Synthesis of 6-(Fluoromethyl)-19-norcholest-5(10)-en-3-ol, a Fluorinated Analogue of NP-59, using the Mild Fluorinating Reagent, TBAF(Pinacol)$_2$

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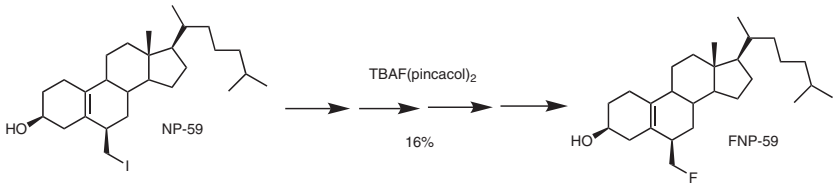

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Abstract For 45 years, efforts to prepare a fluorinated analogue of the scintiscanning/SPECT agent 6-(iodomethyl)-19-norcholest-5(10)-en-3-ol (NP-59) for development of a PET imaging agent have failed due to undesired elimination reactions and unexpected rearrangements observed while utilizing a wide variety of fluorinating conditions (e.g., cesium fluoride, silver fluoride, (2-chloro-1,1,2-trifluoroethyl)diethylamine (FAR), diethylaminosulfur trifluoride (DAST), and hexafluoropropene diethylamine FPA). Herein, we report the full synthesis of NP-59, followed by the four-step synthesis of 6-(fluoromethyl)-19-norcholest-5(10)-en-3-ol (FNP-59) using a recently developed mild fluorinating reagent, less prone to producing elimination reactions in the preparation of primary fluorides, TBAF(pinacol)$_2$, with an overall yield of 16% (four steps). Also included is an evaluation of the TBAF(pinacol)$_2$ reagent on eight test substrates to investigate its scope.

Key words steroids, PET, fluorination, cholesterol, adrenal

The scintiscanning agent 6-(iodomethyl)-19-norcholest-5(10)-en-3-ol (NP-59) was first reported in 1975 as part of an effort to develop a cholesterol analogue for imaging diseases associated with the adrenal glands such as Cushing's syndrome, aldosteronism, and identification of adrenal remnants following adrenalectomy procedures.[1] NP-59 was identified as an impurity in the preparation of 19-iodocholesterol.[2] It was discovered that 19-iodocholesterol, upon heating as part of the isotopic exchange reaction to incorporate iodine-131, would rearrange to give NP-59. Adrenal uptake of NP-59 was greater with a better tissue to background ratio compared to 19-iodocholesterol, and NP-59 showed improved stability to deiodination.

Interest in utilizing NP-59 for cortical adrenal imaging has continued with efforts made to improve the agent by using alternate iodine isotopes to prepare NP-59 for use in single-photon emission computed tomography (SPECT) imaging (^{123}I, ^{125}I)[3] or in positron emission tomography (PET) imaging (^{124}I).[4] [^{131}I]NP-59 is limited to scintigraphy and SPECT methods that have lower spatial resolution than PET, which limits the diagnostic utility of the agent. The PET imaging agent has the benefit of coincidence detection for better resolution but is limited by the low positron output of iodine-124 (^{124}I decays by β^+ 26% vs. ^{18}F, 97%) leading to noise that lowers image quality, and requires undesirably high radiation dosimetry to the patient. A fluorine-18 analogue will improve the imaging characteristics, by providing a PET imaging cholesterol analogue with better spatial resolution.

Additionally, NP-59 has a relatively long biological half-life, necessitating multiday imaging protocols, where injection occurs on one day with the patient returning on a later day for scanning, which is not ideal for the patient and limits the extent of quantitation that can be performed with the imaging data. It is common for fluorine analogues to have improved metabolic stability and other pharmacokinetic parameters. For instance, the biological half-life of metaiodobenzylguanidine is estimated at 34 hours, whereas its fluorine analogue metafluorobenzylguanidine has a 2-hour biological half-life.[5]

Reflecting these advantages as well as a wider interest in the use of radiofluorinated steroids for imaging purposes,[6] there have been efforts for decades to prepare a fluorinated analogue of NP-59, as well as the corresponding ^{18}F-isotopologue. However, common fluorinating reagents have overwhelmingly led to elimination (e.g., cesium fluoride, (2-chloro-1,1,2-trifluoroethyl)diethylamine (FAR), diethylaminosulfur trifluoride (DAST), and hexafluoropropene diethylamine (FPA)), ring expansion, rearrangement, and other undesired products.[7] While other steroids prone to unwanted side reactions have been successfully fluorinated with 1-butyl-3-methylimidazolium tetrafluoroborate, such

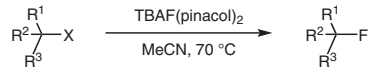
as 7α-(fluoromethyl)dihydrotestosterone, these methods gave low-single-digit yields of the fluorinated products.[8] Recently, the coordination of fluoride species with various alcohols, and the effects of hydrogen bonding on their reactivity have given rise to milder fluorinating reagents, which are less apt to produce unwanted byproducts. One of these alcohol coordinated reagents, tetra-*N*-butylammonium fluoride bis-pinacol (TBAF(pinacol)$_2$), proved particularly promising at producing primary fluorides while minimizing byproduct formation.[9] However, this reagent was evaluated on only one test substrate, so it was necessary to vet it on a series of model compounds prior to its application as the penultimate step of a multistep steroid synthesis.

To determine the potential utility of TBAF(pinacol)$_2$, including for the fluorination of NP-59, a representative group of primary, secondary, and tertiary tosylates and alkyl bromides were prepared. Each was then stirred with TBAF(pinacol)$_2$ at 70 °C in acetonitrile for 2 hours, and an extract was removed. To these extracts, an equimolar amount of 4-fluorobenzonitrile was added as an internal standard, and the conversion into the fluorinated product was determined by ^{19}F NMR spectroscopy. The process was then repeated with a second reaction, and extracts were taken after 18 hours to determine the time dependency of the reactions (Table 1).

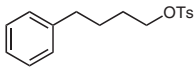
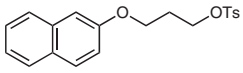
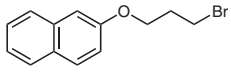
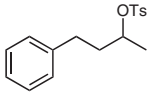
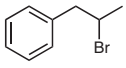
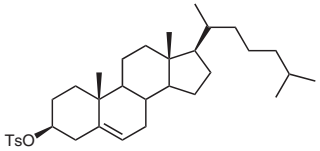
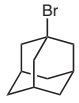
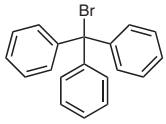
Comparing the substrates by their degree of substitution shows that TBAF(pinacol)$_2$ performs best in the synthesis of primary fluorides (Table 1, entries 1–3), although secondary (entries 4–6) and tertiary (entries 7 and 8) fluorides were also accessible, albeit with lower conversions into fluoride product. Alkyl bromides showed higher conversions into product after 18 hours compared with 2 hours, whereas there were no significant differences between 2- and 18-hour conversions when using tosylates. This substrate scope study suggests tosylates are better leaving groups for use with TBAF(pinacol)$_2$.

To prepare NP-59, we started from cholesterol (Scheme 1). The synthesis of **1**–**4** was conducted according to reported procedures, with some optimization for scale and time.[10] Cholesterol was protected at the 3-position by treating it with acetic anhydride in the presence of pyridine to give **1**, the acetylated intermediate. Compound **1** was then stirred with *N*-bromoacetamide under acidic conditions under foil to block light to give bromohydrin **2**. Compound **2** was heated with lead tetraacetate and iodine to give **3**, the cyclized intermediate, which was then treated with zinc powder in acetic acid to give alcohol **4**. Intermediate **4** was then treated with *p*-toluenesulfonsyl chloride in the presence of dimethylaminopyridine to give tosylate **5**. While there are various methods for accessing NP-59 from protected tosylate intermediate **5**, we expected that deprotection and a subsequent one-step iodination/rearrangement would be the most straightforward and reliable.[2,11] Thus, compound **5** was deprotected at the 3-position by stirring it in a solution of K_2CO_3 to yield **6**, which was immediately heated with KI to promote the iodination/rearrangement reported by Maeda and colleagues.[2b] Analysis showed that, after 7 h, the product was approximately a 1:1 mixture of the unrearranged 19-iodocholesterol and NP-59. As such, the mixture was resuspended in MeCN and heated for an additional 2 h to give only NP-59.

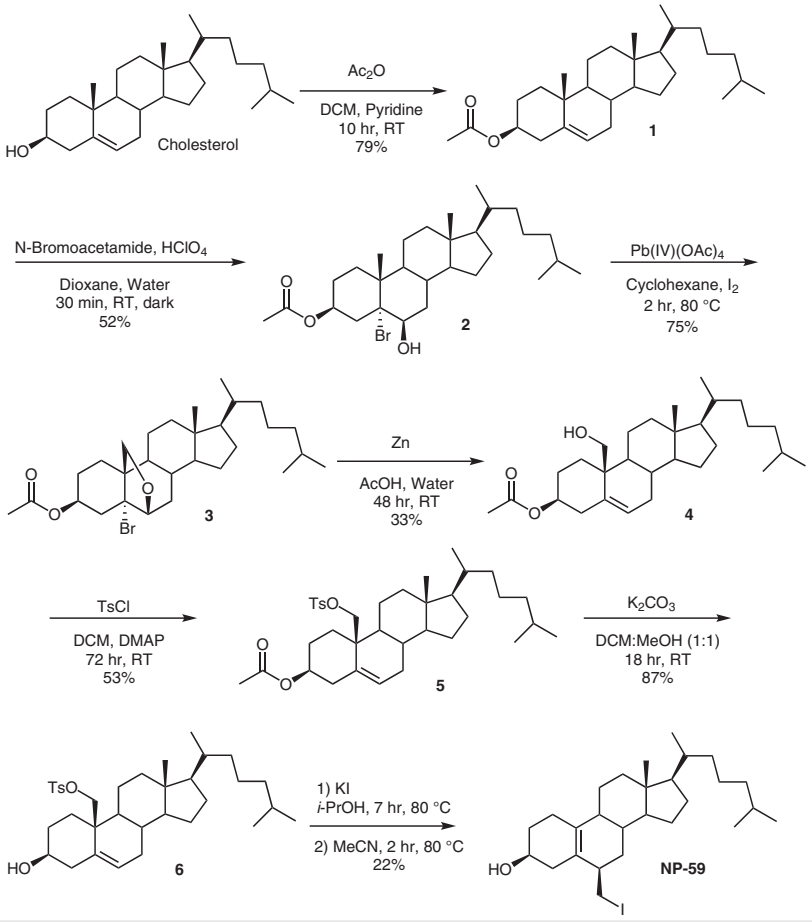
Table 1 TBAF(pinacol)$_2$ Substrates and ^{19}F NMR Yields[a]



X = OTs or Br; R = H or alkyl group

Entry	Substrate	Conversion (%)[b]	
		2 h	18 h
1		63	57
2		68	64
3		61	72
4		21	9
5		2	10
6		7	7
7		5	4
8		19	44

[a] Starting material (0.2 mmol) was dissolved in acetonitrile (0.8 mL), TBAF(Pinacol)$_2$ (0.4 mmol) was then added. The reaction was heated at 70 °C for 2 or 18 hours.
[b] Non-isolated conversion determined by ^{19}F NMR spectroscopic analysis.



Scheme 1 Synthesis of NP-59 from cholesterol

Lastly, we investigated the conversion of NP-59 into FNP-59 (Scheme 2). To produce the intermediate for fluorination, NP-59 was initially protected as the acetate at the 3-position by treating it with acetic anhydride in the presence of 4-(dimethylamino)pyridine to form **7**. We initially explored whether treating **7** directly with TBAF(pinacol)$_2$ could produce the desired product, but this resulted in a complex mixture. Therefore, **7** was instead heated with AgOTs to yield **8**. In the penultimate step **8** was heated with TBAF(pinacol)$_2$ in acetonitrile to give **9**, the protected fluoride, in 67% yield. Treatment of **9** with K_2CO_3 in a mixture of MeOH/CH_2Cl_2 (1:1) yielded FNP-59.

In summary, an updated synthesis of NP-59, along with spectroscopic characterization of all intermediates has been conducted. NP-59 was then converted into FNP-59 via a four-step synthesis in an overall yield of 16% using TBAF(pinacol)$_2$ in the key fluorination step. With FNP-59 in hand, toxicity studies are under way, and a method for the radiosynthesis of [^{18}F]FNP-59 is being developed.

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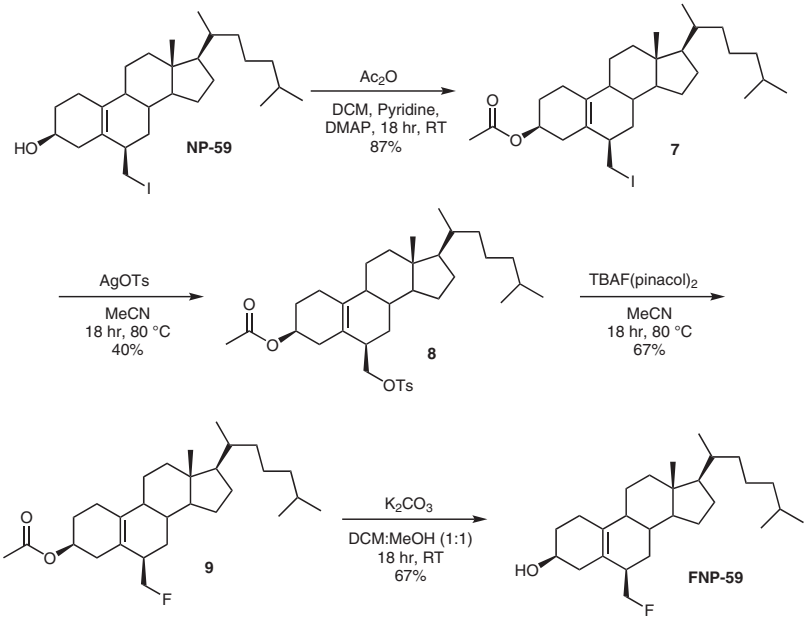
Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1611845.



Scheme 2 Synthesis of FNP-59 from NP-59

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