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Chemo-enzymatic synthesis of polyhydroxyazepanes

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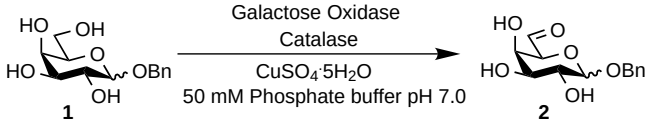
Abstract—Galactose oxidase (EC 1.1.3.9, GAO) is an extracellular copper-containing enzyme that utilizes molecular oxygen to convert the C6-primary hydroxyl moiety of D-galactopyranosides to hydrated aldehydes. Subsequent dehydratative coupling with hydroxylamines produces oximes (**3a**–**f**), which, when subjected to conditions of hydrogenolysis, give rise to polyhydroxyazepanes (**11**–**17**).

There is much current interest surrounding the utility of polyhydroxyazepanes due to their significance in various biological systems. These and related compounds (iminocyclitols or imino sugars with a basic nitrogen instead of an oxygen in the ring) have attracted considerable attention from synthetic and medicinal chemists, biologists, and clinical researchers in recent years as a result of their potent inhibition of glycoprotein and glycolipid processing enzymes such as glycosidases and glycosyltransferases.[1] They have demonstrated great potential as drugs in the treatment of carbohydrate-mediated diseases such as diabetes, and viral infections including HIV.[2,3] Most recently, polyhydroxylated azepanes were reported as new motifs for DNA minor groove binding agents (MGBLs) that were shown to have modest growth inhibitory activity in cancer cell lines and are capable of binding to DNA even at low pH.[4]

Polyhydroxyazepanes have several properties that make them potentially useful as drug candidates. The flexibility of the seven-membered ring (compared with five- or six-membered rings) allows the hydroxyl groups to adopt a variety of positions increasing the probability of forming hydrogen bonds with structural motifs found within DNA framework, or within the active sites of various proteins. The primary advantage of the level of hydroxylation in polyhydroxyazepanes is their high water solubility, allowing them to circumvent the problem of poor bioavailability.[5] The chirality associated with polyhydroxyazepanes can be controlled, allowing access to a range of diastereomers, which could allow structural activity relationship (SAR) studies for improved selectivity.

Recently, a tetrahydroxyazepane was found to exhibit noteworthy glycosidase inhibitory properties against a broad range of glycosidases.[6] The inhibition was rationalized by superpositioning the functional groups of the azepanes, and those of a range of proven powerful glycosidase inhibitors, which in many of the cases, were found to match nicely. Iminocyclitols have also been introduced. These are based on seven-membered rings, having the same carbon content as the azasugars but endowed with the conformational advantage of a more flexible azepane ring that could lead to favorable binding to the active site of the enzyme, which have been introduced.[7] However, the syntheses of these compounds have suffered from lengthy procedures. In many of the cases starting materials were extremely expensive, albeit the synthesis was only a few steps, or starting materials were cheap with a lengthy synthesis.

This report describes a chemo-enzymatic approach to polyhydroxyazepanes that incorporates inexpensive starting materials, employs only a few short synthetic steps, utilizes aqueous media and is capable of proceeding on gram quantities.



(1)

Keywords: aldehyde; aminosugar; azepane; chemo-enzymatic; galactose oxidase; oxidation; oxime; polyhydroxy.

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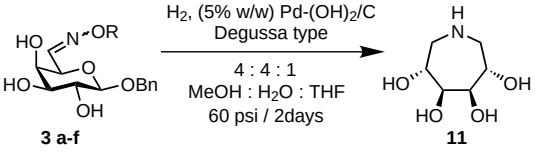
Galactose oxidase (GAO) selectively oxidizes exposed primary hydroxyl groups in nonreducing, terminal galactose and *N*-acetyl galactosamine residues to the corresponding aldehyde (Eq. (1)).[8] The GAO/$NaBH_3CN$ method is well-known and widely used as a technique to label cell-surface glycoconjugates.[9] GAO also has broad substrate specificity for a variety of galactose- and nongalactose-based compounds.[10] The native reaction on galactose yields a C-6 aldehyde, which has been shown to undergo spontaneous Schiff base formation in the presence of amines.[11]

Benzyl pyranosides were easily prepared from aldopyranoses according to two different procedures in good to excellent yields.[12] The enzymatic oxidation reaction was monitored via NMR until completion.[13] DEPT analysis provided the evidence for reaction completion due to a new ^{13}C peak at $\delta \cong 88$ ppm (corresponding hydrated aldehyde) **2** and the disappearance of ^{13}C (CH_2) at $\delta \cong 60$ ppm (corresponding primary alcohol) **1**.[14]

When the evidence suggested 100% conversion, the enzymes were filtered out by first heating at 100°C for 5 min, centrifugation to remove denatured protein, and then the supernatent was filtered through a 0.20 μm Fisherbrand nylon filter. To test the feasibility for generating seven membered rings, a variety of benzyl-D-galactose C6 oximes were synthesized (Table 1, entries 1–6). Initially they were prepared by measuring a 1:1 w/w mixture of the aldehyde:oxime using a standard MeOH/pyridine protocol and purified from silica gel column chromatography using a 17:3:1 (EtAc/MeOH/H_2O) elution system. After purification, the benzyl-D-galactopyranoside-oxime was subjected to hydrogenolysis for 2 days at 60–70 psi in a 4:4:1 MeOH/H_2O/THF solution. Upon passing the mixture through a thin pad of celite, concentration under reduced pressure and lyophilization to remove the remaining traces of water, the polyhydroxylazepane **11** was afforded in high purity and excellent yield (Table 1).

Table 1. Hydrogenolysis of benzyl-D-galactopyranosides-*O*-R-hydroxylamines to form 3(*R*),4(*S*),5(*R*),6(*S*)-tetrahydroxyazepane (**11**)



Entry[a]	Compound	R	Yield (%)[d]
1	**3a**[c]	H	98
2	**3b**	Me	98
3	**3c**	Et	98
4	**3d**	^{t}Bu	98
5	**3e**	Bn	98
6[b]	**3f**	Allyl	90

[a] Oximes were purified using a 17:3:1 (ethylacetate:methanol:water) eluent on silica gel.
[b] Hydrogenolysis halted at 24 h.
[c] Completed on a 5 g scale.
[d] Yield based on collected product without further purification.

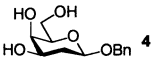
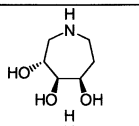
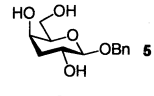
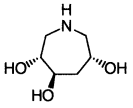
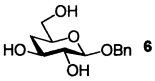
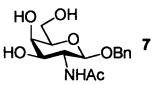
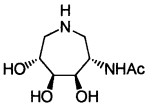
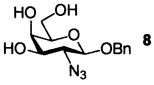
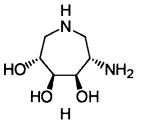
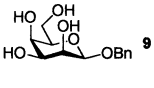
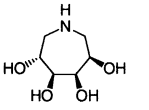
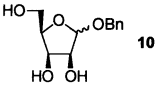
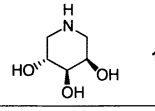
The formation of compound **11** was successfully accomplished by using a variety of *O*-substituted oximes.[16] It is presumed that the bi-product of each entry produced an alcohol and toluene that was evaporated under reduced pressure to afford pure polyhydroxyazapane. The use of Degussa type catalyst (5% w/w) was imperative for the reaction to proceed. Dry Pd/C did not furnish the desired compound but did yield the 6-deoxy-6-oxime debenzylated-D-galactopyranoside. High pressure for the system was also critical for complete conversion as well as a prolonged exposure to H_2. The polyhydroxyazepane **11** was purified (entry 6) by forming the HCl salt in THF and ether. Concentration for hydrogenolysis was kept to 10 mg/mL to negate the possibility of polymerization.

In order to further the utility of the reaction, a survey of solvents and oxime forming conditions were observed so that purification steps could be eliminated. It was observed that the formation of the oxime (**3a**) would proceed to completion in a buffered solution at pH 5.2. The synthesis of seven-membered polyhydroxy iminocitols was accomplished in a simplified procedure to further facilitate the preparation of these bioactive heterocycles. A typical procedure is as follows: For 100 mg of starting material; once the enzymatic oxidation went to completion, the pH was adjusted to 5.2 at which time the hydroxylamine hydrochloride salt was added. The mixture was allowed to stir for 6 h or when TLC evidence indicated completion, and then concentrated down to approximately 25 mg/mL (4 mL of phosphate buffer pH 5.2). MeOH (4 mL), THF (1 mL) and 5% mol $Pd(OH)_2/C$ were added and subjected to hydrogenolysis at 60 psi for 2 days. The mixture was filtered through a pad of Celite and then reduced down with low pressure and then lyophilized to remove the remaining traces of water.

Table 2 shows the conversion of GAO substrates to polyhydroxyazepanes following the simplified protocol. Compounds **4**, **5**,[15] **7**–**10** were excellent candidates for the GAO oxidation and the reaction proceeded to complete conversion within 24 h. When compound **6**[15] was subjected to enzymatic oxidation conditions, the DEPT analysis did not show a new ^{13}C peak at $\delta \cong 88$ ppm, instead only starting material was recovered. This supports the fact that GAO is specific for galactose-like substrates with an axial hydroxyl in the 4′ position. The 3′-deoxy-benzyl-D-galactopyranoside **5** was converted to the polyhydroxyazepane indicating that the 3′ OH was not necessary for GAO recognition.

In conclusion, we have demonstrated the feasibility of a chemo-enzymatic synthesis of polyhydroxyazepanes. Their potential in biological systems has yet to be fully explored. This methodology, although limited in scope to GAO substrates, has shown that the use of GAO negates the use of protecting group chemistry, that polyhydroxyazepanes are easily obtainable and the reaction is capable of proceeding on a gram scale.

Table 2. Substrate conversions to polyhydroxyazepanes[16]

Entry	Substrate		Product		$[\alpha]_D^{20}$	Yield (%)[c]
1		4		12	+40	94
2		5		13	-16	92
3		6	NR[a]		--	--
4		7		14[b]	-9	80
5		8		15[b]	-14	75
6		9		16	+30	97
7		10		17	-23	95

[a]Recovered starting material.

[b]Purified using reverse phase silica gel (70:30; MeOH/H_2O).

[c]Yield based on collected product without further purification.

Acknowledgements

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13. General procedure for GAO:
    In a typical experiment, a mixture of benzyl-D-galactopyranoside **1** (1 g), 500 units of GAO and 6000 units of catalase in 40 mL phosphate buffer (50 mM pH 7.0) containing 6 mg of $CuSO_4 \cdot 5H_2O$ was stirred gently at 4°C with a continuous stream of air (air pump). After the starting material was completely oxidized, as indicated by DEPT NMR, the reaction mixture was heated at 100°C for 5 min to denature the enzymes. Centrifugation at 3200 rpm for 30 min followed by filtration through a 0.20

μm Fisherbrand nylon filter gave the filtrate, which was lyophilized to produce a white solid **2** (990 mg).

14. Compound **1**: A mixture of 20 g of D-galactose in 100 mL of benzyl alcohol was cooled in an ice-bath and saturated with hydrogen chloride by bubbling the gas through for about 20 min. The mixture was allowed to shake overnight at room temperature during which crystallization occurred. 250 mL of ethyl ether was then added slowly with constant mixing, and the mixture left at 5°C for approximately 14 h to complete crystallization. The crystals were collected by filtration, washed with cold ethyl ether and dried in air. The yield was 20 g (70%). recrystallization was carried out from absolute ethanol (50 g/L) to give 15 g (white powder): mp 123°C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.43–7.25 (m, 5H), 4.92 (d, $J=11.6$ Hz, 1H), 4.66 (d, $J=12$ Hz, 1H), 4.31 (d, $J=7.6$ Hz, 1H), 3.83 (d, $J=3.2$, 1H), 3.79–3.71 (m, 2H), 3.58 (t, $J=8$ Hz, 1H), 3.51–3.45 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 137.99, 128.08, 128.02, 127.47, 102.71, 75.57, 73.79, 71.38, 70.48, 69.15, 61.38; DEPT δ 70.48 CH_2, 61.38 CH_2; MS FAB m/z 292.95 $(M+Na)^+$, MS FAB m/z 308.91 $(M+K)^+$.
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16. Compound **11**: ^{1}H NMR (500 MHz, D_2O) δ 4.01 (m, 2H), 3.95 (d, $J=6.5$ Hz, 2H), 3.31 (dd, $J=3.5$, 14.0 Hz, 2H), 3.16 (dd, $J=5.0$, 14.0 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 72.62, 66.88, 48.30; DEPT δ 48.30 CH_2; MS ES$^+$ m/z 164.04 $(M+H)$. Compound **12**: ^{1}H NMR (500 MHz, D_2O) δ 4.02 (dt, $J=2.0$, 10 Hz, 1H), 3.85 (dtdd, $J=2.0$, 5.5, 5.5, 22.5 Hz, 1H), 3.73–3.65 (m, 2H), 3.17–2.98 (m, 2H), 2.85–2.70 (m, 2H), 2.25–2.17 (m, 1H), 1.82–1.75 (m 1H); ^{13}C NMR (125 MHz, D_2O) δ 75.72, 69.23, 68.60, 45.77, 41.90, 27.35; DEPT δ 45.77 CH_2, 41.90 CH_2, 27.35 CH_2; MS ES$^+$ m/z 147.91 $(M+H)$. Compound **13**: ^{1}H NMR (500 MHz, D_2O) δ 3.99 (dd, $J=2.0$, 10 Hz, 1H), 3.92 (t, $J=7.0$ Hz, 1H), 3.75 (t, $J=3.5$ Hz, 1H), 3.85 (d, $J=14$ Hz, 1H), 3.21–3.16 (m, 1H), 3.14–3.06 (m, 2H), 2.13–2.07 (m, 1H), 1.78–1.75 (m 1H); ^{13}C NMR (125 MHz, D_2O) δ 74.99, 68.69, 67.27, 44.57, 41.12, 25.30; DEPT δ 44.57 CH_2, 41.12 CH_2, 25.30 CH_2. MS ES$^+$ m/z 147.87 $(M+H)$. Compound **14**: ^{1}H NMR (400 MHz, D_2O) δ 4.22 (t, $J=8.0$ Hz, 1H), 3.98 (dt, $J=4.0$, 9.0 Hz, 1H), 3.89–3.8 (m, 2H), 3.73–3.62 (m, 2H), 3.44 (dd, $J=6.0$, 8.0 Hz, 1H), 3.35 (d, $J=7.0$ Hz, 1H), 2.15 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 70.13, 68.76, 66.62, 63.51, 56.05, 51.86, 28.09; MS ES$^+$ m/z 227.92 $(M+Na)^+$. Compound **15**: ^{1}H NMR (400 MHz, D_2O) δ 4.17 (t, $J=7.5$ Hz, 1H), 3.79 (dt, $J=3.5$, 9.0 Hz, 1H), 3.76–3.62 (m, 2H), 3.55–3.50 (m, 2H), 3.48 (dd, $J=6.0$, 8.0 Hz, 1H), 3.35 (d, $J=7.0$ Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 75.13, 72.76, 66.62, 51.51, 47.05, 45.86; MS ES$^+$ m/z 185.78 $(M+Na)^+$. Compound **16**: ^{1}H NMR (500 MHz, D_2O) δ 3.95 (ddd, $J=2.0$, 4.0, 8.0 Hz, 1H), 3.91 (d, $J=6.5$ Hz, 1H), 3.69 (m, 2H), 3.57 (q, $J=6.0$ Hz, 2H), 3.16 (dd, $J=5.0$, 14.0 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 75.18, 74.30, 72.9, 70.8, 49.70, 49.30; DEPT δ 49.70, 49.30 CH_2; MS ES$^+$ m/z 186.04 $(M+Na)^+$. Compound **17**: ^{1}H NMR (500 MHz, D_2O) δ 3.91 (ddd, $J=2.0$, 4.0, 8.0 Hz, 1H), 3.79 (ddd, $J=4.0$, 8.0, 9.5 Hz, 1H), 3.49 (m, 1H), 3.21 (q, $J=15.0$ Hz, 1H), 3.16 (dd, $J=5.0$, 14.0 Hz, 1H), 2.95 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 72.08, 66.54, 65.5, 49.68, 49.35; DEPT δ 49.68, 49.35 CH_2; MS ES$^+$ m/z 156.36 $(M+Na)^+$.