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Report

The Structure-Activity Relationship of MPBD and Dictyoquinone Analogs in *Dictyostelium discoideum* Cell Aggregation

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4-Methyl-5-pentylbenzene-1,3-diol (MPBD) (1) and dictyoquinone (DQ or 2-hydroxy-5-methyl-6-pentyl-1,4-benzoquinone) (2) are two polyketides involved in the cell development of *Dictyostelium discoideum*. We synthesized several MPBD and DQ analogs and tested their ability to recover the cell aggregation delay in the *stlA* null strain. According to the findings, the alkyl side chain of both compounds facilitates their activity. The parent *n*-pentyl side chain is required for the cell aggregation.

Key words slime mold, *Dictyostelium discoideum*, cell aggregation, differentiation factor, MPBD, dictyoquinone

INTRODUCTION

Although *Dictyostelium discoideum* is a simple eukaryote organism, its complex cell development system has been studied for years as a model for understanding various cell functions. Signal transduction, chemotaxis, and cell differentiation are a few examples.[1] *D. discoideum* cell development begins when nutrient resources are depleted. To avoid starvation, the single-cell amoeba sends chemoattractant signals to the surrounding amoebas, causing cell aggregation, the formation of a multicellular mound, and the formation of a motile slug. The slug then migrates to a better location to form a fruiting body with fully differentiated spores and stalk cells.[2] Cell development is thought to be aided by signaling molecules such as cAMP, 4-methyl-5-pentylbenzene-1,3-diol (MPBD), and dictyoquinone (2-hydroxy-5-methyl-6-pentyl-1,4-benzoquinone: DQ).[3] During the developmental stages, the cAMP serves several functions. It may act as a chemoattractant during cell aggregation,[4] inducing stalk formation[5] and regulating spore cell development.[6] Adenylyl cyclase A (ACA), encoded by the *acaA* gene, was known to be the protein responsible for cAMP expression during cell aggregation.[7] The cAMP-dependent protein kinase A (PKA) aided in the activation of cAMP.[8]

MPBD and DQ are polyketides isolated from *D. discoideum* (Fig. 1).[9,10] MPBD was also obtained from *D. mucoroides*.[11] Because of their structural similarity, MPBD and DQ were assumed to be produced by the same biosynthetic pathway, and oxidizing MPBD with Frémy's salt resulted in DQ.[10]

MPBD was found to be involved in cell aggregation, spore maturation, and the stalk formation.[9,11–13] SteelyA, the hybrid-type polyketide synthase was employed in synthesizing MPBD *in vivo*. The absence of this enzyme in the *stlA* null strain resulted in early defects such as delayed cell aggregation and the formation of small aggregation regions. The defect was discovered to be caused by the lower ACA expression to produce cAMP when compared to the wild-type strain. Later on, the *stlA* null mutant exhibits an impaired spore cell. The addition of MPBD was able to recover these defects.[12,13] As with MPBD, the addition of DQ to the *stlA* null mutant restored the cell aggregation defect. However, unlike MPBD, the addition of DQ does not allow the spore cells to mature. It is reported that cell aggregation and spore maturation are probably controlled by a separate mechanism. DQ was also discovered to be inducing the *acaA* gene, which is responsible for expressing ACA, which results in the production of cAMP. Induction was discovered to be superior to MPBD. As a result, it

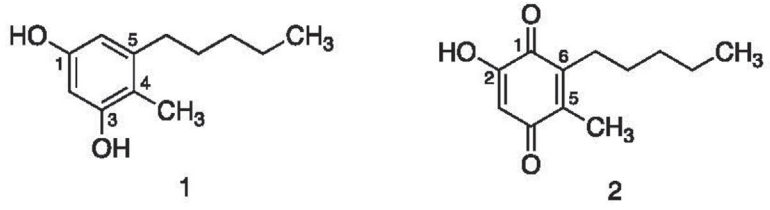

Fig. 1. MPBD (**1**) and DQ (**2**)

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is assumed that MPBD is being oxidized to produce DQ in order to regulate chemotactic cell aggregation during the early stages of development.[14] In this study, we synthesized several MPBD and DQ analogs to gain a better understanding of the structure-activity relationship of MPBD and DQ in *D. discoideum* cell aggregation. The side alkyl chain at position C-5 of MPBD and C-6 of DQ was significantly modified in the analogs from the parental compounds. This position was chosen because previous research showed that the side alkyl chain at the C-6 position had a significant impact on DQ's prespore cell-inducing activity. The DQ isomer 2-hydroxy-6-methyl-5-pentyl-1,4-benzoquinone exhibited no prespore cell-inducing activity.[10] As a result, it was assumed that the side alkyl chain played a significant role in *D. discoideum* cell development. The analog compounds were then tested for their ability to restore the *stlA* null mutant's cell aggregation delay.

Furthermore, various secondary metabolites from *Dictyostelium* sp., including MPBD (**1**), have been shown to inhibit the cell growth of human leukemia K-562[11,15,16] and HL-60.[11,17] The mechanism is ascribed to the fact that the compounds induce cell differentiation from undifferentiated cells.[15] There is no known relationship between tumor cell suppressive activity and aggregation recovery activity against *D. discoideum* cells. Compounds with higher aggregation recovery potency, on the other hand, are expected to show more proliferative activity via the common phenomenon of differentiation, providing a potential new type of antitumor compound. From this perspective, it is critical to investigate the contribution of the analogs' side chains to cell activity when exploring future applications of cellular slime molds.

MATERIALS AND METHODS

General ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance III HD600 spectrometer equipped with a cryoprobe Prodigy. Mass spectra were recorded using Exactive Plus Orbitrap DART mass spectrometer (Thermo Scientific, Waltham, MA, USA). FT-IR spectra were observed using JASCO FT/IR-410. UV-vis spectra were obtained by JASCO V-560 UV/VIS Spectrophotometer. Column chromatographic separations were conducted on silica-gel (BW-820MH, Fuji Silysia, Aichi, Japan). All chemicals were analytical-grade products of Tokyo Chemical Industry (TCI, Tokyo, Japan), Kanto Chemical Company (Tokyo, Japan) and Sigma-Aldrich (St. Louis, MO, USA).

Synthesis of Ethylphosphonium Ylide (3a) A mixture of triphenylphosphine (1.3 g, 5.0 mmol) and ethyl iodide (2.2 g, 14.1 mmol) in $CHCl_3$ was refluxed for 3.5 h. The reaction mixture was then cooled to room temperature, and a white precipitate was obtained by adding diethyl ether. The precipitate was filtered in vacuo to yield **3a** (2.0 g, yield 96%).

Synthesis of Phosphonium Ylide (3b–3g) A mixture of triphenylphosphine (1.5 eq) and alkyl bromide (for **3b**–**3g**) (1.0 eq) was heated at 120°C for 3.5 h. The reaction mixture was then cooled to room temperature to obtain a white precipitate. The precipitate was filtered in vacuo to yield **3b**–**3g**.

Synthesis of 1-alkenyl-3,5-dimethoxybenzene (4a–4d) 0.6 M Potassium bis(trimethylsilyl)amide (KHMDS) in toluene (3.0 eq) was added to the suspension of the phosphonium ylide (**3a**–**3d**) (2.5 eq) in THF under argon at 0°C. After 40 min at 5°C, 3,5-dimethoxybenzaldehyde (1.0 eq) was added to the mixture, followed by stirring for 5 h. Silica-gel column chromatography afforded 1-alkenyl-3,5-dimethoxybenzene (**4a**–**4d**). Compounds **4a**, **4b** and **4d** were obtained as mixtures of *E*- and *Z*-isomers (**4a**: *E*/*Z* = 2.5:1, **4b**: *E*/*Z* = 1.4:1 and **4d**: *E*/*Z* = 1:11.5), whereas **4c** was a single *Z*-isomers.

Synthesis of 1-alkenyl-3,5-dimethoxybenzene (4e–4g) A mixture of 3.5-dimethoxybenzaldehyde (1.0 eq), phosphonium bromide (**3e**–**3g**) (1.1 eq) and potassium carbonate (2.0 eq) in DMSO-H_2O (10:1) was stirred at 120°C for 24 h. Products (**4e**–**4g**) were extracted with ethyl acetate followed by purification by a silica-gel column chromatography. Compounds **4e**–**4g** were obtained as mixtures of *E*- and *Z*-isomers (**4e**: *E*/*Z* = 1.7:1, **4f**: *E*/*Z* = 1.4:1, and **4g**: *E*/*Z* = 1.6:1).

Synthesis of 1-alkyl-3,5-dimethoxybenzene (5a–5g) 5% Pd/C (70 mg) was added to a solution of **4a**–**4g** in ethyl acetate. Next, the mixture was then stirred under a hydrogen atmosphere for 24 h at room temperature. The Pd/C was filtered, followed by the purification of the crude product using silica-gel column chromatography to afford **5a**–**5g**.

Synthesis of 2-alkyl-4,6-dimethoxybenzaldehyde (6a–6g) $POCl_3$ (25 eq) was added dropwise into anhydrous DMF at 0°C followed by 20 min of stirring at room temperature. Next, **5a**–**5g** (1.0 eq) in DMF was added dropwise into the solution. The mixture was then stirred for 4 h at 75°C. The product was extracted with ethyl acetate, and purified by silica-gel column chromatography to afford **6a**–**6g**.

Synthesis of 1-alkyl-3,5-dimethoxy-2-methylbenzene (7a–7g) A mixture of **6a–6g** (1.0 eq) and $NaBH_3CN$ (7.5 eq) in THF was adjusted to a pH of 3 using 1 M HCl. After 24 h, the mixture was extracted with ethyl acetate. The resulted crude product was purified by silica-gel column chromatography to afford **7a–7g**.

Synthesis of 5-alkyl-4-methyl-1,3-benzenediol (1 and 8–13) 1 M BBr_3 (8 eq) in dichloromethane was added to a solution of **7a–7g** (1.0 eq) in dichloromethane under argon at –78°C. The temperature was gradually increased to 0°C within 1.5 h. The solution was further kept at room temperature for 19 h. After adding methanol, the mixture was evaporated, followed by purification using a silica-gel column chromatography to afford **1** and **8**–**13**.

4-Methyl-5-pentyl-1,3-benzenediol (MPBD, 1) A colorless oil. The NMR data is consistent with previous studies.[9] IR (film) cm^{-1}: 3250, 2928, 2870, 1608, 1470, 1307, 1145. UV λ_{max} (EtOH) nm (log ε): 203 (4.62), 225 (3.85), 279 (3.53). DART-MS *m*/*z* 195.1375 ([M+H]$^+$) (Calcd for $C_{12}H_{19}O_2^+$: 195.1380).

4-Methyl-5-propyl-1,3-benzenediol (8) A white solid, mp 55–56°C. ^{1}H NMR (MeOD) δ: 6.16, 6.14 (each 1H, d, *J* = 2.3 Hz), 2.49 (2H, t, *J* = 7.5 Hz), 2.04 (3H, s), 1.56 (2H, sext, *J* = 7.5 Hz), 0.97 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (MeOD) δ: 155.7, 154.8, 142.4, 113.1, 107.1, 99.6, 35.5, 23.3, 12.9, 9.4. IR (KBr) cm^{-1}: 3224, 2929, 2871, 1613, 1471, 1305, 1145. UV λ_{max} (EtOH) nm (log ε): 203 (4.71), 226 (3.94), 277 (3.19). DART-MS *m*/*z* 167.1067 ([M+H]$^+$) (Calcd for $C_{10}H_{15}O_2^+$: 167.1063).

5-Butyl-4-methyl-1,3-benzenediol (9) A white solid, mp 55–56°C. ^{1}H NMR (MeOD) δ: 6.15, 6.14 (each 1H, d, *J* = 2.3 Hz), 2.51 (2H, t, *J* = 7.6 Hz), 2.04 (3H, s), 1.50 (2H, quint, *J* = 7.6 Hz), 1.41 (2H, sext, *J* = 7.6 Hz), 0.97 (3H, t, *J* = 7.6 Hz). ^{13}C NMR (MeOD) δ: 157.1, 156.2, 144.0, 114.5, 108.4, 101.0, 34.5, 34.0, 23.7, 14.3, 10.8. IR (KBr) cm^{-1}: 3240, 2928, 2857, 1608, 1469, 1306, 1145. UV λ_{max} (EtOH) nm (log ε): 203 (4.60), 225 (3.88), 282 (3.21). DART-MS *m*/*z* 181.1223 ([M+H]$^+$) (Calcd for $C_{11}H_{17}O_2^+$: 181.1223).

5-Heptyl-4-methyl-1,3-benzenediol (10) A colorless oil.

^{1}H NMR (MeOD) δ: 6.15, 6.14 (each 1H, d, J = 2.3 Hz), 2.50 (2H, t, J = 7.4 Hz), 2.04 (3H, s), 1.53 (2H, quint, J = 7.4 Hz), 1.39–1.26 (8H, m), 0.92 (3H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 154.5, 153.7, 143.9, 113.9, 108.6, 100.2, 33.8, 31.8, 30.4, 29.6, 29.2, 22.7, 14.1, 10.5. IR (film) cm^{-1}: 3310, 2920, 2852, 1609, 1457, 1279, 1140. UV λ_{max} (EtOH) nm (log ε): 203 (4.60), 226 (3.92), 284 (3.43). DART-MS *m/z* 223.1693 ([M+H]$^+$) (Calcd for $C_{14}H_{23}O_2^+$: 223.1693).

4-Methyl-5-nonyl-1,3-benzenediol (11) A white solid, mp 61–63°C. ^{1}H NMR (MeOD) δ: 6.15, 6.14 (each 1H, d, J = 2.3 Hz), 2.50 (2H, t, J = 7.4 Hz), 2.04 (3H, s), 1.53 (2H, quint, J = 7.4 Hz), 1.40–1.28 (12H, m), 0.92 (3H, t, J = 7.4 Hz). ^{13}C NMR (MeOD) δ: 157.2, 156.3, 144.2, 114.6, 108.6, 101.1, 34.9, 33.2, 31.8, 30.8, 30.6, 23.8, 14.5, 10.9. IR (KBr) cm^{-1}: 3303, 2921, 2851, 1612, 1471, 1282, 1143. UV λ_{max} (EtOH) nm (log ε): 204 (4.70), 226 (4.08), 285 (3.51). DART-MS *m/z* 251.2006 ([M+H]$^+$) (Calcd for $C_{16}H_{27}O_2^+$: 251.2006).

4-Methyl-5-undecyl-1,3-benzenediol (12) A white solid, mp 74–75°C. ^{1}H NMR (MeOD) δ: 6.15, 6.14 (each 1H, d, J = 2.4 Hz), 2.50 (2H, t, J = 7.4 Hz), 2.04 (3H, s), 1.53 (2H, quint, J = 7.4 Hz), 1.38–1.26 (16H, m), 0.92 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 154.5, 153.7, 143.9, 113.9, 108.6, 100.2, 33.8, 31.9, 30.4, 29.7, 29.6, 29.6, 29.6, 29.4, 22.7, 14.1, 10.5. IR (KBr) cm^{-1}: 3298, 2920, 2851, 1598, 1467, 1287, 1143. UV λ_{max} (EtOH) nm (log ε): 204 (4.76), 227 (4.05), 283 (3.55). DART-MS *m/z* 279.2318 ([M+H]$^+$) (Calcd for $C_{18}H_{31}O_2^+$: 279.2319).

4-Methyl-5-tridecyl-1,3-benzenediol (13) A white solid mp 73–75°C. ^{1}H NMR (MeOD) δ: 6.13, 6.12 (each 1H, d, J = 2.5 Hz), 2.48 (2H, t, J = 7.8 Hz), 2.02 (3H, s), 1.50 (2H, quint, J = 7.5 Hz), 1.33–1.28 (20H, m), 0.89 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 154.5, 153.7, 143.9, 113.9, 108.6, 100.2, 33.8, 31.9, 30.4, 29.7, 29.7, 29.6, 29.6, 29.4, 22.7, 14.1, 10.5. IR (KBr) cm^{-1}: 3360, 2919, 2849, 1595, 1465, 1291, 1137. UV λ_{max} (EtOH) nm (log ε): 202 (4.60), 225 (3.92), 283 (3.36). DART-MS *m/z* 307.2634 ([M+H]$^+$) (Calcd for $C_{20}H_{35}O_2^+$: 307.2632).

Synthesis of 6-alkyl-2-hydroxy-5-methyl-1,4-benzoquinone (2 and 14–19) 0.2 mM KH_2PO_4 was added into a solution of 1-alkyl-3,5-dihydroxy-2-methylbenzene (**1** and **8**–**13**) (1.0 eq) in acetone followed by the addition of Frémy's salt (2.5 eq). The solution was then stirred for 20 minutes at room temperature. Next, another portion of Frémy's salt (2.5 eq) was added to the mixture. After 6 h, the mixture was extracted using ethyl acetate followed by purification with silica-gel column chromatography to afford products (**2** and **14–19**).

2-Hydroxy-5-methyl-6-pentyl-1,4-benzoquinone (2) A yellow solid, mp 76–77°C. The ^{1}H NMR data is consistent with previous studies.[10] IR (film) cm^{-1}: 3276, 2956, 2923, 2859, 1658, 1633, 1611, 1395, 1227. UV λ_{max} (EtOH) nm (log ε): 210 (3.97), 278 (3.82), 493 (2.80). DART-MS *m/z* 209.1172 ([M+H]$^+$) (Calcd for $C_{12}H_{17}O_3^+$: 209.1172).

2-Hydroxy-5-methyl-6-propyl-1,4-benzoquinone (14) A yellow solid, mp 102–103°C. ^{1}H NMR ($CDCl_3$) δ: 6.05 (1H, s), 2.49 (2H, t, J = 7.6 Hz), 2.07 (3H, s), 1.48 (2H, sext, J = 7.6 Hz), 0.98 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$) 187.8, 183.9, 154.0, 144.0, 140.7, 107.5, 28.0, 21.8, 14.2, 12.5. IR (KBr) cm^{-1}: 3250, 2961, 2927, 2872, 1660, 1632, 1612, 1395, 1226. UV λ_{max} (EtOH) nm (log ε): 210 (4.23), 279 (3.94), 494 (3.07). DART-MS *m/z* 181.0855 ([M+H]$^+$) (Calcd for $C_{10}H_{13}O_3^+$: 181.0859).

6-Butyl-2-hydroxy-5-methyl-1,4-benzoquinone (15) A yellow solid, mp 94–95°C. ^{1}H NMR ($CDCl_3$) δ: 6.07 (1H, s), 2.53 (2H, t, J = 7.4 Hz), 2.09 (3H, s), 1.41 (4H, m), 0.96 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 187.8, 183.8, 154.0, 143.8, 141.0, 107.5, 30.6, 25.9, 22.9, 13.8, 12.5. IR (KBr) cm^{-1}: 3258, 2954, 2919, 2856, 1657, 1632, 1611, 1394, 1231. UV λ_{max} (EtOH) nm (log ε): 212 (4.35), 280 (4.15), 493 (3.21). DART-MS *m/z* 195.1016 ([M+H]$^+$) (Calcd for $C_{11}H_{15}O_3^+$: 195.1016).

6-Heptyl-2-hydroxy-5-methyl-1,4-benzoquinone (16) A yellow solid, mp 73–74°C. ^{1}H NMR ($CDCl_3$) δ: 6.07 (1H, s), 2.52 (2H, t, J = 7.8 Hz), 2.09 (3H, s), 1.44 (2H, quint, J = 7.3 Hz), 1.39–1.26 (8H, m), 0.91 (3H, t, J = 7.3 Hz).^{13}C NMR ($CDCl_3$) δ: 187.8, 183.8, 153.9, 143.8, 141.0, 107.5, 31.7, 29.7, 29.0, 28.5, 26.2, 22.6, 14.1, 12.5. IR (KBr) cm^{-1}: 3288, 2954, 2922, 2855, 1658, 1632, 1612, 1394, 1227. UV λ_{max} (EtOH) nm (log ε): 210 (4.14), 280 (3.91), 499 (3.03). DART-MS *m/z* 237.1485 ([M+H]$^+$) (Calcd for $C_{14}H_{21}O_3^+$: 237.1485).

2-Hydroxy-5-methyl-6-nonyl-1,4-benzoquinone (17) A yellow solid, mp 76–78°C. ^{1}H NMR ($CDCl_3$) δ: 6.07 (1H, s), 2.52 (2H, t, J = 7.8 Hz), 2.08 (3H, s), 1.44 (2H, quint, J = 7.3 Hz), 1.39–1.29 (12H, m), 0.90 (3H, t, J = 7.3 Hz).^{13}C NMR ($CDCl_3$) δ: 187.8, 183.8, 154.0, 143.7, 141.0, 107.5, 31.9, 29.8, 29.5, 29.4, 29.3, 28.5, 26.2, 22.7, 14.1, 12.5. IR (KBr) cm^{-1}: 3291, 2947, 2920, 2850, 1657, 1628, 1607, 1394, 1230. UV λ_{max} (EtOH) nm (log ε): 202 (4.40), 278 (4.33), 493 (3.17). DART-MS *m/z* 265.1799 ([M+H]$^+$) (Calcd for $C_{16}H_{25}O_3^+$: 265.1798).

2-Hydroxy-5-methyl-6-undecyl-1,4-benzoquinone (18) A yellow solid, mp 79–81°C. ^{1}H NMR ($CDCl_3$) δ: 6.07 (1H, s), 2.52 (2H, t, J = 7.7 Hz), 2.08 (3H, s), 1.44 (2H, quint, J = 7.0 Hz), 1.39–1.28 (16H, m), 0.90 (3H, t, J = 7.0 Hz).^{13}C NMR ($CDCl_3$) δ 187.8, 183.8, 154.0, 143.7, 141.0, 107.5, 31.9, 29.8, 29.6, 29.6, 29.5, 29.4, 29.3, 28.5, 26.2, 22.7, 14.1, 12.5. IR (KBr) cm^{-1}: 3292, 2947, 2919, 2849, 1656, 1627, 1606, 1393, 1232. UV λ_{max} (EtOH) nm (log ε): 201 (4.52), 280 (4.01), 492 (3.09). DART-MS *m/z* 293.2115 ([M+H]$^+$) (Calcd for $C_{18}H_{29}O_3^+$: 293.2111).

2-Hydroxy-5-methyl-6-tridecyl-1,4-benzoquinone (19) A yellow solid, mp 80–82°C. ^{1}H NMR (MeOD) δ: 5.89 (1H, s), 2.52 (2H, t, J = 7.7 Hz), 2.03 (3H, s), 1.45–1.31 (22H, m), 0.92 (3H, t, J = 7.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.8, 183.8, 154.0, 143.7, 141.0, 107.5, 31.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 28.5, 26.2, 22.7, 14.1, 12.5. IR (KBr) cm^{-1}: 3299, 2946, 2919, 2849, 1657, 1628, 1607, 1393, 1229. UV λ_{max} (EtOH) nm (log ε): 202 (4.51), 279 (4.28), 493 (3.19). DART-MS *m/z* 321.2423 ([M+H]$^+$) (Calcd for $C_{20}H_{33}O_3^+$: 321.2424).

Synthesis of 2-methoxy-5-methyl-6-propyl-1,4-benzoquinone (20) MeI (20 μL, 0.32 mmol) and 30 mg of K_2CO_3 were added to a solution of **2** (1.1 mg, 5 μmol) in 3 mL DMF at 0°C. The mixture was stirred for 4 h while gradually raising its temperature until room temperature was reached under an oxygen atmosphere. Ethyl acetate and sodium thiosulfate were then added to the reaction mixture, followed by purification using silica-gel column chromatography to afford **20** (0.9 mg) as a yellow solid (yield 76%), mp 56–58°C. ^{1}H NMR ($CDCl_3$) δ: 5.99 (1H, s), 3.82 (3H, s), 2.52 (2H, t, J = 7.7 Hz), 2.04 (3H, s), 1.44 (2H, m), 1.37 (4H, m), 0.94 (3H, t, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$) δ: 189.2, 183.4, 160.0, 144.1, 142.4, 107.9, 56.8, 33.1, 29.2, 27.0, 23.4, 14.3, 12.1. IR (film) cm^{-1}: 2957, 2923, 2853, 1673, 1646, 1607, 1361, 1230. UV λ_{max} (EtOH) nm (log ε): 201 (3.05), 272 (3.14), 361 (1.34). DART-MS *m/z* 223.1327 ([M+H]$^+$) (Calcd for $C_{13}H_{19}O_3^+$: 223.1329).

Cell Aggregation Recovery Assay The *D. discoideum stlA* null strain (Strain ID: DBS0236953) was grown in an axenic medium (HL-5) supplemented with 10 μg/mL blasticidin S at 22°C.[18,19] To evaluate the effects of DQ and MPBD analogs on the chemotactic cell aggregation defect in the *stlA* null mutant, SM agar plates (1.5% agar in SM)[20] containing

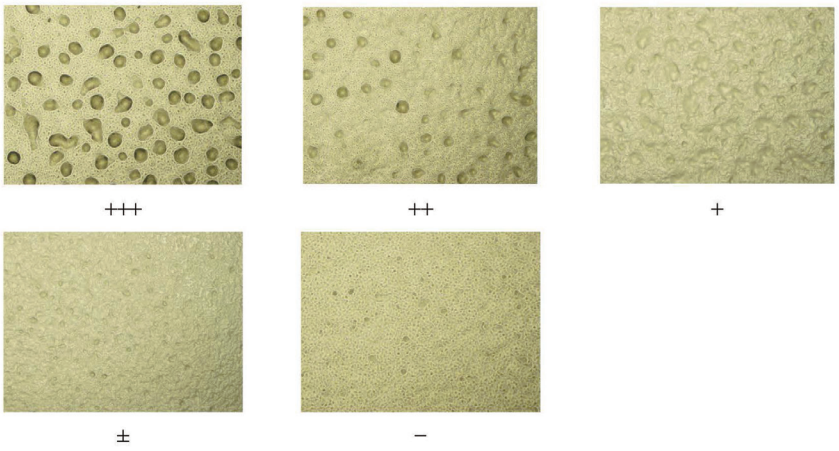


Fig. 2. The Assessment Criteria of the Cell Aggregation Activity

200 nM of each test compound were prepared. Here, 200 nM was the minimum concentration of MPBD required to recover the spore maturation defect in the *stlA* null strain,[12] and in this concentration cell aggregation defect was also recovered.[13] The axenically grown cells were harvested during the log phase, washed twice in SM buffer, and plated on the prepared SM agar plates at a density of 2.5×10^5 cells/cm^2. The morphogenesis was observed at 8 h after inoculation. The assessment of the cell aggregation activity was evaluated by the following criteria by the morphological observation shown in Fig. 2:

+++: complete recovery as the wild type

++: recovery was weaker than the wild-type, but with mounds formation

+: mounds seem to be formed

±: slight non-homogeneity was observed with the likelihood of dense patches

–: no change

RESULTS AND DISCUSSION

Synthesis of MPBD and DQ Analogs Several MPBD and DQ analogs were synthesized as shown in Figs. 3 and 4.[9,10,21] Furthermore, 3,5-dimethoxybenzaldehyde and various alkyl phosphonium bromides (**3a**–**g**) were coupled to afford **4a**–**g**[22,23] as mixtures of *E*- and *Z*-isomers. The alkenyl products (**4a**–**g**)

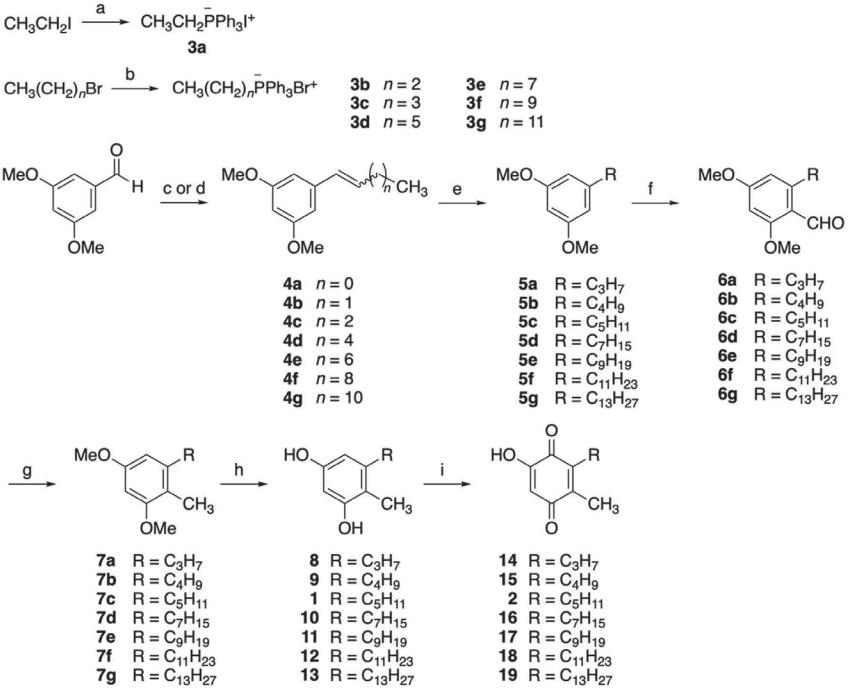


Fig. 3. Synthesis of MPBD (**1**), DQ (**2**), and Their Analogs (**8**–**19**)

Reagents and conditions: (a) PPh_3, $CHCl_3$, reflux, 3.5 h; (b) PPh_3, 120°C, 3.5 h; (c) for **4a**–**4d**: **3a**–**3d**, KHMDS, THF, 0°C to 5°C, 24 h; (d) for **4e**–**4g**: **3e**–**3g**, K_2CO_3, DMSO/H_2O, 120°C, 24 h; (e) H_2, 5% Pd/C, EtOAc, rt, 24 h; (f) $POCl_3$, DMF, rt to 75°C, 4 h; (g) $NaBH_3CN$, 1M HCl, THF, rt, 24 h; (h) BBr_3, CH_2Cl_2, –78°C to rt, 19 h; (i) Frémy's salt, KH_2PO_4, acetone, rt, 6 h.

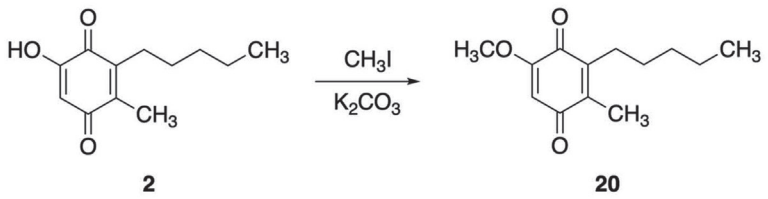


Fig. 4. Methylation of DQ (**2**)

were reduced into **5a**–**g** followed by formylation by Vilsmeier-Haack reaction[9] with some modifications to yield **6a**–**g**. The products were then treated with $NaBH_3CN$ in an acidic environment to convert the aldehyde into a methyl, followed by the demethylation of the methoxy group by BBr_3. Finally, MPBD (**1**) and its analogs (**8**–**13**) were obtained. Each product underwent oxidation using Frémy's salt to afford DQ (**2**) and its analogs (**14**–**19**) (Fig. 3). Furthermore, to investigate the contribution of the hydroxy group at the C-2 of DQ (**2**) to the activity, we converted it into a methoxy group (**20**) using iodomethane (Fig. 4).

Chemotactic Cell Aggregation The effect of MPBD and DQ analogs on the recovery of chemotactic cell aggregation defect in the *stlA* null mutant was investigated. The assay was conducted by observing the mound formation on the SM agar plate with or without the test compounds. MPBD (**1**) and DQ (**2**) showed the recoverability for the cell aggregation delay, resulting in a clear mound formation after 8 h incubation. The results were comparable to a wild-type strain Ax2.

Compounds **8**, **9**, **14**, and **15** bear shorter alkyl chains (propyl and butyl) than the natural compounds **1** and **2**, and compounds **10**–**13** and **16**–**19** have longer alkyl chains ranging from 7 to 13 carbons. These findings revealed that cell aggregation potential decreased gradually with the shorter and longer alkyl side chains than the parent compounds (**1** and **2**). This was evident by the observation of unclear aggregation mounds compared to those obtained by MPBD (**1**) and DQ (**2**). Furthermore, the 11 and 13 carbon alkyl chains (compounds **12**, **13**, **18**, and **19**) failed to recover cell aggregation defects (Table 1). These results indicate that the length of the alkyl side chain is essential in inducing cell aggregation. The analog containing methoxy (**20**) at the C-2 of DQ was also inactive, suggesting that the hydroxy group is critical in cell aggregation.

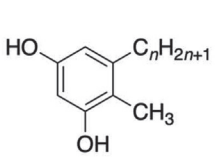
The receptor of MPBD and DQ is still unknown, but the importance of the alkyl side chain length in cell aggregation activity is attributable to the binding receptor of *D. discoideum*. Therefore, the alkyl side chain should be considered when investigating the receptor of MPBD (**1**) and DQ (**2**).

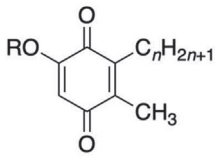
CONCLUSION

In this study, we investigated on the effects of MPBD and DQ analogs with various length of linear side chains on the recovery of cell aggregation delay in the *stlA* null mutant. As a result, the significance of the alkyl side chain length in recovering cell aggregation delay in the *stlA* null mutant was demonstrated by the synthesized analogs. The saturated, five carbons alkyl side chain afforded the best activity for the recovery by both MPBD and DQ. As future research, we are investigating compounds with other types of side chains.

Conflict of interest The authors declare no conflict of interest.

Table 1. Cell Aggregation Recovery Activity in *stlA* Null Strain by MPBD and DQ Analogs





MPBD type			DQ type			
Compds	*n*	Cell Aggregation	Compds	*n*	R	Cell Aggregation
8	3	+	**14**	3	H	+
9	4	++	**15**	4	H	++
1	5	+++	**2**	5	H	+++
10	7	++	**16**	7	H	++
11	9	±	**17**	9	H	±
12	11	–	**18**	11	H	–
13	13	–	**19**	13	H	–
			20	5	CH_3	±

No cell aggregation was observed by DMSO as a vehicle.

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