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# Tartaric acid-based amphiphilic macromolecules with ether linkages exhibit enhanced repression of oxidized low density lipoprotein uptake

Dalia S. Abdelhamid [a, 1, 2], Yingyue Zhang [a, 1], Daniel R. Lewis [b], Prabhas V. Moghe [b, c], William J. Welsh [d], Kathryn E. Uhrich [a, *]

[a] Department of Chemistry and Chemical Biology, Rutgers University, NJ, USA
[b] Department of Chemical and Biochemical Engineering, Rutgers University, NJ, USA
[c] Department of Biomedical Engineering, Rutgers University, NJ, USA
[d] Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Rutgers University, Piscataway, NJ, USA

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ABSTRACT

Cardiovascular disease initiates with the atherogenic cascade of scavenger receptor- (SR-) mediated oxidized low-density lipoprotein (oxLDL) uptake. Resulting foam cell formation leads to lipid-rich lesions within arteries. We designed amphiphilic macromolecules (AMs) to inhibit these processes by competitively blocking oxLDL uptake via SRs, potentially arresting atherosclerotic development. In this study, we investigated the impact of replacing ester linkages with ether linkages in the AM hydrophobic domain. We hypothesized that ether linkages would impart flexibility for orientation to improve binding to SR binding pockets, enhancing anti-atherogenic activity. A series of tartaric acid-based AMs with varying hydrophobic chain lengths and conjugation chemistries were synthesized, characterized, and evaluated for bioactivity. 3-D conformations of AMs in aqueous conditions may have significant effects on anti-atherogenic potency and were simulated by molecular modeling. Notably, ether-linked AMs exhibited significantly higher levels of inhibition of oxLDL uptake than their corresponding ester analogues, indicating a dominant effect of linkage flexibility on pharmacological activity. The degradation stability was also enhanced for ether-linked AMs. These studies further suggested that alkyl chain length (i.e., relative hydrophobicity), conformation (i.e., orientation), and chemical stability play a critical role in modulating oxLDL uptake, and guide the design of innovative cardiovascular therapies.



## 1. Introduction

Cardiovascular diseases are the leading causes of mortality in developed countries [1,2]. Atherosclerosis, characterized as the buildup of lipid rich plaques within the vascular intima, is the primary pathology underlying these conditions. Atherosclerosis starts with the accumulation of low-density lipoprotein (LDL) within blood vessel walls, where LDL undergoes oxidative modification [3]. The oxidized LDL (oxLDL) then triggers monocyte recruitment and differentiation into macrophages, which subsequently uptake oxLDL via scavenger receptors (SRs). This unregulated uptake of oxLDL leads to the formation of lipid-laden macrophages called foam cells and the secretion of inflammatory mediators [4]. The atherogenic accumulation of foam cells results in plaque buildup within artery walls, which can lead to myocardial infarction, stroke, or peripheral arterial disease.

Traditional cardiovascular therapies focus on lowering the hepatic synthesis of LDL to reduce the vascular lipid burden and mitigate atherosclerosis, however, they fail to target lesion sites and suffer from severe off-target effects [5]. An alternative approach to address atherosclerosis is through direct inhibition of oxLDL uptake via macrophage SRs, thus preventing the ensuing inflammatory

* Corresponding author. Department of Chemistry and Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854, USA. Tel.: +1 848 445 0361; fax: +1 732 445 7036.
*E-mail address:* keuhrich@rci.rutgers.edu (K.E. Uhrich).
[1] These authors contributed equally to this work.
[2] Current address: Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, Minia, Egypt.

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response. Previously, amphiphilic macromolecules (AMs) comprised of a branched hydrophobic domain and a hydrophilic tail (poly(ethylene glycol), PEG) were developed by our research group as biocompatible micelle-forming amphiphiles (representative structures shown in Fig. 1A). These AMs have shown promising bioactivity as anti-atherogenic agents [6–9] by selectively blocking uncontrolled oxLDL uptake by macrophages [7]. By varying specific structural motifs of the AMs, the influence of several important features on anti-atherogenic activity was identified. These structural modifications included variations in the free carboxylic acid (location and numbers), nature of the backbone (stereochemistry), hydrophobic chains (number of branches and chain length), and PEG (molecular weight (Mw) and branching) [9,10]. Detailed studies have shown that seemingly minor changes in the stereochemistry, degree of branching, and/or hydrophobicity of the aliphatic chains have a considerable effect on bioactivity [9,11,12]. Molecular modeling studies further suggested that AMs competitively inhibit oxLDL uptake by SRs via electrostatic and hydrophobic interactions with SR binding domains [13]. The 3D conformation of AMs in aqueous conditions, specifically the presentation of the hydrophobic arms, appears to be critically important for this inhibition efficacy [14].

While the influence of chain length and hydrophobic domain branching on bioactivity has been well established, the potential role of the linkage type between hydrophobic chains and backbones has not been systematically investigated. The ester linkages (Fig. 1A) between the linear backbone and hydrophobic chains of previously investigated AMs are characterized by partial double bond character that limits freedom of movement. This conformational rigidity may govern the nature of AMs' corresponding three-dimensional conformation, which ultimately affects the binding affinity of AMs to the SRs. We hypothesized that replacing ester linkages with ether linkages would increase the flexibility and overcome the rigidity challenges due to partial double bond resonance. We envisioned that the increased conformational flexibility afforded by the ether linkage would allow tighter alignment of the hydrophobic arms, rendering a better fit within the SR binding pocket and enhanced binding affinity.

Furthermore, susceptibility to esterase hydrolysis is an important consideration in drug design [15]. As the aforementioned ester linkages are vulnerable to esterase-catalyzed hydrolysis and degradation [16], we examined whether AMs with more stable ether linkages (Fig. 1B) could overcome this potential limitation and thereby exhibit enhanced bioactivity. Bioavailability of ester conjugated AMs can be significantly reduced during blood circulation given the abundant presence of lipase (a subclass of esterases) in human serum [17], leading to compromised drug efficacy. As such, it is critical to design molecules that resist rapid degradation; this need also inspired our investigation of ether bonds, which are less susceptible to enzymatic hydrolysis.

In previous work, the most potent AM was based on a mucic acid backbone with ester-linked alkyl chains: M12P5 (Fig. S1). As a proof of concept, in this study we designed novel ether-containing AM analogs based on a tartaric acid backbone with two hydrophobic chains (Fig. 1B). Thus, for direct comparison and to clearly delineate the ester/ether effect, we also synthesized a tartaric acid-based ester-linked AM (Fig. 1A). Herein, we present the synthetic strategy for preparing ether-linked AMs along with a detailed comparison of ether- and ester-linked AMs' physicochemical properties and biological activity. The enzymatic degradation stability of ether-linked AMs was also compared to ester-linked AMs, with the expectation that replacing ester linkages with more robust ether linkages would provide AMs with enhanced *in vivo* stability.

## 2. Materials and methods

### 2.1. Materials

Reagents tartaric acid, zinc chloride, lauroyl chloride, octyl chloride, decanoyl chloride, sodium hydride (NaH), bromooctane, bromodecane, bromododecane, dimethyl amino pyridine (DMAP), *p*-toluene sulphonic acid, N,N'-dicyclohexylcarbodiimide (DCC), monomethoxy-poly(ethylene glycol) (mPEG, Mn = 5000 Da), lipase from porcine pancreas, phosphate buffered saline (PBS, pH = 7.4), and HPLC grade solvents were purchased from Sigma–Aldrich (Milwaukee, WI) and used directly unless otherwise mentioned. Lipase activity was determined using olive oil as a substrate following manufacturer protocols. (+) Dibenzyl L-tartrate was purchased from TCI (Duncan, SC). Dimethyl amino pyridine *p*-toluene sulphonate (DPTS) was prepared as previously published [18]. Polytetrafluoroethylene (PTFE) syringe filters were purchased from Fisher Scientific (Fair Lawn, NJ). Before use, 5k

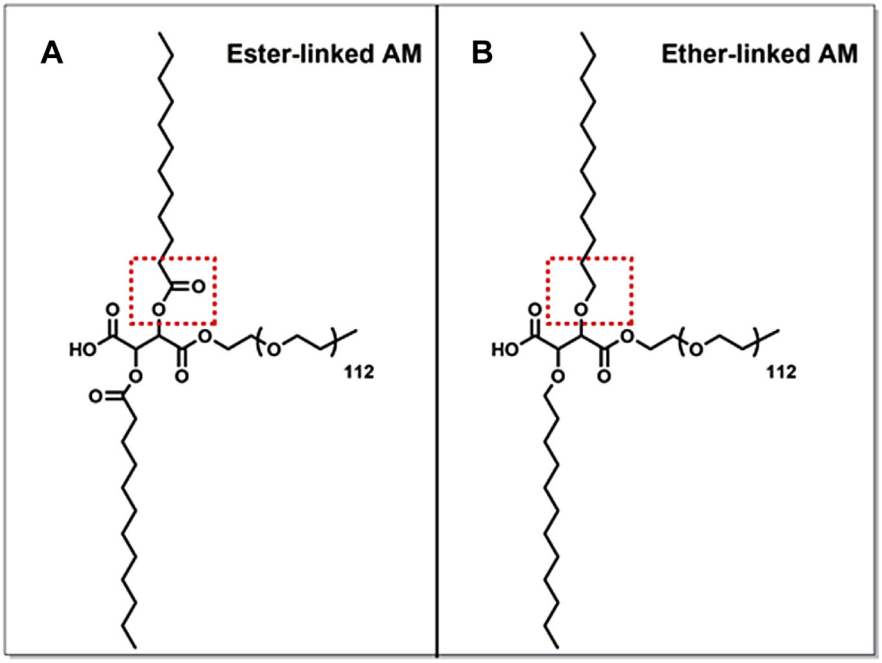


**Fig. 1.** Representative chemical structure of AMs based on tartaric acid backbone with ester linkages (A) and corresponding analogs with ether linkages (B).