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Solid-Supported Synthesis of Highly Functionalized Tripodal Peptides with Flexible but Preorganized Geometry: Towards Potential Serine Protease Mimics

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Tripodal scaffold **1** has been used in the synthesis of a representative member of a library of serine protease mimics, possessing three independent functionalized peptide chains on a central core. Each peptide chain contains one residue of the classical catalytic triad (serine, histidine and aspartate) found in the active site of the serine protease α-chymotrypsin. A particular feature of the novel tripodal design is its essentially flexible yet preorganized structure as deduced from molecular modeling studies. The choice of suitable scaffold and side-chain protecting groups was intensively studied and optimized to ensure complete orthogonality. Inclusion of a photolabile linker enabled the release of intermediates and final structures from the solid-support polymer allowing positive evaluation of the present strategy for the synthesis of future tripodal libraries of serine protease mimics.

Introduction

Mimicking the efficiency and the specificity of enzymes has already been the subject of many investigations.[1] The catalytic power of the serine protease α-chymotrypsin in the cleavage of amide bonds is a typical example.[2] However, the development of efficient catalysts for this process still remains a challenge in organic chemistry. Past efforts to develop α-chymotrypsin models relied on the design of a unique non-peptidic[3] or peptidic[4] molecule possessing a suitable geometric arrangement of functionalities for catalysis. Several years ago, combinatorial chemistry was introduced as a method to speed up the search for catalytic activity.[5] Therefore, the traditional approach of carefully designing one target molecule was replaced by the design and synthesis of libraries of molecules with the hope of increasing the chance of finding a catalyst.

To explore this field, a long-term program was set-up in our laboratory to develop a potentially catalytic hydrolytic system in which three independent peptide chains are constructed, each containing one residue of the catalytic triad, i.e. serine, histidine and aspartate. Initial efforts focussed on the construction of dipodal model systems based on a cholic acid template.[6] These were followed by the synthesis of tripodal construct **1** as a scaffold.[7] In contrast to the other scaffolds described, which are mostly based on a rigid structure,[8] the present design concerns the presentation of the different functionalities necessary for the generation of independent strands on an essentially flexible core unit. How-

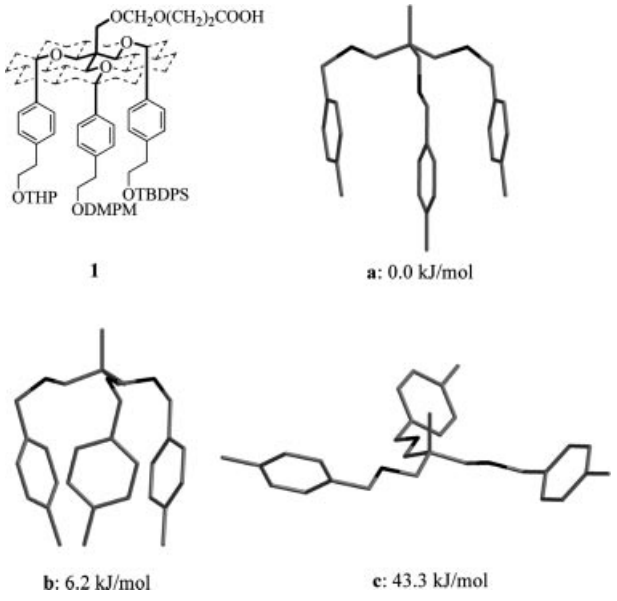

Figure 1. Result of molecular modeling studies on orthogonally protected tripodal scaffold **1** in H_2O (frontal view): **a**) minimum energy conformation, **b**) conformation according to diamond lattice structure **1** with benzylic chains in "axial" positions and **c**) conformation according to diamond lattice structure **1** with benzylic chains in "equatorial" positions. Top views are included in the supporting information.

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ever, despite this flexibility, molecular modeling studies have shown that scaffold **1** preferentially adopts a fairly preorganized structure in which the three benzylic chains are oriented in parallel.[9] As illustrated in Figure 1, the global minimum (**a**) at 0 kJ/mol illustrates a conformation where the three benzylic chains are perfectly parallel. Other conformations presenting the three benzylic ethers in the same direction (such as **b**) are also populated. The corresponding "all-equatorial" conformation (**c**) is considerably less stable as evidenced by its higher relative energy value. Both in water and in vacuo the same trend is observed (see Supporting Information). This important feature is expected to favor the proximity and interactions between the catalytic residues present in each appended peptide strand. However, the usefullness of this scaffold for the generation of multifunctional serine protease mimic libraries remains to be proven. In this work we report on our current efforts aimed at the use of the flexible yet preorganized scaffold **1** in the generation of tripodal libraries with potential serine protease like activity. The validity of the approach is demonstrated by the synthesis and detailed characterisation of one possible library member.

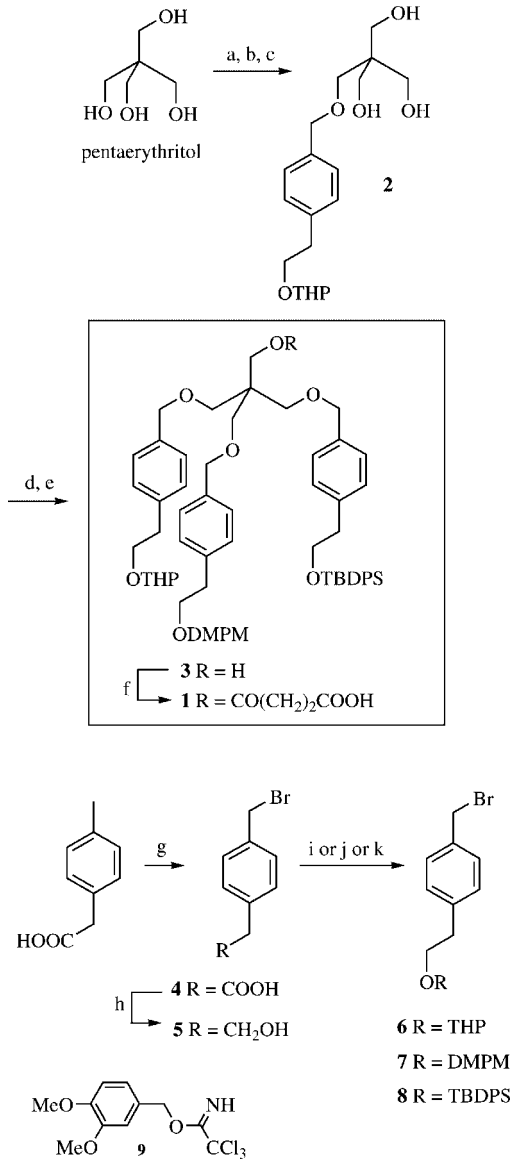
Results and Discussion

The synthesis of tripodal scaffold **1**, in its racemic form, started from pentaerythritol by consecutive Williamson ether syntheses (Scheme 1). The first benzylic chain was introduced by a three-step sequence involving, in first instance, the temporary protection of one of the hydroxy functions as a *tert*-butyldiphenylsilyl ether. Subsequently, classical ether formation with building block **6** followed by deprotection of the silyl ether led to the triol **2**. The other two benzylic chains were introduced by similar procedures leading to the alcohol **3**. Subsequently, the remaining hydroxy function was treated with a large excess of succinic anhydride to afford scaffold **1** in 90% yield. The succinic moiety serves as a spacer for the attachment to the solid-phase. The benzylic bromides **6**, **7** and **8** were readily prepared from the commercially available 4-(methyl)phenylacetic acid by bromination followed by the reduction of the carboxylic acid function with a borane–methyl sulfide complex to form the alcohol **5**.

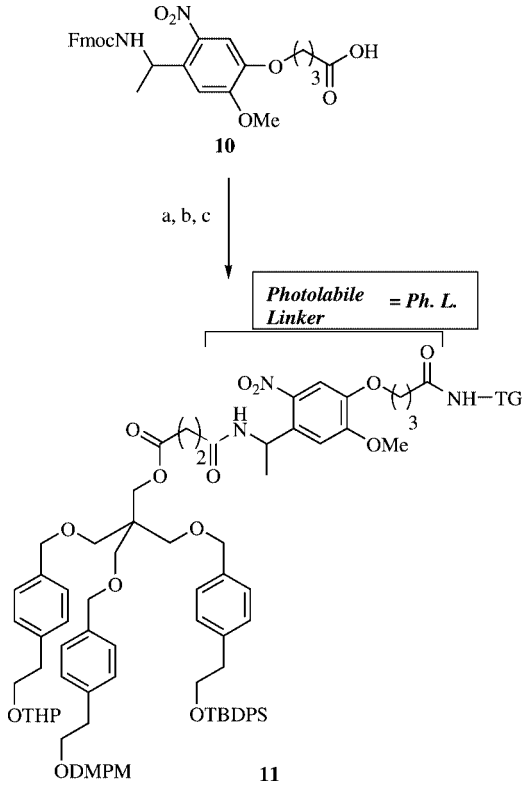
The introduction of the three different protective groups was carried out according to standard procedures.[10] It was further shown[7] that after immobilisation on TentaGel-NH_2 via the use of a photocleavable linker,[11] selective removal of the protecting groups was possible. In this way scaffold **1** is ideally suited for the construction of libaries of serine protease mimics containing three differently functionalised parallel peptide chains.

Condensation of the polymer support TentaGel-NH_2 with the carboxylic acid **10** was followed by Fmoc deprotection, after which tripod **1** was attached to the free amino function affording the polymer supported scaffold **11** (Scheme 2).

Prior to the generation of large libraries, the synthetic processes need to be carefully checked and optimized in order to ensure the absence of error sequences and missing members, especially in view of the high degree of functionalisation of the envisaged library. Therefore a representative tripodal member was synthesized in order to evaluate different parameters. The stability of the benzylic ethers present in scaffold **1** as well as the stability of the ester linkages to the peptide chains were examined. Moreover, the orthogonality and stability of all protecting groups present, either on the scaffold or on the side-chain of the catalytic residues (serine, histidine and aspartic acid), had to be verified. In addition, these protecting groups should be easily and smoothly removable at the end of the synthesis. In view of



Scheme 1. Convergent synthesis of tripodal scaffold **1**. Reagents: (a) TBDPSCl, imidazole, DMF, 68%; (b) **6**, NaH, Bu_4NI, THF, 43%; (c) TBAF, THF, 83%; (d) **7**, NaH, Bu_4NI, THF, 30%, (e) **8**, NaH, Bu_4NI, THF, 49%; (f) succinic anhydride, DMAP, CH_2Cl_2, 90%; (g) Br_2, AIBN, CCl_4, 62%; (h) $BH_3.SMe_2$, THF, 86%; (i) PPTS, dihydropyran, CH_2Cl_2, 88%; (j) trichloroacetimidate **9**, PPTS, CH_2Cl_2, 69% and (k) TBDPSCl, DIPEA, DMF, 71%.



Scheme 2. Synthesis of polymer bound scaffold **11**. Reagents: (a) TentaGel-NH_2, DIC, HOBt, DMF; (b) 20% pip./DMF and (c) **1**, EDC, DMAP, NMP.

the ester attachment of the peptide chains, special attention needs to be made during deprotection to ensure no transesterification takes place.

The synthesis of tripodal member **21** is outlined in Scheme 3 (see Supporting Information for ES-MS spectra of all intermediates and final compounds).

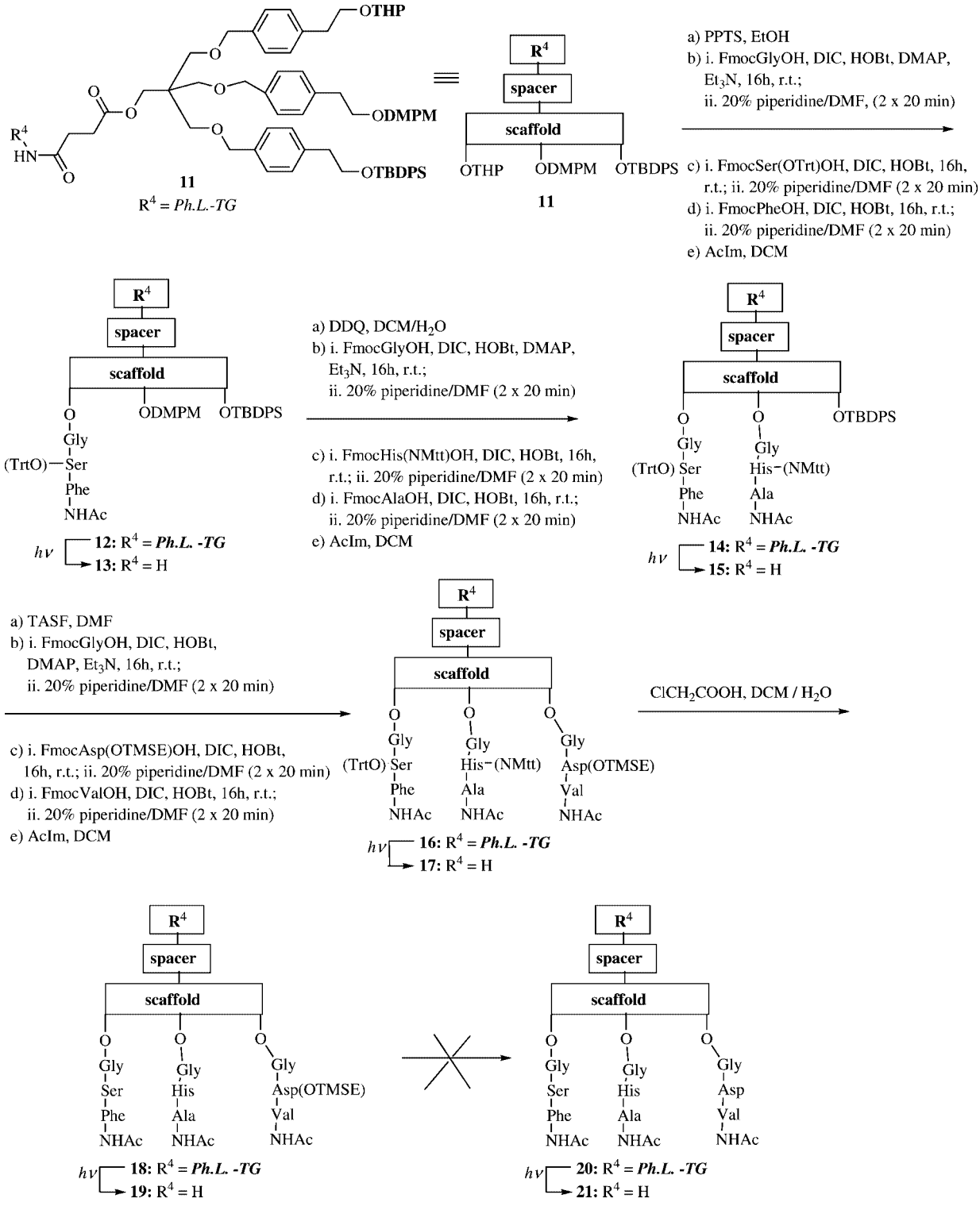
Following subsequent deprotection of the tripodal polymer-supported scaffold **11**, three different tripeptides were constructed on the liberated hydroxy functions. On each peptidic chain, one catalytic residue was introduced. The Fmoc/*t*Bu strategy was applied, thus Nα-Fmoc protected amino acids containing an acid-labile side-chain protecting group were selected. This implies that the tetrahydropyranyl (THP) group, which is cleaved under acidic conditions, needs to be removed first. In a previous communication, the selective deprotection of the THP acetal group was carried out with a solution of $AcOH/CH_2Cl_2/H_2O$ in a 80:15:5 ratio at 60 °C and monitored using gel phase ^{13}C NMR spectroscopy.[7] Nevertheless, in the synthesis of member **21**, as we chose to follow and verify reactions by a cleavage/MS analysis protocol, we found that, using this more sensitive and accurate approach, the earlier reaction conditions described cause an esterification side-reaction leading, to a small extent, to concurrent acetylation of the liberated hydroxy group (which was not observed using the less sensitive gel phase ^{13}C NMR). As an alternative, pyridinium *para*-toluenesulfonate removed the THP protecting group without problems.

Subsequently, a first peptide strand Scaffold-Gly-Ser-(OTrt)-Phe-NHAc (C→N) was generated using a classical peptide synthesis protocol. First attempts to obtain a quantitative coupling of Fmoc-L-glycine on the free hydroxy function, used the reactive reagent FmocGlyCl in a large excess. Since this reagent needed to be freshly prepared before each coupling, the method was replaced by the easy and efficient use of *N*,*N*′-diisopropylcarbodiimide/1-hydroxy-1*H*-benzotriazole (DIC/HOBt) in presence of an excess of diisopropylethylamine and a catalytic amount of 4-(dimethylamino)pyridine.[12] The efficiency of this coupling reaction was monitored using UV/Vis spectroscopy.[13] For the remaining peptide couplings, the *N*-methylmorpholine/(benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (NMM/PyBOP) strategy was initially tested.[14] The success of each coupling reaction was monitored by two colorimetric tests, TNBS[15] and NF31[16] and by exposure of a sample of the resin (1 mg) to UV light at 365 nm for 3 h in acetonitrile followed by ES-MS analysis (see Exp. Sect. and Supporting Information). After final Fmoc deprotection, the free amine was acylated using a large excess of acetylimidazole. The structure of the monopodal member was confirmed after submission of a sample of resin **12** to photolytic cleavage. The structure of the resulting compound **13** was confirmed by ^1H NMR and ES-MS. Accordingly, the second tripeptide was built. A slight excess of dichlorodicyanoquinone at 0 °C for 1 h was used to remove the 3,4-dimethoxyphenylmethyl (DMPM) protecting group. In this step, the reaction time and the temperature were particularly crucial. Cleavage of the benzyl ethers present in the tripodal scaffold was observed when longer reaction times and/or higher temperatures were applied. The functionalised sequence Scaffold-Gly-His(NMtt)-Ala-NHAc (C→N) was generated from the free hydroxy function using similar coupling conditions as before.

After removal of the side-chain trityl ethers in compound **14** with chloroacetic acid, photolytic cleavage in acetonitrile of a sample (1 mg) of the intermediate followed by ES-MS analysis revealed a mixture of the desired compound and a side-product with a lower molecular weight. Surprisingly, characterisation of this side-product showed the cleavage of the first peptide strand that was introduced. This side-reaction was easily avoided by replacing the NMM/PyBOP methodology by the DIC/HOBt coupling strategy.[17]

Using the new coupling strategy, the dipodal peptide **15** was obtained and its structure confirmed after photolysis of resin **14** and characterisation by ^1H NMR spectroscopy. Further analysis of **14** was performed by ES-MS after removal of the side-chain protecting groups on serine and histidine with a solution of chloroacetic acid in dichloromethane. Although a solution of 5% trifluoroacetic acid[18] is usually applied for these deprotections, we chose this milder alternative since the tripodal scaffold is sensitive under harsh acid conditions.

Finally, the *tert*-butyldiphenylsilyl (TBDPS) ether needed to be removed to allow the introduction of the third peptide chain including aspartic acid. Several known methods to



Scheme 3. Synthesis of tripodal member **16**.

deprotect the silyl ether were screened. The standard procedure using tetrabutylammonium fluoride (TBAF) can cause problems during subsequent photolytic cleavage due to the destruction of the *o*-nitrobenzyl unit present in the photolabile linker **10**, a problem that was observed earlier by Nestler.[19] The addition of a stoichiometric amount of acetic acid to the reaction mixture suppresses this side-reaction but when the combination of TBAF/AcOH was used to remove the silyl ether in **14**, partial deprotection of the acid-labile side-chain protecting groups (trityl and methyltrityl) occurred. Similarly, the widely used conditions involving hydrogen fluoride/pyridine were apparently too acidic since the trityl and methyltrityl were removed.[20] As an alternative, the use of ammonium fluoride was tested.

Unfortunately, this led to partial hydrolysis of the ester linkages present in compound **14** as side-reaction.[21] Finally, the use of the less common reagent TASF [tris(dimethylamino)sulfonium (trimethylsilyl)difluoride] was successful in removing the TBDPS group and led to a clean deprotection after 8h of reaction.[22] It should be noted that the quality of the TASF reagent used is important for the success of this reaction. Older samples of reagent gave rise to incomplete deprotections. Subsequently, a last tripeptide Scaffold-Gly-Asp($COO(CH_2)_2SiMe_3$)-Val-NHAc (C→N) was built using the same DIC/HOBt protocol. The most frequently used side-chain protected aspartic acid derivatives include benzyl, allyl, adamantyl or *tert*-Bu esters. These protecting groups are removed under hydrogenolysis, palladium treatment or in the presence of a strong acid.[23] All these deprotection conditions are incompatible with the benzyl ethers in scaffold **1**. Therefore, in order to assure full orthogonality, a trimethylsilylethyl ester (TMSE) protection was chosen for the aspartic acid side-chain. The required Fmoc-protected Asp(OTMSE) was prepared according to literature procedures and was successfully incorporated in the last peptide strand.[24] Despite the well-known occurrence of aspartimide formation during synthesis of Asp-Gly sequences, the desired tripodal member was synthesized without problems.

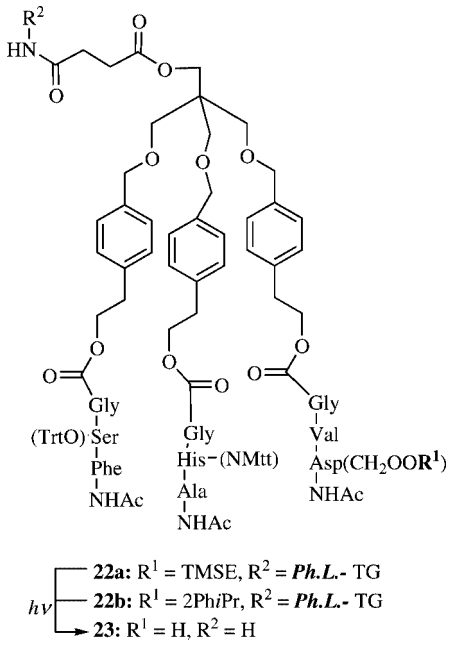
This is supported by the structure analysis by 2D NMR assignment techniques at 500 MHz of the fully protected tripodal member **17**, obtained after photolytic cleavage of a sample of resin **16** (DQF-COSY, TOCSY and ROESY spectra are included in the Supporting Information). Combined analysis of DQF-COSY and TOCSY allowed the straightforward identification of the single Ala and Val residues, as well as the three Gly residues from their typical connectivity patterns.[25] For assignment of the Asp, His, Phe and Ser residues, the amide to aliphatic area of the ROESY spectrum was analyzed for the presence of strong and unambiguous αH_i-NH_j and βH_i-NH_j NOE contacts connecting pairs of amino acid spin systems *i* and *j* identified previously. This allows the assignment and confirmation of the identity of the Val-Asp($COO(CH_2)_2SiMe_3$)-Gly and Ala-His-Gly[2] tripeptides. For the remaining tripeptide the N-terminal residue was identified from a NOE of its NH to the preceding *N*-acetyl methyl resonance and established as a Phe, from the observation of the 75 ms TOCSY spectrum of weak but clear correlations indicative of long-range coupling between resonances in the aromatic region and both βH resonances. The middle residue features the most downfield βH_i resonances, at a position consistent with a serine-type side-chain, thereby establishing the third tripeptide as Phe-Ser(OTrt)-Gly (the detailed analysis procedure is outlined in the Supporting Information). Notwithstanding the fact that **17** still features complete functional group protection as well as the complete scaffold and taking sequential incorporation of each tripeptide onto the scaffold into account, our NMR analyses provides conclusive evidence for the incorporation of the various residues at the desired positions in the polymer bound precursor **16**.

The final deprotection of the side-chain protecting groups of serine, histidine and aspartic acid was then tackled. The trityl protection on the serine and the methyltrityl protection on the histidine were smoothly removed using a solution of chloroacetic acid in dichloromethane to give compound **18**. Following cleavage from the support, intermediate **19** was characterized by ES-MS (see Supporting Information, spectrum 11). However, during the aspartic acid deprotection difficulties were encountered. The usual method described in the literature to remove the TMSE group involves the use of TBAF in DMF.[24] No removal of the TMSE group was observed upon use of TBAF/AcOH. As for the deprotection using TASF, concurrent aspartimide formation took place, as evidenced by the observation of the dehydrated product in the ES-MS spectrum. Aspartimide formation is known to be a problem during the synthesis and deprotection of aspartic acid containing peptides[26] and can usually be suppressed by using sterically hindered protections on the aspartyl side chain such as *tert*-butyl or adamantyl esters.[27] These are to be removed under strongly acidic conditions which, as earlier described, are not compatible with the structure of our tripodal scaffold. Even when the latter is not an issue, difficulties have been encountered during the deprotection of *tert*-butyl or adamantyl protected aspartic acid residues and additional amide backbone protection seems to be necessary to completely exclude aspartimide formation.[28] However, in earlier reports of the synthesis of Asp containing combinatorial peptide libraries this problem was not mentioned,[29] although it is clear from the current discussion that the complete absence of aspartimide formation can only be secured via rigourous quality control at all steps of the synthesis. Also in our case, the desired product can be synthesized and fully characterised in the protected form (vide supra no aspartimide nor β-aspartyl residues were detected during NMR analysis) and problems only arise during the final deprotection step.

For this reason we resynthesized test member **22a** reversing the order in the third strand to give an **Asp-Val-Gly** sequence where the more sterically hindered valine residue should provide protection against aspartimide formation (Scheme 4).

The trityl and methyltrityl protections on Ser and His were smoothly removed with the usual chloroacetic acid solution. Nevertheless, using the fluoride-based reagent TASF for TMSE removal from Asp, dehydration products resulting from aspartimide formation during TMSE deprotection, could still be observed via ES-MS (see Supporting Information, spectrum 14). We therefore decided to systematically explore a variety of deprotecting reagents taking both the lability of the TMSE group and the stability of the scaffold into account. An overview of the conditions tested is given in Table 1.

It is clear from the presented data that no satisfying conditions could be found for the TMSE deprotection. Applying HF/pyridine, after trityl and methyltrityl removal, showed that deprotection of **22a** under these conditions proceeds by concurrent ester cleavage. Use of boron trifluo-



Scheme 4. Structure of the new **Asp-Val-Gly** member.

ride etherate ($BF_3{\cdot}OEt_2$) gave rise to a considerable amount of side products resulting from concurrent cleavage of the various esters and benzyl ethers present in the tripodal peptide.

In view of the difficulties encountered during the final deprotection of the TMSE ether, another side chain protecting group for the aspartic residue had to be selected. Deprotection studies on a representative test sequence showed the Asp(*O*-allyl) protection to cause even more problems related to aspartimide formation, occurring here during Fmoc removal and capping of the sequence. The 2-phenylisopropyl protective group is reported to be removed with 1% TFA,[30] conditions which are still too harsh for the described tripodal scaffold, as evidenced from preliminary tests. However, during test reactions on the protected amino acid building block, it was shown that deprotection could be readily achieved by treatment with chloroacetic acid, conditions previously used for the removal of the trityl and methyltrityl protections on serine and histidine, respectively. Thus, member **22b** (Scheme 4) was synthesized using Fmoc-Asp(O-2-Ph*i*Pr)-OH in the final coupling step. After Fmoc removal and capping, the resin was treated with a 52 wt.-% solution of chloroacetic acid in DCM/H_2O in a 3:1 ratio. In this way, efficient removal of all side-chain protections was achieved as evidenced by the ES-MS spectrum of **23** where the corresponding aspartimide (M-18) could not be detected (see Supporting Information).

From the study presented here, it is clear that for the production of highly-funtionalised tripodal peptide libraries envisaged, aspartimide formation is a serious concern. Therefore, the synthesis strategy should be carefully designed in order to avoid problems of aspartimide formation and more specifically avoid sequences known to be particularly prone to cyclization such as Asp-Gly, Asp-Ala, Asp-Ser and Asp-Asn.[26] Furthermore, although the Asp-OTMSE or Asp-*O*-allyl protective groups in principle allow orthogonal methods to be used for their deprotection, care should be taken since in the current design, using these protections, aspartimide formation could not be avoided. The Asp(O-2-Ph*i*Pr) protection has proven to be the ideal solution for the current orthogonality problem and moreover provides complete protection against unwanted aspartimide formation. The synthesis of libraries of potential serine protease models based on the strategy developed and described here is currently under study.

Conclusion

In conclusion, a new tripodal scaffold has been used in the construction of a single potential member of a library of serine protease mimics, containing the catalytic residues serine, histidine and aspartic acid. In this study, suitable protecting groups were selected and their compatibility was demonstrated. Particular attention has been paid to the analysis and characterisation of the compounds throughout the synthesis. It has been shown that the design of the synthesis strategy deserves special attention when aspartic acid residues are to be included. Only through extensive quality control, e.g. by using ES-MS at every step of the synthesis, the integrity of the final products can be assured. The development of highly functionalised tripodal peptide libraries can now be envisaged and will be reported upon in due course.

Experimental Section

General Remarks: DMF and MeOH were HPLC grade and were provided by Riedel-De-Haën. The coupling reactions were per-

Table 1. Overview of deprotection tests for TMSE removal.

Reagent	Scaffold Stability	TMSE Deprotection
TBAF	**Ph.-L.** not stable	n.t.[a]
TBAF/HOAc	+	SM[b]
TASF	+	aspartimide formation
HF/pyridine	complete ester cleavage	fast deprotection
$BF_3{\cdot}OEt_2$	partial ester and benzyl ether cleavage depending on concentration and reaction time	deprotection observed without aspartimide formation; completion dependent on concentration, temp. and reaction time
$ClCH_2COOH$ 52 wt.-%, DCM/H_2O, 3:1	+	SM

[a] n.t. = not tested. [b] SM = only starting material present.

formed in dry DMF purchased from Biosolve. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride and 1,4-dioxane was distilled from $NaBH_4$ under nitrogen. TentaGel-S-NH_2 resin, Fmoc-amino acids and HOBt were purchased from Novabiochem. Diisopropylcarbodiimide (DIC) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were obtained from Fluka. All reagents and solvents were used as received without further purification. NF31 was synthesized as described previously.[16]

1D ^{1}H NMR and regular DEPT ^{13}C NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively on a Varian Gemini-200 spectrometer, or at 500 MHz and 125.77 MHz, respectively on a Bruker Avance DRX-500 MHz spectrometer. NMR structure analysis of the fully protected tripodal member **17** was performed on 3 mg of the compound dissolved in [D_6]DMSO. A 2D DQF-COSY spectrum and 2D TOCSY spectrum with a 75 ms MLEV17 spin-lock was recorded using standard sequences available from the Bruker pulse program library. The off-resonance 2D ROESY[31] spectrum was recorded with 300 ms mixing time to obtain sufficiently intense NOE cross-peaks for analysis. For all 2D NMR experiments, 512 t1 increments with 4 K data points each were recorded with typically 16 to 64 scans. Following apodisation by a squared cosine bell along both dimensions and zero-filling along F1 to 2 K datapoints a double fourier transformation was executed, followed by a 3rd order polynomial baseline correction along both dimensions.

Mass-spectra were recorded using a Finnigan MAT LCQ mass spectrometer equipped with an ESI source (Thermofinnigan, San José, CA). All measurements were carried out in the positive mode. Deprotection of Nα-Fmoc was performed using 20% piperidine in DMF (2×20 min). Washing volumes were about 2 times the volume used for the reactions. Each coupling and deprotection step was monitored using the TNBS and NF31 tests. The coupling of the free hydroxy function was monitored by Fmoc quantitation using UV/Vis spectroscopy on a Varian Cary 3E spectrophotometer at 300 nm. Resin photolyses were carried out at a distance of 1 cm using a 450-W Hg arc lamp, medium pressure, set at 365 nm. For small quantities of resin (1 mg), photolysis was performed at a distance of 1 cm using a 4 W bioblock Scientific compact UV lamp set at 365 nm (relative intensity at 15 cm of 340 μW/cm^2). All resin bound compounds were analysed by ES-MS after photolytic cleavage (see Supporting Information for spectra of all intermediate and final products).

Synthesis of Triol 2

2-[(*tert*-Butyldiphenylsiloxy)methyl]-2-(hydroxymethyl)propane-1,3-diol: See procedure (a) in Scheme 1. To a solution of pentaerythritol (25 g, 0.184 mol) and imidazole (13.8 g, 0.202 mol) in dimethylformamide (1.2 L), *tert*-butyldiphenylsilyl chloride (26.8 g, 0.098 mol) was added dropwise. The mixture was stirred for 60 h at room temperature. After addition of water, the solution was extracted with ethyl acetate. The organic phase was washed with water, dried with anhydrous magnesium sulfate, filtered and concentrated. The remaining dimethylformamide was removed by kugelrohr distillation under reduced pressure. The oil was purified by HPLC (ethyl acetate/isooctane, 1:1) to provide the mono-protected triol **2** (25 g, 68%). M. p. 50 °C. R_f = 0.25 (ethyl acetate/isooctane, 1:1). IR (KBr): ($\tilde{\nu}$) = 3386, 2930, 2857, 1472, 1428, 1390, 1361, 1113, 1036, 824, 740, 702, 504 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.07 (s, 9 H), 2.75 (s, 3 H), 3.63 (m, 2 H), 3.71 (m, 6 H), 7.41 (m, 6 H), 7.64 (dd, 4 H, J = 8.0, 1.3 Hz) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$, 25 °C): δ = 19.1 (CH_2), 26.9 (CH_3), 45.6 (C), 64.3 (CH_2), 64.7 (CH_2), 65.3 (CH_2), 127.9 (CH), 130.0 (CH), 132.5 (C), 135.6 (CH) ppm. MS: m/z (%) = 233 (10), 199 (100), 181 (19), 139 (76), 105 (25), 91 (91), 57 (51).

2-[(*tert*-Butyldiphenylsiloxy)methyl]-2-{4-(2-tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzyloxymethyl}propane-1,3-diol: See procedure (b) in Scheme 1. Sodium hydride (3.8 g, 128 mmol) (80% dispersion in mineral oil) in THF (250 mL) was added to a solution of mono-TBDPS-protected pentaerythritol (64.0 g, 171 mmol) in dry THF (100 mL). The mixture was stirred for 30 min at 40 °C. After cooling to 10 °C, benzyl bromide **6** (38.5 g, 128 mmol) in THF (60 mL) was added dropwise over a period of 20 min followed by the addition of tetrabutylammonium iodide (0.2 g, 54 mmol). The mixture was then refluxed overnight. The THF was evaporated after addition of an aqueous solution of ammonium chloride (200 mL). The aqueous phase was extracted with EtOAc. The organic phase was washed with water and then dried with anhydrous magnesium sulfate. After filtration and evaporation of the solvent under reduced pressure, the crude compound was purified by column chromatography (toluene/EtOAc, 8:2 to pure EtOAc) affording 34 g (48%) of a yellow oil. R_f = 0.57 (toluene/EtOAc, 1:1). IR (neat): $\tilde{\nu}$ = 3438 (b), 2941 (s), 2864 (s), 1514 (m), 1467 (m), 1110 (s), 1029 (s) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.05 (s, 9 H), 1.50–1.61 (m, 4 H), 1.70 (m, 1 H), 1.81 (m, 1 H), 2.45 (m, 2 H), 2.91 (t, 2 H, J = 7.2 Hz), 3.46 (m, 1 H), 3.55 (s, 2 H), 3.61 (m, 1 H), 3.70 (s, 6 H), 3.76 (m, 1 H), 3.94 (m, 1 H), 4.45 (s, 2 H), 4.60 (m, 1 H), 7.18 (2 H, AB-d, J = 7.9 Hz), 7.21 (2 H, AB-d, J = 7.9 Hz), 7.36–7.45 (m, 6 H), 7.64 (d, 4 H, J = 6.9 Hz) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 19.2, 19.5, 25.4, 26.8, 30.6, 36.0, 45.6, 62.9, 64.8, 64.9, 68.2, 71.6, 73.5, 98.7, 127.6, 127.8, 129.1, 129.9, 132.8, 136.5, 138.7 ppm. MS (ES): m/z 615 [M + Na$^+$]. $C_{35}H_{48}O_6Si$ (592.32): calcd. C 70.91, H 8.15; found C 70.91, H 8.19.

2-Hydroxymethyl-2-{4-[2-(tetrahydro-2*H*-pyran-2-yloxy)-ethyl]benzyloxymethyl}propan-1,3-diol (2): See procedure (c) in Scheme 1. Tetrabutylammonium fluoride (70 mL, 1 M in THF) was added to a solution of diol (36.0 g, 61 mmol) in THF (250 mL). The mixture was stirred at room temperature for 16 h. The solvent was then evaporated under reduced pressure and the oil was purified by column chromatography (toluene/EtOAc, 9:1 to pure EtOAc) affording 18 g of the desired triol (84%). R_f = 0.31 (CH_2Cl_2/MeOH, 93:7). IR (neat): $\tilde{\nu}$ = 3394 (b), 2933 (s), 2861 (s), 1651 (m), 1507 (m), 1456 (m), 1359 (s), 1318 (m), 1256 (m), 1200 (w), 1133 (s), 1113 (s), 1072 (s), 1026 (s) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.47–1.58 (m, 4 H); 1.70 (m, 1 H), 1.80 (m, 1 H), 2.42 (t, 3 H, J = 5.5 Hz), 2.91 (t, 2 H, J = 7.2 Hz), 3.46 (m, 1 H), 3.50 (s, 2 H), 3.61 (dt, 1 H, J = 9.7, 7.2 Hz), 3.72 (d, 6 H, J = 5.3 Hz), 3.77 (m, 1 H), 3.94 (dt, 1 H, J = 9.7, 7.2 Hz), 4.48 (s, 2 H), 4.59 (t, 1 H, J = 3.6 Hz), 7.22 (2 H, AB-d, J = 8.5 Hz), 7.23 (2 H, AB-d, J = 8.5 Hz) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 19.5, 25.4, 30.6, 36.0, 44.9, 62.3, 64.5, 68.2, 72.2, 73.6, 98.7, 127.7, 129.2, 135.4, 138.9 ppm. MS (ES): m/z 377 [M + Na$^+$]. $C_{19}H_{30}O_6$ (354.20): C 64.39, H 8.53; found C 64.41, H 8.47.

Synthesis of Alcohol 3

2-{4-[2-(3,4-Dimethoxybenzyloxy)ethyl]benzyloxymethyl}-2-{4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzyloxymethyl}propan-1,3-diol: See procedure (d) in Scheme 1. Sodium hydride (1.7 g, 56 mmol) (80% dispersion in mineral oil) in dry THF (140 mL) was added to a solution of triol **2** (20 g, 56 mmol) in dry THF (40 mL). The mixture was stirred for 30 min at 40 °C. After cooling to 10 °C, benzyl bromide **7** (20.4 g, 56 mmol) in dry THF (100 mL) was added dropwise over a period of 20 min followed by the addition of tetrabutylammonium iodide (0.2 g, 54 mmol). The solution was then refluxed overnight. After addition of a solution of

ammonium chloride, the THF was evaporated. The aqueous phase was extracted with EtOAc. The organic phase was washed with water and dried with anhydrous magnesium sulfate. After filtration and evaporation, the crude mixture was purified by column chromatography (toluene/EtOAc, 9:1 to pure EtOAc) to give 14 g of an oil (37%). R_f = 0.26 (toluene/EtOAc, 1:1). IR (neat): $\tilde{\nu}$ = 3470 (b), 2939 (s), 2865 (s), 1606 (m), 1594 (m), 1556 (s), 1464 (s), 1420 (m), 1261 (s), 1030 (s) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.48–1.59 (m, 4 H), 1.70 (m, 1 H), 1.79 (m, 1 H), 2.63 (m, 2 H), 2.90 (t, 2 H, J = 6.9 Hz), 2.92 (t, 2 H, J = 6.9 Hz), 3.46 (m, 1 H), 3.54 (s, 2 H), 3.55 (s, 2 H), 3.60 (m, 1 H), 3.66 (m, 6 H), 3.75 (m, 1 H), 3.85 (s, 3 H), 3.87 (s, 3 H), 3.94 (m, 1 H), 4.46 (s, 6 H), 4.59 (t, 1 H, J = 3.4 Hz), 6.82 (m, 3 H), 7.21 (m, 8 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 19.5, 25.4, 30.6, 36.0, 44.8, 55.8, 55.9, 62.2, 65.0, 68.2, 70.8, 72.0, 72.9, 73.6, 98.7, 110.8, 110.9, 120.1, 127.5, 127.6, 128.9, 129.0, 129.1, 130.8, 135.6, 135.7, 138.6, 138.8, 148.7, 148.9 ppm. MS (ES): calcd. for $C_{37}H_{50}O_9Na$: 661, found m/z: 661 [$M + Na^+$].

3-{4-[2-(*tert*-Butyldiphenylsilyloxy)ethyl]benzyloxy}-2-{4-[2-(3,4-dimethoxybenzyloxy)ethyl]-benzyloxymethyl}-2-{4-[2-[tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzyloxymethyl}propan-1-ol (3): See procedure (e) in Scheme 1. Sodium hydride (1.1 g, 36.5 mmol) (80% dispersion in mineral oil) in dry THF (140 mL) was added to a solution of diol (23.3 g, 36.5 mmol) in dry THF (40 mL). The mixture was stirred for 30 min at 40 °C. After cooling to 10 °C, benzyl bromide **7** (16.56 g, 36.5 mmol) in dry THF (60 mL) was added dropwise over a period of 20 min followed by the addition of tetrabutylammonium iodide (0.2 g, 54.0 mmol). The mixture was then refluxed overnight. The THF was then evaporated after addition of a solution of ammonium chloride (200 mL). The aqueous phase was extracted with EtOAc. The organic phase was washed with water and then dried with anhydrous magnesium sulfate. After filtration and evaporation the crude compound was purified by column chromatography (toluene/EtOAc, 1:1) to afford 11.8 g of a yellow oil (32%). R_f = 0.16 (isooctane/EtOAc, 65:35). IR (neat): $\tilde{\nu}$ = 3518 (b), 2934 (s), 1862 (s), 1513 (s), 1482 (s), 1359 (m), 1261 (s), 1108 (s), 1087 (s) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.02 (s, 9 H), 1.54–1.61 (m, 4 H), 1.70 (m, 1 H), 1.81 (m, 1 H), 2.84 (t, 2 H, J = 6.8 Hz), 2.86 (t, 1 H, J = 5.7 Hz), 2.90 (t, 2 H, J = 7.0 Hz), 2.91 (t, 2 H, J = 7.0 Hz), 3.46 (m, 1 H), 3.54 (s, 4 H), 3.55 (s, 2 H), 3.60 (m, 1 H), 3.66 (t, 2 H, J = 7.1 Hz), 3.75 (d, 2 H, J = 6.3 Hz), 3.77 (m, 1 H), 3.83 (t, 2 H, J = 7.0 Hz), 3.85 (s, 3 H), 3.87 (s, 3 H), 3.94 (m, 1 H), 4.44 (s, 6 H), 4.46 (s, 2 H), 4.59 (t, 1 H, J = 3.5 Hz), 6.83 (m, 3 H), 7.10 (2 H, AB-d, J = 8.0 Hz), 7.17 (2 H, AB-d, J = 8.0 Hz), 7.19 (m, 8 H), 7.33–7.42 (m, 6 H), 7.59 (d, 4 H, J = 6.8 Hz) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 19.2, 19.5, 25.4, 26.8, 30.7, 36.1, 38.4, 45.0, 55.8, 55.9, 62.2, 65.1, 66.2, 68.2, 70.9, 72.9, 73.4, 98.7, 110.8, 111.0, 120.1, 127.4, 127.5, 127.6, 128.9, 129.0, 129.2, 129.6, 129.9, 130.8, 133.7, 135.6, 136.0, 136.1, 136.2, 138.3, 138.5, 146.5, 148.9 ppm. MS (ES): m/z 1028 ($M + NH_4^+$). $C_{62}H_{78}O_{10}Si$ (1010.5): calcd. C 73.63 H 7.78; found C 73.53, H 8.07.

Synthesis of Tripodal Scaffold Acid 1: See procedure (f) in Scheme 1. To a solution of alcohol **3** (0.5 g, 0.50 mmol) in CH_2Cl_2 (35 mL), succinic anhydride (0.513 g, 4.95 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.73 g, 5.93 mmol) were added. The solution was stirred at room temperature for 4 h. The mixture was extracted with an saturated aqueous solution of ammonium chloride. The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The resulting oil was then purified by column chromatography (isooctane/EtOAc, 6:4) to provide 0.5 g of acid **1** (90%). R_f = 0.34 (EtOAc/isooctane, 4:6). IR (neat): $\tilde{\nu}$ = 3400 (s), 2922 (s), 2853 (s), 1738 (w), 1712 (w), 1656 (w), 1630 (w), 1512 (w), 1456 (w), 1425 (w), 1359 (w), 1256 (w), 1153 (w), 1092 (w), 10125 (w) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.02 (s, 9 H), 1.55 (m, 4 H), 1.70 (m, 1 H), 1.80 (m, 1 H), 2.45 (2 H, AB-t, J = 5.3 Hz), 2.48 (2 H, AB-t, J = 5.3 Hz), 2.83 (t, 2 H, J = 7.0 Hz), 2.89 (t, 2 H, J = 7.1 Hz), 2.90 (t, 2 H, J = 6.8 Hz), 3.47 (s, 2 H), 3.49 (s, 4 H), 3.60 (m, 1 H), 3.66 (m, 1 H), 3.66 (t, 2 H, J = 7.0 Hz), 3.79 (m, 1 H), 3.82 (t, 2 H, J = 7.0 Hz), 3.84 (s, 3 H), 3.87 (s, 3 H), 3.92 (m, 1 H), 4.22 (s, 2 H), 4.41 (s, 3 H), 4.42 (s, 3 H), 4.47 (s, 2 H), 4.61 (t, 1 H, J = 3.3 Hz), 6.82 (m, 3 H), 7.09 (d, 2 H, J = 7.9 Hz), 7.18 (m, 10 H), 7.33–7.41 (m, 6 H), 7.60 (dd, 4 H, J = 7.8, 1.2 Hz) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 19.1, 19.4, 26.3, 26.7, 28.2, 28.6, 29.0, 30.6, 35.9, 38.9, 44.5, 55.6, 55.8, 62.1, 64.0, 65.0, 68.2, 68.9, 70.8, 72.7, 73.1, 98.7, 110.8, 110.9, 120.1, 127.4, 127.4, 128.7, 128.8, 129.0, 129.5, 130.8, 133.6, 135.4, 136.3, 138.0, 138.2, 148.4, 148.8, 171.7, 176.6 ppm. MS (ES): m/z 1128 ($M + NH_4^+$). $C_{66}H_{82}O_{13}Si$ (1110.6): calcd. C 71.36, H 7.44; found C 70.72, H 7.63.

Synthesis of Building Block 6, 7 and 8

Bromination of Commercially Available 2-(4-Methylphenyl)acetic Acid. Synthesis of Acid 4: See procedure (g) in Scheme 1. To a solution of 2-(4-methylphenyl)acetic acid (25 g, 0.17 mol) in carbon tetrachloride (300 mL), bromine (8.7 mL, 0.17 mol) and 2,2′-azobis(isobutyronitrile) (catalytic amount) were added. The solution was irradiated using a sodium lamp (400 W) and was heated to 40 °C. After 5 min, white crystals precipitated. The mixture was cooled to 0 °C and the crystals were filtered off and washed with carbon tetrachloride. The residue was recrystallized from ethyl acetate to provide the product **4** as white crystals (24 g, 62%). M. p. 180 °C. R_f = 0.20 (toluene/ethyl acetate, 8:2). IR (KBr): $\tilde{\nu}$ = 3027, 2972, 1699, 1516, 1408, 1343, 1244, 1184, 1093, 894, 838, 780, 675, 602 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 25 °C): δ = 3.60 (s, 2 H), 4.50 (s, 2 H), 7.26 (m, 2 H), 7.36 (m, 2 H) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$ + a few drops of [D_6]DMSO, 25 °C): δ = 33.0 (CH_2), 40.6 (CH_2), 128.8 (CH), 129.4 (CH), 134.6 (C), 135.9 (C), 173.0 (C) ppm. MS: m/z (%) = 230 ($M^{+\cdot}Br^{81}$), 228 ($M^{+\cdot}Br^{79}$), 150 (13), 150 (19), 149 (100), 131 (5), 104 (42), 77 (26), 63 (9), 45 (20).

Reduction of Carboxylic Acid 4 into Alcohol 5: See procedure (h) in Scheme 1. To a cooled (0 °C) solution of carboxylic acid **4** (20.0 g, 0.087 mol) in dry tetrahydrofuran (120 mL) borane–dimethyl sulfide complex (11.6 mL, 0.13 mol) was added dropwise. The solution was stirred at 0 °C for 2 h and at room temperature for 2 h. Water was added slowly and tetrahydrofuran was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate. The combined organic phases were dried with anhydrous magnesium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (ethyl acetate/isooctane, 4:6) to afford alcohol **5** (16 g, 86%). M. p. 76 °C. R_f = 0.28 (isooctane/ethyl acetate, 6:4). IR (KBr): $\tilde{\nu}$ = 3395, 3333, 2950, 1513, 1477, 1418, 1228, 1201, 1100, 1044, 836, 766 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 2.87 (t, 2 H, J = 6.5 Hz), 3.86 (t, 2 H, J = 6.5 Hz), 4.49 (s, 2 H), 7.22 (m, 2 H), 7.35 (m, 2 H) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$, 25 °C): δ = 33.4 (CH_2), 38.8 (CH_2), 63.4 (CH_2), 129.2 (CH), 129.4 (CH), 135.9 (C), 138.9 (C) ppm. MS: m/z (%) = 216 ($M^{+\cdot}Br^{81}$), 214 ($M^{+\cdot}Br^{79}$), 135 (100), 105 (63), 104 (54), 91 (10), 77 (22), 63 (10), 51 (13).

Synthesis of Building Block 6: See procedure (i) in Scheme 1. Dihydropyran (5.10 mL, 0.056 mol) and pyridinium *p*-toluenesulfonate (1.25 g, 0.005 mol) were added to a solution of alcohol **5** (10 g, 0.047 mol) in dry dichloromethane (40 mL). The solution was stirred overnight at room temperature. The mixture was extracted with a saturated aqueous solution of sodium hydrogen carbonate

and then with brine. The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (isooctane/ethyl acetate: 9:1) providing building block **6** (12.4 g, 88%). R_f = 0.69 (toluene/ethyl acetate, 8:2). IR (KBr): $\tilde{\nu}$ = 2941, 2868, 1514, 1439, 1352, 1229, 1200, 1120, 1077, 1031, 972, 907, 869, 814, 606 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.54 (m, 4 H), 1.69 (m, 1 H), 1.80 (m, 1 H), 2.91 (t, 2 H, J = 7.1 Hz), 3.45 (m, 1 H), 3.62 (dt, 1 H, J = 9.7, 7.2 Hz), 3.73 (m, 1 H), 3.94 (dt, 1 H, J = 9.7, 7.2 Hz), 4.48 (s, 2 H), 4.59 (m, 1 H), 7.22 (2 H, *A*B-d, J_{AB} = 8.0 Hz), 7.31 (2 H, A*B*-d, J_{AB} = 8.0 Hz) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$, 25 °C): δ = 19.4 (CH_2), 25.3 (CH_2), 30.5 (CH_2), 33.6 (CH_2), 35.9 (CH_2), 62.1 (CH_2), 67.9 (CH_2), 98.6 (CH), 128.9 (CH), 129.3 (CH), 135.5 (C), 139.5 (C) ppm. MS: *m*/*z* (%) = 299 ($M^{+\cdot}Br^{81}$), 297 ($M^{+\cdot}Br^{79}$), 117 (80), 85 (100), 67 (71), 41 (30).

Synthesis of Building Block 7

Synthesis of Trichloroacetimidate 9: A solution of 3,4-dimethoxybenzyl alcohol (25 g, 0.149 mol) in tetrahydrofuran (30 mL) was slowly added to a suspension of sodium hydride (60% dispersion in oil) (1.19 g, 0.030 mol) in tetrahydrofuran (30 mL). The solution was then cooled to 0 °C and trichloroacetonitrile (17.6 mL, 0.175 mol) was added dropwise. The mixture was stirred for 1 h at 0 °C and 2 h at room temperature. Pentane (50 mL) containing methanol (1 mL) was added followed by activated carbon. The mixture was stirred for 1 h before being filtered through celite. The celite was then washed with pentane. The organic phase was concentrated under reduced pressure affording the trichloroacetimidate, which was used without further purification (45 g, 97%). R_f = 0.59 (toluene/ethyl acetate, 8:2). IR (KBr): $\tilde{\nu}$ = 3330, 2935, 1662, 1593, 1518, 1463, 1420, 1267, 1240, 1160, 1079, 1028, 828, 796, 648 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 3.88 (s, 6 H), 5.28 (s, 2 H), 6.86 (d, 1 H, J = 8.1 Hz), 6.98 (m, 2 H), 8.37 (s, 1 H) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$, 25 °C): δ = 55.8 (CH_3), 70.7 (CH_2), 91.4 (C), 110.8 (CH), 111.1 (CH), 120.6 (CH), 127.8 (C), 148.9 (C), 149.0 (C), 162.4 (C) ppm.

Synthesis of Building Block 7: To a solution of alcohol **5** (6.5 g, 0.030 mol) and pyridinium *p*-toluenesulfonate (3.8 g, 0.015 mol) in dichloromethane (48 mL) was added trichloroacetimidate **9** (18.8 g, 0.06 mol). The solution was stirred overnight at room temperature. After addition of a saturated aqueous solution of sodium hydrogencarbonate, the mixture was extracted with dichloromethane, dried with magnesium sulfate and filtered. The residue was purified by column chromatography (20% ethyl acetate in toluene/pentane, 1:1) to afford building block **6** (7.6 g, 69%). R_f = 0.61 (toluene/ethyl acetate, 8:2). IR (KBr): $\tilde{\nu}$ = 2935, 2857, 1593, 1516, 1464, 1419, 1264, 1237, 1157, 1094, 1028, 809, 607 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 2.91 (2 H, J = 6.9 Hz), 3.66 (t, 2 H, J = 6.9 Hz), 3.84 (s, 3 H), 3.87 (s, 3 H), 4.45 (s, 2 H), 4.48 (s, 2 H), 6.82 (m, 3 H), 7.20 (2 H, *A*B-d, J_{AB} = 8.0 Hz), 7.31 (2 H, A*B*-d, J_{AB} = 8.0 Hz) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): δ = 33.5 (CH_2), 35.9 (CH_2), 55.7 (CH_3), 55.8 (CH_3), 70.5 (CH_2), 72.8 (CH_2), 110.7 (CH), 120.0 (CH), 128.9 (CH), 129.3 (CH), 130.7 (C), 135.6 (C), 139.5 (C), 148.4 (C), 148.9 (C) ppm. MS: *m*/*z* (%) = 366 ($M^{+\cdot}Br^{81}$), 364 ($M^{+\cdot}Br^{79}$), 151 (100), 104 (18), 77 (12), 65 (8).

Synthesis of Building Block 8: *tert*-Butyldiphenylsilyl chloride (25.6 g, 0.093 mol) and diisopropylethylamine (28.4 mL, 0.163 mol) were added to a solution of alcohol **5** (10 g, 0.047 mol) in dimethylformamide (50 mL). The mixture was stirred at room temperature for 4 h. The mixture was added to ice and extracted with diethyl ether. The organic phase was dried with anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The crude oil was purified by column chromatography (diethyl ether/isooctane, 1:9) to give building block **8** (15 g, 71%). R_f = 0.58 (diethyl ether/isooctane, 1:9). IR (KBr): $\tilde{\nu}$ = 3047, 2930, 2857, 1742, 1427, 1266, 1111, 823, 702, 505 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.04 (s, 9 H), 2.87 (t, 2 H, J = 6.8 Hz), 3.85 (t, 2 H, J = 6.8 Hz), 4.59 (s, 2 H), 7.16 (2 H, *A*B-d, J_{AB} = 8.0 Hz), 7.30 (2 H, A*B*-d, J = 8.0 Hz), 7.37 (m, 4 H), 7.42 (m, 2 H), 7.60 (dd, 4 H, J = 6.6, 1.4 Hz) ppm. ^{13}C NMR/DEPT (50 MHz, $CDCl_3$, 25 °C): δ = 19.1 (C), 26.8 (CH_2), 38.9 (CH_2), 46.9 (CH_2), 64.9 (CH_2), 127.6 (CH), 128.5 (CH), 129.6 (CH), 133.7 (C), 135.3 (C), 135.5 (CH), 139.6 (C) ppm. MS: *m*/*z* (%) = 351 (57), 247 (20), 217 (100), 181 (42), 117 (64), 91 (54), 57 (44).

Synthesis of Monopodal Member 13

Coupling of the Photocleavable Linker 10 onto TentaGel-NH_2: Diisopropylcarbodiimide (DIC) (27 μL, 0.174 mmol) and 1-hydroxybenzotriazole (HOBt) (27 mg, 0.200 mmol) were added to a solution of linker **10** (90.5 mg, 0.174 mmol) in dry DMF (3 mL). The mixture was stirred for 30 min at room temperature. After adding the solution to the resin (300 mg, 0.21 mmol/g, 0.063 mmol), the suspension was shaken overnight at room temperature. The resin was washed with DMF (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL). To monitor the completeness of the coupling reaction, two colorimetric tests (TNBS and NF31) were performed and led to colourless beads.

TNBS: To a few beads, 3 drops of a solution of 10% diisopropylethylamine (DIPEA) in DMF and 3 drops of trinitrobenzenesulfonic acid (TNBS) are added. The appearance of an orange colour shows the presence of remaining uncoupled amino functions whereas colourless beads indicate completeness of the coupling.

NF 31: A few beads are suspended in NF31 (100 μL, 0.002 M) in acetonitrile. After heating for 10 min at 70 °C, the beads are thoroughly washed with DMF, MeOH and DCM. Beads containing free amino functions appear red, while completely coupled beads remain colourless.

General Procedure for the Deprotection of the Fmoc Group: The resin was treated with a solution of 20% piperidine in DMF (2 × 20 min). The resin was then washed with DMF (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL). Both colorimetric tests were carried out to confirm deprotection.

Synthesis of Construct 11: 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (67 mg, 0.348 mmol) and 4-(dimethylamino)pyridine (DMAP) (2 mg, 0.016 mmol) were added to a solution of scaffold **1** (140 mg, 0.126 mmol) in CH_2Cl_2 (3 mL). After activation of the acid for 30 min at room temperature, the solution was added to the resin (300 mg, 0.060 mmol, 0.20 mmol/g). The suspension was shaken overnight at room temperature. The resin was washed with DMF (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL). Both colorimetric tests were carried out and showed colourless beads. Gel-phase ^{13}C NMR (50 MHz, 100 mg resin suspended in CD_2Cl_2, 25 °C): δ = 20.3, 22.9, 26.3, 28.0, 30.4, 31.7, 31.8, 32.1, 33.6, 37.3, 40.3, 40.6, 45.8, 47.5, 63.3, 64.6, 65.0, 66.5, 71.5 (polyethylene glycol peak), 73.9, 74.5, 99.9, 110.6, 112.4, 112.5, 121.3, 128.9, 129.0, 129.3, 129.4, 130.2, 130.5, 130.8, 131.0, 131.1, 132.5, 135.0, 136.8, 137.0, 137.8, 139.8, 139.9, 141.6, 148.1, 148.2, 150.4, 155.2, 172.2, 173.3, 174.1 ppm.

Removal of the THP-Protecting Group: Pyridinium *p*-toluenesulfonate (0.066 g, 0.265 mmol) was added to a suspension of resin **11** (0.300 g, 0.049 mmol, 0.163 mmol/g) in dry ethanol (3.2 mL). The mixture was heated overnight at 55 °C. After filtration, the resin was washed with ethanol (3 × 5 mL), dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). A sample of resulting resin (2 mg, 0.33 μmol, 0.163 mmol/g) was

suspended in 100 μL acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analysed by ES-MS. ES-MS: m/z = 1048 $[M+Na]^+$ (see also Supporting Information, spectrum 1).

Coupling of Fmoc-L-glycine: To a suspension of resin (0.30 g, 0.050 mmol, 0.165 mmol/g) in dry dimethylformamide (3 mL) were added FmocGlyOH (125 mg, 0.42 mmol), 1-hydroxybenzotriazole (56.7 mg, 0.42 mmol), 4-(dimethylamino)pyridine (2.6 mg, 0.021 mmol), diisopropylcarbodiimide (67 μL, 0.42 mmol) and diisopropylethylamine (73 μL, 0.42 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). The yield of the coupling was determined by UV/Vis spectroscopy monitoring at 300 nm (102%) (see also Supporting Information, ES-MS spectrum 2 of a representative sample upon cleavage). The Fmoc-protecting group was removed according to the general procedure.

Coupling of FmocSer(OTrt)OH: FmocSer(OTrt)OH (0.144 g, 0.252 mmol) was activated with diisopropylcarbodiimide (40.0 μL, 0.252 mmol) and 1-hydroxybenzotriazole (34.0 mg, 0.252 mmol). This solution was stirred for 30 min at room temperature and then added to the resin (0.300 g, 0.049 mmol, 0.164 mmol/g). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). A sample of the resin was submitted to both colorimetric tests (TNBS and NF31) and colourless beads were observed. A sample of the resulting resin (2 mg, 0.320 μmol, 0.150 mmol/g) was suspended in 100 μL acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS. ES-MS: m/z = 1656 $[M+Na]^+$, 1672 $[M+K]^+$ (see also Supporting Information, ES-MS spectrum 3). The Fmoc-protecting group was removed according to the general procedure.

Coupling of FmocPheOH: FmocPheOH (81.4 mg, 0.210 mmol) was activated with diisopropylcarbodiimide (33.0 μL, 0.210 mmol) and 1-hydroxybenzotriazole (29.0 mg, 0.210 mmol). This solution was stirred for 30 min at room temperature and then added to the resin (0.30 g, 0.047 mmol, 0.155 mmol/g). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). A sample of the resin was submitted to both colorimetric tests (TNBS and NF31) and colourless beads were observed. A sample of 2 mg of the resin (2 mg, 0.300 μmol, 0.147 mmol/g) was suspended in 100 μL acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS. ES-MS: m/z = 1804 $[M+Na]^+$, 1820 $[M+K]^+$ (see Supporting Information, spectrum 4).The Fmoc-protecting group was removed according to the general procedure.

Capping of the Free Amine: Acetylimidazole (77 mg, 0.700 mmol) was added to a suspension of resin (0.250 g, 0.038 mmol, 0.152 mmol/g) in dry dichloromethane (3 mL). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). A sample of the resin **12** was submitted to both colorimetric tests (TNBS and NF31) and colourless beads were observed. IR (KBr): $\tilde{\nu}$ = 3572, 3278, 2860, 1734, 1669, 1511, 1453, 1370, 1264, 1073, 947, 841, 698 cm^{-1}. ^{13}C NMR (50 MHz, CD_2Cl_2, 25 °C): δ = 174.2, 173.3, 172.3, 171.4, 155.5, 150.0, 147,9, 145.1, 141.9, 140.2, 138.1, 137.1, 135.4, 131.2, 130.8, 130.4, 130.2, 129.9, 129.3, 129.0, 121.6, 112.8, 112.7, 111.0, 110.9, 88.3, 74.7, 74.2, 73.7, 72.1 (polyethylene glycol peak), 71.2, 71.1, 70.2, 67.1, 66.8, 65.0, 57.9, 57.4, 47.7, 46.2, 40.9, 40.5, 37.6, 36.2, 34.0, 32.0, 28.3, 26.6, 24.4, 23.1, 20.6 ppm.

Characterisation of 13: A sample of resin **12** (34.2 mg, 0.005 mmol, 0.152 mmol/g) in suspension in 1,4-dioxane (1 mL) containing 1% of dimethyl sulfoxide was irradiated with UV light for 4 times 2 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethyl sulfoxide in a kugelrohr, the sample was purified by column chromatography (ethyl acetate) to provide 3.7 mg of the cleaved product **13** (45%). R_f = 0.53 (ethyl acetate). ^1H NMR (500 MHz, CD_2Cl_2, 25 °C): δ = 1.01 (s, 9 H), 1.88 (s, 3 H), 2.35 (2 H, *A*B-t, J = 7.0 Hz), 2.50 (2 H, A*B*-t, J = 7.0 Hz), 2.84 (t, 2 H, J = 6.7 Hz), 2.87 (t, 2 H, J = 6.7 Hz), 2.93 (t, 2 H, J = 7.0 Hz), 2.93 (dd, 1 H, J = 7.5, 14.0 Hz), 3.06 (dd, 1 H, J = 6.8, 14.0 Hz), 3.18 (dd, 1 H, J = 5.1, 9.0 Hz), 3.49 (m, 6 H), 3.52 (m, 1 H), 3.65 (t, 2 H, J = 7.0 Hz), 3.78 (s, 3 H), 3.80 (s, 3 H), 3.83 (t, 2 H, J = 7.0 Hz), 3.84 (m, 1 H), 4.03 (1 H, AB-d, J = 7.78 Hz), 4.16 (s, 2 H), 4.32 (t, 2 H, J = 7.0 Hz), 4.41 (m, 8 H), 4.52 (dd, 1 H, J = 4.9 Hz), 4.58 (dd, 1 H, J = 7.2 Hz), 6.81 (m, 3 H), 7.27 (m, 38 H), 7.58 (dd, 4 H, J = 1.4, 7.9 Hz) ppm. ES-MS: m/z = 1624 $[M+Na]^+$, 1639 $[M+K]^+$ (see Supporting Information, spectrum 5).

Synthesis of Dipodal Member 14

Removal of the DMPM Protecting Group: To a cooled (0 °C) suspension of resin **12** (0.15 g, 0.023 mmol, 0.152 mmol/g) in a mixture of dichloromethane/water (1.4 mL/0.1 mL) was added dichlorodicyanoquinone (14.3 mg, 0.063 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with an aqueous saturated solution of sodium hydrogencarbonate (3 × 5 mL), phenol/methanol, 1:1 (3 × 5 mL), toluene (3 × 5 mL), acetonitrile (3 × 5 mL), dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL). A sample of the resulting resin (2 mg, 0.300 μmol, 0.15 mmol/g) was suspended in acetonitrile (100 μL) and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS. ES-MS: m/z 1473 $[M+Na]^+$ (see Supporting Information, spectrum 6).

Synthesis and Capping of the Tripeptide Strand: The coupling of FmocGlyOH (Supporting Information, spectrum 7), FmocHis(NMtt)OH (Supporting Information, spectrum 8) and FmocAlaOH was performed as explained for the first peptide strand. After final Fmoc deprotection the free amine was capped using AcIm as described above.

Characterisation of 15: A sample of resin **14** (72 mg, 0.010 mmol, 0.142 mmol/g) in 1,4-dioxane (1 mL) containing 1% of dimethyl sulfoxide was irradiated with UV light for 4 times 2 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethyl sulfoxide with the kugelrohr, the sample was purified by column chromatography (dichloromethane/methanol) to provide the cleaved product **15**. R_f(dichloromethane/methanol) = 9:1. IR (KBr): $\tilde{\nu}$ = 3281, 2953, 2855, 1736, 1675, 1649, 1551, 1536, 1512, 1452, 1419, 1256, 1093, 1022, 799, 668 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 25 °C): δ = 0.96 (s, 3 H), 1.27 (d, 3 H, J = 7.6 Hz), 1.83 (s, 3 H), 1.91(s, 3 H), 2.29 (s, 3 H), 2.40 (2 H, *A*B-t, J = 7.0 Hz), 2.47 (2 H, A*B*-t, J = 7.0 Hz), 2.83 (m, 7 H), 2.91 (dd, 1 H, J = 7.0, 14.8 Hz), 3.00 (dd, 1 H, J = 5.7, 14.0 Hz), 3.09 (dd, 1 H, J = 5.7, 14.0 Hz), 3.34 (dd, 1 H, J = 5.5, 9.3 Hz), 3.39 (1 H, J = 5.5, 9.3 Hz), 3.44 (s, 2 H), 3.44 (s, 2 H), 3.45 (s, 2 H), 3.79 (m, 4 H), 3.84 (1 H, *A*B-d, J = 17.8 Hz), 3.93 (1 H, A*B*-d, J = 17.8 Hz), 4.13 (s, 2 H), 4.14 (t, 2 H, J = 7.1 Hz), 4.23 (m, 1 H), 4.24 (t, 2 H, J = 7.2 Hz), 4.35 (s, 2 H), 4.36 (s, 2 H), 4.39 (s, 2 H), 4.60 (m, 2 H), 4.65 (dd, 1 H, J = 6.1, 9.4 Hz), 6.76 (s, 1 H), 7.18 (m, 53 H), 7.51 (dd, 4 H, J = 1.3, 7.9 Hz) ppm.

In view of the limited mass range ($m/z < 2000$) of the available ES-MS equipment mass spectrometric analysis of **15** and subsequent products requires removal of the side-chain protection on serine (*O*-trityl) and histidine (*N*-methyltrityl).

Therefore, a sample of resin **14** (10 mg, 1.4 μmol, 0.142 mmol/g) was treated overnight at room temperature with a solution of chloroacetic acid (1.32 g) in a mixture of dichloromethane/H_2O (3:1, 1 mL). The procedure was repeated 3 times within two hours. A sample of the resulting resin (2 mg, 0.280 μmol, 0.140 mmol/g) was suspended in acetonitrile (100 μL) and was irradiated with UV light (365 nm) for 3 h. The solution was analysed by ES-MS. ES-MS: m/z = 1538 $[M + Na]^+$ (Supporting Information, spectrum 9).

Synthesis of Tripodal Member 18

Removal of the TBDPS Protecting Group: To a suspension of resin **14** (150 mg, 0.021 mmol, 0.142 mmol/g) in dimethylformamide (0.36 mL) was added TASF (0.0975 M) [tris(dimethylamino)sulfur (trimethylsilyl)difluoride] in dimethylformamide (0.82 mL, 0.084 mmol). The suspension was shaken for 3 h at room temperature. After filtration, the resin was washed with dimethylformamide (3 × 5 mL), methanol (3 × 5 mL) and dichloromethane (3 × 5 mL).

Synthesis of the Tripeptide Strand: The coupling of FmocGlyOH (Supporting Information, spectrum 10, after Trt and Mtt removal), FmocAsp[$COO(CH_2)SiMe_3$]OH and FmocValOH was performed as explained for the first peptide strand. After final Fmoc deprotection the free amine was capped using AcIm as described above.

Characterisation of 17: A sample of resin **16** (36.7 mg, 0.005 mmol, 0.141 mmol/g) in suspension of 1,4-dioxane (1 mL) containing 1% of dimethyl sulfoxide was irradiated with UV light for 3 times 2 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethyl sulfoxide with the kugelrohr, the sample was purified by column chromatography (ethyl acetate) to provide 5.6 mg of compound **17** (51%). MW = 2188 g/mol, R_f = 0.52 (methanol/dichloromethane, 1:9). See Results and Discussion for ^1H NMR characterisation.

Deprotection of *O*-Trityl (Ser) and *N*-Methyltrityl (His): A sample of resin **16** (10 mg, 1.4 μmol, 0.140 mmol/g) was treated with a solution of chloroacetic acid (1.32 g) in a mixture dichloromethane/H_2O (3:1, 1 mL) overnight at room temperature. The procedure was repeated 3 times two hours. A sample of the resulting resin **18** (2 mg, 0.280 μmol, 0.140 mmol/g) was suspended in acetonitrile (100 μL) and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS. ES-MS: m/z = 869 $[M + 2Na]^{2+}$, 1713 $[M + Na]^+$ (see Supporting Information, spectrum 11).

Synthesis of Tripodal Member 22a: The synthesis was performed as described for **18** changing the order of introduction of amino acids in the third strand to Fmoc-Gly-OH (Supporting Information spectrum 10, after Trt and Mtt removal), Fmoc-Val-OH (Supporting Information spectrum 12, after Trt and Mtt removal) and Fmoc-Asp(OTMSE)-OH. After final Fmoc deprotection the free amine was capped using AcIm as described above. The Ser-OTrt and His-NMtt protections were removed with a solution of chloroacetic acid as described above for resin **16** (Supporting Information spectrum 13). Subsequent treatment with TASF clearly caused aspartimide formation. ES-MS analysis proved dehydration to be faster than deprotection as evidenced from the signals at 1573 ($M - TMSE - H_2O + H^+$) and 1691 ($M + H^+$, still containing OTMSE) (see Supporting Information, spectrum 14).

Synthesis of Tripodal Member 23: Resin **14** was deprotected as described above. Coupling of Fmoc-Gly-OH (Supporting Information spectrum 10, after Trt and Mtt removal), Fmoc-Val-OH (see Supporting Information, spectrum 12, after Trt and Mtt removal) and Fmoc-Asp(O2Ph*i*Pr)-OH was performed as explained before. Resin **22b** was then treated treated with a solution of chloroacetic acid (1.32 g) in a mixture dichloromethane/H_2O (3:1, 1 mL) overnight at room temperature. The procedure was repeated 3 times within two hours. A sample of the resulting resin was suspended in acetonitrile (100 μL) and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS. ES-MS: m/z = 1591 $[M + H]^+$ (see Supporting Information, spectrum 15).

Supporting Information (see also the footnote on the first page of this article): Detailed Table with energy values for the Molecular Modeling calculations related to Figure 1. Top view pictures of relevant conformations. 2D NMR assignment at 500 MHz of the fully protected tripodal member **17**. ES-MS spectra of all intermediate and final compounds after cleavage from solid support.

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[1] a) Y. Murakami, J. Kikuchi, O. Hayashida, *Chem. Rev.* **1996**, *96*, 721–758; b) W. B. Motherwell, M. J. Bingham, Y. Six, *Tetrahedron* **2001**, *57*, 4663–4686.
[2] A. J. Kirby, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 707–724.
[3] a) J. M. Lehn, C. Sirlin, *J. Chem. Soc., Chem. Commun. J. Chem. Soc., Chem. Commun* **1978**, 949–951; b) D. J. Cram, P. Y. Lam, P. S. Ho, *J. Am. Chem. Soc.* **1986**, *108*, 839–841; c) V. T. D'Souza, M. L. Bender, *Acc. Chem. Res.* **1987**, *20*, 146–152; d) R. Breslow, S. Chung, *Tetrahedron Lett.* **1989**, *30*, 4353–4356; e) R. C. F. Jones, M. Tankart, A. M. Higton, *Bioorg. Med. Chem. Lett.* **1991**, *1*, 353–356; f) L. G. Mackay, R. S. Wylie, J. K. M. Sanders, *J. Am. Chem. Soc.* **1994**, *116*, 3141–3142; g) R. Breslow, *Acc. Chem. Res.* **1995**, *28*, 146–153; h) B. S. Lele, M. G. Kulkarni, R. A. Mashelkar, *React. Funct. Polym.* **1999**, *39*, 37–52.
[4] a) K. W. Hahn, W. A. Klis, J. M. Stewart, *Science* **1990**, *248*, 1544–1547; b) M. Z. Atassi, T. Manshouri, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8282–8286; c) B. Walse, M. Ullner, C. Lindbladh, L. Bulow, T. Drakenberg, O. Teleman, *J. Comput.-Aided Mol. Des.* **1996**, *10*, 11–22; d) R. A. Buono, N. Kucharczyk, M. Neuenschwander, J. Kemmik, L. Y. Hwang, J. L. Fauchere, C. A. Venanzi, *J. Comput.-Aided Mol. Des.* **1996**, *10*, 213–232; e) Y. Fukushima, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2269–2274; f) A. Stavrakoudis, I. N. Demetropoulos, C. Sakarellos, M. Sakarellos-Daitsiotis, V. Tsikeris, *Lett. Pept. Sci.* **1997**, *4*, 481–487.
[5] M. L. Snapper, A. H. Hoveyda, *Combinatorial Chemistry, practical approach*, Oxford University Press, **2003**, pp. 433–457.
[6] a) H. De Muynck, A. Madder, N. Farcy, J. P. De Clercq, M. N. Pérez-Payàn, L. M. Öhberg, A. P. Davis, *Angew. Chem. Int. Ed.* **2000**, *39*, 145–148; b) A. Madder, L. Liu, H. De Muynck, N. Farcy, D. Van Haver, F. Fant, G. Vanhoenacker, P. Sandra, A. P. Davis, J. P. De Clercq, *J. Comb. Chem.* **2002**, *4*, 552–562; c) G. Vanhoenacker, L. Liu, F. Lynen, A. Madder, P. De Clercq, P. Sandra, *J. Sep. Sci.* **2002**, *25*, 671–676.
[7] N. Farcy, H. De Muynck, A. Madder, N. Hosten, J. P. De Clercq, *Org. Lett.* **2001**, *3*, 4299–4301.
[8] a) M. Pátek, B. Drake, M. Lebl, *Tetrahedron Lett.* **1994**, *35*, 9169–9172; b) P. Ko*cis*, O. Issakova, N. Sepetov, M. Lebl, *Tetrahedron Lett.* **1995**, *36*, 6623–6626; c) G. Wess, K. Bock, H. Kleine, M. Kurz, W. Guba, H. Hemmerle, E. Lopez-Calla, K.-H. Baringhaus, H. Glombik, A. Enhsen, W. Kramer, *Angew.*

Chem. Int. Ed. Engl. **1996**, *35*, 2222–2224; d) T. Wunberg, C. Kallus, T. Opatz, S. Henke, W. Schmidt, H. Kunz, *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2503–2505; e) J. F. Barry, A. P. Davis, M. N. Pérez-Payan, *Tetrahedron Lett.* **1999**, *40*, 2849–2852; f) C. Kallus, T. Opatz, T. Wunberg, W. Schmidt, S. Henke, H. Kunz, *Tetrahedron Lett.* **1999**, *40*, 7783–7786; g) M. Pattarawarapan, K. Burgess, *Angew. Chem. Int. Ed.* **2000**, *39*, 4299–4302; h) T. Opatz, R. M. J. Liskamp, *Org. Lett.* **2001**, *3*, 3499–3502; i) C. Chamorro, R. M. J. Liskamp, *J. Comb. Chem.* **2003**, *5*, 794–801; j) For other examples of flexible scaffolds, see: P. Virta, M. Leppänen, H. Lönnberg, *J. Org. Chem.* **2004**, *69*, 2008–2016.

[9] A full conformational analysis was performed using MacroModel V6.0, force field MM2*. To reduce calculation times the carboxylic acid linker and the protected hydroxyethyl chains were simplified as methylgroups.

[10] a) **THP**: N. Miyashita, A. Yoshikoshi, P. A. Grieco, *J. Org. Chem.* **1977**, *42*, 3772–3774; b) **DMPM**: N. Nakajima, M. Saito, M. Ubukata, *Tetrahedron Lett.* **1988**, *29*, 5565–5568; c) **TBDPS**: Y. Guindon, Y. S^{t} Denis, S. Daigneau, H. E. Morton, *Tetrahedron Lett.* **1986**, *27*, 1237–1240.

[11] a) C. P. Holmes, D. G. Jones, *J. Org. Chem.* **1995**, *60*, 2318–2319; b) S. J. Teague, *Tetrahedron Lett.* **1996**, *37*, 5751–5754; c) C. P. Holmes, *J. Org. Chem.* **1997**, *62*, 2370–2380.

[12] H. B. Lee, S. Balasubramanian, *J. Org. Chem.* **1999**, *64*, 3454–3460.

[13] a) J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lambros, R. C. Makofske, C.-D. Chang, *Int. J. Prot. Res.* **1979**, *13*, 35–42; b) C.-D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell, J. D. Haug, *Int. J. Prot. Res.* **1980**, *15*, 59–66.

[14] J. Coste, D. Le-Nguyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205–208.

[15] W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260–264.

[16] A. Madder, N. Farcy, N. G. C. Hosten, H. De Muynck, P. J. De Clercq, J. Barry, A. P. Davis, *Eur. J. Org. Chem.* **1999**, 2787–2791.

[17] D. Hudson, *J. Org. Chem.* **1988**, *53*, 617–624.

[18] K. Barlos, D. Gatos, S. Koutsogianni, W. Schäfer, G. Stavropoulos, Y. Wenging, *Tetrahedron Lett.* **1991**, *32*, 471–474.

[19] H. P. Nestler, *Mol. Div.* **1996**, *2*, 35–40.

[20] J. C. M^{c} Auliffe, O. Hindsgaul, *Synlett* **1998**, 307–309.

[21] W. Zhang, M. J. Robins, *Tetrahedron Lett.* **1992**, *33*, 1177–1180.

[22] K. A. Scheidt, H. Chen, B. C. Followsn, S. R. Chemler, D. S. Coffey, W. R. Roush, *J. Org. Chem.* **1998**, *63*, 6436–6437.

[23] T. W. Greene, P. G. M. Wuts, *Protective groups, in: Organic Synthesis*, 3rd ed., **1999**.

[24] a) P. Sieber, *Helv. Chim. Acta.* **1977**, *60*, 2711–2716; b) C. K. Marlowe, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 437–440.

[25] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, USA, **1986**.

[26] a) M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluščec, O. Kocy, *Biochemistry* **1968**, *7*, 4069–4075; b) M. Bodanszky, S. Natarajan, *J. Org. Chem.* **1975**, *40*, 2495–2499.

[27] a) E. Nicolás, E. Pedroso, E. Giralt, *Tetrahedron Lett.* **1989**, *30*, 497–500; b) Y. Okada, S. Iguchi, *J. Chem. Soc., Perkin Trans. 1* **1988**, 2129–2136.

[28] a) R. Dölling, M. Beyermann, J. Haenel, F. Kernchen, E. Krause, P. Franke, M. Brudel, M. Bienert, *J. Chem. Soc., Chem. Commun.* **1994**, 853–854; b) M. Quibell, D. Owen, L. C. Packman, T. Johnson, *J. Chem. Soc., Chem. Commun.* **1994**, 2343–2344; c) M. Mergler, F. Dick, P. Weiler, T. Vorherr, *J. Pept. Sci.* **2003**, *9*, 36–46; d) M. Mergler, F. Dick, B. Sax, C. Stähelin, T. Vorherr, *J. Pept. Sci.* **2003**, *9*, 518–526.

[29] a) T. Opatz, R. M. J. Liskamp, *J. Comb. Chem.* **2002**, *4*, 275–284; b) C. Chamorro, R. M. J. Liskamp, *J. Comb. Chem.* **2003**, *5*, 794–801.

[30] C. Yue, *Tetrahedron Lett.* **1993**, *34*, 323–326.

[31] H. Desvaux, P. Berthault, N. Birlirakis, M. Goldman, *J. Magn. Reson. A* **1994**, *108*, 219–229.

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