Synthesis of dianionic and trianionic chiral, chelating ligands based on amino acids

Madeleine Schultz*, Jakov Kulis, Julie Murison and Genevieve W. Andrews
School of Physical and Chemical Sciences, Queensland University of Technology, 2 George St, Brisbane QLD 4001 Australia
madeleine.schultz@qut.edu.au

Abstract: The synthesis of two new families of amino acid-containing chiral ligands, based on methyliminodiacetic acid and nitrilotriacetic acid cores, has been accomplished using a simple protection, solution phase amide coupling and deprotection strategy. The amino acids glycine, leucine, aspartic acid and phenylalanine were used to demonstrate the versatility of the synthetic route, and that no epimerisation occurs. Thus, the tridentate ligands bear C_3 symmetry, while the bidentate ligands have C_1 symmetry.

Introduction: Amino acids form a cheap and ubiquitous source of chirality, and use has been made of amino acids as metal-binding components of ligands for classical coordination chemistry.[1-4] Free amino acids and derivatives thereof have also been used extensively as ligands and chiral auxiliaries in a variety of asymmetric syntheses; this field has been reviewed.[5-7] For example, chiral amino acids have been incorporated into phosphine and phosphite ligands for asymmetric catalysis.[8, 9] However, in many cases the amino acids are not incorporated into the ligand via amide bonds, and most of these ligands contain other functional groups; also, many bear the chiral amino acids remote from the metal. In this project, we sought to prepare ligands which resemble peptides in only having amide bonds as functional groups, with the goal that these ligands should be biologically compatible. The ligands are designed to bind to the metal through the deprotonated acid groups of their amino acids.

Incorporation of more than one amino acid into the ligand leads to multiple chiral centres and also to multiple charges. Our final goal is to prepare neutral complexes of divalent and trivalent metals. Thus, we require dianionic and trianionic ligands. For dianionic ligands, IDA (iminodiacetic acid), which resembles glycine, was selected as a basis for the core of the ligands (Figure 1A); however, the NH bond can participate in peptide coupling and so the methyl substituted MIDA (methyliminodiacetic acid) (Figure 1B) was prepared. MIDA structurally resembles half an EDTA molecule and was reported 60 years ago.[10] The chelate NTA (nitrilotriacetic acid) (Figure 1C) was selected as the core for trianionic ligands, because it is extremely cheap, readily available, and also structurally resembles

glycine. Thus, amino acids protected at their C-terminus can be coupled to MIDA and NTA. We have adopted the nomenclature A-[X(OY)]$_n$ where A is NTA or MIDA, X is the amino acid, Y is the protecting group or H for the free acid, and n = 2 or 3 for MIDA and NTA, respectively.

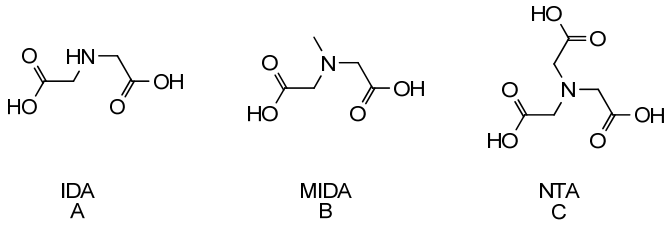


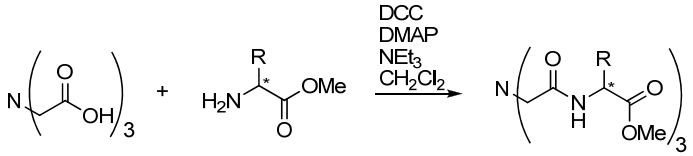
Figure 1. Cores of ligands synthesised.

IDA has previously been used as the core of small peptide mimics with several different groups as the third substituent on the central nitrogen atom, such as oxycarbonyls.[11, 12] However, until now MIDA has not been used as a ligand core. NTA has previously been used as the core of some C_3 symmetric ligands based on a similar synthetic procedure to that reported here, with further synthetic steps to form oxazolines[13-15] or to reduce the amide groups to amines.[16] A NTA-based methyl protected ester of valine, very similar to some of the compounds prepared in this work, NTA-[Val(OMe)]$_3$, has been reported as an intermediate *en route* to the corresponding oxazoline;[13] the phenylalanine and alanine analogues appear in a PhD thesis.[15] Other C_3 symmetric tripodal peptide bundles have been prepared, using ammonia as the core,[17] and used as metalloenzyme mimics.[18] The current interest in C_3 symmetric compounds as biomimetic ligands and catalysts "inspired by nature" has been reviewed.[19] Achiral cryptands have recently been reported using NTA in Ugi-type multiple multicomponent macrocyclizations.[20, 21] However, simple amino acid derivatives such as those reported here have not been used as ligands. The protected ligands and the acid ligands described here are previously unreported, although the achiral NTA-[Gly(OH)]$_3$ has been mentioned in a 1992 patent without any characterisation data.[22]

In order to allow access to a wide range of amino acid-containing ligands, solution phase coupling of the free carboxylic acids of the cores MIDA and NTA with C-protected amino acids was adopted. Both methyl and benzyl protected esters were used in coupling reactions with NTA, and benzyl esters were coupled to MIDA. Standard coupling conditions from solid phase peptide synthesis were adapted for the coupling reactions. The protected esters were fully characterised and deprotected by standard techniques to form the free di- and tri-acids, which have also been fully characterised. Our synthesis

has the advantages of using only cheap and safe starting materials, giving access to a small library of di- and trianionic chiral ligands, and was conducted by undergraduate students.

Results and Discussion: Typical solution phase peptide coupling conditions (DCC or HBTU, DMAP, NEt_3, CH_2Cl_2 solution, overnight, room temperature) were successfully used to couple the methyl and benzyl C-protected amino acids to the carboxylic acids of NTA and MIDA (Scheme 1). Use of DCC as a coupling agent was successful for the methyl esters; however, for the benzyl esters HBTU was adopted because DCC coupling led to low yields. This is presumably due to the steric bulk of the benzyl ester, limiting access of the N-terminus of the amino acid to the activated ester for coupling.



(1)

(2)

(3)

Scheme 1. Solution phase coupling to form protected ligands (1) NTA-$[X(OMe)]_3$; (2) MIDA-$[X(OBz)]_2$; (3) NTA-$[X(OBz)]_3$. R = H (Gly); CH_2CHMe_2 (Leu); CH_2Ph (Phe); CH_2COOMe (AspOMe).

The protected products, other than NTA-$[Gly(OMe)]_3$, are soluble in organic solvents such as CH_2Cl_2, and were purified by silica gel chromatography and characterised by NMR and IR spectroscopy and high resolution mass spectrometry. The ^1H NMR spectra of these compounds show well-resolved coupling constants and all resonances can be unambiguously assigned using a combination of 1 and 2D spectra; Figure 2 shows a typical example. This indicates that no epimerisation occurs under the coupling conditions. This is to be expected using conditions adapted from solid phase peptide synthesis. Methyl-protected D,L phenylalanine was used in one reaction to verify that an epimerised

amino acid leads to a mixture of diastereomers after coupling. In that case the ^{1}H NMR spectrum of the resulting NTA-[Phe(OMe)]$_3$ was very complicated and the coupling constants were not resolved.

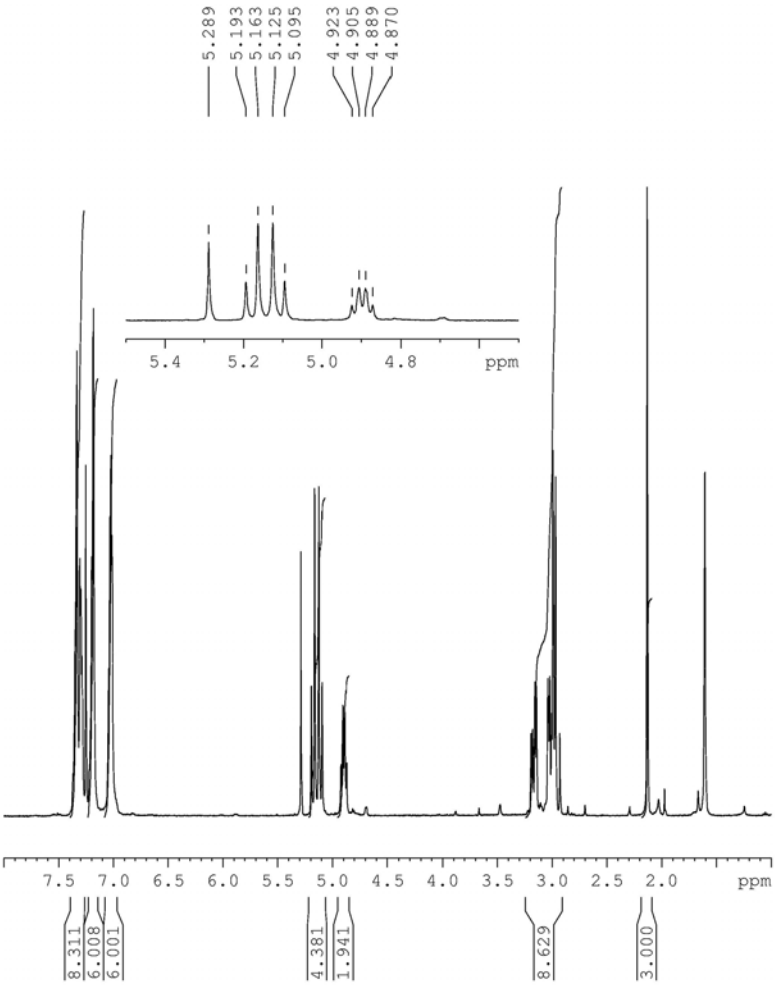
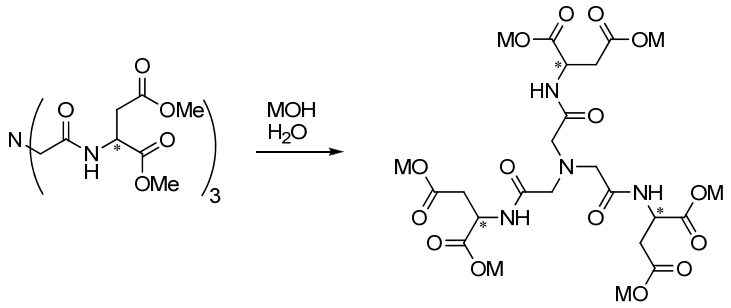


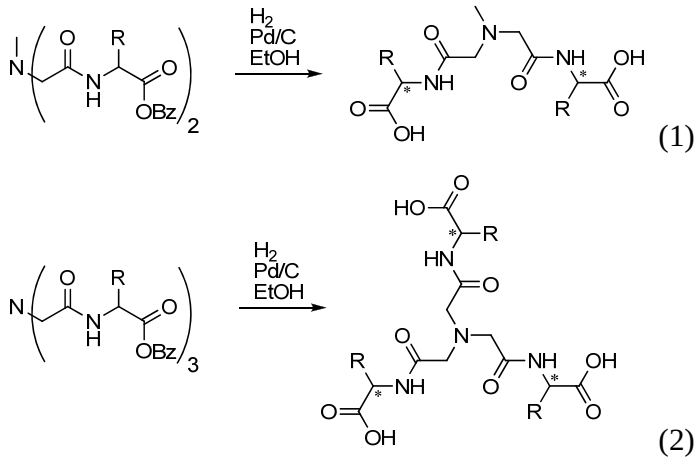
Figure 2. ^{1}H NMR spectrum of NTA-[Leu(OBz)]$_3$ in $CDCl_3$. The inset shows the resolved coupling of the benzylic protons and the α-proton of the amino acid. The resonance at 5.29 is residual CH_2Cl_2.

Deprotection of the six methyl esters of the protected NTA-[Asp(OMe)(OMe)]$_3$ was achieved using 1.0M NaOH or LiOH solution (Scheme 2) to form the alkali metal salt of the C_3 ligand. These salts were not suitable for use in metal complexation studies with higher valent metals because the alkali metals were difficult to remove. Thus, the methyl esters were not pursued further.



Scheme 2. Deprotection of NTA-[Asp(OMe)(OMe)]$_3$ with NaOH or LiOH (M = Na or Li).

The benzyl esters were removed by hydrogenolysis over Pd/C (Scheme 3) in ethanol; the protected phenyl alanine derivatives MIDA-[Phe(OBz)]$_2$ and NTA-[Phe(OBz)]$_3$ are only sparingly soluble in ethanol but this did not affect the reaction. The solubility of the products of hydrogenolysis is substantially different from the protected compounds; none are soluble in CH_2Cl_2, and all are somewhat soluble in water. The glycine derivatives MIDA-[Gly(OH)]$_2$ and NTA-[Gly(OH)]$_3$ are extremely hygroscopic and could only be isolated as powders when not exposed to air; upon air exposure, they became sticky oils.



Scheme 3. Deprotection of benzyl protected ligands by hydrogenolysis to form free acids (1) MIDA-[X(OH)]$_2$; (2) NTA-[X(OH)]$_3$.

The NMR spectra of the free acids were recorded in D_2O, acetone-d^6 or DMSO-d^6. The resolved coupling constants observed in the ^{1}H NMR spectra indicate that no epimerisation occurred during hydrogenolysis, so the newly prepared ligands have C_3 and C_1 symmetry for the NTA and MIDA cores, respectively.

Conclusion: We have demonstrated a cheap, simple and reliable route to the synthesis of a small library of chiral C_1 dianionic and C_3 trianionic chelating ligands, based on MIDA and NTA cores respectively, with amino acid substituents. Benzyl esters were the most suitable protecting groups for the amino acids because upon deprotection, the free acids are formed. The metal complexation behaviour and acidity of these ligands are currently under investigation.

Experimental Section:

General:

HBTU, DCC, Leu(OBz).TsOH and Phe(OBz).HCl were purchased from NovaBioChem. All other chemicals and solvents including dimethylaminopyridine (DMAP), triethylamine (NEt_3) and palladium over carbon (Pd/C) were purchased from Aldrich. Gly(OBz).TsOH was prepared according to the reported method using BzOH and TsOH in C_6H_6.[23] The hydrochloric acid salts of the methyl protected amino acids Gly(OMe).HCl, Leu(OMe).HCl, Phe(OMe).HCl and Asp(OMe)(OMe).HCl were prepared from the free amino acids by stirring overnight at room temperature in methanol with two equivalents of thionyl chloride, followed by removal of all volatile material under vacuum. Silica gel 60, 0.04 - 0.06 mm (230-400 mesh) from Scharlau was used for flash column chromatography. Eluents were optimised by TLC on silica gel 60 F_{254} sheets from Merck. A PMA in EtOH dip followed by charring with a hot air gun was used to visualise TLC plates. NMR spectra were collected on Bruker AMX-300, DRX-400 and DRX-500 instruments at room temperature in the solvent specified and are referenced to residual protons of the proteosolvent for ^1H spectra, or to the carbon of the solvent for $^{13}C\{^1H\}$ spectra. Assignments are based on a combination of 1 and 2D spectra. Infrared spectra were collected on a Perkin-Elmer 1600 or 1000 FTIR using CsF plates or in solution as specified, and only carbonyl stretches are reported. High resolution mass measurements were obtained from a Finnigan MAT 900 XL-Trap electrospray (ESI) mass spectrometer with a Finnigan API III electrospray source. Low resolution ESI mass spectrometry was conducted on a Quattro II triple-quadruple liquid chromatography-mass spectrometer (Micromass, Manchester, UK) or a Finnigan MAT 900 XL-Trap electrospray mass spectrometer with a Finnigan API III electrospray source.

Synthesis of MIDA (modification of a reported technique[10]):

Iminodiacetic acid (6.65 g, 0.05 mol) was weighed into a round-bottomed flask equipped with a magnetic stirrer bar. Water (6.7 mL, 0.38 mol), formic acid (3.8 mL, 0.10 mol) and formaldehyde (7.5 mL, 0.27 mol) were added and the mixture was stirred overnight at reflux. Ethanol (50 mL) was added

to the resulting pale yellow solution, leading to the formation of a white precipitate, which was collected by filtration and washed with ethanol. Yield: 4.18 g (57%).

^{1}H NMR (500 MHz, 298 K, D_2O): 3.83 (s, 4H, MeNC$\underline{H}_2$), 2.87 (s, 3H, N$\underline{Me}$) (carboxylic acid protons not observed).

^{13}C{^{1}H} NMR (125 MHz, 298 K, D_2O): 169.1 ($\underline{C}$OOH), 57.3 (MeN$\underline{C}H_2$), 42.4 (N$\underline{Me}$).

Typical conditions for coupling benzyl-protected amino acids to MIDA:

MIDA (1.10 g, 6.8 mmol) and HBTU (5.69 g, 15 mmol, 2.2 equiv) were weighed into a flask equipped with a magnetic stirrer bar and dichloromethane (50 mL) was added, followed by DMAP (0.138 g, 1.5 mmol, 0.22 equiv), NEt_3 (4.17 mL, 30 mmol, 4.4 equiv) and the benzyl ester of the amino acid to be coupled (15 mmol, 2.2 equiv). The mixture was stirred overnight at room temperature, after which it was a yellow solution. If a solid precipitated, this was removed by filtration. The product was purified using silica gel chromatography (CH_2Cl_2:MeOH 96:4). Yield: 45-60% after chromatography. The later column fractions were sometimes contaminated with tetramethylurea, a byproduct of coupling by HBTU.

$Me_2NCONMe_2$: ^{1}H NMR (500 MHz, 298 K, $CDCl_3$): 2.71 (s, N$\underline{Me}_2$). ^{13}C{^{1}H} NMR (126 MHz, 298 K, $CDCl_3$): 165.4 ($\underline{C}$O), 38.3 (N$\underline{Me}_2$).

MIDA-[Gly(OBz)]$_2$:

IR (CsF): 1747, 1660 cm^{-1}.

^{1}H NMR (400 MHz, 298 K, $CDCl_3$): 7.40 (t, 2H, $^{3}J_{HH}$ = 5.8 Hz, N$\underline{H}$), 7.33 - 7.30 (m, 10H, $CH_2C_6\underline{H}_5$), 5.12 (4H, C$\underline{H}_2C_6H_5$), 4.09 (d, 4H, $^{3}J_{HH}$ = 5.8 Hz, NHC$\underline{H}_2$), 3.17 (s, 4H, MeNC$\underline{H}_2$), 2.44 (s, 3H, N$\underline{Me}$).

^{13}C{^{1}H} NMR (101 MHz, 298 K, $CDCl_3$): 170.3 ($\underline{C}$O), 170.0 ($\underline{C}$O), 135.0 (*ipso*-C of C_6H_5), 128.6, 128.5, 128.3 (*ortho*, *meta*, *para*-C of C_6H_5), 67.2 ($\underline{C}H_2C_6H_5$), 61.2 (MeN$\underline{C}H_2$), 43.9 (N$\underline{Me}$), 40.8 (NH$\underline{C}$H).

M/S (ESI+): *m/z* 480 [M+K], 464 [M+Na], 442 [M+H].

HRESI-MS: *m/z* Calculated for $C_{23}H_{28}N_3O_6$ 442.1973 [M+H]; found 442.1980.

MIDA-[Leu(OBz)]$_2$:

^{1}H NMR (500 MHz, 298 K, $CDCl_3$): 7.28 (m, 10H, Bz), 7.20 (br d, 2H, $^{3}J_{HH}$ = 8.6 Hz, N$\underline{H}$), 5.12 (d, 2H, $^{2}J_{HH}$ = 27 Hz, C$\underline{H}_2$Ph), 5.07 (d, 2H, $^{2}J_{HH}$ = 27 Hz, C$\underline{H}_2$Ph), 4.64 (m, 2H, NHC$\underline{H}$), 3.15 (d, 2H, $^{2}J_{HH}$ = 15 Hz, MeNC$\underline{H}_2$), 3.02 (d, 2H, $^{2}J_{HH}$ = 15 Hz, MeNC$\underline{H}_2$), 2.36 (s, 3H, N$\underline{Me}$), 1.61 (m, 2H, C$\underline{H}$Me$_2$), 1.55 (m, 4H, CHC$\underline{H}_2$CH), 0.87 (d, 6H, $^{3}J_{HH}$ = 1.7 Hz, CH$\underline{Me}$), 0.86 (d, 6H, $^{3}J_{HH}$ = 1.7 Hz, CH$\underline{Me}$).

$^{13}C\{^{1}H\}$ NMR (125 MHz, 298 K, $CDCl_3$): 172.6 (COOCH$_2$Ph), 169.7 (CONH), 135.1 (*ipso*-C of C_6H_5), 128.1, 127.9, 127.7 (*ortho*, *meta*, *para*-C of C_6H_5), 66.5 ($CH_2C_6H_5$), 60.7 (MeNCH$_2$), 50.1 (NHCH), 43.2 (NMe), 40.4 ($CHCH_2CH$), 24.5 ($CHMe_2$), 22.5 (CHMe), 21.2 (CHMe).
M/S (ESI+): *m/z* 1129 [2M+Na], 576 [M+Na], 554 [M+H].
HRESI-MS: *m/z* Calculated for $C_{31}H_{43}N_3O_6Na$: 576.3050 [M+Na]; found 576.3047.

MIDA-[Phe(OBz)]$_2$:
IR (CsF): 1741, 1663 cm^{-1}.
^{1}H NMR (400 MHz, 298 K, $CDCl_3$): 7.33 - 7.01 (m, 22H, Ph and NH), 5.17 (d, 2H, $^{2}J_{HH}$ = 12 Hz, OCH$_2$Ph), 5.11 (d, 2H, $^{2}J_{HH}$ = 12 Hz, OCH$_2$Ph), 4.90 (m, 2H, NHCH), 3.19 - 2.92 (m, 8H MeNCH$_2$ and CHCH$_2$Ph), 2.13 (s, 3H, NMe).
$^{13}C\{^{1}H\}$ NMR (101 MHz, 298 K, $CDCl_3$): 171.6 (COOCH$_2$Ph), 169.4 (CONH), 135.9 and 135.1 (*ipso*-C of C_6H_5), 129.1, 128.6, 128.6, 128.5, 128.5, 127.0 (*ortho*, *meta*, *para*-C of C_6H_5), 67.3 ($OCH_2C_6H_5$), 61.2 (MeNCH$_2$), 52.8 (NHCH), 43.6 (NMe), 37.6 ($CHCH_2Ph$).
M/S (ESI+): *m/z* 622.4 [M+H].
HRESI-MS: *m/z* Calculated for $C_{37}H_{39}N_3O_6Na$: 644.2731 [M+Na]; found 644.2729.

Typical coupling conditions of benzyl esters to NTA:
The solids NTA (0.15 g, 0.76 mmol), and HBTU (0.96 g, 2.54 mmol. 3.3 equiv) were placed in a flask equipped with a magnetic stirrer bar. Dichloromethane (50 mL) was added, followed by DMAP (0.062 g, 0.51 mmol, 0.67 equiv), NEt_3 (1.42 mL, 10.2 mmol, 13 equiv) and the benzyl protected amino acid (2.54 mmol, 3.3 equiv). The mixture was stirred overnight at room temperature, then washed with dilute HCl (1.0 M) and $NaHCO_3$ (1.0 M) followed by distilled water. The product was purified by silica gel chromatography (CH_2Cl_2:MeOH 96:4). Yield: 40 - 60% after chromatography. The later column fractions were sometimes contaminated with tetramethylurea, a byproduct of coupling by HBTU.

NTA-[Gly(OBz)]$_3$:
^{1}H NMR (300 MHz, 298 K, $CDCl_3$): 7.67 (t, 3H, $^{3}J_{HH}$ = 6.0 Hz, NH), 7.31 (m, 15H, $CH_2C_6H_5$), 5.12 (s, 6H, $CH_2C_6H_5$), 4.06 (d, 6H, $^{3}J_{HH}$ = 6.0 Hz, NHCH$_2$), 3.37 (s, 6H, NCH$_2$).
$^{13}C\{^{1}H\}$ NMR (125 MHz, 298 K, $CDCl_3$): 170.5 (NHCO and COOBz), 135.1 (*ipso*-C of C_6H_5), 128.7, 128.6, 128.3 (*ortho*, *meta*, *para*-C of C_6H_5), 67.3 ($CH_2C_6H_5$), 58.6 (NCH$_2$), 41.1 (NHCH$_2$).
M/S (ESI+): *m/z* 655 [M+Na].

HRESI-MS: *m/z* Calculated for $C_{33}H_{36}N_4O_9Na$: 655.2380 [M+Na]; found 655.2371.

NTA-[Leu(OBz)]$_3$:

IR (CsF): 1746, 1666 cm^{-1}.

^{1}H NMR (300 MHz, 298 K, $CDCl_3$): 7.77 (d, 3H, $^3J_{HH}$ = 8.4 Hz, NH), 7.35 (m, 15H, $CH_2C_6H_5$), 5.20 (d, 3H, $^2J_{HH}$ = 12 Hz, CH_2Ph), 5.11 (d, 3H, $^2J_{HH}$ = 12 Hz, CH_2Ph), 4.65 (m, 3H, NHCH), 3.41 (d, 3H, $^2J_{HH}$ = 15 Hz, NCH_2), 3.25 (d, 3H, $^2J_{HH}$ = 15 Hz, NCH_2), 1.73 - 1.57 (m, 9H, $CHMe_2$ and $CHCH_2CH$), 0.92 (d, 9H, $^3J_{HH}$ = 3.4 Hz, CHMe), 0.90 (d, 9H, $^3J_{HH}$ = 3.4 Hz, CHMe).

^{13}C{^{1}H} NMR (125 MHz, 298 K, $CDCl_3$): 174.7 (NHCO and COOBz), 140.1 (*ipso*-C of C_6H_5), 128.7, 127.8, 127.4 (*ortho*, *meta*, *para*-C of C_6H_5), 63.8 ($CH_2C_6H_5$), 58.5 (NCH_2), 52.9 (NHCH), 39.6 ($CHCH_2CH$), 24.0 ($CHMe_2$), 21.8 (CHMe), 20.8 (CHMe).

M/S (ESI+): *m/z* 823 [M+Na], 801 [M+H].

HRESI-MS: *m/z* Calculated for $C_{45}H_{61}N_4O_9$: 801.4433 [M+H]; found 801.4422.

NTA-[Phe(OBz)]$_3$:

IR (CsF): 1732, 1660 cm^{-1}.

^{1}H NMR (400 MHz, 298 K, $CDCl_3$): 7.60 (d, 3H, $^3J_{HH}$ = 8.4 Hz, NH), 7.35 - 7.12 (m, 30H, C_6H_5), 5.19 (d, 3H, $^2J_{HH}$ = 12.2 Hz, OCH_2Ph), 5.13 (d, 3H, $^2J_{HH}$ = 12.2 Hz, OCH_2Ph), 4.90 (m, 3H, NHCH), 3.19 - 2.91 (m, 12H, NCH_2 and $CHCH_2$Ph).

^{13}C{^{1}H} NMR (100 MHz, 298 K, $CDCl_3$): 172.6 (COOBz), 170.1 (NHCO), 136.1 and 135.0 (*ipso*-C of C_6H_5), 129.0, 128.5, 128.4, 128.4, 128.2, 126.9 (*ortho*, *meta*, *para*-C of C_6H_5), 67.3 ($OCH_2C_6H_5$), 57.6 (NCH_2), 53.4 (NHCH), 37.3 ($CHCH_2$Ph).

M/S (ESI+): *m/z* 903 [M+H].

HRESI-MS: *m/z* Calculated for $C_{54}H_{55}N_4O_9$: 903.3964 [M+H]; found 903.3962.

Typical coupling conditions of methyl esters to NTA:

The solids NTA (0.27 g, 1.41 mmol), and DCC (1.05 g, 5.10 mmol. 3.6 equiv) were placed in a flask equipped with a magnetic stirrer bar under nitrogen. Distilled dichloromethane (50 mL) was added, followed by DMAP (0.12 g, 0.98 mmol, 0.7 equiv), triethylamine (1.29 mL, 9.27 mmol, 6.6 equiv) and the methyl protected amino acid (4.63 mmol, 3.3 equiv). The mixture was stirred under nitrogen overnight at room temperature, then filtered and washed with dilute acid followed by distilled water. The product was purified by silica gel chromatography (CH_2Cl_2:MeOH 96:4). Yield: 40 - 60% after chromatography.

NTA-[Gly(OMe)]$_3$:

^{1}H NMR (500 MHz, 298 K, $CDCl_3$): 7.77 (br t, 3H, $^3J_{HH}$ = 3.4 Hz, NH), 4.04 (d, 6H, $^3J_{HH}$ = 3.6 Hz, NHC$\underline{H}_2$), 3.71 (s, 9H, O$\underline{Me}$), 3.39 (s, 6H, NC$\underline{H}_2$).

^{1}H NMR (500 MHz, 298 K, D_2O): 3.98 (s, 6H, NHC$\underline{H}_2$), 3.67 (s, 9H, O$\underline{Me}$), 3.44 (s, 6H, NC$\underline{H}_2$).

^{13}C{^{1}H} NMR (125 MHz, 298 K, $CDCl_3$): 171.2 ($\underline{C}$OOMe), 170.9 (NH$\underline{C}$O), 58.7 (N$\underline{C}H_2$), 52.4 (COO$\underline{Me}$), 40.8 (NH$\underline{C}H_2$).

^{13}C{^{1}H} NMR (125 MHz, 298 K, D_2O): 173.1 ($\underline{C}$OOMe), 171.4 (NH$\underline{C}$O), 57.1 (N$\underline{C}H_2$), 52.3 (COO$\underline{Me}$), 40.5 (NH$\underline{C}H_2$).

M/S: *m/z* 427 [M+Na].

NTA-[Phe(OMe)]$_3$ (mixture of diastereomers) (reported in a PhD thesis[15]):

IR (CH_2Cl_2 solution): ν_{CO} = 1738, 1680 cm^{-1}.

^{1}H NMR (300 MHz, 298 K, $CDCl_3$): 7.36 - 7.08 (m, 18H, N$\underline{H}$ and $C_6\underline{H}_5$), 4.79 (m, 3H, NHC$\underline{H}$), 3.72 (s, 9H, O$\underline{Me}$), 3.25 - 2.90 (m, 12H, C$\underline{H}_2$Ph and NC$\underline{H}_2$).

M/S (ESI+): *m/z* 697 [M+Na], 675 [M+H].

HRESI-MS: *m/z* Calculated for $C_{36}H_{42}N_4O_9Na$ [M+Na]: 697.2849; Found 697.2839.

NTA-[Leu(OMe)]$_3$:

IR (MeOH solution): ν_{CO} = 1749, 1664 cm^{-1}.

^{1}H NMR (300 MHz, 298 K, $CDCl_3$): 7.71 (br d, 3H, $^3J_{HH}$ = 8.5 Hz, N$\underline{H}$), 4.60 (m, 3H, NHC$\underline{H}$), 3.71 (s, 9H O$\underline{Me}$), 3.41 (d, 3H, $^2J_{HH}$ = 15 Hz, NC$\underline{H}_2$), 3.20 (d, 3H, $^2J_{HH}$ = 15 Hz, NC$\underline{H}_2$), 1.74 - 1.56 (m, 9H, C$\underline{H}$Me$_2$ and CHC$\underline{H}_2$CH), 0.92 (d, 9H, $^3J_{HH}$ = 2.4 Hz, CH$\underline{Me}$), 0.90 (d, 9H, $^3J_{HH}$ = 2.3 Hz, CH$\underline{Me}$).

^{13}C{^{1}H} NMR (75 MHz, 298 K, $CDCl_3$): 174.9 ($\underline{C}$OOMe), 170.5 ($\underline{C}$ONH), 58.2 (N$\underline{C}H_2$), 52.4 (O$\underline{Me}$), 50.6 (NH$\underline{C}$H), 40.3 (CH$\underline{C}H_2$CH), 24.8 ($\underline{C}$HMe$_2$), 23.0 (CH$\underline{Me}$), 21.3 (CH$\underline{Me}$).

M/S (ESI+): *m/z* 1167 [2M+Na], 1145 [2M+H], 595 [M+Na], 573 [M+H].

HRESI-MS: *m/z* Calculated for $C_{27}H_{48}N_4O_9Na$ [M+Na]: 595.3319; Found 595.3306.

NTA-[Asp(OMe)(OMe)]$_3$:

IR (CH_2Cl_2 solution): ν_{CO} = 1737, 1681 cm^{-1}.

^{1}H NMR (500 MHz, 298 K, $CDCl_3$): 7.72 (d, 3H, $^3J_{HH}$ = 8.6 Hz, NH), 4.92 (m, 3H, NHC$\underline{H}$), 3.71 (s, 9H, O$\underline{Me}$), 3.66 (s, 9H, O$\underline{Me}$), 3.46 (d, 3H, $^2J_{HH}$ = 15 Hz, NC$\underline{H}_2$), 3.24 (d, 3H, $^2J_{HH}$ = 15 Hz, NC$\underline{H}_2$),

2.99 (dd, 3H, $^{2}J_{HH}$ = 17 Hz, $^{3}J_{HH}$ = 6.0 Hz, CHC<u>H</u>$_2$CO), 2.88 (dd, 3H, $^{2}J_{HH}$ = 17 Hz, $^{3}J_{HH}$ = 4.7 Hz, CHC<u>H</u>$_2$CO).

^{13}C{^{1}H} NMR (125 MHz, 298 K, $CDCl_3$): 171.5 (<u>C</u>OOMe), 171.2 (<u>C</u>OOMe), 169.8 (NH<u>C</u>O), 58.4 (NC<u>H</u>$_2$), 52.8 (O<u>Me</u>), 52.1 (O<u>Me</u>), 48.4 (NH<u>C</u><u>H</u>), 35.8 (CH<u>C</u>H$_2$CO).

M/S (ESI+): *m/z* 643 [M+Na], 621 [M+H].

HRESI-MS: *m/z* Calculated for $C_{24}H_{36}N_4O_{15}Na$ [M+Na]: 643.2075; Found 643.2082.

<u>Typical deprotection conditions for benzyl esters:</u>

A small amount of Pd/C (ca. 2 mg) was added to an ethanol (50 mL) solution or suspension of the compound (ca. 400 mg). The mixture was subjected to a hydrogen atmosphere from a balloon with stirring (NTA-[Gly(OBz)]$_3$, NTA-[Leu(OBz)]$_3$, MIDA-[Leu(OBz)]$_2$), or using a Parr hydrogenator, 30 psi were applied (NTA-[Phe(OBz)]$_3$, MIDA-[Gly(OBz)]$_2$, MIDA-[Phe(OBz)]$_2$). After stirring for 1 hour, the solution was filtered through Celite and the solvent was removed *in vacuo*. The free acids of the glycine derivatives MIDA-[Gly(OH)]$_2$ and NTA-[Gly(OH)]$_3$ are extremely hygroscopic so no yield was recorded. For the other free acids, yields were above 80%.

MIDA-[Gly(OH)]$_2$:

^{1}H NMR (300 MHz, 298 K, D_2O): 4.01 (s, 4H, C<u>H</u>$_2$COOH), 3.80 (s, 4H, MeNC<u>H</u>$_2$), 2.88 (s, 3H, N<u>Me</u>).

^{13}C{^{1}H} NMR (100 MHz, 298 K, D_2O): 175.0 (<u>C</u>OOH), 166.1 (<u>C</u>ONH), 57.6 (MeN<u>C</u>H$_2$), 43.5 (N<u>Me</u>), 42.7 (NH<u>C</u>H$_2$).

M/S (ESI+): *m/z* 561 [2M+K], 545 [2M+Na], 284 [M+Na], 262 [M+H].

HRESI-MS: *m/z* Calculated for $C_9H_{15}N_3O_6Na$: 284.0859 [M+Na]; Found: 284.0868.

Due to the hygroscopic nature of this compound, no infrared spectrum could be obtained.

MIDA-[Leu(OH)]$_2$:

^{1}H NMR (300 MHz, 298 K, D_2O): 8.49 (br d, 2H, $^{3}J_{HH}$ = 12 Hz, N<u>H</u>), 4.17 (m, 2H, NHC<u>H</u>), 3.96 (s, 4H, MeNC<u>H</u>$_2$), 2.86 (s, 3H, N<u>Me</u>), 1.49 (m, 6H, C<u>H</u>Me$_2$ and CHC<u>H</u>$_2$CH), 0.77 (d, 6H, $^{3}J_{HH}$ = 10 Hz, CH<u>Me</u>), 0.74 (d, 6H, $^{3}J_{HH}$ = 10 Hz, CH<u>Me</u>).

^{13}C{^{1}H} NMR (75 MHz, 298 K, D_2O): 177.7 (<u>C</u>OOH), 164.8 (<u>C</u>ONH), 56.8 (MeN<u>C</u>H$_2$), 52.9 (NH<u>C</u>H), 42.8 (N<u>Me</u>), 39.7 (CH<u>C</u>H$_2$CH), 24.5 (<u>C</u>HMe$_2$), 22.2 (CH<u>Me</u>), 20.6 (CH<u>Me</u>).

M/S (ESI+): *m/z* 769 [2M+Na], 747 [2M+H], 396 [M+Na], 374 [M+H].

HRESI-MS: *m/z* Calculated for $C_{17}H_{31}N_3O_6Na$: 396.2111 [M+Na]; Found: 396.2116.

MIDA-[Phe(OH)]$_2$:

IR (CsF): 1667 cm^{-1}.

^{1}H NMR (400 MHz, 298 K, DMSO-*d*^{6}): 8.25 (br, 2H, N<u>H</u>), 7.20 (m, 10H, C$_6$<u>H</u>$_5$), 4.50 (ddd, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 4.8 Hz, 2H, NHC<u>H</u>), 3.11 (dd, 2H, $^2J_{HH}$ = 13.8 Hz, $^3J_{HH}$ = 4.8 Hz, C<u>H</u>$_2$Ph), 3.01 (br, 4H, NCH$_2$), 2.91 (dd, 2H, $^2J_{HH}$ = 13.8 Hz, $^3J_{HH}$ = 9.7 Hz, C<u>H</u>$_2$Ph), 2.03 (br s, N<u>Me</u>) (COO<u>H</u> not observed).

^{13}C{^{1}H} NMR (100 MHz, 298 K, DMSO- *d*^{6}): 172.9 (<u>C</u>ONH), 169.2 (br, <u>C</u>OOH), 137.7 (*ipso*-C of <u>C</u>$_6$H$_5$), 129.2, 128.3, 126.5, (*ortho*, *meta*, *para*-C of <u>C</u>$_6$H$_5$), 59.9 (br, N<u>C</u>H$_2$), 53.2 (NH<u>C</u>H), 42.2 (NMe), 36.6 (<u>C</u>H$_2$Ph).

M/S (ESI+): *m/z* 442.2 [M+H].

HRESI-MS: *m/z* Calculated for C$_{23}$H$_{28}$N$_3$O$_6$: 442.1973 [M+H]; Found: 442.1965.

NTA-[Gly(OH)]$_3$:

IR (THF solution): 1752, 1684 cm^{-1}.

^{1}H NMR (300 MHz, 298 K, D$_2$O): 3.92 (s, 6H, NH<u>C</u>H$_2$), 3.50 (s, 6H, NC<u>H</u>$_2$) (COO<u>H</u> not observed).

M/S (ESI+): *m/z* 385 [M+Na].

NTA-[Leu(OH)]$_3$:

^{1}H NMR (500 MHz, 298 K, D$_2$O): 7.32 (m, 3H, N<u>H</u>), 4.31 (m, 3H, NHC<u>H</u>), 3.39 (s, 6H, NC<u>H</u>$_2$), 1.62 - 1.52 (m, 9H, C<u>H</u>Me$_2$ and CHC<u>H</u>$_2$CH), 0.83 (d, 9H, $^3J_{HH}$ = 3.6 Hz, CH<u>Me</u>), 0.79 (d, 9H, $^3J_{HH}$ = 3.5 Hz, CH<u>Me</u>) (COO<u>H</u> not observed).

^{13}C{^{1}H} NMR (125 MHz, 298 K, D$_2$O): 176.3 (<u>C</u>OOH), 172.7 (<u>C</u>ONH), 57.7 (N<u>C</u>H$_2$), 51.4 (NH<u>C</u>H), 39.3 (CH<u>C</u>H$_2$CH), 24.4 (<u>C</u>HMe$_2$), 22.2 (CH<u>Me</u>), 20.4 (CH<u>Me</u>).

M/S (TOF+): 531 [M+H].

NTA-[Phe(OH)]$_3$:

IR (CsF): 1659 cm^{-1}.

^{1}H NMR (400 MHz, 298 K, acetone-*d*^{6}): 8.1 (br, not integrated, N<u>H</u>), 7.23 - 7.08 (m, 15H, C$_6$<u>H</u>$_5$), 4.67 (dd, 3H, $^3J_{HH}$ = 9.6 Hz, $^3J_{HH}$ = 4.5 Hz, NHC<u>H</u>), 3.19 (dd, 3H, $^2J_{HH}$ = 13.9 Hz, $^3J_{HH}$ = 4.5 Hz, C<u>H</u>$_2$Ph), 2.91 (m, 9H, C<u>H</u>$_2$Ph and NC<u>H</u>$_2$) (COO<u>H</u> not observed).

^{1}H NMR (400 MHz, 298 K, DMSO-d^6): 8.32 (br, 3H, NH), 7.22 - 7.12 (m, 15H, C_6H_5), 4.54 (br dd, 3H, NHCH), 3.12 (dd, 3H, $^2J_{HH}$ = 13.7 Hz, $^3J_{HH}$ = 4.3 Hz, CH_2Ph), 2.87 (br, 9H, NCH_2 and CH_2Ph) (COOH not observed).

^{1}H NMR (400 MHz, 298 K, D_2O): 7.22 (m, 15H, C_6H_5), 4.65 (br dd, 3H, NHCH), 3.30 (dd, 3H, $^2J_{HH}$ = 14.2 Hz, $^3J_{HH}$ = 4.1 Hz, CH_2Ph), 2.91 (dd, 3H, $^2J_{HH}$ = 13.7 Hz, $^3J_{HH}$ = 10.3 Hz, CH_2Ph), 2.57 (d, 3H, $^2J_{HH}$ = 16.8 Hz, NCH_2), 2.51 (d, 3H, $^2J_{HH}$ = 16.1 Hz, NCH_2) (NH and COOH not observed).

^{13}C{^{1}H} NMR (100 MHz, 298 K, acetone- d^6): 174.1 (COOH), 171.1 (CONH), 138.3 (*ipso*-C of C_6H_5), 130.0, 129.1, 127.4 (*ortho*, *meta*, *para*-C of C_6H_5), 58.3 (NCH_2), 54.3 (NHCH), 37.8 (CH_2Ph).

M/S (ESI+): *m/z* 655.4 [M+Na].

HRESI-MS: *m/z* Calculated for $C_{33}H_{36}N_4O_9Na$: 655.2376 [M+Na]; Found: 655.2367.

Deprotection conditions for NTA-[Asp(OMe)(OMe)]$_3$:

0.50 g (0.81 mmol) of the methyl ester was weighed into a round bottomed flask and 10 mL of distilled water were added. The ester was not completely soluble. Six equivalents of the metal hydroxide (NaOH or LiOH) per NTA were weighed into a separate flask and dissolved in water. The base was added to the ester and the mixture was stirred overnight at room temperature. The water was removed under vacuum leaving a hygroscopic white solid which was insoluble in alcohols or THF.

NTA-[Asp(ONa)(ONa)]$_3$:

^{1}H NMR (500 MHz, 298 K, D_2O): 4.30 (dd, 3H, $^3J_{HH}$ = 9.9 Hz, $^3J_{HH}$ = 3.9 Hz, NHCH), 3.30 (s, 6H, NCH_2), 2.54 (dd, 3H, $^2J_{HH}$ = 16 Hz, $^3J_{HH}$ = 4.0 Hz, $CHCH_2$), 2.35 (dd, 3H, $^2J_{HH}$ = 16 Hz, $^3J_{HH}$ = 10 Hz, $CHCH_2$).

^{13}C{^{1}H} NMR (125 MHz, 298 K, D_2O): 178.8 (COONa), 178.5 (COONa), 172.3 (CONH), 56.6 (NCH_2), 53.0 (NHCH), 39.5 ($CHCH_2$).

Acknowledgements: JK acknowledges UniChe for a summer scholarship. We thank Dr Joanne Blanchfield for helpful discussions.

References:

[1] J. Balla, P. V. Bernhardt, P. Buglyo, P. Comba, T. W. Hambley, R. Schmidlin, et al., *J. Chem. Soc., Dalton Trans.* **1993**, 1143-1149.
[2] R. Marchelli, E. Dradi, A. Dossena, G. Casnati, *Tetrahedron* **1982,** *38*, 2061-2067.
[3] S. Utsuno, H. Miyamae, S. Horikoshi, I. Endo, *Inorg. Chem.* **1985,** *24*, 1348-1354.
[4] B. Dangel, M. Clarke, J. Haley, D. Sames, R. Polt, *J. Am. Chem. Soc.* **1997,** *119*, 10865-10866.

[5] A. Mori, H. Abe, S. Inoue, *Appl. Organomet. Chem.* **1995,** *9*, 189-197.
[6] M. Falorni, *Trends Org. Chem.* **1993,** *4*, 351-369.
[7] A. Mortreux, F. Petit, G. Buono, G. Peiffer, *Bull. Chem. Soc. Fr.* **1987**, 631-639.
[8] D. Jayasinghe, H. B. Kraatz, in *Methodologies in Asymmetric Catalysis*, **2004,** pp 175-187 (ACS: Washington, DC).
[9] L. Eberhardt, D. Armspach, D. Matt, L. Toupet, B. Oswald, *Eur. J. Inorg. Chem.* **2007**, 4153-4161.
[10] A. F. Childs, L. J. Goldsworthy, G. F. Harding, F. E. King, A. W. Nineham, W. L. Norris, et al., *J. Chem. Soc.* **1948**, 2174-2177.
[11] S. N. Khattab, A. El-Faham, A. M. El-Massry, E. M. E. Mansour, M. M. Abd El-Rahman, *Lett. Pep. Sci.* **2001,** *7*, 331-345.
[12] C. Abalain, M. Langlois, *Eur. J. Med. Chem.* **1998,** *33*, 155-160.
[13] T. H. Chan, G. Z. Zheng, *Can. J. Chem.* **1997,** *75*, 629-633.
[14] K. Kawasaki, S. Tsumura, T. Katsuki, *Synlett* **1995**, 1245-1246.
[15] M. A. C. Campos, PhD thesis, Eindhoven: Technische Universiteit Eindhoven; **2005**.
[16] X. Liu, P. Ilankumaran, I. A. Guzei, J. G. Verkade, *J. Org. Chem.* **2000,** *65*, 701-706.
[17] G. Trojandt, K. Polborn, W. Steglich, *Tetrahedron Lett.* **1995,** *36*, 857-860.
[18] U. Herr, W. Spahl, G. Trojandt, W. Steglich, F. Thaler, R. van Eldik, *Bioorg. Med. Chem.* **1999,** *7*, 699-707.
[19] S. E. Gibson, M. P. Castaldi, *Angew. Chem., Int. Ed. Engl.* **2006,** *45*, 4718-4720.
[20] D. G. Rivera, L. A. Wessjohann, *J. Am. Chem. Soc.* **2006,** *128*, 7122-7123.
[21] D. G. Rivera, O. E. Vercillo, L. A. Wessjohann, *Synlett* **2007**, 308-312.
[22] K. W. Chiu, W. Hussain, J. R. Thornback, *EP19910401697* **1992**.
[23] M. Bodanszky, A. Bodanszky, *The Practice of Peptide Synthesis*. **1984** (Springer Verlag: Berlin).