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Gram Scale Synthesis of the Glucuronide Metabolite of ABT-724

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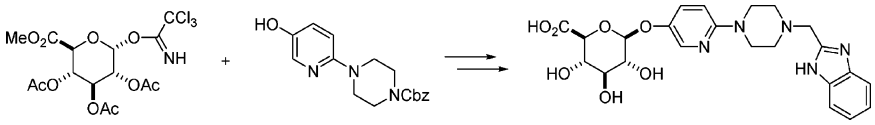


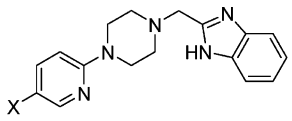
A gram scale synthesis of the glucuronide metabolite of ABT-724 is reported. Glycosidic coupling between a trichloroacetimidate glucuronyl donor and a Cbz-protected hydroxypyridylpiperazine glycosyl acceptor is the key step in the synthesis, since attempts to directly glucuronidate the aglycon, aglycon derivatives, and other truncated glycosyl acceptors were unsuccessful. The route was used to produce 2.1 g of metabolite in eight steps from 2-chloro-5-hydroxypyridine in 21% overall yield.

Introduction

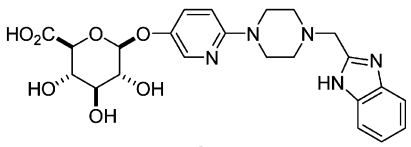
Glucuronidation is a major pathway for drug metabolism.[1] As a drug candidate advances through clinical development, a synthesis of its glucuronide metabolite(s) often becomes necessary to verify its structure, to provide an analytical standard for use in quantification of metabolite levels in clinical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

ABT-724 (**1**), a potent selective D_4 dopamine receptor agonist, has been identified as a promising treatment for erectile dysfunction.[3] ABT-724 is oxidatively metabolized to the hydroxy analogue **2**, which is subsequently glucuronidated to give **3**.[4] As part of the pharmacological evaluation of ABT-724, a synthesis of glucuronide **3** became necessary to confirm its structure, to provide an analytical standard, and to supply gram quantities of material for further pharmacological studies.

The synthesis of **3** presented significant challenges. In general, the use of glucuronic acid derived glycosyl donors is often



1 X = H
2 X = OH



3

inefficient due to the destabilizing effect the C-6 electron-withdrawing group has on the glycosidic bond forming event.[2] In addition, glycosyl acceptors such as **2** can be inefficient coupling partners because of the presence of multiple basic functional groups.[5] Furthermore, we required a glycosidic

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(1) For a review of the importance of glucuronides in regulation of the biological activity of pharmaceuticals, see: Mulder, G. J. *J. Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 25−49.

(2) For reviews on the chemical synthesis of glucuronides, see: (a) Stachulski, A. V.; Jenkins, G. A. *Nat. Prod. Rep.* **1998**, 173−186. (b) Kaspersen, F. M.; van Boeckel, C. A. A. *Xenobiotica* **1987**, *17*, 1451−1471.

(3) (a) Brioni, J. D.; Moreland, R. B.; Cowart, M.; Hsieh, G. C.; Stewart, A. O.; Hedlund, P.; Donnelly-Roberts, D. L.; Nakane, M.; Lynch, J. J., III; Kolasa, T.; Polakowski, J. S.; Osinski, M. A.; Marsh, K.; Andersson, K. E.; Sullivan, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 6758−6763. (b) Cowart, M.; Latshaw, S. P.; Bhatia, P.; Daanen, J. F.; Rohde, J.; Nelson, S. L.; Patel, M.; Kolasa, T.; Nakane, M.; Uchic, M. E.; Miller, L. N.; Terranova, M. A.; Chang, R.; Donnelly-Roberts, D. L.; Namovic, M. T.; Hollingsworth, P. R.; Martino, B. R.; Lynch, J. J., III; Sullivan, J. P.; Hsieh, G. C.; Moreland, R. B.; Brioni, J. D.; Stewart, A. O. *J. Med. Chem.* **2004**, *47*, 3853−3864.

(4) Patel, M. V.; Kolasa, T.; Mortell, K.; Matulenko, M.; Hakeem, A.; Rohde, J.; Nelson, S.; Cowart, M.; Nakane, M.; Miller, L.; Uchic, M.; Terranova, M.; El-Kouhen, O. F.; Donnelly-Roberts, D. L.; Namovic, M. T.; Hollingsworth, P.; Chang, R.; Martino, B.; McVey, J.; Marsh, K.; Martin, R.; Darbyshire, J. F.; Gintant, G.; Hsieh, G.; Moreland, R. B.; Sullivan, J.; Brioni, J. D.; Stewart, A. O. *J. Med. Chem.* Accepted for publication.

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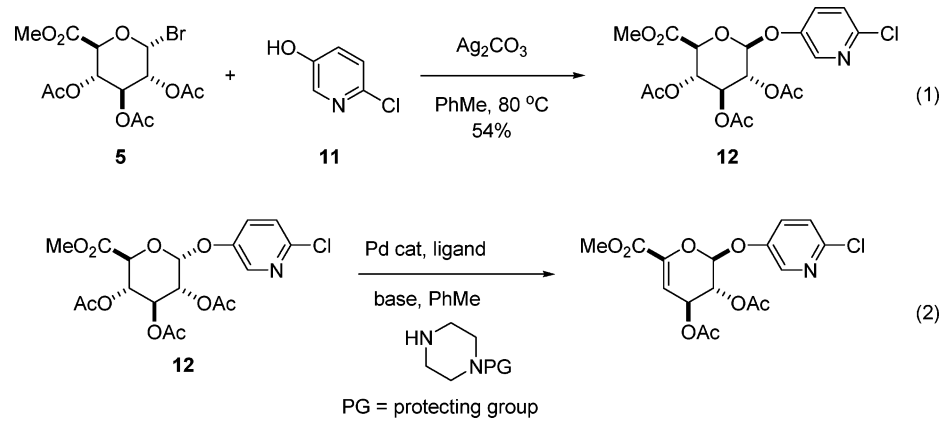
SCHEME 1

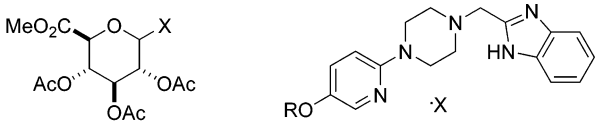


coupling that is selective for the β-anomer, a donor that is readily available in gram quantities, and a practical method for the isolation of the fully deprotected metabolite.

Results and Discussion

1,2,3,4-Tetra-*O*-acetyl-β-D-glucuronic acid methyl ester (**4**) was chosen as a convenient glucuronyl donor starting material for several reasons. Glycosyl donors containing C-2 ester protecting groups often result in β-selective glycosidic bond formation.[2] In addition, **4** is commercially available in significant quantities[6] and the 1-α-bromide (**5**), hemiacetal (**6**), and 1-α-trichloroacetimidate (**7**) derivatives could all be prepared from **4** in one or two steps with literature procedures.[7−9] Easy access to these four distinct glucuronyl donors would allow a wide range of coupling methods and conditions to be quickly surveyed. Bromide **5** can be activated for glycosidic coupling by using a variety of heavy metal salts, whereas acetate **4** and trichloroacetimidate **7** can be activated with acid catalysts.[10] Hemiacetal **6** can be used as a glucuronyl donor under either acidic[10] or Mitsonobu[11] coupling conditions.

The most direct route to glucuronide **3** would use aglycon **2** as the glycosyl acceptor. However, glycosidic coupling between **2** and **4**, **5**, **6**, or **7** failed under all conditions tested. The poor reactivity of **2** as the glycosyl acceptor was attributed to the presence of multiple basic functional groups and its poor solubility in common organic solvents. Bis-borane complex **8** and zinc complex **9** were prepared to attenuate the basicity of the acceptor, while tributylstannane **10**[12] was intended to increase the solubility. Unfortunately, **8**, **9**, and **10** all failed to couple with **4**, **5**, **6**, or **7** under a variety of typical coupling conditions.



4 X = β-OAc
5 X = α-Br
6 X = OH
7 X = α-OC(=NH)CCl3

8 R = H, X = 2 BH3
9 R = H, X = ZnCl2
10 R = SnBu3, no X

Truncated versions of aglycon **2** were then evaluated. 2-Chloro-5-hydroxypyridine **11** was coupled with **5** using Ag_2CO_3 to give **12** in approximately 54% yield (Scheme 1, eq 1). However, Hartwig−Buchwald amination[13] of **12** led exclusively to elimination of the C-4 acetate group to give the α,β-unsaturated ester[14] despite repeated attempts with different piperazines, bases, ligands, and catalyst loadings (Scheme 1, eq 2).

Glycosidic coupling with piperazine-containing acceptors was then evaluated. *N*-Protected hydroxypyridylpiperazines **13**, **14**, and **15** were synthesized and tested in various coupling reactions with donors **4**−**7**. Glycosyl acceptors **13** and **14**, containing the Boc and benzyl protecting groups, respectively, failed to give desired product in greater than 10% yield with any of the four donors. Cbz-protected acceptor **15** likewise did not couple with **4**, **5**, and **6** in useful yield. However, reaction of **15** with 1 equiv

(5) For the synthesis of other structurally related glucuronides, see: (a) Kim, S.; Wu, J. Y.; Zhang, Z.; Tang, W.; Doss, G. A.; Dean, B. J.; DiNinno, F.; Hammond, M. L. *Org. Lett.* **2005**, *3*, 411−414. (b) Liu, C.-H.; Liu, H.; Han, X.-Y.; Wu, B.; Zhong, B.-H.; Gong, Z.-H. *Synth. Commun.* **2005**, *5*, 701−710. (c) Stazi, F.; Palmisano, G.; Turconi, M.; Clini, S.; Santagostino, M. *J. Org. Chem.* **2004**, *4*, 1097−1103. (d) Kawamura, K.; Horikiri, H.; Hayakawa, J.; Seki, C.; Yoshizawa, K.; Umeuchi, H.; Nagase, H. *Chem. Pharm. Bull.* **2004**, *6*, 670−671. (e) Kuo, F.; Gillespie, T. A.; Kulanthaivel, P.; Lantz, R. J.; Ma, T. W.; Nelson, D. L.; Threlkeld, P. G.; Wheeler, W. J.; Yi, P.; Zmijweski, M. *Bioorg. Med. Chem. Lett.* **2004**, *13*, 3481−3486. (f) Wang, Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. *Bioorg. Med. Chem.* **2003**, *7*, 1569−1576. (g) Hasuoka, A.; Nakayama, Y.; Adachi, M.; Kamiguchi, H.; Kamiyama, K. *Chem. Pharm. Bull.* **2001**, *12*, 1604−1608. (h) Rukhman, I.; Yudovich, L.; Nisnevich, G.; Gutman, A. L. *Tetrahedron* **2001**, *6*, 1083−1092 and references therein. (i) Brown, R. T.; Carter, N. E.; Mayalarp, S. P.; Scheinmann, F. *Tetrahedron* **2000**, *56*, 7591−7594. (j) Suzuki, T.; Mabuchi, K.; Fukazawa, N. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 659−662. (k) Dodge, J. A.; Lugar, C. W.; Cho, S.; Osborne, J. J.; Philips, D. L.; Glasebrook, A. L.; Frolik, C. A. *Bioorg. Med. Chem. Lett.* **1997**, *8*, 9993−996.

(6) 50 g obtained from Toronto Research Chemicals, Inc.

(7) Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310−3315.

(8) Nudelman, A.; Herzig, J.; Gottlieb, H. E.; Keinan, E.; Sterling, J. *Carbohydr. Res.* **1987**, *162*, 145−152.

(9) Jacquinet, J. C. *Carbohydr. Res.* **1990**, *199*, 153−181.

(10) Use of bromide **5**, acetate **4**, trichloroacetimidate **7**, and hemiacetal **6** in glucuronidation has been extensively reviewed. For leading references, see refs 2a and 2b.

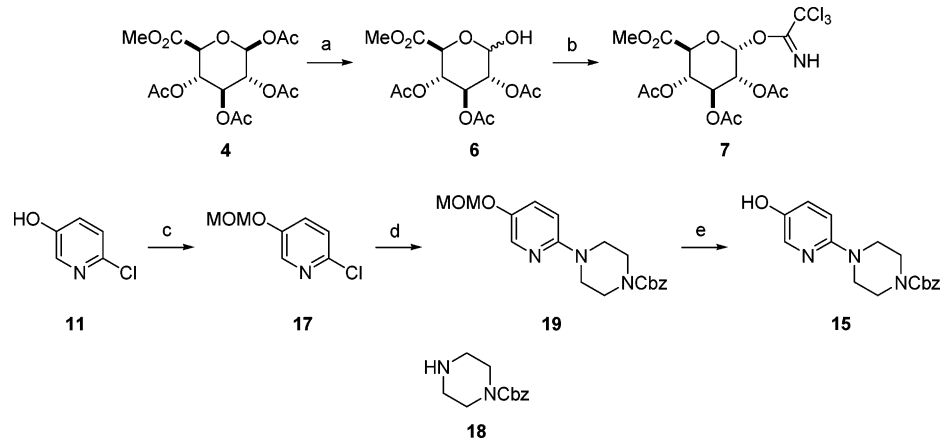
(11) Badman, G. T.; Green, D. V. S.; Voyle, M. *J. Organomet. Chem.* **1990**, *338*, 117−121.

(12) For use of tributyltin phenoxides as glycosyl acceptors, see: (a) Clerici, F.; Gelmi, M. L.; Mottadelli, S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 985−988. (b) Hannessian, S.; Saavedra, O. M.; Xie, F.; Amboldi, N.; Battistini, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 439−442.

(13) (a) Yang, B. H.; Buchwald, S. L. *J. Organomet. Chem.* **1999**, *576*, 125−146. (b) Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215−7216.

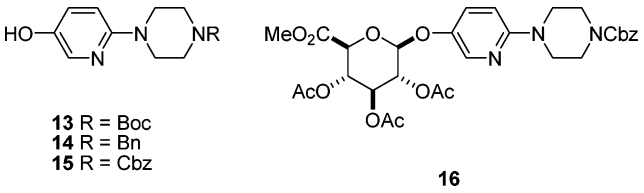
(14) For an example of intentional elimination of the C-4 acetate group with strong base, see: Adamczyk, M.; Chen, Y.-Y.; Fishpaugh, J. R. *Org. Prep. Proced. Int.* **1992**, *24*, 546−548.

SCHEME 2[a]



[a] Reagents and conditions: (a) Bu_3SnOMe, THF, 60 °C, ref 8; (b) Cl_3CCN, DBU, DCE, rt, ref 9; (c) MOMCl, K_2CO_3, DMF, 0 °C to rt, 72%; (d) 2.0 mol % of $Pd(OAc)_2$, 4.4 mol % of Et_3N, 2.4 mol % of *rac*-BINAP, **18**, NaO*t*Bu, PhMe, rt, 95%; (e) 2M HCl_{aq},THF, 55 °C, 91%.

of trichloroacetimidate **7** promoted by $BF_3{\cdot}OEt_2$ in CH_2Cl_2 gave the desired product **16** in approximately 50% yield. While



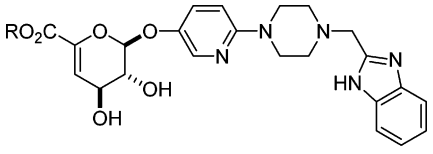
reaction of **13** with **7** resulted in a complex mixture due to the instability of the Boc group to BF_3, reaction of **14** with **7** was prevented by formation of an unreactive precipitate that is presumably a complex formed by reaction of the tertiary piperazine nitrogen with BF_3. It appears that reaction between **15** and **7** succeeds because the Cbz group in **15** is stable under the reaction conditions and attenuates the basicity of the piperazine. Use of **15** as the glycosyl acceptor is therefore the key to gaining synthetic access to glucuronide **3**, the complete synthesis of which is detailed below.

Gram scale synthesis of **3** began with the synthesis of coupling partners **7** and **15** (Scheme 2). Trichloroacetimidate **7** was prepared from acetate **4** in two steps according to literature procedures (Scheme 2).[8,9] To prepare glycosyl acceptor **15**, the phenolic hydroxyl group of 2-chloro-5-hydroxypyridine (**11**)[15] was first protected as a methoxymethyl ether (**17**) in 72% yield. Hartwig−Buchwald amination of **17** with Cbz-protected piperazine **18**, which proceeded at room temperature with just a 2% catalyst loading, afforded pyridyl piperazine **19** in 95% yield. Removal of the methoxymethyl ether with aqueous HCl gave glycosyl acceptor **15** in 91% yield and 62% overall yield from **11**.

Under optimized conditions, acceptor **15** was glycosylated by using 2 equiv of glucuronyl donor **7** promoted by 4 equiv of $BF_3{\cdot}OEt_2$ to give glucuronide **16** in 75% yield[16] with the required anomeric configuration (Scheme 3).[17] The product was isolated in approximately 80% purity[18] after silica gel chromatography and was contaminated with a significant amount of hydrolyzed donor (**6**) and several other unidentified impurities. Fortunately, this mixture could be taken on to the next step without further purification.

Removal of the Cbz group under hydrogenolysis conditions gave amine **20**[19] in 83% isolated yield (Scheme 3). The product was isolated by silica gel chromatography in 96% purity[18] free of **6** carried over from the previous reaction. Alkylation of amine **20** with 2-(chloromethyl)benzimidazole (**21**) in NMP with triethylamine as base gave a 77% yield[16] of protected glucuronide **22**. After aqueous workup to remove NMP, the product was purified by silica gel chromatography and then recrystallized from ethyl acetate to give **22** in 62% isolated yield from amine **20**.

Removal of the acetate groups from **22** was accomplished by using catalytic LiOH in methanol at −10 °C with warming to 23 °C, which minimized formation of the α,*β*-unsaturated ester side product **24** and gave methyl ester **23**.[20] Methyl ester hydrolysis was then accomplished by addition of 1.9 equiv of LiOH. Fortunately, α,*β*-unsaturated methyl ester **24** hydrolyzed at a significantly slower rate than methyl ester **23**. Because of this rate difference, the formation of α,*β*-unsaturated acid **25** could be minimized by a timely quench of the reaction mixture with 2 equiv of acetic acid. After addition of water, chloroform extraction removed the small amount of remaining unhydrolyzed methyl esters **23** and **24**. The aqueous phase was then treated



with Dowex hydroxide resin to adsorb the carboxylate of **3**.[21] Filtration and washing of the resin with methanol and water removed the lithium salts. The product was recovered from the

(15) For the synthesis of 2-chloro-5-hydroxypyridine, see: Lynch, J. K.; Holladay, M. W.; Ryther, K. B.; Bai, H.; Hsiao, C.; Morton, H. E.; Dickman, D. A.; Arnold, W.; King, S. A. *Tetrahedron*: *Asymmetry* **1998**, *9*, 2791−2794.

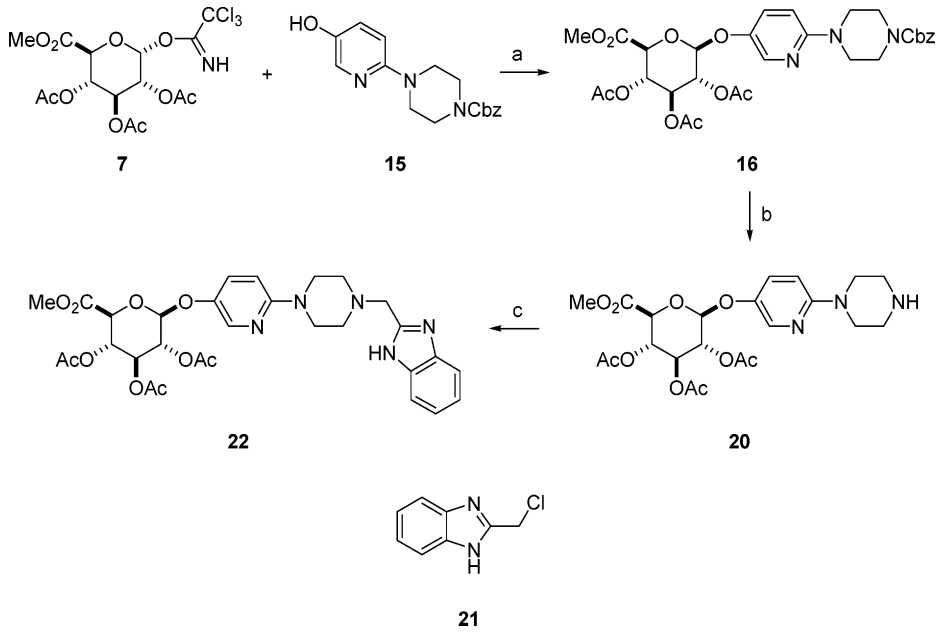
(16) Yield was determined by HPLC weight percent assay using a pure standard.

(17) The α-anomer was not isolated or identified.

(18) Purity was determined by HPLC weight percent assay versus a pure standard.

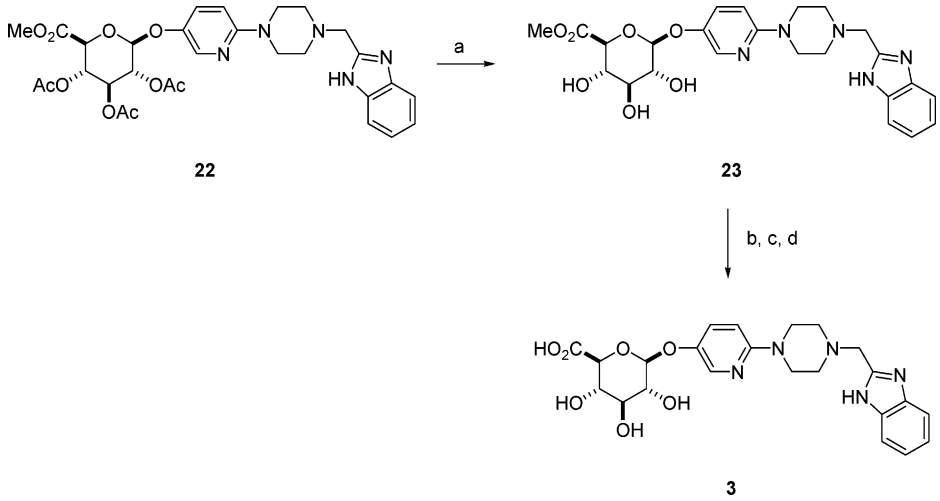
(19) The relative stereochemistry of **20** was confirmed by a rOe experiment.

SCHEME 3[a]



[a] Reagents and conditions: (a) 2.0 equiv of **7**, 1.0 equiv of **15**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −60 °C to rt, 41 h, 75%; (b) 5% Pd/C, H_2, MeOH, rt, 83%; (c) (1) **21**, Et_3N, NMP, 0 °C to rt, (2) EtOAc, rt, 62% from **20**.

SCHEME 4[a]



[a] Reagents and conditions: (a) 0.1 equiv of LiOH, MeOH, −10 °C to rt; (b) 1.9 equiv of LiOH, MeOH, rt; (c) 2.0 equiv of AcOH, H_2O, $CHCl_3$ wash; (d) Dowex hydroxide resin, filtration, wash, then $AcOH_{aq}$, 87% from **22**.

resin by treatment with aqueous acetic acid, which was then removed by azeotropic distillation with 2-propanol and heptane. This deprotection and isolation procedure gave glucuronide **3** as an amorphous solid[22] in 87% overall yield. The current synthesis was used to produce 2.1 g of glucuronide **3** in 21% isolated yield from 2-chloro-5-hydroxypyridine **11** (8 chemical steps, average yield of 82% per step).

(20) For another mild deprotection method that uses Na_2CO_3 in aqueous methanol, see: Brown, R. T.; Scheinmann, F.; Stachulski, A. V. *J. Chem. Res.* (*S*) 1997, 370−371.

(21) Use of hydroxide resin was critical for removal of lithium because the carboxylate form of **3** would not adsorb to chloride resin. For a table of selectivity coefficients of various anions on Dowex anion-exchange resins, consult www.dowex.com.

(22) Attempts to crystallize **3** were unsuccessful. The final amorphous solid was contaminated with 1−2% of α,β unstaurated acid **25** as measured by HPLC at 260 nm.

Conclusions

In summary, although direct glucuronidation of the aglycon **2** and its derivatives was unsuccessful, synthetic access to **3** was realized by development of a three-step synthesis of appropriately protected glycosyl acceptor **15** and its efficient glycosidation, using the readily available glucuronyl donor **7**. Subsequent transformations installed the benzimidazole moiety and a carefully controlled final deprotection and isolation sequence allowed gram quantities of glucuronide **3** to be produced for pharmacological evaluation.

Experimental Section

2-Chloro-5-methoxymethoxypyridine (17). To a 500 mL round-bottom flask is charged 2-chloro-5-hydroxypyridine **11** (20.00 g, 154 mmol, 1.0 equiv) followed by DMF (200 mL). The solution is

cooled to −10 °C and milled K_2CO_3 (42.68 g, 309 mmol, 2.0 equiv) is added. Two minutes after addition of the K_2CO_3, chloromethyl methyl ether (MOMCl, 11.7 mL, 154 mmol, 1.0 equiv) is charged. After the reaction has been allowed to warm to ambient temperature over 19 h, more MOMCl (5.9 mL, 77 mmol, 0.5 equiv) is added, followed by another portion of MOMCl (4.7 mL, 62 mmol, 0.4 equiv) 1 h later. After an additional 2 h the reaction is complete as determined by HPLC analysis (greater than 95% conversion of **11**). H_2O (100 mL) is added followed by EtOAc (100 mL). This mixture is transferred to a separatory funnel and diluted with more H_2O (400 mL) and EtOAc (400 mL). The layers are separated and the organic layer is washed once with H_2O (250 mL). The organic layer is dried with Na_2SO_4, filtered, and concentrated, and the residue is purified by silica gel chromatography (100% hexanes grading to 5% EtOAc in hexanes in 1% increments) to afford the product **17** (19.12 g, 71.5%) as a clear colorless liquid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, J = 3.0 Hz, 1H), 7.51 (dd, J = 8.8, 3.2 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 5.23 (s, 2H), 3.47 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 154.0, 143.7, 138.9, 128.0, 125.5, 95.8, 56.5; FTIR (microscopic technique) 2960, 2910, 1570, 1460, 1375, 1255, 1230, 1205, 1155, 1115, 1085, 1015, 980, 925, 830 cm^{-1}; EI HRMS for $C_7H_8ClNO_2$ (M + H$^+$) calcd 173.0244, found 173.0246.

4-(5-Methoxymethoxypyridin-2-yl)piperazine-1-carboxylic Acid Benzyl Ester (19). To an oven-dried 500 mL three-necked round-bottom flask under an atmosphere of N_2 is charged $Pd(OAc)_2$ (590 mg, 2.63 mmol, 0.020 equiv), racemic BINAP (1.97 g, 3.16 mmol, 0.024 equiv), and toluene (50 mL). Triethylamine (0.81 mL, 5.79 mmol, 0.044 equiv) is added and the mixture is allowed to stir for 90 min before charging benzyl 1-piperazinecarboxylate **18** (25.4 mL, 132 mmol, 1.2 equiv), **17** (19.04 g, 110 mmol, 1.0 equiv), and toluene (114 mL). After an additional 10 min, sodium *tert*-butoxide (12.65 g, 132 mmol, 1.2 equiv) is added followed by toluene (100 mL). The reaction exotherms slowly from 23 to 35 °C over approximately 30 min and is stirred for an additional 19 h at ambient temperature until **17** is completely consumed as determined by HPLC analysis. Aqueous NaCl (50%, 125 mL) solution is added slowly. The mixture is transferred to a separatory funnel and more toluene (100 mL) and 50% aqueous NaCl solution (125 mL) are added. The layers are separated and the organic layer is washed once with 20% aqueous NaCl solution (250 mL). The organic layer is then dried with Na_2SO_4, filtered, and concentrated, and the residue is purified by silica gel chromatography (100% hexanes grading to 30% EtOAc in hexanes in 5% increments) to afford the product **19** (28.70 g, 95.4%) as a crystalline off-white solid: mp 59 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 3.0 Hz, 1H), 7.33 (m, 6H), 6.80 (d, J = 9.2 Hz, 1H), 5.14 (s, 2H), 5.09 (s, 2H), 3.60 (br s, 4H), 3.45 (s, 3H), 3.40 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.3, 153.9, 145.5, 136.3, 136.0, 127.9, 127.4, 127.1, 126.9, 107.8, 94.9, 66.1, 55.4, 45.3, 43.1; FTIR (microscopic technique) 2880, 2830, 1685, 1265, 1250, 1155, 1125, 980, 735, 695 cm^{-1}; FAB HRMS for $C_{19}H_{23}N_3O_4$ (M + H$^+$) calcd 357.1689, found 357.1676.

4-(5-Hydroxypyridin-2-yl)piperazine-1-carboxylic Acid Benzyl Ester (15). A 500 mL round-bottom flask is charged with **19** (25.00 g, 69.9 mmol) followed by THF (50 mL), aqueous 2 M HCl (110 mL), and more THF (55 mL). The reaction is heated to 55 °C for 2 h and is complete when all of **19** is consumed as determined by HPLC analysis. After cooling to room temperature, the reaction mixture is poured into saturated aqueous $NaHCO_3$ solution (300 mL) and stirred for 15 min. The mixture is transferred to a separatory funnel, CH_2Cl_2 (300 mL) is added, and the layers are separated. The aqueous layer is washed once with 100 mL of CH_2Cl_2. The combined organics are dried with Na_2SO_4, filtered, and concentrated. The residue is transferred to a 250 mL round-bottom flask with EtOAc (100 mL) and concentrated to an oil. EtOAc (50 mL) is added to the residue and the resulting suspension is stirred for 16 h, during which time crystals may form. Heptane (150 mL) is added over 30 min to precipitate out the product. The suspension is cooled to 0 °C for 1 h and then filtered. The wet cake is washed once with heptane (50 mL). As determined by HPLC assay, 1.00 g (7.0%) of **15** is lost to the combined filtrate and washes. The wet cake is dried in a vacuum oven at 50 °C and 20 mmHg for 48 h to afford the product **15** (20.58 g, 97.0% purity, 91.2% purity adjusted yield) as an off-white crystalline solid: mp 92 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 7.74 (d, J = 3.0 Hz, 1H), 7.33 (m, 5H), 7.07 (dd, J = 8.9, 3.0 Hz, 1H), 6.73 (d, J = 8.9 Hz, 1H), 5.10 (s, 2H), 3.49 (br s, 4H), 3.29 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.8, 152.5, 146.1, 136.3, 133.6, 127.9, 127.4, 127.1, 124.9, 108.3, 66.1, 45.9, 43.2; FTIR (microscopic technique) 3350, 2850, 1675, 1490, 1440, 1365, 1270, 1240, 1130, 935, 760, 700 cm^{-1}; FAB HRMS for $C_{17}H_{20}N_3O_3$ (M + H$^+$) calcd 314.1505, found 314.1518.

1-β-(4-(5-Hydroxypyridin-2-yl)-piperazine-1-carboxylic acid benzyl ester)-2,3,4-tri-*O*-acetyl-D-glucuronic Acid Methyl Ester (16). To a 200 mL flame-dried Schlenk flask under an atmosphere of N_2 is added trichloroacetimidate **7** (19.16 g, 40.0 mmol, 2.0 equiv) and **15** (6.27 g, 20.0 mmol, 1.0 equiv). Toluene (50 mL) is added and the solvent is removed in vacuo to azeodry the solids. After drying for 1 h under vacuum, CH_2Cl_2 (192 mL) is added and the solution is cooled to −60 °C. $BF_3{\cdot}OEt_2$ (10.15 mL, 80.1 mmol, 4.0 equiv) is added and the reaction is allowed to warm to −20 °C over 1 h. The reaction is then warmed from −20 to 23 °C over 16 h at approximately 3 deg per hour and then stirred for an additional 24 h at ambient temperature. When 99% of **15** has been consumed as determined by HPLC assay, the reaction mixture is poured into 50% saturated aqueous $NaHCO_3$ solution (200 mL) to quench the remaining $BF_3{\cdot}OEt_2$. The mixture is transferred to a separatory funnel and diluted with CH_2Cl_2 (100 mL), and the layers are separated. The aqueous layer is washed once with CH_2Cl_2 (50 mL). The combined organic layers are washed once with 50% saturated NaCl solution (200 mL), the layers are separated, and the aqueous layer is washed once with CH_2Cl_2 (50 mL). The combined organic layers are dried with Na_2SO_4, filtered, concentrated, and redissolved in EtOAc. As determined by HPLC assay, the EtOAc solution contains 9.40 g (74.7%) of **16**. The solution is concentrated and the residue purified by silica gel chromatography (100% hexanes grading to 55% EtOAc in hexanes in 5% increments) to afford the product **16** (11.86 g, 79.3% purity, 74.7% purity adjusted yield) as an off-white foam that is taken on to the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 3.0 Hz, 1H), 7.34 (m, 6H), 6.79 (d, J = 9.2 Hz, 1H), 5.42 (t, J = 9.4 Hz, 1H), 5.24 (d, J = 7.8 Hz, 1H), 5.18 (t, J = 9.6 Hz, 1H), 5.14 (m, 3H), 4.41 (d, J = 10.0 Hz, 1H), 3.71 (s, 3H), 3.60 (br s, 4H), 3.44 (m, 4H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 168.5, 168.4, 166.4, 155.0, 153.8, 145.0, 136.3, 136.2, 127.9, 127.5, 127.4, 127.1, 107.7, 98.4, 70.9, 70.7, 70.3, 68.8, 66.1, 52.5, 45.1, 43.0, 20.5, 20.4, 20.3; FTIR (microscopic technique) 2950, 1755, 1700, 1485, 1430, 1370, 1225, 1125, 1075, 1045, 980 cm^{-1}; FAB HRMS for $C_{30}H_{36}N_3O_{12}$ (M + H$^+$) calcd 630.2299, found 630.2316.

1-β-(4-(5-Hydroxypyridin-2-yl)piperazine)-2,3,4-tri-*O*-acetyl-D-glucuronic Acid Methyl Ester (20). To a hydrogenation vessel is added **16** (9.40 g, 11.86 g of 79.3% purity, 14.9 mmol) followed by MeOH (94 mL). The reactor is purged with N_2 and 5 wt % Pd on carbon (940 mg) is added. The reactor is pressurized to 40 psi with H_2. After 8 h at ambient temperature and 99% conversion of **16** as determined by HPLC assay, the reaction is filtered and the wet cake washed twice with MeOH (10 mL portions). The organic solution is concentrated and the residue is purified by silica gel chromatography (100% CH_2Cl_2 grading to 5% MeOH in CH_2Cl_2 in 1% increments followed by grading to 20% MeOH in CH_2Cl_2 in 5% increments) to afford the product **20** (6.45 g, 96.0% purity, 83.7% purity adjusted yield) as a yellow foam that is taken on to the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 3.0 Hz, 1H), 7.35 (dd, J = 9.1, 3.0 Hz, 1H), 6.78 (d, J = 9.2 Hz, 1H), 5.42 (t, J = 9.4 Hz, 1H), 5.24 (d, J = 7.8 Hz, 1H), 5.19 (t, J = 9.6, 1H), 5.15 (dd, J = 9.5, 7.8 Hz, 1H), 4.42 (d, J = 9.9 Hz, 1H), 3.72 (s, 3H), 3.44 (m, 4H), 3.96 (m,

4H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); an rOe experiment showed significant cross-peaks between the anomeric proton, H_3, and H_5; ^{13}C NMR (100 MHz, CD_3OD) δ 170.7, 170.5, 170.3, 168.3, 157.4, 147.2, 137.9, 129.4, 109.3, 100.9, 73.1, 72.9, 72.4, 70.6, 53.3, 47.2, 45.9, 20.7, 20.7, 20.6; FTIR (microscopic technique) 2950, 2830, 1760, 1490, 1380, 1225, 1140 cm^{-1}; FAB HRMS for $C_{22}H_{30}N_3O_{10}$ ($M + H^+$) calcd 496.1931, found 496.1917.

1-β-Hydroxy-ABT-724-2,3,4-tri-*O*-acetyl-D-glucuronic Acid Methyl Ester (22). A 100 mL round-bottom flask is charged with **20** (6.19 g, 6.45 g of 96.0% purity, 12.5 mmol, 1.0 equiv) followed by NMP (31 mL). The solution is cooled to 0 °C and 2-(chloromethyl)benzimidazole **21** (2.08 g, 12.5 mmol, 1.0 equiv) is added. The reaction is warmed to ambient temperature. After 1 h, the reaction is cooled to 0 °C and triethylamine (1.74 mL, 12.5 mmol, 1.0 equiv) is added. After 30 min, the reaction is warmed to ambient temperature. Ninety minutes after reaching ambient temperature more triethylamine (0.35 mL, 2.5 mmol, 0.20 equiv) is added. The reaction is stirred for an additional 15 h at ambient temperature. When 95% of **21** has been consumed as determined by HPLC assay, the reaction is diluted with H_2O (120 mL), transferred to a separatory funnel, and diluted with EtOAc (120 mL). The layers are separated and the organic layer is washed first with 10% saturated aqueous NaCl solution (100 mL) and then 5% saturated aqueous NaCl solution (100 mL). As determined by HPLC assay, 0.28 g (3.6%) of **15** is lost to the combined aqueous washes. The organic layer is dried with Na_2SO_4 and filtered. As determined by HPLC assay, the EtOAc solution contains 6.04 g (77.3%) of product **22**. The solution is concentrated and the residue purified by silica gel chromatography (100% EtOAc to 5% MeOH in EtOAc in 1% increments) to afford the product **22** (6.15 g, 93.4% purity, 73.5% purity adjusted yield) as an off-white solid. To the solids is added EtOAc (48 mL). After 6 h the suspension is filtered, washed once with 25% EtOAc in hexanes (48 mL), and dried at 50 °C and 20 mmHg for 3 d to give **22** (4.93 g, 100% purity, 63.0% isolated yield from **20**) as a semicrystalline white powder: mp 93 °C; ^1H NMR (400 MHz, CD_3OD) δ 7.90 (d, $J = 2.9$ Hz, 1H), 7.53 (br s, 2H), 7.33 (dd, $J = 9.1$, 3.0 Hz, 1H), 7.21 (m, 2H), 6.77 (d, $J = 9.2$ Hz, 1H), 5.41 (t, $J = 9.4$ Hz, 1H), 5.22 (d, $J = 7.8$ Hz, 1H), 5.18 (t, $J = 9.7$ Hz, 1H), 5.14 (dd, $J = 9.3$, 7.8 Hz, 1H), 4.41 (d, $J = 9.9$ Hz, 1H), 3.84 (s, 2H), 3.71 (s, 3H), 3.49 (m, 4H), 2.65 (m, 4H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.8, 168.5, 168.3, 166.4, 155.3, 150.9, 144.8, 142.4, 136.3, 133.9, 127.4, 121.4, 120.5, 118.0, 110.8, 107.5, 98.4, 70.9, 70.7, 70.3, 68.8, 55.7, 52.5, 52.5, 45.2, 20.4, 20.4, 20.3; FTIR (microscopic technique) 3400, 2950, 2830, 1755, 1490, 1455, 1410, 1385, 1230, 1095, 1055 cm^{-1}; FAB HRMS for $C_{30}H_{36}N_5O_{10}$ ($M + H^+$) calcd 626.2462, found 626.2469; $[\alpha]^{23}_D$ −16.7 (*c* 2.235, MeOH).

1-β-Hydroxy-ABT-724-D-glucuronic Acid (3). A 500 mL round-bottom flask is charged with **22** (3.00 g, 4.80 mmol, 1.0 equiv) followed by MeOH (120 mL). The solution is cooled to −10 °C, lithium hydroxide monohydrate (0.0201 g, 0.480 mmol, 0.10 equiv) is added, and the reaction is gradually warmed to ambient temperature over 1 h. After 4 h at ambient temperature and when greater than 99% of **22** has been consumed as determined by HPLC assay, additional lithium hydroxide monohydrate (0.382 g, 9.12 mmol, 1.9 equiv) is added at ambient temperature. After 19 h and when greater than 99% of **23** has been consumed as determined by HPLC assay, acetic acid (0.27 mL, 9.6 mmol, 2.0 equiv) is added. The reaction is diluted with H_2O (60 mL) and $CHCl_3$ (120 mL) and transferred to a separatory funnel. The 500 mL round-bottom flask is rinsed with additional H_2O (60 mL) and $CHCl_3$ (120 mL), which are also transferred to the separatory funnel. The layers are separated. The aqueous layer is washed with chloroform (240 mL). As determined by HPLC assay, no product **3** is lost to the $CHCl_3$ washes. The aqueous layer is transferred to a 250 mL round-bottom flask. The separatory funnel is rinsed four times with MeOH (10 mL portions) and the rinses transferred to the 250 mL round-bottom flask. DOWEX 550A OH anion-exchange resin (30.0 g) is added and the reaction is stirred at ambient temperature for 18 h. The resin is collected by filtration and washed sequentially with MeOH (100 mL) and H_2O (100 mL). As determined by HPLC assay, 0.017 g (0.7%) of **3** is lost to the combined filtrate and washes. The resin is washed from the filter into a 500 mL round-bottom flask twice with H_2O (60 mL portions) and twice with acetic acid (60 mL portions). The reaction is stirred for 74 h at ambient temperature and then filtered. The resin is washed twice with H_2O (100 mL portions) and the combined filtrate and washes contained 2.13 g (91.4%) of product **3**. The acetic acid and water are removed by azeotropic distillation with *i*PrOH and heptane at 25 Torr and not more than 45 °C. The resulting solids are dried for 14 d at 75 °C and 20 mmHg to give **3** (2.33 g, 86.8% purity, 86.8% purity adjusted yield) as an amorphous tan powder. The material is further purified by trituration with 30 mL of MeOH to give material of 95.0% purity: mp 190 °C dec; ^1H NMR (400 MHz, pyridine-d_5) δ 8.50 (d, $J = 2.9$ Hz, 1H), 7.85 (m, 2H), 7.60 (dd, $J = 9.1$, 3.0 Hz, 1H), 7.33 (m, 2H), 6.58 (d, $J = 9.2$ Hz, 1H), 5.58 (d, $J = 7.3$ Hz, 1H), 4.78 (d, $J = 9.7$ Hz, 1H), 4.71 (t, $J = 9.0$ Hz, 1H), 4.44 (t, $J = 9.7$ Hz, 1H), 4.40 (d, $J = 9.3$ Hz, 1H), 3.97 (s, 2H), 3.45 (m, 4H), 2.60 (m, 4H); ^{13}C NMR (100 MHz, CD_3CO_2D) δ 174.4, 152.2, 146.8, 145.4, 135.4, 132.7, 130.4, 125.3, 115.5, 112.2, 101.2, 76.3, 75.1, 73.5, 72.2, 52.6, 52.5, 45.3; FTIR (microscopic technique) 3300, 2850, 1605, 1495, 1460, 1410, 1245, 1070 cm^{-1}; FAB HRMS for $C_{23}H_{28}N_5O_7$ ($M + H^+$) calcd 486.1989, found 486.2007; $[\alpha]^{23}_D$ −45.5 (*c* 2.099, DMF).

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Supporting Information Available: Copies of ^1H NMR and ^{13}C NMR spectra for compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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