Synthesis and *In Vitro* Antibacterial Activity of Catechol-spiramycin Conjugates

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The first synthesis of siderophore conjugates of two macrolide antibiotics, spiramycin **1** and neospiramycin **2**, which are unable to penetrate the outer membrane of Gram-negative bacteria are described. These novel conjugates were prepared by regioselective acylation of a hydroxyl function of **1** and **2** with a dihydroxybenzoic Fe(III) complexing ligand linked *via* a carboxyl group containing spacer to the macrolide antibiotics. The preliminary biological evaluation of these novel conjugates under standard and iron depleted conditions has shown that their antibacterial activity was comparable to that of spiramycin **1** and neospiramycin **2**.

Infections due to various bacteria and viruses are a major cause of mortality worldwide. It has been demonstrated that some of the bacteria involved in these infections produce iron(III) complexing compounds of low molecular weight called siderophores, which contribute to the virulence by depriving the host of iron[1].

Bacteria are gradually becoming more and more resistant to antibacterial agents. Thus antibacterials with increased activity are constantly needed. Antibiotics such as macrolides are unable to penetrate the outer membrane of Gram-negative bacteria while being active on the ribosomal target.

Recently, several groups have demonstrated that the adjunction of iron complexing ligands to β-lactam antibiotics greatly improves their biological activity against Gram-negative bacteria[2,3]. This astute application was inspired by the existence of a few natural compounds like the ferrimycins[4] and the albomycins[5] which combine strong iron chelators with an antibiotic moiety within the same molecular assembly.

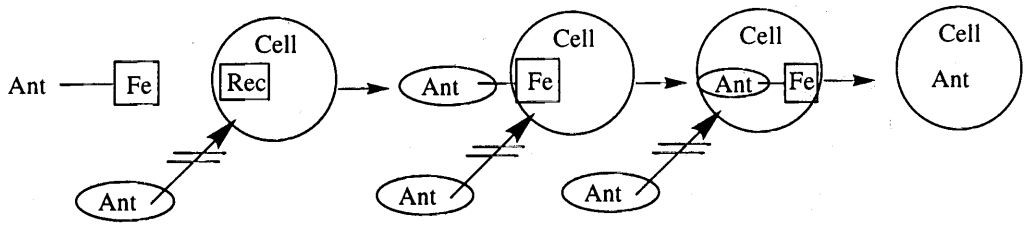
These results and our own interest in this field[6] encouraged us to apply this approach (the "Trojan horse concept"[3]-Scheme 1) to macrolide antibiotics like spiramycin **1** and neospiramycin **2**.

Chemistry

Spiramycin **1** is a natural 16-membered macrolide produced by *Streptomyces ambofaciens*[7]. While the hydrogenation of the two conjugated double bonds does not seriously affect the biological activity of **1** and **2**[8], the aldehyde function and the presence of the amino-sugars mycaminose and forosamine are essential for their antibacterial activity (Scheme 2).

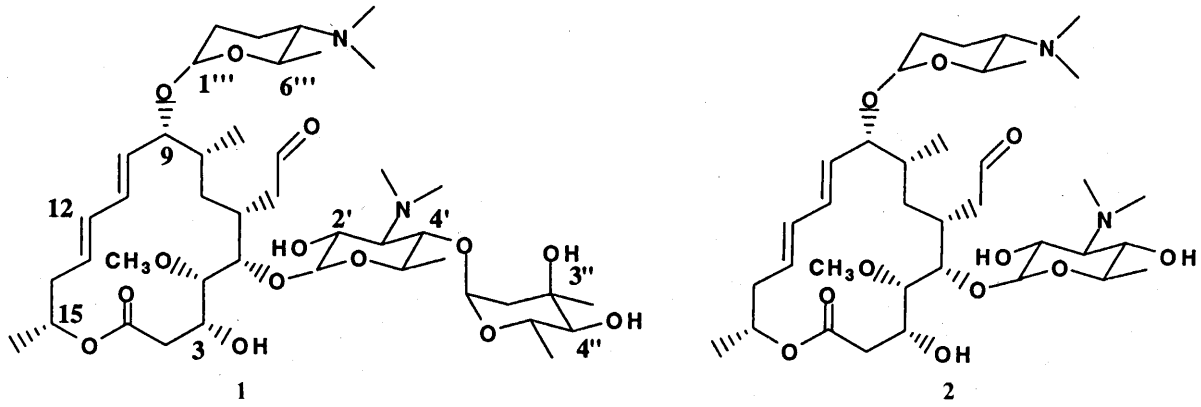
Previous studies have shown that the acylation of **1** at position 4″ does not alter significantly its activity against bacteria[8]. Compound **1** possesses four hydroxyl groups and their reactivity towards esterification decreases in the order 2′, 4″, 3 to 3″. Taking these facts into account,

Scheme 1.

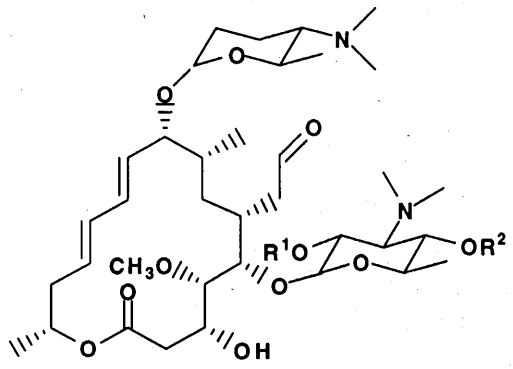


Trojan horse concept. Ant: antibiotic; Rec; Fe-transport receptor.

Scheme 2.



Scheme 3.



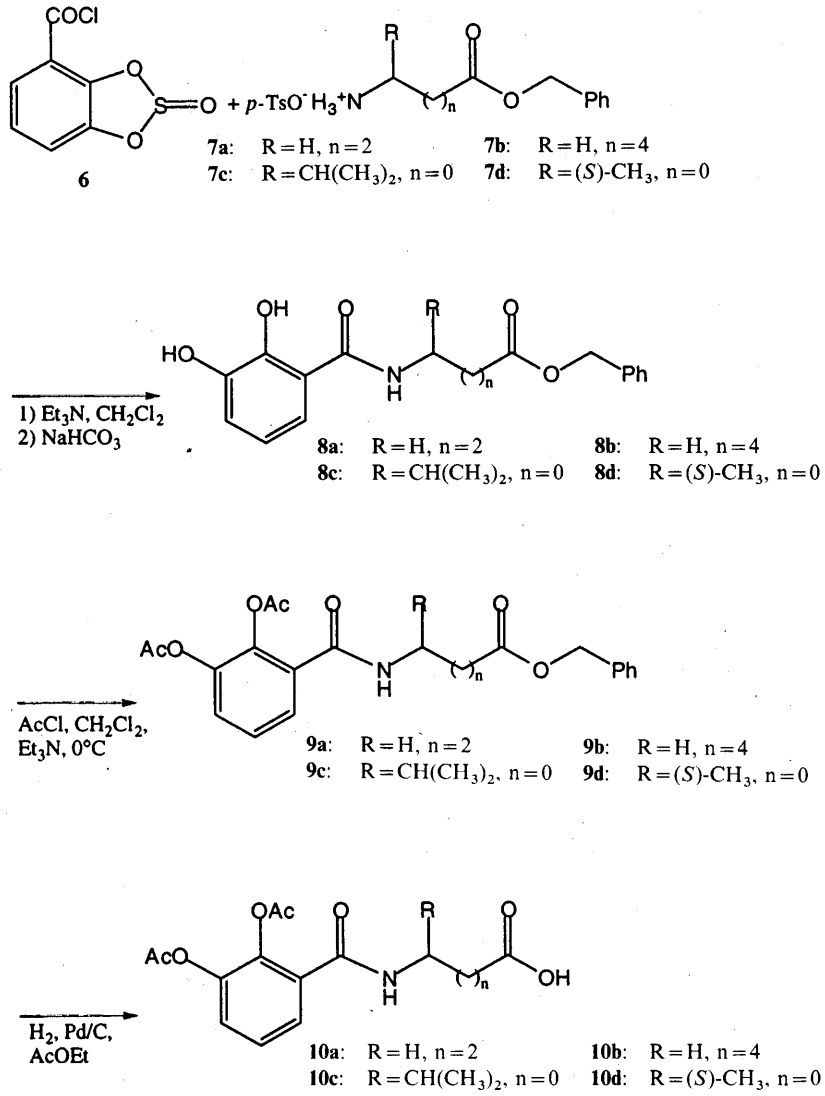
3a: R^1 = Ac; R^2 = Mycarose
3b: R^1 = Ac;
R^2 = Mycarose 4″OCO$(CH_2)_3$CONH(2,3-OAc-C_6H_3)
3c: R^1 = CO(3,4-OAc-C_6H_3); R^2 = Mycarose
3d: R^1 = CO(3,4-OH-C_6H_3); R^2 = Mycarose
3e: Iron(III) complex of **3d**
3f: Gallium(III) complex of **3d**
3g: R^1 = CO(2,3-OH-C_6H_3); R^2 = Mycarose
3h: R^1 = CO$(CH_2)_3$CONH(2,3-OAc-C_6H_3);
R^2 = Mycarose
3i: R^1 = COCH-(*S*)-(CH_3)-NHCP(2,3-OAc-C_6H_3);
R^2 = Mycarose
3j: R^1 = CO$(CH_2)_3$NHCO(2,3-OAc-C_6H_3);
R^2 = CO$(CH_2)_3$NHCO(2,3-OAc-C_6H_3)

acylation at position 2′ providing the intermediate **3a** turned out to be the method of choice to protect the 2′ position. 4″-*O*-Acylation of **3a** followed by treatment of the resulting 2′-*O*-acetyl-4″-*O*-acyl derivative with guanidine[9] furnished 2′-hydroxyl-4″-*O*-acyl derivatives under mild conditions.

Catechols and hydroxamates are the prevalent ligands among natural bacterial siderophores. They generally involve 3 divalent complexing sites in order to allow for the octahedral geometry around the Fe(III) cation. Our earlier attemps to synthesize conjugates able to transport antibiotics *via* the active iron transport system provided interesting results with some mono- and dicatechol adducts of pristinamycin I_A[6].

Protection of the catechol unit turned out to be a major and crucial problem for the success of our investigations. Methylation of the phenolic hydroxyl groups was excluded since their cleavage with BBr_3

Scheme 4.



destroys inevitably the spiramycin moiety, as well as hydrogenolysis of benzyl protected derivatives due to the presence of the double bonds in the macrolide ring. On the other hand, although phenolic acetates are rapidly hydrolyzed in the presence of bases, particularly amines, necessitating a careful choice of reaction conditions, they possess the decisive advantage of being easily hydrolyzed under biological conditions as demonstrated by R. REISSBRODT *et al.*[10].

We chose various natural amino acids as spacers to link an hydroxyl group of the antibiotic to a dihydroxybenzoic acid for two major reasons: the eventual control of the release by enzymatic hydrolysis *in vivo* of the spacer and the absence of any forseeable toxicity of their metabolites.

Thus, several amino acids were protected as benzyl esters **7a**~**7d** by heating them in a solution of benzyl alcohol and *p*-toluene sulfonic acid in toluene. Reaction with 2,3-dioxosulfinyl benzoyl chloride **6**[11] (which can be easily prepared from dihydroxybenzoic acid and thionylchloride) gave compounds **8a**~**8d** (Scheme 4). Acetylation then deprotection by catalytic hydrogenation provided the building blocks (ligand + spacer) **10a**~**10d**. Finally, the introduction of the ligand moieties **10a**~**10b** was realized with classical coupling reagents (DCC or CDI) using either 2′-acetyl-spiramycin **3a** to give the 4″-*O*-acylated derivative **3b**, or on spiramycin **1** leading to 2′-acylated compounds **3c**, **3g**, **3h**, **3i**. Reaction of neospiramycin **2** with **10c** under the same conditions furnished a 2′,4″-diacylated derivative **3j**. The major difficulty encountered with these products was their final purification: chromatography over silicagel gave extremely poor yields of recovered material (<3%). Fortunately MPLC over reversed phase (Lichroprep

RP-18) using a slightly acidic solvent (pH=3) as eluent allowed the purification of the desired adducts.

The Fe(III) and Ga(III) complexes **3e** and **3f** were synthesized from **3d** by treatment with either $Fe(acac)_3$ or gallium nitrate in the presence of triethylamine followed by purification over Sephadex LH-20.

Antibacterial Activity

The MICs were determined by the standard agar dilution method. Two-fold dilutions of antibiotic in sterile distilled water were prepared from stock solutions and incorporated into melted Mueller-Hinton agar supplemented with 25 mg/liter Mg^{++} and 50 mg/liter Ca^{++}. A Denley Multipoint inoculator was used to apply spots of 1 μl (10^4 bacteria) of each bacterial strain studied to plates. Inoculated plates were incubated for 18 hours at 37°C. For iron depleted conditions, the seed medium consisted of 6 g of K_2HPO_4 (Merck 5101), 3 g of KH_2PO_4 (Merck 4873), 0.2 g of $MgSO_4 \cdot 7H_2O$ (Prolabo 25165.292), 1 g of $(NH_4)_2SO_4 \cdot 7H_2O$ (Prolabo 21333.296), 4 g of succinic acid (Merck 682) and 15 g of agar (Difco). The pH was adjusted to 7.0 by addition of concentrated sodium hydroxyde (Prolabo 28252.293).

Several Gram-negative strains like *Pseudomonas aeruginosa* Z61, *Escherichia coli* D22 and *Salmonella typhimurium* TA 1535 possess a permeable outer membrane. This feature allowed us to check wether our new adducts were still capable of binding to the ribosomes of the bacterial cells after the passage through the outer membrane. As a matter of course, spiramycin **1** was chosen as the reference compound.

Under Mueller-Hinton conditions (Table 1), the siderophore receptor proteins are not expressed and no active transport is possible. Spiramycin **1** and the novel adducts **3b** to **3j** are only active on the strains possessing a permeable outer membrane and show similar MICs with the exception of compound **3b**, which is only active against *Salmonella typhimurium* TA 1535. It is interesting

Table 1. MIC (mg/liter) of **1** and the adducts **3** on different strains under Mueller-Hinton growth conditions.

	1	**3b**	**3c**	**3d**	**3e**	**3g**	**3h**	**3i**	**3j**
Staphylococcus aureus ATCC25923	8	32	>128	128	128	64	16	16	64
Staphylococcus aureus 209P	4	16	128	64	64	32	8	4	64
Pseudomonas aeruginosa ATCC27853	>128	>128	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa Z61[a]	16	128	128	>128	>128	128	32	16	64
Escherichia coli NIHJ-JC2	>128	>128	>128	>128	>128	>128	>128	>128	>128
Escherichia coli D22[a]	4	>128	64	32	32	16	2	4	32
Salmonella typhimurium IPL	>128	>128	>128	>128	>128	>128	>128	>128	>128
Salmonella typhimurium TA 1535[a]	8	16	64	64	64	32	4	4	64

[a] Gram-negative strain possessing a permeable outer membrane.

Table 2. MIC (mg/liter) of **1** and the adducts **3** on different strains under iron depressed conditions.

	1	**3b**	**3c**	**3d**	**3e**	**3g**	**3h**	**3i**	**3j**
Staphylococcus aureus ATCC25923	—	—	—	—	—	—	—	—	—
Staphylococcus aureus 209P	—	—	—	—	—	—	—	—	—
Pseudomonas aeruginosa ATCC27853	>128	>128	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa Z61[a]	16	32	64	128	64	32	8	16	32
Escherichia coli NIHJ-JC2	>128	>128	>128	>128	>128	>128	128	>128	>128
Escherichia coli D22[a]	—	—	—	—	—	—	—	—	—
Escherichia coli ATCC25922	64	>128	>128	>128	128	128	64	128	>128
Escherichia coli N51	32	64	128	>128	128	128	32	32	>128
Escherichia coli 642	>128	>128	>128	>128	>128	>128	64	64	>128
Escherichia coli 722	128	128	>128	>128	>128	128	64	64	>128
Escherichia coli 823	64	128	>128	>128	>128	128	64	128	>128
Salmonella typhimurium IPL	128	128	>128	>128	>128	128	64	64	>128
Salmonella typhimurium TA 1535[a]	—	—	—	—	—	—	—	—	—

[a] Gram-negative strain possessing permeable outer membrane. —: no bacterial growth.

to note that compounds **3h** and **3i** which are acylated in position 2′ of the mycarose unit of spiramycin with the spacer-ligand units **10a** and **10d**, show similar MICs when compared to the parent compound **1**.

Under ferric stress conditions, when bacterial growth was allowed (Table 2), no significiant decrease of the MICs was observed. A few hypotheses can be proposed in order to explain these results:

The adducts may be enzymatically hydrolyzed before reaching their target within the bacterial cell.

The receptor site at the surface of the bacterial cell wall may not recognize mono- or dicatechols.

Finally, even if these "Trojan horses" are recognized by the receptors, their mere size (MW close to 3000, see Exp. Part) may not allow them to pass through the membrane channels.

The synthesis of tricatechol and trihydroxamate conjugates of spiramycin **1** of lower molecular weight , less prone to enzymatic hydrolysis by introducing steric hindrance around the ester linkage, is in due course in our laboratory.

Experimental

NMR

To characterize our adducts **3**, all the hydrogen and carbon atoms of spiramycin were attributed using 2D NMR spectroscopy and their chemical shifts were in agreement with published chemical shifts[12]. Therefore, only characteristic and significant chemical shifts are given in this section.

NMR spectra were recorded on BRUKER instruments: AM 200, AM 250 or AM 400 at respectively 200 MHz, 250 MHz or 400 MHz for ^1H and 50 MHz, 62 MHz or 100 MHz for ^{13}C with deuterated chloroform as internal reference (7.24 ppm for ^1H and 77 ppm for ^{13}C).

MS

For compounds with a molecular weight under 700 mass units, a Riber-Mag R-10-10 apparatus was used in the direct introduction mode with chemical ionization by NH_3. The higher molecular weights were analyzed by ES-MS or FAB-MS on a Platform (VG Fisons) quadrupole instrument. Calibration was performed using a cesium iodide solution (10 ng/ml) for the weight range of 250~1400 with a precision of 0.5 mass units. The samples were dissolved in methanol and diluted in 0.2% formic acid solution (pH=3)/acetonitrile (50 : 50) with a concentration of 20 to 100 ng/ml.

HPLC

HPLC analyses were performed on a Perkin Elmer apparatus equipped with a biocompatible LC250 binary pump and an ultraviolet diode LC 235C. The analytical column was a reversed phase Inertsil IN100D$_2$-25F, 250 mm length and 4.6 mm internal diameter.

MPLC

MPLC was effected using SDS Chromagel 40~60 μm (230~400 mesh) silicagel or 40~60 μm LiChroprep RP-18 (Merck).

THF and ether were freshly distilled over benzophenone-sodium just before use. Toluene was distilled over Na, pyridine over KOH and methylene chloride over P_2O_5. All the manipulations were effected under argon atmosphere.

2,3-Diacetoxybenzoic Acid **5**

A suspension of 2,3-dihydroxybenzoic acid (5 g; 32.5 mmol) in 7 ml of acetic anhydride was stirred at room temperature and two drops of concentrated sulfuric acid were added. After five minutes, a precipitate formed, 35 ml of anhydrous ether were added and the solution was stirred for 12 hours at room temperature. Extraction with CH_2Cl_2, washing with cold water, drying over $MgSO_4$ and evaporation of the solvent yielded 7.04 g of the title compound (91%).

$C_{11}H_{10}O_6$ MW 238.20; ^1H NMR (200 MHz, $CDCl_3$) δ 2.3 (6H, s), 7.2~7.5 (2H, m) 7.9 (1H, dd); DIC/NH_3-MS m/z 256 $(M+18)^{+\cdot}$, m/z 239 $(M+H)^{+\cdot}$; mp 158°C.

Anal Calcd for $C_{11}H_{10}O_6$: C 55.47, H 4.23.
Found: C 55.34, H 4.34.

2,3-Dioxosulfinyl Benzoyl Chloride **6**

1.54 g of 2,3-dihydroxybenzoic acid (5 mmol) was heated at 40°C for 3 hours in 5 ml of $SOCl_2$ with a catalytic amount of urea. The reaction mixture was evaporated several times with toluene at a temperature below 55°C to yield 1.06 g of the title compound (97%).

$C_7H_3O_4SCl$ MW 218.61; ^1H NMR (200 MHz, $CDCl_3$) δ 7.3 (1H, t), 7.5 (1H, d), 7.9 (1H, d); IE-MS m/z 219 $(M+2)^{+\cdot}$, $m/z=218$ $(M)^{+\cdot}$; mp 85°C.

Anal Calcd for $C_7H_3O_4SCl$:
C 38.46, H 1.38, S 14.67, Cl 16.22.
Found: C 39.41, H 1.51, S 13.83, Cl 16.15.

ω-Aminobenzyl Esters **7**

1.03 g (100 mmol) GABA, 19.4 g (1.02 equiv) of *p*-toluene sulfonic acid and 40 ml of benzyl alcohol were heated for 1 hour under reflux in 20 ml of toluene using a Dean-Stark apparatus. After cooling, 200 ml of freshly

distilled ether were added and the title compound **7a** precipitated at low temperature (yield 84%). Compounds **7b**, **7c** and **7d** were prepared using the same procedure.

7a: $C_{18}H_{23}NO_5S$, MW 365.44; Yield 84%; (DIC/NH_3)-MS m/z 204 $(M+H)^{+\cdot}$ (amino compound).

7b: $C_{20}H_{27}NO_5S$, MW 393.50; Yield 85%; (DIC/NH_3)-MS m/z 222 $(M+H)^{+\cdot}$ (amino compound).

7c: $C_{19}H_{25}NO_5S$, MW 379.47; Yield 60%; (DIC/NH_3)-MS m/z 216 $(M+H)^{+\cdot}$ (amino compound).

7d: $C_{17}H_{21}NO_5S$, MW 351.42; Yield 68%; (DIC/NH_3)-MS m/z 188 $(M+H)^{+\cdot}$ (amino compound).

N-(2,3-Dihydroxybenzoyl)aminobenzylic Esters **8**

To a stirred, cooled solution of 1.38 g (6.33 mmol) 2,3-dioxosulfinyl benzoyl chloride **6** (1.2 equiv) and 3.73 g of *p*-toluene sulfonate benzylic ester **7a** (9.5 mmol) in 100 ml of CH_2Cl_2, 1.5 ml of Et_3N (1.5 equiv) in 10 ml CH_2Cl_2 was added dropwise. The reaction was followed by TLC and quenched by a saturated solution of $NaHCO_3$, extracted with CH_2Cl_2, dried, evaporated and chromatographed by MPLC (CH_2Cl_2/Et_2O 5%) to give the title compound **8a**. Products **8b**, **8c** and **8d** were prepared using the same procedure.

8a: $C_{18}H_{19}NO_5$, MW 329.35; Yield 62%; ^1H NMR (200 MHz, $CDCl_3$) δ 2.0 (2H, m), 2.5 (2H, t) 3.5 (2H, q), 5.1 (2H, s), 6.8 (1H, t), 6.9 1H, d), 7.0 (1H, d), 7.4 (5H, s); (DIC/NH_3)-MS $m/z=330$ $(M+H)^{+\cdot}$.

8b: $C_{20}H_{23}NO_5$, MW 357.41; Yield 28%; ^1H NMR (250 MHz, $CDCl_3$) δ 1.3 (2H, m), 1.6 (4H, m), 2.3 (2H, t), 3.4 (2H, q), 5.1 (2H, s), 6.8 (1H, t), 6.9 (1H, d), 7.0 (1H, d), 7.4 (5H, s); (DIC/NH_3)-MS $m/z=358$ $(M+H)^{+\cdot}$.

8c: $C_{19}H_{21}NO_5$, MW 343.38; Yield 33%; ^1H NMR (200 MHz, $CDCl_3$) δ 0.95 (3H, d), 1.0 (3H, d), 2.3 (1H, m), 4.75 (1H, dd), 5.1 (2H, s), 6.8 (1H, t), 7.0 (1H, d), 7.1 (1H, d), 7.4 (5H, s); (DIC/NH_3)-MS $m/z=344$ $(M+H)^{+\cdot}$.

8d: $C_{17}H_{17}NO_5$, MW 315.33; Yield 67%; ^1H NMR (250 MHz, $CDCl_3$) δ 1.5 (3H, d), 4.8 (1H, q), 5.2 (2H, s), 6.8 (1H, t), 6.9 (1H, d), 7.1 (1H, d), 7.4 (5H, s); (DIC/NH_3)-MS $m/z=316$ $(M+H)^{+\cdot}$.

N-(2,3-Diacetoxybenzoyl)aminobenzylic Esters **9**

To a stirred solution of **8a** (1.06 mmol) in 60 ml CH_2Cl_2, 0.5 ml of Et_3N was added. Then, the reaction mixture was cooled to 0°C and 0.15 ml of acetyl chloride (2.12 mmol) in 5 ml CH_2Cl_2 was added dropwise. The reaction mixture was allowed to warm to room temperature and analyzed by TLC. After quenching with water, the organic layer was washed, dried and evaporated to yield 630 mg of the title compound **9a**. Products **9b**, **9c** and **9d** were prepared using the same procedure.

9a: $C_{22}H_{23}NO_7$, MW 413.43; Yield 99%; ^1H NMR (200 MHz, $CDCl_3$) δ 1.9 (2H, m), 2.3 (6H, s), 2.4 (2H, t), 3.4 (2H, m), 5.1 (2H, s), 6.4 (1H, t), 6.9 (2H, m), 7.3 (5H, s), 7.4 (1H, m); (DIC/NH_3)-MS $m/z=414$ $(M+H)^{+\cdot}$.

Anal Calcd for $C_{22}H_{23}NO_7$: C 63.91, H 5.61, N 3.39.
Found: C 63.03, H 5.45, N 3.49.

9b: $C_{24}H_{27}NO_7$, MW 441.48; Yield: 91%, ^1H NMR (250 MHz, $CDCl_3$) δ 1.35 (2H, m), 1.55 (2H, m), 1.65 (2H, m), 2.25 (6H, s), 2.3 (2H, t), 3.35 (2H, q), 5.1 (2H, s), 6.2 (1H, t), 7.2 (2H, m), 7.3 (5H, s), 7.5 (1H, dd); (DIC/NH_3)-MS $m/z=442$ $(M+H)^{+\cdot}$.

Anal Calcd for $C_{24}H_{27}NO_7$: C 65.29, H 6.16, N 3.17.
Found: C 65.19, H 6.31, N 2.99.

9c: $C_{23}H_{25}NO_7$, MW 427.45; Yield 78%; ^1H NMR (200 MHz, $CDCl_3$) δ 0.9 (3H, d), 0.95 (3H, d), 2.3 (1H, m), 2.3 (3H, s), 2.35 (3H, s), 4.8 (1H, dd), 5.2 (2H, s), 6.9 (1H, d), 7.3 (6H, m), 7.7 (1H, dd); (DIC/NH_3)-MS $m/z=428$ $(M+H)^{+\cdot}$.

Anal Calcd for $C_{23}H_{25}NO_7$: C 64.62, H 5.90, N 3.28.
Found: C 62.89, H 5.57, N 3.23.

9d: $C_{21}H_{21}NO_7$, MW 399.40; Yield 94%; ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (3H, d), 2.3 (3H, s), 2.4 (3H, s), 4.8 (1H, q), 5.2 (2H, s), 7.3 (7H, m), 7.7 (1H, dd); (DIC/NH_3)-MS $m/z=400$ $(M+H)^{+\cdot}$.

Anal Calcd for $C_{21}H_{21}NO_7$: C 63.15, H 5.30, N 3.51.
Found: C 61.76, H 5.26, N 3.23.

N-(2,3-Diacetoxybenzoyl) Acids **10**

588 mg (1.33 mmol) of benzylic ester **9a** was stirred in 10 ml ethyl acetate under atmospherique pressure of H_2 with 20% equiv wt of 10% Pd/C 10% (118 mg). The reaction mixture was stirred for 12 hours, then the catalyst was filtered and the solvent was evaporated. Products **10b**, **10c** and **10d** were prepared using the same procedure.

10a: $C_{15}H_{17}NO_7$, MW 323.30; Yield 84%; ^1H NMR (200 MHz, $CDCl_3$) δ 1.9 (2H, m), 2.3 (6H, s), 2.45 (2H, t), 3.4 (2H, q), 6.4 (1H, t), 7.3 (2H, m), 7.4 (1H, dd); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.14, 20.26, 24.04, 30.96, 38.91, 125.45, 125.98, 126.22, 130.00, 139.78, 142.58, 165.62, 168.06, 168.19, 177.10; (DIC/NH_3)-MS m/z 324 $(M+H)^{+\cdot}$, $m/z=341$ $(M+18)^{+\cdot}$.

Anal Calcd for $C_{15}H_{17}NO_7$: C 63.91, H 5.61, N 3.39.
Found: C 63.03, H 5.45, N 3.49.

10b: $C_{17}H_{21}NO_7$; MW 351.36; Yield 82%; ^1H NMR (200 MHz, $CDCl_3$) δ 1.4 (2H, q), 1.65 (4H, m), 2.4 (6H,

s), 2.4 (2H, m), 3.4 (2H; q), 6.2 (1H, t), 7.2 (2H, m), 7.6 (1H, dd); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.14, 20.23, 23.96, 25.88, 28.35, 33.50, 39.44, 125.32, 125.98, 126.21, 130.23, 139.64, 142.55, 165.28, 167.90, 168.08, 177.93; (DIC/NH_3)-MS $m/z=338$ $(M+H)^{+\cdot}$, $m/z=355$ $(M+18)^{+\cdot}$.

Anal Calcd for $C_{17}H_{21}NO_7$: C 58.10, H 6.03, N 3.99.
Found: C 56.55, H 6.29, N 3.32.

10c: $C_{16}H_{19}NO_7$, MW 337.33; Yield 90%; ^1H NMR (250 MHz, $CDCl_3$) δ 1.0 (6H, m), 2.20 (1H, m), 2.28 (3H, s), 2.33 (3H, s), 4.77 (1H, dd), 6.9 (1H, d), 7.3 (1H, m), 7.7 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.49, 18.79, 20.34, 20.50, 31.0, 57.38, 126.27, 126.58, 127.21, 129.14, 139.88, 142.63, 164.90, 168.05, 168.14, 175.19; (DIC/NH_3)-MS $m/z=338$ $(M+H)^{+\cdot}$, m/z 355 $(M+18)^{+\cdot}$.

Anal Calcd for $C_{16}H_{19}NO_7$: C 56.97, H 5.68, N 4.15.
Found: C 56.78, H 5.73, N 4.09.

10d: $C_{14}H_{15}NO_7$, MW 309.28; Yield 81%; ^1H NMR (200 MHz, $CDCl_3$) δ 1.5 (3H, d), 2.3 (3H, s), 2.4 (3H, s), 4.75 (1H, q), 7.3 (2H, m), 7.7 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.69, 20.08, 20.26, 48.31, 126.16, 126.37, 126.94, 128.65, 139.82, 142.51, 164.47, 167.88, 168.10, 175.30; (DIC/NH_3)-MS $m/z=310$ $(M+H)^{+\cdot}$, $m/z=327$ $(M+18)^{+\cdot}$.

Anal Calcd for $C_{14}H_{15}NO_7$: C 54.37, H 4.89, N 4.53.
Found: C 54.89, H 5.61, N 2.99.

2′-*O*-Acetyl Spiramycin **3a**

The literature procedure[12] yielded 93% of the title compound **3a**. HPLC analysis showed a purity superior to 95%. $C_{45}H_{76}N_2O_{15}$, MW 885.11; ^1H NMR (400 MHz, $CDCl_3$) δ 2.2 (3H, $COCH_3$, s), 4.9 (1H, $H_{2'}$, dd); ^{13}C NMR δ 205.67 ($COCH_3$); (DIC/NH_3)-MS m/z 886 $(M+H)^{+\cdot}$, ES-MS $m/z=885.6$ $(M+H)^{+\cdot}$, $m/z=741.6$ $(M-\text{mycarose})^{+\cdot}$.

Anal Calcd for $C_{45}H_{76}N_2O_{15}$: C 61.07, H 8.65, N 3.16.
Found: C 58.66, H 8.05, N 3.43.

2′-*O*-Acetyl-4″-*O*-[4-*N*-(2,3-diacetoxybenzoyl)]-butanoyl Spiramycin **3b**

238 mg of compound **10a** (0.73 mmol; 1.2 equiv) were dissolved in 10 ml of CH_2Cl_2 at room temperature and 152 mg of DCC (0.73 mmol; 1.2 equiv) and catalytic amount of PPY (4-pyrrolidinopyridine)[14] were added and stirred for one hour. 544 mg (0.61 mmol; 1 equiv) of 2′-*O*-acetyl spiramycin **3a** and the mixture was stirred overnight. The precipitate of dicyclohexylurea was filtered. The filtrate was washed with water and the organic layer was dried over $MgSO_4$. The solvent was evaporated and the crude reaction product was chromatographed by MPLC on reversed phase LiChroprep RP-18 (Merck) with H_2O (citric acid pH=3, 450 mg/liter) 74%/CH_3CN 26% as elution system. After extraction 145 mg of product **3a** were obtained (20%).

$C_{60}H_{91}N_3O_{21}$, MW 1190.4; ^1H NMR (200 MHz, $CDCl_3$) δ 4.6 (1H, $H_{4''}$, d), 4.9 (1H, $H_{2'}$, dd), 7.55 (1H, m), 7.7 (1H, m); 8.0 (1H, m); ES-MS $m/z=1190.5$ $(M+H)^{+\cdot}$, $m/z=1148.5$, $m/z=1106.5$, $m/z=927.5$ (McLafferty rearrangement), $m/z=885.5$ $(M-\text{substituent in position 4''})^{+\cdot}$; mp 74~76°C.

Anal Calcd for $C_{60}H_{91}N_3O_{21}$: C 60.54, H 7.71, N 3.53.
Found: C 58.41, H 8.40, N 2.38.

2′-*O*-(3,4-Diacetoxybenzoyl)spiramycin **3c**

The same procedure as for compound **3b** gave the title compound with 14% yield. $C_{54}H_{82}N_2O_{19}$, MW 1063.26; ^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 4.9 (1H, $H_{2'}$, dd), 7.3 (1H, d), 7.8 (1H, d), 7.9 (1H, dd); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.82, 167.58 ($COCH_3$); 162.88 (COAr); ES-MS m/z 1063.5 $(M+H)^{+\cdot}$, m/z 1021.5, m/z 979.5, m/z 843.5 $(M-\text{substituent in position 2'})^{+\cdot}$; mp: 128~130°C.

Anal Calcd for $C_{54}H_{82}N_2O_{19}$: C 61.0, H 7.77, N 2.63.
Found: C 59.24, H 7.44, N 2.74.

2′-*O*-(3,4-Dihydroxybenzoyl)spiramycin **3d**

230 mg (10 mmol) of sodium was added under argon to 8 ml of anhydrous methanol at 0°C. After dissolving, 960 mg (10 mmol) of guanidine hydrochloride were added and the solution was stirred during a few minutes and the precipitate of NaCl was filtered. 130 mg of 2′-*O*-(3,4-diacetoxybenzoyl)spiramycin **3c** were dissolved in 5 ml EtOH-CH_2Cl_2 9/1. 150 ml of the above solution was introduced (1 equiv guanidine) and the solution was stirred 15 minutes at room temperature. Then, the solvent was evaporated, the product was dissolved in CH_2Cl_2 and washed with water. After drying and evaporation of the solution, 119 mg of compound **3d** were obtained (99%). $C_{50}H_{78}N_2O_{17}$, MW 979.18; ^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 4.9 ppm (1H, $H_{2'}$, dd), 6.6 ppm (1H, m), 7.3 (2H, m); ES-MS m/z 978.8 $(M+H)^{+\cdot}$; mp: 166°C.

Iron Complex **3e**

343 mg (0.35 mmol) of 2′-*O*-(3,4-dihydroxybenzoyl)-spiramycin **3d** was dissolved in 10 ml anhydrous MeOH, 43 mg (0.12 mmol) $Fe(acac)_3$ and 0.1 ml of triethylamine. The reaction mixture was stirred 3 hours at room temperature. The crude product was filtered on Sephadex LH-20 to yield 230 mg of complex **3e** (33%). $C_{150}H_{228}N_6O_{51}Fe$, MW 2987.34; ES-MS m/z 2984

$(M+H)^{+\cdot}$ (for Mcalc 2985.48); UV: **3e** λ_{max} (MeOH) nm 290, 537 (Fe-O), **3d** λ_{max} (MeOH) nm 282, 290; $Fe(acac)_3$ nm 279, 430 (Fe-O); since iron(III) is paramagnetic the analogous Ga(III) complex **3f** was synthetized using $Ga(NO_3)_3$.

^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 4.9 (1H, $H_{2'}$, dd), 6.44 (1H, d), 7.11 (1H, s), 7.16 (1H, d).

2′-*O*-(2,3-Dihydroxybenzoyl)spiramycin **3g**

The same procedure as for **3d** but using CDI instead of DCC yielded 10% of the title compound. $C_{50}H_{78}N_2O_{17}$, MW 979.18; ^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 5.1 (1H, $H_{2'}$, dd), 6.7 (1H, m), 7.2 (2H, m); ES-MS *m/z* 978.6 $(M+H)^{+\cdot}$.

Anal Calcd for $C_{50}H_{78}N_2O_{17}$: C 61.33, H 8.03, N 2.86.
Found: C 60.81, H 7.97, N 4.48.

2′-*O*-[4-*N*-(2,3-Diacetoxybenzoyl)]butanoyl-spiramycin **3h**

The same procedure as for **3c** but using CDI instead of DCC yielded 27% of the desired compound **3h**. $C_{58}H_{89}N_3O_{20}$, MW 1148.36; ^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 4.9 (1H, $H_{2'}$, dd), 6.85 (1H, m), 7.2 (2H, m); ES-MS *m/z* 1147.80 $(M+H)^{+\cdot}$.

Anal Calcd for $C_{58}H_{89}N_3O_{20}$: C 60.66, H 7.81, N 3.66.
Found: C 58.60, H 7.64, N 3.32.

2′-*O*-[*N*-(2,3-Diacetoxybenzoyl)-(L)-alanyl]spiramycin **3i**

The same procedure as for **3h** yielded 24% of compound **3i**. $C_{57}H_{87}N_3O_{20}$, MW 1134.34; ^1H NMR (400 MHz, $CDCl_3$) δ 2.9 (1H, $H_{4''}$, d), 4.9 (1H, $H_{2'}$, dd), 6.8 ~ 7.3 (3H, m).

Anal Calcd for $C_{57}H_{87}N_3O_{20}$: C 60.36, H 7.73, N 3.70.
Found: C 56.71, H 7.33, N 3.56.

Neospiramycin **2**

1 g (1.19 mmol) of spiramycin was dissolved in 20 ml of HCl 1 N. The reaction mixture was stirred 8 hours at 40°C. The pH was ajusted to 9 ~ 10 and the product was extracted by CH_2Cl_2. The solvent was evaporated and the crude mixture was chromatographed by MPLC on reversed phase LiChroprep RP-18 (Merck) with H_2O (citric acid pH = 3, 450 mg/liter) 74%/CH_3CN 26% as elution system. After extraction 270 mg of neospiramycin **2** were obtained (33%). $C_{36}H_{62}N_2O_{11}$, MW 698.43; in ^1H NMR, the 4″ proton signal at δ = 2.9 ppm and the 1″ proton signal at δ = 5.0 ppm disappeared, 2.2 and 2.5 N(CH_3); ES-MS *m/z* 698.6 $(M+H)^{+\cdot}$, *m/z* 557.3 (M − forosamine)$^{+\cdot}$.

Anal Calcd for $C_{36}H_{62}N_2O_{11}$: C 61.87, H 8.94, N 4.01.
Found: C 59.19, H 8.59, N 3.12.

2′,4′-Di-*O*-[4-*N*-(2,3-diacetoxybenzoyl)butanoyl]neospiramycin **3j**

549 mg (1.70 mmol; 2.4 equiv) of compound **10a** were stirred in 20 ml CH_2Cl_2. After addition of 278 mg (1.70 mmol; 2.4 equiv) of CDI the reaction mixture was stirred at room temperature for 1 hour. 495 mg (0.71 mmol) of neospiramycin in 20 ml de CH_2Cl_2 were added dropwise and the reaction mixture was heated under reflux for 8 hours. The solvent was evaporated and the crude product was chromatographed by MPLC on reversed phase LiChroprep RP-18 (Merck) with H_2O (citric acid pH = 3, 450 mg/liter) 74%/CH_3CN 26% as elution system. After extraction 67 mg of product **3j** were obtained (7%). $C_{66}H_{92}N_4O_{21}$, MW 1309.48; ^1H NMR (400 MHz, $CDCl_3$) δ 6.8 ~ 7.8 (6H, m); ES-MS *m/z* 1310.77 $(M+H)^{+\cdot}$.

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References

1) COHEN, M. L.: Antimicrobial resistance: Progress for Public Health. Trends in Microbiology 2: 422 ~ 425, 1994
2) WOOLDRIDGE, K. G. & P. H. WILLIAMS: Iron uptake mechanisms of pathogenic bacteria. F.E.M.S. Microbiol. Rev. 12: 325 ~ 348, 1993
3) MILLER, M. J. & F. MALOUIN: The Development of Iron Chelators for Clinical Use. *Ed.*, B. J. BERGERON, pp. 275 ~ 306, CRC Press, Boca Raton, FL, U.S.A., 1994
4) BICKEL, H.; E. GAUMANN, G. NUSSBERGER, P. REUSSER, E. VISCHER, W. VOSSER, A. WETTSTEIN & H. ZÄHNER: Über die Isolierung und Charakterisierung der Ferrimycine A_1 und A_2, neuer Antibiotika der Sideromycin-Gruppe. Helv. Chim. Acta 43: 2105 ~ 2118, 1960
5) BENZ, G.; T. SCHRODER, J. KURZ, C. WUNSCHE, W. KARL, G. STEFFENS, J. PFITZNER & D. SCHMIDT: Konstitution der Desferriform der Albomycine δ_1, δ_2, ε. Angew. Chem. Suppl. 1322 ~ 1335, 1982
6) BRICARD, L.; F. RAMIANDRASOA, G. KUNESCH, A. GAUDEMER, C. LOUBEYRE & A. ANDREMONT: French Patent N°9405792, 1994
7) PINNERT-SINDICO, S.; L. NINET, J. PREUD'HOMME & C. COSAR: Antibiotics Annual, pp. 724 ~ 725, 1954 ~ 1955
8) OMURA, S.: Macrolide Antibiotics: Chemistry, Biology and Practice, Academic Press, Orlando, FL, U.S.A., 1984
9) KUNESCH, N.; C. MIET & J. POISSON: Utilisation de la guanidine comme agent désacétylant sélectif: Une méthode de désacétylation instantanée applicable aux sucres. Tetrahedron Lett. 28: 3569 ~ 3572, 1987

10) REISSBRODT, R.; L. HEINISCH, U. MOLLMANN, W. RASCH & H. ULBRICHT: Growth promotion of synthetic catecholate derivatives on Gram-negative bacteria. Biometals 6: 155~162, 1993
11) HUSSON, A.; R. BESSELIEVRE & H.-P. HUSSON: A short and efficient synthesis of phenolcarboxamides. Tetrahedron Lett. 24: 1031~1034, 1983
12) RAMU, K.; S. SHRINGARPURE, S. COOPERWOOD, J. M. BEALE & J. S. WILLIAMSON: ^{1}H-NMR and ^{13}C-NMR spectral assignments of spiramycins I and III. Pharmaceutical Research 11: 458~465, 1994
13) SANO, H.; H. TANAKA, K. YAMASHITA, R. OKACHI & S. OMURA: Synthesis and *in vitro* and *in vivo* activities of 3″,4″-diacylates and 3,3″,4″-triacylates of spiramycin I. J. Antibiotics 37: 760~772, 1984
14) SCRIVEN, E. F. V.: 4-Dialkylaminopyridines: Super Acylation and Alkylation Catalysts. Chem. Soc. Rev. 12: 129~161, 1983