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Solid-Phase Synthesis of New *S*-Glycoamino Acid Building Blocks

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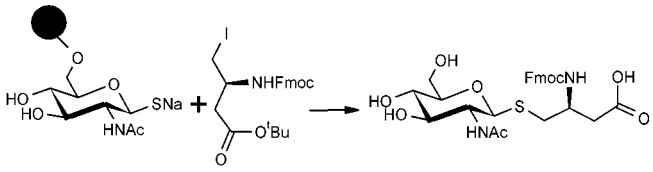
ABSTRACT



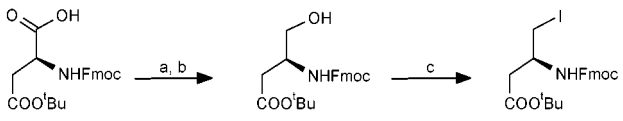
Efficient synthesis of unprotected *S*-glycoamino acid building blocks in the solid phase by coupling a sugar 1-thiolate with iodine activated fluoren-9-ylmethoxycarbonyl (Fmoc) protected amino acids.

Glycoconjugates have been implicated in many biological events important in inflammation, immune response, and tumor metastasis.[1] Of special interest are glycoproteins containing modified glycosyl amino acids, thus exhibiting new properties. While *S*-glycopeptides have been isolated from nature,[2] the driving force behind the synthesis of *S*-glycosides has been the production of glycopeptidomimetics with enhanced stability toward chemical and enzymatic degradation.[3] Several attempts have been made to introduce the S-linkage by chemical synthesis.[4] A variety of glycosylation methods have been applied including Koenigs−Knorr,[5] glycosyl fluorides,[6] Lewis acid-catalyzed glycosylation,[7] trichloroacetimidates[8] and isothiouronium salts.[9] These methods generally use protected carbohydrates and cysteine derivatives. We present here the solid-phase synthesis of new *S*-glycoamino acid building blocks. The key feature of this method is that a nucleophilic sugar 1-thiolate without protective groups is used for coupling with an iodine activated fluoren-9-ylmethoxycarbonyl (Fmoc)/*t*-Bu protected amino acid.

Hummel and Hindsgaul[10] described the use of a sugar-1-thiolate without protective groups in the solid phase as the nucleophile for coupling with trifluoromethanesulfonate (triflate)-activated glycosides. Since the use of triflates with Fmoc-protected amino acids is not compatible, we decided to prepare amino acid iodo derivatives for the synthesis of *S*-glycoamino acid building blocks.

First the free acid functions of the *N*-Fmoc/*t*-Bu ester protected glutamic and aspartic acid derivatives were reduced to the corresponding alcohols **2**, **4**, **6**, and **8** (Scheme 1,

Scheme 1[a]



1 2 3

[a]Reagents and conditions:(a) $ClCO_2C_2H_5$, Et_3N, THF, -30°C, 30 min; (b) $NaBH_4$, H_2O, THF, 0°C to 20°C, 4 h, 74%; (c) I_2, PPh_3, imidazole, $PhCH_3$, 120°C, 20 min, 80% .

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shown for Fmoc-Asp(O^{t}Bu)) using ethyl chloroformate and sodium borohydride in tetrahydrofuran (yields are given in Table 1).

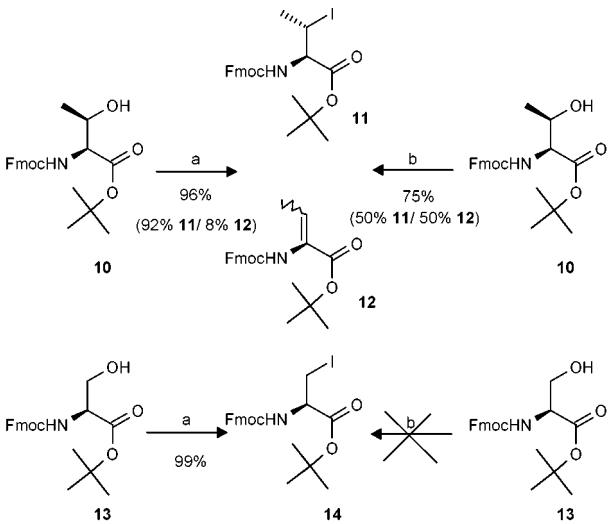
Table 1

amino acids	reduction to alcohol	conversion into iodide
Fmoc-Asp(O^{t}Bu)-OH	**2** (74%)	**3** (80%)
Fmoc-Asp-O^{t}Bu	**4** (81%)	**5** (81%)
Fmoc-Glu(O^{t}Bu)-OH	**6** (86%)	**7** (95%)
Fmoc-Glu-O^{t}Bu	**8** (83%)	**9** (76%)

Treatment of the different alcohols **2**, **4**, **6**, and **8** with triphenylphosphine−iodine−imidazole[11] in toluene at 120 °C afforded the corresponding iodo derivatives **3**, **5**, **7**, and **9** in 76−95% yields after purification by flash chromatography on silica gel.

The same procedure applied to Fmoc-Thr-O^{t}Bu **10** and Fmoc-Ser-O^{t}Bu **13**[12] gave only low yields with threonine and no product with serine (Scheme 2). In the case of

Scheme 2[a]

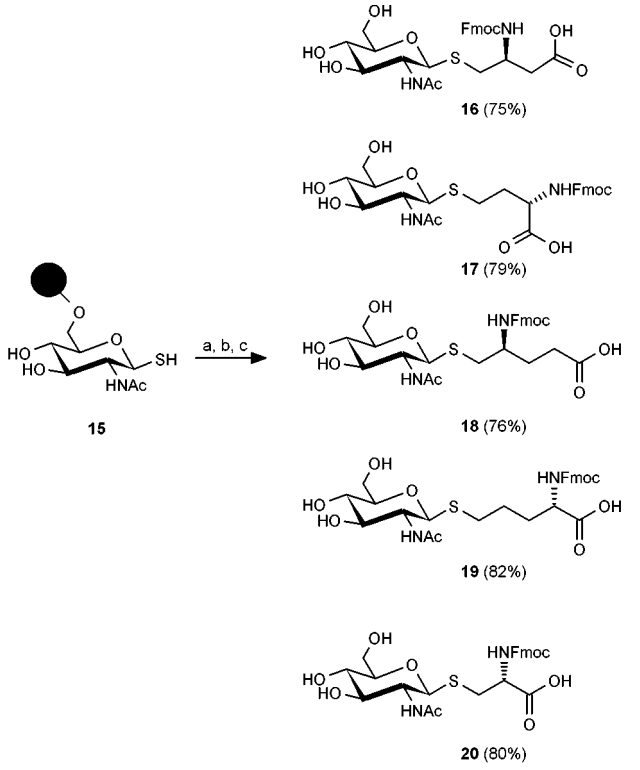


[a]Reagents and conditions: (a) PDPI, imidazole, CH_2Cl_2, reflux; (b) I_2, PPh_3, imidazole, $PhCH_3$, 120°C, 20 min

threonine, we observed the formation of elimination product **12** and iodo compound **11** in a ratio of 1:1. No better results could be obtained by decreasing reaction time or temperature; therefore we tried different conditions. The use of polystyryl diphenylphosphine−iodine (PDPI) complex was previously described[13] for the conversion of *N*-Fmoc *β*-amino alcohols into their corresponding iodides in high yields and without any detectable epimerization of the chiral center. Similar reaction of the alcohols **10** and **13** with triphenylphosphine−iodine complex in the presence of imidazole in anhydrous dichloromethane gave the iodides **11** (with inversion of configuration) and **14** in high yield (**11** 88% and **14** 99%) under very mild conditions (3 h reflux for Thr **11** and 10 min for Ser **14**). No elimination product was observed with Ser and only 7% in the case of Thr. Both iodo compounds were used without further purification in the following steps.

Thiol **15** immobilized on a trityl chloride derivatized polystyrene resin was obtained after reduction of the disulfide[10] (Scheme 3). The loading of the thiol on the solid

Scheme 3[a]



[a]Reagents and conditions: (a) NaOMe, THF, 20°C, 2 h; (b) [15] Crown-5, iodo derivatives **3**-**6**, THF, 45°C, 3 d; (c) 50% TFA, CH_2Cl_2, 20°C, 30 min.

support was determined by elemental analysis (sulfur content) and was 1.2 mmol/g. **15** was treated for 2 h with NaOMe/THF to enhance its nucleophilicity[10] and washed with a mixture of MeOH/THF to eliminate traces of NaOMe. The resulting sodium thiolate was coupled with amino acid iodo derivatives **3**, **5**, **7**, **9**, and **14** in THF at 45 °C in the presence of [15]crown-5 as complexing agent. After 3 days, the resin was washed and the glyco amino acids were cleaved from the resin by treatment with 50% TFA in CH_2Cl_2. All final derivatives **16**−**20** were obtained after reverse-phase HPLC purification in good yields (75 to 82%).

Reaction of the immobilized thiolate with threonine derivative **11** however gave only poor yields (<5%).

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To achieve better results we studied the influence of different conditions such as solvent (THF or DMF), temperature (20 or 45 °C), and complexing agent ([15]crown-5 or Kryptofix 221). The results are summarized in Table 2.

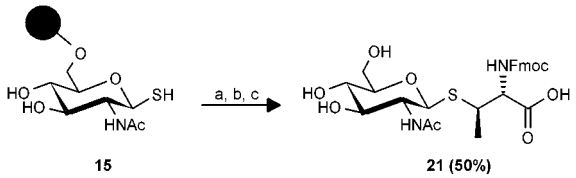
Table 2

temperature	solvent	complexing agent	yield, %
20 °C	THF	[15]crown-5	<20
		Kryptofix 221	
20 °C	DMF	[15]crown-5	30
		Kryptofix 221	
45 °C	THF	[15]crown-5	<5
		Kryptofix 221	
45 °C	DMF	[15]crown-5	50
		Kryptofix 221	<10

Best yields were obtained using DMF at 45 °C in the presence of [15]crown-5 as complexing reagent (Scheme 4). We observe that compound **21** reverts back to its threo configuration after the substitution.

In conclusion, we present here an efficient synthesis of new *S*-glycoamino acid building blocks in the solid phase which are ideal building blocks for the solid-phase synthesis of *S*-glycopeptides using the Fmoc strategy. All glycosides were obtained stereoselectively and in high yields. Using iodine-activated amino acids gives the corresponding *S*-glycosides under very mild conditions with retention of configuration (double inversion) if the iodine is part of an asymmetric carbon atom.

Scheme 4[a]



15 **21 (50%)**

[a]Reagents and conditions: (a) NaOMe, THF, 20°C, 2 h; (b) [15] Crown-5, **11**, DMF, 45°C ; (c) 50% TFA, CH_2Cl_2, 20°C, 30 min.

Supporting Information Available: Experimental procedures for preparation of compounds **2**−**9**, **11**, **14**, and **16**−**21** and analytical data for **2**−**9**, **11**, **14**, and **16**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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