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Multi-gram synthesis of a nucleotide-competing reverse transcriptase inhibitor

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ABSTRACT

An efficient multi-gram synthesis of a nucleotide-competing reverse transcriptase inhibitor is reported. The synthesis features a chiral auxiliary-assisted alcohol resolution, a Mitsunobu reaction involving a carbamate, followed by a lithium-iodide exchange/Weinreb ketone synthesis tandem. These chemical transformations were optimized in order to increase the yield of the synthesis. The route is concluded by a late-stage palladium-catalyzed cyanation followed by a pyrimidine-2-one ring formation.

1. Introduction

According to the World Health Organization, an estimated 34 million people were infected with HIV worldwide in 2010, mostly in Sub-Saharan Africa.[1] Fortunately, progress in highly active antiretroviral therapy over the past 20 years has reduced the mortality caused by the AIDS epidemic.[2] While a vaccine cure aimed at the eradication of the disease is the most desirable goal, supplementing the antiviral arsenal with novel drugs aiming at highly validated viral targets is also needed. In particular, inhibition the HIV reverse transcriptase is a key component in reducing viral load and suppressing the infection.[3]

Our research group focused on a new class of reverse transcriptase inhibitors, nucleotide-competitive reverse transcriptase inhibitors (NcRTIs).[4] These non-nucleosidic molecules bind at, or very close to, the active site of the enzyme,[5] thereby competing with incoming deoxyribonucleotide triphosphates (dNTP). This mode of inhibition prevents elongation of the proviral DNA strand, but does not lead to chain termination. As such, NcRTIs could complement currently available NRTI and NNRTI regimens, especially in the context of the emergence of drug-resistant HIV strains.

Following a high-throughput screening campaign, a new chemotype exhibiting NcRTI mechanism was discovered. Extensive lead optimization led to the discovery of inhibitor **1** that demonstrated excellent antiviral potency, favorable cross-resistance profile, and good pharmacokinetic profile. Based on its favorable in vitro and in vivo profiles, this molecule was selected as a candidate for toxicological and pharmacological studies, requiring a multi-gram synthesis of the active pharmaceutical in high purity. However, the highly functionalized polycyclic molecule **1** posed several synthetic challenges (Fig. 1). Starting from azabenzofuran **2**, the following chemical steps were required: mono-coupling of pyrazole at the C-8 position, cyanation at position C-7, introduction of optically pure 2-ethyltetrahydropyran-4-yl at N-1, without erosion of stereochemical integrity, and introduction of an *N*-methylpyrazole at the C-4 position. The present communication describes the optimization of these key transformations, leading to the multi-gram synthesis of **1**.

2. Synthesis of optically pure tetrahydropyran 4

In order to develop an efficient synthesis of tetrahydropyran alcohol **4**, we decided to embark on a racemic synthesis followed by a chiral auxiliary-assisted separation. Strict timelines, combined with synthetic challenges and the need for a high level of optical purity (>98.5% ee) precluded the pursuit of a stereoselective synthesis.

The epimer of alcohol **4** (***epi*-4**) was prepared using a Prins reaction between propionaldehyde and 3-buten-1-ol, according to a previously reported procedure, in good yield (Scheme 1).[6] The *cis* diastereomer was obtained with 95:5 selectivity; the undesired *trans* isomer could be removed by chromatography during the enantioresolution process (vide infra). The stereochemistry of ***epi*-4** at the alcohol center had to be inverted to obtain the desired

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† This article is dedicated to the memory of Dr. Louis Morency (1979–2012), fellow scientist, colleague and friend.

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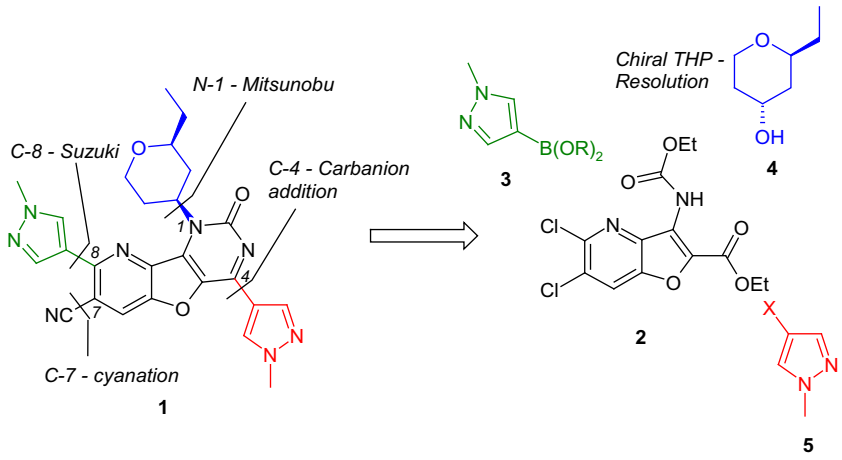
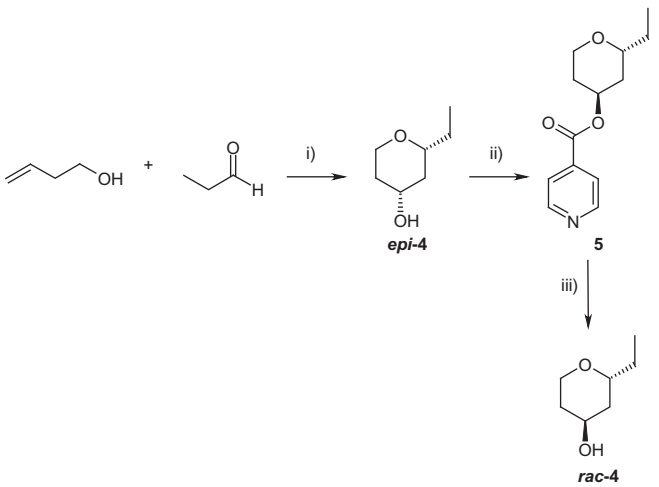


Figure 1. Retrosynthetic approach to compound **1**.

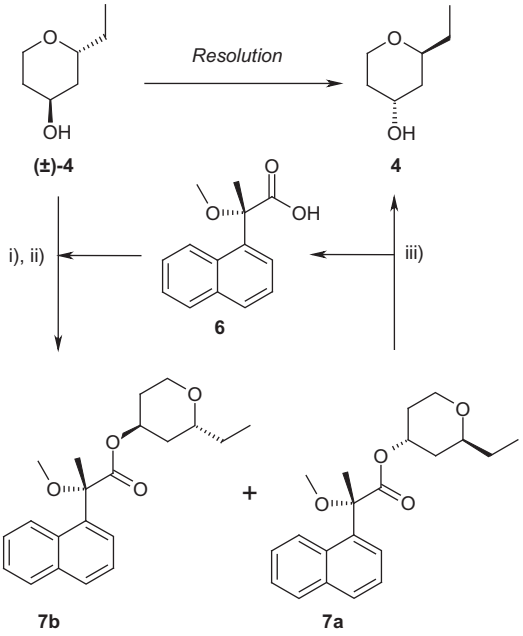
stereochemical outcome in the subsequent Mitsunobu reaction involving carbamate **2** (vide infra). To this end, a Mitsunobu reaction with isonicotinic acid led to complete inversion of stereochemistry. A simple two-step acid–base extraction was performed, affording compound **5**. Isonicotinate ester **5** was hydrolyzed under basic conditions followed by vacuum distillation to afford hydroxypyran ***rac*-4** as a racemic mixture.

We planned on obtaining optically pure tetrahydropyran **4** through chromatographic separation after coupling of a chiral auxiliary. Several chiral auxiliaries (amino acids, Mosher's ester, Trost's ester) were screened, but no practical level of separation of the enantiomeric mixture could be achieved. Finally, Harada's chiral auxiliary **6** was selected for its demonstrated enantioresolution capability[7] (Scheme 2). Due to the fact that the element imparting chirality is far removed from the chiral auxiliary, the separation of **7a** and **7b** proved challenging using standard 40–63 μm silica. However, the diastereomeric mixture comprising **7a** and **7b** was successfully separated with the help of medium-pressure chromatography, using high performance silica cartridges.[8]

After cleavage of the ester under basic conditions, an acid–base separation was performed, at which point chiral auxiliary **6** was isolated and recycled, with recovery greater than 80%. The optically pure tetrahydropyran was obtained in good yield, typically between 60% and 70%.



Scheme 1. Synthesis of ***rac*-4**. Reagents and conditions: (i) H_2SO_4; 80%; (ii) isonicotinic acid, triphenylphoshine, DIAD, THF; (iii) NaOH, MeOH; >90% (two-steps).



Scheme 2. Enantioresolution of chiral tetrahydropyran **4**. Reagents and conditions: (i) DCC (1.1 equiv), DMAP (0.5 equiv), CSA (0.1 equiv), DCM; (ii) separation of diastereomers (highly spherical silica), 25:75 EtOAc:Hex; (iii) NaOMe, 70 °C, then water, 80 °C; 60–70% yield.

The absolute configuration of **4** was determined by comparison of the ^{1}H NMR spectral data of compounds **7a** and **7b**. Chiral auxiliary **6** is known to induce strong, unambiguous anisotropic shifts. In the case of **7a** and **7b**, $\Delta\delta$ values were consistent with (2*S*,4*R*) and (2*R*,4*S*) configurations, respectively.[7,9]

3. Study of the Mitsunobu reaction

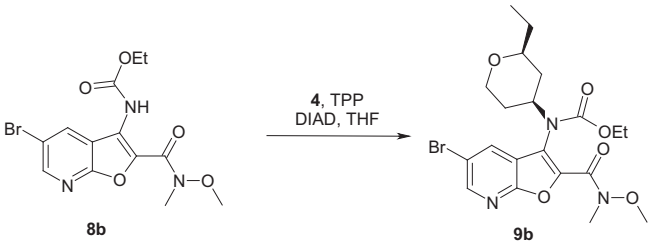
The Mitsunobu reaction represents an efficient way of creating carbon–heteroatom bonds with complete preservation of optical purity.[10] This transformation was clearly the procedure of choice

for the introduction of tetrahydropyran **4** on the core of the molecule. However, in previous studies conducted on different intermediates, we realized that this key connection presented a significant challenge (Scheme 3). Due to the weakly acidic nature of the carbamate proton, the Mitsunobu reaction was slow and subject to side reactions.

Subtle electronic effects had a critical impact on the outcome of the Mitsunobu reaction (Scheme 3). In the case of substrate **8a** bearing an ester at the 2-position of the azabenzofuran, the reaction proceeded quite efficiently over 16 h. In contrast, Weinreb amide **8b** reacted at a much lower rate, reaching less than 40% conversion. Finally, the electron-withdrawing ketone **8c** did not show any reactivity under these conditions. ^{1}H NMR analysis of the carbamate proton of **8a** showed a chemical shift of 9.47 ppm, while the poor susbtrates **8b** and **8c** showed chemical shifts at 9.76 ppm and 10.06 ppm, respectively.[11] This observation was indicative of the relevance of electronic effects in the Mitsunobu reaction.

Due to the slow rate of the Mitsunobu reaction involving carbamates of type **8**, tetrahydropyran alcohol **4** was subject to a non-constructive reaction pathway, leading to low levels of conversion. In order to overcome that difficulty, the stoichiometry of the alcohol was adjusted for problematic substrate **8b**. It was found that 1.2 equiv of tetrahydropyran alcohol **4** yielded a 40% level of conversion (Table 1, entry 2), representing the optimal conditions after significant efforts. While the remaining starting material **8b** could be isolated and re-submitted to the reaction conditions, it was realized that the only path forward to a multi-gram synthesis of inhibitor **1** was to use a substrate of type **8a**, bearing an ester at the 2-position. The key findings from the optimization study (Scheme 3 and Table 2) were used to allow an efficient synthesis of inhibitor **1**.

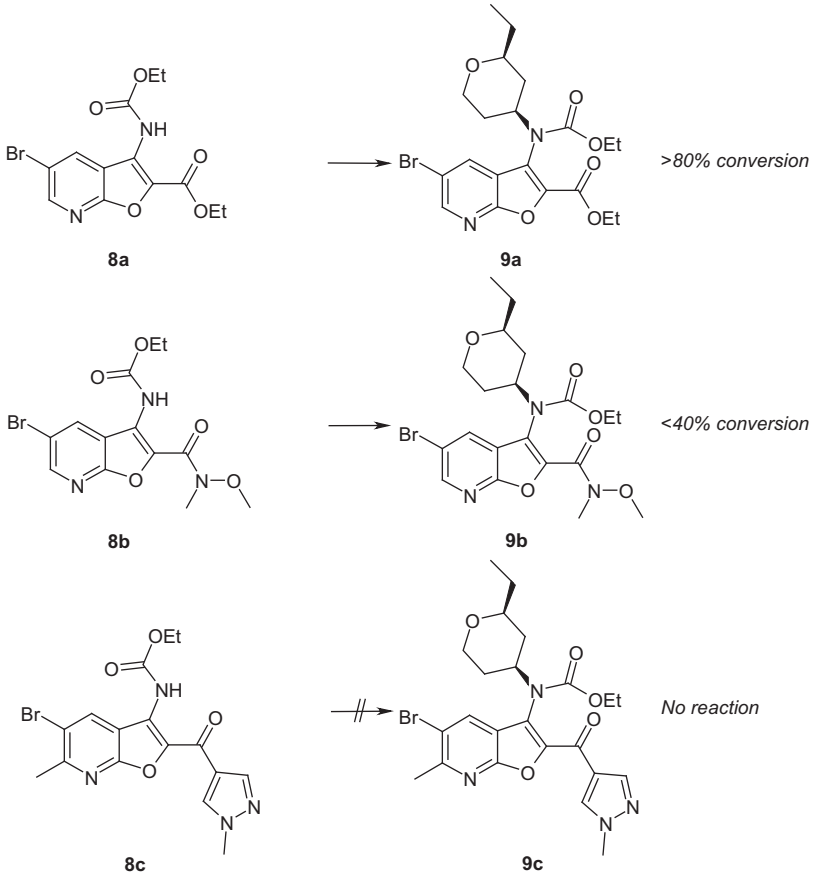
Table 1
Mitsunobu reaction optimization



Entry	Tetrahydropyran (equiv)	% Conversion after 17 h
1	1.0	32
2	1.2	40
3	1.5	28
4	1.8	28
5	2.0	16

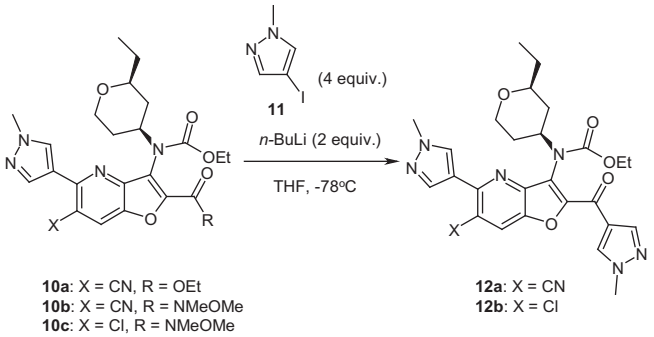
4. Introduction of C-4 pyrazole

Initially, we planned to introduce the C-4 pyrazole by the addition of an organolithium reagent to an ester. Early on, we realized that the preformation of the lithiated pyrazole species was not a viable option, due to the poor outcome of the reaction. The pyrazole anion was believed to be unstable, hence, the order of addition



Scheme 3. Impact of electronic effects on the conversion level of Mitsunobu reaction. Reagents and conditions: Tetrahydropyran **4**, triphenylphosphine, DIAD (dropwise addition), THF, 0 °C.

Table 2
Optimization of the C-4 addition



Entry	R	X	Yield[a] (%)
1[b]	OEt	CN	decomp.
2	OEt	CN	60
3	NMeOMe	CN	63
4	NMeOMe	Cl	74
5[c]	NMeOMe	Cl	74

[a] Isolated yields after silica gel chromatography.
[b] Reaction performed with 4-bromo-1-methylpyrazole.
[c] Reaction performed with *tert*-BuLi (4 equiv).

of the reagents was adjusted accordingly, with the lithiating agent (*n*-butyllithium) being added dropwise to a mixture of ethyl ester **10a** and halopyrazole **11**[12]. The lithiated species underwent nuleophilic addition as it was formed, minimizing the problem of decomposition and affording a more efficient transformation.

In the case of compound **10a**, the bromopyrazole proved ineffective in the reaction, leading to extensive decomposition (Table 2, entry 1). Previous work showed that the use of the iodopyrazole, which undergoes lithium–halogen exchange more readily than bromide, increased the yield significantly. When iodopyrazole **11** was used, product **12a** was formed in 60% yield. However, the over-addition by-product was also formed, in up to 25% (Entry 2). This side reaction raised concerns about the scalability and the reproducibility of the reaction. In addition, chromatographic purification of **12a** was complicated by the presence of this by-product.

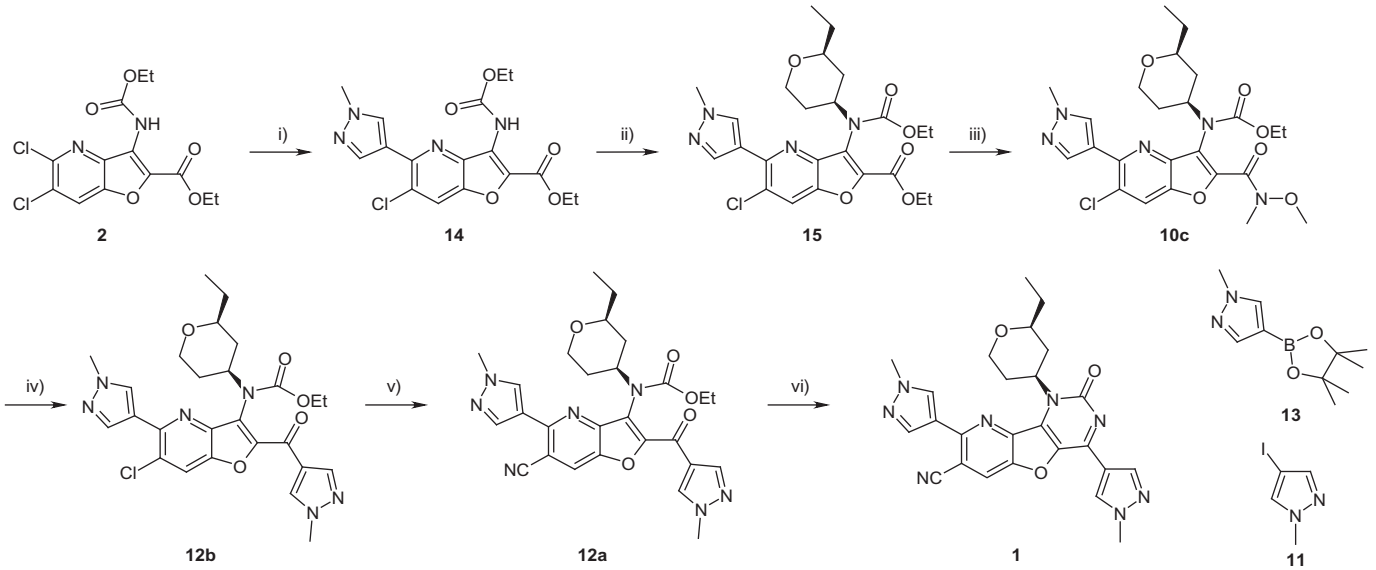
Based on experiments previously conducted on similar scaffolds, we expected that the use of a Weinreb amide would remedy the bis-addition issue.[13] It had been demonstrated that the addition of an organolithium species to a Weinreb amide led to the formation of a stable chelate which did not undergo further reaction.[14] Unfortunately, the reaction of the lithiated pyrazole on **10b** provided only a marginal increase in yield (entry 3). It was inferred from mass spectrometry data that the electrophilic nitrile group at the C-7 position was interfering with the reaction. The order of the synthetic steps was thus reordered: the pyrazole lithiation-addition was performed on the cyanation precursor, chloride **10c**. Substrate **10c** reacted cleanly with the lithiated pyrazole to afford **12b** with a 74% yield (entry 4). Subsequently, it was found that *tert*-butyllithium provided superior reproducibility (entry 5) and provided the best path forward to multi-gram scale up.

5. Multi-gram synthesis of advanced lead 1

A multi-gram synthesis of the advanced lead **1** was undertaken to support toxicological studies (Scheme 4). Extensive optimization of the key chemical steps performed previously allowed for an efficient process (vide supra).

Starting from 70 g of dichloro azabenzofuran **2**,[13] a Suzuki–Miyaura cross-coupling with boronate **13** was completed. The reaction was performed in three separate batches to afford 55 g of intermediate **14**. Coupling of the pyrazole proceeded exclusively at the pyridine-activated C-8 position. The Mitsunobu reaction to introduce tetrahydropyran **4** was performed in two batches of 27.5 g. The transformation proceeded smoothly despite the limited solubility of **14** in THF. Purification of **15** presented a significant bottleneck, since four separate chromatographic separation runs had to be undertaken. A total of 58 g of **15** with >90% purity were obtained.

Ester **15** was satisfactorily converted into Weinreb amide **10c** using the preformed lithium anion of *N*,*O*-dimethylhydroxylamine.



Scheme 4. Synthesis of **1**. Reagents and conditions: (i) Boronate **13** (1.6 equiv), cesium fluoride (0.25 equiv), sodium bicarbonate (2.4 equiv), $Pd(OAc)_2$. (7 mol %), triphenylphosphine (15 mol %), dioxane:water (4:1), 90 °C, 16 h; 69–83%; (ii) tetrahydropyran **4**, triphenylphosphine, DIAD (dropwise add.), THF, 0 °C, 16 h; 67–73%; (iii) *N*,*O*-dimethylhydroxylamine hydrochloride (3.5 equiv), *n*-butyllithium (2.5 M in hexanes, 7 equiv), THF, −60 °C, 15 min, then **15** in THF added, −60 °C, 30 min; 83%; (iv) iodide **11** (5 equiv), *tert*-butyllithium (1.7 M in pentane, 4 equiv), THF, −78 °C, 45 min; 74%; (v) $Zn(CN)_2$ (4.6 equiv), $Pd(P(t\text{-}Bu)_3)_2$ (20 mol %) DMA, microwave 15 °C, 30 min; 74%; (vi) ammonia (gas), ammonium acetate (60 equiv), NMP, 130 °C, 4 h; 70%.

Weinreb amide **10c** was then submitted to the optimized lithium–halogen exchange/addition protocol to afford ketone **12b**. The largest scale on which this protocol was performed was 8 g of **10c**. Considering the necessity for cryogenic conditions and the high reactivity of the organolithium species, we deemed this transformation too delicate to risk a further increase in reaction scale. After combination and chromatographic purification, 37 g of compound **12b** was obtained.

The palladium-catalyzed cyanation of the aryl chloride was done using $Zn(CN)_2$ as the cyanide source. The *bis*(tri-*tert*-butylphoshine)palladium catalyst was used because of its high effectiveness with aryl chlorides and its ease of handling on a large scale.[15] The reaction required microwave heating to proceed efficiently. It was absolutely necessary to achieve near 100% conversion for the cyanation reaction, since the separation of **12b** and **12a** was tedious. After chromatographic purification, the material could be further purified by trituration, to achieve a yield of 27 g of **12a** with >97% purity.

The final cyclization step to form the pyrimidine-2-one ring was performed using ammonium acetate in *N*-methylpyrrolidinone at 130 °C. The reaction mixture was degassed thoroughly with nitrogen prior to heating. Ammonium acetate is known to decompose to acetamide and water at high temperatures,[16] so a relatively short reaction time was desirable. Ammonia gas was bubbled into the reaction mixture to ensure high concentrations of ammonia. After reaction completion, the compound was precipitated by addition of water to the reaction mixture and filtered. Trituration of the crude material with diethyl ether followed by treatment with activated charcoal (SX-Ultra, 100% w/w) afforded inhibitor **1**. We provided 17.4 g of purified (>98% homogeneity by HPLC) active pharmaceutical ingredient for pre-development pharmacological and toxicological profiling. The overall yield of **1** starting from carbamate **2** was 17% over six-steps, for an average yield of 74% per synthetic step.

In conclusion, optically pure tetrahydropyran **4** was obtained through an enantioresolution using an effective chiral auxiliary. This pyran alcohol was introduced into carbamate **2** through a Mitsunobu reaction. A lithium–halogen exchange/Weinreb ketone synthesis protocol was optimized to allow the efficient introduction of a pyrazole at the C-4 position. All these advances allowed for an efficient multi-gram synthesis of advanced lead **1,** which was used for pre-development pharmacological and toxicological profiling.

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Supplementary data

Supplementary data (detailed experimental procedures and spectroscopic data for compounds **7a**, **7b**, **14** and **1**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01.129. These data include MOL files and InChiKeys of the most important compounds described in this article.

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