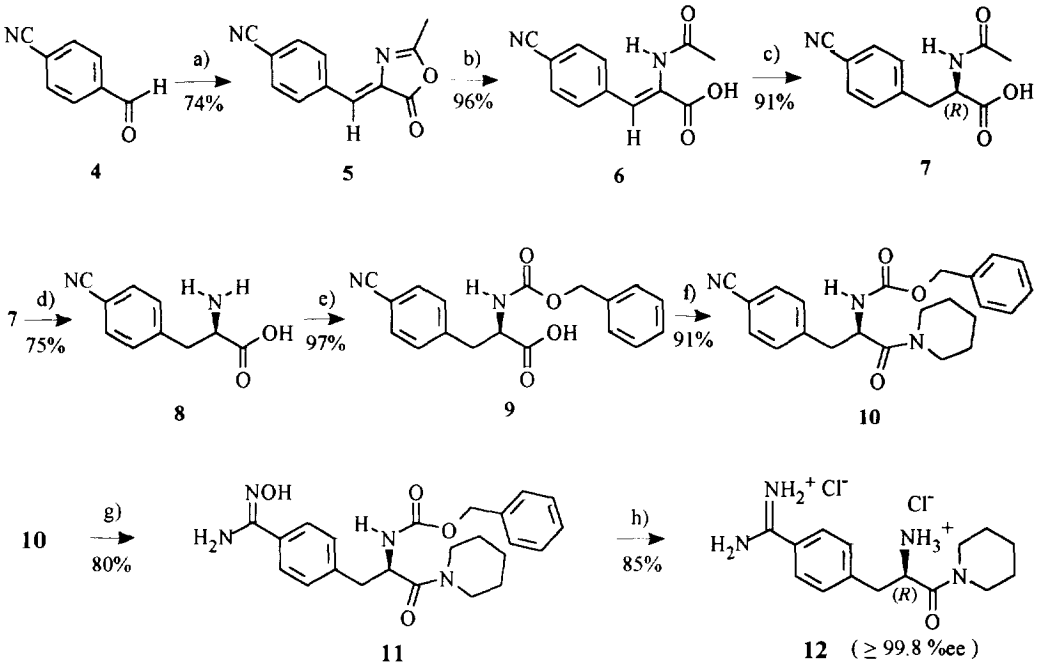
sulfonamido groups, very lipophilic residues (piperidinyl and methoxy-trimethylphenyl), and a principially epimerizable stereocenter in the Asp unit, renders any straight phase (silica, alumina)- , reversed phase (C18-silica)- or adsorption (HP20)- chromatography impossible or totally unpractical. Sephadex chromatography is feasible, however unsuitable for a kg-scale due to enormous sephadex / substrate- and eluent / substrate- ratios. The basic amidino group allows for the precipitation of amidinium salts from suitable solvents, and it was soon realized that this would be the only way for a purification on a large scale. Since the purification efficiency of salt precipitations is clearly limited, we concluded that the final steps of the process (leading to amorphous carboxyl protected and then to unprotected CRC 220) must be exceptionally clean reactions in order to obtain a product of acceptable quality. The transformation by known methodology [9] of a nitrile into an amidino goup is *not* a particularly clean reaction. Initially we therefore concluded that the nitrile should not be transformed to the amidine at the end of the synthesis, but at an earlier stage. However, in the course of elaboration of a process for CRC 220, we uncovered a novel preparation of amidines by hydrogenolysis of amidoximes.[5] It furnishes amidines in up to 90% overall yield from respective nitriles,[5] and the amount of by-products is small enough to be removed by optimized salt precipitation.

Similar reasoning decided on the preferred protecting group for the Asp-β-carboxyl. The mildest method for cleavage of a *tert.*-butyl ester is a moderately acidic hydrolysis.[10] Preliminary tests with the *tert.*-butyl ester of **2** indicated that it is quantitatively cleaved in a 1.2 *N* solution of HCl (3 equiv.) in glacial acetic acid at 20 °C within 4 h. Precipitation of the product furnishes crude **2** in 89% yield with 87% purity (HPLC), the impurities not being easily removed. Allyl esters are usually cleaved by treatment with palladium-phosphine complexes.[10] Only 64% yield could be achieved with the allyl ester of **2**, and traces of palladium were not easily removed. On the contrary, hydrogenolysis of the benzyl ester [10] of **2** could be expected to proceed without significant formation of by-products, since Adf-pip is prepared in excellent yield under very similar conditions by simultaneous hydrogenolysis of a benzyloxycarbonyl (Cbz) and an amidoxime group. Indeed, **2** isolated from the hydrogenolysis of its benzyl ester is of higher purity than its educt.

Results and Discussion

The asymmetric synthesis of Adf-pip dihydrochloride **12** is outlined in Scheme 1. Azlactone **5** is prepared from commercial *p*-cyanobenzaldehyde **4** according to the conventional method with *N*-acetylglycine in acetic anhydride.[11] Hydrolysis of **5** in hot aqueous NaOH solution provides *N*-acetyl-dehydro-*p*-cyanophenylalanine **6**. Asymmetric homogeneous hydrogenation of **6** with the neutral *in situ* catalyst from bis-(1,5-cyclooctadiene)-dirhodium(I) dichloride and (-)-BPPM (substrate to catalyst ratio1300) furnishes (*R*)-*N*-acetyl-*p*-cyanophenylalanine **7** with 96% ee. Acidic cleavage of the acetyl group with boiling 2 *N* aqueous HCl, followed by adjustment to pH 4 with triethylamine gives crystalline (*R*)-*p*-cyanophenylalanine **8** of >99% ee. Cbz protection of its amino group with benzyl chloroformate in THF / water at constant pH 9 furnishes crystalline **9.** Reaction of the carboxylic acid with 2 equiv. of piperidine, supported by methyl-ethyl-phosphonic anhydride (MEPA) in ethyl acetate at ambient temperature leads to the piperidide **10** as a viscous oil. Conversion of the

Scheme 1 Synthesis of (*D*)-4-amidinophenylalanine piperidide hydrochloride (Adf-pip hydrochloride) **12**

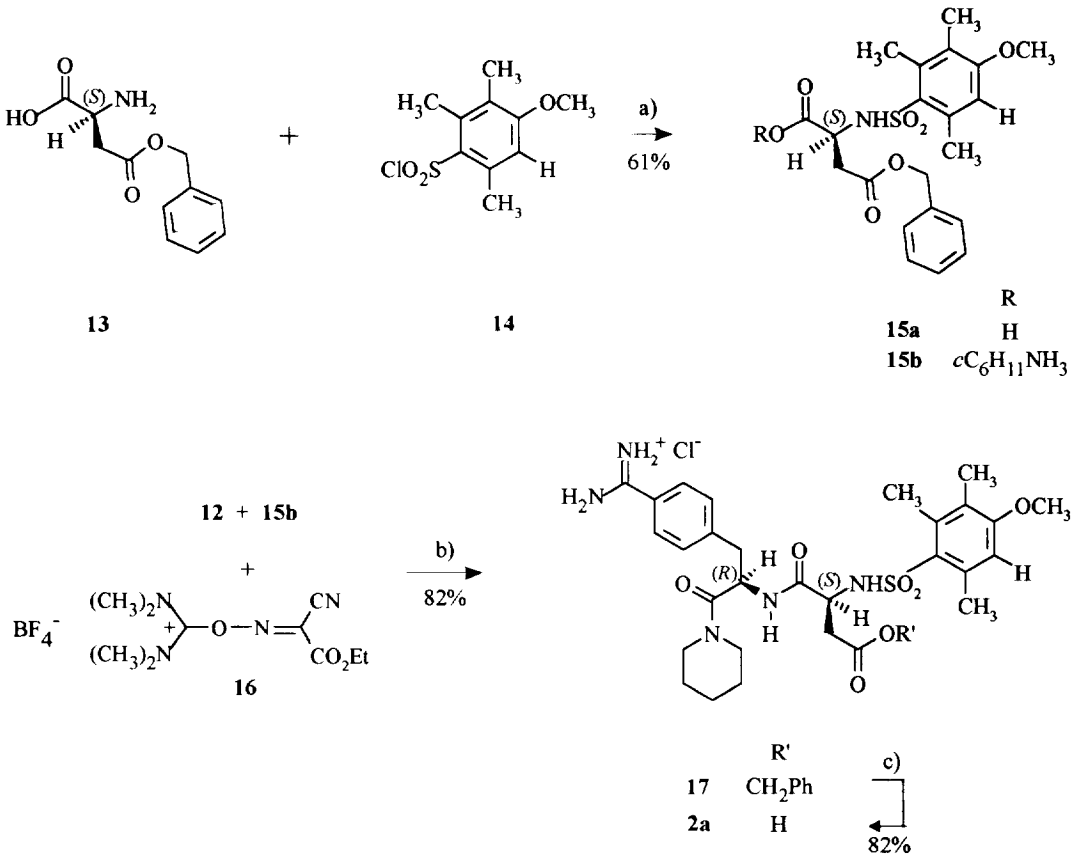


(a) Ac_2O, NaOAc, *N*-acetylglycine, acetone, reflux ; (b) NaOH, H_2O, pH < 10, 80 °C ; (c) 10 bar H_2, (-)-BPPM, $[Rh(COD)Cl]_2$, NEt_3, MeOH, C_6H_5Me, 15 °C ; (d) 1. 2 *N* HCl, reflux , 2. NEt_3 → pH 4, EtOH ; (e) $ClCO_2CH_2Ph$, NaOH, THF, H_2O, 22 °C ; (f) piperidine, MEPA, EtOAc, 22 °C ; (g) $HONH_3^+$ Cl^-, Et_3N, EtOH, reflux ; (h) 1. 10 bar H_2, 10% Pd/C , AcOH, 50 °C , 2. HCl_{gas} , *i*PrOH, 20 °C .

nitrile with hydroxylamine hydrochloride in refluxing ethanol in the presence of 1.2 equiv. of triethylamine furnishes the crystalline amidoxime **11.** Hydrogenolysis, catalyzed by palladium on charcoal, followed by treatment with an *iso*propanolic HCl solution provides homochiral amidinium salt **12.**

Scheme 2 summarizes the preparation of the *N*-terminal Mtr-Asp-*β*-Bzl **15** and its coupling with the *C*-terminal amidinium salt **12** to give target compound **2**. Commercial (*L*)-aspartic acid *β*-benzylester **13** reacts with Mtr-chloride **14** at pH 9 and 0 °C in DMF / water to give, after acidification to pH 3, crude sulfonamide **15a** of 91% purity (HPLC) as a viscous oil. Its cyclohexylammonium salt **15b** is precipitated from an ethyl acetate solution in 99% purity [12] with 61% yield based on **13**. Salt **15b** is coupled with Adf-pip dihydrochloride **12** in DMF solution at 0 °C, using *O*-[cyano(ethoxycarbonyl)methylenamino]-*N,N,N',N'*-tetramethyl-uronium tetrafluoroborate (TOTU) **16** [13] as coupling reagent and *N*-methylmorpholine as a base that binds the excess equivalent of hydrogen chloride, to give the neutral inner salt of **17** (deprotonated sulfonamido- , protonated amidino-group) as a colorless solid of 90-92% purity (HPLC) in quantitative yield.

Scheme 2 Synthesis of Mtr-Asp-β-Bzl and its coupling with Adf-pip

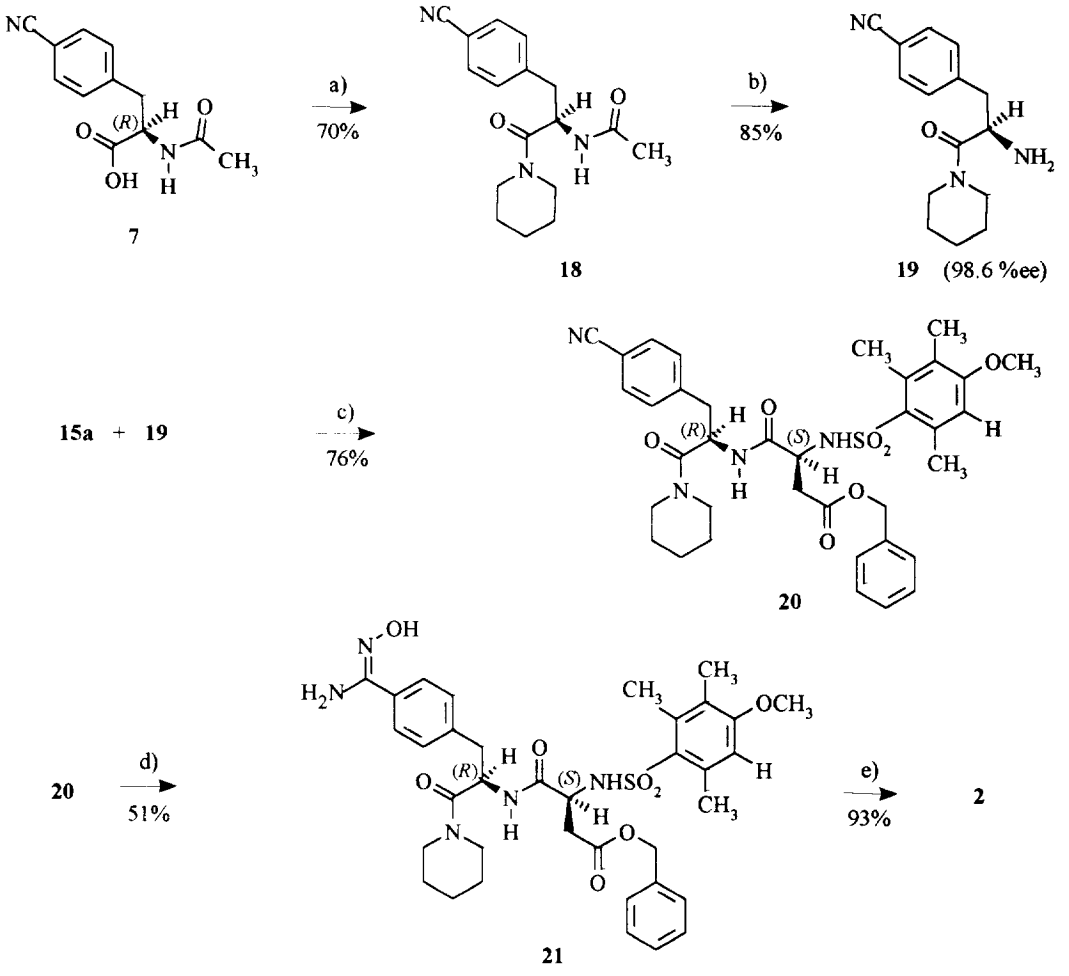


(a) 1. iPr$_2$NEt, DMF, H_2O, 0 °C (91 %) , 2. cyclohexylamine, EtOAc, 0 °C (67%) ; (b) 1. TOTU **16**, *N*-methylmorpholine, DMF, 0 °C , 2. HCl_{gas}, EtOH, iPr$_2$O ; (c) 1. 1 bar H_2, 10% Pd/C, DME, H_2O, 20 °C , 2. HCl_{gas}, THF, MTB, 20 °C , 3. freeze drying of aqueous solution.

It is dissolved in 1 *N* ethanolic HCl and the hydrochloride **17** is precipitated with 93-95% purity by pouring the solution into diisopropyl ether. Hydrogenolysis of the benzyl ester in 1,2-dimethoxyethane (DME) and water furnishes crude hydrochloride **2a**. It is isolated with 95-97% purity by filtration of the catalyst, evaporation of the DME, and freeze-drying of the remaining aqueous suspension. For purification the crude solid is dissolved in a solution of HCl in THF and **2a** is precipitated in 97-98% purity (HPLC) by pouring into methyl-*tert.*-butyl ether (MTB). This product retains ~5 weight-% of MTB, that cannot be removed by extended drying in high vacuo at 25 °C. MTB is however easily released when a stream of nitrogen or argon is bubbled through the aqueous solution of **2a**. Freeze-drying furnishes **2a** on a kg-scale as an amorphous colourless solid in 82% yield based on **17** (67% yield based on **15b**). The overall yield from *p*-cyanobenzaldehyde **4** (10 steps) is 20% of

theory, from (*L*)-Asp-β-Bzl **13** (3 steps) it is 46% of theory. This procedure is the result of considerable optimization.[14]

Scheme 3 Alternative approach to compound **2**

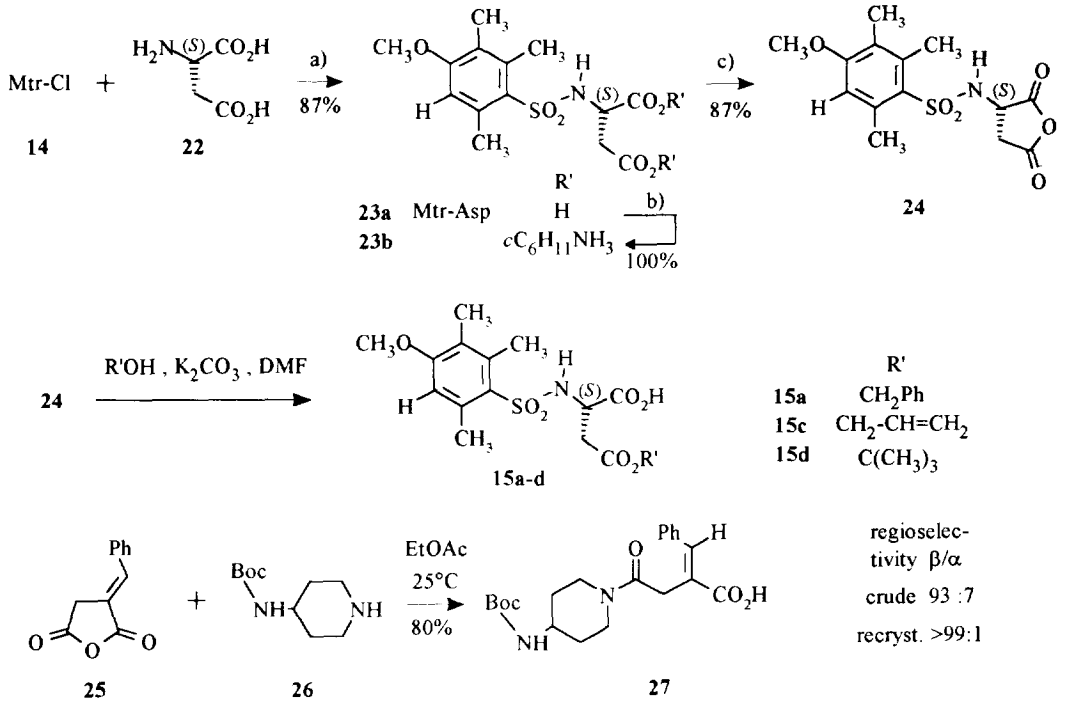


(a) Piperidine, MEPA, EtOAc, 25 °C ; (b) 1. 2 *M* aq. HCl, 80 °C , 2. NaOH → pH 9.0 ; (c) MEPA, EtOAc, 25 °C ; (d) $HONH_3^+$ Cl^-, NEt_3, EtOH, reflux ; (e) 10 bar H_2, 10% Pd/C, AcOH, 50 °C.

Nevertheless, laboratory results (g-scale) indicate that the modified synthesis depicted in Scheme 3 is a competitive alternative. Instead of the amidine **12** the nitrile **19** is employed for the key peptide coupling of the *N*-terminal **15**. Contrary to the amorphous **17**, coupling product **20** and amidoxime **21** can easily be purified by recrystallization. **21** is then converted by clean and simultaneous hydrogenolyses of the amidoxime and the benzyl group directly to CRC 220. The overall yield from **4** (8 steps) of this sequence is currently only 14% of

theory. It has however not yet been optimized to a similar degree than the synthesis depicted in Schemes 1 and 2. It is shorter, and **2** of high purity is easier attained due to the crystalline nature of **20** and **21**.

Scheme 4 Preparation of β-carboxyl protected Mtr-Asp by regioselective alcoholysis of cyclic anhydride **24**



(a) 1. *i*Pr$_2$NH, DMF, H_2O, 0 °C , 2. 2 *N* aq. HCl → pH 1.5 ; (b) cyclohexylamine, acetone, 0 °C ; (c) $SOCl_2$, EtOAc, 0 °C → 20 °C.

The preparation of Mtr-Asp protected at the β-carboxyl, *i.e.* of an *N*-terminal building block like **15a** or **15b**, was also attempted by highly regioselective alcoholysis of cyclic Mtr-Asp anhydride **24** (Scheme 4). In the course of process research for a renin inhibitor we have recently found that a solution of 4- *tert.*-butyloxycarbonylamino-piperidine **26** reacts with phenylitaconic anhydride **25** highly regioselective at the less shielded β-carbonyl to furnish pure **27** in 80% yield after recrystallization.[7b] We envisioned analogous alcoholysis of **24** by benzyl alcohol, allyl alcohol, or *tert.*-butanol, leading with similar regioselectivity to β-protected Mtr-Asp **15a**, **15c**, or **15d**, respectively. Unprotected Mtr-Asp **23a** is prepared by coupling of (*L*)-aspartic acid **22** with Mtr chloride **14** in a DMF / water solution. The viscous foam is conveniently purified by precipitation of its bis(cyclohexylammonium) salt **23b** from an acetone solution. An ethyl acetate solution of pure dicarboxylic acid **23a**, liberated from its salt **23b** by acidification with 1 *N* sulfuric acid, reacts with excess

thionyl chloride to form cyclic anhydride **24** that is purified by trituration with di*iso*propyl ether. Alcoholysis of a DMF solution of anhydride **24** at 15 - 20 °C by benzyl alcohol in the presence of potassium carbonate is only moderately regioselective and gives in virtually quantitative yield the β-benzyl ester **15a** and the α-isomer in a ratio of 4 - 5 : 1 (^{1}H NMR, HPLC). Precipitation with di*iso*propyl ether of the benzyl ester in the presence of 1 equiv. of DMF (60% yield) improves the β / α - ratio to 10 - 17 : 1, but sufficiently pure **15a** could not be obtained without chromatography. Amazingly, the regioselectivity appears to *deteriorate*, if the alcoholysis is conducted at lower temperature (-10 °C: β : α = 2 : 1). Allyl alcohol is more regioselective and provides a crude allyl ester **15c** in which significant amounts of the α-ester are not indicated by ^{1}H NMR and HPLC (β/α - ratio > 20). Alcoholysis of anhydride **24** by solutions of *tert.*-butanol or potassium *tert.*-butoxide in DMF produced *tert.*-butyl ester **15d** in unacceptably low yield. Thus, only in case of allyl alcohol **26** the regioselective alcoholysis of cyclic anhydride **24** is of synthetic utility. Due to the disadvantages of deprotection of an allyl ester, this approach is not competitive with the ones depicted in Schemes 1, 2, and 3.

In summary, we have described the first practical large-scale approaches to a potent thrombin inhibitor. The processes summarized in Schemes 1 + 2 and Scheme 3, respectively, are both convergent, efficient and devoid of any chromatographic purification. The process depicted in Schemes 1 + 2 has been applied to furnish target compound **2** on a kg-scale.

EXPERIMENTAL

Reagents, Instrumentation and General Methods *N*-Acetylglycine (Fluka, >99 %), *L*-aspartic acid [Aldrich, 99 %, $[\alpha]_D^{20}$ = + 28.3 (*c* = 13.2 in 1 *N* aq. HCl)], *L*-aspartic acid β-benzylester **13** [Propeptide, >99 %, $[\alpha]_D^{20}$ = + 27.7 (*c* = 1 in 1 *N* aq. HCl)], BPPM (Fluka, >98 %), di-μ-chloro-bis[(cycloocta-1*c*,5*c*-diene)-rhodium(I)] (Aldrich, 98 %), *p*-cyanobenzaldehyde **4** (Fluka, >97 %), MEPA (Hoechst, 96 %), Mtr chloride **14** (Hoechst, 96 % [12]), TOTU **16** (Hoechst, >98 %) were used as purchased. The reactions depicted in Schemes 1 and 2 were conducted under an atmosphere of nitrogen in stainless steel reactors that were enameled, if acidic reaction conditions prevailed. The reactions summarized in Schemes 3 and 4 were run under nitrogen or argon in dry-glass apparatus. Our analytical equipment and techniques have been described elsewhere.[15] *p*-Nitrobenzylalcohol (NBA) or *o*-nitrophenyl-octyl ether (NPO), respectively, was utilized as the matrix for MS by "fast atom bombardment" positive ionization (FAB).

N-[*N'*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-α-*L*-aspartyl]-4-amidino-*D*-phenylalanine piperidide (Mtr-Asp-*D*-Adf-pip) (2).

a) Hydrochloride **2a** prepared by hydrogenolysis of amidino benzyl ester **17**:

Beginning at 5 °C, during 15 min 1,2-dimethoxyethane (33.7 L) is added to a stirred suspension of **17** (1.50 kg, 2.06 mol) in water (38.7 L). Due to heat of mixing, the temperature climbs to 20 °C and a milky-turbid solution

without significant solid particles is obtained. The solution is transferred into a 125 L- stainless steel autoclave. The autoclave is purged three times with N_2 (3 bar of N_2 pressed in, then slowly released). In a 10 L round bottom flask water (6.2 L) is deaerated for 15 min by bubbling-through a stream of argon. 10% Pd on charcoal (235g, Degussa type E 101 R/D) is added and the resulting suspension is deaerated for 15 min. This suspension is added under N_2 to the solution in the autoclave. The autoclave is again purged three times with N_2 , then with H_2 , and the hydrogenolysis is conducted under 1 bar of H_2, the consumption being measured with a Büchi pressflow gas controller bpc 9901. The theoretical amount is consumed within 15 min. The autoclave is purged with N_2 and the contents are pressed with N_2 through a pressure nutsche that is topped by a slurry of ®Celite (9.3 kg) in water / DME (2:1). The Celite plug is rinsed with water (2.0 L), and the combined filtrates are concentrated *in vacuo*. The resulting suspension is freeze-dried in HV to give a nearly colourless, very voluminous solid [1.19 kg, 2% H_2O, purity 96% (HPLC)]. This crude solid is added within 15 min at 18 °C under argon atmosphere to a vigorously stirred solution of HCl (gas) in THF (1.3*N*, 7.0 L, < 0.2% H_2O) to give a nearly clear solution. It is filtered through a clarifying pad and the filtrate is added within 5 min to vigorously stirred MTB (77.0 L). The precipitate is filtered off under N_2 on a pressure nutsche, washed immediately with MTB (4 x 15 L) and blown as dry as possible by a stream of N_2. The colourless powder is dried for 6 d at 25 °C in HV (1.13 kg, ≈ 5 weight-% MTB, purity 97% (HPLC)]. It is dissolved in water (5.7 L), filtered, and a finely divided stream of argon is bubbled through the filtrate, utilizing a gas-entry tube with a frit tip. After 15 h, MTB can no longer be detected (GC) in the exit gas. The solution is freeze-dried in HV for 14 d (1.075 kg, 82% yield based on **17**), 0.76 weight-% H_2O, ≤ 0.05% MTB, ≤ 1 ppm Pd; purity 97.2% (HPLC: 250 x 4 mm Nucleosil 120 C18 7 µm; eluent A: 450 mL H_2O , 1800 mL MeCN, 5.5 g $NH_4H_2PO_4$, adjusted to pH 3.0 with H_3PO_4 ; eluent B: 1600 mL H_2O, 900 mL MeCN, 5.5 g $NH_4H_2PO_4$, adjusted to pH 2.5 with H_3PO_4; eluent C: 2000 mL H_2O, 500 mL MeCN, 5.5 g $NH_4H_2PO_4$, adjusted to pH 2.5 with H_3PO_4 ; gradient: within 16 min linearly from 100% C to 100% B, then 5 min isocratic with 100% B, then within 16 min linearly to 100% A, then 8 min isocratic with 100% A; 1.0 mL/min, 22 °C, det. 200 nm; t_{ret} **2** 17.50 min), M.p. 180-183 °C; $[\alpha]_D^{20}$ = -21.5 (*c* = 1 in methanol); ^{1}H NMR (270 MHz, [D$_4$]MeOH): δ = 1.26 - 1.67 (m, 6H, CH_2), 2.14 (s, 3H, CH_3), 2.46 (d, *J* = 6 Hz, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 2.90 (dd, *J* = 13 and 8 Hz, 1H, CH_2), 3.10 (dd, *J* = 13 and 7 Hz, 1H, CH_2), 3.33 - 3.58 (m, 4H, CH_2), 3.86 (s, 3H, CH_3), 4.02 (t, *J* = 7 Hz, 1H, CH), 4.82 (s, 7H, NH, NH_2 and CO_2H), 5.08 (t, *J* = 7 Hz, 1H, CH), 6.75 (s, 1H, CH), 7.41 (d, *J* = 9 Hz, 2H, CH), 7.69 (d, *J* = 9 Hz, 2H, CH); ^{13}C NMR (67.93 MHz, [D$_4$]MeOH; multiplicity determined by DEPT 135°): δ = 12.20 (1C, CH_3), 18.39 (1C, CH_3), 24.56 (1C, CH_3), 25.32 (1C, CH_2), 26.66 (1C, CH_2), 27.46 (1C, CH_2), 37.67 (1C, CH_2), 39.33 (1C, CH_2), 44.53 (1C, CH_2), 47.95 (1C, CH_2), 51.16 (1C, CH), 54.16 (1C, CH_3), 56.23 (1C, CH), 113.46 (1C, CH), 126.51 (1C), 127.94 (1C), 129.02 (2C, CH), 130.26 (1C), 131.78 (2C, CH), 140.30 (1C), 145.02 (1C), 161.04 (1C), 168.20 (1C), 169.82 (1C), 171.82 (1C), 173.82 (1C); IR (KBr): ν = 3700 - 2400 cm^{-1} (br, N-H and CO_2H), 1725 cm^{-1} (sh, C=O), 1680 cm^{-1} (C=O), 1620 cm^{-1} (C=N); UV/Vis (H_2O): λ_{max} (ε) = 243 nm (23850) , no absorption at $\lambda \geq$ 300 nm ; MS (ESI, free amidine $C_{29}H_{39}N_5O_7S$ has M =

601): *m/z* (%) = 602 (100) [M + H$^+$], no fragments with ≥ 2.5% intensity indicated; $C_{29}H_{40}ClN_5O_7S$ (638.2): calcd C 54.58, H 6.32, Cl 5.56, N 10.97, S 5.02 ; found C 54.55 , H 6.25, Cl 5.50, N 10.75, S 4.90. X-ray powder diffraction patterns indicate, that it is amorphous. Potentiometric titration with 0.1*N* NaOH solution: in MeOH / H_2O (6:1) one of the three acidic functional groups (CO_2H) is titrated with a base consumption of 92% of theory ; in DMSO / H_2O (3:1) two acidic functional groups are titrated with a base consumption of 104% of theory.

b) Compound **2** prepared by hydrogenolysis of amidoximo benzyl ester **21**:

Palladium on charcoal (1.7 g, 10% ; Degussa type E 10 R/W, 50% H_2O) is added to the deaerated solution of **21** (17.8 g, 25.2 mmol) in glacial acetic acid (300 mL). The mixture is hydrogenated at 50 °C under 10 bar of H_2. The uptake ceases after 18 h (1.30 L, 58.0 mmol, 115% of theory). The catalyst is filtered off and the filtrate is concentrated *in vacuo*. The residue is dissolved in THF (50 mL) and this solution is added dropwise to vigorously stirred MTB (500 mL). The precipitate is suction-filtered, washed with MTB (50 mL), and dried at 40 °C in HV [18.0 g, 119% of theory]. The suspension of the crude product in *i*PrOH (280 mL) and EtOH (140 mL) is quickly heated to reflux to give a clear, colourless solution that is immediately cooled in an ice bath. Seeding crystals of **2** are added. The suspension is stirred for 30 min at 0 °C and the precipitate is suction-filtered and dried in HV (14.1 g, 93% yield), purity: 98.2% (HPLC), 97.1% (capillary electrophoresis). ^{1}H NMR data correspond to that of structure **2**, no resonance for an acetate counterion being indicated. X-ray diffraction of the powder indicates that it is amorphous. A hydrochloride **2a** prepared from this product is identical in ^{1}H- and ^{13}C-NMR, IR, MS with a product prepared from **17** (*vide supra*).

(*Z*)-[4-*p*-Cyanophenylmethylene]-2-methyl-oxazol-5-one (5). To acetone (32.0 L) is added *p*-cyanobenzaldehyde **4** (6.00 kg, 45.8 mol), followed consecutively by *N*-acetylglycine (7.66 kg, 65.5 mol), anhydrous sodium acetate (3.76 kg, 45.8 mol), and acetic anhydride (13.8 L, 140 mol). The mixture is refluxed for 1 h and then cooled to 50 °C. Ice (30 kg) and water (15 L) is added and the mixture is stirred for 1 h without heating. The precipitate is filtered and washed successively with water (30 L), *i*PrOH (15 L), and with iPr$_2$O (30 L). It is dried at 40 °C in HV to furnish yellow crystals (7.18 kg, 74% yield), M.p. 192-193 °C decomp.; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 2.42 (s, 3H, CH_3), 7.30 (s, 1H, CH), 7.96 (d, J = 8 Hz, 2H, CH), 8.33 (d, J = 8 Hz, 2H, CH), only one isomer is indicated and presumed [11c,d] to have *Z*-configuration; IR (KBr): ν = 2223 cm^{-1} (-C≡N), 1805 and 1778 cm^{-1} (C=O), 1660 cm^{-1} (N=C or C=C), 1598 cm^{-1}, 1258 and 1167 cm^{-1} (C-O), 898 cm^{-1} (=C-H); MS (DCI): *m/z* (%) = 213 (100) [M + H$^+$].

2-Acetylamino-(*Z*)-[*p*-cyanocinnamic acid] (6). A solution of NaOH (1.35 kg, 33.75 mol) in water (4.0 L) is added dropwise at 80 - 90 °C and pH ≤ 9 to a suspension of azlactone **5** (7.0 kg, 33.0 mol) in water (35.0 L) until the pH remains constant at pH 9.0 (circa 3 h). The mixture is stirred for further 30 min at 80 °C and then filtered (while hot) through a clarifying pad that had been topped by active charcoal (0.5 kg). The filtrate (25 °C) is adjusted to pH 2.0 with a solution of conc. HCl (3.4 L, 34 mol) in water (4 L). The thick, colourless precipitate is stirred for 12 h, readjusted to pH 2.0 if necessary, suction-filtered, and washed with water (50 L). It is dried at 40 °C in HV to constant weight (7.28 kg, 96% yield); M.p. 236-238 °C; ^{1}H NMR (200 MHz,

[D_6]DMSO): δ = 1.97 (s, 3H, CH_3), 7.18 (s, 1H, CH), 7.79 (AA'BB' system, 4H, CH), 9.62 (s, 1H, NH), 12.94 (br s, 1H, CO_2H), only one isomer is indicated and presumed [11c,d] to have the *Z*-configuration; IR (KBr): ν = 3600 - 2300 cm^{-1} (br, N-H and CO_2H), 2223 cm^{-1} (C≡N), 1696 cm^{-1} (CO_2H), 1669 cm^{-1} (N-C=O), 1640 cm^{-1} (C=C), 1515 cm^{-1} (N-C=O), 1500 cm^{-1} (aryl C-C) ; MS (DCI): *m/z* (%) = 231 (100) [M+H^+], 213 (52) [M+H^+ - H_2O], 188 (13) [M+H^+ - CH_3CO].

***N*-Acetyl-*D*-[*p*-cyanophenylalanine] (7).** A solution of **6** (7.08 kg, 30.78 mol) in MeOH (50 L), NEt_3 (2.2 L, 15.78 mol) and toluene (100 L) is filled into a shaker autoclave that has been purged with N_2 before. N_2 (10 bar) is pressed in, the mixture is stirred for 15 min, and the pressure is then released. This procedure is repeated two times. In the meantime the catalyst solution is prepared in a glass flask by dissolving $[Rh(COD)Cl]_2$ (3.0 g, 12.17 mmol Rh) and (-)-BPPM (6.8 g, 12.28 mmol) in deaerated MeOH (4.0 L) and stirring of the resulting solution for 15 min under argon. The catalyst solution is pressed into the autoclave with N_2. The autoclave is then purged with H_2 , 10 bar of H_2 is pressed in, and the contents are shaken at constant H_2-pressure at 10-15 °C (cooling with tap water). H_2 (720 L, 104% of theory) is absorbed within 20 h. The solvents are evaporated *in vacuo*. The solid residue is dissolved in EtOAc (50 L) and water (30 L) is then added. The pH is adjusted to 1.5 with conc. HCl (1.6 L). The layers are separated and the aqueous phase is extracted with EtOAc (3 x 20 L). The combined organic phases are washed with water (3 x 20 L) and with brine (20 L). They are dried ($MgSO_4$) and then concentrated (to 10 L) *in vacuo*. MTB (20 L) is added and the mixture is stirred at 10 °C for 15 h. The precipitate is suction-filtered and dried at 40 °C in HV to constant weight (6.50 kg, 91% yield), M.p. 187-189 °C; $[\alpha]_D^{20}$ = -50.6 (*c* = 1.0 in MeOH); ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 1.77 (s, 3H, CH_3), 2.92 (dd, *J* = 14 and 10 Hz, 1H, CH_2), 3.14 (dd, *J* = 14 and 5 Hz, 1H, CH_2), 4.46 (m, 1H, CH), 7.42 (d, *J* = 9 Hz, 2H, CH), 7.76 (d, *J* = 9 Hz, 2H, CH), 8.22 (d, *J* = 8 Hz, 1H, NH), 12.96 (s, 1H, CO_2H) ; IR (KBr): ν = 3600 - 2400 cm^{-1} (br, N-H and CO_2H), 2230 cm^{-1} (C≡N), 1744 cm^{-1} (CO_2H), 1728 cm^{-1} (N-C=O), 1610 cm^{-1} (N-C=O); MS (DCI): *m/z* (%) = 233 (100) [M+H^+], 215 (10) [M+H^+ - H_2O], 187 (10) [M^+ - CO_2H]. The enantiomeric purity is determined by GC analysis on a 30 m Chirasil *L*-Val fused silica capillary column at 150 °C, inj. 220 °C, det. (FID) 220 °C, 1.0 bar helium carrier gas (t_{ret} **7** 16.10 min, *ent*-**7** 16.52 min, no baseline separation), or alternatively by derivatization of **7** with (*R*)-(+)-1-phenylethylamine, supported by MEPA, to give diastereomeric amides that are separated by HPLC [250 x 4 mm RP 18 Nucleosil 120 5 µm, eluent water / MeOH (63:37), 1.0 mL / min, 40 °C, det. 204 nm]. The amide of **7** is eluted with t_{ret} = 21.97 min, the amide of *ent*-**7** with t_{ret} = 19.74 min (baseline separation). 96 ± 2% ee of the (*R*)-configuration (*D*-configuration) is indicated by both methods. Chemical purity is indicated as > 99% by HPLC (125 x 4 mm RP18 LiChrospher 100K 5 µm, eluent: water / MeOH 95.2 + 4.8 + 0.1% NH_4OAc, 1.0 mL / min, 40 °C, det. 236 nm, t_{ret} 9.14 min). Potentiometric titration of a methanolic solution of **7** with 1 *N* NaOH indicates 97% of theory presence of acid.

***D*-(*p*-Cyanophenylalanine) (8).** Conc. HCl (7.0 L, 36%, 84 mol) is added at 60 °C to a suspension of **7** (6.50 kg, 28 mol) in water (30.5 L) to give a clear solution that is stirred at reflux (97 °C). The reaction progress is monitored by HPLC [RP 18 LiChrospher 100 5 µm cartridge, eluent A: water / MeOH (4+1) +

0.1% NH_4OAc, eluent B: water + 0.1% NH_4OAc, gradient: 100% B for 8 min, then linearly to 0% B within 22 min; 40 °C, det. 236 nm, t_{ret} **8** 10.93 min) that indicates quantitative acetyl cleavage after 4 - 6 h. The mixture is allowed to stand 16 h at ambient temperature. Active charcoal (0.5 kg) is added and the mixture is stirred for 30 min. The charcoal is filtered off and the pH of the filtrate is adjusted to 4.5 with NEt_3 (13 L). The suspension is stirred with EtOH (20 L), filtered again, and the solid is dried at 60 °C *in vacuo* to furnish colourless crystals (3.95 kg, 74% yield), M.p. 255 °C decomp.. The chemical purity is indicated as > 99% by HPLC (*vide supra*), the enantiomeric purity is indicated as ≥ 99.5% ee by GC [30 m Chirasil *L*-Val, 80 °C for 4 min, then linearly by 8 °C/min to 180 °C, inj. 200 °C, det. (MS) 200 °C, 0.55 bar helium carrier gas, t_{ret} **8** 19.43 min, *ent*-**8** 20.50 min]. ^1H NMR (200 MHz, D_2O, 10 mg of **8** and 10 mg of $NaHCO_3$): δ = 3.17 (dd, J = 15 and 7 Hz, 1H, CH_2), 3.27 (dd, J = 15 and 6 Hz, 1H, CH_2), 3.97 (dd, J = 7 and 6 Hz), 4.74 (s, HOD), 7.32-7.83 (m, 4H, CH); IR (KBr): ν = 3600 - 2300 cm^{-1} (NH_2 and CO_2H), 2237 cm^{-1} (C≡N), 1640 cm^{-1} (shoulder, CO_2H, intramolec. H-bonded), 1613 cm^{-1} (CO_2^-), 1570 cm^{-1} (NH_2), 1510 (NH_3^+); MS (DCI): *m/z* (%) = 191 (100) [$M+H^+$], 145 (8) [M^+ - CO_2H], 74 (17) [H_2N-CH-CO_2H^+]. The hydrochloride of **8** shows $[\alpha]_D^{20}$ = -20.1 (*c* = 1.0 in MeOH).

***N*-Benzyloxycarbonyl-*D*-(*p*-cyanophenylalanine) (9).** *D*-*p*-Cyanophenylalanine **8** (3.135 kg, 16.5 mol) is suspended in water (23.5 L) and THF (23.5 L). The pH is adjusted to 9.0 by addition of NaOH pellets (420 g, 10.5 mol). Benzyl chloroformate (3.394 kg, 19.9 mol) is added dropwise within 2 h at 20-25 °C to the resulting clear solution , and the pH is kept at constant 9.0 by addition of aqueous NaOH (4 *M*, 5 L). The mixture is stirred for 1 h at pH 9.0. THF is evaporated at ≤ 40 °C *in vacuo*. Water (31 L) is added to the residue and the solution is washed with iPr_2O (3 x 10 L). Residual iPr_2O that remains in the aqueous phase is removed *in vacuo*. The aqueous solution is then stirred for 15 min with active charcoal (300 g) and the mixture is suction-filtered through a clarifying pad. The filtrate is acidified at 10 °C to pH 1.5 with 6 *N* HCl (3.7 L). The thick suspension is stirred for 12 h at 10 °C. The precipitate is collected by filtration, washed with water (5 L) and dried at 60 °C *in vacuo* to give a solid resin (5.21 kg, 97% yield), M.p. 125-130 °C; ^1H NMR (200 MHz, [D_6]DMSO): δ = 2.92 (dd, J = 14 and 10 Hz, 1H, CH_2), 3.18 (dd, J = 14 and 5 Hz, 1H, CH_2), 4.21 (m, 1H, CH), 4.94 (s, 2H, CH_2), 7.10-7.40 (m, 5H, CH), 7.43 (d, J = 8 Hz, 2H, CH), 7.54 (d, J = 9 Hz, 1H, NH), 7.71 (d, J = 8 Hz, 2H, CH), 11.0-15.0 (br s, 1H, CO_2H); IR (KBr): ν = 3600 - 2300 cm^{-1} (br, N-H and CO_2H), 2230 cm^{-1} (C≡N), 1730 cm^{-1} (sh), 1692 cm^{-1} (C=O), 1532 cm^{-1} (N-C=O); MS (DCI): *m/z* (%) = 325 (100) [$M+H^+$], 91 (60) [$C_7H_7^+$]. The enantiomeric purity is determined by derivatization with (*R*)-(+)-1-phenylethylamine, supported by MEPA, to give diastereomeric amides that are separated by HPLC [125 x 4 mm RP8 LiChrospher 60 Select B, eluent water / MeCN (60:40), 1.2 mL/min, 22 °C, det. 236 nm]. The amide of **9** is eluted with t_{ret} = 16.86 min, the amide of *ent*-**9** with t_{ret} = 17.97 min (baseline separation). 99.5 ± 0.5% ee is indicated. The chemical purity of the product is 90% according to HPLC [125 x 4 mm RP18 LiChrospher 100 5 µm; eluent A: water + 0.1% NH_4OAc, eluent B: MeCN / water (80:20) + 0.1% NH_4OAc; gradient: from 100% A within 25 min linearly to 20% A + 80% B; 1.0 mL/min, 40 °C, det. 236 nm; t_{ret} **9** 12.30 min, main impurity 16.63 min]. It is used without purification in the next step.

***N*-Benzyloxycarbonyl-*D*-(*p*-cyanophenylalanine) piperidide (10).** *CAUTION : MEPA is very toxic!* A suspension of **9** (5.20 kg, 16.05 mol) in EtOAc (65 L) is stirred for 1 h at 50 °C. It is cooled to 20 °C, piperidine (3.20 L, 32.35 mol) is added at once, and the mixture is stirred for 2 h at 22 °C. A solution of MEPA (4.78 kg, 24.4 mol) in EtOAc (5 L) is added within 10 min, and the mixture is stirred for 2 h at 22 °C. HPLC [125 x 4.6 mm Hypersil MOS 5 µm; eluent A: MeCN / water (70:30) + 0.1% CF_3CO_2H, eluent B: water + 0.1% CF_3CO_2H; A:B = 45:55; 1 mL/min, 25 °C, det. 215 nm, t_{ret} **9** 2.90 min, **10** 5.40 min] indicates quantitative reaction of **9** and formation of **10**. Water (20 L) is added and the mixture is stirred for 16 h at 20 °C. The aqueous phase is separated, the organic phase is diluted with EtOAc (20 L) and washed with water (3 x 12 L), 0.1 *M* NaOH (2 x 10 L), water (12 L), 0.1 *M* HCl (2 x 12 L), water (3 x 12 L), and with brine (12 L). It is dried (Na_2SO_4) and the solvent is evaporated at $\leq$ 40 °C *in vacuo* to furnish a pale-yellow oil (5.70 kg, 91% yield); ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.20 - 1.63 (m, 6H, CH_2), 2.93 (m, 2H, CH_2), 3.30 - 3.60 (m, 4H, CH_2), 4.68 (qua, J = 7-8 Hz, 1H, CH), 4.92 (AB-system, J = 11 Hz, 2H, CH_2), 7.13 - 7.40 (m, 5H, CH), 7.46 (d, J = 8 Hz, 2H, CH), 7.70 (d, J = 8 Hz, 2H, CH); IR ($CHCl_3$): ν = 3290 cm^{-1} (N-H), 2228 cm^{-1} (C$\equiv$N), 1720 cm^{-1} (C=O), 1633 cm^{-1} (C=O); MS (ESI): *m/z* (%) = 392 (100) [M+H$^+$], 91 (34) [$C_7H_7^+$]; enantiomeric purity (250 x 4.6 mm DNBPG-Bakerbond, eluent: *n*-hexane / EtOH 5:1, 1.0 mL/min, 40 °C, det. 236 nm): only one peak, t_{ret} 8.74 min ; chemical purity (125 x 4 mm RP8 LiChrospher 60 Select B 5 µm; eluent A: water + 0.1% NH_4OAc + 0.1% CF_3CO_2H, eluent B: MeOH / water 80:20 + 0.1% NH_4OAc + 0.1% CF_3CO_2H; gradient: within 30 min linearly from 40% B to 100% B, 1.0 mL/min, 40 °C, det. 210 nm, t_{ret} **10** 19.13 min): 85%. The product is used without purification in the next step.

***N*-Benzyloxycarbonyl-*D*-(*p*-amidoximo-phenylalanine) piperidide (11).** Hydroxylamine hydrochloride (1.43 kg, 20.6 mol), followed by NEt_3 (3.4 L, 24.5 mol) is added within 10 min at 25-30 °C to a solution of **10** (5.70 kg, 14.5 mol) in EtOH (50 L). The mixture is refluxed, being monitored by HPLC. Precipitation of a solid is noticed after 30 min and quantitative reaction is indicated after 4 h. The suspension is allowed to stir at ambient temperature for 20 h. The precipitate is suction-filtered, washed with EtOH (5 L), MTB (5 L), and dried at 40 °C *in vacuo* to furnish colourless crystals (4.91 kg, 80% yield), M.p. 223 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.05 - 1.60 (m, 6H, CH_2), 2.84 (m, 2H, CH_2), 3.20 - 3.55 (m, 4H, CH_2), 4.65 (qua, J = 7-8 Hz, 1H, CH), 4.96 (AB-system, J = 14 Hz, 2H, CH_2), 5.74 (s, 2H, NH_2), 7.13 - 7.40 (m, 7H, CH), 7.56 (d, J = 8 Hz, 2H, CH), 7.64 (d, J = 9 Hz, 1H, NH), 9.57 (s, 1H, OH); ^{13}C NMR (67.93 MHz, [D$_6$]DMSO ; multiplicity determined by DEPT 135°): δ = 23.87 (1C, CH_2), 25.16 (1C, CH_2), 25.74 (1C, CH_2), 37.16 (1C, CH_2), 42.44 (1C, CH_2), 45.82 (1C, CH_2), 51.63 (1C, CH), 65.21 (1C, CH_2), 125.01 (2C, CH), 127.45 (2C, CH), 127.62 (1C, CH), 128.20 (2C, CH), 128.99 (2C, CH), 131.38 (1C), 136.94 (1C), 138.39 (1C), 150.55 (1C), 155.57 (1C), 168.96 (1C). ^{1}H and ^{13}C NMR indicate the presence of only one diastereomer of **11**. The OH group is presumed to be anti to the NH_2 group. IR (KBr): ν = 3493 cm^{-1} (N-H), 3380 cm^{-1} (N-H), 3270 cm^{-1} (br, O-H), 1703 cm^{-1} (C=O), 1645 cm^{-1} (C=N), 1618 cm^{-1} (C=O); MS (ESI): *m/z* (%) = 425 (100) [M + H$^+$], 381 (9) [M$^+$ - HNCO], 340 (7) [M + H$^+$ - piperidine]; HPLC [125 x 4 mm RP8 LiChrospher 60 Select B 5 µm; eluent A:

water + 0.1% NH_4OAc + 0.1% CF_3CO_2H, eluent B: MeOH / water (60:40) + 0.1% NH_4OAc + 0.1% CF_3CO_2H; A:B = 20:80; 1.0 mL/min, 40 °C, det. 230 nm; t_{ret} 4.57 min): 97.0 % purity.

4-Amidinophenylalanine piperidide dihydrochloride (Adf-pip x 2 HCl) (12). A solution of amidoxime **11** (1.468 kg, 3.46 mol) in glacial acetic acid (18.0 L) is filled into a stainless steel shaker autoclave. The suspension of 10% palladium on charcoal (150 g, containing 50% water) in acetic acid (1 L) is added, the autoclave is closed and deoxygenated as described for **2**. The hydrogenolysis is conducted at 50 °C under 10 bar of H_2 until the uptake ceases (18 h). The catalyst is filtered off and washed with acetic acid (4.0 L). *Iso*propanolic HCl (1.7 L, 4.9 *N*) is added to the filtrate and all volatiles are evaporated at $\leq$ 40 °C *in vacuo*. The suspension of the crude hydrochloride (1.036 kg) in acetone (4.6 L) is stirred for 45 min at 20 °C under N_2. The solid is filtered off, washed with acetone (1.4 L) and dried in HV to furnish an amorphous, colourless product (1.022 kg, 85% yield); M.p. 106 °C, 124-125 °C decomp.; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 0.95 - 1.60 (m, 6H, CH_2), 2.90 - 3.60 (m, 6H, CH_2), 4.70 (t, J = 7 Hz, 1H, CH), 7.47 (d, J = 8 Hz, 2H, CH), 7.87 (d, J = 8 Hz, 2H, CH), 8.42 (br s, 3H, NH_3^+), 9.28 (s, 2H, NH_2), 9.48 (s, 2H, NH_2^+); ^{13}C NMR (67.93 MHz, [D$_6$]DMSO ; multiplicity determined by DEPT 135°): δ = 23.46 (1C, CH_2), 24.93 (1C, CH_2), 25.40 (1C, CH_2), 36.42 (1C, CH_2), 42.39 (1C, CH_2), 45.79 (1C, CH_2), 49.23 (1C, CH), 126.49 (1C), 128.02 (1C, CH), 130.24 (1C, CH), 140.91 (1C), 164.82 (1C), 165.76 (1C); IR (KBr): ν = 3700 - 2400 cm^{-1} (N-H), 1680 (C=O), 1640 (C=N); MS (ESI, free base Adf-pip $C_{15}H_{22}N_4O$ has M = 274): *m/z* (%) = 275 (77) [M + H$^+$], 162 (73) [M$^+$ - pip-C=O], 145 (27) ["162" - NH_3], 86 (100). The enantiomeric purity is determined by derivatization of a sample of **12** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (Mosher reagent) in the presence of *N*-ethyl-morpholine, followed by HPLC analysis (250 x 4 mm Si60 LiChrosorb 5 µm, eluent: cyclohexane / $CHCl_3$ / 1,2-dimethoxyethane 8:1:1, 1.0 mL/min, 25 °C, det. 306 nm). Only one peak is indicated. When the derivatization is conducted with a 1:1- mixture of (*S*)-(+)- and (*R*)-(-)- Mosher reagent, two peaks (t_{ret} 17.36 and 19.23 min) are indicated in a ratio of 1:1. It is concluded, that the enantiomeric purity of **12** is $\geq$ 99.8% ee. The chemical purity is indicated as 98.1% by capillary electrophoresis (70 cm x 75 µm TSP-capillary; injection: electrokinetic 15.0 kV / 3.0 sec ; buffer: 30 *mM* KH_2PO_4 + 10% *i*PrOH, pH 4.00, 1 *mM* 3-cyclohexylaminopropane sulfonic acid (CAPS); current strength 40 µA, voltage: 23 kV; 20 °C, det. 205 nm, t_{ret} 11.79 min) and as 98.4% by HPLC [125 x 4 mm RP8 LiChrospher 60 Select B 5 µm; eluent A: water + 0.1% NH_4OAc + 0.1% CF_3CO_2H, eluent B: water / MeOH (60:40) + 0.1% NH_4OAc + 0.1% CF_3CO_2H; gradient: 70% A + 30% B for 8 min, then within 17 min linearly to 40% A + 60% B, 1.0 mL/min, 40 °C, det. 210 nm, t_{ret} 5.37 min]. Argentometric titration with a $AgNO_3$ solution indicates 5.1 mmol Cl$^-$ / g **12**, *i.e.* 89% of theory for a dihydrochloride. Potentiometric titration with a 0.1 *N* NaOH solution produces a curve with two inflection points located at pH 8.1 and pH 11.4. Consumption of base corresponds to 101% of theory for a dihydrochloride.

β-(*O*-Benzyl) [*N*-(4-methoxy-2,3,5-trimethylphenylsulfonyl)-*L*-aspartate] (Mtr-Asp-β-Bzl) (15a).

a) Compound **15a** prepared by reaction of β-*O*-benzyl aspartate **13** with Mtr chloride **14**:

L-(+)-[*β*-(*O*-benzyl)-aspartate] **13** (2.00 kg, 8.96 mol) is suspended at 0 °C in DMF (28.0 L) and water (14.0 L). *N*-Ethyl-di*iso*propylamine (2.90 kg, 22.44 mol) is added at 0 °C within 15 min (mildly exothermic), followed by Mtr chloride **14** (2.73 kg, 11.0 mol) in one batch. The suspension is stirred for 4 h at 0 °C, turning into a virtually clear, yellow solution of pH 9-10. HPLC (250 x 4 mm Nucleosil 120 C18 7 µm; eluent A: 1400 mL MeCN / 600 mL water / 0.1% (2g) NH_4OAc; eluent B: 25 mL MeCN / 500 mL water / 0.1% (0.525 g) NH_4OAc; gradient: 100% B for 6 min, then linearly to 100% A within 15 min, finally 100% A for 10 min; 1.0 mL/min, 20 °C, det. 220 nm, t_{ret} DMF 2.43 min, **13** 13.45 min, Mtr-OH 14.70 min, **15a** 19.10 min) indicates quantitative reaction. The mixture is stirred for further 17 h at 0 °C (no change indicated by HPLC). The mixture is poured on ice-water (100 L), the aqueous phase is washed with MTB (3 x 20 L) and these washings are discarded. At 0 °C the aqueous phase is acidified to pH 3.0 with 2*N* HCl. It is then extracted with MTB (3 x 35 L). The combined extracts are dried (Na_2SO_4), filtered, and the solvent is evaporated *in vacuo*. The residue is dried in HV with slow rotation to provide a very viscous, yellow oil, that retains 1 equiv. of DMF and 0.3 - 0.5 equiv. of MTB (3.8 kg, 97% yield), purity 91% (HPLC); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.06 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.82 (dd, J = 17 and 5 Hz, 1H, CH_2), 3.00 (dd, J = 17 and 4 Hz, 1H, CH_2), 3.82 (s, 3H, CH_3), 4.10 (m, 1H, CH), 5.06 (AB-system, 2H, CH_2), 5.75 (d, J = 7 Hz, 1H, NH), 6.08 (br s, 1H, CO_2H), 6.54 (s, 1H, CH), 7.27 - 7.43 (m, 5H, CH).

b) Compound **15a** prepared by regioselective alcoholysis of anhydride **24** with benzyl alcohol :

Ground K_2CO_3 (4.22 g, 30.53 mmol) is added at 15 °C to a solution of **24** (9.8 g, 29.94 mmol) in DMF (18 mL). After 5 min benzyl alcohol (3.9 g, 36.07 mmol) is added at 15 °C and the mixture is stirred for 4 h at 20 °C. It is poured into ice-water (200 mL) and washed with MTB (3 x 80 mL, washings discarded). The aqueous layer is acidified at 10 °C with 2 *N* HCl (45 mL) and then extracted with MTB (3 x 80 mL). The combined extracts are washed with water (80 mL), dried ($MgSO_4$), the solvent is evaporated *in vacuo* and the residue is dried in HV to furnish a yellow oil (12.7 g, 97% yield) that according to ^{1}H NMR and HPLC (conditions as described for **2a**, t_{ret} **15a** 33.62 min, *α*-benzyl ester 17.31 min) consists of *β*-benzyl ester **15a** and regioisomeric *α*-benzyl ester in the ratio 5 : 1.

To this crude **15a** (12.43 g, 28.54 mmol) is added DMF (2.08 g, 28.54 mmol) and after 40 min *i*Pr_2O (100 mL). Seeding crystals of **15a** (50 mg) are added to the vigorously stirred mixture. The suspension is stirred for 2.5 h. The colourless solid is collected by filtration, washed with *i*Pr_2O (20 mL), and dried in HV (7.83 g, 60 % yield). ^{1}H NMR and HPLC indicate a *β*/*α*-ratio of 12 : 1 (in three analogous experiments ratios between 10:1 and 17:1 were obtained), ^{1}H NMR further indicates that the product is a solvate containing 1 mol of DMF per mol **15a**; M.p. 93-96 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.10 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.85 (dd, J = 17 and 5 Hz, 1H, CH_2), 2.88 (s, 3H, CH_3 of DMF), 2.97 (s, 3H, CH_3 of DMF), 3.00 (dd, J = 17 and 4 Hz, 1H, CH_2), 3.83 (s, 3H, CH_3), 4.10 (m, 1H, CH), 4.40-5.60 (very br., 1H, CO_2H), 5.04 (AB system, 2H, CH_2), 5.88 (d, J = 7 Hz, 1H, NH), 6.55 (s, 1H, CH), 7.16-7.37 (m, 5H, CH), 8.02 (s, 1H, CH of DMF) , resonances for the regioisomeric *α*-benzyl ester are at δ = 2.12, 2.59 and 2.68 for the methyl-singlets of

the Mtr group, at δ = 5.73 for the NH doublet and at δ = 6.58 for the aryl-H; MS (FAB): *m/z* (%) = 436 (100) [M + H$^+$], 213 (53) [Mtr$^+$].

α-Cyclohexylammonium [*N*-(4-methoxy-2,3,5-trimethylphenylsulfonyl)-β-(*O*-benzyl)] aspartate (15b). At 0 °C a solution of cyclohexylamine (1.27 kg, 12.8 mol) in EtOAc (2.0 L) is added within 30 min to a solution of crude **15a** (3.80 kg) in EtOAc (26.0 L). Since the very thick suspension becomes difficult to stir EtOAc / MTB (1:1, 3.0 L) is added and the mixture is stirred for 2 h at 0 °C. The precipitate is suction-filtered, washed with EtOAc / MTB (1:1, 22.0 L) and dried in HV at 25 °C (2.92 kg, 61% yield based on **13**), M.p. 169-171 °C; $[\alpha]_D^{20}$ = +35.9 (*c* = 1.02 in acetone); purity (HPLC): 99.3% [12] ; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.98 - 1.34 (m, 5H, CH_2), 1.57 (m, 1H, CH_2), 1.67 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 2.71 (dd, *J* = 12 and 5 Hz, 1H, CH_2), 2.87 (dd, *J* = 12 and 3 Hz, 1H, CH_2), 2.90 (m, 1H, CH), 3.77 (t, *J* ≈ 4 Hz, 1H, CH), 3.78 (s, 3H, CH_3), 4.94 (AB-system, *J* = 9 Hz, 2H, CH_2), 5.0 - 8.0 (very br s, 4H, NH and NH_3^+), 6.51 (s, 1H, CH), 7.20 - 7.38 (m, 5H, CH); IR (KBr): ν = 3600 - 2400 cm^{-1} (br, NH_3^+), 3317 cm^{-1} (N-H), 1740 cm^{-1} (C=O), 1630 cm^{-1} (CO_2^-), 1330, 1310, and 1180 cm^{-1} (SO_2N); $C_{27}H_{38}N_2SO_7$ (534.68): calcd C 60.65, H 7.16, N 5.24, S 5.99 ; found C 60.60, H 7.05, N 5.05, S 5.85.

β-(*O*-Allyl) [*N*-(4-methoxy-2,3,5-trimethylphenylsulfonyl)-*L*-aspartate] (Mtr-Asp-β-allyl) (15c). Ground K_2CO_3 (1.4 g, 10.1 mmol) is added at 15 °C to the solution of **24** (3.3 g, 10.1 mmol) in DMF (7.0 mL). After 5 min allyl alcohol (670 mg, 11.5 mmol) is added and the mixture is stirred for 2 h at 20 °C. It is poured into ice-water (15 mL) (resulting pH is 9-10) and washed with MTB (2 x 15 mL, discarded). The pH is adjusted to 1.5 with 2 *N* HCl (10 mL), and the product is extracted with MTB (2 x 15 mL). The extracts are dried ($MgSO_4$) and the solvent is evaporated *in vacuo* to give a yellow resin (3.9 g) that according to ^{1}H NMR and HPLC contains MTB and DMF, however is does not contain significant amounts of the regioisomeric α-allyl ester. Rigorous purification was not attempted. ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 2.07 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.60 (m, 2H, CH_2), 4.03 (m, 1H, CH), 4.30 (m, 2H, CH_2), 5.10-5.30 (m, 2H, CH_2), 5.58-5.82 (m, 1H, CH), 6.78 (s, 1H, CH), 8.12 (d, *J* = 9 Hz, 1H, NH), 12.52 (br s, 1H, CO_2H).

***N*-[*N'*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-β-(*O*-benzyl)-α-*L*-aspartyl]-4-amidino-*D*-phenylalanine piperidide hydrochloride Mtr-Asp(ß-Bzl)-*D*-Adf-pip x HCl (17).** Solid Adf-pip dihydrochloride **12** (870 g, 2.50 mol) is added to the solution of cyclohexylammonium salt **15b** (1.19 kg, 2.23 mol) in DMF (23.5 L) to give a clear, virtually colourless solution, that is then cooled to 0 °C. *N*-Methylmorpholine (255 g, 2.52 mol) is added within 5 min, followed by TOTU **16** (780 g, 2.39 mol) within 15 min. The reaction has no significant heat tonality. The resulting pale-yellow, slightly turbid solution is stirred for 15 min at 0 °C. The solution is transferred to the distillation flask of a technical rotary evaporator and the DMF is then evaporated at ≤ 35 °C bath temperature at 0.5 - 1.5 mbar to give an oil (4.4 kg). *Vacuo* is released with N_2, iPr$_2$O (17.0 L) is sucked in, and the mixture is rotated for 10 min. The turbid iPr$_2$O layer is siphoned off from the undissolved oil. This procedure is repeated two times with fresh iPr$_2$O (2 x 17.0 L). Traces of residual iPr$_2$O are evaporated *in vacuo* and the semisolid mass is allowed to stand at 0 °C under N_2 overnight. EtOAc (30.0 L) is sucked into

the rotating flask to give a turbid solution, that is transferred into a separation funnel with stirrer. It is washed with aqueous $KHCO_3$ solution (1*M*, 3 x 8 L). The clear, yellow organic layer is washed with aqueous $KHSO_4$ solution (5%, 3 x 8 L) leading to virtually complete decolourization. It is dried (Na_2SO_4, 3.0 kg), and the filtrate is evaporated at $\leq$ 35 °C *in vacuo* and then dried in HV in the evaporator flask to give a colourless solid (1.70 kg). At 0 °C, ethanolic HCl (1*N*, 5.1 L) is sucked into the quickly rotating flask to give a clear solution within 25 min. It is pumped with exclusion of moisture within 20 min at 15 °C into anhydrous, vigorously stirred iPr_2O (80.0 L). The colourless precipitate is filtered through a pressure nutsche, washed with iPr_2O (2 x 25 L) and blown dry by a stream of N_2. (Hydrochloride **17** is hygroscopic as long as it contains mother liquor. In the presence of moisture, the initially granular solid becomes deliquescent and difficult to filter). The solid is dried at 25 °C in HV (1.50 kg, 92 % yield, 82 % yield based on **12**); ^{1}H NMR (200 MHz, [D$_4$]MeOH): δ = 1.26 (m, 6H, CH_2), 2.12 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.56 (AB part of ABX system, partially superimposed by two methyl singlets, 2H, CH_2), 2.63 (s, 3H, CH_3), 2.88 (dd, J = 14 and 7 Hz, 1H, CH_2), 3.10 (dd, J = 14 and 6 Hz, 1H, CH_2), 3.32 - 3.58 (m, 4H, CH_2), 3.82 (s, 3H, CH_3), 4.10 (t, J = 7 Hz, 1H, CH), 4.82 (s, 5H, NH and NH_2), 4.91 (AB-system, J = 13 Hz, 2H, CH_2), 5.07 (m, 1H, CH), 6.72 (s, 1H, CH), 7.20 - 7.36 (m, 5H, CH), 7.41 (d, J = 8 Hz, 2H, CH), 7.68 (d, J = 8 Hz, 2H, CH), 7.92 (d, J = 9 Hz, 1H, NH); ^{13}C NMR (67.93 MHz, [D$_4$]MeOH ; multiplicity determined by DEPT 135°): δ = 12.22 (1C, CH_3), 18.40 (1C, CH_3), 24.57 (1C, CH_3), 25.31 (1C, CH_2), 26.66 (1C, CH_2), 27.47 (1C, CH_2), 38.05 (1C, CH_2), 39.33 (1C, CH_2), 44.52 (1C, CH_2), 47.94 (1C, CH_2), 51.16 (1C, CH), 54.28 (1C, CH_3), 56.22 (1C, CH), 67.63 (1C, CH_2), 113.47 (1C, CH), 126.47 (1C), 127.96 (1C), 129.02 (4C, CH), 129.25 (1C, CH), 129.55 (2C, CH), 130.39 (1C), 131.78 (2C, CH), 137.13 (1C), 140.28 (1C), 144.99 (1C), 161.01 (1C), 168.12 (1C), 169.77 (1C), 171.45 (1C), 171.61 (1C); IR (KBr): ν = 3700 - 2700 cm^{-1} (br, N-H), 1735 cm^{-1} (C=O), 1680 cm^{-1} (C=O), 1623 cm^{-1} (C=N); MS (FAB, free amidine $C_{36}H_{45}N_5SO_7$ has M = 691): *m/z* (%) = 692 (100) [M + H$^+$] ; purity: 94% (HPLC, as described for **2a**; t_{ret} **12** 2.12 min, TOTU **16** 4.47 min, **17** 31.30 min, **15b** 33.34 min).

***N*-Acetyl-*D*-(*p*-cyanophenylalanine) piperidide (18).** Piperidine (146.5 g, 1.75 mol) is added to a suspension of carboxylic acid **7** (200.0 g, 0.862 mol) in EtOAc (2.0 L). A clear solution is obtained for a short while , followed by a precipitation of the piperidinium salt of acid **7** as a thick slurry. MEPA (256.0 g, 1.29 mol) is added at once, leading to the formation of a clear solution and an increase of the reaction temperature to 35 °C. The mixture is stirred at 25 °C for 1 h. Water (1.2 L) is added and the mixture is stirred at 25 °C for an additional h. A pH of 6-7 is indicated by a glass electrode. The aqueous phase is separated and the organic phase is washed with water (1.2 L), 1*M* HCl (1.2 L), and water (4 x 800 mL). It is dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The oily residue is dried in HV (180.0 g, 70% yield); purity : 95.2% (HPLC, 125 x 4 mm RP18 LiChrospher 100 5 µm cartridge; eluent A: H_2O / MeCN 90:10 + 0.1% NH_4OAc + 0.1% CF_3CO_2H, eluent B: MeCN / H_2O 90:20 + 0.1% NH_4OAc + 0.1% CF_3CO_2H ; gradient: linearly from 0% B to 60% B within 25 min; 1.0 mL/min, 40 °C, det. 234 nm; t_{ret} **18** 12.96 min, main impurity 14.50 min); ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.15-1.60 (m, 6H, CH_2), 1.74 (s, 3H, CH_3), 2.83 (dd, J = 14 and 9 Hz, 1H, CH_2), 3.01 (dd, J = 14 and 6 Hz, 1H, CH_2), 3.22 - 3.56 (m, 4H, CH_2), 4.94 (qua, J = 8 Hz, 1H, CH), 7.42 (d, J = 8 Hz,

2H, CH), 7.72 (d, J = 8 Hz, 2H, CH), 8.32 (d, J = 9 Hz, 1H, NH); IR ($CHCl_3$): ν = 3295 cm^{-1} (N-H), 2230 cm^{-1} (C≡N), 1625 cm^{-1} (C=O); MS (DCI): *m/z* (%) = 300 (100) [M + H^+].

***D*-(*p*-Cyanophenylalanine) piperidide (19).** A solution of **18** (180.0 g, 0.60 mol) in aqueous HCl (2*M*, 2.0 L) is stirred at 80 °C until the educt has disappeared (2 h). The mixture is cooled to 20 °C and washed with EtOAc (2 x 300 mL). The aqueous phase is adjusted to pH 9.0 with NaOH. The product is then extracted with EtOAc (3 x 700 mL) and the combined extracts are washed with water (2 x 300 mL) and with brine (100 mL). The solution is dried ($MgSO_4$) and concentrated *in vacuo*. The oily residue is triturated with iPr_2O (500 mL) to furnish colourless crystals, that are collected by filtration and dried *in vacuo* (131.5 g, 85% yield); purity (conditions *cf.* **18**; t_{ret} 9.73 min): 98.7 %; ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 1.08 - 1.60 (m, 6H, CH_2), 1.72 (s, 2H, NH_2), 2.68 (dd, J = 13 and 7 Hz, 1H, CH_2), 2.83 (dd, J = 13 and 6 Hz, 1H, CH_2), 3.20 - 3.53 (m, 4H, CH_2), 3.92 (t, J = 7 Hz, 1H, CH), 7.42 (d, J = 9 Hz, 2H, CH), 7.73 (d, J = 9 Hz, 2H, CH); IR (KBr): ν = 3620 - 3140 cm^{-1} (br, N-H), 2228 cm^{-1} (C≡N), 1627 cm^{-1} (C=O); MS (DCI): *m/z* (%) = 258 (100) [M + H^+], 145 (16) [M^+ - piperidyl-C≡O]. The enantiomeric purity is determined by derivatization of a sample of **19** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride in the presence of *N*-ethyl-morpholine, followed by HPLC analysis (250 x 4 mm Si 60 LiChrosorb 5 µm, eluent: cyclohexane / $CHCl_3$ / 1,2-dimethoxyethane 8:1:1, 1.0 mL/min, 25 °C, det. 234 nm), to give a main peak (t_{ret} = 24.06 min, 99.3%) corresponding to *D*-**19** and a trace peak (t_{ret} = 25.76 min, 0.7%) corresponding to *L*-**19**. When the derivatization is conducted with the (*R*)-(-)-acid chloride the same peaks are obtained with an exactly reversed ratio. When a racemic acid chloride is employed they are obtained in an 1:1 ratio. 98.6% ee is thus indicated for **19**.

***N*-[*N'*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-β-(*O*-benzyl)-α-*L*-aspartyl]-4-cyano-*D*-phenylalanine piperidide (20).** MEPA (44.0 g, 222 mmol) is added at once to a solution of **19** (38.0 g, 148 mmol) and **15a** (liberated from **15b** with aqueous $KHSO_4$ solution, followed by extraction with EtOAc; 70.9 g, 163 mmol) in EtOAc (300 mL). The pale-yellow clear solution is stirred at 25 °C for 16 h, the reaction progress being monitored by HPLC (250 x 4.6 mm Nucleosil Phenyl 7 µm, eluent A: CH_3CN / H_2O 70 : 30 + 0.1% CF_3CO_2H, eluent B: H_2O + 0.1% CF_3CO_2H, A:B = 80 : 20, 1.0 mL / min, 25 °C, det. 215 nm, t_{ret} **19** 3.23 min, **15a** 5.61 min, **20** 9.06 min). Water (1.0 L) is added and the mixture is stirred for 1 h. HPLC indicates that the aqueous phase contains small amounts of unreacted **19**, the EtOAc phase contains the excess of **15a** and product **20**. The organic phase is washed with water (2 x 500 mL), the pH is adjusted to 9.0 with 2 *M* aqueous NaOH, and the organic phase is washed again with water (2 x 500 mL) and with brine. HPLC now indicates that the excess **15a** is completely contained in the aqueous washings and product **20** (> 98% purity) is contained in the EtOAc layer. The organic phase is dried ($MgSO_4$), filtered, and the solvent is evaporated *in vacuo*. The residue is dried in HV to leave a colourless, solid foam (76.0 g, 76% yield); ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 1.18-1.60 (m, 6H, CH_2), 2.03 (s, 3H, CH_3), 2.28 (dd, J = 15 and 8 Hz, 1H, CH_2), 2.42 (s, 3H, CH_3), ~2.48 (dd, superimposed by resonances of solvent and two methyl singlets, 1H, CH_2), 2.57 (s, 3H, CH_3), 2.72 (dd, J = 13 and 8 Hz, 1H, CH_2), 2.95 (dd, J = 13 Hz and 6 Hz, 1H, CH_2), 3.20-3.53 (m, 4H, CH_2), 3.78 (s, 3H, CH_3), 4.00

(m, 1H, CH), 4.78 (d, J = 13 Hz, 1H, CH_2), 4.90 (m, 1H, CH), 4.92 (d, J = 13 Hz, 1H, CH_2), 6.76 (s, 1H, CH), 7.20-7.40 (M, 7H, CH), 7.65 (d, J = 8 Hz, 2H, CH), 7.82 (d, J = 10 Hz, 1H, NH), 8.07 (d, J = 9 Hz, 1H, NH); IR (KBr): ν = 3500-3100 cm^{-1} (br, N-H), 2228 cm^{-1} (C≡N), 1740 cm^{-1} (C=O), 1680 cm^{-1} (sh) and 1640 cm^{-1} (C=O), 1310 cm^{-1} (SO_2N); MS (FAB): m/z (%) = 675 (100) ($M + H^+$), 213 (65).

***N*-[*N'*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-*β*-(*O*-benzyl)-*α*-*L*-aspartyl]-4-amidoximo-*D*-phenylalanine piperidide (21).** A solution of **20** (33.7 g, 50 mmol), hydroxylamine hydrochloride (5.2 g, 75 mmol) and NEt_3 (7.6 g, 75 mmol) in EtOH (150 mL) is refluxed (bath 80 °C) for 4 h. Since HPLC (conditions as described for **20**; t_{ret} **21** 4.52 min) still indicates unreacted substrate **20**, more NH_2OH x HCl (5.2 g, 75 mmol) and NEt_3 (7.6 g, 75 mmol) is added, and the mixture is refluxed for an additional h. The solvent is evaporated *in vacuo*. The residue is taken up in EtOAc (500 mL) and washed with water (2 x 300 mL). Water (300 mL) is added and the pH is adjusted to 2.0 with 2 *M* aqueous HCl. The organic layer is washed with water (2 x 300 mL) and then concentrated *in vacuo*. The residue is triturated with *i*PrOH (400 mL) and seeded with crystals of **21**. The precipitate is suction-filtered, washed with iPr_2O (100 mL) and dried in HV (18.0 g, 51% yield), M.p. 133-134 °C; purity: 94.7% (HPLC); ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 1.10-1.60 (m, 6H, CH_2), 2.03 (s, 3H, CH_3), 2.33 (dd, J = 16 Hz and 8 Hz, 1H, CH_2), 2.44 (s, 3H, CH_3), ~2.48 (dd, superimposed by resonances of solvent and two methyl singlets, 1H, CH_2), 2.55 (s, 3H, CH_3), 2.60 (dd, J = 13 and 7-8 Hz, 1H, CH_2), 2.85 (dd, J = 13 and 8 Hz, 1H, CH_2), 3.15-3.52 (m, 4H, CH_2), 3.78 (s, 3H, CH_3), 4.07 (m, 1H, CH), 4.78 (d, J = 13 Hz, 1H, CH_2), 4.86 (m, 1H, CH), 4.93 (d, J = 13 Hz, 1H, CH_2), 5.72 (s, 2H, NH_2), 6.74 (s, 1H, CH), 7.08 (d, J = 8 Hz, 2H, CH), 7.23-7.42 (m, 5H, CH), 7.52 (d, J = 8 Hz, 2H, CH), 7.83 (d, J = 9 Hz, 1H, NH), 8.02 (d, J = 8 Hz, 1H, NH), 9.57 (s, 1H, OH); IR (KBr): ν = 3660-2700 cm^{-1} (br., N-H and O-H), 1745 cm^{-1} (C=O), 1640 cm^{-1} (C=N), 1625 cm^{-1} (C=O), 1310 and 1178 cm^{-1} (SO_2N); MS (FAB): m/z (%) = 708 (100) [$M + H^+$], 623 (11) [$M + H^+$ - piperidine], 213 (20).

***N*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-*L*-aspartic acid Mtr-Asp (23a).** To a suspension of *L*-(+)-aspartic acid **22** (310 g, 2.33 mol) in DMF (2.4 L) is added dropwise at 5 °C within 20 min ice-water (2.4 L), followed within 10 min by iPr_2NH (1.67 kg, 12.9 mol). The suspension is stirred 10 min at 0 °C. Mtr chloride **14** (580 g, 2.32 mol) is added at once and the mixture is stirred 4 h at 0 °C. A small amount of undissolved solid is removed by filtration. The filtrate is concentrated *in vacuo* (~30 mbar) at 40-45 °C bath temperature. Ice-water (6.0 L) is added to the viscous, pale-yellow oil (1.93 kg) and the pH is adjusted to 1.5 with 2 *N* HCl (800 mL). The mixture is extracted with MTB (4 x 2.5 L). The combined extracts are washed with water (1 L) and dried ($MgSO_4$). The solvent is evaporated *in vacuo* and the solid foam is dried for 3 d in HV (773 g, 96 % yield). HPLC (250 x 4.0 mm Nucleosil 120 C18 7μm, eluent: 1600 mL H_2O, 900 mL CH_3CN, 5.5 g $NH_4H_2PO_4$, adjusted to pH 3.5 with H_3PO_4, 1.0 mL/min, 20°C, det. 250 nm, t_{ret} 4.54 min) indicates 94.4 % purity; ^{1}H NMR indicates a solvent content of 32 mol-% (68 % **23a**, 18 % MTB, 14 % DMF). The product content of crude **23a** is thus 668 g (83 % corrected yield); ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 2.07 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.52 (AB part of ABX system, ^2J not readable , hidden by resonances of

[D_6]DMSO, ^3J = 7 Hz, 2H, CH_2), 2.57 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 3.92 (qua, J = 7-8 Hz, 1H, CH), 6.77 (s, 1H, CH), 7.80 (br d, J = 8 Hz, 1H, NH), 12.54 (br s, 2H, CO_2H); IR ($CHCl_3$): ν = 3680 - 2340 cm^{-1} (br, N-H and O-H), 1750 cm^{-1} (C=O), 1588 and 1564 cm^{-1} (aromatic C=C). Pure **23a** is obtained by acidification of cyclohexylammonium salt **23b**:

1 *N* H_2SO_4 (3.7 L) is added dropwise at 10 °C within 1 h to the suspension of **23b** (925 g, 1.70 mol) in CH_2Cl_2 (9.0 L) to give a slightly turbid two-phase solution. The organic layer is washed with 1 *N* H_2SO_4 (5 x 1 L) and with water (0.5 L), dried ($MgSO_4$), filtered, and the solvent is evaporated *in vacuo* to furnish **23a** as colourless crystals (540 g, 92 % yield based on **23b**, 76 % yield based on **22**), M.p. 90-92 °C decomp., purity (HPLC): 98.6 %. ^{1}H NMR data correspond to that of crude **23a**, however the α-H resonance is now a triplet (δ = 3.90, J = 7 Hz), and the NH resonance is a broad singlet (δ = 7.78).

Bis-cyclohexylammonium [*N*-(4-methoxy-2,3,5-trimethylphenylsulfonyl)-*L*-aspartate] (23b). Crude dicarboxylic acid **23a** (773 g, content of **23a** : 1.93 mol) is dissolved in acetone (10 L). Cyclohexylamine (600 mL, 5.25 mol) is added dropwise at 0-5 °C within 15 min. The suspension is stirred for 30 min at this temperature. The precipitate is suction-filtered, washed with cold acetone (3 L) and dried *in vacuo* to furnish colourless crystals (1.05 kg, 100 % yield for precipitation, 83 % yield based on **22**); M.p. 203-204 °C decomp.; HPLC (conditions as given for **23a**): 97 %; ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 0.94-1.90 (m, 22H, CH_2 and CH), 2.09 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64-2.80 (m, 2H, CH_2), 3.17 (dd, J = 8 and 6 Hz, 1H, CH), 3.83 (s, 3H, CH_3), 5.97 (br s, 7H, NH), 6.82 (s, 1H, CH); IR (KBr): ν = 3600 - 2300 cm^{-1} (N-H and NH_3^+), 1635 cm^{-1} (CO_2^-), 1560 cm^{-1} (aromatic C=C), 1390 cm^{-1} , 1308 and 1178 cm^{-1} (SO_2N); $C_{26}H_{45}N_3SO_7$ (543.7): calcd C 57.43, H 8.34, N 7.73, S 5.90; found C 57.00, H 8.20, N 7.85 ,S 6.30.

3-(*S*)-[*N*-(4-Methoxy-2,3,5-trimethylphenylsulfonyl)-amino]-tetrahydrofuran-2,5-dione (24). Thionyl chloride (366 g, 3.07 mol) is added dropwise at 0 °C (cooling bath -15 °C) within 30 min to a solution of purified **23a** (104 g, 0.30 mol) in EtOAc (1.3 L). The cooling bath is then removed and the mixture is allowed to slowly (1.5 h) warm up to 20 °C and to remain at this temperature for 45 min. All volatiles are removed *in vacuo* (5 mbar, bath 30 °C). The solid residue is dried in HV for 1 h (89.2 g). It is stirred for 1 h in $i$$Pr_2O$ (1.0 L), filtered, and dried in HV to furnish pale-grey crystals (85.4 g, 87 % yield), M.p. 172-175 °C, 180-183 °C decomp.; $[\alpha]_D^{25}$ = +36.0 (c = 1.0 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.16 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.02 (dd, J = 19 and 9 Hz, 1H, CH_2), 3.26 (dd, J = 19 and 10 Hz, 1H, CH_2), 3.85 (s, 3H, OCH_3), 4.37 (ddd, J = 10 ,9 and 4 Hz, 1H, CH), 5.41 (d, J = 4 Hz, 1H, NH), 6.62 (s, 1H, CH); IR (KBr): ν = 3356 cm^{-1} (br, N-H), 1869 cm^{-1} (C=O), 1792 / 1780 cm^{-1} (C=O), 1310 and 1178 cm^{-1} (SO_2N), 918 cm^{-1} (C-O-C); MS (FAB, DMF / NPO): *m/z* (%) = 346 (40) [M + H_2O + H^+], 327 (92) [M^+], 300 (16) [M^+ - CO + H^+], 213 (100) [Mtr^+]; $C_{14}H_{17}NO_6S$ (327.3): calcd C 51.37, H 5.23, N 4.28, S 9.79; found C 51.50, H 5.20, N 4.20, S 9.75.

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This paper is dedicated to Prof. Richard Neidlein on the occasion of his 65th birthday.

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12. The impurity (1%) is the regioisomeric α-cyclohexylammonium 2-methoxy-3,4,6-trimethylsulfonyl-Asp-β-Bzl , due to 3-4% of 2-methoxy-3,4,6-trimethylphenylsulfonyl chloride contained in commercial Mtr chloride **14**.
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14. The hydrogenolysis of **17** must not be conducted in alcoholic solvents. For the precipitation of hydrochloride **2** from MTB, crude **2** must not be dissolved in alcoholic solutions (MeOH, EtOH, *i*PrOH) of HCl. For the precipitation of hydrochloride **17** from di*iso*propyl ether, the inner salt of **17** must not be dissolved in a solution of HCl in EtOH or MeOH (instead of *i*PrOH), since under all these conditions there is considerable (5-15%) esterification of the carboxyl group of the resulting **2**. Obviously, the amidinium- and (or) the sulfonamido-group present in **2** and **17** efficiently catalyses esterification and transesterification, respectively.
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The Synthesis of Polyamide Nucleic Acids using a Novel Monomethoxytrityl Protecting-Group Strategy

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Abstract: The preparation of novel monomethoxytrityl (Mmt) protected monomers for the synthesis of polyamide nucleic acids (PNAs) is described. The use of base-labile acyl-type nucleobase protecting groups and of a succinyl-linked solid-support offers a synthetic strategy similar to standard oligonucleotide synthesis conditions. This strategy has been successfully applied for the synthesis of PNAs of mixed base sequence.

In 1991, Nielsen *et. al* [1] developed a new class of oligonucleotide analogues, known as Polyamide (or Peptide) Nucleic Acids (PNAs). These are oligomers of nucleobase-derivatized N-(2-aminoethyl)glycine which recognize and bind strongly to specific DNA or RNA sequences. PNA oligomers have a number of properties which make them potentially extremely useful as antisense therapeutics and as diagnostic tools.[2]

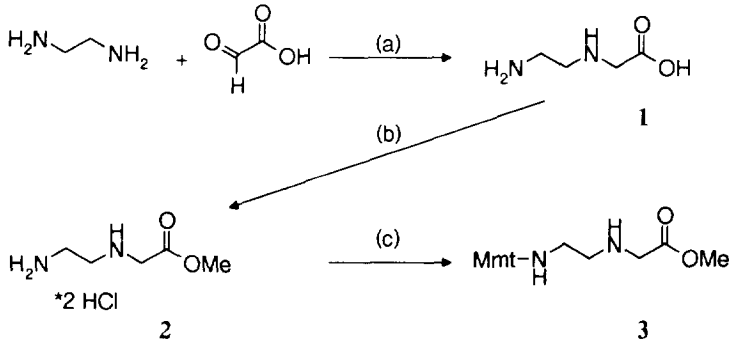
Until recently the only reported synthetic strategy for PNA synthesis was Merrifield solid-phase synthesis using a Boc/benzyloxycarbonyl protecting group strategy.[3,4,5] The repeated treatment with TFA required for Boc deprotection, and the harsh HF or TFMSA treatment required for cleavage from the resin and deprotection render this strategy incompatible with the synthesis of many types of modified PNAs, especially the synthesis of PNA-DNA chimerae due to the sensitivity of DNA to strong acids. Very recently Thomson *et al.* reported the synthesis of Fmoc/ benzyloxycarbonyl protected PNA monomers and outlined methods for their oligomerisation.[6] This Fmoc strategy may be combined with Fmoc peptide synthesis to allow the preparation of PNA-peptide conjugates. However, using this method, a strong acid deprotection step is also required at the end of synthesis.

In search of an alternative strategy which would open the way to a combination of PNA and oligonucleotide synthesis we have developed novel PNA monomers with orthogonal protecting groups in which the Mmt group is used as an N-terminal temporary protecting group and the exocyclic amino functions of the nucleobases are protected by base-labile acyl protecting groups. The Mmt group can be removed under mild acidic conditions (3% trichloroacetic acid), and the nucleobase protecting groups are removed at the end of synthesis using conc. aqueous ammonia. A solid support suitable for use in this synthetic strategy has also been synthesized.

RESULTS AND DISCUSSION

Monomer Synthesis

For the large-scale preparation of N-(2-aminoethyl)glycine **1** we found a very simple and effective method in the reductive amination of glyoxylic acid using an excess of 1,2-diaminoethane in alcohol/water mixtures and hydrogen with palladium on charcoal as reducing agent (Scheme 1). This procedure gives N-(2-aminoethyl)glycine in excellent purity and good yield from readily available starting materials. Methyl N-(2-aminoethyl) glycinate dihydrochloride **2** was obtained from **1** according to the literature procedure.[9] This was then monomethoxytritylated with (4-methoxyphenyl)-diphenylmethyl chloride (Mmt-Cl) in DMF / triethylamine. The Mmt group reacted predominantly on the less hindered primary amine to give **3** as the major product. The bis-Mmt by-product was easily separated by silica gel chromatography. The position of the Mmt group in **3** was confirmed by Nuclear Overhauser Effect NMR experiments.



Reagents and Conditions: (a) H_2, Pd/C, methanol; (b) Methanol/ HCl, reflux; (c) Mmt chloride/ DMF, NEt_3.

Scheme 1: Synthesis of N-(2-aminoethyl)glycine and its Mmt-protected derivative.

Carboxymethylated thymine was synthesized according to the procedure of Kosynkina *et al.*[7] The acyl-protected carboxymethyl nucleobases were synthesized according to *Scheme 2*. Thus cytosine was acylated with *tert*-butylbenzoyl chloride in DMF in the presence of triethylamine to give **4**. Originally we synthesized a benzoyl protected cytosine monomer, however the tendency of this monomer to precipitate out of solution during PNA synthesis led us to chose the more lipophilic *tert*-butylbenzoyl protecting group. **4** was then converted to its sodium salt using NaH in DMF and alkylated using methyl bromoacetate. The resulting methyl ester **5** was saponified using NaOH in dioxane-water. The pH of this reaction solution was carefully controlled to prevent hydrolysis of the N^4-protecting group. The desired product **6** was isolated by pH dependent precipitation.