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Synthesis of novel *gem*-difluorinatedcyclopropane hybrids: Applications for DNA cleavage agents switched by photo irradiation

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Abstract

A strong DNA cleavage property switched by photo irradiation was found for 9-anthracenecarboxylic acid substituted *gem*-difluorocyclopropane derivatives; in particular, a clear contrast in the activity was observed between the enantiomers of (*S*,*S*)- and (*R*,*R*)-3-aminomethyl-2,2-difluorocyclopropylmethyl 9-anthracenecarboxylate.

Keywords: *gem*-Difluorinatedcyclopropane–anthracene hybrids; DNA cleavage agents; Photo irradiation; Enantiomer

1. Introduction

Interest has been growing about DNA damage induced by various types of natural and unnatural compounds, in particular, the search for organic compounds that possess DNA cleavage property switched by photo irradiation [1]. Anthryl or anthraquinone derivatives have been reported to be potent DNA cleavage agents: Schuster and co-workers reported anthraquinonecarboxylic amide caused GG-selective cleavage of duplex DNA [2], and Kumar et al. reported anthracene substituted alkylamine derivatives showed potent DNA cleavage properties [3]. Further, Toshima reported a quinoxaline-carbohydrate hybrid to be a GG-selective DNA cleaving agent with DNA cleaving and binding abilities which are dependent on the structure of the sugar moiety [4].

During the course of our study to establish the stereochemistry of 1,3-bishydroxymethyl-2,2-difluorocyclopropane (**1**)[5] using CD spectroscopic analysis of the corresponding 9-anthracenecarboxylic diester **2** [6] (Fig. 1), we found the interesting facts that the esterification of **1** with 9-anthracencarboxylic acid chloride must be carried out in a flask with a blackout shield, and that the produced diester **2** was so unstable in dichloromethane solution under sunlight that it formed unidentified complex compounds by opening the cyclopropane ring. We expected that our anthracene–*gem*-difluorocyclopropane compounds might display DNA cleavage activity by photo irradiation because it was reported that decomposition of a *gem*-difluorocyclopropane ring proceeded via a radical intermediate [7], and it has been suggested that DNA damage was caused by radical species [8]. In fact, we found that (*S*,*S*)-**2** showed a weak DNA cleavage activity; 23% of supercoiled ϕX174 DNA (Form I) was converted to the relaxed form (Form II) by photo irradiation in the presence of 200 μM of (*S*,*S*)-**2**. On the contrary, no DNA cleavage activity was found for *gem*-difluorocyclopropne (*S*,*S*)-**1** or diacetate of (*S*,*S*)-**1**. Acting on this result, we attempted to increase the DNA cleavage activity by modification of the anthracene–difluorocyclopropane hybrid derivatives. Here we wish to report that anthracene–difluorocyclopropane hybrids worked as potent DNA cleavage agents switched by photo irradiation, and the DNA cleavage property was dependent on the chirality of the cyclopropane ring.

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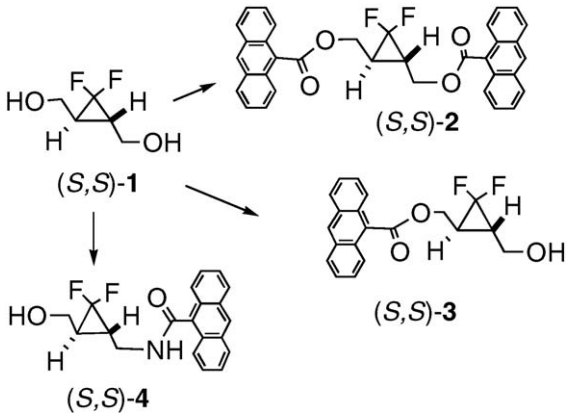


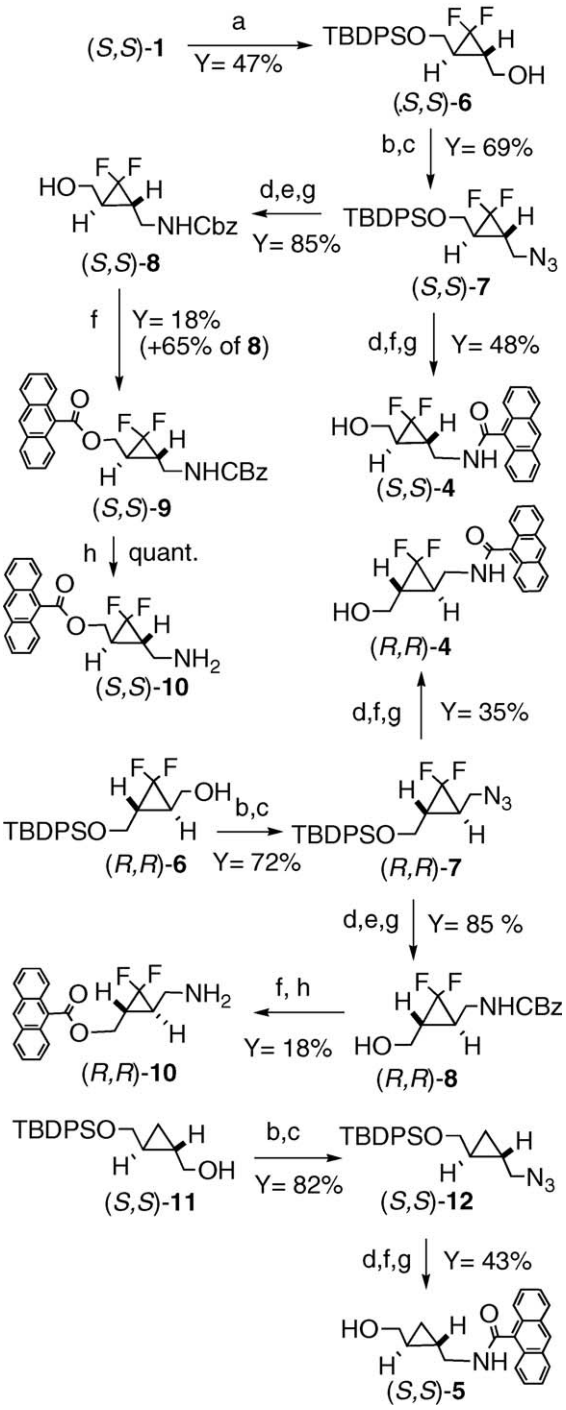
Fig. 1. *gem*-Difluorocyclopropane derivatives.

2. Results and discussion

Although the DNA cleavage property of 9-anthracenecarboxylic ester (*S*,*S*)-**2** was not significant, we expected that enhanced DNA cleavage activity might be obtained by modification of (*S*,*S*)-**2** to convert more hydrophilic derivatives, such as alcohol (*S*,*S*)-**3**, amide (*S*,*S*)-**4** (Fig. 1). Since it was anticipated that the activity may depend on the chirality of the cyclopropane ring because DNA is a chiral array, we synthesized both enantiomers of (*S*,*S*)-and (*R*,*R*)-**4**. Further, we prepared (*S*,*S*)-**5** from (*S*,*S*)-1-(*t*-butyldiphenysilanyloxy)-methyl-2-hydroxymethylcyclopropane [9] (Scheme 1), and investigated its DNA cleavage property by comparing the DNA cleavage activity between difluorocyclopropane derivative and non-fluorinated cyclopropane derivative.

Typically the DNA cleavage test was carried out as follows: a solution of supercoiled ϕX174 plasmid DNA in a phosphate buffer at pH 7.0 was irradiated using a Xe lamp with a polystyrene filter at 365 nm in the presence of a dimethylsulfoxide (DMSO) solution of anthracenecarboxylates. Agarose gel was stained by ethidium bromide and visualized by an UV-B lamp. We tested (*S*,*S*)-**3** and ethyl 9-anthracenecarboxylate as DNA cleavage agent and found that (*S*,*S*)-**3** caused significant cleavage of DNA at a concentration of 250 μM (Fig. 2, lane d), while ethyl 9-anthracenecarboxylate showed no activity at the same concentration (Fig. 2, lane b). Amide 4 showed a significant DNA cleavage activity; (*S*,*S*)-**4** and (*R*,*R*)-**4** caused cleavage of DNA by the photo irradiation and supercoiled ϕX174 DNA (Form I) was converted to the relaxed form (Form II) almost completely in the presence of 100 μM of (*S*,*S*)-**4**; this concentration corresponded to 1.3 equivalent versus the DNA base pair (Fig. 2, lanes e and f). Further, (*S*,*S*)-**5** also caused DNA cleaving (lane g), though the activity was slightly inferior to that of (*S*,*S*)-**4** (lane f) at a lower concentration.

Hence, we investigated the details of DNA cleavage activities for (*S*,*S*)-**3**, (*S*,*S*)-**4**, (*R*,*R*)-**4**, (*S*,*S*)-**5** and *N*-butyl-9-anthracenecarboxamide (BAA) at different concentrations of 25 and 250 μM (Fig. 3). Interestingly, a significant DNA cleavage was observed in the presence of 250 μM of BAA; BAA showed similar DNA cleavage activity with that of (*S*,*S*)-**3** (Fig. 3). Among these four anthracenecarboxylic amide



Scheme 1. (a) TBDPSCl, imidazole, DMF, rt; (b) TsCl, CH_2Cl_2, py, rt; (c) NaN_3, DMF, rt; (d) PPh_3, THF-cat.H_2O, rt; (e) CbzCl, py, dioxane, 55 °C; (f) 9-anthracenecarboxylic acid, EDC, Et_3N, DMAP, CH_2Cl_2, rt; (g) TBAF, THF, rt; (h) H_2, Pd/C, MeOH, rt.

derivatives, stronger activity was observed for 2,2-difluorocyclopropane **4** than for the corresponding non-fluorinated version at a low concentration (25 μM): (*S*,*S*)-**4** (48.1%) showed three times stronger activity than that of (*S*,*S*)-**5** (14.6%). In the Fig. 2, Form III DNA fragment was found at 250 μM for compound (*R*,*R*)-**4**; this may account for the slight lower potency of (*R*,*R*)-**4**. It was thus revealed that fluorinated-cyclopropane derivative **4** showed higher DNA cleavage

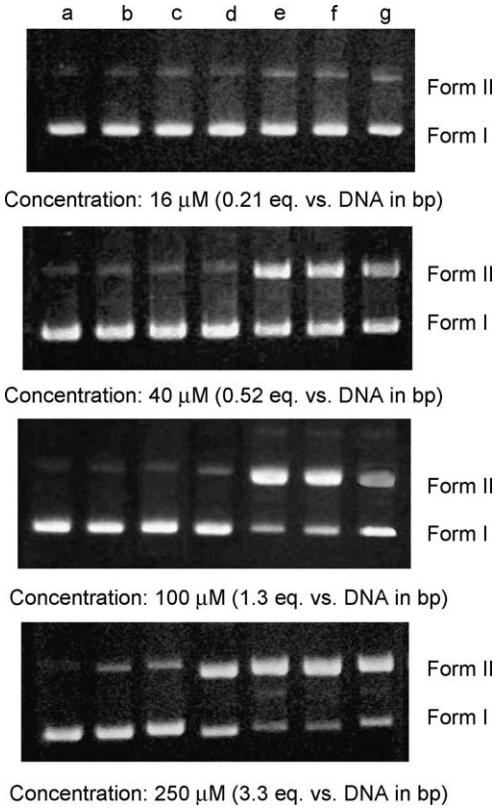


Fig. 2. Photocleavage of supercoiled ϕX174 plasmid DNA by cyclopropane derivatives. Lane a, control; b, ethyl 9-anthracenecarboxylate; c, (*S*,*S*)-**2**; d, (*S*,*S*)-**3**; e, (*R*,*R*)-**4**; f, (*S*,*S*)-**4**; g, (*S*,*S*)-**5**. Reaction buffer: 20 mM sodium phosphate pH 7.0 (20% DMSO). Samples were irradiated at 25 °C for 45 min at a distance of 6 cm using a Xe lamp with a polystyrene filter (365 nm). Electrophoresis (0.8% agarose gel, TAE buffer, 100 V, 40 min). Gel was stained by ethidium bromide and visualized by UV-B lamp (transilluminator).

activity than that of non-fluorinated analogue **5**, in particularly under low concentration conditions. However, no significant difference was found in the activity between (*S*,*S*)-**4** and (*S*,*S*)-**5** at a higher concentration of 250 μM (Fig. 3). Recently it has been suggested that fluorine atom substituted at a position of acetoamide stabilizes a charge separated state of the amide group due to hyper-conjugation effect between the fluorine atom and amide oxygen [10]; this may contribute to enhance DNA cleavage property of compound **4** at low concentration due to stabilization effect of the radical anion which may be induced by photo irradiation on the anthracene amide group. However, (*S*,*S*)-**4** and (*S*,*S*)-**5** showed similar DNA cleavage activity at high concentration.

We anticipated that enhanced DNA cleavage activity might be obtained by introduction of the terminal amino group in our compounds due to increased interaction of the anionic part of DNA. We prepared difluorocyclopropane analogues (*S*,*S*)-**10** and (*R*,*R*)-**10**, which possess the terminal amino group, following the route in Scheme 1, and tested DNA cleavage activity (Fig. 4). The DNA cleaving test gave very interesting results: a clear contrast of the reactivity was found between (*R*,*R*)-**10** and (*S*,*S*)-**10**. Supercoiled ϕX174 DNA was converted to the corresponding relaxed form when photo irradiated in the presence of 85 μM (1.1 eq. versus DNA in base pair) of (*R*,*R*)-**10**, while 1,200 μM (15.6 eq. versus DNA in base pair) was required to show the same activity for the (*S*,*S*)-**10**; (*R*,*R*)-**10** showed more than 10 times stronger activity compare to the corresponding (*S*,*S*)-isomer.

An even more interesting fact was that a significantly reduced activity was obtained for (*S*,*S*)-**10**, though no significant difference of activity was found between amide (*S*,*S*)-**4** and (*R*,*R*)-**4**. We now hypothesize that the terminal amino group may bind to the phosphate group of DNA, and then intercalative or minor groove binding of the anthracenyl group with the DNA sequence takes place [2]; chirality of the *gem*-cyclopropane ring could control the binding fashion of the attached anthracene group with DNA sequence. Although *gem*-difluorocyclopropane decomposition may be not an important factor for the present DNA cleavage activity, the *gem*-difluorocyclopropane group seems to play a very important role in determining the binding fashion with DNA. To confirm this, we are now synthesizing

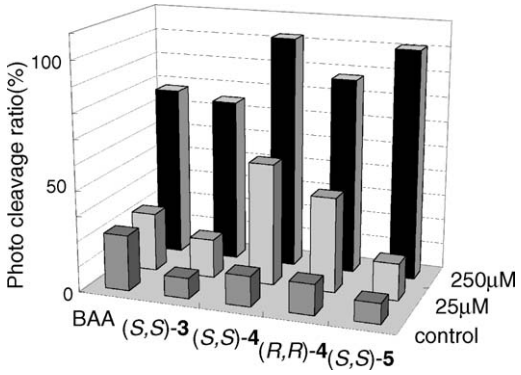


Fig. 3. DNA cleavage activity of various types of cyclopropane–anthracene hybrids. Concentration of photocleavages is 25 μM (0.33 eq. vs. DNA in bp) or 250 μM (3.3 eq. vs. DNA in bp). Reaction buffer: 20 mM sodium phosphate pH 7.0 (20% DMSO). Samples were irradiated at 25 °C for 45 min at a distance of 4 cm using a Xe lamp with polystyrene filter (365 nm).

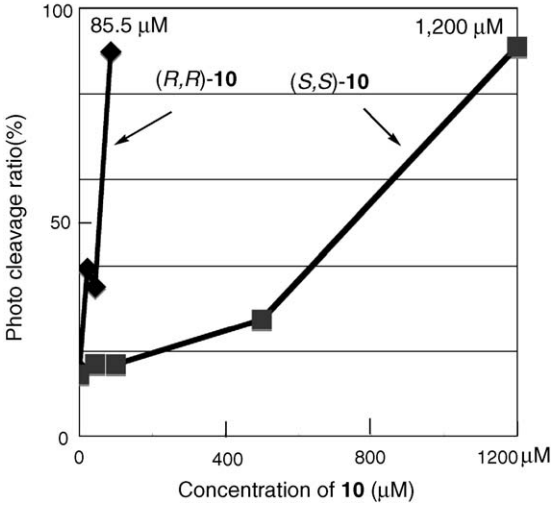


Fig. 4. Photocleavage of supercoiled ϕX174 plasmid DNA by 9-anthracenecarboxylate (*R*,*R*)-**10** and (*S*,*S*)-**10**. Reaction buffer: 20 mM sodium phosphate pH 7.0 (20% DMSO). Samples were irradiated at 25 °C for 40 min at a distance of 3 cm using a Xe lamp with a polystyrene filter (365 nm).

both enantiomers of the corresponding non-fluorinated analogues **10**. This is the novel design of chiral DNA cleavage agents and the first demonstration that chirality of a small cyclopropane derivative could control the DNA cleavage ability of anthracene hybrid compounds.

3. Conclusions

We found that 9-anthracenecarboxylic acid substituted cyclopropane derivatives have strong DNA cleavage property switched by photo irradiation. Our cyclopropane compounds have two hydroxyl groups, thus making it easy to modify the structure by introducing DNA sequence recognition groups such as oligonucleotide [1]. Syntheses of other types of optically active *gem*-difluorinated- and non-fluorinated cyclopropane–anthracene hybrids are now ongoing. Further investigation of the scope and limitation of our cyclopropane derivatives as DNA cleavage agents switched by photo irradiation will make it possible to design an artificial restriction enzyme based on our compounds.

4. Experimental

Reagents and solvents were purchased from common commercial sources and were used as received or purified by distillation from appropriate drying agents. Reactions requiring anhydrous conditions were run under an atmosphere of dry argon. Wako gel C-300 and Wako gel B5F were used for flash column chromatography and thin-layer chromatography (TLC), respectively. Chemical shifts are expressed in δ value (ppm) downfield from tetramethylsilane (TMS) in $CDCl_3$ as an internal reference. ^{19}F NMR spectra were reported in ppm downfield from C_6F_6 as an internal reference.

*4.1. ((1S,3S)-2, 2-difluoro-3-(hydroxymethyl)cyclopropylmethyl anthracene-9-carboxylate (**3**)*

The reaction was carried out in a flask that was covered by aluminum foil to shield the light. To a CH_2Cl_2 (5 mL) solution of (*S*,*S*)-**1** (99% ee) [5b] (49 mg, 0.355 mmol) was added EDC (102 mg, 0.533 mmol), 9-anthracenecarboxylic acid (87 mg, 0.391 mmol) and DMAP (8 mg, 0.07 mmol) at RT and triethylamine (Et_3N, 0.07 mL, 0.533 mmol) was added to the mixture at 0 °C. The mixture was stirred for 4 h at 60 °C. After being cooled to RT, the reaction mixture was diluted with ethyl acetate (30 mL) and the organic layers was washed with aqueous saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Silica gel TLC (hexane/ethyl acetate = 2:1) gave (*S*,*S*)-**3** (27 mg, 0.079 mmol) in 23% yield and diester (*S*,*S*)-**2** [5c] (7.5 mg, 0.014 mmol) in 4% yield.

(*S*,*S*)-**3**: $[\alpha]_D^{25}$ +14.6 (ca. 1.4, $CHCl_3$); rf 0.30 (hexane/ethyl acetate = 2:1); ^1H NMR (270 MHz, ppm, $CDCl_3$, J = Hz) δ 1.65–2.11 (2H, m), 3.20 (1H, brs, OH), 3.70–3.73 (2H, m), 4.53–4.68 (2H, m), 7.38–7.50 (4H, m) 7.92–7.98 (4H, m), 8.45 (1H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, J = Hz) δ 25.30 (t, J_{C-F} = 11.1), 29.38 (t, J_{C-F} = 10.1), 59.96 (d, J_{C-F} = 5.8), 61.77 (d, J_{C-F} = 4.8), 113.85 (t, J_{C-F} = 287.0), 124.76, 125.51, 126.95, 127.15, 128.47, 128.66, 129.71, 130.89, 169.39; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, J = Hz) δ 22.43 (1F, dd, J_{F-F} = 164.1, J_{H-F} = 14.5), 24.26 (1F, dd, J_{F-F} = 165.5, J_{H-F} = 13.0); IR ν (cm^{-1}) 3404, 3057, 1717, 1483, 1145, 1200, 1003, 937, 899. Anal. calcd. for $C_{20}H_{16}F_2O_3$: C, 70.17; H, 4.71. Found: C, 70.02; H, 4.69.

*4.2. N-(1R,3R)-2,2-difluoro-3-(hydroxymethyl)cyclopropylmethyl anthracene-9-carboxamide (**4**)*

To a *N*,*N*-dimethylformamide (DMF) (15 mL) solution of (*R*,*R*)-**1** (99% ee) [5b,5c] (818 mg, 6.00 mmol) and *tert*-butyldiphenylchlorosilan (TBDPSCl) (2.48 g, 9.00 mmol) was added imidazole (612 mg, 9.00 mmol), and the mixture was stirred at RT for 24 h. The reaction was quenched by the addition of crushed ice and extracted with ether (10 mL, four times). The combined organic layers were dried over $MgSO_4$, concentrated, and silica gel flash column chromatography (hexane/ethyl acetate = 50:1 to 4:1) gave (*R*,*R*)-**6** (1.02 g 2.72 mmol) in 45% yield. Bis-TBDPS ether was also obtained (1.64 g, 2.68 mmol) in 45% yield.

To a dichloromethane (CH_2Cl_2) (20 mL) solution of (*R*,*R*)-**6** (1.00 g, 2.65 mmol) and *p*-toluenesulfonyl chloride (*p*-TsCl) (758 mg, 3.97 mmol) was added pyridine (0.67 mL, 8.0 mmol) at 0 °C, and the mixture was stirred at RT for 24 h. The reaction was quenched by addition of water (10 mL) and then extracted with CH_2Cl_2 and ethyl acetate (10 mL, three times). The combined organic layers were dried over $MgSO_4$ and silica gel flash column chromatography (hexane/ethyl acetate = 300:1 to 4:1) gave the corresponding tosylate (1.07 g, 2.01 mmol) in 76% yield. This was used without further purification. To a DMF (8 mL) solution of the tosylate (400 mg, 0.75 mmol) was added sodium azide powder (73 mg, 1.13 mmol) in one portion and the mixture was stirred at RT for 10 h. The reaction was quenched by addition of crushed ice and extracted with ether (10 mL, four times). The combined organic layers were dried over $MgSO_4$ and silica gel flash column chromatography (hexane/ethyl acetate = 50:1 to 10:1) gave (*R*,*R*)-**7** (289 mg, 0.72 mmol) in 96% yield.

(*R*,*R*)-**7** (100 mg, 0.249 mmol) and triphenylphosphine (72 mg, 0.27 mmol) was dissolved in a mixed solvent of tetrahydrofuran (THF) and water (THF/H_2O = 10 mL/40 mg) (5 mL) and the mixture was stirred at RT for 24 h under argon atmosphere, then solvent was removed under vacuum. To the resulting residue was diluted with CH_2Cl_2 (5 mL), and EDC (143 mg, 0.75 mmol), DMAP (3 mg, 0.02 mmol), and 9-anthracenecarboxylic acid (166 mg, 0.747 mmol) were added, then triethylamine (Et_3N) (0.1 mL, 0.747 mmol) was added to the mixture at 0 °C. The resulting mixture was stirred for 4 h at 60 °C. The reaction mixture was being cooled to RT, and diluted with ethyl acetate (30 mL). The organic layers ware washed with $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the corresponding amide (51 mg, 0.088 mmol) after silica gel thin layer chromatography (TLC) (hexane/ethyl acetate = 2:1) in 35%

yield. This was used to the next reaction without further purification. To a THF (1.0 mL) solution of the amide (40 mg, 0.069 mmol) was added a 1.0 M THF solution of tetrabutylammonium fluoride (TBAF) (0.1 mL, 0.103 mmol) at RT and the mixture was stirred at RT for 6 h. To the reaction mixture was added a water and ethyl acetate and was extracted with ethyl acetate (5 mL, four times). The combined organic layers were dried over $MgSO_4$ and purified by silica gel TLC (hexane/ethyl acetate = 1:3) to give (*R*,*R*)-**4** (23 mg, 0.067 mmol) in 98% yield.

(*R*,*R*)-**6**: $[\alpha]_D^{26}$ +3.8 (ca. 1.6, $CHCl_3$); rf 0.30 (hexane/ethyl acetate = 2:1); bp 200 °C/2.4 Torr (Kugelrohr); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, *J* = Hz) δ 1.04 (9H, s), 1.61–1.72 (2H, m), 3.64–3.79 (4H, m), 7.35–7.47 (6H, m), 7.64–7.67 (4H, m); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, *J* = Hz) δ 19.01, 26.71, 28.54 (t, J_{C-F} = 10.1), 28.70 (t, J_{C-F} = 10.1), 59.51 (d, J_{C-F} = 4.8), 60.17 (d, J_{C-F} = 3.9), 114.64 (t, J_{C-F} = 286.5), 127.89, 129.96, 133.29, 133.39, 135.65; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, *J* = Hz) δ 23.06 (1F, dd, J_{F-F} = 162.4, J_{H-F} = 11.1), 23.63 (1F, dd, J_{F-F} = 164.1, J_{H-F} = 11.5); IR ν (cm^{-1}) 3366, 2932, 2858, 1472, 1261, 1184, 1113, 1013, 702. Anal. calcd. for $C_{21}H_{26}F_2O_2Si$: C, 66.99; H, 6.96. Found: C, 66.89; H, 6.98.

(*R*,*R*)-**7**: $[\alpha]_D^{22}$ −14.1 (ca. 1.5, $CHCl_3$); rf 0.70 (hexane/ethyl acetate = 7:1); bp 130 °C/1.6 Torr (Kugelrohr); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, *J* = Hz) δ 1.05 (9H, s), 1.55–1.74 (2H, m), 3.30 (2H, d, J_{C-F} = 6.0), 3.73–3.79 (2H, m), 7.25–7.47 (6H, m), 7.64–7.67 (4H, m); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, *J* = Hz) δ 19.27, 25.22 (t, J_{C-F} = 10.6), 28.86, 29.72 (t, J_{C-F} = 10.1), 48.18 (d, J_{C-F} = 4.8), 59.87 (d, J_{C-F} = 5.0), 113.77 (dd, J_{C-F} = 286.1, 288.9), 127.94, 130.02, 133.22, 133.31, 135.68; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, *J* = Hz) δ 22.82 (1F, dd, J_{F-F} = 160.0, J_{H-F} = 11.5), 24.74 (1F, dd, J_{F-F} = 164.4, J_{H-F} = 14.4); IR ν (cm^{-1}) 2932, 2858, 2098 (N_3), 1458, 1429, 1261, 1186, 1113, 1007, 834, 702. Anal. calcd. for $C_{21}H_{25}F_2N_3OSi$: C, 62.82; H, 6.28; N, 10.47. Found: C, 62.91; H, 6.31; N, 10.46.

(*R*,*R*)-**4**: $[\alpha]_D^{27}$ +8.0 (ca. 0.2, $CHCl_3$); rf 0.35 (hexane/ethyl acetate = 1:3); mp 186–193 °C (recrystallization from hexane–ether); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, *J* = Hz) δ 1.89–2.07 (2H, m), 3.67–3.77 (4H, m), 7.48–7.55 (4H, m), 8.05–8.08 (4H, m), 8.57 (1H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, *J* = Hz) δ 26.57 (t, J_{C-F} = 10.6), 30.23 (t, J_{C-F} = 10.1), 37.37 (d, J_{C-F} = 5.8), 58.70 (d, J_{C-F} = 4.8), 115.64 (t, J_{C-F} = 286.0), 125.35, 125.98, 127.12, 128.60, 128.64, 129.00, 131.95, 132.20, 171.81; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, *J* = Hz) δ 24.70 (2F, dd, J_{F-F} = 364.2, J_{H-F} = 15.8); IR ν (cm^{-1}) 3260, 2926, 2855, 1628, 1549, 1248, 1178, 1115, 1003, 733. Anal. calcd. for $C_{20}H_{17}F_2NO_2$: C, 70.37; H, 5.02; N, 4.10. Found: C, 70.46; H, 5.03; N, 4.09.

(*S*,*S*)-**4** ($[\alpha]_D^{27}$ −7.2 (ca. 0.6, $CHCl_3$)) was prepared in the same method from (*S*,*S*)-**1** in 16% overall yield.

*4.3. (R,R)-[3-(tert-Butyldiphenylsilanyloxymethyl)-2,2-difluorocyclopropylmethyl]carbamic acid benzyl ester (**8**)*

(*R*,*R*)-**7** (494 mg, 1.23 mmol) and triphenylphosphine (354 mg, 1.35 mmol) was dissolved in a mixed solvent of THF and water (THF/H_2O = 10 mL/40 mg) (10 mL), and the mixture was stirred at RT for 24 h and concentrated under vacuum. To the resulting residure was added dioxane (10 mL) solution of benzyloxycarbonyl chloride (CbzCl, 420 mg, 2.46 mmol) and pyridine (0.3 mL, 3.69 mmol) at RT, and the mixture was stirred at 55 °C for 12 h. After being cooled to RT, ethyl acetate (15 mL) and brine (10 mL) were added and extracted with ethyl acetate (15 mL, four times). The combined organic layers were dried over $MgSO_4$, concentrated, and silica gel flash column chromatography (hexane/ethyl acetate = 50:1 to 4:1) gave the corresponding amide (533 mg 1.05 mmol) in 85% yield (two steps). To a THF (5 mL) solution of the amide (300 mg, 0.589 mmol) was added a THF solution of TBAF (0.89 ml, 0.89 mmol) at RT and the mixture was stirred for 20 h. The reaction mixture was diluted with ethyl acetate. The organic layers were concentrated and subsequent silica gel flash column chromatography (hexane/ethyl acetate = 10:1 to 0:1) gave (*R*,*R*)-**8** (160 mg, 0.589 mmol) in quantitative yield.

(*R*,*R*)-**8**: $[\alpha]_D^{27}$ −12.6 (ca. 1.4, $CHCl_3$); rf 0.20 (hexane/ethyl acetate = 2:1); bp 140 °C/3.2 Torr (Kugelrohr); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, *J* = Hz) δ 2.10 (2H, m), 3.18–3.71 (4H, m), 5.03 (2H, s), 7.28 (5H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, *J* = Hz) δ 26.41 (t, J_{C-F} = 9.6), 29.01 (t, J_{C-F} = 9.6), 37.94, 58.54, 66.81, 114.32 (t, J_{C-F} = 278.0), 127.51, 127.95, 128.12, 136.10, 156.64; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, *J* = Hz) δ 23.46 (2F, s); IR ν (cm^{-1}) 3325, 2923, 1699, 1456, 1182, 1130, 1042, 1005. Anal. calcd. for $C_{13}H_{15}F_2NO_3$: C, 57.56; H, 5.57; N, 5.16. Found: C, 57.09; H, 5.58; N, 5.17.

*4.4. (1R,3R)-3-(aminomethyl)-2,2-difluorocyclopropylmethyl anthracene-9-carboxylate (**10**)*

To a CH_2Cl_2 (5 mL) solution of (*R*,*R*)-**8** (65 mg, 0.24 mmol) was added EDC (75 mg, 0.39 mmol), 9-anthracenecarboxylic acid (59 mg, 0.26 mmol), and DMAP (8 mg, 0.072 mmol) at RT, then Et_3N (0.05 mL, 0.39 mmol) was added to the mixture at 0 °C. The mixture was stirred for 4 h at 60 °C in a dark place. After being cooled to RT, the reaction mixture was diluted with ethyl acetate (6 mL) and extracted with ethyl acetate six times (10 mL of each). The combined organic layers were washed with $NaHCO_3$ and brine, and dried over $MgSO_4$. Silica gel TLC (hexane/ethyl acetate = 2:1) give (*R*,*R*)-**9** (20 mg, 0.042 mmol) in 18% yield, while starting (*R*,*R*)-**8** (42 mg, 0.156 mmol) was recovered in 65% yield. A methanol (1.0 mL) solution of (*R*,*R*)-**9** (18 mg, 0.038 mmol) was stirred at RT in the presence of Pd/C (5.4 mg, 30 wt.%) under H_2 (1 atom) for 5 days and filtrated though a glass sintered glass filter with a Celite pad. The filtrate was evaporated under reduced pressure to give (*R*,*R*)-**10** (9.0 mg, 0.039 mmol). This compound was gradually decomposed to form unidentical products when it was stored as a methanol or $CHCl_3$ solution at RT. Therefore, it should keep under argon atmosphere in a dark place. Due to the unstable nature of **10**, we gave up to subject it to the elemental analysis.

(*R*,*R*)-**9**: $[\alpha]_D^{27}$ −29.5 (ca. 1.20, $CHCl_3$); rf 0.60 (hexane/ethyl acetate = 2:1); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, *J* = Hz) δ 1.88–2.04 (2H, m), 3.12–3.23 (1H, m), 3.49–3.56 (1H, m), 4.48–4.63 (2H, m) 4.94 (2H, s), 7.20–7.27 (5H, m), 7.38–7.51

(4H, m) 7.93–7.98 (4H, m), 8.46 (1H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, J = Hz) δ 25.82 (t, J_{C-F} = 10.6), 27.28 (t, J_{C-F} = 10.1), 38.12, 61.72, 66.91, 113.77 (t, J_{C-F} = 287.5), 124.72, 125.51, 126.86, 127.12, 127.73, 128.08, 128.18, 128.50, 128.65, 129.75, 130.89, 136.10, 156.19, 169.30; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, J = Hz) δ 22.23 (1F, dd, J_{F-F} = 166.9, J_{H-F} = 14.4), 23.64 (1F, dd, J_{F-F} = 165.6, J_{H-F} = 13.0); IR ν (cm^{-1}) 3342, 3064, 3034, 2964, 1717, 1456, 1201, 1173, 1003, 733. Anal. calcd. for $C_{28}H_{23}F_2NO_4$: C, 70.73; H, 4.88, N, 2.95. Found: C, 70.94; H, 4.87, N, 2.96.

(*R*,*R*)-**10**: $[\alpha]_D^{21}$ +7.1 (ca. 1.6, MeOH), rf 0.1 (hexane/ethyl acetate = 1:10); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, J = Hz) δ 0.89–0.92 (1H, m), 1.21–1.28 (1H, m), 2.0–2.1 (2H, brs, NH), 2.13–2.15 (2H, m), 4.50–4.89 (2H, m), 7.58–7.68 (4H, m0, 7.93–8.09 (4H, m), 8.53 (1H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, J = Hz) δ 24.8 (t, J_{C-F} = 11.6 Hz), 26.9 (t, J_{C-F} = 10.6 Hz), 37.3, 62.3, 114.0 (t, J_{C-F} = 285.5 Hz), 126.3, 129.4, 129.5, 129.6, 132.6, 132.7, 133.4, 170.2; ^{19}F NMR (470.6 MHz, ppm, $CDCl_3$, J = Hz) δ 24.49 (2F, dd, J_{F-F} = 164.1, J_{H-F} = 14.4); IR ν (cm^{-1}) 3348, 2941, 2829, 1732, 1605, 1474, 1261, 1205, 1153, 1024. (*S*,*S*)-10 ($[\alpha]_D^{21}$ −8.1 (ca. 1.1, MeOH)) was also prepared from (*S*,*S*)-**1** following to the Scheme 1.

4.5. (((1S,2S)-2-(azidomethyl)cyclopropyl)methoxy)(tert-butyl)diphenylsilane (**12**)

This compound was prepared from (*S*,*S*)-**11** [9] following to the Scheme 1 in 82% yield (2 steps). $[\alpha]_D^{21}$ −2.8 (ca. 1.0, $CHCl_3$); rf 0.5 (hexane/ethyl acetate = 7:1); bp 200 °C/2.4 Torr (kugelrohr); ^{1}H NMR (270 MHz, ppm, $CDCl_3$, J = Hz) δ 0.34–0.51 (2H, m), 084–0.95 (2H, m), 0.98 (9H, s), 3.01 (2H, dd, J = 16.0, 5.0), 3.48 (1H, dd, J = 11, 5.0), 3.59 (1H, dd, J = 15, 5.5), 7.27–7.39 (6H, m), 7.58–7.62 (4H, m); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, J = Hz) δ 8.24, 15.23, 19.20, 19.37, 26.69, 26.82, 54.86, 65.81, 127.63, 129.61, 133.77, 135.57; IR ν (cm^{-1}) 3071, 2930, 2858, 2093 (N_3), 1427, 1113, 1088, 741. Anal calcd. for $C_{21}H_{27}N_3OSi$: C, 69.00; H, 7.44, N, 11.50. Found: C, 70.66; H, 7.70, N, 9.85.

4.6. N-(((1S,2S)-2-(hydroxymethyl)cyclopropylmethyl anthracene-9-carboxamide (**5**)

This was prepared from (*S*,*S*)-**12** in 43% yield (three steps). $[\alpha]_D^{22}$ −6.4 (ca. 0.8, $CHCl_3$); rf 0.6 (hexane/ethyl acetate = 2:1); mp 176–180 °C (recrystallization from hexane–ether; ^{1}H NMR (270 MHz, ppm, $CDCl_3$, J = Hz) δ 0.59–0.73 (2H, m), 1.16–1.97 (2H, m), 3.20 (1H, brs, OH), 3.50 (2H, dd, J = 7.4, 4.4), 4.41 (2H, dd, J = 8.0, 4.0) 7.39–7.56 (4H, m), 7.99 (4H, dd, J = 17, 8), 8.46 (1H, s); ^{13}C NMR (125.8 MHz, ppm, $CDCl_3$, J = Hz) δ 8.72, 15.95, 20.39, 43.97, 69.13, 124.96, 125.38, 126.92, 126.98, 127.17, 128.39, 128.62, 129.34, 130.97, 135.44, 169.65; IR ν (cm^{-1}) 3427, 2933, 1628 (CO), 738; Anal. calcd. for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27, N, 4.59. Found: C, 78.46; H, 6.40, N, 4.55.

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