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A new synthetic route to oligoribonucleotides based on CpRu-catalyzed deallylation

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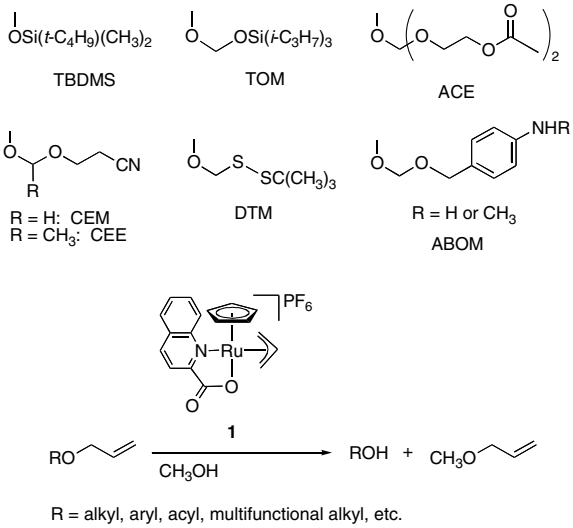
Abstract—A triribonucleotide 3–5 U, which is fully protected by an allyl group at the 2′-hydroxy and phosphoric acid positions was synthesized, the deprotection being quantitatively achieved by use of a catalytic amount of CpRu(η^3-C_3H_5)(2-quinolinecarboxylato) in methanol. The reaction is completed within 30 min at ambient temperature. The utility of the simple allyl protecting group has the potential to open a new pathway in the synthesis of RNA-related compounds.

Supplying oligoribonucleotides via chemical synthesis is of crucial importance to chemical biology.[1] Particularly, recent attention has been paid to siRNA in the development of therapeutic agents for various biomedical applications since Fire's discovery of RNA interference in silencing gene expression.[2] The synthesis, however, is more difficult than that of DNA-related molecules because of the existence of the 2′-hydroxy group, requiring two permanent protecting groups (PPG) together with one temporary PG (TPG) in the chain elongation process: one PPG is for the 2′-OH and the other PPG is for phosphoric acid. As the DMTr group is generally used as the TPG at the 5′-OH position, the key determinant of success or failure is dependent upon the appropriate selection of the PPGs, particularly for 2′-OH protection. The *tert*-butyldimethylsilyl (TBDMS)[3a] group is frequently used, but the high degree of steric demand reduces efficiency in the 5′→3′ elongation step.[3b] 2′–3′ Silyl transfer is also a problem. The development of TOM, ACE, CEM, CEE, DTM, and ABOM[3b–g] (below) has overcome the drawbacks, allowing realization of a practical RNA chemical synthesis.[1] Among such excellent 2′-O-PPGs, the allyl group is of note, as it is simpler than an acetyl group and is stable toward both acidic and basic conditions. The simplicity is attractive, but the high stability causes a critical problem in deprotection. Recently, we have reported that [CpRu(η^3-C_3H_7)(2-C_9H_6COO)]PF_6 (**1**)[4] serves as a catalyst for allyl ether cleavage in alcoholic solvents (typically methanol), without the need for the additional additives, such as metal hydride, amine, or enolate, required for conventional Pd or Ni-based methods.[5] The coproduct is only an easily removable volatile ether. In this letter, the applicability of the highly reactive and chemoselective allyl cleaving catalyst in the synthesis of an RNA-related molecule is demonstrated.



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One of the simplest oligoribonucleotides, 3–5 U (**2** ($n = 1$)), was chosen as the target molecule.[6] The objective was one step deprotection to **2** ($n = 1$) from **3** ($n = 1$), in which the 2′-OH, terminal 3′-OH, and POH are fully protected as PPG by an allyl group. Compound **3** ($n = 1$) was synthesized in 70% total yield from the 2′-*O*-allyl/3′-*O*-P(N(*i*-C_3H_7)$_2$)(OCH$_2$CH=CH$_2$)/5′-*O*-DMTr protected phosphoramidite monomer **4** and the 2′-*O*-allyl/3′-*O*-allyl protected terminal ribonucleoside **5** according to the well established synthetic cycle shown in Figure 1,[7] though $n = 1$ in the present case. Terminal ribonucleoside **5** was coupled with phosphoroamidite **4** in acetonitrile by use of *N*-phenylimidazolium triflate and molecular sieves 3A, followed by treatment with *tert*-butylhydroperoxide, giving the DMTr-protected diribonucleoside ($n = 0$). Deprotection of DMTr TPG with Cl$_2$CHCOOH proceeded quantitatively to give **3** ($n = 0$) in 94% isolated yield in three steps. A second condensation of dimer **3** ($n = 0$) with monomer **4** followed by oxidation completed the synthetic cycle. Removal of the DMTr group afforded **3** ($n = 1$) in 74% isolated yield. The condensation of **4** with **3** proceeds smoothly, completing within 30 min at ambient temperature.

Figure 2 illustrates the synthetic procedure for monomer **4** and the terminal product **5**. The known 2′-*O*-allyluridine[8] was converted to **4** in 82% yield via selective DMTr protection of 5′-OH followed by phosphoramidation of 3′-OH using *O*-allyl-*N,N,N′,N′*-tetraisopropyl phosphorodiamidite. Next, the two hydroxy groups at C(2′) and C(3′) of the 5′-*O* trytylated 4-*O*-(2-nitrophenyl)uridine[9] were allylated by use of allyl ethyl carbonate in the presence of a Pd(0) complex and DPPB (substrate/catalyst = 32, THF, 85 °C, 3 h).[10] Acidic DMTr deprotection of 5′-OH followed by removal of the *o*-nitrophenyl group by aromatic nucleophilic substitution using tetramethylguanidine[11] afforded **5** in 63% three-step yield.

The 2′-*O*-, terminal 3′-*O*-, and *P*-*O*-allylated compound **3** ($n = 1$) was then subjected to [CpRu(η^3-C_3H_5)(C_9H_6-NCOO)]PF$_6$-catalyzed deallylation conditions ([**1**] = 0.5 mM, [**3** ($n = 1$)] = 1 mM, CD$_3$OD, 25 °C). The reaction was completed within 30 min to give 3–5 U (**2** ($n = 1$)) in quantitative yield as shown in Figure 3. The ^{31}P

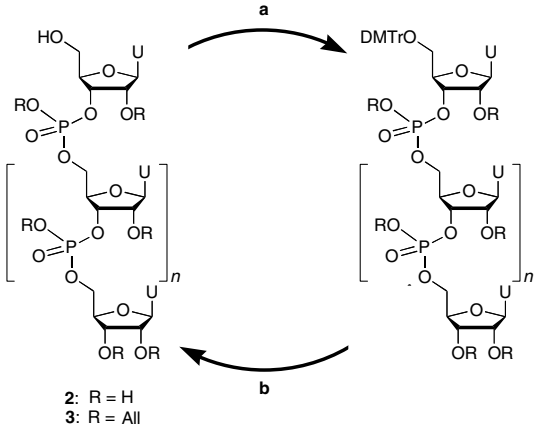

Figure 1. Synthetic cycle for allyl-based RNA synthesis and the simplest target 3–5 U (a: coupling with **4**, b: removal of DMTr).

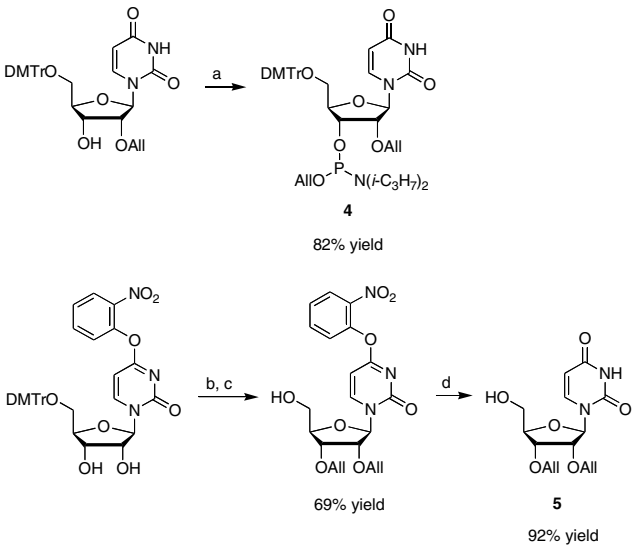

Figure 2. Synthesis of monomer **4** and terminal **5** (a: 4 mol amt *O*-allyl-*N,N,N′,N′*-tetraisopropylphosphoroamidite, 0.5 mol amt diisopropylammonium triazolide, MS 3A, CH$_3$CN, rt, 11 h. b: 5.1 mol amt allyl ethyl carbonate, 0.03 mol amt Pd$_2$(dba)$_3$CHCl$_3$, 0.13 mol amt DPPB, THF, 85 °C, 1 h. c: 5 mol amt Cl$_2$CHCOOH, CH$_2$Cl$_2$, rt, 4 h. d: 10 mol amt (*Z*)-*o*-nitrobenzoxime, 8.9 mol amt ((CH$_3$)$_2$N)$_2$C=NH, CH$_3$CN, rt, 11 h).

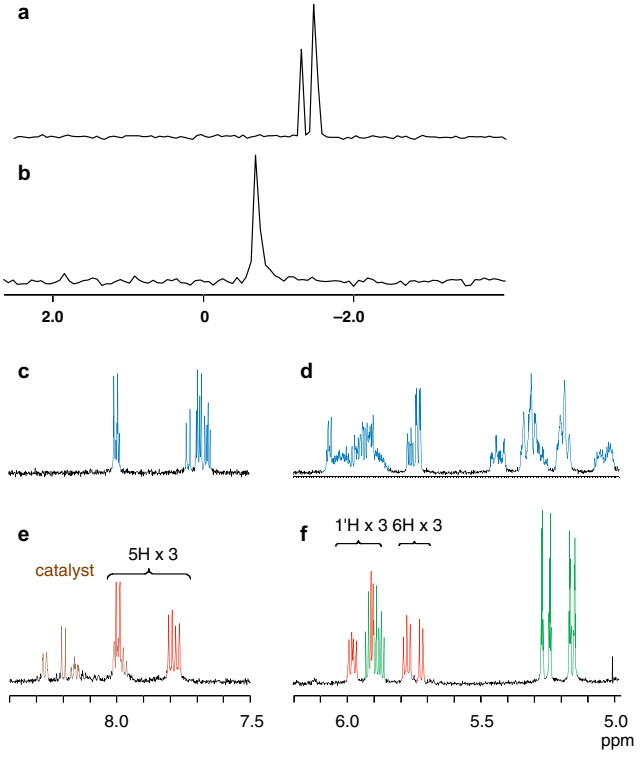

Figure 3. ^{31}P and ^{1}H NMR spectra of 3–5 U deallylation (CD$_3$OD, 25 °C. a: ^{31}P of **3** ($n = 1$), b: ^{31}P of reaction mixture, c and d: ^{1}H of **3** ($n = 1$), e and f: ^{1}H of reaction mixture).

signals of substrate **3** ($n = 1$) resonate at δ −1.41 and −1.54 as broad singlets (Fig. 3a), due to the two phosphorous stereogenic centers generating four possible diastereomers. Upon deallylation, the signals completely disappear, converging into a new single ^{31}P signal at δ −0.76. The ^{31}P NMR data indicates that the stereogenic P-containing phosphoric triester is transformed into a non-stereogenic P-containing phosphoric diester, and that the two phosphorous atoms are in similar magnetic environments. This behavior parallels that of the ^{1}H NMR. Each of the four diastereomers of **3** ($n = 1$) has six allyl groups, making the ^{1}H NMR spectrum complicated, as shown in blue in Figure 3c (uracil region) and 3d (allyl region). The deallylation process leads to a simple spectrum (3e and f). The red-colored signal corresponds to 3–5 U, while the green one is the coproduct, $CH_2{=}CHCH_2OCD_3$. All of allyl signals of **3** ($n = 1$) appearing at δ 5.0–5.5 and δ 5.9 (blue) are lost to give the red and green signals. The signal intensity ratio of all the protons of 3–5 U to the five allyl protons is ca. 1:6, consistent with the ratio expected for full removal of the allyl PPG. The TOF MS shows two peaks at 427.07 and 877.15, which can be assigned to the diphosphorate dianion and its monosodium salt ($M^{2-}/2$: calcd 427.05. MNa^{-}: calcd 877.09. (ESI, negative)). Reverse-phase HPLC analysis also indicates the formation of a single compound, ($t_R = 2.9$ min (ODS-UG 0.45×25 cm, 4:1 CH_3OH–H_2O, 1.0 mL/min)). This experimental data clearly indicates the quantitative deallylation of **3** ($n = 1$) to 3–5 U. The deallylation product was isolated as the diethylammonium salt.[12]

Success in the chemical synthesis of RNA-related molecules depends strongly on the appropriate selection of PPG that causes neither 2′,3′ shift nor deceleration of 3′,5′ chain elongation. The simple and readily available allyl group is attractive, given the availability of a deallylation method operating under very mild conditions. No such ideal catalytic system retards utilization of the allyl group. Although the ratio of **1** to allyl PPG is only 12, even with a simple triribonucleotide at the present stage,[13] the success of the new Ru catalyst opens a new pathway to the chemical synthesis of RNA-related compounds. The search for a more efficient catalytic system is ongoing.

Acknowledgments

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12. 3–5 U diethylammonium salt: ^{31}P NMR δ −0.27, −0.16. ^{1}H NMR δ 1.29 (t, $J = 7.56$ Hz, 12H), 3.09 (q, $J = 7.56$ Hz, 8H), 3.84 (dd, $J = 3.44$ and 12.7 Hz, 1H), 3.93 (dd, $J = 2.75$ and 12.4 Hz, 1H), 4.12–4.17 (m, 2H), 4.24–4.30 (m, 3H), 4.31–4.36 (m, 3H), 4.43–4.49 (m, 3H), 4.55–4.60 (m, 1H), 4.64–4.68 (m, 1H), 5.86–6.00 (m, 6H), 7.90–7.93 (m, 3H). MS ($M^{2-}/2$) calcd: 427.05, obsd: 427.11. HPLC $t_R = 3.07$ min (ODS-10 0.45×25 cm, 3:1 CH_3OH–H_2O, 0.5 mL/min). UV/vis (CH_3OH): λ_{max} (ε) = 260 nm (1460).
13. At an allyl/**1** ratio of 120, the reaction time should be prolonged to 10 h, during which time some side reactions occur. This catalytic system can operate only under neutral or acidic conditions. Basic adenosine, guanosine, and cytidine cannot be utilized at the present stage. Reaction conditions cleaving allyl ethers in the presence of these bases are now under investigation.