# Strategy for the synthesis of multivalent peptide-based nonsymmetric dendrimers by native chemical ligation†

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**A strategy for the synthesis of multivalent peptide-based nonsymmetric dendrimers by native chemical ligation using poly(lysine) dendritic wedges as scaffolds is presented.**

Native chemical ligation (NCL) was first described by Dawson *et al.* for the synthesis of proteins of moderate size, comprising the highly selective and efficient reaction between a C-terminal thioester and an N-terminal cysteine residue.[1] NCL allows the reaction of unprotected peptides under aqueous conditions. Peptides and proteins represent an important class of targeting units, both in the field of molecular imaging and in the field of drug delivery. Peptides have been attached to multivalent scaffolds to obtain a higher affinity for their biological marker through multivalent interactions.[2] By combining multiple targeting units with multiple labels (*e.g.* drug molecules or imaging modalities), more effective agents can be developed.

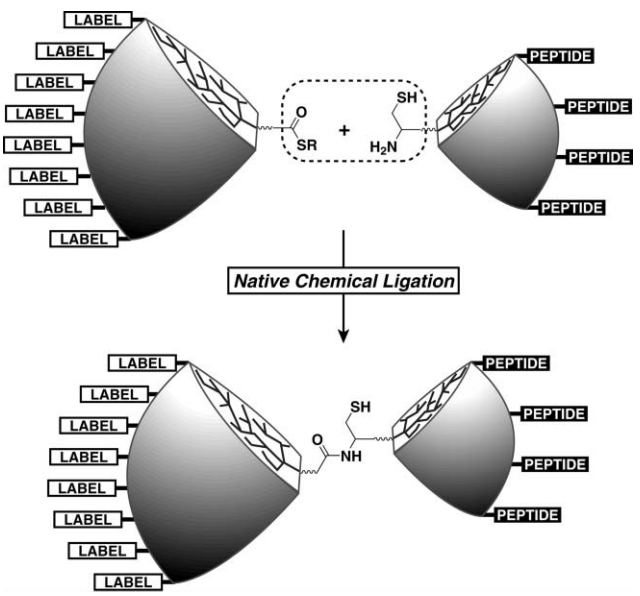
By allowing tuning of the ratio between the number of labels and the number of targeting units in a controlled fashion, the performance of these agents can be optimized. Dendrimers with their well-defined structure and precise number of end groups provide excellent scaffolds to establish this goal. Only very recently, Hawker *et al.* demonstrated this concept by merging a carbohydrate-functionalized dendritic wedge and a bivalent fluorescent label using click chemistry.[3]

In this paper we present a strategy for the synthesis of multivalent peptide-based nonsymmetric dendrimers in aqueous environment employing NCL with poly(lysine) dendritic wedges[4] as scaffolds (Scheme 1). On one end, an oligopeptide containing the arginine-glycine-asparagine-serine (RGDS) sequence, which binds to $\alpha_v\beta_3$ integrins,[5] is used as a model peptide. On the other end, diethylenetriaminepentaacetic acid (DTPA) is used as a ligand for a label. DTPA is a very efficient chelating agent for lanthanide ions, in particular for gadolinium(III) (Gd(III)), which has found broad application in the field of magnetic resonance imaging (MRI).[6]

The key step in this strategy is the ligation of two poly(lysine) dendritic wedges of which one is functionalized with labels (*here*: DTPA) along its periphery and a thioester at its focal point, and the other one with peptides (*here*: RGDS) along its periphery and a cysteine residue at its focal point. For both the dendritic wedge ligation and peptide anchoring NCL will be employed.

The poly(lysine) dendritic wedges were obtained *via* manual solid phase peptide synthesis (SPPS) using the *in situ* neutralization/HBTU activation procedure for *t*Boc chemistry on a *p*-methylbenzhydrylamine (MBHA) resin.[7] In the first steps of SPPS the focal point of the wedge is defined, whereas in the final coupling steps the periphery is determined and an additional functionality can be introduced at the focal point.

To obtain DTPA-functionalized wedges, 1st (**1**) and 2nd (**2**) generation poly(lysine) dendritic wedges with thioester-functionalities at their focal point and sulfhydryl groups along their periphery were synthesized. A glycine spacer consisting of 4 glycine residues was introduced between the thioester at the focal point and the poly(lysine) dendritic wedge to create space for the ligation of the two wedges. The wedges **1** and **2** were used without further purification and were functionalized with DTPA by reacting them in a 0.1 M Tris (aq) buffer at pH 6.5 with the maleimide-functionalized DTPA synthon **3** (Scheme 2). The reaction went to completion within 1 hour. The DTPA-functionalized wedges were purified using preparative reversed phase (RP) HPLC on a C18 column. Subsequent lyophilization rendered **4** and **5** in 26% and 80% yield, respectively.




**Scheme 1** General strategy for the synthesis of multivalent peptide-based nonsymmetric dendrimers employing native chemical ligation.

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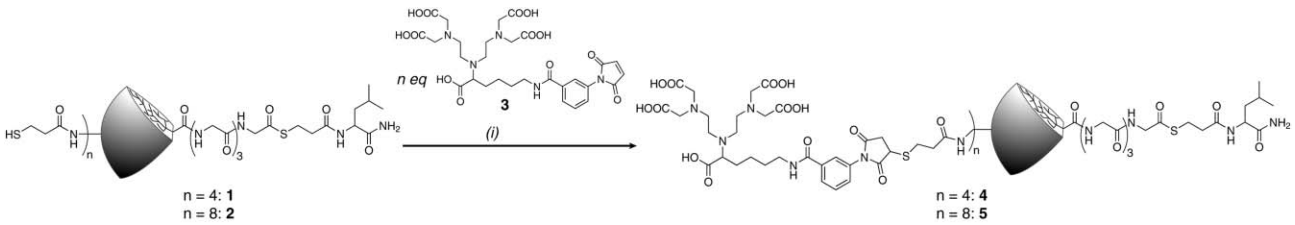
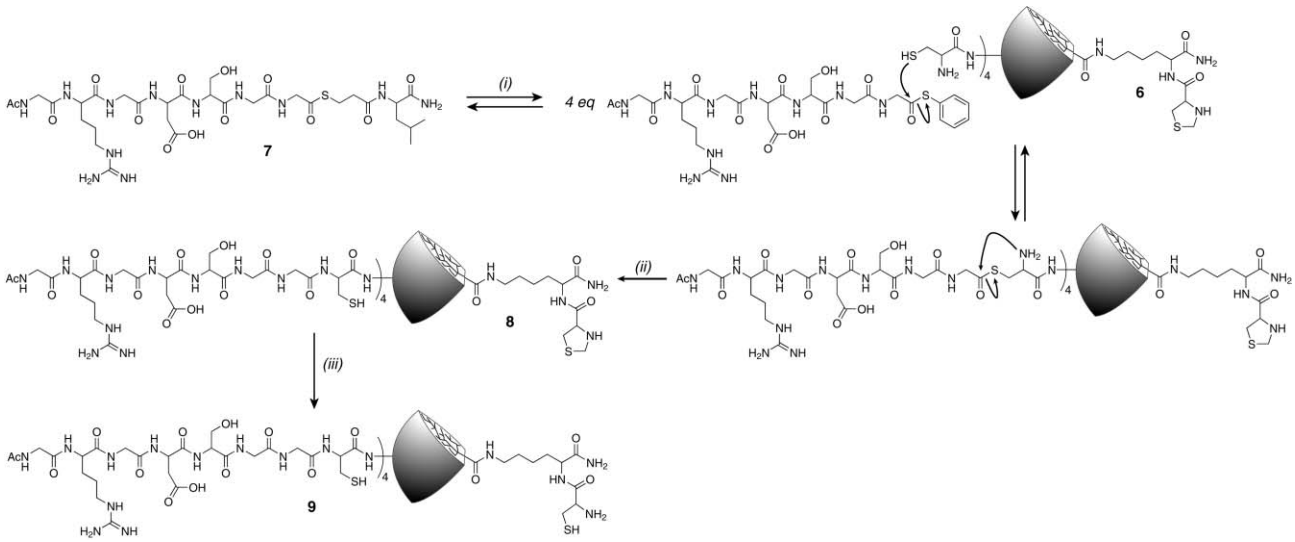
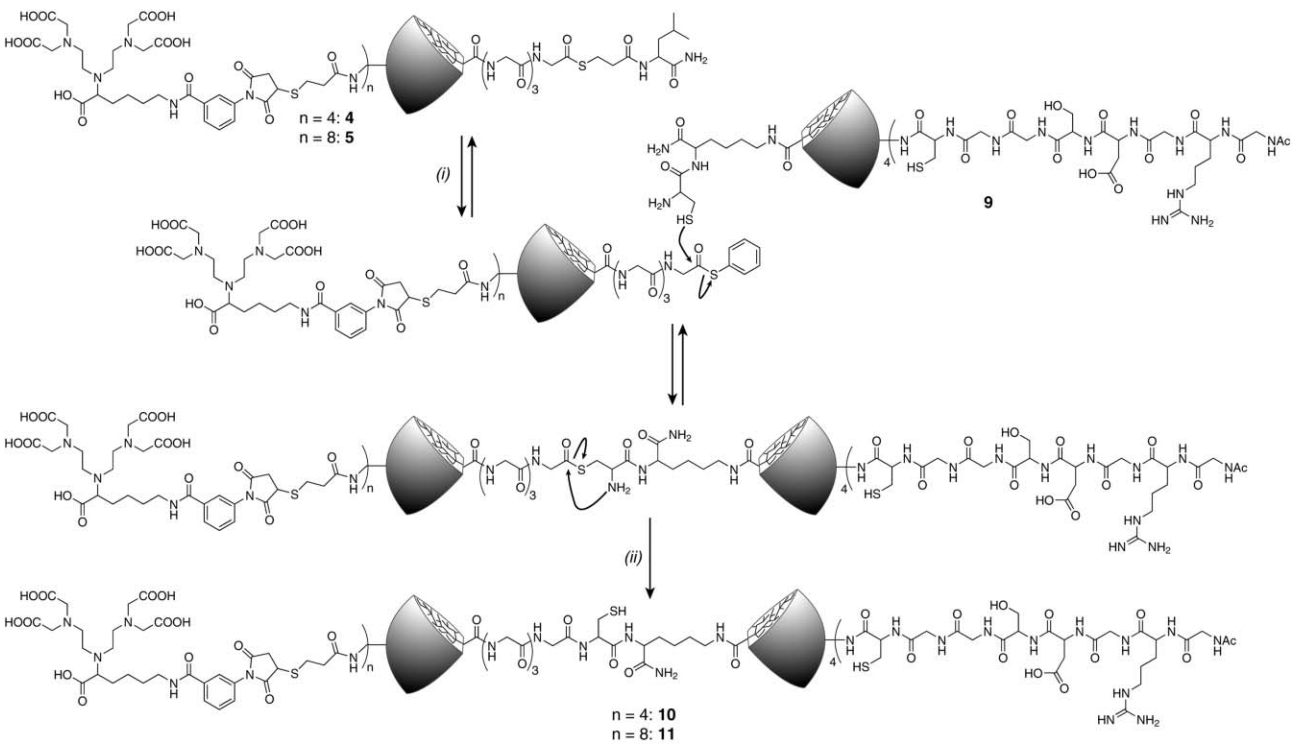
† Electronic supplementary information (ESI) available: Synthesis and characterization of all compounds reported in this paper and a description of the instrumentation used. See DOI: 10.1039/b600286b




**Scheme 2** Synthesis of the DTPA-functionalized poly(lysine) dendritic wedges **4** and **5**. (i) 0.1 M Tris (aq), 1 h, pH 6.5, RT.




**Scheme 3** Synthesis of the RGDS-functionalized poly(lysine) dendritic wedge **9**. (i) 1 vol% Thiophenol, 6 M guanidine in 0.1 M Tris (aq), 1 h, pH ≈ 7, 37°C; (ii) spontaneous rearrangement; (iii) 6 M guanidine in 0.1 M Tris (aq), 0.2 M $CH_3ONH_2$, 2 h, pH ≈ 4, RT.




**Scheme 4** Ligation of two dendritic wedges. (i) 1 vol% Thiophenol, 6 M guanidine in 0.1 M Tris (aq), 1 h, pH ≈ 7, 37 °C; (ii) spontaneous rearrangement.

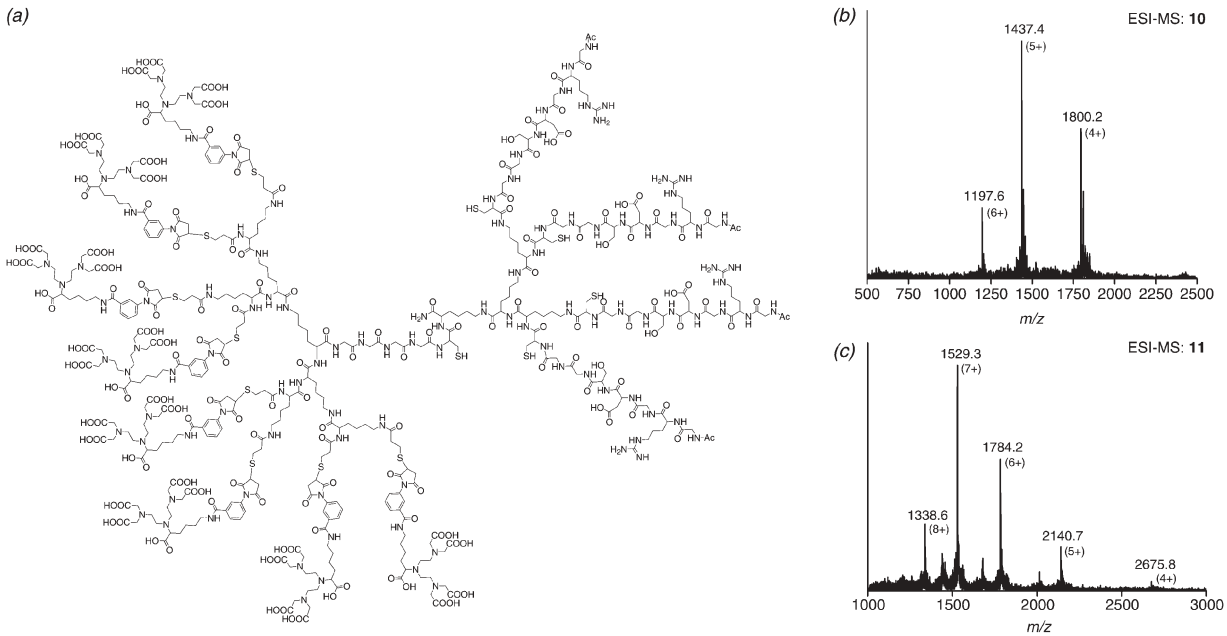



**Fig. 1** (a) Chemical structure of **11** and the ESI-MS spectra of (b) **10** ($[M + H]^+$: calcd. for $C_{285}H_{432}N_{84}O_{116}S_9$ 7179.6, found: 7185.6 $\pm$ 0.7) and (c) **11** ($[M + H]^+$: calcd. for $C_{438}H_{648}N_{112}O_{176}S_{13}$ 10716.3, found: 10699.1 $\pm$ 1.0).

To arrive at the peptide-functionalized wedge, a 1st generation poly(lysine) dendritic wedge with a thioproline residue at its focal point and 4 cysteine residues along its periphery (**6**) was synthesized on the solid phase. After cleavage from the resin with anhydrous HF, **6** was purified by RP HPLC. The periphery of the dendritic wedge was subsequently functionalized with the RGDS peptide using NCL. For this, **6** was reacted with 4 equivalents of the C-terminal thioester of the AcGRGDSGG-MPAL peptide **7** under ligation conditions (Scheme 3). The reaction went to completion within 1 hour. The RGDS-functionalized wedge was purified using preparative RP HPLC on a C18 column. Subsequent lyophilization rendered **8** in 33% yield.

To allow ligation of the RGDS-functionalized wedge **8** to the DTPA-functionalized wedges **4** and **5**, the thioproline residue at the focal point was converted to a cysteine residue by reacting it with a 0.2 M solution of methoxylamine (aq)[8] to give **9** (Scheme 3). After 2 hours full conversion was reached and the reaction mixture was used for the ligation to the DTPA-functionalized wedges without purification.

To demonstrate the concept of using NCL as a synthetic tool to assemble two functional dendritic wedges under aqueous conditions, the ligation of the RGDS-functionalized dendritic wedge **9** with both the 1st generation (**4**) and the 2nd generation (**5**) DTPA-functionalized dendritic wedge was performed on an analytical scale (Scheme 4). The reaction was monitored employing analytical RP HPLC using a C18 column for separation coupled to UV-Vis ($\lambda_{probe}$ = 214 nm). Analysis with electrospray ionization mass spectrometry (ESI-MS) confirmed the formation of a dendrimer consisting of 4 RGDS peptide units and 4 (**10**) or 8 (**11**) DTPA ligands (Fig. 1). It is important to emphasize that the glycine spacer introduced in the case of the DTPA-functionalized dendritic wedges **4** and **5** is essential to allow ligation with the RGDS-functionalized dendritic wedge **9**. No ligation product was observed in the absence of the glycine spacer in **11**.

The system presented in this communication demonstrates the extended applicability of native chemical ligation as a synthetic tool to arrive at well-defined multivalent peptide-based nonsymmetric dendrimers. Our current studies focus on the development of multivalent target-specific MRI contrast agents based on peptides and proteins relevant for *in vivo* imaging of cardiovascular disease using this specific strategy.

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