

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

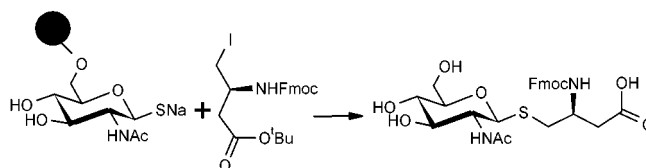
Laurence Jobron and Gerd Hummel*

Jerini Bio Tools GmbH, Rudower Chaussee 29, 12489 Berlin, Germany

hummel@jerini.de

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ABSTRACT



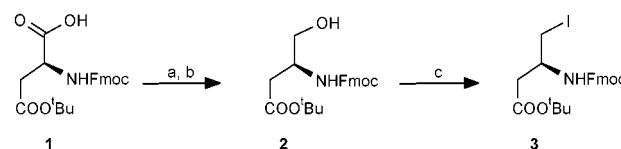
Efficient synthesis of unprotected S-glycoamino acid building blocks in the solid phase by coupling a sugar 1-thiolate with iodine activated fluoren-9-ylmethoxycarbonyl (Fmoc) protected amino acids.

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 trichloroacetimidates⁸ and isothiuronium 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 Hindsgaul¹⁰ 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

^aReagents and conditions: (a) $\text{ClCO}_2\text{C}_2\text{H}_5$, 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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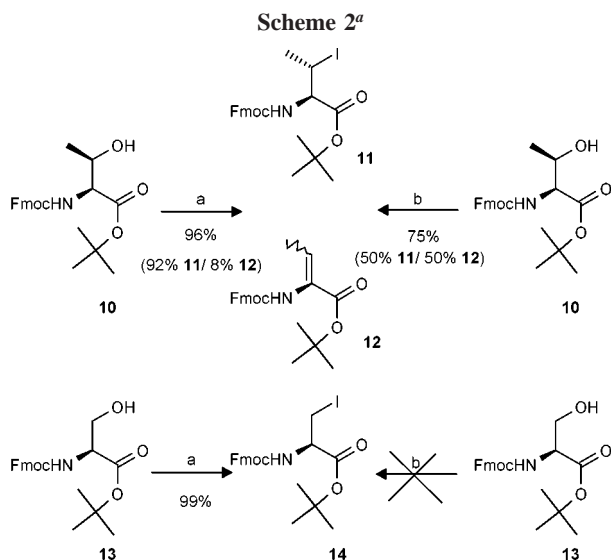
shown for Fmoc-Asp(O^tBu)) using ethyl chloroformate and sodium borohydride in tetrahydrofuran (yields are given in Table 1).

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–imidazole¹¹ 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^tBu **10** and Fmoc-Ser-O^tBu **13**¹² gave only low yields with threonine and no product with serine (Scheme 2). In the case of

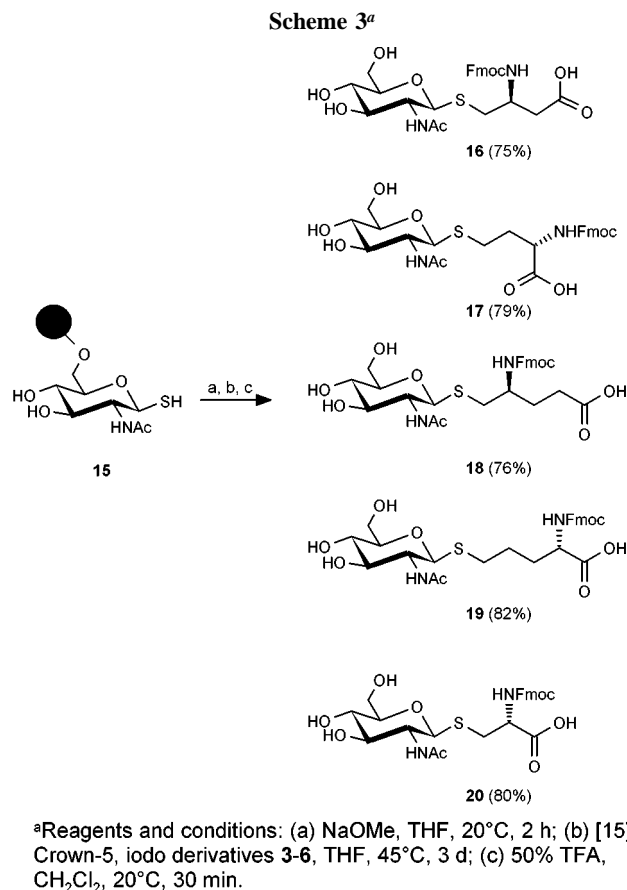


^aReagents and conditions: (a) PDPI, imidazole, CH₂Cl₂, reflux; (b) I₂, PPh₃, imidazole, PhCH₃, 120 °C, 20 min

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 described¹³ 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 disulfide¹⁰ (Scheme 3). The loading of the thiol on the solid



^aReagents 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, CH₂Cl₂, 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 nucleophilicity¹⁰ 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 CH₂Cl₂. 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.

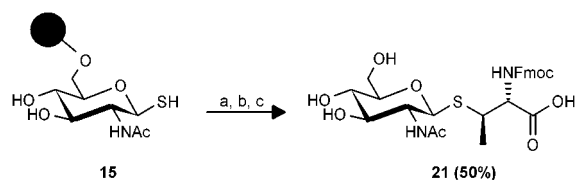
Table 2

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

Scheme 4^a



^aReagents and conditions: (a) NaOMe, THF, 20°C, 2 h; (b) [15] Crown-5, **11**, DMF, 45°C; (c) 50% TFA, CH₂Cl₂, 20°C, 30 min.

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.

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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