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Synthesis of amicetose by three enantioselective methods

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Abstract

Three methods for the synthesis of the deoxy sugar amicetose (2,3,6-trideoxy-D-*erythro*-hexopyranoside) are described. All three utilize the known dihydropyran 2-isobutoxy-6-methyl-2,3-dihydro-4*H*-pyran as an intermediate. Asymmetric hydroboration of the dihydropyran with $IpcBH_2$ gave enantiomerically enriched isobutyl α and β-amicetosides. Hydroboration with borane–tetrahydrofuran followed by derivatization of the major product (β-anomer) with *R*-(−)-1-(1-naphthyl)ethylisocyanate gave diastereomeric carbamates which were separated and converted to isobutyl β-D and β-L-amicetosides having high optical purity. Racemic isobutyl β-amicetosides were also resolved by enzymatic transesterification using lipase and an acyl transfer reagent. Porcine pancreatic lipase and lipases from *Candida rugosa* and *Pseudomonas* sp. were evaluated in the presence of either vinyl acetate, vinyl butyrate, or trifluoroethyl butyrate as acylating agents. A GC-based method for determining enantiomeric purity of amicetose derivatives was developed.

1. Introduction

Amicetose **1** (2,3,6-trideoxy-D-*erythro*-hexose) is a constituent of the antibiotics amicetin[1] and axenomycin,[2] where it is linked to the branched-chain sugar axenose (in axenomycin) or the amino sugar amosamine (in amicetin) resulting in disaccharides **2** and **3**.

As part of our efforts to develop stereoselective methods for the synthesis of oligosaccharides containing deoxy sugars, we required a convenient synthetic route to amicetose from which glycosyl donors/acceptors could be prepared. In the course of this study, we have developed three methods for the synthesis of enantio-enriched amicetosides based on asymmetric hydroboration of a simple enol ether, resolution of diastereomeric carbamate derivatives of amicetose, or lipase-catalyzed transesterification. In addition, we have developed an assay for enantiomeric enrichment of these deoxy sugars and ester derivatives based on capillary gas chromatography.

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The structure of amicetose was first confirmed by synthesis from D-glucal.[3] Since then, several other racemic and enantioselective syntheses of amicetose have been described, from both carbohydrate and non-carbohydrate starting materials.[4] Most of the carbohydrate-based routes required several steps because of multiple deoxygenation steps, and provide access to only the natural D-isomer, while the syntheses that utilize amino acids and other non-carbohydrate precursors typically produce mixtures of D-amicetose and isomeric products. We decided to develop a synthesis based on earlier work by Catelani,[5] with the goal of achieving access to gram quantities of D-amicetose in a minimum number of synthetic steps, for use in the synthesis of oligosaccharides.

The Catelani group synthesized racemic amicetose through a hetero-Diels–Alder reaction followed by hydroboration/oxidation. In subsequent work,[6] they reported the resolution of enantiomers by a sequence involving glycosylation of racemic amicetal with another monosaccharide, HPLC separation of products, and glycoside hydrolysis. The racemic route is efficient in that the ring system and functional groups are assembled in only two steps. We were attracted by the possibility of asymmetric hydroboration of the Diels–Alder adduct, as well as the potential for separating racemic D,L-amicetose by other means, such as the chromatographic separation of diastereomeric carbamate derivatives, and by enzyme-catalyzed transesterification with lipases.

2. Results and discussion

In order to investigate enantioselective syntheses of amicetose from achiral intermediates, it was necessary to first develop an analytical method that would allow the quantitation and identification of enantiomers in reaction products. We have developed two methods which are based on gas chromatography using chiral columns. The first method we developed utilized a Chrompack Chirasil^{TM} L-Val column. As shown in Fig. 1, a clean separation of both racemic hydroboration products was achieved with this column. The relatively short retention times make it the method of choice for analysis of asymmetric hydroboration experiments carried out on the dihydropyran. The method is limited by poor separation of amicetose esters however, making it unsuitable for the analysis of products derived from lipase-catalyzed transesterification. While enantiomeric excess can be measured in the remaining alcohol, values cannot be checked with those obtained for the corresponding esters because of peak overlap. This limitation requires that ester products first be hydrolyzed back to the alcohol before ratios can be determined. Fortunately, we were able to achieve separation of both alcohol and ester in a single sample using a Supelco β-DEX^{TM} 390 column (Fig. 2). This method requires longer times of analysis, but allows enantiomeric excesses for both alcohol and ester to be determined in a single chromatographic run. This feature turned out to be important, since several of the lipase-catalyzed transesterifications used crude enzyme preparations, and side reactions which would convert starting alcohol to other products could not be ruled out. The β-DEX^{TM} 390 column was developed for this study, and it may prove generally useful in the analysis of similar reaction products obtained using lipases.

With the chromatographic methods in place, asymmetric hydroboration of dihydropyran **4** was investigated. The extensive work of Brown provided relevant examples from which an approach could be developed.[7] Asymmetric hydroborations with monoisopinocamphenylborane (IpcBH$_2$, derived from (+)-α-pinene) have been carried out with substituted cyclohexenes,[8] including dihydropyran,[9] albeit in low enantiomeric excess in the latter case. Based on these results, we predicted that asymmetric hydroboration of **4** with this reagent would provide the configuration needed for D-amicetose. Application of the procedure described by Brown[10] to **4** using IpcBH$_2$ gave a ratio of α-anomer to β-anomer of 85:15, surprising in that the racemic hydroboration gave the β-anomer as the major product (Scheme 1). Both

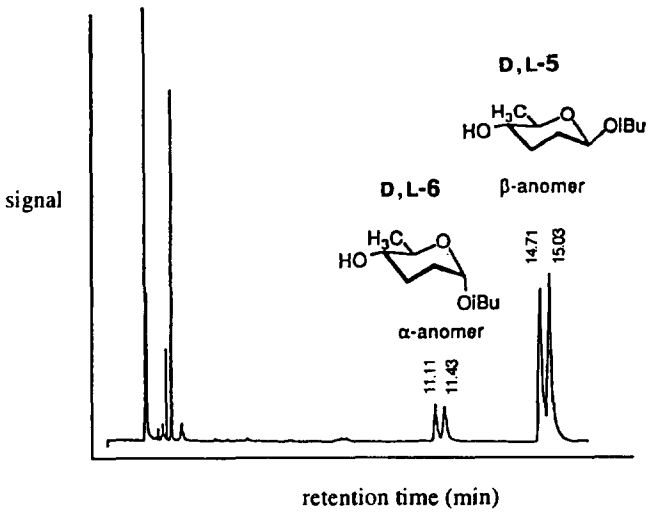


Fig. 1. Separation of isobutyl α- and β-D,L-amicetosides **5** by chiral GC using a Chrompak Chirasil L-Val column

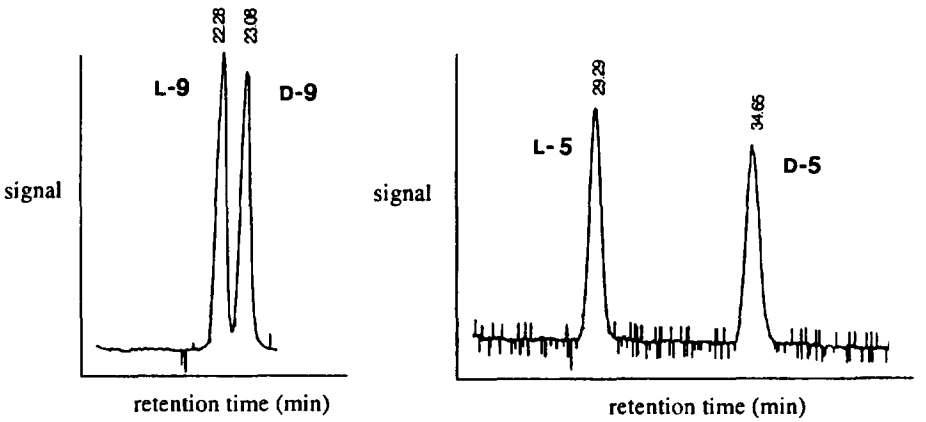
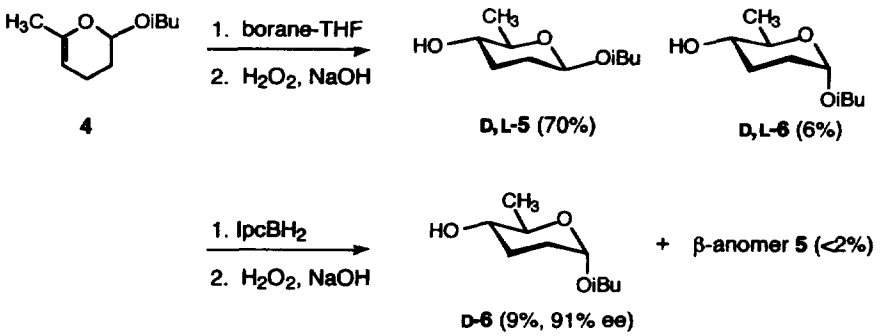


Fig. 2. Separation of isobutyl β-D,L-amicetosides **5** and esters **9** (R=Me) by chiral GC using a Supelco β-DEX 390 column

products were isolated in a combined yield of 11% and the enantiomeric purity was established for each using the GC method (Chirasil L-Val column). The low yield is perhaps due to the presence of two lone-pair donor oxygens in **4**, which could hinder attack of the reagent on the double bond.

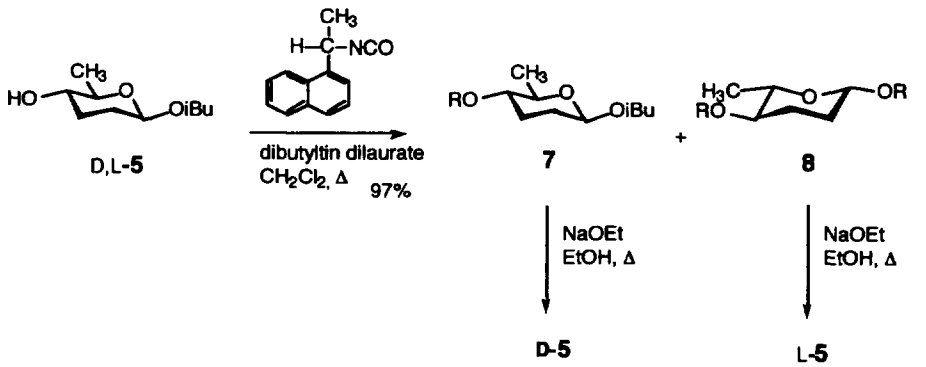


Scheme 1. Achiral and asymmetric hydroborations of **4**

The major product obtained from the $IpcBH_2$ hydroboration was obtained in 91% ee, and tentatively

assigned as the α-D-isomer because of its large positive rotation. To confirm the assignment of absolute stereochemistry, a small sample was hydrolyzed to give amicetose having a specific rotation that matched the value reported by Catelani[6] for D-amicetose (see Experimental). Enantiomeric analysis of the β-anomer revealed only a minor enrichment of 69:31 (38% ee); the assignment of absolute stereochemistry to the products was not carried out. Although the chemical yield of enantio-enriched α-anomer was low, the simple experimental protocol and two-step preparation from readily available starting materials satisfied the requirements for a straightforward route to D-amicetose on a gram scale. Attempts at improving the yield of the hydroboration were unsuccessful, so attention was directed towards alternative methods of asymmetric synthesis.

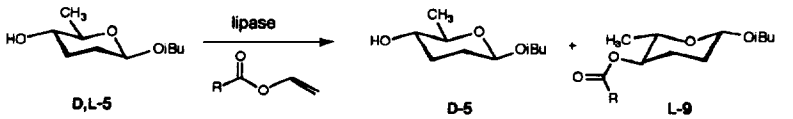
Resolution of the racemic amicetosides obtained by hydroboration (borane–THF) was attempted by derivatization of the products with isocyanates. Both *R*-(−)-1-(1-naphthyl)ethyl isocyanate and *R*-(−)-1-phenylethyl isocyanate were used to convert racemic isobutyl β-D,L-amicetoside into its diastereomeric carbamate derivatives.[11] Carbamates derived from the phenylethyl isocyanate were inseparable, while those derived from the naphthyl reagent could be separated partially by HPLC (Beckman Si, 10 mm×25 cm) or by repetitive flash chromatography. The best conditions for the preparation of naphthyl carbamates **7** and **8** were found to be treatment of the alcohol with the isocyanate in the presence of dibutyltin dilaurate in dichloromethane as solvent (Scheme 2).[12] The naphthyl carbamate method, while giving enantiomeric excesses of 92% or greater, was not suitable for scale-up to mmol quantities due to the tedious nature of the separation involved and expense of the reagents. A larger scale HPLC separation by this method would be an attractive possibility, but the approach was not pursued further in this study.



Scheme 2. Resolution of amicetosides via carbamate derivatives

The resolution of secondary alcohols has been carried out with lipases,[13,14] operating in either the hydrolytic mode on racemic ester, or in transesterification reactions using the racemic alcohol and an acylating agent such as vinyl acetate. Porcine pancreatic lipase (PPL), *Candida rugosa* lipase, and *Pseudomonas* sp. lipase (Amano PS30) were selected for this study based on their previous applications to the resolution of racemic alcohols[15] and their ability to selectively acylate carbohydrate-derived substrates.[16] A study reported by Ciuffreda and coworkers[17] in which the regioselective acylation of 6-deoxy-L- and D- hexopyranosides through lipase-catalyzed transesterification reactions was of particular interest because it was shown that the D- and L-rhamnopyranosides exhibited different regioselectivity in transesterifications with PPL and CCL. The possibility of achieving enantioselective esterifications that would produce D- or L- esters based on the choice of enzyme was an attractive one for our work on amicetose glycosides, since the unnatural enantiomer could be accessed either as unreacted alcohol or by conversion to ester by switching enzymes.

The resolution of isobutyl β-D,L-amicetosides by lipase-catalyzed transesterification was investigated



Entry	Lipase (mg/mmol)	Solvent (mL/mmol)	(mmol)	Time (h)	Conversion (%)	Ester Isolated Yield (%)	Ester ee (%)	Alcohol Isolated Yield (%)	Alcohol ee (%)	E
1	Amano PS 2500		VB 103	17	70	77	30	79	99	∞
2	Amano PS 2500		VA 141	12	62	75	38	74	92	11.1
3	*Candida Rugosa* 2500		VB 102	0.5	77	55	40	53	86	4.0
4	Amano PS 2500	THF/VB1:1 13		16	95	37	28	37	84	2.0

VA = Vinyl Acetate, VB = Vinyl Butyrate

Fig. 3. Lipase-catalyzed transesterification of isobutyl-β-D,L-amicetoside **5**

using porcine pancreatic lipase (PPL) and lipases from *Candida rugosa* (CCL) and *Pseudomonas* sp. (Amano PS lipase) in the presence of vinyl acetate, vinyl butyrate, or trifluororethyl butyrate as acylating agents, and in the presence or absence of solvent. The β-anomer was used in this study because it is the major product of the hydroboration of **4**. The bulky isobutyl group was considered a suitable aglycone on the basis of the preference exhibited by lipases for large hydrophobic groups on substrates reported previously.[14,16]

Initial experiments revealed that PPL, while exhibiting a different enantioselectivity than the other two enzymes, was impractical due to the long reaction times required for conversion. Results of the transesterification method using Amano PS lipase and *Candida rugosa* lipase are shown in Fig. 3. Both of these enzymes exhibit a preference in rate for selective acylation of the L-substrate. Yields shown in the table are based on percent conversion, and the E factor was calculated using the method reported by Sih.[18] Vinyl acetate, vinyl butyrate, and trifluoroethyl butyrate were found to be suitable reagents for the transesterification; however, the GC analysis was more efficient in the case of the acetate esters due to their shorter retention times. Unreacted, and enriched alcohol was readily separated from the esters by flash chromatography. Samples of the esters were hydrolyzed and the product alcohols were again checked for enantiomeric purity by chiral GC. Hydrolysis of the aglycone from enriched isobutyl β-D-amicetoside was carried out and the optical rotation of the amicetose obtained matched that reported previously for D-amicetose. Highly enriched isobutyl β-D-amicetoside was obtained by the lipase method; however, the enrichment of the esters was unexpectedly low, indicating that acyl transfer also occurs to the less reactive enantiomer. In summary, the lipase method is suitable at present for preparing small quantities of enriched D-amicetosides from inexpensive and readily available materials. Further studies are in progress to optimize the procedure for larger scale synthesis.

3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian XL200 spectrometer at 200.06 and 50.3 MHz, respectively, or on a Bruker ACF-300WB instrument at 300 and 75.4 MHz, in deuteriochloroform solu-

tion. Proton chemical shifts are relative to tetramethylsilane (0.00 ppm), and carbon chemical shifts are relative to deuteriochloroform (76.91 ppm). Elemental analyses were obtained from Robertson Microlit Laboratories, Inc. High resolution mass spectra were measured at the University of Pennsylvania under CI conditions using ammonia as the reagent gas. Chiral GC analyses were performed on Hewlett–Packard 5790A and 5890A gas chromatographs using Chrompack Chirasil^{TM} L-Val or Supelco β-Dex^{TM} 390 fused silica capillary columns. Polarimetry was conducted on a Perkin–Elmer 241 polarimeter using a 10 cm cell. Flash chromatography was performed on Merck silica gel 60 using mixtures of ethyl acetate and hexane as indicated. Visualization of TLC plates was carried out with ammonium molybdate–ceric sulfate in sulfuric acid. Anhydrous THF was purchased from Aldrich Chemical Co.

3.2. *2-Isobutoxy-6-methyl-2,3-dihydro-4*H-*pyran (4)*

Dihydropyran **4** was prepared by the procedure of Smith,[19] using modifications reported by Catelani. The hetero-Diels–Alder reaction was carried out in a 300 mL Parr pressure reactor (190°C, 1 h). From methyl vinyl ketone (28 g, 0.40 mol) and isobutyl vinyl ether (100 mL, 0.77 mol) in toluene (100 mL) and hydroquinone (1.0 g) there was obtained 45.68 g (67%) of **4** after purification by vacuum distillation: bp 77.5–83°C at 20 mmHg (lit. bp 89–93°C at 40 mmHg); ^{1}H NMR (300 MHz) δ 0.90 (d, 3H, J=6.7 Hz, CH_3), 0.91 (d, 3H, J=6.7 Hz, CH_3), 1.73 (m, 3H, CH_3C=), 1.74–1.96 (m, 4H, CH_2), 2.00–2.22 (m, 1H, C*H*CH_2), 3.28 (dd, 1H, J=6.8 Hz, 9.4 Hz, OCH_2), 3.56 (dd, 1H, J=6.8 Hz, 9.4 Hz, OCH_2), 4.54 (m, 1H, H–C=), 4.98 (dd, J=3.5 Hz, 2.9 Hz, O–CH–O); ^{13}C NMR δ 16.76 (CH_2), 19.17 (CH_3), 19.85 (CH_3), 26.26 (CH_2), 28.42 (CH_3), 74.76 (CH_2), 96.09 (CH), 97.40 (CH), 147.23 (C). The preparation of **4** was also carried out on smaller scales (30 mmol of MVK) in a screw-capped glass pressure tube, at 140°C for 24 h. Product was obtained in yields of 40–50% under these conditions after purification by flash chromatography with 2% ethyl acetate–hexane as eluant: R_f=0.59.

3.3. *Isobutyl 2,3,6-trideoxy-α- and β-*D,L-erythro-*hexopyranosides (*D,L-***5** *and* D,L-***6***)

Hydroboration of **4** was carried out by the procedure of Catelani[5] with slight modifications. The solvent used was THF instead of hexane and the hydroborating reagent was borane–THF instead of borane–methyl sulfide. The reaction was complete in 24 h. Following workup by the prescribed method, the crude product was purified by flash chromatography using 7% ethyl acetate–hexane as eluant. From 3.50 g (20.56 mmol) of **4** there was isolated 2.44 g (70%) of β-anomer (**5**) and 0.212 g (6%) of α-anomer (**6**). α-Anomer: R_f=0.57 (40% ethyl acetate–hexanes); ^{1}H NMR (300 MHz) δ 0.91 (dd, 6H, J=6.3 Hz, CH_3), 1.25 (d, 3H, J=6.2 Hz, H-6), 1.54 (bs, 1H, OH), 1.70–1.90 (m, 5H, CH, H-2, H-3), 3.15 (dd, 1H, J=9.3 Hz, 6.2 Hz, OCH_2), 3.19–3.28 (m, 1H, H-4), 3.39 (dd, 1H, J=9.3 Hz, 6.2 Hz, OCH_2), 3.55–3.65 (m, 1H, H-5), 4.70 (d, 1H, J=1.6 Hz, H-1); ^{13}C NMR δ 17.93 (C-6), 19.41 (CH_3), 19.53 (CH_3), 27.75 (C-3), 28.46 (CH), 29.75 (C-2), 69.37 (C-5), 72.25 (C-4), 73.78 (CH_2), 96.19 (C-1). β-Anomer: R_f=0.50 (40% ethyl acetate–hexanes); ^{1}H NMR (300 MHz) δ 0.91 (dd, 6H, J=6.3 Hz, CH_3), 1.31 (d, 3H, J=6.2 Hz, H-6), 1.40–1.70 (m, 3H, OH, H-2_a, H-3_a), 1.80–1.95 (m, 2H, CH, H-2e), 3.16 (dd, 1H, J=9.3 Hz, 6.2 Hz, OCH_2), 3.20–3.35 (m, 2H, H-4, H-5), 3.66 (dd, 1H, J=9.3 Hz, 6.2 Hz, OCH_2), 4.42 (dd, 1H, J=1.6 Hz, J=9.6 Hz, H-1); ^{13}C NMR δ 18.10 (C-6), 19.28 (CH_3), 19.41 (CH_3), 28.47 (CH), 30.70 (C-2), 31.26 (C-3), 71.45 (C-5), 75.80 (C-4), 75.96 (CH_2), 101.23 (C-1).

3.4. *Isobutyl 2,3,6-trideoxy-α-*D-erythro-*hexopyranoside (*D-**6**) *and isobutyl 2,3,6-trideoxy-β-*D-*or β-*L-erythro-*hexopyranoside (**5**)*

Into a 300 mL three-necked flask, under argon, was placed R-alpine boranamine™ (20.8 g, 0.050 mol, Aldrich) in THF (75 mL), and with mechanical stirring, $BF_3 \cdot Et_2O$ (11.54 mL, 0.10 mol) was added and the reaction mixture stirred for 1 h at room temperature. The reaction was cooled to −30°C and **4** (9.0 g, 0.053 mol) was added at a rate to maintain the reaction temperature at or beneath −25°C. After stirring for 4 days at −30°C (during which time a precipitate formed), the reaction was warmed to 0°C and water (5 mL) was added (caution: foaming) followed by NaOH 50 mL of 25% aqueous NaOH). After cooling to 0°C, H_2O_2 (15 mL of 30%) was added slowly while maintaining the temperature between 0 and 10°C (caution: exothermic). After refluxing for 1 h, the reaction was cooled and diluted with ethyl acetate (300 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (100 mL). The combined organic layers were washed with water and saturated NaCl solution, dried (Na_2SO_4), and concentrated under reduced pressure. The products were isolated and purified by flash chromatography on silica gel (2×400 g) eluting first with 12.5% ethyl acetate–hexane followed by 25% ethyl acetate–hexane to afford first the α-anomer (1.00 g, 9%), $[\alpha]_D^{22}$ +112.8 (*c* 1.0, chloroform) followed by the β-anomer (0.22 g, 2%).

3.5. *2,3,6-Trideoxy-*D-erythro-*hexopyranose (**1**)*

A mixture of isobutyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (D-**5**) (37.50 mg, 0.199 mmol), H_2O (1 mL), THF (0.5 mL), and 1 M HCl (40 mg) was stirred at 50°C for 2.5 h. The reaction was treated with Ag_2CO_3 (100 mg) and after stirring for an additional 15 min, solids were removed by filtration and the solvent was removed under reduced pressure to afford D-amicetose **1** (26 mg, 98%): $[\alpha]_D^{22}$ +36.9 (*c* 1.0, acetone), lit.[6] $[\alpha]_D^{22}$ +37.2 (*c* 1.2, acetone).

3.6. *Isobutyl 4-*O*-(1-naphthyl)ethylcarbamoyl-2,3,6-trideoxy-β-*D,L-erythro-*hexopyranosides (7 and 8)*

The procedure of Greenwald was followed.[12] A 45 mL pressure tube reactor was charged with a solution of isobutyl 2,3,6-trideoxy-β-D,L-*erythro*-hexopyranoside **5** (0.2 g, 1.06 mmol) in dichloromethane (20 mL), *R*-(−)-(1-naphthyl)ethyl isocyanate (0.374 mL, 2.12 mmol) and dibutyltin dilaureate (0.63 mL, 1.06 mmol). The tube was sealed under nitrogen and stirred for 2.5 h at 60°C. After cooling, hexane (50 mL) was added followed by a solution of ethanolamine (0.64 mL) in methanol (6 mL). The mixture was diluted with dichloromethane (20 mL) and washed with water, and the organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to afford 0.4 g of crude product. The desired carbamates had nearly identical R_f values (0.54 and 0.52) in ethyl acetate:hexane (25:75 v/v) and the two by-products had R_f values of 0.41 (major) and 0.15 (minor). The crude mixture was fractionated by flash chromatography to remove the by-products with 4% ethyl acetate–hexane. The diastereomeric amicetoside carbamates were then separated by flash chromatography with a solvent gradient of ethyl acetate:hexane (7:93 to 15:85 v/v) to give 0.105 g of each carbamate (R_f=0.52 and 0.54); yield, 51% for each. The product (**8**) with R_f=0.54 had mp 113–114°C and $[\alpha]_D^{22}$ +0.94 (*c* 0.32, chloroform), and was determined to be derived from the L-amicetoside by treatment of a 4 mg sample with ethanolic sodium ethoxide (3.6 M, 10 mL, reflux 2.5 h) and analysis of the product by chiral capillary gas chromatography (Supelco β-DEX™ 390 column temperature=130°C, sample volume=1 μL, product ratio=97.3:2.7, enantiomeric excess=94.6%): ^{1}H NMR (200 MHz) δ 0.89 (d, 3H, J=6.6 Hz, CH_3), 0.895 (d, 3H, J=6.6 Hz, CH_3), 1.28 (d, 3H, $J_{5,6}$=6.3 Hz, H-6), 1.65 (d, 3H, J=6.4 Hz, CH_3), 2.22–1.74 (m, 5H, CH, CH_2), 3.13 (bdd,

Table 1

conditions	Chrompack Chirasil ™L-val fused silica column (25m x 0.25mm)	Supelco β-DEX™ 390 fused silica column (30m x 0.25mm)
injector T (°C)	200	220
FID detector T (°C)	250	300
column T (°C)	90	130
carrier gas (column pressure, psi)	25	25
auxillary gas (He,psi)	45	30
hydrogen (psi)	23	23
sample solvent	ethyl acetate	ethyl acetate
sample size	1 μL	1 μL
sample concentration	1.5 mg/mL	1.5 mg/mL

1H), 3.41 (m, 1H, H-5), 3.64 (bdd, 1H), 4.40 (m, 1H, H-4), 4.97 (m, 1H, $J_{1,2a}$=7.6 Hz, H-1), 5.66 (m, 1H), 7.90–7.40 (m, 10 H, Ph–H), 8.11 (d, 1H, NH); ^{13}C NMR (50.3 MHz) δ 18.05, (C-6), 19.16, 19.28, 21.50, 29.93, 28.32, 27.53, 46.43, 73.28 (C-5), 73.62 (C-4), 75.77, 101.60 (C-1), 133.8–122.0 (Ph), 154.86 (C=O). The product (7) with R_f=0.52 had mp 117–118°C and $[\alpha]_D^{22}$ +8.1 (*c* 0.26, chloroform), and was determined to be derived from the D-amicetoside by the same method; product ratio=95.9:4.1, enantiomeric excess=91.8%: ^{1}H NMR (200 MHz) δ 0.89 (d, 3H, J=6.7 Hz, CH_3), 0.90 (d, 3H, J=6.7 Hz, CH_3), 1.20 (d, 3H, $J_{5,6}$=6.2 Hz, H-6), 1.66 (d, 3H, J=6.8 Hz, CH_3), 2.67–1.77 (m, 5H, CH, CH_2), 3.13 (dd, 1H, J=9.3, 7.3 Hz), 3.37 (dq, 1H, $J_{4,5}$=8.7 Hz, $J_{5,6}$=6.2 Hz, H-5), 3.65 (dd, 1H, J=9.3, 6.4 Hz), 4.41 (m, 1H, H-4), 4.95 (m, 1H, $J_{1,2a}$=7.4 Hz, H-1), 5.64 (m, 1H), 7.90–7.40 (m, 10H, Ph–H), 8.10 (d, 1H, J=7.2 Hz, NH); ^{13}C NMR (50.3 MHz) δ 17.96 (C-6), 19.15, 19.28, 21.34, 29.98, 28.33, 27.62, 46.52, 73.06 (C-5), 73.37 (C-4), 75.76, 101.59 (C-1), 133.8–122.0 (Ph), 154.80 (C=O). Anal. Calcd for $C_{23}H_{31}NO_4$: C, 71.66; H, 8.11; N, 3.65. Found: C, 71.47; H, 8.02; N, 3.57.

3.7. Enantiomeric analysis of amicetosides and amicetoside esters by capillary gas chromatography

Determinations of enantiomeric enrichments of the isobutyl α- and β-D,L-amicetosides and their acetate and butyrate esters were carried out on a Hewlett–Packard 5790A capillary gas chromatograph, equipped with an HP 3392A integrator, a split/splitless injector (split with packing, ratio 100:1), and a flame ionization detector. Conditions for the two columns used are given in Table 1.

Analyses of isobutyl amicetosides and acetate esters were conducted isothermally. Butyrate esters were injected at initial column temperatures equal to those in the isothermal analyses, and the temperature was then increased at a rate of 5°C per minute to final column temperatures of 110°C (Chirasil column) or 145°C (β-DEX 390 column).

3.8. General procedure for lipase-catalyzed transesterification of isobutyl β-D,L-amicetoside with vinyl acetate or vinyl butyrate on an analytical scale

Racemic isobutyl β-D,L-amicetoside (0.030 g, 0.159 mmol) was dissolved in vinyl acetate or vinyl butyrate (2 mL) in a 4-dram screwcapped vial. In runs containing solvent, the starting material was dissolved in 2 mL of a mixture containing acylating agent (1 mL) and tetrahydrofuran. Lipase (0.40 g of Amano PS-30, PPL, or CCL) was added and the vial was sealed tightly with a cap and a Teflon-coated septum. The reaction was placed in a Brunswick environment-controlled shaker at 45°C and shaken at

265 rpm. Aliquots of 0.10 mL were taken at prescribed intervals and diluted with ethyl acetate (0.9 mL) in a 2 cc syringe fitted with a Gelman Sciences Acro LC 35 HPLC filter (0.45 μ). The suspension was passed through the filter to remove the enzyme and the filtrate was analyzed by capillary GC as described above. After analyses were completed, the remaining reaction mixture was filtered through a pad of Celite using a fritted glass funnel and the solid was washed with ethyl acetate several times. Concentration of the filtrate gave a crude mixture which was separated by flash chromatography on a 1×6 cm column (10% ethyl acetate–hexane) to give enriched alcohol and ester.

3.9. General procedure for lipase-catalyzed transesterification of isobutyl β-D,L-amicetoside with vinyl acetate or vinyl butyrate on a mmol scale

Racemic isobutyl β-D,L-amicetoside (0.188 g, 1 mmol) was dissolved in vinyl acetate or vinyl butyrate (13 mL) in a 1 oz. screwcapped bottle. In runs containing solvent, the starting material was dissolved in 13 mL of a mixture containing acylating agent (6.5 mL) and tetrahydrofuran (6.5 mL). Lipase (2.5 g of Amano PS-30, PPL, or CCL) was added and the vial was sealed tightly with a cap covered with Teflon tape and Parafilm M. The reaction was placed in a Brunswick environment-controlled shaker at 45°C and shaken at 265 rpm. After this time period had elapsed, the reaction mixture was filtered through a pad of Celite in a sintered glass funnel and the solid cake was washed with ethyl acetate. Concentration of the filtrate gave a crude mixture which was separated by flash chromatography on a 2×10 cm column and 10% ethyl acetate–hexane to give first the enantioenriched isobutyl β-L-amicetoside ester followed by enantioenriched isobutyl β-D-amicetoside $[\alpha]_D^{22}$ −32.3 (*c* 2.3, chloroform).

Results are shown in Fig. 3.

3.10. General procedure for ester cleavage

To a mixture of the desired ester (acetate or butyrate, 0.125 mmol) obtained from the transesterification and anhydrous methanol (3 mL) was added freshly prepared 1 M sodium methoxide solution (100 μL) The reaction was stirred overnight at room temperature and neutralized with Amberlite IRC-50H resin. The resin was removed by filtration through a plug of glass wool and washed with ethyl acetate. Solvent was removed under reduced pressure and the product was analyzed by capillary GC using the Chrompack Chirasil L-Val column without further purification.

3.11. Isobutyl 4-O-acetyl-2,3,6-trideoxy-α-D,L-erythro-hexopyranoside 9 (R=Me)

To a solution of racemic **5** (0.150 g, 0.797 mmol) in dichloromethane (0.3 mL) and pyridine (0.36 mL) at 0°C was added a solution of acetic anhydride (0.162 g, 1.6 mmol, 0.15 mL) in dicholormethane (0.3 mL) and the reaction mixture was stirred for 24 h. Dichloromethane (3 mL) was added and the mixture was washed with 1 M HCl, water, saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography on Florisil with 7% ethyl acetate–petroleum ether to afford 0.1295 g (71%) racemic ester **9** (R=Me): R_f=0.72 (20% ethyl acetate–hexanes); $[\alpha]_D^{22}$ −9.4 (*c* 0.66, chloroform); ^{1}H NMR (200 MHz) δ 0.89 (d, 3H, CH_3), 0.93 (d, 3H, CH_3), 1.22 (d, 3H, H-6), 1.26–2.18 (m, 4H, H-2, H-3), 2.05 (s, 3H, CH_3CO), 3.16 (dd, 1H, OCH_2), 3.45–3.53 (m, 2H, H-4, H-5), 3.67 (dd, 1H, OCH_2), 4.44 (dd, 1H, H-1); ^{13}C NMR δ 17.31 (C-6), 18.49 (CH_3), 18.62 (CH_3), 20.38 (CH_3), 26.56 (C-2), 27.68 (CH), 29.29 (C-3), 72.21 (C-5), 72.35 (C-4), 75.13 (CH_2), 100.98 (C-1), 169.47 (CO). Anal. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.82; H, 9.65.

3.12. *Isobutyl 4-O-butyryl-2,3,6-trideoxy-α-D,L-erythro-hexopyranoside 9 (R=n-Bu)*

To a solution of racemic **5** (0.298 g, 1.5 mmol) in dichloromethane (0.6 mL) and pyridine (0.72 mL) at 0°C was added a solution of butyryl chloride (0.339 g, 3.2 mmol, 0.33 mL) in dicholormethane (0.6 mL) and the reaction mixture was stirred for 24 h. Dichloromethane (5 mL) was added and the mixture was washed with 1 M HCl, water, saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography on Florisil with 7% ethyl acetate–hexanes or on silica gel with 10% ethyl acetate–hexanes to afford 0.274 g (69%) racemic ester **9** (R=*n*-Bu): R_f=0.74 (20% ethyl acetate–hexanes; $[\alpha]_D^{22}$ −35.0 (*c* 0.2, chloroform); ^{1}H NMR (200 MHz) δ 0.89 (d, 3H, CH_3), 0.93 (d, 3H, CH_3), 0.95 (t, 3H, CH_3), 1.22 (d, 3H, H-6), 1.26–2.19 (m, 3H, CH, CH_2), 1.56–1.93 (m, 4H, H-2, H-3), 2.27 (t, 2H, CH_2), 3.16 (dd, 1H, OCH_2), 3.43–3.56 (m, 2H, H-4, H-5), 3.67 (dd, 1H, OCH_2), 4.44 (dd, 1H, H-1); ^{13}C NMR δ 13.28 (CH_2), 17.78 (C-6), 18.17 (CH_3), 19.07 (CH_3), 19.95 (CH_3), 27.111 (C-2), 28.16 (CH), 29.81 (C-3), 36.04 (CH_2), 72.37 (C-5), 72.85 (C-4), 75.55 (CH_2), 101.46 (C-1), 172.48 (CO). Anal. Calcd for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14. Found: C, 65.26; H, 10.24.

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