Notes

9-β-Melibiosyladenine. Protection of the 1 → 6-Glycosidic Linkage of Melibiose with Benzoate Ester Blocking Groups

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Nucleosides derived from disaccharides have been reported by Wolfrom and co-workers, who were successful in the synthesis of the adenine and 2,6-diaminopurine nucleosides of lactose,[1] cellobiose,[2] and maltose.[2] A nucleoside derivative of gentiobiose, namely, 2,6,8-trichloro-9-(hepta-*O*-acetyl-β-gentiobiosyl)purine, has been reported.[3] The nucleoside antibiotic, amicetin, has been shown to be a cytosine disaccharide derivative.[4] During a study to develop a synthetic route to amicetin, Stevens and Blumbergs[4] succeeded in the synthesis of a number of pyrimidine disaccharide nucleosides, including 1-β-lactosyl- and 1-β-cellobiosylcytosine.

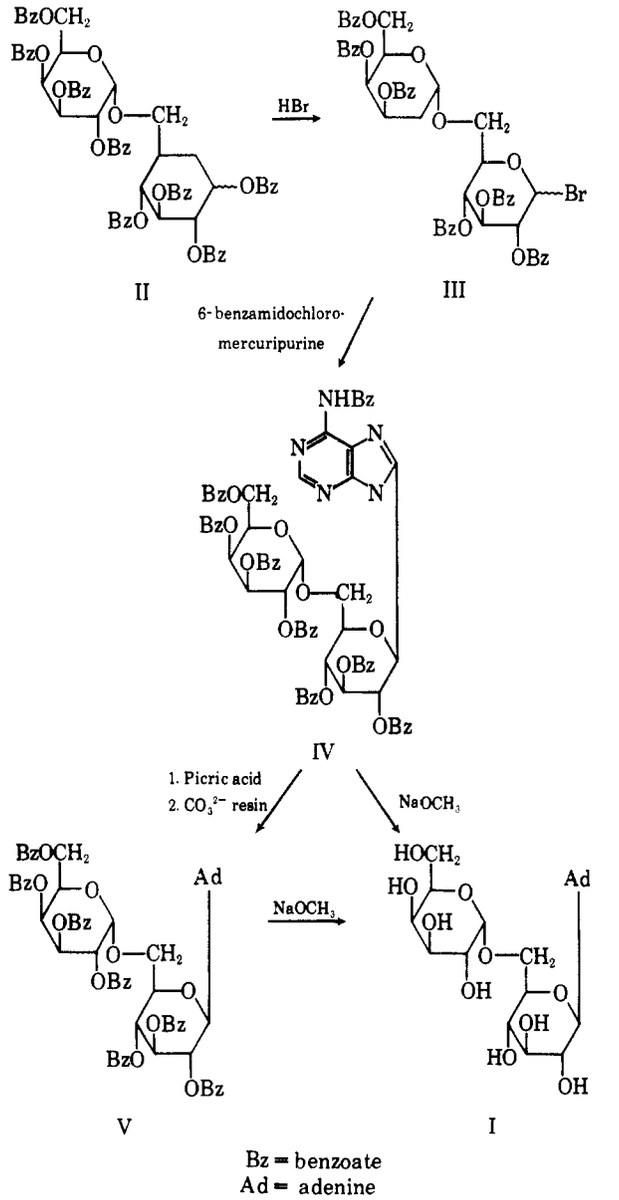
As part of an investigation into the preparation of disaccharide nucleosides, the author decided to synthesize the unreported 9-β-melibiosyladenine (9-[6-*O*-α-D-galactopyranosyl-β-D-glucopyranosyl]adenine) (I) as an exercise in organic chemistry in order to gain experience in working with this class of compounds.

Octa-*O*-benzoylmelibiose (II) was converted to the crude bromide by reaction with hydrogen bromide in acetic acid. When the bromide was coupled with 6-benzamidochloromercuripurine in refluxing xylene,[1,5] the reaction mixture became black in about 15 min, an indication that a good deal of degradation had occurred. In comparison to this, after 5 hr of refluxing at the cooler temperature of toluene the reaction mixture had only a pale orange color. The blocking groups were removed and isolation *via* the picrate[6] gave a 38.5% yield of the product I. The presence of some 9-β-D-glucopyranosyladenine was also indicated by paper chromatography.

The desired product I was also prepared by an alternative pathway. After the halogenose III and the base had been coupled, this time in refluxing xylene, the N-benzoyl group was removed in boiling 10% ethanolic picric acid,[7] and the intermediate product, 9-(hepta-*O*-benzoyl-β-melibiosyl)adenine (V), was isolated as a pure crystalline compound in a 22% yield. Removal of the blocking groups afforded I.

The configuration at the anomeric carbon of the glucose moiety is assigned as β because of the directive effect due to the ester at C-2 of the sugar, in accord with the *trans* rule.[8]

The benzoyl blocking groups of melibiose apparently have a protective effect at the 1 → 6-glycosidic linkage. Attempts to prepare the title compound I using octa-*O*-acetylmelibiose under the conditions herein described for the benzoate met with failure due to considerable cleavage of the 1 → 6-glycosidic bond between galactose and glucose during the hydrogen bromide–acetic acid reaction. The success with the benzoate ester is probably due to a steric hindrance of the attack by bromide ion because of the bulky size of the esters. The steric role of acetyl groups at C-2 and



(1) M. L. Wolfram, P. McWain, F. Shafizadeh, and A. Thompson, *J. Am. Chem. Soc.*, **81**, 6080 (1959).

(2) M. L. Wolfrom, P. McWain, and A. Thompson, *ibid.*, **82**, 4353 (1960).

(3) N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965).

(4) C. Stevens and P. Blumbergs, *ibid.*, **30**, 2723 (1965), and references cited therein.

(5) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

(6) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957).

(7) J. R. Parikh, M. E. Wolff, and A. Burger, *J. Am. Chem. Soc.*, **79**, 2778 (1957); M. L. Wolfrom, A. B. Foster, P. McWain, W. von Bebenburg, and A. Thompson, *J. Org. Chem.*, **26**, 3095 (1961).

(8) B. R. Baker, in Ciba Foundation Symposium, Chemistry and Biology of Purines, G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown, and Co., Boston, Mass., 1957, p 120.

C-6 of hexopyranoses is well documented in the literature.[9] It is expected that the larger size of the benzoyl groups would afford an even greater steric protection at the glycosidic carbon. These results suggest that benzoate esters are advantageous as blocking groups of disaccharides, especially in those cases where there is a danger that the glycosidic bond can be broken.

Experimental Section[10]

Octa-*O*-benzoylmelibiose (II).—The procedure used to prepare this substance was based upon that used by Thiel, *et al.*,[11] for the preparation of octa-*O*-benzoylmaltose.

α-Melibiose monohydrate (Pfanstiehl Labs, 5 g) was suspended in 50 ml of dry pyridine and the stirred suspension was cooled in an ice bath. Benzoyl chloride (20 ml) was added dropwise and after the suspension had been stirred for several hours complete solution occurred. The mixture stood at room temperature for 4 days and then was heated on a steam bath for 2.5 hr. The dark orange reaction mixture was poured into ice water and when the ice had melted the water was decanted from the syrup which had formed. The syrup was triturated with cold water and the water was decanted. This process was repeated several times.

The syrupy product was dissolved in boiling absolute ethanol, seeded,[12] and chilled in the refrigerator. The mass of crystals was broken up with a rod and collected by filtration to yield 12.3 g of II, mp 110–113°. A second crop was afforded by concentration of the mother liquor, 1.7 g (total yield, 72%).

The product (2 g) was filtered through a short column containing 40 g of silicic acid (Mallinkrodt, 100 mesh) to remove a yellow colored contaminant. After recrystallization from ethanol the product had mp 111–112°, $[\alpha]^{27}D$ +174° (*c* 4.55, $CHCl_3$). The product was homogeneous on tlc in solvent D,[13,14] R_f 0.36.

Anal. Calcd for $C_{68}H_{54}O_{19}$: C, 69.48; H, 4.64. Found: C, 69.32; H, 4.55.

The crude crystalline material was satisfactory for the following steps.

6-Benzamido-9-(hepta-*O*-benzoyl-β-melibiosyl)purine (IV).—Octa-*O*-benzoylmelibiose (17 g, 14.5 mmoles) was dissolved in 29 ml of chloroform, and 63 ml of a saturated solution of hydrogen bromide in acetic acid was added. The mixture was shaken until it was homogeneous and the reaction was allowed to proceed at room temperature for 2.5 hr. Dry toluene (120 ml) was added and the mixture was concentrated to an orange syrup. Fifty-milliliter portions of toluene were added and evaporated three more times to remove traces of acetic acid and hydrogen bromide.

The syrup obtained was coupled with 6-benzamidochloromercuripurine[5] (8.5 g, 14.5 mmoles) in the presence of Celite-545 (8.5 g) and cadmium carbonate[1] (4.5 g) using toluene (300 ml) as the refluxing solvent. After the usual work-up[1,5] a syrup was obtained which was dissolved in a minimum amount of warm benzene and placed aside for several hours. The 6-benzamidopurine that precipitated was removed by filtration (80 mg, mp 238–240°). The benzene was evaporated to afford 27 g of a hard, orange syrup containing IV. This material was used directly for the next step.

9-β-Melibiosyladenine (I).—The syrup IV was dissolved in 250 ml of absolute methanol; 3.5 g of sodium methoxide was added and the mixture was refluxed for 3 hr. The methanol was removed by evaporation and the residue was dissolved in 100 ml of water which contained 3.7 ml of glacial acetic acid. The aqueous solution was extracted once with 100 ml of chloroform and the aqueous solution was then concentrated to about 30 ml. To this solution was added 225 ml of 10% methanolic picric acid solution and the flask was chilled in an ice bath for 30 min. The yellow precipitate was removed by suction filtration and washed with methanol and with water.

The crude picrate was suspended in 550 ml of continuously stirred warm water and Bio-Rad AG1-X8 (CO_3^{2-}) resin was added in small portions until the yellow color was discharged.[6] Stirring was continued for 30 min, the resin was removed by filtration, and it was washed with 200 ml of water. The water was evaporated to afford a clear gum which was triturated with ethanol and the solvent was removed by evaporation. A repeat of this procedure caused the gum to solidify. This solid material was dissolved in 500 ml of boiling methanol, the solution was concentrated to 150 ml and then was chilled in the refrigerator. The white product was isolated by filtration: 2.18 g (31.5%), mp 187–188°. A second crop (0.38) was obtained by concentration of the mother liquor (total yield, 38.5%). A trace of 9-β-D-glucopyranosyladenine[5] was present as revealed by paper chromatography. Two recrystallizations from methanol at room temperature yielded the analytical sample: mp 186–188° with a soft appearance at 180°; $[\alpha]^{25}D$ +65.2° (*c* 1.15, H_2O); ultraviolet and infrared spectra $\lambda_{max}^{H_2O}$ 259 mμ (ε 13,500) and λ_{max}^{KBr} (cm^{-1}) 3370 (OH, NH), 1640 (NH_2-C=N), 1610, 1590 (purine ring), 1080, 1030 (COC, CO—). The product was homogeneous on paper chromatograms,[13] R_{AD} 2.13 (solvent A), 0.04 (solvent B).

Anal. Calcd for $C_{17}H_{25}N_5O_{10}$: C, 44.44; H, 5.48; N, 15.25. Found: C, 44.54; H, 5.56; N, 15.38.

A small sample of the nucleoside was hydrolyzed in 1 *N* hydrochloric acid solution at 110° for 2.5 hr in a sealed tube. After adjusting to neutral pH, the contents were spotted on tlc plates and developed with solvent C.[13] Adenine, glucose, galactose, and a trace of melibiose were all readily identified.

9-(Hepta-*O*-benzoyl-β-melibiosyl)adenine (V).—6-Benzamidochloromercuripurine (5 g) was coupled with III (prepared from 10 g of II) in the same manner as described above except that the reaction solvent was xylene.[1,5] A dark brown syrup (12.1 g) was obtained which was dissolved in 45 ml of absolute ethanol. A solution of 10% ethanolic picric acid (70 ml) was added and the mixture was refluxed for 1 hr.[7] An orange oil formed. The solvent was decanted and the oil was triturated twice with ethanol and the ethanol was decanted. The oil could not be induced to crystallize. It was, therefore, dissolved in 360 ml of acetone; 80 ml of water was added and the color was discharged by addition of Bio-Rad AG1-X8 (CO_3^{2-}) resin. A small amount of Darco G-60 was used to remove a brown colored impurity and the mixture was filtered through Whatman No. 42 filter paper using suction. The clear, colorless filtrate was evaporated to yield a syrup which solidified after two evaporations with absolute ethanol. Crystallization of this substance from hot ethanol (an air stream was required to induce crystallization) afforded 1.41 g of white crystals, mp 145–147°. Two recrystallizations from warm methanol (air stream) gave the analytical sample, mp 150–151°, $[\alpha]^{24}D$ +112° (*c* 1.00, $CHCl_3$). The substance was homogeneous on tlc with ethyl acetate as the developing solvent, R_f 0.54. Ultraviolet and infrared spectra gave λ_{max}^{EtOH} 257 mμ and λ_{max}^{film} 3440–3340 (broad NH, NH_2), 1730 (benzoate ester), 1640, 1610, 1590 (phenyl and purine ring), 1265 (benzoate COC), 1090, 1070, 1030 (sugar CO—), 710 cm^{-1} (monosubstituted phenyl).

Anal. Calcd for $C_{66}H_{53}N_5O_{17}$: C, 66.73; H, 4.50; N, 5.90. Found: C, 66.84; H, 4.60; N, 5.83.

The mother liquors from the above were pooled and concentrated to dryness. The resulting syrup (5 g) was dissolved in a small amount of chloroform and applied to the top of a column

(9) F. W. Newth and G. O. Phillips, *J. Chem. Soc.*, 2896, 2900, 2904 (1953).

(10) Elementary analyses were performed by the Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were determined on a Fisher-Johns block and are corrected. Optical rotations were determined on a Rudolph Model 200 spectropolarimeter, ultraviolet spectra were determined on a Beckman DK-2 spectrophotometer, and the infrared spectra were determined on a Perkin-Elmer Model 21 spectrophotometer. All evaporations were performed *in vacuo* with a bath temperature of 40–45°. Chloroform used in polarimetry and chromatography contained 0.75% ethanol. Chloroform used in reactions was passed through a column of activated alumina prior to use.

(11) I. M. Thiel, J. O. Deferrari, and R. A. Codenas, *J. Org. Chem.*, **31**, 3704 (1966).

(12) Seed crystals were obtained after chromatographing 0.5 g of the syrup on 10 g of silicic acid with chloroform as the elution solvent.

(13) Thin layer chromatography (tlc) was performed on silica gel G or HF (E. Merck, AG, Darmstadt) with Desaga equipment. Spots were located with a chromic acid spray followed by careful application of heat from an open flame. Ultraviolet absorbing material was located with an ultraviolet lamp before the plates were sprayed. Paper chromatograms were obtained by descending techniques on Whatman No. 1 paper. Spots were visualized with an ultraviolet lamp. The solvents used in this investigation were 5% aqueous disodium hydrogen phosphate (solvent A), 1-butanol–water (86:14) (solvent B), 1-butanol–acetic acid–ethyl ether–water (9:6:3:1)[14] (solvent C); and benzene–ethyl acetate (95:5) (solvent D). The expression R_{Ad} refers to the ratio of the distance that the nucleoside migrated to the distance that adenine migrated. Free sugars were chromatographed on tlc plates with solvent C.

(14) G. W. Hay, B. A. Lewis, and F. Smith, *J. Chromatog.*, **11**, 479 (1963).

13 cm long and 6 cm wide of activated silicic acid (Merck). The column was eluted with chloroform (400 ml), chloroform–ether (1:1) (600 ml), ether (400 ml), and ether–acetone (1:1) (400 ml). The chloroform–ether fraction contained the largest amount of material (2.7 g) which had the component V of R_f 0.54. This fraction was rechromatographed on 70 g of silicic acid (Mallinkrodt) with ethyl acetate as the elution solvent and 25-ml fractions were collected. Fractions 4–6 contained the desired substance. They were combined, the solvent was evaporated, and the product was crystallized to give another 0.60 g of V, total yield 22%.

A small sample of V was deacylated with methanolic sodium methoxide in the usual manner. The product obtained was identical in every respect with I.

Registry No.—I, 13673-75-1; II, 13673-76-2; IV, 13673-77-3; V, 13673-78-4.

Pseudo-Halogens. IX.[1] Reaction of Iodine Isocyanate with Dienes and Acetylenes

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The reaction of iodine isocyanate (INCO) with monoolefinic compounds has received considerable attention in our laboratory[4] and elsewhere.[5] The addition products are useful intermediates in the preparation of carbamates, aziridines, ureas, 2-oxazolidones, and β-iodoamines.

Strikingly absent from the literature, however, are detailed reports on the reaction of INCO with dienes, acetylenes, and allenes. The recently published paper of Hassner, Lorber, and Heathcock,[5c] in which brief mention was made of the reaction of INCO with di- and triolefins and acetylenes, prompts us to report our results with conjugated and nonconjugated aliphatic and alicyclic dienes, acetylenes, and methylallene. Our work has dealt mainly with the relationship between the structure of dienes or acetylenes and moles of INCO consumed, although we have also studied experimental techniques and product isolation.

Typically, INCO additions (eq 1) are conducted in a stirred heterogeneous system containing the monounsaturated compound, iodine, and silver cyanate in ether at low temperatures (0 to −30°). INCO is consumed as it is generated (*in situ* method). In the

$$AgOCN + I_2 + >C{=}C< \xrightarrow[0 \text{ to } -30^\circ]{Et_2O} >\underset{I}{C}\!-\!\!-\!\underset{NCO}{C}< + AgI \quad (1)$$

initial studies with acetylenes, the *in situ* method was found to be unsatisfactory; diiodo compounds formed preferentially thus removing both the acetylene and iodine from the reaction system and also contaminated desired addition products. In all of the work to be described in this Note, therefore, preformed solutions of INCO of known concentration were prepared in tetrahydrofuran (THF) at −30 to −50° by our published procedure[4b] and these solutions were employed for the addition reactions. Such solutions were not entirely free of iodine, however, as about 5% of it could not be converted to INCO. This probably caused some complications and may account, in part, for the difficulty experienced in obtaining analytically pure derivatives (see Experimental Section). The stoichiometric quantity of INCO was used, that is, 2 moles for each mole of diene, methylallene, or acetylenic compound.

Disappearance of INCO was followed iodometrically[4b] and in all cases attempts were made to isolate and characterize the primary reaction products or convert them to carbamates, ureas, or amine hydrochlorides by reaction with methanol, ammonia, or 17% hydrochloric acid, respectively, at or below room temperature. Except in the few cases described in the Experimental Section, well-characterized products with correct elemental analyses could not be obtained, although infrared and nuclear magnetic resonance spectra suggested that INCO addition had occurred in a predictable way. Products obtained were usually high in iodine content suggesting that iodine addition was the main competing reaction.

Addition of INCO to Aliphatic Dienes with Isolated Double Bonds.—Dienes with at least one $-CH_2-$ group between the double bonds are included in this category. Table I summarizes the results. The most noteworthy feature is the high rate even at −28 to −45°. In every case, over 90% of the INCO was consumed between the time the diene was added to the stirred INCO solution in THF and removal of the first analytical sample (15 min or less). The times listed in the table are those at which INCO consumption had ceased for all practical purposes. The high reaction rate precluded a detailed kinetic study.

TABLE I
ADDITION OF INCO TO ALIPHATIC DIENES WITH ISOLATED DOUBLE BONDS[a]

Diene	Reacn temp, °C	Reacn time, min	Moles of INCO consumed per mole of diene
1,4-Pentadiene	−35	15	1.74
1,5-Hexadiene	−28	45	1.82
2,5-Dimethyl-1,5-hexadiene	−45	15	1.60
1,7-Octadiene	−45	30	1.80
4-Vinylcyclohexene	−38	60	1.58

[a] 0.1 mole of INCO/0.05 mole of diene in THF.

As anticipated, all the listed dienes had the stoichiometry of approximately 2 moles of INCO reacting with 1 mole of diene. An excess of INCO was not employed in any of the experiments. The adducts obtained after evaporation of the THF had the expected characteristic band of organic isocyanates in the infrared spectrum (~2280 cm^{-1}). On treatment of the adducts with methanol at room temperature, the band disappeared and was replaced by bands at 3310 (>NH), 1720 (>C=O), and 1550 (amide-II) cm^{-1}, typical of carbamates.

In only one case, from 2,5-dimethyl-1,5-hexadiene,

(1) Pseudo-Halogens. VIII: T. A. Foglia and D. Swern, *J. Org. Chem.*, **32**, 75 (1967).

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(4) (a) C. G. Gebelein and D. Swern, *Chem. Ind.* (London), 1462, 1965. (b) S. Rosen and D. Swern, *Anal. Chem.*, **38**, 1392 (1966). (c) C. G. Gebelein, Ph.D. Thesis, and S. Rosen, M.A. Thesis, Temple University, February 1967.

(5) (a) L. Birckenbach and M. Linhard, *Ber.*, **63**, 2544 (1930); *ibid.*, **64**, 961, 1076 (1931). (b) G. Drefahl and K. Ponsold, *Chem. Ber.*, **93**, 519 (1960). G. Drefahl, K. Ponsold, and G. Köllner, *J. Prakt. Chem.*, **28**, 136 (1964). (c) A. Hassner, M. E. Lorber, and C. Heathcock, *J. Org. Chem.*, **32**, 540 (1967), and references contained therein to earlier work of this group.