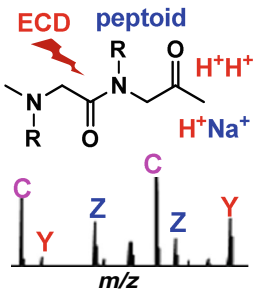
J. Am. Soc. Mass Spectrom. (2014) 25:1202–1216
DOI: 10.1007/s13361-014-0869-0

RESEARCH ARTICLE

Electron Capture Dissociation Studies of the Fragmentation Patterns of Doubly Protonated and Mixed Protonated-Sodiated Peptoids

Bogdan Bogdanov,[1] Xiaoning Zhao,[1] David B. Robinson,[2] Jianhua Ren[1]

[1]Department of Chemistry, University of the Pacific, Stockton, CA 95211, USA
[2]Sandia National Laboratories, Livermore, CA 94550, USA



Abstract. The fragmentation patterns of a group of doubly protonated ($[P + 2H]^{2+}$) and mixed protonated-sodiated ($[P + H + Na]^{2+}$) peptide-mimicking oligomers, known as peptoids, have been studied using electron capturing dissociation (ECD) tandem mass spectrometry techniques. For all the peptoids studied, the primary backbone fragmentation occurred at the N-C_α bonds. The N-terminal fragment ions, the C-ions (protonated) and the C′-ions (sodiated) were observed universally for all the peptoids regardless of the types of charge carrier. The C-terminal ions varied depending on the type of charge carrier. The doubly protonated peptoids with at least one basic residue located at a position other than the N-terminus fragmented by producing the $Z^{\bullet}$-series of ions. In addition, most doubly protonated peptoids also produced the Y-series of ions with notable abundances. The mixed protonated-sodiated peptoids fragmented by yielding the $Z^{\bullet\prime}$-series of ions in addition to the C′-series. Chelation between the sodium cation and the amide groups of the peptoid chain might be an important factor that could stabilize both the N-terminal and the C-terminal fragment ions. Regardless of the types of the charge carrier, one notable fragmentation for all the peptoids was the elimination of a benzylic radical from the odd-electron positive ions of the protonated peptoids ($[P + 2H]^{\bullet+}$) and the sodiated peptoids ($[P + H + Na]^{\bullet+}$). The study showed potential utility of using the ECD technique for sequencing of peptoid libraries generated by combinatorial chemistry.

Keywords: ECD, Radical assisted fragmentation, Odd-electron negative ion, Peptide-mimicking oligomer, Poly(N-substituted glycine)

Received: 21 July 2013/Revised: 9 February 2014/Accepted: 14 February 2014/Published online: 21 May 2014

Introduction

Peptoids are a class of bio-inspired polymers that are constructed based on the structure of poly(N-substituted glycine) [1–4]. It has been demonstrated that peptoids can form peptide-like secondary structures: helices, sheets, and coils, and with certain constructs, they can also form helix-bundles [5–12]. Studies have shown that peptoids exhibit excellent bio-compatibility and potential biological activities [13, 14]. Unlike peptides, peptoids are resistant to protease digestion. This property enables a high metabolic stability [15, 16]. Because of their diverse structures and tunable properties, peptoids have been an attractive molecular model for biophysical research and have also been considered to be a major candidate as peptide-mimicking therapeutic agents, ligands for proteins, and scaffolds in the search for new properties [2, 17–24]. Most of these studies rely on the creation of diverse peptoid libraries [25–27]. Peptoids can easily be constructed via a solid-phase synthesis strategy that incorporates a two-step monomer addition cycle [28]. Bromoacetic acid is carbodiimide-coupled to the end of the growing peptoid, and then primary amines are used as the second sub-monomers to create the side-chain groups in the peptoid polymer. The hundreds of commercially available and tested primary amines enable the generation of large number of peptoids with diverse sequences [2, 5]. In connection to this, an efficient analytical method to analyze the sequence and to characterize the structures of the peptoids can greatly facilitate the discovery of functional peptoids via combinatorial chemistry.

Electronic supplementary material The online version of this article (doi:10.1007/s13361-014-0869-0) contains supplementary material, which is available to authorized users.

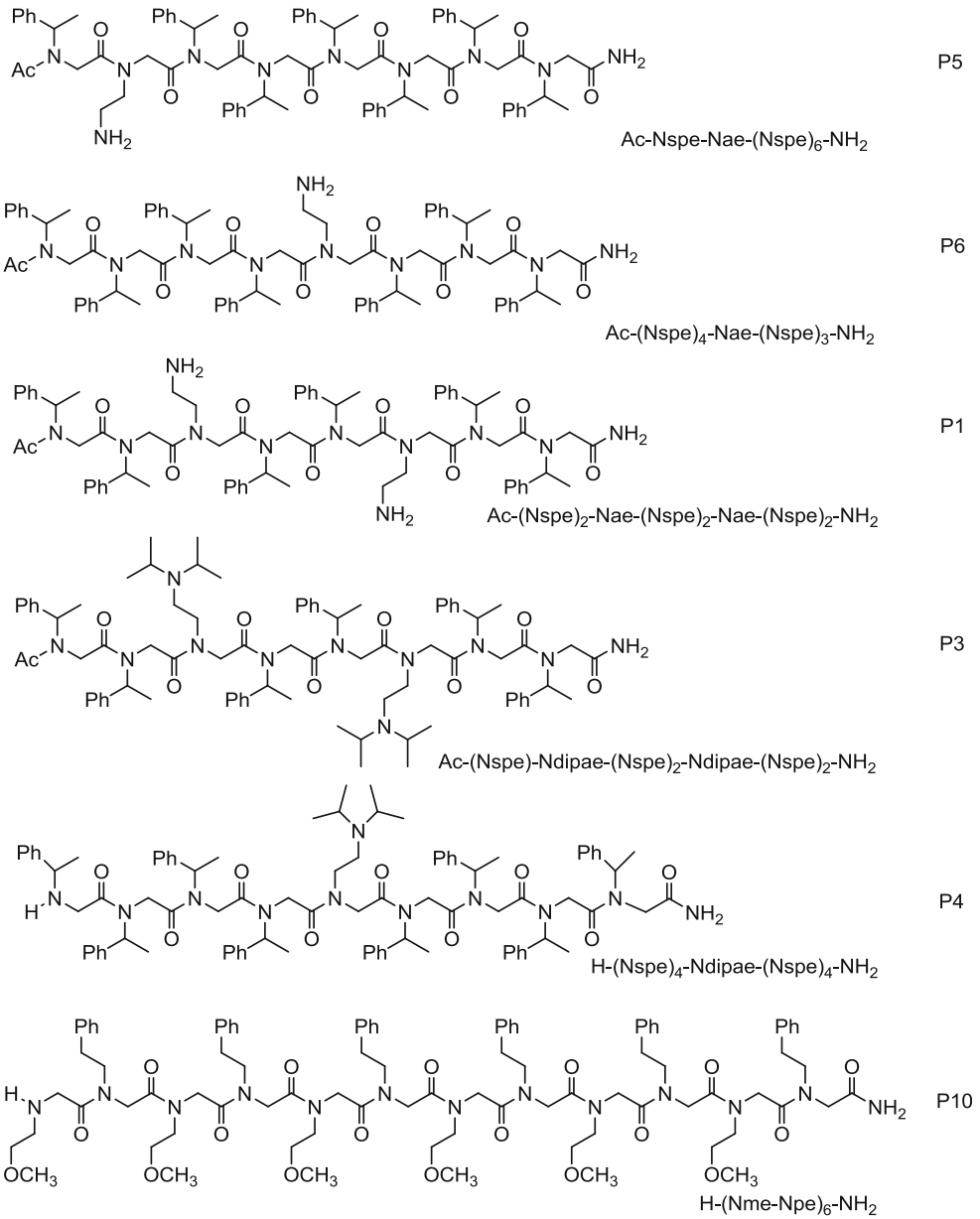
Correspondence to: Jianhua Ren; *e-mail:* jren@pacific.edu

Direct sequencing of peptoids on the solid resin by Edman degradation has been a common practice. However, this method is extremely time-consuming, as it requires the synthesis and analysis of standards for each unnatural residue [29–31]. Since the introduction of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), tandem mass spectrometry (MS/MS) has been applied as a robust technique to routinely characterize the sequences and structures of peptides and proteins [32–35]. This technique has also shown promise as the method of choice for analyzing the sequences and structures of peptoids [27, 36–42]. Early studies using fast atom bombardment (FAB) ionization combined with high-energy collision-induced dissociation (CID) as well as nano-electrospray ionization (nESI) combined with low-energy CID have shown that the protonated peptoids fragmented in a manner similar to those found in peptides [39, 41, 42]. The predominant fragments corresponded to the dissociation at the amide bonds. A mass spectrometry method based on the isotopic patterns of bromine-containing C–terminal ions was used to rapidly sequence the one-bead-one-compound peptoid library [37]. In addition, a method that combined partial Edman degradation and mass spectrometry analysis was used for high-throughput sequencing of a peptoid library [36]. Recently, MALDI-time-of-flight (TOF) mass spectrometry was applied to analyze the sequence of a library of azapeptoids [43]. We have recently studied the fragmentation patterns of a group of protonated and metallated model peptoids in the gas phase. We have found that the protonated peptoids dissociated by producing mainly the C–terminal fragment ions, whereas the alkali metal-peptoid adducts fragmented by producing both the C–terminal and the N-terminal fragment ions [44]. These results further demonstrated the utility of using mass spectrometry methods for de-novo sequencing of peptoid libraries generated by combinational chemistry.

In addition to the conventional CID technique, electron capture dissociation (ECD) and electron transfer dissociation (ETD) have emerged as promising tandem mass spectrometry techniques for characterizing the sequence information of peptides and proteins [45–56]. Unlike the CID experiment, which largely cleaves the peptide backbone at the amide bonds, both ECD and ETD experiments selectively cleave the peptide backbone at the N–C_α bonds [49, 57]. Thus ECD and ETD experiments can provide complementary sequence information to that produced by CID experiments.

There have been several concurrent mechanistic hypothesis proposed to explain the fragmentations observed in ECD experiments [46, 49, 56, 58, 59]. The two most compelling ones are the Cornell mechanism and the Utah-Washington mechanism, which have been used to explain the peptide fragmentations, especially the cleavage of the N–C_α bonds, observed in both ECD and ETD experiments. We will discuss how these mechanisms may apply to peptoids. To introduce the concepts, in the *Cornell mechanism* [46], an electron is thought to be initially captured at a positively charged site to form a hypervalent species. This is followed by ejecting a hydrogen atom from the charge site. The ejected hydrogen atom attaches to a backbone carbonyl group to form an "aminoketyl" radical intermediate with the radical site at the carbonyl carbon. The radical then induces a homolytic dissociation at the neighboring N–C_α bond to form an enol-imine structure at the N-terminal side and a new radical site at the C_α atom of the C-terminal side. If the remaining proton resides at the N-terminal side, a C-ion forms. If the proton resides at the C-terminal side, a Z-ion forms. In the *Utah-Washington mechanism* [58–60], an electron is thought to be captured directly into the amide π^* orbital to form an "amide superbase," an odd-electron negative ion with the negative charge at the oxygen and the radical site at the carbon atom. Alternatively, the captured electron initially enters into one Rydberg orbital at a positively charged site and subsequently transfers to an amide π^* orbital to form the "amide superbase"[61]. This follows by two possible paths (the "chicken or egg" mechanism) [62]. In one path, a proton transfers to the "amide superbase" to form the "aminoketyl" radical intermediate followed by N–C_α cleavage. In the other path, the "amide superbase" odd-electron negative ion directly induces the homolytic cleavage of the neighboring N–C_α bond to form an "imine superbase" at the amide group, $[CO = NH]^-$, and a radical site at the C_α atom (which would form the Z-ion). Upon transferring a proton to the "imine superbase," a C-ion would form [62, 63].

We have studied the fragmentation patterns of a group of model peptoids under ECD conditions. The structures and the corresponding names of the model peptoids are shown in Scheme 1. The peptoid number is assigned based on our peptoid library sequence. Except Peptoid 10 (P10), all other peptoids contain the poly-(S)-N-(1-phenylethyl)glycine (poly-Nspe) as the backbone structure. Nspe has a chiral side group and the structure of poly-Nspe is expected to be relatively rigid. The basic residue N-(2-aminoethyl)glycine (Nae) or N-(2-diisopropylaminoethyl)glycine (Ndipae) is placed at different sites of the peptoid chain. Peptoids 5 and 6 (P5 and P6) are Nspe-based isomers with the Nae residue placed at the second position from the N-terminus and in the middle of the peptoid chain, respectively. Peptoid 1 (P1) is an analogue of P5 and P6, but it contains two Nae residues. Peptoid 3 (P3) is essentially an analogue of P1, such that the two Nae residues are replaced by two Ndipae units. Ndipae has a relatively bulky side group. Peptoid 4 (P4) has a structure similar to P3, but with only one Ndipae residue. In addition, P4 has a free amino group at the N-terminus. Peptoid 10 (P10) consists of alternating N-(2-methoxyethyl)glycine (Nme) and N-(2-phenylethyl)glycine (Npe) residues. The side groups of P10 are less bulky and are achiral, which allows greater conformational flexibility. P10 has a free amino group at the N-terminus as well. These model peptoids allow us to examine the effects of the backbone structure, the location and the type of the basic



Scheme 1.

residue, and the acetylation at the N-terminus on the fragmentation patterns. In this paper, we present the results of the ECD experiments on the doubly protonated peptoids and the mixed protonated-sodiated peptoids.

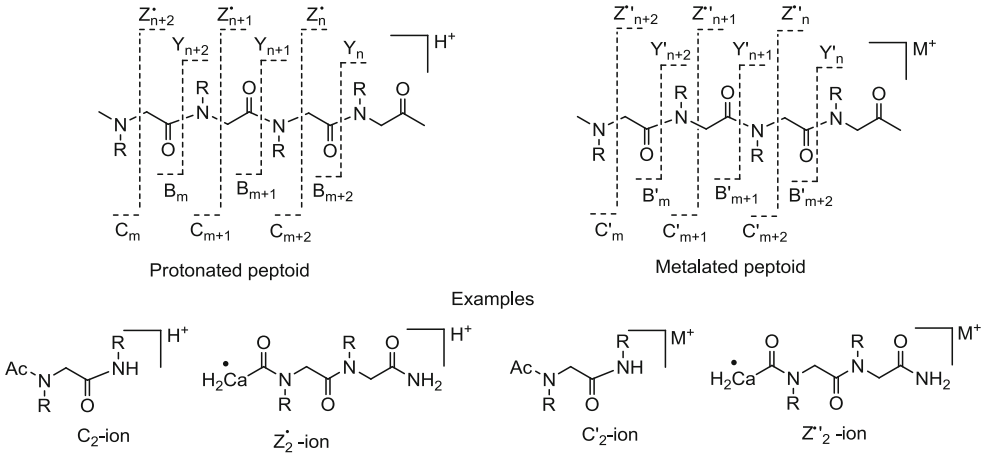
Experimental

Nomenclature of Peptoid Fragment Ions

Peptoids fragmented similar to peptides under tandem mass spectrometry conditions. The main fragment ions observed in the CID experiments corresponded to the dissociation of the amide bonds of the peptoid backbone, which would produce the Y- and the B-series of ions (the Roepstorff nomenclature [64]). Similar nomenclature was used by Liskamp and coworkers to assign the peptoid fragment ions [39, 41, 42]. Dissociation of the N–C_α bonds could be accomplished in the ECD and ETD experiments, which would produce the C- and the $Z^{\bullet}$-series of ions. The nomenclature we used to characterize the fragment ions and the sample structures are shown in Scheme 2. Similar to those for peptides, the $Z^{\bullet}$-ions and $Z^{\bullet\prime}$-ions are radical ions (odd-electron positive ion).

Mass Spectrometry Measurements

The ECD experiments were performed on a Thermo Finnigan 7 T LTQ-FT instrument (Thermo Finnigan, Bremen, Germany). All peptoid sample solutions were prepared by dissolving 1 mg



Scheme 2.

of solid peptoid in 1 mL of mixed solvent of water:methanol (1:1 v:v). The sample solutions were diluted in the mixed solvent to a final concentration of 10 μM. Doubly protonated or mixed protonated-sodiated peptoids were generated by electrospray ionization (ESI) using the standard Thermo ESI ion source by infusing the peptoid sample at a flow-rate of 5 μL/min. The ESI and ion optics voltages plus the ECD conditions were optimized for the 2+ charge state of Substance P (*m/z* 674.37135). The following experimental ECD parameters were applied: *m/z* 50–2000, AGC target = 2×10^5, maximum accumulation time = 100 ms, FTICR resolving power = 100,000 @ *m/z* = 400, precursor ion isolation width = 10 *m/z* units, ECD energy = 10.0 V, delay time = 0 ms, ECD irradiation time = 10 ms, and each ECD spectrum was an average of 2 min data acquisition of 1 μscan. To assign the ECD fragment ions in the ECD MS/MS spectra, the *m/z* values of the monoisotopic peaks of all A-, B-, C-, X-, Y-, and Z-type ions of the six peptoids were calculated based on elemental compositions in Excel. In addition, the *m/z* values after the losses of H_2O, NH_3, and various neutral molecules and radicals from the peptoid monomer side groups were calculated. For the ECD spectra, Decon2LS was used to deconvolute the spectra and to determine the monoisotopic peaks of all detected fragment ions. Decon2LS used the TRASH algorithm and the following parameters were applied: peak fit type = quadratic, minimum S/N = 3, minimal background ratio = 5, maximum fit = 0.1, threshold intensity for detection = 10, averagine, no apodization and zero-fill. All spectra and assignments were manually checked and were based on a ±5 ppm mass measurement deviation.

The ETD experiments were performed using Thermo LTQ XL and LTQ-Orbitrap XL instruments equipped with an ETD/CI ion source. The ESI and ion optics voltages plus the ETD conditions were optimized for the 2+ charge state of Substance P (*m/z* 674.37135). ETD conditions recommended in the hardware manuals were used to generate fluoranthene odd-electron negative ions that reacted with the doubly-charged peptoids in the LTQ. The following experimental ETD parameters were applied: *m/z* 50–2000, AGC target = 1×10^4 (LTQ), 3×10^5 (ETD reagent ion) or 2×10^5 (Orbitrap); maximum accumulation time = 50 (ETD reagent ion) or 1000 ms (ESI ions), FTICR resolving power = 60,000 @ *m/z* = 400 (1 spectrum/s), precursor ion isolation width = 3 (LTQ) or 10 *m/z* (Orbitrap), ETD reaction time = 100 ms, and each ETD spectrum was an average of 2 min data acquisition of three (LTQ) or one (Orbitrap) μscan.

Peptoid Synthesis

All peptoids studied in this work were synthesized using the solid-phase strategy on Rink amide resin (EMD Chemicals, Gibbstown, NJ, USA). A slightly modified Aapptec Apex 396 synthesizer was employed for the synthesis [65]. They were purified by HPLC, and then freeze-dried. A detailed synthesis procedure was described in our previous publication [44].

Results

Peptoid-5

P5 is a polymer of Nspe with one Nae residue inserted at the second position from the N-terminus. The ECD spectra of the doubly charged P5 with two protons $[P + 2H]^{2+}$ or with one proton plus one sodium cation $[P + H + Na]^{2+}$ are given in Supplementary Figures S1a and S1b, respectively. The abundances of some fragment ions were much lower than the others. For a better view, the portions of the spectrum with low abundance ion were magnified accordingly. The scaled spectra are presented in Fig. 1a and b, respectively. Shown in Fig. 1a, the peaks at *m/z* 644 and 1287 corresponded to the ions of the doubly and singly protonated P5, respectively. The dominant fragments were the C-series of ions from C_4 to C_8. The ion C_8 was much more abundant than the other fragment ions. The corresponding $Z^{\bullet}$-ions

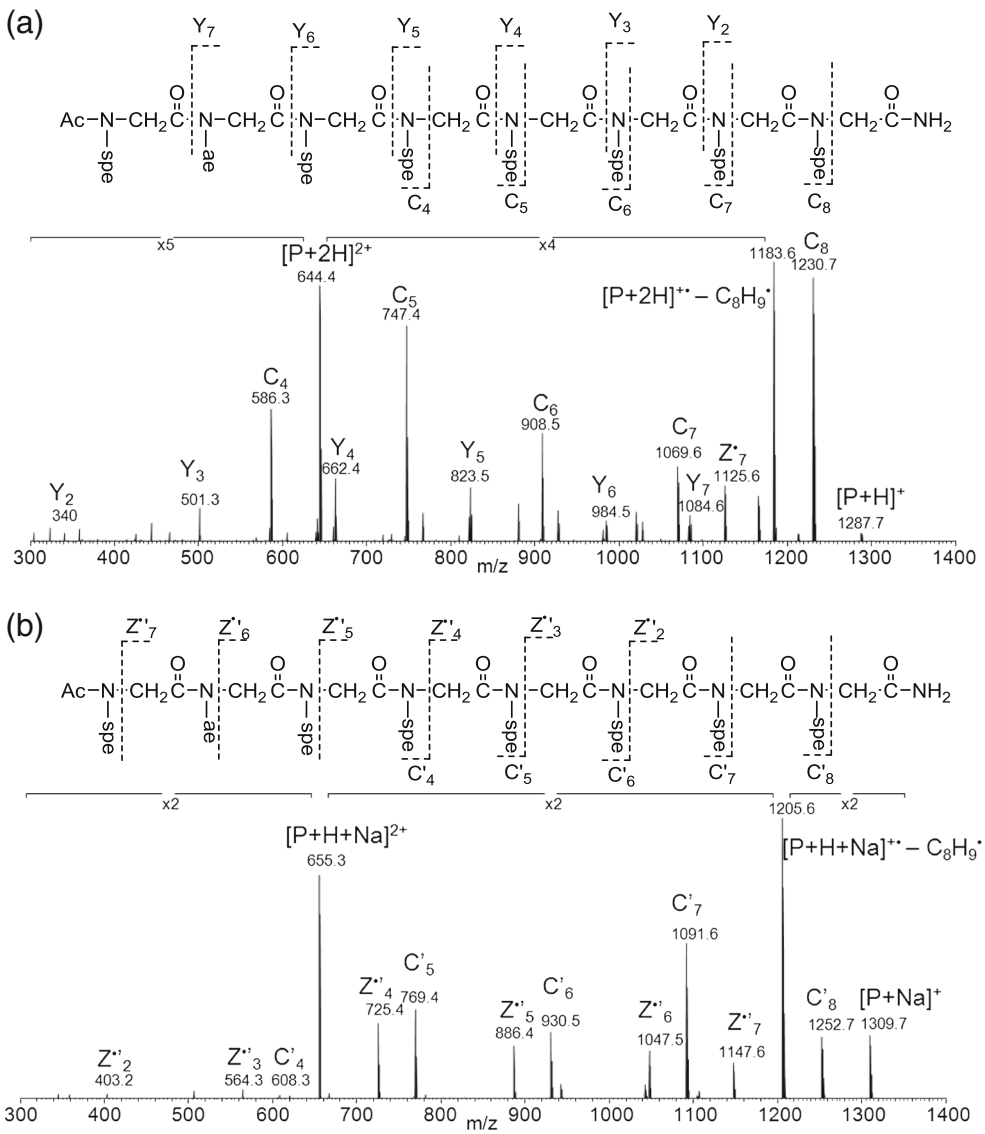


Figure 1. The ECD spectra of the doubly charged P5, **(a)** $[P5 + 2H]^{2+}$, and **(b)** $[P5 + H + Na]^{2+}$

were not present, except a low abundant $Z^{\bullet}_7$ at *m/z* 1125. Instead, the Y-series of ions, from Y_2 to Y_7, were observed, but the intensities were relatively low compared with the C-series. An ion at *m/z* 1183 was the most abundant one among all the fragments. This ion corresponded to the loss of a side-chain group, $[Ph\text{-}CH\text{–}CH_3]^{\bullet}$ ($C_8H_9^{\bullet}$, 105 u), from the odd-electron positive ion of the doubly protonated peptoid, $[P + 2H]^{+\bullet}$. In Fig. 1b, the peaks at *m/z* 655 and 1309 were for the doubly charged precursor ion, $[P + H + Na]^{2+}$ and the singly charged sodium cation adduct of the peptoid, $[P + Na]^{+}$, respectively. In this spectrum, both the sodiated C′-series (C'_4 to C'_8) and the sodiated $Z^{\bullet}$′-series of ions ($Z^{\bullet}{}'_2$ to $Z^{\bullet}{}'_7$) were present. The intensities of the lower mass ions, C'_4 and $Z'_{2–3}$, were relatively weak. The ion at *m/z* 1205, corresponding to the loss of the side group from the radical cation precursor ($[P + H + Na]^{+\bullet} – C_8H_9^{\bullet}$), was the most abundant ion in the spectrum. Neither the B′- nor the Y′-series of ion were observed.

Peptoid-6

P6 is an isomer of P5. The structural difference between these two peptoids is the location of the basic Nae residue. In P5 the Nae is close to the N-terminal side and in P6 it is in the middle of the molecule. The scaled ECD spectra of the doubly charged P6 are shown in Fig. 2, and the unscaled spectra are given in Supplementary Figure S2. The doubly protonated P6 (Fig. 2a) produced three series of ions, the C-series, the Y-series, and the $Z^{\bullet}$-series. The C-series of ions were the dominant ones. The ion at *m/z* 1183 (loss of the side group, $C_8H_9^{\bullet}$) was the most abundant fragment. The noticeable difference between P6 and P5 was whether or not the $Z^{\bullet}$-ions were formed. The $Z^{\bullet}$-ions were quite abundant in P6, whereas except for $Z^{\bullet}_7$, other Z-ions were absent in P5. The fragmentation pattern for the sodiated P6 appeared similar to that for the sodiated P5. Both the

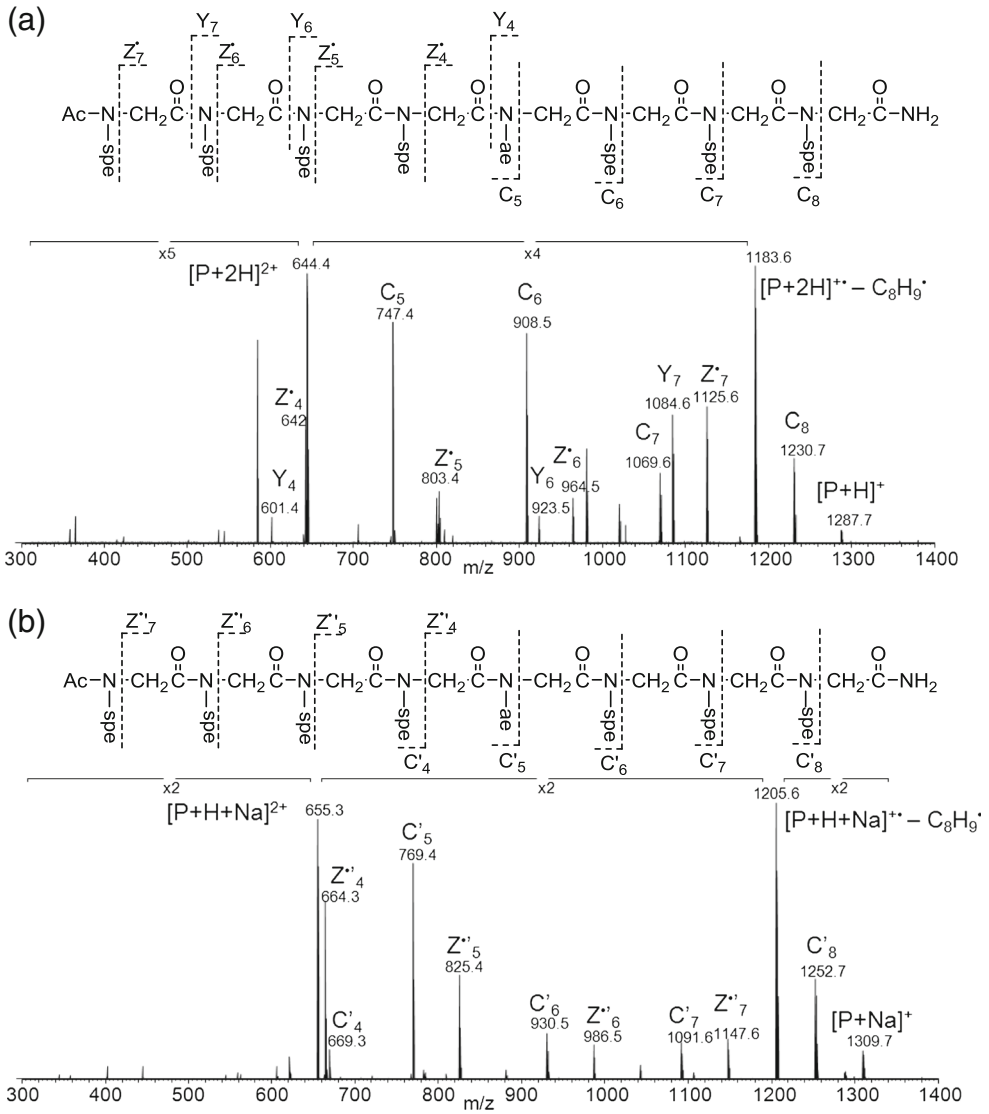


Figure 2. The ECD spectra of doubly charged P6, **(a)** $[P6 + 2H]^{2+}$, and **(b)** $[P6 + H + Na]^{2+}$

C′-series and the Z″-series of ions were present in the spectrum (Fig. 2b). The peak at *m/z* 1205, corresponding to the loss of the side group ($C_8H_9^{\bullet}$), was the base peak in the spectrum.

In order to examine the possibility of sequential fragmentation, the doubly protonated P6 was subjected to electron transfer dissociation (ETD) followed by collision-induced dissociation (CID) on the $Z^{\bullet}_7$ ion (*m/z* 1125). The corresponding spectra are shown in Supplementary Figures S2c and d. The ETD experiment on P6 produced comparable fragmentation as that of the ECD experiment. Upon CID, the $Z^{\bullet}_7$ ion fragmented to yield a series of lower mass $Z^{\bullet}$-ions, from $Z^{\bullet}_4$ to $Z^{\bullet}_6$. No Y-ions were produced from $Z^{\bullet}_7$.

Peptoid-1

P1 is a polymer of Nspe with two Nae residues placed at the third and the sixth positions from the N-terminus. The ECD spectrum of the doubly protonated P1 is shown in Fig. 3 and Supplementary Figure S3. The three types of fragment ions, the C-ions, the Y-ions, and the $Z^{\bullet}$-ions, were all observed. The C-series was more dominant, followed by the $Z^{\bullet}$-series. The Y-series had the lowest relative abundance. The most abundant fragment ion in the spectrum was the ion at *m/z* 1122, corresponding to the loss of the side group from the odd-electron positive ion precursor ($[P + 2H]^{\bullet+} - C_8H_9^{\bullet}$). Two other notable ions were observed at the high mass region, *m/z* 1104 and 1005. Both ions were 18 mass units smaller than the corresponding precursor ions, *m/z* 1122 ($[P + 2H]^{\bullet+} - C_8H_9^{\bullet}$) and *m/z* 1023 ($Y_7$), respectively.

Peptoid-3 and Peptoid-4

P3 is an analogue of P1. In P3, the two Nae residues are replaced by two Ndipae residues. The ECD spectrum of the doubly protonated P3 is shown in Fig. 4a as well as in

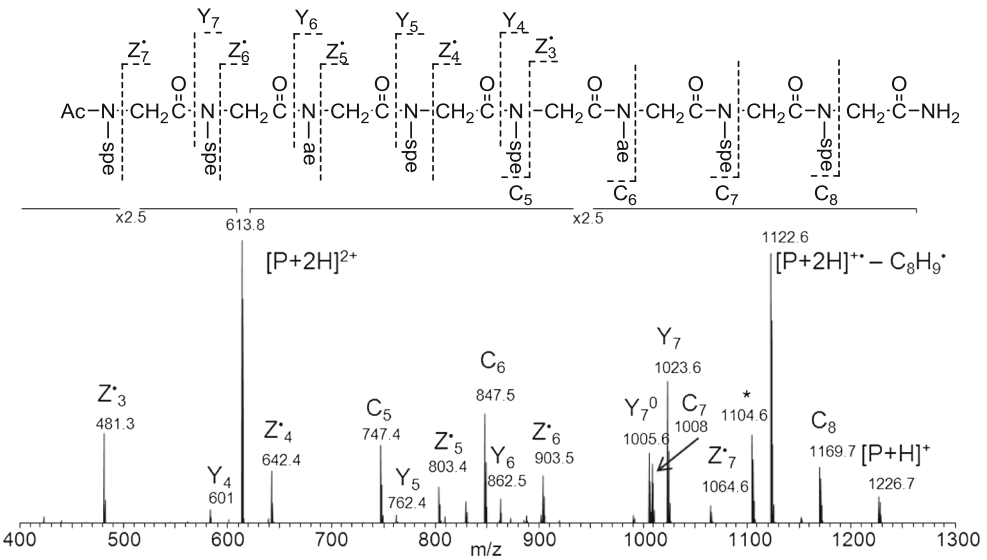


Figure 3. The ECD spectrum of doubly charged P1, $[P1 + 2H]^{2+}$

Supplementary Figure S4a. The most abundant ion was at *m/z* 1290, corresponding to the loss of the side group from the odd-electron positive ion precursor, $[P + 2H]^{\bullet+} - C_8H_9^{\bullet}$. The high mass C-ions ($C_{4-8}$) and the medium mass $Z^{\bullet}$-ions ($Z^{\bullet}_{3-6}$) were observed in the spectrum. Unlike P1 with observable Y-ions, the Y-series of ions were absent for P3. A notable fragment ion at *m/z* 1028 corresponded to the A_6 ion. None of the other A-ions were observed.

P4 is an analogue of P6 with one basic side-chain amine group. In P4, the Ndipae residue is placed in the middle of the peptoid chain, and the N-terminus is a free amine group. The ECD spectrum is shown in Fig. 4b as well as in Supplementary Figure S4b. The most abundant fragment was at *m/z* 1386, corresponding to the loss of a side group from the odd-electron positive ion precursor, $[P + 2H]^{\bullet+} - C_8H_9^{\bullet}$. Two series of ions were clearly present in the spectrum, the C-ions and the $Z^{\bullet}$-ions. The C-ions were relatively more abundant than the $Z^{\bullet}$-ions. After magnifying the spectrum, the Y-series of ions were also observed. In general, the fragmentation pattern for P4 was similar to that for P6.

Peptoid-10

P10 consists of 12 monomer residues with alternating Nme and Npe and does not have any basic side-chain groups. The N-terminus is a secondary amine without acetylation. The ECD spectra of the doubly charged P10 are shown in Fig. 5 and Supplementary Figure S5. Both the C-series and the Y-series of ions were present in the spectrum of the doubly protonated P10 (Fig. 5a). The C_{12}-ion was the most abundant one and the $Z^{\bullet}$-series of ions were not observed. The mixed protonated and sodiated P10 showed a very different picture (Fig. 5b). The ECD experiments produced two series of ions, the C′-ions and the $Z^{\bullet\prime}$-ions, with comparable abundances. The Y′-ions were not observed.

Discussion

To aid the comparison and discussion, the observed fragmentations for all of the model peptoids are summarized in Table 1 and 2. The C-type ions were universally observed for all the doubly charged protonated and mixed protonated-sodiated peptoids. The Z-type ions seemed to be favored over Y-type for all the sodiated peptoids (Table 2) and most of the protonated peptoids except P5 and P10 (Table 1). The Y-type ions were commonly observed for the doubly protonated peptoids, except for P3 (Table 1). The Y-ions were not observed for the sodiated peptoids (Table 2). All the observed fragment ions had medium to high *m/z* values (with four residues and longer). The low mass ions (three residues and shorter) were either absent or at very low abundances. Other type fragments (A-ions, B-ions, and X-ions) were not observed. One interesting fragmentation observed in P1 was the loss of 18 mass units from the corresponding precursor ions. One notable fragmentation from all charged peptoids was the loss of the side-chain group ($[Ph\text{-}CH\text{-}CH_3]^{\bullet}$ or $C_8H_9^{\bullet}$, 105 u) from the peptoid odd-electron positive ions ($[P + 2H]^{\bullet+}$ or $[P + H + Na]^{\bullet+}$).

Doubly Protonated Peptoids

The ECD fragmentation patterns of the doubly protonated peptoids appeared quite different from those observed for most multiply protonated peptides. For peptides, the fragmentations are dominated by the c- and the $z^{\bullet}$-ions. Other backbone fragment ions, such as y- or b-ions, are often absent or in very low abundances [49, 57], whereas for the model peptoids used in this study (Table 1), the $Z^{\bullet}$-ions were absent for P5 and P10, and the Y-ions were common for all the doubly protonated peptoids, except P3.

Both the Cornell and the Utah-Washington mechanisms could be used to explain the cleavage of the N–C_α bonds in

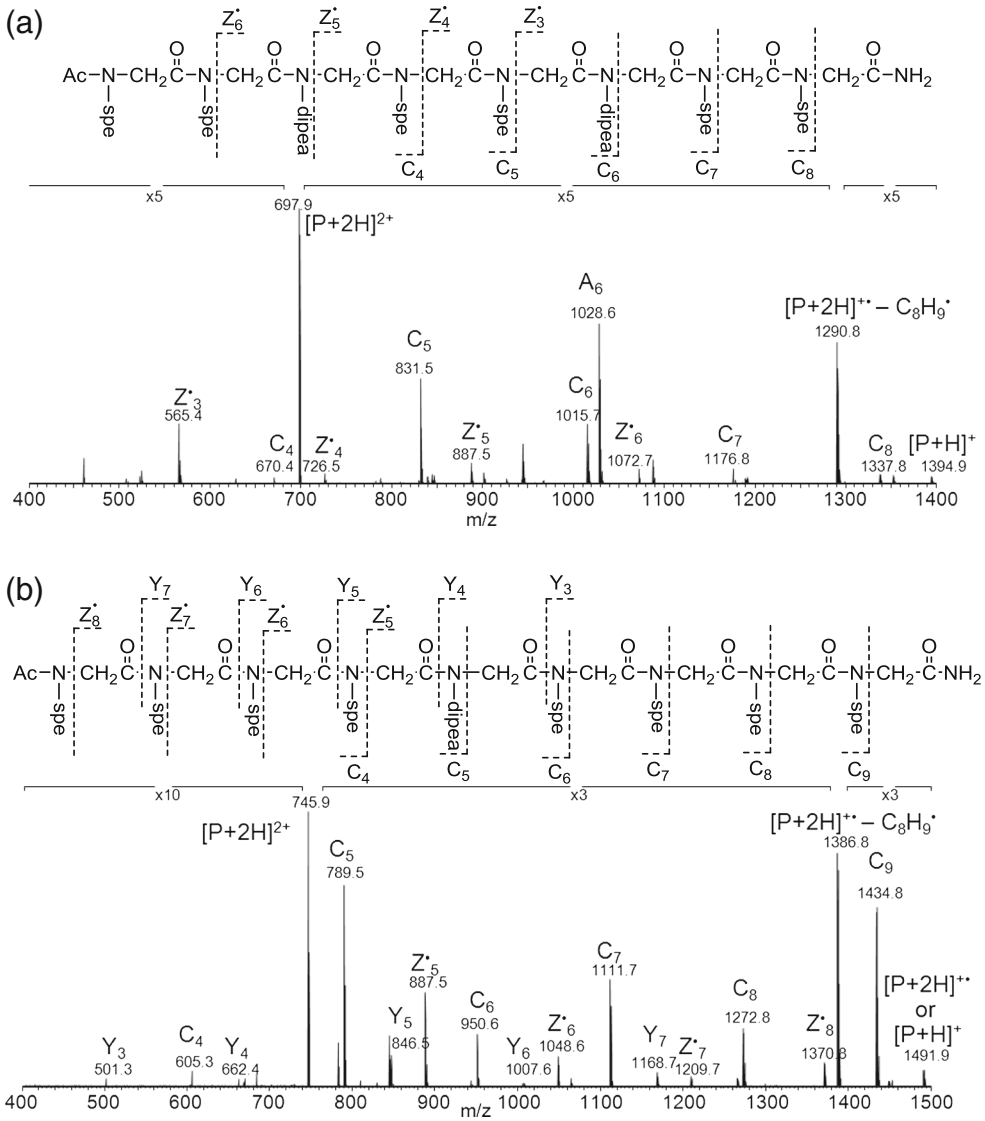


Figure 4. The ECD spectra of doubly charged P3 and P4, **(a)** $[P3 + 2H]^{2+}$, and **(b)** $[P4 + 2H]^{2+}$

peptoids. Some of the fragment ions were the results of charge-remote fragmentation. The Utah-Washington mechanism provides a better explanation for charge-remote fragmentation [63]. Here, we use this mechanism to demonstrate the formation of a C-ion and a $Z^{\bullet}$-ion from peptoid 1, Scheme 3. The vertical dashed line indicates the fragmentation site, and the dashed circle represents the protonation site in the vicinity of the dissociating N–C_α bond. Before the ECD process, the two protons were likely bound to the two side-chain amino groups. Upon the ECD experiment, an electron was captured by the amide carbonyl group adjacent to the dissociating N–C_α bond. Subsequently, a proton from a side-chain amino group transferred to the carbonyl group oxygen to yield an "aminoketyl" radical intermediate. The proton could come from either of the two protonation sites. The radical then induced the homolytic dissociation of the N–C_α bond and the radical site moved to the C_α carbon. If the remaining proton retained at the N-terminal side fragment, a C_5-ion would form. The C_5-ion could have either an enol-imine structure or an amide structure. If the remaining proton transferred to the C-terminal side fragment, a $Z^{\bullet}_3$-ion would form. As shown in Fig. 3, both C_5 and $Z^{\bullet}_3$ ions were observed with comparable abundances.

The experimental results indicate that the formation of the C- and the $Z^{\bullet}$ fragment ions may be associated with the location of the basic residue(s). All the observed C-type ions include at least one basic residue. In P5, the basic Nae is the second residue from the N-terminus, and the smallest C-ion is C_4, whereas in P6, the basic Nae is the fifth residue from the N-terminus, and the smallest C-ion is C_5. Similarly, in P4, the basic Ndipae is the fifth residue from the N-terminus, and the major C-ions are C_5–C_9. Only a trace amount of C_4-ion has been observed. All the observed $Z^{\bullet}$-ions also include at least one basic residue. The basic Nae is the third and the fourth residue from the C-terminus in P1 and P6,

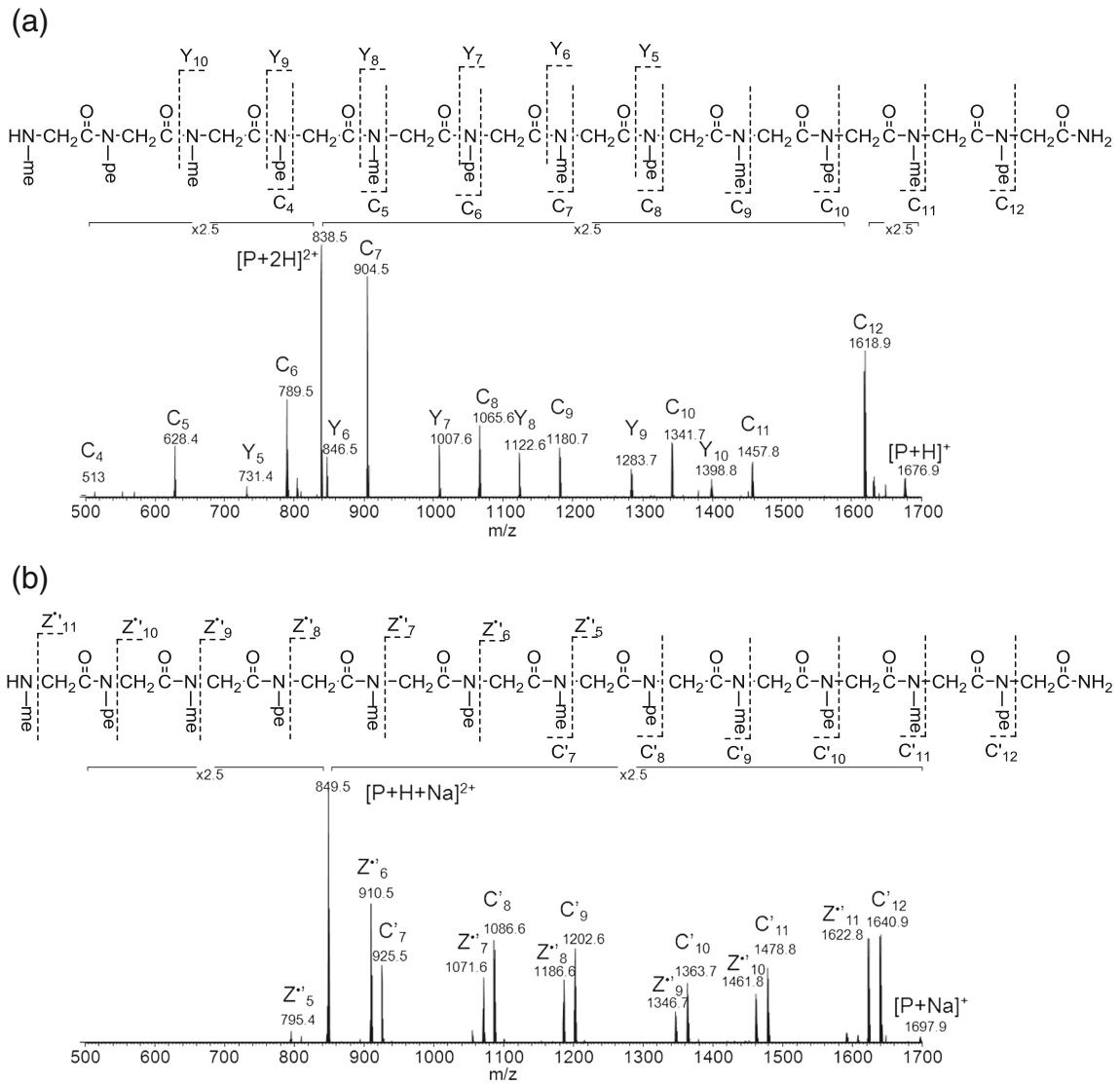


Figure 5. The ECD spectra of doubly charged P10, **(a)** $[P10 + 2H]^{2+}$, and **(b)** $[P10 + H + Na]^{2+}$

respectively. The smallest $Z^{\bullet}$-ions are $Z^{\bullet}_3$ and $Z^{\bullet}_4$, respectively. Similarly, $Z^{\bullet}_5$ is the smallest $Z^{\bullet}$-ion in P4, corresponding to the basic Ndipae at the fifth residue from the C-terminus. Both P5 and P10 have the basic residue near or at the N-terminus, and the $Z^{\bullet}$-ions are absent for both peptoids.

The Y-ions were observed for most protonated peptoids. One question was whether the peptoid Y-ions were formed from the $Z^{\bullet}$-ions via sequential fragmentation. Studies on peptide ion fragmentation have shown that the c- and the $z^{\bullet}$-type ions generated from ETD could fragment further under the CID condition to produce additional backbone cleavaged ions [66]. We performed a test experiment to fragment a $Z_7^{\bullet}$-ion generated by ETD of peptoid 6. The results showed that the $Z_7^{\bullet}$-ion fragmented to produce a series of lower mass $Z^{\bullet}$-ions,

Table 1. Fragmentation Patterns for the Doubly Protonated Peptoids[a], $[P + 2H]^{2+}$

Peptoid	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	$Z^{\bullet}_3$	$Z^{\bullet}_4$	$Z^{\bullet}_5$	$Z^{\bullet}_6$	$Z^{\bullet}_7$	$Z^{\bullet}_8$	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}
P1		x	x	x	x					x	x	x	x	x				x	x	x	x			
P3	x	x	x	x	x					x	x	x	x											
P4		x	x	x	x	x						x	x	x	x		x	x	x	x	x			
P5	x	x	x	x	x											x	x	x	x	x	x			
P6		x	x	x	x						x	x	x	x				x		x	x			
P10	x	x	x	x	x	x	x	x	x										x	x	x	x	x	x

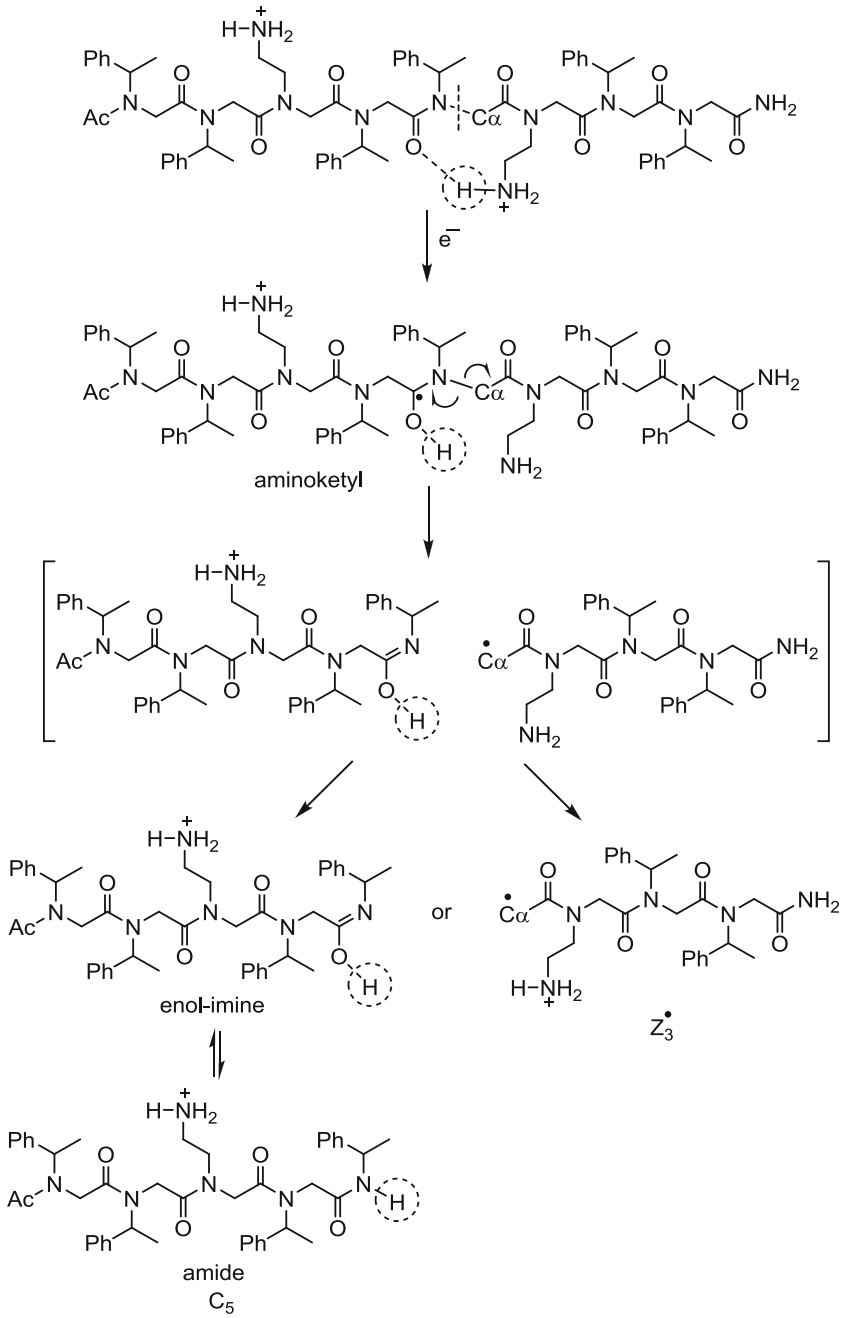
[a] The symbol x indicates the observed ions with the relative abundance of 2% or higher

Table 2. Fragmentation Patterns for the Mixed Protonated-Sodiated Peptoids[a], $[P + H + Na]^{2+}$

Peptoid	C'_4	C'_5	C'_6	C'_7	C'_8	C'_9	C'_{10}	C'_{11}	C'_{12}	$Z^{\bullet}{}'_2$	$Z^{\bullet}{}'_3$	$Z^{\bullet}{}'_4$	$Z^{\bullet}{}'_5$	$Z^{\bullet}{}'_6$	$Z^{\bullet}{}'_7$	$Z^{\bullet}{}'_8$	$Z^{\bullet}{}'_9$	$Z^{\bullet}{}'_{10}$	$Z^{\bullet}{}'_{11}$
P5	x	x	x	x	x					x	x	x	x	x	x				
P6	x	x	x	x	x							x	x	x	x				
P10				x	x	x	x	x	x				x	x	x	x	x	x	x

[a] The symbol x indicates the observed ions with the relative abundance of 2% or higher

but no Y-type ions were observed. Although ETD experiments might yield different results from ECD experiments, our results suggested that the ETD and ECD spectra of P6 were qualitatively comparable. The ETD-CID experiment suggested that the Y-ions were not likely the secondary fragments from the $Z^{\bullet}$-ions. In addition, the ECD spectra of the doubly



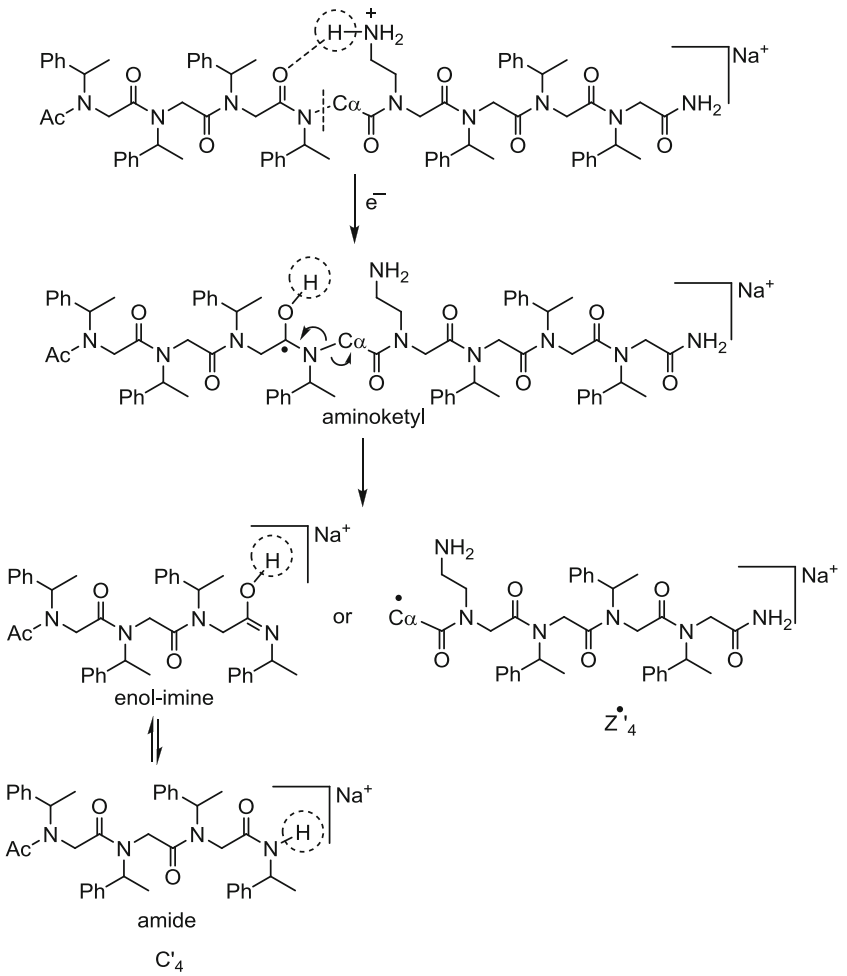
Scheme 3.

protonated P5 and P10 showed the C-ions and Y-ions, but not the Z•-ions, further suggesting that the Y-ions were not likely the results of the Z•-ions.

Our results of the Z_7•-ion fragmentation under the CID condition agreed with a recent study of the cascade dissociations of peptide cation-radicals [67, 68]. That study showed that the Z•-ions formed by ETD could undergo sequential dissociation to produce a series of smaller Z•-ions under both CID and infrared multiphoton dissociation (IRMPD) conditions. The sequential dissociation was thought to be triggered by a side-chain β-hydrogen atom transfer to the z-ion Cα radical site followed by homolytic dissociation of the adjacent Cα–CO bond to form the intermediate x-type cation-radicals that spontaneously dissociated by loss of the HNCO moiety. For peptoid Z•-ions, the mechanism would not involve the X-ion intermediate, since the peptoid side-chain groups were appended at the backbone nitrogen instead of the Cα atoms. The proposed mechanism for the sequential fragmentation of the Z_7•-ion to form the lower mass Z•-ions is given in Supplementary Scheme S1. A side-chain benzylic hydrogen atom was transferred to the Cα radical site and this resulted in a new radical site at the side-chain benzylic carbon. The benzylic radical would induce a homolytic dissociation of the adjacent N–Cα bond to form the Z_6•-ion.

The Y-ions were likely formed from the charge-reduced peptoid, $[P + H]^+$. The charge reduced peptoid was generated via the sequence that the doubly protonated peptoid acquired an electron to form a radical cation, and this was followed by losing a hydrogen atom: $[P + 2H]^{2+} + e^- \rightarrow [P + 2H]^{+\bullet} \rightarrow [P + H]^+ + H^\bullet$. The mechanism to yield Y-ions from $[P + H]^+$ would be similar to the Y-ion formation in the CID process [44]. Formation of non-standard ECD fragment ions, such as the b-ions, was observed for doubly protonated peptides. The b-type fragment series were similar to those shown in the CID spectra of the singly protonated peptide [69, 70]. The formation of the peptide b-ions was attributed to the vibrationally-excited singly protonated peptides generated by electron capture and subsequently hydrogen atom desorption, and not from secondary fragmentation of c-ions. Producing Y-ions were expected to be more favorable for peptoids than for peptides, since the C-terminal fragment of a peptoid is a secondary amine with a higher proton affinity compared with a primary amine formed in a peptide fragment.

The loss of 18 mass units from the precursor ions in P1 might correspond to the loss of a water molecule. Our previous study on peptoid fragmentation under CID condi-



Scheme 4.

tions showed that water molecule loss from the peptoid ion and the Y-ions was a characteristic fragmentation observed in P1 [44]. The mechanism for water loss observed in the ECD experiment should be similar to that observed in the CID experiment [44].

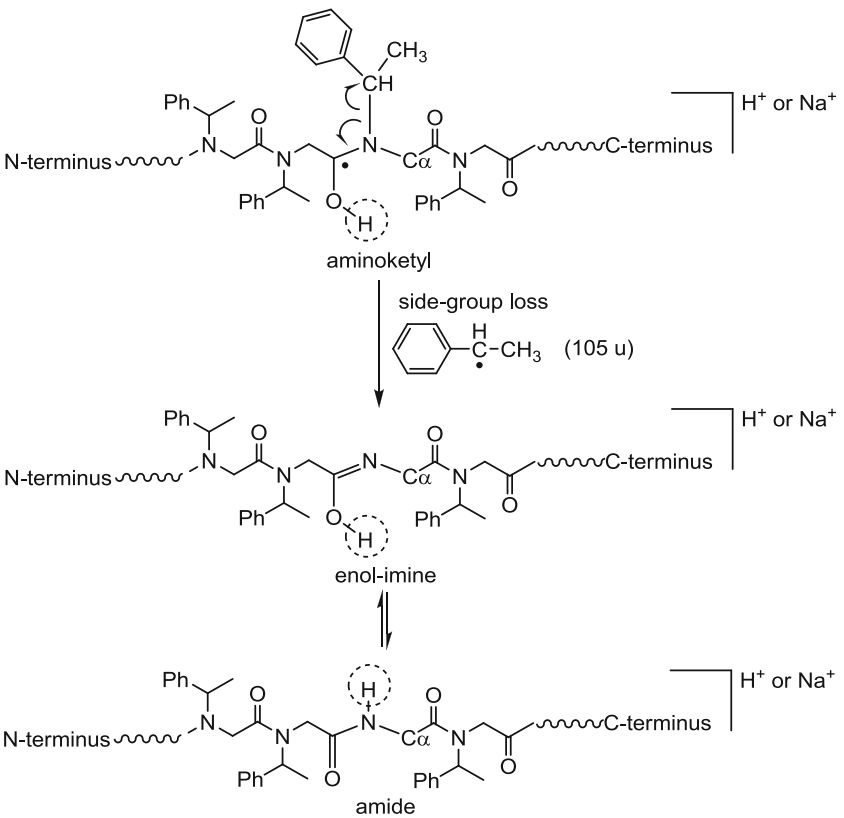
Mixed Protonated-Sodiated Peptoids

The mixed protonated-sodiated peptoids ECD fragmentation led to the generation of medium to high mass C′-ions and $Z^{\bullet}$′-ions with comparable abundances. The fragmentation mechanism is similar to that for the doubly protonated peptoids, except that one proton is replaced by a sodium cation. A sample mechanism for the fragmentation of P6 is shown is Scheme 4, where the vertical dashed line indicates the fragmentation site and the dashed circle represents the protonation site in the vicinity of the dissociating N–C_α bond. Upon capturing an election to the amide carbonyl group, the nearby proton was transferred to the carbonyl group oxygen and formed an "aminoketyl" radical intermediate. The radical induced a homolytic cleavage of the N–C_α bond and the radical site moved to the C_α atom of the C-terminal fragment. The N-terminal fragment would be either an enol-imine structure or an amide structure. If the N-terminal fragment retained the sodium cation a C'_4 ion would form. Likewise, if the C-terminal fragment retained the sodium cation, a $Z^{\bullet}{}'_4$ ion would form. In fact, both of the fragment ions were observed in the ECD spectrum of P6 (Fig. 2).

The relative abundances between the C′-ion and the $Z^{\bullet}$′-ions were comparable for all three mixed protonated-sodiated peptoids, P5, P6, and P10. The similarity in the ion abundance suggested that the chances of acquiring the sodium cation were comparable for both the N-terminal and the C-terminal fragments. Chelation between the sodium cation and the amide groups of the peptoid backbone might be an important factor that can stabilize both types of fragment ions. The chelation effect had been observed in the CID experiments in which both the B′- and Y′-type ions were formed comparably for alkali metallated peptoids [44]. The patterns for the formation of the C′-ions and $Z^{\bullet}$′-ions were quite similar for all three model peptoids, P5, P6, and P10, regardless of the location of the basic residue. The results showed that a basic residue did not seem to play a notable role in determining the observed fragment ions, which further suggested the chelation effect between the sodium cation and the carbonyl groups.

Fragmentation at the Nspe Side-Chain

The loss of a radical side-chain group ($C_8H_9^{\bullet}$) from the protonated ($[P + 2H]^{\bullet+}$) and the sodiated ($[P + H + Na]^{\bullet+}$) peptoid radical precursor ions yielded the most abundant product ions for all Nspe containing peptoids. Peptoid P10



Scheme 5.

was the only one that did not have an Nspe residue and did not give such a product ion corresponding to the loss of $C_8H_9^{\bullet}$. The loss of $C_8H_9^{\bullet}$ most likely occurred upon the doubly charged peptoid captured an electron. The proposed mechanism is shown in Scheme 5. Upon capturing an electron at the carbonyl group and followed by a proton transfer to the carbonyl group (similar process to that shown in Schemes 3 and 4) to form an aminoketyl radical intermediate, the radical induced a homolytic dissociation of the N–CH bond to produce a very stable benzylic radical (105 u) and an enol-imine structure at the amide bond. The enol-imine might subsequently rearrange to an amide.

An earlier computational study on the competitive dissociation of peptide aminoketyl cation radicals suggested that the loss of the phenylalanine side-chain benzyl group via homolytic dissociation of the Cα–C bond was energetically unfavorable and would be much slower than the cleavage of the backbone N–Cα bond [71]. Our study on peptoid cation radicals indicated that the loss of the side-chain benzylic radical group was facile. The difference in the abundance of the side-chain group loss was probably due to the structural difference between peptides and peptoids. In peptides, the side-chain groups were bonded to the Cα atoms, whereas in peptoids the side-chain groups were appended at the nitrogen atoms. Loss of a side-chain group from peptides would require the homolytic dissociation of a C–C bond, and loss of a side-chain group from peptoids would dissociate a N–C bond. In general a N–C bond is weaker than a C–C bond. For example, the bond dissociation energy for $H_2N\text{-}CH_2Ph$ is 71 kcal/mol and the bond dissociation energy for $H_3C\text{-}CH_2Ph$ is 77 kcal/mol [72]. The lower bond dissociation energy and the formation of the stable benzylic radical would facilitate the loss of a side-chain group from the aminoketyl cation radicals of Nspe-containing peptoids.

Conclusions

The fragmentation characteristics of the doubly protonated and mixed protonated-sodiated model peptoids have been studied via ECD tandem mass spectrometry experiments. Although the primary dissociation sites were the backbone $N\text{–}C_\alpha$ bonds, peptoid ions with different charge carriers showed different fragmentation patterns. All doubly protonated peptoids produced the expected C-ions. All peptoids with at least one basic residue in the peptoid chain other than the N-terminus also yielded the expected $Z^{\bullet}$-ions. The two peptoids with the basic amine group located at the N-terminal residue or at the N-terminus did not yield the $Z^{\bullet}$-ions. The formation of the C- and the $Z^{\bullet}$-ions might be associated with the location of the basic residue within the peptoid chain. All observed C- and $Z^{\bullet}$-type fragment ions consisted of a residue with a basic amine side-chain group. A fragment containing a basic residue would have a higher chance to retain the proton. In addition to the C- and the $Z^{\bullet}$-ions, all doubly protonated peptoids, except one, also produced the Y-series of ions. The Y-ions were likely formed from the charge reduced peptoid ions.

The mixed protonated-sodiated peptoids produced comparable sets of the C′- and the $Z^{\bullet}$′-series of ions. Chelation between the sodium cation and the amide groups of the peptoids might be an important factor that could stabilize both the N- and the C-terminal fragments. The location of the basic residue seemed to have minimal influence on the fragmentation patterns. The Y′-ions were not observed for all sodiated peptoid ions. Regardless of the types of the charge carrier, the most abundant fragment ion for all the Nspe-containing peptoids corresponded to the loss of a side-chain group from the protonated or sodiated odd-electron positive ions of the peptoids, $[P + 2H]^{\bullet+}$ or $[P + H + Na]^{\bullet+}$, respectively. The side-chain group was eliminated as a stable benzylic radical, $Ph\text{-}CH^{\bullet}\text{-}CH_3$ and, therefore, this channel was much more favored than other fragment pathways. Fragmentations of doubly protonated and mixed protonated and sodiated peptoids provided complementary sequential information. The characteristic fragmentation patterns of the doubly charged peptoids demonstrate the potential utility of sequencing peptoid libraries using the ECD tandem mass spectrometry technique.

Acknowledgments

The authors thank Dr. Kiran Morishetti (University of the Pacific, currently at Abon Pharmaceuticals LLC) for helping to interpret some of the spectra data, and Dr. Ronald Zuckermann (The Molecular Foundry, Lawrence Berkeley National Laboratory) for providing peptoid-10. J.R. acknowledges the support from the National Science Foundation [CHE-0749737 (prior) and CHE-1301505 (current)]. D.R. acknowledges the support from the Laboratory-Directed Research and Development program at Sandia National Laboratories (DE-AC04-94AL85000). Peptoid synthesis at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Sciences, US Department of Energy (DE-AC02-05CH11231). All ECD experiments were conducted at the Center for Regulatory and Environmental Analytical Metabolomics (CREAM) of the University of Louisville. The authors thank Dr. Shenheng Guan for assisting with the ETD experiments at the mass spectrometry facility of the University of California at San Francisco. They are also thankful for performing some of the ETD experiments in Dr. Joseph Loo's laboratory at the University of California at Los Angeles.

References

1. Hill, D.J., Mio, M.J., Prince, R.B., Hughes, T.S., Moore, J.S.: A field guide to foldamers. Chem. Rev. **101**, 3893–4011 (2001)
2. Sun, J., Zuckermann, R.N.: Peptoid polymers: a highly designable bioinspired material. ACS Nano **7**, 4715–4732 (2013)
3. Simon, R.J., Kania, R.S., Zuckermann, R.N., Huebner, V.D., Jewell, D.A., Banville, S., Ng, S., Wang, L., Rosenberg, S., Marlowe, C.K., Spellmeyer, D.C., Tan, R., Frankel, A.D., Santi, D.V., Cohen, F.E., Bartlett, P.A.: Peptoids: a modular approach to drug discovery. Proc. Natl. Acad. Sci. U. S. A. **89**, 9367–9371 (1992)

4. Farmer, P.S., Ariens, E.J.: Speculations on the design of nonpeptidic peptidomimetics. Trends Pharmacol. Sci. **3**, 362–365 (1982)
5. Paul, B., Butterfoss, G.L., Boswell, M.G., Renfrew, P.D., Yeung, F.G., Shah, N.H., Wolf, C., Bonneau, R., Kirshenbaum, K.: Peptoid atropisomers. J. Am. Chem. Soc. **133**, 10910–10919 (2011)
6. Burkoth, T.S., Beausoleil, E., Kaur, S., Tang, D., Cohen, F.E., Zuckermann, R.N.: Toward the synthesis of artificial proteins. The discovery of an amphiphilic helical peptoid assembly. Chem. Biol. **9**, 647–654 (2002)
7. Kirshenbaum, K., Barron, A.E., Goldsmith, R.A., Armand, P., Bradley, E.K., Truong, K.T.V., Dill, K.A., Cohen, F.E., Zuckermann, R.N.: Sequence-specific polypeptoids: a diverse family of heteropolymers with stable secondary structure. Proc. Natl. Acad. Sci. U. S. A. **95**, 4303–4308 (1998)
8. Wu, C.W., Sanborn, T.J., Huang, K., Zuckermann, R.N., Barron, A.E.: Peptoid oligomers with alpha-chiral, aromatic side chains: sequence requirements for the formation of stable peptoid helices. J. Am. Chem. Soc. **123**, 6778–6784 (2001)
9. Wu, C.W., Sanborn, T.J., Zuckermann, R.N., Barron, A.E.: Peptoid oligomers with alpha-chiral, aromatic side chains: effects of chain length on secondary structure. J. Am. Chem. Soc. **123**, 2958–2963 (2001)
10. Wu, C.W., Kirshenbaum, K., Sanborn, T.J., Patch, J.A., Huang, K., Dill, K.A., Zuckermann, R.N., Barron, A.E.: Structural and spectroscopic studies of peptoid oligomers with alpha-chiral aliphatic side chains. J. Am. Chem. Soc. **125**, 13525–13530 (2003)
11. Lee, B.-C., Zuckermann, R.N., Dill, K.A.: Folding a nonbiological polymer into a compact multihelical structure. J. Am. Chem. Soc. **127**, 10999–11009 (2005)
12. Lee, B.-C., Chu, T.K., Dill, K.A., Zuckermann, R.N.: Biomimetic nanostructures: creating a high-affinity zinc-binding site in a folded nonbiological polymer. J. Am. Chem. Soc. **130**, 8847–8855 (2008)
13. Lee, J., Udugamasooriya, D.G., Lim, H.-S., Kodadek, T.: Potent and selective photo-inactivation of proteins with peptoid–ruthenium conjugates. Nat. Chem. Biol. **6**, 258–260 (2010)
14. Fowler, S.A., Blackwell, H.E.: Structure–function relationships in peptoids: recent advances toward deciphering the structural requirements for biological function. Org. Biomol. Chem. **7**, 1508–1524 (2009)
15. Miller, S.M., Simon, R.J., Ng, S., Zuckermann, R.N., Kerr, J.M., Moos, W.H.: Comparison of the proteolytic susceptibilities of homologous L-amino acid, D-amino acid, and N-substituted glycine peptide and peptoid oligomers. Drug Dev. Res. **35**, 20–32 (1995)
16. Miller, S.M., Simon, R.J., Ng, S., Zuckermann, R.N., Kerr, J.M., Moos, W.H.: Proteolytic studies of homologous peptide and N-substituted glycine peptoid oligomers. Bioorg. Med. Chem. Lett. **4**, 2657–2662 (1994)
17. Nguyen, J.T., Turck, C.W., Cohen, F.E., Zuckermann, R.N., Lim, W.A.: Exploiting the basis of proline recognition by SH3 and WW domains: design of N-substituted inhibitors. Science **282**, 2088–2092 (1998)
18. Kruijtzer, J.A.W., Nijenhuis, W.A.J., Wanders, N., Gispen, W.H., Liskamp, R.M.J., Adan, R.A.H.: Peptoid–peptide hybrids as potent novel melanocortin receptor ligands. J. Med. Chem. **48**, 4224–4230 (2005)
19. Liu, B., Alluri, P.G., Yu, P., Kodadek, T.: A potent transactivation domain mimic with activity in living cells. J. Am. Chem. Soc. **127**, 8254–8255 (2005)
20. Ruijtenbeek, R., Kruijtzer, J.A.W., Van de Wiel, W., Fischer, M.J.E., Fluck, M., Redegeld, F.A.M., Liskamp, R.M.J., Nijkamp, F.P.: Peptoid–peptide hybrids that bind syk SH2 domains involved in signal transduction. Chem. Biochem. **2**, 171–179 (2001)
21. Chongsiriwatana, N.P., Patch, J.A., Czyzewski, A.M., Dohm, M.T., Ivankin, A., Gidalevitz, D., Zuckermann, R.N., Barron, A.E.: Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. Proc. Natl. Acad. Sci. U. S. A. **105**, 2794–2799 (2008)
22. Patch, J.A., Barron, A.E.: Helical peptoid mimics of magainin-2 amide. J. Am. Chem. Soc. **125**, 12092–12093 (2003)
23. Seurynck-Servoss, S.L., Dohm, M.T., Barron, A.E.: Effects of including an N-terminal insertion region and arginine-mimetic side chains in helical peptoid analogues of lung surfactant Protein B. Biochemistry **45**, 11809–11818 (2006)
24. Brown, N.J., Wu, C.W., Seurynck-Servoss, S.L., Barron, A.E.: Effects of hydrophobic helix length and side chain chemistry on biomimicry in peptoid analogues of SP-C. Biochemistry **47**, 1808–1818 (2008)
25. Izzo, I., De Cola, C., De Riccardis, F.: Properties and bioactivities of peptoids tagged with heterocycles. Heterocycles **82**, 981–1006 (2011)
26. Deane, S., Yao, N., Lam, K.S.: On arrows and traps: the use of the one-bead one-compound combinatorial library method to identify cell surface targeting ligands. In: Firer, M.A. (ed) Targeted Drug Delivery in Cancer Therapeutics, pp. 85-125. Research Signpost, Kerala, India (2010)
27. Lam, K.S., Lebl, M., Krchnak, V.: The "one-bead-one-compound" combinatorial library method. Chem. Rev. **97**, 411–448 (1997)
28. Zuckermann, R.N., Kerr, J.M., Kent, S.B.H., Moos, W.H.: Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. J. Am. Chem. Soc. **114**, 10646–10647 (1992)
29. Alluri, P.G., Reddy, M.M., Bachhawat-Sikder, K., Olivos, H.J., Kodadek, T.: Isolation of protein ligands from large peptoid libraries. J. Am. Chem. Soc. **125**, 13995–14004 (2003)
30. Udugamasooriya, D.G., Dineen, S.P., Brekken, R.A., Kodadek, T.: A peptoid antibody surrogate that antagonizes VEGF receptor 2 activity. J. Am. Chem. Soc. **130**, 5744–5752 (2008)
31. Liu, R., Lam, K.S.: Automatic Edman microsequencing of peptides containing multiple unnatural amino acids. Anal. Biochem. **295**, 9–16 (2001)
32. Aebersold, R., Mann, M.: Mass spectrometry-based proteomics. Nature **422**, 198–207 (2003)
33. Paizs, B., Suhai, S.: Fragmentation pathways of protonated peptides. Mass Spectrom. Rev. **24**, 508–548 (2005)
34. Dodds, E.D.: Gas-phase dissociation of glycosylated peptide ions. Mass Spectrom. Rev. **31**, 666–682 (2012)
35. Konermann, L., Stocks, B.B., Pan, Y., Tong, X.: Mass spectrometry combined with oxidative labeling for exploring protein structure and folding. Mass Spectrom. Rev. **29**, 651–667 (2010)
36. Thakkar, A., Cohen, A.S., Connolly, M.D., Zuckermann, R.N., Pei, D.: High-throughput sequencing of peptoids and peptide–peptoid hybrids by partial Edman degradation and mass spectrometry. J. Comb. Chem. **11**, 294–302 (2009)
37. Paulick, M.G., Hart, K.M., Brinner, K.M., Tjandra, M., Charych, D.H., Zuckermann, R.N.: Cleavable hydrophilic linker for one-bead-one-compound sequencing of oligomer libraries by tandem mass spectrometry. J. Comb. Chem. **8**, 417–426 (2006)
38. Gibson, C., Sulyok, G.A.G., Hahn, D., Goodman, S.L., Holzemann, G., Kessler, H.: Nonpeptidic $\alpha v \beta_3$ integrin antagonist libraries: on-bead screening and mass spectrometric identification without tagging. Angew. Chem. Int. Ed. **40**, 165–169 (2001)
39. Ruijtenbeek, R., Versluis, C., Heck, A.J.R., Redegeld, F.A.M., Nijkamp, F.P., Liskamp, R.M.J.: Characterization of a phosphorylated peptide and peptoid and peptoid–peptide hybrids by mass spectrometry. J. Mass Spectrom. **37**, 47–55 (2002)
40. Kruijtzer, J.A.W., Hofmeyer, L.J.F., Heerma, W., Versluis, C., Liskamp, R.M.J.: Solid-phase syntheses of peptoids using Fmoc-protected N-substituted glycines: the synthesis of (retro)peptoids of leu-enkephalin and Substance P. Chem. Eur. J. **4**, 1570–1580 (1998)
41. Heerma, W., Boon, J.-P.J.L., Versluis, C., Kruijtzer, J.A.W., Hofmeyer, L.J.F., Liskamp, R.M.J.: Comparing mass spectrometric characteristics of peptides and peptoids. 2. J. Mass Spectrom. **32**, 697–704 (1997)
42. Heerma, W., Versluis, C., de Koster, C.G., Kruijtzer, J.A.W., Zigrovic, I., Liskamp, R.M.J.: Comparing mass spectrometric characteristics of peptides and peptoids. Rapid Commun. Mass Spectrom. **10**, 459–464 (1996)
43. Sarma, B.K., Kodadek, T.: Submonomer synthesis of a hybrid peptoid-azapeptoid library. ACS Comb. Sci. **14**, 558–564 (2012)
44. Morishetti, K.K., Russell, S.C., Zhao, X., Robinson, D.B., Ren, J.: Tandem mass spectrometry studies of protonated and alkali metalated peptoids: enhanced sequence coverage by metal cation addition. Int. J. Mass Spectrom. **308**, 98–108 (2011)
45. Pitteri, S.J., McLuckey, S.A.: Recent developments in the ion/ion chemistry of high-mass multiply charged ions. Mass Spectrom. Rev. **24**, 931–958 (2005)
46. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron capture dissociation of multiply charged protein cations. A nonergodic process. J. Am. Chem. Soc. **120**, 3265–3266 (1998)
47. Syka, J.E.P., Coon, J.J., Schroeder, M.J., Shabanowitz, J., Hunt, D.F.: Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc. Natl. Acad. Sci. U. S. A. **101**, 9528–9533 (2004)
48. Li, W., Song, C., Bailey, D.J., Tseng, G.C., Coon, J.J., Wysocki, V.H.: Statistical analysis of electron transfer dissociation pairwise fragmentation patterns. Anal. Chem. **83**, 9540–9545 (2011)
49. Zhurov, K.O., Fornelli, L., Wodrich, M.D., Laskay, U.A., Tsybin, Y.O.: Principles of electron capture and transfer dissociation mass spectrometry

applied to peptide and protein structure analysis. Chem. Soc. Rev. **42**, 5014–5030 (2013)
50. Wiesner, J., Premsler, T., Sickmann, A.: Application of electron transfer dissociation (ETD) for the analysis of posttranslational modifications. Proteomics **8**, 4466–4483 (2008)
51. Chi, A., Huttenhower, C., Geer, L.Y., Coon, J.J., Syka, J.E.P., Bai, D.L., Shabanowitz, J., Burke, D.J., Troyanskaya, O.G., Hunt, D.F.: Analysis of phosphorylation sites on proteins from *Saccharomyces cerevisiae* by electron transfer dissociation (ETD) mass spectrometry. Proc. Natl. Acad. Sci. U. S. A. **104**, 2193–2198 (2007)
52. Li, X., Lin, C., Han, L., Costello, C.E., O'Connor, P.B.: Charge remote fragmentation in electron capture and electron transfer dissociation. J. Am. Soc. Mass Spectrom. **21**, 646–656 (2010)
53. Coon, J.J., Shabanowitz, J., Hunt, D.F., Syka, J.E.P.: Electron transfer dissociation of peptide anions. J. Am. Soc. Mass Spectrom. **16**, 880–882 (2005)
54. Cooper, H.J., Hakansson, K., Marshall, A.G.: The role of electron capture dissociation in biomolecular analysis. Mass Spectrom. Rev. **24**, 201–222 (2005)
55. Yoo, H.J., Wang, N., Zhuang, S., Song, H., Hakansson, K.: Negative-ion electron capture dissociation: radical-driven fragmentation of charge-increased gaseous peptide anions. J. Am. Chem. Soc. **133**, 16790–16793 (2011)
56. Turecek, F., Julian, R.R.: Peptide radicals and cation radicals in the gas phase. Chem. Rev. **113**, 6691–6733 (2013)
57. Zubarev, R.A.: Reactions of polypeptide ions with electrons in the gas phase. Mass Spectrom. Rev. **22**, 57–77 (2003)
58. Turecek, F., Chen, X., Hao, C.: Where does the electron go? Electron distribution and reactivity of peptide cation radicals formed by electron transfer in the gas phase. J. Am. Chem. Soc. **130**, 8818–8833 (2008)
59. Sawicka, A., Skurski, P., Hudgins, R.R., Simons, J.: Model calculations relevant to disulfide bond cleavage via electron capture influenced by positively charged groups. J. Phys. Chem. B **107**, 13505–13511 (2003)
60. Syrstad, E.A., Turecek, F.: Toward a general mechanism of electron capture dissociation. J. Am. Soc. Mass Spectrom. **16**, 208–224 (2005)
61. Simons, J.: Mechanisms for N–C_α bond cleavage in peptide ECD and ETD mass spectrometry. Chem. Phys. Lett. **484**, 81–95 (2010)
62. Chamot-Rooke, J., Malosse, C., Frison, G., Turecek, F.: Electron capture in charge-tagged peptides. Evidence for the role of excited electronic states. J. Am. Soc. Mass Spectrom. **18**, 2146–2161 (2007)
63. Neff, D., Sobczyk, M., Simons, J.: Through-space and through-bond electron transfer within positively charged peptides in the gas phase. Int. J. Mass Spectrom. **276**, 91–101 (2008)
64. Roepstorff, P., Fohlman, J.: Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed. Mass Spectrom. **11**, 601 (1984)
65. Figliozzi, G.M., Goldsmith, R., Ng, S.C., Banville, S.C., Zuckermann, R.N.: Synthesis of N-substituted glycine peptoid libraries. Methods Enzymol. **267**, 437–447 (1996)
66. Han, H., Xia, Y., McLuckey, S.A.: Ion trap collisional activation of c and $z^{\bullet}$ ions formed via gas-phase ion/ion electron-transfer dissociation. J. Proteome Res. **6**, 3062–3069 (2007)
67. Chung, T.W., Hui, R., Ledvina, A., Coon, J.J., Turecek, F.: Cascade dissociations of peptide cation-radicals. Part 1. Scope and effects of amino acid residues in penta-, nona-, and decapeptides. J. Am. Soc. Mass Spectrom. **23**, 1336–1350 (2012)
68. Ledvina, A.R., Chung, T.W., Hui, R., Coon, J.J., Turecek, F.: Cascade dissociations of peptide cation-radicals. Part 2. Infrared multiphoton dissociation and mechanistic studies of z-ions from pentapeptides. J. Am. Soc. Mass Spectrom. **23**, 1351–1363 (2012)
69. Cooper, H.J.: Investigation of the presence of b ions in electron capture dissociation mass spectra. J. Am. Soc. Mass Spectrom. **16**, 1932–1940 (2005)
70. Haselmann, K.F., Schmidt, M.: Do b-ions occur from vibrational excitation upon H-desorption in electron capture dissociation? Rapid Commun. Mass Spectrom. **21**, 1003–1008 (2007)
71. Turecek, F., Syrstad, E.A., Seymour, J.L., Chen, X., Yao, C.: Peptide cation-radicals. A computational study of the competition between peptide N–C_α bond cleavage and loss of the side chain in the [GlyPhe-NH2 + 2H]+ cation radical. J. Mass Spectrom. **38**, 1093–1104 (2003)
72. Blanksby, S.J., Ellison, G.B.: Bond Dissociation energies of organic molecules. Acc. Chem. Res. **36**, 255–263 (2003)