Scalable Synthesis of Functional Polyesters that Enable Effective siRNA Delivery to Cancer Cells

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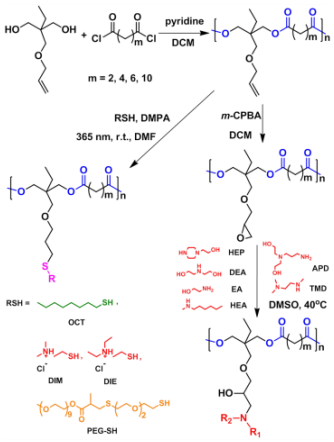
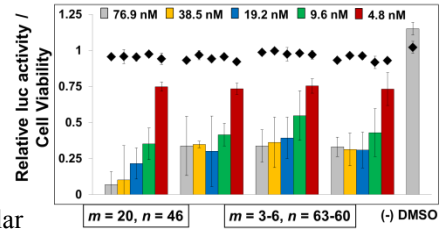
Statement of Purpose: Functional polyester chemistry is well suited to improve delivery of biomacromolecular drugs because it enables tuning of degradation profiles and inclusion of key functional groups (e.g. cationic charges for nucleic acid binding).[1] However, current approaches to introduce functionality suffer from inadequate scalability and modularity. To improve utility of these materials in translational medical applications, there is a need to develop higher yielding synthetic strategies for functional polyesters. We have developed a scalable library of functional polyesters based on the polymerization of trimethylolpropane allyl ether (TPAE) with diacid chlorides (poly(TPAE-*co*-AC)s). The reactive side chains can be easily modified with various functional thiols (e.g. alkyl-, amino-, and PEG-) and amines to generate a diverse set of materials, thereby tuning the chemistry for potential opportunities in drug delivery. The polymerization occurs readily under mild conditions at room temperature with M_w for poly(TPAE-*co*-suberoyl chloride) exceeding 42,000 g/mol. In addition, poly(TPAE-*co*-adipoyl chloride) was polymerized on a 100+ gram scale in 71% isolated yield. Due to the structural similarity to established non-functional polyesters such as PLGA, these new materials may find broad application as functional polyesters for gene delivery, cross-linked hydrogels for tissue engineering,[2] and other applications.

Methods: Polyesters containing -ene groups were prepared via polyesterification of TPAE and diacid chlorides with pyridine as the HCl acceptor in DCM at room temperature. In a typical experiment, 12 mL of DCM, 0.879 mL of TPAE (5 mmol) and 5 mmol of diacid chloride (e.g. 0.928 mL of suberoyl chloride) were added in a 50 mL round-bottom flask equipped with a rubber stopper. 0.892 mL of pyridine (11 mmol) was pumped into the mixture in ~8.5 min under N_2 atmosphere while stirring. The final product with desired molecular weight was obtained by removal of formed salts, followed by 3x precipitation in excess methanol. *Modification of ene-functionalized polyesters:* 0.1 g of P(TPAE-*co*-diACl-C8) with M_w of 24,800 g/mol, M_w/M_n = 1.3, containing 0.32 mmol -ene group, 16.5 mg of DMPA (0.2 equiv. to -ene groups), and 10 equiv. of thiol were added into 2 mL of DMF (6 mL for the modifications with tertiary amines due to their poorer solubility), and stirred for 0.5 h. The mixture was purged with N_2 flow for 15 min, and then irradiated under UV light (365 nm) at r.t. The modified polymers were purified by precipitation in methanol (for alkyl thiol reactions) or dialysis against methanol for 48 h. *In vitro siRNA delivery:* Polyester-siRNA complexes were prepared by adding 2.5 µL of polyester solution (1 g/L in DMSO) into 97.5 µL of siRNA solution (containing 250 ng siRNA) in phosphate buffer (pH 6.8, 10 mM) with a final weight ratio of 10 to 1 (polymer to siRNA) or 2 to 1 for mixed modifications. For each cell type, 10,000 cells and 50 ng siRNA per well (19.2 nM) were used in screening. Dose response was also performed on hit materials.

Results: The optimal conditions for the polycondensation were determined to be TPAE:AC:pyridine of 1:1:2.2 (mol) with addition of the promoter (pyridine) into the mixture of TPAE and diacid chloride in DCM. The polymerization was scalable, and enabled the synthesis of a library of functional polyesters via thiol-ene and epoxy-amine strategies (**right**). Previous studies identified a number of key functional groups that enable siRNA delivery *in vitro* and *in vivo*.[3] Those groups have been incorporated into this new library of materials. Key members of this library including diethyl amine and alkyl chain hydrophobic modifications. These polymers could efficiently deliver siLuc to HeLa cells stably expressing luciferase (generated using a Lentivirus). The delivery exhibited dose response, and was effective at low doses of siRNA (**right**). Indicated concentrations show siRNA molar concentrations. Other members of this library could selectively silence luciferase expression in HeLa Luc, MDA-231 Luc (breast cancer), and A549 Luc (lung cancer) lines. (Data not shown due to space constraints).





Conclusions: We developed a scalable and facile approach for the synthesis of high molecular weight -ene containing polyesters. These polymers are capable of functionalization via thiol-ene and epoxy-amine chemistries. The functional polyesters have been able to successfully deliver siRNA *in vitro* to HeLa, A549, and MDA-231 cell lines. Delivery *in vivo* will also be presented for top performing materials.

References: 1) Tian HY, Tang ZH, Zhuang, XL, Chen XS, Jing XB. *Prog. Polym. Sci.* **2012**; *37*, 237-280.
2) Cheng H, et al. *Adv. Mater.* **2011**, *23*, H95-H100.
3) Siegwart, DJ et al. *Proc. Natl. Acad. Sci. U.S.A.* **2011**; *108*, 12996-13001.