

Research Article

A facile and efficient synthesis of d₃-labelled ReyatazTM

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Summary

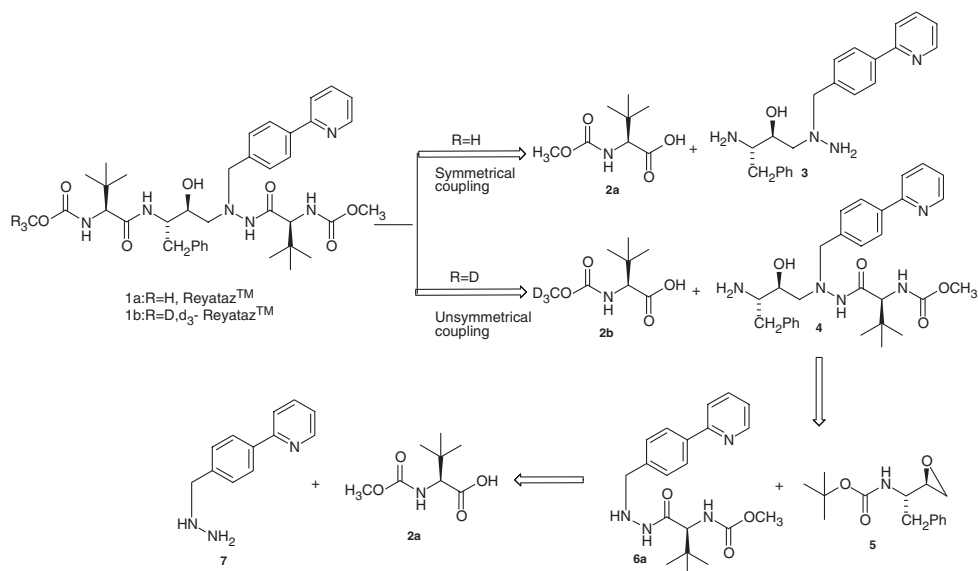
A facile and efficient synthesis of d₃-labelled ReyatazTM is described. The key step of synthesis involved the coupling of *N*-(d₃-methoxycarbonyl)-*L*-*tert*-leucine **2b** with an advanced chiral non-racemic building block **4**. The latter was synthesized in 4 steps from the readily accessible hydrazine derivative **8**. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: ReyatazTM; HIV protease inhibitor; d₃-labelled; chiral non-racemic

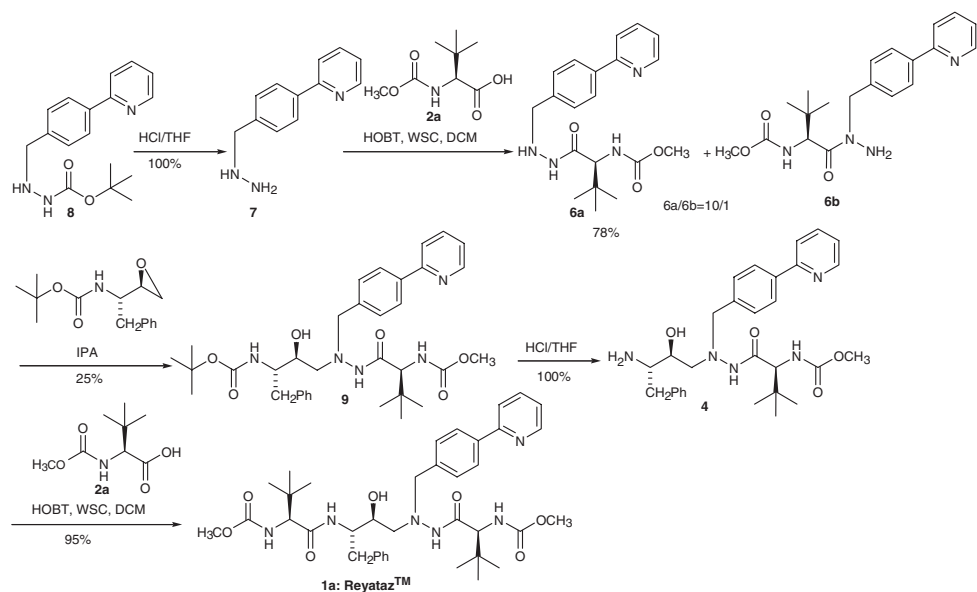
Introduction

ReyatazTM (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 ReyatazTM in humans, d₃-labelled ReyatazTM is required because its physical and chromatographic properties are identical to the unlabelled compound. Several efficient synthetic routes leading to ReyatazTM have been published in the literature.^{2,3} Several of these use the symmetrical coupling of 2 equivalents of *N*-methoxycarbonyl-*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*-(d₃-methoxycarbonyl)-*L*-*tert*-leucine **2b** with a key chiral non-racemic intermediate **4** would give d₃-labelled ReyatazTM (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 d₃-ReyatazTM using this unsymmetrical coupling approach.

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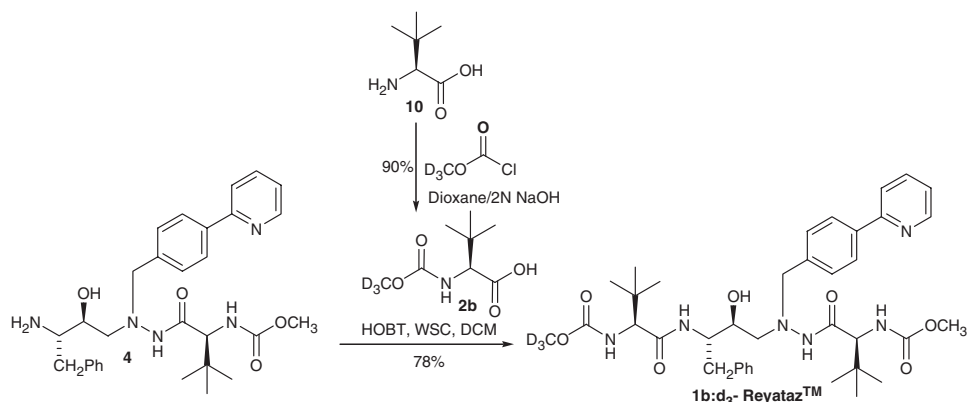
Scheme 1. Retrosynthesis of Reyataz™ and d₃-labelled Reyataz™



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 d₃-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 d₃-methyl chloroformate to give compound **2b** in 90% yield.⁴ Coupling of compound **4** directly with d₃-methyl chloroformate **2b** in the presence of WSC and HOBT gave d₃-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. d₃-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 ^1H 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. ^1H NMR (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}C NMR (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 $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_3$: 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-butyl)-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. ¹H NMR (CDCl₃): 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). ¹³C NMR (CDCl₃): 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 C₃₅H₄₇N₅O₆ · 0.39 H₂O: 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%). ¹H NMR (DMSO-d₆): 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⁺], C₃₀H₃₉N₅O₄ requires 533.3).

*Preparation of N-(d₃-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 d₃-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 Na₂SO₄, 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. ¹H NMR (300 MHz, CDCl₃): δ 9.9 (s, 1H), 5.2 (bs, 1H), 4.1 (bs, 1H), 0.9 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 176.5, 156.9, 62.1, 34.5, 26.4; ESIMS m/z 193.1 ([M + H⁺], C₈H₁₃D₃NO₄ requires 193.12).

Preparation of d_3 -labelled ReyatazTM (**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. 1H 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 ReyatazTM. The synthetic utility of this chiral building block was illustrated in the high yielding one step conversion into d_3 -labelled ReyatazTM, which was a valuable internal standard for an assay designed to quantify the plasma concentration of ReyatazTM taken from subjects in clinical studies.

Appendix A

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

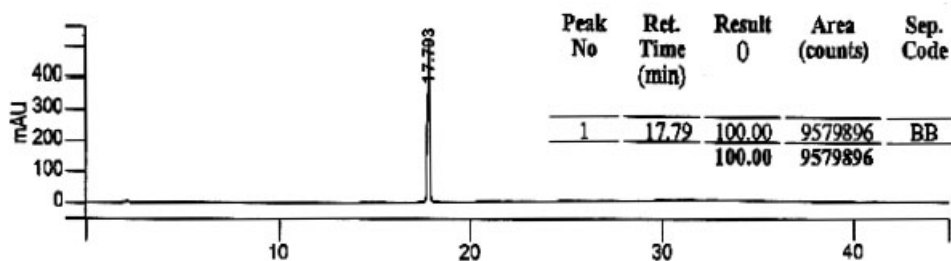


Figure A1. HPLC of d_3 -labelled ReyatazTM (**1b**)

Method: Column: XTERRA C₁₈MS column, 3.5 µm (4.6 × 150 mm).

Mobile phase A: 90% water, 10% CH₃CN, 0.075% TFA.

Mobile phase B: 10% water, 90% CH₃CN, 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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