O-Benzyl-N-*t*-butyloxycarbonyl-L-serine[1]

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In solid-phase peptide synthesis, it is desirable to incorporate both serine and threonine as their O-benzyl-N-acyloxycarbonyl derivatives.[3] Recently, a simple method for preparing O-benzyl-N-*t*-butyloxycarbonyl-L-threonine was reported.[4] On the other hand, methods for preparing O-benzyl-N-*t*-butyloxycarbonyl-L-serine are very laborious.[5,6] The present study reports a simple two-step synthesis of O-benzyl-N-*t*-butyloxycarbonyl-L-serine from L-serine.

O-Benzyl-N-*t*-butyloxycarbonyl-L-serine was obtained directly from the readily available N-*t*-butyloxycarbonyl-L-serine[7] by treatment of the latter compound, in anhydrous liquid ammonia, first with sodium metal and then with benzyl bromide. No detectable racemization was observed. By use of column chromatography for purification, it was possible to recover optically pure N-*t*-butyloxycarbonyl-L-serine for subsequent preparations. No ester was detected in the reaction mixture.

When the same procedure was applied to the synthesis of O-benzyl-N-*t*-butyloxycarbonyl-L-threonine, the maximum yield of this substance from N-*t*-butyloxycarbonyl-L-threonine was 6%.

Experimental Section[8]

O-Benzyl-N-*t*-butyloxycarbonyl-L-serine.—Freshly cut sodium metal (920 mg, 40 mg-atoms) was added to freshly distilled anhydrous ammonia (120 ml) at −70°, and N-*t*-butyloxycarbonyl-L-serine[7] (4.2 g, 20 mmol) was added with stirring under nitrogen. The mixture was vigorously stirred until colorless and then sodium metal (*ca.* 5 mmol) was added, followed by benzyl bromide (3.72 ml, 31 mmol). The turbid solution was stirred for 30–60 min at −50 to −30° to give a clear solution. The ammonia was then removed by slow evaporation and lyophilized. The residue was dissolved in distilled water (20 ml), and the solution was extracted with ether (two 20-ml portions). The aqueous phase was chilled, acidified to pH 3.5 with solid citric acid, saturated with sodium chloride, and extracted with ethyl acetate (four 100-ml portions). The combined organic layers were washed with saturated sodium chloride solution (three 35-ml portions) and dried over anhydrous sodium sulfate. The ethyl acetate was removed *in vacuo* at room temperature to give a colorless oil. The oil was dissolved in chloroform (8 ml), placed on a 3 × 45 cm column of silicic acid (150 g, Baker Analyzed), and eluted with chloroform (800 ml). The chloroform was evaporated *in vacuo* to give O-benzyl-N-*t*-butyloxycarbonyl-L-serine as a clear oil (2.7 g, 45%). Further elutions with methanol yielded 2.7 g of a mixture of N-*t*-butyloxycarbonyl-L-serine and traces of O-benzyl-N-*t*-butyloxycarbonyl-L-serine as an oil. Thin layer chromatography of the oils on silica gel plates in solvent system A against standard reference samples indicated the above structural assignments. An analytical sample of the O-benzyl-N-*t*-butyloxycarbonyl-L-serine was prepared by crystallization of the oil from ether–petroleum ether (bp 30–60°) and a recrystallization from the same solvent mixture: mp 56–58°; $[\alpha]^{25}D$ +19.8° (*c* 2.0, 80% EtOH) [lit.[9] mp 54–63°; $[\alpha]^{25}D$ +20.3° (*c* 2, 80% EtOH)].

Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.12; H, 7.19; N, 4.70.

Recrystallization of the oil from the methanol elution yielded optically pure starting material: mp 86–90°; $[\alpha]^{25}D$ −7.3° (*c* 2.29, 8% EtOH) [lit. mp 84°;[7] $[\alpha]^{25}D$ −7.7°[9] (*c* 2, 8% EtOH)].

Demonstration of Steric Purity.—An aliquot of O-benzyl-N-*t*-butyloxycarbonyl-L-serine prepared by the above procedure was dissolved in 5.4 *N* HBr–HOAc (2 ml). After 1 hr at room temperature, the reaction mixture was evaporated under water aspirator pressure at 20° to yield a residue which was then diluted to 5 ml with 1 *N* HCl for optical rotation determination. This sample showed the same optical rotation as a sample of L-serine similarly treated, $[\alpha]^{25}D$ +14° (*c* 2.1, 1 *N* HCl).

Registry No.—O-Benzyl-N-*t*-butyloxycarbonyl-L-serine, 23578-14-5.

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(8) All melting points were determined in capillaries on a Mel-Temp apparatus and are corrected. Microanalysis was by Spang Microanalytical Laboratory, Ann Arbor, Mich. Thin layer chromatography was performed on silica gel plates using chloroform–methanol–acetic acid (85:10:5)—system A.

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Preparation of 2-Acetamido-2-deoxy-α-glycopyranosides. II[1]

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Notwithstanding the marked influence of temperature on the anomeric equilibrium of glycosides of glucosamine and galactosamine,[2] a single product, phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannopyranoside (**1**),[3] is found to predominate in condensation of mannosamine pentaacetate with phenol, at 150 or 125° with catalysis by zinc chloride, or at 100° with *p*-toluenesulfonic acid. The β anomer (**2**), not reported previously, is isolable in small amount from all three reaction mixtures. A similar preference for formation of the α glycoside has been observed in analogous reactions of mannose derivatives.[4] Formulation of **1** as the α pyranoside[3] was confirmed by nmr studies.[5] The present formulation of **1** and **2** and the derived phenyl 2-acetamido-2-deoxy-α- and -β-D-mannopyranosides (**3** and **4**) as anomeric pairs of pyranosides is supported by their optical-rotation data and by their resistance to acid hydrolysis. For the glycosides **3** and **4**, the value of 2*A* ($\Delta[M]D$) is 43,800; for their tri-O-acetyl esters **1** and **2**, 2*A* is 60,500. For comparison, 2*A* is 47,400 for the phenyl α- and -β-D-mannopyranosides and 58,000 for their tetraacetyl esters.[4] A parallel correspondence has been noted for the glycosides of

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glucose and 2-acetamido-2-deoxyglucose,[2] although the values of $2A$ for these differ appreciably from those found in the mannose series. The sensitivity to acid hydrolysis of **3** and **4** is of the correct order of magnitude for pyranosides (48 and 31% liberation of phenol from 0.01 *M* solutions in 0.05 *M* HCl, heated 20 min at 100°), as shown by comparisons with the behavior of the phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides (15 and 30% liberation of phenol). As reported elsewhere,[6] **3** and **4** are inactive as substrates for α- or β-acetylglucosaminidase or for α-acetylgalactosaminidase.

The crystalline *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranosides (**5** and **6**) are produced by O-deacetylation of the syrupy product from nitration of the previously characterized phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (**7**).[2] The two nitrophenyl glycosides, which are separable by adsorption chromatography on Dowex 50,[7] are valuable test substrates for α-acetylgalactosaminidase (to be published).

Experimental Section

Melting points are corrected. A Perkin-Elmer Model 141 polarimeter was used with 1-dm tubes. Microanalyses were done by Spang Microanalytical Laboratories, Ann Arbor, Mich. Acetate esters were O-deacetylated in warm methanol–chloroform with sodium methoxide catalysis.[2] The orientation of nitro groups in pure glycosides and mixtures was determined by acid hydrolysis and chromatography.[2] Phenol was estimated by the method of Folin and Ciocalteau.[6]

Phenyl 2-Acetamido-2-deoxy-α- and -β-D-mannopyranoside (3 and 4).—Pentaacetyl β-mannosamine, 2 g, was allowed to react with 5 g of phenol and 0.5 g of zinc chloride for 2.5 hr at 125° (50 mm).[2] The reaction product was crystallized from ethyl acetate, yielding 1.18 g of the pure tri-O-acetyl α-glycoside **1**, mp 198–198.5°, $[\alpha]^{23}D$ +72.6° (*c* 0.6, chloroform) [lit.[3] mp 192–193°, $[\alpha]^{30}D$ +74° (chloroform)].

A second crop, 0.63 g, mp 165–180°, $[\alpha]^{23}D$ +34.8°, and a third crop, 0.06 g, mp 156–176°, $[\alpha]^{23}D$ +44.6°, were obtained with the aid of ether and hexane. Systematic fractional crystallization of these materials from ethyl acetate–isopropyl ether and absolute ethanol yielded additional quantities of 1 and 59 mg (3%) of pure phenyl 2-acetamido-tri-O-acetyl-2-deoxy-β-D-mannopyranoside (**2**), mp 184.5–185°, $[\alpha]^{23}D$ −70.2° (*c* 0.6, chloroform).

Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.7; H, 5.95; N, 3.31. Found: C, 56.7; H, 5.96; N, 3.21.

O-Deacetylation of **1** and crystallization of the syrupy product from moist acetone gave the α glycoside **3**, mp 104°, which contained water of hydration not determined with precision. For the monohydrate, a loss of 5.7% was calculated and a loss of 4.1% was found at 110° (0.05 mm). The optical rotation, $[\alpha]^{25}D$ +49.1° (*c* 1.0, ethanol) and +42.9° (*c* 0.8, water), and analyses are reported for the dried substance.

Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.44; N, 4.71. Found: C, 56.6; H, 6.49; N, 4.57.

For anhydrous (?) **3**, the following values were reported: mp 98–99°, $[\alpha]D$ +50° (ethanol).[3]

O-Deacetylation of **2** gave a syrup, crystallized from methanol–ether and recrystallized from hot water to yield the pure β glycoside **4**, mp 184–185°, $[\alpha]^{25}D$ −104.4° (*c* 0.8, water).

Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.44; N, 4.71. Found: C, 56.6; H, 6.32; N, 4.58.

***o*- and *p*-Nitrophenyl 2-Acetamido-2-deoxy-α-D-galactopyranoside (5 and 6).**—A nitration mixture prepared from 2.25 ml of nitric acid (90%) and 7.5 ml of acetic anhydride was added at one time to a stirred solution of 10 g of **7** in 25 ml of acetic acid, and the reaction[2] was allowed to proceed for 2 hr at 37°. After dilution with 60 ml of ice-cold 2 *M* potassium acetate solution and storage for 3 hr at room temperature, the reaction mixture was extracted with chloroform. Washing of the extract with 2 *M* sodium carbonate and water, drying with sodium sulfate, clarification by passage through a small pad of silicic acid, and removal of solvent under reduced pressure left a syrupy mixture of *o*- and *p*-nitrophenyl derivatives, not successfully resolved. O-Deacetylation of the syrup yielded a solid product, recrystallized from absolute ethanol to give 6.1 g of colorless, seemingly homogeneous needles and a second crop, 0.6 g, both shown to be gross mixtures of the *o*- and *p*-nitrophenyl glycosides (**5** and **6**). These were not separated by repeated recrystallizations from absolute ethanol, acetone, or water. The mixture was applied as a 1% solution in 0.001 *M* acetic acid to a column of Dowex 50 × 4-H^+ (200–400 mesh) of bed volume 3.2 l. Development with the same solvent completely resolved two peaks (11.8 and 17.4 l.), as revealed by absorbance measurements at 265 mμ. Concentration *in vacuo* of the pooled fractions of the first peak and recrystallization of the solid residue from absolute ethanol gave the pure *o*-nitrophenyl glycoside **5**: yield 3.6 g; mp 208–209°; $[\alpha]^{25}D$ +244° (*c* 0.5, water); uv max (water) 265 mμ (ε 3640) and 322 (2000); solubility in water at 25°, 0.70%.

Anal. Calcd for $C_{14}H_{18}N_2O_8$: C, 49.1; H, 5.30; N, 8.19. Found: C, 49.0; H, 5.36; N, 8.07.

Similarly, the pooled fractions of the second chromatographic peak gave the pure *p*-nitrophenyl glycoside **6**: yield 2.2 g; mp 266° dec; $[\alpha]^{25}D$ +310° (*c* 0.2, water); uv max 222 mμ (ε 6930) and 305 (10,760); solubility in water at 25°, 0.23%.

Anal. Calcd for $C_{14}H_{18}N_2O_8$: C, 49.1; H, 5.30; N, 8.19. Found: C, 49.3; H, 5.29; N, 8.03.

Registry No.—**2**, 23646-65-3; **3**, 4366-43-2; **4**, 23646-66-4; **5**, 23646-67-5; **6**, 23646-68-6.

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Phosphonic Acids and Esters. XXI.[1] Dimerization and Diels–Alder Reactions of Dialkyl 1-(1,3-Butadienyl)phosphonates

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Previous studies have shown that vinylic[3] and acetylenic[4] phosphonates function as moderately reactive dienophiles in Diels–Alder reactions. Aromatization of the adducts provides a useful synthesis of substituted phenylphosphonates.[3,4] Pudovik and coworkers[5,6] have shown that diethyl 1-(1,3-butadienyl)phosphonate (**1a**) is a comparably effective diene. On heating, **1a** forms

$$CH_2{=}CHCH{=}CHP(O)(OR)_2$$
1a, R = C_2H_5
b, R = CH_3

a dimer, and the reaction of **1a** with acrylonitrile yields a Diels–Alder adduct.[6] Both reactions were apparently directionally specific to yield a single isomer; structures

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