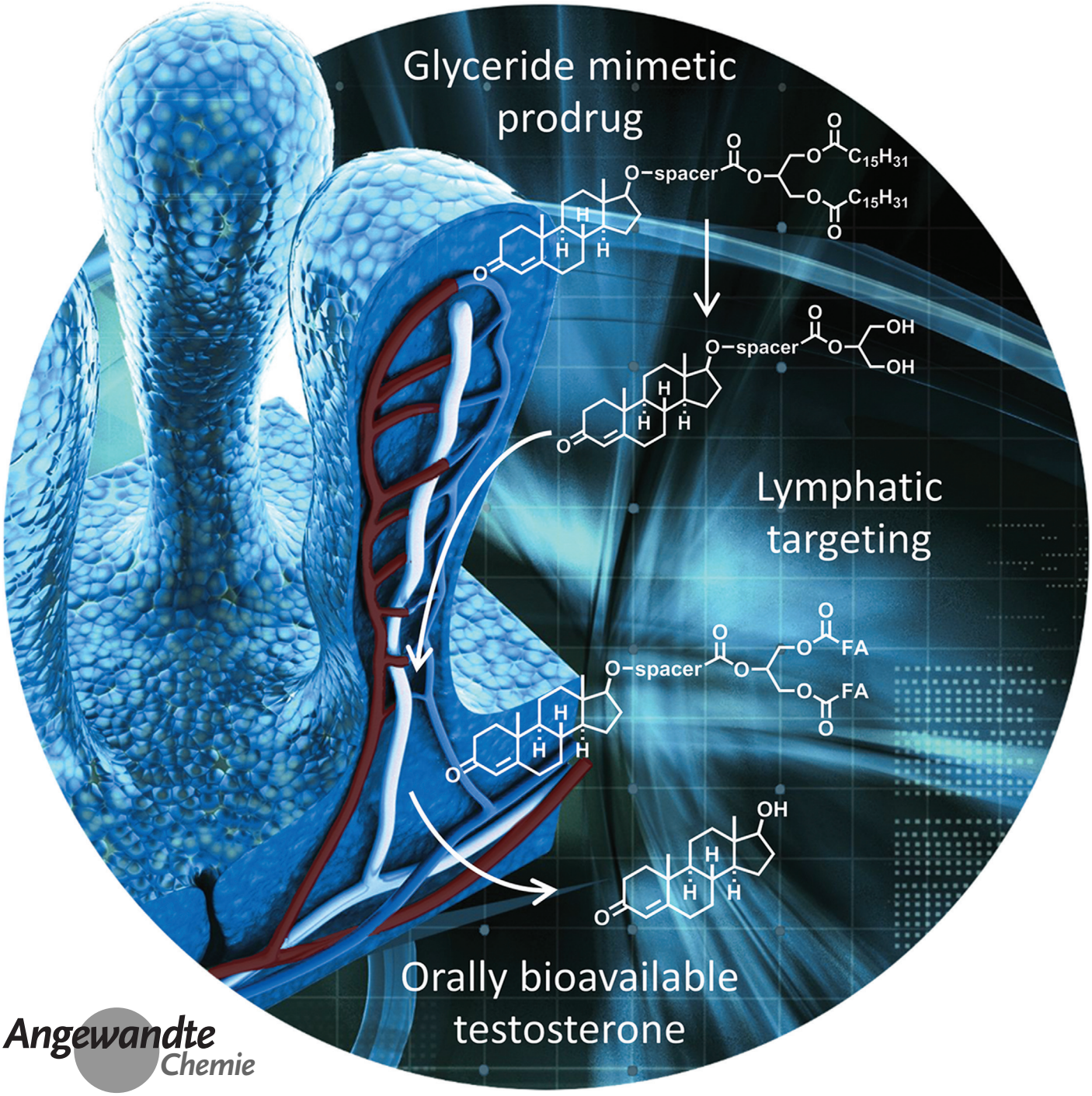
Prodrugs

Deutsche Ausgabe: DOI: 10.1002/ange.201604207
Internationale Ausgabe: DOI: 10.1002/anie.201604207

Glyceride-Mimetic Prodrugs Incorporating Self-Immolative Spacers Promote Lymphatic Transport, Avoid First-Pass Metabolism, and Enhance Oral Bioavailability

Luojuan Hu[+], Tim Quach[+], Sifei Han[+], Shea F. Lim, Preeti Yadav, Danielle Senyschyn, Natalie L. Trevaskis, Jamie S. Simpson, and Christopher J. H. Porter**



Abstract: *First-pass hepatic metabolism can significantly limit oral drug bioavailability. Drug transport from the intestine through the lymphatic system, rather than the portal vein, circumvents first-pass metabolism. However, the majority of drugs do not have the requisite physicochemical properties to facilitate lymphatic access. Herein, we describe a prodrug strategy that promotes selective transport through the intestinal lymph vessels and subsequent release of drug in the systemic circulation, thereby enhancing oral bioavailability. Using testosterone (TST) as a model high first-pass drug, glyceride-mimetic prodrugs incorporating self-immolative (SI) spacers, resulted in remarkable increases (up to 90-fold) in TST plasma exposure when compared to the current commercial product testosterone undecanoate (TU). This approach opens new opportunities for the effective development of drugs where oral delivery is limited by first-pass metabolism and provides a new avenue to enhance drug targeting to intestinal lymphoid tissue.*

After oral administration, first-pass drug metabolism in the liver limits exposure in the general (systemic) circulation and is a significant potential barrier to clinical and commercial success. First-pass metabolism precludes useful oral bioavailability for marketed drugs such as testosterone (TST) and buprenorphine, and limits the progression of many more experimental drug candidates. The problems of first-pass hepatic metabolism may be overcome by re-routing the pathway of drug absorption from the portal blood to the intestinal lymph.[1] The intestinal lymph is responsible for the transport of dietary lipids (primarily triglyceride, TG) from the small intestine to the systemic circulation and drains from the gut through the lymphatic ducts directly into the major veins in the neck (rather than first-passing through the liver).

Dietary TG is digested in the intestine to form diglyceride and ultimately monoglyceride (MG) and fatty acid (FA). After absorption into enterocytes, MG and FA are re-esterified to TG and assembled into lymph lipoproteins (LP). The size of LP (typically 0.1–1 μm) precludes access to the blood across the continuous vascular endothelium and instead promotes specific entry into the more permeable lymphatic capillaries.[2] LP association thus dictates specific access to lymph. Drug association with intestinal LP may occur by partitioning, but this requires very high lipophilicity ($\log Ds > 5$ and TG solubilities $> 50\ mg\,g^{-1}$),[3] a condition met by few drug candidates. Alternatively, prodrugs may be generated that temporarily increase lipophilicity and integrate into intestinal lipid transport pathways to promote lymph uptake, but ultimately revert to the parent drug.

Herein, we describe the synthesis of lymphotropic prodrugs, based on a glyceride template, that mimic the digestion, re-esterification, and lymphatic transport pathways of dietary TG (Figure 1). Unlike the majority of small molecule drugs that are absorbed into the portal blood (Figure 1, ①) and exposed to first pass metabolism in the liver (②), TG-mimetic prodrugs are digested to form a MG-like intermediate (③), absorbed, re-esterified to a TG-derivative (④) and biochemically integrated into LP assembly pathways (⑤). LP containing drug-TG derivatives are subsequently transported through the intestinal lymphatic system (⑥), thereby avoiding first-pass metabolism in the liver. After entry into the systemic circulation, the re-esterified TG prodrug is hydrolyzed by blood- or tissue-resident lipases/esterases (⑦) to release the free drug.

Simple TG-prodrugs have been described previously, but have not been effective in enhancing oral bioavailability, a situation ascribed to poor drug release.[4] The synthetic approaches described herein were therefore designed to trigger drug release in the systemic circulation and to further enhance the utility of TG-based prodrugs by stabilizing drug-MG intermediates in the GI fluids. TST, an androgenic steroid hormone that is used to treat androgen deficiency (a condition present in 1 in 18 men aged 30–79 years[5]), was employed as a model high first-pass drug. TG-mimetic, rather than 2-MG mimetic prodrugs were employed due to stability concerns, in particular the known tendency for acyl group migration to generate the 1-MG equivalent, as has been shown previously when the acyl group is FA.[6] The TG derivative was also expected to be more lipid soluble, assisting in formulation preparation.

Prodrugs were synthesized by bridging drug and glyceride moieties with a C5 linker (Scheme 1). A self-immolative (SI) spacer group was inserted into the linker to promote drug release in the systemic circulation and an alkyl substitution was made β to the glyceride ester to sterically hinder enzymatic lability in the GI tract. After activation, typically by an enzymatic trigger, SI groups undergo spontaneous intramolecular reaction resulting in drug release. SI groups were first described in the prodrug literature[7] and more recently have been adopted to promote rapid fragmentation of polymeric species such as dendrimers.[8] Here, two SI groups (with different release mechanisms: elimination and cyclization) have been employed to promote drug release and to explore whether utility is linker-specific. 1,3-Diacylglycerol **1** was first converted to the acid-terminated TG **2** containing a C5 linker through reaction with glutaric anhydride.[9] Conjugation with TST was then achieved using standard ester-bond forming chemistry to give the simple alkyl glyceride prodrug **4** (TST-C5-TG). To generate prodrugs

[*] Dr. L. Hu,[+] Dr. S. Han,[+] P. Yadav, D. Senyschyn, Dr. N. L. Trevaskis, Prof. C. J. H. Porter
Drug Delivery, Disposition and Dynamics
Monash University
381 Royal Parade, Parkville, Victoria 3052 (Australia)
E-mail: chris.porter@monash.edu
Dr. T. Quach,[+] Dr. S. F. Lim, Dr. J. S. Simpson
Medicinal Chemistry, Monash University
381 Royal Parade, Parkville, Victoria 3052 (Australia)
E-mail: jamie.simpson@monash.edu
Dr. L. Hu,[+] Dr. T. Quach,[+] Dr. S. Han,[+] Dr. S. F. Lim, D. Senyschyn, Prof. C. J. H. Porter
ARC Centre of Excellence in Convergent Bio-Nano Science and Technology
Monash Institute of Pharmaceutical Sciences
Monash University
381 Royal Parade, Parkville, Victoria 3052 (Australia)
[+] These authors contributed equally to this work.
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201604207.

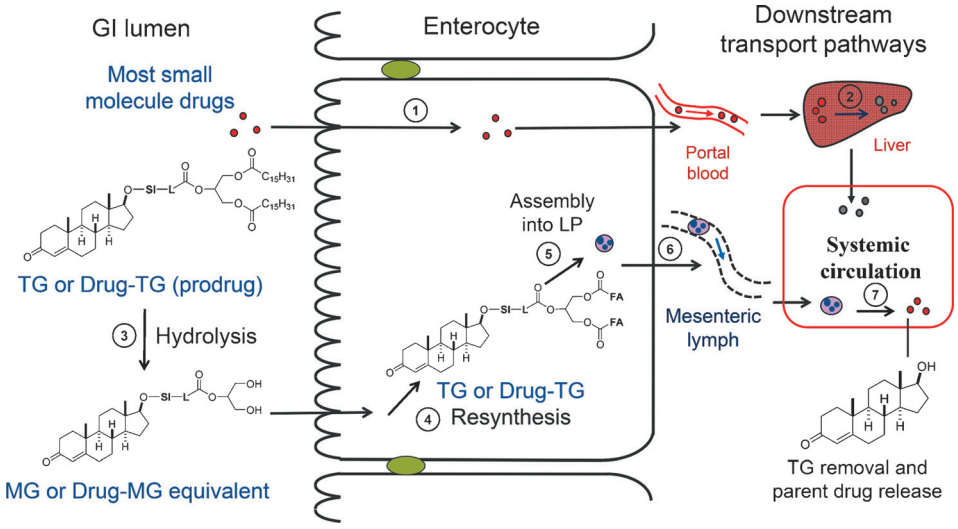
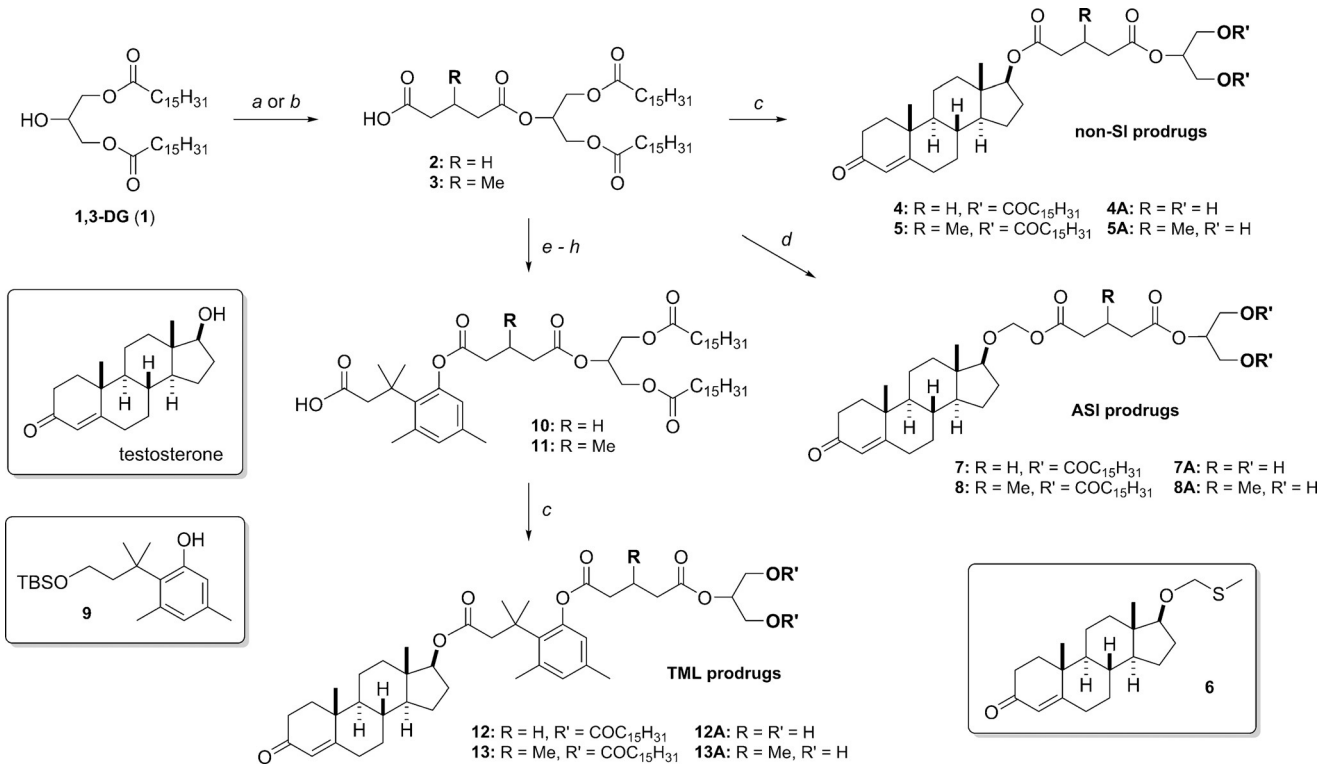


Figure 1. Blood vs. lymph transport pathways following oral drug or prodrug administration (see text for explanation). SI is self-immolative spacer, L is linker, TG is triglyceride, and MG is monoglyceride.

containing an acetal SI (ASI) group (release by elimination), TST was first transformed into (methylthio)methyl (MTM) ether **6** using known methods.[10] Activation of MTM ether **6** using sulfuryl chloride, followed by reaction with acid-TG **2** gave prodrug **7** (TST-ASI-C5-TG). To access prodrugs containing an alternative "trimethyl-lock" (TML) SI group (release by cyclization),[11] acid-TG **2** was coupled with TML phenol **9**,[7c] and the resulting product elaborated into acid **10** over three steps. Final coupling with TST then afforded prodrug **12** (TST-TML-C5-TG). To promote stability in the GI tract the same series of reactions were undertaken using 3-methylglutaryl dichloride as the C5 linker precursor to provide the β-methyl-substituted C5 prodrug (TST-C5-βMe-TG **5**) and the equivalent ASI (TST-ASI-C5-βMe-TG **8**) and TML (TST-TML-C5-βMe-TG **13**) SI group-containing prodrugs.

To investigate prodrug integration into lipid digestion pathways, and to evaluate gastrointestinal stability, prodrugs were incubated with simulated gastric fluid (SGF, pH 1.2) and with simulated intestinal fluid (SIF) containing pancreatic enzymes (Figure 2). The prodrugs were stable in SGF for 3 h (>95% remaining; Supporting Information). In contrast, incubation with SIF resulted in rapid hydrolysis of the fatty acids in the 1′ and 3′ position of the glycerol backbone to generate the drug-MG lipolysis product (**A** suffix in Scheme 1). For the unbranched prodrug



Scheme 1. Synthetic pathway for TST prodrugs. Conditions: a) for R = H: glutaric anhydride, DMAP, pyridine, THF/CH_2Cl_2, 39%; b) for R = Me: 3-methylglutaryl dichloride, pyridine, CH_2Cl_2, 88%; c) testosterone, EDC•HCl, DMAP, CH_2Cl_2, 41% for **4**, 73% for **5**, 44% for **12**, 68% for **13**; d) MTM-ether **6**, SO_2Cl_2, CH_2Cl_2, then add to acid **2** or **3** in PhMe, 70% for **7**, 65% for **8**; e) phenol **9**, EDC•HCl, DMAP, CH_2Cl_2; f) 10-CSA, MeOH/CH_2Cl_2; g) PCC, CH_2Cl_2; h) $KMnO_4$, acetone/H_2O, 37% over 4 steps from **2** to **10**, 55% over 4 steps from **3** to **11**.

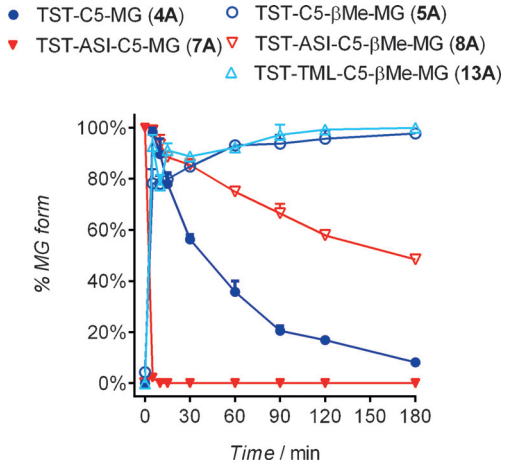


Figure 2. Production of MG derivatives of the TG-mimetic prodrugs upon in vitro incubation with simulated GI fluids containing pancreatic lipase. Mean ($n=3$) ± SEM.

4, concentrations of drug-MG **4A** rapidly increased and then decreased over time, following first-order kinetics ($R^2=0.97$, degradation half-life ≈46 min). Hydrolysis of **4A** was expected to reduce lymphatic transport because it is a key substrate for post-absorptive re-esterification. A methyl substitution was therefore introduced into the C5-linker on the β-carbon to the glyceride ester to form **5**. The design was chosen to sterically stabilize the labile ester between the drug and glyceride moiety and to potentially slow the rate of isomerization in situ that forms the more enzymatically labile 1-MG derivative. The methyl substitution was introduced β rather than α to the glyceride because both are expected to inhibit hydrolysis but β substitution allows further separation from the glycerol backbone, potentially reducing interference with subsequent re-esterification. β-Methylation (βMe) almost completely prevented luminal hydrolysis of **5A**. Realizing the benefit to prodrug utility of enhanced drug release in the systemic circulation, prodrugs were constructed with SI groups between TST and the C5 linker. Addition of the ASI group in **7** led to rapid luminal instability of drug-MG **7A**; however, the combination of either ASI or TML SI groups and βMe protection resulted in prodrugs (**8** and **13**, respectively) with acceptable GI stability of the drug-MG form (Figure 2).

Subsequent studies explored the impact of βMe protection and SI insertion on TST release in plasma (Figure 3). Prodrugs were incubated with rat plasma (supplemented with lipoprotein lipase (LPL)) at 37 °C and TST release was quantified over time. Consistent with the suggestion that βMe may prevent drug release, the plasma stability of **5A** (the drug-MG lipolysis product of **5**) was significantly higher than that of the straight chain analogue **4A**, although in neither case was free TST released. Instead, the final product appeared to be TST-C5-acid resulting from cleavage of the ester link to the glyceride backbone. In contrast, addition of the ASI group in **7** resulted in rapid release of free TST, and this was evident regardless of the inclusion of the βMe group in **8**. Similar data were evident for the TML prodrug **13,** supporting the effectiveness of both SI groups.

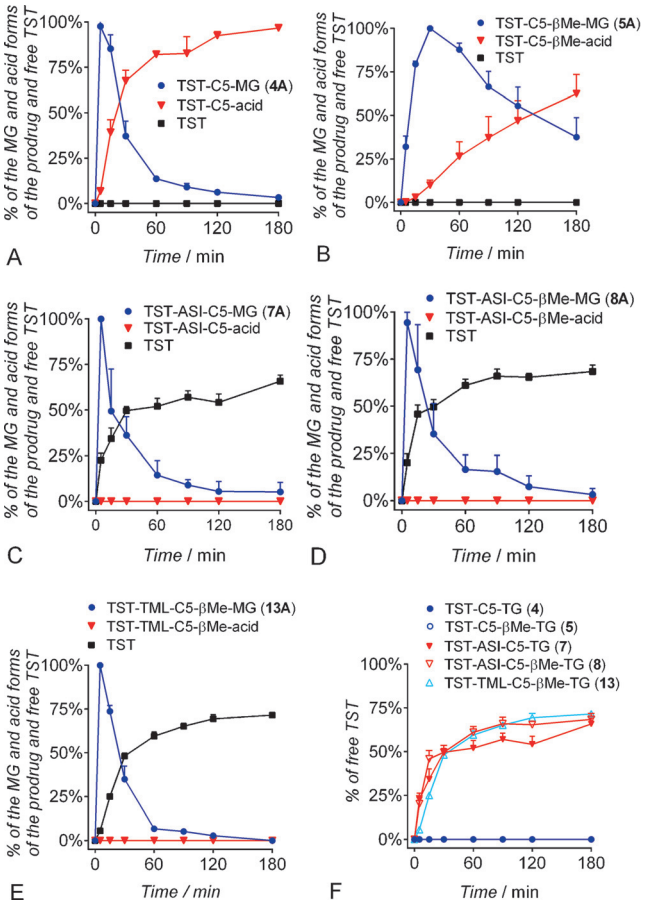


Figure 3. Production of metabolic intermediates and TST during in vitro incubation of TST prodrugs with plasma supplemented with lipoprotein lipase (LPL). A–E) % generation of different species versus time for each prodrug. F) Comparison of % free TST generation across all prodrugs. Mean ($n=3$) ± SEM.

The in vitro data therefore suggest that SI groups are required to promote drug release in plasma (and that elimination- and cyclization-mediated SI groups are effective), but that they may destabilize the prodrug in the GI lumen. However, βMe substitution attenuates the reduction in GI instability resulting from SI group insertion, and retains effective release properties.

The net effect of changes to GI stability and drug release profiles were subsequently evaluated in vivo, firstly by exploring the lymphatic transport of the re-esterified prodrug, and ultimately through evaluation of TST systemic exposure.

Lymphatic transport of parent TST was essentially zero (Figure 4), reflecting a lack of TST affinity for lymph LP. Data was also generated for the only commercial oral TST product, testosterone undecanoate (TU, Andriol®). TU is a fatty acid ester of TST, designed to promote lymphatic transport and increase TST bioavailability. TU is partially successful but is inefficient (≈4–7% oral bioavailability in dogs and humans[12]) and many patients struggle to achieve therapeutic TST levels with oral TU. Consistent with these suggestions, in the current study the lymphatic transport of TU was low (1.9%). In contrast, the simple straight-chain TG-mimetic prodrug **4** significantly increased lymphatic transport when

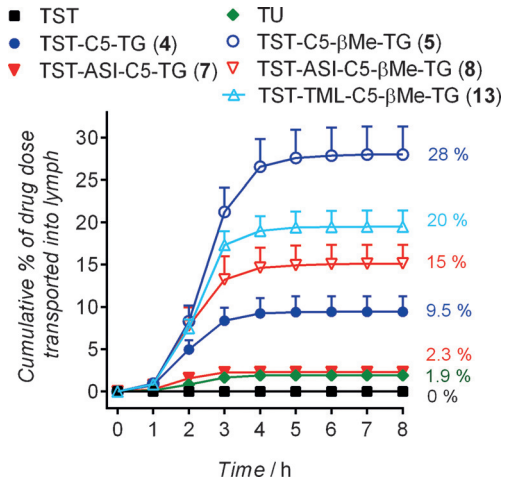


Figure 4. Cumulative lymphatic transport of TST, TU, and TG prodrugs following intraduodenal infusion to anaesthetised, mesenteric lymph-duct-cannulated female rats. Mean ($n \geq 3$) ± SEM.

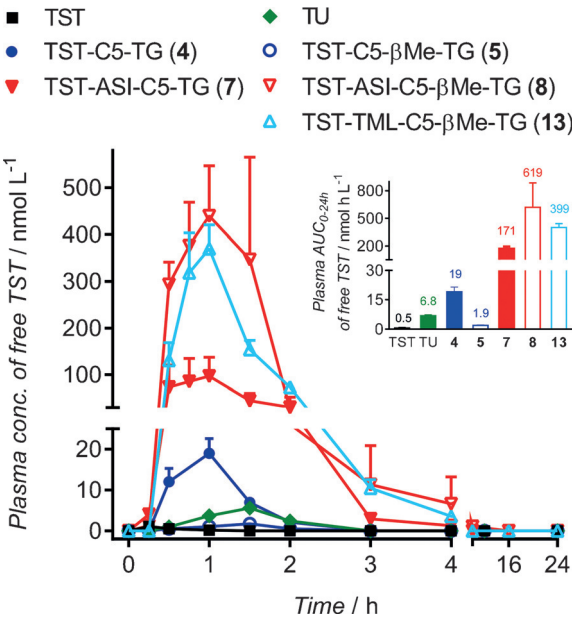


Figure 5. Plasma concentrations (nmol L^{-1}, dose normalized to 2 mg kg^{-1} equivalent dose of TST) of free TST versus time following oral administration of TST (4 mg kg^{-1} dose), TU (2.5 mg kg^{-1} equivalent dose of TST), and TG prodrugs (2 mg kg^{-1} equivalent dose of TST) formulations to carotid-artery-cannulated rats. Mean ($n \geq 3$) ± SEM.

compared to TST or TU, and this was increased again by βMe stabilization in **5**. As expected based on the poor luminal stability of the prodrugs containing the SI groups, lymphatic transport of **7** was low, but combination of the SI groups and βMe stabilization resulted in robust lymphatic transport for both the ASI- and TML-containing prodrugs **8** and **13**.

Finally, systemic exposure of TST was explored after oral administration of TST, TU, and the glyceride-mimetic prodrugs to conscious female rats. Plasma TST exposure (Figure 5) was negligible after oral administration of TST and elevated slightly by administration of TU or the straight-chain prodrug **4**, although in both cases the increases in systemic TST were moderate, consistent with previous studies.[4a] In contrast, the ASI prodrug **7** significantly enhanced plasma exposure and this was enhanced further by βMe stabilization of the ASI and TML prodrugs **8** and **13**. T_{max} was slightly shorter for the better performing TG prodrugs when compared to TU, possibly reflecting more rapid systemic release when compared to TU (< 3 % TST was released from TU under the conditions employed in Figure 3).

In conclusion, we demonstrate prodrug strategies that markedly (10–90 fold) improve the systemic exposure of drugs such as TST where bioavailability is limited due to substantial first-pass metabolism. Glyceride-mimetic prodrugs integrate drugs into endogenous lymph LP transport pathways that deliver the drugs directly into the systemic circulation and avoid first-pass metabolism in the liver. The twin challenges of GI stability and systemic lability may be met using a combination of βMe-branched linkers and SI groups to stabilize against GI hydrolysis and promote systemic drug release, respectively. This marks a considerable advance over previous approaches that rely on direct conjugation[13] or simple alkyl spacers, where GI stability is reduced and drug release is limited.[4b] The technology is adaptable to different SI groups and the conjugation chemistries may be adjusted for application to drugs with hydroxyl, amine, thiol, or carboxylic acid functionality. Beyond promoting oral bioavailability, lymphatic drug transport may also provide advantages for drugs with lymph-resident targets and, in particular, lymphocyte-related targets[14] because more than 90 % of the total lymphocyte pool resides within the lymphatic system and approximately 50 % within the intestinal lymph and lymphoid tissue.[1]

Experimental Section

Materials and prodrug synthesis: See Scheme 1 and the Supporting Information.

In vitro hydrolysis: See the Supporting Information for details. Briefly, hydrolysis experiments were conducted by prodrug incubation (37 °C) with simulated gastric fluid (pH 1.2) or simulated intestinal fluid containing porcine pancreatin (1000 IU mL) to reflect events in the GI tract, and with rat plasma supplemented with lipoprotein lipase (200 IU mL), to evaluate drug release in the systemic circulation. Samples were analyzed for the products of prodrug hydrolysis by LC-MS/MS.

In vivo lymphatic transport and pharmacokinetic studies: See the Supporting Information for details. The mesenteric lymph duct and/or carotid artery were cannulated as described previously for the collection of lymph and blood samples.[15] The rats were dosed via an intraduodenal cannula (for lymphatic transport studies) or oral gavage (for pharmacokinetic studies) with drug or prodrug. Drug content in lymph and plasma samples was determined by LC-MS/MS.

Acknowledgements

We acknowledge Ms. Elise Cheroux for assistance during in vitro hydrolysis experiments, and the ARC (Australian Research Council) Centre of Excellence in Convergent Bio-Nano Science and Technology for partial financial support.

Keywords: drug delivery · lymphatic transport · metabolism · prodrugs · testosterone

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 13700–13705
Angew. Chem. **2016**, *128*, 13904–13909

[1] N. L. Trevaskis, L. M. Kaminskas, C. J. H. Porter, *Nat. Rev. Drug Discovery* **2015**, *14*, 781–803.
[2] a) J. Bernier-Latmani, C. Cisarovsky, C. S. Demir, M. Bruand, M. Jaquet, S. Davanture, S. Ragusa, S. Siegert, O. Dormond, R. Benedito, F. Radtke, S. A. Luther, T. V. Petrova, *J. Clin. Invest.* **2015**, *125*, 4572–4586; b) J. B. Dixon, S. Raghunathan, M. A. Swartz, *Biotechnol. Bioeng.* **2009**, *103*, 1224–1235; c) C. M. Mansbach, S. A. Siddiqi, *Annu. Rev. Physiol.* **2010**, *72*, 315–333.
[3] a) W. N. A. Charman, V. J. Stella, *Int. J. Pharm.* **1986**, *34*, 175–178; b) P. Gershkovich, J. Fanous, B. Qadri, A. Yacovan, S. Amselem, A. Hoffman, *J. Pharm. Pharmacol.* **2009**, *61*, 31–39.
[4] a) J. K. Amory, G. K. E. Scriba, D. W. Amory, W. J. Bremner, *J. Androl.* **2003**, *24*, 716–720; b) G. K. E. Scriba, *Arch. Pharm.* **1995**, *328*, 271–276.
[5] A. B. Araujo, G. R. Esche, V. Kupelian, A. B. O'Donnell, T. G. Travison, R. E. Williams, R. V. Clark, J. B. McKinlay, *J. Clin. Endocrinol. Metab.* **2007**, *92*, 4241–4247.
[6] H. L. Mu, C. E. Hoy, *Prog. Lipid Res.* **2004**, *43*, 105–133.
[7] a) R. Madec-Lougerstay, J. C. Florent, C. Monneret, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1369–1375; b) A. B. Mauger, P. J. Burke, H. H. Somani, F. Friedlos, R. J. Knox, *J. Med. Chem.* **1994**, *37*, 3452–3458; c) K. L. Amsberry, A. E. Gerstenberger, R. T. Borchardt, *Pharm. Res.* **1991**, *8*, 455–461; d) P. L. Carl, P. K. Chakravarty, J. A. Katzenellenbogen, *J. Med. Chem.* **1981**, *24*, 479–480.
[8] a) F. M. H. de Groot, C. Albrecht, R. Koekkoek, P. H. Beusker, H. W. Scheeren, *Angew. Chem. Int. Ed.* **2003**, *42*, 4490–4494; *Angew. Chem.* **2003**, *115*, 4628–4632; b) R. J. Amir, N. Pessah, M. Shamis, D. Shabat, *Angew. Chem. Int. Ed.* **2003**, *42*, 4494–4499; *Angew. Chem.* **2003**, *115*, 4632–4637.
[9] M. Lalanne, A. Paci, K. Andrieux, N. Dereuddre-Bosquet, P. Clayette, A. Deroussent, M. Re, G. Vassal, P. Couvreur, D. Desmaele, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2237–2240.
[10] S. Zavgorodny, M. Polianski, E. Besidsky, V. Kriukov, A. Sanin, M. Pokrovskaya, G. Gurskaya, H. Lonnberg, A. Azhayev, *Tetrahedron Lett.* **1991**, *32*, 7593–7596.
[11] a) M. N. Levine, R. T. Raines, *Chem. Sci.* **2012**, *3*, 2412–2420; b) Y. Zheng, B. Yu, K. Ji, Z. Pan, V. Chittavong, B. Wang, *Angew. Chem. Int. Ed.* **2016**, *55*, 4514–4518; *Angew. Chem.* **2016**, *128*, 4590–4594.
[12] a) D. M. Shackleford, W. A. Faassen, N. Houwing, H. Lass, G. A. Edwards, C. J. H. Porter, W. N. Charman, *J. Pharmacol. Exp. Ther.* **2003**, *306*, 925–933; b) F. M. Kohn, W. B. Schill, *World J. Urol.* **2003**, *21*, 311–315.
[13] S. Han, T. Quach, L. Hu, A. Wahab, W. N. Charman, V. J. Stella, N. L. Trevaskis, J. S. Simpson, C. J. H. Porter, *J. Controlled Release* **2014**, *177*, 1–10.
[14] a) N. L. Trevaskis, W. N. Charman, C. J. H. Porter, *Mol. Pharm.* **2010**, *7*, 2297–2309; b) H. Liu, K. D. Moynihan, Y. Zheng, G. L. Szeto, A. V. Li, B. Huang, D. S. Van Egeren, C. Park, D. J. Irvine, *Nature* **2014**, *507*, 519–522.
[15] S. M. Caliph, E. Cao, J. B. Bulitta, L. Hu, S. Han, C. J. H. Porter, N. L. Trevaskis, *J. Pharm. Sci.* **2013**, *102*, 2395–2408.

Received: April 30, 2016
Revised: June 25, 2016
Published online: August 2, 2016

