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Oxopiperazine capping: Formation of oxopiperazine-containing peptoids *via* *C*-terminal cyclization

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ABSTRACT

An unexpected side reaction was observed in peptoids containing a *C*-terminal carboxamide with a 2-aminoethyl side chain. The reaction proceeded *via* cyclization and release of NH_3, resulting in *C*-terminal oxopiperazine formation, analogous to pyroglutamate formation from *N*-terminal glutamine in peptides. Reaction conditions that promote oxopiperazine formation were developed. In particular, the addition of organic bases accelerated the cyclization, thus providing a simple strategy for the post-synthetic *C*-terminal capping of peptoids.

Introduction

Numerous side reactions in peptides have been reported [1], among which cyclization reactions, such as the formation of aspartimide [2,3] or pyroglutamate [4] (Fig. 1), are frequently encountered during peptide synthesis. Aspartimide formation occurs during solid-phase peptide synthesis (SPPS) *via* Fmoc-chemistry [5,6], repeated exposure of the protected aspartic acid to piperidine causes closure of the five-membered ring (Fig. 1a), and the extent of this ring closure varies depending on the amino acid sequences neighbouring the aspartic acid residue or the bulkiness of the aspartate protecting group. Under physiological conditions, aspartimide is an intermediate during *in vivo* peptide degradation (e.g. the deamination pathway) and is frequently found in aspartate- or asparagine-containing peptide drugs such as exenatide [3]. These side reactions are undesirable but can sometimes prove to be beneficial. In the case of pyroglutamate, *N*-terminal glutamine or glutamate undergoes cyclization spontaneously or enzymatically, resulting in more stable *N*-terminal capped proteins or peptides (Fig. 1b) [4]. Clinical peptide drugs such as leuprorelin and triptorelin contain *N*-terminal pyroglutamate [7]. In addition, many antibodies or therapeutic proteins contain the pyroglutamate residue, which is important for molecular recognition and for stabilization against enzymatic degradation [8,9]. Recently, the formation of a pyroglutamate-like five-membered ring was utilized for the site-specific hydrolysis of peptide bonds, thus providing a new selective cleavage method [10].

Unexpected side reactions are also observed in peptoids (oligo-*N*-substituted glycines) [11]. Under acidic conditions, *N*-terminal acetylated peptoids undergo truncation at the terminal peptoid unit through the formation of an oxazolinium intermediate (Fig. 1c) [12]. An unwanted side chain cleavage was observed when (*S*)-*N*-(1-phenylethyl)glycine (*N*spe, a commonly used aromatic peptoid monomer) with an electron-donating substituent at the *para*-position was exposed to acidic conditions (e.g. resin cleavage with trifluoroacetic acid) (Fig. 1d) [13].

Peptoid side chains are attached to the backbone nitrogen atom; therefore, the peptoid backbone is composed of unnatural tertiary amide bonds that are less likely to be recognized by biological degradation enzymes (e.g. proteases) [14,15]. Because of their physiological stability, peptoids are widely used to develop novel bioactive agents such as antimicrobials [16], lung surfactant protein mimics [17], and cell-penetrating transporters [18]. Modification from the natural secondary amide to the non-natural tertiary amide backbone (from α-peptide to α-peptoid) does not change the chemical preference for cyclization; pyroglutamate- or aspartimide-type side reactions can occur in peptoids under conditions that favour the cyclization. During the synthesis of amine side chain-containing peptoids, we found that the peptoid sequence with a 2-aminoethyl side chain positioned at the *C*-terminus degrades over time and systematically investigated the degradation mechanism. Herein, we demonstrate cyclization of the *C*-terminal 2-aminoethyl side chain and the formation of a

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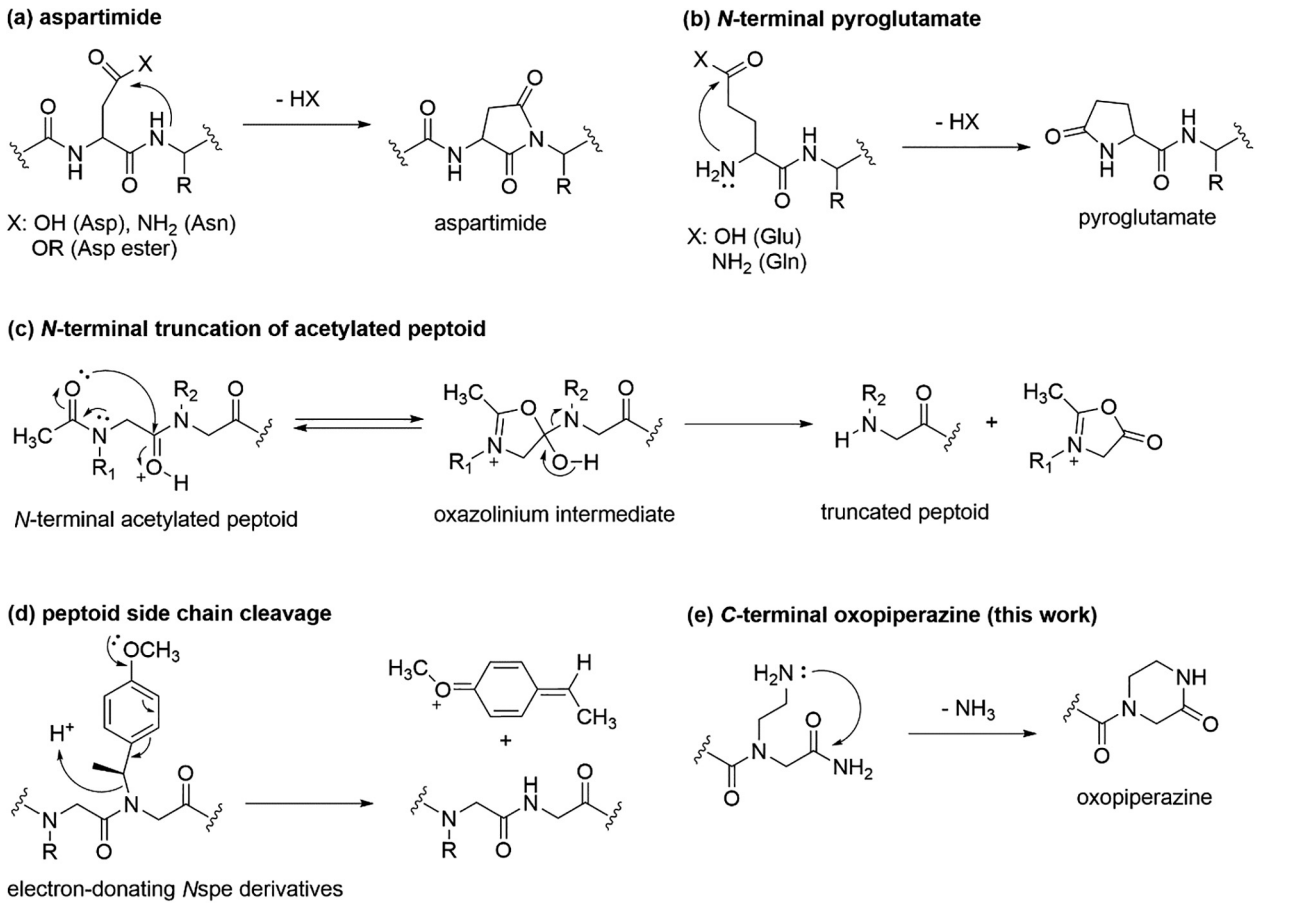

Fig. 1. Formation of (a) aspartimide, (b) *N*-terminal pyroglutamate, (c) *N*-terminal truncated peptoid, (d) side-chain cleaved peptoid, and (e) *C*-terminal oxopiperazine.

C-terminal oxopiperazine (Fig. 1e). Conditions that suppress or promote the *C*-terminal cyclization are also reported; thus, the unwanted side reaction can be prevented, or this chemistry can be utilized for beneficial purposes such as the *C*-terminal capping of peptoids [19–22].

Results and discussion

Model peptoids **1**–**5** with (*S*)-*N*-(1-phenylethyl)glycine (*N*spe), *N*-(2-aminoethyl)glycine (*N*ae), and *N*-(3-aminopropyl)glycine (*N*ap) residues were synthesized according to the solid-phase peptoid submonomer synthesis protocol (Fig. 2) [14]. The peptoids were cleaved from the resin using trifluoroacetic acid (TFA) and purified by preparative high-performance liquid chromatography (HPLC). For peptoid **1**, the purified fraction obtained by preparative HPLC was left at 25 °C for 1 h and for 9 d (Fig. 3); during the 9 d, degradation of **1** (t_R = 16.7 min) occurred, and a new peak appeared at 19.1 min. The new peak was analyzed by high-resolution mass spectrometry (HRMS), and an $[M + H]^+$ peak with m/z = 745.4060 (ESI, Fig. S1) was observed, which corresponded to *C*-terminal

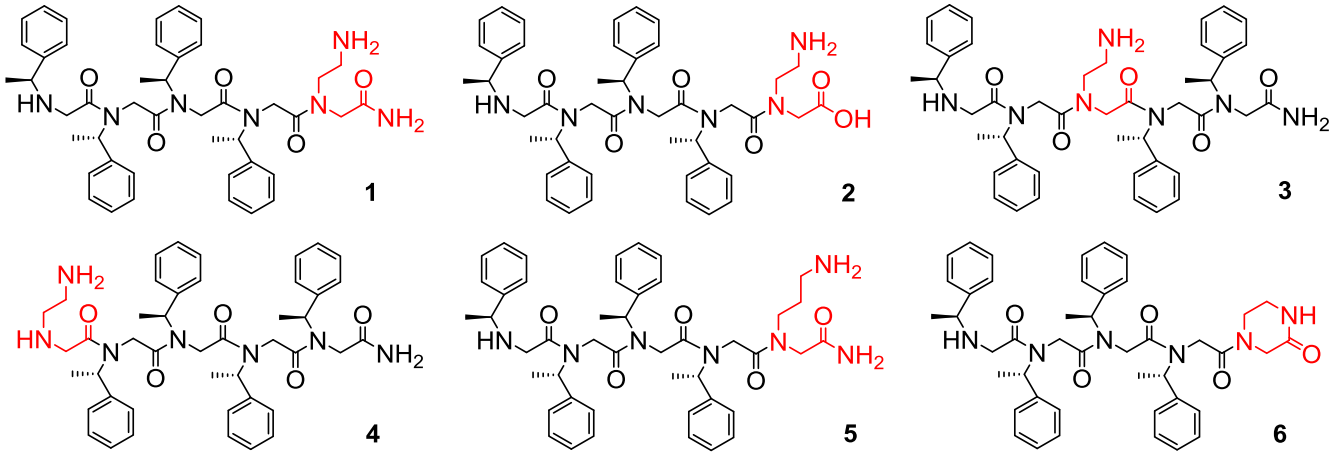

Fig. 2. Structures of peptoids **1**–**6**.