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# Synthesis of Water-Soluble Dendrimers Based on Melamine Bearing 16 Paclitaxel Groups

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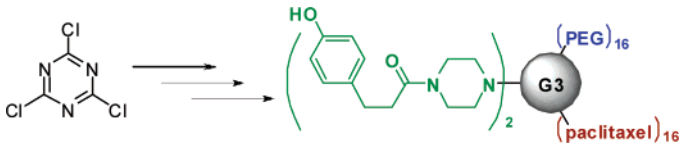
## ABSTRACT




**The design, synthesis, and characterization of triazine dendrimers derivatized with the anticancer agent paclitaxel are described. The precursor generation two dendrimer 1 is prepared in six linear steps in 64% overall yield and presents 16 amines and two groups for radioiodination. This macromolecule is subsequently derivatized with a paclitaxel conjugate to yield a generation three dendrimer, 2, which is then pegylated in two steps. The pegylated final products, 4a and 4b, with molecular weights of 46 and 77 kDa, respectively, solubilize paclitaxel in water. Pegylated dendrimer 4a is 30 wt % paclitaxel, 52 wt % PEG, and 18 wt % dendrimer. Target 4b is 18 wt % paclitaxel, 71 wt % PEG, and 11 wt % dendrimer.**

To date, various types of polymers have been investigated as drug delivery systems.[1] Examples include grafts,[2] stars,[3] multivalent polymers,[4] block copolymers,[5] dendronized polymers,[6] and dendrimers.[7] Dendrimers are promising as drug delivery vehicles by virtue of their multivalency, well-defined and large globular structure, mono- or low polydispersity, and amenability to postsynthetic manipulation. We and others believe that dendrimers may surmount barriers to chemotherapy, including poor solubility of some agents in physiological conditions, high systemic toxicity, poor bioavailability, and low stability. Through rational design, dendrimers might shift in vivo pharmacokinetics of the drug, accelerate cellular uptake, prolong plasma half-life, and even specifically target the diseased cells either actively (via ligands) or passively (via the enhanced permeability and retention (EPR) effect[8] resulting from a leaky vasculature and the lack of lymphatic drainage in solid tumor tissues).

We have chosen paclitaxel (Taxol) as our illustrative drug for four reasons. First, paclitaxel is a clinically relevant broad spectrum anticancer agent useful for treating tumors of the ovaries, breast, head and neck, lung, and AIDS-related Kaposi's sarcoma.[9] Second, it displays low solubility in water (<0.1 $\mu$g/mL). Third, it is amenable to synthetic manipulations (through esterification of the 2′-hydroxyl group) that provide a mechanism for conjugation and release. Finally, the target molecules can be compared to a variety of delivery vehicles which have been described, including emulsions,[10] micelles,[11] liposomes,[12] nanoparticles,[13] and other dendrimers.[14]

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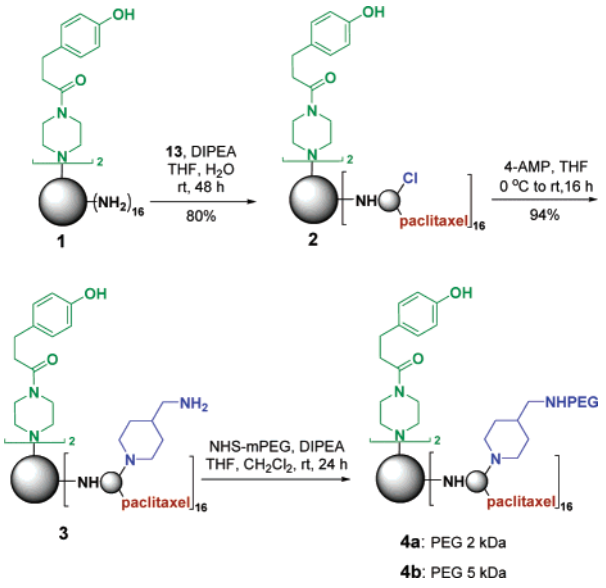
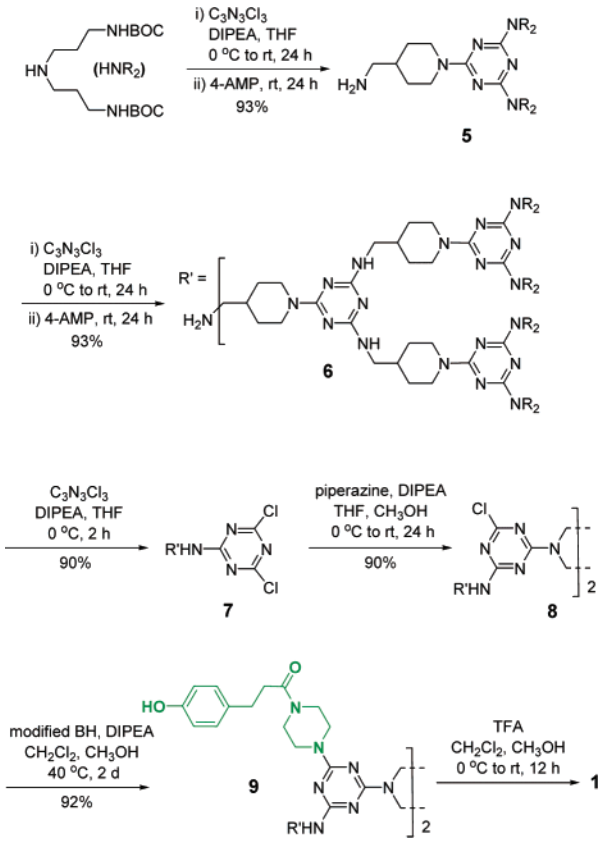
The chemistry that we describe here is outlined in Scheme 1. The benefits of triazine chemistry derive from the stepwise fashion that the chlorine atoms on triazine rings are substituted with amine nucleophiles.[7c,15] The results are products with compositional diversity. Dendrimer **1**, a second generation dendrimer, possesses Bolton−Hunter-type groups amenable to radioiodination.[16] Reaction of **1** with a dichlorotriazine decorated with paclitaxel yields a generation three dendrimer **2**. Pegylation is performed in two steps to convey biocompatibility and solubility to the hydrophobic triazine dendrimer while simultaneously allowing us to tailor its size and, accordingly, its biodistribution kinetics and fates.

**Scheme 1.** Summary of the Chemistries Reported Including Paclitaxel Conjugation and Pegylation




Target **1** is synthesized using a convergent route (Scheme 2).[17] Intermediate **5** is prepared in one pot by the reaction of cyanuric chloride with 2 equiv of the Boc-protected triamine[18] and subsequent reaction with the secondary amine of 4-aminomethyl piperidine (4-AMP). Elaboration of **5** to **6** proceeds in a similar way: cyanuric chloride is first reacted with 2 equiv of **5** and is followed by the addition of 4-AMP.

**Scheme 2.** Synthesis of **1**




Dichlorotriazine **7** is obtained by reacting **6** with cyanuric chloride. Dimerization of **7** with piperazine yields **8**. The radioiodination group (prepared as previously reported)[15d,19] is incorporated to give **9**. Finally, the Boc groups of **9** are quantitatively removed using trifluoroacetic acid (TFA). Overall, **1** is synthesized from the protected amine in 64% yield in six linear steps.

Our strategy for the covalent attachment of paclitaxel to dendrimer **1** follows the precedented esterification of the 2′-hydroxyl group.[20] While both glutaric and succinic esters have been pursued, Safavy and co-workers[20a] reported that the glutaric ester linked conjugates displayed better antitumor activity compared to that of the succinic ester linked conjugate. Our strategy rests on the preparation of a dichlorotriazine modified with paclitaxel that can be used to react with the amines of **1**, giving 16 monochlorotriazines to be further manipulated. The chemistry employed is outlined in Scheme 3.

The conjugate, **13**, is prepared in four steps in 60% overall yield. Paclitaxel is reacted with glutaric anhydride in the presence of pyridine to give 2′-glutarylpaclitaxel **10**. NHS ester **11** is obtained by reaction of **10** with *N*-succinimidyl

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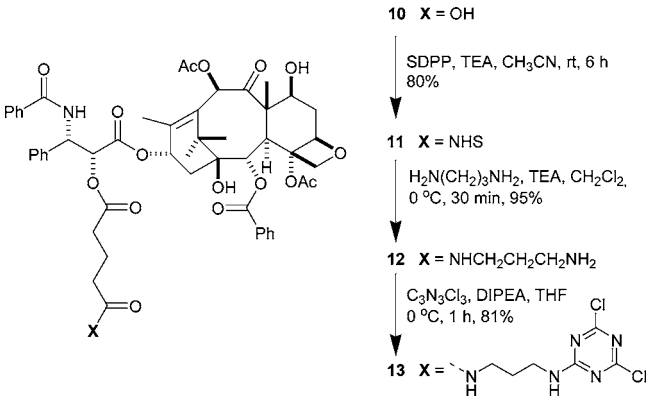
**Scheme 3**




diphenylphosphate (SDPP).[21] This ester is treated with an excess of 1,3-diaminopropane at low temperature to afford the amine **12**. The linear, flexible diamine was chosen to reduce steric hindrance. Finally, the reaction of amine **12** with cyanuric chloride using DIPEA as a base yields the desired dichlorotriazine **13**.

The reaction of dendrimer **1** with **13** gives **2** (Scheme 1). The resulting poly(monochlorotriazine) dendrimer undergoes nucleophilic aromatic substitution with 4-AMP to afford **3**. Steric hindrance prevents complete pegylation of **3**. Pegylation with NHS-mPEG 2 and 5 kDa gives **4a** (MW ∼ 46 kDa for 14 PEG chains) and **4b** (MW ∼ 77 kDa for 12 PEG chains), respectively.

With the exception of reactions involving **1**−**4**, most reactions are readily monitored by thin layer chromatography. NMR spectroscopy and mass spectrometry are useful over the entire range of targets. The MALDI-TOF MS data of intermediates **5**−**13** were consistent with single chemical entities: these spectra showed peaks derived from a single

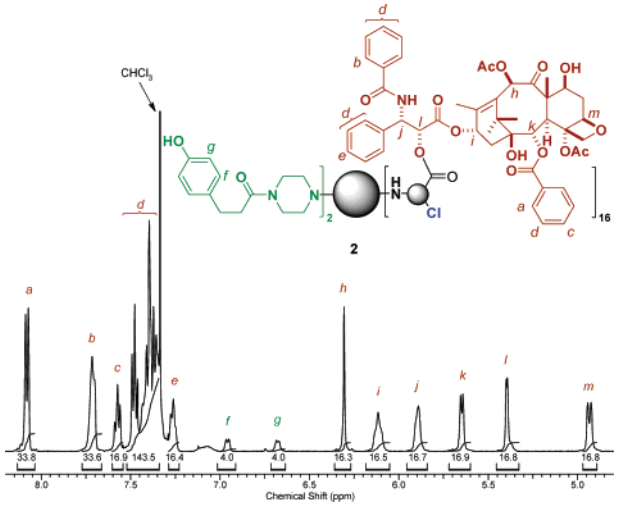



**Figure 1.** $^1$H NMR assignment and integration of **2** in the expanded region of 4.8−8.2 ppm. The spectrum was obtained in $CDCl_3$/MeOH-$d_4$ (10:1).

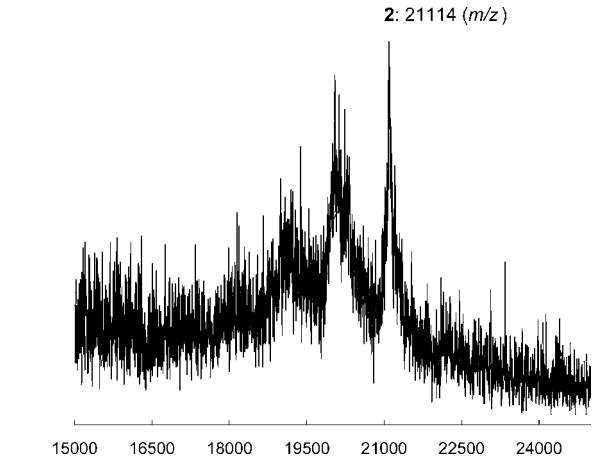



**Figure 2.** MALDI-TOF spectrum of **2**. Peaks appearing at lower *m*/*z* reflect either incomplete reaction (inconsistent with NMR data) or decomposition during ionization.

compound corresponding to $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$. A ladder of peaks from loss of Boc groups during the ionization was observed for **5**−**9**.

Targets **2**−**4** are more difficult to characterize. Figure 1 shows the assignment and integration of the $^1$H NMR spectrum of **2** which suggests 16 molecules of paclitaxel are conjugated. Obtaining consistent MALDI-TOF MS from the hydrophobic dendrimer **2** was challenging. The spectrum in Figure 2 shows a reasonable agreement between the calculated exact mass 21 038 and the observed mass 21 114 (*m*/*z*). Due to resolution in this mass range, the ladder of peaks occurring at lower mass cannot be uniquely attributed to incomplete reaction with **13** or loss of paclitaxel due to the labile ester link during ionization.

Both **4a** and **4b** were analyzed with GPC, $^1$H NMR, and MALDI-TOF MS. The pegylated dendrimers are readily soluble (>20 mg/mL) in aqueous media, allowing purification by dialysis. Separately, we find that pegylation with 0.6

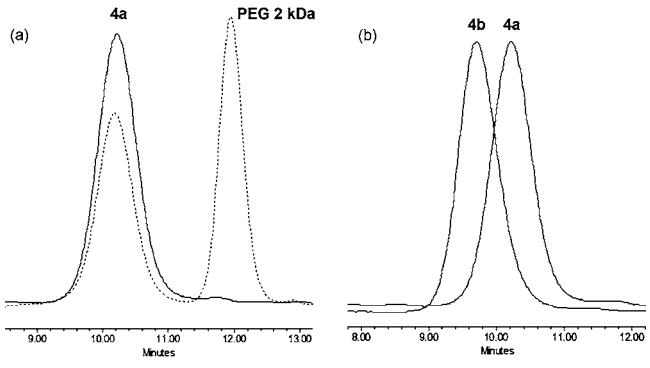



**Figure 3.** The analytical GPC chromatogram obtained using 0.1 M $NaNO_3$ (aq) as an eluent with a RI detector: (a) before (dotted line) and after (solid line) dialysis purification of **4a**; (b) GPC traces of **4a** and **4b**.

and 1.2 kDa PEG groups leads to products that are not water-soluble. The dendrimers were analyzed using GPC in 0.1 M $NaNO_3$ (aq) with a refractive index (RI) detector (Figure 3).

$^1$H NMR spectra of **4a** and **4b** shown in Figure 4 display unique lines for the aromatic protons of paclitaxel ($\delta$ 7.25−

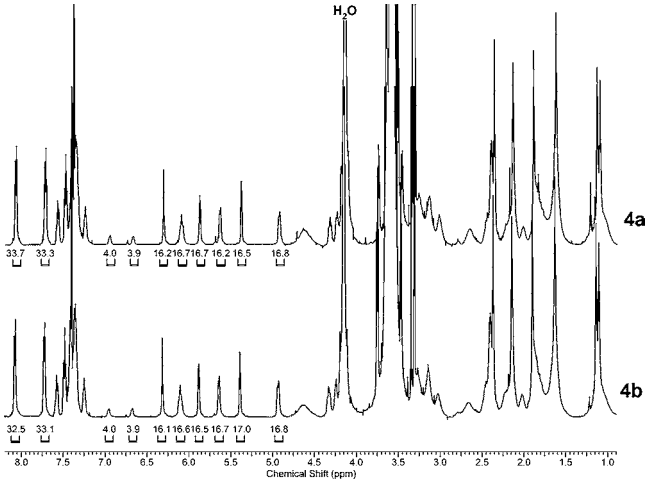



**Figure 4.** $^1$H NMR of the pegylated paclitaxel conjugates **4a** and **4b** purified from dialysis. The spectra were obtained in $CDCl_3$/MeOH-$d_4$ (10:1).

8.09 ppm), the radioiodination group ($\delta$ 6.68 and 6.96 ppm for ortho and meta protons, respectively), and the PEG chains ($\delta$ ~3.6 ppm). The integration values are similar to those of dendrimer **2**, suggesting little hydrolysis of paclitaxel occurs in the intervening steps including dialysis. The molecular weight was determined by MALDI-TOF MS: 46 100 Da for **4a** and 77 400 Da for **4b**. This corresponds to macromolecules that are 30 wt % (**4a**) and 18 wt % (**4b**) paclitaxel.

In summary, we have described a drug delivery vehicle, **2**, that has Bolton−Hunter-type groups to report biodistribution and multiple paclitaxel groups attached by labile ester linkages. The precursor dendrimer **1** was prepared at 64% overall yield from the bisprotected amine, and the modification of paclitaxel proceeded in four steps with 60% overall yield. Both mass spectrometry and NMR spectroscopy of **2** show that the drug conjugate contains 16 molecules of paclitaxel, indicating full coupling of **1** with the modified drug. After pegylation, we obtained two dendrimers **4a** and **4b** with a molecular weight of about 46 and 77 kDa, respectively. The degree of drug loading in **4a** at 20 mg/mL corresponds to the concentrations achieved in Cremophor EL (the clinically relevant castor oil derivative used for solubilization), namely, 6 mg/mL (before the requisite dilution with saline for intravenous delivery).[22]

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**Supporting Information Available:** Detailed experimental procedures, NMR, and mass spectrometry data. This material is available free of charge via the Internet at http://pubs.acs.org.

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