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Convergent syntheses of carbon-13 labeled midazolam and 1′-hydroxymidazolam☆

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Abstract—For the purpose of drug interaction studies, the stable-isotope labeled [$^{13}C_3$]midazolam and its metabolite, 1′-hydroxy-[$^{13}C_3$]midazolam were synthesized in four and five steps in overall yields of 25.5% and 14.2%, from 7-chloro-5-(2-fluorophenyl)-2-(*N*-nitrosomethylamino)-3*H*-1,4-benzodiazepine, respectively, by a convergent synthesis, in which a key imidazoline ring formation was achieved by the facile reaction of [^{13}C]2-aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1*H*-1,4-benzodiazepine with varying ethyl imidate hydrochlorides. The scrambling of C-3 and C-4 labeling in intermediate diamine, and consequently in the final products, as well as the formation of a $\Delta^{4,5}$ isomer of 1′-hydroxy-[$^{13}C_3$]midazolam was observed and explained.

1. Introduction

Many important drugs are subject to oxidation by cytochrome P450. Midazolam, being a substrate and also the inducer of cytochrome P450, is often used for drug interaction study to see how the oxidation of a particular drug may be influenced by the presence of other drugs, which are also subject to oxidation, by the enzyme.[2] Ordinarily the drug interaction study by IV route and by oral route needs to be studied separately. By using labeled midazolam and 1-hydroxymidazolam, the method can be modified so that the effects by the two routes of administration can be determined simultaneously.

Several syntheses of unlabeled midazolam have been reported in literature.[3] Considering the availability and cost of labeled reagents, we aimed to put the label into the molecule as late in the synthetic scheme as possible and to use the least amount of labeled reagents possible. Conceivably, stable-isotope labeled midazolam **5** may be synthesized by an adaptation of Walser's procedure[3a] (Scheme 1), involving the sequential condensation of a 2-*N*-nitrosomethylamino benzodiazepine **1** with nitromethane, catalytic reduction of the resulting unsaturated-nitro compound **2**, cyclization of diamine **3** with triethyl orthoacetate, and oxidation of imidazoline **4** with MnO_2. Such small molecules as nitromethane and ethyl orthoacetate involved in the synthesis could be the source of stable-isotope labeled reagents. However, the synthesis of unlabeled 1′-hydroxymidazolam **8**, reported by the same authors, was achieved by a highly laborious linear synthesis that involved the *N*-oxide formation of midazolam, Polonovsky rearrangement of the 2-*N*-oxide or di-*N*-oxide **6** to 1-acetoxymidazolam or its 5-*N*-oxide analog **7**, and removal of oxygen from any 5-*N*-oxide, followed by saponification of the acetoxy group (Scheme 1). The overall synthesis from diamine **3** thus required six steps with a calculated yield of 1.8%.

We now wish to report the first isotope-synthesis, which is convergent and allows the preparation of both carbon-13 labeled midazolam **13a** and 1′-hydroxymidazolam **14** from the common labeled intermediate diamine **10** (Scheme 2).

2. Synthesis of the common labeled intermediate diamine 10

2-*N*-Nitrosomethylamino benzodiazepine **1** was prepared (Scheme 2) according to a published procedure.[3] Compound **1** was treated with nitro[^{13}C]-methane to

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Keywords: Drug interaction; Isotope labeling; Midazolam; 1′-Hydroxymidazolam; Imidazoline ring formation; Imidate hydrochloride.

☆ See Ref. 1.

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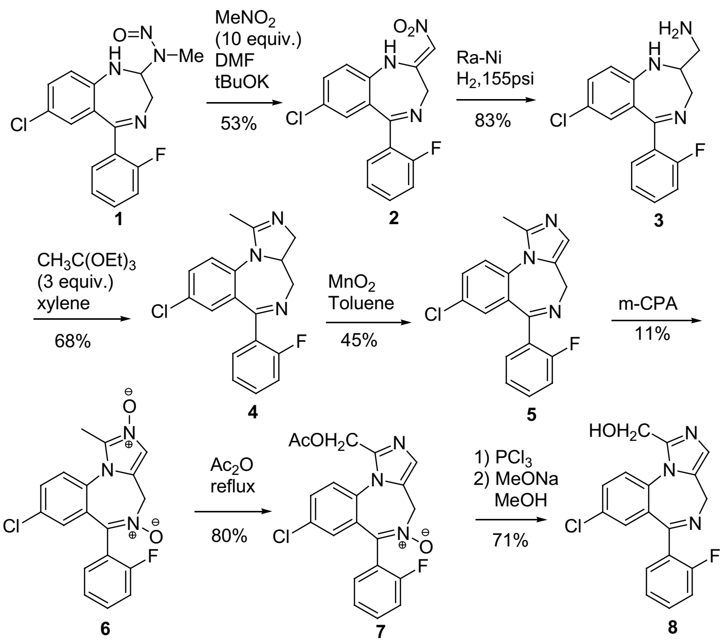
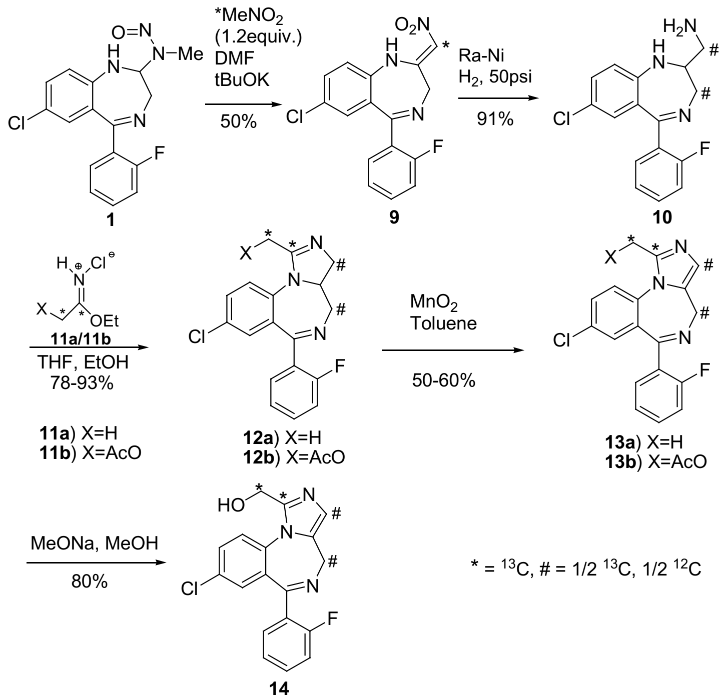


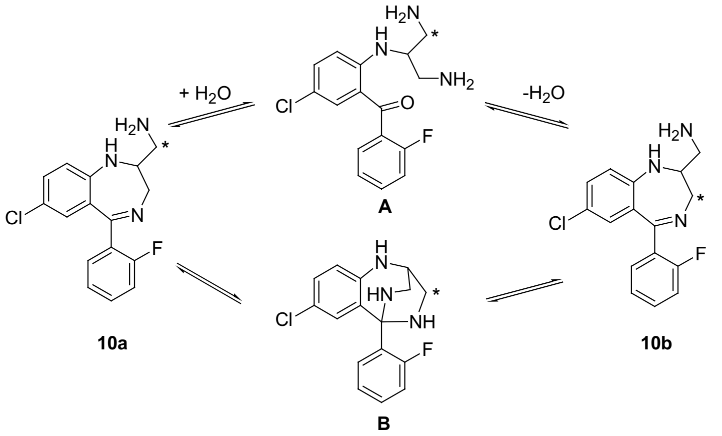
Scheme 1. Original synthesis of midazolam and 1′-hydroxy-midazolam.



Scheme 2. Convergent synthesis of [$^{13}C_3$]midazolam and 1′-hydroxy[$^{13}C_3$]midazolam.

give the nitro derivative **9**, which was then hydrogenated in MeOH–THF in the presence of Raney nickel to provide the crucial common labeled intermediate **10**.

In the literature procedure for the synthesis of unlabeled diamine **3**, a large amount of nitromethane (10 equiv) was used for the reaction. However, by increasing the concentration of reactants in the reaction mixture, we were able to decrease the molar ratio of the labeled nitromethane from 10-fold to 1.25-fold without largely affecting the yield. In the synthesis of **10** through hydrogenation of **9** we initially obtained highly inconsistent results, often encountering poor yields with large number of impurities if the progress of the reduction reaction went slowly. The data showed that achievement of fast initial reduction was necessary to avoid the impurities found in slow reactions. We reasoned that fast initial reaction should be favored by the use of active catalyst, large catalyst/substrate ratio. Indeed, using a high catalyst/substrate ratio (4/1) and fresh catalyst, we were able



Scheme 3. Possible mechanisms for the scrambling of C-3 and C-4 labeling.

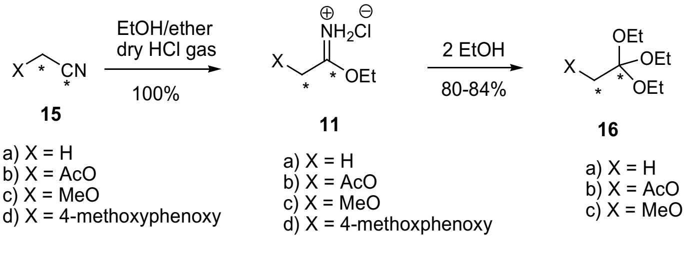
to achieve high yield, fast reaction rate, and pure product. We also found that during hydrogenation, a scrambling of the ^{13}C label occurred. Two possible mechanisms for the scrambling are shown in Scheme 3: one involving ring-opening by water (from Raney nickel), the other resulting from direct nucleophilic attack by the primary amine on the imino carbon in the diazepine ring. Partial de-fluorination (5%) also occurred during the hydrogenation as a side reaction.

3. Imidazoline ring formation

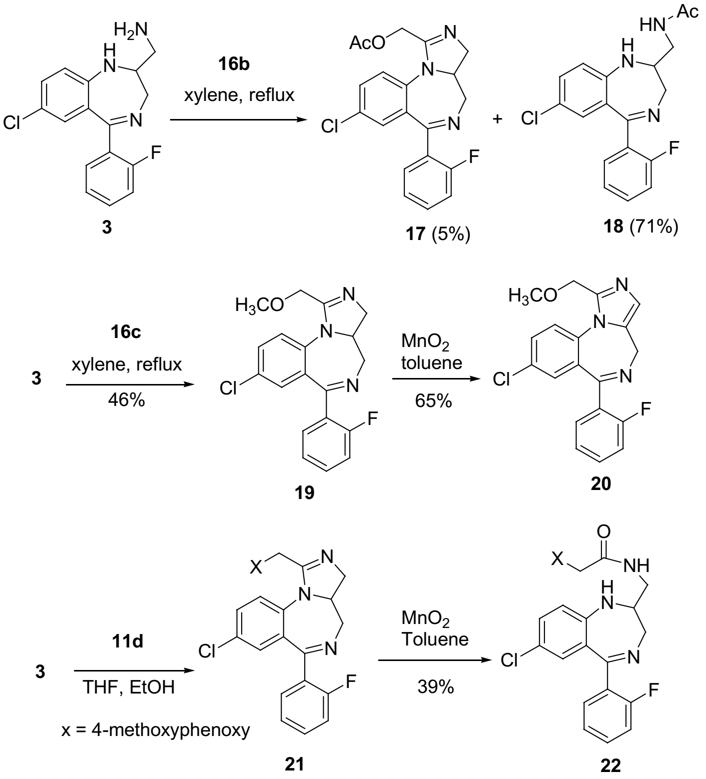
The imidazoline ring formation was initially carried out by the condensation of diamine 3 and triethyl orthoacetate (Scheme 1). The yields were not as good as Walser reported,[3a] and were further decreased when the amount of orthoacetate was reduced from 3 to 2 equiv. We thought that the treatment of diamine **3** with triethyl 2-acetoxyorthoacetate **16b** might produce a precursor **17** of 1′-hydroxymidazolam **8**. However, the condensation provided undesired **18** (71%) rather than the desired compound **17** (Scheme 5), apparently due to the higher reactivity of the acetoxy group in the reagent **16b** (Scheme 4). By changing acetoxy group to methoxy group, we were able to prepare the desired cyclic compound **19**, but could not remove the methyl group without decomposition. An attempt to convert ethyl 2-(*p*-methoxyphenoxy)acetamidate hydrochloride **11d** to the corresponding orthoacetate was unsuccessful (Scheme 4).

We reasoned, however, that the protonated imino group in an iminoester hydrochloride (such as **11a** and **11b**),[4,5] should be much more reactive (than the corresponding orthoesters **16a** and **16b**) to the nucleophilic diamine function in **10**. An initial reaction of the primary amine in **10** would lead to an intermediate **23a** and **23b** still containing a protonated imino group, in a favorable position to react with the γ-aromatic amino group to form an imidazoline ring by eliminating NH_4Cl. Furthermore, the availability of varying substituted iminoester hydrochloride would provide a facile convergent synthesis to various midazolam derivatives. Thus, when ethyl 2-(*p*-methoxyphenoxy)acetamidate hydrochloride **11d** was reacted with diamine **3** (Scheme 5), the desired cyclic compound **21** was produced in 37% yield at rt instead of 150 °C. However, compound **21** was not stable and the ring-opened compound **22** was isolated in the next oxidation reaction. Nevertheless, the results confirmed our reasoning that the reagent, imidate hydrochloride **11**, is much more reactive than triethyl orthoacetate, and prompted us to apply various imidate hydrochlorides **11a** and **11b** to form the imidazoline ring system. The results are partially shown in Table 1. It was found that the treatment of diamine **10** with ethyl imidate hydrochloride **11a** in ethanol/THF at a 1:1.5 molar ratio at 0 °C provided the product **12a** in 93.5% yield.[6] Also similar reaction of diamine with acetoxy imidate hydrochloride **11b** in ethanol/THF gave **12b** in 78% yield.

Because of the significantly higher reactivity of the imidate hydrochlorides **11a/11b** as compared with the

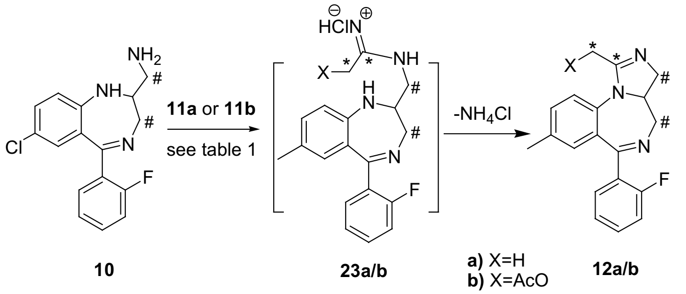


Scheme 4. Preparation of the labeled acetimidate **11a**–**d** and orthoacetate **16a**–**c**.



Scheme 5. Attempted new approaches to 1′-hydroxymidazolam.

Table 1. A novel approach to labeled midazolam and 1′-hydroxymidazolam

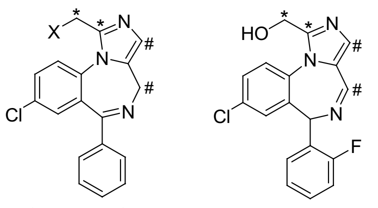


Entry	**10**/**11** (mol)	Conditions	Yields (%)
1	**10**/**11a** = 1/2	MeOH, 0 °C–rt, 10 h	37
2	**10**/**11a** = 1/2	MeOH, THF, 0 °C–rt, 10 h	71
3	**10**/**11a** = 1/1	MeOH, THF, 0 °C–rt, 10 h	32
4	**10**/**11a** = 1/1	EtOH, THF, 0 °C–rt, 10 h	41
5	**10/11a = 1/1.5**	**EtOH, THF, 0 °C–rt, 3 h**	**93.5**
6	**10**/**11b** = 1/1.5	EtOH, THF, 0 °C–rt, 3 h	35
7	**10**/**11b** = 1/1.5	EtOH, THF, 0–10 °C, 4 h	40
8	**10/11b = 1/1.5**	**EtOH, THF, −78–5 °C, 2 h**	**78**

corresponding orthoacetates, and the fact that orthoacetates are generally prepared from the imidates as precursors (Scheme 4), the modified procedure was much more convenient and led to significantly higher yields, especially in the synthesis of 1′-hydroxymidazolam. Thus the latter was prepared in three steps from diamine **9** in 28.4% overall yield, while the reported synthesis via the modification of midazolam required six steps with an overall yield of 1.8% from diamine **9**.

4. Oxidation of midazoline with MnO_2[7]

The conversion of imidazoline to imidazole was studied using several kinds of reagents, such as Pd/C, $BaMnO_4$, DDQ, and MnO_2. Our study showed that only the reaction with MnO_2 produced the desired product. It was found that the source of MnO_2 dramatically affected the yields and material recovery rate. The reaction with the reagent from Aldrich without any treatment gave



24a) X=H, **24b**) X=OH

25

Figure 1. Side products.

poor recovery rate (38% by weight); however, the oxidation with the new reagent prepared by boiling Aldrich's MnO$_2$ in toluene for 5 h afforded high recovery rate even with a high loading of the new MnO$_2$. The results probably indicate that the MnO$_2$ from Aldrich absorbed the product irreversibly, while the new reagent was saturated with toluene, and did not absorb the product. Using the new reagent we were able to convert an unstable compound **12b** to the desired compound **13b** in 50% yield (Scheme 2). In the oxidation of acetoxy derivative **12b**, it was especially essential to use manganese dioxide that had been pretreated with toluene at 130 °C and prepared in neutral and not basic conditions.

5. Side products formation

The defluorinated contaminant (2–5%) in diamine **10**, which was produced during reduction over Raney Ni, was carried to the final products **24a** and **24b** (Fig. 1). Suppression of this side reaction would eliminate subsequent purification problems.

The preparation of **14** was accompanied by the formation of a very insoluble side product **25** (5%). It might have formed by isomerization initiated by methoxide abstraction of the proton at C4.

In summary, stable-isotope labeled midazolam (99.56% purity) and 1′-hydroxymidazolam (98.3% purity) were synthesized using a novel efficient imidate approach. It was found that acetimidate is a fairly stable and more efficient labeling reagent than orthoacetate. Our study also showed that the pretreatment of commercially available MnO$_2$ was necessary for high recovering rate and yields. An interesting scrambling of label position was observed and rationalized. Several by-products were isolated and identified by means of spectroscopy and X-ray crystal structure. These modified syntheses provided much higher yields than those from the literatures.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.01.128.

References and notes

1. Zhang, Y. et al. 15th Annual Central US International Isotope Society Chapter Meeting, Mason, OH, 2001.
2. Gorski, J. C.; Jones, D. R.; Haehner-Daniels, B. D.; Hamman, M. A.; O'Mara, E. M., Jr.; Hall, S. D. *Clin. Pharmacol. Ther.* **1998**, *64*(2), 133–135.
3. (a) Walser, A. et al. *J. Org. Chem.* **1978**, *43*(5), 936; (b) Fryer, R. I. et al. *J. Heterocycl. Chem.* **1991**, *28*(7), 1661; (c) Fryer, R. I. et al. *J. Org. Chem.* **1978**, *43*(23), 1661; (d) Fryer, R. I. et al. *J Org. Chem.* **1978**, *43*(23), 4480; (e) Field, G. F. et al. U.S. Patent 4,194,049, 1980; (f) Khann, J. M. et al. European Patent 0835874, 1998; (g) Huber, J. E. U.S. Patent 5,831,089, 1998; (h) Bender, K. et al. European Patent 768310, 1997; (i) Kowalczyk, E. et al. Pol Patent 162851, 1994; (j) Walser, A. U.S. Patent 4,226,771, 1980.
4. Miyano, M.; Smith, J. N. *J. Heterocycl. Chem.* **1982**, *19*, 659.
5. Reagent **11a** is stable for several months when stored at <0 °C. Reagent **11b** was similarly prepared from 2-acetoxy-[$^{13}C_2$]acetonitrile, which was obtained by treatment of bromo[$^{13}C_2$]acetonitrile with potassium acetate. Reagent **11b** was unstable at room temperature and completely decomposed after about 6 months. Thus it is best to use a freshly prepared reagent or one kept at <0 °C and used within 2 weeks.
6. Typical procedures for imidazoline ring formation: To a solution of the crude compound **10** (12.5 g, 41 mmol) in EtOH (100 mL) and THF (60 mL) was added compound **11a** (13.0 g) in three portions at −10 °C. The resulting mixture was stirred at 0 °C for 1 h, and warmed up slowly to rt during 2 h period. Then the solvent was evaporated under vacuum at 25 °C. To this residue was added 300 mL of CH_2Cl_2 and $NaHCO_3$ saturated solution. The aqueous layer (pH about 7) was extracted with CH_2Cl_2 three times (100 mL × 3). The combined organic layers were washed with brine (50 mL × 3), and then dried over Na_2SO_4. The solvent was evaporated to give the crude product **12a**, which was dried under vacuum and used for next reaction. Mp: 143–145 °C [lit.[3a] 142–145 °C (unlabeled)]. ^1H NMR ($CDCl_3$) δ 7.6–6.9 (m, 7H), 4.6 (m, 1H), 4.1–3.2 (m, 4H), 1.3 (dd, 6.81, 28.7 Hz, 3H).
7. To a suspension of the crude compound **12a** (ca. 41 mmol) in dry toluene (180 mL) was added MnO$_2$ (80.0 g, Aldrich MnO$_2$ was heated in toluene for 5 h, dried under vacuum for 24 h at 60 °C). The mixture was stirred at 110–115 °C for 1.5 h. The solid was filtered off, washed with toluene and CH_2Cl_2. The solvent was evaporated under reduced pressure to give the crude product **13a** (12.4 g), which was applied to flash chromatography (Biotage, 250 mL of CH_2Cl_2/MeOH/Et_3N = 500/10/1 treated the column first, then eluted with 2% MeOH/CH_2Cl_2). The product was further recrystalized from CH_2Cl_2/ether/hexane = 1/2/4. Total weight: 8.0 g (60%). Mp: 159–161 °C. [lit.[3a] 158–160 °C (unlabeled)]. ^1H NMR ($CDCl_3$) δ 7.61 (dt, 1.7, 7.5 Hz, 1H), 7.54 (dd, 2.4, 8.5 Hz, 1H), 7.43 (m, 1H), 7.37 (d, 8.7Hz, 1H), 7.24–7.20 (m, 1H), 7.01 (m, 1H), 6.92 (d, 10.7 Hz, 1H), 6.93 (dd, 190.2, 10.7 Hz, 1H), 4.93 (ddd, 88.2, 76.2, 14.1 Hz, 1H), 4.02 (ddd, 88.1, 77.8, 13.5 Hz, 1H), 2.54 (dd, 129.2, 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 15.0 (d, $^{13}CH_3$), 46.3 ($^{13}CH_2N$=), 124.2 (d, ^{13}CH=N); 144.3 (d, N^{13}CH=N); ^{19}F NMR −112.58; MS 329 [(M+3)+H]$^+$.