SUPPORTING INFORMATION

Synthesis and Chemical and Biological Comparison of Nitroxyl and Nitric Oxide Releasing Diazeniumdiolate-based Aspirin Derivatives

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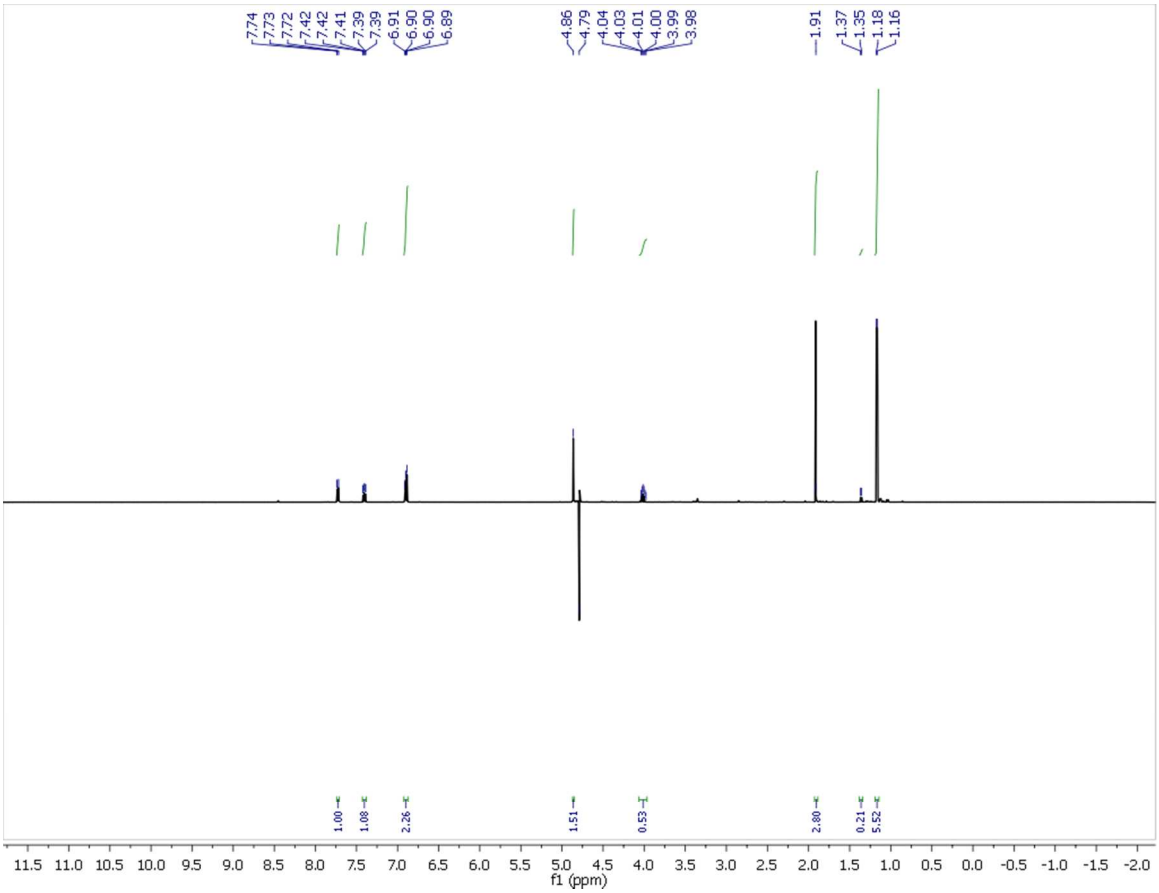


Figure S1. NMR spectra of products generated on hydrolysis of IPA/NO-aspirin after 24 h at pH 10 and 37 °C.

The hydrolysis products of IPA/NO-aspirin were examined by NMR at pH 10 after 24 h of incubation. Accumulation of isopropanol (δ 1.18), formaldehyde (4.86), salicylate (δ 6.89-6.91, 7.39-7.42, 7.72-7.74) and acetate (δ 1.91) is evident. Notably the hydrolysis was complete as apparent from the lack of linker -OCH_2O- protons peak at around 6 ppm. A lack of acetyl CH_3 protons (δ 2.33) suggests negligible accumulation of aspirin. The near lack of isopropylamine (δ 1.07), the byproduct of the NO forming pathway (Scheme 1), indicates decomposition of IPA/NO-aspirin primarily by the pathway involving deprotonation of the amine (Scheme 3).

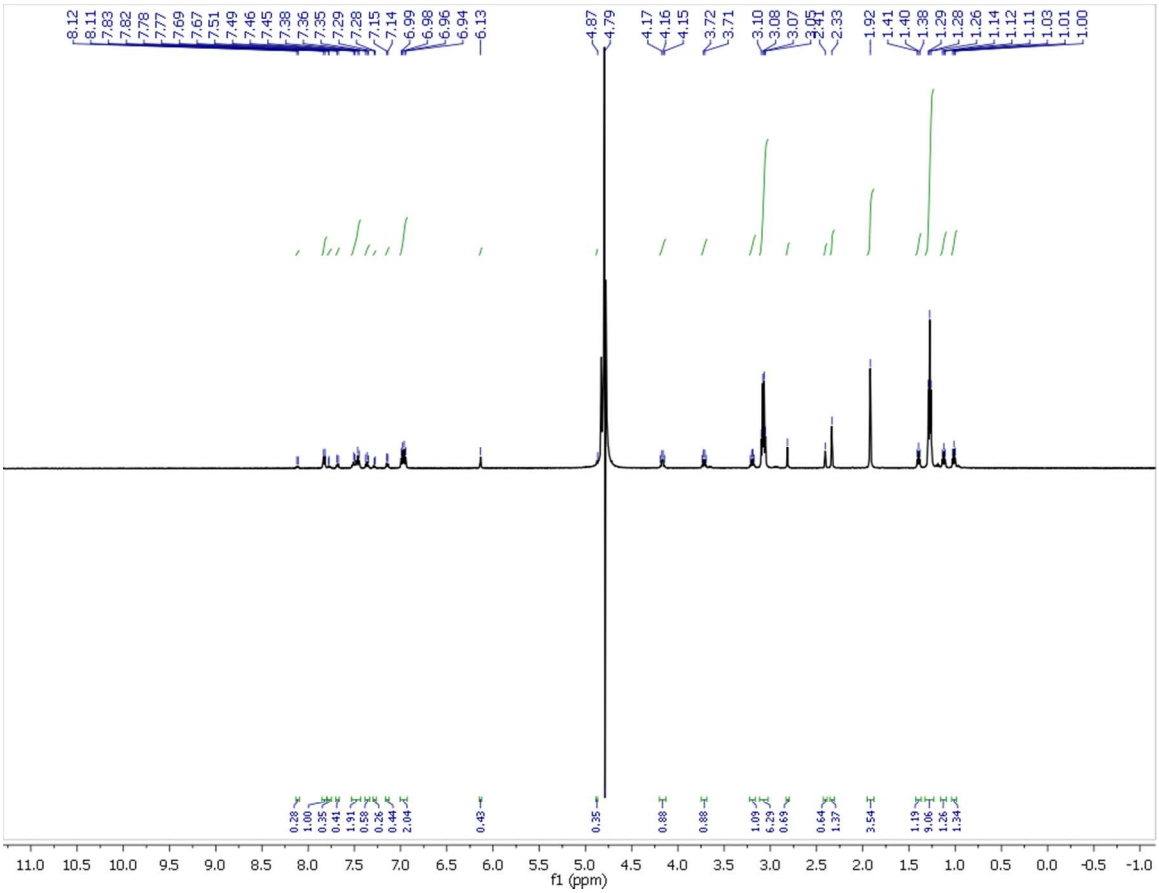


Figure S2. NMR spectra of products generated on hydrolysis of DEA/NO-aspirin after 90 h at pH 10 and 37 °C.

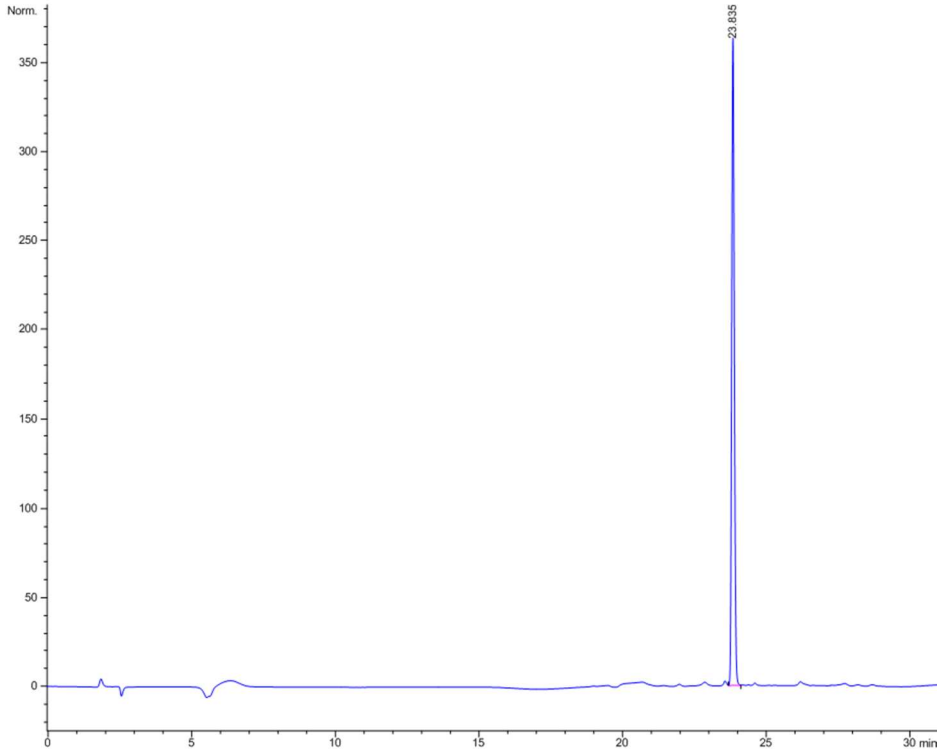
DEA/NO decomposes at pH 10 to produce peaks at 4.17, 3.72, 1.38, 1.27, and 1.12 ppm. These peaks are also present in the NMR spectrum collected after 90 h hydrolysis of DEA/NO-aspirin at pH 10, signifying release of free DEA/NO. The 1.9 ppm peak indicates hydrolysis of the acetyl ester to form acetate. Moreover small peaks for the OCH_2O linker (6.13 ppm) and acetyl group (2.41 ppm) of DEA/NO-aspirin were still present, suggesting incomplete hydrolysis. Although the peak at 2.33 ppm hints at the presence of aspirin, the overlapping peaks in the aromatic region hampers assignment of DEA/NO-salicylate, aspirin or salicylate.

Assessment of compound purity by HPLC

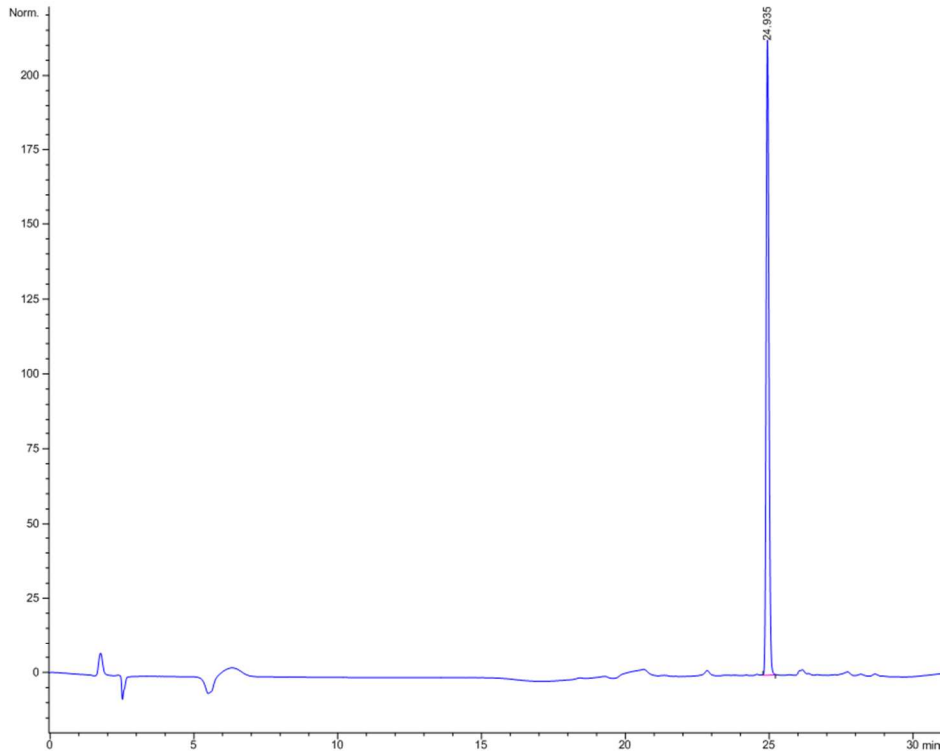
Compounds **3** and **6-8** were determined by HPLC to have a purity of >95%, and thus were used for subsequent biological assays. The HPLC spectra are provided below.

Column: Phenomenex Luna C18 column, 3 μm, 150 × 2.1 mm; mobile phase: water and acetonitrile gradient containing 0.1% formic acid; wavelength: 265 nm; rate: 0.2 mL/min; temperature: 30 °C

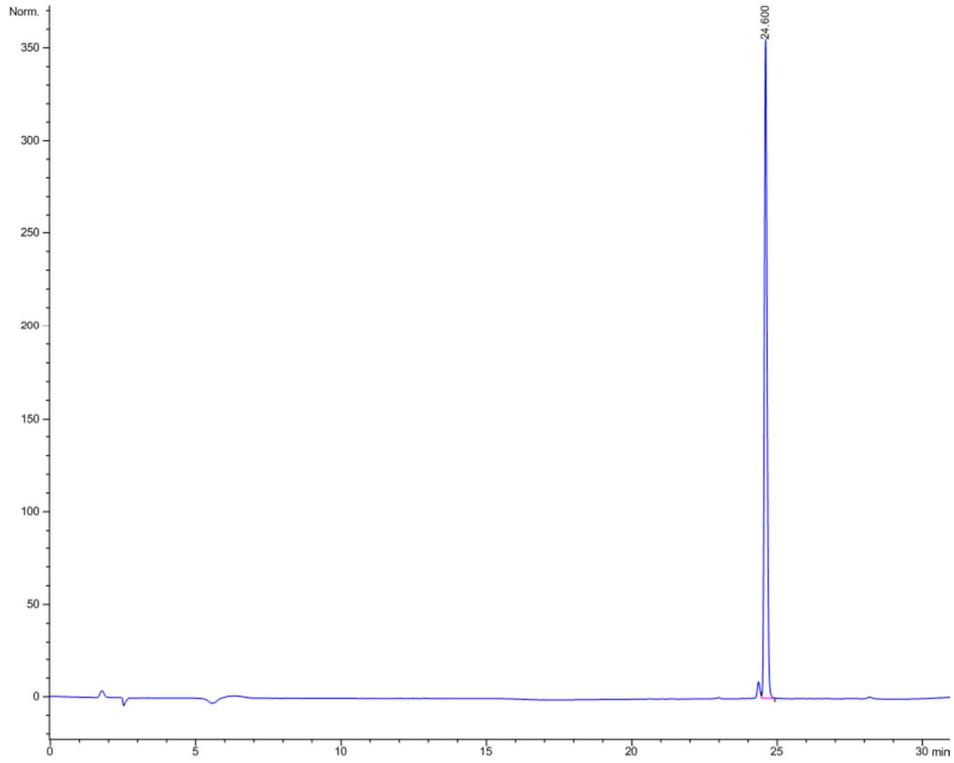
Compound 3, 98.1%



Compound 6, 97.4%



Compound 7, 97.6%



Compound 8, 99.2%