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Tetrahedron Letters 47 (2006) 2627–2630
Tetrahedron Letters

Synthesis of sphingomyelin difluoromethylene analogue

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Received 24 September 2005; revised 30 January 2006; accepted 6 February 2006
Available online 28 February 2006

Abstract—As a new sphingomyelinase inhibitor, a novel sphingomyelin CF_2 analogue was designed and synthesized. One key step of this synthesis is the very mild hydrolysis of the oxazolidinone ring, a suitable intermediate for the introduction of a diethyl difluoromethylphosphonate group, by utilizing the characteristic electron withdrawing nature of the nosyl group at the oxazolidinone ring in an alcoholic solvent to produce the corresponding carbonate attaching at the secondary hydroxy group. The synthesized CF_2 analogue inactivated toward *B. cereus* sphingomyelinase with nearly the same attitude as the nitrogen analogue that we previously reported.

In our project to develop new sphingomyelinase (SMase) competitive inhibitors, which act at the catalytic site of the enzyme, we have already reported the syntheses of carbon,[1] nitrogen,[2] and sulfur analogues[3] that possess a shorter and saturated backbone skeleton than natural sphingomyelin. These backbone skeleton were designed based on the previous results of the initial hydrolytic velocity of sphingomyelin analogues toward *B. cereus* sphingomyelinase.[4,5] In these analogues, one oxygen atom, at which the sphingomyelin was hydrolyzed by SMase, was replaced with a carbon, a nitrogen, or a sulfur atom. In general, SMase is regarded as a key enzyme of the sphingolipid metabolism[6] and catalyzes the hydrolysis of sphingomyelin to produce ceramide, recognized as an essential signal transduction factor in cell differentiation and in programmed cell death (apoptosis) derivation (Fig. 1).[7] Strong and competitive inhibitors against SMase would be valuable for the elucidation of the SMase hydrolytic mechanism.[8]

In this letter, we report the synthesis and inhibitory activity of sphingomyelin analogue **1**, which possesses a difluoromethylene group in the place of the hydrolyzed oxygen atom (Fig. 2). The difluoromethylene group is well regarded as an isoster of the oxygen atom due to similar steric, electrical, and polar properties.[9]

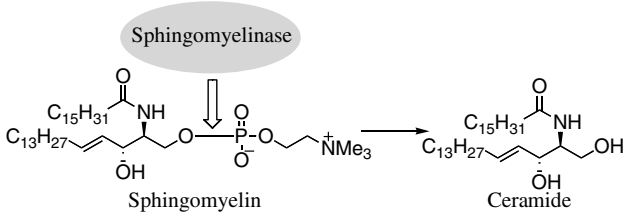

Figure 1. Sphingomyelin catabolite.

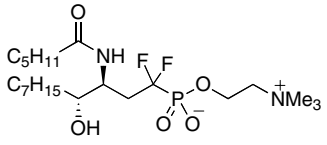

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Figure 2. Difluoromethylene analogue.

The retrosynthesis of sphingomyelin CF_2-analogue **1** is described in Figure 3. Difluoromethylphosphonate **3** would be a suitable intermediate for the synthesis of **1**, and be obtained by the introduction of a difluoromethylphosphonate group[10] to the corresponding triflate of alcohol **2**, which would be prepared in the

Keywords: Sphingomyelin CF_2 analogue; Sphingomyelinase inhibitor.
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doi:10.1016/j.tetlet.2006.02.018

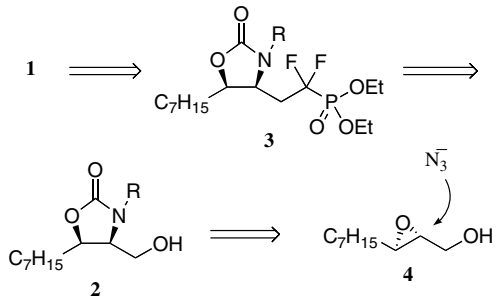
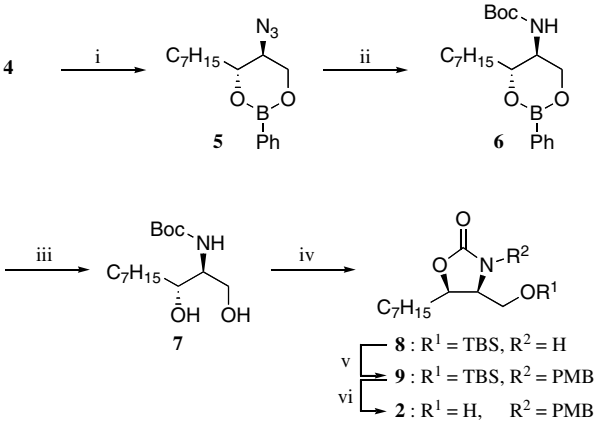


Figure 3. Retrosynthesis of CF_2 analogue **1**.

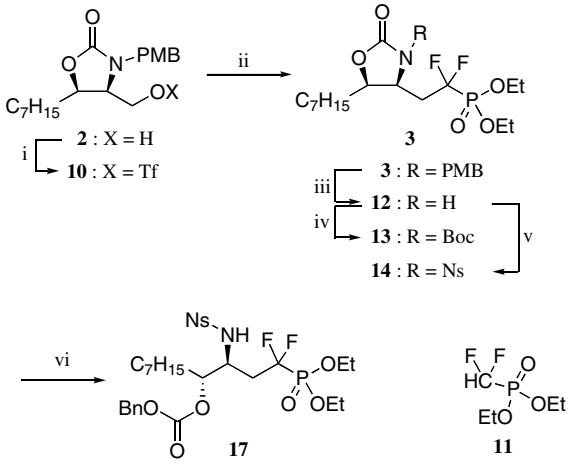
optically pure form from known **4**[9] by regioselective azide introduction followed by oxazolidinone ring formation.[11]

Thus, epoxide ring opening of **4** selectively proceeded at the C-2 position in a 6:1 ratio by treatment with sodium azide in the presence of phenylboric acid to give phenyl boronate **5**,[12] which was hydrogenated in the presence of di-*tert*-butyl dicarbonate in methanol to give the protected amine **6**.[13] Desired oxazolidinone alcohol **2** was obtained in excellent yield by a sequence of the deprotection of boronate **6**, the protection of the resulting primary alcohol of **7**, formation of the oxazolidinone ring, the introduction of a *p*-methoxybenzyl group at the nitrogen atom of **8**, and the subsequent removal of the *tert*-butyldimethylsilyl group. Compound **2** was easily purified by recrystallization (Scheme 1).

With desired oxazolidinone **2** in hand, the introduction of the diethyl difluoromethylphosphonate group at the C-1 position was examined (Scheme 2). The reaction of triflate **10**, which was prepared from **2** by treatment with trifluoromethanesulfonic anhydride and 2,6-lutidine, with a lithium anion of diethyl difluoromethylphosphonate **11**, gave the desired **3** in about 50% yield on a small scale according to the reported procedure.[10] On a 1-g scale, however, the yield of **3**[14] was significantly decreased due to the instability of the anion



Scheme 1. Synthesis of the difluoromethylene analogue. Reagents and conditions: (i) NaN_3, $PhB(OH)_2$, DMF, 86%; (ii) Boc_2O, H_2, Pd–C, MeOH, 86%; (iii) H_2O_2, MeOH, 0 °C, 95%; (iv) (a) TBSCl, imidazole, DMF, 0 °C; (b) NaH, THF, 30 °C, quant. for two steps; (v) PMBCl, NaH, TBAI, THF, rt; (vi) 2 N HCl, MeOH, rt, quant. for two steps.



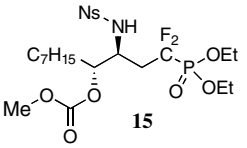
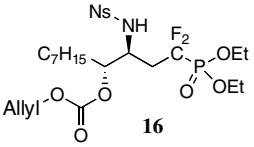
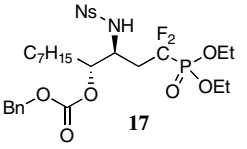
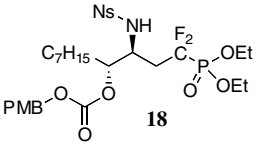
Scheme 2. Introduction of CF_2 group and nosyl group. Reagents and conditions: (i) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C; (ii) **11**, LDA, HMPA, −78 °C, 72% for two steps; (iii) CAN, CH_3CN, H_2O, 0 °C, 74%; (iv) Boc_2O, DMAP, Et_3N, DMF, quant.; (v) *n*-BuLi, NsCl, −78 °C, 78%; (vi) BnOH, K_2CO_3, 18-crown-6, 94%.

generated from **11**.[9b,15] After reinvestigating the reaction conditions, we successfully realized the modification of the reaction conditions to obtain the desired **3** in a 72% yield even on a 5-g scale by the following procedure: a pre-cooled THF solution of the anion derived from **11** at −100 °C was quickly added to a mixture of **10** and HMPA in THF at −78 °C, and then the resulting mixture was rapidly poured into a mixture of 2 N hydrochloric acid and ethyl acetate.

Next is the conversion of **3** to **17**. The PMB group of **3** was replaced by a Boc group to activate the oxazolidinone ring for hydrolysis based on a previous method.[1b,16] Unfortunately, the chemoselective hydrolysis of the oxazolidinone ring of **13** was unsuccessful, and unidentified compounds having higher polarity were produced. A nosyl group was then introduced into oxazolidinone **12** by treatment with *n*-butyllithium and nosyl chloride in THF.[17] Fortunately, the oxazolidinone ring of **14** was cleanly hydrolyzed within 5 min at room temperature by treatment with potassium carbonate in methanol to produce the desired methyl carbonate **17**. To the best of our knowledge, this is one of the mildest conditions for hydrolyzing an oxazolidinone ring.[16,18] Then, we studied this hydrolysis by using other alcohols, which led to the alcoxy part of the resulting carbonate. The obtained results are shown in Table 1. In the case of benzyl alcohol, the addition of 18-crown-6 ether in THF was very effective to obtain the corresponding carbonate in excellent yield. For further elaboration, we chose benzyl carbonate **17**, because the benzyl group could be removed by hydrogenolysis and the resulting alcohol was obtained by the procedure of simple filtration.

The remaining issues for the synthesis of difluoromethylene analogue **1** were the introduction of an acyl group and then a choline group. The acylation of **17** to lead **19** was successful by reaction with hexanoyl chloride in the presence of triethylamine in Et_2O in 83% yield.[19]

Table 1. Hydrolysis of oxazolidinone ring

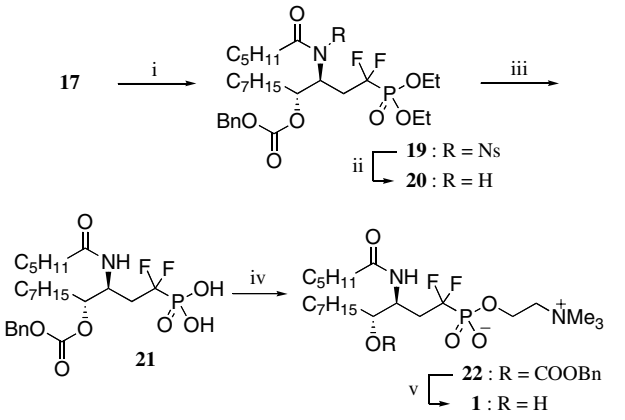
Alcohol	Yield	Products
Ethanol	90	 **15**
Allyl alcohol	94	 **16**
Benzyl alcohol	69[a] 94[b]	 **17**
p-Methoxybenzylalcohol	77	 **18**

[a] THF was used as a solvent.
[b] 18-Crown-6 was added as an additive.

The nosyl group was then removed by treatment with benzenethiol and DBU in acetonitrile[17] to produce ceramide derivative **20**. Treatment of **20** with bromotrimethylsilane produced the corresponding silyl ester, which was hydrolyzed by continuously stirring in methanol to produce acid **21** in 82% yield. A choline group was successfully introduced by three-step procedures. Thus, the reaction of **21** with Boc-protected aminoethanol in the presence of trichloroacetonitrile in pyridine at 60 °C, the removal of the Boc group by treatment with TFA, and then the methylation of the resulting primary amine produced the desired choline compound **22** in 68% yield. Pure **22** was obtained by using reverse phase HPLC. Finally, the benzyl carbonate group was removed by hydrogenolysis with palladium on carbon under a hydrogen atmosphere to produce desired **1**.[20] Thus, we achieved synthesis of difluoromethylene analogue **1** (Scheme 3).

The inhibitory ability of the synthesized CF_2 analogue **1** was tested against SMase from *B. cereus* under the same experimental conditions as the measurements of the methylene, ethylene, and nitrogen analogues.[1a,2,21] As shown in Figure 4, CF_2 analogue **1** showed nearly the same inhibitory ability to that of nitrogen analogue **23** and showed stronger ability than methylene **24** and ethylene **25** analogues. The IC_{50} values of CF_2 analogue **1** and nitrogen analogue **23** were approximately 57 and 53 μM, compared with 120 and 78 μM of the methylene and ethylene analogues, respectively.

In conclusion, we achieved the synthesis of difluoromethylene analogue **1** in an optically pure form. In this



Scheme 3. Synthesis of difluoromethylene analogue **1**. Reagents and conditions: (i) $C_5H_{11}COCl$, Et_3N, Et_2O, 0 °C, 85%; (ii) PhSH, DBU, CH_3CN, 0 °C, 86%; (iii) TMSBr, CH_2Cl_2, then MeOH, 82%; (iv) (a) CCl_3CN, $HO(CH_2)_2NHBoc$, pyridine, quant.; (b) TFA, CH_2Cl_2, 0 °C, 71%; (c) MeI, K_2CO_3, $CHCl_3$, 96%; (v) H_2, Pd/C, MeOH, 83%.

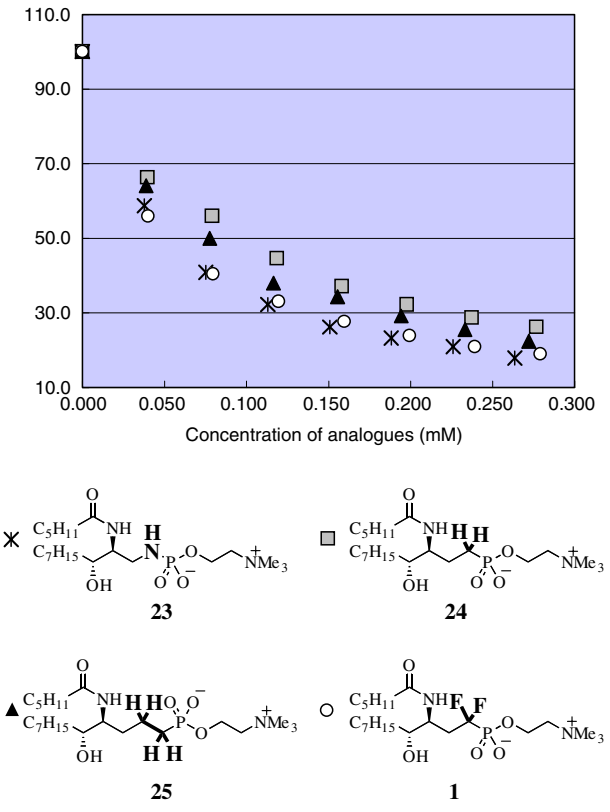


Figure 4. Inhibitory abilities of both **1** and the compounds previously synthesized.

synthesis, we found an extremely mild condition to hydrolyze the oxazolidinone ring by utilizing the strong electron withdrawing effect of a nosyl group. In addition, a choline group was introduced into the phosphonic acid moiety by convenient three-step procedures in excellent yield. Analogue **1** apparently inhibited the hydrolytic ability of *B. cereus* sphingomyelinase with nearly the same attitude to that of nitrogen analogue **24**.[2]

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13. Spectra data of **6**. $[\alpha]_D^{23.0}$ 25.9 (*c* 0.99, $CHCl_3$); IR (KBr disk) 3329, 2930, 2106, 1601, 1522, 1318, 1256, 1167 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (md, $J = 6.6$ Hz, 2H), 7.44 (m, 1H), 7.36 (mtd, $J = 6.8$, 7.6 Hz, 2H), 4.93 (br d, $J = 8.1$ Hz, 1H), 4.26 (dd, $J = 2.9$, 11.0 Hz, 1H), 3.90–4.02 (m, 2H), 3.84 (m, 1H), 1.58–1.69 (m, 2H), 1.40–1.52 (m, 2H), 1.44 (s, 9H), 1.23–1.40 (m, 8H), 0.89 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 134.9, 133.9, 130.9, 127.6, 90.0, 74.9, 62.7, 49.7, 35.4, 31.8, 29.4, 29.2, 28.3, 25.3, 22.6, 14.1.
14. Spectra data of **3**. $[\alpha]_D^{19.5}$ 17.43 (*c* 0.53, $CHCl_3$); IR (NaCl neat) 2930, 2859, 1755, 1514, 1273, 1250, 1177, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.22 (md, $J = 8.8$ Hz, 2H), 6.86 (md, $J = 8.8$ Hz, 2H), 4.72 (d, $J = 15.4$ Hz, 1H), 4.45 (m, 1H), 4.18–4.30 (m, 4H), 4.04 (d, $J = 15.4$ Hz, 1H), 3.97 (dt $J = 2.4$, 7.8 Hz, 1H), 3.80 (s, 3H), 2.24–2.56 (m, 2H), 1.53–1.65 (m, 3H), 1.38 (t, $J = 7.1$ Hz, 3H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.20–1.35 (m, 9H), 0.87 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 158.0, 129.5, 127.9, 120.0 (dt, J_{C-P}, $J_{C-F} = 215.9$, 260.5 Hz), 114.2, 77.5, 64.8, ($J_{C-P} = 6.6$ Hz), 55.2, 51.6 (m), 45.7, 31.7, 31.5 (dt, J_{C-P}, $J_{C-F} = 14.1$, 19.9 Hz), 29.9, 29.2, 29.0, 25.6, 22.6, 16.4 ($J_{C-P} = 5.0$ Hz), 14.0.
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19. When THF was used as a solvent, the desired **20** was produced in 43% yield, while in DMF only an unidentified product was produced.
20. Spectra data of **1** $[\alpha]_D^{26.0}$ 6.10 (*c* 0.32, MeOH); IR (KBr disk) 3395, 2926, 1649, 1561, 1092, 970 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 4.36–4.44 (m, 2H), 4.21 (ddd, $J = 1.7$, 5.4, 10.0 Hz, 1H), 3.61–3.65 (m, 2H), 3.49 (m, 1H), 3.22 (s, 9H), 2.49 (m, 1H), 2.22 (m, 1H), 2.16 (t, $J = 7.6$ Hz, 2H), 1.56–1.60 (m, 2H), 1.47–1.53 (m, 2H), 1.23–1.40 (m, 14H), 0.91 (t, $J = 6.8$ Hz, 3H), 0.90 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.5, 124.0 (dt, J_{C-P}, $J_{C-F} = 201.0$, 260.5 Hz), 75.0, 67.8 (m), 61.2 ($J_{C-P} = 5.8$ Hz), 54.74, 54.71, 54.68, 49.6 (m), 37.3, 34.5, 34.2 (dt, J_{C-P}, $J_{C-F} = 14.1$, 19.9 Hz), 33.0, 32.6, 30.7, 30.4, 27.0, 26.7, 23.7, 23.5, 14.4, 14.3.
21. We tested the ability of CF_2 analogue **1** to inhibit SMase from *B. cereus*. Enzyme activity was measured three times at 37 °C and ionic strength 0.2 with a buffer of 50 mM Tris–HCl buffer (pH 7.5) in the presence of 10 mM $MgCl_2$. The concentration of SMase and 2-hexadecanoylamino-4-nitrophenylphosphocholine used as the substrate were 1.0×10^{-9} M and 1.0 mM, respectively.