



Stereoselective Synthesis, Pro-resolution, and Anti-inflammatory Actions of RvD5_{n-3} DPA

LIPCHEM

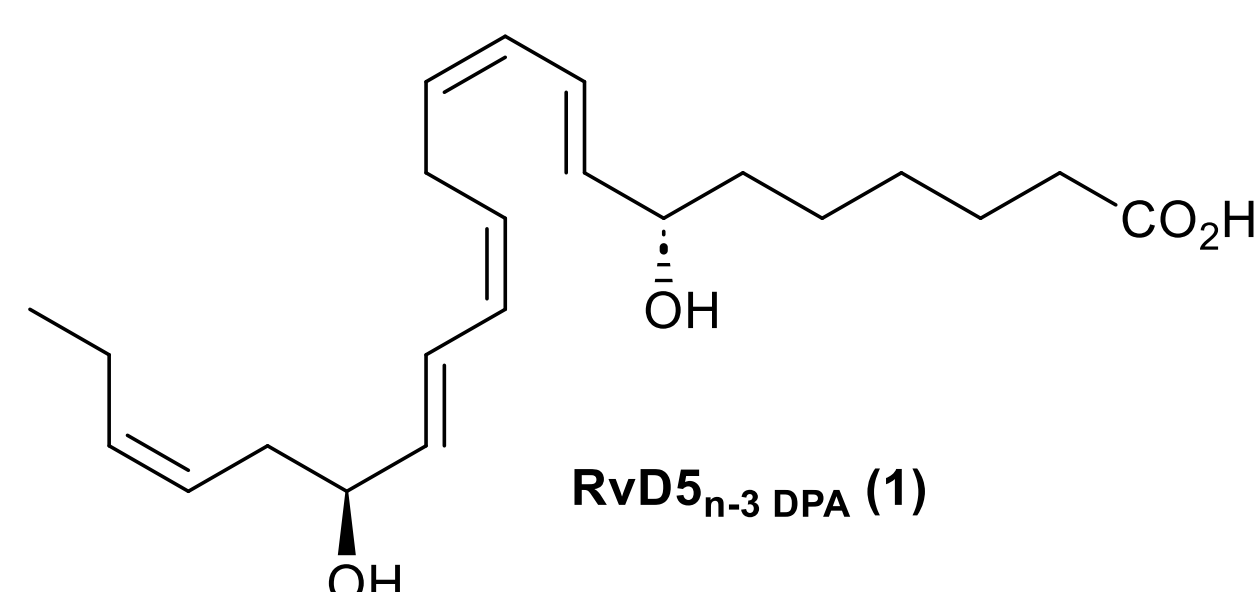
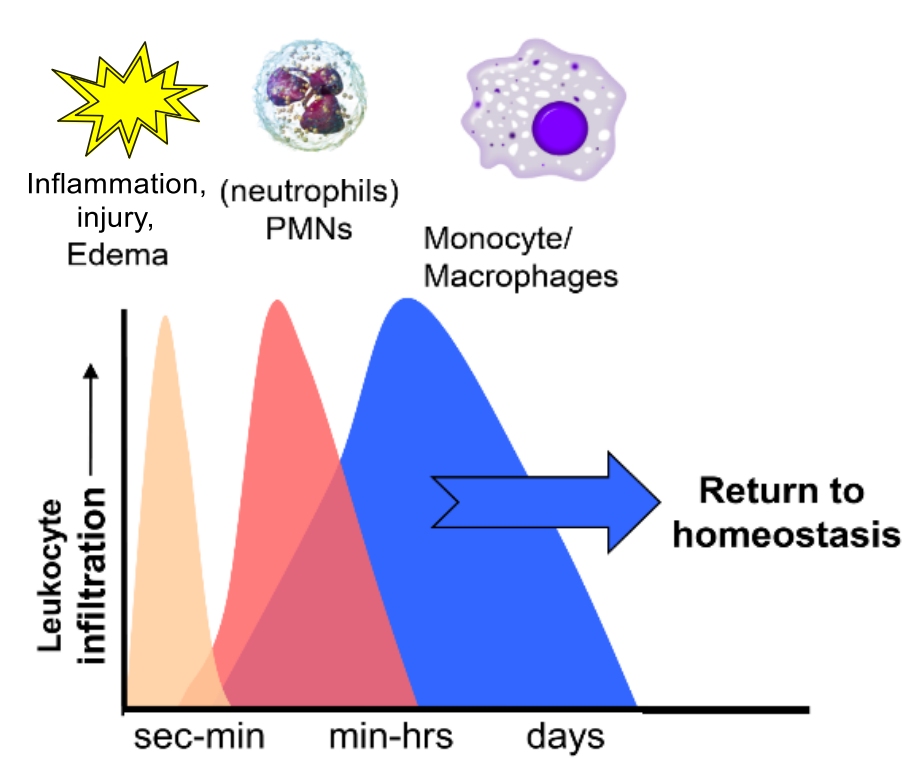
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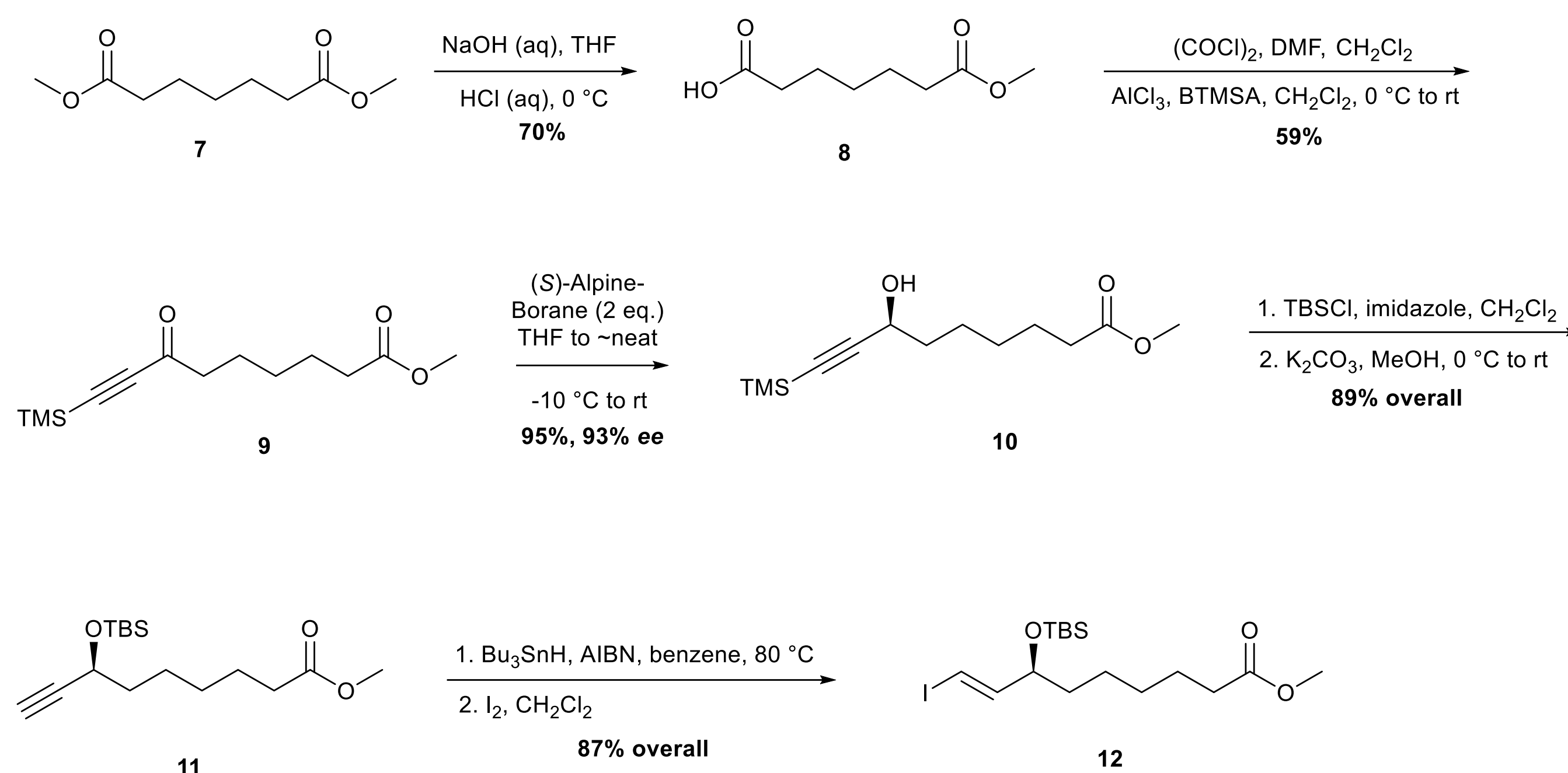
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Introduction

It was for a long time believed that the resolution phase of inflammation was a passive process. Recently, it was discovered that the resolution of inflammation and the return to homeostasis are regulated by active and strictly controlled biosynthesis of several oxygenated polyunsaturated fatty acids (PUFAs), coined specialized pro-resolving mediators (SPMs).¹ The newly discovered SPM RvD5_{n-3} DPA (**1**) might be an excellent biotemplate for the development of new, small molecular anti-inflammatory drugs and immunoresolvents.² Herein, the total synthesis and biological evaluations of the SPM **1** is presented.³

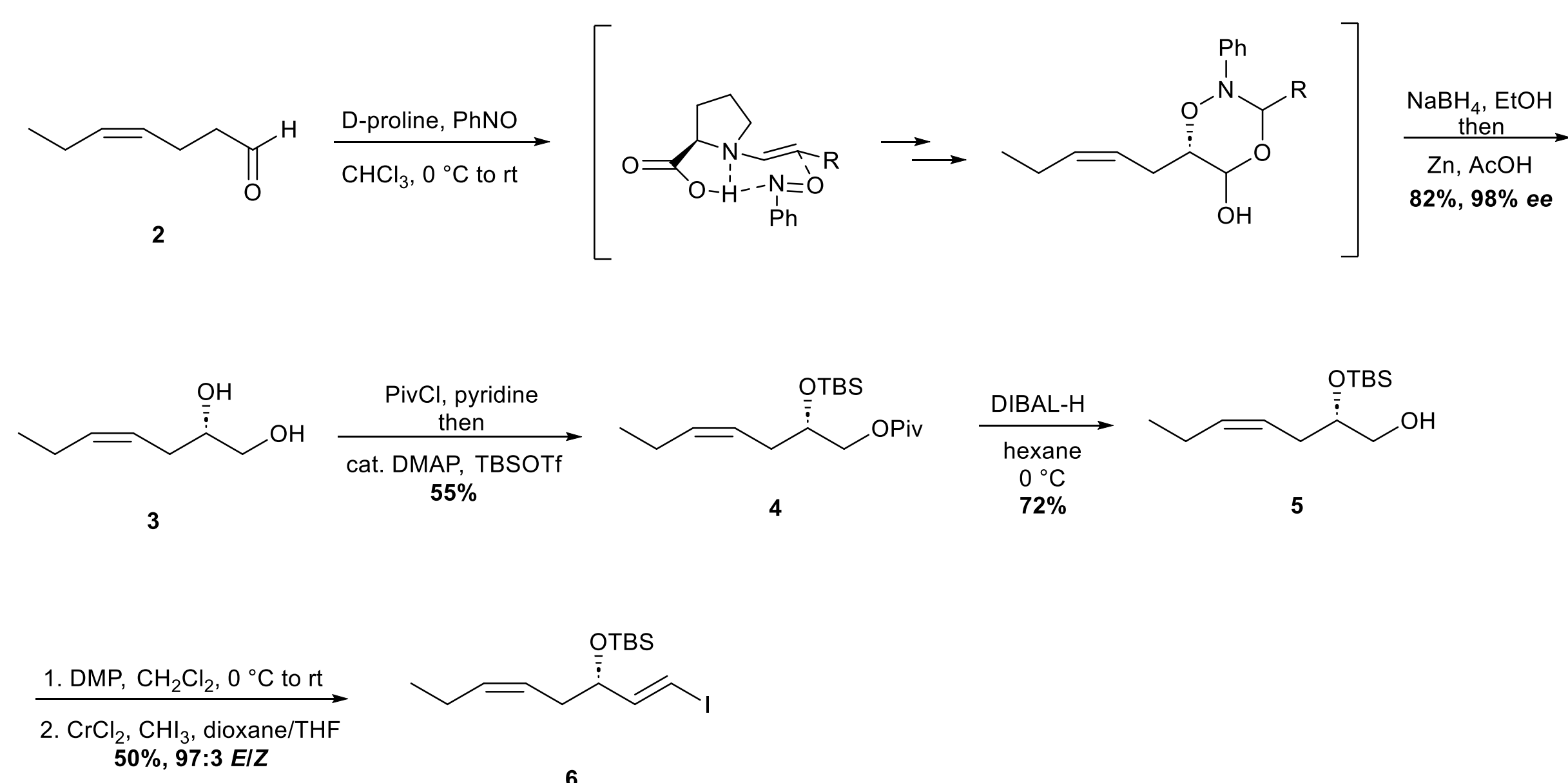


Synthesis of fragment 12



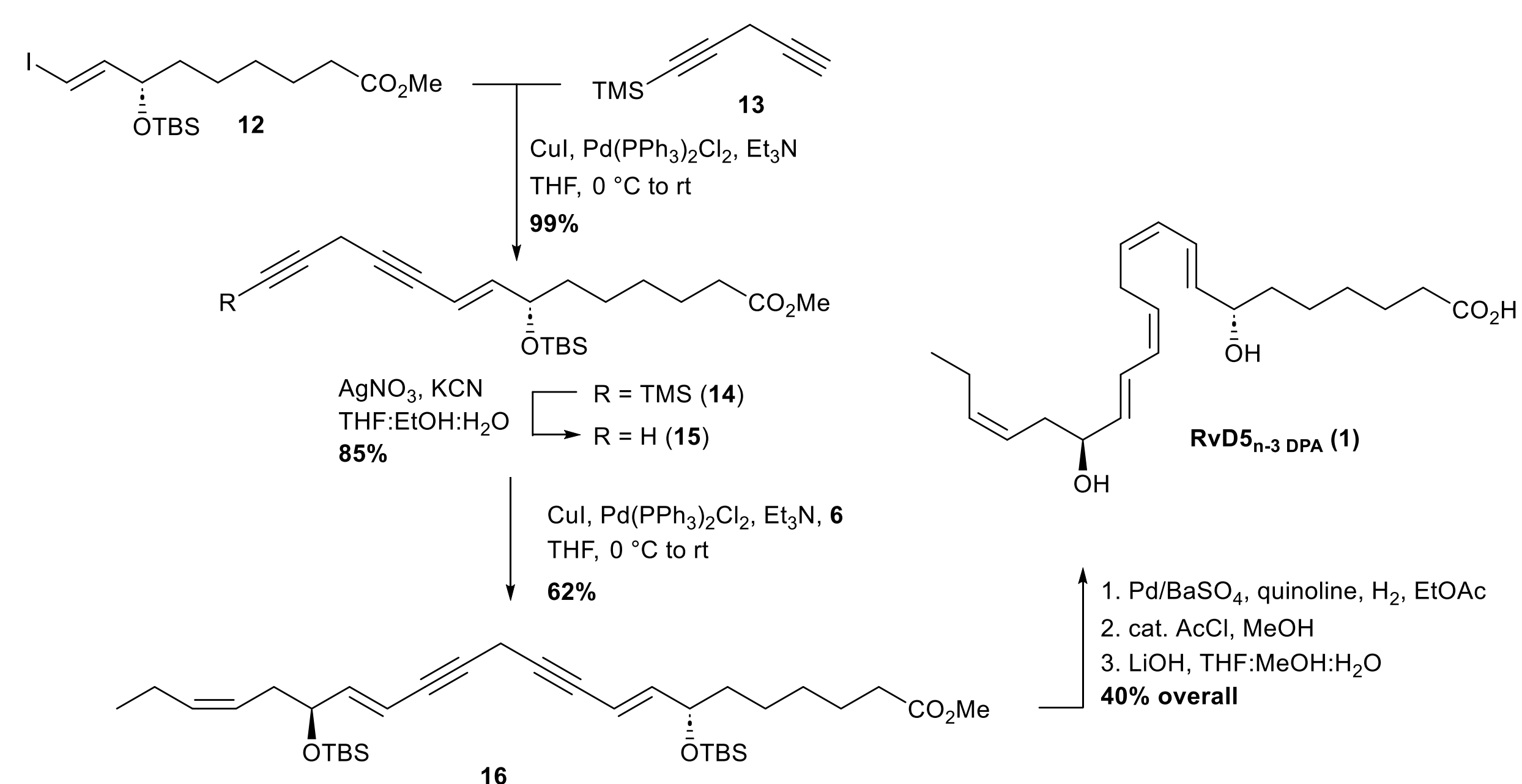
Scheme 2. Fragment **12** was successfully synthesized over seven steps.

Synthesis of fragment 6



Scheme 1. Fragment **6** was synthesized over five steps from commercially available *cis*-4-heptenal (**2**).

Connecting the fragments



Scheme 3. Total synthesis of the SPM RvD5_{n-3} DPA (**1**) utilizing Sonogashira cross-coupling and Rosenmund Z-selective reduction.



RP-LC-MS/MS experiments revealed the same peak from both human serum and mouse infectious exudates as the synthetic **1**, demonstrated in Figure 1. This confirms the physical properties of the synthesized material as the endogenously biosynthesized RvD5_{n-3} DPA.

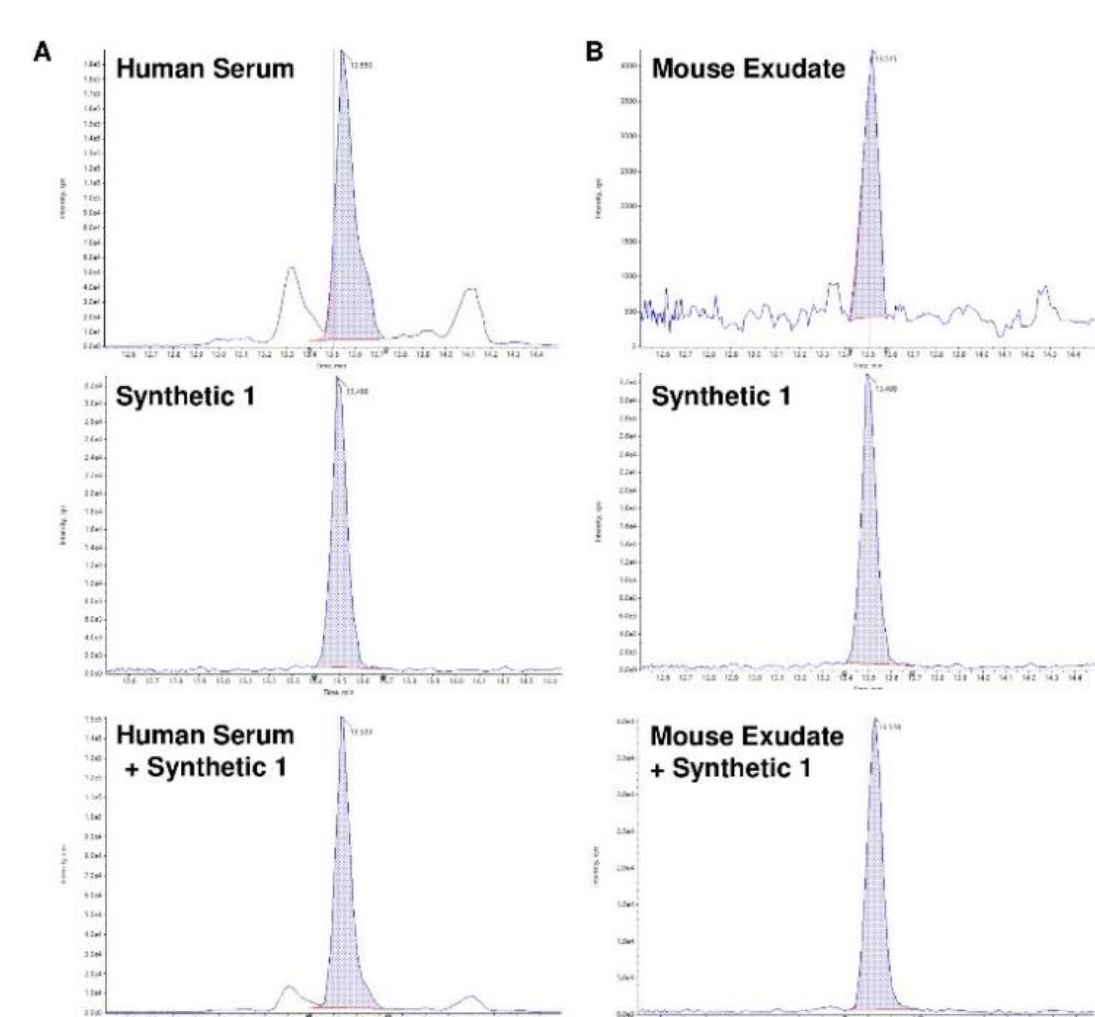


Figure 1. Chromatographic properties of synthetic **1** matched those of biogenetic **1**.

LC-MS/MS matching data and anti-inflammatory actions of RvD5_{n-3} DPA

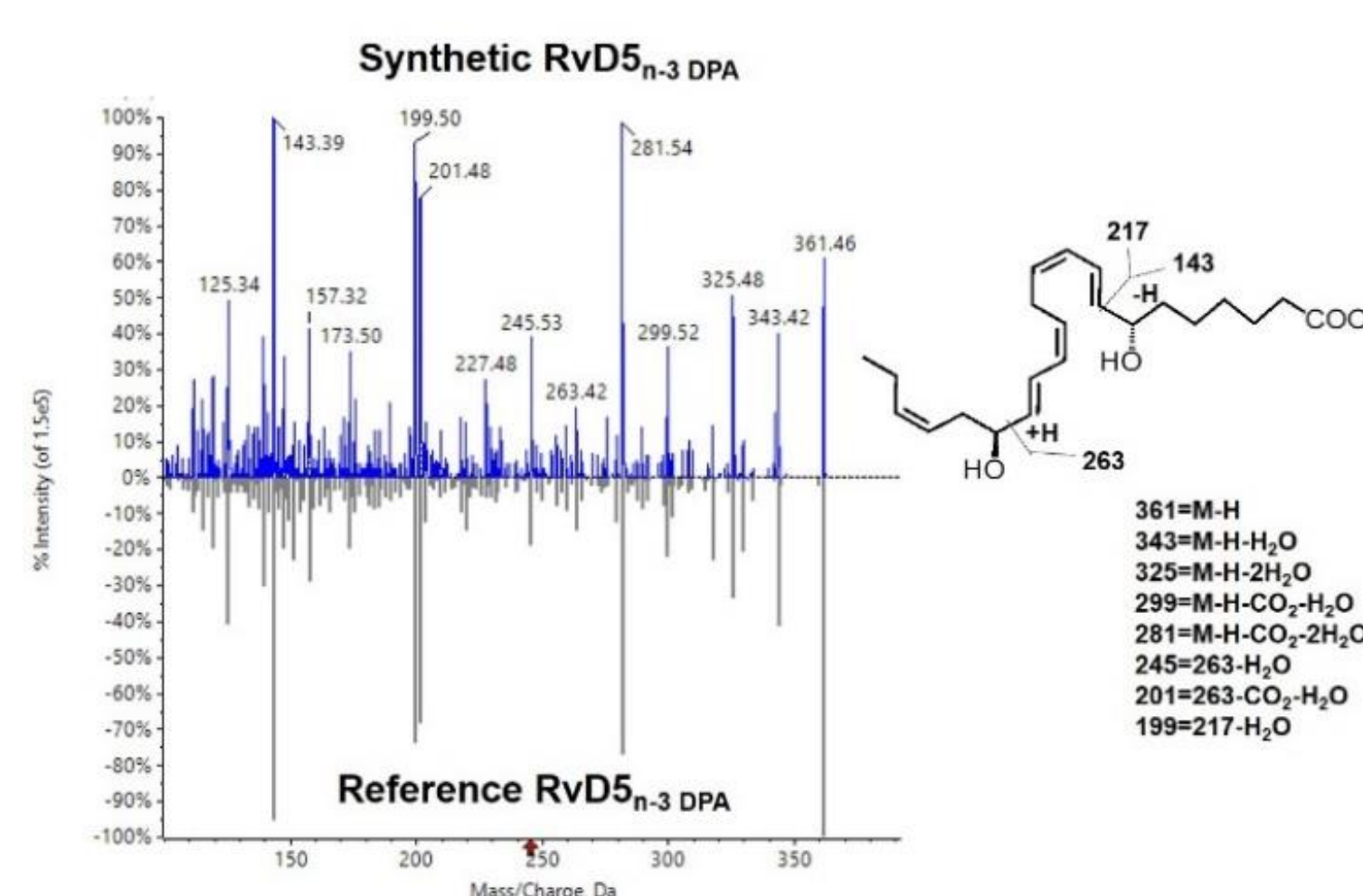


Figure 2. MS/MS fragmentation spectrum.

The MS/MS fragmentation spectrum of synthetic **1** matched that of the reference spectrum for biologically produced RvD5_{n-3} DPA, including key fragments, see Figure 2.

Using an impedance-based assay, it was observed that the SPM **1** potently activated the human orphan receptor GPR101 (Figure 3). A change in impedance in cells overexpressing GPR101 was denoted in the range of 0.1 to 10 nM concentrations of the novel mediator.

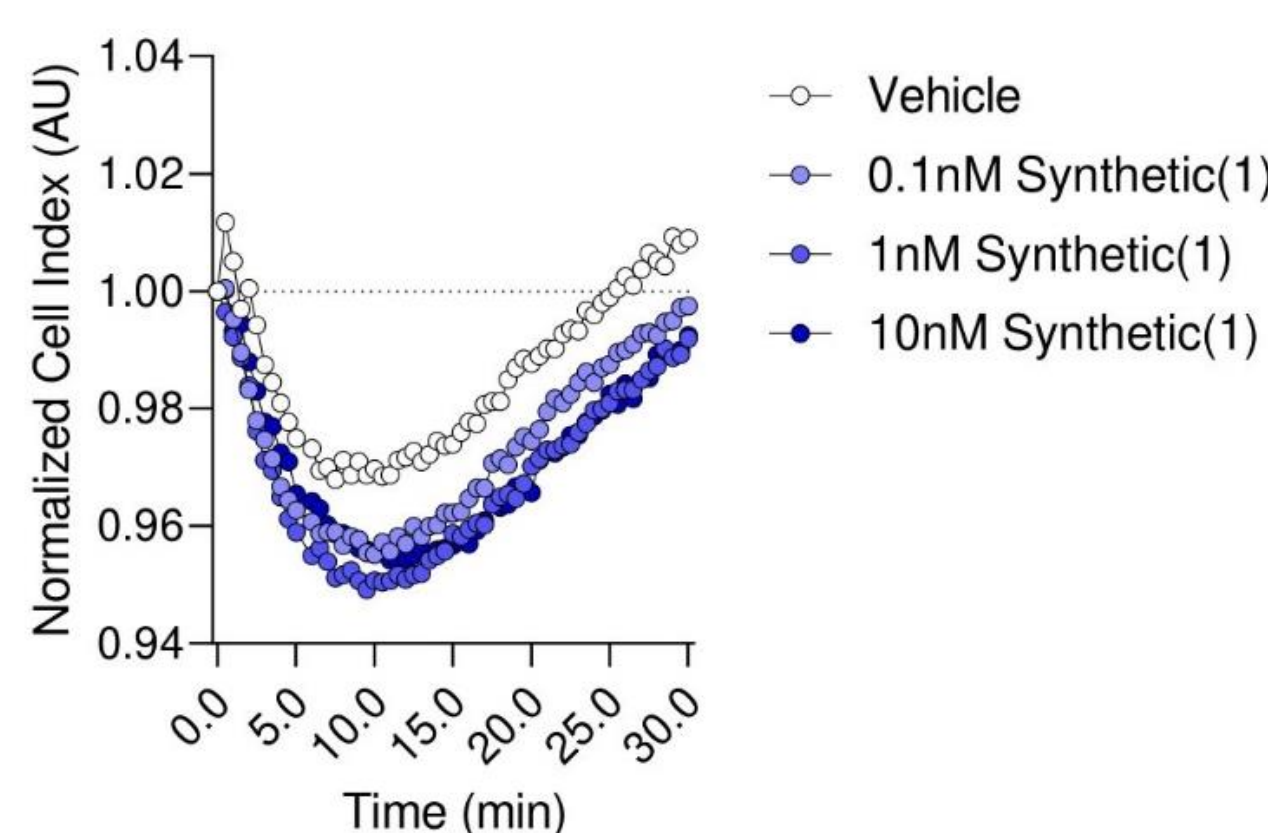


Figure 3. RvD5_{n-3} DPA activates human GPR101.

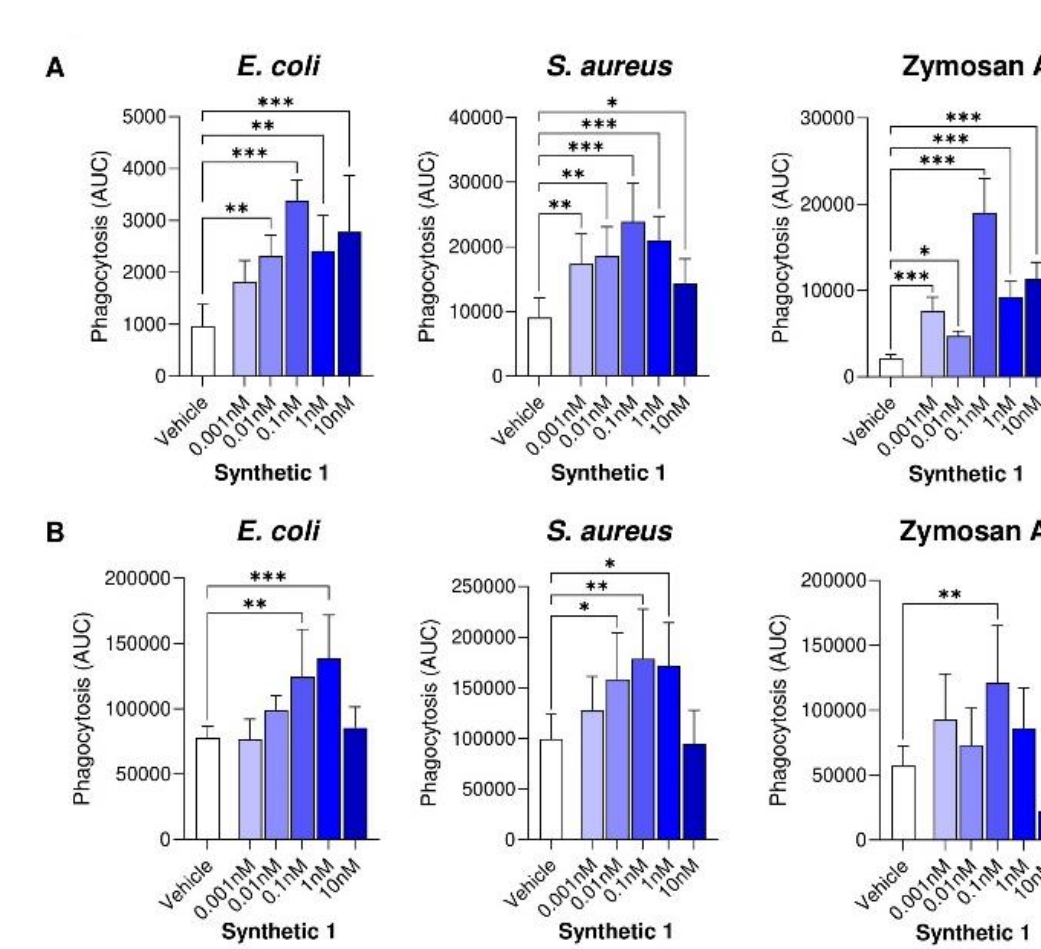


Figure 4. Human neutrophils (A) and monocyte-derived macrophages (B) upregulated phagocytosis in the presence of **1**.

It was observed that the SPM **1** upregulated the clearance of bacterial and fungal particles by human neutrophils and macrophages. RvD5_{n-3} DPA increased the phagocytosis of fluorescently labeled *Escherichia coli*, *Staphylococcus aureus* and zymosan A particles in both cell types, as illustrated in Figure 4. In addition, it was observed a switch in phenotypic markers expressed from macrophages in the presence of **1**, lowering pro-inflammatory markers and upregulating proteins linked with tissue protection.

References

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- Serhan, C. N.; Chiang, N. Resolution phase lipid mediators of inflammation: agonists of resolution. *Curr. Opin. Pharmacol.* **13**, 632-640 (2013).
- Ervik, K.; Reinertsen, A. F.; Koenis, D. S.; Dalli, J.; Hansen, T. V. Stereoselective Synthesis, Pro-resolution, and Anti-inflammatory Actions of RvD5_{n-3} DPA. *J. Nat. Prod.* **86**, 2546-2553 (2023).

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