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Solid-Phase Synthesis of Anandamide Analogues

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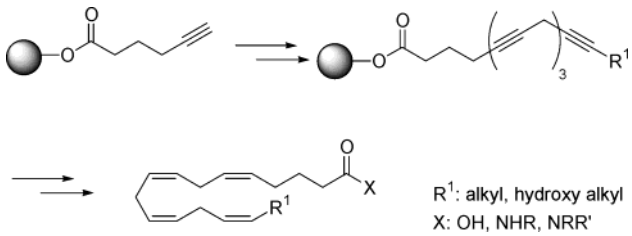
ABSTRACT



The endocannabinoids are amides and esters of arachidonic acid that can mimic the pharmacological properties of Δ⁹-tetrahydrocannabinol (Δ⁹-THC). Anandamide, the most prominent of the endocannabinoids, has been implicated in both metabolic/physiological roles of the central nervous system, making it an attractive medicinal target. As such, we report the first solid-phase methodology that expedites access to various anandamide analogues. Our synthesis features a repetitive Cu-mediated coupling reaction between terminal alkynes and propargyl halides or allylic halides.

Since the discovery of anandamide (arachidonyl-ethanolamide, AEA) **1** in 1992[1] as a member of the family of endocannabinoids, which are endogenous ligands of cannabinoid receptors, a flurry of reports has ensued detailing its physiological importance. Many of these studies have concentrated on elucidation of the endocannabinoids' mechanism of action. Thus far, three types of endogenous cannabinoid−receptor agonists have been identified (Figure 1). They include ethanolamides, consisting of polyunsaturated fatty acids with anandamide **1** as the best-known compound in the amide series; 2-arachidonoyl glycerol (**2**, 2-AG),[2] the only known endocannabinoid in the ester series; and the ether endocannabinoid, 2-arachidonyl glyceryl ether (**3**, noladin, HU-310).[3] The endogenous cannabinoid system (ECS)[4] is primarily responsible for all the effects mediated by cannabinoids, and it consists of two G-protein-coupled receptors, i.e., CB1 and CB2. The ECS is involved in the regulation

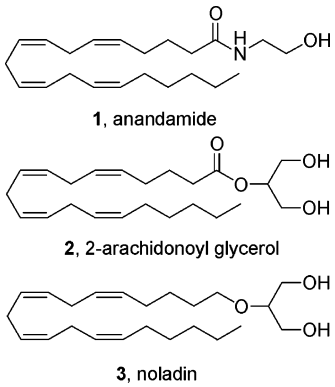


Figure 1. The Three Types of Endocannabinoids.

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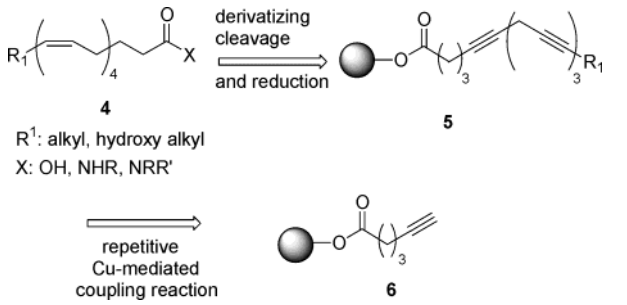
of a wide variety of physiological functions such as antinociception, brain development, memory, retrograde neuronal communication, control of movement, cardiovascular and immune regulation, and cellular proliferation.[5] Small molecules affecting ECS activity have the potential to be therapeutic agents for the treatment of diverse pathologies,[6] including neurodegenerative disorders, nociceptive alterations, and malignant tumors. Hence, structure−activity relationship (SAR) studies of anandamide analogues are beginning to emerge.[7] Most of the structural modifications have focused on alteration of the ethanolamido headgroup. For example, substituents at the 2-position have shown an enhancement of metabolic stability,[8] whereas introduction of electronegative groups, such as halogen atoms or unsaturated three-carbon substituents in the headgroup, results in increased receptor binding affinity but reduced biochemical stability. Reversal of the carbonyl and NH groups results in higher metabolic stability; however, the receptor binding affinity is somewhat decreased compared to that of anandamide.[9] The effect of chain length and branching of the pentyl moiety of AEA has also been reported.[10] More recently, research on analogues of arachidonic acid has revealed that there is no correlation between effects on activity of the fatty acid amidohydrolase (FAAH) and on the anandamide transport system (ANT).

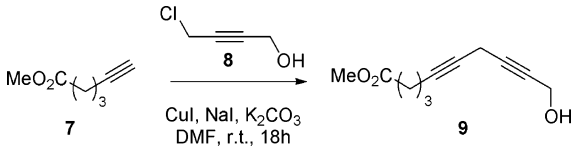
The availability of AEA analogues should prove to be useful for future in vitro and in vivo studies aimed at delineating AEA's mode of action and potential therapeutic applications. Herein we report the first solid-phase synthesis of anandamide analogues, which features copper-mediated coupling reactions[11] between terminal alkynes and propargyl halides and a cleavage step that provides diversification of the headgroup. Scheme 1 shows a retrosynthetic analysis of our approach wherein Wang resin is employed as the solid support. As shown, diversity at the headgroup would be accomplished when the substrate is cleaved by acid, alcohol, or amine to provide acid, ester, or amide, respectively. Noteworthy would be the repetitive Cu-mediated C−C bond formation that was successfully applied in solution-phase reactions to obtain skipped polyyne compounds.

Scheme 1. Retrosynthetic Analysis of the Solid-Phase Synthesis of Anandamide Analogues



Key to our synthesis of AEA analogues was the C−C bond forming reaction to install the consecutive diynes. To explore and mimic this reaction on solid support, we commenced with the solution-phase reaction of ester **7** with 4-chlorobut-2-yn-1-ol (**8**).[12] Using the conditions shown in Scheme 2 afforded diyne **9** in high yield (93%).

Scheme 2. Key Coupling Reaction Mediated by CuI in Solution Phase



Our successful solid-phase synthesis of target **4** is illustrated in Schemes 3 and 4. Thus, 5-hexynoic acid was quantitatively anchored to Wang resin via an ester linkage. The resulting resin-bound alkyne **6** was anticipated to react with alcohol **8** similarly to the solution-phase reaction between **7** and **8**. However, this Cu-mediated coupling reaction on solid-support was sluggish due to the presence of multiple phases. Consequently, alternative conditions were investigated, and subsequently it was found that when 10 equiv of **8** reacted with resin-bound terminal alkyne **6** in the presence of 3 equiv of CuI and 3 equiv of NaI in 9 mL of DMF per gram of resin, yields greater than 90% could be achieved. The resultant propargyl alcohol **10a** was quantitatively converted to the corresponding bromide **10b** using Ph_3P/CBr_4 at temperatures from −40 to 0 °C. The C−C bond-forming reaction was repeated, but now using propargyl alcohol to afford a triynol **11a**, which was transformed to propargyl bromide **11b**. The final alkyne addition was accomplished using the same coupling conditions, however, with various alkynes to provide diversity on the tail ends of tetrayne **5** (Scheme 3).

Attempts to reduce tetrayne **5** with the Schwartz reagent or DIBALH on the resin were not successful. Thus, tetrayne **5** was cleaved using TFA (50% in CH_2Cl_2)[13] and $AlMe_3$/

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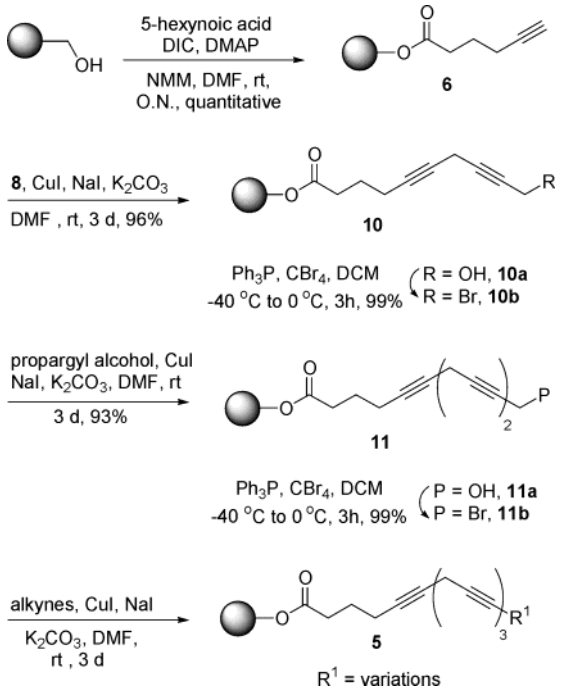
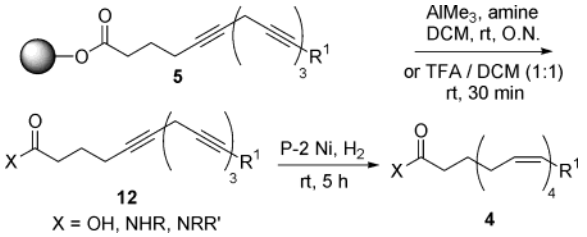
Scheme 3. Solid-Phase Synthesis of Tetrayne **5**



Scheme 4. Synthesis of Anandamide Analogues



amine[14] to provide acids and amides, respectively. Finally, reduction was performed in the solution phase with P−2 Ni catalyst to give the desired structures with up to 65% yield (for eight steps) (Scheme 4).

To illustrate the versatility of our method, a small library was prepared in parallel format (Figure 2). ^{1}H NMR analysis of all compounds indicated that only cis double bonds were obtained. The purity of all compounds was greater than 95% as judged by NMR.

An interesting observation that may be explored further for future studies was the formation of 17-membered macrocyclic ester **13a** in 50% yield by cleavage of the corresponding resin-bound substrate with TFA/CH_2Cl_2 for 1 h at room temperature (Scheme 5).

In summary, we have presented a flexible solid-phase synthesis of anandamide analogues. Our methodology features a repetitive Cu-mediated carbon−carbon bond-forming reaction that provides access to the tetrayne framework in good yield. We also note that the synthesis allows diversification of both the head and tail of the parent molecule as well as control of the chain length. Because of the versatility of our approach, it could prove to be of value in the development of novel anandamide analogues for mechanistic investigations of the mode of action of **1** in the brain.

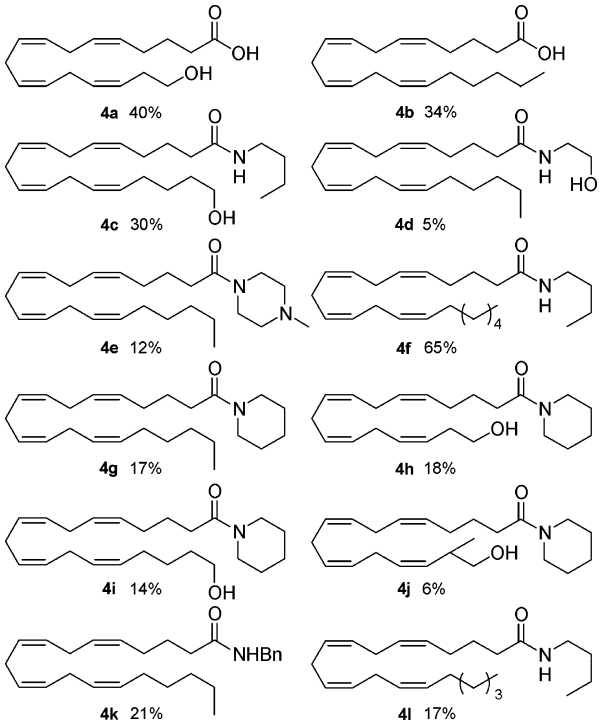
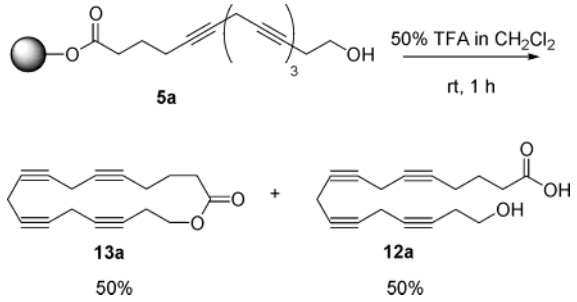


Figure 2. Library of Anandamide Analogues.

Scheme 5. Macrolactonization by TFA-Promoted Cleavage



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Supporting Information Available: Experimental synthetic procedures and NMR, LC/MS, and/or HRMS data for the library members and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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