Synthesis of $^{13}C_6$-glucuronic Acid Drug Metabolites

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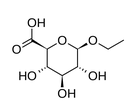
Abstract

Analysis of drugs and metabolites is accomplished via the use of labeled internal standards. However, stable isotope labeling of parent drugs can be synthetically challenging. Since glucuronides are primary metabolites of many drugs, one alternative would be the incorporation of the stable isotope into the glucuronic acid moiety.To this end, a $^{13}C_6$-glucuronic acid sugar donor was synthesized and used in the synthesis of ethynylestradiol and AZT $^{13}C_6$-glucuronides.

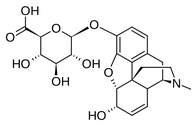
Introduction

- Monitoring and quantitation of drugs and their metabolites in serum, plasma and urine represents an important facet of clinical and pharmacological laboratory work. Therapeutic monitoring of drugs can play an important role in clinical treatment decisions. For drug development and PKDM, identification and quantitation of drug metabolites are key to understanding the distribution, half life and toxicity of new drugs.
- Since many drugs are metabolized *in vivo*, detection of the metabolites is often the primary means of drug quantitation. A common metabolic product is the conjugation of glucuronic acid to the parent drug.
- In the analysis and quantitation of drugs and their metabolites, the use of stable isotopically labeled versions of these compounds as internal standards for GC/MS and LC/MS applications is desirable.
- Traditionally, labeled glucuronides are made by first synthesizing the labeled parent drug followed by glycosylation.
- Labeling of the parent drug can be synthetically challenging. Suitable labeled reagents may not be available or costly. The structure of the drug may not be suitable for introduction of a stable label. A viable alternative would be to incorporate the label into the glucuronic acid moiety.
- A $^{13}C_6$-glucuronic acid sugar donor was synthesized and used to make two metabolites, ethynylestradiol-3-glucuronide-$^{13}C_6$ and AZT-glucuronide-$^{13}C_6$.

Examples of Glucuronide Metabolites

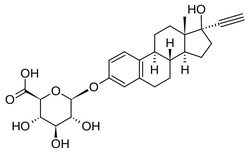


Ethyl glucuronide

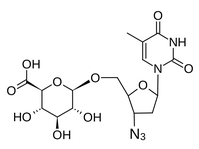


Morphine-3-glucuronide

Labeled versions of the aglycones are readily available, e.g.ethyl-d5 glucuronide and morphine-3-glucuronide



Ethynylestradiol glucuronide



AZT glucuronide

Labeled versions of the aglycones are not available; good candidates for labeling of the glucuronic acid

Cerilliant® Analytical Reference Standards

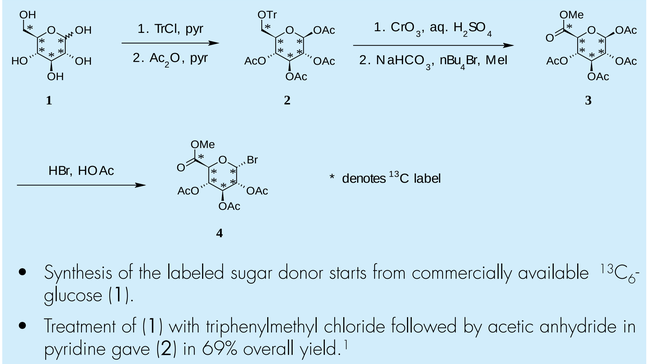
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Labeled Drug Glucuronide Synthesis

Selection of The Isotopic Label: Advantages of A $^{13}C_6$ Glucuronic Acid Sugar Donor

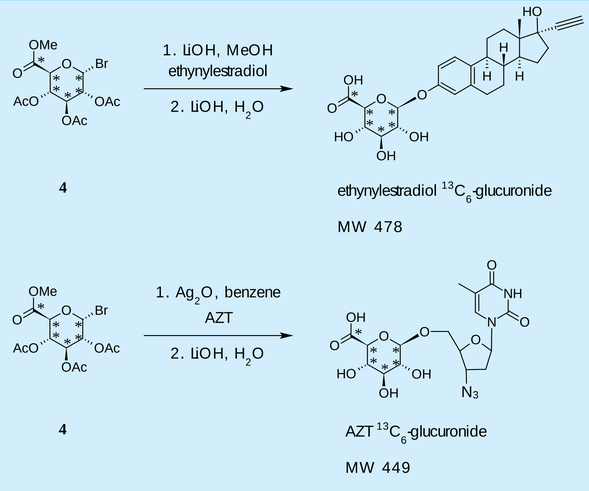
- A key consideration in the synthesis of any stable labeled compound is the availability of labeled precursors.
- For internal standard use, label incorporation of a minimum of three mass units is preferred.
- $^{13}C_6$ glucose is a readily available starting material.
- The chemistry for synthesizing the sugar donor is well-established.
- An alternative to using $^{13}C_6$ glucose is glucose-d_7.
- The drawback to using deuterated glucose is two-fold:
  - Loss of 2 mass units during the oxidation step, resulting in glucuronic-d_5 acid
  - High probability of exchange at the C-5 position, leading to varying amounts of d_4 and d_5
- Use of $^{13}C_6$ glucose precludes these drawbacks.

Synthesis of $^{13}C_6$-glucuronic Acid Sugar Donor



- Synthesis of the labeled sugar donor starts from commercially available $^{13}C_6$-glucose (1).
- Treatment of (1) with triphenylmethyl chloride followed by acetic anhydride in pyridine gave (2) in 69% overall yield.[1]
- Without purification of the intermediates, (2) was successively detritylated, oxidized, and esterified to give (3) in 55% overall yield.[2]
- Sugar donor (4) was obtained in 84% yield by treating (3) with hydrogen bromide in acetic acid.[3]

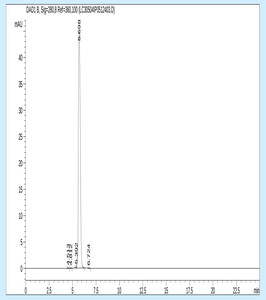
Synthesis of Ethynylestradiol and AZT $^{13}C_6$-glucuronides



- Ethynylestradiol-3-glucuronide-$^{13}C_6$ was synthesized in one step with a 19% yield and an LC purity of 99%.[4]
- AZT-glucuronide-$^{13}C_6$ was synthesized in two steps with a 1% overall yield and an LC purity of 90%.
- LCMS-SIM demonstrated that both compounds had an isotopic purity of $^{12}C_6/^{13}C_6$ < 1%.

Ethynylestradiol-3-glucuronide-$^{13}C_6$

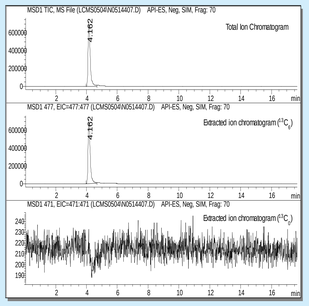
HPLC DATA



Instrument	Agilent 1100 Series HPLC System
Column	Inertsil 5 ODS-3 4.6 x 250 mm
Column Temperature	Ambient
Mobile Phases	A: Acetonitrile B: 0.01M KH_2PO_4 buffer
Gradient	Isocratic, 50:50 = A :B
Flow Rate	0.5 mL/min
Injection Volume	2 µL
Detector	UV/Vis or Photo Diode Array
UV Wavelength	280 nm
Run Time	15 minutes

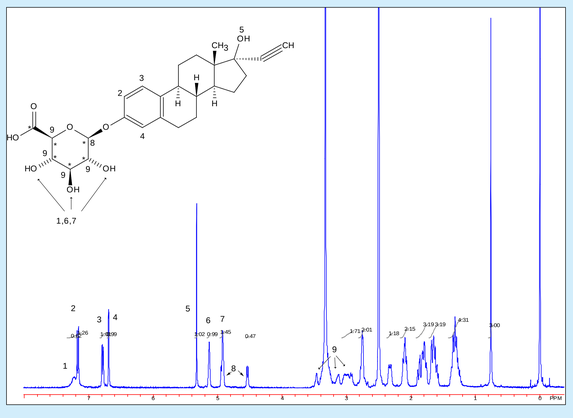
Peak #	Ret Time	Area	Height	Area %
1	4.51	0.88	0.13	0.14
2	4.81	0.58	0.08	0.09
3	5.39	1.55	0.29	0.24
4	5.70	630.49	44.98	99.45
5	6.72	0.49	0.06	0.08

LCMS DATA



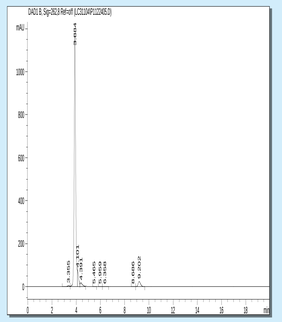
Instrument	Agilent 1100 LCMS
Column	Zorbax C_8, 150*4.6 mm
Column Temperature	Ambient
Mobile Phases	A: 10 mM Ammonium acetate B: Acetonitrile
Gradient	Isocratic, 50:50 = A :B
Flow Rate	0.3 mL/min
Injection Volume	1 µL
UV Wavelength	215 nm
MS Detector Polarity	Negative
MS Detector	AP-ESI
Mass scan range for full scan	100-1000 Da

^{1}H-NMR SPECTRUM (DMSO-d_6)



AZT-3-glucuronide-$^{13}C_6$

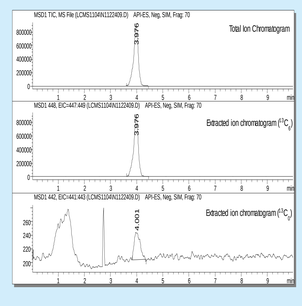
HPLC DATA



Instrument	Agilent 1100 Series HPLC System
Column	Betasil phenyl 4.6 x 250 mm
Column Temperature	Ambient
Mobile Phases	A: Acetonitrile B: 0.01M KH_2PO_4 buffer
Gradient	Isocratic, 20:80 = A :B
Flow Rate	0.5 mL/min
Injection Volume	2 µL
Detector	UV/Vis or Photo Diode Array
UV Wavelength	262 nm
Run Time	20 minutes

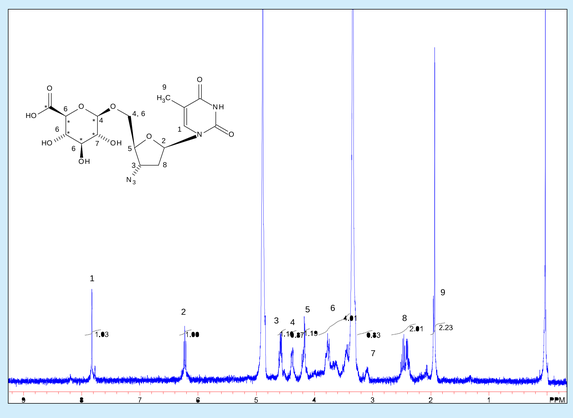
Peak #	Ret Time	Area	Height	Area %
1	3.36	49.62	4.30	0.43
2	3.88	10336.10	1174.23	89.98
3	4.10	524.61	75.89	4.57
4	4.39	209.22	17.15	1.82
5	5.47	1.79	0.28	0.02
6	5.96	3.59	0.37	0.03
7	6.36	8.34	0.65	0.07
8	8.69	4.19	0.37	0.04
9	9.20	350.25	23.92	3.05

LCMS DATA



Instrument	Agilent 1100 LCMS
Column	Zorbax C_8, 150*4.6 mm
Column Temperature	Ambient
Mobile Phases	A: methanol B: water
Gradient	Isocratic, 50:50 = A :B
Flow Rate	0.3 mL/min
Injection Volume	1 µL
UV Wavelength	215 nm
MS Detector Polarity	Negative
MS Detector	AP-ESI
Mass scan range for full scan	100-1000 Da

^{1}H-NMR SPECTRUM (CD_3OD)



CONCLUSIONS

- Labeled versions of the drug metabolites ethynylestradiol-3-glucuronide and AZT-glucuronide have been synthesized wherein the glucuronic acid moiety is $^{13}C_6$-labelled.
- The $^{13}C_6$-sugar donor was prepared in 32% overall yield from D-glucose-$^{13}C_6$.
- Isotopic purity of the synthesized metabolites was >99%.
- This work demonstrates that stable isotope labeling of the glucuronic acid is a feasible approach to drug metabolite internal standard synthesis.
- This work is widely applicable to a range of glucuronide metabolites.

References
1. Helferich, B. and Leete, J. *Ber.* **1929**, *62*, 1552.
2. Vogel, C.; Boye, H.; and Kristen, H. *J. Prakt. Chem.*, **1990**, *332*, 28-36.
3. Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; and Lindquist, J. A. *J. Am. Chem. Soc.*, **1955**, *77*, 3310-3315.
4. Cerilliant procedure.