STRUCTURE-ACTIVITY RELATIONSHIPS IN A SERIES OF PIPERAZINE-2,3-DIONE CONTAINING PENICILLINS AND CEPHALOSPORINS

II. DERIVATIVES SUBSTITUTED AT N(4) OF THE PIPERAZINE RING

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The structure-activity relationships within a series of penicillins and cephalosporins containing an N(4)-substituted piperazine-2,3-dione moiety in the C(6)/C(7)-β-side chain are discussed.

Since the discovery in these laboratories of BRL 36650 (**1**)[1], a C(6)-α-formamidopenicillin containing an N(4)-ethylpiperazine-2,3-dione group in the C(6)-β-side chain, we have continued to search for new C(6)-α-formamidopenicillins/C(7)-α-formamidocephalosporins[2~7] with an expanded spectrum of antibacterial activity when compared with **1**. One outcome has been the synthesis of C(7)-α-formamidocephalosporins of type (**14**)[7]. In common with BRL 36650 (**1**), **14** is a potent, β-lactamase-stable antibiotic, particularly active against *Pseudomonas* species[8]. The structural similarities between **1** and **14** are apparent, not only in the C(6)/C(7)-α-formamido substituents, but also in the C(6)/C(7)-β-side chains, both of which contain a catecholic amino acid residue acylated with the N(4)-ethylpiperazine-2,3-dione radical.

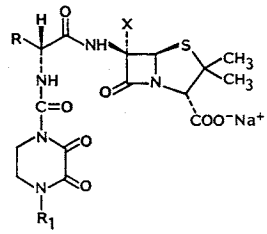
Our early attempts at producing C(6)/C(7)-α-formamido analogues of **1** and **14** with improved biological properties, involved the replacement of the catechol and/or the piperazine-2,3-dione groups[2~5]. No new compounds with significantly enhanced activity against either Gram-positive or Gram-negative bacteria were obtained. Similarly, modification of the piperazine-2,3-dione moiety itself, by substitution at the C(5) or C(6) carbon atoms[9], did not produce any derivatives with advantages over BRL 36650 (**1**).

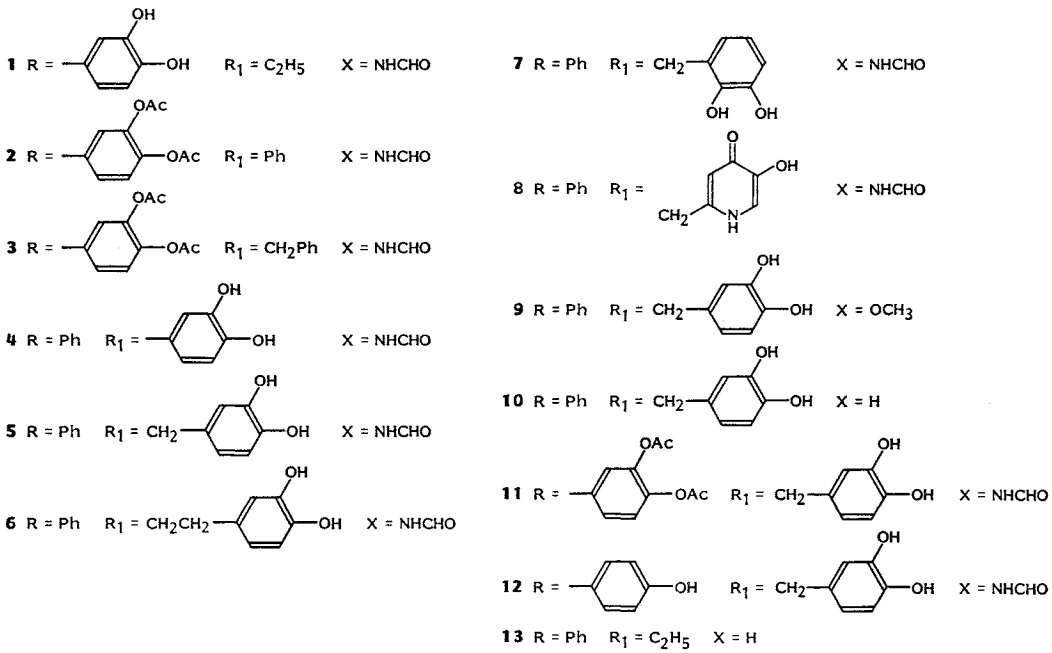
We now report the preparation of analogues of **1** and **14** with improved antibacterial potency and/or an expanded spectrum of biological activity, having varying substituents at N(4) of the piperazine-2,3-dione ring.

Chemistry

The synthesis of the C(6)-α-substituted penicillins (**2~9**, **11** and **12**) is shown in Scheme 1[9,10]. The C(6)-unsubstituted penicillin (**10**) was prepared by a simple Schotten-Baumann acylation of ampicillin[11]. The catecholic hydroxyl groups in the N(4)-substituents were protected as benzyl ethers and deprotected by catalytic hydrogenation as shown. The acetate protecting groups in penicillins (**2** and **3**) are known to be labile[9] and were not removed.

The cephalosporins (**15~17**) were prepared by methods developed in these laboratories[12,13]

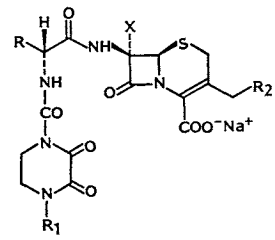


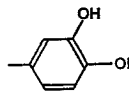


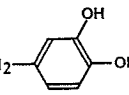
and are illustrated in Scheme 2. The catecholic hydroxyl groups were protected as acetates and, because of the uncertainty of their stability, deprotected by hydrolysis with citrus acetyl esterase at neutral pH[7].

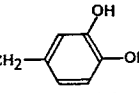
Results and Discussion

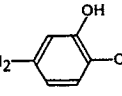
Replacement of the N(4)-ethyl substituent by the more lipophilic phenyl or benzyl groups, gave compounds of similar **2** or slightly lower activity **3** (see Table 1). However, the R and R_1 substituents in **2** could be interchanged to give a new derivative **4** without significantly affecting the antibacterial activity. The higher homologues, **5** and **6**, were likewise potent anti-Gram-negative agents, **5** being slightly more active than BRL 36650 (**1**) itself. The isomeric 2,3-dihydroxy analogue (**7**) and the bioisosteric pyridone (**8**) were also highly active antibiotics, although the latter showed poor activity against *Streptococcus pyogenes* and cefotaxime-resistant *Serratia marcescens* HCN 3956. The incorporation of a second catechol group into the phenylglycyl residue of **5**, gave the analogue **11**, which showed no further improvement in activity over the phenyl (**5**) or 4-hydroxyphenyl (**12**) derivatives. Replacement of the C(6)-α-formamido substituent in **5** with a methoxy group, gave penicillin **9**, which exhibited rather poor activity against *Proteus mirabilis* and cephalosporinase-producing Enterobacteriaceae. However, the necessity of a C(6)-α-substituent for β-lactamase stability, was clearly

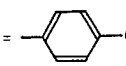


14 R =  $R_1 = C_2H_5$ R_2 =  X = NHCHO

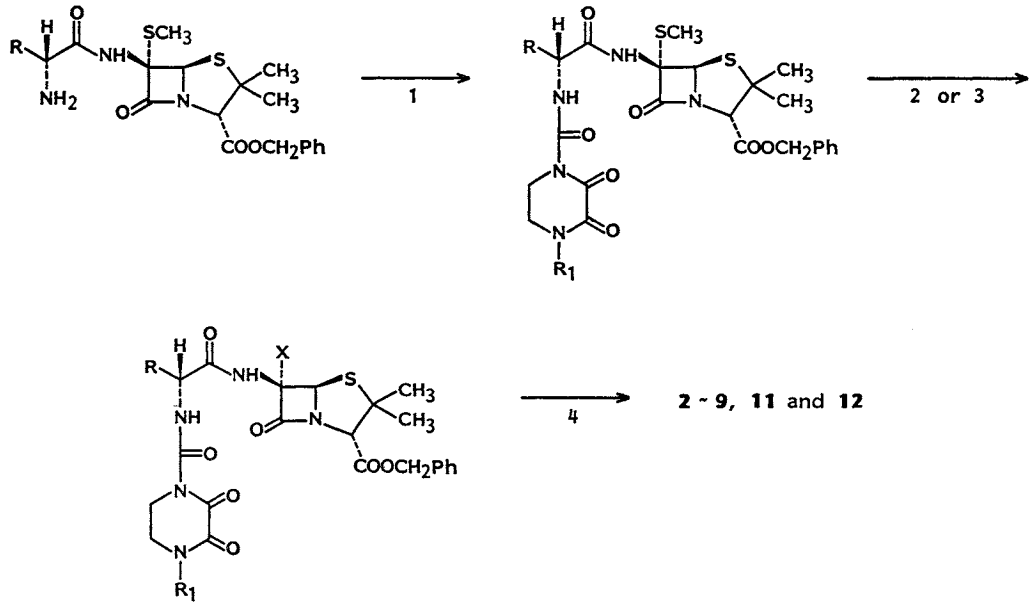
15 R = Ph $R_1 = CH_2$  $R_2 = OCOCH_3$ X = H

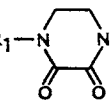
16 R = Ph $R_1 = CH_2$  R_2 =  X = NHCHO

17 R = Ph $R_1 = CH_2$  R_2 =  X = NHCHO

18 R =  $R_1 = C_2H_5$ R_2 =  X = H

Scheme 1.



1) R_1—  —COCl, $N(C_2H_5)_3$, 2) NH_3, $Hg(OAc)_2$ then $HCOOCOCH_3$, pyridine,
3) CH_3OH - $Hg(OAc)_2$, 4) H_2 - Pd/C.

demonstrated by comparing the activities of **5** and **10**.

Similar results were obtained in the cephalosporin series. For example, the antibacterial activity of **16**, or the corresponding *N*-methyltetrazole analogue (**17**), both incorporating a catechol group in the diketopiperazine N(4)-substituent, was similar to that of cephalosporin (**14**), in which the catechol

Scheme 2.

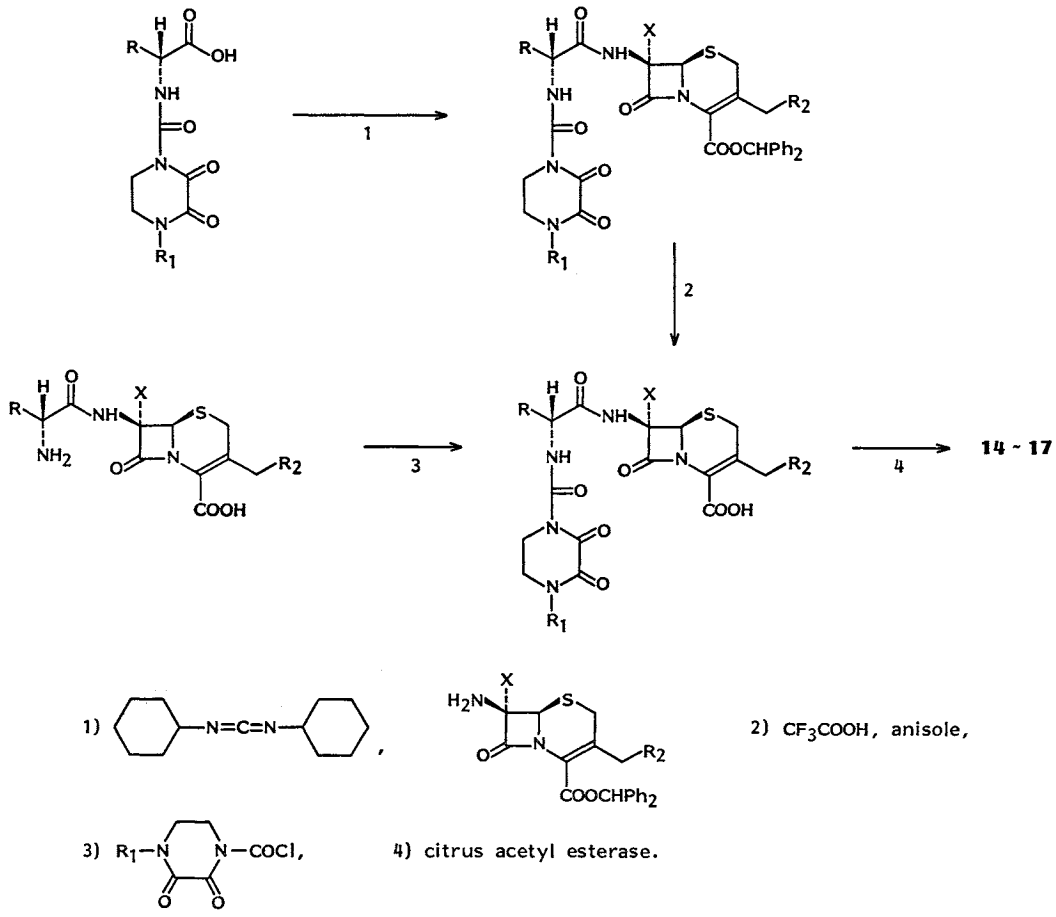


Table 1. *In vitro* activity of catechol containing

Organism	1	2	3	4	5	6	7
Escherichia coli DCO	≤0.06	≤0.06	0.5	≤0.06	≤0.06	0.06	0.12
E. coli DCO RTEM	≤0.06	≤0.06	0.5	≤0.06	≤0.06	0.06	0.12
Klebsiella pneumoniae T167	≤0.06	≤0.06	0.5	≤0.06	≤0.06	0.12	0.25
Enterobacter cloacae P99[b]	0.5	0.25	2.0	0.25	0.12	1.0	0.5
Serratia marcescens US 32	0.5	0.12	0.5	0.25	0.12	1.0	0.25
S. marcescens HCN 3956[b]	1.0	2.0	4.0	8.0	1.0	8.0	4.0
Proteus mirabilis C977	0.5	0.5	4.0	2.0	0.5	4.0	1.0
Pseudomonas aeruginosa 10662	0.12	0.5	2.0	0.25	≤0.06	0.25	0.25
Streptococcus pyogenes CN10	2.0	1.0	2.0	1.0	1.0	1.0	1.0
Staphylococcus aureus Oxford	>64	>64	>64	>64	>64	>64	>64

[a] Serial dilution in Diagnostic Sensitivity Test agar containing 5% defibrinated horse blood inoculated

[b] Constitutive class I β-lactamase producing strain.

resided in the phenylglycyl portion of the C(7)-β-side chain. Indeed, the Gram-positive activity of **16** was slightly improved compared with **14**. The best Gram-positive activity, however, was seen in the cephalosporins lacking a C(7)-α-substituent, **15** and **18**. Unfortunately, **15** and **18** showed poor activity against Gram-negative bacteria producing class I β-lactamases constitutively.

Conclusion

The introduction of a catechol-containing group into the N(4)-substituent of penicillins and cephalosporins containing a piperazine-2,3-dione moiety in the C(6)/C(7)-β-side chain, led to compounds with potent Gram-negative activity.

Experimental

Analytical and general experimental techniques are as described in our earlier paper[9].

Sodium 6β-[D-2-[4-(3,4-Dihydroxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]penicillanate (**10**)

4-(3,4-Dibenzyloxybenzyl)-2,3-dioxopiperazine[13] (416 mg, 1 mmol) was converted to its carbonyl chloride by known methods[9] and used to acylate sodium ampicillin (371 mg, 1 mmol) under standard conditions[12]. The product from above was hydrogenated in saturated, aqueous sodium hydrogen carbonate in the usual manner[9] and the title compound isolated, after treatment with sodium 2-ethylhexanoate in 4-methylpentan-2-one, by filtration (87 mg, 14%): IR (KBr) cm^{-1} 1764, 1712, 1676, 1605; ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 1.41 (3H, s), 1.54 (3H, s), 3.32~3.51 (4H, m), 3.83 (1H, s), 4.41 (2H, br s), 5.24 (1H, d, J=4 Hz), 5.32~5.43 (1H, m), 5.73 (1H, d, J=7 Hz), 6.50~6.70 (3H, m), 7.24~7.50 (5H, m), 9.28 (1H, br d, J=5 Hz, exchangeable), 9.84 (1H, d, J=7 Hz, exchangeable); fast atom bombardment mass spectrum (FAB-MS) (positive ion, thioglycerol) m/z 656 (M+Na, $C_{28}H_{28}N_5O_9SNa$).

Sodium 6β-[D-2-[4-(3,4-Dihydroxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]-6α-formamidopenicillanate (**5**)

4-(3,4-Dibenzyloxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl chloride (478.5 mg, 1 mmol) (prepared as described above) was used to acylate benzyl 6β-[D-2-amino-2-phenylacetamido]-6α-methylthiopenicillanate (485 mg, 1 mmol) in the normal manner[9]. Work up and chromatography (50% EtOAc-cyclohexane) gave benzyl 6β-[D-2-[4-(3,4-dibenzyloxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]-6α-methylthiopenicillanate (650 mg, 70%): IR (KBr) cm^{-1} 1779, 1742, 1714, 1683; ^1H NMR (250 MHz, $CDCl_3$) δ 1.01 (3H, s), 1.23 (3H, s), 2.29 (3H, s), 3.00~3.30 (2H, m), 3.41~3.84 (2H, m), 4.33 (1H, s), 4.41 and 4.58 (2H, ABq, J=12 Hz), 5.10~5.25 (6H, m), 5.50 (1H, d, J=7 Hz), 5.54 (1H, s), 6.66~7.56 (24H, m), 10.02 (1H, d, J=7 Hz, exchangeable); FAB-MS (positive ion,

penicillins and cephalosporins (**1**~**18**) (MIC[a], μg/ml).

8	9	10	11	12	13	14	15	16	17	18
≤0.06	0.25	≤0.06	≤0.06	≤0.06	1.0	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
≤0.06	0.25	>64	≤0.06	≤0.06	64	≤0.06	2.0	≤0.06	≤0.06	1.0
0.06	0.25	2.0	0.06	0.25	8.0	≤0.06	0.12	≤0.06	≤0.06	0.25
2.0	32	16	0.5	0.5	64	0.5	32	0.25	0.5	>64
2.0	64	1.0	0.5	0.25	0.5	<0.06	4.0	0.12	0.06	2.0
>64	>64	64	4.0	4.0	64	1.0	>64	4.0	0.5	>64
1.0	64	2.0	2.0	1.0	0.5	0.25	1.0	0.5	0.5	2.0
1.0	2.0	0.12	0.12	0.25	4.0	0.06	<0.06	0.06	0.06	8.0
>64	8.0	0.25	2.0	2.0	0.12	0.25	0.12	0.06	0.12	0.12
>64	>64	2.0	>64	>64	1.0	8.0	1.0	4.0	8.0	2.0

with 0.001 ml of an overnight broth culture diluted 1/100 (approx 10^4 cfu/spot).

thioglycerol) *m/z* 928 (M+H, $C_{50}H_{49}N_5O_9S_2$).

The above ester was converted by known methods[1] to benzyl 6β-[D-2-[4-(3,4-dibenzyloxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]-6α-formamidopenicillanate (203 mg, 51%) after chromatography (EtOAc): IR (KBr) cm^{-1} 1785, 1740, 1715, 1687; ^1H NMR (250 MHz, $(CD_3)_2CO$) δ 0.90 (3H, s), 1.15 (3H, s), 3.35~3.54 (2H, m), 3.73~3.90 (2H, m), 4.37 (1H, s), 4.48~4.68 (2H, ABq, *J*=14 Hz), 5.11~5.28 (6H, m), 5.59 (1H, s), 5.71 (1H, d, *J*=7 Hz), 6.86~7.07 (3H, m), 7.17~7.63 (20H, m), 8.18 (1H, d, *J*=1 Hz), 8.26 (1H, br s, exchangeable), 8.78 (1H, s, exchangeable), 10.08 (1H, d, *J*=7 Hz, exchangeable); FAB-MS (positive ion, 3-nitrobenzyl alcohol - sodium acetate) *m/z* 947 (M+Na, $C_{50}H_{48}N_6O_{10}S$).

Use of methanol[10] in the above displacement instead of ammonia gave the C(6)-α-methoxy-analogue, which was progressed as described below to give **9**.

The benzyl protecting groups were removed by hydrogenation and the title compound (160 mg, 79%) isolated by filtration, after treatment with sodium 2-ethylhexanoate in 4-methylpentan-2-one: IR (KBr) cm^{-1} 1770, 1710, 1676, 1608; ^1H NMR (250 MHz, D_2O) δ 0.84 (3H, s), 1.23 (3H, s), 3.49~3.61 (2H, m), 3.83~3.96 (4H, 2×m), 4.13 (1H, s), 4.54 (2H, br s), 5.44 (1H, s), 5.56 (1H, s), 6.76 (1H, br d, *J*=8 Hz), 6.84 (1H, br s), 6.88 (1H, d, *J*=8 Hz), 7.36~7.54 (5H, m), 8.09 (1H, s); FAB-MS (positive ion, thioglycerol) *m/z* 677 (M+H, $C_{29}H_{29}N_6O_{10}SNa$).

Penicillins (**4~8**) were prepared as described above. Diacetoxypenicillins (**2**, **3** and **11**) were prepared as stated from benzyl 6β-[D-2-amino-2-(3,4-diacetoxyphenyl)acetamido]-6α-methylthiopenicillanate[9]. Similarly, the *p*-hydroxy analogue (**12**) was prepared from benzyl 6β-[D-2-amino-2-(4-benzyloxycarbonyloxyphenyl)acetamido]-6α-methylthiopenicillanate[10].

Sodium 7β-[D-2-[4-(3,4-Dihydroxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]cephalosporanate (**15**)

Treatment of 1-(3,4-dihydroxybenzyl)-2,3-dioxopiperazine[13] (236 mg, 1.05 mmol) with trichloromethyl chloroformate in the usual manner[9] and reaction of the product with 3-acetoxy-7β-[D-2-amino-2-phenylacetamido]cephalosporanic acid (219 mg, 1 mmol) under standard conditions[11] gave the title compound (419 mg, 61%), after treatment with sodium 2-ethylhexanoate in 4-methylpentan-2-one: IR (KBr) cm^{-1} 1765, 1715, 1685, 1609; ^1H NMR (250 MHz, D_2O) δ 2.04 (3H, s), 3.07~3.54 (4H, m), 3.65~3.98 (2H, m), 4.49 (2H, br s), 4.61 and 4.81 (2H, ABq, *J*=12 Hz), 4.96 (1H, d, *J*=5 Hz), 5.41 (1H, s), 5.62 (1H, d, *J*=5 Hz), 6.68~6.89 (3H, m), 7.34~7.53 (5H, m); FAB-MS (positive ion, thioglycerol) *m/z* 690 (M+H, $C_{30}H_{28}N_5O_{11}SNa$).

Cephalosporin **17** was prepared by the same method from the appropriate aminocephalosporin[13].

Sodium 7β-[D-2-[4-Dihydroxybenyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetamido]-7α-formamido-3-[(1,3,4-thiadiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylate (**16**)

Diphenylmethyl 7β-amino-7α-formamido-3-[(1,3,4-thiadiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylate[12] (217 mg, 0.4 mmol) was acylated with D-2-[4-(3,4-diacetoxybenzyl)-2,3-dioxopiperazin-1-ylcarbonyl]amino-2-phenylacetic acid[13] (200 mg, 0.4 mmol), prepared by standard methods[7], in THF in the presence of *N*,*N*′-dicyclohexycarbodiimide according to the literature[12]. The product was purified by chromatography (90% EtOAc - cyclohexane) and the ester protecting group removed with TFA and anisole[4]. Removal of the acetate protecting groups with citrus acetyl esterase[7], gave the title compound (102 mg, 32%) after chromatography (THF - water, 0 to 50% gradient): IR (KBr) cm^{-1} 1771, 1710, 1677, 1609; ^1H NMR (250 MHz, D_2O) δ 2.79 and 3.37 (2H, ABq, *J*=17 Hz), 3.49~3.62 (2H, m), 3.82~4.01 (2H, m), 3.90 and 4.32 (2H, ABq, *J*=14 Hz), 4.47~4.58 (2H, m), 5.21 (1H, s), 5.48 (1H, s), 6.73~6.86 (3H, m), 7.24~7.56 (5H, m), 8.09 (1H, s), 9.37 (1H, s); FAB-MS (positive ion, thioglycerol) *m/z* 813 (M+Na, $C_{31}H_{27}N_8O_{10}S_3Na$).

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