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Non-catalytic reaction of isonitriles and carboxylic acids en route to amide type linkages

Xuechen Li[†] and Samuel J. Danishefsky^{*,†,‡}

[†]Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10065

[‡]Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, New York 10027

Abstract

This protocol describes the preparation of an *N*-methyl-asparagine linked glycosyl amino acid, based on a reaction between carboxylic acids and isonitriles. Under microwave/thermolysis, carboxylic acids can couple with isonitriles without external catalyst, to afford *N*-formyl-amides, which may be further advanced to the corresponding amides, *N*-methyl amide, and *N*-methyloyl amide. The example reaction of β -galactopyranosyl isonitrile (**7**) with a protected aspartic acid under microwave condition in 30 minutes stereoselectively leads to a β -galactopyranosyl-*N*-formyl-asparagine **9**. Further chemical transformations readily convert **9** into β -galactopyranosyl-*N*-methyl-asparagine (**11**).

Search terms

Synthetic chemistry; amide bond formation; isonitrile chemistry

Introduction

The amide bond represents one of the most abundant linkages found in nature, as exemplified by the peptide and protein. A number of historic methods are commonly employed for the formation of this critical bond, including the Staudinger reaction^{1,2}, Ritter reaction³, Schotten-Baumann reaction⁴, Ugi reaction^{5,6}, and the Passerini reaction^{7,8}. More recently, a multitude of advanced techniques have been developed, which seek to address the demands for improved efficiency and preservation of stereochemical integrity, as well as the desire to minimize economic cost and utilize more practical solvent systems. The majority of efforts have focused on the development of efficient strategies for the activation of the carboxylic acid moiety, either through the introduction of external coupling reagents^{9a} or through the derivatization of the carboxylic acid component as an acyl chloride,^{9b} acyl fluoride,^{9c} or mixed anhydride.^{9d} Although, these methods have been extensively used to construct amidic bonds, acylating hindered or secondary amines can still pose a significant challenge. In addition, each of these protocols depends on the addition of external reagents, which may cause troublesome purification and racemization problems.⁹

In contrast, we recently disclosed a highly efficient coupling protocol of a very different genre, in that it combines two functional groups which have been known from virtually the dawn of organic chemistry – carboxylic acids and isonitriles¹⁰ – without the requirement of an external

catalyst. Thus, as outlined in Figure 1, under this novel protocol, carboxylic acid (**1**) and isonitrile (**2**) substrates are heated in a microwave oven to provide adducts of the type **4**. The reaction sequence is presumed to commence with protonation of the isonitrile component (**2**). Subsequent carboxylate-nitrilium neutralization affords an intermediate **3**, which is formally an *O*-acylated imidic acid. Finally 1,3 *O*→*N* acyl transfer provides the target *N*-formyl amide adducts of the type **4**. This general reaction sequence has been extended to a number of complex substrates, providing adducts in high yields – generally above 80% (entry 1, 3, 4, and 5, Table 1). To our surprise, when a GlcNAc isonitrile is used, instead of producing *N*-formyl amide, an ester was obtained in a stereospecific fashion (entry 6, Table 1). To solve this problem, the C₂ amine needs to be masked as an azido group (entry 5, Table 1). The azide group can be readily advanced to an *N*-acetyl group, by reduction and acetylation.

As outlined in Figure 2, these *N*-formyl amide adducts (cf. **4**) may be further derivatized to provide a number of valuable product types. Thus, the *N*-formyl amide moiety has been shown to be readily converted to various useful functionalities, such as the amide, *N*-methyl amide, and *N*-methyloyl amide.¹⁰ This approach importantly provides access to tertiary amides, which are difficult to access through the conventional route of acylating a secondary amine.

Experimental Design

The sequence of preparation of glycosylamino acid **11** is described in this protocol as an example (Figure 3). The isonitrile **7** is prepared from the previously known glycosyl azide **5**¹¹, via *in situ* formylation of glycosylamine by hydrogenation and dehydration by triphosgene. In the event, the anomeric isonitrile **7** reacts with **8** under microwave reactor (158 °C, 30 min) to produce **9**. These reactions appear to be anomERICALLY specific, i.e. the β-isonitrile produces, correspondingly, the β-*N*-linked glycosyl amino acid. We next describe the sequence for the conversion of **9** to the *N*-methyl amide, **11**. The route commences with chemospecific reduction of the *N*-formyl function of the mixed imide, to provide the “methylo” derivative, **10**. Thus, further reduction of the “methylo” intermediates with triethylsilane in the presence of trifluoroacetic acid affords the corresponding *N*-methyl compound **11**. The hydroxymethyl derivatives of **10** might not be stable enough to purify, thus it is advisable to proceed to the next step in crude form for reaching other amide modifying structures.

Materials

Reagents

Compound **5**, obtained as reported in ref. 11.

Preparation of Compound 5

1. Weigh 220 mg (0.407 mmol) of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (Acros, cat. no. 35394 2500) into a 25-ml round-bottomed flask with a Teflon-coated magnetic stir bar.
2. Add 1 ml of acetic anhydride and 2 ml of pyridine. Stir at room temperature for 2 h.
3. Remove the solvent by rotary evaporation and dry the residue under vacuum.
4. Dissolve above crude material in anhydrous dichloromethane (5 ml).
5. Slowly add TMSN₃ (75 μl, 0.57 mmol) and 0.12 mL SnCl₄ (1 M in dichloromethane, 0.12 mmol).
6. Stir at room temperature for 1 h.

7. Pour the reaction mixture into a separatory funnel filled with 10 ml sat. NaHCO_3 . Extract the organic material with dichloromethane (20 ml $\times$ 2) and combine the organic phases
8. Wash the organic phase with brine (20 ml). Dry the organic phase over anhydrous sodium sulfate (add $\sim$ 1 g, and stir the mixture for 5 min).
9. Decant the solvent carefully into a 100-ml round-bottomed flask, and remove volatiles by rotary evaporation at $\sim$ 40 $^\circ\text{C}$. It takes $\sim$ 30 min.
10. Purify the desired isonitrile by flash chromatography on silica gel (1.5 cm i.d. $\times$ 20 cm length) using 1:8 ethyl acetate/hexanes as eluent. (TLC R_{f1} = 0.45, R_{f2} = 0.40, EtOAc/Hexanes, 1/5, UV lamp) Fraction 1 (34-38%) $\rightarrow$ β -glycosyl azide; Fraction 2 (50-58%) $\rightarrow$ α -glycosyl azide.
11. Evaporate solvents from the eluate using a rotary evaporator at $\sim$ 40 $^\circ\text{C}$. It takes $\sim$ 1 h. Dry under vacuum. Product can be dried overnight under vacuum

TMSN_3 (azidotrimethylsilane) (Aldrich, cat. no. 155071)

SnCl_4 (tin tetrachloride, 1.0 M methylene chloride) (Aldrich, cat. no. 249955)

Et_3N (Aldrich)

Palladium on carbon (10 wt. %) (Aldrich, cat. no. 205699)

Formic acid **CAUTION** corrosive (wear gloves when handling)

Acetic anhydride (Aldrich)

Triphosgene **CAUTION** Very toxic (handle under fume hood; Any object that may have come into contact with triphosgene should be rinsed with 10% NaOH solution)

Sodium borohydride (Aldrich, cat. no. 213462)

Fmoc-Asp-O-*t*-Bu (NovaBiochem, cat. no. 04-12-1080)

TFA **CAUTION** corrosive (wear gloves when handling)

Triethylsilane (Aldrich, cat. no. 230197)

Dichloromethane

Ethyl acetate

Hexane **CAUTION** Flammable

Chloroform (anhydrous, Aldrich, cat. no. 372978)

Methanol **CAUTION** Toxic (do not inhale or drink)

Sodium sulfate, anhydrous

Brine (saturated aqueous NaCl solution)

Saturated aqueous NaHCO_3

10% aqueous NaHSO_4

10% NaOH solution

Celite

Thin-layer chromatography (Silica gel 60A, layer thickness 250 μm , Whatman)

Silica Gel (SilicaFlash F60, 40-63 μm , cat. R100030B, Silicycle)

Equipment

Round-bottomed flasks
Filter Buchner funnel (Kontes, cat. no. K293050-0344)
Dual argon vacuum manifold with vacuum line
Rubber septum
Disposable syringes and injection needles
Rotary evaporator
Chromatographic columns
 ^1H NMR and ^{13}C NMR spectrometers
Biotage microwave reactor (Initiator)
LCMS and HPLC

Reagent Setup

All commercial materials were used as supplied unless otherwise noted. Dry dichloromethane was obtained from a solvent purification system.

Procedure

Synthesis of glycosyl isonitrile (7) TIMING 20 h

- 1 | Weigh 120 mg (0.212 mmol) of glycosyl azide **5**, obtained as reported in ref. ¹¹, into a 10-ml round-bottomed flask.
- 2 | Dissolve in ethyl acetate (3 ml). Add a Teflon-coated magnetic stir bar.
- 3 | Add 88 μl (0.632 mmol) of triethylamine using a syringe.
- 4 | Add 10 mg of palladium on carbon (10% wt) and turn on the magnetic stirrer.
- 5 | Cap the flask and insert a balloon of H_2 . Stir vigorously for 30 min.
- 6 | Remove the balloon and add formic acetic anhydride (1 ml, pre-made by heating 3 ml formic acid and 5 ml acetic anhydride at 60 $^\circ\text{C}$ for 3 h).
- 7 | Stir the reaction mixture for 1 h.
- 8 | Filter the reaction mixture through a filter Buchner funnel packed with 2 mm celite into a 100-ml round-bottomed flask.
- 9 | Remove the volatile materials by rotary evaporation at $\sim 40^\circ\text{C}$. It takes ~ 30 min. Purify the desired formamide by flash chromatography column packed with silica gel (2.5 cm i.d. $\times$ 15 cm length) using 3:1 ethyl acetate/hexanes as eluent (TLC R_f = 0.3 EtOAc/Hexanes, 1/3, UV lamp).
- 10 | Evaporate solvent from the eluent using a rotary evaporator at $\sim 40^\circ\text{C}$. It takes ~ 1 h. Dry under vacuum ($\rightarrow$ **6**, 85-90%).

PAUSE POINT Product **6** can be dried overnight under vacuum.

11 | Dissolve 20 mg (0.035 mmol) of **6** in dry dichloromethane (2 ml) in a 10-ml round-bottomed flask and add a Teflon-coated magnetic stir bar. Cool the flask to 0 °C.

12 | Add 39 µl (0.28 mmol) of triethylamine using a syringe.

13 | Add 10 mg (0.033 mmol) of triphosgene. Stir the resulting mixture at 0 °C for an additional 5 min and warm the reaction mixture to room temperature for 45 min.

CAUTION Triphosgene is very toxic. Special care should be taken when handling it.

14 | Pour the reaction mixture into a separatory funnel filled with 10 ml sat. NaHCO₃. Extract the organic material with dichloromethane (20 ml × 2) and combine the organic phases.

15 | Wash the organic phase with brine (20 ml).

16 | Dry the organic phase over anhydrous sodium sulfate (add ~ 1 g, and stir the mixture for 5 min).

17 | Decant the solvent carefully into a 100-ml round-bottomed flask, and remove volatiles by rotary evaporation at ~ 40 °C. It takes ~30 min.

18 | Purify the desired isonitrile by a flash chromatography column packed with silica gel (1.5 cm i.d. × 20 cm length) using 1:4 ethyl acetate/hexanes as eluent. (TLC R_f = 0.6 EtOAc/Hexanes, 1/3, UV lamp).

19 | Evaporate solvents from the eluate using a rotary evaporator at ~ 40 °C. It takes ~ 1 h. Dry under vacuum (→ **7**, 80-85%).

PAUSE POINT Product **7** can be dried overnight under vacuum

Synthesis of glycosyl N-formyl-L-asparagine derivative (**9**) TIMING 4 h

20 | Weigh 10 mg (0.018 mmol) of glycosyl isonitrile **7** and 11 mg (0.026 mmol) aspartic acid **8** into a 0.5-2.0 ml microwave tube.

21 | Add dry chloroform (0.5 ml) under argon using a syringe.

22 | Cap the tube and place it into the microwave reactor. Set it to 158 °C and 30 min.

CRITICAL STEP the faster the microwave reactor reaches the set temperature, the better the reaction; chloroform can be replaced with 1,2-dichloroethane (anhydrous).

23 | Load the reaction mixture directly onto a flash chromatography column packed with silica gel (1.5 cm i.d. × 20 cm length). Purify the desired imide **9** using 1:3 ethyl acetate/hexanes as eluent (TLC R_f = 0.45 EtOAc/Hexanes, 1/3, UV lamp).

24 | Evaporate the solvents from the eluate using a rotary evaporator at ~ 40 °C. It takes ~ 1 h. Dry under vacuum (→ **9**, 82-90%).

PAUSE POINT Product **9** can be dried overnight under vacuum.

Synthesis of glycosyl N-Methyl asparagines derivative (**11**) TIMING 5 h

25 | Weigh 8.0 mg (8.3 µmol) of imide **9** into a 10-ml round-bottomed flask.

26 | Dissolve in methanol (1 ml). Add a Teflon-coated magnetic stir bar. Cool the flask to 0 °C.

27 | Add 20 mg (0.52 mmol) of sodium borohydride. Stir the reaction mixture for 30 min at 0 °C.

28 | Dilute the reaction mixture with dichloromethane (20 ml). Transfer the resulting solution to a separatory funnel. Wash with aq. 10% NaHSO₄ (10 ml), H₂O (10 ml), and brine (10 ml).

29 | Dry the organic phase over anhydrous sodium sulfate (add ~ 1 g, and stir the mixture for 5 min).

30 | Decant the solvent carefully into a 100-ml round-bottomed flask, and remove volatiles by rotary evaporation at ~ 40 °C. It takes ~ 30 min. Dry under vacuum to give **10**.

PAUSE POINT Product **10** can be dried overnight under vacuum.

31 | Dissolve the resultant residue in 1 ml cocktail (TFA/CH₂Cl₂/Et₃SiH, 0.15/0.70/0.15).

32 | Stir the reaction mixture for 1 h at room temperature.

33 | Blow off the solvent by air. It takes ~ 10 min.

34 | Purify the desired **11** by HPLC (reverse phase C₁₈, 250×21.4 mm, 16 ml/min, MeCN/H₂O/0.04% TFA, gradient, 50%-70%, 30 min).

35 | Lyophilize solvents overnight (→ **11**, 50-55%). The final product can be stored at room temperature.

TIMING

Steps 1-19: 20 h; Steps 20-24: 4 h; Steps 25-35: 5 h

Troubleshooting

Low yield: Repeat reaction with fresh reagents and dry glassware, ensuring that all are anhydrous and that the reaction is maintained under an inert atmosphere. For step 22, the faster the microwave reactor reaches the set temperature, the better the reaction; chloroform can be replaced with 1,2-dichloroethane (anhydrous). If it takes a very long time for the microwave reactor to reach the set temperature (less than 1 min is the best), preheating the microwave might be helpful.

Anticipated Results

Analytical data

2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (7)—Yield 60-70% over 2 steps; ¹H NMR (CDCl₃, 500 MHz) δ 7.39-7.24 (20H, m), 4.94 (1H, d, J = 11.5 Hz), 4.91 (2H, ABd, J = 10.3 Hz), 4.71 (2H, ABd, J = 11.8 Hz), 4.59 (1H, d, J = 11.5 Hz), 4.55 (1H, d, J = 9.0 Hz), 4.43 (2H, ABd, J = 11.7 Hz), 4.04 (1H, t, J = 9.0 Hz), 3.91 (1H, br d, J = 2.6 Hz), 3.61-3.52 (3H, m), 3.47 (1H, dd, J = 2.7, 9.0 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ 161.5, 138.2, 137.8, 137.5, 137.4, 128.0, 127.9, 127.8, 82.1, 81.6, 78.7, 76.4, 76.1, 74.8, 73.7, 73.0, 72.8, 68.2; HRMS calcd for C₃₅H₃₅NO₅Na (M+Na⁺) 572.2413, found 572.2406.

N^α-(Fluoren-9-ylmethyloxycarbonyl)-N^γ-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-N^γ-formyl-L-asparagine-*t*-butyl-ester (9)—Yield 82-90%; ¹H NMR (CDCl₃, 500 MHz) δ 9.3-8.5 (1H, br), 7.68 (2H, d, J = 7.5 Hz), 7.53-7.51 (2H, m), 7.31 (2H, t, J = 7.6 Hz), 7.26-7.11 (22H, m), 5.61 (1H, d, J = 9.0 Hz), 5.48 (1H, d, J = 9.6 Hz), 4.90

(1H, d, J = 10.8 Hz), 4.74 (1H, d, J = 10.3 Hz), 4.65 (2H, ABd, J = 11.7 Hz), 4.54-4.48 (2H, m), 4.43-4.30 (5H, m), 4.23 (1H, t, J = 7.4 Hz), 4.15 (1H, m), 3.94 (1H, s), 3.60-3.59 (2H, m), 3.49 (1H, m), 3.42 (1H, m), 3.32 (1H, m), 3.20 (1H, m), 1.35 (9H, s); ¹³C NMR (CDCl₃, 125 MHz) δ 169.8, 164.5, 162.1, 156.0, 143.9, 143.8, 141.3, 141.3, 138.5, 137.9, 137.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.5, 127.1, 125.2, 125.1, 119.9, 82.4, 77.6, 75.9, 74.9, 74.6, 73.6, 73.5, 73.3, 73.1, 72.6, 68.0, 67.1, 50.7, 47.1, 47.0, 27.8; HRMS calcd for C₅₈H₆₁N₂O₁₁Na (M+H⁺) 961.4275, found 961.4314.

N^α-(Fluoren-9-ylmethyloxycarbonyl)-N^γ-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-N^γ-methyl-L-asparagine (11)—Yield 50-55% over 2 steps; ¹H NMR (CDCl₃, 500 MHz) (two rotamers, 1.0/1.7) 7.73 (2H, m), 7.56 (2H, m), 7.73-7.10 (24H, m), 6.02 and 5.59 (1H, 2 br major and minor, NH), 4.93-4.17 (13H, m), 3.93-3.24 (6H, m), 3.24 and 3.04 (1H, 2 br d, J = 16.0 Hz, major and minor), 2.78 and 2.64 (1H, 2 br dd, J = 10.9, 16.0 Hz, major), 2.60 and 2.38 (3H, 2 s, major and minor, NCH₃); ¹³C NMR (CDCl₃, 150 MHz) δ 173.4, 172.7, 171.6, 156.6, 143.9, 143.6, 141.3, 138.8, 138.6, 137.9, 137.8, 137.6, 137.4, 137.1, 128.6, 128.5, 128.3, 128.0, 127.6, 125.1, 120.0, 83.9, 83.7, 75.3, 75.1, 74.5, 74.4, 73.6, 73.5, 72.9, 72.6, 72.4, 68.2, 67.3, 50.2, 47.1, 47.0, 37.0, 36.4; HRMS calcd for C₅₄H₅₅N₂O₁₀ (M+H⁺) 891.3857, found 891.3884.

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Figure 1.
Amide bond formation through reaction of isonitriles and carboxylic acids.

Figure 2.
Derivatization of *N*-formyl imide adducts.

Figure 3.
Protocol for the synthesis of **11**.

Table 1

1				86%
2				60%
3				85%
4				85%
5				82%
6				50%