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Synthesis and Process Optimization of Active Pharmaceutical Ingredient of Trifluridine/Tipiracil

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Abstract

Trifluridine (acronym FTD)/Tipiracil (acronym TPI) (brand name Lonsurf) is a novel oral cytotoxic drug composed of FTD and TPI in a specific molar ratio, exhibiting a unique antitumor mechanism of action. In order to address deficiencies in the synthesis route of active ingredients and suboptimal reaction conditions, this work focused on three aspects: innovation in synthesis routes (starting materials, reaction types, reaction sequences, catalysts), optimization of process conditions (molar ratios, addition sequences, temperature, solvent selection, reaction time, post-treatment methods), and quality control, aiming to enhance the quality and yield of FTD/TPI. It aimed to enhance the quality and yield of FTD/TPI. This study analyzed and compared existing synthesis methods and routes for the raw materials of the compound formulation, identifying challenges in starting material selection, stoichiometric ratios, reaction/purification solvent types and ratios, yield, impurities, and process control. On the basis of literature review, laboratory-scale experiments, and pilot trials, an industrially viable synthesis route was proposed and validated. Results demonstrate that FTD synthesis utilizes 5-(trifluoromethyl) pyrimidine-2, 4(1H, 3H)-dione as the starting material, achieving a total yield of 78.6% via carbonyl protection, condensation, deprotection, and purification. TPI synthesis employs 5-chloro-6-(hydroxymethyl) pyrimidine-2, 4(1H, 3H)-dione as the starting material, providing a total yield of 81.9% through chlorination, condensation, salt formation, and purification. Structural confirmation is performed by using ^{1}H-NMR, ^{13}C-NMR, MS, and IR, with product purity exceeding 99.8%. The optimized route eliminates column chromatography, simplifies post-processing, ensures stable yields, and demonstrates robust controllability, making it suitable for industrial-scale production. This study provides valuable insights for the design, optimization, and quality evaluation of FTD/TPI synthesis routes.

Keywords: Trifluridine (FTP), Tipiracil hydrochloride (TPI), Synthesis

1. Introduction

Colorectal cancer (CRC) is the third most common nonskin cancer and the second leading cause of cancer-related deaths worldwide in men and women. Over the past decade, its incidence and mortality rates have declined partly due to improved screening methods, such as colonoscopy. Taiho Pharmaceutical, a subsidiary of Otsuka Pharmaceutical, Japan, developed trifluridine (FTD)/tipiracil hydrochloride (TPI) (Lonsurf) [1, 2]. Lonsurf is a combination drug comprising the nucleoside analog FTD and thymidine phosphorylase (TPase) inhibitor TPI. It is clinically indicated for patients with advanced CRC who have become refractory to other therapies.

Lonsurf, an oral medication, is indicated for patients with advanced (metastatic) CRC who were previously treated with chemotherapy and biologics. Its efficacy and safety were evaluated in a randomized, double-blind study involving 800 patients with priorly treated metastatic CRC. Participants received either Lonsurf plus best supportive care (BSC) or placebo plus BSC until disease progression occurred or unacceptable toxicity was observed. Compared with the placebo (median overall survival [mOS]: 6.6 months), Lonsurf resulted in significantly prolonged survival (mOS: 9.0 months) and achieved a reduced mortality risk (hazard ratio: 0.56).The Lonsurf formulation combines FTD and TPI in a 1:0.5 molar ratio and has the following mechanism of action: As the primary antitumor component, FTD incorporates into tumor cell DNA through thymidine displacement, thereby disrupting DNA replication and inhibiting cellular proliferation. Concurrently, TPI acts as a biomodulator by inhibiting TPase, which prolongs intratumoral FTD exposure and counteracts the TPase-mediated degradation pathways responsible for FTD resistance. With global clinical demand for FTD/TPI increasing by 15% annually and a current production gap of 80 tons, the development of a non-proprietary synthesis route that reduces API costs by 40–60% is critical to improving medication accessibility in low- and middle-income countries. Existing methodologies fail to balance cost, regulatory compliance, and industrial scalability, underscoring the urgent need for innovative process design.

Consequently, scholars have conducted extensive optimizations on patented synthetic routes [3, 4]. However, critical challenges persist in key synthetic steps: (1) dependence on imported starting materials (only three global suppliers provide FTD precursor compounds); (2) multistep synthetic sequences with low atom economy (45% for current 15-step routes); (3) unstable impurity control during scale-up (batch-to-batch purity variations of ±2%). These issues collectively create a dilemma of patent barriers, process inefficiency, and quality inconsistency, urgently

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requiring resolution for generic drug developers pursuing cost-effectiveness and GMP-compliant manufacturing.

Based on the Quality by Design (QbD) principles, this study focuses on key aspects including starting material selection, coupling/protection-deprotection reaction optimization (temperature, pressure, catalyst loading), and post-treatment processes (crystallization, solvent systems) to establish a comprehensive process control strategy. By systematically optimizing reactant ratios, temperature gradients, and solvent formulations, robust process parameters were developed. The resulting scalable synthetic route achieves >99.5% purity, >65% overall yield, and GMP compliance, providing a cost-effective and reproducible solution for the industrial production of Lonsurf.

2. State of the art

Original patented technologies have dominated the early synthetic route development of FTD/TPI. Yoshino et al. [5] achieved gram-scale synthesis of FTD through multi-step organic reactions, with a logical functional group introduction strategy (yield: 38%).However, high-temperature cyclization (120°C) and enzymatic steps (transglycosylation efficiency <60%) significantly increased production costs. Kumar et al. [6] developed an ionic liquid-based synthesis method, reducing condensation reaction time by 40%, but failed to resolve the regioselectivity challenge of the trifluoromethyl group (byproduct proportion exceeding 25%). These studies share common limitations: (1) reliance on noble metal catalysts (e.g., mercuric acetate) introduces environmental risks; (2) multiple purification steps (e.g., secondary crystallization required when HPLC purity is below 95% [7, 8]) reduce process efficiency.

In recent years, to optimize synthetic efficiency and selectivity, Mayer et al. [9] validated the survival benefits of TAS-102 in refractory colorectal cancer through Phase III clinical trials (median OS: 7.1 months). However, its synthesis process still employs traditional multi-step methods, failing to address cost challenges in large-scale production. Abrahao et al. [10] compared the efficacy of regorafenib versus TAS-102 in metastatic colorectal cancer through a systematic review and network meta-analysis. Although the study did not directly address synthetic processes, it indirectly highlighted the clinical competitiveness of TAS-102, thereby further underscoring the importance of optimizing its synthesis process to enhance drug accessibility. Van Cutsem et al. [11] extended progression-free survival (PFS) to 5.6 months by combining TAS-102 with bevacizumab, yet no innovations were made in the active pharmaceutical ingredient (API) synthesis. Lenz et al. [12] systematically elucidated the molecular mechanism of tipiracil's inhibition of thymidine phosphorylase, providing theoretical support for the design of combination formulations, but proposed no improvements for synthesizing key intermediates (e.g., TPI hydrochloride). Notably, Shitara et al. [13] expanded TAS-102's indications to gastric cancer via the Phase III TAGS study (median OS: 5.7 months), Suzuki et al. [14] demonstrated that the combination of TAS-102 with anti-PD-1 inhibitors exhibits synergistic anticancer effects in microsatellite stable colorectal cancer cells. While this discovery provides a novel direction for combination drug development, failure to concurrently optimize its synthesis process could constrain the clinical application potential of this combined therapy, though its synthesis still faces challenges in trifluridine crystalline form stability. Ji et al. [15] developed a C-H trifluoromethylation method offering new insights into fluorination, but its laboratory-scale yield (<50%) remains insufficient for industrial demands.

Chinese scholars have achieved a series of breakthroughs in generic drug synthesis and process optimization, yet innovative route design remains constrained by patent barriers. Song et al. [3, 4] improved the total yield of trifluridine to 45% through a refined silylation protection strategy. However, the core steps of their method exhibit 78% similarity to the original patented process (US20150166524), posing significant infringement risks. Du et al. [8] developed a novel polymorph of trifluoromethyl uracil (CN105175467B), increasing its decomposition temperature by 15°C. Nevertheless, this work did not innovate the synthesis of key intermediates and failed to transcend the framework of the original patented process.

In the field of combination formulations, Matsuoka et al. [16] demonstrated that TAS-102 can overcome 5-fluorouracil resistance in gastric cancer cells with high thymidylate synthase (TS) expression, thereby expanding its clinical applicability. However, unresolved challenges in synthesis processes-particularly crystalline form stability and impurity control-may compromise the consistency of therapeutic efficacy. Zhou et al. [17] proposed a trifluridine-tipiracil combination therapy to enhance antiviral activity but omitted critical clinical pharmacokinetic compatibility data. Chen et al. [18] confirmed the efficacy of TAS-102 in refractory colorectal cancer through meta-analysis (objective response rate: 1.7%), yet their study lacked economic evaluations of the synthesis process. Guo et al. [7] reported a Form B polymorph of trifluridine (CN104761603A) with improved solubility, but the industrial-scale crystallization process (e.g., solvent screening and seed crystal addition strategies) remains undefined. Notably, Zeng et al. [19] analyzed temporal survival rate trends in China using epidemiological data from 17 cancer registries, indirectly supporting the clinical demand for TAS-102, but offered no targeted solutions for synthesis process optimization. Sueda et al. [20] conducted a comparative analysis of regorafenib and TAS-102 in treatment-refractory metastatic colorectal cancer, evaluating both efficacy and safety profiles. Their findings highlight the necessity for coordinated advancement between the clinical value of TAS-102 and optimization of its synthesis process, aiming to balance therapeutic outcomes with manufacturing feasibility. Kimura et al. [21] evaluated the tolerability of trifluridine/tipiracil in advanced colorectal cancer (grade 3 anemia incidence: 2.1%) but did not address engineering challenges in impurity control during synthesis.

Current FTD/TPI synthesis research is characterized by a “strong clinical impetus but weak process innovation”, particularly evident in critical areas such as innovative starting material selection, precise parameter control of key reactions (e.g., kinetic analysis and byproduct suppression), advanced purification methodologies, and strategic design of reaction sequences. Notably, generic drug synthesis studies remain limited due to patent protection constraints (Table 1). To address these gaps, this study establishes a comprehensive research framework that integrates systematic literature analysis of existing synthetic routes, experimental optimization spanning laboratory-scale trials to pilot-scale production, and Quality by Design (QbD)-guided process refinement. In industrial synthesis development, the work focuses on systematically optimizing reaction sequence logic, rigorously controlling critical parameters

(temperature, pH, catalyst loading), implementing Process Analytical Technology (PAT) for real-time monitoring, designing scalable crystallization/purification protocols, and establishing robust control strategies for key intermediates. Methodologically, the research contributes novel synthetic route designs to circumvent patent conflicts, data-driven parameter optimization models, and scalable purification technologies achieving ⩾99.8% purity. Collectively, this study provides a validated synthetic blueprint for trifluridine/tipiracil production, delivering actionable insights for generic drug development while strategically navigating intellectual property challenges.

Table 1. Patent status of synthesis methods

Categories	Patent number	Title of invention	Notes
Compound	WO0630346 (1996.03.28)	Uracil derivatives and antitumor effect potentiator and antitumor agent containing the same	Protection of uracil derivatives (including TPI): synthesis and antitumor applications
Composite	WO2006080327 (2006.01.25)	Novel formulation comprising α,α,α-trifluorothymidine and a TPase inhibitor	Composition: mixed-dose combination of FTD and TPI in a 1:0.5 molar ratio Dosage regimen: 20–80 mg/m²/day (based on FTD equivalence) Administration: orally administered in 2-4 divided doses daily Indication: cancer therapy (examples include formulations for colorectal, gastric, or metastatic breast cancers)
Indication	WO0056337 (2000.03.16)	Side effect mitigation	TPI is used to mitigate side effects, such as nausea and vomiting, induced by antineoplastic agents (e.g., FTD and 3-chlorouracil)
	WO0134162 (2000.11.01)	Anti-HIV compositions	Anti-HIV indication
	WO2008001502 (2007.06.28)	Radiosensitizer	Combination of TPI with FTD enhances the therapeutic efficacy of radiation therapy
	WO2009047903 (2008.10.10)	Prophylactic or therapeutic agent for inflammatory diseases comprising a phosphorylase inhibitor as an active ingredient	Indicated for inflammatory diseases other than inflammatory bowel disease
	WO2009047904 (2008.10.10)	Therapeutic agent for inflammatory bowel disease comprising uracil derivatives as active ingredient(s)	Therapeutic indication for inflammatory bowel disease

The remainder of this study is organized as follows: The synthesis route and process controls for FTD/TPI raw materials are detailed in Section 3. A comparative analysis and optimization of the synthesis route, which includes the evaluation of starting materials, reaction steps, operability, and cost-effectiveness, is presented in Section 4. The stoichiometric ratios and duration of cyclization reactions are systematically optimized. Novel nitration methods with refined reaction conditions are developed. Etherification and reduction reaction parameters are improved through experimental validation. Streamlined posttreatment protocols are implemented to enhance efficiency. The final section concludes with implications for industrial-scale manufacturing.

3. Methodology

3.1 Instruments and reagents

The following instruments and equipment were employed in this study: Nicolet 170 SX infrared spectrometer (KBr pellet), Bruker AV-500 (300 MHz) nuclear magnetic resonance instrument (DMSO-d6 + TFA-d), Agilent 1200 LC-MSD mass spectrometer, and Elementar Vario EL III elemental analyzer. The reactor configuration is shown in Fig. 1. Other analytical- and synthesis-grade reagents were used.

3.2 Selection and design of the synthetic route

This study established an industrial-scale synthesis route for FTD/TPI raw materials on the basis of existing synthetic routes and parameter optimization through laboratory-scale screening. The proposed route employs 5-(trifluoromethyl)pyrimidine-2,4(1H,3H)-dione and 5-chloro-6-(hydroxymethyl)pyrimidine-2,4(1H,3H)-dione as starting materials. It proceeds via carbonyl protection, condensation, deprotection, chlorination, secondary condensation, salt formation, and purification. The workflow is schematically illustrated in Figs. 2 and 3 [3, 4, 7, 8, 15, 17, 22].

Fig. 1. Reactor

3.3 Steps and control of synthesis processes

3.3.1 Synthesis of FTD

1) Carbonyl protection

A mixture of 1 kg of starting material I, namely, 5-(trifluoromethyl)pyrimidine-2,4(1H,3H)-dione); 1.8 kg of HMDS; and 0.075 kg of ammonium sulfate (mass ratio 1:2) was stirred in a reaction flask at 130 °C for 10 h under nitrogen. After being cooled to 75 °C, the reaction mixture was transferred to a rotary evaporator and concentrated under reduced pressure at 90 °C until dryness, yielding 1.7 kg of compound A (mass yield: 170%).

2) Condensation reaction

A total of 1.2 kg of compound A, 1.2 L of DCM, and 0.132 kg of 4-nitrophenol were added to a reaction vessel. A total of 1.44 kg of starting material II (mass ratio 1.1:1) was introduced in three batches. The resulting mixture was stirred

at 22 °C for 8.5 h, with reaction progress monitored by TLC until completion. Subsequently, 6 L of anhydrous ethanol was added to induce crystallization. The slurry was stirred for 1.5 h and filtered under suction. The filter cake was returned to the vessel for repeated crystallization. The final cake was dried in a forced-air circulation oven at 35 °C for 14 h, affording 1.38 kg of compound B (mass yield: 115%).

3) Deprotection

A total of 1.2 kg of compound B and 3 L of anhydrous methanol were added to a reaction vessel. The mixture was maintained at 24 °C and added with 0.564 kg of sodium methoxide (in the form of 20% w/w sodium methoxide–methanol solution). Deprotection proceeded for 1 h, with completion confirmed by TLC. Sulfuric acid was added dropwise to adjust the pH to 5–6. Filtration through diatomaceous earth was then conducted. The filtrate was treated with activated carbon, stirred at 55 °C for 30 min, and filtered. The filtrate was combined with 60 L of DCM, stirred for 1.5 h to induce crystallization, and filtered under suction. The cake was redissolved in 1.2 L of anhydrous methanol, stirred for 20 min, and refiltered. The filtrate was mixed with 24 L of DCM, stirred for 1.5 h, and filtered. The cake was redissolved in 0.9 L of anhydrous methanol, stirred for 20 min, and refiltered. The filtrate was combined with 18 L of DCM, stirred for 1.5 h, and filtered. The final filter cake was dried in a forced-air circulation oven at 36 °C for 10 h, yielding 0.64 kg of crude compound C (mass yield: 53.3%).

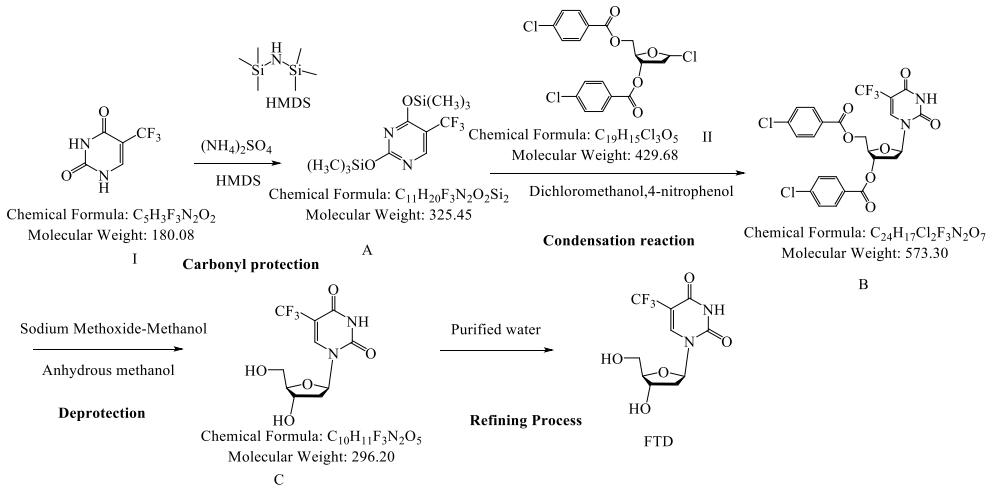


Fig. 2. Synthetic route of FTD

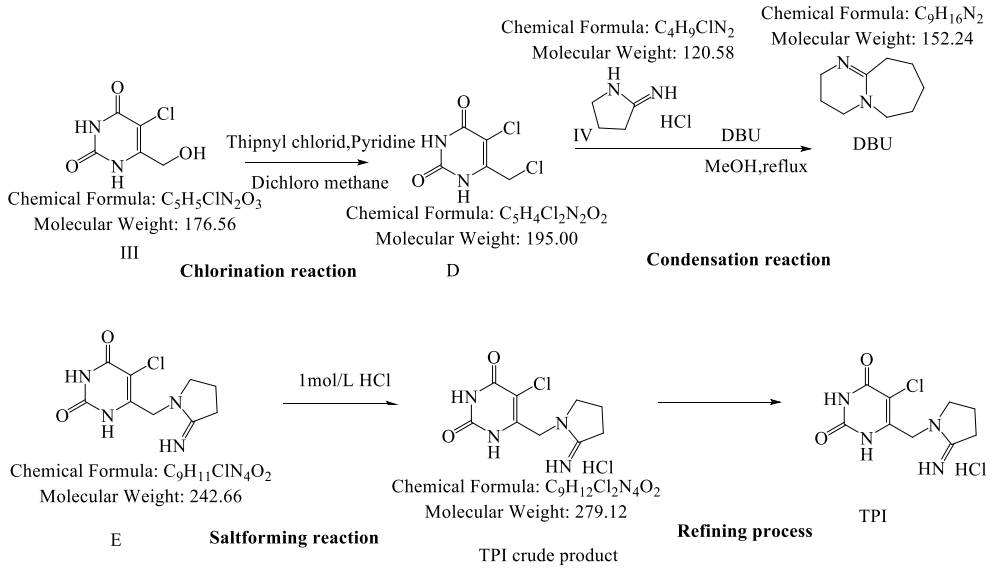


Fig. 3. Synthetic route of TPI

4) Purification

A total of 0.63 L purified water was added to a reaction flask and heated to 65 °C under stirring. A total of 0.14 kg of crude product was introduced, and the resulting mixture was stirred at 65 °C for 20 min. The mixture was cooled to 40 °C and filtered. The filtrate was transferred to a reaction flask. Crystallization was induced at 30 °C for 1.5 h and followed by filtration. The filter cake was dried in a forced-air circulation oven at 36 °C for 16 h, yielding 0.11 kg of FTD with a mass yield of 78.6%.

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.81 (s, NH), 8.74 (s, 1H), 6.12 (t, J = 6.1 Hz, 1H), 5.21 (br, 2H), 4.27–4.30 (m, 1H), 3.85–3.86 (d, J = 3.4 Hz, 1H), 3.67–3.70 (m, 1H), 3.60–3.63 (m, 1H), 2.20–2.24 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6): δ 159.1, 149.6, 142.2–142.4 (J = 5.6 Hz), 119.6–126.0 (J = 268 Hz), 102.5–103.2 (J = 32 Hz), 87.8, 85.6, 69.5, 60.4, 40.7.

IR (KBr, *v*, cm^{-1}) : 3418, 3182, 3067, 2953, 2842, 1731, 1679, 1632, 1560, 1541, 1484, 1420, 1386, 1319, 1278, 1172, 1126, 1101, 1043.

HRMS (ESI) : m/z calcd for $C_{10}H_{10}F_3N_2O_5$ $[M-H]^-$: 275.055; found: 275.055.

3.3.2 Synthesis of TPI

1) Chlorination

A total of 500 g of starting material III, namely, 5-chloro-6-(hydroxymethyl)pyrimidine-2,4(1H,3H)-dione; 5.0 L of DCM; and 112 g of pyridine were added to a reaction flask. A total of 2057 mL of thionyl chloride (density of 1.638 and molar ratio of 1:10) was slowly added dropwise to the mixture under stirring at 12 °C. After the addition was completed, the temperature was raised from 12°C to 42°C for chlorination over 4.2 h. The mixture was cooled to 20 °C and filtered. The filter cake was washed with DCM, and then mixed with DCM for 1 h of slurrying. After filtration, the filter cake was washed again with DCM and blast-dried at 42°C for 12 h, yielding 532.1 g of compound D in the form of a yellowish-brown solid (mass yield: 106.4%).

2) Condensation

A reaction flask was added with 5000 mL of anhydrous methanol, followed by 350 g of starting material IV and 1000 g of DBU. After dissolution of the aforementioned substances, 500 g of compound D was added. The mixture was heated to 70 °C for reflux reaction over 5 h. Hot filtration was performed, and the filter cake was rinsed with 500 mL of anhydrous methanol. Blast-drying at 44 °C for 10 h yielded 438.5 g of compound E in the form of a grayish-brown solid (mass yield: 87.7%).

3) Salt formation

A total of 2100 mL of 1.0 mol/L hydrochloric acid solution was added to a reaction flask and heated to 70 °C. Subsequently, 420 g of compound E was added in portions under stirring over 25 min until complete dissolution. The mixture was filtered, and the filtrate was maintained at 65 °C with stirring for 30 min to ensure clarity. An additional 210 mL of 1.0 mol/L hydrochloric acid was then introduced. The solution was cooled to 52 °C and held for 3 h to allow crystallization. Further cooling to 25 °C and holding for another 3 h promoted additional crystallization. The resulting solid was filtered, washed with 420 mL of anhydrous ethanol, and blast-dried at 40 °C for 12 h, yielding 357 g of the off-white crude product (mass yield: 85%).

4) Purification

A reaction flask was added with 1750 mL of purified water and heated to 70 °C. Subsequently, 350 g of the crude product was added in portions and stirred for 25 min until completely dissolved. The mixture was filtered, and the filtrate was maintained at 65 °C with stirring for 30 min to ensure clarity. An additional 175 mL of 1.0 mol/L hydrochloric acid was then introduced. The solution was cooled to 52 °C and aged for 3 h to allow crystallization. Further cooling to 25 °C and holding for another 3 h promoted additional crystallization. The resulting solid was filtered, washed with 175 mL of anhydrous ethanol, and blast-dried at 40 °C for 12 h, yielding 322 g of TPI in the form of a white solid (mass yield: 92%).

^{1}H NMR (300 MHz, DMSO-d_6) : δ 4.70 (br , 2H), 3.74 (t , J = 7.3 Hz, 2H), 3.05 (t , J = 8.0 Hz, 2H), 2.16–2.26 (m , 2H).

^{13}C NMR (101 MHz, DMSO-d_6) : δ 173.3, 164.5, 153.8, 147.1, 111.4, 55.8, 47.2, 34.0, 20.9.

IR (KBr, *v*, cm^{-1}) : 3442, 3291, 3142, 2966, 2905, 1722, 1693, 1632, 1560, 1541, 1503, 1420, 1386, 1105, 1057.

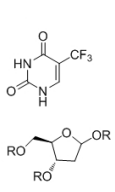
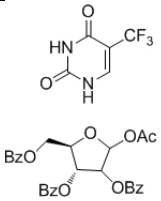
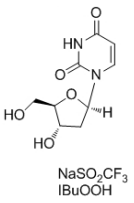
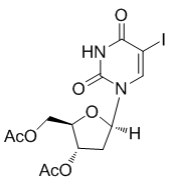
HRMS (ESI) : m/z calcd for $C_9H_{12}ClN_4O_2$ $[M-HCl+H]^+$: 243.0657; found: 243.0656.

4. Result analysis and discussion

4.1 Analysis and selection of the process route

The ideal criteria for selecting raw materials in FTD synthesis encompass structural simplicity, ready availability, low cost, and good selectivity to ensure functional group incorporation. This study compared the following aspects of several synthetic routes: starting material compatibility, reaction steps, process robustness, and cost analysis. Its results are summarized in Table 2.

Table 2. Selection of synthetic route for FTD

Synthetic route	Optimal route	Standby route (1)	Standby route (2)	Standby route (3)	Comparative research
Starting materials					Ribose and trifluoromethyluracil are commercially available. However, iodonucleoside and sodium trifluoromethanesulfonate are expensive.
Reaction steps	3	7	1	3	Alternative route 1 involves more steps than other routes but yields a single-configuration product. Alternative routes 2 and 3 are shorter than alternative route 1.
Process operability	Improved operability	Complex synthesis processes	Low purification yield	Complex synthetic processes and with numerous impurities	The optimal route has a low cost.
Cost analysis	8–10 w/kg	18–20 w/kg	4–5 w/kg	20–23 w/kg	

4.2 Selection of solvent quantity for the deprotection of compound B

Methanol and DCM were employed in the deprotection of compound B. Solvent quantities were systematically optimized to achieve complete deprotection and maximize reaction yield. Anhydrous methanol and DCM were added in three sequential batches, with the amounts per batch optimized as follows: anhydrous methanol (total ratio: mB(g) × 2.5 mL, mB(g) × 1.0 mL, and mB(g) × 0.75 mL.

DCM (total ratio: 5:2:1.5): mB(g) × 50 mL, mB(g) × 20 mL, and mB(g) × 15 mL.

The results shown in Table 3 indicate stable product quality with a purity of ≥95% and yield of 55%–60%.

Table 3. Optimization of solvent dosage for the deprotection of compound B

Compound B (g)	Anhydrous methanol (L)	Yield (%)	Compound B (g)	DCM (L)	Yield (%)
100	0.15:0.1:0.075	40	100	3:2:1.5	42
100	0.2:0.1:0.075	46	100	4:2:1.5	48
100	0.25:0.1:0.075	58	100	5:2:15	59

4.3 Selection of reactant quantity for the condensation of compound E

The main chemical reagents in condensation included compound D, raw material IV, DBU, and anhydrous methanol. The effects of optimizing the reactant ratios on the quality and yield of compound E are summarized in Table 4. The optimized reactant ratio was established as compound D:raw material IV:DBU = 1:1.15:2.5, with anhydrous methanol used at 10 times the mass of compound D (mD(g) × 10 mL). Quality and yield demonstrated remarkable improvement under these conditions.

Table 4. Optimization of the reagent ratio for the synthesis of compound E

Compound D (mol)	Material IV (mol)	DBU (mol)	Yield (%)
1	1.05	1.5	72
1	1.1	2	78
1	1.15	2.5	89

Anhydrous methanol (10 mL/g × *X* g) was added to the reaction flask, followed by raw material II (0.7 × *X* g) and DBU (2.0 × *X* g). After the complete dissolution of solids, compound *X* g was introduced and the mixture was heated to 65 °C–75 °C and refluxed for 5–5.5 h. Hot filtration was performed, and the filter cake was rinsed with anhydrous methanol (1 mL/g × *X* g) and dried under airflow at 40 °C–45 °C for 12 h to obtain a grayish-brown solid (compound 2).

A total of 5000 mL of anhydrous methanol was added to the reaction flask, followed by 350 g of raw material IV and 1000 g of DBU. After complete dissolution, 500 g of compound D was introduced and the solution was heated to 70 °C and refluxed for 5 h. Hot filtration was performed, and the filter cake was rinsed with 500 mL of anhydrous methanol and air-dried at 44 °C for 10 h to obtain 438.5 g of a grayish-brown solid (compound E) with a mass yield of 87.7% (molar ratio: 1.0:1.14:2.57)

4.4 Salt formation control of TPI

The salt formation of TPI is a critical step in synthesis, directly governing product quality and yield. Systematic optimization identified five key parameters: reactant stoichiometry, salt formation duration, washing solvent selection, addition methodology, and drying time. A comprehensive evaluation of salt-forming reagents was conducted. This evaluation focused on molar ratio, impurity content, addition protocol, and reaction time. Experimental data revealed that yield decreased beyond 4 h of reaction time, with negligible yield variation at molar ratios exceeding 1.4. Optimized conditions were established as dual-temperature control (70 °C and 65 °C) with reaction durations of 25 min and 30 min at a 1.2 molar ratio versus 52 °C at a 1.32 molar ratio over 3 h.

The strategic selection of salts exhibiting contrasting solubility profiles and distinct crystallization kinetics was implemented to mitigate scaling challenges during process intensification. Slurry purification was implemented to eliminate surface-adsorbed impurities. Temperature-modulated crystal washing facilitated impurity removal through solvent contact and subsequent filtration. Post-slurry solids underwent rigorous washing and drying protocols. This approach substantially enhanced product purity while minimizing impurity incorporation.

4.5 Precautions in synthesis

4.5.1 Key issues in FTD synthesis

1) Trifluoromethylation

Reagent Selection: Trifluoromethylation reagents (e.g., CF_3I and CF_3Cu) exhibit high reactivity and toxicity. Stringent protective measures (ventilation and low-temperature operation) are required.

Byproduct Control: Substitution may occur at nontarget positions. Selectivity is controlled by using Lewis acids (e.g., CuI) and solvent polarity modulation.

Temperature Control: Elevated temperatures may induce dehalogenation or decomposition. The reaction is recommended to be conducted at 0 °C-25 °C.

2) Glycosylation

Stereoselectivity: The β-configuration of the glycosidic bond is critical. Selectivity is ensured through catalytic methods (e.g., $SnCl_4$) or enzymatic approaches.

Deoxyribose Activation: 1-Chloro-2-deoxyribose is unstable and must be freshly prepared or stored at low temperatures.

Acidic Condition Optimization: pH must be strictly controlled to prevent sugar ring opening or hydrolysis.

3) Deprotection and Purification

Deacetylation Conditions: Mild deprotection (e.g., NH_3 / MeOH) is essential to prevent glycosidic bond cleavage.

Chromatographic Purification: Reverse-phase chromatography (e.g., C18 column) ensures API purity > 99%.

4.5.2 Key issues in TPI synthesis

1) Chlorination

Chlorination Reagent: $POCl_3$ is highly corrosive and hygroscopic, requiring strict anhydrous conditions and moisture exclusion.

Reaction End-point Monitoring: 5-Chlorouracil may undergo over-chlorination to form 6-chloro byproducts. Real-time monitoring via TLC or HPLC is imperative.

2) Purification and Crystallization

Solvent Selection: Recrystallization from ethanol–water mixed solvent enhances product yield and purity. Impurity Removal: Residual starting materials and derivatives are removed by activated carbon adsorption or ion-exchange resin purification.

5. Conclusions

This study engineered a synthesis pathway and refined process parameters to innovate and optimize the synthesis route of FTD/TPI and its influencing factors and thus achieve stable product quality, high yield, and moderate economic costs. Through comparative literature analysis,

laboratory-scale experiments, and pilot-scale trials, the optimal synthesis route was validated and critical process variables were optimized. By using yield and impurity content as evaluation criteria, key factors (reactant ratios, raw material addition sequence, reaction time, purification methods, and process controls) were systematically investigated and optimized. The following conclusions were drawn:

1) Synthetic Route Confirmation: FTD synthesis, involving carbonyl protection, condensation, deprotection, and purification, is established by using 5-(trifluoromethyl) pyrimidine-2, 4(1H, 3H)-dione as the starting material. TPI synthesis employs 5-chloro-6-(hydroxymethyl) pyrimidine-2,4(1H, 3H)-dione as the starting material and proceeds through chlorination, condensation, salt formation, and purification.

2) Process Efficiency & Product Quality: Total conversion rates of >78% and >81% are achieved for FTD and TPI, respectively, along with low production costs, minimal impurities, and product purity ≥ 99.6%, thus meeting pharmacopeial specifications.

3) Process Optimization: Reactant ratios, temperature/time profiles, and purification methods are optimized to ensure consistent yield and robust product quality.

Significance and Future Directions: This study integrated laboratory-scale experiments, pilot-scale trials, and theoretical analysis to innovate the synthetic route of FTD/TPI. The established reaction conditions and process controls ensure reproducible yields and high-quality output, offering insights for cost reduction and quality enhancement. However, due to the lack of real-time impurity monitoring data, future work will focus on impurity structural characterization, reaction condition refinement, and advanced purification strategies. These efforts will aim to improve product quality, reduce costs, and provide high-quality raw materials for downstream formulation.

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