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Primary Structure Control of Oligomers Based on Natural and Synthetic Building Blocks

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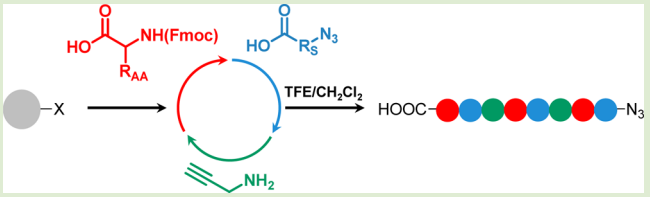
Supporting Information

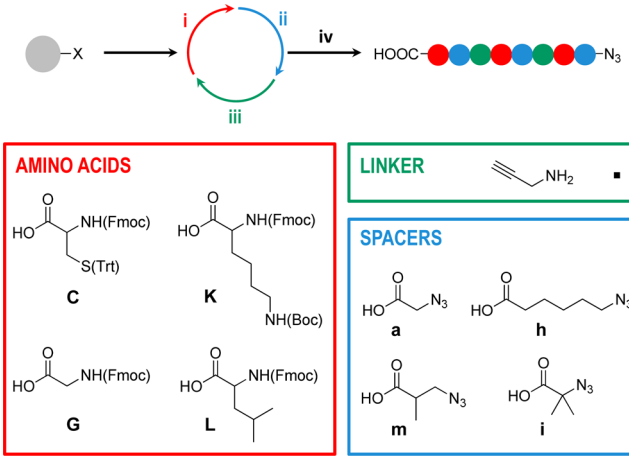
ABSTRACT: Solid-phase synthesis was exploited for the preparation of oligomers constructed from natural and synthetic building blocks by combining the formation of amide bonds and copper-assisted alkyne−azide cycloaddition reactions extending the variety of oligomers with well-defined primary structures accessible through this technique and providing control over the spacing between amino acids.



Proteins offer a large catalogue of functions essential to life such as providing structural support for cells, transporting substances across cell membranes, acting as catalysts in biochemical reactions, and contributing to cell defense mechanisms against foreign objects like bacteria and viruses. The specificity of each of these linear macromolecules produced in nature and based on 20 natural amino acids is induced by the precise control of their primary structures, i.e., the specific sequence of their constituting building blocks along the macromolecule backbone. The development of synthetic strategies to reach comparable control level of the primary structure for the preparation of sequence-controlled oligomers has been in particular pursued by iterative approaches from a solid support.[1] This technique is widely employed for the synthesis of peptides occurring through repeated cycles of addition of *N*-protected amino acids and removal of the protecting group present at the *N*-terminus of the peptide main chain and provides an easier synthetic approach as compared to solution synthesis due to the simplification of the purification steps.[2] This methodology has been successfully applied for the synthesis of oligosaccharides[3] and oligonucleotides[4] and more recently was adapted for the preparation of a wide range of oligomers such as peptoids,[5] oligocarbamates,[6] oligoureas,[7] oligoamides,[8] and triazolamers[9] through the iterative addition of building blocks from a solid support. Furthermore, few research groups have combined different types of orthogonal coupling reactions to build sequence-defined oligomers such as oligo(amide-triazole) prepared by alternated formation of amide bonds and copper-assisted alkyne−azide cycloaddition reactions.[10] Besides, the modification of peptide backbones through the use of triazole moieties as a surrogate for an amide bond has been investigated for its influence on the folding behavior of peptide architectures.[11]

In this work natural and synthetic building blocks were employed to prepare sequence-controlled oligomers. The building blocks considered are natural amino acids introduced as for peptide synthesis through the formation of an amide bond, spacer units functionalized at the alpha position with a carboxylic acid and at the omega position with an azide introduced on the growing oligomer chain using conditions similar to the ones for amino acids, and propargylamine acting as a linker inserted by copper-assisted alkyne−azide cycloaddition reaction (Scheme 1). The main objective of this work is to demonstrate the flexibility of this approach to tune the composition and primary structure of the synthesized oligomers. Furthermore, this synthetic strategy allows achieving the control over the spacing between amino acids without using spacers such as *β*-alanine[12] and 6-aminohexanoic acid,[13] requiring the use of their Fmoc protecting forms, and thus accessing oligomers in a more cost-efficient pathway.

Scheme 1. Synthetic Approach for the Preparation of Oligomers Based on Natural and Synthetic Building Blocks Performed by Solid-Phase Synthesis through Successive Insertion of (i) a Protected Amino Acid, (ii) Spacer, and (iii) Linker before (iv) Cleavage from the Resin



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The investigation started with the synthesis of a library of azidoamino acids constituted of one amino acid and one spacer. The amino acids used had their functional groups on the side chain protected, and the preparation of azidoamino acids was mostly focused here on glycine (G), leucine (L), Trt-protected cysteine (C), and Boc-protected lysine (K). The spacers with various lengths of the aliphatic chain and degree of branching, i.e., acetyl (a), hexyl (h), isobutyryl (i), and methylpropionyl (m) derivatives, were easily afforded through the conversion of commercially available ω-bromocarboxylic acids into their corrresponding ω-azidocarboxylic acids with NaN_3. As an example unimer Gh was prepared by functionalizing a chlorotrityl chloride resin with Fmoc-Gly-OH in the presence of DIPEA in CH_2Cl_2 followed by successively removing the Fmoc protecting group with a solution of 25% piperidine in DMF, coupling 6-azidohexanoic acid to the glycine-functionalized resin using HBTU and HOBt as coupling reagents in the presence of DIPEA in DMF, and cleaving the azidoamino acid from the resin using a 4:1 CH_2Cl_2/2,2,2-trifluoroethanol (TFE) cleavage solution. The structure of Gh was confirmed by ^1H and ^{13}C NMR and ESI-MS (Figure S1, Supporting Information) and exhibited on the IR spectrum an absorbance band at 2100 cm^{-1} characteristic of the asymmetric stretching vibration of azide groups.

As an extension of azidoamino acid syntheses, azidopeptides were prepared similarly by solid-phase synthesis through functionalization of the resin with the first amino acid of the targeted peptide sequence, sequential addition of the other amino acids in the predetermined sequence, and finally capping of the peptide with 6-azidohexanoic acid using the same coupling agents as previously, i.e., HBTU and HOBt in the presence of DIPEA, before recovering the azidopeptide after cleavage from the resin. In this study Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) and Val-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro peptide sequences were used to demonstrate the approach feasibility, which was confirmed by NMR (Figure S2, Supporting Information), ESI-MS, and MALDI-ToF mass spectrometry (Figure 1).

To elaborate more complex oligomers the methodology adopted consisted of performing cycles of successive additions of amino acid, spacer, and linker on a solid support where the last step before cleavage of the oligomer from the resin was always a spacer insertion leading to oligomers bearing at one extremity a carboxylic acid and at the other extremity an azide (Scheme 1). Amino acids and spacers were inserted on the oligomer chain growing from the surface of the resin as previously described, while propargylamine (■) acting as a linker was introduced by copper-assisted alkyne−azide cycloaddition in the presence of copper(I) bromide (CuBr) and *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (PMDETA) in CH_2Cl_2. The model oligomer considered to determine the viability of this synthetic approach was prepared from Fmoc-Lys(Boc)-OH, 6-azidohexanoic acid, and propargylamine up to three cycles. The completion of each cycloaddition reaction and the insertion of a spacer unit were validated by FT-IR through the respective monitoring of the disappearance and appearance of the characteristic absorbance band of azide groups at 2100 cm^{-1} as illustrated in Figure 2. After cleavage from the resin the structural integrity of oligomer Kh■Kh■Kh was supported by ESI-MS ($[M + Na]^+$ calculated 1252.75, found 1252.74) and NMR exhibiting the methylene next to the azide at 3.27 ppm, the alpha proton of the lysine residue at 4.29 ppm, the methylene next to the amide of the hexyl spacer at 2.11 ppm on the ^1H NMR spectrum, and the tertiary carbon of the triazole at 123 ppm on the ^{13}C NMR spectrum (Figure 3). The integration comparison of the oligomer extremity with the building blocks constituting the oligomer assessed the purity of the compound. Besides, the purity of the synthesized oligomer was verified by MALDI-ToF mass spectrometry using 2,4,6-ditrihydroxyacetophenone as the matrix and CsCl as the cationizing agent.[14] Figure 3 demonstrated that each synthetic step is quantitative since the spectrum exhibited only adducts expected for the oligomer Kh■Kh■Kh and the absence of peaks corresponding to possible synthetic intermediates. The functional groups present on the side chain of each amino acid residue were easily retrieved upon removal of their protecting groups using a solution of trifluoroacetic acid (Figure S3, Supporting Information).

A library of oligomers depicted in Figure 4 has been prepared using this synthetic strategy from the list of spacers indicated in Scheme 1 and various protected amino acids, i.e., Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Cys(Trt)-OH, and Fmoc-Val-OH. Starting from the model oligomer Kh■Kh■Kh some hexyl spacers were replaced at various locations on the

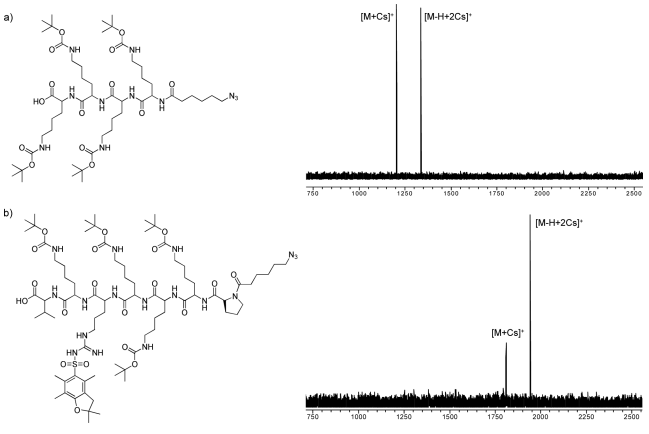

Figure 1. MALDI-ToF mass spectrometry of azidopeptides (a) Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-hexyl-N_3 and (b) Val-Lys-(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro-hexyl-N_3.

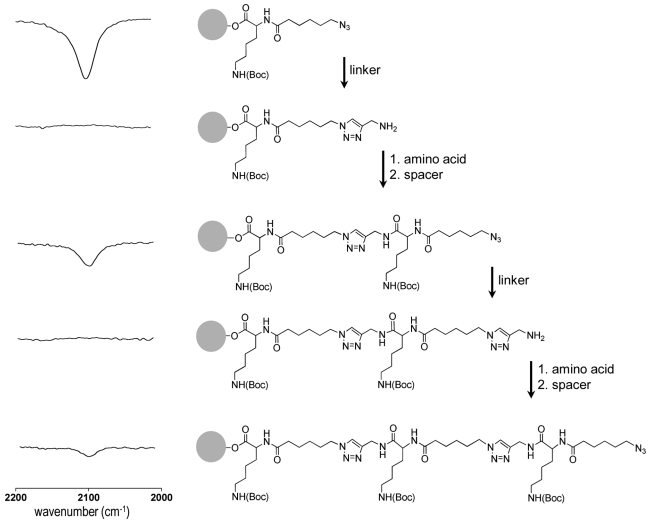

Figure 2. FT-IR monitoring of the iterative synthesis of Kh■Kh■Kh.

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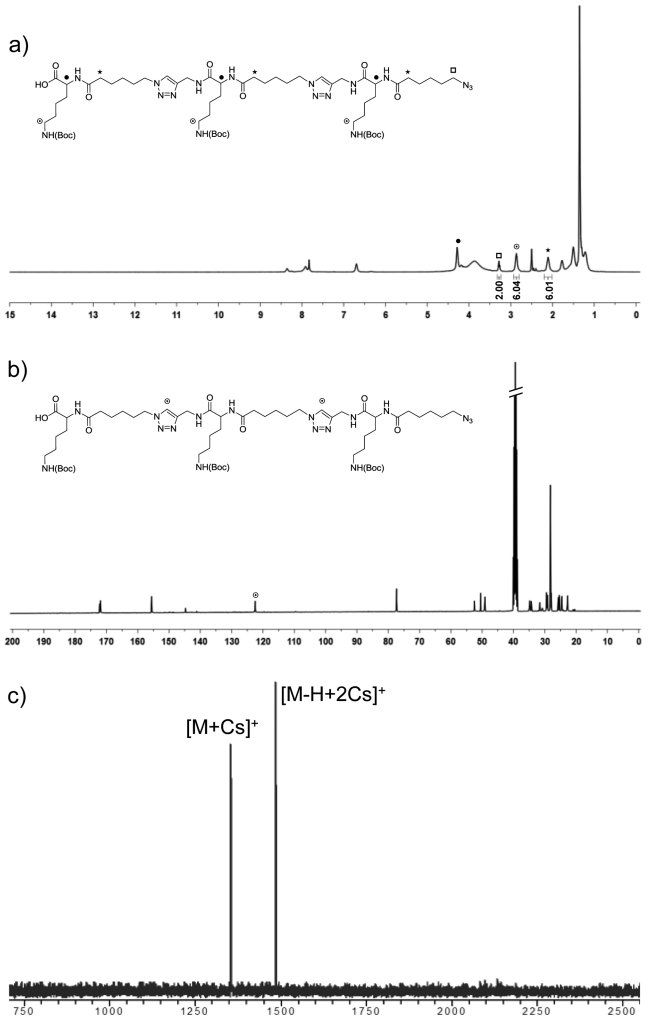

Figure 3. (a) ^{1}H and (b) ^{13}C NMR spectra in DMSO-d_6 and (c) MALDI-ToF of Kh■Kh■Kh.

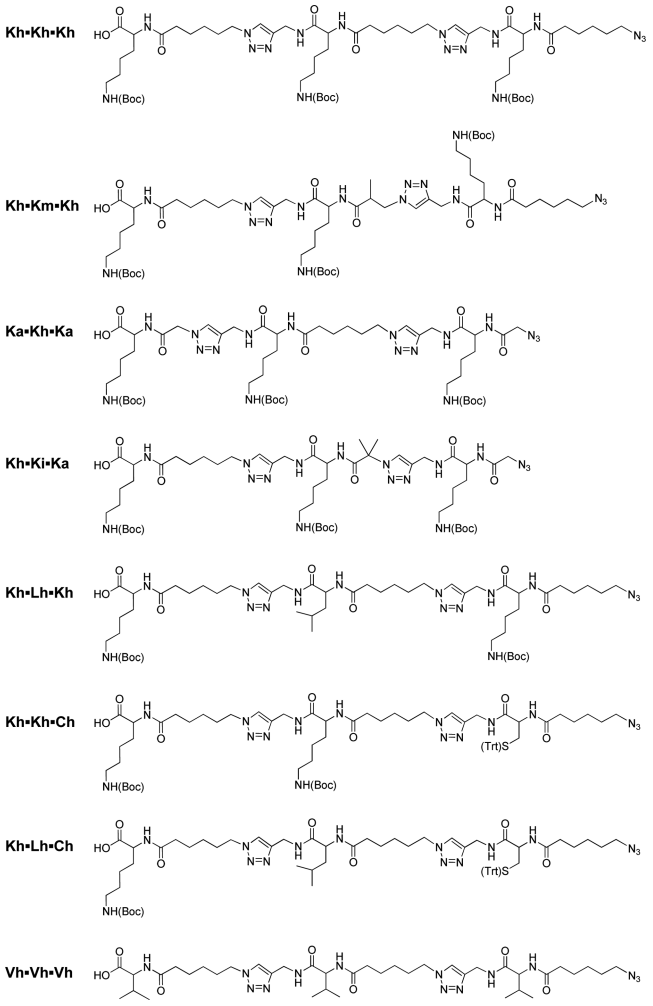

Figure 4. Library of sequence-defined oligomers based on natural and synthetic building blocks.

oligomer backbones by different spacers to demonstrate the flexibility of this technique regarding the controlled positioning of the desired spacers but also the possible control over the distance between amino acids on the oligomer chains through the use of spacers of precise alkyl chain length. For example a different spacer than hexyl was introduced during the second cycle of the oligomer synthesis with the insertion of a methylpropionyl spacer (Kh■Km■Kh), or the oligomer was prepared using a different spacer at each cycle (Kh■Ki■Ka). In a similar manner, sequence-defined oligomers were prepared by replacing one or multiple Lys(Boc) residues by other amino acids. The structural integrity of all the oligomers synthesized was confirmed by NMR (Figures S4 and S5, Supporting Information) and ESI-MS.

The experimental procedure to prepare these oligomers was optimized by reducing the reaction time of each synthetic step and the amount of copper catalyst used for the copper-assisted alkyne−azide cycloaddition step while maintaining a good efficiency regarding the synthesis of these oligomers, i.e., high yield and purity. The synthesis of longer sequence-defined oligomers is currently investigated using these optimized conditions and will be described in a subsequent report.

In summary, combining the formation of amide bonds and copper-assisted alkyne−azide cycloaddition reactions from a solid support permitted the synthesis of a library of sequence-controlled oligomers based on natural amino acids and synthetic building blocks. This strategy will allow extending the choice of building blocks beyond the ones available for peptide synthesis while maintaining the control over the oligomer primary structure as existing in peptides. The results reported in this letter emphasized not only the flexibility of the described synthetic approach to precisely position synthetic building blocks on peptidomimetic oligomers but also the ability to control the distance between amino acid residues. It is noteworthy to mention that this iterative technique was optimized to provide a synthetic approach that would not be more constraining as performing solid-phase peptide synthesis. Besides, the presence of triazole moieties and primary amines, i.e., after removal of the Boc protecting groups for the oligomers bearing lysine residues, on the oligomer chains makes them good candidates as potential antibacterial agents.

■ ASSOCIATED CONTENT

Supporting Information

Experimental section describing the procedure and characterization details for the synthesis of azidocarboxylic acids, azidoamino acids, azidopeptides, and sequence-defined oligomers based on natural and synthetic building blocks and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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