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FOREWORD

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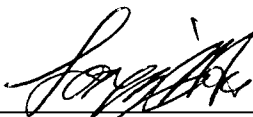
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Table of Contents

	Page Number
Front Cover	
Report Documentation Page	2
Foreword	3
Table of Contents (this page)	4
Introduction	5
Annual Report Body	5
1) Stability of protected Linker-Drug conjugates 1 and 2 of 5-FU	5
2) Synthesis of protected Linker-Drug conjugates 3 and 4 of 5-FU	6
3) Stability of protected Linker-Drug conjugates 3 and 4 of 5-FU	7
4) Cyclization of Linker-Drug conjugates 3 and 4 of 5-FU	7
5) Synthesis of a Peptide-Linker-Drug conjugate of 5-FU	7
6) Experimental Section	9
Key Research Accomplishments	16
Reportable Outcomes	16
Conclusions	16
Appendices	17

Introduction

The goal of this project is to design, synthesize and evaluate peptide-based prodrugs containing an effective clinical anticancer agent such as doxorubicin or 5-fluorouracil for greater tissue-specific activation in order to increase the efficacy and to decrease the systemic toxicity of anticancer drugs used in the treatment of advanced prostate cancer. Peptide-based prodrugs in the form of **Peptide-Linker-Drug** were designed. It was proposed that the peptide portion would be cleaved site-specifically by a prostate tissue-specific enzyme, prostate specific antigen (PSA). After enzymatic cleavage in the targeted prostate cancer tissue, the **Linker-Drug** will undergo a cyclization activation process to release the **Drug**, which will produce the desired cytotoxic effect. We first needed to synthesize the Linker-Drug conjugates to test the cyclization activation process. If the Linker system works, we would continue with the synthesis of **Peptide-Linker-Drug** conjugates and test the activation using enzyme and cell culture assays in vitro.

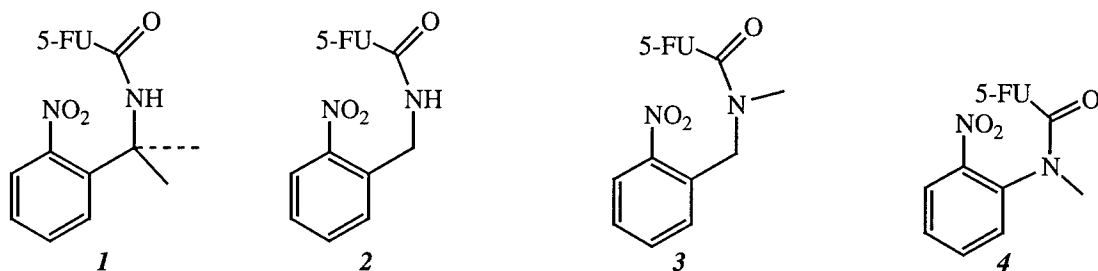
The grant was first awarded when the PI was at the University of Oklahoma Health Sciences Center and transferred with the PI to Rutgers University in March 2000. There was a gap of about six months during the grant transfer. This is the second annual report of this grant. The first annual report was submitted in August of 1999 covering all the work that was done at the University of Oklahoma. This report covers the grant period after the subject grant was transferred to Rutgers University in March 15, 2000. During this period, we focused on the synthesis of **Linker-Drug** and **Peptide-Linker-Drug** conjugates of 5-fluorouracil (5-FU) proposed in the original application. We uncovered a stability problem in our 5-FU conjugates originally designed. We modified the linker portion and increased the stability of the linker conjugates. Some of our work was published. Efforts are now focused on the synthesis of the **Peptide-Linker-Drug** conjugates of 5-FU incorporating the stabilized linker and final evaluation using enzyme and cell culture assays. The remaining work will be done during the coming year, which I have applied for an extension without additional funds.

Annual Report Body

Because of the potential facile cyclization of **Linkers** having a free amino group to form the corresponding cyclic urea, we used synthetic strategies that mask the amino group as an inert nitro group. Reduction to the corresponding amino group will take place only when needed.

1) Stability of protected Linker-Drug conjugates 1 and 2 of 5-FU

In the first year, we synthesized the Linker-Drug conjugates **1** and **2**. During this reporting period, we found that compounds **1** and **2**, were having stability problems. The urea linkage is rather unstable in phosphate buffer at pH 7.4 and 37 °C with half lives of 15.9 and 4.4 min (Figure 1), respectively, making them unstable for incorporation into prodrugs. Thus, our research in this period was focused on the design and synthesis of new analogues with increased stability.



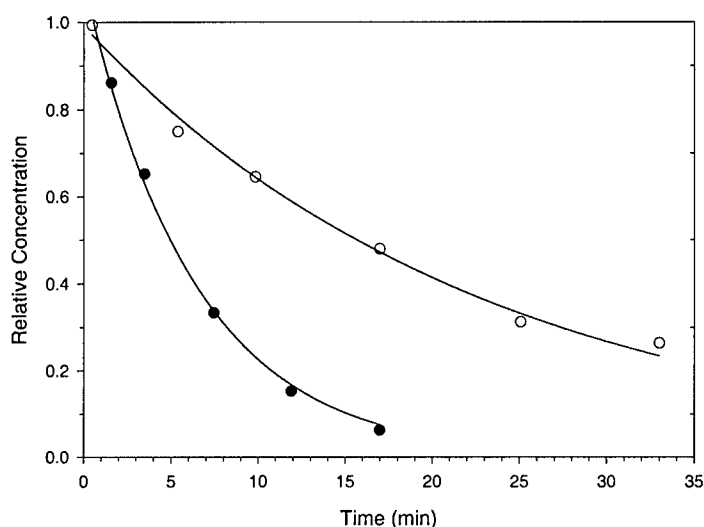
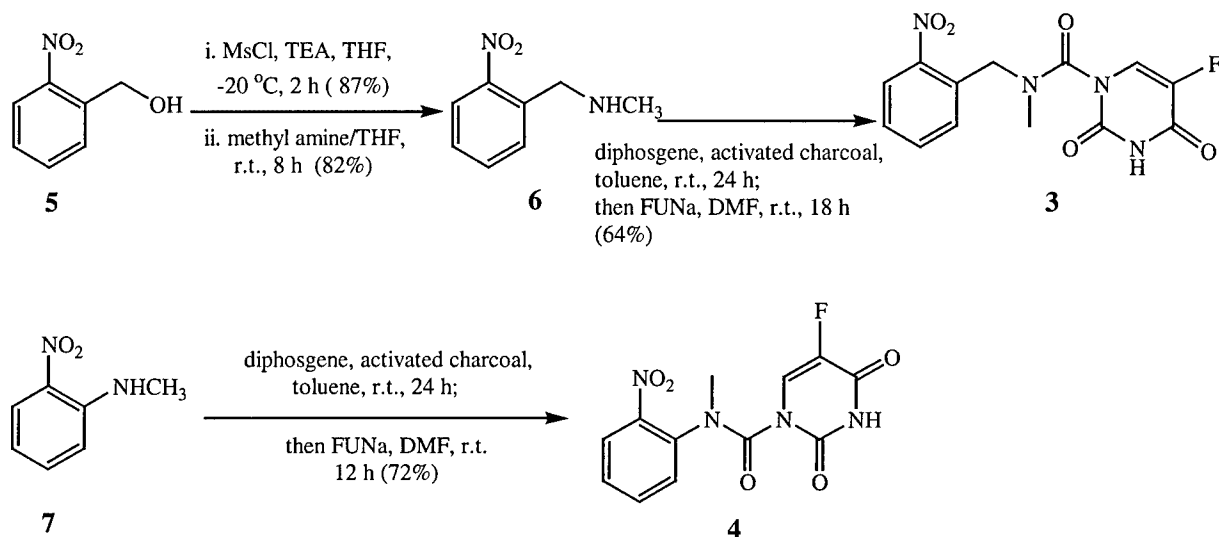


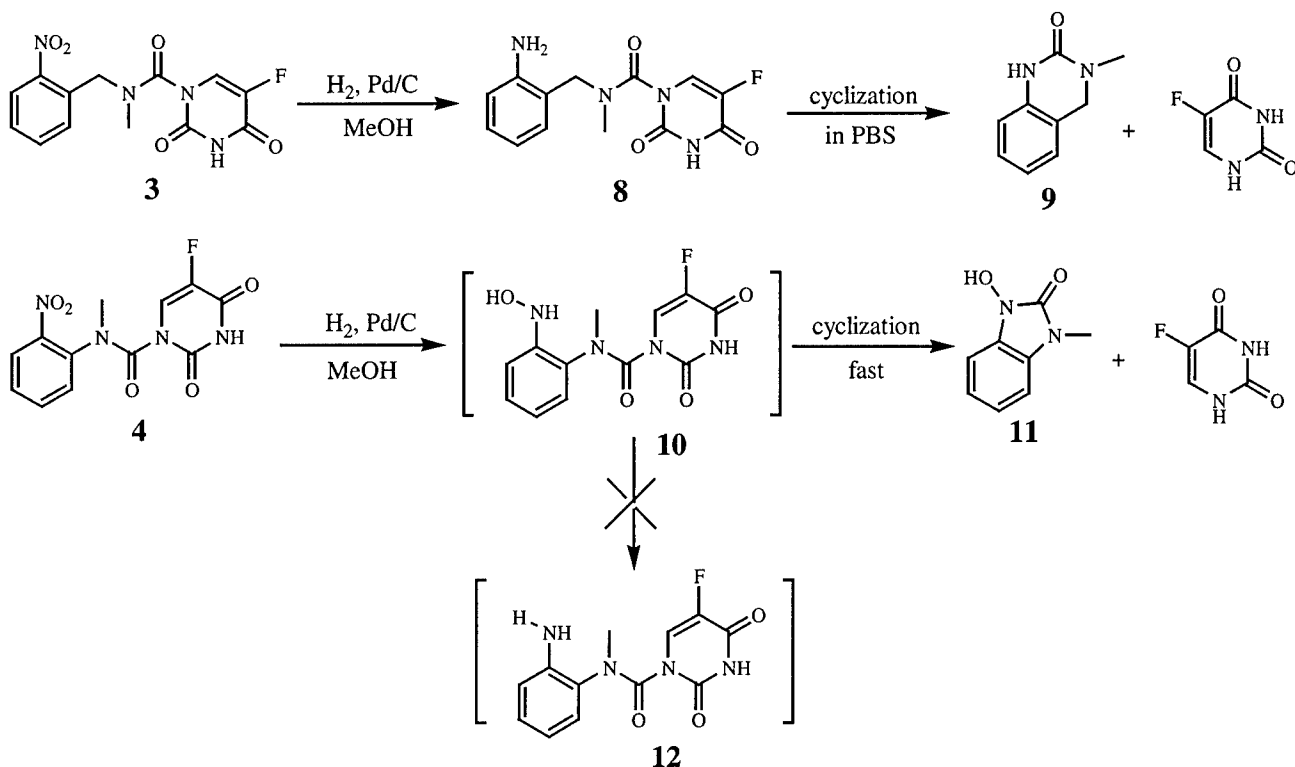
Figure 1. The stability of compounds 1 (●) and 2 (○) in phosphate buffer, pH 7.4 at 37 °C as monitored by HPLC.

2) Synthesis of protected Linker-Drug conjugates 3 and 4 of 5-FU

The synthesis of compounds 3 and 4 are outlined in Scheme 1. *N*-Methyl-2-nitro-benzylamine 6 was prepared by substituting the hydroxyl group on 2-nitrobenzyl alcohol (5) via an activated mesylate in a yield of 72%. The other starting material, *N*-methyl-2-nitroaniline (7), is commercially available. Phosgene chemistry was used to link the amines 6 and 7 with 5-FU. Diposgene is a much safer substitution for phosgene, as it can generate phosgene *in situ* in the presence of activated charcoal. Therefore, the amine was first mixed with diposgene and activated charcoal in toluene to form the carbamoyl chloride. Excess phosgene was purged by a smooth flow of nitrogen, then the carbamoyl

Scheme 1.





Scheme 2. Chemical reductive activation of compounds **3** and **4**.

chloride intermediate was further reacted with 5-FU sodium salt in DMF to afford the desired product. Compounds **3** and **4** were obtained in yields of 64% and 72%, respectively.

3) Stability of protected Linker-Drug conjugates **3** and **4** of 5-FU

Compounds **3** and **4** synthesized above were found to be stable. No significant hydrolysis was observed after three days of incubation in phosphate buffer at pH 7.4 and 37 °C. The introduction of a methyl group on the nitrogen of the urea linkage dramatically increased the stability of 5-FU conjugates.

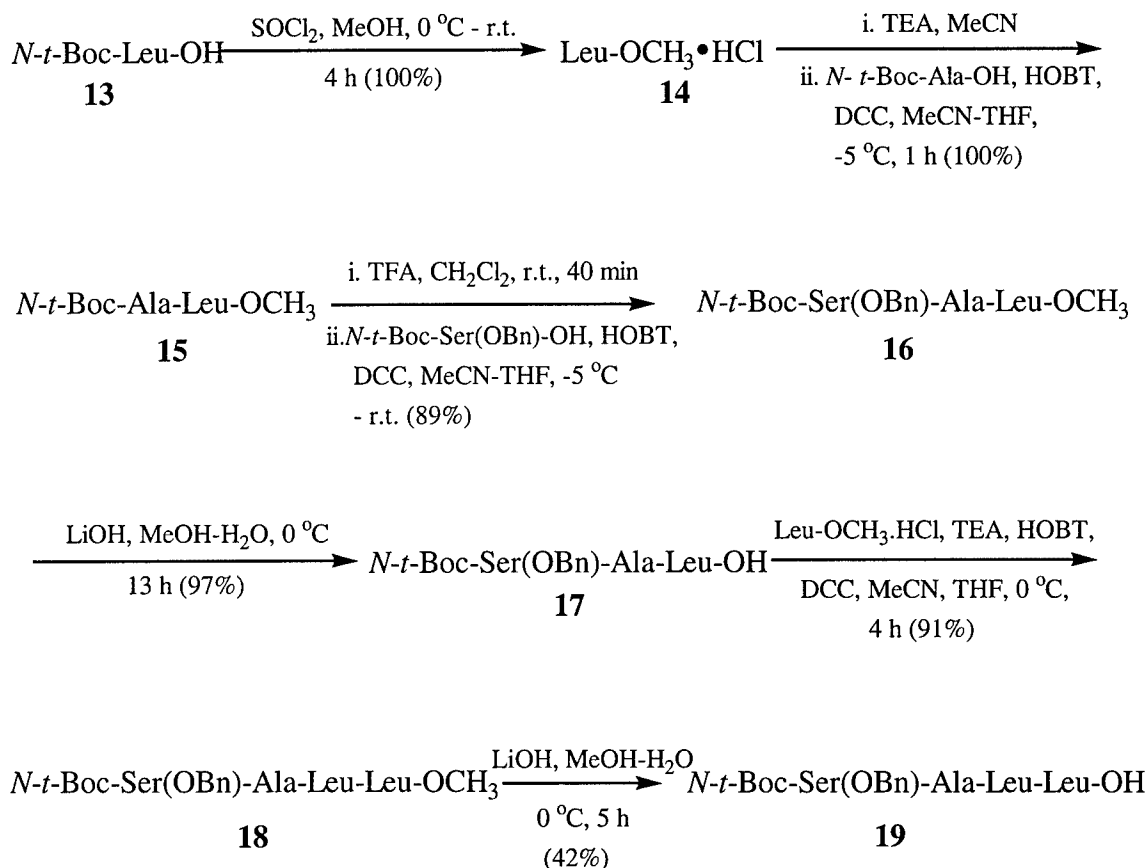
4) Cyclization of Linker-Drug conjugates **3** and **4** of 5-FU

Hydrogenation was employed with the two compounds to test the feasibility of releasing 5-FU (Scheme 2). Amine **8**, obtained from the reduction of compound **3**, cyclized in phosphate buffer to free the active drug with a half-life of 2.86 h. Interestingly, hydrogenation of compound **4** resulted in the formation of compound **11**, which apparently was derived from the hydroxylamine intermediate **10**. The structure of **11** was confirmed by ^1H NMR spectra and HRMS. It was hypothesized that in this case, the hydroxylamine cyclized much faster than its further reduction to form the amine intermediate.

5) Synthesis of a Peptide-Linker-Drug conjugate of 5-FU

The peptide sequence of L-Serine-L-Alanine-L-Leucine-L-Leucine was chosen as the peptide portion for our prodrugs. It is one of the fastest cleaved sequences *N*-terminal to the cleavage site for PSA. Although not specific for PSA, the simple peptide sequence would still enable us to test our activation mechanism, and future studies might involve the structure or sequence modification to make prodrugs as specific substrates for PSA with respect to other serine proteases.

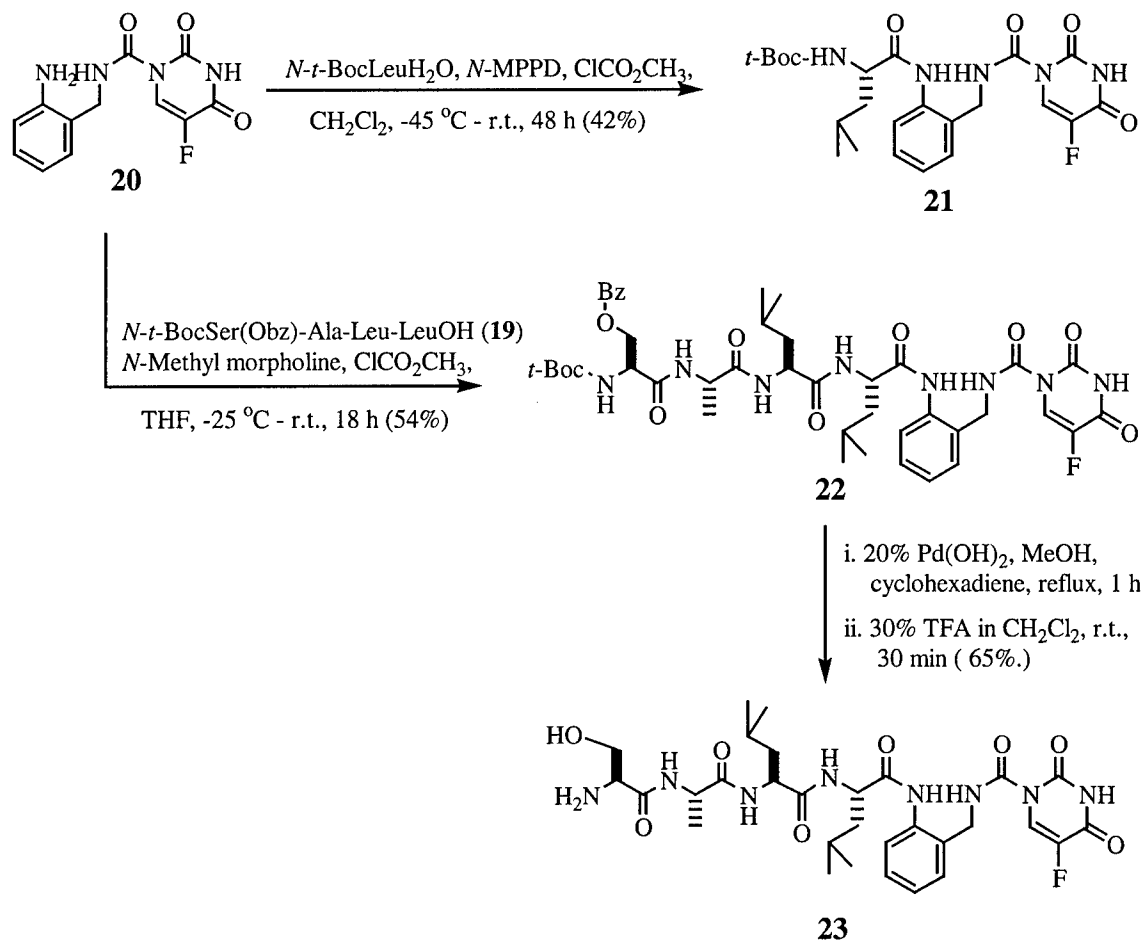
Synthesis of protected peptide 19. The preparation of protected tetrapeptide **19** in solution is summarized in Scheme 3. Treatment of *N*-*tert*-butoxycarbonyl (*t*-Boc) protected L-leucine **13** with



Scheme 3. Synthesis of tetrapeptide **19**.

thionyl chloride in methanol resulted in the protection of the carboxylic acid methyl ester **14**. Removal of the *t*-Boc group under acidic condition and coupling of the resulting amine with *t*-Boc-protected L-alanine via the activation by *N*-hydroxybenzotriazole (HOBT) and 1,3-dicyclohexylcarbodiimide (DCC) gave protected dipeptide **15**. The same method was applied to the coupling of dipeptide **15** with protected L-serine to give protected tripeptide **16**. Lithium hydroxide mediated hydrolysis converted the tripeptide methyl ester **16** to its corresponding carboxylic acid **17**. Preparation of the protected tetrapeptide **18** from the coupling of acid **17** and leucine methyl ester, followed by lithium hydroxide mediated hydrolysis furnished the desired peptide **19** in a total yield of 33%.

Synthesis of initial target 23. The synthesis of our first peptide-linker-drug **23** is outlined in Scheme 4. A model test was first performed to construct the peptide bond between *N*-*t*-Boc protected leucine and the aromatic amino group in compound **20**, which was derived from reduction of the nitro compound **2**. The conditions using HOBT/DCC and *N*-hydroxysuccinimide (HOSU)/DCC failed to accomplish the coupling, and compound **21** could only be produced in 42% yield through an activated anhydride. The difficulty in forming the peptide bond in this case might be due to the weak nucleophilicity of the aromatic amine in **20**. Subsequently, using these conditions, conjugate **22** was obtained in 54% yield from the coupling of the tetrapeptide **19** with the Linker-Drug **20**. In the last step of deprotecting the hydroxyl group and amino group on the serine residue of the peptide, the sequence of deprotection was found to be very important. If the *N*-*t*-Boc group was removed first, the benzyl ether protecting the hydroxyl would not been cleaved by hydrogenation, which might be explained by the poisoning of the palladium catalyst by free amino groups. Therefore, deprotection of the hydroxyl group was first carried out by heating **22** with 20% Pd(OH)₂ catalyst and cyclohexadiene in methanol. Further treatment with a solution of trifluoroacetic acid (TFA) in dichloromethane (30%) removed the *t*-Boc protecting group and afforded the final compound **23** in 65% yield.



Scheme 4. Synthesis of compound 43.

6) Experimental Section

General Methods. Melting points were determined on a Mel-Temp capillary apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer and are reported in wave numbers (cm^{-1}) with broad signals denoted by (br). ^1H NMR spectra were recorded in deuterated solvents at 200 or 300 MHz on Varian Gemini 200 or 300 MHz spectrometer as indicated. ^{13}C NMR spectra were recorded at 50 MHz on a Varian Gemini 200 MHz spectrometer. Coupling constants are reported in hertz (Hz). UV spectra were recorded on HP-8451A diode array spectrophotometer. HPLC analysis was performed on Spectra-Physics HPLC system. Mass spectra (MS) were obtained from mass spectrometry laboratories in University of Oklahoma and University of Kansas. Analytical LCMS spectra were obtained from Department of Pharmaceutics, Rutgers, the State University of New Jersey.

All reactions were stirred magnetically. Moisture-sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon as indicated. Air and moisture-sensitive liquids and solutions were transferred via syringes and were introduced into reaction vessels through rubber septa. Analytical thin-layer chromatography (TLC) was carried out on Whatman TLC plates precoated with silica gel 60 F₂₅₄ (250- μm layer thickness). Flash column chromatography was performed on EM Science silica gel 60 (230–400 mesh) purchased from Aldrich. Organic solutions were concentrated using a Büchi rotary evaporator at 15–20 mmHg.

Tetrahydrofuran (THF), diethyl ether (Et₂O) were distilled from sodium metal/benzophenone. Pyridine, dichloromethane (CH₂Cl₂) and acetonitrile (CH₃CN) were distilled from calcium hydride. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride and stored over 4-Å molecular sieves. Dioxane was distilled from calcium oxide (CaO). Anhydrous toluene was purchased from Aldrich and used directly. 5-Fluorouracil (5-FU) was purchased from ICN Biomedicals Inc. All amino acids were obtained from Advanced Chem. Tech. Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. All other commercially available chemicals were purchased from Sigma and Aldrich Chemical Co., and were used without further purification unless otherwise indicated.

Stability test in phosphate buffer

The masked Linker-5-FU conjugates (1.5 mg) was dissolved in 200 mL of CH₃CN and stored at 0 °C. A solution (10 µL) was withdrawn and quickly added to 190 µL of 100 mM phosphate buffer (pH 7.4) pre-warmed at 37 °C to give a final concentration of 1 mM. The resulting solution was incubated at 37 °C while aliquots (25 µL) of sample were removed at intervals and injected directly into the HPLC injection port. HPLC analysis with C-18 reverse-phase column used either a gradient mobile phase from 28% CH₃CN to 52% CH₃CN over 10 min or isocratic elution with a mobile phase of 40% CH₃CN, at a flow rate of 1 mL/min and detection wavelengths of 220 nm and 280 nm.

General procedure for hydrogenation

A solution of nitro compound (0.05 mmol) in 3 mL of methanol underwent atmospheric hydrogenation in the presence of 10% Pd/C. Both HPLC and TLC were used to monitor the progress of reduction. At the end of reaction, the catalyst was removed by filtration, the residue was washed with methanol (3 × 5 mL). The combined organic phase was condensed *in vacuo*, and the residue was purified by flash column chromatography on silica gel eluted with acetone–hexanes to afford the cyclized lactam, parent drug 1, and/or the amine intermediate. HPLC analysis was performed on a C-18 reversed-phase column (150 × 4.6 mm), using first a isocratic elution of 2% CH₃CN for 5 min followed by a gradient elution from 2% CH₃CN to 70% CH₃CN over 15 min and a final isocratic elution of 70% CH₃CN for 5 min, at a flow rate of 1 mL/min and detection wavelengths of 220 nm and 280 nm.

General procedure for cyclization

A solution of nitro compound (0.05 mmol) in 3 mL of methanol underwent hydrogenation according to procedure A. The product after isolation was the amine intermediate. The amine compound was then incubated in 100 mM phosphate buffer (pH 7.4) at 37 °C for half an hour and monitored by HPLC. At the end of reaction, the solution was extracted with ethyl acetate, and the organic phase was washed with brine, dried over anhydrous Na₂SO₄ and filtered. Removal of the solvent *in vacuo* afforded the lactam. FUDR in the aqueous phase was detected by HPLC.

Methyl-(2-nitrobenzyl)amine (6)

To a solution of 2-nitrobenzylalcohol **5** (2 g, 13 mmol) and triethylamine (2.72 mL, 19.6 mmol) in 40 mL of THF was added methanesulfonyl chloride (1.06 mL, 13.65 mmol) dropwise at –20 °C under argon atmosphere. After being stirred for 2 h at this temperature, the suspension was added 50 mL of ice water and extracted with *t*-butyl methyl ether (3 × 30 mL). The organic phase was washed with 1 N HCl solution (40 mL) and saturated NaHCO₃ solution, dried over anhydrous MgSO₄, and condensed *in vacuo* to afford 2.61 g (87%) of mesylate as a pale yellow solid. mp 95.5–97 °C; ¹H NMR (200 MHz, CDCl₃) δ 8.20–7.53 (m, 4 H), 5.67 (s, 2 H), 3.14 (s, 3 H), 1.70 (br s, 1 H); ¹³C NMR (50 MHz, CDCl₃) δ 134.53, 130.32, 129.86, 129.55, 125.48, 68.25, 37.95; IR (KBr) 3098.3, 3028.9, 3015.5, 1613.7, 1578.3, 1525.1, 1443.2, 1383.4, 1342.1, 1270.9, 1198.6, 1174.0, 1149.8, 1050.4, 1010.1, 989.1, 967.1, 870.2, 805.8, 792.4, 736.4 cm^{–1}; MS (ESI) *m/z* (rel intensity): 135.85 [(M–OMs)⁺, 100].

To a solution of 2 N methylamine in THF (6.72 mL) was added mesylate (2.596 g, 11.2 mmol) as prepared above. The reaction mixture was stirred at room temperature for 8 h and then diluted with *t*-butyl methyl ether. The organic layer was washed with saturated NaHCO₃ solution and brine, dried over anhydrous MgSO₄, and condensed *in vacuo*. The residue was subjected to flash column chromatography on silica gel eluted with methanol–dichloromethane–1% triethylamine (1:40 → 1:30 → 1:10 → 1:5) to afford 1.52 g (82%) of **6** as a brown oil. ¹H NMR (200 MHz, CDCl₃) δ 7.96–7.37 (m, 4 H), 3.98 (s, 2 H), 2.46 (s, 3 H), 1.70 (br s, 1 H); ¹³C NMR (50 MHz, CDCl₃) δ 135.86, 133.62, 131.75, 128.44, 125.23, 53.26, 36.63; IR (neat) 3344.0, 2944.0, 2841.4, 2790.2, 1610.3, 1528.4, 1441.2, 1343.8, 1123.3, 851.5, 784.8, 723.3 cm⁻¹; MS (ESI) *m/z* (rel intensity): 167.01 (MH⁺, 100).

1-(N-Methyl-N-2-nitrobenzylcarbamoyl)-5-fluorouracil (3)

To a suspension of methyl-(2-nitrobenzyl)amine **6** (130 mg, 0.78 mmol) and 20 mg of activated charcoal in 5 mL of anhydrous toluene was added diphosgene (190 μL, 1.57 mmol) dropwise under argon atmosphere. After the reaction mixture was stirred at room temperature for 24 h, argon was bubbled through for 5 min to get rid of the excess phosgene. The activated charcoal was removed by vacuum filtration. After removal of the solvent *in vacuo*, the residue was dissolved in 5 mL of anhydrous DMF, to which was added 5-fluorouracil sodium salt (178 mg, 1.17 mmol) under argon atmosphere. The reaction mixture was stirred for 18 h at room temperature and condensed *in vacuo*. The residue was subjected to column chromatography on silica gel eluted with acetone–hexanes (1:5 → 1:3 → 1:2 → 1:1) to afford 161 mg (64%) of **3** as a white solid. mp 218–220 °C; ¹H NMR (200 MHz, DMSO-*d*₆) δ 12.15 (s, 1 H), 8.21 (d, 1 H, *J* = 6.2 Hz), 8.17–7.57 (m, 4 H), 5.25–4.86 (m, 2 H), 3.01 (s, 3 H); IR (KBr) 3425.6, 3189.7, 3076.9, 2964.1, 2923.1, 1733.3, 1712.8, 1517.9, 1476.9, 1441.0, 1353.8, 1338.5, 1271.8, 1261.5, 1205.1, 1233.3, 1066.7, 928.2, 882.1, 861.5, 841.0, 794.9, 723.1, 702.6, 605.1 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 323.1 (MH⁺, 12), 307.1 (85), 154.1 (100); HRMS calcd for C₁₃H₁₂N₄O₅F (MH⁺) 323.0792 found 323.0814.

1-(N-Methyl-N-2-nitrophenylcarbamoyl)-5-fluorouracil (4)

To a suspension of commercially available *N*-methyl-2-nitroaniline **7** (304.1 mg, 1.99 mmol) and 40 mg of activated charcoal in 10 mL of anhydrous toluene was added diphosgene (483 μL, 3.98 mmol) dropwise under argon atmosphere. After the reaction mixture was stirred at room temperature for 24 h, argon was bubbled through for 5 min to get rid of the excess phosgene. The activated charcoal was removed by vacuum filtration and toluene was evaporated *in vacuo*. The residue was dissolved in 10 mL of anhydrous DMF, to which was added 5-fluorouracil sodium salt (304 mg, 2.0 mmol) under argon atmosphere. The reaction mixture was stirred for 12 h at room temperature and condensed *in vacuo*. The residue was subjected to column chromatography on silica gel eluted with acetone–hexanes (1:5 → 1:3 → 1:2 → 1:1) to afford 443 mg (72%) **4** as a yellow oil. ¹H NMR (200 MHz, CDCl₃) δ 8.09 (d, 1 H, *J* = 8.4 Hz), 7.74–7.48 (m, 4 H), 3.48 (s, 3 H); IR (KBr) 3425.6, 3087.2, 1723.1, 1707.7, 1528.2, 1343.6, 1266.7, 1138.5, 784.6, 702.6 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 309.1 (MH⁺, 29), 179.1 (9), 154.1 (100); HRMS calcd for C₁₂H₁₀N₄O₅F (MH⁺) 309.0635 found 309.0635.

3-Methyl-3,4-dihydro-1H-quinazolin-2-one (9)

A solution of 1-(*N*-methyl-*N*-2-nitrobenzylcarbamoyl)-5-fluorouracil **3** (20 mg, 0.062 mmol) in 5 mL of methanol underwent atmospheric hydrogenation in the presence of 10 % Pd/C for 40 min. The reaction mixture was filtered and condensed *in vacuo* to afford 1-(*N*-methyl-*N*-2-aminobenzylcarbamoyl)-5-fluorouracil **8** as a colorless oil quantitatively after evaporating the solvent *in vacuo*. ¹H NMR (200 MHz, DMSO) δ 11.13 (br s, 1 H), 8.18 (d, 1 H, *J* = 6.2 Hz), 7.15–6.54 (m, 4 H), 5.08 (br s, 2 H), 4.51–3.77 (m, 2 H), 2.83 (d, 3 H, *J* = 11.6 Hz).

1-(*N*-Methyl-*N*-2-aminobenzylcarbamoyl)-5-fluorouracil **8** was incubated in phosphate buffer (pH 7.4, 100 mM) at 37 °C for 5 h and the reaction progress was monitored by HPLC. At the end of the reaction, the mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous Na₂SO₄ and condensed *in vacuo* to afford cyclized compound **9** as a white solid quantitatively. mp 196–198 °C; ¹H NMR (200 MHz, CDCl₃) δ 7.18–6.67 (m, 4 H), 4.47 (s, 2 H), 3.06 (s, 3 H); ¹³C NMR (50 MHz, CDCl₃) δ 154.39, 137.20, 128.40, 125.63, 122.05, 113.67, 51.06, 34.85; IR (KBr) 3435.9, 3210.3, 3128.2, 3066.7, 2923.1, 1671.8, 1610.3, 128.5, 1497.4, 1441.0, 1400.0, 1328.3, 1302.6, 1282.1, 1251.3, 1035.9, 753.8, 717.9 cm⁻¹; MS (ESI) *m/z* (rel intensity): 185.99 (MNa⁺, 10), 162.90 (MH⁺, 100).

1-Hydroxy-3-methyl-1,3-dihydro-benzoimidazol-2-one (**11**)

A solution of 1-(*N*-methyl-*N*-2-nitrophenylcarbamoyl)-5-fluorouracil **4** (20 mg, 0.065 mmol) in 5 mL of methanol underwent atmospheric hydrogenation in the presence of 10 % Pd/C for 15 min. The catalyst was removed by vacuum filtration and product **11** was obtained as a white solid quantitatively after evaporating the solvent *in vacuo*. mp 180–182 °C; ¹H NMR (200 MHz, CDCl₃) δ 10.59 (br s, 0.01 H), 7.38–6.70 (m, 4 H), 3.41 (s, 3 H); ¹³C NMR (50 MHz, CDCl₃) δ 153.21, 128.19, 126.14, 122.39, 121.86, 108.17, 108.06, 27.60; IR (KBr) 3449.3, 3097.4, 2765.4, 1685.5, 1488.0, 1446.2, 1388.1, 1333.4, 1260.8, 1220.5, 1125.7, 984.3, 753.5, 740.5, 715.3 cm⁻¹; MS (ESI) *m/z* (rel intensity): 186.95 (MNa⁺, 12), 164.92 (MH⁺, 100); HRMS calcd for C₈H₉N₂O₂ (MH⁺) 165.0664 found 165.0670.

HCl•Leu-OCH₃ (**14**)

A solution of *N*-*t*-Boc-Leu-OH **13** (3.5 g, 15 mmol) in 20 mL of methanol at 0 °C was added slowly 2.7 mL of thionyl chloride while stirring. After the addition, the reaction mixture was gradually warmed to room temperature and stirred for another 4 h. Removal of the solvent *in vacuo* afforded 2.5 g of **14** as a white foam. ¹H NMR (300 MHz, CDCl₃) δ 3.72 (s, 3 H), 3.48 (dd, 1 H, *J* = 5.7, 5.85 Hz), 1.76 (m, 1 H), 1.57 (ddd, 1 H, *J* = 5.4, 6.6, 7.8 Hz), 1.47 (br s, 2 H), 1.45 (m, 1 H), 0.95 (d, 3 H, *J* = 5.1 Hz), 0.93 (d, 3 H, *J* = 5.4 Hz).

N-*t*-Boc-Ala-Leu-OCH₃ (**15**)

To a solution of *N*-*t*-Boc-Ala-OH (1.9 g, 10 mmol) in 175 mL of THF and 75 mL of CH₃CN were added HCl•Leu-OCH₃ **14** (2.0 g, 11 mmol) and triethylamine (13.4 mL) followed by HOBT•H₂O (2.02 g, 15 mmol). After the addition, the reaction mixture was cooled to –5 °C and charged with a solution of DCC (3.1 g, 15 mmol) in 10 mL of THF. After stirred for 1 h, the reaction mixture was filtered and 50 mL of THF was added to wash. After the combined organic phase was concentrated *in vacuo*, the residue was diluted with CH₂Cl₂, washed with 5% citric acid solution, 5% NaHCO₃ solution and brine, dried over anhydrous Na₂SO₄ and filtered. Removal of the solvent *in vacuo* afforded product **15** as a white foam quantitatively. ¹H NMR (300 MHz, CDCl₃) δ 6.60 (br s, 1 H), 5.06 (br s, 1 H), 4.61 (m, 1 H), 4.19 (br s, 1 H), 3.73 (s, 3 H), 1.67–1.55 (m, 3 H), 1.45 (s, 9 H), 1.35 (d, 3 H, *J* = 7.2 Hz), 0.93 (d, 6 H, *J* = 5.7 Hz); IR (KBr) 3314.8 (br), 2959.9, 2861.5, 1751.2, 1680.2, 1660.4, 1535.0, 1512.8, 1458.3, 1367.1, 1254.7, 1160.3, 1068.5, 854.2 cm⁻¹; MS (ESI) *m/z* (rel intensity) 317.45 (MH⁺, 100).

N-*t*-Boc-Ser(Obz)-Ala-Leu-OCH₃ (**16**)

N-*t*-Boc-Ala-Leu-OCH₃ **15** (10 mmol) was treated with 50 mL of 50% TFA in CH₂Cl₂ at room temperature for 40 min. Removal of the solvent *in vacuo* afforded TFA salt of dipeptide, which was used directly in the next step. To a solution of *t*-Boc-Ser(Obz)-OH (3.2 g, 11 mmol) in 70 mL of THF and 30 mL of CH₃CN were added the solution of TFA salt of dipeptide in 14 mL of triethylamine and HOBT•H₂O (2.02 g, 15 mmol) sequentially with stirring. After the addition, the reaction mixture was cooled to –5 °C and charged with a solution of DCC (3.1 g, 15 mmol) in 10 mL of THF. The reaction mixture was warmed up to room temperature within one hour, and stirred at this temperature for 18 h.

DCU was filtered off and 25 mL of THF was used to wash. After removal of the solvent *in vacuo*, the residue was diluted with CH₂Cl₂. The organic phase was washed with 5% citric acid solution, 5% aqueous NaHCO₃ solution, and brine, dried over anhydrous Na₂SO₄ and filtered. After removal of the solvent *in vacuo*, the residue was subjected to flash column chromatography on silica gel eluted with ethyl acetate–hexanes (1:10 → 1:5 → 1:2) to afford 4.38 g (89%) of **16** as a white foam. ¹H NMR (300 MHz, CDCl₃) δ 7.36–7.35 (m, 5 H), 7.16 (d, 1 H, *J* = 7.5 Hz), 7.01 (d, 1 H, *J* = 8.1 Hz), 5.49 (d, 1 H, *J* = 7.2 Hz), 4.57 (m, 2 H), 4.54 (s, 2 H), 4.32 (br s, 1 H), 3.88 (dd, 1 H, *J* = 4.2, 9.3 Hz), 3.72 (s, 3 H), 3.61 (dd, 1 H, *J* = 6.0, 9.3 Hz), 1.66–1.48 (m, 3 H), 1.42 (s, 9 H), 1.38 (d, 3 H, *J* = 7.2 Hz), 0.91 (d, 6 H, *J* = 5.7 Hz); IR (KBr) 3310.0 (br), 2961.1, 2872.0, 1752.0, 1717.9, 1646.0, 1523.1, 1453.1, 1367.2, 1248.5, 1208.9, 1166.0, 1115.3, 1025.3, 738.2, 698.4 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 494.2 (MH⁺, 20.3), 438.2 (22.3), 146.0 (100).

N-*t*-Boc-Ser(Obz)-Ala-Leu-OH (**17**)

To a solution of *N*-*t*-Boc-Ser(Obz)-Ala-Leu-OCH₃ **16** (100 mg, 0.2 mmol) in 3 mL of methanol and 1 mL of water was added lithium hydroxide (18 mg, 0.75 mmol) at 0 °C with stirring. After the reaction proceeding at this temperature for 13 h, the solvent was removed *in vacuo*. The residue was diluted with water and extracted with ethyl acetate. The aqueous phase was adjusted with 0.2 N HCl solution to pH 3, extracted with ethyl acetate, dried over anhydrous Na₂SO₄ and filtered. Removal of the solvent *in vacuo* afforded 98.7 mg (97%) of **17** as a white foam. ¹H NMR (300 MHz, CDCl₃) δ 7.34–7.25 (m, 5 H), 7.04 (d, 1 H, *J* = 7.5 Hz), 6.95 (d, 1 H, *J* = 8.1 Hz), 5.50 (br s, 1 H), 4.55 (s, 2 H), 4.52 (m, 2 H), 4.32 (br s, 1 H), 3.85 (dd, 1 H, *J* = 4.2, 9.3 Hz), 3.62 (dd, 1 H, *J* = 6.0, 9.3 Hz), 1.75–1.58 (m, 3 H), 1.45 (s, 9 H), 1.39 (d, 3 H, *J* = 7.2 Hz), 0.91 (d, 6 H, *J* = 5.7 Hz); IR (KBr) 3306.0 (br), 2961.9, 2872.6, 1711.5, 1641.9, 1535.5, 1454.5, 1393.0, 1367.9, 1251.6, 1170.1, 1109.1, 1026.4, 738.5, 698.6 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 480.3 (MH⁺, 10.6), 424.2 (7.5), 154.1 (100); HRMS calcd for C₂₄H₃₈N₃O₇ (MH⁺) 480.2710 found 480.2712.

N-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-OCH₃ (**18**)

To a suspension of Leu-OCH₃ HCl salt (350 mg, 1.92 mmol) in 14 mL of THF and 6 mL of CH₃CN were added 2.15 mL of triethylamine, *N*-*t*-Boc-Ser(Obz)-Ala-Leu-OH **17** (837 mg, 1.745 mmol) and HOBT•H₂O (353 mg, 2.62 mmol) sequentially. The reaction mixture was cooled to 0 °C, charged with DCC (500 mg, 2.62 mmol), and stirred for 4 h. After filtration, the reaction mixture was diluted with ethyl acetate. The organic phase was washed by 5% citric acid, 5% NaHCO₃ solution, and brine, dried over anhydrous Na₂SO₄ and filtered. Removal of the solvent *in vacuo* afforded 960 mg (91%) of **18** as a white foam. ¹H NMR (200 MHz, CDCl₃) δ 7.36–7.24 (m, 4 H), 7.24–6.97 (m, 3 H), 5.54 (d, 1 H, *J* = 6.2 Hz), 4.58–4.48 (m, 5 H, singlet at 4.51), 4.30 (d, 1 H, *J* = 5.6 Hz), 3.80 (dd, 1 H, *J* = 4.72, 9.6 Hz), 3.69 (s, 3 H), 3.64 (dd, 1 H, *J* = 5.27, 9.7 Hz), 1.79–1.26 (m, 19 H, singlet at 1.43, doublet at 1.35, *J* = 7.0 Hz), 0.90–0.84 (m, 12 H); ¹³C NMR (50 MHz, CDCl₃) δ 173.54, 172.43, 172.33, 170.90, 156.38, 137.80, 128.99, 128.69, 128.45, 128.17, 81.05, 73.89, 70.22, 55.50, 52.64, 52.13, 51.24, 49.98, 41.37, 41.11, 32.05, 28.73, 25.24, 23.36, 23.31, 23.12, 22.34, 22.21, 18.73, 14.59; IR (KBr) 3290.7 (br), 2958.8, 2872.0, 1749.6, 1719.5, 1641.1, 1542.4, 1452.4, 1367.4, 1252.0, 1209.4, 1166.7, 1114.4, 736.8, 698.4 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 607.4 (MH⁺, 100), 551.3 (32); HRMS calcd for C₃₁H₅₁N₄O₈ (MH⁺) 607.3707 found 607.3689.

N-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-OH (**19**)

To a solution of *N*-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-OCH₃ **18** (3.03 g, 5 mmol) in 60 mL of methanol and 30 mL of water was added lithium hydroxide (1.06 g, 44 mmol) at 0 °C with stirring. After the reaction proceeding at this temperature for 5 h, methanol was removed *in vacuo*. The residue was partitioned between water and ethyl acetate. The aqueous phase was adjusted with 1 N HCl solution to pH 3, and extracted with *t*-butyl methyl ether (3 × 100 mL). The organic phase was dried over anhydrous

MgSO₄, filtered, and condensed *in vacuo* to afford 1.25 g (42%) of **19** as a white foam. ¹H NMR (200 MHz, CDCl₃) δ 7.46–7.27 (m, 6 H), 7.13 (d, 2 H, *J* = 6.58 Hz), 5.51 (m, 1 H), 4.54–4.40 (m, 5 H, singlet at 4.54), 4.27 (m, 1 H), 3.83 (dd, 1 H, *J* = 4.44, 9.54 Hz), 3.67 (dd, 1 H, *J* = 4.76, 9.76 Hz), 1.73–1.53 (m, 6 H), 1.45 (s, 9 H), 1.39 (d, 3 H, *J* = 6.96 Hz), 0.96–0.87 (m, 12 H); ¹³C NMR (50 MHz, CDCl₃) δ 174.60, 173.62, 172.76, 171.32, 156.60, 137.68, 129.05, 128.56, 128.46, 128.39, 128.27, 81.54, 73.98, 73.90, 69.86, 55.75, 52.55, 52.36, 50.46, 41.31, 40.52, 28.73, 25.25, 23.36, 22.18, 18.86; IR (KBr) 3303.8 (br), 2960.3, 2872.3, 1717.9, 1646.9, 1534.3, 1453.2, 1368.1, 1251.7, 1166.5, 736.5, 698.3 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 593.4 (MH⁺, 100), 537.3 (50); HRMS calcd for C₃₀H₄₉N₄O₈ (MH⁺) 593.3550 found 593.3552.

1-(2-Aminobenzylcarbamoyl)-5-fluorouracil (**20**)

A solution of 1-(2-nitrobenzylcarbamoyl)-5-fluorouracil **2** (11 mg, 0.036 mmol) in 3 mL of methanol underwent atmospheric hydrogenation in the presence of 10 % Pd/C for 25 min. The catalyst was removed by vacuum filtration and the filtrate was condensed *in vacuo* to afford **20** as a white solid quantitatively. mp 242–244 °C (acetone/hexanes); ¹H NMR (300 MHz, acetone-*d*₆) δ 9.65 (br s, 1 H), 8.43 (d, 1 H, *J* = 7.8 Hz), 7.18–6.61 (m, 4 H), 4.51 (d, 2 H, *J* = 5.7 Hz); IR (KBr) 3424.0, 3251.3, 3087.2, 1736.0, 1686.3, 1651.3, 1523.2, 1498.0, 1458.7, 1339.7, 1266.2, 1246.2, 1210.3, 815.4, 755.6 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 279.1 (MH⁺, 17.0), 176.0 (44.5), 154.0 (100), 106.0 (48.3); HRMS calcd for C₁₂H₁₂FN₄O₃ (MH⁺) 279.0893 found 279.0855.

N-*t*-Boc-Leu-linker-5-FU conjugate (**21**)

To a solution of *N*-*t*-Boc-Leu•H₂O (30 mg, 0.13 mmol) in 1 mL of CH₂Cl₂ cooled at –45 °C under argon atmosphere, were added *N*-methylpiperidine (15 μL, 0.121 mmol) followed by methyl chloroformate (10 μL, 0.121 mmol). After being stirred at this temperature for 10 min, the reaction mixture was added to 1-(2-aminobenzylcarbamoyl)-5-fluorouracil **20** (33 mg, 0.12 mmol), warmed up to room temperature and stirred for 48 h. After removal of the solvent *in vacuo*, the residue was subjected to flash column chromatography on silica gel eluted with ethyl acetate–petroleum ether (1:10 → 1:5 → 1:2) to afford 25 mg (42%) of **21** as a white foam. ¹H NMR (300 MHz, CDCl₃) δ 9.06 (br s, 0.5 H), 8.47 (d, 1 H, *J* = 7.2 Hz), 7.85 (d, 1 H, *J* = 8.1 Hz), 7.36–7.05 (m, 4 H), 5.05 (m, 1 H), 4.47 (m, 1 H), 4.34 (d, 2 H, *J* = 6.3 Hz), 1.80–1.70 (m, 3 H), 1.44 (s, 9 H), 1.02 (d, 6 H, *J* = 6.0 Hz); IR (KBr) 3424.4 (br), 2964.1, 1720.0, 1702.6, 1525.6, 1456.7, 1369.2, 1338.5, 1249.8, 1164.1, 1112.8, 1066.7, 762.1 cm⁻¹; MS (FAB, *m*-NBA) *m/z* (rel intensity): 492.3 (MH⁺, 8), 262.2 (13.3), 154.1 (100); HRMS calcd for C₂₃H₃₁N₅O₆F (MH⁺) 492.2258, found 492.2255.

N-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-linker-5-FU conjugate (**22**)

To a solution of *N*-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-OH **19** (300 mg, 0.5 mmol) in 15 mL of THF cooled at –25 °C under argon atmosphere, was added *N*-methylmorpholine (66 μL, 0.6 mmol) followed by methyl chloroformate (46 μL, 0.6 mmol). After being stirred at this temperature for 10 min, the reaction mixture was charged with a solution of 1-(2-aminobenzylcarbamoyl)-5-fluorouracil **44** (130 mg, 0.5 mmol) in 6 mL of THF, warmed up to room temperature and stirred for 18 h. After removal of the solvent *in vacuo*, the residue was subjected to flash column chromatography on silica gel eluted with acetone–hexanes (1:10 → 1:5 → 1:4 → 1:2) to afford 116 mg (54%) of **22** as a colorless oil. ¹H NMR (200 MHz, CDCl₃) δ 10.0 (m, 1 H), 9.51 (m, 1 H), 8.80 (s, 1 H), 8.40 (d, 1 H, *J* = 6.88 Hz), 7.72 (m, 1 H), 7.51–7.11 (m, 10 H), 6.83 (m, 1 H), 5.50 (m, 1 H), 4.61–4.23 (m, 7 H, singlet at 4.53), 4.13 (m, 1 H), 3.85–3.68 (m, 2 H), 1.90–1.67 (m, 6 H), 1.50–1.30 (m, 12 H), 0.98–0.82 (m, 12 H); MS (FAB, *m*-NBA) *m/z* (rel intensity): 875.42 (MNa⁺, 98), 853.44 (MH⁺, 15), 629.36 (100); HRMS calcd for C₄₂H₅₈N₈O₁₀F (MH⁺) 853.4260, found 853.4252.

H-Ser-Ala-Leu-Leu-linker-5-FU conjugate (23)

A solution of *N*-*t*-Boc-Ser(Obz)-Ala-Leu-Leu-linker-5-FU conjugate **22** (20 mg, 0.0235 mmol), 5 mg of 20% Pd(OH)₂ and cyclohexadiene (22 μ L, 0.235 mmol) in 1 mL of methanol was heated under refluxing for 1.0 h with stirring. The catalyst was removed by filtration. Removal of the solvent *in vacuo* afforded the crude intermediate containing free hydroxyl group. The residue was treated with a solution of 30% TFA in CH₂Cl₂ (0.5 mL) at room temperature for 30 min. Removal of the solvent *in vacuo* afforded **23** as a yellow oil. ¹H NMR (200 MHz, CD₃OD) δ 8.45 (d, 1 H, *J* = 6.24 Hz), 8.20 (m, 1 H), 7.75–7.20 (m, 9 H), 4.65–4.40 (m, 5 H, singlet at 4.61), 4.10 (m, 1 H), 3.90–3.70 (m, 3 H), 1.78–1.59 (m, 5 H), 1.39–1.24 (m, 6 H), 1.03–0.85 (m, 12 H); IR (KBr) 3424.1 (br), 2959.6, 2871.8, 1717.9, 1655.6, 1538.5, 1527.2, 1455.7, 1410.3, 1214.7, 1092.3, 753.8, 697.4 cm⁻¹; MS (ESI) *m/z* (rel intensity): 645.09 (M⁺–OH, 55), 623.20 (100).

Key Research Accomplishments

- 1) **Synthesis of protected Linker-Drug conjugates of 5-FU**
 - Synthesis of N^1 -[methyl (2-nitrobenzyl)carbamoyl]-5-fluorouracil **3**
 - Synthesis of N^1 -[methyl (2-nitrophenyl)carbamoyl]-5-fluorouracil **4**
- 2) **Synthesis of Peptide-Linker-Drug conjugate of 5-FU**
 - Synthesis of N^1 -[2-(H-Ser-Ala-Leu-Leu-amino)benzylcarbamoyl]-5-fluorouracil **23**
- 3) **Selective reduction and the kinetic analysis of the cyclization-activation process**
 - Selective reduction of N^1 -[methyl (2-nitrobenzyl)carbamoyl]-5-fluorouracil **3**
 - Selective reduction of N^1 -[methyl (2-nitrophenyl)carbamoyl]-5-fluorouracil **4**
 - Kinetic analysis of the cyclization-activation process of N^1 -[methyl (2-aminobenzyl)carbamoyl]-5-fluorouracil **8**

Reportable Outcomes

Bin Liu received her Master's degree in Pharmaceutical Sciences in December 2000. She was in part supported by this grant.

Some of this work and work done in the first year of support was published. The following is a list of publications as a result of research funded in part by this grant.

1. Chengzhi Yu, Bin Liu, and **Longqin Hu** A Simple One-Pot Procedure for the Direct Conversion of Alcohols to Azides via Phosphate Activation. *Org. Lett.* **2000**, 2(13), 1959-1961.
2. Chengzhi Yu, Bin Liu, and **Longqin Hu** A convenient biphasic process for the monosilylation of symmetrical 1,n-primary diols. *Tetrahedron Lett.* **2000**, 41(22), 4281-4285.
3. **Longqin Hu**, Bin Liu, and Douglas Hacking 5'-[2-(2-Nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine as a potential bioreductively activated prodrug of FUDR: synthesis, stability, and reductive activation. *Bioorg. Med. Chem. Lett.* **2000**, 10(8), 197-800.
4. Chengzhi Yu, Bin Liu, and **Longqin Hu** A modified procedure for the deprotection of methoxymethyl ether. *Tetrahedron Lett.* **2000**, 41(6), 819-822.

Conclusions

Two 5-FU Linker-Drug conjugates (**1** and **2**) were found to be unexpectedly unstable under physiological conditions. Thus, two new conjugates were designed and synthesized with modified urea linkers and were found to be stable under the same conditions tested. The new conjugates were synthesized in a protected form (NO₂) and chemically reduced to test the cyclization activation process. It was found that both new linker-drug conjugates of 5-FU could release the drug 5-FU upon conversion to the nucleophilic amino or hydroxylamino group. These two new linker systems will provide the basis for further conjugation with peptide and test the PSA-activation process. We also synthesized a **Peptide-Linker-Drug** conjugate of 5-FU using the unstable linker in **2**. The chemistry developed here would be useful in synthesizing the more stable conjugate of 5-FU using the new linkers developed in the past year.

Appendices

Reprints of the following publications.

1. Chengzhi Yu, Bin Liu, and **Longqin Hu** A Simple One-Pot Procedure for the Direct Conversion of Alcohols to Azides via Phosphate Activation. *Org. Lett.* **2000**, 2(13), 1959-1961.
2. Chengzhi Yu, Bin Liu, and **Longqin Hu** A convenient biphasic process for the monosilylation of symmetrical 1,n-primary diols. *Tetrahedron Lett.* **2000**, 41(22), 4281-4285.
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4. Chengzhi Yu, Bin Liu, and **Longqin Hu** A modified procedure for the deprotection of methoxymethyl ether. *Tetrahedron Lett.* **2000**, 41(6), 819-822.

A Simple One-Pot Procedure for the Direct Conversion of Alcohols to Azides via Phosphate Activation

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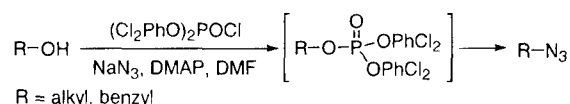
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ABSTRACT



A one-pot procedure was developed to prepare efficiently alkyl azides from alkanols using bis(2,4-dichlorophenyl) phosphate activation. 4-(Dimethylamino)pyridine was used as a base, and phosphorylpyridinium azide is believed to be the activating agent under this condition.

Conversion of an alcohol to its corresponding azide is an important functional group transformation in organic synthesis.¹ Although there are a variety of indirect methods reported in the literature, few direct azidation methods are known. Among the known direct methods, Mitsunobu displacement² using hydrazoic acid as the azide source³ proved to be the most efficient in transforming alkyl, benzylic, and allylic hydroxyls into their corresponding azides. However, the use of highly toxic hydrazoic acid limits the applicability of this method. Alternatives to hydrazoic acid include diphenyl phosphorazidate (DPPA)⁴ and zinc azide/bis-pyridine complex.⁵ Under these conditions, a substrate alcohol is mixed with diethyl azodicarboxylate and triphenyl phosphine prior to the addition of the azide reagent, a procedure that often leads to racemization and olefin formation. A more recent method reported by Thompson et al. for direct conversion of alcohols to azides uses DPPA

and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry toluene,⁶ where DBU acts as a base to help convert the alcohol to the corresponding phosphate intermediate and then, without isolation, the phosphate is displaced by the azide ion. However, few simple alkanols have been successfully converted to their corresponding azides using such direct methods.

In our efforts to synthesize anticancer prodrugs of phosphoramidate mustard and FUDR, we needed to convert the hydroxyl groups in compounds **1**, **2**, and **3** to their corresponding azides. Hydrazoic acid-assisted Mitsunobu reaction successfully converted compound **1** to its corresponding azide in 93% yield, while Thompson's procedure using DPPA/DBU in toluene failed. The latter procedure also failed to convert alkanol **2** and benzylic diol **3** to the desired azide products even upon heating to 45 °C; only the corresponding phosphates **4** and **5** were isolated. This prompted us to develop new direct procedures for the preparation of alkyl azides from such simple and less reactive alcohols. In this Letter, we wish to report a convenient one-pot procedure using bis(2,4-dichlorophenyl) phosphate as a good leaving group to activate hydroxyl groups for the direct conversion

(1) (a) Trost, B. M.; Fleming, I. *Comprehensive Organic Synthesis*; 1991; Pergamon Press: New York, Vol. VI, pp 76–79. (b) Hanessian, S. *Preparative Carbohydrate Chemistry*; Marcel Dekker: New York, 1997; Chapter 5, pp 90–97.

(2) (a) Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1972**, *94*, 679–680. (b) Hughes, D. L. *Org. React.* **1992**, *42*, 358–359.

(3) Loibner, H.; Zbiral, E. *Helv. Chim. Acta* **1977**, *60*, 417–425.

(4) Lal, B.; Pramanik, B. N.; Manhas, M. S.; Bose, A. K. *Tetrahedron Lett.* **1977**, 1977–1980.

(5) Viaud, M. C.; Rollin, P. *Synthesis* **1990**, 130–132.

(6) Thompson, A. S.; Humphrey, G. R.; Demarco, A. M.; Mathre, D. J.; Grabowski, J. J. *J. Org. Chem.* **1993**, *58*, 5886–5888.



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TETRAHEDRON
LETTERS

A convenient biphasic process for the monosilylation of symmetrical 1,*n*-primary diols

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Abstract

A simple and mild biphasic process was developed for the selective protection of one of two chemically equivalent primary hydroxyl groups in 1,*n*-diols using *t*-butyldiphenyl silyl chloride in diisopropyl ethyl amine and dimethyl formamide. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: selective protection; 1,*n*-diol; silylation; biphasic.

Selective derivatization/protection of one of two hydroxyl groups in the same molecule is a very important issue in organic synthesis.¹ Chemically non-equivalent hydroxyl groups such as primary, secondary, and tertiary hydroxy can be readily differentiated from each other by employing common protection and deprotection strategies. However, two chemically equivalent hydroxyl groups such as those in 1,*n*-primary diols are often difficult to differentiate. Derivatization of these diols with a stoichiometric amount of a reagent usually generates a mixture of the unreacted, the monoderivatized and the diderivatized diol in a statistical distribution of 1:2:1.² Selective protection of only one hydroxyl group in a 1,*n*-diol is difficult to achieve. It requires careful control of experimental conditions;³ the cleavage of cyclic intermediates;⁴ or the use of catalysts such as strongly acidic ion-exchange resins,^{1a} inorganic polymer supports,^{1b} and hydrolytic enzymes.⁵ All of these conditions resort to costly and time-consuming recycling procedures. One method of selective protection of 1,*n*-diols employs NaH/*t*-butyldimethyl silyl chloride (TBDMSCl) in THF.⁶ This method gives good yields for the primary diols tested, but the formation of sodium alkoxides in the presence of stoichiometric amounts of NaH requires careful manipulation. Besides, this condition is too strong to be compatible with diols containing base-sensitive functional groups. Therefore, there is still a need to develop a mild and convenient method for the selective protection of 1,*n*-diols.

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References

- (a) Nishiguchi, T.; Fujisaki, S.; Ishii, Y.; Yano, Y.; Nishida, A. *J. Org. Chem.* **1994**, *59*, 1191–1195; (b) Leznoff, C. C. *Acc. Chem. Res.* **1978**, *11*, 327–333; (c) Kata, Y.; Fujiwara, Y.; Asano, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3207–3210. (d) Lalonde, M.; Chan, T. H. *Synthesis* **1985**, 817–845.
- (a) Raederstorff, D.; Shu, A. Y. L.; Thompson, J. E.; Djerassi, C. *J. Org. Chem.* **1987**, *52*, 2337–2346; (b) Kulkarni, B. A.; Chattopadhyay, S.; Chattopadhyay, A.; Mamdapur, V. R. *J. Org. Chem.* **1993**, *58*, 5964–5966.
- (a) Wilkinson, C. G. In *Comprehensive Organic Chemistry*; Stoddart, J. F., Ed.; Pergamon Press: New York, 1979; Vol. 1, pp. 681–687; (b) Furhop, J.; Penzlin, G. *Organic Synthesis*; Verlag Chemie: Weinheim, 1983; pp. 143–151; (c) Ogawa, H.; Chihara, T.; Taya, K. *J. Am. Chem. Soc.* **1985**, *107*, 1365–1369.
- (a) Murahashi, S.; Oda, Y.; Naota, T. *Chem. Lett.* **1992**, 2237, and references cited therein; (b) Griffin, B. E.; Jarman, M.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2301–2313; (c) Ortuño, R. M.; Mercé, R.; Font, J. *Tetrahedron Lett.* **1986**, *27*, 2519–2522.
- (a) Terao, O.; Akamatsu, M.; Achiwa, K. *Chem. Pharm. Bull.* **1991**, *39*, 823–825; (b) Houille, O.; Schmittberger, T.; Uguen, D. *Tetrahedron Lett.* **1996**, *37*, 625–628; (c) Farquhar, D.; Khan, S.; Wilkerson, M. C.; Anderson, B. S. *Tetrahedron Lett.* **1995**, *36*, 655–658.
- McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. D. *J. Org. Chem.* **1986**, *51*, 3388–3390.
- Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley-Interscience: New York, 1999; Chapter 2, pp. 141–142.
- The conditions we tried but failed to give any silylated products include: (a) TBDPSCI (1.05 equiv.), DIEA (10 equiv.), CH₂Cl₂, rt, 36 h; (b) TBDPSCI (1.05 equiv.), Et₃N (10 equiv.), CH₂Cl₂, rt, 24 h; (c) TBDPSCI (1.05 equiv.), Et₃N (10 equiv.)/DMAP (0.05 equiv.), CH₂Cl₂, rt, 24 h; (d) TBDPSCI (1.05 equiv.), DMAP (10 equiv.), CH₂Cl₂, rt, 24 h; (e) TBDPSCI (1.05 equiv.), pyridine, rt, 24 h; (f) TBDPSCI (1.05 equiv.), imidazole (5 equiv.), DMF, rt, 24 h; (g) TBDPSCI (1.05 equiv.), NaH (1 equiv.), THF, 0°C, rt, 3 h.
- Ritchie, C. D.; Lu, S.-Z. *J. Am. Chem. Soc.* **1989**, *111*, 8542–8543.
- In a typical procedure, DIEA (10 mmol, 1.7 mL) and DMF (3 mL) form two immiscible solution phases at room temperature. Using NMR, we found that the top DIEA phase contained about 18% DMF (mol) and the bottom DMF phase contained about 16% DIEA. TLC analysis indicated that reactants used in our experiments were mainly in the bottom DMF phase. We also found that using saturated DIEA solution in DMF as the reaction media decreased both the reaction yield and the selectivity of silylation.
- Acetone–hexane was found to be a better eluting solvent system than the commonly used ethyl acetate/hexane for the purification and structure assignment of the monosilylated 1,*n*-primary diols, since ethyl acetate can be strongly retained in compounds containing hydroxyl groups and it is hard to remove completely in vacuo.
- Selected ¹H NMR (200 MHz, CDCl₃) data: δ ppm compound **1**: 7.60 (dd, 2H, *J* = 7.5, 1.3 Hz), 7.41–7.26 (m, 3H), 4.13–3.89 (m, 6H), 3.08 (t, 2H, *J* = 6.6 Hz), 1.98 (br s, 2H, OH), 1.18 (t, 3H, *J* = 7.1 Hz); compound **2**: 7.76–7.20 (m, 15H), 4.24–4.12 (m, 2H), 4.03–3.90 (m, 4H), 2.55 (dd, 1H, *J* = 5.7, 4.0 Hz, OH), 1.08 (t, 3H, *J* = 7.8 Hz), 1.06 (s, 9H); compound **3**: 7.76–7.67 (m, 10H), 7.44–7.26 (m, 15H), 4.36 (Abq, 2H, Δ*γ* = 14.0 Hz, *J* = 9.5 Hz), 3.65 (q, 2H, *J* = 6.8 Hz), 1.05 (s, 18H), 0.82 (t, 3H, *J* = 7.1 Hz); *mono*-TBDPS-**4**: 7.78–7.73 (m, 4H), 7.49–7.43 (m, 6H), 3.85–3.80 (m, 2H), 3.74 (br s, 2H), 2.45 (br s, 1H, OH), 1.15 (s, 9H); *mono*-TBDPS-**5**: 7.75–7.70 (m, 4H), 7.45–7.42 (m, 6H), 3.88 (t, 2H, *J* = 5.6 Hz), 3.87 (t, 2H, *J* = 5.8 Hz), 2.54 (br s, 1H, OH), 1.80 (q, 2H, *J* = 5.7 Hz), 1.10 (s, 9H); *mono*-TBDPS-**6**: 7.82–7.68 (m, 4H), 7.52–7.38 (m, 6H), 3.60 (d, 2H, *J* = 5.5 Hz), 3.56 (s, 2H), 2.68 (t, 1H, *J* = 5.5 Hz, OH), 1.42–0.75 [m, 23H, including 1.12 (s, 9H), 0.92 (t, 3H, *J* = 6.1 Hz), 0.81 (t, 3H, *J* = 6.5 Hz)]; *mono*-TBDPS-**7**: 7.70–7.65 (m, 4H), 7.45–7.39 (m, 6H), 3.53–3.33 (m, 4H), 1.85 (br s, 2H, NH₂), 1.09 (s, 9H), 1.04 (s, 3H); *mono*-TBDPS-**8**: 7.83–7.79 (m, 4H), 7.50–7.45 (m, 4H), 7.33 (br s, 3H), 4.89 (s, 2H), 4.73 (s, 2H), 3.20 (br s, 1H, OH), 1.17 (s, 9H); *mono*-TBDPS-**9**: 7.76–7.71 (m, 4H), 7.45–7.41 (m, 6H), 3.73 (t, 2H, *J* = 6.2 Hz), 3.64 (t, 2H,

- $J = 5.5$ Hz), 2.05 (br s, 1H, OH), 1.68–1.33 (m, 6H), 1.12 (s, 9H); *mono*-TBDPS-10: 7.75–7.70 (m, 4H), 7.44–7.41 (m, 6H), 3.71 (t, 2H, $J = 6.3$ Hz), 3.66 (Abq, 2H, $\Delta\gamma = 12.2$ Hz, $J = 6.6$ Hz), 1.75 (br s, 1H, OH), 1.61–1.55 (m, 4H), 1.44–1.31 (m, 6H), 1.10 (s, 9H); *mono*-TBDPS-11: 7.73–7.68 (m, 4H), 7.43–7.39 (m, 6H), 3.69 (t, 2H, $J = 6.5$ Hz), 3.65 (t, 2H, $J = 6.4$ Hz), 1.65–1.50 (m, 4H), 1.48–1.25 (m, 10H), 1.08 (s, 9H).
13. (a) Nicolaou, K. C.; Claiborne, C. F.; Paulvannan, K.; Postema, M. H. D.; Guy, R. K. *Chem. Eur. J.* **1997**, *3*, 399–409; (b) Nemoto, H.; Ishibashi, H.; Nagamochi, M.; Fukumoto, K. *J. Org. Chem.* **1992**, *57*, 1707–1712; (c) Feixas, J.; Capdevila, A.; Guerrero, A. *Tetrahedron* **1994**, *50*, 8539–8550; (d) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4743–4763.



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BIOORGANIC &
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5'-[2-(2-Nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine as a Potential Bioreductively Activated Prodrug of FUDR: Synthesis, Stability and Reductive Activation

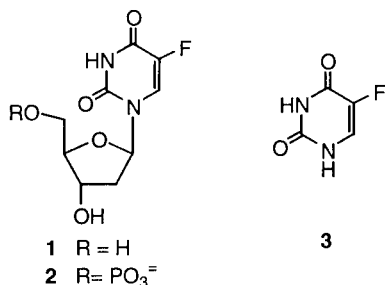
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Abstract—5'-[2-(2-Nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine was synthesized as a potential bioreductively activated prodrug of 5-fluoro-2'-deoxyuridine (FUDR). The target compound was stable in both phosphate buffer and human serum and was found to release quickly the parent drug FUDR in quantitative yield upon mild chemical reduction. © 2000 Elsevier Science Ltd. All rights reserved.

5-Fluoro-2'-deoxyuridine (FUDR, **1**) is a fluoropyrimidine nucleoside used in the treatment of a variety of tumors.¹ It exerts its anticancer effects mainly by suppression of DNA synthesis. It is converted by a single thymidine kinase to the active metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate (**2**), which disrupts DNA synthesis via its inhibition of thymidylate synthase and its incorporation into DNA.² The simplicity of this pathway provides a strong rationale for the use of FUDR in preference to 5-fluorouracil (5-FU, **3**), which undergoes extensive metabolism to both active fluoropyrimidine nucleotides and other inactive degradation products.² Unfortunately, FUDR has not shown consistently superior therapeutic results.³ It suffers from a number of drawbacks including high toxicity, rapid blood clearance, rapid conversion to 5-FU, poor oral activity and lack of selectivity.



Because of the primitive state of tumor vasculature, solid tumors often develop regions of chronic or acute hypoxia as a result of chronic or transient deficiencies of blood flow. Such oxygen deficiency often leads to resistance to ionizing radiation and to many chemotherapeutic drugs.^{4–6} Hypoxia also appears to accelerate malignant tumor progression and increase metastasis.⁶ Hypoxic tumor cells are known to have a greater capacity for reductive reactions as compared to well-oxygenated normal cells.⁶ This unique feature of solid tumors provides an attractive target for selective anticancer chemotherapy. Several bioreductively activated nitro compounds, quinones and aromatic *N*-oxides are currently in clinical trials as hypoxia-selective cytotoxins and could potentially be developed into selective anticancer prodrugs.⁷

Prodrug design is an important strategy that has been proven to work for many drugs in improving their undesirable physico-chemical and biological properties.^{8–10} Several strategies based on intramolecular cyclization reactions have been reviewed.¹¹ Recently, prodrug strategies have also been used in targeted drug delivery including antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT). In these approaches, an enzyme is delivered site-specifically by chemical conjugation or genetic fusion to a tumor specific antibody or by enzyme gene delivery systems into tumor cells. This is then followed by the administration of a prodrug, which is selectively activated by the delivered enzyme at the tumor cells. A number of these systems are in development and have been reviewed.^{12–16} Among the enzymes

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under evaluation is a bacterial nitroreductase from *Escherichia coli* B. This FMN-containing flavoprotein is capable of reducing certain aromatic nitro groups to the corresponding amines or hydroxylamines in the presence of a cofactor NADH or NADPH.^{17–19}

To improve the oral bioavailability and tumor-selectivity of FUDR, we designed and synthesized 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine (**4**) as a bioreductively activated FUDR prodrug that could be used to target hypoxic cells or used in the enzyme prodrug therapies mentioned above. Scheme 1 shows the potential mechanism of activation of the target compound **4**. After reduction in the hypoxic tumor cells or by an enzyme such as the bacterial nitroreductase, the resulting hydroxylamine **5a** (R=OH) or amine **5b** (R=H) could undergo facile cyclization reaction forming the lactam **6a** or **6b** and, at the same time, releasing the active drug FUDR (**1**). The two methyl groups attached to the α -position of the carbonyl are designed to restrict the rotational freedom of the conformation of the molecule and place the carbonyl group in a more favorable position with respect to the nucleophilic amine or hydroxylamine.²⁰ In addition, the two methyl groups might inhibit the esterase-catalyzed hydrolysis reaction of the ester bond and provide the necessary stability in human serum, which is required for the compound to be useful. In this communication, we report the synthesis, stability in phosphate buffer and in human serum, and the reductive activation of target compound **4**.

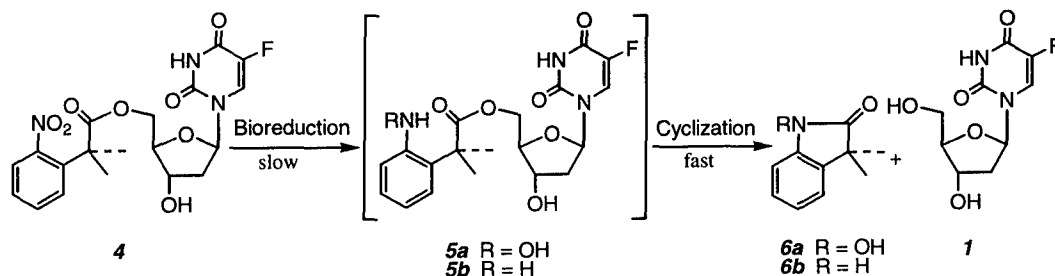
Chemical Synthesis

The synthesis of 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine (**4**) is shown in Scheme 2 starting from the commercially available 2-nitrophenylacetic acid (**7**). After treatment with thionyl chloride

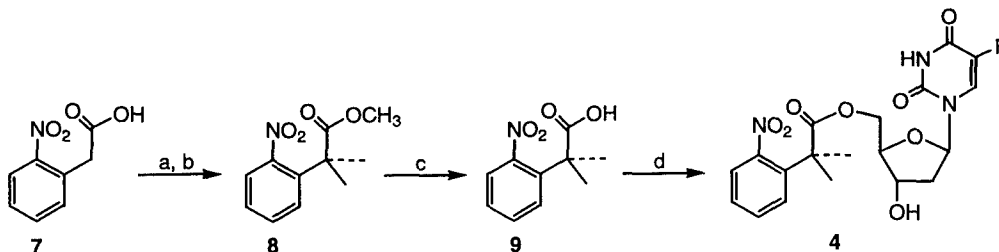
(SOCl₂) in methanol, the corresponding methyl ester was dialkylated using methyl iodide and sodium hydride in the presence of catalytic amount of 18-crown-6 to give methyl 2-nitrophenyl-2-methylpropionate (**8**). Sodium hydroxide-mediated hydrolysis converted the methyl ester **8** to the corresponding acid **9**. Coupling of the acid **9** to the 5'-primary hydroxyl group of FUDR was accomplished by a Mitsunobu reaction (treatment with diethyl azodicarboxylate (DEAD) and triphenyl phosphine (PPh₃)).^{21,22} A major advantage of this approach is that the condensation reaction can be effected under mild and neutral conditions. Furthermore, the reaction is selective for the 5'-primary hydroxyl over the 3'-secondary hydroxyl of the 2'-deoxyribonucleosides, thus avoiding the requirement for protection and subsequent deprotection of the 3'-hydroxyl group.²¹ All new compounds were fully characterized by ¹H NMR and high-resolution MS.²³

Stability Test

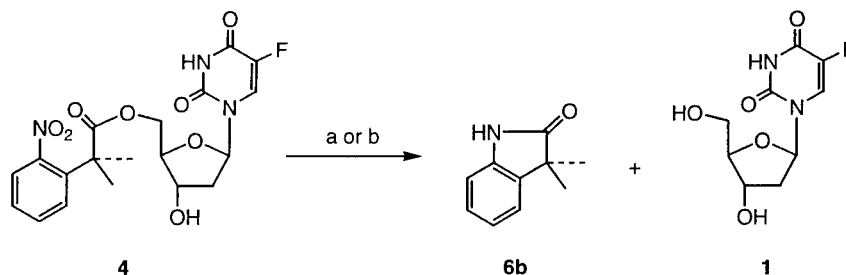
One concern in using compound **4** as a prodrug is the presence of the ester linkage that might make it vulnerable to hydrolysis by esterases present in the blood. We, therefore, tested the stability of compound **4** by incubating it in sodium phosphate buffer (100 mM, pH 7.4) and in human serum at 37°C. Acetonitrile was used to help dissolve compound **4** and the concentration of acetonitrile in the incubation mixture was kept below 5%. At different time intervals, aliquots (25 μ L) were withdrawn and analyzed by HPLC on a C₁₈ reversed phase column using acetonitrile/water/TFA as the mobile phase. In the case of human serum, the esterase activity was quenched with 7% HClO₄ (100 μ L) immediately after the aliquots were withdrawn. Our results indicate that compound **4** is stable in both phosphate buffer and human serum and no significant hydrolysis was observed after three days of incubation. Derivatives



Scheme 1. Proposed mechanism of activation of the FUDR prodrug **4**.



Scheme 2. Synthesis of FUDR prodrug **4**. (a) SOCl₂, MeOH (97%); (b) MeI, NaH, 18-Crown-6, 5°C (89%); (c) NaOH, MeOH (90%); (d) DEAD, PPh₃, FUDR, dioxane, rt (57%).



Scheme 3. Chemical reduction of FUDR prodrug **4**. (a) H_2 balloon, 10% Pd/C, MeOH, h to overnight; (b) NaBH_4 , 10% Pd/C, MeOH- H_2O , 20 min.

without the two methyl groups at the α -position of the ester carbonyl or without the FUDR moiety (such as in a methyl ester) showed the same stability in phosphate buffer, but were significantly less stable than compound **4** in human serum (data not shown). We attribute this unusual stability of the ester bond in **4** to the steric hindrance provided by the two methyl groups α to the ester carbonyl as well as the deoxy-ribose sugar ring in FUDR.

Chemical Reduction

To test the feasibility of reductive release of the anticancer drug FUDR from compound **4**, we selected two mild chemical reduction conditions that mimic the bioreduction in hypoxic tumor cells and the enzymatic action of a nitroreductase. One was hydrogenation under normal atmospheric pressure in the presence of 10% Pd/C and the other was sodium borohydride (NaBH_4) reduction in the presence of 10% Pd/C.²⁴ Both reduction conditions were mild and neutral; and both would allow selective reduction of the nitro group without affecting other functional groups in the molecule. Both of these chemical reduction methods would only convert the nitro group to the final amino group. Bioreduction in hypoxic tumor cells or reduction by a nitroreductase would more likely stop at the intermediate hydroxylamine before reaching the final amino product. Kinetic studies have shown that cyclization of 2-nitroarylamides via the hydroxylamine intermediate is actually faster than that via the amino product.^{25,26} Thus, the hydrogenation and NaBH_4 reduction reactions are simple, practical chemical tests of the cyclization-activation system.

Hydrogenation was found to be slower than sodium borohydride reduction. The former required hours of incubation to complete the reaction while the latter took only 20 min to finish. The only isolated products under both reduction conditions were the lactam **6b** and the parent drug **1** (Scheme 3). Both products were identified by ^1H NMR, MS and by comparison with authentic samples.²⁷ It should be noted that both reduction conditions were very clean and that the presumed intermediate **5b** was not observed. When we used the filtrate directly after partial hydrogenation (4 min) to test the cyclization process in pH 7.4 phosphate buffer at 37°C , the cyclization was so fast that within seconds the absorbance at 249 nm became constant, making the

calculation of a kinetic constant impossible. On the other hand, in a derivative without the two methyl groups, the cyclization process can be accurately monitored by observing the change in absorbance at 249 nm and has a calculated half life of 14 min at 37°C (data not shown). These results suggest that the two methyl groups at the α -position of the ester carbonyl serve to restrict the rotational freedom of the conformation of the molecule and facilitate the intramolecular cyclization process as proposed in Scheme 1.

In summary, 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine (**4**) was synthesized as a potential prodrug of FUDR to target hypoxic tumor cells or to be activated by a nitroreductase. Compound **4** is stable and resists the hydrolysis by human serum esterases present in the blood. The release of FUDR from **4** is very fast after conversion of the nitro group to the amino group. Compound **4** could potentially be used to treat hypoxic solid tumors or used in combination with a nitroreductase in antibody-directed or gene-directed enzyme prodrug therapy to improve the physico-chemical properties and the therapeutic effectiveness of FUDR in the treatment of cancer. Further work is underway to study its cytotoxicity towards hypoxic cancer cells or cancer cells in the presence of a nitroreductase and NAD(P)H.

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References and Notes

- Grem, J. L. In *Cancer Chemotherapy: Principles and Practice*; Chabner, B. A.; Collins, J. M., Ed.; J. B. Lippincott: Philadelphia, 1990; pp 180–224.
- Daher, G. C.; Harris, B. E.; Diasio, R. B. In *Anticancer Drugs: Antimetabolite Metabolism and Natural Anticancer Agents*; Powis, G., Ed.; Pergamon: New York, 1994; pp 55–94.

3. Chabner, B. A. In *Pharmacologic Principles of Cancer Treatment*; Chabner, B. A., Ed.; W. B. Saunders Company: Philadelphia, 1982; pp 183–212.
4. Tercei, M.; Wilson, W. R.; Anderson, R. F.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 1084.
5. Wilson, W. R. In *Cancer Biology and Medicine*; Waring, M. J.; Ponder, B. A. J., Ed.; Kluwer Academic: Lancaster, 1992; 3, pp 87–131.
6. Brown, J. M. *Cancer Res.* **1999**, *59*, 5863.
7. Siim, B. G.; Atwell, G. J.; Anderson, R. F.; Wardman, P.; Pullen, S. M.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1381.
8. Pochopin, N. L.; Charman, W. N.; Stella, V. J. *Int. J. Pharmaceut.* **1995**, *121*, 157.
9. Oliyai, R.; Stella, V. J. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *32*, 521.
10. Bundgaard, H. *Design of Prodrugs*; Elsevier: Amsterdam, 1985; p 357.
11. Shan, D.; Nicolaou, M. G.; Borchardt, R. T.; Wang, B. J. *Pharm. Sci.* **1997**, *86*, 765.
12. McNeish, I. A.; Searle, P. F.; Young, L. S.; Kerr, D. J. *Adv. Drug Delivery Rev.* **1997**, *26*, 173.
13. Niculescu-Duvaz, I.; Springer, C. J. *Adv. Drug Delivery Rev.* **1997**, *26*, 151.
14. Senter, P. D.; Svensson, H. P. *Adv. Drug Delivery Rev.* **1996**, *22*, 341.
15. Sherwood, R. F. *Adv. Drug Delivery Rev.* **1996**, *22*, 269.
16. Springer, C. J.; Niculescu-Duvaz, I. *Adv. Drug Delivery Rev.* **1996**, *22*, 351.
17. Bridgewater, J.; Minton, N. P.; Michael, N. P.; Knox, P.; Springer, C.; Collins, M. *Eur. J. Cancer* **1995**, *31*, 2362.
18. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Coles, B.; Friedlos, F.; Knox, R. J. *Biochem. Pharmacol.* **1992**, *44*, 2289.
19. Knox, R. J.; Friedlos, F.; Sherwood, R. F.; Melton, R. G.; Anlezark, G. M. *Biochem. Pharmacol.* **1992**, *44*, 2297.
20. Atwell, G. J.; Sykes, B. M.; O'Connor, C. J.; Denny, W. A. *J. Med. Chem.* **1994**, *37*, 371.
21. Mitsunobu, O. *Synthesis* **1981**, *1*, 1.
22. Farquhar, D.; Chen, R.; Khan, S. J. *J. Med. Chem.* **1995**, *38*, 488.
23. For compound **4**: ^1H NMR (CDCl_3): δ 1.67 (3H, s, CH_3), 1.72 (3H, s, CH_3), 2.22–2.38 (2H, m, $2'\text{-CH}_2$), 4.11–4.48 (4H, m, $3'\text{-CH}$, $4'\text{-CH}$, $5'\text{-CH}_2$), 6.18 (1H, t, $1'\text{-CH}$), 7.37–7.65 (4H, m, Ar-H), 7.96 (1H, d, $J=7.8$ Hz, 6-CH), 9.09 (b, 1H, -CONHCO-); MS (FAB $^+$): m/z (relative intensity) 438 (MH^+ , 5.4), 308 (13.5), 289 (7.9); HRMS (FAB $^+$) [MH^+] calcd for $\text{C}_{19}\text{H}_{21}\text{FN}_3\text{O}_8$: 438.1313; found: 438.1321.
24. Berry, J. M.; Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1147.
25. Sykes, B. M.; Atwell, G. J.; Denny, W. A.; O'Connor, J. *J. Phys. Org. Chem.* **1995**, *8*, 587.
26. Sykes, B. M.; Atwell, G. J.; Denny, W. A.; McLennan, D. J.; O'Connor, C. J. *J. Chem. Soc. Perkin Trans. 2* **1995**, 337.
27. For compound **6b**: ^1H NMR (CDCl_3): δ 1.41 (6H, s, $\text{C}(\text{CH}_3)_2$), 6.91–7.26 (4H, m, Ar-H), 8.20 (1H, b, -CONH-); MS (EI): m/z (relative intensity) 161 (M^+ , 100), 146 (48); HRMS (FAB $^+$) [MH^+] calcd for $\text{C}_{10}\text{H}_{12}\text{NO}$: 162.0919; Found: 162.0898.

A modified procedure for the deprotection of methoxymethyl ether

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Abstract

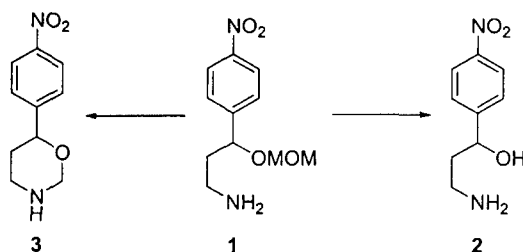
A new modified procedure using a combination of catechol boron bromide with acetic acid was developed to deprotect methoxymethyl group to form 1,3-diols and 1,3-aminoalcohols. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: methoxymethyl ether; catechol boron bromide; deprotection; formyl acetal.

Alkoxymethyl ethers are widely used to protect hydroxyl groups in organic synthesis. They are easily introduced under very mild conditions and are quite stable even in the presence of strong acids and bases. Methoxymethyl ether (MOM) group is the most robust of the alkoxymethyl ethers and plays a pivotal role in protecting group chemistry. A variety of conditions are available for MOM cleavage;¹ however, there are often problems deprotecting MOM due to the unique structural features and functionalities in the substrate. One such problem is the formation of cyclic formyl acetals when a nucleophilic hydroxy or amino group is nearby.²

During our study of anticancer prodrug cyclophosphamides, we wanted to prepare 1,3-aminoalcohol **2** by removing the MOM protecting group in **1**. A number of common conditions were attempted but failed to afford the desired product **2**. Instead, compound **3**³ was found to be the major product after flash silica gel column chromatography. The conditions we tried include: (a) catechol boron bromide (CBB), CH₂Cl₂, –78°C→0°C, 2 h, 87%; (b) HCl, MeOH, 0°C→rt, 30 min, complex; (c) (CH₃)₃SiBr, CH₂Cl₂, 0°C, 1.5 h, 67%; (d) PhSH, BF₃·OEt₂, CH₂Cl₂, 1 h, 61%; (e) TsOH·2H₂O, toluene, reflux, 45 min, 55% (yields given are for compound **3** only). The difficulty in obtaining the 1,3-aminoalcohol **2** prompted us to develop a new procedure to cleave MOM protected 1,3-aminoalcohols or mono-MOM protected 1,3-diols.

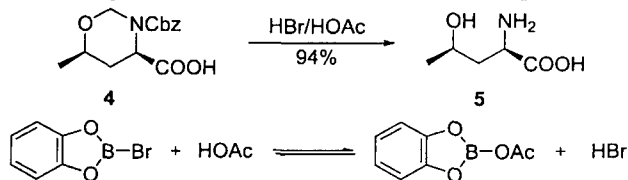
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Under common deprotection conditions, the formation of **3** could be the result of a facile acetal–acetal exchange process where the primary amino group at the β position relative to the MOM ether was nucleophilic and could attack the methylene electrophilic carbon forming the entropically-favored six-membered ring. Similar examples reported in the literature showed that this could also occur when cleaving MOM groups with nearby hydroxyl groups.²

To our knowledge, the only method that can circumvent this problem is using $(i\text{-PrS})_2\text{BBr}$ in methanol.⁴ Deprotection with $(i\text{-PrS})_2\text{BBr}$ affords 1,2 and 1,3-diols without forming the formyl acetals in a single step. Catechol boron halides, particularly the bromide, are similar to $(i\text{-PrS})_2\text{BBr}$ and have been shown to be more effective in cleaving MOM ethers in recent reports.⁵ They are also more selective in multifunctional substrates. According to our experimental results, treatment of **1** using catechol boron bromide gave compound **3** with the highest yield (87%). Therefore, it was reasonable for us to focus on this reagent and explore further the conditions needed to effect the desired deprotection and at the same time convert the cyclic formyl acetal to the desired product.

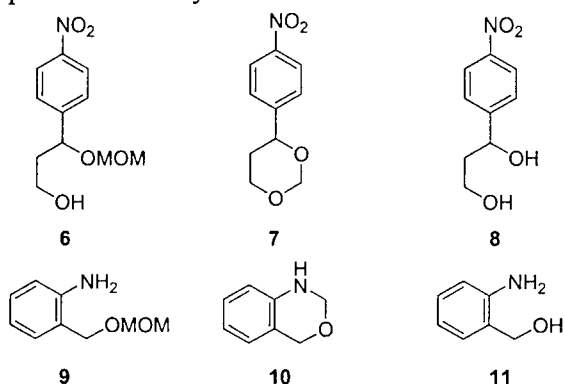
From the literature, we found a procedure using hydrogen bromide in acetic acid to cleave benzyl-oxycarbonyl (Cbz) protecting group while concurrently hydrolyzing the formyl acetal functionality in compound **4** to produce the erythro- γ -hydroxynorvaline **5**.⁶ We reasoned that if acetic acid was added to a mixture of **1** and excess catechol boron bromide, hydrogen bromide would be formed quickly (as depicted below), due to the affinity of electrophilic boron(III) species for acetate anion. In this instance, the reactive hydrogen bromide might cleave the formyl acetal in **3** to produce the desired product **2**.



Based upon this hypothesis, we reinvestigated the deprotection of **1** and found that the deprotection worked very well using the following one-pot procedure: (i) a solution of substrate **1** (1.0 g, 4.2 mmol) in dry methylene chloride (25 mL) was cooled down to -78°C and treated with catechol boron bromide (2.5 equiv., 2.1 g) in methylene chloride (25 mL); (ii) the reaction proceeded for 2 h at -78°C and was allowed to warm up to -20°C before glacial acetic acid (5 equiv., 1.2 mL) was added. The reaction mixture was stirred at ambient temperature for an additional 5 h; (iii) chloroform (100 mL) and 3N aqueous sodium hydroxide (50 mL) were added to quench the reaction, the organic phase was washed with 3N aqueous sodium hydroxide (3×30 mL) until the aqueous phase became colorless and clear. The organic phase was then washed with brine and dried over anhydrous magnesium sulfate. After removal of organic solvents under vacuum, the crude product was purified by flash silica gel column chromatography (chloroform saturated with ammonium hydroxide: methanol, 9:1–8:1) to give **2** (0.63 g, 77%) as a colorless oil, whose structure was confirmed by ^1H NMR, IR and high resolution FAB-MS.⁷ Using thin layer chromatography to monitor the reaction process, we clearly observed that **3** was formed in step (i) and then disappeared quickly in step (ii) with the concomitant appearance of the more polar compound **2**.

This result suggests that the formyl acetal formed during the Lewis acid-catalyzed deprotection of MOM can be completely hydrolyzed by enhancing the acidity of the medium through the addition of acetic acid and excess CBB. It is believed that acetic acid reacted with CBB to produce hydrogen bromide, which is the reagent responsible for the eventual cleavage of cyclic formyl acetal intermediates.

To demonstrate the capability of our new procedure, we compared the two conditions (CBB/CH₂Cl₂, -78°C→0°C versus CBB/CH₂Cl₂ followed by HOAc, -78°C→rt) in the deprotection of compounds **6** and **9**. It was found that CBB deprotection of **6** gave cyclic formal acetal **7** in 25% yield and 1,3-diol **8** in 74% yield while our modified procedure using CBB followed by the addition of acetic acid gave compound **8** as the only product in 96% yield after isolation. Similarly, CBB deprotection of **9** gave a mixture of **10** (28%) and **11** (71%) while CBB followed by acetic acid afforded compound **11** as the only product in 91% yield after isolation.⁸ The difference between these parallel experiments indicates that our modified procedure is better than CBB alone in deprotecting MOM groups when there is another nucleophilic functional group nearby.



In conclusion, the combination of catechol boron bromide with acetic acid effectively cleaves MOM groups, especially in cases where a neighboring hydroxy or amino group might prevent the formation of the desired product using commonly available procedures. Our one-pot procedure presented here is mild and convenient and should be useful in the synthesis of complex natural products.

Acknowledgements

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References

- Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed., Wiley-Interscience: New York, 1999; Chapter 2, pp. 29–33.
- Barot, B. C.; Pinnick, H. W. *J. Org. Chem.* **1981**, *46*, 2981–2983.
- Selected physical and spectral data for compound **3**: IR (neat): ν/cm^{-1} 3200, 2870, 1575, 1485, 1405, 1360, 1300, 1250, 1080, 1000, 920, 750, 675; ¹H NMR (300 MHz, CDCl₃): δ/ppm 8.22–8.20 (m, 2H), 7.70–7.65 (m, 2H), 5.00–4.95 (m, 1H), 4.61 (d, J=6.8 Hz, 1H), 4.50 (d, J=6.8 Hz, 1H), 3.40–3.10 (m, 2H), 2.10–1.85 (m, 2H); MS (FAB, 3NBA): m/z 209.0 (MH⁺, 3.8).

4. Corey, E. J.; Hua, D. H.; Seitz, S. P. *Tetrahedron Lett.* **1984**, 25, 3–6.
5. (a) Paquette, L. A.; Gao, Z.; Ni, Z.; Smith, G. F. *Tetrahedron Lett.* **1997**, 38, 1271–1274; (b) Boeckman, R. K.; Potenza, J. C. *Tetrahedron Lett.* **1985**, 26, 1411–1414.
6. (a) Harding, K. E.; Marman, T. H.; Nam, D.-H. *Tetrahedron Lett.* **1988**, 29, 1627–1630; (b) Bodanszky, M.; Vigneaud, V. *J. Am. Chem. Soc.* **1959**, 81, 5688–5691.
7. Selected physical and spectral data for compound **2**: mp (CHCl₃–MeOH): 126–127.5°C; IR (KBr): ν/cm^{-1} 3330, 3260, 3100, 2880, 2850, 1575, 1490, 1400, 1330, 1300, 1275, 1085, 1075, 1050, 1000, 935, 810, 730, 680; ¹H NMR (300 MHz, CDCl₃): δ/ppm 8.13 (dd, *J*=2.0, 6.9 Hz, 2H), 7.52–7.47 (m, 2H), 5.03 (dd, *J*=2.7, 8.7 Hz, 1H), 3.12–3.06 (m, 1H), 3.07–2.92 (m, 1H), 1.99–1.81 (m, 1H), 1.67–1.41 (m, 1H); MS (FAB, 3NBA): *m/z* 197.1 (MH⁺, 30.5), 181.0 (M–OH, 1.8); HR FAB-MS: calcd for C₉H₁₃N₂O₃ (M+1) 197.0926, found 197.0939.
8. Yields were not optimized.



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