RASĀYAN *J. Chem.*
Vol. 16 | No. 1 |122-128| January - March | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# SYNTHETIC STUDY OF Pc-2, AN ACTIVE COMPOUND FROM INDONESIAN RED BETEL LEAF

**Ritmaleni[1,✉] and Dong-Hwi Kim[2]**
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia 55281
[2]Merck Ltd. Korea, 3 Floor Haesung-2-Bldg, 508 Teheran-ro, Gangnam-gu, Seoul, Republic of Korea, 06178
[✉]Corresponding Author: ritmaleni@ugm.ac.id

**ABSTRACT**
PC-2 is one of the active compounds isolated from Indonesian red betel leaf. The structure of the compound is named 2-allyl-4-(1'-hydroxy-1'-(3",4",5"-trimethoxyphenyl)propan-2'-yl)-3,5-dimethoxycyclohexa-3,5-dienone and included in the neolignan's structure category. This compound was reported to have very good biological activity in giving antiproliferative effects on human breast (T47D) cells. From 2.12 g of its leaf methanolic extract, only 12.1 mg of Pc-2 was collected. This amount is too small to do more studies about its biological activities. The synthetic work should be applied to get a sufficient amount. This paper is aimed to explain the synthetic study and design of the Pc-2 compound. The first approach is by involving the aldol reaction in the synthetic pathway through the disconnection approach. The result showed that this attempt did not work very well. The second approach is to design the new synthetic pathway by using the synthia Organic Retrosynthesis Software by Merck. This software gave a reasonable result to be tried. The result proposed the use of the Suzuki coupling reaction for the new synthetic pathway of Pc-2.
**Keywords:** Active Compound, Disconnection Approach, Indonesian Red Betel Leaf, PC-2, Synthia Retrosynthesis Software, Synthetic Study

RASĀYAN *J. Chem.*, Vol. 16, No.1, 2023

## INTRODUCTION

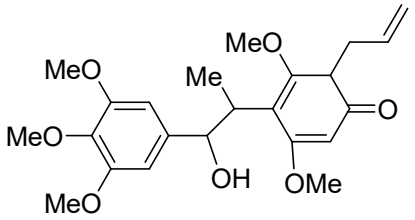
Many plants have been reported to have various kinds of medicinal activities in different traditional medicines in Indonesia. Some of that consist of plants from the genus Piper. The study about the potency of Betel leaves extracts as antibiofilm has been reported against Methicilin Resistant Staphylococcus aureus (MSRA).[1] In Indonesia, one of the genus Piper plants is Red betel (Piper crocatum Ruiz & Pav). This species has red silvery leaves. It is used to use as a medicinal plant for treating various diseases. Its scientific approval also has been investigated and published in many publications around the world.[2] A bioactive compound from red betel leaves was studied in silico for its activity as an anticancer agent against colon cancer marker proteins.[3] Antibacterial activity of the phytochemical compound of this red betel leaf has also been studied against bacteria in dental caries.[4] The methanolic extract of this Red betel (Piper crocatum Ruiz & Pav) was reported as antiproliferative effect on human breast (T47D) cells.[5] The active compound from Red betel also has been isolated from this methanolic extract. It was found that the active compound showed an immunomodulatory effect and histopathological features of the mouse liver and kidney. This isolated compound was then named PC-2. Pc-2 can increase macrophage phagocytosis as well as nitric oxide production but not lymphocyte proliferation. The compound was code as Pc-2 where its chemical name is 2-allyl-4-(1'-hydroxy-1'-(3",4",5"-trimethoxyphenyl)propan-2'-yl)-3,5-dimethoxycyclohexa-3, 5-dienone. 2.12 g of this red betel leaf methanolic extract yielded 12.1 mg of Pc-2 only. The structure of Pc-2 is shown below.[6] This is a neolignan-classified structure compound.[7]

This report is a model study of the synthesis of Pc-2. The Synthetic design of this compound is by cleavage of the bond in the middle that connects both aromatic rings. The band has hydroxyl and methyl groups attached.

Synthon-1 and synthon-2 could be combined through the aldol reaction. This paper explains the study of the main reaction that might be involved in the synthesis of Pc-2. The process will start with the compound-like of synthon-1. Only synthon-1 is being focused on. The general synthetic equivalent of synthon-1 is an

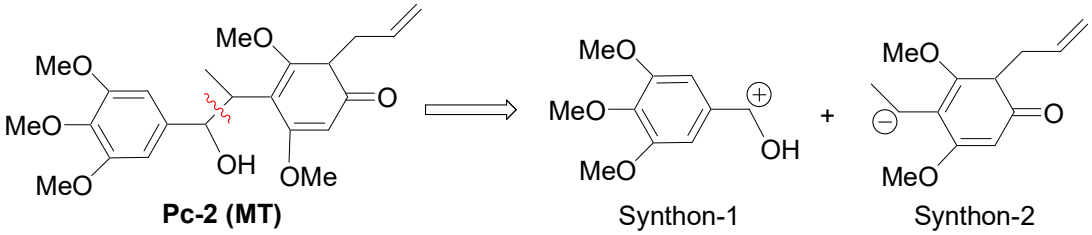

**http://doi.org/10.31788/RJC.2023.1617052**

aldehyde. So, it can be started by using the below disconnection approach as a model. This study only reports the model synthesis of the aldehyde compound of synthon-1.

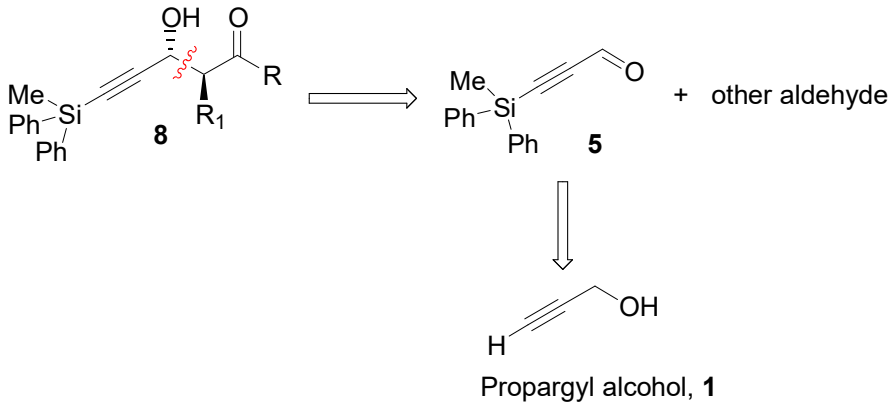



**Pc-2**

Fig.-1: Structure of Pc-2




Scheme-1: Cleavage Between Both Aromatic Rings on Pc-2

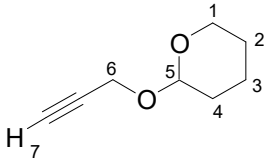



Scheme-2: Disconnection Approach of Compound 8

This research is aimed to synthesize the Pc-2 compound and study the possibility of the synthetic pathway of that compound.

## EXPERIMENTAL

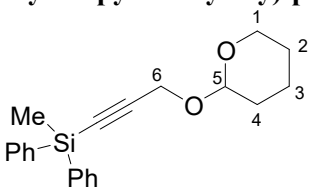
### Synthesis of 2-Prop-2-ynyloxy-tetrahydropyran, 2




To a round bottom flask (2L), propargyl alcohol (10 g; 178 mmol; 1 eq) was added into DCM (900 mL) at 0 °C. Then DHP (40.6 mL; 446 mmol; 2.5 eq) and TsOH (3.066 g; 17.8 mmol; 10 mol%) were added respectively. The color of the reaction mixture was changed from transparent brown to black. The reaction mixture was left for 1.5 h for completion. Then it was quenched with $NaHCO_3$ (aq), extracted with $CHCl_3$ (2 X 200 mL) and the organic phase was evaporated under vacuum. The product was isolated by using flash column chromatography (EtOAc: Hexane = 1: 9), yielded a colorless oil in 80 %, Rf = 0.36 (EtOAc: Hexane = 1: 9); $^1$H-NMR ($CDCl_3$, 400 MHz) δ 1.45-1.55 (1H, m, 3-$CH_2$), 1.55-1.61 (1H, m, 3-$CH_2$), 1.67-1.76 (2H,

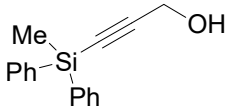
m, 2-$CH_2$), 1.78-1.83 (2H, m, 1-$CH_2$), 2.389 (1 H, s, H-C≡ ), 2.38-3.51, (1H, m, 4-$CH_2$), 3.72-3.86 (1H, m, 4-$CH_2$), 4.16-4.28 (2H, m, 6-$CH_2$), 4.55 (1H, s, 7-H).

**Synthesis of Methyl-diphenyl-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-silane, 3**



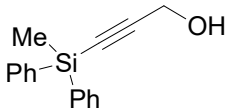

To neck round bottom flask (100 mL), Propargyl-OTHP (7.13 mmol; 1 g) was added to THF (18 mL) at -78 °C and followed by adding of n-BuLi (8.556 mmol; 5.889 mL). After 1 h, the reaction was cooled to -78 °C and Diphenyl-methyl-silane chloride (8.556 mmol; 1.8 mL) was added to the mixture. The yellowish transparent color was changed into a colorless solution in 1 h to reach r.t. The reaction mixture was quenched with buffer (10 mL), extracted with EtOAc (3 x 30 mL), and washed with water (2 x 10 mL) and brine (1 x 10 mL). The organic phase was dried over $Na_2SO_4$, filtered, and evaporated under a vacuum. The product was isolated by using flask column chromatography (EtOAc: Hexane = 1: 15) yielded a colorless oil in 44 %, Rf = 0.36 (EtOAc: Hexane = 1: 15); $^1$H-NMR ($CDCl_3$, 400 MHz) δ 0.7 (3H, s, -$CH_3$), 1.53-1.68 (4H, m, 3-H and 2-H), 1.71-1.87 (2H, m, 1-H), 3.50-3.55 (2H, m, 4-H), 3.83-3.89 (1H, m, 4-H), 4.37 (2H, s, 5-$CH_2$), 7.34-7.43 (6H, m, Ph), 7.63-7.65 (4H, m Ph); $^1$C-NMR ($CDCl_3$, 100 MHz) 19.20, 25.51, 30.42, 55.02, 62.21, 96.99, 128.07, 129.85, 134.66; IR (neat): νmax 2942, 1428, 1117, 1027,792, 728cm$^{-1}$; HRMS (ESI): $[M+Na]^+$ calculated for $[C_{21}H_{24}NaO_2Si]^+$: 359.1443, foun: 359.1443

**Synthesis of 3-(Methyl-diphenyl-silanyl)-prop-2-yn-1-olcohol, 4**




To dimethyl-phenyl-OTHP (4.62 g; 13.73 mmol) in two necks round bottom flask (100 mL), was added amberlyst-15 (20 wt %, 924 mg) and MeOH (28 mL, 0.5 M). The reaction mixture was refluxed for 1.5 h. Product 4 was isolated by flash column chromatography (EtOAc: Hexane = 1: 9, gradually changed to EtOAc: Hexane = 1: 5 ) and yielded a yellowish-colorless oil in 91 %, Rf = 0.09 (EtOAc: Hexane = 1: 9); $^1$H-NMR ($CDCl_3$, 400 MHz) δ 0.73 (3H, s, CH3), 1.77 (1H, s, OH), 4.36 (2H, s, CH2), 7.37-7.45 (6H, m, Ph), 7.62-7.67 (4H, m, Ph); $^{13}$C-NMR ($CDCl_3$, 100 MHz); IR (neat): νmax 3336, 3068, 2176, 1588, 1428, 1114, 1040, 984, 793 cm$^{-1}$; HRMS (ESI) : $[M+Na]^+$ calculated for $[C_{16}H_{16}NaOSi]^+$: 275.0868, foun,: 275.0868

**Synthesis of 3-(Methyl-diphenyl-silanyl)-prop-2-yn-1-olcohol, 5**

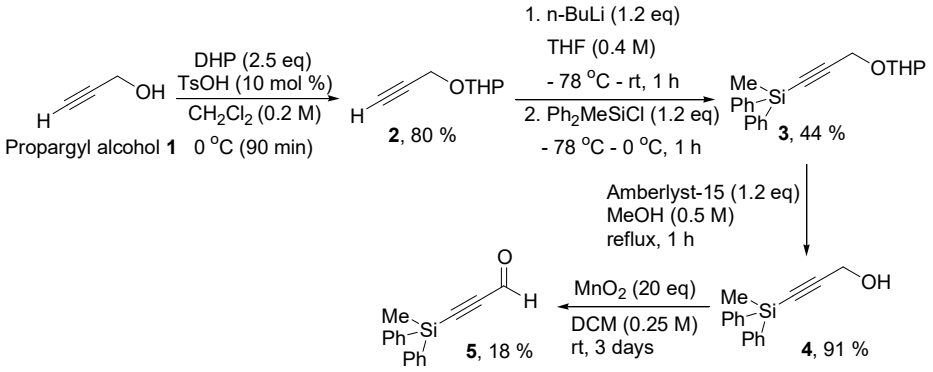



To dimethyl-phenyl-propargyl-OH (2.5 g; 9.91 mmol) in two necks round bottom flask (100 mL), was added $MnO_2$ (20 eq. 17,136 mg) and DCM (40 mL, 0.25 M). The reaction mixture was left for 3 h at room temperature. After filtration and solvent evaporated, product 5 was isolated by using flash column chromatography (EtOAc: Hexane = 1: 9) yielded a colorless oil in 18 %, Rf = 0.41 (EtOAc: Hexane = 1: 9); $^1$H-NMR ($CDCl_3$, 400 MHz) δ 0.82 (3H, s, CH3), 7.48-7.64 (6H, m, Ph), 7.66-7.663 (4H, m, Ph), 9.26 (1H, s, CHO); $^{13}$C-NMR ($CDCl_3$, 100 MHz); IR (neat): νmax 3070, 2153, 1666, 1429, 1387, 1116, 997, 795, 731, 697 cm$^{-1}$; HRMS (ESI) : $[M+Na]^+$ calculated for $[C_{16}H_{14}NaOSi]^+$: 273.0712, foun: 273.0709.

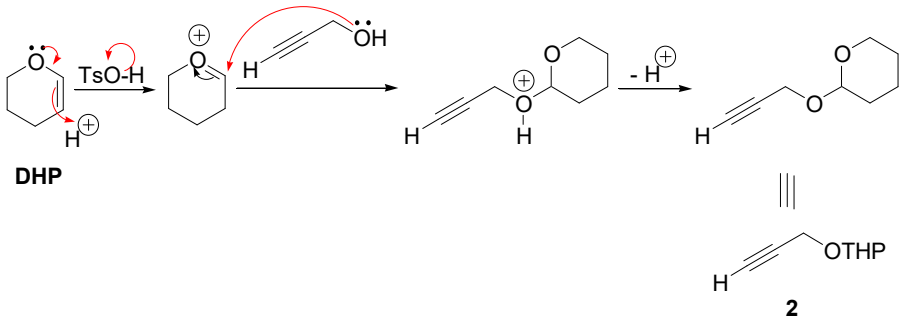
## RESULTS AND DISCUSSION

The whole process of reaction can be seen in schemes 3-7. Propargyl alcohol was transformed into protected propargyl alcohol and then removed from the protecting group to the alcohol group again. This alcohol group was next to be oxidized into an aldehyde.

Starting with the protection of propargyl alcohol, the given condition yielded compound 2 in 80 %. The reaction mechanism is as in Scheme-3. In the beginning, 3,4-DiHydro-2H-pyran (DHP) was protonated by p-Toluenesulfonic acid (TsOH·H2O) resulting in a positive charge on the oxygen of DHP. This species was attacked by Propargyl alcohol which resulted in protected propargyl alcohol.

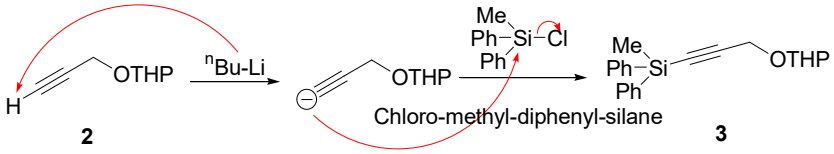



Scheme-3: Synthesis of Aldehyde 5



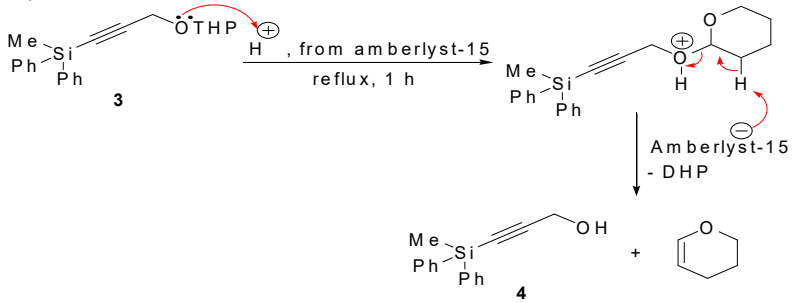

Scheme-4: Reaction Mechanism for the Formation of Compound **2**

Sodium bicarbonate was to quench p-Toluenesulfonic acid. The next reaction is the formation of 3 by reacting compound 2 with chloromethyl diphenyl silane. After one hour, the reaction was stopped and the product was tried to be isolated, which was resulting in failing to be isolated. Buffer was to quench n-butyl lithium. Compound 3 can be successfully isolated in 44 % pure isolated compound. The reaction mechanism is as follows where nBuLi acted as the catalyst.



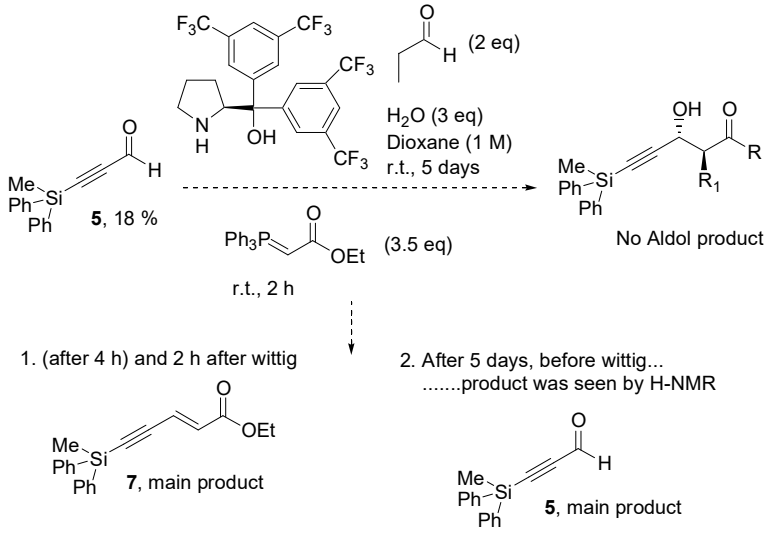

Scheme-5: Reaction Mechanism for the Formation of Compound **3**

Compound 3 was subjected to the next reaction, which yielded 4 in 91 %. The protection reaction was carried out by amberlyst-15 for one hour.



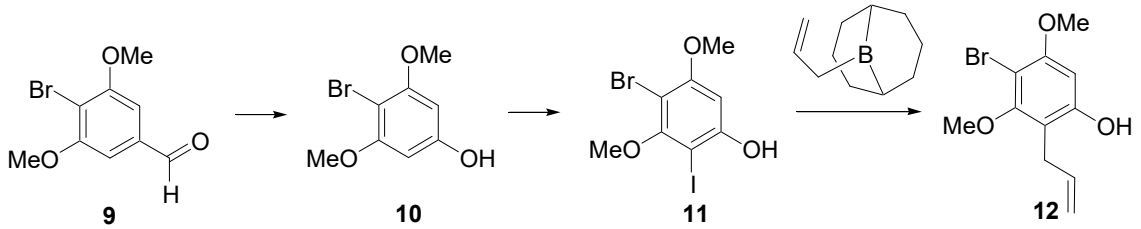

Scheme-6: The Reaction Mechanism for the Formation of Compound **4**

The reaction was continuous to the next step where alcohol was oxidized to yield compound 5 in 18 %. Compound 5 was then reacted with the aldehyde and ester phosphine. So far, no aldol product was obtained even after five days of reaction. Hence, after four hours of reaction without further isolation and the ester phosphine was added. Only product 7 was observed as the main product of the Wittig reaction. The organocatalyst, (R)-diarylprolinol did not help much yet.[8]



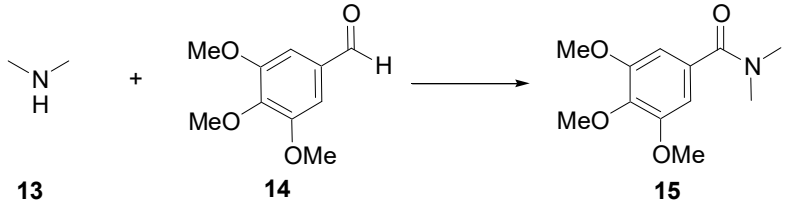

Scheme-7: Attempt to Form the Aldol Product

According to Scheme-3 and Scheme-7, the aldol product, β-methyl alcohol cannot be successfully formed. This pathway was not successful yet when applied to the synthesis of Pc-2. The other proposed synthetic pathway is initiated by the oxidation of aryl aldehydes to phenols/hydroxyheteroaryls via formats (Scheme-8). Compound 4-bromo-3,5-dimethoxy-benzaldehyde 9 is converted to 4-bromo-3,5-dimethoxyphenol 10 with $H_2O_2$ or mCPBA. Iodination of aromatic compounds, 4-bromo-3,5-dimethoxyphenol 10 to $C_8H_8BrIO_3$ 11, by $I_2$ or other iodinating agents e.g. NIS.[9-11] Suzuki coupling of alkyl-9-BBNs with aryl iodides perform $C_8H_8BrIO_3$ 11 and 9-allyl-9-bora-bicyclo[3.3.1]nonane to $C_{11}H_{13}BrO_3$ 12 by using Pd catalyst in a basic solvent.[11-14] Suzuki coupling is the palladium-catalyzed cross-coupling between organoboronic acid and halides. Recently the catalyst and the method have been broadly developed especially the use of 9-BBN.[16-21]



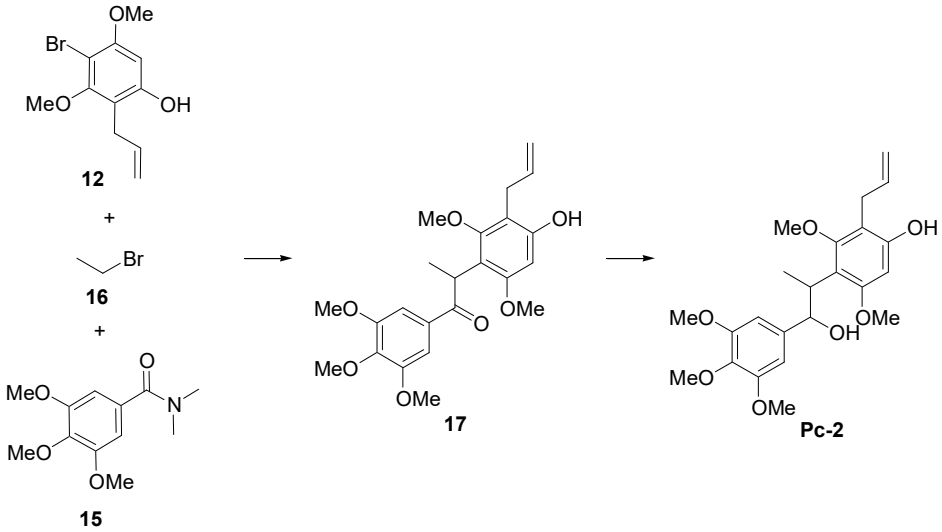

Scheme-8: The Synthesis of Suzuki Coupling Product

The next step (Scheme-9) is the synthesis of secondary amides from aldehydes, 3,4,5-trimethoxy-benzaldehyde 14 and n-methyl-methanamine 13 turn into 3,4,5-trimethoxy-benzoic acid dimethylamide 15 in CuI.MS 4A, toluene, 100 °C and air.[22]




Scheme-9: The Synthesis of the Secondary Amide

One pot arylative synthesis of ketones from benzamides is the further reaction where $C_{11}H_{13}BrO_3$ 12 and 3,4,5-tri methoxy-benzoic acid diethylamide 15 and bromoethane 16 turn into $C_{23}H_{28}O_7$ 17 by $Pd[(PtBu)_3]_2$ as a catalyst in THF at 60 °C.[23] Last reaction is the reduction of ketones with $NaBH_4$, $C_{23}H_{28}O_7$ 17 to $C_{23}H_{30}O_7$ Pc-2 with $NaBH_4$ as the catalyst in MeOH at 0 °C. This is a very common chemistry seen in basic chemistry textbooks (Scheme-10).




Scheme-10: The Synthesis of the Target Molecule, Pc-2

## CONCLUSION

The synthetic study of Pc-2, isolated from Indonesian Red betel leaf, has been studied in two methods of pathways. The first method was carried out in the laboratory. Throughout the whole reaction, the last step of reactions is still not working. This is the attempt to form the product of the aldol reaction. The second method is the proposed pathway by Synthia Retrosynthesis Software and seems that it will work very well.

## ACKNOWLEDGMENTS

This work was partially supported by Prof. Yujiro Hayashi from Tohoku University and Dong-Hwi Kim from Merck for the proposed synthetic pathway using the Synthia program.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Ritmaleni http://orchid.org/0000-0003-4934-7673
Dong-Hwi Kim http://orchid.org/0000-0002-0870-8498



## REFERENCES

1. A. Asnani, P. Ruwielanisa, Hernayanti, D. U., Anjarwati, in Proceedings of *AIP Conference Proceedings* of 6th Joint Conference on Chemistry: Visualizing the Latest Up-Front of Chemistry Research and Education for Better Future, Purwokerto, Indonesia, 020021(2022), https://doi.org/10.1063/5.0103681

2. A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, M. J. Thun, *CA: A Cancer Journal of Clinicians*, **59**, 225(2009), https://doi.org/10.3322/caac.20006
3. M. A. Umar, M. Safitri, R. Pratama, *Hayati Journal of Biosciences*, **30**, 1(2023), https://doi.org/10.4308/hjb.30.1.113-121
4. L. Heliawati, S. Lestari, U. Hasanah, D. Ajiati, *Molecules*, **27**, 9(2022), https://doi.org/10.3390/molecules27092861
5. R. L. Siegel, K. D. Miller, A. Jemal, *CA: A Cancer Journal of Clinicians*, **65**, 5(2015), https://doi.org/10.3322/caac.21254
6. Y. Safdari, M. Khalili, M. A. Ebrahimzadeh, Y. Yazdani, and S. Farajnia, *Pharmacological Research*. **93**, 1(2015), https://doi.org/10.1016/j.phrs.2014.12.004
7. Y. Safdari, M. Khalili, S. Farajnia, M. Asgharzadeh, Y. Yazdani, M. Sadeghi, *Clinical Biochemistry*, **47**, 13(2014), https://doi.org/10.1016/j.clinbiochem.2014.05.066
8. T. M. Brand, and D. L. Wheeler, *Cancer Biology and Therapy*, **11**, 3(2011), https://doi.org/10.4161/cbt.11.3.14696
9. B. T. Hennessy, D. L. Smith, P. T. Ram, Y. Lu, and G. B. Mills, *Nature Reviews Drug Discovery*, **4**, 988(2005), https://doi.org/10.1038/nrd1902
10. A. Joshi, M. Gadhwal, and U. J. Joshi, *International Journal of Pharmacy and Pharmaceutical Sciences*, **7(6)**, 77(2015).
11. P. Erasto, D. S. Grierson, and A. J. Afolayan, *Food Chemistry*, **104(2)**, 636(2007), https://doi.org/10.1016/j.foodchem.2006.12.013
12. A. N. Magpusao, G. Omolloh, J. Johnson, J. Gascon, M. W. Pechzuh, and G. Fenteany, *Chemical Biology*, **10(2)**, 561(2015), https://doi.org/10.1021/cb500665r
13. A. M. Marzouk, and O. B. Abd Elhalim, *Natural Product Research*, **30(7)**, 741(2015), https://doi.org/10.1080/14786419.2015.1062004
14. Hajime Ohigashi,Mitsuo Jisaka,Teruyoshi Takagaki,Hiroshi Nozaki,Toshiji Tada,Michael A. Huffman,Toshisada Nishida,Mikio Kaji and Koichi Koshimizu, *Agricultural and Biological Chemistry*, **55(4)**, 1201(1991), https://doi.org/10.1080/00021369.1991.10870699
15. S. Patel, *Biomedicine & Pharmacotherapy,* **84**, 1036(2016), https://doi.org/10.1016/j.biopha.2016.10.030
16. Jing Wang, Hua Song, Xiaoxue Wu, Shuyi Zhang, Xuemin Gao, Funan Li, Xuan Zhu and Qing Chen, *Molecules*, **23(3)**, 579(2018), https://doi.org/10.3390/molecules23030579
17. R. A. Syahputra, U. Harahap, A. Dalimunthe, P. Nasution, G. Haro, Widodo, D. H. Utomo and D. Satria, *Rasayan Journal of Chemistry,* **13(2)**, 796(2020), https://doi.org/10.31788/RJC.2020.1325638
18. P. A. Z. Hasibuan, P. Sitorus, D. Satria, *Asian Journal of Pharmaceutical Clinical Research,* **10(5)**, 306(2017), https://doi.org/10.22159/ajpcr.2017.v10i5.16931
19. J. Wang, H. Ji, X. Niu, L.Yin, Y. Wang, Y. Gu, D. Li, H. Zhang, M. Lu, F. Zhang and Q. Zhang, *Disease Markers*, Article ID 6103542, 1-12(2020), https://doi.org/10.1155/2020/6103542
20. S. Sigismund, D. Avanzato and L. Lanzetti, *Molecular Oncology*, **12(1)**, 3(2018), https://doi.org/10.1002/1878-0261.12155
21. A. C. de Molo, E. Paulino and A. H. I. Garces, *Oxidative Medicine and Cellular Longevity*, Article ID 4809751, 1-8(2017), https://doi.org/10.1155/2017/4809751
22. Franklyn Nonso Iheagwam, Olubanke Olujoke Ogunlana, Oluseyi Ebenezer Ogunlana, Itunuoluwa Isewon, Jelili Oyelade, *Bioinformatics and Biology Inshights*, 13(2019), https://doi.org/10.1177/1177932218821371
23. J. L. Wang, L. Li, M .B. Hu, B. Wu, W. X. Fan, W. Peng, D. N. Wei, C. J. Wu, *Computational Biology and Chemistry*, **78**, 297(2019), https://doi.org/10.1016/j.compbiolchem.2018.12.021

[RJC-7052/2020]