JOC Article

Synthesis, Isolation, and Characterization of 2′-Paclitaxel Glycinate: An Application of the Bsmoc Protecting Group

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The relevance of the Bsmoc protecting group in the synthesis of moisture- and base-sensitive compounds has been demonstrated by the preparation of 2′-paclitaxel glycinate with use of the solid anhydrous base, 4-piperidinopiperidine in DCM solvent.

The modification of paclitaxel (**1**), a water-insoluble and highly effective anticancer agent,[1] as a 2′-α, *β*, or *γ*-amino acid ester to afford water-soluble acid salts as prodrugs has been carried out by several groups.[2−4] The overriding features observed for the α-series is their instability toward nucleophiles and difficult isolation in a pure state. Modifications employing hindered amino acids such as alanine or α-dimethylamino glycine have yielded the desired paclitaxel esters (prodrugs) only after extensive purification and concomitant loss of yield. The general instability of these esters was attributed to the inductive effect of the (protonated) amino moieties, which aids nucleophilic attack at the 2′-acyl group. In support of this hypothesis, the *β*-series demonstrated greater stability.[2]

It was of interest to us to obtain a pure sample of paclitaxel-2′-glycinate for direct condensation with PEG derivatives, and since the use of the BOC, TCEC, and CBZ protecting groups in the ester synthesis had already been exploited without success for this compound, we focused on a reported method that utilized the Fmoc protecting group, which could be removed under basic conditions.[4] Thus, condensing paclitaxel with Fmoc-glycine in the presence of EDC and DMAP led to the formation of the intermediate, Fmoc-2′-paclitaxel glycinate (**3**). Fmoc deprotection usually requires large amounts of secondary amines, such as piperidine or morpholine.[5] In our hands, complete deprotection of Fmoc from **3** was achieved by using the relatively mild base DMAP (a 3° amine).[5] Thus, removal of the Fmoc group in this case was accomplished by employing 20 equiv of DMAP at 40 °C for 2 h in a DMF/DCM mixed solvent system (1:4 v/v) (Scheme 1). The desired product, **4**, could be detected as a single peak by HPLC. (See Experimental Section.) However, when attempts were made to isolate **4** from the highly basic reaction mixture, 30% decomposition to paclitaxel occurred.

The 1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) amino protecting group recently developed by Carpino and co-workers[6,7] can also be removed by treatment with a 2° amine via a Michael addition that is carried out under extremely mild conditions and results in conversion of the 2° amine into a substituted 3° amine.

Again, using EDC as the coupling reagent, Bsmoc-glycine (**5**)[8] was coupled to paclitaxel without difficulty to give Bsmoc-gly-2′-paclitaxel (**6**) in high yield and purity (>98%). Excess secondary amines (at least 4 equiv), especially piperidine, have previously been used for the deprotection of the Bsmoc group.[7] Compared to the removal of the Fmoc group where piperidine is often used in very large excess (usually 5−20% v/v of the solvent),[5] these conditions can be considered to be mild. Unfortunately even these conditions for removal of Bsmoc were still too harsh. Excess 2° amine (2 to 4 equiv of piperidine) again led to the formation of free paclitaxel from **4** (about 30% by HPLC), and made the isolation of pure ester impossible. This necessitated the consideration and development of new deprotection conditions. On the basis of the known mechanism of Bsmoc deprotection,[6,7] piperidine will covalently add to the benzothiophene dioxide portion of the Bsmoc moiety in Michael fashion. One equivalent of piperidine can be completely removed from the system with this technique (Scheme 2). By using this 1-equiv modification it was found that the reaction was complete after 2 h and that **4** formed in high yield. Isolation of the ester by precipitation with hexane resulted in almost pure paclitaxel 2′-glycinate but a trace of free paclitaxel was still detectable. Since **4** is very sensitive to moisture, it was thought that **1** arose from a small amount of residual water, most likely from the hygroscopic liquid piperidine used in the deprotection step. Attempts at complete water removal from piperi-

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(8) Purchased from Morre-Tec Industries, Inc., Union, NJ.

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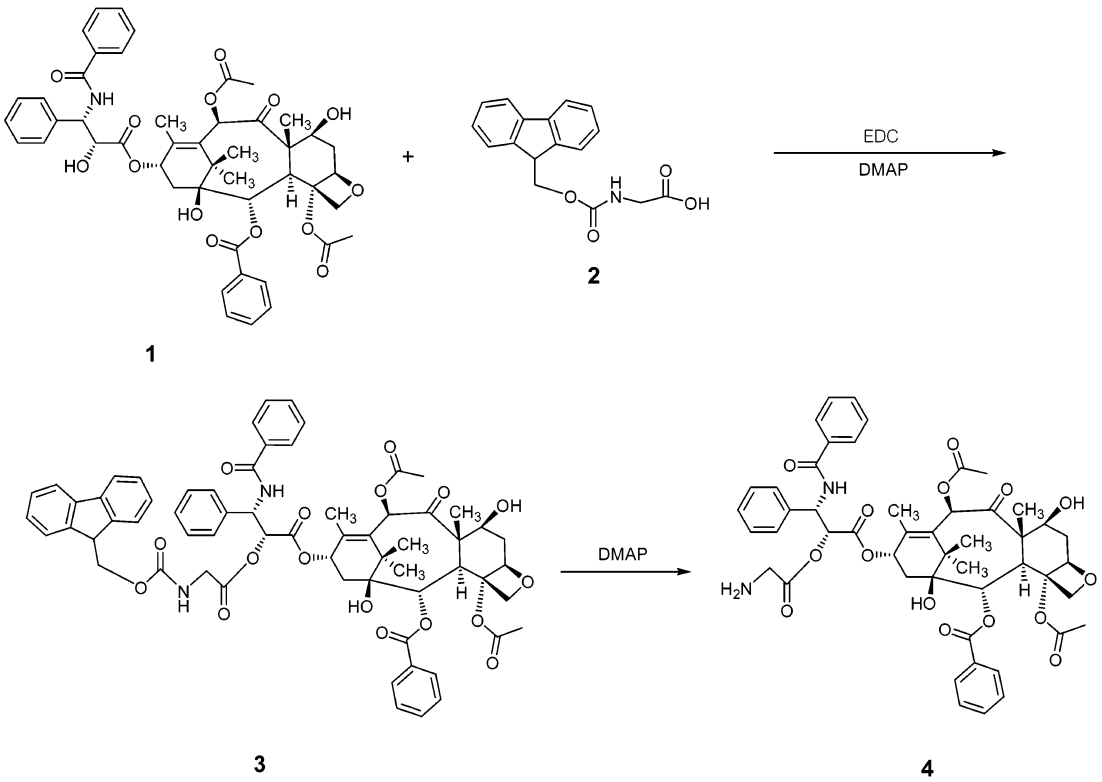
SCHEME 1



1 2 3 4

dine to completely inhibit hydrolysis to **1** could not be achieved. Therefore we turned to the solid 2° amine, 4-piperidinopiperidine (**7**), which is easily dried in vacuo prior to use. This last modification finally resulted in a paclitaxel free product that could be isolated and characterized. The structure of pure **4** was confirmed by ^{1}H and ^{13}C NMR spectra as well as MS spectroscopy, and was stable for prolonged periods of time at −20 °C when protected from moisture.

For PEG conjugation, pure paclitaxel-2′-glycinate did not need to be isolated when employing this last procedure in the synthesis of **10**. The addition of PEG acid (**9**) directly to the reaction mixture in addition to EDC and a catalytic amount of DMAP gave the desired PEG-paclitaxel-2′-glycinate, **10**,[9] without the detection of any PEG-2′-paclitaxel[10] byproduct, which would arise from the presence of free paclitaxel. By using this approach, high-yield conversions of paclitaxel to PEG conjugate **10** were obtained by a one-pot procedure.

We believe that the successful preparation of **4**, an important paclitaxel derivative never before isolated, demonstrates the great utility of the Bsmoc protecting group. The modifications developed in this study (equimolar amounts of solid 2° amine derivatives under anhydrous conditions) should further enhance the use of this versatile reagent for applications to base- and moisture-sensitive compounds.

Experimental Procedures

General Procedures. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation from toluene prior to use. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Mercury 300 NMR spectrometer with deuterated chloroform as the solvent unless otherwise specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). MS was obtained from Yale Cancer Center Mass Spectrometry Resource and W. M. Keck Formulation Biotechnology Resource Laboratory, Yale University, New Haven. The melting point was measured with a Thomas Mel-Temp apparatus.

HPLC Method. The reaction mixtures and the purity of intermediates and final products were monitored by a Beckman Coulter System Gold HPLC instrument employing a ZORBAX 300 SB C-8 reversed-phase column (150 × 4.6 mm) or a Phenomenex Jupiter 300A C18 reversed-phase column (150 × 4.6 mm) with a multiwavelength UV detector, using a gradient of 10−90% of acetonitrile in 0.5% trifluoroacetic acid (TFA) at a flow rate of 1 mL/min.

Paclitaxel Fmoc Derivative: Compound 3. To a solution of **1** (5.0 g, 5.88 mmol), **2** (1.95 g, 6.44 mmol), and DMAP (3.1 g, 25.4 mmol) in anhydrous methylene chloride (DCM, 500 mL) chilled to −8 °C was added EDC (2.5 g, 13.0 mmol). The reaction mixture was stirred at −8 °C for 30 min, then warmed to room temperature with continuous stirring for 6 h. The solution was washed with 0.1 N HCl (300 mL) and water (300 mL), the organic layer dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated to give pure **3** (6.3 g, 5.56 mmol, 95%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.12 (d, J = 7.3 Hz, 1H), 7.73 (t, J = 7.2 Hz, 2H), 7.45−7.61 (m, 2H), 7.28−7.43 (m, 4H), 7.02 (d, J = 9.2 Hz, 1H), 6.28 (s, 1H), 6.23 (t, J = 8.9 Hz, 1H), 5.97 (d & d, J_1 = 8.9 Hz, J_2 = 3.0 Hz, 1H), 5.67 (d, J = 6.9 Hz, 1H), 5.52 (d, J = 3.0 Hz, 1H), 5.36 (t, J = 5.6 HZ, 1H), 4.95 (d, J = 8.2 HZ, 1H), 4.28−4.45 (m, 2H), 4.14 (d & d, J_1 = 30.0 Hz, J_2 = 8.6 Hz, 2H), 3.79 (d, J = 6.9 Hz, 1H), 2.50−2.59 (m, 1H), 2.43 (s, 1H), 2.20 (s, 1H), 1.91 (s, 2H), 1.67 (s, 1H), 1.21 (s, 1H), 1.12 (s, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 203.7, 171.2, 169.8, 169.2, 167.6, 167.2, 166.9, 156.2, 143.6, 142.4, 141.2, 136.6, 133.6, 133.5, 132.8, 132.0, 130.2, 129.1, 128.7, 127.7, 127.1, 127.0, 126.5, 124.9, 120.0, 84.4, 81.0, 79.0, 75.5, 75.0, 74.8, 72.1, 67.3, 58.5, 52.8, 46.9,

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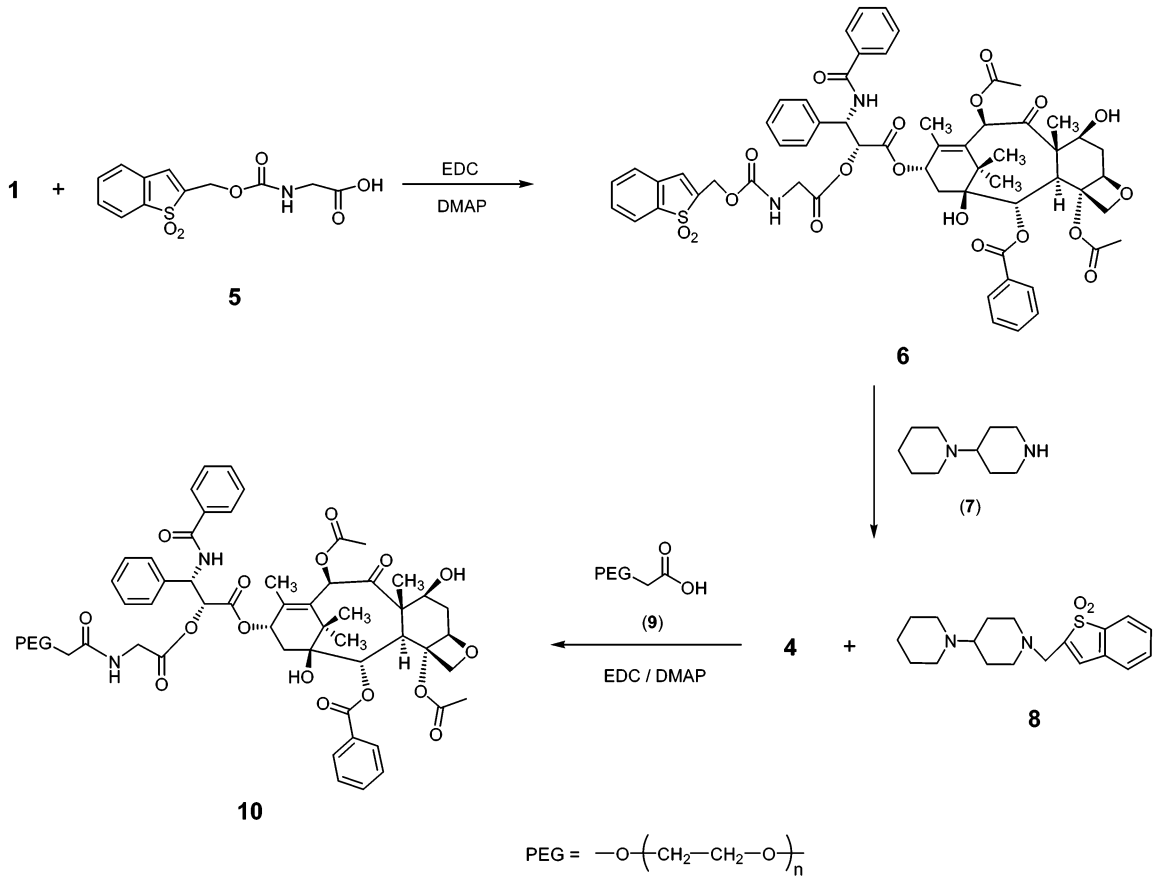
SCHEME 2



45.6, 43.1, 35.5, 26.8, 22.7, 22.1, 20.8, 14.8, 9.6; ESI-MS (ES^+) (*m*/*z*) calcd for $C_{64}H_{65}N_2O_{17}$ 1133.00, found 1133.46 (MH^+).

Paclitaxel Bsmoc Derivative: Compound 6. To a solution of **1** (1.00 g, 1.17 mmol), **5** (0.375 g, 1.34 mmol), and (dimethylamino)pyridine (DMAP, 0.043 g, 0.35 mmol) in methylene chloride (DCM, 100 mL) cooled to 10 °C was added EDC (0.336 g, 1.75 mmol) and the solution was continuously stirred at 10 °C for 50 min before it was warmed to room temperature and stirred for another 30 min. The reaction mixture was washed with 0.1 M HCl (2 × 50 mL) and water (50 mL), dried ($MgSO_4$), and filtered, and solvent was evaporated under reduced pressure to give **6** (1.20 g, 1.05 mmol, 90%). ^{1}H NMR (300.07 MHz, $CDCl_3$) δ 1.14 (s, 3H), 1.22 (s, 3H), 1.68 (s, 3H), 1.83 (s, 2H), 1.90 (s, 3H), 2.22 (s, 3H), 2.30 (s, 1H), 2.43 (s, 3H), 2.56 (s, H), 3.79 (d, J = 6.3 Hz, H), 4.04 (m, H), 4.12 (d, J = 5.7 Hz, H), 4.19 (d, J = 8.1 Hz, H), 4.30 (d, J = 8.4 Hz, H), 4.42 (m, H), 4.96 (d, J = 9.6 Hz, H), 5.06 (s, H), 5.56 (m, H), 5.66 (d, J = 4.2 Hz, H), 6.00 (m, H), 6.22 (t, J = 8.4 Hz, H), 6.29 (s, H), 7.08 (s, H), 7.15 (d, J = 8.7 Hz, H), 7.30−7.70 (m, 16H), 7.77 (d, J = 8.4 Hz, H), 8.13 (d, J = 7.5 Hz, H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 203.5, 171.0, 169.7, 168.7, 167.5, 167.0, 166.8, 155.3, 142.3, 138.8, 136.8, 136.5, 133.6, 133.6, 132.7, 131.9, 130.5, 129.8, 129.0, 128.5, 127.1, 126.8, 126.6, 125.3, 121.4, 84.4, 81.1, 79.1, 76.4, 75.6, 75.0, 74.9, 72.0, 58.5, 57.2, 52.9, 45.7, 43.2, 42.8, 35.6, 26.8, 22.7, 20.9, 14.9, 9.7; ESI-MS (ES^+) (*m*/*z*) calcd for $C_{59}H_{61}N_2O_{19}S$ 1132.94, found 1133.42 (MH^+).

2′-Paclitaxel Glycinate: Compound 4. To a solution of **6** (1.00 g, 0.917 mmol) in DCM (50 mL) was added 4-piperidnopiperidine (0.917 mmol, 0.157 g) and the mixture was stirred for 2 h at room temperature. The solution was concentrated to about 2 mL by rotary evaporation and 50 mL of hexane was added to precipitate the product. The resulting mixture was centrifuged and the supernatant decanted. The hexane wash was repeated twice and the final residue was dried in a desiccator over phosphorus pentoxide to give **4** (0.791 g, 0.862 mmol, 94%). ^{1}H NMR (300.07 MHz, $CDCl_3$) δ 1.14 (s, 3H), 1.23 (s, 3H), 1.68 (s, 3H), 1.95 (s, 3H), 2.22 (s, 3H), 2.46 (s, 3H), 2.58 (s, H), 3.82 (d, J = 6.6 Hz, H), 4.20 (d, J = 8.7 Hz, H), 4.31 (d, J = 8.4 Hz, H), 4.47 (m, H), 4.97 (d, J = 9.3 Hz, H), 5.55 (d, J = 3.3 Hz, H), 5.68 (d, J = 6.9 Hz, H), 6.00 (d, J = 3.3 Hz, H), 6.25 (t, J = 8.4 Hz, H), 6.30 (s, H), 6.96 (s, H), 6.99 (d, J = 8.7 Hz, H), 7.30−7.70 (m, 16H), 7.73 (d, J = 4.2 Hz, H), 8.13 (d, J = 6.9 Hz, H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 203.5, 173.2, 171.0, 169.6, 167.8, 166.9, 166.8, 142.4, 136.7, 133.6, 133.5, 132.8, 131.9, 130.1, 129.1, 129.0, 128.6, 128.4, 127.0, 126.4, 84.4, 81.1, 79.1, 76.4, 75.6, 75.1, 74.3, 72.1, 71.9, 58.5, 54.7, 52.8, 45.6, 43.7, 43.2, 35.6, 26.9, 26.0, 22.8, 22.2, 20.9, 14.9, 9.7; ESI-MS (ES^+) (*m*/*z*) calcd for $C_{49}H_{54}N_2O_{15}$ 911.35, found 911.47 (MH^+); melting point 182−184 °C.

PEG 2′-Paclitaxel Glycinate: Compound 10. To a solution of **6** (2.10 g, 1.85 mmol) in DCM (200 mL) was added 4-piperidinopiperidine (0.281 g, 0.167 mmol) and the reaction was stirred for 3 h at room temperature. To the reaction mixture was added **9**[10] (15.0 g, 0.375 mmol), DMAP (0.186 g, 1.52 mmol), and EDC (0.288 g, 1.50 mmol) and stirring was continued for 12 h. The solution was washed with 0.1 M HCl (2 × 200 mL) and water (200 mL), dried ($MgSO_4$), and filtered, the solvent evaporated under reduced pressure, and the residue crystallized from dimethylformamide/isopropyl alcohol (DMF/IPA = 1:4, 300 mL) to give **10** (12.88 g, 0.284 mmol, 82%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 203.0, 170.4, 169.2, 168.9, 167.2, 166.6, 166.2, 156.0, 141.9, 136.3, 133.1, 132.3, 131.4, 129.6, 128.7, 128.6, 128.2, 128.0, 126.8, 126.3, 83.9, 80.6, 78.5, 75.9, 75.1, 74.6, 74.1, 63.9, 58.0, 52.6, 45.3, 42.8, 35.3, 35.1, 26.4, 22.3, 21.7, 20.5, 14.4, 9.3.

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