Stereoselective Synthesis of Carbon-Linked Analogues of α- and β-Galactoserine Glycoconjugates Using Asymmetric Enolate Methodology[1]

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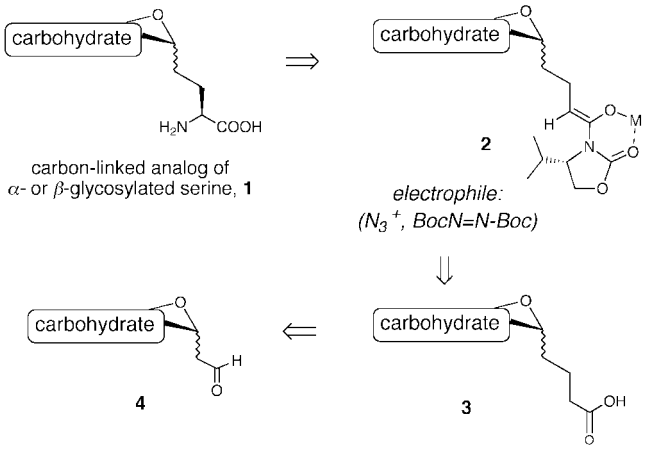
Received January 8, 1998

In recent years, glycopeptides have become the focus of considerable bioorganic/medicinal research due to their involvement in various cellular biological and pathological processes. Glycosylation of proteins not only affects their physical properties (i.e., folding and conformation) but also influences biological function.[3] Since glycosides anchored to proteins form highly branched structures, it is not surprising that they are involved in cell surface recognition processes such as interactions with bacteria, viruses, and toxins as well as tumor metastasis.[4] Glycosylation has also been implicated in the modulation of protein function (i.e., cellular uptake, proteolytic stability) and immune responses.[5]

Oxygen-linked glycosylation (i.e., glycosylation of serine and threonine amino acid moieties) is one of the primary modes for the attachment of glycosides to proteins. However, a fundamental problem in using *O*-glycoconjugate-based therapeutic approaches to the treatment of disease is the inherent lack of in vivo stability of such compounds since native *O*-glycopeptides are easily degraded in both acidic and basic media.[3] A solution to this problem lies in obtaining carbon-linked isosteric derivatives of *O*-glycopeptides.[6] Biologically stable carbon-linked glycopeptides may have applications in the areas of cancer research, immunotherapy, and treatment of inflammatory responses as well as various infection and pathogenic processes.

In 1992, Bednarski et al. reported the first synthesis of carbon-linked β-galactoside serine.[6a] A key step in their approach was the coupling of *C*-glycosyl aldehyde with the chiral Wittig reagent which was obtained from (*S*)-serine in a number of steps. Following Bednarski's approach, a catalytic asymmetric hydrogenation methodology for the synthesis of *C*-glycosyl amino acid derivatives was recently achieved by Toone et al.[7] As an alternative to the previously reported approaches, herein, we describe a novel strategy involving the application of well-established asymmetric enolate methodology for the synthesis of carbon-linked isosteres of α- or β-glycoconjugates bearing the amino acid serine. The key step in our approach involved the reaction of a chiral oxazolidinone enolate with an electrophilic azide source or electrophilic amination using a dialkyl azodicarboxyl ester derivative (Scheme 1: retrosynthetic analysis, **1**−**4**).[8] In either instance, reduction of the resulting α-azido ester or the fundamental functional group manipulation of the hydrazide derivative would furnish the desired C-linked galactoserine. To our knowledge, a chiral auxiliary-based enolate strategy for the synthesis of carbon-linked glycosylated amino acids has never been explored. One significant advantage of this methodology is that it could easily be extended to a variety of electrophiles which allows for the synthesis of other analogues of *C*-glycosyl derivatives. To demonstrate the utility of this approach, we have chosen to develop the methodology using D-galactose.

Scheme 1. Retrosynthetic Analysis



As illustrated in Scheme 2, a two-carbon homologation of α-*C*-galactose derivative **5**, using commercially available (carbethoxymethylene)triphenylphosphorane, produced a mixture of geometric olefin isomers, which upon hydrogenation using Pd/C as a catalyst furnished **6**. After conversion of the acetate protecting groups to benzyl ethers on galactose moiety, the free carboxylic acid derivative was then coupled to (4*S*)-4-isopropyl-2-oxazolidinone to furnish **7** in 55% isolated yield. Surprisingly, reaction of the potassium enolate derived from **7** with trisyl azide failed to produce the expected α-azido product.[9] In contrast, di-*tert*-butyl azodicarboxylate (DBAD) underwent reaction with the enolate of **7** to give the

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(1) (a) NRCC Publication No. 40865. (b) Presented at the 15th American Peptide Symposium, Nashville, TN June 14−19, 1997.

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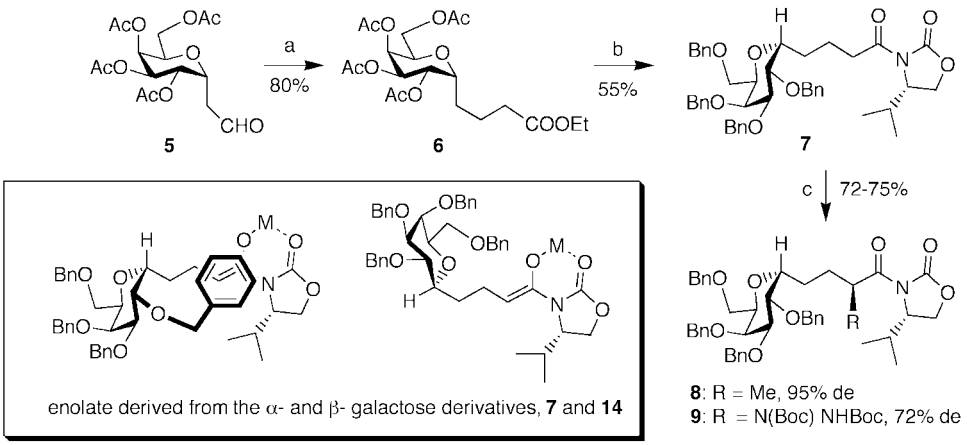
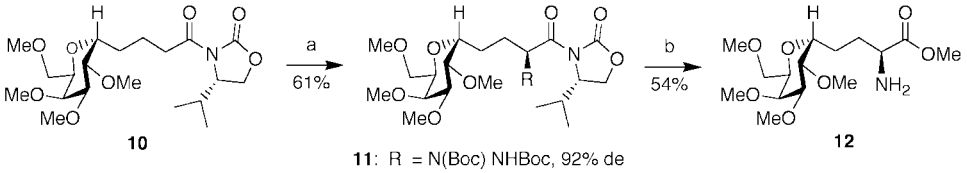
S0022-3263(98)00026-7 CCC: $15.00
Published on Web 06/23/1998

Scheme 2[a]



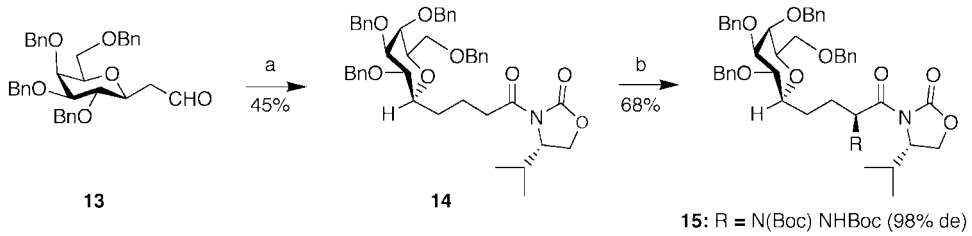
[a] Reagents and conditions: (a) (i) $Ph_3P{=}CHCOOEt$, benzene, reflux, (ii) H_2, 10% Pd/C, EtOH; (b) (i) 1 M LiOH, MeOH/H_2O, (ii) *p*-TSA, MeOH, (iii) NaH, BnBr, Bu_4NI, 15-crown-5, DMF, (iv) 1 M LiOH, H_2O/THF, (v) 2,4,6-trichlorobenzoyl chloride, Et_3N, CH_2Cl_2, for 30 min then lithio-*(S)*-isopropyl-2-oxazolidinone, THF; (c) KHMDS (1.1 equiv), THF, −78 °C, 30 min, MeI (5 equiv) or DBAD (1.3 equiv).

Scheme 3[a]



[a] Reagents and conditions: (a) LDA, THF, −78 °C, 30 min, DBAD (1.3 equiv); (b) (i) 2.5 M LiOH, THF/H_2O, 0 °C, (ii) CH_2N_2, THF, (iii) TFA/CH_2Cl_2 (1:1), (iv) Raney-Ni, H_2, 500 psi.

Scheme 4[a]



[a] Reagents and conditions: (a) (i) $Ph_3P{=}CHCOOEt$, benzene, reflux; (ii) H_2, 10% Pd/C, EtOH, (iii) 1 M LiOH, MeOH/H_2O, (iv) 2,4,6-trichlorobenzoyl chloride, Et_3N, CH_2Cl_2, for 30 min then lithio-(*S*)-isopropyl-2-oxazolidinone, THF; (b) LDA (1.1 equiv), THF, −78 °C, 30 min, DBAD (1.3 equiv).

hydrazide product **9**, but the selectivity of the reaction using DBAD was not very high (72% de). On the basis of literature precedents of similar reactions, the observed low diastereoselectivity was quite surprising.[8] In view of the experimental results, *it seemed probable that the benzyl protecting group at the 2-position of the galactose moiety hindered the approach of the electrophile (see enolate derived from **7**, Scheme 2).* In an effort to enhance the diastereoselectivy of the electrophilic amination reaction, the benzyl group at the 2-position was replaced by the less sterically demanding methyl group to give the permethylated derivative **10**, which was synthesized from **6** (Scheme 3). As anticipated, reaction of the enolate of **10** (generated under standard conditions, LDA, THF, −78 °C) with DBAD furnished **11** with a high diastereoselectivity (92% de).[10] Compound **11** was subjected to reaction with 2.5 M LiOH, esterification with diazomethane, and treatment with TFA to affect the removal of the Boc group. In situ reduction of the hydrazine derivative with Raney-Ni under a hydrogen atmosphere then furnished the desired product **12**.

In a separate attempt to investigate the relative orientation of the enolate and attendant benzyloxy group at the 2-position of the galactose moiety, the perbenzylated derivative of *β*-galactose **14** was synthesized from **13**[11] in a number of steps: (i) two-carbon homologation by coupling with the (carbethoxymethylene)triphenylphosphorane as described earlier for α-galactose derivative **5**, (ii) hydrogenation using Pd/C, (iii) hydrolysis of the carboxyl ester group to obtain free carboxyl acid, and (iv) coupling of the (4*S*)-4-isopropyl-2-oxazolidinone to furnish **14** in 45% isolated yield (Scheme 4). It seemed likely that with the *β*-configuration at the anomeric

(9) The enolate of **7** with methyl iodide gave **8** in excellent yield with a high diastereoselectivity (>95% de).

(10) Diastereomeric excess (% de) was measured using HPLC and HPLC−MS techniques. Structures of all the compounds were fully assigned using ^1H and ^{13}C NMR and MS experiments. In compounds **8**, **11**, and **15**, the stereochemistry of new stereogenic centers was assigned as it would be expected from Evans' asymmetric oxazolidinone enolate methodology.

center, the benzyl group at the 2-position of the saccharide moiety of **14** would be unable to interfere during the reaction of the enolate with di-*tert*-butyl azodicarboxylate in contrast to α-galactose derivative **7** (see Scheme 2 for enolates derived from α- and *β*-galactose derivatives, **7** and **14**). As expected, reaction of the enolate derived from **14** with DBAD gave the expected product **15**, with excellent diastereoselectivity (98% de).

In conclusion, we have successfully demonstrated that chiral auxiliary-based enolate methodology can be utilized to synthesize carbon-linked analogues of both α- and *β*-galactoserine glycoconjugates which are of tremendous biological and medicinal importance. The effect of the saccharide moiety as a remote chiral group on the selectivity of the electrophilic amination is unprecedented. Although the mechanism by which the benzyl protecting group at the 2-position of the α-galactose moiety interferes with the enolate is not clear at this stage, it has the ability to modulate the selectivity of the electrophilic amination reaction. Further work is required to explore and exploit the full potential of this surprising observation. We are currently extending the enolate methodology and investigating other applications of these *C*-linked glycoconjugates.

Experimental Section

General Remarks. Proton NMR spectra were recorded using a Bruker AM-200 or an AMX 400 MHz instrument using $CDCl_3$ as solvent. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Multiplicities are reported as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded using a JEOL JMS-AX 505H mass spectrometer using mNBA as a matrix.

All reactions were carried out under a nitrogen atmosphere using oven-dried glassware, and unless otherwise stated, typical extractive workup procedures were applied in all cases. Chromatographic separations were performed using Merck grade 60 silica gel (230−400 mesh, 60A) in the solvent systems specified, and all yields refer to isolated yields after purification. All solvents were dried prior to use.

(4*S*)-4-Isopropyl-3-[1-oxo-4-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)butyl]-2-oxazolidinone (7). In an oven-dried round-bottom flask under nitrogen the perbenzylated carboxylic acid (188 mg, 0.31 mmol) was placed in 20 mL of THF and cooled to −78 °C. Then diisopropylethylamine (0.06 mL, 1.1 equiv) and 2,4,6-trichlorobenzoyl chloride (0.05 mL, 1.1 equiv) were added. After stirring for 1 h the lithio-(*S*)-isopropyloxazolidinone was added via cannula and the solution was warmed to room temperature over a 2-h period. Typical aqueous workup and extraction with diethyl ether followed by treatment with magnesium sulfate and removal of the solvent under reduced pressure produced a yellow oil. After purification by column chromatography using hexanes−ethyl acetate (2:1) as an elutant, 160 mg (72% yield) of **7** was obtained as a pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 0.85 (q, 6H, *J* = 8, 13 Hz), 1.70 (m, 5H), 2.35 (m, 1H), 2.95 (s, 2H), 3.90−3.50 (m, 3H), 3.95 (s, 2H), 4.30−4.10 (m, 2H), 4.80−4.40 (m, 10H), 7.25 (m, 20H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 18.0, 21.0, 23.2, 25.1, 26.0, 39.5, 63.2, 64.0, 70.0, 76.3, 79.1, 80.5, 81.3, 131.3, 132.1, 133.1, 143.0, 143.2, 157.0, 177.6. MS (ES^+): *m*/*z* (relative intensity) 722.2 (40). HRMS: calcd for $C_{44}H_{51}NO_8$ (M+) 722.3695, found 722.3767.

(4*S*)-4-Isopropyl-3-[2-[*N*-(*N*-butyloxycarbamido]butyloxycarbamido-1-oxo-4-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)butyl]-2-oxazolidinone (9). Compound **7** (55 mg, 0.08 mmol) was placed in a flame-dried round-bottom flask with 25 mL of dry THF under a nitrogen atmosphere. This solution was then cooled to −78 °C, and LDA (1.1 equiv) was added. After 45 min a THF solution of DBAD (22 mg, 1.2 equiv) was added, and the solution was stirred for 5 min after which time glacial acetic acid (0.01 mL, 2.6 equiv) was added and the solution was allowed to warm to room temperature overnight. Extractive workup furnished a crude yellow oil which was purified using hexanes−ethyl acetate (3:1) as an elutant to give 55 mg (72% yield) of **9** as a mixture of diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 0.80 (d, 6H, *J* = 13 Hz), 1.50−1.31 (m, 18H), 1.60 (br s, 1H), 1.80 (br s, 1H), 2.20 (br s, 2H), 3.40 (br s, 1H), 3.80−3.61 (m, 3H), 3.90 (s, 1H), 4.10−3.95 (m, 5H), 4.44 (d, 1H, *J* = 14 Hz), 4.60−4.50 (m, 3H), 4.85−4.65 (m, 4H), 5.71 (br s, 1H), 6.74 (br s, 1H), 7.42−7.10 (m, 20H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 19.2, 21.2, 27.8, 27.9, 28.0, 40.2, 64.0, 73.1, 78.2, 79.1, 80.5, 82.2, 131.8, 132.5, 132.8, 131.0, 144.0, 143.2, 148.3, 150.2, 154.2, 154.8, 154.9, 170.2. MS (ES^+): *m*/*z* (relative intensity) 952.0 (50), 463.5 (28), 346.0 (37), 332 (71), 233 (100).

(4*S*)-4-Isopropyl-3-[1-oxo-4-(2,3,4,6-tetra-*O*-methyl-α-D-galactopyranosyl)butyl]-2-oxazolidinone (10). For a general experimental procedure, refer to **7**. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 0.90 (t, 6H, *J* = 8.6 Hz), 1.90−1.51 (m, 5H), 2.42 (m, 2H), 2.94 (m, 3H), 3.73−3.19 (m, 14H), 3.82 (m, 1H), 4.05 (m, 1H), 4.28 (m, 2H), 4.40 (m, 1H). ^{13}C NMR (50 MHz, CDCl3) *δ*: 32.9, 36.2, 39.0, 43.7, 46.6, 53.5, 76.9, 78.3, 81.6, 88.4, 89.2, 89.9, 93.8, 94.7, 95.3, 95.9, 96.1, 97.2, 172.3, 191.3. MS (ES+): *m*/*z* (relative intensity) 418.2 (100): HRMS calcd for $C_{20}H_{35}NO_8$ (M^+) 417.2362, found 417.2365.

(4*S*)-4-Isopropyl-3-[2-[*N*-(*N*-butyloxycarbamido)butyloxycarbamido]-1-oxo-4-(2,3,4,6-tetra-*O*-methyl-α-D-galactopyranosyl)butyl]-2-oxazolidinone (11). For a general experimental procedure, refer to **9**. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 0.80 (d, 3H, *J* = 10.0 Hz), 0.84 (d, 3H, *J* = 10 Hz), 1.40 (s, 18H), 1.82 (m, 2H), 1.86 (br s, 1H), 2.30 (br s, 2H), 3.60−3.20 (m, 18H), 3.82 (br s, 1H), 3.90 (br s, 1H), 3.95 (q, 1H, *J* = 8.0, 3.1 Hz), 4.10 (m, 2H), 5.38 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 14.5, 17.4, 17.7, 20.6, 23.2, 25.6, 27.9, 32.4, 58.1, 58.7, 60.1, 63.7, 68.3, 70.7, 72.4, 75.8, 78.7, 80.2, 81.6, 153.1, 154.2, 155.7, 160.1. HRMS: calcd for $C_{30}H_{54}N_3O_{12}$ (M^+) 648.3709, found 648.3701.

Methyl 2-Amino-4-(2,3,4,6-tetra-*O*-methyl-α-D-galactopyranosyl)butanate (12). Compound **11** (32 mg, 0.06 mmol) was placed in a round-bottom flask, 8 mL of a TFA−CH_2Cl_2 (1:1) mixture was added, and the reaction was stirred at room temperature. After 1 h Raney-Ni (300 mg, damp weight) was added, and the reaction flask was pressurized (550 psi) with hydrogen gas. After 3 h the solution was filtered through Celite, and the solvent was removed to produce a pale-green oil which was purified by column chromatography to give **12** (10 mg, 54% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.80 (m, 3H), 2.30 (t, 2H, *J* = 5.0 Hz), 2.9 (s, 2H), 3.50−3.31 (m, *J* = 16 Hz), 3.70 (s, 3H), 4.12 (m, 1H), 4.52 (t, 1H, *J* = 6.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 17.7, 18.2, 21.5, 32.8, 33.9, 51.7, 53.2, 59.3, 68.7, 70.2, 71.3, 75.8, 76.4, 128.1, 170.2. MS (ES^+): *m*/*z*: 335 [M+].

2-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl)acetaldehyde (13). For a general experimental procedure describing the preparation of **13** see ref 11. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 2.33 (m, 1H), 2.61 (m, 1H), 3.30 (td, 1H, *J* = 8.0, 9.1 Hz), 3.54 (m, 1H), 3.71 (t, 1H, *J* = 8.0 Hz), 3.98 (d, 1H, *J* = 2.6 Hz), 4.45 (q, 2H, *J* = 10.1, 11.2 Hz), 4.68 (m, 2H), 4.74 (d, 2H, *J* = 11.2 Hz), 4.93 (d, 2H, *J* = 9.0 Hz), 5.06 (m, 2H), 5.91 (m, 1H), 7.30 (m 20H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 36.9, 69.2, 72.8, 73.4, 73.6, 76.1, 76.9, 77.2, 78.1, 79.3, 85.3, 117.3, 128.3, 128.4, 128.7, 128.9, 130.1, 130.3, 132.4, 132.8, 136.2, 138.1, 138.4, 139.0, 139.2.

(4*S*)-4-Isopropyl-3-[1-oxo-4-(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)butyl]-2-oxazolidinone (14). For a general experimental procedure, see **7** and **10**. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.80 (m, 3H), 2.30 (t, 2H, *J* = 5.0 Hz), 2.9 (s, 2H), 3.50−3.31 (m, *J* = 16 Hz), 3.70 (s, 3H), 4.12 (m, 1H), 4.52 (t, 1H, *J* = 6.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 17.7, 18.2, 21.5, 32.8, 33.9, 51.7, 53.2, 59.3, 68.7, 70.2, 71.3, 75.8, 76.4, 128.1, 170.2. MS (ES^+): *m*/*z*: 335 [M+].

(4*S*)-4-Isopropyl-3-[2-[*N*(*N*-butyloxycarbamido)butyloxycarbamido]-1-oxo-4-(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)butyl]-2-oxazolidinone (15). For a general ex-

(11) Synthesis of **13** was achieved from 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride by the reaction with allylmagnesium bromide (0 °C), followed by ozonolysis and the reductive opening of the ozonide. For a similar series of reactions, see: Uchiyama, T.; Woltering, T. J.; Wong, W.; Lin, C.-C.; Kajimoto, T.; Takebayashi, M.; Weitz-Schmidt, G.; Asakura, T.; Noda, M.; Wong, C.-H. *Bioorg. Med. Chem.* **1996**, *4* (7), 1149−1165.

perimental procedure, see **9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (br s, 5H), 1.50−1.05 (m, 19H), 1.84−1.60 (m, 3H), 2.10 (br s, 2H), 2.32 (br s, 1H), 3.22 (br s, 1H), 3.52 (m, 6H), 4.20−3.98 (m, 4H), 4.72−4.42 (m, 6H), 4.95 (m, 2H), 7.43 (m, 20H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 15.0, 18.3, 28.4, 30.1, 59.3, 63.8, 72.5, 73.8, 74.4, 74.9, 75.7, 79.8, 80.8, 81.6, 82.0, 85.0, 127.9, 128.2, 128.5, 128.7, 138.6, 138.9, 139.4, 153.5, 155.0, 155.4, 156.1, 175.0. FAB-MS: *m*/*z* (relative intensity) 974.4 (70), 874.4 (30), 774.3 (20), 617.3 (15). HRMS: calcd for $C_{54}H_{69}O_{12}N_3Na$ ($M^+ + Na$) 974.4778, found 974.4788.

Acknowledgment. Mr. Don Leek and Ms. Malgosia Daroszewska are thanked for providing technical assistance for running NMR and MS.

Supporting Information Available: Spectral data of compounds (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO980026X