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Fluorine-Substitution in Cholesteryl Ester Transfer Protein Inhibitors (CETP-Inhibitors) – Biology, Chemistry, SAR, and Properties

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Abstract: The inhibition of the *c*holesteryl *e*ster *t*ransfer *p*rotein (CETP) provides a method for the elevation of the *h*igh *d*ensity *l*ipoprotein *c*holesterol (HDL-C) level, *i.e.* the 'good' cholesterol. The expected anti-atherogenic effect of this approach is independent of the proven benefits of lowering the *l*ow *d*ensity *l*ipoprotein *c*holesterol (LDL-C) level, *i.e.* the 'bad' cholesterol. A medicinal chemistry project is presented starting from the first screening hit to the second generation development candidate. The structure–activity relationship, the syntheses and the role of fluorine during optimization, as well as the biological activities are discussed.

Keywords: Cholesteryl ester transfer protein · Domino reaction · Hantzsch-reaction · HDL-C elevation · Oxazaborolidine reduction

1. Introduction

*C*oronary *h*eart *d*isease (CHD) is the leading cause of death in the industrialized countries. Angina pectoris and myocardial infarction are clinical manifestations of CHD. They are caused by a stenosis or an occlusion of a coronary artery through atherosclerosis.

Atherosclerosis is the development of fibro-fatty plaques within the inner walls of arteries, resulting in a narrowing of the vessel. There are several non-modifiable and modifiable risk factors for atherosclerosis and eventually CHD. Non-modifiable factors include age, gender, and family or personal history of CHD. Examples of modifiable factors are dyslipidemia, high blood pressure, obesity, diabetes mellitus, dietary factors, lack of exercise, thrombogenic factors, excess alcohol consumption, and smoking. Lipid disorders are key risk factors for atherosclerosis. It is known that high LDL-C (*l*ow *d*ensity *l*ipoprotein *c*holesterol) levels and low HDL-C (*h*igh *d*ensity *l*ipo-protein *c*holesterol) levels are independent risk factors for the development of atherosclerosis [1]. Excess cholesterol in the cell walls of coronary arteries is involved in oxidative events which trigger the invasion of macrophages. The macrophages can degenerate and transform themselves into foam-cells by intake of too much ox-LDL-C. As a consequence atherosclerotic plaques are formed. They consist of proliferated smooth muscle cells, fibrous tissue and the lipid accumulation. Lowering LDL-C by blocking cholesterol biosynthesis with so-called 'statins' is well-established medical practice. Elevation of HDL-C levels offers a new chance for a promising therapy [2], because it is an important player in the reverse cholesterol transport, *i.e.* the transport of cholesterol from periphery, for example cell walls of coronary arteries, towards the liver. This removal of cholesterol from a cell membrane starts with an esterification by action of *l*ecithin *c*holesterol *a*cyl *t*ransferase (LCAT). Typical cholesteryl esters are fatty acid esters, *e.g.* linoleic acid. These very lipophilic compounds are carried through the aqueous bloodstream in the HDL-particles. In the liver cholesterol is stored or metabolized into bile acids but it is also a site of biosynthesis of cholesterol.

An increase of the HDL-C level can be achieved by inhibition of the *c*holesteryl *e*ster *t*ransfer *p*rotein (CETP) [3]. This protein mediates the exchange of cholesteryl esters from HDL to LDL (Fig. 1). The blockade therefore increases the efficiency of the reverse cholesterol transport.

2. Lead Candidate

A medicinal chemistry project aiming at the discovery and optimization of CETP inhibitors was started. The screening for active compounds was performed using a scintillation proximity assay: HDL loaded with radioactively labelled cholesteryl ester is exposed to CETP and tagged LDL. The tagged LDL is scavenged by a fluoro-bead

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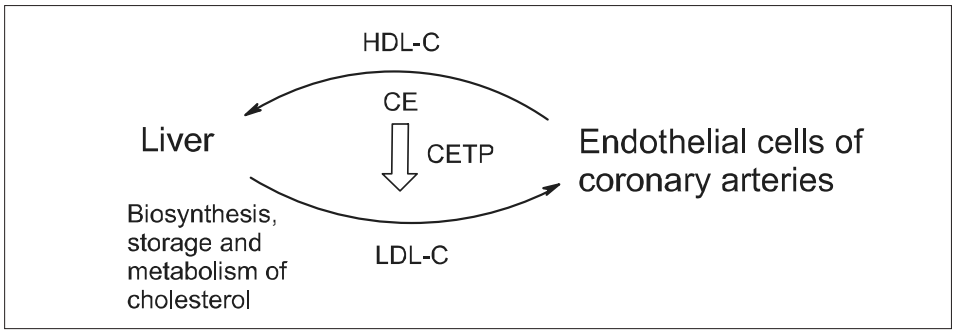


Fig. 1

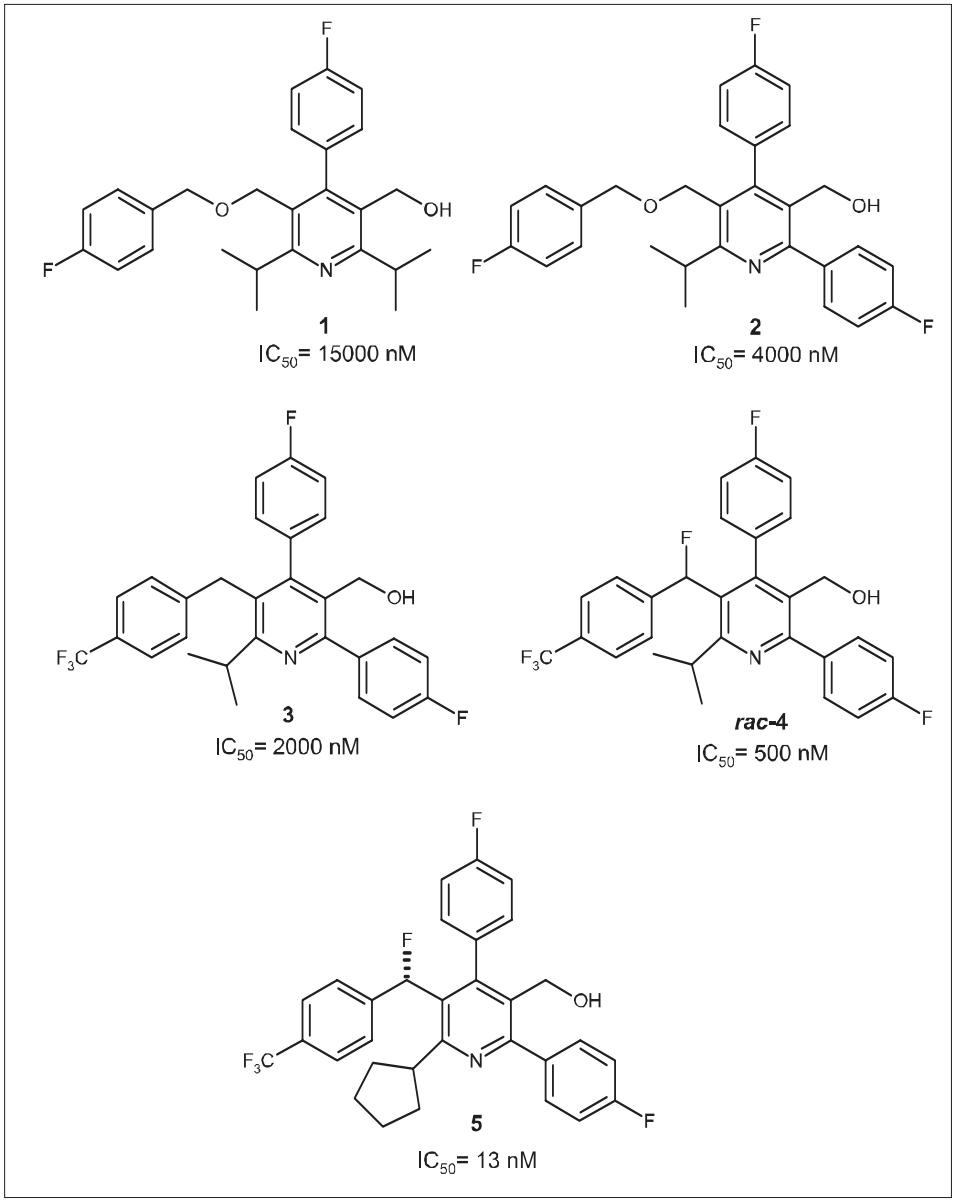


Fig. 2

via the biotin-streptavidin system. If a transfer of cholesteryl ester to the LDL particle has taken place the radioactivity is located in the vicinity of the fluoro-bead. This results in a light-signal which is detected in a scintillation counter. The first screening hit **1** displayed a potency of IC_{50} = 15 000 nM. During the early phase of the project a successful optimization delivered a highly potent lead compound **5**. Fluorine substitution played a key role in this process (Fig. 2).

A significant improvement was achieved by introduction of a second *p*-fluoro-phenyl-substituent instead of an isopropyl group leading to compound **2**. The exchange of the *p*-fluoro-phenyl substituent against a *p*-trifluoromethyl phenyl substituent accompanied by a shortening of the spacer (**3**) resulted in another increase of potency. The introduction of fluorine at the bis-benzylic bridge (***rac*-4**) led to a further improvement and the exchange of the isopropyl *versus* a cyclopentyl moiety produced a highly potent CETP inhibitor. In its enantiomerically pure form lead candidate **5** already has an inhibitory activity at CETP of IC_{50}= 13nM.

3. First Generation CETP-Inhibitors

However, the metabolic stability of this lead compound was insufficient. During the later phase of the project the optimization of this and other parameters was addressed in order to meet the requirements for a development candidate. The metabolism of the primary alcohol was tackled by rigidification in a bicyclic structure (Fig. 3). Although alkyl replacement of the right hand *p*-F-phenyl substituent (***rac*-6**) led to a complete loss of activity the rigidification of the alcohol group through cyclization gave a promising result, albeit at the expense of introducing another stereogenic center: The *anti*-diastereomer displays an IC_{50} of 100 nM as a racemate (***rac*-7**). Based on this new bicyclic core structure an intensive structure–activity screening by chemical derivatization was performed (Fig. 4). The *para*-fluoro substitution at the phenyl-substituent, commonly introduced to prevent metabolic *para*-hydroxylation, improves potency by a factor of three. The fluorine substituent in the bis-benzylic bridge leads to the most potent inhibitors as shown in Fig. 4. Another unique substituent was the trifluoromethyl group in *p*-position of the phenyl substituent. Probably due to optimal size and lipophilicity no other substituent gave a better result regarding potency. A breakthrough was achieved by methyl-substitution at the cyclohexyl moiety in 1,3 position to the hydroxy group (Fig. 4).

To avoid another stereogenic center a geminal dimethyl compound was prepared and proved to be equally active. The enantiomerically pure compound **8** was chosen as the first development candidate for CETP-inhibition (Fig. 5). The configuration of the alcoholic carbon center is crucial for high potency as isomer **9** demonstrates. The inversion of the fluorine bearing center as in compound ***ent*-9** leads to a more than twofold decrease in activity.

In order to provide sufficient quantities of material a technically feasible synthesis was established for candidate **8** (Scheme 1). After preparation of enamine **10** the reaction sequence starts with an unsymmetrical Hantzsch-dihydropyridine synthesis of compound **11**, followed by an oxidation to

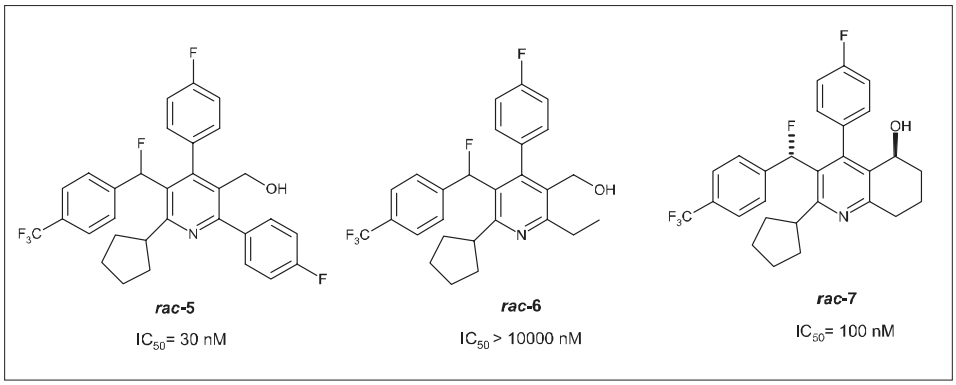


Fig. 3.

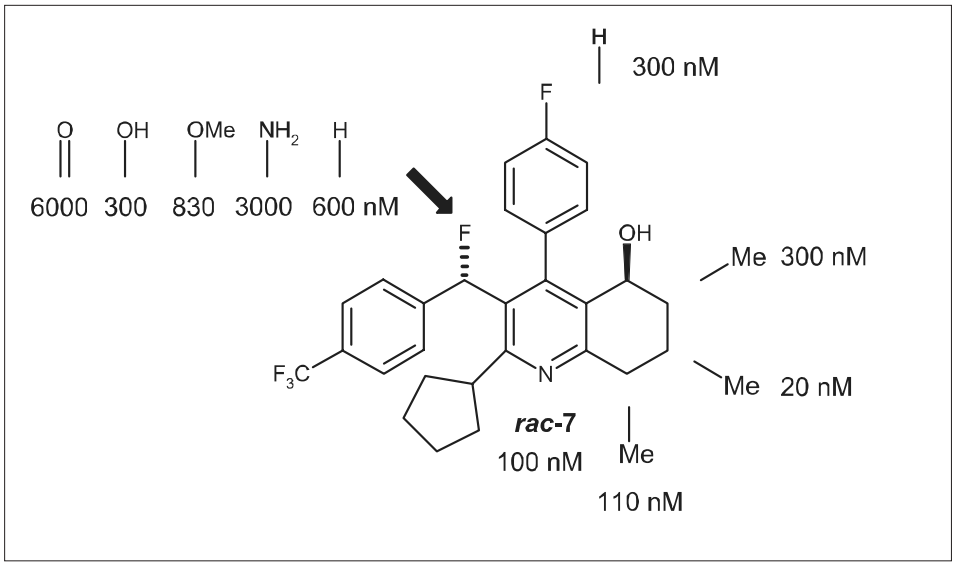


Fig. 4.

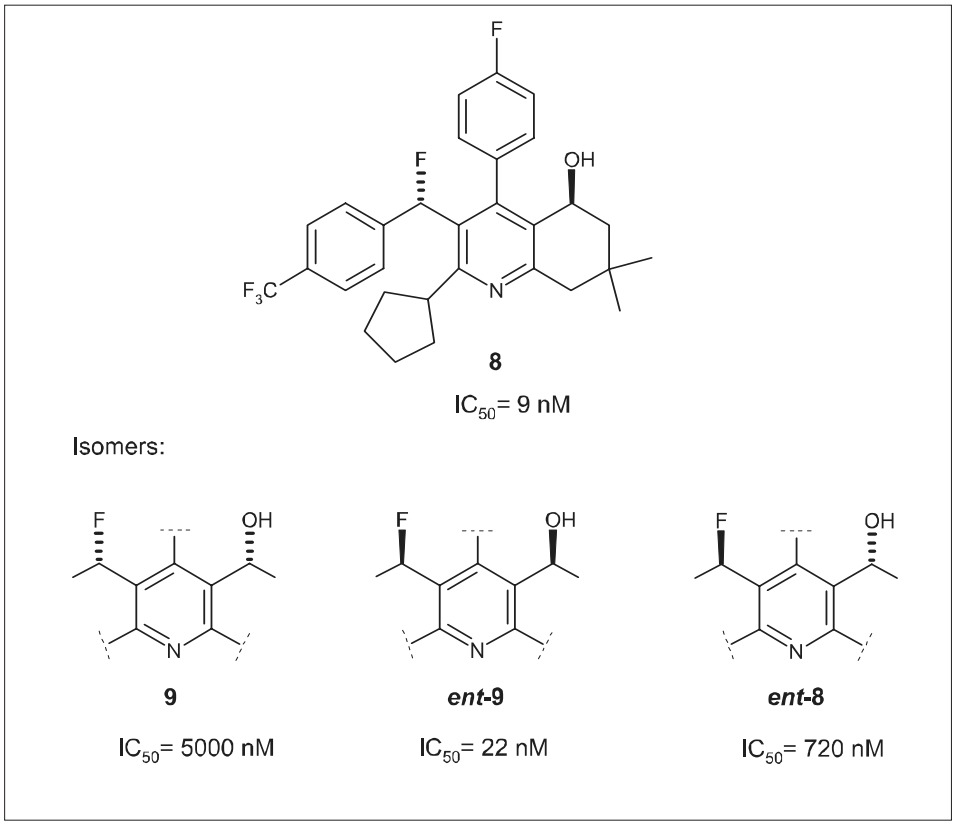


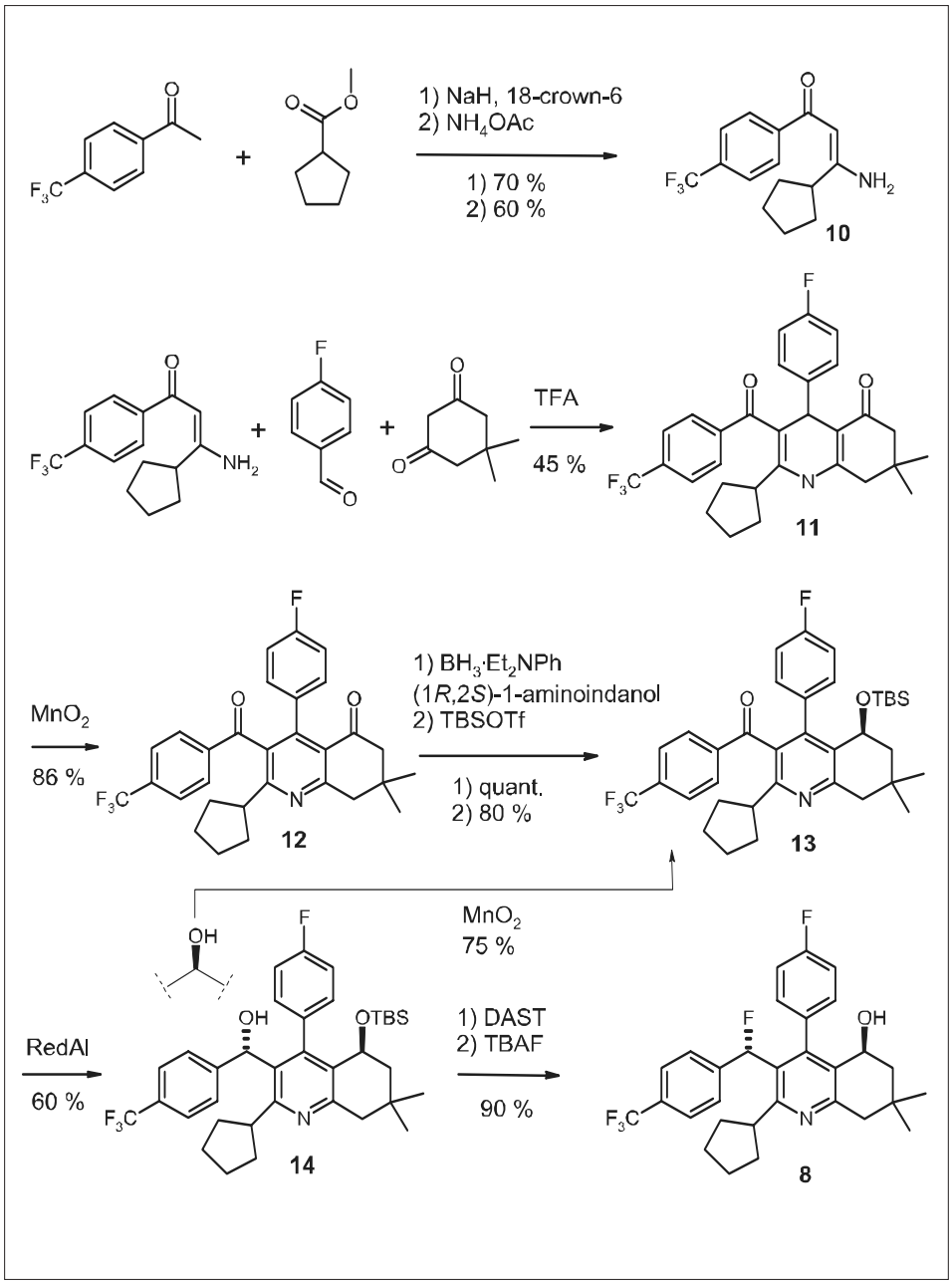
Fig. 5.

the pyridine **12**. Diketone **12** undergoes a completely regioselective CBS-type reduction [4] which in addition is highly enantioselective (95% ee). After TBS-protection of the resultant hydroxy function (**13**), the remaining keto group is reduced diastereoselectively in favour of the desired diastereomer **14** (75% ds) with RedAl®. The desired diastereomerically and enantiomerically pure compound **14** is isolated by crystallization in 60% yield. The mixture of diastereomers remaining in the mother liquor can be oxidatively recycled to the silyloxy ketone **13**. The mechanistic basis for the diastereoselectivity remains to be clarified. The inducing stereogenic center in this fairly rigid system is located at significant distance to the newly generated center. The role of the *p*-F-phenyl-substituent in the conveyance of stereo-information has to be elucidated. The direct and stereoselective replacement of the unprotected hydroxyl group was achieved by using diethylaminosulfur trifluoride (DAST) or the Ishikawa reagent (diethyl-1,1,2,3,3,3-hexa-fluoropropylamine) [5] as fluorinating reagent. In contrast to the usual S_N2 pathway of DAST-reactions, at this sterically very encumbered site, the reaction proceeds with retention of configuration [6]. This was proven by X-ray structures of candidate **8** and a close analog of compound **14**. The mechanism for this reaction is under investigation and will be published subsequently. Final TBAF or acidic silyl-deprotection provided the desired compound **8** in high overall yield.

With this compound in hand, a significant *in vivo* activity was demonstrated after oral administration. An increase in HDL-C by 35% and 50% was obtained in transgenic human-CETP mice after three days of treatment with 5 mg/kg *p.o.* and 10 mg/kg *p.o.*, respectively. The anti-atherogenic effect was proven in New Zealand white rabbits under high fat diet. After three months of treatment with compound **8** in food, a daily dose of 50 mg/kg *p.o.* reduced the atherosclerotic plaque area by 40%, a daily dose of 150 mg/kg *p.o.* reduced the plaque area by 70%. These results pave the way for a new approach towards treatment of atherosclerosis.

4. Second Generation CETP-Inhibitors

During the optimization program, it was revealed that carba-analogs of the pyridine compounds were even more potent CETP-inhibitors. A direct comparison of both classes is shown in Fig. 6. The second generation candidate **17** was therefore selected from this structural class of CETP-inhibitors.



Scheme 1.

The seemingly small chemical change in the core structure resulted in a considerable synthetic challenge. Several pathways were examined including Diels-Alder approaches, a carba-Hantzsch [7] pathway, and other strategies. But most successful was a new domino cyclization process [8] (Scheme 2). The introduction of the stereogenic centers was expected to be achieved analogous to the pyridine case. Therefore, retrosynthesis focused on the corresponding 1,5-diketone **25**.

Since the Mukaiyama-Michael addition is recognized as a very reliable method for the synthesis of 1,5-diketones, the following domino reaction was established for the one-step construction of the complete core structure: the enolate **20**, formed in the Mukaiyama-Michael addition of silyl enol ether **18** to cyclohexenone **19**, is submitted *in situ* to an intermolecular Michael addition to chalcone **21**. The newly generated enolate finally undergoes an intramolecular aldol addition to form a six-membered ring (**22**), thus generating the carbocyclic core in a one-pot procedure from readily accessible starting materials. The cyclohexanol **22** is isolated readily in good yield *via* crystallization as a single diastereomer (all C-substituents equatorial). All stereogenic centers are destroyed in the following aromatization. This is performed in a double halogenation–elimination sequence with the cyclohexadiene intermediate being oxidized *in situ* during the second elimination. The establishment of the stereogenic centers proceeds in analogy to the sequence developed for pyridine **12**, with minor modifications. This synthesis is performed in a kg-scale and is carried out without chromatographic purification. The final product **17** is isolated as a pure diastereomer and enantiomer (>99% ds, >99% ee after crystallization).

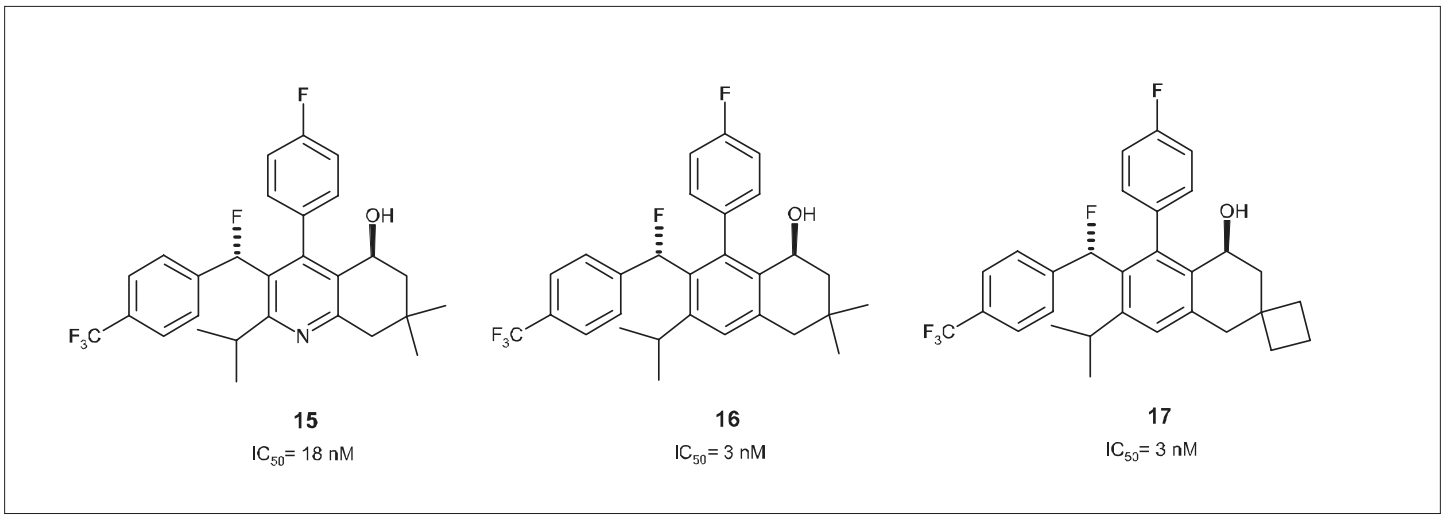
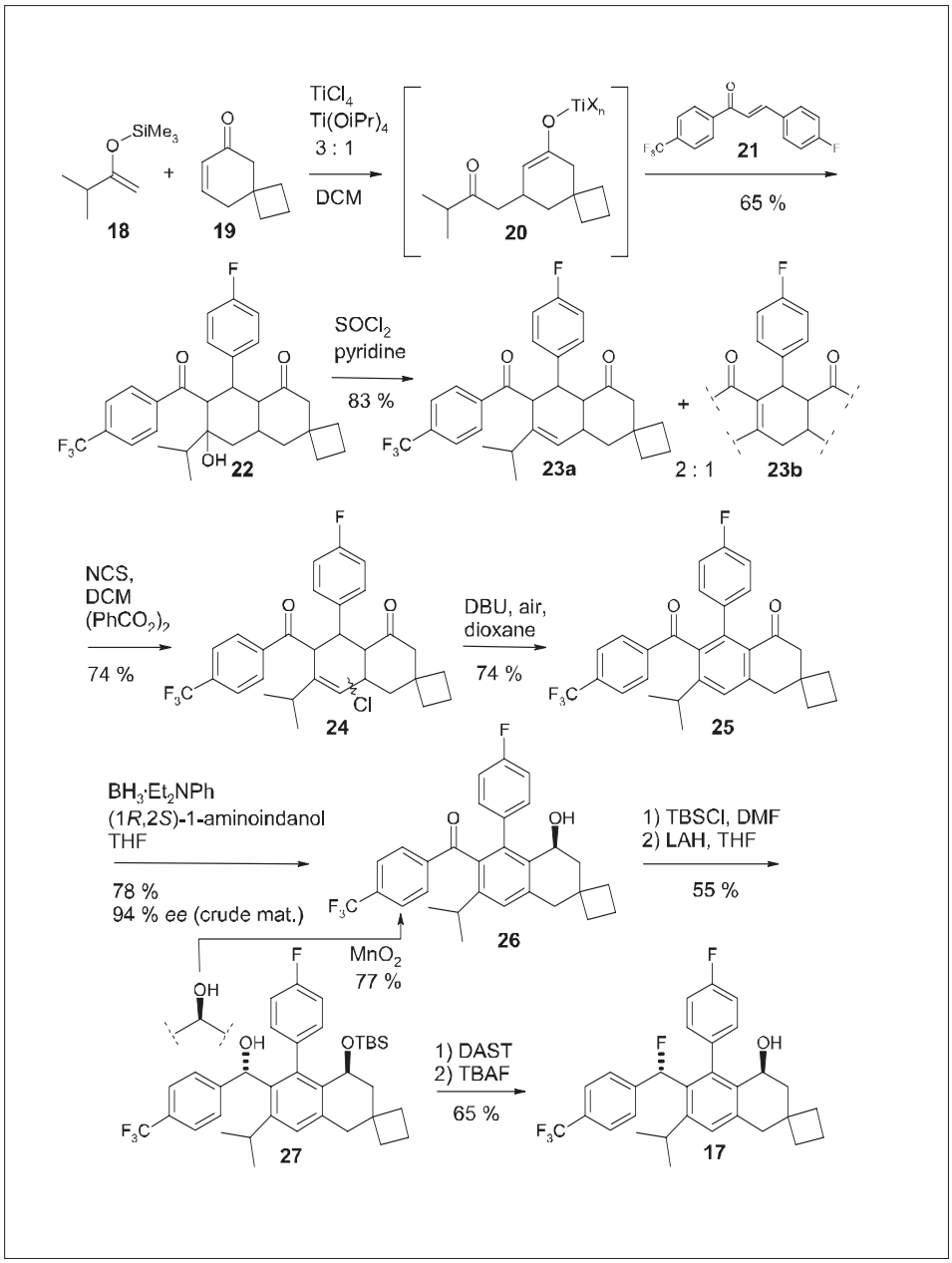


Fig. 6.



Scheme 2.

Significant HDL-C increase in hamsters and hCETP mice at doses of <1 mg/kg *p.o.* are achieved with this compound. A seven day treatment with 0.6 mg/kg of compound **17** administered in the food resulted in a 57% increase in HDL-C in human-CETP mice, proving the impressive *in vivo* potency of the second generation candidate.

5. Conclusion

In summary, CETP inhibition is a promising principle for the elevation of HDL-C and treatment of atherosclerosis. Starting from weak hits, extremely potent CETP inhibitors were discovered with the use of fluorine substitution. Significant synthetic challenges were overcome by newly invented synthetic methods.

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