Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 11, pp. 1209-1219, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00094-8

Support Studies for Installing the Phosphodiester Residues of the Thy-1 Glycoprotein Membrane Anchor

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Abstract—Support studies for late-stage installation of the three different types of phosphodiesters found in the rat brain Thy-1 glycoprotein membrane anchor are described. The strategy is geared towards optimizing convergency and the development of chemoselective procedures including deprotection, phosphorylation, esterification and cysteinylation has been investigated. Some of these procedures are being designed for oligosaccharides containing several unprotected hydroxy groups.

Introduction

There is currently considerable interest in the recently discovered group of glycoconjugates which differ from the usual cellular glycoproteins in that the protein is attached to the cell membrane surface via a glycophosphatidyl-inositol anchor.[1-3] Only two of these structures have been fully characterized thus far, the first being the variant surface glycoprotein (VSG) anchor from *Trypanosoma brucei*[2] **1**, and the Thy-1 glycoprotein anchor from rat brain[3] **2a**. Their representations in Scheme I, combined with scattered information about other membrane anchors currently under investigation, indicate that there is considerable structural homology in this class of compounds. That such homology should be found in species as widely different as single-cell eukaryotes and vertebrates is of immense biological importance and prompts our interest in these systems.

Compounds **1** and **2a** share a *pseudo* pentasaccharide core as highlighted in Scheme I. The core was first synthesized in this laboratory[4] and subsequent reports from the laboratories of Ogawa[5] and van Boom[6] have provided larger segments of, and alternative approaches to **1**, although the entire glycan moiety has not yielded to total synthesis.

Our attention has turned towards the Thy-1 glycoprotein anchor **2a**, being the first isolated from a mammalian source, and we recently reported the synthesis of the heptasaccharide **5**[7] (Scheme I). Compound **2a** possesses different phosphodiester groups at the sites indicated A, B, and C, and our aim is to phosphorylate the oligosaccharide as late into the synthesis as possible, some pertinent operations being carried out on partially protected material. This plan presents unique challenges for chemo- and regioselective control in such derivatizations, and in this manuscript we describe some exploratory studies that relate this objective.

Results and Discussion

Scheme I gives a retrosynthetic analysis for synthesis of **2a**, the sites for the three phosphodiester units being differentiated in the synthetic heptasaccharide **5** so as to facilitate selective introduction of each. In the interest of convergency it would be most efficient to attach each of these phosphodiester units as fully formed as possible so as to minimize handling of the polar products.

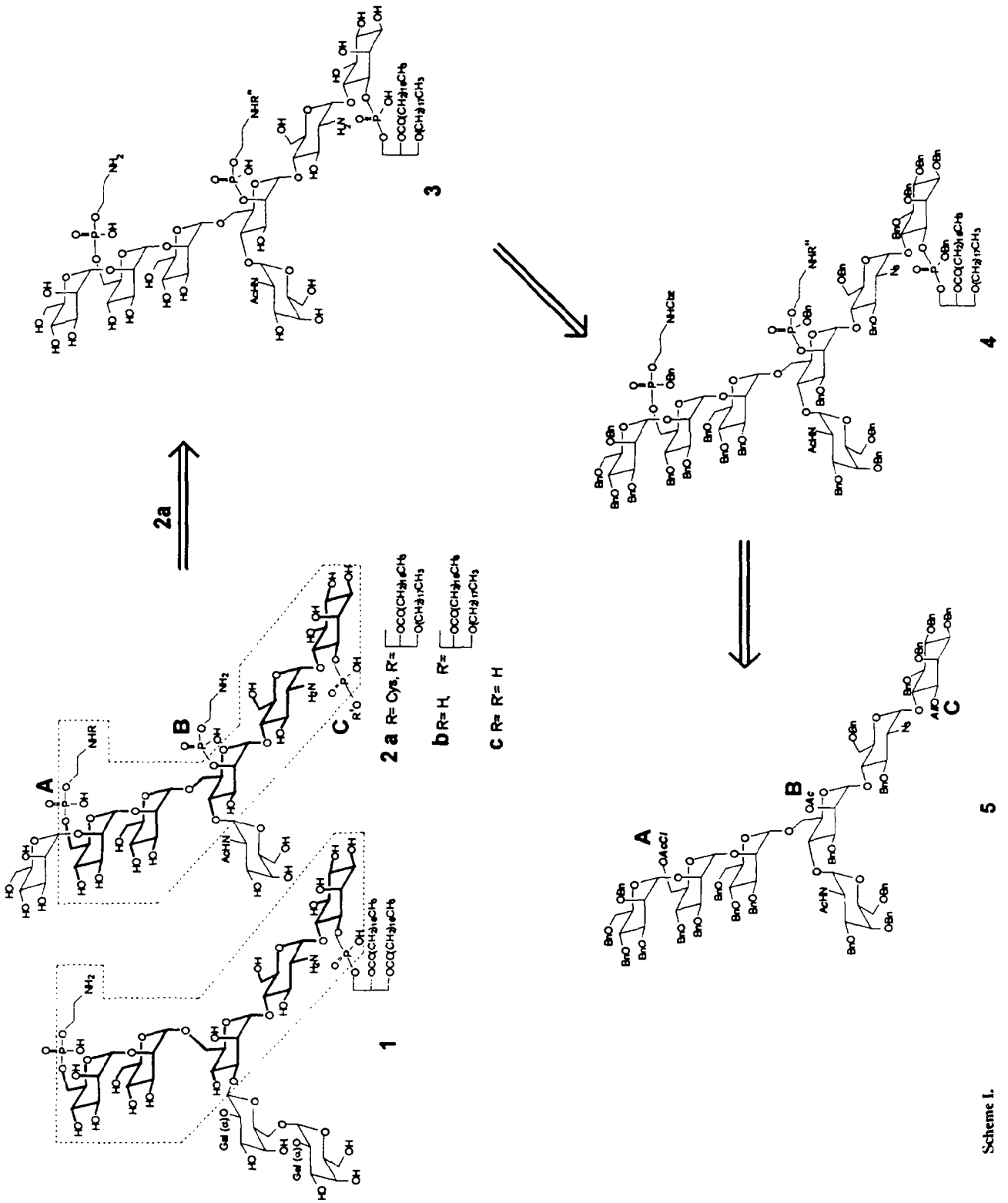
In view of the wealth of experience in several laboratories, including our own, the phosphoramidite protocol was chosen as the method for phosphorylation, since precedents show that a wide range of hydroxyl groups, hindered and reactive, can be derivatized under mild conditions using tetrazole as an acid catalyst.[8]

A variety of N-protected ethanolamines **6a–6c** was therefore prepared under standard conditions[9] (Scheme IIa) and converted to the corresponding phosphoramidites **7a–7c**.

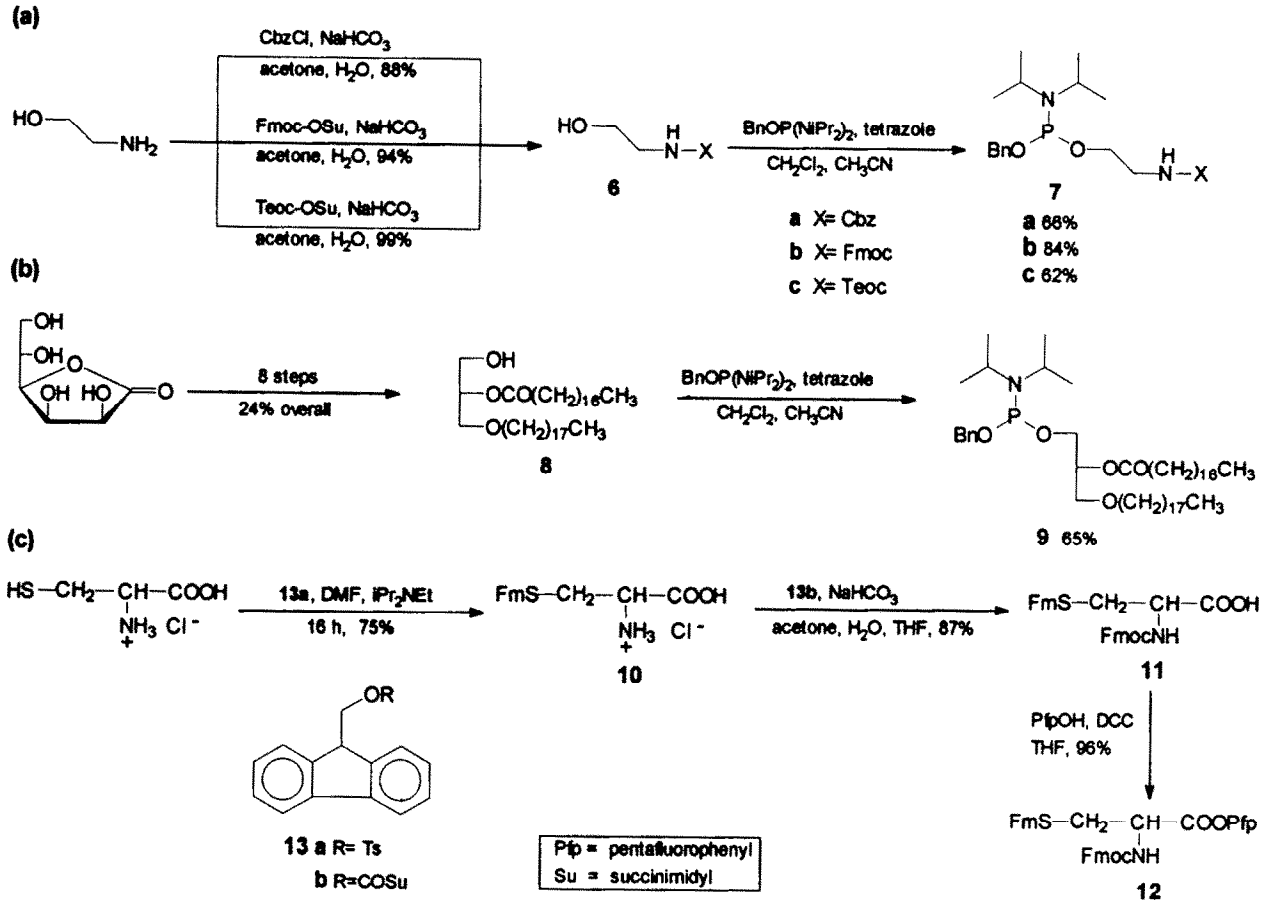
L-Gulonolactone was processed to give the D-glycerol derivative **8** (Scheme IIb) in eight steps by adaptation of known procedures.[10] The phosphoramidite **9** was then prepared.[8g]

With respect to sites A and B of **5**, the phosphoramidite reagents would be differentiated on the primary amine to allow for future coupling of the cysteinyl residue. The timing of this coupling is critical with regard to the requirement for selective liberation of the amino group at site A. Use of a carbobenzyloxy (Cbz) group is an interesting option since it would be removed at the same time as the sugars' benzyl ethers. Our hope would be that the resulting primary amine could be cysteinylated chemospecifically, without affect from the many free hydroxyl groups or the glucosamine nitrogen.

The cysteine residue is a major cause of concern, since removal of the benzyl protecting groups *after* its attachment, either by hydrogenolysis or oxidation would be problematic because catalyst poisoning, cleavage of the sulfur[11] or oxidation to a disulfide[12] could occur. In order to minimize these problems, the benzyl groups would therefore have to be removed *prior* to connecting the suitably protected cysteine residue. The success of this



Scheme 1.



Scheme II.

strategy relies on the higher nucleophilicity of a primary amine *vis-à-vis* primary and secondary hydroxyl groups, and the higher accessibility of the ethanolamine N versus the glucosamine N, in order to allow chemospecific introduction of the cysteine.

The preparation of a suitably protected cysteine was therefore undertaken (Scheme IIc). In order to minimize future deprotections, it seemed ideal that the protecting groups used for amine and sulfur should be removable simultaneously under conditions that did not challenge the rest of the molecule. The 9-fluorenylmethyl (Fm)- based protecting group seemed to answer this need since its derivatives, with a primary amine[13] or a thiol,[14] can be cleaved mildly and efficiently by use of a secondary amine.[13–15]

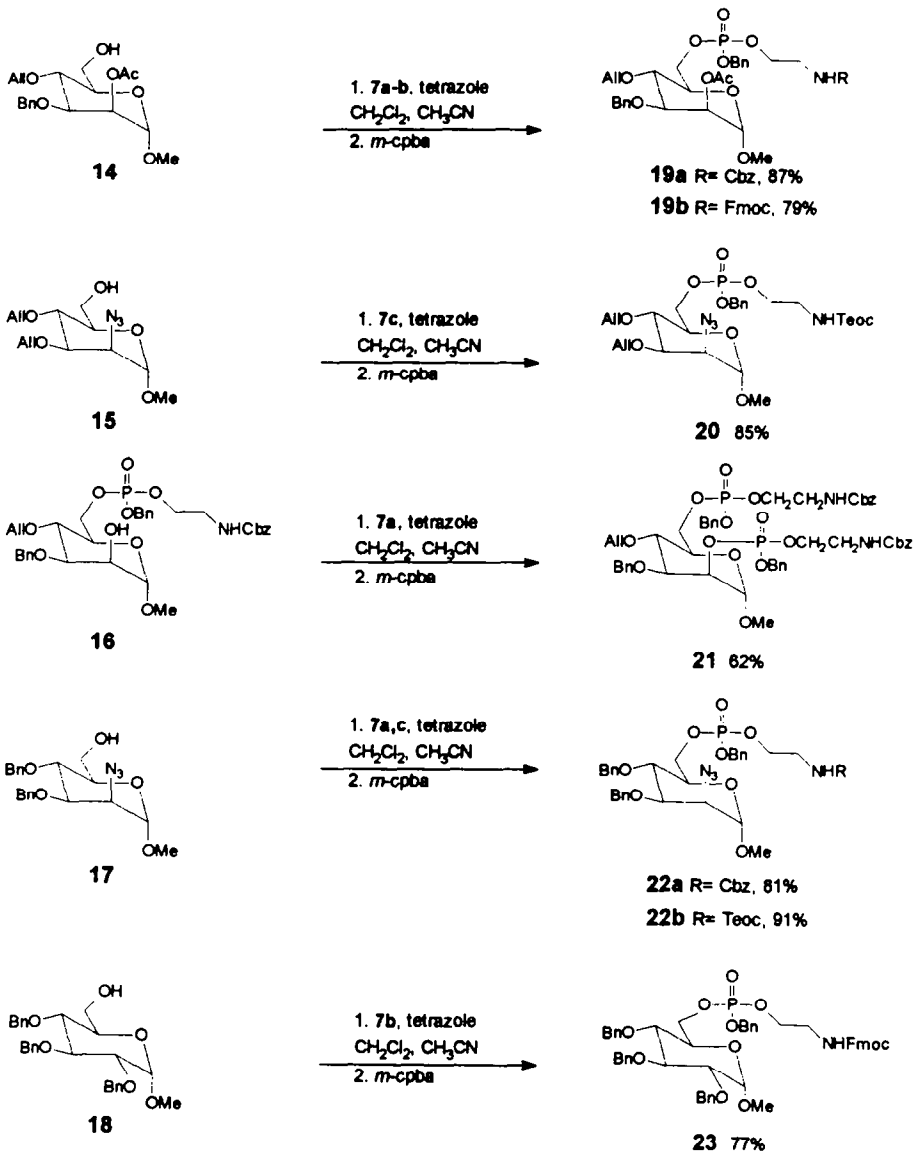
Accordingly cysteine hydrochloride was treated with 9-Fm-*p*-toluene sulfonate **13a**[14b] to give the salt **10** in 75 % yield, which was then allowed to react with the succinimidyl derivative **13b** to give an excellent yield of the di-protected derivative **11**.

With regard to the problem of attaching the cysteine moiety to synthon **3**, one option involved *in situ* activation of the carboxyl group.[12] However, the alternative of using a pentafluorophenyl ester seemed more appealing, since these derivatives are easily stored and yet react readily under appropriate conditions to give amides free of side products.[12,16] Accordingly the carboxyl group of **11** was coupled with pentafluorophenol under the agency of DCC[12,14b,17] to give the fully protected derivative **12** in 96 % yield.

It was necessary to establish that the protecting groups present in synthetic heptasaccharide **5** would be able to withstand the planned phosphorylation procedures. Accordingly model studies were carried out on compounds **14–18**[18] (Table 1) to determine whether the allyl and azido groups, present in **5**, would survive the phosphorylation reactions. With respect to the allyl group, there is evidence in the literature that the group is stable to *in situ* peroxyacid oxidation which would have to be carried out on the phosphite triester formed in the initial phosphoramidite coupling.[8d,e] The results for compounds **14**, **15**, and **16** are in keeping with this precedent. It should be noted that use of rigorously dried MCPBA and extended reaction times are essential for the yields quoted.

Our fear that the azido group might undergo a Staudinger reaction[19] with the phosphoramidite was dispelled by the results with compounds **15** and **17**. Thus the yields for the corresponding phosphotriesters **20**, **22a/22b** are in line with the other yields in Table 1. For example compound **18**

Table 1.



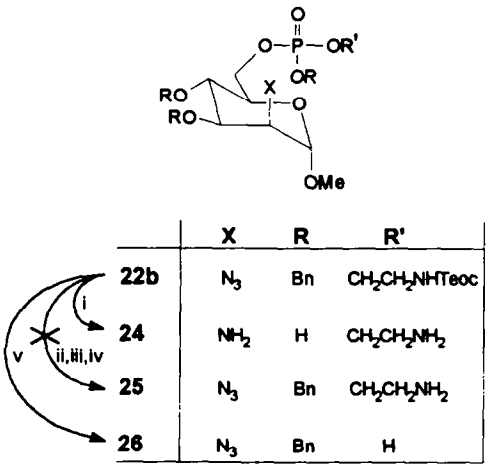
does not contain an allyl or azido group but it was phosphorylated in a yield comparable to those substrates containing such groups.

In our planned strategy, the FMOC group would be required to withstand hydrogenolysis, as might have been expected on the basis of the initial report[13] as well as a recent example.[20] On the other hand there are reports of facile hydrogenolysis of FMOC groups in peptide syntheses[21] and this has led to the suggestion that the quality of the noble metal catalyst is a crucial factor in determining the outcome of the deprotection reaction.[21c] When compound **19b** was subjected to standard hydrogenolytic conditions (3 atm H_2, Pd/C, EtOH) the FMOC group was removed *before* the 3-*O* benzyl group.

As an alternative for amine protection, the 2-(trimethylsilyl)-ethoxycarbonyl (TEOC)[22] group was examined. First, stability to standard hydrogenolytic conditions was established by use of model compound **22b**. The amino diol **24** was obtained quantitatively (Scheme III) under the conditions that had led to rapid loss of the FMOC group in **19b**.

With regard to removal of the TEOC group, fluoride ion has been reported to be very effective.[22,23] However, in our hands compound **22b** was unaffected by, or suffered degradation under procedures involving the use of potassium fluoride (Scheme III). Since it has been observed that traces of water are deleterious to the fluorinolysis reaction, an anhydrous form of tetra-*n*-butylammonium fluoride on silica gel[24] was examined. A clean, albeit slow, reaction occurred whereby a single product was obtained. Unfortunately this proved to be the diester **26** (Scheme III).

Trifluoroacetic acid has been used to remove TEOC protecting groups in complex alkaloid synthesis,[25] and since this reagent is compatible with sugar substrates, as may be judged by its use for deprotecting the anomeric center of 2-trimethylsilylethyl glycosides,[26] its use was examined. However after 30 min at 0 °C with this reagent, compound **22b** had given none of the desired amine **25**.



	X	R	R'
22b	N_3	Bn	$CH_2CH_2NHTeoc$
24	NH_2	H	$CH_2CH_2NH_2$
25	N_3	Bn	$CH_2CH_2NH_2$
26	N_3	Bn	H

Scheme III. (i) H_2, Pd/C, EtOH (ii) Bu_4NCl, KF·H_2O, MeCN (iii) Et_4NCl, $KF_{(anh)}$ (iv) CF_3CO_2H, CH_2Cl_2, 0 °C, 30 min. (v) Bu_4NF·Silica Gel (anh), MeCN, 54 °C, 3 d, 86 % + 9 % recovery of **22b**.

From this survey it would therefore appear that the TEOC group will be unsuitable for nitrogen protection of these ethanolamine phosphate-containing oligosaccharides.

Finally we studied the viability of coupling the activated amino acid derivative **12** to a partially protected sugar substrate containing the ethanolamine phosphate residue by use of model compounds **19b** and **23** (Scheme IV). For liberation of the amino group in these substrates, we examined the use of piperidine[14] which has been recently developed in our laboratory for the N-deacetylation of glycopeptides.[27] In the case of substrate **19b**, the free amines **27** and **28** were obtained (the latter being formed at the expense of the former) by use of a 1:1 solution of piperidine and DMF at room temperature for 4 h. Also obtained was the phosphodiester **29** (Scheme IV). In the case of **23**, the analogues **30** and **31** were obtained, but there was no product corresponding to **27** (Scheme IV).

Each of the amines **27**, **28**, and **31** was then cysteinylated with compound **12** to give glycopeptides **32**, **33** and **34** respectively, the yields being excellent in all cases (see Scheme IV, series i). Each amine was then forced to compete with the glucosamine derivative **35** for the activated cysteine **12** (Scheme IV, series ii).

Based on the percentage yields, the results in Scheme IV are most encouraging. In each case there was only faint evidence (TLC) for coupling of cysteine **12** to the glucosamine **35**. Thus the virtually exclusive reaction was the same, in products as well as percentage yields, as in the absence of glucosamine **35** (i.e. in series i). In the case of **32**, somewhat lower yields in series ii appear to be due to some loss of the Fm group from the initially formed **32** (TLC evidence).

It is interesting to note that the phosphodiesters **28** and **31** reacted with the cysteine slightly faster than did the triester **27**. This observation may prove useful in our future plans.

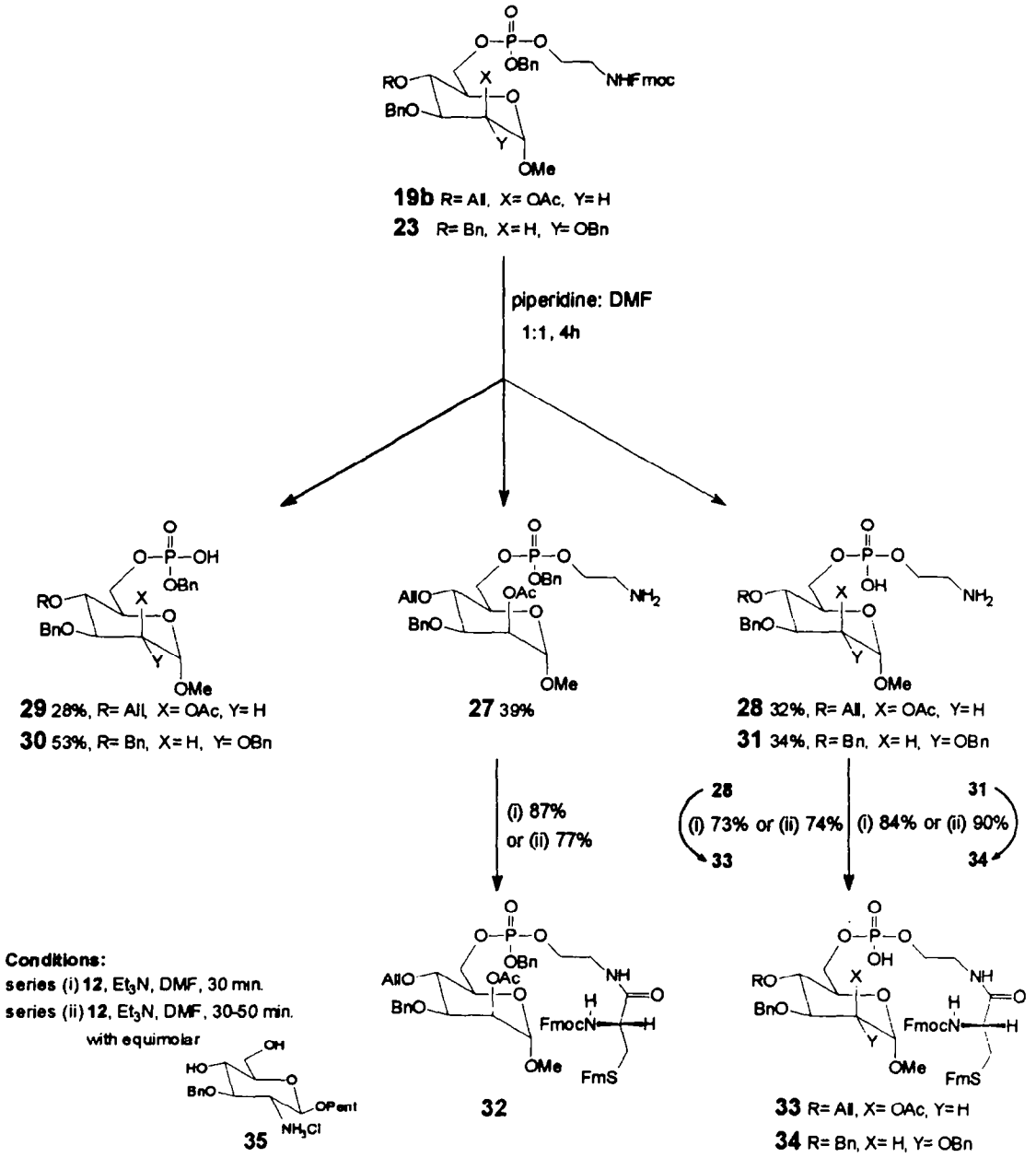
Experimental

Flash column chromatography was carried out on silica gel 60 (230–400 mesh, Merck) with the eluent specified in parentheses. All reactions requiring anhydrous conditions were conducted under a positive pressure argon atmosphere. Organic extracts were dried over $MgSO_4$, and concentrated at aspirator pressure (~ 25 mm Hg) using a rotary evaporator, unless otherwise stated. Dichloromethane, acetonitrile, *N,N*-dimethylformamide (DMF), piperidine, and tetrahydrofuran (THF) were dried and distilled before use using standard methods.[28] *m*-Chloroperbenzoic acid (MCPBA) and tetrazole were purified by literature methods,[28] and all tetra-alkylammonium halide salts were dried *in vacuo* over P_2O_5 prior to use. All ^1H NMR spectra were recorded on a Varian XL-300 spectrometer. ^{13}C NMR were recorded on either a Varian XL-300 or a GE QE-300 spectrometer. ^{13}P NMR were recorded on a Varian XL-300 spectrometer using 85 % H_3PO_4 as external reference. Optical rotations were measured in a Perkin–Elmer 241 instrument. Routine and high resolution mass (HRMS) spectral data were recorded on a JEOL JMS-SX102A mass spectrometer using FAB^+ or FAB^- techniques. Melting points were recorded with a Büchi 510 apparatus and are uncorrected. TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Merck, Art. 5554) and visualized using a solution of ammonium molybdate tetrahydrate and cerium(IV) sulfate tetrahydrate in 10 % aqueous sulfuric acid, or an ethanolic solution of ninhydrin (Aldrich). Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA.

2-Amino ethanol carbamates (6)

General procedure. The procedure reported by Mallams *et al.*[9] was follows for the synthesis of **6a**, and was adapted as followed for production of **6b** and **6c**.

$NaHCO_3$ (5.0 mmol) was dissolved in H_2O (7 mL). Acetone (12 mL) was then added followed by



Scheme IV.

ethanolamine (5.0 mmol). FMOC-OSu (**13b**) or TEOC-OSu[29] was then added in one portion. When the reaction was shown by TLC (5:95, $MeOH:CH_2Cl_2$) to be complete the reaction medium was diluted with CH_2Cl_2 (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the organic extracts were dried, filtered and concentrated.

*2-(Benzyloxycarbonylamino)ethanol (**6a**)*

Recrystallized from ethyl acetate/hexanes, 88 %. Mp 61–62 °C (lit. 53–55 °C,[9] 62–63 °C[30]); δ_H ($CDCl_3$): 3.35 (m, 2H, CH_2N), 3.71 (t, J = 5.7 Hz, 2H), CH_2OH), 5.10 (s, 2H, CH_2Ph), 5.22 (bs, 1H, NH), 7.35 (m, 5H, Ph); δ_C: 43.41, 61.57, 66.79, 128.06, 128.16, 128.54, 136.43, 157.27. Found: C, 61.45; H, 6.72; requires: C, 61.54; H, 6.71.

*2-(9-Fluorenylmethoxycarbonylamino)ethanol (**6b**)*

Recrystallized from ethyl acetate/hexanes, 94 %. Mp 136 °C; δ_H ($CDCl_3$): 3.37 (m, 2H, CH_2N), 3.74 (m, 2H, CH_2OH), 4.22 (t, 1H, 6.8H, H-9), 4.45 (m, 2H, CH_2O), 5.11 (bs, 1H, NH), 7.32 (dt, 2H, J = 0.6 Hz, J = 3.7 Hz, Ph), 7.41 (t, 2H, J = 3.7 Hz, Ph), 7.60 (d, 2H, J = 7.7 Hz,

Ph), 7.78 (d, 2H, J = 7.7 Hz, Ph); δ_C: 43.46, 47.23, 62.31, 66.76, 120.02, 124.95, 127.09, 127.68, 141.33, 143.86, 157.10. Found: C, 72.06; H, 6.08; requires: C, 72.07; H, 6.05.

2-(2-Trimethylsilylethoxycarbonylamino)ethanol (6c)[9]

Colorless oil used without further purification, 99 % (crude). δ_H ($CDCl_3$): 0.02 (s, 9H, CH_3), 0.97 (t, 2H, J = 8.5 Hz, CH_2Si), 3.32 (m, 2H, CH_2N), 3.69 (t, 2H, J = 4.9 Hz, CH_2OH), 4.14 (t, 2H, J = 8.3 Hz, CH_2O), 5.16 (bs, 1H, NH); δ_C: −1.55, 17.65, 43.22, 61.71, 63.09, 157.56. Found: C, 46.70; H, 9.38; requires: C, 46.80; H, 9.33.

Phosphoramidite reagents (7,9)

Typical procedure (adapted from literature procedures[8g,h]). The ethanolamine derivative **6**, (4.0 mmol) or the alkylacylglycerol **8** was dried prior to reaction by repeated co-evaporation with toluene (2 × 2 mL), then taken up in CH_2Cl_2 (25 mL). Benzyloxy[bis-(diisopropylamino)]-phosphine[8a] (4.0 mmol) was added from a syringe, followed immediately by a solution of 1*H*-tetrazole (4.0 mmol) in CH_3CN (15 mL). The reaction was monitored by TLC, as indicated below, until complete (usually ~ 30 min), whereupon it was quenched with saturated $NaHCO_3$ solution (10 mL) and diluted with CH_2Cl_2 (150 mL). The organic layer was washed quickly with saturated $NaHCO_3$ solution (50 mL), dried, filtered and concentrated. The crude product was purified by flash chromatography as indicated below.

Benzyloxy [2-(benzyloxycarbonylamino)ethoxy]-(N,N-diisopropylamino) phosphine (7a)

Flash chromatographed (5:95:5→10:90:5, EtOAc:hexanes: Et_3N) to give a colorless oil in 66 % yield. R_f = 0.80 (20:20:1, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 1.18 (s, 6H, CH_3), 1.20 (s, 6H, CH_3), 3.41 (m, 2H, CH_2N), 3.64 (m, 2H, *i*Pr CH), 3.74 (m, 2H, CH_2CH_2OP), 4.71 (ABX, 2H, CH_2Ph), 5.21 (bs, 1H, NH), 7.33 (m, 10H, Ph); δ_C: 24.62 (d, J = 5.3 Hz, CH_3), 24.70 (d, J = 6.0 Hz, CH_3), 42.30 (d, J = 6.8 Hz, CH_2OP), 43.00 (d, J = 12.8 Hz, *i*Pr CH), 62.64 (d, J = 16.6 Hz, CH_2N), 65.45 (d, J = 18.1 Hz, $PhCH_2OP$), 66.63 ($PhCH_2$), 127.05, 127.17, 127.20, 127.43, 128.07, 128.31, 128.36, 128.40, 128.48, 128.53, 136.66, 139.13, 156.42; δ_P: 148.48. Found: C, 63.58; H, 7.72; requires C, 63.87; H, 7.69.

Benzyloxy [2-(9-fluorenylmethoxycarbonylamino)ethoxy]-(N,N-diisopropylamino)phosphine (7b)

Flash chromatographed (5:95:5, EtOAc: hexanes:Et_3N) to give a colorless oil in 84 % yield. R_f = 0.72 (50:50:1, EtOAc: hexanes:Et_3N). δ_H ($CDCl_3$): 1.19 (s, 6H, CH_3), 1.21 (s, 6H, CH_3), 3.42 (m, 2H, CH_2N), 3.65 (m, 2H, *i*Pr CH), 3.74 (m, 2H, CH_2CH_2OP), 4.21 (t, J = 6.8 Hz, 1H, H-9), 4.37 (m, 2H, CH_2O), 4.72 (ABX, 2H, CH_2Ph), 5.22 (bs, 1H, NH), 7.22–7.46 (m, 9H, Ph), 7.59 (d, J = 6.8 Hz, 2H, Ph), 7.78 (d, J = 7.2 Hz, 2H, Ph); δ_C: 24.63 (d, J = 6.5 Hz, CH_3), 24.71 (d, J = 6.5 Hz, CH_3), 42.24 (d, J = 6.8 Hz, CH_2OP), 43.03 (d, J = 12.5 Hz, *i*Pr CH), 47.22 (C-9), 62.62 (d, J = 16.1 Hz, CH_2N), 65.48 (d, J = 18.7 Hz, OCH_2OP), 66.75 (CH_2O), 119.97, 125.11, 127.03, 127.16, 127.24, 127.33, 127.37, 127.46, 127.66, 128.05, 128.15, 128.25, 128.35, 139.10, 141.30, 144.00, 156.45; δ_P: 148.60.

Benzyloxy [2-(2-trimethylsilylethoxycarbonylamino)-ethoxy]-(N,N-diisopropylamino)phosphine (7c)

Flash chromatographed (20:80:5, EtOAc: hexanes: Et_3N) to give a colorless oil in 55 % yield. R_f = 0.81 (30:70:5, EtOAc: hexanes: Et_3N). δ_H ($CDCl_3$): 0.03 (s, 9H, CH_3), 0.96 (t, J = 8.4 Hz, 2H, CH_2Si), 3.37 (m, 2H, CH_2N), 3.68 (m, 4H, *i*Pr CH, CH_2CH_2OP), 4.13 (t, J = 7.2 Hz, 2H, CH_2O), 4.70 (ABX, 2H, $PhCH_2OP$), 5.04 (bs, 1H, NH), 7.34 (m, 5H, Ph); δ_C: −1.44 (CH_3), 17.75 (CH_2Si), 24.61 (d, J = 5.3 Hz, *i*Pr CH_3), 24.69 (d, J = 6.0 Hz, *i*Pr CH_3) ,42.13 (d, J = 7.0 Hz, CH_2OP), 42.99 (d, J = 12.1 Hz, *i*Pr CH), 62.74 (d, J = 16.8 Hz, CH_2N), 62.94 (CH_2O), 65.48 (d, J = 18.5 Hz, OCH_2Ph), 127.12, 127.22, 127.40, 128.13, 128.18, 128.24, 128.32, 139.65, 156.80; δ_P: 148.38. Found C, 56.43; H, 8.56; requires C, 56.99; H, 8.88.

Benzyloxy (1-O-octadecyl-2-O-stearoyl-sn-glyceryl)-(N,N-diisopropylamino)phosphine (9)

Flash chromatographed (95:4:1, pet. ether:ether:Et_3N) to give a colorless gum in 58 % yield. R_f = 0.84 (8:3:1, pet. ether:ether:Et_3N). δ_H ($CDCl_3$): 0.83 (m, 14H), 1.18 (m, 12H, CH_3), 1.26 (m, 48H), 1.57 (m, 5H), 2.31 (t, J = 7.6 Hz, 2H), 3.42 (m, 3H), 3.59 (d, J = 5.1 Hz, 2H), 3.60–3.88 (m, 4H), 4.69 (m, 2H, CH_2 Ph), 5.13 (m, 1H, glyceryl H-2), 7.33 (m, 5H, Ph); δ_C: 14.16, 22.72, 24.54, 24.60, 24.70, 25.00, 26.11, 29.17, 29.55, 29.40, 29.53, 29.69, 29.74, 31.96, 34.49, 42.93, 43.01, 62.02, 65.42 (d, J = 18.0 Hz, OCH_2Ph), 69.06, 69.16, 71.59, 72.13, 126.92, 127.23, 128.24, 143.03, 145.00, 173.32; δ_P: 148.77, 148.98 (diastereomers).

1-O-Octadecyl-2-O-stearoyl-sn-glycerol (8)

1-*O*-Octadecyl-2-*O*-stearoyl-3-*O*-trityl-*sn*-glycerol[10b] (2.0 g, 24 mmol), prepared in seven steps in 32 % overall yield from L-gulonolactone by combining literature procedures,[10] was added to a stirred solution of BF_3·MeOH (1.16 g of 14 wt % solution in methanol, 2.4 mmol) in CH_2Cl_2 (80 mL) at 0 °C. After 30 min the reaction was stopped by the addition of ice-cold saturated $NaHCO_3$ solution (100 mL). The organic layer was extracted with ice-water (2 × 100 mL), dried, filtered and concentrated. The crude product was flash chromatographed on a short column (6 cm height) (100:0→90:10, pet. ether:ether) to give **8** as a solid in 73 % yield, and less than 5 % of the isomerized 1,3-product. The acyl migration occurred during chromatography. Mp 59–60 °C; $[\alpha]_D^{20}$= −3.78 ° (c = 0.98, $CHCl_3$); R_f = 0.36 (8:3, toluene:ether). δ_H ($CDCl_3$): 0.88 (m, 14H), 1.25 (m, 48H), 1.59 (m, 5H), 2.36 (t, J = 7.5 Hz, 2H), 3.45 (m, 2H), 3.62 (m, 2H), 3.82 (m, 2H), 5.00 (m, 1H, glyceryl H-2); δ_C: 14.15, 22.72, 25.00, 26.06, 29.12, 29.39, 29.50, 29.56, 29.64, 29.73, 31.95, 34.41, 63.03, 70.03, 71.92, 72.80, 173.75. Found: C, 76.40; H, 12.86; requires C, 76.66; H, 12.87.

N-(9-Fluorenylmethoxycarbonyl)-S-(9-fluorenylmethyl)-L-cysteine (11)

$NaHCO_3$ (750 mg, 9.0 mmol) was dissolved in H_2O (15 mL). To this was added **10**[14b] (1.05 g, 3.0 mmol) followed by acetone (15 mL) and THF (15 mL). FMOC-OSu **13b** (1.0 g, 3.0 mmol) was added and the pH of the reaction medium was brought to between 8 and 9 by addition of $NaHCO_3$. The reaction was followed to completion by TLC (90:5:5, CH_2Cl_2:HOAc:MeOH). After 15 h, the medium was acidified to pH 2 by addition of 1 N HCl. The acetone and THF were removed *in vacuo*. The aqueous phase was then extracted with CH_2Cl_2 (3 × 100 mL), and the organic extracts were washed with 1 N HCl (50 mL), dried filtered and concentrated. Flash chromatography (98:2, CH_2Cl_2:HOAc) gave **11** as a white solid (1.46 g, 92 %). A portion of the solid was recrystallized from ethyl acetate/hexanes for analysis. Mp 110–112 °C; $[\alpha]_D^{20}$= +13.8 ° (c = 1.66, $CHCl_3$); R_f = 0.26 (98:2, CH_2Cl_2:HOAC). δ_H ($CDCl_3$): 3.04 (m, 2H, CH_2S), 3.12 (d, J = 6.8 Hz, 2H, CH_2S), 4.09 (t, J = 6.6 Hz, 1H, Fm H-9), 4.20 (bt, J = 6.8 Hz, 1H, CHN), 4.38 (m, 2H, FMOC CH_2), 4.59 (m, 1H, FMOC CH), 5.70 (d, J = 7.4 Hz, 1H, NH), 7.20–7.41 (m, 8H, Ph), 7.52–7.79 (m, 8H, Ph); δ_C: 35.24, 37.09, 46.92, 47.10, 53.83, 67.43, 120.06, 124.86, 125.16, 125.23, 127.18, 127.74, 141.11, 141.35, 143.69, 143.79, 145.70, 156.17, 175.30. Found: C, 71.61; H, 5.42; requires C, 71.22; H, 5.42 (hydrate).

N- (9- Fluorenylmethoxycarbonyl)- S-(9-fluoroenylmethyl)-L-cysteine pentafluorophenyl ester (12)

The cysteine derivative **11** (1.00 g, 1.92 mmol) was dissolved in THF (40 mL), and to this was added a solution of pentafluorophenol (390 mg, 2.11 mmol) in THF (20 mL). The stirred solution was cooled to 0 °C prior to the addition of DCC (435 mg, 2.11 mmol) in THF (20 mL). The solution was diluted with THF (20 mL) to facilitate stirring and the reaction was allowed to proceed for 1 h at 0 °C. The ice-bath was then removed and the reaction was stirred 5 h more, at which time TLC (96:2:2, CH_2Cl_2:MeOH:AcOH) revealed complete reaction. The reaction was concentrated to dryness, and the residue was flash chromatographed (90:10, CH_2Cl_2:cyclohexane) to give the title compound as a white solid (1.30 g, 99 %). Mp 169–170 °C; $[\alpha]_D^{20}$= –13.8 ° (c = 1.66, $CHCl_3$); R_f = 0.45 (90:10, CH_2Cl_2:cyclohexane). δ_H ($CDCl_3$): 3.06 (m, 2H, CH_2S), 3.25 (m, 2H, CH_2S), 4.16 (bt, J = 5.4 Hz, 1H, Fm H-9), 4.23 (t, J = 7.1 Hz, 1H, CHN), 4.48 (m, 2H, FMOC CH_2), 4.89 (m, 1H, FMOC CH), 5.54 (d, J = 7.2 Hz, 1H, NH), 7.22–7.44 (m, 8H, Ph), 7.54–7.82 (m, 8H, Ph); δ_C: 35.27, 37.10, 46.97, 47.05, 53.59, 67.43, 120.08, 124.63, 124.68, 124.93, 125.03 125.06, 125.10, 125.16, 127.15, 127.80, 141.20, 141.33, 145.37, 156.40, 175.24. Found: C, 66.31; H, 3.85 requires C, 66.37; H, 3.81.

Synthesis of glycophosphates 19–23

Typical procedure. The alcohol **14–18** (0.68 mmol) was dried prior to reaction by co-evaporation with toluene (2 × 2 mL), then it was dissolved in CH_2Cl_2 (1 mL). To this was added a solution of the phosphoramidite reagent **7** (0.75 mmol) in CH_2Cl_2 (1 mL), and the syringe rinsed with CH_2Cl_2 (1 mL). Immediately, a solution of 1*H*-tetrazole (2.0 mmol) in CH_3CN (5 mL) was added. The reaction was stirred at rt and monitored by TLC until complete, at which time the medium was cooled to –40 °C, and MCPBA (250 mg, xs) was added. The cold bath was removed and the reaction was stirred for 45 min as the temperature gradually became ambient. The solution was diluted with CH_2Cl_2 (50 mL), extracted with 10 % Na_2SO_3 solution (2 × 10 mL), washed with brine (10 mL), dried, filtered, and concentrated. The crude product was flash chromatographed as indicated below to give a diastereomeric mixture.

19a. 87 % Yield. TLC (40:60:2, EtOAc:hexanes:Et_3N); flash chromatography (50:50:1, EtOAc:hexanes:Et_3N); R_f = 0.20 (20:20:1, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 2.01 (s, 1.5H, CH_3), 2.05 (s, 1.5H, CH_3), 3.23 (s, 1.5H, OCH_3), 3.25 (s, 1.5H, OCH_3), 3.43 (m, 2H), 3.60 (m, 1H), 3.71 (m, 1H), 3.86 (m, 1H), 4.10 (m, 3H), 4.30 (m, 3H), 4.56 (AB, 2H, CH_2Ph), 4.63 (s, 1H, H-1), 5.07 (m, 4H, $PhCH_2OP$, CH_2Ph), 5.18 (m, 2H CH_2=CH), 5.31 (bs, 1H, H-2), 5.50 (bs, 1H, NH), 5.86 (m, 1H, CH_2=C*H*), 7.32 (m, 15H, Ph); δ_C: 20.97, 29.74, 41.88, 55.05, 66.89, 68.37, 69.47, 69.50, 69.53, 70.26, 71.69, 71.71, 73.49, 74.01, 77.71, 77.75, 98.71, 116.97, 117.00, 127.80, 127.98, 128.09, 128.21, 128.23, 128.37, 128.40, 128.45, 128.51, 128.54, 128.56, 128.68, 134.60, 135.61, 136.42, 137.22, 156.38, 170.19; δ_P: –0.29, –0.82; HRMS (FAB$^-$) $C_{36}H_{44}NO_{12}P$, found: 712.2544 (M – H)$^-$, 622.2040 (M – Bn)$^-$, calcd 712.2522 (M – H)$^-$.

19b. 79 % Yield. TLC (40:60:2, EtOAc:hexanes:Et_3N); flash chromatography (20:20:1, EtOAc:hexanes:Et_3N); R_f = 0.25 (20:20:1, EtOAc:hexanes:Et_3N); δ_H ($CDCl_3$): 2.03 (s, 1.5H, CH_3), 2.07 (s, 1.5H, CH_3), 3.26 (s, 1.5H, OCH_3), 3.28 (s, 1.5H, OCH_3), 3.43 (m, 2H, CH_2), 3.61 (dt, J = 3.4 Hz, J = 8.8 Hz, 1H), 3.72 (m, 1H), 3.89 (dd, J = 3.4 Hz, J = 8.8 Hz, 1H), 4.10 (m, 3H), 4.20 (t, J = 6.8 Hz, 1H, FMOC CH), 4.25–4.42 (m, 5H), 4.56 (AB, 2H, CH_2Ph), 4.63 (s, 1H, H-1), 5.01–5.28 (m, 4H, $POCH_2Ph$, CH_2=CH), 5.33 (bs, 1H, H-2), 5.49 (s, 1H, NH), 5.88 (m, 1H, C*H*=CH_2), 7.22–7.42 (m, 14H, Ph), 7.57 (d, J = 7.4 Hz, FMOC Ph), 7.75 (d, J = 7.4 Hz, 2H, FMOC Ph); δ_P: –0.02, –0.17.

20. 85 % Yield. (TLC 40:60:2, EtOAc:hexanes:Et_3N); flash chromatography (40:60:2, EtOAc:hexanes:Et_3N); R_f =0.56 (50:50:2, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 0.01 (s, 9H, $SiCH_3$), 0.95 (t, J = 7.8 Hz, 2H, CH_2Si), 3.30 (s, 3H, OCH_3), 3.39 (m, 10H), 4.61 (s, 1H, H-1), 5.03–5.40 (m, 7H, CH=CH_2), 7.35 (m, 5H, Ph); δ_C: –1.47, 17.70, 17.72, 41.25, 55.08, 61.03, 61.08, 63.15, 66.31, 66.35, 67.14, 67.18, 67.22, 69.38, 69.45 69.47, 69.54, 70.41, 71.24, 73.42, 73.47, 74.02, 74.05, 79.06, 99.03, 117.23, 117.35, 117.38, 127.95, 128.01, 128.64, 134.15, 134.48, 135.64, 156.74, 156.79; δ_P: –0.12, –0.37.

21. 33 % Yield (62 % based on recovered starting material after 5 days reaction time). TLC (75:25:1, EtOAc:hexanes:Et_3N); flash chromatography (75:25:1, EtOAc:hexanes:Et_3N) gave initially a fraction containing a mixture of four diastereomers which was re-chromatographed (5:95:1,

MeOH:CH_2Cl_2:Et_3N) to give two fractions, each containing two diastereomers (21 % and 12 % yields respectively).

Fraction 1: R_f = 0.31 (60:40:2, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 3.22 (m, 3.5H, OCH_3, CH_2), 3.38 (m, 3.5H, OCH_3, CH_2), 3.64 (m, 2H), 3.82 (m, 2H), 3.90–4.11 (m, 4H), 4.24 (m, 4H), 4.50–4.81 (m, 7H, H-1, CH_2Ph), 4.91–5.34 (m, 8H, CH=CH_2, CH_2Ph, NH), 5.82 (m, 1H, CH=CH_2), 7.28 (m, 25H, Ph); δ_P: –0.60, –0.53, –0.36, –0.04; HRMS (FAB^-) $C_{51}H_{60}N_2O_{16}P_2$, found: 1017.3374 $(M-H)^-$, calcd 1017.3340 $(M-H)^-$.

Fraction 2: R_f = 0.28 (60:40:2, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 3.21 (m, 3.5H, OCH_3, CH_2), 3.35 (m, 3.5H, OCH_3, CH_2), 3.65 (m, 2H), 3.80 (m, 2H), 3.91–4.10 (m, 4H), 4.11–4.40 (m, 4H), 4.50–4.84 (m, 7H, H-1, CH_2Ph), 4.91–5.18 (m, 6H, OCH_2Ph, CH=CH_2), 5.30 (m, 2H, NH), 5.82 (m, 1H, CH=CH_2), 7.31 (m, 25H, Ph); δ_P: –0.59, –0.53, –0.36, –0.03; HRMS (FAB^-) $C_{51}H_{60}N_2O_{16}P_2$, found: 1017.3350 $(M-H)^-$, calcd 1017.3340 $(M-H)^-$.

22a. 81 % yield. TLC (40:60:5, EtOAc:hexanes:Et_3N); flash chromatography (25:75:5→50:50:5, EtOAc:hexanes:Et_3N); R_f = 0.22 (50:50:2, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 3.22 (s, 1.5H, OCH_3), 3.26 (s, 1.5H, OCH_3), 3.41 (m, 2H, CH_2CH_2OP), 3.67 (m, 1H, H-5), 3.82 (t, 0.5H, J = 9.6 Hz, H-4), 3.83 (t, 0.5H, J = 9.6 Hz, H-4), 3.88 (bs, 1H, H-2), 4.10 (m, 3H), 4.24 (m, 2H), 4.50–4.94 (m, 5H, OCH_2Ph, H-1), 5.10 (m, 4H, OCH_2Ph), 5.40 (bt, J = 5.3 Hz, 0.5H, NH), 5.52 (bt, J = 5.3 Hz, 0.5H, NH), 7.30 (m, 20H, Ph); δ_C: 41.38, 41.45, 55.05, 60.97, 61.04, 66.50, 66.84, 67.16, 69.44, 69.53, 69.61, 70.43, 70.52, 72.51, 72.53, 73.62, 73.67, 75.37, 79.59, 99.05, 127.90, 127.97, 128.04, 128.18, 128.38, 128.56, 128.68, 135.61, 136.40, 137.56, 137.89, 156.28, 156.42; δ_P: –0.34, –0.01; HRMS (FAB^-) $C_{38}H_{43}N_4O_{10}P$, found: 745.2638 $(M-H)^-$ calcd 745.2638 $(M-H)^-$.

22b. 91 % Yield. TLC, flash chromatography, R_f = 0.22 (40:60:2, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 0.03 (s, 9H, $SiCH_3$), 0.97 (m, 2H, CH_2Si), 3.31 (s, 3H, OCH_3), 3.39 (m, 2H, CH_2CH_2OP), 3.70 (m, 1H), 3.83 (t, J = 9.4 Hz, 0.5H, H-4), 3.84 (t, J = 9.4 Hz, 0.5H, H-4), 3.93 (bs, 1H, H-2), 4.03–4.18 (m, 5H), 4.24–4.31 (m, 2H), 4.53–4.92 (m, 5H, OCH_2Ph, H-1), 5.09 (t, J = 8.1 Hz, 2H, $POCH_2Ph$), 5.24 (bs, 0.5H, NH), 5.35 (bs, 0.5H, NH), 7.30 (m, 15H, Ph); δ_C: –1.48, 17.75, 36.53, 55.09, 60.99, 61.06, 63.16, 66.30, 66.38, 67.22, 67.31, 69.39, 69.47, 69.54, 70.38, 70.46, 72.55, 73.62, 73.66, 75.33, 77.25, 79.59, 99.09, 127.88, 127.94, 128.01, 128.48, 128.56, 128.64, 134.90, 137.56, 137.88, 162.56; δ_P: –0.34, –0.12; HRMS (FAB^-) $C_{36}H_{49}N_4O_{10}PSi$, found: 756.2990 (M^-), calcd 756.2955.

23. 77 % Yield. TLC (30:70:1, EtOAc:hexanes:Et_3N); flash chromatography, R_f = 0.24 (50:50:1, EtOAc:hexanes:Et_3N). δ_H ($CDCl_3$): 3.32 (s, 1.5H, OCH_3), 3.34 (s, 1.5H, OCH_3), 3.39 (m, 2H, CH_2CH_2OP), 3.50 (m, 2H), 3.78 (m, 1H, H-5), 3.95–4.16 (m, 3H), 4.24 (m, 3H), 4.37 (m, 2H, FMOC CH_2), 4.60 (m, 3H, CH_2Ph, H-1), 4.71–4.94 (m, 3H, CH_2Ph), 5.15 (m, 3H, CH_2Ph, $POCH_2Ph$), 5.29 (bt, J = 5.5 Hz, 0.5H, NH), 5.36 (bt, J = 5.5 Hz, 0.5H, NH), 7.30 (m, 24H, Ph), 7.58 (m, 2H, FMOC Ph), 7.77 (d, J = 7.1 Hz, 2H, FMOC Ph); δ_C: 29.76, 41.35, 41.42, 47.17, 55.37, 66.49, 66.56, 66.64, 66.83, 66.97, 67.03, 69.26, 69.29, 69.39, 69.55, 69.63, 73.42, 75.15, 75.77, 77.13, 79.78, 79.81, 81.81, 98.08, 120.03, 125.10, 127.11, 127.76, 127.94, 128.02, 128.11, 128.32, 128.48, 128.53, 128.70, 128.77, 135.58, 135.65, 137.94, 138.00, 138.60, 141.32, 143.87, 156.34; δ_P: –0.03, –0.01; MS (FAB^-) $C_{52}H_{54}NO_{11}P$, found: 900.4 (MH^+), 922.3 (M + Na)$^+$, calcd 900.4 (MH^+).

*Methyl 2-azido-3,4-di-O-benzyl-6-O-(benzyl)phosphoryl-2-deoxy-α-D-mannopyranoside (**26**)*

The TEOC-protected ethanolamine derivative **22b** (25 mg, 0.033 mmol) was dissolved in CH_3CN (2 mL). Tetra-*n*-butylammonium fluoride on silica gel (Aldrich, 104 mg, 0.07–0.10 mmol) was added, and the mixture was stirred at 54 °C for 55 h. Water (2 mL) was added and the reaction mixture was concentrated to dryness *in vacuo*. The residue was flash chromatographed (2:98:1, MeOH:CH_2Cl_2:Et_3N) to give 19 mg (86 %) **26** as its triethylammonium salt, and 9 % recovery of **22b**. R_f = 0.25 (5:95:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 3.27 (s, 3H, OCH_3), 3.66 (m, 1H, H-5), 3.86 (t, J = 9.0 Hz, 1H, H-4), 3.90 (bs, 1H, H-2), 4.01 (dd, J = 9.0 Hz, J = 3.8 Hz, 1H, H-3), 4.18 (m, 2H, H-6), 4.54 (s, 1H, H-5), 4.65 (s, 2H, CH_2Ph), 4.76 (AB, 2H, CH_2Ph), 4.98 (ABX, 2H, $POCH_2Ph$), 7.15–7.40 (m, 15H, Ph); δ_C: 55.87, 61.23, 64.81, 64.87, 67.66, 67.73, 70.94, 71.05, 72.53, 74.19, 75.16, 79.67, 98.86, 127.49, 127.57, 127.61, 127.79, 127.83, 128.04, 128.18, 128.33, 128.41, 128.47, 137.81, 138.22; δ_P: –0.27; HRMS (FAB^-) $C_{28}H_{32}N_3O_8P$, found: 568.1870 $(M-H)^-$, calcd 568.1848 $(M-H)^-$.

*Removal of FMOC protecting group from **19b***

The FMOC-protected ethanolamine derivative **19b** (200 mg, 0.25 mmol) was stirred in a piperidine:DMF (1:1) solution (10 mL) for 4 h. The reaction was monitored by TLC (30:70:1, MeOH:CH_2Cl_2:Et_3N). The reaction medium was concentrated to dryness *in vacuo*, and the residue was subjected to gradient flash chromatography (5:95:1→ 30:70:1, MeOH:CH_2Cl_2:Et_3N). The reaction gave three products, **27** in 39 % yield, and **28** and **29** as their triethylammonium salts in 28 and 32 % yields, respectively.

27. R_f = 0.76 (30:70:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.04 (s, 1.5H, CH_3), 2.10 (s, 1.5H, CH_2), 3.05 (bs, 2H, CH_2), 3.30 (s, 1.5H, OCH_3), 3.31 (s, 1.5H, OCH_3), 3.56 (t, J = 9.4 Hz, 0.5H, H-4), 3.57 (5, J = 9.4 Hz, 0.5H, H-4), 3.71 (m, 1H, H-5), 3.88 (dd, J = 9.4 Hz, J = 2.7 Hz, 1H, H-3), 4.06 (m, 1H), 4.18 (m, 2H), 4.30 (m, 3H), 4.57 (AB, 2H, CH_2Ph), 4.67 (s, 1H, H-1), 5.05–5.28 (m, 4H, CH=CH_2, $POCH_2Ph$), 5.31 (m, 1H, H-2), 5.85 (m, 1H, CH=CH_2), 7.32 (m, 10H, Ph); δ_C: 21.00, 21.07, 40.14, 40.23, 55.14, 64.15, 64.22, 67.27, 67.32, 67.38, 68.30, 69.96, 70.03, 70.19, 70.23, 71.66, 73.39, 73.46, 73.92, 77.71, 98.70, 116.92, 116.96, 127.52, 127.68, 127.76, 127.96, 128.08, 128.39, 128.70, 128.76, 129.10, 134.65, 137.81, 170.17, 170.25; δ_P: –0.50, –0.38.

28. R_f = 0.29 (30:70:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.09 (s, 3H, CH_3), 3.12 (bs, 2H, CH_2), 3.31 (s, 3H, OCH_3), 3.51 (t, J = 9.1 Hz, 1H, H-4), 3.67 (m, 1H, H-5), 3.84 (dd, J = 9.1 Hz, J = 4.0 Hz, 1H, H-3), 4.10 (m, 5H), 4.33 (m, 1H), 4.55 (AB, 2H, CH_2Ph), 4.60 (s, 1H, H-1), 5.17 (m, 2H, CH=CH_2), 5.30 (bs, 1H, H-2), 5.87 (m, 1H, CH=CH_2), 7.28 (m, 5H, Ph); δ_C: 21.08, 40.43, 40.47, 55.04, 62.02, 62.09, 65.13, 65.21, 68.52, 70.91, 71.02, 71.69, 73.82, 74.05, 77.86, 77.94, 98.69, 116.60, 127.72, 127.93, 128.37, 134.95, 137.92, 170.28; δ_P: 0.88.

29. R_f = 0.74 (30:70:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.05 (s, 3H, CH_3), 3.29 (s, 3H, OCH_3), 3.50–3.72 (m, 2H, H-4, H-5), 3.83 (dd, J = 9.3 Hz, J = 3.4 Hz, 1H, H-3), 4.01–4.20 (m, 3H), 4.29 (m, 1H), 4.54 (AB, 2H, CH_2Ph), 4.55 (d, J = 1.1 Hz, 1H, H-1), 4.96 (ABX, 2H, $POCH_2Ph$), 5.12 (m, 2H, CH=CH_2), 5.25 (dd, J = 3.4 Hz, J = 1.1 Hz, 1H, H-2), 5.84 (m, 1H, CH=CH_2), 7.27 (m, 10H, Ph); δ_C: 21.05, 54.81, 64.73, 64.80, 67.15, 67.22, 68.70, 71.00, 71.11, 71.64, 73.85, 74.15, 77.86, 98.45, 116.48, 127.33, 127.42, 127.49, 127.58, 127.80, 128.10, 128.15, 128.18, 128.29, 128.41, 134.98, 138.06, 170.36; δ_P: 0.67; HRMS (FAB$^+$) $C_{26}H_{33}O_{10}P$, found: 535.1750 (M – H)$^-$, calcd 535.1733 (M – H)$^-$.

Removal of FMOC protecting group from 23

Carbamate **23** (83mg, 0.093 mmol) was subjected to identical conditions as those described for deprotection of **19b**. Flash chromatography (5:95:1→20:80:1 MeOH:CH_2Cl_2:Et_3N) of the crude residue gave **30** and **31** as their triethylammonium salts in 53 and 34 % yields respectively.

30. R_f = 0.80 (30:70:1, MeOH:CH_2Cl_2:Et_3N); δ_H ($CDCl_3$): 3.32 (s, 3H, OCH_3), 3.46 (dd, J = 9.7 Hz, J = 3.6 Hz, 1H, H-2), 3.60 (t, J = 8.5 Hz, 1H, H-4), 3.72 (m, 1H, H-5), 3.96 (t, J = 9.2 Hz, 1H, H-3), 4.14 (m, 2H, H-6), 4.50 (d, J = 3.5 Hz, 1H, H-1), 4.60–4.98 (4 × AB, 8H, CH_2Ph), 7.15–7.40 (m, 20H, Ph); δ_C: 55.10, 64.19, 64.27, 67.17, 67.24, 70.03, 70.15, 73.30, 74.94, 75.58, 77.64, 79.66, 81.96, 97.91, 127.27, 127.41, 127.97, 127.56, 127.80, 127.86, 127.43, 128.10, 128.16, 128.33, 128.42, 138.19, 138.41, 138.94; δ_P: 0.94; HRMS (FAB$^-$) $C_{35}H_{39}O_9P$, found: 633.2245 (M – H)$^-$, 543.1776 (M – Bn)$^-$, calcd 633.2253 (M – H)$^-$.

31. R_f = 0.39 (30:70:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.92 (bs, 3H, CH_2N), 3.33 (s, 3H, OCH_3), 3.49 (m, 2H, H-2, H-4), 3.71 (m, 1H, H-5). 3.98 (m, 5H), 4.55 (d, J = 3.4 Hz, 1H, H-1), 4.60–5.00 (3 × AB, 6H, CH_2Ph), 7.15–7.40 (m, 15H, Ph); δ_P: 0.62.

Glycopeptide formation general procedures

(A) Control reactions. The amine **27**, **28** or **31** (~ 0.01 mmol, 1 eq.) was dissolved in DMF (0.5 mL). Triethylamine (~ 0.02 mmol, 2 eq.) and the activated cysteine derivative **12** (~ 0.01 mmol, 1 eq.) were added sequentially. The reaction was stirred at rt and monitored to completion by TLC (5:95:1, MeOH:CH_2Cl_2:Et_3N), usually about 30 min. The reaction was quenched with saturated NH_4Cl solution (1 mL) and concentrated to dryness *in vacuo*. The residue was extracted with CH_2Cl_2 (10 mL), concentrated to dryness and flash chromatographed to give glycopeptides **32**, **33**, and **34** from amines **27**, **28**, and **31** respectively. Any Et_3NHCl contaminant was precipitated from the product by addition of ethyl acetate (3 × 1 mL).

32. 87 % yield. R_f = 0.75 (5:95:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.01 (s, 1.5H, CH_3), 2.05 (s, 1.5H, CH_3), 3.12 (m, 3H), 3.25 (s, 1.5H, OCH_3), 3.28 (s, 1.5H, OCH_3), 3.29 (m, 1H), 3.42 (m, 2H), 3.56 (t, J = 8.5 Hz, 1H, H-4), 3.69 (m, 1H, H-5), 3.87 (dd, J = 8.5 Hz, J = 2.8 Hz, 1H, H-3), 3.97–4.43 (m, 11H), 4.56 (AB, 2H, CH_2Ph), 4.63 (s, 1H, H-1), 5.01–5.25 (m, 4H, $POCH_2Ph$, CH=CH_2), 5.31 (bs, 1H, H-2), 5.75 (bs, 1H, NH), 5.81 (m, 1H, CH=CH_2), 6.72 (bs, 1H, NH amide), 7.22–7.44 (m, 18H, Ph), 7.55–7.82 (m, 8H, Fm Ph); δ_C: 20.97, 21.02, 29.69, 29.73, 35.31, 36.38, 40.08, 40.12, 41.85, 55.08, 66.19, 66.84, 66.87, 66.94, 66.97, 67.16, 67.42, 68.37, 69.51, 69.59, 69.65, 70.26, 70.37, 71.69, 73.50, 73.54, 73.96, 77.25, 77.74, 98.74, 116.91, 119.86, 119.95, 120.02, 120.20, 120.34, 124.35, 124.53, 124.65, 124.70, 124.77, 125.00, 125.05, 125.17, 125.30, 125.40, 125.44, 127.13, 127.30, 127.39, 127.44, 127.58, 127.66, 127.68, 127.77, 127.98, 128.25, 128.39, 128.54, 128.67, 128.73, 128.81, 129.11, 134.15, 134.61, 134.73, 137.81, 140.79, 141.06, 141.29, 143.72, 145.62, 145.75, 163.16, 170.23, 170.33; δ_P: 0.23; HRMS (FAB$^-$) $C_{60}H_{63}N_2O_{13}PS$, found: 1081.3673 (M – H)$^-$, calcd 1081.3710 (M – H)$^-$.

33. 73 % Yield. R_f = 0.42 (15:85:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 2.02 (s, 3H, CH_3), 2.95 (m, 2H, CH_2), 3.08 (m, 4H, CH_2S), 3.28 (s, 3H, OCH_3), 3.45 (m, 1H), 3.54 (m, 1H, H-4), 3.69 (m, 1H, H-5), 3.83 (dd, J = 9.1 Hz, J = 3.6 Hz, 1H, H-3), 3.96–4.42 (m, 10H), 4.55 (AB, 2H, CH_2Ph), 4.58 (s, 1H, H-1), 5.11 (m, 2H, CH=CH_2), 5.30 (bs, 1H, H-2), 5.82 (m, 1H, CH=CH_2), 5.96 (d, J = 7.6 Hz, 1H, NH), 7.21–7.42 (m, 13H, Ph), 7.54–7.79 (m, 9H, NH amide, Fm Ph); δ_C: 21.00, 29.71, 35.89, 36.64, 41.07, 46.84, 47.09, 54.47, 54.54, 54.87, 64.08, 64.14, 64.99, 65.07, 67.17, 68.57, 70.99, 71.10, 71.69, 73.85, 74.10, 77.28, 77.88, 98.63, 116.47, 119.79, 119.94, 124.94, 125.20, 125.30, 127.02, 127.12, 127.52, 127.65, 127.71, 127.90, 128.33, 134.92, 138.01, 140.97, 141.03, 141.23, 143.82, 145.88, 145.93, 155.97, 170.32; δ_P: 0.28; HRMS (FAB$^-$) $C_{53}H_{57}N_2O_{13}PS$, found: 991.3249 (M – H)$^-$, calcd 991.3241 (M – H)$^-$.

34. 84 % Yield. R_f = 0.35 (10:90:1, MeOH:CH_2Cl_2:Et_3N). δ_H ($CDCl_3$): 3.06 (m, 2H, CH_2), 3.31 (s, 3H, OCH_3), 3.48 (m, 5H, H-2, 3, 4, CH_2), 3.71 (m, 1H, H-5), 3.95 (m, 3H), 4.09 (m, 3H), 4.18 (m, 2H), 4.35 (m, 2H), 4.51–4.97 (m, 3 × AB, 8H, CH_2Ph, H-1, CH), 6.04 (m, 1H, NH), 7.13–7.39 (m, 22H, Ph), 7.52–7.76 (m, 9H, FmPh, NH amide); δ_C: 29.70, 35.93, 36.62, 41.08, 46.81, 47.06, 54.55, 55.15, 64.08, 64.53, 67.13, 70.04, 73.26, 74.85, 75.60, 77.34, 79.79, 81.85, 97.93, 119.76, 119.93, 120.05, 124.97, 125.21, 125.32, 127.11, 127.38, 127.50, 127.60, 127.66, 127.83, 128.08, 128.33, 128.40, 128.65, 132.00, 132.13, 134.77, 138.13, 138.38, 138.80, 140.97, 141.20, 143.81, 145.90, 145.95, 170.27, 172.85; δ_P: 1.11; HRMS (FAB$^-$)

$C_{62}H_{63}N_2O_{12}PS$, found: 1089.3784 $(M - H)^-$, calcd 1089.3761 $(M - H)^-$.

(B) Competition reactions. The same procedure as described above for the control experiments was followed except that the glucosamine derivative **35**[18] (~ 0.01 mmol, 1 eq.) was added to the reaction mixture prior to addition of Et_3N and the cysteine derivative. Reaction times were 30–50 min in each case. The products from completion experiments involving amines **27**, **28**, and **31** gave **32**, **33**, and **34** respectively as the sole coupling products. The yields were 77, 74, and 90 %, respectively. See above for data.

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(*Received in U.S.A.* 15 *February* 1994; *accepted* 6 *May* 1994)