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Articles

Practical Synthesis of the High-Quality Antitumor Agent KW-2189 from Duocarmycin B2 Using a Facile One-Pot Synthesis of an Intermediate

Masahiko Kinugawa,[†] Satoru Nagamura,[‡,1] Akihiko Sakaguchi,[†] Yoshiaki Masuda,[†] Hiromitsu Saito,[‡,2] Takehiro Ogasa,*[,†] and Masaji Kasai[†]

Sakai Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1-1-53, Takasu-cho, Sakai, Osaka 590, Japan, and Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-3-6, Asahimachi, Machida, Tokyo 194, Japan

Abstract:

A facile and large-scale preparation process of a potent antitumor agent KW-2189 (2), derived from the antitumor antibiotic duocarmycin B2 (1), has been developed. This new synthetic route required three steps: (i) one-pot carbamoylation and subsequent reduction, (ii) Wagner−Meerwein rearrangement of the methoxycarbonyl group for the production of the pyrrole compound 6, and (iii) formation of the hydrobromide salt 2. The key strategic improvement was to obtain good quality hydroxy compound 4 in a reasonable yield without isolation of the unstable keto intermediate 3a. During commercial-scale production at a scale of about 50 g, this strategy provided high-quality KW-2189 (2) in a 55% overall yield from 1. Potential degradation compounds 7−9 were also synthesized and shown to be absent in the KW-2189 (2) prepared.

Introduction

Duocarmycins (DUMs) (Figure 1) are a new class of antitumor antibiotics isolated from *Streptomyces* species which show antitumor activity against murine lymphocytic P388 transplanted in CDF1 mice and murine Sarcoma 180 in *ddY* mice.[3−9] With the aim of enhancing and broadening the spectrum of antitumor activity and improving their solubility or stability,[10−13] a series of analogues have been synthesized from duocarmycin B2 (**1**, DUMB2), which was more efficiently isolated than other natural DUMs. Among these analogues, KW-2189 (**2**) (Figure 2) displays a remarkably effective and broad spectrum of activities in vivo against various human xenografts including LC-6 (lung), St-4 (stomach), and Co-3 (colon).[14] KW-2189 (**2**) also possesses

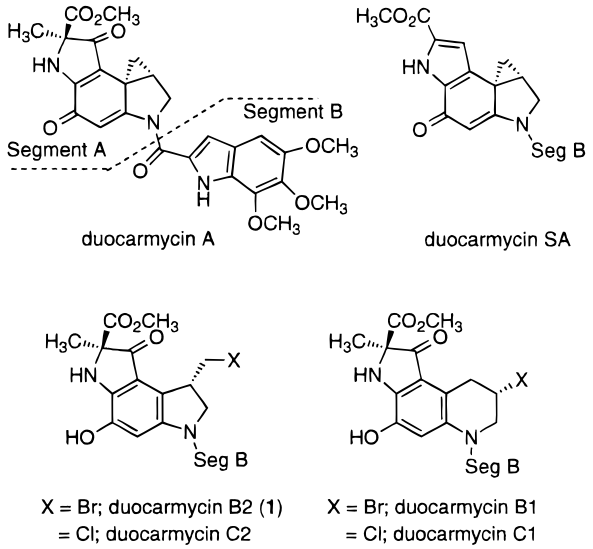

Figure 1. **Structure of duocarmycins isolated from *Streptomyces*.**

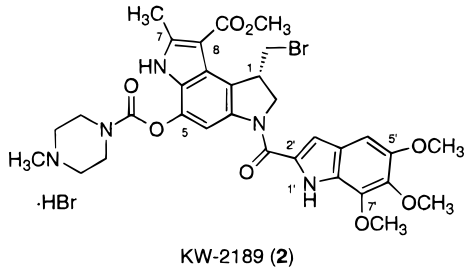

Figure 2. **Structure of KW-2189.**

[†] Sakai Research Laboratories.
[‡] Tokyo Research Laboratories.

(1) Present address: Technical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1-1, Kyowamachi, Hofu, Yamaguchi 747, Japan.
(2) Present address: Fuji Plant, Kyowa Hakko Kogyo Co., Ltd., 1188, Shimotogari, Nagaizumi-cho, Shizuoka 411, Japan.
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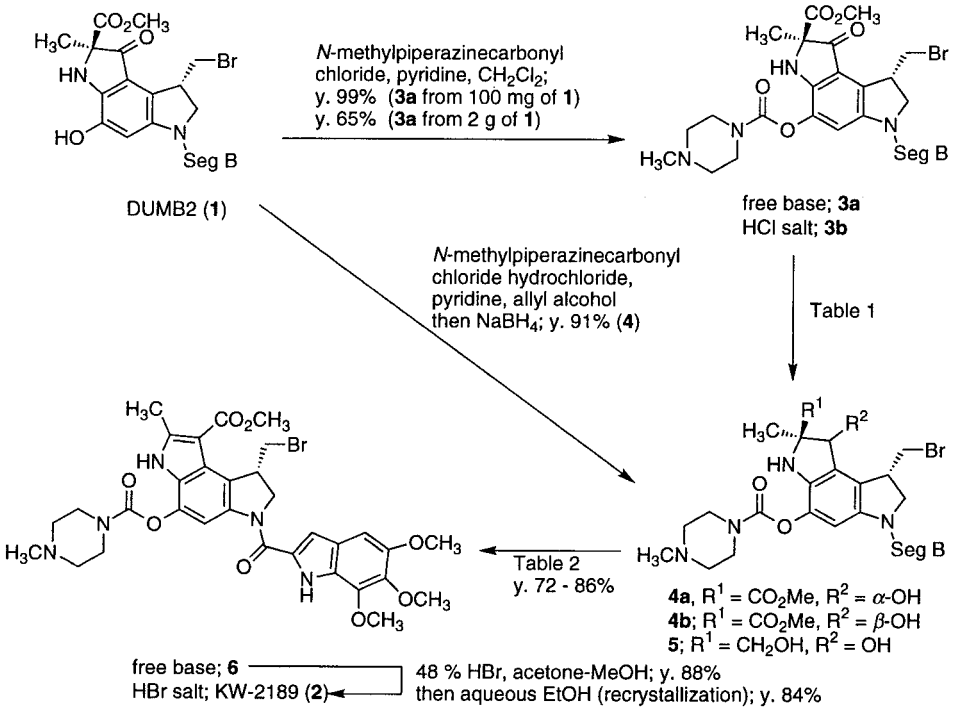
Scheme 1. **Synthesis of KW-2189**



improved stability and solubility in water (10 mg/mL) and has consequently been selected for clinical development.

Under basic conditions, the starting material **1** is easily converted to duocarmycin A (DUMA), followed by decomposition.[13] Consequently, the reported synthetic method of **2** required seven steps (24% overall yield) including tedious protection−deprotection procedures of the phenolic hydroxy group of **1** with the *tert*-butyldimethylsilyl group. For commercial production, more efficient procedures for large-scale operation were required. Our synthetic approach avoided protective group chemistry and proceeded by direct carbamoylation of the phenolic hydroxy group of **1**, followed by reduction and rearrangement to **2**, as shown in Scheme 1. The carbamoyl group might have been unstable under the reduction conditions with sodium borohydride and the subsequent acid treatment, but if this strategy could be followed, this synthetic pathway would not need a protecting group such as the silyl moiety. We report an improved and straightforward synthesis of KW-2189 (**2**).

Results and Discussion

DUMB2 (**1**) was almost quantitatively converted into the carbamate **3a** using *N*-methylpiperazinecarbonyl chloride (Scheme 1).[15] Sodium borohydride was then used for a selective reduction of the carbonyl group at C8 of compound **3a**. With regard to solvents, this reduction was carried out in best yield and stereoselectivity in ice-cold allyl alcohol when the 8α-hydroxy compound **4a** was obtained as the major product in 71% yield.[16] When other solvents were used, selectivity was reduced and the undesirable diols **5**[17] were formed (Table 1). The stereochemistries of compounds **4a** and **4b** at C8 were confirmed by NMR studies.[18]

Table 1. **Solvent effects on the $NaBH_4$ reduction of 3a[a]**

solvent	yield (%)[c] time (h)[b]	4a	4b	5
MeOH	2.0	57	22	10
allyl alcohol	1.0	71	6	8
i-PrOH	5.0	31	9	26
n-PrOH	4.0	31	6	18
$PhCH_2OH$	1.5	57	4	15
DMF	4.0	16	11	12
THF	4.0	24	7	14

[a] All reactions were carried out under ice-cooling using a 3.0 M amount of $NaBH_4$ (based on **3a**). [b] All reactions were quenched after disappearance of **3a** in HPLC analyses. [c] Determined by HPLC analyses. Other byproducts have not been identified.

During further studies on scale-up, it was found that the intermediate **3a** was degraded to the corresponding exo-methylene compound **7** in the isolation (Scheme 2).[19] Generally speaking, the yield of the reaction went down on scale-up; the stability of the product might be responsible. Compound **3a** was unstable in highly concentrated solution even at 20 °C and was presumed to easily convert to **7** in the evaporation process of the workup (Figure 3). The dehydrobromination was thought to be caused by the

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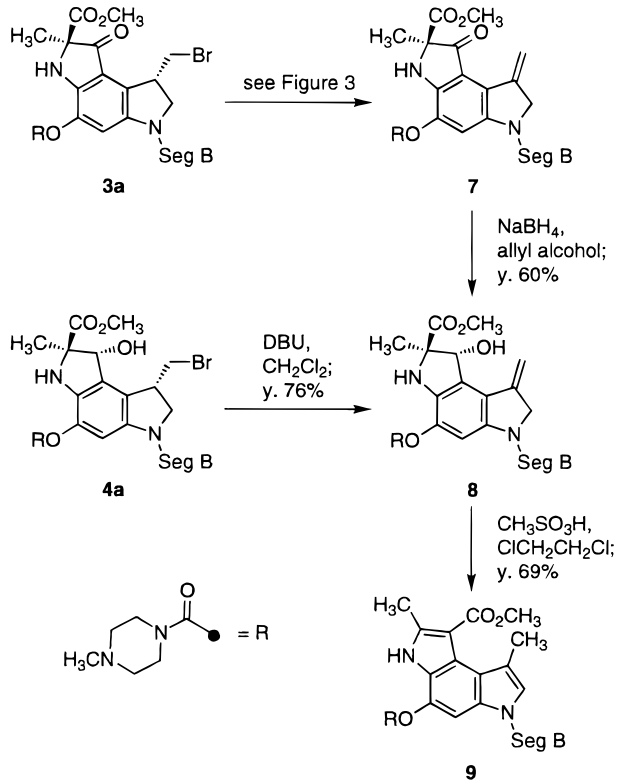
(16) To our knowledge, sodium borohydride reduction using allyl alcohol as reaction solvent has not been investigated.

(17) Diols **5** are the mixture of 8α- and 8β-hydroxy compounds. They are not separated by the various analytical methods such as HPLC and TLC analyses.

(18) More intense NOEs between 8-H and 7-CH_3 and between 8-H and 1-CH_2-Br were observed in **4b** than in **4a**, indicating that the stereochemistry of 8-H is β-face in **4a** and α-face in **4b**.

(19) For an example, a suspension of **1** (2.0 g) was carried out using the similar procedure described in the Experimental Section; **3a** was obtained in 65% yield and the yield of **7** was 34%.

Scheme 2. **A series of degradation compounds 7−9**



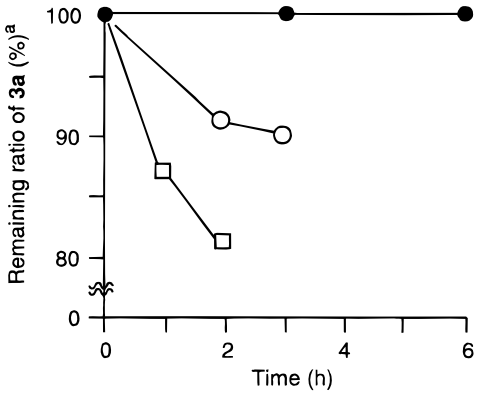


Figure 3. **Stabilities of 3a in highly concentrated solution. Legend: (a) determined by HPLC analyses (see Experimental Section), at 0 °C (●), 20 °C (○), and 35 °C (□).**

N-methylpiperazine fragment of **3a**. Thus, the degradation accelerated in line with the amount of triethylamine used. Decreasing the basicity of the reaction mixture by the addition of acids improved the stability of **3a** and as a result the corresponding hydrochloride salt **3b**, which was prepared from **3a** with hydrogen chloride in ethanol, was quite stable in the same concentrated solution even at 35 °C. However, the solubility of **3b** in chloroform which had been selected as the most appropriate solvent for the extraction of **3a** from the reaction mixture, was very low (less than 1 mg/mL) and the crystallization trials did not succeed as well.

Therefore, we decided to pursue a straightforward synthesis of **4** using the hydrochloride salt of *N*-methylpiperazinecarbonyl chloride without isolation of **3b** by telescoping in the reduction of the carbonyl group at C8. *N*-Methylpiperazinecarbonyl chloride hydrochloride was added to **1** in allyl alcohol in the presence of pyridine, and the mixture

Table 2. **Acid-catalyzed rearrangement of 4**

	conditions			
acid[a]	solvent	temp (°C)	time (h)[b]	yield of **6** (%)[c]
CSA[d]	$ClCH_2CH_2Cl$	50	2.5	83
CSA[d]	toluene	50	2.5	72
$TsOH·H_2O$	$ClCH_2CH_2Cl$	50	1.5	82
CH_3SO_3H	$ClCH_2CH_2Cl$	50	3.0	86
CF_3SO_3H	$ClCH_2CH_2Cl$	25	1.5	81
$BF_3·Et_2O$	$ClCH_2CH_2Cl$	50	1.0	85

[a] A 3 M amount of acid was used (based on **4**). [b] All reactions were quenched after disappearance of **4** in HPLC analyses. [c] Isolated yield. [d] (±)-10-Camphorsulfonic acid.

was stirred at 25 °C until the carbamoylation was complete. The mixture was then cooled, and sodium borohydride was added intermittently to the reaction mixture, until **3** disappeared completely and converted to **4**. This one-pot procedure accounted for the high isolated yield and quality improvement to the key intermediate **4**. The undesirable exomethylene compound **8** was detected at less than 0.2% in the isolated **4**.[20] In a typical experiment, 31.8 g of **4** (**4a** and **4b**) was obtained from 30 g of **1** (91% yield, **4a** 87%, **4b** 4%). This result was critical to the successful scale-up of the synthesis of KW-2189.

The Wagner−Meerwein rearrangement of **4** is the most important route to KW-2189, and up to now this acid-catalyzed rearrangement has been investigated using silyl-protected DUMB2.[13] Lewis acids such as aluminum chloride and boron trifluoride etherate were more effective than *p*-toluenesulfonic acid and methanesulfonic acid.[12] In our hands, the Lewis acid, boron trifluoride etherate, was effective, and Brønsted acids were also shown to be useful for the rearrangement (Table 2). On balance, we selected methanesulfonic acid as the most convenient reagent, because of the high yield, easy workup, and environmental concerns. Thus, **4** [a mixture of **4a** and **4b** (ca. 19:1)] was treated with methansulfonic acid in 1,2-dichloroethane to give **6** in an 86% yield.[21]

The free base **6** was finally converted to the corresponding hydrobromide salt **2** by treating it with 48% hydrobromic acid in a mixture of acetone and methanol when the salt **2** was precipitated from the reaction mixture and isolated easily by filtration (88% yield). A trace amount of **9** generated by acidic rearrangement of **8** was present in the reaction mixture, but was completely removed in the filtrates. To remove a small amount of residual acetone (approximately 2000 ppm), compound **2** was crystallized from aqueous ethanol (84% yield, more than 99.5% purity by HPLC[22]).

Conclusions

The procedure for the commercial production of high quality KW-2189 has been developed. The synthesis was

(20) The compound **4** is more stable than **3a** and the degradation product **8** was not increased even at the high concentration during the workup evaporation process. The compound **8** was obtained from **4a** only using a strong base such as DBU (Scheme 2).

(21) The rearrangement rate of **4b** was faster than that of **4a**. The 3-OH group of **4b** appears to be *pseudo* axial and well overlapped with π-electrons of the aromatic ring, so that the elimination proceeded more easily than in **4a**.

(22) Compound **9** was not detectable in the final compound **2** by HPLC analyses.

accomplished in three steps from DUMB2 (**1**) in 55% overall yield. The improvement in the yield significantly contributed to reduce the quantity of the starting material. It needed less than half of the natural DUMB2 (**1**) compared with the preliminary method.[13] Despite the lower yield, we should select toluene as the reaction solvent instead of 1,2-dichloroethane before launch (Table 2). The degradation compounds **7**−**9** (Scheme 2) were also synthesized and shown to be absent from the KW-2189 (**2**) prepared by this procedure.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded at 270 MHz on a JEOL JNM-GX270 and 300 MHz on a JEOL JNM-GX300 spectrometers, and signals are given in ppm using TMS as an internal standard. IR spectra were recorded on a Shimadzu FTIR-4300 spectrophotometer. SIMS spectra were recorded on a Hitachi M-80B mass spectrometer. HRFABMS were recorded on a JEOL JMS SX-102 mass spectrometer. Elemental analyses were performed using a Yanaco MT-3 CHN apparatus. For column chromatography, silica gel (SiO_2, Wako C-200) was used. All reagents and solvents were of commercial quality.

HPLC Analyses. The yields of **4a**, **4b**, and **5** in Table 1 and the stability of **3a** in Figure 3 were estimated using the following conditions: detector, ultraviolet absorption photometer (wavelength 330 nm); column, GL Sciences Inc. UNISIL PACK 5C18-250A; mobile phase, a mixture of 0.05 M phosphate buffer (pH 5.9) and CH_3CN (1:1); flow rate, 1.0 mL/min; column temperature, 35 °C; t_R (min), **2** and **6** (38), **3a** and **3b** (21), **4a** (9.7), **4b** (9.0), **5** (5.7), **7** (20), **8** (8.2), **9** (30).

Stability in Highly Concentrated Solutions (Figure 3). From the organic layer in the workup process for the synthesis of **3a** (described below), 1.0 mL of solution was exactly weighed out and evaporated to a concentration of 200 mg/mL under reduced pressure and maintained at the corresponding measured temperature. The time-course stability of **3a** was determined by HPLC analyses using 1-nitronaphthalene as the internal standard. In this stability test of **3a**, the only detected degradation product was **7**. Compounds **3b** and **4** were not degraded at 35 °C in the same procedures for **3a**.

Methyl (1*S*,7*R*)-1-(Bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-8-oxo-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylate (3a). To a suspension of **1** (DUMB2, 100 mg, 0.17 mmol) in CH_2Cl_2 (2.0 mL) and pyridine (0.14 mL) was added *N*-methylpiperazinecarbonyl chloride (55 mg, 0.34 mmol), and the mixture was stirred for 2.5 h at 25 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with $CHCl_3$ (5.0 mL). The organic layer was washed with saturated brine (5.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **3a** as a yellow solid: 120 mg (99%); mp 185−190 °C (dec); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.69 (s, 3H), 2.37 (s, 3H), 2.52 (br s, 4H), 3.61 (dd, J = 10.1, 8.9 Hz, 1H), 3.64 (br s, 2H), 3.76 (br s, 2H), 3.78 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 4.04 (dd, J = 10.1, 3.3 Hz, 1H), 4.08 (s, 3H), 4.25 (m, 1H), 4.59 (dd, J = 10.8, 4.5 Hz, 1H), 4.63 (br d, J = 9.3 Hz, 1H), 5.48 (br s, 1H), 6.87 (s, 1H), 6.94 (d, J = 2.3 Hz, 1H), 8.45 (s, 1H), 9.32 (br s, 1H); IR (KBr) υ = 2940, 1710, 1621, 1521, 1493, 1430, 1385, 1289, 1231, 1049, 1002 cm^{-1}; SIMS 716, 714 $(M + H)^+$.

Methyl (1*S*,7*R*)-1-(Bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-8-oxo-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylate Hydrochloride (3b). A solution of **3a** (29 mg, 0.041 mmol) in EtOH (1.5 mL) was treated with anhydrous 5.8 M HCl in EtOH (0.01 mL) at 0 °C for 1 h. The resulting mixture was poured into Et_2O (30 mL), and the whole was vigorously stirred at 0 °C for 1 h. The resulting precipitate was collected by filtration and dried under reduced pressure to afford **3b** as a bright yellow solid: 16 mg (53%); mp 183−195 °C; ^{1}H NMR (DMSO-d_6) δ = 1.51 (s, 3H, 7-CH_3), 2.83 (s, 3H, N-CH_3), 3.24 (br s, 4H, N-$(CH_2)_2$), 3.42 (br s, 4H, N-$(CH_2)_2$), 3.64 (s, 3H, CO_2CH_3*), 3.79 (s, 3H, 5′-OCH_3*), 3.81 (s, 3H, 6′-OCH_3*), 3.90 (dd, J = 10.0, 6.7 Hz, 1H, 1-C*H*HBr), 3.93 (s, 3H, 7′-OCH_3*), 3.97 (dd, J = 10.0, 3.1 Hz, 1H, 1-C*H*HBr), 4.20 (m, 1H, 1-H), 4.34 (dd, J = 10.4, 4.4 Hz, 1H, 2-H), 4.68 (t, J = 10.4 Hz, 1H, 2-H), 5.75 (s, 1H, 6-NH), 6.96 (s, 1H, 4′-H), 7.00 (d, J = 2.2 Hz, 1H, 3′-H), 7.83 (br s, 1H, HCl), 8.30 (s, 1H, 4-H), 11.35 (br s, 1H, 1′-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR (DMSO-d_6) δ = 20.1 (q), 36.5 (t), 41.2 (d), 42.1 (q), 43.9 (t, 2C), 51.7 (t, 2C), 53.0 (q), 54.9 (t), 56.0 (q), 61.0 (q), 61.1 (q), 71.0 (s), 98.0 (d), 106.1 (d), 115.2 (s), 120.3 (d), 123.2 (s), 125.3 (s), 127.1 (s), 130.6 (s), 135.5 (s), 135.9 (s), 139.0 (s), 139.9 (s), 149.2 (s), 151.5 (s), 151.6 (s), 159.6 (s), 168.9 (s), 197.5 (s); IR (KBr) υ = 2930, 1718, 1617, 1522, 1492, 1457, 1430, 1387, 1308, 1234, 1170, 1109 cm^{-1}; HRFABMS calcd for $C_{32}H_{36}{}^{79}BrN_5O_9$ *m*/*z* 714.1775 $(M + H)^+$, found 714.1794.

Methyl (1*S*,7*R*,8*R*)-1-(Bromomethyl)-8-hydroxy-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-tri-methoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo-[3,2-*e*]indole-7-carboxylate (4a). To a suspension of **1** (30.0 g, 51.0 mmol) in allyl alcohol (600 mL) and pyridine (41.2 mL) was added *N*-methylpiperazinecarbonyl chloride hydrochloride (19.3 g, 96.9 mmol), and the mixture was stirred for 4 h at 25 °C. After cooling to −5 to 0 °C, the reaction mixture was treated with $NaBH_4$ (25.0 g, 663 mmol), and then the mixture was stirred for 3.5 h at the same temperature. After the reaction, the pH of the mixture was adjusted to pH 6.95 with citric acid buffer solution (0.2 M, pH 1.9, 900 mL), and the quenched mixture was extracted with EtOAc (300 mL). The organic layer was washed with saturated brine (each 300 mL) twice and dried under reduced pressure, and the resulting residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH as eluents, initially 96:4 then 90:10) to afford **4a** as a slightly yellow solid: 31.8 g (87%); mp 200−205 °C (dec); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.57 (s, 3H, 7-CH_3), 2.17 (s, 1H, 8-OH), 2.34 (s, 3H, N-CH_3), 2.47 (br s, 4H, N-$(CH_2)_2$), 3.43 (dd, J = 10.3, 10.2 Hz, 1H, 1-C*H*HBr), 3.58 (br s, 2H, N-CH_2), 3.69 (br s, 2H, N-CH_2),

3.73 (s, 3H, CO_2CH_3*), 3.89 (s, 3H, 5′-OCH_3*), ca. 3.9 (m, 1H, 1-H), 3.94 (s, 3H, 6′-OCH_3*), 4.06 (s, 3H, 7′-OCH_3*), 4.12 (dd, 1H, J = 10.3, 3.1 Hz, 1-C*H*HBr), 4.50 (dd, J = 10.6, 8.7 Hz, 1H, 2-H), 4.56 (dd, J = 10.6, 4.6 Hz, 1H, 2-H), 4.79 (s, 1H, 6-NH), 5.33 (s, 1H, 8-H), 6.86 (s, 1H, 4′-H), 6.90 (d, J = 2.2 Hz, 1H, 3′-H), 8.03 (s, 1H, 4-H), 9.48 (s, 1H, NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS): δ = 18.9 (q), 36.2 (t), 42.9 (d), 43.9 (t), 44.4 (t), 46.0 (q), 53.0 (q), 54.5 (t), 54.5 (t), 55.8 (t), 56.3 (q), 61.1 (q), 61.5 (q), 71.8 (s), 75.3 (d), 97.7 (d), 106.2 (d), 113.7 (d), 123.7 (s), 125.4 (s), 126.7 (s), 126.9 (s), 129.9 (s), 135.9 (s), 137.6 (s), 138.8 (s), 139.3 (s), 140.4 (s), 150.1 (s), 152.6 (s), 159.4 (s), 175.6 (s); IR (KBr) υ = 1709, 1623, 1522, 1482, 1428, 1387, 1311, 1227, 1151, 1114, 1050 cm^{-1}; SIMS 718, 716 (M + H)$^+$; HRFABMS calcd for $C_{32}H_{38}{}^{79}BrN_5O_9$ *m/z* 716.1931 (M + H)$^+$, found 716.1932.

Methyl (1*S*,7*R*,8*S*)-1-(Bromomethyl)-8-hydroxy-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (4b). To a solution of **3a** (72 mg, 0.10 mmol) in MeOH (3.0 mL) was added $NaBH_4$ (11 mg, 0.30 mmol), and the mixture was stirred for 2 h at 0 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with EtOAc (5.0 mL each) three times. The organic layer was washed with saturated brine (5.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **4a** [49 mg (70%)] and a fraction containing compound **4b**. The latter was further purified by HPLC (ODS, 50:50 CH_3CN/H_2O as an eluent) to give **4b** as a slightly yellow solid: 10 mg (14%); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.53 (s, 3H, 7-CH_3), 2.32 (s, 3H, N-CH_3), 2.44 (br s, 4H, N-$(CH_2)_2$), 3.54 (dd, J = 10.4, 10.0 Hz, 1H, 1-C*H*HBr), 3.64 (br s, 4H, N-CH_2), 3.76 (dd, 1H, J = 10.4, 3.1 Hz, 1-C*H*HBr), 3.84 (s, 3H, CO_2-CH_3*), 3.85 (s, 3H, 5′-OCH_3*), ca. 3.9 (1H, 8-OH), ca. 4.0 (m, 1H, 1-H), 3.93 (s, 3H, 6′-OCH_3*), 4.04 (s, 3H, 7′-OCH_3*), 4.38 (dd, J = 10.7, 5.0 Hz, 1H, 2-H), 4.49 (dd, J = 10.7, 9.2 Hz, 1H, 2-H), 4.71 (s, 1H, 6-NH), 5.06 (s, 1H, 8-H), 6.83 (s, 1H, 4′-H), 6.86 (d, J = 2.2 Hz, 1H, 3′-H), 8.10 (s, 1H, 4-H), 9.36 (s, 1H, NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS) δ = 23.9 (q), 34.2 (t), 41.6 (d), 44.2 (t, 2C), 46.1 (q), 52.7 (q), 54.5 (t, 2C), 55.5 (t), 56.3 (q), 61.1 (q), 61.5 (q), 72.2 (s), 79.7 (d), 97.7 (d), 106.3 (d), 113.6 (d), 123.7 (s), 125.3 (s), 126.1 (s), 126.3 (s), 129.9 (s), 136.5 (s), 137.8 (s), 138.8 (s), 138.8 (s), 140.4 (s), 150.1 (s), 152.7 (s), 159.3 (s), 173.4 (s); IR (KBr) υ = 1716, 1625, 1527, 1458, 1435, 1314, 1232, 1114 cm^{-1}; SIMS 718, 716 (M + H)$^+$; HRFABMS calcd for $C_{32}H_{38}{}^{79}BrN_5O_9$ *m/z* 716.1931 (M + H)$^+$, found 716.1944.

(1*S*,7*R*)-1-(Bromomethyl)-8-hydroxy-7-(hydroxymethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]indole (5). To a solution of **3a** (143 mg, 0.20 mmol) in MeOH (6.0 mL) was added $NaBH_4$ (22 mg, 0.60 mmol), and the mixture was stirred for 2 h at 0 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with EtOAc (10.0 mL each) three times. The organic layer was washed with saturated brine (10.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 90:10 $CHCl_3$/MeOH as an eluent) to afford **5** as a slightly yellow solid: 14 mg (10%); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.29 (s, 3H, 7-CH_3), 2.35 (s, 3H, N-CH_3), 2.53 (br, 4H, N-$(CH_2)_2$), 3.31 (br, 1H, 1-C*H*HBr), 3.35 (s, 2H, 7-CH_2O), 3.50 (br, 2H, N-CH_2), 3.57 (br, 2H, N-CH_2), 3.86 (s, 3H, 5′-OCH_3*), ca. 3.9 (m, 1H, 1-H), 3.92 (s, 3H, 6′-OCH_3*), 4.04 (s, 3H, 7′-OCH_3*), 4.14 (br, 1H, 1-C*H*HBr), 4.44 (br, 2H, 2-H_2), 4.79 (s, 1H, 8-H), 6.83 (s, 1H, 4′-H), 6.85 (br, 1H, 3′-H), 8.14 (s, 1H, 4-H), 9.74 (s, 1H, 1′-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS) δ = 17.1 (q), 36.4 (t), 42.8 (d), 43.8 (t), 44.2 (t), 45.9 (q), 54.3 (t, 2C), 55.8 (t), 56.1 (q), 61.0 (q), 61.4 (q), 67.1 (t), 68.5 (s), 74.6 (d), 97.6 (d), 106.2 (d), 112.7 (d), 123.5 (s), 125.3 (s), 127.1 (s), 128.9 (s), 129.8 (s), 135.8 (s), 136.9 (s), 138.7 (s), 138.8 (s), 140.2 (s), 149.9 (s), 152.6 (s), 159.4 (s); IR (KBr) υ = 3350, 1700, 1445, 1225, 1232 cm^{-1}; SIMS 690, 688 (M + H)$^+$; HRFABMS calcd for $C_{31}H_{38}{}^{79}BrN_5O_8$ *m/z* 688.1982 (M + H)$^+$, found: 688.1982.

Methyl (7*R*)-7-Methyl-1-methylene-5-[(4-methylpiperazinyl)carbonyloxy]-8-oxo-3-[(5,6,7-trimethoxy-2-indolyl)-carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (7). To a solution of **3a** (72 mg, 0.10 mmol) in CH_2Cl_2 (2.0 mL) was added Et_3N (0.042 mL, 0.30 mmol), and the mixture was stirred for 10 h at 25 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with $CHCl_3$ (5.0 mL). The organic layer was washed with saturated brine (5.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **7** as a yellow solid: 57 mg (90%); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.70 (s, 3H, 7-CH_3), 2.37 (s, 3H, N-CH_3), 2.52 (br, 4H, N-$(CH_2)_2$), 3.64 (br, 2H, N-CH_2), 3.76 (br, 2H, N-CH_2), 3.79 (s, 3H, CO_2CH_3*), 3.91 (s, 3H, 5′-OCH_3*), 3.94 (s, 3H, 6′-OCH_3*), 4.07 (s, 3H, 7′-OCH_3*), 5.14 (s, 2H, 2-H_2), 5.48 (s, 1H, 6-NH), 5.49 (br, 1H, 1-C*H*H), 6.86 (s, 1H, 4′-H), 6.94 (d, J = 2.2 Hz, 1H, 3′-H), 6.98 (br, 1H, 1-C*H*H), 8.65 (s, 1H, 4-H), 9.39 (s, 1H, 1′-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS) δ = 22.2 (q), 44.2 (t), 44.7 (t), 46.1 (q), 53.6 (q), 54.5 (t), 54.6 (t), 55.8 (t), 56.3 (q), 61.1 (q), 61.5 (q), 70.7 (s), 97.7 (d), 106.5 (d), 111.3 (t), 116.4 (s), 120.4 (d), 123.8 (s), 125.6 (s), 126.5 (s), 129.5 (s), 138.2 (s), 138.6 (s), 138.7 (s), 140.5 (s), 141.3 (s), 150.2 (s), 152.1 (s), 152.2 (s), 158.9 (s), 169.5 (s), 196.6 (s); IR (KBr) υ = 1702, 1616, 1491, 1441, 1229 cm^{-1}; SIMS 634 (M + H)$^+$; HRFABMS calcd for $C_{32}H_{35}N_5O_9$ *m/z* 634.2513 (M + H)$^+$, found: 634.2508.

Methyl (7*R*,8*R*)-8-Hydroxy-7-methyl-1-methylene-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2,7,8-tetrahydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylate (8). To a solution of **7** (63 mg, 0.10

mmol) in allyl alcohol (3.0 mL) was added $NaBH_4$ (8 mg, 0.20 mmol), and the mixture was stirred for 5 h at 0 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with EtOAc (5.0 mL each) three times. The organic layer was washed with saturated brine (5.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **8** as a slightly yellow solid: 38 mg (60%). Another synthetic method is described below. To a solution of **4a** (72 mg, 0.10 mmol) in CH_2Cl_2 (3.0 mL) was added DBU (0.030 mL, 0.20 mmol), and the mixture was stirred for 10 h at 25 °C. After being quenched with aqueous phosphate buffer (0.05 M, pH 5.9, 5.0 mL), the mixture was extracted with $CHCl_3$ (5.0 mL). The organic layer was washed with saturated brine (5.0 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure. The resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **8** as a slightly yellow solid: 57 mg (90%); ^{1}H NMR ($CDCl_3$/TMS) δ = 1.65 (s, 3H, 7-CH_3), 2.33 (s, 3H, N-CH_3), 2.44 (br, 4H, N-$(CH_2)_2$), 3.53 (br, 4H, N-$(CH_2)_2$), 3.72 (s, 3H, CO_2CH_3), 3.77 (s, 3H, 5′-OCH_3*), 3.93 (s, 3H, 6′-OCH_3*), 4.04 (s, 3H, 7′-OCH_3*), 4.81 (d, J = 15.6 Hz, 1H, 2-H), 4.86 (s, 1H, 6-NH), 4.92 (d, J = 14.7 Hz, 1H, 2-H), 5.21 (br, 1H, 1-C*H*H), 5.47 (s, 1H, 8-H), 5.72 (br, 1H, 1-C*H*H), 6.77 (s, 1H, 4′-H), 6.80 (d, J = 2.2 Hz, 1H, 3′-H), 8.27 (s, 1H, 4-H), 9.38 (s, 1H, 1′-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS) δ = 19.1 (q), 44.1 (t, 2C), 46.1 (q), 53.0 (q), 54.5 (t, 2C), 55.6 (t), 56.1 (q), 61.1 (q), 61.5 (q), 71.6 (s), 75.0 (d), 97.5 (d), 106.4 (d), 106.5 (t), 114.1 (d), 123.8 (s), 124.1 (s), 124.5 (s), 125.3 (s), 139.7 (s), 136.5 (s), 138.8 (s), 139.1 (s), 140.1 (s), 140.2 (s), 140.3 (s), 150.0 (s), 152.7 (s), 158.6 (s), 176.0 (s); IR (KBr) υ = 3440, 1718, 1617, 1455, 1235 cm^{-1}; SIMS 636 $(M + H)^+$; HRFABMS calcd for $C_{32}H_{37}N_5O_9$ m/z 636.2670 $(M + H)^+$, found 636.2666.

Methyl (1*S*)-1-(Bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (6). To a solution of **4** (30.0 g, 41.9 mmol) in $ClCH_2CH_2Cl$ (1,200 mL) was added $MeSO_3H$ (6.80 mL, 105 mmol), and the mixture was stirred for 5 h at 50 °C. After cooling, the reaction mixture was quenched with saturated $NaHCO_3$ (600 mL) and extracted with $CHCl_3$ (300 mL). The organic layer was washed with saturated brine (300 mL) and dried over Na_2SO_4, and the filtrate was dried under reduced pressure, the resulting residue was purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) to afford **6** as a slightly yellow solid: 25.3 g (86%); mp 160−163 °C; ^{1}H NMR ($CDCl_3$/TMS) δ = 2.39 (s, 3H, 7-CH_3), 2.56 (br s, 4H, N-$(CH_2)_2$), 2.60 (s, 3H, N-CH_3), 3.21 (br d, J = 9.6 Hz, 1H, 1-C*H*HBr), 3.63 (br s, 2H, N-CH_2), 3.67 (br s, 2H, N-CH_2), 3.79 (dd, J = 9.6, 2.2 Hz, 1H, 1-C*H*HBr), 3.91 (s, 3H, CO_2CH_3*), 3.95 (s, 3H, 5′-OCH_3*), 3.95 (s, 3H, 6′-OCH_3*), 4.07 (s, 3H, 7′-OCH_3*), 4.49 (dd, J = 10.4, 8.8 Hz, 1H, 2-H), 4.57 (m, 1H, 1-H), 4.74 (dd, J = 10.4, 1.2 Hz, 1H, 2-H), 6.89 (s, 1H, 4′-H), 6.99 (d, J = 2.3 Hz, 1H, 3′-H), 8.12 (s, 1H, 4-H), 9.41 (s, 2H, 6-NH and 1′-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR ($CDCl_3$/TMS): δ = 15.0 (q), 36.6 (t), 44.3 (t), 44.5 (d), 44.8 (t), 46.1 (q), 51.2 (q), 54.5 (t), 54.7 (t), 55.4 (t), 56.3 (q), 61.1 (q), 61.5 (q), 97.8 (d), 104.3 (s), 106.4 (d, 2C), 120.1 (s), 123.7 (s), 124.5 (s), 125.4 (s), 125.6 (s), 130.1 (s), 136.2 (s), 138.7 (s), 138.9 (s), 140.5 (s), 146.6 (s), 150.1 (s), 153.3 (s), 160.0 (s), 165.4 (s). IR (KBr) υ = 2944, 1698, 1491, 1410, 1313, 1217, 1110 cm^{-1}; SIMS 700, 698 $(M + H)^+$; HRFABMS calcd for $C_{32}H_{36}{}^{79}BrN_5O_8$ m/z 698.1825 $(M + H)^+$, found 698.1812.

Methyl (1*S*)-1-(Bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate hydrobromide (2, KW-2189). To a solution of **6** (56.3 g, 80.6 mmol) in acetone (500 mL) and MeOH (2,250 mL) was added 48% hydrobromic acid (13.8 mL, 121 mmol), and the mixture was stirred for 7 h at 25 °C and for 9 h under ice-cooling. The precipitated crystals were filtered, washed with cold MeOH/EtOH mixture (1:1) (300 mL), and dried under vacuum to afford semi-purified **2**: 55.3 g (88%). The semi-purified **2** (52.4 g, 67.2 mmol) was further purified by recrystallization from aqueous EtOH (2,830 mL) and drying under vacuum to afford **2** as a light yellow crystalline powder: 44.0 g (84% from semi-purified **2**); mp 252 °C (dec); ^{1}H NMR (DMSO-d_6/TMS) δ = 2.70 (br s, 3H, 7-CH_3), 2.89 (br s, 3H, N-CH_3), 3.26 (br s, 4H, N-$(CH_2)_2$), 3.40 (br s, 1H, 1-C*H*HBr), 3.53 (br s, 4H, N-$(CH_2)_2$), 3.80 (br s, 1H, 1-C*H*HBr), 3.81 (s, 3H, 6′-OCH_3*), 3.82 (s, 3H, 5′-OCH_3*), 3.86 (s, 3H, CO_2CH_3*), 3.96 (s, 3H, 7′-OCH_3*), 4.44 (br d, J = 10.9 Hz, 1H, 2-H), 4.48 (m, J = 8.6, 2.8 Hz, 1H, 1-H), 4.65 (dd, J = 10.9, 8.6 Hz, 1H, 2-H), 6.98 (s, 1H, 4′-H), 7.02 (br d, J = 2.3 Hz, 1H, 3′-H), 7.94 (br s, 1H, 4-H), 10.0 (br s, 1H, HBr), 11.24 (br s, 1H, 1′-NH), 12.02 (br s, 1H, 6-NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR (DMSO-d_6/TMS) δ = 14.5 (q), 38.2 (t), 41.0 (t, 2C), 42.5 (q), 43.0 (d), 50.9 (q), 52.2 (t, 2C), 55.4 (t), 56.1 (q), 61.0 (q), 61.1 (q), 98.2 (d), 103.3 (s), 105.8 (d), 106.0 (d), 119.6 (s), 123.2 (s), 123.9 (s), 125.2 (s), 125.6 (s), 131.2 (s), 135.6 (s), 138.2 (s), 139.0 (s), 139.8 (s), 146.8 (s), 149.3 (s), 152.1 (s), 160.0 (s), 165.0 (s); IR (KBr) υ = 3460, 2947, 2550, 1718, 1410, 1219, 746 cm^{-1}; UV λ_{max} (CH_3OH) nm (ϵ) 331 (4010); $[\alpha]^{20}_D$ = −27.9° (c 1.5, $CHCl_3$); SIMS 698 $(M + H)^+$. Anal. Calcd for $C_{32}H_{36}BrN_5O_8$·HBr: C, 49.31; H, 4.89; N, 8.96. Found: C, 49.31; H, 4.78; N, 8.98.

Methyl 1,7-Dimethyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)carbonyl]-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (9). Acidic rearrangement of **8** (32 mg, 0.050 mmol) with $MeSO_3H$ (0.008 mL,0.125 mmol) was carried out in $ClCH_2CH_2Cl$ (1.2 mL) at 50 °C to afford **9** in a manner similar to the synthesis of **6**. Compound **9** was isolated and purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) as a slightly yellow solid: 21 mg (69%); ^{1}H NMR (DMSO-d_6/TMS) δ = 2.28 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.49 (br, 4H, N-$(CH_2)_2$), 2.60 (s, 3H, N-CH_3), 3.51 (br, 2H, N-CH_2), 3.76 (br, 2H, N-CH_2), 3.85 (s, 3H, 6′-OCH_3*), 3.85 (s, 3H, 5′-OCH_3*), 3.85 (s, 3H, CO_2CH_3*), 3.99 (s, 3H, 7′-OCH_3*),

7.03 (s, 1H, 4′-H), 7.14 (s, 1H, 2-H), 7.74 (s, 1H, 3′-H), 8.04 (s, 1H, 4-H), 11.81 (s, 1H, NH), 12.00 (s, 1H, NH) {assignments with an asterisk may be interchanged}; ^{13}C NMR (DMSO-d_6/TMS) δ = 13.0 (q), 13.4 (q), 43.8 (t), 44.2 (t), 45.8 (q), 51.1 (q), 54.1 (t, 2C), 55.9 (q), 61.0 (q), 61.1 (q), 98.2 (d), 103.8 (d), 106.4 (s), 110.3 (d), 117.7 (s), 119.0 (s), 120.1 (s), 122.9 (s), 124.8 (s), 125.5 (s), 126.8 (s), 129.4 (s), 131.0 (s), 134.3 (s), 139.1 (s), 140.1 (s), 140.6 (s), 149.5 (s), 152.9 (s), 160.2 (s), 166.7 (s); IR (KBr) υ = 1701, 1419, 1207 cm^{-1}; SIMS 618 $(M + H)^+$; HRFABMS calcd for $C_{32}H_{35}N_5O_8$ *m*/*z* 618.2564 $(M + H)^+$, found 618.2563.

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