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DESIGN AND SYNTHESIS OF PYRROLO[2,1-*c*][1,4]BENZODIAZEPINE (PBD)- POLYAMINOALKYL CONJUGATES BY THE USE OF S_NAr REACTION OF 2-NITRO-5-FLUOROBENZOATE PRECURSOR AS KEY REACTION†

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Abstract–The design and synthesis of a series of pyrrolo[2,1-*c*][l,4]benzodiazepine (PBD)- polyaminoalkyl conjugates as DNA minor groove binders are described. To introduce polyaminoalkyl groups to the pyrrolo[2,1-*c*][1,4]benzodiazepine pharmacophore, S_NAr reactions between 2-nitro-5-fluorophenyl ketone or 2-nitro-3-pehenyl ketone and polyaminoalkyl side chains were developed.

INTRODUCTION

By the completion of the human genome project, it is hoped that many diseases including cancer, hereditary and viral diseases can be understood at the DNA sequence level. Control of specific gene expression may provide ultimate gene therapy.[1] DNA alkylators have potential to provide such gene targeting drugs.[2] In order to alter the DNA-recognition abilities of natural occurring DNA alkylating anitibiotics, various strategies have been proposed for the synthesis of these antibiotics and their

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Dedicated to Professor Leo A. Paquette on the occasion of his 70th birthday

derivatives.[3] One of the successful routes was reported by us based on the concept of cyclopropylpyrroloindole (CPI)-(pyrrole-imidazole polyamide) conjugates; we could obtain optimized CPI-conjugates which bind with predetermined DNA sequences and exhibit 10,000-100,000 times higher potency than CC-1065 (natural CPI compound) against KB human cancer cells.[4] Nearly 40 years have passed since the first pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumor antibiotic, anthramycin, was discovered by Leimgruber.[5] PBDs recognize preferred DNA sequences such as 5'-PyGPu and bind to N_2 of guanine in the minor groove of double helical DNA *via* an acid-labile aminal bond to the electrophilic imine at the N10-C11 position.[6,7] Therefore, PBDs have potential as gene regulators with possible therapeutic application in the treatment of genetic disorders, including some cancers; as selective anti-infective agents; and as probes for use in molecular biology. The N10-C11 carbinolamine form (**A**), may exist in the equivalent imine (**C**), or carbinolamine methyl ether form (**B**), depending on the precise structure of the compound and the method of isolation (Figure 1).[8-10]

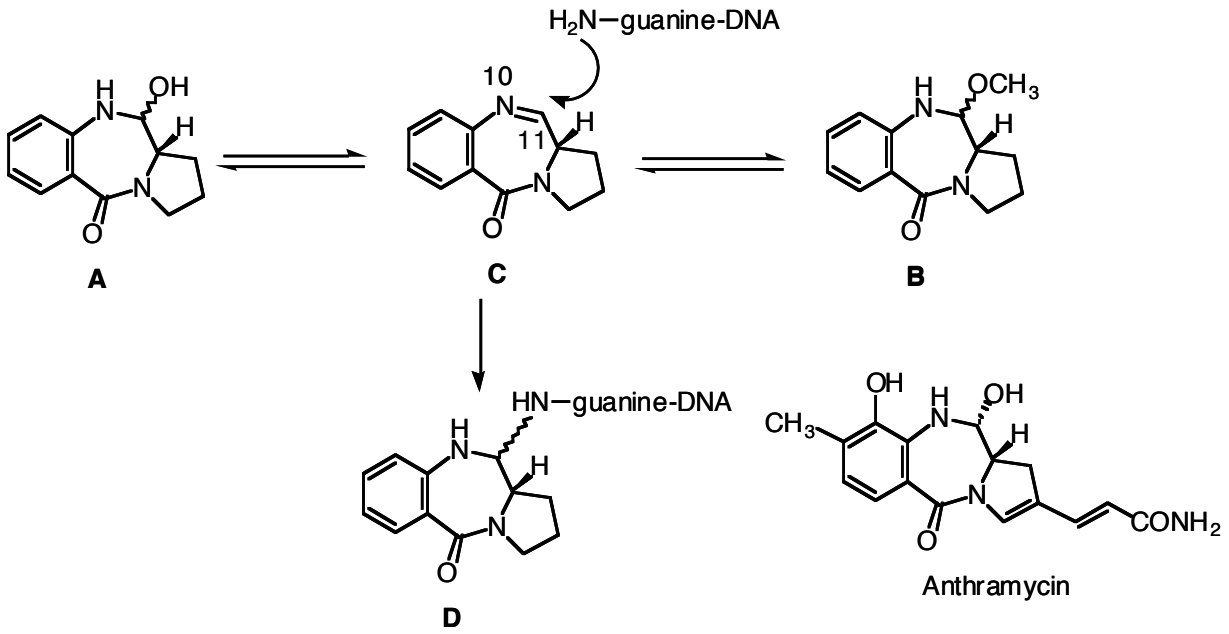


Figure 1. Equilibrium of N10-C11 carbinolamine form (**A**), imine (**C**), and carbinolamine methyl ether (**B**) and possible mechanism of the formation of PBD-DNA adduct. The strucure of anthramycin is also shown in the figure.

In the last few years, various strategies have been proposed for the synthesis of PBDs and have met with varying degrees of success while exhibiting significant limitations.[11-15] In order to alter the DNA-recognition ability and selectivity of PBD, it was considered desirable to introduce, *inter alia*, polyaminoalkyl groups as side-chain to the positions 7 and 9 of the A ring of PBD which is known to interact with DNA reversibly (Figure 2).[16,17] Our retrosynthesis for this purpose is shown as Scheme 1. In

this connection, we described below S_NAr (nucleophilic aromatic substitution) reaction of fluoroaromatics (**3**).

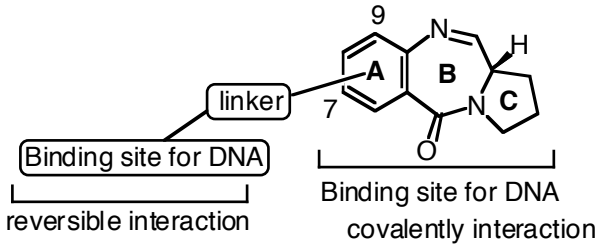
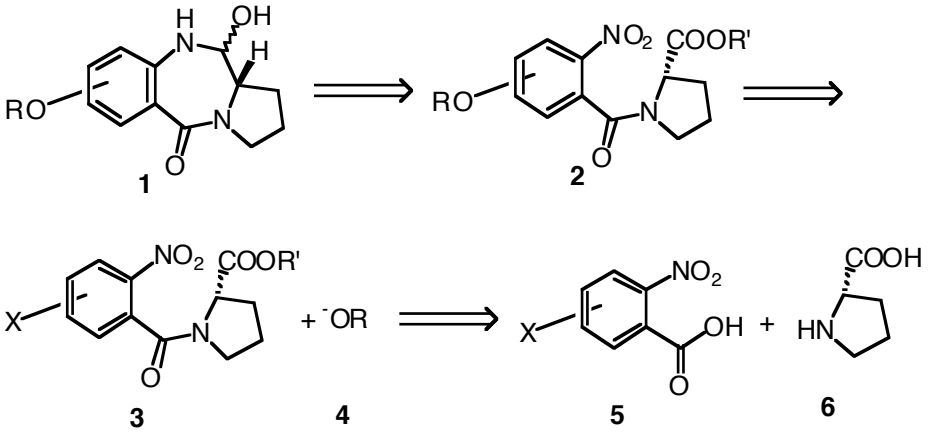


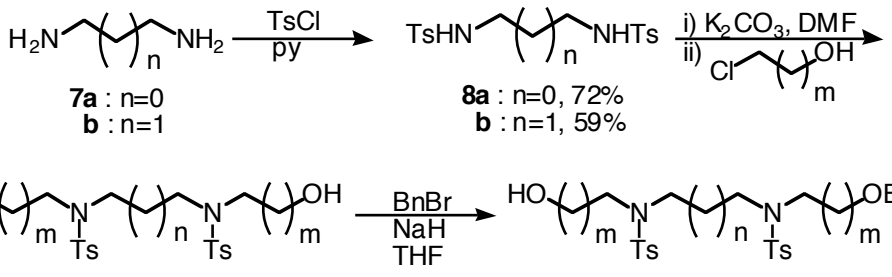
Figure 2



Scheme 1 Retrosynthetic analysis

RESULTS AND DISCUSSION

The straight-chain polyaminoalkanes were chosen for their ready availability as side-chains interacting with DNA electrostatically. The amino part of 1,2-diaminoethane or 1,3-diaminopropane (**7**) was *p*-toluenesulfonylated, metalated, and treated with 2-chloroethanol or 3-chloropropanol, and the diols (**9**) were obtained in moderate to good yields. Then diols (**9**) were selectively monobenzylated with benzyl bromide in THF in the presence of sodium hydride, producing the corresponding monobenzyl alcohols (**10**) (Scheme 2).



7a : n=0
b : n=1

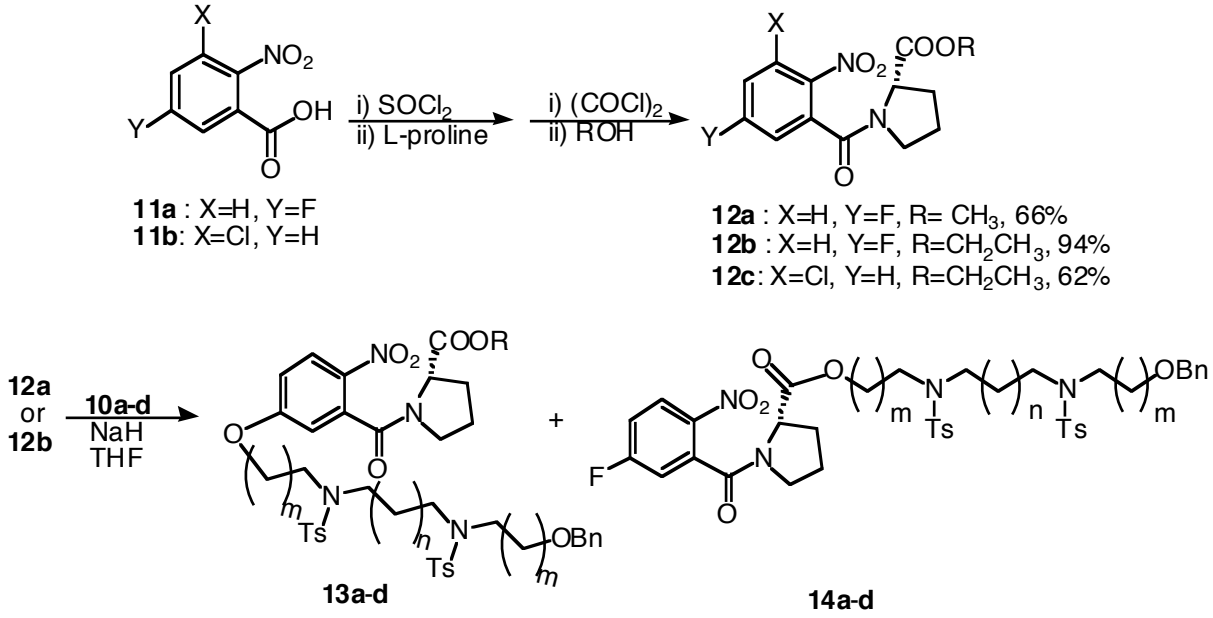
8a : n=0, 72%
b : n=1, 59%

9a : n=0, m=1, 74%
b : n=0, m=2, 59%
c : n=1, m=1, 84%
d : n=1, m=2, 66%

10a : n=0, m=1, 73%
b : n=0, m=2, 69%
c : n=1, m=1, 56%
d : n=1, m=2, 50%

Scheme 2

The intermediates, in which the proline moiety is already present, were prepared as outlined in Scheme 3. The nitrobenzoic acid (**11**) was converted to the corresponding acid chloride by treating with thionyl chloride and then, coupled with L-proline. The carboxylic acid moiety of proline was then converted into the acid chloride by treating with oxalyl chloride. Treatment of the acid chloride with methanol or ethanol produced the corresponding amide esters (**12a**-**c**).



Scheme 3

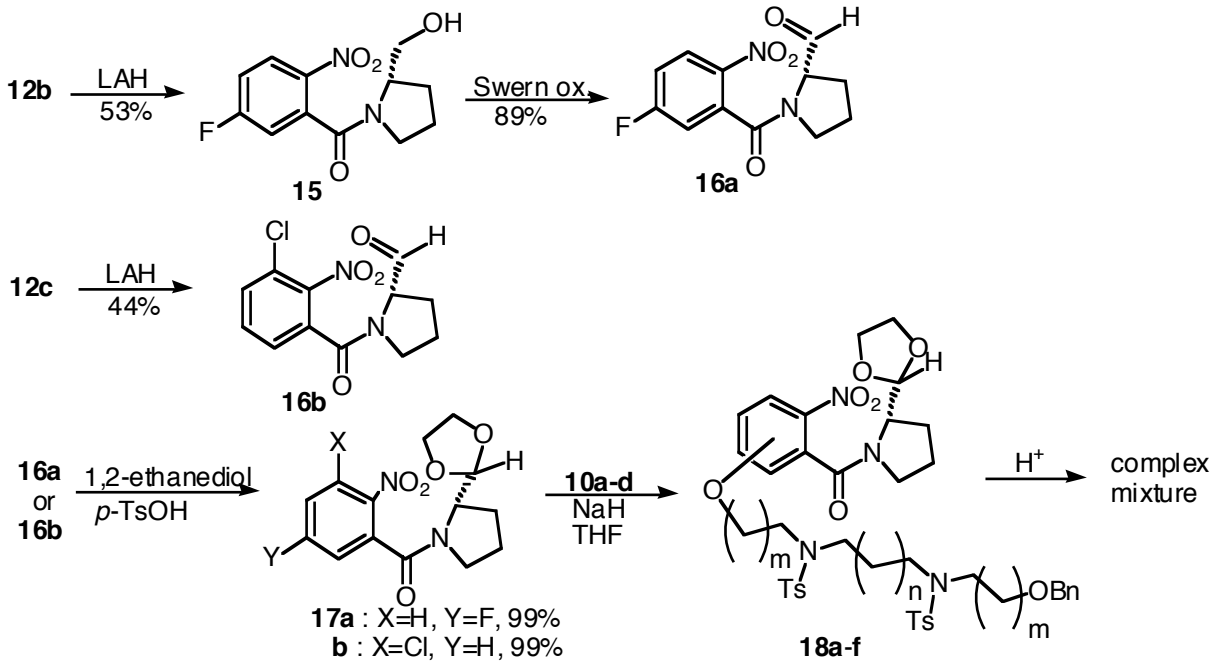
The results of S_NAr reactions of **12a** and **12b** are summarized in Table 1. The reaction did not take place under normal conditions and only the starting materials were recovered. However, in the presence of catalytic amount of 18-crown-6, the S_NAr reaction of **12a**, **b** occurred easily with alocohol (**10a**-**c**) giving the compounds (**13aa**, **13ab**, **13bb** and **13cb**) respectively. In the cases of **10b** and **10d** in which m=2, however, the anticipated products (**13ba** and **13db**) were not obtained, instead reaction afforded the transesterification products (**14ba** and **14db**), respectively.

Table 1. S_NAr and transesterification reaction of **12**

alcohol	benzoate	18-crown-6	**13**	**14**
10a	**12a**	---	no reaction	
a	**b**	---	no reaction	
a	**a**	cat.	67%	0%
a	**b**	cat.	46%	trace
b	**a**	cat.	trace	22%
b	**b**	cat.	20%	0%
c	**b**	cat.	44%	trace
d	**b**	cat.	complex mixture	

In order to prevent such transesterification, the ester portion was protected before S_NAr reaction *e.g.* the nitro ester (**12b**) was reduced to the alcohol (**15**) with LAH at low temperature, followed by Swern

oxidation and subsequent protection producing the aldehyde compound (**16a**). In contrast, the nitro ester (**12c**) was reduced with LAH at low temperature to yield aldehyde compound (**16b**). Subsequent protection using ethanediol produced nitro compounds (**17a**, **b**), respectively. The yields of the S_NAr reaction of **17** with **10** were good to poor to afford acetals (**18a**-**f**) as shown in Scheme 4 and Table 2. Unfortunately, attempts to perform reductive cyclization of the corresponding acetals led to formation of a complex mixture.



Scheme 4

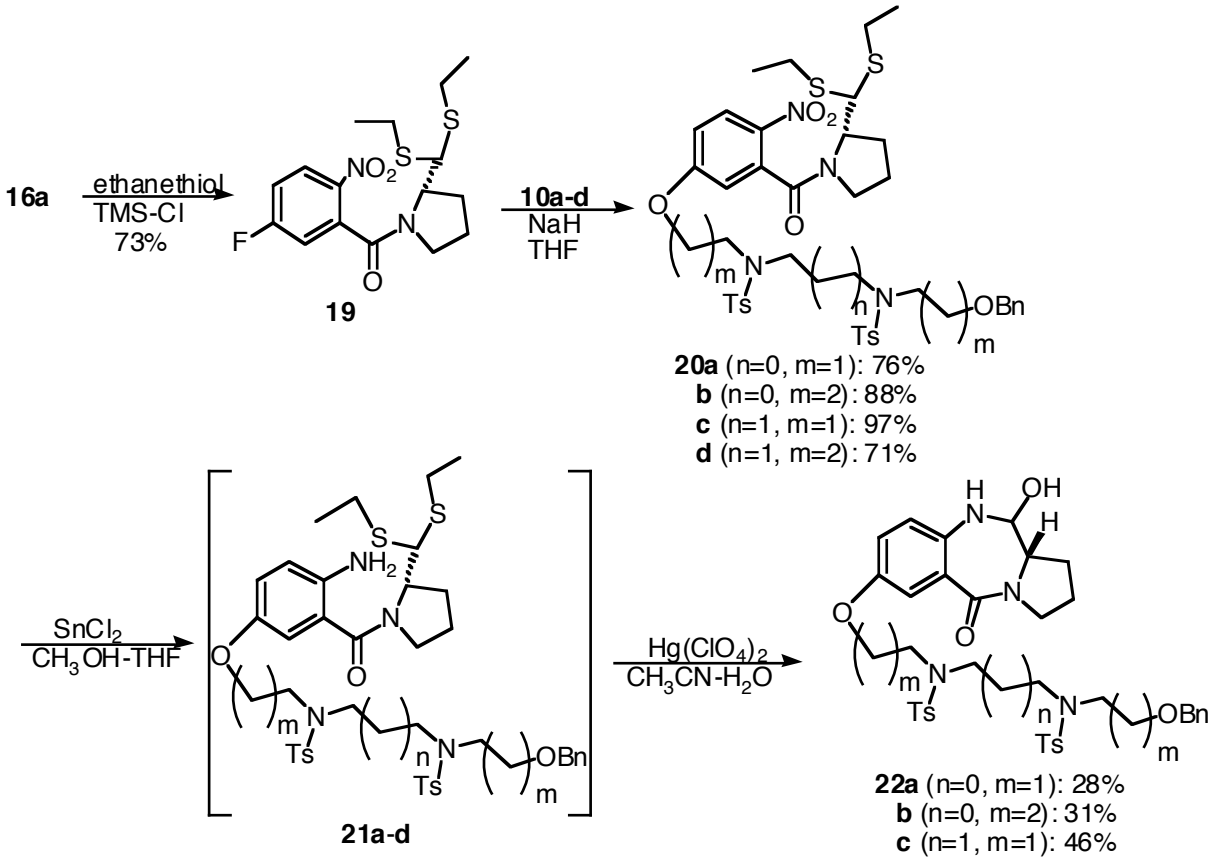
Table 2. S_NAr reaction of **17a** and **17b**

alcohol	benzoate	yield (%)	product
10a	**17a**	76	**18a**
b	**a**	63	**b**
c	**a**	89	**c**
d	**a**	84	**d**
a	**b**	46	**e**
b	**b**	23	**f**

Therefore, aldehyde (**16a**) was protected by the use of ethanethiol to yield the corresponding dithioacetal compound (**19**). The yields of the S_NAr reactions of **19** with **10** were good to moderate to afford nitro compounds (**20a**-**d**) as shown in Scheme 5. The completion of the PBD pharmacophore was achieved on reduction of **20a**-**d** with stannous dichloride to the aniline intermediates (**21a**-**d**) which were converted to the PBD conjugates (**22a**-**d**) on treatment with mercury perchlorate.

In conclusion, we have established a convenient methodology to introduce a linker to the 7 or 9-position of PBD by S_NAr reaction. Recent progress in the development of sequence specific polyamides and other

minor groove binders augurs well for the future of such bioactive conjugates.[18-20] This methodology may apply to the synthesis of these bioactive conjugates.



Scheme 5

EXPERIMENTAL

All reactions were performed under argon or nitrogen environment. Melting points were taken on a Yanagimoto micro melting point apparatus Yanaco-MP and are uncorrected. ^{1}H NMR spectra were measured either on a JEOL JNM-EX270 (270MHz) or on a JNM-ALPHA500 (500MHz) instrument. ^{13}C NMR spectra were recorded either on a JEOL JNM-EX270 or on a JNM-ALPHA500 pulsed Fourier-transform spectrometer operating at 67.80 MHz, and 125.65 MHz, respectively. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. IR spectra were recorded on a Nicolet MAGNA 750 spectrophotometer with a Nic-plane microscope or on a JASCO IR-G. UV and visible absorption spectra were recorded on a Shimadzu UV-3101PC UV-VIS-NIR scanning spectrophotometer. EIMS measurement was performed either on a Kraytos MS 50 or JEOL JMS-DX303 high-resolution mass spectrometer, while fast atom bombardment mass measurement was carried out with AEI MS-9 and MS-50 mass spectrometers using 1,4-dithiothreitol (Cleland's reagent) as the matrix. MS spectroscopy was also measured either on a SHIMADZU/KRATOS (TOF-MS) KOMPACT-MALDI

employing DHD as the matrix or on a SHIMADZU/GCMS-QP5050Ac. Elemental analysis was performed by the department services at the laboratory for organic elemental microanalysis, faculty of pharmaceutical sciences, Kyoto University on Yanaco CHN-CORDER MT-2 or MT-3 or MT-5. All solvents were distilled before use and, where necessary, dried according to literature procedures. Column chromatography on silica gel were carried out using Merck Silica Gel 60 (70-200 mesh) or Merck Silica Gel 9385 (230-400 mesh) or Wacogel C-200 (100-200 mesh) or Wacogel C-300 (200-300 mesh), and column chromatography on alumina using Merck Acid Alumina (activity grade I). Analytical TLC was carried out on Merck Silica Gel 60 G_{254} pre-coated glass sheets. Preparative column chromatography were carried out using Merck pre-coated PLC plates Silica Gel 60F-254. All new compounds had correct elemental and/or high resolution MS spectral analyses.

A general procedure for the prepararion of 8 : To a solution of 1,2-diaminoethane (6.0 g, 0.10 mol) in pyridine (130 mL) was added *p*-toluenesulfonyl chloride (384 g, 0.20 mol) in small portions over a period of 30 min at 0 °C. The mixture was stirred at 60 °C for 6 h. Then water was added and the precipitate was collected, recrystallized from acetone to afford **8a** (27.6 g, 72%) as colorless needles.

8a : mp 164-165 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 2.43 (6H, s), 3.04-3.08 (4H, m), 5.05 (2H, br s), 7.25-7.74 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5 (q), 43.0 (t), 127.1 (d), 129.8 (d), 136.4 (s), 143.8 (s); IR υ_{max} (film): 3286, 1156, 663, 550 cm^{-1}; *Anal*. Calcd for $C_{16}H_{20}N_2O_4S_2$: C, 52.16; H, 5.48; N, 7.61. Found: C, 52.33; H, 5.52; N, 7.59.

8b: 59%, mp 141.0-142.0 °C (ethyl acetate-hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.58-1.72 (2H, m), 2.41 (6H, s), 2.99 (4H, q, *J* = 6.5 Hz), 5.12 (2H, t, *J* = 6.5 Hz), 7.29 (4H, d, *J* = 8.1 Hz), 7.72 (4H, d, *J* = 8.1 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 29.7, 39.8, 127.0, 129.8, 136.7, 143.5; IR υ_{max} (nujol): 3248, 1156 cm^{-1}; *Anal*. Calcd for $C_{17}H_{22}N_2O_4S_2$: C, 53.39; H, 5.80; N, 7.32. Found: C, 53.43; H, 5.80; N, 7.16.

A general procedure for the prepararion of 9 : To a solution of **8a** (13.2 g, 34 mmol) in DMF (80 mL) was added pottasium carbonate (23.4 g, 170 mmol), and the mixture was refluxed for 30 min. Then to the mixture was added a solution of 2-chloroethanol (41.1 g, 51 mL) in DMF (200 mL) at the same temperature, and the mixture was refluxed overnight. Then water was added to the mixture, and the filtrate was collected, recrystallized from ethanol to afford **9a** (11.6 g, 74%) as colorless needles.

9a: mp 155-156 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 2.44 (6H, s), 2.86 (2H, br s), 3.25 (4H, t, *J* = 5.0 Hz), 3.41 (4H, s), 3.81 (4H, t, *J* = 5.0 Hz), 7.31-7.74 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 50.4, 53.1,

61.1, 127.4, 129.9, 135.0, 143.8; IR υ_{max} (film): 3289, 1332, 1155, 717, 549, 516 cm^{-1}; *Anal*. Calcd for $C_{20}H_{28}N_2O_6S_2$: C, 52.62; H, 6.19; N, 6.14. Found: C, 52.35; H, 6.25; N, 6.09.

9b : 59%; mp 138-139 °C; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.74-1.86 (4H, m), 2.17 (2H, br s), 2.44 (6H, s), 3.25 (4H, t, *J* = 6.8 Hz), 3.31 (4H, s), 3.68-3.80 (4H, m), 7.30-7.72 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 31.4, 46.9, 49.4, 58.9, 127.2, 129.9, 135.4, 143.8; IR υ_{max} (film): 3354, 1341, 1154, 1086, 811, 648, 566, 548 cm^{-1}; *Anal*. Calcd for $C_{22}H_{32}N_2O_6S_2$: C, 54.52; H, 6.66; N, 5.78. Found: C, 54.33; H, 6.80; N, 5.52.

9c: 84%, colorless oil; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.99 (2H, quin, *J* = 7.3 Hz), 2.43 (6H, s), 2.83 (2H, t, *J* = 5.4 Hz), 3.14-3.28 (8H, m), 3.77 (4H, q, *J* = 5.4 Hz), 7.30-7.72 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 28.9, 48.2, 52.0, 61.6, 127.2, 129.8, 135.4, 143.6; IR υ_{max} (neat): 3450, 1322, 1149 cm^{-1}; *Anal*. Calcd for $C_{21}H_{30}N_2O_6S_2$: C, 53.60; H, 6.43; N, 5.95. Found: C, 53.41; H, 6.42; N, 5.96; MS 470 (M^+, 470 calcd for $C_{21}H_{30}N_2O_6S_2$).

9d: 66%, colorless oil; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.70-1.96 (6H, m), 2.43 (6H, s), 2.52 (2H, br s, D_2O exchangeable), 3.15 (4H, t, *J* = 7.4 Hz), 3.21 (4H, t, *J* = 6.8 Hz), 3.72 (4H, q, *J* = 5.5 Hz), 7.27-7.70 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 28.9, 31.7, 46.1, 47.5, 59.0, 127.1, 129.8, 135.7, 143.6; IR υ_{max} (neat): 3370, 1327, 1148 cm^{-1}; *Anal*. Calcd for $C_{23}H_{34}N_2O_6S_2$: C, 55.40; H, 6.87; N, 5.62. Found: C, 55.27; H, 6.90; N, 5.68; MS 498 (M^+, 498 calcd for $C_{23}H_{34}N_2O_6S_2$).

A general procedure for the preparartion of 10 : To a solution of diol (**9a**) (912 mg, 2.0 mmol) in THF (20 mL) was added 50 % sodium hydride (96 mg, 2.0 mmol), and the suspension was refluxed for 30 min. To the mixture was added a solution of benzyl bromide (0.29 mL, 2.4 mmol) in THF (10 mL) at the same temperature, and the mixture refluxed overnight. Then water (10 mL) was added to the mixture, and the organic layer was extracted with ethyl acetate (10 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over magnesium sulfate. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (30 g) with ethyl acetate and hexane (1:1, v/v) to afford monobenzyl alcohol (**10a**) (792 mg, 73 %) as colorless needles.

10a : mp 106-107 °C (ethyl acetate-hexane); ^1H NMR (270 MHz, $CDCl_3$) δ: 2.19 (1H, br s, -OH), 2.43 (6H, s), 3.13 (2H, t, *J* = 5.1 Hz), 3.32-3.40 (2H, m), 3.38 (4H, s), 3.63 (4H, t, *J* = 5.1 Hz), 4.48 (2H, s), 7.24-7.72 (13H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 49.6, 49.7, 49.9, 52.7, 61.2, 69.4, 73.2, 127.2, 127.3, 127.8, 127.9, 128.4, 129.8, 129.8, 135.4, 135.7, 143.5, 143.6; IR υ_{max} (film): 3528, 1340, 1158, 1089, 718, 549 cm^{-1}; *Anal*. Calcd for $C_{27}H_{34}N_2O_6S_2$: C, 59.32; H, 6.27; N, 5.12. Found: C, 59.10; H, 6.33;

N, 5.07.

10b: 69%, mp 111-112 °C (etheyl acetate-hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.72-1.93 (4H, m), 2.28 (1H, br s), 2.42 (6H, s), 3.18-3.29 (6H, m), 3.50 (2H, t, *J* = 6.0 Hz), 3.66-3.74 (2H, m), 4.47 (2H, s), 7.25-7.72 (8H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 29.0, 31.3, 46.8, 47.4, 48.8, 49.1, 58.8, 67.1, 72.9, 127.1, 127.2, 127.6, 127.7, 128.4, 129.8, 129.9, 135.4, 135.6, 138.2, 143.6, 143.7; IR υ_{max} (film): 3543, 1341, 1158, 1090, 726, 549 cm^{-1}; *Anal*. Calcd for $C_{29}H_{38}N_2O_6S_2$: C, 60.60; H, 6.66; N, 4.87. Found: C, 60.32; H, 6.67; N, 4.86.

10c : 56%, colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.93 (2H, quint, *J* = 7.0 Hz), 2.41 (3H, s), 2.42 (3H, s), 2.51 (1H, t, *J* = 5.9 Hz, D_2O exchangeable), 3.07-3.16 (4H, m), 3.21-3.30 (2H, m), 3.34 (2H, t, *J* = 5.4 Hz), 3.62 (2H, t, *J* = 5.4 Hz), 3.67 (2H, q, *J* = 5.4 Hz), 4.45 (2H, s), 7.22-7.70 (13H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 28.4, 47.4, 48.2, 48.5, 51.9, 61.5, 69.5, 73.2, 127.1, 127.2, 127.7, 127.8, 128.4, 129.7, 129.8, 135.5, 136.2, 137.7, 143.3, 143.5; IR υ_{max} (neat): 3485, 1330, 1152 cm^{-1}; *Anal*. Calcd for $C_{28}H_{36}N_2O_6S_2$: C, 59.98; H, 6.47; N, 5.00. Found: C, 59.83; H, 6.51; N, 4.85.

10d : 50%, colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.71-1.92 (6H, m), 2.39 (1H, t, *J* = 5.8 Hz, D_2O exchangeable), 2.42 (6H, s), 3.07-3.25 (8H, m), 3.47 (2H, t, *J* = 5.9 Hz), 3.68 (2H, q, *J* = 5.8 Hz), 4.46 (2H, s), 7.25-7.69 (13H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 28.6, 29.3, 31.6, 45.8, 46.4, 46.8, 47.4, 58.7, 67.3, 73.0, 127.1, 127.7, 128.4, 129.7, 129.8, 135.7, 136.2, 138.1, 143.3, 143.5; IR υ_{max} (neat): 3500, 1328, 1150 cm^{-1}; *Anal*. Calcd for $C_{30}H_{40}N_2O_6S_2$: C, 61.20; H, 6.85; N, 4.76. Found: C, 61.11; H, 6.82; N, 4.62.

A general procedure for the preparation of 12 : To a solution of 2-nitro-5-fluorobenzoic acid (**11a**) (925 mg, 5.0 mmol) in benzene (30 mL) was added thionyl chloride (0.73 mL, 10.0 mmol), and the mixture was refluxed for 1 h. The solvent was removed *in vacuo* to afford crude acid chloride.

A solution of this crude acid in THF (5 mL) was dropwised to a solution of L-proline (575 mg, 5 mmol) in THF (10 mL) and water (5 mL) at 0 °C keeping pH between 8.5-9.0 by adding triethylamine. The reaction mixture was stirred for 40 min at rt and THF was removed *in vacuo*. The remained aqueous solution was adjusted to pH 1.0 with conc. HCl, and extracted with ethyl acetate (20 mL, 3 times). The combined organic layer was dried over magnesium sulfate, and the solvent was removed *in vacuo* to afford crude amide acid.

To a solution of this crude acid in dichloromethane (30 mL) was added thionyl chloride (0.58 mL, 7.90 mmol) and the whole was refluxed for 1.5 h. To the mixture was added methanol (10 mL) at 0 °C and the

mixture was stirred for 1 h at rt. Water (10 mL) was added to the mixture, and the organic layer was extracted with dichloromethane (30 mL, 3 times). The combined organic layer was dried over magnesium sulfate, and the solvent was removed *in vacuo*. This residue was purified by column chromatography on silica gel (50 g) with ethyl acetate and hexane (1:2, v/v) to afford amide ethyl ester (**12a**) (1.02 g, 66 %) as colorless oil.

12a : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.90-2.40 (4H, m), 3.17-3.45 (2H, m), 3.82 (3H, s), 4.76 (1H, q, *J* = 4.3 Hz), 7.20-7.36 (2H, m), 8.18-8.30 (1H, m); IR υ_{max} (film) : 1744, 1651, 1586, 1531, 1436, 1347 cm^{-1}; MS (m/z) : 296 (M^+), 237, 168 (100%), 94; HRMS 296.0814 (M^+, 296.0808 calcd for $C_{13}H_{13}N_2O_5F$).

12b : 94%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.33 (3H, t, *J* = 4.6 Hz), 1.90-2.40 (4H, m), 3.20-3.50 (2H, m), 4.27 (2H, q, *J* = 4.6 Hz), 4.73 (1H, q, *J* = 4.3 Hz), 7.20-7.35 (2H, m), 8.10-8.40 (1H, m); IR υ_{max} (film): 1740, 1651, 1586, 1532, 1436, 1347, 1189 cm^{-1}; MS (m/z) : 310 (M^+), 265, 237, 168 (100%), 94; HRMS 310.0962 (M^+, 310.0965 calcd for $C_{14}H_{15}N_2O_5F$).

12c : 62%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.31 (3H, t, *J* = 7.2 Hz), 1.90-2.40 (2H, m), 3.40-3.55 (2H, m), 4.23 (2H, q, *J* = 7.2 Hz), 4.63 (1H, q, *J* = 4.3 Hz), 7.30-7.60 (3H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.0, 24.8, 29.4, 49.2, 58.8, 61.2, 125.9, 126.0, 126.5, 131.7, 131.8, 132.9, 164.0, 171.4; IR υ_{max} (film): 1741, 1647, 1541, 1434, 1412, 1363, 1189 cm^{-1}; MS (m/z) : 326 (M^+), 2281, 253, 183 (100%), 167, 137, 111, 76; HRMS 328.0656 (M^+ 328.0640 calcd for $C_{14}H_{15}N_2O_5{}^{37}Cl$), 326.0667 ($M^+$ 326.0670 calcd for $C_{14}H_{15}N_2O_5{}^{35}Cl$).

A general procedure for S_NAr reaction of 12 : To a solution of benzyl ether (**10a**) (273 mg, 0.5 mmol) in THF (20 mL) was added sodium hydride (50%, 72 mg, 1.5 mmol), 18-crown-6 (26.4 mg, 0.1 mmol), and the mixture was refluxed for 40 min. Then to the suspension was added a solution of amide ester (**12a**) (444 mg, 1.5 mmol) in THF (10 mL), and refluxed overnight. After addition of water (10 mL), the organic layer was extracted with ethyl acetate (15 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo*. This residue was further purified by column chromatography on silica gel (50 g) with ethyl acetate and hexane (1:2, v/v) to afford S_NAr product (**13aa**) (277 mg, 67%) as colorless oil and/or transesterification product (**14**) as colorless oil.

13aa : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.85-2.40 (4H, m), 2.41 (3H, s), 2.42 (3H, s), 3.17-3.48 (10H, m), 3.64 (2H, t, *J* = 5.4 Hz), 3.79 (3H, s), 4.12 (2H, q, *J* = 7.0 Hz), 4.47 (2H, s), 4.74 (1H, q, *J* = 4.3 Hz), 6.89-8.17 (16H, m); IR υ_{max} (film): 1743, 1648, 1580, 1518, 1436, 1340, 1158, 1088 cm^{-1}; FABMS

(m/z) : 823 (M^++H), 669, 529, 155 (100%); HRFABMS 823.2651 (M^++H, 823.2683 calcd for $C_{40}H_{47}N_4O_{11}S_2$).

13ab: 46%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.32 (3H, t, *J* = 7.0 Hz), 1.86-2.12 (4H, m), 2.41 (3H, s), 2.42 (3H, s), 3.32-3.48 (10H, m), 3.64 (2H, t, *J* = 5.1 Hz), 4.24 (2H, q, *J* = 7.0 Hz). 4.47 (2H, s), 4.71 (1H, q, *J* = 4.3 Hz), 6.85-8.15 (16H, m); IR υ_{max} (film): 1740, 1649, 1617, 1580, 1518, 1341, 1289, 1158 cm^{-1}; FABMS (m/z) : 837 (M^++H), 684, 529(100%), 439, 155, 92; HRFABMS 837.2824 (M^++H, 837.2839 calcd for $C_{41}H_{49}N_4O_{11}S_2$).

14ba : 22%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.78-2.20 (8H, m), 2.41 (3H, s), 2.42 (3H, s), 3.16-3.32 (10H, m), 3.50 (2H, t, *J* = 5.7 Hz), 4.20-4.30 (2H, m), 4.47 (2H, s), 4.73 (1H, q, *J* = 4.3 Hz), 7.18-8.27 (16H, m); IR υ_{max} (film): 1742, 1651, 1586, 1531, 1436, 1346, 1159, 1080, 549 cm^{-1}; *Anal.* Calcd for $C_{40}H_{45}N_4O_{10}S_2F$: C, 58.24; H, 5.50; N, 6.79. Found: C, 58.18; H, 5.43; N, 6.77; MS (m/z) : 825 (M^+, 825 calcd for $C_{40}H_{45}N_4O_{10}S_2F$).

13bb : 20%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.33 (3H, t, *J* = 7.0 Hz), 1.85-2.40 (4H, m), 2.41 (3H, s), 2.42 (3H, s), 3.17-3.48 (11H, m), 3.64 (2H, t, *J* = 5.4 Hz), 3.79 (3H, s), 4.12 (2H, q, *J* = 7.0 Hz), 4.28 (2H, q, *J* = 7.0 Hz), 4.47 (2H, s), 4.74 (1H, q, *J* = 4.3 Hz), 6.89-8.17 (16H, m); IR υ_{max} (film): 1740, 1650, 1580, 1518, 1438, 1341, 1288, 1160, 1090, 549 cm^{-1}; FABMS (m/z) : 865 (M^++H), 557,421, 395, 378 (100%); HRFABMS 865.3134 (M^++H 865.3152 calcd for $C_{43}H_{53.}N_4O_{11}S_2$).

13cb : 44%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.32 (3H, t, *J* = 7.0 Hz), 1.85-2.04 (6H, m), 2.41 (6H, s), 3.18-3.28 (6H, m), 3.36 (2H, t, *J* = 4.3 Hz), 3.42 (2H, t, *J* = 6.2 Hz), 3.60 (2H, t, *J* = 5.4 Hz), 4.10-4.22 (2H, m), 4.28 (2H, q, *J* = 7.0 Hz), 4.42 (2H, s), 4.70 (1H,. q, *J* = 4.3 Hz), 6.86-8.18 (16H, m); IR υ_{max} (neat): 1730, 1637, 1572, 1433, 1333, 1152 cm^{-1}; *Anal.* Calcd for $C_{43}H_{51}N_4O_{11}S_2$: C, 59.70; H, 6.06; N, 6.48. Found: C, 59.54; H, 6.02; N, 6.44; MS (m/z) : 851 (M^+, 851 calcd for $C_{42}H_{50}N_4O_{11}S_2$).

***N*-(2-Nitro-5-fluorobenzoyl)pyrrolidine-2-(2-hydroxy)ethane (15)**. To a cooled (-30 °C) solution of ester (**12b**) (1.2 g, 4.05 mmol) in THF (15 mL) was added LAH (308 mg, 8.10 mmol) in small portions over 1 h. The suspension was stirred for more 10 min and 10 % ammonium chloride (5 mL) was added to the mixture at the same temparature. Water (10 mL) was added to the mixture, and organic layer was extracted with ethyl acetate (10 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (30 g) with ethyl acetate and hexane (1:1, v/v) to afford alcohol (**15**) (0.58 g, 53%) as colorless oil.

15 : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.60-2.30 (4H, m), 3.22 (2H, dd, J = 7.6, 5.9 Hz), 3.74-4.15 (3H, m), 4.30-4.45 (1H, m), 7.10-7.40 (2H, m), 8.21-8.28 (1H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 24.4, 28.4, 49.6, 61.6, 65.7, 115.3 ($J_{C\text{-}F}$ = 25.6 Hz), 117.0 ($J_{C\text{-}F}$ = 23.1 Hz), 127.8 ($J_{C\text{-}F}$ = 9.7 Hz), 136.4 ($J_{C\text{-}F}$ = 8.5 Hz), 145.7, 165.2 ($J_{C\text{-}F}$ = 260.7 Hz), 166.7; IR υ_{max} (film) : 3404, 1625, 1586, 1530, 1443, 1346 cm^{-1}; MS (m/z) : 268 (M^+), 237, 168 (100%), 122, 94; HRMS 268.0860 (M^+ 268.0859 calcd for $C_{12}H_{13}N_2O_4F$).

Ethyl *N*-(2-Nitro-5-fluorobenzoyl)pyrrolidine-2-carboxaldehyde (16). To a cooled (-78 °C) solution of oxalyl chloride (0.50 mL, 5.7 mmol) in dichloromethane (20 mL) was added DMSO (0.60 mL, 8.5 mmol) and the mixture was stirred for 10 min. Then to the mixture was added a solution of alcohol (**15**) (758 mg, 2.8 mmol) in dichloromethane (10 mL), and the mixture was stirred for 15 min at the same temperature, then for 15 min at -45°C. To the mixture was added triethylamine (0.97 mL, 7.0 mmol) at 45°C, and the whole was stirred for 20 min at 0 °C. To the mixture was added 10% ammonium chloride (10 mL), and the organic layer was extracted with dichloromethane (20 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate. The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography on silica gel (30 g) with ethyl acetate and hexane (2:1, v/v) to afford amide aldehyde (**16a**) (669 mg, 89 %) as colorless oil.

16a : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.70-2.30 (4H, m), 3.23-3.40 (2H, m), 4.67-4.77 (1H, m), 7.20-7.33 (2H, m), 8.21-8.32 (1H, m), 9.77 (1H, br s); IR υ_{max} (film) : 1732, 1641, 1586, 1530, 1439, 1347 cm^{-1}; MS (m/z) : 237 (M^+-CHO), 168 (100%), 94; HRMS 237.0675 (M^+-CHO 237.0713 calcd for $C_{11}H_{10}N_2O_3F$).

16b : 44%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.80-2.35 (4H, m), 3.42-3.53 (2H, m), 4.63-4.68 (1H, m), 7.27-7.65 (3H, m), 9.64 (1H, d, J = 1.4 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 24.8, 26.3, 49.3, 64.9, 125.9, 127.0, 131.9, 132.0, 132.1, 132.9, 164.9, 198.2; IR υ_{max} (film): 1733, 1637, 1539, 1436, 1412, 1361 cm^{-1}; MS (m/z) : 255 (M^+-CHO), 253, 183 (100%), 149, 76; HRMS 255.0349 (M^+-CHO 255.0351 calcd for $C_{11}H_{10}N_2O_3{}^{37}Cl$), 253.0377 ($M^+$-CHO 253.0380 calcd for $C_{11}H_{10}N_2O_3{}^{35}Cl$).

A general procedure for protection with 1,2-ethanediol of 16 : To a soution of aldehyde (**16b**) (444 mg, 1.57 mmol) in benzene (30 mL) was added ethanediol (0.10 mL, 1.73 mmol) and *p*-toluenesulfonic acid (30 mg, 0.16 mmol), and the mixture was refluxed for 1 h with continuously removing water by Dean-Stark apparatus. Then the solvent was removed *in vacuo*, and residue was purified by flash column chromatography on silica gel (30 g) with ethyl acetate and hexane (2:1, v/v) to afford amide acetal (**17b**) (509 mg, 99 %) as colorless oil.

17b : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.80-2.10 (4H, m), 3.30-3.40 (2H, m), 3.73-4.10 (4H, m), 4.50-4.60 (1H, m), 5.26 (1H, d, *J* = 2.4 Hz), 7.30-7.60 (3H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 24.0, 24.7, 49.8, 58.2, 65.2, 65.4, 102.3, 126.0, 131.6, 134.0, 141.1, 151.7, 152.2, 164.7; IR υ_{max} (film): 1641, 1540, 1432, 1411, 1362, 1142 cm^{-1}; MS (m/z) : 325 (M^+-H), 253, 183, 142, 73 (100%); HRMS 325.0589 (M^+-H 325.0591 calcd for $C_{14}H_{14}N_2O_5{}^{35}Cl$).

17a : 99%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.92-2.22 (4H, m), 3.10-3.28 (2H, m), 3.86-4.06 (4H, m), 4.68 (1H, d, *J* = 3.8 Hz), 5.39 (1H, d, *J* = 1.9 Hz), 7.10-8.26 (3H, m); IR υ_{max} (film): 1644, 1586, 1530, 1435, 1346 cm^{-1}; MS (m/z) : 311 (M^++H), 293, 237, 168, 73 (100%); HRMS 311.1045 (M^++H 311.1043 calcd for $C_{14}H_{16}N_2O_5F$).

A general procedure for S_NAr reaction of 17: To a solution of benzyl ether (**10a**) (163 mg, 0.30 mmol) in THF (10mL) was added 50% sodium hydride (48 mg, 1.0 mmol), and the mixture was refluxed for 1 h. Then to the suspension was added a solution of amide acetal (**17a**) (100 mg, 0.32 mmol) in THF (10mL), and the mixture was refluxed overnight. After addition of water (10 mL), the organic layer was extracted with ethyl acetate (15mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo*. This residue was purified by column chromatography on silica gel (30 g) with ethyl acetate and hexane (1:1, v/v) to afford amide acetal S_NAr (**18a**) (191 mg, 76 %) as colorless oil.

18a : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.72-2.08 (4H, m), 2.35 (6H, s), 3.10-3.50 (10H, m), 3.63 (2H, t, *J* = 5.1 Hz), 3.85-4.15 (6H, m), 4.46 (2H, s), 4.57-4.66 (1H, m), 5.39 (1H, d, *J* = 2.4 Hz), 6.82-8.20 (16H. m); IR υ_{max} (film) : 1642, 1580, 1517, 1435, 1340, 1158 cm^{-1}; MS (m/z) : 838 (M^++2H), 684, 530, 439, 364, 332; *Anal.* Calcd for $C_{41}H_{48}N_4O_{11}S_2$: C, 58.84; H, 5.78; N, 6.69. Found: C, 58.73; H, 5.71; N, 6.64.

18b : 63%; ^{1}H NMR (270 MHz, CDCl3) δ: 1.78-2.14 (8H, m), 2.40 (3H, s), 2.41 (3H, s), 3.15-3.35 (8H, m), 3.50 (2H, t, *J* = 5.9 Hz), 3.88-4.10 (6H, m), 4.47 (2H, s), 4.57-4.70 (1H, m), 5.41 (1H, d, *J* = 2.2 Hz), 6.83-8.20 (16H, m); IR υ_{max} (neat) : 1646, 1572, 1509, 1430, 1338, 1155 cm^{-1}; *Anal.* Calcd for $C_{43}H_{52}N_4O_{11}S_2$: C, 59.70; H, 6.06; N, 6.48. Found: C, 59.68; H, 6.01; N, 6.40; MS (m/z) : 865 (M^+, 865 calcd for $C_{43}H_{52}N_4O_{11}S_2$).

18c : 89%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.88-2.08 (6H, m), 2.41 (6H, s), 3.13-3.27 (6H, m), 3.32 (2H, t, *J* = 5.4 Hz), 3.42 (2H, t, *J* = 5.9 Hz), 3.59 (2H, t, *J* = 5.4 Hz), 3.87-4.06 (4H, m), 4.19 (2H, t, *J* = 5.9 Hz), 4.42 (2H, s), 4.57-4.68 (1H, m), 5.40 (1H, d, *J* = 2.4 Hz), 6.82-8.17 (16H, m); IR υ_{max} (neat): 1633, 1575, 1431, 1335, 1154, 1083 cm^{-1}; *Anal.* Calcd for $C_{42}H_{50}N_4O_{11}S_2$: C, 59.28; H, 5.92; N, 6.58. Found: C, 59.11;

H, 5.90; N, 6.49; MS (m/z) : 851 (M^+, 851 calcd for $C_{42}H_{50}N_4O_{11}S_2$).

18d : 84%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.72-2.14 (8H, m), 2.40 (3H, s), 2.41 (3H, s), 3.07-3.33 (10H, m), 3.46 (2H, t, *J* = 5.9 Hz), 3.88-4.12 (6H, m), 4.45 (2H, s), 4.58-4.72 (1H, m), 5.41 (1H, d, *J* = 2.4 Hz), 6.82-8.20 (16H, m); IR υ_{max} (neat): 1635, 1333, 1155 cm^{-1}; *Anal.* Calcd for $C_{44}H_{54}N_4O_{11}S_2$: C, 60.12; H, 6.19; N, 6.37. Found: C, 59.97; H, 6.21; N, 6.40; MS (m/z) : 879 (M^+, 879 calcd for $C_{44}H_{54}N_4O_{11}S_2$).

18e : 46%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.82-2.04 (4H, m), 2.38 (3H, s), 2.40 (3H, s), 3.30-3.53 (8H, m), 3.61 (2H, t, *J* = 5.4 Hz), 3.64-4.12 (6H, m), 4.43 (2H, s), 4.47-4.54 (1H, m), 5.34 (1H, d, *J* = 2.2 Hz), 7.05-7.74 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.4 (q), 21.4 (q), 23.9 (t), 24.4 (t), 48.9 (t), 49.0 (t), 49.1 (t), 49.4 (t), 49.6 (t), 58.2 (d), 65.0 (t), 65.1 (t), 68.7 (t), 72.9 (t), 73.4 (t), 102.0 (d), 104.2 (d), 125.0 (s), 125.3 (d), 126.3 (d), 127.2 (d), 127.3 (d), 127.5 (d), 127.6 (d), 128.1 (s), 128.2 (d), 129.6 (d), 129.7 (d), 133.9 (s), 136.0 (s), 137.8 (s), 143.2 (s), 143.3 (s), 150.8 (s), 166.6 (s); IR υ_{max} (film): 1631, 1454, 1443, 1344, 1159, 1090, 719, 549 cm^{-1}; MS (m/z) : 837 (M^+, 837 calcd for $C_{41}H_{48}N_4O_{11}S_2$); *Anal.* Calcd for $C_{41}H_{48}N_4O_{11}S_2$: C, 58.84; H, 5.78; N, 6.69. Found: C, 58.75; H, 5.76; N, 6.63.

18f: 23%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.81-2.08 (8H, m), 2.41 (6H, s), 3.12-3.43 (10H, m), 3.51 (2H, t, *J* = 5.9 Hz), 3.80-4.16 (6H, m), 4.48 (2H, s), 4.50-4.57 (1H, m), 5.37 (1H, d, *J* = 2.4 Hz), 7.04-7.74 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5 (q), 21.5 (q), 24.0 (t), 24.5 (t), 29.1 (t), 29.6 (t), 47.3 (t), 47.4 (t), 48.6 (t), 48.7 (t), 48.8 (t), 58.3 (d), 65.2 (t), 65.3 (t), 67.2 (t), 72.0 (t), 72.9 (t), 102.1 (d), 104.2 (d), 125.0 (d), 126.6 (d), 127.2 (d), 127.5 (d), 127.6 (d), 128.1 (d), 128.3 (d), 129.8 (d), 130.9 (d), 131.2 (d), 133.6 (s), 135.8 (s), 137.4 (s), 138.3 (s), 143.4 (s), 143.5 (s), 151.1 (s), 167.0 (s) ; *Anal.* Calcd for $C_{44}H_{54}N_4O_{11}S_2$: C, 60.12; H, 6.19; N, 6.37. Found: C, 60.08; H, 6.20; N, 6.35; MS (m/z) : 879 (M^+, 879 calcd for $C_{44}H_{54}N_4O_{11}S_2$).

***N*-(2-Nitro-5-fluorobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal (19)** : To a solution of aldehyde (**16a**) (2.50 g, 9.40 mmol) in dichloromethane (30 mL) was added ethanethiol (1.53 mL, 20.7 mmol) at rt, and the mixture was stirred for 30 min. Then to the mixture was added chlorotrimethylsilane (3.0 mL, 23.5 mmol), and the mixture was further stirred overnight. The solvent was removed *in vacuo*, and residue was purified by flash column chromatography on silica gel (30 g) with ethyl acetate and hexane (1:4, v/v) to afford amide thioacetal (**19**) (2.55 g, 73 %) as pale yellow oil.

19 : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.25-1.38 (6H, m), 1.75-2.38 (4H, m), 2.65-2.90 (4H, m), 3.20-3.40 (2H, m), 4.65-4.74 (1H, m), 4.84 (1H, d, *J* = 5.4 Hz), 7.10-8.22 (3H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.9, 24.6, 26.2, 26.6, 27.2, 50.4, 52.7, 61.2, 115.4 ($J_{C\text{-}F}$ = 24.4 Hz), 116.7 ($J_{C\text{-}F}$ = 23.1 Hz), 127.6 ($J_{C\text{-}F}$ =

9.8 Hz), 136.8 ($J_{C\text{-}F}$ = 7.3 Hz), 141.0, 164.9, 165.4 ($J_{C\text{-}F}$ = 259.5 Hz); IR υ_{max} (neat): 1634, 1580, 1526, 1428, 1344, 1259 cm^{-1}; *Anal.* Calcd for $C_{16}H_{21}N_2O_3S_2F$: C, 51.59; H, 5.68; N, 7.52. Found: C, 51.54; H, 5.62; N, 7.56; MS (m/z) : 372 (M^+, 372 calcd for $C_{16}H_{21}N_2O_3S_2F$).

A general procedure for S_NAr reaction of 19: To a solution of benzyl ether (**10a**) (172 mg, 0.30 mmol) in THF (15 mL) was added 50% sodium hydride (38 mg, 0.80 mmol), and the mixture was refluxed for 1 h. Then to the suspension was added a solution of (**19**) (93 mg, 0.25 mmol) in THF (10 mL), and the mixture was refluxed overnight. After addition of water (10 mL), the organic layer was extracted with ethyl acetate (15 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo*. This residue was purified by column chromatography on silica gel (30 g) with ethyl acetate and hexane (1:1, v/v) to afford amide thioacetal S_NAr (**20a**) (171 mg, 76 %) as colorless oil.

20a : ^1H NMR (270 MHz, $CDCl_3$) δ: 1.31 (2H, t, *J* = 7.6 Hz), 1.35 (2H, t, *J* = 7.6 Hz), 1.72-2.28 (4H, m), 2.40 (3H, s), 2.41 (3H, s), 2.64-2.86 (4H, m), 3.15-3.50 (10H, m), 3.62 (2H, t, *J* = 5.1 Hz), 4.16 (2H, t, *J* = 5.4 Hz), 4.46 (2H, s), 4.64-4.73 (1H, m), 4.83 (1H, d, *J* = 2.4 Hz), 6.85-8.14 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.9 (q), 15.0 (q), 21.4 (q), 24.5 (t), 26.3 (t), 26.5 (t), 27.1 (t), 48.9 (t), 49.5 (t), 49.8 (t), 49.9 (t), 50.1 (t), 52.8 (d), 61.2 (d), 67.5 (t), 69.2 (t), 73.1 (t), 113.5 (d), 114.7 (d), 127.0 (d), 127.1 (d), 127.7 (d), 128.3 (d), 129.7 (d), 129.8 (d), 135.4 (s), 135.8 (s), 136.3 (s), 137.6 (s), 138.0 (s), 143.6 (s), 143.8 (s), 162.8 (s), 166.1 (s); IR υ_{max} (neat): 1635, 1429, 1338, 1154 cm^{-1}; *Anal.* Calcd for $C_{43}H_{54}N_4O_9S_4$: C, 57.44; H, 6.05; N, 6.23. Found: C, 57.39; H, 6.06; N, 6.10; MS (m/z) : 899 (M^+, 899 calcd for $C_{43}H_{54}N_4O_9S_4$).

20b : 88%; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.33 (2H, t, *J* = 7.0 Hz), 1.35 (2H, t, *J* = 7.0 Hz), 1.74-2.17 (8H, m), 2.40 (3H, s), 2.41 (3H, s), 2.66-2.86 (4H, m), 3.18-3.37 (10H, m), 3.49 (2H, t, *J* = 5.7 Hz), 4.08 (2H, t, *J* = 5.7 Hz), 4.47 (2H, s), 4.65-4.75 (1H, m), 4.86 (1H, d, *J* = 3.5 Hz), 6.85-8.19 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.9 (q), 15.0 (q), 21.4 (q), 24.5 (t), 26.2 (t), 26.4 (t), 27.1 (t), 28.2 (t), 28.9 (t), 46.8 (t), 47.3 (t), 48.6 (t), 48.9 (t), 50.1 (t), 52.8 (d), 61.1 (d), 65.9 (t), 67.0 (t), 72.8 (t), 113.5 (d), 114.6 (d), 127.0 (d), 127.1 (d), 127.5 (d), 128.2 (d), 129.7 (d), 129.8 (d), 135.4 (s), 135.6 (s), 136.2 (s), 137.7 (s), 138.2 (s), 143.6 (s), 143.7 (s), 163.3 (s), 166.2 (s); IR υ_{max} (neat): 1642, 1580, 1518, 1438, 1345, 1160, 1088 cm^{-1}; *Anal.* Calcd for $C_{44}H_{56}N_4O_9S_4$: C, 57.87; H, 6.18; N, 6.14. Found: C, 57.69; H, 6.11; N, 6.17; MS (m/z) : 913 (M^+, 913 calcd for $C_{46}H_{59}O_9N_3S_4$).

20c : 97%; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.29 (2H, t, *J* = 7.3 Hz), 1.32 (2H, t, *J* = 7.3 Hz), 1.70-2.28 (6H, m), 2.40 (6H, s), 2.61-2.88 (4H, m), 3.17-3.25 (10H, m), 3.31 (2H, t, *J* = 5.4 Hz), 3.43 (2H, t, *J* =

5.91 Hz), 3.58 (2H, t, *J* = 5.4 Hz), 4.21 (2H, t, *J* = 5.9 Hz), 4.41 (2H, s), 4.62-4.71 (1H, m), 4.84 (1H, d, *J* = 3.5 Hz), 6.83-8.15 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.7 (q), 14.8 (q), 21.3 (q), 24.4 (t), 26.2 (t), 26.3 (t), 27.0 (t), 28.2 (t), 47.2 (t), 47.7 (t), 48.5 (t), 49.9 (t), 52.7 (d), 61.0 (d), 67.6 (t), 69.12 (t), 72.9 (t), 113.4 (d), 114.5 (d), 126.9 (d), 127.1 (d), 127.4 (d), 127.5 (d), 128.2 (d), 129.5 (d), 129.7 (d), 135.6 (s), 136.0 (s), 136.1 (s), 137.6 (s), 137.8 (s), 143.3 (s), 143.5 (s), 162.7 (s), 166.0 (s); IR υ_{max} (neat): 1639, 1580, 1520, 1435, 1340, 1291, 1160, 1090 cm^{-1}; *Anal.* Calcd for $C_{45}H_{58}N_4O_9S_4$: C, 58.29; H, 6.30; N, 6.04. Found: C, 58.30; H, 6.28; N, 6.02; MS (m/z) : 927 (M^+, 927 calcd for $C_{45}H_{57}O_9N_3S_4$).

20d : 71%; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.30-1.39 (6H, m), 1.73-2.18 (10H, m), 2.40 (3H, s), 2.41 (3H, s), 2.65-2.88 (4H, m), 3.10-3.30 (10H, m), 3.46 (2H, t, *J* = 5.9 Hz), 4.08 (2H, t, *J* = 5.9 Hz), 4.45 (2H, s), 4.65-4.73 (1H, m), 4.86 (1H, d, *J* = 3.5 Hz), 6.83-8.18 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 14.8 (q), 14.9 (q), 21.3 (q), 24.5 (t), 26.2 (t), 26.3 (t), 27.1 (t), 28.4 (t), 29.1 (t), 45.7 (t), 46.3 (t), 46.5 (t), 46.8 (t), 50.1 (t), 52.7 (d), 61.1 (d), 66.0 (t), 67.2 (t), 72.8 (t), 113.5 (d), 114.5 (d), 127.0 (d), 127.1 (d), 127.3 (d), 127.4 (d), 128.2 (d), 129.6 (d), 135.8 (s), 135.9 (s), 136.1 (s), 137.6 (s), 138.1 (s), 143.3 (s), 143.4 (s), 163.3 (s), 166.2 (s); IR υ_{max} (neat): 1641, 1583, 1521, 1441, 1342, 1293, 1163, 1092 cm^{-1} *Anal.* Calcd for $C_{46}H_{60}N_4O_9S_4$: C, 58.70; H, 6.43; N, 5.95. Found: C, 58.64; H, 6.37; N, 5.92; MS (m/z) : 941 (M^+, 941. calcd for $C_{46}H_{60}N_4O_9S_4$).

A general procedure for the preparation of PBD structure: To a solution of thioacetal (**20a**) (77 mg, 0.086 mmol) in THF (5 mL) and methanol (5 mL) was added stannous chloride dihydrate (174 mg, 0.75 mmol), and the mixture was refluxed overnight. Then the reaction mixture was adjusted to pH 8 with saturated sodium hydrogen carbonate solution and extracted with ethyl acetate (15 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo* to afford crude amine (**21a**) as colorless oil.

To a solution of crude amine (**21a**) in acetonitrile (5 mL) and water (2 mL) was added mercuric perchlorate trihydrate (156 mg, 0.34 mmol), and the mixture was stirred at rt for 5 min. The reaction mixture was diluted with ethyl acetate (15 mL) and water (3 mL), and centrifuged at 3000 rpm for 5 min and the supernatant decanted and washed with saturated sodium hydrogen carbonate solution (10 mL, 3 times). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo*. This residue was purified by column chromatography on silica gel (30 g) with ethyl acetate to afford PBD (**22a**) (17 mg, 28 %) as colorless oil.

22a : ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.88-2.10 (2H, m), 2.25-2.35 (2H, m), 2.40 (3H, s), 2.41 (3H, s), 3.32-3.90 (15H, m), 4.10 (1H, dd, *J* = 5.1, 3.5 Hz), 4.47 (2H, s), 6.95-7.72 (17H, m); ^{13}C NMR (67.8

MHz, $CDCl_3$) δ: 21.5, 24.1, 29.6, 46.7, 49.1, 49.3, 49.5, 49.9, 53.5, 67.2, 69.3, 73.1, 113.5, 119.4, 127.2, 127.3, 127.7, 127.8, 128.3, 128.8, 128.9, 129.7, 135.9, 136.0, 137.8, 139.9, 143.4, 143.5, 156.4, 162.7, 164.4; IR υ_{max} (neat): 3380, 1630, 1598, 1498, 1452, 1344, 1158, 1090, 738, 720, 700 cm^{-1}; *Anal.* Calcd for $C_{39}H_{46}N_4O_8S_2$: C, 61.40; H, 6.08; N, 7.34. Found: C, 61.57; H, 6.13; N, 7.29; MS (m/z): 762 (M^+, 762 calcd for $C_{39}H_{46}N_4O_8S_2$).

22b : 31%; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.80-1.92 (2H, m), 1.95-2.10 (4H, m), 2.26-2.35 (2H, m), 2.40 (3H, s), 2.41 (3H, s), 3.20-3.88 (14H, m), 4.02 (1H, q, *J* = 6.1 Hz), 4.48 (2H, s), 7.01-7.70 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 24.1, 28.5, 29.1, 29.6, 46.7, 47.4, 48.6, 53.5, 65.1, 67.2, 72.9, 113.4, 119.6, 127.2, 127.6, 127.7, 128.4, 128.9, 129.8, 135.6, 135.7, 138.3, 139.7, 143.5, 143.5, 156.8, 162.6, 164.5; *Anal.* Calcd for $C_{41}H_{50}N_4O_8S_2$: C, 62.26; H, 6.37; N, 7.08. Found: C, 62.40; H, 6.50; N, 7.00; MS (m/z): 790 (M^+, 790 calcd for $C_{41}H_{50}N_4O_8S_2$).

22c : 46%; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.85-2.10 (4H, m), 2.25-2.35 (2H, m), 2.40 (6H, s), 3.13-3.88 (14H, m), 4.12 (1H, q, *J* = 6.1 Hz), 4.42 (2H, s), 6.90-7.73 (16H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 24.1, 28.2, 28.7, 29.6, 46.7, 47.3, 47.5, 47.6, 48.4, 53.5, 67.1, 69.2, 73.1, 113.7, 119.2, 127.2, 127.7, 127.8, 128.4, 128.9, 129.7, 129.8, 136.3, 136.4, 137.9, 143.3, 143.4, 156.4, 162.4, 164.5; IR υ_{max} (neat): 3350, 1670, 1598, 1495, 1450, 1335, 1158, 1088, 730, 695 cm^{-1}; *Anal.* Calcd for $C_{40}H_{48}N_4O_8S_2$: C, 61.83; H, 6.23; N, 7.21. Found: C, 62.06; H, 6.04; N, 7.33; MS (m/z): 776 (M^+, 776 calcd for $C_{40}H_{48}N_4O_8S_2$).

22d : 46%; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.75-2.10 (8H, m), 2.25-2.35 (2H, m), 2.39 (3H, s), 2.41 (3H, s), 3.08-3.90 (15H, m), 4.01 (1H, q, *J* = 6.2 Hz), 4.46 (2H, s), 6.98-7.70 (17H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 21.5, 24.1, 28.4, 28.7, 29.2, 29.6, 45.8, 46.3, 46.6, 46.7, 53.5, 65.3, 67.3, 72.9, 113.5, 119.3, 127.2, 127.5, 127.6, 128.3, 128.9, 129.7, 129.8, 136.1, 136.3, 138.3, 139.7, 143.2, 143.4, 156.8, 162.6, 164.5; IR υ_{max} (neat): 3400, 1630, 1598, 1498, 1453, 1338, 1158, 1090, 738 cm^{-1}; *Anal.* Calcd for $C_{42}H_{52}N_4O_8S_2$: C, 62.66; H, 6.51; N, 6.96. Found: C, 62.52; H, 6.66; N, 6.83; MS (m/z): 804 (M^+, 804 calcd for $C_{42}H_{52}N_4O_8S_2$).

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REFERENCES AND NOTES

† Preliminary Communications: K. Matsumoto, H. Iida, and J. W. Lown, *Heterocycles*, 2000, **52**, 1015.

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1. H. Iida, G. Jia, and J. W. Lown, *Curr. Opin. Biotechnol*., 1999, **10**, 29.
2. B. S. Reddy, S. K. Sharma, and J. W. Lown, *Current Med. Chem*., 2001, **8**, 475.
3. B. S. P. Reddy, S. M. Sondhi, and J. W. Lown, *Pharmacol. & Ther*., 1999, **84**, 1.
4. H. Iida and J. W. Lown, *Recent Res. Devel. Synth. Organic Chem*., 1998, **1**, 16.
5. (a) W. Leimgruber, A. D. Batcho, and F. Schenker, *J. Am. Chem. Soc*., 1965, **87**, 5793; (b) W. Leimgruber, V. Stefanovic, F. Schenker, A. Karr, and J. Berger, *J. Am. Chem. Soc*., 1965, **87**, 5791.
6. L. H. Hurley and R. L. Petrusek, *Nature (London)*, 1979, **282**, 529.
7. D. E. Thurston, Advances in the study of pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumor antibiotics. In Molecular Aspects of Anticancer Drug - DNA interactions Vol. 1, ed. by S. Niedle and M. Waring, CRC Press, Boca Raton, 1993, p. 54.
8. Y. Damayanthi, B. S. P. Reddy, and J. W. Lown, *J. Org. Chem*., 1999, **64**, 290.
9. J. W. Lown and A. V. Joshua, *Biochem. Biopharmacol*., 1979, **28**, 2017.
10. M. L. Kopka, D. S. Goodsell, I. Baikalov, K. Grzeskowaik, D. Cascio, and R. E. Dickerson, *Biochemistry*, 1994, **33**, 13593.
11. (a) R. Kumar and J. W. Lown, *Oncol. Res.,* 2003, **14**, 221; (b) R. Kumar and J. W. Lown, *Mini-Reviews Med. Chem.,* 2003, **3**, 323; (c) R. Kumar and J. W. Lown, *Heterocyclic Commun*., 2002, **8**, 115; (d) B. S. Reddy, Y. Damayanthi, B. S. Reddy, and J. W. Lown, *Anti-Cancer Drug Desig*n, 2000, **15**, 225.
12. (a) M. Smellie, D. S. Bose, A. S. Thompson, T. C. Jenkins, J. A. Hartley, and D. E. Thurston, *Biochemistry*, 2003, **42**, 8232; (b) S. J. Gregson, P. W. Howard, K. E. Corcoran, T. C. Jenkins, L. R. Kelland, and D. E. Thurston, *Bioorg. Med. Chem. Lett*., 2001, **11**, 2859; (c) S. J. Gregson, P. W. Howard, J. A. Hartley, N. A. Brooks, L. J. Adams, T. C. Jenkins, L. R. Kelland, and D. E. Thurston,

J. Med. Chem., 2001, **44**, 737.

13. (a) A. Kamal, G. Ramesh, N. Laxman, P. Ramulu, O. Srinivas, K. Neelima, A. K. Kondapi, V. B. Sreenu, and H. A. Nagarajaram, *J. Med. Chem.*, 2002, **45**, 4679; (b) A. Kamal, G. S. K. Reddy, K. L. Reddy, and S. Raghavan, *Tetrahedron Lett.*, 2002, **43**, 2103; (c) A. Kamal, M. V. Rao, N. Laxman, G. Ramesh, and G. S. K. Reddy, *Current Med. Chem.: Anti-Cancer Agents*, 2002, **2**, 215.
14. M. Tercel, S. M. Stribbling, H. Sheppard, B. G. Siim, K. Wu, S. M. Pullen, K. J. Botting, W. R. Wilson, and W. A. Denny, *J. Med. Chem.*, 2003, **46**, 2132.
15. H. Iida, Y. Misumi, T. Morishita, K. Matsumoto, and J. W. Lown, *Heterocyclic Commun.*, 1999, **5**, 509.
16. W. D. Wilson, In Organic Synthesis - Concepts, Methods, Starting Materials -Second Revised and Enlarged Edition; ed. by J. Fuhrhop and G. Penzlin, VCH, Weinheim, 1994, p. 341.
17. J. W. Lown, Design of Sequence-specific Agents: Lexitropsins, In Molecular Aspects of Anticancer Drug-DNA Interactions Vol. 1, ed. by S. Neidle and M. Waring, CRC Press, Boca Raton, 1993, p. 322.
18. H. Iida and H. Sugiyama, *J. Synth. Org. Chem. Jpn.*, 2000, **58**, 975.
19. S. M. Sondhi, N. Singhal, M. Johar, B. S. Reddy, and J. W. Lown, *Curr. Med. Chem.*, 2002, **9**, 1045.
20. (a) P. B. Dervan and B. S. Edelso, *Curr. Opin. Struct. Biol.*, 2003, **13**, 284; (b) P. B. Dervan, *Bioorg. Med. Chem.*, 2001, **9**, 2215.