Second-Generation Inhibitors for the Metalloprotease Neprilysin Based on Bicyclic Heteroaromatic Scaffolds: Synthesis, Biological Activity, and X-Ray Crystal-Structure Analysis

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A new class of nonpeptidic inhibitors of the Zn^{II}-dependent metalloprotease neprilysin with IC_{50} values in the nanomolar activity range (0.034 – 0.30 μM) were developed based on structure-based *de novo* design (*Figs. 1* and *2*). The inhibitors feature benzimidazole and imidazo[4,5-*c*]pyridine moieties as central scaffolds to undergo H-bonding to Asn542 and Arg717 and to engage in favorable π-π stacking interactions with the imidazole ring of His711. The platform is decorated with a thiol vector to coordinate to the Zn^{II} ion and an aryl residue to occupy the hydrophobic S1′ pocket, but lack a substituent for binding in the S2′ pocket, which remains closed by the side chains of Phe106 and Arg110 when not occupied. The enantioselective syntheses of the active compounds (+)-**1**, (+)-**2**, (+)-**25**, and (+)-**26** were accomplished using *Evans* auxiliaries (*Schemes 2*, *4*, and *5*). The inhibitors (+)-**2** and (+)-**26** with an imidazo[4,5-*c*]pyridine core are *ca.* 8 times more active than those with a benzimidazole core ((+)-**1** and (+)-**25**) (*Table 1*). The predicted binding mode was established by X-ray analysis of the complex of neprilysin with (+)-**2** at 2.25-Å resolution (*Fig. 4* and *Table 2*). The ligand coordinates with its sulfanyl residue to the Zn^{II} ion, and the benzyl residue occupies the S1′ pocket. The 1*H*-imidazole moiety of the central scaffold forms the required H-bonds to the side chains of Asn542 and Arg717. The heterobicyclic platform additionally undergoes π-π stacking with the side chain of His711 as well as edge-to-face-type interactions with the side chain of Trp693. According to the X-ray analysis, the substantial advantage in biological activity of the imidazopyridine inhibitors over the benzimidazole ligands arises from favorable interactions of the pyridine N-atom in the former with the side chain of Arg102. Unexpectedly, replacement of the phenyl group pointing into the deep S1′ pocket by a biphenyl group does not enhance the binding affinity for this class of inhibitors.

1. Introduction. – In the preceding paper [1], we described a new class of inhibitors of the metalloprotease neprilysin with a central 1*H*-imidazole platform, featuring IC_{50} values (IC_{50}: concentration of inhibitor at which 50% V_{max} is observed) in the low micromolar range. The *de novo* design of these compounds was based on the X-ray crystal structure of NEP complexed with phosphoramidon (Protein Data Bank (PDB) file name 1DMT) [2]. For the design of the second-generation inhibitors, we reverted to an unpublished X-ray crystal structure [3] of NEP complexed with the inhibitor thiorphan [4]. During the analysis, we carefully compared the active sites of the two

different crystal structures (*Fig. 1*). The larger phosphoramidon occupies both the S1′ and the S2′ pockets of the enzyme (blue structure in *Fig. 1*), whereas the smaller thiorphan fills only the S1′ pocket (green structure in *Fig. 1*). In the thiorphan complex, the S2′ pocket is closed by the side chains of Arg110 and Phe106.

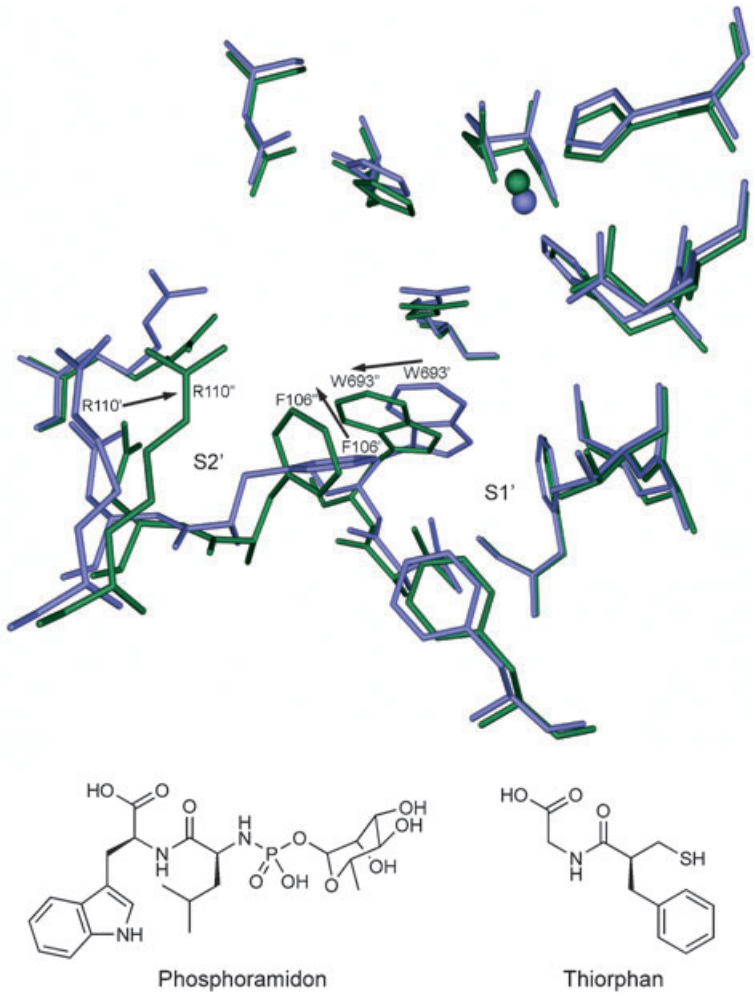


Fig. 1. *Superimposed active-site residues seen in the X-ray crystal structures of NEP complexed with phosphoramidon* (blue) *and thiorphan* (green). The inhibitors are omitted for clarity. The conformational changes leading to the closure of the unoccupied S2′ pocket in the thiorphan complex are indicated by arrows.

Although thiorphan does not occupy the S2′ pocket, it is a very potent neprilysin inhibitor ($K_i = 4.7 \pm 1.2$ nM [4]). Clearly, the occupancy of the S2′ pocket is not necessary for good inhibitor binding [5]. For instance, *De Lombaert et al.* developed very potent inhibitors without substituents in the S2′ pocket [6]. Also, our investigations of the first generation, 1*H*-imidazole-based inhibitors [1] had shown that increasing the size of the S2′ substituent from a Ph to a naphthalenyl residue did

not translate into enhanced binding, which reflects that this pocket is not well-defined (actually, it has been shown that the P2′ and P1′ residues depend on each other: smaller P1′ substituents allow for the presence of both, either smaller or larger P2′ residues [7]). Based on these observations, we designed the two potential inhibitors (+)-**1** and (+)-**2** without a substituent pointing into this pocket (*Fig. 2,a*). The two compounds feature the same vectors to coordinate to the Zn^{II} ion (sulfanyl residue) and to fill the S1′ (Ph residue) as the inhibitors described in the preceding paper [1]. As platforms, which should anchor by H-bonding to the side chains of Asn542 and Arg717, we introduced more-expanded heterocyclic systems, compared with the 1*H*-imidazole core in the first-generation inhibitors. According to modeling studies with the program MOLOC [8], benzimidazole and imidazo[4,5-*c*]pyridine should be able to form the above-mentioned obligatory H-bonds and, additionally, undergo attractive π-π stacking with the side chain of His711 and edge-to-face interactions with the indole ring of Trp693 [9]. Furthermore, we expected formation of an H-bond between the pyridine N-atom in (+)-**2** and the side chain of Arg102 (*Fig. 2,b*). Here, we report the synthesis of (+)-**1** and (+)-**2**, and some analogs, their biological activities, and the X-ray crystal structure of NEP bound to inhibitor (+)-**2** (for a preliminary communication on parts of this work, see [10]).

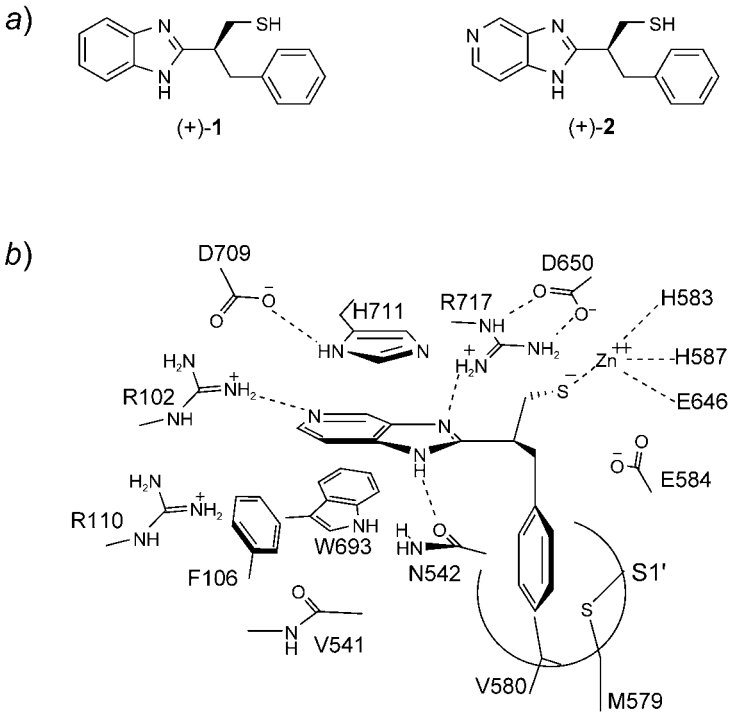
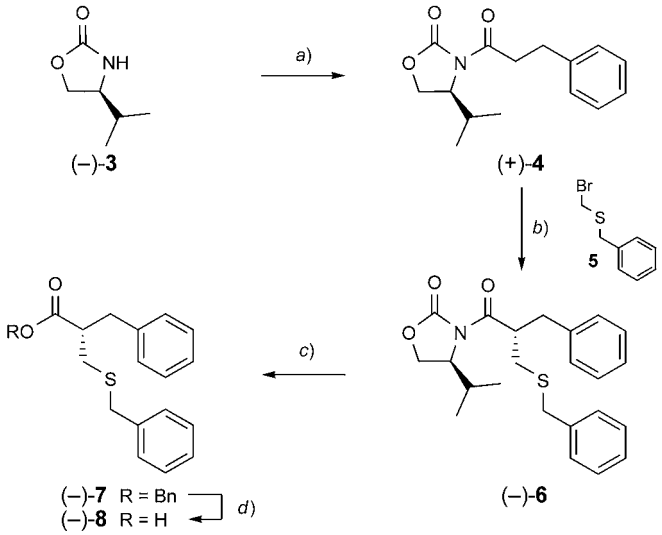


Fig. 2. a) *Structures of the new inhibitors (+)-***1** *and (+)-***2**. b) *Schematic representation of the predicted interactions of (+)-***2** *at the active site of NEP.* A similar binding mode is predicted for ligand (+)-**1**. Potential H-bonds are shown as dashed lines.

2. Results and Discussion. – 2.1. *Synthesis of Inhibitors (+)-***1** *and (+)-***2**. Stereoselective synthesis started from oxazolidinone (−)-**3** derived from commercially available (*S*)-valinol and diethyl carbonate. According to a procedure of *Evans et al.* [11], (−)-**3** was acylated to give (+)-**4**, which, by diastereoselective alkylation with $BnSCH_2Br$ (**5**), provided (−)-**6** (*Scheme 1*). The α-bromo thioether was synthesized according to *Reich et al.* from benzenemethanethiol, HBr, and *s*-trioxane [12]. Oxazolidinone (−)-**6** was subsequently converted *via* benzyl ester (−)-**7** to carboxylic acid (−)-**8** [11].

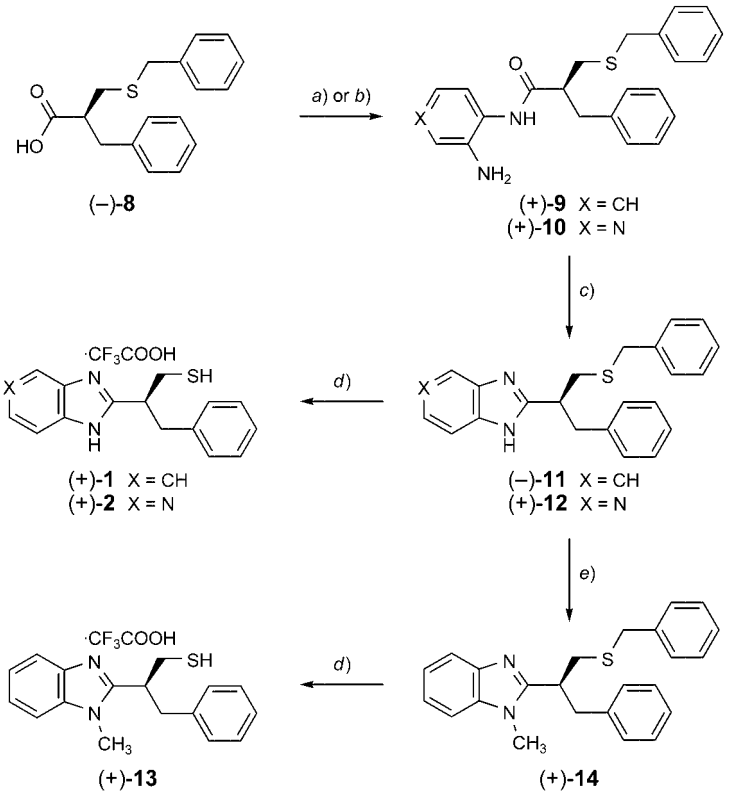
Scheme 1. *Diastereoselective Synthesis of the Carboxylic Acid (−)-***8**



a) BuLi, 3-phenylpropanoyl chloride, THF, −78°→0°, 35 min; 65%. *b*) 1. $(i\text{-Pr})_2NH$, BuLi, THF, 0°→r.t., 15 min; 2. **5**, −78° → −15°, 14 h; 62%, diastereoisomeric ratio dr > 95 : 5. *c*) BnOH, BuLi, THF, −10°→0°, 2 h; 64%. *d*) HBr, AcOH, 50°, 15 min; 82%.

One method to build up 2-substituted benzimidazoles is the condensation of carboxylic acids with arene-1,2-diamines [13]. *Maekawa* and *Ohtani* developed a mild protocol consisting of amide coupling between carboxylic acid and amine, followed by condensation to the heteroaromatic bicycle in AcOH [14]. We used a similar protocol to build up the desired 2-substituted benzimidazole and imidazo[4,5-*c*]pyridine scaffolds, respectively. Amide coupling of carboxylic acid (−)-**8** with benzene-1,2-diamine or pyridine-3,4-diamine was accomplished by forming *in situ* a mixed anhydride with i-BuOCOCl, followed by addition of the amine to give (+)-**9** and (+)-**10**, respectively (*Scheme 2*; for the constitutional assignment, see *Sect. 2.4*) [15][16]. Unfortunately, the yields were low (see *Sect. 2.2* for optimized coupling conditions). The intramolecular condensation of (+)-**9** and (+)-**10** according to *Chen et al.* yielded bicycles (−)-**11** and (+)-**12** in high yields [17]. To prepare control compound (+)-**13**, (−)-**11** was *N*-methylated with NaH and MeI to give (+)-**14**. Final *S*-debenzylation of (−)-**11**, (+)-**12**, and (+)-**14** was accomplished with Na in liquid NH_3 to afford (+)-**1**, (+)-**2**, and (+)-**13**, respectively. The crude products were purified by

Scheme 2. *Synthesis of the Inhibitors (+)-***1** *and (+)-***2** *Starting from (−)-***8**

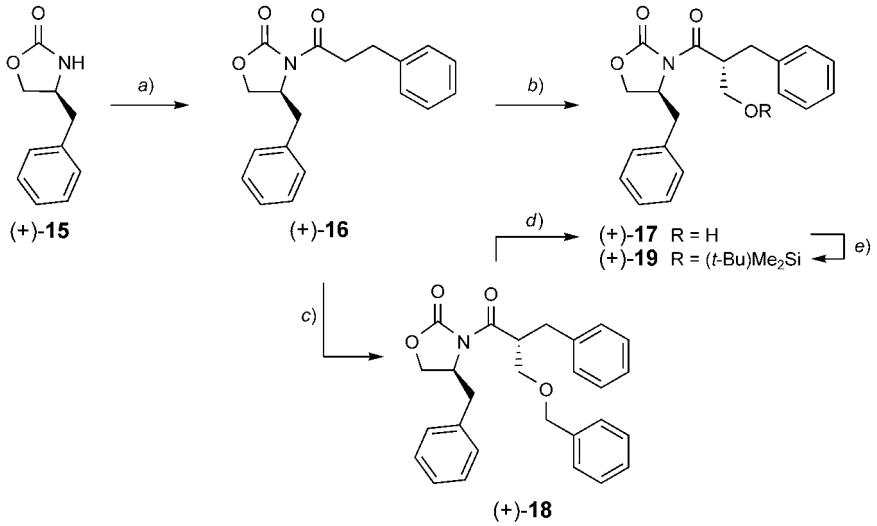


a) Benzene-1,2-diamine, *N*-methylmorpholine, i-BuOCOCl, THF, −20°, 16 h; 25%. *b*) Pyridine-3,4-diamine, *N*-methylmorpholine, i-BuOCOCl, THF, −20°, 16 h; 51%. *c*) AcOH, 65°, 2.5 h; 80% (−)-**11**, 93% (+)-**12**. *d*) Na, NH_3, THF, −78°, 30 min; 33% (+)-**1**, 10% (+)-**2**, 61% (+)-**13**. *e*) NaH, MeI, THF, 0° → r.t., 55 min; 95%.

reversed-phase (RP) HPLC with 0.1% aq. CF_3COOH/MeCN as eluent to give the corresponding trifluoroacetate salts. Disappointingly, the yields of (+)-**1** and (+)-**2** were low to very low, presumably due to isolation problems.

2.2. *Modified Synthesis of Inhibitor* (+)-**2**. Since the synthesis of (+)-**2** described in *Sect. 2.1* was unsatisfactory in terms of yields, a modified synthesis was planned in which the sulfanyl residue is introduced at the end by a *Mitsunobu* reaction. The synthesis started from oxazolidinone (+)-**15**, which was obtained from L-phenylalanine in two steps [18][19]. Treatment of (+)-**15** with BuLi and 3-phenylpropanoyl chloride afforded (+)-**16** (*Scheme 3*) [20]. The direct conversion to (+)-**17** *via* the titanium enolate of (+)-**16**, with *s*-trioxane as electrophile, gave only modest yields. Therefore, the indirect path *via* Bn-protected (+)-**18** [20] was pursued. Debenzylation to (+)-**17** was accomplished by using H_2 and Pd/C [21]. The overall yield of the two-step conversion was substantially higher than that of the one-step protocol. Alcohol (+)-**17** was readily silylated with (*t*-Bu)Me_2SiCl to give (+)-**19**.

Scheme 3. *Diastereoselective Synthesis of (+)-***19**

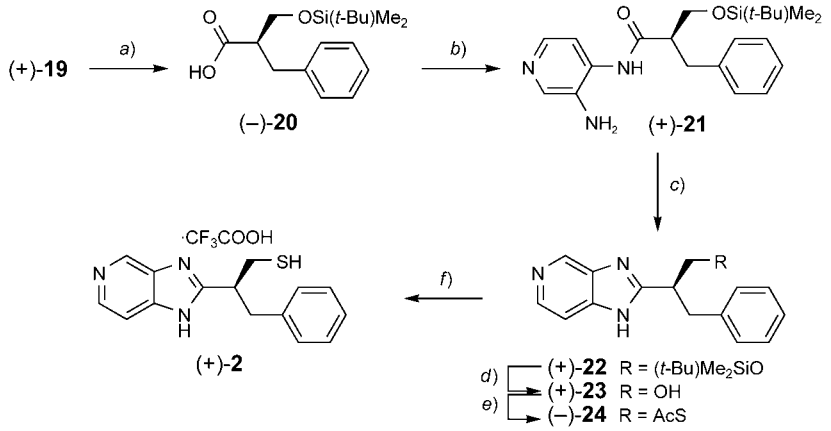


a) BuLi, 3-phenylpropanoyl chloride, THF, $-78° \rightarrow 0°$, 35 min; 77%. *b*) $TiCl_4$, Et_3N, *s*-trioxane, CH_2Cl_2, 0°, 3.5 h; 48%. *c*) $TiCl_4$, $EtN(i\text{-}Pr)_2$, $BnOCH_2Cl$, CH_2Cl_2, 0°, 4.5 h; 80%. *d*) H_2 (1 atm), Pd/C, AcOEt, HCl, r.t., 6 h; 86%. *e*) (*t*-Bu)Me_2SiCl, DMAP, CH_2Cl_2, 16 h, r.t.; 71%. DMAP = 4-(Dimethylamino)pyridine.

The chemoselective hydrolysis of the exocyclic 'amide' group of (+)-**19** was accomplished using LiOOH, generated *in situ* from H_2O_2 and LiOH, to give carboxylic acid (−)-**20** and to regain oxazolidinone (+)-**15** (*Scheme 4*) [22] (for an earlier synthesis of racemic (±)-**20**, see [23]). Since the amide coupling with pyridine-3,4-diamine described in *Sect. 2.1* gave low yields, we established an alternative protocol according to a procedure of *Pigro et al.*, who coupled pyridine-2,6-diamines with carboxylic acids [24]. Acid (−)-**20** was transformed *in situ* into its corresponding acid chloride with $(COCl)_2$, then the amine was added to produce amide (+)-**21**. Treatment with AcOH gave the 2-substituted imidazo[4,5-*c*]pyridine (+)-**22**. Deprotection with Bu_4NF provided the primary alcohol (+)-**23**, which was converted in a *Mitsunobu* reaction with AcSH to thioester (−)-**24** in excellent yield [1][25]. Final deprotection of the acetylated sulfanyl group according to *Zervas et al.* yielded the desired (+)-**2** [26]. The isolated yield of 73% was much higher than that obtained pursuing the Bn-deprotection route (see *Sect. 2.1*). The two pK_a-values of (+)-**2** were determined by potentiometric titrations as 6.19 and 10.59 (for a full experimental description of the pK_a determinations, see [27]).

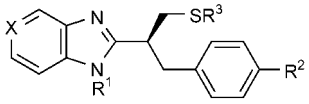
2.3. *Biological Activity of (+)-***1**, *(+)-***2**, *(−)-***11**, *and (+)-***13**. The *in vitro* activity of (+)-**1**, (+)-**2**, (−)-**11**, and (+)-**13** towards neprilysin was determined in a fluorometric assay (*Table 1*; for details, see [1]). Gratifyingly, the IC_{50} values of (+)-**1** and (+)-**2** are in the nanomolar range; these compounds show *ca.* 5–25 times higher affinities towards neprilysin compared to the imidazole-based inhibitors [1]. Interestingly, ligand (+)-**2** ($IC_{50} = 0.040$ μM; the higher value given in the preliminary communication [10] is wrong) is seven times more active than (+)-**1** ($IC_{50} = 0.29$ μM). The enhanced activity seems to support the modeling-based proposal (*Fig. 2*, *b*) that the pyridine N-atom of

Scheme 4. *Modified Synthesis of (+)-***2**



a) H_2O_2 (30%), $LiOH \cdot H_2O$, THF/H_2O, 0°, 3 h; 66%. *b*) 1. $(COCl)_2$, CH_2Cl_2, r.t., 12 h; 2. Pyridine-3,4-diamine, Et_3N, r.t., 12 h; 93%. *c*) AcOH, 65°, 2.5 h; 96%. *d*) Bu_4NF, THF, r.t., 2.5 h; 84%. *e*) DIAD, Ph_3P, AcSH, THF, 0° → r.t., 3 h; 93%. *f*) MeONa, MeOH, H_2, r.t., 75 min; 73%. DIAD = Diisopropyl azodicarboxylate.

Table 1. *Activities of the New Bicyclic Inhibitors towards Neprilysin*



Inhibitor	X	R^1	R^2	R^3	IC_{50} [μM]
(+)-**1**	CH	H	H	H	0.29
(+)-**2**	N	H	H	H	0.040
(−)-**11**	CH	H	H	Bn	30[a])
(+)-**13**	CH	Me	H	H	5.3
(+)-**25**	CH	H	Ph	H	0.30
(+)-**26**	N	H	Ph	H	0.034

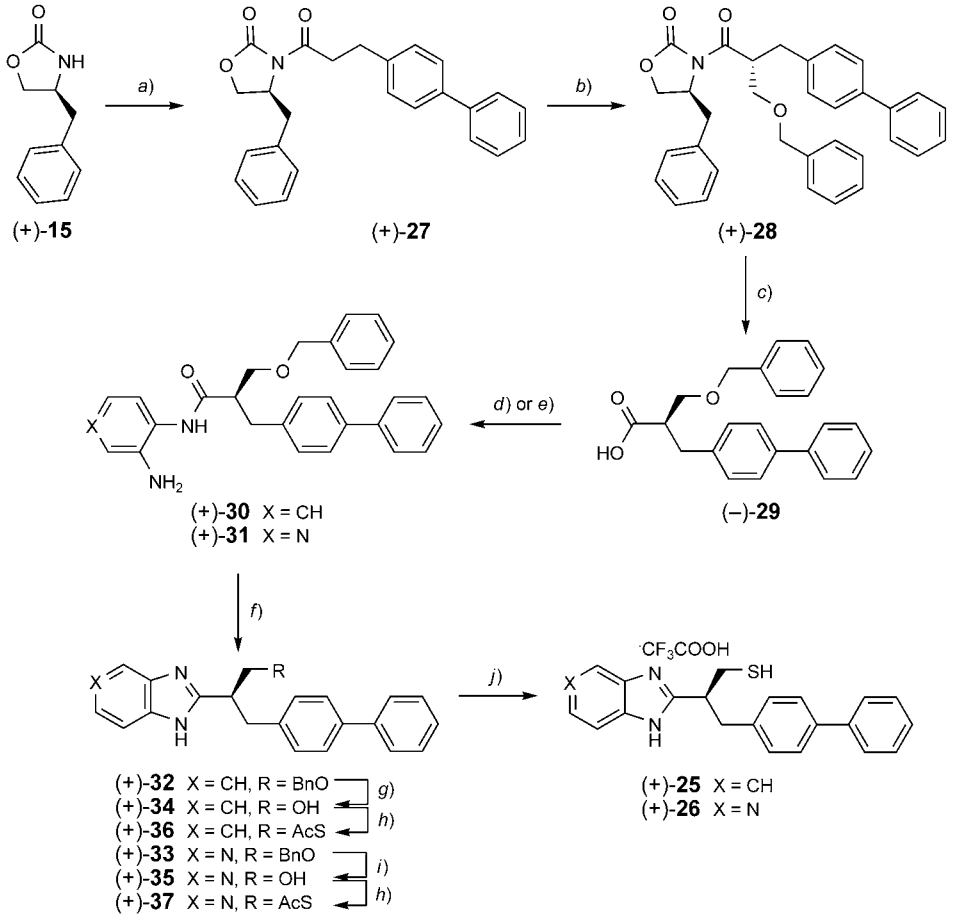
[a]) % Inhibition at 100 μM inhibitor concentration.

(+)-**2** undergoes additional attractive interactions with the enzyme, for instance by H-bonding to the side chain of Arg102 (see also *Sect. 2.6*). As a control, the binding affinity of benzylated (−)-**11** is very weak, although the Bn group is well accommodated in the active site according to the modeling. Clearly, a thiol ligand to coordinate to the Zn^{II} ion is still needed for a good binding. The binding affinity of *N*-methylated benzimidazole (+)-**13** ($IC_{50} = 5.3$ μM) is reduced by a factor of 20, compared to (+)-**1**. This result confirms the assumption that the 1*H*-imidazole moiety in the heterocyclic scaffold of (+)-**1** and (+)-**2** serves as a peptide-bond isoster and undergoes H-bonding to the side chains of Asn542 and Arg717.

2.4. *Synthesis of Inhibitors (+)-***25** *and (+)-***26** *with 1,1′-Biphenyl Substituents to Fill the S1′ Pocket*. The S1′ pocket is very deep and can accommodate large substituents as was shown in different studies [6][7][28][29]. For instance, *De Lombaert et al.* could enhance the affinity of their inhibitors by a factor of 30 by replacing Ph by 1,1′-biphenyl

as the S1′-pocket-filling substituent [28]. To test whether we would score similar gains in binding free enthalpy in our class of ligands, we prepared compounds (+)-**25** and (+)-**26** with 1,1′-biphenyl substituents. Their synthesis started from 3-(1,1′-biphenyl-4-yl)propanoic acid, obtained in two steps from [1,1′-biphenyl]-4-carbaldehyde and malonic acid [30], and oxazolidinone (+)-**15**, which were coupled to yield (+)-**27** (*Scheme 5*) [31]. Diastereoselective alkylation of the titanium enolate of (+)-**27** with $BnOCH_2Cl$ afforded (+)-**28** in excellent yield and high diastereoselectivity. Chemoselective hydrolysis of (+)-**28** with LiOOH gave (−)-**29**. The amide coupling of

Scheme 5. *Synthesis of Inhibitors (+)-**25** and (+)-**26***



a) 1. BuLi, −78°, 30 min; 2. 3-[1,1′-biphenyl-4-yl]propanoic acid, Et_3N, *t*-BuCOCl, −78° → 0°, 1 h; 76%. *b*) $TiCl_4$, $EtN(i\text{-}Pr)_2$, $BnOCH_2Cl$, CH_2Cl_2, 0°, 5 h; 94%. *c*) H_2O_2 (30%), $LiOH \cdot H_2O$, THF/H_2O, 0°, 2.5 h; 73%. *d*) 1. $(COCl)_2$, CH_2Cl_2, r.t., 12 h; 2. Benzene-1,2-diamine, Et_3N, r.t., 12 h; 66%. *e*) 1. $(COCl)_2$, CH_2Cl_2, r.t., 12 h; 2. pyridine-3,4-diamine, Et_3N, r.t., 12 h; 57%. *f*) AcOH, 65°, 2.5 h; 85% (+)-**32**, 100% (+)-**33**. *g*) 1. BCl_3, CH_2Cl_2, 4 h, −78°; 2. MeOH, NH_3, 0° → r.t., 10 min; 34%. *h*) DIAD, Ph_3P, AcSH, THF, 0° → r.t., 3 h; 44% (+)-**36**, 46% (+)-**37**. *i*) 1. BCl_3, CH_2Cl_2, 3 h, −78°; 2. MeOH, $NaHCO_3$, 0° → r.t., 12 h; 3. H_2SO_4, EtOH, 50°, 2 h then r.t. 12 h; 59%. *j*) MeONa, MeOH, H_2, r.t., 75 min; 68% (+)-**25**, 57% (+)-**26**.

carboxylic acid (−)-**29** with benzene-1,2-diamine and pyridine-3,4-diamine gave (+)-**30** and (+)-**31**, respectively, and the subsequent condensations to the heteroaromatic bicycles (+)-**32** and (+)-**33** was accomplished as described in *Sect. 2.2*. The lower yields of the amide couplings are due to formation of the corresponding bisamides.

Crystals of (+)-**31** were obtained by recrystallization from AcOEt and analyzed by X-ray crystallography (*Fig. 3*). The compound crystallizes in the chiral space group $P2_1$. The crystal structure led to the constitutional assignment for (+)-**31**, whose NH_2 group is in *meta*-position to the pyridine N-atom. The same constitution was derived for amides (+)-**10** and (+)-**21** by comparison of the ^{1}H-NMR spectra.

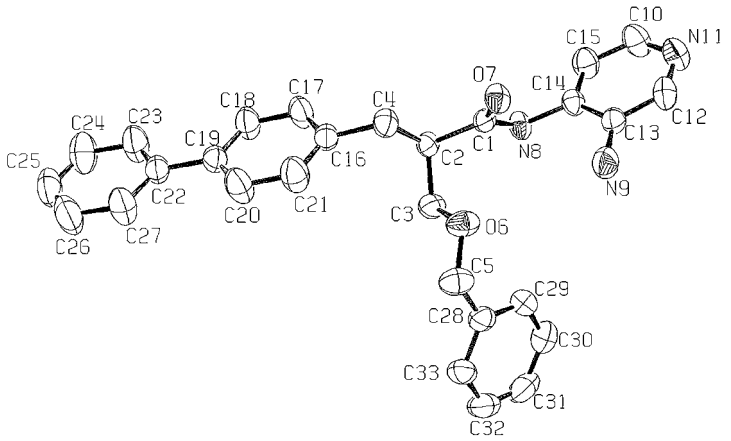


Fig. 3. *Crystal structure of (±)-***31**. The ORTEP representation is shown with atomic-displacement-parameter ellipsoids at the 50% probability level. Arbitrary Numbering.

O-Debenzylation of (+)-**32** and (+)-**33** by hydrogenation with H_2 and Pd/C in MeOH or EtOH was unsuccessful, even at enhanced H_2 pressure and catalyst loading. Deprotection of (+)-**32** was finally accomplished with BCl_3 in CH_2Cl_2 to give (+)-**34** in 34% yield [32]. The low yield is likely due to the problematic chromatographic separation of B-containing impurities. For the debenzylation of (+)-**33**, we also used BCl_3 in CH_2Cl_2, but after workup, the product could not be separated from all impurities. Therefore, the crude product was treated with H_2SO_4 in EtOH to give (+)-**35** in 59% overall yield. The two primary alcohols (+)-**34** and (+)-**35** were transformed into their corresponding thioesters (+)-**36** and (+)-**37** as described in *Sect. 2.2*. Final hydrolysis of the thioesters afforded the desired inhibitors (+)-**25** and (+)-**26**.

2.5. *Biological Activity of (+)-***25** *and (+)-***26**. The IC_{50} values of the two inhibitors (*Table 1*) are in the same range as those of the initial leads (+)-**1** and (+)-**2**. This result shows that, contrary to literature-based expectations, introduction of a 1,1′-biphenyl substituent to fill the S1′ pocket does not lead to higher binding affinities towards neprilysin. It seems likely that the extended heteroaromatic platform and the 1,1′-biphenyl moiety cannot be both positioned in an ideal way in the active site. Inhibitor (+)-**26** is by a factor of *ca.* 9 more potent than (+)-**25**. This finding further supports the assumption that the pyridine N-atom in (+)-**26** undergoes H-bonding with the side chain of Arg102.

2.6. *Crystal Structure of Neprilysin Complexed with (+)-***2**. For inhibitor binding studies, compound (+)-**2** was soaked into NEP crystals at mM concentration (for a detailed protocol, see *Exper. Part* and *Table 2*). The crystal structure (PDB file name 1Y8J) confirms the predicted binding mode (*Fig. 4*). The inhibitor is nestled in the

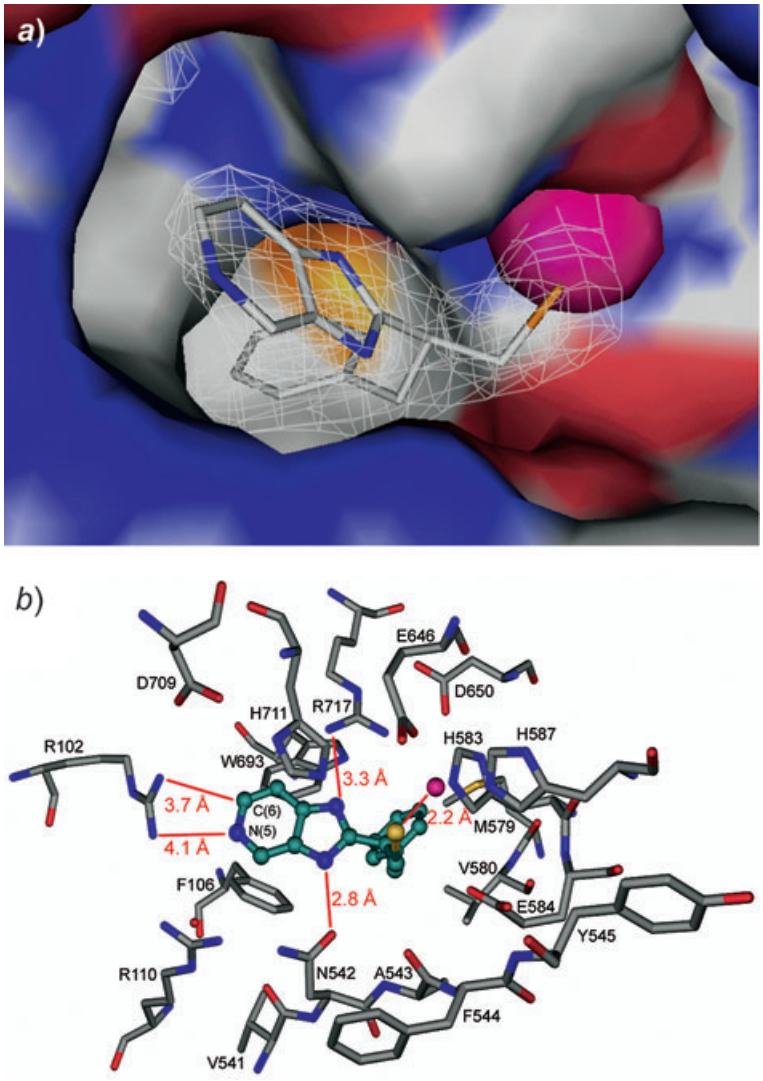


Fig. 4. *Section of the crystal structure of NEP complexed with (+)-***2**. a) *2Fo-Fc omit electron-density map for (+)-***2** *at 2.25 Å, calculated with phases from the refined model.* The map, contoured at 1σ, is shown in light grey mesh. The molecular surfaces of the S1′ and S2′ sub-sites are indicated and colored by electrostatic potential; blue, positive; red, negative. The Zn^{II} ion is shown as a red sphere. The *Figure* was generated using *PyMOL* [33]. b) *Top view of (+)-***2** *bound in the active site of NEP.* Interesting distances discussed in the text are shown in red. c) *Front view showing H-bonds and coordinate bonds* (black dashed lines) *and the aromatic interactions involving the central imidazopyridine scaffold* (red) *of (+)-***2**. A H_2O molecule is indicated as small red sphere. Aromatic contacts are shown as red lines.

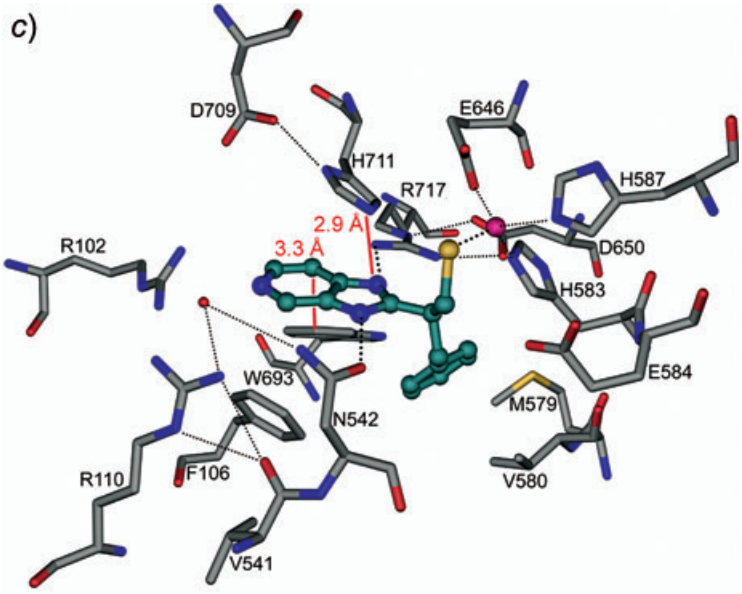


Fig. 4 (cont.)

interior of the enzyme cavity and binds near the conserved residues of the consensus sequences ^{583}HExxH^{587} and ^{646}ExxxD^{650} and the residues of the ^{542}NAFY^{545} motif. *Fig. 4*,*a*, shows the final difference-omit electron-density map for (+)-**2** at 2.25-Å resolution, calculated with phases from the refined model [33]. The inhibitor coordinates the Zn^{II} ion *via* its sulfanyl moiety with an interatomic distance $d(S\cdots Zn) = 2.2$ Å (*Fig. 4*,*b*). The Bn residue expectedly occupies the S1′ pocket, encompassed by Phe106, Ile558, Phe563, Met579, Val580, Val692, and Trp693 (*Fig. 4*,*c*) [1]. The annellated imidazole ring forms the required two H-bonds to the side chains of Asn542 ($d(N\cdots O) = 2.8$ Å) and Arg717 ($d(N\cdots N) = 3.3$ Å; *Fig. 4*,*b*). Additionally, it is nicely sandwiched between the side chains of His711 and Trp693, undergoing π-π interactions at short distance (2.9 Å) with the imidazole ring of the former and edge-to-face-type interactions (shortest $C\cdots C$ distance: 3.3 Å) with the indole ring of the latter. The distances between N(5) and C(6) of the imidazo[4,5-*c*]pyridine and the N-atoms of the side chain of Arg102 are 4.1 Å and 3.7 Å, respectively. At a resolution of 2.25 Å, it is not clearly apparent, which position the N-atom takes in the pyridine ring. Thus, it is also possible that the other tautomeric form (1*H*-imidazo[4,5-*c*]pyridine instead of 3*H*-imidazo[4,5-*c*]pyridine) binds to the enzyme or that both tautomers are present. Indeed, we suggest that the scaffold is complexed as 1*H*-imidazo[4,5-*c*]pyridine tautomer, since this binding mode establishes the short distance (3.7 Å) between the attracting N(pyridine) and N–H(Arg) and the long distance (4.1 Å) between the repulsive C–H(pyridine) and N–H(Arg) fragments.

3. Conclusions. – This paper reports the successful development of new neprilysin inhibitors with heteroaromatic bicyclic scaffolds. The stereoselective synthesis of the

chiral, nonracemic ligands was accomplished using *Evans* auxiliaries. Two different synthetic routes were explored with the most efficient one (*Schemes 4* and *5*) featuring the introduction of the thiol residue (for coordination to the Zn^{II} ion of the metalloprotease) in a *Mitsunobu* reaction at the end of the multi-step sequence. The binding affinities towards neprilysin of the new inhibitors with the heterobicyclic scaffolds were strongly improved (by a factor of 5–25) over the first-generation imidazole-based inhibitors [1]. Inhibitors (+)-**2** and (+)-**26** with an imidazo[4,5-*c*]pyridine core proved to be more active than the corresponding benzimidazole-derived ligands (+)-**1** and (+)-**25** by a factor of *ca.* 8. The binding mode predicted by modeling studies was established by the X-ray-analysis of the complex of neprilysin with (+)-**2** at 2.25-Å resolution (*Fig. 4*). The ligand coordinates with its thiol residue to the Zn^{II} ion, and the Bn residue occupies the S1′ pocket. The 1*H*-imidazole moiety of the central scaffold forms the required H-bonds to the side chains of Asn542 and Arg717. At the same time, the heterobicyclic platform undergoes aromatic interactions with the side chains of His711 and Trp693. According to the X-ray analysis, the substantial advantage in biological activity of the imidazo-pyridine inhibitors over the benzimidazole ligands arises from favorable interactions of the pyridine N-atom in the former with the side chain of Arg102. In future work, we intend to further explore favorable contacts between suitably decorated heterocyclic scaffolds and residues above the S2′ pocket of the protein. Contrary to literature expectations, introduction of 1,1′-biphenyl substituents for occupation of the deep S1′ pocket did not lead to enhanced binding activities and further optimization of the residues for filling this pocket will be required.

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Experimental Part

General. See [1]. The following compounds were prepared according to literature procedures: (−)-**3** [11], (+)-**4** [11], **5** [12], (−)-**7** [11], (−)-**8** [11], (+)-**15** [18], (+)-**16** [20], (+)-**18** [20], 3-[1,1′-biphenyl-4-yl]propanoic acid [30]. Optical rotations: *Perkin-Elmer 241* polarimeter; 1-dm cell, $\lambda = 589$ nm (Na-D-line). Prep. RP-HPLC: *Merck Hitachi* HPLC with HPLC pump *L-7150*; *Vydac 201TP C18* column (2501 × 4 mm, 5 μm, 100 Å).

Biological Assay. See [1]. *General Procedure for the Amide Coupling with Aromatic Amines* (*GP 1*). *Method A:* To a soln. of carboxylic acid (1 equiv.) in THF (0.05M), 4-methylmorpholine (1.5 equiv.) and isobutyl chloroformate (1.1 equiv.) were added dropwise at −20°. After stirring for 15 min, the arom. amine (2 equiv.) was added, and the mixture was stirred for additional 16 h at −20°. The solvent was removed *in vacuo*, the residue was dissolved in AcOEt and washed with half-sat. aq. NH_4Cl soln., half-sat. aq. $NaHCO_3$ soln., and sat. aq. NaCl soln. The org. phases were dried ($MgSO_4$) and concentrated *in vacuo*.

Method B: To a soln. of carboxylic acid (1 equiv.) in CH_2Cl_2 (0.1M), $(COCl)_2$ (1 equiv.) was added at 0°, and the mixture was stirred for 12 h at r.t. The solvent was removed *in vacuo*, and the residue was dissolved in THF (0.1M). The arom. amine (2 equiv.) and NEt_3 (2 equiv.) were added, and the mixture was stirred for 12 h at r.t. After addition of H_2O, THF was removed *in vacuo*, and the residual aq. mixture was extracted (CH_2Cl_2). The org. phases were washed with sat. aq. NaCl soln., dried (Na_2SO_4), and concentrated *in vacuo*.

General Procedure for the Condensation to Bicyclic Heteroaromatics (*GP 2*). The amide (1 equiv.) was dissolved in AcOH, and the mixture was stirred at 65° for 2.5 h. AcOH was removed by adding PhMe (3×), followed by concentration *in vacuo*. The residue was dissolved in CH_2Cl_2, and the soln. was washed with sat. aq. $NaHCO_3$ soln. The aq. phases were extracted (CH_2Cl_2), and the combined org. phases were dried ($MgSO_4$) and concentrated *in vacuo*.

General Procedure for the Benzyl Thioether Cleavage with Na/NH_3 (*GP 3*). To a soln. of thioether (1 equiv.) in THF (0.05M), condensed NH_3 (NH_3/THF 2:1) was added at −78°. The NH_3 (g) was dried before

condensation by streaming through soda lime. Na (6 equiv.) was added in 3 portions, and the mixture was stirred until a deep blue color persisted. After stirring for additional 30 min at $-78°$, NH_4Cl (10 equiv.) was added and the suspension turned yellow. The NH_3 was removed by bubbling N_2 (g) through the suspension at 0°. Et_2O was added to the residue, and the resulting suspension was washed with sat. aq. NH_4Cl soln. The aq. phases were extracted (Et_2O), and the combined org. phases were dried ($MgSO_4$) and concentrated *in vacuo*.

General Procedure for the Replacement of a OH Group with AcSH under Mitsunobu *Conditions* (*GP 4*). To a soln. of Ph_3P (1.5 equiv.) in dry THF (0.1M), DIAD (1.5 equiv.) was added at 0°, and the mixture was stirred for 30 min, until a white solid precipitated. A soln. of the alcohol (1 equiv.) in dry THF (0.16M) was added dropwise to the mixture, followed by addition of freshly distilled AcSH (2 equiv.). The mixture was stirred for 1 h at 0° and for 2 h at r.t. After addition of Et_2O, and washing with H_2O and sat. aq. NaCl soln., the aq. phases were extracted (CH_2Cl_2), and the combined org. phases were dried ($MgSO_4$) and concentrated in vacuo.

General Procedure for Thioester Cleavage (*GP 5*). The thioester (1 equiv.) was dissolved in MeOH (0.05M), and the soln. was degassed under Ar for 30 min. The soln. was added to a suspension of MeONa (10 equiv.) in MeOH (0.03M) that was degassed for 30 min under Ar and for 10 min under H_2. The mixture was stirred for 2 h at r.t. After addition of sat. aq. NH_4Cl soln. and extraction (CH_2Cl_2), the org. phases were dried ($MgSO_4$) and concentrated in vacuo.

*(2*S*)-*N*-(2-Aminophenyl)-2-benzyl-3-(benzylsulfanyl)propanamide* ((+)-**9**). *GP 1*, *Method A*, starting from (−)-**8** (1.00 g, 3.49 mmol) and diamine, gave (+)-**9** (330 mg, 25%) after purification by CC (SiO_2-*60*; CH_2Cl_2/AcOEt 91:9). Colorless oil. $[\alpha]_D^{25} = +6.7$ ($c = 1$, $CHCl_3$). IR (neat): 3251, 3028, 2916, 1651, 1622, 1495, 1453, 1381, 1304, 1155, 1077. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.06–2.48 (*m*, 1 H); 2.65 (*dd*, *J* = 13.4, 4.7, 1 H); 2.81 (*dd*, *J* = 13.1, 5.3, 1 H); 2.91 (*dd*, *J* = 13.1, 10.0, 1 H); 2.92 (*dd*, *J* = 13.4, 10.0, 1 H); 3.51 (br. *s*, 2 H); 3.75 (*s*, 2 H); 6.53 (br. *s*, 1 H); 6.67–6.73 (*m*, 2 H); 6.90 (*dd*, *J* = 7.5, 1.6, 1 H); 7.01 (*dt*, *J* = 7.5, 1.6, 1 H); 7.13–7.35 (*m*, 10 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.1; 37.3; 39.0; 50.9; 117.0; 118.8; 123.0; 126.1; 126.5; 127.1; 127.3; 128.5; 128.8; 128.7; 128.9; 138.5; 138.9; 141.1; 171.8. MALDI-MS (DHB): 399.2 (76, $[M + Na]^+$), 377.2 (100, MH^+), 359.2 (15), 331.2 (12). MALDI-HR-MS (DHB): 377.1687 (MH^+, $C_{23}H_{25}N_2OS^+$; calc. 377.1682).

*2-[(1*S*)-1-Benzyl-2-(benzylsulfanyl)ethyl]-1*H*-benzimidazole* ((−)-**11**). *GP 2*, starting from (+)-**9** (200 mg, 0.53 mmol), gave (−)-**11** (152 mg, 80%) after purification by CC (SiO_2-*60*; pentane/AcOEt 80:20). Colorless solid. M.p. 161–162°. $[\alpha]_D^{25} = -4.0$ ($c = 1$, EtOH). IR ($CHCl_3$): 3451, 3064, 2952, 2364, 1622, 1600, 1527, 1494, 1455, 1424, 1329, 1273. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, 5 drops of CF_3COOH added): 2.93 (*dd*, *J* = 13.8, 5.4, 1 H); 3.00 (*dd*, *J* = 13.8, 9.5, 1 H); 3.14–3.26 (*m*, 2 H); 3.72 (*s*, 2 H); 3.82–3.92 (*m*, 1 H); 7.11–7.27 (*m*, 10 H); 7.51–7.57 (*m*, 2 H); 7.77–7.83 (*m*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 35.3; 37.1; 40.2; 43.0; 122.6; 126.8; 127.4; 128.8; 129.1; 129.3; 138.5; 139.1; 156.0 (3 arom. signals overlapping). MALDI-MS (DHB): 359.2 (100, MH^+), 307.1 (6), 179.6 (7). MALDI-HR-MS (DHB): 359.1581 (MH^+, $C_{23}H_{23}N_2S^+$; calc. 359.1582). Anal. calc. for $C_{23}H_{22}N_2S$ (358.50): C 77.06, H 6.19, N 7.81, S 8.94; found C 76.80, H 6.40, N 7.73, S 8.96.

*2-[(1*S*)-1-Benzyl-2-sulfanylethyl]-1*H*-benzimidazol-3-ium Trifluoroacetate* ((+)-**1**). *GP 3*, starting from (−)-**11** (100 mg, 0.28 mmol), gave a residue that was purified by CC (SiO_2-*60*; CH_2Cl_2/MeOH 98:2) and RP-HPLC (*RP-18* SiO_2; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min) to give (+)-**1** (35 mg, 33%). Colorless oil. $[\alpha]_D^{25} = +15.2$ ($c = 1.17$, EtOH). IR (KBr): 3423, 3120, 3053, 2840, 2678, 1941, 1891, 1813, 1620, 1538, 1491, 1454, 1426, 1328, 1309, 1270. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.35 (*t*, *J* = 8.7, 1 H); 2.85–2.94 (*m*, 1 H); 3.00–3.11 (*m*, 1 H); 3.19 (*dd*, *J* = 13.7, 8.1, 1 H); 3.25 (*dd*, *J* = 13.7, 7.8, 1 H); 3.79–3.89 (*m*, 1 H); 7.02–7.12 (*m*, 5 H); 7.28–7.33 (*m*, 2 H); 7.59–7.65 (*m*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 26.9; 39.1; 44.8; 114.0; 125.7; 127.0; 128.5; 128.7; 131.0; 136.3; 154.5. MALDI-MS (DHB): 291.1 (12, $[M + Na]^+$), 269.1 (100, MH^+). MALDI-HR-MS (DHB): 269.1103 (MH^+, $C_{16}H_{17}N_2S^+$; calc. 269.1107).

*(2*S*)-*N*-(3-Aminopyridin-4-yl)-2-benzyl-3-(benzylsulfanyl)propanamide* ((+)-**10**). *GP 1*, *Method A*, starting from (−)-**8** (751 mg, 2.63 mmol) and pyridine-3,4-diamine, gave (+)-**12** (502 mg, 51%) after purification by CC (SiO_2-*60*; CH_2Cl_2/AcOEt 91:9). Colorless foam. M.p. 45°. $[\alpha]_D^{25} = +4.4$ ($c = 1$, $CHCl_3$). IR (neat): 3028, 2919, 2361, 1661, 1582, 1511, 1455, 1421, 1331, 1300, 1219, 1168. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.44–2.54 (*m*, 1 H); 2.66 (*dd*, *J* = 13.4, 4.7, 1 H); 2.80–2.94 (*m*, 3 H); 3.32 (br. *s*, 2 H); 3.75 (*s*, 2 H); 6.99 (br. *s*, 1 H); 7.10–7.34 (*m*, 11 H); 7.96 (*d*, *J* = 5.0, 1 H); 8.04 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 33.8; 37.4; 38.9; 51.1; 117.3; 126.6; 127.2; 128.6; 128.7; 128.8; 128.8; 131.5; 134.5; 138.3; 138.7; 140.0; 141.3; 171.9. MALDI-MS (DHB): 400.1 (2, $[M + Na]^+$), 378.2 (100, MH^+). MALDI-HR-MS (DHB): 378.1629 (MH^+, $C_{22}H_{24}N_3OS^+$; calc. 378.1635). Anal. calc. for $C_{22}H_{23}N_3OS$ (377.50): C 70.00, H 6.14, N 11.13; found C 70.08, H 6.22, N 10.90.

*2-[(1*S*)-1-Benzyl-2-(benzylsulfanyl)ethyl]-1*H*-imidazo[4,5-*c*]pyridine* ((+)-**12**). *GP 2*, starting from (+)-**10** (400 mg, 1.06 mmol), gave (+)-**12** (356 mg, 93%) after purification by CC (SiO_2-*60*; AcOEt). Colorless oil, which solidified upon standing. M.p. 63°. $[\alpha]_D^{25} = +14.9$ ($c = 1$, $CHCl_3$). IR: 3027, 2920, 1734, 1621, 1587, 1533, 1494, 1454, 1424, 1283, 1238, 1210, 1167. ^{1}H-NMR (300 MHz, $CHCl_3$): 2.91 (*dd*, *J* = 13.4, 5.3, 1 H); 2.97 (*dd*, *J* =

13.4, 7.8, 1 H); 3.09–3.16 (*m*, 1 H); 3.21–3.31 (*m*, 2 H); 3.60 (*s*, 2 H); 7.02–7.27 (*m*, 10 H); 7.40 (br. *s*, 1 H); 8.36 (*d*, *J* = 5.6, 1 H); 8.90 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 35.0; 36.9; 40.3; 43.2; 110.0; 126.6; 127.1; 128.5; 128.7; 128.8; 137.9; 138.4; 140.8 (5 arom. signals not observable due to exchange). MALDI-MS (DHB): 360.2 (100, MH^+). MALDI-HR-MS (DHB): 360.1527 (MH^+, $C_{22}H_{22}N_3S^+$; calc. 360.1529).

*2-[(1S)-1-Benzyl-2-sulfanylethyl]-1*H*-imidazo[4,5-c]pyridin-5-ium Trifluoroacetate* ((+)-**2**). *GP 3*, *Method 1*, starting from (+)-**12** (400 mg, 1.11 mmol), gave a residue that was purified by CC (SiO_2-*60*; AcOEt/MeOH 95:5) and RP-HPLC (*RP-18* SiO_2; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min) to give (+)-**2** (42 mg, 10%). *GP 5*, *Method 2*, starting from (−)-**24** (100 mg, 0.32 mmol), gave (+)-**2** (90 mg, 73%) after purification by RP-HPLC (*RP-18* SiO_2; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min). Colorless oil. $[\alpha]_D^{25} = +23.0$ (c = 1, $CHCl_3$). IR: 3687, 3088, 3035, 2927, 2733, 2372, 2097, 1667, 1526, 1454, 1422, 1363, 1312, 1269. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.49 (*t*, *J* = 8.6, 1 H); 2.99–3.14 (*m*, 2 H); 3.24 (*dd*, *J* = 13.8, 7.9, 1 H); 3.31 (*dd*, *J* = 13.8, 7.3, 1 H); 3.66–3.76 (*m*, 1 H); 7.14–7.25 (*m*, 5 H); 7.97 (*d*, *J* = 6.5, 1 H); 8.23 (*d*, *J* = 6.5, 1 H); 9.27 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.4; 39.6; 46.3; 113.8; 126.8; 128.6; 128.8; 129.4; 131.6; 131.9; 137.2; 137.5; 167.3. MALDI-MS (DHB): 270.1 (100, MH^+), 236.1 (3). MALDI-HR-MS (DHB): 270.1064 (MH^+, $C_{15}H_{16}N_3S^+$; calc. 270.1059).

*2-[(1S)-1-Benzyl-2-(benzylsulfanyl)ethyl]-1-methyl-1*H*-benzimidazole* ((+)-**14**). A soln. of (+)-**11** (55 mg, 0.15 mmol) in THF (3 ml) was added to NaH (12 mg) at 0°, and the mixture was stirred for 15 min. After addition of MeI (11 µl, 0.17 mmol) at 0°, the soln. was stirred for 10 min at 0° and for 30 min at r.t. H_2O was added, the mixture was extracted (CH_2Cl_2), the org. phases were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by CC (SiO_2-*60*; pentane/AcOEt 71:29) to give (+)-**14** (53 mg, 95%). Colorless oil. $[\alpha]_D^{25} = +78.5$ (c = 1, $CHCl_3$). IR ($CHCl_3$): 2949, 1946, 1887, 1809, 1750, 1696, 1602, 1492, 148, 1452, 1320, 1282, 1153, 1073. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.97–3.25 (*m*, 5 H); 3.10 (*s*, 3 H); 3.56 (*d*, *J* = 13.5, 1 H); 3.61 (*d*, *J* = 13.5, 1 H); 6.87–6.92 (*m*, 2 H); 7.11–7.31 (*m*, 11 H); 7.76–7.80 (*m*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 29.1; 36.5; 41.4; 41.6; 109.2; 119.2; 121.8; 121.9; 126.4; 126.9; 128.3; 128.5; 128.8; 128.8; 135.0; 138.6; 139.0; 142.4; 156.2. MALDI-MS (DHB): 395.2 (4, $[M + Na]^+$), 373.2 (100, MH^+). MALDI-HR-MS (DHB): 373.1730 (MH^+, $C_{24}H_{25}N_2S^+$; calc. 373.1733).

*2-[(1S)-1-Benzyl-2-sulfanylethyl]-1-methyl-1*H*-benzimidazol-3-ium Trifluoroacetate* ((+)-**13**). *GP 3*, starting from (+)-**14** (94 mg, 0.25 mmol), gave a residue that was purified by CC (SiO_2-*60*; pentane/AcOEt 80:20) and RP-HPLC (*RP-18* SiO_2-*60*; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min) to give (+)-**15** (60 mg, 61%). Colorless oil. $[\alpha]_D^{25} = +44.8$ (c = 0.5, $CHCl_3$). IR ($CHCl_3$): 3003, 1669, 1522, 1495, 1454, 1417, 1347, 1191, 1139, 1075. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.68 (br. *s*, 1 H); 3.16–3.23 (*m*, 1 H); 3.35 (*d*, *J* = 6.9, 2 H); 3.42 (*s*, 3 H); 3.54–3.66 (*m*, 2 H); 6.88–7.03 (*m*, 2 H); 7.15–7.19 (*m*, 3 H); 7.35–7.38 (*m*, 1 H); 7.45–7.55 (*m*, 2 H); 8.03–8.06 (*m*, 1 H); 10.49 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.7; 30.7; 40.7; 46.1; 110.8; 116.9; 125.7; 126.3; 127.5; 128.8; 129.2; 132.2; 137.5; 154.6 (1 arom. signal overlapping). MALDI-MS (DHB): 283.1 (100, MH^+), 281.1 (55). MALDI-HR-MS (DHB): 283.1262 (MH^+, $C_{17}H_{19}N_2S^+$; calc. 283.1262).

*(4*S*)-4-Benzyl-3-[(2*R*)-2-benzyl-3-hydroxypropanoyl]-1,3-oxazolidin-2-one* ((+)-**17**). *Method 1:* To a soln. of (+)-**16** (6.79 g, 21.95 mmol) in CH_2Cl_2 (100 ml), $TiCl_4$ (2.65 ml, 24.14 mmol) and Et_3N (3.35 ml, 24.14 mmol) were added at 0°. After stirring the mixture for 30 min at 0°, a soln. of *s*-trioxane (2.17 g, 24.14 mmol) in CH_2Cl_2 (15 ml) and then $TiCl_4$ (2.65 ml, 24.14 mmol) were added, and the mixture was stirred for 3 h at 0°. After addition of half-sat. aq. NH_4Cl soln., the mixture was extracted (AcOEt). The org. phases were washed with sat. aq. $NaHCO_3$ soln., H_2O, and sat. aq. NaCl soln., dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by CC (SiO_2-*60*; hexanes/AcOEt 40:60) to give (+)-**17** (3.61 g, 48%).

Method 2: To soln. of (+)-**18** (50 mg, 0.12 mmol) in AcOEt (1 ml), Pd/C (10%, 15 mg) and conc. HCl (37%, 1 drop) were added, and the mixture was stirred for 6 h under H_2 (1 atm). The suspension was filtered over *Celite* and concentrated *in vacuo* to give (+)-**17** (34 mg, 86%). Colorless oil. $[\alpha]_D^{20} = +115.7$ (c = 1, $CHCl_3$). IR (neat): 3514, 2925, 1773, 1692, 1494, 1452, 1385, 1349, 1210, 1109, 1055, 1013, 962. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.29 (br. *s*, 1 H); 2.79 (*dd*, *J* = 13.4, 9.7, 1 H); 2.89 (*dd*, *J* = 13.4, 7.8, 1 H); 3.02 (*dd*, *J* = 13.4, 7.2, 1 H); 3.27 (*dd*, *J* = 13.4, 3.4, 1 H); 3.82 (*dd*, *J* = 11.2, 6.5, 1 H); 3.89 (*dd*, *J* = 11.2, 4.0, 1 H); 3.98 (*dd*, *J* = 8.9, 7.8, 1 H); 4.11 (*dd*, *J* = 8.9, 2.2, 1 H); 4.25–4.34 (*m*, 1 H); 4.51–4.58 (*m*, 1 H); 7.17–7.36 (*m*, 10 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.8; 38.0; 47.1; 55.6; 63.1; 66.2; 126.5; 127.3; 128.4; 128.9; 129.1; 129.4; 135.0; 138.1; 153.2; 175.1. EI-MS: 339.1 (1, M^+), 321.1 (17), 131.0 (78), 91.0 (100). Anal. calc. for $C_{20}H_{21}NO_4$ (339.39): C 70.78, H 6.24, N 4.13; found C 70.93, H 6.26, N 3.94.

*(4*S*)-4-Benzyl-3-((2*R*)-2-benzyl-3-{[(*tert*-butyl)(dimethyl)silyl]oxy}propanoyl)-1,3-oxazolidin-2-one* ((+)-**19**). To a soln. of (+)-**17** (3.6 g, 10.6 mmol) in dry CH_2Cl_2 (25 ml), (*t*-Bu)Me_2SiCl (2.4 g, 15.9 mmol) and DMAP (2.2 g, 18.1 mmol) were added, and the mixture was stirred for 16 h at r.t. After addition of Et_2O, and washing

with H_2O and sat. aq. NaCl soln., the aq. phases were extracted (CH_2Cl_2), and the combined org. phases were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by CC (SiO_2-*60*; hexanes/AcOEt 83:17) to give (+)-**19** (3.45 g, 71%). Colorless oil. $[\alpha]_D^{25} = +70.0$ ($c = 1$, $CHCl_3$). IR (neat): 2930, 2858, 1778, 1760, 1700, 1605, 1494, 1452, 1390, 1349, 1212, 1184, 1099, 1003, 972. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.05 (*s*, 6 H); 0.88 (*s*, 9 H); 2.67 (*dd*, $J = 13.2$, 9.8, 1 H); 2.87 (*dd*, $J = 13.5$, 7.3, 1 H); 2.94 (*dd*, $J = 13.5$, 8.2, 1 H); 3.26 (*dd*, $J = 13.2$, 3.3, 1 H); 3.80 (*dd*, $J = 9.6$, 5.0, 1 H); 3.89 (*dd*, $J = 9.0$, 8.4, 1 H); 3.96 (*dd*, $J = 9.6$, 7.2, 1 H); 4.03 (*dd*, $J = 9.0$, 2.5, 1 H); 4.37–4.46 (*m*, 1 H); 4.48–4.56 (*m*, 1 H); 7.15–7.35 (*m*, 10 H). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.3; 18.4; 26.0; 35.0; 38.1; 47.5; 55.4; 63.6; 65.8; 126.3; 127.2; 128.2; 128.8; 129.0; 129.3; 135.3; 138.6; 152.9; 174.0. EI-MS: 438.3 (1, $[M - Me]^+$), 396.2 (32, $[M - Me_3C]^+$), 219.1 (37), 145.0 (36), 117.0 (100). Anal. calc. for $C_{26}H_{35}NO_4Si$ (453.65): C 68.84, H 7.78, N 3.09; found C 68.91, H 7.83, N 3.29.

*(2*R*)-2-Benzyl-3-{[(*tert*-butyl)(dimethyl)silyl]oxy}propanoic Acid* ((−)-**20**). To a soln. of (+)-**19** (2.63 g, 5.80 mmol) in a mixture of THF/H_2O (75 ml/25 ml), aq. H_2O_2 (30%, 3.55 ml, 34.78 mmol) was added. After addition of a soln. of $LiOH \cdot H_2O$ (487 mg, 11.59 mmol) in H_2O (15 ml) at 0°, the mixture was stirred for 3 h at 0°, then 1.5N aq. Na_2SO_3 soln. (30 ml) was added, and the THF was removed *in vacuo*. The aq. phases were acidified by addition of 1N HCl to a pH of 1–2 and extracted (CH_2Cl_2). The org. phases were dried ($MgSO_4$), concentrated *in vacuo*, and the residue was purified by CC (SiO_2-*60*; pentane/AcOEt 83:17) to give (−)-**20** (1.13 g, 66%). Colorless oil. $[\alpha]_D^{25} = -5.3$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3067, 2954, 1948, 1879, 1806, 1709, 1602, 1470, 1406, 1390, 1360, 1258. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.04 (*s*, 3 H); 0.04 (*s*, 3 H); 0.89 (*s*, 9 H); 2.80–2.89 (*m*, 1 H); 2.86 (*dd*, $J = 16.2$; 7.2, 1 H); 3.02 (*dd*, $J = 16.2$, 10.0, 1 H); 3.73 (*dd*, $J = 10.0$, 5.6, 1 H); 3.78 (*dd*, $J = 10.0$, 4.8, 1 H); 7.16–7.34 (*m*, 5 H). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.3; 18.4; 26.0; 34.0; 49.6; 62.9; 126.7; 128.7; 129.2; 138.9; 178.7. MALDI-MS (DCTB): 339.1 (100, $[M - H + 2\,Na]^+$), 317.2 (59, $[M + Na]^+$), 309.2 (11), 261.1 (18). MALDI-HR-MS (DCTB): 317.1539 ($[M + Na]^+$, $C_{16}H_{26}NaO_3Si^+$; calc. 317.1543). Anal. calc. for $C_{16}H_{26}O_3Si$ (294.46): C 65.26, H 8.90; found C 65.02, H 8.88.

*(2*R*)-*N*-(3-Aminopyridin-4-yl)-2-benzyl-3-{[(*tert*-butyl)(dimethyl)silyl]oxy}propanamide* ((+)-**21**). *GP 1*, *Method B*, starting from (−)-**20** (100 mg, 0.34 mmol) and pyridine-3,4-diamine, gave (+)-**21** (122 mg, 93%) after purification by CC (SiO_2-*60*; pentane/AcOEt 33:67). Colorless solid. M.p. 107°. $[\alpha]_D^{25} = +7.4$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3412, 3299, 2954, 2857, 1951, 1882, 1806, 1691, 1621, 1586, 1511, 1470, 1422, 1298, 1258. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.06 (*s*, 3 H); 0.07 (*s*, 3 H); 0.89 (*s*, 9 H); 2.78–2.87 (*m*, 2 H); 3.03–3.11 (*m*, 1 H); 3.50 (br. *s*, 2 H); 3.84 (*d*, $J = 5.6$, 2 H); 7.21–7.34 (*m*, 6 H); 8.00 (*d*, $J = 5.3$, 1 H); 8.04 (br. *s*, 1 H); 8.09 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.3; 18.4; 26.0; 34.8; 52.0; 63.3; 117.0; 126.6; 128.6; 128.9; 132.0; 134.5; 138.8; 140.3; 141.4; 172.4. MALDI-MS (DCTB): 386.2 (100, *M*H$^+$), 368.2 (3), 254.1 (4). MALDI-HR-MS (DCTB): 386.2254 (*M*H$^+$, $C_{21}H_{32}N_3O_2Si^+$; calc. 386.2258). Anal. calc. for $C_{21}H_{31}N_3O_2Si$ (385.58): C 65.42, H 8.10, N 10.90; found C 65.46, H 7.92, N 10.84.

*2-((1*S*)-1-Benzyl-2-{[(*tert*-butyl)(dimethyl)silyl]oxy}ethyl)-1*H*-imidazo[4,5-*c*]pyridine* ((+)-**22**). *GP 2*, starting from (+)-**21** (72 mg, 0.19 mmol), gave (+)-**22** (66 mg, 96%). Colorless oil. $[\alpha]_D^{25} = +52.6$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3391, 2954, 2857, 1954, 1857, 1618, 1583, 1529, 1460, 1401, 1277, 1258. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.03 (*s*, 3 H); 0.03 (*s*, 3 H); 0.93 (*s*, 9 H); 3.08 (*dd*, $J = 13.7$, 9.3, 1 H); 3.32 (*dd*, $J = 13.7$, 6.2, 1 H); 3.40–3.48 (*m*, 1 H); 3.84 (*dd*, $J = 10.1$, 5.4, 1 H); 3.93 (*dd*, $J = 10.1$, 3.9, 1 H); 7.16–7.31 (*m*, 6 H); 8.39 (*d*, $J = 5.6$, 1 H); 9.02 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 2 drops of CF_3COOH added): −5.5; 18.3; 25.8; 36.2; 44.6; 63.8; 113.6; 127.5; 128.8; 129.1; 131.6; 134.4; 136.8; 147.2; 167.4 (1 arom. signal overlapping). MALDI-MS (DHB): 390.2 (5, $[M + Na]^+$), 368.2 (100, *M*H$^+$), 352.2 (4), 254.1 (8). MALDI-HR-MS (DHB): 368.2148 (*M*H$^+$, $C_{21}H_{30}N_3OSi^+$; calc. 368.2153).

*(2*S*)-2-(1*H*-Imidazo[4,5-*c*]pyridin-2-yl)-3-phenylpropan-1-ol* ((+)-**23**). To a soln. of (+)-**22** (740 mg, 2.01 mmol) in dry THF (25 ml), Bu_4NF soln. (1M in THF, 4 ml, 4.00 mmol) was added dropwise at r.t. The mixture was stirred for 2.5 h at r.t., and the reaction was quenched by addition of H_2O. After extraction (CH_2Cl_2), the org. phases were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by CC (SiO_2-*60*; AcOEt/MeOH 93:7) afforded (+)-**23** (428 mg, 84%). Colorless foam. M.p. 85°. $[\alpha]_D^{25} = +92.4$ ($c = 1$, $CHCl_3$). IR (neat): 2929, 1621, 1589, 1532, 1495, 1455, 1418, 1283, 1201, 1167, 1057. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.13 (*dd*, $J = 13.7$, 8.1, 1 H); 3.26 (*dd*, $J = 13.7$, 7.5, 1 H); 3.39–3.49 (*m*, 1 H); 3.99 (*dd*, $J = 10.9$, 6.2, 1 H); 4.08 (*dd*, $J = 10.9$, 3.7, 1 H); 7.16–7.32 (*m*, 5 H); 7.39 (*d*, $J = 5.8$, 1 H); 8.33 (*d*, $J = 5.8$, 1 H); 8.89 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 37.1; 45.0; 63.8; 109.5; 126.7; 128.7; 129.1; 137.5; 138.0; 138.9; 140.5; 143.0; 160.2. ESI-MS: 276. 1 (28, $[M + Na]^+$), 254.1 (100, *M*H$^+$). ESI-HRMS: 254.1286 (*M*H$^+$, $C_{15}H_{16}N_3O^+$; calc. 254.1288).

S-*[(2*S*)-2-(1*H*-Imidazo[4,5-*c*]pyridin-2-yl)-3-phenylpropyl] Ethanethioate* ((−)-**24**). *GP 4*, starting from (+)-**23** (140 mg, 0.55 mmol), afforded (−)-**24** (160 mg, 93%) after purification by CC (SiO_2-*60*; AcOEt/MeOH/NEt_3 94:5:1). Colorless oil. $[\alpha]_D^{25} = -30.8$ ($c = 1$, $CHCl_3$). IR (neat): 3028, 2922, 1688, 1621, 1587, 1532, 1495, 1455, 1423, 1353, 1282, 1210. ^{1}H-NMR (300 MHz, $CDCl_3$, 4 drops of CF_3COOH added): 2.28 (*s*, 3 H); 3.25 (*dd*,

J = 13.9, 8.6, 1 H); 3.32 (*dd*, J = 13.9, 7.6, 1 H); 3.36 (*dd*, J = 14.0, 8.4, 1 H); 3.46 (*dd*, J = 14.0, 5.3, 1 H); 3.81–3.88 (*m*, 1 H); 7.05–7.23 (*m*, 5 H); 8.19 (*d*, J = 6.5, 1 H); 8.50 (*d*, J = 6.5, 1 H); 9.45 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$; 4 drops of CF_3COOH added): 30.4; 31.9; 39.6; 43.0; 113.1; 127.3; 128.5; 128.8; 131.6; 133.8; 134.3; 136.0; 146.6; 166.0; 197.0. MALDI-MS (DCTB): 312.1 (100, MH$^+$), 236.1 (48), 156.1 (1). MALDI-HR-MS (DCTB): 312.1165 (MH$^+$, $C_{17}H_{18}N_3OS^+$; calc. 312.1165).

*(4*S*)-4-Benzyl-3-(3-[1,1′-biphenyl-4-yl]propanoyl)-1,3-oxazolidin-2-one* ((+)-**27**). To a soln. of 3-(1,1′-biphenyl-4-yl)propanoic acid (6.88 g, 30.42 mmol) and Et_3N (5.13 ml, 36.79 mmol) in dry THF (100 ml), pivaloyl chloride (3.91 ml, 31.81 mmol) was added at −78°. The mixture was stirred for 1 h at 0°. To a soln. of (+)-**15** (4.90 g, 27.66 mmol) in dry THF (60 ml), BuLi (1.6M in hexanes, 19.01 ml, 30.42 mmol) was slowly added at −78°. The latter soln. was added *via* canula to the anhydride soln. at −78°, and the mixture was stirred for 30 min at −78°. The mixture was warmed to 0°, and CH_2Cl_2 and a phosphate-buffer soln. (pH 7) were added. The org. phases were washed with sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and concentrated *in vacuo*. Purification by recrystallization from AcOEt afforded (+)-**27** (8.12 g, 76%). Colorless crystals. M.p. 182°. $[\alpha]_D^{25}$ = +48.3 (c = 1, $CHCl_3$). IR ($CHCl_3$): 2922, 1780, 1699, 1487, 1452, 1384, 1352, 1108, 1048. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.77 (*dd*, J = 13.4, 9.7, 1 H); 3.04–3.10 (*m*, 2 H); 3.23–3.42 (*m*, 3 H); 4.11–4.22 (*m*, 2 H); 4.64–4.72 (*m*, 1 H); 7.17–7.59 (*m*, 14 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 30.0; 37.2; 37.9; 55.2; 66.3; 126.9; 127.0; 127.1; 127.3; 128.6; 128.9; 129.3; 135.1; 139.1; 139.4; 140.8; 172.2 (1 arom. and 1 CO signal overlapping). MALDI-MS (DCTB): 408.2 (33, [M + Na]$^+$), 386.2 (10, MH$^+$), 235.1 (87), 224.1 (100). MALDI-HR-MS (DCTB): 408.1568 ([M + Na]$^+$, $C_{25}H_{23}NNaO_3^+$; calc. 408.1517). Anal. calc. for $C_{25}H_{23}NO_3$ (385.46): C 77.90, H 6.01, N 3.63; found C 77.66, H 5.93, N 3.64.

*(4*S*)-4-Benzyl-3-[(2*R*)-3-(benzyloxy)-2-([1,1′-biphenyl-4-yl]methyl)propanoyl]-1,3-oxazolidin-2-one* ((+)-**28**). To a soln. of (+)-**27** (386 mg, 1.00 mmol) in dry CH_2Cl_2 (10 ml), $TiCl_4$ (0.12 ml, 1.05 mmol) was slowly added at 0°. After stirring for 5 min, EtN(i-Pr)$_2$ (0.19 ml, 1.1 mmol) was added, and the mixture was stirred for 1 h at 0°. After addition of $BnOCH_2Cl$ (0.28 ml, 2 mmol), the mixture was stirred for additional 4 h at 0°. The reaction was cautiously quenched with sat. aq. NH_4Cl soln., and the mixture was extracted (CH_2Cl_2). The org. phases were washed with H_2O, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by CC (SiO_2-*60*; pentane/AcOEt 83:17) afforded (+)-**32** (470 mg, 94%). Colorless crystals. M.p. 72°. $[\alpha]_D^{25}$ = +77.2 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3019, 2954, 1777, 1694, 1599, 1484, 1384, 1349, 1261, 1099, 1008. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.69 (*dd*, J = 13.5, 9.2, 1 H); 2.93 (*dd*, J = 13.5, 7.4, 1 H); 3.03 (*dd*, J = 13.5, 8.3, 1 H); 3.20 (*dd*, J = 13.5, 3.3, 1 H); 3.70 (*dd*, J = 9.2, 4.9, 1 H); 3.89 (*dd*, J = 9.2, 7.6, 1 H); 3.90 (*dd*, J = 9.0, 8.4, 1 H); 4.03 (*dd*, J = 9.0, 2.8, 1 H); 4.52–4.64 (*m*, 4 H); 7.16–7.58 (*m*, 19 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 35.0; 37.9; 45.4; 55.5; 66.1; 70.8; 73.4; 127.2; 127.3; 127.4; 127.5; 127.9; 128.0; 128.6; 129.0; 129.1; 129.7; 129.8; 135.4; 137.8; 138.3; 139.6; 141.0; 153.3; 174.4. EI-MS: 506.2 (2, MH$^+$), 505.2 (8, M^+), 397.2 (42), 220.1 (75), 219.1 (83), 91.1 (100), 69.0 (96). Anal. calc. for $C_{33}H_{31}NO_4$ (505.63): C 78.39, H 6.18, N 2.77; found C 78.26, H 6.04, N 2.78.

*(2*R*)-3-(Benzyloxy)-2-([1,1′-biphenyl-4-yl]methyl)propanoic Acid* ((−)-**29**). To a soln. of (+)-**28** (7.41 g, 14.65 mmol) in a mixture of THF/H_2O (225 ml/75 ml), aq. H_2O_2 soln. (30%, 2.69 ml, 26.37 mmol) and aq. LiOH soln. (0.8M, 35 ml, 29.31 mmol) were added at 0°. The mixture was stirred for 2.5 h at r.t, then 1.5N aq. Na_2SO_3 soln. (90 ml) was added, and the THF was removed *in vacuo*. The aq. phases were acidified by addition of 1N HCl to a pH of 1–2 and extracted (CH_2Cl_2). The org. phases were dried ($MgSO_4$), concentrated *in vacuo*, and the residue was purified by CC (SiO_2-*60*; CH_2Cl_2/pentane/AcOH 66:33:1) to give (−)-**33** (3.71 g, 73%). Colorless solid. M.p. 97°. $[\alpha]_D^{25}$ = −7.4 (c = 1, $CHCl_3$). IR ($CHCl_3$): 2927, 2857, 1712, 1602, 1487, 1449, 1258, 1099. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.92 (*dd*, J = 12.2, 6.9, 1 H); 2.99–3.07 (*m*, 1 H); 3.11 (*dd*, J = 12.2, 6.4, 1 H); 3.65 (*d*, J = 5.3, 2 H); 4.52 (*d*, J = 12.1, 1 H); 4.57 (*d*, J = 12.1, 1 H); 7.23–7.58 (*m*, 14 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.0; 47.5; 69.5; 73.5; 127.2; 127.4; 127.4; 128.0; 128.0; 128.7; 129.0; 129.6; 137.7; 137.9; 139.7; 141.0; 178.8. ESI-MS: 345.1 (100, M^+), 193.1 (16). Anal. calc. for $C_{23}H_{22}O_3$ (345.55): C 79.74, H 6.40; found C 79.78, H 6.69.

*(2*R*)-*N*-(2-Aminophenyl)-3-(benzyloxy)-2-([1,1′-biphenyl-4-yl]methyl)propanamide* ((+)-**30**). *GP 1*, *Method B*, starting from (−)-**29** (1.00 g, 2.89 mmol) and benzene-1,2-diamine, afforded (+)-**30** (0.83 g, 66%) after purification by CC (SiO_2-*60*; CH_2Cl_2/AcOEt 91:9). Yellow solid. M.p. 109°. $[\alpha]_D^{25}$ = +30.1 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3412, 3019, 2857, 1675, 1621, 1503, 1481, 1258, 1099. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.88–2.96 (*m*, 2 H); 3.10–3.19 (*m*, 1 H); 3.48 (br. *s*, 2 H); 3.72–3.76 (*m*, 2 H); 4.57 (*s*, 2 H); 6.68 (*dd*, J = 7.8, 1.6, 1 H); 6.74 (*dt*, J = 7.8, 1.3, 1 H); 7.00 (*dt*, J = 7.8, 1.6, 1 H); 7.06 (*dd*, J = 7.8, 1.3, 1 H); 7.26–7.60 (*m*, 15 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.8; 50.1; 70.6; 73.7; 117.1; 118.9; 123.6; 125.6; 126.9; 127.0; 127.2; 127.2; 127.9; 128.5; 128.7; 129.4; 137.4; 138.0; 139.4; 140.7; 171.9 (2 arom. signals overlapping). MALDI-MS (DCTB): 459.2 (15, [M + Na]$^+$), 437.2 (21, MH$^+$), 420.2 (31), 419.2 (100). MALDI-HR-MS (DCTB): 459.2050 ([M + Na]$^+$, $C_{29}H_{28}N_2NaO_2^+$; calc. 459.2043). Anal. calc. for $C_{29}H_{28}N_2O_2$ (436.55): C 79.79, H 6.46, N 6.42; found C 79.81, H 6.52, N 6.29.

2-[(1S)-2-(Benzyloxy)-1-([1,1′-biphenyl-4-yl]methyl)ethyl]-1H-benzimidazole ((+)-**32**). *GP 2*, starting from (+)-**30** (777 mg, 1.78 mmol), afforded (+)-**32** (638 mg, 85%). Yellow foam. M.p. 60°. $[\alpha]_D^{25} = +40.3$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3418, 3013, 2954, 2863, 2356, 1677, 1621, 1484, 1452, 1411, 1266, 1099. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.17 (*dd*, $J = 13.6$, 10.0, 1 H); 3.37 (*dd*, $J = 13.6$, 5.8, 1 H); 3.49–3.58 (*m*, 1 H); 3.73 (*dd*, $J = 9.2$, 5.5, 1 H); 3.82 (*dd*, $J = 9.2$, 3.6, 1 H); 4.55 (*d*, $J = 12.0$, 1 H); 4.60 (*d*, $J = 12.0$, 1 H); 7.17–7.61 (*m*, 18 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 2 drops of CF_3COOH added): 36.6; 39.8; 67.7; 74.1; 113.9; 126.9; 127.3; 127.4; 127.5; 128.3; 128.7; 128.8; 128.9; 129.1; 129.2; 129.8; 134.7; 136.1; 140.3; 154.6. MALDI-MS (DCTB): 419.21 (100, *M*H$^+$), 209.6 (4). MALDI-HR-MS (DCTB): 419.2123 (*M*H$^+$, $C_{29}H_{27}N_2O^+$; calc. 419.2118). Anal. calc for $C_{29}H_{26}N_2O$ (418.53): C 83.22, H 6.26, N 6.69; found C 83.32, H 6.54, N 6.98.

(2S)-2-(1H-Benzimidazol-2-yl)-3-[1,1′-biphenyl-4-yl]propan-1-ol ((+)-**34**). To a soln. of (+)-**32** (577 mg, 1.38 mmol) in dry CH_2Cl_2 (60 ml), BCl_3 (1M in CH_2Cl_2, 13.8 ml, 13.8 mmol) was slowly added at −78°, and the mixture was stirred for 4 h at −78°. At 0°, MeOH was slowly added, and the solvent was removed *in vacuo*. This was repeated twice. After addition of sat. methanolic NH_3 soln., the mixture was concentrated *in vacuo*, and the residue was purified by CC (SiO_2-*60*; AcOEt) to give (+)-**34** (155 mg, 34%). Colorless solid. M.p. 210°. $[\alpha]_D^{25} = +165.8$ ($c = 0.82$, EtOH). IR (neat): 3053, 3027, 2871, 2050, 1980, 1916, 1651, 1626, 1600, 1538, 1520, 1486, 1455, 1372, 1276, 1223, 1074, 1006. ^{1}H-NMR (300 MHz, CD_3OD): 3.14 (*dd*, $J = 13.7$, 9.0, 1 H); 3.23 (*dd*, $J = 13.7$, 5.6, 1 H); 3.36–3.45 (*m*, 1 H); 3.91 (*dd*, $J = 10.8$, 5.9, 1 H); 3.95 (*dd*, $J = 10.8$, 7.2, 1 H); 7.14–7.53 (*m*, 13 H). ^{13}C-NMR (75 MHz, CD_3OD): 37.3; 46.5; 64.7; 122.7; 127.3; 127.4; 127.6; 129.3; 129.9; 139.2; 140.0; 141.6; 156.8; 164.3; 173.5 (1 arom. signal overlapping). MALDI-MS (DHB): 329.2 (100, *M*H$^+$), 164.6 (7). MALDI-HR-MS (DHB): 329.1645 (*M*H$^+$, $C_{22}H_{21}N_2O^+$; calc. 329.1648).

S-*[(2S)-2-(1H-Benzimidazol-2-yl)-3-[1,1′-biphenyl-4-yl]propyl] Ethanethioate* ((+)-**36**). *GP 4*, starting from (+)-**34** (148 mg, 0.45 mmol), gave (+)-**36** (79 mg, 44%) after double purification by CC (SiO_2-*60*; CH_2Cl_2/AcOEt 91:9 and pentane/AcOEt 83:17). Colorless solid. M.p. 183°. $[\alpha]_D^{25} = +20.5$ ($c = 1$, $CHCl_3$). IR (neat): 2745, 1694, 1539, 1486, 1445, 1271, 1130, 1008, 957. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.32 (*s*, 3 H); 3.20 (*dd*, $J = 13.4$, 5.3, 1 H); 3.33 (*dd*, $J = 13.4$, 7.8, 1 H); 3.42–3.46 (*m*, 3 H); 7.09–7.56 (*m*, 12 H); 7.77 (*d*, $J = 7.2$, 1 H); 8.91 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 30.3; 30.6; 39.2; 41.1; 113.9; 126.8; 126.9; 127.4; 127.6; 128.7; 129.1; 130.3; 134.2; 140.0; 140.4; 153.5; 199.4. MALDI-MS (DHB): 409.1 (17, $[M + Na]^+$); 387.2 (100, *M*H$^+$). MALDI-HR-MS (DHB): 387.1522 (*M*H$^+$, $C_{24}H_{23}N_2OS^+$; calc. 387.1526).

2-[(1S)-2-[1,1′-Biphenyl-4-yl]-1-(sufanylmethyl)ethyl]-1H-benzimidazol-3-ium Trifluoroacetate ((+)-**25**). *GP 5*, starting from (+)-**36** (25 mg, 0.064 mmol), afforded (+)-**25** (20 mg, 68%) after purification by RP-HPLC (*RP-18* SiO_2; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min). Colorless solid. M.p. 79°. $[\alpha]_D^{25} = +52.9$ ($c = 1$, $CHCl_3$). IR (neat): 2635, 1657, 1622, 1567, 1520, 1487, 1461, 1435, 1410, 1300, 1183, 1131. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.26 (br. *s*, 1 H); 2.81 (*dd*, $J = 13.9$, 4.8, 1 H); 3.02 (*dd*, $J = 13.9$, 9.8, 1 H); 3.13 (*dd*, $J = 14.0$, 7.8, 1 H); 3.24 (*dd*, $J = 14.0$, 8.1, 1 H); 3.76–3.86 (*m*, 1 H); 7.08–7.11 (*d*, $J = 8.1$, 2 H); 7.24–7.40 (*m*, 9 H); 7.57–7.62 (*m*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.0; 38.9; 44.7; 114.1; 125.5; 126.7; 127.2; 127.3; 128.6; 128.9; 131.5; 135.4; 139.8; 140.1; 154.6. MALDI-MS (DHB): 367.1 (8, $[M + Na]^+$), 345.1 (100, *M*H$^+$). MALDI-HR-MS (DHB): 345.1424 (*M*H$^+$, $C_{22}H_{20}N_2S^+$, calc. 345.1420).

(2R)-N-(3-Aminopyridin-4-yl)-3-(benzyloxy)-2-([1,1′-biphenyl-4-yl]methyl)propanamide ((+)-**31**). *GP 1*, *Method B*, starting from (−)-**29** (1.00 g, 2.89 mmol) and 3,4-diaminopyridine, afforded (+)-**31** (719 mg, 57%) after purification by CC (SiO_2-*60*; AcOEt). Colorless crystals. M.p. 148°. $[\alpha]_D^{25} = +12.6$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3407, 3326, 2868, 1688, 1618, 1583, 1511, 1484, 1422, 1306. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.89–2.99 (*m*, 2 H); 3.13–3.23 (*m*, 3 H); 3.72 (*dd*, $J = 9.7$, 6.5, 1 H); 3.76 (*dd*, $J = 9.7$, 3.7, 1 H); 4.56 (*s*, 2 H); 7.26–7.59 (*m*, 15 H); 8.01 (*d*, $J = 5.3$, 1 H); 8.03–8.08 (*m*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.7; 50.2; 70.2; 74.1; 116.9; 127.2; 127.6; 128.4; 128.5; 128.9; 129.1; 129.7; 132.6; 134.1; 137.4; 138.0; 139.8; 140.4; 140.8; 142.1; 172.5 (1 arom. signal overlapping). MALDI-MS (DCTB): 438.2 (100, *M*H$^+$), 330.2 (9), 219.1 (5). MALDI-HR-MS (DCTB): 438.2170 (*M*H$^+$, $C_{28}H_{28}N_3O_2^+$; calc. 438.2176). Anal. calc. for $C_{28}H_{27}N_3O_2$ (437.53): C 76.86, H 6.22, N 9.60; found C 76.84, H 6.29, N 9.64. X-Ray: see *Fig. 3*.

2-[(1S)-2-(Benzyloxy)-1-([1,1′-biphenyl-4-yl]methyl)ethyl]-1H-imidazo[4,5-c]pyridine ((+)-**33**). *GP 2*, starting from (+)-**31** (842 mg, 1.92 mmol), afforded (+)-**33** (805 mg, 100%). Colorless foam. M.p. 69°. $[\alpha]_D^{25} = +48.8$ ($c = 0.91$, $CHCl_3$). IR ($CHCl_3$): 3396, 3024, 2965, 1621, 1487, 1452, 1403, 1277, 1102, 903. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.15 (*dd*, $J = 13.5$, 9.5, 1 H); 3.37 (*dd*, $J = 13.5$, 5.5, 1 H); 3.54–3.57 (*m*, 1 H); 3.75 (*dd*, $J = 9.5$, 5.8, 1 H); 3.84 (*dd*, $J = 9.5$, 3.6, 1 H); 4.56 (*d*, $J = 11.7$, 1 H); 4.61 (*d*, $J = 11.7$, 1 H); 7.16–7.58 (*m*, 15 H); 8.40 (*d*, $J = 5.6$, 1 H); 9.02 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 2 drops of CF_3COOH added): 36.4; 42.2; 68.4; 73.9; 113.5; 126.8; 127.4; 127.4; 128.3; 128.5; 128.6; 128.7; 129.1; 131.5; 133.2; 134.2; 135.2; 136.1; 140.1; 140.2; 146.8; 166.6. MALDI-MS (DCTB): 420.21 (100, *M*H$^+$), 390.2 (6). MALDI-HR-MS (DCTB): 420.2065 (*M*H$^+$,

$C_{28}H_{26}N_3O^+$; calc. 420.2070). Anal. calc. for $C_{28}H_{25}N_3O$ (419.50): C 80.16, H 6.01, N 10.02; found C 80.18, H 6.19, N 9.88.

*(2*S*)-3-[1,1′-Biphenyl-4-yl]-2-(1*H*-imidazo[4,5-*c*]pyridin-2-yl)propan-1-ol* ((+)-**35**). To a soln. of (+)-**33** (477 mg, 1.14 mmol) in dry CH_2Cl_2 (50 ml), BCl_3 (1M in CH_2Cl_2, 11.4 ml, 11.4 mmol) was slowly added at $-78°$, and the mixture was stirred for 3 h at $-78°$. At 0°, MeOH and $NaHCO_3$ (*ca.* 5 g) were added, and the mixture was stirred for 12 h. The suspension was filtered, the precipitate was washed with CH_2Cl_2/MeOH, and the combined org. phases were concentrated *in vacuo*. The residue was purified by CC (SiO_2-*60*; AcOEt/MeOH 91:9). Since not all impurities could be removed, the crude product was dissolved in EtOH (12.3 ml) and 96% H_2SO_4 (0.18 ml), and the mixture was stirred for 2 h at 50° and for 12 h at r.t. The mixture was slowly neutralized with sat. aq. $NaHCO_3$ soln. and extracted (CH_2Cl_2). The org. phases were dried ($MgSO_4$) and concentrated *in vacuo* to afford (+)-**35** (220 mg, 59%). Colorless solid. M.p. 113°. $[\alpha]_D^{25} = +138.9$ ($c = 1$, EtOH). IR (neat): 3027, 2925, 2362, 2340, 1622, 1589, 1532, 1487, 1422, 1283, 1202, 1169, 1029. ^{1}H-NMR (300 MHz, CD_3OD): 3.16 (*dd*, $J = 13.7$, 9.0, 1 H); 3.24 (*dd*, $J = 13.7$, 6.2, 1 H); 3.45–3.52 (*m*, 1 H); 3.95–3.98 (*m*, 2 H); 7.15–7.54 (*m*, 10 H); 8.25 (*d*, $J = 5.6$, 1 H); 8.78 (*s*, 1 H). ^{13}C-NMR (75 MHz, CD_3OD): 36.2; 45.7; 63.7; 126.6; 126.8; 127.0; 128.6; 129.2; 138.1; 139.5; 140.5; 140.6; 140.8; 160.1 (3 arom. signals overlapping). MALDI-MS (DHB): 330.2 (100, MH$^+$), 322.3 (22), 165.1 (8). MALDI-HR-MS (DHB): 330.1607 (MH$^+$, $C_{21}H_{20}N_3O^+$; calc. 330.1601).

S-*[(2*S*)-3-[1,1′-Biphenyl-4-yl]-2-(1*H*-imidazo[4,5-*c*]pyridin-2-yl)propyl] Ethanethioate* ((+)-**37**). *GP 4*, starting from (+)-**35** (400 mg, 1.21 mmol), gave (+)-**37** (218 mg, 46%) after purification by CC (SiO_2-*60*; AcOEt/MeOH 99:1). Colorless oil. $[\alpha]_D^{25} = +23.0$ ($c = 1$, $CHCl_3$). IR (neat): 2923, 1979, 1687, 1620, 1587, 1520, 1487, 1422, 1353, 1282, 1130, 1107. ^{1}H-NMR (300 MHz, $CDCl_3$, 4 drops of CF_3COOH added): 2.31 (*s*, 3 H); 3.32–3.49 (*m*, 4 H); 3.87–3.96 (*m*, 1 H); 7.16 (*d*, $J = 8.1$, 2 H); 7.29–7.56 (*m*, 7 H); 8.24 (*d*, $J = 6.5$, 1 H); 8.53 (*d*, $J = 6.5$, 1 H); 9.59 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 4 drops of CF_3COOH added): 30.5; 32.0; 39.1; 43.0; 112.6; 113.9; 126.7; 127.4; 127.5; 127.6; 128.7; 128.8; 128.9; 131.4; 134.9; 140.0; 166.1; 185.0 (1 arom. signal overlapping). MALDI-MS (DHB): 410.1 (11, [M + Na]$^+$); 388.1 (100, MH$^+$); 312.1 (27). MALDI-HR-MS (DHB): 388.1478 (MH$^+$, $C_{23}H_{22}N_3OS^+$; calc. 388.1478).

*2-[(1*S*)-2-[1,1′-Biphenyl-4-yl]-1-(sulfanylmethyl)ethyl]-1*H*-imidazo[4,5-*c*]pyridin-5-ium Trifluoroacetate* ((+)-**26**). *GP 5*, starting from (+)-**37** (40 mg, 0.103 mmol), afforded (+)-**26** (27 mg, 57%) after purification by RP-HPLC (*RP-18* SiO_2; 0.1% aq. CF_3COOH/MeCN 99:1 → 0:100 in 60 min). Colorless solid. M.p. 82°. $[\alpha]_D^{25} = +71.0$ ($c = 0.5$, $CHCl_3$). IR (neat): 3029, 2657, 2111, 1667, 1644, 1522, 1487, 1475, 1411, 1309, 1177, 1128, 1007. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.52 (*t*, $J = 8.4$, 1 H); 3.07–3.16 (*m*, 2 H); 3.28–3.35 (*m*, 2 H); 3.70–3.79 (*m*, 1 H); 7.20–7.51 (*m*, 9 H); 7.94 (*d*, $J = 6.4$, 1 H); 8.16 (*d*, $J = 6.4$, 1 H); 9.26 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.6; 39.4; 46.4; 114.1; 127.1; 127.5; 128.9; 129.5; 131.9; 136.8; 139.8; 140.7; 167.6 (4 arom. signals overlapping). MALDI-MS (DHB): 368.1 (4, [M + Na]$^+$), 346.1 (100, MH$^+$), 322.3 (24). MALDI-HR-MS (DHB): 346.1377 (MH$^+$, $C_{21}H_{20}N_3S^+$; calc. 346.1372).

X-Ray Crystal Structure of (+)-**31**. Crystal data at 298 K for $C_{28}H_{27}N_3O_2$ (M_r 437.54): monoclinic, space group $P2_1$, $D_c = 1.269$ g cm^{-3}, $Z = 2$, $a = 4.79580(10)$, $b = 9.8396(2)$, $c = 24.2769(6)$ Å, $\beta = 91.5860(11)°$, $V = 1145.16(4)$ Å^3. *Bruker-Nonius Kappa CCD* diffractometer, MoK_α radiation, $\lambda = 0.71073$, linear crystal dimensions $0.44 \times 0.2 \times 0.08$ mm. The structure was solved by direct methods (SIR97) [34]. The non-H-atoms were refined anisotropically (SHELXL-97) [35]. The H-atoms were calculated at idealized positions and refined with constrained isotropic displacement parameters. Final $R(F) = 0.0495$, $wR(F^2) = 0.1314$ for 299 parameters, 1 restraint, and 5213 reflections with $I > 2\sigma(I)$ and $0.10 < \theta < 27.49°$. Deposition No. CCDC-258179. Copies of the data can be obtained free of charge on application to *Cambridge Crystallographic Data Centre* (CCDC), 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

X-Ray Crystal Structure of Inhibitor (+)-**2** *Bound to Neprilysin.* Crystals of the soluble extracellular domain (residues 52–749) of human NEP were obtained by vapor diffusion as described by *Oefner et al.* [2]. A binary complex of the glycosidase-treated sNEP was formed with (+)-**2** by soaking experiment. Crystals were transferred into the stabilization soln. for 30 min, which consists of 200 mM ammonium acetate, 25% PEG 3350, 100 mM *Hepes* (pH 7.5), and 30% ethylene glycol and containing inhibitor at a concentration of 10 mM. A crystal was flash-frozen in liquid N_2 at 100 K and belongs to the trigonal space group $P3_221$ with one molecule in the asymmetric unit. Diffraction intensities were measured with CuK_α radiation provided from a *NONIUS FR591* rotating anode generator equipped with an *OSMIC* mirror system and were recorded on a *MAR-Research* image plate area detector. All diffraction data were processed and scaled with DENZO and SCALEPACK [36], and further analyzed with the CCP4 program suite [37]. The structure of the binary complex was solved by using the refined protein coordinates of human sNEP present in a complex with phosphoramidon (PDB file name 1DMT, [2]). Prior to refinement, the phosphoramidon inhibitor and all

solvent molecules were removed from the model. Iterative rounds of model building were performed with MOLOC [8c], stereochemically restrained positional and temp.-factor refinement was done with REFMAC [38], using parameters for ideal stereochemistry as described by *Engh* and *Huber* [39]. A difference *Fourier* map revealed a residual electron density located in the active site corresponding to the bound small molecule. Progressive introduction of solvent molecules with good geometry lead to the binary complex, lacking the *N*-terminal two residues D52 and D53 of human sNEP. Data collection and refinement statistics are summarized in *Table 2*. PDB file name 1Y8J.

Table 2. *Data Collection and Refinement Statistics for the Complex of NEP with (+)-***2**

Cell axes *a*, *c*	107.4 Å, 112.5 Å
Resolution range	20.0–2.25 Å
No. of obs. reflections	93.629
No. of unique reflections	34.323
R_{sym} overall/outer shell[a])	7.8%/47.7% (2.39–2.25 Å)
$I/\sigma(I)$ overall/outer shell	11.9/1.8
Completeness overall/outer shell	95.4%/84.9%
Refinement statistics	
Resolution range [Å]	20–2.25
R_{cryst} (R_{free}) [%][b])	22.6 (29.6)
No. of protein atoms (mean *B* in Å^2)	5595 (29.7)
No. of H_2O molecules	212
No. of ligand atoms (mean *B* in Å^2)	20 (27.3)
No. of NAG atoms (mean *B* in Å^2)	42 (44.1)
Rmsd bonds [Å^2][c])	0.007
Rmsd angles [°]	0.89

[a]) $R_{sym} = \Sigma_h\Sigma_i |I_i(h) - \langle I(h)\rangle| / \Sigma_h\Sigma_i(h)$, where $I_i(h)$ and $\langle I(h)\rangle$ are the ith and mean measurement of the intensity of reflection *h*. [b]) $\Sigma_h ||F_{obs}| - |F_{calc}|| / \Sigma_h |F_{obs}|$, where $|F_{obs}|$ and $|F_{calc}|$ are the observed and calculated structure factor amplitudes for the reflection *h*, applied to the working (R_{cryst}) and test (R_{free}) sets, respectively. [c]) Rmsd: root mean-square deviation from mean.

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