A new synthetic method of 1β- and 2β-hydroxyprovitamin D_3, the precursor of the 1β- and 2β-hydroxyvitamin D_3

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Abstract

A new method was described for the synthesis of 1β- and 2β-hydroxyprovitamins D_3, the photoprecusors of 1β- and 2β-hydroxyvitamin D_3. The key step of stereoselective introduction of C-1 and C-2 hydroxy groups was performed with a very mild method for the hydroxybromination of the D-A cyclo adduct with tribromoisocyanuric acid(TBCA)/water. The newly developed method requires no toxic or expensive reagents and 1β(2β)-hydroxyprovitamin D_3 was obtained with excellent yield and stereoselectivity.

Keywords: Steroids, 1β(2β)-hydroxyvitamin D_3, bromohydrins, stereoselectivity

Introduction

Vitamin D_3 is a very important regulator of calcium and phosphorus metabolism,[1] and it is now established that the active derivatives of parent D_3 mainly includes following three forms: 1α-OH-D_3, 25-OH-D_3 and 1α,25-$(OH)_2$-D_3,[2] among which the 1α,25-$(OH)_2$-D_3 is recognized as showing the highest activity of all.[3] The excellent biological activity of these three derivatives has led to various studies on synthesis and biological testing of other analogues, especially those hydroxylated at different positions of the A ring with various configurations.[4-7]

Despite considerable research into vitamin D_3 derivatives, efficient methods for the synthesis of 1β-OH-D_3 and 2β-OH-D_3 are rarely reported. DeLuca's group first reported a facile three-step method for preparation of 1β-OH-D_3 via oxidation of 1α-OH-D_3, followed by the reduction of the formed 1-oxo-previtamin D_3 and finally by thermal isomerization, to yield the product 1β-

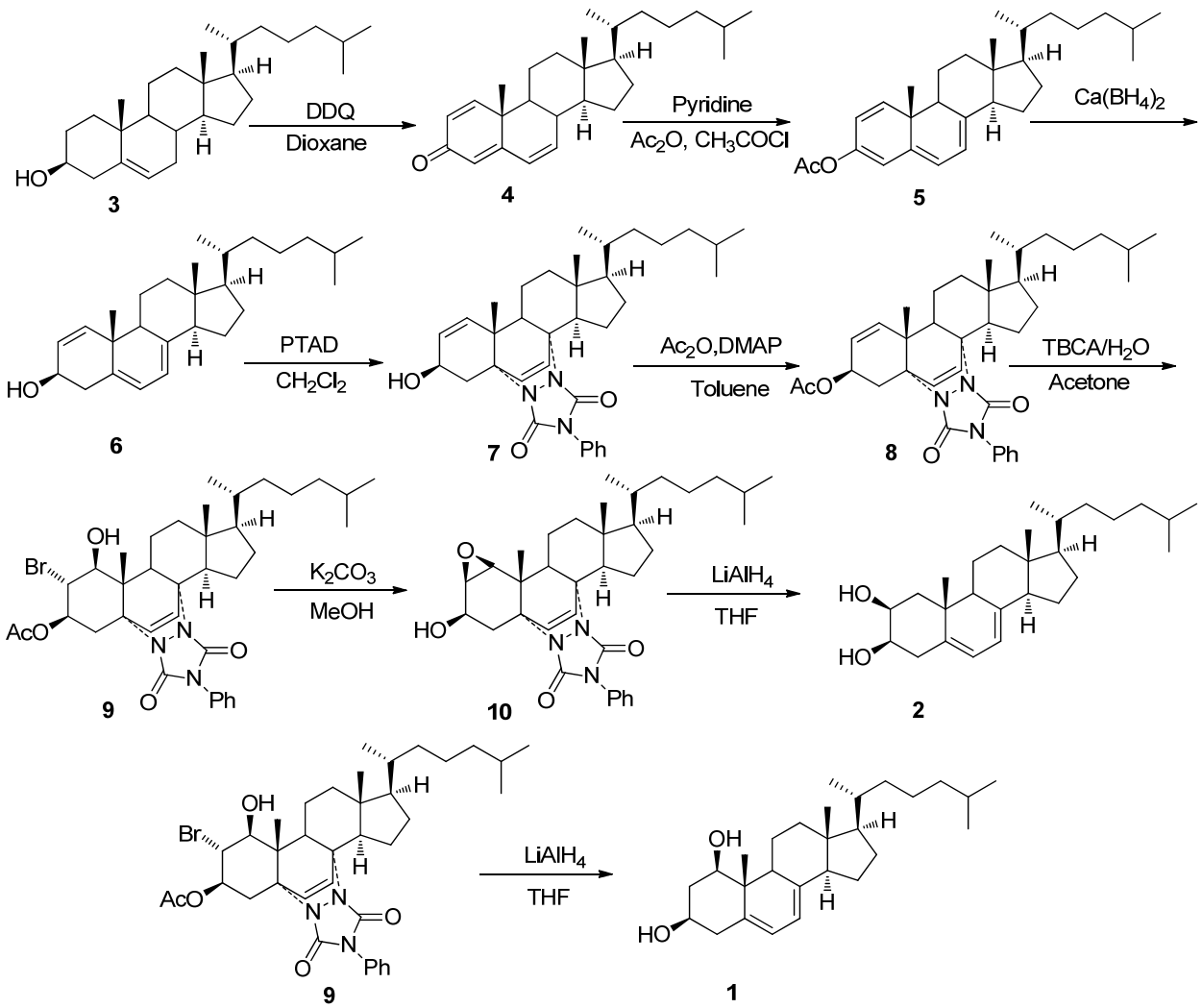
OH-D_3.[5] However, the high cost of the starting material (1α-OH-D_3) significantly devalued its practical application. It is well known that irradiation of 5,7-diene steroids followed by thermal isomerization is widely used in the preparation of vitamin D_3 derivatives. 1β(2β)-hydroxyprovitamin D_3 could be converted into 1β- and 2β-hydroxyvitamin D_3 via routine procedure. The traditional method for preparation of 1β(2β)-hydroxyprovitamin D_3 was that reported by Kaneko's group.[8] Cholesta-1,4,6-trien-3-one **4**, which could be easily prepared from cholesterol **3** by treatment with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), was converted into 3β-hydroxy-cholesta-l,5,7-triene **6** via the deconjugation procedure using t-BuOK in DMSO, followed by the reduction with $Ca(BH_4)_2$. The 5,7-diene system of **6** was protected as D-A cyclo adduct **7** by treatment with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). However, the next step, epoxidation of **7** with *m*-chloroperbenzoic acid gave a mixture of the 1β,2β-epoxide and the 1α,2α-epoxide in the ratio 3:2. Reduction of the 1β,2β-epoxide with $LiAlH_4$ under reflux in THF afforded the 2β-hydroxyprovitamin D_3 **2** and 1β-hydroxyprovitamin D_3 **1** in the ratio 8:1. The stereo-unselective epoxidation in this route would lead to a tedious workup process and also a poor overall yield, which limited the application of this method.

Searching for an efficient and eco-friendly method for the synthesis of 1β(2β)-hydroxyprovitamin D_3 is the key step in preparation of 1β- and 2β-hydroxyvitamin D_3. Our group here reports a new synthesis of 1β-hydroxyprovitamin D_3 (**1**) and 2β-hydroxyprovitamin D_3 (**2**) in a higher yield and selectivity than formerly (Scheme 1). In addition, our method also avoids defects of the traditional method, such as a tedious workup procedure, low yield, and non-selectivity. The key step of regio- and stereo-selective introduction of C-1 hydroxy group was performed by a very mild method of the hydroxybromination of the ester **8** with TBCA/water, followed by intramolecular nucleophilic substitution to obtain the epoxide **10** and then with $LiAlH_4$ reduction to yield the product **2**. By treatment with $LiAlH_4$, the bromohydrin **9** can be converted into product **1** directly.

Results and Discussion

In the synthesis of cholesterol derivatives, the commercial cholesterol **3** was often chosen as a starting material. The whole synthetic route from cholesterol was shown in Scheme 1. Our research was initiated by oxidation of cholesterol with DDQ in dioxane under reflux. The cholesta-1,4,6-trien-3-one **4** could be obtained as pale yellow crystals in 63% yield (Scheme 1).

In the next sequence of reactions, **4** was transformed into the 3-acetoxy-1,3,5,7-cholestatetraene **5** (Scheme 1). It has been reported that the enol acetylation of the trienone **4** in the presence of catalytic amount of *p*-toluenesulfonic acid could yield the tetraene **5**.[9] However, when **4** was treated with this system, the desired product tetraene **5** was obtained in only 56% yield. This stimulated us to search for a much more efficient method.



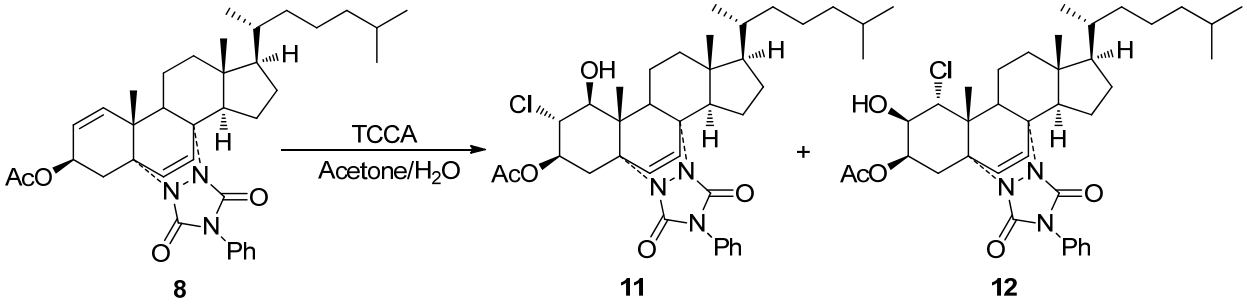
Scheme 1. Synthesis of 1β(2β)-hydroxyprovitamin D_3 with cholesterol **3** as starting material.

We next tested the transformation of trienone with a mixture of acetyl chloride, acetic anhydride and pyridine, which was another system of enol acetylation reported by Toh's group.[10] We found that the reaction proceeded well employing this system and the product **5** was obtained in higher yield (85%), after crystallization from methanol. Next, the cholesta-1,5,7-trien-3β-ol (**6**) was carried out by reduction of tetraene **5** with $Ca(BH_4)_2$ in ether at 0 °C (Scheme 1).

Cholesta-1,5,7-trien-3β-ol (**6**) was converted into its Diels-Alder cycloadduct **7** (Scheme 1). We tested the transformation of compound **6** with PTAD, which is reported to have been used in protection of the 5,7-diene system in steroids.[8] It was observed that the spot of **6** monitored on TLC disappeared immediately after addition of a slight excess of PTAD (1.1 eq.) at 25 °C. After the usual workup and chromatographic purification, the D-A cycloadduct **7** was obtained as white crystals in excellent yield. The next step, acetylation of the 3-hydroxy group, was easily achieved by treating the adduct **7** with acetic anhydride in the presence of DMAP at 60 °C in toluene (Scheme 1). After chromatography, the 3-protected ester **8** was obtained as white crystals

in good yield (91%).

Next, the ester **8** was converted to the halohydrin derivative **9** (Scheme 1). The halohydrination of a double bond has been widely reported using N-halogen species such as trichloroisocyanuric acid (TCCA),[11] tribromoisocyanuric acid (TBCA),[12] *N*-bromosuccinimide (NBS) [13,14] and *N*-chlorosuccinimide (NCS).[15] Our research focused on searching for an effective N-halogen reagent for the halohydrination of the ester **8**. Various N-halogen species, including NCS, TCCA, 1,3-dichloro-5,5-dimethylhydantoin (DCDMH), NBS, TBCA and 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) were evaluated for this halohydrin reaction in acetone : H_2O (10:1) (Table 1). It was observed that the reaction proceeded well only when TBCA was used as the halogenation reagent and when performed at a relatively high temperature (Table 1, entry 13). Compared with TBCA, when TCCA was used as halogenation reagent, we found that the desired product **11** was accompanied by the formation of a side product **12** (Scheme 2) in the ratio of 7:3 (Table 1, entry 4), and the structure of **12** was confirmed by $LiAlH_4$ reduction (in reflux THF for 2 h) affording 2β-hydroxyprovitamin D_3 (**2**). On account of their structural similarity it is hard to separate **11** and **12** by chromatography and the ratio of **11:12** was determined by ^1H NMR spectroscopy. However, the reaction did not proceed satisfactorily or at all when other N-halogen reagents used, no matter whether it was performed at a low or elevated temperature (Table 1, entries 1-3, 5-12). In addition, various solvents such as acetone, THF, hexane, CH_2Cl_2 and EtOAc, in combination with water were also tried for this halohydrin reaction. Among these, a mixture of acetone and water in a 15:1 ratio was the best solvent for the halohydrin formation (Table 1, entry 19). When hexane, CH_2Cl_2 and EtOAc in combination with water was used, the reaction did not proceed well even by extending the reaction time to 6 h, which could be attributed to their insolubility in water, leading to the reaction proceeding in a two-phase system (Table 1, entries 15-17). Furthermore, we found that the amount of water also significantly affected the yield of the product in this process. A significant decrease trend was observed if the ratio of solvent and water was adjusted from 20:1 to 50:1 (Table 1, entries 20-22).



Scheme 2. The reaction between Ester **8** and TCCA.

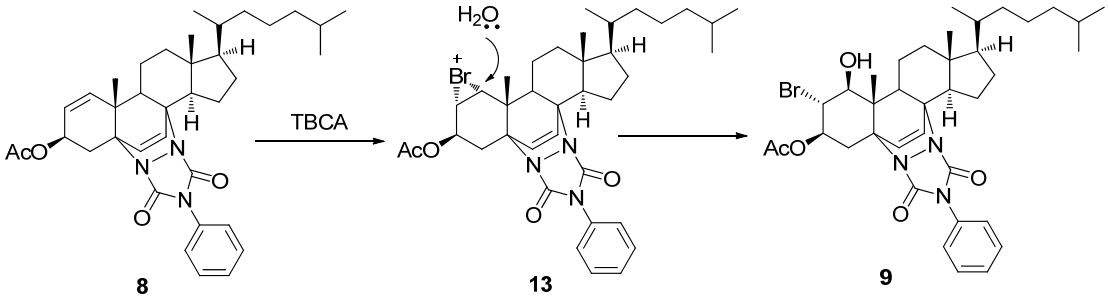
Table 1. Optimization of halohydrin reaction [a]

Entry	N-halogen reagent	Solvent	Temp (°C)	Yield (%)[b]
1	NCS	Acetone:H_2O (5:1)	0	N.R.
2	NCS	Acetone:H_2O (5:1)	25	N.R.
2	NCS	Acetone:H_2O (5:1)	50	N.R.
3	TCCA	Acetone:H_2O (5:1)	0	trace
4	TCCA	Acetone:H_2O (5:1)	25	86% (7/3) [c]
5	NBS	Acetone:H_2O (5:1)	0	N.R.
6	NBS	Acetone:H_2O (5:1)	25	N.R.
7	NBS	Acetone:H_2O (5:1)	50	trace
8	DBDMH	Acetone:H_2O (5:1)	0	N.R.
9	DBDMH	Acetone:H_2O (5:1)	25	N.R.
10	DBDMH	Acetone:H_2O (5:1)	50	trace
11	TBCA	Acetone:H_2O (5:1)	0	N.R.
12	TBCA	Acetone:H_2O (5:1)	25	trace
13	TBCA	Acetone:H_2O (5:1)	50	84%
14[d]	TBCA	THF:H_2O (5:1)	50	82%
15[d]	TBCA	Hexane:H_2O (5:1)	50	14%
16[d]	TBCA	CH_2Cl_2:H_2O (5:1)	50	18%
17[d]	TBCA	EtOAc:H_2O (5:1)	50	22%
18	TBCA	Acetone:H_2O (10:1)	50	87%
19	TBCA	Acetone:H_2O (15:1)	50	90%
20	TBCA	Acetone:H_2O (20:1)	50	80%
21	TBCA	Acetone:H_2O (30:1)	50	52%
22	TBCA	Acetone:H_2O (50:1)	50	30%

[a] Reagents and conditions: halogenation reagent (2.4 mmol), ester **8** (2 mmol), 2 h. [b]Isolated yield. [c] The ratio of **11/12** was determined by ^{1}H NMR spectroscopy. [d] Extending reaction time to 6 h

A probable mechanistic pathway to explain the excellent stereo-selectivity of the bromohydrin **9** is shown in Scheme 3. Because of the steric hindrance of C-3 acetoxy group and C-19 methyl, an endo-three-membered bromonium ion **13** was generated initially via electrophilic addition of Br^+ on to the **8**, followed by nucleophilic trans-addition of water to the intermediate **13**, to obtain

the bromohydrins **9**. The C-6 double bond did not react during this process, which could be attributed to the steric hindrance of diene-protecting group.



Scheme 3. Proposed mechanism for the stereoselective hydroxybromination.

Subsequently, by action of K_2CO_3 on the bromohydrins product **9** in methanol at room temperature, the epoxide **10** was obtained in excellent yield (92%). Finally, transformation of the epoxide **10** to the 2β-hydroxyprovitamin D_3 **2** was achieved in one step by treatment with an excess amount of lithium aluminum hydride in THF with 72% yield. With the same $LiAlH_4$ reduction, the bromohydrins product **9** could be converted to 1β-hydroxyprovitamin D_3 **1** in one step in 86% yield.

Conclusions

In conclusion, we here reported an efficient and environmentally-friendly method for the synthesis of 1β(2β)-hydroxyprovitamin D_3, the photoprecursors of 1β- and 2β-hydroxyvitamin D_3. The key procedure was rapid, performed in acetone:H_2O (15:1) by using TBCA at 50 °C to give bromohydrins product in good yield. To the best of our knowledge, there are no reports of the synthesis of 1β-hydroxyprovitamin D_3 **1** and 2β-hydroxyprovitamin D_3 **2** employing our methods. This method also avoids the defects existed in previous methods, such as poor yield and non-stereoselectivity.

Experimental Section

General. Melting points were determined using a digital melting point apparatus and uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian spectrometer at working frequencies 400 and 100 MHz respectively in $CDCl_3$ using TMS as internal standards. All chemical shifts were reported as δ values (ppm) relative to TMS and observed coupling constants (*J*) are given in Hertz (Hz). Mass spectra were measured with a HRMS-ESI instrument or a low-resolution MS instrument using ESI ionization. Cholesterol was provided by Hangzhou Xiasha

Biochemical Science and Technology Co., Ltd, and all other reagents were purchased from commercial source and without prior purification. Column chromatography was performed on silica gel (200-300 mesh) and the elution was performed with *n*-hexane / ethyl acetate.

Preparation of cholesta-1,4,6-trien-3-one (4). The cholesta-1,4,6-trien-3-one was prepared according to the literature:[16] a solution of the cholesterol **3** (5 g, 12.95 mmol) and DDQ (11.8 g, 51.8 mmol) in dioxane (125 mL) was refluxed for 24 h under nitrogen (TLC control, petroleum ether/EtOAc (4:1, v/v)). Then the reaction mixture was cooled down and the resulting precipitate was filtered off and washed with dichloromethane (20 mL × 3). The filtrate was rotary evaporated to dryness and the dark brown residue was purified by column chromatography on silica gel (ethyl acetate/*n*-hexane, 1:10) to afford trienone **4** (3.1 g, 63%).

Cholesta-1,4,6-trien-3-one (4). White crystals, mp 88.5-90.3 °C (lit.[16] 89.5-91.5 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (d, *J* 10.0 Hz, 1H), 6.26-6.15 (m, 2H), 6.07-5.94 (m, 2H), 2.25 (m, 1H), 2.08 (m, 1H), 1.99-1.20 (m, 13H), 1.19 (s, 3H), 1.17-0.95 (m, 5H), 0.92 (d, *J* 6.8 Hz, 3H), 0.86 (d, *J* 6.4 Hz, 6H), 0.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.1, 162.5, 152.8, 138.6, 127.9, 127.3, 123.5, 56.0, 53.6, 48.4, 43.1, 41.3, 39.5, 38.3, 36.2, 35.8, 28.2, 28.1, 23.9, 23.8, 22.9, 22.7, 21.9, 20.8, 18.7, 12.1, MS (ESI) *m/z* 381.2.7 $[M+H]^+$, 403.3 $[M+Na]^+$.

Preparation of 3-acetoxy-1,3,5,7-cholestatetraene (5). A solution of trienone **4** (2.5 g, 6.58 mmol) in acetic anhydride (30 mL), acetyl chloride (12 mL) and pyridine (2 mL) was heated under reflux for 3 h with stirring (TLC control, petroleum ether/EtOAc (4:1, v/v)). Then the excess reagents were removed in vacuo and the 3-acetoxy-1,3,5,7-cholestatetraene **5** was purified by crystallization from MeOH (2.4 g, 85%).

3-acetoxy-1,3,5,7-cholestatetraene (5). Yellow crystals, mp 119.2-121.5 °C (lit.[17] 122-124 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.98-5.75 (m, 4H), 5.60 (m, 1H), 2.31-1.99 (m, 5H), 1.96-0.92 (m, 17H), 0.89 (d, *J* 8.0 Hz, 3H), 0.80 (d, *J* 8.0 Hz, 6H), 0.72 (s, 3H), 0.57 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4, 143.9, 138.0, 137.1, 134.4, 122.5, 119.7, 117.4, 115.0, 56.2, 55.2, 51.5, 44.3, 42.1, 39.8, 39.4, 36.5, 36.4, 28.6, 28.5, 24.4, 23.4, 23.3, 23.0, 22.2, 21.6, 19.4, 12.7; HRMS: $C_{29}H_{42}NaO_2$ $[M+Na]^+$; calculated: 445.3077, found: 445.3092.

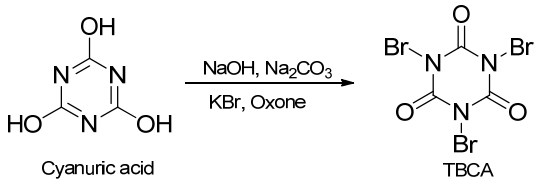
Preparation of D-A cyclo adduct (7). A solution of tetraene **5** (2.11 g, 5.0 mmol) in ether (40 mL) was added dropwise at 0 °C to a stirred solution of calcium borohydride in ethanol-methanol (from calcium chloride (6.3 g) in methanol (50 ml) and sodium borohydride (3.2 g) in ethanol (60 ml). Then the reaction mixture was allowed to stir at 0 °C for 3 h (TLC control, *n*-hexane/EtOAc (4:1, v/v)). The reaction mixture was filtered and HCl solution (10%wt.) (50 mL) was slowly added to the filtrate (note: take care! vigorous reaction). Then the mixture was extracted with CH_2Cl_2 (50 mL × 3) and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give the crude product **6**. The crude **6** (1.53 g, 4.0 mmol) was then dissolved in CH_2Cl_2 (20 mL) and 4-phenyl-1,2,4-triazoline-3,5-dione (0.77 g, 4.4 mmol) in CH_2Cl_2 (15 mL) was added slowly at room temperature until a faint pink colour persisted. The solvent was removed under reduced pressure

and the residue was chromatographed on silica gel (hexane:AcOEt, 2:1) to yield compound **7** (2.18 g, 78%, from tetraene **5**).

D-A cyclo adduct (**7**). **W**hite solid, mp 166.5-168.7 °C (lit.[17], 169-170 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.21 (m, 5H), 6.43 (d, *J* 8.0 Hz, 1H), 6.25 (d, *J* 8.0 Hz, 1H), 5.72 (m, 2H), 5.02 (m, 1H), 3.35 (m, 1H), 2.47-1.89 (m, 8H), 1.62-1.11 (m, 12H), 1.07 (s, 3H), 0.93 (d, *J* 6.4 Hz, 3H), 0.86 (d, *J* 6.4 Hz, 6H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.4, 145.8, 135.0, 134.9, 131.6, 129.7, 128.7, 128.0, 127.6, 126.0, 65.5, 64.6, 64.4, 60.4, 55.3, 51.4, 49.1, 44.0, 43.2, 39.5, 38.3, 35.9, 35.3, 33.5, 29.7, 28.1, 27.6, 23.7, 23.3, 22.9, 22.7, 22.4, 19.0, 12.9; HRMS: $C_{35}H_{47}N_3NaO_3$ $[M+Na]^+$; calculated: 580.3510, found: 580.3501.

Preparation of the ester (**8**)**.** To a solution of D-A cyclo adduct **7** (1.11 g, 2.0 mmol) in toluene (15 mL) was added DMAP (12 mg, 0.1 mmol) and acetic anhydride (0.41 g, 4.0 mmol). The mixture was allowed to stir at 60 °C for 2 h. After cooling, the reaction mixture was washed with HCl solution (10%wt.), saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane:AcOEt, 5:1) to yield compound **8** (1.09 g, 92%).

Ester (**8**): white crystals, mp 86.2-89.5 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.25 (m, 5H), 6.48 (d, *J* 8.4 Hz, 1H), 6.26 (d, *J* 8.4 Hz, 1H), 5.97 (m, 1H), 5.88 (d, *J* 10.0 Hz, 1H), 5.78 (m, 1H), 3.47 (m, 1H), 2.56-2.32 (m, 3H), 2.12-2.06 (m, 1H), 2.05 (s, 3H), 2.03-1.87 (m, 2H), 1.72-1.12 (m, 13H), 1.10 (s, 3H), 1.07-0.99 (m, 1H), 0.93 (d, *J* 6.4 Hz, 3H), 0.86 (d, *J* 6.8 Hz, 6H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 147.9, 145.6, 137.7, 134.6, 131.6, 130.1, 128.6, 127.6, 126.0, 124.4, 67.9, 64.9, 64.3, 55.5, 51.7, 49.1, 44.0, 43.2, 39.5, 38.3, 35.9, 35.3, 30.7, 29.8, 28.1, 27.6, 23.7, 23.2, 23.1, 22.9, 22.7, 21.8, 21.4, 19.0, 13.0; HRMS: $C_{37}H_{49}N_3NaO_4$ $[M+Na]^+$; calculated: 622.3615, found: 622.3611.



Scheme 4. Preparation of TBCA.

Preparation of tribromoisocyanuric acid.[12] To a solution of cyanuric acid (1.61 g, 12.5 mmol), NaOH (1.50 g, 37.5 mmol), Na_2CO_3 (1.99 g, 18.75 mmol), and KBr (4.46 g, 37.5 mmol) in H_2O (180 mL) coold in an ice bath was added dropwise a solution of Oxone (23.1 g, 37.5 mmol) in H_2O (150 mL). A white solid precipitate appeared during the addition of the oxidant solution and the mixture was stirred for 24 h. The product was isolated by vacuum filtration, washed with cold H_2O, and then dried with P_2O_5 to give the product (85% yield). The m.p. was not determined because it decomposed upon heating.

Preparation of bromohydrins (**9**)**.** To a stirred solution of the ester **8** (1.20 g, 2.0 mmol) in acetone:water (15:1) (32 mL), TBCA (0.87 g, 2.4 mmol) was added. The mixture was allowed to

stir at 50 °C for 2 h. Thereafter, water (30 mL) was added and the mixture was extracted with CH_2Cl_2 (30 mL × 3), and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (hexane:AcOEt, 2:1) to yield compound **9** (1.26 g, 91%).

Bromohydrins (9). White crystals, mp 105.6-108.2 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.20 (m, 5H), 6.43 (d, *J* 8.4 Hz, 1H), 6.28 (d, *J* 8.4 Hz, 1H), 5.59 (m, 1H), 5.27 (m, 1H), 4.46 (d, *J* 2.4 Hz, 1H), 3.11 (m, 1H), 2.70 (m, 1H), 2.54 (m, 1H), 2.38 (m, 2H), 2.11 (s, 3H), 2.09-1.93 (m, 2H), 1.68-1.27 (m, 10H), 1.19 (s, 3H), 1.16-0.99 (m, 4H), 0.93 (d, *J* 6.4 Hz, 3H), 0.86 (d, *J* 6.8 Hz, 6H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 146.2, 144.6, 135.6, 131.5, 129.4, 128.7, 127.8, 126.3, 74.8, 64.4, 63.7, 62.8, 55.8, 55.2, 50.8, 48.7, 44.4, 43.8, 39.5, 38.0, 35.9, 35.3, 30.4, 29.8, 28.1, 27.5, 23.6, 23.3, 22.9, 22.7, 22.5, 21.7, 21.3, 19.0, 12.9; HRMS: $C_{37}H_{50}BrN_3NaO_5$ $[M+Na]^+$; calculated: 718.2826, found: 718.2822.

Preparation of 1β-hydroxyprovitamin D$_3$ (1). To the THF solution (20 mL) of $LiAlH_4$ (0.76 g, 20 mmol), bromohydrins **9** (1.39 g, 2.0 mmol) in THF (25 mL) was added dropwise at 0 °C. The mixture was then heated under reflux for 4 h. After cooling, diluted hydrochloric acid was added for decomposition of the excess $LiAlH_4$ and the mixture became a clear solution. The solution was extracted with CH_2Cl_2 (40 mL × 3), and the combined organic layers were washed with saturated $NaHCO_3$ solution, brine, dried over anhydrous sodium sulphate and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (hexane:AcOEt, 3:1) to yield compound **1** (0.69 g, 86%).

1β-Hydroxyprovitamin D$_3$ (1). White crystals, mp 175.2-178.7 °C (lit.[8], 176-180 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.60 (m, 1H), 5.39 (m, 1H), 4.06 (m, 1H), 3.72 (m, 1H), 2.69 (m, 1H), 2.26-1.14 (m, 22H), 1.09 (s, 3H), 0.94 (d, *J* 6.4 Hz, 3H), 0.87 (d, *J* 6.8 Hz, 6H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.3, 139.4, 119.3, 116.3, 71.2, 70.0, 55.9, 54.5, 46.9, 43.0, 39.6, 39.2, 36.5, 36.2, 34.2, 29.8, 28.2, 28.1, 24.0, 23.1, 22.9, 22.8, 22.7, 21.1, 19.0, 12.0. HRMS: $C_{27}H_{44}NaO_2$ $[M+Na]^+$; calculated: 423.3234, found: 423.3233.

Preparation of the epoxide (10). To a stirred solution of the bromohydrins **9** (1.39 g, 2.0 mmol) in methanol (25 mL), K_2CO_3 (1.38 g, 10 mmol) was added. The mixture was allowed to stir at 25 °C for 2 h. Thereafter, water (30 mL) was added and the mixture was extracted with CH_2Cl_2 (25 mL × 3), and the combined organic layers were washed with brine, dried over anhydrous sodium sulphate and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (hexane:AcOEt, 4:1) to yield compound **10** (1.03 g, 93%).

Epoxide (10). White crystals, mp 169.5-171.2 °C (lit.[8], 172-173 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-7.17 (m, 5H), 6.39 (d, *J* 8.0 Hz, 1H), 6.21 (d, *J* 8.0 Hz, 1H), 4.99 (m, 1H), 3.52 (m, 1H), 3.21 (m, 1H), 3.09 (m, 1H), 2.52 (m, 1H), 2.34 (m, 1H), 2.25-1.77 (m, 5H), 1.68-1.05 (m, 13H), 1.01 (s, 3H), 0.95 (d, *J* Hz, 3H), 0.86 (d, *J* 6.4 Hz, 6H), 0.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.6, 148.0, 133.5, 131.2, 129.0, 128.7, 127.9, 125.9, 66.3, 65.4, 61.2, 61.1, 56.3, 54.8, 50.2, 49.5, 43.9, 41.3, 39.5, 37.9, 35.9, 35.4, 30.5, 29.8, 28.1, 27.5, 23.7, 23.5, 22.9,

22.6, 22.1, 19.2, 16.7, 13.0. HRMS: $C_{35}H_{47}N_3NaO_4$ $[M+Na]^+$; calculated: 596.3459, found: 596.3445.

Preparation of 2β-hydroxyprovitamin D_3 (2). To the THF solution (20 mL) of $LiAlH_4$ (0.76 g, 20 mmol), epoxide **10** (1.11 g, 2.0 mmol) in THF (25 mL) was added dropwise at 0 °C. The mixture was then heated under reflux for 2 h. After cooling, diluted hydrochloric acid was added for decomposition of the excess $LiAlH_4$ and the mixture became a clear solution. The solution was extracted with CH_2Cl_2 (40 mL×3), and the combined organic layers were washed with saturated $NaHCO_3$ solution, brine, dried over anhydrous sodium sulphate and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (hexane:AcOEt, 7:3) to yield compound **2** (0.58 g, 72%).

2β-Hydroxyprovitamin D_3 (2). White crystals, mp 176.5-178.9 °C (lit.[8], 176-180 °C), ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.62 (m, 1H), 5.31 (m, 1H), 3.83-3.52 (m, 2H), 2.47-1.07 (m, 23H), 1.03 (s, 3H), 0.92 (d, *J* 6.4 Hz, 3H), 0.87 (d, *J* 6.8 Hz, 6H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.9, 135.6, 121.1, 114.6, 77.6, 67.6, 56.2, 55.3, 46.1, 43.6, 43.1, 41.3, 41.1, 40.1, 39.6, 36.3, 36.2, 29.8, 28.1, 25.1, 24.0, 23.0, 22.7, 19.0, 12.1, 12.0. HRMS: $C_{27}H_{44}NaO_2$ $[M+Na]^+$; calculated: 423.3234, found: 423.3219.

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