JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 123–130.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.905

Research Article

Synthesis of $^{13}C_6$-labeled Reyataz™ (BMS-232632)

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Summary

An efficient synthesis of $^{13}C_6$-labeled Reyataz™ (BMS-232632) is described. $^{13}C_6$-labeled Reyataz™ was synthesized in eight steps from commercially available [*ring*-$^{13}C_6$]-L-phenylalanine. The overall yield of the synthesis was 8% and the purity of the final product was greater than 98%. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: Reyataz™; Atazanavir; HIV protease inhibitor; carbon-13

Introduction

Reyataz™ (BMS-232632) is a potent human immunodeficiency virus (HIV) protease inhibitor with a half-life that is suitable for once-daily dosing.[1] Reyataz™ has been approved by the FDA to treat patients with HIV. An important step in the development of Reyataz™ was the validation of rapid, accurate, and selective assays to quantify the concentration of the drug in blood samples taken from subjects in clinical studies.[2] These assays use LC-MS-MS to measure the concentration of Reyataz™ relative to an internal standard. The internal standard chosen was $^{13}C_6$-labeled Reyataz™ (**1**). Isotopically labeled Reyataz™ is an excellent internal standard because its properties and chromatography are identical to the unlabeled compound. The only difference is that Reyataz™ has a molecular weight of 704.86 g/mol while $^{13}C_6$-labeled Reyataz™ (**1**) has a molecular weight of 710.86 g/mol. This

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Received 7 October 2004
Accepted 12 October 2004

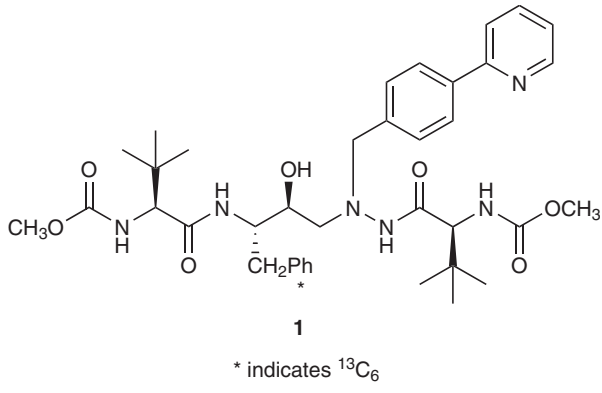


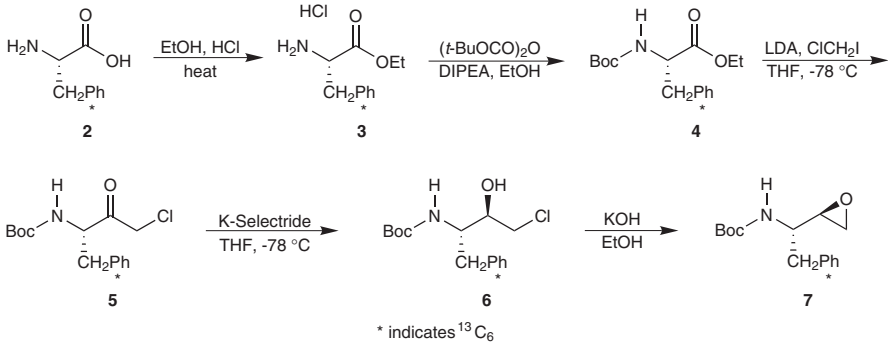
Figure 1. Structure of $^{13}C_6$-labeled Reyataz^{TM} (1)

difference in molecular weight allows the two compounds to be easily distinguished by LC–MS–MS. In this paper we describe an efficient synthesis of $^{13}C_6$-labeled Reyataz^{TM} (**1**) (Figure 1).

Results and discussion

The route used to synthesize $^{13}C_6$-labeled Reyataz^{TM} ([*ring*-$^{13}C_6$]-1-[4-(Pyridin-2-yl)phenyl]-5(*S*)-2,5-bis{[*N*-(methoxy-carbonyl)-L-*tert*-leucinyl]amino}-4(*S*)-hydroxy-6-phenyl-2-azahexane (**1**)) took advantage of some existing methodologies developed by Bristol–Myers Squibb's Discovery Chemistry department and Process Research & Development department.[3,4] The key to the synthesis was the use of [*ring*-$^{13}C_6$]-L-phenylalanine as the starting material. This $^{13}C_6$-labeled compound is commercially available with high enantiomeric and isotopic purity.

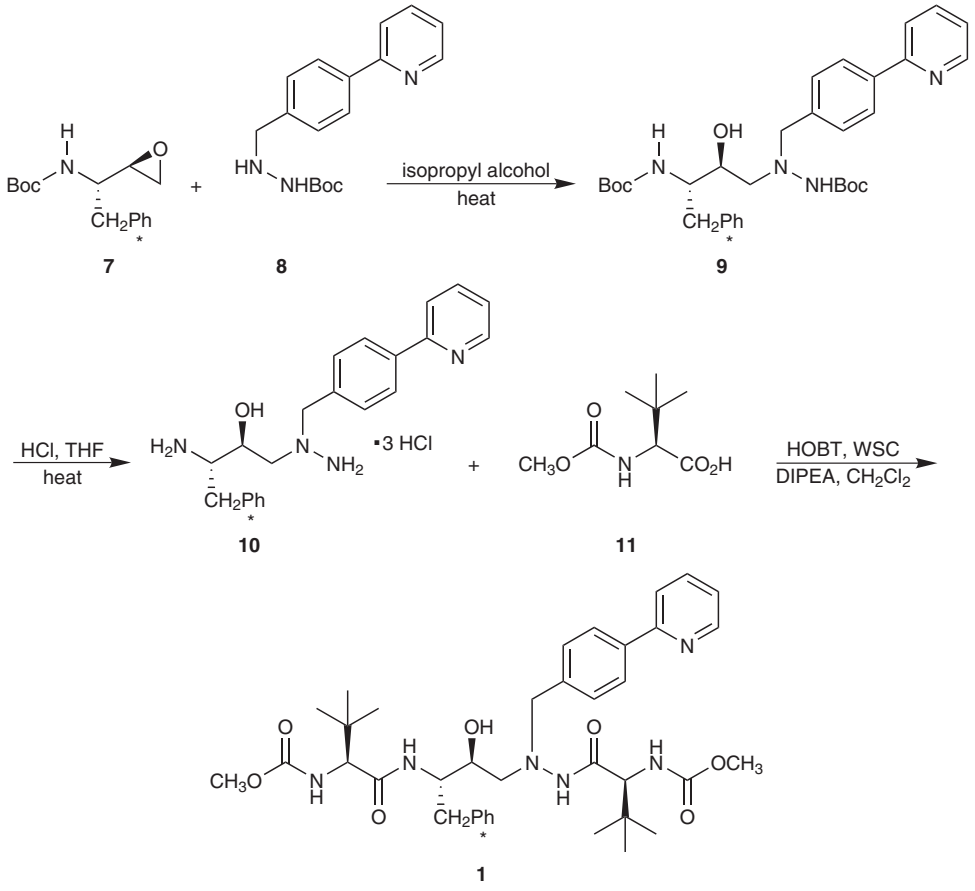
The synthesis of epoxide **7** was conducted as outlined in Scheme 1. The amino acid [*ring*-$^{13}C_6$]-L-phenylalanine (**2**) was converted to the corresponding



Scheme 1.

ethyl ester (**3**) and then Boc-protected to produce **4**.[5] Treatment of **4** with $ClCH_2I$ and LDA gave the chloromethyl ketone **5**.[3] Reduction of chloromethyl ketone **5** with K-selectride yielded an 8:1 mixture of diastereomeric alcohols. The major diastereomer (**6**) was isolated by column chromatography. Alcohol **6** was treated with KOH to give epoxide **7**.

$^{13}C_6$-labeled Reyataz^{TM} (**1**) was synthesized from epoxide **7** as outlined in Scheme 2. Epoxide **7** was opened with hydrazine derivative **8** to produce the chiral alcohol **9**.[4] Deprotection of **9** with aqueous HCl gave diamine **10**.[4] Diamine **10** was coupled with carboxylic acid **11** to yield $^{13}C_6$-labeled Reyataz^{TM} (**1**).[4] $^{13}C_6$-labeled Reyataz^{TM} (**1**) was synthesized in an 8% overall yield with a purity greater than 98%.



Scheme 2.

Experimental procedure

All reagents were obtained from Aldrich Chemical Company and used without further purification unless otherwise stated. Tetrahydrofuran ultra low water was obtained from J. T. Baker and used without further purification. [*ring*-$^{13}C_6$]-L-Phenylalanine (**2**) was obtained from Cambridge Isotope Laboratories with a chemical and enantiomeric purity of greater than 98%. The hydrazine derivative **8** and carboxylic acid **11** were obtained from Bristol–Myers Squibb's Process Research & Development Department.

All experimental procedures were optimized using unlabeled materials. All glassware was dried and purged with nitrogen or argon before use. All reactions were monitored by HPLC using the following conditions: YMC Pack Pro column s-3 μm 4.6 × 150 mm. Solvent: A = water with 0.05% TFA; B = acetonitrile with 0.05% TFA. Gradient: 100% A 0–10 min, 100–0% A 10–30 min, 0% A 30–35 min, 0–100% A 35–40 min. Flow: 1 ml/min, Wavelength 215 nm). HPLC analyses were performed using a Rainin Dynamax system (model SD-200) equipped with a Varian ProStar PDA detector (model 330). All ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer using $CDCl_3$ as the solvent.

*[ring-$^{13}C_6$]-L-Phenylalanine ethyl ester hydrochloride (**3**)*

Absolute ethanol (110 ml) and [*ring*-$^{13}C_6$]-L-phenylalanine (**2**, 3.01 g, 17.59 mmol) were added to a round-bottomed flask.[5] Anhydrous hydrochloric acid was bubbled in for 20 min and the solution was heated to 60°C. The mixture was stirred at 60°C under nitrogen for 15.5 h. The reaction mixture was cooled to room temperature and anhydrous hydrochloric acid was bubbled in for 5 min. The solution was heated to 60°C and stirred for 5 h. At that time the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The concentrate was dried under vacuum for 16 h to give 3.98 g (96% yield) of [*ring*-$^{13}C_6$]-L-phenylalanine ethyl ester hydrochloride (**3**) as a white solid. The white solid coeluted with an authentic sample of L-phenylalanine ethyl ester hydrochloride when it was analyzed by HPLC (98% pure).

*[ring-$^{13}C_6$]-(tert-Butyloxycarbonyl)-L-phenylalanine ethyl ester (**4**)*

Absolute ethanol (50 ml), [*ring*-$^{13}C_6$]-L-phenylalanine ethyl ester hydrochloride (**3**, 3.98 g, 16.87 mmol), di-*tert*-butyl dicarbonate (3.68 g, 16.87 mmol) and diisopropylethylamine (6.47 ml, 4.80 g, 37.11 mmol, 2.2 eq) were added to a round-bottomed flask. The solution was stirred for 16 h at room temperature under argon. The reaction mixture was concentrated and the concentrate was dissolved in EtOAc (60 ml). The solution was washed with H_2O (25 ml), 10% $KHSO_4$ (25 ml) and brine (25 ml). The organic layer was dried ($MgSO_4$),

filtered and concentrated to obtain a viscous oil. The crude material was purified by column chromatography (silica, 10% EtOAc/hexanes, TLC R_f = 0.53 (ninhydrin) using 30% EtOAc/hexanes) to give 4.59 g (91% yield) of **4** as a white solid. The white solid was analyzed by HPLC (98% pure) and ^{1}H NMR: δ 7.66–6.77 (m, 5 H), 5.04–4.89 (m, 1 H), 4.62–4.49 (m, 1 H), 4.16 (q, J= 7.2 Hz, 2 H), 3.14–3.02 (m, 2 H), 1.41 (s, 9 H), 1.22 (t, J= 7.2 Hz, 3 H).

*[ring-$^{13}C_6$]-(1(S)-Benzyl-3-chloro-2-oxo-propyl)-carbamic acid tert-butyl ester (**5**)*

Anhydrous THF (80 ml), chloroiodomethane (10.81 g, 61.28 mmol) and **4** (4.59 g, 15.32 mmol) were added to a round-bottomed flask.[3] The solution was cooled to −78°C and stirred under argon. An LDA solution (freshly prepared from *n*-butyllithium (30.6 ml, 76.59 mmol, 2.5 M solution in hexanes) and diisopropylamine (11.8 ml, 8.53 g, 84.25 mmol)) at −78°C was added dropwise to the reaction flask over 40 min via a cannula. As LDA was added, the reaction mixture changed from a bright yellow suspension to an orange solution to a brown suspension. The reaction mixture was stirred at −78°C for an additional 15 min after the addition of LDA was complete. The reaction was quenched at −78°C by dropwise addition of AcOH:THF (1:2, 75 ml) over 30 min. The reaction mixture was stirred for 10 min at −78°C and then allowed to warm to room temperature. Brine (50 ml) was added and the mixture was partially concentrated under reduced pressure. The concentrate was diluted with EtOAc (200 ml) and the aqueous layer was removed. The organic layer was washed with saturated $NaHCO_3$ (2 × 75 ml), 10% $NaHSO_3$ (2 × 75 ml) and brine (75 ml). The organic solution was dried ($MgSO_4$), filtered and concentrated to yield a brown solid. The crude brown solid was partially purified by column chromatography (silica, 10% EtOAc/hexanes, TLC R_f = 0.49 (ninhydrin) using 30% EtOAc/hexanes) to give 3.31 g of **5** as a yellow solid. The chloroketone **5** coeluted with an authentic sample when it was analyzed by HPLC (71 + % pure).

*[ring-$^{13}C_6$]-(1(S)-Benzyl-3-chloro-2(R)-hydroxy-propyl)-carbamic acid tert-butyl ester (**6**)*

Anhydrous THF (30 ml) and **5** (3.31 g, 10.89 mmol) were added to a round-bottomed flask and cooled to −78°C. *K*-Selectride (21.8 ml, 21.8 mmol, 1.0 M solution in THF) was added to a second flame-dried flask and cooled to −78°C. The *K*-selectride solution was added dropwise over 10 min to the solution of **5** using a cannula. The reaction mixture was stirred under argon for 30 min at −78°C. At that time the mixture was quenched by the addition of 10% $KHSO_4$ (50 ml). The aqueous solution was extracted with EtOAc (4 × 50 ml). The combined organic layers were washed with brine (20 ml), dried ($MgSO_4$), filtered and concentrated to obtain 6.57 g of crude product (ratio of

"*R*" to "*S*" alcohol by HPLC, 8:1). The crude material was purified by column chromatography (silica, 10% EtOAc/hexanes, TLC $R_f = 0.37$ (ninhydrin) using 30% EtOAc/hexanes) to give 1.38 g of **6** as a yellow oil (32% yield for the two steps based on recovered **4**). The yellow oil was analyzed by HPLC (94% pure) and ^{1}H NMR: δ 7.69–6.86 (m, 5 H), 4.93–4.81 (m, 1 H), 3.91–3.69 (m, 2 H), 3.60–3.47 (m, 2 H), 3.10–2.88 (m, 2 H), 1.41 (s, 9 H).

*[ring-$^{13}C_6$]-N-(tert-Butyloxycarbonyl)-2(S)-amino-1-phenyl-3(R)-3,4-epoxy-butane (**7**)*

Absolute ethanol (10 ml), **6** (1.38 g, 4.52 mmol) and KOH (0.30 g, 5.42 mmol, dissolved in absolute ethanol (20 ml)) were added to a round-bottomed flask.[3] The solution was stirred at room temperature under argon for 1 h. At that time the reaction mixture was concentrated and the concentrate was diluted with water (50 ml). The aqueous solution was extracted with EtOAc (3×25 ml). The combined organic layers were washed with brine (25 ml), then dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield 1.12 g of crude **7** as an orange oil. The epoxide **7** coeluted with an authentic sample when it was analyzed by TLC ($R_f = 0.41$ (ninhydrin) using 30% EtOAc/hexanes) and HPLC (90% pure).

*[ring-$^{13}C_6$]-1-[4-(Pyridin-2-yl)phenyl]-5(S)-2,5-bis[(tert-butyloxy-carbonyl)-amino]-4(S)-hydroxy-6-phenyl-2-azahexane (**9**)*

Anhydrous isopropyl alcohol (15 ml), **7** (1.12 g, 4.17 mmol) and **8** (1.25 g, 4.17 mmol) were added to a round-bottomed flask.[4] The mixture was heated to 75°C and stirred for 12 h under argon. At that time the solution was cooled to room temperature and concentrated under reduced pressure to give a yellow wax. Column chromatography (silica, 30% EtOAc/hexanes, TLC $R_f = 0.11$ (ninhydrin) using 30% EtOAc/hexanes) gave **9** contaminated with a small amount of **8**. The product was further purified by washing with TBME (20 ml) to give 1.10 g of **9** as a white solid (43% for the two steps). The white solid was analyzed by HPLC (95% pure) and ^{1}H NMR: δ 8.85 (brs, 1 H), 8.17–7.83 (m, 4 H), 7.64–7.39 (m, 5.5 H), 7.06–6.83 (m, 2.5 H), 5.39–4.93 (m, 2 H), 4.16–3.83 (m, 2 H), 3.76–3.54 (m, 2 H), 3.04–2.77 (m, 3 H), 2.61–2.44 (m, 1 H), 1.39 (s, 9 H), 1.33 (s, 9 H).

*[ring-$^{13}C_6$]-1-[4-(Pyridin-2-yl)phenyl]-5(S)-2,5-bis{[N-(methoxy-carbonyl)-L-tert-leucinyl]amino}-4(S)-hydroxy-6-phenyl-2-azahexane (**1**)*

Tetrahydrofuran (7 ml), **9** (1.10 g, 1.94 mmol) and aqueous HCl (0.77 ml 9.20 mmol, 12 N solution) were added to a round-bottomed flask.[4] The solution was heated to 60°C and stirred for 3 h. Gas evolution was observed during the first 30 min. The reaction mixture was cooled to room temperature

and concentrated to give crude **10** as a yellow oil. The diamine **10** coeluted with an authentic sample, when it was analyzed by HPLC (92% pure).

Anhydrous CH_2Cl_2 (5 ml), **11** (0.92 g, 4.84 mmol), 1-hydroxybenzotriazole hydrate (0.68 g, 5.04 mmol, 2.6 eq) and 1-[3-(dimethylamino)-propyl]-3-ethyl-carbodiimide hydrochloride (0.97 g, 5.04 mmol) were added to a round-bottomed flask.[4] Crude **10** (assume 1.94 mmol) and diisopropylethylamine (1.55 g, 2.10 ml, 12.03 mmol) were dissolved in anhydrous CH_2Cl_2 (5 ml) and added to the solution prepared above. The mixture was stirred at room temperature under argon for 17 h. At that time the reaction mixture was diluted CH_2Cl_2 (75 ml) and then washed with water (25 ml), saturated $NaHCO_3$ (25 ml), and brine (25 ml). The organic solution was concentrated under reduced pressure to give crude **1** as a yellow foam. The crude material was partially purified by column chromatography (silica, 10% hexanes/EtOAc, TLC $R_f = 0.31$ (UV short wave) using 100% EtOAc, 90% pure by HPLC). A second purification by column chromatography (silica, 25% hexanes/EtOAc) gave 0.899 g of **1** as a white solid (65% yield the two steps). The white solid was analyzed by HPLC (98.5% pure) and ^{1}H NMR: δ 8.74–8.67 (m, 1 H), 8.01–7.92 (m, 2 H), 7.85–7.67 (m, 2 H), 7.43–6.84 (m, 8 H), 6.56–6.33 (m, 2 H), 5.37–5.12 (m, 2 H), 4.81 (brs, 1 H), 4.15–3.89 (m, 3 H), 3.83–3.72 (m, 1 H), 3.67 (s, 3 H), 3.63 (s, 3 H), 3.65–3.49 (m, 1 H), 3.02–2.77 (m, 3 H), 2.59–2.48 (m, 1 H), 0.87 (s, 9 H), 0.79 (s, 9 H). The material was also analyzed by HRMS (ES) calculated for $^{12}C_{32}$ $^{13}C_6$ H_{53} N_6 O_7 $(M+H)^+$: 711.4177, found 711.4183. The isotopic distribution was determined to be: M + 6 (91.4%), M + 5 (7.0%), M + 4 (1.7%), M + 3 (< 0.1%), M + 2 (< 0.1%), M + 1 (< 0.1%), M + 0 (< 0.1%).

Conclusion

$^{13}C_6$-Labeled Reyataz™ was synthesized in eight steps from commercially available [*ring*-$^{13}C_6$]-L-phenylalanine with an overall yield of 8%. This $^{13}C_6$-labeled material was used successfully as an internal standard in an assay designed to quantify the concentration of Reyataz™ in blood samples taken from subjects in clinical studies. The validation of this assay was an important step in the development of Reyataz™.

Acknowledgements

We thank Samuel Bonacorsi Jr for helpful conversations and Richard Gedamke for performing the high resolution MS analysis.

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