Parallel Synthesis of a Vitamin D_3 Library in the Solid-Phase

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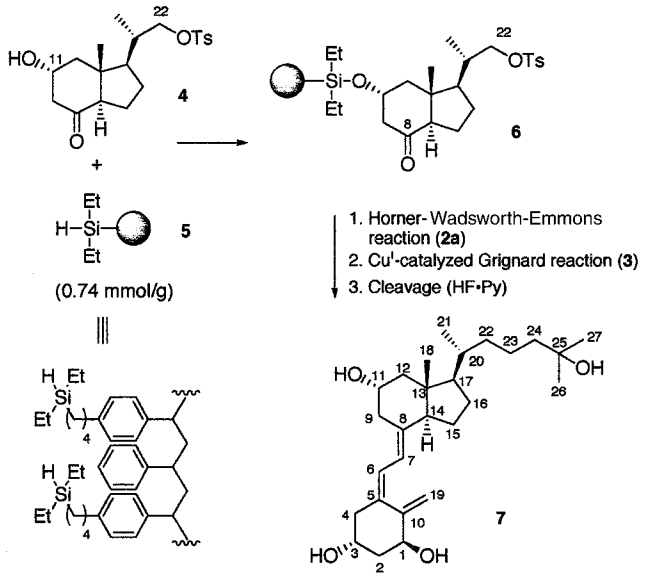
Abstract: A highly efficient synthesis of the vitamin D_3 system on solid support is described. Two synthetic strategies for the solid-phase synthesis of vitamin D_3 were developed. One is for 11-hydroxy analogues, and the other is for most other synthetic analogues. In the latter strategy, the sulfonate-linked CD-ring **58** was initially immobilized on PS-DES resin to give solid-supported CD-ring **63** (Scheme 10). Similarly, solid-supported CD-ring **63** was prepared by attachment of the CD-ring **10** to the chlorosulfonate resin **64**. The vitamin D_3 system was synthesized by Horner−Wadsworth−Emmons reaction of the A-ring phosphine oxide to a solid-supported CD-ring, followed by simultaneous introduction of the side chain and cleavage from resin with a Cu^I-catalyzed Grignard reagent. Parallel synthesis of the vitamin D_3 analogues was accomplished by a split and pool methodology utilizing radio frequency encoded combinatorial chemistry, and a manual parallel synthesizer for side chain diversification and deprotection. Additionally, we demonstrated the synthesis of various A-rings in a similar protocol for efficient preparation of building blocks.

Introduction

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol) (**8**, Scheme 2), the biologically active metabolite of vitamin D_3, is recognized as a steroid hormone.[1] The natural hormone exhibits a variety of physiological activities, such as regulation of calcium and phosphorus metabolism, cell differentiation and proliferation, and the immune system.[2] Recently, to separate these activities, especially cell differentiation and proliferation activity from calcemic activity, and to enhance the specific activities, a number of analogues have been synthesized by many laboratories.[3] Most of these have been modified in either the A-ring or the side chain, while a few derivatives were altered in the CD-ring, or in both the A-ring and the CD-ring side chain, so-called "hybrid" analogues[3c−e] by Posner et al. These analogues have been synthesized one by one, in various discrete manners, many of which cannot be considered as consistently convergent methods for analogue synthesis. In contrast, we have recently reported the solid-phase synthesis of the 11-hydroxyvitamin D_3 system in four steps by combining three components, i.e. the A-ring, the CD-ring, and the side chain (in our nomenclature, the CD-ring includes the carbons at the 20, 21, and 22 positions, steroidal numbering, see **7** in Scheme 1).[4] In recent years, it has been proven that applications of solid-phase chemistry, capable of generating combinatorial natural small molecule libraries, to the drug discovery process is extremely effective in terms of searching a wide range of analogues rapidly.[5] Therefore, it seems to be of great value to employ

Scheme 1. Synthetic Study of the 11-Hydroxyvitamin D_3 System on Solid-Support (X^I = OH, Strategy I)



(1) (a) Norman, A. W.; Bouillon, R.; Thomasset, M., Eds. *Vitamin D: Gene Regulation, Structure Function Analysis and Clinical Application*; Walter de Gruyter and Co.: Berlin, 1991. (b) Yamamoto, K.; Masuno, H.; Choi, M.; Nakashima, K.; Taga, T.; Ooizumi, H.; Umesuno, K.; Scinska, W.; VanHooke, J.; Deluca, H. F.; Yamada, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1467.

(2) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200.

(3) (a) Zhu, G.-D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877. (b) Ikekawa, N. *Med. Res. Rev.* **1987**, *7*, 333. For recent examples on the synthesis of the vitamin D analogues, see: (c) Posner, G. H.; Wang, Q.; Han, G.; Lee, J. K.; Crawford, K.; Zand, S.; Brem, H.; Peleg, S.; Dolan, P.; Kensler, T. W. *J. Med. Chem.* **1999**, *42*, 3425. (d) Posner, G. H.; Lee, J. K.; White, M. C.; Hutchings, R. H.; Dai, H.; Kachinski, J. L.; Dolan, P.; Kensler, T. W. *J. Org. Chem.* **1997**, *62*, 3299. (e) Fujishima, T.; Konnno, K.; Nakagawa, K.; Kurobe, M.; Okano, T.; Takayama, H. *Bioorg. Med. Chem.* **2000**, *8*, 123. (f) Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **1998**, *41*, 4662. (g) Shimizu, M.; Iwasaki, Y.; Yamada, S. *Tetrahedron Lett.* **1999**, *40*, 1697. (h) Mikami, K.; Osawa, A.; Isaka, A.; Sawa, E.; Shimizu, M.; Terada, M.; Kubodera, N.; Nakagawa, K.; Tsugawa, N.; Okano, T. *Tetrahedron Lett.* **1998**, *39*, 3359. (i) Courtney, L. F.; Lange, M.; Uskokovic, M. R.; Wovkulich, P. M. *Tetrahedron Lett.* **1998**, *39*, 3363. (j) Oshida, J.; Okamoto, M.; Ishizuka, S.; Azumi, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 381. For a recent report for modification at the 6-position, see: (k) Addo, J. K.; Swamy, N.; Ray, R. *Steroid* **1999**, *64*, 273.

(4) Doi, T.; Hijikuro, I.; Takahashi, T. *J. Am. Chem. Soc.* **1999**, *121*, 6749.

(5) For reviews, see: (a) Dolle, R. E. *J. Comb. Chem.* **2000**, *2*, 383. (b) Dolle, R. E.; Nelson, K. H., Jr. *J. Comb. Chem.* **1999**, *1*, 235. (c) Schreiber, S. L. *Science* **2000**, *287*, 1964.

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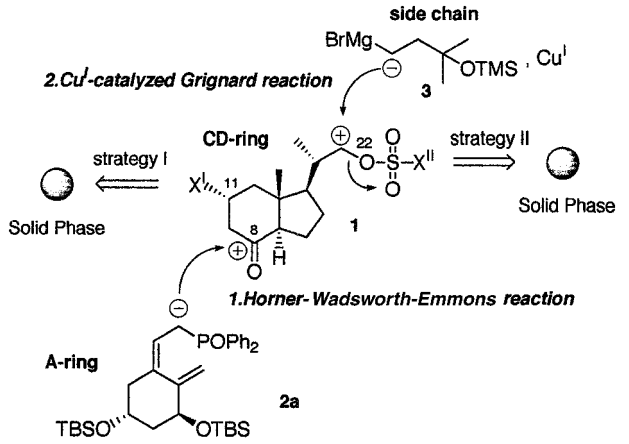
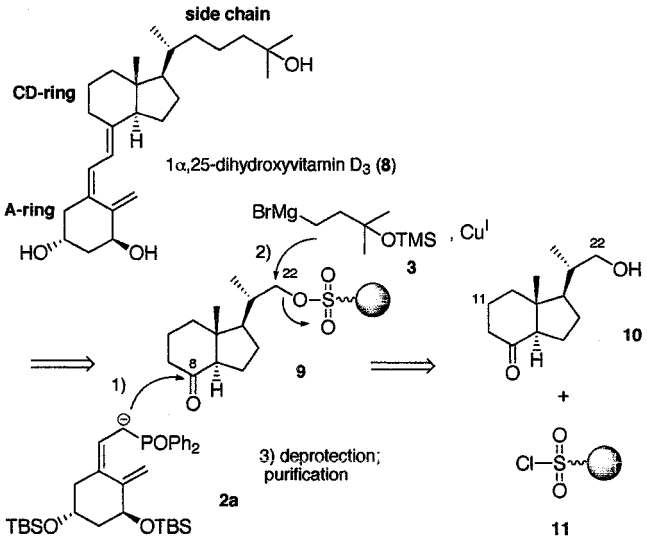


Figure 1. Strategy of the vitamin D_3 system on the solid-phase.

Scheme 2. Retrosynthesis of the Vitamin D_3 System on a Solid Support (Strategy II)



solid-phase chemistry to construct a combinatorial vitamin D_3 library including analogues previously synthesized and to investigate their structure−activity relationships.

In this article, we predominantly describe the improved solid-phase synthesis of the vitamin D_3 system (strategy II, Figure 1) that is applicable to most synthetic analogues and the preparation of its library utilizing radio frequency encoded combinatorial (REC) chemistry,[6] which is a useful technique for the identification of members in a "split and pool" combinatorial library. In addition, we wish to report an efficient synthesis of various A-ring moieties by means of the Pd(0)-catalyzed intramolecular Mizoroki−Heck reaction.

Solid-Phase Synthesis Strategy

Consideration of the structure of the vitamin D_3 system (see **8** in Scheme 2) readily led us to the assembly of the three components: the A-ring, the CD-ring, and the side chain for molecular diversification. Our strategy for the solid-phase synthesis of vitamin D_3 is shown in Figure 1. The linkage between the A-ring and the CD-ring at the 8-position is accomplished by Horner−Wadsworth−Emmons methodology.[7] The Horner−Wadsworth−Emmons methodology, pioneered by Lythgoe et al.,[7a] is the most desirable as it could proceed with high chemo- and stereoselectivity and in high yield, and the unreacted excess A-ring moiety **2a** could be recovered by simple purification after quenching of the reaction with water. On the other hand, the linkage between the CD-ring and the side chain is accomplished by Cu^I-catalyzed Grignard reaction.[8] The substitution reaction of the C-22 sulfonyloxy group with the side chain via Cu^I-catalyzed Grignard reagent **3**, previously underutilized in vitamin D_3 synthesis,[4,9] would be most desirable for direct coupling of the side chain.[10] Hence, it is important which component is initially immobilized on a solid support. In solid-phase nucleophilic reactions, addition of excess nucleophiles to a solid-supported electrophile is apparently desirable to complete the reactions. Thus, attachment of the electrophilic CD-ring **1** to a solid support is essential. The solid-supported CD-ring has two electrophilic sites, which are required to be compatible during the two carbon−carbon bond formations, without protecting groups and/or functional group manipulation, which is undesirable for a practical combinatorial synthesis. On the basis of these constraints, the Horner−Wadsworth−Emmons reaction of an A-ring moiety to the polymer-supported 8-keto CD-ring is performed prior to the alkylation of the sulfonyloxy group with Grignard reagents to avoid Grignard addition at the 8-position and facile epimerization at the 14-position adjacent to the carbonyl group. Alkylation of a sulfonyloxy group at the 22-position of the polymer-supported CD-ring with a Grignard reagent would afford the vitamin D_3 system, if this could be performed at sufficiently low temperature (<30 °C) to avoid isomerization of the preformed triene system. In our previous investigation[4] shown in Scheme 1 (strategy I in Figure 1), we selected the hydroxy group at the 11-position as the loading site of the CD-ring **4** on the solid support and PS-DES resin (**5**).[11] Although there was steric influence from not only the substituent at the 11-position but also the polystyrene chain with a four-carbon alkyl chain from the diethylsilyl group, we anticipated that it would not affect the subsequent two steps, i.e. (1) Horner−Wadsworth−Emmons reaction of A-ring moieties **2a** to the polymer-supported 8-keto CD-ring **6** and (2) alkylation of the polymer-supported tosylate at the 22-position with the Grignard reagent **3**. Furthermore, PS-DES resin (**5**) is appropriate as a polymer support because silyl protection has proven to be effective in the synthesis of various vitamin D_3 analogues and is readily cleaved even in the presence of the unstable triene moiety. Finally, treatment of the polymer-supported vitamin D_3 with HF·Py in THF would release the desired vitamin D_3 system **7** after aqueous workup and simple

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(7) (a) Lythgoe, B.; Moran, T. A.; Nambudiry, M. E. N.; Tideswell, J.; Wright, P. W. *J. Chem. Soc., Perkin Trans. 1* **1978**, 590. (b) Toh, H. T.; Okamura, W. H. *J. Org. Chem.* **1983**, *48*, 1414. (c) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098.

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(10) For another approach to formation of the side chain of the vitamin D_3 system, see: (a) Partrige, J. J.; Faber, S.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 764. (b) Lythgoe, B.; Roberts, D. A.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2608. (c) Tachibana, Y.; Yokoyama, S.; Tsuji, M. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2599. (d) Torneiro, M.; Fall, Y.; Castedo, L.; Mourino, A. *Tetrahedron Lett.* **1992**, *33*, 105.

(11) (a) Hu, Y.; Porco, J. A., Jr.; Labadie, J. W.; Gooding, O. W.; Trost, B. M. *J. Org. Chem.* **1998**, *63*, 4518. (b) Hu, Y.; Porco, J. A., Jr. *Tetrahedron Lett.* **1998**, *39*, 2711. (c) Hu, Y.; Porco, J. A., Jr. *Tetrahedron Lett.* **1999**, *40*, 3289.

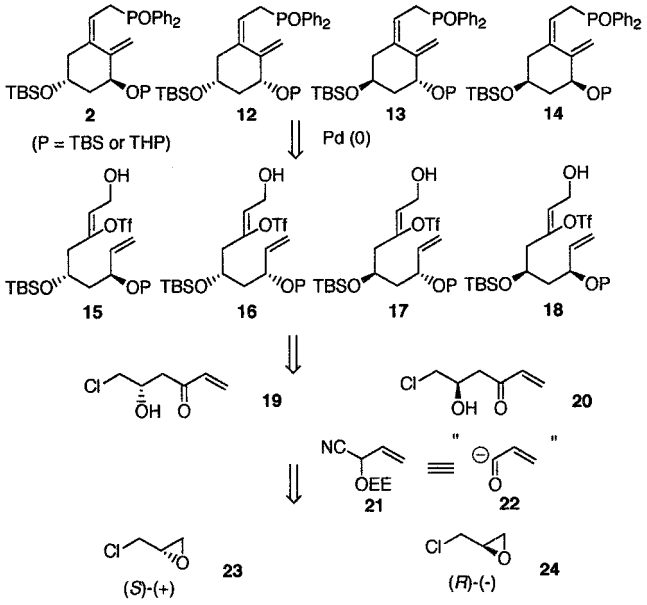
filtration through silica gel to remove silyl residues. Following the above procedure, we performed the synthesis of twelve 11-hydroxyvitamin D_3 analogues.[12] This strategy promises to overcome the required two carbon−carbon bond-forming steps on the solid phase, resulting in the consecutive coupling of the CD-ring with the A-ring and side chain moieties. However, these analogues are limited to "11-hydroxy"[10d,13] analogues, and it is also difficult to prepare various 11-hydroxy CD-rings. Therefore, it is necessary to develop an alternative strategy to cover vitamin D_3 analogues beyond those encompassed by our earlier design. To this end, as shown in Scheme 2 (strategy II in Figure 1), attachment of the C-22 hydroxy group of the CD-ring **10** to a sulfonate-linked resin **11**[14] would be acceptable in terms of not requiring the C-11 hydroxy group. The solid-supported sulfonated CD-ring **9** would be available not only for Cu^I-catalyzed Grignard reagents,[4,9] but also other various nucleophiles, such as lithium acetylide,[10a] α-lithiated sulfone,[10c] α-lithiated cyanide,[10d] etc. As a consequence, we adopted the Horner−Wadsworth−Emmons reaction of A-ring moiety **2a** with 8-keto CD-ring **9**, followed by simultaneous alkylation and cleavage from the solid support by Cu^I-catalyzed Grignard reaction with **3**. Finally, deprotection and simple purification of the crude cleavage product would afford vitamin D_3 analogues **8**. In the present strategy, there are many impurities in the cleavage solution, therefore we should modify the workup procedure and purification for parallel synthesis of a number of analogues.

Preparation of Various A-Ring Moieties

The modified CD-rings and the side chain moieties are readily available from the Inhoffen−Lythgoe diol (**46**, Scheme 7)[15] and bromo esters,[16] respectively, whereas preparation of the modified A-ring moieties[3a,c−i] is somewhat laborious. Thus, generating a library of vitamin D_3 analogues requires an efficient synthesis of A-ring moieties via a similar protocol.

One of our strategies, which could produce all possible A-ring diastereomers of 1,25-$(OH)_2D_3$,[17] is described in Scheme 3. A-ring moieties **2** and **12**−**14** can be prepared from the corresponding enol triflates **15**−**18** through Pd(0)-catalyzed intramolecular Mizoroki−Heck reaction. The enol triflates **15**−**18** can be synthesized by diastereoselective reductions and various functional group transfomations of the β-hydroxy ketones **19** and **20**. Enantiomerically pure β-hydroxy ketones **19**[18] and **20** can be prepared by coupling of optically active epichlorohydrins **23** and **24** with the protected cyanohydrin **21**

Scheme 3. Retrosynthesis of Four Possible A-Ring Diastereomers of 1,25-$(OH)_2D_3$



as a synthon for the acyl anion of acrolein **22**. The protected cyanohydrins have been developed by Stork et al.[19] and allowed our group to accomplish the total syntheses of several natural products.[20] Chemoselective alkylation of the lithiated protected cyanohydrin of acrolein **21** with (*S*)-epichlorohydrin (**23**) (Scheme 4) followed by treatment with a catalytic amount of copper(II) sulfate in methanol and water at 60 °C and shaking with aqueous $NaHCO_3$ gave crude enantiomerically pure β-hydroxy ketone **19**. The 1,3-*anti* diastereoselective reduction of the β-hydroxy ketone **19** with $Me_4NBH(OAc)_3$[21] in acetic acid and acetonitrile followed by neutralization of the acetic acid with aqueous NaOH afforded epoxy alcohol **25**[22] in 63% yield from starting material **23**. The 1,3-*syn* diastereoselective reduction of **19** with $Et_3B-NaBH_4$[23] was also accomplished to give diol **27**,[22] which potentially could be converted to the corresponding phosphine oxide **12** (see Scheme 3).

THP protection of the hydroxy group of **25** gave epoxide **26**, which was then transformed to acid **29a** by alkylation with potassium cyanide, TBS protection, reduction with DIBAL, and oxidation. Treatment of acid **29a** with carbonyl diimidazole, followed by the addition of magnesium ethyl malonate, provided the corresponding β-keto ester,[24] which was converted to alcohol **15a** by trapping the (*Z*)-enolate from the above β-keto ester with Tf_2NPh,[25] and reduction of the ethyl ester. Palladium(0)-catalyzed cyclization of **15a** was carried out as previously reported.[4,25a,26] The reaction proceeded smoothly at room

(12) For the structures and spectral data of these analogues, see the Supporting Information.

(13) For examples of C-11 substituted 1α,25-$(OH)_2D_3$, see: (a) D'Halleweyn, C.; Van Haver, D.; Van der Eycken, J.; De Clercq, P.; Vandewalle, M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 477. (b) Bouillon, R.; Allewaert, K.; van Leeuwen, J. P. T. M.; Tan, B.-K.; Xiang, D. Z.; De Clercq, P.; Vandewalle, M.; Pols, H. A. P.; Bos, M. P.; Van Baelen, H.; Birkenhäger, J. C. *J. Bio. Chem.* **1992**, *267*, 3044. (c) Zhu, G.-D.; Van Haver, D.; Jurriaans, H.; De Clercq, P. J. *Tetrahedron* **1994**, *50*, 7049. (d) Kobayashi, N.; Higashi, T.; Shimada, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 269.

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(16) Wilson, S. R.; Zhao, H.; Dewan, J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 341.

(17) For a study on a similar concept, see ref 3e.

(18) Trost, B. M.; Hanson, P. R. *Tetrahedron Lett.* **1994**, *35*, 8119.

(19) Stork, G.; Moldnado, L. *J. Am. Chem. Soc.* **1971**, *93*, 5286.

(20) (a) Takahashi, T.; Kanda, Y.; Nemoto, H.; Kitamura, K.; Tsuji, J. *J. Org. Chem.* **1986**, *51*, 3393. (b) Takahashi, T.; Shimizu, K.; Doi, T.; Tsuji, J. *J. Am. Chem. Soc.* **1988**, *110*, 2674. (c) Takahashi, T.; Tsukamoto, H.; Kurosaki, M.; Yamada, H. *Synlett* **1997**, 1065.

(21) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560.

(22) The stereochemistries of **27** and **28** were confirmed by ^{13}C NMR analysis of the *gem*-dimethyl group of the corresponding acetonides (δ 19.8, 30.0 from **27**; δ 24.9, 25.2 from **28**); see: Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945.

(23) Chen, K.-M.; Gunderson, K. G.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Chem. Lett.* **1987**, 1923.

(24) Brooks, D. W.; Lu, L. D.-L.; Masamune, S. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 72.

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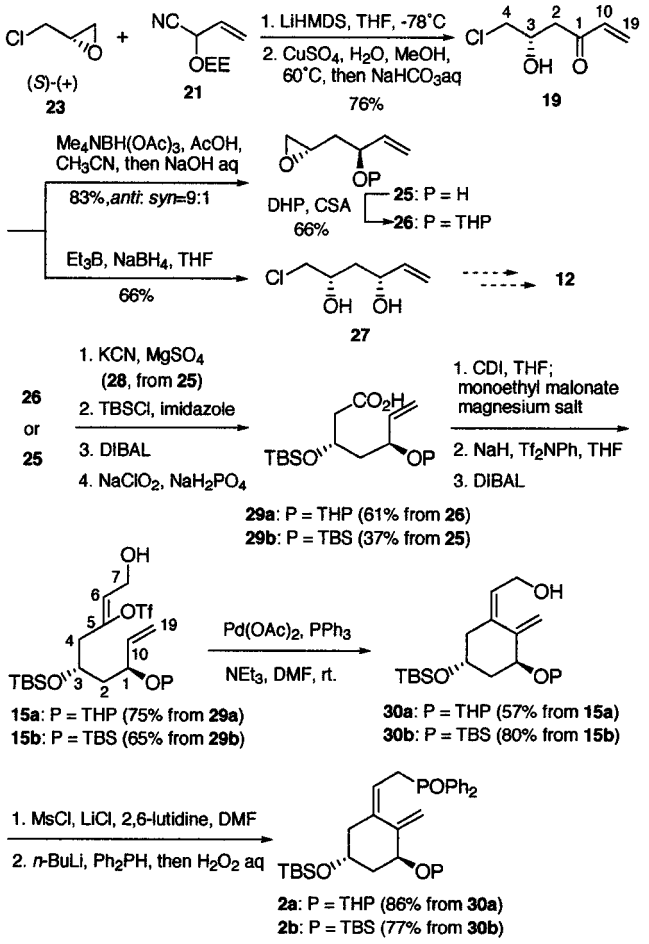
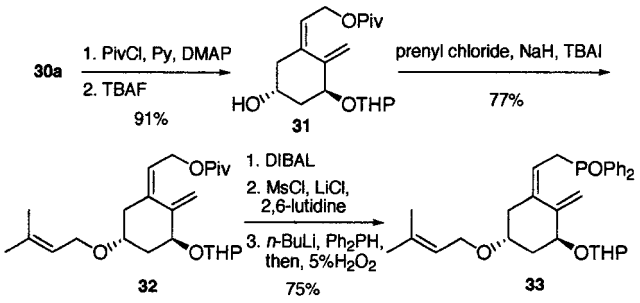
Scheme 4. Synthesis of A-Ring Moieties **2**, and 1,3-*syn*-Diol **27** for A-Ring Moiety **12**



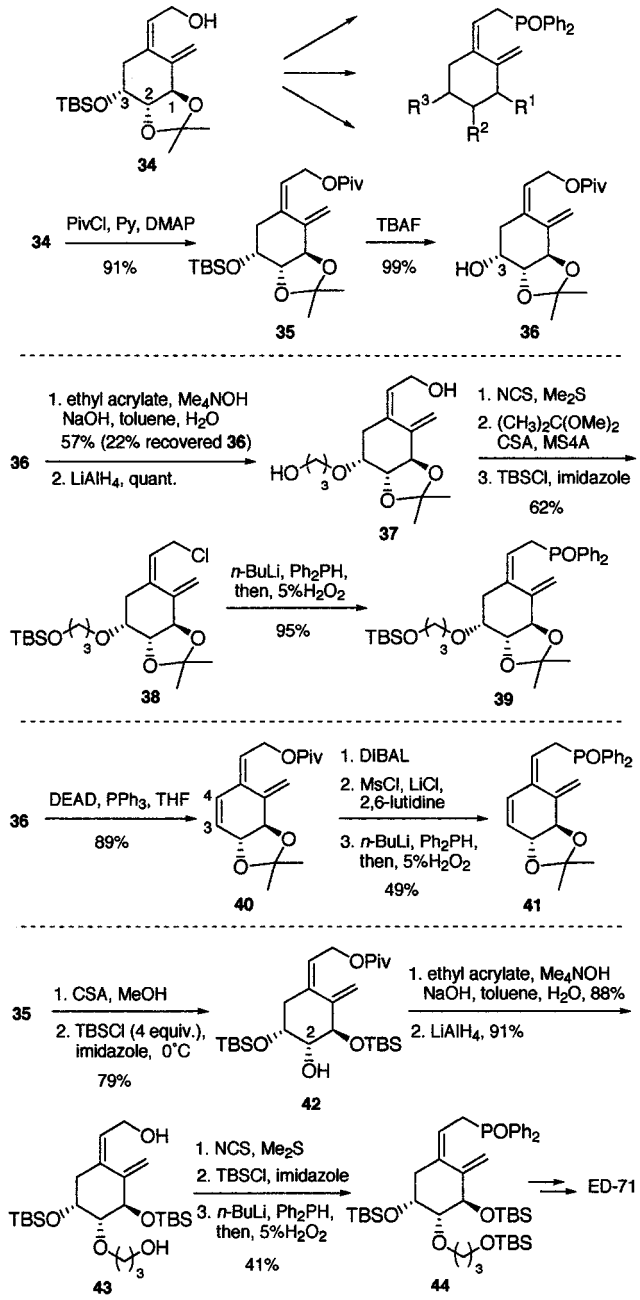
temperature to furnish cyclized (*Z*)-diene **30a** in 57% yield with slight formation (<5%) of the corresponding (*E*)-diene via *syn* Pd−H β-elimination. Dienyl alcohol **30a** was chlorodehydroxylated and then converted to the phosphine oxide **2a** according to the literature procedure.[7b] The phosphine oxide **2b**, previously synthesized by other groups,[7c,27] was also prepared from **25** in a similar manner.

THP-protected cyclic compound **30a** is a key intermediate that can lead to a variety of A-ring moieties by selective deprotection at the C-1 or C-3 position. For example, deprotection at the 3-position allowed for the subsequent etherification, substitution, inversion, and reduction, etc. of the C-3 hydroxy group. As one of these modifications, the etherified A-ring moiety **33** was synthesized (Scheme 5). The allylic hydroxy group of **30a** was protected as its pivalate, which was converted to alcohol **31** by desilylation with tetrabutylammonium fluoride. The etherification of alcohol **31** with prenyl chloride in the presence of tetrabutylammonium iodide provided ether **32**. Deprotection of the pivaloyl group and several transformations

Scheme 5. Preparation of A-Ring Moiety **33**



Scheme 6. Synthesis of A-Ring Moieties **39**, **41**, and **44**

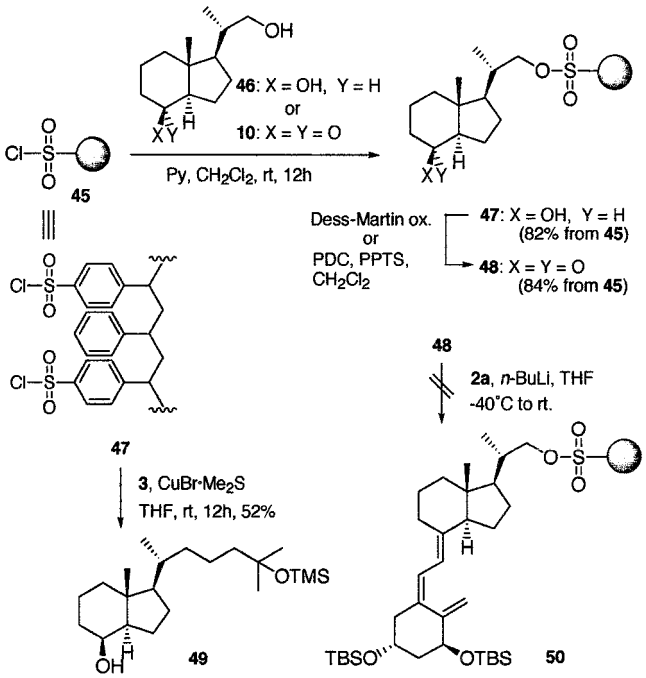


as shown in Scheme 5 gave phosphine oxide **33**. Another strategy is indicated in Scheme 6. We previously reported the synthesis of the 2-hydroxy A-ring moiety **34**,[4,26] which can also be a candidate to produce various A-ring phosphine oxides. Michael addition to ethyl acrylate of intermediate **36**, prepared from **34** (Scheme 6), afforded the ester,[28] which was reduced

(26) Takahashi, T.; Nakazawa, M. *Synlett* **1993**, 37.

(27) (a) Posner, G. H.; Kinter, C. M. *J. Org. Chem.* **1990**, *55*, 3967. (b) De Shrijver, J.; De Clercq, P. J. *Tetrahedron Lett.* **1993**, *34*, 4369. (c) Kobayashi, S.; Shibata, J.; Shimada, M.; Ohno, M. *Tetrahedron Lett.* **1990**, *31*, 1577. (d) Stork, G.; Hutchinson, D.; Okabe, M.; Parker, D.; Ra, C.; Ribereau, F.; Suzuki, T.; Zebovitz, T. *Pure Appl. Chem.* **1992**, *64*, 1809. (e) Mascarenas, J. L.; Garcia, M.; Castedo, L.; Mourino, A. *Tetrahedron Lett.* **1992**, *33*, 4365. (f) Nagasawa, K.; Ishihara, H.; Zako, Y.; Shimizu, I. *J. Org. Chem.* **1993**, *58*, 2523. (g) Tazumi, K.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* **1994**, 1903. (h) Hatakeyama, S.; Numata, H.; Osanai, K.; Takano, S. *J. Org. Chem.* **1989**, *54*, 3515. (i) Batty, D.; Crich, D. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2894.

Scheme 7. Initial Study of the Vitamin D_3 Synthesis (strategy II) on a Directly Sufonylchloride-Attached Polystylene Resin



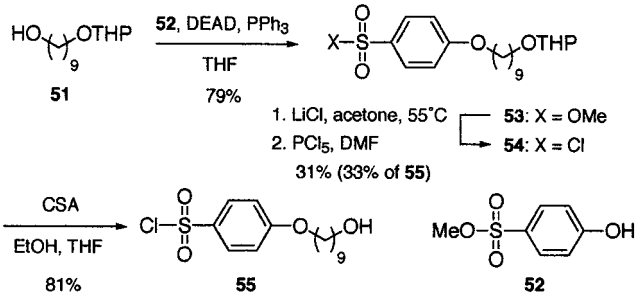
to alcohol **37**. Alcohol **37** was transformed to the allylic chloride with NCS and dimethyl sulfide.[29] Under these conditions approximately half of the isopropylidene group was removed by a chloronium cation; therefore, the crude allylic chloride was reprotected with 2,2-dimethoxy propane. TBS protection of the primary hydroxy group afforded **38**, which was converted to phosphine oxide **39** as previously described. The A-ring synthon **44**,[30] an intermediate for the vitamin D_3 analogue ED-71,[31] was assembled in a manner similar to the synthesis of **39**.

The intermediate **36** was also converted to triene **40** by treatment with DEAD and PPh_3. Phosphine oxide **41** was readily synthesized from **40**. Unfortunately, phosphine oxide **41** did not react with the CD-ring synthon **56** (Scheme 9) even at room temperature, presumably because of the stabilization of the intermediate carbanion that is highly delocalized due to the internal olefin.

Initial Study of the Solid-Phase Vitamin D_3 Synthesis

In our initial investigation of the solid-phase vitamin D_3 synthesis (strategy II), we selected commercially available TsCl resin (**45**, 1.58 mmol/g)[32] as a solid support. It has chlorosulfonyl groups attached to a polystyrene chain directly, as shown in Scheme 7. Attachment of the CD-rings **46** and **10** was performed according to the literature.[32] Simultaneous alkylation and cleavage from resin **47** by Cu^I-catalyzed Grignard reaction proceeded successfully to give **49** in 52% isolated yield. The solid-supported CD-ring **47** also provided **48** by Dess−Martin oxidation[33] or PDC oxidation.[10d,34] However, the solid-bound CD-ring **48** failed to couple with phosphine oxide **2a**. We attributed this failure to steric interferance by the polystyrene chains. We decided to devise our own sulfonate linker to obtain a solid-supported triene.

Scheme 8. Preparation of the Sulfonate-Linkers **54** and **55**



Preparation of Sulfonate-Linked CD-Ring Derivatives

The sulfonate linker is an attractive linker on solid-phase synthesis as a traceless linker that can be displaced with various nucleophiles.[14] We designed the sulfonate linker **55** that has an appropriate spacer with sufficient length to avoid steric interference with the polystyrene chains of the solid support. As illustrated in Scheme 8, Mitsunobu reaction of alcohol **51** with methyl 4-hydroxybenzenesulfonate (**52**) provided 4-alkoxybenzenesulfonate **53**. The sulfonate ester **53** was hydrolyzed with lithium chloride in refluxing acetone to give the sulfonic acid lithium salt, which was converted to the sulfonyl chloride **54** by PCl_5 in DMF.[14c,d] THP-deprotection of **54** with CSA in EtOH and THF provided hydroxy sulfonyl chloride **55** in 81% yield.[35]

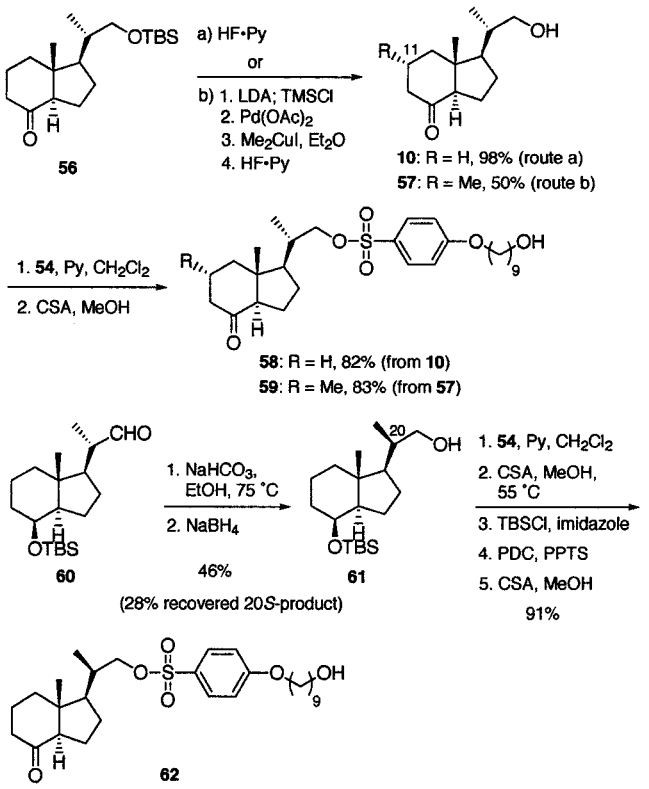
Coupling of the sulfonyl chloride **54** with various CD-rings afforded the corresponding sulfonate-linked CD-rings, three of which were prepared as shown in Scheme 9. Sulfonylation of **54** with the CD-ring **10** prepared from **56** and subsequent removal of the THP protecting group afforded the sulfonate-linked CD-ring **58**. The 11-methyl[13a,b] sulfonate-linked CD-ring **59** was also prepared from **57** (via route b) in the same manner. The 20-epi[3e,36] sulfonate-linked CD-ring **62** was synthesized as follows. Treatment of aldehyde **60** with $NaHCO_3$ in ethanol at 75 °C gave the 20-epimers whose ratio was 62:38 in favor of the desired 20*R*-epimer.[36] The mixture was reduced with $NaBH_4$ to alcohols, which could be separated on silica gel to yield the pure desired epimer **61** in 46% yield in two steps.[3e] Coupling of epimer **61** with sulfonyl chloride **54** followed by deprotection of THP and TBS, selective protection of the primary alcohol by TBS, oxidation of the C-8 secondary alcohol, and TBS-deprotection afforded the 22-epi sulfonate-linked CD-ring **62**.

Solid-Phase Synthesis of the Vitamin D_3 System

The sulfonate-linked CD ring **58** was loaded onto chlorinated PS-DES resin (**5**, 0.74 mmol/g)[11] as shown in Scheme 10. The loading yield of **63** was determined to be 95% by gravimetric analysis. Subsequently, we developed another preparation of **63** by attachment of the CD-ring **10** with the sulfonyl chloride

(28) Kubodera, N.; Watanabe, H.; Kawanishi, T.; Matsumoto, M. *Chem. Pharm. Bull.* **1992**, *40*, 1494.
(29) Corey, E. J.; Kim, C. U.; Takeda, M. *Tetrahedron Lett.* **1972**, 4339.
(30) For a precedent report, see: Hatakeyama, S.; Iwabuchi, Y.; Irie, H.; Kawase, A.; Kubodera, N. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2871.
(31) (a) See Supporting Information for our synthesis of ED-71. (b) Ono, Y.; Kawase, A.; Watanabe, H.; Shiraishi, A.; Takeda, S.; Higuchi, Y.; Sato, K.; Yamauchi, T.; Mikami, T.; Kato, M.; Tsugawa, N.; Okano, T.; Kubodera, N. *Bioorg. Med. Chem.* **1998**, *6*, 2517. (c) Ono, Y.; Watanabe, H.; Shiraishi, A.; Takeda, S.; Higuchi, Y.; Sato, K.; Tsugawa, N.; Okano, T.; Kobayashi, T.; Kubodera, N. *Chem. Pharm. Bull.* **1997**, *45*, 1626. (d) Okano, T.; Tsugawa, N.; Masuda, S.; Takeuchi, A.; Kobayashi, T.; Takita, Y.; Nishii, Y. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 1444.
(32) This material and reaction conditions are available from Argonaut Technologies. Home page: http://www.argotech.com.
(33) (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.
(34) Leyers, G. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 6099.
(35) Alternative use of MeOH as a solvent resulted in affording 40% methyl sulfonate byproduct with 45% desired product.
(36) Calverley, M. J. *Tetrahedron* **1987**, *43*, 4609.

Scheme 9. Preparation of the Sulfonate-Linker Loaded CD-Rings **58**, **59**, and **62**



resin **64** in the presence of DMAP in >90% yield, which is a more general method for loading alcohols on a solid support via a sulfonate-linker.[14] It should be noted that use of DMAP is essential for not only accelerating the reaction but also ensuring attachment of the sulfonate-linked CD-ring.[37] Sulfonyl chloride resin **64** was prepared in one step by immobilizing hydroxy sulfonyl chloride **55** to PS-DES resin in 83−87% yield, practically without substitution of the chlorosulfonyl group. Horner−Wadsworth−Emmons reaction of **63** with lithiated **67** in THF at −40 to −10 °C for 3 h yielded triene **65**. Sequential one-pot coupling of immobilized sulfonate **65** and cleavage from the polymer-support by Cu^I-catalyzed Grignard reaction of **68** at room temperature for 6 h gave the crude alkylation product. This crude protected product was immediately subjected to CSA solution in methanol and water at room temperature for 6 h to afford vitamin D_3 analogue **66** in 47% yield from **63** (via route a, b). These solid-phase reactions were monitored by ^{13}C SR-MAS (swollen resin−magic angle spinning) NMR[38] and FT-IR in comparison with the corresponding TES-protected compounds prepared in solution. Thus, we have developed a solid-phase synthesis of the vitamin D_3 system, not requiring cleavage from the resin for analysis.

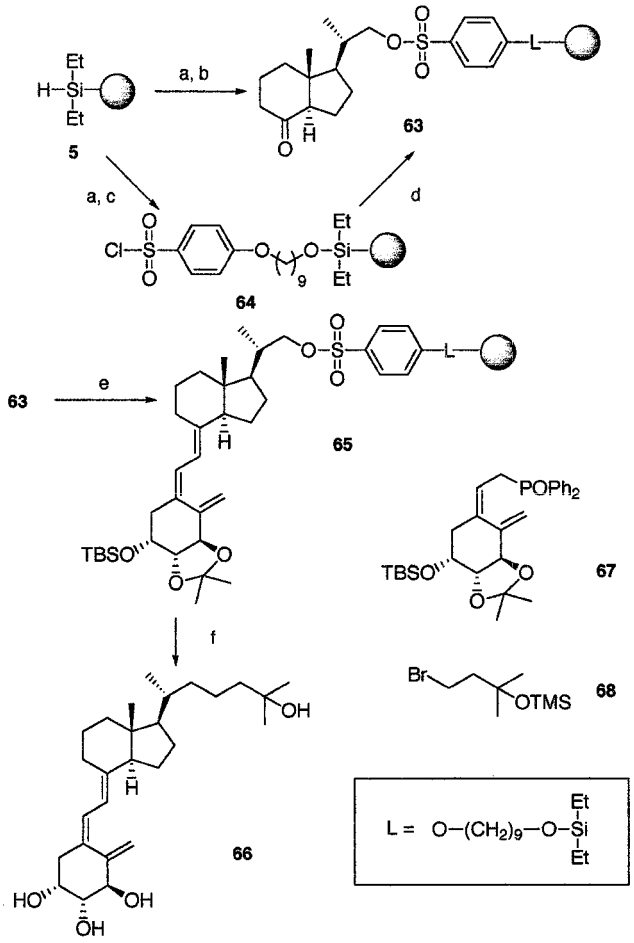
Synthesis of the Vitamin D_3 Library

On the basis of Scheme 10, we constructed a vitamin D_3 combinatorial library utilizing Radiofreqency Encoded Combinatorial (REC) chemistry.[6] Building blocks of a 72-member vitamin D_3 library are summarized in Figure 2. The 72 microreactors each containing ca. 25 mg of PS-DES resin (ca.

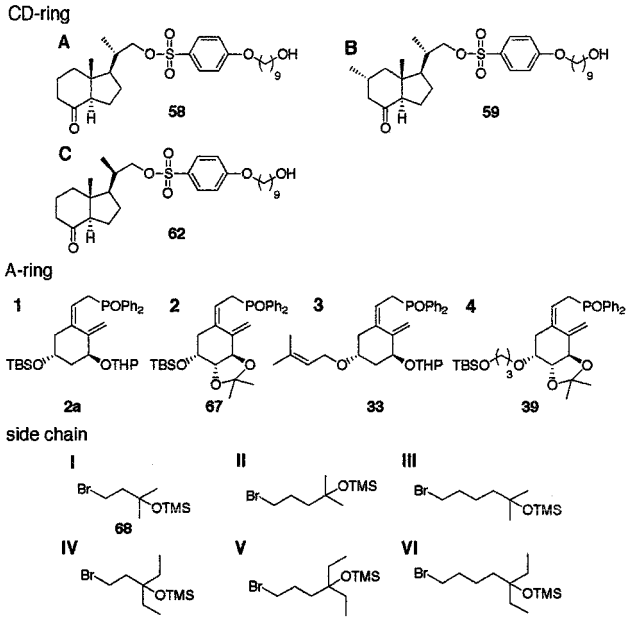
(37) In treatment with pyridine as a base, the sulfonate-CD ring was cleaved from diethylsilyl resin by a generated pyridine hydrochloride. Use of triethylamine did not complete the reaction within 12 h, and 2,6-lutidene was not effective to do the reaction at all.

(38) Kobayashi, S.; Akiyama, R.; Furuta, T.; Moriwaki, M. *Molecules Online* **1998**, *2*, 35.

Scheme 10. Solid-Phase Synthesis of the Vitamin D_3 System (**66**)[a]



[a](a) 1,3-Dichloro-5,5-dimethylhydantoin (3 equiv), CH_2Cl_2, rt, 1h; (b) **58** (3 equiv), imidazole (4 equiv), CH_2Cl_2, rt, 6h, 95% convergent yield; (c) **55** (3 equiv), DIEA (6 equiv), CH_2Cl_2, rt, 2h, 83-87% convergent yield; (d) **10** (3 equiv), DMAP (4 equiv), CH_2Cl_2, rt, 2h, >90% convergent yield; (e) **67** (8 equiv), *n*-BuLi (7.5 equiv), THF, -40 to -10°C, 3h; (f) **68** (15 equiv), Mg (15 equiv), CuBr•Me_2S (1 equiv), THF, rt, 3h; CSA, MeOH, H_2O, 30°C, 6h, 47% from **63** (via a, b).



[a] **A**, **B**, **C**: CD-ring, **1**, **2**, **3**, **4**: A-ring, **I**, **II**, **III**, **IV**, **V**, **VI**: side chain were named as radiofrequency codes for library synthesis.

Figure 2. Building blocks of a 72-member vitamin D_3 library.

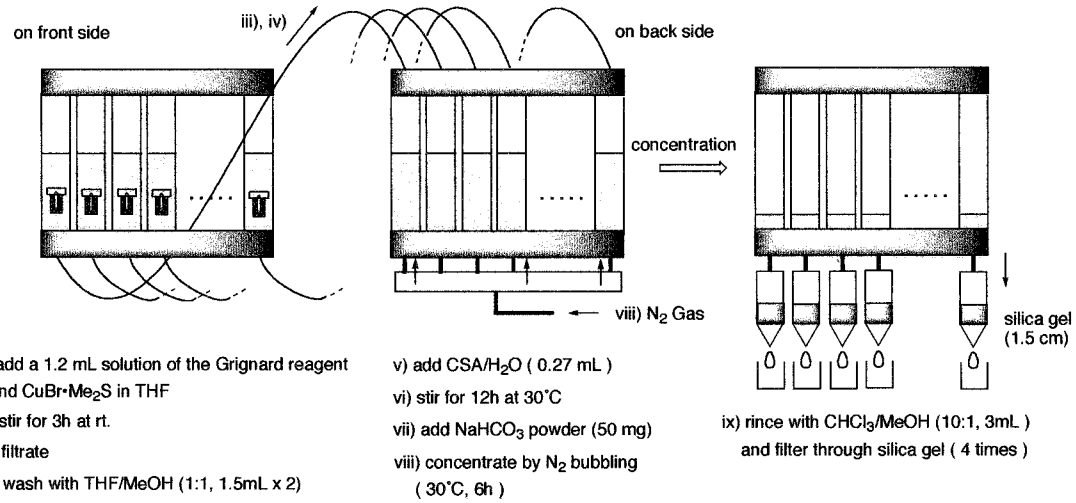


Figure 3. Flow chart of simultaneous coupling and cleavage from resin by Cu^I-catalyzed Grignard reaction and deprotection in parallel using a manual synthesizer.

18 μmol) were encoded and split into individual flasks (**A**, **B**, **C**) according to radio frequency signals. After treatment of the sulfonate-linked CD-rings with imidazole and recovery of excess reagents individually, microreactors were pooled together for washing and drying. These microreactors were decoded and split, followed by coupling with A-rings in individual flasks (**1**, **2**, **3**, **4**). After recovery of the excess A-ring reagents individually, microreactors were pooled together again for washing and drying.

In this parallel synthesis, sequential one-pot coupling and cleavage from the resin with Cu^I-catalyzed Grignard reagent and deprotection in solution were crucial. It is impractical to line up many vessels on stirrers and work up the reaction mixture in a conventional manner, i.e. via transfer of the mixture with pipets and aqueous purification, and concentration on a rotary evaporator. To overcome these difficulties, we utilized a manual parallel synthesizer[39] equipped with 20 vessels where agitation and reaction temperature can be controlled, and that can be connected to another vessel as shown in Figure 3. Side chain diversification and deprotection in parallel was performed according to the flowchart in Figure 3. In the Grignard substitution with side chain IV, CuBr•Me_2S powder was placed in vessels because steric interference with its neighbor diethyl group prevented prompt formation of the cuprate. The filtrates in vials were concentrated together on a centrifugal evaporator. Finally, purification by GPC with chloroform for 30 min per compound afforded a series of vitamin D_3 analogues (2−9 mg) in good purity.[40] This sequence of manipulations was very useful, especially when the cleavage product is a mixture and is subject to further reaction. HRMS spectral data of all compounds and ^1H,^{13}C NMR spectral data of five compounds selected randomly confirmed these structures.[41]

(39) Quest 210, Argonaut Technologies, San Carlos, CA 94070.

(40) On the way toward the 72-member library synthesis, we found that addition of $NaHCO_3$ powder (see operation vii in Figure 3) before concentration of the reaction mixtures prevented decomposition of the products.

Conclusion

In the vitamin D field, we have described the first generation of a combinatorial library via solid-phase chemistry. We have developed two synthetic strategies for a three-component library in only four steps including two nucleophilic additions. The latter library was constructed in a split and pool methodology utilizing REC chemistry.[6] Our sulfonate linker as a traceless linker for molecular diversification is effective for simultaneous alkylation and cleavage from resin. We also demonstrated the workup and purification in parallel using a manual parallel synthesizer for efficient construction of a library. Additionally, preparation of various A-ring moieties by a common protocol suggested the desirability of such a parallel synthesis. These results may prove useful for the general development of combinatorial libraries, as well as that of the vitamin D_3 system. Currently, biological evaluation is in progress, and the structure−activity relationships will be reported in due course.

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Supporting Information Available: Experimental information including spectral date of 10 "11-hydroxy" vitamin D_3 analogues, 270 MHz ^1H NMR and 67.8 MHz ^{13}C NMR spectra, 100 MHz ^{13}C SR-MAS NMR spectra of resins **63**, **64**, and **65**, and details on the preparation of ED-71 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA003976K

(41) See Supporting Information.