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RESEARCHARTICLE

A SHORT PROTOCOL FOR THE CONVERSION OF THE 2-CHLORO-9-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL) HYPOXANTHINE TO 9-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL) GUANINE (araF-G)

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ABSTRACT

The current protocol was aimed to describe step by step synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) guanine from 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) hypoxanthine as masked guanine precursor. The method described here is straight forward coupling of 3, 5-di-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide **1**(Elzagheid *et al*., 2002)with a silylated 2-chlorohypoxanthine base **2**(Elzagheid *et al*., 2003).Subsequent treatment of N9-glycoside **3** with methanolic ammonia (150^{o}C, 6 h) afforded 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) guanine **4** in more than 60%yield. This procedure can be applied in large scale synthesis of this nucleoside.

INTRODUCTION

In recent years a number of methods for the synthesis of 2'-deoxy-2'-fluoronucleosides have been introduced (Howell *et al.,* 1988; Wild *et al*., 2000; Elzagheid *et al*., 2003) as building blocks for the promising antisense oligonucleotides (Souleimanian *et al*., 2012) such as 2'-deoxy-2' fluoro-arabinonucleic acids(Viazovkina *et al*., 2002)or as nucleic acids mimics for structure and stability studies (Wild *et al*., 2000; Pintado *et al*., 2013) or as starting materials for the synthesis of 2′-Deoxy-2′,4′-difluoroarabinose-Modified Nucleic Acids (Montero *et al.,* 2015) and also as nucleic acid therapeutics for hematologic malignancies (Oplalinska *et al.,* 2006).Although I have reported a short description for the synthesis of araF-G (Elzagheid *et al*., 2003) and I (Elzagheid *et al*., 2002) and others (Yamada *et al*., 2009) also reported a full multi-step protocol for the synthesis of araF-G by the chemo-enzymatic approach using adenosine deaminase, a chemical approach protocol for araF-G was not reported. This current protocol will describe a two-step protocol by chemical approach. A step-by-step preparationand critical parameters will also be thoroughly elaborated.

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The main step of this synthesis involves coupling of 2-deoxy-2-fluoro-3, 5-di-O-benzoyl-α-D-arabinofuranosyl bromide **1** with silylated 2-chloro hypoxanthine **2** to afford 2-chloro-β-araF-I **3** that was transformedto araF-G **4** by treatment with methanolic ammonia in high yield (Scheme 1).

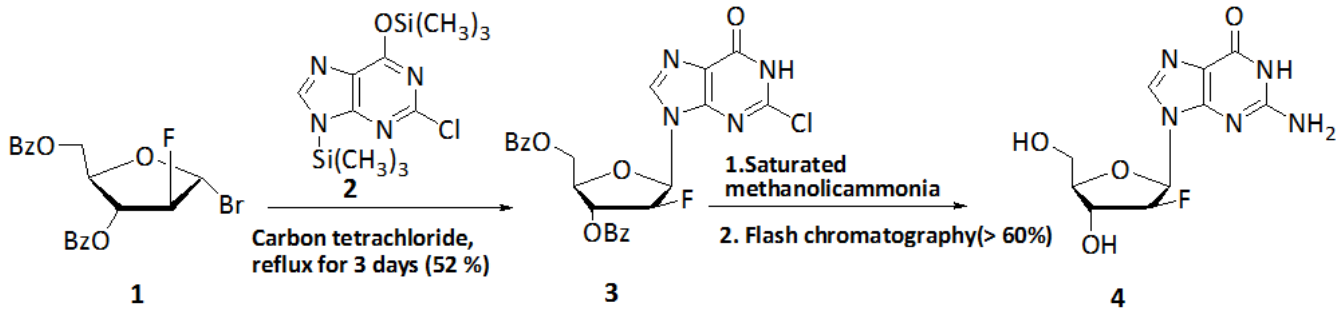
MATERIALS AND METHODS

Glassware

Oven-dried glassware round-bottom flasks, separatory funnels, and reflux condenserChromatography columns: 5x50 cm and 3x20 cm

Materials, Reagents and Solvents

Source of nitrogen gas
Anhydrous carbon tetrachloride (CCl_4)
Dichloromethane and Methanol
Anhydrous sodium sulfate
Chlorotrimethylsilane (TMSCl)
Hexamethyldisilazane (HMDS)
2-deoxy-2-fluoro-3,5-di-O-benzoyl-α-**D**-arabinofuranosyl bromide
Merck thin-layer chromatography silica plates
Silylated 2-chlorohypoxanthine



Scheme 1. Synthesis of araF-G

Solutions

9:1 (v/v) Dichloromethane/methanol
5%, 10%, 15%, and 25% (v/v) methanol in dichloromethane
0% to 25% (v/v) gradient of methanol in dichloromethane

Equipment

Oil bath, 60°C to 70°C
Rotary evaporator equipped with a vacuum pump or water aspirator
UV-lamp

Synthesis of 9-(2-deoxy-2-fluoro-3, 5-di-O-benzoyl-β-D-arabinofuranosyl)-2-chlorohypo- xanthine3: condensation ofsilylated 2-chlorohypoxanthine2 with 2-Deoxy-2-fluoro-3, 5-di-O-benzoyl-β-D-arabinofuranosyl bromide 1

Step 1: In an oven-dried 250-mL round-bottom flask equipped with a reflux condenser, stirbar, and nitrogen gas source, add 4 mL TMSCl to a suspension of 2.56 g (15 mmol) 2-chlorohypoxanthine**2** in 40 mL HMDS. Reflux at 120°C for 2 to 3 hrs. Cool down, andevaporate to dryness in the rotary evaporator.

Step 2: Co-evaporate the residue with 50 mL dry CCl_4. To this residue,add 3.2 g (7.5mmol) 1in 100 mL dry $CCl_4$and reflux the resulting solution at 77 °Cfor 72 hrs.

Step 3: Analyze the reaction by TLC.The starting material should be run alongside the reaction for comparison. The plates aredeveloped using 9:1 (v/v) methylene chloride/methanol, and the spots are visualized by UVshadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. The typical R_f value of the desired product, the N9-β-isomer**3**is in the range of 0.42-0.46

Step 4: Cooland evaporate the reaction mixture. Dissolve the residue in 200 mL dichloromethane, and wash it carefully with300 mL saturated sodium bicarbonate solution.Dry the dichloromethane layers over anhydrous Na_2SO_4, filter, and evaporate todryness. The crude mixture can either be purified by column chromatography or subjected to next step without purification.

^{1}H-NMR (400MHzDMSO-d_6): 8.10–7.46 (10H, m, Bz), 8.15 (1H, d, H-8), 6.50 (1H, dd, H-1'), 5.85 (1H, 2 dd, H-3'), 5.70 (1H, 2dd, H-2'), 4.80–4.65 (3H, m, H-4', H-5', 5''); **^{13}C-NMR (100.61MHZ, DMSO-d_6)**:166 (C-6), 165 (C-4), 148.49 (C-2), 139.92 (d, C-8), 134.54–129.14 (Bz), 123.09 (C-5), 92.70 (d, C-2'), 83.19 (d, C-1'), 79.26(d, C-4'), 77.21 (d, C-3'), 64.48 (C-5'); **APCI-MS:** 512.9 ($M+H^+$), 535 ($M+Na^+$).

Synthesis of 9-(2-deoxy-2-fluoro-3, 5-di-O-benzoyl-β-D-arabinofuranosyl)-guanine 4

Step 5: The crude product was treated with saturated methanolic ammonia (in steel bomb) for 6 hours at 150°C. The solution was evaporated and the resulting residue wad applied to silica gel flash column. Elution with dichloromethane/ methanol (gradient 1:0 to 4:1) gave the desired nucleoside as a white powder (> 60%).

^{1}H-NMR (400 MHz, DMSO-d_6): 10.62 (1H, s, N-H), 7.77 (1H, d,H-8),6.51 (2H, br, s, NH_2), 6.11 (1H, dd, H-1'), 5.91 (1H, d,HO-C2'), 5.00 and 5.15 (1H, dt or ddd, H-2'), 5.05 (1H, t, HO-C5'), 4.32 (1H, m, H-3'), 3.77 (1H, m, H-4'), 3.57 (2H, m,H-5' and H-5''); **FAB-MS (NBA-matrix):** 286 [$M+H^+$].

RESULTS AND DISCUSSION

Originally, araF-G was synthesized (Chu *etal.,* 1989) from riboguanosine (ribo-G). Coupling the purines to the fluorinatedarabinose sugar **1**, however, largely improvesyields and minimizes the number of steps. Silylation of the masked base enhances the coupling step. Coupling of guanine to arabinoside 1was not successful and gave non nucleosidic products but coupling of2-chloro-6-hydroxypurine (2-chlorohypoxanthine) gave the anticipated N9-β-anomers in more than 50 % yield. Displacement of the chloro function with 2N sodium hydroxide in dioxane (Hanna et al., 1988) or a mixture of sodium methoxide/ mercaptoethanol/ water (Cheriyan*et al*., 1982; Ma*et al*., 1997) were not successful and gave only modified starting material (not isolated). In contrast, the treatment of N9-glycoside **3** with methanolic ammonia (150°C, 6 h) afforded araF-G **4**in more than 60% yield.

Practical Considerations

During the synthesis of araF-G **4** we found out that purification of the fluoronucleosides **3**is very important in order to get good yields of araF-G during the final step. Coupling of arabinoside**1**with silylated 2-chloro hypoxanthine **2**in carbon tetrachloride gave better results than in dichloromethane or dichloroethane.

Conclusion

The quality of the desired araF-Gwas excellent after simple purification. I believe that this chemical approach is most useful for the synthesis of the guanine 2'-fluoroarbinonucleoside.

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