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PREPARATION OF AMINATED TAXOL SIDE CHAIN PRECURSORS. A SIMPLE APPROACH TO 2,3-DIAMINO ACIDS USING THE β-LACTAM SYNTHON METHOD.

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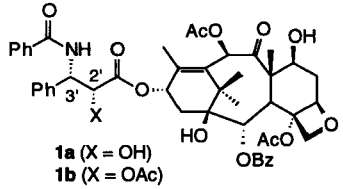
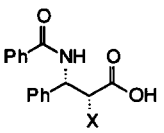
Abstract. Coupling-ready aminated side chain analog precursors of the anticancer drug taxol were prepared through the β-lactam synthon method. The procedure described represents an easy connection between β-lactams and 2,3-diamino acids, is highly stereospecific, and causes no racemization due to vicinal group participation.

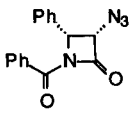
Taxol (**1a**), a complex diterpene alkaloid first described in 1971,[1] is one of the most promising new anticancer agents, currently used for the treatment of ovarian and breast cancers.[2] It binds and stabilizes microtubules, preventing the formation of a functional mitotic spindle apparatus and inhibiting cell division,[3] making it unique among tubulin poisons. Although extensive structure activity relationship studies for the drug have been reported,[4,5] the specific roles of its functional groups in microtubule binding are poorly understood.



1a (X = OH)
1b (X = OAc)
1c (X = NH_2)



2a (X = N_3)
2b (X = NH_2)
2c (X = NHtBoc)



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In a previous report, we investigated the conformations of the inactive analog 2'-acetyltaxol (**1b**) in lipophilic and lipophobic solvent systems that can mimic different cellular environments.[6] Our findings indicated that the 2'-hydroxyl functionality is crucial for microtubule binding, perhaps acting as a hydrogen bond donor. Studies of other inactive taxol analogs with no hydrogen bond donors at the 2'-position support our theory.[7,8] To test this hypothesis, analogs bearing hydrogen bond donors at this position need to be prepared. Since the amino can participate in the formation of hydrogen bonds in proteins,[9] we decided to prepare an aminated taxol analog, 2'-aminotaxol (**1c**). As taxol can be prepared by coupling its diterpene portion, Baccatin III, to a side chain precursor,[10-13] the synthesis of 2'-aminotaxol requires only the preparation of compounds **2a-c**, or derivatives of the closely related azetidinone **3**. Similar oxygenated compounds have been successfully used in the semisynthetic preparation of taxol and taxol analogs.

In this communication we wish to report the application of the β-lactam synthon method[14] to the preparation of compounds **3** and **2a-c**. The synthesis of 2,3-diamino acids **2b-c** reported here can be easily extended to the preparation of other *erythro* and *threo* diamino acids, and provides a simple connection between these compounds and β-lactams.

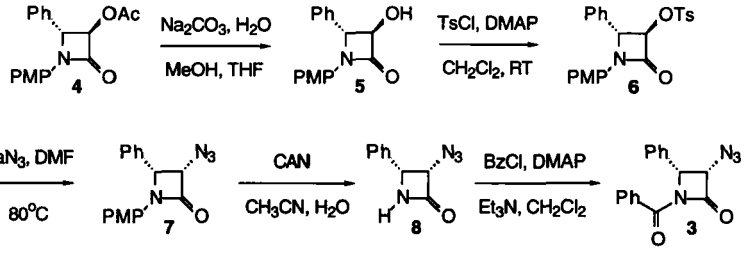
Results and Discussion

Although the preparation of an aminated taxol side chain precursor has been published recently,[15] its synthesis was based on an oxazolidinone, and the authors reported that racemization during the nitrogen incorporation reaction could occur under certain reaction conditions. This was explained to be caused by vicinal group participation of the oxazolidinone nitrogen.[16] In azetidinones, the nitrogen atom is blocked and the β-lactam amide bond can be hydrolyzed to the corresponding β-amino acid, making them attractive starting materials for our purposes. Our attention was focused on 1-(4-methoxyphenyl)-3-acetoxy-4-phenylazetidin-2-one (**4** - Scheme I), a compound that can be conveniently obtained in its *cis* and *trans* forms,[17] and whose 3-acetoxy group can be transformed into a nitrogen functionality by common procedures. Added to this is the posibility of optical resolution of this compound with lipases, leading to optically pure starting materials.[18]

Following the synthetic steps outlined in Scheme I, **4** was transformed into **6** by hydrolysis of the acetate and subsequent reaction with tosyl chloride, in 68 % overall yield. The tosyl group was cleanly displaced with sodium azide in DMF to afford compound **7**, which had complete inversion of configuration at C-3 as shown by its ^{1}H-NMR spectrum. Oxidative removal of the p-methoxyphenyl (PMP) group with ceric ammonium nitrate (CAN) in aqueous acetonitrile at 0°C, afforded compound **8**, which, due to its poor stability, was converted to **3** without purification. Thus, treatment of **8** with benzoyl chloride, triethylamine and 4-dimethylamino pyridine (DMAP) in dichloromethane, gave compound **3** in 49 % yield from **6**. This compound has all the functionality and the correct *cis* configuration needed in the final aminated analog.

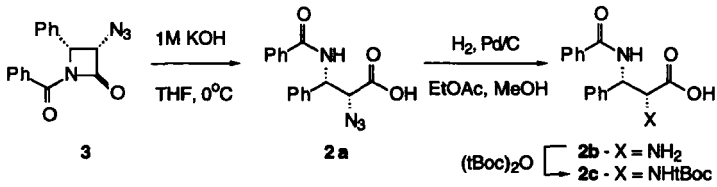
Although azetidinone **3** can be used directly as acylating agent in the coupling with Baccatin III as mentioned earlier, the availability of other precursors suitable for different coupling

Scheme I. Preparation of aminated taxol side chain precursor **3** (PMP - p-methoxyphenyl).



conditions was desirable. Another disadvantage of compound **3** is the presence of an azide functionality. Due to its poor stability, this group may not be appropriate for the coupling reaction in any of the conditions reported so far. For these reasons we prepared open forms of the side chain, and transformed the azide group to a protected amine. According to the steps outlined in Scheme II, the activated β-lactam bond in azetidinone **3** was hydrolyzed with aqueous base to afford 2-azido-3-amino acid **2a** in excellent yield. The azide was then hydrogenated over Pd/C to afford the 2,3-diamino acid **2b**, which upon treatment with di-*tert*-butyl dicarbonate and aqueous base gave the differentially protected 2,3-diamino acid **2c**.

Scheme II. Preparation of 2-azido-3-amino acid **2a** and 2,3-diamino acids **2b-c**.



Conclusions

The β-lactam synthon method has been successfully applied to a number of complex synthetic targets, and the preparation of azetidinones is well documented.[14,19] The method reported here for the preparation of aminated taxol side chain precursors provides a simple connection between azetidinones and 2,3-diamino acids.

We are presently studying which of the aminated taxol side chain precursors (**3**, **2a-c**), together with appropiate side chain coupling conditions, are suitable for the preparation of 2'-aminotaxol in reasonable yields. The results from these investigations will be reported elsewhere.

Experimental Section

NMR spectra were recorded on a Bruker ARX-500 instrument operating at 500 MHz for ^1H and 125 MHz for ^{13}C, with $CDCl_3$ as solvent unless otherwise specified. Chemical shifts (δ) are

indicated in ppm and coupling constants (J) in Hz. Melting points were obtained with a MEL-TEMP apparatus and are uncorrected. FAB-MS were aquired on a VG Analytical 70S high resolution mass spectrometer.

(±)-*trans*-1-(4-Methoxyphenyl)-3-acetoxy-4-phenylazetidin-2-one (**4**) was prepared following reported procedures.[17] mp. 101-103°C (lit. 103-104°C). ^{1}H-NMR: δ 2.17 (s, 3H), 3.72 (s, 3H), 4.89 (d, 1H, J=1.5), 5.36 (d, 1H, J=1.5), 6.73 (d, 2H, J=9.0), 7.22 (d, 2H, J=9.0), 7.31-7.36 (m, 5H). ^{13}C-NMR: δ 20.5, 55.4, 63.7, 82.5, 114.3, 118.9, 126.3, 129.0, 129.1, 130.2, 135.0, 156.5, 161.1, 169.7. FAB-MS: 311 (M), 312 (M+H).

(±)-*trans*-1-(4-Methoxyphenyl)-3-hydroxy-4-phenylazetidin-2-one (**5**): Azetidinone **4** (2.5 g, 8 mmol) was dissolved in a THF-MeOH-H_2O mixture (2:6:2, 80 ml) and cooled to 0°C. A solution of Na_2CO_3 (3.4 g, 32 mmol) in H_2O (20 ml) was added dropwise, and the resulting heterogeneous mixture was stirred vigorously at 0°C for 3 hours. After that time, it was concentrated, diluted with H_2O (50 ml) and extracted with CH_2Cl_2 (4 x 50 ml). The combined organic extracts were washed with H_2O (1 x 50 ml), brine (1 x 50 ml), dried over $MgSO_4$ and the solvent evaporated in vacuo. The resulting solid was recrystallized from CH_2Cl_2-hexanes to yield **5** (1.7 g, 6.3 mmol, 79 %) as white needles. mp 174-176°C. ^{1}H-NMR: δ 3.71 (s, 3H), 4.70 (d, 1H, J=1.7), 4.85 (d, 1H, J=1.7), 6.72 (d, 2H, J=9.0), 7.17 (d, 2H, J=9.0), 7.30-7.45 (m, 5H). ^{13}C-NMR: δ 54.8, 65.1, 83.1, 113.7, 118.5, 125.6, 128.2, 128.6, 129.7, 135.4, 155.9, 165.7. FAB-MS: 269 (M), 270 (M+H).

(±)-*trans*-1-(4-Methoxyphenyl)-3-tosyl-4-phenylazetidin-2-one (**6**): Azetidinone **5** (1.7 g, 6.3 mmol) was dissolved in CH_2Cl_2 (100 ml) together with DMAP (1.15 g, 9.5 mmol) and cooled to 0°C. Tosyl chloride (2.4 g, 12.6 mmol) was added, and the solution was allowed to warm to room temperature. After 4 hours the reaction mixture was washed with 1N HCl (1 x 30 ml), saturated $NaHCO_3$ (1 x 30 ml), H_2O (1 x 30 ml), dried over $MgSO_4$ and the solvent removed in vacuo. The crude product was recrystallized from EtOH to afford **6** (2.3 g, 5.4 mmol, 86 %) as pale yellow needles. mp. 126-127°C. ^{1}H-NMR: δ 2.42 (s, 3H), 3.69 (s, 3H), 5.02 (d, 1H, J=1.5), 5.09 (d, 1H, J=1.5), 6.74 (d, 2H, J=9.0), 7.16 (d, 2H, J=9.0), 7.25 (m, 2H), 7.27 (d, 2H, J=8.2), 7.35 (m, 3H), 7.68 (d, 2H, J=8.2). ^{13}C-NMR: δ 21.2, 54.8, 63.0, 84.6, 113.8, 118.5, 125.9, 127.7, 128.6, 128.7, 129.3, 129.5, 131.4, 133.7, 145.2, 156.2, 158.0. FAB-MS: 423 (M), 424 (M+H).

(±)-*cis*-1-(4-Methoxyphenyl)-3-azido-4-phenylazetidin-2-one (**7**): Azetidinone **6** (2.3 g, 5.4 mmol) was dissolved in anhydrous DMF (20 ml) together with NaN_3 (2.1 g, 32.4 mmol). The resulting suspension was heated at 80°C under argon for 5 days. The solution was then allowed to cool to room temperature, diluted with H_2O (80 ml), and extracted with EtOAc (5 x 50). The combined organic extract was washed with H_2O (3 x 50), dried over $MgSO_4$ and the solvent evaporated. The crude product was purified by column chromatography with hexanes-EtOAc (8:1)

as eluant, to yield **7** (1.15 g, 3.9 mmol, 72 %) as a white powder. mp. 115-116°C. ^{1}H-NMR: δ 3.73 (s, 3H), 5.01 (d, 1H, J=5.3), 5.27 (d, 1H, J=5.3), 6.78 (d, 2H, J=9.1), 7.25 (d, 2H, J=9.1), 7.31 (m, 2H), 7.39 (m, 3H). ^{13}C-NMR: δ 54.9, 60.2, 66.9, 113.9, 118.2, 127.0, 128.4, 128.6, 129.6, 132.1, 156.1, 160.3. FAB-MS: 294 (M), 295 (M+H).

(±)-*cis*-3-azido-4-phenylazetidin-2-one (**8**): Azetidinone **7** (1.1 g, 3.7 mmol) was dissolved in CH_3CN (30 ml) and cooled to 0°C. A solution of ceric ammonium nitrate (CAN - 6.1 g, 11.1 mmol) in H_2O (30 ml) was added over a period of 10 minutes. The solution was stirred for an additional 30 minutes at 0°C, diluted with H_2O (60 ml), extracted with EtOAc (4 x 50 ml), and the organic extract was washed with 5 % $NaHCO_3$ (50 ml), and the aqueous extract was again extracted with EtOAc (1 x 20 ml). The organic extracts were combined, washed with 5 % $NaHCO_3$ (4 x 20 ml), 10 % $Na_2S_2O_3$ until the aqueous layer remained colorless, dried over $MgSO_4$ and the solvent removed in vacuo, to afford **8** (580 mg, 3.1 mmol, 83 %) as an oil that solidified on cooling. ^{1}H-NMR: δ 4.93 (dd, 1H, J=5.1, 2.1), 5.01 (d, 1H, J=5.1), 6.78 (bs, 1H), 7.36-7.48 (m, 5H). ^{13}C-NMR: δ 56.8, 68.5, 126.6, 128.2, 128.4, 134.2, 164.7. FAB-MS: 189 (M+H).

(±)-*cis*-*N*-Benzoyl-3-azido-4-phenylazetidin-2-one (**3**): Azetidinone **8** (570 mg, 3.0 mmol) was dissolved in CH_2Cl_2 (30 ml) together with triethylamine (750 mg, 6.0 mmol), DMAP (45 mg, 0.3 mmol), cooled to 0°C and treated with benzoyl chloride (780 mg, 4.5 mmol) for 2 hours. The reaction mixture was then washed with 1N HCl (1 x 30 ml), H_2O (1 x 40 ml), 5 % $NaHCO_3$ (1 x 30 ml), dried over $MgSO_4$ and the solvent evaporated. Column chromatography using hexanes-EtOAc (6:1) as eluant yielded **3** (740 mg, 2.5 mmol, 83 %) as a pale yellow oil that solidified upon standing. ^{1}H-NMR: δ 5.00 (d, 1H, J=6.5), 5.54 (d, 1H, J=6.5), 7.34-7.43 (m, 5H), 7.51 (dd, 2H, J=7.7, 7.6), 7.63 (tt, 1H, J=7.7, 0.8), 8.05 (dd, 2H, J=7.6, 0.8). ^{13}C-NMR: δ 58.0, 65.4, 126.2, 127.9, 128.3, 128.5, 129.5, 130.8, 131.9, 133.4, 160.5, 165.3. HRFAB-MS: 293.1039 (M+H - $C_{16}H_{12}N_4O_2$+H requires 293.1038).

(±)-*threo*-3-Benzoylamino-2-azido-3-phenylpropionic acid (**2a**): Azetidinone **3** (200 mg, 0.68 mmol) was dissolved in THF (20 ml) and cooled to 0°C. 1M KOH (6.8 ml, 6.8 mmol) was added, and the mixture was stirred for 10 minutes, diluted with H_2O (10 ml) and the organic solvent evaporated. The aqueous solution was washed with CH_2Cl_2 (10 ml), acidified to pH 2, and extracted with EtOAc (2 x 30 ml). The combined organic extract was dried over $MgSO_4$ and the solvent evaporated in vacuo. Recrystallization from CH_2Cl_2 yielded **2a** (204 mg, 0.64 mmol, 93 %) as a pale yellow solid. mp. 152-154°C (dec.). ^{1}H-NMR (DMSO-d_6): δ 4.53 (d, 1H, J=8.6), 5.55 (dd, 1H, J=8.6, 9.1), 7.30 (tt, 1H, J=7.2, 1.2), 7.37 (dd, 2H, J=7.4, 7.2), 7.47-7.51 (m, 4H), 7.57 (tt, 1H, J=7.2, 1.0), 7.86 (dd, 2H, J=7.3, 7.2), 9.00 (d, 1H, J=9.1).^{13}C-NMR (DMSO-d_6): δ 53.8, 65.2, 127.4, 127.5, 127.7, 128.2, 128.3, 131.4, 133.9, 138.6, 166.1, 169.4. HRFAB-MS: 311.1154 (M+H - $C_{16}H_{14}N_4O_3$+H requires 311.1144).

(±)-*threo*-3-Benzoylamino-2-amino-3-phenylpropionic acid (**2b**): Azido acid **2a** (200 mg, 0.64 mmol) was dissolved in EtOAc-MeOH (1:1, 20 ml), together with 10 % Pd/C catalyst (50 mg). The mixture was hydrogenated at atmospheric pressure, room temperature, for 16 hours, filtered through celite, and the solvent evaporated to dryness. The crude product was triturated with THF and filtered to yield **2b** (125 mg, 0.44 mmol, 68 %) as a white solid. mp. 204-206°C (dec.). ^{1}H-NMR (DMSO-d_6): δ 3.71 (d, 1H, J=5.3), 5.35 (dd, 1H, J=7.6, 5.3), 7.30 (m, 3H), 7.42 (bd, 2H, J=7.3), 7.51 (dd, 2H, J=7.6, 7.3), 7.57 (tt, 1H, J=7.3, 1.2), 7.83 (dd, 2H, J=7.6, 1.2), 10.38 (d, 1H, J=7.6). ^{13}C-NMR (DMSO-d_6): δ 52.6, 55.6, 126.7, 127.4, 127.8, 128.1, 128.5, 131.4, 134.1, 138.4, 164.4, 168.2. HRFAB-MS: 285.1240 (M+H - $C_{16}H_{16}N_2O_3$+H requires 285.1239).

(±)-*threo*-3-Benzoylamino-2-*tert*-butoxycarbonylamino-3-phenylpropionic acid (**2c**): Diamino acid **2b** (100 mg, 0.35 mmol) was dissolved in a mixture of saturated $NaHCO_3$-THF (2:1, 20 ml), and treated with a solution of di-*tert*-butyl dicarbonate (110 mg, 0.52 mmol) in THF (5 ml). After the evolution of CO_2 ceased (4 hours), the mixture was diluted with H_2O (30 ml) and washed with CH_2Cl_2 (5 ml). The aqueous layer was acidified to pH 2, and extracted with EtOAc (2 x 20). The organic layers were combined, dried over $MgSO_4$ and the solvent evaporated in vacuo. The crude product was triturated with Et_2O to afford **2c** (93 mg, 0.24 mmol, 68 %) as a white solid. mp. 176-179°C (dec.) ^{1}H-NMR (DMSO-d_6): δ 1.26 (s, 9H), 4.58 (dd, 1H, J=9.6, 4.4), 5.80 (dd, 1H, J=9.2, 4.4), 7.23 (t, 1H, J=6.9), 7.32-7.39 (m, 5H), 7.50 (dd, 2H, J=7.6, 7.2), 7.57 (t, 1H, J=7.2), 7.84 (d, 2H, J=7.6), 8.75 (d, 1H, J=9.6). ^{13}C-NMR (DMSO-d_6): δ 28.1, 53.5, 57.9, 78.4, 126.6, 127.0, 127.4, 128.1, 128.4, 131.6, 134.1, 139.5, 155.5, 166.2, 171.6. HRFAB-MS: 407.1607 (M+Na - $C_{16}H_{16}N_2O_3$+Na requires 407.1583).

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