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Synthesis of 7- and 10-spermine conjugates of paclitaxel and 10-deacetyl-paclitaxel as potential prodrugs

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Abstract—Efficient syntheses of two taxol analogs bearing the linear polyamine spermine at 7- and 10-positions of paclitaxel and 10-deacetyl-paclitaxel have been developed. These polyamine-taxol-conjugates were isolated as water soluble difluoride salts. The aim of the present work was to introduce a chemical modification into taxol skeleton in order to increase drug selectivity toward tumor cells. The cytotoxic activity of these conjugates was evaluated in MCF7 and MCF7-R cell lines. The observed low cytotoxicity suggests that these conjugates could act as potential prodrugs.

1. Introduction

The naturally occurring anticancer agent paclitaxel and its analogs are currently recognized as the most important available drugs for treatment of solid tumors especially ovarian, breast, and lung cancers.[1] Unfortunately, these compounds present relevant drawbacks, such as poor solubility and lack of selectivity toward tumor cells.[2] Thus, water-soluble paclitaxel prodrugs, incorporating acids or amino acids at the 2′-position, which are readily hydrolyzed to taxol, have been synthesized.[3] In order to improve drug selectivity toward tumor cells, many efforts have been made to convert taxol into compounds with a considerably lower cytotoxicity but which maintain their chemical integrity under physiological conditions and can be rapidly cleaved by tumors expressed proteolytic enzymes.[4] For example, tripartite prodrugs were prepared by coupling reactions between taxol and a tripeptide moiety through a 2′-carbonate, or carbamate linkage in order to assure stability against ubiquitous enzymatic activity. The tripeptide is cleaved by the tumor-associated proteases, such as plasmin, which plays a key role in tumor invasion and metastasis.[5] Furthermore, taxol prodrugs were designed containing a monoclonal antibody (Mab), which selectively bind to antigens expressed on tumor cell and were activated after internalization into the tumor cell via an enzymatic hydrolysis according to ADEPT (antibody directed enzyme prodrug therapy) strategy.[6]

An interesting approach for drug deliver is the use of polyamine based transporters. The ubiquitous polyamines, such as cadaverine, putrescine, spermidine, and spermine are biosynthesized in humans and play an essential role as regulators of cell growth and differentiation.[7] Polyamines exist in vivo as polycations since the nitrogen is protonated at physiological pH. During transport, the cationic polyamines bind electrostatically to intracellular polyanions to modulate their function and stimulate the synthesis of some proteins.[8] Nevertheless, more data on these transporter features are required to make possible the design of prodrugs, which comprise a cytotoxic constituent attached to a polyamine. Although information regarding mammalian polyamine transporters are at an early stage,[9] several attempts to design a polyamine vector have been made. These attempts are based on structure–activity studies,[10] which suggested that minor structural changes in the polyamine skeleton gave rise to marked differences in

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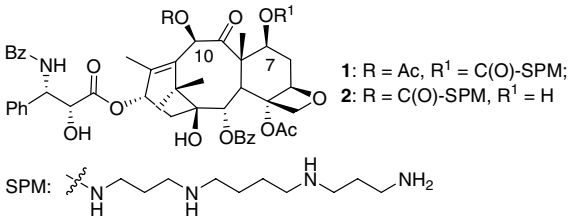


Figure 1.

their transport behavior, and that linear polyamines were superior vectors over their branched counterpart. Selected examples of anticancer agents delivered by polyamine were DNA intercalators,[10] amino acids,[11] nitroimidazole,[12] porphyrin,[13] and binuclear platinum complexes.[14]

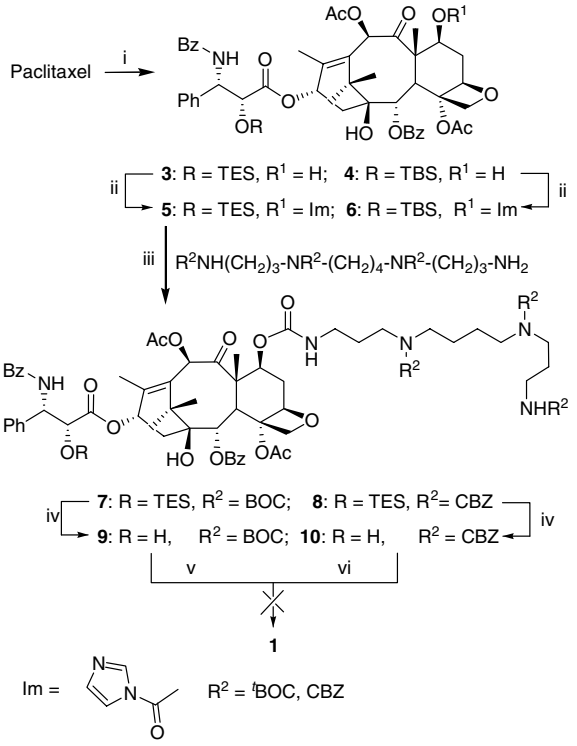
This report illustrates the synthesis of new conjugates between the linear polyamine spermine, as the transport system, and the antitumor drug paclitaxel. The majority of the efforts for the development of water-soluble paclitaxel prodrugs were focused on the synthesis of their 2′-acyl derivatives, such as esters or carbamates, since it was expected the prodrug to lose its cytotoxic activity by modification of the 2′-hydroxyl functionality.[15] In our case a serious drawback for the synthesis of the 2′-carbamate linked paclitaxel-spermine conjugate was the possible instability of the 2′-acyl bond, as it has been observed in a tripartite prodrug of paclitaxel and a tripeptide bearing a monosubstituted carbamate group in the linker. A release of baccatin III was observed due to an addition–elimination sequence of the carbamate nitrogen to the acyl group of the isoserine appendant.[15] We reasoned that this unspecific hydrolysis by ubiquitous extra-cellular esterases could be avoided by forming the carbamate linkage at the 7- and 10-positions. In particular, we have attached the spermine to the hydroxy groups at the 7-position of paclitaxel and at the 10-position of 10-deacetyl-paclitaxel by formation of a carbamate bond (compounds **1** and **2**, Fig. 1).

2. Chemistry

Paclitaxel was used as the starting reagent for the semisyntheses of compounds **1** and **2**, which are reported in Sections 1 and 2, respectively.

2.1. Synthesis of compound 1

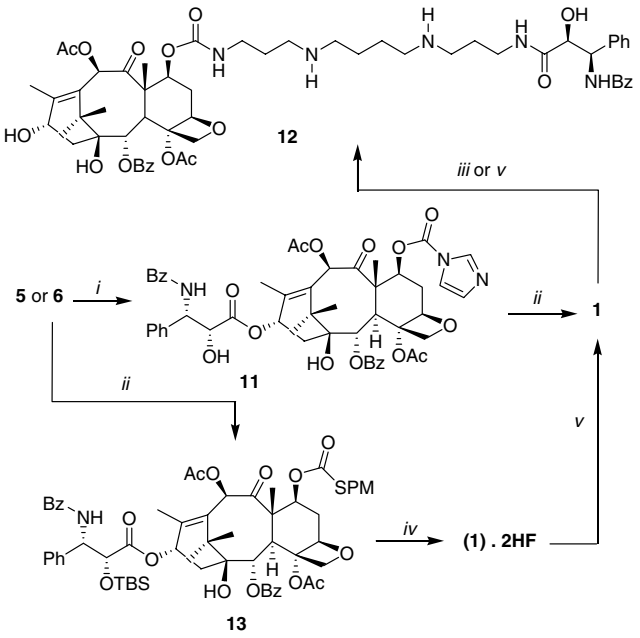
Different approaches were investigated for the synthesis of **1** based on coupling reactions of a 2′-protected-7-imidazolide paclitaxel derivative to polyamine spermine (SPM-H) in its free or N^1,N^4,N^9-protected form (Scheme 1). The synthesis of 7-imidazolides of paclitaxel requires an initial protection of the 2′-hydroxy group. Thus, paclitaxel was converted into the corresponding 2′-triethylsilyl (TES) ether **3**[16] and the 2′-*tert*-butyldimethylsilyl (TBS) ether **4**,[17] which were reacted with carbonyldiimidazole (DCI) and a catalytic amount of 4-dimethylaminopyridine (DMAP)[18] to afford the imidazolides **5** and **6**, respectively. The coupling of **5** with



Scheme 1. Reagents and conditions: (i) TES-Cl or TBDMSCl/imidazole/DMAP; (ii) DCI/DMAP; (iii) CH_2Cl_2/^{i}PrOH/reflux; (iv) HF/Py; (v) CF_3CO_2H/NH_4OH (R_2 = BOC); (vi) H_2/Pd (R_2 = CBZ).

triprotected N^1,N^4,N^9-tri-*tert*-butoxycarbonyl-[19] and N^1,N^4,N^9-tri-benzyloxycarbonyl-spermines[19] required reflux conditions (4 days) in a 3:4 ^{i}PrOH/CH_2Cl_2 mixed solvent, affording the corresponding spermine-paclitaxel conjugates **7** and **8** in 80% and 84% yields, respectively. HF/Py induced selective deprotection of the 2′-oxygen of **7** and **8** yielded compounds **9** and **10** in 90% isolated yields. Attempted deprotection of the spermine moiety of **9** with CF_3CO_2H, or **10** by reduction with Me_3SiI or H_2/Pd in the presence of ammonium formiate, failed to give the target **1** since the harsh reaction conditions favored uncontrolled side-reactions involving the taxane skeleton.

Hence, the use of unprotected spermine as the coupling partner was probed. Deprotection of the 2′-oxygen of compounds **5** or **6** with HF/Py yielded the 7-imidazolide derivative **11** in 78% yield. This compound displayed a high stability at 25 °C and no hydrolysis of the imidazolide group was observed when its purification was performed by silica gel column chromatography. The coupling of spermine to the imidazolide **11** occurred under milder conditions with respect to those required for N^1,N^4,N^9-triprotected spermines (20 °C, 2:1 ^{i}PrOH/CH_2Cl_2 mixed solvent, 6 h). ^{1}H and ^{13}C NMR spectroscopic analysis of the reaction mixture showed the presence of compound **1** as the major product (Scheme 2). Surprisingly, this conjugate was unstable on standing at 20 °C since it rearranged to the baccatin derivative **12** within 24 h. Most likely an addition–elimination reaction occurred between the nucleophilic unsubstituted terminal nitrogen of the spermine moiety



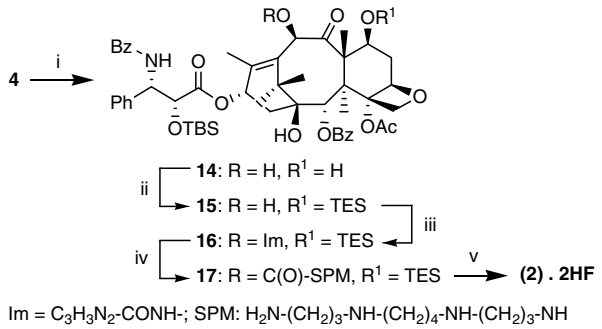
Scheme 2. Reagents and conditions: (i) HF/Py; (ii) SPM-H (^{i}PrOH/CH_2Cl_2, 4:1); (iii) 20 °C, 24 h; (iv) $Et_3N{\cdot}3HF$ (1.2 equiv, 20 °C, 15 h); (v) $NaHCO_3$.

and the acyl group of the isoserine appendant of **1**. Compound **12** displayed high stability and could be separated in 71% yields by chromatography.

We reasoned that reversing the reaction sequence, the desired spermine-conjugated derivative would have been more stable, due to the steric hindrance exerted by the 2′-OTBDMS group, which inhibits the nucleophilic attack of the terminal nitrogen of the spermine to the carbonyl group of the isoserine side chain. Accordingly, 2′-OTBS derivative **6** was reacted with spermine following the standard protocol. The resulting conjugate **13** was stable at 85 °C and it was purified by silica gel column chromatography (82%). The desilylation of **13** was performed with the complex $Et_3N{\cdot}3HF$, which yielded the target compound **1**.[20] ESI-MS/MS experiments (Micromass type Waters ZQ 4000 quadrupole) showed that compound **1** was formed as the di-fluoride salt since the spectrum appears with a number of charges of $z = 2$. In fact, only the peak of the bis-cation at the m/z value of 542 $[(\mathbf{1}+2H^+)/2]^+$ is shown. Satisfying, this salt was stable in water solutions at 60 °C after several days. Only the hydrolysis under basic conditions ($NaHCO_3$) favored its conversion into the baccatin derivative **12**.

2.2. Synthesis of compound 2

The synthesis of compound **2** (Scheme 3) required initial deprotection of the 10-position by hydrazinolysis of the 10-acetate of the 2′-TBS derivative **4**, which afforded the 10-deacetyl derivative **14** in 79% yield.[17] This was followed by selective protection of the position 7 of **14** as triethylsilyl ether **15**[17] (85%) and carbonylation of the 10-OH of **15** with DCI, which afforded 10-imidazolide **16** in 86% yield. Coupling of **16** with unprotected spermine was carried out as reported above providing the 2′-OTBS conjugate **17** in 52% isolated yield. Target compound **2** was obtained as difluoride salt in 63% yield by $Et_3N{\cdot}3HF$ induced desilylation of **17**.[21]



Scheme 3. Reagents and conditions: (i) N_2H_2; (ii) TESCl/imidazole/DMAP; (iii) DCI/DMAP; (iv) SPM-H (^{i}PrOH/CH_2Cl_2, 4:1); (v) $Et_3N{\cdot}3HF$.

Table 1. In vitro profile (IC50, nM)[a] for compounds **1**, **2** and taxol, in MCF7 and MCF7-R cell lines

Compound	Taxol[a]	**1**	**2**
MCF7	1.7	1928 ± 38	894 ± 85
MCF7-R	299	⩾3000	⩾3000

[a] See Ref. 23.

3. Growth inhibition effects of taxoid-spermine conjugates 1–2

The fluoride salts of the paclitaxel-spermine conjugates **1** and **2** were stable in water solutions. After 3 days of incubation no degradation products were detected. Their cytotoxic activity was evaluated by in vitro growth inhibition experiments conducted on MCF7 sensitive and MCF7-R resistant human breast cancer cell lines.[22] These experiments showed a three order decrease in cytotoxicity with respect to paclitaxel in the sensitive cell line (Table 1), while no activity (⩽3000) was found toward resistant cell line. Hence, these water soluble, low-toxic, and extra-cellular stable conjugates could be suitable candidates as tumor specific prodrug after their internalization provided that the carbamate group is cleaved by tumor-associated enzymes.[23] In vivo studies on the activity of these compounds are currently underway.

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20. Compound [**1**·2HF]. Compound **13** (0.31 g, 0.26 mmol) was reacted with 1.2 equiv of $Et_3N{\cdot}3HF$ in THF at 20 °C for 15 h. The solvent was evaporated and the residue was washed with Et_2O and recrystallized to afford 0.21 g (0.19 mmol, 73%) of compound **1** as the difluoride salt: ^1H NMR (400 MHz, D_2O, 60 °C): δ 8.06 (m, 2H), 7.80–7.77 (m, 3H), 7.70–7.40 (m, 10H), 7.46–7.40 (br, 1H, $J = 9.0$ Hz, NH), 6.36 (s, 1H, H10), 6.07 (t, 1H, $J = 9.0$ Hz, H13), 5.56 (d, 1H, $J = 7.0$ Hz, H3′), 5.47 (d, 1H, $J = 6.8$ Hz, H2), 5.30 (m, 1H, H7), 5.10 (m, 1H, $J_1 = 9.5$ Hz, $J_2 = 2.0$ Hz, H5), 4.87 (d, 1H, H2′), 4.36 (d, 1H, $J = 8.2$ Hz, H20), 4.22 (d, 1H, H′20), 3.79 (d, 1H, H3), 3.30–3.00 (m, 14H, 7 CH_2), 2.55–2.45 (m, 1H, H6), 2.35 (s, 3H, Me), 2.20 (s, 3H, Me), 2.10–1.90 (m, 3H, H14, H14′, and H6′), 1.90–1.60 (m, 12H, 2Me, 3CH_2), 1.14 (s, 3H, Me), 1.10 (s, 3H, Me). ^{13}C NMR (100 MHz, D_2O) δ 206.1, 174.0, 173.1, 172.0, 171.3, 167.6, 157.0, 141.1, 137.1, 134.7, 133.5, 132.9, 132.5, 130.1, 129.3, 129.1, 129.0, 128.9, 128.8, 127.4, 127.2, 84.0, 80.6, 78.4, 76.5, 75.7, 74.7, 73.6, 72.5, 71.4, 57.4, 56.3, 47.2, 47.0, 45.3, 44.7, 43.0, 37.7, 36.7, 34.8, 32.7, 25.9, 25.6, 24.0, 23.0, 22.5, 21.1, 20.3, 13.8, 10.6. ESI-MS m/z 542 $[(\mathbf{1}+2H^+)/2]^+$.
21. Compound [**2**·2HF]. Compound **13** (0.47 g, 0.40 mmol) was reacted with 1.2 equiv of $Et_3N{\cdot}3HF$ in THF at 20 °C for 15 h. The solvent was evaporated and the residue was washed with Et_2O, then recrystallized from THF/Et_2O to afford 0.29 g (0.27 mmol, 66%) of compound **2** as the difluoride salt: ^1H NMR (400 MHz, $CDCl_3$, 60 °C): δ 8.18 (m, 2H), 7.92 (m, 3H), 7.80–7.72 (m, 2H), 7.75–7.68 (m, 1H), 7.65–7.50 (m, 7H), 7.40–7.33 (br, 1H, NH), 6.31 (s, 1H, H10), 6.14 (t, 1H, $J = 9.0$ Hz, H13), 5.63 (d, 1H, $J = 6.5$ Hz, H3′), 5.54 (d, 1H, $J = 6.5$ Hz, H2), 5.19 (d, 1H, $J = 9.7$ Hz, H7), 4.91 (m, 1H, $J_1 = 9.0$ Hz, $J_2 = 1.5$ Hz, H5), 4.75 (m, 1H, H2′), 4.16 (d, 1H, $J = 8.0$ Hz, H20), 4.04 (d, 1H, H20), 3.55 (d, 1H, $J = 7.0$ Hz, H3), 3.10–2.90 (m, 14H, 7 CH_2), 2.68–2.60 (m, 1H, H6), 2.41 (s, 3H, Me), 2.24–21.10 (m, 3H, H14, H14′, and H6′), 2.0–1.70 (m, 12H, 2Me, 3CH_2), 1.22 (s, 3H, Me), 1.18 (s, 3H, Me); ^{13}C NMR (100 MHz, D_2O) δ 204.5, 174.2, 173.0, 171.3, 167.7, 156.9, 140.8, 137.0, 134.6, 133.4, 133.3. 132.5, 130.1, 129.3, 129.1, 129.0, 128.9, 128.8, 127.4, 127.2, 84.6, 80.9, 78.4, 76.5, 76.4, 75.0, 73.6, 71.4, 71.0, 68.0, 57.9, 57.4, 47.1, 47.0, 45.1, 44.6, 43.0, 39.7, 37.4, 36.6, 35.5, 26.1, 25.1, 23.9, 22.9, 22.8, 22.5, 21.1, 13.8, 9.7. ESI-MS m/z 521 $[(\mathbf{2}+2H^+)/2]^+$.
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