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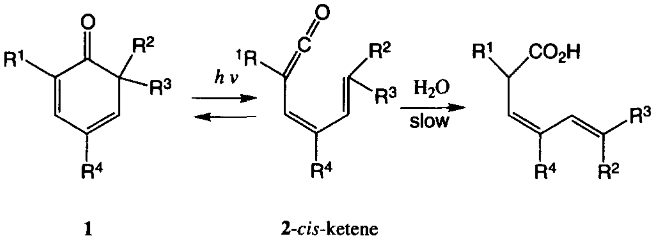
# PHOTOCHEMICALLY INDUCED SYMMETRICAL COUPLING REACTIONS BETWEEN CYCLOHEXA-2,4-DIENONES AND α,ω-DIAMINES. A NEW APPROACH TO THE SELECTIVE LABELLING OF PEPTIDES AND PROTEINS

Derek H.R. Barton,[a] Sung Kee Chung,[b] Young Mee Kim,[c] and Tae Woo Kwon[c*]

[a]*Department of Chemistry, Texas A&M University, College Station, TX 77843-3255, U.S.A.*
[b]*Department of Chemistry, Pohang University of Science & Technology, Pohang 790-784, Korea*
[c]*Department of Chemistry, Kyungsung University, Pusan 608-736, Korea*

**Abstract**: 2,4,6-Trimethylcyclohexa-2,4-dien-1-one derivatives undergo ring cleavage to furnish symmetrical amides on irradiation with visible light in the presence of various α,ω-diamines. Five different symmetrical amides containing a substituted diene moiety were synthesized in 65-85% yield.

On photolysis ortho-type cyclohexa-2,4-dien-1-ones **1** in water undergo a slow conversion to acids via nucleophilic trapping of the intermediate cis-ketene, **2**.[1] Besides the capture of the ketene **2** with water, a number of other nucleophiles have also been successfully employed in excellent yields. The mechanism is shown in Scheme I.[1-4]



**Scheme I**

Recently we reported the preparation of several water soluble cyclohexa-2,4-dienones of type **1** ($R^1$, $R^2 = CH_3$, $R^3 = CH_2SO_2Ph$ or $CH_2SO_2CH_3$, $R^4 = CH_2COOH$ or $CH_2P(O)(OCH_3)_2$) and the cleavage of the ring to form *cis*-ketenes by photolysis using a conventional mercury lamp (175W). Coupling reactions between cyclohexa-2,4-dienones and various amino acids and dipeptides (glycine, glycyl-glycine, glycyl-L-leucine) showed the preference of the *cis*-ketene for the amino-function rather than for the solvent water.[5]

Since the use of mercury lamps for the ketene formation might have some disadvantage due to damage of tryptophan and similar functions, we examined the possibility of ring cleavage of cyclohexa-2,4-dienones using other lamps and found that the use of visible light (tungsten lamp) was an excellent light source for the photochemical cleavage of 2,4,6-trimethylcyclohexa-2,4-dien-1-one derivatives.[6]

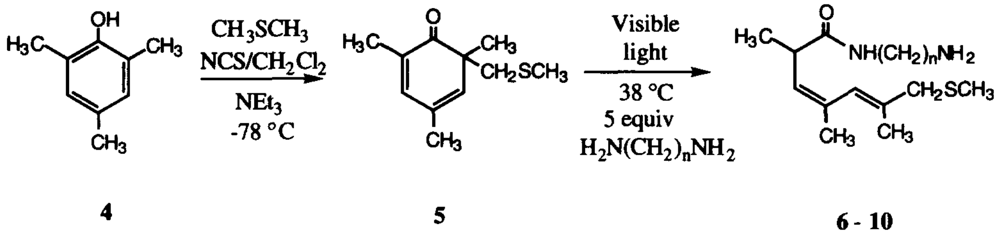
One application of this methodology is to prepare a series of difunctional molecules that would furnish traps for pairs of basic functions on a polypeptide or protein. Before preparing this new class of compounds, we decided to see if excess (5 equiv) of di-basic functionality such as in α,ω-diamines (n = 2, 3,...) could be suitably coupled with 1 equiv of cyclohexa-2,4-dienones without polymerization and then if 2 equiv of cyclohexa-2,4-dienones could be used to produce symmetrical bis-amides.

Herein we report that the photochemical coupling reactions between cyclohexadienone and various diamines, such as 1,4-diaminobutane, 1,8-diaminoctane, 1,10-diaminodecane, were successfully carried out using visible light irradiation to give the various amide products **6-10** in ethanol or ethanol-DMSO solvent below 38 °C.

The dienone **5** was easily prepared from the reaction of **4** with dimethylsulfide, *N*-chlorosuccinimide and triethylamine in $CH_2Cl_2$ at -78 °C.[7] The dienone **5** was very sensitive to acidic conditions and we were unable to purify it by using silica gel column chromatography. Such sensitivity is already known.[8] However, Kugelrohr vacuum distillation gave good quality dienone **5** (bath temperature = 130 °C/0.2 mm Hg). The reaction of the solvent (alcohol functional group) with dienone **5** was negligible. This result is in agreement with the previous report[6] in which the ketene intermediate captures the amine much faster than the alcohol functional group.

To carry out this irradiation, cold water with a magnetic stirrer in a large beaker (1 L) was used as external cooling. Ethyl alcohol (**6**, **7** and **8**) and a 1:1 ratio of ethyl alcohol:DMSO (**9** and **10**) were used as solvents since the long chain diamines were generally not soluble in diethyl ether or THF. Light from a tungsten lamp was employed for the dienone and diamine mixtures contained in standard Pyrex glassware. The solution was irradiated and the temperature kept below 38 °C. The reactions are summarized in Table 1.

**Table 1. Photolytic Reactions of Dienone 5 and Excess of Various α,ω-Diamines at 38-40 °C.**



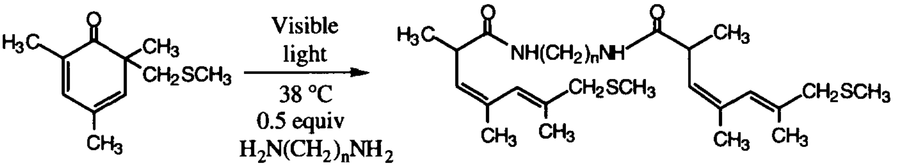
| Product | α,ω Diamine[a] (n) | Time (h) | Solvent[b] | $R_f$[c] | Yield (%)[d] |
|---|---|---|---|---|---|
| **6** | 2 | 2 | EtOH | 0.14 | 84 |
| **7** | 3 | 2 | EtOH | 0.17 | 86 |
| **8** | 4 | 2 | EtOH | 0.10 | 69 |
| **9** | 8 | 3 | EtOH:DMSO 1:1 | 0.14 | 75 |
| **10** | 10 | 3 | EtOH : DMSO 1:1 | 0.17 | 70 |

[a] The diamines were purchased from Aldrich and used without further purification. [b] Absolute ethanol was purified by standard methods. [c] 30% MeOH/70% ethyl acetate; TLC plates were made from E. Merck AG Darmstadt Silica gel 60 F254. [d] Yield of isolated product; all products were characterized by their 300 MHz $^1H$ NMR, $^{13}C$ NMR, FT-IR and high-resolution mass spectra.

We also have examined the photolysis reaction of **5** with diamines using a different ratio of reagents. When 1 equiv of diamine and 2 equiv of dienone were used, **11-15** were obtained as major products in ethyl alcohol. The reactions were usually completed within 5-6 h and gave 65-85% yield. The reaction time for **11-15** was approximately 2 times longer than that for **6-10**. One amine group required 2.5-3 h to capture the one ketene intermediate. The latter is, of course, formed reversibly.[14] The results are summarized in Table 2.

These experiments suggest that an approach to the selective functionalization of proteins should now be possible. If we construct a molecule that contains two blocked cyclohexa-2,4-dien-1-one units, the measurement of distance between functional groups should be possible. The rate of photolysis to give ketene will depend upon the intensity of the light. With low light intensity, one ketene will be formed before the other. Thus, the first formed ketene will react with a nitrogen nucleophile on (say) a protein. When the second ketene is formed, it will be tethered to the amide function which was first formed. It will be obliged to seek out a second nitrogen nucleophile at a distance which is fixed within the limits of the tether. When both ends are bonded to the same protein, the distance between the two nucleophiles will, within limits, be defined. Of course, if the tether is a fixed rod-like structure, then the distance between the two nucleophiles would indeed be defined. Thus we would have constructed molecular measuring rods.

**Table 2. Photolytic Reactions of Excess Dienone 5 and Various Diamines in EtOH at 38-40 °C.**



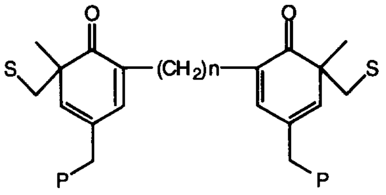
**5**

**11–15**: Major products
**6–10**: Minor products

| Product | (n) | Time (h) | $R_f$[a] | Yield (%)[d] | High-resolution Mass Spectrum | |
|---|---|---|---|---|---|---|
| | | | | | Calculation;$[M+H]^+$ | Found |
| **11** | 2 | 5.5 | 0.17[b] | 65 | $C_{24}H_{40}N_2O_2S_2$;453.26095 | 453.2610 |
| **12** | 3 | 5 | 0.25[b] | 77 | $C_{25}H_{42}N_2O_2S_2$;467.2766 | 467.2769 |
| **13** | 4 | 6 | 0.21[b] | 85 | $C_{26}H_{44}N_2O_2S_2$;481.2922 | 481.2903 |
| **14** | 8 | 3 | 0.20[c] | 70 | $C_{30}H_{52}N_2O_2S_2$;537.3548 | 537.3532 |
| **15** | 10 | 5 | 0.56[c] | 68 | $C_{32}H_{56}N_2O_2S_2$;565.3861 | 565.3836 |

[a]TLC plates were made from E. Merck AG Darmstadt Silica gel 60 F254. [b]100% $Et_2O$. [c]2% MeOH/98% $CH_2Cl_2$. [d]Yield of isolated product; all products were characterized by their 300 MHz, $^1$H NMR, FT-IR and high-resolution mass spectra.

To begin with, we plan to synthesize molecules of type **16** where P = dimethylphosphonyl and S = methyl- or phenyl-sulfonyl. Such molecules should be water soluble. The dimethylphosphonyl will make it possible to measure, by quantitative $^{31}$P NMR, the number of residues attached to a protein. If need be, mild hydrolysis would afford phosphonic acid residues and make the molecules more water soluble.



**16**

**Scheme 2**

More advanced molecular measuring rods would have the flexible $(CH_2)_n$ chain replaced by more rigid groups to define distances more precisely. So far, we have not mentioned DNA. The same arguments apply here. If the basic residues that react are guanine derived, then distance between these residues could also be defined.

## Experimental

A typical experimental procedure for tungsten irradiation photolysis of 2,4,6-trimethyl-6-methylthiomethylenecyclohexa-2,4-dien-1-one **5** is as follows: Light from a tungsten lamp (220 W) was employed to irradiate the dienone solution **5** (150 mg, 0.781 mmol, 1 equiv) in 3 mL of freshly distilled ethyl alcohol in the presence of 1,2-ethylene diamine (234 mg, 3.90 mmol, 5 equiv). The solution was then irradiated at a distance of 2 cm and the temperature kept below 38 °C by external water cooling. The reactions were monitored by TLC and were completed within 2 h. The volatiles were removed in vacuo and the crude products were diluted with 20 mL of $CH_2Cl_2$ and washed with water (2 × 10 mL). The organic phases were dried over $MgSO_4$, filtered, and the $CH_2Cl_2$ was removed in vacuo. The residue was subjected to flash chromatography (MeOH:ethyl acetate, 3:7) over silica gel; $C_{13}H_{24}N_2OS$; $[M+H]^+$ 257.16876/Found 257.1703.

Typical experimental procedure for tungsten irradiated production of **11** is the same as above except for the ratio of **5** and 1,2-ethylene diamine. A tungsten lamp (220 W) was employed to irradiate the dienone solution **5** (150 mg, 0.781 mmol, 1 equiv) in 3 mL of freshly distilled ethyl alcohol in the presence of 1,2-ethylene diamine (23.4 mg, 0.390 mmol, 0.5 equiv). The reaction was completed within 5.5 h. The volatiles were removed in vacuo and the crude product was diluted with 20 mL of $CH_2Cl_2$ and washed with 10% HCl (2 × 10 mL). After drying over $MgSO_4$, the residue was subjected to flash chromatography (100% $Et_2O$) over silica gel.

## Acknowledgments

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