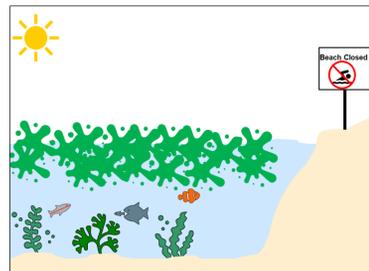


# Enhanced Structural Elucidation of Microcystins by Electron Activated Dissociation (EAD)

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## Introduction

- The structural diversity of microcystins (MCs) stems from variable amino acid substitutions within the cyclic peptide structures of MC and nodularin (NOD).
- Conventional immunoassay-based methods lack the specificity to unambiguously identify individual congeners
- CID-based MS/MS produces non-selective fragments at a common side chain
- Unique and alternative dissociation pathways are critical for identifying and differentiating known and new MCs
- EAD fragmentation in the ZenoTOF 7600 system enhances structural elucidation through production of unique fragments



### CE and KE ramping for MC-YR

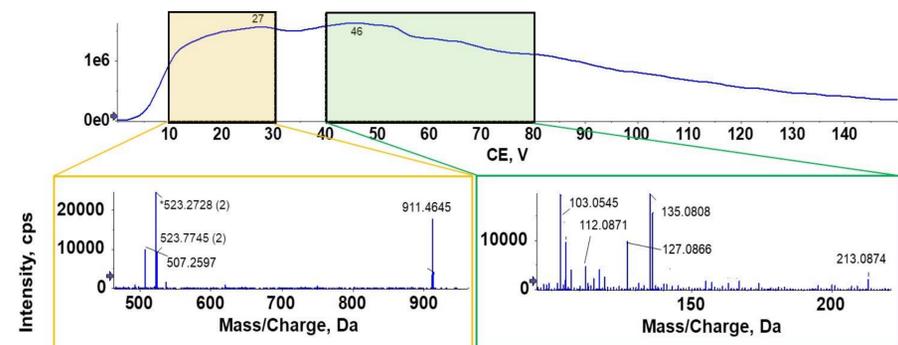


Figure 1. Collision energy ramping from 0 V to 150 V using CID fragmentation mode for the MC-YR congener.

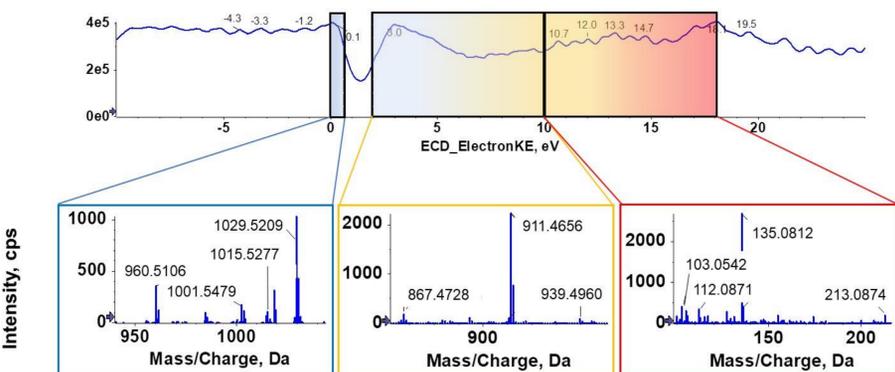


Figure 2. Kinetic energy ramping from -10 to 25 eV using EAD fragmentation mode for the MC-YR congener.

## Tunable kinetic energy (KE) in EAD reveals 80% more unique and alternative dissociation pathways not observed with CID

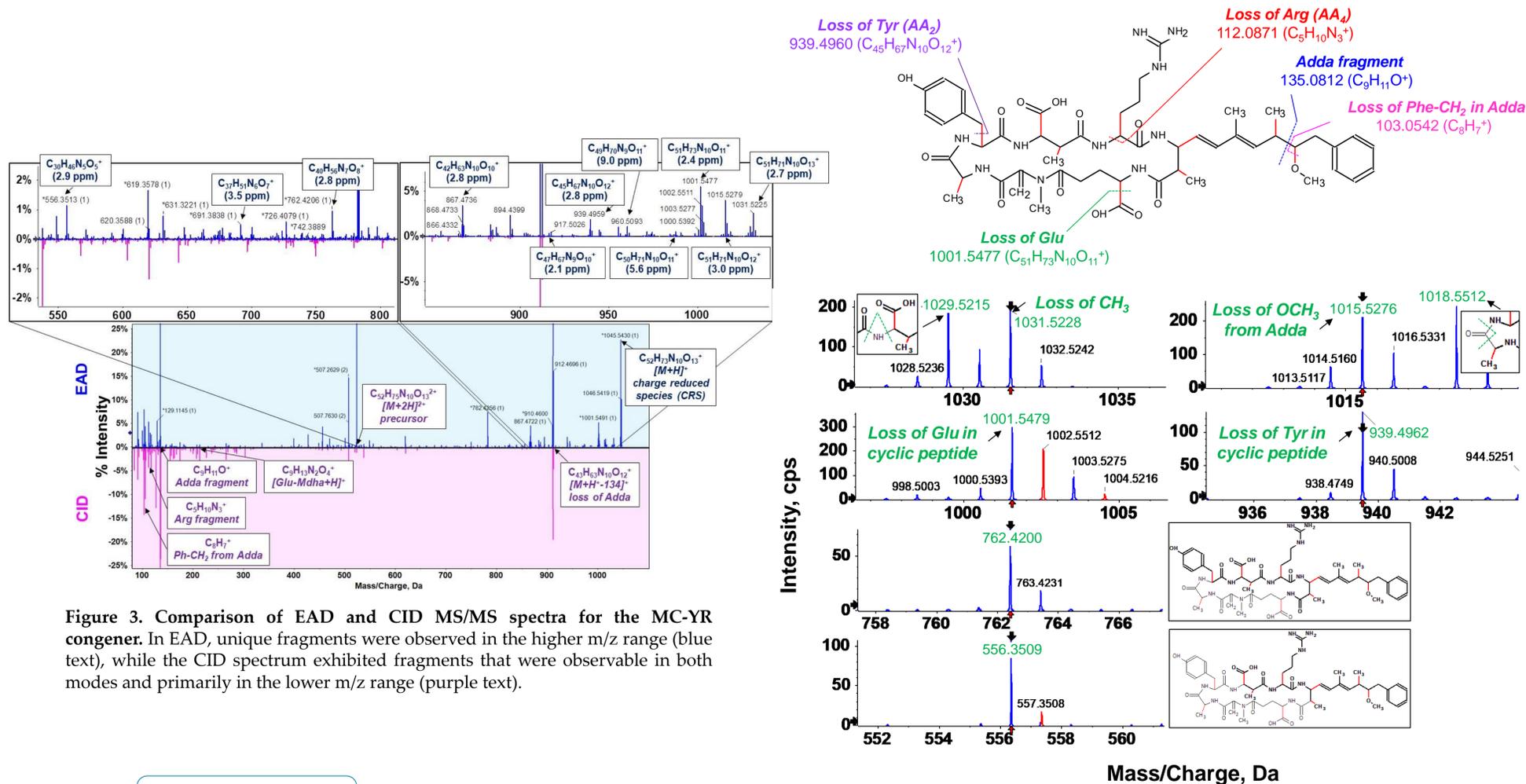
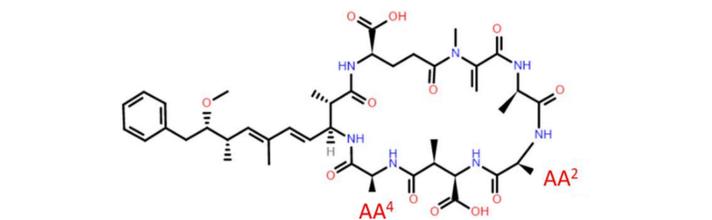


Figure 3. Comparison of EAD and CID MS/MS spectra for the MC-YR congener. In EAD, unique fragments were observed in the higher m/z range (blue text), while the CID spectrum exhibited fragments that were observable in both modes and primarily in the lower m/z range (purple text).



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MC-LR AA <sup>2</sup> = Leu, AA <sup>4</sup> = Arg		MC-RR AA <sup>2</sup> = Arg, AA <sup>4</sup> = Arg		MC-YR AA <sup>2</sup> = Tyr, AA <sup>4</sup> = Arg	
Predicted formula	EAD CID	Predicted formula	EAD CID	Predicted formula	EAD CID
<b>C<sub>49</sub>H<sub>75</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>49</sub>H<sub>76</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓	<b>C<sub>52</sub>H<sub>73</sub>N<sub>10</sub>O<sub>13</sub><sup>+</sup></b>	✓
<b>C<sub>48</sub>H<sub>75</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>48</sub>H<sub>74</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓	<b>C<sub>51</sub>H<sub>71</sub>N<sub>10</sub>O<sub>13</sub><sup>+</sup></b>	✓
<b>C<sub>48</sub>H<sub>74</sub>N<sub>10</sub>O<sub>10</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>48</sub>H<sub>74</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓	<b>C<sub>52</sub>H<sub>74</sub>N<sub>9</sub>O<sub>13</sub><sup>+</sup></b>	✓
<b>C<sub>47</sub>H<sub>73</sub>N<sub>10</sub>O<sub>8</sub><sup>+</sup></b>	✓	<b>C<sub>48</sub>H<sub>77</sub>N<sub>13</sub>O<sub>11</sub><sup>+</sup></b>	✓	<b>C<sub>51</sub>H<sub>74</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓
<b>C<sub>47</sub>H<sub>71</sub>N<sub>10</sub>O<sub>9</sub><sup>+</sup></b>	✓	<b>C<sub>48</sub>H<sub>74</sub>N<sub>13</sub>O<sub>11</sub><sup>+</sup></b>	✓	<b>C<sub>51</sub>H<sub>71</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓
<b>C<sub>40</sub>H<sub>63</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>48</sub>H<sub>76</sub>N<sub>13</sub>O<sub>10</sub><sup>+</sup></b>	✓	<b>C<sub>51</sub>H<sub>73</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓
<b>C<sub>39</sub>H<sub>63</sub>N<sub>10</sub>O<sub>9</sub><sup>+</sup></b>	✓	<b>C<sub>48</sub>H<sub>74</sub>N<sub>13</sub>O<sub>9</sub><sup>+</sup></b>	✓	<b>C<sub>50</sub>H<sub>71</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓
		<b>C<sub>47</sub>H<sub>74</sub>N<sub>13</sub>O<sub>9</sub><sup>+</sup></b>	✓	<b>C<sub>49</sub>H<sub>70</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓
		<b>C<sub>47</sub>H<sub>72</sub>N<sub>10</sub>O<sub>10</sub><sup>+</sup></b>	✓	<b>C<sub>49</sub>H<sub>70</sub>N<sub>9</sub>O<sub>11</sub><sup>+</sup></b>	✓
		<b>C<sub>47</sub>H<sub>70</sub>N<sub>10</sub>O<sup>+</sup></b>	✓	<b>C<sub>48</sub>H<sub>68</sub>N<sub>10</sub>O<sub>11</sub><sup>+</sup></b>	✓
		<b>C<sub>46</sub>H<sub>70</sub>N<sub>13</sub>O<sub>11</sub><sup>+</sup></b>	✓	<b>C<sub>48</sub>H<sub>67</sub>N<sub>10</sub>O<sub>13</sub><sup>+</sup></b>	✓
		<b>C<sub>45</sub>H<sub>71</sub>N<sub>13</sub>O<sub>10</sub><sup>+</sup></b>	✓	<b>C<sub>45</sub>H<sub>67</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓
		<b>C<sub>40</sub>H<sub>66</sub>N<sub>13</sub>O<sub>11</sub><sup>+</sup></b>	✓	<b>C<sub>43</sub>H<sub>63</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓
		<b>C<sub>40</sub>H<sub>65</sub>N<sub>10</sub>O<sub>12</sub><sup>+</sup></b>	✓	<b>C<sub>42</sub>H<sub>63</sub>N<sub>10</sub>O<sub>10</sub><sup>+</sup></b>	✓
		<b>C<sub>40</sub>H<sub>57</sub>N<sub>9</sub>O<sub>10</sub><sup>+</sup></b>	✓	<b>C<sub>40</sub>H<sub>56</sub>N<sub>9</sub>O<sub>8</sub><sup>+</sup></b>	✓
		<b>C<sub>39</sub>H<sub>64</sub>N<sub>10</sub>O<sub>9</sub><sup>+</sup></b>	✓	<b>C<sub>30</sub>H<sub>46</sub>N<sub>5</sub>O<sub>5</sub><sup>+</sup></b>	✓
		<b>C<sub>39</sub>H<sub>59</sub>N<sub>9</sub>O<sub>9</sub><sup>+</sup></b>	✓		
		<b>C<sub>39</sub>H<sub>59</sub>N<sub>9</sub>O<sub>9</sub><sup>+</sup></b>	✓		
<b>C<sub>9</sub>H<sub>11</sub>O<sup>+</sup></b>	✓ ✓	<b>C<sub>9</sub>H<sub>11</sub>O<sup>+</sup></b>	✓ ✓	<b>C<sub>9</sub>H<sub>11</sub>O<sup>+</sup></b>	✓ ✓
<b>C<sub>5</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>5</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>5</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓
<b>C<sub>8</sub>H<sub>7</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>8</sub>H<sub>7</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>8</sub>H<sub>7</sub><sup>+</sup></b>	✓ ✓
<b>C<sub>4</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>4</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓	<b>C<sub>4</sub>H<sub>10</sub>N<sub>3</sub><sup>+</sup></b>	✓ ✓

Table 1. Comparison of diagnostic fragments produced by EAD and CID for structural elucidation of different MC congeners. Molecular formulas in bold represent the parent ions.

## Summary

- EAD fragmentation on the ZenoTOF 7600 provides complementary MS/MS data for the structural elucidation of MCs
- KE ramping in EAD induces different dissociation pathways to produce unique fragmentation profiles in the ECD, hot ECD and EIEIO regions
- EAD yields unique product ions that reveal additional fragmentation pathways, such as side-chain modifications and ring-opening bond breakages within the cyclic peptide structures

