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Effective irreversible alkylating reagents based on the structure of clavulones

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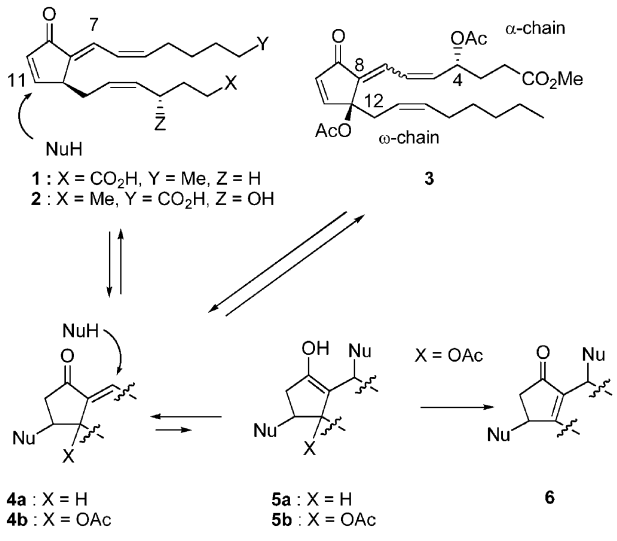
Abstract—We describe the design and synthesis of alkylating reagents based on the structure of clavulones. They are composed of cross-conjugated dienone system and irreversibly reacted with two nucleophiles under mildly basic conditions via β-elimination. Hydroxyl derivative **7b** showed the highest reactivity toward thiols and showed the strongest cytotoxicity in Hela S3 cells among the three derivatives having a different protecting group at the *tert*-hydroxyl group.

Chemical genetics is a research approach that uses biologically active small molecules as probes to study protein function in cells or organisms.[1] Chemical irreversible modification on specific proteins with the small molecules should be effective not only to elucidate function of the binding proteins, but also to identify the target proteins from living cells or isolated protein mixtures.[2] Therefore, selectively and strongly alkylating agents are required as chemical probes.

Cross-conjugated dienone prostanoids such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) (**1**) and Δ^7-prostaglandin A_2 (**2**) display varied biological activities.[3] Their mechanism of action is based on reversible and selective alkylation with specific proteins at their C11 position to provide thermodynamically stable adducts **4a**.[4] Recently, Oliva J. L. and Rojas J. M. et al. have reported 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (**1**) selectively elicited H-Ras activation by formation of a covalent bond.[5] Additionally, Suzuki and Noyori et al. have elucidated that the metabolic stability of prostaglandin A_2 (**2**) was dependant on the structure of the side chain.[6]

Marine prostanoid clavulone I (**3**) isolated from the Okinawan soft coral features the same cross-conjugate dienone system along with a *tert*-acetoxyl group at the C12 position and shows strong cytotoxicity.[7,8] The cross-conjugated dienone **3** could undergo sequential irreversible alkylation to provide the di-coupling



Scheme 1.

Keywords: Michael reactions; Clavulones; Prostanoids; Alkylating reagents; Aldol reactions.
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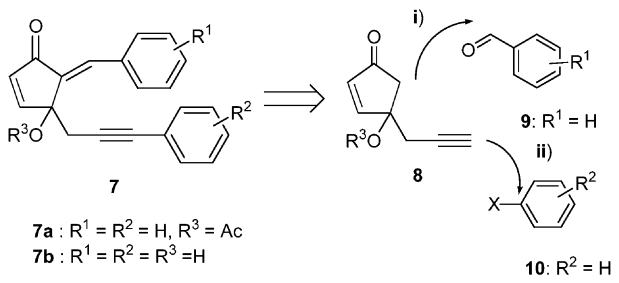


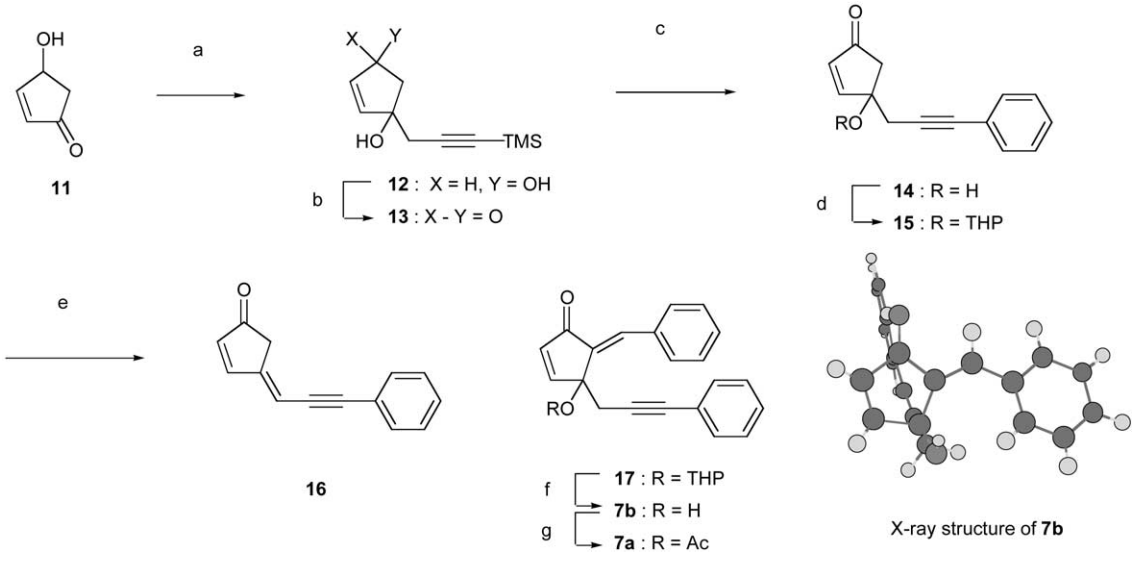
Scheme 2. Strategy for the synthesis of **7**.

product **5b**, followed by β-elimination of the C12 acetoxyl group to provide enone **6**. The irreversible alkylation would be more effective to inhibit protein functions than the reversible one.[9] Therefore, clavulone derivatives having appropriate side-chains could become useful chemical probes. In this report, we describe the synthesis of simple clavulone derivatives with two aromatic side-chains, and the relationship between reactivity of the double Michael acceptors and their cytotoxicity (Scheme 1).

Cross-conjugated dienones **7** bearing two aromatic side-chains were designed as irreversible alkylating reagents (Scheme 2).[9] Substituents on the aromatic side-chains would be effective not only to vary their electrostatic and steric parameters for the selective alkylation to specific proteins, but also to tune the reactivity of the second alkylation reaction. Electron withdrawing protecting groups at the C12 *tert*-hydroxyl group should enhance the subsequent β-elimination. However, its bulkiness could reduce the reactivity of Michael acceptor at C11 position.

Our strategy for the synthesis of **7** involves the coupling of terminal acetylene **8** and aryl halide **10** with palladium catalyst, followed by aldol condensation of **9**, and would be effective to diversify the two side-chains. At the first stage of the project, we planned to prepare hydroxyl and acetoxyl derivatives **7a** and **7b** bearing two phenyl side-chains.

The preparation of the di-phenyl derivatives **7a** and **7b** is shown in Scheme 3. Treatment of 4-hydroxy-cyclopentenone (**11**) with lithiated 1-trimethylsilylpropyne at −78 °C provided diol **12** in 58% yield.[10] Selective oxidation of the secondary alcohol in diol **12** with MnO_2 afforded enone **13** in 71% yield. Introduction of the aromatic ring at the ω-chain was achieved by one-pot silyl deprotection and Sonogashira coupling reaction[11] of **13** with phenyl iodide in the presence of tetrabutyl ammonium fluoride and a catalytic amount of $Pd(PPh_3)_4$ and CuI to give phenyl acetylene **14** in 99% yield. Subsequent protection of the *tert*-alcohol with 3,4-dihydro-2*H*-pyran (DHP) in the presence of pyridinium *p*-toluenesufonate (PPTS) provided enone **15** in 89% yield. Aldol condensation of enone **15** with benzaldehyde to form the cross-conjugated dienone was investigated. Exposure of **15** to several bases to generate the enolate resulted in β-elimination of the protected *tert*-hydroxyl group to give dienone **16** as the major product. The dianion enolate from β-hydroxyl ketone **14** was readily decomposed at −78 °C. Further examination of the aldol condensation reaction revealed that treatment of ketone **15** with an equivalent of KHMDS in THF at −78 °C for 15 min, followed by addition of benzaldehyde at the same temperature afforded the desired cross-conjugated dieone **17** in 8% yield along with dienone **16** (32%) and the starting material **15** (38%). Removal of the tetrahydropyranyl ether of **17** under acidic conditions provided alcohol **7b**[12] in 99% yield. X-ray crystallographic analysis of alcohol **7b** showed that the newly formed double bond had the (*E*)-configuration.[13] Acetylation of alcohol **7b** with Ac_2O in the presence of pyridine provided acetate **7a** in 99% yield.[14]



Scheme 3. Reactions and conditions: (a) 1-trimethylsilyl-1-propyne, BuLi, −78 °C, 58%; (b) MnO_2, Et_2O, 71%; (c) $Pd(PPh_3)_4$, CuI, phenyl iodide, Bu_4NF, 99%; (d) DHP, PPTS, 89%; (e) KHMDS, −78 °C, then benzaldehyde, 32% for **11**, 8% for **12**, and 38% for recovery of **10**; (f) 5%TFA/CH_2Cl_2, 99%; (g) Ac_2O, pyridine, CH_2Cl_2, 99%.

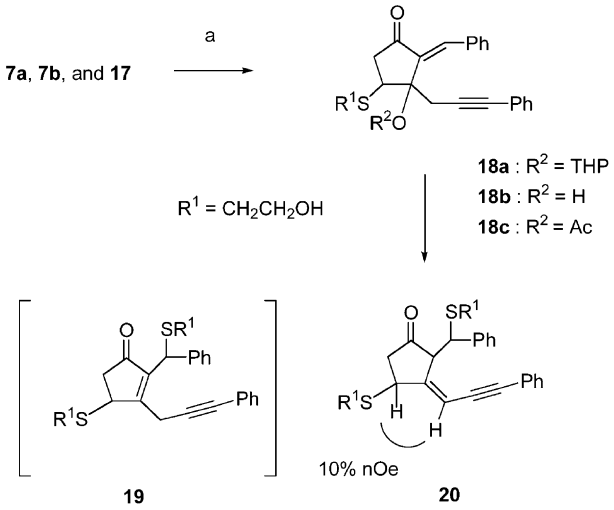
Table 1. Michael addition of cross-conjugate dienones **7a**, **7b** and **17**

Entry	Substrate	Recovered starting material (%)	Yield of **18** (%)	Yield of **20** (%)
1	**7a**	14	0	78
2	**7b**	0	14	65
3	**17**	85	0	6

Next, alkylation reaction of thiols with the cross-conjugated dinenones **7a**, **7b** and **17** was investigated (Scheme 4 and Table 1). Treatment of the cross-conjugated dienone **7a** with mercaptoethanol (2.0 equiv) in the presence of triethylamine (2.0 equiv) at room temperature for 24 h provided bis-thioethers **20** in 78% yield as two diastereomers (1:1), instead of the expected cyclopentenone **19**, along with the recovered starting material **7a** in 14% yield. The highly conjugated phenyl acetylene moiety at the ω-chain could promote the unexpected β-elimination to form the exocyclic double bond, or isomerization of **19** to **20** would occur because of sever steric repulsion among substituents on the conjugated enone. Structure determination of bis-thioether **20** was achieved by analysis of 1D and 2D-NMR spectra of each of the separated isomers **20a**[15] and **20b**.[16] ^{1}H NOE experiment shows the *exo*-double bond had the (*E*)-configuration. The relative stereochemistry of the two isomers **20a** and **20b** were not determined. Under the reaction conditions, mono-adduct intermediate **18c** was not observed. Exposure of alcohol **7b** to the same reaction conditions resulted in the disappearance of starting material **7b** and yielded the bis-thioether **20** in 65% yield along with mono-adduct **18b**[17] in 14% yield as a single stereoisomer. On the other hand, reaction of the THP derivative **17** with thiol under the same conditions provided only bis-thioether **20** in 6% yield along with dienone **17** (85%). These results indicate that sequential Michael reactions were initiated by conjugate addition of thiol to the *endo*-enone and steric hindrance of the protecting group at 12 position could block both two Micheal reactions.

Random alkylation reaction to biomolecules in the cells would result in antiproliferative effects. To test the applicability of the sequential Michael reaction to biomolecules in the cell, the cytotoxicity of the derivatives **7a**, **7b**, **17**, **18b**, **20a**, and **20b** in HeLa S3 cells (uterocervical carcinoma) was tested[18] (Table 2). Alcohol **7b** showed the strongest activity (IC_{50} = 15.0 nM) among the three dienones **7a**, **7b** and **17**. Cytotoxicity of mono-adduct **18b** was almost the same as dienone **7b**. On the other hand, bis-thioethers **20a** and **20b** did not show cytotoxicity. These results suggested that retro-Michael reaction of **18b** proceeded to generate the cross-conjugate dienone **7b** in situ. Furthermore, the highly reactivity of the Michael acceptor would be essential for the strong biological activity. These results indicate that their biological activity should be tunable by the steric hindrance of the protecting group.

In conclusion, we have reported the synthesis of cross-conjugated dienones **7a**, **7b**, and **17** bearing two



Scheme 4. Reactions and conditions: $HOCH_2CH_2SH$ (2.0 equiv), NEt_3 (2.0 equiv) CH_2Cl_2, rt.

Table 2. Cytotoxicity of cross-conjugated dienone derivatives **7a**, **7b**, **17**, **18b**, **20a**, and **20b** in HeLa S3 cells[a]

Entry	Compd	IC_{50} (nmol/L)
1	**7a**	24
2	**7b**	15
3	**17**	1.1×10^3
4	**18b**	21
5	**20a**	$>1.0\times10^6$
6	**20b**	$>1.0\times10^6$

[a] Cell proliferation assay was carried out using Cell Counting Kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). In brief, cells were plated in triplicate in 96-well plates at a density of 10×10^3 cells/well in EMEM medium. Following overnight culture, compounds **7a**, **7b**, **17**, **18b**, **20a**, and **20b** were added and the cells were incubated for 72 h. After 72 h, WST-8 solution was added and incubated for 1 h. The plates were read at a wavelength of 450 nm using Microplate Reader Model 3550 (Bio-Rad, Richmond, CA). Results are presented as the mean ±S.D. of three wells.

aromatic side-chains. Dieones **7a**, **7b**, and **17** reacted with two equivalents of thiol at room temperature. Alcohol **7b** had the highest reactivity in the Michael reaction and showed the strongest biological activity in comparison with acetate **7a** and THP ether **17**. The protecting group of the *tert*-hydroxyl affected the reactivity of the Michael acceptor with thiol and their cytotoxicity. The synthesis of a combinatorial library of cross-conjugated derivatives varying at the two aromatic rings is in progress.

Acknowledgements

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12. Spectra **7b** : ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.81 (brs, 1H), 2.90 (d, 1H, $J=17.0$ Hz), 3.28 (d, 1H, $J=17.0$ Hz), 6.51 (d, 1H, $J=6.8$ Hz), 7.27–7.30 (m 3H), 7.33–7.39 (m 2H), 7.33–7.39 (m 2H), 7.41–7.45 (m 3H), 7.52 (s 1H), 7.66 (d, 1H, $J=6.8$ Hz), 7.98 (brd, 2H, $J=8.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.2, 160.8, 136.1, 135.3, 134.4, 133.4, 132.1, 131.6, 130.0, 128.8, 128.3, 128.2, 122.8, 84.1, 84.0, 78.1, 27.4; IR (neat) 3418, 3074, 2910, 1681, 1589 cm^{-1}; MS(ESI-TOF) 301 $[M+H]^+$.
13. Crystallographic data (excuding structure factors) for the structure of **7b** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 197097; 146 °C.
14. Spectra **7a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.71 (s, 3H), 3.04 (d, 1H, $J=16.9$ Hz), 3.42 (d, 1H, $J=16.9$ Hz), 6.64 (d, 1H, $J=6.30$ Hz), 7.27–7.30 (m, 5H), 7.33–7.39 (m, 3H), 7.53 (s, 1H), 7.68 (d, 1H, $J=6.3$ Hz), 7.98 (brd, 2H, $J=8.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.7, 168.8, 157.0, 135.5, 134.2, 133.8, 133.3, 131.5, 131.1, 129.9, 128.8, 128.2, 128.1, 122.8, 84.2, 83.6, 83.0, 27.2, 21.3; IR (neat): 3074, 1751, 1705, 1632 cm^{-1}; MS(ESI-TOF) 343 $[M+H]^+$.
15. Spectra **20a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40–2.60 (m, 3H), 2.98 (dd, 1H, $J=6.8$, 19.3 Hz), 3.13 (t, 2H, $J=6.3$ Hz), 3.53 (t, 2H, $J=6.3$ Hz) 3.84 (t, 2H, $J=6.3$ Hz), 3.92 (s, 1H), 3.99 (s, 1H), 4.04 (brd, 1H, $J=6.8$ Hz), 6.59 (s, 1H), 7.15 (m, 2H), 7.27–7.31 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.5, 162.4, 152.6, 142.6, 141.0, 134.5, 129.1, 129.0, 127.7, 127.3, 127.0, 126.8, 113.3, 61.4, 60.2, 53.4, 45.0, 44.0, 42.0, 34.0, 32.3; IR (neat): 3360, 2920, 1668, 1615, 1533 cm^{-1}; MS (ESI-TOF) 439 $[M+H]^+$.
16. Spectra **20b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.53 (dd, 2H, $J=1.9$, 18.8 Hz), 2.76 (t, 2H, $J=6.3$ Hz), 2.94 (dd, 1H, $J=6.8$, 18.8 Hz), 3.05–3.20 (m, 2H), 3.81 (t, 2H, $J=6.3$ Hz), 3.83 (t, 2H, $J=5.7$ Hz), 3.89 (s, 1H), 4.01(s, 1H), 4.10 (brd, 1H, $J=6.8$ Hz), 6.48 (s, 1H), 7.20–7.33 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.5, 163.3, 152.1, 142.1, 141.0, 134.3, 129.1, 129.0, 127.7, 127.4, 127.1, 127.0, 114.1, 61.3, 60.2, 53.6, 44.9, 44.6, 42.6, 34.0, 32.8; IR (neat): 3384, 2855,1687, 1618, 1527 cm^{-1}; MS (ESI-TOF) 439 $[M+H]^+$.
17. Spectra **18b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.71 (dd, 1H, $J=6.8$, 18.8 Hz), 2.89 (m, 1H), 2.96 (m, 1H), 3.04 (dd, 1H, $J=8.2$, 18.8 Hz), 3.07 (m, 2H), 3.82 (brs, 3H), 3.91 (dd, 1H, $J=6.8$, 8.2 Hz), 7.26–7.32 (m, 5H), 7.39–7.43 (m, 3H), 7.72 (s, 1H), 7.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.1, 140.8, 137.2, 133.3, 132.2, 131.5, 131.4, 130.3, 128.3, 128.2, 122.8, 84.8, 83.8, 79.0, 61.4, 50.3, 43.9, 36.0, 28.6; IR (neat): 3412, 2924, 1713, 1610 cm^{-1}; MS(ESI-TOF) 379 $[M+H]^+$.
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