EurJOC
European Journal of Organic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201800601

Link to VoR: http://dx.doi.org/10.1002/ejoc.201800601

Supported by ACES Asian Chemical Editorial Society

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New tri-orthogonal scaffolds for the solid-phase synthesis of peptides

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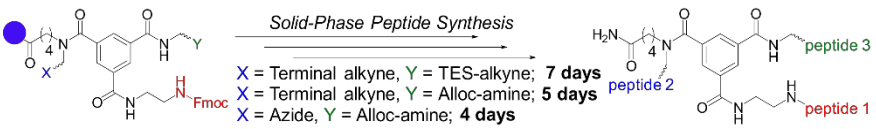
Supporting information for this article is available on the www under http://dx.doi.org/ and consists of: Experimental Section for the synthesis of compounds **1**-**12** (including Scheme S1), additional analytical data (^{1}H and ^{13}C NMR and IR) for compounds **13**-**30** and compound **39**, description of the thermal cleavage of the Fmoc protecting group (including Scheme S2) and HPLC chromatograms for compounds **16**, **29**, **30**, **38**, **39**, **45**, **51** and **57** (Figures S1-S8).

Graphical Abstract



Three tripodal scaffolds were developed and tested for the step-by-step synthesis of three different peptides on the same scaffold by the solid-phase synthesis. Such compounds with three differents peptides attached to a central core could mimic large proteins and find applications in modulating protein-protein interactions.

Key Topic: multifunctional scaffolds, solid-phase peptide synthesis

Abstract

Multi-orthogonal scaffolds can be useful for the attachment of several different compounds to the same central skeleton. Such compounds can find applications in the development of protein

mimics for their potential to mimic several distant epitopes in the protein structure. Based on the structure of our previously published scaffold (Vaněk et al., Eur. J. Org. Chem. 17, 3689-3701, 2015), we developed three new tri-orthogonal variants of this scaffold, which are suitable for the solid-phase synthesis of three different peptides on the same skeleton. We combined different chemical moieties for the phased attachment of amino acids to the scaffolds: Fmoc- or Alloc-protected amine, free or TIPS-protected alkyne or azido group. We prepared and characterized several model compounds and compared the suitability of new scaffolds for a peptide synthesis. All three scaffolds provided peptides with satisfactory yields and purities, making them suitable for a synthesis of libraries of compounds.

Introduction

The majority of biological processes in living organisms is mediated by actions of peptides and proteins, which often interact with other peptides and proteins, creating complicated networks of protein-protein interactions (PPIs).[1] Targeting PPIs with specific inhibitors or activators can provide potentially useful tools for the treatment of various disorders.[2] However, targeting PPIs with small molecules is extremely difficult, because protein interaction surfaces are usually large, exposed to solvent, shallow and featureless and noncontiguous.[3],[4] For this reason, many important proteins involved in PPIs are classified as "undruggable targets".[5] However, the majority of free energy for protein–protein interactions is often mediated by what are called protein hot spots, which are groups of a few residues that confer to a majority of free energy for the interaction.[6] Simultaneous targeting of a few separated hot spots in a protein of interest could result in the potent inhibition or activation of some biological event.

For this purpose, we previously developed two tripodal scaffolds, which enable stepwise attachment of three different moieties to individual scaffold arms by Cu(I)-catalyzed click chemistry (**scaffold I**)[7] or by a reaction of aldehydes with a hydrazide group of **scaffold Ia**[8] (Figure 1). Recently, we synthesized a combinatorial library of 1 000 compounds based on the structure of **scaffold I** modified with 30 different azides. The library was tested for binding to the receptor for insulin. We identified few weak insulin receptor binders and activators.[9] Nevertheless, it was clear that much larger compound diversity and complexity would be necessary for the discovery of more potent insulin receptor binders. In this respect, amino acids represent convenient types of building blocks for the modification of tripodal scaffold arms, because they offer sufficient structural variety. There are only a few reports about the stepwise

solid-phase synthesis of three different peptides on specifically and orthogonally protected molecular scaffolds of different chemical origins (Figure 1).[10]

In general, solid-phase peptide synthesis starts with the attachment of the first amino acid by its carboxylic group to a reactive moiety on the solid support. The amino group represents a convenient reactive partner for the carboxylate. For this reason, our general plan consisted of the adaptation of the structure of **scaffold I** to a tri-orthogonal solid-phase peptide synthesis, i.e. the introduction of amino groups to individual arms of **scaffold I.** In addition, we designed and synthesized new **scaffolds II-IV** (Figure 1). Finally, we synthesized model peptides and compared the suitability of each scaffold for the solid-phase peptide synthesis (SPPS).

Results

Synthesis of scaffolds

The synthesis of **16** (**scaffold II**) from trimesic acid is shown in Scheme 1 and was inspired by our previous work.[7] Briefly, during the first two reaction steps (steps a and b in Scheme 1), both thermodynamic aspects (excess of acid and slow addition of the amine) and kinetic factors (addition of the amine at 0 °C) affected significantly the yield of the reaction as unwanted multi-acylations occurred. During the introduction of the third Fmoc-protected amino "arm", we expected the primary amine of salts **4** or **5** to be quickly generated, as the reaction mixture contained an excess of DIPEA. Such primary amine could cleave the Fmoc protecting group. To avoid this, we tested different modes of addition of the Fmoc-protected amines (in solution or as a solid, in one or several portions), two different salts (trifluoroacetate **4** or chlorohydrate **5**), two different solvents (CH_3CN or DMF) and an alternative DIC-mediated chemistry, which does not require a base. We obtained the best yield (88 %) when salt **4** was added in one portion to the PyBroP pre-activated acid **14** in CH_3CN. Finally, the *tert*-butyl protecting group in compound **15** was removed by treatment with 50 % TFA/DCM to afford **scaffold II** (**16**).

For the synthesis of **scaffold III** (**29**) and **scaffold IV** (**30**), we chose a different strategy (Scheme 2). Coupling the first arm to free trimesic acid leads to a mixture of products; unreacted trimesic acid, desired monoamide, di- and triamide by-products and not easily removable tripyrrolidinophosphinoxid. Previously, in the case of intermediate **13** (Scheme 1), this problem was successfully solved by extensive and repetitive washings and triturations. Here, this work-up was bypassed by introducing suitable protecting groups.

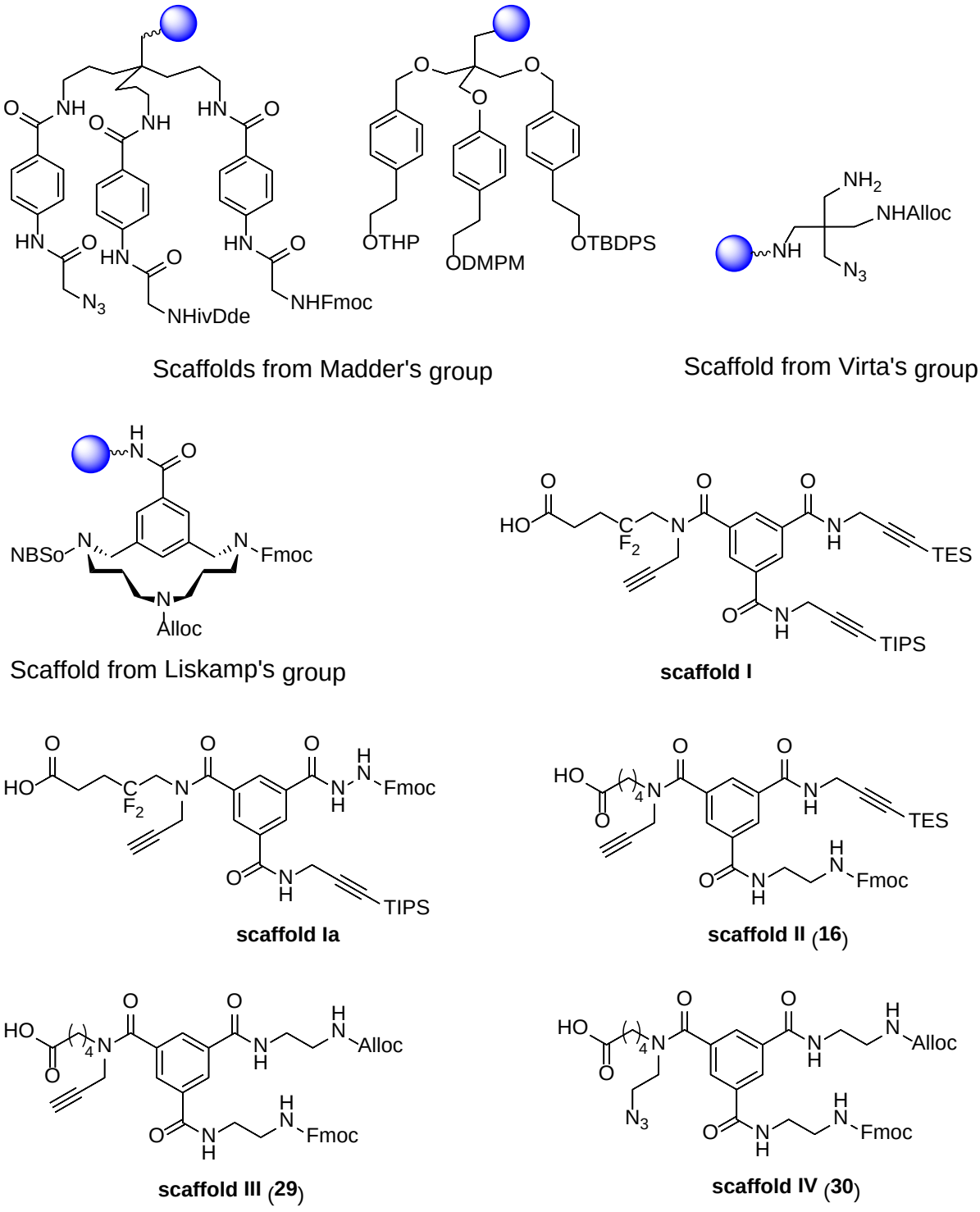
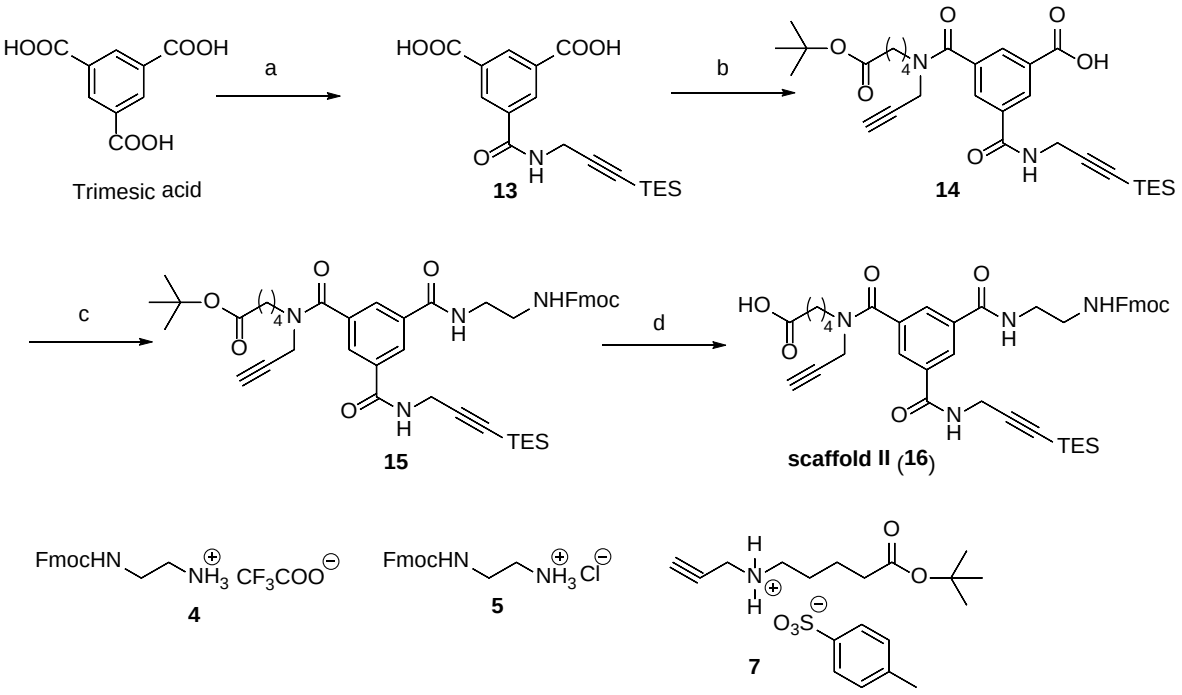


Figure 1. Scaffolds developed by other groups, scaffolds from our previous works (**scaffolds I** and **Ia**) and new scaffolds prepared in this work (**scaffolds II-IV**).

Thus, trimesic acid was esterified with methanol to give trimethylester **17**, which was converted to **18** by a saponification of only one methoxycarbonyl moiety. Reaction between **18** and salt **1** afforded intermediate **19**, which was easily isolated by a silica gel column

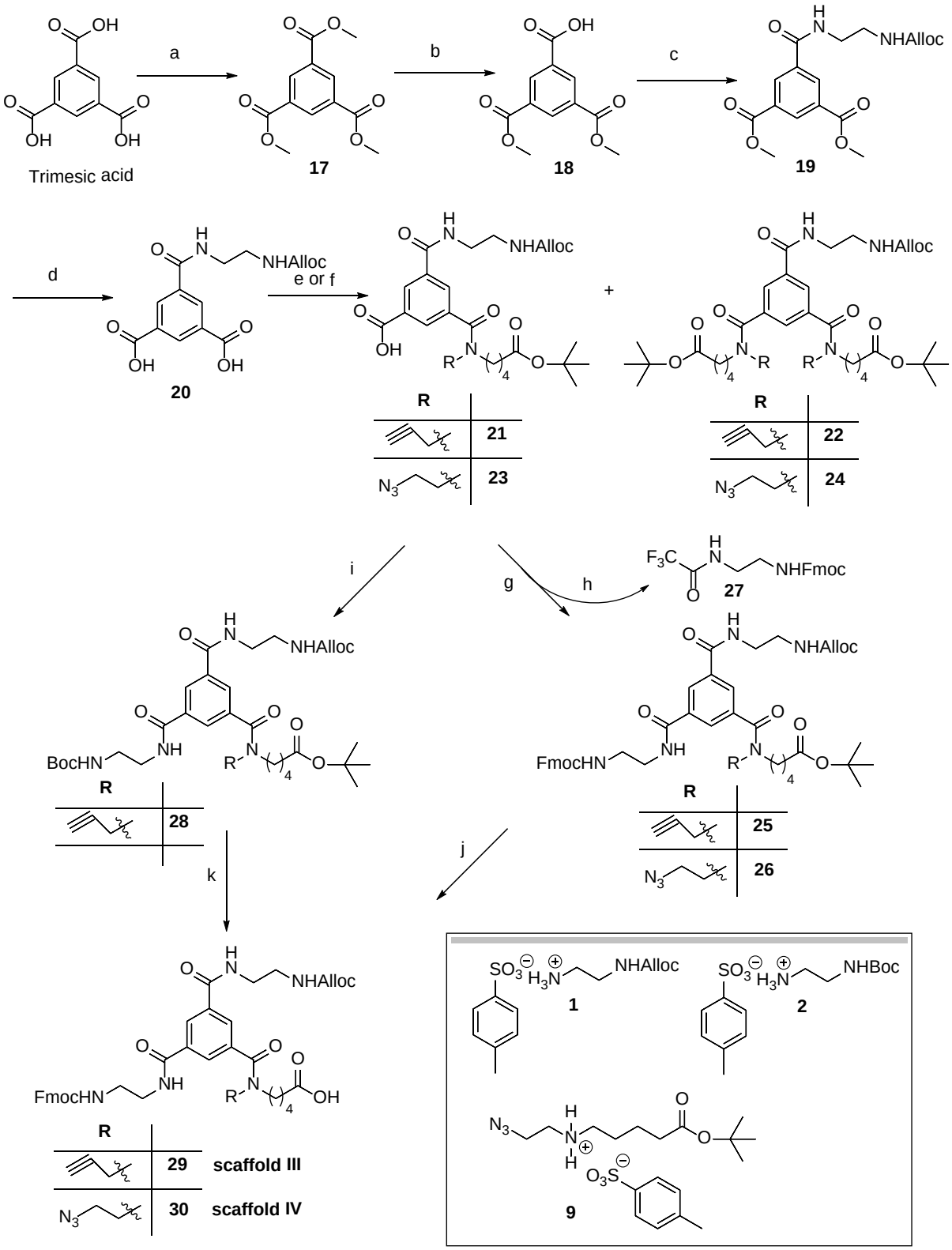
chromatography. Both ester functionalities were removed by basic hydrolysis to give diacid **20**. The second "arm" was attached by amidation with amines **7** or **9** to give the desired compounds **21** or **23**, as well as the by-products **22** or **24**. The yields were satisfactory: 77 % for **21** and 55 % for **23**.



Scheme 1: Reagents, conditions, yields: (a) $NH_2CH_2C{\equiv}C$-TES, TEA, PyBroP, DMF, 0 °C to rt for 16 h (53 %); (b) **7**, TEA, PyBroP, DMF, 0 °C to rt for 16 h (40 %); (c) **4**, DIPEA, PyBroP, CH_3CN, rt, 5 h (88 %); (d) TFA, DCM, rt, 1.5 h (82 %).

The third arm was attached similarly. Surprisingly, however, reaction of **21** with trifluoroacetate salt **4** gave trifluoroacetamide **27** and starting acid **21**. Treatment of **4** with an excess of TEA released the corresponding free amine, which reacts faster with trifluoroacetate species than with carboxylate group of **21**. When trifluoroacetate salt **4** was replaced with chlorohydrate **5**, expected products **25** and **26** were isolated. We assumed that the presence of a bulky Fmoc moiety caused the low yields observed during the third coupling. For this reason, we used the Boc derivative **2** instead of the Fmoc derivative **5**. However, the yields were similar. A plausible explanation could be the steric hindrance of the two-arm intermediates **21** and **23**. TFA-mediated deprotection of acido-labile *tert*-butyl group afforded the desired **scaffold III** (**29**) and **scaffold IV** (**30**). Compound **29** was obtained, also starting from **28**; both acido-labile protecting groups were removed in one step, followed by the introduction of the Fmoc group

instead of the Boc group. HPLC analyses of **scaffolds II**, **III** and **IV** confirmed their high chemical purity (see chromatograms in the Supporting Information).



Scheme 2. Reagents, conditions, yields: (a) MeOH, H_2SO_4, reflux, overnight (91 %); (b) NaOH, methanol and water, rt, overnight (76 %); (c) **1**, PyBroP, TEA, DMF, rt, overnight

(67 %); (d) NaOH, MeOH and water, rt, overnight (92 %); (e) **7**, PyBroP, TEA, DMF, rt, overnight (77 % for **21**, 33 % for **22**); (f) **9**, PyBroP, TEA, DMF, rt, overnight (55 % for **23**, 12 % for **24**); (g) **5**, PyBroP, DIPEA, DMF, rt, 4.5 h (20 % for **25**, 29% for **26**); (h) **4**, PyBroP, TEA, DMF, rt, overnight (39 %); (i) **2**, PyBroP, TEA, DMF, rt, overnight (33 %); (j) TFA, DCM, rt, 3.5 h (85 % for **29**, 90 % for **30**); (k) TFA, DCM, rt, 3.5 h, then Fmoc-Osu, $NaHCO_3$, water, dioxane, rt, overnight (42 % over two steps).

Synthesis of peptides on the scaffolds

For the synthesis of peptides on the scaffolds, we first examined the sequential clicking of azides **11** and **12** to the resin-bound **scaffold I** as shown in Scheme 3. These azides possess the Fmoc-protected amino group, which enables the synthesis of peptides directly on the resin-bound scaffold. For the solid-phase synthesis, we used the PEG-based ChemMatrix resin[11] with a highly acido-labile Ramage linker.[12] We used only half-maximum loading to the resin, to limit the possible steric hindrance which we had previously encountered.[7]

The synthesis of the **scaffold I**-attached tripeptides proceeded without any problem with apolar amino acids (compounds **38** and **39** in Scheme 3), but gave multi-component mixtures with amino acids containing side-chain protections (Asp(tBu), Lys(Boc), His(Trt) etc.). We found that *tert*-butyl ammonium fluoride (TBAF), used for the deprotection of the triisopropylsilyl (TIPS) group, cleaves the protecting groups from the amino acids on the first two arms. We did not find any convenient alternative TIPS-cleaving agent to fluoride (TBAF) and therefore investigated peptide syntheses within **scaffolds II**, **III** and **IV** (Figure 1) with the TIPS-alkynyl arm replaced with a Fmoc-amino group.

Next, as shown in Scheme 4, we synthetized compound **45** on the resin-bound **scaffold II**. After loading **scaffold II** on the resin with PyBrop/DIPEA chemistry, we acetylated the unreacted amines of the resin and obtained the resin-bound compound **40**. The first model dipeptide (Ile-Asp) was then built on the Fmoc-amino group-containing arm of the scaffold using HATU/DIPEA activation. Thereafter, the *N*-terminal amines were acetylated with acetic anhydride to give the resin-bound compound **41**. Azide **11** was then selectively clicked to the free alkyne arm via CuAAC protocol[13] to afford the resin-bound compound **42**. The second peptide (Met-Lys) was synthesized and capped as described for the first arm to give the resin-bound compound **43**.

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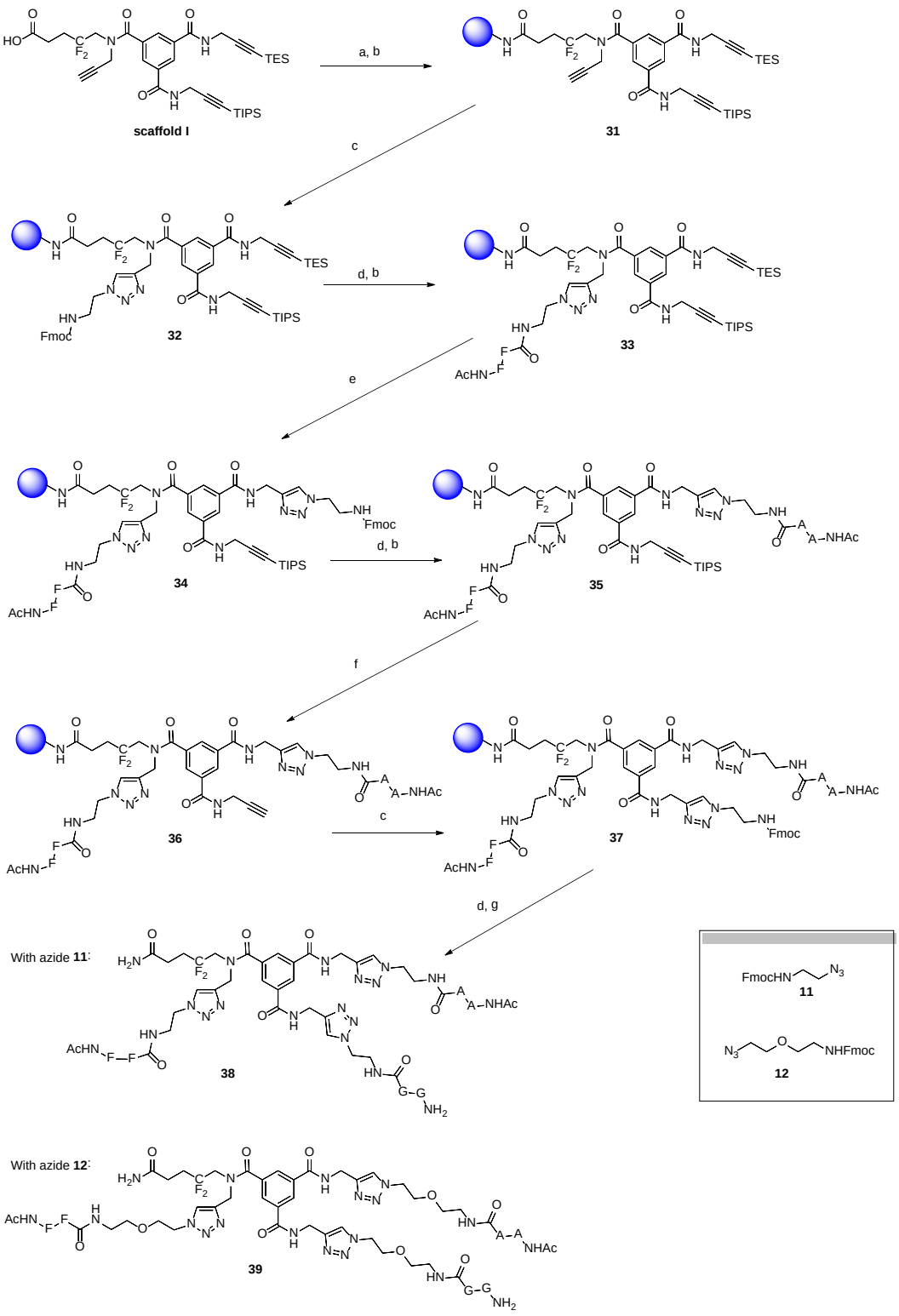


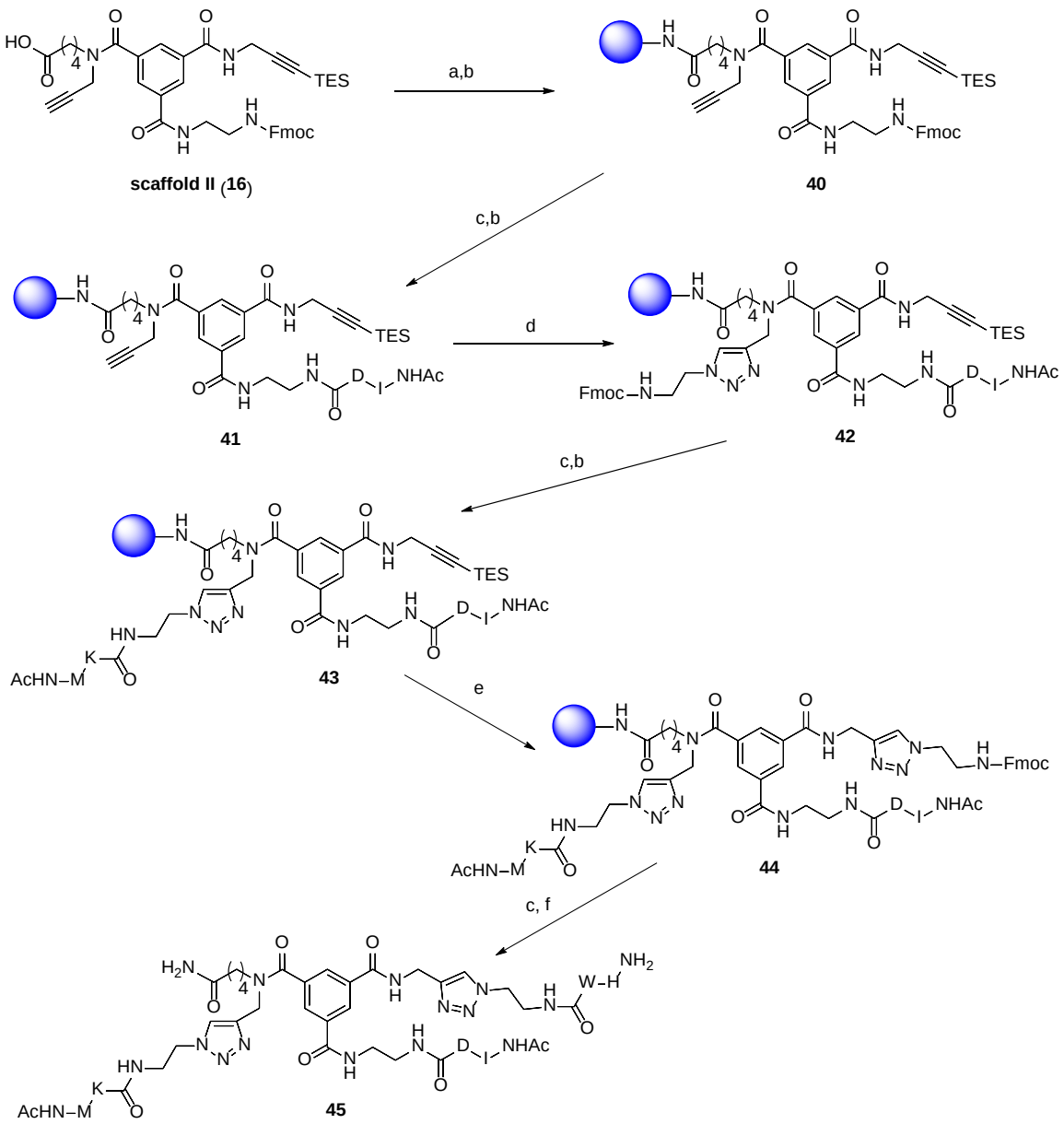
Scheme 3. Reagents and conditions: (a) Ramage ChemMatrix (2 equiv), DIPEA, PyBroP, DMF, rt, 5 h; (b) 5 % Ac_2O (v/v), 5 % DIPEA (v/v) in NMP/DCM (1:1), rt, 2×15 min; (c) Azide **11** or **12** (5 equiv), $CuSO_4.5H_2O$ (0.1 equiv), sodium ascorbate (0.5 equiv), DMF/H_2O (6:4), rt, 16 h; (d) Peptide synthesis (HATU/DIPEA); (e) Azide **11** or **12** (5 equiv), $CuSO_4.5H_2O$ (5 equiv), sodium ascorbate (10 equiv), DMF/H_2O (6:4), rt, 3×16 h; (f) TBAF (5 equiv) in DMF,

3×1 h; (g) TFA/H_2O, scavengers, rt, 2×1 h. Amino acids are shown in one-letter codes: A for alanine, F for phenylalanine and G for glycine.

We previously reported the one-pot TES-deprotection/CuAAC of a TES-protected alkyne using high-copper(I) loadings.[7] Here we employed this strategy and transformed the TES-alkyne group of compound **43** to the triazole **44** in one step. Next, we removed remaining copper ions[14] by washing the resin with a solution of 20% 4-methylpiperidine in DMF. The TES-deprotection with copper is slow and we obtained only a 70 % conversion after 16 h of the reaction. After repeating the reaction for a further 16 h, the conversion increased to about 90 %. Eventually, a third treatment allowed the full conversion (> 98%). Next, we built the third peptide (His-Trp) on the resin-bound compound **44**, and then cleaved the compound from the resin altogether with side chain protecting groups, using a TFA/scavengers mixture. Finally, the compound **45** was isolated from the crude mixture by HPLC.

Scaffold III has the TES-alkyne arm replaced by an Alloc-protected amine. This substitution significantly shortens the synthesis on **scaffold III**. The synthesis of the first two arms of **49** (Scheme 5) was the same as in the case of **scaffold II.** The Alloc-protecting group was removed from **49** by treatment with $Pd(PPh_3)_4$ and in the presence of $(CH_3)_2NH.BH_3$ scavenger, according to the literature.[15] The last dipetide (Trp-His) was built on the third arm of **50** and, after acidic cleavage from support and simultaneous removal of all protecting groups, we isolated the final product **51**.

The synthesis of compound **57** (Scheme 6) was similar to the preparation of compound **51** (Scheme 5). The only different step (Step d) was the reduction of the azide. To reduce the azido group, we first used phosphines: triphenyl-, tributyl- or tris(2-carboxyethyl)phosphine (TCEP). Triphenylphosphine was unreactive, tributylphosphine led to complex reaction mixtures, but TCEP gave a pure product after 2 h of reaction in a mixture of THF and water. We also tried gaseous hydrogen sulfide as an alternative reducing agent. Hydrogen sulfide gas was blown into the syringe containing the resin in a pyridine/H_2O mixture. After three treatments of 2 h each, the azido group was cleanly reduced to the corresponding amine.



Scheme 4. Reagents and conditions: (a) Ramage ChemMatrix, DIPEA, PyBroP, DMF, rt, 5 h; (b) 5 % Ac_2O (v/v), 5 % DIPEA (v/v) in NMP/DCM (1:1), rt, 2×15 min; (c) Peptide synthesis (HATU/DIPEA); (d) Azide **11** (5 equiv), $CuSO_4.5H_2O$ (0.1 equiv), sodium ascorbate (0.5 equiv), DMF/H_2O (6:4), rt, 16 h ; (e) Azide **11** (5 equiv), $CuSO_4.5H_2O$ (5 equiv), sodium ascorbate (10 equiv), DMF/H_2O (6:4), rt, 3×16 h; (f) TFA/H_2O, scavengers, rt, 2×1 h. Amino acids are shown in one-letter codes: D for aspartic acid, I for isoleucine, H for histidine, M for methionine, K for lysine and W for tryptophane.

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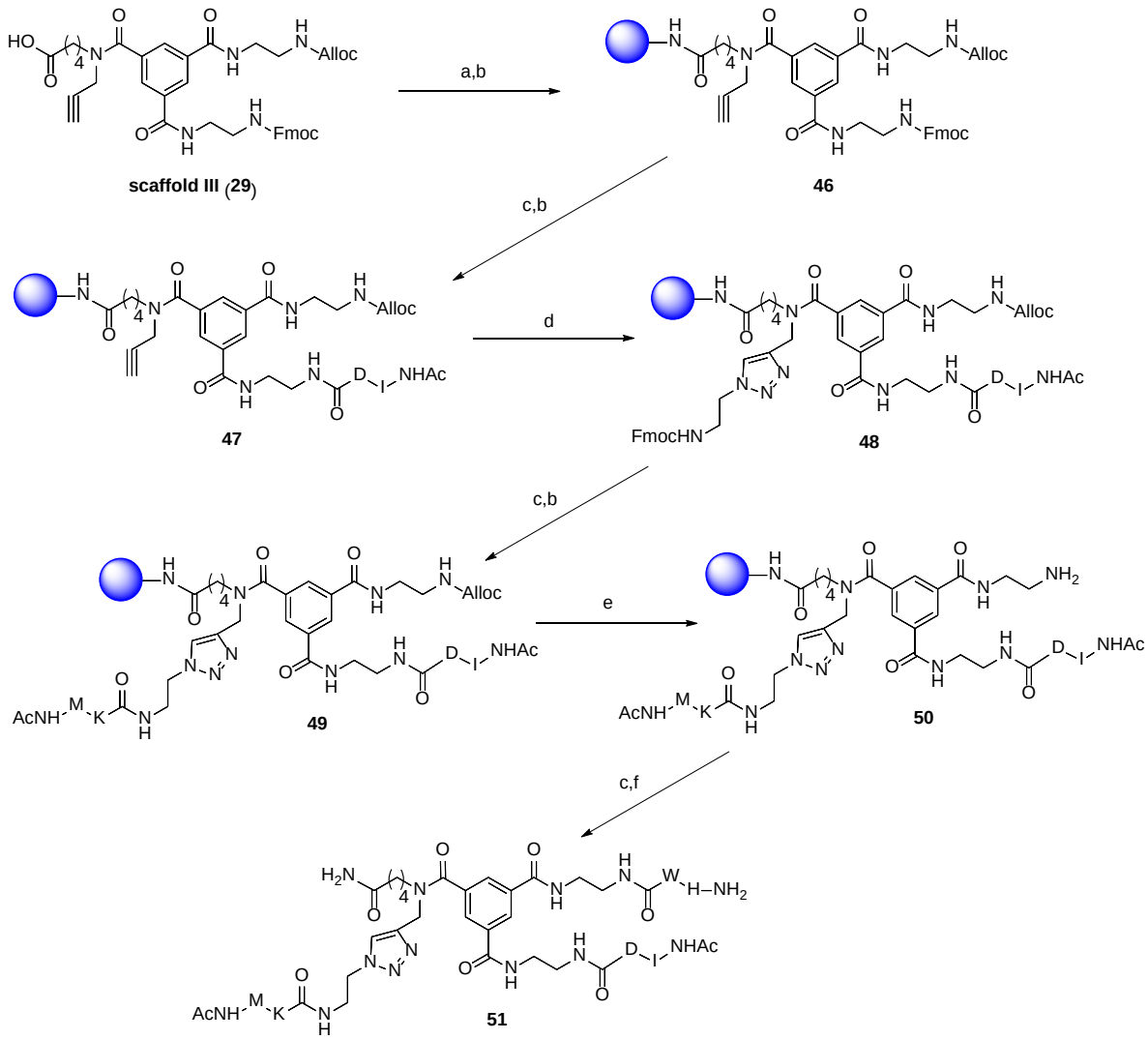
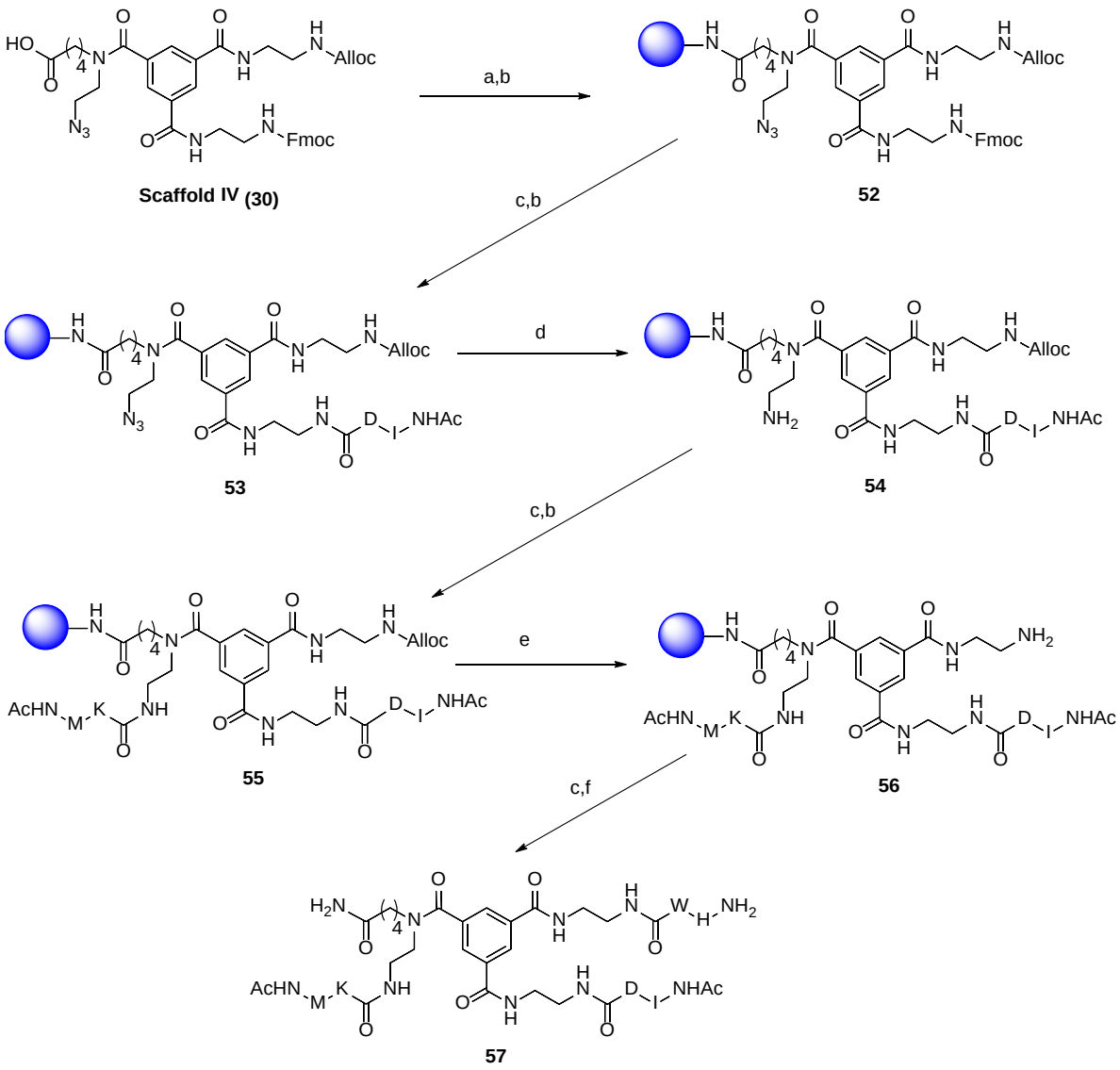


Scheme 5. Reagents and conditions: (a) Ramage ChemMatrix, DIPEA, PyBroP, DMF, rt, 5 h; (b) 5 % Ac_2O (v/v), 5 % DIPEA (v/v) in NMP/DCM (1:1), rt, 2×15 min; (c) Peptide synthesis (HATU/DIPEA); (d) Azide **11** (5 equiv), $CuSO_4.5H_2O$ (0.1 equiv), sodium ascorbate (0.5 equiv), DMF/H_2O (6:4), rt, 16 h; (e) $Pd(PPh_3)_4$ (0.15 equiv), $(CH_3)_2NH.BH_3$ (20 equiv), NMP, N_2 purging, rt, 1.5 h; (f) TFA/H_2O, scavengers, rt, 2×1 h.



Scheme 6. Reagents and conditions: (a) Ramage ChemMatrix, DIPEA, PyBroP, DMF, rt, 5 h; (b) 5 % Ac_2O (v/v), 5 % DIPEA (v/v) in NMP/DCM (1:1), rt, 2×15 min; (c) Peptide synthesis (HATU/DIPEA); (d) TCEP, H_2O/THF (2:1), rt, 2 h (e) $Pd(PPh_3)_4$ (0.15 equiv), $(CH_3)_2NH.BH_3$ (20 equiv), NMP, N_2 bubbling, rt, 1.5 h; (f) TFA/H_2O, scavengers, rt, 2×1 h.

Discussion

Here we describe the development of three tripodal scaffolds compatible with the solid-phase peptide synthesis. Despite their similar structures, their preparation differed. **Scaffold II** was prepared by a direct and sequential amidation of trimesic acid, adapting our protocol for the synthesis of **scaffold I**.[7] We believe that the presence of a bulky and lipophilic TES group in **scaffold II** resulted in very different solubilities of its monoamidate and di- or triamidate derivatives in aqueous or organic media. Consequently, we were able to isolate the monoamide

13 from the crude mixture by successive extractions and washings. On the contrary, during the synthesis of **scaffolds III** and **IV**, we were not able to isolate monoamidation products from the crude reaction mixtures. **Scaffolds III** and **IV** required the full esterification, followed by the mono-saponification of trimesic acid.

Scaffold I is not compatible with SPPS, because the fluoride TBAF agent used to deprotect the TIPS group also cleaves the acid-labile protections of the amino acids' side-chains. Some research groups have already reported the cleavage of carbamate protecting groups with TBAF (reviewed in Ref.[16]) For instance, Coudert and co-workers reported the complete deprotection of *tert*-butyl carbamates at room temperature in a few hours.[17] Gea et al. faced a similar problem when they performed the deprotection of a *tert*-butyldiphenylsilyl ether from a tripodal scaffold bearing protected amino acids.[10c] There, TBAF caused the cleavage of acid-labile side-chain protections. They eventually succeeded with tris(dimethylamino)sulfonium (trimethylsilyl)difluoride, one of the few sources of anhydrous fluoride. We chose to abandon the TIPS-alkyne group and preferred the use of a Fmoc-amino moiety in **scaffolds II**, **III** and **IV**. The solid-phase synthesis on **scaffold II** was straightforward and we obtained compound **45** in an acceptable yield (30%, after HPLC purification).

As discussed above, the one-pot TES deprotection/CuAAC was slow and, for that reason, we preferred to incorporate an Alloc-amino moiety to **scaffold III**. We tested different allyl scavengers, p-toluenesulfinic acid,[18] phenylsilane and borane dimethylamine complexes[15] for the catalytic deallylation with tetrakis(triphenylphosphine)palladium. The borane dimethylamine complex gave the best result, yielding the pure product in 1.5 h. Finally, we eventually modified **scaffold III** by replacing the alkyne arm by an azido moiety in **scaffold IV**. As explained above, the reduction with tributylphosphine or triphenylphosphine in THF/water mixture failed, but the TCEP worked well.

This means that an important factor for success could be the water solubility of the phosphine. Other groups also failed at reducing a solid-supported azide with triphenylphosphine because the reactions stopped after the formation of the iminophosphorane intermediate.[10g, 10h] They eventually succeeded by using the more reactive trimethylphosphine. In a different approach, we used gaseous hydrogen sulfide as the reducing agent. The reaction was slower than with TCEP, but was also highly satisfactory. Nevertheless, we prefer the TCEP approach, which is more convenient and safer.

Finally, we compared the solid-phase syntheses on **scaffolds II-IV,** using three model dipeptides (Ile-Asp, Met-Lys and His-Trp). We selected these amino acids to effectively test our methodology as they represent bulky amino acids, are prone to oxidations or alkylations,

and contain protecting groups. We were also curious whether Met would poison the Pd(0) catalyst used during the deallylation. We considered three criteria that we judged relevant for our future combinatorial processes: purity, time of the synthesis and feasibility of procedures. The HPLC chromatograms of the crude compounds **45**, **51** and **57** are shown in Figure 2. The HPLC purity (integration at 218 nm) of the desired product (P) in the crude mixtures varied from 53 % (for compound **51**) to 63 % (for compound **57**). In all three crude reaction mixtures, we obtained a major and similar side product (S) that was identified as an oxidation product (M+16 by mass spectrometry), probably resulting from the partial oxidation of methionine, which is not surprising. **Scaffolds II** and **IV** gave more pure crude products (compounds **45** and **57**, respectively), whereas the crude compound **51** prepared from **scaffold III** contained almost 30% of the oxidation side product. In general, even if all three scaffolds provide products of satisfactory purities, **scaffolds II** and **IV** clearly represent the more optimal tools for further applications in hit discovery and combinatorial applications.

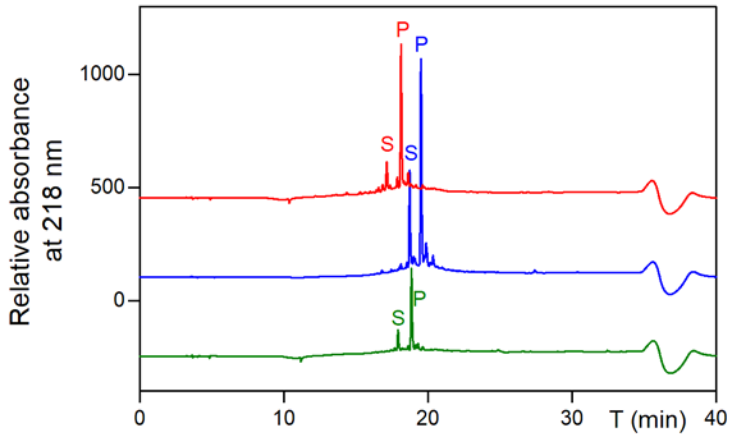


Figure 2. RP-HPLC chromatograms of the crude compounds **45** (in green), **51** (in blue) and **57** (in red). The peaks of the desired products are labeled as P and the peaks of major side-products, which were identified as oxidation products, are labeled as S.

Our general strategy was to replace the slow CuAACs and TES deprotection by two faster reactions: Pd-catalysed deallylation and azide reduction. Besides its slowness in the solid phase (presumably due to the slow diffusion of the active species within the resin), the CuAAC is hardly compatible with an automatic synthesizer. On the contrary, the azido reduction by phosphine can be automated,[10g] which is an advantage regarding planned combinatorial processes. Here a possible limitation of **scaffold IV** appears; the Pd-catalyzed deallylation cannot be automated, because the reaction must be performed using fresh and homogenous

solutions of palladium or borane and because the resin suspension has to be stirred by a strong nitrogen flow.

Considering the various parameters, **scaffold IV** appears to be the most suitable one for potential combinatorial synthesis in the solid phase. However, **scaffold II** could enable an alternative diversity of the structures of the final compounds by using different non-peptide azides during the two CuAACs. This could also be useful, e.g. for introducing more rigidity to final compounds.

In that respect, we are aware that peptide compounds derived from **scaffolds II-IV** will have a somewhat flexible behavior. In order to examine this, we assigned the proton and carbon NMR spectra of the compound **39** and measured intra-molecular NOEs as well. We observed important line broadening, as well as a lack of inter-arm NOEs. These observations suggest a rather extended conformation of the three arms of compound **39,** with fairly important conformational freedom. In the context of drug discovery, such a flexible scaffold allows for the exploration of a fairly large chemical space. On the other hand, they can suffer from unfavorable entropic effects, which can decrease the overall binding energy to the target. For this reason, we are currently developing more constrained tripodal scaffolds. We believe that both approaches (flexible vs constrained scaffolds) could be complementary when starting a hit discovery process.

Overall, we developed three variants of tripodal scaffolds, which enable an efficient stepwise synthesis of three different peptides on the same scaffold. Here we may mention that we also attempted to modify **scaffold I** with pre-prepared tripeptides containing azido-amino acids.[19] This strategy was lengthy and rather unsuccessful because the CuAAC with azido-peptides was inefficient, especially on the second and third arms. This underlines the advantage of the peptide synthesis directly on the scaffolds. The methodology could also be useful for a facile synthesis of potential proteomimetics in the form of combinatorial libraries.

In the accompanying article,[20] all three new scaffolds were further validated by the preparation of new tripodal compounds with tetra to hexapeptides derived from the insulin structure, or octapeptides derived from previously developed insulin peptide mimetics.[21] The compounds were tested for their ability to bind and activate receptors for insulin and IGF-1.

Experimental Section

General. The conditions and methods for purification and analyses of compounds as well as NMR and IR analytical data and HPLC chromatograms are provided in the Supporting information.

For the synthesis of compounds **1-12**, refer to the Supporting information (Scheme S1 and below). For compounds **16**, **25**, **29**, a thermal cleavage of the Fmoc protection group[22] was observed at 90 °C and details are shown in the Scheme S2.

5-((3-(triethylsilyl)prop-2-yn-1-yl)carbamoyl)isophthalic acid (13): PyBrop (29.3 g, 62.8 mmol, 1.4 equiv) was added to a solution of trimesic acid (56.6 g, 269.3 mmol, 6 equiv) and TEA (43.8 mL, 314.2 mmol, 7 equiv) in DMF (300 mL) at 0 °C. A solution of 3-(triethylsilyl)prop-2-yn-1-amine[7] (7.6 g, 44.9 mmol, 1 equiv) in DMF (150 mL) was added dropwise (exothermic process). After complete addition of the amine, the ice bath was removed and the resulting mixture was stirred overnight at room temperature. TLC analysis performed on a pretreated plate with gaseous NH_3 in the system (IPA : conc. aq. ammonia : water 7 : 1 : 2) showed completion of the reaction (Rf = 0.73). The solvent was evaporated at 70 °C *in vacuo* and the solid residue was suspended in an AcOH-acetone mixture (60 mL + 240 mL). The resulting slurry was stirred for 20 minutes and filtered through an S3 frit. The solid was transferred back to the round bottom flask, stirred in acetone (50 mL) and filtered as above. The solid (mostly unreacted trimesic acid) was then discarded and the filtrate was evaporated *in vacuo* to give a pale yellow residue. This residue was partitioned between Et_2O (500 mL) and DMF-H_2O (1:1, 500 mL in total), and the aqueous layer was further extracted with Et_2O (5 x 200 mL). The combined organic layers were then washed successively with 50 % aqueous DMF (3 x 300 mL) to remove the rest of the unreacted trimesic acid and brine (300 mL), dried over anhydrous sodium sulfate, evaporated *in vacuo*, co-evaporated with 100 mL of MeOH, and evaporated to dryness. The crude product was obtained as a pale yellow semi-solid (19.0 g), containing the desired monoamide **13**, the diamide and tri(pyrrolidin-1-yl)phosphine oxide (recognized by ^{1}H NMR). The solid was crushed, transferred to a flask containing 300 mL of toluene, and heated to reflux for 1 h under intensive stirring. After cooling overnight at 5 °C, the slurry was filtered through an S4 frit, and the solid was washed with a small amount of hexane and dried to dryness to give the monoamide **13**. Analysis by NMR and HPLC revealed complete disappearance of phosphinoxide and diamide. Yield 8.6 g, 53 %. White solid. HRMS (*m/z*): calcd for $C_{18}H_{22}O_5NSi$ $(M+H)^+$ 360.1273, found 360.1276.

3-((5-(*tert*-butoxy)-5-oxopentyl)(prop-2-yn-1-yl)carbamoyl)-5-((3-(triethylsilyl)prop-2-yn-1-yl)carbamoyl)benzoic acid (14): PyBrop (6.8 g, 14.6 mmol, 1.1 equiv) was added to a solution of monoamide **13** (4.8 g, 13.3 mmol, 1 equiv) and Triethylamine (11.1 mL, 79.7 mmol, 6 equiv) in DMF (40 mL) at 0 °C. A solution of the tosylate salt **7** (5.3 g, 13.9 mmol, 1.05

equiv) in DMF (40 mL) was added dropwise. After complete addition, the ice bath was removed and the resulting mixture was stirred overnight at room temperature. A TLC plate, pretreated with gaseous NH_3, showed in the system (ethyl acetate : acetone : ethanol : water 4 : 1 : 1 :1) completion of reaction (Rf = 0.60). The solvent was then evaporated at 70 °C *in vacuo* and the residue was partitioned between Et_2O (150 mL) and an AcOH-H_2O mixture (10 mL + 120 mL). The aqueous layer was further extracted with Et_2O (2 x 100 mL). The combined organic layers were washed successively with H_2O (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate and evaporated to dryness to give 12.4 g of brown oil. The crude product was purified by flash chromatography on silica gel (elution with a linear gradient of 1 % AcOH/EtOAc (v/v) in toluene) to give the diamide **14** as a white foam. Yield 2.96 g, (40 %). HRMS **(***m/z***):** calcd for $C_{30}H_{42}O_6N_2NaSi$ $(M+Na)^+$ 577.2704, found 577.2705.

tert*-butyl 5-(3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(prop-2-yn-1-yl)-5-((3-(triethylsilyl)prop-2-yn-1-yl)carbamoyl)benzamido)pentanoate (15):** DIPEA (3.10 mL, 17.8 mmol, 4.0 equiv) was added to a solution of diamide **14** (2.96 g, 5.3 mmol, 1.2 equiv) in CH_3CN (40 mL) in a water bath at room temperature, followed by PyBrop (3.10 g, 6.7 mmol, 1.5 equiv). The resulting solution was stirred for 10 minutes and trifluoroacetate amine **4** (1.41 g, 4.4 mmol, 1.0 equiv) was added in one portion. The resulting mixture was stirred at room temperature for 5 h. TLC analysis performed in the system (ethyl acetate : toluen 1 :1) on (Rf = 0.39) showed completion. The solvent was evaporated at 40 °C *in vacuo* and the residue was partitioned between ethyl acetate (100 mL) and 2 % AcOH/H_2O (v/v, 100 mL in total). The organic layer was then washed successively with saturated NH_4Cl (80 mL), H_2O (80 mL) and brine (80 mL), dried over anhydrous sodium sulfate and evaporated to dryness to give 7.4 g of brown oil. The crude product was purified by flash chromatography on silica gel (elution with a linear gradient of ethyl acetate in toluene) to give triamide **15** as a white foam. Yield 3.2 g (88 %)**.** HRMS **(m/z***):** calcd for $C_{47}H_{58}O_7N_4NaSi$ $(M+Na)^+$ 841.3967, found 841.3968.

5-(3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(prop-2-yn-1-yl)-5-((3-(triethylsilyl)prop-2-yn-1-yl)carbamoyl)benzamido)pentanoic acid 16 (Scaffold II): Trifluoroacetic acid (3 mL) was added to a solution of triamide **15** (3.20 g, 3.91 mmol) in DCM (3 mL) at 0 °C. The reaction mixture was stirred for 2 h at room temperature and the solvents were evaporated at 40 °C *in vacuo*. The residue was purified by flash chromatography on silica gel (elution with a linear gradient of 10 % MeOH/$CHCl_3$ (v/v) in $CHCl_3$) to give a pale

yellow foam. This foam was lyophilized from 1,4-dioxane to afford **16** as a white solid. The RP-HPLC analysis of compound **16** is shown in the Supporting Information. Yield 2.40 g (82 %). RP-HPLC (Method A): 95.8 %. HRMS **(***m/z*): calcd for $C_{43}H_{50}O_7N_4NaSi$ $(M+Na)^+$ 785.3341, found 785.3343.

Trimethyl 1, 3, 5-benzenetricarboxylate (17): Trimesic acid (30 g, 0.143 mol) with concentrated sulfuric acid (6 mL) was refluxed overnight in methanol (350 mL). After cooling at -20 °C, the mass of crystals was filtered and washed with 500 mL of chilled methanol. Crystallization from methanol afforded a pure product. Yield 32.6 g (91 %). White solid, m.p. 140-143 °C (methanol) R_f = 0.84 (50 % toluene / 50 % ethyl acetate). HRMS (EI) calc for $C_{12}H_{12}O_6$ $[M]^+$ 252.0634, found: 252.0632.

3,5-bis(Methoxycarbonyl)benzoic acid (18): A partial saponification was performed by the reaction of **17** (32.4 g, 0.128 mol) with 1M aqueous NaOH solution (4.6 g, 0.115 mol) in methanol (2 L), using the protocol described in the literature.[23] Yield 23.3 g (76 %). White solid, m.p. 136-137 °C (ethyl acetate – petroleum ether) R_f = 0.69 (ethyl acetate methanol acetone water 6/2/1/0.5). HRMS (ESI) calc for $C_{11}H_{10}O_6Na$ $[M+Na]^+$ 261.0370, found: 261.0370.

Dimethyl-5-(2-(((allyloxycarbonyl)amino)ethyl)carbamoyl)-1,3-benzendicarboxylate (19): TEA (18 mL, 128.8 mmol) was added in one portion to a solution of salt **1** (10.2 g, 32.2 mmol), **17** (7.7 g, 32.2 mmol) and PyBroP (21.2 g, 45.1 mmol) in DMF (200 mL). The mixture was allowed to react overnight at room temperature. 500 mL of water was added and the product was extracted with ethyl acetate (4 x 150 mL). The combined organic phases were washed with water (150 mL), brine (3 x 150 mL), dried over anhydrous sodium sulphate, filtrated and evaporated. The brown residue was purified by flash chromatography, using a linear gradient of ethyl acetate in toluen. Evaporation of the fractions containing the product afforded a solid, which was crystalized from a mixture of ethyl acetate-hexane. Yield 7.6 g (67 %). White solid, m.p. 132-134 °C. R_f = 0.69 (ethyl acetate). HRMS (ESI) calc for $C_{17}H_{21}O_7N_2$ $[M+1]^+$ 365.1343, found: 365.1344.

5-(2-(((allyloxycarbonyl)amino)ethyl)carbamoyl)-1,3-benzendicarboxylic acid (20): Diester **19** (7 g, 19.2 mmol) was suspended in methanol (30 mL) and NaOH (3.1 g ; 76.9 mmol) in water (50 mL) was added. The mixture was stirred overnight at room temperature. Thereafter,

1M HCl was added to the clear solution until pH value was approx. ~ 6. Methanol was evaporated and the flask with the resulting aqueous solution was immersed in an ice bath, with the pH adjusted by 1 M HCl to pH ~ 1. The slurry was kept at 5 °C for 30 minutes. The precipitate was filtered in a Büchner funnel, washed with 100 mL of chilled water and dried under a deep vacuum over P_2O_5. Yield 6 g (92 %). White solid, m.p. 252-255 °C. R_f = 0.66 (conc. ammonia isopropyl alcohol water 6/2/1). HRMS (ESI) calc for $C_{15}H_{15}O_7N_2$ $[M+1]^+$ 335.0885, found: 335.0886.

3-((((2-Allyloxycarbonyl)amino)ethyl)carbamoyl) - 3 - (((5-(*tert*-butoxy)-5-oxopentyl)prop-2-yn-1-yl)carbamoyl)benzoic acid (21)

1-((((2-Allyloxycarbonyl)amino)ethyl)carbamoyl) - 3,5-bis(((5-(*tert*-butoxy)-5-oxopentyl)prop-2-yn-1-yl)carbamoyl)benzene (22): PyBroP (8.2 g, 17.6 mmol) and TEA (15 mL, 112 mmol) were added within 10 minutes to a solution of **20** (5.4 g, 16 mmol) in DMF (150 mL), followed by **7** (6.1 g, 16 mmol), which was added in three equal portions. The reaction mixture was stirred overnight at room temperature. Afterwards, 300 mL of water was added. The resulting mixture was acidified with glacial acetic acid until pH 5 was reached. The aqueous phase was extracted with ethyl acetate (4 x 150 mL). The combined organic layers were washed with water (100 mL), brine (3 x 100 mL), and dried over anhydrous sodium sulfate. Evaporation of the filtrate afforded a brown residue, which was subjected to flash chromatography. Less polar, unwanted diamide **22** was eluated with ethyl acetate; the desired product **21** was then eluted with a linear gradient of ethyl acetate / MeOH / acetone (6 : 2 :1) in ethyl acetate.

21. Yield 6.6 g (77 %). Bright yellow oil. R_f = 0.49 (ethyl acetate : MeOH : acetone : water 6 : 2 : 1 : 0.5). HRMS (ESI) calc for $C_{27}H_{34}O_8N_3$ $[M+1]^+$ 528.2351, found: 528.2342.

22. Yield 3.8 g (33 %). Colorless oil. R_f = 0.76 (ethyl acetate). HRMS (ESI) calc for $C_{39}H_{54}O_9N_4Na$ $[M+Na]^+$ 745. 3783, found: 745.3785.

3-((((2-Allyloxycarbonyl)amino)ethyl)carbamoyl) - 5 - ((2-azidoethyl-5-(*tert*-butoxy)-5-oxopentyl)carbamoyl)benzoic acid (23) and 1-((((2-Allyloxycarbonyl)amino)ethyl)carbamoyl) - 3,5-bis((2-azidoethyl-5-(*tert*-butoxy)-5-oxopentyl)carbamoyl)benzene (24): Compounds **23** and **24** were prepared by a reaction of **20** (3.6 g, 10.7 mmol), PyBroP (5.5 g, 11.8 mmol), **9** (4.4 g, 10.7 mmol) and TEA (10.4 mL, 74.9 mmol) in DMF (100 mL), using the protocol described for **21** and **22**.

23: Yield 3.3 g (55 %). Colorless oil. R_f = 0.61 (ethyl acetate : MeOH : acetone : water 6 : 2 : 1 : 0.5). HRMS (ESI) calc for $C_{26}H_{35}O_8N_6$ $[M+1]^+$ 559.25219 found: 559.2524.
24: Yield 1 g (12 %). Colorless oil. R_f = 0.43 (ethyl acetate). HRMS (ESI) calc for $C_{37}H_{56}O_9N_{10}Na$ $[M+Na]^+$ 807.41239 found: 807.4125.

***Tert*-butyl 5-3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-5-((2-(((allyloxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(prop-2-yn-1-yl)benzamido)pentanoate (25):** PyBroP (7.3 g, 15.7 mmol) and DIPEA (7.2 ml, 41.6 mmol) were added to the solution of **21** (6.6 g, 12.5 mmol) in DMF (100 mL), followed by the addition of **5** (3.3 g, 10.4 mmol) in one portion. The reaction mixture was stirred for 4.5 h at room temperature. Thereafter, water (200 mL) was added, and the resulting solution was acidified with 1 M solution of citric acid until pH 5 was reached. The aqueous phase was extracted with ethyl acetate (4 x 150 mL) and the combined organic layers were washed with water (100 mL), brine (3 x 100 mL) and dried over anhydrous sodium sulfate. Evaporation of the filtrate afforded a crude product, which was purified by flash chromatography, using a linear gradient of ethyl acetate / MeOH / acetone (6 : 2 :1) in ethyl acetate. Yield 1.9 g (20 %). White foam. R_f = 0.47 (ethyl acetate). HRMS (ESI) calc for $C_{44}H_{51}O_9N_5Na$ $[M+Na]^+$ 816.3579, found: 816.3580.

***tert*-butyl 5-(3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-5-((2-(((allyloxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(2-azidoethyl)benzamido)pentanoate (26):** Intermediate **26** was prepared as described for **25** by a reaction of **23** (2.8 g, 5 mmol), PyBroP (3.5 g, 7.5 mmol), DIPEA (3.5 g, 20 mmol) and **5** (1.6 g, 5 mmol) in DMF (100 mL). Yield 1.2 g (29 %). White foam. R_f = 0.42 (ethyl acetate). HRMS (ESI) calc for $C_{43}H_{52}O_9N_8Na$ $[M+Na]^+$ 847.37495, found: 847.3752.
Using the protocol described above, the reaction of **21** (2.5 g, 4.7 mmol), PyBroP (6.6 g, 14.1 mmol), TEA (2.6 ml, 18.8 mmol) and **4** (1.9 g, 4.7 mmol) in DMF (100 mL) gave the starting compound **21** and *N*-trifluoroacetyl-*N´*-(9*H*-fluoren-9-yl)methoxy)carbonyl)ethylenediamine **27**. Yield 0.7 g (39 %). Colorless crystals, m. p. 198-199 °C. R_f = 0.68 (ethyl acetate : toluen 50 : 50). Anal. calc. (%): C 60.32, H 4.53, 7.40 N, 15.06 F. Found (%): C 60.35, 4.63 H, 7.16 N, 14.80 F. HRMS (ESI) calc for $C_{19}H_{18}O_3N_2F_3$ $[M+1]^+$ 379.12640, found: 379.1266.

***Tert*-butyl 5-(3-((2-(((allyloxy)carbonyl)amino)ethylcarbamoyl)-5-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-*N*-(prop-2-yn-1-yl)benzamido)pentanoate (28):** Boc-protected intermediate **28** was prepared as described for **25** by the reaction of **21** (6.6 g,

12.5 mmol), PyBroP (17.5 g, 37.5 mmol), TEA (7 mL, 50 mmol) and **2** (8.3 g, 25 mmol) in DMF (70 mL). Yield 2.8 g (33 %). White foam. R_f = 0.41 (ethyl acetate). HRMS (ESI) calc for $C_{34}H_{49}O_9N_5Na$ $[M+Na]^+$ 694.3423, found: 694.3425.

5-(3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-5-((2-(((allyloxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(prop-2-yn-1-yl)benzamido)pentanoic acid 29 (Scaffold III) and 5-(3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)carbamoyl)-5-((2-(((allyloxy)carbonyl)amino)ethyl)carbamoyl)-*N*-(2-azidoethyl)benzamido)pentanoic acid 30 (Scaffold IV): 25 (1.8 g, 2.3 mmol) or **26** (1.1 g, 1.33 mmol) was stirred in DCM (3 mL) and TFA (6 mL) for 3.5 h at room temperature. TLC analysis revealed the full deprotection of the *tert*-butyl protecting group. The volatile material was evaporated under reduced pressure and the residual brown oil was purified by flash chromatography with a linear gradient of ethyl acetate / MeOH / acetone (6 : 2 :1) in ethyl acetate. The product was dissolved in 10 mL dioxane and lyophilized. The RP-HPLC analyses of **scaffold III** (**29**) and **scaffold IV** (**30**) are shown in the Supporting Information.

Analogously, **28** (2.8 g, 4.2 mmol) was stirred DCM (3 mL) and TFA (6 mL). After 3 h at room temperature, the solvents were removed by evaporation. The residual oil was emulgated in a solution of $NaHCO_3$ (1.4 g ; 8.4 mmol) in water (50 mL). The flask was placed in an ice-cooling bath. A solution of Fmoc-Osu (1.4 g ; 4.2 mmol) in dioxane (20 mL) was added dropwise under vigorous stirring. The reaction mixture was stirred overnight at room temperature. 1M citric acid was then added until pH ≈ 3 was reached. The reaction mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic phases were washed in brine (3 x 50 mL), dried over anhydrous sodium sulfate and filtered. The solvent was evaporated and the resulting brown oil was subjected to flash chromatography with a linear gradient of ethyl acetate / MeOH / acetone (6 : 2 :1) in ethyl acetate. The title compound was dissolved in 10 mL dioxane and lyophilized to afford 1.3 g (42 %) of the required product as a white solid.

29. Yield 1.4 g (85 %). White solid. R_f = 0.71 (ethyl acetate : MeOH : acetone 6 : 2 : 1). RP-HPLC (Method A): purity = 96 %. HRMS (ESI) calc for $C_{40}H_{43}O_9N_5Na$ $[M+Na]^+$ 760.2953, found: 760.2954.

30. Yield 0.9 g (90 %). White solid. R_f = 0.69 (ethyl acetate : MeOH : acetone 6 : 2 : 1). RP-HPLC (Method A): 98 %. HRMS (ESI) calc for $C_{43}H_{44}O_9N_8Na$ $[M+Na]^+$ 791.3124, found: 791.3126.

General description for the solid-phase synthesis of 25, 38, 39, 45, 51 and 57

Peptide synthesis was performed manually in a PP syringe equipped with a Teflon frit.

Loading to the resin (31, 40, 46, 52): In a 10 mL-fritted polypropylene syringe, Ramage ChemMatrix® resin (213 mg, 100 µmol - Aldrich 727792, lot # BCBV2122, loading = 0.47 mmol/g) with free amino groups was swelled in MeOH, DCM, and DMF (10 minutes each, 5 mL of each solvent). The resin was then washed with 3×3 mL DMF. A solution of **scaffold I**, **scaffold II** (**16**), **scaffold III** (**29**) or **scaffold IV** (**30**) (50 µmol, 1.0 equiv), PyBroP (47 mg, 100 µmol, 2.0 equiv), and DIPEA (26 µL, 150 µmol, 3.0 equiv) in DMF (1.5 mL) was added to the resin. The syringe was rotated at room temperature for 5 h. The resin was successively washed with 3×3 mL of DMF, MeOH, DCM and NMP/DCM (1:1). The unreacted amino groups of the resin were capped by two treatments of 5 % Ac_2O (v/v), 2 % DIPEA (v/v) in NMP/DCM (1:1) (2.5 mL and 15 min for each treatment). The resin was washed with 3×3 mL of NMP/DCM (1:1), MeOH, DCM and DMF to give the resin-bound compound **31**, **40**, **46** and **52**.

Peptide synthesis (compounds 33, 35, 38, 39, 41, 43, 45, 47, 49, 51, 53, 55 and 57):

Fmoc deprotection: The resin was washed with 3×3 mL DMF and treated with 20 % 4-methylpiperidine (v/v) in DMF (2.5 mL for each treatment, two treatments of 5 + 20 min). The resin was washed with 3×3 mL DMF, DCM, MeOH and DMF.

Peptide coupling: The resin was washed with 3×3 mL NMP or DMF. To the resin was added a solution of Fmoc-amino acid (3 equiv), HATU (3 equiv) and DIPEA (4 equiv) in NMP or DMF (1.5 mL). The coupling was performed for 40 to 50 minutes at room temperature. The resin was washed with 3×3 mL NMP or DMF and the coupling was repeated once as above. The resin was finally washed with 3×3 mL of DMF, MeOH, DCM and DMF.

Capping: After completion of the first or the second arm of the scaffold, the *N*-terminal end of the peptide was acetylated. The resin was washed with 3×3 mL NMP/DCM (1:1) and treated with 5 % Ac_2O (v/v), 5 % DIPEA (v/v) in NMP/DCM (1:1) (2.5 mL for each treatment, two treatments of 15 min). The resin was washed with 3×3 mL of NMP/DCM (1:1), MeOH, DCM and DMF.

CuAAC with a low copper load (**32**, **37**, **42** and **48**): The resin was washed with 3×3 mL DMF. The following solutions were sequentially added to the resin: i) azide (250 µmol, 5.0 equiv) in 0.9 mL of DMF; ii) sodium ascorbate (50 µL of a freshly prepared 0.5 M aqueous solution, 25 µmol, 0.5 equiv) in 0.25 mL of H_2O; iii) copper(II) sulfate pentahydrate (50 µL of a freshly prepared 0.1 M aqueous solution, 5 µmol, 0.1 equiv) in 0.25 mL of H_2O. The syringe was

agitated at room temperature for 16 h and the resin was washed with 3x3 mL of DMF/water (1:1), MeOH, DCM and DMF to give the triazole compound.

One-pot TES-deprotection/CuAAC with a high copper load (**34** and **44**): The resin was washed with 3×3 mL DMF. The following solutions were sequentially added to the resin: i) azide (250 µmol, 5.0 equiv) in 3 mL of DMF; ii) sodium ascorbate (99 mg, 500 µmol, 10 equiv) in 1 mL of H_2O; iii) copper(II) sulfate pentahydrate (62.5 mg, 250 µmol, 5 equiv) in 1 mL of H_2O. The syringe was agitated at room temperature for 16 h and the resin was washed with 3x3 mL DMF/water (1:1), water, MeOH, DCM and DMF. The coupling was repeated once, using the same conditions. The resin was finally washed with 3x3 mL DMF/water (1:1), water, MeOH, DCM and DMF to give the triazole compound. After these washings, the resin still contained some copper, which was washed out from the resin during the following Fmoc deprotection with 20 % 4-methylpiperidine (v/v) solution in DMF.

Removal of Alloc protecting group (**50** and **56**): The resin was washed with 5 x 5 ml of N_2-degazed NMP and transferred with 4 ml of degazed NMP to a 15-ml polypropylene centrifuge tube. A long needle was introduced tightly above the bottom of the tube and the slurry was deoxygenated with a stream of dry nitrogen for 30 minutes. $Pd(PPh_3)_4$ (0.15 equiv) and $(CH_3)_2NH.BH_3$ (20 equiv) were added as solutions in degazed NMP (0.3 mL). The mixture was allowed to react for 1.5 h at room temperature under vigorous bubbling of the dry nitrogen. The resin was transferred back to the syringe, washed with 5 x 5 ml NMP, 3 x 5 ml 20 % 4-methylpiperidine in DMF and 5 x 5 ml DMF.

Reduction of azide (**54**): The resin was washed 3 x 3 ml H_2O/THF (2:1, v/v) and TCEP (20 equiv) and DIPEA (12 equiv) in 2 mL H_2O/THF (2:1, v/v) were added. The syringe was rotated for 2 h at room temperature. The resin was washed with 3x3 mL of mL H_2O/THF (2:1, v/v), MeOH, DCM and DMF.

Alternatively, gaseous hydrogen sulfide was aspirated in a syringe containing the resin in a pyridine/H_2O mixture (2:1, 2 mL). The syringe was left at room temperature for 2 h and the resin was washed with 3x3 mL pyridine/H_2O (2:1). This operation was repeated twice. The resin was washed with 3x3 mL pyridine/H_2O (2:1), MeOH, DCM and DMF. CAUTION: hydrogen sulfide is a very toxic gas that should be handled with care and under a fume hood.

*Cleavage from the resin (***38**, **39**, **45**, **51**, **57**): The resin was washed with 3×3 mL DCM, transferred to a fritted glass reactor and dried under reduced pressure. The following cleavage mixture was added to the reactor: 95 % TFA/H_2O (10 mL) + 2 % TIS (v/v), + 2 % DODT (v/v), + 2 % thioanisole (v/v). Two cleavages of 1 h each were performed. The combined filtrates were evaporated under reduced pressure at 40 °C. The oily residue was then triturated twice

with 25 mL Et_2O. The resulting solid was dissolved in 40 % ACN/H_2O and lyophilized. The crude product was analyzed by RP-HPLC and purified on preparative RP-HPLC, as described in the General section. The crude purity, the yield of the synthesis, the final purity and the HRMS of compounds **38**, **39**, **45**, **51** and **57** are given in Table 1. The RP-HPLC profiles of the isolated product of compounds **38**, **39**, **45**, **51** and **57** are shown in the Supporting Information.

Table 2: Purities of crude compounds **38**, **39**, **45**, **51** and **57**, yields after isolation by RP-HPLC, purities of the isolated products and HRMS of purified compounds. Purities were determined by integration of peaks at 218 nm.

Compound	**Purity of the Crude Compound**	**Purity of HPLC purified compound**	**Yield after HPLC purification**	**HR-MS** (calculated) $(M+H)^+$	**HR-MS** (experimental)
38	82 %	> 95 %	42 %	1349.6210	1349.6215
39	76 %	> 95 %	38 %	1481.6997	1481.7001
45	63 %	> 95 %	30 %	1492.7379	1492.7385
51	53 %	> 95 %	28 %	1411.7052	1411.7045
57	60 %	> 95 %	22 %	1330.6725	1330.6726

Acknowledgements

This work was supported by the European Regional Development Fund; OP RDE; Project: "Chemical Biology for drugging undruggable targets (ChemBioDrug)" (No. CZ.02.1.01/0.0/0.0/16_019/0000729), by the Grant Agency of the Czech Republic (Grant 14-17305S), by the Academy of Sciences of the Czech Republic (Research Project RVO:6138963, support to the Institute of Organic Chemistry and Biochemistry, and partially by the Medical Research Council (Grant MR/K000179/1).

Keywords: Scaffold. Peptide synthesis. Solid-phase synthesis. Click chemistry.

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