Intercepting the synthesis of triazine dendrimers with nucleophilic pharmacophores: a general strategy toward drug delivery vehicles[†]

Vincent J. Venditto^a, Kimberly Allred^b, Clinton D. Allred^b, and Eric E. Simanek^a

Clinton D. Allred: callred@ag.tamu.edu; Eric E. Simanek: simanek@tamu.edu

^aDepartment of Chemistry, Texas A&M University, College Station, TX 77843, USA. Fax: +1-979-845-9452; Tel: +1-979-845-4242

^bDepartment of Nutrition and Food Science, Texas A&M University, College Station, TX 77843, USA. Fax: +1-979-862-7782; Tel: +1-979-845-0863

Abstract

The camptothecin ester of isonipecotic acid is installed on a triazine dendrimer intermediate obtained through an iterative, scalable route to ultimately yield cationic and PEGylated targets with activities in cell culture comparable to free drug.

Macromolecular drug delivery agents are of interest in cancer therapy due to their extended lifetime within the circulatory system as compared to their small molecule counterparts and opportunities to tailor release rates.¹ The extended half-life provides opportunities for passive tumor targeting through the EPR (enhanced permeability and retention) effect for localized tumor therapy.² Various architectures have been investigated for this purpose,³ but recently much attention has focused on the use of dendrimers due to their monodispersity and ease of manipulation at their multivalent periphery.⁴

We recently completed the kilogram-scale synthesis of a second generation dendrimer⁵ that has attributes of a “universal” drug delivery vehicle. That is, this dendrimer can potentially be derivatized with a range of drugs in a rapid fashion through interception of one of two intermediates obtained during iterative synthesis (Scheme 1). We have shown that sophisticated, accessible, electrophilic dichlorotriazines bearing paclitaxel could be incorporated into the synthesis (light arrow).⁶ Here, we describe the interception of the poly(monochlorotriazine) containing dendrimer with nucleophiles (dark arrow) using camptothecin as a model.

Camptothecin (CPT) and its derivatives, notably irinotecan and topotecan, have been prescribed to patients with ovarian and colorectal cancers, respectively.⁷ Both derivatives possess broad spectrum anti-cancer effects⁸ and are most commonly prescribed to patients with late stage metastatic cancers. The success of CPT, however, is limited by lactone hydrolysis at physiological conditions that can be retarded by esterification of the C²⁰ hydroxyl group.⁹ Greenwald and co-workers have shown that hydrolysis of the lactone of CPT occurs above pH 9 upon esterification or alkylation.¹⁰ Davis *et al.* employ glycine esters for attachment of CPT to a PEG-cyclodextrin polymer that releases the drug slowly.¹¹

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Correspondence to: Clinton D. Allred, callred@ag.tamu.edu; Eric E. Simanek, simanek@tamu.edu.

Other macromolecular constructs containing CPT esters have been described including PEGylated drug,¹² micelles,¹³ dendrimers,¹⁴ and polymers of caprolactone,¹⁵ lactic acid,¹⁶ dextran,¹⁷ hydroxypropyl-methacrylamide,¹⁸ and glutamic acid.¹⁹

Our synthesis of the ester derivative of CPT (Scheme 2) utilizes 1-BOC-isonipetric acid under standard coupling conditions to afford **3** in 97% yield after methanol crystallization. Subsequent deprotection with trifluoroacetic acid gives the TFA salt of **4** in 82% after precipitation with methanol. Installation of the constrained secondary amine is a critical design element. These amines have been shown to be highly reactive towards monochlorotriazines.²⁰

Scheme 3 shows the route for elaboration to the final products. Reaction of **4** with **1** was accomplished in *N,N*-dimethylformamide in the presence of *N,N*-diisopropyl-ethylamine at 50 °C for 12 days to yield dendrimer **5**. While higher temperatures may accelerate this reaction, accelerated hydrolysis of the camptothecin ester led us to pursue this more conservative approach. Reaction progress could be followed with MALDI-TOF mass spectrometry with the desired species, **5**, appearing initially at day 4. While the spectra produced depend on matrix and ionization conditions, the reaction was continued until only lines corresponding to product, loss of BOC, and loss of a single CPT were observed. Purification was accomplished using Sephadex LH-20 size exclusion chromatography. While mass spectrometry shows the presence of dendrimer bearing 5 CPT groups, ¹H and ¹³C NMR cannot be used to unambiguously quantify purities of these species due to the broad signals inherent in these dendrimers. We estimate that the average loading of CPT–dendrimer is >5.5 based on mass spectrometry.

Dendrimer **5** was deprotected using trifluoroacetic acid to afford **6** in nearly quantitative yield. PEGylation of **6** using 2000 Da NHS-mPEG leads to dendrimer **7**. Estimates of the degree of PEGylation based on integration of a broad and overlapping NMR spectra (and unsatisfying data from mass spectrometry) lead us to conclude that **7** bears between 6 and 12 PEG chains. While both targets **6** and **7** are water soluble, **7** was purified by ultrafiltration using a YM3 regenerated cellulose membrane in an Amicon stirred cell. PEGylation significantly changes the molecular weight values of the targets (**6** is 5 kDa mol⁻¹; **7** is between 17 and 29 kDa mol⁻¹). In addition to potentially affecting the activity *in vivo*, the presence of nucleophilic, basic amines in **6** could impact release of CPT and toxicity.

The cytotoxicity data are summarized in Table 1. Irinotecan was selected as a control due to its clinical relevance and solubility in water. Treatment of MCF-7 (human breast) and HT-29 (colon cancer) cells provided dose dependent response curves for all analytes. The IC₅₀ value for CPT was 0.2 μM regardless of cell line, while the IC₅₀ for irinotecan varied with line, 52 μM or 33 μM. Targets **6** and **7** show similar behavior to CPT with values that reflect that approximately 10% of the drug is likely released over the course of the study. Curiously, a drug-free dendrimer control bearing piperidine groups instead of isonipetric esters showed no toxicity up to 10 μM in MCF-7 cells, but an IC₅₀ value of 2 μM in HT-29 cells. At this time it is unclear why there is a pronounced difference in toxicity between cell lines. Similar cell specific cytotoxicity has been observed using cyclic peptides.²¹ Given the success of these constructs *in vitro*, further optimization of the synthetic strategy could offer routes to scales of material required for additional inquiry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

Strategy used for kilogram-scale dendrimer synthesis and further exploitation of intermediates for drug conjugation.

Scheme 2.

Installation of BOC-Inp on 20-(*S*)-camptothecin through a hydrolyzable ester linkage.

Scheme 3.
Elaboration of **1** to amine and PEGylated targets.

Table 1

IC₅₀ values (μM) determined by MTT assay and corrected for CPT concentration, parenthetically, assuming 6 CPT–dendrimer

Cells	CPT	Irinotecan	G2-NH ₂	6	7
MCF-7	0.2	52	2.3	1.5 (9.0)	2.2 (13)
HT-29	0.2	33	> 10	1.3 (8.0)	0.5 (3)