# *Studies on Antibiotics and Related Substances. XVII. Syntheses of trans-2-Aminocyclohexyl-D-glucosaminides*

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As a model compound of simpler structure of kanamycin[1], 2-aminocyclohexyl-D-glucosaminides has been studied in our laboratory[2]. In the present report a further investigation has been accomplished to characterize *l*- and *d-trans*-2-aminocyclohexyl-D-glucosaminides.

*d,l-trans*-2-Aminocyclohexanol was prepared by the method of Osterberg and Kendal[3]. A literature method[4] for a resolution of *d,l-trans*-2-aminocyclohexanol with L-(+)-tartaric acid by a fractional recrystallization in 80% ethanol proved to be good for the preparation of *l-trans* isomer. Then, by applying 95% aqueous methanol for a fractional recrystallization, a better result for isolating *d-trans* isomer was obtained.

By treatments of L-(+)-tartrates of *l*- and *d-trans*-2-aminocyclohexanol with alkali, *l*- and *d-trans*-2-aminocyclohexanol were obtained respectively in the optically active forms.

*N*-Carbobenzoxy-*l-trans*-2-aminocyclohexanol (I) and *N*-carbobenzoxy-*d-trans*-2-aminocyclohexanol (V) were prepared directly by carbobenzoxylation of L-(+)-tartrate of *l*- and *d-trans*-2-aminocyclohexanol respectively in good yields.

The condensation of I with $\alpha$-1-bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine[5] in the presence of mercuric cyanide gave $\beta$-(*N*-carbobenzoxy-*l-trans*-2-aminocyclohexyl)-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide (II) melting at 217~217.5°C in 37% yield. Also it was noticed that a considerable amount of 4, 5-(3, 4, 6-triacetyl-D-glucopyranosyl)oxazolone-2[10] was formed which was recovered from the mother liquor of II as a by-product.

Deacetylation of II with methanol saturated with ammonia gave $\beta$-(*N*-carbobenzoxy-*l-trans*-2-aminocyclohexyl)-*N*-carbobenzoxy-D-glucosaminide (III) melting at 224~225.5°C in 81% yield.

A catalytic hydrogenolysis of III yielde $\beta$-(*l- trans* -2-aminocyclohexyl)-D-glucosaminide (IV) melting at 159~161°C (decomp.) in 60% yield.

The reaction sequences employed in the synthesis of $\beta$-(*d-trans*-2-aminocyclohexyl)-D-glucosaminide (VIII) were similar to those of IV.

The optical rotations of the products were listed on Table I.

TABLE I. SPECIFIC ROTATION OF *trans*-2-AMINOCYCLOHEXANOLS AND THEIR DERIVATIVES

| Derivative | $[\alpha]_D$ *l* | $[\alpha]_D$ *d* |
|---|---|---|
| *trans*-2-Aminocyclohexanol | −37.6° | +38.4° |
| *N*-Benzoyl-*trans*-2-aminocyclohexanol | −39.8° | +41.1° |
| *N*-Carbobenzoxy-*trans*-2-aminocyclohexanol | −20.1° | +20.3° |
| $\beta$-(*N*-Carbobenzoxy-*trans*-2-aminocyclohexyl)-3, 4, 6-triacety-*N*-carbobenzoxy-D-glucosaminide | +5.6° | +22.9° |
| $\beta$-(*N*-Carbobenzoxy-*trans*-2-aminocyclohexyl)-*N*-carbobenzoxy-D-glucosaminide | −28.1° | +10.3° |
| $\beta$-(*trans*-2-Aminocyclohexyl)-D-glucosaminide | −72.6° | +4.6° |

Designation of IV and VIII as $\beta$-anomers was based on their optical rotations and infrared spectra. The optical rotations of IV and VIII were less dextrorotatory, compared with that of ($\alpha$-D-6-amino-6-deoxyglucopyranosyl)deoxystreptamine[6] and paromamine[7]. The infrared spectra of IV and VIII showed characteristic absorption bands for derivatives of $\beta$-glucopyranose at 895 $cm^{-1}$ (type 2b)[8], and for cyclohexane ring deformation at 975 and 1044 $cm^{-1}$ [9] in agreement with the assigned structures.

1) H. Umezawa, M. Ueda, K. Maeda, K. Yagishita, S. Kondo, Y. Okami, R. Utahara, Y. Osato, K. Nitta and T. Takeuchi, *J. Antibiotics, Ser. A*, **10**, 181 (1957).
2) T. Suami, S. Ogawa and S. Umezawa, This Bulletin, **35**, 474 (1962).
3) A. E. Osterberg and E. C. Kendall, *J. Am. Chem. Soc.*, **42**, 2616 (1920).
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7) T. H. Haskell, T. C. French and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3481 (1959).
8) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *Chem. & Ind.*, **1953**, 196; *J. Chem. Soc.*, **1954**, 171.
9) L. W. Morrison, *J. Chem. Soc.*, **1951**, 1614.

Considering from the optical rotation of *trans*-2-aminocyclohexyl-D-glucosaminides in Table I, β-(d, *l-trans*-2-aminocyclohexyl)-D-glucosaminide in the previous paper[2] had to be corrected to β-(*l-trans*-2-aminocyclohexyl)-D-glucosaminide and *d,l-tsans*-2-aminocyclohexyl-D-glucosaminide must be a crude product of β-(*d-trans*-2-aminocyclohexyl)-D-glucosaminide.

In addition to the above mentioned syntheses of *l-* and *d-trans*-2-aminocyclohexyl-D-glucosaminides, the attempt to prepare glucosaminides via trityl derivative of aminocyclohexanol was made. *N*-Carbobenzoxy-*d,l-trans*-2-aminocyclohexanol was tritylated with trityl chloride in anhydrous pyridine to give *N*-carbobenzoxy-*O*-trityl-*d,l-trans*-2-aminocyclohexanol (IX) melting at 126.5~128°C in 37% yield. Then, IX was condensed with α-l-bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine in the presence of silver perchlorate to obtain a mixture of II and VI. The recrystallization of the mixture resulted in obtaining II in a considerably pure state, but VI was not obtained in a pure state.

## Experimental

***l-trans*-2-Aminocyclohexanol.**—According to the procedure of Godchot and Mousseron[4], *l-trans*-2-aminocyclohexanol L-(+)-tartrate melting at 175~176°C, $[\alpha]_D^{22}$ −4.8° (*c* 3.2, water) [lit.[4] m. p. 177~178°C, $[\alpha]_D$ −2.73°] was obtained in 10% yield from *d, l-trans*-2-aminocyclohexanol L-(+)-tartrate. A mixture of *l-trans*-2-aminocyclohexanol L-(+)-tartrate and 10% potassium hydroxide solution was extracted with ether. The ethereal solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to yield *l-trans*-2-aminocyclohexanol melting at 84~86°C, $[\alpha]_D^{22}$ −37.6° (*c* 1.30, water) [lit.[4] m. p. 83~84°C, $[\alpha]_D$ −40.2°].

*N*-Benzoyl derivative was prepared by shaking 1.0 g. of *l-trans*-2-aminocyclohexanol L-(+)-tartrate with 0.8 g. of benzoyl chloride in 11 ml. of 6.5% sodium hydroxide solution. The amide separating from the reaction mixture was collected by filtration. After three recrystallizations from benzene, the product melted at 168~169°C, $[\alpha]_D^{11}$ −39.8° (*c* 2.9, ethanol).

Fonud: C, 71.33; H, 7.61; N, 6.24. Calcd. for $C_{13}H_{17}NO_2$ (219.3): C, 71.20; H, 7.82; N, 6.39%.

***N*-Carbobenzoxy-*l-trans*-2-aminocyclohexanol (I).**—A solution of 4.3 g. of *l-trans*-2-aminocyclohexanol L-(+)-tartrate and 5.5 g. of sodium bicarbonate in 30 ml. of water was vigorously agitated and 5.3 g. of carbobenzoxy chloride was added to it in toluene (78% solution) in twenty minutes at room temperature. After agitation for two hours, the mixture was kept in a refrigerator overnight. The crystalline precipitate was collected and washed with cold water. The crude product weighed 4.3 g. (98%). The product was recrystallized repeatedly from a water-methanol mixture (3:2) to yield 2.8 g. (66%) of *N*-carbobenzoxy-*l-trans*-2-aminocyclohexanol melting at 115.5~116.5°C, $[\alpha]_D^{14}$ −20.1° (*c* 1.2, ethanol).

Found: C, 67.44; H, 7.75; N, 5.66. Calcd. for $C_{14}H_{19}NO_3$ (249.3): C, 67.44; H, 7.68; N, 5.62%.

**β-(*N*-Carbobenzoxy-*l-trans*-2-aminocyclohexyl)-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide (II).**—A mixture of 1.9 g. of α-l-bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine and 1.0 g. of I in 22 ml. of dry benzene was heated under reflux for 2 hr. with 1.7 g. of mercuric cyanide. The mixture was cooled and filtered to remove an insoluble substance. The filtrate was diluted with 70 ml. of chloroform and then washed twice with 10% sodium chloride solution and three times with cold water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to give a crystalline residue. The residue was washed with anhydrous ether to obtain 1.75 g. of the product. The crude product was recrystallized from absolute ethanol to yield 0.95 g. (37%) of crystals melting at 217~217.5°C, $[\alpha]_D^{25}$ +5.6° (*c* 2, chloroform). A further recrystallization did not raise its melting point.

Found: C, 61.25; H, 6.44; N, 4.06. Calcd. for $C_{34}H_{42}N_2O_{12}$ (670.7): C, 60.88; H, 6.31; N, 4.18%.

The infrared spectrum (KBr) of the product showed absorption at 1751 (ester carbonyl); 1708, 1542, 1294 (amide); 975, 1049 (cyclohexane); 902 (type 2b of β-D-glucopyranose) and 752, 695 $cm^{-1}$ (monosubstituted benzene).

The mother liquor of the above product was evaporated under reduced pressure to half the volume and placed in a refrigerator to yield 0.28 g. of a crystalline product. The product was recrystallized from ethyl acetate to obtain fine crystals melting at 170~170.5°C.

Mixed with an authentic sample of 4, 5-(3,4,6-triacetyl-D-glucopyranosyl)oxazolone-2[10], this product did not show a depression of the melting point. The infrared spectrum of the product was superimposable with that of an authentic sample.

**4, 5-(3, 4, 6-Triacetyl-D-glucopyranosyl)oxazolone-2.**—According to the procedure of Konstas et al.[10], 1, 3, 4, 6-tetraacetyl-*N*-carbobenzoxy-D-glucosamine, m. p. 148.5~149°C, $[\alpha]_D^{12}$ +20.4° (*c* 1.2, pyridine) [lit.[11] m.p. 150~151°C, $[\alpha]_D$ +21.5°], was treated with aluminum chloride and phosphorus pentachloride in dry chloroform to obtain 4, 5-(3, 4, 6-triacetyl-D-glucopyranosyl)oxazolone-2 melting at 170.5~171°C, $[\alpha]_D^{24}$ +23.7° (*c* 2, chloroform) in 36% yield. [lit.[10] m. p. 174~176°C, $[\alpha]_D$ +50.3° (*c* 2, chloroform)].

Found: C, 47.53; H, 5.21; N, 4.17. Calcd. for $C_{13}H_{17}O_9$ (331.3): C. 47.11; H, 5.18; N, 4.23%.

**β-(*N*-Carbobenzoxy-*l-trans*-2-aminocyclohexyl)-*N*-carbobenzoxy-D-glucosaminide (III).**—A mixture of 1.31 g. of II and 50 ml. of methanol saturated with ammonia was kept below 5°C for 3 hr. to obtain a clear solution. After it was placed in a refrigerator overnight, the solution was evaporated under reduced pressure to dryness. The residue was washed with 20 ml. of ethyl aceteate and

10) S. Konstas, I. Photaki and L. Zervas, *Chem. Ber.*, **92**, 1288 (1959): see also S. Umezawa, Y. Ito and S. Koto, This Bulletin, **36**, 186 (1963).

11) A. Neuberger and R. P. Rivers, *J. Chem. Soc.*, **1939**, 122.

recrystallized from isopropanol to yield 0.87 g. (81%) of the product melting at 224~225.5°C. A further recrystallization did not show any change in the melting point. $[\alpha]_D^{27}$ −28.1° (*c* 1.8, pyridine).

Found: C, 61.60; H, 6.58; N, 5.28. Calcd. for $C_{28}H_{36}N_2O_9$ (544.6): C, 61.75; H, 6.66; N, 5.14%.

The infrared spectrum (KBr) of the product showed absorption at 3400 broad (OH); 1690, 1553, 1260 (amide); 975, 1049 (cyclohexane); 896 (type 2b of β-D-glucopyranose) and 775, 696 $cm^{-1}$ (mono-substituted benzene).

**β-(*l-trans*-2-aminocyclohexyl)-D-glucosaminide (IV).**—A suspension of 0.72 g. of III in 30 ml. of dioxane- water mixture (1 : 1) was added to a prehydrogenated suspension of 0.2 g. of palladium black in 10 ml. of the same solvent. The mixture was hydrogenated for 3 hr. at room temperature (31~32°C) with mechanical shaking. At the end of the reaction, 53 ml. of hydrogen was absorbed. The mixture was filtered to remove the catalyzer and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in 7 ml. of hot absolute methanol and added with 5 ml. of anhydrous ether. The solution was placed in a refrigerator for a few days to obtain crystals. The crystals were collected by filtration and dried in a vacuum desiccator to yield 0.22 g. (60%) of the product melting at 159~161°C (decomp.). $[\alpha]_D^{30}$ −72.6° (*c* 1.3, water).

Found: C, 51.90; H, 8.77: N, 10.27. Calcd. for $C_{12}H_{24}N_2O_5$ (276.3): C, 52.16; H, 8.75; N, 10.14%.

The infrared spectrum (KBr) of the product showed absoption at 3590 (OH); 3355, 3300, 1593 (amine); 975, 1044 (cyclohexane) and 895 $cm^{-1}$ (type 2b of β-D-glucopyranose).

***d-trans*-2-Aminocyclohexanol.**—From the mother liquor of *l-trans*-2-aminocyclohexanol L-(+)-tartrate, an attempt to isolate *d-trans*-2-aminocyclohexanol L-(+)-tartrate with 80% ethanol was not successful. The suitable solvent for separating *d-trans*-2-amino-cyclohexanol L-(+)-tartrate was found to be 95% methanol. The mother liquor of *l-trans*-2-amino-cyclohexanol L-(+)-tartrate was evaporated under reduced pressure to dryness. The residue was recrystallized repeatedly from 95% methanol to obtain the crystals melting at 169.5~170°C in 30% yield. $[\alpha]_D^{28}$ +36.5° (*c* 2.3, water). (Found: C, 45.33; H, 7.23, N, 5.26%). [lit.[4] m. p. 170~171°C, $[\alpha]_D$ +31.2°].

A further recrystallization did not show any change in the melting point and the specific rotation.

*d-trans*-2-Aminocyclohexanol was obtained by the same procedue of *l-trans* aminoalcohol from the corresponding tartrate. The *d-trans* aminoalcohol melted at 84~86°C. $[\alpha]_D^{10}$ +38.4° (*c* 1.8, water). [lit[4]. m. p. 83~84°C, $[\alpha]_D$ +40.2°].

*N*-Benzoyl derivative melting at 168~169°C was obtained by the Schotten-Baumann technique. $[\alpha]_D^{12}$ +43.5° (*c* 2.0, ethanol). Found: C, 71.33; H, 7.61; N, 6.25. Calcd. for $C_{13}H_{17}NO_2$ (219.3): C, 71.20; H, 7.82; N, 6.39%.

***N*-Carbobenzoxy-*d-trans*-2-aminocyclohexanol (V).**—To 5.3 g. portion of *d-trans*-2-aminocyclo-hexanol L-(+)-tartrate in 30 ml. of water was added 6.7 g. of sodium bicarbonate. To the mixture 6.6 g. of carbobenzoxy chloride (80% toluene solution) was added under mechanical agitation. The precipitate was treated similarly as I to obtain 3.3 g. (66%) of the crystals melting at 115.5~116.5°C. $[\alpha]_D^{14}$ +20.3° (*c* 1.1, ethanol).

Found: C, 67.30; H, 7.68; N, 5.68. Calcd. for $C_{14}H_{19}NO_3$ (249.3): C, 67.44; H, 7.68; N, 5.62%.

**β-(*N*-Carbobenzoxy-*d-trans*-2-aminocyclohexyl)-3, 4, 6, -triacetyl-*N*-carbobenzoxy-D-glucosaminide (VI).**—A mixture of 2.2 g. of α-l-bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine and 1.1 g. of V in 25 ml. of anhydrous benzene was refluxed for 2 hr. with 1.9 g. of mercuric cyanide. Then the mixture was treated similarly to a corresponding derivative of *l*-form, II, to obtain 1.1 g. (38%) of the crystalline product melting at 191~192°C. $[\alpha]_D^{17}$ +22.9° (*c* 1.4, chloroform).

Found: C, 60.94; H, 6.19; N, 4.30. Calcd. for $C_{34}H_{42}N_2O_{12}$ (670.7): C, 60.88; H, 6.31; N, 4.18%.

The infrared spectrum of the product showed the same pattern as that of II.

**β-(*N*-Carbobenzoxy-*d-trans*-2-aminocyclohexyl)-*N*-carbobenzoxy-D-glucosaminide (VII).**— Upon treatment of 2.7 g. of VI with 130 ml. of methanol saturated with ammonia in a refrigerator overnight, the clear solution was obtained. The solution was treated similarly to III to yield 1.6 g. (76%) of crystals melting at 199~200°C. $[\alpha]_D^{11}$ +10.30 (*c* 3.1, pyridine).

Found: C, 61.42; H, 6.66; N, 4.91. Calcd. for $C_{28}H_{36}N_2O_9$ (544.6): C, 61.75; H, 6.66; N, 5.14%.

The infrared spectrum of the product was superimposable with that of III.

**β-(*d-trans*-2-Aminocyclohexyl)-D-glucosaminide (VIII).**—A 1.39 g. portion of VII was hydrogenated with 0.4 g. of palladium black in 65 ml. of dioxane-water mixture (1 : 1). The mixture was treated similarly to IV to yield 0.35 g. (57%) of the crystalline product melting at 195.5~196.5°C (decomp.). $[\alpha]_D^{11}$ +4.9° (*c* 1.4, water).

Found: C, 52.21; N, 8.57; N, 10.12. Calcd. for $C_{12}H_{24}N_2O_5$ (276.3): C, 52.16; H, 8.75; N, 10.14%.

The infrared spectrum of the product was superimposable with that of IV.

***N*-Carbobenzoxy-*O*-trityl-*d, l-trans*-2-amino-cyclohexanol (IX).**—To a solution of 6.0 g. of *N*-carbobenzoxy-*d, l-trans*-2-aminocyclohexanol in 43 ml. of dry pyridine was added 7.4 g. of trityl chloride. The mixture was heated on a boiling water bath for 20 hr. After placed overnight at room temperature, the mixture was filtered to remove a precipitate.

The filtrate was poured into 180 ml. of ice cold water to give an oily product, which began to crystallize by scratching a wall of the vessel with a glass rod under ice cooling.

The crystals were collected by filtration and then dissolved in ether. The ethereal solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield a crystalline residue. The residue was recrystallized from methanol to yield 6.29 g. (53%) of the product melting at 111~115°C. The crude product was recystallized again

from absolute ethanol to yield 4.36 g. (37%) of crystals melting at 126.5~128°C.

Found: C, 80.52; H, 6.56; N, 2.68. Calcd. for $C_{33}H_{33}NO_3$ (491.6): C, 80.62; H, 6.77; N, 2.85%.

The second crop of the product (0.15 g.) melting at 126~128°C was recovered from the mother liquor.

**β-(*N*-Carbobenzoxy-*l-trans*-2-aminocyclohexyl)-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide.**—A solution of 1.1 g. of silver perchlorate and 18 ml. of anhydrous nitromethane was added with 1.5 g. of anhydrous calcium sulfate. Then, 2.4 g. of IX and 2.9 g. of α-l-bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine were added to the mixture under ice cooling. The mixture was vigorously shaken at room temperature for 2 hr. After standing overnigth at room temperature, the mixture was filtered to remove a precipitate. The filtrate was washed twice with saturated sodium bicarbonate solution and five times with cold water. The organic solvent layer was filtered again to remove triphenylcarbinol and then diluted with 50 ml. of chloroform. The solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield 5.1 g. of a crystalline residue. The residue was washed with anhydrous ether to obtain 2.6 g. of a crude mixture of II and VI melting at 107~167°C. The crude product was repeatedly recrystallized from absolute ethanol to yield 230 mg. (7.0%) of the product melting at 215~216°C. The mixed melting point with II did not show any depression.

From the mother liquor, VI was not obtained in a pure state.

**Bioassays.**—IV and VIII showed almost no antimicrobial activity against *E. coli* in a paper disk method.

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