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A Convenient Protocol for the Synthesis of Fatty Acid Amides

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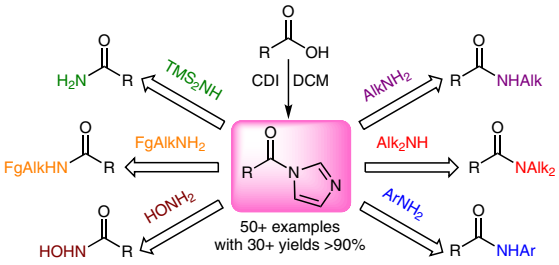

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Abstract Several classes of biologically occurring fatty acid amides have been reported from mammalian and plant sources. Many amides conjugated with fatty acids of mammalian origin exhibit specific activation of individual receptors. Their potential as pharmacological tools or as lead compounds towards the development of novel therapeutics is of great interest. Hence, access to such amides by a practical, high-yielding and scalable protocol without affecting the geometry or position of sensitive functionalities is needed. A protocol that meets all these requirements involves activation of the corresponding acid with carbonyl diimidazole (CDI) followed by reaction with the desired amine or its hydrochloride. More than fifty compounds have been prepared in generally high yields.

Key words fatty acid amides, CDI, AM404, ethanolamide, *N*-acylethanolamines (NAEs), *N*-acyl dopamines (NAPs), *N*-acyl amino acids (NAAs), anandamide

Fatty acid amides form the basis of a diverse collection of metabolites. The capsaicinoids and ceramides have been known for more than a century. In the mid 1950s, the anti-inflammatory compound *N*-palmitoylethanolamine (**1**) was isolated from egg yolk,[1] followed by reports of related *N*-acylethanolamides in the following decades.[2] However, with the discovery of *N*-arachidonylethanolamide, known as anandamide (**2**), interest in such compounds has increased tremendously.[3] Today, fatty acid amides (FAAs) from several different classes are known, and the members show a broad spectrum of biological activity. Examples are shown in Figure 1.

Formally the FAAs are products of a *de facto* transacylation process, with many aspects relating to their origin via a nonoxidative pathway still being subject of investigation. Recently, it has been demonstrated that primary fatty acid amides (PFAMs) are biosynthesized through the involvement of a glycine *N*-acetyltransferase in selected cell lines.[4] However, for higher-order amides such as *N*-acylethanolamines (NAEs), *N*-acyl dopamines (NAPs), and *N*-acyl amino acids (NAAs), many details of the *en route* metabolism seems currently to evade conclusive elucidation.[5]

In terms of therapeutic potential, the causative effect of FAAs has in recent years also received a surge of attention, as they are able to modulate core responses in mammalian

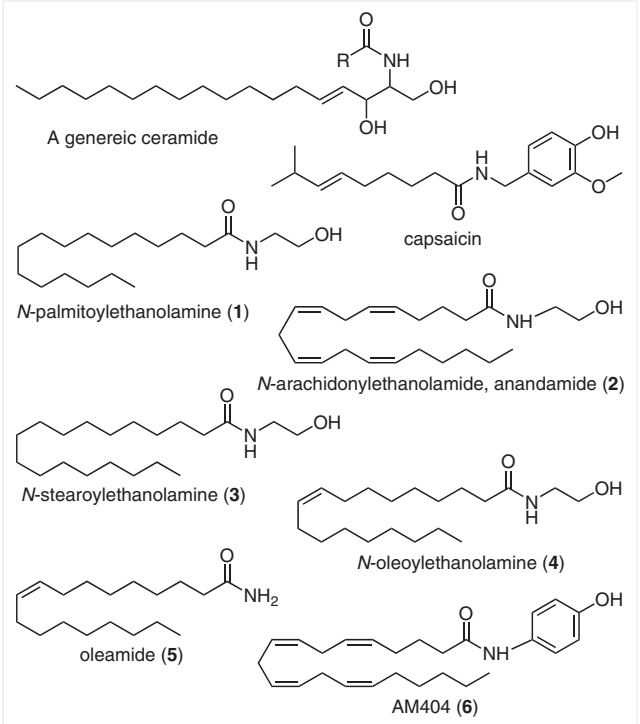


Figure 1 Examples of pharmacologically active polyunsaturated fatty acid amides

nervous systems and control a series of basal physiological processes, either directly or indirectly.[6] Thus, these FAAs can exert their action either as neurotransmitters or behave as hormones.[7]

FFAs can affect the immune system, energy balance and food intake, metabolic homeostasis, fertility, anxiety and depression.[8] Furthermore, some FAAs have been shown to affect cell proliferation, and are therefore of interest in cancer research.[9] It has also been observed that *N*-acylethanolamines increase in animal models of tissue degeneration.[10]

In particular, anandamide has received attention for its ability to bind to druggable receptors connected to the endocannabinoid and the endovanilloid systems[3,11] that may have clinical application in the treatment of afflictions such as neuropathic pain.[5e]

N-Stearoylethanolamine (**3**) shows proapoptotic activity,[12] while *N*-oleoylethanolamine (**4**) is an anorexic mediator.[13] Nanomole quantities of the primary amide of oleic acid, oleamide (**5**) have been shown to induce physiological sleep in rats.[14] *N*-Arachidonoylaminophenol, also known as AM404 (**6**), is one of the active metabolites of paracetamol and has been reported to be responsible for part of its effect as an analgesic and anticonvulsant.[15]

A different aspect of their latent pharmacology is the ability of FAAs to act as xenobiotics in the treatment of bacterial and insecticidal infections. Thus, certain synthetic FAAs have been demonstrated to exhibit antitubercular activity on resistant strains.[16] Another trial set of FAAs has been shown to possess larvicidal activity against the zoonotic roundworm *Toxocara canis*, which is associated with the debilitating and potentially fatal condition named 'visceral larval migrans syndrome'.[17]

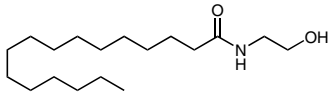
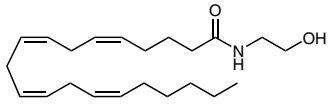
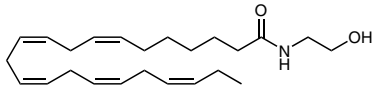
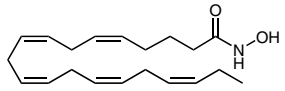
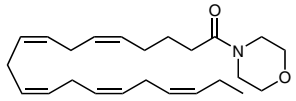
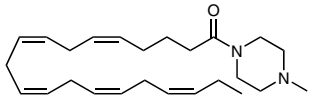
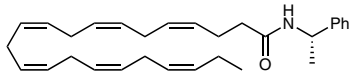
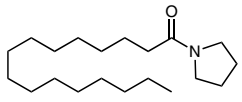
Based on the above biological effects, it is clear that FAAs, as a structural class, have attracted much interest within biomedical research. Novel FAAs continue to be isolated and structurally elucidated, resulting in an increased focus with regard to their chemistry and biology. Thus, efficient synthetic access to these FAAs and their analogues becomes necessary in order to advance their pharmacological understanding. Often, these compounds are only present in microgram quantities in neuronal cells.

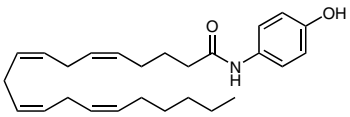
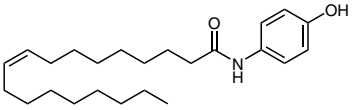
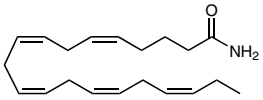
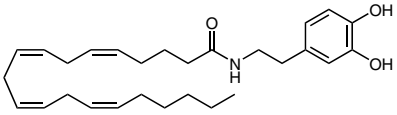
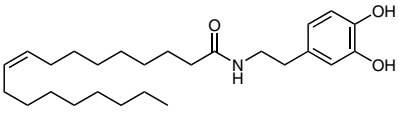
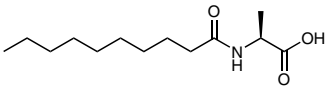
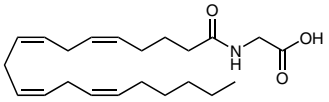
As the relatively low reactivity of carboxylic acids has long been recognized, amide synthesis has traditionally been performed via the corresponding acyl chloride or anhydride derivatives.[18]

The introduction of *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling agent, marked the entrance of the first mild amide coupling protocol, transforming the acid into an acyl equivalent in the presence of a nonhalogen-based dehydrating agent.[19] While many of these protocols are efficient and versatile, they also have some drawbacks such as cost, complex reagent handling procedures, and problems with removal of reagent byproducts from the resulting amide.[20] The use of carbonyl diimidazole (CDI) to activate acids is a well-known methodology,[21] but often overlooked in favor of other methods. We find this surprising as CDI is cheaper, less toxic, and gives fewer toxic byproducts than the more endorsed DCC. Following our continued interested in PUFAs, which are both sensitive and expensive compounds, CDI stood out as the best candidate for mild and efficient activation in amide coupling.

After tuning the reaction conditions, we found a simple and reliable one-pot procedure to activate fatty acids with CDI and react them with the desired amine. The reactions are carried out at room temperature with dichloromethane as solvent. The workup is simple as there were found to be no, or only trace amounts of, side products or starting material (for a general procedure, see ref. 21). In particular, the protocol is also applicable to the direct coupling of amine hydrochloride salts at ambient temperature, rendering it very attractive for the preparation of sensitive FAAs. The conversions and reaction yields are excellent for most of the substrates explored. Representative examples are shown in Table 1 and data for all other prepared compounds can be found in the Supporting Information.

Table 1 Representative Examples of Compounds Prepared[a,21]

Entry		Compd	Yield (%)
1		**1**	>96
2		**2**	>96
3		**9**	>95
4		**11**	>96
5		**12**	>96
6		**13**	>96
7		*S*-**14**	>96
8		**15**	>96

Entry		Compd	Yield (%)
9		**6**	61
10		**16**	86
11		**17**	>96
12		**18**	77
13		**19**	58
14		L-**20**	>96
15		**21**	>96

[a] See Supporting Information for a complete list of compounds.

We prepared the *N*-acyletanolamines from palmitic acid (**1**), arachidonic acid (AA, **2**), stearic acid (**3**), eicosapentaenoic acid (EPA, **7**), docosahexaenoic acid (DHA, **8**), clupanodonic acid (n-3 DPA, **9**), and linoleic acid (**10**). These are all reported with yields higher than 95%. A significant improvement in the total synthesis of clupanodonic acid should also be noted. See Supporting Information for details.

Given the range of biological activities of the *N*-acyletanolamines, it is interesting to investigate the importance of the distance between the amide and hydroxyl functionalities. Two *N*-acyl propanolamines and two *N*-acyl hydroxylamines were prepared (*e.g.*, compound **11** in Table 1). *N*-Acyl hydroxylamines are known to be involved in a broad range of biological activities, due to their ability to act as ligands.[22] Yields were quantitative for all four compounds.

Several synthetic *N*-benzyl FFAs and secondary amides (from pyrrolidine, piperidine, piperazine, and morpholine), with antituberculotic properties and activity against *Toxocara canis*, have been reported by D'Oca and co-workers.[16,17] As we found these data to be noteworthy, we prepared more than 35 new amides with similar functionalities (*e.g.*, compounds **12–15** in Table 1). Interestingly, we found that the yields are somewhat lower for the saturated fatty acids than for their corresponding polyunsaturated fatty acids (PUFAs). We tested other solvents to investigate whether the problem was related to solubility, perhaps due to the aggregation/deaggregation of the fatty acids as dimers or oligomers, but we were not fully able to rationalize these findings. From arachidonic acid we prepared *N*-arachidonoylaminophenol (**6**) with a yield of 61%. The oleic acid analogue **16** was also prepared, but with a somewhat higher yield of 86%. We investigated the use of protecting groups to increase the yield but obtained comparable results.

Preparation of primary amides proved to be more difficult than the previously described classes as none of the tested ammonia salts reacted due to the presence of water. One possible solution is to bubble ammonia gas through the solution, or use liquid ammonia at low temperature, but the use of acid chlorides is probably a better alternative in such cases. However, we found that bis(trimethylsilyl)amine (HMDS) worked well as a masked version of NH_3 when applied to EPA. The silyl groups are readily cleaved off during the workup to provide the primary amide, (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenamide (**17**) in quantitative yield. Similar results were seen for DHA. When we applied this protocol to the monounsaturated and saturated acids, such as oleic acid, linoleic acid, stearic acid, and palmitic acid, the yields dropped significantly to only afford between 10% and 35% yields of the corresponding amides. *N*-Arachidonoyldopamine (**18**) and *N*-oleoyldopamine (**19**), both found in the mammalian brain,[23] were prepared from the corresponding FFA and dopamine hydrochloride in yields of 77% and 58%, respectively.

The procedure also works well with amino acids. However, in contrast to the previously listed results, the amino acids needed to be derivatized as their esters. We used the hydrochloride methyl esters of the amino acids with great success. The ester group can subsequently be hydrolyzed with ease, using LiOH in MeOH/THF/H_2O.[24] The naturally occurring derivatives are often from short length, saturated fatty acids. Several of these were prepared in high yields, including both the D- and L-form of *N*-decanoylalanine (**20**), along with *N*-arachidonylglycine (**21**), which is known to bind to GPR18.[25] Analytical data for all prepared compounds can be found in the Supporting Information.

In conclusion, the protocol presented herein enables efficient access to FAAs by an experimentally simple and convenient procedure. More than fifty FAAs have been prepared in multimilligram scale. To the best of our knowledge, we describe the first synthesis for several of these. The reported protocol has been demonstrated to work well with a broad range of FAA classes. Several are currently undergoing biological screening and the results from these tests will be reported in due course.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1611939.

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(21) **General Procedure for Amide Coupling**

To a stirred solution of the fatty acid (1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) was added CDI (0.178 g, 1.1 mmol, 1.1 equiv.). After 30 min at room temperature, the amine (1.1 mmol, 1.1 equiv.) was added. After 12 h, CH_2Cl_2 (25 mL) was added, followed by saturated aqueous NH_4Cl. The mixture was acidified to pH 2 by addition of HCl, the organic phase was separated, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 10 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*, to give the amide.

Example 1: (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-*N*-[(*R*)-1-phenylethyl]icosa-5,8,11,14,17-pentaenamide (*R*-28)

Starting with EPA (0.302 g, 1.0 mmol, 1.0 equiv.) and (*R*)-α-methylbenzylamine (0.133 g, 0.14 mL, 1.1 mmol, 1.1 equiv.). Yield 0.376 g, 93%; TLC (hexane/EtOAc 75:25, visualized by UV and $KMnO_4$ stain): R_f = 0.20; $[\alpha]_D^{20}$ +5.2 (*c* = 0.2, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.49–7.06 (m, 5 H), 5.65 (d, *J* = 8.2 Hz, 1 H), 5.47–5.27 (m, 10 H), 5.15 (p, *J* = 7.1 Hz, 1 H), 2.91–2.73 (m, 8 H), 2.18 (dd, *J* = 8.3, 6.6 Hz, 2 H), 2.15–2.02 (m, 4 H), 1.79–1.67 (m, 2 H), 1.50 (d, *J* = 6.9 Hz, 3 H), 0.98 (t, *J* = 7.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 143.2, 132.0, 129.1, 128.7 (2 × CH),

128.6, 128.5, 128.2, 128.2, 128.1, 128.0, 127.8, 127.3, 126.9, 126.1(2 × CH), 48.6, 36.1, 26.6, 25.6, 25.5, 25.4, 21.6, 20.5, 14.2. IR(ATR): ν_{max} = 3300, 3014, 2964, 1644, 1544 cm^{-1}. HRMS (EI^+): exact mass calcd for $C_{28}H_{39}NO$ $[M]^+$ m/z = 405.3032; found: 405.3051.

Antipodal *S*-28

Yield 0.403 g, >96%; $[\alpha]_D^{20}$ –5.4 (*c* = 0.3, MeOH).

Example 2: (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-*N*-[(*R*)-1-phenylethyl]-docosa-4,7,10,13,16,19-hexaenamide (*R*-14)

Starting with DHA (0.328 g, 1.0 mmol, 1.0 equiv.) and (*R*)-α-methylbenzylamine (0.133 g, 0.14 mL, 1.1 mmol, 1.1 equiv.). Yield 0.417 g, >96%; TLC (hexane/EtOAc 75:25, visualized by UV and $KMnO_4$ stain): R_f = 0.23; $[\alpha]_D^{20}$ +6.5 (*c* = 0.2, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.21 (m, 5 H), 5.68 (d, *J* = 7.9 Hz, 1 H), 5.47–5.26 (m, 12 H), 5.15 (p, *J* = 7.0 Hz, 1 H), 2.91–2.77 (m, 10 H), 2.46–2.38 (m, 2 H), 2.27–2.21 (m, 2 H), 2.13–2.03 (m, 2 H), 1.49 (d, *J* = 7.0 Hz, 3 H), 0.98 (t, *J* = 7.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 143.1, 132.0, 129.3, 128.6 (2 × CH), 128.5, 128.2 (2 × CH), 128.2 (2 × CH), 128.1, 128.0, 128.0, 127.8, 127.3, 127.0, 126.2 (2 × CH), 48.6, 36.5, 25.6, 25.6, 25.6, 25.5, 23.4, 21.7, 20.5, 14.2. IR(ATR): ν_{max} = 3294, 3014, 2964, 1644, 1544 cm^{-1}. HRMS (EI^+): exact mass calcd for $C_{30}H_{41}NO$ $[M]^+$ m/z = 431.3188; found: 431.3164.

Antipodal *S*-14

Yield 0.430 g, >96%; $[\alpha]_D^{20}$ –6.2 (*c* = 0.2, MeOH).

Example 3: (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaen morpholide (12)

Starting with EPA (0.302 g, 1.0 mmol, 1.0 equiv.) and morpholine (0.096 g, 0.095 mL, 1.1 mmol, 1.1 equiv.). Yield 0.417 g, >96%; TLC (hexane/EtOAc 40:60, visualized by UV and $KMnO_4$ stain): R_f = 0.32. ^1H NMR (400 MHz, $CDCl_3$): δ = 5.45–5.25 (m, 10 H), 3.66 (dd, *J* = 5.7, 3.9 Hz, 4 H), 3.62 (d, *J* = 4.9 Hz, 2 H), 3.45 (d, *J* = 4.9 Hz, 2 H), 2.90–2.75 (m, 8 H), 2.34–2.28 (m, 2 H), 2.18–2.02 (m, 4 H), 1.72 (p, *J* = 7.5 Hz, 2 H), 0.98 (t, *J* = 7.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5, 132.0, 129.2, 128.7, 128.5, 128.2, 128.2, 128.1, 128.0, 127.8, 126.9, 66.9, 66.6, 45.9, 41.8, 32.3, 26.7, 25.6, 25.6, 25.5, 24.9, 20.5, 14.2. IR (ATR): ν_{max} = 3009, 2964, 2931, 2863, 1656, 1432 cm^{-1}. HRMS (EI^+): exact mass calcd for $C_{24}H_{37}NO_2$ $[M]^+$ m/z = 371.2824; found: 371.2833.

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