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Synthesis of the glucuronide metabolite of ABT-751

Kenneth M. Engstrom,* Rodger F. Henry and Ian Marsden

Abbott Laboratories, 1401 Sheridan Road, North Chicago, IL 60064-6290, USA

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Abstract—A linear synthesis of the glucuronide metabolite of ABT-751 was replaced with a convergent synthesis that features direct glycosidic coupling between the aglycone and a trichloroacetimidate glucuronyl donor. Structural elucidation of a unique and unexpected difluoroboron complex of the desired glycosidic coupling product along with optimization of the synthetic steps is described.

Glucuronidation is a major pathway for drug metabolism.[1] As a drug candidate advances through clinical development, a synthesis of its glucuronide metabolite(s) often becomes necessary to verify its structure, to provide an analytical standard for use in quantification of metabolite levels in clinical samples, and to provide material for further pharmacological evaluation. The synthesis of structurally complex glucuronides is not always straightforward.[2] The efficiency and scalability of such syntheses is often limited by low yielding or unselective glycosidic couplings, complex protecting group strategies, tedious isolations, or impractical enzymatic reactions.

ABT-751 (**1**, formerly E7010[3]) is currently being evaluated as a treatment for pediatric neuroblastoma.[4] Compound **1** is metabolized in humans to both sulfate **2** and glucuronide **3** and therefore a synthesis of both compounds was recently required to support Phase II clinical studies (Fig. 1). The initial synthesis of glucuronide **3** used *p*-nitrophenol and 1-bromo-2,3,4-triacetylglucuronic acid methyl ester **4** to form the key glycosidic bond followed by five steps to reach the target molecule (Scheme 1).[5] Although this route provided the quantities of material initially required for Phase I clinical studies, a more convergent synthesis that uses the aglycone as the glucuronyl acceptor would be more efficient for supplying larger amounts since **1** is readily available in multi-kilogram quantities.

Glucuronidation of glycosyl acceptors similar to **1** can be particularly problematic due to the presence of multiple basic functional groups.[6] However, we were aware that the use of the Schmidt trichloroacetimidate methodology might overcome this difficulty.[7] Indeed, 2.0 equiv of trichloroacetimidate **5**[8] reacts with **1** promoted by 4.0 equiv of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 to give nearly quantitative conversion of **1** to a 1:1 mixture of two products as determined by HPLC analysis (Scheme 2). After quench of the reaction mixture with aqueous sodium bicarbonate, the two products were separated by silica gel chromatography. One product proved to be the desired compound **6**, whose structure was assigned through X-ray crystallography (Fig. 2).[9] Unfortunately, all attempts to produce crystals of the other product suitable for X-ray crystallography were unsuccessful. However, its structure was determined to be boron complex **7** as deduced from NMR data.

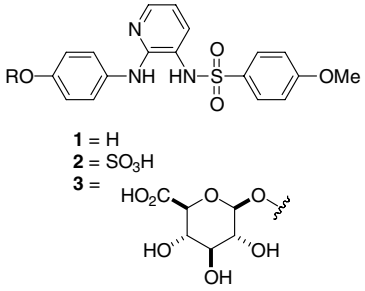

Figure 1. ABT-751 (**1**) and its metabolites (**2** and **3**).

The NMR spectra of **6** and **7** in DMSO-d_6 are significantly different and initially it was assumed that a difference in the glucuronide regiochemistry was responsible for the different NMR spectra.[10] However, a full assignment of the two compounds using 2D NMR methods

* Corresponding author. Tel.: +1 847 938 3183; fax: +1 847 938 2258; e-mail: kenneth.engstrom@abbott.com

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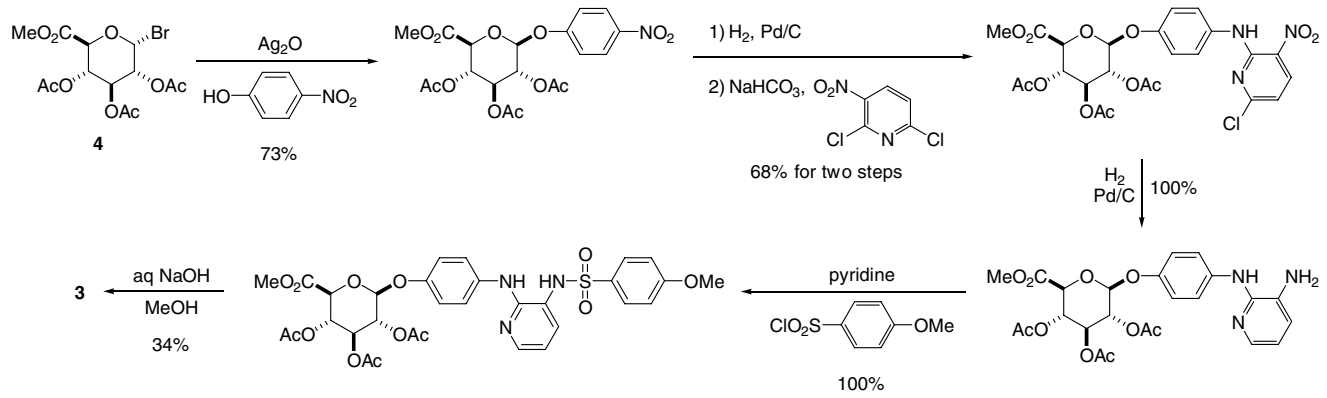
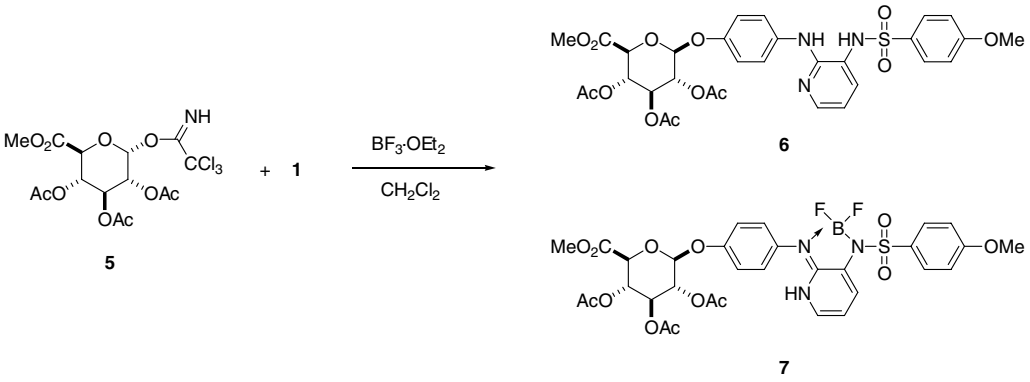


Scheme 1. Initial route to **3**.



Scheme 2. Glucuronidation of **1**.

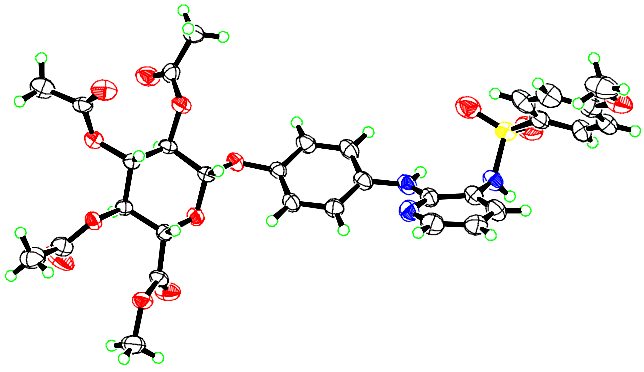


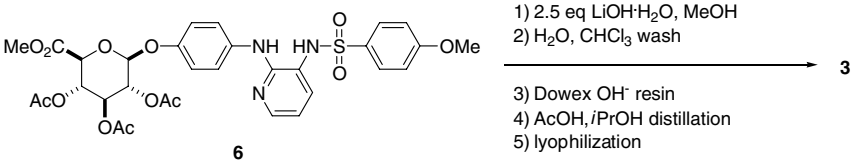
Figure 2. ORTEP of **6** (black = C, green = H, red = O, blue = N, yellow = S).

showed that the glucuronide was attached to the phenolic hydroxyl moiety in both molecules and also that the rest of the carbon skeleton was the same. This led to the hypothesis that **7** might be a boron-fluoro adduct, which was subsequently confirmed by the observation of resonances in the ^{19}F and ^{11}B NMR spectra. The ^{11}B spectrum showed a triplet in CD_3CN ($^1J_{BF} \sim 26$ Hz), while the ^{19}F spectra showed two closely spaced doublets. These data suggest the presence of a BF_2 moiety.

The ^1H and ^{13}C spectra for **6** and **7** show the greatest chemical shift changes ($\Delta\delta$ ppm) for resonances in the pyridine moiety and only one exchangeable NH resonance is observed for **7** in DMSO-d_6 (vide infra), in contrast to **6** where two are observed. Therefore, it is postulated that the structure of **7** is a difluoroboron complex with the BF_2 moiety coordinated to both of the exocyclic nitrogen atoms on the central pyridine ring. This is also consistent with the chemical shift values of the ^{11}B (3.97 ppm in CD_3CN) and ^{19}F (−137.2 and −137.3 ppm in CD_3CN)[10] coordinated to nitrogen, since similar values were observed in a BF_2 moiety complexed to two nitrogen heterocycles in modified BODIPY dyes.[11] Such structures are common for BF_2 complexed to substituted dipyrromethene derivatives (BODIPY), which are typically used as laser dyes[12] or fluorophores in molecular probes.[13] However, this structure is somewhat unusual given that the complexation occurs between two exocyclic nitrogen atoms as opposed to nitrogen heterocycles as is the case of BODIPY dyes and pyridylpyrrolide complexes.[14]

Several tautomers for **7** can be envisaged. However, the structure shown above is likely the lowest energy structure since the only exchangeable proton observed in the spectrum resides primarily on the pyridine nitrogen (12.99 ppm),[10] as shown by observed coupling between this proton and the adjacent proton on the pyridine ring when the sample is cooled in CD_3CN.

Under optimized conditions, greater than 99% conversion of **1** was achieved using 1.3 equiv of trichloroacetimidate **5** and 3.0 equiv of $BF_3{\cdot}OEt_2$ (Scheme 2).



Scheme 3. Deprotection of **6**.

After the reaction was complete, addition of 1.5 equiv of water converted boron complex **7** into the desired product **6**. By HPLC analysis, no significant impurities or isomers were detected in the crude reaction mixture. It is possible that the excess equivalents of BF_3 form a complex with **1**, which effectively deactivates the reactive nitrogen functionality and prevents formation of N-linked isomers. Aqueous sodium bicarbonate workup quenched and removed the boron side products, while silica gel chromatography and recrystallization from methanol removed excess hydrolyzed **5** to give an 83% isolated yield of **6**.

Removal of the acetate groups and hydrolysis of the methyl ester in **6** were accomplished using 2.5 equiv of $LiOH{\cdot}H_2O$ in methanol (Scheme 3).[7] Addition of water and subsequent washing with chloroform removed partially deprotected impurities if any. Treatment with Dowex hydroxide resin adsorbed the carboxylate of **3** and filtration and washing of the resin with water removed the lithium salts. The product was then recovered from the resin by treatment with acetic acid, followed by azeotropic removal of acetic acid with isopropanol. Repeated lyophilization from water removed residual solvents to give amorphous **3** in 72% isolated yield.

In summary, synthesis of the ABT-751 glucuronide **3** using the Schmidt trichloroacetimidate methodology for the direct glucuronidation of the readily available aglycone **1** resulted in an increase in overall yield from 16% using the linear route to 60% using this more convergent route. This increase in efficiency and throughput allowed for more rapid synthesis of the multigram quantities of this metabolite required for clinical use.

Acknowledgements

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9. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC627190. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
10. Data for **6**: ^{1}H NMR (DMSO-d_6 (2.50 ppm), 500 MHz): δ 9.48 (s, 1H, NH), δ 7.94 (dd, $J = 4.9$, 1.7 Hz, 1H), δ 7.85 (s, 1H, NH), δ 7.59 (d, $J = 9.0$ Hz, 2H),δ 7.33 (d, $J = 9.2$ Hz, 2H), δ 7.25 (dd, $J = 7.7$, 1.7 Hz, 1H), δ 6.96 (d, $J = 9.1$ Hz, 2H), δ 6.88 (d, $J = 9.1$ Hz, 2H), δ 6.70 (dd, $J = 4.9$, 7.7 Hz, 1H), δ 5.53 (d, $J = 7.9$ Hz, 1H), δ 5.48 (t, $J = 9.6$ Hz, 1H), δ 5.07 (dd, $J = 9.6$, 7.8, 1H), δ 5.06 (t, $J = 9.6$ Hz, 1H), δ 4.68 (d, $J = 9.9$, 1H) δ 3.71 (s, 3H), δ 3.65 (s, 3H), δ 2.04 (s, 3H), δ 2.01 (s, 3H), δ 2.00 (s, 3H). ^{13}C NMR (DMSO-d_6 (39.5 ppm), 125 MHz): δ 170.0, 169.8, 169.5, 167.6, 163.0, 151.4, 151.4, 145.7, 136.5, 135.3,

131.2, 129.4, 120.9, 118.6, 117.1, 114.9, 114.7, 98.4, 71.6, 71.4, 71.1, 69.6, 56.0, 53.0, 20.8, 20.8, 20.7. Data for **7**: ^{1}H NMR (DMSO-d_6 (2.50 ppm), 500 MHz): δ 12.99 (s, 1H, NH), δ 7.94 (d, $J = 9.1$ Hz, 2H), δ 7.34–7.31 (m, 3H), δ 7.13–7.08 (m, 5H), δ 7.64 (t, $J = 7.1$ Hz, 1H), δ 6.72 (d, $J = 7.9$ Hz, 1H), δ 5.49 (t, $J = 9.7$ Hz, 1H), δ 5.13 (dd, $J = 7.9$, 9.7 Hz, 1H), δ 5.08 (t, $J = 9.7$ Hz, 1H), δ 4.75 (d, $J = 9.9$ Hz, 1H), δ 3.82 (s, 3H), δ 3.64 (s, 3H), δ 2.03 (s, 3H), δ 2.02 (s, 3H), δ 2.01 (s, 3H). ^{13}C NMR (DMSO-d_6 (39.5 ppm), 125 MHz): δ 169.5, 169.3, 169.0, 167.1, 162.5, 154.9, 146.7, 131.6, 131.4, 129.3, 126.8, 124.5, 117.3, 116.5, 114.2, 111.4, 96.8, 71.0, 70.9, 70.4, 69.0, 67.0, 55.6, 52.6, 20.3, 20.3, 20.2.

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