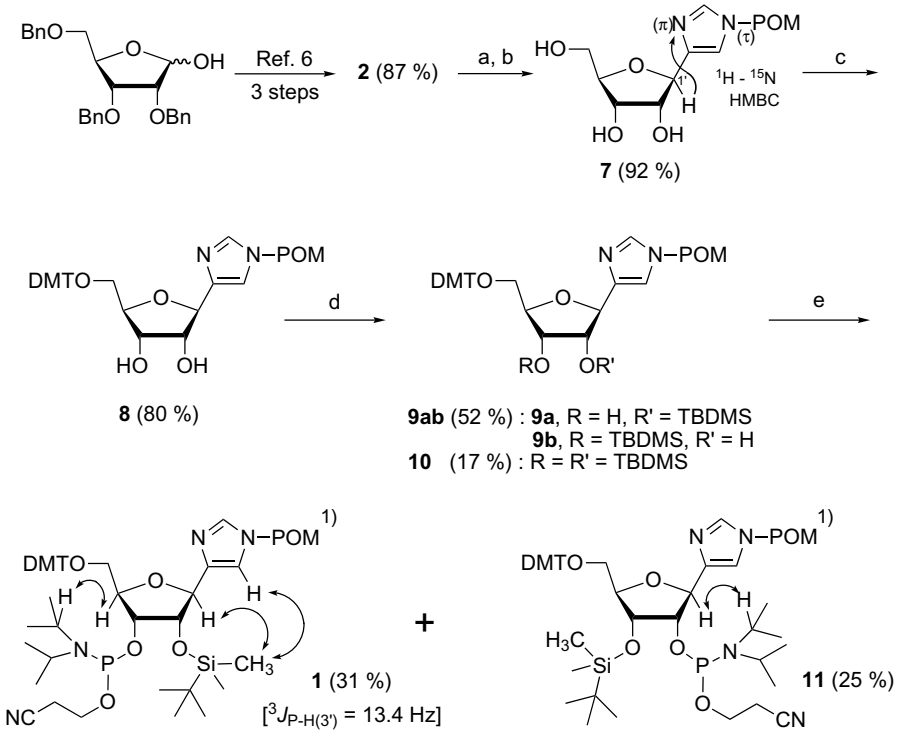
4b - e **3a, 5 - 7**

Entry	Conditions	Product	Yield (%)
1	**4c** (Troc), H_2/5% Pd–C (1 kg/cm^2), 6 h	**3a** (R^1, R^2, R^3 = H)	Quant
2	**4d** (An), H_2/5% Pd–C (3 kg/cm^2), 16 h	**3a**	Quant
3	**4e** (POM), H_2/10% Pd–C (3 kg/cm^2), 16 h	**5** (R^1 = POM, R^2, R^3 = Bn) **6** (R^1 = POM, R^2 = H, R^3 = Bn) or (R^1 = POM, R^2 = Bn, R^3 = H)	22 32[a]
4	**4e** (POM), 20% Pd $(OH)_2$–C, cyclohexene, reflux, 4 h	**7** (R^1 = POM, R^2, R^3 = H)	Quant

[a] Yield as a mixture of two isomers.

(ppm) ^{15}N (τ) 176.1, ^{15}N (π) 247.1, and ^{1}H (C1′) 4.66] (Scheme 3).[17]

The utility of the POM group may be further pointed out in RNA synthesis: (1) In the capping step, unreacted 5′-hydroxy group is acetylated with acetic anhydride to prevent the growing oligonucleotide chain with a base deletion,[7] whereas activated N^{im}-carbonyl groups (as in **4b**–**d**) may be susceptible to potential exchange-reactions at this stage, leading to complexities. (2) Base-protecting groups are conventionally removed in the final step of RNA synthesis by ammonia and ethanol mixture [28% aq NH_3–EtOH (3:1, v/v)] at 60 °C for 16 h. As the POM group can be removed under faster and milder



Scheme 3. Reagents and conditions: (a) Table 1, run 4; (b) Table 2, run 4; (c) DMTCl (1.5 equiv), Et_3N (1.5 equiv), DMAP (0.025 equiv), py., rt, overnight; (d) TBDMSOTf (1.1 equiv), py., MS4A, −40 °C, 5 min; (e) 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphodiamidite (2.5 equiv × 2), DIPT (1.2 equiv × 2), $ClCH_2CH_2Cl$, 40 °C, 62 h; 1) Arrows indicate interactions between sets of two protons in NOESY experiments.

conditions, it is particularly attractive to sensitive RNA such as Cy5 labeled RNA where deprotection is recommended at room temperature to minimize the destruction of cyanine dye. (3) In addition, the POM group has not been employed as a protecting group for imidazoles to date.[12] Hence, the N-POM group is the most suitable and practical protecting group for the imidazole ribonucleosides.

DM-tritylation of **7** thus obtained successfully afforded 5′-*O*-DMT-derivative **8** in 80% yield. Therefore, synthesis of 5′-*O*-DMT-2′-*O*-*tert*-butyldimethylsilyl (TBDMS)-3′-*O*-cyanoethyldiisopropylphosphoramidite **1** was carried out via the N-POM-imidazole **7** (Scheme 3).

Selective protection of the 2′-hydroxy group of **8** using TBDMSCl did not proceed in the presence of $AgNO_3$ and pyridine in THF,[18] but treatment of **8** with TBDMSOTf in pyridine led to an inseparable 1:1 mixture **9ab** (52%) of 2′-*O*-TBDMS and 3′-*O*-TBDMS isomers **9a** and **9b**, together with a 2′,3′-bis-*O*-substituted derivative **10** (17%). As compound **9a** could not be isolated owing to easy isomerizaton between **9a** and **9b**,[19] the mixture **9ab** was subjected to phosphitylation. Treatment of **9ab** with 2-cyanoethyl tetraisopropylphosphodiamidite in the presence of diisopropylammonium tetrazolide (DIPT) in dichloromethane at rt yielded 3′- and 2′-phosphoramidites **1** and **11**, but the yields were variable and less than satisfactory. The problem was resolved by use of 1,2-dichloroethane as the solvent at 40 °C to provide **1**[20] (31%) and unstable **11** (25%). The structure of **1** was assigned by NOESY experiments of the respective phosphoramidites **1** and **11**, as illustrated in Scheme 3. It was further confirmed by observation of a ^{31}P–^{1}H (C3′) coupling constant [$^{3}J_{P\text{–}H\ (C3')} = 13.4$ Hz] with the aid of ^{31}P-decoupled ^{1}H NMR spectrum of **1** [^{31}P NMR: δ 148.9 ppm (CD_3CN) as a singlet-like peak for two P-diastereoisomers].

Further work on application of **1** is under way and will be published in due course.

Acknowledgements

We are grateful to Dr. Y. Hari, Graduate School of Pharmaceutical Sciences, Nagoya City University, for helpful discussions. Financial support by a Grant-in-Aid for High Technology Research from the Ministry of Education, Science, Sports, and Culture, Japan is acknowledged.

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14. A solution of **4e** (20 mg, 0.035 mmol) in MeOH (1.5 mL) and 28% NH_4OH (0.5 mL) was stirred for 3 h at rt to give **2** (15 mg, 92%) as a colorless oil.
15. **7**: colorless oil; IR (neat, cm^{-1}) 1730 (COO); ^{1}H NMR (200 MHz, CD_3OD) δ 1.18 (s, 9H), 3.52–4.19 (m, 5H), 4.66 (d, $J = 5.4$ Hz, 1H), 6.02 (s, 2H), 7.45 (s, 1H), 8.36 (s, 1H); HRMS (SIMS) m/z $(M+1)^+$ calcd for $[C_{14}H_{21}O_6N_2+H]^+$ 315.1555, found, 315.1552.
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17. Conditions of ^{1}H–^{15}N HMBC spectrum of **7**: CD_3OD, 25 °C, 500 MHz for ^{1}H and 51 MHz for ^{15}N; δ (ppm) relative to external DMF (103.2 ppm).
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20. **1**: white powder; IR (neat, cm^{-1}) 1730 (COO); ^{1}H NMR (500 MHz, CD_3 CN) δ − 0.09 (s, 3H), −0.16 (s, 3H), 0.81 (s, 9H), 0.96 (d, $J = 5.14$ Hz, 6H), 1.11 (s, 9H), 1.12 (d, $J = 5.1$ Hz, 6H), 2.60–2.66 (m, 2H), 3.00 (dd, $J = 9.0$, 4.5 Hz, 1H), 3.28 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.47–3.60 (m, 2H), 3.75 (s, 6H), 3.72–3.92 (m, 2H), 4.06–4.10 (m, 1H), 4.21 (ddd, $J = 13.4$, 4.5, 2.3 Hz, 1H) (3′-H), 4.52 (dd, $J = 5.8$, 3.9 Hz, 1H), 4.68 (d, $J = 5.8$ Hz, 1H), 5.78 (s, 2H), 6.80–6.87 (m, 4H), 7.14–7.30 (m, 5H), 7.33–

7.41 (m, 3H), 7.47–7.54 (m, 2H), 7.62 (d, $J = 1.6$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ −4.8, −4.7, 18.2, 20.4, 24.5, 24.6, 24.65, 24.7, 25.9, 26.9, 27.0, 29.7, 38.7, 42.8, 42.9, 55.2, 58.6, 58.7, 64.0, 67.7, 73.9, 74.0, 76.5, 78.5, 82.7, 86.2, 113.0, 113.1, 117.7, 118.4, 126.6, 127.7, 128.4, 130.25, 130.3, 136.1, 136.2, 138.0, 141.7, 145.0, 158.4, 177.6; ^{31}P NMR (202 MHz, CD_3CN) δ 148.9; MALDI-TOF (THAP): $[M+Na]^+$ calcd 953.46, found 953.52.