# DEOXY DERIVATIVES OF BUTIROSIN A AND 5''-AMINO-5''-DEOXYBUTIROSIN A, AMINOGLYCOSIDE ANTIBIOTICS RESISTANT TO BACTERIAL 3'-PHOSPHORYLATIVE ENZYMATIC INACTIVATION
## SYNTHESIS AND NMR STUDIES

PETER W. K. WOO* and THEODORE H. HASKELL

Warner Lambert/Parke Davis Pharmaceutical Research Division, Warner Lambert Company, Ann Arbor, Michigan 48105, U.S.A.

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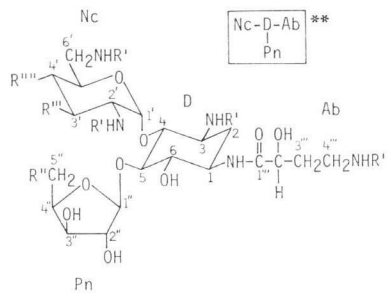
3'-Deoxybutirosin A (**4**), 5''-amino-3',5''-dideoxybutirosin A (**6**), and 5''-amino-4',5''-dideoxybutirosin A (**7**) were prepared by deoxygenation of the appropriate hydroxyl in suitably protected derivatives of butirosin A, using sequentially trifluoromethylsulfonylation, displacement with benzenethiolate, and hydrogenolysis. The structures of the compounds were confirmed by NMR spectroscopy, using $^{13}C$ NMR and $^{1}H$ NMR at up to 600 MHz. The compounds are broad-spectrum antibiotics active against resistant microorganisms which inactivate butirosin and related aminoglycosides by 3'-phosphorylation.

Chemical modification of butirosin A (**1**),[1] a broad-spectrum aminoglycoside antibiotic with notable activities against Gram-negative opportunistic organisms, have, in our laboratories, led to the synthesis of 5''-amino-5''-deoxybutirosin A (**2**)[2] and 5''-amino-3',4',5''-trideoxybutirosin A (**3**)[3,4] and given support to certain structure-activity relationships. First, the replacement of the 5''-hydroxyl group of butirosin A by an amino group enhances the potency, especially against certain strains of *Pseudomonas* and *Serratia*. Secondly, deoxygenation of the 3'-hydroxyl circumvents enzymatic 3'-phosphorylation, (*e. g.*, by kanamycin-neomycin phosphotransferase, APH(3'), from certain *E. coli* carrying R factors), a mechanism by which certain resistant organisms inactivate butirosin A and related aminoglycoside antibiotics.[5,6] However, this inactivation may also be circumvented by deoxygenation




| | R' | R'' | R''' | R'''' |
|---|---|---|---|---|
| **1*** | H | OH | OH | OH |
| **2** | H | $NH_2$ | OH | OH |
| **3** | H | $NH_2$ | H | H |
| **4** | H | OH | H | OH |
| **5** | H | OH | OH | H |
| **6** | H | $NH_2$ | H | OH |
| **7** | H | $NH_2$ | OH | H |

* (*S*)-*O*-2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-*O*-[β-D-xylofuranosyl-(1→5)]-$N^1$-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine.

** "D", "Nc", "Pn", and "Ab" are abbreviated notations for the four structural units, or their derivatives, the exact structures of which may vary according to context.

of the 4′-hydroxyl alone, since the latter may be necessary for enzyme attachment in the 3′-phosphorylation process.[7] Hence it was of interest to deoxygenate the 3′-hydroxyl and the 4′-hydroxyl individually, in butirosin as well as in its more active 5″-amino-5″-deoxy analog. The absence of the 4′-hydroxyl function should offer additional protection against any possibility of enzymatic inactivation by 4′-adenylation (*cf.* AAD(4)′, which inactivates kanamycin, *etc.*), and the absence of 5″-hydroxyl function, by 5″-phosphorylation (*cf.* APH (5″), which inactivates ribostamycin).

Accordingly, we have synthesized 3′-deoxybutirosin A (**4**),[8~12] 5″-amino-3′,5″-dideoxybutirosin A (**6**),[13~15] and 5″-amino-4′,5″-dideoxybutirosin A (**7**),[16,17] utilizing an alcohol-dehydrogenation method which we developed.[18] These compounds have also been synthesized independently by others using different synthetic routes.[8~17]

## Chemistry

Our approach in the deoxygenation of butirosin derivatives consists of the initial conversion of a specific hydroxyl to an excellent leaving group, namely, the *O*-trifluoromethylsulfonyl function (OTf, or triflate), giving, *e.g.*, **8** (R=Tf), followed by displacement with a highly potent nucleophile such as the benzenethiolate or benzeneselenate anion, and eventual replacement of the nucleophile-derived portion with hydrogen by catalytic hydrogenolysis, sodium in liquid ammonia, or tributyl tin hydride.[18~20] Attempted displacement of the analogous tosylate (**8**, R=tosyl) with sodium iodide in acetylacetone or *N*,*N*-dimethylformamide, under conditions feasible for the deoxygenation of kanamycin B and ribostamycin,[21,22] was unsuccessful, giving products which contained no iodine.

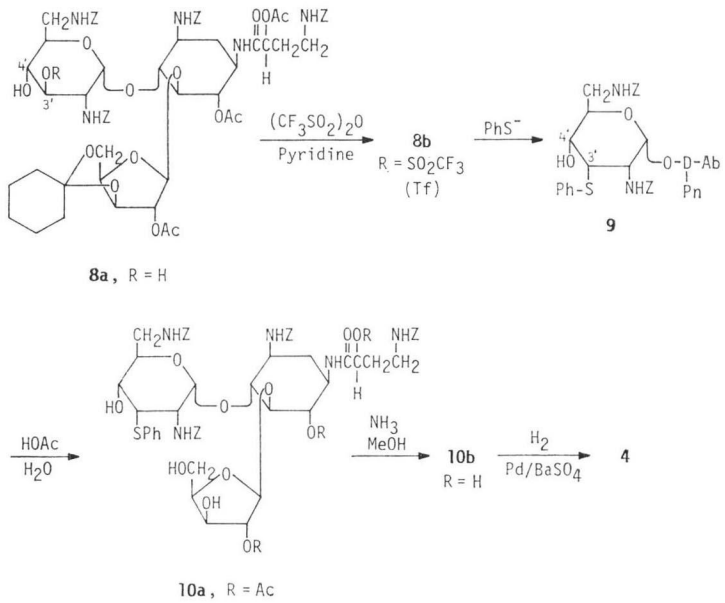
The synthesis of 3′-deoxybutirosin (**4**) proceeded from the suitably protected derivative 2″,2‴,6-tri-*O*-acetyl-3″,5″-*O*-cyclohexylidenetetrakis-*N*-[(phenylmethoxy)carbonyl]butirosin A (**8a**), prepared by procedures similar to those described by SAEKI *et al.*[23] Chromatographically purified **8a** was treated with trifluoromethanesulfonic anhydride in the presence of pyridine in methylene chloride at −10°C to give the 3′-*O*-Tf derivative **8b** as the major product and trace amounts, if any, of the 4′-substituted or 3′,4′-disubstituted products. The crude product was allowed to react with benzenethiol sodium salt in DMF at −5°C for one day to give the corresponding 3′-phenylthio derivative **9**, purified by chromatography. Subsequent hydrolysis of the 3″,5″-cyclohexylidene with acetic acid containing 36% water at 45°C for one day, followed by de-*O*-acetylation with methanolic ammonia or sodium methoxide in methanol, gave the 3′-phenylthio derivative **10b**, purified by chromatography.

The (phenylmethoxy)carbonyl and the phenylthio groups in **10b** were removed by sodium in liquid ammonia to give 3′-deoxybutirosin (**4**), together with appreciable quantities of side products. Alternatively, **4** was obtained relatively free from side products by hydrogenolysis of **10b** in methanol containing aqueous acid in the presence of about six times its weight of 20% palladium on barium sulfate, which was added in various portions during the hydrogenolysis over 50 hours. The crude product **4** was purified by gradient elution chromatography over CM-Sephadex C-25 in the ammonium form, using aqueous ammonia as eluant, and freeze-dried to give **4** in base form.

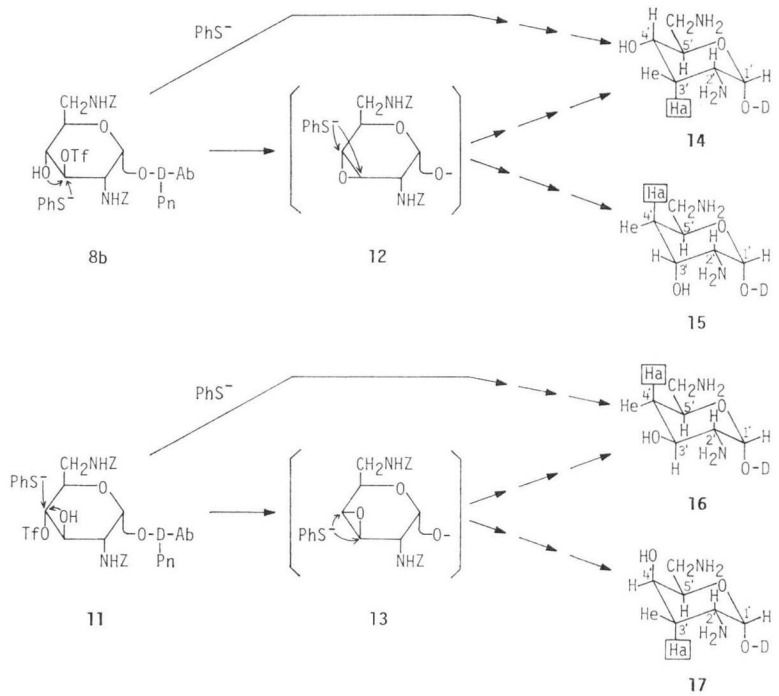
The mass spectrum of the tetra-*N*-acetyl-hexa-*O*-trimethylsilyl derivative of **4** showed a peak at 301 (21%), corresponding to the "Nc"[24] fragment, in this instance 2,6-bis-*N*-acetyl-3-deoxy-4-*O*-trimethylsilylneosamine C-yl, arising from C-1′-*O* bond cleavage. Thus, the newly introduced deoxy function was in the neosamine C moiety.

The assignment of the product as 3′-deoxybutirosin (**4**) instead of 4′-deoxybutirosin (**5**) was based on two reasonable assumptions. First, in analogy with the tosylation of similar kanamycin B and ribo-

Scheme 1. Synthesis of 3′-deoxybutirosin A (**4**).



Scheme 2. Product formation (**14**～**17**) from hypothetical epoxide intermediates (**12**, **13**).



stamycin derivatives,[21,22] the major product in triflation of **8a** was the 3′-substituted analog **8b**. Secondly, in analogy with model compounds,[18] the benzenethiolate anion displaced the OTf group in **8b** in a straight forward $S_N2$ manner without the prior formation of a 3′,4′-epoxide intermediate (**12** or **13**, from intramolecular displacement) which subsequently reacted with the benzenethiolate anion at either

Table 1. $^{13}C$ Chemical-shift assignments[a,b] for butirosin A (**1**), 3′-deoxybutirosin A (**4**), butirosin A sulfate (**1**·$2H_2SO_4$), 3′-deoxybutirosin A hydrochloride (**4**·4HCl), methyl 2-acetylamino-2-deoxy-α-D-glucopyranoside (**19**), and methyl 2-acetylamino-2,3-dideoxy-α-D-glucopyranoside (**18**).

| | **1** | **4** | **1**·$2H_2SO_4$ | **4**·4HCl | **19** | **18** |
|---|---|---|---|---|---|---|
| 1 | 50.30[c] | 50.30[c] | 49.8[c] | 49.82[c] | | |
| 2 | 34.66 | 35.41[d] | 31.9 | 32.01[c] | | |
| 3 | 50.89[c] | 50.95[c] | 50.2[c] | 50.09[c] | | |
| 4 | 82.61 | 82.07[c] | 76.8 | 77.76 | | |
| 5 | 85.47 | 85.99 | 87.0 | 87.14 | | |
| 6 | 75.44 | 75.38 | 75.4[c] | 75.28[c] | | |
| 1′ | 99.71 | 98.58 | 95.7 | 94.05 | 98.96 | 97.50 |
| 2′ | 56.34 | 50.14 | 54.8 | 48.79 | 54.51 | 48.52 |
| 3′ | 73.55[c] | 35.04[d] | 69.3[c] | 30.90 | 72.04 | 32.72 |
| 4′ | 72.26 | 67.45 | 72.1 | 65.62 | 70.91 | 64.97 |
| 5′ | 73.98[c] | 74.23 | 70.1[c] | 70.74[c] | 72.58 | 73.40 |
| 6′ | 42.43 | 42.37 | 41.5 | 40.97 | 61.47 | 61.52 |
| 1″ | 110.88 | 111.30 | 112.7 | 112.82 | | |
| 2″ | 81.37 | 81.48[c] | 81.7 | 81.64 | | |
| 3″ | 75.44 | 75.38 | 74.8[c] | 74.79[c] | | |
| 4″ | 82.61 | 82.83 | 83.7 | 83.58 | | |
| 5″ | 61.25 | 61.14 | 61.5 | 61.47 | | |
| 1‴ | 176.90 | 177.06 | 176.4 | 176.31 | | |
| 2‴ | 70.64 | 70.69 | 70.6 | 70.48[c] | | |
| 3‴ | 34.66 | 34.82[d] | 31.9 | 31.80[c] | | |
| 4‴ | 37.57 | 37.62 | 37.6 | 37.52 | | |
| $OCH_3$ | | | | | 56.02 | 55.70 |
| $(C{=}O)\ CH_3$ | | | | | 175.28 | 174.31 |
| $(C{=}O)\ CH_3$ | | | | | 22.79 | 22.63 |

[a] Chemical shifts (δ) are reported as ppm from TMS with 1,4-dioxane (δ 67.40 ppm) as internal reference and deuterium oxide as solvent.

[b] Assignments are mainly based on: (1) spectral comparison with related compounds, and (2) absorption shifts of carbon atoms β to (two bonds from) amino groups at various pD's.[29~32]

[c] Group assignment; two peaks with closely similar δ.

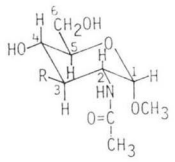
[d] Group assignment; three peaks with closely similar δ.

the 3′- or 4′-position. The implications of these two assumptions in terms of product formation (**14**, **15**, **16**, **17**) are illustrated in Scheme 2.

The structural assignment of the product as **4** was fully confirmed by spin-decoupling $^1H$ NMR spectroscopy. Compound **4** was first hydrolyzed (2 N HCl, 5 hours) to 3′-deoxyneamine (**14**).[25] The spectrum of **14** in $D_2O$ shows the signal of H-3′a appearing as a quartet (partially masked by H-4a from the deoxystreptamine moiety at 90 MHz, but fully revealed at 220 MHz) at δ 1.64, $J_{2',3'a}$, $J_{3'a,3'e}$, and $J_{3'a,4'}$ all equal to about 11.5 Hz.[26] Spin-decoupling studies show that the quartet is coupled to the masked signals of H-4′, α to oxygen, at δ 3.52, and to the masked signals of H-2′, α to amino, at δ 2.82 to 3.10, the H-2′ signals in turn being coupled, as expected, to the H-1′ doublet at δ 5.17. These data are inconsistent with those expected from the axial methylene proton in **15** (H-4′a), **16** (H-4′a), and **17** (H-3′a), which are therefore readily eliminated as structural possibilities.

The presence of the deoxy function at C-3′ of **4** is also consistent with $^{13}C$ NMR data (Table 1). Relative to butirosin (**1**) (or **1**·$2H_2SO_4$), the C-3′ peak of **4** (or **4**·4HCl) shows a large upfield shift (*ca.*

39 ppm), while both the C-2′ and C-4′ peaks show a smaller upfield shift (*ca.* 6 ppm). The magnitudes of these shifts ($\Delta$) are similar to the substituent effects of an equatorial hydroxyl on a cyclohexane ring: $\alpha$-carbon, 42.6 ppm; $\beta$-carbon, 8.0 ppm.[27] Entirely analogous shifts are shown by methyl 2-acetylamino-2,3-dideoxy-$\alpha$-D-*ribo*hexopyranoside (**18**) relative to methyl 2-acetylamino-2-deoxy-$\alpha$-D-glucopyranoside (**19**): $\Delta$C-3, 39.3 ppm; $\Delta$C-2, 6.0 ppm; $\Delta$C-4, 5.9 ppm (*cf.* reference 28).



18 R = -H
19 R = -OH

The assignment of the methylene carbon peak at *ca.* 35 ppm to C-3′ of **4** (rather than C-4′ of **5**) is confirmed by the upfield shift of *ca.* 4.1 ppm observed upon protonation of **4**, similar to those shown by other carbon atoms which are $\beta$ to amino groups,[29~32] namely, C-2, 4, 1′, 3′, 5′ and 3‴ (3.0~4.5 ppm). On the other hand, C-4′ of **5**, being $\gamma$ to the amino group, should show negligible shift, although actually a small shift of 1.83 ppm is observed.

The synthesis of 5″-amino-3′,5″-dideoxybutirosin A (**6**) and 5″-amino-4′,5″-deoxybutirosin A (**7**) utilized a reaction series entirely analogous to that used for the synthesis of **4**. The suitably protected derivative, 2″, 2‴, 3″, 6-tetra-*O*-acetyl-5″-amino-5″-deoxypentakis-*N*-[phenylmethoxy(carbonyl)]-butirosin A (**20**), previously reported by Woo,[3] was allowed to react with trifluoromethanesulfonic anhy-

Scheme 3. Preparation of 5″-amino-3′,5″-dideoxybutirosin A (**6**) and 5″-amino-4′,5″-dideoxybutirosin A (**7**).

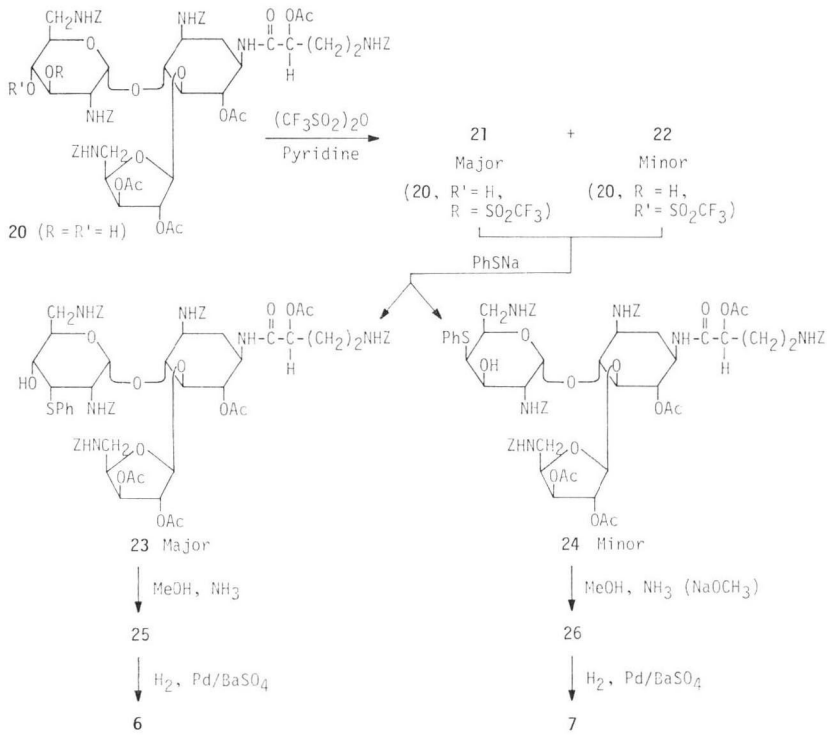


Fig. 1. A. A portion of the normal spectrum of **7** (BBK 137) in deuterium oxide saturated with carbon dioxide at 600.2 MHz.

B. The corresponding difference spectrum, *i.e.*, spectrum of the same sample spin-decoupled from the proton having signals at 2472 Hz ($\delta$ 4.119) (H-3′) and substracted from the normal spectrum. The inverted signals originated from the spin-decoupling spectrum. Signals from protons unaffected by spin-decoupling disappeared.

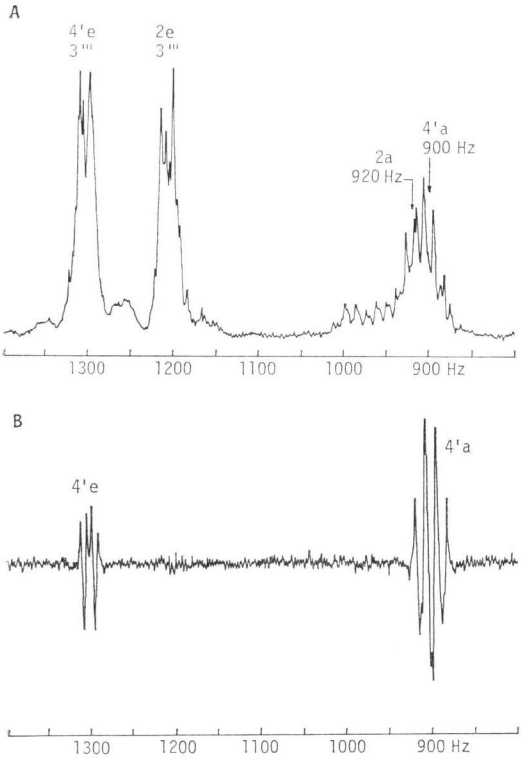



Table 2. Comparative NMR data ($\delta$) from crucial protons in BBK 137, BBK 137 carbonate, and **7** carbonate at 600.2 MHz.

| H | BBK-137 | BBK-137 $+CO_2$ | **7** $+CO_2$ |
|---|---|---|---|
| 1′ | 5.430 | 5.591 | 5.561 |
| 2′ | 2.631 | 3.282 | 3.149 |
| 3′ | 3.839 | 4.119 | 4.065 |
| 4′-a | 1.381 | 1.500 | 1.485 |
| 4′-e | 2.026 | 2.166 | 2.166 |
| 5 | 2.821 | | |
| 1 | 3.864 | 3.890 | 3.880 |
| 2a | 1.388 | 1.533 | 1.534 |
| 2e | 1.949 | 2.006 | 1.999 |
| 3 | 2.823 | | |
| 2‴ | 4.249 | 4.315 | 4.319 |
| 3h‴ | 1.824 | 2.006 | 1.999 |
| 3l‴ | 1.949 | 2.166 | 2.166 |
| 4‴ | 2.799 | 3.171 | 3.171 |
| 1″ | 5.292 | 5.488 | 5.465 |
| 2″ | 4.245 | | |
| *J* | | | |
| 1′, 2′ | 3.6 | 3.7 | 3.8 |
| 2′,3′ | 10.2 | ~10.5 | ~11 |
| 3′,4′e | | 4.6 | 4.6 |
| 3′,4′a | ~12.0 | ~11.5 | ~11.5 |
| 4′a,4′e | ~12.0 | ~12.2 | ~12 |
| 4′a,5 | ~12.0 | ~12.2 | ~12 |
| 1,2a | ~12.5 | ~12.3 | |
| 2a,2e | ~12.5 | ~12.3 | |
| 2a,3 | ~12.5 | ~12.3 | |
| 1″, 2″ | 1.4 | singlet | singlet |

dride in pyridine to give a mixture consisting of the 3′-*O*-Tf **21** as the major component and the 4′-*O*-Tf **22** as the minor component. In contrast to the triflation of **8a** in the previous series, a higher proportion of the minor component was produced. The mixture of **21** and **22** was allowed to react with benzenthiol sodium salt to give the corresponding mixture of 3′- and 4′-phenylthio derivative (**23** and **24**), which were separated by silica gel chromatography. The purified **23** and **24** were individually de-*O*-acetylated with methanolic ammonia or sodium methoxide in methanol, then treated with sodium in liquid ammonia, or preferably, with hydrogen in the presence of large excess of palladium on barium sulfate to give **6** and **7**, respectively.

The structural assignment of the major product as **6** (3′-deoxy) and the minor product as **7** (4′-deoxy) was based on analogy with the parallel synthetic sequence leading to **4**, the structure of which was fully substantiated as described above. Further confirmation was obtained by spin-decoupling $^1$H NMR data obtained at 600 MHz. The high magnetic field, together with the technique of recording the spin-decoupling data as a "difference spectrum" (*cf.* Fig. 1, discussed below), was found to be highly advanta-

Table 3. Comparative *in vitro* activities of butirosin A, derivatives of butirosin A, and gentamicin.

| | MIC (Minimal inhibitory concentration, $\mu$g/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** Butirosin A sulfate | **2** 5″-Amino-5″-deoxy | **4** 3′-Deoxy, carbonate | **6** 5″-Amino-3′,5″-dideoxy, carbonate | **7** 5″-Amino-4′,5″-dideoxy, carbonate | **3** 5″-Amino-3′,4′,5″-trideoxy | Gentamicin sulfate |
| *Staphylococcus aureus* S18713 | 50 | 25 | 6.3 | 3.1 | 6.3 | 3.1 | 3.1 |
| *Serratia marcescens* IMM-5 | 25 | 6.3 | 12.5 | 25 | 12.5 | 12.5 | 6.3 |
| *Pseudomonas aeruginosa* | | | | | | | |
| Aquilar | 200 | 200 | 25 | 12.5 | 25 | 6.3 | 25 |
| GNT[a] resistant[b] | 31.3 (3) | 10.0 (3) | 12.5 (3) | 6.3 (2) | 6.3 (2) | 3.9 (3) | 126 (3) |
| Others[b] | 13.8 (7) | 4.2 (7) | 9.3 (7) | 5.5 (7) | 4.7 (7) | 1.9 (7) | 3.1 (7) |
| *Escherichia coli* | | | | | | | |
| JR76.2 | 200 | 200 | 3.1 | 25 | 25 | 12.5 | 200 |
| CW | 6.3 | 6.3 | 3.1 | — | — | 3.1 | 100 |
| Others[b] | 4.4 (2) | 6.2 (2) | — | — | — | 2.2 (2) | 1.6 (2) |

[a] Gentamicin. [b] Geometric means (Number of strains).

geous and informative. The analysis was also aided by carbonate induced shifts, allowing some crucial protons to be revealed, free enough from interfering signals, although the presence of carbon dioxide was also accompanied by formation of extraneous peaks difficult to explain.

In the spectra of **6**, H-3′a and H-2a appear as two overlapping quartets ($\delta$ 1.515, $\delta$ 1.427), each showing large a-a couplings (*ca.* 12 Hz each) with the geminal equatorial proton and with the two axial protons on the adjacent carbon atoms, the quartet patterns expected being analogous to those shown in Fig. 1. In a solution saturated with carbon dioxide, H-2′ appears as doublet of triplet at $\delta$ 3.615, coupled to H-1′ at $\delta$ 5.435 with an a-e coupling ($J_{1',2'} = 3.5$ Hz), to H-3e′ with an a-e coupling ($J_{2',3e'} = 4.7$ Hz) and to H-3a′ with an a-a coupling ($J_{2',3a'} = ca.\ 12.7$ Hz).

In the spectra of **7** in deuterium oxide saturated with carbon dioxide, H-3′ appears as a doublet of triplet at $\delta$ 4.119, coupled to H-4′e with an a-e coupling ($J_{3',4'e} = 4.6$ Hz), to H-4′a with an a-a coupling ($J_{3',4'a} = ca.\ 11.5$ Hz), and to H-2′ with an a-a coupling ($J_{2',3'} = ca.\ 10.5$ Hz). Comparison of the crucial NMR absorptions of **7** with the same compound (BBK 137, NAITO *et al.*[17]) synthesized by a different route shows virtual identity (Table 2), with small differences probably accounted for by degree of carbon dioxide saturation.

The technique of recording spin-decoupling data as a difference spectrum, *i.e.*, decoupled spectrum subtracted from the normal spectrum, with signals from the former appearing as negative, inverted signals, and from the latter as positive signals, allowed easy recognition of the signals affected by the decoupling. A portion of the difference spectrum ($\delta$ 1.416~2.249), obtained when H-3′ of **7** was irradiated, was shown in Fig. 1.*

## Biological Activities

A comparison of the *in vitro* activities of the various deoxy derivatives **3**, **4**, **6**, and **7**, with butirosin A (**1**), 5″-amino-5″-deoxybutirosin A (**2**), and gentamicin, are shown in Table 3. The deoxy derivatives

* It is noted that when the decoupled signal consists of small couplings and is extensively overlapped by other signals, the correct splitting patterns may not be obtained in the difference spectrum. Thus, in Fig. 1, while H-4′a shows the correct splitting patterns (quartet[26] decoupled to triplet), H-4′e does not show the correct splitting patterns (two pair of quartets[26] decoupled to two pairs of doublets).

show improved activities over butirosin or gentamicin against certain organisms. They are significantly more active than butirosin against *Pseudomonas aeruginosa* Aquilar and *Staphylococcus aureus* S18713, and against *E. coli* JR76.2, a known carrier of kanamycin-neomycin phosphotransferase which inactivates butirosin and related aminoglycosides by 3′-phosphorylation. The advantageous effect of the 5′-amino function and that of the deoxy function, when combined in the same molecule, appear to be cummulative against some, but not all, of the organisms studied.

## Experimental

TLC was generally performed using glass plate precoated with silica gel (Quanta QIF, 10 cm length, Quantum Industries, Fairfield, New Jersey), 12.5% aqueous solution of ammonium molybdate containing 5% phosphoric acid and 5% sulfuric acid as spray, and detection by heating at 120°C. Column chromatography was performed using silica gel (E. Merck 60, 0.063~0.20 mm). Compositions of solvent mixtures are expressed in volume %; *e. g.*, 12% M in C means 12 ml of methanol (M) diluted to 100 ml with chloroform (C) (which contains *ca.* 0.75% ethanol). Analysis of the purity of aminoglycosides by VPC was performed using the *N*-TFA-*O*-TMS (poly-*N*-trifluoroacetyl-poly-*O*-trimethylsilyl) derivatives and a column with OV-17 as the liquid phase at a temperature of 290°C.[2]

2″,2‴,6-Tri-*O*-acetyl-3″,5″-*O*-cyclohexylidenetetrakis-*N*-[(phenylmethoxy)carbonyl]-3′-*O*-[(trifluoromethyl)sulfonyl]butirosin A (**8b**)

A solution of 2.56 ml (14.4 mmole) of trifluoromethanesulfonic anhydride in 33 ml of dried methylene chloride was added dropwise with stirring, during 25 minutes, to a cooled (−10°C) solution of 4.445 g of **8a**[23] (3.42 mmole; *cf.* Woo and Haskell[16,18]) in 50 ml of dried methylene chloride and 16 ml of pyridine. After 20 minutes at −10°C, TLC showed the presence of a major reaction product (Rf 0.49 in 3% M in C), trace amounts of two faster moving materials (Rf 0.56 and 0.64), and trace amount, if any, of the starting material **8a** (Rf 0.30). After an additional 50 minutes at −10°C, 15 ml of water was added with stirring and the organic layer washed successively with dilute hydrochloric acid, water, aqueous sodium bicarbonate, and water. After drying (sodium sulfate) and evaporation, 4.69 g (3.28 mmole, 96%) of essentially pure **8b** was obtained.

3′-*Epi*-2″,2‴,6-tri-*O*-acetyl-3″,5″-*O*-cyclohexylidene-3′-deoxytetrakis-*N*-[(phenylmethoxy)carbonyl]-3′-(phenylthio)butirosin A (**9**)

To a stirred suspension of 1.690 g (36.8 mmole) of sodium hydride (50% in mineral oil) in 10.0 ml of dried DMF (*N*,*N*-dimethylformamide), cooled to 0°C, was added 5.5 ml (58 mmole) of benzenethiol dropwise during 40 minutes. The solution was cooled to −6°C and added to a solution of 4.64 g (3.24 mmole) of **8b** in 7.0 ml of dried DMF at −6°C. After stirring for 16 hours at −6°C (temperature up to +10°C has been used in other runs), TLC of aliquots treated with glacial acetic acid showed that the reaction was complete. A solution of 4.7 ml glacial acetic acid in 13 ml of ether was added, and the mixture was evaporated *in vacuo* (mostly 25°C, briefly up to 45°C) and further dried under high vacuum to give 13.5 g of residue. A solution of the residue in 250 ml of chloroform was washed three times with water, dried over sodium sulfate, and evaporated as before to give 7.4 g of crude product. Chromatographic purification on 60 g of silica gel (elution with 1.5% M in C) afforded 1.3 g (0.93 mmole, 29%) of purified **9** (Rf 0.53, 2.5% M in C).

3′-*Epi*-2″,2‴,6-tri-*O*-acetyl-3′-deoxytetrakis-*N*-[(phenylmethoxy)carbonyl]-3′-(phenylthio)butirosin A (**10a**)

To a solution of 1.142 g (0.82 mmole) of **9** in 25 ml of glacial acetic acid was added dropwise with swirling 14.1 ml of water. After 19.5 hours at 45°C, the solution contained essentially homogeneous **10a** (Rf 0.21, 2.5% M in C) and was evaporated *in vacuo* to give 1.075 g (100%) of **10a**, which was used without further purification.

3′-*Epi*-3′-deoxytetrakis-*N*-[(phenylmethoxy)carbonyl]-3′-(phenylthio)butirosin A (**10b**)

(a) Methanolic Ammonia Method: An ice-cold solution of 1.074 g (0.82 mmole) of **10a** in 70 ml

of absolute methanol, saturated with anhydrous ammonia, was kept at 0°C for 21 hours, then evaporated to dryness *in vacuo*. The residue was chromatographed over 13 g of silica gel. Elution with chloroform containing methanol (0% increasing to 2.5%) yielded 0.423 g of pure **10b** (Rf 0.30, 8% M in C, developed two times) and 0.382 g of less pure material (trace impurities of Rf 0.80, 0.24, or 0.14). The latter was further purified by preparative TLC to give an additional 0.280 g of pure **10b**. Total yield, 0.59 mmole, 72%.

(b) Sodium Methoxide Method: A solution of 1.3 g of **10a** in 40 ml of dry MeOH was cooled to 5°C, and 0.050 g of $NaOCH_3$ was added with stirring. The mixture was allowed to stand at 5°C overnight and then neutralized by the addition of 1 ml of aqueous 2 N acetic acid. The mixture was evaporated to dryness *in vacuo* and triturated with ice water. Filtration and drying *in vacuo* afforded 1.18 g of **10b** (100%).

3′-Deoxybutirosin A (**4**)

(a) Hydrogen and Palladium Method: Hydrogen was bubbled into a stirred methanolic mixture (15~20 ml) containing 0.687 g (0.58 mmole) of **10b**, 1.5 ml of 2 N acetic acid, and palladium catalyst prepared by freshly hydrogenating 0.2 g of 20% palladium oxide in methanol. Fresh portions of catalyst in methanol was added in intervals of two to three hours. A total of 4.3 g of the 20% palladium was used, together with an additional 3 ml of 1 N acetic acid, during 51 hours, to achieve complete hydrogenolysis. Workup in the usual manner afforded 0.454 g of lyophilized crude product, which was then purified chromatographically on CM-Sephadex C-25 (medium, ammonia form, 36 ml) by gradient elution with aqueous ammonia (0~0.45 M) to yield 0.276 g (88%) of **4** as a lyophilized free base. The product was characterized as the carbonate salt, $[\alpha]_\lambda^{23}$ ($\lambda$ in nm) (*c* 0.9, water): +15.4° (589), +15.6° (578), +17.6° (546), +28.6° (436), +41.4° (365).

*Anal.* Calcd. for $C_{21}H_{41}N_5O_{11}\cdot 2H_2CO_3\cdot H_2O$: C 40.52, H 6.95, N 10.28.
Calcd. for $C_{21}H_{41}N_5O_{11}\cdot 2H_2CO_3\cdot H_2O$ with 2.24% inert ash: C 39.61, H 6.79, N 10.05.
Found (after drying *in vacuo* at room temperature overnight): C 39.58, H 6.36, N 9.86, ash 2.24.

Mass spectrum of the poly-*N*-Ac-poly-*O*-TMS derivative (70 e.v., 260°C, Finnigen 1015 Quadruple) showed prominent peaks at 301 (21%) (**15**, Nc fragment), 259 (21%) (Pn fragment−TMSOH), and 188 (26%) ($^+CH(OTMS)CH_2CH_2NHAc$, from Ab fragment).[24]

(b) Sodium and Liquid Ammonia Method: Compound **10b** (0.09 g) in 40 ml of liquid ammonia was reduced with metallic sodium in the usual manner. Isolation (Amberlite IRC-50, $NH_4^+$ form, 10ml) and purification (CM-Sephadex, *cf.* above) afforded 0.025 g (61%) of **4**, identical with the sample obtained from method (a) according to VPC.

3′-Deoxyneamine (**14**)

A sample of 0.09 g of **4** was hydrolyzed (3 ml of 2 N hydrochloric acid, reflux 5.5 hours). Evaporation to dryness *in vacuo* and purification with Amberlite IRC-50 (ammonium form, 1.2 ml) afforded 0.042 g of **14**, characterized as the crystalline tetra-*N*-acetyl derivative, mp 282~287°C (dec.)

2″,2‴,3″,6-Tetra-*O*-acetyl-5″-amino-5″-deoxypentakis-*N*-[(phenylmethoxy)carbonyl]butirosin A (**20**)

Compound **20** was prepared according to the procedure of Woo.[8] $[\alpha]_\lambda^{23}$ ($\lambda$ in nm) (*c* 1.03, methanol): +16.9° (589), +17.5° (578), +19.8° (546), +34.2° (436), +54.7° (365).

Tetra-*O*-acetyl-5″-amino-5″-deoxypentakis-*N*-[(phenylmethoxy)carbonyl]-3′-*O*-(trifluoromethanesulfonyl)butirosin A (**21**) and Tetra-*O*-acetyl-5″-amino-5″-deoxypentakis-*N*-[(phenylmethoxy)-carbonyl]-4′-*O*-(trifluoromethanesulfonyl)butirosin A (**22**)

According to the procedure described for the synthesis of **8b**, 6.66 g of 2″,2‴,3″,6-tetra-*O*-acetyl-5″-aminopentakis-*N*-[(phenylmethoxy)carbonyl]-5″-deoxybutirosin A (**20**)[8] was treated with 2.18 ml of trifluoromethanesulfonic anhydride and 12 ml of pyridine, yielding a crude product consisting of a major proportion of **21** and a minor proportion of **22**.

3′-*Epi*-tetra-*O*-acetyl-5″-amino-3′,5″-dideoxypentakis-*N*-[(phenylmethoxy)carbonyl]-3′-(phenylthio)butirosin A (**23**) and 4′-*Epi*-tetra-*O*-acetyl-5″-amino-4′,5″-dideoxypentakis-*N*-[(phenylmethoxy)carbonyl]-4′-(phenylthio)butirosin A (**24**)

According to the procedure described for the synthesis of **9**, 1.96 g (1.41 mmole) of the above mixture of **21** (major) and **22** (minor), was treated with 0.88 g (18 mmole) of 50% sodium hydride and 3.1 ml (33 mmole) of benzenethiol. The crude product was chromatographed on 40 g of silica gel to yield 0.32 g (15%) of **24** (Rf 0.55, 2% M in C), 0.744 g (35.6%) of **23** (Rf 0.50), and 0.457 g (21.9%) of intermediate fractions containing both, from which an additional 0.110 g (5.3%) of **24** was obtained by preparative TLC.

3′-*Epi*-5″-amino-3′,5″-dideoxypentakis-*N*-[(phenylmethoxy)carbonyl]-3′-(phenylthio)butirosin A (**25**)

Anhydrous ammonia was bubbled into a solution of 0.307 g of **23** in 20 ml of anhydrous methanol at 0°C for 8 minutes. The solution was allowed to stand at 0°C for 18 hours, then evaporated at reduced pressure to give a residue of **25**.

5″-Amino-3′,5″-dideoxybutirosin A (**6**)

Compound **25**, 0.318 g (0.242 mmole), was hydrogenolyzed with hydrogen and palladium as described for the synthesis of **4**. Purification by chromatography on 26 ml of CM-Sephadex, using gradient elution with aqueous ammonia (0.2~0.8 N), afforded **6** as 0.081 g (62%) of lyophilized free base. The product was characterized as the carbonate salt, $[\alpha]_\lambda^{23}$ ($\lambda$ in nm) (*c* 1, water): +18° (589), +18.5° (578), +22.4° (546), +36.4° (436), +48.4° (365).

*Anal.* Calcd. for $C_{21}H_{42}N_6O_{10}\cdot 2H_2CO_3\cdot 2H_2O$: C 37.95, H 6.76, N 11.06.
Found: C 37.58, H 6.42, N 10.79, ash 0.38.

An aqueous solution of 35.4 mg of **6** free base was adjusted to pH 6.28 with 0.1 N sulfuric acid and freeze-dried to 42.5 mg of the sulfate salt. $[\alpha]_\lambda^{23}$ ($\lambda$ in nm) (*c* 0.94, water): +13.8° (589), +15.5° (578), +17.6° (546), +28.8° (436), +42.3° (365).

*Anal.* Calcd. for $C_{21}H_{42}N_6O_{10}\cdot 2.5H_2SO_4\cdot 3H_2O$ (837.86): C 30.10, H 6.38, N 10.03, S 9.57.
Found: C 29.68, H 6.03, N 9.83, S 9.04, ash 0.65.

4′-*Epi*-5″-amino-4′,5″-dideoxypentakis-*N*-[(phenylmethoxy)carbonyl]-4′-(phenylthio)butirosin A (**26**)

Compound **26** was prepared from 0.097 g of **24** by treatment with methanolic ammonia (*cf.* preparation of **25**, above).

5″-Amino-4′,5″-dideoxybutirosin A (**7**)

Hydrogenolysis of 0.097 g (0.082 mmole) of **26**, using 0.59 g of 20% palladium oxide on barium sulfate (*cf.* preparation of **4**, above) afforded 0.069 g of crude **7**, which was purified on *ca.* 27 ml of Sephadex CM-25. The compound was isolated as the carbonate (*ca.* 60% yield; 95% pure by VPC), $[\alpha]_\lambda^{23}$ ($\lambda$ in nm) (*c* 1, water): +30.6° (589), +30.9° (578), +35.5° (546), +58.7° (436), +88.0° (365). Rf 0.44 (chloroform - methanol - 15 M ammonia - water, 20: 10: 6: 4); Rf of **1** 0.48, **3** 0.44, **2** 0.38.

*Anal.* Calcd. for $C_{21}H_{42}N_6O_{10}\cdot 2.5\,H_2CO_3\cdot H_2O$ (711.68): C 39.66, H 6.94, N 11.81.
Found (after drying *in vacuo* at room temperature overnight): C 39.67, H 6.98, N 11.82, ash 0.00.

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