SYNTHETIC STUDIES TOWARDS THE SYNTHESIS OF
WESTERN AND EASTERN CHLOROPEPTIN I, II SUBUNITS

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Abstract - The western subunit (16-membered ring) was synthesized by the intramolecular $S_{N}Ar$ reaction while the first 16-membered ring compound was obtained as a model of the eastern subunit via an intramolecular Ni$_{0}$ mediated coupling reaction.

Chloropeptins I and II are antibiotics produced by Streptomyces sp. WK-3419,1 whose most important biological activity is related to their ability to inhibit both the cytopathic effect in HIV-1 infected MT 4 cells and the syncytium formation in co-cultured HIV-1 infected MOLT-4 cells. Chloropeptin II, also referred to in the literature as complestatin, was reported to strongly inhibit the hemolysis of complement system sensitized erythrocytes.2
The 16-membered cyclotripeptide BODC (western subunit) common to chloropeptin I and chloropeptin II is characterized by an \textit{endo} biaryl ether bond connecting tyrosine B to the central 4-hydroxyphenylglycine D. This amino acid is linked to position 6 or 7 of tryptophane F by an \textit{endo} carbon-carbon bond, forming the 17-membered macrocycle of chloropeptin I, or the 16-membered ring of chloropeptin II (eastern subunit). The non-proteinogenic 3, 5-dichloro-4-hydroxyphenylglycine is found in both western and eastern subunits as amino acids C and E. From acidic degradation\textsuperscript{3} and studies combining computer modeling with NMR analysis,\textsuperscript{4} relative and absolute configurations\textsuperscript{6} have been recently assigned as (S), (R), (R) to amino acids B, C and D.

We report here our contribution which includes the synthesis and incorporation of the very racemization prone amino acid C in a BOD 16-membered polypeptide ring as a model of the western subunit and the first synthesis of a 16-membered ring macropolypeptide DEF containing an arylindole key component, as a simplified model of the eastern subunit of chloropeptin II.

**WESTERN SUBUNIT**

The very efficient intramolecular $S_N$Ar reaction recently developed in our group and successfully used in the synthesis of monocyclic,\textsuperscript{5a-d} bicyclic\textsuperscript{5e-g} and tricyclic polypeptides\textsuperscript{5h} containing respectively one, two or three \textit{endo} biaryl ether bonds was employed for ring closure of a linear peptide ABCD whose terminal aromatic rings carry the functionalities essential for the intramolecular $S_N$Ar reaction leading to the desired ABODC cyclised compound.

The synthesis of the precursor ABCD involved use of commercially available products: 3-hydroxyphenylacetic acid in place of the central amino acid D carrying the nucleophilic chromophore and (R)-4-methoxyphenylglycine methyl ester A while non-proteinogenic amino acids: (S)-phenylalanine derivative

\[
\begin{align*}
\text{A} & \quad \text{CH}_{3}	ext{OOC} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{CH}_{3} \\
\text{C} & \quad \text{CH}_{3}	ext{OOC} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{CH}_{3} \\
\text{B} & \quad \text{NO}_{2} \\
\text{D} & \quad \text{CH}_{3}	ext{OOC} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{CH}_{3} \\
\text{H} & \quad \text{CH}_{3}	ext{OOC} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{CH}_{3} \\
\text{F} & \quad \text{NO}_{2} \\
\text{OH} & \quad \text{CH}_{3}	ext{OOC} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{CH}_{3} \\
\end{align*}
\]
B carrying the activated nucleofuge on proper position, and 3,5-dichloro-4-methoxyphenylglycine C had to be synthesized.

Methylation of (R)-N-Boc-4-hydroxyphenylglycine by classical procedure to get 1a did not go to completion, but in acetone and in the presence of tetrabutylammonium iodide, the expected product was obtained in 90% yield. The extent of epimerization (less than 10%) was deduced from the ratio of the OMe signals in the 1H NMR spectrum of the mixture of peptides (3a+3b), obtained by coupling 2a with (S)-N-Boc-alanine (Scheme 2).

The non-proteinogenic amino acid derivative, (S)-N-Boc-N-methyl-4-fluoro-3-nitrophenylalanine methyl ester (6a) was obtained by methylation of N-Boc-4-fluoro-3-nitrophenylalanine methyl ester (5b) resulting from alkylation of the (S)-Schollkopf bislactim ether with 4-fluoro-3-nitrobenzyl bromide (4c). This electrophilic reagent was prepared in 74% overall yield from 4-fluorobenzaldehyde by nitration to give 4a, reduction of the formyl function to the alcohol (4b) and bromination. (S)-N-Boc-4-fluoro-3-nitrophenylalanine methyl ester (5b) was efficiently N-methylated to give 6a, the hydrolysis of which provided then the protected N-methyl amino acid ((S)-6b) (Scheme 3).
The second non-proteinogenic amino acid, \( (R-N-Boc-3,5\text{-dichloro-4-methoxyphenylglycine methyl ester} \) ((\( R \))-11) was prepared by standard Strecker methodology.\(^8\) 3-5-Dichloro-4-methoxybenzaldehyde (8) obtained by conventional methods from 3-5-dichloro-4-hydroxybenzoic acid was treated with \((R)\)-phenylglycinol as chiral agent and subsequently with TMSCN to afford a mixture of two diastereomers (9a+9b) (70/30). The low selectivity of this step was partly compensated by the high chemical yield. The major compound (9a) was isolated in 64% yield by column chromatography and its absolute configuration was deduced from the \(^1\)H NMR spectrum\(^9\) where the benzylic proton appears at higher field than that of the diastereomer ((\( S,S \))-9b). The conditions used for converting 9a to 10a led to a diastereomeric mixture (83/17) of amino esters (10a+10b) which was separated by silica gel column chromatography to afford pure 10a (73%) and 10b (15%). Oxidative cleavage of the chiral auxiliary afforded the respective amino esters hydrochlorides ((\( R \))-lla) and ((\( S \))-llb). The \((R)\) configuration of lla was confirmed by dehalogenation leading to \((R)\)-hydroxyphenylglycine derivative (1b) ([\( \alpha \])\(_D\)=-117°, \( \lambda \),\(^l\) [\( \alpha \])\(_D\)=-100°). N-Boc protection of lla and llb gave pure 12a and 12b, whose hydrolysis under controlled basic conditions led to the corresponding NH-Boc protected amino acid ((\( R \))-13a) and ((\( S \))-13b) (Scheme 4).

Reagents and conditions. a: i) \( K_2\text{CO}_3, (\text{CH}_3)_2\text{SO}_4 \), DMF; ii) LiAlH\(_4\), THF, 88%; b: PCC, CH\(_2\)Cl\(_2\), 91%; c: \((S)\)-phenylglycinol, TMSCN, CHCl\(_3\), 0°C, 90%; d: CH\(_3\)OH-HCl, 76%; e: Pb(OAc)\(_4\), CH\(_2\)Cl\(_2\), CH\(_3\)OH, 0°C, 82%; f: Boc\(_2\)O, \( N(\text{C}_2\text{H}_5)\)\(_2\), THF, 92%; g: K\(_2\)CO\(_3\), CH\(_3\)OH, 67%; h: H\(_2\), Pd/C, CH\(_3\)OH, 81%.
The synthesis of the tripeptide (15a) was easily achieved by coupling (R)-2a containing less than 10% of its epimer ((S)-2b) with (S)-N-Boc-4-fluoro-3-nitrophenylalanine (6b) to give (R,S)-14a. A simple TLC separation afforded pure (R,S)-14a and some amount of the (S,S)-diastereomer (14a'). Mild deprotection of 14a provided 14b in high yield whose coupling with N-Boc-(R)-3,5-dichloro-4-hydroxyphenylglycine (13a) under classical conditions provided an isomeric mixture of tripeptides ((R,S,R)-15a) and ((R,S,S)-16a) which was separated by chromatography. The compound (15a) was obtained in better yield, without appreciable racemisation by using bromo-tris(pyrro1idino)phosphonium hexafluorophosphate (PyBrop) as a coupling agent.11 After deprotection, coupling of 15b with 3-hydroxyphenylacetic acid gave the expected linear precursor ((R,S,R)-17a); similarly (R,S,S)-17b was prepared from 16a for the purpose of comparative macrocyclisation studies (Scheme 5).

Reagents and conditions. a: (S)-6b, HOBT, EDC, N(C6H5)3, CH2Cl2; b: TMSCI, NaI, CHCl3; c: 13a, PyBrOP, CH2Cl2, 0°C; d: TMSCI, NaI, CHCl3; e: 3-hydroxyphenylacetic acid, HOBT, EDC, CH2Cl2.
Macrocyclisation studies

The first reaction (entry 1, Table 1) of 17a under the previously established conditions proceeded slowly. A prolonged reaction time (20 h) was necessary to convert 80% of the starting material, yielding a mixture of six compounds in place of the pair of atropisomers (18a) and (18a') normally expected. Comparison of $^1$H NMR spectrum of the crude mixture with that of the open chain precursor (17a) showed several signals between 5.9 and 6.1 ppm, *i.e.* in the range of the upfield shifted H-21 characteristic of 16-membered macropolypeptides containing an *endo* biaryl ether function observed in synthetic compounds$^{5a-h}$ and in natural products$^{3,12}$ (Table 2). The ratios of the six cyclized compounds remained unchanged after 40 h (entry 2). An interesting observation came from the $^1$H NMR analysis of the outcome of a reaction carried out with the diastereomeric precursor ((R,S,S)-17b) which gave the same six products but in different ratios (entry 3). We were thus led to assume that among the four unexpected macrocycles, two at least (a pair of atropisomers of a diastereomeric macrocycle) might originate from racemization of amino acid C occurring under the slightly basic reaction conditions applied to the linear peptides (17a) and (17b).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Tetrapeptide</th>
<th>Conditions</th>
<th>Conversion %$^a$</th>
<th>Cyclized Products$^a$</th>
<th>18 %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a'</td>
</tr>
<tr>
<td>1</td>
<td>17a</td>
<td>K$_2$CO$_3$, DMF, 20 h</td>
<td>80</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>17a</td>
<td>K$_2$CO$_3$, DMF, 40 h</td>
<td>90</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>17b</td>
<td>K$_2$CO$_3$, DMF, 40 h</td>
<td>70</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>17b</td>
<td>K$_2$CO$_3$, DMF$^b$</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17b</td>
<td>Li$_2$CO$_3$, THF$^b$</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>17a</td>
<td>KHCO$_3$, THF$^c$, 40h</td>
<td>70</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>17b</td>
<td>KHCO$_3$, THF$^c$, 2h</td>
<td>100; 60$^d$</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>17c</td>
<td>KHCO$_3$, THF$^c$, 40h</td>
<td>100</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$Ratio obtained from $^1$H NMR. $^b$12-Crown-4. $^c$18-Crown-6. $^d$Yield of isolated products.

Several reaction parameters were then studied in order to determine the minimal basic conditions compatible with cyclisation. With Li$_2$CO$_3$ as a base in DMF (entry 4), or in THF where 12-crown-4 ether was added (entry 5), the linear peptide ((R,S,S)-17b) did not cyclize. Using KHCO$_3$ in THF where 18-crown-6 was added (entry 6), the linear peptide ((R,S,R)-17a) was observed to undergo cyclization.
giving an almost identical mixture of six products. In contrast, under the same conditions, the intramolecular SNAr reaction of 17b took place much faster (entry 7) to give only four products.

Preparative thin layer chromatography allowed isolation of pure products whose structures were established as pairs of (R,S,R) atropisomers (18a) and (18a') (natural configuration) and (R,S,S) 18b and 18b' in almost equal amount. This experiment clearly showed that amino acid C had undergone racemization. A separate experiment, in which no racemization of the cyclized product (18a) took place under the cyclization conditions clearly indicated that 17a had racemized prior to cyclization (Figure 2).

Table 2. Characteristic Chemical Shifts in $^1$H NMR of 18, and of Chloropeptins I and II

<table>
<thead>
<tr>
<th>Tetrapeptide</th>
<th>Cyclized Compounds 18</th>
<th>Chloropeptins</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,S,R) 17a</td>
<td>18a: R$_1$=H; R$_2$=NO$_2$</td>
<td>18b: R$_1$=H; R$_2$=NO$_2$</td>
</tr>
<tr>
<td>(R,S,S) 17b</td>
<td>18a': R$_1$=NO$_2$; R$_2$=H</td>
<td>(S,S,R) 18c</td>
</tr>
<tr>
<td>(R,S,R) 17c</td>
<td>2.98  6.74</td>
<td>2.59  6.08</td>
</tr>
<tr>
<td>(R,S,S) 17d</td>
<td>2.93  6.81</td>
<td>2.65  5.91</td>
</tr>
<tr>
<td>(S,S,R) 17e</td>
<td>2.74  6.76</td>
<td>2.97  6.07</td>
</tr>
<tr>
<td>(S,S,S) 17f</td>
<td>2.79  5.69</td>
<td>3.01  5.69</td>
</tr>
</tbody>
</table>
With the $^1$H NMR spectra of pure $18a$, $18a'$, $18b$ and $18b'$ in hand, we were able to identify four over the six products formed in previous reactions of $17a$ (entries 1, 2, 6), and furthermore, to detect unambiguously the epimerized $(R,S,S)$ precursor $(17b)$ in the crude mixture, demonstrating that indeed epimerization of 3,5 dichloro-4-methoxyphenylglycine had occurred prior to cyclisation when the intramolecular SNAr reaction was particularly slow. Finally, macrocyclisation of the peptide $(S,S,R)-17c$ (entry 8) which was slightly faster than that of $17a$ gave two major diastereomeric products in almost equal amounts, namely $(S,S,R)-18c$ and $(S,S,S)-18d$ (whose atropisomers could not be detected by $^1$H NMR) along with very minor amounts of $(R,S,R)-18a$ and $(R,S,S)-18b$. It became then possible to establish ($^1$H-NMR) that $18c$ and $18d$ were the two minor components present in the mixture of six products resulting from the reactions of the linear peptide $(R,S,R)-17a$.

**EASTERN SUBUNIT**

The eastern subunit of chloropeptine I differs from chloropeptine II by the position of the *endo* carbon-carbon linkage between the central hydroxyphenylglycine D with the indole component. Indeed, the former (at position C-7) results in a 16-membered ring macropolypeptide, while the latter (at position C-6) gives a 17-membered ring macropolypeptide. In a previous paper,$^{13}$ we have reported the synthesis of a simplified 17-membered ring macropolypeptide of kistamycin which is distinct of that of chloropeptin I by amino acid E only. Therefore only the synthesis of a simplified 16-membered macropolypeptide DFE encountered in chloropeptin II eastern subunit is here reported.

The macrolactamisation approach requires a linear precursor containing the 7-phenylindole subunit which turned to be hardly available due to the very poor yield$^{13}$ or the failure$^{14}$ reported for arylation of the indole at position C-7, and we focused our efforts on the macrocyclisation approach.

Reactions selected to effect ring closure by carbon-carbon linkage were those already reported in a preliminary study,$^{14}$ namely the intramolecular versions of cross-coupling Pd catalysed Suzuki reaction$^{15}$ and Ni$^{0}$ catalysed reaction according to Semmelhack.$^{16}$

The simplified precursors needed for performing the key intramolecular biaryl cross coupling reaction at position C-7 were easily prepared from 7-bromoindolepropionic acid (22b), itself synthesized from 7-bromoindole (17).$^{17}$ Formylation under Vilsmeier-Hack conditions led to 3-formylindole (20)$^{18}$ which was treated with ethyl monomalonate$^{19}$ to give ethyl indoleacrylate (21) whose selective reduction$^{20}$ gave the 7-bromo-3-indolepropionic acid ethyl ester (22a). The acid (22b) resulting from saponification was
then coupled with glycine methyl ester in place of amino acid E to give the peptide (23a), readily saponified to 23b (Scheme 6).

Reagents and conditions. a: POCI,, DMF at 0°C; b: HOOCCH2COOC2H5, pyridine, piperidine, 50°C, 60%; c: NaBH4, BiCl3, C2H5OH, 0°C, 40%; d: NaOH, CH3OH-H2O, 98%; e: glycine methyl ester hydrochloride, N(C2H5)2, HOBT, EDC, DMF, 58%; f: NaOH, C2H5OH-H2O, 84%; g: 3-aminomethylphenylboronic acid, CH2Cl2; h: Pd(OAc)2, Ba(OH)2, C2H5OH, DME; i: 3-bromobenzylamine hydrochlordide, N(C2H5)3, HOBT, EDC, DMF, 98%; j: Ni(Ph3P)2Cl2, Zn, Ph3P, DMF, 1%. 

Scheme 6

The linear precursor required for the Suzuki reaction was easily obtained by coupling (23b) with 3-aminomethylphenylboronic acid14 to give 24a. When a 0.01M solution of 24a in degassed C2H5OH/DME was treated with Pd(OAc)2 and Ba(OH)2, the only product which could be isolated and characterized was 24c.

The linear precursor (24b) carrying bromine instead of boronic acid on the benzylamine component needed for carrying out intramolecular Semmelhack type reaction was prepared by coupling the peptide (23b) with 3-bromobenzylamine under standard conditions. Treatment of a 0.01M solution of 24b with Ni0 (prepared apart according to the procedure described by Kende21) at 50°C lead after consumption of the starting material to a mixture of several products which were separated by preparative thin layer chromatography. The major product was once again 24c, but in the 1H NMR spectrum of a more polar and minor fraction the H-8 signal of 24b was shifted from 7.35 to 5.85 ppm, a value close to that of chloropeptin I (5.81).12 Further purification led finally to pure 25 whose structure was established by physical methods.

The yield was very poor (1%), but no efforts were made to increase it since chloropeptin I is probably an
artefact. Indeed, it has been very recently reported\textsuperscript{22} that chloropeptin II (also called complestatin) was quantitatively converted to chloropeptin I under acidic conditions (CF$_3$COOH), so that, the synthesis of the fully functionalized 17-membered ring eastern part, provides an access to the 16-membered ring compound. We are therefore now focusing our efforts on chloropeptin II which is more interesting than chloropeptin I from a biological point of view.

CONCLUSION

We have described an access to a 16-membered ABOD ring model of the western subunit of chloropeptins I, II via an S$_{\text{N}}$Ar cyclisation reaction and the first synthesis of a 16-membered ring macropolypeptide DFE containing an endo C-C bond, as model of the eastern subunit of chloropeptin I. These results represent an important step towards the total synthesis of chloropeptin I and chloropeptin II.

EXPERIMENTAL SECTION

Melting points were determined with a Richter apparatus. IR spectra were recorded on a Nicolet-205 spectrometer. $[\alpha]_D$ were recorded on a Perkin-Elmer 141 polarimeter. $^1$H NMR spectra were recorded on Brucker WP 200 SY (200MHz), AC-250 (200MHz), AC-300 (300MHz) and Brucker WM-400 (400MHz) spectrometers with tetramethylsilane as internal standard ($\delta$ ppm), using CDCl$_3$, CD$_3$OD, CD$_3$COCD$_3$ as solvents. All reactions requiring anhydrous conditions or inert atmosphere were conducted under argon. Analytical and preparative TLC were performed on SiO$_2$ plates.

(R)-N-tert-Butoxycarbonyl-4-methoxyphenylglycine methyl ester (1a)\textsuperscript{23}: To a solution of (R)-N-tert-Butoxycarbonyl-4-hydroxyphenylglycine (0.50 g, 1.87 mmol) in acetone (25 mL) were added K$_2$CO$_3$ (0.77 g, 5.61 mmol), TBAI (50 mg, 0.19 mmol) and (CH$_3$)$_2$SO$_4$ (0.39 mL) and the mixture was stirred at reflux. After 6 h, hydrolysis, extraction (AcOEt) and flash chromatography (SiO$_2$, heptane/AcOEt=9/1) gave 1a as an oil (0.50 g, 90%): $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 1.42 (s, 9H, Boc), 3.70 (s, 3H), 3.78 (s, 3H), 5.25 (d, 1H, $J$=7.3 Hz), 5.54 (d, 1H, $J$=7.3 Hz), 6.86 (d, 2H, $J$=8.7 Hz), 7.26 (d, 2H, $J$=8.7 Hz); $^{13}$C NMR $\delta$ 28.31, 52.59, 55.27, 57.06, 80.50, 114.51, 128.41, 131.35, 155.01, 159.70, 171.95.
N-[(R)-(4-Methoxyphenylacetic acid methyl ester)-(2S)-2-tert-butyloxycarbonylamino]propionamide (3a): To a solution of 1a (0.55 g, 1.80 mmol) in CH₃CN (4 mL), was added HCl (0.60 mL) and the mixture was kept at rt for 2 h. Evaporation of volatiles gave the hydrochloride salt (2b) (0.38 g, 89%). 2b (50 mg, 0.22 mmol) dissolved in anhydrous CH₂Cl₂ (5 mL) in the presence of N(C₅H₅)₃ (45 mL, 0.32 mmol) was kept for 15 min at rt and concentrated under vacuum. A solution of (S)-N-Boc-alanine (41 mg, 0.22 mmol), EDC (46 mg, 0.24 mmol) and HOBT (30 mg, 0.32 mmol) in CH₂Cl₂ (5 mL) was then added and stirred at rt for 4 h. Hydrolysis followed by extraction (AcOC₂H₅) and flash chromatography (heptane/AcOC₂H₅, 1:1) yielded 3a as an oil (74 mg, 93%): ¹H NMR (300 MHz, CDC₁₃) δ 1.34 (d, 3H, J=7.0Hz), 1.44 (s, 9H), 3.71 (s, 3H), 3.87 (s, 3H), 4.18-4.26 (m, 1H), 5.25 (br s, 1H, NH), 5.47 (d, 1H, J=7.0 Hz), 6.87 (d, 2H, J=8.6 Hz), 7.22 (br s, 1H, NH), 7.27 (d, 2H, J=8.6 Hz); ¹³C NMR δ 17.98, 28.34, 49.91, 52.72, 55.35, 55.91, 80.51, 114.41, 128.02, 128.51, 155.01, N-(tert-Butoxycarbonyl)-(S)-(4-fluoro-3-nitro)-phenylalanine methyl ester (5b): To a solution of compound 5a (261 mg, 1.08 mmol) in anhydrous THF (10 mL) were added BOqO (258 mg, 1.18 mmol) and N(C₅H₅)₃ (302 mL, 2.15 mmol) and the mixture was stirred at rt for 16 h. Solvent evaporation, extraction (AcOC₂H₅) and flash chromatography (SiO₂, heptane/AcOC₂H₅, 9:1) gave 5b (198 mg, 0.58 mmol, 54 %): mp 89-90° C (CH₂Cl₂/heptane); [α]D = +39° (c= 0.1, CHCl₃); ¹H NMR (200 MHz, CDC₁₃, CDCl₃) δ 1.42 (s, 9H), 3.05 (dd, 1H, J₁=6.5, J₂=13.9 Hz), 3.26 (dd, 1H, J₁=5.4, J₂=13.9 Hz), 3.77 (s, 3H), 4.59 (ddd, 1H, J₁=6.5, J₂=5.4 Hz), 5.13 (d, 1H, J₁=6.5 Hz), 7.22 (dd, J₁=8.6, J₂=10.4 Hz), 7.39-7.47 (m, 1H), 7.84 (dd, 1H, J₁=2.0, J₂=6.9 Hz); ¹³C NMR δ 27.97, 37.04, 52.33, 54.07, 79.96, 118.22 (d, J = 21.2 Hz), 126.56 (d, J = 2.4 Hz), 133.73 (d, J = 5.5 Hz), 136.40 (d, J = 7.9 Hz), 136.78 (d, J = 6.9 Hz), 154.35 (d, J = 262.3 Hz), 154.88, 171.50; MS (CI, isobutene) m/z 287 [M-57]+, 243.

(S)-N-(tert-Butoxycarbonyl)-N-methyl-(S)-(4-fluoro-3-nitro)phenylalanine methyl ester (6a): A mixture of 5b (0.30 g, 0.89 mmol), CH₃I (4.5 mL, 71.3 mmol) and Ag₂O (2.0 g, 8.92 mmol) in DMF (6 mL), was heated (60° C) in a sealed tube for 60 h. Usual work up and preparative TLC (heptane/AcOC₂H₅, 9:1) gave 6a (0.260 g, 83%) as a colorless oil: [α]D = -16° (c= 0.24, CHCl₃); ¹H NMR (200 MHz, CDC₁₃) δ 1.38 (s, 9H), 2.74 (s, 3H), 3.08 (dd, 1H, J₁=10.6, J₂=14.5 Hz), 3.36 (dd, 1H, J₁=5.3, J₂=14.5 Hz), 3.76 (s, 3H), 4.53 and 4.88 (2 dd, 1H, J₁=5.3, J₂=10.6 Hz), 7.17-7.22 (m, 1H), 7.40-7.46 (m, 1H), 7.88 (dd, 1H, J₁=2.0, J₂=7.0 Hz); ¹³C NMR δ 28.26, 34.05, 34.65, 52.52,
59.43, 80.77, 118.47 (d, J = 20.4 Hz), 126.38, 134.92, 136.23 (d, J = 8.7 Hz), 136.80, 154.47 (d, J = 263.3 Hz), 154.54, 171.04; MS (Cl, isobutene) m/z 357 [M+H]+, 301, 257; Anal. Calcd for C_{16}H_{21}N_{2}O_{6}F: C, 53.92; H, 5.94; N, 7.86. Found: C, 54.09; H, 5.84; N, 7.72.

(S)-N-(tert-Butoxycarbonyl)-N-methyl-(S)-(4-fluoro-3-nitro)phenylalanine (6b): Compound (6a) (230 mg, 0.65 mmol) in CH_{3}OH (16 mL) was treated at rt for 6 h with H_{2}O (2 mL) and K_{2}CO_{3} (133 mg, 0.97 mmol). Acid-base workup gave 6b (199 mg, 90%): mp 176.1-178°C (acetonelheptane); [α]D = -12° (c=0.1, CH_{3}OH); {^1}H NMR (200 MHz, CDCl_{3}, δ 1.32 (s, 9H), 2.73 and 2.75 (2s, 3H), 3.23 (dd, 1H, J_{1}=10.9, J_{2}=14.4 Hz), 3.39 (dd, 1H, J_{1}=5.0, J_{2}=14.4 Hz), 4.81 and 4.96 (2dd, 1H, J_{1}=5.0, J_{2}=10.9 Hz), 7.42 (dd, 1H, J_{1}=8.7, J_{2}=10.8 Hz), 7.71-7.74 (m, 1H), 8.04 (dd, 1H, J_{1}=1.6, J_{2}=7.0 Hz); {^{13}C} NMR δ 28.20, 33.78, 34.35, 59.76, 81.20, 118.48 (d, J = 20.5 Hz), 126.05, 134.77 (d, J = 4.2 Hz), 136.23 (d, J = 8.7 Hz), 137.15 (d, J = 6.9 Hz), 154.44 (d, J = 262.5 Hz), 155.10, 174.58; MS (Cl, isobutene) m/z 343 [M+H]+, 287, 243.

3,5-Dichloro-4-methoxybenzyl alcohol (7): To a solution of methyl 3,5-dichloro-4-methoxybenzoate (5 g, 21.3 mmol) in anhydrous THF (50 mL) was slowly added LiAlH_{4} (1.0 g, 26.3 mmol) in anhydrous THF (20 mL) and the solution was stirred at rt for 4 h. Hydrolysis by succesive addition of H_{2}O (2 mL), 15% NaOH (2 mL) and H_{2}O (8 mL), filtration, evaporation and extraction (CH_{2}Cl_{2}) gave 7 (3.87 g, 88%): mp 40°C (MeOH/(C_{2}H_{5})_{2}O) lit.,24 42-44°C; {^1}H NMR (200 MHz, CDCl_{3}) δ 2.39 (br s 1H), 3.87 (s, 3H), 4.58 (s, 2H), 7.26 (s, 2H); {^{13}C} NMR δ 60.82, 63.64, 127.14, 129.35, 138.51, 151.30.

3,5-Dichloro-4-methoxybenzaldehyde (8): To a suspension of PCC (6.0 g, 28.0 mmol) in CH_{2}Cl_{2} (20 mL) was added 7 (2.9 g, 14.0 mmol) in CH_{2}Cl_{2} (30 mL) and the reaction mixture was stirred at rt for 4 h. The liquid phase was removed and the solid residue washed thoroughly with CH_{2}Cl_{2}. Evaporation and chromatography (SiO_{2}, CH_{2}Cl_{2}/ether. 9:1) gave 8 (2.61 g, 91%): mp 55-56°C (CH_{2}Cl_{2}/heptane); lit.,25 58°C; {^1}H NMR (200 MHz, CDCl_{3}) δ 4.00 (s, 3H), 7.85 (s, 2H), 9.83 (s, 1H, ); {^{13}C} NMR δ 61.13, 130.15, 131.33, 133.24, 157.37, 188.77.

[(R)-(3,5-Dichloro-4-methoxyphenyl)-(S)-(2-hydroxy-1-phenylethylamino)]acetonitrile (9a): A solution of 8 (8.34 g, 40.66 mmol) in CHCl_{3} (47 mL) added with (S)-phenylglycinol (5.57 g, 40.66
mmol) was stirred at rt for 5 h, cooled to 0°C and then successively added with dry MeOH (2 mL) and TMSCN (8.1 mL, 61.0 mmol). After stirring for 15 h at rt, concentration and flash chromatography (SiO2, heptane/AcOCH5, 9:1) gave two compounds, (R,S)-9a and (S,S)-9b.

(R,S)-9a (9.22 g, 64%) colorless oil: [α]D = -15° (c = 0.13, CHCl3); IR (CHCl3) ν 3331, 1560, 1486, 1457, 1421; 1H NMR (300 MHz, CDCl3) δ 1.61 (br s, OH exchange with D2O), 2.60 (d, 1H, J = 10.6 Hz), 3.67 (dd, 1H, J1 = 9.5, J2 = 10.6 Hz), 3.85 (dd, 1H, J1 = 3.9, J2 = 10.6 Hz), 3.90 (s, 3H), 4.22 (dd, 1H, J1 = 3.9, J2 = 10.6 Hz), 7.35-7.43 (m, 5H), 7.48 (s, 2H); 13C NMR δ 50.79, 60.92, 63.37, 67.34, 117.86, 127.47, 127.77, 127.83, 128.71, 128.83, 129.29, 130.05, 132.35, 137.57, 152.91; MS (CI, isobutene) m/z 382, 380 [M-HCN+57]+, 326, 324 [M-HCN+H]+; CIHRMS m/z 324.0539/326.0537 (C17H16N2O2Cl2-HCN+H+ requires 324.0558/326.0551).

(S,S)-9b (3.71 g, 26%) colorless oil: [α]D = +35° (c = 0.16, CHCl3); IR (CHCl3) ν 3331, 1708, 1560, 1483, 1456, 1428; 1H NMR (300 MHz, CDCl3) δ 1.97 (br s, OH), 2.51 (br s, 1H), 3.65-3.85 (m, 2H), 3.89 (s, 3H), 3.90-3.95 (m, 1H), 4.76 (br s, 1H), 7.25-7.35 (m, 8H); 13C NMR δ 50.14, 60.79, 62.27, 66.91, 118.35, 127.66, 127.71, 127.80, 128.08, 128.45, 128.90, 129.20, 129.96, 132.33, 138.48, 152.80; MS (CI, isobutene) m/z 382, 380 [M-HCN+57]+, 326, 324 [M-HCN+H]+; CIHRMS m/z 384.0761/386.0717 (C18H19N2O4Cl2+H+ requires 384.0769/386.0739).

[(R)-(3,5-Dichloro-4-methoxyphenyl)-(S)-(2-hydroxy-1-phenylethylamino)]acetic acid methyl ester (10a): A solution of aminonitrile (9a) (0.60 g, 1.71 mmol) in saturated solution of gaseous hydrochloric acid in dry methanol (10 mL) was stirred at rt for 15 h. Evaporation of solvent, neutralization (buffer phosphate solution), extraction (CH2Cl2) and column chromatography (SiO2, heptane/AcOCH5, 3:1) gave a mixture of 10a and 10b (10%). A further purification on TLC afforded a pure sample of 10a (0.442 g, 76%) as a colorless oil: [α]D = -21° (c = 0.13, CHCl3); IR (CHCl3) ν 1741, 1557, 1475, 1450, 1421, 1402; 1H NMR (300 MHz, CDCl3) δ 3.57-3.69 (m, 1H), 3.70 (s, 3H), 3.75-3.80 (m, 1H), 3.70 (s, 3H), 4.20 (br s, 1H), 7.21-7.33 (m, 8H); 13C NMR δ 52.6, 60.66, 61.68, 63.56, 67.31, 127.58, 127.76, 128.29, 128.72, 129.47, 135.78, 139.41, 151.94, 173.17; MS (CI, isobutene) m/z 442, 440 [M+57]+, 386, 384 [M+H]+; CIHRMS m/z 384.0761/386.0717 (C18H19N2O4Cl2+H+ requires 384.0769/386.0739).

(R)-(3,5-Dichloro-4-methoxy)phenylglycine methyl ester hydrochloride (11a): To a solution of 10a
(0.500 g, 1.3 mmol) in CH₂Cl₂ (10 mL) and CH₃OH (5 mL) was added Pb(OAc)₄ (0.634 g, 1.43 mmol) and the solution was stirred at 0° C for 10 min, diluted with phosphate buffer (pH 7) and then stirred for another 30 min. After filtration through Celite and water addition, extraction (CH₂Cl₂) gave crude aldimine which was dissolved in ether (10 mL) and IN HCl and stirred at rt for 3 h. Ether extraction of the aqueous phase to remove the neutral material, and evaporation gave the pure hydrochloride salt of 11a (0.323 g, 82%): mp 175° C (CH₃OH/C₂H₅)₂0; [α]D= +104.3° (c= 0.25, 0.1 N, HCl); ¹H NMR (200 MHz, D₂O) δ 3.83 (s, 3H), 3.95 (s, 3H), 5.28 (s, 1H), 7.52 (s, 2H); ¹³C NMR δ 53.26, 57.94, 61.22, 127.95, 130.09, 137.99, 152.52, 173.85; MS (CI, isobutene) m/z 322, 320 [M+57]+, 266, 264 [M+H]+; CIHRMS m/z 364.0693/366.0610 (C₁₅H₁₉N₀₅C₁₂ + H⁺ requires 364.0718/366.0688).

N-(tert-Butoxycarbonyl)-(R)-(3,5-dichloro-4-methoxy)phenylglycine methyl ester (12a): To a solution of compound (11a) (0.200 g, 0.66 mmol) in anhydrous THF (10 mL) were added Boc₂O (0.159 g, 0.73 mmol) and N(CZH~)₂ (186 mL, 1.33 mmol) and the solution was stirred at rt for 5 h. Solvent evaporation and extraction (AcO₂C₂H₅) gave pure 12a (0.223 g, 92%) oil: [α]D= -54° (c= 0.12, CHCl₃); ¹H NMR (300 MHz, CDC₁₃) δ 1.43 (s, 9H), 3.74 (s, 3H), 3.88 (s, 3H), 5.25 (d, 1H, J = 6.7 Hz), 5.80 (d, 1H, J = 6.7 Hz), 7.32 (s, 2H); ¹³C NMR δ 28.25, 53.08, 56.39, 60.63, 80.55, 127.55, 129.70, 134.65, 152.29, 154.63, 170.49; MS (CI, isobutene) m/z 366, 352 [M+H]+; 310, 308, 296, 266, 264; CIHRMS m/z 364.0693/366.0667 (C₁₅H₁₉N₀₅C₁₂ + H⁺ requires 364.0718/366.0688).

N-(ter-Butoxycarbonyl)-(R)-(3,5-dichloro-4-methoxy)phenylglycine (13a): Compound (12a) (200 mg, 0.55 mmol) in CH₃OH (7 mL), was treated at rt for 2 h with an aqueous solution of K₂CO₃ (115 mg, 0.82 mmol). Acid-base workup gave 13a (129 mg, 67%): mp 66-68° C (CHCl₃/heptane); [α]D= +4° (c= 0.1, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 1.26 (s, 9H), 3.89 (s, 3H), 5.03 (d, 1H, J = 4.2 Hz), 7.36 (s, 2H), 8.06 (d, 1H, J = 4.2 Hz); ¹³C NMR δ 28.11, 57.80, 60.83, 82.68, 127.71, 129.47, 134.65, 152.9, 154.63, 170.49; MS (CI, isobutene) m/z 366, 352 [M+H]+, 296, 294, 252, 250; CIHRMS m/z 350.0573/352.0510 (C₁₄H₁₇N₀₅C₁₂ + H⁺ requires 350.0562/352.0532).

[(2S)-2-(tert-Butoxycarbonylmethylamino)-3-(4-fluoro-3-nitrophenyl)propionylamino]-(R)-(4-methoxyphenyl)methyl ester (14a): To a solution of 2a (145 mg, 0.63 mmol) in CH₂Cl₂ (4 mL), were
added successively N(C₆H₅)₃ (132 mL, 0.94 mmol), 6b (211 mg, 0.62 mmol), EDC (118 mg, 0.62 mmol), HOBT (125 mg, 0.92 mmol) and the solution was stirred for 2 h at rt and extracted (C₂H₅OAc). The organic phase was washed with brine, dried (Na₂SO₄) and evaporated. Purification by preparative TLC (heptane/AcOCH₅, 99:1) gave 14a (295 mg, 92%): mp 40-42°C (CHCl₃/heptane); [α]D = -113.0° (c= 0.1/CHCl₃); ¹H NMR (250 MHz, CDCl₃) δ 1.42 (s, 9H), 2.84 (s, 3H), 2.96 (dd, 1H, J₁ = 8.9, J₂ = 14.2 Hz), 3.33-3.41 (m, 1H), 3.71 (s, 3H), 3.79 (s, 3H), 4.91-4.97 (m, 1H), 5.40 (d, 1H, J = 6.7 Hz), 6.85 (d, 2H, J = 8.6 Hz), 7.12-7.16 (m, 1H), 7.18-7.22 (d, 2H, J = 8.6 Hz in m, 1H), 7.43-7.51 (m, 1H), 7.88 (dd, 1H, J₁ = 2.2, J₂ = 7.0 Hz); ¹³C NMR 6 28.25, 30.79, 32.94, 52.79, 55.36, 56.12, 58.90, 81.28, 114.50, 118.41 (d, J = 25.5 Hz), 126.43, 128.39, 128.57, 134.96, 136.33 (d, J = 8.0 Hz), 136.46, 154.36 (d, J = 261.7 Hz), 155.06, 159.96, 169.38, 171.23; MS (CI, isobutene) m/z 520 [M+H]+, 464, 420; CIHRMS m/z 520.2070 (C₂₇H₃₀N₃O₈F + H+ requires 520.2095).

[3-(4-Fluoro-3-nitrophenyl)-(2S)-2-methylaminopropionylamino]-(R)-(4-methoxyphenyl)acetic acid methyl ester (14b): To a solution of 14a (300 mg, 0.58 mmol) in CHCl₃, (5 mL) were added NaI (105 mg, 0.69 mmol) and TMSCl (90 mL, 0.69 mmol). The reaction mixture was stirred for 10 min at rt, then quenched by CH₃OH (5 mL) and neutralized with KHCO₃. Extraction (C₂H₅OAc) and preparative TLC (CH₂Cl₂/CH₃OH, 99:1) gave 14b (230 mg, 94%): mp 82°C (CHCl₃/heptane); [α]D = -89° (c= 0.11/CHCl₃); IR (CHCl₃) ν 1774, 1672, 1542, 1513, 1441, 1354; ¹H NMR (250 MHz, CDCl₃) δ 1.18-1.28 (m, 1H), 2.33 (d, 3H, J = 6.0 Hz), 2.99 (dd, 1H, J₁ = 7.5, J₂ = 14.0 Hz), 3.13 (dd, 1H, J₁ = 5.3 Hz, J₂ = 14.0 Hz), 3.25-3.27 (m, 1H), 3.71 (s, 3H), 3.80 (s, 3H), 5.48 (d, 1H, J = 7.6 Hz), 6.87 (d, 2H, J = 8.6 Hz), 7.19 (dd, 1H, J₁ = 8.8, J₂ = 10.4 Hz), 7.22 (d, 2H, J = 8.6 Hz), 7.43-7.46 (m, 1H), 7.74 (d, 1H, J = 7.6 Hz), 7.88 (dd, 1H, J₁ = 2.1, J₂ = 6.9 Hz); ¹³C NMR δ 35.11, 37.51, 52.76, 55.33, 55.50, 65.12, 114.43, 118.55 (d, J = 20.3 Hz), 126.54, 128.31, 128.53, 134.63 (d, J = 3.2 Hz), 136.48 (d, J = 7.9 Hz), 137.13 (d, J = 6.9 Hz), 154.55 (d, J = 262.7 Hz), 159.79, 171.38, 172.07; MS (CI, isobutene) m/z 420 [M+H]+, 390; CIHRMS m/z 420.1548 (C₂₇H₂₂N₃O₆F + H+ requires 420.1570); Anal. Calcd for C₂₇H₂₂N₃O₆F: C, 57.27; H, 5.28; N, 10.01. Found: C, 57.19; H, 5.54; N, 9.37.

[2-{(R)-(tert-Butoxycarbonylamino-(3,5-dichloro-4-methoxyphenyl)acetyl]methylamino}-(3S)-3-(4-fluoro-3-nitrophenyl)propionylamino]-(R)-(4-methoxyphenyl)acetic acid methyl ester (15a): To a solution of 14b (126 mg, 0.30 mmol) in CH₂Cl₂ (2 mL) were added 13a (105 mg, 0.30 mmol) and
PyBrOP (186 mg, 0.41 mmol). The reaction mixture was stirred for 2 h at 0° C and then for 4 h at rt. Extraction (AcOC2H5) and preparative TLC (CH2Cl2/CH3OH, 99:1) gave a mixture of 15a, containing 16a (less than 10%).

15a Oil (146 mg, 65 %): \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 1.31 (s, 9H), 2.08-2.97 (m, 1H), 3.03 (s, 3H), 3.33-3.40 (m, 1H), 3.72 (s, 3H), 3.79 (s, 3H), 3.88 (s, 3H), 5.35 (d, 1H, J = 6.8 Hz), 5.43 (d, 1H, J = 6.8 Hz), 5.59 (d, 1H, J = 7.2 Hz), 5.82 (d, 1H, J = 7.2 Hz), 6.85 (d, 2H, J = 8.6 Hz), 7.03-7.16 (m, 3H), 7.25 (d, 2H, J = 8.6 Hz), 7.35 (br s, 2H), 7.40-7.44 (m, 1H), 7.76 (dd, 1H, J = 7.2, J = 8.5 Hz), 5.35 (d, 1H, J = 6.8 Hz), 5.43 (d, 1H, J = 6.8 Hz), 5.59 (d, 1H, J = 7.2 Hz), 5.82 (d, 1H, J = 7.2 Hz), 6.85 (d, 2H, J = 8.6 Hz), 7.03-7.16 (m, 3H), 7.25 (d, 2H, J = 8.6 Hz), 7.35 (br s, 2H), 7.40-7.44 (m, 1H), 7.76 (dd, 1H, J = 7.2, J = 8.5 Hz); \(^{13}\)C NMR \(\delta\) 28.22, 30.98, 32.74, 52.78, 55.33, 56.45, 56.92, 60.81, 80.55, 114.52, 118.44 (d, J = 21.7 Hz), 126.29, 128.14, 128.23, 128.42, 128.96, 133.81, 134.26, 135.85 (d, J = 7.4 Hz), 136.26 (d, J = 8.6 Hz), 152.74, 154.34 (d, J = 8.6 Hz), 155.28, 159.95, 167.63, 168.40, 171.34; FABMS (thio/Na\(^+\)) m/z 775, 773 [M+Na\(^+\)), 753, 751 [M+H\(^+\)], 697, 695, 653, 651.

[2-((R)-(3,5-Dichloro-4-methoxyphenyl)acetyl]methylamino)-(3S)-3-(4-fluoro-3-nitrophenyl)propionylamino]-(R)-(4-methoxyphenyl)acetic acid methyl ester (15b): To a solution of 15a (145 mg, 0.193 mmol) in CHCl\(_3\), (5 mL), were added NaI (35 mg, 0.23 mmol) and TMSCl (44 \(\mu\)L, 0.35 mmol) and the solution was stirred for 6 h at rt and then quenched by CH3OH (5 mL). Neutralization with KHCO\(_3\), extraction (C\(_2\)H\(_5\)OAc) and preparative TLC (CH2Cl2/CH3OH, 99:1) gave 15b (103 mg, 82%): mp 60-62° C (CHCl\(_3\)/heptane); [\(\alpha\)]\(_D\) = -136° (c=0.16, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 2.93 (br s, 4H), 3.26 (dd, 1H, J = 6.9, J = 14.5 Hz), 3.71 (s, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 4.70 (br s, 1H), 5.43 (d, 1H, J = 7.3 Hz), 5.54 (dd, 1H, J = 7.3, J = 8.5 Hz), 6.86 (d, 2H, J = 8.6 Hz), 7.06 (br s, 3H), 7.24 (d, 3H, J = 8.6 Hz), 7.32-7.38 (m, 1 H), 7.76 (dd, 1H, J = 2.1, J = 6.9 Hz); \(^{13}\)C NMR \(\delta\) 30.93, 32.73, 52.91, 55.42, 55.93, 56.16, 57.00, 60.82, 114.61, 118.58 (d, J = 21.7 Hz), 126.42, 127.35, 128.03, 128.70, 130.03, 133.66, 135.94 (d, J = 7.8 Hz), 139.90, 137.96 (d, J = 8.6 Hz), 152.40, 154.43 (d, J = 262.5 Hz), 160.04, 168.44, 171.51, 174.43; MS (Cl, isobutene) m/z 652, 650 [M+H\(^+\)].

[2-[(R)-(3,5-Dichloro-4-methoxyphenyl)acetyl]methylamino]-(3S)-3-(4-fluoro-3-nitrophenyl)propionylamino]-(R)-(4-methoxyphenyl)acetic acid methyl ester (17a): To a solution of 16b (24.0 mg, 0.037 mmol) in CH2Cl2 (0.5 mL) were added 3-hydroxyphenylacetic acid (5.6 mg, 0.037 mmol), EDC (7.1 mg, 0.037 mmol), HOBT (7.5 mg, 0.055 mmol) and
the solution was stirred for 30 min at rt and then extracted (C₂H₅OAc). Purification by preparative TLC (CH₃OH/CH₂Cl₂, 99:1) gave 17a (23.9 mg, 83 %): mp 89-91°C (CH₂Cl₂/heptane); [α]D = +96° (c=0.08, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 2.84 (dd, 1H, J₁ = 5.3, J₂ = 14.7 Hz, H-15), 2.98 (s, 3H, H-34), 3.28 (dd, 1H, J₁ = 7.2, J₂ = 14.7 Hz, H-15'), 3.34 (s, 2H, H-8), 3.71 (s, 3H, H-26), 3.76 (s, 3H, H-33), 3.86 (s, 3H, H-41), 5.46 (d, 1H, J = 6.7 Hz, H-24), 5.49 (d, 1H, J = 7.2 Hz, H-14), 5.62 (d, 1H, J = 6.6 Hz, H-11), 6.58-6.62 (m, 2H, H₄, H-6), 6.74 (d, 2H, J = 8.6 Hz, H-29, H₃₁, 1H, H-21), 6.92 (d, 1H, J = 6.6 Hz, H-10), 7.03 (dd, 1H, J₁ = 8.6, J₂ = 10.4 Hz, H-19), 7.05 (s, 2H, H-36, H-40), 7.12 (t, 1H, J = 7.7 Hz, H-5), 7.15 (d, 2H, J = 8.6 Hz, H-28, H-32), 7.26-7.31 (m, 1H, H-20), 7.55 (d, 1H, J = 6.7 Hz, H-23), 7.74 (dd, 1H, J₁ = 2.2, J₂ = 6.9 Hz, H-17); ¹³C NMR δ 31.33, 33.04, 42.83, 53.12, 53.49, 55.43, 56.36, 57.33, 60.88, 114.40, 114.99, 116.21, 118.52 (d, J = 21.6 Hz), 121.26, 126.25 (d, J = 2.8 Hz), 127.45, 128.20, 129.06, 130.05, 130.30, 133.17, 133.65 (d, J = 4.6 Hz), 135.44, 135.92, (d, J = 7.8 Hz), 137.02 (d, J = 8.6 Hz), 152.76, 154.40 (d, J = 263.8 Hz), 156.84, 159.90, 168.26, 170.84, 171.05, 171.95; FABMS (thio/Na⁺) m/z 788, 786 [M+H]+, 592, 590.

[2-((S)-(3,5-Dichloro-4-methoxyphenyl)-(2-(3-hydroxyphenyl)acetylamino)acetylmethylamino)propionylamino]-(R)-(4-methoxyphenyl)acetic acid methyl ester (17b): To a solution of 16b (21.5 mg, 0.033 mmol) in CH₂Cl₂ (0.5 mL) were added 3-hydroxyphenylacetic acid (5 mg, 0.03 mmol), EDC (6.4 mg, 0.03 mmol) and HOBT (6.7 mg, 0.049 mmol) and the solution was stirred for 45 min at rt. Extraction (C₂H₅OAc) and preparative TLC (CH₃OH/CH₂Cl₂, 99:1) gave 17b as an oil (21 mg, 81 %):¹H NMR (300 MHz, CDCl₃) δ 2.86-2.90 (m, 1H, H-15'), 2.93 (s, 3H, H-34), 3.63 (dd, 1H, J₁ = 7.9, J₂ = 14.6 Hz, H-15'), 3.47 (s, 2H, H-8), 3.60 (s, 3H, H-26), 3.79 (s, 3H, H-33), 3.87 (s, 3H, H-41), 5.24 (d, 1H, J = 7.5 Hz, H-14'), 5.29 (d, 1H, J = 7.0 Hz, H-24'), 5.68 (d, 1H, J = 7.0 Hz, H-11'), 6.62 (br s, 1H, H-23), 6.70-6.73 (m, 2H, H-4, H-6), 6.81 (d, 2H, J = 8.6 Hz, H-29, H-31, 1H, H-21), 6.94 (br s, 1H, H-10), 7.03 (d, 2H, J = 8.6 Hz, H-28, H-32), 7.07 (dd, 1H, J₁ = 8.8, J₂ = 10.5 Hz, H-19), 7.17 (t, 1H, J = 7.7 Hz, H-5), 7.29 (s, 2H, H-36, H-40), 7.33-7.38 (m, 1H, H-20), 7.31 (dd, 1H, J₁ = 2.0, J₂ = 7.2 Hz, H-17).

[2-((S)-(3,5-Dichloro-4-methoxyphenyl)-(2-(3-hydroxyphenyl)acetylamino)acetylmethylamino)propionylamino]-(S)-(4-methoxyphenyl)acetic acid methyl ester (17c): To a solution of 16c (2.0 mg, 3 µmol) in CH₂Cl₂ (0.2 mL) were added 3-hydroxyphenylacetic acid
(0.5 mg, 3 μmol), EDC (0.6 mg, 3 μmol) and HOBT (0.7 mg, 4 μmol) and the solution was stirred for 20 min at rt. Extraction (C2H5OAc) and preparative TLC (CH3OH/CH2Cl2, 99:1) gave 17c as an oil (1.5 mg, 66 %): 1H NMR (300 MHz, CDCl3) δ 2.74 (s, 3H, H-34), 2.87 (dd, 1H, J1 = 10.1, J2 = 14.7 Hz, H-15), 3.29 (dd, 1H, J1 = 5.9, J2 = 14.7 Hz, H-15'), 3.51 (d, 2H, J = 5.3 Hz, H-8), 3.74 (s, 3H, H-26), 3.79 (s, 3H, H-34), 3.86 (s, 3H, H-41), 4.58 (br s, 1H, H-14), 5.60 (d, 1H, J = 7.0 Hz, H-24 and 1H, H-11), 6.67-6.72 (m, 1H, H-4 and 1H, H-6), 6.76 (br s, 1H, H-21), 6.79 (br s, 1H, H-10), 6.87 (d, 2H, J = 8.8 Hz, H-29, H-31), 7.01 (s, 2H, H-36, H-40), 7.08 (t, 1H, J = 9.4 Hz, H-19), 7.18 (t, 1H, J = 7.7 Hz, H-5), 7.23 (d, 2H, J = 8.8 Hz, H-28, H-32), 7.26 (br s, 1H, H-23), 7.30-7.36 (m, 1H, H-20), 7.39 (s, 3H, H-34), 7.48 (br s, 1H, H-21), 5.60 (d, 1H, H-14), 6.08 (br s, 1H, H-21), 6.45 (d, 1H, J = 7.2 Hz, H-23), 6.70 (d, 2H, J = 8.7 Hz, H-29, H-31), 6.87 (d, 2H, J = 8.7 Hz, H-28, H-32), 6.89 (d, 1H, J = 7.9 Hz, H-6), 6.96 (d, 1H, J = 7.5 Hz, H-10), 6.99 (d, 1H, J = 8.3 Hz, H-19), 7.15 (2dd, 2H, J1 = 2.0, J2 = 8.3 Hz, H-4, H-20), 7.29 (t, 1H, J = 7.9 Hz, H-5), 7.36 (s, 2H, H-36, H-40), 8.03 (d, 1H, J = 2.0 Hz, H-17); NOESY: H-21/H-19, H-10, H-8'; H-17/H-14, H-15'; H-20/H-15; H-14/H-15', H-23; H-23/H-24; H-8/H-6; H-10/H-11, H-8'; H-11/H-14; MS (Cl, isobutene) m/z 767, 765 [M+H]+; CI-HRMS m/z 765.1711/767.1689 (C37H34N4010Cl H+ requires 765.1730/767.1703).

Compound (18): Compound(17b)(2.0 mg) in anhydrous THF (0.5 mL) was stirred at rt for 2 h in the presence of KHCO3 (0.5 mg) and 18-crown-6. After filtration and solvent evaporation, purification by preparative TLC (CH2Cl2/MeOH, 99:1) gave a mixture of four products (1.2 mg), 18a 30%, 18a' 15%, 18b 37%, 18b' 18% (1H NMR). Pure samples were obtained by preparative HPLC of the pooled outcomes of several experiments.

(R,S,R)-18a: mp 86-88°C (CH3OH / CH2Cl2); [α]D = +4°, (c=0.13, CHCl3); IR (CHCl3) v 1738, 1673, 1647, 1609, 1493, 1351; 1H NMR (CDCl3, 300 MHz) δ 2.59 (s, 3H, H-34), 2.99 (dd, 1H, J1 = 12.5, J2 = 14.3 Hz, H-15), 3.27 (dd, 1H, J1 = 3.9, J2 = 14.3 Hz, H-15'), 3.31 (d, 1H, J = 14.0 Hz, H-8), 3.49 (d, 1H, J = 14.0 Hz, H-8'), 3.72 (s, 3H, H-26), 3.76 (s, 3H, H-33), 3.89 (s, 3H, H-41), 5.40 (d, 1H, J = 7.2 Hz, H-14), 5.47 (d, 1H, J = 7.5 Hz, H-11), 5.57 (dd, 1H, J1 = 3.9, J2 = 12.4 Hz, H-14), 6.08 (br s, 1H, H-21), 6.45 (d, 1H, J = 7.2 Hz, H-23), 6.70 (d, 2H, J = 8.7 Hz, H-29, H-31), 6.87 (d, 2H, J = 8.7 Hz, H-28, H-32), 6.89 (d, 1H, J = 7.9 Hz, H-6), 6.96 (d, 1H, J = 7.5 Hz, H-10), 6.99 (d, 1H, J = 8.3 Hz, H-19), 7.15 (2dd, 2H, J1 = 2.0, J2 = 8.3 Hz, H-4, H-20), 7.29 (t, 1H, J = 7.9 Hz, H-5), 7.36 (s, 2H, H-36, H-40), 8.03 (d, 1H, J = 2.0 Hz, H-17); NOESY: H-21/H-19, H-10, H-8'; H-17/H-14, H-15'; H-20/H-15; H-14/H-15', H-23; H-23/H-24; H-8/H-6; H-10/H-11, H-8'; H-11/H-14; MS (Cl, isobutene) m/z 767, 765 [M+H]+; CI-HRMS m/z 765.1711/767.1689 (C37H34N4010Cl H+ requires 765.1730/767.1703).

(R,S,R)-18a': Oil. 1H NMR (300 MHz, CDCl3) δ 2.65 (s, 3H, H-34), 3.03 (dd, 1H, J1 = 12.5, J2 = 14.5 Hz, H-15), 3.32 (dd, 1H, J1 = 4.3, J2 = 14.5 Hz, H-15'), 3.41 (br s, 2H, H-8), 3.71 (s, 3H, H-26), 3.77
(s, 3H, H-33), 3.88 (s, 3H, H-41), 5.40 (d, 1H, J = 7.1 Hz, H-24), 5.49 (d, 1H, J = 7.1 Hz, H-11), 5.67 (dd, 1H, J1 = 4.3, J2 = 12.5 Hz, H-14), 5.91 (br s, 1H, H-21), 6.43 (d, 1H, J = 7.1 Hz, H-23), 6.71 (d, 2H, J = 8.7 Hz, H-29, H-31), 6.82 (d, 1H, J = 8.1 Hz, H-6), 6.86 (d, 1H, J = 7.1 Hz, H-10), 6.89 (d, 2H, J = 8.7 Hz, H-28, H-32), 7.13 (2dd, 2H, J1 = 2.1, J2 = 8.3 Hz, H-4, H-20), 7.23 (d, 1H, J = 8.3 Hz, H-19), 7.28 (t, 1H, J = 8.1 Hz, H-5), 7.39 (s, 2H, H-36, H-40), 7.63 (d, 1H, J1 = 2.1 Hz, H-17); FABMS (thio/Na+) m/z 789, 787 [M+Na]+, 767, 765 [M+H]+.

(R,S)-18b: mp 76-78°C (CH3OH/CH2Cl2); [α]D = +2° (c= 0.33, CHCl3); IR (CHCl3) υ 1731, 1679, 1654, 1609, 1538, 1493, 1351; 1H NMR (CDCl3, 300 MHz) δ 2.97 (s, 3H, H-34), 2.99 (dd, 1H, J1 = 12.5, J2 = 14.4 Hz, H-15), 3.33 (d, 1H, J = 14.1 Hz, H-8), 3.39 (dd, 1H, J1 = 3.9, J2 = 14.4 Hz, H-15'), 3.45 (d, 1H, J = 14.1 Hz, H-8'), 3.60 (s, 3H, H-26), 3.83 (s, 3H, H-33), 3.89 (s, 3H, H-41), 5.33 (d, 1H, J = 6.8 Hz, H-24), 5.56 (dd, 1H, J1 = 3.9, J2 = 12.5 Hz, H-14), 5.59 (d, 1H, J = 7.5 Hz, H-11), 6.05 (d, 1H, J1 = 2.3, J2 = 8.2 Hz, H-6), 6.98 (d, 1H, J = 7.5 Hz, H-10), 7.02 (d, 1H, J = 8.3 Hz, H-19), 7.06 (d, 2H, J = 8.7 Hz, H-28, H-32), 7.15 (dd, 1H, J1 = 2.3, J2 = 8.2 Hz, H-4), 7.23 (dd, 1H, J1 = 2.1, J2 = 8.3 Hz, H-20), 7.29 (t, 1H, J = 8.2 Hz, H-5), 7.35 (s, 2H, H-36, H-40), 7.99 (d, 1H, J = 2.1 Hz, H-17); NOESY: H-21/H-19, H-10, H-11, H-8'; H-17/H-14, H-15'; H-20/H-15, H-14/H-15', H-24; H-23/H-15, H-24; H-8/H-6; H-10/H-8', H-11; FABMS (thio/Na+) m/z 789, 787 [M+Na]+, 767, 765 [M+H]+.

(R,S)-18b+: mp 64-66°C; [α]D = -1° (c= 0.13, CHCl3); IR (CHCl3) υ 1731, 1679, 1647, 1602, 1493, 1351; 1H NMR (CDCl3, 300 MHz) δ 3.00 (s, 3H, H-34), 3.04 (dd, 1H, J1 = 12.5, J2 = 14.4 Hz, H-15), 3.42 (s, 2H, H-8, dd, 1H, J1 = 4.3, J2 = 14.4 Hz, H-15'), 3.62 (s, 3H, H-26), 3.83 (s, 3H, H-33), 3.89 (s, 3H, H-41), 5.34 (d, 1H, J = 6.9 Hz, H-24), 5.59 (d, 1H, J = 7.3 Hz, H-11), 5.64 (dd, 1H, J1 = 4.3, J2 = 12.5 Hz, H-14), 5.91 (br s, 1H, H-21), 6.07 (d, 1H, J = 6.9 Hz, H-23), 6.82-6.84 (m, 2H, H-10, H-6), 6.87 (d, 2H, J = 8.7 Hz, H-29, H-39), 7.07 (d, 2H, J = 8.7 Hz, H-28, H-32), 7.14 (dd, 1H, J1 = 2.4, J2 = 8.2 Hz, H-4), 7.21 (d, 1H, J = 8.4 Hz, H-19), 7.29 (t, 1H, J = 8.0 Hz, H-5), 7.39 (s, 2H, H-36, H-40), 7.60 (dd, 1H, J1 = 2.1, J2 = 8.4 Hz, H-20), 7.74 (d, 1H, J = 2.1 Hz, H-17); NOESY: H-21/H-19, H-10, H-11; H-17/H-15; H-20/H-15'; H-14/H-15, H-15'; H-23, H-10; H-23/H-15, H-24, H-14; H-8/H-6; FABMS (thio/Na+) m/z 789, 787 [M+Na]+, 767, 765 [M+H]+.
3-(7-Bromo-3H-indol-3-yl)acrylic acid methyl ester (21): Compound (20), (90 mg, 0.408 mmol), monoethyl malonate (64 mg, 0.48 mmol), dry pyridine (1 mL) and dry piperidine (2 drops) were heated on an oil-bath at 50 °C for 24 h. Evaporation of the volatile and preparative TLC (CH₂Cl₂/heptane 10:1) gave 21 (70 mg, 60%): mp 146-147 °C (ether/pentane); ¹H NMR (300 MHz, CDCl₃) δ 1.34 (t, 3H, J = 7.1 Hz), 4.27 (q, 2H, J = 7.1 Hz), 6.43 (d, 1H, J = 16.0 Hz), 7.09 (d, 1H, J = 7.9 Hz), 7.39 (d, 1H, J = 7.9 Hz), 7.50 (d, 1H, J = 2.7 Hz), 7.71 (d, 1H, J = 7.9 Hz), 7.87 (d, 1H, J = 16.0 Hz), 8.82 (br s, 1H); ¹³C NMR δ 14.38, 60.29, 105.28, 114.59, 119.60, 122.57, 125.68, 126.55, 128.59, 135.77, 137.60, 167.98; MS (CI, isobutene) m/z 296, 294.

3-(7-Bromo-3H-indol-3-yl)propionic acid methyl ester (22a): To an ice water cooled solution of 21 (632 mg, 2.14 mmol) in 95% ethanol (25 mL) and BiCl₃ (336 mg, 1.07 mmol) was added portionwise NaSH₄ (316 mg, 8.03 mmol) and the solution was stirred for 4 h at 0°C. Evaporation of the volatile and preparative TLC (CH₂Cl₂) gave 22a (253 mg, 40%): mp 80°C (CH₃OH/pentane); ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, 3H, J = 7.1 Hz), 2.69 (t, 2H, J = 7.6 Hz), 3.07 (t, 2H, J = 7.6 Hz), 4.13 (q, 2H, J = 7.1 Hz), 6.99 (d, 1H, J = 7.6 Hz), 7.02 (s, 1H), 7.33 (d, 1H, J = 7.6 Hz), 7.54 (d, 1H, J = 7.6 Hz), 8.17 (br s, 1H); ¹³C NMR δ 14.30, 20.83, 34.99, 60.53, 104.87, 116.43, 118.09, 120.60, 122.16, 124.46, 128.53, 135.05, 173.31; MS (CI, isobutene) m/z 298, 296.

3-(7-Bromo-3H-indol-3-yl)propionic acid (22b): Compound (22a) (170 mg, 0.57 mmol) in CH₃OH/H₂O 1:1 (10 mL) was treated with H₂O (2 mL) and NaOH (120 mg, 3 mmol) at rt for 4 h. Evaporation of the volatile and acid-base workup gave 22b (150 mg, 98 %): mp 129-130°C (CH₃OH/CH₂Cl₂); ¹H NMR (300 MHz, acetone-D₆) δ 2.81 (t, 2H, J = 7.5 Hz), 3.15 (t, 2H, J = 7.5 Hz), 7.01 (t, 1H, J = 7.8 Hz), 7.23 (s, 1H), 7.38 (d, 1H, J = 7.8 Hz), 7.60 (d, 1H, J = 7.8 Hz), 10.40 (br s, 1H); ¹³C NMR δ 21.95, 35.65, 105.76, 117.30, 119.49, 121.81, 125.35, 130.59, 136.50, 174.88; MS (CI, isobutene) m/z 269, 267.

[3-(7-Bromo-3H-indol-3-yl)propionylamino]acetic acid methyl ester (23a): A solution of glycine methyl ester hydrochloride (37 mg, 0.30 mmol) in DMF (5 mL), containing N(C₂H₅)₃ (42 µL, 0.30 mmol) was stirred at rt for 15 min and was successively added with EDC (68 mg, 0.36 mmol), HOBT (40 mg, 0.30 mmol) and 22b (80 mg, 0.30 mmol). After 8 h, dilution with aqueous 1N HCl (pH=3),
extraction (AcOC2H5) and preparative TLC (CH2Cl2/CH3OH, 8:2) gave 23a (58 mg, 58%): mp 124 °C (CH3OH-ether/pentane); 1H NMR (300 MHz, acetone-d6) δ 2.72 (t, 2H, J = 7.6 Hz), 3.16 (t, 2H, J = 7.6 Hz), 3.74 (s, 3H), 4.04 (d, 2H, J= 5.8Hz), 7.01 (t, 1H, J = 7.8 Hz), 7.26 (d, 1H, J = 2.4 Hz), 7.35 (d, 1H, J = 7.8 Hz), 7.45 (br s, 1H), 7.63 (d, 1H, J = 7.8 Hz), 9.96 (br s, 1H); 13C NMR δ 21.74, 37.02, 41.45, 51.95, 105.04, 117.10, 118.85, 120.69, 124.17, 124.56, 130.01, 136.50, 171.50, 173.30; MS (CI, isobutene) m/z 341, 339. Anal. Calcd for C14H15N2O5Br: C, 49.57; H, 4.46; N, 8.25. Found: C, 50.33; H, 4.81; N, 7.71.

[3-(7-Bromo-3H-indol-3-yl)propionylamino]acetic acid (23b): Compound (23a) (1.5 g, 4.4 mmol) in CH3OH (80 mL), was treated with H2O (20 mL) and NaOH (0.44 g, 11 mmol) at rt for 5 h. Evaporation of the volatile and acid-base workup (AcOC2H5) gave 23b (1.2 g, 84%): mp 118-120°C (CH2Cl2); 1H NMR (300 MHz, acetone-d6) δ 2.61 (t, 2H, J= 7.6 Hz), 3.05 (t, 2H, J = 7.6 Hz), 3.93 (d, 2H, J= 4.9 Hz), 6.96 (t, 1H, J = 7.7 Hz), 7.25 (s, 1H), 7.30 (d, 1H, J = 7.7 Hz), 7.34 (br s, 1H), 7.61 (d, 1H, J = 7.7 Hz), 10.11 (br s, 1H); 13C NMR δ 21.92, 37.14, 41.60, 104.90, 118.02, 119.02, 120.84, 124.10, 124.80, 130.06, 137.10, 173.09; MS (CI, Isobutene) m/z 327, 325.

N-[(3-Bromobenzylcarbamoyl)methyl]-3-(7-bromo-3H-indol-3-yl)propionamide (24b): A solution of 3-bromobenzylamine hydrochloride (71 mg, 0.326 mmol) and N(C2H5)3 (45 pL, 0.326 mmol) in DMF (2 mL) was stirred at rt for 10 min. To this mixture were added EDC (68 mg, 0.391 mmol), HOBT (42 mg, 0.326 mmol) and 23b (106 mg, 0.326 mmol) and the solution was stirred at rt for 10 min. After 15 h the reaction was quenched with water (10 mL) and extracted (AcOC2H5). Preparative TLC (CH2Cl2/CH3OH 9:1) gave 24b (60 mg, 38%): mp 159 °C (CH3OH-ether/pentane); 1H NMR (300 MHz, CDCl3) δ 2.57 (t, 2H, J = 7.2 Hz), 3.01 (t, 2H, J = 7.2 Hz), 3.84 (d, 2H, J = 5.2 Hz), 4.24 (d, 2H, J = 6.0 Hz), 6.74 (br s, 1H), 6.94 (t, 2H, J = 7.8 Hz), 7.08-7.13 (m, 3H), 7.29-7.35 (m, 3H), 7.46 (d, 1H, J = 7.8 Hz), 10.14 (br s, 1H); 13C NMR δ 22.31, 37.70, 43.28, 43.61, 105.53, 116.34, 118.82, 120.85, 123.36, 124.23, 124.91, 127.10, 130.03, 131.17, 131.21, 136.37, 142.37, 171.78, 176.28; MS (CI, isobutene) m/z 495, 493, 491.

Compound (25): Into a flamed dried 25 mL round bottom flask were introduced (TPP)2NiCl2 (40 mg, 0.061 mmol), triphenylphosphine (32 mg, 0.12 mmol), and zinc powder (4.0 mg, 0.061 mmol). A septum
cap was placed on the flask and of dry, O₂-free DMF (1 mL) was added. The flask was evacuated and filled with N₂ three times by means of a syringe needle connected with tygon tubing to a vacuum line and another syringe needle connected to nitrogen line. After 1 h compound (24b) (30 mg, 0.061 mmol) in 1 mL of dry, O₂-free DMF was added via syringe with careful exclusion of air and the reaction mixture was stirred under nitrogen at 50°C for 2 h. It was then cooled, poured into aqueous 5 % HCl (100 mL), extracted with AcOC₂H₅(20 mLx3) and the extract was washed with distilled water and brine, dried over Na₂SO₄. Removal of solvents and TLC chromatography (CH₂C₁₂/CH₃OH 9: 1) gave 25 (0.2 mg, 1%) as a yellow solid; MS (EI) m/z 333; CIHRMS m/z 334.1562 (C₂₀H₁₉N₃O₂ + H⁺ requires 334.1555).

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REFERENCES AND NOTES


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