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Dose-dependent antithrombotic activity of an orally active tissue factor/factor VIIa inhibitor without concomitant enhancement of bleeding propensity

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Abstract—The discovery of a highly potent and selective tissue factor/factor VIIa inhibitor is described. Upon oral administration of its double prodrug in the guinea pig, a dose-dependent antithrombotic effect is observed in an established model of arterial thrombosis without prolonging bleeding time. The pharmacodynamic properties of this selective inhibitor are compared to the behaviour of a mixed factor VIIa/factor Xa inhibitor.

1. Introduction

Thromboembolic disorders are among the leading causes for morbidity and mortality in the industrialized world. Thrombosis of either the venous or the arterial vascular system may cause pulmonary embolism, myocardial infarction or ischaemic stroke.[1] The development of new anticoagulant therapies therefore represents an important medical need. For a long period of time the coumarins have been the only orally active anticoagulants interfering with the coagulation cascade which have been available on the market. They act indirectly, inhibiting the γ-carboxylation in the biosynthesis of coagulation factors prothrombin, VII, IX and X. This mechanism leads to a slow on-set of action. Additionally, the coumarins suffer from substantial food and drug interaction and high protein binding[2], rendering it very difficult to maintain a balanced plasma exposure. Careful and regular monitoring of the patient is therefore required.[3] During the last decades substantial efforts have been devoted to the search for an orally bioavailable replacement of the coumarins. Out of numerous research programmes, the only compound to have reached the market is Ximelagatran from AstraZeneca, which is a double prodrug of the direct thrombin inhibitor Melagatran.[4] Ximelagatran has been launched in France in 2004 under the tradename Exanta, but in the meantime has been withdrawn from the market due to concerns about long-term liver damage and possible risk of heart attacks.

It is known that inhibition of the coagulation cascade at the final stages (factor Xa, thrombin) can lead to bleeding complications.[5] An alternative target would be the inhibition of the tissue factor/factor VIIa (TF/F.VIIa) complex which is the main trigger of thrombotic events.[6] This complex is part of the extrinsic pathway of the coagulation cascade and causes the activation of factor X and factor IX, ultimately resulting in the generation of thrombin and the thrombin-mediated conversion of fibrinogen to fibrin as well as platelet activation. Proof-of-concept experiments in animal models from our laboratories[7] and from other research groups[8] demonstrate that specific inhibition of the TF/F.VIIa complex results in an antithrombotic effect without enhancing bleeding propensity. These results demonstrated that selective inhibition of the extrinsic pathway of the coagulation might be a very promising mechanism for a novel anticoagulant and therefore stimulated many discovery programmes in the pharmaceutical industry aiming at the identification of low molecular weight TF/F.VIIa inhibitors.[9]

Recently, we have described the design, synthesis and profiling of the first highly potent, orally bioavailable

Keywords: Anticoagulant; Coagulation cascade; Tissue factor/factor VIIa inhibitor; Bleeding time prolongation; Double prodrug.
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TF/F.VIIa inhibitors from our laboratories.[10] Despite their promising oral activity and good half-life, a plasma exposure sufficient to achieve a sustained anticoagulant effect was not reached with these compounds. Here we report the design and profiling of an orally active TF/F.VIIa inhibitor which exhibits dose-dependent antithrombotic activity in a guinea pig model of recurrent arterial thrombosis. The efficacy and safety of this selective TF/F.VIIa inhibitor is compared with the pharmacodynamic behaviour of a competitor compound with mixed TF/F.VIIa and factor Xa (F.Xa) inhibitory activity.

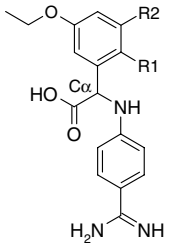
2. Results and discussion

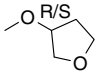
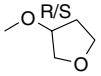
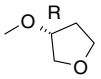
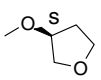
In a previous publication[10] compound **1** (Table 1) was described as a TF/F.VIIa inhibitor with moderate in vitro inhibitory activity and good selectivity against related serine proteases. It has moderate functional activity in the PT assay which is a measure for the ability of an inhibitor to prevent clotting via the extrinsic pathway of the coagulation cascade.[11] It is characterized by a promising pharmacokinetic profile in the rat (low clearance and volume of distribution, long half-life) and, when administered as amidoxime/ethylester double prodrug, by an excellent bioavailability ($F = 100\%$). Despite this excellent bioavailability, a very high dose would be required to cover the plasma exposure needed for sustained anticoagulant activity in vivo because the activity of the parent compound **1** is relatively low.

Extensive efforts focused on improving the functional activity of the phenylglycine class while maintaining their selectivity. The targeted value for PT activity lies around 2 μM. With respect to selectivity an aPTT/PT ratio above 4 was regarded as optimal to warrant low bleeding propensity and thus a large safety window. The aPTT (2x prolongation of activated partial thromboplastin time) serves as a measure for the ability of the inhibitor to interfere with the coagulation cascade via the intrinsic pathway.[11]

It was observed that upon introduction of a fluorine substituent in position 2 (compound **2**, Table 1), the in vitro binding affinity for F.VIIa remains unchanged, while the functional activity (2x PT) is improved by a factor 2. Previously it was found that a tetrahydrofuranyloxy substituent results in good binding affinity and functional activity as illustrated by compound **3**. Combination of both the fluorine substituent and the tetra-

Table 1. Inhibition of TF/F.VIIa by phenylglycine derivatives **1**–**6**



Compound	Configuration at C_α	R1	R2	K_i (μM)				Thrombin / F.VIIa	2x PT[a] (μM)	2x aPTT[b] (μM)	aPTT/PT
				F.VIIa	Thrombin	F.Xa	Trypsin				
1	RS	H	EtO	0.19	>35	5.6	16.9	>184	9.1	36.8	4.0
2	RS	F	EtO	0.20	8.8	3.0	6.8	44	4.4	21.7	4.9
3	RS	H		0.14	10.7	2.9	>6.8	77	5.5	20.6	3.7
4	RS	F		0.088	7.1	1.2	3.1	80	2.5	10.4	4.2
5	R	F		0.081	4.5	1.9	3.2	55	2.0	10.8	5.4
6	R	F		0.035	3.7	0.41	1.5	105	1.1	3.6	3.3

[a] Human citrated plasma is spiked with at least six concentrations of inhibitor. Clotting is initiated by addition of exogenous tissue factor (Innovin). Clotting time is determined by a turbidity measurement. The concentration of inhibitor necessary to double control clotting time is determined by fitting the data to an exponential regression.[11]

[b] Human citrated plasma is spiked with at least 6 concentrations of inhibitor. Clotting via the intrinsic pathway is initiated by addition of Actin® FS (ellagic acid in soy phosphatides). Clotting time is determined by a turbidity measurement. The concentration of inhibitor necessary to double control clotting time (EC_{50}) is determined by fitting the data to an exponential regression.[11]

hydrofuranyloxy moiety resulted in compound **4** with a functional activity approaching the targeted value. Compound **4** consists of 2 diastereomeric racemates which were separated into the 4 individual stereoisomers. The two isomers with configuration *S* at C_α of the phenylglycine moiety were almost inactive (data not shown). The two isomers with configuration *R* at C_α (compounds **5** and **6**) were both potent inhibitors and had excellent functional activity. The plasma potency of compound **6** even exceeds the targeted value of 2 μM for 2x PT prolongation. Its selectivity in the functional assays (aPTT/PT ratio) however is somewhat lower than that for compound **5**. This might be explained by the residual factor Xa inhibitory activity (K_i (F.Xa) = 410 nM) of compound **6**. Since F.Xa is located at the intersection of the intrinsic and the extrinsic pathways, its inhibition prolongs both PT and aPTT.

Since compound **5** fulfills both targeted requirements for functional activity and selectivity, it was chosen for further profiling. Its absolute configuration was corroborated by an X-ray structural analysis.

Figure 1 shows the X-ray analysis of compound **5** bound to the active site of TF/F.VIIa. The amidine forms the expected salt bridge with the carboxylate of Asp 189 at the bottom of the S1 pocket. A hydrogen bond is formed between the aniline NH and the hydroxyl group of the active site serine (Ser 195). The ethoxy group in position 5 of the phenylglycine moiety is located in the small S2 pocket of F.VIIa. It is arranged in an extended conformation. The fluorine points towards the solvent and is not involved in any specific interaction. Rather its presence most likely influences the conformation of the tetrahydrofuranyloxy substituent in such a way that it is rotated away from the fluorine substituent and thus is accommodated optimally in the shallow S3 pocket. A detailed discussion of the influence of the fluorine substituent on the in vitro and functional activity of the phenylglycine TF/F.VIIa inhibitors will be the subject of a further publication. The carboxylate is located in close proximity of the side chain of Lys 192 and might thus be engaged in a favorable electrostatic interaction, although Lys 192 is quite disordered in most X-ray crystal structures of complexes between F.VIIa and inhibitors.

The PK profile upon iv administration of compound **5** in the rat and the guinea pig (Table 2) is characterized by a long half-life (≈6 h) in the rat, a low clearance and a moderate volume of distribution (corresponding

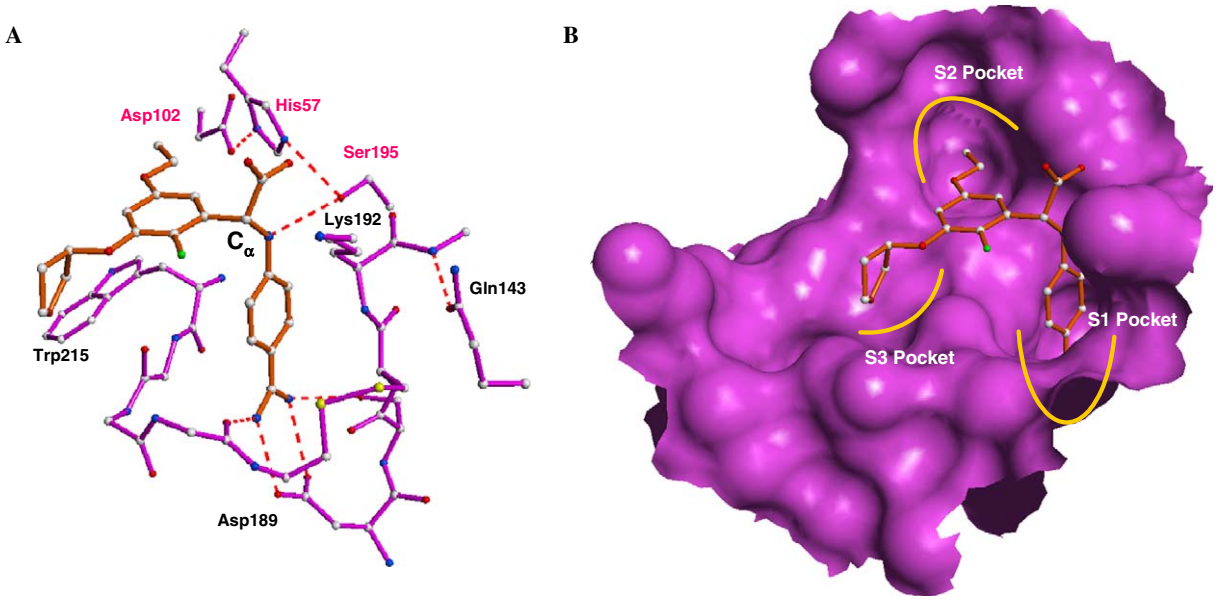


Figure 1. Crystal structure (2bz6.pdb) of compound **5** bound to the active site of F.VIIa. (A) Ball-and-stick representation. (B) Surface representation. Crystals of compound **5** in complex with short form recombinant F.VIIa were produced as described.[10] A crystal was frozen and measured on the Swiss Norwegian Beam Lines (SNBL) at the ESRF, Grenoble, to a resolution of 1.47 Å. Experimental details were: temperature 100 K, wavelength 0.80 Å, detector MAR300, pixel size 0.15 mm, detector distance 260 mm, frame size 0.5° and number of frames 173. Data were processed with XDS2000.[12] The unit cell is P41212, with $a = b = 94.93$ Å, $c = 115.64$ Å. Ice rings were observed at 3.90, 3.67, 3.44, 2.67, 2.25 and 1.91 Å and removed using the EXCLUDE_RESOLUTION_RANGE command. To 1.5 Å resolution, for 482714 observations of 69242 unique reflections the overall merging *R*-factor is 12.5% with 81.6% completeness and mean Intensity/standard deviation of 10.15, and in the outer shell from 1.6 to 1.5Å the *R*-factor is 80.7% with 36.5% completeness and mean Intensity/standard deviation of 1.78. The structure was refined, starting from coordinates of other in-house structures, initially using CNX[13] and then using Refmac5[14] and ARP/wARP[15] from the CCP4 program suite[16] with cycles of model building using Moloc.[17] The outer resolution limit was finally reduced to 1.6 Å and the inner cut-off of the outer ice-ring reduced in resolution from 1.92 to 1.94 Å. The final refinement cycles with Refmac5 used TLS and restrained refinement, and hydrogens were included at riding positions for amino acids as calculated by Refmac5, and for the chemical ligand as calculated by Moloc, but not for water molecules. For 307 amino acids (10 with alternative conformations), the ligand, one sulfate ion, one calcium ion and 500 water molecules there were 2434 hydrogen atoms and 2985 non-hydrogen atoms. For 58180 reflections (90.2% completeness to 1.60 Å), overall crystallographic *R*-factors were 16.6% (working) and 19.3% (free), with values in the outer shell (1.64–1.60 Å) of 25.3% and 27.4%, respectively. The inhibitor density is clear. The peptide Lys192-Gly193 is in the 'inactive' conformation previously reported.[10] Coordinates have been deposited in the Protein Data Bank as 2bz6.pdb.

to extracellular water) in both rat and guinea pig. As expected for a zwitterionic compound the bioavailability of **5** in the rat is very low. As described previously for compound **1**, charged functional groups (amidine, carboxylic acid) can be masked resulting in the amidoxime/ethyl ester double prodrug **7**. Such prodrug was shown to exhibit 20% oral bioavailability in the rat. The conversion efficiency from double prodrug to parent upon iv administration of **7** amounts to 50%.

A dose-dependent plasma concentration of **5** was observed upon po administration of **7** in the guinea pig. High doses between 60 and 500 mg/kg had to be administered to see a pharmacological effect in the guinea pig (Fig. 2) since the functional activity of **5** in guinea pig plasma is much lower than in human plasma (Table 3). Nevertheless a dose-dependent PT prolongation was observed as an in vivo effect, while aPTT remained almost unchanged even at the highest dose.

Compounds **5** and **7** were characterized in a guinea pig model of recurrent arterial thrombosis. Furthermore their propensity to enhance bleeding was assessed. In parallel both experiments were also performed with a mixed F.VIIa/F.Xa inhibitor, namely compound **8** from Ono (Table 3)[18], in order to confirm that selective F.VIIa inhibition ensures a large safety window. In this study, an established model of arterial thrombosis was used.[7a] Bleeding propensity was quantified by determining the nail cuticle bleeding time as previously described.[7a]

Upon continuous iv infusion of **5** and po administration of **7**, dose-dependent prolongation of PT was observed, while aPTT remains unaffected. Furthermore, a dose-dependent reduction of the thrombosis index, which is a measure for antithrombotic efficacy[7a], is observed, while nail cuticle bleeding is only slightly prolonged at the highest doses.

Upon continuous iv infusion of the Ono compound **8**, both PT and aPTT were prolonged to the same extent. An antithrombotic effect comparable to that of compound **5** was observed. The nail cuticle bleeding time however was prolonged substantially at higher doses.

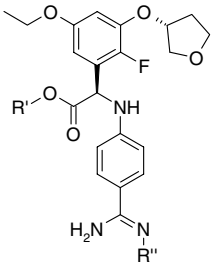
These results confirm previous findings from analogous experiments with an inactive tissue factor mutant hTFAA[7] and a small molecule inhibitor from Pfizer[8a,b] which demonstrated that a specific inhibitor of TF/F.VIIa can produce an antithrombotic effects, while only minimally disturbing hemostasis. It therefore seems that TF/F.VIIa is an especially promising target for the development of an anticoagulant drug. Furthermore, it was demonstrated that it is possible to produce an antithrombotic effect upon po administration of a double prodrug of an active TF/F.VIIa inhibitor which by itself is unsuitable for oral dosing. It remains to be shown how a double prodrug TF/F.VIIa inhibitor would perform in clinical investigations.

3. Chemistry

The fluorinated diethoxy derivative **2** was synthesized starting with the *tert*-butyldimethylsilyl protection of 4-fluorophenol (Scheme 1). Regioselective lithiation in the ortho position of the fluorine atom followed by reaction with trimethylborate and subsequent oxidation with hydrogen peroxide led to phenol **9**. The phenol was again protected with a *tert*-butyldimethyl silyl group. Regioselective lithiation in the ortho position of the fluorine substituent followed by quenching with DMF provided benzaldehyde **10**. In a one-pot procedure both silyl groups were removed with potassium fluoride and then alkylated with ethyl iodide. Benzaldehyde **11** was reacted in a Lewis-acid catalyzed condensation with benzylisonitrile and 4-aminobenzonitrile. The intermediate iminoether **12** was treated in situ with an excess of water and hydrolyzed to phenylglycine ester **13**. Conversion of the nitrile into the respective amidine by a Pinner reaction and hydrolysis of the ester functionality led to the zwitterionic phenylglycine derivative **2**.

The synthesis of the unfluorinated tetrahydrofuranyloxy derivative **3** started with the monoethylation of 5-hydroxymethyl-benzene-1,3-diol, followed by a benzylation of the remaining phenol and oxidation of the hydroxymethyl group to give benzaldehyde **14** which was condensed with 4-amino-benzonitrile and benzylisonitrile to

Table 2. Pharmacokinetic parameters of parent compound **5** as well as of the corresponding prodrug **7**



Compound	R′	R″	Rat					Guinea pig			
			Dose (mg/kg)	$T_{1/2}$ iv (h)	Cl (ml/min/kg)	V_{dss} (L/kg)	F (%)	Dose (mg/kg)	$T_{1/2}$ iv (h)	Cl (ml/min/kg)	V_{dss} (L/kg)
5	H	H	3	6.3	7.2	1.2	2	1	3.4	3.8	0.7
7	Et	OH					20				

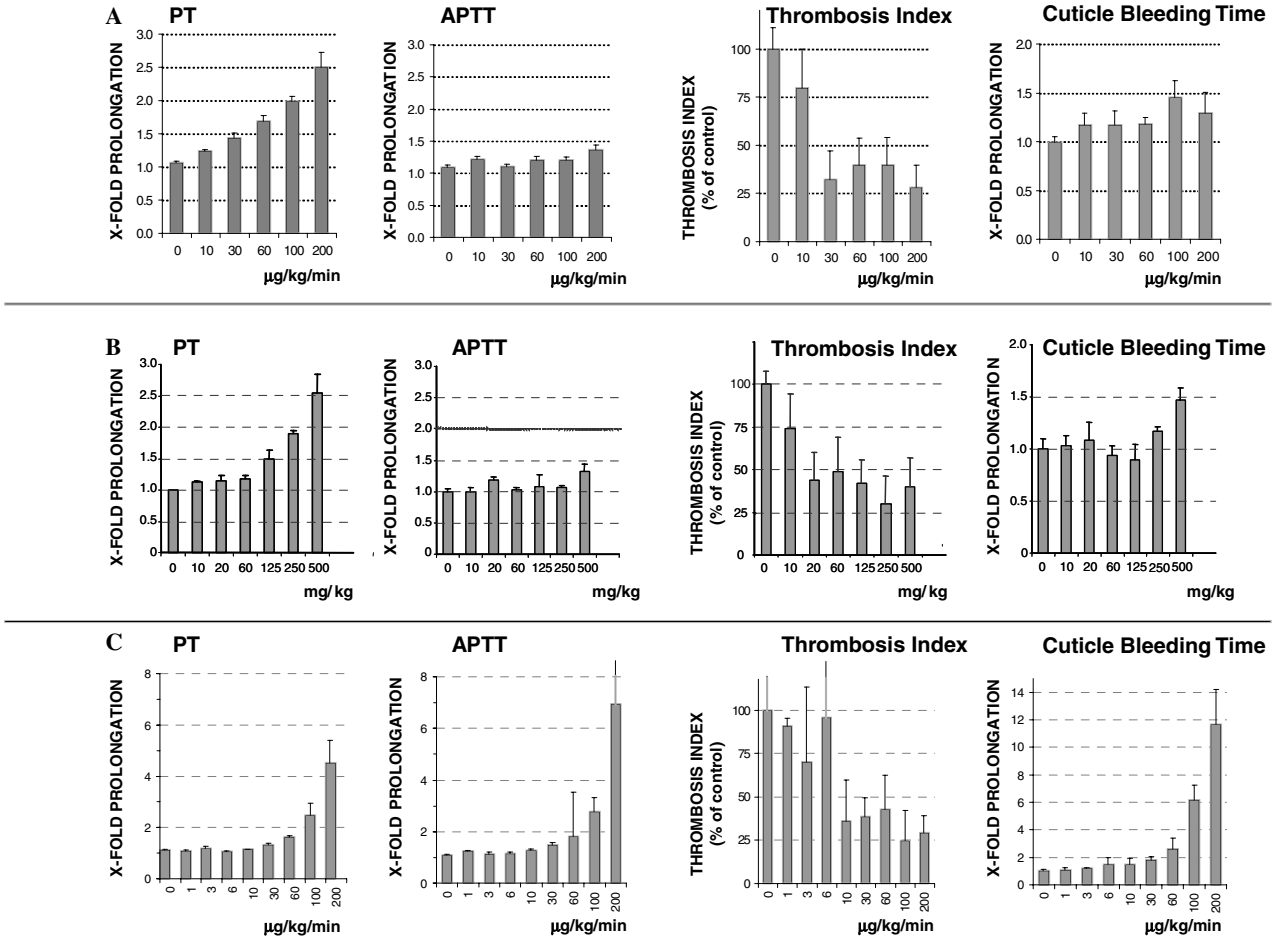
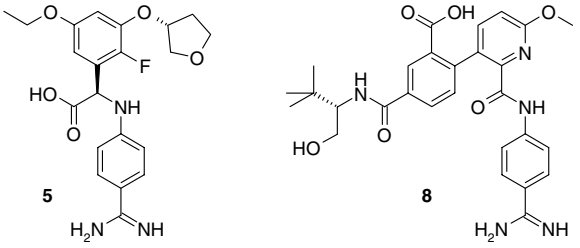


Figure 2. Dose-dependent pharmacodynamic effects (PT and aPTT prolongation), antithrombotic efficacy (expressed by percent reduction of thrombosis index[7]) and nail cuticle bleeding time reduction[7] in guinea pig ($n = 6$ per dose level) upon (A) iv administration of compound **5**; (B) po administration of compound **7**; (C); iv administration of compound **8**.

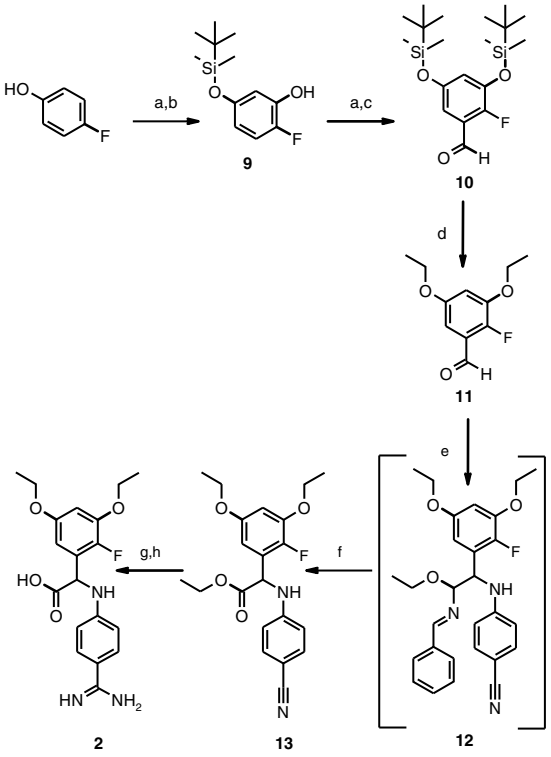
Table 3. Inhibitory activity of compounds **5** and **8** (mixed F.VIIa/F.Xa inhibitor from Ono) towards TF/F.VIIa and related serine proteases as well as in human and guinea pig plasma



Compound	K_i (μM)				Human plasma			Guinea pig plasma		
	F.VIIa	Thrombin	F.Xa	Trypsin	2x PT (μM)	2x aPTT (μM)	aPTT/PT	2x PT (μM)	2x aPTT (μM)	aPTT/PT
5	0.081	4.5	1.9	3.2	2.0	10.8	5.4	17	61	3.6
8	0.010	4.3	0.15	0.24	1.8	1.4	0.8	3.5	1.9	0.5

furnish phenylglycine ester **16** (Scheme 2). After removal of the benzyl group, phenol **17** was derivatized by a Mitsunobu reaction with (*RS*)-3-hydroxyfurane. Conversion of the nitrile into the respective amidine by a Pinner reaction and hydrolysis of the ester functionality led to the zwitterionic phenylglycine derivative **3**.

The synthesis of the fluorinated tetrahydrofuranyloxy compounds **4**–**7** started with the regioselective lithiation of 4-ethoxyfluorobenzene in the ortho position of the fluorine atom, quenching with trimethylborate and subsequent oxidation with hydrogen peroxide to give phenol **18**. After protection as *tert*-butyldimethyl silyl



Scheme 1. Preparation of fluorinated diethoxy derivative **2**. Reagents and conditions: (a) *t*-BDMSCl, imidazole, DMF, 0 °C; (b) i—*sec*-butyl lithium (1.3 M in hexanes), THF, −78 °C, 30 min; ii—$B(OMe)_3$, −78 °C, 30 min; iii—HOAc, H_2O_2, 0 °C → rt, overnight; (c) *sec*-butyl lithium (1.3 M in hexanes), DMF, THF, −78 °C; (d) ethyl iodide, KF, DMF, rt; (e) i—4-aminobenzonitrile, EtOH; ii—benzylisonitrile, BF_3–OEt_2, 0 °C; (f) 20 equiv H_2O; (g) HCl(gas), $CHCl_3$/MeOH 3:1, −10 °C; evaporate, then 2N NH_3 in MeOH; (h) LiOH, THF.

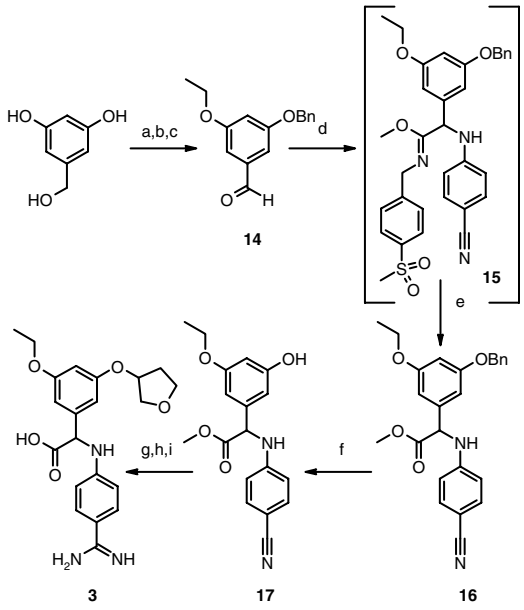
ether, compound **19** was lithiated regioselectively in the ortho position of the fluorine atom and quenched with DMF. Lewis-acid catalyzed condensation of benzaldehyde **20** with 4-aminobenzonitrile and 2-morpholinoethylisocyanide gave iminoether **21** which was hydrolyzed in situ with an excess of water to give phenylglycine ester **22**. The silyl-protecting group was lost under these reaction conditions.

Mitsunobu reaction of phenol **22** with (*RS*)-3-hydroxytetrahydrofurane, conversion to the respective amidine by a Pinner reaction and ester hydrolysis provided the zwitterionic product **4**.

The analogous reaction sequence using (*S*)-3-hydroxytetrahydrofurane in the Mitsunobu step led to derivative **24** which was separated into the active inhibitor **5** and its corresponding inactive epimer by chiral HPLC.

In an analogous manner using (*R*)-3-hydroxytetrahydrofuran in the Mitsunobu step the active inhibitor **6** and its corresponding inactive epimer were obtained.

Reaction of the nitrile **23** with hydroxylamine hydrochloride and subsequent separation of the epimeric mixture by chiral HPLC led to the double prodrug **7**.



Scheme 2. Preparation of unfluorinated tetrahydrofuranyl derivative **3**. Reagents and conditions: (a) ethylbromide, K_2CO_3, DMF, 60 °C; (b) benzylbromide, K_2CO_3, DMF, 65 °C; (c) MnO_2, 1,2-dichloroethane, 50 °C; (d) i—**14**, 4-aminobenzonitrile, MeOH; ii—toluene-4-sulfonylisocyanide, BF_3-OEt_2, 0 °C; (e) 20 equiv H_2O; (f) H_2, Pd/C, THF, EtOH; (g) 3-hydroxy-tetrahydrofurane, PPh_3, DEAD, THF; (h) HCl(gas), $CHCl_3$/MeOH 3:1, −10 °C; evaporate, then 2 N NH_3 in MeOH; i—LiOH, THF.

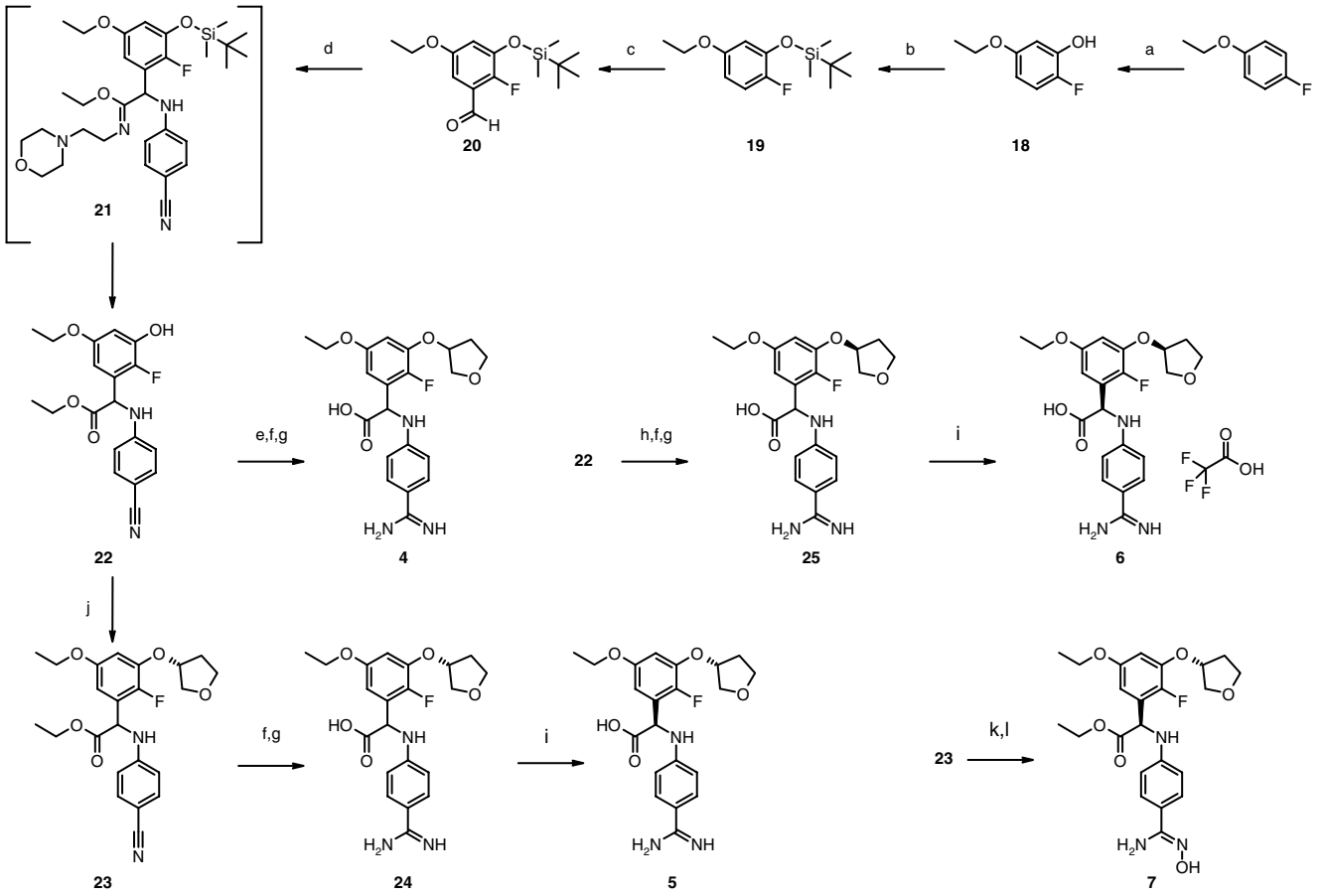
4. Experimental

4.1. General

All reagents and solvents were purchased from Fluka and used directly without further purification. Column chromatography was performed on Merck Kieselgel 60 (230–400 mesh) silica gel. ^{1}H NMR spectra were obtained using a Bruker Avance 300 MHz instrument. Chemical shifts are reported in parts per million (δ) relative to TMS using residual chloroform or dimethyl sulfoxide as an internal reference. Coupling constants (J) are reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartett; m, multiplet; br, broad. ESI mass spectra were recorded on an Sciex API 150 instrument from Applied Biosystems using Analyst 1.3 software. EI mass spectra were recorded on a Finnigan MAT SSQ 7000 instrument using Xcalibur software.

4.2. Synthesis of (*RS*)-(4-carbamimidoyl-phenylamino)-(3,5-diethoxy-2-fluoro-phenyl)-acetic acid (2) (Scheme 1)

4.2.1. 5-(*tert*-Butyl-dimethyl-silanyloxy)-2-fluoro-phenol (9). A solution of 4-fluorophenol (50 g, 0.45 mol) in DMF (225 ml) was cooled under an argon atmosphere to 0 °C and treated with *tert*-butyldimethylsilyl chloride (73.86 g, 0.49 mol). Then imidazole (33.36 g, 0.49 mol) was added slowly. A colourless precipitate formed. The reaction mixture was stirred for 1 h. Then H_2O (360 ml) was added. The solution was extracted with *n*-hexane. The organic layers were washed with water, 10% Na_2CO_3 solution and again water, then dried over



Scheme 3. Preparation of fluorinated tetrahydrofuranyl derivatives **4**–**7**. Reagents and conditions: (a) i—pentamethyldiethylentriamine, *n*-butyllithium (1.6 M in hexanes), THF, −78 °C, 2 h; ii—$B(OMe)_3$, −78 °C → rt, 2 h; iii—AcOH, 0 °C, 30 min; H_2O_2 (aq, 35%), 0 °C → rt, overnight; (b) *t*-BDMSCl, imidazole, DMF, 0 °C; (c) i—pentamethylethylenetriamine, *n*-butyllithium (1.6 M in hexanes), THF, −78 °C → −50 °C, 5 h; ii—DMF, −78 °C → rt, overnight; (d) i—**20**, 4-aminobenzonitrile, EtOH, 1 h, rt; ii—2-morpholinoethylisocyanide, then BF_3–OEt_2, 0 °C → rt, 3 h; iii—20 equiv H_2O, 50 °C, overnight; (e) (*RS*)-3-hydroxy-tetrahydrofurane, PPh_3, DEAD, THF; (f) HCl(gas), $CHCl_3$/MeOH 3:1, −10 °C; evaporate, then 2 N NH_3 in MeOH; (g) LiOH, THF; (h) (*R*)-3-hydroxy-tetrahydrofurane, PPh_3, DEAD, THF; (i) separation of epimers on ChiralPak AD with heptane/isopropanol/trifluoroacetic acid 75:25:0.2 as eluent; (j) (*S*)-3-hydroxy-tetrahydrofurane, PPh_3, DEAD, THF; (k) H_2N-OH hydrochloride, TEA, EtOH, rt; (l) separation of epimers on ChiralPak AD with heptane/isopropanol/trifluoroacetic acid 70:30:0.2 as eluent.

$MgSO_4$ and filter. The filtrate was concentrated and dried to give *tert*-butyl-(4-fluoro-phenoxy)-dimethyl-silane (92.22 g, 91%) as a colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.01 (6 H, s, CH_3), 0.80 (9H, s, *t*-butyl), 6.59 (2H, dd, J = 4.6 Hz, 9.1 Hz, 2-CH, 2′-CH), 6.73 (2H, t, J = 9.1 Hz, 3-CH, 3′-CH).

A solution of *tert*-butyl-(4-fluoro-phenoxy)-dimethyl-silane (50.0 g, 0.22 mol) in THF (220 ml) was cooled under an argon atmosphere to −65 °C and treated within 30 min with *sec*-butyllithium in hexane (1.3 M, 188.5 ml, 0.245 mol). The obtained off-white suspension was stirred for 30 min at −65 °C. Then a solution of trimethylborate (25 ml, 0.224 ml) in THF (45 ml) was added within 30 min. The reaction mixture was stirred for 30 min at −65 °C, then warmed to 0 °C and treated with acetic acid (19 ml, 0.332 mol). Hydrogen peroxide solution (35% in water, 21 ml, 0.244 mol) was added within 30 min. A white precipitate formed. After the addition of H_2O (500 ml), the obtained solution was extracted with Et_2O. The combined organic layers were washed with H_2O, 10% NaOH solution and again H_2O, then dried over $MgSO_4$. The crude product was purified by chromatography on silica gel using cyclohexane/EtOAc 18:1 as eluent. The title compound **9** (38.86 g, 73%) was obtained as a yellow liquid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.00 (6H, s, CH_3), 0.77 (9H, s, *t*-butyl), 6.01 (1H, dt, J = 3.2 Hz, 8.7 Hz, 4-CH), 6.28 (dd, J = 2.9 Hz, 7.6 Hz, 6-CH), 6.81 (1H, dd, J = 8.7 Hz, 11.2 Hz, 3-CH), 9.61 (1H, s br, OH); MS (ESI) *m*/*z* 240.5 ([M−H]$^-$, 100%).

4.2.2. 3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-2-fluoro-benzaldehyde (10). Using the same procedure as described for the preparation of *tert*-butyl-(4-fluoro-phenoxy)-dimethyl-silane (Section 4.2.1, first reaction step), compound **9** (4.5 g, 18.6 mmol) was reacted with *tert*-butyldimethylsilyl chloride (3.1 g, 20.4 mmol) and imidazole (1.4 g, 20.4 mmol) in DMF (10 ml) to give 2,4-bis-(*tert*-butyl-dimethyl-silanyloxy)-1-fluoro-benzene (6.54 g, 99%) as a light yellow liquid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.00 (6H, s, CH_3), 0.01 (6H, s, CH_3), 0.77 (9H, s, *t*-butyl), 0.79 (9H, s, *t*-butyl), 6.24 (1H, dd, J = 2.7 Hz, 7.4 Hz, 3-CH), 6.27 (dt,

J = 3.0 Hz, 8.5 Hz, 5-CH), 6.91 (1H, dd, J = 8.7 Hz, 10.8 Hz, 6-CH); MS (ESI) m/z 355.2 ($[M-H]^-$).

A solution of 2,4-bis-(*tert*-butyl-dimethyl-silanyloxy)-1-fluoro-benzene (6.3 g, 17.7 mmol) in THF (18 ml) was cooled to −70 °C and treated within 1 h with *sec*-butyllithium solution in hexane (1.3 M, 15 ml, 19.4 mmol). Then DMF (1.5 ml) in THF (3 ml) was added within 30 min. Stirring was continued for 1 h at −70 °C and 90 min at rt, followed by the addition of H_2O (30 ml). The reaction mixture was extracted with Et_2O. The combined organic layers were washed with H_2O, dried over $MgSO_4$, filter and concentrated to give **10** (6.3 g, 92%) as a yellow liquid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.18 (6H, s, CH_3), 0.20 (6H, s, CH_3), 0.93 (9H, s, *t*-butyl), 0.96 (9H, s, *t*-butyl), 6.78 (2H, m), 10.12 (1H, s, CHO); MS (ESI) m/z 385.2 2 ($[M+H]^+$).

4.2.3. 3,5-Diethoxy-2-fluoro-benzaldehyde (11). A solution of **10** (3.0 g, 7.8 mmol) in DMF (25 ml) was treated with potassium fluoride (1.81 g, 31.2 mmol) and ethyl iodide (1.51 ml, 18.72 mmol). The reaction mixture was stirred for 2 h at rt, and then quenched with H_2O (25 ml). The obtained suspension was extracted with Et_2O. The combined organic layers were washed with H_2O, dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography on silica gel using a gradient of cyclohexane/EtOAc 18:1 → 1:1 as eluent to give **11** (904 mg, 55%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.33 (3H, t, J = 7.0 Hz, CH_3), 1.35 (3H, t, J = 7.0 Hz, CH_3), 4.05 (2H, q, J = 7.0 Hz, CH_2), 4.14 (2H, q, J = 7.0 Hz, CH_2), 6.77 (1H, dd, J = 3.0 Hz, 4.2 Hz, ar-CH), 7.03 (1H, dd, J = 3.0 Hz, 7.4 Hz, ar-CH), 10.19 (1H, s, CHO).

4.2.4. (4-Cyano-phenylamino)-(3,5-diethoxy-2-fluoro-phenyl)-acetic acid ethyl ester (13). To a solution of **11** (880 mg, 4.2 mmol) in 15 ml EtOH were added under an argon atmosphere 4-aminobenzonitrile (493 mg, 4.2 mmol) and 4 Å molecular sieves. The reaction mixture was cooled to 0 °C and treated with benzyl isonitrile (0.51 ml, 4.2 mmol), followed by the slow addition of 1.57 ml of boron trifluoride diethyl etherate (1.57 ml, 12.5 mmol) while maintaining a temperature between 0 °C and 5 °C. The reaction was stirred for 15 min at 0 °C and for 90 min at rt, then filtered and concentrated. The obtained iminoether **12** was dissolved in EtOH (15 ml) and treated with H_2O (1.5 ml). After stirring at rt overnight, a precipitate had formed. It was filtered off, washed with cold EtOH/H_2O 1:1 and dried to give **13** (1.15 g, 72%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.15 (3H, t, J = 7.1 Hz, CH_3), 1.30 (3H, t, J = 7.0 Hz, CH_3), 1.35 (3H, t, J = 7.0 Hz, CH_3), 3.98 (2H, q, J = 7.0 Hz, CH_2), 4.09 (4H, m, 2× CH_2), 5.49 (1H, d, J = 8.2 Hz, CH_α), 6.51 (1H, dd, J = 2.9 Hz, 4.6 Hz, ar-CH), 6.66 (1H, dd, J = 2.7 Hz, 6.9 Hz, ar-CH), 6.76 (2H, d, J = 8.7 Hz, ar-CH), 7.33 (1H, d, J = 8.2 Hz, NH), 7.48 (2H, d, J = 8.7 Hz, ar-CH); MS (EI) 386 ($[M]^+$).

4.2.5. (4-Carbamimidoyl-phenylamino)-(3,5-diethoxy-2-fluoro-phenyl)-acetic acid (2). A solution of **13** (500 mg, 1.3 mmol) in $CHCl_3$/EtOH 3:1 (5 ml) was cooled to −15 °C. A stream of dry HCl gas was passed through the solution for 15 min. The flask was stoppered well and stored at 4 °C overnight. Then, the reaction mixture was concentrated. The residue was taken up in EtOH (4 ml) and treated with 2 M NH_3 in EtOH (5.2 ml, 10.4 mmol). The mixture was heated for 2 h at 60 °C and then concentrated. The crude product was purified by chromatography on silica gel using a gradient of CH_2Cl_2/MeOH 19:1 → 2:1 as eluent to give (4-carbamimidoyl-phenylamino)-(3,5-diethoxy-2-fluoro-phenyl)-acetic acid ethyl ester hydrochloride (535 mg, 94%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.14 (3H, t, J = 7.0 Hz, CH_3), 1.28 (3H, t, J = 6.8 Hz, CH_3), 1.33 (3H, t, J = 6.8 Hz, CH_3), 3.95 (2H, q, J = 7.0 Hz, CH_2), 4.09 (4H, m, 2× CH_2), 5.56 (1H, d, J = 8.2 Hz, CH_α), 6.53 (1H, s br, ar-CH), 6.66 (1H, d, J = 6.5 Hz, ar-CH), 6.82 (2H, d, J = 8.4 Hz, ar-CH), 7.39 (1H, d, J = 8.2 Hz, NH), 7.64 (2H, d, J = 8.4 Hz, ar-CH), 8.77 (4H, br, H_2N^+=C–NH_2); MS (ESI) m/z 404.5 ($[M+H]^+$, 100%).

To a suspension of (4-carbamimidoyl-phenyl-amino)-(3,5-diethoxy-2-fluoro-phenyl)-acetic acid ethyl ester hydrochloride (320 mg, 0.73 mmol) in THF (4 ml) was added 1 N LiOH solution (3.6 ml). The reaction mixture was stirred for 3 h at rt and then neutralized with 1 N HCl solution. The precipitate was filtered off and dried to give **2** (234 mg, 86%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) ^{1}H NMR δ = 1.28 (3H, t, J = 7.0, CH_3), 1.34 (3H, t, J = 7.0, CH_3), 3.95 (2H, q, J = 7.0 Hz, CH_2), 4.08 (2H, q, J = 7.0, CH_2), 5.46 (1H, s, CH_α), 6.53 (1H, t br, ar-CH), 6.64 (1H, dd, J = 2.5 Hz, 6.7 Hz, ar-CH), 6.86 (2H, d, J = 8.7 Hz, ar-CH), 7.64 (2H, d, J = 8.7 Hz, ar-CH), 8.72 (<2H, s, H_2N^+=C–NH_2), 8.93 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 376.5 ($[M+H]^+$, 100%).

4.3. Synthesis of (4-carbamimidoyl-phenylamino)-[3-ethoxy-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid (3) (Scheme 2)

4.3.1. 3-Benzyloxy-5-ethoxy-benzaldehyde (14). To a solution of 3,5-dihydroxy benzylalcohol (125 g, 0.89 mol) in DMF (1250 ml) were added under an argon atmosphere K_2CO_3 (246.6 g, 1.78 mol) and ethylbromide (73.2 ml, 0.98 mol). The suspension was stirred for 4 h at 65 °C, then filter. The filtrate was concentrated. The residue was taken up in EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by chromatography on silica gel using hexane/EtOAc 3:1 as eluent to give 3-ethoxy-5-hydroxymethyl-phenol (45.8 g, 31%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.28 (3H, t, J = 7.0 Hz, CH_3), 3.93 (2H, q, J = 7.0 Hz, CH_2), 4.35 (2H, d br, J = 4.2 Hz, CH_2-OH), 5.06 (1H, t br, J = 5.3, *HO*-CH_2), 6.15 (1H, t, J = 2.2 Hz, ar-CH), 6.30 (2H, m, ar-CH), 9.27 (1H, s br, ar-OH). MS (EI) m/z 168 ($[M]^+$).

To a solution of 3-ethoxy-5-hydroxymethyl-phenol (45.8 g, 0.27 mol) in DMF (470 ml) were added under an argon atmosphere K_2CO_3 (75.3 g, 0.54 mol) and ben-

zylbromide (35.6 ml, 0.30 mol). The yellow suspension was stirred at 65 °C for 4 h. The reaction mixture was concentrated, then taken up in EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by chromatography using hexane/EtOAc 4:1 as eluent to give (3-benzyloxy-5-ethoxy-phenyl)-methanol (58.7 g, 83%) as a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.39 (3H, t, J = 7.0 Hz, CH_3), 1.67 (1H, t, J = 6.0, OH), 4.01 (2H, q, J = 7.0 Hz, CH_2), 4.62 (2H, d, J = 6.0 Hz, -CH_2-OH), 5.04 (2H, s, Bn-CH_2), 6.46 (1H, t, J = 2.3 Hz, ar-CH), 6.52 (1H, m, ar-CH), 6.60 (1H, m, ar-CH), 7.31–7.44 (5H, m br, Bn-CH); MS (EI) m/z 258 ([M]$^+$).

A solution of (3-benzyloxy-5-ethoxy-phenyl)-methanol (58.7 g, 0.23 mol) in 1,2-dichloroethane (1180 ml) was treated under an argon atmosphere with manganese(IV) oxide (71.1 g, 0.81 mol). The black suspension was stirred overnight at 50 °C and then filtered over Celite. The filtrate was concentrated to give 47.3 g (81%) of **14** as a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.43 (3H, t, J = 7.0 Hz, CH_3), 4.06 (2H, q, J = 7.0 Hz, CH_2), 5.10 (2H, s, Bn-CH_2), 6.78 (1H, t, J = 2.3 Hz, ar-CH), 7.01 (1H, m, ar-CH), 7.08 (1H, m, ar-CH), 7.34–7.41 (5H, m br, Bn-CH), 9.89 (1H, s, CHO); MS (EI) m/z 256 ([M]$^+$).

4.3.2. (3-Benzyloxy-5-ethoxy-phenyl)-(4-cyano-phenylamino)-acetic acid methyl ester (16). A solution of **14** (47.3 g, 0.18 mol) in MeOH (900 ml) was treated under an argon atmosphere with 4-aminobenzonitrile (21.8 g, 0.18 mol). The yellow solution was stirred for 2 h at rt, then treated with toluene-4-sulfonylisocyanide (36.0 g, 0.18 mol) and cooled to 0 °C. Then, boron trifluoride diethyl etherate (69.5 ml, 0.55 mol) was added within 20 min, maintaining the temperature below 5 °C. The reaction mixture was stirred at 0 °C for 10 min and at rt for 1 h, then concentrated. The residue was taken up in 660 ml MeOH and 66 ml H_2O, heated until the material was completely dissolved and then cooled to rt. The suspension was stirred for 1 h and then filter. The remaining solid was recrystallized from MeOH/hexane to give **16** (49.1 g, 64%) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.30 (3H, t, J = 6.9 Hz, CH_3), 3.65 (3H, s, CH_3-O), 3.99 (2H, q, J = 6.9 Hz, CH_2), 5.06 (2H, s, Bn-CH_2), 5.32 (1H, d, J = 7.8, CH_α), 6.52 (1H, t, J = 2.1, ar-CH), 6.64 (1H, t br, ar-CH), 6.73 (1H, t br, ar-CH), 6.78 (2H, d, J = 8.7, ar-CH), 7.31–7.47 (7H, m, ar-CH, Bn-CH).

4.3.3. (4-Cyano-phenylamino)-(3-ethoxy-5-hydroxy-phenyl)-acetic acid methyl ester (17). To a solution of **16** (24.9 g, 59.8 mmol) in EtOH/THF 1:1 (700 ml) was added Pd/C 10% (2.5 g). The reaction mixture was hydrogenated at 1.1 bar and rt for 5 h. The catalyst was filtered off and washed with EtOH. The filtrate was concentrated. The crude product was purified by chromatography n silica gel using hexane/EtOAc 3:1 as eluent to give **17** (13.0 g, 67%) as a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.39 (3H, t, J = 7.0, CH_3(OEt)), 3.76 (3H, s, CH_3-O), 3.97 (2H, q, J = 7.0 Hz, CH_2), 4.95 (1H, d, J = 5.4 Hz, CH_α), 4.97 (1H, s, OH), 5.51 (1H, d, J = 5.4 Hz, NH), 6.35 (1H, t, J = 2.3 Hz, ar-CH), 6.49 (1H, m, ar-CH), 6.51 (2H, d, J = 8.8 Hz, ar-CH), 6.58 (1H, m, ar-CH), 7.37 (2H, d, J = 8.8 Hz, ar-CH); MS (ESI) m/z 327.3 ([M+H]$^+$, 100%).

4.3.4. (4-Carbamimidoyl-phenylamino)-[3-ethoxy-5-(tetra-hydrofuran-3-yloxy)-phenyl]-acetic acid (3). A solution of **17** (1.50 g, 4.6 mmol), 3-hydroxy-tetrahydrofurane (0.51 ml, 6.3 mmol) and triphenylphosphine (1.87 g, 7.1 mmol) in THF (30 ml) was treated with diethyl azodicarboxylate (1.11 ml, 7.1 mmol). The reaction mixture was stirred for 5 h and then concentrated. The crude product was purified by column chromatography using cyclohexane/EtOAc 4:1 as eluent to give (4-cyano-phenylamino)-[3-ethoxy-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid methyl ester (2.0 g, 110%, contains EtOOC-NH-NH-COOEt as impurity). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.30 (3H, t, J = 6.9 Hz, CH_3), 1.92 (1H, m, tetrahydropyrane-CH_2), 2.22 (1H, m, THP-CH_2), 3.53–3.94 (4H, m, THP-CH_2O), 3.99 (2H, q, J = 6.9, CH_2), 5.04 (1H, t br, J = 4.8 Hz, THP-CH), 5.33 (1H, d, J = 8.0 Hz, CH_α), 6.40 (1H, t, J = 2.1 Hz, ar-CH), 6.61 (1H, t br, ar-CH), 6.64 (1H, t br, ar-CH), 6.78 (2H, d, J = 8.7 Hz, ar-CH), 7.32 (1H, d, J = 8.2 Hz, NH), 7.46 (2H, d, J = 8.7 Hz, ar-CH).

A solution of (4-cyano-phenylamino)-[3-ethoxy-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid methyl ester (2.0 g, 5.0 mmol) in $CHCl_3$/EtOH 3:1 (22 ml) was cooled to −15 °C. A stream of dry HCl gas was passed through the solution for 15 min. The flask was stoppered well and stored at 4 °C overnight. Then, the reaction mixture was concentrated. The residue was taken up in EtOH (24 ml) and treated with 2 M NH_3 in EtOH (7.0 ml, 14.0 mmol). The mixture was heated for 5 h at 65 °C and then concentrated. The crude product was purified by chromatography on silica gel using CH_2Cl_2/MeOH 19:1 as eluent to give (4-carbam-imidoyl-phenylamino)-[3-ethoxy-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid methyl ester hydrochloride (800 mg, 35%) as an off-white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.35 (3H, t, J = 6.8 Hz, CH_3), 2.05–2.19 (2H, m, THP-CH_2), 3.73 (3H, s, CH_3), 3.73–3.98 (6H, m, CH_2-O), 4.87 (1H, m, CH_α), 5.00 (1H, m, THP-CH), 5.78 (1H, m, NH), 6.31 (1H, s, ar-CH), 6.51–6.58 (4H, m, ar-CH), 7.67 (2H, d, J = 8.4 Hz, ar-CH), 8.10 (<2H, s br, H_2N^+=C–NH_2), 8.72 (<2H, s br, H_2N^+=C–NH_2); MS (ESI) m/z 414.4 ([M+H]$^+$, 100%).

To a suspension of (4-carbamimidoyl-phenylamino)-[3-ethoxy-5-(tetrahydrofuran-3-yloxy)-phenyl]-acetic acid methyl ester hydrochloride (800 mg, 1.8 mmol) in THF (8 ml) was added 1N LiOH solution (5.3 ml). The reaction mixture was stirred for 1 h at rt, then neutralized with 1 N HCl solution and then concentrated. The crude product was purified by chromatography on RP-18 silica gel using a gradient of 0–40% MeCN in H_2O to give **3** (330 mg, 47%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.30 (3H, t, J = 6.9 Hz, CH_3), 1.93 (1H, m, tetrahydropyrane-CH_2), 2.21 (1H, m, THP-CH_2), 3.70–3.90 (4H,

m, THP-CH_2O), 3.98 (2H, q, J = 6.9, CH_2), 4.98 (1H, t br, THP-CH), 5.26 (1H, s, CH_α), 6.39 (1H, t br, ar-CH), 6.64 (1H, t br, ar-CH), 6.67 (1H, t br, ar-CH), 6.82 (2H, d, J = 8.8 Hz, ar-CH), 7.62 (2H, d, J = 8.8 Hz, ar-CH), 8.68 (<2H, s, H_2N^+=C–NH_2), 8.91 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 400.5 ($[M+H]^+$, 100%).

4.4. Synthesis of (4-cyano-phenylamino)-(5-ethoxy-2-fluoro-3-hydroxy-phenyl)-acetic acid ethyl ester (22) (Scheme 3)

4.4.1. 5-Ethoxy-2-fluoro-phenol (18). A solution of 4-ethoxy-1-fluoro-benzene (56.3 g, 0.4 mol) in THF (200 ml) was treated under an argon atmosphere with pentamethyl-diethylentriamine (84 ml, 0.4 mol) and cooled to −78 °C. *n*-Butyllithium solution (1.6 M in hexane, 251 ml, 0.4 mol) was added slowly. The reaction mixture was stirred for 3 h at −78 °C. Then, trimethylborate (89.6 ml, 0.8 mol) was added slowly. The mixture was stirred for 15 min at −78 °C, then for 2 h at rt, then cooled to 0 °C and treated with acetic acid (63.2 ml, 1.1 mol). Hydrogen peroxide solution (30% in water, 68.3 ml, 0.6 mol) was added slowly at 0 °C. The reaction was warmed to rt and stirred overnight. After cooling to 0 °C, saturated Na_2SO_3 solution (200 ml), then 300 ml H_2O and 500 ml hexane were added. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, filtered and concentrated to give **18** (60.3 g, 96%) as a light brown liquid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.28 (3H, t, J = 7.0 Hz, CH_3), 3.91 (2H, q, J = 7.0 Hz, CH_2), 6.29 (1H, dt, J = 3.2 Hz, 8.9 Hz, 4-CH), 6.47 (1H, dd, J = 3.0 Hz, 7.6 Hz, 6-CH), 7.00 (1H, dd, J = 8.9 Hz, 11.2 Hz, 3-CH), 9.79 (1H, s, OH).

4.4.2. *tert*-Butyl-(5-ethoxy-2-fluoro-phenoxy)-dimethyl-silane (19). Using the same procedure as described for the preparation of *tert*-butyl-(4-fluoro-phenoxy)-dimethyl-silane (Section 4.2.1, first reaction step), compound **18** (75.5 g, 0.48 mol) was reacted with *tert*-butyldimethylsilyl chloride (80.2 g, 0.53 mol) and imidazole (36.2 g, 0.53 mol) in DMF (250 ml) to give **19** (127.0 g, 97%) as a light brown liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.00 (3H, s, CH_3-Si), 0.01 (3H, s, CH_3-Si), 0.80 (s, 9H, *tert*-butyl-CH_3), 1.19 (3H, t, J = 7.0 Hz, CH_3), 3.75 (2H, q, J = 7.0 Hz, CH_2), 6.21 (1H, dt, J = 3.2 Hz, 9.0 Hz, 4-CH), 6.27 (1H, dd, J = 2.9 Hz, 7.1 Hz, 6-CH), 6.73 (1H, dd, J = 8.9 Hz, 10.3 Hz, 3-CH).

4.4.3. 3-(*tert*-Butyl-dimethyl-silanyloxy)-5-ethoxy-2-fluoro-benzaldehyde (20). To a solution of **19** (43.3 g, 0.16 mol) in THF (160 ml) was added under an argon atmosphere pentamethyl-diethylentriamine (66.9 ml, 0.32 mol). After cooling the mixture to −78 °C, *n*-butyllithium solution (1.6 M in THF, 200 ml, 0.32 mol) was added within 1 h. The obtained viscous suspension was stirred for 5 h at a temperature between −50 and −60 °C. After cooling again to −78 °C, DMF (24.7 ml, 0.32 mol) was added within 20 min. The clear yellow solution was stirred overnight, warming to rt. The reaction was quenched with ice and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated to give **20** (51.4 g, 107%, contains impurities) as a light yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.22 (6H, s, CH_3-Si), 1.01 (9H, s, *t*-butyl-CH_3), 1.40 (3H, t, J = 7.0 Hz, CH_3), 3.95 (2H, q, J = 7.0 Hz, CH_2), 6.83 (2H, m, ar-CH), 10.28 (1H, s, CHO).

4.4.4. (4-Cyano-phenylamino)-(5-ethoxy-2-fluoro-3-hydroxy-phenyl)-acetic acid ethyl ester (22). To a solution of **20** (16.9 g, 56.6 mmol) was added 4-aminobenzonitrile (6.7 g, 56.6 mmol). The reaction mixture was stirred for 1 h at rt, then treated with 2-morpholinoethyl isocyanide (7.8 ml, 56.6 mmol) and cooled to 0 °C. Boron trifluoride diethyl etherate (28.4 ml, 226.3 mmol) was added slowly, maintaining the temperature between 0 °C and 5 °C. The reaction mixture was stirred for 15 min at 0 °C and for 3 h at rt. Then, 20 ml H_2O was added. The mixture was heated to 50 °C overnight and then concentrated. The residue was taken up in water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filter and concentrated. The crude product was purified by chromatography on silica gel using cyclohexane/EtOAc 2:1 as eluent to give **22** (12.9 g, 64%) as a viscous oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.14 (3H, J = 7.0 Hz, CH_3), 1.27 (3H, t, J = 6.9 Hz, CH_3), 3.88 (2H, q, J = 6.9 Hz, CH_2), 4.07–4.21 (2H, m, CH_2), 5.45 (1H, d, J = 8.0 Hz, CH_α), 6.38 (1H, t br, J = 3.6 Hz, ar-CH), 6.45 (1H, dd, J = 2.8 Hz, 7.0 Hz, ar-CH), 6.76 (2H, d, J = 8.5 Hz, ar-CH), 7.31 (1H, d, J = 8.2 Hz, NH), 7.48 (2H, d, J = 8.2 Hz, ar-CH), 10.02 (1H, s, OH); MS (ESI) m/z 357.1 ($[M-H]^-$)

4.5. Synthesis of (4-carbamimidoyl-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydro-furan-3- yloxy)-phenyl]-acetic acid (4) (Scheme 3)

A solution of **22** (358 mg, 1.0 mmol), 3-hydroxy-tetrahydrofurane (106 mg, 1.2 mmol) and triphenylphosphine (315 mg, 1.2 mmol) in THF (10 ml) was cooled under an argon atmosphere to 0 °C and treated with diethyl azodicarboxylate (0.187 ml, 1.2 mmol). The reaction mixture was stirred for 3 h and then concentrated. The crude product was purified by column chromatography using cyclohexane/EtOAc 1:1 as eluent to give (4-cyano-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid ethyl ester (405 mg, 95%, contains according to ^{1}H NMR EtOOC-NH-NH-COOEt (H_2-DEAD) as impurity). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.14 (3H, t, J = 6.9, CH_3), 1.17 (t, J = 6.9, H_2-DEAD-CH_3), 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.22 (1H, m, THP-CH_2), 3.71–3.91 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 4.03 (q, J = 6.9 Hz, H_2-DEAD-CH_2), 4.15 (2H, m, CH_2), 5.09 (1H, t br, THP-CH), 5.49 (1H, d, J = 8.2 Hz, CH_α), 6.55 (1H, dd, J = 2.9 Hz, 4.6 Hz, ar-CH), 6.65 (1H, dd, J = 2.8 Hz, 6.9 Hz, ar-CH), 6.77 (2H, d, J = 8.7 Hz, ar-CH), 7.33 (1H, d, J = 8.3 Hz, ar-CH), 7.48 (2H, d, J = 8.7 Hz, ar-CH), 8.98 (1H, H_2-DEAD-NH); MS (EI) m/z 428.1 ($[M]^+$).

A solution of (4-cyano-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid ethyl ester (400 mg, 0.93 mmol) in $CHCl_3$/EtOH 3:1 was

cooled under an argon atmosphere to −10 °C. A stream of dry HCl gas was passed through the solution for 15 min. The flask was stoppered well and stored at 4 °C overnight. Then, the reaction mixture was concentrated. The residue was taken up in EtOH (2 ml) and treated with 2 M NH_3 in EtOH (2.4 ml, 4.7 mmol). The mixture was heated for 2 h at 60 °C and then concentrated. The product was isolated by chromatography on silica gel using $CHCl_3$/MeOH 4:1 as eluent to give (4-carbamimidoyl-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid ethyl ester hydrochloride (135 mg, 30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.14 (3H, t, J = 6.9 Hz, CH_3), 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.71–3.89 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 4.16 (2H, m, CH_2), 5.09 (1H, t br, THP-CH), 5.56 (1H, d, J = 8.2 Hz, CH_α), 6.57 (1H, t br, ar-CH), 6.66 (1H, dd, J = 2.8 Hz, 6.9 Hz, ar-CH), 6.83 (2H, d, J = 8.7 Hz, ar-CH), 7.40 (1H, d, J = 8.3 Hz, NH), 7.64 (2H, d, J = 8.7 Hz, ar-CH), 8.64 (<2H, s, H_2N^+=C–NH_2), 8.89 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 446.3 ([M+H]$^+$, 100%).

A solution of (4-carbamimidoyl-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydro-furan-3-yloxy)-phenyl]-acetic acid ethyl ester hydrochloride (95 mg, 0.20 mmol) in THF (2.5 ml) was cooled to 0 °C and treated with 1 N LiOH solution (0.98 mg, 0.98 mmol). The reaction mixture was stirred for 2 h and then neutralized with 1 N HCl. The precipitate was filtered and dried to give **4** (62 mg, 76%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.72–3.90 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.46 (1H, s, CH_α), 6.57 (1H, m br, ar-CH), 6.63 (1H, dd, J = 2.8 Hz, 6.7 Hz, ar-CH), 6.80 (2H, d, J = 8.7 Hz, ar-CH), 7.63 (2H, d, J = 8.7 Hz, ar-CH), 8.67 (<2H, s, H_2N^+=C–NH_2), 8.91 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

4.6. Synthesis of (*R*)-(4-carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*R*)-(tetrahydro-furan-3-yl)oxy]-phenyl}-acetic acid (5) (Scheme 3)

4.6.1. (4-Cyano-phenylamino)-{3-ethoxy-2-fluoro-5-[(*R*)-(tetrahydro-furan-3- yl)oxy]-phenyl}-acetic acid ethyl ester (23). Using the same procedure as described for the preparation of (4-cyano-phenylamino)-[3-ethoxy-2-fluoro-5-(tetrahydrofuran-3-yloxy)-phenyl]-acetic acid ethyl ester (Section 4.5, first reaction step), compound **22** (7.38 g, 20.6 mmol) was reacted with (*S*)-3-hydroxytetrahydrofurane (1.68 ml, 24.7 mmol), triphenylphosphine (6.48 g, 24.7 mmol) and diethyl azodicarboxylate (3.84 ml, 24.7 mmol) in THF (220 ml) to give **23** (9.37 g, >100%, contains according to ^{1}H NMR EtOOC-NH-NH-COOEt (H_2-DEAD) as impurity). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.14 (3H, t, J = 6.9 Hz, CH_3), 1.17 (t, J = 6.9 Hz, H_2-DEAD -CH_3), 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.22 (1H, m, THP-CH_2), 3.71–3.91 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9, CH_2), 4.03 (q, J = 6.9 Hz, H_2-DEAD-CH_2), 4.15 (2H, m, CH_2), 5.09 (1H, t br, THP-CH), 5.49 (1H, d, J = 8.2 Hz, CH_α), 6.55 (1H, dd, J = 2.9 Hz, 4.6 Hz, ar-CH), 6.65 (1H, dd, J = 2.8 Hz, 6.9 Hz, ar-CH), 6.77 (2H, d, J = 8.7 Hz, ar-CH), 7.33 (1H, d, J = 8.3 Hz, NH), 7.48 (2H, d, J = 8.7 Hz, ar-CH), 8.98 (1H, H_2-DEAD -NH); MS (EI) m/z 428.1 ([M]$^+$).

4.6.2. (4-Carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*R*)-(tetrahydro-furan-3-yl)oxy]-phenyl}-acetic acid (24). Using the same procedure as described for the preparation of **4** (Section 4.5, second and third reaction step), **23** (6 g, 14 mmol) was converted to **24** (3.13 g, 54% over two steps). ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.72–3.90 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.46 (1H, s, CH_α), 6.57 (1H, m br, ar-CH), 6.63 (1H, dd, J = 2.8 Hz, 6.7 Hz, ar-CH), 6.80 (2H, d, J = 8.7 Hz, ar-CH), 7.63 (2H, d, J = 8.7 Hz, ar-CH), 8.67 (<2H, s, H_2N^+=C–NH_2), 8.91 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

4.6.3. (*R*)-(4-Carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*R*)-(tetra-hydro-furan-3-yl)-oxy]-phenyl}-acetic acid (5). The epimeric mixture **24** (3.0 g, 7.2 mmol) was separated by chiral HPLC, using ChiralPak AD as stationary phase and heptane/isopropanol/TFA 75:25:0.2 as eluent. The two fractions containing the respective epimers were worked up separately. After distilling off the eluent, the residue was taken up several times in H_2O and evaporated completely to remove residual TFA. Then, the remaining solid was taken up in water and neutralized with 1N LiOH solution. The precipitate was collected, washed with water and diethyl ether and dried to give **5** (first eluting fraction, 922 mg, 31%) as a white solid, along with its epimer (*S*)-(4-carbamimidoyl-phenylamino)-{3-etho-xy-2-fluoro-5-[(*R*)-(tetrahydro-furan-3-yl)-oxy]-phenyl}-acetic acid (second eluting fraction, 1.20 g, 40%) as a white solid.

Compound **5**: ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.72–3.90 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.46 (1H, s, CH_α), 6.57 (1H, m br, ar-CH), 6.63 (1H, dd, J = 2.8 Hz, 6.7 Hz, ar-CH), 6.80 (2H, d, J = 8.7 Hz, ar-CH), 7.63 (2H, d, J = 8.7 Hz, ar-CH), 8.67 (<2H, s, H_2N^+=C–NH_2), 8.91 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

4.7. Synthesis of (*R*)-(4-carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*S*)-(tetra- hydrofuran-3-yl)oxy]-phenyl}-acetic acid trifluoro acetate (6) (Scheme 3)

4.7.1. (4-Carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*S*)-(tetrahydro- furan-3-yl)oxy]-phenyl}-acetic acid (25). Using the same procedure as described for the preparation of **24** (Section 4.5), compound **22** was first reacted with (*R*)-3-hydroxytetrahydrofurane and then converted to **25**. White solid. ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.29 (3H,

t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.72–3.90 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.46 (1H, s, CH_α), 6.57 (1H, m br, ar-CH), 6.63 (1H, dd, J = 2.8 Hz, 6.7 Hz, ar-CH), 6.80 (2H, d, J = 8.7 Hz, ar-CH), 7.63 (2H, d, J = 8.7 Hz, ar-CH), 8.67 (<2H, s, H_2N^+=C–NH_2), 8.91 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

4.7.2. (*R*)-(4-Carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*S*)-(tetra-hydro-furan-3-yl)-oxy]-phenyl}-acetic acid trifluoro acetate (6). The epimeric mixture **25** (128 mg, 0.31 mmol) was separated by chiral HPLC, using ChiralPak AD as stationary phase and heptane/isopropanol/TFA 75:25:0.2 as eluent. The two fractions containing the respective epimers were worked up separately. After distilling off the eluent, the residue was taken up several times in H_2O and evaporated completely to remove residual TFA. Then, the remaining solid was taken up in water and lyophilized to give **6** (first eluting fraction, 66 mg, 41%) as a white amorphous solid, along with its epimer (*S*)-(4-carbamimidoyl-phenylamino)-{3-ethoxy-2-fluoro-5-[(*S*)-(tetrahydro-furan-3-yl)-oxy]-phenyl}-acetic acid trifluoro acetate (second eluting fraction, 80 mg, 49%) as a white solid.

Compound **6**: ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop DCl 20 wt% in D_2O) δ = 1.29 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.72–3.90 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.46 (1H, s, CH_α), 6.57 (1H, dd, J = 2.9 Hz, 4.8 Hz, ar-CH), 6.63 (1H, dd, J = 2.9 Hz, 6.8 Hz, ar-CH), 6.81 (2H, d, J = 8.9, ar-CH), 7.64 (2H, d, J = 8.9 Hz, ar-CH), 8.73 (<2H, s, H_2N^+=C–NH_2), 8.93 (<2H, s, H_2N^+=C–NH_2); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

4.8. Synthesis of (*R*)-[5-ethoxy-2-fluoro-3-[(*R*)-tetrahydro-furan-3-yloxy]-phenyl]-[4-(*N*- hydroxycarbamimidoyl)-phenyl-amino]-acetic acid ethyl ester (7) (Scheme 3)

To a solution of **24** (12.46 g, 29.1 mmol) in EtOH (500 ml) were added hydroxylamine hydrochloride (20.21 g, 291 mmol) and triethylamine (81.07 ml, 582 mmol). The reaction mixture was stirred overnight at rt and then concentrated. The crude product was purified by chromatography on silica gel using CH_2Cl_2/MeOH 20:1 as eluent to give [5-ethoxy-2-fluoro-3-[(*R*)-tetrahydro-furan-3-yloxy]-phenyl]-[4-(*N*-hydroxy-car-bamimidoyl)-phenyl-amino]-acetic acid ethyl ester (7.64 g, 58%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.13 (3H, t, J = 6.9 Hz, CH_3), 1.22 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.71–3.89 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.41 (1H, d, J = 8.7 Hz, CH_α), 5.55 (2H, s br, H_2N), 6.55 (1H, d, J = 8.7 Hz, NH), 6.58–6.65 (4H, m, ar-CH), 7.38 (2H, d, J = 8.7 Hz, ar-CH), 9.26 (1H, s, OH); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

The epimeric [5-ethoxy-2-fluoro-3-[(*R*)-tetrahydro-fu-ran-3-yloxy]-phenyl]-[4-(*N*-hydroxycarbamimidoyl)-phenyl-amino]-acetic acid ethyl ester (8.1 g, 17.6 mmol) was separated by chiral HPLC, using ChiralPak AD as stationary phase and heptane/isopropanol/TFA 70:30:0.2 as eluent. The two fractions containing the respective epimers were worked up separately. After distilling off the eluent, the residue was taken up in EtOAc and washed with 10% $KHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, concentrated and dried to give **7** (first eluting fraction, 3.0 g, 37%) as an off-white amorphous solid, along with its epimer (*S*)-[5-ethoxy-2-fluoro-3-[(*R*)-tetrahydro-furan-3-yloxy]-phenyl]-[4-(*N*-hydroxycarbamimidoyl)-phenyl-amino]-acetic acid ethyl ester (second eluting fraction, 3.21 g, 40%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.13 (3H, t, J = 6.9 Hz, CH3), 1.22 (3H, t, J = 6.9 Hz, CH_3), 1.98 (1H, m, THP-CH_2), 2.23 (1H, m, THP-CH_2), 3.71–3.89 (4H, m, THP-CH_2O), 3.95 (2H, q, J = 6.9 Hz, CH_2), 5.08 (1H, t br, THP-CH), 5.41 (1H, d, J = 8.7 Hz, CH_α), 5.55 (2H, s br, H_2N), 6.55 (1H, d, J = 8.7 Hz, NH), 6.58–6.65 (4H, m, ar-CH), 7.38 (2H, d, J = 8.7 Hz, ar-CH), 9.26 (1H, s, OH); MS (ESI) m/z 418.3 ([M+H]$^+$, 100%).

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