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Enzymatic approach toward the synthesis of isotopically labeled (*R*)-9-(2-hydroxypropyl)adenine

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ABSTRACT

Isotopically labeled compounds have numerous applications in drug discovery and development. (*R*)-9-(2-Hydroxypropyl)adenine **1** is an important intermediate in the synthesis of various acyclic nucleoside phosphonates including tenofovir. We have developed a short synthetic method to prepare deuterium and tritium labeled **1** using a ketoreductase enzyme catalyzed reduction as the key step to introduce the label in a highly efficient and enantioselective way.

Introduction

Isotopically labeled compounds have numerous applications in drug discovery and development. Stable isotope labeled (SIL) compounds (i.e., compounds containing non-radioactive isotopes such as ^{13}C, ^{15}N and ^{2}H) are most commonly used as internal standards for mass spectroscopy studies for the accurate determination of drug concentration in biological samples.[1] For this application, the SIL internal standard is added to the biological matrix to correct for variability in dilution, evaporation, degradation, recovery, adsorption and derivatization. SIL compounds have also been used in the assessment of drug pharmacology to determine the pharmacokinetic profile or mode of action of a drug substance; for the assessment of drug products or drug delivery systems by determination of parameters such as the bioavailability or the release profile; and for assessment of patient-specific drug treatment.[2] In addition, radioactive isotopes of drug candidates (e.g., ^{14}C, ^{3}H isotope analogs) are required during the course of pre-clinical and clinical development. Because radioactive compounds, such as tritium-labeled isotopes, can be detected and quantified at nanomolar levels of concentration, they are indispensable tools for drug metabolism and pharmacokinetic (DMPK) studies such as initial metabolism studies, binding assays, receptor occupancy and plasma-protein binding assays.[3]

(*R*)-9-(2-Hydroxypropyl)adenine **1** is an important intermediate in the synthesis of various acyclic nucleoside phosphonates. Most notably it is a key intermediate in the synthesis of tenofovir **2** which has been shown to display broad activity against viral infections. The corresponding prodrug, tenofovir disoproxil fumarate, has been approved as an anti-HIV and anti-Hepatitis B drug.[4] Other compounds of this class which have been synthesized and evaluated for antiviral activity include the boranophosphonate nucleoside **3**[5] and the thiophosphonate nucleoside **4**[6] (Fig. 1). We were interested in developing an efficient synthetic method to prepare SIL-**1** intermediate which can be further elaborated to SIL drug candidates to be used as mass spectrometry internal standard. In addition, we would like to apply the same synthetic methodology to prepare a tritium labeled analog of **1**.

Results and discussion

Synthetically viable labeling strategies to prepare isotopically labeled **1** include incorporation of the label into the adenine core or into the hydroxypropyl side chain. A number of synthetic methods for preparing ^{13}C and ^{15}N labeled adenines have been published.[7] One can consider converting these SIL adenines to the desired SIL-**1** via alkylation with various electrophiles following known procedures developed for the preparation of unlabeled **1**.[8] Alternatively, a deuterium labeled side chain can be prepared following a procedure previously described by Czarnik.[9]

Biocatalysis is a growing field and has numerous applications in the synthesis of pharmaceutical compounds. For example,

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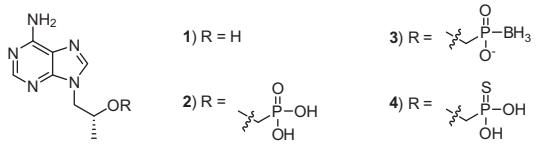
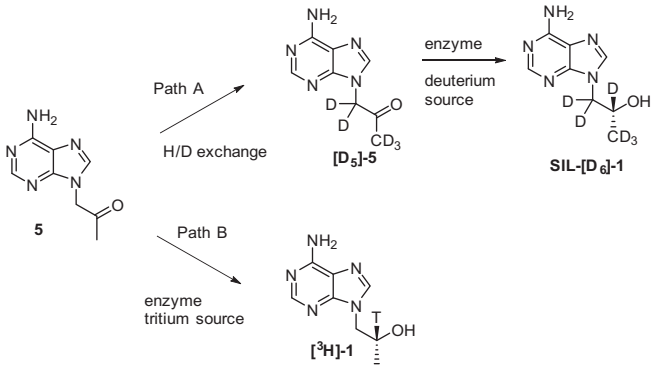


Figure 1. (*R*)-9-(2-Hydroxypropyl)adenine **1** and (*R*)-9-(2-hydroxypropyl)adenine containing compounds.

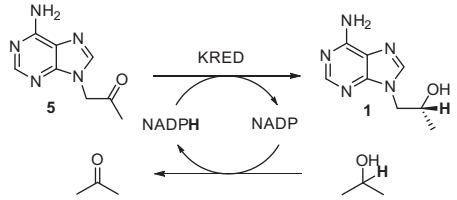
ketoreductase (KRED) enzymes have been widely used in our laboratories for the preparation of chiral alcohols.[10] Furthermore, KRED enzymes have been used to prepare deuterium labeled alcohols from ketones.[11] We envisioned that a short and direct route to **SIL**-[**D$_6$**]-**1** and [**^{3}H**]-**1** would be through a biocatalytic enantioselective reduction of a ketone intermediate (Scheme 1).[12] For SIL synthesis (Path A), the acidic protons of ketone **5** would allow for facile H/D exchange followed by an enzymatic reduction with an appropriate deuterium source to give the desired **SIL**-[**D$_6$**]-**1**. A similar approach (Path B) would provide tritium labeled alcohol [**^{3}H**]-**1** via a direct enzymatic reduction of ketone 5 with an appropriate tritium source.

With this synthetic approach in mind, we first sought to identify conditions to reduce ketone **5**[13] enzymatically. A number of KRED enzymes[14] were screened using NADP as a cofactor and iPrOH to regenerate the cofactor (Scheme 2). The representative results summarized in Table 1 show that either enantiomer can be accessed in very high enantioselectivities and conversions (entries 1 and 4). With the optimal enzyme identified (entry 1, P1B02 enzyme), the reaction conditions were optimized with respect to enzyme and cofactor loading, reaction concentration, and amount of iPrOH required. Typically with these reactions, in order to drive the reaction equilibrium in favor of the desired product, an excess amount of iPrOH is used. Furthermore, the acetone byproduct can be vented to drive the product formation.

In practice SIL compounds to be used as internal standards generally require a molecular weight increase of at least 4 atomic mass units (amu). When the difference is less than 3 amu the isotope peaks mass signal of the analyte may interfere with the signal of the internal standard.[15] In addition, as a general guideline, we typically require that the unlabeled content (D_0) cannot be above 0.1%. For this particular substrate, we targeted a mass increase of 6 amu. With these specifications in mind, we began the synthesis of **SIL**-[**D$_6$**]-**1** with hydrogen–deuterium exchange of the acidic protons of ketone **5**. After some optimization with regard to deuterated solvents used, pH and temperature, we found that the exchange can be accomplished by heating ketone **5** in a mixture of D_2O/MeOD/ACN at 50 °C to give ketone [**D$_5$**]-**5** in very high isotopic incorporation with [D_5] as the most abundant isotope as shown in Table 2.[16]



Scheme 1. Enzymatic synthesis of isotopically labeled (*R*)-9-(2-hydroxypropyl) adenine.



Scheme 2. Enzymatic asymmetric reduction with KRED and NADP with a cofactor recycling system using isopropanol.

Table 1
Representative KRED reduction enzyme screen results

Entry	Enzyme	% conversion[a]	% ee[b]
1	P1B02	97.3	>99 (*R*)
2	P1B10	30	97.7 (*R*)
3	P1H10	12.2	>99 (*R*)
4	P2C02	99.7	97.5 (*S*)
5	P2D11	44.6	97.5 (*R*)
6	KRED 101	2.3	68.4 (*R*)
7	KRED 119	23.3	40.6 (*R*)
8	KRED 130	19.2	81.6 (*R*)
9	KRED NADH 101	11.9	92.8 (*S*)

[a] Conversion, as area % of alcohol **1** relative to ketone **5**, was determined by HPLC analysis.
[b] ee was determined by chiral HPLC.

Table 2
Isotopic distribution of ketone [**D$_5$**]-**5**

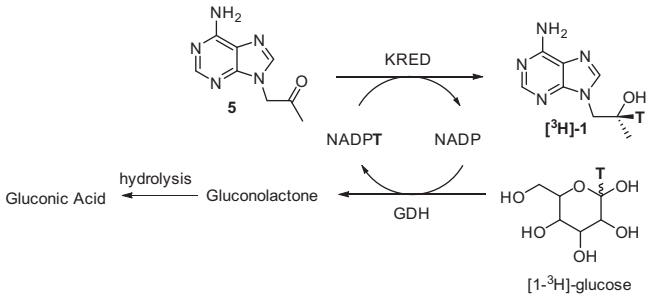
m/*z*	Isotopic species	% distribution
192	D_0	0.01
193	D_1	0.02
194	D_2	0.17
195	D_3	2.38
196	D_4	19.10
197	D_5	67.87
198	D_6	10.47

Some minor H/D exchange was observed at the C-8 position in the adenine core resulting in [D_6] isotope species with *m*/*z* = 198.

The final deuterium was introduced enzymatically using [2-^{2}H]-iPrOH as the deuterium source. We initially established that a single deuterium transfer experiment using P1B02 enzyme with unlabeled ketone **5** and [2-^{2}H]-iPrOH gave the corresponding [D_1]-labeled alcohol with 100% deuterium incorporation as measured by MS analysis. Thus, taking ketone [**D$_5$**]-**5** and subjecting it to KRED reduction conditions gave the desired isotopically labeled alcohol **SIL**-[**D$_6$**]-**1** in high enantiopurity (>99% ee) and yield (90%). More importantly, the isotopic distribution is such that D_0 is less than 0.1% as shown in Table 3, with [D_6] as the most abundant isotope. It is important to note that due to the propensity of the (acidic) deuterium labels of ketone [**D$_5$**]-**5** to back exchange to hydrogen, the enzymatic reduction was carried out in

Table 3
Isotopic distribution of alcohol **SIL**-[**D$_6$**]-**1**

m/*z*	Isotopic species	% distribution
194	D_0	0.01
195	D_1	0.03
196	D_2	0.04
197	D_3	0.29
198	D_4	2.25
199	D_5	16.79
200	D_6	58.80
201	D_7	21.79



Scheme 3. Enzymatic asymmetric reduction with KRED and NADP with a cofactor recycling system using glucose dehydrogenase and [1-^{3}H]-glucose.

deuterated aqueous buffer system. During the course of the reduction with deuterated aqueous buffer, an increase in deuteration at the C-8 position occurred giving rise to a [D_7] isotope with m/z = 201.

Following the same KRED strategy used for the SIL synthesis, our initial attempt to prepare [**^{3}H**]-**1** from ketone **5** using [2-^{3}H]-iPrOH (prepared by reduction of acetone with $NaBT_4$)[17] resulted in less than 5% conversion of **5** to [**^{3}H**]-**1** as measured by HPLC-UV. We attributed this low conversion to the fact that the [2-^{3}H]-iPrOH that was used contained acetone (3:1 ratio acetone relative to iPrOH) which caused the reaction equilibrium to shift toward the starting material. Due to the volatility of the tritium reagent and the microscale amounts that we were operating in, a nitrogen sweep which can be implemented to remove excess acetone was not practical in this case. Thus, we sought to investigate other cofactor recycling systems that are typically used for KRED reductions. After a series of experiments, we found that the sodium formate/Formate Dehydrogenase (FDH) system gave no reaction. Fortunately, on the other hand, the glucose/glucose dehydrogenase (GDH) system gave very high conversion of ketone **5** to the desired alcohol **1**. The main advantages with the glucose/GDH system over the previous iPrOH system is that [1-^{3}H]-glucose is non-volatile thus safer to handle and the byproduct of the reduction, gluconolactone, spontaneously hydrolyzes to gluconic acid in a non-reversible process (Scheme 3). Furthermore, in contrast to the iPrOH system, we were able to use glucose as the limiting reagent. Thus, treatment of [1-^{3}H]-glucose[18] with 1.1 equiv of ketone **5** resulted to complete consumption of the tritium reagent and 90% conversion of **5** to [**^{3}H**]-**1** was observed**.** After purification using reverse phase preparative chromatography, [**^{3}H**]-**1** was obtained in good yield (54%) and very high ee (>99%). ^{3}H NMR confirmed the tritium location as expected.

Conclusion

We have developed efficient enzymatic routes to access both deuterium and tritium labeled (*R*)-9-(2-hydroxypropyl)adenine **1**. **SIL**-[**D_6**]-**1** was prepared via H/D exchange followed by enzymatic reduction with [2-^{2}H]-iPrOH in very high yield and enantiopurity. More importantly, the isotope distribution of the resulting product was such that the unlabeled content [D_0] was less than 0.1%. Similarly, a method to prepare the tritiated analog [**^{3}H**]-**1** was also developed using the same enzyme but with [1-^{3}H]-glucose as a non-volatile tritium source. Although the enzymatic methodology presented here is used to prepare isotopically labeled **1**, it should be emphasized that it can be used to make non-labeled (*R*)-9-(2-hydroxypropyl)adenine which is a key intermediate to tenofovir and other pharmaceutically interesting acyclic nucleoside phosphonates.

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Supplementary data

Supplementary data (experimental procedures and data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.087.

References and notes

1. Elmore, C. S. *Annu. Rep. Med. Chem.* **2009**, *44*, 515–534.
2. Schellekens, R.; Stellaard, F.; Woerdenbag, H.; Frijlink, H.; Kosterink, J. *Br. J. Clin. Pharmacol.* **2011**, *72*, 879–897.
3. Isin, E.; Elmore, C.; Nilsson, G.; Thompson, R.; Weidolf, L. *Chem. Res. Toxicol.* **2012**, *25*, 532–542.
4. (a) Arimilli, M. N.; Kim, C. U.; Dougherty, J.; Mulato, A.; Oliyai, R.; Shaw, J. P.; Cundy, K. C.; Bischofberger, N. *Antiviral Chem. Chemother.* **1997**, *8*, 557; (b) Naessens, L.; Bischofberger, N.; Augustijns, P.; Annaert, P.; Van Den Mooter, G.; Arimilli, M. N.; Kim, C. U.; De Clerq, E. *Antimicrob. Agents Chemother.* **1998**, *42*, 1568; (c) Robbins, B.; Srinivas, R. V.; Kim, C.; Bischofberger, N.; Fridland, A. *Antimicrob. Agents Chemother.* **1998**, *42*, 612.
5. Barral, K.; Priet, S.; Sire, J.; Neyts, J.; Balzarini, J.; Canard, B.; Alvarez, K. *J. Med. Chem.* **2006**, *49*, 7799–7806.
6. Roux, L.; Priet, S.; Payrot, N.; Weck, C.; Fournier, M.; Zoulim, F.; Balzarini, J.; Canard, B.; Alvarez, K. *Eur. J. Med. Chem.* **2013**, *63*, 869–881.
7. For example: (a) Laxer, A.; Major, D.; Gottlieb, H.; Fischer, B. *J. Org. Chem.* **2001**, *66*, 5463–5481; (b) Jain, M.; Tsao, Y.-P.; Ho, N.-L.; Cheng, J.-W. *J. Org. Chem.* **2001**, *66*, 6472–6475; (c) Chiriac, M.; Axente, D.; Palibroda, N.; Craescu, C. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 377–385.
8. (a) Holy, A.; Masojidkova, M. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1196–1212; (b) Barral, K.; Priet, S.; Sire, J.; Neyts, J.; Balzarini, J.; Canard, B.; Alvarez, K. *J. Med. Chem.* **2006**, *49*, 7799; (c) Schultze, L. M.; Chapman, H. H.; Dubree, N. J. P.; Jones, R. J.; Kent, K. M.; Lee, T. T.; Louie, M. S.; Postich, M. J.; Prisbe, E. J.; Rohloff, J. C.; Yu, R. H. *Tetrahedron Lett.* **1853**, *1998*, 39; (d) Brown Ripin, D. H.; Teager, D. S.; Fortunak, J.; Mahaboob Basha, S.; Bivins, N.; Boddy, C. N.; Byrn, S.; Catlin, K. K.; Houghton, S. R.; Jagadeesh, S. T.; Kumar, K. A.; Melton, J.; Muneer, S.; Rao, L. N.; Rao, R. V.; Ray, P. C.; Reddy, N. G.; Reddy, R. M.; Shekar, K. C.; Silverton, T.; Smith, D. T.; Stringham, R. W.; Subbaraju, G. V.; Talley, F.; Williams, A. *Org. Process Res. Dev.* **2010**, *14*, 1194; (e) Roux, L.; Priet, S.; Payrot, N.; Weck, C.; Fournier, M.; Zoulim, F.; Balzarini, J.; Canard, B.; Alvarez, K. *Eur. J. Med. Chem.* **2013**, *63*, 869.
9. Czarnik, A. WO 2008/157657.
10. (a) Pollard, D.; Truppo, M.; Pollard, J.; Chen, C-y.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554–559; (b) Kosjek, B.; Tellers, D. M.; Biba, M.; Farr, R.; Moore, J. C. *Tetrahedron: Asymmetry* **2006**, *17*, 2798–2803.
11. For example (a) Edegger, K.; Gruber, C.; Poessl, T.; Wallner, S.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402–2404; (b) Kanamarlapudi, R.; Weissman, S.; Eisenruich, E.; Liu, X. WO 2012/031073.
12. A highly efficient Ru catalyzed asymmetric reduction of ketone **5** was recently published: Zhang, Q.; Ma, B.-W.; Wang, Q.-Q.; Wang, X.-X.; Hu, X.; Xie, M.-S.; Qu, G.-R.; Guo, H.-M. *Org. Lett.* **2014**, *16*, 2014–2017.
13. CN 101230065.
14. Enzymes were obtained from Codexis (Redwood City, CA, U.S.A.).
15. Stokvis, E.; Rosing, H.; Beijnen, J. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 401–407.
16. Isotope distribution was calculated based on mass spectrometry data using IsoPat^2. Excel-Worksheet for deconvolution of MS-patterns (D,^{17}O,^{13}C,^{15}N) © Christian C. Gruber, Wolfgang Kroutil 2006.; Changes and additions to the original and excellent IsoPat^2 have been made by W J S Lockley (Mod21) to facilitate its use by isotopic chemists. For details of the original spreadsheet see: Gruber, C.; Oberdorfer, G.; Voss, C.; Kremsner, J.; Kappe, C.; Kroutil, W. *J. Org. Chem.* **2007**, *72*, 5778–5783. The original IsoPat^2 Excel spreadsheet can be downloaded from ftp://biocatalysis.uni-graz/pub/IsoPat2/..
17. [2-^{3}H]-^{i}PrOH was prepared for us by Moravek Biochemicals (577 Mercury Lane, Brea, California 92821 U.S.A.) following the method described by: Agrawal, N.; Kohen, A. *Anal. Biochem.* **2003**, *322*, 179–184.
18. [1-^{3}H]-d-glucose was purchased from Moravek Biochemicals. It can also be prepared the procedure described in: Evans, E.; Sheppard, H.; Turner, J.; Warrell, D. *J. Labelled Compd. Radiopharm.* **1974**, *10*, 569–587.