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Toward the Selective Inhibition of G Proteins: Total Synthesis of a Simplified YM-254890 Analog

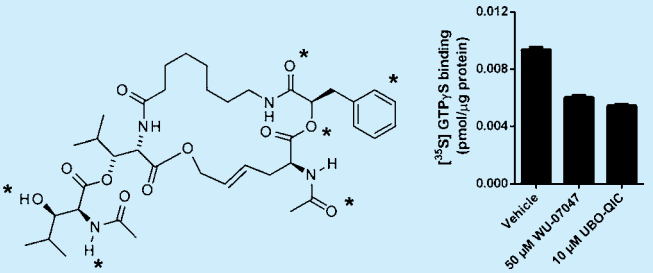
Derek T. Rensing,[†] Sakshi Uppal,[†] Kendall J. Blumer,[‡] and Kevin D. Moeller*[,†]

[†]Department of Chemistry, Washington University, St. Louis, Missouri 63130, United States

[‡]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110, United States

Supporting Information

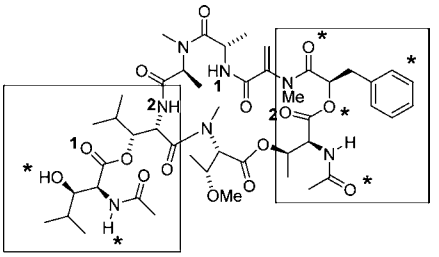
ABSTRACT: A simplified analog (WU-07047) of the selective Gαq/11 inhibitor YM-254890 has been synthesized, and an initial probe of its activity conducted. In the analog, the two peptide-based linkers in the cyclic YM-254890 have been replaced with hydrocarbon chains. This enables a convergent approach to the synthesis of the analog. Biochemical assays showed that while the simplified analog is not as potent as YM-254890, it does still inhibit Gq.



G protein coupled receptors (GPCRs) and their associated G proteins play critical roles in an extraordinary range of cellular, physiological, and disease processes.[1−3] In these events, hundreds of GPCRs signal through heterotrimeric G proteins that are made by combining one of 16 G α subunits, with one of 5 G β isoforms and one of 13 G γ subunits. The picture is complicated by the fact that each GPCR often couples to more than one G protein and that several GPCRs often work in concert with each other and a set of G proteins control a given process. The combination of these features leads to a great deal of diversity, and we do not yet know the functional consequences of this complexity. Besides using pertussis toxin to inhibit activation of Gi/o class G proteins,[4−7] the biochemical community relies on analyzing G protein function genetically. However, this approach can be complicated by redundancy between closely related G protein subunits or by cellular adaptations such as rewiring signaling pathways when one piece has been removed by deletion or knockdown. By comparison, chemical biology approaches to investigate G protein signaling pathways offer an opportunity to determine the acute function of specific G proteins. For this reason, small molecule ligands that directly target selected G proteins to modulate their activity are potentially very valuable as probes of biological function and as avenues to develop therapeutics.

The first such small molecule G protein inhibitor is YM-254890 (Figure 1, **1**), which is produced by an isolate of the chromobacterium sp. QS3666.[8] YM-254890 (YM) potently (IC_{50} = 0.15 nM) and specifically inhibits the α subunit of the G protein Gq.[9−11] Gq is a key signal transducer for many GPCRs,[3] and animal models have shown that YM reduces blood pressure,[12] inhibits thrombosis,[13] and blunts neointima formation following vascular injury.[12] All three effects are consistent with phenotypes of mice lacking Gq.[3]

It appears that YM inhibits Gq by binding a pocket in the α-subunit of Gq (Gαq) that stabilizes the protein in an inactive conformation[9] and prevents activation of the protein by GPCRs. The selectivity of YM for Gq is thought to depend on the unique features of the binding pocket for YM in Gαq. The binding pocket itself is found in all G-protein subtypes. Hence, analogs of YM that were designed to fit the pockets of other G-protein subtypes might provide selective inhibitors of those G-proteins. In this way, the development of YM analogs has the potential to provide a powerful collection of inhibitors for probing the role of specific G-proteins in various physiological or disease processes.



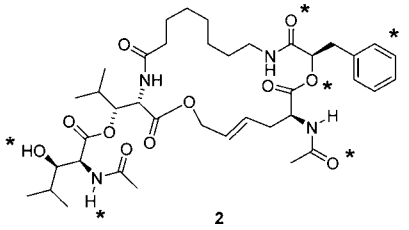


Figure 1. YM-254890 and proposed analog.

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To date, two main complications have blocked efforts to develop a family of G-protein inhibitors based on the YM structure. First, YM is not readily available which impedes efforts to use the natural product itself as a platform for building derivatives. Second, YM has a complex cyclic depsipeptide core structure that has thwarted efforts to obtain a series of analogs by means of total synthesis.[14] With this in mind, we report here the synthesis and initial biological testing of the simplified YM analog **2** (Figure 1).

Structure **1** (Figure 1) contains two regions that are highlighted along with several asterisks and a pair of numbers. The boxes highlight the regions of YM that bind to Gαq in an X-ray crystal structure of the YM-Gαq complex.[9] The points in YM that make contact with Gαq are marked with the asterisks, and the numbers denote hydrogen bonds that impart stability to the YM-conformation involved in binding Gαq. Between the two regions responsible for YM-Gαq binding are two bridging groups. It was hoped that these two bridging groups could be replaced by simplified chains that would make the synthesis of YM analogs easier while allowing for the molecule to still bind and inhibit Gq. For a first attempt, the bridge at the top of the molecule was replaced with an alkyl chain and the bridge at the bottom was replaced with an alkene unit. The alkene would arise from a metathesis reaction used to construct the macrocycle. The trans double bond was located in the same position as the "s-cis" ester located in the bottom bridge of YM in the YM-Gαq complex.[9]

The synthesis of the simplified YM-analog **2** was initially proposed to follow the overall plan suggested in Figure 2. The

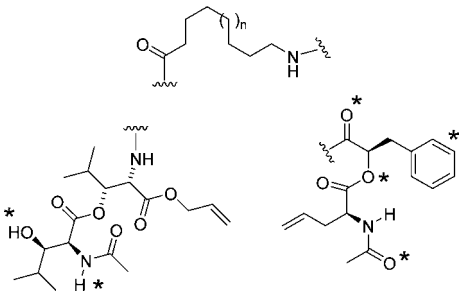
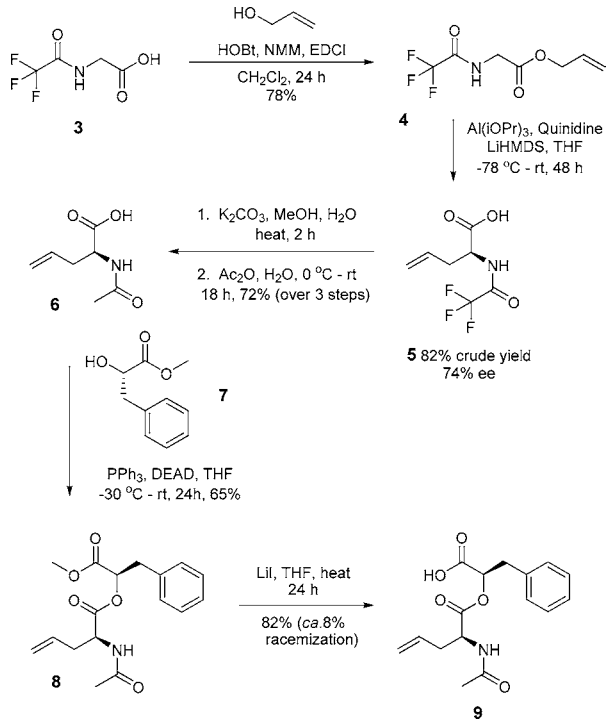


Figure 2. YM-analog synthetic plan.

strategy called for the construction of three building blocks that would be assembled into an acyclic version of the molecule, which could then be cyclized with an olefin metathesis reaction. The left-hand building block is a dimer of β-hydroxy leucine with a C-terminal allyl ester, and the right-hand building block appeared to be a readily assembled coupling product from a 2-hydroxy-3-phenylpropionic acid derivative and a protected allylglycine. The top bridge was to be a simple extended amino acid derivative.

The effort began with construction of the right-hand building block (Scheme 1). Key to this effort was an asymmetric Ireland–Claisen rearrangement that converted allyl ester **4** into allylglycine **5**.[15] The reaction proceeded in superior ee when the nitrogen of the glycine was protected with a trifluoroacetyl group. Exchange of this protecting group for an acetate afforded the allylglycine that was coupled to methyl (*S*)-2-hydroxy-3-phenylpropionate with the use of a Mitsunobu reaction. The reaction led to a mixture of diastereomers (87:13) because of the isomers generated during the Ireland–Claisen reaction. The mixture could be separated, and only the pure major

Scheme 1. Synthesis of Right Hand Building Block



diastereomer was carried forward. Cleavage of the methyl ester then led to the right-hand building block **9**. Analysis of the product by proton NMR indicated that about 8% of the material racemized during the final deprotection step. Still, after recrystallization 50% of the pure building block could be obtained. Another 30% of the building block could be isolated that was contaminated with the other diastereomer.

Synthesis of the left-hand building block proved to be more challenging. After employing the known methodology for the construction of *syn*-β-hydroxyleucine **10**,[16] our initial plan was to dimerize the amino acid derivative and then couple the dimer to the top bridge of the molecule (**11**). Numerous efforts were made along these lines, but each met with failure. Eventually, an alternative strategy that first coupled the top bridge of the molecule to the hydroxyleucine monomer was pursued (Scheme 2). To this end, a mixed anhydride of the Boc-protected 8-aminooctanoic acid was prepared and then treated with **10** in the presence of sodium hydroxide. This resulted in the formation of an amide that was then converted into a C-terminal allyl ester (**12**).

The final protection step introduced the double bond needed for the final olefin metathesis reaction. At this point, the amino and alcohol functionality in **10** needed to be protected so that the acid in **13** could be coupled to the hydroxyl functionality in **12**. After significant experimentation, the strategy shown for the conversion of **10** into **13** shown in Scheme 3 was developed. In this approach, the amine in **10** is protected with a Troc-group. The carboxylic acid is then protected as an allyl ester followed by silylation of the alcohol. Pd(0)-catalyzed cleavage of the allyl ester then afforded the desired **13**.

The pieces of the molecule were then assembled into the final product as outlined in Scheme 3. The effort began by coupling the two β-hydroxyleucine building blocks **13** and **12** using EDCI and DMAP in dichloromethane to afford 59% of the complete left-hand building block. The Troc group on the amine was required for the coupling reaction to be successful.

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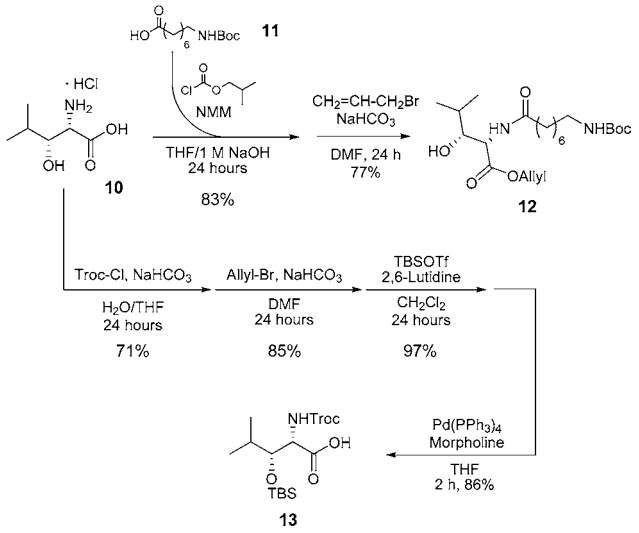
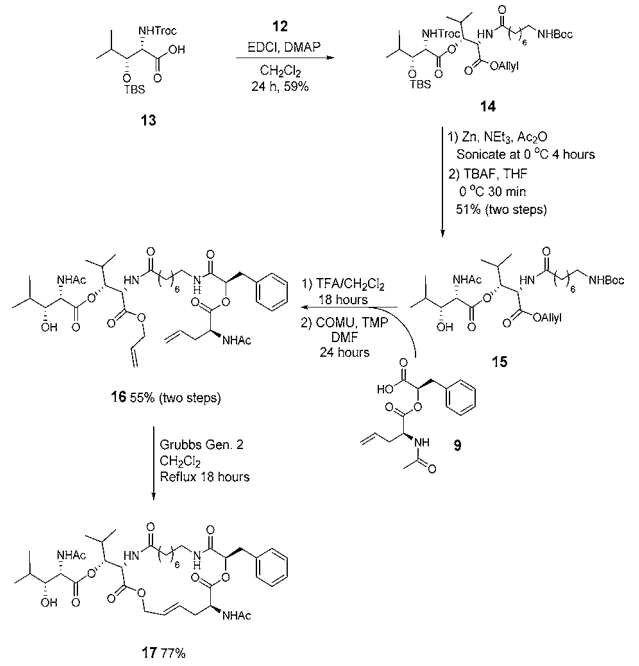
Scheme 2. Synthesis of Left Hand Building Block



Scheme 3. Assembly of the Final Product



An alternative strategy that placed the acetate group needed for the final product on the nitrogen at this point in the synthesis was not successful. In this case, it appeared that the reaction led to the formation of an alkyl oxazolone ring as is often the case in similar circumstances.[17] Because the acetate group could not be added to the molecule prior to the coupling reaction, the Troc group in **14** was exchanged for the acetate following the coupling reaction. Since the right-hand portion of the molecule is more difficult to scale, it was more efficient to conduct the exchange at this point in the synthesis rather than waiting to accomplish the transformation after assembly of the whole molecule. The TBS protecting group was then removed (again to avoid the additional step following construction of the whole molecule) followed by deprotection of the N-terminus of the molecule and coupling of the resulting amine to the right-hand building block **9** to afford cyclization substrate **16**.

The olefin metathesis reaction was conducted with a second generation Grubbs catalyst.[18] The reaction ran very well and afforded a 77% yield of the 22-membered ring. The result was a convergent synthesis with a longest linear sequence of 10 steps with an overall yield of 6.4%. The nature of the convergent synthesis should allow for variations to the core structure of the analog.

With the simplified YM analog (WU-07047) in hand, attention was turned toward determining its inhibitory activity in a receptor-assisted GTPγS nucleotide exchange assay (Figure 3).[19,20,9] Upon receptor activation, the α subunit of the Gq

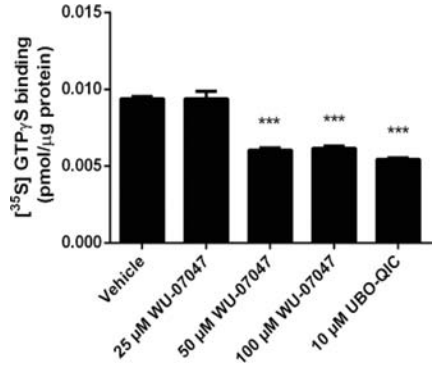


Figure 3. Receptor-assisted GTPγS nucleotide exchange assay. Quantification of receptor-assisted nucleotide exchange assay. The data represents mean (±SEM) counts from three independent experiments (n = 3). Statistical significance is marked by ***, where $p < 0.0001$ with respect to vehicle.

heterotrimer exchanges bound GDP for GTP to establish an active conformation of the protein. By using a GTP analog (GTPγS) with its γ-phosphate radiolabeled with 35S, this reaction was monitored over time to obtain conditions of half-maximal exchange. Under these conditions, the ability of WU-07047 to inhibit nucleotide exchange was compared to that of another natural product closely related to YM (UBO-QIC),[21] relative to a vehicle (DMSO) control. WU-07047 inhibited receptor-driven nucleotide exchange on Gαq in a concentration-dependent manner. Results indicated that WU-07047 and UBO-QIC displayed similar efficacy (up to 40% inhibition of nucleotide exchange), but WU-07047 was less potent. So while WU-07047 is a greatly simplified version of YM that retained none of the functionality in the tethers connecting the regions of the molecule thought to be associated with its activity, it did retain the ability to inhibit Gq activation. This finding suggests that WU-07047 can provide a good starting point for exploring new Gq inhibitors and learning more about the factors that impart selectivity to YM. For example, current efforts are working to reinstall the hydrogen bond represented by the number 1's in Figure 1 in order to determine its role in the selectivity and activity of the analog and to determine if removal of the double bond in the bottom bridge improves activity.

In conclusion, we have developed a synthetic approach to a simplified analog of the selective Gq inhibitor YM. While this analog is not as potent as YM, it inhibits Gq and provides a starting point for further studies of YM analogs. The convergent nature of the synthetic route developed will allow us to develop rapid screening methods for assessing the binding of additional YM variants to a variety of Gα-subtypes and in subsequent studies to probe determinants of potency, G

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protein selectivity and PK/PD associated with YM. A collection of G protein subtype-selective inhibitors would provide powerful tools for probing the functions of specific G proteins in diverse biological processes and diseases.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and characterization (^{1}H and ^{13}C NMR, IR, and HRMS data) of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: moeller@wustl.edu.

Notes

The authors declare no competing financial interest.

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