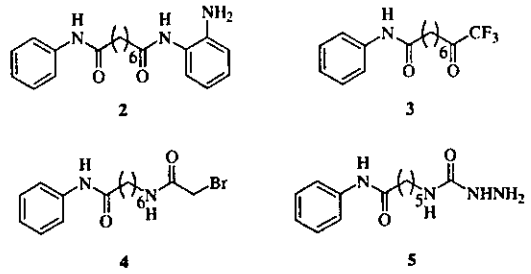


Figure 2. SAHA-based nonhydroxamate HDAC inhibitors.

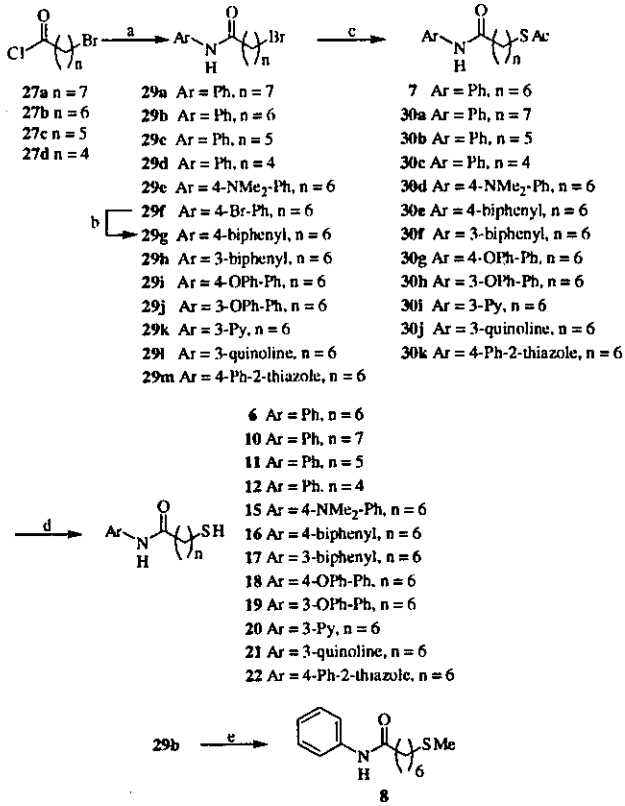
replacement groups for hydroxamic acid with the goal of drug discovery as well as finding new tools for biological research.

In the search for a suitable hydroxamic acid replacement, thiols seemed to be reasonable targets, because they have been reported to inhibit zinc-dependent enzymes such as angiotensin converting enzyme[20] and matrix metalloproteinase.[21] Recently, Furumai et al. demonstrated that the disulfide bond of FK228 (Fig. 1), a cyclic peptide HDAC inhibitor, is reduced in the cellular environment, releasing the free thiol analogue as the active species,[22] and Nishino et al. reported that cyclic tetrapeptides bearing disulfide group such as **1** (Fig. 1) inhibit HDACs under reductive conditions.[23] These reports prompted us to report on the synthesis and HDAC inhibition of thiol-based small molecule analogues.

The compounds prepared for this study are shown in Tables 1–3. Syntheses were accomplished as illustrated in Schemes 1–3. Compounds **6–8**, **10–12**, **15–21**, and **22** were synthesized from the corresponding acid chlorides **27a–d** by the route shown in Scheme 1. The amino group of aromatic amines **28** was acylated with an appropriate acid chloride **27** to give the amides **29a–f** and **29h–m**. Suzuki coupling[24] of bromobenzene **29f** with phenylboronic acid provided the biphenyl **29g**. Bromides **29** were treated with potassium thioacetate to give compounds **7** and **30a–k**, after which hydrolysis of the thioacetates under alkaline conditions gave the desired compounds **6**, **10–12**, **15–21**, and **22**. Sulfide **8** was obtained by the alkylation of sodium methanethiolate with bromide **29b**.

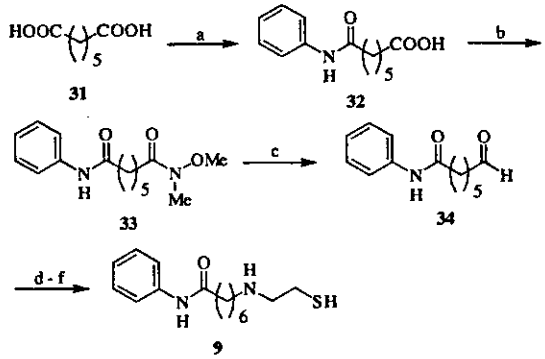
Synthesis of aminoethanethiol **9** was accomplished via aldehyde **34** (Scheme 2). The condensation of dicarboxylic acid **31** with an equivalent amount of aniline gave mono-anilide **32**. The carboxylic acid **32** was converted to Weinreb amide[25] **33** in the presence of EDCI and HOBt. Compound **33** was allowed to react with lithium aluminum hydride at 0 °C to give aldehyde **34** and subsequent reductive amination afforded aminoethanethiol **9**.

Compounds **13**, **14**, **23–25**, and **26** were prepared from alcohol **35** or **36** by the procedure outlined in Scheme 3. Treatment of bromide **35** with phenol in the presence of K_2CO_3 gave ether **38a**, and condensation of amine **36** with an appropriate aromatic carboxylic acid **37** afforded amides **38b–f**. Alcohols **38a–f** were converted to thiols **13**, **14**, **23–25**, and **26** in a three-step sequence: conversion of the alcohols to bromides, treatment of the bromides with potassium thioacetate, and hydrolysis of the resulting thioacetates.

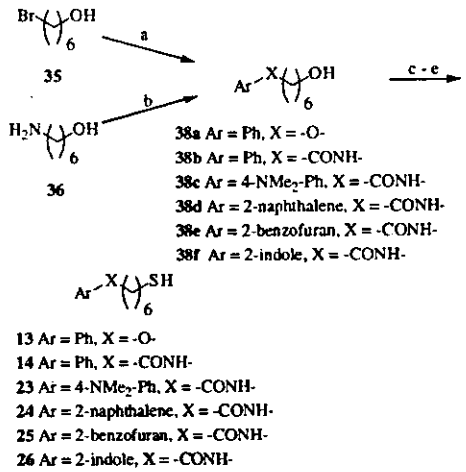


Scheme 1. Reagents and conditions: (a) $ArNH_2$ (**28**), Et_3N, CH_2Cl_2, rt, 25–99%; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, 1-methyl-2-pyrrolidinone, H_2O, 80 °C, 18%; (c) AcSK, EtOH, 60 °C, 84–99%; (d) 2 N aq NaOH, EtOH, THF, rt, 47–99%; (e) 15% aq NaSMe, EtOH, rt, 99%.

The compounds synthesized in this study were tested with an in vitro assay using a HeLa nuclear extract rich in HDAC activity.[26] The results are summarized in Tables 1–3 as IC_{50} values.



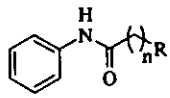
Scheme 2. Reagents and conditions: (a) Aniline, 180 °C, 43%; (b) *N,O*-dimethylhydroxylamine hydrochloride, Et_3N, EDCI, HOBt, DMF, rt, 94%; (c) $LiAlH_4$, THF, 0 °C, 72%; (d) 2-aminoethanol, $NaBH(OAc)_3$, THF, AcOH, rt; (e) $(Boc)_2O$, Et_3N, rt; (f) TFA, CH_2Cl_2, rt, 44% (three steps).

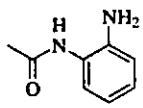


Scheme 3. Reagents and conditions: (a) Phenol, K_2CO_3, DMF, 80 °C, 96%; (b) ArCOOH (37), EDCI, HOBt, DMF, rt, 61–96%; (c) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 25–99%; (d) AcSK, EtOH, 60 °C, 47–99%; (e) 2 N aq NaOH, EtOH, THF, rt, 28–74%.

As seen in Table 1, the IC_{50} values of SAHA, *o*-aminoanilide **2**, bromoacetamide **4**, and semicarbazide **5** were 0.28, 120, 14, and 150 μM, respectively (entries 1, 2, 4, and 5). Trifluoromethyl ketone **3** was reported previously to inhibit HDACs with an IC_{50} of 6.7 μM (entry 3).[15] In our study, changing the hydroxamic acid of SAHA to thiol yielded fruitful results. A pronounced inhibitory effect ($IC_{50} = 0.21$ μM) was observed with thiol **6**, which was about 30–700-fold more active than the previously reported nonhydroxamates, and as potent as SAHA (entry 6). To confirm that the thiol group plays an important role in anti-HDAC activity, thioacetate **7**, and sulfide **8** were tested. As expected, thiol transformation into thioacetate and sulfide led to an inhibitor that was about 30-fold less potent and a compound devoid of anti-HDAC activity, respectively (entries 7 and 8). These results suggest that although the inherent affinity of a monodentate thiol ZBG is less than that of bidentate ZBGs such as hydroxamate and hydrated electrophilic ketone, its ease of ionization makes thiol **6** as powerful as SAHA.[27] In order to find more potent ZBGs, we examined the activity of aminoethanethiol **9**, which was expected to coordinate zinc ion in a bidentate fashion. Although aminoethanethiols have been reported to inhibit zinc proteins,[28] this functional group led to a loss of HDAC inhibitory activity (entry 9).

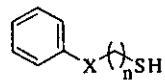
Table 1. HDAC inhibition data for SAHA and SAHA-based nonhydroxamates[a]



Entry	Compd	*n*	R	IC_{50} (μM)
1	SAHA[b]	6	-CONHOH	0.28
2	2[c]	6		120
3	**3**	6	$-COCF_3$	6.7[d]
4	4[e]	6	$-NHCOCH_2Br$	14
5	5[e]	5	$-NHCONHNH_2$	150
6	**6**	6	-SH	0.21
7	**7**	6	-SAc	7.1
8	**8**	6	-SMe	>100
9	9[f]	6	$-NHCH_2CH_2SH$	>100

[a] Values are means of at least three experiments.
[b] Prepared as described in Ref. 31.
[c] Prepared as described in Ref. 13.
[d] Data taken from the literature (Ref. 15).
[e] Prepared as described in Ref. 18.
[f] Trifluoroacetic acid salt.

Table 2. Effect of linker variation on HDAC inhibitory activity of thiols[a]



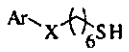
Entry	Compd	X	*n*	IC_{50} (μM)
1	**6**	-NHCO-	6	0.21
2	**10**	-NHCO-	7	1.5
3	**11**	-NHCO-	5	0.37
4	**12**	-NHCO-	4	6.2
5	**13**	-O-	6	11
6	**14**	-CONH-	6	0.36

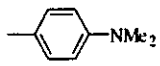
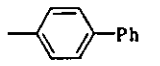
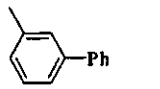
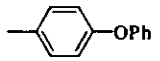
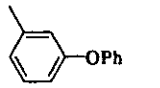
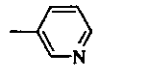
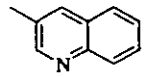
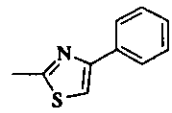
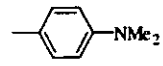
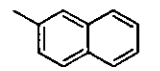
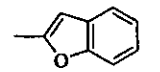
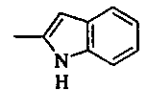
[a] Values are means of at least three experiments.

We next examined the effect of linker parts of thiol **6**. The results are shown in Table 2. HDAC inhibition was distinctly dependent on chain length, with $n = 7$ (**10**) and $n = 4$ (**12**) resulting in less potent inhibitors. However, compound **11**, in which $n = 5$, proved to be equally effective to **6**, in which $n = 6$ (entries 1–4). The similar structure–activity relationship (SAR) between thiols and hydroxamates, with $n = 6$ optimal,[29,30] indicates that thiols inhibit HDACs in a binding mode similar to that of hydroxamates. As for the group attaching the phenyl moiety, ether **13** displayed a moderate activity, whereas the activity of the reversed amide **14** was maintained (entries 5 and 6).

Having investigated the requirements for the ZBGs and linker parts, we next turned our attention to aromatic groups (Table 3). In the amide-linked series (entries 1–9), 4-substituted phenyl compounds tended to reduce the potency. Specifically, compounds **15** (Ar = 4-NMe_2-Ph), **16** (Ar = 4-biphenyl), and **18** (Ar = 4-PhO-Ph) showed about a 3–10-fold decrease in potency when compared to the parent thiol **6** (entries 2, 3, and 5). In contrast, when a phenyl group was introduced at the 3-position of the phenyl group of **6**, the IC_{50} of compound **17** was improved and reached 0.075 μM (entry 4). In addition, 3-phenoxy compound **19** was equipotent with compound **6** (entry 6). Next, we investigated the effect of the replacement of the phenyl group of compound **6** with heteroaryl rings (entries 7–9). While pyridine **20** and phenylthiazole **22** retained the potency of compound **6**, quinoline **21** was about 3-fold more active than compound **6** ($IC_{50} = 0.072$ μM). The reversed amide-linked series (entries 11–14) exhibited potencies similar to or

Table 3. Effect of aromatic group variation on HDAC inhibitory activity of thiols[a]



Entry	Compd	Ar	X	IC_{50} (μM)
1	**6**	-Ph	-NHCO-	0.21
2	**15**		-NHCO-	1.2
3	**16**		-NHCO-	1.1
4	**17**		-NHCO-	0.075
5	**18**		-NHCO-	0.62
6	**19**		-NHCO-	0.21
7	**20**		-NHCO-	0.11
8	**21**		-NHCO-	0.072
9	**22**		-NHCO-	0.17
10	**14**	-Ph	-CONH-	0.36
11	**23**		-CONH-	0.61
12	**24**		-CONH-	0.085
13	**25**		-CONH-	0.079
14	**26**		-CONH-	0.1

[a] Values are means of at least three experiments.

greater than the parent thiol **14**, except for **23** (Ar = 4-NMe_2-Ph), which resulted in a slightly less potent inhibitor. In particular, the reversed amides **24** with a naphthalene substituent and **25** with a benzofuran substituent showed stronger inhibition of HDACs with IC_{50}s of 0.085 and 0.079 μM, respectively. As a result, IC_{50}s in the double-digit nanomolar range were observed with 3-biphenyl **17**, quinoline **21**, naphthalene **24**, and benzofuran **25**, which were approximately 3–4-fold more potent than SAHA.

In summary, in order to find novel nonhydroxamate HDAC inhibitors, we designed and prepared a series of thiol-based SAHA analogues, and evaluated their inhibitory effect on HDACs. Compound **6**, in which the hydroxamic acid of SAHA was replaced by a thiol, was found to be as potent as SAHA. We have shown that the potency is related to chain length, with $n = 6$ optimal, and the amide and reversed amide were preferred as the group attaching the phenyl moiety. The conversion of the phenyl group of compound **6** to other aromatic groups led to the identification of inhibitors more potent than SAHA. The SAR results within the thiol series indicate that thiols inhibit HDACs in a manner similar to that of hydroxamates. As far as we could determine, this is the first report of nonmacrocyclic thiol inhibitors of HDACs. These small molecule thiols may be useful as tools for biological research and as orally bioavailable anticancer drugs. Currently, further detailed SAR studies and the next stage of evaluations are under way.

Acknowledgements

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Design and synthesis of cyclic urea compounds: a pharmacological study for retinoidal activity

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Abstract—Retinoids are natural and synthetic analogues of all-*trans* retinoic acid (ATRA). Cancer and other serious hyperproliferative diseases are attractive therapeutic targets for retinoids. We report here the design and synthesis of novel cyclic urea compounds with retinoidal activity. YR105 exhibited potent differentiation-inducing ability toward human promyelocytic leukemia HL-60 cells at the concentration of 10^{-9} M: its potency was almost equal to that of the native ligand, all-*trans* retinoic acid.

Retinoids, natural and synthetic analogues of all-*trans* retinoic acid (ATRA), have a variety of potent biological activities, such as induction of cell differentiation, proliferation, and apoptosis, as well as developmental changes.[1]

Retinoids also have potential chemotherapeutic and chemopreventive applications in the fields of dermatology and oncology.[2] Retinoic acid has a remarkable remedial effect on acute promyelocytic leukemia (APL).[3] Further, the inhibitory effect of retinoids on IL-6 production suggests their possible usefulness in various IL-6 associated diseases, including psoriasis and rheumatoid arthritis.[4]

It has been shown that the biological effects of retinoids are mediated by the activation of retinoic acid receptors (RARs), which are ligand-dependent gene transcription factors. There are three distinct receptor subtypes (RARα, β, γ), which possess considerable homology in their ligand binding domains.[5]

We report here the design and synthesis of novel cyclic urea compounds, which have retinoidal activity.

In earlier studies, a number of synthetic retinoid analogues were prepared, and a few of them showed potential biological effects several times higher than all natural retinoids (Fig. 1).[6] Some were selected as promising lead compounds for retinoidal activity. All the compounds consisted of two parts: a lipophilic portion fused with a hydrophilic benzoic acid moiety via amide or alkene or keto linkage. The biological activity depended on the linker type as well as the presence or absence of lipophilic moieties. Considering these aspects we selected the lipophilic moieties and the amido or alkene was changed to a urea linker, which may act as a suitable ligand for retinoid receptors. however Ur80,[7]

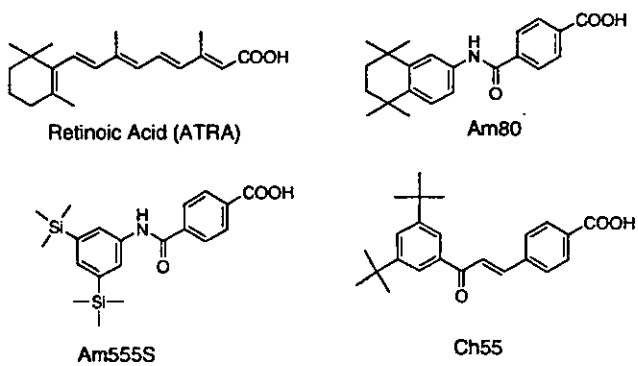

Figure 1. Structures of typical retinoid agonists.

Keywords: Cyclic urea; Retinoid; Cell differentiation; Drug design.
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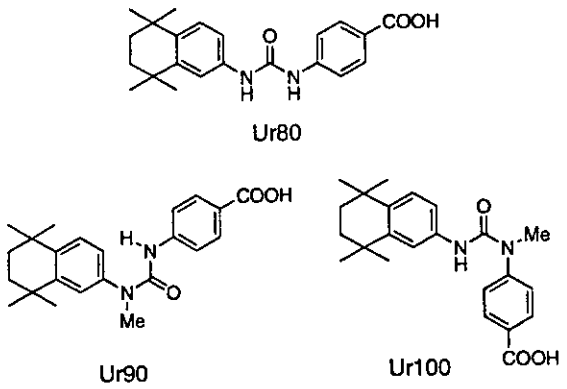


Figure 2. Conformations of methylated Ur80.

which has a urea linker, exhibited low bioactivity. Introduction of substitutes to urea nitrogen would cause flipping of stereochemistry to form Ur90 and Ur100 like Am80[8] (Fig. 2). Because of fix of conformation and lipophilicity, we chose a cyclic urea structure as the linker (Fig. 3).

A very simple method was developed to synthesize all desired cyclic urea derivatives. The synthesis of urea derivatives (**5a–h**) was accomplished via the following reaction sequences (Scheme 1). A number of different aromatic amines (**1a–d**) were utilized as the starting materials for the preparation of the desired cyclic urea compounds.

Various primary aromatic amines (**1a–d**) alkylated with 1,2-dibromoethane or 1,3-dibromopropane in acetonitrile resulted in formation of the respective secondary amines (**2a–h**). The synthesized secondary aromatic

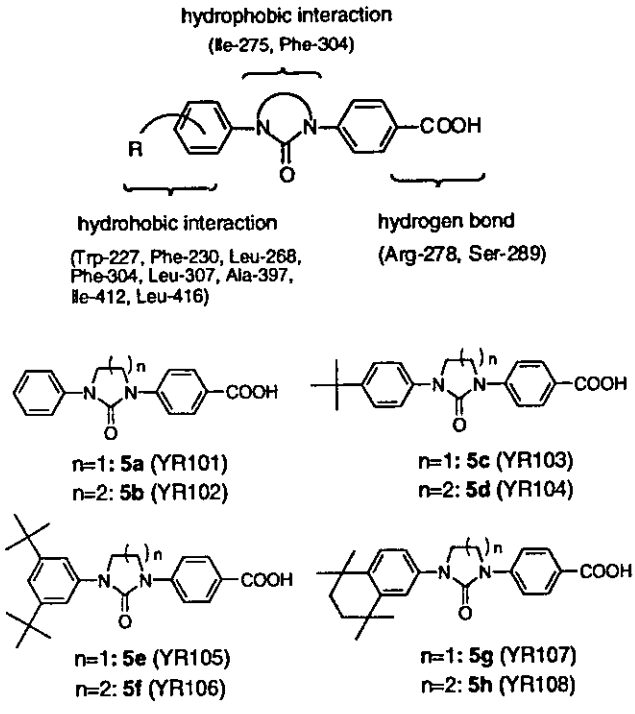
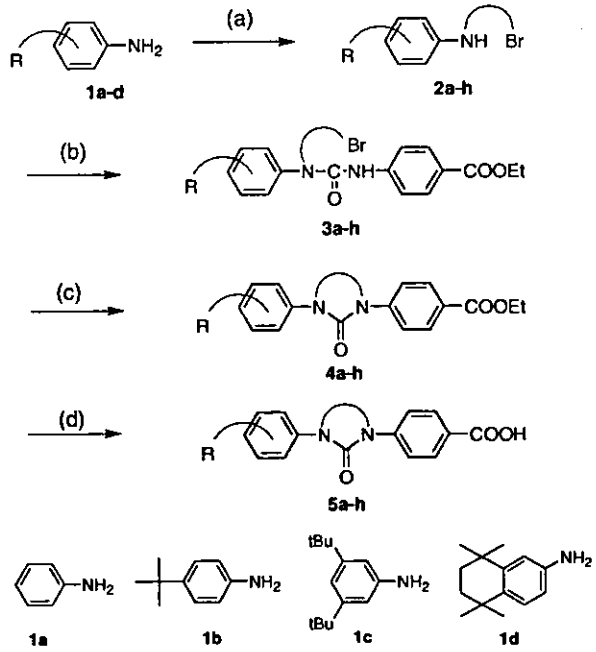


Figure 3. Design of cyclic urea compounds.



Scheme 1. Reagents and conditions: (a) 1,2-dibromoethane or 1,3-dibromopropane, CH_3CN, 50–70 °C, 72 h, 25–60%; (b) ethyl 4-isocyanatobenzoate, benzene, 60–70 °C, 48 h, 60–80%; (c) NaH, THF, rt, 3 h, 75–97%; (d) 5% NaOH, MeOH–H_2O (7:3), 60 °C, 1 h, 90–96%.

amines were allowed to react with ethyl 4-isocyanobenzoate in anhydrous benzene to form urea derivatives (**3a–h**). The intramolecular cyclization of the urea derivatives was performed utilizing sodium hydride as the base to obtain cyclic urea derivatives (**4a–h**). Finally, the ethyl ester of cyclic urea derivatives was converted to free acid form (**5a–h**) under basic conditions.

The biological activities of compounds **5a–h** were evaluated in terms of induction of differentiation of HL-60 cells into mature granulocytes. The results are summarized in Table 1. YR105 (**5e**) exhibited potent differentiation-inducing activity toward HL-60 cells, with an EC_{50} value of 8.3×10^{-9} M. The activity of YR105 was one order weaker than that of retinobenzoic acid Am80, and comparable to that of all-*trans* retinoic acid. YR106

Table 1. HL-60 differentiation-inducing activity of cyclic urea compounds (**5a–h**)

Compound	Activity (ED_{50}) (M)
5a (YR101)	Inactive
5b (YR102)	Inactive
5c (YR103)	Inactive
5d (YR104)	Inactive
5e (YR105)	8.3×10^{-9}
5f (YR106)	4.9×10^{-7}
5g (YR107)	1.2×10^{-7}
5h (YR108)	Inactive
Retinoic acid	2.4×10^{-9}
Am80	7.9×10^{-10}
Ur80	$>10^{-6}$

Inactive means there was no activity at 10^{-6} M, and $>10^{-6}$ M means there was slight activity at 10^{-6} M.

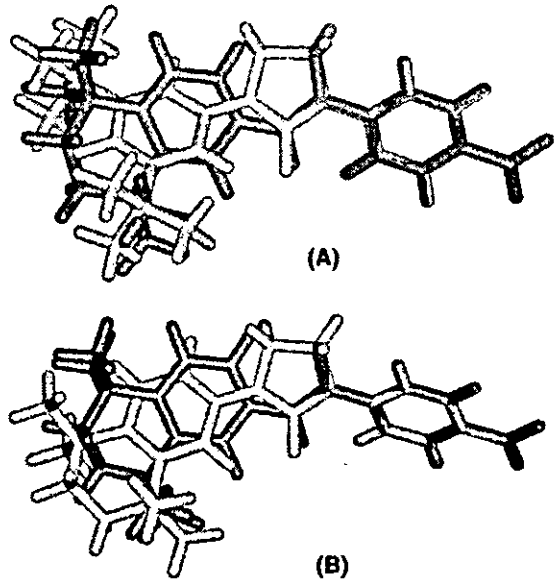


Figure 4. (A) Superimposition of energy-minimized conformations of YR105 (green) and Am80 (red). (B) Superimposition of energy-minimized conformations of YR107 (green) and Am80 (red).

(5f), YR107 (5g) decreased the activity by two orders of magnitude, compared with YR105.

Figure 4 shows superimposition of the energy-minimized structures of YR105 (5e) and YR107 (5g) on the energy-minimized structure Am80. YR105 is better overlapped with Am80 than YR107.

A docking model of YR105 (5e) bound to RARγ (1EXA) was constructed by molecular dynamics (MD) simulation at high temperature (1000 K) and molecular mechanics (MM) energy minimization. AMBER* was used as force field. Calculations were performed by *MacroModel* (ver. 6.5 and 8.0).[9] YR105 was well fitted to the cavity of the ligand binding domain (LBD) of RARγ, as shown in Figure 5. The bulky alkylated phenyl moiety fits well to the hydrophobic region of the LBD (Trp-227, Phe-230, Leu-268, Phe-304, Leu-307, Ala-397, Ile-412, Leu-416). The carboxylate group of the ligand interacted with Arg-278, Ser-289 by hydrogen bonds.

In conclusion, we have discovered a new class of cyclic urea compounds that exhibit retinoidal activity.

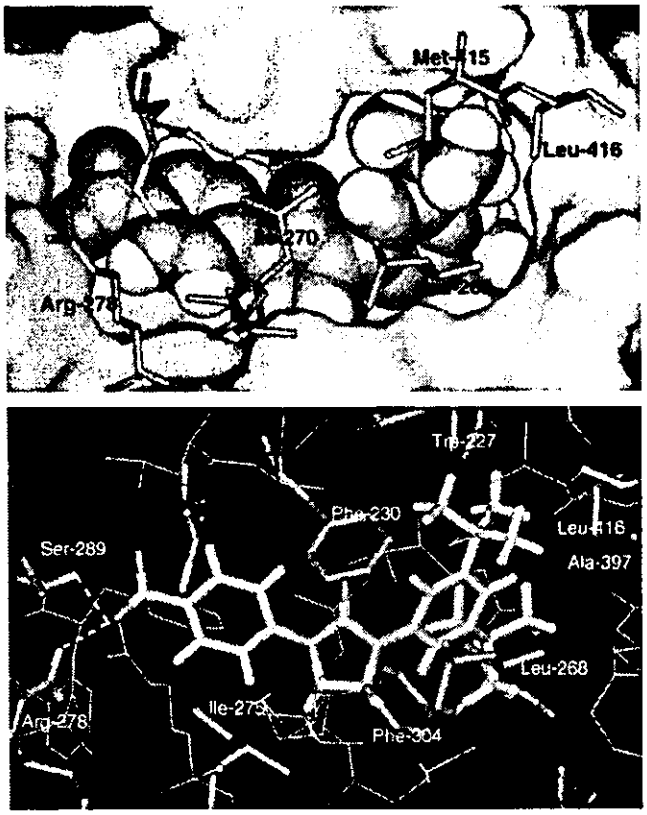


Figure 5. Stable docking model of 5e (YR105) in the RARγ simulated from the crystal structure of RAR-BMS270394 complex (1EXA).

Acknowledgements

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Synthesis of C_{60} derivatives for photoaffinity labeling

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Abstract—In order to study the interaction of fullerenes with biological molecules, a novel photoaffinity labeling agent derived from C_{60} was designed and synthesized. As photosensitive functional groups, azide group, and aziridine group are utilized. A convenient synthetic route via fulleropyrrolidine **2** was employed to obtain compounds labeling agents **5** and **9**.

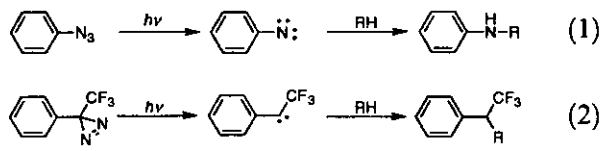
The biological activities of fullerenes have attracted considerable attention due to their potential medicinal applications.[1–3] Their novel and unexploited properties stem from their bulky hydrophobic shape and their photosensitivity[4–7] and radical-generating[8–11]/-quenching[12,13] activities enabled by highly conjugated π-electron system.

As a most remarkable activity, direct inhibition of enzymes by C_{60} has been reported. The first example, HIV-1 protease inhibition by a water soluble fullerene derivative, was reported in 1993[14–16] by Wudl, Wilkins, et al. Independently, Toniollo et al. has reported C_{60}-peptide conjugates and identified activity of these compounds against HIV-1 protease and chemotatic activity against human monocytes.[17] Separately, we have developed new procedures for solubilizing C_{60} in water[18] and assayed unfunctionalized C_{60} for direct enzymatic inhibtion. These studies led to the discovery that aqueous solutions of C_{60} inhibit glutathione-*S*-transferase (GST).[19]

The ability of C_{60}, which is large (7 Å id) hydrophobic molecules, to bind to biological compounds, was initially surprising and several groups have attempted to identify and calculate the binding sites. Based on a computer simulated docking study, Wudl, Wilkins, et al. speculated that the C_{60} core was enclosed in the cylindrical active site, which consists primarily of hydrophobic amino acid residues, of HIV-1 protease. In our own work, we calculated that C_{60} binds to GST at a cleft between two subunits of the enzyme, although the specific residues, which make up the active site are unclear.[20]

In order to clarify the more detailed binding site of C_{60}, two solutions are possible as follows. One is to isolate pure enzyme–fullerene complex and determine the structure by NMR or crystallographic methods. Another potential method for identifying the active site area is photoaffinity labeling, which is particularly useful for identifying the active site in solution under physiological conditions.

We now report the design and synthesis of the first C_{60}-derived photoaffinity labeling reagents. Our synthetic route to photoaffinity reagents **5** and **9** provide a concise, flexible route to fullerenes functionalized with photoreactive pendant groups such as phenylazide and phenyldiazirine, which generate aryl nitrene and aryl carbene, respectively (Eqs. 1 and 2).[21]



(1)

(2)

In order to develop an efficient and flexible synthetic method, which would allow the late-stage introduction of a variety of photoaffinity labels, we chose to utilize dimethylfulleropyrrolidine (**2**). This C_{60} derivative

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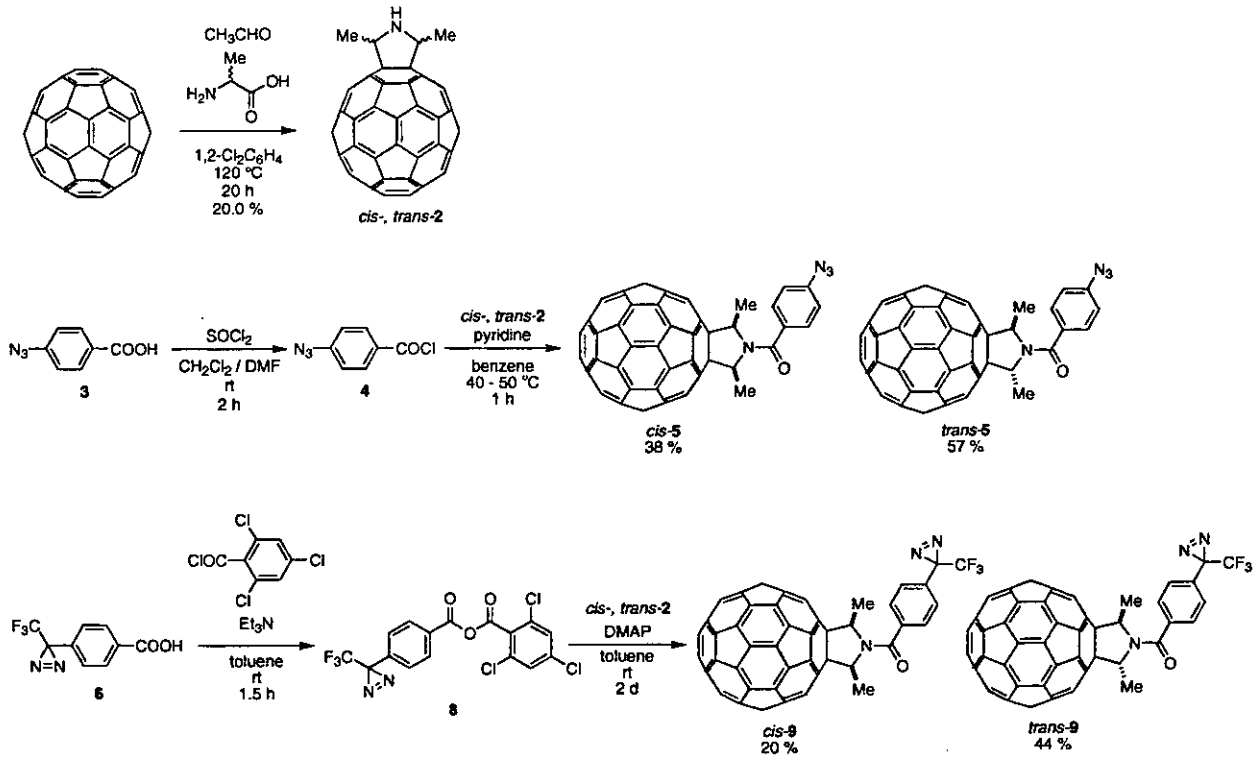

Scheme 1. Synthesis of C_{60} derivatives with phenylazide (**5**) and phenylaziridine (**9**) group.

is readily prepared by the method of Prato and co-workers[22] and Wilson and co-workers.[23,24] This route provides a convenient approach to C_{60} derivatives with a secondary amine as an ideal site for the incorporation of further functionalization.

The synthesis of phenylazide derivative of fullerene was achieved as shown in Scheme 1. Dimethyl fulleropyrrolidine **2** (*cis*- and *trans*-mixture) was prepared by 1,3-dipolar cycloaddition[25] and then acylated with acid chloride **4** to give *cis*- and *trans*-C_{60}-phenylazide derivatives **5**, which can be easily separated by silica gel column chromatography.[26]

To synthesize the C_{60}-phenyldiazirine derivative **9**, we first attempted the reaction of fulleropyrrolidine **2** with an acid chloride, but this reaction did not give useful amounts of the desired product. Despite attempts to activate the acyl moiety by a succinimide group using 4-(3-trifluoromethylazirino)benzoic succinimide, product formation was not observed. In sharp contrast, however, the use of Yamaguchi reagent **8** to couple **6** and **2** gave good yields of *cis*- and *trans*-C_{60}-phenyldiazirine derivatives **9**.[27] These stereoisomers are readily separated by silica gel chromatography. Compounds **5** and **9** were characterized by spectroscopic methods.[28] The *cis*- and *trans*-stereochemistry of each compounds were determined according to the reported studies.[23,24]

In addition to the potential utility of fullerene-derived photoaffinity labels for elucidating the active site of C_{60} binding to enzymes such as GST and HIV-1 protease, the ability to selectively tag a protein or enzyme with fullerene may offer a new approach to the detection of biological molecules with high sensitivity. For example, an acidic isozyme of GST is specified as cancer expressing marker in liver cancers.[29,30] The ability to selectively tag such diagnostic enzymes with C_{60}, which has unique and useful chemical and photophysical properties, may offer a novel and rapid detection method for identifying trace amounts of enzyme present in a biological sample. These and other applications of the reported photoaffinity labeling reagents currently in progress.

In conclusion, we have described a concise and flexible route to fullerene-derived photoaffinity labels with potential utility in enzyme tagging and the elucidation of the binding sites of protein to C_{60}.

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25. To a solution of C_{60} (36 mg, 0.05 mmol) and D,L-alanine (9.2 mg, 0.10 mmol) in 1,2-dichlorobenzene (10 mL), acetaldehyde (11 mg, 0.25 mmol) was added and the mixture stirred at 120 °C for 20 h. The reaction process was checked by HPLC [silica gel column, solvent: benzene–EtOAc (10:1)]. The reaction mixture was purified by silica gel column chromatography (hexane–benzene–EtOAc) to give brown solid **2** (9.0 mg, 0.011 mmol, $y = 22\%$) as a *cis*- and *trans*-mixture.
26. To a solution of 4-azidobenzoic acid **3** (1.84 g, 11 mmol) in CH_2Cl_2 (5 mL), $SOCl_2$ (4.0 mL, 6.5 g, 55 mmol) in CH_2Cl_2 (5 mL) was added under argon atmosphere. Subsequently, dry DMF (1.5 mL) was added dropwise under Ar. After stirring for 2 h under Ar, the generation of acid chloride **4** was checked by TLC [solvent: hexane–EtOAc (1:1)] and then reaction mixture was filtered and concentrated in vacuo. To a solution of dimethyl fulleropyrrolidine **2** (*cis*- and *trans*-mixture, 20 mg, 0.025 mmol) in benzene (10 mL), acid chloride **4** (100 mg, 0.55 mmol) and pyridine 1 mL were added and the mixture stirred at 50 °C for 1 h. The reaction process was checked by TLC [benzene–EtOAc (1:1)], and then small amount of Et_3N was added. The products (*cis*- and *trans*-isomers) were separated by silica gel column chromatography (hexane–benzene–EtOAc) to give *cis*-**5** (8.9 mg, 9.5 μmol, $y = 38\%$) and *trans*-**5** (13.3 mg, 14.2 μmol, $y = 57\%$).
27. To a solution of 4-(3-trifluoromethylazirino)benzoic acid **6**, (9.7 mg, 0.042 mmol) with Et_3N (10 μL) in toluene (2 mL), 2,4,6-trichlorobenzoyl chloride (10 μL) was added and stirred under Ar at room temperature for 1.5 h. The reaction process was monitored by TLC [hexane–EtOAc (1:1)]. Subsequently, dimethyl fulleropyrrolidine **2** (10 mg, 12.6 μmol), DMAP 7 mg in toluene (4 mL) was added and then stirred under Ar at room temperature in dark condition for 2 days. The reaction process was monitored by TLC [benzene–EtOAc (1:1)] and then reaction mixture was purified by silica gel column chromatography (hexane–benzene–CH_2Cl_2) to give *cis*-**9** (2.5 mg, 2.5 μmol, $y = 20\%$) and *trans*-**9** (5.6 mg, 5.6 μmol, $y = 44\%$).
28. Selected spectroscopic data for *cis*-**5**: ^{1}H NMR ($CDCl_3$, 300 MHz): 2.28 (d, $J = 6.9$, 6H), 6.14 (q, $J = 6.9$, 2H), 7.23 (d, $J = 8.7$, 2H), 7.81 (d, $J = 8.7$, 2H); MALDI-TOF-MS (negative, matrix: DTT): 936 ($[M-1]^-$), 720. *trans*-**5**: 2.21(d, $J = 6.0$, 6H), 5.74 (q, $J = 6.6$, 2H), 7.22 (d, $J = 8.4$, 2H), 7.99 (d, $J = 8.4$, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 19.9 (CH), 65.3 (CH3), 119.5 (CH), 130.2 (CH), 133.3–154.6 (C_{60}), 173.1 (CO); MALDI-TOF-MS (negative, matrix: DTT): 936 ($[M-1]^-$), 720; FT-IR (KBr): 2122 (N_3), 1670 (CO), 1600, 1260, 1182, 842, 756, 527 cm^{-1}. *cis*-**9**: ^{1}H NMR ($CDCl_3$, 300 MHz): 2.27 (d, $J = 6.7$, 6H), 6.08 (q, $J = 6.7$, 2H), 7.40 (d, $J = 8.5$, 2H), 7.81 (d, $J = 8.5$, 2H); MALDI-TOF-MS (negative, matrix: DTT): 1003 ($[M-1]^-$), 720. *trans*-**9**: 2.23 (d, $J = 6.9$, 6H), 5.17 (q, $J = 6.9$, 2H), 7.40 (d, $J = 8.3$, 2H), 7.81 (d, $J = 8.3$, 2H); MALDI-TOF-MS (negative, matrix: DTT): 1003 ($[M-1]^-$), 720.
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JOC Article

Design and Efficient Synthesis of 2α-(ω-Hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3 Analogues, Including 2-*epi*-ED-71 and Their 20-Epimers with HL-60 Cell Differentiation Activity

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A concise and efficient synthetic approach to 2α-(ω-hydroxyalkoxy)-1α,25-dihydroxyvitamin D_3 (**4a**–**c**), including 2-*epi*-ED-71, was developed starting from D-glucose as a chiral template for the construction of the 2α-modified A-ring precursors (**11a**–**c**). It was found that the best ligand for the bovine thymus vitamin D receptor (VDR) in this series is **4b**, which has 1.8 times greater binding affinity for the bovine thymus VDR than that of the natural hormone **1**. Interestingly, potency in the induction of HL-60 cell differentiation for **4a**–**c** was almost the same or weaker than that of **1** despite the strong binding affinity for the VDR. Next, we were interested in the "double modification" of **1** based on **4a**–**c** with C20-epimerization, affording 2α-(ω-hydroxyalkoxy)-20-*epi*-1α,25-dihydroxyvitamin D_3 (20-*epi*-**4a**–**c**). All three 2α-substituted 20-*epi* analogues of **1** (20-*epi*-**4a**–**c**) exhibited stronger binding affinities for the VDR, and their conformations in the ligand binding domain of VDR were analyzed by molecular modeling. Double-modified analogues of 20-*epi*-**4a**–**c** showed marked HL-60 cell differentiation activity, and 20-*epi*-**4a** possesses an activity 58-fold higher than that of the natural hormone **1**.

Introduction

The physiologically active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$, **1**], is the nuclear hormone that regulates cellular growth, differentiation, and apoptosis in addition to its classical role in calcium homeostasis and bone mineralization.[1−4] Most of the biological effects of 1α,25$(OH)_2D_3$ are considered to be mediated via binding to the specific intracellular receptor, vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily acting as a ligand-dependent transcription factor with coactivators.[5] Ubiquitous distribution of VDR makes this hormone a potentially useful therapeutic agent for certain cancers, skin diseases, and immune disorders, and in fact, **1** and some synthetic analogues of **1** are clinically used in the treatment of bone diseases, secondary hyperparathyroidism, and psoriasis.[3] Therefore, it is interesting to design and synthesize analogues of **1** with high VDR affinity in terms of new drug development. To investigate the structure−activity relationships of the natural hormone, we systematically developed the A-ring-modified analogues, such as 2-methyl-,[6] 2α-alkyl-, and 2α-(ω-hydroxyalkyl)-1α,25$(OH)_2D_3$ (Figure 1).[7−10]

One of the striking results was that 2α-methyl-1α,25-$(OH)_2D_3$ (**2a**) showed a VDR binding potency that was 4-fold higher than that of **1**.[6] This simple A-ring modification afforded for the first time an analogue, having the natural side chain, with a VDR binding activity significantly higher than that of the parent hormone **1**; as a

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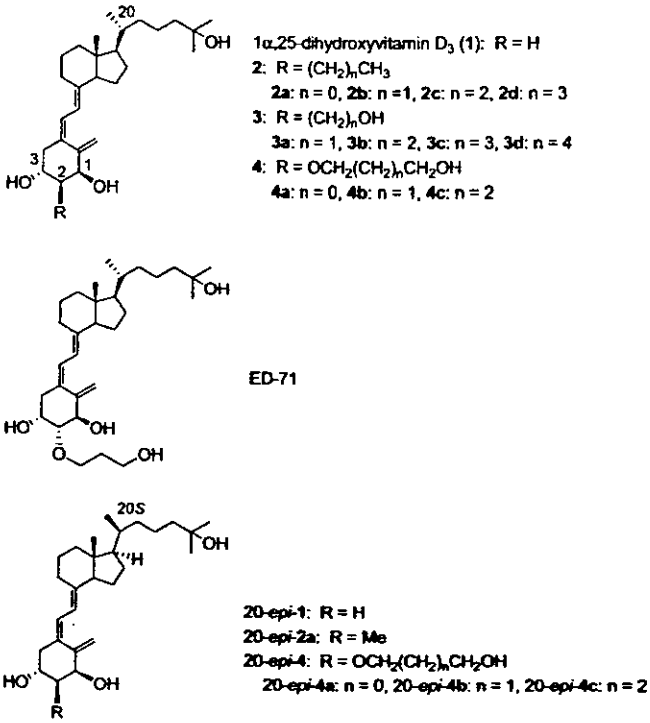

FIGURE 1. Structures of 1α,25-dihydroxyvitamin D_3 (**1**) (and its 2α-substituted analogues **2**–**4**), ED-71, and 20-*epi*-**1** (and its 2α-substituted analogues 20-*epi*-**2a** and 20-*epi*-**4a**–**c**).

result, **2a** shows a marked potency of transactivation of target genes, induction of HL-60 cell differentiation, and elevation of rat serum calcium concentration.[11] Elongation of the 2α-alkyl chain, as in **2b**–**d**, however, caused a decrease in the agonistic activity for VDR.[7a] In regard to the modification with the 2α-(ω-hydroxyalkyl) group, it was found that **3c** with the 2α-(3-hydroxypropyl) group on **1** best fits the cavity of the ligand binding domain (LBD) of VDR among the 2α-hydroxyalkyl series of **3** and the binding activity of **3c** is 3-fold higher than that of **1**.[7] On the other hand, Chugai Pharmaceutical Co. Ltd. developed 2β-(3-hydroxypropoxy)-1α,25$(OH)_2D_3$ (ED-71) as a promising candidate for the treatment of osteoporosis.[3,12] Although ED-71 shows high calcemic activity and a long half-life in plasma due to its strong affinity for vitamin D binding protein (DBP, twice the affinity of 1α,-25$(OH)_2D_3$),[12,13] its binding affinity for bovine thymus VDR is weaker than that of the natural hormone (13–93%).[12] We anticipated that 2α-(ω-hydroxyalkoxy)-1α,-25$(OH)_2D_3$ compounds (**4a**–**c**), including 2-*epi*-ED-71, could be better ligands for VDR and have potent vitamin D_3 activities.[14] Furthermore, we were interested in a structural cross talk in the vitamin D skeleton toward biological activity, between the A-ring and the CD-ring side chains through VDR binding, which would affect the biological activity profile of 1α,25$(OH)_2D_3$.[7d,15] Among the various synthetic 1α,25$(OH)_2D_3$ analogues, the side-chain structure of 20-*epi*-**1** is especially noteworthy because 20-*epi*-**1** possesses a more potent activity in cell differentiation and an immunosuppressive effect than the natural hormone, despite a practically unchanged calcemic activity.[16] The VDR binding potency of 20-*epi*-**1** relative to that of 1α,25$(OH)_2D_3$ is 4–5 times higher.[16b,17] Thus, 20-*epi* analogues of **4a**–**c** were also synthesized based on Trost's convergent method,[18] and VDR binding affinities and the inducing effects of HL-60 cell differentiation of these compounds were evaluated to understand the details of the structure–activity relationships of 1α,25$(OH)_2D_3$ analogues.

Results and Discussion

Synthesis. Convergent synthesis, in particular, Trost's A-ring/CD-ring connective strategy,[18] seemed to be most useful for synthesizing our target molecules. For the synthesis of A-ring precursor enynes **11a**–**c**, we chose D-glucose as a chiral template for the desired stereochemistry (1α,2α,3β; steroidal numbering) of the A-ring. Methyl α-D-glucoside was converted to the known epoxide **5**,[19] and the regiospecific ring opening[20] by an appropriate alkanediol[12a] at C3 under basic conditions gave the altrose configuration, in which the chiralities of C2, C3,

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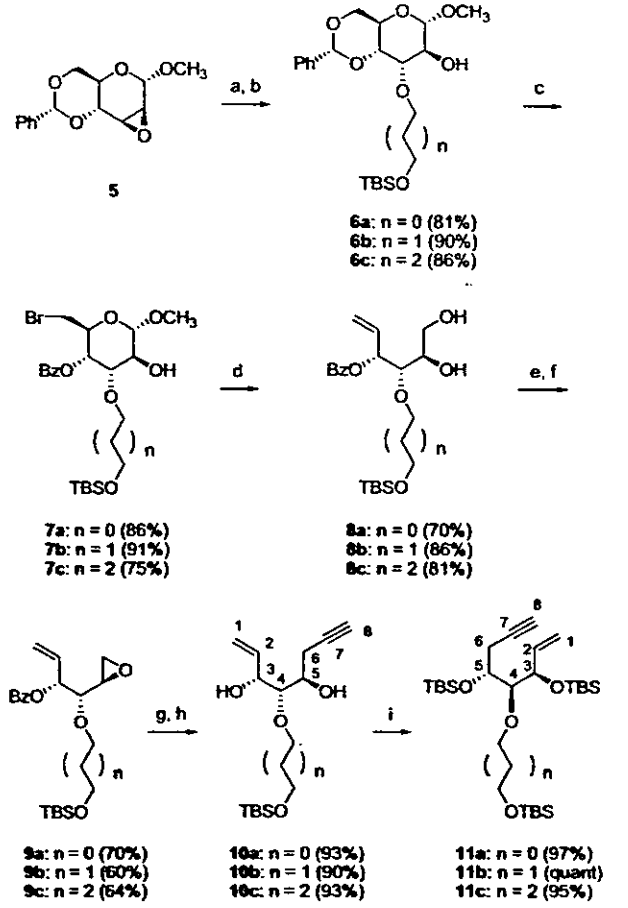
and C4 satisfy the 3β, 2α, and 1α stereochemistries of the target molecules **4**, respectively. Treatment of epoxide **5** with 1,2-ethanediol, 1,3-propanediol, or 1,4-butanediol in the presence of KO^{t}Bu with heat followed by O-silylation afforded protected methyl 3-*O*-(ω-hydroxyalkoxy)altropyranosides **6a**–**c** in 81–90% yield. NBS treatment[21] of benzylidene acetals **6a**–**c** gave bromides **7a**–**c** in 75–91% yield. Previously, we exchanged the resulting benzoyl group of **7** with the TBS group;[14] however, the ester group is resistant to the later steps being exposed under basic conditions, and it has made the process three steps shorter. Reaction of bromides **7a**–**c** with activated zinc powder and $NaBH_3CN$ provided alcohols **8a**–**c** in 70–86% yield.[22] The diols were converted to epoxides **9a**–**c** through sulfonylation of the primary alcohol followed by LiHMDS treatment in 60–70% yield. Ethynylation of **9a**–**c** using lithium TMS acetylide in the presence of BF_3·OEt_2 in THF and subsequent solvolysis in K_2CO_3/MeOH supplied enynes **10a**–**c** in 90–93% yield. Persilylation with TBSOTf/2,6-lutidine afforded the desired protected enynes **11a**–**c** for the palladium coupling in excellent yield (Scheme 1).

The B-*seco* steroidal structure was constructed by Trost's palladium-catalyzed alkylative cyclization with bromoolefin of the CD-ring counterpart (**12**),[18] and subsequent deprotection furnished the target 2α-(ω-hydroxyalkoxy)-1α,25(OH)$_2$D$_3$ in 32–66% yield in two steps (Scheme 2). Next, each enyne was connected to the 20-*epi*-CD-ring counterpart (**14**), which was synthesized from vitamin D$_2$ by our reported method,[17] in the same manner to yield 20-*epi* analogues (20-*epi*-**4a**–**c**) in 45–57% yield (Scheme 3). All analogues were purified with reversed-phase HPLC (recycled) for biological evaluations.

Biological Evaluations. The VDR binding affinities and potencies of induction of HL-60 cell differentiation of the newly synthesized analogues (**4a**–**c** and 20-*epi*-**4a**–**c**) are summarized in Table 1 in comparison with those of the natural hormone **1** and 20-*epi*-**1**.[16,17] In the VDR binding assays[23] using the bovine thymus VDR, **4a** and **4b** showed a greater binding affinity for the VDR (entries 2 and 3) and **4b** reaches a peak in these three analogues with the natural side chain (20*R*).

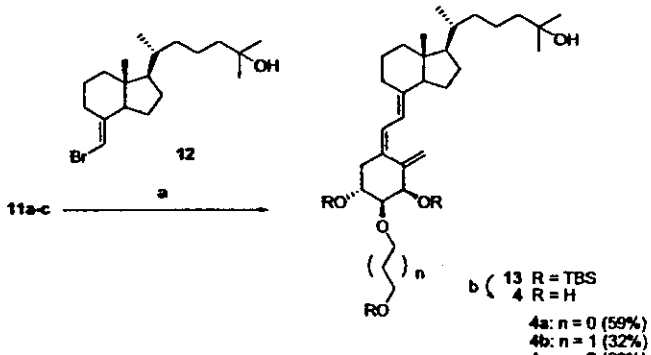
Docking Studies. We investigated a three-dimensional structure of 2α-(3-hydroxypropoxy) analogue **4b** docking in the VDR ligand binding domain (LBD) based on the crystal structure established by Moras et al.[24] All of the important hydrogen bonds, which make the ligand anchor in the LBD, between 1α-OH and both Ser-237 and Arg-274, 3β-OH and both Tyr-143 and Ser-278, and 25-OH and both His-305 and His-397 can remain as in the original X-ray structure, and an additional hydrogen

SCHEME 1. Synthesis of the A-Ring Precursors[a]



[a] Reagents: (a) $HOCH_2(CH_2)_nCH_2OH$, KO^{t}Bu, 110 °C; (b) TBSCl, Et_3N, DMAP, CH_2Cl_2; (c) NBS, $BaCO_3$, CCl_4, reflux; (d) Zn powder, $NaBH_3CN$, 1-propanol/H_2O (9/1), 95 °C; (e) 2,4,6-trimethylbenzenesulfonyl chloride, pyridine; (f) LiHMDS, THF, −78 to 0 °C; (g) TMSCCH, BuLi, BF_3·OEt_2, THF, −78 °C to room temperature; (h) K_2CO_3, MeOH; (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C.

SCHEME 2. Synthesis of 2α-(ω-Hydroxyalkoxy)-1α,25(OH)$_2$D$_3$[a]



[a] Reagents: (a) catalyst $(Ph_3P)_4Pd$, Et_3N/toluene (1/1), reflux, n = 0 (75%), 1 (52%), and 2 (69%); (b) Bu_4NF, THF, n = 0 (78%), 1 (61%), and 2 (96%).

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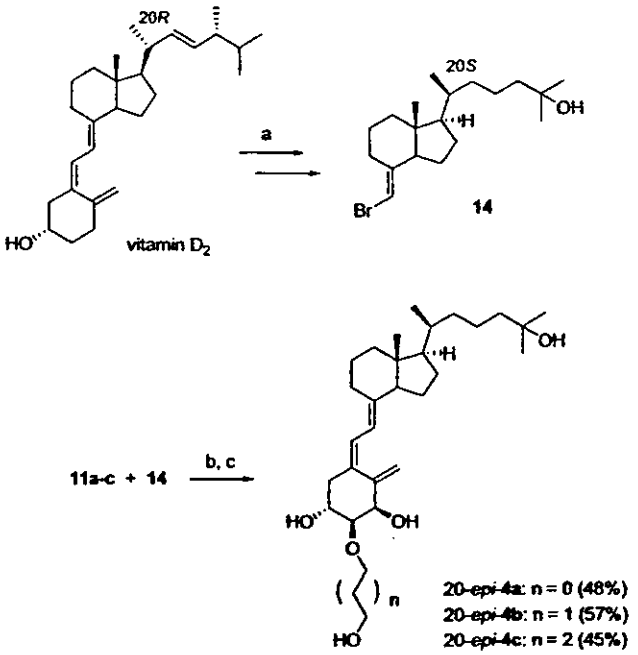
SCHEME 3. Synthesis of 2α-(ω-Hydroxyalkoxy)-20-*epi*-1α,25(OH)$_2$D$_3$[a]



[a] Reagents: (a) see ref 17; (b) catalyst $(Ph_3P)_4Pd$, Et_3N/toluene (1/1), reflux; (c) HF/MeCN, n = 0 (48%), 1 (57%), and 2 (45%) in two steps.

TABLE 1. Relative Binding Affinity for Bovine Thymus VDR and HL-60 Cell Differentiation Activity[a]

compound	VDR	HL-60
1	100	100
4a	120	100
4b	180	70
4c	40	40
20-*epi*-1	400	1810
20-*epi*-4a	260	5820
20-*epi*-4b	165	2120
20-*epi*-4c	100	2770

[a] The potency of 1 is normalized to 100. The data are the mean of three separate experiments[26] (see the Supporting Information).

FIGURE 2. Molecular modeling of **4b** in the LBD of VDR.[24,25]

bonding network from the C2α terminal hydroxyl group to Asp-144 and Tyr-236 would be properly formed (Figure 2).

However, the inducing effect of HL-60 cell differentiation[26] was not correlated to the binding affinity, and it was between 70 and 100% of that of **1** despite the stronger affinity for the VDR (Table 1). It could be explained by a possibly weaker stability of the ligand (**4a**–**c**)–VDR with coactivator(s) complex, which switches on the process in transactivation of the target genes.[27]

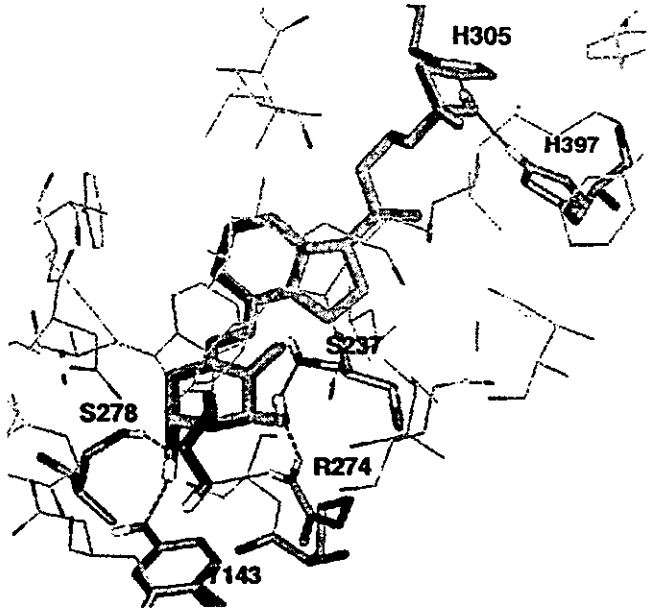


FIGURE 3. Modeled structure of 20-*epi*-**4a** (red) with Moras' X-ray structure of the VDR–20-*epi*-**1** (green) complex.[28]

As noted in the Introduction, 20-*epi*-**1**, itself, exhibits a VDR affinity 4–5 times stronger than that of **1**; however, introduction of the 2α-(3-hydroxypropoxy) group, which strengthens the affinity for VDR in the case of having the 20*R* natural side chain, causes an affinity weaker than that of 20-*epi*-**1**, while these are still better ligands if compared to **1**. The docking study utilizing Moras' crystal structure of the 20-*epi*-**1**–VDR complex[28] explains the different positions of the two hydroxyls (1α,3β) on the A-ring of 20-*epi*-**4a** in the LBD of the VDR from the originals that would be located at the ideal positions for the binding of 20-*epi*-**1** (Figure 3).[25] Similar results were obtained when we synthesized 2α-(ω-hydroxyalkyl)-20-*epi*-1α,25-dihydroxyvitamin D_3 derivatives and tested the VDR binding affinity.[7d] When the 2α substituent was introduced, which also strengthens VDR binding affinity in the case of having the natural side chain (20*R*), into the 20-*epi* analogues of **1**, the binding affinity to VDR decreased, compared with that of 20-*epi*-**1**.[7d]

HL-60 cell differentiation activity[26] was markedly high with the 20-*epi* series compared to that of the natural side-chain analogues **4a**–**c** (Table 1).

Conclusions

We have developed an efficient synthetic route to the novel biologically active 2α-(ω-hydroxyalkoxy)-1α,25-(OH)$_2$D$_3$ complexes (**4a**–**c**) and their 20-*epi* counterparts through the new A-ring precursors derived from D-glucose. It was found that the VDR binding affinities of **4a**, **4b**, 20-*epi*-**4a**, and 20-*epi*-**4b** are stronger than that

(27) Masuno, H.; Yamamoto, K.; Wang, X.; Choi, M.; Ooizumi, H.; Shinki, T.; Yamada, S. *J. Med. Chem.* **2002**, *45*, 1825–1834.
(28) Tocchini-Valentini, G.; Rochel, N.; Wultz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491–5496.

of the natural hormone. We investigated the potency of inducing HL-60 cell differentiation and found the two 2α-(ω-hydroxyalkoxy)-1α,25(OH)$_2$D$_3$ compounds (**4b,c**) exhibit a rather lower effect, and all their 20-*epi* counterparts (20-*epi*-**4a−c**) showed higher potency. So far, in many cases, 20-*epi* analogues of **1** are more potent in cell growth and differentiation than the corresponding compounds with the natural C20 stereochemistry, and 2α-substituted 20-*epi*-**4a−c** analogues are no exception. We propose that double modification on the 2α position and the side chain would provide for the design and development of new B-*seco* steroidal drugs for the treatment of rickets, osteoporosis, psoriasis, certain cancers, and so forth.[2−4] These results would contribute to the understanding of the detail of structure−activity relationships on the A-ring with variations of the CD-ring side chain. Further biological testing is underway in our laboratories.

Experimental Section

Methyl 4,6-*O*-Benzylidene-3-*O*-[2-{(*tert*-butyldimethylsilyl)oxy}ethyl]-α-D-altropyranoside (6a). To a suspension of **5** (1.5 g, 5.7 mmol) in ethylene glycol (25 mL) was added KO^{t}Bu (2.1 g, 19 mmol), and the mixture was stirred at 110 °C for 24 h. The mixture was diluted with CH_2Cl_2, and the organic layer was washed with saturated NH_4Cl aqueous solution, saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. To a solution of the crude product (1.9 g) in CH_2Cl_2 (14 mL) were added TBSCl (1.1 g, 7.5 mmol), Et_3N (2.4 mL, 17 mmol), and DMAP (210 mg, 1.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. To the mixture was added water, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 5/1−3/1) to give **6a** (2.0 g, 81% in two steps) as a colorless oil: $[\alpha]^{25}_D$ +58.4° (*c* 0.92, $CHCl_3$); IR (neat) 3480, 1649, 1095 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 1.90 (d, *J* = 6.1 Hz, 1H), 3.39 (s, 3H), 3.65−3.90 (m, 5H), 3.92 (dd, *J* = 2.9, 2.9 Hz, 1H), 3.96 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.05 (ddd, *J* = 5.9, 2.9, 0.7 Hz, 1H), 4.25−4.35 (m, 2H), 4.59 (s, 1H), 5.55 (s, 1H), 7.32−7.40 (m, 3H), 7.45−7.52 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −5.1, 18.5, 26.0, 55.6, 58.5, 63.1, 69.4, 70.3, 73.2, 77.0, 77.1, 101.9, 102.2, 126.1, 128.1, 128.8, 137.5; EI−LRMS *m/z* 440 (M$^+$), 351, 305, 259, 121. EI−HRMS calcd for $C_{22}H_{36}O_7Si$ 440.2231. Found 440.2227.

Methyl 4,6-*O*-Benzylidene-3-*O*-[3-{(*tert*-butyldimethylsilyl)oxy}propyl]-α-D-altropyranoside (6b). In a manner similar to that for the synthesis of **6a** from **5**, a crude product, which was obtained from **5** (1.4 g, 5.1 mmol), KO^{t}Bu (1.9 g, 17 mmol), and 1,3-propanediol (25 mL), was dissolved in CH_2Cl_2 (20 mL). To the solution were added Et_3N (2.1 mL, 15 mmol), TBSCl (1.2 g, 7.6 mmol), and DMAP (63 mg, 0.51 mmol), and the mixture was stirred at room temperature for 3 h. After the usual workup, the crude product was purified by column chromatography on silica gel (hexane/AcOEt = 5/1−2/1) to give **6b** (2.1 g, 90% in two steps) as a colorless oil: $[\alpha]^{20}_D$ +68.8° (*c* 3.9, $CHCl_3$); IR (neat) 3463 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 3H), 0.03 (s, 3H), 0.87 (s, 9H), 1.78−1.82 (m, 2H), 1.93 (br s, 1H), 3.39 (s, 3H), 3.65−3.80 (m, 6H), 3.95 (dd, *J* = 8.8, 2.7 Hz, 1H), 3.99−4.00 (m, 1H), 4.26−4.33 (m, 2H), 4.89 (s, 1H), 5.55 (s, 1H), 7.34−7.37 (m, 3H), 7.47−7.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.4, 18.3, 25.9, 33.3, 55.5, 58.6, 60.2, 68.5, 69.4, 70.0, 76.4, 76.9, 102.0, 102.3, 126.0, 126.2, 128.2, 129.0, 137.7; EI−LRMS *m/z* 454 (M$^+$). EI−HRMS calcd for $C_{23}H_{38}O_7Si$ 454.2387. Found 454.2387.

Methyl 4,6-*O*-Benzylidene-3-*O*-[4-{(*tert*-butyldimethylsilyl)oxy}butyl]-α-D-altropyranoside (6c). In a manner similar to that for the synthesis of **6a** from **5**, a crude product, which was obtained from **5** (2.5 g, 9.5 mmol), KO^{t}Bu (3.5 g, 31 mmol), and 1,4-butanediol (45 mL), was dissolved in CH_2Cl_2 (19 mL). To the solution were added Et_3N (6.6 mL, 47 mmol), TBSCl (2.9 g, 19 mmol), and DMAP (116 mg, 0.95 mmol), and the mixture was stirred at room temperature for 19 h. After the usual workup, the crude product was purified by column chromatography on silica gel (hexane/AcOEt = 3/1) to give **6c** (3.9 g, 86% in two steps) as a colorless oil: $[\alpha]^{20}_D$ +60.7° (*c* 7.7, $CHCl_3$); IR (neat) 3472 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.88 (s, 9H), 1.57−1.65 (m, 4H), 1.82 (d, *J* = 5.8 Hz, 1H), 3.40 (s, 3H), 3.58−3.64 (m, 3H), 3.70−3.78 (m, 2H), 3.80 (t, *J* = 3.1 Hz, 1H), 3.96 (dd, *J* = 9.5, 3.1 Hz, 1H), 4.01 (dd, *J* = 6.1, 3.1 Hz, 1H), 4.28−4.33 (m, 2H), 4.59 (s, 1H), 5.55 (s, 1H), 7.34−7.37 (m, 3H), 7.46−7.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, 18.5, 26.1, 26.6, 29.6, 55.7, 58.7, 63.1, 69.4, 70.2, 71.7, 76.2, 77.2, 102.0, 102.3, 126.1, 128.1, 128.9, 137.6; EI−LRMS *m/z* 468 (M$^+$). EI−HRMS calcd for $C_{24}H_{40}O_7Si$ 468.2543. Found 468.2540.

Methyl 4-*O*-Benzoyl-6-bromo-3-*O*-[2-{(*tert*-butyldimethylsilyl)oxy}ethyl]-6-deoxy-α-D-altropyranoside (7a). To a solution of **6a** (1.9 g, 4.3 mmol) in CCl_4 (44 mL) were added $BaCO_3$ (518 mg, 2.6 mmol) and NBS (817 g, 4.6 mmol) at room temperature, and the mixture was refluxed for 1 h. After the mixture was filtered, to the filtrate were added 10% $Na_2S_2O_3$ aqueous solution and saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 5/1) to give **7a** (2.0 g, 86%) as a colorless oil: $[\alpha]^{25}_D$ +25.4° (*c* 1.0, $CHCl_3$); IR (neat) 3459, 1724, 1361, 1095 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 3H), 0.03 (s, 3H), 0.86 (s, 9H), 3.03 (br s, 1H), 3.50 (s, 3H), 3.55−3.65 (m, 3H), 3.68−3.83 (m, 4H), 3.99 (dd, *J* = 8.2, 3.7 Hz, 1H), 4.32 (m, 1H), 4.71 (d, *J* = 8.0 Hz, 1H), 5.47 (dd, *J* = 6.4, 4.3 Hz, 1H), 7.45 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.58 (dd, *J* = 7.8, 7.8 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, 18.5, 26.0, 32.9, 56.0, 62.9, 69.6, 70.5, 70.6, 72.6, 77.9, 103.0, 128.3, 129.4, 129.7, 133.2, 165.6; EI−LRMS *m/z* 461 (M − ^{t}Bu)$^+$, 429, 308, 179, 105. EI−HRMS calcd for $C_{18}H_{26}O_7{}^{79}BrSi$ (M − ^{t}Bu) 461.0631. Found 461.0627.

Methyl 4-*O*-Benzoyl-6-bromo-3-*O*-[3-{(*tert*-butyldimethylsilyl)oxy}propyl]-6-deoxy-α-D-altropyranoside (7b). In a manner similar to that for the synthesis of **7a** from **6a**, a crude product, which was obtained from **6b** (3.6 g, 7.9 mmol), NBS (1.5 g, 8.3 mmol), and $BaCO_3$ (940 mg, 4.8 mmol) in CCl_4 (79 mL), was purified by column chromatography on silica gel (hexane/AcOEt = 5/1) to give **7b** (3.8 g, 91%) as a colorless oil: $[\alpha]^{20}_D$ +46.0° (*c* 4.2, $CHCl_3$); IR (neat) 1725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04 (s, 3H), −0.03 (s, 3H), 0.83 (s, 9H), 1.67−1.73 (m, 2H), 2.52 (br s, 1H), 3.50 (s, 3H), 3.55−3.70 (m, 6H), 3.73 (dd, *J* = 7.3, 4.0 Hz, 1H), 3.97 (dd, *J* = 7.3, 3.3 Hz, 1H), 4.35 (dt, *J* = 3.7, 7.0 Hz, 1H), 4.70 (d, *J* = 3.3 Hz, 1H), 5.45 (dd, *J* = 7.0, 4.0 Hz, 1H), 7.43−7.47 (m, 2H), 7.58 (m, 1H), 8.03−8.08 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.5, 18.2, 25.9, 32.7, 32.9, 55.9, 60.0, 67.9, 69.3, 69.8, 69.9, 76.9, 102.7, 128.5, 129.5, 129.8, 133.3, 165.7; EI−LRMS *m/z* 475 (M − ^{t}Bu)$^+$. EI−HRMS calcd for $C_{19}H_{28}{}^{79}BrO_7Si$ (M − ^{t}Bu)$^+$ 475.0787. Found 475.0786.

Methyl 4-*O*-Benzoyl-6-bromo-3-*O*-[4-{(*tert*-butyldimethylsilyl)oxy}butyl]-6-deoxy-α-D-altropyranoside (7c). In a manner similar to that for the synthesis of **7a** from **6a**, a crude product, which was obtained from **6c** (1.0 g, 2.1 mmol), NBS (460 mg, 2.6 mmol), and $BaCO_3$ (240 mg, 1.2 mmol) in CCl_4 (25 mL), was purified by silica gel column chromatography on silica gel (hexane/AcOEt = 5/1) and gave **7c** (874 mg, 75%) as a colorless oil: $[\alpha]^{20}_D$ +36.0° (*c* 7.7, $CHCl_3$); IR (neat) 3465, 1725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.86 (s, 9H), 1.46−1.62 (m, 4H), 2.37 (d, *J* = 3.7 Hz, 1H), 3.51 (s, 3H), 3.53−3.65 (m, 6H), 3.75 (dd, *J* = 4.0, 7.3 Hz, 1H), 3.99 (dt, *J* = 3.7, 7.3 Hz, 1H), 4.38 (dt, *J* = 3.7, 6.7 Hz, 1H), 4.71 (d, *J* = 3.7 Hz, 1H), 5.47 (dd, *J* = 6.7, 4.0 Hz, 1H), 7.45−7.48 (m, 2H),

7.60 (m, 1H), 8.04–8.07 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, 18.3, 26.1, 26.4, 29.4, 32.9, 56.1, 62.8, 69.0, 69.9, 70.7, 76.9, 102.6, 128.4, 129.3, 129.7, 133.3, 165.5; EI–LRMS *m/z* 546 (M^+). EI–HRMS calcd for $C_{24}H_{39}{}^{79}BrO_7Si$ 546.1648. Found 546.1640.

(2*R*,3*S*,4*R*)-4-Benzoyloxy-3-[2-(*tert*-butyldimethylsilyloxy)ethoxy]hex-5-ene-1,2-diol (8a). To a solution of **7a** (2.8 g, 5.3 mmol) in 1-propanol/H_2O (9/1, 53 mL) were added activated Zn dust (24 g, 373 mmol) and $NaBH_3CN$ (3.7 g, 59 mmol) at 95 °C, and the mixture was stirred at the same temperature for 1 h. After the mixture was filtered, to the filtrate was added saturated NH_4Cl aqueous solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 4/1–2/1) to give **8a** (1.5 g, 70%) as a colorless oil: $[\alpha]^{25}_D$ +1.47° (*c* 2.3, $CHCl_3$); IR (neat) 3438, 1719, 1649, 1271, 1109 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.91 (s, 9H), 2.51 (m, 1H), 2.61 (br s, 1H), 3.62 (ddd, *J* = 10.6, 8.4, 3.0 Hz, 1H), 3.68–3.88 (m, 6H), 4.03 (ddd, *J* = 10.6, 3.7, 3.2 Hz, 1H), 5.35 (d, *J* = 10.6 Hz, 1H), 5.44 (d, *J* = 17.2 Hz, 1H), 5.68 (dd, *J* = 6.7, 3.9 Hz, 1H), 6.07 (ddd, *J* = 17.2, 10.6, 6.7 Hz, 1H), 7.45 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.58 (dd, *J* = 7.8, 7.8 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, 18.5, 26.0, 62.9, 63.4, 71.2, 74.4, 75.2, 82.5, 119.2, 128.3, 129.5, 132.4, 133.0, 165.2; EI–LRMS *m/z* 353 ($M - {}^tBu)^+$, 278, 219, 105. EI–HRMS calcd for $C_{17}H_{25}O_6Si$ ($M - {}^tBu)^+$ 353.1420. Found 353.1430.

(2*R*,3*S*,4*R*)-4-Benzoyloxy-3-[3-(*tert*-butyldimethylsilyloxy)propoxy]hex-5-ene-1,2-diol (8b). In a manner similar to that for the synthesis of **8a** from **7a**, a crude product, which was obtained from **7b** (3.6 g, 6.8 mmol), activated zinc dust (13 g, 203 mmol), and $NaBH_3CN$ (2.1 g, 34 mmol) in 1-propanol/H_2O (9/1, 68 mL), was purified by flash column chromatography on silica gel (hexane/AcOEt = 3/1) to give **8b** (2.5 g, 86%) as a colorless oil: $[\alpha]^{23}_D$ +26.1° (*c* 3.4, $CHCl_3$); IR (neat) 3406, 1722, 1643, 1271, 1095 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 1.70–1.88 (m, 2H), 2.26 (br s, 1H), 3.05 (br s, 1H), 3.59–3.81 (m, 7H), 3.96 (dt, *J* = 9.1, 5.7 Hz, 1H), 5.34 (ddd, *J* = 10.6, 1.2, 1.2 Hz, 1H), 5.43 (ddd, *J* = 17.3, 1.2, 1.2 Hz, 1H), 5.68 (dddd, *J* = 6.3, 3.9, 1.2, 1.2 Hz, 1H), 6.05 (ddd, *J* = 17.3, 10.6, 6.3 Hz, 1H), 7.45 (dd, *J* = 7.9, 7.9 Hz, 2H), 7.58 (dd, *J* = 7.8, 7.8 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −5.1, 18.4, 26.0, 33.0, 60.0, 63.7, 69.5, 71.1, 74.9, 81.0, 118.8, 128.3, 129.5, 129.8, 132.4, 133.1, 165.3; EI–LRMS *m/z* 367 ($M - {}^tBu)^+$, 293, 263, 233, 185, 105. EI–HRMS calcd for $C_{18}H_{27}O_6Si$ ($M - {}^tBu)^+$ 367.1577. Found 367.1583.

(2*R*,3*S*,4*R*)-4-Benzoyloxy-3-[4-(*tert*-butyldimethylsilyloxy)butoxy]hex-5-ene-1,2-diol (8c). In a manner similar to that for the synthesis of **8a** from **7a**, a crude product, which was obtained from **7c** (2.7 g, 4.8 mmol), activated zinc dust (9.5 g, 145 mmol), and $NaBH_3CN$ (1.5 g, 24 mmol) in 1-propanol/H_2O (9/1, 48 mL), was purified by column chromatography on silica gel (hexane/AcOEt = 3/1) to give **8c** (1.7 g, 81%) as a colorless oil: $[\alpha]^{18}_D$ +21.4° (*c* 1.4, $CHCl_3$); IR (neat) 3424, 1722, 1645, 1603, 1271, 1097 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.88 (s, 9H), 1.50–1.73 (m, 4H), 2.17 (m, 1H), 2.78 (d, *J* = 4.9 Hz, 1H), 3.50–3.65 (m, 4H), 3.68–3.82 (m, 3H), 3.86 (ddd, *J* = 9.0, 6.5, 6.5 Hz, 1H), 5.34 (d, *J* = 10.6 Hz, 1H), 5.43 (d, *J* = 17.2 Hz, 1H), 5.71 (dd, *J* = 6.2, 4.4 Hz, 1H), 6.03 (ddd, *J* = 17.2, 10.6, 6.4 Hz, 1H), 7.45 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.58 (dd, *J* = 7.5, 7.5 Hz, 1H), 8.05 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, 18.5, 26.1, 26.7, 29.5, 62.8, 63.9, 70.9, 72.5, 74.8, 80.5, 118.8, 128.3, 129.6, 129.7, 132.4, 133.1, 165.3; EI–LRMS *m/z* 381 ($M - {}^tBu)^+$, 259, 187, 179, 105. EI–HRMS calcd for $C_{19}H_{29}O_6Si$ ($M - {}^tBu)^+$ 381.1733. Found 379.1726.

(3*R*,4*R*,5*R*)-3-Benzoyloxy-4-[2-(*tert*-butyldimethylsilyloxy)ethoxy]-5,6-epoxyhex-1-ene (9a). To a solution of **8a** (380 mg, 0.93 mmol) in pyridine (0.93 mL) was added mesitylenesulfonyl chloride (TmCl) (224 mg, 1.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 24 h. To the mixture was added water, and the aqueous layer was extracted with Et_2O, washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The crude product was dissolved in THF (9.3 mL). To the solution was added LiHMDS (1.0 M solution in THF, 1.1 mL, 1.1 mmol) at −78 °C, and the mixture was allowed to warm to 0 °C over 1 h. After the mixture was stirred at 0 °C for 30 min, to the mixture was added saturated NH_4Cl aqueous solution. The aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 20/1) to give **9a** (255 mg, 70% in two steps) as a colorless oil: $[\alpha]^{18}_D$ +33.0° (*c* 1.1, $CHCl_3$); IR (neat) 1725, 1647, 1603, 1269, 1109 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 2.65 (dd, *J* = 4.9, 2.8 Hz, 1H), 2.77 (dd, *J* = 4.6, 4.6 Hz, 1H), 3.12 (ddd, *J* = 6.6, 3.8, 2.8 Hz, 1H), 3.29 (dd, *J* = 6.6, 5.3 Hz, 1H), 3.65–3.85 (m, 4H), 5.31 (d, *J* = 10.5 Hz, 1H), 5.40 (d, *J* = 17.3 Hz, 1H), 5.68 (dd, *J* = 6.1, 5.6 Hz, 1H), 6.05 (ddd, *J* = 17.3, 10.5, 6.1 Hz, 1H), 7.45 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.58 (dd, *J* = 7.8, 7.8 Hz, 1H), 8.06 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, 18.4, 26.0, 43.6, 52.1, 62.7, 72.3, 74.8, 82.6, 118.1, 128.3, 129.5, 129.7, 132.8, 133.0, 165.0; EI–LRMS *m/z* 392 (M^+), 376, 335, 305, 179, 105. EI–HRMS calcd for $C_{21}H_{32}O_5Si$ 392.2019. Found 392.2016.

(3*R*,4*R*,5*R*)-3-Benzoyloxy-4-[3-(*tert*-butyldimethylsilyloxy)propoxy]-5,6-epoxyhex-1-ene (9b). In a manner similar to that for the synthesis of **9a** from **8a**, a crude product, which was obtained from **8b** (2.5 g, 5.8 mmol) and TmCl (1.5 g, 6.7 mmol) in pyridine (5.8 mL), was dissolved in THF (48 mL). To the solution was added LiHMDS (1.0 M solution in THF, 7.2 mL, 7.2 mmol) at −78 °C, and the mixture was allowed to warm to 0 °C over 1 h. After the usual workup, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 30/1) to give **9b** (1.4 g, 60% in two steps) as a colorless oil: $[\alpha]^{18}_D$ +29.4° (*c* 4.8, $CHCl_3$); IR (neat) 1724, 1651, 1601, 1269, 1109 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.87 (s, 9H), 1.80 (tt, *J* = 6.4, 6.4 Hz, 2H), 2.62 (dd, *J* = 4.9, 2.7 Hz, 1H), 2.76 (dd, *J* = 4.6, 4.3 Hz, 1H), 3.10 (ddd, *J* = 6.8, 3.9, 2.7 Hz, 1H), 3.18 (dd, *J* = 6.8, 5.1 Hz, 1H), 3.60–3.75 (m, 3H), 3.83 (dt, *J* = 9.5, 6.1 Hz, 1H), 5.31 (d, *J* = 10.7 Hz, 1H), 5.41 (d, *J* = 17.3 Hz, 1H), 5.66 (dd, *J* = 6.1, 5.1 Hz, 1H), 6.04 (ddd, *J* = 17.3, 10.7, 5.1 Hz, 1H), 7.45 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.58 (dd, *J* = 7.8, 7.8 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, 18.3, 26.0, 33.2, 43.6, 52.2, 59.8, 67.5, 74.7, 82.4, 118.2, 128.2, 129.4, 129.8, 132.8, 132.9, 165.0; EI–LRMS *m/z* 406 (M^+), 390, 349, 316, 179, 105. EI–HRMS calcd for $C_{22}H_{34}O_5Si$ 406.2175. Found 406.2177.

(3*R*,4*R*,5*R*)-3-Benzoyloxy-4-[4-(*tert*-butyldimethylsilyloxy)butoxy]-5,6-epoxyhex-1-ene (9c). In a manner similar to that for the synthesis of **9a** from **8a**, a crude product, which was obtained from **8c** (536 mg, 1.2 mmol) and TmCl (320 mg, 1.5 mmol) in pyridine (1.2 mL), was dissolved in THF (11 mL). To the solution was added LiHMDS (1.0 M solution in THF, 1.2 mL, 1.2 mmol) at −78 °C, and the mixture was warmed to 0 °C over 1.5 h. After the usual workup, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 25/1) to give **9c** (328 mg, 64% in two steps) as a colorless oil: $[\alpha]^{18}_D$ +31.4° (*c* 2.1, $CHCl_3$); IR (neat) 1725, 1647, 1603, 1269, 1101 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.88 (s, 9H), 1.55–1.70 (m, 4H), 2.68 (dd, *J* = 4.8, 2.7 Hz, 1H), 2.77 (dd, *J* = 4.8, 3.9 Hz, 1H), 3.10 (ddd, *J* = 6.9, 3.9, 2.7 Hz, 1H), 3.17 (dd, *J* = 6.9, 5.1 Hz, 1H), 3.55–3.65 (m, 3H), 3.76 (dt, *J* = 9.3, 5.9 Hz, 1H), 5.31 (ddd, *J* = 10.6, 1.2, 1.2 Hz, 1H), 5.41 (ddd, *J* = 17.2, 1.2, 1.2 Hz, 1H), 5.66 (dddd, *J* = 6.3, 5.1, 1.2, 1.2 Hz, 1H), 6.04 (ddd, *J* = 17.2, 10.6, 6.3 Hz, 1H), 7.45 (dd, *J* = 7.7, 7.7 Hz, 2H), 7.58 (dd, *J* = 7.7, 7.7 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, 18.5, 26.1, 26.5, 29.5, 43.7, 52.4, 62.9, 71.0, 74.8, 82.4,

118.3, 128.3, 129.5, 129.8, 132.8, 133.0, 165.1; EI−LRMS *m/z* 420 (M^+), 405, 363, 241, 217, 179, 105. EI−HRMS calcd for $C_{23}H_{36}O_5Si$ 420.2232. Found 420.2235.

(3*R*,4*S*,5*R*)-4-[2-(*tert*-Butyldimethylsilyloxy)ethoxy]oct-1-en-7-yne-3,5-diol (10a). To a solution of trimethylsilylacetylene (0.35 mL, 2.5 mmol) in THF (2 mL) was added ^{n}BuLi (1.5 M solution in hexane, 1.5 mL, 2.3 mmol) at −78 °C, and the mixture was stirred at the same temperature for 30 min. To the mixture were added a solution of **9a** (384 mg, 0.98 mmol) in THF (6 mL) and BF_3·OEt_2 (0.14 mL, 1.1 mmol) at −78 °C, and the resulting mixture was warmed to 0 °C over 2 h. To the mixture was added saturated NH_4Cl aqueous solution, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was dissolved in MeOH (3 mL). To the solution was added K_2CO_3 (406 mg, 2.9 mmol), and the mixture was stirred at room temperature for 2 h. To the mixture was added H_2O, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 4/1) to give **10a** (285 mg, 93% in two steps) as a colorless oil: $[\alpha]^{16}_D$ −7.91° (*c* 1.1, $CHCl_3$); IR (neat) 3438, 3314, 2122, 1645, 1255, 1103 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.91 (s, 9H), 2.02 (t, *J* = 2.7 Hz, 1H), 2.46 (ddd, *J* = 16.6, 5.9, 2.7 Hz, 1H), 2.55 (ddd, *J* = 16.6, 7.1, 2.7 Hz, 1H), 3.18 (br d, *J* = 3.9 Hz, 1H), 3.24 (d, *J* = 5.4 Hz, 1H), 3.53 (dd, *J* = 3.7, 3.7 Hz, 1H), 3.70−3.85 (m, 4H), 3.93 (m, 1H), 4.43 (m, 1H), 5.27 (d, *J* = 10.5 Hz, 1H), 5.40 (d, *J* = 17.0 Hz, 1H), 5.96 (ddd, *J* = 17.0, 10.5, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, −5.1, 18.5, 23.5, 26.0, 63.0, 69.9, 70.4, 72.7, 73.2, 80.6, 82.2, 116.6, 136.1; EI−LRMS *m/z* 257 ($M - {}^tBu)^+$, 239, 183, 171, 119. EI−HRMS calcd for $C_{12}H_{21}O_4Si$ ($M - {}^tBu)^+$ 257.1210. Found 257.1197.

(3*R*,4*S*,5*R*)-4-[3-(*tert*-Butyldimethylsilyloxy)propoxy]-oct-1-en-7-yne-3,5-diol (10b). In a manner similar to that for the synthesis of **10a** from **9a**, a crude product, which was obtained from **9b** (1.2 g, 2.9 mmol), lithium trimethylsilylacetylide [prepared from trimethylacetylene (1.1 mL, 7.4 mmol) and ^{n}BuLi (1.52 M solution in hexane, 4.4 mL, 6.7 mmol)], and BF_3·OEt_2 (0.41 mL, 3.24 mmol) in THF (24 mL), was treated with K_2CO_3 (1.2 g, 8.8 mmol) in MeOH (2.9 mL). After the usual workup, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 3/1) to give **10b** (861 mg, 90% in two steps) as a colorless oil: $[\alpha]^{21}_D$ −10.5° (*c* 1.1, $CHCl_3$); IR (neat) 3393, 2121, 1647, 1255, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.06 (s, 6H), 0.09 (s, 9H), 1.81 (tt, *J* = 5.7, 5.7 Hz, 2H), 2.01 (t, *J* = 2.6 Hz, 1H), 2.40−2.60 (m, 2H), 2.86 (d, *J* = 5.5 Hz, 1H), 2.96 (d, *J* = 5.5 Hz, 1H), 3.41 (dd, *J* = 4.4, 2.4 Hz, 1H), 3.60−3.90 (m, 4H), 4.00 (m, 1H), 4.48 (m, 1H), 5.27 (d, *J* = 10.5 Hz, 1H), 5.42 (d, *J* = 17.1 Hz, 1H), 5.95 (ddd, *J* = 17.1, 10.5, 5.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.2, 18.3, 23.6, 26.0, 33.0, 59.7, 68.0, 69.8, 70.3, 72.2, 80.6, 80.7, 116.1, 136.8; EI−LRMS *m/z* 271 ($M - {}^tBu)^+$, 241, 185, 171, 133, 75. EI−HRMS calcd for $C_{13}H_{23}O_4Si$ ($M - {}^tBu)^+$ 271.1365. Found 271.1356.

(3*R*,4*S*,5*R*)-4-[4-(*tert*-Butyldimethylsilyloxy)butoxy]oct-1-en-7-yne-3,5-diol (10c). In a manner similar to that for the synthesis of **10a** from **9a**, a crude product, which was obtained from **9c** (855 mg, 2.0 mmol), lithium trimethylsilylacetylide [prepared from trimethylacetylene (0.72 mL, 5.1 mmol) and ^{n}BuLi (1.52 M solution in hexane, 3.1 mL, 4.7 mmol)], and BF_3·OEt_2 (0.28 mL, 32 mmol) in THF (24 mL), was treated with K_2CO_3 (842 mg, 6.1 mmol) in MeOH (4 mL). After the usual workup, the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt = 4/1) to give **10c** (647 mg, 93% in two steps) as a colorless oil: $[\alpha]^{18}_D$ −6.30° (*c* 0.98, $CHCl_3$); IR (neat) 3314, 2122, 1645, 1255, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.89 (s, 9H), 1.55−1.73 (m, 4H), 2.01 (t, *J* = 2.6 Hz, 1H), 2.50 (dd, *J* = 7.1, 2.6 Hz, 2H), 2.76 (br d, *J* = 4.2 Hz, 1H), 2.93 (br d, *J* = 5.8 Hz, 1H), 3.38 (dd, *J* = 4.8, 2.1 Hz, 1H), 3.55 (dt, *J* = 9.1, 6.4 Hz, 1H), 3.63 (t, *J* = 6.1 Hz, 2H), 3.75 (dt, *J* = 9.1, 6.3 Hz, 1H), 5.62 (m, 1H), 4.48 (m, 1H), 5.27 (ddd, *J* = 10.6, 1.6, 1.6 Hz, 1H), 5.43 (ddd, *J* = 17.2, 1.6, 1.6 Hz, 1H), 5.93 (ddd, *J* = 17.2, 10.6, 5.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.12, 18.4, 23.8, 26.0, 26.6, 29.4, 62.8, 69.8, 70.3, 71.4, 72.4, 80.5, 80.7, 116.2, 136.9; EI−LRMS *m/z* 285 ($M - {}^tBu)^+$, 211, 187, 147, 89. EI−HRMS calcd for $C_{14}H_{25}O_4Si$ ($M - {}^tBu)^+$ 285.1522. Found 285.1525.

(3*R*,4*S*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-4-[2-(*tert*-butyldimethylsilyloxy)ethoxy]oct-1-en-7-yne (11a). To a solution of **10a** (273 mg, 0.87 mmol) in CH_2Cl_2 (2.9 mL) were added 2,6-lutidine (0.31 mL, 2.7 mmol) and TBSOTf (0.5 mL, 2.2 mmol) at 0 °C, and the mixture was stirred at the same temperature for 3 h. To the mixture was added H_2O, and the aqueous layer was extracted with Et_2O. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 30/1) to give **11a** (456 mg, 97%) as a colorless oil: $[\alpha]^{14}_D$ +0.24° (*c* 1.2, $CHCl_3$); IR (neat) 2124, 1647, 1255, 1098 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.054 (s, 6H), 0.068 (s, 3H), 0.087 (s, 3H), 0.10 (s, 3H), 0.89 (s, 18H), 0.90 (s, 9H), 1.95 (t, *J* = 2.7 Hz, 1H), 2.34 (ddd, *J* = 16.8, 6.0, 2.7 Hz, 1H), 2.52 (ddd, *J* = 16.8, 5.4, 2.7 Hz, 1H), 3.40 (dd, *J* = 5.3, 3.5 Hz, 1H), 3.61 (m, 1H), 3.71 (t, *J* = 5.6 Hz, 2H), 3.78 (m, 1H), 3.90 (dd, *J* = 5.6, 5.6 Hz, 1H), 4.32 (dddd, *J* = 6.8, 3.7, 1.5, 1.2 Hz, 1H), 5.12 (ddd, *J* = 10.3, 1.7, 1.2 Hz, 1H), 5.21 (ddd, *J* = 17.3, 1.7, 1.5 Hz, 1H), 5.97 (ddd, *J* = 17.3, 10.3, 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.06, −5.00, −4.47, −4.06, −3.90, −3.87, 18.2, 18.3, 18.5, 24.1, 26.0, 26.1, 62.7, 69.9, 71.7, 73.8, 74.5, 82.1, 85.3, 115.9, 138.7; EI−LRMS *m/z* 542 (M^+), 485, 371, 327, 233, 183, 171, 159. EI−HRMS calcd for $C_{28}H_{58}O_4Si_3$ 542.3643. Found 542.3654.

(3*R*,4*S*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-4-[3-(*tert*-butyldimethylsilyloxy)propoxy]oct-1-en-7-yne (11b). In a manner similar to that for the synthesis of **11a** from **10a**, a crude product, which was prepared from **10b** (104 mg, 0.32 mmol), TBSOTf (0.18 mL, 0.78 mmol), and 2,6-lutidine (0.11 mL, 0.94 mmol) in CH_2Cl_2 (3 mL), was purified by flash column chromatography on silica gel (hexane/AcOEt = 50/1) to give **11b** (177 mg, quantitative) as a colorless oil: $[\alpha]^{21}_D$ +0.3° (*c* 0.9, $CHCl_3$); IR (neat) 2122 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.05 (s, 6H), 0.07 (s, 3H), 0.09 (s, 3H), 0.10 (s, 3H), 0.89 (s, 18H), 0.90 (s, 9H), 1.74−1.80 (m, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 2.35 (ddd, *J* = 16.9, 5.5, 2.6 Hz, 1H), 2.49 (ddd, *J* = 16.9, 5.5, 2.6 Hz, 1H), 3.35 (dd, *J* = 5.5, 3.5 Hz, 1H), 3.60−3.76 (m, 4H), 3.88 (dt, *J* = 9.1, 5.5 Hz, 1H), 4.30 (dd, *J* = 7.0, 3.5 Hz, 1H), 5.12 (br d, *J* = 10.3 Hz, 1H), 5.20 (dt, *J* = 17.2, 1.3 Hz, 1H), 5.95 (ddd, *J* = 17.2, 10.3, 7.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.5, −4.1, −3.9, 18.2, 18.3, 18.5, 24.2, 26.0, 26.1, 33.7, 60.6, 69.5, 69.8, 71.6, 74.5, 82.1, 85.1, 115.8, 138.7; EI−LRMS *m/z* 499 ($M - {}^tBu)^+$. EI−HRMS calcd for $C_{25}H_{51}O_4Si_3$ ($M - {}^tBu)^+$ 499.3096. Found 499.3074.

(3*R*,4*S*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-4-[3-(*tert*-butyldimethylsilyloxy)butoxy]oct-1-en-7-yne (11c). In a manner similar to that for the synthesis of **11a** from **10a**, a crude product, which was obtained from **10c** (615 mg, 1.8 mmol), TBSOTf (1.0 mL, 4.5 mmol), and 2,6-lutidine (0.65 mL, 5.6 mmol) in CH_2Cl_2 (3.6 mL), was purified by column chromatography on silica gel (hexane/AcOEt = 30/1) to give **11c** (971 mg, 95%) as a colorless oil: $[\alpha]^{20}_D$ −2.1° (*c* 6.3, $CHCl_3$); IR (neat) 2122 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.04 (s, 6H), 0.07 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.89 (s, 18H), 0.90 (s, 9H), 1.24−1.28 (m, 2H), 1.57−1.60 (m, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 2.35 (ddd, *J* = 16.8, 5.5, 2.6 Hz, 1H), 2.49 (ddd, *J* = 16.8, 5.5, 2.6 Hz, 1H), 3.36 (dd, *J* = 5.5, 3.7 Hz, 1H), 3.55−3.69 (m, 4H), 3.86 (dt, *J* = 9.1, 5.5 Hz, 1H), 4.30 (dd, *J* = 3.7, 6.9 Hz, 1H), 5.12 (d, *J* = 10.4 Hz, 1H), 5.20 (d, *J* = 17.4 Hz, 1H), 5.96 (ddd, *J* = 17.4, 10.4, 6.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.5, −4.1, −3.9, −3.8, 18.3, 18.4, 18.5, 24.2, 26.0, 26.1, 26.2, 26.8, 29.7, 63.2, 69.8, 71.7, 72.5, 74.6,

82.1, 85.0, 115.8, 138.7; EI−LRMS *m/z* 513 (M − ^{t}Bu)$^+$. EI−HRMS calcd for $C_{26}H_{53}O_4Si_3$ (M − ^{t}Bu)$^+$ 513.3252. Found 513.3251.

2α-(3-Hydroxyethoxy)-1α,25-dihydroxyvitamin D_3 (4a). To a solution of **12** (50 mg, 140 μmol) and **11a** (50 mg, 92 μmol) in toluene (1 mL) were added Et_3N (1 mL) and $Pd(PPh_3)_4$ (32 mg, 28 μmol), and the mixture was stirred at 120 °C for 2 h. The mixture was filtered through a silica gel pad. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (hexane−hexane/AcOEt = 95/5) to give the coupling product **13a** (57 mg, 75%) as a colorless oil, which was used without any further purification. To the THF (3 mL) solution of **13a** (30 mg, 37 μmol) was added TBAF (1 M solution in THF, 0.18 mL, 0.18 mmol), and the mixture was stirred at room temperature for 4 days. After the solution was concentrated, the residue was purified by a preparative TLC (10% MeOH in CH_2Cl_2) to give **4a** (14 mg, 78%) as a white powder. Further purification of **4a** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{20}_D$ +59.1° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} 269 nm; ^{1}H NMR (400 MHz, $CDCl_3/D_2O$) δ 0.54 (s, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 1.21 (s, 6H), 1.25−1.80 (m, 14H), 1.83−1.89 (m, 1H), 1.96−2.01 (m, 2H), 2.23 (dd, *J* = 13.0, 9.5 Hz, 1H), 2.67 (dd, *J* = 13.0, 4.8 Hz, 1H), 2.83 (m, 1H), 3.38 (dd, *J* = 8.1, 3.3 Hz, 1H), 3.72 (ddd, *J* = 9.5, 4.8, 2.7 Hz, 1H), 3.77−3.84 (m, 4H), 4.07 (ddd, *J* = 9.5, 7.9, 4.8 Hz, 1H), 4.43 (d, *J* = 3.4 Hz, 1H), 5.10 (d, *J* = 1.7 Hz, 1H), 5.38 (d, *J* = 1.7 Hz, 1H), 6.01 (d, *J* = 11.1 Hz, 1H), 6.43 (d, *J* = 11.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.8, 20.8, 22.2, 23.5, 27.7, 29.1, 29.2, 29.4, 36.1, 36.4, 40.5, 41.2, 44.4, 45.9, 56.4, 56.6, 62.0, 68.6, 71.1, 71.4, 72.5, 85.0, 116.6, 117.1, 125.6, 131.4, 143.7, 144.1; EI−LRMS *m/z* 476 (M$^+$), 458, 440. EI−HRMS calcd for $C_{29}H_{48}O_5$ (M$^+$) 476.3503. Found 476.3527.

2α-(2-Hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (4b). In a manner similar to that for the synthesis of **4a** from **11a** and **12**, a crude product, which was obtained from **12** (219 mg, 0.39 mmol), **11b** (130 mg, 0.36 mmol), and $Pd(PPh_3)_4$ (125.8 mg, 0.109 mmol) in toluene/Et_3N (1/1, 10 mL), was purified by silica gel column chromatography (hexane−hexane/AcOEt = 95/5) to give the coupling product **13b** (157 mg, 52%) as a colorless oil, which was used without any further purification. The coupling product **13b** (157 mg, 0.19 mmol) was subjected to desilylation by TBAF (1 M solution in THF, 0.94 mL, 0.94 mmol) in THF (3 mL) at room temperature for 4 days. After the usual workup, the crude product was purified by preparative TLC (10% MeOH in CH_2Cl_2) to give **4b** (56 mg, 61%) as a white powder. Further purification of **4b** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{25}_D$ +46.4° (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max} 267 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (s, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 1.21 (s, 6H), 1.25−2.10 (m, 23H), 2.24 (dd, *J* = 13.4, 9.2 Hz, 1H), 2.69 (dd, *J* = 13.4, 4.4 Hz, 1H), 2.82 (m, 1H), 3.38 (dd, *J* = 7.5, 3.2 Hz, 1H), 3.75−3.91 (m, 5H), 4.05 (m, 1H), 4.44 (br d, *J* = 2.8 Hz, 1H), 5.10 (d, *J* = 1.8 Hz, 1H), 5.39 (br s, 1H), 6.01 (d, *J* = 11.3 Hz, 1H), 6.42 (d, *J* = 11.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.8, 20.8, 22.2, 23.5, 27.7, 29.1, 29.2, 29.4, 31.9, 36.1, 36.4, 40.5, 41.0, 44.4, 45.9, 56.4, 56.6, 61.2, 68.4, 68.5, 71.1, 71.9, 84.5, 116.1, 117.1, 125.5, 131.5, 143.6, 144.3; EI−LRMS *m/z* 490 (M$^+$), 472, 454. EI−HRMS calcd for $C_{30}H_{50}O_5$ 490.3660. Found 490.3638.

2α-(3-Hydroxybutoxy)-1α,25-dihydroxyvitamin D_3 (4c). In a manner similar to that for the synthesis of **4a** from **11a** and **12**, a crude product, which was obtained from **11c** (26 mg, 0.05 mmol), **12** (52 mg, 0.15 mmol), and $Pd(PPh_3)_4$ (16 mg, 14 μmol) in toluene/Et_3N (1/3, 2.5 mL), was purified by preparative TLC (hexane/AcOEt = 4/1) to give the coupling product **13c** (26.5 mg, 69%) as a colorless oil, which was used without any further purification. The coupling product **13c** was subjected to desilylation by TBAF (1 M solution in THF, 0.2 mL, 0.2 mmol) in THF (3 mL) at room temperature for 36 h. After the usual workup, the crude product was purified by preparative TLC (10% MeOH in CH_2Cl_2) to yield **4c** (15.1 mg, 66%, two steps from **11c**). Further purification of **4c** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{20}_D$ −22.1° (*c* 0.054, $CHCl_3$); UV (MeOH) λ_{max} 267 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (s, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 1.21 (s, 6H), 1.25−2.00 (m, 25H), 2.23 (dd, *J* = 13.3, 9.3 Hz, 1H), 2.68 (dd, *J* = 13.3, 4.6 Hz, 1H), 2.83 (m, 1H), 3.35 (dd, *J* = 7.6, 3.2 Hz, 1H), 3.61 (dt, *J* = 9.5, 6.0 Hz, 1H), 3.68−3.77 (m, 4H), 4.05 (ddd, *J* = 8.6, 7.6, 4.6 Hz, 1H), 4.42 (d, *J* = 3.0 Hz, 1H), 5.10 (d, *J* = 2.1 Hz, 1H), 5.39 (br s, 1H), 6.02 (d, *J* = 11.3 Hz, 1H), 6.42 (d, *J* = 11.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.8, 20.8, 22.2, 23.5, 26.9, 27.6, 29.1, 29.2, 29.4, 29.7, 36.1, 36.4, 40.5, 40.8, 44.4, 45.9, 56.4, 56.5, 62.6, 68.2, 70.0, 71.1, 71.8, 84.6, 116.2, 117.1, 125.5, 131.5, 143.6, 144.2; EI−LRMS *m/z* 504 (M$^+$), 486, 468. EI−HRMS calcd for $C_{31}H_{52}O_5$ 504.3817. Found 504.3823.

20-*epi*-2α-(2-Hydroxyethoxy)-1α,25-dihydroxyvitamin D_3 (20-*epi*-4a). To a solution of **14** (31 mg, 87 μmol) and **11a** (71 mg, 0.13 mmol) in toluene (2 mL) were added Et_3N (2 mL) and $Pd(PPh_3)_4$ (30 mg, 26 μmol), and the mixture was stirred at 110 °C for 1.5 h. After the mixture was filtered through a silica gel short column (hexane/AcOEt = 10/1), the filtrate was concentrated to give the crude product 20-*epi*-vitamin D_3 (45 mg). To a solution of the crude vitamin D_3 in MeCN (1 mL) was added HF/MeCN (1/9, 1 mL) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the mixture was added saturated $NaHCO_3$ aqueous solution at 0 °C, and the aqueous layer was extracted with AcOEt. The organic layer was washed with saturated NaCl aqueous solution, dried over Na_2SO_4, and concentrated. The residue was purified by preparative TLC (AcOEt) to give 20-*epi*-**4a** (20 mg, 48% in two steps) as a colorless oil. Further purification of 20-*epi*-**4a** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{19}_D$ +12.4° (*c* 0.82, $CHCl_3$); UV (MeOH) λ_{max} 266 nm; IR (neat) 3374, 1647, 1074 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (s, 3H), 0.84 (d, *J* = 6.4 Hz, 3H), 1.21 (s, 6H), 1.10−2.05 (m, 21H), 2.24 (dd, *J* = 12.5, 11.5 Hz, 1H), 2.67 (dd, *J* = 12.5, 4.6 Hz, 1H), 2.83 (m, 1H), 3.37 (dd, *J* = 7.9, 3.0 Hz, 1H), 3.70 (m, 1H), 3.73−3.85 (m, 4H), 4.07 (m, 1H), 4.43 (d, *J* = 2.9 Hz, 1H), 5.09 (d, *J* = 1.5 Hz, 1H), 5.37 (d, *J* = 1.5 Hz, 1H), 6.01 (d, *J* = 11.1 Hz, 1H), 6.42 (d, *J* = 11.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 18.7, 21.0, 22.2, 23.6, 27.4, 29.2, 29.3, 29.4, 35.5, 36.1, 40.5, 41.4, 44.4, 46.0, 56.2, 56.4, 61.9, 68.5, 71.1, 71.3, 72.5, 85.1, 116.6, 117.1, 125.3, 131.3, 143,3, 143.8; EI−LRMS *m/z* 476 (M$^+$), 458, 440, 396, 378. EI−HRMS calcd for $C_{29}H_{48}O_5$ 476.3502. Found 476.3503.

20-*epi*-2α-(3-Hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (20-*epi*-4b). In a manner similar to that for the synthesis of 20-*epi*-**4a** from **14** and **11a**, a crude product, which was obtained from **14** (22 mg, 60 μmol), **11b** (51 mg, 91 μmol), and $Pd(PPh_3)_4$ (21 mg, 18 μmol) in toluene/Et_3N (1/1, 4 mL), was dissolved in MeCN. To the solution was added HF/MeCN (1/9, 1 mL) at 0 °C, and the mixture was stirred at room temperature for 1.5 h. After the usual workup, the crude product was purified by preparative TLC (AcOEt) to give 20-*epi*-**4b** (17 mg, 57% in two steps) as a colorless oil. Further purification of 20-*epi*-**4b** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{21}_D$ +11.0° (*c* 1.31, $CHCl_3$); UV (MeOH) λ_{max} 267 nm; IR (neat) 3389, 1647, 1265, 1076 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (s, 3H), 0.84 (d, *J* = 6.6 Hz, 3H), 1.20 (s, 6H), 1.10−1.73 (m, 15H), 1.75−1.90 (m, 4H), 1.90−2.00 (m, 2H), 2.22 (dd, *J* = 13.4, 9.2 Hz, 1H), 2.64 (br s, 3H), 2.66 (dd, *J* = 13.4, 4.8 Hz, 1H), 2.81 (m, 1H), 3.36 (dd, *J* = 7.4, 3.3 Hz, 1H), 3.70−3.90 (m, 4H), 4.04 (m, 1H), 4.43 (br d, *J* = 3.2 Hz, 1H), 5.08 (d, *J* = 2.0 Hz, 1H), 5.37 (s, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 6.40 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.3, 18.5, 20.6, 22.1,

23.5, 27.3, 29.0, 29.2, 31.8, 35.4, 36.0, 40.4, 41.0, 44.3, 45.9, 56.1, 56.4, 61.1, 68.3, 68.4, 71.1, 71.9, 84.5, 116.1, 117.1, 125.4, 131.6, 143.4, 144.3; EI−LRMS *m/z* 490 (M^+), 473, 472, 396, 267. EI−HRMS calcd for $C_{30}H_{50}O_5$ 490.3660. Found 490.3676.

20-*epi*-2α-(2-Hydroxybutoxy)-1α,25-dihydroxyvitamin D_3 (20-*epi*-4c). In a manner similar to that for the synthesis of 20-*epi*-**4a**, a crude product, which was obtained from **14** (40 mg, 0.11 mmol), **11c** (96 mg, 0.17 mmol), and Pd(PPh_3)$_4$ (39 mg, 34 μmol) in toluene/Et_2N (1/1, 4 mL), was dissolved in MeCN (1 mL). To the solution was added HF/MeCN (1/9, 1 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. After the usual workup, the crude product was purified by preparative TLC (AcOEt) to give 20-*epi*-**4c** (25 mg, 45% in two steps) as a colorless oil. Further purification of 20-*epi*-**4c** for biological assays was conducted by reversed-phase recycle HPLC (YMC−Pack ODS column, 20 × 150 mm, 9.9 mL/min, CH_3CN/H_2O = 6/4): $[\alpha]^{16}_D$ +8.1° (*c* 1.82, $CHCl_3$); UV (MeOH) λ_{max} 269 nm; IR (neat) 3376, 1645, 1078 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.53 (s, 3H), 0.84 (d, *J* = 6.6 Hz, 3H), 1.10 (s, 6H), 1.08−2.05 (m, 25H), 2.23 (dd, *J* = 13.4, 9.3 Hz, 1H), 2.50 (br s, 1H), 2.67 (dd, *J* = 13.4, 4.5 Hz, 1H), 2.83 (m, 1H), 3.34 (dd, *J* = 7.6, 3.1 Hz, 1H), 3.60 (dt, *J* = 9.5, 5.8 Hz, 1H), 3.68 (t, *J* = 5.7 Hz, 2H), 3.74 (dt, *J* = 9.5, 5.9 Hz, 1H), 4.05 (ddd, *J* = 8.8, 7.6, 4.6 Hz, 1H), 4.41 (br d, *J* = 3.1 Hz, 1H), 5.09 (d, *J* = 1.6 Hz, 1H), 5.38 (d, *J* = 1.6 Hz, 1H), 6.02 (d, *J* = 11.2 Hz, 1H), 6.41 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 18.7, 21.0, 22.2, 23.6, 27.0, 27.4, 29.2, 29.3, 29.4, 29.7, 35.5, 36.1, 40.5, 41.0, 44.4, 46.0, 56.2, 56.4, 62.3, 68.2, 69.9, 71.1, 71.8, 84.5, 116.1, 117.1, 125.3, 131.4, 143.2, 144.0; EI−LRMS *m/z* 504 (M^+), 486, 396, 378. EI−HRMS calcd for $C_{31}H_{52}O_5$ 504.3815. Found 504.3814.

Binding Assays to the Bovine Thymus VDR. Bovine thymus VDR was obtained from a commercial supplier, and the affinity was evaluated according to the literature.[23]

Assays for Induction of HL-60 Cell Differentiation. The activity was estimated by superoxide anion production as previously described.[26] The superoxide radicals reduce the cytochrome *c*, and the reduced cytochrome *c* is measured by spectrophotometry at 550 nm.

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Supporting Information Available: General experimental details, ^1H NMR and ^{13}C NMR spectra for all new compounds (**6a−11a**, **6b−11b**, **6c−11c**, **4a−c**, 20-*epi*-**4a−c**), charts of VDR binding assays of compounds **4a−c** and 20-*epi*-**4a−c**, and a chart for assays of induction of HL-60 cell differentiation activity of compounds **4a−c** and 20-*epi*-**4a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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