Synthesis of Novel Streptonigrin 2-Amide Derivatives with 3,3'-(Phenylphosphoryl)bis(1,3-thiazolidine-2-thione)

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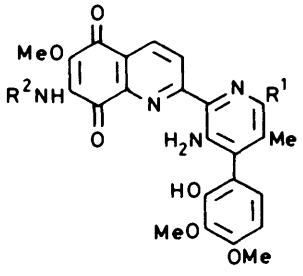
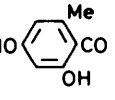
Novel streptonigrin 2-amide derivatives have been efficiently synthesized without protection by using 3,3'-(phenylphosphoryl)bis(1,3-thiazolidine-2-thione) (PPBTT) as a chemoselective condensing agent.

Because there is much current interest in the binding of antitumour drugs to their putative target in cancer cells, DNA,[1] our recent research interests have been focussed on the construction of a molecule in which both a DNA binding unit (*i.e.* a polyamine which binds to DNA electrostatically, or some intercalating functionality) and a reactive moiety are connected. In recent years, a number of natural or synthetic compounds, which are reactive to DNA, have been reported.[2] Among these compounds, streptonigrin, isolated by Rao and Cullen[3] as a metabolite of *Streptomyces flucculus*, is one of most effective agents for the treatment of human cancer.[4] Its structure was brilliantly deduced as a unique tetracyclic pyridylquinoline quinone structure, (1), by Rao, Biemann, and Woodward in 1963,[5] and this was later confirmed by *X*-ray crystallography.[6] Recently, two total syntheses of streptonigrin have been achieved by Weinreb[7] and Kende.[8]



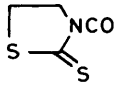
Streptonigrin (1) $R^1 = CO_2H$, $R^2 = H$

(2) $R^1 = CO_2Me$, $R^2 = H$

(3) $R^1 = CO_2H$, R^2 = 

(5) $R^1 = COCl$, $R^2 = H$

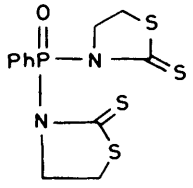
(6) $R^1 = CONHR$, $R^2 = H$

(7) R^1 =  , $R^2 = H$

(8) $R^1 = CO_2^-[H_2NC(:NH)NH_2]\cdot H^+$, $R^2 = H$

(9) $R^1 = CO_2^-Na^+$, $R^2 = H$

(10) $R^1 = CONHCH_2CO_2^-Na^+$, $R^2 = H$



(4) PPBTT

There is evidence that streptonigrin exerts antitumour action *via* interference with cell respiration and disruption of cell replication;[9] with oxygen and cuprous ions[10] as agents it causes single strand breaks in DNA. Recent work by Lown[11] has shown that a single-strand scission of PM2 ccc-DNA by quinoline-5,8-quinones, including streptonigrin, correlates well with inhibition of Walker carcinoma. Although, streptonigrin has shown clinical promise as an antitumour drug, it has a number of undesirable side effects, including severe bone marrow depression, which limit its wide application in cancer chemotherapy.[12]

Considerable efforts have been made to prepare streptonigrin analogues, the ultimate goal being the preparation of a compound having high activity but showing attenuated toxicity.[12] Such a goal is however still a major challenge because of streptonigrin's unique structural features and high degree of functionalization. Thus, relatively few derivatives of the antibiotic have been reported and only two are efficient due to partial *in vivo* hydrolysis to streptonigrin: these are streptonigrin methyl ester (2)[13] and the amide derivative (3).[12] The latter has been obtained by Rosazza by a high-yield microbial synthesis using a strain of *Streptomyces griseus*; a chemical synthesis of the compound has however not yet been achieved.

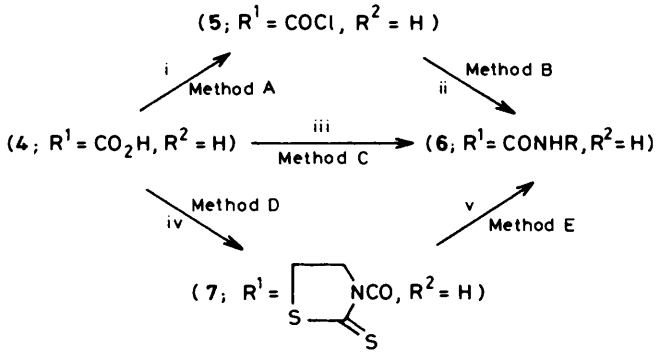
Recently we have described chemoselective amide[14] and peptide syntheses[15] and the stereoselective total synthesis of parabactin,[16] a spermidine-containing microbial iron-transporting compound. In the course of this work, we found that 3,3'-(phenylphosphoryl)bis(1,3-thiazolidine-2-thione) (PPBTT) (4) is chemoselective and effective condensing agent.[16] Here we describe the synthesis of novel streptonigrin 2-amide derivatives by using PPBTT.

Results and Discussion

We have synthesized several kinds of streptonigrin 2-amides (see Scheme 1); the results of our work are summarized in the Table. Schotten-Baumann conditions[17] were used to prepare the amides. Typically, the carboxylic acid chloride (**5**), readily prepared with $SOCl_2$ and one drop of DMF, was dissolved in THF, and added to the solution of amine and an excess of K_2CO_3 in water at 0 °C with vigorous stirring; stirring was continued for 30 min at room temperature, after which work-up, and purification by silica-gel chromatography gave the amide. The amides (**6a**, **f**, **i**, and **j**) were thus prepared in moderate or low yield. The amides (**6d**, **e**, **g**, and **j**) however could not be prepared in spite of exhaustive efforts (see Table). Subsequently, we investigated direct condensation of streptonigrin with

Table. Synthesis of streptonigrin 2-amide derivatives (**6**)

Amide (R)	Method	Time (h)	(Method)	Yield (%)
	A—B	30 min	(B)	40
(**6a**) $(CH_2)_2OH$	C	2	(C)	49
	D—E	1	(E)	72
(**6b**) $(CH_2)_3NMe_2$	C	2	(C)	51
	D—E	10 min	(E)	83
(**6c**) CH_2CO_2Me	C	2	(C)	50
	D—E	22	(E)	81
(**6d**) $(CH_2)_3NH(CH_2)_4$	C	5	(C)	27
	D—E	30 min	(E)	84
(**6e**) $(CH_2)_3NH(CH_2)_4NH(CH_2)_3$	C	3	(C)	32
	D—E	30 min	(E)	60
(**6f**) H	A—B	30 min	(B)	27
	D—E	1	(E)	63
(**6g**) NH_2	C	4	(C)	21
	D—E	30 min	(E)	67
(**6h**) OH	C	7	(C)	10
	D—E	7	(E)	47
(**6i**) $NHCONH_2$	A—B	30 min	(B)	27
	C	2	(C)	27
(**6j**) $NHCSNH_2$	A—B	2	(B)	12
	C	6	(C)	9

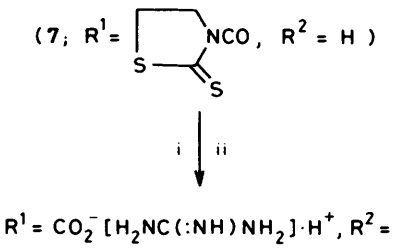


Scheme 1. *Reagents:* i, $SOCl_2$, DMF, reflux; ii, RNH_2, K_2CO_3, THF–H_2O; iii, PPBTT (**4**), RNH_2, Et_3N, MeCN, reflux; iv, PPBTT (**4**), Et_3N, MeCN, reflux; v, RNH_2, CH_2Cl_2

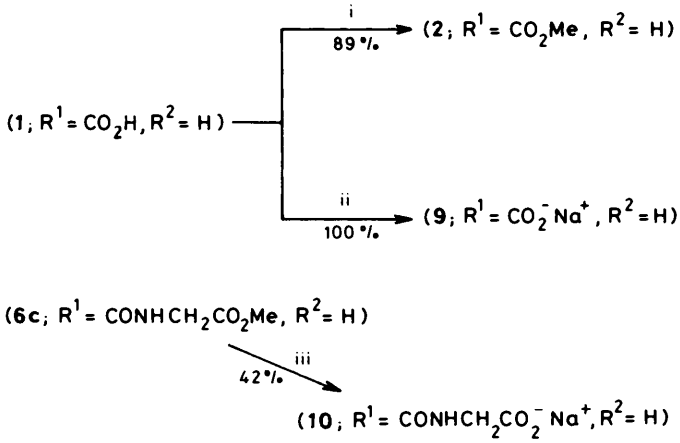
amines using PPBTT (**4**). Thus, treatment of streptonigrin and amines with PPBTT–Et_3N in MeCN under reflux for 2—7 h, gave the desired amides (**6a**—**c**) in (**6d**, **e**, **g**—**j**) in low to moderate yields (see Table).

Although the condensation is a single one-step reaction, it is difficult to introduce volatile NH_3 into the reaction mixture, and even the secondary amino groups of spermidine and spermine are reactive under reflux.[14] Therefore, the preparation of the 1,3-thiazolidine-2-thione derivative of streptonigrin (**7**) was examined next. This is a difficult compound to prepare chemoselectively, the yield of product being low by the usual condensing methods. However, it was found that treatment of streptonigrin with PPBTT–Et_3N (2.4 equiv.) and D-glucosamine·HCl (1.2 equiv.) in MeCN under reflux for 2 h, gave (**7**) (58% yield). Optimization of the reaction was achieved when an excess of Et_3N (>2 equiv.) was added to the reaction mixture. Thus streptonigrin with PPBTT–Et_3N (2.2 equiv.) in MeCN under reflux for 5 h by column chromatography gave (**7**) in 84% yield (Scheme 1). Subsequent aminolysis of the 3-acyl-1,3-thiazolidine-2-thione[14] gave the desired amides (**6a**—**h**) in good yields (see Table).

Unfortunately, treatment of (**7**) with guanidine in THF–H_2O (1:1) even under reflux for 4 h, gave the guanidinium salt of streptonigrin (**8**) (74% yield), rather than the expected amide (Scheme 2). The streptonigrin methyl ester (**2**), and the sodium salts (**9**) and (**10**) were also prepared (see Scheme 3).



Scheme 2. *Reagents:* i, $NH_2C(:NH)NH_2 \cdot 2HCl$, Na_2CO_3, THF–H_2O; ii, chromatography



Scheme 3. *Reagents:* i, MeOH, BF_3, reflux; ii, Na_2CO_3, $CHCl_3$–MeOH–H_2O; iii, Na_2CO_3, $CHCl_3$–MeOH–H_2O, reflux

The structures of these compounds were confirmed by 400 MHz ^1H n.m.r., e.i., and f.a.b. (fast atom bombardment) mass spectroscopy. Finally, streptonigrin amide derivatives have been efficiently and chemoselectively synthesized, without protection, by using PPBTT (**4**); the condensation occurs *via* a concerted mechanism,[18] a result of the low nucleophilicity of the amino groups of streptonigrin arising from steric hindrance. The biological activity of these compounds is now being examined, and the results will be described elsewhere.[10]

Experimental

M.p.s were determined with a Yanagimoto microapparatus. I.r. spectra were run using KBr plates on a JASCO A-202 spectrophotometer. E.i. and f.a.b. mass spectra were recorded on a JEOL JMS DX300 mass spectrometer. ^1H N.m.r. spectra were recorded on a JEOL JNM-JX400 spectrometer in $CDCl_3$–CD_3OD (1:1) unless otherwise stated with $SiMe_4$ as internal standard. Extracts were dried over Na_2SO_4. Silica gel (100—200 mesh) (KANTO Chemical Co., Inc) and Sephadex LH 20 (Pharmacia Fine Chemicals) were used for column chromatography.

Typical Preparation of the Amide Derivatives (**6**) *from a Carboxylic Acid Chloride* (**5**): *Method A—B.*—One drop of DMF was added to a suspension of streptonigrin (30 mg, 0.06 mmol) in $SOCl_2$ (2 ml) and the reaction mixture was refluxed for 30 min under N_2; excess of $SOCl_2$ was then evaporated off. The oily residue was dissolved in THF (2 ml), and added to a

solution of 2-aminoethanol (5.4 mg, 0.09 mmol) and K_2CO_3 (82 mg, 0.6 mmol) in water (10 ml) at 0 °C. The reaction mixture was stirred for 30 min at room temperature after which it was extracted with EtOAc (100 ml). The extract was washed with 5% aqueous Na_2CO_3 and brine, dried, and the solvent evaporated off under reduced pressure to give an oily residue, which was chromatographed on silica gel with MeOH–$CHCl_3$ (1:9) to afford the amide (**6a**) (12 mg, 40%).

Direct Condensation Reaction Using 3,3′-(*Phenylphosphoryl*)-*bis*(1,3-*thiazolidine*-2-*thione*) (*PPBTT*) (**4**): *Method C.*—To a stirred suspension of streptonigrin (30 mg, 0.06 mmol) and PPBTT (24 mg, 0.072 mmol) in MeCN (3 ml), a solution of 2-aminoethanol (4.3 mg, 0.072 mmol) and Et_3N (7.2 mg, 0.072 mmol) in MeCN (2 ml) was added. The reaction mixture was refluxed for 2 h under N_2, and then worked up as described above to give the amide (**6a**) (16 mg, 49%).

Typical Preparation of 3-*Amino*-2-(5,8-*dioxoquinolin*-2-*yl*)-4-(2-*hydroxy*-3,4-*dimethyl*)-5-*methyl*-6-(2-*thioxothiazolidin*-2-*ylcarbonyl*)*pyridine, the* N-*Acylthiazolidine* (**7**) *from Streptonigrin.*—To the suspension of streptonigrin (253 mg, 0.5 mmol) and PPBTT (258 mg, 0.75 mmol) in MeCN (50 ml), Et_3N (0.15 ml, 1.1 mmol) was added and the reaction mixture was refluxed for 5 h under N_2. It was then extracted with EtOAc (100 ml) and the extract was washed with, 5% aqueous Na_2CO_3, and brine, and dried. Evaporation of solvent under reduced pressure gave an oily residue which was column chromatographed on Sephadex LH 20 ($CHCl_3$–MeOH, 3:7) to afford (**7**) (256 mg, 84%).

Typical Preparation of the Amide Derivatives (**6**) *from the* N-*Acylthiazolidine* (**7**): *Methods D—E.*—To a stirred solution of (**7**) (20 mg, 0.033 mmol) in CH_2Cl_2 (5 ml), a solution of 2-aminoethanol (2.2 mg, 0.036 mmol) in CH_2Cl_2 (5 ml) was added. The reaction mixture was stirred at room temperature until (**7**) disappeared (checked by t.l.c.), and was then treated as before to give the amide (**6a**) (13 mg, 72%).

Physical Data for Streptonigrin Amide Derivatives (**6**).—*Streptonigrin* 2-N-(2-*hydroxyethyl*)*amide:* 5-*Amino*-6-(7-*amino*-6-*methoxy*-5,8-*dioxoquinolin*-2-*yl*)-4-(2-*hydroxy*-3,4-*dimethoxyphenyl*)-N-(2-*hydroxyethyl*)-3-*methylpyridine*-2-*carboxamide* (**6a**). Dark brown needles, m.p. 255—256 °C ($MeOH–CHCl_3$); $\nu_{max.}$ 3 440 and 1 605 cm^{-1}; δ 2.37 (3 H, s), 3.59 (3 H, m), 3.81 (2 H, t, *J* 5.4 Hz), 3.92, 3.95, and 3.98 (each 3 H, s), 6.69 and 6.79 (2 H, ABq, *J* 8.3 Hz), and 8.38 and 8.89 (2 H, ABq, *J* 8.8 Hz) (Found: C, 58.85; H, 5.0; N, 12.6% M^+, 549.185. $C_{27}H_{27}N_5O_8$ requires C, 59.0; H, 4.95; N, 12.75%; *M*, 549.185).

Streptonigrin 2-N-(N,N-*dimethylaminopropyl*)*amide* (**6b**). Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 300, 1 630sh, and 1 605 cm^{-1}; δ 1.82—2.00 (2 H, m), 2.36 (3 H, s), 2.56 (6 H, s), 2.75—2.82 (2 H, m), 3.48—3.56 (2 H, m), 3.92, 3.95, and 3.97 (each 3 H, s), 6.70 and 6.80 (2 H, ABq, *J* 8.5 Hz), and 8.38 and 8.93 (2 H, ABq, *J* 8.4 Hz) (Found: C, 60.75; H, 5.9; N, 14.1%; M^+, 590.253. $C_{30}H_{34}N_6O_7$ requires C, 61.0; H, 5.8; N, 14.25%; *M*, 590.249).

Streptonigrin 2-N-(*methylcarboxymethyl*)*amide* (**6c**). Dark brown needles, m.p. 273.5—274.5 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 300, 1 735, 1 650sh, and 1 602 cm^{-1}; δ 2.40 (3 H, s), 3.55 (2 H, t, *J* 7.3 Hz), 3.83 (3 H, s), 3.92, 3.95, and 3.99 (each 3 H, s), 4.24 (2 H, s), 6.70 and 6.79 (2 H, ABq, *J* 8.5 Hz), and 8.43 and 8.94 (2 H, ABq, *J* 8.5 Hz) (Found: C, 58.0; H, 4.8; N, 12.0%; M^+, 577.181. $C_{28}H_{27}N_5O_9$ requires C, 58.2; H, 4.7; N, 12.15%; *M*, 577.181).

1,10-*Bis-streptonigrin substituted spermidine* (**6d**). Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460— 3 230 and 1 605 cm^{-1}; δ 1.79—1.95 (4 H, m), 2.40—2.54 (2 H, m), 2.37 (6 H, s), 3.02—3.16 (4 H, m), 3.44—3.62 (4 H, m), 3.89—4.01 (18 H, m), 6.64—6.82 (4 H, m), 8.28—8.40 (2 H, m), and 8.72—8.79 (2 H, m) [Found: C, 60.75; H, 5.4; N, 13.6%; m.s. (f.a.b.) $(M + 6H)^+$, 1 127. $C_{57}H_{59}N_{11}O_{14}$ requires C, 61.0; H, 5.3; N, 13.75%; *M*, 1 121].

1,14-*Bis-streptonigrin substituted spermine* (**6e**). Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 250 and 1 605 cm^{-1}; δ 1.60—2.08 (9 H, m), 2.35 (6 H, s), 2.28—2.42 (4 H, m), 2.82—3.08 (4 H, m), 3.87—4.01 (18 H, m), 6.62—6.81 (4 H, m), and 8.22 and 8.73 (4 H, ABq, *J* 8.3 Hz) [Found: C, 60.75; H, 5.7; N, 13.9%; m.s. (f.a.b.) $(M + H)^+$, 1 179. $C_{60}H_{66}N_{12}O_{14}$ requires C, 61.0; H, 5.6; N, 14.0%; *M*, 1 178].

Streptonigrin 2-*amide* (**6f**). Dark brown needles, m.p. 172—174 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460, 3 370, 1 655sh, and 1 605 cm^{-1}; δ 2.39 (3 H, s), 3.92, 3.96, and 3.98 (each 3 H, s), 6.69 and 6.89 (2 H, ABq, *J* 8.8 Hz), and 8.40 and 8.87 (2 H, ABq, *J* 8.5 Hz) [Found: C, 59.2; H, 4.7; N, 13.7%; m.s. (f.a.b.) $(M + 3H)^+$, 508. $C_{25}H_{23}N_5O_7$ requires C, 59.4; H, 4.6; N, 13.85%; *M*, 505].

Streptonigrin 2-*hydrazide* (**6g**). Dark brown needles, m.p. 214—218 °C (decomp.) (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 200 and 1 607 cm^{-1}; δ 2.39 (3 H, s), 3.92, 3.96, and 3.99 (each 3 H, s), 6.69 and 6.79 (2 H, ABq, *J* 8.6 Hz), and 8.42 and 8.97 (2 H, ABq, *J* 8.6 Hz) (Found: C, 57.5; H, 4.8; N, 16.0%; M^+, 520.169. $C_{25}H_{24}N_6O_7$ requires C, 57.7; H, 4.65; N, 16.15%; *M*, 520.171).

Streptonigrin 2-*hydroxamic acid* (**6h**). Dark brown needles, m.p. 168—172 °C (decomp.) (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 200 and 1 607 cm^{-1}; δ 2.32 (3 H, s), 3.92, 3.95, and 3.99 (each 3 H, s), 6.69 and 6.79 (2 H, ABq, *J* 8.6 Hz), and 8.42 and 8.94 (2 H, ABq, *J* 8.4 Hz) [Found: C, 57.4; H, 4.5; N, 13.35%; $(M + H)^+$, 520. $C_{25}H_{23}N_5O_8$ requires C, 57.6; H, 4.45; N, 13.45%; *M*, 521].

Streptonigrin 2-*semicarbazide* (**6i**). Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 250 and 1 605 cm^{-1}; δ 2.37 (3 H, s), 3.92, 3.96, and 3.98 (each 3 H, s), 6.70 and 6.79 (2 H, ABq, *J* 8.6 Hz), and 8.39 and 8.93 (2 H, ABq, *J* 8.6 Hz) [Found: C, 55.25; H, 4.6; N, 17.3%; m.s. (f.a.b.) $(M + 3H)^+$, 566. $C_{26}H_{25}N_7O_8$ requires C, 55.4; H, 4.5; N, 17.4%; *M*, 563].

Streptonigrin 2-*thiosemicarbazide* (**6j**). Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 460—3 300 and 1 605 cm^{-1}; δ 2.37 (3 H, s), 3.91, 3.95, and 3.98 (each 3 H, s), 6.70 and 6.79 (2 H, ABq, *J* 8.5 Hz), 8.43 and 9.02 (2 H, ABq, *J* 8.5 Hz) (Found: C, 53.7; H, 4.5; N, 16.8%; M^+, 579. $C_{26}H_{25}N_7O_7S$ requires C, 53.9; H, 4.35; N, 16.9%; *M*, 579).

N-*Acylthiazolidine* (**7**).—Dark brown needles, m.p. >300 °C (from $MeOH–CHCl_3$); $\nu_{max.}$ 3 440, 3 330, 1 675, and 1 603 cm^{-1}; δ($CDCl_3$) 1.40—1.90 (2 H, br s), 2.13 (3 H, s), 3.66 (2 H, m), 3.94, 3.97, and 4.07 (each 3 H, s), 4.69 (2 H, t, *J* 7.3 Hz), 5.18 (2 H, br s), 5.91 (1 H, br s), 6.65 and 6.83 (2 H, ABq, *J* 8.4 Hz), and 8.39 and 8.80 (2 H, ABq, *J* 8.4 Hz) [Found: C, 55.3; H, 4.3; N, 13.6%; m.s. (f.a.b.) $(M + 3H)^+$, 610. $C_{28}H_{25}N_5O_7S_2$ requires C, 55.35; H, 4.15; N, 11.55%; *M*, 607].

Guanidinium Salt of Streptonigrin (**8**).—To the solution of (**7**) (20 mg, 0.033 mmol) in THF (5 ml), a solution of guanidine·2HCl (5 mg, 0.048 mmol) in Na_2CO_3 (14 mg, 0.13 mmol) was added. The reaction mixture was refluxed for 4 h. The solvent was evaporated under reduced pressure to give an oily residue, which was chromatographed on Sephadex LH 20 ($CHCl_3$–MeOH, 3:7) to afford the dark brown, hygroscopic and amorphous salt (**8**) (18 mg, 74%); $\nu_{max.}$ 3 370, 1 650, and 1 605sh cm^{-1}; δ 2.19 (3 H, s), 3.86 (3 H, s), 3.92, (6 H, s-like), 6.72 and 6.77 (2 H, ABq, *J* 8.4 Hz), and 8.35 and 8.93 (2 H, ABq, *J* 8.3 Hz) [Found: m.s. (f.a.b.) $(M -$ guanidine $+ 3H)^+$, 509. $C_{26}H_{27}N_7O_8$ requires *M*, 565].

Streptonigrin Methyl Ester (**2**).—Streptonigrin methyl ester was prepared according to the method reported by Weinreb,[7]

and purification by column chromatography on Sephadex LH 20 ($CHCl_3$–MeOH, 3:7) gave the desired ester (89% yield) as dark brown needles, m.p. 254.5—255.5 °C (from MeOH–$CHCl_3$); $\nu_{max.}$ 3 470—3 250, 1 710, and 1 580 cm^{-1}; δ($CDCl_3$) 2.32 (3 H, s), 3.00 (2 H, br d), 3.95, 3.99, and 3.99 (each 3 H, s), 4.08 (3 H, s), 5.02 (2 H, br s), 5.59 (1 H, br s), 6.66 and 6.81 (2 H, ABq, *J* 8.5 Hz), and 8.42 and 8.99 (2 H, ABq, *J* 8.4 Hz) [Found: C, 59.8; H, 4.8; N, 10.7%; M^+, 520.159. $C_{26}H_{24}N_4O_8$ requires C, 60.0; H, 4.65; N, 10.75%; *M*, 520.159].

Sodium Salt of Streptonigrin (**9**).—To a stirred solution of streptonigrin (30 mg, 0.06 mmol), Na_2CO_3 (7 mg, 0.066 mmol) in water (1 ml) was added. The reaction mixture was stirred at room temperature for 30 min after which the solvent was evaporated off under reduced pressure to give an oily residue. The residue was purified by column chromatography on Sephadex LH 20 ($CHCl_3$–MeOH, 3:7) to afford the salt (**9**) (31 mg, 100%) as dark brown plates, m.p. 300 °C (from MeOH–$CHCl_3$); $\nu_{max.}$ 3 460—3 300 and 1 600 cm^{-1}; δ(CD_3OD) 2.17 (3 H, s), 3.86, 3.91, and 3.93 (each 3 H, s), 6.65 and 6.74 (2 H, ABq, *J* 8.3 Hz), and 8.34 and 8.98 (2 H, ABq, *J* 8.5 Hz) [Found: m.s. (f.a.b.) $(M + 3\ H)^+$, 531. $C_{25}H_{21}N_4NaO_8$ requires *M*, 528].

Sodium Salt of Streptonigrin 2-N-(*Methylcarboxymethyl*)-*amide* (**10**).—To a solution of the ester (**6c**) (29 mg, 0.05 mmol) in $CHCl_3$–MeOH (1:4) (10 ml), a solution of Na_2CO_3 (6 mg, 0.055 mmol) in water (1 ml) was added. The reaction mixture was refluxed for overnight and treated as described above to give the salt (**10**) as dark brown needles, m.p. >300 °C (from MeOH–$CHCl_3$); $\nu_{max.}$ 3 460—3 350 and 1 605 cm^{-1}; δ 2.35 (3 H, s), 3.87, (3 H, s), 3.93 (6 H, s-like), 4.04 (2 H, s), 6.73 and 6.77 (2 H, ABq, *J* 8.8 Hz), 8.43 (1 H, m), and 9.04 (1 H, m) [Found: m.s. (f.a.b.) $(M + 3\ H)^+$, 588. $C_{27}H_{24}N_5NaO_9$ requires *M*, 585].

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