THE SYNTHESIS OF OLIGOSACCHARIDE–L-ASPARAGINE COMPOUNDS. PART IV*. 2-ACETAMIDO-*N*-(L-ASPART-4-OYL)-2-DEOXY-6-*O*-α-D-MANNOPYRANOSYL-β-D-GLUCOPYRANOSYLAMINE†

MOHAMMED A. E. SHABAN‡ AND ROGER W. JEANLOZ§

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (U. S. A.)

(Received February 15th, 1972)

ABSTRACT

The title compound, used for the chemical and biochemical synthesis of glycopeptides and as a reference substance in the structure elucidation of glycoproteins, was synthesized *via* two different routes. In one of these syntheses, condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**1**) with 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide, or with the 3-*O*-acetyl azide analog, followed by acetylation, gave 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranosyl azide (**4**) in 76 and 74% yields, respectively. Catalytic reduction of **4** with Adams' catalyst, and condensation of the resulting amine with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate, gave the protected derivative **10** of the title compound in 67% yield. In the other synthesis, **10** was obtained in 66% yield by condensation of **1** with 2-acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine. Removal of the protective *O*-acetyl, *N*-(benzyloxycarbonyl), and benzyl groups gave the title compound.

INTRODUCTION

As part of our program of synthesis of oligosaccharide–L-asparagine compounds[1–3], 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-6-*O*-α-mannopyranosyl-β-D-glucopyranosylamine (**13**) was synthesized. This compound is of interest for the identification of the chemical structure of the "core" of the carbohydrate chains of glycoproteins, as a D-mannosyl-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine struc-

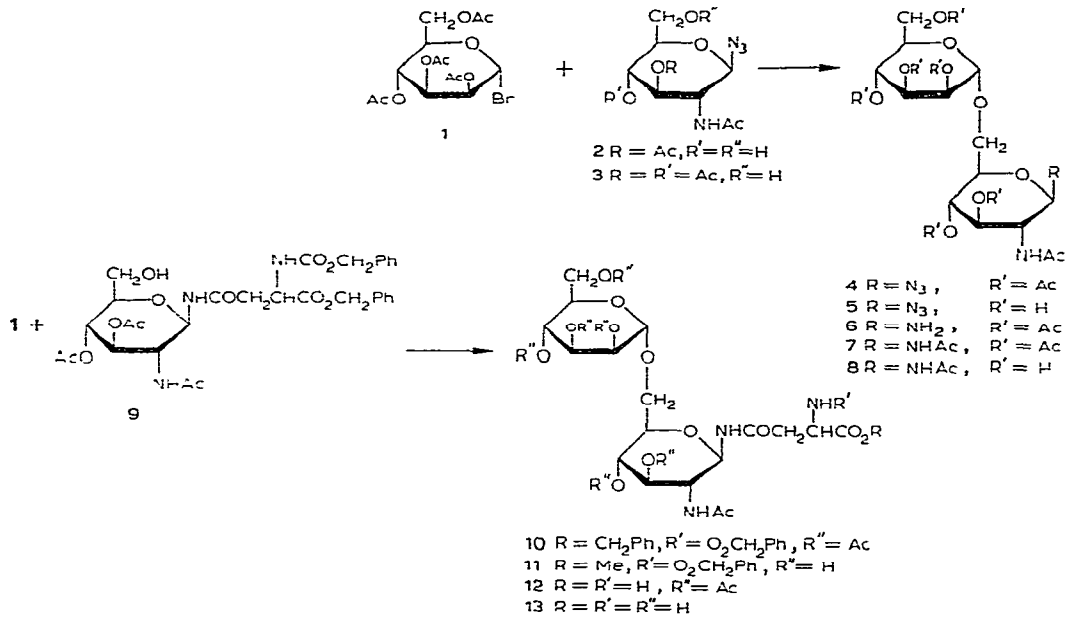
*For parts I, II, and III, see refs. 1–3.

†Amino Sugars LXXIX. This is publication No. 564 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This work was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases (AM-03564-12), National Institutes of Health, U. S. Public Health Service.

‡On leave of absence from the Faculty of Science, Alexandria University, Alexandria, Egypt.

§To whom inquiries should be sent.

ture has been proposed for some glycoproteins[4]. It is intended that compound **13** will also be used as a starting material for the synthesis of glycopeptides that can be tested as receptors in the study of the biosynthesis of glycoproteins. Finally, compound **13** is of interest for a study of the inhibition of concanavalin A in relation to the structure of the receptor sites at the surface of cancer cells and virus-transformed cells[5,6].



DISCUSSION

In the synthesis[3] of 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-3-*O*-α-D-mannopyranosyl-β-D-glucopyranosylamine, the (1→3) analog of compound **13**, it was shown that derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[7] were the most convenient starting-materials, as these compounds are stable and crystalline, and can readily be transformed into glycosylamine derivatives.

In the first synthesis of **13**, 2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**) was prepared from the known 3-*O*-acetyl-4,6-*O*-benzylidene derivative[3] by treatment with acetic acid. Compound **2** was then condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[8] (**1**) in the presence of mercuric cyanide to give, after acetylation, crystalline 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranosyl azide (**4**) in 74% yield. No evidence for the formation of the (1→4) disaccharide was observed, a result that agrees with the lack of reactivity, previously observed[9,10], of the 4-hydroxyl group of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue. The assigning of the α-D configuration to the D-mannopyranosyl residue was based on the results of the previous

syntheses[3,10,11]. The structure of the disaccharide azide **4** was ascertained by its preparation in 76% yield starting from the D-mannopyranosyl bromide **1**, which was condensed with 2-acetamido-3,4-di-*O*-acetyl-β-D-glucopyranosyl azide[12] (**3**). De-*O*-acetylation of the azide **4** gave the amorphous derivative **5**, whereas reduction of **4** in the presence of Adams' catalyst led to the rather unstable amine **6**. This amine was characterized by the crystalline peracetyl derivative **7** and by the di-*N*-acetyl derivative **8**. Condensation of the amine **6** with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[13] in the presence of *N,N'*-dicyclohexylcarbodiimide gave the crystalline, protected, disaccharide–L-asparagine compound **10** in 67% yield.

In the second route of synthesis, the disaccharide **10** was obtained directly, in 66% yield, by condensation of the D-mannopyranosyl bromide derivative **1** with 2-acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine[2] (**9**) in the presence of mercuric cyanide. De-*O*-acetylation of **10** with sodium methoxide in dry methanol simultaneously transesterified the benzyl ester group to give **11**. Removal of the benzyl and *N*-(benzyloxycarbonyl) groups by hydrogenolysis, and then of the *O*-acetyl group of **10** by treatment with lithium hydroxide gave, in good yield, the crystalline disaccharide–L-asparagine derivative **12** and the title compound **13**.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting points". Optical rotations were determined, for solutions in 1-dm semimicrotubes, with a Perkin–Elmer Model 141 polarimeter; the chloroform used was analytical-reagent grade and contained ~0.75% of ethanol. Infrared spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. The nuclear magnetic resonance spectrum was recorded with a Varian A-60 n.m.r. spectrometer for a solution in chloroform-*d*, with tetramethylsilane as the internal standard. Column chromatography was performed on Silica Gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The proportion of weight of substance to weight of silica gel was 1:80 to 1:120. The ratio of diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2–3 ml per mg of the material to be chromatographed. The homogeneity of the nonpolar compounds was verified by thin-layer chromatography (ascending) on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany). The zones were detected by spraying with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol, followed by heating on a hot plate for a few min. Evaporations were conducted *in vacuo*, at a bath temperature below 45°. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

2-Acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**2**). — A suspension of 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide[3] (2 g)

in 60% acetic acid (20 ml) was heated for 30 min at 100°. The clear solution was evaporated under diminished pressure, and the residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from methanol–ether–pentane gave 1.3 g (84%) of prismatic needles, m.p. 164–165° (dec.); $[\alpha]_D^{20}$ −86° (*c* 0.8, methanol); i.r. data: ν_{max}^{KBr} 1655 (CONH), 1710 (OAc), 3290 (NH), and 3440 cm^{-1} (OH); t.l.c. in 7:3 benzene–methanol: R_F 0.42.

Anal. Calc. for $C_{10}H_{16}N_4O_6$: C, 41.65; H, 5.59; N, 19.42; O, 33.30. Found: C, 41.72; H, 5.63; N, 19.42; O, 33.29.

*2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-glucopyranosyl azide* (**4**). — (*a*) *From* **2**. A mixture of compound **2** (900 mg) and finely powdered mercuric cyanide (1.5 g) in dry, 1:1 benzene–nitromethane (180 ml) was concentrated to 130 ml at atmospheric pressure, and then cooled to room temperature. To this mixture was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[8] (**1**, 2 g) in dry benzene (10 ml), and the mixture was stirred for 48 h at room temperature. It was then diluted with chloroform (200 ml) and filtered, and the filtrate was successively washed with water, saturated sodium hydrogen carbonate solution, and water, dried (sodium sulfate), and evaporated to a residue that failed to give crystalline fractions after chromatography on a column of silica gel, with 19:1 chloroform–ethanol.

The fractions were recombined and evaporated, and the dry residue was acetylated with pyridine (20 ml) and acetic anhydride (30 ml) for 16 h at room temperature. Evaporation of the mixture gave a syrup which was chromatographed on a column of silica gel with 19:1 chloroform–ethanol. Fractions having R_F 0.36 on t.l.c. in the same solvent mixture were combined and evaporated, to give 1.525 g (75%) of **4**, which crystallized from chloroform–ether in needles, m.p. 145–147°; $[\alpha]_D^{20}$ +6.0° (*c* 1.2, chloroform); i.r. data: ν_{max}^{KBr} 1660 (CONH), 1750(OAc), 2130 (N_3), and 3325 cm^{-1} (NH); n.m.r. data (chloroform-*d*): τ 3.35 (one-proton doublet, *J* 9.0 Hz, NH), and 7.83, 7.92, 7.98 (21 protons, NAc and 6 OAc).

Anal. Calc. for $C_{26}H_{36}N_4O_{16}$: C, 47.29; H, 5.49; N, 8.48; O, 38.76. Found: C, 47.14; H, 5.40; N, 8.48; O, 38.73.

(*b*) *From* **3**. 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[12] (**3**, 970 mg) was condensed with compound **1** (2 g) in the same way as described in (*a*). Chromatography of the resulting syrup on a column of silica gel with 19:1 chloroform–ethanol gave 1.46 g of **4** (76%), m.p. and mixed m.p. with the compound described under (*a*): 145–147°.

*2-Acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-β-D-glucopyranosyl azide* (**5**). — A solution of **4** (100 mg) in methanol (15 ml) was treated with 0.1M sodium methoxide solution in methanol (2 ml) and kept for 16 h at 4°. The solution was de-ionized by passage through a bed of Dowex 50 (H^+) ion-exchange resin, and then evaporated. The residue could not be crystallized, and was obtained as an amorphous solid (54 mg; 87%) by precipitation from a methanolic solution with ether; $[\alpha]_D^{20}$ +7.0° (*c* 0.7, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 2130 (N_3), and 3350 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{14}H_{24}N_4O_{10}$: C, 41.18; H, 5.92; N, 13.72; O, 39.17. Found: C, 41.10; H, 6.02; N, 13.60; O, 39.14.

*2-Acetamido-*N*-acetyl-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-glucopyranosylamine* (**7**). — A solution of **4** (155 mg) in abs. ethanol (30 ml) was hydrogenated at atmospheric pressure in the presence of Adams' catalyst (50 mg) for 4 h. The catalyst was filtered off, and the solution, which gave a positive ninhydrin reaction, was treated with acetic anhydride (10 ml) for 30 min at room temperature. Evaporation gave a residue which crystallized from chloroform–ether–pentane to give 112 mg (74%) of **7**, m.p. 185–186°; $[\alpha]_D^{20}$ +35° (*c* 0.5, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1745 (OAc), and 3300 cm^{-1} (NH); t.l.c. in 9:1 chloroform–ethanol: R_F 0.44.

Anal. Calc. for $C_{26}H_{38}N_2O_{16}$: C, 49.21; H, 6.04; N, 4.42; O, 40.33. Found: C, 49.03; H, 5.87; N, 4.17; O, 39.98.

*2-Acetamido-*N*-acetyl-2-deoxy-6-*O*-α-*D*-mannopyranosyl-β-*D*-glucopyranosylamine* (**8**). — To a solution of **7** (120 mg) in methanol (15 ml) was added 0.1M sodium methoxide solution in methanol (2 ml), and the solution was kept for 3 h at room temperature, de-ionized by passage through Dowex 50 (H^+) ion-exchange resin, and evaporated. Crystallization from methanol–ethyl acetate gave 72 mg (89%) of **8**, m.p. 161–163°; $[\alpha]_D^{20}$ +52° (*c* 0.6, methanol); i.r. data: ν_{max}^{KBr} 1655 (CONH) and 3350 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{16}H_{28}N_2O_{11}$: C, 45.28; H, 6.65; N, 6.60; O, 41.47. Found: C, 45.18; H, 6.55; N, 6.42; O, 41.46.

*2-Acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-glucopyranosylamine* (**10**). — (*a*) *From* **4**. A solution of **4** (750 mg) in absolute ethanol (150 ml) was hydrogenated in the presence of platinum oxide, as described for the preparation of **7**. After removal of the catalyst by filtration, the filtrate was evaporated at room temperature, and the last traces of ethanol were removed by repeated addition and distillation of dichloromethane. The residue was dissolved in dry dichloromethane (20 ml), and 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[13] (430 mg) and *N,N'*-dicyclohexylcarbodiimide (350 mg) were added. The mixture was stirred for 16 h at room temperature, and then treated with 10 drops of glacial acetic acid, and stirred for a further 30 min. The resulting *N,N'*-dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, and fractions having an R_F of 0.36 on t.l.c. in the same solvent mixture were combined and evaporated. The residue was crystallized from methanol–ether–pentane to give 740 mg (67%) of prismatic needles, m.p. 192–193°, $[\alpha]_D^{20}$ +27° (*c* 0.6, chloroform); i.r. data: ν_{max}^{KBr} 1660 (CONH), 1750 (C=O ester), and 3300 cm^{-1} (NH).

Anal. Calc. for $C_{45}H_{55}N_3O_{21}$: C, 55.49; H, 5.69; N, 4.31; O, 34.49. Found: C, 55.42; H, 5.63; N, 4.25; O, 34.47.

(*b*) *From 2-acetamido-3,4-di-*O*-acetyl-*N*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-2-deoxy-β-*D*-glucopyranosylamine* (**9**). — A mixture of **9** (ref. 2) (450 mg)

and finely powdered mercuric cyanide (300 mg) in dry, 1:1 benzene–nitromethane (120 ml) was concentrated at atmospheric pressure to 100 ml, and then cooled to room temperature. To this mixture was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[8] (500 mg) in dry benzene (5 ml). After being stirred for 48 h at room temperature, the mixture was filtered, and the filtrate evaporated. The residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol. Fractions having an R_F of 0.36 on t.l.c. in the same solvent mixture were combined and evaporated. The residue was crystallized from methanol–ether–pentane to give 450 mg (66%) of prismatic needles, m.p. 185–187°, showing no depression of the m.p. on admixture with the compound just described and the same i.r. spectrum as that compound.

Anal. Calc. for $C_{45}H_{55}N_3O_{21}$: C, 55.49; H, 5.69; N, 4.31; O, 34.49. Found: C, 55.48; H, 5.70; N, 4.32; O, 34.65.

2-Acetamido-2-deoxy-6-O-α-D-mannopyranosyl-N-[1-methyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-β-D-glucopyranosylamine (**11**). — A solution of **10** (100 mg) in methanol (10 ml) was treated with M sodium methoxide in methanol (1 ml) and kept for 2 h at room temperature. The solution was de-ionized by passage through a column of Dowex 50 (H^+) cation-exchange resin, and then evaporated. The residue was obtained as an amorphous solid (57 mg; 87%) by precipitation of a methanolic solution with ether; $[\alpha]_D^{20}$ +38° (*c* 0.31, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1720 (CO_2Me), and 3350 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{27}H_{39}N_3O_{15}$: C, 50.23; H, 6.09; N, 6.51; O, 37.17. Found: C, 50.22; H, 6.14; N, 6.29; O, 36.82.

2-Acetamido-3,4-di-O-acetyl-N-(L-aspart-4-oyl)-2-deoxy-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-glucopyranosylamine (**12**). — A solution of **10** (200 mg) in 80% acetic acid (30 ml) was hydrogenolyzed at atmospheric pressure for 2 h in the presence of palladium-on-charcoal (150 mg). The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was crystallized from methanol–ether–pentane to give 128 mg (83%) of **12**, m.p. 177–179°; $[\alpha]_D^{20}$ +25° (*c* 0.9, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1740 (CO_2H and OAc), and 3400 cm^{-1} (broad, NH, OH).

Anal. Calc. for $C_{30}H_{43}N_3O_{19}$: C, 48.06; H, 5.78; N, 5.60; O, 40.55. Found: C, 47.94; H, 5.78; N, 5.65; O, 40.53.

2-Acetamido-N-(L-aspart-4-oyl)-2-deoxy-6-O-α-D-mannopyranosyl-β-D-glucopyranosylamine (**13**). — Compound **10** (200 mg) was treated with a solution of lithium hydroxide (80 mg) in water (10 ml), and kept for 1 h at room temperature. The solution was de-ionized by passage through a column of Amberlite IRC-50 (H^+) ion-exchange resin, and then evaporated. The residue was crystallized from water–methanol to give 109 mg (83%) of **13** as microcrystals that started to decompose, without melting, at 217° (effervescence); $[\alpha]_D^{20}$ +26° (*c* 0.55, 50% methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1720 (CO_2H), and 3350 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{18}H_{31}N_3O_{13}$: C, 43.46; H, 6.28; N, 8.45; O, 41.81. Found: C, 43.39; H, 6.35; N, 8.18; O, 41.58.

REFERENCES

1 M. SPINOLA AND R. W. JEANLOZ, *J. Biol. Chem.*, 245 (1970) 4138.
2 M. SPINOLA AND R. W. JEANLOZ, *Carbohyd. Res.*, 15 (1970) 361.
3 M. SHABAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 21 (1972) 347.
4 See, for example, E. EYLAR AND R. W. JEANLOZ, *J. Biol. Chem.*, 237 (1962) 622; P. W. WAGH, I. BORNSTEIN, AND R. J. WINZLER, *ibid.*, 244 (1969) 658; G. DAWSON AND R. J. CLAMP, *Biochem. J.*, 107 (1969) 341.
5 M. INGBAR AND L. SACHS, *Proc. Nat. Acad. Sci. U. S.*, 63 (1969) 1418; *Nature*, 223 (1969) 710.
6 M. M. BURGER AND K. D. NOONAN, *Nature*, 228 (1971) 512.
7 F. MICHEEL AND H. WULFF, *Chem. Ber.*, 89 (1956) 1521.
8 E. A. TALLEY, D. D. REYNOLDS, AND W. L. EVANS, *J. Amer. Chem. Soc.*, 65 (1943) 575.
9 E. S. RACHAMAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 10 (1969) 435.
10 M. SHABAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 17 (1971) 411.
11 M. SHABAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 17 (1971) 193.
12 E. WALKER AND R. W. JEANLOZ, unpublished results.
13 M. BERGMANN, L. ZERVAS, AND L. SALZMANN, *Ber.*, 66 (1933) 1288.