·论 文·

Improved synthesis of raltegravir

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Abstract Aim: To develop a practical synthetic route of raltegravir, a drug for HIV treatment. Methods: Raltegravir was synthesized through an eight-step process including aminonitrile formation, protection with benzyloxycarbonyl group, conversion of the nitrile to the amidoxime, cyclization to form hydroxypyrimidinone, *N*-methylation, amidation with microwave-assistance, deprotection, amidation with acyl chloride. Results: The overall yield of the eight-step synthesis is about 12.0% and the structure of the target compound was confirmed by ^{1}H NMR, ^{13}C NMR, LR-MS and HR-MS. Conclusion: The reported synthetic process of raltegravir highlights the advantages in terms of readily available starting materials, convenient operation and low cost.

Key words HIV-1 integrase; raltegravir; synthesis; process research

CLC Number TQ460.31 Document code A Article ID 1000 - 5048(2009)04 - 0297 - 05

Raltegravir的合成工艺研究

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摘 要 **目的**:研究治疗获得性免疫缺陷综合症药物 raltegravir的合成工艺。**方法**:经过成氨基腈,苄氧羰基保护氨基,从腈成氨肟,环合成二羟基嘧啶,氮甲基化,微波催化成酰胺,脱保护,再经酰化缩合成酰胺等 8步反应制备 raltegravir。**结果**:经过对合成工艺的改进得到了目标产物,其结构经 ^{1}H NMR, ^{13}C NMR, LR-MS和 HR-MS确证,总收率为 12.0%。**结论**:此方法原料易得、操作简单、成本低。

关键词 HIV-1整合酶; raltegravir;合成;工艺研究

Acquired immunodeficiency syndrome (AIDS) is one of the greatest challenges to humankind. AIDS and HIV infection represent global health hazards, and complex scientific puzzles, thus becoming obvious targets for drug discovery and vaccination, with enormous social, economical and ethical ramifications[1]. The UN and WHO currently estimate that 33.2 million people are living with HIV/AIDS worldwide and approximately 2.1 million people died from AIDS in 2007[2]. Combination therapy using reverse transcriptase and protease inhibitors has been highly successful, but the emergence of drug resistance prompts a search for alternative treatments. A rapidly expanding area of HIV/AIDS research targets a third enzyme, integrase, the catalyst responsible for the integration of proviral DNA into the host cell chromosome[3-4]. In the past 15 years, a range of natural and synthetic compounds have been identified as inhibitors of recombinant integrase enzyme in biochemical assays, and some of them have been developed and are

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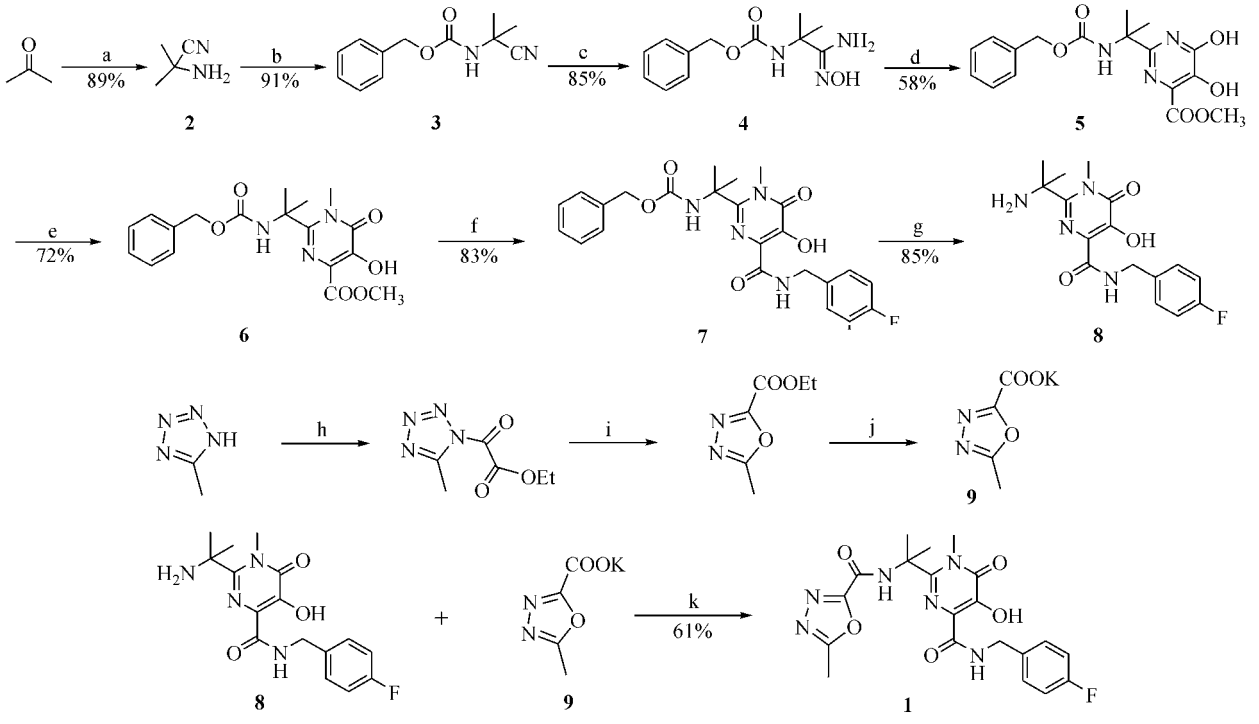
Foundation Item The research work was supported by the National High Technology Research and Development Program of China (863 Program) (No. 2006AA020602)

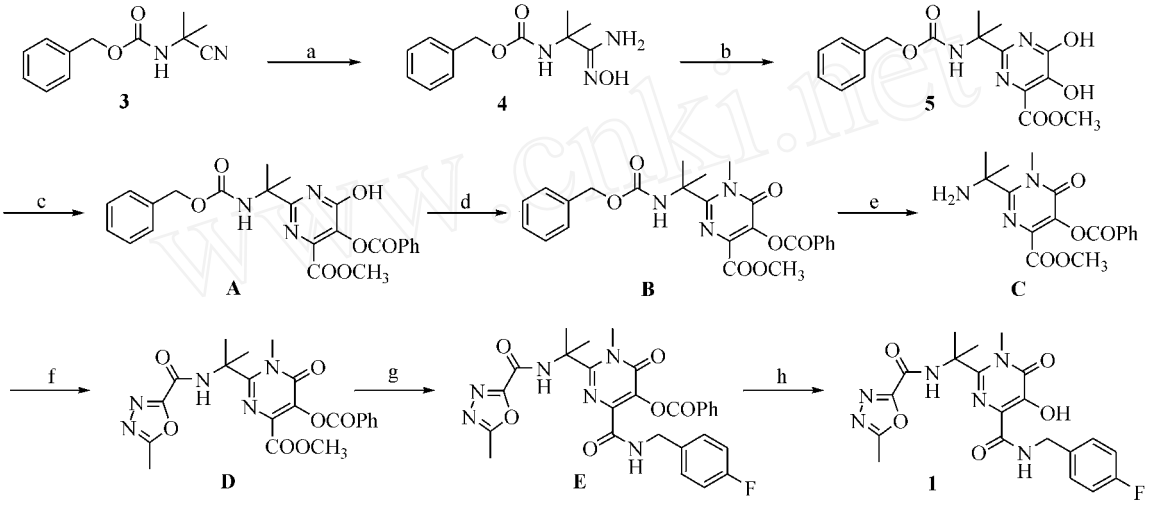
in the phase of clinical trials. Research at Merck & Co., Inc. has led to the identification of raltegravir potassium[5], a well-tolerated HIV-1 integrase inhibitor that targets strand transfer, the second of two catalytic cycles mediated by the integrase enzyme[6]. In this paper, we detailed the exploration and improvement of synthetic route for the preparation of raltegravir (MK-0518, Isentress), the first HIV-integrase inhibitor approved by FDA for the treatment of HIV infection.

The synthetic routes of raltegravir were reported by Merck & Co., Inc., which are respectively viable for use in laboratory research[7] and larger industrial scales[8]. After a thorough analysis of the two synthetic routes, we reported an eight-step synthetic route for raltegravir preparation utilizing successively improved and optimized modifications of the synthesis, including aminonitrile formation, addition of benzyloxycarbonyl protective group, conversion of nitrile to amidoxime, cyclization to hydroxypyrimidinone, *N*-methylation, amidation with microwave-assistant, deprotection, amidation with acyl chloride. The synthetic route is outlined in Scheme 1. In general, intermediate **2** could be prepared by reacting with ammonia, NH_4Cl, acetone and NaCN at room temperature. Treatment of intermediate **2** with benzylchloroformate in THF and $NaHCO_3$ saturated aqueous solution gave **3**, which could be converted into amidoxime **4** by reacting with hydroxylamine aqueous solution. Amidoxime **4** was reacted with dimethylacetylene dicarboxylate (DMAD) to provide a mixture. The mixture was not isolated and heated at 90 ℃ for 2 h and 120 ℃ for 2 h, and then refluxed in xylene for 12 h under nitrogen to yield the desired compound **5**. Using this method, the side-product imidazole[9] could be reduced and the yield of the product could be improved. Compound **6** could be formed using iodomethane with the presence of $Mg(OMe)_2$ in DMSO. With microwave-assistant, compound **7** could be prepared by reacting with 4-fluorobenzylamine in ethanol within 1 h at 90 ℃. Hydrogenation of compound **7** with Pd/C as a catalyst gave compound **8**, which was then converted into the target compound raltegravir (**1**) by reacting with the acyl chloride prepared by potassium salt (**9**) and methanesulfonyl chloride with 4-dimethylaminopyridine as a catalyst. The total yield is 12.0%.



Scheme 1 Reagents and conditions: (a) NaCN, NH_4Cl, ammonia (30%), H_2O, r. t.; (b) Cbz-Cl, THF, $NaHCO_3$; (c) NH_2OH, IPA; (d) 1: DMAD, MeOH, r. t.; 2: xylene, refluxed; (e) MeI, $Mg(OMe)_2$, DMSO; (f) 4-fluorobenzylamine, EtOH, microwave 90 ℃; (g) H_2, Pd/C, MeOH, r. t.; (h) ClCOCOOEt, TEA, toluene; (i) heated, loss of N_2; (j) KOH, EtOH, H_2O; (k) MsCl, DMAP, CH_2Cl_2

The synthetic route of raltegravir reported by the Merck Research Laboratories[7] to be used in laboratory research is outlined in Scheme 2. There are six steps from intermediate **5** to the target compound raltegravir(**1**), but through our synthetic route, raltegravir (**1**) could be obtained from intermediate **5** with four-step reactions. Furthermore, amidoxime **4** was reacted with DMAD and the reaction was refluxed overnight, after being evaporated, taken into xylene and heated at 145 ℃ for 48 h to get intermediate **5**. The reaction time was long but the yield was only 41%. Benzoylation of 5-hydroxyl and the subsequent methylation afforded compound **B**, hydrogenation and amidation with acyl chloride, amidation with 4-fluorobenzylamine refluxed for 14 h to get compound **E**, and finally deprotection to give raltegravir(**1**). Compared with this synthetic route, our synthetic process highlights some advantages. The starting materials are commercially available. The side-products could be reduced and the yield of the product could be improved in the reaction of amidoxime **4** being converted into intermediate **5**, and the reaction time could be reduced to 24 h. Amidation with 4-fluorobenzylamine under microwave irradiation could shorten the reaction time with convenient operation.



Scheme 2 Reagents and conditions: (a) $NH_2OH\cdot HCl$, KOH, MeOH; (b) i: DMAD, $CHCl_3$, 60 ℃; ii: xylene, 150 ℃; (c) Bz_2O, pyridine, rt; (d) Me_2SO_4, LiH, dioxane, 60 ℃; (e) H_2, Pd/C, MeOH, rt; (f) RCOCl, Et_3N, CH_2Cl_2; (g) 4-Fluorobenzylamine, MeOH, refluxed; (h) 6 mol/L HCl

In summary, an economical and practical synthetic route of raltegravir was developed with such advantages as readily available starting materials, convenient operation and low cost.

The reagents (chemicals) were purchased from commercial sources (Alfa, Acros, Sigma Aldrich and Shanghai Chemical Reagent Company), and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15- 0.2 mm thickness, Yantai Huiyou Company, China). The microwave reactor, Chempower 18920570, was manufactured by Shanghai Chubo Instrument Co., Ltd. Column chromatography was performed with CombiFlash Companion system (Teledyne Isco, Inc.). The products were characterized by their NMR and MS. ^1H and ^{13}C NMR spectra were obtained on Varian Mercury-300 and Varian Mercury-400 spectrometers (TMS as IS). Low- and high-resolution mass spectra (LR-MS and HR-MS) were measured on Finnigan Mat 95 and LCQ-DE-CA mass spectrometer.

2.1 *2-Amino-2-methylpropanenitrile* (**2**)

To a stirred solution of NH_4Cl (9.6 g, 0.18 mol), acetone (10.2 g, 13 mL, 0.17 mol) and ammonia (30% aq, 10 mL) in 20 mL H_2O, NaCN (8.6 g, 0.17 mol) was added at 0 ℃. The mixture was stirred at room temperature for 60 h, extracted with dichloromethane. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the white oil (12.8 g, 89%): LC/MS *m/z* 85 $(M+H)^+$.

2.2 *Benzyl 1-cyano-1-methylethylcarbamate* (**3**)

A mixture of 2-amino-2-methylpropanenitrile (**2**,

8.4 g, 0.1 mol), 160 mL THF and 310 mL $NaHCO_3$ saturated aqueous solution, was stirred at 0 ℃ for 15 min. Benzylchloroformate (17 mL, 0.12 mol) was added dropwise, and then the reaction mixture was stirred for 18 h at room temperature, adjusted to pH 1 with 1 mol/L HCl and extracted with EtOAc. The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the white solid (19.8 g, 91 %): LC/MS *m/z* 219 $(M+H)^+$.

2.3 *Benzyl 2-amino-2-(hydroxyimino)-1, 1-dimethylethylcarbamate* (**4**)

The above resulting product (**3**, 19.8 g, 91 mmol) was dissolved in 40 mL isopropanol, then 6.3 mL 50% hydroxylamine aqueous solution was added dropwise, the mixture was stirred for 3 h at 60 ℃. 50 mL *n*-heptane was added dropwise. The resulting mixture was stirred for 2 h at 0 ℃, filtered. The cake was washed with isopropanol and *n*-heptane, dried to afford the white solid (19.3 g, 85 %): ^{1}H NMR (CD_3OD) δ: 7.34 (m, 5H), 5.04 (s, 2H), 1.47 (s, 6H); LC/MS *m/z* 252 $(M+H)^+$.

2.4 *Methyl 2-(1-{[(benzyloxy) carbonyl]amino}-1-methylethyl)-5, 6-dihydroxy pyrimidine-4-carboxylate* (**5**)

Benzyl 2-amino-2-(hydroxyimino)-1, 1-dimethylethylcarbamate (2.0 g, 8.0 mmol) was dissolved in 30 mL MeOH, dimethyl acetylenedicarboxylate (DMAD, 1.1 mL, 9.0 mmol) was added slowly. The mixture was stirred for 2 h at room temperature, and then concentrated under reduced pressure to get white oil. The mixture of the oil and 80 mL xylene was stirred at 90 ℃ for 2 h and 120 ℃ for 2 h, and then refluxed for 12 h under nitrogen, concentrated to yield the crude product. The crude product was recrystallized with 2 mL MeOH and 10 mL *tert*-butyl methyl ether to afford the yellow solid (1.7 g, 58%): ^{1}H NMR (DMSO-d_6) δ: 7.35 (m, 5H), 5.00 (s, 2H), 3.83 (s, 3H), 1.48 (s, 6H); ESI-MS *m/z* 360 $(M)^-$, 384 $(M+Na)^+$.

2.5 *Methyl 2-(2-(benzyloxycarbonyl) propan-2-yl)-5-hydroxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carboxylate* (**6**)

The above resulting product (**5**, 1.1 g, 3.0 mmol) was dissolved in 10 mL anhydrous DMSO, and 11 mL $Mg(OMe)_2$ (7%-8% in MeOH) was added, then the mixture was evaporated under reduced pressure to remove MeOH. Iodomethane (MeI, 4.8 mL) was added, then the tube was sealed under nitrogen. The mixture was stirred for 2 h at room temperature, and then stirred in 70 ℃ oil-bath overnight, extracted with EtOAc. The organic phase was washed with $Na_2S_2O_3$ saturated aqueous solution, dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude product. Then the crude product was recrystallized with EtOH and *tert*-butyl methyl ether to afford the white solid (0.82 g, 72 %): ^{1}H NMR (CD_3OD) δ: 7.32 (s, 5H), 5.01 (s, 2H), 3.99 (s, 3H), 3.67 (s, 3H), 1.66 (s, 6H); LC/MS *m/z* 376 $(M+H)^+$.

2.6 *Benzyl 1-(4-{[(4-fluorobenzyl) amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1, 6-dihydropyrimidin-2-yl)-1-methylethylcarbamate* (**7**)

The above resulting product (**6**, 750 mg, 2.0 mmol) and 0.8 mL 4-fluorobenzylamine were dissolved in 15 mL EtOH. The vial was sealed and the mixture was irradiated for 50 min at 90 ℃ in the microwave reactor. The resulting mixture was concentrated under reduced pressure, extracted with EtOAc. The organic phase was washed with NH_4Cl saturated aqueous solution and brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude product. The desired product (780 mg, 83 %) was obtained after trituration with EtOAc and ethyl ether. ^{1}H NMR (CD_3OD) δ: 7.38 (m, 2H), 7.31 (s, 5H), 7.06 (m, 2H), 5.00 (s, 2H), 4.56 (s, 3H), 3.58 (s, 3H), 1.66 (s, 6H); LC/MS *m/z* 469 $(M+H)^+$.

2.7 *2-(1-Amino-1-methylethyl)-N-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carboxamide* (**8**)

The above resulting product (**7**, 600 mg, 1.3 mmol) was dissolved in 15 mL MeOH, and 90 mg 10% Pd/C was added. Then the mixture was stirred overnight under a hydrogen atmosphere, filtered through celite, and the filtrate was concentrated. The white solid (360 mg, 85%) was obtained after trituration with EtOAc and ethyl ether. ^{1}H NMR (DMSO-d_6) δ: 7.35 (m, 2H), 7.15 (m, 2H), 4.47 (s, 2H), 3.56 (s, 3H), 1.62 (s, 6H). ESI-MS *m/z* 333 $(M)^-$, 335 $(M+H)^+$.

2.8 *5-Methyl-1, 3, 4-oxadiazole-2-carboxylate potassium salt* (**9**)

3.3 mL ethyl oxalyl chloride was added dropwise to the mixture of 2.4 g 5-methyl-1*H*-tetrazole, 4.0 mL triethylamine and 50 mL toluene at 0 ℃. The mixture

was stirred at this temperature for 1 h, and filtered. The filtrate was added to 50 mL toluene. The resulting mixture was stirred for 1 h at 65 ℃ to release N_2, concentrated under reduced pressure to remove most of toluene. 40 mL EtOH and 8 mL 20 % KOH aqueous solution were added dropwise at 0 ℃. The resulting slurry was stirred for 30 min at room temperature, and filtered. The cake was washed with EtOH and *tert*-butyl methyl ether, and dried to afford the white solid (4.3 g, 91%).

2.9 *N-[(4-fluorophenyl) methyl]-1, 6-dihydro-5-hydroxy-1-methyl-2-[1-methyl-1-[[(5-methyl-1, 3, 4-oxadiazol-2-yl) carbonyl] amino] ethyl]-6-oxo-4-pyrimidine carboxamide* (**1**)

A mixture of potassium salt (**9**, 120 mg), 20 mL anhydrous dichloromethane and 4-dimethylaminopyridine (DMAP, 200 mg) was stirred for 10 min, and then methanesulfonyl chloride (MsCl, 150 mL) was added slowly at 0 ℃. Intermediate **8** (120 mg) was added, and then the mixture was stirred overnight at room temperature, extracted with dichloromethane. The organic layer was washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (dichloromethane/methanol = 20∶1) to yield the expected product (98 mg, 61%): ^{1}H NMR (CD_3OD) δ: 7.40 (m, 2H), 7.04 (m, 2H), 4.56 (s, 2H), 3.46 (s, 3H), 2.65 (s, 3H), 1.83 (s, 6H); ^{13}C NMR (CD_3OD) δ: 168.4, 164.8, 163.2, 162.0, 161.9, 160.1, 155.3, 145.8, 136.0, 134.9, 131.0, 116.7, 116.6, 60.2, 43.8, 41.3, 34.8, 27.6, 11.4; ESI-MS *m/z* 443 $(M)^-$; LR-MS (EI) *m/z* 444 $(M)^+$; HR-MS (EI) *m/z* $C_{20}H_{21}FN_6O_5$ $(M)^+$ calcd. 444.1557, found 444.1542.

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