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N-Benzoyl-(2*R*,3*S*)-3-Phenylisoserine Methyl Ester; A Facile and Convenient Synthesis and Resolution by Entrainment

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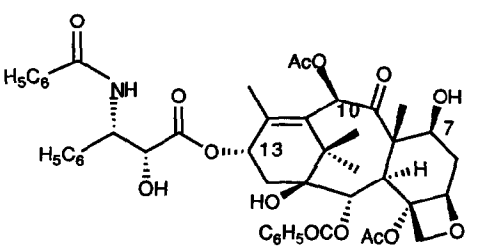
Abstract: The importance of *N*-benzoyl-(2*R*,3*S*)-phenylisoserine (**3**), also known as the "taxol side chain", for the strong antitumor activity of taxol (**1**), a potent anticancer drug, has prompted numerous efforts towards the development of a practical taxol side chain synthesis. Here we describe a highly practical and inexpensive synthesis of the taxol side chain methyl ester **4** and resolution via entrainment based on the observation that compound **4** exhibits conglomerate crystal structure.

INTRODUCTION

The clinical utility of taxol (**1**), a natural product isolated in extremely low yield from *Taxus brevifolia*,[1] in advanced cancer chemotherapy[2,3] has prompted a prodigious effort to obtain this complex molecule synthetically.[4] The chemical complexity of taxol[5] dictates that its commercial production by total synthesis is not



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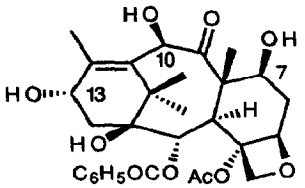
likely to be economical. The naturally derived 10-deacetylbaccatin III (**2**) is readily available in relatively higher yield (1g/kg).[6] Preparation of quantities of taxol economically by a semisynthetic approach which involves the condensation of **2** with *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine (**3**), provides an alternative source of this important natural product.

While several methods,[7a-n] have been reported for the synthesis of compound **3** in optically active form, almost all of them involve the use of either a chiral auxiliary or resolving agents which increase the cost of

production of commercial quantities of taxol. The observation[8] that the taxol side chain methyl ester (**4**) exhibits conglomerate behavior, prompted us to develop a new, facile and inexpensive synthetic route to compound **4**.



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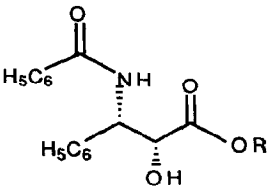


3: R = H
4: R = CH_3

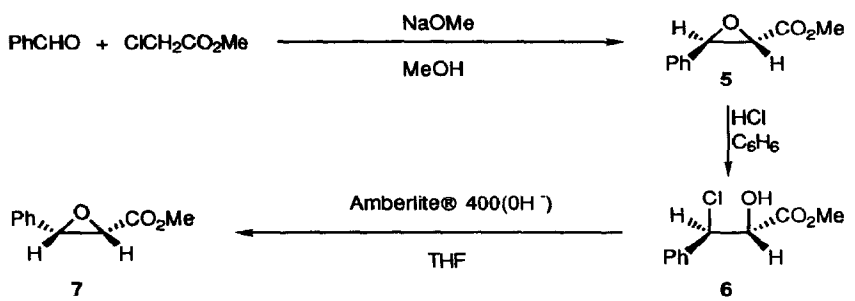
The present report describes a highly practical and efficient synthesis of **4** in terms of production cost and handling of the reaction process.

RESULTS AND DISCUSSION

Our strategy begins with benzaldehyde as starting material and has the following salient features: (a) One pot synthesis of trans-methyl 3-phenylglycidate from benzaldehyde via Darzen's procedure, (b) conversion of the trans-epoxide to the cis-isomer, (c) ammonolysis of the cis-epoxide to introduce the taxol sidechain skeleton, (d) resolution of the racemic taxol side chain methyl ester via the entrainment procedure.

Benzaldehyde was subjected to a Darzen's reaction with methyl chloroacetate to provide trans-epoxide **5** (Scheme I). Reaction of trans-epoxide **5** with HCl gas in benzene[9] furnished the highly stereoselective ring opened product **6** which upon treatment with inexpensive Amberlite® 400 (OH^-) resin gave cis-epoxide **7** (Scheme I). The use of resin was advantageous over other alkaline reagents because of high stereoselectivity in

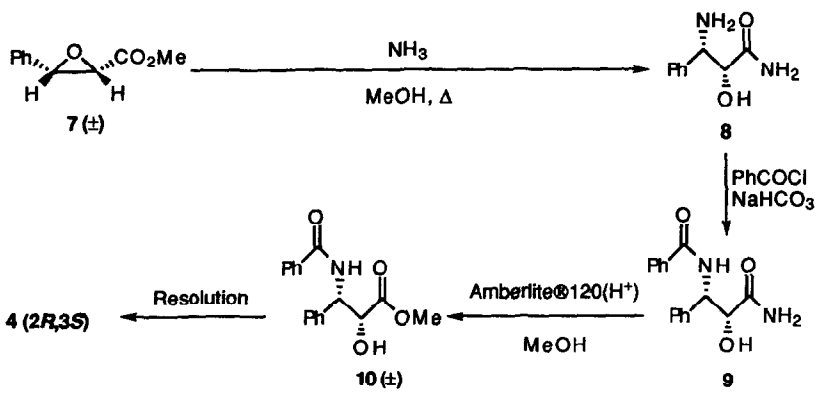
Scheme I



the formation of the reaction product, good reagent recovery and relatively higher yield in comparison to other bases used so far. Ammonolysis of **7** by methanolic ammonia under high pressure provided threo-isoserineamide **8** as the major product (Scheme II). Any serine byproduct was easily removed by washing with

methanol. However, no formation of erythro-isoserine isomer was observed which allowed us to omit several recrystallization steps as compared to earlier reports.[7d] The amide **8** was then *N*-benzoylated using the Schotten-Baumann procedure to produce the new intermediate **9** which was converted into methyl ester **10** in one step by treating with easily recoverable Amberlite® IR-120 (H^+) resin in the presence of methanol (Scheme II). This reaction was of great utility as it not only saved one step in the well known method of methyl ester preparation[7f] but also would be compatible with large scale reactions. The racemic taxol side chain methyl ester **10**, thus obtained, was then resolved into pure (2*R*,3*S*) isomer **4** using the entrainment procedure. The enantiomeric purity of **4** was determined to be >95% by ^1H NMR analysis with Eu(hfc)3 as chiral shift reagent.

Scheme II



In summary, the route outlined in Schemes I-II. provides a practical, inexpensive synthesis of the taxol side chain methyl ester which does not employ any chiral auxiliary or resolving agent or any chromatographic separations.

EXPERIMENTAL SECTION

General: Melting points were obtained in open capillary tubes and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded at 300 MHz on a Varian VXR 300 instrument and the chemical shifts are reported as δ units (ppm) relative to tetramethylsilane as an internal standard. IR spectra were obtained with a Perkin Elmer 281 B spectrophotometer, while the optical rotations observed at the Na-D line were determined at 25 °C with a Perkin Elmer 141 polarimeter. Elemental analyses were performed by Oneida Research Services, Inc. New York. Thin layer chromatography (TLC) was performed on Merck 0.25 mm glass plates of silica gel 60 F_{254} and visualization was achieved with UV light.

The commercially available resin was rinsed with methanol until the washes were neutral. Unless otherwise noted, all reagents and solvents were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

trans-Methyl 3-phenylglycidate (5). To an ice cooled solution of sodium metal (12.7 g, 0.55 g atom) in methanol (165 mL) was added a mixture of benzaldehyde (38.92 g, 0.37 mol) and methyl chloroacetate

(59.78 g, 0.55 mol) dropwise. The resulting mixture was stirred at room temperature overnight. The solution was concentrated to approximately 15 mL and poured into water (250 mL). The aqueous solution was extracted with diethylether (4x50 mL) and the organic phases were combined, dried over anhyd. $MgSO_4$ and evaporated. The crude residue thus obtained was vacuum distilled (108-111 °C, 0.6 mm Hg) to yield pure trans-epoxide[7k] **5** (24 g, 73%) : ^1H NMR ($CDCl_3$) δ 3.50 (d, J = 1.7 Hz, 1H), 3.79 (s, 3H), 4.08 (d, J = 1.7 Hz, 1H), 7.25-7.36 (m, 5H).

threo-3-Choloro-2-hydroxy-3-phenyl-methylpropanoate (6). Dry HCl gas was bubbled into a solution of **5** (44.6 g, 0.25 mol) in dry benzene (500 mL) for 3 h and the excess HCl was removed under partial vacuo. The solvent was evaporated and the residue was triturated with petroleum ether (12 mL) to provide a white solid which was recrystallized from benzene-pet. ether (20:80) to furnish pure crystalline product **6** (37.5 g, 70%) : mp 77-79 °C ; IR (KBr) 3500, 1740 cm^{-1} ; ^1H NMR ($CDCl_3$) δ 3.29 (d, J = 7.5 Hz, 1H), 3.87 (s, 3H), 4.55 (dd, J = 2.3 Hz, 1H), 5.33 (d, J = 2.3 Hz, 1H), 7.36-7.52 (m, 5H).

cis-Methyl 3-phenylglycidate (7). A suspension of chlorohydrin **6** (32 g, 149.1 mmol) and Amberlite® 400 (OH^-) resin (240 g) in dry THF (510 mL) was stirred at room temperature for 3 h under N_2 atmosphere. The resin was filtered and washed with THF (3x950 mL). The solvent was evaporated and the oily residue was dried under high vacuo to give the cis epoxide[7f] **7** (16 g, 60%) which was pure enough for the subsequent step. ^1H NMR ($CDCl_3$) δ 3.51 (s, 3H), 3.81 (d, J = 4.6 Hz, 1H), 4.23 (d, J = 4.6 Hz, 1H), 7.27-7.41 (m, 5H).

threo-3-Phenylisoserineamide (8). A solution of cis-epoxide **7** (15 g, 82.5 mmol) in methanol saturated with ammonia (prepared by passing anhydrous NH_3 through methanol at –20 °C) was sealed in a steel bomb and heated at 100 °C with constant stirring for 3 h. The solution was cooled to room temperature and further stirred for 12 h. A white solid preciptated out which was filtered and washed with minimal amounts of methanol to yield 10.5 g (69%) of threo-amide **8** : mp 208-210 °C (lit.[7d] mp 192-193 °C); IR (KBr) 3400, 3360, 3306, 1660 cm^{-1}; FABHRMS calcd for $C_9H_{13}N_2O_2$ 181.0977, obsvd. 181.0976; ^1H NMR (DMSO-d_6/ D_2O) δ 3.91 (d, J = 3.3 Hz, 1H), 4.10 (d, J = 3.3 Hz, 1H), 7.15-7.37 (m, 5H); ^{13}C NMR (DMSO-d_6/ D_2O) δ 57.46, 75.85, 126.94, 127.41, 128.24, 144.16, 175.73; Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.98; H, 6.71; N, 15.55. Found: C, 59.92; H, 6.65; N, 15.44.

***N*-Benzoyl-threo-3-phenylisoserineamide (9).** To a cooled solution of amide **8** (7.5 g, 41.62 mmol) in 10% aqueous $NaHCO_3$ (1L) was added benzoyl chloride (15 mL, 125 mmol) and the mixture stirred at 4 °C for 6 h. The solution was acidified by 5N HCl to pH = 1 and then extracted with CH_2Cl_2-THF (1:4, 3x1.25 L). The organic phases were combined and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave the crude residue which was crystallized by acetone-hexane (5:95) to furnish 8.7 g (74%) of the desired product as white solid : mp 184-186 °C; IR (KBr) 3400, 3360, 3200, 1665, 1625 cm^{-1}; MS, m/e 285 (M^+ + 1); ^1H NMR (DMSO-d_6) δ 4.21 (dd, J = 6.9, 3.4 Hz, 1H), 5.42 (dd, J = 8.5, 3.2 Hz, 1H), 5.78 (d, J = 6.9 Hz, 1H), 7.24-7.58 (m, 10H), 7.83-7.86 (m, 2H), 8.45 (d, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 55.83, 73.78, 126.83, 127.31, 127.96, 128.41, 131.42, 134.42, 140.81,165.89, 174.13; Anal. Calcd for $C_{16}H_{16}N_2O_3.1/2\ H_2O$: C, 65.52; H, 5.84; N, 9.55. Found: C, 65.50; H, 5.45; N, 9.45.

***N*-Benzoyl-threo-3-phenylisoserine methyl ester (10).** *N*-Benzoyl-3-phenylisoserineamide **9** (5 g, 17.59 mmol) and Amberlite® IR-120 H.C.P. resin[10] (H^+, 75 g) were combined with methanol (200 mL) and the mixture was heated at reflux for 24 h. The mixture was transferred to a small column and the product eluted with a solution of methanolic ammonia (prepared by diluting a saturated NH_3 in methanol

solution with six volumes of methanol). The solvent was evaporated and $CHCl_3$ (200 mL) was added to precipitate the resin impurities. After filtration, the filtrate was concentrated to approximately 20 mL volume and triturated with hexane (80 mL) to yield the methyl ester **10** (3.7 g, 70%) : mp 156-158 °C (lit.[11] 157-158 °C); ^1H NMR ($CDCl_3$) δ 3.40 (b, 1H), 3.84 (s, 3H), 4.64(d, *J* = 2Hz, 1H), 5.75 (dd, *J* = 9.3, 2.0 Hz, 1H), 7.02 (d, *J* = 9.2 Hz, 1H), 7.30-7.54 (m, 8H), 7.75-7.78 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 53.25, 54.82, 73.21, 126.88, 127.04, 127.92, 128.62, 128.72, 131.75, 134.03, 138.66, 166.87, 173.66.

***N*-Benzoyl-(2*R*,3*S*)-3-phenylisoserine methyl ester (4).** Racemic threo-methyl ester **10** (2 g) and the (2*R*,3*S*) enantiomer[12] (0.08 g) were dissolved in 95% dimethylsulfoxide (1.5 mL) at an elevated temperature (90 °C) to prepare a super saturated solution. The solution was cooled slowly to 16-20 °C, seeded with fine crystals of (2*R*,3*S*) enantiomer (0.007 g) and stirred for 10-20 min at the same temperature. The precipitated crystals were collected by filtration and dried under high vacuo to yield 0.16 g of the (2*R*,3*S*) enantiomer. Additional racemic methyl ester (0.16 g) was added to the filtrate and heated at an elevated temperature (90 °C). A small amount of 95% dimethylsulfoxide (0.1-0.2 mL) was added. The solution thus obtained was cooled to 16-20 °C and seeded with (2*S*,3*R*) enantiomer (0.007 g) and stirred at the same temperature. After 10-20 min, precipitated crystals were filtered and dried to furnish 0.15 g of (2*S*,3*R*) enantiomer. By repeating this cycle 9 times, 1.15 g of (2*R*,3*S*) enantiomer (optical purity 60-95%) and 0.6 g of (2*S*,3*R*) enantiomer (optical purity 50-60%) were obtained. The optically impure (2*R*,3*S*) enantiomer (1.15 g) was further dissolved in 95% dimethylsulfoxide(0.75 mL) at an elevated temperature (90 °C) and cooled to 16-20 °C. After stirring for 30 min at the same temperature, the precipitated crystals were collected by filtration and dried under high vacuo to furnish optically pure taxol side chain methyl ester **4** [(2*R*,3*S*), 0.7 g, 19.5% overall yield] : mp 184-186 °C (lit.[7f] mp 184-185 °C); $[\alpha]^{25}_D$ –49.1 (c 0.92, CH_3OH) {lit.[7f] $[\alpha]^{26}_D$ –48.0 (c 0.92, CH_3OH)}. The ^1H NMR spectrum was identical to that of the racemic methyl ester **10**. The ee of the (2*R*,3*S*) enantiomer was determined to be >95% by ^1H NMR analysis with Eu(hfc)3 as chiral shift reagent.

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