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Solid-phase synthesis of novel inhibitors of Farnesyl Transferase

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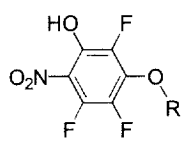
Abstract:
A novel diphosphate mimic, the 2,3,6-trifluoro-5-hydroxy-4-nitrophenoxy group (**1**), has been employed as the template in the solid-phase synthesis of novel Farnesyl Transferase inhibitors using the Mitsunobu reaction. The most potent inhibitor (farnesyloxy-5-hydroxy-2,3,6-trifluoro-4-nitrobenzene) displayed an IC_{50} of 6.3 μM versus Farnesyl Transferase.

Farnesyl Transferase (FTase) catalyses the transfer of the farnesyl moiety from farnesyl diphosphate (FPP) to the Ras protein.[1] This post-translational modification enables anchoring of the protein to the cell membrane which is necessary for cell transformation.[2] Mutant Ras is found in many human colon (50%) and pancreatic (90%) cancers,[3] thus inhibition of FTase is an attractive therapeutic target for new anticancer agents and has been the subject of vigorous research activity.[4,5] Recently, potent diphosphate-competitive inhibitors of Ftase have been reported.[6]

Previous work carried out in our laboratories showed the ability of the 2,3,6-trifluoro-5-hydroxy-4-nitrophenoxy group to mimic the diphosphate (dpp) residue.[7] In this paper, we describe the preparation of the dpp mimic **1** and its use as a template in a solid-phase synthesis of potential FPP-related inhibitors of FTase.



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Chemistry

The synthesis of the protected dpp mimic **4** is illustrated in **Scheme 1**. Pentafluoronitrobenzene reacts with nitrite ion in DMSO[8] to give the phenol **2** in 80% yield. The phenol was then protected as the allyl ether **3** (100% yield) followed by nucleophilic

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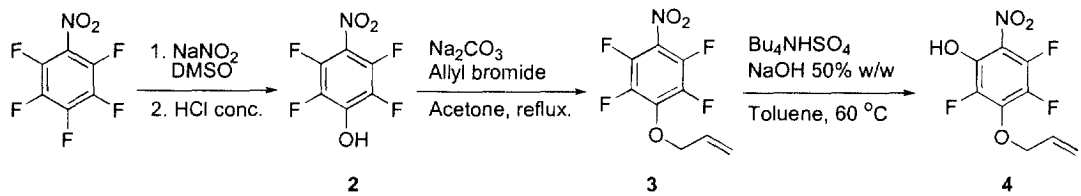
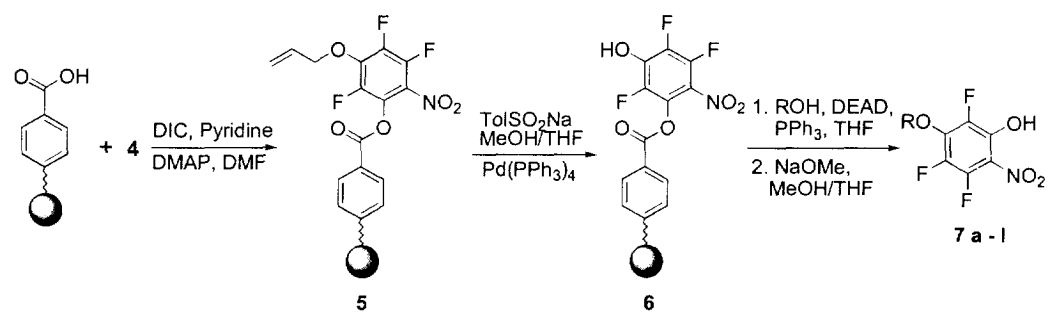
aromatic substitution (SNAr) of the fluorine with NaOH (50% w/w) under phase transfer catalysis conditions[9] to afford the phenol **4** in 82% yield.

Scheme 1



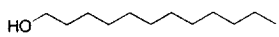
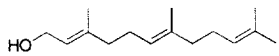
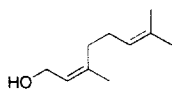
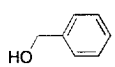
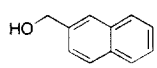
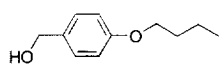
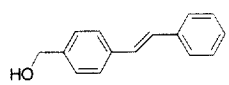
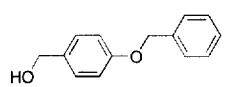
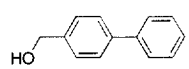
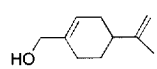
The solid-phase strategy is shown is **Scheme 2**. Template **4** was attached to carboxypolystyrene resin (Novabiochem; 0.98 mmol/g) using standard solid-phase coupling protocols (DIC/py/DMAP in DMF; calc. loading 0.66 mmol/g). The deprotection of the polymer bound allyl ether **5** was accomplished with sodium toluenesulfinate and palladium catalyst[10] to afford phenol **6**. The loaded resin was stored at –20 °C as it proved to be unstable during prolonged storage at room temperature.

Scheme 2



The Mitsunobu etherification of **6** was attempted under a variety of conditions; these included several Mitsunobu type reagents, i.e. TMAD/Bu_3P[11], di-tert-butyl-azocarboxylate/PPh_3, DIAD/PPh_3,[12] and reaction conditions. The best results were obtained when a 10 fold excess of DEAD and triphenylphosphine were employed along with a 20 fold excess of the alcohol in THF.[13] A range of alcohols was used and the results are summarised in **Table 1**. In general, benzylic alcohols performed better than aliphatic alcohols (which presented 'alkylated DEAD' side-products despite the reaction being carried out at low temperatures).[12] The final product was cleaved by exposure to THF/sat. NaOMe in MeOH (8:1) for 20 minutes,[14] acidified with 1M HCl and extracted with ethyl acetate.

Table 1. Ethers Prepared by Polymer-Supported Mitsunobu Reaction.

ROH	COMPD.	ISOLATED YIELD[a] %	PURITY[b]%	HPLC-ESMS DATA[c] Calcd/Found [M-H]$^-$
	**7a**	72	99	236/236
	**7b**	—	12	264/264
	**7c**	73	82	376/376
	**7d**	62	14	412/412
	**7e**[d]	85	40	344/344
	**7f**	75	85	298/298
	**7g**	94	94	348/348
	**7h**[d]	50	70	370/370
	**7i**	64	65	400/400
	**7j**[d]	76	72	404/404
	**7k**	82	90	374/374
	**7l**	82	83	342/342

Notes: a) From calcd. loading; b) Analysis by HPLC (C18 reverse phenomenex. Analytical column 250x4.60 mm; Gradient elution 60/40 to 0/100 H_2O/MeCN containing 0.1% TFA, 1.0 ml/min for 35 min) by area integration at 254 nm; c) Obtained using Finnigan TSQ 700 triple sector quadruple mass spectrometer; d) Unstable.

In vitro biological evaluation

Stable compounds with a purity of greater than 75% [15] were screened for biological activity against FTase and geranylgeranyl protein transferase-I (GGTase I), a closely related isoprenyl protein transferase enzyme that may be a useful target in its own right.[16] The results of *in vitro* biochemical assays are shown in **Table 2**. Rat brain cytosol was used as the source of the enzyme. *In vitro* IC_{50}s were determined as previously described.[17]

Table 2. IC_{50} values of compounds **4** and **7**.

COMPOUND	IC_{50} VALUES (μM)	
	FTase	GGTase I
4	>200	>200
7c	>100	>100
7d	6.3 ± 1.1	12.5 ± 2.0
7f	>200	58.0 ± 17
7g	200 ± 27	>200
7k	200 ± 15	>200
7l	120 ± 23	85 ± 14

The farnesyl ether **7d,** displays good inhibitory activity against both FTase and GGTase I with IC_{50}s of 6.3 and 12.5 μM respectively, this demonstrates the ability of the nitroperfluorophenol **1** to mimic the dpp group. In order to improve both inhibitory potency, selectivity and chemical stability, we are currently working on a combinatorial library synthesis of these novel FTase inhibitors .

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12. Krchnák, V.; Flegelová, Z.; Weichsel, A. S.; Lebl, M. *Tetrahedron Letters.* **1995**, *36*, 6193-6196.
13. The resin (50 μmol) was washed with dry THF (3 x 2 ml). A solution of PPh_3 (10 eq.) and the alcohol (20 eq.) in THF (1 ml) was added under an Ar and the slurry was cooled to -15°C. Then, DEAD (10 eq.) in THF (500 μl) was added dropwise over 30 minutes. The mixture was kept at -15°C for 1 hour, then shaken at r.t. for 24 hours.
14. Longer cleavage periods resulted with S_NAr of the methoxy ion into the perfluoroaryl group.
15. The IC_{50} value for **7d** was obtained using an analytically pure sample.
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