A practical synthesis of N-allyl/propargyl-substituted 5-fluorouracils

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Abstract

Monosubstituted N-allyl/propargyl-5-fluorouracils are versatile intermediates for the structural modification of 5-fluorouracil derivatives. However, the regioselective synthesis of these monosubstituted 5-fluorouracils is a challenge. Thus, in the current research work, a practical method for synthesizing \(N_1/N_3\)-allyl/propargyl-5-fluorouracils was developed with di-tertbutyl dicarbonate acting as a protective reagent. The process is easy to operate, gives a good regioselectivity, satisfying yields and a simple post-treatment.

Keywords: 5-Fluorouracil, allyl, propargyl, regioselective synthesis

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5-Fluorouracil (5-FU, 1) is an antimetabolite acting as a bioisostere of the natural uracil in living body, and has been widely used in the treatment of colorectal cancer and several solid tumors.\(^1\) 5-FU derivatives incorporating pharmacologically active natural or synthetic molecules, such as 5-FU linked with podophyllotoxin, colchicine, parthenolide, coumarins, thymoquinone, emodin, camptothecin, cisplatin, oxaliplatin, tamibarotene and chalcone, have attracted much attention of many researchers in the field of medicinal chemistry.\(^2\) Recently, many structural modifications have been focused on merging a particular pharmacologically active molecule at the $N_1$ or $N_3$ position of 5-FU using a triazole linkage in order to achieve a higher bioactivity. For example, 5-FU derivatives, incorporating the bioactive molecules parthenolide \(3a\), thymoquinone \(3b\), or glucose derivative \(3c\) incorporating the click triazole linker, have shown potential anticancer activities.\(^3\)–\(^5\) Moreover, 5-FU with triazole-linked polyheterocyclic compound \(3d\) has been reported to exhibit a superior antibacterial activity (Figure 1).\(^6\)

**Figure 1.** Chemical structures of 5-FU, 1-propargyl-3H-5-FU, and its triazole derivatives.

Since triazole moieties have been developed as versatile pharmacophoric linkers, connecting two biological units into one molecule called hybrids,\(^7\)–\(^8\) alkyne or alkene-decorated 5-FUs, especially 1-propargyl-5-FU \(2\), have been used as a key intermediate to furnish various isoxazole or triazole-functionalized 5-FU derivatives \(3\) via 1,3-dipolar cycloaddition reactions with nitrile oxides or azide dipoles.

Given the proved applications of allyl or propargyl-containing 5-FUs in the development of the bioactive 5-FU derivatives, extensive research has been focused on the synthesis of 1-propargyl-5-FU \(2\) and 1-allyl-5-FU \(4\). A common method for the preparation of $N_1$-substituted 5-FUs \(2\) and \(4\) was through nucleophilic substitution of 5-FU with bromopropyne or bromopropylene in the presence of a base such as K$_2$CO$_3$, NaH or DBU (Scheme 1, eq 1).\(^3\)\(^,\)\(^9\)–\(^12\) Although this method provides a simple and direct route to propargyl or allyl-monosubstituted 5-FUs, the poor regioselectivity and low reaction yields are still challenging problems. In a modified method, the combination of Pd(PPh$_3$)$_4$/1,1'-Bis(diphenylphosphino)ferrocene(DPPF) and allyl acetate was used instead of bromopropylene, giving $N_1$-allyl-5-FU \(4\) in a moderate yield.\(^13\) Alternatively, a multiple-step pathway could be used to synthesize the monosubstituted 5-FUs, where 5-FU was first transformed into the key intermediates featuring $N_1$-CH$_2$OCOOBn or $N_3$-CH$_2$OCOOBn by multiple steps, followed by alkylation and deprotection to give allyl-substituted 5-FUs \(4\) and \(5\) (Scheme 1, eq 2).\(^14\) Although multiple methods have been utilized for the synthesis of propargyl/allyl-substituted 5-FUs, there are some disadvantages such as low reaction
yields,\textsuperscript{10-12} using an expensive catalyst\textsuperscript{13} and complex synthetic procedures.\textsuperscript{14} Furthermore, few of these methods were involved in the preparation of \textit{N}_3-substituted 5-FUs (7 and 5). Therefore, it is still of interest to develop a general and efficient method for the synthesis of allyl/propargyl-mono-substituted 5-FUs.

\textbf{Scheme 1.} 1) One-step synthesis of 5-FUs 2 and 4, and 2) multi-step synthesis of 5-FUs 4 and 5.

\textbf{Results and Discussion}

The methods reported to synthesize 1-propargyl-5-FU 2 from the reaction of 5-FU and bromopropyne using K$_2$CO$_3$ as a base in DMF have problems of a low regioselectivity and a poor yield.\textsuperscript{3,9} To improve the regioselectivity and yield, the reaction conditions of 5-FU and bromopropyne with K$_2$CO$_3$ (1 equiv to 5-FU) in DMF were optimized (Table S1, see supporting information); however, a single product \textit{N}$_1$, \textit{N}$_3$-dipropargyl-5-FU 6 was obtained instead of \textit{N}$_1$-propargyl-5-FU 2. Interestingly, no trace of compound 2 was detected by $^1$H-NMR analysis of the isolated product (Scheme 2).

\textbf{Scheme 2.} The reaction of 5-FU and bromopropyne with K$_2$CO$_3$ as a base.
Usually, the proton $N_1\text{-H}$ of 5-FU is much more reactive than that of the $N_3$ position. A plausible reaction mechanism was assumed, where the proton at the $N_1$ position of 5-FU was firstly replaced by propargyl, and the resulting intermediate significantly improved the reaction activity of the proton at the $N_3$ position. Therefore, there was no single substitution product 2, but always the double substitution product 6. While this reaction was carried out with 5-FU and bromopropylene, it was observed that $N_1, N_3$-diallyl-5-FU and $N_1$-allyl-5-FU were both generated. In this reaction mechanism, $N_1$-allyl-5-FU is formed in the first step, and then a double substituted product is generated.

Scheme 3. Synthetic route of $N$-monosubstituted allyl/propargyl-5-FU.

Then, the synthesis of the monosubstituted 5-FUs by a multi-step method was evaluated with a strategy of protection/alkylation/deprotection. Since the $N$-protecting groups were commonly used to prepare pyrimidine derivatives, such as benzoyl, trichloroethoxyformyl, benzyloxyformyl, benzyl and diphenylmethyl moieties, different $N$-protecting methods were tested and ultimately di-tertbutyl dicarbonate (Boc$_2$O) was selected as the $N$-protecting reagent of the starting material 5-FU 1. After several optimizations of the reaction conditions, $N_1$-Boc-substituted intermediate 11 was directly obtained in 65% yield, by the regioselective reaction of 5-FU 1 and 1.0 equiv of Boc$_2$O, with $\text{Et}_3\text{N}$ as a base. In contrast, $N_3$-monosubstituted 5-FU 9 needed a reaction sequence of $N_1,N_3$-diBoc protection and regioselective deprotection of $N_1$-Boc group. In detail, $N_1,N_3$-diBoc-5-FU 8 was provided by reacting 5-FU 1 and 3.0 equiv of Boc$_2$O with pyridine as a base, followed by the regioselective
deprotection of \(N_1\)-Boc group to give the desired \(N_2\)-Boc-5-FU 9. Then the subsequent propargylation of intermediates 11 and 9 were performed with bromopropyne, and finally, the desired \(N_2\)-propargyl-5-FU and \(N_1\)-propargyl-5-FU (7 and 2) were prepared after deprotection of the Boc group, with total yields of 29% and 20%, respectively (Scheme 3). Using the same procedure, when bromopropylene was used instead of bromopropyne, the corresponding allyl-substituted 5-FUs, 4 and 5, were obtained with total yields of 20% and 23%, respectively (Scheme 3).

Conclusions

In summary, the developed method uses 5-FU as starting material and Boc anhydride as a protective reagent to regioselectively produce \(N_1/N_3\)-Boc-5-FU, and provides a practical synthesis of allyl/propargyl-monosubstituted 5-FUs. This method is easy to operate, leads to a good regioselectivity, satisfying yield and simple post-treatment. The produced allyl/propargyl-functionalized 5-FUs can have wide applications in the subsequent preparation of diverse 5-FU derivatives with specific pharmacological activities.

Experimental Section

General. Melting points were recorded using the XRC-1 apparatus and are uncorrected. IR spectra were obtained on a Thermo Nicolet Avatar 370 FT-IR spectrophotometer. \(^1\)H and \(^{13}\)C NMR spectra were recorded on a Bruker AVANCE III spectrometer at 600 MHz using TMS as internal standard. HRMS spectra were recorded on a Waters GCT Premier instrument with EI mode. All the chemicals and solvents were analytical reagents and commercially available and used as received.

**Synthesis of 1-Boc-5-FU (11).** A mixture of 5-FU (13.0 g, 100 mmol), Boc anhydride (22.0 g, 100 mmol), and triethylamine (43.4 mL) in DCM (200 mL) was stirred at 20 °C until 5-FU disappeared by TLC monitoring. The solvent was concentrated under vacuum, and the crude product was purified by column chromatography to obtain 11, white solid, 15.0 g, yield 65%, mp 148-150 °C [lit\(^{15}\) 149-150 °C]. \(^1\)H NMR (600 MHz, DMSO-\(d_6\) δ 11.95 (br, 1H, NH), 8.19 (d, \(J\ 7.2\ Hz\), 1H, ArH), 1.52 (s, 9H, C(CH\(3\))\(_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\) δ 157.5 (d, \(J\ 27.0\ Hz\), 147.9, 146.6, 140.3 (d, \(J\ 234.3\ Hz\)), 125.0 (d, \(J\ 37.1\ Hz\)), 86.5, 27.8.

**Synthesis of 1-Boc-3-propargyl-5-FU (12).** A mixture of 5-FU (4.6 g, 20 mmol) was mixed with 60% NaH (0.96 g, 24 mmol) in DMF (50 mL) and stirred for 45 min at 0–5 °C. Bromopropyne (2.4 g, 20 mmol) was added dropwise and then stirred at rt until no reactant was detected by TLC. The mixture was poured into ice-water, extracted with ethyl acetate, and washed with water. The organic layer was dried with anhydrous magnesium sulfate, concentrated under vacuum, and purified by column chromatography to obtain 12, white solid, 2.7 g, yield 50%, mp 125-127 °C; IR (KBr) cm\(^{-1}\): 215, 2984, 1712, 1683, 1356, 1211, 863, 670; \(^1\)H NMR (600 MHz, DMSO-\(d_6\) δ 8.34 (d, \(J\ 6.6\ Hz\), 1H, ArH), 4.53 (s, 2H, N-CH\(_2\)-C), 3.22 (s, 1H, C\(=\)CH), 1.55 (s, 9H, C(CH\(3\))\(_3\)); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\) δ 156.2 (d, \(J\ 27.2\ Hz\)), 147.8, 146.2, 139.7 (d, \(J\ 232.7\ Hz\)), 124.5 (d, \(J\ 37.0\ Hz\)), 87.2, 78.6, 74.2, 31.2, 27.7; HRMS calcd for C\(_{12}\)H\(_{13}\)FN\(_2\)O\(_4\): 268.0859, found 268.0857.

**Synthesis of 3-propargyl-5-FU (7).** A mixture of 1-Boc-3-propargyl-5-FU (1.5 g, 5.6 mmol), saturated NaHCO\(_3\) (10 mL) in methanol (20 mL) was stirred at 45 °C for 45 min. The solvent was concentrated under vacuum to remove methanol and extracted with DCM (25 mL x 3). The organic layer was dried with anhydrous magnesium sulfate, concentrated under vacuum, and purified by column chromatography to obtain 7, as white solid, 0.83
Synthesis of 1-Boc-3-allyl-5-FU (13). Similar procedure with the synthesis of 12 using bromopropylene (2.4 g, 20 mmol) instead of bromopropane to obtain 1-Boc-3-allyl-5-FU 13, white solid, 2.2 g, yield 40%; mp 70-72 °C; IR (KBr) cm⁻¹: 3230, 2976, 1715, 1658, 1467, 1223, 805, 770; ¹H NMR (600 MHz, DMSO-ᴅ₂) δ 11.29(br, 1H, NH), 7.92(d, J 5.8 Hz, 1H, ArH), 4.50(d, J 2.2 Hz, 2H, N-CH₂-C), 3.16(t, J 2.4 Hz, 1H, C=CH). ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 157.0(d, J 26.0 Hz), 149.6, 139.7(d, J 226.7 Hz), 126.0(d, J 31.6 Hz), 79.1, 73.6, 30.2; HRMS calcd for C₁₇H₁₅FN₂O₂: 270.1016, found 270.1018.

Synthesis of 3-allyl-1-5-FU (5).⁷ Similar procedure with the synthesis of 7 using 1-Boc-3-allyl-5-FU 13 (2.2 g, 8.0 mmol) instead of 1-Boc-3-propargyl-5-FU 12 to obtain 5, white solid, 1.2 g, yield 88%; mp 67-68 °C; IR (KBr) cm⁻¹: 3181, 3020, 2957, 1758, 1723, 1610, 1376, 642; ¹H NMR (600 MHz, DMSO-ᴅ₂) δ 8.36(d, J 7.3 Hz, 1H, ArH), 1.54(s, 9H, C(CH₃)₃), 1.53(s, 9H, C(CH₃)₃); ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 154.9(d, J 25.5 Hz), 150.0, 139.8(d, J 226.1 Hz), 132.5, 125.6(d, J 31.6 Hz), 117.1, 42.7; HRMS calcd for C₁₇H₁₅FN₂O₂: 270.0492, found 270.0499.

Synthesis of 1,3-diBoc-5-FU (8).⁸ A mixture of 5-FU (13.0 g, 100 mmol), acetonitrile (100 mL), Boc anhydride (66.0 g, 300 mmol), and pyridine (8 mL) was stirred at 55 °C until no 5-FU was detected by TLC. After concentration under vacuum, the residue was poured into water (50 mL) and extracted with DCM (20 mL x 3), dried over anhydrous magnesium sulfate. The filtrate was concentrated and purified by column chromatography to obtain 8, white solid, 25.0 g, yield 75%, mp 111-113 °C; IR (KBr) cm⁻¹: 3181, 3020, 2957, 1758, 1723, 1610, 1376, 642; ¹H NMR (600 MHz, DMSO-ᴅ₂) δ 8.36(d, J 7.3 Hz, 1H, ArH), 1.54(s, 9H, C(CH₃)₃), 1.53(s, 9H, C(CH₃)₃); ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 154.9(d, J 29.0 Hz), 147.2, 146.8, 144.6, 139.5(d, J 234.8 Hz), 125.9(d, J 37.3 Hz), 117.1, 87.7, 87.5, 27.7, 27.5; HRMS calcd for C₁₇H₁₅FN₂O₂: 330.1227, found 330.1235.

Synthesis of 3-Boc-5-FU (9).⁸ 1,3-diBoc-5-FU 8 (10.0 g, 30 mmol) was dissolved in dioxane (50 mL), and the mixture was added with 10 mL aqueous K₂CO₃ (6.2 g, 45 mmol) dropwise. After stirring for 8 h at rt, the mixture was concentrated under vacuum to remove dioxane. Then, water (50 mL) was added and extracted with DCM (20 mL x 3). The organic layer was dried, concentrated and purified by column chromatography to obtain 9, white solid, 2.4 g, yield 31%, mp 128-130 °C; ¹H NMR (600 MHz, DMSO-ᴅ₂) δ 11.52(br, 1H, NH), 7.98(d, J 6.1 Hz, 1H, ArH), 1.52(s, 9H, C(CH₃)₃); ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 155.6(d, J 27.8 Hz), 147.9, 147.5, 139.5(d, J 228.7 Hz), 127.7(d, J 31.9 Hz), 87.0, 27.5; IR (KBr) cm⁻¹: 3197, 3099, 2832, 1732, 1621, 1153, 842, 612; HRMS calcd for C₁₇H₁₅FN₂O₄: 230.0703, found 230.0694.

Synthesis of 3-Boc-1-propargyl-5-FU (10). A similar procedure as the synthesis of 12 using 3-Boc-5-FU 9 (0.5 g, 2.0 mmol) to obtain 10, white solid, 0.48 g, yield 90%, mp 113-114 °C; IR (KBr) cm⁻¹: 3287, 2964, 1710, 1685, 1452, 1356, 856, 786; ¹H NMR (600 MHz, DMSO-ᴅ₂) δ 8.34(d, J 6.6 Hz, 1H, ArH), 4.53(d, J 2.2 Hz, 2H, N-CH₂-C), 3.54(t, J 2.2 Hz, 1H, C=CH), 1.53(s, 9H, C(CH₃)₃); ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 155.0(d, J 27.9 Hz), 147.2, 147.1, 139.5(d, J 231.7 Hz), 130.4(d, J 34.3 Hz), 87.7, 77.9, 77.3, 38.4, 27.5; HRMS calcd for C₁₇H₁₅FN₂O₄: 268.0859, found 268.0855.

Synthesis of 1-propargyl-5-FU (2).⁵ A similar procedure as the synthesis of 7 using 3-Boc-1-propargyl-5-FU 10 (0.48 g, 1.8 mmol) to obtain 2, colorless crystal, 0.28 g, yield 93%; mp 168-170 °C; IR (KBr) cm⁻¹: 3359, 3012, 1670, 1605, 1280, 1136, 923, 806; ¹H-NMR (600MHz, DMSO-ᴅ₂) δ: 11.94(br, 1H, NH), 8.13(d, J 6.6 Hz, 1H, ArH), 4.47(d, J 2.2 Hz, 2H, N-CH₂-C), 3.44(t, J 2.2 Hz, 1H, C=CH); ¹³C NMR (151 MHz, DMSO-ᴅ₂) δ 157.3(d, J 25.9 Hz), 149.0, 139.9(d, J 230.6 Hz), 128.8(d, J 34.0 Hz), 78.0, 76.1, 36.9; HRMS Calcd for C₁₇H₁₅FN₂O₂: 168.0393, found 168.0333.
Synthesis of 3-Boc-1-allyl-5-FU (14). Similar procedure with the synthesis of 10 using 3-Boc-5-FU 9 (0.5 g, 2.0 mmol) and bromopropylene (2.4 g, 20 mmol), to obtain 3-Boc-1-allyl-5-FU 14, white solid, 0.5 g, yield 93%, mp 123–124 °C; IR (KBr) cm⁻¹: 3053, 2932, 1689, 1396, 1257, 862, 724; ¹H NMR (600 MHz, DMSO-d₆) δ 8.22(d, J 6.8 Hz, 1H, ArH), 5.94-5.87(m, 1H, CH=C), 5.26-5.24(m, 2H, C=CH₂), 4.30(d, J 5.5 Hz, 2H, N-CH₂-C), 1.52(s, 9H, C(CH₃)₃); ¹³C NMR (151 MHz, DMSO-d₆) δ 155.1(d, J 27.8 Hz), 147.5, 147.3, 139.4(d, J 230.7 Hz), 132.4, 131.1(d, J 33.6 Hz), 119.1, 87.4, 50.7, 27.5; HRMS calcd for C₁₂H₁₃FN₂O₄: 270.1016, found 270.1019.

Synthesis of 1-allyl-5-FU (4).³ Similar procedure with the synthesis of 2 using 3-Boc-1-allyl-5-FU 14 (0.5 g, 2.0 mmol) instead of 1-Boc-3-propargyl-5-FU 10 to obtain 4, colorless needle crystal, 0.31 g, yield 91%; mp 125–126 °C [lit ¹⁰ 101 °C]; IR (KBr) cm⁻¹: 3382, 3200, 1705, 1663, 1573, 1328, 1149, 756; ¹H NMR (600 MHz, DMSO-d₆) δ 11.82(br, 1H, NH), 8.02(d, J 13.2 Hz), 118.3, 119.8, 119.6, 119.2, 118.2, 49.8; HRMS calcd for C₇H₇FN₂O: 170.0492, found 170.0500.

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Supplementary Material

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References

[http://dx.doi.org/10.1016/j.bioorg.2017.01.010](http://dx.doi.org/10.1016/j.bioorg.2017.01.010)

[https://doi.org/10.1016/j.ejmech.2019.111700](https://doi.org/10.1016/j.ejmech.2019.111700)


[https://doi.org/10.3389/fchem.2014.00056](https://doi.org/10.3389/fchem.2014.00056)

[https://doi.org/10.26434/chemrxiv.7327904.v1](https://doi.org/10.26434/chemrxiv.7327904.v1)

[https://doi.org/10.1038/ncomms4277](https://doi.org/10.1038/ncomms4277)

[https://doi.org/10.1016/j.tet.2004.11.019](https://doi.org/10.1016/j.tet.2004.11.019)

[https://doi.org/10.1246/cl.1988.1381](https://doi.org/10.1246/cl.1988.1381)

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[https://doi.org/10.1021/acs.joc.9b02353](https://doi.org/10.1021/acs.joc.9b02353)

[https://doi.org/10.1039/P19860000515](https://doi.org/10.1039/P19860000515)

[http://dx.doi.org/10.1002/adsc.201400646](http://dx.doi.org/10.1002/adsc.201400646)

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