

Liposomal Circular Dichroism. Assignment of Remote Stereocenters in Plakinic Acids K and L from a *Plakortis*–*Xestospongia* Sponge Association

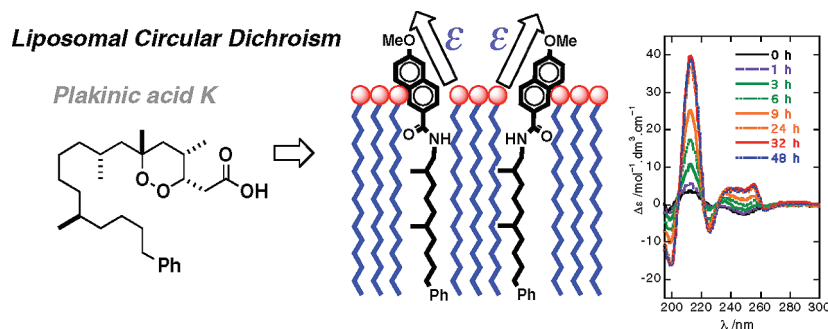
Doralyn S. Dalisay,^{†,§} Tim Quach,[†] and Tadeusz F. Molinski^{*,†,‡}

Department of Chemistry and Biochemistry and Skaggs School of Pharmacy and
Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive,
La Jolla, California 92093

tmolinski@ucsd.edu

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ABSTRACT



Two new ω -phenyl polyketide peroxides, plakinic acids K and L, were isolated from a two-sponge association of *Plakortis halichondroides* and *Xestospongia deweerdtae*. The absolute configurations of the remote dimethyl-branched stereocenters in plakinic acid K were assigned by degradation of plakinic acid K to a long-chain naphthamide and analysis by liposomal circular dichroism (L-CD) and comparison with synthetic standards.

The marine sponges of the genera *Plakortis* and *Plakinas-trella*¹ are prolific producers of cyclic polyketide peroxides that exhibit a broad spectrum of biological properties, including antifungal,¹ antimalarial,² antiprotozoal,³ and im-

munosuppressant⁴ activities. Sponge-derived cyclic peroxides may occur as 1,2-dioxane or 1,2-dioxolane ring systems.⁵ We recently reported the stereochemical assignments of two ω -phenyl polyketide peroxides, plakinic acids I (**1**) and J

[†] Department of Chemistry and Biochemistry.

[‡] Skaggs School of Pharmacy and Pharmaceutical Sciences.

[§] Present address: Department of Chemistry, University of British Columbia, Vancouver, BC, Canada V6T 1Z1.

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(2) (Figure 1), from the symbiotic two-sponge association *Plakortis halichondroides*–*Xestospongia deweerdtae* Lehnert & van Soest, 1999,⁶ which showed differential inhibition against haplodeficient *lag1ΔLAG1* strains of *Saccharomyces cerevisiae*.⁷ The configurations of the remote methyl-branched C8/C7 stereocenters of **1** and **2** were solved by the application of liposomal circular dichroism (L-CD),⁸ a very sensitive technique (limit of detection ~16 nmol) that amplifies CD Cotton effects (CEs) through ordering of the long-chain lipids in highly uniform, unilamellar liposomes.⁷ Here, we show a remarkable long-range perturbation of naphthamide chromophores by a *single methyl branch, over eight bonds* away, that discriminates between spectroscopically indistinguishable diastereomers. L-CD was used to assign configurations of both proximal C7/C8 and distal C12/C11 methyl-branched centers in two new ω -phenyl polyketide peroxides, plakinic acids **3** and **4**.

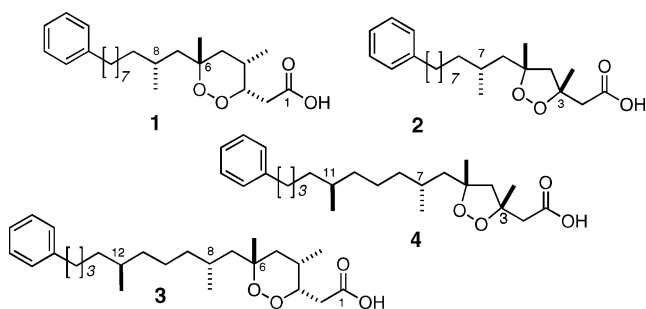


Figure 1. Structures of plakinic acids **1** (**1**), **2** (**2**), **3** (**3**), and **4** (**4**).

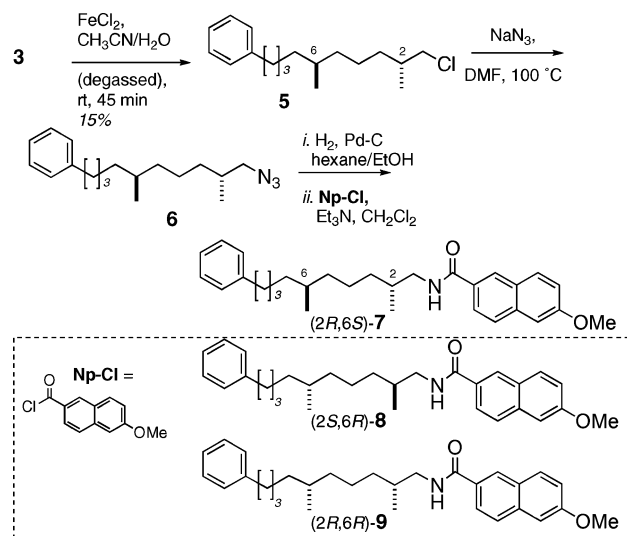
P. halichondroides–*X. deweerdtae*, collected from the Bahamas, was extracted with MeOH/CH₂Cl₂ (1:1) to yield a dark-brown residue which was sequentially extracted with *n*-hexane, chloroform, *n*-butanol, and water. Antifungal bioassay-guided fractionation revealed that the CHCl₃-soluble extract exhibited potent activity against *S. cerevisiae lag1ΔLAG1* strain. This fraction was subjected to flash chromatography (silica, 0–100% MeOH–CHCl₃, stepped gradient) and the active fraction further purified by HPLC (RP-C₁₈ CH₃CN/H₂O) to yield known compounds **1** and **2**,⁷ new methyl-branched plakinic acids **3** (**3**) and **4** (**4**), and plakinic acid **M**, a linear homologue of **1**.⁹

Plakinic acid **3** (**3**) was isolated as an optically active colorless oil (4.74 × 10^{−3} % yield, w/w, wet weight), [α]_D −137 (*c* 1.31, CHCl₃), of molecular formula C₂₆H₄₄O₄ as determined by HREIMS [M⁺] *m/z* 432.3239, with 14 mass units greater than **1**. The UV, IR, and ¹H and ¹³C NMR spectra of **3** were almost identical to those of **1**, except for

an additional methyl group (δ_H 0.82, d, *J* = 6.4 Hz; δ_C 19.9, CH₃). Inspection of the COSY and HMBC spectra established the position of the second methyl branch at C12 (see the Supporting Information). Using a similar analysis, plakinic acid **L** (**4**, 7.5 × 10^{−3} % yield w/w wet weight, [α]_D −26.2 (*c* 1.90, CHCl₃) C₂₅H₄₀O₄; HREIMS [M⁺] *m/z* 404.2918) was shown to be a homologue of **2**.

The common origin of **1**–**4**, similar specific rotations and ¹H NMR suggest that the respective stereocenters at C3, C4, and C6 have the same configurations as those of **1** and **2**.¹⁰ The configurations of the *proximal* C8/C7 stereocenters in **1** and **2** were assigned as *R* by interpretation of the intense CEs in L-CD of the derived 6-methoxy-2-naphthamide (in contrast, CD of the naphthamides in isotropic media, such as methanol solutions, showed only baseline).⁷ We had proposed⁷ that the strong, bisignate CEs displayed in L-CD spectra arise from pairwise *intramolecular* exciton coupling of naphthamides within the liposomal bilayers. Phospholipid bilayers, comprised of saturated long chains, promote hexagonal close packing of extended CH₂ groups.

Scheme 1. Degradation of **3** and Conversion to **7**



The dimensions of the problem expand significantly for assignment of the *remote* C12/C11 methyl-branched stereocenters in **3** and **4**. We surmised the remote methyl branch in **3** and **4** may alter the chain packing and influence the *magnitude* and *form* of the naphthamide CEs in L-CD. This would constitute net transmission of stereochemical information from the remote C12 center to the naphthamide group; however, it was not certain the effect would be large enough to be readily observable in the CD spectrum.

(6) While *n*- and isoalkyl cyclic peroxides are found in free-living *P. halichondroides*, we found only the *P. halichondroides*–*X. deweerdtae* association produces ω -phenyl-terminated polyketide peroxides.

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(8) (a) MacMillan, J. B.; Molinski, T. F. *J. Am. Chem. Soc.* **2004**, *126*, 9944–5. (b) MacMillan, J. B.; Linington, R. G.; Andersen, R. J.; Molinski, T. F. *Angew. Chem., Int. Ed.* **2004**, *43*, 5946–51.

(9) See S1, Supporting Information, for complete characterization.

(10) The absolute configurations at stereocenters around the 1,2-dioxane ring in **1** were solved conventionally by integrated analysis of ROESY spectra and the modified Mosher's ester method. The absolute configurations of C4 and C5 within the 1,2-dioxolane ring of **2** were assigned by comparison of the [α]_D with those of synthetic "plakinates" of defined configuration. Dai, P.; Trullinger, T. K.; Liu, X.; Dussault, P. H. *J. Org. Chem.* **2006**, *71*, 2283–92, and ref 7.

In order to test this hypothesis, **3**¹¹ was degraded (Scheme 1) by cleavage of the C6–C7 bond using Fe(II)-promoted reductive fragmentation (FeCl₂, aq CH₃CN–H₂O, N₂-sparged) to give primary alkyl chloride **5**,¹² which was subsequently transformed by a three-step sequence⁷ to give naphthamide **7**.

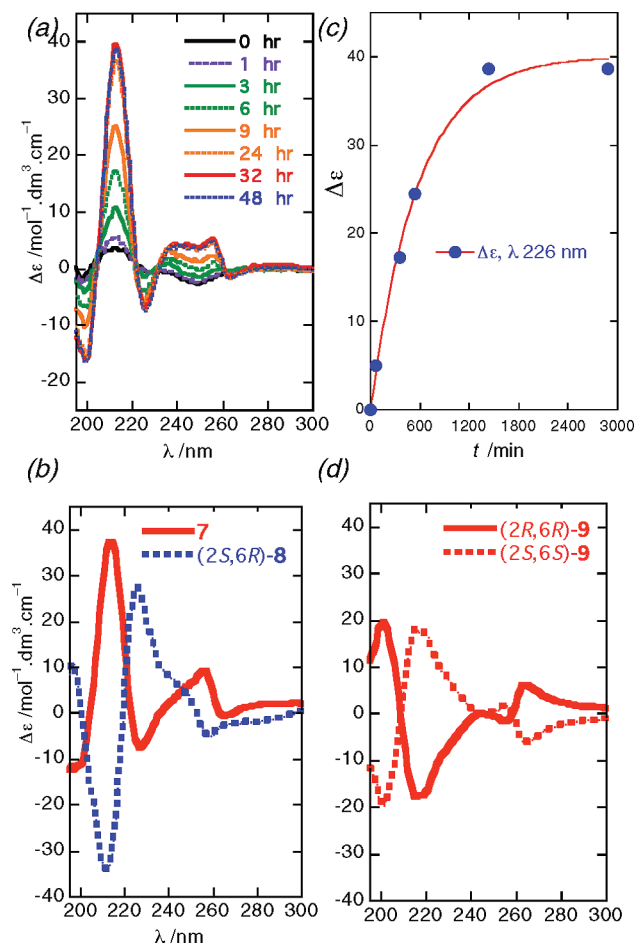


Figure 2. Liposomal circular dichroism (L-CD) spectra ($T = 296$ K): (a) **7** from $t = 0$ to 48 h after formulation of liposomes (H₂O, distearoyl-*sn*-glycero-3-phosphocholine, 2 mg/mL; molar ratio of phospholipid:**7** = 20:1); (b) **7**, $c = 2.24 \times 10^{-4}$ M and (2*S*,6*R*)-**8**, $c = 2.25 \times 10^{-4}$ M; (c) time dependence of the Cotton effect of **7** ($\lambda = 226$ nm), postformulation; (d) (2*R*,6*R*)-**9**, $c = 2.47 \times 10^{-4}$ M and *ent*-**9** (calcd).

Compound **7** was formulated into a highly uniform, unilamellar liposomes of distearoyl-*sn*-glycero-3-phosphocholine (DSPC) as previously described,¹³ (phospholipid:**7** ratio = 20:1) prior to measurement of the CD spectrum.

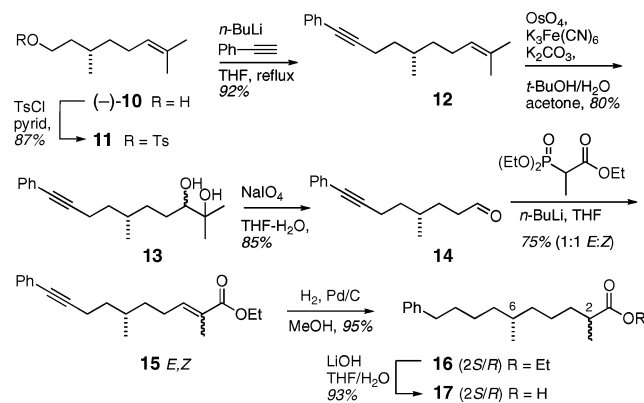
(11) Plakinic acid **L** (**4**) also gave **5** under the same conditions.

(12) Compound **5** arises from C6–C7 bond scission initiated by a “chloro-Fenton” reaction that captures Cl from the Fe ligand sphere by radical rebound. See ref 7 and: Sawyer, D. T.; Hage, J. P.; Sobkowiak, A. *J. Am. Chem. Soc.* **1995**, *117*, 106–9.

(13) The crude liposome suspension, prepared by shell evaporation of a solution of naphthamides and DSPC in CHCl₃ (2 mg/mL), followed by sonication in water and thermal annealing, was repeatedly extruded under pressure through a 100 nm pore polycarbonate membrane to give uniform diameter, unilamellar liposomes.⁸

Remarkably, no signal appeared in the CD spectrum of **7** (Figure 2a) immediately after formulation in liposomes, but over 24 h intense, complex CEs gradually appeared that were stable (>30 days) and reproducible [λ 196 nm ($\Delta\epsilon -11.6$), 213 (+ 37.4), 226 (−6.5), 255 (+ 5.6)]. Fitting the time-dependence of the CE at $\lambda = 226$ nm in the L-CD spectrum of **7** (Figure 2c) to an exponential function gave $t_{1/2} = 385$ min. Evidently, the kinetics of lipid chain reorganization of liposomal **7** at room temperature are considerably slower than the corresponding naphthamides derived from **1** and **2** in which less complex CEs appeared essentially immediately.¹⁴ The assignment of configuration in **7** was completed by comparison with synthetic model compounds (2*S*,6*R*)-**8** and (2*R*,6*R*)-**9**, which were prepared as follows.

Scheme 2. Synthesis of an Epimeric Mixture of Acids **17**



Tosylation of (*S*)-(-)-citronellol¹⁵ **10** gave **11**¹⁶ in 87% yield (Scheme 2), which upon treatment with lithium phenylacetylide (reflux, THF), afforded enyne **12** in 92% yield.

Two-step Johnson–Lemieux oxidation¹⁷ of **12** provided aldehyde **14** (Scheme 2), which was subjected to Horner–Wadsworth–Emmons olefination to give α,β -unsaturated ester **15** as an inseparable 1:1 mixture of *E/Z* isomers. Hydrogenation of enyne **15** (Pd/C) afforded saturated ester **16**, which was saponified (LiOH, THF/water) to give acid **17** as a 1:1 mixture of diastereomers, epimeric at C2. Acid **17** was coupled with (*S*)-phenethylamine (HATU, *i*-Pr₂NEt) (Scheme 3) to provide a mixture of diastereomeric amides (2*S*,6*R*)-**18** and (2*R*,6*R*)-**19**, which were separated by silica gel chromatography.¹⁸ Amides **18** and **19** were individually treated with BH₃·THF to afford secondary amines **20** and

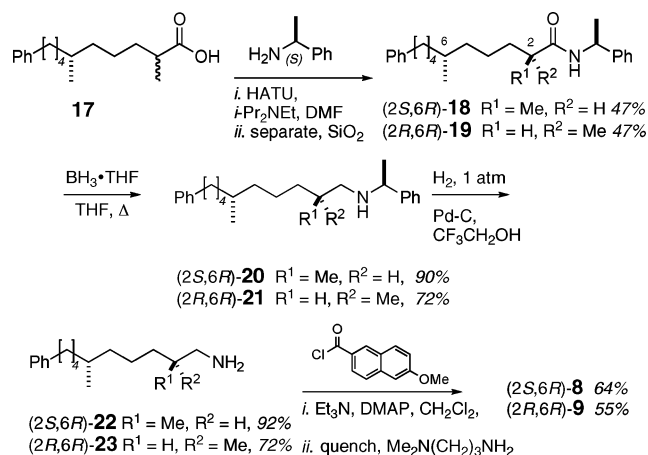
(14) The higher order complexity of the L-CD spectrum of **7** (which is the subject of our ongoing investigations) is indicative of delocalized intermolecular excitons, or *J*-arrays: Fidler, H.; Knoester, J.; Wiersma, D. A. *J. Chem. Phys.* **1993**, *98*, 6564–66.

(15) (-)-Citronellol (**10**) obtained from TCI-EP (lot GM01) was of low optical purity (72% ee, [α]_D²⁰ −3.42 (neat) [lit. [α]_D²⁰ −4.76 (neat)]: Rienäcker, R.; Ohloff, G. *Angew. Chem.* **1961**, *73*, 240). Consequently, pure (-)-**10** (98% ee, [α]_D²⁰ −5.18 (neat)) was obtained by reduction (NaBH₄, MeOH) of (-)-citronellal (Takasago, 98% ee).

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(17) Pappo, R.; Allen Jr, D. S.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478–9.

Scheme 3. Synthesis of Standards, Naphthamides **8** and **9**



21. Hydrogenolysis of **20** and **21** (Pd/C, $\text{CF}_3\text{CH}_2\text{OH}$) provided primary amines **22** and **23** in 92% and 72% yield, respectively. Separate acylation of **22** and **23** (6-methoxy-2-naphthoyl chloride, Et_3N , DMAP) gave naphthamides (2*S*,6*R*)-**8** and (2*R*,6*R*)-**9**.

Comparisons of the L-CD spectra of three stereoisomers are shown in Figure 2: **7** derived from **3** or **4** and synthetic compounds (2*S*,6*R*)-**8** and (2*R*,6*R*)-**9**. The signs and magnitudes of the CEs are primarily dominated by perturbation of the naphthamide chromophore by the proximal stereocenter C2 within the first sphere of asymmetry. It is also evident that longer range perturbation from the remote methyl branch alters the forms of L-CD spectra of (2*S*,6*R*)-**8** and (2*R*,6*R*)-**9** as a function of the C6 configuration (Figures 2b and d). Whereas the diastereomers (2*S*,6*R*)-**8** and (2*R*,6*R*)-**9** were indistinguishable by NMR,¹⁹ they were clearly differentiated by the CEs and fine structure of their L-CD spectra. In order to evaluate the effect of the distal C6 stereocenter, the L-CD of (2*S*,6*R*)-**8** was compared with the inverted L-CD of (2*R*,6*R*)-**9** (Figure 2d) which corresponds to (2*S*,6*S*)-**9**. The CEs in **8** [λ_{max} 196 ($\Delta\epsilon + 11.0$), 213 (-34.0), 226 ($+ 27.3$), 258 (-4.9)] were changed in **9**, particularly λ 217 nm ($\Delta\epsilon + 17.5$) and λ 256 nm ($\Delta\epsilon - 6.2$) (see Table S4 (Supporting Information) for complete data).

The L-CD of **7** did not match either enantiomer of **9**: it was equal in magnitude and form but opposite in sign to that of (2*S*,6*R*)-**8** (Figure 2b). Therefore, **7** and (2*S*,6*R*)-**8** are enantiomers, and the complete configurations of **3** and **4** are 3*S*,4*S*,6*R*,8*R*,12*S* and 3*R*,5*R*,7*R*,11*S*, respectively.

Compounds **1–4** were assayed for antifungal activity against strains of *Candida albicans*, *C. glabrata*, *C. krusei*, and *Cryptococcus neoformans* (Table 1). All compounds were exceedingly potent antifungal agents ($\text{MICs} \leq 0.5 \mu\text{g/mL}$) against all seven strains. The monomethyl-branched ω -phenyl polyketide peroxides **1** and **2** were about twice as

Table 1. Antifungal Activities of Plakinic Acids I–L (**1–4**) (Minimum Inhibitory Concentration, MIC ($\mu\text{g/mL}$))^a

	1	2	3	4
<i>C. albicans</i> , ATCC 14053	0.25	0.25	0.50	0.50
<i>C. albicans</i> 96–489 ^b	0.06	<0.03	0.12	0.12
<i>C. albicans</i> UCD-FR1 ^b	0.25	0.12	0.50	0.50
<i>C. glabrata</i>	0.12	0.06	0.12	0.12
<i>C. krusei</i>	0.06	0.12	0.12	0.12
<i>Cryptococcus neoformans</i> var. <i>gattii</i>	0.12	0.25	0.12	0.50
<i>C. neoformans</i> var. <i>grubii</i>	0.12	0.25	0.12	0.50

^a The in vitro susceptibilities were determined by the microbroth dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). ^b Fluconazole-resistant ($\text{MIC} > 64 \mu\text{g/mL}$).

active as plakinic acids **K** (**3**) and **L** (**4**). Compound **2** showed the most potent activity ($\text{MIC} < 0.12 \mu\text{g/mL}$) against *C. albicans* 96–489, *C. albicans* UCD-FR1, and *C. glabrata* suggesting that the 1,2-dioxolane ring and a monomethyl-branch in the ω -phenylalkyl side chain are key determinants of antifungal activity.

In conclusion, liposomal CD differentiates long-chain methyl-branched naphthamides, with epimeric configurations at a stereocenter eight bonds removed from the chromophore. Comparison of the L-CD spectrum of the naphthamide, obtained by degradation of plakinic acid **K** (**3**), with those of diastereomeric synthetic models, allowed assignment of C8/C7 and C12/C11 of plakinic acids **K** (**3**) and **L** (**4**), respectively. This remarkable long-range propagation of stereochemical information is made possible by amplification of the Cotton effects through ordering of lipid chains within the liposomal bilayer. The current work also provides CD references for interrogation of remote double methyl-branched polyketides by L-CD. Additional applications of L-CD are the subject of ongoing investigations in our laboratories.

Acknowledgment. We thank A. Marcus (University of Oregon) for helpful discussions, S. Zea (Universidad Nacional de Colombia) for identification of the sponges, and C. Green (Takasago International Corp.) for a generous gift of (S)-(–)-citronellal. We are grateful to B. Morinaka (UC San Diego) and A. Jansma (UC San Diego) for assistance with NMR experiments and J. Pawlik (University of North Carolina, Wilmington) and the crew of the *RV Seward Johnson* for logistics of sponge collection. EI HRMS data were provided by Y. Su (UC San Diego). The 500 MHz NMR spectrometers were purchased with funds from the NSF (CRIF, CHE0741968). Financial support of this work was provided by the National Institutes of Health (CA1225601 and AI039987).

Supporting Information Available: Full isolation and characterization of **3**, **4**, and plakinic acid **M** (**S1**), degradation of **3** and **4**, synthetic procedures, ¹H and ¹³C NMR spectra of **3–23**, and CD spectra of **7–9**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(18) The assignment of the C2 configurations in **18** and **19** followed from ¹H NMR comparisons with analogues of known configuration: Nicholas, G. M.; Molinski, T. F. *Tetrahedron* **2000**, *56*, 2921–27.

(19) ¹³C NMR (CDCl_3 , 125 MHz): $\sum [\Delta\delta(\text{8–9})]^2 < 0.07 \text{ ppm}$.