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Synthesis of 4,5-Dianilinophthalimide and Related Analogues for Potential Treatment of Alzheimer's Disease via Palladium-Catalyzed Amination

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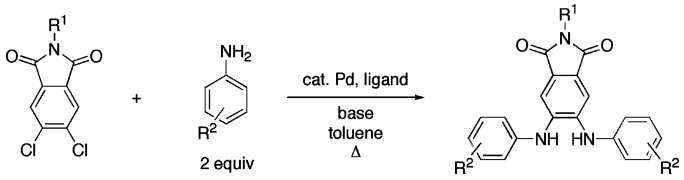

DAPH (4,5-dianilinophthalimide) has previously been shown to reverse the formation of neurotoxic fibrils associated with Alzheimer's disease. We have developed a synthetic route to DAPH and structurally related analogues that employs palladium-catalyzed amination as the key bond-forming step. The requisite substrates are easily obtained, and their coupling with substituted anilines proceeds in generally high yields. Thus, a variety of DAPH analogues can be quickly accessed in a modular fashion. In addition, the route described herein should also be amenable to the incorporation of other classes of nucleophiles into the molecular framework.

Introduction

Despite intensive research efforts over recent decades, Alzheimer's disease is still an incurable affliction that affects many elderly patients. The trademark pathological markers of Alzheimer's, observed in post-mortem analysis of the brains of affected patients, are β-amyloid plaques and neurofibrillary tangles.[1] While the exact role of these lesions in the pathogenesis of the disease (or whether they are merely symptomatic of the underlying illness) is unknown, several hypotheses have been put forth to explain their formation.

The β-amyloid plaques are primarily composed of short peptides (denoted Aβ) that are derived from a much larger transmembrane protein, amyloid precursor protein (APP). It has been found that normal APP catabolism occurs at a site within the Aβ region, and thus, the intact Aβ produced in patients with Alzheimer's disease is derived from abnormal APP processing.[2]

The neurotoxicity of Aβ peptides, which are typically comprised of 40−42 amino acids (Aβ40 and Aβ42, respectively), has been documented.[3] These short peptides aggregate into a fibrillar form with high β-sheet content, and it is these fibrils that are believed to be toxic (through their interaction with receptors on neurons). For example, it has been demonstrated that Aβ fibrils cause an influx of Ca^{2+} ions into neuronal cells.[4] This disruption of calcium homeostasis can have many deleterious effects within the cell, eventually leading to cell death. Moreover, aggregated Aβ42 has been shown to cause prolonged depolarization of neuronal cell membranes.[5] This drastically affects the excitability of the neurons and may be an underlying cause for the cognitive deterioration associated with Alzheimer's.

Among the many potential therapies aimed at curing Alzheimer's disease, biologists at MIT have hypothesized that by preventing or reversing the formation of β-sheets in the short peptides Aβ40 and Aβ42 the toxicity associated with the fibrillar forms will not manifest itself. Indeed, they have found that Aβ42 must be preincubated for at least 24 h before being able to induce Ca^{2+} ion influx into neuronal cells, an occurrence that correlates with the β-sheet content of the peptide.[6] Accordingly, this group has explored the use of physiologically stable "decoy peptides", derived from D-amino acids, that interfere with the aggregation of Aβ peptides into their

(1) Ferreira, A.; Lu, Q.; Orecchio, L.; Kosik, K. S. *Mol. Cell. Neurosci.* **1997**, *9*, 220.
(2) Sisodia, S. S.; Koo, E. H.; Beyreuther, K.; Unterbeck, A.; Price, D. L. *Science* **1990**, *248*, 492.
(3) Yankner, B. A.; Duffy, L. K.; Kirschner, D. A. *Science* **1990**, *250*, 279.
(4) Mattson, M. P.; Cheng, B.; Davis, D.; Bryant, K.; Lieberburg, I.; Rydel, R. E. *J. Neurosci.* **1992**, *12*, 376.
(5) Blanchard, B. J.; Thomas, V. L.; Ingram, V. M. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 1197.
(6) Blanchard, B. J.; Chen, A.; Rozeboom, L. M.; Stafford, K. A.; Weigele, P.; Ingram, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14326.

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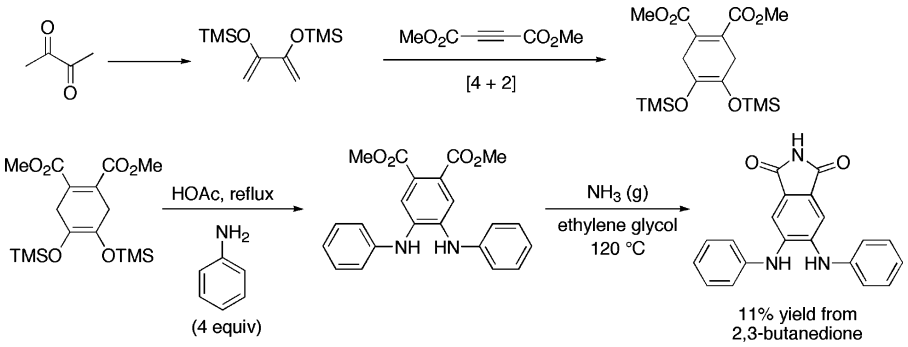
SCHEME 1. Prior Synthesis of DAPH[8b]



neurotoxic forms.[7] Several of these decoy peptides have been identified that exhibit promising activity in this regard.

More recently, the MIT biology team has expanded their focus to small molecule inhibitors of β-sheet formation as potential therapeutic agents for Alzheimer's disease. In particular, a screen of a number of compounds known to have other types of biological activity was conducted, monitoring for a decrease in the β-sheet content of preaggregated Aβ-42.[6] Of the molecules studied, the most promising lead was 4,5-dianilinophthalimide (DAPH, Figure 1). This compound demonstrated a

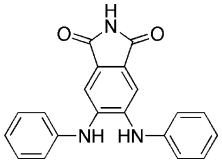
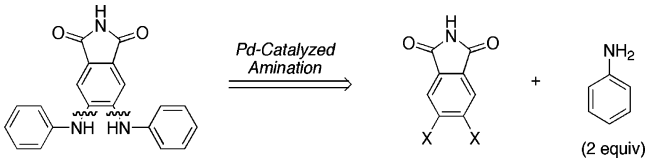


FIGURE 1. 4,5-Dianilinophthalimide (DAPH).

strong reversal of β-sheet formation (IC_{50} ~15 μm), as well as the ability to shut down the Ca^{2+} ion influx associated with Aβ fibrils (IC_{50} ~0.7 μm).

4,5-Dianilinophthalimide was first synthesized at CIBA Pharmaceuticals as a part of a study on tyrosine kinase inhibitors.[8] In this work, DAPH and its related analogues were assembled as shown in Scheme 1. Bis-silylation of 2,3-butanedione followed by [4 + 2] cycloaddition with dimethylacetylene dicarboxylate has been shown to afford the dienyl diester shown.[9] Treatment of this compound with an excess of aniline in refluxing acetic acid afforded a relatively low yield of the dimethyl dianilinophthalate, which upon treatment with gaseous ammonia in hot ethylene glycol affords the desired compound. Overall, this process is relatively low-yielding (11% from the starting dione for DAPH), and more importantly only allows for the introduction of nitrogenous nucleophiles onto the aromatic ring. Other groups (such as thiols) cannot be installed by direct reaction with the diene,[8b] and thus, a separate synthetic sequence must be used.

SCHEME 2. Retrosynthetic Disconnection of C−N Bonds in DAPH



Given the promising result obtained by the MIT biologists, it was obvious that there would be a need for lead optimization to identify a druglike small molecule for potential Alzheimer's therapy. However, the initially published synthetic sequence to this class of compounds is not amenable to significant variations in terms of introduction of other nucleophiles at the 4- and 5-positions. Moreover, the number of steps and overall low yields further detract from the applicability of this route.

An alternative route to dianilinophthalimides that would be considerably more attractive is the formation of the C−N bonds through palladium-catalyzed aryl amination, a reaction pioneered and developed in our group over the past decade.[10] Specifically, the target compounds could be constructed from a 4,5-dihalophthalimide derivative and the corresponding aniline (Scheme 2). Moreover, given the precedence for the formation of aromatic C−C, C−O, and C−S bonds using palladium catalysis, the potential exists for a diversity of related analogues that are readily accessible from convenient starting materials.

Given our experience and understanding of the palladium-catalyzed amination process, we entered into a collaborative effort with the MIT biology group headed by Professor Vernon Ingram. It is our hope that the work presented herein is only the beginning of a fruitful relationship, one which may very well have an impact on future research into potential Alzheimer's therapies.

Results and Discussion

Amination on 4,5-Dichlorophthalic Acid Dimethyl Ester. Our initial ventures into the synthesis of DAPH analogues using palladium-catalyzed amination focused on a more expedient synthesis of the dimethyl 4,5-dianilinophthalate intermediates used in the originally published route. We hypothesized that these would be

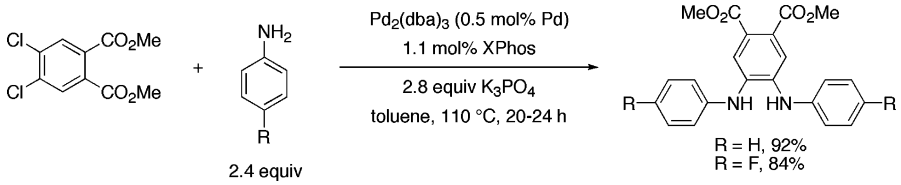
(7) Blanchard, B. J.; Konopka, G.; Russell, M.; Ingram, V. M. *Brain Res.* **1997**, *776*, 40.

(8) (a) Buchdunger, E.; Trinks, U.; Mett, H.; Regenass, U.; Müller, M.; Meyer, T.; McGlynn, E.; Pinna, L. A.; Traxler, P.; Lydon, N. B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2334. (b) Trinks, U.; Buchdunder, E.; Furet, P.; Kump, W.; Mett, H.; Meyer, T.; Müller, M.; Regenass, U.; Rihs, G.; Lydon, N.; Traxler, P. *J. Med. Chem.* **1994**, *37*, 1015.

(9) Matlin, S. A.; Barron, K. *J. Chem. Res., Synop.* **1990**, 246.

(10) Jiang, L.; Buchwald, S. L. Palladium-Catalyzed Aromatic Carbon−Nitrogen Bond Formation. In *Metal-Catalyzed Cross Coupling Reactions*, 2nd ed.; de Meijere, A., Diedrich, F., Eds.; Wiley-VCH: Weinheim, 2004; pp 699−760.

SCHEME 3. Amination Reactions on 4,5-Dichlorophthalic Acid Dimethyl Ester



easily accessible from commercially available 4,5-dichlorophthalic acid and that the subsequent closure of the phthalimide ring could be performed a number of ways.

The palladium-catalyzed amination has been conducted under a wide variety of reaction conditions, with seemingly countless permutations of palladium precatalyst, phosphine ligand, stoichiometric base, and solvent.[10] However, based on the considerable research efforts expended by our group on both synthetic[11] and mechanistic[12] aspects of the amination, we chose to start our studies using 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos, Figure 2) as the ligand for palladium. This phosphine has proven to be superior for amination in most synthetic applications. We also chose $Pd_2(dba)_3$, a convenient Pd^0 source, as the precatalyst since the substrate combinations we planned to employ were not amenable to in situ reduction of a Pd(II) precatalyst. Last, we decided to avoid the use of sodium *tert*-butoxide as the stoichiometric base due to the potential for transesterification, which would hamper subsequent formation of the phthalimide ring.

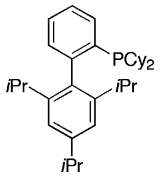
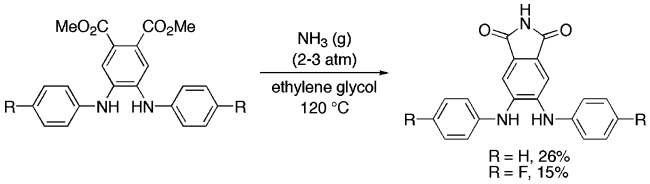


FIGURE 2. XPhos (2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl).

Gratifyingly, by heating a toluene solution of 4,5-dichlorophthalic acid dimethyl ester (obtained in high yield by treatment of the diacid with thionyl chloride in methanol) and aniline in the presence of potassium phosphate and catalytic quantities of $Pd_2(dba)_3$ and XPhos, the desired dimethyl 4,5-dianilinophthalate can be obtained in excellent yield (Scheme 3). Due to the low solubility of the product in nonpolar organic media as well as water, isolation is simplified by pouring the entire reaction into a mixture of diethyl ether and water (to dissolve any unreacted starting reagents as well as any inorganic material) and filtration of the insoluble product. The *p*-fluoroanilino analogue can be made in an analogous fashion (Scheme 3).

Given the ease with which dimethyl 4,5-dianilinophthalates could be assembled, we were disappointed to find that the closure of the phthalimide ring was a nontrivial problem despite the variety of protocols examined. For example, heating a solution of the diester with urea and sodium methoxide, a procedure commonly used for this transformation in cases of more simple phthalates,[13] was not successful when applied to these compounds (nor was it when conducted under acid-catalyzed conditions). Alternatively, no desired phthalimides were observed when heating the diesters in the presence of excess hexamethyldisilazane (commonly used as an ammonia equivalent)[14] and Lewis acids. Difficulties in the formation of this ring were also discussed in the initially published synthesis (Scheme 1).[8b] When applying a modification of the published ammonolysis protocol, we were pleased to observe formation of the desired phthalimides albeit in disappointingly low yields (Scheme 4). While the published procedure typically affords yields of 40−60% for this step, it requires larger quantities of gaseous ammonia (a steady stream for a duration of 16 h) that were not immediately available in our laboratory at the time of this work. Instead, we conducted the reactions in sealed reaction vessels pressurized with ammonia gas under otherwise identical conditions.

SCHEME 4. Synthesis of Phthalimide Ring via Ammonlysis



Due to the difficulties encountered in formation of the phthalimide ring (both in our hands and in the previously published route), we sought to develop an alternative approach to the desired DAPH analogues by conducting the palladium-catalyzed amination reactions on substrates that already contained the phthalimide ring.

Amination on 4,5-Dichlorophthalimide Derivatives. The most attractive and intuitively straightforward protocol for the synthesis of DAPH analogues would be to conduct the palladium-catalyzed amination directly on commercially available 4,5-dichlorophthalimide. Such a route would obviate the need to use protecting groups for the acidic phthalimide nitrogen and would afford the desired compounds directly. However, while a variety of reaction conditions were explored for this coupling, we were unable to effect the amination on the unprotected substrate.

We were pleased to observe that the amination reaction does proceed on 4,5-dichlorophthalimides in which the nitrogen is protected. For example, amination on the *p*-methoxyphenyl (PMP)-protected substrate proceeds

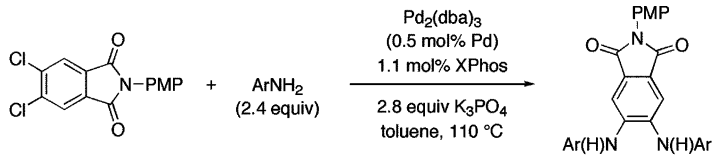
(11) For example, see: (a) Huang, X.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 6653. (b) Harris, M. C.; Huang, K.; Buchwald, S. L. *Org. Lett.* **2002**, *4*, 2885. (c) Huang, X.; Buchwald, S. L. *Org. Lett.* **2001**, *3*, 3417.

(12) (a) Strieter, E. R.; Blackmond, D. G.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 13978. (b) Singh, U. K.; Strieter, E. R.; Blackmond, D. G.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 14014.

(13) For example, see: Rosowsky, A.; Vaidya, C. M.; Bader, H.; Wright, J. E.; Teicher, B. A. *J. Med. Chem.* **1997**, *40*, 286.

(14) For example, see: Pellegata, R.; Italia, A.; Villa, M.; Palmisano, G.; Lesma, G. *Synthesis* **1985**, 517.

TABLE 1. Synthesis of *p*-Methoxyphenyl-Protected DAPH Analogues



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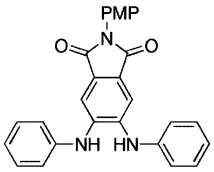
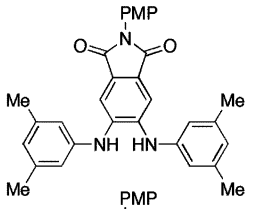
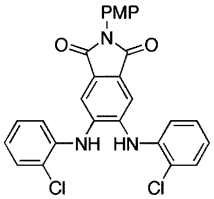
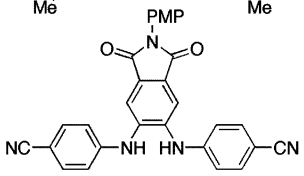
1		97%[a]	3		68%[b]
2		55%[b]	4		74%[c]

[a] Product isolated by silica gel chromatography. [b] Product isolated by recrystallization of crude material. [c] Product isolated by direct crystallization from reaction mixture.

cleanly under the aforementioned conditions to give the corresponding DAPH analogues (Table 1).

Unfortunately, we were unable to find a way to effectively remove the *p*-methoxyphenyl group from these protected phthalimides under standard conditions. In hindsight, however, a PMP protecting group is far from ideal for this class of products. Since removal of this moiety is typically performed under oxidative conditions (for example, using ceric ammonium nitrate),[15] one can imagine a host of unwanted side reactions occurring at the diarylamino motif instead, especially in cases where the DAPH analogues are derived from electron-rich anilines.

Given the difficulty in liberating the desired compounds from the PMP-protected analogues, we set about surveying other types of commonly used nitrogen protecting groups. Unfortunately, there is little precedent for the protection of the phthalimide nitrogen; usually the phthalimide ring *itself* is the protecting group that is cleaved![15] We found that the amination reaction proceeds cleanly on *N*-benzyl-4,5-dichlorophthalimide, although again we encountered difficulties in removing the protecting group. Hydrogenolysis (Pd/C) was ineffective, and we worried that more forcing reductive conditions would also result in the reduction of one of the carbonyl groups of the phthalimide ring (which are known to be reduced under relatively mild conditions).[16] We also ruled out the possibility of using carbonyl-based protecting groups, as phthalimide ring-opening (with the aniline used in the amination) should be facile at the temperatures employed.[17]

The logical solution to the problem seemed to reside in the use of silicon-based protecting groups. While silicon reagents are more commonly used to protect hydroxyl groups in organic synthesis, bulkier groups have found applications in protection of the amide nitrogen.[15] Moreover, the weak N–Si bond was expected to translate into easy removal of the protecting group. Indeed, we were encouraged by the report of a stable, crystalline *N*-*tert*-butyldimethylsilyl (TBS)-protected phthalimide, from which the parent N–H compound is easily liberated by treatment with a fluoride source.[18] The analogous *N*-TBS-protected 4,5-dichlorophthalimide is easily synthesized, but unfortunately, the amination reaction fails (presumably due to desilylation of the substrate). We were able to synthesize the analogous *N*-triisopropylsilyl (TIPS)-protected 4,5-dichlorophthalimide, and this substrate undergoes the amination reaction without any noticeable decomposition. Thus, employing reaction conditions similar to those described above (with the exception that Cs_2CO_3 was used as the stoichiometric base), the TIPS-protected DAPH analogues can be accessed in short order (Table 2).

It should be pointed out that the amination reaction employing 4-nitroaniline as the nucleophile requires the use of a weaker base (K_2CO_3); employing Cs_2CO_3 or K_3PO_4 resulted in ineffective coupling. Also noteworthy about this particular example is that the product is insoluble in toluene and crystallizes as it is formed. As a result, the crystallization trapped some toluene that could not be removed under high vacuum, resulting in an observed yield higher than the theoretical maximum. As expected, the silicon protecting group is easily removed from the desired DAPH analogues, either by heating with aqueous acetic acid or by treatment with methanolic KF (Scheme 5).

Thus, we have developed an effective route to DAPH analogues employing palladium-catalyzed amination to form the 4,5-carbon–nitrogen bonds. A number of previ-

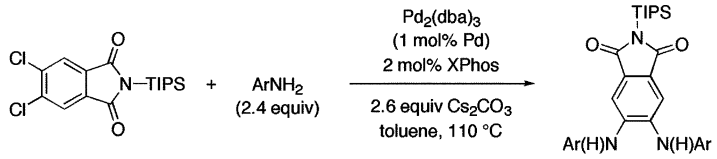
(15) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991; pp 358–9, 399, 400.

(16) Brewster, J. H.; Fusco, A. M.; Carosino, L. E.; Corman, B. G. *J. Org. Chem.* **1963**, *28*, 498 and references therein.

(17) (a) Nefkens, G. H. L.; Tesser, G. I. *J. Am. Chem. Soc.* **1961**, *83*, 1263. (b) Einhorn, C.; Einhorn, J.; Marcadal-Abbadi, C. *Synth. Commun.* **2001**, *31*, 741.

(18) Chun, Y.-J.; Park, J.-H.; Oh, G.-M.; Hong, S.-I.; Kim, Y.-J. *Synthesis* **1994**, 909.

TABLE 2. Synthesis of TIPS-Protected DAPH Analogues



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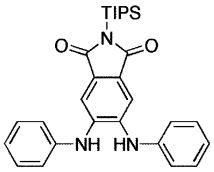
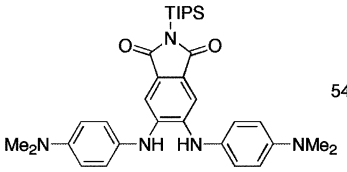
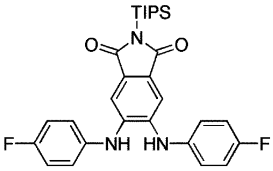
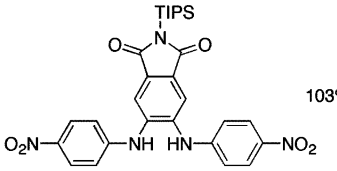
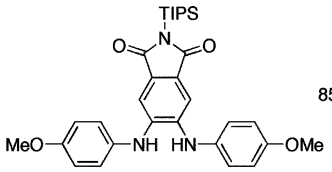
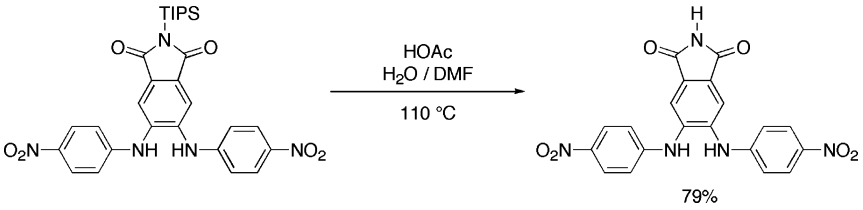
1		92%	4		54%
2		80%	5		103%[a,b]
3		85%			

[a] 2.6 equiv of K_2CO_3 used as base. [b] Product crystallizes directly from reaction mixutre; reported yield includes solvent that could not be removed in vacuo.

SCHEME 5. Deprotection of TIPS-Protected DAPH Analogues



ously known as well as novel compounds have been accessed through this methodology, and biological screening of the ability of these compounds to reverse β-sheet formation of aggregated Aβ42 is currently underway.

Conclusions

We have developed a synthetic route to 4,5-dianilinophthalimide (DAPH) and structurally related analogues that employs palladium-catalyzed amination as the key bond-forming step. The requisite dihalogenated substrates are easily obtained, often in a single step, from commercially available materials. The coupling of these precursors with a host of substituted anilines proceeds in generally high yields. In the case of *N*-TIPS-protected compounds, subsequent removal of the silicon protecting group has proven to be facile. Thus, a variety of DAPH analogues can be quickly accessed in a modular fashion.

In addition to the related analogues synthesized thus far, the route described herein should be amenable to the incorporation of other classes of nucleophiles at the DAPH 4- and 5-positions, given the wealth of palladium-catalyzed cross-coupling reactions developed in the recent decades. For example, one can imagine the installment of carbon-, oxygen-, and sulfur-based motifs into the DAPH structure using these reactions.

Biological assays using the DAPH analogues synthesized are currently underway, and preliminary data indicate possible directions for further lead optimization. While a small molecule-based therapy for Alzheimer's disease based on the reversal of β-sheet formation is likely years away, the promising results obtained thus far validate the concept of such a treatment and will serve as a foundation for future work in the area.

Acknowledgment. We thank Professor Vernon Ingram (Department of Biology, MIT) and co-workers for their extensive contributions to this collaboration. Financial support for this work was provided by the NIH (GM58160). We also acknowledge Merck for unrestricted funding.

Supporting Information Available: Experimental procedures as well as characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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