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# SUGAR-SUBSTITUTED 2-AZETIDINONE CHOLESTEROL ABSORPTION INHIBITORS: ENHANCED POTENCY BY MODIFICATION OF THE SUGAR

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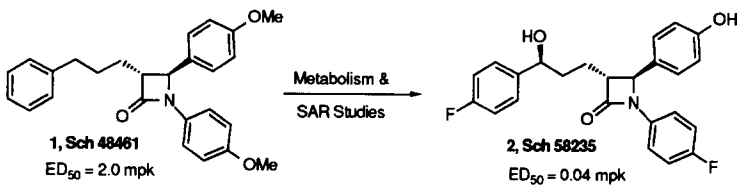
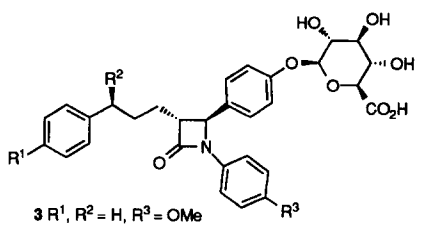
**Abstract:** A glucuronide conjugate of the potent 2-azetidinone cholesterol absorption inhibitor Sch 58235 was synthesized to confirm the structure of a metabolite isolated from in vivo sources. A series of 2-azetidinone glycosides was prepared via Schmidt trichloroimidate methodology. Enhanced cholesterol absorption inhibition was achieved by modification of the sugar moiety. 

A combination of metabolism and SAR studies of the 2-azetidinone cholesterol absorption inhibitor **1**, Sch 48461[1] led to the discovery of the highly potent analog **2**, Sch 58235.[2] Analog **2** is 50 times as potent as **1** in reducing liver cholesterol esters when given orally in the seven day cholesterol fed hamster assay.




These studies also found that both **1** and **2** are rapidly converted in vivo to the corresponding glucuronides **3** and **4**.[3] Sufficient quantities of **3** and **4** were required to confirm the structures of metabolites isolated from in vivo sources.




## Chemistry

We recently confirmed the structure of **3** by independent synthesis.[4] The key glycosidal linkage of **3** was forged by a modified Mitsunobu coupling protocol by treatment of phenol **5** and the benzyl protected sugar **6** with tributylphosphine and 1,1'-(azodicarbonyl)dipiperdine (ADDP) in THF. Subsequent hydrogenolysis

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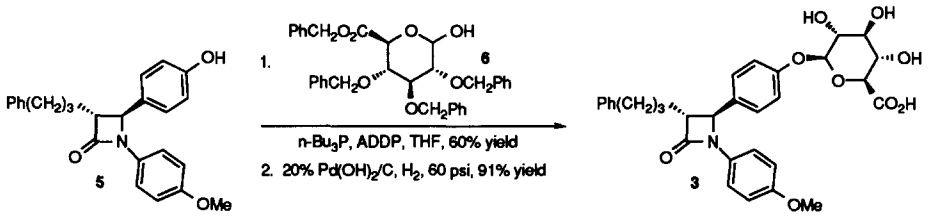
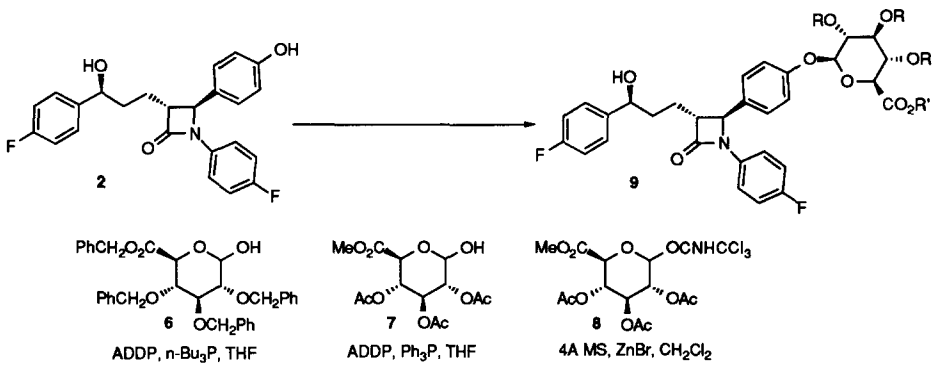
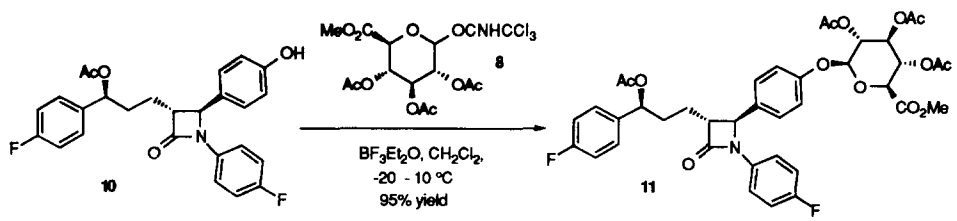
with Pearlman's catalyst provided the β-glucuronide **3**, identical by NMR, MS and HPLC to **3** isolated from in vivo sources.



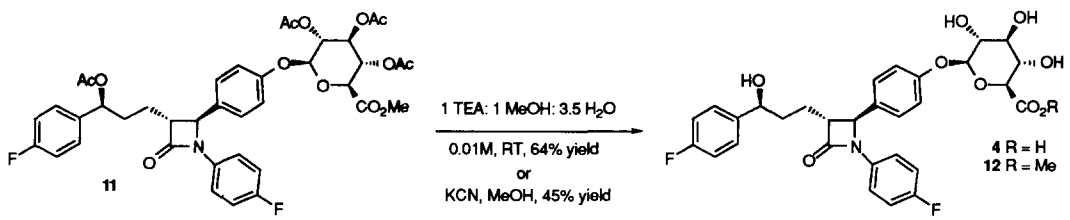

We assumed that **4** could be readily prepared from **2** by similar Mitsunobu methodology. However, treatment of **2** with **6**[5], ADDP and tributylphosphine or **7**[6], ADDP and triphenylphosphine failed to afford **9**. These disappointing results led us to explore other modes of glycosylation. We next explored the possibility of employing Schmidt trichloroimidate mediated glycosylation to prepare **4**.[7] Zinc bromide promoted Schmidt coupling of **2** and **8**[8] from 0 °C to reflux resulted in either no reaction or complex mixtures of products.[9]




We speculate that the failure to prepare **9** via Schmidt or Mitusunobu glycosylation of **2** may be due to interference of the benzylic OH group. The benzylic OH of **2** was protected as the corresponding acetate **10** by bisacetylation of **2** ($Ac_2O$, $CH_2Cl_2$, DMAP, $Et_3N$, 100%) and subsequent selective cleavage of the phenolic acetate (guanidine, MeOH, RT, 60%).[10] Several attempts to promote glycoside formation of **10** and **8** with zinc bromide were unsuccessful. Coupling was finally achieved under more traditional Schmidt conditions, treatment of a -20 °C solution of **10** and **8** in $CH_2Cl_2$ with catalytic boron trifluoride etherate, which upon warming to room temperature afforded **11** in 95% yield.[11]




Hydrolysis of the ester groups was accomplished by stirring a dilute solution of **11** in a mixture of $Et_3N$:$H_2O$:MeOH (1:1:3.5, 0.01 M with respect to **11**) overnight to afford **4**.[12] Alternately, the acetate groups of **11** could be selectively removed with KCN in methanol to provide the ester **12**.[13]




Synthetic **4** was found to be identical by NMR, MS, and HPLC with **4** isolated from in vivo sources.[14] The stereochemistry at the anomeric center was determined to be beta by NMR studies, ($J_{\text{anomeric H}}$ = 7.3 Hz). The Schmidt trichloroimidate coupling protocol and described deprotection procedures were employed to prepare compounds **14** - **16** presented in Table 1.

**Biological Results**

The glucuronic acid **4** was found to be less potent than **2** when given orally in the seven day cholesterol fed hamster assay. As was observed with analogs of **3**, variation of the C-6 substitutent of the sugar modulates cholesterol absorption inhibition. Both the ester **12** and the alcohol **14** are more potent than **4**. Cholesterol absorption inhibition appears to be tolerant of modification of the sugar moiety as evidenced by the activity of the variety of functionalized sugars presented in the table. Of particular note is the disaccharide **16**, which is 4 times as potent as **2**. The disaccharide portion of **16** is found in naturally occurring and synthetic saponins such as **17**, which are reported to be inhibitors of cholesterol absorption.[15] Disaccharide **16** ($ED_{50}$: 0.01 mg/kg) appears to be considerately more potent than **17** (reported $ED_{50}$: 2 mg/kg). However, we have not assessed compounds **16** and **17** head to head in the cholesterol fed hamster assay.

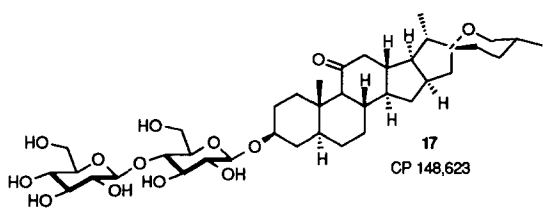
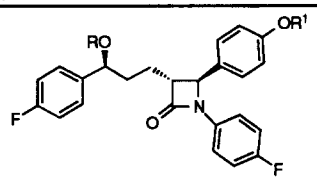
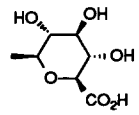
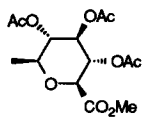
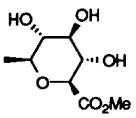
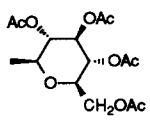
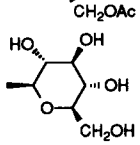
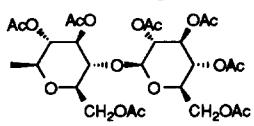
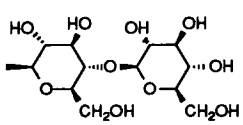


Table 1: Cholesterol Absorption Inhibition Activity of Sugar Substituted 2-Azetidinones in Orally Dosed Seven Day Cholesterol Fed Hamsters.*



| Compound | R | $R^1$ | Serum Cholesterol (% reduction) | Liver Cholesterol Esters (% reduction) | Dose (mg/Kg/day) | $ED_{50}$ (mg/Kg/day) |
|---|---|---|---|---|---|---|
| **2** | H | H | -43 | -93 | 3 | 0.04 |
| **4** | H |  | -50 | -95 | 3 | 0.17 |
| **11** | Ac |  | -53 | -96 | 3 | 0.09 |
| **12** | H |  | -48 | -89 | 3 | 0.04 |
| **13** | Ac |  | -14 | -58 | 1.0 | ND[a] |
| **14** | H |  | -48 | -92 | 1.0 | 0.08 |
| **15** | Ac |  | -43 | -75 | 6.0 | ND[a] |
| **16** | H |  | -36 | -90 | 1.0 | 0.01 |

*Compounds were evaluated in the cholesterol fed hamster model at the indicated dose (n = 6/group).[16] All compounds were statistically different from the cholesterol fed control group (n = 6/group). The compounds were evaluated in separate studies hence, direct statistical comparisons among the compounds was not performed. (a) ND = Not Determined.

## Conclusions

The synthetic β-glucuronide **4** was found to be identical with **4** isolated from in vivo sources. Although metabolism studies have shown that **2** is rapidly converted to **4** in vivo, **4** was found to be less potent than **2** when orally dosed in the seven day cholesterol fed hamster assay. Cholesterol absorption activity approaching that of **2** can be restored by variation of the C-6 substituent of the sugar. The ester **12** and alcohol **14** are more potent than the acid **4**. The disaccharide **16**, was the most potent cholesterol absorption inhibitor identified in this study $ED_{50}$: 0.01 mg/kg/day and is four times as potent as **2**. Cholesterol absorption inhibition appears to be tolerant of substitution of the sugar moiety. However, since we are presently restricted to an in vivo assay, interpretation of the cholesterol absorption activity of the compounds in Table 1 may not be straight forward. The observed cholesterol absorption inhibition may be a reflection of a compound's bioavailability and/or ease of conversion to active metabolites and not its intrinsic cholesterol absorption activity.

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11. **Representative procedure for Schmidt coupling: 2,3,4-Tri-*O*-acetyl-1-*O*-[4-[Trans-(3*R*,4*S*)-3-[3-[(*S*)-acetyloxy-3-(4-fluorophenyl)propyl-1-(4-fluorophenyl)-2-oxo-4-azetidinyl]phenyl]-β-D-gluco-pyranuronic Acid Methyl Ester 11:** Boron trifluoride etherate (0.091 mL, 0.74 mmol) was added to a -25 °C solution of the **7** (3.33 g, 7.38 mmol) and **10** (4.24 g, 8.86 mmol) in methylene chloride (74 mL). The reaction was maintained at -20 °C for 2 h. TLC monitoring (50% EtOAc/hexanes) indicated formation of two new spots. The reaction was allowed to warm to 10 °C over 2 h. TLC indicated consumption of starting material and the formation of a single spot. The mixture was quenched with saturated ammonium chloride, diluted with ethyl acetate, transferred to a separatory funnel, washed with saturated ammonium chloride, water and brine, dryed over anhydrous sodium sulfate and concentrated onto enough silica such that a free flowing powder was obtained. The resulting powder was loaded onto a chromatography column packed with 40% EtOAc/hexanes. Elution with the same solvent provided 5.39 g (95%) as a white foam. **$^1$HMR** (400 MHz, $CDCl_3$): 7.26 (4H, m), 7.21 (2H, m), 7.01 (4H, m), 6.93 (2H, t, $J$ = 8.4 Hz), 5.69 (1H, t, $J$ = 6.7 Hz), 5.34 (2H, m), 5.29 (1H, m), 5.15 (1H, d, $J$ = 7.2 Hz), 4.56 (1H, d, $J$ = 2.1 Hz), 4.17 (1H, m),

3.73 (3H, s), 3.02 (1H, dt, $J$ = 7.6, 2.3 Hz), 2.07 (14H, m), 1.85 (2H, m). **HRMS** (FAB): calcd for M + H: $C_{39}H_{40}NO_{13}F_2$, 768.2468, found 768.2460.

12. **1-*O*-[4-[Trans-(3*R*,4*S*)-1-(4-fluorophenyl)-2-oxo-3-[3-[(*S*)-hydroxy-4-fluorophenyl)propyl]]-4-azetidinyl]-phenyl]-β-D-glucuronic Acid 4:** **11** (5.08 g, 6.98 mmol) was dissolved in a mixture of methanol (127 mL) and triethylamine (127 mL) at room temperature. Water (445 mL) was added slowly via an addition funnel over 10 min in order to maintain a homogeneous solution. The resulting clear yellow solution was stirred over night. A small aliquot of the reaction mixture was quenched into a vial containing 1 M HCl and ethyl acetate. TLC (5% HOAc/20% MeOH/75% $CH_2Cl_2$) of the ethyl acetate layer indicated consumption of starting material. The methanol and triethylamine were removed on a rotory evaporator. The remaining solution was made acidic with 1M HCl, diluted with ethyl acetate, transfered to a separatory funnel and extracted with ethyl acetate. The extracts were combined, washed with 1M HCl, water and brine, dryed over anhydrous sodium sulfate and concentrated to a white solid 3.81 g (93%). The solid was redissolved in methylene chloride, concentrated onto enough silica such that a free flowing powder was obtained. The resulting powder was loaded onto a chromatography column packed with silica and 15% MeOH/$CH_2Cl_2$. Elution with 5% HOAc/15% MeOH/80% $CH_2Cl_2$ provided 2.95 g of pure **4** and 0.36 g of the slightly impure **4**. The 2.95g of pure **4** was azeotroped first with toluene (3X) and then methanol (5X). The resultant solid was heated to 60°C overnight under vacuum to remove any residual solvent and provide the title compound as a white solid 2.6g (64%). $^1$**HMR** (500 MHz, $CD_3OD$): 7.31 (4H, m), 7.26 (2H, m), 7.09 (2H, d, $J$ = 8.5 Hz), 6.99 (4H, m), 4.96 (1H, anomeric, d, $J$ = 7.3 Hz), 4.78 (1H, d, $J$ = 2.1 Hz), 4.59 (1H, dd, $J$ = 5.3, 6.5 Hz), 3.96 (1H, d, $J$ = 9.7 Hz), 3.59 (1H, m), 3.48 (2H, m), 3.08 (1H, m), 1.88 (4H, m). **CMR** (400 MHz, $CD_3OD$): 172.56, 169.81, 163.72, 160.51, 159.14, 142.37, 135.36, 133.19, 128.92, 128.71, 126.08, 120.05, 118.61, 116.83, 102.27, 77.43, 76.66, 74.67, 73.86, 73.15, 62.13, 61.36, 37.60, 26.23. **HRMS** (FAB): calcd for M + H: $C_{30}H_{30}NO_9F_2$, 586.1889, found 586.1883. $[\alpha]^{21.4}\,^{\circ}C_D$ -73° ( 5.88 mg/2 mL MeOH). **HPLC**: Metachem Inertsil C8 column (1.0 mL/min, solvent gradient 70% 0.2 M $NH_4Ac$ pH 6 Buffer/30% Acetonitrile gradient to 100% acetonitrile over 40 min.) Rt **4**: 9 min. Rt **2**: 40 min.

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16. For a discussion of the seven day cholesterol fed hamster model see ref.[1]