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# Asymmetric Total Synthesis and Structural Elucidation of NFAT-68

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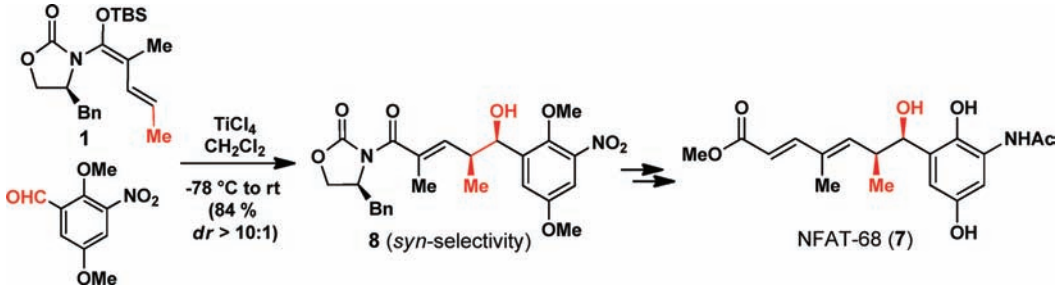
## ABSTRACT




**Total synthesis of NFAT-68 (7) has been achieved and its relative stereochemistry has been determined. A key step thereof is the utilization of the chelation-controlled vinylogous Mukaiyama aldol reaction (VMAR) to stereoselectively synthesize the *syn*-aldol product 8. This developed chemistry is anticipated to have wider application in total syntheses of many other natural products.**

Developing efficient strategies to access optically pure compounds is at the forefront of synthetic organic chemistry. The Evans-aldol reaction has been regarded as one of the premier methods to achieve that goal.[1] Many efforts were observed in designing an extension of the Evans-aldol reaction.[2] Among those, Evans' chiral auxiliary derived vinylogous Mukaiyama aldol reaction (VMAR), which was developed by Kobayashi and co-workers,[3] turned out to be able to stereoselectively construct δ-hydroxyl-α,γ-dimethyl-α,β-unsaturated units. The latter are seen as common substructures in many natural products as well as important synthetic intermediates.[4]

Recently, our group has been devoted to undertaking the diversity-oriented synthesis of maytansinoids, a family of 19-membered macrolides with potent antimitotic activity, by way of disrupting microtubule assembly.[5] We desired to identify an efficient way to generate structurely diverse δ-hydroxyl-α,γ-dimethyl-α,β-unsaturated units via the VMAR. In this context, our experiments revealed that the stereochemistry of VMAR adducts could be regulated by either adding additives[6] or changing the chelation state of sub-

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(1) (a) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1. (b) Heathcock, C. H. In *Comprehensive Carbonion Chemistry*; Buncel, E., Durst, T., Eds.; Elsevier: New York, 1984; Vol. 5B, p 177. (c) Kim, B. M.; Williams, S. F.; Masumune, S. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Heathcock, C. H., Eds.; Pergamon: Oxford, UK, 1991; Vol. 2, Chapter 1.7, p 239.

(2) Carreira, E. M. In *Catalytic Asymmetric Synthesis*, 2nd ed.; Ojima, I., Ed.; Wiley-VCH: Weinheim, Germany, 2000; Chapter 8B2.

(3) (a) Shirokawa, S.-I.; Kamiyama, M.; Nakamura, T.; Okada, M.; Nakazaki, A.; Hosokawa, S.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 13604. (b) Shinoyama, M.; Shirokawa, S.-i.; Nakazaki, A.; Kobayashi, S. *Org. Lett.* **2009**, *11*, 1277. (c) Nakamura, T.; Shirokawa, S. I.; Hosokawa, S.; Nakazawk, A.; Kobayashi, S. *Org. Lett.* **2006**, *8*, 677. (d) Yamaoka, M.; Nakazaki, A.; Kobayashi, S. *Tetrahedron Lett.* **2009**, *50*, 3849. (e) Yamaoka, M.; Fukatsu, Y.; Nakazaki, A.; Kobayashi, S. *Tetrahedron Lett.* **2009**, *50*, 6764. (f) Yamaoka, M.; Nakazaki, C.; Kobayashi, S. *Tetrahedron Lett.* **2010**, *51*, 287.

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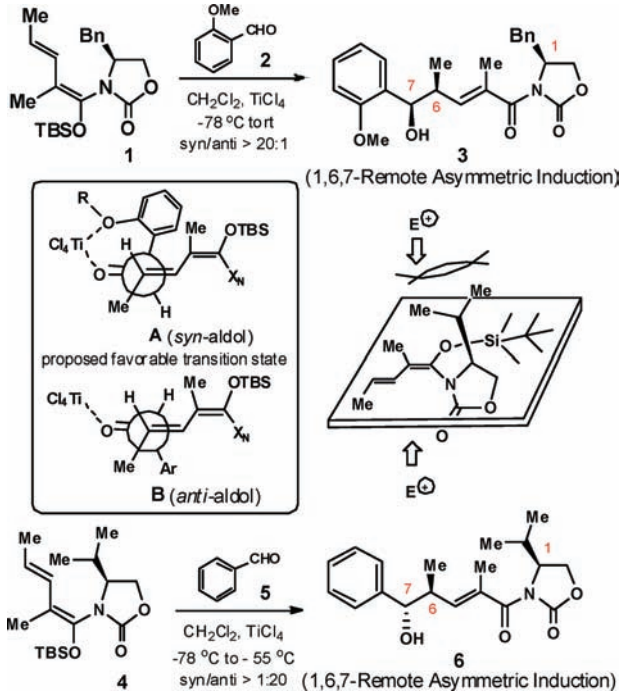
strates.[7] We further found that by incorporating a chelation element at the ortho-position of benzaldehydes, the *syn*-aldol adduct **3** could be obtained as the major product through transition state **A** (Scheme 1). In constrast, according to the

**Scheme 1.** Vinylogous Mukaiyama Aldol Reaction




report by Kobayashi and co-workers,[3a] when benzaldehyde was employed as the substrate, the *anti*-aldol adduct **6** would be formed predominantly through transition state **B** (Scheme 1).

(4) (a) O'Hagan, D. *The Polyketide Metabolites*; Ellis Horwood: Chichester, U.K., 1991. (b) O'Hagan, D. *Nat. Prod. Rep.* **1995**, *12*, 1. (c) Katz, D.; Donadio, S. *Annu. Rev. Microbiol.* **1993**, *47*, 875. (d) Higashide, E.; Asai, M.; Ootsu, K.; Tanida, S.; Kozai, Y.; Hasegawa, T.; Kishi, T.; Sugino, Y.; Yoneda, M. *Nature (London)* **1977**, *270*, 721. (e) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Hoto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031. (f) Mandala, S. M.; Thornton, R. A.; Rosenbach, M.; Milligan, J.; Garcia-Calvo, M.; Bull, H. G.; Kurtz, M. B. *J. Biol. Chem.* **1997**, *272*, 32709. (g) Nakamura, N.; Takahashi, Y.; Naganawa, H.; Nakae, K.; Imoto, M.; Shiro, M.; Matsumura, K.; Watanabe, H.; Kitahara, T. *J. Antibiot.* **2002**, *55*, 442. (h) Hong, Y.-S.; Lee, D.; Kim, W.; Jeong, J.-K.; Kim, C.-G.; Sohng, J. K.; Lee, J.-H.; Paik, S.-G.; Lee, J. J. *J. Am. Chem. Soc.* **2004**, *126*, 11142. (i) Aldrich, C. C.; Venkatraman, L.; Sherman, D. H.; Fecik, R. A. *J. Am. Chem. Soc.* **2005**, *127*, 8910. (j) Hosokawa, S.; Ogura, T.; Togashi, H.; Tatsuta, K. *Tetrahedron Lett.* **2005**, *46*, 333. (k) Hosokawa, S.; Yokota, S.; Imamura, K.; Suzuki, Y.; Kawarasaki, M.; Tatsuta, K. *Tetrahedron Lett.* **2006**, *47*, 5415. (l) Hosokawa, S.; Kuroda, S.; Imamura, K.; Tatsuta, K. *Tetrahedron Lett.* **2006**, *47*, 6183. (m) Andrus, M. B.; Wong, Y.; Liu, J.; Beebe, K.; Neckers, L. M. *Tetrahedron Lett.* **2009**, *50*, 6701. (n) Hosokawa, S.; Matsushita, K.; Tokimatsu, S.; Toriumi, T.; Suzuki, Y.; Tatsuta, K. *Tetrahedron Lett.* **2010**, *51*, 5532.

(5) (a) Kupchan, S. M.; Branfman, A. R.; Sneden, A. T.; Verma, A. K.; Dailey, R. G., Jr.; Komoda, Y.; Nagao, Y. *J. Am. Chem. Soc.* **1975**, *97*, 5294. (b) Sneden, A. T.; Beemsterboer, G. L. *J. Nat. Prod.* **1982**, *45*, 624. (c) Remillard, S.; Rebhum, L. I.; Howie, G. A.; Kupchan, S. M. *Science* **1975**, *189*, 1002. (d) Huang, A. B.; Lin, C. M.; Hamel, E. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 1239.

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In this paper, we report our recent progress about the asymmetric total synthesis and structure elucidation of NFAT-68 (**7** in Figure 1) via the chelation-controlled VMAR as a key step.

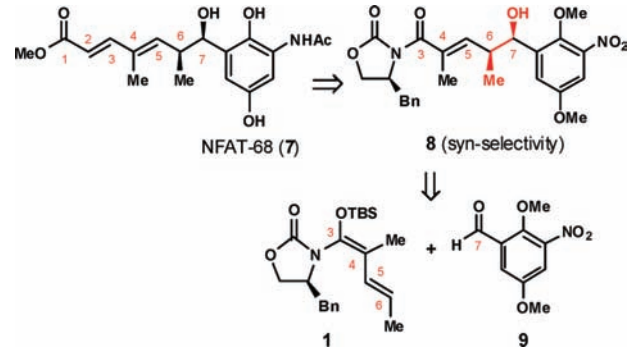



**Figure 1.** Retrosynthetic analysis.

NFAT-68 (**7** in Figure 1), a polyketide from the fermentation broth and mycelia of two *Streptomyces* sp. was discovered with NFAT-/lacZ $\beta$-galactosidase reporter gene construct assay.[8]

The primary screening indicates that NFAT-68 is a potent immunosuppressant with the $IC_{50}$ concentrations less than 1 $\mu$g/mL, and that no apparent toxicity is observed at this concentration. Therefore, NFAT-68 may become a useful probe for studying signaling pathways of early T cell activation.

The structure of NFAT-68 (**7**) was assigned based on analyzing its NMR spectra. However, neither its stereochemistry nor optical rotation was reported in the original article.[8] Hence in the meantime of pursuing the total synthesis of NFAT-68 (**7**), our other mission is to validate its structure.

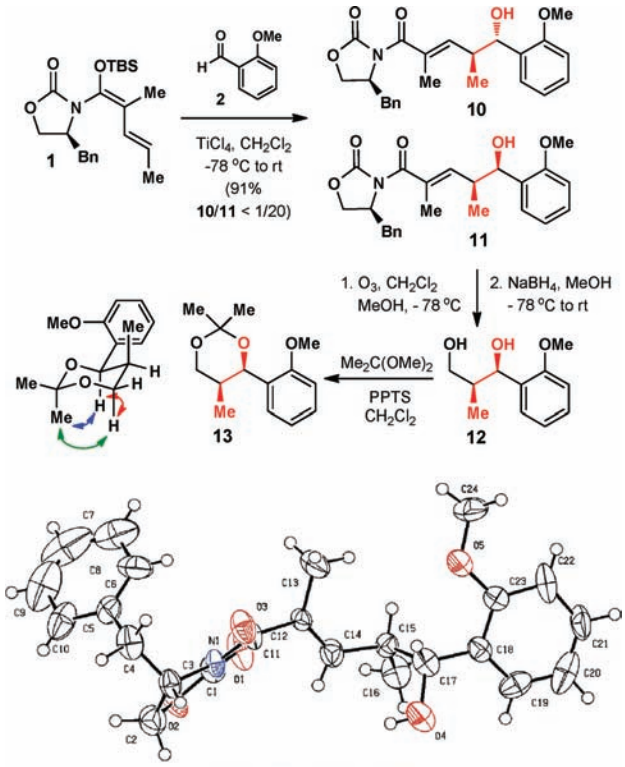
To resolve the structural issue of NFAT-68, we initially assumed that the stereochemistry at C6 and C7 in NFAT-68 is a *syn*-relationship. We therefore expected that our recently developed chelation-controlled VMAR could potentially be applied to the synthesis of the $\delta$-hydroxyl-$\alpha,\gamma$-dimethyl-$\alpha,\beta$-unsaturated unite in natural product NFAT-68. Our retrosynthetic analysis of NFAT-68 is outlined in Figure 1. We envisaged that NFAT-68 could be derived from intermediate **8**, which in turn could be generated from **1** and **9** via the chelation-controlled VMAR (Figure 1).

Our synthesis began with investigating the chelation effect of substrate on the diastereoselectivity of the aldol adducts of VMARs. To this end, vinylketene silyl N,O-acetal **1**[7] was reacted with 2-methoxybenzaldehyde **2** under the conditions listed in Scheme 2. Delightfully, adducts **10** and **11** were obtained in 91% combined yield, and the ratio of **10**/**11** is 1/20 (Scheme 2).

The stereochemistry for **11** was established by both NMR and X-ray single crystal analysis. These studies demonstrate

(8) Burres, N. S.; Premachandran, U.; Hoselton, S.; Cwik, D.; Hochlowski, J. E.; Ye, Q.; Sunga, G. N.; Karwowski, J. P.; Jackson, M.; Whittern, D. N.; McAlpine, J. B. *J. Antibiot.* **1995**, *48*, 380.

**Scheme 2.** Syntheses of Compounds **10**, **11**, and **13**



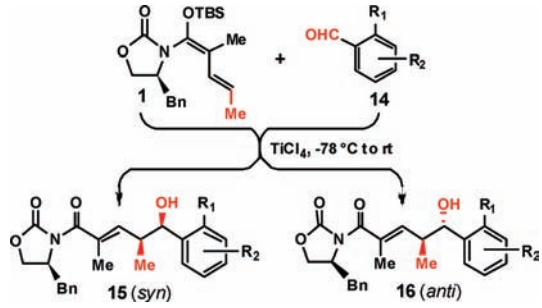

that the chelation-controlled VMAR is an effective method to enable the diastereoselective synthesis of *syn*-aldol adducts.

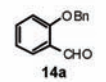
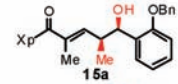
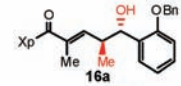
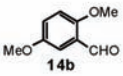
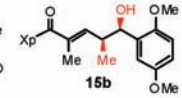
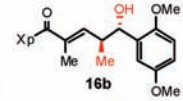
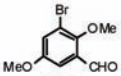
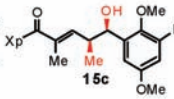
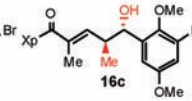
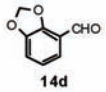
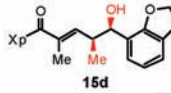
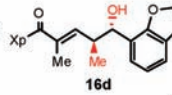
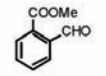
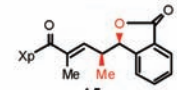
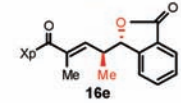
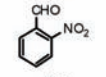
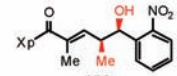
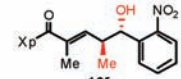
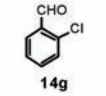
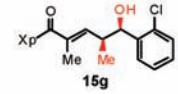
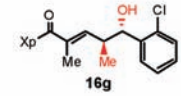
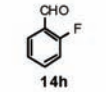
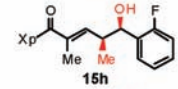
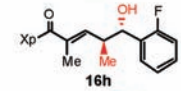
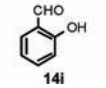
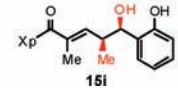
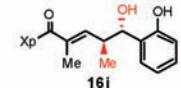
To profile the scope of the chelation-controlled VMAR, several ortho-substituted aryl aldehydes were reacted with vinylketene silyl N,O-acetal **1**, and their coupling results are listed in Table 1. The stereochemistry of the coupling products was first analyzed by modified Mosher method,[9] then those compounds were converted to their cooresponding acetonides to further verify their sterochemistry by way of 1D-NOE study (Scheme 2).

We derive the following observations from Table 1: (1) both benzyloxy and methoxy groups are effective chelation elements in this chelation-controlled VMARs to give the *syn*-aldol adducts; (2) adding both electron-donating and electron-withdrawing groups to the aromatic ring of the α-substituted aryl aldehyde affords the *syn*-aldol adducts in high yield and good diastereoselectivity; (3) benzo[*d*][1,3]dioxane-4-carbaldehyde (entry 4) does not give the expected high *syn*-selectivity aldol adduct, presumbly because its rigid 1,3-dioxane ring prevents formation of the chelation complex **A** (Scheme 1); (4) the ester, nitro group, and halogens (entries 5−8) are not chelation elements in this chelation-controlled VMAR, and no significant diaselectivity is observed in their VMARs; and (5) phenol cannot be utilized as a chelation element, as 2-hydroxybenzaldehyde (entry 9) does not give any aldol adduct under the conditions listed in Table 1. It is

(9) For review of the Mosher method, see: Seco, J. M.; Quiñoá, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915.

**Table 1.** Profile of VMAR



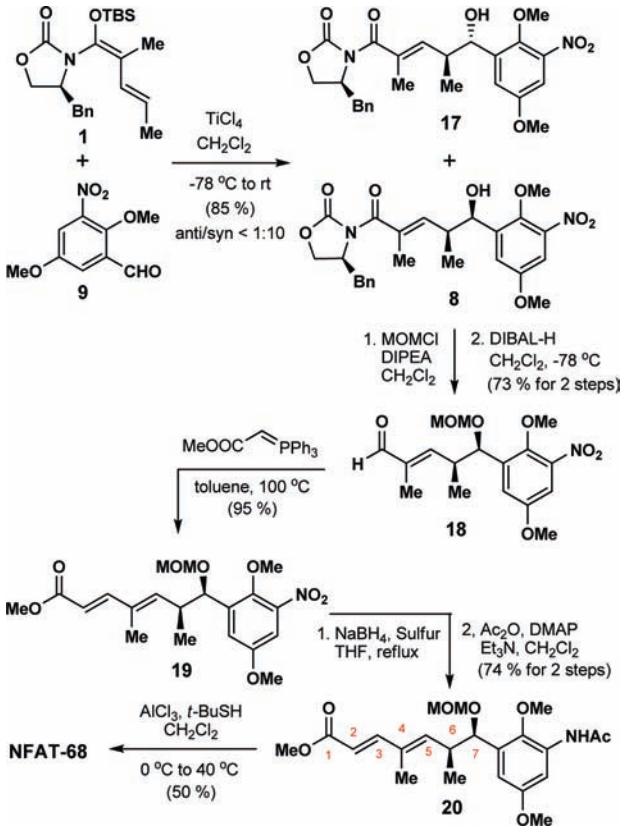

| entry[a] | aldehyde | 15 | 16 | yield % | dr[b] |
|---|---|---|---|---|---|
| 1 |  14a |  15a |  16a | 87 | > 20:1 |
| 2 |  14b |  15b |  16b | 85 | > 20:1 |
| 3 |  14c |  15c |  16c | 85 | > 9:1 |
| 4 |  14d |  15d |  16d | 78 | 1.5:1 |
| 5 |  14e |  15e |  16e | 69 | 1.3:1 |
| 6 |  14f |  15f |  16f | 37 | 1.9:1 |
| 7 |  14g |  15g |  16g | 90 | 1.4:1 |
| 8 |  14h |  15h |  16h | 85 | 1.4:1 |
| 9 |  14i |  15i |  16i | | |

[a] Reactions were conducted with silylketene (60 mg, 1.0 equiv), aldehyde (2.0 equiv), and $TiCl_4$ (1.0 equiv). [b] Diastereo selectivity was detemined by $^1H$ NMR (300 or 400 MHz).

worthwhile mentioning that in the event of replacing MeO or BnO with other protecting groups, such as AcO, MOMO, and BOMO, the desired VMAR adducts could not form at low temperature, and the starting materials were decomposed when the reactions were carried out at room temperature, presumably because of the existence of $TiCl_4$.

Upon completion of the development of a method for stereoselective synthesis of *syn*-aldol adducts via VMAR, we then applied this methodology to the total synthesis of NFAT-68 (**7**). To this end, aldehyde **9** (Scheme 3) was reacted with chiral vinylketene silyl N,O-acetal **1** to give aldol

**Scheme 3.** Total Synthesis of NFAT-68




adducts **17** and **8** in 85% combined yield, and the ratio of **17**/**8** was ca. 1:20. **8** was then subjected to the action of protection−reduction protocols, as shown in Scheme 3, which resulted in aldehyde **18** in 73% yield in two steps. Aldehyde **18** was further reacted with $Ph_3P{=}CHCOOMe$ in toluene at 100 °C,[10] affording ester **19** in 95% yield. To complete the total synthesis, a sequential reduction[11]−acetylation event on **19** delivered **20** in 74% yield in two steps. Thus, after global deprotection by treatment of **20** with $AlCl_3$/*t*-BuSH, the proposed NFAT-68 was obtained in 50% yield. The data of $^1H$ NMR and $^{13}C$ NMR were identical with the published data,[8] indicating that the relative stereochemistry of natural product NFAT-68 (**7**) is the *syn*-relationship at C6 and C7. The optical rotation of the synthesized NFAT-68 is −65.5 ($[\alpha]^{20}_D$ 0.5, $CHCl_3$).

To elucidate the absolute stereochemistry of NFAT-68 (**7**), the enantiomer of NFAT-68 (**7**) was made following the same chemistry shown in Scheme 3, the synthetic details of which are provided in the Supporting Information. To further determine the absolute stereochemistry of NFAT-68, we will conduct biological testing of the both synthesized enantiomers of NFAT-68. We wish such an effort might help us to decide the absolute stereochemistry of the naturally occurring NFAT-68.

In summary, our group has developed a concise synthetic strategy to construct the scaffold of NFAT-68 (**7**). Both enantiomers were asymmetrically synthesized via the chelation-controlled VMAR as a key step. This developed chemistry is anticipated to have wider application in the total synthesis of many other natural products.

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**Supporting Information Available:** Details of experimental procedures and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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