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Site selective syntheses of [^{3}H]omeprazole using hydrogen isotope exchange chemistry

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Omeprazole (Prilosec®) is a selective and irreversible proton pump inhibitor used to treat various medical conditions related to the production of excess stomach acids. It functions by suppressing secretion of those acids. Radiolabeled compounds are commonly employed in the drug discovery and development process to support efforts including library screening, target identification, receptor binding, assay development and validation and safety assessment. Herein, we describe synthetic approaches to the controlled and selective labeling of omeprazole with tritium via hydrogen isotope exchange chemistry. The chemistry may also be used to prepare tritium labeled esomeprazole (Nexium®), the active pure (S)-enantiomer of omeprazole.

Keywords: omeprazole; esomeprazole; Prilosec; Nexium; tritium; hydrogen isotope exchange

Introduction

Omeprazole (Prilosec®, **1**, Figure 1) is a member of a class of drugs known as proton pump inhibitors that function by decreasing the production of stomach acids via a selective and irreversible inhibition of the H^+/K^+ ATPase system found at the secretory surface of gastric parietal cells.[1] This inhibition allows for the treatment of a number of conditions including gastroesophageal reflux disease, peptic ulcers and erosive esophagitis. Omeprazole is a racemic mixture of (S)- and (R)-sulfoxides with the (S)-enantiomer being the active form. A chiral shift occurs *in vivo* which converts the inactive (R)-enantiomer to the active (S)-enantiomer, thus, potentially doubling the concentration of the active form. This transformation is effected by the CYP 2C19 isozyme of the cytochrome P450 enzyme. That enzyme is not found equally in human populations[2] and those who do not metabolize the drug effectively are called 'poor metabolizers'. Their distribution is: Caucasians 10%–Asian 20%–South Pacific Islanders 70%. This discovery led to the development and marketing of esomeprazole (Nexium®, **2**), the pure (S)-enantiomer.

The use of radiolabeled compounds is commonplace within and essential to drug discovery and development.[3] One of the many uses of radiotracers is in the development of assays for safety assessment and de-risking of potential clinical candidates. That type of development requires compounds that are deemed safe, such as omeprazole (**1**), to be used as controls. Because of the mechanism of action and metabolism of omeprazole,[4] a request was made to prepare two distinct radiotracers (**3** and **4**, Scheme 1) with a label on either half of the molecule (pyridyl and benzimidazole rings, respectively). For the assay being developed, either a ^{14}C or ^{3}H label was deemed acceptable as high specific activity was not required. A review of the literature showed that [^{14}C]omeprazole had been previously prepared (along with ^{35}S and various stable isotope variants) with labeled atoms on either side of the molecule.[5] However, each of those approaches would require significant costs in terms of materials (as well as lead time to acquire) and manpower. There are also reports on the use of [^{3}H]omeprazole in the literature,[6] but they are limited to the [^{3}H]pyridyl ring analog (**3**) and no conditions were reported for its preparation. Fortunately, the synthesis of unlabeled omeprazole is well-explored, and its primary synthetic building blocks (**5** and **6**, Scheme 1), as well as most other intermediates, are readily available. Those fragments, or appropriate precursors, appeared excellent candidates for a variety of labeling strategies. Thus, we felt we would be able to quickly assess the potential for success of each of these strategies and define the desired chemistry.

Classically, there are a number of methodologies that may be applied to labeling a molecule with tritium.[7] The structure in question will often determine which are most appropriate and/or likely to succeed. Typically, as is the case for omeprazole, the simplest and fastest of these approaches would appear to be either direct incorporation of tritium into the API (e.g. **1** or **2**) via a hydrogen isotope exchange (HIE) reaction[8] or reduction of a suitable halogenated precursor. This halogenated precursor is often prepared from the API itself, although, it must be noted that using a halogenated compound does not always result in site specific incorporation as capricious, non-specific labeling may occur. In the event those attempts fail, a *de novo* synthesis of the molecule is required. Omeprazole can be quickly prepared via alkylation of 2-thiobenzimidazole **5** with chloromethylpydine **6**. A simple oxidation completes the synthesis. While both fragments **5** and **6** are commercially available, a labeled version of each would need to be prepared to couple with the other to complete the synthesis.

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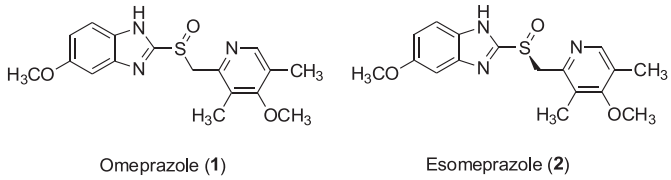
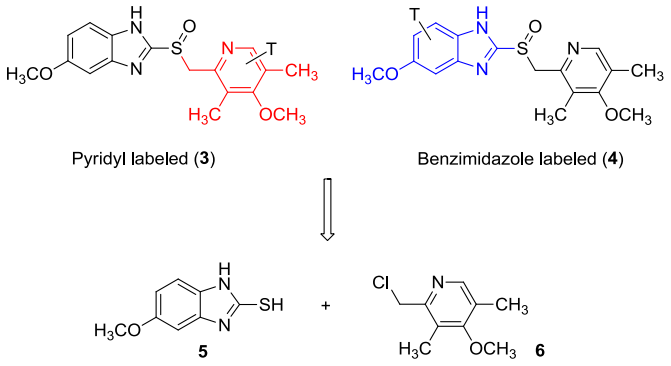


Figure 1. Labeling targets.

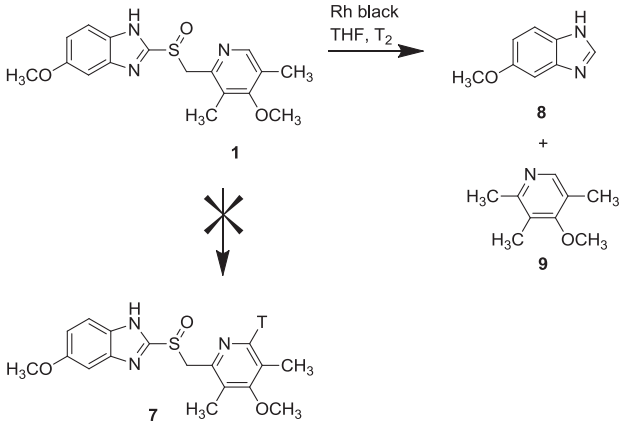


Scheme 1. Retrosynthetic approach to labeling omeprazole.

Results and discussion

[^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)sulfinyl)-1H-benzo[d]imidazole 7

We began our efforts by first attempting to prepare omeprazole labeled with tritium on the pyridine ring (e.g. **3**). It is well-known that tritium can be incorporated at the 2- or 4-positions of pyridine rings by use of rhodium black and tritium gas at sub-atmospheric pressures.[7] Unfortunately, the hydrogen isotope exchange reaction with rhodium black on omeprazole, even under varying pressures (100–350 Torr) of T_2, only resulted in the consumption of starting material and production of fragments **8** and **9** (Scheme 2), as shown by LCMS, consistent with desulfurization. This result indicated that there would be a low probability of success in using a halogenated API to access tritiated material as the conditions (Pd or Pt, T_2) necessary to effect reduction would likely result in a similar fragmentation.



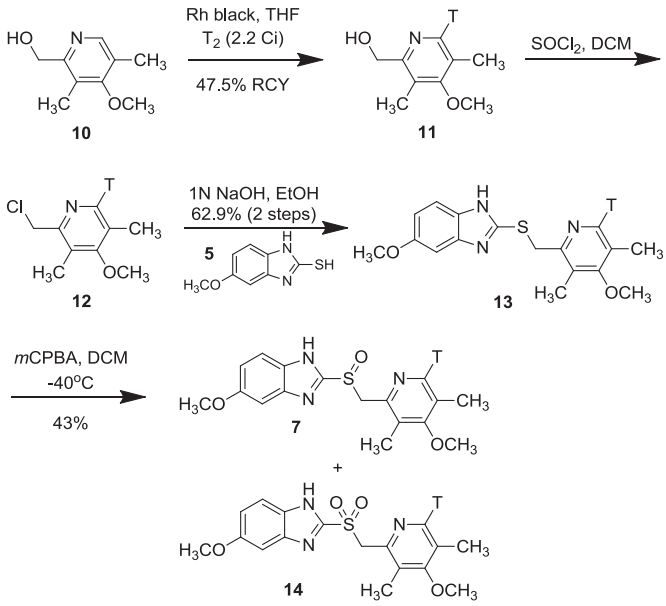
Scheme 2. Attempted direct labeling of API.

As a result, it was necessary to begin the synthesis with a simpler, non-sulfur containing, substrate. One of the published syntheses of omeprazole employed readily available pyridinemethanol **10** whose conversion to omeprazole has been demonstrated.[9] This material was readily labeled using tritium gas (~2.2 Ci) and rhodium black to produce [^{3}H]pyridinemethanol **11**, without purification, in large quantity (~1 Ci, 47.5% radiochemical yield), high purity (95% radiochemical purity) and excellent SA (10 Ci/mmol). This provided sufficient material to explore and complete the synthesis. Following along with the published synthetic route, alcohol **11** was converted to chloride **12** in quantitative yield by treatment with thionyl chloride (Scheme 3). This material could be used directly, without purification, in the subsequent alkylation of thiol **5** to give sulfide **13** in good overall yield and purity (62.9% yield, 99% radiochemical purity). Purification of sulfide **13** by semi-preparative HPLC was required in order for the subsequent reaction to proceed. Completion of the synthesis was effected by oxidation of sulfide **13** with *m*CPBA to give a 2:1 mixture of [^{3}H]omeprazole (**7**) and [^{3}H]sulfone **14**. Examination of the literature showed that over-oxidation is a known problem.[10] As the impurity was easily separable from the desired product, and we were able to produce sufficient quantities (~200 mCi), no additional efforts were made to decrease the amount of sulfone **14** formed.

Tritium NMR (Figure 2) analysis confirmed that labeling of compound **7** was exclusive to the C6-postion of pyridine ring. This, therefore, represents an effective route for the production of pyridyl labeled [^{3}H]omeprazole **(7)**. Additionally, we were able to isolate esomeprazole (Nexium®) using chiral HPLC to separate the active (S)-enantiomer from the racemic mixture using conditions reported in the literature for unlabeled material.[11]

[^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)sulfinyl)-1H-benzo[d]imidazole 29

As with the pyridine labeled tracer, we desired to prepare a benzimidazole labeled [^{3}H] omeprazole in the most expedient



Scheme 3. Preparation of omeprazole labeled with tritium on pyridine ring.

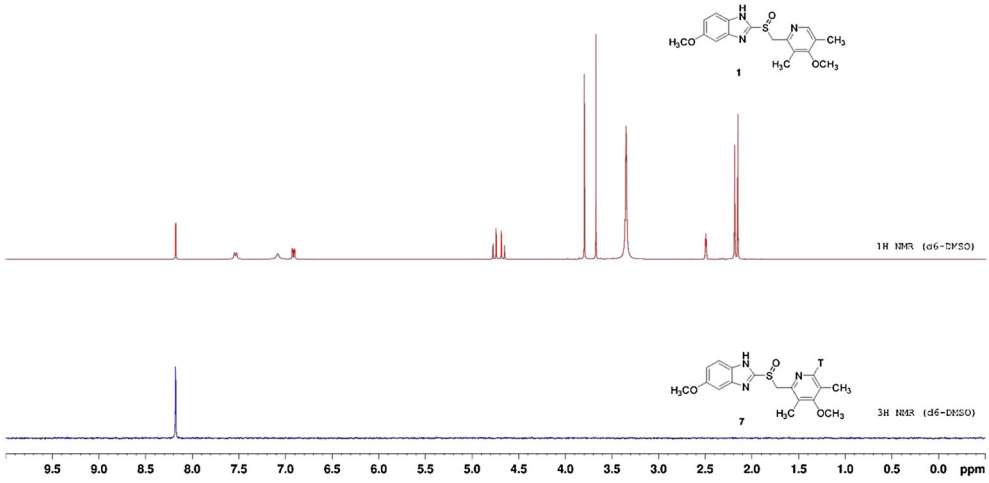
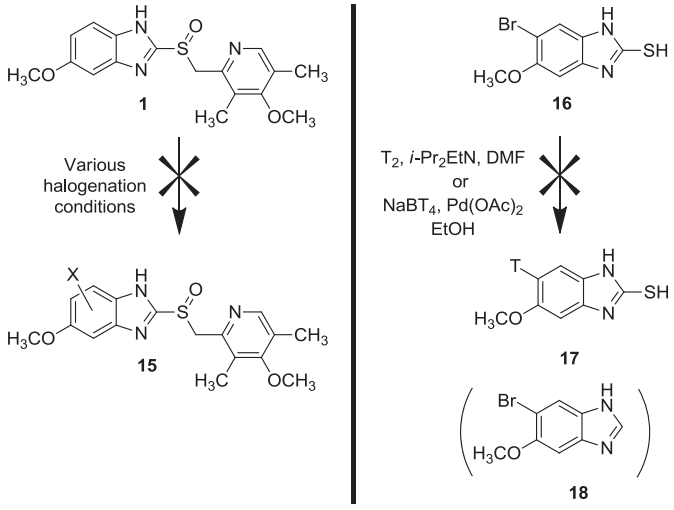
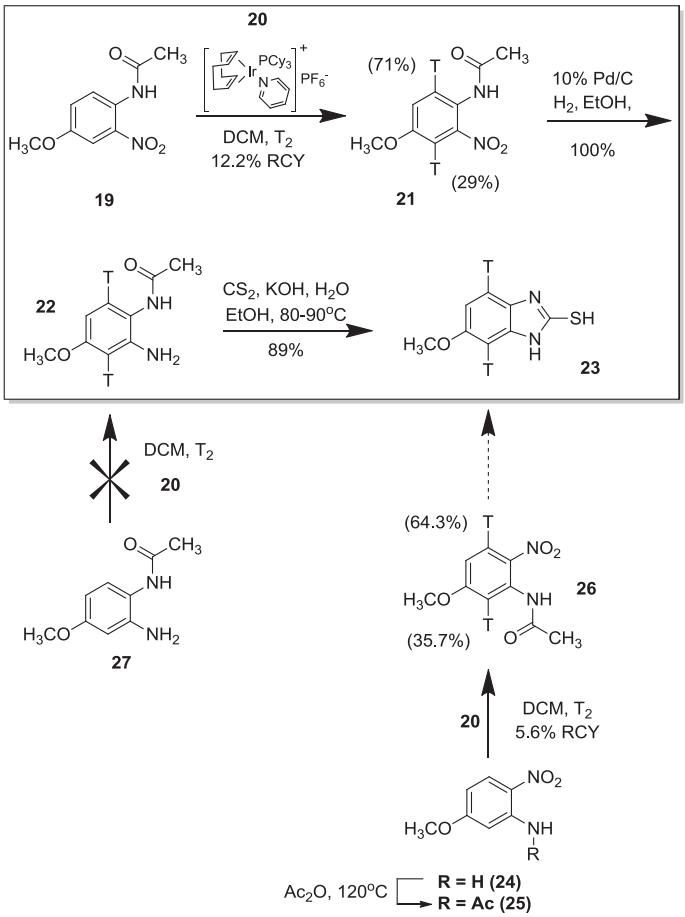


Figure 2. Tritium NMR of omeprazole labeled on the pyridine ring.



Scheme 4. Attempted halogenation strategies to label benzimidazole ring with tritium.



Scheme 5. Preparation of tritium labeled benzimidazole fragment.

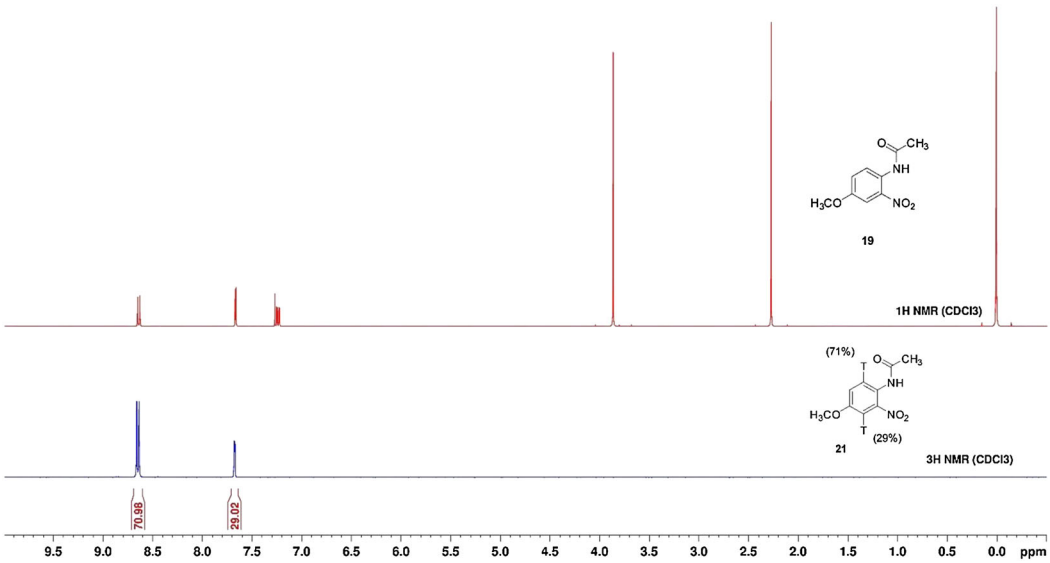


Figure 3. NMR of labeled and unlabeled nitro compounds 19 and 21.

manner possible. As previously discussed, a strategy of halogenation/tritio-dehalogenation is often chosen as a means of accessing tritium labeled material. It was hoped that the methoxybenzimidazole ring would be sufficiently electron rich to allow for direct halogenation of the API. Regioselectivity in the halogenation was not a concern as any mixture of halogen products could, theoretically, be converted to [^{3}H]omeprazole. Unfortunately, all attempts to produce halogenated omeprazole **15** (see Scheme 4) failed utilizing a variety of electrophilic halogenating agents (NIS, NBS, bromine, iodine, Iodo-Gen, etc.).

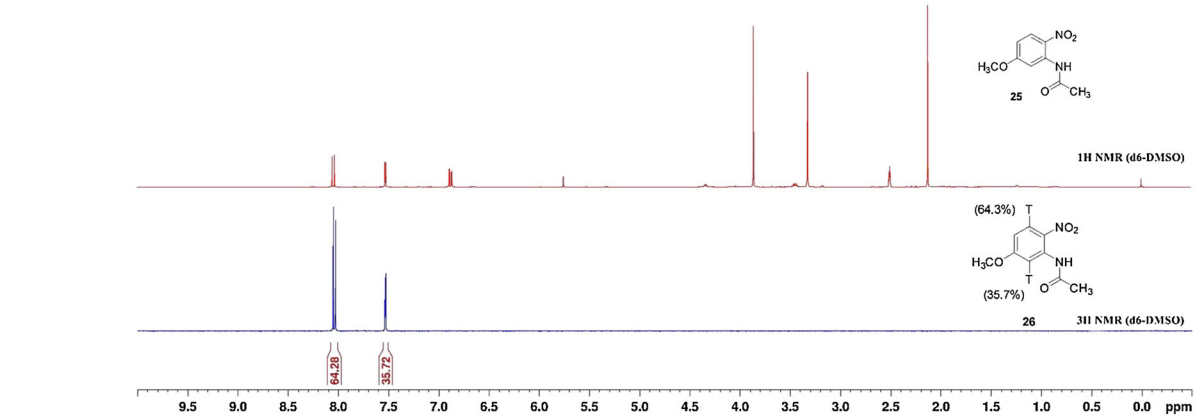
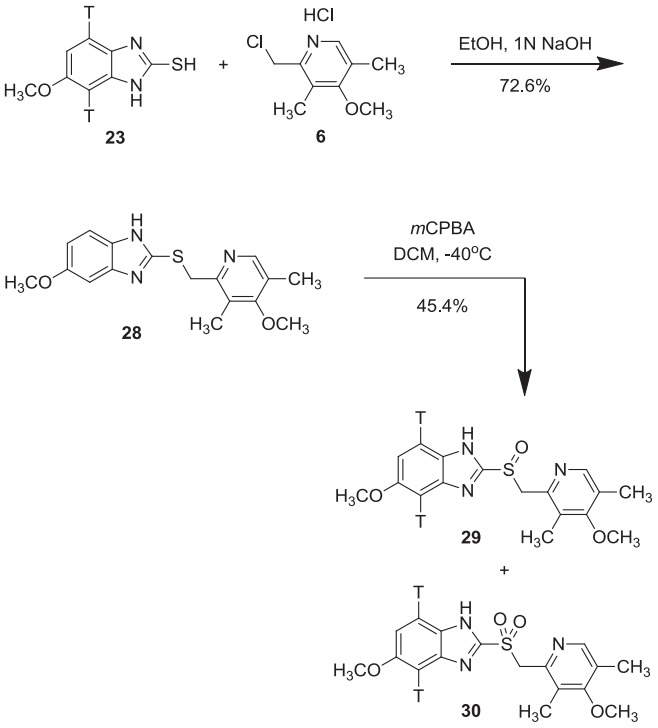


Figure 4. NMR of labeled and unlabeled nitro compounds 25 and 26.



Scheme 6. End-game for preparation benzimidazole labeled [^{3}H]omeprazole.

Figure 5. Tritiation manifold set-up used in labeling experiments.

We either observed no reaction or, using more forcing conditions, the API was consumed in a non-productive manner.

One potential solution for this problem was to prepare a halogenated fragment of the molecule that could itself be tritiated or possibly converted to a halogenated omeprazole as a precursor for tritiation. The latter strategy seemed less likely to succeed because of the results from the attempted **HIE** reaction on omeprazole with rhodium black. The brominated thiol **16**[12] (Scheme 4) was subjected to various tritio-dehalogenation conditions including sodium borotritide/palladium acetate.[13] No incorporation of tritium (or debromination) occurred to give the desired labeled compound (**17**); only the product resulting from desulfurization (**18**) was observed. These results collectively highlighted the need for an alternative approach to the requisite tritiated thiobenzimidazole.

A review of methods for the preparation of unlabeled thiobenzimidazole **5** indicated that methoxyacetanilide **19** might be a suitable starting material for labeling with tritium.[14] Being both electron rich and containing a suitable coordinating/directing group (acetanilide), it appeared to be potentially suitable for an HIE reaction using Crabtree's catalyst[15] (**20**). This catalyst is well known to effect hydrogen isotope exchange. When subjected to standard conditions for using Crabtree's catalyst, [^{3}H]acetanilide **21** (see Scheme 5 and Figure 3) was produced with an acceptable specific activity of ~5 Ci/mmol (12.2% radiochemical yield). Incorporation was observed not only ortho to the amide, as is normally the case, but ortho to the nitro group as well, accounting for 29% of total tritium incorporation. With tritiated nitroacetanilide **21** in hand, conversion to the requisite [^{3}H]thiobenzimidazole **23** was accomplished by hydrogenolytic reduction of the nitro group followed by condensation with carbon disulfide under basic conditions in excellent overall yield (89% over 2 steps) with no loss of specific activity. Interestingly, when an HIE reaction (Crabtree's catalyst) was attempted on unlabeled aminoacetanilide **27** to prepare the corresponding [^{3}H]aminoacetanilide **22** directly, the reaction resulted in no incorporation of tritium. That result was presumably because of non-productive binding of the catalyst. However, higher catalyst loading did not result in tritium incorporation.

An alternative approach to [^{3}H]benzimidazole **23** was explored that employed isomeric acetanilide **25**, prepared from nitroaniline **24**.[16] When subjected to the same conditions used in the preparation of **21**, acetanilide **25** produces material (**26**; see Figure 4) with a specific activity (3.75 Ci/mmol, 5.6% radiochemical yield) similar to that of the previous case (5 Ci/mmol). However, the distribution of tritium was very different with 64.3% of incorporated tritium located ortho to the nitro group. Higher incorporation for that position might simply be the result of increased accessibility with respect to the more hindered position ortho to the amide. Because use of this approach required an additional step, the specific activity was lower and there were no obvious benefits, it was decided to use the more readily available acetanilide **19** for the synthesis of the benzimidazole labeled omeprazole.

The synthesis was completed in a manner identical to that of the pyridine labeled tracer **7** via alkylation of pyridyl chloride **6** to give sulfide **28** (72.6% yield) followed by oxidation to produce desired [^{3}H]omeprazole **29** labeled on the benzimidazole ring along with sulfone byproduct **30** (**Scheme 6**). The reaction proceeded in reasonable yield (45.4%) and produced a large quantity (~100 mCi) of material with high radiochemical purity (99%).

Conclusion

In conclusion, we have demonstrated efficient and robust strategies for the synthesis of two selectively tritium labeled variants of omeprazole and esomeprazole. Both strategies effectively utilize hydrogen isotope exchange reactions to install tritium and take advantage of both substrate availability and well-demonstrated chemistry.

Experimental

General

All commercially available reagents and solvents were used as received without purification. Catalysts (Crabtree's, rhodium black and palladium on carbon) were purchased from Strem Chemicals (Newburyport, MA, USA). HPLC purifications were carried out using a Varian ProStar® HPLC System (Model 210 pumps and Model 330 PDA detector—now owned by Agilent, Santa Clara, CA, USA). Analytical analyses/method development were conducted using an Agilent 1200 HPLC with an attached Perkin Elmer (Waltham, MA, USA) Radiomatic 625TR flow scintillation analyzer. HPLC-MS analyses were performed on an Agilent 1100 HPLC-MSD instrument in API-ES positive ionization mode using an Ascentis® Express C18 column (2.7 μm, 4.6 × 100 mm, 5% to 95% ACN/2 mM aqueous ammonium formate buffer, 1 ml/min). HPLC columns were purchased from either Phenomenex (Torrance, CA, USA) or Sigma Aldrich (St. Louis, MO, USA). ^{1}H and ^{3}H NMR (400 MHz and 427 MHz, respectively) were obtained on a Bruker Ultrashield 400-MHz magnet with an AVIII console using TopSpin™ 3.0 and a TXO probe (^{3}H/^{13}C dually tuned on the inner coil) in $CDCl_3$ or DMSO-d_6. Radioactivity was counted on a calibrated PerkinElmer Tri-Carb® liquid scintillation counter. Specific activity was determined through MS analysis via comparison of isotopic abundance of labeled and unlabeled samples.[17] Tritium gas (carrier free) was purchased (in 50cc stainless steel cylinders with Swagelok® VCR fitting for connection to the tritiation manifold) from American Radiolabeled Chemicals (St. Louis, MO, USA). Sep-Pak® SPE cartridges were purchased from Waters (Milford, MA, USA) and conditioned prior to use by rinsing with absolute ethanol followed by an equal amount of water. All unlabeled products have previously been fully characterized elsewhere where noted in the preceding paragraphs.

Gaseous tritiations were performed using a TRI-SORBER Tritiation Manifold® (LabLogic, Brandon, FL USA) fitted with three activated uranium beds for recovering unused tritium (Figure 5). Tritium used in hydrogen isotope exchange reactions was recovered onto a separate bed (because of use of dichloromethane or tetrahydrofuran, which can poison the bed and result in dilution of the tritium with hydrogen from the substrates) than the one(s) used for hydrogenolysis (halogen reduction) or hydrogenation (olefin reduction) reactions. This ensures a stable bed loaded with carrier free tritium that may also be used to regenerate tritium for routine labeling experiments. The attached picture (Figure 5) shows the setup used for tritiations described in this paper. The manifold block (green-handled quarter turn diaphragm valves) on the right-side allows connection of the reaction bulb to either the TRI-SORBER (vacuum or uranium beds) or the U-tube containing tritium for filling the reaction. The block has a permanently attached absolute pressure transducer (MKS Instruments, Andover, MA, USA) for constant monitoring of reaction pressure. Reaction bulbs were attached to the manifold block by means of an ultra-torr fitting (Swagelok, Solon, OH, USA). Reaction bulbs used with the tritiation manifold were custom items purchased from Chemglass (Vineland, NJ, USA) and were of the following dimensions: 15.25-mm O.D. Bulb, 1/4″ O.D. × 58-mm length neck—bulb volume of ~1.1 ml and head space of 0.75 ml. The U-tube (~11-ml volume) connecting the tritium gas cylinder and the reaction block was used to fill the reaction with tritium gas. Upon installation of a new cylinder, an empty reaction tube is attached to the system, and both the reaction vessel and U-tube are evacuated via high-vacuum pump (open both diaphragm valves). Closing off the system to vacuum allows use of the pressure transducer to check the system for leaks. The U-tube is then closed off to the reaction block and opened to the tritium gas cylinder and then reclosed. The tritium gas in the U-tube is used to fill the reaction vessel. A reaction bulb with reactants is attached to the system, cooled in an ethanol/dry ice bath, evacuated using a high vacuum pump (close valve when evacuation is complete) and finally opened to the U-tube while still cooled. The tritium gas cylinder is never directly opened to a reaction. The valve to the U-tube is closed, and the reaction is stirred at room temperature for the required time. Residual tritium is adsorbed, after the reaction is complete, onto a uranium bed (with the reaction cooled in ethanol/dry ice) by opening the front diaphragm valve to the TRI-SORBER (bed and intervening system previously evacuated under high vacuum). The reaction is then warmed to ambient temperature, removed from the system and concentrated to remove residual volatile radioactive material.

Preparation of [^{3}H](4-methoxy-3,5-dimethylpyridin-2-yl)methanol (11)

To a borosilicate reaction bulb with a flea stir bar was added rhodium black (8.4 mg, 0.082 mmol) and a solution of (4-methoxy-3,5-dimethylpyridin-2-yl) methanol (**10**) (18 mg, 0.108 mmol) in 1.1-ml THF. The vessel was cooled in an ethanol/CO_2 bath, degassed under high vacuum and filled with T_2 gas (425 Torr, ~2.2 Ci). The suspension was stirred at ambient temperature for 18 h. The reaction was then cooled in an ethanol/CO_2 bath, and excess tritium was transferred onto one of the tritiation manifold's uranium beds. The reaction was warmed to ambient temperature, transferred with aid of ethanol from the reaction vessel, filtered through a Whatman® Autovial™ syringeless filter to remove catalyst, washed with ethanol (10 ml) and concentrated *in vacuo* on a rotary evaporator. Additional ethanol (10 ml) was added and the evaporation repeated two times to ensure removal of any volatile radioactive material. Analysis of the isolated product, [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d] imidazole (**11**) (~1.1 Ci; 47.5% radiochemical yield), by HPLC-MS, showed a specific activity of 10.0 Ci/mmol (59.6 mCi/mg) with analytical HPLC (Develosil RP Aqueous C30, 5u, 10 × 250 mm, 5 ml/min, 20% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t = 11.8 min) showing 100% chemical purity (by UV) with a radiochemical purity of 95%. The product can be stored, at −80°C, as a solution in ethanol (<20–25 mCi/ml) or used directly in the next step. Further purification by HPLC (using a

10 × 250 mm version of the column used for analysis) was avoided as poor recovery occurs and, also, the following reaction is not enhanced by the use of material with a higher radiochemical purity. The labeled material has the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection).

Preparation of [^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (13)

The [^{3}H]-(4-methoxy-3,5-dimethylpyridin-2-yl)methanol **(11)** (~1.1 Ci, 95% RCP; 0.1 mmol), prepared previously, was dissolved in 3.0 ml of dichloromethane and added to a 20-ml vial with stir bar. Excess thionyl chloride (0.15 ml; 2 mmol) was added, and the solution stirred at ambient temperature for 1 h. HPLC-MS showed complete conversion to [^{3}H]-2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine **(12)** (specific activity = 10 Ci/mmol). The mixture was concentrated *in vacuo*, then dissolved in ethanol (2.5 ml) and added to a 4-ml vial containing 5-methoxy-1H-benzo[d]imidazole-2-thiol (**5**) (23 mg, 0.12 mmol). A solution of 1 M sodium hydroxide (0.25 ml, 0.25 mmol) was added, and the reaction was stirred at ambient temperature for 2 h. The reaction was diluted with water (10 ml), loaded onto a 5-g C18 Sep-Pak™ SPE cartridge (conditioned with 20 ml of ethanol then 20 ml of water), rinsed with water (50 ml) and then ethanol (50 ml). The ethanol fraction was concentrated *in vacuo* and purified by semi-preparative reversed phase HPLC (Curosil PFP, 5 μm, 10 × 250 mm, 5 ml/min, 40% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t = 13.6 min) to give 680 mCi (67.5%) of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d] imidazole **(13)** (specific activity = 30.3 mCi/mg, radiochemical purity = 99%). The labeled material has the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection).

Preparation of [^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]imidazole (omeprazole, Prilosec®) (7)

To a solution of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)thio)-1H-benzo[d]imidazole **(13)** (450 mCi; 10 Ci/mmol; 14.3 mg, 0.043 mmol) in 5-ml DCM, at ~ −40°C to −45°C (acetonitrile/CO_2 bath), was added 3-chloroperoxybenzoic acid (9.6 mg, 0.043 mmol; 77% purity) was added as a solid in one portion. Stir at −40°C for 1 h then an additional equivalent of 3-chloroperoxybenzoic acid (9.6 mg, 0.043 mmol; 77% purity) was added and continued stirring for 1 h at −40°C. HPLC-MS showed a 2:1 mixture of desired sulfoxide and undesired sulfone from over-oxidation. The reaction was quenched by the addition of 1 ml of concentrated ammonium hydroxide, warmed to ambient temperature and then diluted with dichloromethane (20 ml) and saturated aqueous sodium bicarbonate (5 ml). The dichloromethane layer was separated and concentrated *in vacuo*. The crude material was purified by reversed phase HPLC (Develosil RP Aqueous C30, 5 μm, 10 × 250 mm, 30% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t = 14.6 min) to give (after solvent switch of product containing fractions to an ethanol solution using C18 Sep-Pak® SPE cartridges) 194 mCi (43%) of omeprazole **(7)** (specific activity = 10.0 Ci/mmol, 28.92 mCi/g; radiochemical purity = 99%.) The labeled material has the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection). Material is stored in absolute ethanol at a concentration of ~2 mCi/ml at −80°C. Note: the final product has a shelf-life of 3–6 months at −80°C in solution and is very sensitive to acid catalyzed degradation. The sulfide precursor may be a more viable candidate for longer term storage.

Preparation of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]imidazole (esomeprazole, Nexium®) (2)

[^{3}H]esomeprazole was isolated from [^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]imidazole (omeprazole) **(7)** (91.5 mCi) by use of semi-preparative normal phase Chiral HPLC (ChiralPak® AD-H, 5 μm, 10 × 250 mm, 3.5 ml/min, 60% ethanol/heptane, PDA detector, R_t on corresponding analytical column [0.7 ml/min, 5u, 4.6 × 250 mm] is 13.12 min for the desired (S)-isomer and 20.67 min for the (R)-isomer.) The identity and order of elution were taken from a published procedure[10]—modified by use of heptane instead of hexane. A solvent switch of the product containing fractions from the HPLC solution to 100% ethanol using either C18 or silica gel Sep-Pak® SPE cartridges afforded 30.5 mCi (33.3%/50%) of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)sulfinyl)-1H-benzo[d]imidazole (esomeprazole) **(2)** (specific activity = 10.0 Ci/mmol (28.98 mCi/mg; radiochemical purity > 99%)).

Preparation of [^{3}H]N-(4-methoxy-2-nitrophenyl)acetamide (21)

To a tritiation reaction bulb with flea stir bar was added Crabtree's catalyst (**20**) (7.2 mg, 0.009 mmol) and a solution of 4-methoxy-2-nitroacetanilide (**19**) (5.15 mg, 0.025 mmol) in ~ 1.1-ml dichloromethane. The vessel was cooled in an ethanol/CO_2 bath, degassed under high vacuum and filled with T_2 (260 Torr, ~1.36 Ci). The solution was stirred at ambient temperature for 18 h. The reaction was cooled in an ethanol/CO_2 bath, and excess tritium is transferred onto one of the tritiation manifold's uranium beds. The reaction was warmed to ambient temperature, transferred with the aid of dichloromethane from the reaction bulb and concentrated *in vacuo* on a rotary evaporator. Ethanol (10 ml) was added, and the evaporation repeated two times to ensure removal of volatile radioactive material. HPLC-MS analysis of the crude showed product with a specific activity of 5.57 Ci/mmol (26.5 mCi/mg). The reaction was repeated, under the same general conditions, with Crabtree's catalyst (7.2 mg, 0.009 mmol), a solution of 4-methoxy-2-nitroacetanilide (12 mg, 0.057 mmol) in ~ 1.1-ml dichloromethane and under an atmosphere of T_2 (263 Torr; ~1.38 Ci) for 18 h. After an identical workup of the reaction, HPLC-MS of the crude showed product with a specific activity of 5.07 Ci/mmol (24.11 mCi/mg). The material from both reactions was combined and purified by semi-preparative reversed phase HPLC (Luna C18(2), 5 μm, 10 × 250 mm, 5 ml/min, 25% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t on analytical column [5u, 4.6 × 250 mm, 1 ml/min] is 11.5 min using 30% acetonitrile/water + 20 mM NH_4OAc—sample dissolved in 1:1 acetonitrile/water + 20 mM NH_4OAc; 1.4 ml total; 200-μl injections) to give (after solvent switch of product containing fractions to an ethanol solution using C18 Sep-Pak® SPE cartridges) 333 mCi (12% radiochemical yield) of [^{3}H]-N-(4-methoxy-2-nitrophenyl)acetamide **(21)** (specific activity = 5.3 Ci/mmol, 25.2 mCi/mg). The labeled material had the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection).

Preparation of [^{3}H] N-(2-amino-4-methoxyphenyl)acetamide (22)

To a 25-ml one-neck flask with stir bar was added 10 wt % Pd/C (10 mg, 0.0094 mmol). The vessel was purged with nitrogen and then add a solution [^{3}H]-N-(4-methoxy-2-nitrophenyl)acetamide **(21)** (330 mCi, 13.22 mg, 0.07 mmol) in 5-ml ethanol. The flask was purged twice with hydrogen gas, via a balloon, and then stirred under a balloon of hydrogen gas for 2 h. HPLC-MS analysis showed complete conversion to product. The reaction was filtered through a Whatman® Autovial™ and washed with 1:1 tetrahydrofuran:ethanol (30 ml). The filtrate was concentrated *in vacuo* to give [^{3}H]-N-(4-methoxy-2-nitrophenyl)acetamide **(22)** (~330 mCi; 100%, specific activity = 5.3 Ci/mmol, 29.84 mCi/mg). The labeled material had the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection). The material required no further purification and was used directly in the next step.

Preparation of [^{3}H] 5-methoxy-1H-benzo[d]imidazole-2-thiol (23)

To a 4-ml vial with stir bar was added potassium hydroxide (17 mg, 0.303 mmol), water (0.5 ml), carbon disulfide (0.2 ml, 3.31 mmol) and a solution of [^{3}H]-N-(2-amino-4-methoxyphenyl)acetamide (**22**) (330 mCi; 11 mg, 0.060 mmol) in 1.0-ml ethanol. The vial was capped and heated at 80°C in an aluminum heating block for 2 h. HPLC-MS analysis showed complete consumption of the starting material. The reaction was cooled, diluted with ethanol (10 ml) and to it was added 1 ml of 1 M hydrochloric

acid solution. The mixture was then concentrated *in vacuo*. This crude material was diluted with water and the salts removed by adsorption of compound onto two stacked Waters C18 Sep-Pak® Plus long cartridges (820-mg sorbent each) [conditioned with 10-ml ethanol and then 10-ml water]. The SPE cartridges were then washed with water (20 ml), and the flask used for concentrating the reaction was rinsed with ethanol (10 ml) and flushed through cartridges followed by an additional 20-ml ethanol. Negligible radioactivity was observed in the water wash. The ethanol wash contained 321 mCi (89% accounting for radiochemical purity) of [^{3}H]-5-methoxy-1H-benzo[d]imidazole-2-thiol **(23)** (91.4% radiochemical purity; specific activity = 5.23 Ci/mmol, 29.0 mCi/mg.) The labeled material has the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection). The material required no further purification and was used directly in the next step (synthesis of compound **28**).

Preparation of N-(5-methoxy-2-nitrophenyl)acetamide (25)

To a 20-ml vial with stir bar was added 2-nitro-5-methoxyaniline (**24**) (3 g, 17.8 mmol) and acetic anhydride (5.40 ml, 57.1 mmol). The vial was capped and heated at 120°C in an aluminum heating block (heating block temp) overnight. The reaction was cooled and a solid precipitated. The suspension was poured into 150-ml water then filtered followed by washing with water (50 ml) and then 100-ml chloroform that was collected separately. The chloroform fraction was washed with saturated aqueous sodium chloride solution (100 ml), water (50 ml), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 3.56 g of a yellow solid. The crude product was purified by silica gel chromatography (Biotage SNAP 100-g cartridge, column conditioned with hexanes, sample loaded in dichloromethane, elute three column volumes (CV) of hexanes then gradient from hexanes to 30% ethyl acetate/hexanes over 6 CV, hold at 30% ethyl acetate/hexanes for 6 CV) to give N-(5-methoxy-2-nitrophenyl)acetamide (**27**) (1.39 g, 6.62 mmol, 37.1% yield) as a yellow solid. ^{1}H NMR is consistent with reported values.[15]

Preparation of [^{3}H]-N-(5-methoxy-2-nitrophenyl)acetamide (26)

To a tritiation reaction bulb with a flea stir bar was added Crabtree's catalyst (5 mg, 0.0062 mmol) and a solution of N-(5-methoxy-2-nitrophenyl)acetamide (**25**) (4.5 mg, 0.021 mmol) in 1.1-ml dichloromethane. The vessel was cooled in an ethanol/CO_2 bath, degassed under high vacuum and filled with T_2 (160 Torr, ~0.84 Ci). The solution was then stirred at ambient temperature for 18 h. The reaction was cooled in an ethanol/CO_2 bath, and excess tritium was transferred onto one of the tritiation manifold's uranium beds. The reaction was warmed to ambient temperature, transferred with the aid of dichloromethane from the reaction bulb and concentrated *in vacuo* on a rotary evaporator. Ethanol (10 ml) was added and the evaporation repeated two times to ensure removal of volatile radioactive material. The crude material was purified by semi-preparative reversed phase HPLC (Luna C18(2), 5u, 10 × 250 mm, 5 ml/min, 35% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t on corresponding analytical column [5 μm, 4.6 × 250 mm, 1 ml/min, 40% acetonitrile/water + 20 mM NH_4OAc] = 10.9 min) to give (after solvent switch of product containing fractions to an ethanol solution using C18 Sep-Pak® SPE cartridges) 46.8 mCi (5.6% radiochemical yield) of [^{3}H]-N-(5-methoxy-2-nitrophenyl)acetamide (**26**) (specific activity = 3.76 Ci/mmol, 17.8 mCi/mg.) The labeled material had the same R_t as the unlabeled standard as seen by both HPLC and HPLC-MS analyses (individually and co-injection).

Preparation of [^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (28)

To a 4-ml vial with stir bar was added 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride **(6)** (16.0 mg, 0.072 mmol), a solution of [^{3}H]-5-methoxy-1H-benzo[d]imidazole-2-thiol **(23)** (10.1 mg, 0.056 mmol, 321mCi, 91.4% radiochemical purity) in 2-ml ethanol and 1 M aqueous sodium hydroxide solution (200 μl, 0.2 mmol). After stirring at ambient temperature for 2 h, HPLC-MS analysis of the reaction showed > 95% conversion to the desired sulfide. HPLC of the crude showed product at 90% radiochemical purity (Curosil PFP, 5 μm, 4.6 × 250 mm, 1 ml/min 40% acetonitrile/water + 20 mM NH_4OAc, R_t = 13.6 min).The reaction was concentrated *in vacuo* and purified by semi-preparative reversed phase HPLC (Curosil PFP, 5u, 10 × 250 mm, 5 ml/min 40% acetonitrile/water + 20 mM NH_4OAc) to give (after solvent switch of product containing fractions to an ethanol solution using C18 Sep-Pak® SPE cartridges) 210 mCi (72.6%) of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole **(28)** (specific activity = 5.2 Ci/mmol, 15.7 mCi/mg; radiochemical purity > 98%.) The labeled material had the same R_t as the unlabeled as seen by both HPLC and HPLC-MS analyses (individually and co-injection).

Preparation of [^{3}H]5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]imidazole (omeprazole) (29)

To a solution of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)thio)-1H-benzo[d]imidazole **(28)** (210 mCi, 13.4 mg, 0.040 mmol) in 2.5-ml DCM, at ~ −40°C to −45°C (acetonitrile/CO_2 bath), was added 3-chloroperoxybenzoic acid (9 mg, 0.04 mmol; 77% purity) as a solid in one portion. The reaction was stirred at −40°C for 1 h, then an additional equivalent of 3-chloroperoxybenzoic acid (9 mg, 0.04 mmol; 77% purity) was added and stirring was continued for 1 h at −40°C. HPLC-MS analysis showed a mixture 2:1 of desired sulfoxide and sulfone (**30**) from over-oxidation. The reaction was quenched by the addition of 1 ml of concentrated ammonium hydroxide, warmed to ambient temperature and then diluted with dichloromethane (20 ml) and saturated aqueous sodium bicarbonate (5 ml). The dichloromethane layer was separated and concentrated *in vacuo*. The crude material was purified by reversed phase HPLC (Develosil RP Aqueous C30, 5 μm, 10 × 250 mm, 30% acetonitrile/water + 20 mM NH_4OAc, PDA detector, R_t = 14.6 min) to give (after solvent switch of product containing fractions to an ethanol solution using C18 Sep-Pak® SPE cartridges) 95.3 mCi (45.4%) of [^{3}H]-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]imidazole (omeprazole) **(29)** with acceptable specific activity (5.4 Ci/mmol, 15.63 mCi/mg) and radiochemical purity (>99%). The labeled material had the same R_t as the unlabeled as seen by both HPLC and HPLC-MS analyses (individually and co-injection). No loss in specific activity was observed throughout the course of the synthesis.

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Conflict of interest

The authors did not report any conflict of interest.

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