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A new dendrimer scaffold for preparing dimers or tetramers of biologically active molecules

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Abstract—Synthesis of a new scaffold derived from iminodipropionic acid for the preparation of peptide dimers and tetramers is described.

1. Introduction

Enhanced potency can be achieved by multivalent display of ligands as antagonists or agonists of biological processes.[1] Constructs bearing multiple copies of a ligand often display dramatically increased affinity and specificity relative to the monomer due to avidity and cooperativity effects.[2,3] Recently, increased emphasis has been placed on utilizing constructs capable of multiple interactions with either different epitopes of a single molecule of a biomolecular target or with the same epitope on more than one molecule of the target. The strategy has been applied to problems in cancer imaging,[4] cancer therapy,[5] cancer radiotherapy,[6] and as immunogens,[7,8] biosensors,[9] and artificial proteins.[10] Biosynthetic methods have been recently disclosed to prepare the scaffolds that can carry several copies of the peptides in immunotherapy and gene therapy for cancer.[11]

The delivery of ligands to the binding site as polyamino-amide (PAMAM) dendrimers is well documented.[12] We describe here an efficient synthesis of a scaffold that utilizes a polyamide approach for dimeric or tetrameric presentation of ligands, with the added ability to append a detectable tag, such as Oregon green (for binding studies). We applied this scaffold to the antagonist, TKPPR,[13] of the Tuftsin receptor.[14]

2. Synthesis of the scaffold (Scheme 1)

We chose iminodipropionic acid,[15] as the central core for the dendrimer. Bisaddition of benzylamine to *t*-butylacrylate under standard conditions was unsuccessful, but a neat Michael addition reaction of benzylamine to *t*-butyl acrylate in the presence of 1 mol% $Yb(OTf)_3$,[16] enabled this transformation in 90% yield, to provide **1a**. Debenzylation of **1a**, to obtain **1b**, followed by protection with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide yielded the protected amine **1c**. Alternatively, the amine **1b** was coupled to Fmoc-Gly-OH to furnish the amide **2a** which in turn was selectively deprotected to give the acid **3**. The *t*-butyl groups were removed from **1c** to provide **1d**. Crude acids **1d** and **3** were purified by recrystallization from acetonitrile and both were obtained as colorless solids. Bisacid **3** was coupled with 2.2 equiv of **2b** to yield the corresponding tetraester **4a** which was smoothly converted to tetraacid **4b**. The intermediates **1d** and **4b** were set for coupling to the protected TKPPR peptide to provide a dimer or tetramer construct, respectively.

3. Preparation of protected Tuftsin receptor antagonist H-Adoa-Thr(*t*-Bu)-Lys(Boc)Pro-Pro-Arg(Pmc)-O*t*-Bu (7)

The fully protected core sequence Fmoc-Thr(*t*-Bu)-Lys-(Boc)-Pro-Pro-Arg(Pmc)-O*t*-Bu (**5**), was prepared as follows: H-Arg(Pmc)-O*t*-Bu was coupled to Z-Pro-OH using HATU/$(i\text{-Pr})_2$NEt. Removal of the Z-group by hydrogenation followed by another coupling with Z-Pro-OH provided Z-Pro-Pro-Arg(Pmc)-O*t*-Bu. Z-Lys(Boc)-OH and Fmoc-Thr(*t*-Bu)-OH were then

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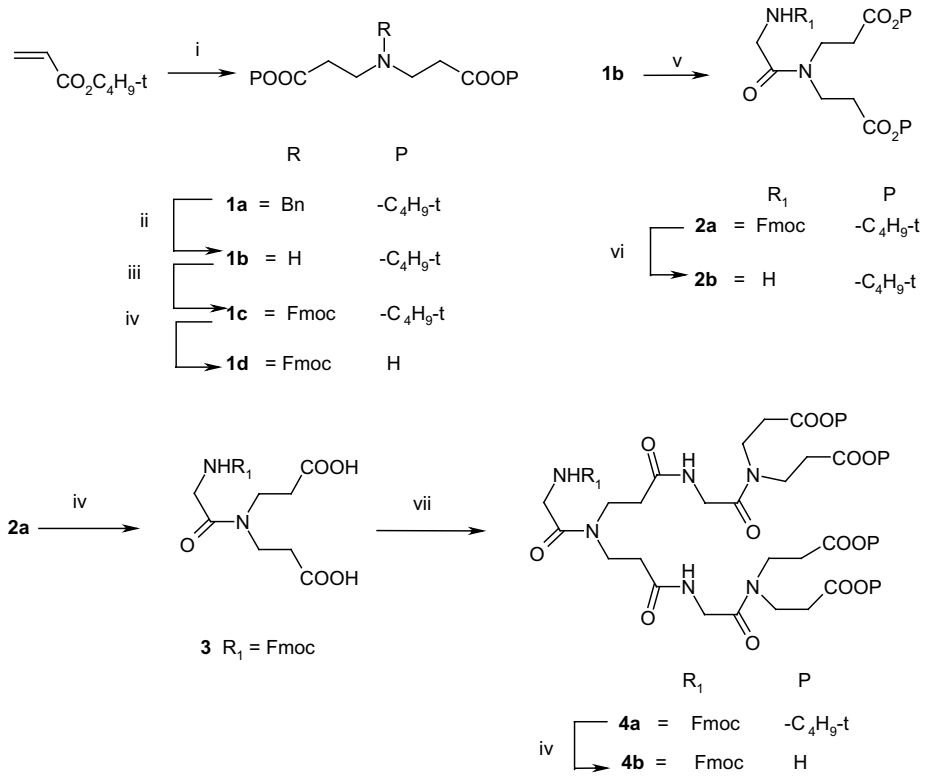
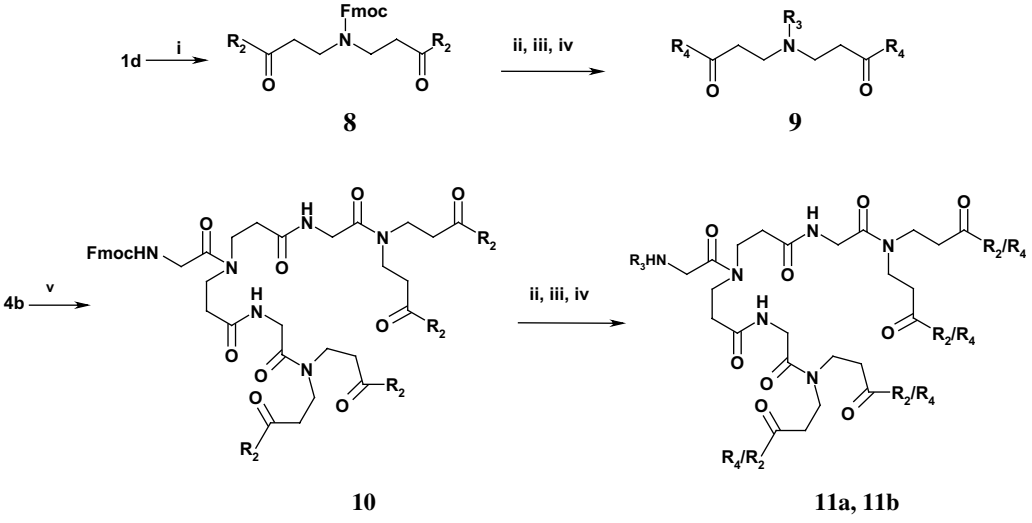


Scheme 1. Reagents and conditions: i) $BnNH_2$, 0.45 eq; $Yb(OTf)_3$, 1.0 mol%, 100 °C, 2 h, 90%; ii) H_2, $Pd(OH)_2$, MeOH–water (95:5), 100%; iii) Fmoc-OSu; THF/water, 65%; iv) TFA/CH_2Cl_2 (1:1); 2 h, 75%; v) Fmoc-Gly-OH, HATU, $(iPr)_2NEt$, CH_2Cl_2; vi) 10% piperidine in acetonitrile, 30 min., 85%; vii) **2b**, HATU, $(iPr)_2NEt$, CH_2Cl_2, 65%.

sequentially coupled to the growing peptide chain. At this stage the fully protected peptide **5** was purified by silica gel column chromatography [50% overall yield; DCM–MeOH (9:1)]. Treatment of **5** with piperidine in acetonitrile afforded H-Thr(*t*-Bu)-Lys(Boc)-Pro-Pro-Arg(Pmc)-O*t*-Bu, which was acylated with 8-Fmoc-



8, 10, 11a R_2= Adoa-Thr(*t*Bu)-Lys(Boc)-Pro-Pro-Arg(Pmc)-O*t*Bu **9, 11b** R_4= Adoa -Thr-Lys-Pro-Pro-Arg-OH	R_3 =

Scheme 2. Reagents and conditions: i) **7** (2.2 eq.), HATU, $(iPr)_2NEt$, CH_2Cl_2; ii) 10% piperidine in acetonitrile, 30 min; iii) Oregon Green-OSu, DMF; iv) TFA/anisole/water (95:5:1); v) **7** (4.4 eq.), HATU, $(iPr)_2NEt$, CH_2Cl_2.

amino-3,6-dioxaoctanoic acid (Fmoc-Adoa) to give Fmoc-Adoa-Thr(*t*-Bu)-Lys(Boc)Pro-Pro-Arg(Pmc)-O*t*-Bu (**6**). Removal of the Fmoc—protecting group from **6** resulted in H-Adoa-Thr(*t*-Bu)-Lys(Boc)Pro-Pro-Arg-(Pmc)-O*t*-Bu (**7**). Both **6** and **7** were purified by silica gel column chromatography (9:1—DCM/MeOH for **6** and 8:2—DCM/MeOH for **7**). This procedure provided rapid access to the suitably protected peptide **7** for coupling to a dimeric/tetrameric cores.

4. Synthesis of dimeric and tetrameric peptide ligands

The linker acid **1d** was coupled to amine **7** to give the protected dimer **8**, whose Fmoc group was removed (piperidine/acetonitrile). The fluorescent tracer Oregon Green™ was appended to the secondary amine function using Oregon Green-OSu ester. The protecting groups were removed under standard conditions and the crude product was purified by preparative HPLC to furnish the required dimer **9** in 48% overall yield for the final three steps. The structure of fluorescent dimer **9** was confirmed by MS, NMR, and elemental analysis (Scheme 2).

The tetra acid **4b** when subjected to coupling with amine **7**, following similar protocols as above, resulted in **10**, which was purified by silica gel column chromatography. Generation of the amine from **10** followed by incorporation of the fluorescent tag with Oregon Green-OSu ester yielded compound **11a** (R_2 = Adoa-Thr(*t*-Bu)-Lys-(Boc)-Pro-Pro-Arg(Pmc)-O*t*-Bu, R_3 = Oregon Green) the penultimate protected peptide tetramer bearing the reporter. Deprotection of **11a** under standard conditions resulted in the desired fluorphore-labeled peptide tetramer **11b**. Purification of crude **11b** by preparative HPLC yielded the pure compound, containing four units of the biologically active peptide, in 24% overall yield. The structure was confirmed by NMR, MS, and analytical data.

Thus, we have increased the versatility of the iminodipropionic acid-based scaffold as a carrier for multiple ligands. We are currently exploring other routes to increase the number of displayed peptide units in a controlled manner and applications to the Tuftsin receptor antagonist and other bioactive molecules.

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