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SUGAR-SUBSTITUTED 2-AZETIDINONES AS CHOLESTEROL ABSORPTION INHIBITORS

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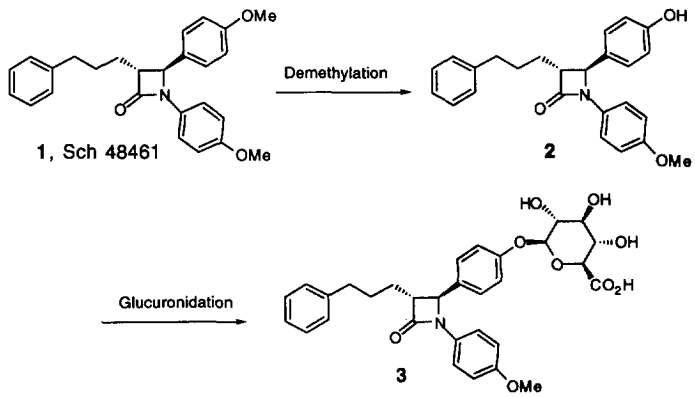
Abstract: The asymmetric synthesis of a glucuronide conjugate of the 2-azetidinone cholesterol absorption inhibitor Sch 48461 was accomplished to confirm the structure of a metabolite isolated from in vivo sources. Key features of this article include the asymmetric synthesis of 2-azetidinones by Evan's chiral oxazolidinone methodology and glucuronide formation by a Mitsunobu protocol.

Metabolism studies identified the glucuronide **3** as a major metabolite of the potent 2-azetidinone cholesterol absorption inhibitor **1** (Sch 48461).[1,2] Glucuronide **3** presumably arises from demethylation of **1** to give the phenol **2** which undergoes subsequent glucuronidation. A sufficient quantity of **3** was required to confirm the structure of the glucuronide isolated from in vivo sources and to further our studies in determining the roles of the metabolites of **1** in cholesterol absorption inhibition.



Chemistry

The requisite phenol **2** was prepared as shown below. 5(*S*)-phenyloxazolidinone **4** was acylated with 5-phenylvaleryl chloride to provide **5**. Treatment of **5** with titanium tetrachloride generated the corresponding titanium enolate and subsequent addition of imine **6** provided a mixture of β-amino amides as a 4:1 ratio of diastereomers. A single recrystallization of the mixture from ethyl acetate/hexanes gave **7** in enantiomerically pure form (33% yield, unoptimized). Silylation of **7** with bis(trimethylsilyl)acetamide followed by fluoride catalyzed cyclization gave the 2-azetidinone **8** in a one pot operation. HPLC analysis indicated that **8** was optically pure when compared with racemic **8**.[3] The absolute stereochemistry of **8** was assigned as 3*R*,4*S* by analogy to **1** by relative HPLC retention times and biological activity.[4] Hydrogenolysis of **8** provided phenol **2**.[5]

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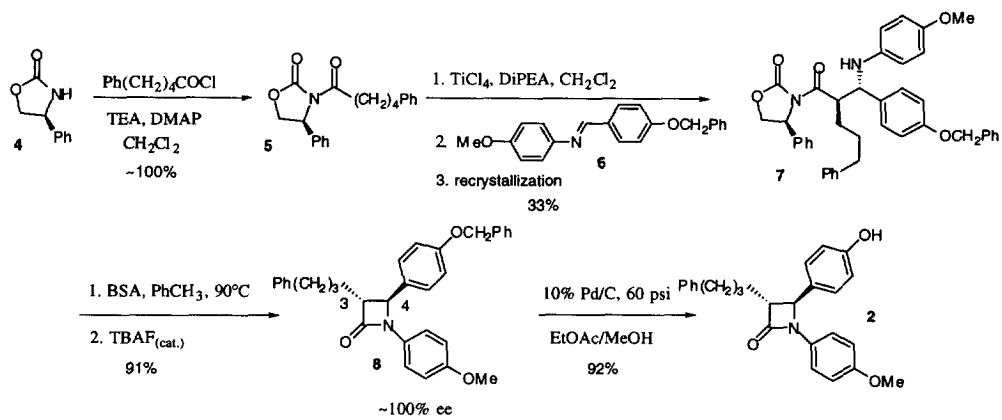
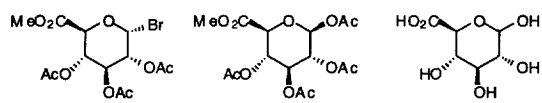
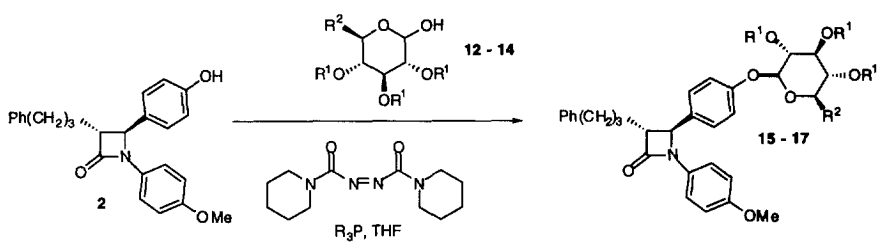


Initial attempts to prepare the glucuronide **3** by coupling the phenol **2** and commercially available glucuronic acid derivatives **9–11** under a variety of conditions were unsuccessful.[6–9]



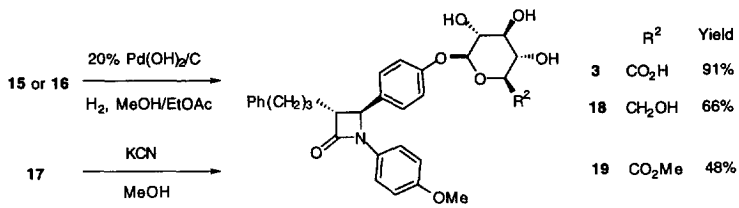
9 **10** **11**

The key glycosidal linkage was ultimately forged by a modified Mitsunobu procedure.[10,11] Treatment of a THF solution of **2** and benzyl-protected sugar derivatives **12**[10a] or **13**[12] with 1,1′-(azodicarbonyl)dipiperdine and tributylphosphine provided **15** and **16** in good (60%) and excellent (~100%) yields, respectfully. However, with acetate-protected sugars such as **14**,[13] the presence of tributylphosphine resulted in rapid transacetylation of phenol **2**. Transacetylation could be prevented by substituting triphenylphosphine for tributylphosphine, although the yield of coupled product **17** was greatly reduced (20%).

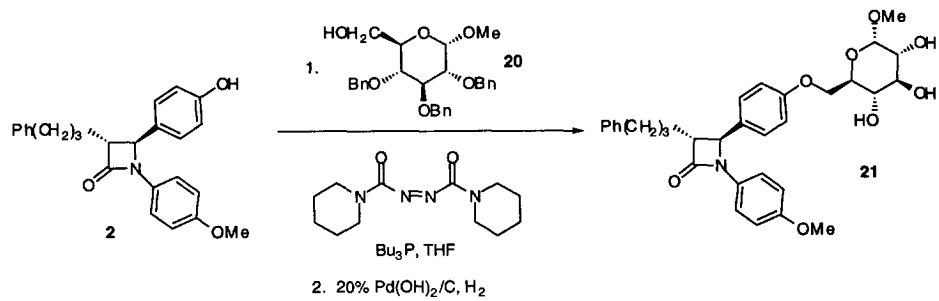


Sugar	Prod	R^1	R^2	R_3P	Yield
12	15	Bn	CO_2Bn	Bu_3P	60%
13	16	Bn	CH_2OBn	Bu_3P	100%
14	17	Ac	CO_2Me	Ph_3P	20%

Debenzylation of **15** was sluggish with 10% Pd/C under a hydrogen atmosphere (15–60 psi) in various solvent mixtures or under hydrogen transfer conditions. Compounds **15** and **16** were successfully debenzylated with Pearlman's catalyst (20% $Pd(OH)_2/C$, H_2(1 atm), 50% MeOH/EtOAc) to give compounds **3** and **18**.[14] Treatment of compound **17** with KCN/MeOH provided the methyl ester **19**.[15] Synthetic **3** was found to be identical to **3** isolated from in vivo sources by HPLC, MS, and NMR.[2] The stereochemistry of **3** at the anomeric center was assigned as beta by NMR studies ($J_{\text{anomeric H}} = 7.5$ Hz).[16]



In a similar fashion connection of the C-6 position of sugar **20** and phenol **2** was achieved to afford **21**.



Biological Results

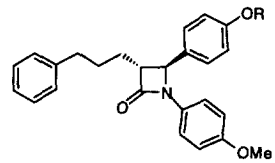
Both the phenol **2** and the glucuronide **3** are less active than **1** when given orally in the seven day cholesterol-fed hamster assay (Table).[17] Variation of the C-6 substitutent of the sugar was found to modulate cholesterol absoroption activity. The alcohol **18** and ester **19** have similar potencies whereas the acid **3** is less potent. Attachment of the phenol **2** need not be limited to the 2-position of the sugar as demonstrated by compound **21**, wherein attachment is made at the 6-position of the sugar and cholesterol absorption inhibition activity is retained.

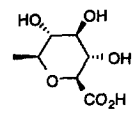
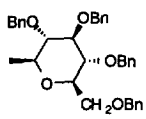
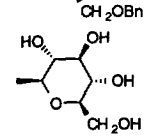
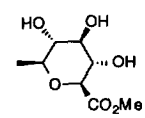
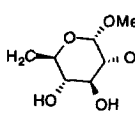
Conclusions

The synthetic β-glucuronide **3** was found to be identical with **3** isolated from in vivo sources.[2] Although metabolism studies have shown that **1** is rapidly converted to **3** in vivo, both **3** and the postulated intermediate, phenol **2** were found to be less potent than **1** when given orally in the cholesterol fed hamster assay. Cholesterol absorption inhibition activity approaching that of **1** can be restored by variation of the C-6 position of the sugar.

The alcohol **18** and ester **19** are more potent than the acid **3** in the cholesterol fed hamster assay. Additionally, cholesterol absorption inhibition appears to be tolerant of position of attachment as well as substitution of the sugar moiety. Since we are limited to using an in vivo assay, interpertation of the cholesterol absorption inhibition of the compounds presented in the table may be clouded. 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 inhibition activity. The methods described for the synthesis of 2-azetidinones and their sugar substituted analogs allow for ready variation of both the 2-azetidinone and sugar moieties.

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



Compound	R	Dose (mg/Kg/day)	Serum Cholesterol (% reduction)	Liver Cholesterol Esters (% reduction)
1	Me	10	–43	–93
2	H	10	–25	–70
3		10	–10	–9
16		10	–9	–21
18		10	–29	–86
19		8	–23	–62
21		10	–10	–48

*Compounds were evaluated in the cholesterol-fed hamster model at the indicated dose (n = 6/group).[17] 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.

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3. **1-(Methoxyphenyl)-3(*R*)-(3-phenylpropyl)-4(*S*)-(4-benzyloxyphenyl)-2-azetidinone 8: HPLC**: Analytical Chiracel OD column (3% isopropanol/hexanes, 1.0 mL/min), Rt = 17.53 min. (racemic **8** entiomer A Rt = 17.58 min, enantiomer B Rt = 22.05 min). **PMR** (400 MHz, $CDCl_3$): 7.38 (5H, m). 7.21 (9H, m), 6.96 (2H, d, J = 8.6 Hz), 6.76 (2H, d, J = 9.1 Hz), 5.04 (2H, s), 4.55 (1H, d, J = 2.2 Hz), 3.06 (1H, m), 2.64 (2H, m), 1.97 (1H, m), 1.82 (3H, m).

4. Burnett, D. A. *Tetrahedron Lett.* **1994**, *35*, 7339. The stereochemistry of **1**, Sch 48461 was determined to be 3*R*,4*S* by independent chiral synthesis and by X-ray analysis of a crystalline analog. The stereochemical assignment of phenol **2** was confirmed by independent synthesis from **1**, boron tribromide demethylation of **1** gave a phenol which was identical to **2** by NMR and chiral HPLC.

5. **1-(Methoxyphenyl)-3(*R*)-(3-phenylpropyl)-4(*S*)-(4-hydroxyphenyl)-2-azetidinone 2: PMR** (400 MHz, $CDCl_3$): 7.26 (2H, m). 7.20 (5H, m), 7.14 (2H, m), 6.83 (2H, d, J = 8.6 Hz), 6.76 (2H, d, J = 9.1 Hz), 5.61 (1H, s), 4.54 (1H, d, J = 2.0 Hz), 3.72 (3H, s), 3.04 (1H, m), 2.62 (2H, t, J = 7.4 Hz), 1.94 (1H, m), 1.81 (3H, m). **Anal.** $C_{25}H_{25}NO_3$, calcd. C = 77.49, H = 6.50, N = 3.61; found C = 77.48, H = 6.85, N = 3.73. **MS** (CI): 388 (M+1, 100). HRMS (FAB) calcd for M+H: $C_{25}H_{26}NO_3$, 388.1913, found 388.1905. $[\alpha]^{22.6}_D$ –31.0° (18.96 mg/2 mL, $CHCl_3$)

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7. Attempted coupling of **2** and **9**, $Hg(CN)_2$, CH_3NO_2/PhH: (a) Smith, A. B.; Rivero, R. A.; Hale, K. J.; Vaccaro, H. A. *J. Am. Chem. Soc.* **1991**, *113*, 2092. (b) Ag_2CO_3, $AgClO_4$, $PhCH_3$: Smith, A. B.; Rivero, R. A.; Hale, K. J.; Vaccaro, H. A. *J. Am. Chem. Soc.* **1991**, *113*, 2092. (c) $AgSO_3CF_3$, CH_2Cl_2 (d) NaH, DMF (e) Cs_2CO_3, DMF. (f) LiOH, THF: Carrupt, P.-A.; Testa, B.; Belchalany, A.; Tayer, N. E.; Descas, P.; Perrissoud, D. *J. Med. Chem.* **1991**, *34*, 1272.

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11. **Representative procedure for Mitsunubu coupling: benzyl 2,3,4-tri-*O*-benzyl-1-*O*-[4-[*trans*-(3*R*,4*S*)-1-(4-methoxyphenyl)-2-oxo-3-[3-(phenyl)propyl]]-4-azetidinyl]phenyl]-b-D-glucuronate** **15**: 1,1′-(Azodicarbonyl)dipiperdine (1.47 g, 7.27 mmol) was added to a 0 °C solution of **2** (0.194 g, 5.0 mmol), **12** (2.52 g, 4.54 mmol) and tributylphosphine (1.81 mL, 7.27 mmol) in THF (20 mL). After 5 min, a thick precipitate formed. Additional THF (20 mL) was added to facilitate stirring, and the mixture was allowed to come to room temperature overnight. The mixture was diluted with 20% EtOAc/hexanes, filtered through celite, and the filter cake was well washed with 20% EtOAc/hexanes. The filtrate was 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 20% EtOAc/hexanes. Elution with the same solvent mixture provided 2.51 g (60%) of the title compound as a thick syrup. **PMR** (400 MHz, $CDCl_3$): 7.22 (29H, m), 7.01 (2H, d, J = 8.7 Hz), 6.77 (2H, d, J = 9.1 Hz), 5.15 (2H, app. d, J = 3.8 Hz), 5.01 (anomeric H, 1H, d, J = 7.2 Hz), 4.97 (1H, d, J = 11 Hz), 4.90 (1H, d, J = 11 Hz), 4.80 (2H, d, J = 11 Hz), 4.74 (1H, d, J = 10.7 Hz), 4.56 (1H, d, J = 2.2 Hz), 4.50 (1H, d, J = 10.7 Hz), 4.04 (1H, d, J = 9.6 Hz), 3.93 (1H, t, J = 8.6 Hz), 3.73 (5H, m), 3.05 (1H, m), 2.65 (2H, t, J = 7.6 Hz), 1.96 (1H, m), 1.83 (3H, m). **TLC**: Rf = 0.29 (30% EtOAc/hexanes) **HRMS** (FAB): calcd for M+H: $C_{59}H_{58}NO_9$ 924.4112, found 924.4119. **HPLC**: Analytical Chiracel OD column (20% isopropanol/hexane, 1.0 mL/min, Rt = 15.13 min).

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14. **Representative procedure for Hydrogenolysis:1-*O*-[4-[*trans*-(3*R*,4*S*)-1-(4-methoxyphenyl)-2-oxo-3-(3-phenylpropyl)-4-azetidinyl]phenyl]-b-D-glucuronic acid 3. 15** (0.57 g, 0.62 mmol) was dissolved in methanol (20 mL), and the solution was diluted with ethyl acetate (20 mL) and purged with nitrogen. 20% $Pd(OH)_2$ on carbon (0.62 g) was added. The resulting mixture was purged with hydrogen (3X) and then stirred under a balloon of hydrogen. TLC (20% MeOH/CH_2Cl_2) after 5 h indicated consumption of starting material. The mixture was filtered through celite, and the filter cake was well washed with 50% MeOH/EtOAc. The filtrate was concentrated to give the title compound as a clear glass 0.32 g (91%). **PMR** (400 MHz, CD_3OD): 7.18 (11H, m), 6.79 (2H, m), 4.95 (1H, anomeric, d, J = 7.5 Hz), 4.76 (1H, d, J = 2 Hz), 3.96 (1H, d, J = 9.5 Hz), 3.69 (3H, s), 3.59 (1H, m), 3.48 (2H, m), 3.06 (1H, m), 2.64 (2H, t, J = 7 Hz), 1.84 (4H, m). **CMR** (300 MHz, CD_3OD): 173.8, 171.0, 160.3, 159.2, 144.6, 134.8, 133.6, 130.8, 130.7, 130.0, 128.3, 121.2, 119.8, 116.7, 103.6, 78.7, 77.9, 76.0, 74.4, 63.4, 62.7, 57.3, 38.0, 31.6, 30.7. **HRMS** (FAB): calcd for M+H: $C_{31}H_{34}NO_9$ 564.2234, found 564.2242. **TLC**: Rf = 0.31 (5% HOAc/20% MeOH/75% CH_2Cl_2). $[\alpha]^{22.8}_D$ −54.3° (9.36 mg/2 mL, MeOH). **HPLC**: Metachem Inertsil C8 column (1.0 mL/min, solvent gadient 70% 0.2 M NH_4Ac pH 6 Buffer/30% Acetonitrile gradient to 100% acetonitrile over 40 min.) Rt **3**: 10 min. Rt **1**: 43 min.

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17. For a discussion of the seven day cholesterol fed hamster assay see ref 1.