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Stereocontrolled Backbone Connection of Peptides by C═C-Double Bonds

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Dedicated to Professor Rolf Huisgen on the occasion of his 80th birthday

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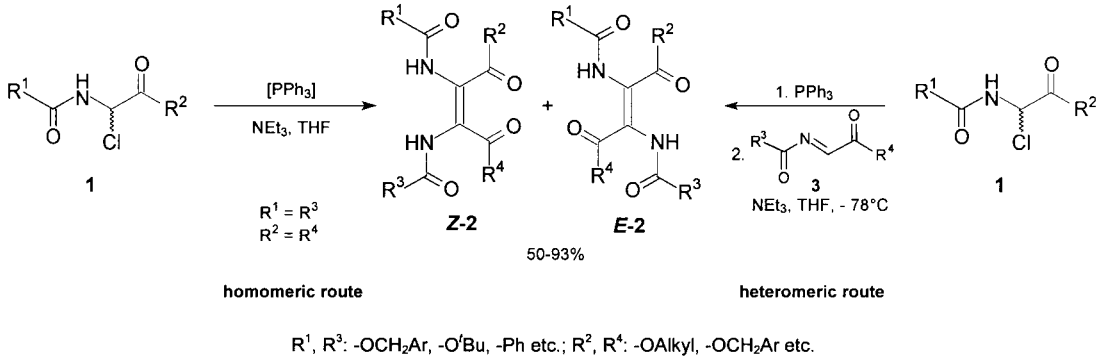
Abstract—Treatment of peptides containing an α-chloroglycine residue with triethylamine and catalytic amounts of triphenylphosphine constitutes an efficient method for the stereoselective synthesis of amino acid and peptide dimers bridged by a C═C-double bond. The dimers can be converted into novel peptide structures by standard methods of peptide synthesis.

The disulfide bridge is the most common cross-link found in proteins formed by oxidative coupling of two cysteine residues. This reaction is important in stabilising the three-dimensional folding of the backbone chain in proteins. Partially rigidified structures can also result from amide respectively ester linkage of glutamic acid residues with lysine or serine moieties or by oxidative phenolic coupling of tyrosine units.[1] In addition, bridging by diamino dicarboxylic acids like lanthionine and *meso*-1,6-diaminopimelic acid is observed in several biological active peptides.[2,3] Recently, a number of bridged peptides have been synthesised to study their importance to conformational constraints.[4,5]

Much less is known about peptides cross-linked at backbone positions.[6,7] As part of our ongoing investigations on the modification of oligopeptides,[8] we describe now a simple method for the preparation of dimers **2** in which two amino acids or peptides are connected by a C═C-double bond.

The reaction relies on the chemistry of α-halogenoglycine derivatives **1** that are easily available from the corresponding seryl or α-(ethylthio)glycyl compounds.[8] Treatment of an amino acid or peptide derivative of type **1** with triethylamine and catalytic amounts of triphenylphosphine in THF affords the dimers ***Z*-2** and (or) ***E*-2** in moderate to excellent yields.[9] This mild method allows not only the synthesis of homomers but also of heteromers containing two different peptide chains (Scheme 1).

In these cases the triphenylphosphonium salt preformed from one component is subsequently treated with an acylimine **3** generated from a second α-chloroglycine peptide. For this procedure stoichiometrical amounts of triphenylphosphine are required.



Scheme 1. Dimerisation of α-chloroglycine derivatives **1**.

Keywords: peptide analogues; dimerisation; olefins; stereoselectivity; phosphines.

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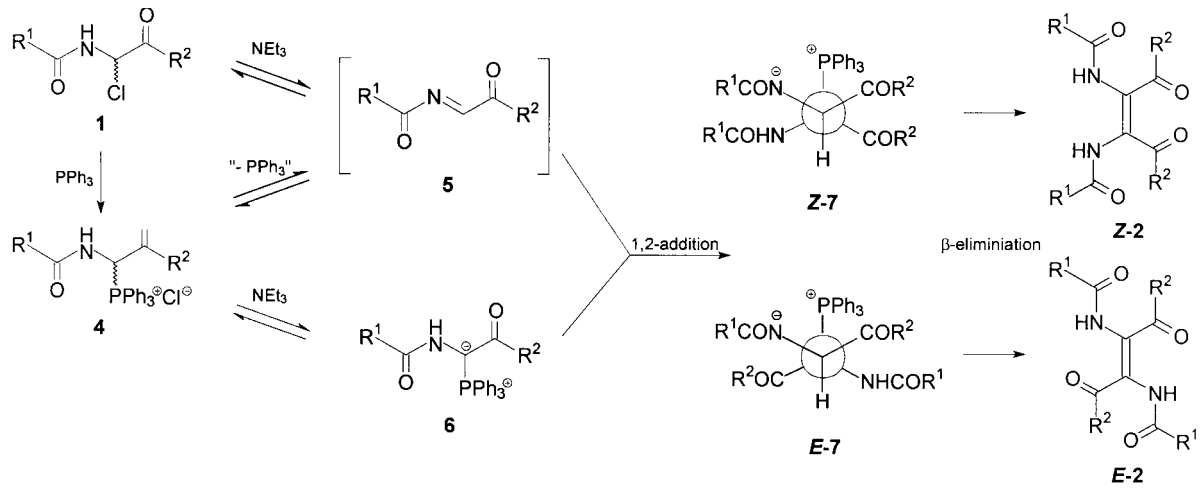


Scheme 2. Proposed course for the formation of olefins **2**.

The catalytic activity of triphenylphosphine can be explained by the reactions depicted in Scheme 2. We assume that the triphenylphosphonium salt **4** initially formed from chloroglycine **1** and triphenylphosphine, affords both the ylide **6** and the acylimine **5** on treatment with base. Reaction of the two reactive intermediates **5** and **6** to the diastereomeric betaines **7** followed by β-elimination yields the dimers ***Z*-2** and ***E*-2**, respectively.

For protected di- and tripeptides the olefinic linkage can be established at any position of the peptide chain (Table 1). The stereochemical outcome of the reaction, however, depends on the position of the activated glycine residue.

N-Acylamino-α-chloroglycinates or *N*-acylpeptide esters with a *C*-terminal chloroglycine residue afford the *cis*-dimers ***Z*-2** with high stereoselectivity. Thus, Boc-Gly-Gly(Cl)-OBn furnished ***Z*-2c** in 61% yield under standard conditions. The corresponding *E*-isomer could not been detected in the reaction mixture. In contrast, peptides with a chloroglycine residue at the *N*-terminus yield predominantly the *trans*-dimers ***E*-2**. For example, Z-Gly(Cl)-Ala-OMe afforded ***E*-2e** in 60% yield in addition to 8% of ***Z*-2e**. The position-dependent control of product stereochemistry is also observed for tripeptides. Compounds with an activated glycine residue at the *C* and *N*-termini yield preferably the *Z*- and *E*-dimers, respectively (examples **2f** and **2g**), whereas an activated glycine residue at the centre of the peptide chain induces a mixture of both diastereomers which can be separated by silica gel chromatography. Like in the case of peptides activated at the *N*-terminal position the major product shows *trans*-configuration (examples **2h** and **2i**).

Dimers with two different peptide chains (heteromers) can be easily prepared by the two-component procedure described above (Scheme 1). For example, Boc-Val-Gly(Cl)-OMe is first converted to the corresponding phosphonium salt by treatment with equimolar amounts of triphenylphosphine and then treated with *Z*-Gly-Gly(Cl)-OMe and triethylamine. Chromatography of the products afforded the dimer ***Z*-2k** in 54% yield (Fig. 1).

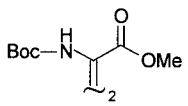
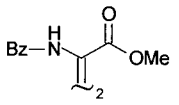
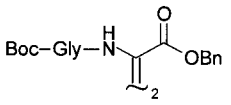
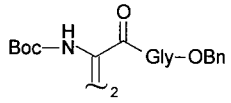
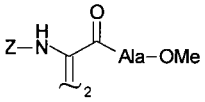
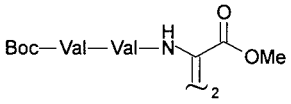
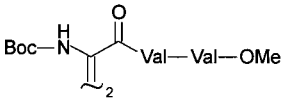
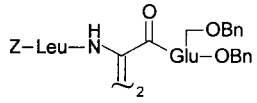
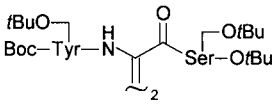
Analogous cross-coupling of Boc-Gly($PPh_3^+Cl^-$)ValOBn and Boc-Gly(Cl)-Gly-OMe yielded 63% of ***E*-2l**. In the formation of heteromers the same stereochemical preferences are observed as for homomeric compounds. The occurrence of small amounts of homomers as by-products can be explained through exchange of the triphenylphosphine residues in the pre-equilibrium according to the reaction sequence proposed in Scheme 2.

The configurational assignment of the olefinic compounds **2** is based on X-ray structure analyses of numerous dimers.[10] As an example, the X-ray structure of compound ***E*-2d** is presented in Fig. 2.[11] In cases where both stereoisomers are known, the *E*-dimers exhibit a greater λ_{max} in their UV spectra (between 1 to 5 nm) and higher R_f values on silica gel in chromatographic separations. The conventional comparison of the ^{13}C NMR shifts of the olefinic carbons (cf. maleic and fumaric acid) for determining the stereochemistry is prevented in many cases by a large broadening of these signals.[12]

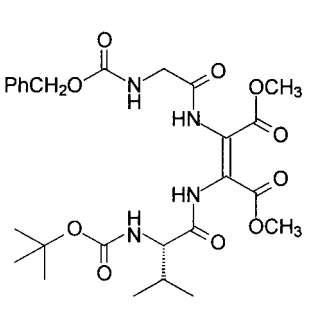
The peptide dimers **2c–2l** are stable against acids and bases and exhibit high configurational stability.[13,14] They can therefore be used for the construction of more complex compounds by standard methods of peptide synthesis. This is illustrated by the synthesis of the triple dimeric peptide structure ***Z,E,Z*-10** (Scheme 3). The carboxylic acid component ***E*-8** was obtained by hydrogenolysis of the benzyl esters from the orthogonally protected homomeric dimer ***E*-2d**. ***E*-8** was then coupled under standard conditions with two equivalents of the selectively Boc-deprotected heteromeric amino component ***Z*-9** to yield the pseudododecapeptide ***Z,E,Z*-10** in which three dimeric units are connected by peptide bonds.

Some restrictions exist in the use of dimers **2** for further peptide syntheses. *C*-terminally bridged homomers **2** (R^1=R^3; R^2=R^4) create problems in carboxyl activation because of anhydride formation with the neighbouring carboxyl group. Furthermore, deprotection of dimers **2** in direct neighbourhood to the bridging double bond leads to an enediamine moiety that is unsuitable for peptide couplings.

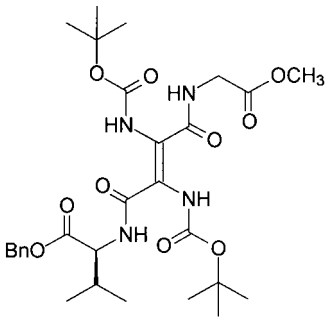
Table 1. Symmetrical dimers of type **2** (R^1=R^2=R^3=R^4)

Substrate 1	Dimer 2	Ratio *Z*/*E*	Yield[a]
Boc–Gly(Cl)–OMe		> 99 : 1[b]	96% ***Z*-2a**
Bz–Gly(Cl)–OMe		> 99 : 1[b]	80% ***Z*-2b**
Boc–Gly–Gly(Cl)–OBn		> 99 : 1[b]	61% ***Z*-2c**
Boc–Gly(Cl)–Gly–OBn		1 : 13	5% ***Z*-2d** 63% ***E*-2d**
Z–Gly(Cl)–Ala–OMe		2 : 15	8% ***Z*-2e** 60% ***E*-2e**
Boc–Val–Val–Gly(Cl)–OMe		6 : 1	42% ***Z*-2f** 7% ***E*-2f**
Boc–Gly(Cl)–Val–Val–OMe		2 : 7	17% ***Z*-2g** 61% ***E*-2g**
Z–Leu–Gly(Cl)–Glu(OBn)–OBn		2 : 15	8% ***Z*-2h** 58% ***E*-2h**
Boc–Tyr(*t*BuO)–Gly(Cl)–Ser(O*t*Bu)–O*t*Bu		1 : 5	12% ***Z*-2i** 58% ***E*-2i**

[a] Isolated yields after purification on silica gel.
[b] Determined through ^{1}H NMR analysis (only one diastereomer detectable).



***Z*-2k**



***E*-2l**

Figure 1. Heteromeric peptide structures.

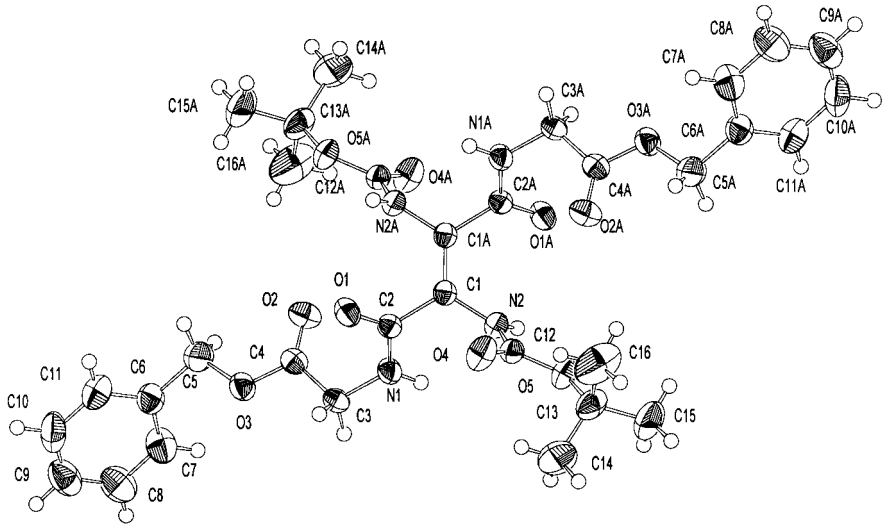
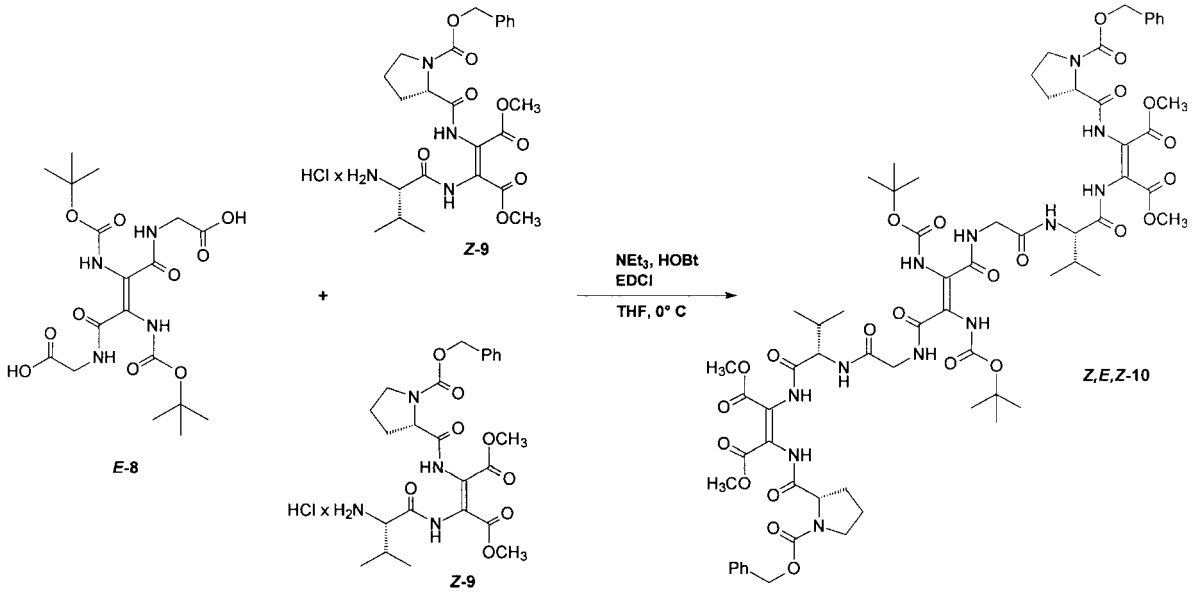


Figure 2. X-Ray structure of ***E*-2d**.[11]



Scheme 3. Synthesis of a triple dimeric peptide structure ***Z,E,Z*-10**.[15]

Catalytic hydrogenation of the rather inert double bond of dimers **2** to generate additional peptides, bridged at glycine residues by a C–C single bond, is also an option. Experiments to study the optimal reaction conditions and the stereochemistry of these hydrogenations are in progress.

Our new dimerisation reaction can also be extended to related systems. We found that α-chlorinated α-acylamino-ketones afford (*E*)-acylamino ketone dimers of type ***E*-11**[16] (R^1=Boc, Bz, Z) in high yields and with excellent stereoselectivity. The use of *N*-arylsulfonyl derivatives instead of the *N*-acylamino-α-chloroglycinates yields preferably *trans*-dimers like ***E*-12** (Fig. 3).[16]

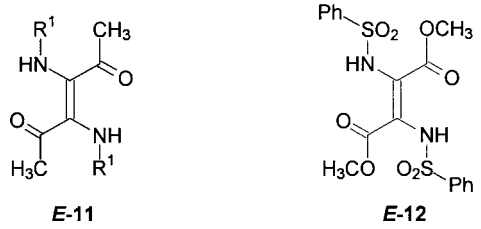


Figure 3. Olefinic bridged α-acylamino ketones **11** and *N*-sulfonylamino acid ester **12**.

In conclusion, the new dimerisation method offers an efficient way for the synthesis of novel peptidic and non-peptidic structures.

Experimental

General

IR: Perkin–Elmer FTIR spectrophotometer FT 1000 or 1420 IS. UV/Vis: Perkin–Elmer Lambda 16 Spectrophotometer.

NMR: Bruker AMX 600 and ARX 300 with solvent peak as internal reference. TLC: Silica gel Merck G plates. Column chromatography: Silica gel Merck 60. Petroleum ether (40–60°C) was used in all cases. FAB MS and ESI MS, HR-FAB MS and HR-ESI MS were performed on Finnigan MAT 90 and 95 instruments. The X-ray diffraction analyses were carried out on a Enraf–Nonius CAD4 diffractometer at 296(2) K using Mo K_α (λ=0.71069 Å) radiation. CH_2Cl_2 was distilled from Sicapent® and THF from potassium/benzophenone. Sulfuryl chloride was purchased as a 1 M solution in CH_2Cl_2 from Aldrich. All reactions were carried out under an argon atmosphere using oven-dried glassware. All α-(ethylthio)glycine derivatives were prepared according to standard procedures.[8] Microanalyses were performed by the microanalytical laboratory of our department.

General procedure for the synthesis of α-chloroglycine derivatives 1

To a solution of the α-(ethylthio)glycyl peptide (1.0 mmol)[8] in CH_2Cl_2 (30 mL) was added a 1 M solution of SO_2Cl_2 (1.1 mL, 1.1 mmol) in CH_2Cl_2 at 0°C. After 30 min stirring, the solvent and all volatile by-products were evaporated (cool trap), and the resulting residue was dried in high vacuo. The resulting α-chloropeptides were used for the next step without further purification.

General procedure for the synthesis of homomeric dimers 2

To a solution of the α-chloroglycyl peptide **1** (1.0 mmol) and PPh_3 (0.2 mmol) in THF (50 mL) was added NEt_3 (0.15 mL, 1.1 mmol) in THF (30 mL) dropwise during 5 h. After continued stirring overnight, petroleum ether (200 mL) was added and the mixture filtered through Celite. Evaporation of the filtrate in vacuo yielded an oily residue which was purified by column chromatography on silica gel (petroleum ether/EtOAc).

2,3-Bis(*N,N′*-*tert*-butyloxycarbonylamino)maleic acid dimethyl ester (*Z*-2a). Yield 96%, colourless foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=270.03 nm (10816). IR(KBr): $\tilde{\nu}$ = 3411 (m, br.), 2981 (m), 1723 (s), 1627 (s), 1494 (m), 1436 (m), 1393 (m), 1369 (s), 1326 (m), 1242 (s), 1154 (s), 1049 (w), 1022 (w), 866 (w), 831 (w), 772 (w), 746 (w), 602 (w) cm^{-1}. ^1H NMR ([D_6]DMSO, 300 MHz): δ=8.85 (br. s, 2H, NH), 3.62 (s, 6H, $COOCH_3$), 1.38 (s, 18H, $C(CH_3)_3$). ^{13}C NMR ([D_6]DMSO, 75 MHz): δ=164.3 (*C*$OOCH_3$), 152.5 (O*C*ONH), 123.2 (br., C=C), 79.8 (*C*$(CH_3)_3$), 52.1 ($COOCH_3$), 28.9 ($C(CH_3)_3$). ESI MS *m/z* (%)=772 (1) $[2M+H+Na]^+$, 771 (4) $[2M+Na]^+$, 399 (3) $[MH_2+Na]^+$, 398 (17) $[MH+Na]^+$, 397 (100) $[M+Na]^+$, 341 (12), 303 (8), 285 (4). HR-ESI MS *m/z* found 397.1599, calcd for $C_{16}H_{26}N_2O_8Na$ $[M+Na]^+$ 397.1587. Anal. calcd for $C_{16}H_{26}N_2O_8$: C, 51.33; N, 7.48; H, 7.00; Found: C, 50.93; N, 7.39; H, 7.01.

2,3-Bis(*N,N′*-benzoylamino)maleic acid dimethyl ester (*Z*-2b). Yield 80%, colourless solid. UV (CH_3OH): λ_{max}=294, 234, 204 nm. IR(KBr): $\tilde{\nu}$ = 3320 (m), 2982 (m), 1730 (s), 1708 (s), 1656 (s), 1613 (m), 1596 (m), 1496 (s), 1472 (s), 1438 (m), 1386 (m), 1362 (m), 1330 (s), 1260 (s), 1113 (m), 1068 (m), 846 (m), 792 (m), 705 (m), 690 (m) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ=9.49 (br. s, 2H, NH), 7.84 (d, *J*=7.2 Hz, 4H, Ph), 7.50–7.30 (m, 6H, Ph), 3.80 (s, 6H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=166.0 (*C*$OOCH_3$), 164.7 (*C*ONH), 133.2 (arom. CH), 132.9 (arom. q.C), 129.3 (arom. CH), 128.2 (arom. CH), 123.0 (C=C), 53.6 ($COOCH_3$). ESI MS *m/z* (%)=596 7 (3), 596 (17), 407 (1) $[MH_2+Na]^+$, 406 (16) $[MH+Na]^+$, 405 (100) $[M+Na]^+$, 262 (1). HR-ESI MS *m/z* found 405.1066, Calcd for $C_{20}H_{18}N_2O_6Na$ $[M+Na]^+$ 405.1062.

2,3-Bis(*N,N′*-*tert*-butyloxycarbonyl-glycylamino)maleic acid dibenzyl ester (*Z*-2c). Yield 61%, colourless foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=276.25 nm (13597). IR(KBr): $\tilde{\nu}$= 3420 (m), 2979 (m), 2933 (w), 1711 (s), 1629 (m), 1500 (s), 1456 (m), 1380 (m), 1368 (m), 1328 (m), 1251 (m), 1164 (s), 1052 (m), 1029 (w), 944 (w), 863 (w), 751 (w), 698 (s), 582 (w) cm^{-1}. ^1H NMR ([D_6]DMSO, 300 MHz): δ=9.72 (br. s, 2H, NH), 7.47 (s, 10H, Ph), 5.42 (br. t, 2H, *J*=5.6 Hz, NH), 4.99 (s, 4H, CH_2-Ph), 3.77 (d, *J*=5.6 Hz, 4H, NH–CH_2–CO), 1.39 (s, 18H, $C(CH_3)_3$). ^{13}C NMR ([D_6]DMSO, 75 MHz): δ=168.8 (CONH), 163.0 (*C*$OOCH_2$), 155.8 (OCONH), 135.3 (arom. q.C), 128.3 (arom. CH), 128.0 (arom. CH), 123.4 (C=C), 78.1 (*C*$(CH_3)_3$), 66.6 ($COOCH_2$), 42.9 (NH–*C*H_2–CO), 28.2 ($C(CH_3)_3$). ESI MS *m/z* (%)=1319 (6) $[2M+K]^+$, 1305 (8) $[2MH+Na]^+$, 1304 (74) $[2M+H+Na]^+$, 1303 (100) $[2M+Na]^+$, 983 (6), 925 (5), 679 (12) $[M+K]^+$, 665 (4) $[MH_2+Na]^+$, 664 (20) $[MH+Na]^+$, 663 (58) $[M+Na]^+$, 529 (2). HR-ESI MS *m/z* found 663.2646, calcd for $C_{29}H_{40}N_4O_{10}Na$ $[M+Na]^+$ 663.2642. Anal. Calcd for $C_{29}H_{40}N_4O_{10}$: C, 59.99; N, 8.74; H, 6.29; Found: C, 60.09; N, 8.76; H, 6.51.

1,1′-[2,3-Bis(*N,N′*-*tert*-butyloxycarbonylamino)-maleoyl]-bis-glycine benzyl ester (*Z*-2d). Yield 8%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=267.80 nm (12771). IR(KBr): $\tilde{\nu}$ = 3409 (s), 2979 (m), 2933 (m), 1740 (s), 1653 (s), 1498 (s), 1456 (m), 1392 (m), 1367 (m), 1249 (m), 1165 (s), 1071 (w), 1017 (w), 740 (m), 697 (m), 578 (w) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ=7.74 (br. s, 2H, NH), 7.39–7.16 (m, 12H, NH and Ph), 5.12 (s, 4H, CH_2-Ph), 4.10 (d, *J*=5.3 Hz, 4H, NH–CH_2–CO), 1.43 (s, 18H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=169.9 (*C*$OOCH_2$), 165.1 (CONH), 153.5 (OCONH), 135.9 (arom. q.C), 129.3 (arom. CH), 129.1 (arom. CH), 129.0 (arom. CH), 124.8 (br., C=C), 82.5 (*C*$(CH_3)_3$), 67.7 ($COOCH_2$), 42.7 (NH–*C*H_2–CO), 28.7 ($C(CH_3)_3$). ESI MS *m/z* (%)=1305 (15) $[2MH+Na]^+$, 1304 (40) $[2M+H+Na]^+$, 1303 (54) $[2M+Na]^+$, 981 (1), 806 (4), 679 (13) $[M+K]^+$, 665 (7) $[MH_2+Na]^+$, 664 (35) $[MH+Na]^+$, 663 (100) $[M+Na]^+$, 498 (6), 476 (9), 364 (5), 304 (5). HR-ESI MS *m/z* found 663.2637, calcd for $C_{29}H_{40}N_4O_{10}Na$ $[M+Na]^+$ 663.2642. Anal. Calcd for $C_{29}H_{40}N_4O_{10}$: C, 59.99; N, 8.74; H, 6.29; Found: C, 60.08; N, 8.77; H, 6.43.

1,1′-[2,3-Bis(*N,N′*-*tert*-butyloxycarbonylamino)-fumaroyl]-bis-glycine benzyl ester (*E*-2d).[11] Yield 58%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=270.47 nm (14381). IR(KBr): $\tilde{\nu}$ = 3412 (s), 2980 (m), 1742 (s), 1703 (s), 1646 (m), 1498 (m), 1455 (m), 1392 (m), 1369 (m), 1248 (m), 1194 (s), 1154 (s), 1051 (w), 753 (w), 699 (w), 577 (w)

cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.39 (br. s, 2H, NH), 7.40–7.24 (m, 10H, Ph), 6.78 (br. t, J=5.8 Hz, 2H, NH), 5.18 (s, 4H, CH_2-Ph), 4.13 (d, J=5.8 Hz, 4H, NH–CH_2–CO), 1.41 (s, 18H, C(CH_3)$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=169.8 (*C*OOCH$_2$), 165.6 (CONH), 153.8 (OCONH), 135.7 (arom. q.C), 129.3 (arom. CH), 129.2 (arom. CH), 129.0 (arom. CH), 82.4 (*C*(CH$_3$)$_3$), 67.9 (COO*C*H$_2$), 42.1 (NH–*C*H$_2$–CO), 28.7 (C(*C*H$_3$)$_3$). No C=C-signal was observable. ESI MS *m/z* (%)=1319 (18) [2M+K]$^+$, 1305 (29) [2MH+Na]$^+$, 1304 (73) [2M+H+Na]$^+$, 1303 (100) [2M+Na]$^+$, 999 (2), 743 (9), 742 (23), 697 (22), 681 (60) [M+Na+H$_2$O]$^+$, 679 (19) [M+K]$^+$, 665 (3) [MH$_2$+Na]$^+$, 664 (14) [MH+Na]$^+$, 663 (39) [M+Na]$^+$, 563 (1), 322 (5), 278 (10). HR-ESI MS *m/z* found 663.2647, Calcd for $C_{29}H_{40}N_4O_{10}Na$ [M+Na]$^+$ 663.2642. Anal. Calcd for $C_{29}H_{40}N_4O_{10}$: C, 59.99; N, 8.74; H, 6.29; Found: C, 59.88; N, 8.79; H, 6.27.

1,1′-[2,3-Bis(*N*,*N*′-benzyloxycarbonylamino)-maleoyl]-bis-L-alanine methyl ester (*Z*-2e). Yield 8%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=264.01 nm (13099). IR(KBr): $\tilde{\nu}$ = 3279 (s), 2991 (m), 2952 (m), 1741 (s), 1648 (s), 1512 (s), 1454 (m), 1217 (s), 1148 (m), 1091 (m), 1056 (m), 1002 (w), 898 (w), 844 (w), 748 (m), 697 (m), 579 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=7.66 (br. s, 2H, NH), 7.59 (br. d, J=7.0 Hz, 2H, NH), 7.47–6.97 (m, 10H, Ph), 5.07 (s, 4H, COOCH$_2$), 4.52 (psq, J=7.0 Hz, 2H, C*H*–CH$_3$), 3.64 (s, 6H, COOCH$_3$), 1.32 (d, J=7.0 Hz, 6H, CH–C*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=173.5 (*C*OOCH$_3$), 164.0 (CONH), 154.3 (OCONH), 136.2 (arom. q.C), 129.1 (arom. CH), 128.9 (arom. CH), 128.8 (arom. CH), 125.3 (C=C), 68.4 (CH_2-Ph), 52.9 (COO*C*H$_3$), 49.4 (*C*H–CH$_3$), 18.0 (CH–*C*H$_3$). ESI MS *m/z* (%)=1776 (29) [3M+H+Na]$^+$, 1207 (22), 1193 (18) [2MH+Na]$^+$, 1192 (58) [2M+H+Na]$^+$, 1191 (100) [2M+Na]$^+$, 688 (17), 623 (9), 609 (1) [MH$_2$+Na]$^+$, 608 (3) [MH+Na]$^+$, 607 (36) [M+Na]$^+$, 423 (2), 331 (7), 304 (12). HR-ESI MS *m/z* found 607.2027, Calcd for $C_{28}H_{32}N_4O_{10}Na$ [M+Na]$^+$ 607.2016. Anal. Calcd for $C_{28}H_{32}N_4O_{10}$: C, 57.53; N, 9.58; H, 5.52; Found: C, 57.61; N, 9.53; H, 5.73.

1,1′-[2,3-Bis(*N*,*N*′-benzyloxycarbonylamino)-fumaroyl]-bis-L-alanine methyl ester (*E*-2e). Yield 60%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=267.54 nm (17419). IR(KBr): $\tilde{\nu}$ = 3338 (s), 2954 (m), 1747 (s), 1671 (s), 1647 (s), 1499 (s), 1451 (s), 1375 (m), 1308 (m), 1207 (s), 1158 (m), 1044 (m), 982 (w), 890 (w), 850 (w), 801 (w), 743 (m), 697 (m), 579 (w), 470 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.75 (br. s, 2H, NH), 7.39–7.16 (m, 10H, Ph), 6.82 (br. d, J=7.2 Hz, 2H, NH), 5.16–5.07 (m, 4H, COOCH$_2$), 4.55 (ps-q, J=7.2 Hz, 2H, C*H*–CH$_3$), 3.68 (s, 6H, COOCH$_3$), 1.35 (d, J=7.2 Hz, 6H, CH-C*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=173.4 (*C*OOCH$_3$), 164.6 (CONH), 154.6 (OCONH), 136.1 (arom. q.C), 129.1 (arom. CH), 129.0 (arom. CH), 128.8 (arom. CH), 68.5 (CH_2Ph), 53.1 (COO*C*H$_3$), 48.8 (*C*H–CH$_3$), 18.4 (CH–*C*H$_3$). No C=C-signal was observable. ESI MS *m/z* (%)=1777 (28) [3M+2H+Na]$^+$, 1481 (2), 1207 (4), 1193 (20) [2MH+Na]$^+$, 1192 (61) [2M+H+Na]$^+$, 1191 (100) [2M+Na]$^+$, 1152 (13), 862 (1), 688 (1), 644 (9), 608 (2) [MH+Na]$^+$, 607 (13) [M+Na]$^+$, 412 (9). HR-ESI MS *m/z* found 607.2003, Calcd for $C_{28}H_{32}N_4O_{10}Na$ [M+Na]$^+$ 607.2016. Anal. Calcd for $C_{28}H_{32}N_4O_{10}$: C, 57.53; N, 9.58; H, 5.52; Found: C, 57.42; N, 9.52; H, 5.51.

2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonyl-L-valyl-L-valylamino) maleic acid dimethyl ester (*Z*-2f). Yield 42%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=276.08 nm (14016). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.69 (br. s, 2H, NH), 6.84 (br. d, J=5.4 Hz, 2H, NH), 5.37 (br. s, 2H, NH), 4.45–4.28 (m, 2H, C*H*–CH(CH$_3$)$_2$), 3.86 (ps-t, J=6.9 Hz, 2H, C*H*–CH(CH$_3$)$_2$), 3.75 (s, 6H, COOCH$_3$), 2.28–1.78 (m, 4H, CH–C*H*(CH$_3$)$_2$), 1.40 (s, 18H, C(CH$_3$)$_3$), 0.99–0.92 (m, 24H, CH–CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 173.5 (CONH), 170.6 (CONH), 163.9 (*C*OOCH$_3$), 156.9 (OCONH), 124.6 (C=C), 81.0 (*C*(CH$_3$)$_3$), 61.5 (*C*H–CH(CH$_3$)$_2$), 59.4 (*C*H–CH(CH$_3$)$_2$), 52.3 (COO*C*H$_3$), 31.3 (CH–*C*H(CH$_3$)$_2$), 30.9 (CH–*C*H(CH$_3$)$_2$), 28.9 (C(*C*H$_3$)$_3$), 20.0, 19.9, 18.9, 18.4 (CH–CH(*C*H$_3$)$_2$). ESI MS *m/z* (%)=1565 (3) [2MH+Na]$^+$, 1564 (6) [2M+H+Na]$^+$, 1563 (8) [2M+Na]$^+$, 1055 (35), 838 (4), 795 (10) [MH$_2$+Na]$^+$, 794 (42) [MH+Na]$^+$, 793 (100) [M+Na]$^+$, 671 (2), 579 (8), 481 (2). HR-ESI MS *m/z* found 793.4321, Calcd for $C_{36}H_{62}N_6O_{12}Na$ [M+Na]$^+$ 793.4323. Anal. Calcd for $C_{36}H_{62}N_6O_{12}$: C,56.09; N,10.90; H,8.11; Found: C, 56.03; N, 10.67; H, 8.26.

2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonyl-L-valyl-L-valylamino) fumaric acid dimethyl ester (*E*-2f). Yield 7%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=277.52 nm (17144). IR(KBr): $\tilde{\nu}$ = 3341 (m), 2967 (m), 2934 (m), 1734 (s), 1657 (s), 1506 (s), 1392 (m), 1369 (m), 1239 (m), 1154 (s), 1047 (w), 1021 (w), 889 (w), 814 (w), 769 (w), 605 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.17 (br. s, 2H, NH), 6.89 (br. d, J=8.2 Hz, 2H, NH), 6.06 (br. d, J=6.9 Hz, 2H, NH), 4.36 (dd, J=8.2 Hz; J=5.6 Hz, 2H, C*H*–CH(CH$_3$)$_2$), 4.32 (ps-t, J=7.0 Hz, 2H, C*H*–CH(CH$_3$)$_2$), 3.79 (s, 6H, COOCH$_3$), 2.45–2.25 (m, 2H, CH–C*H*(CH$_3$)$_2$), 2.16–2.03 (m, 2H, CH–C*H*(CH$_3$)$_2$), 1.39 (s, 18H, C(CH$_3$)$_3$), 1.09–0.90 (m, 24H, CH–CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=174.1 (CONH), 170.7 (CONH), 164.0 (*C*OOCH$_3$), 157.5 (OCONH), 124.3 (C=C), 80.9 (*C*(CH$_3$)$_3$), 61.3 (*C*H–CH(CH$_3$)$_2$), 60.3 (*C*H–CH(CH$_3$)$_2$), 53.4 (COO*C*H$_3$), 31.8 (CH–*C*H(CH$_3$)$_2$), 31.1 (CH–*C*H(CH$_3$)$_2$), 29.0 (C(*C*H$_3$)$_3$), 19.5, 19.3, 18.2, 17.7 (CH–CH(*C*H$_3$)$_2$). ESI MS *m/z* (%)=1565 (5) [2MH+Na]$^+$, 1564 (10) [2M+H+Na]$^+$, 1563 (12) [2M+Na]$^+$, 844 (1), 795 (11) [MH$_2$+Na]$^+$, 794 (43) [MH+Na]$^+$, 793 (100) [M+Na]$^+$, 693 (1), 481 (1). HR-ESI MS *m/z* found 793.4321, Calcd for $C_{36}H_{62}N_6O_{12}Na$ [M+Na]$^+$ 793.4323. Anal. Calcd for $C_{36}H_{62}N_6O_{12}$: C, 56.09; N, 10.90; H, 8.11; Found: C, 55.79; N, 10.60; H, 8.48.

1,1′-[2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonylamino)-maleoyl]-bis-L-valyl-L-valine methyl ester (*Z*-2g). Yield 17%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$= 267.49 nm (10330). IR(KBr): $\tilde{\nu}$ = 3351 (m), 2967 (m), 2876 (m), 1735 (s), 1663 (s), 1497 (s), 1393 (m), 1369 (m), 1314 (m), 1248 (s), 1156 (s), 1049 (w), 1020 (w), 890 (w), 772 (w), 605 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=7.73 (br. d, J=8.2 Hz, 2H, NH), 7.12 (br. d, J=8.3 Hz, 2H, NH), 7.01 (br. s, 2H, NH), 4.51 (dd, J=8.3 Hz; J=5.7 Hz, 2H, C*H*–CH(CH$_3$)$_2$), 4.42 (dd, J=8.2 Hz; J=5.4 Hz, 2H, C*H*–CH(CH$_3$)$_2$), 3.72 (s, 6H, COOCH$_3$), 2.33–2.16 (m,

4H, CH–C*H*(CH_3)$_2$), 1.42 (s, 18H, C(CH_3)$_3$), 0.97–0.93 (m, 24H, CH–CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 173.1 (CO), 171.5 (CO), 164.9 (*C*ONH), 153.6 (OCONH), 126.2 (C=C), 82.4 (*C*(CH_3)$_3$), 60.2 (*C*H–CH(CH_3)$_2$), 58.2 (*C*H–CH(CH_3)$_2$), 52.7 (COO*C*H$_3$), 31.5 (CH–*C*H(CH_3)$_2$), 31.2 (CH–*C*H(CH_3)$_2$), 28.7 (C(*C*H$_3$)$_3$), 19.8, 19.7, 18.9, 18.5 (CH–CH(*C*H$_3$)$_2$). FAB MS *m/z* (%)=793 (100) $[M+Na]^+$, 737 (3), 634 (2), 619 (3), 593 (26), 541 (31), 485 (12), 429 (63), 385 (10), 226 (24). HR-ESI MS *m/z* found 793.4344, Calcd for $C_{36}H_{62}N_6O_{12}Na$ $[M+Na]^+$ 793.4323. Anal. Calcd for $C_{36}H_{62}N_6O_{12}$: C, 56.09; N, 10.90; H, 8.11; Found: C, 55.98; N, 10.60; H, 8.51.

1,1′-[2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonylamino)-fumaroyl]-bis-L-valyl-L-valine methyl ester (*E*-2g). Yield 61%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=273.28 nm (13820). IR (KBr): $\tilde{\nu}$ = 3341 (m), 2967 (m), 2934 (m), 1734 (s), 1657 (s), 1506 (s), 1392 (m), 1369 (m), 1239 (m), 1154 (s), 1047 (w), 1021 (w), 889 (w), 814 (w), 769 (w), 605 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.57 (br. s, 2H, NH), 6.67 (br. d, *J*=8.1 Hz, 2H, NH), 6.58 (br. d, *J*=8.4 Hz, 2H, NH), 4.50 (dd, *J*=8.4 Hz; *J*=5.3 Hz, 2H, C*H*–CH(CH_3)$_2$), 4.32 (dd, *J*=8.1 Hz; *J*=5.8 Hz, 2H, C*H*–CH(CH_3)$_2$), 3.74 (s, 6H, COOCH_3), 2.28–2.16 (m, 4H, CH–C*H*(CH_3)$_2$), 1.42 (s, 18H, C(CH_3)$_3$), 1.01–0.91 (m, 24H, CH–CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=172.7 (CO), 171.0 (CO), 165.3 (CONH), 153.9 (OCONH), 82.4 (*C*(CH_3)$_3$), 59.5 (*C*H–CH(CH_3)$_2$), 58.1 (*C*H–CH(CH_3)$_2$), 52.7 (COO*C*H$_3$), 31.7 (CH–*C*H(CH_3)$_2$), 31.5 (CH–*C*H(CH_3)$_2$), 28.7 (C(*C*H$_3$)$_3$), 19.8, 19.6, 18.7, 18.6 (CH–CH(*C*H$_3$)$_2$). No C=C-signal was observable. ESI MS *m/z* (%)=1565 (40) $[2MH+Na]^+$, 1564 (84) $[2M+H+Na]^+$, 1563 (100) $[2M+Na]^+$, 1541 (3), 1241 (4), 1001 (2), 811 (9), 795 (3) $[MH_2+Na]^+$, 794 (16) $[MH+Na]^+$, 793 (39) $[M+Na]^+$, 671 (2), 571 (1), 441 (1). HR-ESI MS *m/z* found 793.4334, Calcd for $C_{36}H_{62}N_6O_{12}Na$ $[M+Na]^+$ 793.4323. Anal. Calcd for $C_{36}H_{62}N_6O_{12}$: C, 56.09; N, 10.90; H, 8.11; Found: C, 55.83; N, 10.82; H, 8.40.

1,1′-[2,3-Bis-(*N*,*N*′-benzyloxycarbonyl-L-leucylamino)-maleoyl]-bis-L-glutaminic acid dibenzylester (*Z*-2h). Yield 8%, colourless oily foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.67 (br. s, 2H, NH), 8.09 (br. d, *J*=6.8 Hz, 2H, NH), 7.29–7.26 (m, 30H, Ph), 5.49 (br. d, *J*=6.5 Hz, 2H, NH), 5.14–4.95 (m, 12H, CH_2-Ph), 4.67–4.62 (br. m, 2H, C*H*–CH_2CH_2), 4.22 (br. s, 2H, C*H*–CH_2–CH(CH_3)$_2$), 2.44–2.38 (br. m, 4H, $CH_2CH_2CO_2$Bn), 2.25–2.21, 2.07–1.96 (m, 4H, CH(NH)CH_2), 1.67–1.60 (m, 4H, CH_2–CH(CH_3)$_2$), 1.54–1.51 (m, 2H, CH_2–C*H*(CH_3)$_2$), 0.92 (d, *J*=4.9 Hz, 12H, CH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 150 MHz): δ=173.3 (CO), 172.3 (CO), 171.5 (CO), 164.1 (CONH), 157.2 (OCONH), 136.7, 136.5, 136.0 (arom. q.C) 129.2, 129.1, 129.0, 128.9, 128.8, 128.7 (arom. CH), 126.8 (C=C), 67.9, 66.9 (OCH_2Ph), 54.8, 53.1 (NH–*C*H–CH), 41.0 (*C*H_2–CH(CH_3)$_2$), 30.6, 27.3 (*C*H_2–*C*H_2COOBn), 25.4 (*C*H(CH_3)$_2$), 23.7, 22.2 (CH(*C*H_3)$_2$). ESI MS *m/z* (%)=2559 (13) $[2M+K]^+$, 2541 (37) $[2M+Na]^+$, 2053 (6), 1395 (24), 1299 (33) $[M+K]^+$, 1282 (100) $[M+Na]^+$, 933 (4), 653 (8). HR-ESI MS *m/z* found 1281.5380, Calcd for $C_{70}H_{78}N_6O_{16}Na$ $[M+Na]^+$ 1281.5372.

1,1′-[2,3-Bis-(*N*,*N*′-benzyloxycarbonyl-L-leucylamino)-fumaroyl]-bis-L-glutamic acid dibenzyl ester (*E*-2h). Yield 58%, colourless oily foam. UV (CH_3OH): λ_{max}=276, 238 nm. IR(KBr): $\tilde{\nu}$ = 3400 (s, br.), 3065 (m) 3034 (m), 2957 (m), 2871 (w), 1736 (vs, sh.), 1646 (s), 1524 (s), 1498 (s), 1455 (s), 1388 (m), 1333 (m), 1260 (s), 1215 (s), 1168 (s), 1123 (m), 1081 (w), 697 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ=9.76 (br. s, 2H, NH), 7.36–7.26 (m, 30H, Ph), 7.12–7.08 (m, br., 2H, NH), 5.21 (br. s, 2H, NH), 5.18–5.00 (m, 12H, CH_2–Ph), 4.66–4.63 (br. m, 2H, C*H*–CH_2CH_2), 4.15 (br. s, 2H, C*H*–CH_2–CH(CH_3)$_2$), 2.46–2.42 (br. m, 4H, $CH_2CH_2CO_2$Bn), 2.24–2.23, 2.08–2.06 (m, 4H, CH(NH)CH_2), 1.67–1.62 (m, 4H, CH_2–CH(CH_3)$_2$), 1.52–1.51 (m, 2H, CH_2–C*H*(CH_3)$_2$), 0.89 (d, *J*=6.0 Hz, 12H, CH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=173.1 (CO), 173.0 (CO), 172.5 (CO), 165.0 (CONH), 156.9 (OCONH), 136.5, 136.2, 135.7 (arom. q.C) 129.0, 128.9, 128.8, 128.6, 128.5 (arom. CH), 125.3 (C=C), 67.8, 66.8 (OCH_2Ph), 54.6, 52.6 (NH–*C*H–CH), 40.5 (*C*H_2–CH(CH_3)$_2$), 30.6, 27.0 (*C*H_2–*C*H_2COOBn), 25.1 (*C*H(CH_3)$_2$), 23.4, 21.9 (CH(*C*H_3)$_2$). FAB MS *m/z* (%)=1259 (2) $[M+H]^+$,775(1), 685 (1), 328 (5), 181 (4), 105 (2), 91 (100), 77 (3), 55 (3). HR-FAB MS *m/z* found 1259.5552, Calcd for $C_{70}H_{79}N_6O_{16}$ $[M+H]^+$ 1259.5621. Anal. Calcd for $C_{70}H_{79}N_6O_{16}$: C, 66.76; N, 6.67; H, 6.24; Found: C, 66.59; N, 6.47; H, 6.17.

1,1′-[2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonyl-*O*-*tert*-butyl-L-tyrosylamino)-maleoyl]-bis-*O*-*tert*-butyl-L-serine *tert*-butyl ester (*Z*-2i). Yield 12%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=221.26 nm (22975), 273.42 (10270) nm. IR(KBr): $\tilde{\nu}$ = 3432 (s, br.), 2933 (s), 1716 (s, sh.), 1507 (s, sh.), 1392 (m), 1366 (s), 1247 (m),1240 (m), 1165 (vs), 1101 (w), 1051 (w), 1027 (w), 900 (w), 848 (w) 765 (w), 568 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=9.92 (br. s, 2H, NH), 7.09 (d, *J*=8.4 Hz, 4H, Ph), 6.95 (br. s, 2H, NH), 6.88 (d, *J*=8.4 Hz, 4H, Ph), 5.48 (br. s, 2H, NH), 4.58–4.55 (m, 4H, NH–*C*H–CH), 3.79 (dd, *J*=2.9, 8.8 Hz, 2H, CH_2O*t*Bu), 3.57 (br. d, *J*=6.6 Hz, 2H, CH_2O*t*Bu), 3.30 (br. d, *J*=12.2 Hz, 2H, CH_2Ph), 2.86 (br. p-t, *J*=11.5 Hz, 2H, CH_2Ph), 1.49 (s, 18H, C(CH_3)$_3$), 1.32 (s, 18H, C(CH_3)$_3$), 1.30 (s, 18H, C(CH_3)$_3$), 1.13 (s, 18H, C(CH_3)$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=172.3, 170.0 (CO), 164.6 (CONH), 156.1 (OCONH), 154.7, 132.5 (arom. q.C), 130.5 (arom. CH), 125.5 (C=C), 124.8 (arom. CH), 82.9, 80.4, 78.9, 74.1 (*C*(CH_3)$_3$), 62.4 (O*C*H_2), 56.4, 54.3 (NH–*C*H–CH), 37.8 (*C*H_2Ph), 29.5, 28.9, 28.8, 28.0 (C(*C*H_3)$_3$). ESI MS *m/z* (%)=1205 (14) $[M+Na]^+$, 1200 (3) $[M+NH_4]^+$, 1183 (45) $[M+H]^+$, 966 (100), 910 (3). HR-ESI MS *m/z* found 1183.7082 Calcd for $C_{62}H_{99}N_6O_{16}$ $[M+H]^+$ 1183.7117.

1,1′-[2,3-Bis-(*N*,*N*′-*tert*-butyloxycarbonyl-*O*-*tert*-butyl-L-tyrosylamino)-fumaroyl]-bis-*O*-*tert*-butyl-L-serine *tert*-butyl ester (*E*-2i). Yield 58%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=222.01 nm (25715), 275.87 (20420). IR(KBr): $\tilde{\nu}$ = 3432 (m), 2977 (s), 2932 (m), 1720 (s, sh), 1646 (m), 1507 (s), 1476 (m), 1457 (m), 1392 (m), 1366 (s), 1238 (s), 1164 (s), 1101 (w), 1052 (w), 1022 (w), 900 (w) 849 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=8.74 (br. s, 2H, NH), 7.90 (br. s, 2H, NH), 7.09 (d, *J*=8.4 Hz, 4H, Ph), 6.90 (d, *J*=8.4 Hz, 4H, Ph), 5.48 (br. d, *J*=8.0 Hz, 2H, NH), 4.60–4.58 (m, 2H, NH–*C*H–CH), 4.37 (s, 2H, NH–*C*H–CH), 3.74 (dd, *J*=3.3, 8.6 Hz, 2H, CH_2O*t*Bu), 3.59 (d d, *J*=3.3, 4.3 Hz, 2H, CH_2O*t*Bu), 3.20 (br. dd, *J*=4.9, 14 Hz, 2H, CH_2Ph), 2.83–2.91 (br. m, *J*=11.5 Hz, 2H, CH_2Ph),

1.45 (s, 18H, $C(CH_3)_3$), 1.35 (s, 18H, $C(CH_3)_3$), 1.31 (s, 18H, $C(CH_3)_3$), 1.14 (s, 18H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=171.2, 169.4 (CO), 163.7 (CONH), 156.0 (OCONH), 154.8, 132.1 (arom. q.C), 130.4 (arom. CH), 126.5 (C=C), 125.0 (arom. CH), 82.5, 80.9, 79.1, 73.7 ($C(CH_3)_3$), 62.5 (OCH_2), 56.9, 55.0 (NH–*C*H–CH), 37.6 (CH_2Ph), 29.5, 28.9, 28.7, 28.0 ($C(CH_3)_3$). FAB MS *m/z* (%)=1205 (37) $[M+Na]^+$, 1200 (61) $[M+NH_4]^+$, 1183 (100), $[M+H]^+$, 1127 (16). HR-ESI MS *m/z* found 1183.7130, Calcd for $C_{62}H_{99}N_6O_{16}$ $[M+H]^+$ 1183.7117.

(3E)-3,4-Bis(*N*,*N*′-*tert*-butyloxycarbonylamino)hexene-2,5-dione (*E*-11).[16] Yield 70%, colourless solid. UV (CH_3CN): $\lambda_{max}(\epsilon)$=289.17 nm (13055). IR(KBr): $\tilde{\nu}$ = 3327 (s), 2976 (m), 2932 (w), 1737 (s), 1725 (s), 1654 (m), 1587 (s), 1497 (s), 1442 (s), 1393 (w), 1370 (m), 1360 (m), 1265 (s), 1250 (s), 1048 (w), 1033 (w), 991 (w), 875 (w), 784 (w), 762 (w), 581 (w) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ=8.38 (br. s, 1H, NH), 2.29 (s, 6H, $COCH_3$), 1.45 (s, 9H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=200.4 (CO), 154.2 (OCONH), 82.9 ($C(CH_3)_3$), 29.2 ($COCH_3$), 28.8 ($C(CH_3)_3$). No C=C signal was observable. ESI MS *m/z* (%)=365 (15) $[M+Na]^+$, 333 (11), 297 (12), 265 (44), 197 (50), 165 (100). HR-ESI MS *m/z* found 381.1401, Calcd for $C_{16}H_{26}N_2O_6K$ $[M+K]^+$ 381.1428. Anal. Calcd for $C_{16}H_{26}N_2O_6$: C, 56.13; N, 8.18; H, 7.65. Found: C, 55.68; N, 7.98; H, 7.82.

2,3-Bis(*N*,*N*′benzenesulfonylamino)fumaric acid dimethyl ester (*E*-12).[16] Yield 57%, colourless foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=217.84 (1495), 272.88 (923) nm. IR(KBr): $\tilde{\nu}$ = 3436 (s), 2926 (w), 1740 (m), 1711 (m), 1624 (m), 1448 (m), 1439 (m), 1345 (w), 1276 (s), 1163 (s), 1090 (m), 1018 (w), 849 (w), 789 (m), 757 (m), 725 (m), 688 (m), 593 (m, sh.) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ=7.90–7.43 (m, 10H, Ph), 7.15 (br. s, 2H, NH), 3.56 (s, 6H, OCH_3). ^{13}C NMR ([D_6]Acetone, 150 MHz): δ=164.0 (CO), 141.4 (arom. q. C), 134.8, 130.7, 128.9 (arom. CH), 126.5 (C=C). ESI MS *m/z* (%)=477 (100) $[M+Na]^+$, 393 (4).

General procedure for the synthesis of heteromeric dimers 2

To a solution of an α-chloroglycyl peptide (1.0 mmol) in CH_2Cl_2 (40 mL) was added PPh_3 (1.0 mmol) and the mixture stirred at room temperature overnight. Removal of the solvent afforded the phosphonium salt in quantitative yield. The salt was dissolved in THF (100 mL) and cooled to −78°C (solution I).

A solution of the second α-chloroglycyl peptide (1.5 mmol) in THF (50 mL) was transferred to an addition funnel with cooling (CO_2/acetone) and NEt_3 (2.5 mmol) was added at −78°C (solution II).

Solution II was added dropwise at −78°C to the stirred solution I, and the mixture was allowed to rise to room temperature overnight. After addition of petroleum ether and filtration through Celite the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc).

3-(*N*-Benzyloxycarbonyl-glycylamino)-2-(*N*-*tert*-butyloxycarbonyl-L-valyl-amino)-maleic acid dimethyl ester (*Z*-2k). Yield 58%, colourless oily foam. UV (CH_3CN): $\lambda_{max}(\epsilon)$=275.32 nm (15526). IR(KBr): $\tilde{\nu}$ = 3407 (m), 2968 (m), 1702 (s), 1630 (m), 1511 (s), 1456 (m), 1436 (m), 1392 (m), 1367 (m), 1335 (m), 1210 (m), 1162 (m), 1051 (w), 1016 (w), 977 (w), 871 (w), 839 (w), 740 (w), 698 (w), 577 (w) cm^{-1}. ^1H NMR ([D_6]DMSO, 300 MHz): δ=9.69 (br. s, 1H, NH), 9.63 (br. s, 1H, NH), 7.51 (t, *J*=5.7 Hz, 1H, NH), 7.43–7.24 (m, 5H, Ph), 6.68 (d, *J*=9.1 Hz, 1H, NH), 5.04 (s, 2H, CH_2–Ph), 4.13–4.08 (m, 1H, C*H*–$CH(CH_3)_2$), 3.91–3.78 (m, 2H, NH–CH_2–CO), 3.63 (s, 3H, $COOCH_3$), 3.61 (s, 3H, $COOCH_3$), 2.12–2.00 (m, 1H, CH–C*H*$(CH_3)_2$), 1.39 (s, 9H, $C(CH_3)_3$), 0.97, 0.92 (2d, *J*=6.6 Hz, 6H, CH–$CH(CH_3)_2$). ^{13}C NMR ([D_6]DMSO, 75 MHz): δ=171.0 (*C*$OOCH_3$), 168.4 (*C*$OOCH_3$), 163.4 (2 CONH), 156.5 (OCONH), 155.6 (OCONH), 136.9 (arom. q.C), 128.3 (arom. CH), 127.7 (arom. CH), 123.6 (C=C), 123.4 (C=C), 78.2 ($C(CH_3)_3$), 65.6 (CH_2-Ph), 58.7 (*C*H–$CH(CH_3)_2$), 52.2 ($COOCH_3$), 52.1 ($COOCH_3$), 43.2 (NH–*C*H_2–CO), 30.7 (CH–*C*H$(CH_3)_2$), 28.1 ($C(CH_3)_3$), 19.1, 17.2 (CH–$CH(CH_3)_2$). ESI MS *m/z* (%)=1153 (1) $[2MH+Na]^+$, 1152 (3) $[2M+H+Na]^+$, 1151 (5) $[2M+Na]^+$, 953 (2), 952 (5), 866 (2), 865 (2), 773 (2), 623 (1), 603 (5), 589 (5) $[MH_2+Na]^+$, 588 (29) $[MH+Na]^+$, 587 (100) $[M+Na]^+$, 584 (2), 531 (4), 487 (9), 484 (3), 465 (4). HR-ESI MS *m/z* found 587.2331, Calcd for $C_{26}H_{36}N_4O_{10}Na$ $[M+Na]^+$ 587.2329.

[2,3-Bis(*N*,*N*′-*tert*-butyloxycarbonylamino)-fumaroyl]-1-L-valine-benzylester-4-glycine methyl ester (*E*-2l). Yield 63%, colourless oily foam. IR(KBr): $\tilde{\nu}$ = 3422 (s), 2967 (m), 2931 (m), 1736 (s), 1669 (s), 1497 (m), 1392 (m), 1368 (m), 1312 (w), 1248 (m), 1154 (s), 1051 (w), 889 (w), 822 (w), 750 (m), 698 (m), 583 (w) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ=8.98 (br. s, 1H, NH), 8.52 (br. s, 1H, NH), 7.41–7.18 (m, 5H, Ph), 6.94 (br. s, 1H, NH), 5.30–4.86 (m, 3H, NH; CH_2-Ph), 4.27–3.89 (m, 3H, C*H*–$CH(CH_3)_2$; NH–CH_2–CO), 3.73 (s, 3H, $COCH_3$), 2.23–1.92 (m, 1H, CH–C*H*$(CH_3)_2$), 1.42 (s, 9H, $C(CH_3)_3$), 1.40 (s, 9H, $C(CH_3)_3$), 0.86, 0.75 (2d, *J*=6.8 Hz, 6H, CH–$CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=172.7 (COO), 169.7 (COO), 165.2 (CONH), 163.1 (CONH), 155.7 (OCONH), 151.3 (OCONH), 134.9 (arom. q.C), 128.6 (arom. CH), 128.5 (arom. CH), 128.3 (arom. CH), 82.3 ($C(CH_3)_3$), 79.9 ($C(CH_3)_3$), 67.4 (CH_2Ph), 59.6 (*C*H–$CH(CH_3)_2$), 52.3 ($COOCH_3$), 41.4 (NH–*C*H_2–CO), 30.5 (CH–*C*H$(CH_3)_2$), 28.2 ($C(CH_3)_3$), 27.9 ($C(CH_3)_3$), 19.1, 17.2 (CH–$CH(CH_3)_2$). No C=C signals were observable. ESI MS *m/z* (%)=1238 (21) $[2MH+Na]^+$, 1237 (63) $[2M+H+Na]^+$, 1236 (100) $[2M+Na]^+$, 1234 (24), 1214 (5), 1128 (8), 885 (1), 666 (5), 630 (1) $[MH+Na]^+$, 629 (3) $[M+Na]^+$, 607 (1), 403 (1). HR-ESI MS *m/z* found 629.2820, Calcd for $C_{29}H_{42}N_4O_{10}Na$ $[M+Na]^+$ 629.2799.

Tetramethyl-2,2′-(N^{α},$N^{\alpha'}$-{*N*,*N*′-[2,3-bis(*tert*-butyloxycarbonylamino)fumaroyl]diglycyl}bis-L-valine amido)-bis{3-[1-(benzyloxycarbonyl)-L-proline amido]-maleate} (*Z*,*E*,*Z*-10). *N*-terminal deprotection of 2(*N*-benzyloxycarbonyl-L-prolylamino)-3(*N*-*tert*-butyloxycarbonyl-L-valyl-amino)-maleic acid dimethylester (**Z-9**) (108 mg, 0.18 mmol) was achieved by treatment with a 2 M solution of

HCl in ethyl acetate (4 mL). The amino compound of ***Z*-9** was isolated as hydrochloride.

C-terminal deprotection of ***E*-2d** (41 mg, 0.09 mmol) was carried out in dry ethanol (10 mL) with 1,4-cyclohexadiene (0.85 mL, 1.80 mmol) and palladium on charcoal (90 mg) to yield the acid ***E*-8**.

To a suspension of ***Z*-9** and ***E*-8** in THF (10 mL) were added triethylamine (0.05 mL, 0.36 mmol), HOBt (32 mg, 0.24 mmol) and EDC×HCl (35 mg, 0.18 mmol) at 0°C. After stirring overnight, the solvent was removed in vacuo and the resulting oil suspended in EtOAc. The solution was washed successively with 1 M $KHSO_4$, saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting oily residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH 15:1 as eluent. Yield 25%, colourless solid. IR(KBr): $\tilde{\nu}$ = 3412 (s), 2980 (m), 1742 (s), 1703 (s), 1646 (m), 1498 (m), 1455 (m), 1392 (m), 1369 (m), 1248 (m), 1194 (s), 1154 (s), 1051 (w), 753 (w), 699 (w), 577 (w) cm^{-1}. ^1H NMR ([D_6]DMSO, 600 MHz): δ=9.73 (d, J=10.3 Hz, 4H, NH), 9.57 (d, J=11.7 Hz, 2H, NH), 9.37 (s, br., 2H, NH), 8.15 (s, br., 2H, NH), 7.88 (d, J=7.0 Hz, 2H, NH), 7.36–7.30 (m, 10H, Ph), 5.13–4.96 (m, 4H, OCH_2-Ph), 4.52–4.40 (m, 4H, 2 C*H*–CH(CH_3)$_2$, 2 CH-Pro), 3.91–3.70 (m, 4H, NH–C*H*$_2$–CO), 3.61 (s, 12H, COOC*H*$_3$), 3.43–3.31 (m, 4H, CH_2-Pro), 2.13–1.81 (m, 10H, 2 CH–C*H*(CH_3)$_2$, 4 CH_2-Pro), 1.37 (s, 18H, C(CH_3)$_3$), 0.89 (ps-t, J=6.5 Hz, 12H, CH–CH(C*H*$_3$)$_2$). ^{13}C NMR ([D_6]DMSO, 150 MHz): δ=168.7, 168.5, 168.4 (CONH), 164.7, 163.5, 163.3 (2 *C*OOCH$_3$, CONH), 154.1, 152.6 (OCONH), 136.8 (arom. q.C), 128.4 (arom. CH), 127.0 (arom. CH), 126.5 (arom. CH), 125.0, 122.5, 121.1 (C=C), 80.1 (*C*(CH_3)$_3$), 66.0 (CH_2-Ph), 59.5 (CH-Pro), 57.5 (*C*H–CH(CH_3)$_2$), 52.2 (COO*C*H$_3$), 52.0 (COO*C*H$_3$), 47.0 (NCH_2-Pro), 42.4 (NH–*C*H$_2$–CO), 31.0 (CH–*C*H(CH_3)$_2$), 29.8 (CH_2-Pro), 27.8 (C(*C*H$_3$)$_3$), 23.7 (CH_2-Pro), 19.0, 17.7 (CH–CH(*C*H$_3$)$_2$). For the proline sidechain doubling of signals is observed. ESI MS *m/z* (%)=1456 (100) $[M+Na]^+$, 1377 (4), 1321 (5), 1299 (7), 1039 (6), 856 (7), 780 (5), 771 (6), 755 (19), 617 (7), 406 (4). HR-ESI MS *m/z* found 1455.5926, Calcd for $C_{66}H_{88}N_{12}O_{24}Na$ $[M+Na]^+$ 1455.5932.

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11. (a) Crystal data for ***E*-2d**: $C_{29}H_{40}N_4O_{10}$, M=640.69; 2.96<θ<24.97; triclinic, *P*-1; a=5.676 (2) Å, b=12.308 (3) Å, c=13.975 (3) Å, α=63.58 (2)°, β=78.22 (2)°, γ=82.87 (3)°; V=855.3 (4) Å^3; λ 0.71069 Å; Z=1; D_c=1.244 Mg/m^3; μ=0.093 mm^{-1}; crystal size: 0.57×0.40×0.27 mm^3; full-matrix least-squares, $R1$=0.0444, $wR2$=0.1014 for observed 3016 reflections [$I>2\sigma(I)$] (CCDC 143354); the structure was solved with SHELXS 86 and refined by SHELXL 93 program.[17] (b) Further details of the X-ray analyses (see also Ref. 16) are available on request from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).
12. Details concerning the unusual NMR phenomena exhibited by the dimers **2** will be published elsewhere.
13. (a) Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962. (b) Crombie, L. *Q. Rev. Chem. Soc.* **1952**, 6, 101–104. (c) Sonnet, P. E. *Tetrahedron* **1980**, 36, 557–604.
14. In contrast, the amino acid dimers **2a** and **2b** are equilibrated with bases to yield mixtures of both diastereomers in a ratio of about 1:1 (Ref. 10).
15. Compound ***Z*-9** was prepared according to the general procedure for heteromers from Boc-Val-Gly(Cl)-OMe and Z-ProGly($PPh_3^+Cl^-$)-OMe; for details concerning deprotection and peptide coupling see Experimental.
16. The configuration of the compounds ***E*-11** and ***E*-12** was confirmed by X-ray structure analyses (CCDC 143355 and CCDC 143356).[11b]
17. (a) Sheldrick, G. M. SHELXS 86; Universität Göttingen, 1986. (b) Sheldrick, G. M. SHELXS 93; Universität Göttingen, 1993.