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Diastereoselective Synthesis of γ-Amino-δ-hydroxy-α,α-difluorophosphonates: A Vehicle for Structure−activity Relationship Studies on SMA-7, a Potent Sphingomyelinase Inhibitor

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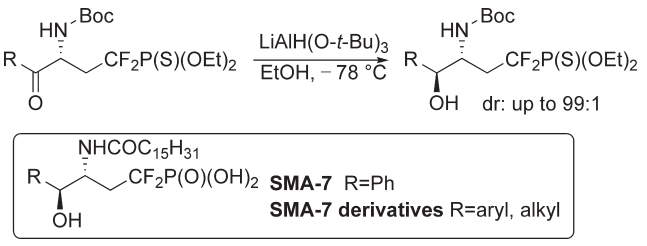

A highly diastereoselective synthesis of 2-amino alcohol derivatives bearing a difluoromethylphosphonothioate group at the 3-position was achieved through LiAlH(O-*t*-Bu)$_3$-mediated reduction of the corresponding α-amino ketones. The phosphonothioate moiety of the product was readily converted into the corresponding phosphonate by oxidation with *m*-CPBA, followed by aqueous workup. The developed methods should be useful for SAR studies of **SMA-7**, a potent inhibitor of SMases.

Sphingolipids are known as secondary messengers in mammalian cells and cell members.[1] It is now well accepted that sphingolipids play key roles in cellular signal transmission pathways. Ceramide, the primary sphingomyelin metabolite, is generated through the action of a lysosomal acid sphingomyelinase (A-SMase)[2] or a membrane-bound neutral sphingomyelinase (N-SMase), believed to be an essential signal transduction factor in cell differentiation and in programmed cell death derivation.[1] However, direct links between SMases and specific signaling systems have not been fully elucidated yet. Potent SMase inhibitors are believed to be useful probes to establish a clear picture of metabolic links.[3] In addition, the SMase inhibitor is expected to have some clinical value for the treatment of ceramide-mediated pathogenic states such as inflammation[4] and AIDS.[5]

During our studies directed toward the discovery of novel inhibitors for SMases, we carried out chemical modifications of sphingomyeline (SM) by replacement of the phosphocoline moiety with a metabolically stable difluoromethylenephosphonate (DFMP) group. In these modifications, we found that **SMA-7**, a short chain SM-analogue having a phenyl group at the terminal position, inhibits noncompetitively N-SMase in bovine brain microsomes with IC_{50} values of 3.3 μM (Figure 1).[6,7] Our biological studies revealed that **SMA-7** had the ability to suppress tumor necrosis factor (TNF) α-induced apoptosis of PC-12 neurons at a low concentration of 0.1 μM.[8]

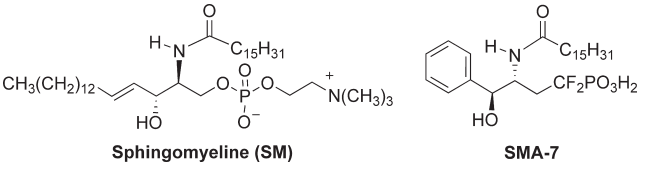

FIGURE 1. Structues of SM and SMA-7.

Brief structure activity relationship (SAR) studies have suggested that the stereochemistry of **SMA-7** may be a critical factor in biological activity and we found (*S*,*S*)-stereochemistry is a better inhibition motif than (*R*,*R*)-stereochemistry corresponding to natural SM regarding 2-amino alcohol moiety.[6b] However, there are many ambiguous issues remaining in SAR studies of **SMA-7**. In particular, SAR studies focusing on a phenyl group have remained to be solved, since the previous synthesis of **SMA-7** and its enantiomer relies on commencing from commercially available either (1*S*,2*S*)-2-amino-1-phenyl-1,3-propanediol or its enantiomer.[6b] To examine detailed

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(2) For a review of SMase, see: Montes, L. R.; Goni, F. M.; Alonso, A. *Sphingolipids Cell Funct.* **2006**, 53.

(3) For select examples on the preparation of SMase inhibitors, see: (a) Kornhuber, J.; Tripal, P.; Reichel, M.; Terfloth, L.; Bleich, S.; Wiltfanf, J.; Gulbins, E. *J. Med. Chem.* **2008**, *51*, 219. (b) Inoue, M.; Yokota, W.; Katoh, T. *Synthesis* **2007**, 622. (c) Wascholowski, V.; Giannis, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 827 and references cited therein.

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(7) Katsumura and co-workers independently reported that the DFMP analogue of sphingomyelin inhibits SMase competitively, see: Hakogi, T.; Yamamoto, T.; Fujii, S.; Ikeda, K.; Katsumura, S. *Tetrahedron Lett.* **2006**, *47*, 2627.

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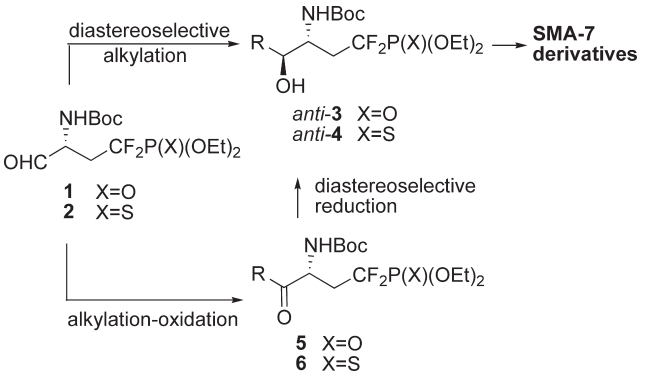
SCHEME 1. Strategies for Stereoselective Synthesis of SMA-7 Derivatives with High Diversity



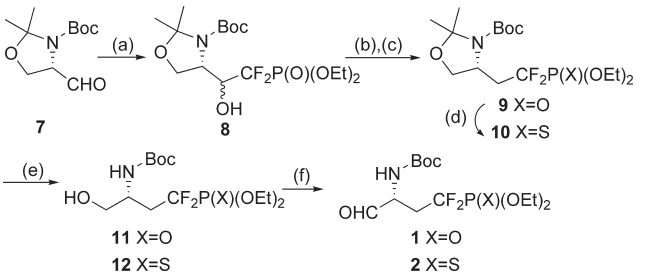
SAR studies on the phenyl moiety of **SMA-7**, a new methodology for incorporating a variety of functional groups into the terminal position with high diversity is required. In this context, we planned to examine two synthetic routes directed toward divergent synthesis of **SMA-7** derivatives from readily available α-amino aldehydes **1** and **2** bearing a DFMP or a difluoromethylphosphonothioate (DFMPT) group as shown in Scheme 1. In this paper, we wish to describe these experimental results.

Requisite α-amino aldehyde derivatives **1** and **2** were prepared through the method of Otaka[9a] and Berkowitz[9b] as shown in Scheme 2. Accordingly, (*S*)-Garner aldehyde **7** was treated with the lithium anion of diethyl difluoromethylphosphonate to give adduct **8**, which was subjected to Barton deoxygenation[10] furnishing **9** in a good overall yield. Compound **9** was transformed to the phosphonothioate analogue **10** through thioation, using Lawesson's reagent under conventional conditions.[11] The removal of isopropylydene groups of **9** and **10**, followed by Swern oxidation of the resulting alcohols **11**[12] and **12**, provided **1** and **2**, respectively. Both compounds were utilized for the next reactions without silica gel column chromatography to avoid racemization.

Having requisite aldehydes **1** and **2** in hand, first, the reactivity of **1** toward phenyl nucleophiles was examined (Scheme 3). When **1** was treated with 2 equiv of phenyl magnesium bromide in THF at 0 °C for 2 h, the alkylation reaction proceeded slowly to give **3a** in a low yield (11%) accompanied by a large amount of unidentified byproduct. The yield slightly increased to 26% upon increasing the amounts of phenyl magnesium bromide to 4 equiv. However, both reactions were found to be almost nonstereoselective. Similar results were obtained in a phenylation reaction with a phenyl lithium reagent in place of phenyl magnesium bromide. These poor results might be attributed to the significant decomposition of the DFMP moiety of **1** arising from its high coordination ability to the metallic reagents.[13]

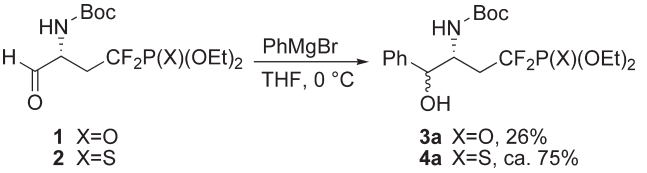
Next, we turn our attention to the phenylation of phosphonothioate analogue **2**, since our previous research revealed

SCHEME 2. Preparation of α-Amino Aldehyde Derivatives 1 and 2

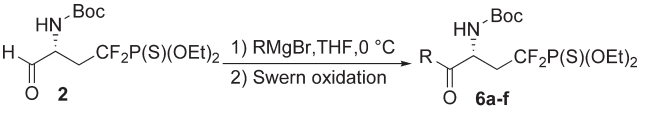


[a]Reagents and conditions: (a) LDA, $HCF_2PO_3Et_2$, THF, −78 °C, 93%; (b) *n*-BuLi, ClC(S)OPh, THF, −78 to 0 °C; (c) *n*-Bu_3SnH, AIBN, toluene, reflux, 80% for two steps; (d) Lawesson's reagent, toluene, reflux, 60%; (e) Amberlyst **15**, MeOH, rt, 72% for **11**, 70% for **12**; and (f) DMSO, $(COCl)_2$, Et_3N, −78 °C.

SCHEME 3. Comparison of Reactivity between 1 and 2 in Phenylation with PhMgBr



SCHEME 4. Preparation of α-Amino Ketones 6a−f from 2



6a: R= C_6H_5 (40%); **6b**: R= 4-ClC_6H_4 (43%); **6c**: R= 4-$MeOC_6H_4$ (31%); **6d**: R= Me (16%); **6e**: R= *c*-C_6H_{11} (19%); **6f**: R= CH_2=CH (21%)

that the stability of the DFMPT group was much better than that of the DFMP group under reduction conditions in the presence of metal hydride reagents.[13] Thus **2** was treated with phenyl magnesium bromide (6 equiv) in THF at 0 °C for 2 h (Scheme 3). While the diastereoselectivity of this phenylation reaction was quite low (1:1.3), the reaction gave **4a** in modest yield (ca. 75%)[14,15] accompanied by unidentified byproduct, amounts of which were apparently suppressed in comparison with the case of phenylation of phosphonate **1** as expected.

These results prompted us to examine the alkylation−oxidation−reduction sequence starting from aldehyde **2** through α-amino ketone **6** as an alternative method (Scheme 1).[16,17]

(9) (a) Otaka, A.; Miyoshi, K.; Burke, T. R., Jr.; Roller, P. P.; Kurobe, H.; Tamamura, H.; Fujii, N. *Tetrahedron Lett*. **1995**, *36*, 927. (b) Berkowitz, D. B.; Eggen, M.; Shen, Q.; Shoemarker, R. K. *J. Org. Chem*. **1996**, *61*, 4666.

(10) Martin, S. M.; Dean, D. W.; Wagman, A. S. *Tetrahedron Lett*. **1992**, *33*, 1839.

(11) (a) Ozturk, T.; Ertas, E.; Mert, O. *Chem. Rev*. **2007**, *107*, 5210. (b) Jesberger, M.; Davis, T. P.; Barner, L. *Synthesis* **2003**, 1929. (c) Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.

(12) Yokomatsu, T.; Sato, M.; Shibuya, S. *Tetrahedron: Asymmetry* **1996**, *7*, 2743.

(13) Murano, T.; Yuasa, Y.; Kobayakawa, H.; Yokomatsu, T.; Shibuya, S. *Tetrahedron* **2003**, *59*, 10223.

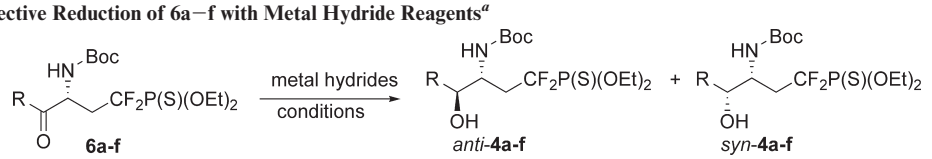
(14) Yields refers to those from crude aldehyde **2** and not optimized.

(15) An accurate yield was not caluculated because of some inseparable impurities included.

(16) For the preparation of amino ketones **5**, we have synthesized the Weinreb amide derived from alcohol **11** and examined the reaction with a large excess of vinyl magnesium bromide (THF/0 °C to rt). However, the Weinreb amide was found to be inert to the Grignad reagent under our experimental conditions. In a series of the phosphonothiate analogues, we failed to prepare the correponding carboxylate due to significant decomposition of **12** during oxidation. Therefore, we could not examine the reactivity of the Weinreb amide, derived from alcohol **12**, to the Grignard reagent.

(17) To obtain the required amino alcohol derivertives in a stereoselective manner, an alternative method may be available on the basis of sequential DIBAL-mediated reduction and alkylation with a Grignard reaction toward amino esters in one pot. (a) Polt, R.; Peterson, M. A.; DeYong, L. *J. Org. Chem*. **1992**, *57*, 5469. (b) Polt, R.; Peterson, M. A. *Tetraherdon Lett*. **1990**, *31*, 4985. However, in the present studies, we focused on the alkylation−oxidation−reduction sequence starting from aldehydes **1** and **2**.

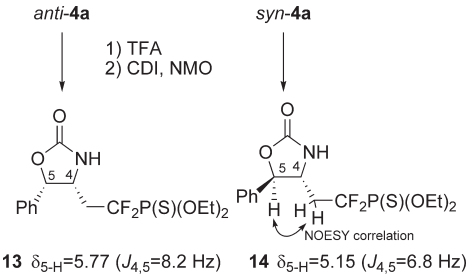
TABLE 1. Stereoselective Reduction of 6a−f with Metal Hydride Reagents[a]



entry	substrate	R	metal hydride (equiv)	time, min	solvent	*anti*:*syn*	yield of **4a**−**f**,[d] %
1	**6a**	C_6H_5	$NaBH_4$ (2)	30	EtOH	52:48[b]	53
2	**6a**	C_6H_5	DIBAL-H (2)	150	toluene	89:11[b]	44
3	**6a**	C_6H_5	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	30	EtOH	98:2[b]	99
4	**6a**	C_6H_5	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	180	THF	42:58[b]	53
5	**6a**	C_6H_5	L-Selectride (4)	60	THF	5:95[b]	71
6	**6b**	$4\text{-}ClC_6H_4$	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	20	EtOH	>99:1[c]	99
7	**6c**	$4\text{-}MeOC_6H_4$	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	60	EtOH	>99:1[c]	71
8	**6d**	CH_3	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	40	EtOH	>99:1[c]	87
9	**6e**	$c\text{-}C_6H_{11}$	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	40	EtOH	>99:1[c]	85
10	**6f**	$CH_2{=}CH$	$LiAlH(O\text{-}t\text{-}Bu)_3$ (8)	20	EtOH	>99:1[c]	97

[a]All reactions were carried out at −78 °C under a nitrogen atmosphere. [b]The ratios were determined by HPLC analysis (Inertsil PREP-SIL (GL Sciences Inc.), hexane-EtOAc). [c]The ratio was determined by ^{31}P NMR ($CDCl_3$, 121 MHz) analysis. [d]Combined yields for *anti*- and *syn*-isomers.

SCHEME 5. Determination of Stereochemistry of *anti*-4a and *syn*-4a



Thus, **2** was respectively treated with aryl magnesium bromides (C_6H_5MgBr, $4\text{-}ClC_6H_5MgBr$, $4\text{-}MeOC_6H_4MgBr$) and alkyl magnesium bromides (MeMgBr, $c\text{-}C_6H_{11}MgBr$, $CH_2{=}CH\text{-}MgBr$) in THF at 0 °C for 1 h, followed by Swern oxidation of the resulting adducts without purification, to give α-amino ketones **6a**−**f** in 43−16% yields[14,15] for two steps (Scheme 4).

Aiming at overcoming the diastereoselectivity problem, next, reductions of α-amino ketones **6a**−**f** were examined with use of representative metal hydride reagents[18] (Table 1). Although reduction of **6a** with $NaBH_4$ in EtOH was almost nondiastereoselective (entry 1), DIBAL-H-mediated reduction was found to proceed with somewhat moderate *anti*-selectivity (89:11) (entry 2). The *anti*-selectivity was significantly improved to 98:2 to provide *anti*-**4a** in a 99% yield, when $LiAlH(O\text{-}t\text{-}Bu)_3$ was employed in EtOH[18] (entry 3). Using EtOH as a solvent was critical for high selectivity, hence a similar reaction in THF resulted in poor diastereoselectivity (42:58) (entry 4). It should be noted that the reduction with L-Selectride in THF proceeded to give *syn*-**4a** in a ratio of 5:95 (entry 5). Reduction of **6b**−**f** with $LiAlH(O\text{-}t\text{-}Bu)_3$ showed excellent diastereoselectivity independently of substituent R (entries 6−10).

The product *anti*-**4a** was confirmed to be almost optically pure by ^{31}P NMR ($CDCl_3$, 162 MHz) analysis of the corresponding (*R*)- and (*S*)-MTPA esters, indicating no racemization proceeded in these sequences. The relative configurations of *anti*-**4a** and *syn*-**4a** were verified after transformation to the corresponding oxazolidinones **13** and **14** on the basis of the *vic*-coupling constants and the selected NOESY correlation as shown in Scheme 5.

High *anti*-selectivity observed in the reaction with $LiAlH(O\text{-}t\text{-}Bu)_3$ in EtOH may be explained by considering the formation of the intermediate **15**, wherein the carbamate nitrogen is binding to aluminum and the carbonyl oxygen is chelating to the aluminum (Figure 2).[18] A similar chelate model and the effectiveness of EtOH on the formation of the chelate intermadiate have been reported by Hoffman.[18]

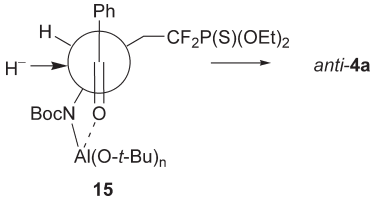


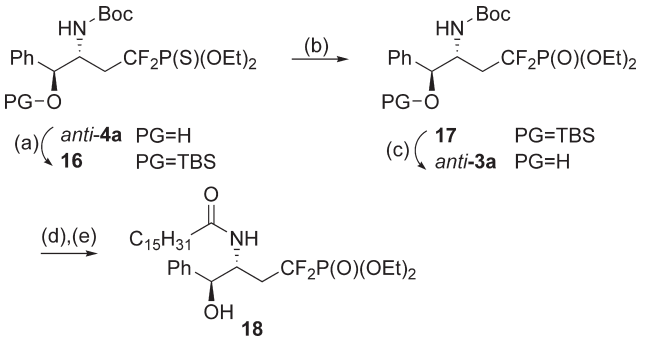
FIGURE 2. A possible transition state for the reduction of **6a**.

Having established the diastereoselective approach to *anti*-**4a**−**f**, preparation of a **SMA-7** precursor from *anti*-**4a** was next explored (Scheme 6). For this purpose, the DFMPT moiety of *anti*-**4a** must be converted into DFMP. A direct conversion of *anti*-**4a** into the corresponding phosphonate *anti*-**3a** was attempted with *m*-CPBA as an oxidant.[19] However, any desired product was not obtained because of the significant decomposition of the substrate. Therefore, *anti*-**4a** was protected with TBS on the hydroxy group and subsequently was treated with *m*-CPBA to provide DFMP derivative **17** in a 92% yield after aqueous workup. The TBS protecting group of **17** were removed by TBAF to afford phosphonate *anti*-**3a**, which was subjected to deprotection of the Boc group with TFA and subsequent palmitoylation of the resulting amine to give **18**. The product **18** has already been elucidated to be a useful precursor of **SMA-7**.[6b]

(18) (a) Hoffman, R. V.; Maslouh, N.; Cervantes-Lee, F. *J. Org. Chem.* **2002**, *67*, 1045. (b) Davis, F. A.; Gaspari, P. M.; Nolt, B. M.; Xu, P. *J. Org. Chem.* **2008**, *73*, 9619.

(19) Lopin, C.; Gautier, A.; Gouhier, G.; Piettre, S. R. *J. Am. Chem. Soc.* **2002**, *124*, 14668.

SCHEME 6. Conversion of *anti*-4a into a Precursor of SMA-7



[a]Reagents and conditions: (a) TBSCl, Et_3N, DMAP, DMF, 61%; (b) *m*-CPBA, CH_2Cl_2, then aq $NaHCO_3$, 92%; (c) TBAF, THF, 95%; (d) TFA, CH_2Cl_2; and (e) Et_3N, DMAP, $C_{15}H_{31}COCl$, CH_2Cl_2, 69% (two steps).

In conclusion, we have developed a new stereoselective method for the synthesis of 2-amino alcohols bearing a DFMPT group through LiAlH(O-*t*-Bu)$_3$-mediated reduction of the corresponding α-amino ketones. The DFMPT group of the product could be converted into the corresponding DFMP group. This method features high diversity in the introduction of the terminal substituent and high generality in stereocontrolling the stereogenic center, and should be useful for SAR studies of **SMA-7**, a potent inhibitor of SMases. The elucidation of SAR of **SMA-7** is ongoing.

Experimental Section

Typical Procedure for the Reduction of α-Amino Ketones: The Reaction of 6a with LiAlH(O-*t*-Bu)$_3$. To a stirred suspension of LiAlH(O-*t*-Bu)$_3$ (208.7 mg, 0.80 mmol, 97% purity) in EtOH (1.2 mL), under a nitrogen atmosphere, was added a solution of **6a** (44.6 mg, 0.1 mmol) in EtOH (1.2 mL) slowly at −78 °C. After being stirred for 20 min at the same temperature, the mixture was diluted with aqueous 10% citric acid and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. The removal of the solvent gave a residue, which was chromatographed on silica gel (hexane:EtOAc 4:1) to give *anti*-**4a** (44.5 mg, 99%). Colorless crystals; mp 90−91 °C; $[\alpha]^{25}_D$ + 0.49 (*c* 0.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.25 (5H, m), 5.02 (1H, br s), 5.00 (1H, br d, J = 5.9 Hz), 4.25−4.06 (5H, m), 2.33−2.16 (2H, m), 1.45 (9H, s), 1.25 (6H, dt, J = 4.6, 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 140.4−126.1 (aromatic carbons), 121.2 (dt, J_{CP} = 177.8 Hz, J_{CF} = 264.2 Hz), 80.1, 76.2, 64.52 (d, J_{CP} = 6.6 Hz), 64.37 (d, J_{CP} = 6.8 Hz), 51.8, 31.2−31.0 (m), 28.3 (3 carbons), 16.05 (d, J_{CP} = 2.9 Hz), 15.99 (d, J_{CP} = 3.0 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −45.01 (1F, dddd, J_{FH} = 8.9 Hz, J_{FH} = 25.7 Hz, J_{FP} = 111.4 Hz, J_{FF} = 284.9 Hz), −48.22 (1F, dddd, J_{FH} = 12.9 Hz, J_{FH} = 23.5 Hz, J_{FP} = 111.4 Hz, J_{FF} = 284.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 75.01 (t, J_{PF} = 111.4 Hz); IR (KBr) 3356, 1660, 1171, 1022 cm^{-1}; ESIMS *m*/*z* 454. Anal. Calcd for $C_{19}H_{30}NO_5F_2PS$: C, 50.32; H, 6.67; N, 3.09. Found: C, 50.16; H, 6.67; N, 2.99.

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Supporting Information Available: Experimental details and spectral data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.