Regioselective Allylations of Pyrimidine Ribonucleosides Using Pd(0) Catalyst†

Vaijayanti KUMAR, Vidhya GOPALAKRISHNAN, and K. Nagappa GANESH*
Bio-organic Chemistry Unit, Division of Organic Chemistry,
National Chemical Laboratory, Pune 411008, India
(Received October 17, 1991)

A novel procedure for regiospecific *O*-allylation of pyrimidine ribonucleosides is reported by using $Pd(PPh_3)_4$–allyl ethyl carbonate reagent to synthesize 2′-*O*-allyluridine and 2′-*O*-allylcytidine. 3-*N*-allylation of the pyrimidine ring is prevented by protection of uridine imide function by 4-*O*-(2,5-dimethylphenyl) group which can be transformed to 4-oxo function of uridine or exocyclic amino function of cytidine.

2′-*O*-Alkyl oligoribonucleotides have recently emerged as important antisense probes because of their hybridizing capability, nuclease resistance, and better membrane penetration.[1] Among the various alkyl substituents, 2′-*O*-allyl substitution has been found to be superior to 2′-*O*-methyl and 2′-*O*-(1-methyl-2-butenyl)analogues.[2] Consequently, efficient approaches to synthesis of 2′-*O*-allyl ribonucleosides assumes importance. A major drawback of most alkylations of ribonucleosides is the undesirable but preferential ring *N*-alkylations.[3] *N*-Alkylation of purines were avoided by reaction of appropriate 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-6-(2,6-dichlorophenoxy)purine nucleosides with an alkyl bromide and a hindered base, to obtain 2′-*O*-alkyl derivatives of adenosine and guanosine.[4] Unfortunately, this reaction cannot be satisfactorily extended to pyrimidine nucleosides since it is known to result in 3-*N*-allylation product.[5] Allylation reactions using $Pd(PPh_3)_4$, although common,[6] have not yet found routine application in nucleoside chemistry. Recently, it has been reported[7] that Pd(0) catalysis gives better yields of 2′-*O*-allyl purine nucleosides and the strategy can be extended for the preparation of pyrimidine nucleosides as well, but it neither gives any experimental details nor the product characterizations. In this note, we report the regioselective formation of 2′-*O*-allyl pyrimidine ribonucleosides using Pd(0) catalyst and the spectroscopic analysis of the *N*-allyl, *N*,2′-*O*-diallyl, *N*,3′-*O*-diallyl and exclusive 2′-*O*-allyl pyrimidine ribonucleosides.

The protected ribonucleosides 5′-*O*-4,4′-dimethoxytrityl (DMT) uridine (**1a**) and *N*-benzoyl-5′-*O*-DMT-cytidine (**2**) were treated with stoichiometric amounts of allyl ethyl carbonate (AEC) (1 equiv and 2.5 equiv) in anhydrous tetrahydrofuran (THF) in presence of catalytic amounts of insitu generated $Pd(PPh_3)_4$. The products were isolated and purified by silica-gel column chromatography and the product analysis is shown in Table 1. From the results it is seen that (i) molar equivalents of allylating reagent leads exclusively to ring 3-*N*-allylation (Entry 1,3), (ii) with 2.5 equivalents of the reagent, 2′/3′-*O*-allylation is observed in addition to *N*-allylation (Entry 2,4), (iii) regioselectivity in favor of 2′-*O*-allyl isomer is seen in all cases though the degree of selectivity varies with the substrate, and (iv) *N*-allylation can not be avoided (except Entry 5) under any of these conditions.

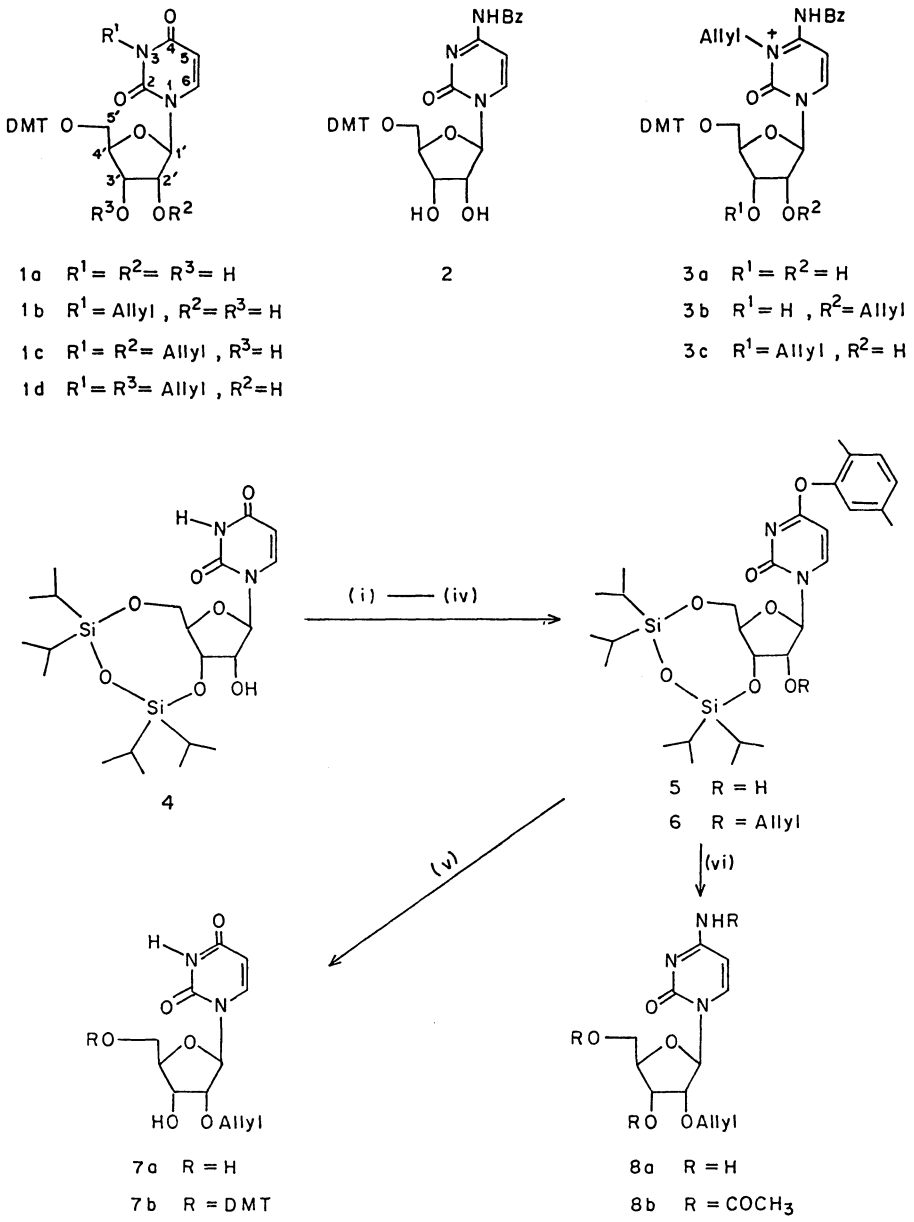
For this reaction to be useful, two objectives have to be met: (i) Avoidance of ring 3-*N*-allylation and (ii) exclusive formation of 2′-*O*-allyl isomer. These were realized by a synthetic route in which the 4-*O*-substituted uridine **5** was allylated to obtain 2′-*O*-monoallyl product **6** (Scheme 1). 2,5-Dimethylphenyl group was chosen as 4-*O*-protector as this is known[8] to give uridine and cytidine in quantitative yields on treatment with either oximate or aqueous ammonia respectively.

The 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (**4**)[9] was 2′-*O*-acylated and converted to the 4-(2,5-dimethylphenoxy)derivative by sulfonylation/displacement.[8] 2′-*O*-Acetyl group was then hydrolyzed by treatment with saturated ammonia[10] for 30 min at 30 °C to obtain **5** which was subsequently allylated using 1.5 equiv of AEC/Pd(0) to give exclusive 2′-*O*-monoallyl derivative **6** in 80—85% yield. No 3-*N*-allylation was observed. The compound **6** was then converted to the 2′-*O*-allyluridine (**7a**), by treatment with 4-nitrobenzaldoximate (0.1 M, 1 M=1 mol dm^{-3}) followed by tetrabutylammonium fluoride (TBAF) in THF (1 M). Alternatively, **6** was transformed to 2′-*O*-allylcytidine (**8a**) by reaction with aqueous ammonia at 50 °C overnight, followed by TBAF/THF (1 M) treatment. The products **7a** and **8a** were then characterized by (i) derivatization to **7b** and **8b** respectively and (ii) ^{1}H and ^{13}C NMR.

Table 1. Product Ratios in Allylation Reactions of Ribonucleosides

Entry	Substrate	AEC	Product ratios	2′:3′
1	**1a**	1.0	**1b**(90%)	—
2	**1a**	2.5	**1b**(10%), **1c**(60%), **1d**(30%)	2:1
3	**2**	1.0	**3a**(80%)	—
4	**2**	2.5	**3a**(10%), **3b**(60%), **3c**(15%)	4:1
5	**5**	1.5	**6** (80%)	—

† NCL Communication No. 4939.



Scheme 1. Reagents: (i) Ac_2O/Pyridine. (ii) MsCl (2.5 equiv), TEA (3 equiv), DMAP (0.2 equiv), in DCM; 2,5-dimethylphenol (7 equiv), TEA (10 equiv), DABCO (0.5 equiv). (iii) sat. MeOH–NH_3. (iv) $Pd(dba)_2$ (5 mol%), PPh_3 (40 mol%), AEC (1.5 equiv) in THF (1 ml). (v) 4-nitrobenzaldoximate ions (0.1 M) in dioxane: water (5 : 1), TBAF in THF (1 M). (vi) aq NH_3, 50 °C, 17 h; TBAF in THF (1 M).

Unambiguous proof for 2′-*O*, 3′-*O*- or 3-*N*-allylation came from ^{13}C NMR data (Table 2). Allylation of ring nitrogens in **1b**—**d** and **3a**—**c** was confirmed by the presence of N–CH_2 signal around 42—45 ppm. The assignments of 2′/3′-*O*-allyl isomers **1c**/**1d** and **3b**/**3c** were based on ^{13}C chemical shifts of 2′-C and 3′-C. It is known[11] that in free nucleosides 3′-C is downfield shifted compared to 2′-C by about 5 ppm. 2′-*O*-Alkylation leads to shifts of 10—12 ppm downfield for 2′-C accompanied by 4—6 ppm upfield shifts for 3′-C. The observed ^{13}C NMR data largely complied with these facts for **1c**, **3b**, **7b**, **8b**. Further we noticed that in case of 3′-*O*-allylation (**1d** and **3c**), the shifts are 5 ppm downfield for both 3′-C and 2′-C. The individual assignments were substantiated by a distortionless enhancement by polarization transfer (DEPT) experiment. *O,N*-Diallyl compounds (**1c,d** and **3b,c**) showed signals for O–CH_2 around 70—72 ppm and N–CH_2 around 45 ppm in addition to that of sugar 5′-OCH_2. The absence of *N*-allylation in **6**, **7b**, and **8b** was confirmed by nonobservence of ^{13}C signal in 42—45 ppm region.

This note reports the synthesis of 2′-*O*-allyl pyrimidine nucleosides (both uridine and cytidine) by a route

Table 2. Selected ^{13}C Chemical Shifts[a]

Compound	NCH_2	OCH_2	2-C′	3′-C
1a	—	—	69.70	74.00
1b	44.0	—	70.18	74.75
1c	42.59	71.25	81.24	68.10
1d	42.89	71.56	73.90	76.35
2	—	—	69.30	74.30
3a	44.75	—	69.47	74.55
3b	44.90	71.37	80.64	68.13
3c	44.85	70.99	74.41	79.04
6	—	71.05	80.60	67.60
7b	—	71.30	81.20	68.60
8b	—	71.30	78.70	69.03

a) All recorded on Bruker MSL 300 at 80 MHz for ^{13}C. Solvent: $CDCl_3$–10% Pyridine-d_5.

which avoids the undesirable *N*-allylation. It is shown that the ring *N*-allylation can be efficiently prevented by decreasing electron density in the ring through 4-O derivatization rather than a steric block strategy. Prevention of 3-N allylation was also attempted by protection of imide function with 2-nitrophenylsulphenyl (NPS) group in **1a** and exocyclic amino function in **2** with bulky protecting groups such as pivaloyl, DMT and 9-fluorenylmethoxycarbonyl (Fmoc) followed by allylation. NPS and Fmoc groups were unstable under the reaction conditions (liberation of EtO^-) whereas, even the steric bulk of pivaloyl and DMT protecting groups could not prevent *N*-allylation. We also observed that allylations of free *cis*-2,3-diol system using Pd(0) shows a substantial preference for formation of 2′-*O*-allyl isomer which may be due to the higher nucleophilicity of 2′-OH.[13] The reported strategy for regiospecific synthesis of 2′-*O*-isomer will be valuable for the synthesis of 2′-*O*-allyl oligoribonucleotides and also to explore the potential of allyl group as a 2′-*O*-protector in RNA synthesis.

Experimental

General Procedure: A mixture of a nucleoside (0.5 mmol), palladium dibenzylidineacetone (0.013 mmol), and triphenylphosphine (0.1 mmol) in anhydrous THF was treated with stoichiometric amounts of AEC under nitrogen atmosphere at 60—65 °C for about 2 h. The reaction mixture was concentrated and products purified by column chromatography on silica gel. All the products were characterized by a combination of UV,[12] ^1H and ^{13}C NMR spectroscopy and gave satisfactory elemental analysis. The 3-*N*-allylcytidine derivatives **3a**—**c** exhibited a UV absorption band at 280 nm characteristic of ring N-protonated nucleoside.[12] The presence of *cis*-diol system in **1b** and **3a** was indicated by a positive benzidine spray test.

6, (Yield=80%), R_f=0.85 (10% MeOH-CH_2Cl_2), ^1H NMR δ=1.0 (28H, brs, overlap with a multiplet, 4×$CH(CH_3)_2$), 2.1 (3H, s, $ArCH_3$), 2.32 (3H, s, $ArCH_3$), 3.91 (2H, brs, 5′-H), 4.01 (1H, m, 4′-H), 4.22 (2H, brs, OCH_2), 4.42 (2H, m, 2′-H and 3′-H), 5.26 (2H, dd, $=CH_2$), 5.78 (1H, d, J=8 Hz, 5-H), 5.8 (1H, brs, 1′-H), 6.0 (1H, m, =CH), 7.0 (3H, m, ArH), 8.0 (1H, d, J=8 Hz, 6-H). ^{13}C NMR δ=59.5 (5′-C), 67.6 (3′-C), 71.05 (OCH_2), 80.6 (2′-C), 81.7 (4′-C), 89.9 (1′-C), 93.9 (5-C), 117.0 ($=CH_2$), 134.5 (=CH), 143.7 (6-C), 150.1 (2-C).

7b, R_f=0.6 (10% MeOH-CH_2Cl_2), ^1H NMR δ=3.54 (2H, dd, J=3 and 12 Hz, 5′-H), 3.79 (6H, s, 2×OCH_3), 4.05 (2H, m, OCH_2), 4.26 (1H, m, 4′-H), 4.44 (2H, m, overlap of 2′-H and 3′-H), 5.25 (1H, d, J=8.5 Hz, 5-H), 5.31 (2H, dd, $=CH_2$), 5.8 (1H, m, =CH), 5.96 (1H, J=1.8 Hz, 1′-H), 8.03 (1H, d, J=8.5 Hz, 6-H). ^{13}C NMR δ=55.15 (OCH_3), 61.4 (5′-C), 68.5 (3′-C), 71.3 (OCH_2), (OCH_2), 81.2 (2′-C), 83.1 (4′-C), 87.5 (1′-C), 102.0 (5-C), 118.4 ($=CH_2$), 133.2 (=CH).

8b, R_f=0.7 (10% MeOH-CH_2Cl_2), ^1H NMR δ=2.1—2.2 (9H, 3s, $COCH_3$), 4.21 (2H, m, OCH_2), 4.32 (1H, d, J=5 Hz, 5′-H), 4.43 (2H, m, 4′-H, 5′-H), 4.55 (1H, m, 2′-H), 4.81 (1H, m, 3′-H), 5.24 (2H, m, $=CH_2$), 5.83 (1H, m, =CH), 5.92 (1H, s, 1′-H), 7.5 (1H, d, J=8 Hz, 5-H), 8.15 (1H, d, J=8 Hz, 6-H). ^{13}C NMR δ=20.1, 20.4 ($OCOCH_3$), 24.3 ($NCOCH_3$), 61.5 (5′-C), 69.03 (3′-C), 71.3 (OCH_2), 78.7 (4′-C), 79.1 (2′-C), 90.4 (1′-C), 95.9 (5-C), 117.2 ($=CH_2$), 133.5 (=CH), 144.1 (6-C), 153.8 (2-C), 162.7 (4-C).

One of us (V.G.) thanks CSIR, India, for the award of a research fellowship.

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