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Design and synthesis of potent, orally active, inhibitors of carboxypeptidase U (TAFIa)

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Abstract—A series of 3-mercapto-propionic acid derivatives that function as reversible inhibitors of carboxypeptidase U have been prepared. We present a successful design strategy using cyclic, low basicity guanidine mimetics resulting in potent, selective and bioavailable inhibitors of carboxypeptidase U (TAFIa).

1. Introduction

Venous and arterial thromboembolism is the largest cause of disease and death in the Western world and more than 30% of the population will once experience a thromboembolic episode. Therapy available today includes thrombolytics, anticoagulants and antiplatelets. However, none of these therapies seems to be optimal. The only thrombolytics on the market are the plasminogen activators, which are parenterally administered and associated with more or less severe bleeding complications. Among the anticoagulants, Warfarin requires closely monitored therapy because of the high risk of bleeding. Heparin on the other hand is not orally available. The antiplatelets are also associated with bleeding complications. Clearly, there is a need for an orally active antithrombotic or thrombolytic drug that is clinically safe and requires less monitoring.

Procarboxypeptidase U[1] (proCPU, EC 3.4.17.20, thrombin activatable fibrinolysis inhibitor, TAFI,[2] procarboxypeptidase R[3] and plasma procarboxypeptidase B[4,5]) is a metallopeptidase, circulating in plasma. The function of this enzyme in fibrinolysis was elucidated in the mid 1990s.[6] After activation, the enzymatic activity of CPU is to cleave off C-terminal basic amino acids, that is, lysine and arginine residues, from peptides and proteins (Chart 1).

During fibrinolysis, C-terminal lysine and arginine residues are formed by the action of plasmin on the fibrin network and thereby high-affinity binding sites for plasminogen and tPA (tissue-type plasminogen activator) are created. Upon binding to the clot, plasminogen is efficiently converted to plasmin and an acceleration in the rate of fibrinolysis is achieved. Through the action of CPU on partly degraded fibrin, the C-terminal lysine residues are cleaved off. This diminishes the amount of plasminogen and tPA able to bind to fibrin and thereby the amount of plasmin formed. This proposed mechanism for suppression of fibrinolysis provides the rationale for inhibiting CPU in order to enhance clot lysis. Furthermore, since the

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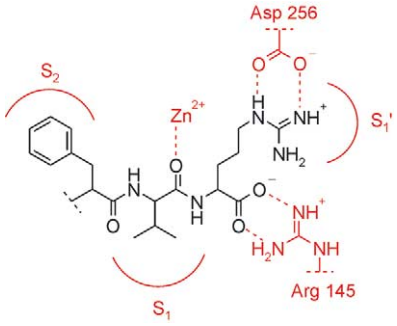


Chart 1. Binding of a substrate (black) in the CPU active site (red).

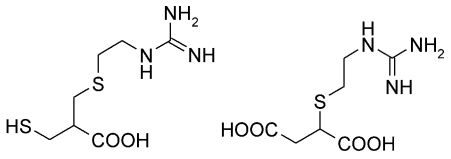
coagulation is unaffected, CPU inhibition may result in less bleeding complications than conventional therapy.[7] This fact may contribute to the interest in the pharmaceutical industry for CPU as a potential drug target.[8] The concept of CPU inhibition for stimulation of fibrinolysis is supported by in vitro studies in human blood and plasma as well as in vivo studies in animals.[9] A high molecular weight peptide CPU inhibitor, potato carboxypeptidase inhibitor (PTCI), has been shown to be active in rabbit venous and arterial thrombosis models.[10–12]

So far, only a few low molecular weight inhibitors of CPU, for example, compound **1**[13] and **2**[14] with $IC_{50} = 20$ μM and 270 μM respectively, have been described (Chart 2). Both compounds where originally reported as carboxypeptidase N (CPN) inhibitors (CPN $IC_{50} = 200$ nM and 17 μM respectively) and both compounds have also been reported to inhibit pancreatic carboxypeptidase B (CPB) with $IC_{50} = 8$ μM.[15,16]

In this paper, we report the design and synthesis of the first potent, selective and orally active inhibitors of CPU.[17]

2. Results and discussion

CPU has a very low solubility and is also a very unstable enzyme in vitro, factors probably contributing to the fact that no X-ray crystallographic structure has been published. The X-ray structure of several complexes between CPA and different inhibitors has been reported[18] while for CPB only the X-ray coordinates for the backbone atoms has been reported.[19] In order to obtain a more relevant starting point for drug design, the structure of compound **2** bound to porcine CPB was solved using X-ray crystallography to a resolution of 1.55 A (Fig. 1). As expected, one of the carboxylates in compound **2** interacts with the zinc atom while the other carboxylate interacts in a bidentate fashion with Arg145 and in a monodentate fashion with Arg124 through charge–charge



1 2

Chart 2. Commercial carboxypeptidase inhibitors **1** and **2**.

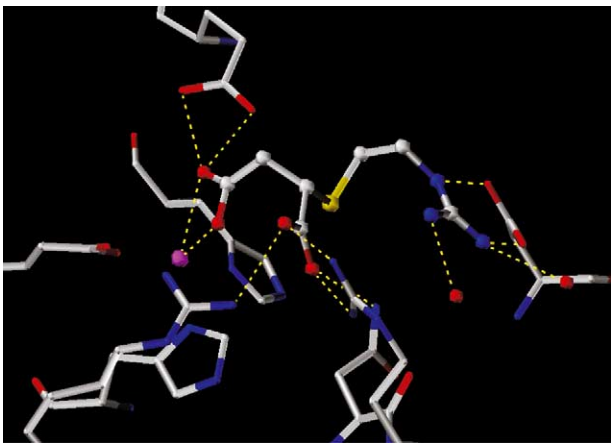


Figure 1. X-ray structure of compound **2** in complex with CPB. Zink atom in magenta, water molecules in red and hydrogen bonds in dotted yellow lines.

interactions (CPB numbering[20]). The guanidine forms a bidentate charge–charge interaction with Asp256 in the specificity pocket. Although racemic **2** was used for the crystallisation, the configuration of the inhibitor found in the X-ray structure correspond to the unnatural form of arginine.

Compound **1** and **2** have two major drawbacks from a drug discovery point of view. Firstly, both compounds are potent inhibitors of CPN. CPN is believed to have an important function in plasma as an inactivator of anaphylatoxins and in the processing of peptide hormones.[21] Consequently, selectivity towards CPN was an important parameter to consider during optimisation. Secondly, both compounds are extremely polar resulting in low oral bioavailability. Our approach was to improve bioavailability simultaneously with potency and selectivity towards CPN. The strongly basic guanidine group was identified as the major obstacle for achieving a high bioavailability and we reasoned that a low basicity guanidine replacement should solve that problem. Furthermore, if cyclic guanidine replacements where used, increased potency and selectivity may be an additional bonus. It was decided to use the neutral thiol moiety as zinc binding group since charged alternatives, for example, imidazole, carboxylate or phosphinate groups would reduce the possibility to obtain compounds with good drug metabolism and pharmacokinetic (DMPK) properties.

A homology model of CPU was considered to be sufficient for the first design steps and such a model was constructed using the protein structure analysis program WHAT IF[22] based on the X-ray structure of porcine CPB (49% sequence identity) in complex with compound **2**. The CPU homology model indicated only small backbone deviations from the CPB structure.

In the first design cycle six different cyclic basic groups was incorporated using the CPA inhibitor 2-mercaptomethyl-3-phenyl-propionic acid as a template.[23] Although no crystal structure has been reported for 2-mercaptomethyl-3-phenyl-propionic acid we reasoned that the binding conformation should be similar to the

conformation reported for benzylsuccinic acid in complex with CPA.[24] Superimposing the X-ray structures of compound **2** in complex with CPB with benzylsuccinic acid in complex with CPA indicate that basic groups could be merged with the 2-mercaptomethyl-3-phenylpropionic acid template (Fig. 2).

The resulting inhibitors **3–8** were energy minimised in the active site of the CPU homology model using the docking programs DOCK[25] and GOLD.[26] The predicted binding conformations for all compounds looked reasonable with the thiol interacting with the zinc atom, the carboxylate interacting with Arg124 and Arg145 and the basic group interacting with Asp256 (CPB numbering[27]).

Compounds **3–5** (Chart 3), containing five-membered ring basic groups, with calculated pK_a values of 11.3, 10.8 and 5.4 respectively, gave valuable information (Table 1). Compounds **3–5** where equally potent, with CPU IC_{50} values around 1 μM but the selectivity towards CPN differed significantly with CPN IC_{50} values of 0.5 μM, 8 μM and 40 μM, respectively. Interestingly, the basicity does not seem to influence CPU binding affinity indicating that the interaction with Asp256 may be of hydrogen bond character and not a charge–charge interaction.[28] However, compound **5** has the possibility to interact in a bidentate fashion with Asp256, possibly compensating for the effect of lower basicity. Compound **6** had a similar profile as the pyrrolidine **3**, with a CPU IC_{50} of 3 μM and a CPN IC_{50} of 0.5 μM, while the 2-aminopyridine **7**, showed reversed selectivity with a CPU IC_{50} of 3 μM and a CPN IC_{50} of 6 μM. The regioisomer, aminopyridine **8** proved to be the best of the six compounds in the first design cycle. This compound was potent with an IC_{50} of 0.2 μM (K_i = 22 nM) with 15 times selectivity toward CPN. Furthermore, the weakly basic 2-aminopyridine (calculated pK_a = 7) should result in a higher bioavailability than the guanidine-containing starting points, **1** and **2**. The X-ray structure of compound **8** complexed to bovine CPB shows that this compound binds very much as expected. The thiol interacts with the zinc atom in a tetragonal fashion while the carboxylate interact in a bidentate fashion with Arg143 and in a monodentate fashion with Arg125. The aminopyridine forms a bidentate interaction with Asp257 in the specificity pocket. The positions of the aminopyridine nitrogens are identical to the guanidine nitrogens of compound **2** in the X-ray structure.

With the potent and fairly selective compound **8** as a starting point, we tried to increase selectivity towards CPN further. The CPU homology model indicated that there is some space around the pyridine ring, especially around the 2- and 3-position, and our next step was to investigate this area. Compounds **9–11** was first designed to evaluate this (Chart 4).

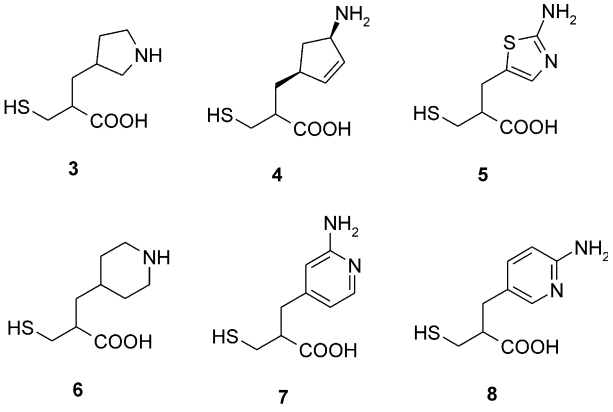


Chart 3. Inhibitors **3–8**.

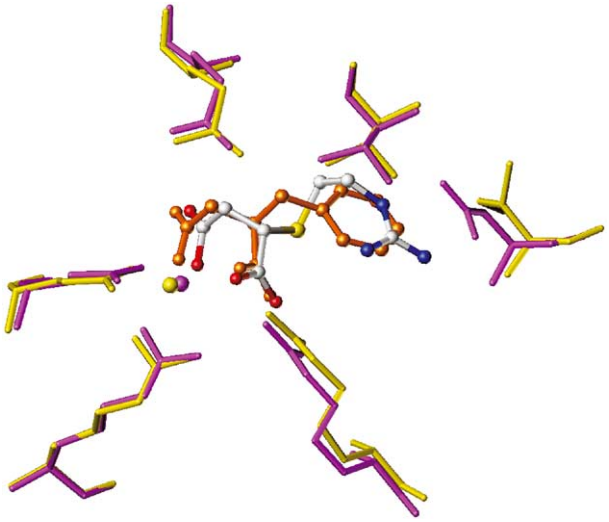


Figure 2. Superimposition of the X-ray structures of compound **2** in complex with CPB (magenta), and benzylsuccinic acid (orange) in complex with CPA (yellow).

Table 1. Enzyme inhibition data on compounds **1–19**

Compd	CPU[a] IC_{50} (μM)	CPN[a] IC_{50} (μM)	CPB[a] IC_{50} (μM)	pK_a[b] calcd
1	20	0.2	8	12.7
2	270	17	8	12.7
3	1.6	0.5	3.2	11.3
4	1	8	3.2	10.2
5	1.3	40	0.2	5.9
6	3.2	0.5	7.9	11.3
7	3.2	6.3	10	7.3
8	0.2	3.2	0.13	6.8
9	7.9	5	5	7.4
10	6.3	1.3	4.0	7.0
11	1	50	0.13	7.1
12	10	>2000	0.8	4.0
13	13	398	4	6.2
14	250	500	40	6.8
15	83	>1000	41	7.0
16	0.9	72	0.8	6.9
17	0.5	20	0.05	6.6
18	0.6	16	0.5	6.8
19	1.6	200	0.2	7.2

[a] The assays was performed as described earlier[30] at pH 7.4 (CPU and CPN) and at pH 6.0 (CPB).
[b] pK_a was calculated with ACD/Labs 6.00 from Advanced Chemistry Development Inc., Canada.

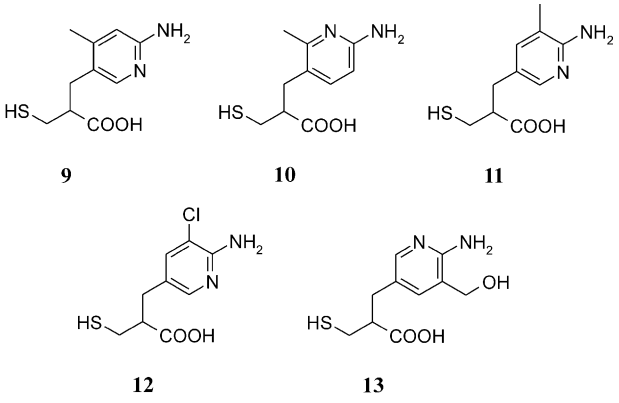


Chart 4. Inhibitors **9**–**13**.

Methyl substitution at the 4- and 6-position, compound **9** and **10** respectively, resulted in decreased CPU affinity. On the other hand, methyl substitution at the 3-position resulted in an increased selectivity towards CPN with only a slight decrease in CPU affinity. Chloro substitution at the 3-position, compound **12**, resulted in significantly decreased CPU affinity. This could be an effect of the low basicity (calculated $pK_a = 4.3$).

In the X-ray structure of compound **2** complexed to CPB, two water molecules can be seen close to the guanidine nitrogens (Fig. 1). The well-tolerated methyl group in compound **11** served as a handle and compound **13** was designed with the intention to replace or interact with these water molecules. This attempt resulted however, in a decrease in binding affinity of more than two orders of magnitude. In the CPB crystal structures as well as in the CPU homology model, there was a small pocket below the carboxylate in the inhibitors, close to the zinc atom. Attempts to reach this pocket from the α-position showed that only small substituents where tolerated, with ethyl (compound **14**) and hydroxymethyl (compound **15**) being detrimental for activity (Chart 5). The smaller substituents hydroxy, fluoro and methyl (compounds **16**, **17** and **18**, respectively) resulted in only slightly decreased binding affinity. Compound **18** showed an increased selectivity towards CPN and the combination of α-methyl and 2-methyl substitution (compound **19**) resulted in more than two orders of magnitude selectivity towards CPN. As expected from the homology model, the compounds show no selectivity towards CPB.

The most potent inhibitor, compound **8**, was selected for further studies. DMPK studies in the rat show a good bioavailability (74%) and a medium clearance (27 mL/min/kg), indicating that our design strategy was indeed successful. In a disseminated intravascular coagulation model in the rat, the compound increases the rate of thrombus dissolution with an ED_{50} value of around 1 μmol/kg after intravenous or oral administration.[29]

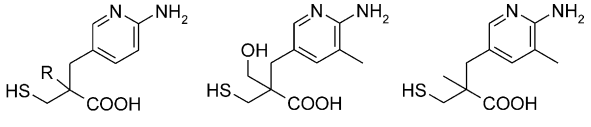


Chart 5. Inhibitors **14**–**19**.

3. Inhibitor synthesis

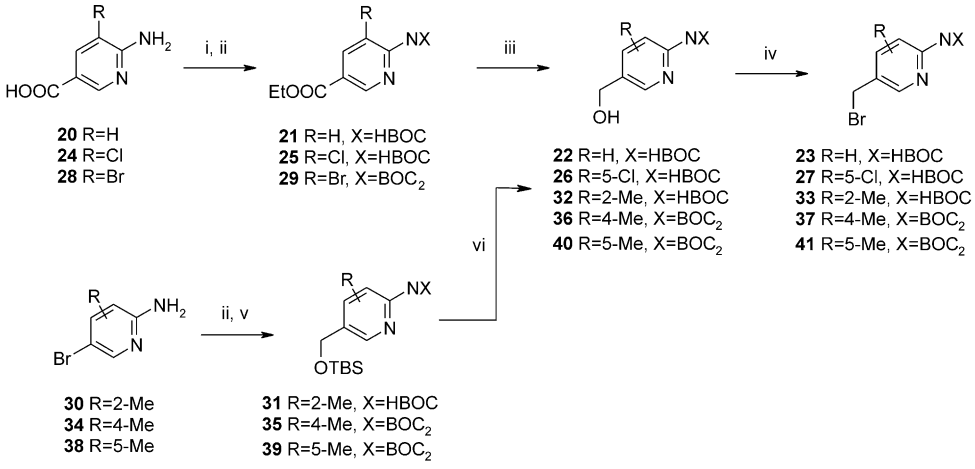
The presence of the 3-mercaptopropanoic acid fragment in the proposed inhibitors led us to consider a synthetic strategy based on malonate alkylation chemistry. This would provide the possibility to investigate different basic groups using one synthetic approach. One of the key intermediates, bromide **23**, was synthesized from 6-amino-nicotinic acid (**20**) in four steps. Esterification and BOC protection followed by reduction to the alcohol and finally bromination using Ph_3P and CBr_4 in methylene chloride gave bromide **23** in 30% overall yield. While the 5-chloro analogue (**27**) could be synthesized using the same route, the methyl-substituted analogues **33**, **37** and **41** had to be synthesized using another route. These bromides was conveniently accessed from the corresponding aryl bromides via a palladium catalyzed coupling with a hydroxymethyl equivalent, $TBSOCH_2$-$SnBu_3$, followed by deprotection of the TBS group and bromination (Scheme 1).

With the key bromides at hand, alkylation with diethyl malonate/NaH followed by careful ester hydrolysis to form the monoacid could be accomplished. This was followed by a consecutive decarboxylation/Mannich reaction to give the alkenoic esters **43**, **46**, **49**, **52** and **55**. Michael addition of thioacetic acid followed by exhaustive deprotection using boiling concentrated hydrochloric acid gave compounds **8**–**12** as the hydrochloride salts (Scheme 2).

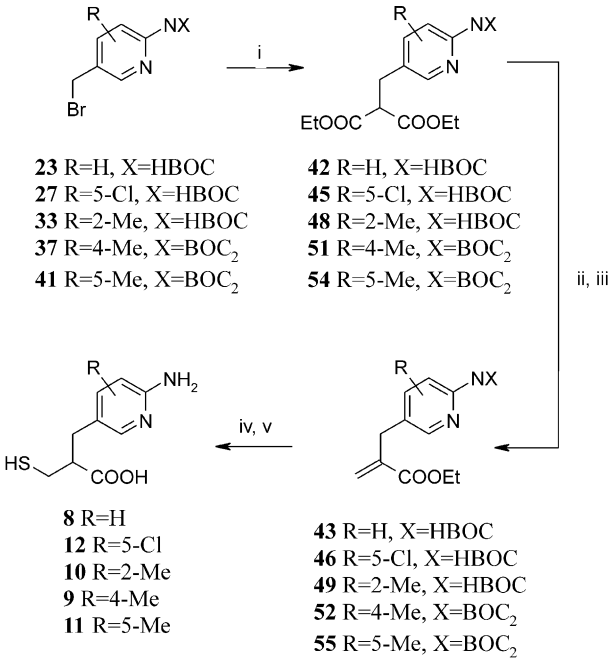
Compound **7** was also synthesized using this method, starting from 2-acetylamino-isonicotinic acid. Esterification and deacetylation was accomplished using BF_3*OEt_2 in EtOH followed by Boc protection of the amine function and reduction of the ester using $LiAlH_4$. The obtained alcohol was then brominated ($Ph_3P/CBr_4/CHCl_2$) and subsequently converted to compound **7** using the same procedure as for compound **8**–**12** (Scheme 3).

Compound **4** was synthesized starting from *cis*-(4-hydroxymethyl-cyclopent-2-enyl)-carbamic acid *tert*-butyl ester (**62**), via mesylation and bromination using LiBr in acetone. The bromide was then converted to compound **4** using the standard route (Scheme 4).

The synthesis of compound **3** started from 1-benzyl-5-oxo-pyrrolidine-3-carboxylic acid (**65**), with concomitant reduction of the acid and the lactam carbonyl group. After debenzylation and reprotection as the BOC derivative, the alcohol was converted to the bromide (**67**) via the triflate. The synthesis was completed using the standard procedures (Scheme 5).



Scheme 1. Conditions: (i) $SOCl_2$, EtOH; (ii) Boc_2O, DMAP, *t*BuOH/acetone; (iii) $LiAlH_4$, THF; (iv) Ph_3P, CBr_4, $CHCl_2$; (v) $TBSOCH_2SnBu_3$, $(Ph_3P)_2PdCl_2$, 1,2-dichloroethane; (vi) Bu_4NF, THF.

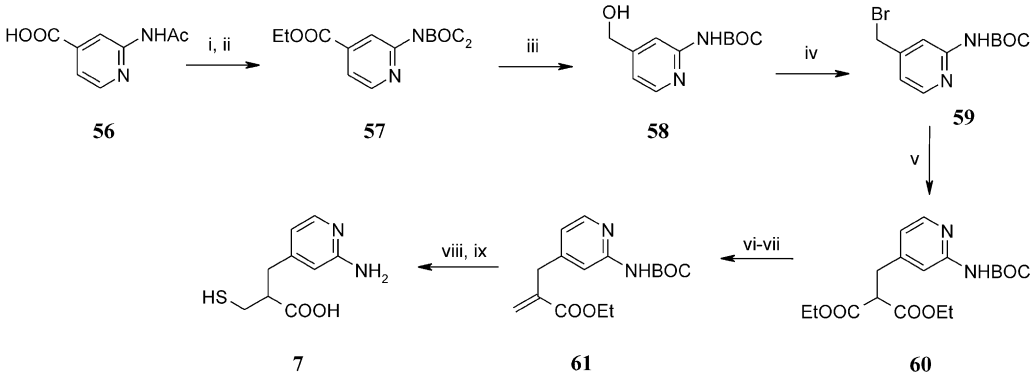


Scheme 2. Conditions: (i) $(EtOCO)_2CH_2$, NaH, THF; (ii) KOH, $EtOH/CH_2Cl_2$; (iii) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (iv) Et_3N, AcSH; (v) $HCl_{(aq)}$.

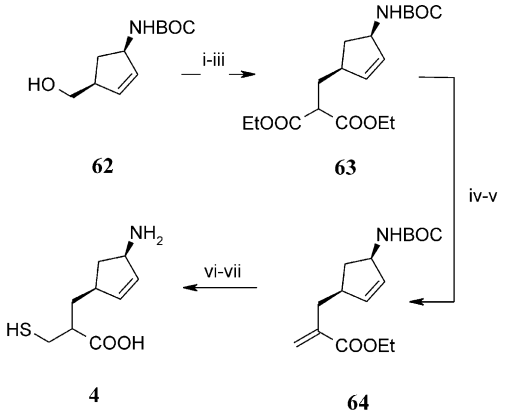
Compounds **17** and **18** were synthesised from bromide **23** and the fluoro- and methyl-substituted malonates, respectively. In this case, ester hydrolysis was followed by reduction of the acid to give the corresponding alcohols (**72** and **73**) via the mixed anhydride (MeOCOCl, Et_3N then $NaBH_4$). The alcohol was then converted to the thiol using the Mitsunobu reaction followed by exhaustive deprotection (Scheme 6).

A similar route was used for compounds **14** and **19**, but ethyl- or methyl-substituted Meldrums acid was used instead of the corresponding malonate. Alkylation of the Meldrums acid derivatives using triethyl amine/DMSO and bromide **23** or **41**, gave compound **74** and **75**, respectively. Treatment with NaOEt in EtOH resulted in ring opening giving the monoacids in good yield. The acid was then reduced to the alcohol via the mixed anhydride method. The alcohol was then converted to thiols **14** and **19** using the Mitsunobu reaction followed by removal of the protecting groups using concentrated aqueous HCl (Scheme 7).

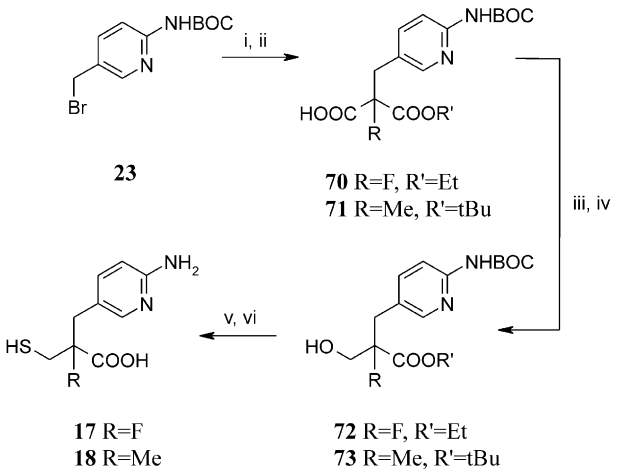
The synthesis of compound **15** was accomplished using this route as well, but starting from the malonate. Double alkylation, first with bromide **41** as earlier, and then with $BnOCH_2Cl$ gave the disubstituted malonate,



Scheme 3. Conditions: (i) $BF_3{*}OEt_2$, EtOH; (ii) Boc_2O, DMAP, *t*BuOH/acetone; (iii) $LiAlH_4$, THF; (iv) Ph_3P, CBr_4, $CHCl_2$; (v) $(EtOCO)_2CH_2$, NaH, THF; (vi) KOH, $EtOH/CH_2Cl_2$; (vii) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (viii) Et_3N, AcSH; (ix) $HCl_{(aq)}$.



Scheme 4. Conditions: (i) MsCl, Et_3N, CH_2Cl_2; (ii) LiBr, acetone; (iii) $(EtOCO)_2CH_2$, NaH, THF; (iv) KOH, $EtOH/CH_2Cl_2$; (v) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (vi) Et_3N, AcSH; (vii) $HCl_{(aq)}$.



Scheme 6. Conditions: (i) *t*BuOCOCHMeCOOEt, NaH, DMF or $(EtOCO)_2CHF$, NaH, DMF; (ii) NaOH, EtOH/THF; (iii) MeOCOCl, Et_3N, THF; (iv) $NaBH_4$, THF; (v) AcSH, DEAD, Ph_3P, THF; (vi) $HCl_{(aq)}$.

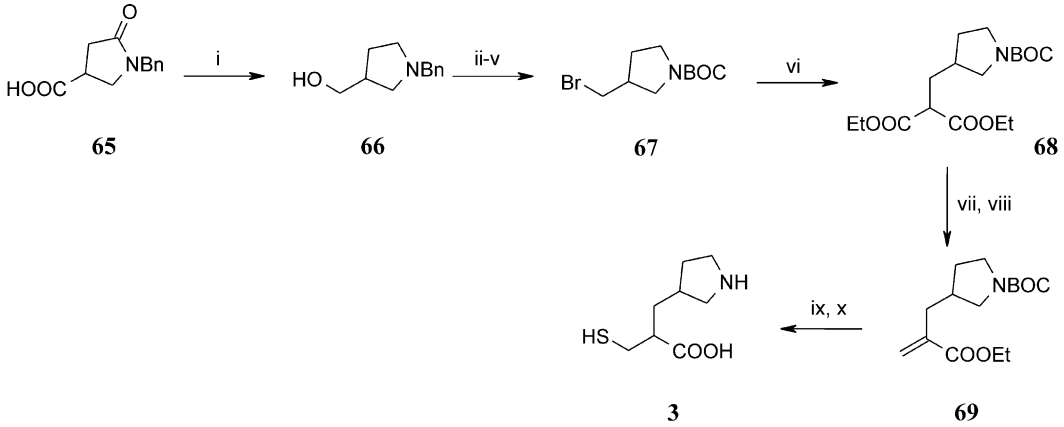
which was then hydrolysed to the monoacid. Reduction of the carboxylic acid to the corresponding alcohol could not be accomplished in good yields using our standard procedure on this compound. After some experimentation, it was found that activation of the acid using Bop/$Et(iPr)_2N$ in THF reproducibly gave the active ester. It was then necessary to exchange the solvent to ethanol and to use $LiBH_4$ instead of $NaBH_4$ in order to obtain the alcohol in good yield. The benzyl ether was then deprotected using catalytic hydrogenation to give the symmetric diol (**79**). One of the hydroxy groups was then converted to the thiol using the Mitsunobu reaction. This procedure gave the mono-thiol in 45% yield after standard deprotection (Scheme 8).

The synthesis of compound **13** was accomplished using the same method as for compounds **8**. However, the hydroxymethyl group could not be introduced using $TBSOCH_2SnBu_3$ and instead $Bu_3SnCHCH_2$ was used as a protected hydroxymethyl equivalent and coupling partner for aryl bromide **29**. After conversion to the thioacetate **81**, the vinyl group was cleaved using ozonolysis with reductive workup. This procedure resulted in a mixture of alcohols containing unchanged thioacetate as well as some free thiol. This material was immediately acetylated in order to facilitate purification. Standard deprotection then gave compound **13** (Scheme 9).

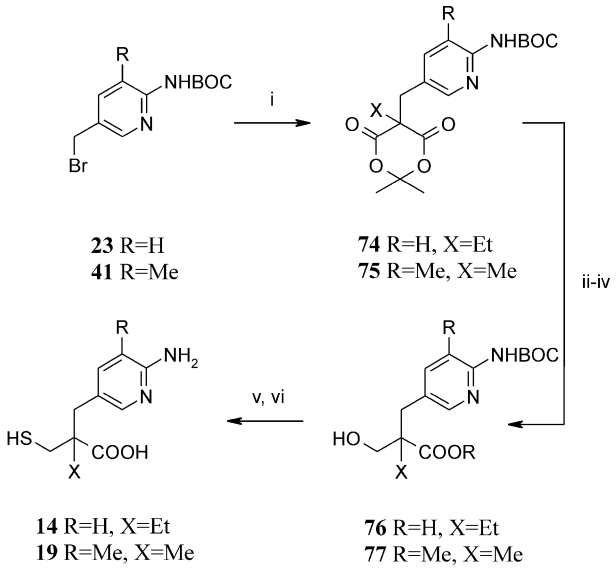
The tertiary alcohol **16** was synthesized starting from intermediate **43**. Osmiumtetroxide catalyzed dihydroxylation followed by introduction of the thioacetate using Mitsunobu conditions and subsequent deprotection gave compound **16** in good yield (Scheme 10).

Aldehyde **83** was used as starting material for the synthesis of compound **5**. A Knoevenagel reaction with diethylmalonate followed by reduction of the double bond and BOC protection of the amine gave the required intermediate. The standard procedure via Mannich and Michael sequences gave compound **5** after deprotection (Scheme 11).

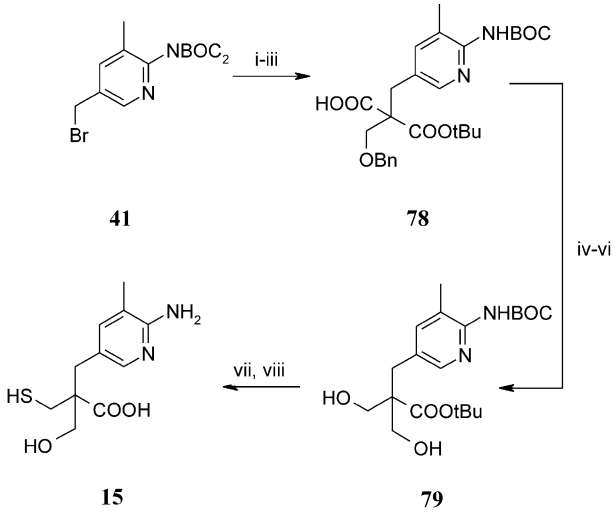
Compound **6** was synthesized using another strategy starting from pyridine **86**. Concomitant hydrogenation of the olefin and the pyridine ring gave a piperidine that was subsequently BOC protected. Carboxylic acid **87** was then alpha-alkylated using $BrCH_2SBn$ as a thiomethyl



Scheme 5. Conditions: (i) Red-Al, THF; (ii) NH_4O_2CH, Pd/C, MeOH; (iii) Boc_2O, K_2CO_3, THF/H_2O; (iv) TfCl, Et_3N, CH_2Cl_2; (v) LiBr, acetone; (vi) $(EtOCO)_2CH_2$, NaH, THF; (vii) KOH, $EtOH/CH_2Cl_2$; (viii) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (ix) Et_3N, AcSH; (x) $HCl_{(aq)}$.



Scheme 7. Conditions: (i) 5-alkyl-2,2-dimethyl-1,3-dioxane-4,6-dione, Et_3N, DMSO; (ii) NaOEt, EtOH; (iii) MeOCOCl, Et_3N, THF; (iv) $NaBH_4$, THF; (v) AcSH, DEAD, Ph_3P, THF; (vi) $HCl_{(aq)}$.

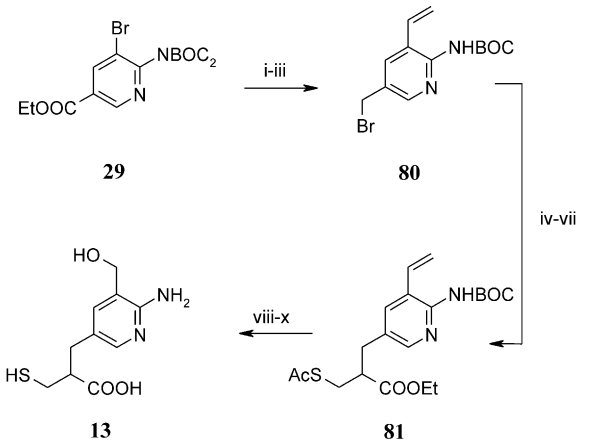


Scheme 8. Conditions: (i) *t*BuOCOCH$_2$COOEt, NaH, THF; (ii) BnOCH$_2$Cl, LDA, THF; (iii) KOH, EtOH; (iv) Bop, Et(iPr)$_2$N, THF; (v) $LiBH_4$, EtOH; (vi) H_2, Pd/C, EtOH/HCl; (vii) AcSH, DEAD, Ph_3P, THF; (viii) $HCl_{(aq)}$.

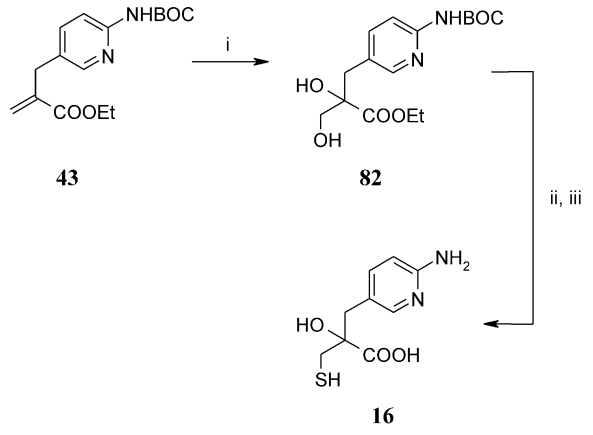
equivalent. Benzyl deprotection using sodium in liquid ammonia followed by BOC deprotection gave compound **6** (Scheme 12).

4. Conclusion

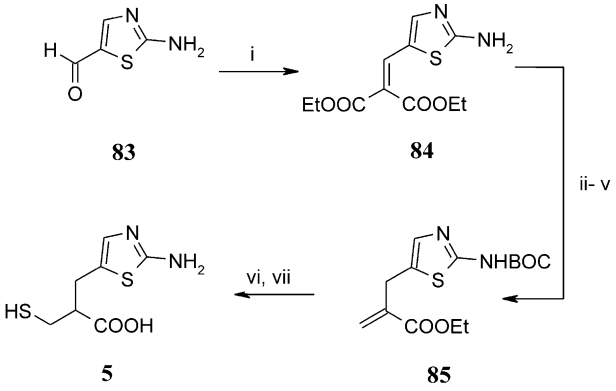
The data shown in this paper suggest that the 3-mercapto-propionic acid scaffold is useful for preparing potent inhibitors of carboxypeptidase U (TAFIa). The introduction of cyclic, low basicity guanidine mimetics, for example, 2-amino-pyridine, resulted in good oral bioavailability and several compounds show a good selectivity towards CPN.



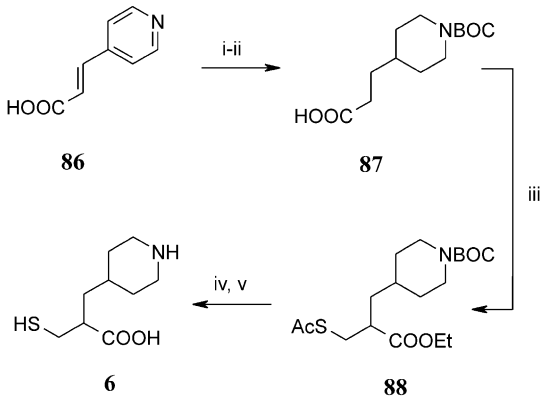
Scheme 9. Conditions: (i) $Bu_3SnCHCH_2$, $(Ph_3P)_4Pd$, 1,2-dichloroethane; (ii) DIBAL, THF; (iii) Ph_3P, CBr_4, $CHCl_2$; (iv) (EtOCO)$_2$CH$_2$, NaH, DMF; (v) KOH, EtOH/CH_2Cl_2; (vi) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (vii) Et_3N, AcSH; (viii) O_3, EtOH, then $NaBH_4$, water; (ix) Ac_2O, $KHCO_3$; (x) $HCl_{(aq)}$.



Scheme 10. Conditions: (i) OsO_4, *N*-methyl morpholine *N*-oxide, acetone/water; (ii) DEAD, Ph_3P, AcSH; (iii) $HCl_{(aq)}$.



Scheme 11. Conditions: (i) (EtOCO)$_2$CH$_2$, piperidine, MS 4A, DMF/CH_2Cl_2; (ii) $NaCNBH_3$, HCl(aq), EtOH; (iii) Boc_2O, Et_3N, DMAP, CH_2Cl_2; (iv) KOH, EtOH/THF; (v) Et_2NH, $CH_2O_{(aq)}$, CH_2Cl_2; (vi) Et_3N, AcSH; (vii) $HCl_{(aq)}$.



Scheme 12. Conditions: (i) H_2 (60 bar), Ru (5% on alumina), NH_3/H_2O; (ii) Boc_2O, $NaHCO_3$, DMAP, THF/H_2O; (iii) LDA, $BrCH_2SBn$, THF; (iv) Na, $NH_{3(l)}$, THF; (v) TFA, Et_3SiH, CH_2Cl_2.

5. Experimental

5.1. General

Mass spectra were recorded on a VG Platform II mass spectrometer equipped with an electrospray interface (LC-MS). ^{1}H NMR measurements were performed on Varian UNITY plus 400, 500 and 600 spectrometers, operating at ^{1}H frequencies of 400, 500 and 600 MHz respectively. NMR spectra were recorded in DMSO, D_2O, CD_3CN or mixtures thereof. Chemical shifts are given in ppm with the solvent as internal standard. Chromatography separations were performed using Merck Silica gel 60 (0.063–0.200 mm). The compounds named below were named using ACD/Name version 6.06/11 June 2002 available from advanced chemistry development inc., Canada.

5.2. 2-Mercaptomethyl-3-pyrrolidin-3-yl-propionic acid (3)

5.2.1. (1-Benzyl-pyrrolidin-3-yl)-methanol (66). Red-Al (160 mL of a 3.5 M solution in toluene, 560 mmol) was added to a solution of 1-benzyl-5-oxo-pyrrolidine-3-carboxylic acid (20 g, 91 mmol) in dry THF (650 mL) under argon. The reaction mixture was refluxed for 2.5 h and then poured onto a mixture of crushed ice and NaOH (20%). The phases were separated, the aqueous phase was extracted with toluene and the combined organic phases were dried and concentrated under reduced pressure to give crude **66** as a yellow oil 17.9 g. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.6–1.8 (m, 1H), 1.9–2.1 (m, 1H), 2.2–2.4 (m, 2H), 2.4–2.55 (m, 1H), 2.55–2.7 (m, 1H), 2.75–2.9 (m, 1H), 3.48–3.55 (m, 1H), 3.58 (br s, 2H), 3.63–3.7 (m, 1H), 7.14–7.35 (m, 5H).

5.2.2. 3-Hydroxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester. Pd (10% on carbon, 6.1 g) and ammonium formate (10g, 158 mmol) were added to a solution of crude **66** (6.1 g, 32 mmol) in methanol (220 mL) under argon. After reflux for 15 min the reaction mixture was filtered while warm through a pad of Celite, the Celite was further washed with methanol, and the combined organic phases were concentrated. The residue was dissolved in THF (35 mL) and water (35 mL), the solution was cooled to 0 °C and K_2CO_3 (22 g, 159 mmol) and di-*tert*-butyl dicarbonate (6.95 g, 32 mmol) were added. The reaction mixture was stirred at room temperature overnight. The THF was removed under reduced pressure, water added and the aqueous phase was extracted with EtOAc. The combined organic phases were dried and concentrated under reduced pressure to give 4.64 g of the crude product. Flash chromatography (Heptane/EtOAc: 1/0→68/32) of the crude product afforded 3-hydroxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (3.82 g, 60%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (br s, 9H), 1.55–2.05 (m, 3H), 2.3–2.5 (m, 1H), 3.0–3.2 (m, 1H), 3.2–3.7 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.6, 79.2, 64.2, 48.7, 48.2, 45.4, 45.0, 41.3, 40.5, 28.1, 27.4.

5.2.3. 3-Trifluoromethanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester. Methanesulfonyl chloride (0.4 mL, 5.17 mmol) was added dropwise to a solution of 3-hydroxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (1 g, 4.97 mmol) and triethyl amine (1.04 mL, 7.46 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. After filtration CH_2Cl_2 was added, and the organic phase was washed with 1 M HCl, dried and concentrated under reduced pressure to yield 3-trifluoromethanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (1.4 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (br s, 9H), 1.65–1.78 (m, 1H), 1.98–2.1 (m, 1H), 2.55–2.68 (m, 1H), 3.0 (s, 3H), 3.1–3.2 (m, 1H), 3.3–3.6 (m, 3H), 4.1–4.25 (m, 2H).

5.2.4. 3-Bromomethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (67). A mixture of 3-trifluoromethanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (3.85 g, 13.8 mmol) and LiBr (3.61 g, 42 mmol) in dry acetone (30 mL) was refluxed overnight. The reaction mixture was allowed to cool to room temperature, filtered and concentrated. The residue was dissolved in CH_2Cl_2 and washed with water, dried and concentrated under reduced pressure to give 6 g of the crude product. Purification by flash chromatography (Heptane/EtOAc: 1/0→68/32) afforded compound **67** (2.84 g, 78%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (br s, 9H), 1.65–1.8 (m, 1H), 2.0–2.1 (m, 1H), 2.5–2.65 (m, 1H), 3.05–3.15 (m, 1H), 3.26–3.65 (m, 5H).

5.2.5. 2-(1-*tert*-Butoxycarbonyl-pyrrolidin-3-ylmethyl)-malonic acid diethyl ester (68). Diethyl malonate (1.93 mL, 12.7 mmol) was added dropwise to a solution of NaH (60%; 0.51 g, 12.8 mmol) in dry THF (15 mL) at 0 °C. The mixture was stirred at room temperature for 1 h after which it was added to a refluxed mixture of compound **67** (2.8 g, 10.6 mmol) in dry THF (30 mL). The reaction mixture was further refluxed for 19 h, and then concentrated to almost dryness. Water (1 L) was added, and the product was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated under reduced pressure to yield 3.3 g of the crude product. Purification by flash chromatography (CH_2Cl_2/EtOAc: 1/0→68/32) afforded compound **68** (1.64 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, $J = 7$ Hz, 6H), 1.44 (br s, 9H), 1.46–1.55 (m, 1H), 1.95–2.05 (m, 3H), 2.05–2.2 (m, 1H), 2.8–2.95 (m, 1H), 3.16–3.3 (m,

1H), 3.3–3.4 (m, 1H), 3.4–3.6 (m, 2H), 4.2 (q, $J=7$ Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.1, 169.0, 154.4, 79.1, 61.5, 51, 50.8, 45.3, 36.6, 31.9, 31.1, 28.5, 14.

5.2.6. 2-(1-*tert*-Butoxycarbonyl-pyrrolidin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.26 g; 4.6 mmol) in ethanol (7 mL) was added to a solution of compound **68** (1.52 g; 4.4 mmol) in ethanol (7 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight, concentrated under reduced pressure and the residue was dissolved in water (500 mL). The aqueous layer was washed with diethyl ether, acidified to pH 3 by 0.5 M HCl, and extracted with diethyl ether. The organic phase was dried and concentrated under reduced pressure to yield 2-(1-*tert*-butoxycarbonyl-pyrrolidin-3-ylmethyl)-malonic acid monoethyl ester (1.13 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (t, $J=7$ Hz, 3H), 1.45 (br s, 9H), 1.47–1.6 (m, 1H), 1.95–2.1 (m, 1H), 2.1–2.25 (m, 3H), 2.85–2.95 (m, 1H), 3.2–3.3 (m, 1H), 3.35–3.6 (m, 3H), 4.22 (q, $J=7$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 172.5, 169.2, 169.1, 154.7, 79.6, 61.8, 51, 50.5, 45.5, 36.5, 32.0, 31.0, 28.5, 14.0.

5.2.7. *tert*-Butyl 3-[2-(ethoxycarbonyl)prop-2-en-1-yl]-pyrrolidine-1-carboxylate (69). Diethyl amine (0.34 mL; 3.3 mmol) was added to a mixture of 2-(1-*tert*-butoxycarbonyl-pyrrolidin-3-ylmethyl)-malonic acid monoethyl ester (0.69 g, 2.19 mmol) in 36% aqueous solution of formaldehyde (0.27 mL, 3.5 mmol), CH_2Cl_2 (1.6 mL) and water (1.6 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight, poured onto ice-water (500 mL) and extracted with CH_2Cl_2. The combined organic phases were washed with 5% $NaHCO_3$, dried and concentrated under reduced pressure to yield compound **69** (0.55 g, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.3 (t, $J=7$ Hz, 3H), 1.45 (br s, 9H), 1.47–1.65 (m, 1H), 1.9–2.0 (m, 1H), 2.37 (m, 3H), 2.85–3.0 (m, 1H), 3.2–3.3 (m, 1H), 3.35–3.6 (m, 2H), 4.2 (q, $J=7$ Hz, 2H), 5.55 (s, 1H), 6.18 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 154.5, 139.0, 125.9, 79, 60.7, 51.1, 50.8, 45.5, 45.1, 37.7, 36.9, 35.5, 31.4, 30.6, 28.5, 14.2.

5.2.8. 3-(3-Acetylsulfanyl-2-ethoxycarbonyl-propyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester. Thioacetic acid (5 mL), which had been cooled to 0 °C, was added to a mixture of 3-(2-ethoxycarbonyl-allyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (0.72 g; 2.54 mmol) and triethyl amine (0.37 mL; 2.67 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, at room temperature for 23 h and then poured onto ice-water (400 mL). The aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with saturated $NaHCO_3$, dried and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/EtOAc: 1/0→68/32) to yield 3-(3-acetylsulfanyl-2-ethoxycarbonyl-propyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (0.72 g, 79%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, $J=7$ Hz, 3H), 1.44 (br s, 9H), 1.53–1.7 (m, 2H), 1.73–1.85 (m, 1H), 1.95–2.1 (m, 1H), 2.1–2.25 (m, 1H), 2.32 (s, 3H), 2.53–2.65 (m, 1H), 2.8–2.9 (m, 1H), 2.95–3.15 (m, 2H), 3.2–3.3 (m, 1H), 3.4–3.5 (m, 1H), 3.5–3.6 (m, 1H), 4.16 (q, $J=7$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.1, 173.9, 173.8, 154.4, 79.0, 60.8, 51.1, 45.4, 44.6, 35.3, 35.2, 30.7, 30.6, 30.5, 28.5, 14.2.

5.2.9. 2-Mercaptomethyl-3-pyrrolidin-3-yl-propionic acid (3). A solution of 3-(3-acetylsulfanyl-2-ethoxycarbonyl-propyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (0.52 g; 1.45 mmol) in concentrated HCl (15 mL) was refluxed under argon for 1 h. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure to afford a diastereomeric mixture of compound **3** as the hydrochloride salt (0.33 g; 100%). ^{1}H NMR (400 MHz, D_2O): δ 1.6–1.95 (m, 3H), 2.2–2.5 (m, 2H), 2.65–3.0 (m, 4H), 3.25–3.35 (m, 1H), 3.4–3.6 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ 178.8, 50.2, 50.1, 48.1, 45.5, 36.0, 33.8, 30.2, 29.9, 25.8, 25.6. MS (+) 190 (M+1). HRMS (ESI) calcd for $C_8H_{15}NO_2S$ 189.0824, found 189.0850.

5.3. 3-(*cis*-4-Amino-cyclopent-2-enyl)-2-mercaptomethyl-propionic acid (4)

5.3.1. *cis*-Methanesulfonic acid 4-*tert*-butoxycarbonyl-amino-cyclopent-2-enylmethyl ester. Methanesulfonyl chloride (0.76 mL, 9.8 mmol) was added to a solution of compound **62** (2 g, 9.4 mmol) and triethyl amine (1.96 mL, 14.1 mmol) in CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. After filtration CH_2Cl_2 was added, and the organic phase was washed with 1 M HCl, dried and concentrated under reduced pressure to yield *cis*- methanesulfonic acid 4-*tert*-butoxycarbonylamino-cyclopent-2-enylmethyl ester (2.64 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.3–1.4 (m, 1H), 1.44 (s, 9H), 2.53–2.65 (m, 1H), 3.0 (br s, 4H), 4.12–4.22 (m, 2H), 4.55–4.8 (m, 2H), 5.75–5.85 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.1, 134.7, 132.4, 79.4, 72.0, 56.0, 44.0, 37.4, 34.7, 28.4.

5.3.2. *cis*-(4-Bromomethyl-cyclopent-2-enyl)-carbamic acid *tert*-butyl ester. A mixture of *cis*-methanesulfonic acid 4-*tert*-butoxycarbonylamino-cyclopent-2-enylmethyl ester (2.51 g, 8.6 mmol) and LiBr (2.24 g, 25.8 mmol) in dry acetone (20 mL) was refluxed overnight. The reaction mixture was allowed to cool to room temperature, filtered and concentrated. The residue was dissolved in CH_2Cl_2 and washed with water, dried and concentrated under reduced pressure to give *cis*-(4-bromomethyl-cyclopent-2-enyl)-carbamic acid *tert*-butyl ester (2.23 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26–1.36 (m, 1H), 1.45 (s, 9H), 2.55–2.68 (m, 1H), 3.02–3.13 (m, 1H), 3.36–3.48 (m, 2H), 4.5–4.8 (m, 2H), 5.74–5.83 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.1, 134.6, 133.9, 79.3, 56.1, 46.3, 38.2, 37.1, 28.4.

5.3.3. 2-(*cis*-4-*tert*-Butoxycarbonylamino-cyclopent-2-enylmethyl)-malonic acid diethyl ester (63). Diethyl malonate (1.29 mL, 8.5 mmol) was added to a mixture of NaH (60%, 0.34 g; 8.5 mmol) in DMF (10 mL). After stirring at room temperature for 15 min a solution of *cis*-(4-bromomethyl-cyclopent-2-enyl)-carbamic acid *tert*-butyl ester (1.95 g, 7.1 mmol) in DMF (12 mL) was added, and the reaction mixture was stirred at 60 °C for 19 h. EtOAc was added and the organic phase was

extracted with water and brine, dried and concentrated under reduced pressure to give 2.44 g of the crude product. Purification by flash chromatography (CH_2Cl_2/EtOAc: 1/0→68/32) afforded of compound **63** (1.47 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05–1.15 (m, 1H), 1.24–1.30 (m, 6H), 1.44 (s, 9H), 1.85–1.95 (m, 1H), 2.02–2.12 (m, 1H), 2.53–2.65 (m, 2H), 3.34–3.40 (m, 1H), 4.15–4.25 (m, 4H), 4.5–4.75 (m, 2H), 5.65–5.70 (m, 1H), 5.75–5.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 155.1, 136.3, 132.4, 79.2, 61.4, 56.3, 50.4, 42.2, 38.4, 35.1, 28.4, 14.0.

5.3.4. 2-(*cis*-4-*tert*-Butoxycarbonylamino-cyclopent-2-enylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.19 g; 3.4 mmol) in ethanol (6 mL) was added to a solution of compound **63** (1.15 g; 3.2 mmol) in ethanol (6 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight, concentrated and ice-water (400 mL) was added. The aqueous phase was washed with diethyl ether (the emulsion formed during the extraction was treated with brine in order to get good phase separation), acidified to pH 3 with 0.5 M HCl, and extracted with diethyl ether. The organic phase was dried and concentrated under reduced pressure to afford 2-(*cis*-4-*tert*-butoxycarbonylamino-cyclopent-2-enylmethyl)-malonic acid monoethyl ester (0.86 g, 81%) as white crystals. ^{1}H NMR (500 MHz, dioxane-d_8): δ 1.0–1.1 (m, 1H), 1.22–1.27 (m, 3H), 1.41 (s, 9H), 2.4–2.65 (m, 3H), 3.39–3.45 (m, 1H), 4.12–4.20 (m, 2H), 4.55–4.65 (m, 1H), 5.64–5.70 (m, 1H), 5.74–5.80 (m, 1H), 5.80–5.88 (m, 1H). ^{13}C NMR (100 MHz, dioxane-d_8): δ 170.5, 169.7, 155.4, 136.3, 133.5, 78.4, 61.4, 56.9, 50.5, 42.9, 38.9, 35.9, 28.5, 14.1.

5.3.5. 2-(*cis*-4-*tert*-Butoxycarbonylamino-cyclopent-2-enylmethyl)-acrylic acid ethyl ester (64). Diethyl amine (0.31 mL; 3.0 mmol) was added to a mixture of 2-(*cis*-4-*tert*-butoxy-carbonylamino-cyclopent-2-enylmethyl)-malonic acid monoethyl ester (0.66 g, 2.0 mmol) in 36% aqueous solution of formaldehyde (0.25 mL, 3.2 mmol), CH_2Cl_2 (1.6 mL) and water (1.6 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight, poured onto ice-water (400 mL) and extracted with CH_2Cl_2. During the extraction, phase separation was improved by the addition of brine. The combined organic phases were washed with 5% $NaHCO_3$, dried and concentrated under reduced pressure to yield compound **64** (0.56 g, 94%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08–1.17 (m, 1H), 1.26–1.32 (m, 3H), 1.43 (s, 9H), 2.28–2.60 (m, 3H), 2.75–2.88 (m, 1H), 4.15–4.24 (m, 2H), 4.45–4.75 (m, 2H), 5.52 (br s, 1H), 5.62–5.70 (m, 1H), 5.75–5.82 (m, 1H), 6.17 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 155.2, 139.1, 137.0, 131.8, 125.7, 79.2, 60.7, 56.3, 43.0, 38.6, 38.2, 28.4, 14.2.

5.3.6. 2-Acetylsulfanylmethyl-3-(*cis*-4-*tert*-butoxycarbonylamino-cyclopent-2-enyl)-propionic acid ethyl ester. Thioacetic acid (4 mL), which had been cooled to 0 °C, was added to a mixture of 2-(*cis*-4-*tert*-butoxycarbonylamino-cyclopent-2-enylmethyl)-acrylic acid ethyl ester (0.56 g, 1.9 mmol) and triethyl amine (0.28 mL, 2.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, at room temperature for 19 h and then poured onto ice-water (400 mL). The aqueous layer was extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$, dried and concentrated under reduced pressure to give 1.7 g of the crude product. Purification by flash chromatography (CH_2Cl_2/EtOAc: 1/0→68/32) afforded acetylsulfanylmethyl-3-(*cis*-4-*tert*-butoxycarbonylamino-cyclopent-2-enyl)-propionic acid ethyl ester (0.46 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04–1.15 (m, 1H), 1.24–1.31 (m, 3H), 1.46 (s, 9H), 1.60–1.95 (m, 2H), 2.34 (s, 3H), 2.55–2.72 (m, 3H), 3.01 (dd, J_{AB} = 13.5 Hz, J_{AX} = 9 Hz, 1H), 3.14 (dd, J_{AB} = 13.5 Hz, J_{AX} = 5 Hz, 1H), 4.10–4.22 (m, 2H), 4.5–4.8 (m, 2H), 5.65–5.71 (m, 1H), 5.75–5.83 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.2, 174.1, 155.1, 136.8, 136.5, 132.3, 132.1, 79.2, 60.8, 56.4, 44.3, 44.2, 42.2, 38.8, 38.7, 30.8, 30.7, 30.5, 28.4, 14.2, 14.1.

5.3.7. 3-(*cis*-4-Amino-cyclopent-2-enyl)-2-mercaptomethyl-propionic acid (4). A solution of 2-acetylsulfanylmethyl-3-(*cis*-4-*tert*-butoxycarbonylamino-cyclopent-2-enyl)-propionic acid ethyl ester (86 mg, 0.23 mmol) in concentrated HCl (5 mL) was refluxed under argon for 1 h. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure to afford a diasteromeric mixture of compound **4** as the hydrochloride salt (65 mg, 100%). ^{1}H NMR (400 MHz, D_2O): δ 1.35–1.47 (m, 2H), 1.60–1.72 (m, 1H), 1.74–1.92 (m, 2H), 1.92–2.04 (m, 1H), 2.67–2.92 (m, 10H), 4.34–4.43 (m, 2H), 5.79–5.85 (br s, 2H), 6.12 (d, 1H), 6.18 (d, 1H). ^{13}C NMR (100 MHz, D_2O): δ 179.3, 141.6, 141.4, 127.0, 126.9, 56.9, 48.2, 48.0, 43.0, 42.9, 37.4, 37.2, 35.0, 25.9, 25.6. HRMS (ESI) calcd for $C_9H_{15}NO_2S$ 201.0824, found 201.0854.

5.4. 3-(2-Amino-thiazol-5-yl)-2-mercaptomethyl-propionic acid (5)

5.4.1. 2-(2-Amino-thiazol-5-ylmethylene)-malonic acid diethyl ester (84). To a solution of 2-amino-thiazole-5-carbaldehyde (6 g, 23 mmol) in CH_2Cl_2 (30 mL) and DMF (30 mL), was added 4A molecular sieves, diethyl malonate (3.5 mL, 23 mmol), piperidine (1.1 mL, 11.5 mmol) and acetic acid (0.7 mL, 11.5 mmol). The reaction mixture was stirred at room temperature for 96 h. Then EtOAc was added, and the reaction mixture was filtered through Celite in order to remove the precipitate formed. EtOAc (500 mL) was added to the filtrate, and the organic phase was washed with $NaHCO_3$ and brine. The organic phase was dried and concentrated to yield 4.9 g of the crude product. Addition of petroleum ether to a solution of the crude product in ethanol followed by filtration afforded compound **84** (1.13 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.38 (m, 6H), 4.2–4.35 (m, 4H), 7.43 (s, 1H), 7.73 (s, 1H).

5.4.2. 2-(2-Amino-thiazol-5-ylmethyl)-malonic acid diethyl ester. $NaCNBH_3$ (1.88 g; 29.9 mmol) was added to a stirred solution of compound **84** (1.13 g, 4.2 mmol) in ethanol at 0 °C. The pH of the solution was monitored by addition of a small amount of bromocresol Green to the solution. Concentrated HCl was added dropwise until the solution turned yellow. The ice bath was removed, and the reaction mixture was stirred at room

temperature for 5 h. Water was added and the product was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to yield 2-(2-amino-thiazol-5-ylmethyl)-malonic acid diethyl ester (1 g, 87.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.30 (m, 6H), 3.24 (d, J = 7 Hz, 2H), 3.56 (t, J = 7 Hz, 1H), 4.14–4.26 (m, 4H), 6.81 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 167.5, 136.6, 123.6, 61.7, 53.5, 26.3, 14.0.

5.4.3. 2-(2-*tert*-Butoxycarbonylamino-thiazol-5-ylmethyl)-malonic acid diethyl ester. Di-*tert*-butyl-dicarbonate (0.6 g, 27.5 mmol) was added to a solution of triethyl amine (0.4 mL; 30.1 mmol), 4-(dimethylamino)pyridine (0.34 g, 27.8 mmol) and 2-(2-amino-thiazol-5-ylmethyl)-malonic acid diethyl ester (0.75 g, 27.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. Additional di-*tert*-butyl-dicarbonate (0.15 g, 0.7 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. CH_2Cl_2 was added, and the organic phase was extracted with 0.3 M $KHSO_4$ and brine. The organic phase was dried and concentrated to yield 0.78 g of the crude product. NMR indicated that approximately 40% of 2-(2-amino-thiazol-5-ylmethyl)-malonic acid diethyl ester remained. Di-*tert*-butyl-dicarbonate (0.6 g, 27.5 mmol) was added to a solution of the crude product (0.78 g), triethyl amine (0.4 mL, 30.1 mmol), 4-(dimethylamino)pyridine (0.34 g, 27.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight and the work up procedure was repeated. The crude product (1 g) was purified by flash chromatography (Heptane/EtOAc, 1:1) and HPLC to afford 2-(2-*tert*-butoxycarbonylamino-thiazol-5-ylmethyl)-malonic acid diethyl ester (184 mg, 17.9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.28 (m, 6H), 1.56 (s, 9H), 3.31 (d, J = 7 Hz, 2H), 3.59 (t, J = 7 Hz, 1H), 4.13–4.26 (m, 4H), 7.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.1, 161.1, 152.8, 134.7, 126.4, 81.7, 61.6, 53.4, 28.2, 25.9, 13.9.

5.4.4. 2-(2-*tert*-Butoxycarbonylamino-thiazol-5-ylmethyl)-malonic acid monoethyl ester. 2-(2-*tert*-Butoxy-carbonylamino-thiazol-5-ylmethyl)-malonic acid diethyl ester (158 mg, 0.43 mmol) was dissolved in ethanol (1 mL) and THF (0.5 mL), and a solution of KOH (24 mg, 0.43 mmol) in ethanol (0.14 mL) was added at 0 °C. The reaction mixture was stirred at room temperature for 96 h, and then poured onto ice-water. The aqueous phase was extracted with diethyl ether, acidified to pH 3 by addition of 0.5 M HCl, and extracted with diethyl ether. The combined organic phases were dried and concentrated to yield 2-(2-*tert*- butoxycarbonylamino-thiazol-5-yl-methyl)-malonic acid monoethyl ester (97 mg, 66.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, J = 7 Hz, 3H), 1.54 (s, 9H), 3.23–3.42 (m, 2H), 3.57–3.68 (m, 1H), 4.23 (q, J = 7 Hz, 2H), 7.02 (s, 1H).

5.4.5. 2-(2-*tert*-Butoxycarbonylamino-thiazol-5-ylmethyl)-acrylic acid ethyl ester (85). To a mixture of 2-(2-*tert*-butoxycarbonylamino-thiazol-5-ylmethyl)-malonic acid monoethyl ester (94 mg, 0.27 mmol), a 36% aqueous solution of formaldehyde (36 μL, 1.2 mmol), CH_2Cl_2 (0.2 mL) and water (0.2 mL) was added at 0 °C diethyl amine (30 μL, 0.40 mmol). The reaction mixture was stirred at room temperature overnight, poured onto ice-water and extracted with CH_2Cl_2. The combined organic phases were washed with 5% $NaHCO_3$, dried and concentrated to yield compound **85** (69 mg, 80.9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (t, J = 7 Hz, 3H), 1.43 (s, 9H), 3.72 (s, 2H), 4.21 (q, J = 7 Hz, 2H), 5.63 (s, 1H), 6.26 (s, 1H), 7.08 (s, 1H).

5.4.6. 2-Acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonyl-amino-thiazol-5-yl)-propionic acid ethyl ester. Triethyl amine (32 μL, 0.23 mmol) was added to a solution of compound **85** (67 mg; 0.21 mmol) in thioacetic acid (0.4 mL) at 0 °C. The reaction mixture was stirred at room temperature for 48 h, and then poured onto ice-water. The aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with saturated $NaHCO_3$, dried and concentrated to yield 190 mg of the crude product. The crude product was purified by flash chromatography (Heptane/EtOAc, 1:0→68:32) to yield 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonyl-amino-thiazol-5-yl)-propionic acid ethyl ester (43 mg, 51.6%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, J = 7 Hz, 3H), 1.56 (s, 9H), 2.33 (s, 3H), 2.82–2.92 (m, 1H), 2.98–3.20 (m, 4H), 4.14 (q, J = 7 Hz, 2H), 7.05 (s, 1H).

5.4.7. 3-(2-Amino-thiazol-5-yl)-2-mercaptomethyl-propionic acid (5). A solution of 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-thiazol-5-yl)-propionic acid ethyl ester (43 mg, 0.11 mmol) in concentrated HCl (1.5 mL) was refluxed under argon for 1.5 h. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure to yield 30 mg of the crude product. The crude product was purified by preparative HPLC to afford the title compound **5** (8 mg, 21%) as the hydrochloride salt. ^{1}H NMR (500 MHz, D_2O): δ 2.75–3.1 (m, 5H), 7.0 (br s, 1H). MS (+) 219 (M + 1). HRMS (ESI) calcd for $C_7H_{10}N_2O_2S_2$ 218.0184, found 218.0237.

5.5. 2-Mercaptomethyl-3-piperidin-4-yl-propionic acid (6)

5.5.1. 3-Piperidin-4-yl-propionic acid. A solution of 3-pyridin-4-yl-acrylic acid (**86**) (4.20 g, 28.0 mmol) in water (50 mL) and ammonia (aq, 25%, 4 mL) was hydrogenated at 60 bar in a high-pressure steel autoclave in presence of ruthenium (5% on alumina, 439 mg). When hydrogen pressure remained constant (3 days) the catalyst was removed from the reaction mixture by filtration. The catalyst was washed with ethanol and water, and the ethanol was removed from the solution on a rotavapor and the aqueous solution was freeze dried to give 3-piperidin-4-yl-propionic acid (4.30 g, 100%). ^{1}H NMR (300 MHz, D_2O): δ 1.25 (m, 2H), 1.47 (m, 3H), 1.82 (m, 2H), 2.13(m, 2H), 2.80 (m, 2H), 3.25 (m, 2H).

5.5.2. 4-(2-Carboxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (87). A solution of 3-piperidin-4-yl-propionic acid (4.79 g, 30.5 mmol), di-*tert*-butyl-dicarbonate (6.98 g, 32.0 mmol) and $NaHCO_3$ (2.69 g, 32.0 mmol) in THF/water (1:1, 50 mL) were stirred at room temperature for 22 h. Another portion of di-*tert*-butyl-dicarbonate (2.00 g, 9.10 mmol) was added together with a catalytic amount of DMAP, the resulting mixture was stirred for

another four days. THF was removed under reduced pressure and the aqueous phase was extracted with CH_2Cl_2. The aqueous was then acidified to pH 2 with 1M HCl and the acid extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried and concentrated in vacuo to yield compound **87** as a white solid (6.36 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.0–1.2 (m, 2H), 1.45 (s + m, 10H), 1.6 (m, 4H), 2.39 (m, 2H), 2.65 (m, 2H), 4.10 (m, 2H).

5.5.3. 4-(3-Benzylsulfanyl-2-carboxy-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (88). BuLi (1.6 M, 15.3 mL, 24.4 mmol) was added to a solution of diisopropylamine (3.43 mL, 24.4 mmol) in THF (3 mL) at −78 °C under argon. After a few min the solution was allowed to warm up to room temperature over a period of 15 min. The resulting LDA solution was slowly added to a solution of compound **87** (3.07 g, 11.9 mmol) in THF (7 mL) at −78 °C. The resulting solution was stirred at −78 °C for 10 min, THF (20 mL) was added during that time in order to dissolve the anion, which had solidified. The dianion was cooled to −7 8 °C and bromomethyl thiobenzylether (2.72 g, 12.5 mmol) was added as a solution in THF (3 mL), the solution was stirred at −78 °C for 30 min, at 0 °C for 30 min and then allowed to warm up to room temperature and stirred overnight. The reaction mixture was acidified with 1 M HCl, diluted with EtOAc and the organic phase was washed with water and dried. The crude product was purified by flash chromatography (MeOH/$CHCl_3$, 1:9) to yield compound **88** as a pale yellow oil (3.12 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.9–1.1 (m, 2H), 1.40 (m, 1H), 1.45 (s, 9H), 1.60 (m, 3H), 2.45 (m, 1H), 2.65 (m, 4H), 3.70 (s, 2H), 4.05 (bs, 2H), 7.27 (m, 5H).

5.5.4. 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester. Sodium metal (513 mg, 22.5 mmol) was added in portions during 5 min to a solution of compound **88** (0.9 g, 2.29 mmol) in THF (45 mL) and liquid ammonia (50 mL) at −60 °C under argon. After stirring for 15 min ammonium chloride (1.7 g, 31.5 mmol) was added in portions. The cooling bath was removed and the ammonia was evaporated using a stream of argon. 0.5 M NaOH was added and the mixture was washed with heptane. The aqueous phase was acidified with 2 M HCl and extracted with methylene chloride. The organic phase was washed with brine, dried and concentrated under reduced pressure to give crude 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.7 g, 100%). This material was used directly in the next step.

5.5.5. 2-Mercaptomethyl-3-piperidin-4-yl-propionic acid (6). To a solution of crude 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.7 g, 2.29 mmol) in methylene chloride (8 mL) under argon was added triethylsilane (731 μL, 4.58 mmol) followed by TFA (4 mL). The reaction mixture was stirred for 60 min and then concentrated under reduced pressure. Purification by HPLC (10→30% acetonitrile, 0.1% TFA in water) gave compound **6** as the TFA salt (447 mg, 61%). ^{1}H NMR (400 MHz, D_2O): δ 1.34–1.50 (m, 2H), 1.54–1.76 (m, 3H), 1.90–1.99 (m, 1H), 2.0–2.1 (m, 1H), 2.9–3.05 (m, 5H), 3.38–3.48 (m, 2H). MS (+) 204 (M + 1).

5.6. 2-(2-Amino-pyridin-4-ylmethyl)-3-mercapto-propionic acid (7)

5.6.1. 2-Amino-isonicotinic acid ethyl ester. 2-Acetylamino-isonicotinic acid (56) (10.8 g, 60.0 mmol) was suspended in ethanol (150 mL) and $BF_3{\cdot}OEt_2$ (22 mL, 138 mmol) was added. The mixture was refluxed overnight, and after cooling to room temperature 10% $NaHCO_3$ (250 mL) was added. The product was extracted with chloroform and the combined organic extracts were washed with water and dried. Filtering and concentration afforded 2-amino-isonicotinic acid ethyl ester (7.46 g, 79%) as pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 3H), 4.35 (q, 2H), 4.60 (bs, 2H), 7.05 (m, 1H), 7.15 (m, 1H), 8.15 (m, 1H).

5.6.2. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-isonicotinic acid ethyl ester (57). To a solution of 2-amino-isonicotinic acid ethyl ester (5.00 g, 30 mmol) in *t*-BuOH (45 mL) and acetone (15 mL) was added DMAP (50 mg, 0.41 mmol) and di-*t*-butyl dicarbonate (16.4 g, 75.0 mmol). The reaction was stirred at room temperature overnight and hexane (60 mL) was added. The reaction mixture was cooled in a refrigerator for 3 h and filtered to give compound **57** (8.71 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 3H), 1.50 (s, 18H), 4.40 (q, 2H), 7.75 (m, 1H), 7.80 (m, 1H), 8.60 (m, 1H).

5.6.3. (4-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (58). A solution of to compound **57** (35.0 g, 95.5 mmol) in THF (350 mL) was treated with $LiAlH_4$ (7.25 g, 191 mmol) and refluxed for 1 h under nitrogen. The reaction mixture was poured carefully onto crushed ice and the product extracted several times with $CHCl_3$ and $CHCl_3$:MeOH (9:1). The combined organic extracts were dried, filtered and concentrated under reduced pressure to give compound **58** (18.5 g, 86%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H), 4.60 (s, 2H), 7.00 (m, 1H), 7.90 (m, 1H), 8.25 (m, 1H), 8.60 (bs, 1H).

5.6.4. (4-Bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (59). Compound **58** (8.00 g, 35.6 mmol) was dissolved in CH_2Cl_2 (150 mL) and treated with PPh_3 (11.2 g, 42.8 mmol) under nitrogen. The reaction flask was cooled in an ice bath and CBr_4 (14.2 g, 42.8 mmol) was added in small portions. The ice bath was removed after 30 min and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and acetonitrile (50 mL) was added. The reaction flask was placed in a refrigerator for 3 h and the precipitate filtered and washed with cold acetonitrile. The white solid was dried in vacuo giving compound **59** (8.38 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H), 4.40 (s, 2H), 7.00 (m, 1H), 8.05 (m, 1H), 8.30 (m, 1H), 8.90 (bs, 1H).

5.6.5. 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid diethyl ester (60). To a solution of NaH (80%, 0.17 g, 4.00 mmol) in THF (5 mL) at 0 °C under

argon was added diethyl malonate (0.64 g, 4.00 mmol). After the mixture was stirred for 15 min the mixture was added to a refluxed mixture of compound **59** (1.00 g, 3.48 mmol) in THF (10 mL), and the mixture was refluxed for 2 h. The mixture was concentrated under reduced pressure and the residue was partitioned between water and chloroform. The organic layer was washed with water and brine and dried. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (MeOH/ CH_2Cl_2, 1:100) to give compound **60** (0.80 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 6H), 1.55 (s, 9H), 3.22 (d, 2H), 3.70 (t, 1H), 4.20 (q, 4H), 6.90 (d, 1H), 7.88 (s, 1H), 8.19 (d, 1H), 8.42 (bs, 1H).

5.6.6. 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.19 g, 3.43 mmol) in ethanol (5 mL) was added to a solution of compound **60** (1.20 g, 3.27 mmol) in ethanol (5 mL) and methylene chloride (5 mL) at 0 °C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and water was added to the residue. The aqueous layer was washed with diethyl ether, acidified to pH 4 by 1M HCl, and extracted with methylene chloride. The organic layer was washed with water, brine and dried. After filtration and evaporation in vacuo, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:20) to yield 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester (0.90 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.55 (s, 9H), 3.22 (m, 2H), 3.78 (t, 1H), 4.21 (q, 2H), 6.95 (d, 1H), 8.05 (m, 2H), 9.00 (bs, 1H).

5.6.7. 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-acrylic acid ethyl ester (61). A solution of diethylamine (0.26 g, 2.67 mmol) in methylene chloride (4 mL) was added to a mixture of 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester (0.90 g, 2.66 mmol) and 37% aq solution of formaldehyde (0.24 g, 3.00 mmol) at 0 °C. The mixture was stirred for 5 h at room temperature and the mixture was poured onto ice-water and extracted with methylene chloride. The organic layer was washed with 5% $NaHCO_3$ and dried. The crude product was purified by flash chromatography (1% methanol in CH_2Cl_2) to yield compound **61** (0.58 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.55 (s, 9H), 3.62 (s, 2H), 4.20 (q, 2H), 5.55 (s, 1H), 6.30 (s, 1H), 6.80 (d, 1H), 7.85 (s, 1H), 8.10 (d, 1H), 8.65 (bs, 1H).

5.6.8. 2-Acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonyl-amino-pyridin-4-yl)-propionic acid ethyl ester. A solution of compound **61** (0.48 g, 1.57 mmol) and triethylamine (0.17 g, 1.64 mmol) was added to thioacetic acid (3 mL) at 0 °C under nitrogen. The mixture was stirred at room temperature for 4 h. The mixture was poured onto ice-water and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ (aq) and dried. The crude product was purified by flash chromatography (2.5% MeOH in CH_2Cl_2) to give 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-pyridin-4-yl)-propionic acid ethyl ester (0.60 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (t, 3H), 1.51 (s, 9H), 2.30 (s, 3H), 2.95 (m, 3H), 3.09 (m, 2H), 4.10 (q, 2H), 6.70 (d, 1H), 7.78 (s, 1H), 8.15 (d, 1H), 8.30 (s, 1H).

5.6.9. 2-Acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester. TFA (0.5 mL) was added to a solution of 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-pyridin-4-yl)-propionic acid ethyl ester (50 mg, 0.13 mmol) in methylene chloride under argon. The solution was stirred for 60 min and concentrated under reduced pressure to give crude 2-acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester (52 mg, 100%). ^{1}H NMR (500 MHz, CD_3OD_3): δ 1.15 (t, 3H), 2.32 (s, 3H), 2.73–2.83 (m, 2H), 2.86–2.93 (m, 1H), 3.01–3.07 (dd, 1H), 3.12–3.18 (dd, 1H), 4.03–4.12 (m, 2H), 6.39 (s, 1H), 6.43 (d, 1H), 7.77 (d, 1H).

5.6.10. 2-(2-Amino-pyridin-4-ylmethyl)-3-mercapto-propionic acid (7). 2-Acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester (52 mg, 0.13 mmol) was dissolved in concentrated HCl (2 mL) under argon. The solution was heated to reflux for 1 h. Concentration under reduced pressure gave compound **7** as the hydrochloride salt (32 mg, 100%). ^{1}H NMR (500 MHz, CD_3OD): δ 2.70 (bs, 2H), 2.85–3.0 (m, 3H), 6.76 (bs, 1H), 6.81 (bs, 1H), 7.67 (bs, 1H). MS (+) 213 (M + 1).

5.7. 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-propionic acid (8)

5.7.1. 6-Amino-nicotinic acid ethyl ester. 2-Amino-5-pyridinecarboxylic acid (**20**) (25.0 g, 181 mmol) was suspended in ethanol (190 mL) and $SOCl_2$ (15 mL, 206 mmol) was added. The mixture was refluxed for 10 h and more $SOCl_2$ (16 mL) was added. After 3 days with reflux (and more $SOCl_2$ (10 mL) added each day), the reaction mixture was cooled to room temperature and diethyl ether was added. After 24 h at −20 °C the mixture was filtered. The crude salt was dissolved in methanol (214 mL) and a solution of NaOH (40.0 g, 23.5 mmol) in methanol (90 mL) was added. The reaction mixture was stirred for 1 h and THF (270 mL) was added. The reaction mixture was filtered through a plug of silica (THF/MeOH) and concentrated under reduced pressure to give 6-amino-nicotinic acid ethyl ester (36.2 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 3H), 4.35 (q, 2H), 4.60 (bs, 2H), 7.05 (m, 1H), 7.15 (m, 1H), 8.15 (m, 1H).

5.7.2. 6-*tert*-Butoxycarbonylamino-nicotinic acid ethyl ester (21). To a solution of 6-amino-nicotinic acid ethyl ester (36.0 g, 217.0 mmol) in *t*-BuOH (308 mL) and acetone (103 mL) was added DMAP (0.53 g, 4.34 mmol) and di-*t*-butyl dicarbonate (72.0 g, 330 mmol). The reaction was stirred at room temperature for 10 h followed by addition of more di-*t*-butyl dicarbonate (2.60 g). After 10 h stirring at room temperature hexane (470 mL) was added. The reaction mixture was cooled to −20 °C for 2 h and filtered. The filtrate was washed with hexane/dichloromethane (3:1) and dried in vacuo to give compound **21** (40.5 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 3H), 1.50 (s, 18H), 4.40 (q, 2H), 7.75 (m, 1H), 7.80 (m, 1H), 8.60 (m, 1H).

5.7.3. (5-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (22). To a stirred solution of compound **21** (3.50 g, 13.1 mmol) in THF (20 mL) under nitrogen was added $LiAlH_4$ (0.91 g, 24.0 mmol) in THF (20 mL) over a period of 2 h. The reaction mixture was stirred for 6 h, and then NH_4Cl (satd) was added (carefully) until neutral solution. The mixture was filtered and concentrated under reduced pressure to give compound **22** (2.00 g, 68%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.52 (s, 9H), 4.62 (s, 2H), 7.68 (d, 1H), 7.98 (d, 1H), 8.19 (bs, 1H), 8.29 (s, 1H).

5.7.4. (5-Bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (23). Triphenylphosphine (8.70 g, 33.1 mmol) and carbontetrabromide (17.0 g, 51.2 mmol) were added to a suspension of compound **22** (7.00 g, 31.2 mmol) in CH_2Cl_2 (200 mL) at room temperature. Stirring was continued for 5 h followed by evaporation of the solvent. Acetonitrile (200 mL) was added and the mixture was cooled to −20 °C for 2 h. The mixture was then filtered and the crystalline residue washed with cold acetonitrile (2×10 mL), to give compound **23** (5.96 g, 67%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 4.41 (s, 2H), 7.68 (d, 1H), 7.98 (d, 1H), 8.32 (s, 1H), 9.00 (bs, 1H).

5.7.5. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid diethyl ester (42). To a suspension of NaH (0.49 g, 16.3 mmol, 80%) in THF (15 mL) at 0 °C was added diethyl malonate (2.61 g, 16.3 mmol). The mixture was stirred for 15 min and was then added dropwise to a refluxed mixture of compound **23** (3.90 g, 13.6 mmol) in THF (25 mL), and the resulting solution was refluxed for 15 min. After evaporation of the solvent, the crude product was purified by flash chromatography (methanol/CH_2Cl_2, 1:100→2.5:100) to give compound **42** (2.18 g, 44%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.20 (t, 6H), 1.52 (s, 9H), 3.15 (d, 2H), 3.55 (t, 1H), 4.15 (q, 4H), 7.55 (d, 1H), 7.70 (bs, 1H), 7.90 (d, 1H), 8.10 (s, 1H).

5.7.6. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.37 g, 6.54 mmol) in ethanol (5 mL) was added to a solution of compound **42** (2.18 g, 5.95 mmol) in ethanol (25 mL) and methylene chloride (10 mL) at 0 °C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. The aqueous layer was washed with ether, acidified to pH 4 by 1M HCl and extracted with methylene chloride. The organic layer was washed with water, brine and dried. After filtration and concentration under reduced pressure, the crude product was purified by flash chromatography (methanol/CH_2Cl_2, 1:20) to yield 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.00 g, 50%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, 3H), 1.52 (s, 9H), 3.10 (m, 2H), 3.55 (t, 1H), 4.25 (q, 2H), 7.55 (d, 1H), 7.85 (m, 1H), 8.05 (m, 1H), 9.20 (bs, 1H).

5.7.7. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-acrylic acid ethyl ester (43). Diethylamine (0.29 g, 3.00 mmol), water (2 mL) and methylene chloride (2 mL) was added to a mixture of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.00 g, 2.96 mmol) and 37% aq solution of formaldehyde (0.25 g, 3.05 mmol) at 0 °C. The mixture was stirred for 16 h at room temperature and then poured onto ice-water and extracted with methylene chloride. The organic layer was washed with 5% $NaHCO_3$ and dried. Filtration and concentration under reduced pressure gave compound **43** (0.75 g, 83%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.52 (s, 9H), 3.55 (s, 2H), 4.15 (q, 2H), 5.45 (s, 1H), 6.22 (s, 1H), 7.50 (d, 1H), 7.85 (d, 1H), 8.10 (s, 1H), 8.20 (bs, 1H).

5.7.8. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester. Compound **43** (0.49 g, 1.60 mmol) and triethylamine (0.17 g, 1.64 mmol) were added to thioacetic acid (3 mL) at 0 °C. The mixture was stirred at room temperature for 6 h. The mixture was poured onto ice-water and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ and dried. The crude product was purified by flash chromatography (MeOH/CH_2Cl_2, 2.5:100) to give 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester (0.36 g, 61%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.52 (s, 9H), 2.30 (s, 3H), 2.85 (m, 3H), 3.09 (m, 2H), 4.05 (q, 2H), 7.45 (d, 1H), 7.90 (d, 1H), 8.09 (s, 1H), 8.50 (s, 1H).

5.7.9. 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-propionic acid (8). 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester (38 mg, 0.096 mmol) was dissolved in concd HCl (2.0 mL) under argon. The solution was stirred at room temperature for 1 h and then heated to reflux for 1 h. Concentration under reduced pressure gave compound **8** (25.7 mg, 100%) as the hydrochloride salt. ^1H NMR (500 MHz, CD_3OD): δ 2.74–2.78 (m, 2H), 2.84–2.94 (m, 3H), 7.02 (d, 1H), 7.72 (s, 1H), 7.89 (d, 1H). MS (+) 213 (M + 1). HRMS (ESI) calcd for $C_9H_{12}N_2O_2S$ 212.0619, found 212.0678.

5.8. 3-(6-Amino-4-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid (9)

5.8.1. 2-Amino-5-bromo-4-methylpyridine (34). 2-Amino-4-methylpyridine (110 g, 1.02 mol) in hydrobromic acid (1 L, 48%) was stirred at 70 °C and hydrogen peroxide (300 mL, 15% in water) was added dropwise over a one h at such a rate that the temperature of the reaction mixture remained at 70–80 °C. The mixture was stirred for 90 min at 70 °C and poured onto crushed ice. The pH was adjusted to 4–5 with sodium carbonate and the precipitated solid (containing mostly dibrominated products) was filtered off and discarded. The pH was subsequently raised to 9 and the precipitated product collected by filtration. Recrystallization from toluene gave compound **34** (76.3 g, 40%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H), 4.35 (s, 2H), 6.38 (s, 1H), 8.07 (s, 1H).

5.8.2. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-bromo-4-methylpyridin. Compound **34** (5.70 g, 30.5 mmol) in chloroform was treated with di-*tert*-butyl dicarbonate (20.0 g, 91.60 mmol) and DMAP (0.60 g, 4.91 mmol).

The reaction mixture was left at ambient temperature overnight and was then concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 95:5) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-4-methylpyridin (8.02 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 18H), 2.40 (s, 3H), 7.25 (s, 1H), 8.49 (s, 1H).

5.8.3. 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-4-methylpyridin (35). A solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-4-methylpyridin (15.0 g, 38.70 mmol), *tert*-butyl-dimethyl-tributylstannanylmethoxy-silane (25.4 g, 58.3 mmol), and bis(triphenylphosphine)palladium(II) dichloride (0.90 g, 1.42 mmol) in 1,2-dichloro-ethane (50 mL) was stirred at 90 °C for two days. The mixture was cooled to 0 °C and diethyl ether (200 mL) was added followed by saturated aqueous potassium fluoride (40 mL). The mixture was stirred vigorously for 30 min and filtered. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 95:5) gave compound **35** (10.0 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.10 (s, 6H), 0.91 (s, 9H), 1.44 (s, 18H), 2.32 (s, 3H), 4.72 (s, 2H), 7.00 (s, 1H), 8.40 (s, 1H).

5.8.4. 5-Bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]- 4-methylpyridin (37). Tetrabutylammonium fluoride (13.9 g, 44.1 mmol) was added to a solution of compound **35** (10.0 g, 24.3 mmol) in THF (100 mL). The reaction mixture was stirred for 3 h at room temperature. Concentration under reduced pressure followed by flash chromatography (hexane/EtOAc, 50:50) gave compound **36** (5.0 g, 67%). Triphenylphosphine (4.69 g, 17.9 mmol) and CBr_4 (4.89 g, 14.8 mmol) was added to a solution of compound **36** (5.00 g, 22.0 mmol) in dichloromethane (130 mL) at 0 °C. The reaction mixture was stirred for 3 h and was then diluted with dichloromethane. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 80:20) gave compound **37** (5.35 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 18H), 2.42 (s, 3H), 4.47 (s, 2H), 7.12 (s, 1H), 8.34 (s, 1H).

5.8.5. 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (51). To a suspension of NaH (0.24 g, 6.0 mmol, 60%) in DMF (5 mL) was added diethyl malonate (0.91 mL, 6.0 mmol) and the mixture was stirred for 15 min. A solution compound **37** (2.0 g, 5.0 mmol) in DMF (5 mL) was added and the resulting solution stirred for 120 min at 60 °C. Ethyl acetate was added and the mixture was washed with water and brine and dried. After evaporation of the solvent, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:100→1:20) to give a pure fraction of compound **51** (1.15 g, 48%) together with an impure fraction (1.1 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23, (t, 6H), 1.44, (s, 18H), 2.36 (s, 3H), 3.23, (d, 2H), 3.61 (t, 1H), 4.10–4.25 (m, 4H), 7.03 (s, 1H), 8.22 (s, 1H).

5.8.6. 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (141 mg, 2.52 mmol) in ethanol (2 mL) was added to a solution compound **51** (1.1 g, 2.29 mmol) in ethanol (10 mL) and methylene chloride (4 mL) at 0 °C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. Ethyl acetate was added and the organic layer was washed with 0.5 M HCl, water, brine and dried. After filtration and concentration under reduced pressure gave crude 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 97%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.19, (t, 3H), 1.36, (s, 18H), 2.41 (s, 3H), 3.24, (d, 2H), 3.66 (t, 1H), 4.05–4.16 (m, 2H), 7.14 (s, 1H), 8.18 (s, 1H).

5.8.7. 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (52). Diethylamine (0.26 g, 2.67 mmol) was added a mixture of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 2.2 mmol) and 37% aq solution of formaldehyde (0.24 g, 3.00 mmol) in methylene chloride (2 mL) at 0 °C. The mixture was stirred for 16 h at room temperature and ethyl acetate was added. The organic layer was washed with water and 5% $NaHCO_3$ and dried. Concentration under reduced pressure followed by flash chromatography (toluene/ethyl acetate, 3:1→1:1) gave compound **52** (0.81 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30, (t, 3H), 1.53, (s, 18H), 2.26 (s, 3H), 3.62 (s, 2H), 4.22 (q, 2H), 5.16 (s, 1H), 6.23 (s, 1H), 7.04 (s, 1H), 8.19 (s, 1H).

5.8.8. 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester. Triethylamine (0.279 mL, 2.0 mmol) was added to a solution of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.8 g, 1.9 mmol) in thioacetic acid (3 mL) at 0 °C. The mixture was stirred at room temperature for 16 h. Ethyl acetate was added and the organic phase was washed with water, saturated $NaHCO_3$ and brine and dried. The crude product was purified by flash chromatography (toluene/ethyl acetate, 3:1→1:2) to give pure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (200 mg, 21%) and slightly impure 2-acetyl-sulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester (0.68 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17, (t, 3H), 1.44, (s, 18H), 2.35 (s, 3H), 2.85-3.17 (m, 5H), 4.02-4.12 (m, 2H), 7.04 (s, 1H), 8.17 (s, 1H).

5.8.9. 3-(6-Amino-4-methyl-pyridin-3-yl)-2-mercapto-methyl-propionic acid (9). 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester (36 mg, 0.072 mmol) was dissolved in concentrated HCl (3.0 mL). The solution was heated to reflux for 1 h. Concentration under reduced pressure gave compound **9** (18.7 mg, 98%) as the hydrochloride salt. ^{1}H NMR (500 MHz, CD_3OD): δ 2.42 (s, 3H), 2.72–2.95 (m, 5H), 6.81 (s, 1H), 7.58 (s, 1H). MS (+) 227 (M+1). HRMS (ESI) calcd for $C_{10}H_{14}N_2O_2S$ 226.0776, found 226.0801.

5.9. 3-(6-Amino-2-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid (10)

5.9.1. (5-Bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester. Compound **30** (25.0 g, 133.7 mmol) in THF/*tert*-butanol (1:10, 550 mL) was treated with di-*tert*-butyl dicarbonate (39.3 g, 180.0 mmol) and DMAP (2.40 g, 19.6 mmol). The reaction mixture was stirred for 4 h at 40 °C and concentrated under reduced pressure. Flash chromatography (methylene chloride) gave (5-bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (17.0 g, 44%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 9H), 2.50 (s, 3H), 7.40 (bs, 1H), 7.65 (d, 1H), 7.70 (d, 1H).

5.9.2. [5-(*tert*-Butyl-dimethyl-silanyloxymethyl)-6-methyl-pyridin-2-yl]-carbamic acid *tert*-butyl ester (31). A solution of (5-bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (27.5 g, 95.8 mmol), *tert*-butyl-dimethyl-tributylstannanylmethoxy-silane (43.5 g, 100.2 mmol), and bis(triphenylphosphine)palladium(II) dichloride (1.00 g, 1.40 mmol) in 1,2-dichloroethane (350 mL) was stirred at reflux for 48 h. Additional bis(triphenylphosphine)-palladium(II) dichloride (1.00 g, 1.40 mmol) was added every 12 h. The mixture was cooled to 0 °C and diethyl ether (300 mL) was added followed by saturated aqueous potassium fluoride (100 mL). The mixture was stirred vigorously for 60 min and filtered. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (MeOH/CH_2Cl_2, 1:99) compound **31** (15 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.07 (s, 6H), 0.90 (s, 9H), 2.35 (s, 3H), 4.62 (s, 2H), 7.25 (bs, 1H), 7.62 (d, 1H), 7.68 (d, 1H).

5.9.3. (5-Hydroxymethyl-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (32). Tetrabutylammonium fluoride (19.6 g, 62.4 mmol) was added to a solution of compound **31** (10.5 g, 31.23 mmol) in THF (100 mL) and stirred at room temperature overnight. Water was added and the product extracted with chloroform. The organic phase was dried and concentrated under reduced pressure. Flash chromatography (MeOH/CH_2Cl_2, 2.5:77.5) gave compound **32** (6.0 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51 (s, 9H), 2.45 (s, 3H), 4.65 (s, 3H), 7.27 (bs, 1H), 7.62 (d, 1H), 7.70 (d, 1H).

5.9.4. 5-Bromomethyl-2-[*N*,*N*-bis(*tert*-butoxycarbonyl)-amino]-pyrimidin (33). Triphenylphosphine (9.83 g, 37.5 mmol) and CBr_4 (17.7 g, 53.5 mmol) was added to a solution of compound **32** (8.50 g, 35.7 mmol) in dichloromethane (30 mL) at 0 °C. The reaction mixture was stirred for 3 h at room temperature and was then diluted with dichloromethane. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2) gave compound **33** (4.05 g, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (s, 9H), 2.48 (s, 3H), 4.45 (s, 2H), 7.53 (bs, 1H), 7.56 (d, 1H), 7.70 (d, 1H).

5.9.5. 2-(6-*tert*-Butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (48). A solution of diethyl malonate (1.21 mL, 7.97 mmol) in DMF (2 mL) was added dropwise to a suspension of NaH (348 mg, 7.97 mmol, 55% in mineral oil) in DMF (5 mL) at 0 °C under argon. The reaction mixture was stirred for 45 min and a solution of compound **33** (2.0 g, 6.64 mmol) in DMF (5 mL) was added dropwise. The mixture was stirred overnight (0 °C→20 °C). EtOAc was added and the solution was washed with water and brine, dried and concentrated under reduced pressure. Flash chromatography (heptane/EtOAc, 4:1) gave compound **48** (1.87 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23, (t, 6H), 1.49, (s, 9H), 2.45 (s, 3H), 3.17, (d, 2H), 3.59 (t, 1H), 4.11–4.24 (m, 4H), 7.42 (d, 1H), 7.56 (broad s, 1H), 7.66 (d, 1H).

5.9.6. 2-(6-*tert*-Butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (300 mg, 5.35 mmol) in EtOH (4 mL) was added to a solution of compound **48** (1.85 g, 4.86 mmol) in EtOH/methylene chloride (2:1, 21 mL) at 0 °C. The mixture was stirred for 40 h at room temperature and EtOAc was added. The mixture was washed with 0.5 M HCl and brine, dried and concentrated under reduced pressure to give crude 2-(6-*tert*-butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.45 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25, (t, 3H), 1.51, (s, 9H), 2.61 (s, 3H), 3.13–3.27 (m, 2H), 3.61 (t, 1H), 4.14–4.25 (m, 2H), 7.87 (d, 1H), 8.11 (d, 1H).

5.9.7. 2-(6-*tert*-Butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (49). Diethylamine (359 mg, 4.90 mmol) was added dropwise to a solution of 2-(6-*tert*-butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.44 g, 4.09 mmol) and formaldehyde (464 mg, 5.72 mmol, 37% in water) in methylene chloride (35 mL) at 0 °C under argon. The mixture was stirred at room temperature over night. Methylene chloride was added and the solution was washed with Na_2CO_3 and brine, dried and concentrated under reduced pressure to give compound **49** (1.03 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30, (t, 3H), 1.49, (s, 9H), 2.38 (s, 3H), 3.57 (s, 2H), 4.22 (q, 2H), 5.25 (d, 1H), 6.23 (d, 1H), 7.40 (d, 1H), 7.71 (d, 1H), 8.10 (broad s, 1H).

5.9.8. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonyl-amino-2-methyl-pyridin-3-yl)-propionic acid ethyl ester. Triethylamine (0.556 mL, 3.99 mmol) was added to a solution of 2-(6-*tert*-butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (1.23 g, 3.84 mmol) in thioacetic acid (10 mL) at 0 °C under argon. The mixture was stirred at room temperature for 64 h. EtOAc was added and the solution was washed with Na_2CO_3 and brine, dried and concentrated under reduced pressure. Flash chromatography (toluene/EtOAc, 5:1→1:1) gave 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-2-methyl-pyridin-3-yl)-propionic acid ethyl ester (1.33 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.09, (t, 3H), 1.21, (s, 9H), 2.12 (s, 3H), 2.19 (s, 3H), 2.71–2.90 (m, 3H), 2.96–3.12 (m, 2H), 3.95–4.02 (m, 2H), 7.35 (d, 1H), 7.62 (d, 1H), 8.25 (broad s, 1H).

5.9.9. 3-(6-Amino-2-methyl-pyridin-3-yl)-2-mercapto-methyl-propionic acid (10). Concentrated hydrochloric acid (2 mL) was added to 2-acetylsulfanylmethyl-3-(6-

tert-butoxycarbonylamino-2-methyl-pyridin-3-yl)-propionic acid ethyl ester (77 mg, 0.19 mmol) under argon. The reaction was heated to reflux for 110 min and was then concentrated under reduced pressure to give compound **10** (39 mg, 76%) as the hydrochloride salt. ^{1}H NMR (500 MHz, D_2O): δ 2.49 (s, 3H), 2.73–2.92 (m, 5H), 6.81 (d, 1H), 7.77 (d, 1H). MS (+) 227 (M+1). HRMS (ESI) calcd for $C_{10}H_{14}N_2O_2S$ 226.0776, found 226.0835.

5.10. 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid (11)

5.10.1. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-bromo-3-methylpyridin. A solution of compound **38** (15.0 g, 80.2 mmol) in *tert*-butanol (500 mL) was treated with di-*tert*-butyl dicarbonate (43.6 g, 200 mmol) and dimethylaminopyridine (0.60 g, 4.91 mmol). The reaction mixture was stirred overnight and concentrated under reduced pressure. Hexane was added and the product precipitated as a solid. Filtering afforded 22.0 g (71%) of 2-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-3-methylpyridin. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 18H), 2.22 (s, 3H), 7.72 (s, 1H), 8.40 (s, 1H).

5.10.2. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-hydroxymethyl-3-methylpyridin (40). A solution of (*tert*-butyldimethylsilyloxymethyl)tri-*n*-butyltin (47.6 g, 109 mmol), 2-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-3-methylpyridin (26.0 g, 67.1 mmol) and bis(triphenylphosphine)palladium(II) dichloride (0.90 g, 1.42 mmol) in 1,2-dichloroethane (80 mL) was stirred at 90 °C for two days. The mixture was cooled to 0 °C and ether (200 mL) was added followed by saturated aqueous potassium fluoride (40 mL). The mixture was stirred vigorously for 30 min, filtered and the organic phase separated and washed with water. The organic phase was dried, filtered and evaporated. The crude product was purified by flash chromatography (hexane, then hexane:ethyl acetate, 95:5) to afford compound **39** (18.0 g, 59%). A solution of compound **39** (18.0 g, 39.8 mmol) in 150 mL dry THF was treated with tetrabutylammonium fluoride (25.1 g, 79.6 mmol) at room temperature overnight. Concentration under reduced pressure followed by flash chromatography (hexane:ethyl acetate, 1:1) afforded compound **40** (8.00 g, 59%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 18H), 2.22 (s, 3H), 4.70 (s, 2H), 7.60 (s, 1H), 8.27 (s, 1H).

5.10.3. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-bromomethyl-3-methyl-pyridin (41). Compound **40** (8.00 g, 23.6 mmol) and triphenylphosphine (7.43 g, 28.3 mmol) were dissolved in dichloromethane (220 mL) and cooled to 0 °C. Carbon tetrabromide (9.49 g, 28.6 mmol) was added and the reaction mixture allowed to reach room temperature. Stirring was continued for 3 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (hexane:ethyl acetate, 80:20) to afford compound **41** (8.00 g, 77%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 18H), 2.22 (s, 3H), 4.44 (s, 2H), 7.60 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.1, 27.9, 28.9, 83.1, 131.2, 133.6, 139.9, 146.5, 150.7, 151.2.

5.10.4. 2-(6-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (54). To a suspension of NaH (0.24 g, 6.0 mmol, 60%) in DMF (5 mL) was added diethyl malonate (0.91 mL, 6.0 mmol) and the mixture was stirred for 15 min. A solution compound **41** (2.0 g, 5.0 mmol) in DMF (5 mL) was added and the resulting solution stirred for 120 min at 60 °C. Ethyl acetate was added and the mixture was washed with water and brine and dried. After evaporation of the solvent, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:100→1:20) to give compound **54** (1.2 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, 6H), 1.38(s, 18H), 2.19 (s, 3H), 3.20 (d, 2H), 3.60 (t, 1H), 4.10–4.20 (m, 4H), 7.44 (s, 1H), 8.20 (s, 1H).

5.10.5. 2-(6-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (154 mg, 2.75 mmol) in ethanol (2 mL) was added to a solution compound **54** (1.2 g, 2.50 mmol) in ethanol (10 mL) and methylene chloride (4 mL) at 0 °C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. Ethyl acetate was added and the organic layer was washed with 0.5 M HCl, water, brine and dried. After filtration and concentration under reduced pressure gave crude 2-(6-[*N*,*N*-bis(*tert*-butoxy- carbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, 3H), 1.37 (s, 18H), 2.08 (s, 2H), 2.20 (s, 3H), 3.24 (d, 2H), 3.64 (t, 1H), 4.12–4.22 (m, 2H), 7.53 (s, 1H), 8.25 (s, 1H).

5.10.6. 2-(6-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (55). Diethylamine (0.26 g, 2.67 mmol) was added a mixture of 2-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 2.2 mmol) and 37% aq solution of formaldehyde (0.24 g, 3.00 mmol) in methylene chloride (2 mL) at 0 °C. The mixture was stirred for 16 h at room temperature and ethyl acetate was added. The organic layer was washed with water and 5% $NaHCO_3$ and dried. Concentration under reduced pressure followed by flash chromatography (toluene/ethyl acetate, 3:1→1:2) gave compound **55** (0.68 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, 3H), 1.44 (s, 18H), 2.24 (s, 3H), 3.66 (s, 2H), 4.22 (q, 2H), 5.50 (s, 1H), 6.31 (s, 1H), 7.46 (d, 1H), 8.24 (d, 1H).

5.10.7. 2-Acetylsulfanylmethyl-3-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester. Triethylamine (0.234 mL, 1.68 mmol) was added to a solution of compound **55** (0.68 g, 1.61 mmol) in thioacetic acid (3 mL) at 0 °C. The mixture was stirred at room temperature for 16 h. Ethyl acetate was added and the organic phase was washed with water, saturated $NaHCO_3$ and brine and dried. The crude product was purified by flash chromatography (toluene/ethyl acetate, 3:1→1:2) to give pure 2-acetylsulfanylmethyl-3-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (489 mg, 61%) and slightly impure 2-acetylsulfanylmethyl-3-(6-[*N*,*N*-bis(*tert*-butoxy-

carbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (0.34 g, 43%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, 3H), 1.41 (s, 18H), 2.22 (s, 3H), 2.36 (s, 3H), 2.88-3.17 (m, 5H), 4.06-4.16 (m, 2H), 7.43 (s, 1H), 8.17 (s, 1H).

5.10.8. 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid (11). 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (17 mg, 0.034 mmol) was dissolved in concentrated HCl (3.0 mL). The solution was heated to reflux for 1 h. Concentration under reduced pressure gave compound **11** (8.9 mg, 100%) as the hydrochloride salt. ^{1}H NMR (500 MHz, CD_3OD): δ 2.26 (s, 3H), 2.72–2.75 (m, 2H), 2.83–2.91 (m, 3H), 7.60 (s, 1H), 7.77 (s, 1H). MS (+) 227 (M + 1). HRMS (ESI) calcd for $C_{10}H_{14}N_2O_2S$ 226.0776, found 226.082.

5.11. 3-(6-Amino-5-chloro-pyridin-3-yl)-2-mercaptomethyl-propionic acid (12)

5.11.1. 6-Amino-5-chloro-nicotinic acid ethyl ester. *N*-Chlorosuccinimide (21.7 g, 0.162 mol) was added to a suspension of 6-amino-nicotinic acid ethyl ester (18.0 g, 0.108 mol) in acetonitrile (270 mL) and the mixture was refluxed for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane, washed with water and dried. Flash chromatography (2.5% MeOH in CH_2Cl_2) gave pure 6-amino-5-chloro-nicotinic acid ethyl ester (17.23 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (t, 3H), 4.34 (q, 2H), 5.38 (br s, 2H, NH_2), 8.08 (s, 1H), 8.62 (s, 1H).

5.11.2. (3-Chloro-5-hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (26). DMAP (0.11 g, 0.9 mmol) and di-*tert*-butyl dicarbonate (21.54 g, 99 mmol) was added to a solution of 6-amino-5-chloro-nicotinic acid ethyl ester (9.0 g, 45 mmol) in dichloromethane (250 mL). The reaction mixture was stirred for 24 h. DMAP (0.02 equiv) and di-*tert*-butyl dicarbonate (3×0.5 equiv) was added during the reaction time. The reaction mixture was washed with water and dried. The crude product was washed with hexane to give pure compound **25** (11.87 g, 66%). $LiAlH_4$ (2.4 g, 63.2 mmol) was added in portions over a period of 3.5 h to solution of compound **25** (11.5 g, 28.6 mmol) in THF (70 mL) at 0 °C. The reaction mixture was stirred in room temperature over night, and then saturated aqueous NH_4Cl was added carefully followed by water. The solution was filtered, dried and concentrated under reduced pressure to yield crude compound **26** (5.86 g, 79%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.58 (s, 9H), 2.09 (s, 1H), 4.66 (2, 2H), 7.47 (s, 1H), 7.76 (d, J = 2.0 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H).

5.11.3. (5-Bromomethyl-3-chloro-pyridin-2-yl)-carbamic acid *tert*-butyl ester (27). Triphenylphosphine (2.61 g, 9.7 mmol) followed by carbontetrabromide (4.58 g, 13.8 mmol) was added to a suspension of compound **26** (2.38 g, 9.2 mmol) in CH_2Cl_2 (60 mL) at 0 °C. The mixture was stirred at room temperature for 5 h and concentrated under reduced pressure. Acetonitrile (40 mL) was added and the mixture was kept to −20 °C overnight. The mixture was then filtered and the crystalline residue washed with cold acetonitrile. The filtrate was concentrated under reduced pressure and another crop of bromide was obtained as described above. Compound **27** (1.86 g, 63%) was obtained as white crystals.%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (s, 9H), 4.41 (s, 2H), 7.30 (bs, 1H), 7.71 (d, J = 1.8 Hz), 8.35 (d, J = 1.8 Hz).

5.11.4. 2-(6-*tert*-Butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-malonic acid diethyl ester (45). Diethyl malonate (1.87 mL, 12.31 mmol) was added to a suspension of NaH (0.54 g, 12.31 mmol, 55% in mineral oil) in dry DMF (15 mL) at −8 °C. This mixture was stirred for 15 min. before it was added dropwise to a solution of compound **27** (3.30 g, 10.26 mmol) in dry DMF (50 mL) at 0 °C. The resulting solution was stirred for 40 min at 0 °C, then saturated aqueous NH_4Cl (5 mL) was added carefully. Stirring at room temperature overnight and concentration under reduced pressure gave a residue, which was dissolved in water/CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were dried, filtered and concentrated under reduced pressure. Flash chromatography (1% MeOH in CH_2Cl_2) gave compound **45** (2.45, 60%) as a sticky clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, 6H), 1.53 (s, 9H), 3.16 (d, 2H), 3.57 (t, 1H), 4.1–4.25 (m, 4H), 7.67 (s, 1H), 8.22 (s, 1H).

5.11.5. 2-(6-*tert*-Butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.44 g, 6.72 mmol, 85%) in ethanol (5 mL) was added to a solution of compound **45** (2.45 g, 6.11 mmol) in ethanol (25 mL) and methylene chloride (10 mL) at 0 °C. The mixture was stirred for 18 h at room temperature. The solvent was concentrated under reduced pressure and the residue dissolved in water. The aqueous layer was washed with ether, acidified to pH 4 by 1 M HCl and extracted with methylene chloride and ethyl acetate. The combined organic layers were washed with water and brine and dried. Filtration and concentration under reduced pressure gave the crude product which was purified by flash chromatography (10% MeOH in CH_2Cl_2) giving 2-(6-*tert*-butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.41 g, 62%) as a yellow-white glassy foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, 6H), 1.53 (s, 9H), 3.16 (d, 2H), 3.57 (t, 1H), 4.1–4.25 (m, 4H), 7.67 (s, 1H), 8.22 (s, 1H).

5.11.6. 2-(6-*tert*-Butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-acrylic acid ethyl ester (46). Diethylamine (3.67 mL, 3.67 mmol) was added dropwise followed by water (2.5 mL) and CH_2Cl_2 (2.5 mL) to a mixture of 2-(6-*tert*-butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.40 g, 3.64 mmol) and formaldehyde (0.29 mL, 3.75 mmol, 37% in water) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred for 20 h at room temperature and then poured onto ice-water and extracted with methylene chloride. The organic layer was washed with 5% $NaHCO_3$, dried and concentrated under reduced pressure. Flash chromatography (1→2.5% methanol in CH_2Cl_2) yielded compound **46** (0.81 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t,

3H), 1.53 (s, 9H), 3.56 (s, 2H), 4.17 (q, 2H), 5.53 (d, 1H), 6.26 (s, 1H), 7.19 (br s, NH), 7.53 (d, 1H), 8.28 (br s, 1H).

5.11.7. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-propionic acid ethyl ester. Thioacetic acid (4 mL) was added to a suspension of compound **46** (0.732 g, 2.16 mmol) and triethylamine (0.31 mL, 2.23 mmol) at 0 °C. The mixture was stirred at room temperature under argon overnight, poured onto ice-water and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ until gas evolution ceased and then dried. The crude product was purified twice with flash chromatography (CH_2Cl_2, 1–2.5% MeOH in CH_2Cl_2 and Hexane/EtOAc, 5:2→1:1) to give pure 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-chloro-pyridin-3-ylmethyl)-propionic acid ethyl ester (0.79 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (t, 3H), 1.52 (s, 9H), 2.32 (s, 3H), 2.78–2.98 (m, 3H), 3.0–3.15 (m, 2H), 4.08 (q, 2H), 7.21 (br s, NH), 7.52 (d, 1H), 8.16 (d, 1H).

5.11.8. 3-(6-Amino-5-chloro-pyridin-3-yl)-2-mercaptomethyl-propionic acid (12). A solution of 2-acetylsulfanylmethyl-3-(6-tert-butoxycarbonylamino-5-chloro-pyridin-3-yl)-propionic acid ethyl ester (55mg, 0.132 mmol) in concentrated aqueous HCl (4 mL) was refluxed for 90 min. The reaction was cooled and concentrated under reduced pressure to give compound **12** as the HCl salt (36mg, 96.4%). ^{1}H NMR (400 MHz, D_2O): δ 2.70–2.97 (m, 5H), 7.73 (s, 1H), 8.09 (s, 1H). MS (+) 248 (M + 1). HRMS (ESI) calcd for $C_9H_{11}N_2O_2ClS$ 246.0230, found 246.0286.

5.12. 3-(6-Amino-5-hydroxymethyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid (13)

5.12.1. 6-bis(*tert*-Butoxycarbonyl)amino-5-vinyl-nicotinic acid ethyl ester. A mixture of compound **29** (4.50 g, 10.1 mmol), vinyltributyltin (3.52 g, 11.1 mmol) and tetrakispalladium triphenyphosphine(0.50 g, 0.40 mmol) in THF (15 mL) was stirred at reflux for 24 h. Tetrakispalladium triphenyphosphine(0.50 g) was added and after 24 h at reflux the reaction mixture was cooled and diluted with dichloromethane (100 mL). Saturated aqueous KF (25 mL) was added and the solution stirred for 1 h. Water was added and the product extracted with dichloromethane, the organic phase was dried and concentrated under reduced pressure. Flash chromatography (1% MeOH in dichloromethane) gave 6-bis(*tert*-butoxycarbonyl)-amino-5-vinyl-nicotinic acid ethyl ester 3.20 g, (81%).^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (s, 18H), 1.36 (t, J = 7.0 Hz, 3H), 4.38 (q, J = 7.0 Hz, 2H), 5.46 (d, J = 10.8 Hz, 1H), 5.83 (d, J = 17.3 Hz, 1H), 6.65 (dd, J = 10.8, 17.3 Hz, 1H), 8.40 (d, J = 1.8 Hz, 1H), 8.92 (d, J = 1.8 Hz, 1H).

5.12.2. (5-Hydroxymethyl-3-vinyl-pyridin-2-yl)carbamic acid tert-butyl ester. DIBAL (25 mL, 1M in hexane) was added dropwise to a solution of 6-bis(*tert*-butoxycarbonyl)amino-5-vinyl-nicotinic acid ethyl ester (2.00 g, 5.1 mmol) in THF (40 mL) at 0 °C. The mixture was stirred at room temperature for 1 h. Saturated aqueous NH_4Cl was added carefully followed by water, and the mixture was concentrated under reduced pressure. The residue was suspended in MeOH/dichloromethane (5:95) and filtered through silica gel. The filtrate was dried and concentrated under reduced pressure to give (5-hydroxymethyl-3-vinyl-pyridin-2-yl)carbamic acid *tert*-butyl ester (1.00 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.72, (bs, 1H), 4.70 (s, 3H), 5.45 (d, J = 11.6 Hz, 1H), 5.78 (d, J = 17.8 Hz, 1H), 6.78 (dd, J = 11.6, 17.8 Hz, 1H), 7.96 (d, J = 1.8 Hz, 1H), 8.29 (d, J = 1.8 Hz, 1H).

5.12.3. (5-Bromomethyl-3-vinyl-pyridin-2-yl)carbamic acid *tert*-butyl ester (80). To a stirred suspension of (5-hydroxymethyl-3-vinyl-pyridin-2-yl)carbamic acid *tert*-butyl ester (10.0 g, 40.0 mmol) in CH_2Cl_2 (150 mL) and THF (60 mL) was added triphenylphosphine (11.5 g, 44.0 mmol) followed by carbontetrabromide (19.9 g, 60.0 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure. Acetonitrile (100 mL) was added and the mixture was kept at −20 °C overnight. The mixture was then filtered and the crystalline residue washed with cold acetonitrile. The product was purified by flash chromatography (MeOH/dichloromethane, 1:99) to give compound **80** (4.5 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.72, (bs, 1H), 4.42 (s, 2H), 5.49 (d, J = 11.0 Hz, 1H), 5.78 (d, J = 17.0 Hz, 1H), 6.78 (dd, J = 11.0, 17.0 Hz, 1H), 7.91 (d, J = 1.8 Hz, 1H), 8.32 (d, J = 1.8 Hz, 1H).

5.12.4. 2-(6-*tert*-Butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid diethyl ester. Diethyl malonate (2.30 g, 14.3 mmol) was added to a suspension of NaH (0.61 g, 14.3 mmol, 55%) in dry DMF (75 mL) at 0 °C. The mixture was stirred for 15 min and then added dropwise to a solution of compound **80** (4.50 g, 14.3 mmol) in dry DMF (100 mL) at 0 °C. The resulting solution was stirred for 40 min at 0 °C, and then saturated aqueous NH_4Cl (30 mL) was added carefully. Concentration under reduced pressure gave a residue, which was dissolved in water/CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 and the combined extracts were dried, filtered and concentrated under reduced pressure. Flash chromatography (MeOH/CH_2Cl_2, 1:99) gave 2-(6-*tert*-Butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (4.30 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (t, J = 7.1 Hz, 6H), 1.48 (s, 9H), 3.19 (d, J = 7.7 Hz, 2H), 3.58 (t, J = 7.7 Hz, 1H), 4.15 (q, J = 7.1 Hz, 2H), 4.17 (q, J = 7.1 Hz, 2H), 5.42 (d, J = 11.0 Hz, 1H), 5.72 (d, J = 17.5 Hz, 1H), 6.72 (dd, J = 11.0, 17.5 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 8.00 (s, 1H), 8.17 (d, J = 2.4 Hz, 1H).

5.12.5. 2-(6-*tert*-Butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester. A solution of KOH (0.71 g, 12.6 mmol, 85%) in ethanol (10 mL) was added to a solution of 2-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (4.30 g, 11.0 mmol) in ethanol (25 mL) and dichloromethane (10 mL) at 0 °C. The mixture was stirred for 6 h at room temperature. The solvent was concentrated

under reduced pressure and the residue dissolved in water. The aqueous layer was washed with ether, acidified to pH 4 by 1 M HCl and extracted with dichloromethane. The organic layer was washed with water and brine and dried. Filtration and concentration under reduced pressure followed by flash chromatography ($MeOH/CH_2Cl_2$, 1:9) gave 2-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (3.05 g, 76%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, $J=7.1$ Hz, 3H), 1.48 (s, 9H), 3.21 (m, 2H), 3.62 (t, $J=7.5$ Hz, 1H), 4.20 (q, $J=7.1$ Hz, 2H), 5.38 (d, $J=11.1$ Hz, 1H), 5.72 (d, $J=17.4$ Hz, 1H), 6.66 (dd, $J=11.1$, 17.4 Hz, 1H), 7.83 (d, $J=1.8$ Hz, 1H), 8.00 (s, 1H), 8.05 (d, $J=1.8$ Hz, 1H).

5.12.6. 2-(6-*tert*-Butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)- acrylic acid ethyl ester. Diethylamine (0.85 mL, 8.80 mmol) was added dropwise to a mixture of 2-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (3.05 g, 8.37 mmol) and formaldehyde (0.71 g, 8.80 mmol, 37% in water) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred for 3 h at room temperature and then poured onto ice-water and extracted with dichloromethane. The organic layer was washed with 5% $NaHCO_3$ and dried. Flash chromatography ($MeOH/CH_2Cl_2$, 1:99) yielded 2-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (1.70 g, 61%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, $J=7.4$ Hz, 3H), 1.48 (s, 9H), 3.61 (s, 2H), 4.18 (q, $J=7.4$ Hz, 2H), 5.42 (d, $J=11.4$ Hz, 1H), 5.55 (s, 1H), 5.72 (d, $J=18.0$ Hz, 1H), 6.26 (s, 1H), 6.73 (dd, $J=11.4$, 18.0 Hz, 1H), 7.72 (d, $J=1.9$ Hz, 1H), 8.16 (d, $J=1.9$ Hz, 1H).

5.12.7. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-yl)-propionic acid ethyl ester (81). Thioacetic acid (4 mL) was added to a suspension of 2-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.73 g, 2.16 mmol) in triethylamine (0.31 mL, 2.23 mmol) at 0 °C. The mixture was stirred at room temperature under argon overnight, poured onto icewater and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ until gas evolution ceased and then dried. Flash chromatography ($MeOH/CH_2Cl_2$, 1:99) gave compound **81** (0.51 g, 70%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, $J=6.6$ Hz, 3H), 1.48 (s, 9H), 2.34 (s, 3H), 2.80–3.15 (br m, 5H), 4.09 (m, 2H), 5.42 (d, $J=10.8$ Hz, 1H), 5.75 (d, $J=17.8$ Hz, 1H), 6.73 (dd, $J=10.8$, 17.8 Hz, 1H), 7.68 (d, $J=2.2$ Hz, 1H), 8.19 (d, $J=2.2$ Hz, 1H).

5.12.8. 3-(6-*tert*-Butoxycarbonylamino-5-hydroxymethyl-pyridin-3-yl)-mercaptomethyl-propionic acid ethyl ester. Ozone was bubbled through a solution of 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-vinyl-pyridin-3-yl)-propionic acid ethyl ester (0.65 g, 1.60 mmol) in ethanol (25 mL) at −78 °C. O_2 was then bubbled through the mixture for 5 min followed by N_2 bubbling for 15 min. A mixture of $NaBH_4$ 0.30 g, 8.00 mmol) in water was carefully added to the mixture at −78 °C, and the reaction mixture allowed to reach 0 °C. Stirring was continued for 3 h. Acetone (10 mL) was added and the reaction mixture evaporated to 1/3 of the initial volume. 50% aqueous NaCl was added and the mixture was extracted with dichloromethane, dried and concentrated under reduced pressure to give crude 3-(6-*tert*-butoxy-carbonylamino-5-hydroxymethyl-pyridin-3-yl)-mercaptomethyl-propionic acid ethyl ester (0.38 g, 63%).^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, $J=7.1$ Hz, 3H), 1.49 (s, 9H), 2.07 (s, 1H), 2.32 (s, 3H), 2.81–3.14 (b m, 5H), 4.07 (q, $J=7.1$ Hz, 2H), 5.09 (s, 2H), 7.58 (d, $J=2.4$ Hz, 1H), 8.16 (d, $J=2.4$ Hz, 1H).

5.12.9. Ethyl 3-{5-[(acetyloxy)methyl]-6-[(*tert*-butoxycarbonyl)amino]pyridin-3-yl}-2-[(acetylthio)methyl]propanoate. A mixture of crude 3-(6-*tert*-butoxycarbonylamino-5-hydroxymethyl-pyridin-3-yl)-mercaptomethyl-propionic acid ethyl ester (0.38 g, 1.0 mmol) and $KHCO_3$ (0.11 g, 1.1 mmol) in acetic acid anhydride (1 mL) was stirred at room temperature for 5 h. Saturated aqueous NH_4Cl and water was then added. The mixture was extracted with dichloromethane, dried and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2, 2.5% MeOH in CH_2Cl_2) gave ethyl 3-{5-[(acetyloxy)methyl]-6-[(*tert*-butoxycarbonyl)amino]pyridin-3-yl}-2-[(acetylthio)methyl]propanoate (0.23 g, 60%).^1H NMR (300 MHz, $CDCl_3$):? δ?1.17 (t, $J=7.1$ Hz, 3H), 1.51 (s, 9H), 2.11 (s, 3H), 2.34 (s, 3H), 2.84–3.03 (m, 3H), 3.09 (dd, $J=4.2$, 6.0 Hz, 2H), 4.08 (q, $J=7.1$ Hz, 2H), 5.11 (s, 2H), 7.66 (s, 1H), 8.17 (s, 1H).

5.12.10. 3-[6-Amino-5-(hydroxymethyl)pyridin-3-yl]-2-(mercaptomethyl)propanoic acid (13). A solution of ethyl 3-{5-[(acetyloxy)methyl]-6-[(*tert*-butoxycarbonyl)-amino]pyridin-3-yl}-2-[(acetylthio)methyl]propanoate (50 mg, 135 mmol) in concentrated aqueous HCl (2 mL) was refluxed for 60 min. The reaction was cooled and concentrated under reduced pressure to give compound **13** as the HCl salt (37mg, 98.3%). ^1H NMR (400 MHz, D_2O): δ 2.70–2.95 (m, 5H), 4.65 (s, 2H), 7.68 (s, 1H), 7.88 (s, 1H). MS (+) 244 (M + 1). HRMS (ESI) calcd for $C_{10}H_{14}N_2O_3S$ 242.0725, found 242.0785.

5.13. 2-(6-Amino-pyridin-3-ylmethyl)-2-mercaptomethyl-butyric acid (14)

5.13.1. [5-(5-Ethyl-2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester (74). Compound **23** (1.0 g, 3.48 mmol) was added to a solution of 2,2-dimethyl-5-ethyl-1,3-dioxane-4,6-dione (600 mg, 3.48 mmol) and triethylamine (0.51 mL, 3.66 mmol) in dimethyl sulfoxide (40 mL) under nitrogen. The reaction mixture was stirred over night and water (100 mL) was added. Filtration of the precipitate gave compound **74** (1.15 g, 87%). ^1H NMR (500 MHz, $CDCl_3$): δ 0.96 (s, 3H), 1.02 (t, 3H), 1.53 (s, 9H),1.63 (s, 3H), 2.20 (q, 2H), 3.27 (s, 2H), 7.56 (d, 1H), 7.92 (m, 1H), 8.07 (m, 1H).

5.13.2. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester. A solution of sodium metal (140 mg, 6.08 mmol) in ethanol (20 mL) was added to a solution of compound **74** (1.15 g, 3.04 mmol) in ethanol (10 mL). The reaction was stirred for 90 min and methylene chloride was then added. The mixture was washed with 0.5 M HCl, dried and concentrated

under reduced pressure to give 2-(6-*tert*-butoxy-carbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester (1.05 g, 95%). ^{1}H NMR (400 MHz, CD_3SOCD_3): δ 0.85 (t, 3H), 1.15 (t, 3H), 1.62 (m, 2H), 3.03 (m, 2H), 4.04–4.14 (m, 2H), 7.49 (dd, 1H), 7.62 (d, 1H), 7.96 (d, 1H), 9.88 (s, 1H), 13.0 (bs, 1H).

5.13.3. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-hydroxymethyl-butyric acid ethyl ester (76). Methyl chloroformate (150 μL, 1.95 mmol) was added dropwise to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester (700 mg, 1.91 mmol) and Et_3N (275 μL, 1.97 mmol) in THF (15 mL) at-20 °C under nitrogen. The reaction mixture was stirred for 50 min., filtered and added dropwise to a suspension of $NaBH_4$ (80 mg, 2.1 mmol) in THF (15 mL) at-20 °C. The reaction was stirred for 16 h at room temperature. 0.2 M HCl was added followed by methylene chloride. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromatography (toluene/EtOAc, 3:1→1:3) gave compound **76** (300 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H), 1.27 (t, 3H), 1.47–1.53 (m, 1H), 1.54 (s, 9H),1.61–1.73 (m, 1H), 2.83 (d, 1H), 3.03 (d, 1H), 3.46 (d, 1H), 3.73 (d, 1H), 7.56 (d, 1H), 7.92 (m, 1H), 8.07 (m, 1H).

5.13.4. 2-Acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester. Diisopropyl azodicarboxylate (296 μL, 1.53 mmol) was added dropwise to a solution of triphenylphosphine (402 mg, 1.53 mmol) in THF (4 mL) at 0 °C under argon and the reaction was stirred for 30 min. A solution of thioacetic acid (109 μL, 1.53 mmol) and compound **76** (0.27 g, 0.77 mmol) in THF (2 mL) was added dropwise during 10 min. The reaction was stirred for 60 min at 0 °C and then for 16 h at room temperature. Concentration under reduced pressure followed by chromatography (heptane/EtOAc, 10:1→1:1) gave 2-acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester (193 mg, 61%). ^{1}H NMR (500 MHz, CD_3OD): δ 0.88 (t, 3H), 1.23 (t, 3H), 1.52 (s, 9H),1.56–1.78 (m, 2H), 2.35 (s, 3H), 2.86 (dd, 2H), 3.15 (dd, 2H), 4.12 (q, 2H), 7.47 (dd, 1H), 7.74 (d, 1H), 7.96 (d, 1H).

5.13.5. 2-(6-Amino-pyridin-3-ylmethyl)-2-mercaptomethyl-butyric acid (14). 2-Acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester (12.3 mg, 30 μmol) was dissolved in concentrated HCl (2 mL) under argon. The solution was heated to reflux for 24 h. Concentration under reduced pressure gave compound **14** as the hydrochloride salt (8.3 mg, 100%). ^{1}H NMR (500 MHz, CD_3OD): δ 0.91 (t, 3H), 1.71 (m, 2H), 2.68 (m, 2H), 2.92 (m, 2H), 6.96 (d, 1H), 7.65 (bs, 1H), 7.82 (dd, 1H). MS (−) 239 (M-1).

5.14. 3-(6-Amino-5-methylpyridin-3-yl)-2-(hydroxymethyl)-2-(mercaptomethyl)-propanoic acid (15)

5.14.1. 2-(6-bis-*tert*-Butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester. A mixture of sodium hydride (33 mg, 0.82 mmol, 60% in mineral oil) in tetrahydrofuran (10 mL) was cooled to −70 °C. *Tert*-butyl ethyl malonate (154 mg, 0.82 mmol) was added and the reaction mixture was stirred for 60 min. A solution of compound **41** (0.3 g, 0.75 mmol) in tetrahydrofuran (10 mL) was then added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was cooled to 0 °C and quenched with water. The aqueous layer was extracted with ethyl acetate and the organic phase was washed with brine and dried. Concentration under reduced pressure followed by flash chromatography (hexane/ethyl acetate, 3:1) yielded 2-(6-bis-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester (178 mg, 59% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (m, 3H), 1.39 (s, 9H), 1.43 (s, 3H), 2.19 (s, 3H), 3.16 (d, 2H), 3.52 (t, 1H), 4.16 (m, 2H), 7.44 (d, 1H), 8.20 (d, 1H).

5.14.2. 2-Benzyloxymethyl-2-(6-bis-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester. A solution of 2-(6-bis-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester (200 mg, 393 mmol) in tetrahydrofuran (5 mL) was cooled to 0 °C and lithium diisopropylamide (2.16 mL, 0.43 mmol, 2 M solution in heptane/THF/ethylbenzene) was added. The mixture was stirred for 0.5 h and benzyl chloromethyl ether (66.90 mg, 0.393 mmol) was added. This mixture was stirred for 1 h at 0 °C and then allowed to warm to room temperature overnight. Water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried and concentrated under reduced pressure to afford 0.23 g of crude product. Purification by flash chromatography (ethyl acetate/hexane, 1:5), yielded 2-benzyloxymethyl-2-(6-bis-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester (149 mg, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (m, 3H), 1.37 (s, 9H), 1.43 (s, 9H), 2.12 (s, 3H), 3.33 (d, 2H), 3.62 (s, 2H), 4.15 (m, 2H), 4.51 (s, 2H), 7.30 (m, 6H), 8.11 (d, 1H).

5.14.3. 2-Benzyloxymethyl-2-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid mono-*tert*-butyl ester (78). KOH (14 g, 250 mmol) was added slowly to a solution of 2-benzyloxymethyl-2-(6-bis-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-ylmethyl)-malonic acid *tert*-butyl ester ethyl ester (3.2 g, 5 mmol) in CH_2Cl_2 (10 mL), EtOH (200 mL) and H_2O (100 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 days. The reaction was quenched by slow addition of 1 M HCl at 0 °C until pH = 2–3 and was then extracted with CH_2Cl_2. The combined organic layer was dried and concentrated under reduced pressure. Filtration through a plug of silica (EtOAc) yielded compound **78** (1.9 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.50 (s, 9H), 2.13 (s, 3H), 3.25–3.29 (m, 2H), 3.44 (d, J = 9.6 Hz, 1H), 3.93 (d, J = 9.3 Hz, 1H), 4.51 (d, J = 12.3 Hz, 1H), 4.63 (d, J = 12.3 Hz, 1H), 7.25–7.40 (m, 7H), 7.78 (s, 1H).

5.14.4. 2-Benzyloxymethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester. BOP (975 mg, 2.2 mmol) was added to a solution of compound **78** (1g, 2 mmol) in THF (80

mL). DIPEA (470 μL, 2.4 mmol) was added and the solution was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and EtOH (60 mL) was added. To this mixture $LiBH_4$ (175 mg) was added in portions. The reaction mixture was stirred for 40 min and saturated aqueous NH_4Cl was added followed by extraction with EtOAc. The combined organic layer was dried and concentrated under reduced pressure. Flash chromatography (EtOAc/hexane, 1:9 to 3:7) yielded 2-benzyloxymethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester (700 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H), 1.50 (s, 9H), 2.22 (s, 3H), 2.89 (s, 2H), 3.54 (dd, 2H), 3.75 (q, 2H, $J = 13.2$ Hz), 4.50 (s, 2H), 7.25–7.4 (m, 6H), 8.07 (s, 1H).

5.14.5. 3-(6-*tert*-Butoxycarbonylamino-5-methyl-pyridin-3-yl)-2,2-bis-hydroxymethyl-propionic acid *tert*-butyl ester (79). A suspension of 2-benzyloxymethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester (0.6 g, 1.23 mmol), Pd (0.15 g, 10% on carbon, 50% wet) and concentrated HCl (0.4 mL) in ethanol (30 mL) was shaken under hydrogen atmosphere (50 psi) for 18 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. Filtration through a plug of silica (dichloromethane to MeOH/dichloromethane, 1:9) yielded compound **79** (398 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 1.5 (s, 9H), 2.17 (s, 3H), 2.32 (s, 2H), 2.83 (s, 2H), 3.6–3.82 (m, 4H), 7.26–7.47 (m, 2H), 8.12 (s, 1H). MS: (M +) 397.8 (M + 1).

5.14.6. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester. Triphenyl phosphine (215 mg, 0.82 mmol) and DEAD (130 μL, 0.82 mmol) was added to a solution of compound **79** (200 mg, 0.41 mmol) in THF (15 mL) After stirring for 30 min, thiolacetic acid (63 μL, 0.82 mmol) was added. After stirring for 5 h, the solvent was removed under reduced pressure and saturated aqueous $NaHCO_3$ (1 mL) was added. The mixture was extracted with EtOAc, dried and concentrated under reduced pressure. Flash chromatography (EtOAc/hexane, 1:9 to 3:7) yielded 60 mg pure product and 100 mg of mixture product/reduced DEAD. This fraction was repurified to provide 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester in a total yield of 100 mg (45%). FT–IR (cm^{-1}): 3342, 1720, 1367, 1244 and 1156. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H), 1.5 (s, 9H), 2.24 (s, 3H), 2.40 (s, 3H), 2.85 (dd, 2H), 3.18 (q, 2H), 3.52–3.59 (m, 2H), 6.70 (s, 1H), 7.36 (s, 1H), 8.05 (s, 1H). ^{13}C NMR (75.45 MHz, $CDCl_3$) δ 17.80, 27.98, 28.21, 30.5, 31.48, 35.78, 53.57, 62.44, 80.64, 82.34, 125.94, 128.81, 141.19, 146.97, 148.53, 152.48, 172.12, 198.49. MS: (M +) 455.3 (M + 1). Elemental analysis: Anal. calcd for $C_{22}H_{34}N_2O_6S*H_2O$: C, 55.91; H, 7.68; N, 5.93. Found: C, 55.79; H, 7.21; N, 5.95.

5.14.7. 3-(6-Amino-5-methylpyridin-3-yl)-2-(hydroxymethyl)-2-(mercaptomethyl)-propanoic acid (15). 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-5-methyl-pyridin-3-yl)-2-hydroxymethyl-propionic acid *tert*-butyl ester (380 mg, 0.84 mmol) was dissolved in concd HCl (4.0 mL) under argon. The solution was then heated to reflux for 3 h. Concentration under reduced pressure gave compound **15** (251 mg, 100%) as the hydrochloride salt. ^{1}H NMR (500 MHz, CD_3OD): δ 2.24 (s, 3H), 2.74 (d, 1H), 2.83 (d, 1H), 2.91 (s, 2H), 3.72 (d, 1H), 3.83 (d, 1H), 7.58 (s, 1H), 7.68 (s, 1H). HRMS (ESI) calcd for $C_{11}H_{16}N_2O_3S$ 256.0882, found 256.0942.

5.15. 2-Mercaptomethyl-3-(6-amino-pyridin-3-yl)-2-hydroxy-propionic acid (16)

5.15.1. 2-Hydroxymethyl-3-(6-*tert*-butyoxycarbonylamino-pyridin-3-yl)-2-hydroxy-propionic acid ethyl ester (82). To a solution of compound **43** (2.5 g, 8.16 mol) in Acetone/H_2O (4/1, 200 mL) at 0 °C was added *N*-methyl morpholine *N*-oxide (1.0 g, 8.5 mmol) followed by OsO_4 (12 mg). The reaction mixture was stirred at rt for 16 h. $Na_2S_2O_5$ (1.0 g) was then added and the mixture was stirred at rt for 2 h. The mixture was extracted with EtOAc, washed with brine and dried to afford crude compound **82** (2.5 g, 90%) as a white solid . ^{1}H NMR (300 MHz, DMSO): δ 1.52 (t, $J = 6.9$ Hz, 3H), 1.47 (s, 9H), 2.73–2.87 (m, 2H), 3.39–3.60 (m, 2H), 4.01–4.08 (m, 2H), 4.91–4.95 (m, 1H), 5.11 (s, 1H), 7.54 (dd, $J = 8.7$, 2.1 Hz, 1H), 7.67 (d, $J = 8.7$ Hz, 1H), 8.03 (s, 1H), 9.68 (s, 1H). ^{13}C NMR (300 MHz, DMSO): δ 14.09, 28.02, 37.01, 60.38, 66.87, 78.45, 79.44, 111.39, 126.24, 139.52, 148.72, 150.85, 152.76, 179.43. MS (APCI): 341.0 (M + 1).

5.15.2. 2-Acetylsulfanylmethyl-3-(6-*tert*-butyoxycarbonylamino-pyridin-3-yl)-2-hydroxy-propionic acid ethyl ester. To a solution of compound **82** (2g, 5.9 mmol) in THF (120 mL) was added triphenylphosphine (3.1 g, 11.8 mmol) and diethylazodicarboxylate (2 g, 11.8 mmol). After stirring for 0.5 h, thiolacetic acid (0.9 mL) was added. The reaction mixture was stirred for 60 h. The mixture was diluted with EtOAc, then quenched with saturated aqueous $NaHCO_3$ (10 mL). A white solid formed, which was filtered and washed with EtOAc. The organic layer was washed with water and brine, and dried. Concentration under reduced pressure followed by flash chromatography (EtOAc/hexane, 1:9 to 3:7) gave 720 mg product with 90% purity and 480 mg impure product. The 720 mg batch was purified by recrystallization from diethyl ether to provide 580 mg pure 2-Acetylsulfanylmethyl-3-(6-*tert*-butyoxycarbonylamino-pyridin-3-yl)-2-hydroxy-propionic acid ethyl ester. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, 3H), 1.53 (s, 9H), 2.34 (s, 3H), 3.02 (m, 2H), 3.3 (d, 1H), 3.53 (d, 1H), 3.79 (s, 1H), 4.16–4.18 (m, 2H), 7.52 (d, $J = 8.7$, 1H), 7.86 (d, $J = 8.7$, 1H), 8.11 (s, 1H), 8.38 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 14.12, 28.29, 30.43, 37.56, 40.69, 62.56, 80.80, 111.63, 125.03, 139.76, 148.80, 151.27, 152.51, 173.53, 194.42. Elemental analysis: Anal. calcd C 54.21%, H 6.52%, N 7.03%; found: C 54.34%, H 6.64%, N 7.01%. MS (CI): 399 (M +).

5.15.3. 2-Mercaptomethyl-3-(6-amino-pyridin-3-yl)-2-hydroxy-propionic acid (16). 2-Acetylsulfanylmethyl-3-(6-*tert*-butyoxycarbonylamino-pyridin-3-yl)-2-hydroxy-

propionic acid ethyl ester (47 mg, 118 μmol) was dissolved in concentrated HCl (2 mL) under argon. The solution was heated to reflux for 18 h. Concentration under reduced pressure yielded compound **16** as the hydrochloride salt (31 mg, 99%). ^{1}H NMR (500 MHz, D_2O): δ 2.87 (d, 1H), 2.98 (d, 1H), 3.08 (d, 1H), 3.11 (d, 1H), 6.99 (d, 1H), 7.68 (s, 1H), 7.85 (d, 1H). HRMS (ESI) calcd for $C_9H_{12}N_2O_3S$ 228.0569, found 228.0616.

5.16. 2-Mercaptomethyl-3-(6-amino-pyridin-3-yl)-2-fluoro-propionic acid (17)

5.16.1. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-fluoro-malonic acid diethyl ester. To a suspension of NaH (1.2 g, 60% in mineral oil, 30.2 mmol) in DMF (100 mL) was added diethyl fluoromalonate (4.3 g, 24.1 mmol) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 0.5 h. A slurry of compound 23 (6.9 g, 24.1 mmol) in DMF (100 mL) was then added dropwise at 0 °C. The reaction mixture was then stirred at room temperature for 16 h. Saturated aqueous ammonium chloride (70 mL) was added slowly and the mixture was extracted with EtOAc. The organic layers was washed with brine and dried. Concentration under reduced pressure followed by flash chromatography (Hexane/EtOAc, 5:1) gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-fluoro-malonic acid diethyl ester (7.0 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 6.9 Hz, 6H), 1.53 (s, 9H), 3.37 (s, 1H), 3.45 (s, 1H), 4.26 (q, J = 7.2 Hz, 4H), 7.57 (d, J = 8.7 Hz, 1H), 7.79 (s, 1H), 7.89 (d, J = 8.7 Hz, 1H), 8.13 (s, 1H). MS (CI): 384.8 (M +).

5.16.2. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-fluoro-malonic acid monoethyl ester (70). A solution of KOH (300 mg, 5.7 mmol) in EtOH (30 mL) was added dropwise to a suspension of 2-(6-*tert*-butoxycarbonyl-amino-pyridin-3-ylmethyl)-2-fluoro-malonic acid diethyl ester (2.0 g, 5.2 mmol) in EtOH (60 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and at room temperature for 6 h. The reaction was quenched at 0 °C using slow addition of 5% HCl (60 mL). The mixture was extracted with EtOAc, washed with brine and dried. Concentration under reduced pressure gave compound **70** (1.5 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (t, J = 7.2 Hz, 3H), 1.51 (s, 9H), 3.31 (t, J = 7.2 Hz, 1H), 3.73 (dd, J = 7.2 Hz, 1H), 4.39 (m, 2H), 7.99 (d, J = 8.7 Hz, 1H), 7.40–7.52 (m, 1H), 8.06 (s, 1H), 8.25 (d, J = 9.0 Hz, 1H). MS (CI): 356.9 (M +).

5.16.3. 3-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-2-fluoro-2-hydroxymethyl-propionic acid ethyl ester (72). *N*-methyl morpholine (38.6 mg, 0.38 mmol) was added to a solution of compound **70** (100 mg, 0.28 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred for 30 min. Isobutylchloroformate (40 mg, 0.31 mmol) was then added dropwise at 0 °C and the reaction mixture was stirred for 1 h. $NaBH_4$ (12 mg, 0.32 mmol) was then added in portions and the mixture was stirred at room temperature for 16 h. Saturated aqueous ammonium chloride (10 mL) was added and the mixture was extracted with EtOAc. The organic layer was washed with brine (10 mL), dried and concentrated under reduced pressure. Flash chromatography (Hexane/EtOAc, 9:1) gave compound **72** (30 mg, 32%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, J = 7.2 Hz, 3H), 1.54 (s, 9H), 3.08 (s, 1H), 3.18 (s, 1H), 3.79–4.02 (m, 2H), 4.25 (q, J = 7.2 Hz, 2H), 7.53 (dd, J = 1.8, 8.7 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 1.8 Hz 1H), 8.44 (s, 1H). MS (CI): 342.8 (M +).

5.16.4. Ethyl-2-(acetylthiomethyl)-3-{6-[(*tert*-butoxy) carbonylamino](3-pyridyl]-2-fluoropropanoate. Diethyl azodicarboxylate (74.8 mg, 0.43 mmol) was added to a solution of compound **72** (70 mg, 0.21 mmol) and PPh_3 (113 mg, 0.43 mmol) in THF (5 mL). The reaction mixture was stirred for 10 min where after thiolacetic acid (39 mg, 0.52 mmol) was added. The reaction mixture was stirred for 16 h. Saturated aqueous $NaHCO_3$ was added and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried. Concentration under reduced pressure followed by flash chromatography (Hexane/EtOAc, 9:1) gave ethyl-2-(acetylthiomethyl)-3-{6-[(*tert*-butoxy) carbonylamino](3-pyridyl]-2-fluoropropanoate (30 mg, 37%) as a white solid. m.p.; 113.5–114.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (t, J = 7.4 Hz, 3H), 1.52 (s, 9H), 2.38 (s, 3H), 3.12 (s, 1H), 3.19 (d, J = 3.9 Hz, 1H), 3.43–3.57 (m, 2H), 4.14 (q, J = 7.5 Hz, 2H), 7.46 (s, 1H), 7.53 (dd, J = 1.5, 8.4 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 8.07 (d, J = 2.4 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 193.50, 169.11, 168.78, 152.74, 151.95, 148.87, 140.01, 123.61, 112.00, 96.95, 94.38, 80.95, 62.09, 38.91, 38.62, 34.73, 34.42, 30.25, 28.21, 13.90. MS (CI): 400 (M +). FT-IR: 1159,1534, 1721, 2987, 3190 cm^{-1}. Elemental analysis: Anal. calcd C, 53.99; H, 6.29; N, 7.00; Found: C, 53.74; H, 6.17; N, 6.89.

5.16.5. 2-Mercaptomethyl-3-(6-amino-pyridin-3-yl)-2-fluoro-propionic acid (17). Ethyl-2-(acetylthiomethyl)-3-{6-[(*tert*-butoxy) carbonylamino](3-pyridyl]-2-fluoropropanoate (53 mg, 132 μmol) was dissolved in concentrated HCl (2 mL) under argon. The solution was heated to reflux for 1 h. Concentration under reduced pressure yielded compound **17** as the hydrochloride salt (39 mg, 99%). ^{1}H NMR (500 MHz, D_2O): δ 3.05-3.22 (m, 4H), 6.98 (d, 1H), 7.68 (s, 1H), 7.82 (d, 1H). HRMS (ESI) calcd for $C_9H_{12}N_2O_2SF$ 231.0604, found 231.0617.

5.17. 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid (18)

5.17.1. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester. A solution of *tert*-butyl ethyl methylmalonate (457 mg, 2.26 mmol) in DMF (4 mL) was added dropwise to a suspension of NaH (90 mg, 2.26 mmol, 60% in oil) in DMF (4 mL). The reaction mixture was stirred for 20 min. A solution of compound **23** (500 mg, 1.74 mmol) in DMF (2.5 mL) was added and the reaction was stirred for 70 min. EtOAc was added and the mixture was washed with water and brine, dried and concentrated under reduced pressure. Chromatography (Heptane/EtOAc, 3:1→1:3) gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester (437 mg, 61% yield). ^{1}H NMR

(500 MHz, $CDCl_3$): δ 1.30 (t, 3H), 1.35 (s, 3H), 1.48 (s, 9H), 1.56 (s, 9H), 3.15 (q, 2H), 4.22 (q, 2H), 7.60 (d, 1H), 7.97 (d, 1H), 8.06 (s, 1H).

5.17.2. 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid mono-*tert*-butyl ester (71). 1M NaOH (2 mL) was added to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester (0.42g, 1.03 mmol) in THF/EtOH (4 mL, 1:1). The reaction mixture was stirred at 50 °C for 16 h. CH_2Cl_2 was added and the mixture was washed with 0.5 M HCl and brine and dried. Concentration under reduced pressure gave compound **71** (348 mg, 89%). ^{1}H NMR (500 MHz, CD_3SOCD_3): δ 1.11 (s, 3H), 1.37 (s, 9H), 1.45 (s, 9H), 2.96 (dd, 2H), 7.50 (d, 1H), 7.66 (d, 1H), 8.01 (s, 1H), 9.63 (s, 1H).

5.17.3. 3-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid *tert*-butyl ester (73). Methyl chloroformate (75 μL, 0.92 mmol) was added dropwise to a solution of compound **71** (348 mg, 0.915 mmol) and Et_3N (123 μL, 0.92 mmol) in THF (6 mL). The reaction mixture was stirred for 20 min., filtered and added dropwise to a suspension of $NaBH_4$ (39 mg, 1.04 mmol) in THF (6 mL) at 0 °C. The reaction was stirred for 16 h at room temperature. 0.2 M HCl was added followed by EtOAc. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromatography (toluene/EtOAc, 3:1→1:3) gave compound **73** (190 mg, 57%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.09 (s, 3H), 1.43 (s, 9H), 1.53 (s, 9H), 2.69 (d, 1H), 2.88 (d, 1H), 3.50 (d, 1H), 3.59 (d, 1H) 7.58 (dd, 1H), 7.74 (d, 1H), 8.04 (d, 1H).

5.17.4. 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester. Diethyl azodicarboxylate (160 μL, 1.01 mmol) was added dropwise to a solution of 3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid *tert*-butyl ester (180 mg, 0.49 mmol) and triphenylphosphine (266 mg, 1.01 mmol) in THF (6 mL) and the reaction was stirred for 5 min. Thiolacetic acid (96 μL, 1.34 mmol) was added and the reaction was stirred for 16 h. Concentration under reduced pressure followed by chromatography (toluene/EtOAc, 10:1→1:1) gave 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester (137 mg, 65%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.10 (s, 3H), 1.42 (s, 9H), 1.55 (s, 9H), 2.35 (s, 3H), 2.79 (d, 1H), 2.94 (d, 1H), 3.12 (d, 1H), 3.21 (d, 1H) 7.56 (d, 1H), 7.78 (d, 1H), 8.02 (s, 1H).

5.17.5. 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid (18). 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester (4 mg, 9.4? μmol) was dissolved in concentrated aqueous HCl under argon. The solution was heated to reflux for 1 h. Concentration under reduced pressure yielded compound **18** as the hydrochloride salt (2.5 mg, 100%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.20 (s, 3H), 2.62 (d, 1H), 2.76–2.83 (m, 2H), 2.95 (d, 1H), 6.94 (d, 1H), 7.64 (d, 1H), 7.80 (dd, 1H). MS (+) 227 (M+1). HRMS (ESI) calcd for $C_{10}H_{14}N_2O_2S$ 226.0776, found 226.0776.

5.18. 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid (19)

5.18.1. 2-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-3-Methyl-5-(2,2,5-trimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin (75). Compound **41** (1.6 g, 4.0 mmol) was added to a solution of 2,2,5-trimethyl-1,3-dioxane-4,6-dione (630 mg, 4.0 mmol) and triethylamine (0.58 mL, 4.2 mmol) in dimethyl sulfoxide (40 mL). The reaction mixture was stirred overnight and water (100 mL) was added. The mixture was extracted with EtOAc, the combined organic phases washed with water and brine and dried. Concentration under reduced pressure gave crude compound **75** (2.06 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.27 (s, 3H), 1.42 (s, 18H), 1.70 (s, 3H), 1.81 (s, 3H), 2.22 (s, 3H), 3.36 (s, 2H), 7.48 (s, 1H), 8.21 (s, 1H).

5.18.2. 2-(6-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester. A solution of sodium metal (184 mg, 8.0 mmol) in ethanol (20 mL) was added to a solution of crude compound **75** (2.06 g, ~4.0 mmol) in ethanol (20 mL) under argon. The reaction was stirred for 60 min. and methylene chloride was then added. The mixture was washed with 0.5 M HCl and brine, dried and concentrated under reduced pressure to give crude 2-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester (1.9 g). ^{1}H NMR (500 MHz, CD_3SOCD_3): δ 1.20 (t+s, 6H), 1.35 (s, 18H), 2.11 (s, 3H), 3.13 (q, 2H), 4.13 (m, 2H), 7.52 (d, 1H), 8.09 (d, 1H), 13.11 (bs, 1H).

5.18.3. 3-(6-[*N*,*N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid ethyl ester (77). Methyl chloroformate (338 μL, 4.4 mmol) was added dropwise to a solution of crude 2-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester (1.9 g) and Et_3N (641 μL, 4.6 mmol) in THF (30 mL) at −20 °C. The reaction mixture was stirred for 50 min., filtered and added dropwise to a suspension of $NaBH_4$ (182 mg, 4.8 mmol) in THF (30 mL) at −20 °C. The reaction was stirred for 16 h at room temperature. 0.5 M HCl was added followed by methylene chloride. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromatography (toluene/EtOAc, 10:1→1:3) gave compound **77** (885 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (s, 3H), 1.24 (t, 3H), 1.39 (s, 18H), 2.09 (s, 3H), 2.70 (broad s, 1H), 2.95 (dd, 2H), 3.55 (dd, 2H), 4.18 (q, 2H), 7.42 (s, 1H), 8.13 (s, 1H).

5.18.4. 2-Acetylsulfanylmethyl-3-(6-[*N*,*N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester. Diisopropyl azodicarboxylate (755 μL, 3.91 mmol) was added dropwise to a solution of triphenylphosphine (1.026 g, 3.91 mmol) in THF (10 mL) at 0 °C and the reaction was stirred for 30 min. A solution of thiolacetic acid (279 μL, 3.91 mmol) and compound **77** (885 mg, 1.96 mmol) in THF (5 mL) was added dropwise during 10 min. The reaction was stirred for 60 min. at 0 °C and then for 16 h at room temperature. Concentration under reduced pressure followed by

chromatography (heptane/EtOAc, 10:1 1:3) gave impure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (1.46 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 3H), 1.15 (t, 3H), 1.62 (m, 2H), 3.03 (m, 2H), 4.04–4.14 (m, 2H), 7.49 (dd, 1H), 7.62 (d, 1H), 7.96 (d, 1H), 9.88 (s, 1H), 13.0 (bs, 1H).

5.18.5. 2-Acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester. Crude 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (1.46g) was dissolved in TFA (5 mL) and stirred for 60 min. Concentration under reduced pressure followed by chromatography (toluene/EtOAc, 1:1→1:10→0:1) gave slightly impure 2-acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (696 mg, 84%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.16 (s, 3H), 1.23 (t, 3H), 1.58 (s, 3H), 2.23 (s, 3H), 2.76 (d, 1H), 2.94 (d, 1H), 3.19 (dd, 2H), 4.11 (m, 2H), 7.52 (s, 1H), 7.61 (s, 1H).

5.18.6. 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid (19). 2-Acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (17 mg, 40 μmol) was dissolved in concd HCl (2 mL) under argon. The solution was heated to reflux for 150 min. Concentration under reduced pressure gave compound **19** as the hydrochloride salt (10.7 mg, 96%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.20 (s, 3H), 2.23 (s, 3H), 2.61 (d, 1H), 2.79 (2d, 2H), 2.94 (d, 1H), 7.55 (m, 1H), 7.69 (m, 1H). MS (+) 241 (M + 1).

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