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Original article

Structure–activity relationships of imidazole-derived 2-[*N*-carbamoylmethyl-alkylamino]acetic acids, dual binders of human insulin-degrading enzyme

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ABSTRACT

Insulin degrading enzyme (IDE) is a zinc metalloprotease that degrades small amyloid peptides such as amyloid-â and insulin. So far the dearth of IDE-specific pharmacological inhibitors impacts the understanding of its role in the physiopathology of Alzheimer's disease, amyloid-â clearance, and its validation as a potential therapeutic target. Hit **1** was previously discovered by high-throughput screening. Here we describe the structure-activity study, that required the synthesis of 48 analogues. We found that while the carboxylic acid, the imidazole and the tertiary amine were critical for activity, the methyl ester was successfully optimized to an amide or a 1,2,4-oxadiazole. Along with improving their activity, compounds were optimized for solubility, lipophilicity and stability in plasma and microsomes. The docking or co-crystallization of some compounds at the exosite or the catalytic site of IDE provided the structural basis for IDE inhibition. The pharmacokinetic properties of best compounds **44** and **46** were measured *in vivo*. As a result, **44 (BDM43079)** and its methyl ester precursor **48 (BDM43124)** are useful chemical probes for the exploration of IDE's role.

Abbreviations used: AcOH, acetic acid; ANP, Atrial natriuretic peptide; Boc, tert-butoxycarbonyl; CH_3CN, acetonitrile; DCE, dichloroethane; DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EDCI, N-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Et_3N, triethylamine; EtOAc, ethyl acetate; EtOH, ethanol; hIDE, human insulin-degrading enzyme; HOBt, N-hydroxybenzotriazole; MeOH, methanol; PBS, phosphate buffered saline; PTSA, para-toluenesulfonic acid; rt, room temperature; SAR, structure–activity relationship; TFAA, Trifluoroacetic anhydride; THF, tetrahydrofuran.

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1. Introduction

Insulin-degrading enzyme (IDE) is a zinc protease of the M16 family that is linked to type-2 diabetes and Alzheimer's disease [1,2]. In addition to hydrolyse insulin or IGF-II [3], IDE was shown to degrade numerous small size peptide substrates [4] including amyloid-β (Aβ) [5] and ubiquitin [6]. Interestingly, IDE-*ko* rodent models display elevated brain Aβ [7], while transgenic over-expression of IDE in neurons results in reduced brain Aβ levels [8]. Moreover, *Ide* gene was linked Alzheimer's disease (AD) in humans [9]. In addition to being involved in the clearance of peptides, IDE may have additional functions such as the regulation of the proteasome complex [10], the refolding of amyloid-forming peptides by serving as a chaperone [11] or the elimination of $A\beta_{1\text{-}40}$ across the blood–brain barrier by capillary endothelial cells [12].

Structures of human IDE have revealed the molecular basis for the preference of IDE to degrade amyloidogenic peptides below 8 kDa [13,14]. IDE has a sizable and enclosed catalytic chamber that is delimited by the N-terminal and C-terminal halves joined by a loop [15]. Upon opening, the enzyme encapsulates the substrates that primarily bind an exosite, 30 Å away from the catalytic zinc ion. This binding promotes a conformational change of the substrate to allow the regions that can adapt the β-strand structure to enter the catalytic cleft for zinc-ion-mediated cleavage [16,17]. While larger substrates need to enter into the catalytic chamber via a large open-closed conformational switch of IDE, shorter peptides could also enter the catalytic chamber by the displacement (swinging-door) of a subdomain of IDE that creates an 18 Å opening [18].

The first substrate-based zinc-binding hydroxamate inhibitors of IDE [19] display both a hydroxamate group [20] and an arginine residue that limit their use as pharmacological probes. Other compounds that behave as activators were also published [21].

We previously reported reversible, partial, competitive inhibitors of IDE discovered by high-throughput screening of a 2000-member library on amyloid-beta hydrolysis [22]. We showed that these compounds are dual binding inhibitors of IDE. Indeed, they bind a permanently formed exosite and the catalytic site formed upon conformational switch of the N- and C-terminal halves from the open to closed state and stabilisation of the swinging door [22]. A few analogues leading to cell-active compounds were disclosed. Herein, we describe the full structure–activity relationships in the series. We performed additional studies for the interaction of IDE with inhibitors both by X-ray analysis and docking. Finally best compounds were evaluated for their *in vivo* pharmacokinetic properties.

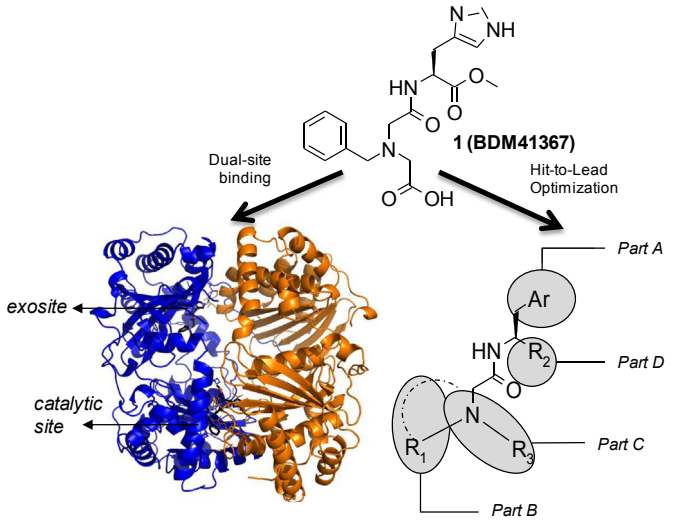


Fig. 1. Structures of hit **1** discovered by screening, binding to hIDE (PDB code 4DTT) and hit-to-lead optimization strategy.

2. Chemistry

A few analogues were synthesized to explore the replacement of the imidazole ring of histidine (part A) (Fig. 1). Also we explored the benzyle replacement by either alkyl groups, homologues of benzyle or substituted benzyle. The impact of the nature of the linker between the nitrogen and the phenyl ring was investigated, as well as the removal of the tertiary amine function (part B) (Fig. 1). Several analogues were designed to evaluate the importance of the carboxylic acid function (part C) (Fig. 1) or the methyl ester group (part D) (Fig. 1). Finally, a few analogues that combine several modifications were synthesized.

2.1. Synthesis of analogues modified at part A

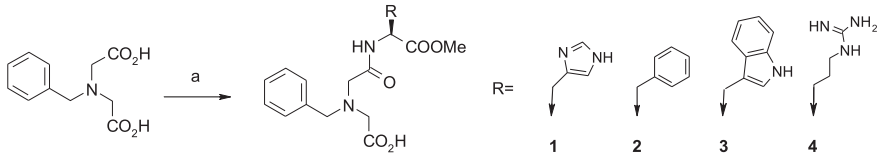
The synthesis of analogues **2**–**4** of hit **1** derived from different L-amino-acid methyl esters was performed using a two-step procedure: cyclization of commercially available iminodiacetic precursor with TFAA in acetic anhydride, then anhydride opening in DMF (Scheme 1).

2.2. Synthesis of analogues modified at part B

The synthesis of analogues **5**–**23** proceeded as depicted in Scheme 2. Non commercial iminodiacetic precursors **5a**–**20a** were prepared by alkylation of iminodiacetic acid with bromides. **20a**–**22a** were prepared by acylation of the dimethyl ester of iminodiacetic, using acid chlorides or activated carboxylic acids. Reaction of iminodiacetic with Boc_2O or benzylchloroformate in 2 N NaOH solution allowed diacid **17a** and **23a** respectively. Synthesized iminodiacetic acid precursors (**5a**–**16a**, **20a**–**23a**) and commercial analogues (**1a** and **18a**–**19a**) were converted *in situ* to the corresponding cyclic anhydride with trifluoroacetic anhydride in acetic anhydride. **17a** was converted to the corresponding cyclic anhydride with DCC (Scheme 2). The anhydride then reacted with histidine derivatives to give final amide compounds **1, 5**–**23** after a deprotection step if needed (Scheme 2). Branched analogue **15** was obtained via a different synthetic route from 1-methyl-3-phenylpropylamine and L-His(Trt)-OMe. First L-His(Trt)-OMe was converted to chloroacetamide **15a**. 1-methyl-3-phenylpropylamine reacted with *tert*-butylbromoacetate to give secondary amine **15b**. Both amine **15b** and halide **15a** reacted in refluxed DMF in the presence of $NaHCO_3$ to give **15** after a deprotection step of the *tert*-butyl group (Scheme 3). Cyclic analogue **24** (Scheme 3) was obtained in three steps from 1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid, *tert*-butylbromoacetate and L-His(Trt)-OMe.

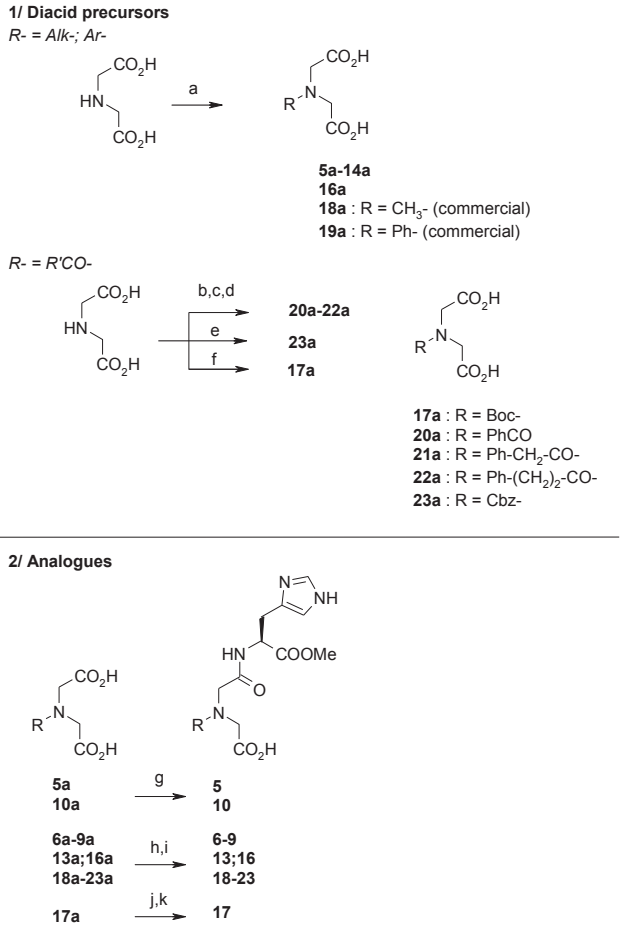
2.3. Synthesis of analogues modified at part C

Compound **25** deprived of a tertiary amine function derived from diacid **25c** obtained by chain homologation from diethylbenzylmalonate [23] (Scheme 4). Briefly, ester functions were reduced to alcohols by LAH, then converted in tosyle group to give **25a**. Reaction with KCN allowed to get the bis-nitrile **25b**. Hydrolysis of the nitrile functions in H_2SO_4 provided the desired diacid **25c** (Scheme 4). Analogues **26**–**31** required the synthesis of precursors **26a**–**27a**, **29a**–**31a** (Scheme 5). N-benzyl-iminodiacetic acid was first converted to cyclic anhydride then treated either with ammonia or with O-trityl-hydroxylamine to give respectively precursors **27a** or **30a**. Homologue **29a** was obtained in 3 steps from N-



Scheme 1. Synthesis of analogues **1**–**4**. Reagents and conditions: (a) 1) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h 2) L-aminoacid methyl esters, anhydrous DIEA, anhydrous DMF, Argon, room temp., overnight.

benzylglycine. First N-benzylglycine was converted to its methyl ester using $SOCl_2$ in methanol. Then, the nitrogen was alkylated using tert-butyl-3-bromo-propionate. Finally, methyl ester was saponified to give **29a**. Compound **26a** was directly obtained from N-benzylglycine using Boc_2O. Squaric derivative **31a** was synthesized using previously published conditions [24]. In order to avoid the formation of squaramide, commercially available diethyl squarate was dissymmetrized using potassium tert-butoxide in dry THF [25]. This dissymmetric diester then reacted with N-benzylglycine to give compound **31a.** Intermediates **26a**–**27a**, **29a**–**31a** reacted with the desired amine and gave compounds **26**–**31** after deprotection if necessary (Scheme 5). Methyl ester **28** was obtained directly from **1** by treatment of the acid by thionyl chloride in methanol. Tetrazole analogue **32** was obtained in three steps. First carboxylic acid **27a** was reacted with amine **35a**. Then the primary amide function was converted to cyano group using trifluoroacetic acid in pyridine. Then the nitrile function was converted to the tetrazole using NaN_3 to give **32** (Scheme 6) [26].



Scheme 2. Synthesis of analogues **5**–**14, 16**–**23**. Reagents and conditions: (a) R–Br, MeOH, DIEA, room temp., 2–12 h, 27–76%; (b) $SOCl_2$, MeOH, 0°c then room temp., 18 h; (c) RCOCl, DIEA, DCM or RCOOH, EDCI, HOBt, DIEA, DCM, room temp., 18 h; (d) NaOH, MeOH, H_2O, 1–6 h, room temp.; (e) benzylchloroformate, NaOH 2 N, 0 °C then room temp., 2 h, 64%; (f) Boc_2O, dioxane, NaOH 2 N, 0 °C then room temp., 18 h, 67%. (g) 1) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h 2) L-Histidine methyl ester dihydrochloride, anhydrous DIEA, anhydrous DMF, Argon, room temp., overnight. (h) 1) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h 2) H-His(1-*Trt*)-OMe HCl, anhydrous DIEA, anhydrous DMF, Argon, room temp., overnight. (i) TFA, TIS, DCM, room temp., 5min-4 h. (j) 1) DCC, THF, overnight, room temp. 2) L-Histidine methyl ester dihydrochloride, DIEA, THF, 5 h, room temp. (k) HCl(g), DCM, 2 h, room temp.

2.4. Synthesis of analogues modified at part D

Dicarboxylic acid **33** was obtained from **1** by saponification of the methyl ester (Scheme 7). For other analogues, amine precursors (**34a**–**38a**, **40a**–**42a**, **46a**) were synthesized as depicted in Scheme 7. First, amine **40a** was obtained from L-H-His(Trt)-OMe by direct reaction with methylamine. Then trityle protection was removed using TFA. L-histidine was activated with $SOCl_2$ then coupled with isobutanol to give the corresponding isobutyl ester **36a**. Similarly, Boc-L-histidine was reacted with isopropanol in the presence of EDCI to give the isopropyl ester **35a** after deprotection of the amine function. Dimethylamide (**42a**) and benzylamine derivatives **(41a)** were obtained using classical conditions. Bioisosteric replacement of the methyl ester with a 1,2,4-oxadiazole (compound **46a**) was performed using methylamidoxime [27]. Analogues **34**–**46** were synthesized by reacting either 3-benzyl-glutaric acid-anhydride or 3-(3-phenylpropyl)-glutaric acid-anhydride generated *in situ* from the corresponding iminodiacetic acids with synthesized amine precursors or commercial histamine **34a**, L-histidine(Trt)-OtBu **37a**, histidinol **38a**. **39** was obtained by reaction of **1** with gaseous ammonia in a dioxane/methanol mixture (Scheme 7).

3. Results and discussion

3.1. Structure–activity relationships

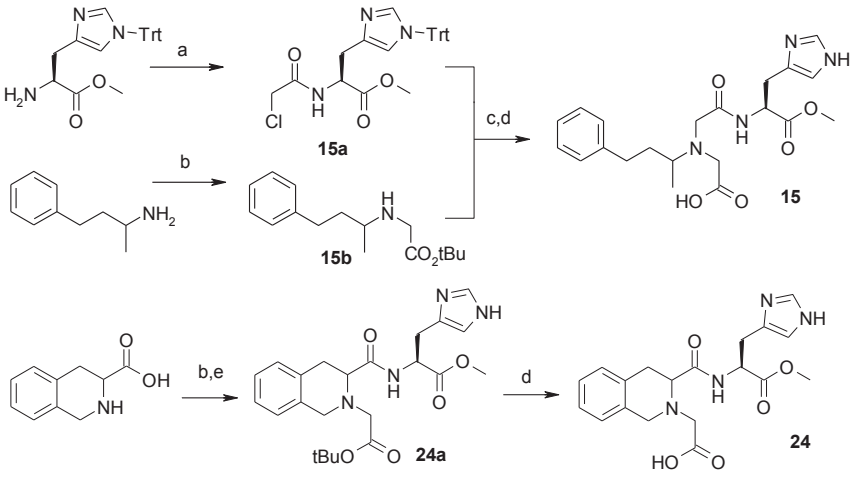
All the compounds reported here are partial inhibitors of the enzyme for the degradation of amyloid-beta peptide [22]. Analogues were assayed for their ability to inhibit amyloid-$\beta_{16\text{-}23}$ hydrolysis by *h*IDE (Tables 1–5).

3.1.1. Impact of imidazole ring on activity

We first evaluated the replacement of the imidazole ring of **1** by a phenyl (**2**) or an indole (**3**). Such modification in Part A of the hit is deleterious for activity while introducing a guanidine like in arginine derivative (**4**) allows retaining some activity (Table 1).

3.1.2. Impact of tertiary amine and chain elongation on activity

Table 2 shows the impact of modifications at Part B of hit **1** such as substitution, chain elongation, charge deletion of the benzylamino group. First, substitution in *para* with either electroattractor or electrodonor substituents did not have a large impact on the activity (**5**–**8**). Isosteric replacement of the phenyl ring by a pyridine leads to a loss of activity (**9**) whereas hydrophobic naphthyl or indole analogues **10**–**11** are slightly more active. Best activities are



Scheme 3. Synthesis of analogues **15** and **24**. Reactants and conditions a) chloroacetylchloride, $NaHCO_3$, DCM, room temp., 10 min; b) *tert*-butylbromoacetate, DIEA, THF, 0 °C then room temp., overnight; c) $NaHCO_3$, DMF, reflux, 24 h; d) TFA, TIS, DCM, room temp. 2 h; e) L-Histidine methyl ester dihydrochloride, EDCI, HOBt, DIEA, DCM, room temp., 18 h.

obtained with longer-chain homologues (**12**–**14**) (phenylbutyl > phenylpropyl > phenethyl > benzyl). A branched chain is tolerated, as exemplified with **15**. Interestingly, replacement of the phenylalkyl group (like in **14**) by an *n*-hexyl group provides equipotent compound **16**. Consistent with the positive effect of chain elongation, truncated analogues are less active. Indeed, secondary amine **17** and methyl derivative **18** are inactive. Also removal of the methylene of the benzyl moiety (**19**) results in complete loss of activity. This could be due to both steric constrains or the loss of the charge on the adjacent nitrogen. Also, all amide or carbamate analogues with various chain lengths (**20**–**23**) are inactive. These results show the critical role of the tertiary amine function. Finally, cyclization of **1** into a tetrahydroquinoleine result in slightly equipotent compound **24**.

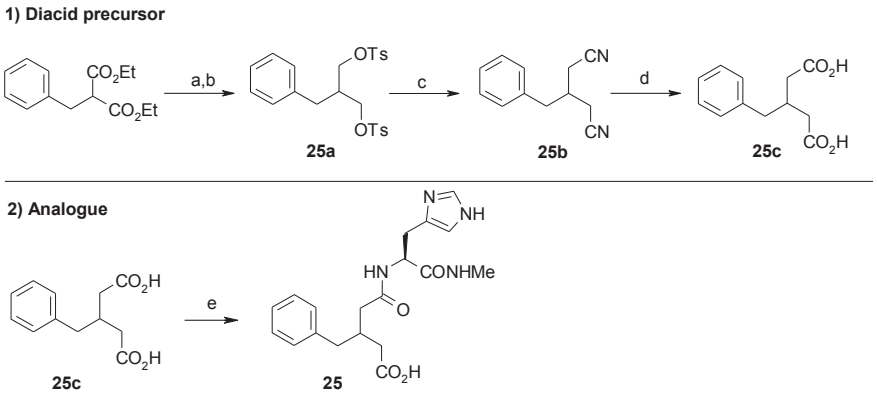
3.1.3. Isosteric replacement of methyl ester and impact on plasma stability

Methyl ester function of **1** (Part D) was hydrolyzed rapidly in mouse plasma giving the corresponding carboxylic acid **33** which is inactive on the target. We evaluated the impact of both isosteric replacement of the methyl ester and increasing ester size on activity and stability (Table 3). Complete removal of the methyl ester results in inactive compound **34**. Interestingly, bulkier esters **35**–**37** are 1.7–4.7 more active than **1**, *iso*-propyl ester (**35**) being the most active in the series with an IC_{50} of 0.3 μM. Their plasma half-life was ranked as *tert*-butyle > *iso*-butyle > methyl > *iso*-propyle, consistently with reported metabolism of esters by carboxyesterases [28]. Thus *tert*-butyle ester **37** is the best compromise between a good activity and a good stability compared to **1**.

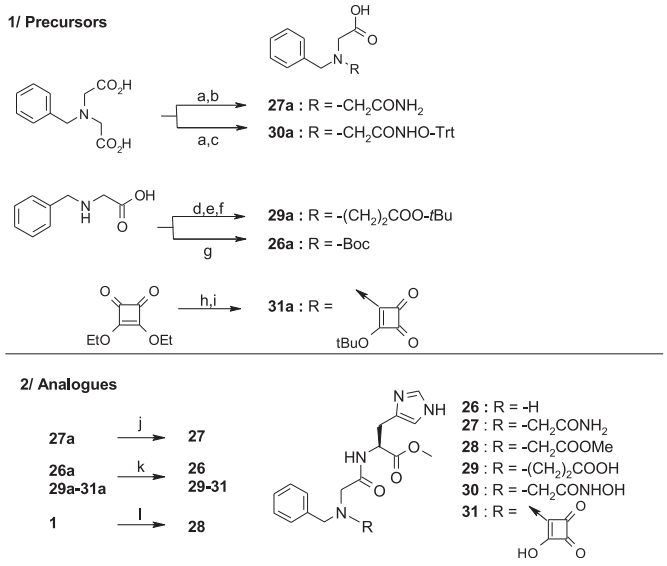
Alcohol **38** analogue is equipotent to **1** and stable in plasma. Interestingly, primary amide **39** or dimethyl amide **42** are less active than methylamide **40**. This shows the importance of an acceptor of hydrogen bond at this position. Interestingly, introducing a larger benzyle group on the amide decreases activity (**41** vs **40**).

3.1.4. Impact of carboxylic acid isosteric replacement on activity

Compounds **25**–**32** (Table 4) were synthesized to evaluate the importance of the amino-methyl-carboxylic acid function (Part C) for the activity of **1** and methylamide analogue **40**. Isosteric replacement of the nitrogen by a CH (**25** vs **40**) abolishes activity, illustrating, as shown in Table 2, the critical role of the tertiary amine function. Deletion of the acetic acid resulted in inactive compound **26**. Replacement of the carboxylic function by amide (**27**) or methyl ester (**28**) is deleterious for activity. Elongation of the chain is not efficient (**29**). Introduction of hydroxamate or squaric isosters allows retaining some activity (**30** and **31** vs **1**). Replacement of the carboxylic function of **40** by a tetrazole moiety



Scheme 4. Synthesis of analogue **25**. Reagents and conditions: (a) LAH, THF, 0 °C then reflux, 18 h, 70%; (b) TsCl, TEA, DCM, room temp., 18 h, 44%; (c) KCN, DMSO, 80 °C, 3.5 h; (d) H_2SO_4, H_2O, reflux, 3 days, 40% (e) 1) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h or DCC, THF, 5 h, room temp. 2) L-H-Histidine-methylamide, anhydrous DIEA, anhydrous DMF, Argon, room temp., overnight.



Scheme 5. Synthesis of analogues **26**–**31**. Reagents and conditions: (a) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h (b) ammoniac, DMF 100%; (c) TritylO-NH_2, DIEA, DMF, room temp., 18 h, 93%; (d) 20% $SOCl_2$/MeOH, room temp., 18 h, 100%; (e) $Br(CH_2)_2COOtBu$, K_2CO_3, KI, acetone, reflux, 36 h; (f) NaOH, MeOH, 5 days, 40 °C; (g) Boc_2O, NaOH 2 N, dioxane, room temp.overnight; (h) potassium *tert*-butylate 1 M in THF, diethyl squarate, THF, 4 °C, 15 min, 55%; (i) N-Benzylglycine hydrochloride, TEA, MeOH, room temp., 18 h, 70%. (j) EDCI, HOBt, DIEA, DCM, L-His-OMe.2HCl, room temp., 18 h, or i. chlorure d'oxalyle, DCM, DMF cat., 0 °C, 30 min; ii. L-(1-Trt)-His-OMe.HCl, DIEA, DCM, room temp., 18 h, 38%; (k) TFA, TIS, DCM, room temp., 5 min-4 h. (l) $SOCl_2$, MeOH, room temp., 2 h.

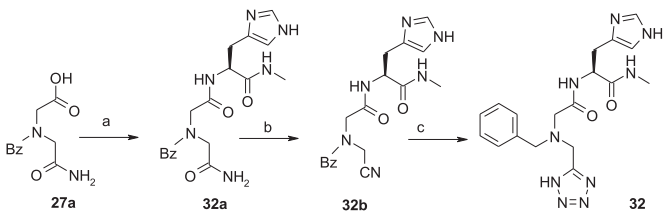
resulted in inactive compound **32.** All these data suggest an important interaction between the carboxylic acid function and the target.

3.1.5. *Identification of lead **44 (BDM43079)***

Chain elongation at Part B was beneficial for activity. As well, isosteric replacement of methylester at Part D proved to enhance both plasma stability of the compounds and activity. Several analogues with these two modifications (**43**–**45**; Table 5) are more active than their respective benzyl analogues (**37**–**41**), with IC_{50} values ranging from 100 nM to 1.6 μM. Best compound in the series is **44** (**BDM43079**), with an IC_{50} of 0.1 μM on the target. We also evaluated in the phenylpropyl series two more isosteric replacements of the methyl ester by the corresponding 1,2,4-oxadiazole (**46**) or methanol analogue (**47**). Interestingly, while alcohol analogue is less active than amide counterpart **44**, oxadiazole analogue is almost equipotent.

3.2. *Binding of analogues*

We previously disclosed the binding of **1** obtained by X-Ray diffraction of crystallized IDE-**1** complex [22]. Surprisingly, **1** **(BDM41367)** has two possible binding sites in the crypt: either the exosite or the catalytic site. This binding mode is consistent with observed SAR.



Scheme 6. Synthesis of **32**. Reagents and conditions: (a) EDCI, HOBt, DIEA, DMF, L-H-Histidine-methylamide, room temp., 18 h, 80%; (b) TFAA/pyridine, THF, 0 °C; (c) NaN_3, ammonium chloride, DMF, 90 °C, 60 h.

At the exosite (Fig. 2), the imidazole ring forms a hydrogen bond with Glu341 side chain a key residue that binds the N-terminus of IDE substrates. The backbone of Leu359 also interacts with the imidazole ring. The amide function of inhibitors interacts with Gly361 while the hydrophobic benzyl or phenylpropyl is in the hydrophobic region of Ile374 and Lys364. Interestingly, inactive analogues **22** and **33** bind to the exosite.

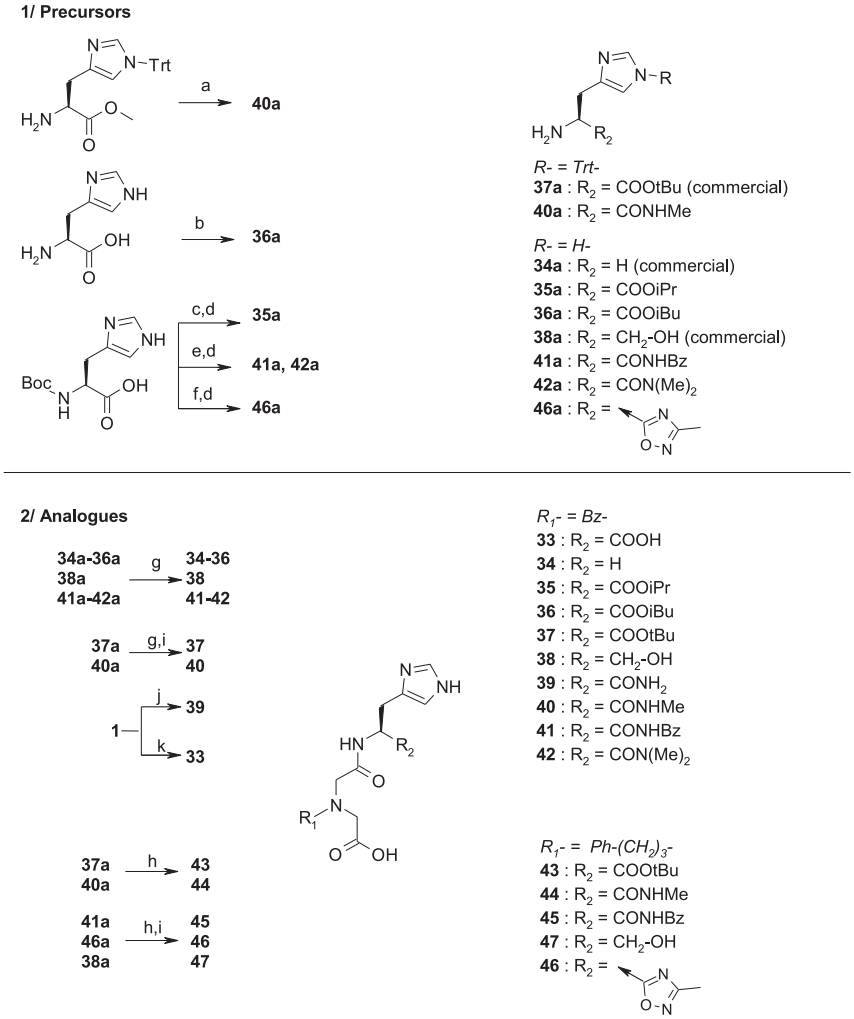
At the catalytic site, the carboxylic acid function of all three inhibitors (**1**, **44**, **46**) completes the zinc coordination sphere formed by His108, His112 and Glu198 (Fig. 3). Interestingly they interact with residues from both N- and C-terminal domains, keeping the enzyme in a closed conformation. The amide function is implicated in two hydrogen bonds with the side chains of Tyr831 (with NHCO) and Asn139 (with NHCO). Furthermore, the imidazole ring interacts with the backbone of Val833. Finally, the phenyl ring makes stacking with Phe115. The negatively charged E111 and E182 are in the vicinity of the imino function of the inhibitors. This explains why analogue **24** and **22** deprived of this positively nitrogen are inactive. In particular **22** binds the exosite (Fig. 2) but should not be able to adequately bind the catalytic site resulting in inactivity. The critical involvement of E111-amine interaction is also supported by the fact that when the IDE E111Q mutant is used for co-crystallisation, the compounds only bind the exosite [22]. The better activities of analogues **44** and **46** (Fig. 3, panels B and C respectively) over hit **1** can be explained by the better fitting of the phenylpropyl ring into the hydrophobic pocket defined by Phe115 and Phe820. Also, the carbonyl of amides is a better hydrogen bond acceptor than the carbonyl of esters [29] resulting in stronger hydrogen bond between Asn139 and the carbonyl group in **44**. The oxygen of 1,2,4-oxadiazole **46** is a good acceptor too [30]. **46** is 5 times less active than **44** but this could be attributed to the extra-methyl group. Finally, diacid **33**, though binding the exosite (Fig. 2) may be inactive because the anionic charge in the vicinity of the imidazole ring may prevent the imidazole to correctly interact with Val833 in the C-term domain.

3.3. *Vitro and vivo pharmacokinetic properties*

As shown in Table 5, compounds in the series are highly hydrophilic with Log*D* ranging from −2.29 to −0.58 and aqueous solubilities above 150 μM. As expected chain elongation enhances Log D (**13** vs **1**, and **44** vs **40**). Replacement of the methyl ester group by a methyl amide surprisingly lowers Log*D*, this may be due to the introduction of a new hydrogen-bond donor while having moderate impact on solubility (**40** vs **1**; **44** vs **13**). Further replacement of the methyl ester function of **1** by either the corresponding alcohol (**47**) or oxadiazole (**46**) has no dramatic impact on solubility and Log*D*. As expected all compounds except methyl esters are stable in plasma. Some compounds were evaluated for microsome stability and showed a good half-life (>40 min).

Methyl ester precursors **48** and **49** were obtained in two steps from 3-(3-phenylpropyl)-glutaric acid **13a** (Scheme 8). At last, suppression of the acid function (**28**, **48**, **49**) leads to inactive compounds and allows to reach moderate Log*D* that may be better for cell permeation. As expected, these compounds give readily the active carboxylic acid in plasma and/or microsomes.

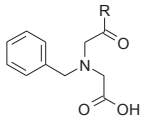
Given the preliminary *in vitro* ADME data and activity, both carboxylic acids **44** and **46** were selected, along with their methyl esters precursors **48** (**BDM43124**) and **49**, for pharmacokinetic experiment in mice following intraperitoneal dosing at 10 mg/kg (n = 3/group). Results are presented in Table 6. All 4 compounds display good AUC ranging from 0.2 to 3.6 h μg/mL. Surprisingly,



Scheme 7. Synthesis of compounds **33**–**47**. Reactants and conditions: (a) i. $MeNH_2$, EtOH, MeOH, reflux; (b) $SOCl_2$, iBuOH, 60 °C, 48 h; (c) EDCI, DMAP, isopropanol, DCM, reflux, 24 h, 56% (d) HClg, DCM, 1 h, 100%; (e) amine, EDCI, HOBt, TEA, DMF, room temp., 16 h; (f) i. TBTU, DIEA, DMF, 10 min, room temp.; ii. amidoxime, DMF, room temp. 4.5 h then reflux 1.5 h (g) i) *N*-benzyl-iminodiacetic acid, TFAA 2% in acetic anhydride, 50–70 °C, 5 h, ii) amine, anhydrous DIEA, anhydrous DMF, argon, room temp., overnight; (h) i) diacid **13a**, TFAA 2% in acetic anhydride, 50–70 °C, 5 h, ii) amine, anhydrous DIEA, anhydrous DMF, argon, room temp., overnight, (i) TFA/DCM 50/50, TIS; (j) NH_3g, dioxane/MeOH (5/2), room temp. 10 h, (k) NaOH, H_2O, MeOH, room temp., 4 h.

mice are better exposed to the carboxylic derivatives that the methyl esters. This may suggest the implication of transporters for the highly hydrophilic compounds **44** and **46**. Nevertheless, in the series, half-lives of the series are short for both methyl esters and carboxylic acids. This may be due to rapid elimination due to high polarity. Also, mice that were given methyl esters **48** or **49** were dosed also for carboxylic acid metabolites. These were detected instantaneously. This is coherent with *in vitro* data on plasmatic stability.

Table 1
Impact of imidazole replacement on inhibition of $A\beta_{16-23}$ hydrolysis by *h*IDE (**1**–**4**).



Cpd	-R	IC_{50} (μM)[a]
1	-L-H-His-OMe	2.9
2	-L-H-Phe-OMe	–[b]
3	-L-H-Trp-OMe	–[b]
4	-L-H-Arg-OMe	36.3

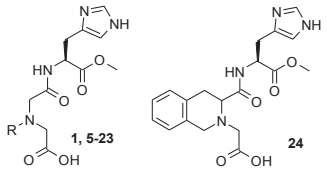
[a] Values are means of 2 experiments minimum, standard deviations are ±10%.
[b] %inhibition below 10 at 100 μM.

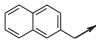
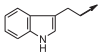
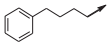
4. Conclusion

The effect of lead **44** (**BDM43079**) on the hydrolytic profile of IDE was further assessed using full unlabelled native substrates [22]. Expectedly **44** inhibits the hydrolysis of amyloid-β_{1-40} by IDE. However, it moderately promotes the hydrolysis of insulin, in contrast with pan-inhibitors like EDTA [22]. **44** (**BDM43079**) was shown to be selective of *h*IDE versus a panel of metalloproteases including *h*NEP [31] and *h*ECE two other metalloproteases implicated in the hydrolysis of amyloid-β. Cellular effects of **48** (**BDM43124**) methyl ester precursor of the lead **44** were assessed in SH-SY5Y [22]. Cells treated with **48**, display a dose-dependent increase in levels of both amyloid-β_{1-40} and β_{1-42}.

Our compounds differentiate from the pan-substrate inhibitor [19a] and are deprived of guanidinium function known to slow down cell membrane permeation. Fig. 4 summarizes the structure–activity relationships in the series in the light of the binding of

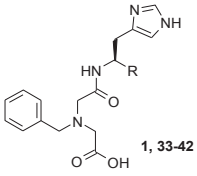
Table 2
Impact of tertiary amine replacement and chain elongation on inhibition of $A\beta_{16-23}$ hydrolysis by *h*IDE (**1**, **5**–**24**).



Cpd	R-	IC_{50} (μM)[a]	Cpd	R-	IC_{50} (μM)[a]
1		2.9	**15**		0.6
5		2.5	**16**		0.4
6		2.0	**17**	H-	–[b]
7		1.7	**18**	CH_3-	–[b]
8		2.9	**19**		–[b]
9		6.3	**20**		–[b]
10		1.2	**21**		–[b]
11		1.2	**22**		–[b]
12		1.4	**23**		–[b]
13		1.0	**24**	–	3.6
14		0.6			

[a] Values are means of 2 experiments minimum, standard deviations are ±10%.
[b] %inhibition below 10 at 100 μM.

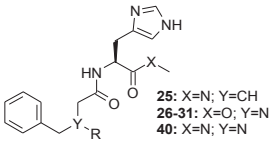
Table 3
Impact of isosteric replacement of methyl ester on inhibition of $A\beta_{16-23}$ hydrolysis by *h*IDE and plasmatic stability (**1**, **33**–**42**).

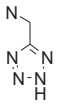


Cpd	-R	IC_{50} (μM)[a]	Mouse plasma stability $t_{1/2}$ (h)
1	$-CO_2Me$	2.9	6.4
33	$-CO_2H$	–[b]	–[c]
34	-H	–[b]	–[c]
35	$-CO_2iPr$	0.3	0.2
36	$-CO_2iBu$	0.6	8.7
37	$-CO_2tBu$	0.8	>24
38	$-CH_2OH$	4.1	>24
39	$-CONH_2$	1.4	>24
40	-CONHMe	0.6	>24
41	-CONH-Bz	1.4	–[c]
42	$-CON(Me)_2$	6.9	–[c]

[a] Values are means of 2 experiments minimum, standard deviations are ±10%.
[b] % inhibition below 10 at 100 μM.
[c] Not determined.

Table 4
Impact of carboxylic acid replacement on inhibition of $A\beta_{16-23}$ hydrolysis by *h*IDE for compounds (**1**, **25**–**32**, **40**).

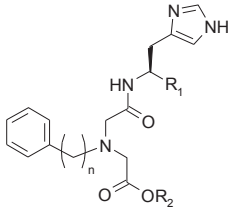


	Y-R	IC_{50} (μM)[a]
1	$-N-CH_2COOH$	2.9
40	$-N-CH_2COOH$	0.6
25	$-CH-CH_2COOH$	–[b]
26	-NH	–[b]
27	$-NCH_2CONH_2$	–[b]
28	$-NCH_2COOMe$	–[b]
29	$-N(CH_2)_2COOH$	–[b]
30	$-NCH_2CONHOH$	3.2
31		12.5
32		–[b]

[a] Values are means of 2 experiments minimum, standard deviations are ±10%.
[b] %inhibition below 10 at 100 μM.

compound **1** to IDE. We evidenced the key role of chain length and methyl ester replacement both on activity and plasma stability. In particular, compound **44** (IC_{50} = 100 nM) is a promising analogue that showed cellular activity. This series is highly soluble in aqueous media, and both methyl amide and 1,2,4-oxadiazole derivatives displayed interesting bioavailability. Further optimization

Table 5
Inhibition of $A\beta_{16-23}$ hydrolysis by *h*IDE and LogD, solubility, plasmatic and microsome stability for some analogues of **44**.



Cpd	n	-R1	-R2	IC_{50} (μM)[a]	Mouse plasma stability $t_{1/2}$ (h)	Log$D_{7.4}$[b]	Sol. (μM)[b]	Mouse liver microsome $t_{1/2}$ (min)
1	1	$-CO_2Me$	-H	2.9	6.4	−2.05	154	–[c]
40	1	-CONHMe	-H	0.6	>24	−2.29	151	>40
13	3	$-CO_2Me$	-H	1.0	1.3	−0.78	199	–[c]
43	3	$-CO_2tBu$	-H	0.4	>24	–[c]	–[c]	–[c]
44	3	-CONHMe	-H	0.1	>24	−1.36	170	>40
45	3	-CONHBz	-H	0.4	>24	−0.58	179	–[c]
46	3		-H	0.5	>24	−0.84	198	>40
47	3	$-CH_2OH$	-H	1.6	>24	−1.30	180	>40
28	1	$-CO_2Me$	-Me	>100	–[c]	0.82	191	–[c]
48	3	-CONHMe	-Me	–[c]	0.1	1.07	179	26
49	3		-Me	–[c]	–[c]	1.52	172	19

[a] Values are means of 2 experiments minimum, standard deviations are ±10%.
[b] Solubility and Log*D* are measured from a DMSO stock solution.
[c] Not determined.

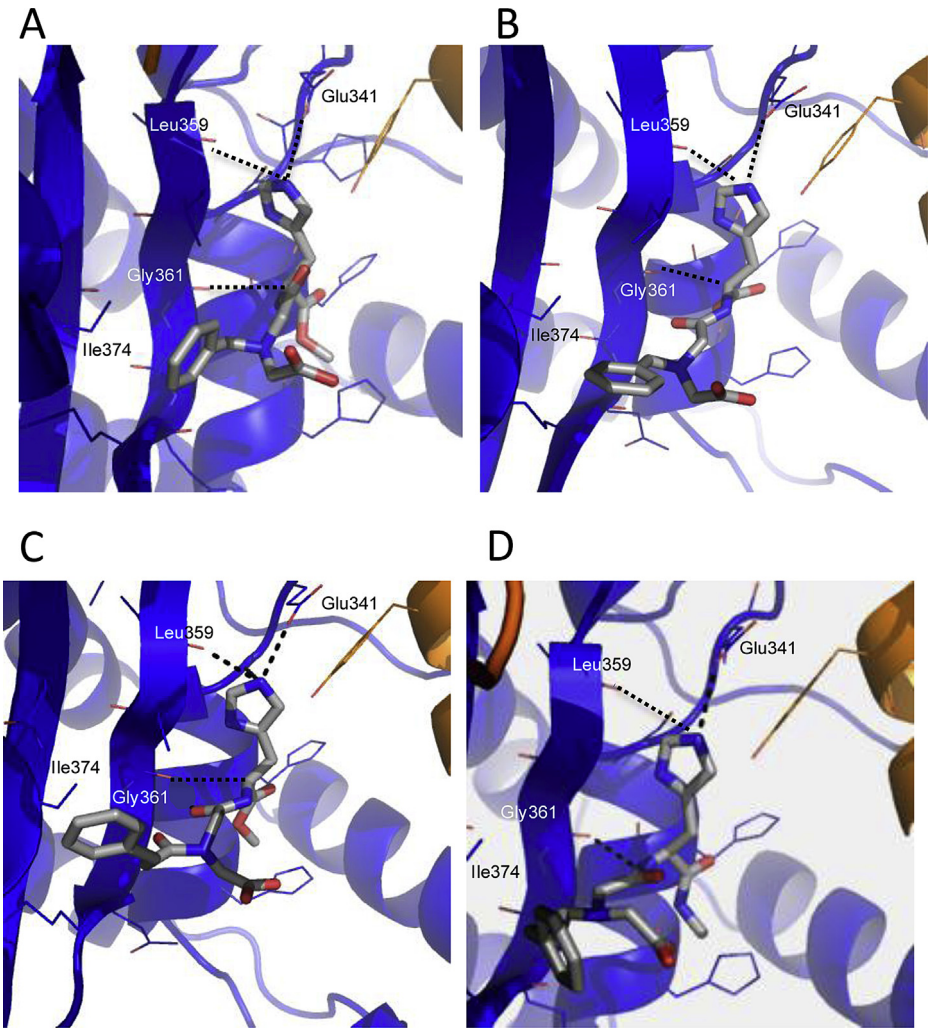


Fig. 2. Binding of **1** and analogues **22**, **33** and **44** at the exosite of *h*IDE-CF-E111Q. O (red); N (blue); C (blue for IDE N-terminal domain, orange for IDE C-terminal domain, grey for inhibitors); hydrogen contacts in dotted lines. A) **1**: X-Ray structure PDB code: 2YPU; B) **33** X-Ray structure PDB code: 4QIA; C) **22** X-Ray structure PDB code: 4GSF; D) **44** X-Ray structure PDB code: 4GS8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of pharmacokinetic parameters in the series will focus on optimizing both cell permeation and elimination half-life. In summary, we have successfully developed and optimized a series of inhibitors of amyloid-beta hydrolysis by Insulin-degrading enzyme.

5. Experimental section

5.1. Biology

5.1.1. In vitro IDE activity assay

In vitro IDE activity was measured with a quenched substrate ATTO 655- Cys-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Trp. Human recombinant IDE was cloned and purified as previously described. Briefly, human IDE (1.87 ng/μL) was incubated 10 min with compound in Hepes 50 mM, NaCl 100 mM, pH 7.4 and the enzymatic reaction is started by adding the substrate (final concentration 5 μM). After 30 min at 37 °C, samples (1% DMSO final) are excited at 635 nm and fluorescence emission at 750 nm is measured on a Victor3 V1420 Perkin Elmer spectrophotometer. All measurements were carried out as 8-point dose response curves and are reported as the average of at least two independent measurements. EDTA was used as a reference inhibitor (100% inhibition at 2 mM). Results were expressed as percentage of inhibition and IC_{50} values were calculated from concentration-response curves by a non-linear regression analysis at four parameters (Hill equation) using XLfit™ software.

5.1.2. Solubility/LogD measurements

The analysis was performed using an LC-MS/MS system (Varian 1200L) under SIM detection using the parameters optimized for each compounds. HPLC analysis was performed using a Luna C18 (50*2.1 mm, 5 μm); the gradient and the mobile phase (flow rate 600 μL min^{-1}) used are determined in order to detect the compound of interest with satisfying retention time and peak shape. Acquisition and analysis of data were performed with MS WorkstationTM software (version 6.3.0 or higher). 10 μL of a 10 mM solution in DMSO of the compound are diluted either in 490 μL of PBS pH 7.4 or in organic solvent MeOH in a 700 μL-microtube (in triplicate). The tubes are gently shaken 24 h at room temperature, then centrifuged for 5 min at 4000 rpm. The mixtures are filtered over 0.45 μm filters (Millex-LH Millipore). 20 μL of sample are diluted in 180 μL of MeOH. The solubility is determined by the ratio of mass signal area PBS/organic solvent. 40 μL of a 10 mM solution in DMSO of the compound were diluted in 1.960 mL of a 1/1

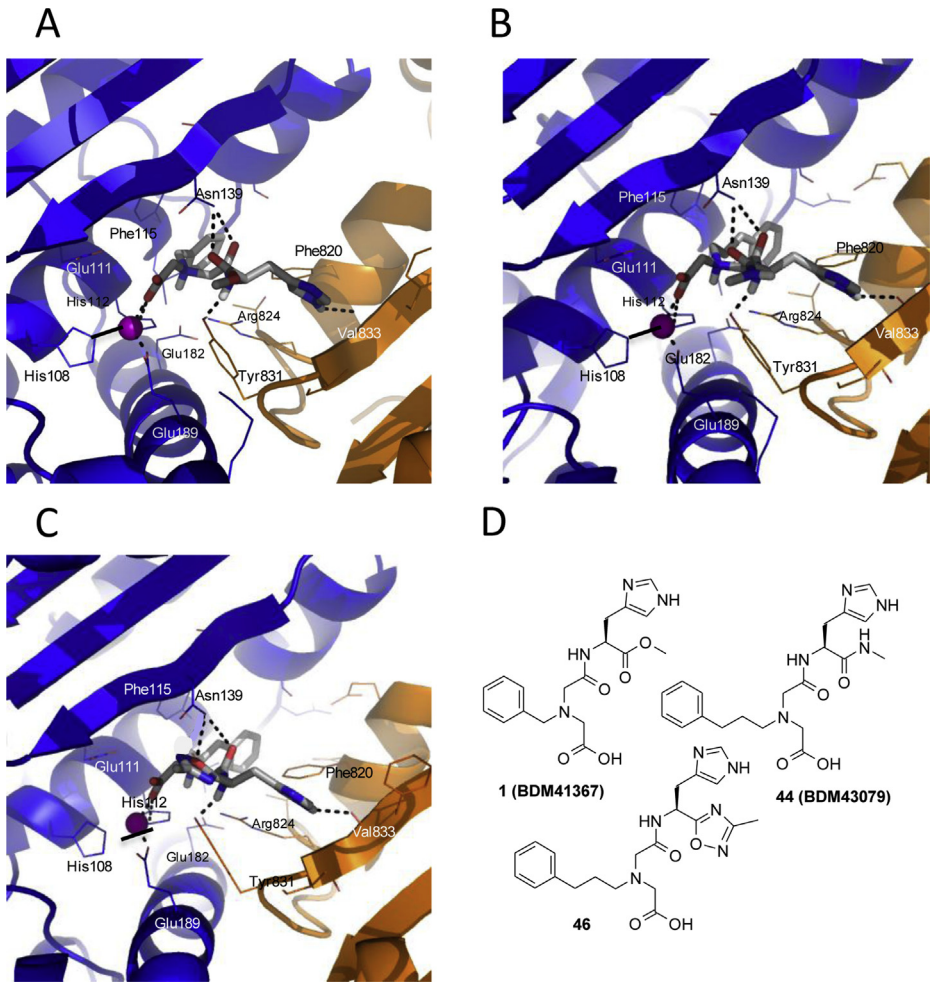
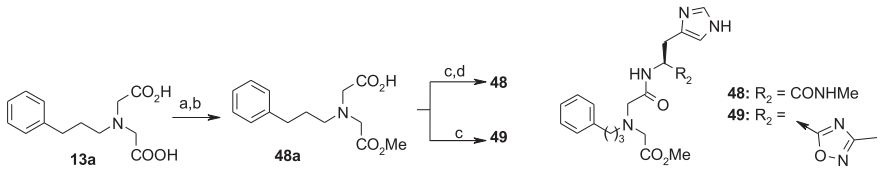


Fig. 3. Binding of **1** and analogues **44** and **46** at the catalytic site of *h*IDE-CF. Zn (magenta sphere); O (red); N (blue); C (blue for IDE N-terminal domain, orange for IDE C-terminal domain, grey for inhibitors); hydrogen contacts in dotted lines. A) **1**: X-Ray structure PDB code: 4DTT; B) Docking of **44** in PDB code: 4DTT; C) Docking of **46** in PDB code: 4DTT; D) Structures of **1**, **44** and **46**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

octanol/PBS at pH 7.4 mixture. The mixture was gently shaken 2 h at room temperature. 20 μL of each phase was diluted in 480 μL of MeOH and analyzed by LC-MS. Each compound is tested in triplicate. Log D was determined as the logarithm of the ratio of concentration of product in octanol and PBS respectively, determined by mass signals.

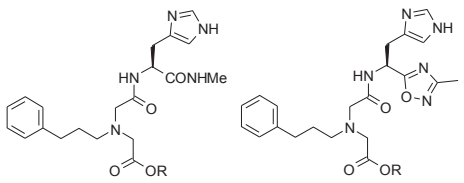
5.1.3. Stability in plasma

Incubations were performed in duplicate in Eppendorf tubes. The mouse plasma (Mouse Plasma Lithium Heparine from Sera Laboratories International Ltd) or the human plasma (mixed gender) was pre-incubated 5 min at 37 °C before the addition of test compounds to a final concentration of 10 μM (1% DMSO maximum). At the defined time points, 50 μL from each tube were removed to another tube containing 450 μL of cold CH_3CN + internal standard (1 μM). After centrifugation (10 min at 10,000 rpm), supernatants are analyzed. Analysis and quantification used an LC-MS/MS triple-quadrupole system (Varian 1200L) under MRM detection using the parameters optimized for each compounds. HPLC analysis was performed using a Luna C_{18} (50*2.1 mm, 5 μm); the gradient and the mobile phase (flow rate 600μL/min-1) used are determined in order to detect the compound of interest with satisfying retention time and peak shape. Acquisition and analysis of data were performed with MS Workstation™ software (version 6.3.0 or higher). The degradation half-life (t1/2) values were calculated using the following equation: $t1/2 = 0.693/k$ where k is the first-order degradation rate constant. The degradation rate constant (k) was estimated by one-phase exponential decay non-linear regression



Scheme 8. Synthesis of **48**–**49**. Reagents and conditions: (a) trifluoroacetic anhydride 2% in acetic anhydride, 50–70 °C, 5 h; (c) MeOH, DIEA, reflux, overnight; (c) amine, DMF, HOBt, EDCI, NMM, room temp. 22 h; (d) TFA/DCM TIS, room temp. 4 h.

Table 6
Intraperitoneal pharmacokinetic parameters for **44, 46, 48, 49** in mouse (30 mpk).[a]



44: R = H
48: R = Me

46: R = H
49: R = Me

Cpd	Cmax (μg/mL)	Tmax (min)	$T_{1/2}$ (min)	AUC (h.μg/mL)
48	3.8	10	7	1.0
49	1.0	10	6	0.2
44	11.0	10	9	3.6
46	5.8	10	14	1.9

[a] Analogues were dosed in male Black mice ($n = 3$) as a PBS solution.

analysis of the degradation time course data using Xlfit™ software (version 2.1.2 or higher) from IDBS.Ltd.

5.1.4. Microsomal stability

Male murine liver microsomes were purchased from BD Gentest (Le Pont de Claix, France). All incubations were performed in triplicates in a shaking water bath at 37 °C. The incubation mixture were prepared in polypropylene tubes and contained 1 μM test compound (1% acetonitrile), human liver microsomes (0.6 mg of microsomal protein/ml), 5 mM $MgCl_2$, 1 mM NADP, 5 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase and 50 mM potassium phosphate buffer pH 7.4 in a final volume of 0.5 mL. Sampling points were taken at 10, 20, 30 and 40 min and reactions were terminated by adding ice-cold acetonitrile containing 1 μM internal standard (4 vol). The samples were centrifuged for 10 min at 10,000 g, 4 °C to pellet precipitated microsomal protein, and the supernatant was subjected to LC-MS/MS analysis. Control incubations were performed with denaturated microsomes with acetonitrile containing 1 μM internal standard and sampling points were taken at 0 min and 40 min (to evaluate the compound chemical stability in the experimental conditions). Remaining compound was quantified by converting the corresponding analyte/internal standard peak area ratios to percentage drug remaining, using the initial ratio values in control incubations as 100%. The degradation half-life ($t_{1/2}$) values were calculated using the following equation: $t_{1/2} = 0.693/k$ where k is the first-order degradation rate constant. The degradation rate constant (k) was estimated by one-phase exponential decay non-linear regression analysis of the degradation time course data using Xlfit™ software (version 2.1.2 or higher) from IDBS.Ltd.

5.1.5. Pharmacokinetics in mice

Compounds were dissolved in either in 100% PBS (**48** and **49**) or in 10% cyclodextrin HP in PBS (Keptose, Roquette) (**44** and **46**), and were administered at 30 mg per kg body weight by intraperitoneal route to male C57Bl6/N 8-week old mice (env 25–30 g) (Charles River). Three mice per time point were anesthetized with isoflurane and aliquots taken from retroorbital sinus sampling at 10 min, 20 min, 30 min, 1 h, 2 h, and 4 h after administration of a single dose of ligands were put in heparinated tubes (4 °C). The blood samples were centrifuged (5000 *g*, 15 min) for plasma separation.

Plasma samples were thawed on ice. Aliquots of 50 μL were precipitated with 450 μL of ice cold acetonitrile containing **1** (1 μM) used as internal standard. The samples were vigorously mixed with a Vortex and centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatants were transferred into Matrix tubes for LC–MS–MS analysis (Table S2). Spiked standard solutions (10, 50, 100, 500, 1000, 5000, 10 000, and 50 000 nM) were prepared the same way. The half-life (t1/2) values were calculated using the following equation: $t1/2 = 0.693/k$ where k is the first-order degradation rate constant. The rate constant (k) was estimated by one-phase exponential decay non-linear regression analysis of the degradation time course data using Xlfit™ software (version 2.1.2 or higher) from IDBS.Ltd. AUC is the area under the concentration versus time curve extrapolated to infinity.

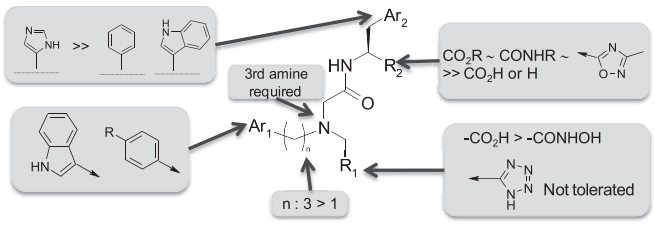


Fig. 4. Summary of structure–activity relationships.

5.2. Molecular modelling

The analogues **44** and **46** were docked into the binding pocket of hIDE using MOE[32] using the Protein DataBank (PDB) under pdb code 4DTT. Protein protonation was made with the Protonate3D process within MOE 10.2012. The docking used was based on the Induced Fit protocol of MOE, using a Triangle Matcher placement, a first rescoring process using the London dG routine and a force field-based refinement process and a second rescoring process using GBVI/WSA dG keeping in the end the best docking pose for each ligand. No restraints were used during docking but the binding modes were chosen based on both the most favoured predicted interaction energy with IDE and a proper binding to the Zinc ion by the carboxylic acid.

5.3. Crystallization and data process

The complexes of IDE-CF-E111Q with compounds **1**, **22**, **33** were crystallized by hanging drop vapour diffusion at 18 °C, using 1 μl of 10 mg/ml IDE and 1 μL of mother liquor (10–13% PEG MME 5000, 100 mM HEPES pH 7.0, 4–14% Tacsimate, 10% dioxane). IDE-CF in complex with **1** (BDM41367) was crystallized under the same condition except the addition of 200 μM of given compound in the crystallization drop. IDE crystals were equilibrated in cryo-protective buffer containing mother liquor with 30% glycerol and flash frozen in liquid nitrogen. Diffraction data were collected at 100 K at the Advance Photon Source 19-ID beamline at Argonne National Laboratory. The data sets were processed using HKL2000 [33] and the structures were solved by molecular replacement using software Phaser [34] and the IDE-CF-E111Q portion of insulin-bound IDE-CF-E111Q structure as a search model (PDB:2WBY). Structure refinement and rebuilding were performed using software REFMAC and Coot [32,35]. The extra electron density at the catalytic chamber of IDE-CF in the structures of IDE in complex with compound were clearly visible based on σ_A-weighted Fo-Fc map calculated by software CNS [36] and manually built. The refinement statistics are summarized in supplemental information Table S3. Figures were generated using software PyMol [37].

5.4. Chemistry

5.4.1. General information

NMR spectra were recorded on a Bruker Avance 300 spectrometer. Chemical shifts are in parts per million (ppm) and were

referenced to the residual proton peaks in deuterated solvents. The assignments were made using one dimensional (1D) ^{1}H and ^{13}C spectra (classical or Jmod) and two-dimensional (2D) HSQC, HMBC, ROESY and COSY spectra. Mass spectra were recorded with an LC–MS–MS triple–quadrupole system (Varian 1200ws) or an LCMS (Waters Alliance Micromass ZQ 2000). LCMS analysis was performed using a C18 TSK-GEL Super ODS (2 μm particle size column, dimensions 50 mm × 4.6 mm). A gradient starting from 100% H_2O/0.1% formic acid and reaching 20% H_2O/80% CH_3CN/0.08% formic acid within 10 min at a flow rate of 1 mL/min was used. Preparative HPLC were performed using a Varian PRoStar system using an OmniSphere 10 column C18 250 mm × 41.4 mm Dynamax from Varian, Inc. A gradient starting from 20% CH_3CN/80% H_2O/0.1% formic acid and reaching 100% CH_3CN/0.1% formic acid at a flow rate of 80 mL/min or 20% MeOH/80% H_2O/0.1% formic acid reaching 100% MeOH/0.1% formic acid was used. Purity (%) was determined by reversed phase HPLC, using UV detection (215 nm), and all compounds showed purity greater than 95%. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. All commercial reagents and solvents were used without further purification. Organic layers obtained after extraction of aqueous solutions were dried over $MgSO_4$ and filtered before evaporation under reduced pressure. Purification yields were not optimized. The LC-MS/MS system consisted of a Varian 1200L (Varian, Les Ulis, France) a Prostar 430 autosampler, a (Varian, Les Ulis, France) triple quadrupole mass spectrometry equipped with an electrospray ionisation source, with a Prostar 325 detector. The HPLC separation uses a gel TSK C18 Super-ODS, 5 μm, 50 × 4.6 mm column (Interchim, Montluçon France) with mobile phase solvents: (A) 0.01% formic acid in water; (B) 0.01% formic acid in acetonitrile for with the following mobile phase gradient: 0% B during 30 s, 0–98% (B) in 3′; hold at 98% (B) for 1 min; 98%–0% B in 0.30 min; 0% B hold for 1 min, at a flow rate of 1 mL/min, or Luna C18, 5 μm, 50 × 2.1 mm column (Phenomenex) with mobile phase solvents: (A) 5 mM ammonium formate pH 9,2 in water; (B) 5 mM ammonium formate pH 9,2 in acetonitrile with the following mobile phase gradient: 0% B during 30 s, 0–98% (B) in 2′30; hold at 98% (B) for 1 min; 98%–0% B in 0′10; 0% B hold for 1 min, at a flow rate of 600 μL/min. The injection volume was 40 μL. The pressures of drying and nebulising gas are respectively 19 and 50 psi; the curtain gas pressure is 2 mTorr. Source temperature, declustering potential, collision energy and observed transitions were respectively individually optimized for each compound.

5.4.2. General procedure A for anhydride opening by amines

1) Obtention of anhydrides: iminodiacetic acid analogue (5 mmol) was dissolved in trifluoracetic anhydride 2% in acetic anhydride (5 mL). The reaction mixture was stirred for 4 h at room temperature or 70 °C if product was not soluble at room temperature and then evaporated under reduced pressure. The crude product was directly used in the next step. In the case of iminodiacetic acids **9a** and **18a**, a stirred solution of diacid (5 mmol) in THF (10 mL) was added DCC (6 mmol). The resulting mixture was stirred at room temperature for 5 h and evaporated. 2) anhydride opening by amines: The crude anhydride (1 mmol) was solubilised in anhydrous DMF (5 mL). Then corresponding amine (1 mmol) (free base) and DIEA (4 mmol) was added. The mixture is stirred at room temperature under argon overnight. The solvent is removed under reduced pressure and the crude product is purified by preparative HPLC. Trityle protections were removed using TFA/DCM/TIS (5/90/5 v/v) mixtures and final compounds were purified by preparative HPLC if needed.

5.4.3. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester ***(1)***

BDM41367 as its formiate salt was synthesized following general procedure A from commercial N-benzyliminodiacetic acid and L-Histidine methyl ester dihydrochloride. White solid. Yield 20%. ^{1}H NMR (DMSO-d_6) δ ppm:, 8.34 (d, 1H, NH), 8.26 (d, $J = 1.1$ Hz, 1H), 7.30 (m, 5H), 7.09 (d, $J = 1.1$ Hz, 1H), 4.65 (m, 1H), 3.77 (d, $J = 13.4$ Hz, 1H), 3.71 (d, $J = 13.4$ Hz, 1H), 3.59 (s, 3H), 3.27 (s, 2H), 3.23 (s, 2H), 2.99 (d, $J = 6.3$ Hz, 2H), LC $t_R = 2.30$ min, MS (ESI+): $m/z = 375$ $(M + H)^+$. ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 171.8, 170.4, 138.2, 137.9, 129.0, 128.4, 127.3, 115.9, 57.5, 56.7, 53.6, 52.1, 51.7, 28.4. $t_{R,LCMS} = 2.30$ min; Purity 100%; MS (ESI+): $m/z = 375$ $(M+H)^+$; HRMS (m/z): (M^+) calcd. for $C_{18}H_{23}O_5N_4$, 375.1668; found, 375.1665.

5.4.4. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-phenyl-propionic acid methyl ester ***(2)***

Compound **(2)** was synthesized from L-phenylalanine methyl ester hydrochloride and N-benzyliminodiacetic acid following general procedure A. Colourless oil. Yield 40%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.13 (d, $J = 8.10$ Hz, 1H), 7.31–7.16 (m, 10H), 4.58 (m, 1H), 3.69 (d, $J = 13.2$ Hz, 1H), 3.64 (d, $J = 13.2$ Hz, 1H), 3.61 (s, 3H), 3.22 (s, 2H), 3.16 (s, 2H), 3.08 (dd, $J = 5.4$ and 13.8 Hz, 1H), 2.96 (dd, $J = 8.7$ and 13.8 Hz, 1H); $t_{R,LCMS} = 4.39$ min, Purity 100%; MS (ESI+): $m/z = 385$ $(M+H)^+$.

5.4.5. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(1H-indol-3-yl)-propionic acid methyl ester ***(3)***

Compound **(3)** was synthesized following general procedure A from L-tryptophan methyl ester dihydrochloride and N-benzyliminodiacetic acid. White solid. Yield: 25%. ^{1}H NMR (DMSO-d_6) δ ppm: 10.9 (s, 1H), 8.10 (d, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 7.8$ Hz, 1H), 7.36 (d, $J = 8.1$ Hz, 1H), 7.20–7.22 (m, 3H), 7.16 (d, $J = 2.1$ Hz, 1H), 7.08–7.10 (m, 2H), 7.08 (td, $J = 1.2$ and 7.8 Hz, 1H), 6.98 (td, $J = 1.2$ and 8.1 Hz, 1H), 4.60 (dd, $J = 7.2$ and 15.0 Hz, 1H), 3.68 (d, $J = 13.2$, 1H), 3.62 (d, $J = 13.2$, 1H), 3.59 (s, 3H), 3.14–3.27 (m, 6H), ^{13}C NMR (DMSO-d_6) δ ppm: 172.8, 172.6, 170.7, 138.4, 136.7, 128.8, 128.2, 127.1, 124.2, 121.7, 119.2, 118.2, 111.9, 109.3, 58.0, 57.2, 54.2, 52.4, 52.1, 27.4. $t_{R,LCMS} = 4.42$ min, Purity 99%; MS (ESI+): $m/z = 424$ $(M+H)^+$.

5.4.6. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-5-guanidino-pentanoic acid methyl ester ***(4)***

Compound **(4)** was synthesized following general procedure A from commercial N-benzyliminodiacetic acid and L-Arginine methyl ester dihydrochloride. White solid. Yield 98%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.39 (d, $J = 7.8$ Hz, 1H), 7.97 (m, 1H), 7.26–7.35 (m, 5H), 4.30 (ddd, $J = 4.8$ Hz, 8.4 Hz and 8.1 Hz, 1H), 3.78 (s, 2H), 3.63 (s, 3H), 3.34 (s, 2H), 3.26 (s, 2H), 3.07–3.14 (m, 2H), 1.70–1.79 (m, 2H), 1.44–1.51 (m, 2H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.7, 172.2, 170.6, 157.0, 138.2, 128.8, 128.2, 127.2, 57.6, 56.4, 54.5, 51.9, 51.2, 41.5, 28.1, 24.9. $t_{R,LCMS} = 2.51$ min, Purity 99%; MS (ESI+): $m/z = 394$ $(M+H)^+$.

5.4.7. General procedure for the synthesis of N-substituted iminodiacetic acids from corresponding halides (***5a–14a, 16a***)

To a solution of iminodiacetic acid hydrochloride (2 mmol) in MeOH (10 mL) were added NEt_3 (6 mmol) and the corresponding bromide derivative (2 mmol). The mixture was stirred overnight at room temperature. The crude product was purified by preparative HPLC.

5.4.8. (Carboxymethyl-(4-fluoro-benzyl)-amino)-acetic acid ***(5a)***

White solid. Yield 55%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.30 (m, 2H), 7.12 (m, 2H), 3.77 (s, 2H), 3.31 (s, 4H). $t_{R,LCMS} = 1.93$ min, Purity 100%; MS (ESI+): $m/z = 242$ $(M + H)^+$.

*5.4.9. (Carboxymethyl-(4-trifluoromethyl-benzyl)-amino)-acetic acid **(6a)***

White solid. Yield 44%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.68 (d, J = 7.8 Hz, 2H), 7.58 (d, J = 7.8 Hz, 2H), 3.91 (s, 2H), 3.42 (s, 4H). $t_{R,LCMS}$ = 3.17 min; Purity 98%; MS (ESI+): m/z = 292 $(M+H)^+$.

*5.4.10. (Carboxymethyl-(4-methyl-benzyl)-amino)-acetic acid **(7a)***

White solid. Yield 31%. ^{1}H NMR (DMSO-d_6) δ ppm: 12.27 (s, 3H), 3.34 (s, 2H), 7.10 (d, J = 7.8 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H). $t_{R,LCMS}$ = 2.38 min; Purity 90%; MS (ESI+): m/z = 238 $(M+H)^+$.

*5.4.11. ((4-Tert-Butyl-benzyl)-carboxymethyl-amino)-acetic acid **(8a)***

White solid. Yield 43%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.34 (d, J = 8.4 Hz, 2H),7.23 (d, J = 8.4 Hz, 2H), 3.76 (s, 2H), 3.37 (s, 4H), 1.28 (s, 9H). $t_{R,LCMS}$ = 3.88 min; Purity 90%; MS (ESI+): m/z = 280 $(M+H)^+$.

*5.4.12. Carboxymethyl-pyridin-4-ylmethyl-amino)-acetic acid **(9a)***

White solid. Yield 95%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.48 (d, J = 5.7 Hz, 2H), 7.29 (d, J = 5.7 Hz, 2H), 3.83 (s, 2H), 3.35 (s, 4H); $t_{R,LCMS}$ = 0.87 min; Purity 98%; MS (ESI-): m/z = 223 $(M-H)^-$.

*5.4.13. (Carboxymethyl-naphthalen-2-ylmethyl-amino)-acetic acid **(10a)***

White solid. Yield 57%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.89–7.85 (m, 3H), 7.77 (s, 1H), 7.57 (dd, J = 1.5 and 8.4 Hz, 1H), 7.51–7.45 (m, 2H). $t_{R,LCMS}$ = 3.13 min; Purity 95%; MS (ESI+): m/z = 274 $(M+H)^+$.

*5.4.14. (Carboxymethyl-(2-(1H-indol-3-yl)ethyl)-amino)-acetic acid) **(11a)***

White solid. Yield 58%. ^{1}H NMR (DMSO-d_6) δ ppm: 10.77 (s, NH), 7.49 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 8.04 Hz, 1H), 7.16 (d, J = 2.16 Hz, 1H), 7.04 (dt, J = 1.1 Hz, 7.1 Hz and 8.04 Hz, 1H), 6.94 (dt, J = 1.1 Hz, 7.1 Hz and 7.7 Hz, 1H), 3.50 (s, 4H), 2.88 (m, 4H); $t_{R,LCMS}$ = 3.28 min; Purity 98%; MS (ESI+): m/z = 277 $(M+H)^+$.

*5.4.15. (Carboxymethyl-phenethyl-amino)-acetic acid **(12a)***

White solid. Yield 36%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.22 (m, 5H), 3.46 (s, 4H), 2.86 (m, 2H), 2.70 (m, 2H). $t_{R,LCMS}$ = 1.12 min, Purity 100%; MS (ESI+): m/z = 238 $(M+H)^+$.

*5.4.16. (Carboxymethyl-(3-phenyl-propyl)-amino)-acetic acid **(13a)***

White solid. Yield 60%. ^{1}H NMR (DMSO-$d_6$6) δ ppm: 7.13–7.26 (m, 5H), 3.41 (s, 4H), 2.65 (t, J = 7.5 Hz, 2H), 2.56 (t, J = 7.5 Hz, 2H), 1.67 (dt, J = 7.5 Hz, 2H). $t_{R,LCMS}$ = 2.76 min; Purity 99%; MS (ESI+): m/z = 252 $(M+H)^+$.

*5.4.17. (Carboxymethyl-(4-phenyl-butyl)-amino)-acetic acid **(14a)***

White solid. Yield 76%. NMR (DMSO-d_6) δ ppm: 7.26–7.12 (m, 5H), 3.39 (s, 4H), 2.65 (t, J = 7.1 Hz, 2H), 2.55 (t, J = 7.1 Hz, 2H), 1.54 (m, 2H), 1.39 (m, 2H). $t_{R,LCMS}$ = 3.59 min; Purity 99%; MS (ESI+): m/z = 266 $(M+H)^+$.

*5.4.18. (Carboxymethyl-hexyl-amino)-acetic acid (**16a**)*

Oil directly used in the next step $t_{R,LCMS}$ = 2.90 min; MS (ESI+): m/z = 218 $(M+H)^+$.

*5.4.19. (tert-Butoxycarbonyl-carboxymethyl-amino)-acetic acid **(17a)***

To a stirred solution of iminodiacetic acid (2 g, 15 mmol) in a dioxane/H_2O (3/1, 40 mL) mixture, were added at 0 °C, Boc_2O (3.93 g, 18 mmol) and 10 mL of 2 N NaOH solution. The mixture was stirred at room temperature and dioxane was evaporated under reduced pressure. The aqueous layer was acidified with a 20% citric acid solution and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give **17a** (67%), as a white solid directly used in the next step.

*5.4.20. (Benzoyl-carboxymethyl-amino)-acetic acid **(20a)***

Iminodiacetic acid was stirred overnight in an 80/20 MeOH/$SOCl_2$ mixture. The diester obtained after evaporation under reduced pressure (300 mg, 1.52 mmol) was dissolved in CH_2Cl_2 (10 mL) and DIEA (792 μL, 4.56 mmol) and benzoylchloride (176 μL, 1.52 mmol) were added. The mixture was stirred overnight at room temperature and washed with HCl 0.5 N and $NaHCO_3$ 10% solutions. The organic layer was dried over $MgSO_4$ and evaporated to give the desired diester as a colourless oil (99%). The former compound (400 mg, 1.5 mmol) was saponified with NaOH (6 mmol) in MeOH (5 mL) and H_2O (1 mL). After evaporation of MeOH under reduced pressure, HCl 1 N was added to the residue and the aqueous layer was extracted 3 times with AcOEt to give (benzoyl-carboxymethyl-amino)-acetic acid **(20a)** directly used in the next step. White powder. Yield 98 Purity 98%; MS (ESI+): m/z = 238 $(M+H)^+$.

*5.4.21. (Carboxymethyl-phenylacetyl-amino)-acetic acid **(21a)***

Iminodiacetic acid was stirred overnight in an 80/20 MeOH/$SOCl_2$ mixture and evaporated. To the diester obtained (591 mg, 3.0 mmol) in solution in CH_2Cl_2 (15 mL) were added DIEA (2.08 mL, 12 mmol), phenylacetic acid (490 mg, 3.6 mmol), EDCI.HCl (747 mg, 3.9 mmol), HOBt (597 mg, 3.9 mmol). The mixture was stirred overnight at room temperature and washed with HCl 1 N solution, $NaHCO_3$ 10% solution and H_2O. The organic layer was dried over $MgSO_4$ and evaporated to give the diester as a colourless oil. The diester was saponified with NaOH (15 mmol) in MeOH (10 mL) and H_2O (1 mL). MeOH was evaporated, H_2O was added and the aqueous layer was extracted with CH_2Cl_2 (3 times). The aqueous layer was acidified to pH 2 and extracted 4 times with AcOEt to give the **21a**. White solid. Yield 70% over two steps. ^{1}H NMR (DMSO-d_6) δ ppm: 7.27 (m, 5H), 4.22 (s, 2H), 3.99 (s, 2H), 3.65 (s, 2H). $t_{R,LCMS}$ = 2.83 min, Purity 98%; MS (ESI+): m/z = 252 $(M+H)^+$.

*5.4.22. (Carboxymethyl-(3-phenyl-propionyl)-amino)-acetic acid **(22a)***

Was synthesized following procedure described for **20a** from iminodiacetic acid and hydrocinnamoyl chloride. White solid. Yield 99%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.23 (m, 5H), 4.18 (s, 2H), 3.98 (s, 2H), 2.79 (t, J = 8.1 Hz, 2H), 2.56 (t, J = 8.1 Hz, 2H). $t_{R,LCMS}$ = 3.41 min, Purity 97%; MS (ESI+): m/z = 266 $(M+H)^+$.

*5.4.23. (Benzyloxycarbonyl-carboxymethyl-amino)-acetic acid **(23a)***

To a solution of iminodiacetic acid (266 mg, 2.0 mmol) in 2 N NaOH solution in an ice bath, was added benzylchloroformate (337 μL, 2.4 mmol). The mixture was stirred at room temperature for 2 h and extracted twice with Et_2O. The aqueous layer was acidified to pH 2 and extracted with Et_2O (3 times). The organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give **24a** as a colourless oil directly used in the next step. Yield 64%. $t_{R,LCMS}$ = 3.49 min, Purity 98%; MS (ESI^-): m/z = 266 $(M-H)^-$.

*5.4.24. (S)-2-(2-(Carboxymethyl-(4-fluoro-benzyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(5)***

Compound **(5)** was synthesized according to general procedure A from **5a** and L-histidine methyl ester dihydrochloride. White solid. Yield 37%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.38 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 0.9 Hz, 1H), 7.35–7.30 (m, 2H), 7.15–7.09 (m, 2H), 6.84 (d, J = 0.9 Hz, 1H), 4.57 (m, 1H), 3.75 (d, J = 13.2 Hz, 1H), 3.69 (d, J = 13.2 Hz, 1H), 3.58 (s, 3H), 3.23 (s, 2H), 3.22 (s, 2H), 2.98 (dd,

J = 6.9 and 15 Hz, 1H), 2.92 (dd, J = 5.4 and 14.7 Hz, 1H)). $t_{R,LCMS}$ = 2.54 min, Purity 100%; MS (ESI+): m/z = 393 $(M+H)^+$.

*5.4.25. (S)-2-(2-(Carboxymethyl-(4-trifluoromethyl-benzyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(6)***

First N-4-(trifluoromethyl)benzyliminodiacetic acid **6a** and H-His(1-trt)-OMe HCl reacted as described in general procedure A to give the trityle protected intermediate (S)-2-(2-(carboxymethyl-(4-trifluoromethyl-benzyl)-amino)-acetylamino)-3-(1-trityl-1H-imidazol-4-yl)-propionic acid methyl ester. White solid. Yield 75%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.50 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.1 Hz, 2H), 7.5 (d, J = 8.1 Hz, 2H), 7.36 (m, 9H), 7.25 (d, J = 1.2 Hz, 1H), 7.00–7.04 (m, 6H), 6.65 (d, J = 1.2 Hz, 1H), 4.59 (m, 1H), 3.88 (s, 2H), 3.51 (s, 3H), 3.29 (s, 4H), 2.95 (dd, J = 6.5 and 14.6 Hz, 1H), 2.87 (dd, J = 5.4 and 14.6 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.0, 171.6, 169.9, 143.3, 142.1, 137.9, 136.4, 129.9, 129.6, 128.6, 128.5, 126.41, 125.4, 119.2, 74.5, 56.9, 56.8, 53.3, 51.8, 29.5 $t_{R,LCMS}$ = 5.87 min, Purity 99%; MS (ESI+): m/z = 685 $(M+H)^+$. Deprotection using TFA/DCM in the presence of triisopropylsilane allowed **6** as an oil (TFA salt). Yield 100%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.97 (s, 1H), 8.42 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.37 (s, 1H), 4.80 (m, 1H), 3.85 (s, 2H), 3.63 (s, 3H), 3.35 (s, 2H), 3.28 (s, 2H), 3.18 (dd, J = 5.3 and 15.3 Hz, 1H), 3.08 (dd, J = 9.1 and 15.3 Hz, 1H) ^{13}C NMR (DMSO-d_6) δ ppm: 172.5, 171.3, 170.6, 143.4, 132.4, 130.0, 129.6, 126.4, 125.5, 117.5, 57.4, 56.6, 54.4, 52.8, 51.2, 26.6 $t_{R,LCMS}$ = 4.68 min, Purity 99%; MS (ESI+): m/z = 443 $(M+H)^+$.

*5.4.26. (S)-2-(2-(Carboxymethyl-(4-methyl-benzyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(7)***

Compound **(7)** was synthesized following general procedure A from **7a** and H-His(1-trt)-OMe HCl to give the trityle protected intermediate as a white solid. Yield 40%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.57 (d, J = 8.4 Hz, 1H), 7.35–7.39 (m, 9H), 7.26 (d, J = 1.2 Hz, 1H), 7.21 (d, J = 7.9 Hz, 2H), 7.01–7.04 (m, 6H), 6.95 (d, J = 7.9 Hz, 2H), 6.64 (d, J = 1.2 Hz, 1H), 4.58–4.64 (m, 1H), 3.71 (s, 2H), 3.51 (s, 3H), 3.25 (s, 2H), 3.18 (s, 2H), 2.95 (dd, J = 6.2 and 14.5 Hz, 1H), 2.87 (dd, J = 5.0 and 14.5 Hz, 1H), 2.21 (s, 3H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 171.6, 170.2, 142.1, 137.7, 136.2, 135.1, 129.2, 128.9, 128.7, 128.3, 128.2, 119.1, 74.5, 57.1, 57.0, 53.2, 51.8, 29.6, 20.7. $t_{R,LCMS}$ = 5.28 min (10 min gradient), Purity 95%; MS (ESI+): m/z = 631 $(M+H)^+$. Then deprotection using TFA/DCM/TIS allowed **7** as ist TFA salt. White solid. Yield 100%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.98 (d, J = 1.2 Hz, 1H), 8.54 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 1.2 Hz, 1H). 7.20 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 4.70 (m, 1H), 3.83 (s, 2H), 3.65 (s, 3H), 3.45 (s, 2H), 3.41 (s, 2H), 3.19 (dd, J = 5.4 and 15.3 Hz, 1H), 3.08 (dd, J = 9.0 and 15.3 Hz, 1H), 2.29 (s, 3H), ^{13}C NMR (DMSO-d_6) δ ppm: 171.0, 170.7, 169.7, 158.0, 137.1, 133.7, 132.7, 129.5, 129.1, 129.0, 117.1, 57.3, 55.5, 53.6, 52.4, 50.9, 26.2, 20.7. $t_{R,LCMS}$ = 2.86 min, Purity 99%; MS (ESI+): m/z = 389 $(M+H)^+$.

*5.4.27. (S)-2-(2-((4-tert-Butyl-benzyl)-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(8)***

First N-4-(tert-butyl)benzyliminodiacetic acid **8a** and H-His(1-trt)-OMe HCl reacted as described in general procedure A to give the protected intermediate (S)-2-(2-((4-*tert*-butyl-benzyl)-carboxymethyl-amino)-acetylamino)-3-(1-trityl-1H-imidazol-4-yl)-propionic acid methyl ester. White solid. Yield 79%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.47 (d, J = 8.1 Hz, 1H), 7.37 (m, 10H), 7.26 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 7.03 (m, 6H), 6.65 (s, 1H), 4.62 (m, 1H), 3.74 (s, 2H), 3.52 (s, 3H), 3.22 (s, 2H), 3.16 (s, 2H), 2.92 (m, 2H), 1.19 (s, 9H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.1, 171.6, 170.1, 149.5, 142.1, 137.9, 136.3, 135.0, 129.2, 128.6, 128.2, 128.0, 124.9, 119.1, 74.5, 56.6, 56.5, 52.6, 34.1, 31.1, 29.9. $t_{R,LCMS}$ = 6.32 min Purity 94%; MS (ESI+): m/z = 673 $(M+H)^+$. Deprotection using TFA/DCM in the presence of triisopropylsilane allowed **8** as colourless oil (TFA salt). Yield 84%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.96 (s, 1H), 8.40 (d, J = 8.1 Hz, 1H), 7.36 (s, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 4.70 (m, 1H), 3.71 (s, 2H), 3.65 (s, 3H), 3.30 (s, 2H), 3.26 (s, 2H), 3.19 (dd, J = 5.6 and 15.4 Hz, 1H), 3.09 (dd, J = 8.8 and 15.4 Hz, 1H),1.27 (s, 9H) ^{13}C NMR (DMSO-d_6) δ ppm: 172.5, 171.3, 170.7, 150.2, 134.9, 134.2, 129.6, 129.3, 125.5, 117.6, 57.5, 56.7, 54.1, 52.8, 51.2, 34.7, 31.6, 26.7. $t_{R,LCMS}$ = 4.13 min, Purity 95%; MS (ESI+): m/z = 431 $(M+H)^+$.

*5.4.28. (S)-2-(2-(Carboxymethyl-pyridin-4-ylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(9)***

First trityle protected intermediate was synthesized from **9a** and H-His(1-trt)-OMe HCl following general procedure A. Yield 41%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.65 (d, J = 4.8 Hz, 0.3 CONH), 8.62 (d, J = 4.8 Hz, 0.6 CONH), 8.48 (d, J = 5.7 Hz, 1H), 8.36 (d, J = 5.7 Hz, 1H), 8.2 (s, HCOOH), 7.36–7.18 (m, 15H(Trt)+1H), 7.04–7.01 (m, 2H), 6.85 (s, 0.3H), 6.65 (s, 0.6H), 4.56 (m, 1H), 3.81 (s, 2H), 3.55 (s, CO_2Me), 3.27 (s, 2H), 3.24 (s, 2H), 2.95 (m, 2H); $t_{R,LCMS}$ = 4.59 min, Purity 95%; MS (ESI+): m/z = 618 $(M+H)^+$. Deprotection using TFA/DCM/TIS mixture allowed **9** as ist formiate salt after preparative HPLC purification. Yield 75%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.5–8.47 (m, CONH+2H), 8.2 (s, HCOOH), 7.59 (d, J = 1.2 Hz, 1H), 7.33 (d, J = 5.7 Hz, 2H), 6.85 (d, J = 1.2 Hz, 1H), 4.55 (m, 1H), 3.80 (s, 2H), 3.58 (s, CO_2Me), 3.27 (s, 2H), 3.24 (s, 2H), 2.96 (m, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.5, 171.9, 170.3, 163.8, 149.6, 147.8, 135.6, 123.8, 116.8, 57.0, 56.6, 54.3, 52.1, 52.0, 28.8; $t_{R,LCMS}$ = 0.79 min (5 min gradient), Purity 99%; MS (ESI+): m/z = 376 $(M+H)^+$.

*5.4.29. (S)-2-(2-(Carboxymethyl-naphthalen-2-ylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(10)***

Compound **(10)** was synthesized according to general procedure A from **10a** and L-histidine methyl ester dihydrochloride. White solid. 45%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.44 (d, J = 8.1 Hz, 1H), 8.14 (s, 1H, HCOOH), 7.82–7.90 (m, 3H), 7.78 (s, 1H), 7.71 (d, J = 0.9 Hz, 1H), 7.49–7.56 (m, 3H), 6.91 (d, J = 0.9 Hz, 1H), 4.59 (m, 1H), 3.95 (d, J = 13.5 Hz, 1H), 3.90 (d, J = 13.5 Hz, 1H), 3.59 (s, 3H), 3.32 (s, 2H), 3.29 (s, 2H), 2.99 (m, 2H), $t_{R,LCMS}$ = 3.46 min, Purity 100%; MS (ESI+): m/z = 425 $(M+H)^+$.

*5.4.30. (S)-2-(2-(Carboxymethyl-(2-(1H-indol-3-yl)-ethyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(11)***

The compound was synthesized according to general procedure A starting from **11a** and H-His(1-trt)-OMe HCl to give protected intermediate (S)-2-(2-(Carboxymethyl-(2-(1H-indol-3-yl)-ethyl)-amino)-acetyl amino)-3-(1-trityl-1H-imidazol-4-yl)-propionic acid methyl ester. White solid. Yield 45%. $t_{R,LCMS}$ = 6.93 min, Purity 98%; MS (ESI+): m/z = 670 $(M+H)^+$. Deprotection using TFA/DCM in the presence of triisopropylsilane allowed **11** as a white solid (TFA salt). Yield 86%. ^{1}H NMR (DMSO-d_6) δ ppm: 10.92 (sl, NH), 8.97 (s, 1H), 8.93 (m, CONH), 7.53 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.17 (d, J = 1.8 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 6.98 (t, J = 7.8 Hz, 1H), 4.71 (m, 1H), 3.96 (s, 2H), 3.87 (s, 2H), 3.62 (s, CO_2Me), 3.22–2.98 (m, 6H); ^{13}C NMR (DMSO-d_6) δ ppm: 170.5, 169.4, 166.6, 136.3, 134.6, 128.8, 126.8, 123.3, 121.3, 118.5, 118.2, 117.3, 111.6, 109.5, 55.7, 54.4, 54.9, 52.5, 51.4, 26.1, 21.0; $t_{R,LCMS}$ = 4.17 min (at pH 3.8), Purity 97%; MS (ESI+): m/z = 428 $(M+H)^+$.

*5.4.31. (S)-2-(2-(Carboxymethyl-phenethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(12)***

Compound **(12)** was synthesized from **12a** and L-histidine methyl ester dihydrochloride following general procedure A, as its formiate salt. White solid. Yield 52%. ^{1}H NMR (DMSO-d_6) δ ppm:8.22 (d, J = 7.8 Hz, 1H), 8.14 (s, 1H, HCOOH), 7.56 (d,

J = 1.2 Hz, 1H), 7.28–7.22 (m, 2H), 7.18–7.13 (m, 3H), 6.82 (d, J = 1.2 Hz, 1H), 4.52 (m, 1H), 3.58 (s, 3H), 3.38 (s, 2H), 3.25 (s, 2H), 2.92 (m, 2H), 2.76 (m, 2H), 2.66 (m, 2H); $t_{R,LCMS}$ = 2.63 min, Purity 96%; MS (ESI+): m/z = 389 $(M + H)^+$. HRMS (m/z): (M^+) calcd. for $C_{19}H_{25}O_5N_4$, 389.1825; found, 389.1820.

5.4.32. (S)-2-(2-(Carboxymethyl-(3-phenyl-propyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (13)

The compound was synthesized according to general procedure A starting from **13a** and H-His(1-trt)-OMe HCl to give the protected intermediate. Yield 57%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.49 (d, J = 8.2 Hz, 1H), 6.99–7.38 (m, 21H), 6.62 (s, 1H), 4.58 (ddd, J = 6 Hz, 8.1 Hz, 1H), 3.51 (s, 3H), 3.28 (s, 2H), 3.19 (s, 2H), 2.88 (m, 2H), 2.59 (t, J = 7.4 Hz, 2H), 2.5 (m, 2H), 1.62 (dt, J = 7.4 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.7, 171.7, 170.6, 142.1, 137.6, 136.3, 129.2, 128.2, 128.0, 127.8, 127.5, 125.6, 119.0, 57.8, 55.1, 53.8, 51.8, 32.7, 29.8, 29.3, $t_{R,LCMS}$ = 5.21 min, Purity 92%; MS (ESI+): m/z = 645 $(M + H)^+$. Deprotection of the trityle group using TFA/DCM/TIS mixture allowed **13** as its formiate salt after purification by preparative HPLC. White solid. Yield 55%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.98 (s, 1H), 8.86 (d, J = 7.2 Hz, H), 7.41 (s, 1H), 7.17–7.30 (m, 5H), 4.70 (m, 1H), 3.78 (s, 2H), 3.70 (s, 2H), 3.62 (s, 3H), 3.11 (m, 2H), 2.90 (m, 2H), 2.57 (m, 2H), 1.79 (m, 2H). ^{13}C NMR (DMSO-d_6) δ ppm: 170.6, 169.7, 167.5. 141.1, 133.7, 128.9, 128.4, 128.3, 126.0, 117.2, 55.7, 54.8, 54.7, 52.4, 51.2, 32.2, 26.8, 26.1. $t_{R,LCMS}$ = 3.06 min; Purity 99%; MS (ESI+): m/z = 403 $(M + H)^+$; HRMS (m/z): (M^+) calcd. for $C_{20}H_{27}O_5N_4$, 403.1981; found, 403.1978.

5.4.33. (S)-2-(2-(Carboxymethyl-(4-phenyl-butyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (14)

Was prepared from **14a** and L-histidine methyl ester dihydrochloride following general procedure A. White solid. Yield 73%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.28 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 1.2 Hz, 1H), 7.28–7.12 (m, 5H), 7.01 (d, J = 1.2 Hz, 1H), 4.59 (m, 1H), 3.59 (s, 1H), 3.30 (s, 2H), 3.19 (s, 2H), 3.02 (dd, J = 6.1 Hz, J = 15.2 Hz, 1H), 2.93 (dd, J = 7.3 Hz, J = 15.2 Hz, 1H), 2.56 (m, 4H), 1.52 (m, 2H), 1.40 (m, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.5, 171.4, 170.6, 142.2, 134.7, 131.9, 128.3, 128.2, 125.6, 116.3, 57.6, 55.0, 54.0, 52.0, 51.5, 35.0, 28.5, 28.0, 26.7. $t_{R,LCMS}$ = 3.41 min, Purity 99%; MS (ESI+): m/z = 417 $(M + H)^+$.

5.4.34. (S)-2-(2-Chloro-acetylamino)-3-(1-trityl-1H-imidazol-4-yl)-propionic acid methyl ester (15a)

To a stirred solution of L-His(Trt)-OMe (895 mg, 2 mmol) and $NaHCO_3$ (420 mg, 5 mmol) in DCM (10 mL) was added dropwise chloroacetylchloride (159 μL, 2 mmol). The mixture was stirred at room temperature 10 min. The insoluble was filtered and the filtrate evaporated to give the halide **15a** quantitatively. ^{1}H NMR (DMSO-d_6) δ ppm: 8.63 (d, J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.47–7.36 (m, 9H), 7.11–7.06 (m, 6H), 6.82 (s, 1H), 4.55 (m, 1H), 4.09 (s, 2H), 3.58 (s, 1H), 2.96 (dd, J = 5.7 Hz, J = 14.7 Hz, 1H), 2.88 (dd, J = 8.3 Hz, J = 14.7 Hz, 1H); $t_{R,LCMS}$ = 2.81 min, Purity 97%; MS (ESI+): m/z = 488 $(M + H)^+$.

5.4.35. (1-Methyl-3-phenyl-propylamino)-acetic acid tert-butyl ester (15b)

To a stirred solution of 1-methyl-3-phenylpropylamine (321 μL, 2 mmol) and DIEA (870 μL, 5 mmol) in THF (5 mL) was added dropwise at 0 °C tert-butylbromoacetate (322 μL, 2 mmol). The resulting mixture was stirred at room temperature overnight. The insoluble was filtrated and filtrate was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography to give **15b**. Yield 63%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.25–7.15 (m, 5H), 3.30 (m, 1H), 3.20 (s, 2H), 2.58 (m, 2H), 1.71–1.51 (m, 2H), 1.41 (s, 9H), 0.99 (d, J = 6.2 Hz, 2H); $t_{R,LCMS}$ = 4.73 min, Purity 97%; MS (ESI+): m/z = 264 $(M + H)^+$.

5.4.36. (S)-2-(2-(Carboxymethyl-(1-methyl-3-phenyl-propyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (15)

To a stirred solution of **31** (102 mg, 0.2 mmol) and $NaHCO_3$ (66 mg, 0.6 mmol) in anhydrous DMF (1 mL) was added **33** (61 mg, 0.23 mmol). The mixture was stirred at room temperature for 1 h then refluxed 20 h. KI (15 mg, 0.09 mmol) was then added and the resulting mixture was refluxed 1 h. The solvent was evaporated under reduced pressure and the residue was precipitated in water. The resulting solid was purified by preparative HPLC to give protective intermediate as an oil. Yield 45%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.43 (d, J = 8.4 Hz, 1H), 7.37–6.99 (m, 21H), 6.60 (s, 1H), 4.60 (m, 1H), 3.69–3.61 (m, 7H), 3.20–3.09 (m, 3H), 2.61 (m, 2H), 1.80–1.46 (m, 2H), 0.97 (m, 3H); $t_{R,LCMS}$ = 3.94 min, Purity 98%; MS (ESI+): m/z = 715 $(M + H)^+$. Then protections were removed using TFA/DCM (50/50) in the presence of triisopropylsilane. Solvents were evaporated under reduced pressure and the crude product was precipitated in Et_2O/pentane to give quantitatively **15** as its TFA salt. ^{1}H NMR (DMSO-d_6) δ ppm: 8.98 (s, 1H), 8.92 (d, J = 8.1 Hz, 1H), 7.4 (s, 1H), 7.37–7.17 (m, 5H), 4.72 (m, 1H), 3.69–3.61 (m, 7H), 3.20–3.01 (m, 3H), 2.57 (t, J = 7.9 Hz, 2H), 1.85 (m, 1H), 1.59 (m, 1H), 1.13 (m, 3H); ^{13}C NMR (DMSO-d_6) δ ppm: 170.5, 158.6, 158.1, 141.4, 135.14, 128.7, 128.3, 128.2, 125.9, 117.2, 52.4, 51.2, 33.7, 31.8, 26.3, 26.4, 14.3; $t_{R,LCMS}$ = 2.22 min, Purity 96%; MS (ESI+): m/z = 417 $(M + H)^+$.

5.4.37. (S)-2-(2-(Carboxymethyl-(n-hexyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (16)

The compound was prepared using procedure A starting from **16a** and H-His(1-trt)-OMe HCl to give trityle-protected intermediate as a white solid. Yield 52%. $t_{R,LCMS}$ = 4.93 min; Purity 83%; MS (ESI+): m/z = 611 $(M + H)^+$. Then deprotection using a TFA/DCM/TIS mixture allowed **16** as its TFA salt. Yield 100%.

^{1}H NMR (DMSO-d_6) δ ppm: 8.99 (s, 1H), 8.80 (m, CONH), 7.41 (s, 1H), 4.70 (m, 1H), 3.76 (s, 2H), 3.65 (s, 5H(Ph)), 3.19 (dd, J = 5.7 Hz and 15.3 Hz, 1H), 3.07(dd, J = 9.3 Hz and 15.3 Hz, 1H), 2.85 (m, 2H), 1.45 (m, 2H), 1.22 (m, 6H), 0.85 (t, J = 6.3 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ ppm: 171.1, 170.6, 168.5, 134.5, 129.0, 117.1, 56.1, 54.9, 54.7, 52.4, 51.1, 30.9, 26.5, 26.1, 25.9, 21.9, 13.9; $t_{R,LCMS}$ = 3.03 min, Purity 98%; MS (ESI+): m/z = 369 $(M + H)^+$.

5.4.38. (S)-2-(2-(Carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (17)

To **17a** (1 g, 4.29 mmol) in solution in dry THF (20 mL) was added DCC (0.88 g, 4.29 mmol). The mixture was stirred overnight at room temperature and L-histidine methyl ester dihydrochloride (1.14 g, 4.67 mmol) and DIEA (2.1 mL) were added. After stirring at room temperature during 5 h, the precipitate was filtrated and the filtrate was evaporated. The crude product was purified by preparative HPLC to give (S)-2-(2-(*tert*-butoxycarbonyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester as a white solid. Yield 40% over 2 steps. ^{1}H NMR (DMSO-d_6) δ ppm: 8.95 (d, J = 7.4 Hz, 0.5H), 8.90 (d, J = 7.4 Hz, 0.5H), 7.74 (d, J = 0.9 Hz, 0.5H), 7.72 (d, J = 0.9 Hz, 0.5H), 6.88 (s, 1H), 4.50 (m, 1H), 3.85 (m, 2H), 3.83 (s, 2H), 3.58 (s, 3H), 2.95 (dd, J = 5.7 and 14.9 Hz, 1H), 2.87 (dd, J = 8.1 and 14.9 Hz, 1H), 1.33 (s, 4.5H), 1.28 (s, 4.5H); $t_{R,LCMS}$ = 2.65 min, Purity 99%; MS (ESI+): m/z = 385 $(M + H)^+$. The latter compound (300 mg, 0.78 mmol) was deprotected in presence of HClg in DCM for 2 h at room temperature. DCM was evaporated and the product was precipitated in Et_2O to give **17** as its white dihydrochloride salt. Yield 77%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.36 (d, J = 7.5 Hz, 1H), 9.09 (d, J = 1.2 Hz, 1H), 7.48 (s, 1H), 4.66 (m, 1H), 3.85

(s, 2H), 3.82 (s, 2H), 3.65 (s, 3H), 3.20 (dd, J = 5.2 and 15.2 Hz, 1H), 3.10 (dd, J = 9.0 and 15.4 Hz, 1H); $t_{R,LCMS}$ = 0.68 min, Purity 100%; MS (ESI+): m/z = 285 $(M + H)^+$.

*5.4.39. (S)-2-(2-(Carboxymethyl-methyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(18)***

H-His(trityl)OMe.HCl and commercial N-methyliminodiacetic acid (**18a**) reacted following general procedure A to give intermediate (S)-2-(2-(Carboxymethyl-methyl-amino)-acetylamino)-3-(1-trityl-1H-imidazol-4-yl)-propionic acid methyl ester as a white solid. Yield 33%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.43 (d, J = 1.5 Hz, 1H), 7.42–7.37 (m, 9H), 7.16–7.11 (m, 6H), 6.76 (d, J = 1.5 Hz, 1H), 4.73 (m, 1H), 3.65 (s, 3H), 3.61 (s, 2H), 3.48 (s, 2H), 3.09 (dd, J = 5.4 and 15.0 Hz, 1H), 2.98 (dd, J = 7.8 and 14.7 Hz, 1H), 2.64 (s, 3H). $t_{R,LCMS}$ = 4.21 min, Purity 98%; MS (ESI+): m/z = 541 $(M + H)^+$. Deprotection allowed **22** as the TFA salt. Colourless oil. Yield 100%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.16 (d, J = 7.8 Hz, 1H), 8.99 (d, J = 1.2 Hz, 1H), 7.43 (d, J = 1.2 Hz, 1H), 4.70 (m, 1H), 4.03 (s, 2H), 3.97 (s, 2H), 3.66 (s, 3H), 3.19 (dd, J = 5.4 and 15.3 Hz, 1H), 3.06 (dd, J = 8.7 and 15.3 Hz, 1H), 2.80 (s, 3H), $t_{R,LCMS}$ = 0.70 min, Purity 98%; MS (ESI+): m/z = 299 $(M + H)^+$.

*5.4.40. (S)-2-(2-(Carboxymethyl-phenyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(19)***

Compound **(19)** was synthesized from commercial N-phenyliminodiacetic acid (**19a**) and L-H-histidine methyl ester dihydrochloride as a white solid. Yield 33%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.05 (d, J = 7.6 Hz, CONH), 7.63 (d, J = 1.1 Hz, 1H), 7.15 (dd, J = 7.2 Hz and 8.6 Hz, 2H), 6.77 (s, 1H), 6.68 (t, J = 7.2 Hz, 1H), 6.42 (d, J = 8.1 Hz, 2H), 4.50 (m, 1H), 4.13 (s, 2H), 4.02 (s, 2H), 3.57 (s, CO_2Me), 2.94 (dd, J = 5.3 Hz and 14.6 Hz, 1H), 2.83 (dd, J = 8.7 Hz and 14.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 173.5, 171.9, 171.6, 147.5, 135.1, 133.2, 129.4, 117.4, 116.7, 111.9, 56.0, 54.6, 52.3, 49.0, 29.0; $t_{R,LCMS}$ = 2.94 min, Purity 98%; MS (ESI+): m/z = 361 $(M+H)^+$.

*5.4.41. (S)-2-(2-(Benzoyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(20)***

The diacid **20a** (0.74 mmol) was dissolved in trifluoracetic anhydride 2% in acetic anhydride (3 mL). The reaction mixture was stirred for 4 h at room temperature then evaporated. To a stirred solution of L-histidine methyl ester dihydrochloride (188 mg, 0.77 mmol) in DMF (8.0 mL) were added DIEA (515 μL) and the anhydride (0.74 mmol) in DMF (1.0 mL). The mixture was stirred overnight at room temperature and the solvent was evaporated. The crude product was purified by preparative HPLC to give **20** as a white solid. Yield: 26%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.89 (J = 7.2 Hz, 0.4H), 8.78 (d, J = 7.5 Hz, 0.6H), 7.74 (s, 0.4H), 7.69 (s, 0.6H), 7.31–7.44 (m, 5H), 6.91 (s, 0.4H), 6.86 (s, 0.6H), 4.55 (m, 1H), 4.08 (s, 0.8H), 4.02 (s, 1.2H), 3.88 (s, 2H), 3.64 (s, 1.8H), 3.59 (s, 1.2H), 2.85–3.02 (m, 2H). $t_{R,LCMS}$ = 2.48 min; Purity 98%; MS (ESI+): m/z = 389 $(M + H)^+$.

*5.4.42. (S)-2-(2-(Carboxymethyl-phenylacetyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(21)***

Compound **(21)** was synthesized following general procedure A from **21a** and L-H-Histidine methyl ester dihydrochloride as a yellow solid. Yield 77%. 2 conformers (30/70). ^{1}H NMR (DMSO-d_6) δ ppm: 8.87 (d, J = 7.5 Hz, 0.7H, CONH), 8.84 (d, J = 7.5 Hz, 0.3 CONH), 7.80 (s, 0.4H), 7.68 (s, 0.6H), 7.30–7.13 (m, 5H(Ph)), 6.92 (s, 0.3H), 6.89 (s, 0.7H), 4.56 (m, 0.7H), 4.46 (m, 0.3H), 4.13 (s, 0.6H), 4.10 (s, 1.4H), 3.95 (s, 2H), 3.63 (s, 0.6H), 3.61 (s, 2.1 CO_2Me), 3.57 (s, 0.9 CO_2Me), 3.56 (1.4H), 2.96 (m, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 171.6, 171.3, 171.1, 168.7, 135.2, 134.9, 134.7, 132.9, 129.3, 128.1, 126.3, 116.5, 52.2, 52.0, 51.8, 51.1, 49.3, 38.8, 38.7, 28.5. $t_{R,LCMS}$ = 2.81 min, Purity 99%; MS (ESI+): m/z = 403 $(M+H)^+$.

*5.4.43. (S)-2-(2-(Carboxymethyl-(3-phenyl-propionyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(22)***

Compound **(22)** was obtained from **22a** as depicted for **20**, as a white solid. Yield 36%.^{1}H NMR (DMSO-d_6) δ ppm: 8.89 (d, J = 7.2 Hz, 1H), 7.74 (s, 0.4H), 7.61 (s, 0.6H), 7.28–7.13 (m, 5H), 6.89 (s, 0.4H), 6.84 (s, 0.6H), 4.48 (m, 1H), 4.06 (s, 2H), 3.93 (s, 2H), 3.58 (s, 1.2H), 3.54 (s, 1.8H), 2.95 (m, 2H), 2.82 (m, 2H), 2.50 (m, 2H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.7, 172.5, 171.6, 171.5, 168.9, 141.2, 134.9, 134.7, 128.3, 128.2, 125.8, 116.8, 116.5, 52.5, 52.4, 51.9, 51.8, 49.5, 33.5, 30.3, 28.5, 28.4. $t_{R,LCMS}$ = 3.25 min, Purity 98%; MS (ESI+): m/z = 417 $(M+H)^+$.

*5.4.44. (S)-2-(2-(Benzyloxycarbonyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(23)***

The diacid **23a** (1.2 mmol) was dissolved in trifluoracetic anhydride 2% in acetic anhydride (5 mL). The reaction mixture was stirred for 4 h at room temperature then evaporated. To a stirred solution of L-histidine methyl ester dihydrochloride (290 mg, 1.2 mmol) in DMF (10 mL) were added DIEA (834 μL) and the anhydride (1.2 mmol) in DMF (2 mL). The mixture was stirred overnight at room temperature and the solvent was evaporated. The crude product was purified by preparative HPLC to yield **23** as a white solid. Yield 32%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.00 (J = 7.2 Hz, 0.5H), 8.93 (d, J = 7.5 Hz, 0.5H), 7.79 (s, 0.5H), 7.75 (s, 0.5H), 7.25–7.34 (m, 5H), 6.91 (s, 0.5H), 6.88 (s, 0.5H), 5.02 (s, 1H), 5.01 (s, 1H), 4.50 (m, 1H), 3.92 (m, 4H), 3.58 (s, 1.5H), 3.54 (s, 1.5H), 2.88–2.97 (m, 2H). $t_{R,LCMS}$ = 3.15 min, Purity 98%; MS (ESI+): m/z = 419 $(M + H)^+$.

*5.4.45. 3-(((S)-1-Methoxy-1-oxo-3-imidazol-2-yl)carbamoyl)-1,2,3,4-tetrahydroisoquinoline-2-ethanoic acid **(24)***

To a stirred solution of 1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid hydrochloride (426 mg, 2 mmol) and DIEA (695 μL, 2 eq) in H_2O (10 mL) and dioxane (5 mL) was added dropwise at 0 °C tert-butylbromoacetate (322 μL, 2 mmol) in dioxane (5 mL). The resulting mixture was stirred at room temperature overnight. The insoluble was filtrated and filtrate was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was directly used in the next step. To a solution of carboxylic acid (2 mmol) in DCM (10 mL) were added EDCI (422 mg, 2.2 mmol), HOBt (334 mg, 2.2 mmol), DIEA (1390 μL, 8 mmol) and L-histidine methyl ester dihydrochloride (482 mg, 2 mmol). The mixture was stirred at room temperature overnight. Then, the mixture was washed with a 5% $NaHCO_3$ aqueous solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by preparative HPLC to give the *tert*-butyl protected intermediate **24a**. Yield 33% (two-steps). $t_{R,LCMS}$ = 2.79 and 2.87 min, Purity 96%; MS (ESI+): m/z = 443.1 $(M+H)^+$. Then *tert*-butyl protection was removed using TFA/DCM (50/50) in the presence of triisopropylsilane. Solvents were evaporated under reduced pressure and the crude product was precipitated in Et_2O/pentane to give quantitatively **24** as a mixture of diastereoisomers (TFA salts). Yield: 95%. ^{1}H NMR (DMSO-*d6*) δ ppm: ^{1}H NMR (DMSO-d_6) δ ppm: 9.1 (br s, 1H, CONH), 9.0 (d, J = 3.0 Hz, 1H); 7.33 (d, J = 6.0 Hz, 1H); 7.12–7.25 (m, 5H), 4.64–4.68 (m, 1H), 4.15–4.36 (m, 3H), 3.84 (dd, J = 7.50 Hz, J = 10.5 Hz, 2H), 3.64 (s, 3H), 2.99–3.22 (m, 4H). ^{13}C NMR (DMSO-d_6) δ ppm: 170.5, 158.4, 158.3, 128.9, 128.1, 127.5, 126.7, 126.6, 114.1, 60.7, 52.5; 51.3, 17.9. t_R $t_{R,LCMS}$ = 1.74 and 1.84 min, Purity 98%; MS (ESI+): m/z = 387 $(M+H)^+$.

*5.4.46. 3-Benzyl-4-((S)-2-(1H-imidazol-4-yl)-1-methylcarbamoyl-ethylcarbamoyl)-butyric acid **(25)***

To a stirred solution of LAH (600 mg, 15 mmol) in THF (4 mL) at

0 °C were added dropwise a solution of diethyl benzylmalonate (940 μL, 4 mmol) in THF (4 mL). The mixture was then refluxed overnight. The excess of LAH was quenched with wet Na_2SO_4. The solid was eliminated and the filtrate evaporated to give 2-*b*enzyl-propane-1,3-diol. Yield 70%. $t_{R,LCMS}$ = 1.93 min, Purity 98%; MS (ESI+): *m/z* = 167 $(M+H)^+$. To a stirred solution of the diol (623 mg, 3.75 mmol) in DCM (15 mL) were added *p*-toluenesulfonyl chloride (2.86 g, 15 mmol) and triethylamine (1.56 μL, 11.25 mmol). The mixture was stirred at room temperature overnight and washed with water. The organic layers were then washed with a saturated solution of Na_2CO_3. The organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography to yield the 2-*b*enzyl-propane-1,3-ditosylate **25a**. Yield 44%. ^{1}H NMR (DMSO-d_6) δ ppm:7.70 (d, *J* = 8.4 Hz, 4H), 7.46 (d, *J* = 8.4 Hz, 4H), 7.19–7.155 (m, 3H), 6.95–6.92 (m, 2H), 3.91 (dd, *J* = 10.2 Hz and 4.8 Hz, 2H), 3.80 (dd, *J* = 10.2 Hz and 6 Hz, 2H), 2.52 (m, 2H), 2.43 (s, 6H), 2.25 (m, 1H), $t_{R,LCMS}$ = 3.45 min (5 min gradient), Purity 98%; MS (ESI+): *m/z* = *475* $(M + H)^+$. To a stirred solution of **25a** (792 mg, 1.6 mmol) in DMSO (6 mL) were added KCN (458 mg, 7 mmol). The mixture was heated at 80 °C for 3.5 h. The mixture was diluted with a solution of NaCl and extracted with DCM. The organic layers were dried over $MgSO_4$ and the solvent evaporated to give 3-*b*enzyl-pentanedinitrile **25b** directly in the next reaction. $t_{R,LCMS}$ = 2.58 min (5 min gradient), Purity 97%; MS (ESI+): *m/z* = 186 $(M + H)^+$. To a stirred solution of dinitrile **25b** (1.6 mmol) in water (4 mL) were added H_2SO_4 96% (4 mL). The mixture was refluxed 3 days. The mixture was then extracted with DCM. The organic layers were extracted with a diluted solution of NH_4OH. The aqueous layers were frozen dry to give 3-benzyl-pentanedioic acid **25c** as an orange powder, directly used in the next step. Yield 40%. $t_{R,LCMS}$ = 2.18 min (5 min gradient), Purity 98%; MS (ESI+): *m/z* = 223 $(M+H)^+$. The compound was prepared using general procedure A starting from **25c** (134 mg, 0.6 mmol) and L-H-Histidine-methyl-amide **40a** (145 mg, 0.6 mmol). The crude product was purified by preparative HPLC to give **25** as a white powder. Yield 45%. ^{1}H NMR (CD_3OD) δ ppm: 8.54 (s, CONH), 7.61 (s, 1H) 7.26–7.14 (m, 5H (Ph)) 6.88 (s, 1H), 4.59 (m, 1H), 3.11–2.86 (m, 2H), 2.70 (s, 3H), 2.60–2.49 (m, 2H+1H), 2.31–2.06 (m, 4H); ^{13}C NMR (CD_3OD) δ ppm:175.2, 173.9, 141.4, 136.2, 130.4, 129.2, 127.0, 118.0, 54.7 41.3, 41.0, 36.8, 36.6, 30.1, 26.4; $t_{R,LCMS}$ = 1.92 min (5 min gradient), Purity 98%; MS (ESI+): *m/z* = 373 $(M + H)^+$.

5.4.47. General procedure B for the synthesis of compounds **26–7, 29–31**

To a solution of carboxylic acid **26a–27a**, **29a–31a** (0.5 mmol) in DCM (10 mL) were added EDCI (105 mg, 0.55 mmol), HOBt (85 mg, 0.55 mmol), DIEA (412 μL, 2.5 mmol) and L-histidine methyl ester dihydrochloride (145 mg, 0.6 mmol). The mixture was stirred at room temperature overnight. Then, the mixture was washed with a 5% $NaHCO_3$ aqueous solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by preparative HPLC. Tert-butyl, Boc- and Trityle protections were removed using TFA/DCM/TIS (5–50% TFA v/v) mixtures and final compounds were purified by preparative HPLC if needed.

5.4.48. (S)-2-(2-Benzylamino-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(26)**

To a stirred solution of N-benzylglycine hydrochloride (402 mg, 2 mmol) in a H_2O/dioxane mixture (1/4, 5 mL) were added, at 0 °C, Boc_2O (524 mg, 2.4 mmol) and 2 mL of 2 N NaOH solution. The mixture was stirred overnight at room temperature and dioxane was evaporated. The aqueous layer was acidified with a 20% citric acid solution and extracted by AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give *(Benzyl-tert-butoxycarbonyl-amino)-acetic acid* **(26a)** as a colourless oil. Yield 100%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.23–7.35 (m, 5H), 4.4 (s, 1.1H), 4.38 (s, 0.9H), 3.83 (s, 0.9H), 3.74 (s, 1.1H), 1.38 (s, 1.8H), 1.34 (s, 9.9H); $t_{R,LCMS}$ = 5.4 min, Purity 99%; MS (ESI-): *m/z* = 264 $(M-H)^-$.**26a** reacted with L-Histidine methyl ester dihydrochloride following general procedure B to give Boc-protected intermediate (S)-2-(2-(Benzyl-tert-butoxycarbonyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester as a white solid. Yield 51%. ^{1}H NMR (DMSO-*d6*) δ ppm: 11.84 (s, 1H), 8.30 (s, 1H), 7.52 (s, 1H), 7.33–7.18 (m, 5H), 6.82 (s, 1H), 4.52 (m, 1H), 4.35 (m, 2H), 3.74 (m, 2H), 3.59 (s, 3H), 2.88 (m, 2H), 1.34 (s, 9H); $t_{R,LCMS}$ = 4.06 min, Purity 100%; MS (ESI+): *m/z* = 417 $(M + H)^+$. The latter compound was deprotected in presence of HClg in dioxane during 30 min at room temperature. Dioxane was evaporated and the product was precipitated in Et_2O to give the **26** as its white hydrochloride salt. Yield 74%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.26 (d, *J* = 7.5 Hz, 1H), 9.04 (d, *J* = 1.2 Hz, 1H), 7.52–7.38 (m, 6H), 4.67 (m, 1H), 4.11 (s, 2H), 3.71 (d, *J* = 15.9 Hz, 1H), 3.70 (d, *J* = 15.9 Hz, 1H), 3.65 (s, 3H), 3.20 (dd, *J* = 5.4 and 15.3 Hz, 1H), 3.08 (dd, *J* = 8.4 and 15.0 Hz, 1H). $t_{R,LCMS}$ = 1.91 min, Purity 100%; MS (ESI+): *m/z* = 317 $(M + H)^+$.

5.4.49. (S)-2-(2-(Benzyl-carbamoylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(27)**

N-benzyliminodiacetic acid (342 mg, 1.53 mmol) was dissolved in trifluoracetic anhydride 2% in acetic anhydride (5 mL). The reaction mixture was heated for 15 min and then stirred for 5 h at room temperature and evaporated. The crude product was dissolved in DMF (3 mL). The mixture was saturated with gaseous ammoniac and stirred overnight at room temperature. Then the solvent was evaporated to yield *(Benzyl-carbamoylmethyl-amino)-acetic acid* **(27a)** as yellow oil. Yield 98%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.36–7.25 (m, 5H + 1 $CONH_2$), 7.15 (s, 1 $CONH_2$), 3.76 (s, 2H), 3.32 (s, 2H), 3.16 (s, 2H). $t_{R,LCMS}$ = 2.14 min (5 min gradient), Purity 96%; MS (ESI+): *m/z* = 223 $(M + H)^+$. **27a** reacted with L-histidine methyl ester dihydrochloride following general procedure B as a white solid (formiate salt). Yield 10%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.73 (d, *J* = 7.8 Hz, 1H), 7.63 (s, 1H), 7.59 (s, 1H), 7.30 (m, 5H), 7.23 (s, 1H), 6.89 (s, 1H), 4.57 (m, 1H), 3.65 (d, *J* = 13.4 Hz, 1H), 3.57 (d, *J* = 13.4 Hz, 1H), 3.57 (s, 3H), 3.08 (s, 2H), 3.02–2.96 (m, 4H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.2, 171.7, 169.9, 137.3, 135.2, 133.8, 129.2, 128.2, 127.3, 115.8, 57.9, 57.5, 56.9, 51.9, 51.8, 28.6. $t_{R,LCMS}$ = 2.77 min, Purity 99%; MS (ESI+): *m/z* = 374 $(M+H)^+$.

5.4.50. (S)-2-(2-(Benzyl-methoxycarbonylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(28)**

1 (60 mg, 0.16 mmol) was stirred overnight at room temperature in a 20% $SOCl_2$ solution in MeOH (2 mL) and the solvent was evaporated. The crude product was purified by preparative HPLC to give **28** as colourless oil. Yield 73%. ^{1}H NMR (CD_3OD) δ ppm: 7.87 (s, 1H), 7.33–7.25 (m, 5H), 6.97 (s, 1H), 7.75 (m, 1H), 3.77 (d, *J* = 13.2 Hz, 1H), 3.71 (d, *J* = 13.2 Hz, 1H), 3.73 (s, 3H), 3.67 (s, 3H), 3.36 (s, 2H), 3.31 (s, 2H), 3.21 (dd, *J* = 4.5 and 14.1 Hz, 1H), 3.13 (dd, *J* = 7.5 and 15.0 Hz, 1H). $t_{R,LCMS}$ = 3.42 min, Purity 100%; MS (ESI+): *m/z* = 389 $(M+H)^+$.

5.4.51. (S)-2-(2-(Benzyl-(2-carboxy-ethyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester **(29)**

To a stirred solution of N-benzylglycine (820 mg, 4 mmol) and methanol (8 mL) was added thionyle chloride (2 mL) dropwise at 0 °C. The mixture was stirred for 18 h. Then the solvent was evaporated under reduced pressure to give the compound benzylamino-acetic acid methyl ester as a white solid. Yield 100%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.55–7.58 (m, 2H), 7.41–7.43 (m, 3H), 4.16 (s, 2H), 3.99 (s, 2H), 1.72 (s, 3H). $t_{R,LCMS}$ = 2.01 min; Purity 99%; MS (ESI+): *m/z* = 180 $(M + H)^+$. To a stirred solution of benzylamino-acetic acid methyl ester (222 mg, 1.03 mmol) in

acetone (10 mL) were added *tert*-butyl 3-bromopropionate (3*834 μL, 3*5 mmol), K_2CO_3 (3*290 mg, 3*2.09 mmol) and KI (174 mg, 1.04 mmol) and the mixture was refluxed overnight. The solvent was evaporated and the residue was solubilized in ethyle acetate. The organic layer was washed with water and brine then dried over $MgSO_4$ and concentrated under reduced pressure to give a white powder, directly used in the next step. $t_{R,LCMS}$ = 4.76 min; Purity 99%; MS (ESI+): m/z = 308 $(M + H)^+$. To a stirred solution of the methyl ester (349 mg, 1.13 mg) in methanol (5 mL) was added NaOH (74 mg, 1.85 mmol). The mixture was stirred at room temperature for 5 days. The solvent was evaporated and the residue was solubilised in water, then the aqueous layer was acidified with a solution of HCl 1 N, and extracted with DCM. The organic layers were washed with brine, dried over $MgSO_4$ and concentrated to give the corresponding 3-(benzyl-methoxycarbonylmethyl-amino)-propionic acid tert-butyl ester (**29a**), directly used in the next step. Yield 45%. $t_{R,LCMS}$ = 3.76 min; Purity 95%; MS (ESI+): m/z = 294 $(M+H)^+$. To a solution of the latter carboxylic acid **29a** (132 mg, 0.4 mmol) and oxalyl chloride (50 μL, 0.58 mmol) in DCM (3 mL) was added at 0 °C one drop of DMF. The mixture was stirred for 30 min. Then the solvent was evaporated and the crude compound was solubilized in DCM (3 mL). Then L-His-(Trt)-OMe (181 mg, 0.4 mmol) and DIEA (313 μL, 1.8 mmol) were added, the mixture was stirred overnight. The solvent was evaporated and the crude product was purified by preparative HPLC to give the tritile-protected intermediate. Yield 40%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.43 (d, J = 8.10 Hz, 1H), 7.39–7.32 (m, 11H), 7.27 (d, J = 1.2 Hz, 1H), 7.17 (m, 3H), 7.02 (m, 6H), 6.63 (d, J = 1.2 Hz, 1H), 4.60 (m, 1H), 3.62 (d, J = 13.5 Hz, 1H), 3.54 (d, J = 13.5 Hz, 1H), 3.51 (s, 3H), 3.32 (s, 2H), 3.03–2.89 (m, 2H +2H), 2.64 (m, 2H), 1.31 (s, 9H); $t_{R,LCMS}$ = 6.96 min; Purity 99%; MS (ESI+): m/z = 687 $(M + H)^+$. Deprotection with a TFA/DCM/TIS mixture allowed **29** as its white TFA salt. Yield 82%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.98 (s, 1H), 8.90 (s, 1H), 7.40 (m, 6H), 4.68 (m, 1H), 4.1, (s, 2H), 3.65 (s, 3H), 3.56 (s, 2H), 3.16–3.03 (m, 2H+2H), 2.67 (t, J = 7.5 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 170.5, 133.9, 130.5, 128.9, 128.7, 117.3, 57.2, 53.8, 52.4, 51.3, 49.3, 29.7, 26.1. $t_{R,LCMS}$ = 2.39 min; Purity 99%; MS (ESI+): m/z = 389 $(M + H)^+$.

5.4.52. (S)-2-(2-(Benzyl-hydroxycarbamoylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (30)

To a stirred solution of O-trityl hydroxylamine (550 mg, 2.0 mmol) in DMF (10 mL) were added DIEA (660 μL) and N-benzyliminodiacetic anhydride acid (410 mg, 2.0 mmol) in DMF (2.0 mL). The mixture was stirred overnight at room temperature under argon and the solvent was evaporated. The crude product was dissolved in DCM and washed with water. Intermediate **30a** was obtained as orange oil and used directly in the next step. Yield 93%. Then **30a** was reacted with L-histidine methyl ester dihydrochloride following general procedure B to give the protected intermediate. White solid. Yield 51%. $t_{R,LCMS}$ = 5.9 min, Purity 100%; MS (ESI+): m/z = 633 $(M+H)^+$. Deprotection using a TFA 2%/TIS 5%/DCM mixture allowed **30** as its TFA salt. Yield 98%. ^{1}H NMR (DMSO-d_6) δ ppm: 10.66 (s, 1H), 8.97 (d, J = 1.2 Hz, 1H), 8.68 (d, J = 8.1 Hz, 1H), 7.38–7.30 (m, 5H), 4.71 (m, 1H), 3.72 (s, 2H), 3.64 (s, 3H), 3.22–3.15 (m, 5H), 3.08 (dd, J = 9.2 and 15.3 Hz, 1H). $t_{R,LCMS}$ = 2.6 min, Purity 100%; MS (ESI+): m/z = 390 $(M+H)^+$.

5.4.53. (S)-2-(2-(Benzyl-(2-hydroxy-3,4-dioxo-cyclobut-1-enyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid methyl ester (31)

A solution of potassium *tert*-butylate (17.6 mL, 17.6 mmol, 1 eq.), 1 M in tetrahydrofuran was added at once to a solution of diethyl squarate (3 g, 17.6 mmol, 1 eq.) in 50 mL of tetrahydrofuran at 4 °C. After 15 min of stirring, the reaction was quenched and acidified with 1 N HCl (1 eq., pH after hydrolysis: 2–3). The mixture was extracted three times with ether. The combined organic layers were washed with saturated aqueous NaCl, dried over magnesium sulphate, filtered and solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (eluent: cyclohexane/AcOEt (97/3)) to give ethyl *tert*-butyl squarate (1.95 g, 55%). ^{1}H NMR ($CDCl_3$) δ ppm: 4.762 (q, J = 7.2 Hz, 2H), 1.60 (s, 9H), 1.48 (t, J = 7.2 Hz, 3H). $t_{R,LCMS}$ = 5.06 min, Purity 99%; MS (ESI+): m/z = 143 (M-*t*Bu+2H)$^+$ and 115 (M-*t*Bu-Et+2H)$^+$. To a solution of N-Benzylglycine hydrochloride (305 mg, 1.51 mmol) and NEt_3 (424 μL, 302 mmol) in MeOH (10 mL), was added ethyl *tert*-butyl squarate (300 mg, 1.51 mmol). The mixture was stirred at room temperature overnight and MeOH was evaporated. The crude product was purified by preparative HPLC to give **31a** as a colourless oil. Yield 70%. $t_{R,LCMS}$ = 5.28 min, Purity 100%; MS (ESI-): m/z = 316 $(M-H)^-$. Then **31a** was reacted with L-histidine methyl ester dihydrochloride following general procedure B. The crude product was purified by preparative HPLC to give protected intermediate as a white solid. Yield 37%. $t_{R,LCMS}$ = 4.3 min, Purity 100%; MS (ESI+): m/z = 469 $(M+H)^+$. The latter intermediate was dissolved in dichloromethane (2 mL) and cooled at 4 °C in an ice/water bath. After 15 min of stirring, trifluoroacetic acid (2 mL) was added and the mixture was stirred at 4 °C for 30 min then 10 μL of distilled water were added. The reaction mixture was concentrated under reduced pressure and the crude product was precipitated in Et_2O and filtrated to give **31** as a purple solid. Yield 99%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.97 (d, J = 1.5 Hz, 1H), 8.57 (d, J = 8.1 Hz, 1H), 7.40–7.22 (m, 5H), 4.70–4.66 (m, 1H), 4.65 (d, J = 14.7 Hz, 1H), 4.58 (d, J = 14.7 Hz, 1H), 4.00 (d, J = 16.5 Hz, 1H), 3.95 (d, J = 16.5 Hz, 1H), 3.67 (s, 3H), 3.16 (dd, J = 5.1 and 15.0 Hz, 1H), 3.03 (dd, J = 9.0 and 15.0 Hz, 1H). $t_{R,LCMS}$ = 3.02 min, Purity 100%; MS (ESI+): m/z = 413 $(M+H)^+$.

5.4.54. (S)-2-(2-(Benzyl-(1H-tetrazol-5-ylmethyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-N-methyl-propionamide (32)

First, (*S*)-2-(2-(Benzyl-carbamoylmethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-N-methyl-propionamide **(32a)** was synthesized from **27a** and L-H-Histidine-methylamide following general procedure B as a yellow oil. Yield 80%. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.46 (d, J = 8.3 Hz, CONH), 7.71 (q, J = 4.6 Hz, CONHMe), 7.52 (d, J = 1.0 Hz, 1H); 7.36–7.23 (m, 5H(Ph)), 6,76 (s, 1H), 4.42 (m, 1H), 3.64 (d, J = 13.4 Hz, 1H), 3.57 (d, J = 13.4 Hz,1H), 3.14–2.85 (m, CONHMe), 2.54 (d, J = 4.6 Hz, 3H); $t_{R,LCMS}$ = 1.47 min (5 min gradient), Purity 95%; MS (ESI+): m/z = 373 $(M + H)^+$.To a stirred solution of **32a** (588 mg, 1.57 mmol) in THF (10 mL) at 0 °C were added pyridine (319 μL, 3.94 mmol) and TFAA (241 μL, 1.73 mmol) in three times over 3 h. Water was added and the mixture was extracted with ethyl acetate. The combined organic layers were washed successively with a saturated solution of $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was evaporated and the crude material was purified by preparative HPLC to give *(S)-2-(2-(Benzyl-cyanomethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-N-methyl-propionamide* **(32b)** as an orange oil. Yield 58%. ^{1}H NMR (CD_3OD) δ (ppm): 7.91 (d, J = 1.1 Hz, 1H), 7.36–7.30 (m, 5H(Ph)), 6.97 (s, 1H), 4.67 (ddd, J = 8.2 Hz and 5.4 Hz, 1H), 3.72 (d, J = 12.9 Hz, 1H), 3.66 (d, J = 12.9 Hz, 1H), 3.62 (d, J = 17.5 Hz, 1H), 3.54 (d, J = 17.5 Hz, 1H), 3.16 (dd, J = 14.9 Hz and 5.3 Hz, 1H), 3,03 (dd, J = 14.9 Hz and 8.2 Hz, 1H), 3.28 (s, 2H), 2.73 (s, CONHMe); $t_{R,LCMS}$ = 1.77 min (5 min gradient), Purity 100%; MS (ESI+): m/z = 355 $(M + H)^+$. To a stirred solution of **32b** (108 mg, 0.3 mmol) in DMF were added sodium azide (198 mg, 3.04 mmol) and ammonium chloride (163 mg, 3.04 mmol). The mixture was heated at 90 °C for 60 h. The solvent was evaporated and the crude material was diluted in EtOH and filtered. The filtrate was concentrated under vacuum to give yellow

oil. Yield 97%. ^{1}H NMR (CD_3OD) δ (ppm): 8.07 (d, J = 1.1 Hz, 1H), 7.32–7.25 (m, 5H(Ph) + 1H), 6.95 (s, 1H), 4.63 (dd, J = 8.2 Hz and 5.2 Hz, 1H), 3.95 (d, J = 14.1 Hz, 1H), 3.88 (d, J = 14.1 Hz, 1H), 3.65 (s, 2H), 3.21–3.04 (m, 4H), 2.74 (s, CONHMe); ^{13}C NMR (CD_3OD) δ (ppm): 172.2, 171.8, 167.6, 158.4, 137.2, 134.4, 131.5, 129.1, 128.1, 127.2, 117.2, 58.6, 56.4, 52.5, 28.2, 25.1; $t_{R,LCMS}$ = 1.95 min (5 min gradient), Purity 95%; MS (ESI+): m/z = 398 $(M + H)^+$.

*5.4.55. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid **(33)***

To a stirred solution of **1** (125 mg, 0.30 mmol) in MeOH (3 mL) were added NaOH (36 mg, 0.90 mmol) and 50 μL of distilled water. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to give **33** as its white sodium salt. Yield 99%. ^{1}H NMR (CD_3OD) δ ppm: 7.45 (d, J = 0.9 Hz, 1H), 7.33–7.21 (m, 5H), 6.81 (s, 1H), 4.52 (dd, J = 4.5 and 7.2 Hz, 1H), 3.78 (d, J = 13.2 Hz, 1H), 3.66 (d, J = 13.2 Hz, 1H). 3.25 (m, 2H), 3.19 (dd, J = 11.1 and 15.6 Hz, 1H), 3.13 (d, J = 16.5 Hz, 1H), 3.09 (dd, J = 7.8 and 15.3 Hz, 1H) 3.05 (d, J = 16.5 Hz, 1H). $t_{R,LCMS}$ = 1.86 min, Purity 94%; MS (ESI+): m/z = 361 $(M + H)^+$.

*5.4.56. (Benzyl-((2-(1H-imidazol-4-yl)-ethylcarbamoyl)-methyl)-amino)-acetic acid **(34)***

Compound **(34)** was obtained following general procedure A from histamine **34a** and N-benzyliminodiacetic anhydride acid. Colourless oil. Yield 47%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.94 (t, J = 5.7 Hz, 1H), 7.67 (d, J = 1.2 Hz, 1H), 7.33–7.22 (m, 5H), 6.85 (d, J = 0.6 Hz, 1H), 3.72 (s, 2H), 3.32 (m, 2H), 3.26 (s, 1H), 3.18 (s, 1H), 2.63 (t, J = 6.9 Hz, 2H). $t_{R,LCMS}$ = 1.99 min, Purity 99%; MS (ESI+): m/z = 317 $(M + H)^+$.

*5.4.57. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid isopropyl ester **(35)***

First to a stirred solution of Boc-His-OH (1.27 g, 5 mmol) in DCM (30 mL) were added *i*PrOH (5 mL, 65 mmol), DMAP (671 mg, 5.5 mmol), EDCI (1.05 g, 5.5 mmol) and DIEA (956 μL, 5.5 mmol). The mixture was heated at 50 °C for 28 h. Then the mixture was washed with a saturated solution of NaHCO3. The organic layer was dried with MgSO4 and evaporated. The crude product was purified by preparative HPLC to yield L-Boc-histidine isopropyl ester as colourless oil. Yield 56%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.58 (s, 1H), 7.14 (d, J = 7.5 Hz, CONH), 6.80 (s, 1H), 4.83 (sept, J = 6.2 Hz, 1H), 4.13 (m, 1H), 2.83 (m, 2H), 1.35 (s, 9H(Boc)), 1.13 (d, J = 6.3 Hz, 3H), 1.09 (d, J = 6 Hz, 3H); $t_{R,LCMS}$ = 3.60 min; Purity: 98%; MS: (ESI+) m/z = 298 $(M + H)^+$. Then deprotection using HClg in DCM during 30 min at room temperature allowed *L-H-Histidine isopropyl ester* **(35a)** as its white dihydrochloride salt. Yield 77%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.08 (d, J = 1.2 Hz, 1H), 7.53 (d, J = 1.2 Hz, 1H), 4.93 (sept, J = 6.2 Hz, 1H), 4.13 (m, 1H), 2.83 (m, 2H), 1.35 (s, 9H(Boc)), 1.13 (d, J = 6.3 Hz, 3H), 1.09 (d, J = 6 Hz, 3H); $t_{R,LCMS}$ = 0.65 min; Purity: 98%; MS: (ESI+) m/z = 198 $(M + H)^+$.N-Benzyliminodiacetic acid and **35a** reacted as described in general procedure A to give **35** as a colourless oil. Yield 40%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.41 (d, J = 8.10 Hz, CONH), 7.65 (s, 1H), 7.33–7.27 (m, 5H(Ph)), 6.87 (s, 1H), 4.83 (sept, J = 6.30 Hz, 1H), 4.51 (m, 1H), 3.80 (d, J = 13.2 Hz, 1H), 3.74 (d, J = 13.2 Hz, 1H), 3.27 (s, 2H), 3.24 (s, 2H), 2.98 (dd, J = 6.9 Hz and 14.7 Hz, 1H), 2.92 (dd, J = 5.4 Hz and 14.7 Hz, 1H), 1.08 (m, 6H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.2, 170.6, 170.1, 138.1, 135.1, 133.5, 128.8, 128.3, 127.2, 68.0, 57.4, 56.6, 53.3, 51.8, 28.7, 21.4; $t_{R,LCMS}$ = 3.23 min; Purity: 98%; MS: (ESI+) m/z = 403 $(M + H)^+$.

*5.4.58. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(3H-imidazol-4-yl)-propionic acid isobutyl ester **(36)***

First, to a stirred solution of L-histidine (463 mg, 2.98 mmol) in *i*BuOH (5 mL) was added at 0 °C thionyl chloride (2 mL). The resulting solution was heated at 60 °C for 2 days and the solvent was evaporated to give **36a** directly used in the next step. N-benzyliminodiacetic acid and **36a** reacted following general procedure A to give **36**.White solid. Yield 8%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.44 (d, J = 7.7 Hz, 1H), 7.60 (s, 1H), 7.29–7.22 (m, 5H), 6.85 (s, 1H), 4.57 (m, 1H), 3.78 (m, 2H+2H), 3.26 (s, 2H), 3.24 (s, 2H), 3.00 (d, J = 6.7 and 14.7 Hz, 1H), 2.93 (dd, J = 5.4 and 14.7 Hz, 1H), 1.78 (m, 1H), 0.79 (d, J = 7.16 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ ppm: 172.2, 171.3, 170.1, 138.1, 135.4, 134.0, 129.9, 128.3, 127.2, 115.6, 70.3, 57.5, 56.6, 53.4, 51.9, 28.9, 27.2, 18.7. $t_{R,LCMS}$ = 3.52 min, Purity 99%; MS (ESI+): m/z = 417 $(M + H)^+$.

*5.4.59. (S)-2-(2-(Benzyl-carboxymethyl-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid tert-butyl ester **(37)***

N-Benzyliminodiacetic acid and commercial L-H-Histidine-(1-trityl) tert-butylester **37a** reacted as described in general procedure A to give the trityle –protected intermediate as a white powder. Deprotection using TFA/DCM/TIS mixture allowed **37** as an oil (formiate salt oil) after preparative HPLC. Yield 41%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.38 (s, 1H), 8.32 (d, J = 8.1 Hz, CONH), 7.28 (m, 5H(Ph)), 7.14 (s, 1H), 4.50 (m, 1H), 3.76 (s, 2H), 3.29 (s, 2H), 3.22 (s, 2H), 3.02 (m, 2H), 1.33 (s, 9H(tBu)); ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 170.2, 169.9, 138.1, 134.7, 131.2, 128.9, 128.3, 127.3, 116.5, 81.1, 57.5, 56.5, 53.7, 51.8, 27.5, 27.4; $t_{R,LCMS}$ = 3.41 min, Purity 98% MS (ESI+): m/z = 417 $(M + H)^+$.

*5.4.60. (Benzyl-(((S)-2-hydroxy-1-(1H-imidazol-4-ylmethyl)-ethylcarbamoyl)-methyl)-amino)-acetic acid **(38)***

Compound **(38)** was synthesized from N-Benzyliminodiacetic acid and histidinol **38a** following general procedure A, as its formiate salt. Yield 47%. ^{1}H NMR (DMSO-d_6) δ ppm: 7.81 (d, J = 8.7 Hz, 1H), 7.71 (s, 1H), 7.27 (m, 5H), 6.83 (s, 1H), 3.98 (m, 1H), 3.71 (s, 2H), 3.37 (dd, J = 4.5 and 10.5 Hz, 1H), 3.29 (dd, J = 6.0 and 10.5 Hz, 1H), 3.24 (s, 2H), 3.16 (s, 2H), 2.77 (dd, J = 6.0 and 15.0 Hz, 1H), 2.67 (dd, J = 7.5 and 15.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.5, 169.7, 138.2, 134.6, 133.4, 128.9, 128.4, 127.3, 117.0, 62.4, 57.7, 57.1, 54.3, 50.2, 28.0.; $t_{R,LCMS}$ = 2.24 min, Purity 98% MS (ESI+): m/z = 347 $(M + H)^+$.

*5.4.61. (Benzyl-(((S)-1-carbamoyl-2-(1H-imidazol-4-yl)-ethylcarbamoyl)-methyl)-amino)-acetic acid **(39)***

To a stirred solution of **1** (150 mg, 0.5 mg) in dioxane/MeOH (5 mL/2 mL) were added by portion gaseous NH_3. When the reaction is total, the solvent was evaporated. The crude product was purified by preparative HPLC to give **39** as its formiate salt. Yield 79%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.95 (d, J = 1.4 Hz, 1H), 8.34 (d, J = 8.7 Hz, 1H), 7.54 (s, 2H), 7.32 (m, 5H), 7.29 (s, 1H), 4.57 (m, 1H), 3.88 (s, 2H), 3.47 (s, 2H), 3.45 (s, 2H), 3.15 (dd, J = 4.3 and 15.4 Hz, 1H), 2.96 (dd, J = 8.5 and 15.4 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ ppm: 171.5, 158.6, 158.1, 135.4, 134.4, 129.8, 129.4, 128.5, 128.0, 116.7, 57.8, 55.8, 53.7, 51.1, 27.2. $t_{R,LCMS}$ = 1.98 min (5 min gradient), Purity 98% MS (ESI+): m/z = 360 $(M + H)^+$.

*5.4.62. (Benzyl-(((S)-2-(1H-imidazol-4-yl)-1-methylcarbamoyl-ethylcarbamoyl)-methyl)-amino)-acetic acid **(40)***

To a stirred solution of L-H-histidine-(1-trityl) methyl ester hydrochloride (2.5 g, 5.5 mmol) in methanol (10 mL) were added 4 mL of a solution of methylamine in ethanol (33%). The mixture was refluxed overnight. The solvent was evaporated and the crude product was precipitated in water and filtrated. The crude product was purified by preparative HPLC to give *L-H-Histidine-(1-trityl)-methyl amide* **(40a)** as a white powder. Yield 63%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.30 (s, 1H), 7.95 (q, J = 4.8 Hz, CONHMe), 7.06–7.43 (m, 15H (Trt)), 6.63 (s, 1H), 3.53 (dd, J = 5.7 Hz and 7.2 Hz, 1H), 2.77 (dd, J = 5.7 Hz and 14.4 Hz, 1H), 2.63 (dd, J = 5.7 Hz and 14.4 Hz,

1H), 2.54 (d, $J = 4.8$ Hz, CONHMe); $t_{R,LCMS} = 4.79$ min; Purity: 74%; MS: (ESI+) $m/z = 411$ $(M + H)^+$. **40** was synthesized following general procedure A from N-Benzyliminodiacetic acid and **40a** to give first the trityle protected intermediate after precipitation in water and filtration. Yield 100%. $t_{R,LCMS} = 4.5$ min, Purity 97% MS (ESI+): $m/z = 616$ $(M + H)^+$. Deprotection using a TFA/DCM/TIS mixture allowed **40** as an oil (formiate salt) after purification by preparative HPLC. Yield 70%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.95 (s, 1H), 8.39 (d, $J = 8.1$ Hz, CONH), 8.00 (q, $J = 4.5$ Hz, CONHMe), 7.33 (m, 5H(Ph)), 7.28 (s, 1H), 4.57 (m, 1H), 3.91 (s, 2H), 3.51 (s, 2H), 3.49 (s, 2H), 3.14 (dd, $J = 5.4$ Hz and 15.3 Hz, 1H), 2.93 (dd, $J = 8.1$ Hz and 15.3 Hz, 1H), 2.60 (d, $J = 4.5$ Hz, CO_2Me); ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 169.8, 168.5, 133.9, 135.3, 129.3, 129.8, 128.5, 128.1, 116.8, 57.9, 55.9, 53.7, 51.3, 27.3, 25.7; $t_{R,LCMS} = 2.55$ min, Purity 98% MS (ESI+): $m/z = 374$ $(M + H)^+$; HRMS (m/z): (M+) calcd. for $C_{18}H_{24}O_4N_5$, 374.1828; found, 374.1823.

5.4.63. (Benzyl-(((S)-1-benzylcarbamoyl-2-(1H-imidazol-4-yl)-ethylcarbamoyl)-methyl)-amino)-acetic acid **(41)**

First, to a stirred solution of L-Boc-Histidine (500 mg, 1.95 mmol) in DMF (10 mL) were added benzylamine (213 μL, 1.95 mmol), HOBt (264 mg, 1.95 mmol), EDCI (455 mg, 2.38 mmol) and DIEA (2 mL, 11.7 mmol). The mixture was stirred overnight. The solvent was evaporated. The residue was solubilised in DCM and washed with a saturated solution of $NaHCO_3$ then with brine. The organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified by preparative HPLC to give L-Boc-Histidine benzyl amide. Yield 64%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.28 (t, $J = 6$ Hz, CONHBz), 7.57 (s, 1H), 7.29–7.11 (m, 5H(Ph)), 6.98 (d, $J = 8.1$ Hz, CONHBoc), 6.79 (s, 1H), 4.25 (d, $J = 6$ Hz, 2H), 4.18 (m, 1H), 2.89–2.75 (m, 2H), 1.36 (s, 9H(Boc)); $t_{R,LCMS} = 2.54$ min, Purity 98% MS (ESI+): $m/z = 345$ $(M + H)^+$. Deprotection was performed using HClg in dichloromethane during 30 min at room temperature. Solvent was evaporated to give L-H-Histidine benzyl amide **41a** as its dihydrochloride salt directly in the next step. $t_{R,LCMS} = 0.75$ min (5 min gradient), Purity 98% MS (ESI+): $m/z = 245$ $(M + H)^+$. Then **41a** and N-Benzyliminodiacetic acid reacted following general procedure A to give **41** as an oil (formiate salt). Yield 10%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.37 (t, $J = 5.96$ Hz, CONHBz), 8.32 (d, $J = 7.95$ Hz, CONH), 7.56 (s, 1H), 7.39–7.11 (m, 10H(Ph)), 6.78 (s, 1H), 4.54 (m, 1H), 4.26 (d, $J = 5.7$ Hz, 2H), 3.75 (s, 2H), 3.33–3.20 (m, 4H), 2.92 (m, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 170.8, 170.0, 139.3, 138.1, 134.7, 128.9, 128.2, 128.1, 127.2, 126.8, 126.6, 116.3, 57.6, 57.0, 53.8, 52.5, 41.9, 29.8; $t_{R,LCMS} = 1.82$ min (5 min gradient), Purity 97% MS (ESI+): $m/z = 450$ $(M + H)^+$.

5.4.64. (Benzyl-(((S)-1-dimethylcarbamoyl-2-(1H-imidazol-4-yl)-ethylcarbamoyl)-methyl)-amino)-acetic acid **(42)**

To a stirred solution of L-Boc-Histidine (765 mg, 3 mmol) in DMF (20 mL) were added dimethylamine. HCl (293 mg, 3.6 mmol), HOBt (551 mg, 3.6 mmol), EDCI (690 mg, 3.6 mmol) and TEA (1 mL, 6.8 mmol). The mixture was stirred overnight. The solvent was evaporated. The crude product was solubilised in DCM and washed with a saturated solution of $NaHCO_3$. The organic layers were dried over $MgSO_4$ and evaporated to give L-Boc-histidine dimethyl amide. The latter compound (198 mg, 0.7 mmol) was deprotected in presence of gaseous HCl in dichloromethane during 30 min at room temperature. The solvent was evaporated to give L-histidine dimethyl amide **42a** used directly in next step. **42a** and N-Benzyliminodiacetic acid reacted following general procedure A to give **42** as a colourless oil (formiate salt) after preparative HPLC purification. Yield 25%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.17 (s, 2HCOOH), 8.12 (d, $J = 8.4$ Hz, CONH), 7.62 (s, 1H), 7.32–7.23 (m, 5H (Ph)), 6.78 (s, 1H), 4.96 (m, 1H), 3.72 (s, 2H), 3.26 (s, 2H), 3.18 (s, 2H), 2.93 (s, 3H ($CONMe_2$)), 2.90–2.70 (m, 3H ($CONMe_2$) + 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.3, 170.5, 169.5, 138.1, 134.7, 132.6, 128.9, 128.3, 127.2, 117.0, 57.6, 56.7, 54.0, 48.4, 36.4, 35.2, 29.5; $t_{R,LCMS} = 2.60$ min, Purity 98% MS (ESI+): $m/z = 388$ $(M + H)^+$.

5.4.65. (S)-2-(2-(Carboxymethyl-(3-phenyl-propyl)-amino)-acetylamino)-3-(1H-imidazol-4-yl)-propionic acid tert-butyl ester **(43)**

Compound **(43)** was synthesized from commercially available L-H-histidine-(1-trityl)tert-butylester **37a** and **13a** following general procedure A. First trityle protected intermediate was obtained. Yield 48%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.72 (d, $J = 8.1$ Hz, CONH), 8.40 (s, 4HCOOH), 7.36–6.99 (m, 15H(Trt)+5H(Ph)+1H), 6.65 (s, 1H), 4.42 (m, 1H), 3.16 (s, 2H), 3.12 (s, 2H), 2.85 (m, 2H), 2.60–2.54 (m, 4H), 1.60 (qt, $J = 7.5$ Hz, 2H7), 1.29 (s, 9H(tBu)); $t_{R,LCMS} = 6.02$ min, Purity 97% MS (ESI+): $m/z = 686$ $(M + H)^+$. Deprotection using a TFA/DCM/TIS mixture allowed **43** as ist formiate salt after preparative HPLC purification. Yield 68%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.54 (d, $J = 8.6$ Hz, CONH), 8.32 (s, 2HCOOH), 7.48 (s, 1H), 7.27–7.14 (m, 5H(Ph)), 6.81 (s, 1H), 4.41 (m, 1H), 3.24 (s, 2H), 3.16 (s, 2H), 2.88 (m, 2H), 2.60–2.54 (m, 4H), 1.60 (qt, $J = 7.5$ Hz, 2H), 1.29 (s, 9H(tBu)); ^{13}C NMR (DMSO-d_6) δ ppm: 173.2, 170.7, 170.3, 164.6, 142.1, 134.9, 133.0, 128.3, 128.2, 125.6, 116.5, 80.5, 58.0, 56.1, 54.2, 52.5, 32.7, 29.3, 29.0, 27.6; $t_{R,LCMS} = 4.19$ min, Purity 99% MS (ESI-): $m/z = 443$ $(M-H)^-$.

5.4.66. ((((S)-2-(1H-imidazol-4-yl)-1-methylcarbamoyl-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid **(44)**

(BDM43079) was synthesized as depicted for **40**, from **13a** and **40a**, as its TFA salt. Yield 58%. ^{1}H NMR (DMSO-d_6) δ ppm: 9.05 (d, $J = 8.7$ Hz, 1H), 9.01 (s, 1H), 8.22 (q, $J = 4.8$ Hz, 1H), 7.38 (s, 1H), 7.32–7.19 (m, 5H), 4.58 (m, 1H), 4.13 (m, 2H+1H), 4.08 (d, $J = 6.6$ Hz, 1H), 3.17–3.13 (m, 2H+1H), 2.96 (dd, $J = 8.7$ Hz and $J = 14.8$ Hz, 1H),2.60–2.57 (m, 3H+2H), 1.92 (qt, $J = 7.8$ Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 169.5, 167.7, 165.2, 140.5, 134.1, 129.2, 128.4, 128.3, 126.1, 116.8, 55.3, 54.9, 54.1, 52.1, 31.8, 27.0, 25.7, 25.3; $t_{R,LCMS} = 3.35$ min, Purity 98% MS (ESI+): $m/z = 402$ $(M + H)^+$; HRMS (m/z): (M^+) calcd. for $C_{20}H_{28}O_4N_5$, 402.2141; found, 402.2137.

5.4.67. ((((S)-1-Benzylcarbamoyl-2-(1H-imidazol-4-yl)-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid **(45)**

Compund **(45)** was obtained following the procedure of obtention of **41**, as its formiate salt. Yield 84%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.38 (t, $J = 5.5$ Hz, CONHBz), 8.30 (d, $J = 8.5$ Hz, CONH), 8.28 (s, HCOOH), 7.5 (s, 1H), 7.11–7.29 (m, 10H(Ph)), 6.76 (s, 1H), 4.55 (m, 1H), 4.24 (d, $J = 5.5$ Hz, 2H), 3.27 (s, 2H), 3.21 (d, $J = 16.6$ Hz, 1H), 3.14 (d, $J = 16.6$ Hz, 1H), 2.94 (dd, $J = 5.5$ Hz and 15 Hz, 1H), 2.87 (dd, $J = 7.4$ Hz and 15 Hz, 1H), 2.52 (m, 4H), 1.64 (dt, $J = 7.5$ Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 173.0, 170.9, 170.6, 164.2, 142.1, 139.3, 134.7, 133.4, 128.3, 128.25, 128.2, 126.8, 126.6, 125.7, 125.6, 58.1, 55.9, 54.3, 52.9, 41.9, 32.7, 29.8, 29.7; $t_{R,LCMS} = 5.02$ min (10 min gradient), Purity 98% MS (ESI+): $m/z = 478$ $(M+H)^+$.

5.4.68. ((((S)-2-(1H-imidazol-4-yl)-1-(3-methyl-(1,2,4)oxadiazol-5-yl)-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid **(46)**

To a stirred solution of hydroxylamine hydrochloride (3.82 mg, 55 mmol) in a solution of ethanol/water (80/20) were added 2.6 mL (50 mmol) of acetonitrile and 2.2 mg (55 mmol) of NaOH. The mixture was refluxed overnight. The solvent was evaporated and the residue was solubilized in 80 mL of ethanol, the suspension was filtrated and the filtrate was evaporated. The crude product was

recrystallized from *i*PrOH to give N-Hydroxy-acetamidine as a white powder. Yield 47%. Purity: 99%; ^{1}H NMR (DMSO-d_6) δ ppm: 8.66 (sl, 1H), 5.34 (sl, 2H), 1.61 (s, 3H). To a stirred solution of L-Boc-Histidine (510 mg, 2 mmol) in DMF (10 mL) were added TBTU (702 mg, 2.2 mmol) and DIEA (1 mL, 6 mmol). The resulting yellow mixture was stirred for 5 min. *N*-Hydroxy-acetamidine (150 mg, 2 mmol) was added and the mixture was stirred for 4.5 h since the intermediate product was formed. The solution was then refluxed 1.5 h. The solvent was evaporated and the residue was solubilized in AcOEt washed with a saturated solution of $NaHCO_3$. The organic layers were dried with $MgSO_4$ and evaporated. The crude product was purified by preparative HPLC to give ((S)-2-(1H-Imidazol-4-yl)-1-(3-methyl-(1,2,4)oxadiazol-5-yl)-ethyl)-carbamic acid tert-butyl ester as a brown oil. Yield 73%. $t_{R,LCMS}$ = 3.56 min; Purity: 98%; MS: (ESI+) m/z = 294 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ ppm: 8.19 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.54 (s, 1H), 6.76 (s, 1H), 5.03 (m, 1H), 3.02 (m, 2H), 2.29 (s, 3H), 1.35 (s, 9H). *Tert*-butoxycarbonyl groups were removed in presence of gaseous HCl in dichloromethane during 30 min at room temperature. Solvent was evaporated to give the expected product *(S)-2-(1H-Imidazol-4-yl)-1-(3-methyl-(1,2,4) oxadiazol-5-yl)-ethylamine.2HCl* **(46a)** as its dihydrochloride salt used directly in next reaction. $t_{R,LCMS}$ = 0.66 min (5 min gradient); Purity: 98%; MS: (ESI+) m/z = 194 $(M + H)^+$. **46** was synthesized as its formiate salt from **13a** and **46a** following general procedure A. Yield 50%.^{1}H NMR (DMSO-d_6) δ ppm: 8.97 (t, J = 7.8 Hz, 1H), 8.27 (s, 1H), 7.5 (s, 1H), 7.28–7.12 (m, 5H), 6.79 (s, 1H), 4.23 (m, 1H), 3.28 (s, 2H), 3.22 (s, 2H), 3.12 (m, 2H), 2.69–2.50 (m, 4H), 2.26 (s, 3H), 1.64 (m, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 178.8, 173.1, 170.9, 166.1, 164.1, 142.0, 135.0, 132.9, 128.2, 125.6, 119.1, 57.9, 55.9, 46.3, 32.7, 32.6, 30.3, 29.1, 11.0; $t_{R,LCMS}$ = 3.68 min, Purity 98% MS (ESI+): m/z = 427 $(M + H)^+$.

5.4.69. ((((S)-2-Hydroxy-1-(1H-imidazol-4-ylmethyl)-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid **(47)**

Compound **(47)** was obtained as depicted for compound **38** from **13a** and **38a**, as a colourless oil (formiate salt) after preparative HPLC purification. Yield 30%. ^{1}H NMR (DMSO-d_6) δ ppm: 8.31 (s, 2HCOOH), 8.07 (d, J = 8.5 Hz, CONH), 7.50 (d, J = 1.2 Hz, 1H), 7.28–7.15 (m, 5H (Ph)), 6.76 (d, J = 1.2 Hz, 1H), 3.92 (m, 1H), 3.35 (dd, J = 4.8 Hz and 10.6 Hz, 1H), 3.28 (dd, J = 6.1 Hz and 10.6 Hz, 1H), 3.19 (s, 2H), 3.10 (s, 2H), 2.75 (dd, J = 5.7 Hz and 14.6 Hz, 1H), 2.63 (dd, J = 7.3 Hz and 14.6 Hz, 1H), 2.5 (m, 4H), 1.62 (qt, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 173.2, 170.3, 164.5, 142.1, 134.6, 133.4, 128.3, 128.2, 125.6, 117.8, 62.4, 58.5, 56.6, 54.4, 50.4, 32.7, 29.1, 28.2; $t_{R,LCMS}$ = 3.08 min, Purity 98% MS (ESI+): m/z = 375 $(M + H)^+$.

5.4.70. ((((S)-2-(1H-imidazol-4-yl)-1-methylcarbamoyl-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid methyl ester **(48)**

13a (751 mg, 2.99 mmol) was dissolved in trifluoracetic anhydride 2% in acetic anhydride (7.5 mL). The reaction mixture was stirred for 4 h at 70 °C and then evaporated. The residue was dissolved in methanol (6 mL) and DIEA (1.56 mL, 8.97 mmol) was added. The mixture was stirred overnight at reflux under argon. The solvent was evaporated under reduced pressure. The crude product was dissolved in DCM and washed with water. The organic layer was dried with $MgSO_4$ and evaporated to give (Methoxycarbonylmethyl-(3-phenyl-propyl)-amino)-acetic acid **48a** as an orange oil. Yield 78%. $t_{R,LCMS}$ = 3.96 min, Purity 97%; MS (ESI+): m/z = 266 $(M + H)^+$. To a stirred solution of L-H-Histidine-(1-trityl) methyl amide **40a** (738 mg, 1.47 mmol) in DMF (7.5 mL) were added acid **48a** (390 mg, 1.47 mmol), HOBt (397 mg, 2.94 mmol), EDCI (338 mg, 1.76 mmol) and N-methylmorpholine (484 µL, 4.41 mmol). The mixture was stirred 22 h. The solvent was evaporated. The crude product was purified by preparative HPLC to give the trityle protected compound. Yield 30%. $t_{R,LCMS}$ = 2.00 min, Purity 81%; MS (ESI+): m/z = 658 $(M+H)^+$. To a stirred solution of TIS and DCM (1.0 mL/8.2 mL) was added the protected compound (339 mg, 0.52 mmol). Then 1.0 mL of TFA was added. The mixture was stirred at room temperature during 16 h. Then the solvent was evaporated and the crude product was purified by preparative HPLC to give **48 (BDM43124)** as a yellow oil (formiate salt). Yield 94%. ^{1}H NMR (CD_3OD) δ ppm: 8.63 (1, H, HCOOH), 7.27–7.15 (m, 6H), 4.74 (dd, J = 5.0 Hz and J = 9.0 Hz, 1H), 3.70 (s, 3H), 3.49 (s, 2H), 3.30 (s, 2H), 3.27 (dd, J = 5.0 Hz and J = 15.0 Hz, 1H), 3.10 (dd, J = 9.0 Hz and J = 15.0 Hz, 1H), 2.73 (s, 3H), 2.64 (t, J = 7.5 Hz, 2H), 2.57 (t, J = 7.5 Hz, 2H), 1.70 (m, 2H); ^{13}C NMR (CD_3OD) δ ppm: 172.4, 172.1, 171.0, 141.7, 129.8, 128.0, 125.5, 118.8, 117.0, 114.9, 58.0, 55.5, 55.3, 51.7, 51.0, 32.8, 29.0, 27.2, 25.1; $t_{R,LCMS}$ = 2.00 min, Purity 100%; MS (ESI+): m/z = 416 $(M+H)^+$. HRMS (m/z): (M^+) calcd. for $C_{21}H_{30}O_4N_5$, 416.2298; found, 416.2284.

5.4.71. ((((S)-2-(1H-imidazol-4-yl)-1-(3-methyl-(1,2,4)oxadiazol-5-yl)-ethylcarbamoyl)-methyl)-(3-phenyl-propyl)-amino)-acetic acid methyl ester **(49)**

Compound **(49)** was synthesized as described for compound **48**, from **46a** and acid **48a**. Yield 40%. ^{1}H NMR (CD_3OD) δ ppm: 7.73 (s, 1H), 7.28–7.14 (m, 5H), 6.95 (s, 1H), 5.51 (dd, J = 6.0 Hz and J = 8.0 Hz, 1H), 3.70 (s, 3H), 3.45 (s, 2H), 3.30 (m, 4H), 2.65–2.55 (m, 4H), 2.32 (s, 3H), 1.71 (m, 2H); ^{13}C NMR (DMSO-d_6) δ ppm: 178.3, 172.8, 172.4, 167.2, 141.9, 135.1, 132.2, 128.1, 125.5, 116.8, 58.0, 55.3, 54.7, 50.8, 46.4, 32.9, 29.7, 29.4, 10.0; $t_{R,LCMS}$ = 2.28 min, Purity 98%; MS (ESI+): m/z = 441 $(M + H)^+$.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2014.12.005. These data include MOL files and InChiKeys of the most important compounds described in this article.

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