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Directed Library of Anilinogeranyl Analogues of Farnesyl Diphosphate via Mixed Solid- and Solution-Phase Synthesis

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ABSTRACT

A directed library of anilinogeranyl diphosphate analogues of the isoprenoid farnesyl diphosphate has been prepared by solid-phase organic synthesis using a traceless linker strategy in moderate yield in three steps: reductive amination, bromination, and treatment with ((*n*-Bu)$_4$N)$_3$HP$_2$O$_7$.

Members of the Ras family of signal transduction proteins require posttranslational prenylation for their proper membrane localization and activity.[1−5] Protein farnesyltransferase (FTase) catalyzes the transfer of a farnesyl group from farnesyl diphosphate (FPP, **1**, Figure 1) to the cysteine residue in a carboxy terminal CAAX box of Ras.[2,6] Prenylation is obligatory for the oncogenic effects of mutant Ras, and these observations have led to the development of a number of FTase inhibitors (FTIs) currently in phase I, II, and III clinical trials.[7−9] Altering the downstream biological function of the prenylated protein by modifying CAAX box-containing proteins with unnatural analogues of FPP is an attractive antineoplastic strategy. Synthetic modifications to the farnesyl moiety of FPP have generated both FTIs and alternative substrates transferable by FTase to target peptides.[10−16] Definition of the structural features responsible

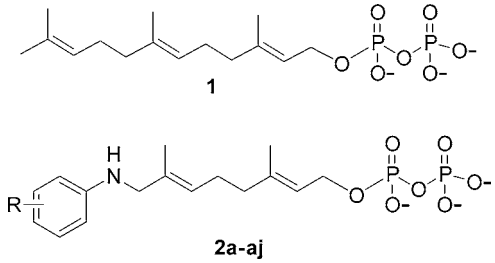

Figure 1. FPP and anilinogeranyl diphosphates.

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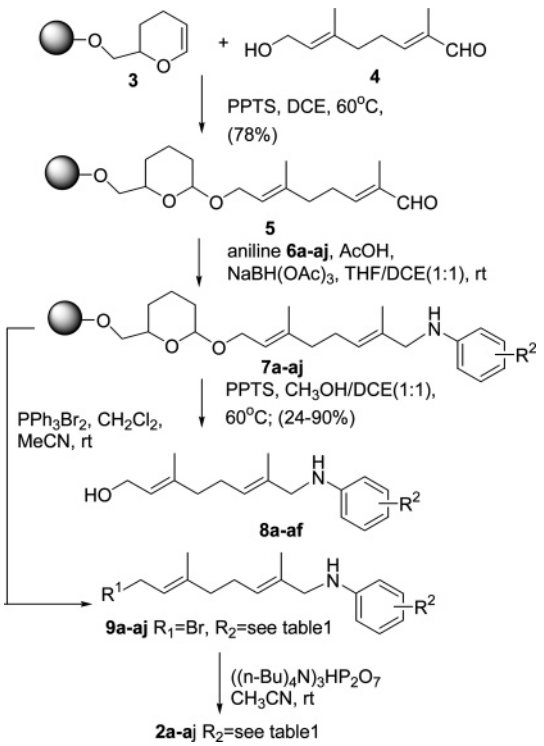
for efficient transfer of unnatural analogues to Ras requires the synthesis of additional, structurally diverse FPP analogues.

Parallel synthesis of directed libraries is an effective strategy for rapidly introducing focused diversity into a target template.[17−20] Directed libraries are particularly useful for generating series of closely related molecules to explore structure−activity relationships.[21,22] We have previously prepared a limited set of alternative substrates for FTase by replacing the terminal isoprene of FPP with substituted anilines in an effort to define some of the structural features responsible for efficient transfer of unnatural analogues to Ras. These 8-anilinogeranyl diphosphates (Figure 1) were prepared by solution-phase synthesis where the key step was reductive amination of an α,β-unsaturated aldehyde to form the anilinogeranyl skeleton. This approach was appealing because it was carried out under mild conditions. However, all intermediates and products required purification by chromatography.

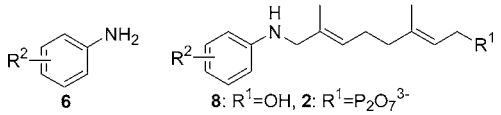
We now report a mixed solid-phase organic synthesis (SPOS)−solution-phase route to a directed library of 8-anilinogeranyl alcohols **8a**−**af** and diphosphates **2a**−**aj** (Scheme 1, Table 1).

Scheme 1. Synthesis of Anilinogeranyl Alcohols and Diphosphates



We have adapted our solution methods to provide an SPOS route to anilinogeranyl FPP analogues (Scheme 1). The advantage of an SPOS approach is that it allows for the rapid preparation of additional analogues and reduces the number of tedious purification steps needed. The THP resin **3** was prepared from the Merrifield-Cl resin as previously described.[23] Attachment of synthetic intermediates to the resin

Table 1. Preparation of 8-Anilinogeraniols and Diphosphates



entry	aniline	R^2 group on aniline	yield of **8** (%)[a]	yield of **2** (%)[b]
1	**6a**	H	**8a** (68)	**2a** (53)
2	**6b**	2-Me	**8b** (64)	**2b** (30)
3	**6c**	3-Me	**8c** (37)	**2c** (36)
4	**6d**	4-Me	**8d** (44)	**2d** (38)
5	**6e**	2-Cl	**8e**(45)	**2e** (23)
6	**6f**	3-Cl	**8f** (65)	**2f** (31)
7	**6g**	4-Cl	**8g** (81)	**2g** (44)
8	**6h**	2-Pr^i	**8h** (59)	**2h** (23)
9	**6i**	4-Pr^i	**8i** (62)	**2i** (38)
10	**6j**	2-OPh	**8j** (51)	**2j** (24)
11	**6k**	3-OPh	**8k** (46)	**2k** (28)
12	**6l**	4-OPh	**8l** (43)	**2l** (32)
13	**6m**	2-OMe	**8m** (45)	**2m** (23)
14	**6n**	3-OMe	**8n** (63)	**2n** (34)
15	**6o**	4-OMe	**8o** (43)	**2o** (31)
16	**6p**	2-Ph	**8p** (60)	**2p** (21)
17	**6q**	4-Ph	**8q** (53)	**2q** (29)
18	**6r**	4-CO_2Me	**8r** (72)	**2r** (18)
19	**6s**	4-NO_2	**8s** (90)	**2s** (67)
20	**6t**	2-CH_2Ph	**8t** (46)	**2t** (24)
21	**6u**	3-CH_2Ph	**8u** (52)	**2u** (29)
22	**6v**	4-CH_2Ph	**8v** (52)	**2v** (30)
23	**6w**	2-F	**8w** (60)	**2w** (27)
24	**6x**	3-F	**8x** (58)	**2x** (25)
25	**6y**	4-F	**8y** (51)	**2y** (33)
26	**6z**	2-CF_3	**8z** (24)	**2z** (17)
27	**6aa**	3-CF_3	**8aa** (65)	**2aa** (22)
28	**6ab**	2-Bu^t	**8ab** (24)	**2ab** (22)
29	**6ac**	4-Bu^t	**8ac** (35)	**2ac** (27)
30	**6ad**	2-OCF_3	**8ad** (39)	**2ad** (19)
31	**6ae**	3-OCF_3	**8ae** (41)	**2ae** (32)
32	**6af**	4-OCF_3	**8af** (36)	**2af** (30)
33	**6ag**	2-Br	c	**2ag** (18)
34	**6ah**	3-Br	c	**2ah** (17)
35	**6ai**	4-Br	c	**2ai** (21)
36	**6aj**	3,5-dimethyl	c	**2aj** (24)

[a] Yield of pure compound after isolation by silica gel column chromatography. [b] Purified by HPLC monitored at 254 nm. [c] Corresponding alcohols were not made.

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through the THP group provides a traceless linker for the alcohols **8a**−**af** and the final compounds **2a**−**aj**. Preparation of resin **5** was achieved by combining a 5-fold excess of 8-oxo-geraniaol **4** with support **3** in the presence of 0.2 equiv of PPTS at 60 °C. Under these conditions, excess alcohol **4** can be recovered for reuse. The optimized reaction conditions gave a resin loading of 72% for the solid support-linked α,β-unsaturated aldehyde **5**. Aldehyde loading was determined by cleavage and recovery of alcohol **4** by treatment of resin **5** with PPTS/MeOH/DCE at 60 °C. Attempts to generate resin-bound aldehyde **4** connected via a silyl linker to the solid support were unsuccessful.

With resin **5** in hand, we employed a solid-phase parallel synthetic strategy to prepare a directed library of the anilinogeranyl farnesol analogues **8a**−**af**. Diversity was introduced into the library by selecting the commercially available substituted anilines **6a**−**aj** listed in Table 1. Optimum yields of the desired resin-bound anilinogeraniols **7a**−**aj** were obtained by performing the reductive amination with 10 equiv of the anilines and 12 equiv of acetic acid in 1:1 THF/DCE and subsequent reduction with 10 equiv of $NaBH(OAc)_3$. Increasing the number of equivalents of acetic acid or substituting PPTS or other Lewis acids both reduced the yield of the desired anilinogeraniols and led to additional uncharacterized byproducts. Moreover, reducing the number of equivalents of anilines for imine formation or decreasing the fold excess of $NaBH(OAc)_3$ employed for the subsequent reduction resulted in longer reaction times that gave reduced yields. Cleavage of the anilinogeraniols **8a**−**af** from the resin-bound amines **7a**−**af** was achieved by treatment of the solid support with DCE/MeOH/PPTS at 60 °C. Methanol, ethanol, and *n*-butanol were all found to be equally effective in promoting the cleavage reaction. The final yields of the anilinogeraniols **8a**−**af** after silica gel column chromatography are reported in Table 1. Anilinogeraniol **8a** and *p*-nitroanilinogeraniol **8s** have previously been prepared by solution methods in 85 and 64% yield, respectively. This is in contrast to results from the SPOS where the presence of an electron-withdrawing group in the para position leads to higher yield from the reductive amination (Table 1, entries 7, **18** and **19**). The optimum yield of anilinogeraniols prepared by SPOS reductive amination requires both elevated temperatures and increased reaction times relative to solution methods.

Integration of the ^1H NMR spectra of amines **8a**−**af** indicated that a mixture of cis/trans isomers about the 6,7 double bond were formed in an approximately 1:9 ratio. This result was expected since similar cis/trans mixtures were observed for unsubstituted and para-substituted anilinogeranyl acetates prepared by solution methods. Interestingly, bulky ortho substituents on anilines **6h**, **6j**, **6p**, **6t**, and **6ab** did not lead to any alteration of the cis/trans ratio for the corresponding anilinogeraniols **8h**, **8j**, **8p**, **8t**, and **8ab**. As is typical of the previously prepared anilinogeraniols, we were unsuccessful in separating the cis/trans isomers of **8a**−**af** by HPLC methods.

In solution, FPP analogues are typically prepared by sequential conversion of the allylic alcohols into corresponding chlorides followed by diphosphorylation to provide diphosphates.[13,24,25] Utilizing this approach reduces some of the advantages of SPOS, as it requires two solution-phase manipulations after release of the anilinogeraniols **8a**−**af** from the resin to produce the desired diphosphates. In previous solution-phase work, we employed an excess of Ph_3PCl_2 to convert alcohol **8a** to its corresponding chloride, which was immediately transformed to the diphosphate **2a**.[13]

Direct cleavage of the resin-linked THP ethers to the corresponding allylic chlorides or bromides would reduce the number of solution steps to one, diphosphorylation. We observed that addition of Ph_3PCl_2 to the resin-bound ethers and subsequent diphosphorylation resulted in poor yield of diphosphates. Alternatively, stirring the THP resin **7a**−**aj** with 3 equiv of Ph_3PBr_2 in CH_2Cl_2 for 4 h followed by addition of 10 equiv of tris(tetra-*n*-butylammonium)hydrogendiphosphate in CH_3CN gave the desired diphosphates **2a**−**aj** in moderate yield. Employing Ph_3PBr_2 not only reduced the reaction time but also increased the yield of the desired diphosphates. We observed that the yield of the diphosphates falls precipitously if the bromination is allowed to continue for longer times. We attribute these observations to the formation of undesired side products due to the enhanced reactivity of the Ph_3PBr_2. The release of bromides **9a**−**aj** from the THP resin by Ph_3PBr_2 provides a traceless linker path to the FPP analogues **2a**−**aj**. In practice, bromides **9a**−**aj** were not isolated but were converted directly to the anilinogeranyl diphosphates **2a**−**aj** by addition of tris(tetra-*n*-butylammonium) hydrogen diphosphate[25] in dry CH_3CN in the same reaction vessel.

The resulting diphosphates **2a**−**aj** were first converted to the NH_4^+ form by ion exchange chromatography and then purified by reverse-phase HPLC. Complete removal of the tetra-*n*-butylammonium counterions from the sample prior to reverse-phase HPLC was required for effective purification. However, efficient recovery of the NH_4^+ form of the diphosphates **2a**−**aj** from the ion exchange chromatography was highly dependent on the buffer conditions employed. This observation is unsurprising, as the various diphosphates **2a**−**aj** are expected to have a wide range of solubilites in aqueous buffer related to the structure of the parent anilines **6a**−**aj**. Optimization of the ion exchange conditions by altering the concentration of NH_4HCO_3 and including compound-dependent proportions of MeCN cosolvent had a profound influence on the yield of individual diphosphates **2a**−**aj** recovered. However, the yields of diphosphates **2a**−**aj** reported in Table 1 are for a single, uniform, ion exchange condition utilized to maximize throughput. The mixed SPOS−solution-phase route outlined here provides sufficient material for biochemical screening and is shorter and more convenient than what we have previously reported.

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In conclusion, we have prepared an exploratory library of anilinogeranyl alcohols and diphosphates by introducing diversity into the aromatic moiety, which replaces the terminal isoprene of FPP. We employed reductive amination as the key step in the solid-phase synthesis of the library in good yields. The penultimate allylic bromides were released from the THP ether-linked resin by treatment with dibromotriphenylphosphorane followed by solution-phase conversion to the diphosphates. Results from the ongoing biological evaluation of this directed library will be reported elsewhere.

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Supporting Information Available: Detailed experimental procedure and spectroscopic data of **8a**−**af** and **2a**−**aj**; ^{1}H spectra and HRMS data sheet of **8a**−**af**; and ^{1}H, ^{31}P, and LRMS spectra of **2a**−**aj**. This material is available free of charge via the Internet at http://pubs.acs.org.

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