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# Structure–Activity Relationship Studies of Flavopiridol Analogues

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**Abstract**—Cyclin dependent kinases (CDKs) along with the complementary cyclins form key regulatory checkpoint controls on the cell cycle. Flavopiridol is a synthetic flavone that shows potent and selective cyclin-dependent kinase inhibitory activity. In this paper, we report modifications of the 3-hydroxy-1-methylpiperidinyl (D ring) of flavopiridol and their effect on CDK inhibitory activity. 

The family of cyclin-dependent kinases (CDKs) are important regulators that control the timing and coordination of the progression of the cell cycle.[1] CDKs form reversible complexes with their obligate cyclin partners to control transition through key junctures in the cell cycle. For example the activated CDK4-cyclin D1 complex controls progression through the G1 phase while the CDK1-cyclin B1 complex controls entry into the mitotic phase of the cell cycle.[2] Endogenous cyclin dependent kinase inhibitory proteins (CDKIs) are known which bind either the CDK or cyclin component and inhibit the kinase activity.[3] In many tumors such as melanomas, pancreatic and esophaegal cancers these natural CDKIs are either absent or mutated.[4] Thus selective CDK inhibitors may prove to be effective chemotherapeutic agents.

Flavopiridol **(1)** (NSC 649890, L86-8275) [*cis*-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-1-benzopyran-4-one] is a synthetic flavone that has been shown to have antitumor activity against various tumor cell lines such as human lung carcinoma, breast carcinoma, and also inhibits tumor growth in xenograft models.[5] It has been shown to induce arrest in both the G1 and G2 phases of the cell cycle.[6] Flavopiridol, which is currently in clinical trials as an anticancer therapeutic,[7] is a potent and selective inhibitor of the CDKs (Fig. 1), and its antitumor activity is related to its CDK inhibitory activity.[8] Studies have shown that its tumor cell growth inhibitory activity occurs in a cell cycle specific manner.[6a] In addition, kinetic studies have shown that flavopiridol binds at the ATP binding site of the CDKs.[6b] The recently reported X-ray crystal structure of des-chloro flavopiridol, **1a**, bound to CDK2 also confirms that the ATP binding pocket of the enzyme is occupied by the flavone nucleus.[9] The total synthesis of flavopiridol[10] and some SAR around flavopiridol have been reported.[8] Two naturally occurring polyhydroxylated flavonoids quercetin and genistein (Fig. 1) are potent tyrosine kinase inhibitors but have poor CDK inhibitory activity.[6b,11] Structurally both quercitin and genistein lack the 3-hydroxy-1-methylpiperidinyl ring (D-ring) present in flavopiridol. Therefore, we explored the SAR around the D-ring of flavopiridol to determine the key structural requirements for CDK inhibitory activity.

Flavopiridol **1** and its *trans* isomer **2** were synthesized by methods described previously.[10] The D ring analogues *cis* and *trans* 8-[3-(4-hydroxy-1-methyl)-piperidinyl], **4a** and **4b**, and *cis*-8-[2-hydroxycyclohexyl], **5**, were synthesized by routes analogous to the synthesis of flavopiridol (Scheme 1). Condensation of 1,3,5- trimethoxybenzene with 1-methyl-3-piperidinone in acetic acid saturated with hydrogen chloride provided olefin **20**. Hydroboration of olefin **20** via the in situ generation of borane ($BF_3$-$OEt_2$, $NaBH_4$) followed by an oxidative work up afforded the *trans*-alcohol **21**. Inversion of the alcohol stereochemistry was accomplished in two steps: Swern oxidation to the corresponding ketone followed by

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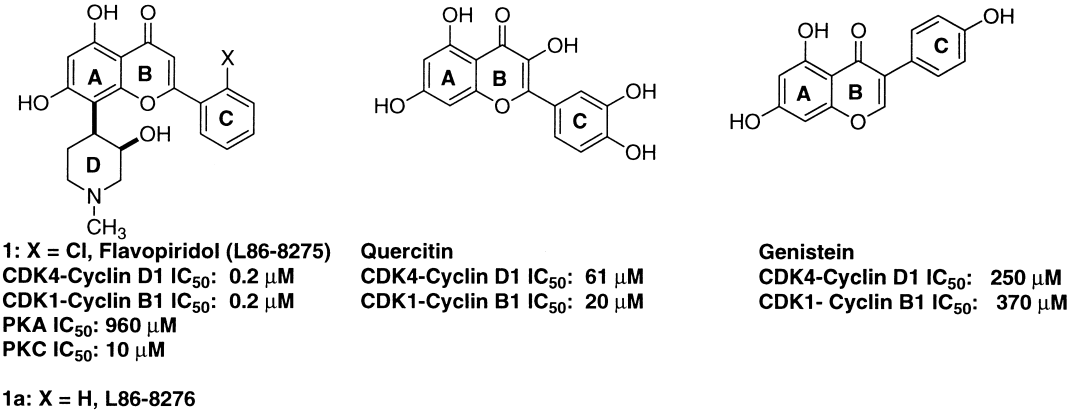
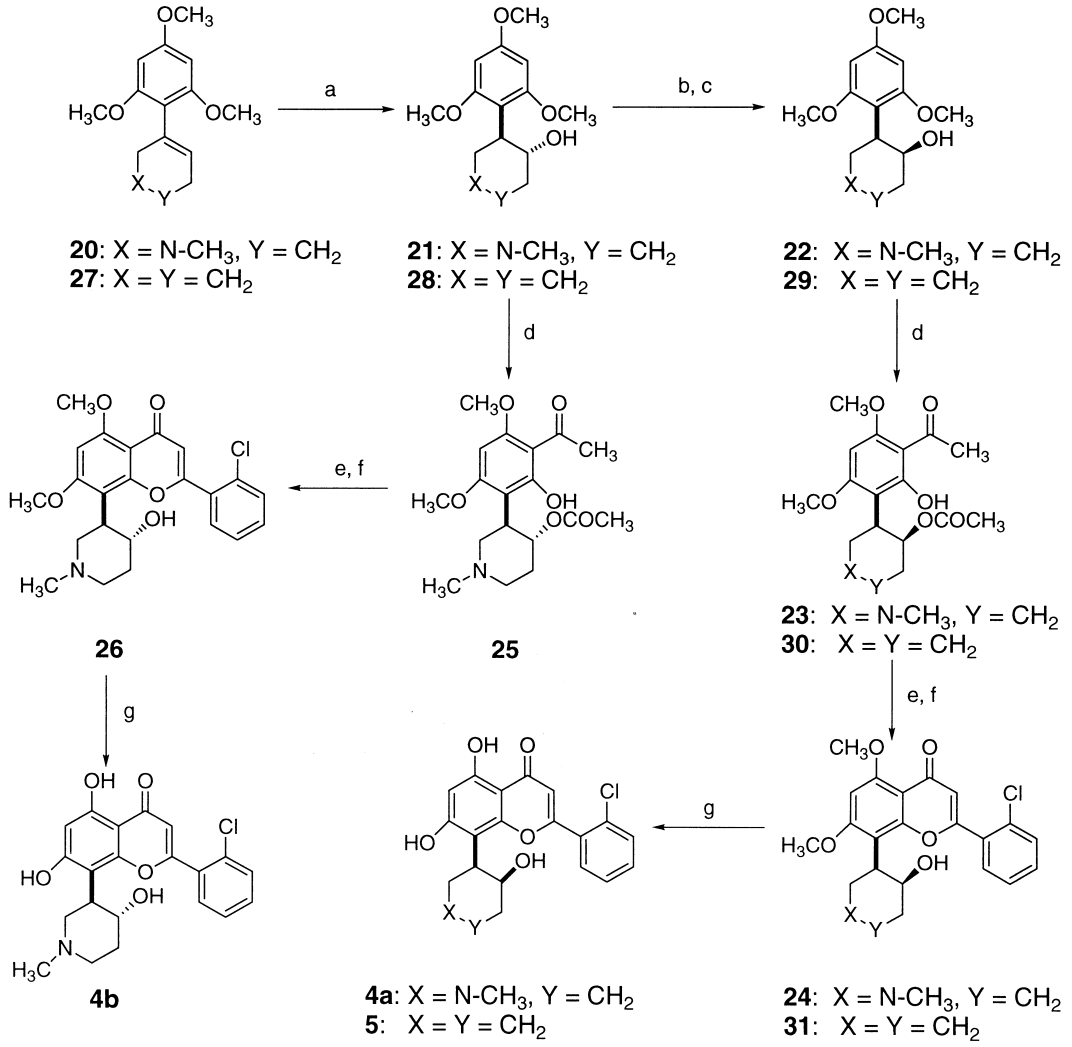



**Figure 1.**



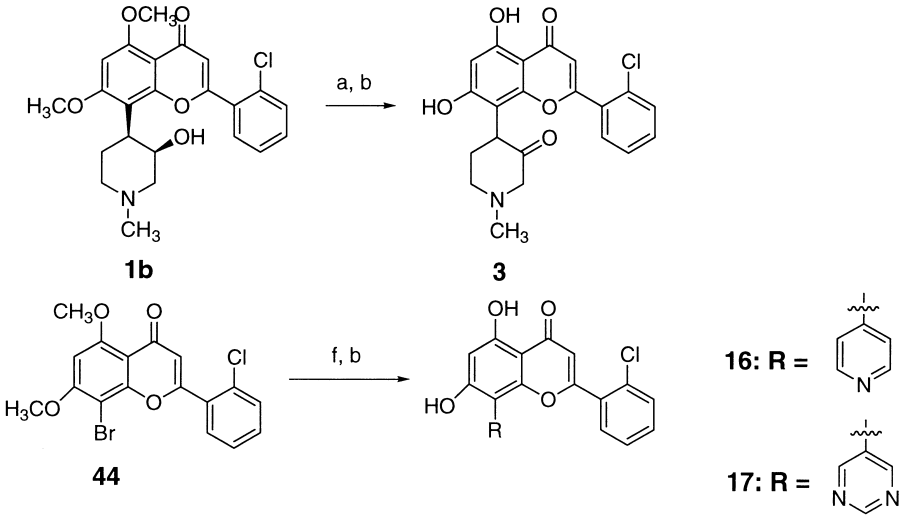

**Scheme 1.** (a) (i) $BF_3$-OEt, $NaBH_4$, THF; (ii) conc HCl, then NaOH, $H_2O_2$; (b) $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$; (c) $NABH_4$, EtOH; (d) $BF_3$-OEt, $Ac_2O$, $CH_2Cl_2$; (e) 2′-Cl-benzoylchloride, pyridine; (f) (i) NaH, THF, (ii) HCl gas, (iii) $Na_2CO_3$; 9g) pyridinium HCl, 180 °C.

reduction with $NaBH_4$ in methanol to produced a mixture of *cis*-alcohol **22** and the *trans*-alcohol **21** in a 1:7 ratio. The alcohols **21** and **22** were separated by silica gel chromatography. Selective demethylation followed by a Fries rearrangement of *cis*-alcohol **22** gave hydroxyacetophenone (**23**). Benzoylation of **23** with 2-chlorobenzoyl chloride followed by cyclization (NaH/THF, HCl) provided the 5,7-dimethoxyflavone (**24**). Finally, demethylation was accomplished by heating **24** with pyridinium hydrochloride under melting conditions (180 °C). The corresponding *trans*-alcohol, **4b** and the cyclohexyl analogue **5**, were prepared in a similar manner. The only notable

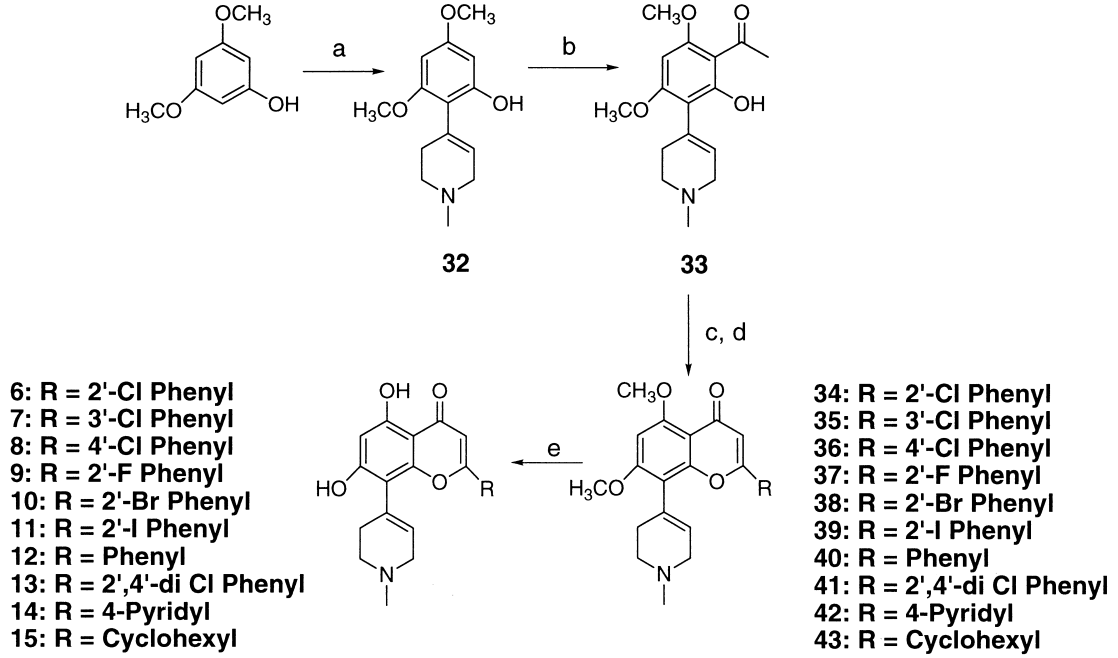
difference in the synthesis of the **6**, is that the olefin derivative **27**, was prepared by the reaction of 2-lithio-1,3,5-trimethoxybenzene with cyclohexanone.

The syntheses of ketone **3**, and analogues **16** and **17** are shown in Scheme 2. Swern oxidation of the dimethoxy flavone **1b**, followed by demethylation with pyridinium hydrochloride provide ketone **3**. The Stille cross-coupling protocol was employed to introduce aromatic rings in place of the piperidinyl moiety. Reaction of the appropriate aryl stannanes with **44** followed by demethylation resulted in analogues **16** and **17**. The synthesis of olefin analogues **6**–**15** is shown in Scheme 3. Condensation of 3,5-dimethoxy phenol with 1-methyl-4-piperidinone in acetic acid saturated with hydrogen chloride provided olefin **32**. Acylation of the aromatic nucleus was accomplished by reaction with acetic anhydride followed by $BF_3$-OEt promoted Fries rearrangement. Modification of the C-ring could easily be accomplished by acylation of **33** with various acid chlorides followed by cyclization (NaH/THF, HCl) to generate flavones **34**–**43**. Finally, demethylation with pyridinium hydrochloride provided flavones **6**–**15**.

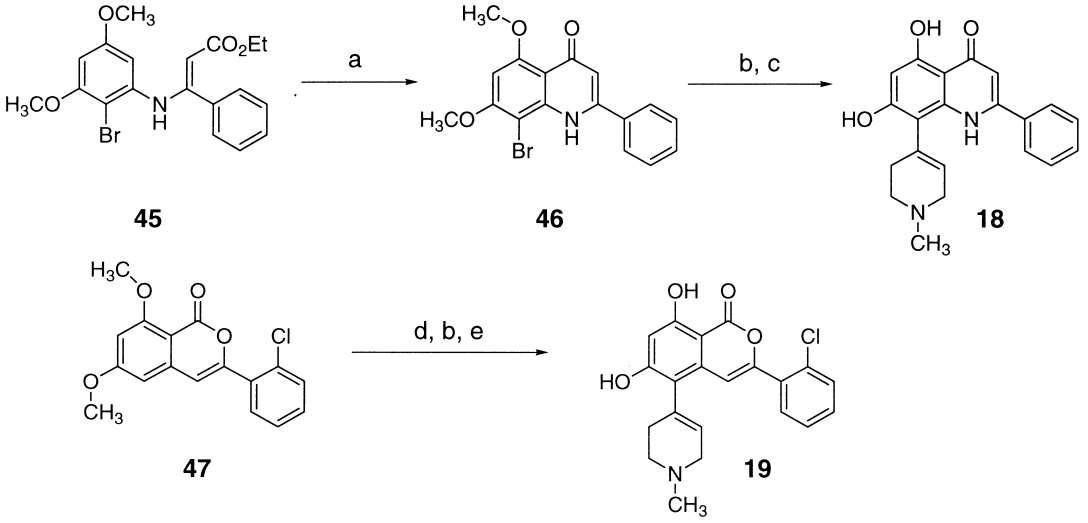
The synthesis of the quinolone and isocoumarin analogues **18** and **19** is shown in Scheme 4. Condensation of 2-bromo-3,5-dimethoxy aniline with ethyl benzoylacetate gave ester **45**.[12] Thermal cyclization in diphenyl ether, Stille cross coupling with vinyl stannane **48**,[13] followed by demethylation provided **18**. Dimethoxy




**Scheme 2.** (a) $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$; (b) pyridinium HCl, 180 °C; (c) $ArSnBu_3$, $Pd(PPh_3)_2Cl_2$, DMF, 110 °C.




**Scheme 3.** (a) *N*-methypiperidinone, $CH_3CO_2H$, HCl gas; (b) $BF_3$-OEt, $Ac_2O$, $Ch_2Cl_2$; (c) RCOCl, pyridine; (d) (i) NaH, THF, (ii) HCl gas, (iii) $Na_2CO_3$; (e) pyridinium HCl, 180 °C.




**Scheme 4.** 9a) $Ph_2O$, 230 °C; (b) **48**, $Pd(PPh_3)_2Cl_2$, DMF 100 °C; 9c) pyridinium HCl, 180 °C; (d) $Br_2$, $CHCl_3$; (e) $BBr_3$, DCE, reflux.

isocoumarin **47** was synthesized as described previously.[14] Bromination of **47**, cross-coupling with **48** and demethylation gave analogue **19**. The kinase assays were carried out as reported previously.[15]

The biological results for compounds **1**–**19** are shown in Table 1. In flavopiridol, the hydroxyl group and the flavone ring substituents on the piperidine ring (D ring) have a *cis*-orientation. Inverting the hydroxyl substituent to give the *trans*-isomer **2** results in >1000-fold loss in activity against both CDK4/cyclin D and CDK1/cyclinB kinases.

Similarly, oxidation of the alcohol to the ketone analogue **3** also results in a significant loss of activity. The importance of the piperidinyl nitrogen was explored with the cyclohexyl D ring analogue **5**. The cyclohexyl D ring analogue **5** is more than an order of magnitude less active than flavopiridol **1**. Thus, the presence of the nitrogen atom on the D ring is very important for CDK inhibitory activity. Next we studied the position of the nitrogen atom on the D ring with the 4-hydroxy-1-methyl-piperidinyl analogues **4a** and **4b**. The *cis*- and *trans*- hydroxyl isomers **4a** and **4b** which are derived from 1-methyl-3-piperidinone are completely inactive against CDK4/cyclin D. Thus the positioning of the nitrogen atom on the D ring is also very important for CDK inhibitory activity.

The olefin analogue **6**, which lacks the hydroxyl group results only in a 4- to 5-fold loss in activity against both the CDKs. The X-ray crystal structure[9] of des-chloro flavopiridol (**1b**) with CDK2 shows that the hydroxy piperidine D ring partially occupies the phosphate binding region. The structure shows interactions between the the piperidine nitrogen and Asp 145 and the hydroxyl group and Lys 33 respectively. Our SAR studies indicate that the piperidine nitrogen-Asp 145 interaction seems to be very critical for CDK inhibitory activity. Presumably the lack of activity of compounds **4a**, **4b**, and **5** is due to the loss of the piperidine nitrogen-Asp 145 interaction. The modest loss in activity with the olefin **6** suggests that the hydroxyl group-Lys 33 interaction may not be very critical for CDK inhibitory activity as long as the nitrogen-Asp 145 interaction is maintained. The major loss in activity of trans flavopiridol **2** and ketone **3** could be due to a loss in one or both hydrogen bonding interactions because of conformational changes of the hydroxy piperidine ring. Replacement of the 3-hydroxy-1-methyl-piperidinyl ring with aryl rings such as pyridyl (compound **16**) and pyrimidyl (compound **17**) also results in a major loss of CDK inhibitory activity.

Since the tetrahydropyridyl analogue (**6**), did not result in a major loss of CDK inhibitory activity we pursued additional SAR studies of this series involving changes in the flavone nucleus and the C-ring. As shown in Table 1, the SAR for the tetrahydropyridine series diverges from the data that has been reported for flavopiridol. Modification of the C-ring generally leads to a

**Table 1.** CDK Inhibitory activity of flavopiridol D-ring analogues

| Compound | CDK4/ Cyc D1 (μM) | CDK1/ Cyc B (μM) | MFC-7 (μM) |
|---|---|---|---|
| **1** | 0.2 | 0.2 | 0.5 |
| **2** | 56 | 49 | NT[a] |
| **3** | 8 | 10 | NT[a] |
| **4a** | 120 | NT[a] | NT[a] |
| **4b** | 250 | NT | NT[a] |
| **5** | 31 | 12 | NT[a] |
| **6** | 0.8 | 1.1 | 0.75 |
| **7** | 2.4 | 1.2 | 1 |
| **8** | 0.55 | 1.7 | 3 |
| **9** | 1.8 | 2.3 | 1 |
| **10** | 0.65 | 0.98 | 1 |
| **11** | 2.5 | NT[a] | NT[a] |
| **12** | 1.0 | NT[a] | NT[a] |
| **13** | 1.2 | 1.7 | 1.5 |
| **14** | 0.8 | NT[a] | NT[a] |
| **15** | 7 | NT[a] | NT[a] |
| **16** | 21 | 22 | NT[a] |
| **17** | 160 | 85 | NT[a] |
| **18** | 44 | NT[a] | NT[a] |
| **19** | 23 | NT[a] | NT[a] |

[a]Not tested.

loss of CDK inhibitory activity in flavopiridol.[8] Removal of the 2′-chlorine atom from flavopiridol, **1a**, results in about a 10-fold loss in activity; however, a similar modification has little effect in the olefin series (compare **6** and **12**). Other variations of the halogen substituent (**7**–**11**,**13**) on the aromatic ring do not seem to impair the CDK inhibitory activity. Heterocyclic modifications such as pyridyl, **14**, also seem to be well tolerated. It is noteworthy that the cyclohexyl analogue, **15**, results in a loss of activity. Modification of the flavone nucleus was more consistent with the flavopiridol SAR. The C-5 and C-7 hydroxyl groups are critical for kinase inhibitory activity. Thus, the corresponding dimethoxy analogues, **34**–**43**, are devoid of CDK inhibitory activity. Our results are consistent with the X-ray crystal structure of **1a** with CDK2 which indicates that the C-5 hydroxyl and the flavone carbonyl are involved in critical hydrogen bonds with E81 and L83 in the adenine binding pocket.[9] In addition, the C-ring is solvent exposed and does not make appreciable protein interactions. It is noteworthy, that in the present study modication of the D-ring seems to attenuate these binding interactions to some degree. While in the flavopiridol series, modification of the C-ring results in at least a 10-fold loss in activity, the tetrahydropyridine series seems quite tolerant of changes in the C-ring (**7**–**15**). On the other hand, the interactions with E81 and L83 seem to be retained, since methylation abrogates CDK inhibitory activity. The olefin analogues **6**–**10** and **13** were tested against the MCF-7 tumor cell line to measure growth inhibition and showed comparable activities for both CDK inhibition and inhibition of cell growth (Table 1). Finally alteration of the flavone nucleus to either a quinol-4-one, **18**, or an isocoumarin nucleus, **19**, resulted in reduced activity. This may reflect ineffective interactions in the adenine binding pocket.

Our SAR studies on flavopiridol have shown that both the presence and the position of the nitrogen moiety on the D ring are critical requirements for CDK inhibitory activity. We have also identified a simpler analogue, olefin **6**, which shows good CDK inhibitory activity. Our SAR studies on olefin **6** have shown that the C ring tolerates substitutions without a major loss in activity. We also found that the quinolone and the isocoumarin rings are not effective flavone replacements.

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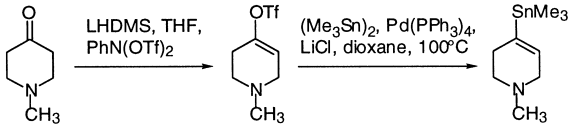
13. Vinyl stannane **48** was prepared as shown below.


**48**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