Synthesis and Biological Evaluation of QRSTUVWXYZA′ Domains of Maitotoxin

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§1. Introduction

1–1. Maitotoxin (MTX)

1976: First isolation from *Ctenochaetus striatus* (a kind of fish) by T. Yasumoto

1988: Examination of chemical properties by A. Yokoyama, M. Murata,Y. Oshima, T. Iwashita and T. Yasumoto

1996: Determination of complete structure by T. Nonomura, M.Sasaki, N. Matsumori, M. Murata, K. Tachibana, Y. Kishi and T. Yasumoto

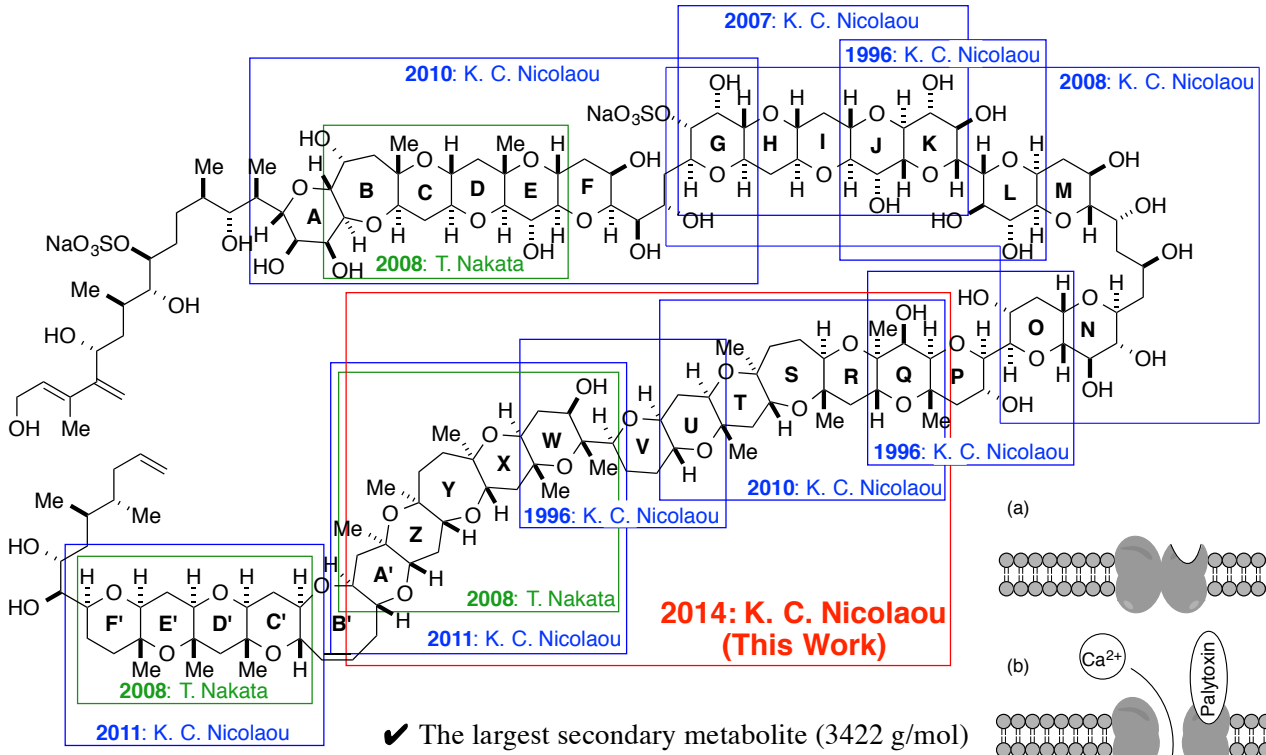


Figure 1. Structure of **MTX**

- ✔ The largest secondary metabolite (3422 g/mol)
- ✔ The most potent neurotoxins (50 ng/kg)
- ✔ 32 rings and 98 asymmetric carbons

Figure 2. (a) Free Na^+–K^+–ATPase **NKA** (b) Complex with Palytoxin

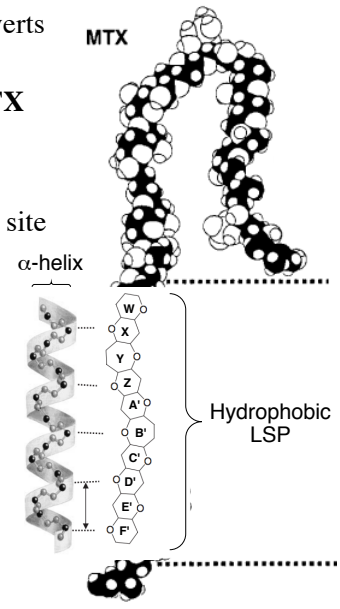
1–2. Hypothetical Bioactivities (**Exact mechanism is still under investigation*)

♦ Sinkins' Hypothesis[1]…**MTX** converts the Ca^{2+} pump into the Ca^{2+}–permeable nonselective cation channel.

- The structurally related marine toxin, **Palytoxin**, binds to the Na^+–K^+–ATPase and converts the Na^+ pump into a nonselective cation channel (***Figure 2***).
- Ca^{2+}–ATPase overexpressed insect cells and human kidneys cells were subjected to **MTX** and showed increase in **MTX**–induced whole cell membrane current.

♦ Murata's Hypothesis[2]…W–F' domain works as an anchor that binds to the α–helix active site

- **Yessotoxin**'s Ladder–Shaped Polyether structure, which is similar to W–F' domain of **MTX**, binds to the α–helix peptide active site of Ca^{2+}–ATPase.
- The average distance of ether oxygen atoms on one side matches the α-helix pitch of the active site (***Figure 4***).



? **MTX** binds to active site of Ca^{2+}–ATPase with W–F' domain and converts it to Ca^{2+}–permeable nonselective cation channel.

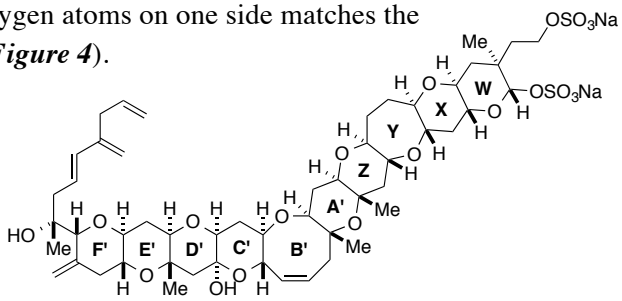


Figure 3. **YTX**, Names of rings correspond to similar rings of **MTX**.

Figure 4. Possible structure of **MTX** interacting with active site

1–3. Remaining Challenges and This Work

✗ Total synthesis has not yet been achieved => Fragments coupling entails huge importance to accomplish total synthesis

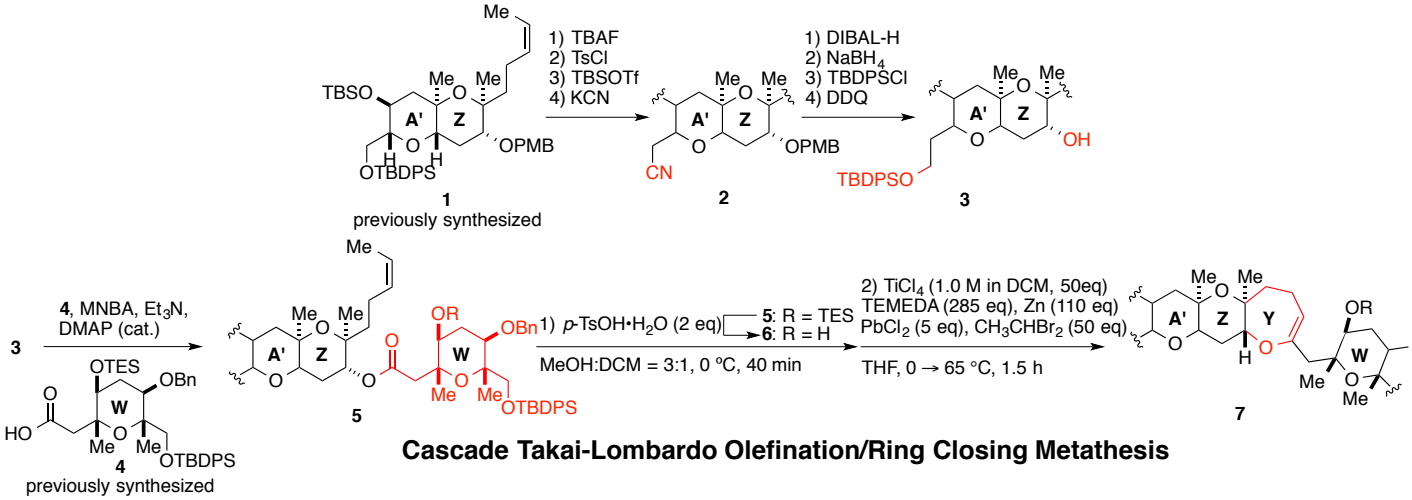
✗ Mechanism of bioactivity is unknown => Q–A' domain contains the fragment promisingly binds to the active site

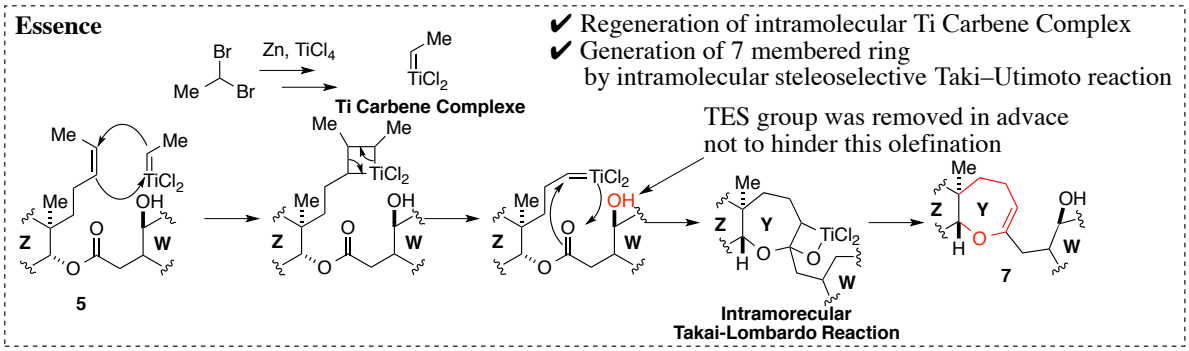
=> Understanding bioactivity provides insights into anticancer drug discovery

§2. Results and Discussion *(*Some parts are omitted accordingly for clarity and space limitation)*

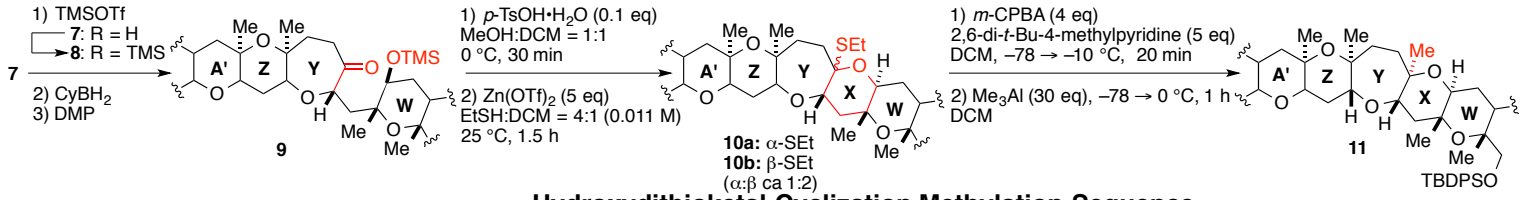
2–1. Synthesis of WXYZA' domain, Synthesis of WXYZA' Ketophosphonate 13

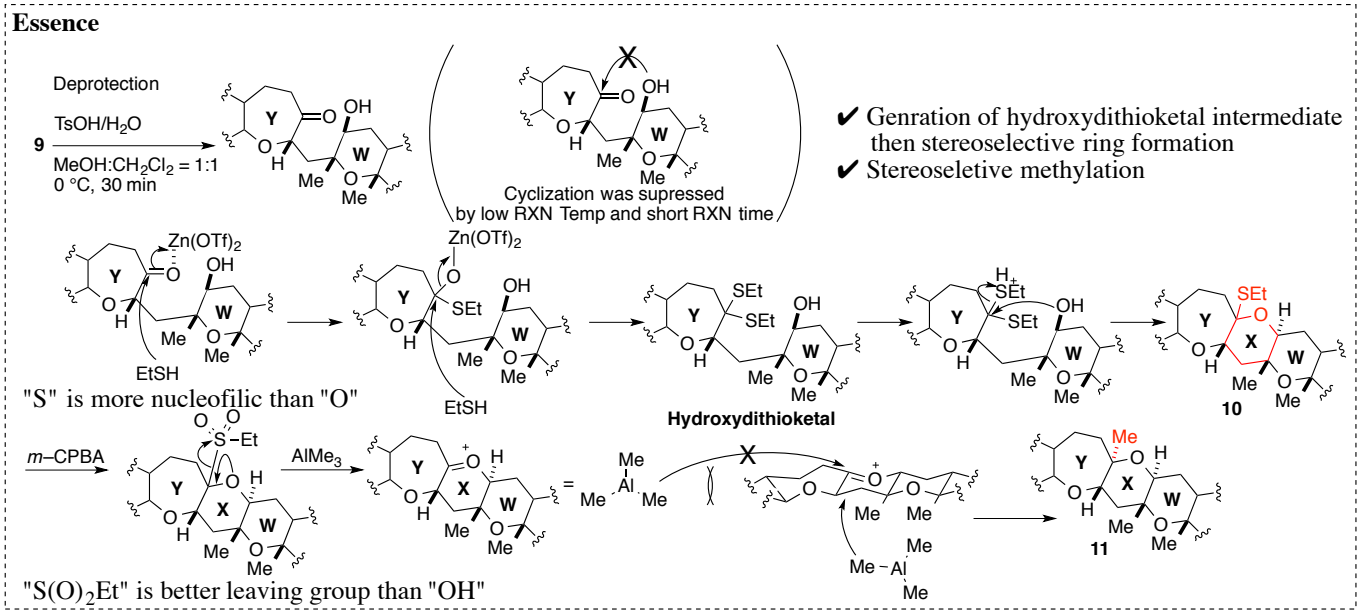
Scheme 1. Synthesis of **1**–**7**, Cascade Takai-Lombardo Olefination/Ring Closing Metathesis and its essence



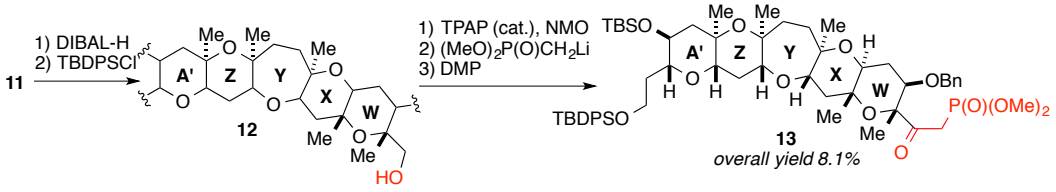


Scheme 2. Synthesis of **9**–**11**, hydroxydithioketal cyclization methylation sequence and its essence [3]



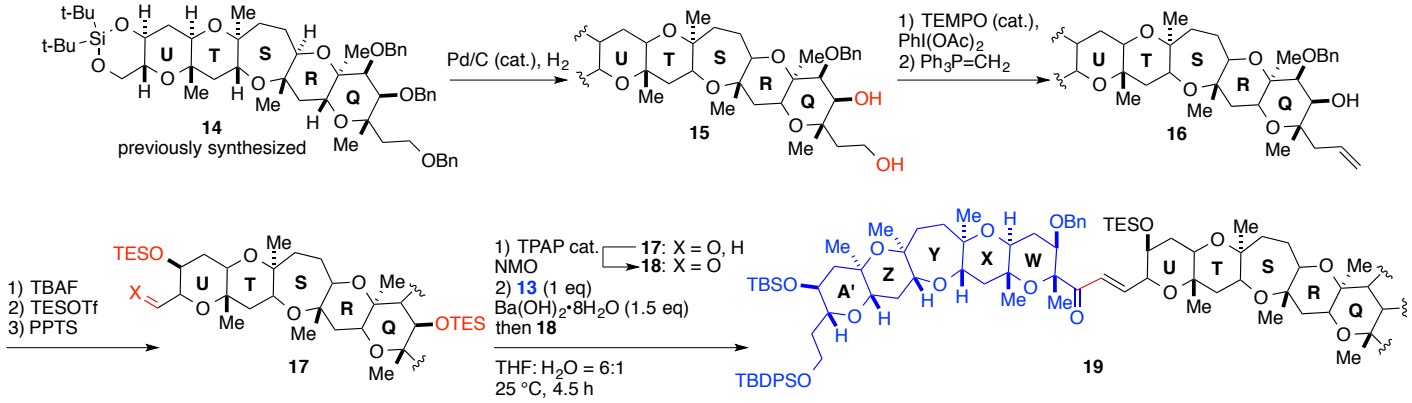


Scheme 3. Synthesis of **12–13**, completion of W–A' domain

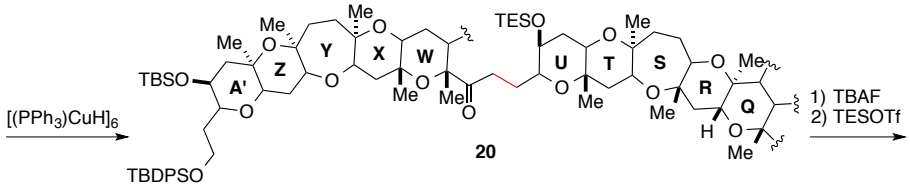


2–2. Synthesis of QRSTU Aldehyde 18, Fragment Coupling and Completion of QRSTUVWXYZA' Domain

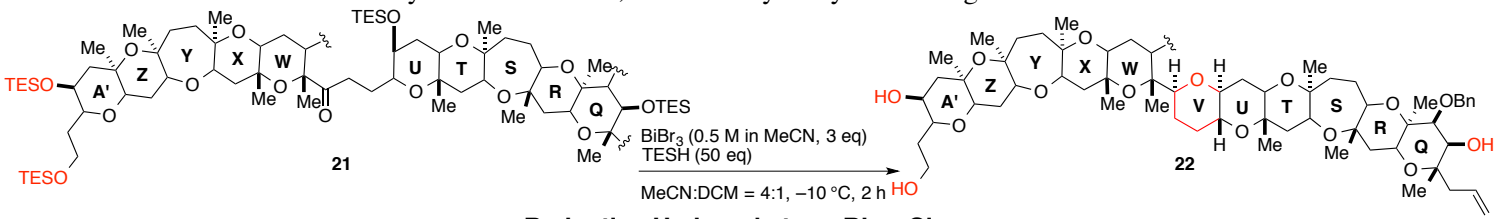
Scheme 4. Synthesis of **14–20,** coupling of Q–U domain and W–A' domain by Honor–Wadsworth Emmons Coupling



Horner–Wadsworth–Emmons Coupling



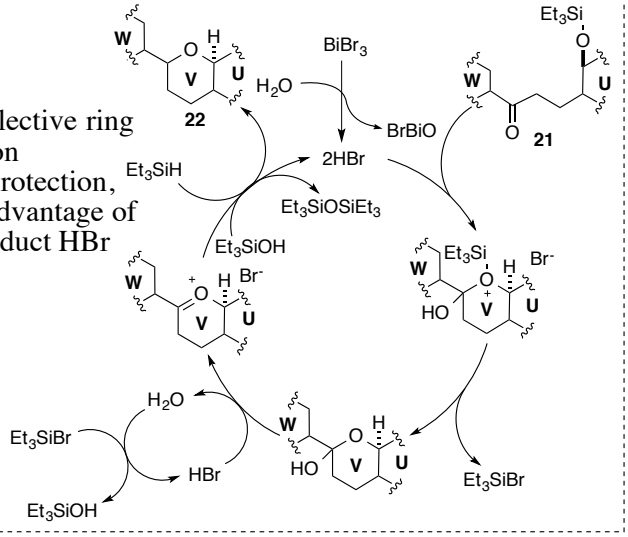
Scheme 5. Synthesis of **21–24**, reductive hydroxyketone ring closure and its essence [4]

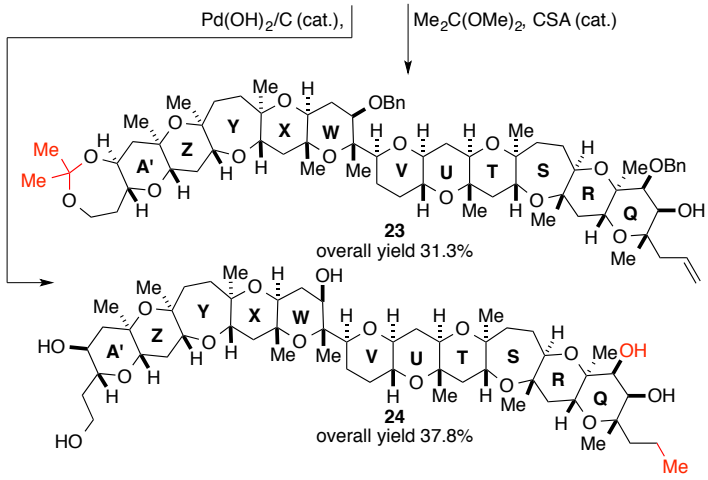


Reductive Hydroxyketone Ring Closure

Essence

- ✔ Stereoselective ring formation
- ✔ TES deprotection, taking advantage of a byproduct HBr





Confirmed by NOE and ^{13}C NMR

2–3. Biological Evaluation

• 19 different fragments, including previously synthesized A–E, A–G, Q–U, Q–A', W–A' and C'–F' domains and its analogs, were subjected to rat glioma C6 (a kind of cancer) cells (***Figure 5***) and human tumor cells.

=> Compound **24**, **25** (Q–A') and **26** (C'–F') (***Figure 6***) gave a positive reaction to the Ca^{2+} influx examination.

=> Compound **24** exhibited significant growth inhibition against 10 different humane tumor cells.

Others, A–E, A–G, Q–U domains and its analogs were completely inactive or slightly active.

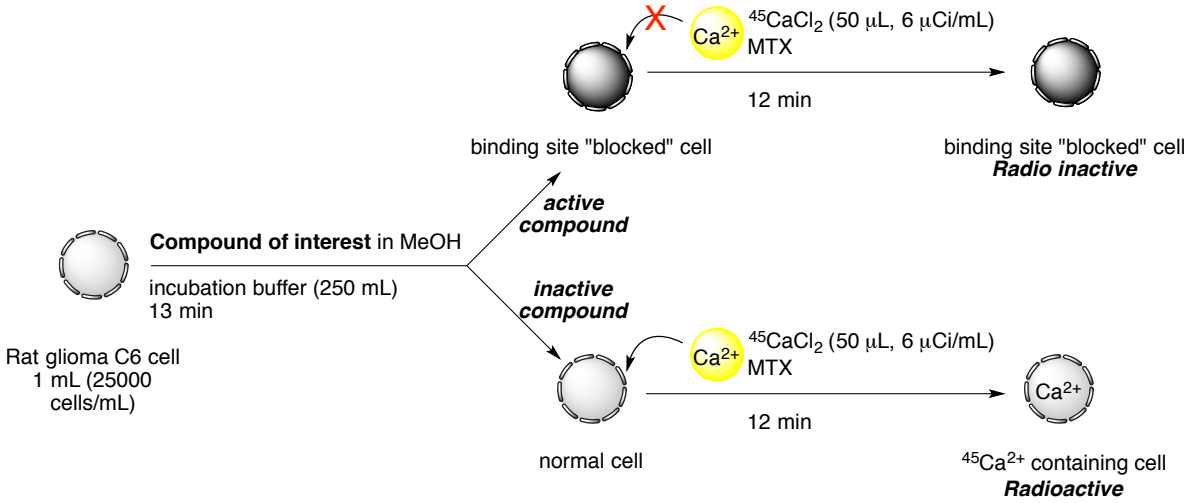


Figure 5. Process of the biological evaluation

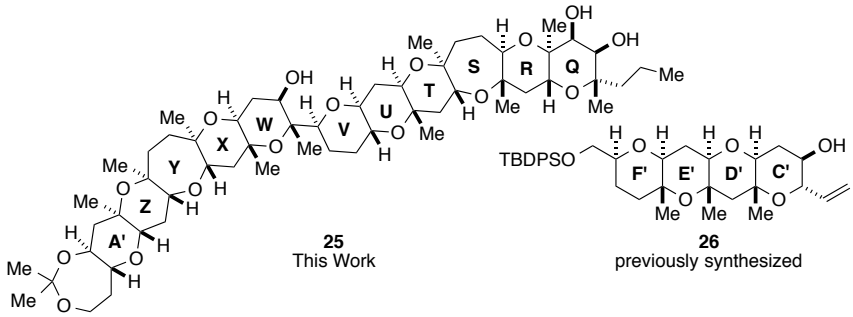


Figure 6. Partial structures of **MTX** that induced Ca^{2+} influx

✔ Some domains bound to the active site, while all domains did not induce Ca^{2+} influx.

=> Binding domains and Ca^{2+} influx inducing domains are not the same.

=> More than 2 domains play a role of converting Ca^{2+}–ATPase to Ca^{2+}–permeable nonselective cation channel.

✔ Q–A' domain and C'–F' domain effectively bound to the active sites (consistent with Murata's hypothesis).

✔ W–A' domain nor S–U domain were not active (inconsistent with Murata's Hypothesis).

=> Both S–U domain and W–A' domain are necessary to bind to active sites.

§3. Conclusion

✔ Succeeded in synthesizing QRSTUVWXYZA' domains, which was remarkable advance towards total synthesis

✔ Evaluated bioactivity of different 19 domains and got new insight into the mechanism of bioactivity of **MTX**

✔ Promising anticancer activity was observed

§4. References

[1] W. G. Sinkins *et al. Am. J. Physiol. Cell Physiol.* **2009**, *297*, C1533–C1543.

[2] M. Murata *et al. Bull. Chem. Soc. Jpn.* **2008**, *81*, 307–319.

[3] K. C. Nicolaou *et al. J. Am. Chem. Soc.* **1989**, *111*, 5321–5330.

[4] P. A. Evans *et al. J. Am. Chem. Soc.* **2003**, *125*, 11456–11457.

Abbreviations:

MNBA = 2,6-methylnitrobenzoyl anhydride; TPAP = tetra-*n*-propylammonium perruthenate; NMO = *N*-methylmorpholine-*N*-oxide; PPTS = pyridineium *p*-toluene sulfonate; CAS = (±)-camphor-10-sulfonic acid