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Syntheses and NMR characterizations of epimeric 4-deoxy-4-fluoro carbohydrates

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ABSTRACT

The synthesis and NMR characterizations of 1,2,3,6-tetra-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside, the 4-deoxy-4-fluoro epimer of an intermediate in the synthesis of a drug substance, needed for use as a potential impurity standard and to confirm the stereoselectivity of a key fluorination step, are described.

1. Introduction

The strategic incorporation of fluorine atoms as a means to optimize pharmacology, safety, and metabolic stability of small molecules is a well established technique in drug design. While replacing hydrogen with fluorine during analoging is a common practice, replacing hydroxy with fluorine is also effective because of similarities in van der Waals radii and electronegativity of fluorine and oxygen.[1] In this regard, 4-deoxy-4-fluoro carbohydrates were of immediate interest. For example, SAR7226 (**1**), a molecule containing a 4-fluoroglucopyranose glycon, progressed in our company as a promising SGLT1/SGLT2 inhibitor, potentially useful for the treatment of diabetes.[2] Scheme 1 illustrates a retrosynthetic, convergent approach to **1** that features the coupling of two advanced intermediates, 4-fluoroglycosyl donor **5** and hydroxypyrazole **2**, to afford the SAR7226 backbone structure.[3] Glycosyl donor **5** was derived from D-galactose in three chemical transformations; namely, selective benzoylation to give the tetrabenzoate **3**, followed by fluorination with inversion at the 4-position, and finally manipulation at the anomeric position to give the desired glycosyl bromide **5**. Hydroxypyrazole **2** was derived from mono-alkylation of ethyl 4,4,4-trifluoroacetoacetate with 4-methoxybenzyl chloride followed by treatment with benzylhydrazine.

During the development of the fluorination step via either direct fluorination of **3** or by way of the corresponding triflates or nonaflates, several reagent systems were evaluated, including bis(2-methoxyethyl)aminosulfur trifluoride (BAST), diethylaminosulfur trifluoride (DAST), Bu_4NF, $Et_3N{\cdot}HF$, and SO_2F_2. The formation of the corresponding olefin **6** was a competing side reaction, and in some cases the major pathway (Scheme 2). Of these reagent systems, BAST was the most effective reagent, and provided an 85:15 mixture of **4**:**6** (1.3 equiv BAST, 20 °C → 55 °C, 2-methyltetrahydrofuran as a solvent). The identification of 1-butanol as an ideal antisolvent was key in developing an efficient and simple isolation of **4**. Thus, after quenching with water and extractive workup in toluene, the final organic phase was treated with 1-butanol to crystallize **4**. After filtration, **4** was isolated as a white solid in 74–78% isolated yield and with purity ⩾98.5 A% (area % UV absorption by HPLC assay). The olefin and other impurities were predominately retained in the filtrate. This simple isolation of **4** was an important aspect of an efficient overall synthesis of **5** that was demonstrated on a multi-kilogram pilot-plant scale.

It appeared that the BAST fluorination occurred with inversion of configuration as was widely reported for DAST.[4] BAST was a preferred replacement for DAST for the fluorination because of its process safety advantages.[5] However, to more firmly establish the stereoselectivity of the BAST fluorination step, to confirm whether any of the C-4 epimer formed even in low levels, and to help establish the impurity profiles of the SAR7226 synthetic route, the corresponding 4-F epimer, 1,2,3,6-tetra-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**9**), was independently synthesized and characterized as described below.

2. Results and discussion

4-Deoxy-4-fluoro epimer **9** was prepared in three steps starting with selective tetrabenzoylation of D-glucose to tetrabenzoyl-β-D-

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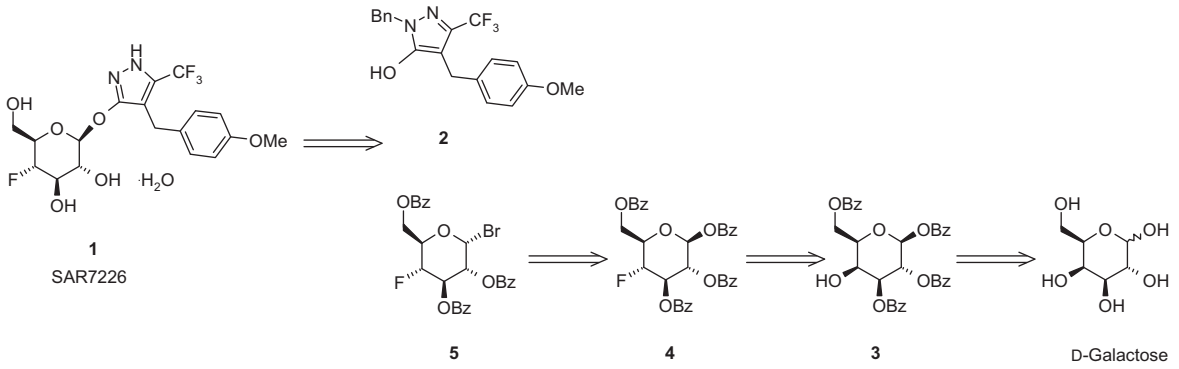
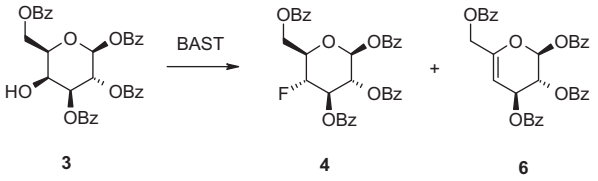


Scheme 1. Retrosynthesis of SAR7226.

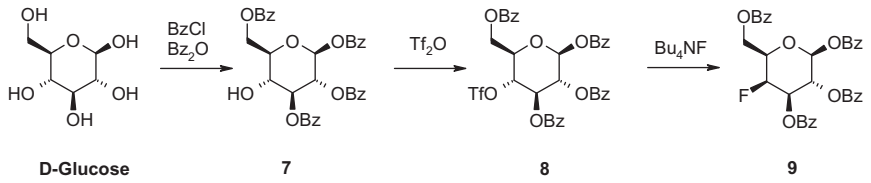


Scheme 2. Fluorination of **3**.[3]

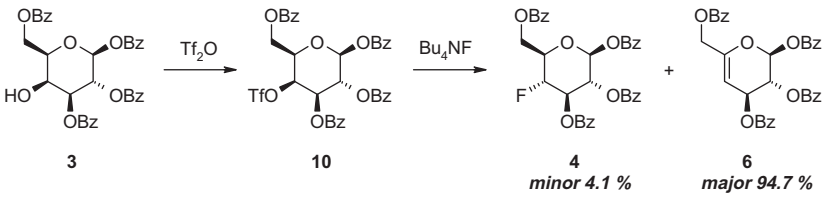
glucopyranose **7** followed by formation of triflate (**8**) and fluorine substitution with tetrabutylammonium fluoride (Scheme 3).[6] For the first step, tetrabenzoylation methodology used previously for galactose[3] was adapted. However, because the equatorial 4-OH in D-glucopyranose is more reactive than the axial 4-OH in D-galactopyranose, it was anticipated that the desired selectivity of 1,2,3,6-tetrabenzoylation would be more difficult to achieve.[7] Indeed, benzoylation of glucose led to a complex mixture of products containing only about 30 A% of **7** (HPLC) together with anomeric mixtures of positional isomers and over- and under-benzoylated species. However, because the intent of the exercise was to isolate an analytical reference standard of epimer **9**, not to develop a scalable process that was required for **4**, this poor conversion was satisfactory for current purposes. Purification of **7** (100 A% HPLC) was achieved by column chromatography. The transformation to the triflate **8** proceeded in high yield (99.7%). The last step, fluoride displacement of triflate using Bu_4NF, proceeded at room temperature.[6] After diluting the reaction mixture with 2-MeTHF and quenching with water, the organic layer was partially concentrated, and **9** was isolated in 91% yield (99.5 A% purity) after crystallization upon addition of 1-butanol.

As expected, **8** produced less olefin **6** (2.5 A%) during fluorination than the corresponding BAST-activated galacto-intermediate due to less favorable *syn*- versus *anti*-elimination.[8] Similarly, fluorination of the analogous triflate **10** derived from tetrabenzoylgalactose **3** with tetrabutylammonium fluoride gave olefin **6** as the major product (94.7 A%) due to more favorable elimination with *anti*-H compared to *syn*-H in **8**[8] (Scheme 4). Olefin **6** was isolated and found to be consistent (^{1}H NMR) with material that was synthesized independently.[9]

NMR spectra are consistent with the proposed structures and relative stereochemistries (Table 1). For example, large (8–10 Hz) vicinal H–H couplings throughout the ring are consistent with chair-like conformation and all-equatorial substituents in **4**. In contrast, the small ($\leqslant$2.5 Hz) vicinal couplings of H-4 in epimer **9** are consistent with equatorial H-4 and axial fluorine. Similarly, the larger vicinal H-F couplings in **9** (J_{HF} = 27 and 26 Hz) versus those in **4** (J_{HF} = 14 and 2.5 Hz) are also consistent with axial fluorine in **9** and equatorial fluorine in **4**. Finally, differences in the ^{1}H and ^{19}F chemical shifts of **4** and **9** are consistent with assignments. For example, the downfield shift of H-2 in epimer **9** can be rationalized by its spatial proximity to fluorine as a result of their 1,3-diaxial relationship; and the H-4 and fluorine chemical shifts follow a typical pattern of being shifted downfield in an equatorial position versus a corresponding axial position (H-4_{eq} in **9** is shifted downfield relative to H-4_{ax} in **4**, and F_{eq} in **4** is shifted downfield relative to F_{ax} in **9**).

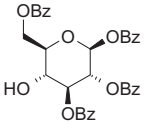
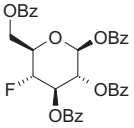
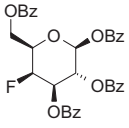


Scheme 3. Synthesis of 4-deoxy-4-fluoro epimer **9**.



Scheme 4. Fluorination by displacement of triflate **10**.

Table 1
^{1}H NMR assignments for **7**, **4**, and **9**

	 **7**	 **4**	 **9**
^{1}H NMR (300 MHz)			
H-1	6.20 d, *J* = 8.0	6.24 d, *J* = 8.3	6.25 d, *J* = 8.3
H-2	5.75 dd, *J* = 9.5, 8.0	5.78 dd, *J* = 9.5, 8.3	6.11 dd, *J* = 10.3, 8.3
H-3	5.64 dd, *J* = 9.5, 8.5	5.99 ddd, *J* = 9.5, 9.0; J_{HF} = 14	5.59 ddd, *J* = 10.3, 2.5; J_{HF} = 27
H-4	3.99 ddd, *J* = 10.0, 8.5, 4.0	4.94 ddd, *J* = 9.5, 9.0; J_{HF} = 50	5.24 br dd, *J* = 2.5; J_{HF} = 50
H-5	4.05 ddd, *J* = 10.0, 3.5, 2.0	4.30 dddd, *J* = 9.5, 4.5, 2.5; J_{HF} = 2.5	4.39 br ddd, *J* = 6.5, 6.5; J_{HF} = 26
H-6,6′	4.89 dd, *J* = 12.5, 3.5	4.75 ddd, *J* = 12.5, 2.5; J_{HF} = 1.5	4.65 AB-m, 2H
	4.63 dd, *J* = 12.5, 2.0	4.64 ddd, *J* = 12.5, 4.5; J_{HF} = 1.0	
Ar-H	7.2–7.6 m, 12H	7.3–7.6 m, 12H	7.2–7.6 m, 12H
	7.8–8.2 m, 8H	7.9–8.1 m, 8H	7.8–8.1 m, 8H
OH	3.60 d, *J* = 4.0		
^{13}C NMR (75 MHz)			
	167.5, 167.2, 165.5, 164.9, 134.0, 133.7, 130.4, 130.2, 130.0, 129.6, 129.1, 129.0, 128.8, 128.6, 93.0, 76.2, 75.7, 70.7, 69.3, 63.2	166.3, 165.7, 165.4, 164.8, 134.2, 133.8, 133.6, 130.4, 130.1, 129.7, 129.1, 128.8, 128.7, 128.6, 92.8, 88.4, 85.9, 72.9, 70.6, 62.5	166.3, 166.0, 165.4, 164.9, 134.1, 134.0, 133.7, 133.6, 130.5, 130.2, 130.1, 130.0 129.6, 129.1, 128.9, 93.0, 87.6, 85.9, 72.4, 68.5, 61.9, 53.7
^{19}F NMR (282 MHz)			
		−199.0 br dd, *J* = 50, 14	-216.3 ddd, *J* = 50, 27, 26

In conclusion, 4-deoxy-4-fluoro epimer **9** was synthesized and its stereochemistry was confirmed by NMR analyses. With pure **9** in hand as an analytical standard sample and HPLC marker, it was then confirmed that the BAST fluorination of **3** proceeded with inversion of configuration at C-4 to give **4** highly selectively. Analyses of isolated product **4** and its concentrated filtrates did not indicate the presence of **9** (HPLC and ^{19}F NMR), thus helping establish the purity profile of SAR7226 and its intermediates.

3. Experimental

3.1. General

HPLC analyses were performed on Agilent 1100 HPLC using Zorbax Eclipse XDB-C8, 5 μm, 4.6 × 150 mm column. Mobile phase A: H_2O (0.1% TFA), B: acetonitrile (0.1% TFA). System: 0–1 min 40/60 A/B, then gradient to 20/80 for 16 min, flow rate 1 mL/min, UV detection at 220 nm.

NMR studies were performed on a Varian 300 spectrometer in $CDCl_3$. Chemical shifts are given in ppm relative internal TMS (^{1}H and ^{13}C) and external $CFCl_3$ (^{19}F). Coupling constants (*J*) are reported in Hz.

The preparations of compounds **3**, **4**, and **5** have been previously described.[3]

3.1.1. 1,2,3,6-Tetra-*O*-benzoyl-β-D-glucopyranose (7)

A three-necked, 500-mL reaction flask equipped with a mechanical stirrer, thermocouple, addition funnel, condenser, and nitrogen inlet was charged with D-glucose (22.9 g, 127 mmol, 1.0 equiv), *N*-methylpyrrolidone (NMP) (200 mL), and pyridine (56.4 mL, 697 mmol, 5.5 equiv). The suspension was cooled to ca. 5 °C and benzoyl chloride (44.9 mL, 387 mmol, 3.0 equiv) was slowly added over 25 min keeping the temperature below 20 °C (all solids dissolved by the end of addition). Cooling and stirring were continued (solids started to precipitate at ca. 13 °C). Benzoic anhydride[10] (43.0 g, 190 mmol, 1.5 equiv) was added and the reaction mixture was heated to 50 °C (clear solution at 45 °C). After heating at 50 °C for 18 h, the reaction mixture contained about 30 A% (HPLC) of **7**. The solution was cooled to 20 °C, then was diluted with toluene (200 mL) and water (250 mL). The biphasic solution was stirred for 1 h at 20 °C, and then the phases were separated. The organic phase was washed with water (1 × 160 mL), aq 6% $NaHCO_3$ (3 × 150 mL) and water (2 × 150 mL). The organic layer was concentrated (50 torr/45 °C), redissolved at 50 °C in toluene (100 mL) and crystallized by the addition of *n*-heptane (100 mL). The solid (62.6 g, ca. 33 A% product by HPLC) was collected by filtration, then was redissolved in CH_2Cl_2 (50 mL) and chromatographed on 210 g of silica gel (60–230 mesh) using CH_2Cl_2 as an eluent. Fractions containing product were combined and concentrated to give 8.95 g of **7** as a white solid (91.8 A% product by HPLC). The solid was redissolved in CH_2Cl_2 (50 mL), heated to gentle reflux, and *n*-heptane (80 mL) was slowly added. The product crystallized after ca. 5 min at 40 °C. The suspension was allowed to cool to 20 °C and then was filtered yielding 7.57 g (12.7 mmol) (100 A% by HPLC, 10% yield from glucose) of **7** as a white solid. The structural assignment of **7**, as the β-anomer, is fully supported by NMR data provided in Table 1. The synthesis of **7** has previously been reported.[11]

HPLC retention time: **7** (9.93 min) (purity 100% at 220 nm).

^{1}H NMR ($CDCl_3$), ^{13}C NMR: see Table 1.

MS (ES^+): 619.18 $(M+Na)^+$.

Anal. Calcd for $C_{34}H_{28}FO_{10}$: C, 68.45; H, 4.73. Found: C, 68.36; H, 4.52.

3.1.2. 1,2,3,6-Tetra-*O*-benzoyl-4-(1,1,1-trifluoromethanesulfonyl)-β-D-glucopyranose (8)

A 250-mL flask was charged at 20 °C with **7** (6.7 g, 10.4 mmol, 1.0 equiv), CH_2Cl_2 (65 mL) and pyridine (2.5 mL, 131.2 mmol, 3.0 equiv). The solution was stirred and cooled in an ice bath, and triflic anhydride (2.5 mL, 14.6 mmol, 1.4 equiv) was added via syringe while keeping the temperature below 5 °C. The bath was removed and the reaction was allowed to warm to 20 °C. After 30 min, the reaction was judged to be complete (by HPLC). The solvent was evaporated to give **8** (99.7% purity by HPLC at 220 nm) as a white solid which was used directly in the next step.

HPLC retention times: **7** (9.93 min), **8** (16.34 min).

3.1.3. 1,2,3,6-Tetra-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranose (9)

A 250-mL flask was charged with crude **8** (7.6 g, 10.4 mmol, 1.0 equiv, based on quantitative trifylation) and 2-methyltetrahydrofuran (35 mL). The solution was cooled in an ice bath and tetrabutylammonium fluoride (1.0 M solution in THF, 90 mL, 120 mmol, 8.6 equiv) was added over 5 min while keeping the temperature below 12 °C. The cooling bath was removed and the reaction mixture was allowed to warm to room temperature. After 1 h, 37 A% (HPLC) starting material remained. Additional tetrabutylammonium fluoride solution (30 mL, 2.9 equiv) was added. After 1 h (<0.3 A% **8**, 92.7 A% **9**), the reaction mixture was diluted with 2-methyltetrahydrofuran (150 mL) and quenched with water (60 mL). The organic layer was washed with water (1 × 60 mL), 6% aq $NaHCO_3$ (2 × 60 mL) and water (2 × 60 mL). The organic layer was partially concentrated (50 Torr/24 °C) to a volume of ca. 70 mL and the product was crystallized by addition of 1-butanol (100 mL). The suspension was partially concentrated to ca. half of the volume (50 Torr/50 °C), then was cooled in an ice bath and filtered. The solid was washed with 1-butanol (50 mL) and dried (50 Torr/42 °C/N_2 bleed) to yield 5.7 g of **9** (91% yield, 99.5 A% purity) as a white solid.

HPLC retention times: triflate **8**: 16.34 min, product **9**: 14.17 min, olefin **6**: 14.59 min.

^1H NMR, ^{13}C NMR, ^{19}F NMR ($CDCl_3$): see Table 1.

MS (ES^+): 621.17 $(M+Na)^+$.

Anal. Calcd for $C_{34}H_{27}FO_9$: C, 68.22; H, 4.55; F, 3.17. Found: C, 67.98; H, 4.28; F, 3.17.

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