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Functionalization of Tripodal Scaffold Molecules on Solid Support

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A versatile synthetic approach is described for the functionalization of tripodal scaffold molecules on solid support. Intrinsic problems related to the attachment of tripodal scaffolds to a resin (for example mono- vs. polyadducts and intramolecular cyclizations) are studied and solutions are provided. The synthetic approach relies on the use of specific protecting groups in the critical steps of the synthesis. Importantly, however, protecting groups on the scaffold molecule are never used, which significantly facilitates scaffold variation, for instance in combinatorial studies.

Introduction

Tripodal molecular structures are increasingly applied in the fields of catalysis,[1] recognition,[2] sensing,[3] and biomimetics.[4] The interest is driven by the observation that functional groups, such as acids/bases, metal-binding sites, H-bond donors/acceptors, oriented towards a central cavity enable selective substrate recognition,[5] and also enzyme-like catalysis[6] via a cooperative interplay between the functional groups. Alternatively, the divergent orientation of the head groups enables binding to large objects, such as protein and even cell surfaces, via multipoint interactions, resulting in remarkably strong complexes.[7]

Having molecular weights typically ranging from 0.5–3 kDa, tripodal structures belong to a distinct class of compounds intermediating between small molecules and macromolecular objects like dendrimers and polymers. As such, syntheses of tripodal structures are often accompanied with specific challenges and pitfalls, which require the development of new synthetic methodology. Generally, tripodal structures are synthesized in solution following a one-compound-one synthesis scheme, which often requires optimisation steps for each different structure synthesized.

In addition, whereas C_3-symmetrical structures can be prepared in one step via a threefold introduction of the functional group on the scaffold, the introduction of different functions requires orthogonally compatible protecting groups on the scaffolds' anchoring points. For these applications, scaffold functionalization on solid support offers significant advantages. Solid-phase synthesis allows reactions to be driven to completeness using an excess of reagents, offers a relatively easy purification, and also permits the preparation of large combinatorial libraries of tripodal structures.

Efforts in this direction generally involve the use of scaffold molecules with an $\mathbf{AB_3}$-functionalization pattern with **A** the chemical group used for anchoring the scaffold to resin and **B** the anchoring points for scaffold functionalization carrying orthogonally compatible protecting groups (Figure 1, top).[8] For instance, impressive libraries of tripo-

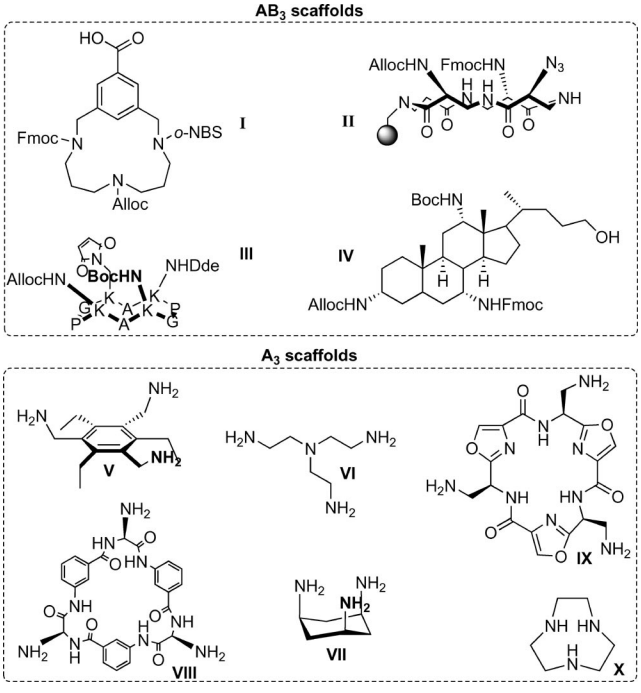

Figure 1. Examples of $\mathbf{AB_3}$-type scaffolds with orthogonally compatible functional groups on the anchoring points (top) and illustrative examples of amine terminated $\mathbf{A_3}$-type scaffolds (bottom). The synthesis of the scaffolds can be found in the following references: **I**[8f], **II**,[8a] **III**,[8h] **IV**,[8g] **V**,[12c] **VII**,[11c] **VIII**,[11a] **IX**.[11b] Scaffolds **VI** [tris(2-aminoethyl)amine], and **X** (triazacyclononane) are commercially available compounds.

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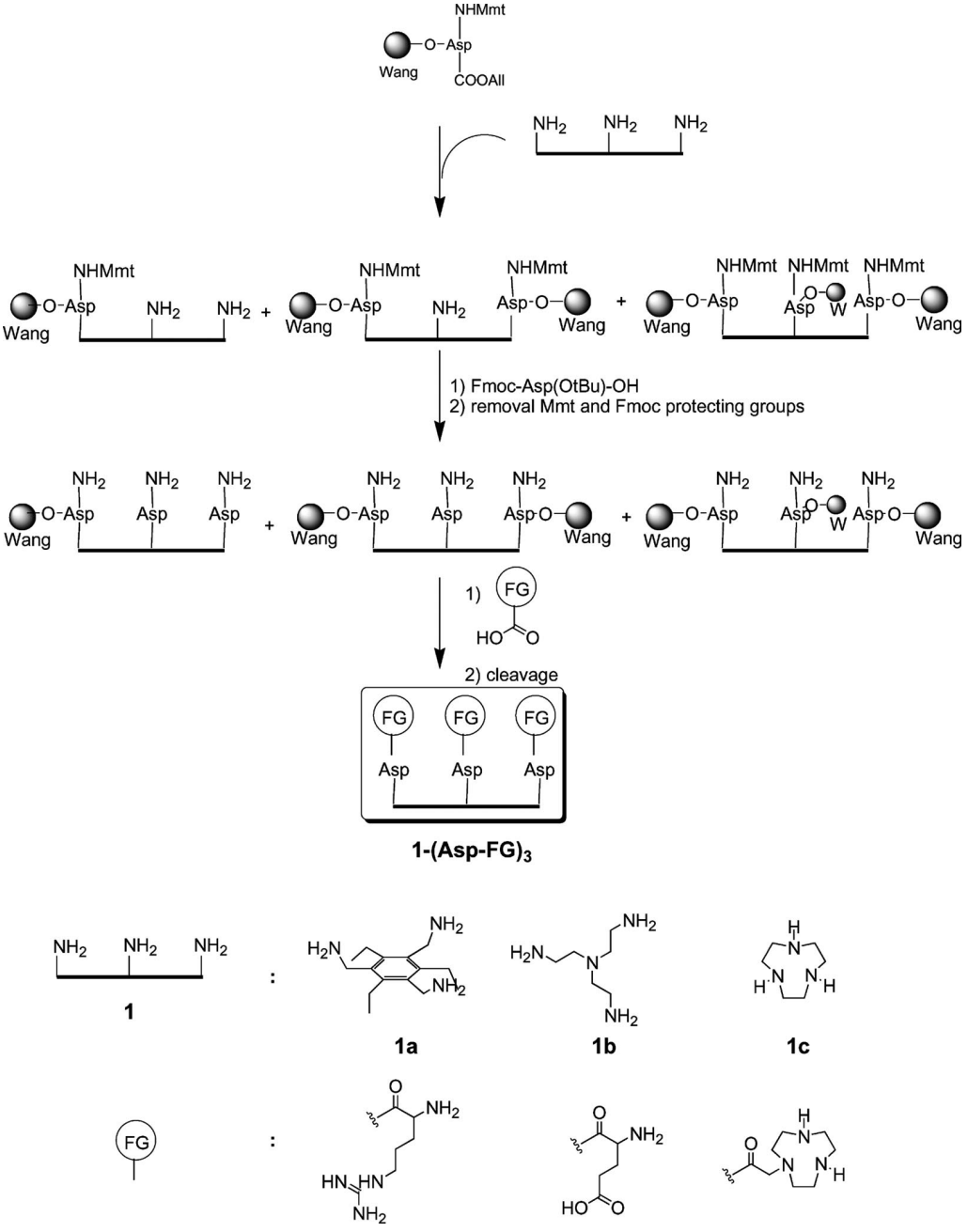
dal receptors containing up to 27000 members have been prepared and screened for binding affinity for Fe^{III}[8f] and small peptides[8c] showing the high potential of this approach. However, two drawbacks can be identified related to the use of $\mathbf{AB_3}$-type scaffolds. Firstly, only a limited number of synthetically easily accessible $\mathbf{AB_3}$-type scaffolds is available. Consequently, there is hardly any possibility of scaffold variation, which is essential in order to explore a larger chemical space. Secondly, the extensive use of orthogonal protecting groups requires a demanding synthesis of the appropriately protected $\mathbf{AB_3}$-scaffold and, subsequently, a series of deprotection–coupling steps to give the final product.

Currently we are working on the development of methodologies that eliminate these issues, thus allowing the functionalization of a wide variety of scaffolds on solid-support *without using protecting groups*. Recently, we have shown that the catalytic activity of heterofunctionalized tripodal structures can be determined directly from small mixtures, simply obtained in a one-pot synthesis without the use of any protecting group.[9] In this proof-of-principle study only a minimal four-component library was studied, with the expansion towards larger libraries hampered by the limitations of solution-phase chemistry. Therefore we are now developing synthetic methodology that allows the functionalization of $\mathbf{A_3}$-type scaffolds on solid support.[10] A wide vari-



Scheme 1. Schematic presentation of the protocol for the synthesis of compounds **1**-(Asp-FG)$_3$ on Wang resin.[10]

ety of $\mathbf{A_3}$-type scaffolds are commercially available or can be synthesized via short synthetic routes (Figure 1, bottom).[8a,8f–8h,11,12c] This means that the use of $\mathbf{A_3}$-type scaffolds significantly enhances the freedom of scaffold-variation with respect to those of type $\mathbf{AB_3}$. The use of $\mathbf{A_3}$-type scaffolds in solid-phase synthesis requires a conceptually different synthetic strategy since the chemical handle **B** is absent. As a result of the absence of synthetic methodology this type of scaffold has rarely been used in solid-phase synthesis. A notable exception in this respect is the work of Anslyn and co-workers, which strongly relies on the use of the 1,3,5-tris(aminoethyl)-2,4,6-triethylbenzene scaffold **1a**, for the development of sensing arrays on solid support.[12] In another isolated example small peptide trimers were prepared in modest yield on resin via threefold coupling to a 1,3,5-trisubstituted benzene-based scaffold.[13] However, despite the potential of $\mathbf{A_3}$-type scaffolds, a systematic study that addresses the functionalization of $\mathbf{A_3}$-type scaffolds on solid support is so far completely absent.

Our previously reported approach was based on the addition of $\mathbf{A_3}$-type scaffold molecules **1** containing multiple amino groups, to Wang resin previously functionalized with an Asp residue via its side-chain (Scheme 1).[10] This resulted in scaffolds linked to the resin one, two, or even three times. Key step in the procedure was the subsequent reaction of the remaining amino groups of the scaffold with Fmoc-Asp(O*t*Bu)-OH, restoring C_3-symmetry and yielding three terminal amino groups accessible for further functionalization. The generality of this approach was illustrated by the functionalization of three structurally diverse scaffold molecules **1a**–**c** with a variety of different functional groups **FG**. Advantages of this approach are its simplicity and the freedom to functionalize any desirable scaffold molecule, without the use of protecting groups or special functionalization patterns. On the other hand, we identified the following limitations/problems that are addressed in this paper:

– *Synthesis of heterofunctionalized scaffold molecules.* Although C_3-symmetrical structures currently find a large interest in the fields of recognition and catalysis, for a series of applications the ability to differentiate between the functional groups is essential. Here we demonstrate how one of the functional groups of scaffold molecules **1** can be differentiated from the other two without using any prior (partial) protection of the amino groups on **1**.

– *Position of the Asp residue.* In the original procedure, the Asp residue acts as a spacer between the scaffold and the desired functional group. This can pose a problem especially in cases where one is looking for synergetic effects between the functional groups for instance in (biomimetic) catalysis. We show that the protocol can be modified in order to freely choose the position of the Asp residue, enabling the possibility to directly connect the functional groups to the scaffold molecule.

– *Yield optimisation.* It was noticed that the original procedure in some cases gave rise to the formation of significant amounts of side products (in some cases up to 20–30%), especially when the flexible scaffold molecules **1b** or **1c** were used. Here we show that the formation of these side products can be completely suppressed by switching from a Wang resin to a DHPP resin.

– *Alternative for the Asp residue.* The use of Wang resin results in the introduction of carboxylic acids in the final structure (originating from the Asp residues). For several applications this may be undesirable. It is shown how the same strategy can be applied on a 2-chlorotrityl resin, which results in the incorporation of an amino group in the final product.

The solution of these problems transforms our procedure in a very versatile protocol for the homo- or heterofunctionalization of $\mathbf{A_3}$-type scaffolds.

Results and Discussion

Synthesis of Heterofunctionalized Scaffold Molecules

We have shown before that the addition of scaffold molecule **1a** to Mmt-Asp(OWang)-OH resulted in the formation of three products **1a**-[Asp(OWang)-Mmt]$_x$ (with x = 1–3) on the resin, evidenced (HPLC, see Figure 2, a) by the fact that cleavage from the resin gives compounds **1a**-(Asp-NH_2)$_x$ (with x = 1–3) in a 48:35:17 ratio, respectively. This was contrary to the observations by Anslyn and co-workers who in a much related synthetic protocol exclusively observed the monocoupling of the identical scaffold **1a**.[12d] We hypothesized that this difference might be related to the difference in spacer length between the scaffold and the resin. In order to verify this, we incremented the distance between the resin and the Asp residue inserting either a Gly residue or a GlyPro dimer at a constant resin loading of 0.44 mmol/g. It was observed that the insertion of one Gly residue was already sufficient to cause the quantitative mono-coupling of scaffold molecule **1a** to the resin. In fact, resin cleavage after the addition of the scaffold gave compound **1a**-[Asp(Gly-OH)-NH_2] as the exclusive product as evidenced by HPLC-MS and ESI-MS (Figure 2, b); compound **1a**-[Asp(Gly-OH)-NH_2]$_2$, the di-coupled adduct, was not observed at all. Analogously, when the GlyPro dimer was used as spacer, exclusively the mono-coupled product **1a**-[Asp(ProGly-OH)-NH_2] was observed.

These results can be rationalized by taking into account that the second coupling between scaffold and resin bears resemblance of an "intramolecular" reaction, leading to the formation of a cycle (with the resin constituting part of that cycle). Studies of intramolecular ring closure reactions have shown that the effectiveness of the intramolecular reaction is inversely related to the size of the ring.[14] Thus, increasing the "ring size" by inserting the Gly- or GlyPro spacer disfavors the intramolecular reaction and consequently diminishes the amount of di- or tricoupled scaffold molecules. Presumably, the number of bonds formed between scaffold and resin is also inversely related to the resin loading. At low resin loadings the formation of multiple bonds between scaffold and resin is less likely because of the larger distal separation of anchoring points on the resin. However, working with reduced resin loadings is highly unfavorable because of the obvious decrease in yield per gram of resin

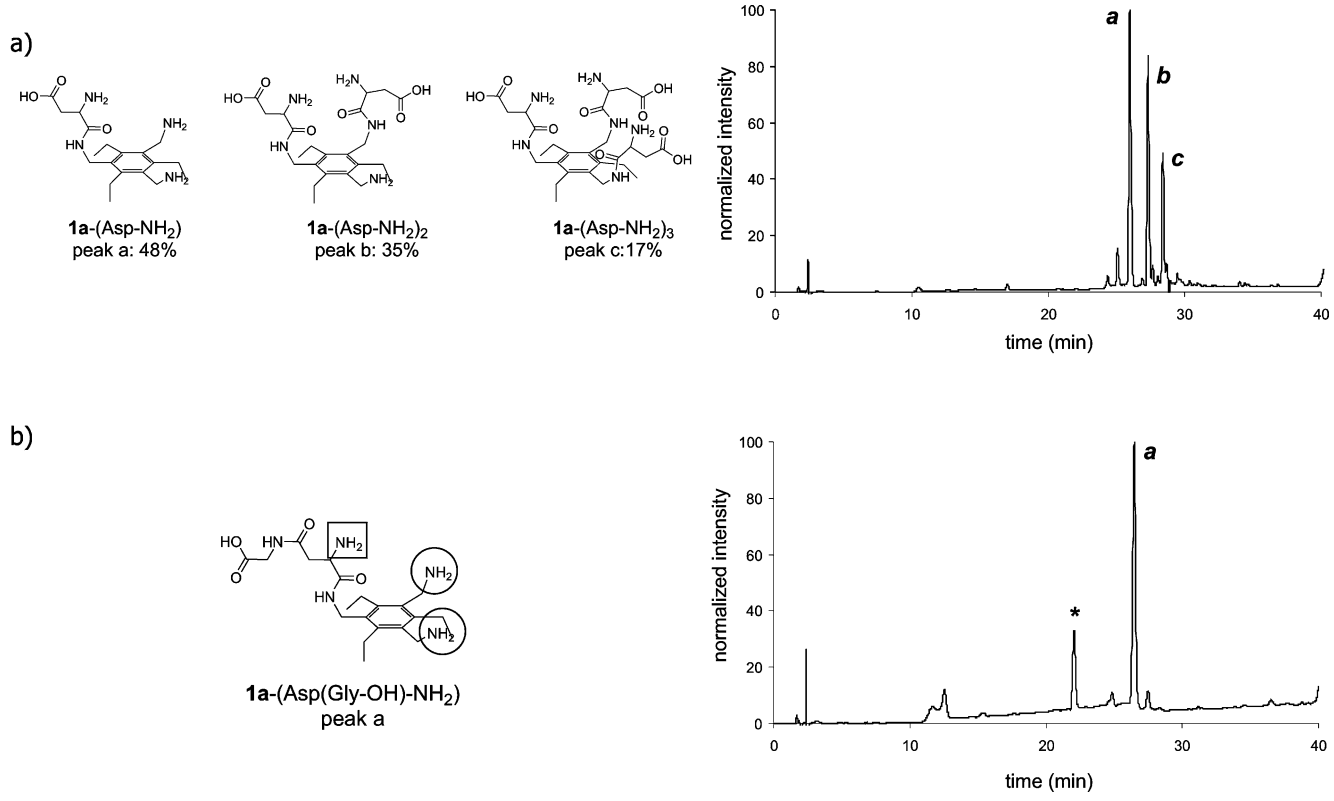


Figure 2. (a) The three products formed after addition of scaffold **1a** to Mmt-Asp(OWang)-OH and (b) the product formed after the addition of **1a** to Mmt-Asp(Gly-OWang)-OH. The HPLC chromatograms show the composition of the crude mixture after cleavage. Peaks were assigned using ESI-MS. The peak marked with an asterisk is a low-molecular weight impurity structurally not related to the scaffold molecules.

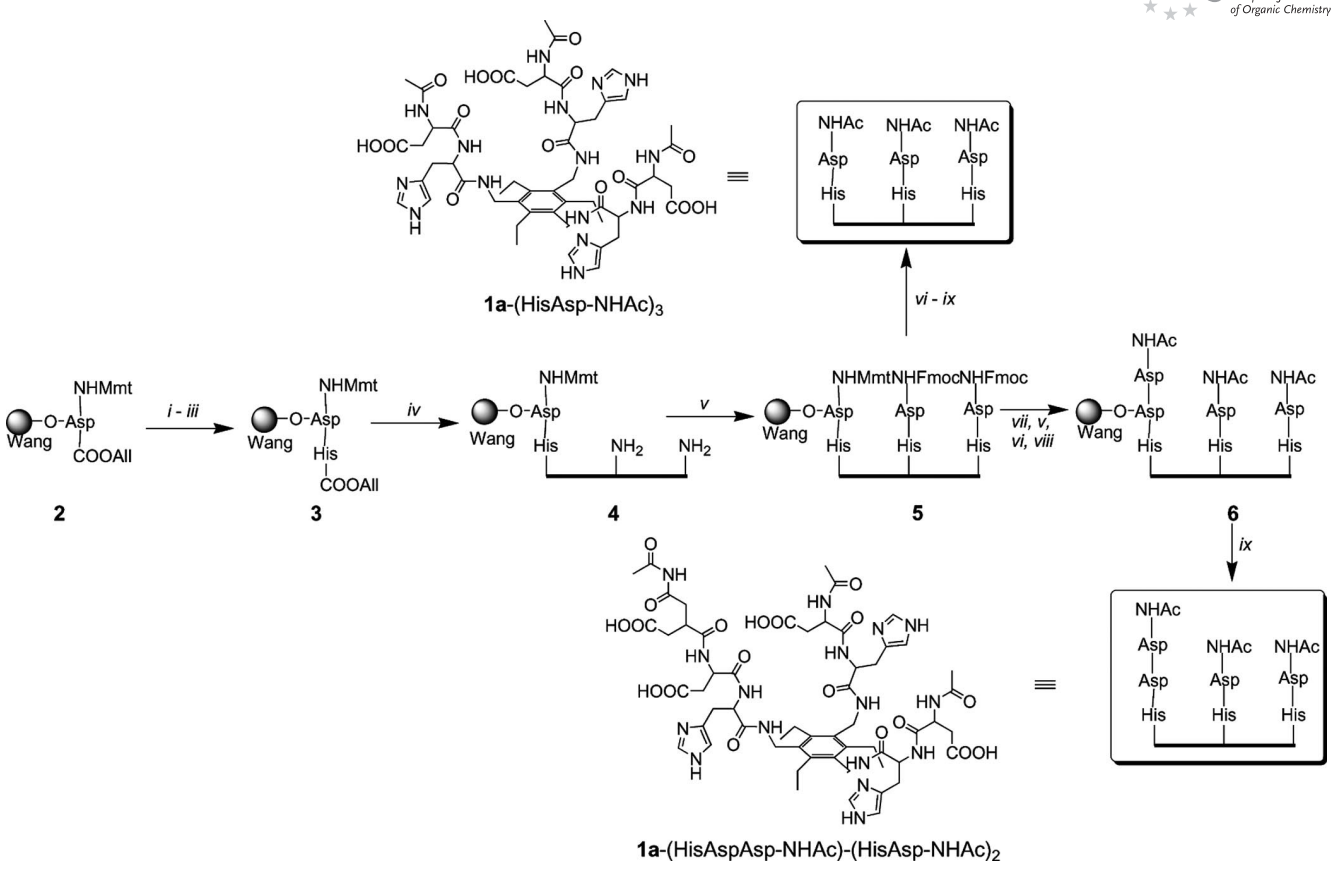
and therefore we did not consider this to be a direction with practical application.

The ability to couple scaffolds **1** using exclusively one of the three amines is very important, since it allows one arm to be functionalized in a diverse manner with respect to the other two (for an example see later). The sites that can be selectively functionalized are indicated in Figure 2 (b) with a square and circles, respectively. It should be emphasized that no prior protection of any of the amines of scaffold **1a** is required. Anslyn and co-workers have exploited this for the preparation of a combinatorial library on Tentagel resin by using two sites for the insertion of (identical) tripeptides and the remaining site for the insertion of a fluorophore that served as a reporter unit to indicate binding.[12d]

The Position of the Asp Residue

An obvious consequence of our synthetic approach is the presence of one or more Asp residues in the final compounds, positioned as spacers between the scaffold **1** and the functional groups FG (Scheme 1). This may not be an ideal situation, especially in case one is interested in synergetic interactions between the functional groups. The insertion of spacers in those cases should be avoided, as they increase the distance between the functional groups. Therefore, we developed a synthetic procedure that allows control over the position of the Asp residue, which relies on an initial N→C growth on resin (Scheme 2).[15] As a first step, the OAll protecting group of the resin bound Asp residue **2** was removed using standard reductive conditions and the resulting carboxylic acid activated with PyBOP. Next, an OAll-protected His residue [H_2N-His(Boc)-OAll] was coupled via the free NH_2 group to give **3**. After subsequent deprotection of the His-OAll group and activation, scaffold molecule **1a** was added. Subsequently, adduct **4** was symmetrized in two cycles using His and Asp, respectively, in the regular C→N direction using Fmoc-based chemistry. At this point, on a small amount of resin both the Mmt- and Fmoc-protecting groups were cleaved, the terminal amines acetylated and the products cleaved from resin. The HPLC chromatogram of the cleavage mixture (Figure 3, a) showed the clean formation of compound **1a**-(HisAsp-NHAc)$_3$ as confirmed by ESI-MS. One of the critical issues of N→C growth on resin is the possibility of amino acid racemization upon activation of the carboxylic acids. However, the NMR spectrum of compound **1a**-(HisAsp-NHAc)$_3$ shows the exclusive presence of a C_3-symmetrical species indicating that racemization has only occurred to a negligible extent (if not at all). This is in line with literature reports in which similar coupling conditions were used.[15b]

Symmetrization of the scaffold resulted in **5**, in which one Asp residue has the Mmt-protecting group, whilst the other two Asp residues have an Fmoc-protecting group. As discussed before, the orthogonality of these protecting



Scheme 2. Synthetic protocol for controlling the position of the Asp residue; *i)* $Pd^0(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, *ii*) PyBOP, DIEA, DMF/CH_2Cl_2, *iii*) H_2N-His(Boc)-OAll, DIEA, DMF, NMP, *iv*) **1a**, DIEA, DMF/CH_2Cl_2, *v*) Fmoc-Asp(O*t*Bu)-OH, PyBOP, DIEA, DMF, NMP, *vi*) piperidine 20% in NMP, *vii*) acetic acid, TFE, CH_2Cl_2, *viii*) acetic anhydride, DIEA, CH_2Cl_2, *ix*) TFA (95%).

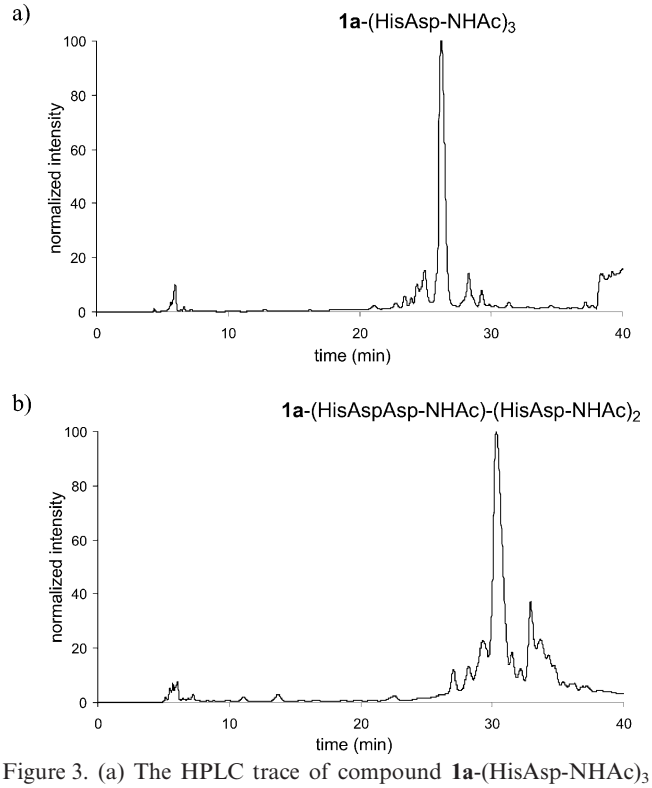
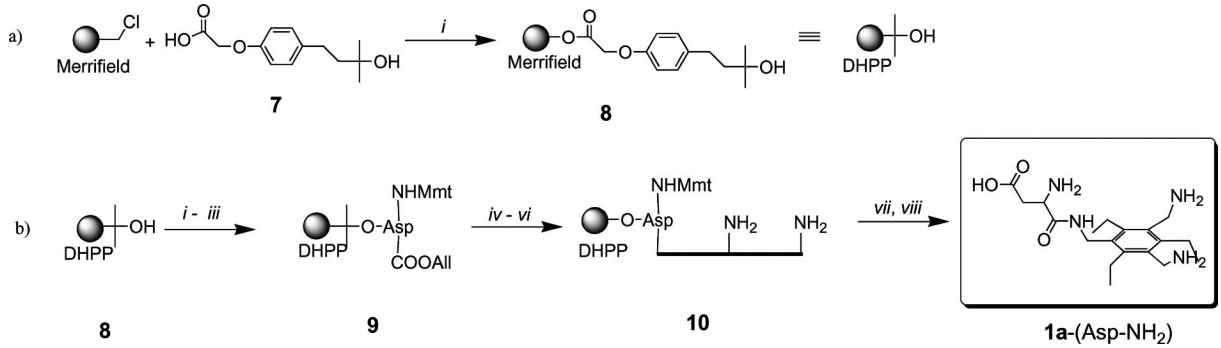


Figure 3. (a) The HPLC trace of compound **1a**-(HisAsp-NHAc)$_3$ obtained from the crude cleavage mixture. (b) The HPLC trace of compound **1a**-(HisAspAsp-NHAc)-(HisAsp-NHAc)$_2$ obtained from the crude cleavage mixture.

groups can be used to selectively functionalize one arm with respect to the other. This was demonstrated by removing the Mmt group under slightly acidic conditions and coupling of a new Asp residue. Cleavage from resin after acetylation of the terminal amines resulted in the formation of compound **1a**-(HisAspAsp-NHAc)(HisAsp-NHAc)$_2$ in reasonable yield and purity as observed from the HPLC chromatogram of the crude cleavage mixture (Figure 3, b).

Yield Optimisation

As mentioned earlier, products using our original procedure were in some cases obtained in a modest yield (down to 50%), especially when the flexible scaffolds **1b** (Tren) or **1c** (Tacn) were used. The HPLC chromatograms after cleavage showed significant amounts of side products, with masses corresponding to the desired products minus 18 or 36 amu, signifying the loss of one or two water molecules. Other side products, for instance lacking one or more amino acid residues were never observed, leading to the conclusion that the symmetrization and functionalization steps occur cleanly and quantitatively. Given the characteristic masses of the side products, we hypothesized that these should result from an intramolecular reaction involving the ester bonds connecting the Asp residues to the Wang resin. Likely candidates as nucleophiles were either the terminal amino groups (after Fmoc deprotection) or the amides, re-



Scheme 3. (a) Preparation of the DHPP resin via coupling of 4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetyl to Merrifield resin; *i*) KI, DMF. (b) Synthesis of compound **1a**-(Asp-NH_2) on DHPP resin; *i*) Fmoc-Asp(F)-OAll, DIEA, DMF/CH_2Cl_2, *ii*) piperidine 20% in DMF, *iii*) Mmt-Cl, DIEA, CH_2Cl_2, *iv*) $Pd^0(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, *v*) PyBOP, DIEA, DMF/CH_2Cl_2, *vi*) **1a**, DIEA, DMF/CH_2Cl_2, *vii*) acido acetico, TFE, CH_2Cl_2, *viii*) TFA (95%).

sulting in diketopiperazine or aspartimide formation, respectively, both well-known side products in solid-phase peptide synthesis. Considering the fact that formation of side products was also observed when the scaffolds were functionalized with diBOC-protected TACN (which were removed upon cleavage from resin, implying that free terminal amines were never present) we ruled out the first possibility and postulated that the side products were the result of aspartimide formation. Only this hypothesis was compatible with the formation of products incorporating all amino acids, but lacking fragments corresponding to one or two water molecules. Since the scaffolds are connected to the resin via one, two, or three ester bonds (see before) at maximum two cyclizations can be observed. An eventual third cyclization would lead to a complete cleavage from the resin and removal during the washing steps. In addition, the observation that the extent of side product formation strongly depends on the type of scaffold used, made us conclude that cyclization does not occur within one of the three branches. In such a case, the amount of side product would have been independent of the type of scaffold used.

So, since side product formation most presumably involves a nucleophilic attack on the benzyl ester connecting Asp to the resin, we reasoned that the use of an ester less prone to nucleophilic attack should improve the yield. Therefore, we decided to use the DHPP [4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetyl] handle **7** for attaching the Asp residue as a *tert*-butyl ester.[16] Generally, the DHPP handle is connected to Merrifield resin via the formation of a benzyl ester (which resists TFA treatment) and then exposes a *tert*-butyl alcohol for further resin functionalization (Scheme 3, a). The attachment of Asp to DHPP requires harsher coupling conditions compared to Wang resin. Accordingly, Fmoc-Asp(OH)-OAll was converted into its acyl fluoride using cyanuric fluoride and reacted with the DHPP resin at 50 °C for 1 night.[17] In order to verify our synthetic protocol on DHPP resin, first the well-performing scaffold **1a** was coupled to **9** using the same procedure as described before (Scheme 3, b). We were satisfied to observe that after sample cleavage from the resin, exclusively the mono adduct **1a**-(Asp-NH_2) was observed in the HPLC chromatogram (Figure 4, a), indicating that the DHPP linker has the additional advantage of suppressing the formation of doubly and triply connected scaffolds (at similar resin loadings: 0.32 mmol/g for the DHPP resin vs. 0.44 mmol/g for the Wang resin). In order to test whether the formation of side products is suppressed on DHPP resin, the synthesis of **1c**-(AspArg-NH_2)$_3$ was repeated. The synthesis of this compound on Wang resin gave the product with a modest purity of 70% based on analysis of the HPLC chromatogram and large amounts of a side product with a difference in mass of –18 amu were detected.[10] On DHPP resin, however, the same compound was obtained with a much im-

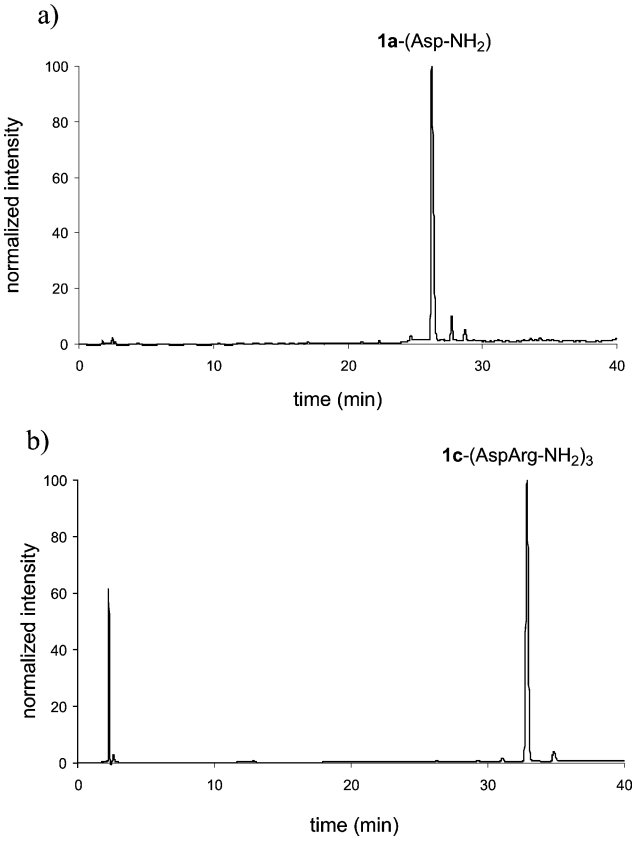


Figure 4. HPLC chromatograms of the crude mixtures obtained after cleaving (a) **1a**-(Asp-NH_2) and (b) **1c**-(Asp-Arg-NH_2)$_3$ from DHPP resin.

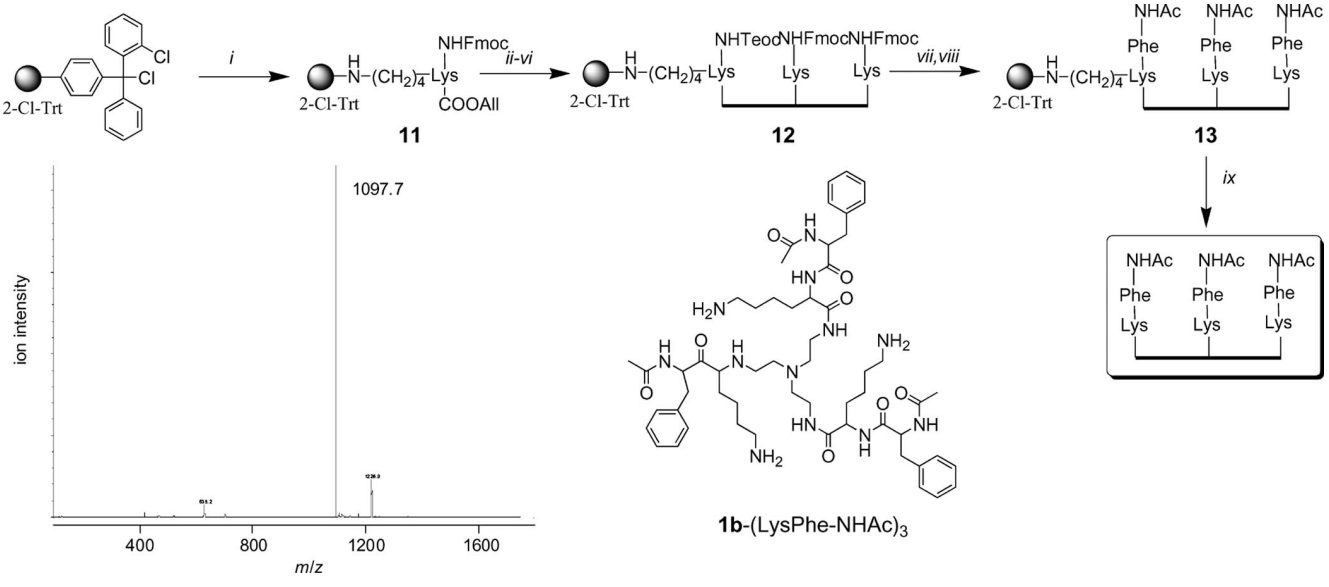
proved purity of 95% as evidenced by the HPLC chromatogram obtained after cleavage from resin (Figure 4, b).

Lysine as an Alternative for the Asp Residue

A direct consequence of functionalizing the scaffolds **1** on either Wang or DHPP resin is the introduction of one or more Asp residues in the final structure, and, consequently, a corresponding number of carboxylic acids. This may be undesirable, when an acidic function (or a negatively charged carboxylate at neutral pH) negatively interferes with the supposed activity of the functionalized scaffold (e.g. metal binding, anion receptor, etc.). In order to widen the scope of our approach, we therefore decided to explore whether also other amino acids then Asp can be used as linkers. Since the nature of the functional group is strictly related to the type of resin, we developed an analogous synthetic scheme for 2-Cl-trityl resin. Compounds are cleaved from this resin as an amine, which is highly complementary to the carboxylic acid residue.

The chemical bond connecting the scaffold to the resin determines which kind of orthogonal protecting groups can be used. In this respect, the protecting group on the amino acid used for coupling the scaffold is highly demanding in our protocol. During our initial attempts on Wang resin we kept an Fmoc group on the Asp residue, but found rather quickly that this group was not compatible with the coupling step with the scaffold. After coupling to Asp, the remaining free amines of the scaffold caused a rapid deprotection of the Fmoc group with concomitant irreversible transfer of the 9-fluorenylmethyl group. A wide variety of side products was observed. Consequently, the protecting group for the Asp amino group required stability against nucleophiles/bases (the scaffold), reductive conditions (required for deprotecting OAll), and strongly acidic conditions (resin cleavage). This excluded a large variety of protecting groups, except for those based on trityl. On Wang resin the only protecting group permitting a clean synthesis was the Mmt-protecting group, which is cleaved under very mild acidic conditions (a 1:2:6 mixture of acetic acid/TFE/DCM), which does not cause cleavage of the products from resin.

Synthesis on 2-Cl-trityl-resin liberates compounds with a terminal NH_2 group after cleavage. Intramolecular cyclization reactions as observed when using Wang resin are not likely to occur, because of the bulkiness of the trityl group. For obvious reasons, the Mmt-protecting group or any other acid sensible protecting group has to be rigorously excluded. Therefore, we were constricted to search for an alternative amino protecting group, which now required stability against nucleophiles/bases, reductive conditions, strong *and* weak acidic conditions. This excludes most of the commonly used protecting groups.[18] Eventually, we obtained excellent results using the Teoc (trimethylsilylethylcarbamate) protecting group. This protecting group is stable under all requested conditions and is generally removed by the strongly nucleophilic F^- ion (TBAF, TASF, etc.) On Wang resin this protecting group cannot be used because, contrary to the trityl-resin, quantitative cleavage from Wang resin is observed during the deprotection step. So, instead of changing the Fmoc group on Fmoc-Asn(Rink amide)-OAll by Mmt, the Teoc group was introduced via reaction with TeocONP at 50 °C for 6 hours. Following the synthetic protocol given in Scheme 4, we synthesized compound **1b**-(LysPhe-NHAc)$_3$ in good yield (80%) and purity as evidenced by the HPLC chromatogram and ESI-MS spectra (Scheme 4).



Scheme 4. Synthesis of compound **1b**-(LysPhe-NHAc)$_3$ on 2-Cl-trityl resin; *i*) Fmoc-Lys-OAll.TFA, DIPEA, CH_2Cl_2; *ii*) piperidine 20% in DMF; *iii*) Teoc-ONp, DIPEA, CH_2Cl_2, 50 °C; *iv*) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2; *v*) Tren, PyBOP, NMM, DMF/CH_2Cl_2; *vi*) Fmoc-Lys(Boc)-OH, PyBOP, NMM, DMF/CH_2Cl_2; *vii*) TBAF, DCM, 50 °C; *viii*) N-Ac-Phe-OH, HBTU, HOBT, NMM, DMF/CH_2Cl_2; *ix*) TFA, CH_2Cl_2, TIPS, H_2O. The ESI MS spectrum of the crude cleavage mixture is shown.

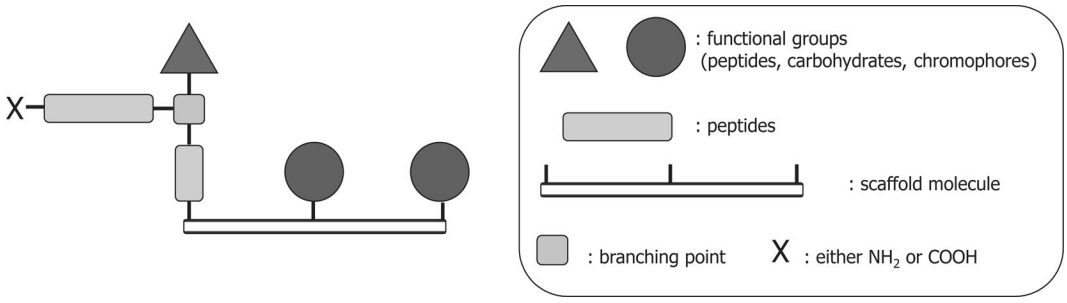


Figure 5. Schematic representation of the versatility of the synthetic procedure.

Conclusions

In this article we have presented a versatile synthetic procedure for the functionalization of tripodal scaffold molecules on solid support. The general type of molecules that are accessible using our approach is summarized in Figure 5. The type of solid support determines whether the final compound is terminated with an amino or carboxylic acid group. A first peptide fragment can be inserted before the branching point by conventional peptide chemistry and after the branching point by peptide growth in the N→C direction. Subsequently, any C_3-symmetrical scaffold containing NH_2 terminal groups can be attached exclusively on one position introducing the advantage of a controlled subsequent functionalization of the scaffold with two different moieties. Alternatively, C_3-symmetrical tripodal scaffolds can be prepared by inserting the peptide, branching point, and identical functional group on the remaining two positions of the scaffold. It should be emphasized that there are no restrictions on the structure of either one of the subcomponents, including the scaffold, which illustrates the versatility of the methodology. Our studies have shown that the coupling of tripodal scaffold molecules to a resin can give rise to very specific problems, such as polyadducts, intramolecular cyclizations, etc. The purity of the products is strongly determined by the choice of resin and the protecting group on the branching unit.

It is our expectation that the synthetic procedures described here will be very useful for the synthesis and screening of multivalent, heterofunctionalized structures for application in the fields of (bio)recognition and catalysis.

Experimental Section

General: All starting materials, solvents, and resins were obtained from commercial sources and used without further purification. Commercially available resins with a loading of 0.93 mmol/g (Wang), 1.1 mmol/g (Merrifield), and 1.0 mmol/g (2-Cl-Trityl) were used. Standard resin loading protocols were used for Wang and 2-Cl-tritylresin. Solid-phase synthesis was performed using standard Fmoc-based peptide chemistry (either using HBTU/HOBT or PyBOP activation in the presence of DIPEA) and the specific procedures reported below.[10] The DHPP linker[16b] was synthesized according to a literature procedure. Characterization data for **1c**-$(AspArg\text{-}NH_2)_3$ were identical to those already reported.[10] ^1H NMR spectra were recorded on a Bruker AC-300 (300.13 MHz) spectrometer operating at 301 K. HPLC chromatograms were recorded using a Shimadzu LC-10AT dual pump system and a Shimadzu SPD-10A UV–Vis detector. HR ESI-mass spectra were obtained using a Perspective Biosystem Mariner spectrometer equipped with a TOF analyzer.

General Synthetic Procedures

Substitution of the Fmoc Group for Mmt (on Wang and DHPP resin): The Fmoc group on Fmoc-Asp(OWang)-OAll or Fmoc-Asp-(ODHPP)-OAll was removed using a solution of 20% piperidine in NMP (2 × 10 min). After washing with DMF (2×) and CH_2Cl_2 (2×), 4-methoxytriphenylchloromethane (Mmt-Cl, 6 equiv.) and *N*,*N*-diisopropylethylamine (DIEA, 15 equiv.) were added in CH_2Cl_2 and the mixture was agitated for 2 h.

Substitution of the Fmoc Group for Teoc (on Cl-Tritylresin): The Fmoc group on Fmoc-Lys(Cl-Trit)-OAll was removed using a solution of 20% piperidine in DMF (2 × 10 min). The Teoc group was introduced by the reaction of the resin with Teoc-ONp [2-(trimethylsilyl)ethyl-4-nitrophenyl carbonate, 3.5 equiv.] in CH_2Cl_2/DIPEA, 9:1 for 3 h at 50 °C. The mixture was then cooled down and the reaction continued overnight at room temperature. The completion of the reaction was confirmed by the Kaiser test.

Removal of the Allyl Group and Activation: The allyl group was removed under reductive conditions as reported.[19] Tetrakis-(triphenylphosphane)palladium(0) (0.2 equiv.) and phenylsilane (15 equiv.) in CH_2Cl_2 were added and the mixture was stirred for 2 h under an inert N_2 atmosphere. After the washing steps [CH_2Cl_2 (2×), NMP/DIEA = 4:1 (2 × 20 min), NMP (2×) and CH_2Cl_2 (2×)], the carboxylic acid was activated by adding PyBOP (5 equiv.) and *N*-methylmorpholine (10 equiv.) in DMF/CH_2Cl_2 = 1:1 for 1 hr.

Removal of the Mmt-Protecting Group (on Wang and DHPP resin): The resin was washed with CH_2Cl_2 (3×) and treated with a mixture of acetic acid/trifluoroethanol/CH_2Cl_2 = 1:2:6 for 1 h and washed again with CH_2Cl_2 (3×).

Removal of the Teoc-protecting group (on Cl-tritylresin). The Teoc-protecting group was removed by treatment of the resin suspended in DCM with 10 equiv. of tetrabutylammonium fluoride (1 M in THF) for 4 h at 50 °C (3×).

Attachment of the DHPP Linker to Merrifield Resin: 4-(1′,1′-Dimethyl-1′-hydroxypropyl)phenoxyacetyl (DHPP) (4 equiv.) was dissolved in EtOH (2 ml/mmol) and water (0.5 ml/mmol), after which the pH was adjusted to 7 by adding Cs_2CO_3. The solution was evaporated to dryness and after subsequent adding of dioxane the evaporation was repeated. The Cs^+ salt of DHPP was added to a suspension of Merrifield resin (0.055 mmol end groups) in DMF for 12 hours at 50 °C in the presence of KI (0.1 equiv.) as catalyst. Next, the resin was washed with MeOH/DMF/DIEA (1:1:0.1) (4×), 20% piperidine in DMF (2 × 10 min), DMF (2×), MeOH (2×) and dried under vacuum.

Attachment of Fmoc-Asp(F)-OAll to DHPP resin. Fmoc-Asp(F)-OAll (5 equiv.) was added to DHPP resin in DMF in the presence of DIEA (15 equiv.) and the mixture was agitated for about 10 h (overnight) at room temperature.

Synthesis and Characterization

Fmoc-Asp(F)-OAll: Fmoc-Asp(OH)-OAll (0.19 g, 0.48 mmol) is dissolved in CH_2Cl_2 (5 mL) and cooled down to 0 °C, after which cyanuric fluoride (76 μL, 0.74 mmol) and pyridine (0.1 mL, 1.4 mmol) are added. After stirring for 1.5 h the reaction mixture is quickly extracted with H_2O (3×) and dried on Na_2SO_4, after which the solvent is evaporated under reduced pressure and dried under high vacuum. The product is obtained as a white solid (0.17 g, 91%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 3.16 (m, 2 H), 4.23 (t, J = 6.8 Hz, 1 H), 4.43 (d, J = 6.9 Hz, 1 H), 4.67 (dd, J = 5.5, J = 14.8 Hz, 2 H), 5.29 (d, J = 10.2 Hz, 2 H), 5.38 (s, 1 H), 5.72 (d, J = 7.2 Hz, 1 H), 5.88 (dd, J = 5.5, J = 11.3 Hz, 1 H), 7.37 (td, J = 7.2 Hz, 4 H), 7.58 (d, J = 7.3 Hz, 2 H), 7.77 (d, J = 7.5 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz): δ = 47.02, 49.80, 66.99, 67.36, 119.63, 120.01, 124.97, 127.05, 127.78, 130.86, 141.28, 143.45 ppm. ESI MS [M + H$^+$] requires 398.1, found 398.2.

1a-[Asp(Gly-OH)-NH$_2$]: White solid (1.6 mg, 85%) after purification with RP-HPLC [Jupiter Proteus-C18; gradient: H_2O/TFA (0.1%)→CH_3CN/TFA (0.1%) from 3% to 25% in 30 min, UV detection 226 nm]: 26.7 min (100%). ^{1}H NMR (CD_3OD, 250 MHz): δ = 1.1–1.3 (m, 9 H), 2.6–2.8 (m, 6 H), 3.05 (m, 2 H), 3.74 (dd, 1 H), 4.23 (s, 4 H), 4.3–4.5 (m, 4 H) ppm. ^{13}C NMR (CD_3OD, 63 MHz): δ = 15.58, 16.29, 24.08, 24.44, 24.62, 30.95, 37.98, 39.27, 51.14, 129.42, 129.86, 132.84, 133.02, 169.94, 174.34, 174.77 ppm. ESI-MS [M + H]$^+$ requires 422.2689, found 422.2678.

1a-[Asp(ProGly-OH)-NH$_2$]: White solid (1.8 mg, 61%) after purification with RP-HPLC [Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)→CH_3CN/TFA (0.1%) from 5% to 27% in 30 min, UV detection 226 nm]: 27.5 min (100%). ^{1}H NMR (CD_3OD, 250 MHz): δ = 1.1–1.3 (m, 9 H), 1.9–2.2 (m, 4 H), 2.6–2.8 (m, 6 H), 3.0 (br.s, 2 H), 3.5–3.7 (m, 2 H), 3.75 (dd, 1 H), 4.21 (s, 4 H), 4.3–4.5 (m, 5 H) ppm. ^{13}C NMR (CD_3OD, 63 MHz): δ = 15.59, 16.28, 24.06, 24.41, 24.48, 24.57, 25.64, 30.93, 37.94, 39.33, 42.25, 51.12, 61.61, 129.48, 129.89, 132.90, 133.05, 169.89, 169.94, 174.34, 174.77 ppm. ESI-MS [M + H]$^+$ requires 519.3217, found 519.3227.

1a-(HisAsp-NHAc)$_3$: White solid (12 mg, 91%) after precipitation with diethyl ether. HPLC [Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)→CH_3CN/TFA (0.1%) from 13% to 30% in 30 min, UV detection 226 nm]: 26.8 min (100%). ^{1}H NMR (CD_3OD, 250 MHz): δ = 1.19 (t, J = 7.4 Hz, 9 H), 1.95 and 2.05 (2s, 9 H), 2.6–2.8 (m, 12 H), 3.0–3.3 (m, 6 H), 4.43 (s, 6 H), 4.5–4.6 (m, 3 H), 4.7–4.8 (m, 3 H), 7.35 (s, 3 H), 8.80 (s, 3 H) ppm. ^{13}C NMR (CD_3OD, 63 MHz): δ = 16.72, 20.89, 22.64, 27.99, 36.73, 39.17, 51.95, 53.66, 118.64, 131.24, 131.29, 132.61, 135.14, 171.53, 173.66, 174.74, 174.04 ppm. ESI-MS [M + H]$^+$ requires 1132.5098, found 1132.5021.

1a-(HisAspAsp-NHAc)-(HisAsp-NHAc)$_2$: White solid (14 mg, 72%) after precipitation with diethyl ether. HPLC [Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)→CH_3CN/TFA (0.1%) from 12% to 28% in 30 min, UV detection 226 nm]: 30.5 min (100%). ^{1}H NMR (CD_3OD, 250 MHz): δ = 1.19 (t, J = 7.4 Hz, 9 H), 1.9–2.0 (m, 9 H), 2.6–2.8 (m, 14 H), 3.0–3.3 (m, 6 H), 4.42 (s, 6 H), 4.5–4.6 (m, 4 H), 4.7–4.8 (m, 3 H), 7.35 (s, 3 H), 8.80 (s, 3 H) ppm. ESI-MS [M + H]$^+$ requires 1247.5367, found 1247.5349.

1b-(LysPhe-NHAc)$_3$: White solid (2.6 mg, 80%) after precipitation from diethyl ether. HPLC [Agilent Eclipse XDB-C18; gradient: H_2O/TFA (0.1%)→CH_3CN/TFA (0.1%) 5% for 5 min from 5% to 50% in 20 min, UV detection 226 nm]: 19.9 min (100%). ^{1}H NMR (CD_3OD 250 MHz): δ = 0.89 (m, 6 H), 1.45 (m, 6 H), 1.68 (m, 6 H), 1.96 (d, 9 H), 2.92 (m, 12 H), 3.41 (m, 6 H), 3.58 (m, 6 H), 4.09–4.24 (m, 3 H), 4.39–4.53 (m, 3 H), 7.28 (m, 15 H) ppm. ESI-MS [M + H]$^+$ requires 1098.6750, found 1098.6742.

Abbreviations

ONBS: 2-nitrophenylsulfonyl, Fmoc: (9-fluorenyl)methoxycarbonyl, Aloc: allyloxycarbonyl, Dde: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, Mmt: 4-methoxyphenyldiphenylmethyl, Teoc: trimethylsilylethylcarbamate, OAll: allyloxy, Boc: *tert*-butoxycarbonyl, O*t*Bu: *tert*-butoxy, PyBOP: [(benzotriazole-1-yl)oxy]tripyrrolidinophosphonium hexafluorophosphate, HOBT: 1-hydroxybenzotriazole, HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DIEA: *N*,*N*-diisopropylethylamine, DMF: dimethylformamide, NMP: *N*-methylpyrrolidone, TFE: 2,2,2-trifluoroethanol, TFA: trifluoroacetic acid, Tren: tris(2-aminomethyl)amine, Tacn: triazacyclononane, DHPP: 4-(1′,1′-dimethyl-1′-hydroxypropyl) phenoxyacetyl, Wang resin: *p*-benzyloxybenzyl alcohol resin, Merrifield resin: chlormethylstyrene-divinylbenzene resin, Rink amide: 4-(2′,4′-dimethoxyphenyl)-Fmoc-aminomethyl-phenoxy-acetamido-norleucyl aminomethyl resin, TBAF: tetrabutylammonium fluoride, NMM: *N*-methylmorpholine, TIPS: triisopropylsilane, TASF: tris(dimethylamino)sulfonium difluorotrimethylsilanide.

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[1] For reviews: a) L. M. Berreau, *Eur. J. Inorg. Chem.* **2006**, 273–283; b) J. Zhou, Y. Tang, *Chem. Soc. Rev.* **2005**, *34*, 664–676; c) G. Licini, L. J. Prins, P. Scrimin, *Eur. J. Org. Chem.* **2005**, 969–977; d) C. Moberg, *Angew. Chem. Int. Ed.* **1998**, *37*, 248–268.

[2] For reviews: a) S. E. Gibson, M. P. Castaldi, *Chem. Commun.* **2006**, 3045–3062; b) C. Moberg, *Angew. Chem. Int. Ed.* **2006**, *45*, 4721–4723; c) J. Yoon, S. K. Kim, N. J. Singh, S. K. Kim, *Chem. Soc. Rev.* **2006**, *35*, 355–360; d) Y. Singh, G. T. Dolphin, J. Razkin, P. Dumy, *ChemBioChem* **2006**, *7*, 1298–1314.

[3] For reviews: a) B. Kuswandi Nuriman, W. Verboom, D. N. Reinhoudt, *Sensors* **2006**, *6*, 978–1017; b) B. T. Nguyen, E. V. Anslyn, *Coord. Chem. Rev.* **2006**, *250*, 3118–3127.

[4] For reviews: a) S. E. Gibson, M. P. Castaldi, *Angew. Chem. Int. Ed.* **2006**, *45*, 4718–4720; b) G. Parkin, *Chem. Commun.* **2000**, 1971–1985.

[5] For selective examples on cation recognition: a) E. A. Dertz, J. Xu, A. Stinti, K. N. Raymond, *J. Am. Chem. Soc.* **2006**, *128*, 22–23; b) J. D. Badjic, V. Balzani, A. Credi, S. Silvi, J. F. Stoddart, *Science* **2004**, *303*, 1845–1849. Anion recognition: c) A. P. Davis, *Coord. Chem. Rev.* **2006**, *250*, 2939–2951; d) L. Fabbrizzi, F. Foti, A. Taglietti, *Org. Lett.* **2005**, *7*, 2603–2606; e) C. Schmuck, M. Schwegmann, *J. Am. Chem. Soc.* **2005**, *127*, 3373–3379; f) S. L. Tobey, B. D. Jones, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 4026–4027. Peptide recognition: g) S. Valente, M. Gobbo, G. Licini, A. Scarso, P. Scrimin, *Angew. Chem. Int. Ed.* **2001**, *40*, 3899–3902; h) M. C. F. Monnee, A. J. Brouwer, L. M. Verbeek, A. M. A. van Wageningen, R. J. M. Liskamp, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1521–1525; i) J. Rao, J. Lahiri, L. Isaacs, R. M. Weis, G. M. Whitesides, *Science* **1998**, *280*, 708–711. Carbohydrate recognition: j) H. Abe, Y. Aoyagi, M. Inouye, *Org. Lett.* **2005**, *7*, 59–61; k) A. Vacca, C.

Nativi, M. Cacciarini, R. Pergoli, S. Roelens, *J. Am. Chem. Soc.* **2004**, *126*, 16456–16465.

[6] For examples: a) A. Scarso, G. Zaupa, F. B. Houillon, L. J. Prins, P. Scrimin, *J. Org. Chem.* **2007**, *72*, 376–385; b) J. R. Morrow, O. Iranzo, *Curr. Opin. Chem. Biol.* **2004**, *8*, 192–200; c) L. Bonfa, M. Gatos, F. Mancin, P. Tecilla, U. Tonellato, *Inorg. Chem.* **2003**, *42*, 3943–3949; d) A. Scarso, I. Scheffer, M. Gobel, Q. B. Broxterman, B. Kaptein, F. Formaggio, C. Toniolo, P. Scrimin, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5144–5149; e) P. Molenveld, W. M. G. Stikvoort, H. Kooijman, A. L. Spek, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem.* **1999**, *64*, 3896–3906.

[7] For selective examples, see: a) L. L. Kiesling, J. E. Gestwicki, L. E. Strong, *Angew. Chem. Int. Ed.* **2006**, *45*, 2348–2368; b) Y. Ye, S. Block, B. Xu, S. Achilefu, *J. Med. Chem.* **2006**, *49*, 2268–2275; c) R. N. De Oliviera, D. Sinou, R. M. Srivastava, *J. Carbohydr. Chem.* **2006**, *25*, 407–425; d) D. Boturyn, J.-L. Coll, E. Garanger, M.-C. Favrot, P. Dumy, *J. Am. Chem. Soc.* **2004**, *126*, 5730–5739; e) G. Thumshirn, U. Hersel, S. L. Goodman, H. Kessler, *Chem. Eur. J.* **2003**, *9*, 2717–2725; f) Y. Nishida, T. Tsurumi, K. Sasaki, K. Watanabe, H. Dohi, K. Kobayashi, *Org. Lett.* **2003**, *5*, 3775–3778; g) P. Scrimin, P. Tecilla, U. Tonellato, A. Veronese, M. Crisma, F. Formaggio, C. Toniolo, *Chem. Eur. J.* **2002**, *8*, 2753–2763; h) M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2755–2794; i) H. Fuji, H. Komazawa, H. Mori, M. Kojima, I. Itoh, J. Murata, I. Azuma, I. Saiki, *Biol. Pharm. Bull.* **1995**, *18*, 1681–1688.

[8] a) P. Virta, M. Karskela, H. Lönnberg, *J. Org. Chem.* **2006**, *71*, 1989–1999; b) A. Gea, N. Farcy, N. Roqué i. Rossell, J. C. Martins, P. J. De Clercq, A. Madder, *Eur. J. Org. Chem.* **2006**, 4135–4146; c) C. Chamorro, J.-W. Hofman, R. J. M. Liskamp, *Tetrahedron* **2004**, *60*, 8691–8697; d) V. Del Amo, L. Siracusa, T. Markidis, B. Baragaña, K. M. Bhattarai, M. Galobardes, G. Naredo, M. N. Pérez-Payán, A. P. Davis, *Org. Biomol. Chem.* **2004**, *2*, 3320–3328; e) S. Lebreton, S.-E. How, M. Buchholz, B.-E. Yingyongnarongkul, M. Bradley, *Tetrahedron* **2003**, *59*, 3945–3953; f) T. Opatz, R. J. M. Liskamp, *J. Comb. Chem.* **2002**, *4*, 275–284; g) X.-T. Zhou, A. Rehman, C. Li, P. B. Savage, *Org. Lett.* **2000**, *2*, 3015–3018; h) P. Dumy, I. M. Eggleston, S. Cervigni, U. Sila, X. Sun, M. Mutter, *Tetrahedron Lett.* **1995**, *36*, 1255–1258.

[9] G. Zaupa, M. Martin, L. J. Prins, P. Scrimin, *New J. Chem.* **2006**, *30*, 1493–1497.

[10] C. Guarise, L. J. Prins, P. Scrimin, *Tetrahedron* **2006**, *62*, 11670–11674.

[11] a) P. H. Rasmussen, J. Rebek Jr, *Tetrahedron Lett.* **1999**, *40*, 3511–3514; b) D. Mink, S. Mecozzi, J. Rebek Jr, *Tetrahedron Lett.* **1998**, *39*, 5709–5712; c) T. Bowen, R. P. Planalp, M. W. Brechbiel, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 807–810.

[12] a) A. T. Wright, E. V. Anslyn, *Chem. Soc. Rev.* **2006**, *35*, 14–28; b) S. C. McCleskey, M. J. Griffin, S. E. Schneider, J. T. McDevitt, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 1114–1115; c) G. Hennrich, E. V. Anslyn, *Chem. Eur. J.* **2002**, *8*, 2219–2224; d) S. E. Schneider, S. N. O'Neill, E. V. Anslyn, *J. Am. Chem. Soc.* **2000**, *122*, 542–543.

[13] M. A. Ashraf, J. K. Notta, J. S. Snaith, *Tetrahedron Lett.* **2003**, *44*, 9115–9119.

[14] a) R. Cacciapaglia, S. Di Stefano, L. Mandolini, *Acc. Chem. Res.* **2004**, *37*, 113–122; b) L. Mandolini, *Adv. Phys. Org. Chem.* **1986**, *22*, 1.

[15] a) S. Brase, J. H. Kirchhoff, J. Kobberling, *Tetrahedron* **2003**, *59*, 885–939; b) N. Thieriet, F. Guibé, F. Albericio, *Org. Lett.* **2000**, *2*, 1815–1817; c) B. Henkel, L. Zhang, E. Bayer, *Liebigs Ann. Recl.* **1997**, 2161; d) R. Léger, R. Yen, M. W. She, V. J. Lee, S. J. Hecker, *Tetrahedron Lett.* **1998**, *39*, 4171; e) R. Matsueda, H. Maruyama, E. Kitazawa, H. Takahagi, T. Mukaiyama, *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3240; f) A. M. Felix, R. B. Merrifield, *J. Am. Chem. Soc.* **1970**, *92*, 1385; g) R. L. Letsinger, M. J. Kornet, *J. Am. Chem. Soc.* **1963**, *85*, 3045.

[16] a) J. Kochansky, R. M. Wagner, *Tetrahedron Lett.* **1992**, *33*, 8007–8010; b) K. Akaji, Y. Kiso, L. A. Carpino, *J. Chem. Soc., Chem. Commun.* **1990**, 584–586.

[17] K. Akaji, H. Tanaka, H. Itoh, J. Imai, Y. Fujiwara, T. Kimura, Y. Kiso, *Chem. Pharm. Bull.* **1990**, *38*, 3471–3472.

[18] T. W. Greene, P. G. M. Wuts, *Protective groups in organic synthesis*, 3rd ed., John Wiley & Sons, New York, **1999**.

[19] M. Dessolin, M. G. Guillerez, N. Thieriet, F. Guibe, A. Loffet, *Tetrahedron Lett.* **1995**, *36*, 5741–5744.

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