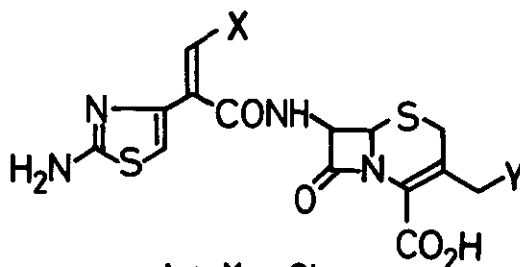


SYNTHESIS OF NOVEL FLUORINE-CONTAINING CEPHALOSPORINS

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Abstract - Cephalosporins with (E)- and (Z)-7 β -[2-(2-aminothiazol-4-yl)-4,4,4-trifluoro-2-butenamido] side chain were synthesized. Of these, the cephalosporin 12e having pyridiniomethyl group at the 3-position exhibited excellent activity against most of microorganisms tested.

As a part of our program aimed at the research and development of novel cephalosporin antibiotics, we have synthesized a new class of cephalosporins (formula A) possessing (Z)-2-(2-aminothiazol-4-yl)-3-chloro-2-propenamido group at the 7-position in the cephem nucleus.¹ They showed an excellent activity of broad spectrum against microorganisms, as well as good stability to various types of β -lactamases.² Substitution of the chlorine of these antibiotics with fluorine or trifluoromethyl will be much more interesting due to their high lipophilicity and also in view of their mimic effect to hydrogen analogues.³ This paper describes the synthesis of trifluoromethyl substituted cephalosporins (formula C) and their antibacterial activities, as well as the attempted synthesis of the fluorine analogue B.



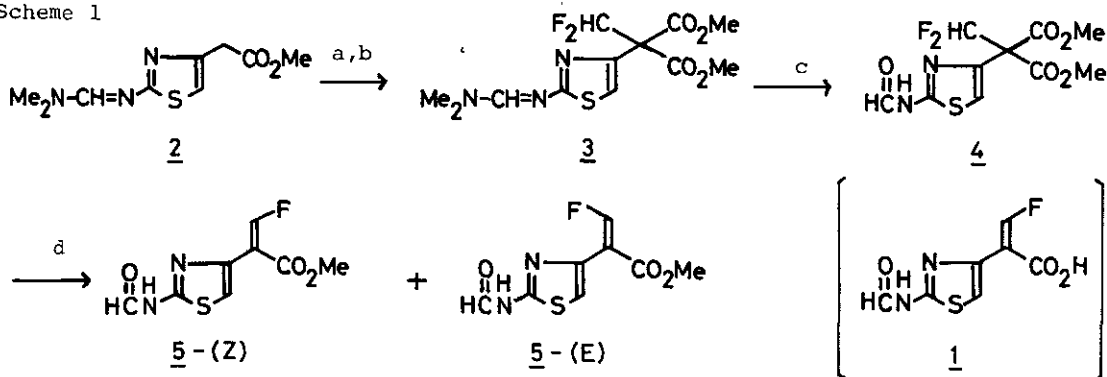
A : X = Cl

B : X = F

C : X = CF₃

At first, our efforts were focused on the synthesis of the fluorine containing side chain acid (1). (Scheme 1)

Scheme 1

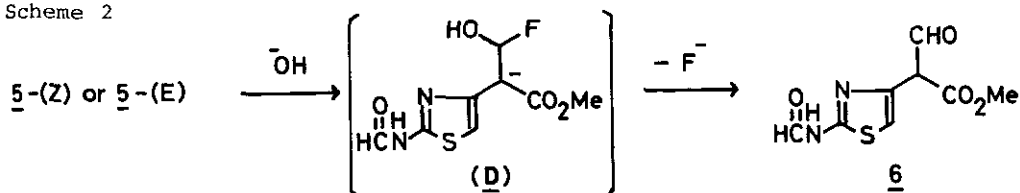


a) $\text{NaH}-(\text{MeO})_2\text{CO}$, DMF b) CHClF_2 c) $\text{HCO}_2\text{H}-\text{Ac}_2\text{O}$ d) $\text{LiI}-\text{DMF}$, 160°C

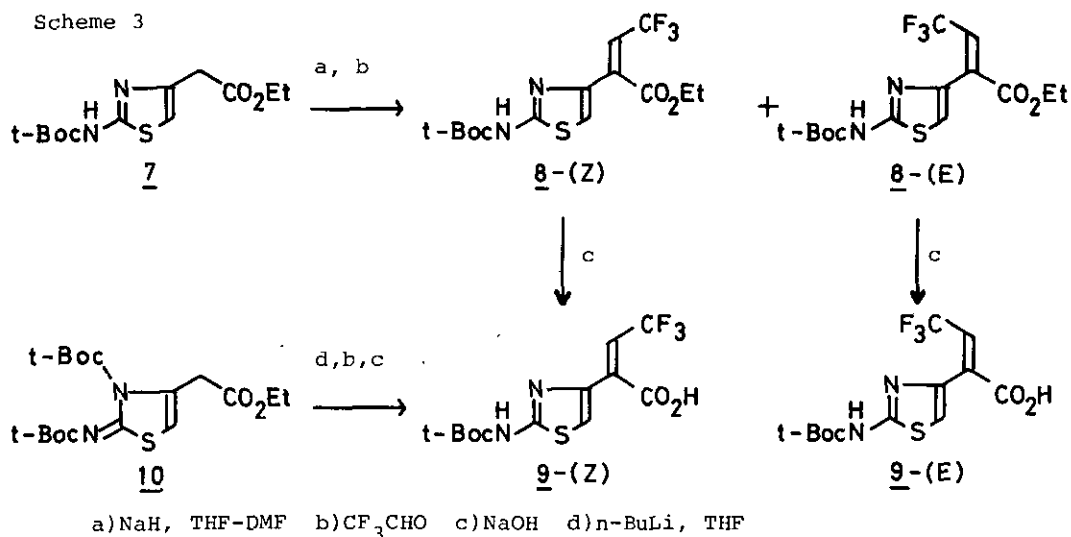
Treatment of methyl 2-[2-(N,N-dimethylaminomethylene)aminothiazol-4-yl]acetate (2)¹ with sodium hydride in N,N-dimethylformamide (DMF) in the presence of dimethyl carbonate, followed by addition of chlorodifluoromethane⁵ at 0°C , gave dimethyl malonate derivative 3⁴ in 46% yield. Thus both methoxycarbonylation⁶ and difluoromethylation⁷ were achieved in one pot reaction. After conversion of N,N-dimethylaminomethylene compound 3 into formyl derivative 4⁴ (88%), dealkoxycarbonylative elimination⁸ of HF from the resulting 4 with lithium iodide in DMF⁹ gave 3-fluoro-2-propenoates 5-(Z)¹⁰ and 5-(E)¹¹ in 14 and 52% yields, respectively. Their configurations were assigned on the basis of ¹H-nmr spectra and ¹³C - H coupling constants.¹²

Attempted alkaline hydrolysis of ester 5-(Z) under various reaction conditions gave methyl 3-oxo-2-(2-formylaminothiazol-4-yl)propanoate (6)¹ and no trace of 1. Similar treatment of 5-(E) resulted to give the same product 6. These results were in contrast with the behavior of chloromethylene derivatives which underwent normal alkaline hydrolysis to afford the corresponding (E)- and (Z)-carboxylic acids.¹ These phenomena¹³ seem to be ascribable to the strong inductive effect¹⁴ of fluorine atom which stabilizes the β -anion in the intermediate (D). (Scheme 2)

Scheme 2



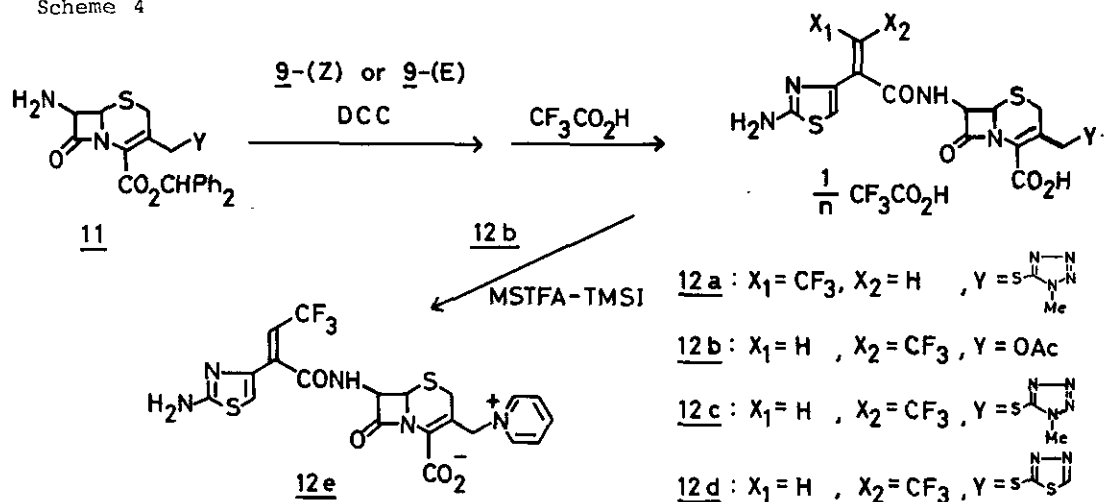
Accordingly, we have designed the synthesis of carboxylic acids 9 having trifluoromethyl group which is expected to improve the biological activity¹⁵ and does not behave as a leaving group.



Aldol condensation of 7 with trifluoroacetaldehyde¹⁶ in the presence of sodium hydride, followed by chromatographic separation, gave 8-(Z)⁴ and 8-(E)⁴ in 10 and 15% yields, respectively. Hydrolysis of 8-(E) proceeded smoothly at room temperature to afford 9-(E)¹⁷ in 96% yield. However, 8-(Z) was intact under the above hydrolysis conditions and necessitated heating at 50°C to give 9-(Z)¹⁸ in 90% yield. Alternatively, we used ethyl 2-(2-t-butoxycarbonylimino-3-t-butoxycarbonyl-4-thiazolin-4-yl)acetate (10)¹⁹ as the starting material. After similar aldol condensation of 10 with trifluoroacetaldehyde, the crude residue was treated with aqueous NaOH solution at room temperature to hydrolyze ester 8-(E), and the remaining ester 8-(Z) was then hydrolyzed by heating at 60°C to afford 9-(Z) in 45% overall yield. (Scheme 3)

Finally, we synthesized the novel cephalosporins 12(a-d)²⁰ by the coupling reaction of 9 with 7β-aminoceph-3-em-4-carboxylates 11 possessing typical substituents at the 3-position, followed by subsequent removal of the t-butoxycarbonyl and benzhydryl groups. In addition, nucleophilic substitution of the acetoxy group in 12b with pyridine was performed by using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) - trimethylsilyl iodide (TMSI) method²¹ to afford the pyridinium compound 12e²² in 11% yield.²³ (Scheme 4)

Scheme 4



In the Table, the minimum inhibitory concentrations (MIC) of the cephalosporins 12(a-e) against several microorganisms are summarized and compared with those of cefotaxime (CTX).

Table : Antibacterial activity (MIC $\mu\text{g/ml}$) of fluorine-containing cephalosporins

Comp. No.	<i>S. aureus</i> FDA 209P JC-1	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> NIHJ JC-2	<i>S. typhi</i> 901	<i>K. pneumoniae</i> PCI 602	<i>P. vulgaris</i> OX 19	<i>P. aeruginosa</i> IFO 3445
<u>12a</u>	3.125	3.125	25	12.5	0.20	0.20	>100
<u>12b</u>	1.56	0.78	0.39	0.78	≤ 0.025	≤ 0.025	>100
<u>12c</u>	1.56	1.56	0.78	0.78	≤ 0.025	≤ 0.025	>100
<u>12d</u>	0.78	0.78	0.78	0.78	≤ 0.025	≤ 0.025	>100
<u>12e</u>	0.39	0.39	0.39	0.39	≤ 0.025	≤ 0.025	12.5
CTX	3.125	0.39	0.10	1.56	≤ 0.025	≤ 0.025	25

The cephalosporins 12(a-e) showed higher antibacterial activity against *S. aureus* than CTX. The (Z)-isomer 12c exhibited higher activity than the corresponding (E)-isomer 12a against gram-positive and gram-negative bacteria. A similar tendency was observed with chloromethylene¹ and methoxyimino cephem derivatives.²⁴ The activity of the pyridinium compound 12e was significantly higher than those of the acetoxymethyl derivative 12b, the other heteroaromatic thiomethyl derivatives 12c, d, and CTX against most of the microorganisms tested, especially against *S. aureus*.

As a result of our investigations, we have succeeded in synthesizing novel fluorine-containing cephalosporins (C) and found that the trifluoromethyl substituted methylene moiety is associated with the potent antibacterial activities.

ACKNOWLEDGMENTS

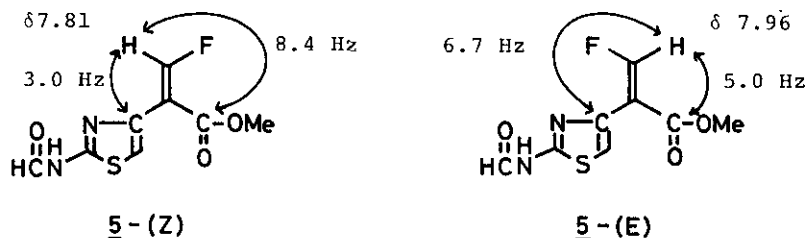
The authors wish to thank M. Takanashi, A. Ohno and their coworkers, Tobishi Pharmaceutical Co., Ltd., for the antimicrobial tests, T. Toshioka and O. Sakuma, the same company, for ^{13}C -nmr data, and T. Inokuma, research student from Kitasato University, for technical assistance. They also express their gratitude to Central Glass Co., Ltd. for providing trifluoroacetaldehyde ethyl hemiacetal.

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2. D. Tunemoto, T. Kobori, K. Nishide, K. Kondo, T. Toshioka, M. Takanashi, A. Ohno, and S. Goto, 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, La, 1986, Abstract, 1302.
3. I. Kumadaki, J. Synth. Org. Chem. Jpn., 1984, 42, 786 ; 'Biomedical Aspects of Fluorine Chemistry', ed. by R. Filler and Y. Kobayashi, Kodansha Ltd., Tokyo, 1982.
4. For these compounds correct elemental analyses and spectroscopic data are in accordance with the structures suggested.
5. P. Bey, J. Fozard, J. M. Lacoste, I. A. McDonald, M. Zreika, and M. G. Palfreyman, J. Med. Chem., 1984, 27, 9 and references cited therein.
6. Ethoxycarbonylation of ethyl 2-[2-(N,N-dimethylaminomethylene)aminothiazol-4-yl]acetate with $\text{NaH}-(\text{EtO})_2\text{CO}$ in DMF gave diethyl [2-(N,N-dimethylaminomethylene)aminothiazol-4-yl]malonate in 77% yield. On the other hand, ethyl 2-[2-(t-butoxycarbonyl)aminothiazol-4-yl]acetate gave the corresponding ethoxycarbonylated product in 11% yield under the same reaction conditions. Thus, the protection of two protons of amino group in alkyl 2-(2-aminothiazol-4-yl)acetate is desirable to this alkoxycarbonylation.

7. Difluoromethylation of diethyl [2-(N,N-dimethylaminomethylene)aminothiazol-4-yl]malonate described above with NaH-CHClF₂ in THF-DMF (1:2) at room temperature gave diethyl difluoromethyl[2-(N,N-dimethylaminomethylene)aminothiazol-4-yl]malonate in 50% yield.
8. The reactions of 4 with KOH-MeOH, t-BuOK-DMSO, or Me₃SiI gave unsuccessful results.
9. A. P. Krapcho, *Synthesis*, 1982, 805, 893 and references cited therein.
10. 5-(Z) : mp 159-160°C (AcOEt) . IR (KBr); ν 3300, 1695(sh), 1690, 1550, 1260 cm⁻¹. ¹H-NMR(DMSO-d₆); δ 3.83(s,3H), 7.33(s,1H), 7.81(d, J_{F-H} = 80 Hz), 8.52(s,1H), 12.41(br s,1H,NH). ¹⁹F-NMR(DMSO-d₆:CFCl₃ standard); 115.5 ppm(d, J_{F-H} = 80 Hz). ¹³C-NMR (DMSO-d₆); 52.3, 112.0, 114.6, 140.1, 155.1, 156.4, 159.8, 163.7 ppm. MS m/z (rel intensity); 230(M⁺,51), 203(10), 202(100), 199(11), 171(15), 170(46), 151(19), 150(62), 115(12), 101(20), 97(15), 59(14), 57(56), 45(24), 43(11), 15(28). Anal. Calcd for C₈H₇FN₂O₃S: C;41.74, H;3.06, N;12.17. Found: C;42.03, H;3.00, N;11.94.
11. 5-(E) : mp 143-145°C (AcOEt) . IR (KBr); ν 3350, 1720, 1700, 1560, 1315 cm⁻¹. ¹H-NMR (DMSO-d₆) ; δ 3.75(s,3H), 7.41(s,1H), 7.96(d, J_{F-H} = 78 Hz,1H), 8.51(s,1H), 12.44(br s,1H,NH). ¹⁹F-NMR (DMSO-d₆ : CFCl₃ standard); 110.0 ppm(d, J_{F-H} = 78 Hz). ¹³C-NMR(DMSO-d₆); 52.2, 114.2, 115.0, 138.3, 155.5, 158.8, 159.6, 165.1 ppm. MS m/z (rel intensity); 230(M⁺,29), 203(10), 202(100), 170(32), 151(17), 150(56), 101(16), 97(15), 69(11), 59(14), 57(52), 45(26), 43(11). Anal. Calcd for C₈H₇FN₂O₃S: C;41.74, H;3.06, N;12.17. Found: C;41.77, H;3.00, N;12.18.

12. The chemical shifts of vinylic protons and ¹³C-H coupling constants are as follows:



13. There is a precedent for this type of reaction: S. Kogure, H. Nakai, and M. Kurono, *Prostaglandins*, 1979, 18, 737.
14. R. D. Chambers, 'Fluorine in Organic Chemistry', John Wiley & Sons, New York, 1973, p.64.

15. For recent papers of synthetic pyrethroid analogs having trifluoromethyl group in place of chlorine atom see: M. Fujita and T. Hiyama, Tetrahedron Lett., 1986, 27, 3655 and references cited therein.
16. D. G. Hooper, L. F. Loucks, and M. T. H. Liu, J. Chem. Educ., 1975, 52, 131.
17. 9-(E): mp 182°C(dec). $^1\text{H-NMR}$ (acetone- d_6); δ 1.51(s,9H), 5.2-6.4(br s, NH , CO_2H , 2H), 6.84(br q, $J_{\text{F-H}} = 8.5$ Hz, 1H), 7.24(br s, 1H). $^{19}\text{F-NMR}$ (acetone- d_6); 57.1 ppm(br d, $J_{\text{F-H}} = 8.5$ Hz). MS m/z (rel intensity); 282(1), 238(10), 194(18), 151(10), 125(20), 59(16), 57(39), 56(53), 55(14), 44(100), 41(85), 39(33). Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{F}_3\text{N}_2\text{O}_4\text{S}$: C;42.60, H;3.87, N;8.28. Found: C;42.36, H;3.74, N;8.00.
18. 9-(Z): $^1\text{H-NMR}$ (acetone- d_6); δ 1.55(s,9H), 5.9-7.1(br s, NH , CO_2H , 2H), 6.52 (br q, $J_{\text{F-H}} = 8.5$ Hz, 1H), 7.31(br s, 1H). MS m/z (rel intensity); 338(M^+ , 5), 282(6), 238(31), 220(10), 194(17), 151(34), 125(31), 82(9), 69(9), 59(29), 58(16), 57(100), 56(14), 45(25), 44(21), 43(12), 41(67), 39(24).
19. Bayer A.-G., Japan Kokai Tokkyo Koho, JP 58-92672(Chem. Abstr., 1983, 99, 122170v).
20. 12a: Yield 25%. IR (KBr); ν 1785 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6); δ 3.6-3.9(AB, 2H), 4.69(s, 3H), 4.27(AB d, $J = 13.4$ Hz, 1H), 4.42(AB d, $J = 13.4$ Hz, 1H), 5.16(d, $J = 4.5$ Hz, 1H), 5.75(dd, $J = 8.0$ and 4.5 Hz, 1H), 6.32(q, $J_{\text{F-H}} = 8.5$ Hz, 1H), 6.81(s, 1H), 7.2(br s, 2H, NH_2), 9.50(br d, $J = 8.0$ Hz, 1H, NH). $^{19}\text{F-NMR}$ (DMSO- d_6 : CFCl_3 standard) 55.5 ppm(d, $J_{\text{F-H}} = 8.5$ Hz, CF_3), 73.5 ppm(s, $\text{CF}_3\text{CO}_2\text{H}$) (3:1). $n = 3$.
- 12b: Yield 54%. IR (KBr); ν 1780, 1740 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6); δ 2.03(s, 3H), 3.3-3.8(AB, 2H), 4.70(AB d, $J = 12.8$ Hz, 1H), 5.00(AB d, $J = 12.8$ Hz, 1H), 5.18(d, $J = 4.8$ Hz, 1H), 5.81(dd, $J = 7.9$ and 4.8 Hz, 1H), 6.0-8.0(br s, 3H, NH_2 , CO_2H), 6.29(q, $J_{\text{F-H}} = 9.0$ Hz, 1H), 6.71(s, 1H), 9.69(d, $J = 7.9$ Hz, 1H, NH). $^{19}\text{F-NMR}$ (DMSO- d_6 : CFCl_3 standard); 57.5 ppm (d, $J_{\text{F-H}} = 9.0$ Hz, CF_3) and 73.9 ppm (s, $\text{CF}_3\text{CO}_2\text{H}$) (3:1). $n = 3$.
- 12c: Yield 31%. IR (KBr); ν 1785 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6); δ 3.55-3.90(AB, 2H), 3.95(s, 3H), 4.26(AB d, $J = 13.5$ Hz, 1H), 4.40(AB d, $J = 13.5$ Hz, 1H), 5.19(d, $J = 5.0$ Hz, 1H), 5.83(dd, $J = 8.0$ and 5.0 Hz, 1H), 6.32(q, $J_{\text{F-H}} = 9.0$ Hz, 1H), 6.74(s, 1H), 7.28(br s, 2H, NH_2), 9.76(br d, $J = 8.0$ Hz, 1H, NH). $^{19}\text{F-NMR}$ (DMSO- d_6 : CFCl_3 standard); 57.3 ppm (d, $J_{\text{F-H}} = 9.0$ Hz, CF_3) and 73.6 ppm(s, $\text{CF}_3\text{CO}_2\text{H}$) (5:1). $n = 5$.

12d : Yield 26%. IR (KBr); ν 1780 cm^{-1} . $^1\text{H-NMR}$ (DMSO-d_6); δ 3.63(AB d, $J = 18.0$ Hz, 1H), 3.78(AB d, $J = 18.0$ Hz, 1H), 4.31(AB d, $J = 13.1$ Hz, 1H), 4.61(AB d, $J = 13.1$ Hz, 1H), 5.20(d, $J = 4.5$ Hz, 1H), 5.82(dd, $J = 8.0$ and 4.5 Hz, 1H), 6.52(q, $J_{\text{F-H}} = 9.0$ Hz, 1H), 6.74(s, 1H), 7.26(br s, 2H, NH_2), 9.58(s, 1H), 9.72(br d, $J = 8.0$ Hz, 1H). $^{19}\text{F-NMR}$ (DMSO-d_6 : CFCl_3 standard); 57.6 ppm (d, $J_{\text{F-H}} = 9.0$ Hz, CF_3) and 73.3 ppm (s, $\text{CF}_3\text{CO}_2\text{H}$) (6:1). $n = 6$.

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22. 12e : IR (KBr); ν 1780 cm^{-1} . $^1\text{H-NMR}$ (DMSO-d_6); δ 3.10(AB d, $J = 17.8$ Hz, 1H), 3.53(AB d, $J = 17.8$ Hz, 1H), 5.10(d, $J = 5.0$ Hz, 1H), 5.13(AB d, $J = 12.8$ Hz, 1H), 5.68(AB d, $J = 12.8$ Hz, 1H), 5.69(dd, $J = 9.0$ and 5.0 Hz, 1H), 6.24(q, $J_{\text{F-H}} = 9.0$ Hz, 1H), 6.60(s, 1H), 7.20(br s, 2H, NH_2), 8.15(br t, $J = 6$ Hz, 2H), 8.59(br t, $J = 6$ Hz, 1H), 9.46(br d, $J = 6$ Hz, 2H), 9.60(d, $J = 9.0$ Hz, 1H, NH). $^{19}\text{F-NMR}$ (DMSO-d_6 : CFCl_3 standard); 57.5 ppm (d, $J_{\text{F-H}} = 9.0$ Hz, CF_3).

23. The crude 12e was subjected to Diaion HP-20 chromatography.

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