Efficient Synthesis of New 5-Substituted Uracil Nucleosides Useful for Linker Arm Incorporation

Hiroaki Sawai,* Akiko Nakamura, Sumie Sekiguchi, Keisuke Yumoto, Masakazu Endoh and Hiroaki Ozaki

Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376, Japan

5-Substituted uracil nucleosides useful for the attachment of linker arms to nucleic acids are prepared from arabinoaminooxazoline and dimethyl α-bromomethylfumarate in a short reaction sequence without using any protecting groups, and incorporated into oligodeoxyribonucleotides.

Uridine and related pyrimidine nucleosides substituted with various functional groups at the C-5 position have found a wide variety of applications as antiviral agents[1] and as constituents of modified nucleic acids.[2] They are distributed in nature as modified nucleosides of transfer RNA.[3] As the C-5 position of pyrimidine is not involved in hydrogen bonding and faces outward in the major groove of the double helix when a double-stranded DNA helix is formed, the C-5 position of uridine is an appropriate site for the attachment of linker arms to functional groups such as fluorophores,[2,4,5] enzymes,[2,6] biotin[2,5] and metal chelates for specific DNA cleavage.[7]

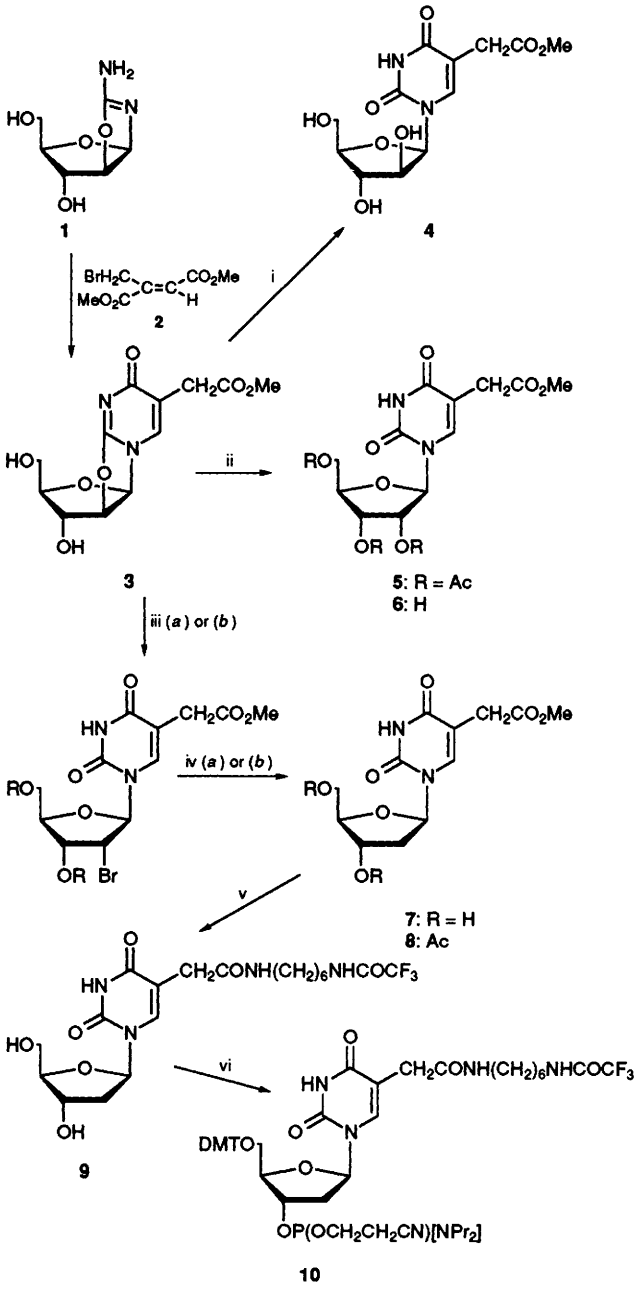
The Heck reaction or modified Heck reaction, coupling of an aryl halide or arylmercuric halide with an alkene or alkyne by an organopalladium complex, has been commonly used for the synthesis of C-5 substituted pyrimidine nucleosides.[2,8] Either C-5 mercurated or C-5 halogenated pyrimidine nucleosides are required as starting materials for the synthesis. Moreover, the palladium-mediated coupling reaction frequently gives side products that may result from participation by solvent molecules, or by a nucleophilic group on the nucleosides.

We now report a new and very simple synthetic method for 5-substituted uracil nucleosides, useful for the attachment of various functional groups. The key step for the synthesis involves the addition reaction of dimethyl α-bromomethylfumarate to arabinoaminooxazoline,[9] derived from D-arabinose and cyanamide, and subsequent cyclization in a one-flask reaction. Dimethyl α-bromomethylfumarate **2** was synthesized (72% yield) from dimethyl itaconate by bromination followed by dehydrobromination with triethylamine. Compound **2** was found to react with arabinoaminooxazoline **1** in the presence of 1.15 equiv. of triethylamine in methanol under reflux for 2 h. After evaporation of the solvent, the concentrated solution was poured into acetone to precipitate triethylamine hydrobromide, which was removed by filtration. The filtrate was evaporated and applied to column chromatography on silica gel, using 15% methanol in dichloromethane as eluent, to afford purified 2,2′-anhydro-1-β-D-arabinofuranosyl-5-methoxycarbonylmethyluracil **3**† in 50% yield. The β-anomer was exclusively formed in this reaction. The allylic bromide of **2** was subjected to nucleophilic substitution with aminooxazoline, giving an intermediate adduct which underwent double-bond migration and spontaneous cyclization to give the 2,2′-anhydro nucleoside **3**.

Hydrolysis of **3** with methanolic ammonia at room temp. led to the corresponding arabinosyl nucleoside **4** in high yield, as is already known for the hydrolysis of 2,2′-β-anhydro pyrimidine nucleoside.[9,10]

Ring opening of 2,2′-anhydro pyrimidine nucleoside with nucleophiles such as I^-, Br^-, Cl^-, F^-, and N_3^- has been reported for the synthesis of various 2′-substituted-2′-deoxynucleosides.[9,11] We attempted the ring opening reaction of **3** for the synthesis of 5′-substituted uridine and deoxyuridine. When heated under reflux in acetic acid containing a small amount of acetic anhydride, **3** was converted to 2′,3′,5′-tri-O-acetyl-5-methoxycarbonylmethyluridine **5** in 62% yield. Hydrolysis of **5** with methanolic ammonia at room temp. for 2 h yielded quantitatively 5-methoxycarbonylmethyluridine **6**, one of the modified nucleosides of transfer RNA.[3]

Bromination of **3** with HBr was accomplished in CF_3CO_2H at 40 °C overnight to give 5-methoxycarbonylmethyl-2′-bromo-2′-deoxyuridine in 74% yield. The 2′-bromo nucleoside was converted to 5-methoxycarbonylmethyl-2′-deoxyuridine **7** in 73% yield by hydrogenation on Pd–black catalyst in



Scheme 1 *Reagents and conditions*: i, MeOH–NH_3; ii, AcOH–Ac_2O, then MeOH–NH_3; iii, (*a*) HBr, CF_3CO_2H, or (*b*) AcBr, MeCN, NaOAc; iv, (*a*) H_2–Pd-black, or (*b*) Bu^n_3SnH–AIBN, C_6H_6; v, $H_2N(CH_2)_6NH_2$, then CF_3CO_2Et; vi, DMTCl, then $ClP(OCH_2CH_2CN)[NPr_2]$

the presence of sodium acetate in aqueous methanol at room temperature for 4 h. Alternatively, without purification, treatment of crude **3** with acetyl bromide in acetonitrile under reflux for 1 h produced 3′,5′-di-O-acetyl-5-methoxycarbonylmethyl-2′-bromo-2′-deoxyuridine in 37% yield from **1**. Reduction of the 2′-bromo derivative with Bu^n_3SnH–AIBN in benzene under reflux for 1 h furnished 3′,5′-O-diacetyl-5-methoxycarbonylmethyl-2′-deoxyuridine **8** in 79% yield.

Various linker arms and functional molecules containing amino groups are easily introduced to the 5-position of **6**, **7** and **8** *via* amide linkages by ester–amide exchange reactions of 5-carbonylmethyl esters. Thus the reaction of **8** with 1,6-hexanediamine in methanol in the presence of dimethylaminopyridine at 50 °C overnight gave 5-[*N*-(6-aminohexyl)carbamoylmethyl]-2′-deoxyuridine, the terminal amino group of which was protected with trifluoroacetyl group by reaction with ethyl trifluoroacetate in methanol, to afford 5-[*N*-(6-trifluoroacetylamidohexyl)carbamoylmethyl]-2′-deoxyuridine **9** in 90% yield from **8**. The nucleoside **9** was converted to the protected nucleoside phosphoramidite by the usual method for oligodeoxyribonucleotide synthesis. The reaction of **9** with dimethyoxytrityl chloride (DMTCl) in the presence of dimethylaminopyridine in pyridine at room temp. afforded the 5′-DMT protected nucleoside, which was phosphitylated with diisopropylamino-β-cyanoethoxychlorophosphine in dichloromethane at room temperature to give the 5′-DMT nucleoside phosphoramidite **10** in 77% yield. Oligodeoxyribonucleotides (25mer; 5′-ACATGCATCCCG*T*GGTCCTATCCGG-3′) containing the C-5 substituted 2′-deoxyuridine in place of one thymidine residue was synthesized on a DNA synthesizer using commercially available normal nucleoside phosphoramidites and **10**. After the normal deprotection and cleavage from the support, the modified oligodeoxyribonucleotide, bearing an amino group at the 5-position of one thymidine residue was isolated by reverse-phase HPLC. The amino group of the oligomer was further reacted with fluorescein isothiocyanate in DMF–carbonate buffer (pH 9.0) quantitatively to furnish the fluorescence-labelled oligodeoxyribonucleotide, which eluted later than the amino-modified oligonucleotide and gave a fluorescence emission at 520 nm.

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Footnote

† *Selected data* for **3**: mp 111–113°; ^{1}H NMR (D_2O) δ 7.96 (1H, s, 6-H), 6.56 (1H, d, 1′-H), 5.47 (1H, d, 2′-H), 4.67 (1H, s, 3′-H), 4.41 (1H, t, 4′-H), 3.73 (3H, s, CH_3), 3.57 (2H, m, 5′-H), 3.49 (2H, s, CH_2); UV λ_{max}/nm 253, 223; MS(FD) *m/z* 298 (M^+).

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