

Molecular Isotopic Engineering (MIE): Industrial Manufacture of Naproxen of Predetermined Stable Carbon-Isotopic Compositions for Authenticity and Security Protection and Intellectual Property Considerations

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ABSTRACT

Molecular Isotopic Engineering (MIE) is the directed stable-isotopic synthesis of chemical products for reasons of product identification and of product security, and also for intellectual property considerations. We report here a generally excellent correspondence between the observed and predicted stable carbon-isotopic ($\delta^{13}\text{C}$) results for a successful directed synthesis of racemic mixture from its immediate precursors. The observed results are readily explained by the laws of mass balance and isotope mass balance. Oxygen- and hydrogen isotopic results which require an additional assessment of the effects of O and H exchange, presumably due to interaction with water in the reaction solution, are addressed elsewhere.

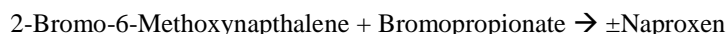
A previous, cooperative study with the US FDA-DPA showed that individual manufacturers of naproxen could readily be differentiated by their stable-isotopic provenance ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and δD ; ref. 1). We suggest that MIE can be readily employed in the bio/pharmaceutical industry without alteration of present manufacturing processes other than isotopically selecting and/or monitoring reactants and products.

Keywords: Stable isotopes, carbon isotopes, intellectual property, security, authenticity, naproxen.

1. INTRODUCTION

Product identity, product security, and intellectual property protection remain major concerns in the bio/pharmaceutical industry (2-4). The directed stable-isotopic synthesis of chemical products allows the predetermination of the stable-isotopic composition of materials to address these challenges (5). Product identification is typically performed at three levels: overt, covert, and forensic levels (*e.g.*, ref. 2). We focus here on a forensic or analytical approach: the analysis of natural-abundance stable isotopes in these products. Natural-abundance stable isotopes are natural tracers that occur in all matter (*e.g.*, ref. 6).

Early work in cooperation with the US FDA on the product characterization of naproxen revealed manufacturer-level isotopic provenance of this small analgesic molecule (Wokovich *et al.*, 2005), which was referred to as “The Manufacturer’s Fingerprint.” This isotopic provenance represented the convergence of the effects of the stable-isotopic compositions of starting materials and isotopic effects of the synthetic process. Rather than merely accepting the random effects of variable sourcing and synthetic process on the stable-isotopic compositions of products, here we take a proactive approach to purposefully direct the stable-isotopic composition of bio/pharmaceutical products. The main rationale for MIE is to pro-actively design the isotopic ranges of products for reasons of product identification and of product security, and also for intellectual property considerations. As an example of MIE, we analyzed the isotopic products of a later step of naproxen synthesis:



Pre-selection of three different stable-isotopic compositions of the starting material, 2-Bromo-6-methoxynaphthalene, respectively yielded racemic naproxen products of three discrete stable-isotopic ranges. The resulting MIE naproxen is very different from a naproxen molecule that has merely been substituted at a single position with a different isotope as in deuterium labeling (ref. 7 and refs. therein). Our directed isotopic synthesis is just one example of MIE to predetermine the discrete isotopic ranges of bio/pharmaceutical products. In principle, the MIE approach should be readily adapted to existing bio/pharmaceutical manufacturing units. The only adjustment to an existing manufacturing process would be the use of starting materials or synthetic intermediates of pre-measured stable-isotopic compositions. The manufacturing apparatus would remain unchanged. This approach could have broad application in securing drug identity/provenance from manufacturing plant to consumer.

By way of background, Molecular Isotope Technologies LLC has developed four patented or patent-pending generations of stable-isotopic methods and technologies: (i) product characterization (for both small molecules and biologics) (*e.g.*, 1, 8-9); (ii) process characterization (notably, process patent protection), (8,10), (iii) in-process (continuous) analysis (12), and now (iv) molecular isotopic engineering or MIE (12). Early work in cooperation with the US FDA on the product characterization of naproxen revealed manufacturer-level isotopic provenance of this small analgesic molecule (1) which was referred to as “The Manufacturer’s Fingerprint.” This isotopic provenance represented the convergence of the effects of the stable-isotopic compositions of starting materials and isotopic effects of the synthetic process. Rather than merely accepting the random effects of variable sourcing and synthetic process on the stable-isotopic compositions of products, we take a proactive approach to purposefully predetermine the stable-isotopic composition of bio/pharmaceutical products. The main rationale for MIE is to design the isotopic ranges of products for reasons of product identification and of product security, and also for intellectual property considerations.

2. EXPERIMENTAL

Samples: Three groups of samples were analyzed here to examine the natural-abundance stable-isotopic compositions for naproxen synthesis, including the two reactants (2-bromo-6-methoxynaphthalene and bromopropionic acid) and the end product (racemic naproxen).

2.1. Naproxen Synthesis

A late-stage synthesis of naproxen was performed for us by IsoSciences, LLC (King of Prussia, Pennsylvania, USA; Fig. 1)

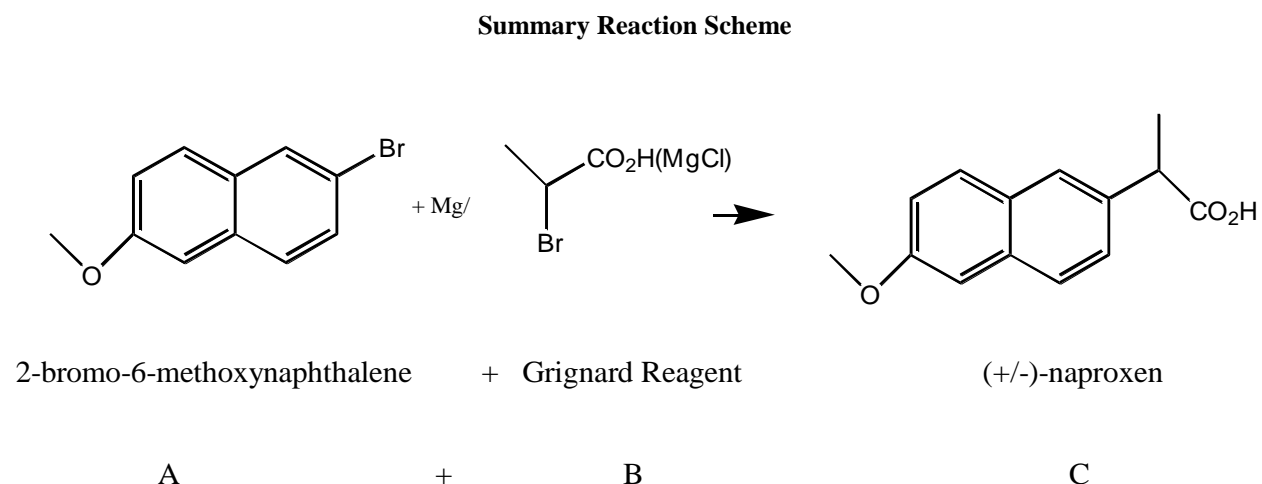


Fig. 1. Late-stage synthetic pathway of naproxen examined here.

2.2. Representative synthetic details

Reactants. Eight samples of 2-bromo-6-methoxynaphthalene were collected from a worldwide selection of suppliers (Table 1). A Grignard reagent (bromopropionic acid) was acquired from Sigma-Aldrich (St. Louis, Missouri USA). Three of the 2-bromo-6-methoxynaphthalene samples, from CombiBlocks, Matrix, and Aesar, were selected for this study based on their differing ^{13}C compositions: one high, one low, and one intermediate ^{13}C composition. Samples of the product (racemic naproxen) were synthesized from the three different starting materials.

Grignard Formation: 2-Bromo-6-methoxynaphthalene was dissolved in a round bottom flask of anhydrous toluene and anhydrous tetrahydrofuran (THF) with heating and degassing. The bromonaphthalene solution was added dropwise to the magnesium via addition funnel. The reaction was allowed to cool to room temperature under nitrogen.

Magnesium Salt Formation on Bromopropionic Acid: Alpha-bromopropionic acid was dissolved in anhydrous THF. The solution was cooled in a dry ice/acetone bath to -15°C and methyl magnesium chloride was added via syringe while maintaining the temperature below 0°C . The temperature was kept below 0°C until the solution was used.

Coupling Reaction: The Grignard solution was transferred into a two-neck round bottom flask with a thermometer and a septum via cannula, then was degassed. The solution was cooled in an ice bath and the mixed magnesium halide complex was added via cannula maintaining the temperature at $15\text{-}20^{\circ}\text{C}$. The reaction was stopped after 2hr. It was cooled in an ice bath and a solution of 10mL of 12N HCl in 75 mL of water was added. After stirring for 5 min, the biphasic mixture was filtered, and the filter was washed with 25 mL of toluene and 25 mL of water. The layers were separated and the organic phase was extracted with 2x75 mL of 10% NaOH solution. The basic extracts were combined, washed with toluene (~25 mL) and filtered. To the filtrate was added 7.5 mL of methanol and 6mL of toluene. This mixture was then acidified with concentrated HCl to pH 5. The resulting slurry was heated to reflux for 1hr and allowed to cool overnight with stirring. The product was washed with 10 mL of water, 2x2 mL of toluene and 2x2 mL of hexane and then dried to give an off-white solid. After drying under high vacuum for 48hr there was 5.8828 g (53% yield).

2.3. Stable-Isotopic Analyses

Three stable-isotopic measurements ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and δD) were made of each of the components of this study. In the starting-material survey study, three stable-isotope ratios were measured on each of the eight samples of 2-bromo-6-methoxynaphthalene in triplicate analysis (*i.e.*, 8 batches \times 3 isotope ratios \times 3 replication = 72 measurements) to assess analytical precision (13,14). Nine analogous isotopic measurements were made on the bromopropionic acid reagent (3 isotope ratios \times 3 replication). Triplicate analyses were also performed for each of the three isotope ratios of the five batches of naproxen synthesized here, yielding 45 isotope measurements. Thus, a total of 126 stable-isotopic measurements of the samples were performed in this study. Only the carbon-isotopic results are addressed in this abbreviated report.

2.3.1 Carbon Isotope Analyses

As detailed elsewhere (16), carbon ($\delta^{13}\text{C}$) isotopic analyses were performed respectively on (i) a Carlo Erba 1108 Elemental Analyzer interfaced using a Conflo III interface to a Thermo Scientific Delta V isotope ratio mass spectrometer (EA/IRMS) and (ii) a Finnigan Thermal Conversion/Elemental Analyzer (TCEA) interfaced to Finnigan Delta V Plus isotope-ratio mass spectrometer (thus a TCEA/IRMS).

2.3.2 Units of Stable Isotopic Measurement

Carbon isotopic results are expressed in δ -values (‰ = parts per thousand differences from international standards) defined as:

$$\delta (\text{‰}) = \left(\frac{R_{\text{smpl}}}{R_{\text{std}}} - 1 \right) \times (1000) \quad (1)$$

where R_{smpl} = the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample material and R_{std} = the $^{13}\text{C}/^{12}\text{C}$ ratio of an International Atomic Energy Authority standard [IAEA, known as “VPDB” (Vienna Pee Dee Belemnite) whose $^{13}\text{C}/^{12}\text{C}$ ratio has been defined as the official zero point of the carbon-isotopic scale]. $^{18}\text{O}/^{16}\text{O}$ and D/H values are given relative to IAEA Vienna Standard Mean Ocean Water (VSMOW) standard which gives the zero points of the oxygen and hydrogen-isotopic scales.

2.4. Estimates of Uncertainty

Since all measurements in this study were made in triplicate, the averages and 1σ -standard deviations are reported here for the observed isotopic data in Tables 1 and 2. Two sigma standard deviations are shown for the deviations from mass balance and isotope mass balance.

Characteristic one sigma (1σ) standard deviations for the isotope measurements reported in this study were: $\delta^{13}\text{C}$ ($\pm 0.03\text{‰}$), $\delta^{18}\text{O}$ ($\pm 0.09\text{‰}$), and δD ($\pm 1.0\text{‰}$) as shown in Table 1.

3. RESULTS

3.1. Stable-Isotopic Composition of Reactants

The $\delta^{13}\text{C}$ compositions of eight samples of the reactant 2-bromo-6-methoxy-naphalene measured in triplicate are shown in Table 1 as those of bromopropionic acid is shown in Table 2.

Sample Name	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{13}\text{C}$	$\pm 1\sigma$ Std dev.	$\delta^{18}\text{O}$	$\pm 1\sigma$ Std dev.	δD	$\pm 1\sigma$ Std dev.
	‰ vs VPDB	‰ vs VSMOW	‰ vs VSMOW	‰ vs VPDB	‰	‰ vs VSMOW	‰	‰ vs VSMOW	‰
Matrix Scientific / W12M - 1	-23.97	24.24	-116.6						
Matrix Scientific / W12M - 2	-24.02	24.31	-114.3	-24.01	0.04	24.24	0.07	-115.1	1.3
Matrix Scientific / W12M - 3	-24.04	24.17	-114.3						
AK Scientific / LC33871 - 1	-23.97	24.17	-115.7						
AK Scientific / LC33871 - 2	-23.99	24.27	-116.4	-24.01	0.05	24.25	0.07	-116.2	0.5
AK Scientific / LC33871 - 3	-24.06	24.30	-116.6						
Oakwood Chemical / D13F - 1	-24.48	13.77	-64.2						
Oakwood Chemical / D13F - 2	-24.53	13.76	-68.0	-24.49	0.04	13.77	0.02	-65.9	1.9
Oakwood Chemical / D13F - 3	-24.46	13.80	-65.6						
Sigma Aldrich / MKBR4254V - 1	-24.51	13.86	-70.6						
Sigma Aldrich / MKBR4254V - 2	-24.49	13.95	-68.6	-24.50	0.01	13.96	0.10	-69.2	1.2
Sigma Aldrich / MKBR4254V - 3	-24.49	14.07	-68.6						
Combi-Blocks / L74583 - 1	-28.76	0.36	-65.4						
Combi-Blocks / L74583 - 2	-28.70	0.54	-65.0	-28.73	0.03	0.45	0.09	-64.6	1.0
Combi-Blocks / L74583 - 3	-28.75	0.45	-63.5						
Tokyo Chemical Industry Co., Ltd. (TCI) / GJ01-CSBE - 1	-29.67	-2.45	-144.9						
Tokyo Chemical Industry Co., Ltd. (TCI) / GJ01-CSBE - 2	-29.65	-2.38	-146.4	-29.67	0.03	-2.42	0.04	-145.9	0.8
Tokyo Chemical Industry Co., Ltd. (TCI) / GJ01-CSBE - 3	-29.71	-2.44	-146.3						
Apollo Scientific Limited / AS447149 - 1	-29.82	3.84	-116.5						
Apollo Scientific Limited / AS447149 - 2	-29.85	3.89	-116.5	-29.85	0.02	3.80	0.11	-116.7	0.4
Apollo Scientific Limited / AS447149 - 3	-29.87	3.68	-117.1						
Alfa Aesar / 10137505 - 1	-29.90	13.79	-109.0						
Alfa Aesar / 10137505 - 2	-29.84	14.13	-111.0	-29.88	0.04	14.07	0.25	-110.2	1.1
Alfa Aesar / 10137505 - 3	-29.91	14.28	-110.5						

Sample Name	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	δD
	‰ vs VPDB	‰ vs VSMOW	‰ vs VSMOW
2-bromopropionic acid / 1	-30.05	11.01	-19.0
2-bromopropionic acid / 2	-30.13	10.84	-20.8
2-bromopropionic acid / 3	-30.21	11.35	-21.1
Average	-30.13	11.07	-20.3
StDev	0.08	0.26	1.1

4. DISCUSSION

4.1. The Stable-Isotopic Records of Naproxen Synthesis

The directed stable-isotopic synthesis of naproxen is discussed in two parts: the mass-balance / isotope-mass balance (MB/IMB) component, and then the deviations (if any significant) from MB/IMB. Since these results are compared to the MB/IMB frame of reference, that topic is briefly described here.

4.1.1. Frame of Reference: Comparison of Observed versus Predicted Isotopic Values

The laws of mass balance and isotope mass balance (*e.g.*, ref. 12) are a primary frame of reference for assessing the results of the naproxen isotopic synthesis. The basic mathematics of MB/IMB (detailed in ref. 8 and refs. therein) are summarized here:

$$\text{Mass Balance:} \quad n_A + n_B = n_C \quad (2)$$

$$\text{Isotope Mass Balance:} \quad n_A\delta_A + n_B\delta_B = n_C\delta_C \quad (3)$$

Isotopic Fractionation (one component in excess):

$$n_A(\delta_A + \Delta_A) + n_B\delta_B = n_C\delta_C \quad (4)$$

$$\delta_A + \Delta_A = (n_C\delta_C - n_B\delta_B)/n_A \quad (5)$$

$$\Delta_A = [(n_C\delta_C - n_B\delta_B)/n_A] - \delta_A \quad (6)$$

where,

n_A , n_B , n_C = number of moles of compounds A, B, and C;

δ_A , δ_B , and δ_C = isotopic compositions of compounds A, B, and C; and

Δ_A = isotopic fractionation of compound A.

4.1.2. Carbon Isotopes

The observed carbon isotopic results and the predicted MB/IMB results for the naproxen synthesis are shown in Fig. 2a. Observed and predicted values align well and will be further examined below.

4.2. Mass Balance and Isotope Mass Balance: Correspondence and Deviations

The correspondence to and deviations from MB/IMB (Figs. 2-3) are examined here to account for those isotopic relationships only for the carbon isotope ratios examined here.

4.2.1. Carbon: No Significant Deviation from MB/IMB

A plot of the observed $\delta^{13}\text{C}$ values versus the predicted values on the basis of MB/IMB is shown in Fig. 3. The excellent correspondence indicates that the carbon-isotopic synthesis is consistent with the MB/IMB model.

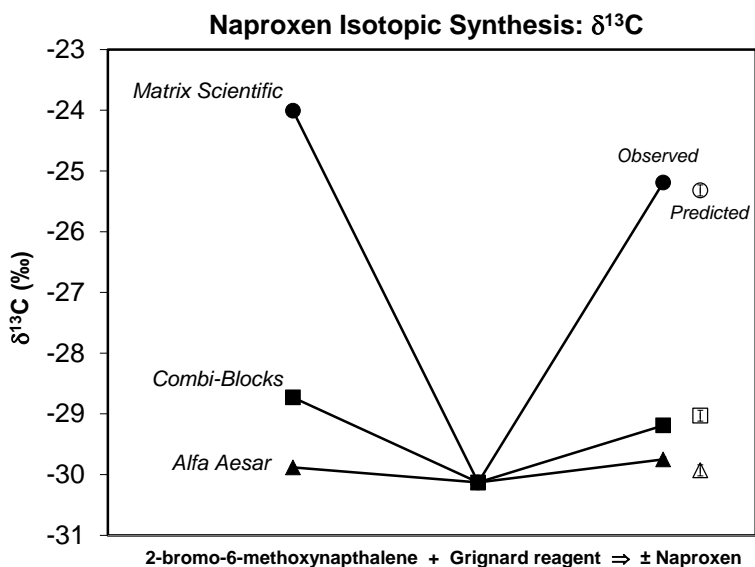


Fig. 2. Carbon-isotopic composition ($\delta^{13}\text{C}$) as a function of either reactant or product number (n). The mass balance / isotopic mass balance-predicted values of racemic (\pm) naproxen are shown adjacent to the observed values for comparison. Deviations from the predicted values are discussed below.

4.3. Product Identification, Product Security, and Intellectual Property Considerations

Molecular Isotopic Engineering (MIE) allows unprecedented stable-isotopic definition of chemical products from isotopically-known starting materials. In fact, the present naproxen synthesis permits the precision of compound production to within a few tenths of a permil for carbon and oxygen and approximately one permil for hydrogen when the ranges of starting materials may span tens of a permil. Such narrow delimitation of products' isotopic fingerprint decreases their vulnerability to various intellectual-property infringements. MIE thus allows for the design and synthesis of drug molecules with discrete stable isotopic composition for a wide range of stable isotopes. Starting with a small survey suite of readily-available reactants, various chemical products can be produced via existing chemical processes. The only difference from pre-existing processes is that the stable-isotopic compositions of the reactants and products are now measured either offline or online. The major result of MIE is to generate chemical products of narrowly-delimited isotopic ranges as compared to the seemingly random distribution of typically-produced products in which no explicit effort is made to delimit their compositions. In other words, MIE allows for the design of a unique and characteristic isotopic array or internal "bar code" or "fingerprint" for a drug molecule. The implications for product identification, supply chain custody, and security, and anti-counterfeiting are enormous.

Furthermore, because MIE designed drug molecules are essentially new chemical entities, MIE has some potentially interesting intellectual property implications. Consider for example the situation of a conventionally-synthesized, but isotopically-labeled drug molecule, where the resulting product molecule is a new entity that was not previously found in nature. In contrast, with MIE we are able to go beyond merely positionally-labeling a drug molecule with an isotope to now rationally and selectively design new molecules with far more complex – multipositional – and thus highly-specific isotopic fingerprints.

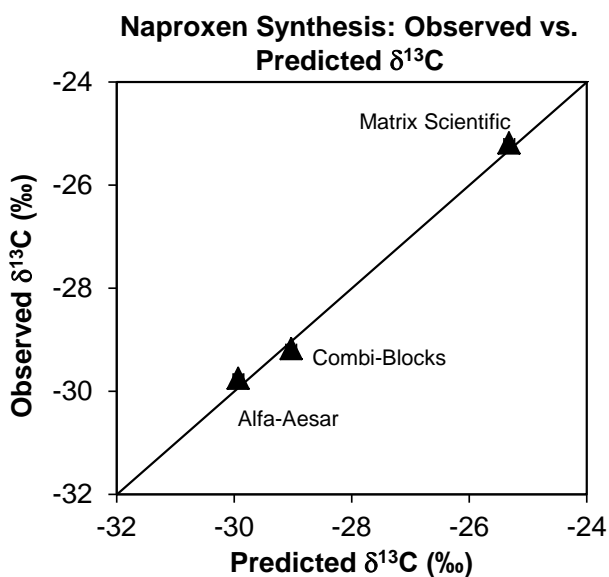


Fig. 3. Observed versus predicted carbon-isotopic compositions of racemic (\pm) naproxen values demonstrating excellent (± 1 s.d.) correspondence between mass-balance/isotope-mass balance estimation and observed values.

5. CONCLUSIONS

Consistent with principles of Mass Balance and Isotope Mass Balance, directed stable-isotopic synthesis (or, “Molecular Isotopic Engineering”) permitted the production of racemic naproxen of pre-determined carbon-isotopic compositions for reasons of identity, security, and IP determination. A small, worldwide survey of a key Naproxen intermediate (2-bromo-6-methoxynaphthalene) gave a wide range of C isotopic values for the present starting material. Mass balance and Isotope Mass Balance (MB/IMB) account for the carbon-isotopic relationship between the reactants and product naproxen very well. In addition to MB/IMB considerations, the equilibration between O and H and naproxen is readily accounted for by equilibrium isotopic exchange with reaction water. In general, the use of existing synthetic manufacturing methods indicate that MIE should generate products in predetermined isotopic ranges for reasons of product identity and security and may present a new mode of pharmaceutical patenting the isotopic Composition of Matter.

NOTE: Pending patent applications are on file with respect to the technology.

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