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Synthesis of a potential photoactivatable anandamide analog

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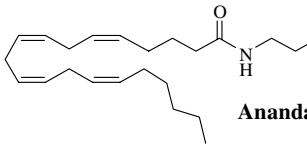
Abstract—A potential photoreactive analog of anandamide was synthesized via selective hydrogenation of a skipped tetrayne intermediate. This compound might be a useful tool to search for new cannabinoid receptors.

Several unsaturated fatty acid derivatives have been identified as the endogenous ligands for the two cannabinoid receptor subtypes cloned thus far: the CB1 and CB2 receptors. These lipid mediators are referred to as endocannabinoids (eCBs) in order to distinguish them from the natural cannabinoid, Δ^9-tetrahydrocannabinol (THC), derived from the *Cannabis sativa* plant.[1] The eCBs identified to date include[2,3] the eicosanoids; *N*-arachidonoylethanolamide (anandamide), 2-arachidonoylglycerol (2-AG), 2-arachidonylglyceryl ether (noladin ether), *O*-arachidonoylethanolamide (virohamine), and *N*-arachidonoyl-dopamine, together with the ethanolamides of docosatetraenoic and homo-γ-linolenic acids. They have been suggested to participate in several physiological and pathological conditions in mammals.[4,5] Several excellent reviews on endocannabinoids and their synthesis, metabolism, and functions have appeared during the last three years.[6] However, despite the recent considerable advances and the extensive studies undertaken over the past 14 years, the eCB system is still not completely understood.[7]

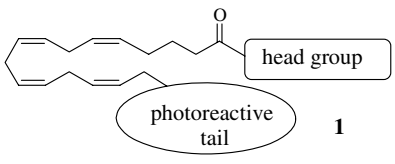
While the anandamide cellular uptake mechanism is currently the source of much controversial debate,[8,9] numerous contemporary experiments suggest that eCBs cause some of their effects independently of the known CB1 and CB2 receptors.

Recently, it was reported that GPR55, an orphan G-protein-coupled receptor, might represent a new CB receptor.[10] In addition, thanks to the development of selective CB1 or CB2 antagonists and to the availability of gene knock-out and double knock-out mice ($CB1^{-/-}$, $CB2^{-/-}$, and $CB1^{-/-}/CB2^{-/-}$), there is emerging evidence suggesting that novel cannabinoid receptors may exist for anandamide in various tissues.[11,12] Interestingly, some of these molecular targets are not sensitive to plant-derived cannabinoids.[13,14] To date, none of these putative receptors have been cloned.

Within this context, we reasoned that an anandamide-derived and potentially photoactivatable tool might be useful for the identification of non-CB1, non-CB2 eCB receptors. We therefore focussed our attention on the synthesis of an aryl azide probe of anandamide. Our design featured a flexible synthetic strategy allowing us to readily change the head and/or tail group at a later stage **1**.



Anandamide



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Since little is known about the targeted putative receptors, structural requirements are rather unpredictable at this time. However, it was clearly established that methylation at the alpha position to the nitrogen atom significantly enhances the metabolic stability toward the FAAH enzyme (and also its CB1 binding affinity).[15] In addition, SAR with both CB receptors highlights the importance of the number of double bonds and their location on the carbon skeleton.[16] Thus, we have chosen to introduce the potentially photoactivatable group in the tail of (*R*)-methanandamide. This will allow us also to infer the influence of the terminal bulky group on the affinity of anandamide analogs for CB1 and CB2 receptors, a type of information that is limited to date.

We report herein the total synthesis of compound **2**. The 2-azido-5-iodobenzoate ester group has already been successfully used in photolabeling experiments.[17] Its radiolabeled analog (^{125}I) is readily obtainable.[26] Moreover, besides their greater stability, aryl azides have the advantage over alkyl azides that their irradiation can be performed with an activation wavelength higher (>300 nm) than the ultraviolet absorption for most proteins[18,19] (thus limiting protein damage). It is hypothesized[20] that the irradiation of the aryl azide by UV light will generate a nitrene intermediate which would bind to putative non-CB1, non-CB2 eCB receptors, establish an irreversible cross-link to these proteins, and permit the isolation and mapping of the receptor site.

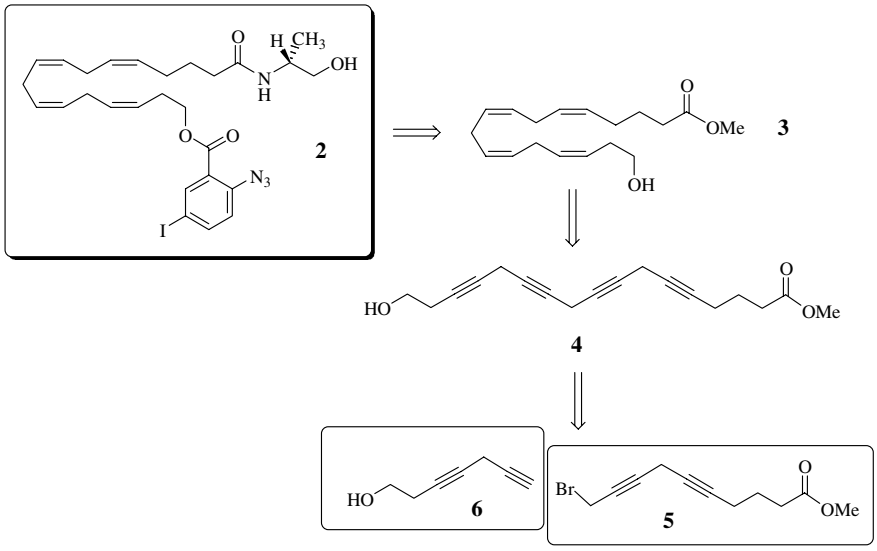
Retrosynthetically, the strategy involves the saponification of the ester function of the key intermediate **3** to introduce the anandamide head group, and esterification of the ω-hydroxy function with 2-azido-5-iodobenzoic acid (Scheme 1).

The tetraene backbone comes from selective hydrogenation of the tetrayne **4**. The three skipped diynes of tetrayne **4** were assembled using cross-coupling between copper(I) alkynides and propargylic halides. Instead of the previously[21,22] described linear strategy which built consecutively the diyne, triyne, and then tetrayne backbone, we have developed a convergent route to the skipped tetrayne **4**, by coupling the two skipped diynes **5** and **6**.

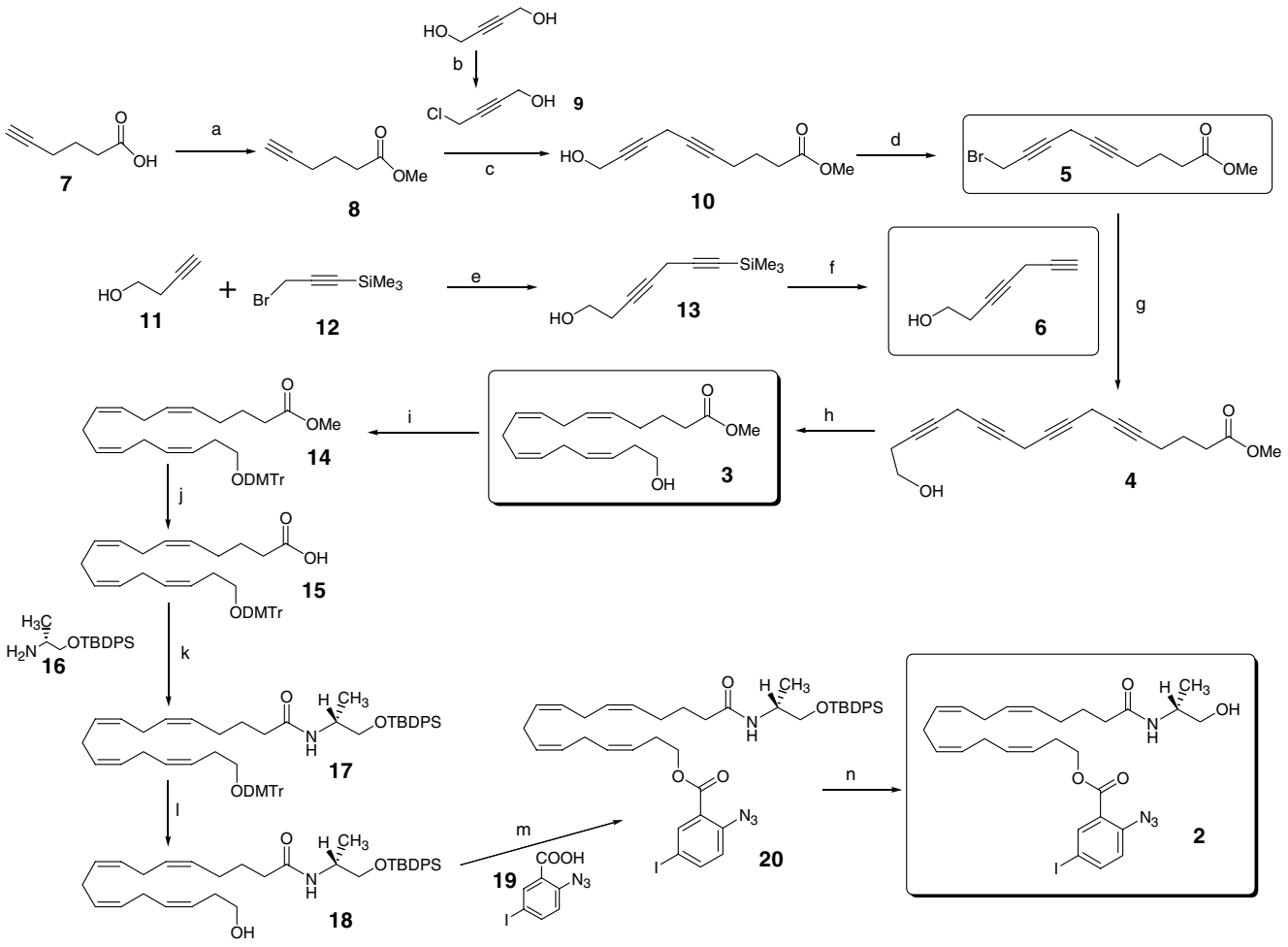
The synthesis of **2** is depicted in Scheme 2.

The precursor **5** was prepared according to Razdan.[23] Thus, starting from the commercially available 5-hexynoic acid **7**, esterification followed by a cross-coupling reaction with 4-chlorobut-2-yn-1-ol **9** and subsequent bromination with CBr_4, in the presence of PPh_3, gave the expected bromide **5** in 73% yield. The synthesis of precursor **6** was achieved in two steps in 73% yield via a coupling reaction between the commercially available 3-bromo-1-(trimethylsilyl)-1-propyne **12**, and 3-butyn-1-ol **11** and subsequent deprotection of the silyl group with fluoride. The key coupling reaction between the precursors **5** and **6** was carried out under mild conditions[24] using Cs_2CO_3 and afforded tetrayne **4** in 45% yield. In our hands, this skipped tetrayne **4** was found to be unstable when stored in a freezer under argon. However, if used immediately its selective reduction using the P2-nickel catalyst subsequently yielded the expected all *cis* key tetraene **3**[25] (47% yield).

The ω-hydroxyl function of tetraene **3** was protected as a dimethoxytrityl group. Upon saponification of the resulting tetraene **14**, the amide coupling reaction with the O-silylated (*R*)-(−)-2-amino-1-propanol **16**, using the mixed anhydride procedure, provided the expected amide **17** along with its corresponding ω-free hydroxyl analog **18**. This partial deprotection of the dimethoxytrityl group occurred during the purification step by column chromatography using Florisil. The remaining protected material **17** was converted into the desired alcohol **18** by treatment with hexafluoro-2-propanol. Steglich esterification of the key tetraene **18** with 2-azido-5-iodobenzoic acid **19** (prepared as described by Perrier et al.[26]) followed by removal



Scheme 1. Key advanced intermediates to probe **2**.



Scheme 2. Reagents and conditions: (a) APTS, MeOH, CH_2Cl_2, reflux, 24 h, 96%; (b) $SOCl_2$, PhH, 55%; (c) CuI, NaI, K_2CO_3, DMF, 30 °C, 20 h, 85%; (d) CBr_4, PPh_3, CH_2Cl_2, 1 h, 90%; (e) CuI, NaI, Cs_2CO_3, DMF, 30 °C, 18 h, 77%; (f) TBAF, THF, 0 °C, 1 h, 95%; (g) CuI, NaI, Cs_2CO_3, DMF, 35 °C, 18 h, 45%; (h); P-2 Ni, H_2, EtOH, 8 h, 13 °C, 47%; (i) DMTrCl, DMAP, NEt_3, CH_2Cl_2, rt, 15 h, 81%; (j) LiOH, MeOH, 55 °C, 22 h, 96%; (k) *i*-BuOCOCl, NMN, MeCN, −5 °C, 3 h; (l) HFIP, 12 h, rt, 65%; (m) DCC, DMAP, CH_2Cl_2, rt, 15 h, 82%; (n) TBAF, THF, rt, 1 h 30 min, 75%.

of the silyl group with TBAF, afforded the expected compound **2**[27] in 61% yield.

Compound **2** was tested for its affinity for human recombinant CB1 and CB2 receptors using a previously described procedure and employing membranes from COS cells overexpressing either receptor and [^{3}H]CP55-940 as the high affinity ligand.[28] The compound exhibited K_i (μM) = 0.57 ± 0.05 and 0.22 ± 0.03 for CB1 and CB2 receptors, respectively (means ± SE, $N = 3$). In the same conditions, anandamide shows K_i (μM) = 0.07 ± 0.03 and 0.18 ± 0.02, respectively. Thus, these assays showed, for the first time, that a cumbersome 'tail' group reduces the binding of putative ligands to CB1 but not CB2 receptors. This residual affinity for the CB2 receptor might suggest that compound **2** might be used as a tool for yet-to-be characterized new eCB receptors. On the other hand, the reduced affinity for CB1 receptors as compared to anandamide also suggests that compound **2** might turn out to be a rather selective tool for the putative new receptor(s).

In summary, probe **2** was found to bind to CB1 and, particularly, CB2 receptors with affinities that should allow its use to identify other non-CB1, non-CB2 cannabinoid receptors for anandamide. Its synthesis was achieved in 11 steps at a 1.6 mmol scale. The skipped tetrayne backbone was obtained using a convergent strategy starting from four commercially available reagents; that is, 5-hexynoic acid **7**, 1,4-butynol, 3-bromo-1-(trimethylsilyl)-1-propyne **12** and 3-butyn-1-ol **11**. We think that the synthesis of different endocannabinoid probes will be attainable in a similar fashion replacing 2-(*R*)-aminopropan-1-ol with a suitable alternative head group (for example, dopamine, aminoacids, etc., or alcohols such as glycerol, ethanolamine, etc.). The synthesis of these novel probes together with their binding affinities at CB1 and CB2, and photoaffinity labeling experiments with probe **2** are in progress and will be reported in due course.

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