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A convenient microwave-assisted synthesis of *N*-glycosyl amino acids

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Abstract—Optimization of coupling reactions of glycosylamines with Fmoc-protected aspartic acid, by microwave approach, is described. Different reaction conditions, quantities of substrates and solvents were tested to develop simple and reproducible methodologies. The best results were obtained using new triazine-based coupling reagents with a monomode microwave Discover® BenchMate™ instrument (CEM). The *N*-glycosyl amino acids were then deprotected to achieve final products for SPPS.

Growing evidences indicate that N-glucosylation is a post-translational modification (PTM) that, either native or aberrant, may play a fundamental role in a large number of biological events.[1] By an innovative 'chemical reverse approach' we demonstrated for the first time that N-glucosylation is possibly triggering autoantibody response in Multiple Sclerosis (MS).[2] Therefore, we developed a synthetic β-hairpin peptide structure optimally exposing on the tip of the β-turn the minimal epitope Asn(Glc). This *N*-glucopeptide is the first specific synthetic probe able to detect autoantibodies in MS patients' sera by an immunoenzymatic assay. The hypothesis that aberrant PTMs trigger autoantibodies in autoimmune diseases is also valid in Guillain-Barré Syndrome (GBS). In fact, bacteria or viruses can elicit an antibody response to specific glycoepitopes cross-reactive with host gangliosides.[3]

Therefore, synthetic glycopeptide probes are useful structural mimic tools of these highly complex ganglioside epitopes able to neutralize or remove autoantibodies in GBS. As a consequence, it is important to set up convenient reactions, in terms of yield and reaction time, leading to glycosyl asparagine residues orthogonally protected for solid-phase peptide synthesis (SPPS) of *N*-glycopeptides by Fmoc/*t*-Bu strategy.[4]

Currently, the synthesis of these building blocks containing glycosyl moieties, linked to the carboxyl function of aspartic acid by an amide bond, typically requires glycosylamines and coupling reagents usually employed in SPPS.[5] Alternatively, Lansbury reported for a single glycosyl amine[6] its direct coupling by a convergent approach to protected peptides containing Asp.[7] There are no doubts, however, that the most versatile methodology in chemical synthesis of *N*-glycopeptides is the building-block approach. Therefore, we focused our attention on optimization of the key step of pathways to large scale synthesis of glycosylated asparagine residues, via glycosylamines.[8]

Although microwave-assisted reactions are widely applied in other domains of organic synthesis, their use in glycopeptide synthesis has been rather limited because of the low stability of carbohydrates to thermal degradation.[9] Generally speaking, the microwave energy should mainly be related to heat effects, and many reports have focused on 'improvement of reactions'. The results show enhanced reaction rates and

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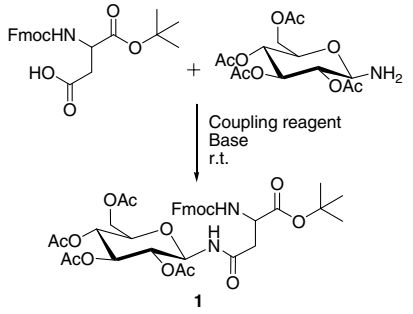
higher product yields as compared to conventional approaches.[10,11]

In this Letter, we report optimization of the coupling reactions between aspartic acid side chain and a series of protected aminosugars using microwave irradiation. Up to now, it is described that this coupling reaction is performed in a long reaction time, usually 24 h, to achieve an almost quantitative yield.[12] We initially investigated the condensation reaction of glucosamine, protected on the hydroxyl functions as acetyl derivative, with the side chain of Fmoc-aspartic acid *tert*-butyl ester, to give Fmoc-L-Asn(GlcAc4)-O*t*-Bu (**1**) (Scheme 1).

First, glucosamine was coupled to the carboxyl function of Fmoc-L-Asp-O*t*-Bu by a multimode Ethos Micro-SYNTH instrument (Milestone), using a sealed vessel, DMF as solvent and HATU[13] as coupling reagent, in the presence of NMM. By this way, we did not obtain satisfying yields (Table 1).

We performed the same reaction using our new efficient triazine-based coupling reagent (TBCR), DMT-NMM/BF_4[14] in CH_3CN. Activation of carboxylic acids by TBCRs is particularly effective because of the formation of triazine 'superactive esters'. The usefulness of TBCRs as coupling reagents has been recently confirmed in the synthesis of protected dipeptides and esters, sterically hindered amino acids, in manual and automated SPPS of difficult peptide sequences, and head-to-tail constrained cyclopeptide analogues, using small excesses of protected amino acids (1 equiv).[15,16] A comparative study of the coupling reaction between glucosamine and aspartic acid was performed using DMT-NMM/BF_4 (in situ activation) or CDMT (pre-activation) and



Scheme 1. Coupling reaction to Fmoc-L-Asn(GlcAc4)-O*t*-Bu (**1**).

Table 1. Coupling of glucosamine to aspartic acid side chain to obtain product **1**, by multimode microwave instrument, via HATU activation

Method	Yield[a] (%)
30 s, 75 W (×2), $T_{max} = 40$ °C	22
6 min, 40 W, $T_{max} = 55$ °C	20

Reagents: Fmoc-L-Asp-O*t*-Bu (0.1 mmol), GlcAc4-NH_2 (1 equiv), HATU (1 equiv), NMM (1 equiv), DMF (3 ml). Protocol is described in the Supplementary data. Characterization of product **1** is in accordance with the literature.[12]

[a] After re-crystallization from EtOH.

Table 2. Coupling of glucosamine to aspartic acid side chain to obtain product **1**

Coupling reagent	Yield (%)
CDMT	70[a]
DMT-NMM/BF_4	84[a]
TBTU	75[b]
HATU	70[b]
BOP	58[b]

Reagents: Fmoc-L-Asp-O*t*-Bu (0.5 mmol), GlcAc4-NH_2 (1 equiv), coupling reagent (1 equiv), NMM (1 equiv), CH_3CN or DCM (10 ml), rt, 18 h. Protocol is described in the Supplementary data. Characterization of product **1** is in accordance with the literature.[12]

[a] After re-crystallization from EtOH.

[b] After precipitation from AcOEt/hexane.

different coupling reagents usually employed in amide bond formation, such as TBTU, HATU, and BOP.[17] The use of TBCRs, but particularly in situ activation by DMT-NMM/BF_4, led to higher yields in the final product (Scheme 1) even after re-crystallization that was in any case easier to be performed compared to the other coupling reagents (Table 2).

To decrease the above mentioned coupling reaction time maintaining high yields and to perform reproducible microwave irradiation experiments, we decided to employ a commercially available monomode microwave instrument equipped with a sealed reactor dedicated to chemical synthesis. CEM Discover® BenchMate™ has the advantage to focus the microwave irradiation on the vessel in order to avoid dispersion. Moreover, the focused reactor has the ability to maintain the target temperature while subjecting the reaction mixture to continuous irradiation with microwaves by cooling the reaction vessel with a stream of nitrogen.

We selected DMT-NMM/BF_4 as activator because of the good results reported in Table 2 to obtain Fmoc-L-Asn(GlcAc4)-O*t*-Bu (**1**) by a microwave approach (Table 3). The reaction was complete in less than 5 min of microwave irradiation (100 W), maintaining the vessel temperature at 70 °C.

In this short time we obtained products characterized by a high level of purity with a yield as good as that reported in the literature.[12] We studied this coupling reac-

Table 3. Coupling of glucosamine to aspartic acid side chain, to obtain product **1**, by monomode microwave instrument, via DMT-NMM/BF_4 activation

Method	Yield[a] (%)
6 min, 40 W, $T_{max} = 55$ °C	26
0.5 + 2 min, 100 W, $T_{max} = 40$ °C	44
3.5 + 3.5 min, 100 W, $T_{max} = 40$ °C	61
1.5 + 3 min, 100 W, $T_{max} = 50$ °C	52
1.5 + 3 min, 100 W, $T_{max} = 60$ °C	68
1.5 + 3 min, 100 W, $T_{max} = 70$ °C	80

Reagents: Fmoc-L-Asp-O*t*-Bu (0.1–1.5 mmol), GlcAc4-NH_2 (1 equiv), DMT-NMM/BF_4 (1 equiv), NMM (1 equiv), CH_3CN (2–7 ml). Protocol is described in the Supplementary data. Characterization of product **1** is in accordance with the literature.[12]

[a] After re-crystallization from EtOH.

tion testing different concentrations of reagents and analyzing the influence of temperature on the microwave-assisted process. We were able to produce gram quantities of the desired product. Actually, we demonstrated that the yields significantly increased with the temperature (70 °C). Moreover, we performed a control experiment, where the coupling reaction of glucosamine to aspartic acid side chain, via DMT-NMM/BF_4 activation, was carried out at 70 °C (without microwave assistance), using reaction conditions and reagents reported in Table 3. After work-up, product **1** was obtained in a very low yield (<10%) compared to microwave-assisted synthesis (80%), confirming the fundamental role of microwaves in increasing yield using very short time reactions.

The optimized reaction conditions by microwaves were applied to the synthesis of aspartic acid building blocks containing different mono and disaccharides orthogonally protected for SPPS. In particular, we selected some sugars most abundant in nature such as galactose, mannose, and cellobiose (Table 4).

Glycosyl amino acids were then deprotected on the carboxylic function to achieve the amino acid derivatives orthogonally protected for SPPS **6**–**10** to be introduced in stepwise peptide synthesis (Fig. 1).

In conclusion, the use of microwave irradiation allowed a rapid and high-yield preparation of *N*-glycosyl amino acids orthogonally protected for SPPS. We carried out the coupling of glucosamine with aspartic acid by conventional protocol as well as by microwave irradiation. Dramatic improvements in the reaction rates were observed under the latter conditions. Both multimode and monomode microwave instruments were used for the experiments. Different reaction conditions, quantities of substrates and solvents were tested to develop a simple and reproducible coupling methodology. Our interest was to optimize a large-scale synthesis of products to be used in glycopeptide synthesis and to demonstrate that the method is suitable for a variety of sugar moieties. The best results were obtained using the tri-

Table 4. Coupling of different glycosylamines to aspartic acid side chain by monomode microwave instrument, via DMT-NMM/BF_4 activation

Sugar (1 mmol)	Yield[a] (%)	Product
GalAc4-NH_2	73	2
ManBz4-NH_2	75	3
GlcAc4β-4GlcAc3-NH_2	75	4

Reagents: Fmoc-L-Asp-O*t*-Bu (1 equiv), DMT-NMM/BF_4 (1 equiv), NMM (1 equiv), CH_3CN (5 ml). Method: 1.5 + 3 min, 100 W; T_{max} = 70 °C. Protocol is described in the Supplementary data. Characterization of product **2**–**4** is in accordance with the literature.[12] The microwave-assisted method applied in Table 4 was used for the coupling of glucosamine with glutamic acid side chain to obtain Fmoc-L-Gln(GlcAc4)-O*t*-Bu (**5**), yield 82%. Characterization of product **5** is in accordance with the literature.[12] The synthesis of mannosyl derivative ManBz4-NH_2 was performed following the procedure reported in the literature.[18] The use of benzoyl protective groups is reported as a high-yielding strategy.

[a] After re-crystallization from EtOH.

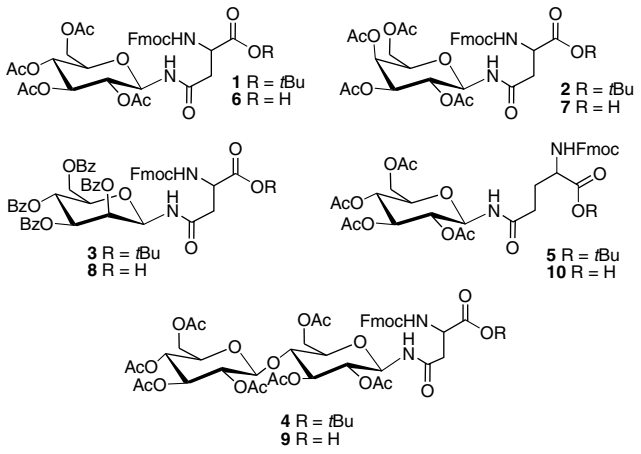


Figure 1. *N*-Glycosyl amino acids **1**–**10**.

azine-based coupling reagents in a monomode microwave instrument (Tables 3 and 4). The reaction times were reduced from 16–24 h to 5 min allowing speeding up of the synthesis of long peptide sequences.

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Supplementary data

In the Supplementary data experimental procedures and characterization data of the final products are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.087.

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