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Research Article

A facile and efficient synthesis of d_3-labelled Reyataz™

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Summary
A facile and efficient synthesis of d_3-labelled Reyataz™ is described. The key step of synthesis involved the coupling of *N*-(d_3-methoxycarbonyl)-L-*tert*-leucine **2b** with an advanced chiral non-racemic building block **4**. The latter was synthesized in 4 steps from the readily accessible hydrazine derivative **8**. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: Reyataz™; HIV protease inhibitor; d_3-labelled; chiral non-racemic

Introduction
Reyataz™ (Atazanavir) is a potent human immunodeficiency virus (HIV) protease inhibitor and is the first once-daily protease inhibitor approved by the FDA to treat patients with HIV.[1] For bioequivalence studies of Reyataz™ in humans, d_3-labelled Reyataz™ is required because its physical and chromatographic properties are identical to the unlabelled compound. Several efficient synthetic routes leading to Reyataz™ have been published in the literature.[2,3] Several of these use the symmetrical coupling of 2 equivalents of *N*-methoxycarbony-L-*tert*-leucine **2a** with a diamine derivative **3** (Scheme 1) as a general approach. Retrosynthetic analysis suggested that a strategy involving the unsymmetrical coupling of *N*-(d_3-methoxycarbonyl)-L-*tert*-leucine **2b** with a key chiral non-racemic intermediate **4** would give d_3-labelled Reyataz™ (Scheme 1). The chiral non-racemic building block **4** can be obtained by the regioselective aminolysis of epoxide **5** with hydrazide **6a**, which can be synthesized by the coupling of phenyl hydrazine derivative **7** with *N*-methoxycarbonyl-L-*tert*-leucine **2a**. Herein we describe the design and synthesis of d_3-Reyataz™ using this unsymmetrical coupling approach.

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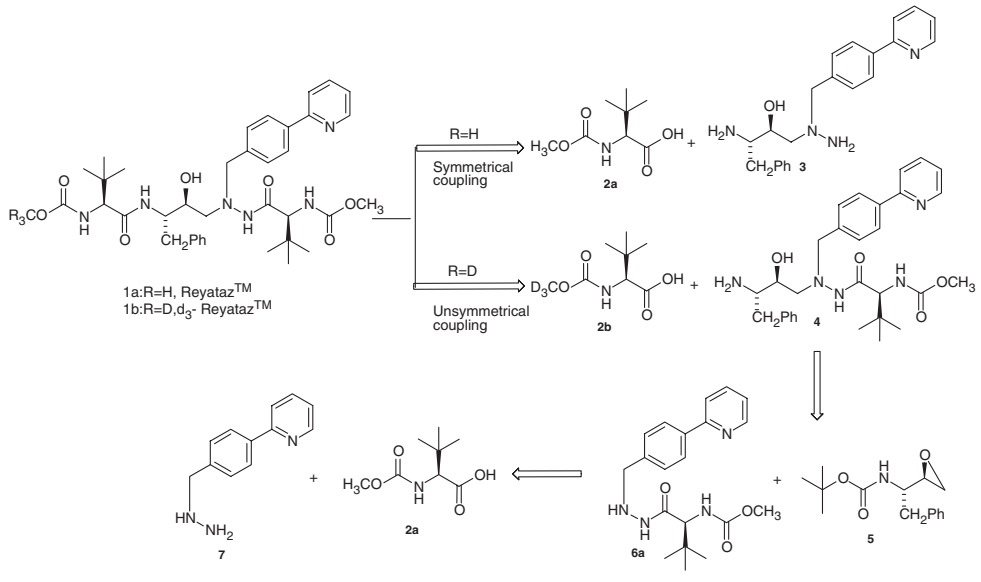
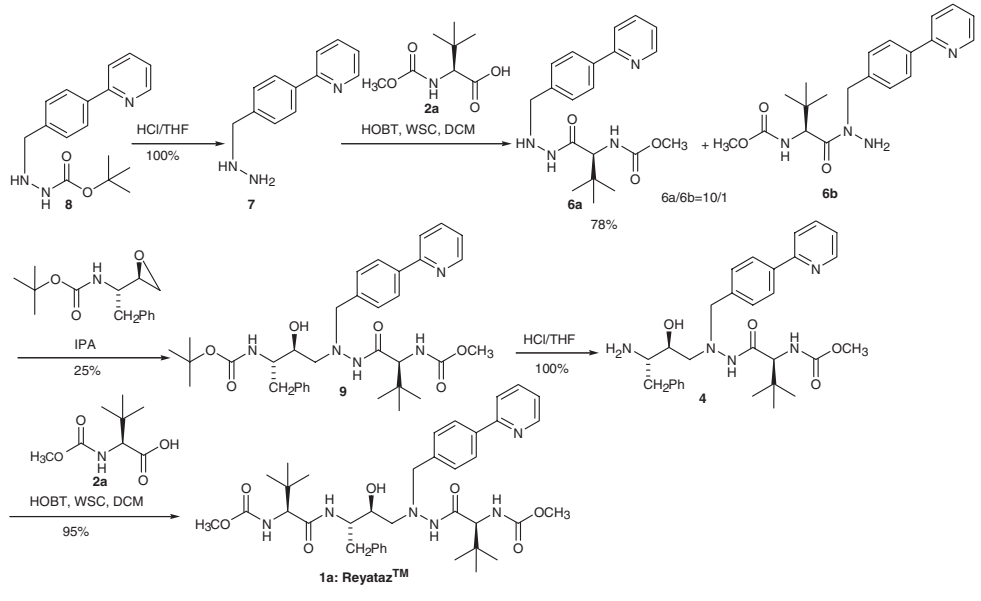


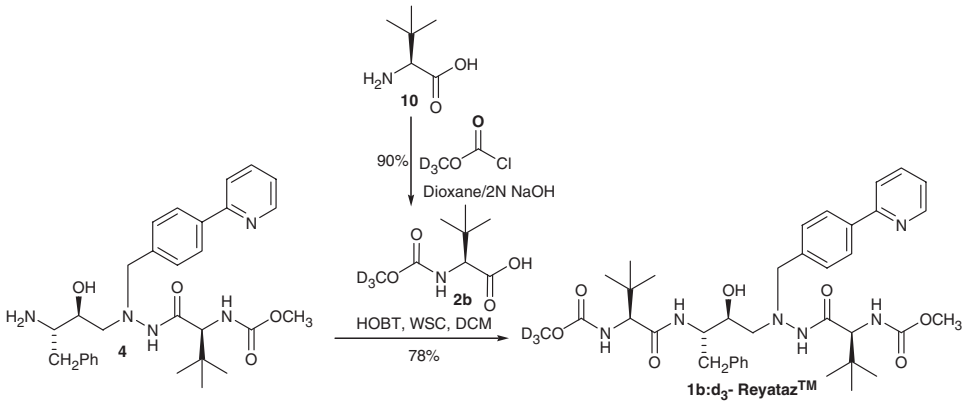
Scheme 1. Retrosynthesis of Reyataz™ and d_3-labelled Reyataz™



Scheme 2. Synthesis of intermediate 4 and Reyataz™

Results and discussion

The key amino intermediate **4** was synthesized as outlined in Scheme 2. Acid hydrolysis of hydrazinocarbamate **8** gave the hydrochloride salt of phenyl



Scheme 3. Synthesis of d_3-labelled Reyataz™

hydrazine derivative **7**. Compound **7** was treated with diisopropylethylamine and added to a mixture of *N*-methoxycarbonyl-L-*tert*-leucine **2a**, water-soluble carbodiimide [WSC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] and 1-hydroxybenzotriazole (HOBT) in dichloromethane to yield a crude solid containing the desired hydrazide **6a** and undesired hydrazide **6b** in 10:1 HPLC area ratio. Subsequent recrystallization from EtOH gave the desired hydrazide **6a** in 78% yield. Treatment of compound **6a** with epoxide **5** in refluxing IPA for 24 h gave compound **9**. Removal of the protecting group using HCl in THF gave the requisite precursor **4** in quantitative yield. Since compound **4** was a novel intermediate, it was further converted to the known free base of Reyataz™ to confirm the structure assignment. Treatment of compound **4** with *N*-methoxycarbonyl-L-*tert*-leucine, WSC and HOBT in dichloromethane yielded the free base of Reyataz™ **1a** in 95% yield. The spectroscopic and physical data for synthetic free base of Reyataz™ were identical to that reported previously.[3]

As shown in Scheme 3, the incorporation of deuterium into *N*-methoxycarbonyl-L-*tert*-leucine was achieved by the acylation of L-*tert*-leucine with d_3-methyl chloroformate to give compound **2b** in 90% yield.[4] Coupling of compound **4** directly with d_3-methyl chloroformate **2b** in the presence of WSC and HOBT gave d_3-labelled Reyataz™ in 78% yield.

Experimental

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. d_3-Methyl chloroformate was obtained from Cambridge Isotope Laboratories with a chemical and isotopic abundance of greater than 98%. The hydrazine derivative **8**, carboxylic acid **2a** and epoxide **5** were obtained from Process Research & Development Department of Bristol Myers Squibb Company.

All glassware was dried and purged with nitrogen or argon before use. All reactions were monitored by HPLC using a Shimadzu system (model SCL-10A) equipped with a Shimadzu PDA detector (model SPD-10AV) or a Rainin Dynamax system (model SD-200) equipped with a Varian ProStar PDA detector (model 330). All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent.

*Preparation of 1-(4-(pyridin-2-yl)benzyl)hydrazine (**7**)*

To a stirring solution of compound **8** (15 g, 50 mmol) in THF (70 ml) was added HCl (12 M, 10 ml) under nitrogen at room temperature. The reaction mixture was heated to 50°C, stirred at this temperature for 3 h, then cooled to room temperature. THF was decanted from the mixture and the resulting yellow solid washed with THF (2 × 20 ml) and dried *in vacuo* to give the product (**7**) (10 g, 100%). The product was used for the next step without further purification.

*Preparation of {(S)-2,2-dimethyl-1-[N′-(4-pyridin-2-yl-benzyl)-hydrazinocarbonyl]-propyl}-carbamic acid methyl ester(**6a**)*

To a stirring solution of compound **2a** (10.4 g, 50 mmol) in dichloromethane (250 ml) was added HOBT (9.44 g, 70 mmol) and WSC (9.74 g, 50 mmol). After the mixture was stirred at room temperature under nitrogen for 20 min, compound **7** (9.95 g, 50 mmol) in dichloromethane (60 ml) and diisopropylethylamine (19.2 g, 150 mmol) was added. The reaction was carried out for 1.5 h and was quenched with H_2O (125 ml). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 150 ml). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to give crude product (25 g) with a HPLC area ratio of **6a**/**6b** = 10/1. The crude product was recrystallized from 150 ml of EtOH/hexane (50/50) to give pure compound (**6a**) (14.43 g, 78%) as a white solid. ^{1}HNMR ($CDCl_3$): 8.67(d, J = 6.2 Hz, 1H), 8.28 (s, 1H), 7.93(d, J = 8.15 Hz, 2H), 7.72(m, 2H), 7.43(d, J = 8.18 Hz, 2H), 7.21(m, 1H), 3.97(m, 3H), 3.18 (s, 3H), 0.96 (s, 9H). ^{13}CNMR ($CDCl_3$): 170.7, 157.5, 157.4, 150.0, 139.0, 138.6, 137.1, 129.6, 127.7, 127.3, 122.5, 120.8, 61.5, 55.9, 52.7, 34.9, 26.8. Analytically calculated for $C_{20}H_{26}N_4O_3$: C, 64.84; H, 7.07; N, 15.12; found: C, 64.86; H, 7.16; N, 15.25.

*Preparation of {(1S,2S)-1-benzyl-2-hydroxy-3-[N′-((S)-2-methoxycarbonylamino-3,3-dimethyl-butyryl)-N-(4-pyridin-2-yl-benzyl)-hydrazino]-propyl}-carbamic acid tert-butyl ester (**9**)*

The stirring solution of compound **6a** (11.3 g, 30 mmol) in IPA (80 ml) was heated to reflux and compound **5** (8.67 g, 33 mmol) was added. The mixture was refluxed for 24 h, cooled to room temperature and concentrated *in vacuo*

to give a syrup which was purified by column chromatography on silica gel (elution with 40% EtOAc/hexanes, TLC $R_f = 0.2$) to give the desired product (**9**) (4.97 g, 25%) as a white solid. ^{1}H NMR ($CDCl_3$): 8.67(d, $J = 4.8$ Hz, 1H), 7.92(d, $J = 8.1$ Hz, 2H), 7.74(m, 2H), 7.42(d, $J = 8.0$ Hz, 2H), 7.21(m, 6H), 5.3(bs, 1H), 5.1(bs, 1H), 4.8(bs, 1H), 4.1(d, $J = 13.9$ Hz, 1H), 3.91 (d, $J = 13.8$ Hz, 1H), 3.62(m, 6H), 2.90(m, 3H), 2.63(d, $J = 11.6$ Hz, 1H), 2.10(bs, 1H), 1.34(s, 9H), 0.72 (s, 9H). ^{13}C NMR ($CDCl_3$): 171.3, 157.3, 157.2, 156.3, 150.0, 139.3, 138.8, 137.1, 136.9, 129.8, 129.6, 128.6, 127.4, 126.5, 122.5, 120.8, 79.4, 67.3, 62.8, 61.9, 61.7, 53.5, 52.8, 39.6, 34.6, 28.7, 26.5. Analytically calculated for $C_{35}H_{47}N_5O_6$ .0.39 H_2O: C, 65.60; H, 7.52; N, 10.93; found: C, 65.60; H, 7.57; N, 10.84.

*Preparation of {(S)-1-[N′-((2S,3S)-3-amino-2-hydroxy-4-phenyl-butyl)-N′-(4-pyridin-2-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (**4**)*

To a stirring solution of compound **9** (2.20 g, 3.47 mmol) in THF (20 ml) was added HCl (12 M, 1.0 ml) under nitrogen at room temperature. The reaction mixture was heated to 50°C, stirred for 5 h at that temperature, and cooled to room temperature. The THF was decanted from the mixture and the resulting yellow solid was washed with THF (2 × 20 ml) and dried *in vacuo* to give the product (**4**) (1.85 g, 100%). ^{1}HNMR (DMSO-d_6): 8.61(d, $J = 5.5$ Hz, 1H), 8.32(t, $J = 8.2$ Hz, 1H), 8.15(m, 1H), 7.86(d, $J = 8.2$ Hz, 3H), 7.71 (t, $J = 6.6$ Hz, 1H), 7.31(d, $J = 8.2$ Hz, 2H), 7.04(m, 3H), 6.81(m, 1H), 3.93(bs, 1H), 3.70(d, $J = 3.7$ Hz, 1H), 3.38(m, 4H), 3.16(s, 3H), 2.76(m, 3H), 1.5(bs, 3H), 0.37(s, 9H) ESIMS m/z 534.3 ([M + H$^+$], $C_{30}H_{39}N_5O_4$ requires 533.3.

*Preparation of N-(d_3-methoxycarbonyl)-L-tert-leucine (**2b**)*

A stirred solution of L-*tert*-leucine **10** (11.4 g, 87.2 mmol) in dioxane (40 ml) and sodium hydroxide (2 M, 145 ml) was cooled in ice and d_3-methyl chloroformate (17 g, 174.3 mmol) was added dropwise over 45 min, maintaining the internal temperature between 15 and 18°C. The reaction flask was warmed to 60°C, stirred at this temperature for 18 h, and extracted with dichloromethane (2 × 50 ml). The organic solution was acidified to pH = 2 by aqueous HCl (6 M) and extracted with ethyl acetate (3 × 50 ml). The extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was crystallized from hexanes (80 ml) to give the product (**2b**) (14.2 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.9 (s, 1H), 5.2 (bs, 1H), 4.1 (bs, 1H), 0.9 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5, 156.9, 62.1, 34.5, 26.4; ESIMS m/z 193.1 ([M + H$^+$], $C_8H_{13}D_3NO_4$ requires 193.12.

*Preparation of d_3-labelled Reyataz™ (**1b**)*

A solution of *N*-(d_3-methoxycarbonyl)-L-*tert*-leucine (331 mg, 1.72 mmol) in dichloromethane (10 ml) was added HOBT (253 mg, 1.87 mmol) and WSC (343 mg, 1.79 mmol), stirred at room temperature for 2 h, and treated with compound **4** (1.0 g, 1.56 mmol) in aqueous K_2HPO_4 (1.14 M, 10 ml). The mixture was stirred at this temperature for 12 h. The organic phase was separated, washed with aqueous NaH_2PO_4 (1 M, 2 × 10 ml, pH 1.5), stirred with aqueous NaOH (0.5 M, 10 ml) for 2 h, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue (967 mg, 88%) was crystallized from dichloromethane/toluene to give the desired product (**1b**) (849 mg, 78%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.1 (s, 1H), 8.6 (d, $J = 4.6$ Hz, 1H), 8.0 (d, $J = 8.2$ Hz, 2H), 7.9 (m, 2H), 7.5 (d, $J = 9.1$ Hz, 1H), 7.4 (d, $J = 8.2$ Hz, 2H), 7.3 (m, 1H), 7.2 (s, 4H), 7.1 (m, 1H), 6.9 (d, $J = 9.3$ Hz, 1H), 6.8 (d, $J = 9.3$ Hz, 1H), 4.04 (m, 1H), 3.9 (m, 2H), 3.8 (d, $J = 9.3$ Hz, 1H), 3.6 (m, 2H), 3.5 (s, 3H), 2.7 (m, 4H), 0.8 (s, 9H), 0.6 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 170, 156.4, 156, 149.4, 138.9, 138.6, 137.4, 137.1, 129, 128.7, 128.2, 127.9, 126.1, 125.8, 122.3, 119.9, 68.1, 63, 61.2, 60.8, 51.6, 51.4, 37.7, 33.5, 33.3, 26.6, 26.2; ESIMS *m/z* 708.5 ([M + H$^+$], $C_{38}H_{50}D_3N_6O_7$ requires 708.41).

Conclusion

A versatile advanced chiral non-racemic building block **4** was synthesized in 4 steps from the readily accessible hydrazine derivative **8**. The structure assignment was established by the conversion into the free base of Reyataz™. The synthetic utility of this chiral building block was illustrated in the high yielding one step conversion into d_3-labelled Reyataz™, which was a valuable internal standard for an assay designed to quantify the plasma concentration of Reyataz™ taken from subjects in clinical studies.

Appendix A

HPCL of d_3-labelled Reyataz™ (**1b**) is given in Figure A1.

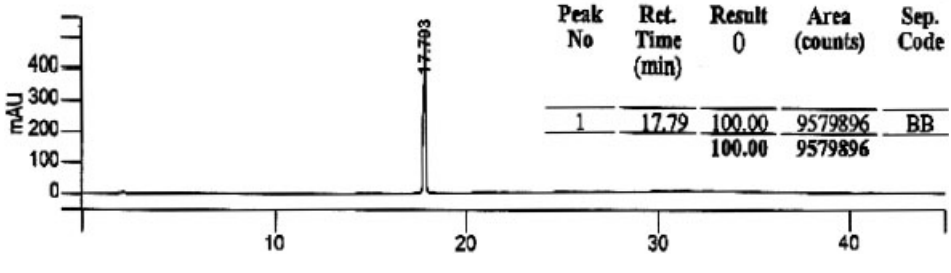


Figure A1. HPLC of d_3-labelled Reyataz™ (1b)

Method: Column: XTERRA C_{18}MS column, 3.5 μm (4.6 × 150 mm).

Mobile phase A: 90% water, 10% CH_3CN, 0.075% TFA.

Mobile phase B: 10% water, 90% CH_3CN, 0.075% TFA.

Program: Isocratic (100% A) 10 min, Gradient (100% B) 10 min. Isocratic (100% B) 10 min, Gradient (100% A) 5 min, flow rate: 1 ml/min, injection size 10 μl.

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