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$(\eta^5\text{-}C_5H_5)Fe(CO)_2$-complexes of uridine and thymidine

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Abstract

The photochemical reaction of $(\eta^5\text{-}C_5H_5)Fe(CO)_2I$ (FpI) with 2′,3′,5′-tri-*O*-acetyluridine (**1a**), 3′,5′-di-*O*-acetylthymidine (**1b**) and 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**1c**) in benzene containing excess of diisopropylamine afforded corresponding Fp-complexes of *N*(3)-deprotonated nucleosides, **2a**–**c** in moderate (45–75%) yields. Attempted removal of acetyl groups from **2a**–**b** (K_2CO_3, MeOH–H_2O, rt, 1 h or 25% NH_3 aq., MeOH, rt, 2 h) gave unseparable mixtures of 3-Fp-uridine or 3-Fp-thymidine (**3a**–**b**) with uridine or thymidine. On the other hand, removal of the 4,4′-dimethoxytrityl groups from **2c** proceeded smoothly in 80% acetic acid affording 3-Fp-thymidine (**3b**) in quantitative yield.

Keywords: Nucleotide; Uridine; Thymidine; Iron; Cyclopentadienyl; Carbonyl

1. Introduction

Incorporation of transition metal-containing moieties to oligonucleotides or DNA has been used for the study of DNA-mediated energy and electron transfer processes [1–3] as well as the development of DNA hybridisation probes or sensors [4–13]. For example, redox-active ferrocene-modified oligonucleotides, undoubtedly the most extensively used, allowed recently sensitive electronic detection of single-base dismatches in DNA [11] and bioelectrocatalytic detection of viral DNA [13].

Another sensitive labelling/detection tool, introduced by Jaouen et al is the use of metallocarbonyl labels and Fourier Transform Infrared Spectroscopy as a detection method [14–17]. This technique takes advantage of the very intense and narrow ($v_{1/2} \sim 10\ cm^{-1}$) IR absorption bands displayed by transition metal carbonyl complexes (M–CO), which fall into a spectral window (1900–2150 cm^{-1}), where virtually all biomolecules and biological matrices are transparent. It has been used for labelling of proteins (e.g. antibodies) or haptens (Carbonyl Metallo Immunoassay, CMIA) and hormones to study their interactions with receptors [16,17]. By contrast, examples of the labelling of nucleosides, nucleotides or DNA with metal carbonyl complexes are few [18–20]. In this communication we report synthesis of $(\eta^5\text{-}C_5H_5)Fe(CO)_2$,-labelled uracil nucleosides, uridine and thymidine, potential monomers for syntheses of labelled oligonucleotides.

2. Results and discussion

We reported earlier [20] that the photochemical reaction of $(\eta^5\text{-}C_5H_5)Fe(CO)_2I$ (FpI) with triacetyl 6-azauridine in the presence of diisopropylamine (dipa) leads to the Fp-complex of the *N*(3)-deprotonated nucleoside in 51% yield (Eq. (1)).

We attempted to extend this reaction for unprotected uracil nucleosides: 6-azauridine, uridine and thymidine. Unfortunately, all these attempts failed because of almost total insolubility of these nucleosides in solvents usually used for this reaction, benzene or dichloromethane. Only traces of yellow, strongly polar complexes, supposed to be the desired Fp-nucleoside complexes were formed and we were unable to isolate and purify them.

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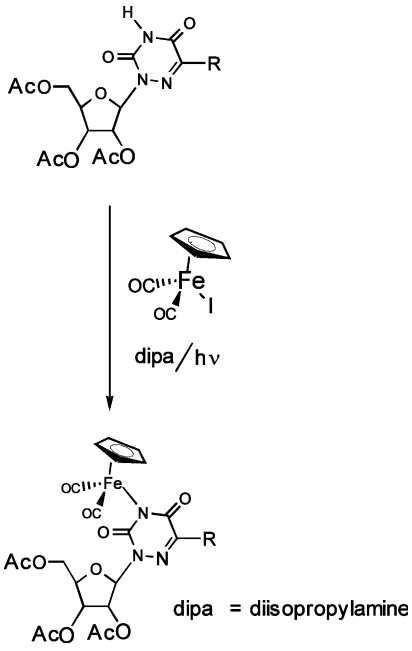
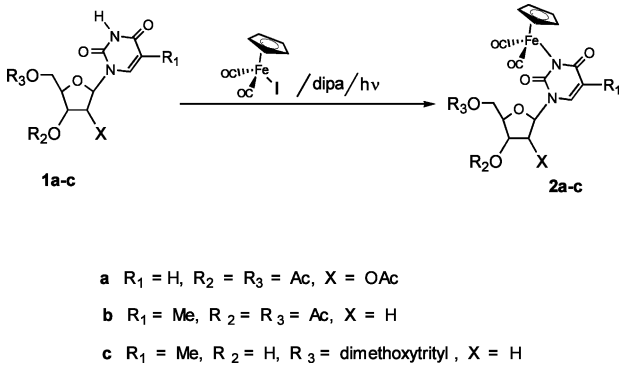


(1)

It has been therefore decided to prepare Fp-complexes of the *O*-protected nucleosides, better soluble in non-polar solvents, and then to remove protecting groups. Photolysis of FpI with 2′,3′,5′-tri-*O*-acetyluridine (**1a**), 3′,5′-di-*O*-acetylthymidine (**1b**) and 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**1c**) in benzene containing excess of dipa afforded corresponding Fp-complexes **2a**–**c** in moderate (45–75%) yields (Scheme 1).

Coordination of the Fp moiety to the deprotonated *N*(3) was confirmed by IR spectra, displaying two characteristic uracil CO bands at 1649 and 1573 cm^{-1}. Furthermore, in the ^1H NMR spectra of **2a**–**c** a high-field shift of olefinic protons resonances (relative to those in **1a**–**c**) was observed, due to the replacement of the *N*(3)-hydrogen by the Fp moiety [20].

It is worthy noting that the presence of unprotected OH group in **2c** does not significantly hamper its photoreaction with the FpI/dipa system. In our opinion, the lack of success with unprotected nucleosides is not due to the competitive reactivity of free OH groups but rather to insolubility of these species.



Scheme 1.

Deprotection of the acetyl groups in **2a**–**b** (Scheme 2) was carried out using two standard procedures [21]:

A) K_2CO_3, MeOH, H_2O, rt, 1 h
B) 25% NH_3 aq., MeOH, rt, 2 h

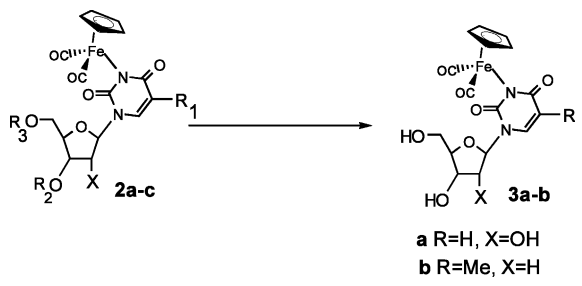
However, we have found that according to ^1H NMR in both cases the mixtures of expected **3a**–**b** with Fp-deprotected nucleosides i.e. uridine or thymidine were formed. We were unable to separate **3a**–**b** from these mixtures by column chromatography. The Fe–N bond in **2a**–**b** is therefore not stable under above mentioned reaction conditions and other approaches should be found to obtain pure **3a**–**b**.

On the other hand, removal of the dimethoxytrityl group in **2c** proceeded smoothly (80% acetic acid, rt, 20 min [21]) without concomitant loss of the Fp moiety and afforded **3b** in quantitative yield. The structure of this complex was confirmed by spectral data (IR, ^1H NMR, CI MS) The CI (NH_3) mass spectrum of this complex shows an intense peak at *m*/*e* 419 $(M+H)^+$. The Fe–N bonds in **2c** and **3b** are therefore stable under acidic conditions.

To our knowledge, compound **3b** is the first metallocarbonyl complex of unprotected thymidine nucleoside. It is relatively air-stable and water-soluble yellow oil. Attempts on applications of this compound in oligonucleotide synthesis are underway.

3. Experimental

All reactions were carried out under argon. Benzene was distilled over sodium-benzophenone. Other solvents were reagent grade and were used without prior purification. FpI, 2′,3′,5′-tri-*O*-acetyluridine (**1a**), 3′,5′-di-*O*-acetylthymidine (**1b**) and 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**1c**) were synthesised according to earlier published procedures [22–24]. Chromatographic separations were carried out using silica gel 60 (Merck, 230–400 mesh ASTM) ^1H NMR spectra were recorded on a Varian Gemini 200BB (200 MHz) and Bruker DRX500



Scheme 2.

(500 MHz). Chemical ionisation mass spectra were run on a NERMAG R1010C spectrometer. IR spectra were recorded on a Biorad 175C apparatus.

3.1. Synthesis of **2a–c**

Solutions of FpI (0.281 g 0.9 mmol) and **1a–c** (0.150 g 0.4 mmol) in benzene (20 ml) containing dipa (4 ml) were irradiated (visible light; 4×150 W domestic tungsten lamps) for 2 h at 0 °C under argon. The resulting yellow solid was filtered and washed repeatedly with ether until the filtrates were colourless. Removal of solvent from the combined benzene and ether filtrates and chromatography ($CHCl_3$–MeOH 50:2) of the residue afforded unreacted FpI followed by yellow **3a–c**. Crystallization from methyl chloride: *n*-hexane afforded analytically pure samples.

2a. Yield 55%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ7.14 (1H, d, $J = 7$ Hz, H-6), 6.09 (1H, s, broad, H-1′), 5.66 (1H, d, $J = 7$ Hz, H-5), 5.31 (2H, broad s, H-2′ H-3′), 5.02 (5H, s, Cp), 4.33 (3H, broad s, H-4′, H-5′), 2.12 (9H, s, CH_3CO). IR (KBr, cm^{-1}): 2048, 2002, 1746, 1649, 1580. Elemental analysis: Calculated for $C_{22}HN_2O_{11}Fe$: C 48.37%, H 4.06%, N 5.13%. Found: C 48.32%, H 4.10%, N 5.07%

2b.Yield 75%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ7.46(1H, s, H-6), 6.26(1H, dd, $J = 9.3$ and 5.4 Hz, H-1′), 5.01(5H, s, Cp), 5.00 (1H, m, H-3′), 4.32 (1H, m, H-4′), 4.19 (2H, broad s, H-5′), 3.39 (2H, m, H-2′), 2.17 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 1.26 (3H, s, CH_3-5). IR (KBr, cm^{-1}): 2041, 1990, 1743, 1670, 1580. MS ICP/NH_3/, *m*/*z*: 503 $[M+H]^+$. Elemental analysis: Calculated for $C_{21}H_{22}N_2O_9Fe$: C 50.22%, H 4.41%, N 5.58%. Found: C 50.16%, H 4.45%, N 5.64%.

2c. Yield 45%. ^{1}H-NMR (500 MHz, $CDCl_3$): δ7.29-6.80 (14H, complex signal, aromatic and H-6), 6.46 (1H, broad t, $J = 5$ Hz, H-1′), 5.01(5H, s, Cp), 4.49 (1H, broad s, H-3′), 3.99 (1H, m, H-4′), 3.78 (6H, s, OCH_3), 3.38 (2H, m, H-5′), 2.23 (2H, m, H-2′), 1.26 (3H, s, CH_3). (IR KBr, cm^{-1}) 2041, 1992, 1668, 1568, 1540. Elemental analysis: Calculated for $C_{37}H_{36}N_2O_9Fe$: C 62.72%, H 5.12%, N 3.95%. Found: C 61.90%, H 5.08%, N 4.05%.

3.2. Removal of acetyl groups from **2a** and **2b**

3.2.1. Method A

2a or **2b** (0.5 mmol) and K_2CO_3 (386 mg, 2.7 mmol) were dissolved in a mixture of methanol (3 ml) and water (2 ml) at rt and stirred 1 h in the darkness. After evaporation to dryness the residue was subjected to column chromatography. A yellow fraction eluted with methanol was collected. According to ^{1}H NMR it was a mixture of deprotected Fp-nucleoside and uridine or thymidine in approximately 1:1 ratio.

3.2.2. Method B

2a or **2b** (1 mmol) was dissolved in a mixture of methanol and 25% aq. NH_3 and stirred 2 h at rt. The same workup afforded yellow oil, which according to ^{1}H NMR was a mixture of **3a** or **3b** and uridine or thymidine in approximately 4:1 ratio.

3.3. Removal of the 4,4′-dimethoxytrityl group from **2c**

2c (168 mg, 0.2 mmol) was dissolved in 80% AcOH (10 ml) and stirred 15 min at rt. Evaporation and column chromatography (silica gel, chloroform-ethanol 5:1) afforded **3b** as an yellow oil. Yield 86 mg (100%). ^{1}H-NMR (200 MHz, methanol-d_4): δ7.56 (1H, s, H-6), 6.28 (1H, t, $J = 6.9$ Hz, H-1′), 5.11 (5H, s, Cp), 4.36 (1H, m, H-3′), 3.86 (1H, m, H-4′), 3.34 (2H, m, H-5′), 2.15 (2H, m, H-2′), 1.81 (3H, s, CH_3-5). IR (KBr, cm^{-1}): 3375, 2045, 1995, 1672, 1636, 1575, 1544. MS ICP/NH_3/, *m*/*z*: 419$[M+H^+]$ 243$[M+H^+]$-Fp.

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