

# Oligo(lactic acid)<sub>n</sub>-Paclitaxel Prodrugs for Poly(ethylene glycol)-*block*-poly(lactic acid) Micelles: Loading, Release, and Backbiting Conversion for Anticancer Activity

Yu Tong Tam, Jieming Gao, and Glen S. Kwon\*

Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison, 777 Highland Avenue, Madison, Wisconsin 53705-2222, United States

## Supporting Information

**ABSTRACT:** Poly(ethylene glycol)-*block*-poly(D,L-lactic acid) (PEG-*b*-PLA) micelles are nanocarriers for poorly water-soluble anticancer agents and have advanced paclitaxel (PTX) to humans due to drug solubilization, biocompatibility, and dose escalation. However, PEG-*b*-PLA micelles rapidly release PTX, resulting in widespread biodistribution and low tumor exposure. To improve delivery of PTX by PEG-*b*-PLA micelles, monodisperse oligo(L-lactic acid), o(LA)<sub>8</sub> or o(LA)<sub>16</sub>, has been coupled onto PTX at the 7-OH position, forming ester prodrugs: o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX, respectively. As expected, o(LA)<sub>n</sub>-PTX was more compatible with PEG-*b*-PLA micelles than PTX, increasing drug loading from 11 to 54%. While *in vitro* release of PTX was rapid, resulting in precipitation, o(LA)<sub>n</sub>-PTX release was more gradual:  $t_{1/2}$  = 14 and 26 h for o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX, respectively. Notably, o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX in PEG-*b*-PLA micelles resisted backbiting chain end scission, based on reverse-phase HPLC analysis. By contrast, o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX degraded substantially in 1:1 acetonitrile:10 mM PBS, pH 7.4, at 37 °C, generating primarily o(LA)<sub>2</sub>-PTX. The IC<sub>50</sub> value of o(LA)<sub>2</sub>-PTX was ~2.3 nM for A549 human lung cancer cells, equipotent with PTX *in vitro*. After weekly IV injections at 20 mg/kg as PEG-*b*-PLA micelles, o(LA)<sub>8</sub>-PTX induced tumor regression in A549 tumor-bearing mice, whereas PTX delayed tumor growth. Surprisingly, o(LA)<sub>8</sub>-PTX caused less toxicity than PTX in terms of change in body weight. In conclusion, o(LA)<sub>n</sub> acts as a novel promoiety, undergoing backbiting conversion without a reliance on metabolizing enzymes, and o(LA)<sub>n</sub>-PTX improves PTX delivery by PEG-*b*-PLA micelles, providing a strong justification for clinical evaluation.

Poly(ethylene glycol)-*block*-poly(D,L-lactic acid) (PEG-*b*-PLA) micelles have been widely studied in anticancer drug development and delivery due to a capacity for drug solubilization, and this class of injectable nanocarriers has progressed to humans for paclitaxel (PTX) as a safer alternative to Cremophor EL and ethanol found in Taxol (PTX) injection. Termed Genexol-PM and Cynviloq in Asia and the U.S.A., respectively, PEG-*b*-PLA micelles permit dose escalation of PTX with less induction of life-threatening hypersensitivity reactions relative to Taxol.<sup>1</sup> Genexol-PM is approved in Asia

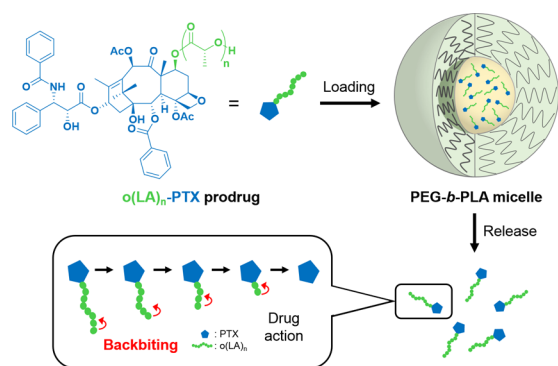
and in a phase 3 clinical trial, the bioequivalence of Cynviloq and Abraxane (nanoparticle albumin-bound PTX) is being evaluated in the U.S.A.<sup>2</sup> Thus, PEG-*b*-PLA micelles possess a proven track record of safety, sterility, and scale-up for clinical translation. However, low drug loading and poor stability against precipitation, e.g.,  $\beta$ -lapachone, limit the broader usage of PEG-*b*-PLA micelles for drug delivery. Further, burst drug release for PEG-*b*-PLA micelles is commonplace *in vivo*, limiting desirable pharmacokinetic (PK) properties, such as a reduced volume of distribution for reduced toxicity and tumor targeting by the enhanced permeability (EPR) effect for increased antitumor efficacy.<sup>3</sup>

To improve PK properties of anticancer agents and broaden the range of accessible anticancer agents for clinical translation, lipophilic acyl ester prodrugs have been synthesized for polymeric micelles. Forrest et al. have stably loaded a 7-hexanoate ester of PTX in PEG-*block*-poly( $\epsilon$ -caprolactone) micelles and revealed equipotent *in vitro* cytotoxicity, lower clearance, and higher exposure of PTX vs Taxol.<sup>4</sup> A 2'-acyl prodrug attached on PTX by a diglycolate linkage has also been stably loaded in PEG-*block*-poly(styrene) micelles by Mayer et al. While prodrugs were 1–2 orders of magnitude less potent than PTX *in vitro*, a long-circulating diglycolate PTX prodrug exerted higher antitumor efficacy than PTX at the maximum tolerated dose (MTD) of 60 and 20 mg/kg, respectively, in a HT29 human colon carcinoma model.<sup>5</sup> More recently, a diacyl ester of  $\beta$ -lapachone has been synthesized and stably loaded in PEG-*b*-PLA micelles by Gao et al., resulting in increases in tumor exposure in an orthotopic A549 non-small cell lung cancer tumor model and survival at the MTD (70 mg/kg) vs  $\beta$ -lapachone at its MTD (25 mg/kg).<sup>6</sup> In summary, lipophilic acyl ester prodrugs have higher loading and slower release from polymeric micelles in comparison to parent drugs, resulting in increases in MTD, tumor exposure, and antitumor efficacy. However, lipophilic ester prodrugs are less cytotoxic *in vitro* and *in vivo*, reflecting esterase-dependent hydrolytic conversion, which may vary according to type of esterase, substrate specificity, and interindividual differences in enzyme levels.<sup>7</sup>

For PEG-*b*-PLA micelles, we hypothesize that o(LA)<sub>n</sub>-PTX will have higher loading and slower release in comparison to PTX (Figure 1), and upon release, o(LA)<sub>n</sub>-PTX will undergo conversion by a backbiting mechanism: intramolecular attack of

Received: April 19, 2016

Published: July 3, 2016

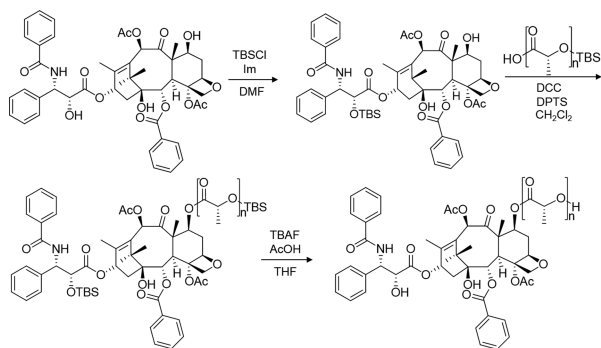


**Figure 1.** Oligo(lactic acid)<sub>n</sub>-paclitaxel prodrugs for PEG-*b*-PLA micelles: Loading, release, and backbiting conversion for anticancer activity.

the terminal-hydroxyl of o(LA)<sub>n</sub> on the penultimate ester bond, loss of lactoyllactate, and stepwise chain end scission. We note three distinct features of o(LA)<sub>n</sub> as a pro-moiety for PTX: (1) Release of o(LA)<sub>n</sub>-PTX from PEG-*b*-PLA micelles may be tuned by adjusting the chain length of o(LA)<sub>n</sub>; (2) conversion of o(LA)<sub>n</sub>-PTX may be preprogrammed as a function of chain length of o(LA)<sub>n</sub>, contrasting with earlier lipophilic acyl ester prodrugs that rely on esterases; and (3) lactoyllactate, major degradation product of o(LA)<sub>n</sub>, hydrolyzes into two units of nontoxic D,L-lactic acid. In the research of Tong and Cheng, the 2'-OH of PTX was used to polymerize D,L-lactic acid, forming PLA-PTX conjugates of varied chain length for the preparation of novel conjugate nanoparticles.<sup>8</sup> Although the 2'-OH of PTX is the most reactive and the most common site for prodrug synthesis, complete removal of the pro-moiety is a requirement for bioactivity.<sup>9</sup> Alternatively, esterification of the 7-OH of PTX has been shown to minimally impact the assembly of microtubules, and this site was chosen for the coupling of o(LA)<sub>n</sub> on PTX, characterization of o(LA)<sub>n</sub> conversion, and evaluation of o(LA)<sub>n</sub>-PTX bioactivity *in vitro* and *in vivo*.

TBS-PTX was esterified at the 7-OH position with TBS-o(LA)<sub>8</sub> or TBS-o(LA)<sub>16</sub> by 1,3-dicyclohexylcarbodiimide coupling, followed by deprotection, resulting in o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX prodrugs (Scheme 1). An orthogonal synthetic strategy afforded monodisperse o(LA)<sub>n</sub> for detailed structure–activity relationships for o(LA)<sub>n</sub>-PTX prodrugs.<sup>10</sup> The chemical structures of o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX prodrugs were supported by <sup>1</sup>H NMR spectroscopy and electrospray mass spectroscopy analysis (Figure S1). Selective

**Scheme 1. Synthetic Scheme for o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX Prodrugs**



reduction of o(LA)<sub>8</sub>-PTX at the 13-ester group produced (1S,2R)-N-1-(1-phenyl-2,3-dihydroxypropyl)benzamide (DPPB) and o(LA)<sub>8</sub>-baccatin III, based on reverse-phase HPLC analysis and mass spectroscopy (Figures S2–S5), providing support for coupling of o(LA)<sub>8</sub> or o(LA)<sub>16</sub> solely at the 7-OH position of PTX.

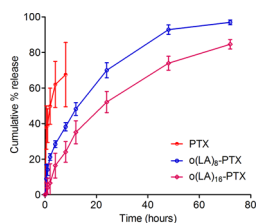
Physicochemical properties of PEG-*b*-PLA micelles containing PTX, o(LA)<sub>8</sub>-PTX, or o(LA)<sub>16</sub>-PTX prodrug are summarized in Table 1. PEG-*b*-PLA micelles increased the water solubility of PTX from ~10 mg/L to 0.9 mg/mL, forming micelles with an average hydrodynamic diameter at 30.5 nm and 8.6% drug loading. However, an increase in the water solubility of PTX was not realized by a 6-fold increase in the initial level of PTX used in drug loading. Instead, loading efficiency of PTX was low, ~21%, and drug loading for PEG-*b*-PLA micelles leveled off 11.2% drug loading. Notably, PEG-*b*-PLA micelles containing PTX were unstable at room temperature, precipitating in <2 h. By contrast, drug loading of o(LA)<sub>8</sub>-PTX prodrug for PEG-*b*-PLA micelles increased from 8.7 to 37.1 and 54.5% with a 6- and 12-fold increase in the initial level of prodrug, respectively, and loading efficiency was ~100%. The hydrodynamic diameter of PEG-*b*-PLA micelles containing o(LA)<sub>8</sub>-PTX prodrug at 37.1 and 54.5% increased to 58.8 and 100 nm, respectively. Drug loading of o(LA)<sub>16</sub>-PTX prodrug was also higher than PTX for PEG-*b*-PLA micelles, ~39% and 6.2 mg/mL in water. Notably, PEG-*b*-PLA micelles containing o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrug were stable at 37 °C >72 h, indicating thermodynamic stability for o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrug solubilization. At 9% drug loading and particle size at 30 nm, PEG-*b*-PLA micelles rapidly released PTX *in vitro*, resulting in precipitation of PTX <4 h (Figure 2). By contrast, *in vitro* release of o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrug from PEG-*b*-PLA micelles was gradual, with *t*<sub>1/2</sub> = 14.2 and 26.5 h, respectively, indicating control of prodrug release by tuning of o(LA)<sub>n</sub> chain length. In summary, o(LA)<sub>8</sub> or o(LA)<sub>16</sub> as a pro-moiety acts as a compatibilizer between o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrugs and PEG-*b*-PLA micelles, resulting in improvements in drug loading, physical stability, and drug release in comparison to PTX.

Prior research by Hennink et al. showed that o(LA)<sub>n</sub> with a free hydroxyl-containing chain end degrades by chain end scission by a backbiting mechanism, as opposed to a random chain scission mechanism observed for high molecular weight PLA.<sup>11</sup> In a 1:1 mixture of acetonitrile and PBS buffer (pH 7.4, 10 mM), used to gain solubility, o(LA)<sub>8</sub>-PTX prodrug eluted at ~23 min, and upon conversion, it produced a series of well-defined peaks with shorter elution times, approaching the elution time of PTX, ~12 min (Figure 3). The major peaks were assigned to even number degradation products of o(LA)<sub>8</sub>-PTX upon the loss of lactoyllactate upon backbiting: o(LA)<sub>6</sub>-PTX, o(LA)<sub>4</sub>-PTX, o(LA)<sub>2</sub>-PTX, and PTX, whereas noticeably smaller peaks corresponded to odd number degradation products from random hydrolysis. The relative area (%) of o(LA)<sub>8</sub>-PTX prodrug, even number degradation products, and o(LA)<sub>1</sub>-PTX were plotted vs time (Figure 4). The *t*<sub>1/2</sub> for the conversion of o(LA)<sub>8</sub>-PTX prodrug was ~7.3 h, producing o(LA)<sub>2</sub>-PTX as the major species and to a lesser extent o(LA)<sub>1</sub>-PTX and PTX over 300 h (Figure S6). Similarly, o(LA)<sub>16</sub>-PTX prodrug generated a backbiting degradation profile based on reverse-phase HPLC analysis (Figures S7–S8): *t*<sub>1/2</sub> = 7.4 h and even number degradation products, mostly o(LA)<sub>2</sub>-PTX. On the other hand, conversion of o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrugs in PEG-*b*-PLA micelles slowed considerably: *t*<sub>1/2</sub> =

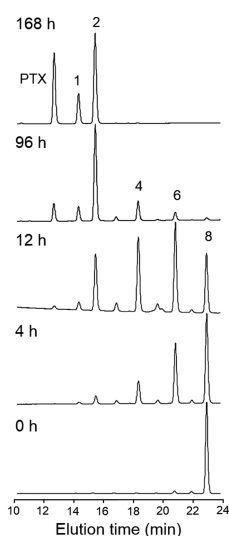
**Table 1. Physicochemical Properties of PEG-*b*-PLA Micelles Containing PTX, o(LA)<sub>8</sub>-PTX, or o(LA)<sub>16</sub>-PTX Prodrug**

drug	initial level of PTX (mg) <sup>a</sup>	drug loading efficiency (%)	drug loading (%)	size (nm)	apparent solubility (mg/mL)	stability (h)
PTX	1	93.8 ± 12.8	8.6 ± 1.1	30.5 ± 0.3	0.9 ± 0.1	<2
	6	21.0 ± 2.3	11.2 ± 1.1	37.0 ± 5.7	1.2 ± 0.1	<2
o(LA) <sub>8</sub> -PTX	1	94.9 ± 3.6	8.7 ± 0.4	32.7 ± 0.6	0.9 ± 0.4	>72
	6	98.4 ± 10.0	37.1 ± 2.3	58.8 ± 0.3	5.9 ± 0.6	>72
o(LA) <sub>16</sub> -PTX	12	100.7 ± 10.0	54.5 ± 4.3	100.0 ± 1.3	12 ± 1.0	>72
	1	96.4 ± 3.3	8.8 ± 0.3	31.0 ± 0.2	1.0 ± 0.3	>72
	6	104 ± 5.0	38.6 ± 1.0	87.7 ± 0.8	6.2 ± 0.2	>72
	12	45.8 ± 3.6	35.4 ± 1.8	89.0 ± 6.3	5.5 ± 0.4	>72

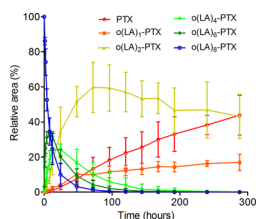
<sup>a</sup>10 mg of PEG-*b*-PLA was used in each formulation. (mean ± SD, *n* = 3) ; *M<sub>n</sub>* of PEG = 4000 g/mol; *M<sub>n</sub>* of PLA = 2200 g/mol.



**Figure 2.** *In vitro* release of PTX, o(LA)<sub>8</sub>-PTX, or o(LA)<sub>16</sub>-PTX prodrug from PEG-*b*-PLA micelles (mean ± SD, *n* = 3).



**Figure 3.** Reverse-phase HPLC chromatograms of o(LA)<sub>8</sub>-PTX prodrug and its backbiting conversion products after incubation in 1:1 CH<sub>3</sub>CN/10 mM PBS at 37 °C, pH 7.4 at 0, 4, 12, 96, and 168 h.

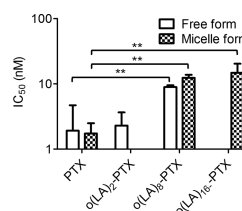


**Figure 4.** Backbiting conversion of o(LA)<sub>8</sub>-PTX prodrug into o(LA)<sub>6</sub>-PTX, o(LA)<sub>4</sub>-PTX, o(LA)<sub>2</sub>-PTX, and PTX (mean ± SD, *n* = 3).

157 and 315 h, respectively (Figures S9–S10), consistent with hindered backbiting reaction in a nonpolar environment (PLA core).<sup>11,12</sup> In summary, conversion of o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrugs in an aqueous solution proceeds rapidly by a backbiting reaction, but slowly in PEG-*b*-PLA micelles in water. Thus, PEG-*b*-PLA micelles can stably carry o(LA)<sub>8</sub>-PTX or

o(LA)<sub>16</sub>-PTX prodrug, and upon release, o(LA)<sub>8</sub>-PTX or o(LA)<sub>16</sub>-PTX prodrug undergoes rapid backbiting, primarily generating o(LA)<sub>2</sub>-PTX and to a lesser extent o(LA)<sub>1</sub>-PTX and PTX.

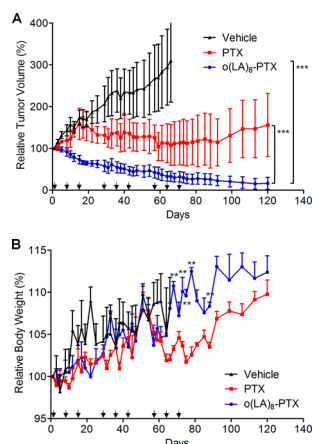
PTX is a potent anticancer agent as a microtubule stabilizer, and it plays a central role in the treatment of non-small cell lung cancer. Accordingly, PTX had a low IC<sub>50</sub> value of 2.0 nM for the human A549 non-small cancer cell line (Figure 5). The IC<sub>50</sub>



**Figure 5.** *In vitro* cytotoxicity of PTX, o(LA)<sub>2</sub>-PTX, o(LA)<sub>8</sub>-PTX, or o(LA)<sub>16</sub>-PTX prodrug against human A549 non-small lung cancer cells. Columns, mean of quadruplicate determinations; bars, SD; \*\*, *p* < 0.01 for o(LA)<sub>8</sub>-PTX compared to PTX for free and micelle forms.

value of o(LA)<sub>8</sub>-PTX in the free form has a slighter higher value of 8.9 nM, reflecting the time needed for conversion. The IC<sub>50</sub> values of o(LA)<sub>8</sub>-PTX and o(LA)<sub>16</sub>-PTX as micelles were about 7-fold higher, ~15 nM, reflecting the time needed for release from PEG-*b*-PLA micelles (over 72 h). Notably, o(LA)<sub>2</sub>-PTX, the major species generated from backbiting, was equipotent with PTX *in vitro*. Thus, 2 lactic acid units at the 7-OH position of paclitaxel does not interfere with microtubule stabilization, defining o(LA)<sub>2</sub>-PTX, o(LA)<sub>1</sub>-PTX, and PTX as bioactive species. By contrast, 2'-OH ester prodrugs require full conversion back to PTX for cytotoxicity. In summary, backbiting of o(LA)<sub>8</sub>-PTX prodrug generates cytotoxic species, primarily o(LA)<sub>2</sub>-PTX, without a reliance on converting esterases, enabling a novel prodrug strategy for PEG-*b*-PLA micelles.

The *in vivo* anticancer efficacy of PEG-*b*-PLA micelles containing PTX or o(LA)<sub>8</sub>-PTX prodrug was evaluated in an A549 xenograft model after weekly tail vein injection at a dose of 20 mg/kg (Figure 6a). It is noted that the MTD for Taxol and Genexol-PM is 20 and 60 mg/kg, respectively, and the MTD for o(LA)<sub>8</sub>-PTX prodrug has yet to be defined. Further, a weekly IV injection schedule for PTX or o(LA)<sub>8</sub>-PTX prodrug was evaluated because of its clinical relevance (3 weekly injections and 1 week off × 3 cycles). With PEG-*b*-PLA micelles containing PTX at 20 mg/kg, growth of A549 tumors paralleled the tumor growth of the vehicle control for about 2 weeks, followed by tumor growth inhibition during treatment over 71 days and delayed tumor growth. By contrast, PEG-*b*-



**Figure 6.** (A) *In vivo* antitumor efficacy PTX or o(LA)<sub>8</sub>-PTX prodrug (20 mg/kg) as PEG-*b*-PLA micelles (9% loading) in an A549 xenograft tumor model. Mice received 3 weekly injections followed by 1 week off for 3 cycles (mean  $\pm$  SEM,  $n = 3-4$ ). Bars, SEM; \*\*\*,  $p < 0.001$ . (B) Relative body weight of mice treated with PTX or o(LA)<sub>8</sub>-PTX prodrug (20 mg/kg) as PEG-*b*-PLA micelles (9% loading). Bars: SEM; \*\*,  $p < 0.01$ .

PLA micelles containing o(LA)<sub>8</sub>-PTX prodrug at 20 mg/kg decreased tumor volumes during weekly treatment over 71 days without relapse up to 120 days (Figure 6a). Surprisingly, o(LA)<sub>8</sub>-PTX prodrug was also less toxic than PTX in terms of body weight change (Figure 6b).

PTX ester prodrugs are often water-soluble and less toxic but less active as anticancer agents. However, o(LA)<sub>8</sub>-PTX prodrug with PEG-*b*-PLA micelles is a unique anticancer nanomedicine in terms of backbiting conversion, physical stability, lower toxicity, and higher antitumor efficacy in an A549 xenograft model. Given slower *in vitro* release and improved physical stability of o(LA)<sub>8</sub>-PTX prodrug micelles vs PTX micelles, o(LA)<sub>8</sub>-PTX prodrug may circulate longer in bloodstream, increase AUC, reduce the distribution of PTX into nontarget tissue, increase tumor exposure (EPR effect), and undergo intratumoral conversion by backbiting. We note that the small size of PEG-*b*-PLA micelles containing o(LA)<sub>8</sub>-PTX prodrug (~30 nm) is favorable for the EPR effect,<sup>13</sup> and the low  $C_{\max}$  of PTX brought about o(LA)<sub>8</sub>-PTX prodrug favors low host toxicity, especially in comparison to PEG-*b*-PLA micelles containing PTX. The contribution of esterases on o(LA)<sub>8</sub>-PTX conversion *in vivo* awaits characterization. In the current context of a phase 3 clinical trial on Cynviloq and Abraxane in the U.S.A., o(LA)<sub>8</sub>-PTX prodrug using proven PEG-*b*-PLA micelle technology may represent a significant advance in PTX delivery and cancer treatment. Lastly, o(LA)<sub>8</sub> as pro-moiety may be expanded to other poorly water-soluble anticancer agents and enhance delivery by PEG-*b*-PLA micelles.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03995.

Experimental procedures, synthesis, and characterization of o(LA)<sub>*n*</sub>-PTX prodrug, backbiting conversion of o(LA)<sub>*n*</sub>-PTX prodrug in PEG-*b*-PLA micelles (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

\*glen.kwon@wisc.edu

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported in part by NIH (R01AI01157). The purchase of the LCT was partially funded by NSF award no. CHE-9974839.

## ■ REFERENCES

- (1) (a) Xiao, R. Z.; Zeng, Z. W.; Zhou, G. L.; Wang, J. J.; Li, F. Z.; Wang, A. M. *Int. J. Nanomedicine* **2010**, *5*, 1057. (b) Kim, S. C.; Kim, D. W.; Shim, Y. H.; Bang, J. S.; Oh, H. S.; Kim, S. W.; Seo, M. H. *J. Controlled Release* **2001**, *72*, 191–202. (c) Kim, T. Y.; Kim, D. W.; Chung, J. Y.; Shin, S. G.; Kim, S. C.; Heo, D. S.; Kim, N. K.; Bang, Y. J. *Clin. Cancer Res.* **2004**, *10*, 3708. (d) Lee, K. S.; Chung, H. C.; Im, S. A.; Park, Y. H.; Kim, C. S.; Kim, S. B.; Rha, S. Y.; Lee, M. Y.; Ro, J. *Breast Cancer Res. Treat.* **2008**, *108*, 241–250.
- (2) Cho, H.; Gao, J.; Kwon, G. S. *J. Controlled Release*, in press, **2015** <http://dx.doi.org/10.1016/j.jconrel.2015.12.015>.
- (3) (a) Ma, X.; Huang, X.; Huang, G.; Li, L.; Wang, Y.; Luo, X.; Boothman, D. A.; Gao, J. *Adv. Healthcare Mater.* **2014**, *3*, 1210–1216. (b) Miller, T.; Breyer, S.; van Colen, G.; Mier, W.; Haberkorn, U.; Geissler, S.; Voss, S.; Weigandt, M.; Goepferich, A. *Int. J. Pharm.* **2013**, *445*, 117–124. (c) Owen, S. C.; Chan, D. P.; Shoichet, M. S. *Nano Today* **2012**, *7*, 53–65.
- (4) Forrest, M. L.; Yáñez, J. A.; Remsberg, C. M.; Ohgami, Y.; Kwon, G. S.; Davies, N. M. *Pharm. Res.* **2008**, *25*, 194–206.
- (5) Ansell, S. M.; Johnstone, S. A.; Tardi, P. G.; Lo, L.; Xie, S.; Shu, Y.; Harasym, T. O.; Harasym, N. L.; Williams, L.; Bermudes, D.; Liboiron, B. D.; Saad, W.; Prud'homme, R. K.; Mayer, L. D. *J. Med. Chem.* **2008**, *51*, 3288–3296.
- (6) Ma, X.; Huang, X.; Moore, Z.; Huang, G.; Kilgore, J. A.; Wang, Y.; Hammer, S.; Williams, N. S.; Boothman, D. A.; Gao, J. *J. Controlled Release* **2015**, *200*, 201–211.
- (7) Yang, Y. H.; Aloysius, H.; Inoyama, D.; Chen, Y.; Hu, L. Q. *Acta Pharm. Sin. B* **2011**, *1*, 143–159.
- (8) Tong, R.; Cheng, J. *Angew. Chem.* **2008**, *120*, 4908–4912.
- (9) (a) Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. J. *J. Med. Chem.* **1992**, *35*, 145–151. (b) Kingston, D. G. *Trends Biotechnol.* **1994**, *12*, 222–227.
- (10) Takizawa, K.; Nulwala, H.; Hu, J.; Yoshinaga, K.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 5977–5990.
- (11) (a) De Jong, S. J.; Arias, E. R.; Rijkers, D. T. S.; Van Nostrum, C. F.; Kettenes-Van den Bosch, J. J.; Hennink, W. E. *Polymer* **2001**, *42*, 2795–2802. (b) van Nostrum, C. F.; Veldhuis, T. F.; Bos, G. W.; Hennink, W. E. *Polymer* **2004**, *45*, 6779–6787.
- (12) (a) Van de Wetering, P.; Zuidam, N. J.; Van Steenberg, M. J.; Van der Houwen, O. A. G. J.; Underberg, W. J. M.; Hennink, W. E. *Macromolecules* **1998**, *31*, 8063–8068. (b) Villermaux, S.; Villermaux, J.; Gibert, R. *J. Chim. Phys.* **1966**, *63*, 1356.
- (13) Cabral, H.; Matsumoto, Y.; Mizuno, K.; Chen, Q.; Murakami, M.; Kimura, M.; Terada, Y.; Kano, M. R.; Miyazono, K.; Uesaka, M.; Nishiyama, N.; Kataoka, K. *Nat. Nanotechnol.* **2011**, *6*, 815–823.