UDP-Glucose Analogues as Inhibitors and Mechanistic Probes of UDP-Glucose Dehydrogenase

Robert E. Campbell and Martin E. Tanner*

Department of Chemistry, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

Received July 7, 1999

UDP-glucose dehydrogenase catalyzes the NAD⁺-dependent 2-fold oxidation of UDP-glucose to give UDP-glucuronic acid. The putative aldehyde intermediate is not released from the active site and is presumably tightly bound. We have prepared UDP-7-deoxy-α-D-glucopyranosyl-6-ol, 5, that contains a methyl ketone at C-6 and cannot be further oxidized by the enzyme. Ketone 5 was found to be a competitive inhibitor of the dehydrogenase from Streptococcus pyogenes with a Ki value of 6.7 μM. We have also prepared the secondary alcohols UDP-6S-6C-methylglucose, 4a, and UDP-6R-6C-methylglucose, 4b. Compound 4a, but not 4b, was found to be a slow substrate for the dehydrogenase and was converted into the ketone inhibitor 5. This is consistent with the notion that the pro-R hydride is transferred in the first oxidation step of the normal enzymatic reaction.

Introduction

UDP-glucose dehydrogenase catalyzes the irreversible 2-fold oxidation of UDP-glucose to produce UDP-glucuronic acid (Figure 1).1 UDP-glucuronic acid serves as an activated donor of glucuronic acid residues in a variety of biochemical pathways throughout nature. In mammals, glucuronic acid residues are found as components of glycosaminoglycans (heparin, hyaluronic acid, and chondroitin sulfate), which form the “ground substance” or extracellular medium of connective tissues.2,3 They are also used to solubilize waste materials targeted for excretion in a process known as glucuronidation.4 In many strains of pathogenic bacteria such as group A streptococci and Streptococcus pneumoniae type 3, they are found as components of the polysaccharide capsule.5,6 The capsule is thought to act as a “sugar coating” that masks the normal cell surface features from the immune system of the host. It is well established that virulent strains of encapsulated bacteria become avirulent when capsule formation is disrupted.7–9 An understanding of the mechanism used by the bacterial UDP-glucose dehydrogenase may allow the design of inhibitors in an effort toward antibiotic design.

The mechanism of the dehydrogenase reaction appears to involve an initial oxidation of UDP-glucose to generate

![Figure 1. Proposed mechanism for the reaction catalyzed by UDP-glucose dehydrogenase.](image)

The bound aldehyde intermediate 1 (Figure 1)10–15 This putative intermediate is not released from the active site to any appreciable extent and is inaccessible to external aldehyde trapping reagents present in the solution.12,14,15 Compound 1 has been independently synthesized and was found to be a kinetically competent substrate for the dehydrogenase reaction (Kcat = 1.0 s⁻¹, KM = 14 μM).11

This supports the notion that aldehyde 1 is a true intermediate in the reaction and is effectively sequestered or tightly bound in the active site of the enzyme. This binding is likely in the form of a covalent thiohemiacetal linkage with an active site cysteine residue. Mechanistic

References:

studies indicate that the second oxidation proceeds directly from the thiohemiacetal 2 to give a thioester intermediate, 3. A final hydrosis step generates the product UDP-glucuronic acid and the free enzyme. Support for the involvement of covalent catalysis via cysteine comes from inactivation studies with thiol-directed reagents including UDP-chloroacetate. In the case of the enzyme from Streptococcus pyogenes, mutation of a conserved cysteine residue led to a dramatic reduction in catalytic activity. Furthermore, it was shown that the Cys-to-Ser mutant underwent the first two oxidation steps of the reaction to generate the ester intermediate presumably because the acidic conditions promoted the formation of a tertiary carbocation at C-6 and led to the decomposition of the compound.

In this paper we describe studies aimed at generating a mechanism-based reversible inhibitor of the dehydrogenase from group A streptococci. We reasoned that a UDP-glucose analogue such as ketone 5 should mimic the aldehyde intermediate 1 and be tightly bound by the dehydrogenase via a thiohemiacetal linkage (Figure 2). Ketone 5 could be produced in the active site of the enzyme by the oxidation of UDP-6S-6C-methylglucose, 4a, and/or UDP-6R-6C-methylglucose, 4b. A second oxidation presumably could not take place since it would require the cleavage of a carbon–carbon bond. We have found that ketone 5 is a competitive inhibitor of the enzyme with a Ki value of 6.7 μM. In addition, alcohol 4a is slowly oxidized by the enzyme to give ketone 5, whereas alcohol 4b is not a substrate. This is consistent with the notion that the pro-R hydrid is transferred in the first oxidation step of the normal enzymatic reaction.

Results

(A) Synthesis of Analogues. UDP-6S-6C-methylglucose, 4a, and UDP-6R-6C-methylglucose, 4b, were prepared from the corresponding methylglucose diastereomers 6a and 6b that had been described previously by Blériot et al. (Scheme 1). In their synthesis a nonstereoselective route is reported that allows one to prepare both diastereomers from glucuronolactone in seven steps. The diastereomers may be chromatographically separated when protected as diacetonides. In this work the free sugars 6a and 6b were first peracetylated with sodium acetate in acetic anhydride. In each case, it was possible to crystallize the α-anomer 7a or 7b from the anomeric mixture (α:β = 1:3) that was initially isolated. To confirm the stereochemical assignment made in the previous work, the structure of 7b was determined by X-ray crystallographic analysis and was found to contain an R-configuration at C-6 as expected. The peracetylated sugars were subjected to a modified Mac-Donald procedure using neat phosphoric acid at 55 °C and then deprotected using NaOMe/MeOH to give the α-phosphates 8a and 8b in 30% yield. These compounds were coupled to UMP-morpholidate in the presence of 1H-tetrazole to give UDP-6S-6C-methylglucose, 4a (36% yield), and UDP-6R-6C-methylglucose, 4b (26% yield).

Ketone 5 was prepared from the known alcohol 9 that is stereoselectively synthesized from glucuronolactone in six steps (Scheme 2). Alcohol 9 was oxidized under Swern conditions to give the crude ketone that was directly converted to alkene 10 using the Takai procedure (53% yield from 9). Alkene 10 was deprotected with TFA to give 11 and then peracylated using acetic anhydride and sodium acetate to give 12 as a mixture of anomers (α:β = 1:2). A protected version of compound 11 has been previously prepared via an alternate route. It was possible to selectively crystallize the β-anomer of 12, and an attempt was made to directly introduce the α-phosphate by submitting this anomer to the Mac-Donald procedure. This proved to be unsuccessful presumably because the acidic conditions promoted the formation of a tertiary carbocation at C-6 and led to the decomposition of the compound. Instead the anomeric mixture of 12 was treated with hydrazine acetate to give the free hemiacetal 13. This material was dibenzylphosphorylated, oxidized/deprotected by ozonolysis, hydroxylated over Pd/C, and finally deacylated using 10% triethylamine in methanol/H2O to give phosphate 14 as a mixture of 15a and 15b.

References

a mixture of anomers ($\alpha:\beta = 3:2$) in a 40% yield. To introduce the terminal uridine monophosphate group, a mild coupling reaction that operates under nonbasic conditions was desired. In previous work, UDP-glucose pyrophosphorylase was used to prepare aldehyde 1, indicating that the enzyme accepted glucose 1-phosphate analogues with some structural modification at C-6. It also seemed likely that the enzyme would selectively accept only the $\alpha$-anomer of 14 and remove the need for a separation of the anomeric mixture. Indeed, when 14 was treated with UDP-glucose pyrophosphorylase, UTP, and inorganic pyrophosphatase, the target ketone 5 was cleanly generated as analyzed by HPLC and could be isolated with a 15% yield. It is likely that the pyrophosphorylase coupling reaction could also have been employed in the preparation of 4a and 4b; however, this was not attempted.

(B) Enzymatic Studies. Ketone 5 was tested as an inhibitor of the dehydrogenase reaction by following the initial rate of oxidation of UDP-glucose as a function of substrate concentration at six different fixed inhibitor concentrations. The resulting double reciprocal plot intersected on the Y-axis, indicating that the inhibition was competitive in nature (Figure 3). A replot of the slopes against $[5]$ gave a linear plot showing a $K_I$ value of 6.7 $\mu$M (Figure 4, inset). No evidence for slow, tight binding or irreversible inhibition was detected.

UDP-6S-6C-methylglucose, 4a, and UDP-6R-6C-methylglucose, 4b, were tested as substrates by incubating them with UDP-glucose dehydrogenase and NAD$^+$ and monitoring the production of NADH using UV spectroscopy at 340 nm. In the case of UDP-6R-6C-methylglucose, 4b, no reaction could be detected even at very high enzyme concentrations (1.4 mg/mL). In the case of UDP-6S-6C-methylglucose, 4a, however, a slow reaction did occur that reached equilibrium after less than 10% of the substrate was consumed.

To further establish the notion that 4a, but not 4b, was oxidized to give 5 in the previous experiment, a sample of 5 was treated with NADH to look for the formation of 4a via the reverse reaction. The reaction was followed by ion-paired reversed-phase HPLC as shown in Figure 4. Prior to addition of the enzyme (Figure 4a), peaks corresponding to NAD$^+$ (present as a minor impurity in NADH) and ketone 5 are observed (NADH itself is not shown in Figure 4). In separate runs it was shown that 4a coelutes with NAD$^+$ under all conditions tested and that 4b coelutes with ketone 5. After incubation with NADH, the NADH peak decreased and a new peak with the retention time of 4a appeared.

(24) Due to the slow rate of the enzymatic reaction and the fact that the equilibrium favored 4a, it was not possible to obtain accurate steady-state kinetic constants. The equilibrium could be shifted toward the product 5 by the addition of 25 mM NH$_2$OH, and under these conditions the value of $k_{cat}/K_M$ for the oxidation of 4a was found to be 10 000 times lower than that for the oxidation of UDP-glucose.

tion with the dehydrogenase (Figure 4b), the peak eluting at the retention time of 5/4b had disappeared and the peak corresponding to NAD+/4a had increased in intensity. This indicates that ketone 5 had been converted solely to UDP-6S-6C-methylglucose, 4a. Appropriate spiking experiments with 4a and 4b confirmed this assignment (traces not shown).

**Discussion**

The inability to detect any released aldehyde 1 during the course of the normal UDP-glucose dehydrogenase reaction led us to suspect that this compound was tightly bound by the enzyme and that UDP-glucose analogues with carboxyl functionalities at C-6 might serve as potent inhibitors of the enzyme. The observation that ketone 5 is a competitive inhibitor with a Ki value of 6.7 μM, whereas the Km value for UDP-glucose is 20 μM, indicates that any potential improvement in binding affinity is moderated by the introduction of the methyl group. The methyl group could be causing steric problems in the active site as well as stabilizing the carbonyl form of the compound and thereby lowering the propensity for addition of the active-site thiol to generate a thioheicacetal (relative to an analogous additional to the aldehyde). The latter problem may be addressed by preparing the trifluromethyl ketone analogue of 5 that should greatly increase the amount of thioheicacetal formed and possibly lower the value of Ki.

During the course of the normal UDP-glucose dehydrogenase reaction, both the pro-R and the pro-S hydrogens are sequentially transferred to two molecules of NAD⁺ (Figure 1). In the UDP-6C-methylglucose distereomers 4a and 4b, these hydrogens have been selectively replaced with methyl groups, and the compounds may be used as a tool to probe the order of the hydride-transfer steps. The observation that 4a, which retains the "pro-R-like" hydrogen, is a substrate (albeit a slowly reacting one) and 4b is not suggests that the pro-R hydride is transferred during the first oxidation of alcohol to aldehyde in the normal S. pyogenes dehydrogenase reaction (Figure 2). This observation is in agreement with studies done on the UDP-glucose dehydrogenase from beef liver which report that the pro-R hydride is transferred in the first oxidation step.

**Experimental Section**

**General Procedures.** Reactions were performed under argon unless otherwise noted. All chemicals were purchased from Sigma, Aldrich, or Lancaster. Preparative thin-layer chromatography (TLC) was performed on glass-backed plates of either 1 or 2 mm thick silica gel 60F254. Amberlite IR-120(plus) resin was used for all counterion-exchange (sodium form) and neutralization (hydrogen or pyridinium form) after O-deacetylation or hydrogenation. 7-Deoxy-L-glycerol-1,6-dideoxy-7-heptopyranose, 6a, 7-deoxy-L-glycerol-1,6-dideoxy-7-heptopyranose, 6b, and 7-deoxy-1,2,3,5-di-O-isopropylidene-L-glycerol-1,6-dideoxy-7-heptopyranose, 9, were prepared as previously described. Elemental analyses were performed by the Microanalytical Lab of the University of British Columbia Chemistry Department.

12,3,4,6-Pentadecyl-7-deoxy-L-glycerol-1,6-dideoxy-7-heptopyranose (7a). To a solution of 6a (0.277 g, 1.43 mmol) in AcO (12 mL) was added NaOAc (0.79 g, 9.6 mmol), and the solution was refluxed for 1 h, cooled to rt, and once again heated to reflux. The solution was poured into saturated NaHCO₃ and extracted with EtOAc. The organic layer washed with brine (dried with Na₂SO₄) and the solvent removed in vacuo. Flash chromatography (50% EtOAc/50% hexanes) yielded 7a. The crude product was purified by preparative TLC (10% CHCl₃/50% hexanes) as a white solid (21 mg, 29%).


**Uridine 5′-{(7-deoxy-L-glycerol-1,6-dideoxy-7-heptopyranosyl Diphosphate), Sodium Salt (4a)}**

Compound 8a (22 mg, 0.074 mmol) was converted to its pyridinium counterion form and maintained in vacuo overnight. Trioctylamine (33 μL, 0.076 mmol) and pyridine (2–5 mL) were added, and the solvent was removed in vacuo. The process of dissolution in pyridine, solvent removal, and equalization with argon was repeated twice. UMP-morpholide (84 mg, 0.12 mmol) and 1H-tetrazole (17 mg, 0.24 mmol) were added to the flask, dissolved, and concentrated to dryness twice from pyridine, and the final volume of pyridine (0.15 mL) was added. After 2 days at rt the solution was removed in vacuo and the residue dissolved in water and washed twice (Et₂O). The crude product was applied to a column (1.5 cm × 18 cm) of DE-52 anion-exchange resin and eluted with a linear gradient from 0 to 300 mM LiCl with detection at 254 nm. The relevant fraction (30–40 mL) was applied to a column (110 × 2.5 cm) of Biogel P-2 (200–400 mesh) and eluted with distilled water. Fractions containing product were pooled, concentrated in vacuo to 2–3 mL, and applied to the Biogel P-2 column. Counterion exchange to sodium and lyophilization yielded 4a as a white powder (17 mg, 36%). The crude product was applied to a column (110 × 2.5 cm) of Biogel P-2 (200–400 mesh) and eluted with distilled water. Fractions containing product were pooled, concentrated in vacuo to 2–3 mL, and applied to the Biogel P-2 column. Counterion exchange to sodium and lyophilization yielded 4a as a white powder (17 mg, 36%).
Inhibitors of UDP-Glucose Dehydrogenase

2.03 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.98 (s, 1H, J 7.2, 2.4), 3.90 (dd, 1H, J 6.7, 2.3), 3.76 (dd, 1H, J = 10.1, 2.4), 2.09 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.23 (d, 3H, J = 6.7); 13C NMR (75 MHz, CDCl3) 170.2, 170.0, 169.6, 169.3, 169.3, 168.9, 91.7, 75.4, 72.9, 70.2, 68.7, 68.5, 21.1, 20.8, 20.6, 20.5, 20.5, 13.5; DCI(+)(MS (NH4)2) m/z 422 (M + NH4)+, 100%. Anal. Calcd for C17O11H24: C, 50.48; H, 5.99. Found: C, 50.56; H, 6.05.

7-Deoxy-o-glycero- ß-D-gluco-heptopyranosyl phosphate, Sodium Salt (8b). Compound 7b (100 mg, 0.25 mmol) was treated according to the modified MacDonald procedure described for the preparation of TLE. Water gave the acetylated free acid of 8b (73 mg), which was immediately subjected to O-deacetylation and purification as described. This procedure gave 8b as a white powder (23 mg, 31%): 1H NMR (400 MHz, CDCl3) δ 5.43 (dd, 1H, J = 7.1, 3.7), 4.09 (dq, 1H, J = 6.7, 2.4), 3.90 (dd, 1H, J = 10.1, 2.3), 3.71 (dd, 1H, J = 9.4, 9.4), 3.48–3.45 (m, 1H), 3.32 (dd, 1H, J = 10.0, 9.3), 1.16 (d, 3H, J = 6.8); 13C NMR (75 MHz, CDCl3) δ 94.4 (d, 3J = 9.6, 9.3), 9.52 (dd, 1H, J = 9.8, 3.3, 3.1), 3.39 (dd, 1H, J = 10.1, 9.2), 1.21 (d, 3H, J = 6.6); 13C NMR (75 MHz, CDCl3) δ 166.6, 152.1, 141.8, 102.9, 95.8 (d, J = 6.5), 88.6, 83.5 (d, J = 8.9), 74.9, 74.0, 73.3, 71.8 (d, J = 8.2), 70.8, 69.9, 66.8, 65.2 (d, J = 5.0), 15.6; 31P NMR (121.5 MHz, CDCl3) δ 121.3 (s); [31P NMR (121.5 MHz, H2O)] as a solid white foam (225 mg, 97%): 1H NMR (400 MHz, CDCl3) δ 7.90 (d, 1H, J = 8.3), 5.23 (dd, 1H, J = 9.5, 9.5), 5.07 (dd, 1H, J = 9.5, 8.3), 5.03 (dd, 1H, J = 9.7, 9.6), 4.94 (s, 2H), 3.95 (dd, 1H, J = 9.8), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.70 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 170.0, 169.9, 169.6, 168.8, 139.2, 117.0, 91.5, 78.8, 72.6, 70.4, 68.9, 20.8, 20.5, 20.5, 16.6; DCI(+)(MS (NH4)2) m/z 376 (M + NH4)+, 30%. Anal. Calcd for C10H16O13: C, 53.63; H, 6.19. Found: C, 53.71; H, 6.07.

7-Deoxy- ß-glycero- ß-D-gluco-hept-6-enopyranosyl phosphate, Sodium Salt (14). To a stirring solution of 13 (82 mg, 0.26 mmol) in CH2Cl2 (5 mL) were added 1H-tetrazole (73 mg, 1.0 mmol) and dibenzyl N,N-diisopropylphosphoramidite (0.22 mmL, 0.65 mmol). After 3 h at rt, the reaction mixture was diluted (EtOAc), washed (ice-cold brine), and dried (Na2SO4), and solvent was removed in vacuo. Flash chromatography (60% hexanes/40% EtOAc) gave the free sugar 13 (45 mg, 85%): 1H NMR (400 MHz, CDCl3) δ 5.54 (dd, 0.7H, J = 9.9, 9.8), 5.42 (dd, 0.7H, J = 3.6), 5.24 (dd, 0.3H, J = 9.6, 9.6), 5.03–4.94 (m, 3H), 4.87 (dd, 0.7H, J = 7.4, 3.6), 4.84 (dd, 0.3H, J = 9.7, 8.0), 4.73 (d, 0.3H, J = 10.1), 4.42 (d, 0.7H, J = 10.1), 3.88 (d, 0.3H, J = 9.9), 3.30 (broader, 1H, J = 2.06 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.74 (s, 0.9H), 1.72 (s, 1.1H); 1H NMR (75 MHz, CDCl3) δ 170.0, 170.3, 170.2, 169.6, 161.8, 161.8, 140.3, 139.8, 116.7, 116.6, 95.3, 90.1, 78.4, 73.4, 73.2, 72.1, 71.3, 69.6, 69.6, 69.4, 20.7, 20.7, 20.6, 16.7; DCI(+)(MS (NH4)2) m/z 334 (M + NH4)+, 38%. Anal. Calcd for C10H18O8: C, 53.16; H, 6.37. Found: C, 53.32; H, 6.47.
sulfide (1 mL) was added, and the solution was stored overnight at \(-20^\circ C\). Solvent was removed in vacuo, the residue was redissolved in 50% MeOH/50% EtOAc (20 mL), and 10% Pd/C (40 mg) was added. The solution was repeatedly degassed in vacuo, with H2 being used to equalize the pressure each time. After the solution was stirred for 1 h under H2, a small amount of cation-exchange resin (pyridinium form) was added, and the residue was dissolved and filtered in vacuo. The residue was dissolved in 1.1 M MeOH/H2O containing 10% NEt3, and the reaction was stirred at rt for 3 h. Two successive rounds of preparative TLC (45% MeOH/45% CHCl3/10% H2O), exchange to a sodium counterion, and lyophilization gave 14 as a white powder (3x2)/ by 1H NMR, 33.1 mg, 40%. Material prepared in this manner contained a persistent impurity (10 mol% by 1H NMR) displaying spectral properties consistent with 1-PrNPO3Na2: 1H NMR (400 MHz, D2O) \(\delta\) 5.48 (dd, 0.60H, \(J = 7.3\), 3.5), 4.92 (dd, 0.40H, \(J = 8.0\), 7.3), 4.41 (d, 0.60H, \(J = 10.2\)), 4.06 (d, 0.40H, \(J = 9.7\)), 3.78 (dd, 0.60H, \(J = 9.5\), 9.3), 3.58–3.44 (m, 2H), 3.52 (d, 1.3H, \(J = 10.6\), (Me\(\text{CH}_3\))NPO3Na2), 3.35 (dd, 0.40H, \(J = 8.7\), 8.2), 2.31 (s, 3H); 13C NMR (75 MHz, D2O) \(\delta\) 211.0, 210.1, 97.7 (d, \(J = 3.7\)), 94.8 (d, \(J = 4.4\)), 79.9, 76.4, 75.8, 74.1 (d, \(J = 5.4\)), 73.2, 71.8 (d, \(J = 5.8\)), 71.6, 71.4, 52.8 (d, \(J = 4.1\), (Me\(\text{CH}_3\))NPO3Na2), 28.3, 28.1; 31P NMR (121.5 MHz, D2O) \(\delta\) 3.07 (s, 0.4P), 2.56 (s, 0.1P), 2.06 (s, 0.6P); HR-LSI(–) MS (thioglycerol matrix) m/z calcd for C16H22N2O17P2Na 599.0291, found 599.0268.

Enzymatic Studies. UDPGDH (1.5 unit/mg) was prepared as previously reported. All enzyme kinetic experiments were performed at 30 °C in 50 mM triethanolamine/ HCl (pH 8.7), with 2 mM dithiothreitol. Initial rates were determined by following the reduction of NAD+ at 340 nM with a Varian Cary 3E UV–vis spectrophotometer followed by least-squares analysis with Cary 3 software version 3.0. For the K1 determination of 5, the enzymatic reaction was initiated by adding UDPGDH (final concentration 0.011 mg/mL) to assay buffer (1.0 mL) containing 500 μM NAD+ and varying concentrations of both 5 and UDPG. The standard error of the reported K1 value is less than 10%. To test 4a and 4b as substrates, UDPGDH (final concentration 1.4 mg/mL) was added to assay buffer (0.60 mL) containing either 4a or 4b and 10.0 mM NAD+. Both 4a and 4b were also tested as substrates with 25 mM NH2OH in the assay buffer. To measure the kinetic constants for turnover of 4a, UDPGDH (final concentration 0.48 mg/mL) was added to assay buffer (0.60 mL) containing 25 mM NH2OH, 10 mM NAD+, and varying concentrations of 4a. Figure 3 was constructed using the program Grafit.

Ion-Paired Reversed-Phase HPLC. HPLC experiments were performed with a Radial-pak C-18 column with detection at 260 nM. The column was eluted by washing for 10 min with 100 mM K2PO4 (pH 6.9) and 5.0 mM tetrabutylammonium hydrogen sulfate followed by a linear gradient of 0–50% acetonitrile in the same buffer. To determine the product of the enzymatic reduction of 5, UDPGDH (final concentration 1.1 mg/mL) was incubated at 30 °C with 10.0 mM NADH and 1.0 mM 5 in a total volume of 0.380 mL of assay buffer. A control sample without UDPGDH was also prepared. Aliquots were removed at timed intervals and analyzed by ion-paired reversed-phase HPLC as described. Peaks were identified by spiking aliquots with 5, 4a, 4b, or NAD+.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support. R.E.C. was supported by an NSERC Graduate Student Fellowship.

Supporting Information Available: 1H NMR spectra for compounds 4, 5, 8, and 14 and X-ray crystal structure data for compound 7b. This material is available free of charge via the Internet at http://pubs.acs.org.