A Simple One-Pot Dehydration Process to Convert *N*-acetyl-D-glucosamine into a Nitrogen-Containing Compound, 3-acetamido-5-acetylfuran

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Abstract

An efficient process for converting N-acetyglucosamine (NAG) into 3-acetamido-5-acetylfuran (3A5AF) is reported. 3A5AF is proposed as a N-containing platform chemical, which contains renewable nitrogen. In the reported method, NAG in the presence of boric acid ($B(OH)_3$) and sodium chloride (NaCl) produces 58% yield 3A5AF in dimethylacetamide (DMA) under microwave irradiation (220 °C, 15 min). A maximum yield of 62% was obtained in the presence of 4 equiv NaCl. Performing ICP-MS analysis on NAG from different chemical suppliers highlighted the importance of Cl and B levels in this process. Trace impurities are therefore important considerations in biomass transformations. This solution-phase process produces approximately 30 times more 3A5AF than via a previously reported pyrolysis route.

Introduction

Renewable, bio-sourced feedstocks are now being widely studied for the production of both fuels and chemical precursors.[1-3] These processes typically afford chemicals containing only C, H and O. There is growing interest in the production of renewable chemicals that contain other heteroatoms. For example, recently, caprolactam has been prepared in a four-step process from 5-hydroxymethylfurfural (5-HMF) and ammonia.[4] Inspired by such research, we wondered whether amino-sugars could be used as precursors to new *N*-containing platform chemicals. Our initial studies in this area are presented herein.

N-acetyl-D-glucosamine (NAG), Figure 1a, is an amino sugar and also known as *N*-acetyl-2-amino-2-deoxy-D-glucose. Chitin is a polysaccharide made up of NAG monomers. It is available from crustaceans' shells such as crab, lobster and shrimp (fisheries' waste). It is also found in the cell walls of fungi and insects.[5] Chitin is produced on a large scale annually from a range of sources,[6] and is the second most abundant biopolymer after cellulose. It is readily available, non-toxic and environmentally benign. Also, it may be available as a feedstock in regions of the world that do not have easy access to waste cellulosic biomass feed. Partial hydrolysis of chitin in HCl followed by neutralization, filtration, decolorization and salt removal affords 13.5 wt% NAG.[7] Chitin hydrolysis using enzymes is another approach to produce NAG e.g. cellulase has been used to produce 40 wt% NAG from chitin.[8] Chitosan is the partially deacetylated form of chitin, which is also non-toxic and readily available. Hydrolysis of chitosan (22% deacetylated) using hemicellulase can produce 6.5 wt% of NAG.[5] Two studies have been reported where NAG has been used as the starting material to yield low molecular weight organic products through decomposition pathways. Franich *et al.* produced 3-acetamido-5-acetylfuran (3A5AF), Figure 1b, from NAG. The pyrolysis of NAG was performed in a glass tube apparatus, which was heated in an oven at 400 °C. The yield of 3A5AF obtained through this process was 2%,[9] which is currently the highest reported in the literature. In another study, NAG was mixed with anhydrous disodium hydrogen phosphate and quartz sand in a stainless steel vessel. The pyrolysis of this mixture was performed in an oil bath at 200 °C for 30 min. After work-up, the major product was 3A5AF (0.04% yield).[10] In our study, we present a novel method to produce 3A5AF from NAG using microwave irradiation to heat up the reaction mixture and also there is no need to adjust the pH of the mixture prior to extracting the 3A5AF. The extraction procedure is performed using ethyl acetate, which is more environmentally friendly than chloroform, methanol and dichloromethane that were used in previous studies.[11]

The method reported herein produces 30 times the yield of the Franich *et al.* procedure. This novel process will allow 3A5AF to become available as a starting material for more complex chemical products. For example, polyamides proximicins A, B and C, Figure 2, are naturally occurring compounds, which have been isolated from marine actinomycete of the genus *Verrucosispora*.[12] Recently, proximicins A-C were studied as antitumor and antibiotic drugs.[13] Due to the similarity in structure between our furan product and sub-units in proximicins, we propose that NAG or other amino-sugars are the biosynthetic precursors to such complex natural products.

Results and Discussion

Solvent screening

At the beginning of this study, dimethylformamide (DMF) was used as a solvent in this process. The reaction mixture (50 mg NAG and 2 mL DMF) was microwave heated at 207 °C for 15 min. This temperature was selected based on the maximum safe working temperature for microwave-heating acetonitrile in our instrument. Acetonitrile was one of the screened solvents. The %yield of 3A5AF that was produced from this reaction using DMF was the best among the solvents studied, Figure S1. We examined different solvents hoping to replace DMF with a greener solvent and produce more 3A5AF. The selected solvents have different “greenness”, some of them have few and some have major environmental, health and safety issues associated with them.[14] TBME, CPME, DEA and EG did not yield any 3A5AF. Figure S1 shows that in ester solvents (TBOAc, IPOAc and EtOAc) small amounts of 3A5AF were produced (average 1.7%) but in the related solvent EL 6.3% was obtained. Interestingly, 3A5AF did not form in EG, but 8.1% yield 3A5AF was produced in PEG. However, overall with respect to product yield, dipolar aprotic solvents (DMF, NMP, CH_3CN and DMSO) performed the best with 24.6% yield of 3A5AF produced in DMF. Clearly, in order to produce a significant quantity of 3A5AF under the conditions studies, a dipolar aprotic solvent is required. Dimethylacetamide (DMA) is less dangerous than DMF, in terms of physical properties including boiling point, flashpoint and acute toxicity. Therefore, DMA was used and a yield of 31.3% was obtained. This yield was ca. 15 times greater than the amount produced via pyrolysis reported in the literature. Unfortunately, we were unable to find any correlation between solvent polarity (and other solvent parameters) and the yields obtained in this solvent-screening study. However, it should be noted that no catalyst or other additive was used in these initial experiments.

Catalyst/additive screening

After determining that DMA will be the best solvent to use, we then attempted to find the best catalyst or additive, Table 1. The reaction mixtures consisted of 10 wt% NAG of the total reaction mass and 6 mol% catalyst. In the case of reactions in the presence of LiBr, it was added at 10 wt% of the total mass. LiBr was added because bromide is a good nucleophile that has been shown to interact with glucose and fructose in DMA to increase the productivity of 5-HMF.[15] Table 1 shows that in presence of LiBr, the %yield 3A5AF is reduced. This contrasts with results using glucose and fructose.[15] $CrCl_3$ has been shown to facilitate the isomerization of glucose into fructose to produce a high yield of 5-HMF.[16] $CrCl_3$ has also been used in the conversion of cellulose into levulinic acid.[17] Therefore, it was tested in this study but failed to significantly increase the yield (entries 3 and 4). This might be due to strong nitrogen coordination (from the reagent, product or intermediate) to the chromium centre preventing catalytic turnover. The best catalysts were basic ones (entries 6-11). Despite NH_3 not giving the best results in the screening, it was selected to study via factorial design for optimizing the method because it is cheap and relatively benign.

2^5 Factorial Design (FD)

Although studying one factor at a time to optimize reaction conditions is a common approach, it has some limitations such as neglecting the interaction between factors. Using FD, a researcher can study not only one factor at a time but also the effects of factors' interactions on the result.[18] Therefore, we studied the effects of five factors with each factor at two levels. These factors were NAG, DMA, amount of ammonia, temperature and time. The factors and levels are shown in table S1. The maximum %yield of 3A5AF obtained was 42.6%. This yield was achieved using 0.2356 g NAG, 4.5 mL DMA, 0.26 mmol NH_3, 217 °C and 15 min. The results in this table were analyzed using Minitab software. The normal plot of the effects (Figure 3) shows that all effects are significant. In fact, in this case the individual effects (i.e. each factor alone) are more significant than combined effects/factors.

We tested the most significant effects at different levels, e.g. increased T, Figure S2, but none of these improved on the amount of 3A5AF produced (42.6%) during the FD experiments.

Testing NAG from different chemical suppliers

The results described above were obtained using NAG purchased from Toronto Research Chemicals (TRC). When NAG from AK Scientific or Alfa Aesar was used, we were disappointed to obtain yields of 14.7% and 6.2%, respectively. The challenge that faced us was to answer the following question; why did changing the supplier/source of NAG cause such a dramatic decrease in yield? After studying the impurity levels in the NAG from each supplier using ICP-MS, boron (B) and chloride (Cl) concentrations stood out as being significantly higher in the NAG from TRC, Table 2.

Table 2. ICP-MS analyses for B and Cl in NAG from three suppliers.

Chemical Supplier	B[a]	Cl[a]
TRC	27.9	20681
AK Scientific	4.4	819
Alfa Aesar	0.3	391

[a] mg B or Cl per Kg NAG

Cl^- ions have been shown to have a significant and positive effect on biomass conversions processes[15,17,20] Therefore, different amounts of NaCl were added to reaction mixtures to test the effect of Cl^- concentration on 3A5AF production using NAG from AK Scientific and Alfa Aesar. Figure 4 shows that NaCl at 30-50 mol% with respect to NAG produced the highest % yield of 3A5AF.

NAG from Alfa Aesar consistently produced less 3A5AF compared with AK Scientific and we propose that this is because it contains less boron (see Table 2). Therefore, boron was added to the reaction mixture in the form of boric acid ($B(OH)_3$). $B(OH)_3$ is a weak acid, non-toxic, inexpensive and readily available. Boric acid with NaCl has been used previously to increase 5-HMF production from hexoses.[19, 20] Yields of up to 42% from glucose and as much as 66% from sucrose have been reported.

Figure 5 shows the effect of adding $B(OH)_3$ at a 2:1 $B(OH)_3$:NAG mol ratio on 3A5AF production in the presence and absence of NaCl (30 mol% with respect to NAG), and in the presence and absence of ammonia (24.4 mol% of NAG). In all example reactions, Figure 5, the NAG from AK scientific produced more 3A5AF, which we propose is because of its initially higher B and Cl content, Table 2. Figure 5 emphasizes the significance of adding Cl^- to the reaction mixture. The importance of $B(OH)_3$ and NaCl in dehydrating NAG led us to study 3^2 FD to determine the optimum amounts that should be added. Table S2 shows 3^2 FD using NaCl at three levels 10, 30 and 60 mol% with respect to NAG and $B(OH)_3$ at 0.5:1, 1:1, 2:1 mole ratios with respect to NAG. The other parameters for the reactions were kept constant at 0.2356 g NAG, 4.5 mL DMA, 220 °C and 15 min. The results were analyzed using Minitab software. The interaction plot for 3A5AF (Figure S3) shows that at each level of $B(OH)_3$ the amount of 3A5AF produced increased with increasing NaCl concentration. The interaction plot also clearly shows that a 1:1 mole ratio of $B(OH)_3$:NAG produced the highest yield of 3A5AF.

Figure 6 shows the %yields of 3A5AF at different NaCl levels using a fixed amount of 1:1 $B(OH)_3$:NAG (mol ratio). 57.7% yield 3A5AF was produced at 2:1 NaCl:NAG mol ratio in 15 min. This compares well with studies using fructose and glucose, which produce 5-HMF. Using NaCl and $B(OH)_3$, fructose has been shown to produce 60% yield 5-HMF and glucose produced 14% yield 5-HMF.[20]

Therefore, at the present time, we assume that the reaction mechanism is similar to those reported for conventional carbohydrates.

Different Cl^- sources were tested ($SnCl_4.5H_2O$, $MnCl_2.4H_2O$, $MgCl_2.6H_2O$, KCl, $CaCl_2.2H_2O$, $CaCl_2$, NaCl and LiCl) Figure 7. NaCl is the most benign and the cheapest chemical among them. Compared with NaCl (57.7% yield), every chemical produced less 3A5AF except LiCl, which produced 59.2% yield. DMA-LiCl (10%) as a solvent in the presence of CuCl and 1-ethyl-3-methylimidazolium chloride ([EMIm]Cl) has been shown to produce 83% yield 5-HMF from fructose.[15] $CaCl_2$ produced 54.5% yield 3A5AF and $CaCl_2.2H_2O$ 50.7% yield, which shows that unfortunately water inhibits the production of 3A5AF in our system. In order to further study the effect of adding water to the reaction, several amounts of water were added (1, 2, 4, 6, 8, 10, 15 and 20 %v/v in 4.5 mL DMA). Figure S4 shows that the %yield of 3A5AF produced decreases as the amount of added water increases and is further evidence for water inhibiting this process. If dry, distilled DMA was used in reactions, no significant increase in 3A5AF yield was observed.

The effect of time on the reaction was studied at 0, 5, 10, 15, 30, 45, 60, 75, 90 min. Figure S5 shows an approximately linear increase in 3A5AF production between 0 and 15 min but the amount of 3A5AF does not change significantly between 15 and 45 min. However, the %yield starts to decrease after 45 min, which is probably due to thermal decomposition of 3A5AF. The effect of temperature on the reaction was studied by performing reactions at 160, 180, 200, 220, 240 and 250 °C for 15 min, Figure 8. It should be noted that temperature monitoring in commercial microwave instruments is not always accurate and care must be taken to ensure that the instrument is calibrated at regular intervals. The amount of 3A5AF produced increased linearly between 160 and 220 °C. Above 220 °C, 3A5AF %yield decreased presumably due to product decomposition. However, decomposition products were not observed in the chromatograms from these reactions and, therefore, the by-products are likely insoluble in ethyl acetate (Figure S6).

Preliminary studies towards scaling up the reaction were performed in a 300 mL autoclave (Parr® reactor 5500 series). The following conditions were used: 5.00 g NAG, 2.67 g NaCl, 1.40 g $B(OH)_3$, 100 mL DMA, 220 °C, 15 min. It should be noted that it took 30 min to achieve the desired reaction temperature and that the pressure at 220 °C was approximately 6 bar. Under these reaction conditions, 42.8% yield of 3A5AF was obtained. Further studies are needed to optimize scale-up of this reaction.

Conclusions

Figure 9 shows the optimum reaction conditions that produce 58% yield 3A5AF from NAG. This is 30 times more 3A5AF than produced using previously reported pyrolysis methods. NaCl and $B(OH)_3$ are very important additives in this reaction and markedly increase 3A5AF production. This study will allow the chemistry of 3A5AF, a carbohydrate-derived amide, to be studied further and it may find use either as a platform chemical, a source of renewable amines or as a high-value precursor to proximicins and other biologically active compounds. Some possible reactions of 3A5AF are shown in Figure 10. Due to the multifunctional nature of this compound (amide and ketone groups), selective transformations will be challenging but would also provide many opportunities for accessing a wide range of products.

In addition to looking at the reactivity of this compound, future studies will involve investigating the kinetics of this reaction, further scale-up studies and attempting to find (i) a more environmentally benign solvent to use and (ii) ways to re-use the NaCl and $B(OH)_3$ components.

Acknowledgements

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Keywords: *Biomass · Amines · Carbohydrates · Catalysis*

References

[1] J. J. Bozell, G. R. Petersen, *Green Chem.* **2010**, *12*, 539-554.

[2] P. N. R. Vennestrøm, C. M. Osmundsen, C. H. Christensen, E. Taarning, *Angew. Chem.* **2011**, *123*, 10686–10694; *Angew. Chem. Int. Ed*. **2011**, *50*, 10502–10509.

[3] Introduction to chemicals from biomass (Eds.: J. H. Clark, F. E. I. Deswarte), John Wiley & Sons, Chichester, **2008**

[4] T. Buntara, S. Noel, P. H. Phua, I. Melián-Cabrera, J. G. de Vries, H. J. Heeres, *Angew. Chem*. **2011**, *123*, 7221–7225; *Angew. Chem. Int. Ed.* **2011**, *50*, 7083–7087.

[5] R. A. A. Muzzarelli, M. G. Peter, *Chitin Handbook,* European Chitin Society, Grottammare, Italy, **1997**.

[6] Y. Xu, C. Gallert, J. Winter, *Appl. Microbiol. Biotechnol*. **2008**, *79*, 687-697.

[7] S. Sato, K. Minamikawa (Nankai Kagaku Kogyo K. K., Japan). Process for the preparation of natural *N*-acetylglucosamine. JP 2009191001, August 27, **2009**.

[8] H. Zhu, E. Muraki, S. Aiba. *Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30,* **2000**, 69CLAC.

[9] R. A. Franich, S. J. Goodin, *J. Anal. Appl. Pyrolysis* **1984**, *7*, 91–100.

[10] J. Chen, M. Wang, C. Ho, *J. Agric. Food Chem*. **1998**, *46*, 3207–3209.

[11] F. M. Kerton, *Alternative Solvents for Green Chemistry*, RSC Publishing, Cambridge, **2009.**

[12] F. E. Wolter, K. Schneider, B. P. Davies, E. R. Socher, G. Nicholson, O. Seitz, R. D. Süssmuth, *Org. Lett.* **2009**, *11*, 2804–2807.

[13] K. Schneider, S. Keller, F. E. Wolter, L. Röglin, W. Beil, O.Seitz, G. Nicholson, C. Bruntner, J. Riedlinger, H. Fiedler, R. D. Süssmuth, *Angew. Chem.* **2008**, 120, 3302–3305; *Angew. Chem. Int. Ed.* **2008**, *47*, 3258–3261.

[14] R. K. Henderson, C. Jimenez-Gonzalez, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks, A. D. Curzons, *Green Chem*. **2011**, *13*, 854–862.

[15] J. B. Binder, R. T. Raines, *J. Am. Chem. Soc.* **2009**, *131*, 1979–1985.

[16] H. Zhao, J. E. Holladay, H. Brown, Z. C. Zhang, *Science* **2007**, *316*, 1597–1600

[17] J. Potvin, E. Sorlien, J. Hegner, B. DeBoef, B. L. Lucht, *Tetrahedron Lett.* **2011**, *52*, 5891–5893.

[18] D.C. Montgomery, *Design and Analysis of Experiments*, John Wiley & Sons, Hoboken, NJ, **2009**.

[19] T. Ståhlberg, S. Rodriguez-Rodriguez, P. Fristrup, A. Riisager, *Chem. Eur. J,* **2011**, *17*, 1456–1464.

[20] T.S. Hansen, J. Mielby, A. Riisager, *Green Chem*. **2011**, *13*, 109–114.

Suggested text for table of contents

Harbour not Haber Nitrogen: An amido-substituted furan can be obtained in up to 58% yield through heating an amino-sugar, which can be obtained from fishery waste, in the presence of NaCl and boric acid. The product represents a structural motif within biologically active natural products.

Figure Captions

Figure 1. a) *N*-acetyl-D-glucosamine (NAG) and b) 3-acetamido-5-acetylfuran (3A5AF).

Figure 2. Chemical structure of proximicin A.

Figure 3. Normal plot of the effects for 3A5AF production. This plot was produced using Minitab software. Reaction conditions (See Table S1).
Figure 4. Effect of adding NaCl on yield of 3A5AF from NAG supplied by AK Scientific and Alfa Aesar. Solid circles = Alfa Aesar, Hollow circles = AK Scientific. Reaction conditions: 0.2356 g NAG, 0-50 NaCl (mol% of NAG), 4.5 mL DMA, ammonia (24.4 mol% of NAG), MW, 217 °C, 15 min.

Figure 5. Effect of added $B(OH)_3$, NaCl and NH_3 on 3A5AF production. Black = NAG from Alfa Aesar. Grey = NAG from AK Scientific. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA in the presence and absence of NaCl (30 mol% with respect to NAG), NH_3 (24.4 mol% with respect to NAG), 2:1 $B(OH)_3$:NAG mol ratio, MW, 217 °C, 15 min. Yield determined using GC-MS.

Figure 6. Effect of adding different levels of NaCl at 1:1 $B(OH)_3$:NAG (mol ratio). Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 10-400 NaCl (mol% of NAG), 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min. 3A5AF was quantified using GC-MS.

Figure 7. Effect of chloride sources on 3A5AF production. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 Cl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min. 3A5AF was quantified using GC-MS.

Figure 8. Effect of temperature on 3A5AF production. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 NaCl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 160-250 °C, 15 min. Yield was determined using GC-MS.

Figure 9. The optimum reaction conditions for dehydrating NAG into 3A5AF.

Figure 10. Some potential future transformations of 3A5AF.

Figure 1

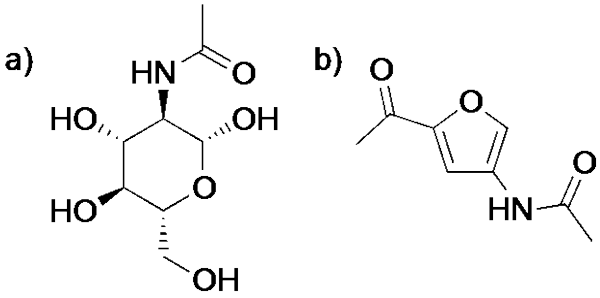


Figure 2

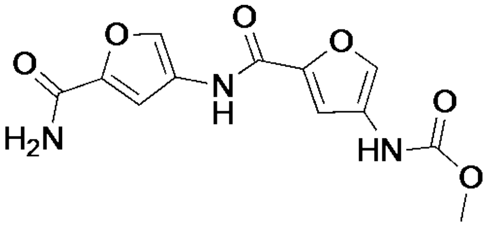


Figure 3

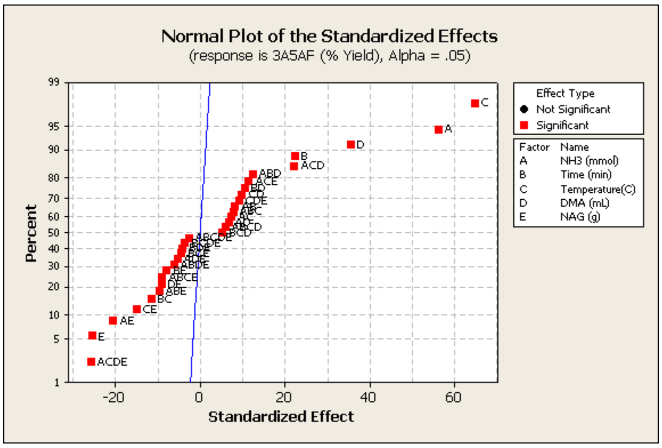

Normal Plot of the Standardized Effects
(response is 3A5AF (% Yield), Alpha = .05)
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
-20
0
20
40
60
Standardized Effect
C
A
D
B
ACD
ABD
ACE
BD
CD
ACDE
ABC
BCD
ABCDE
ABDE
BE
ABCE
DE
ABE
BC
CE
AE
E
ACDE
Effect Type
● Not Significant
■ Significant
Factor Name
A NH3 (mmol)
B Time (min)
C Temperature(C)
D DMA (mL)
E NAG (g)

Figure 4

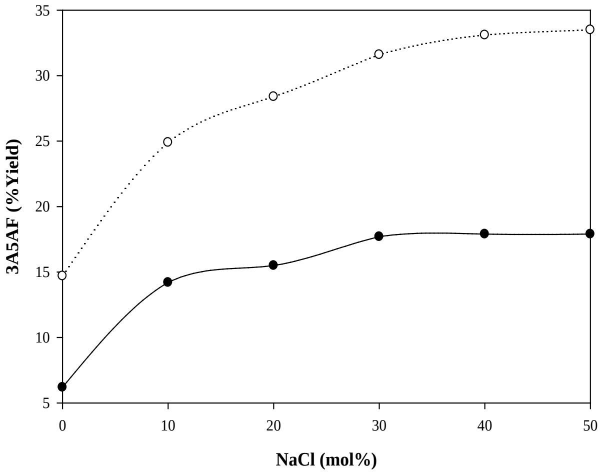
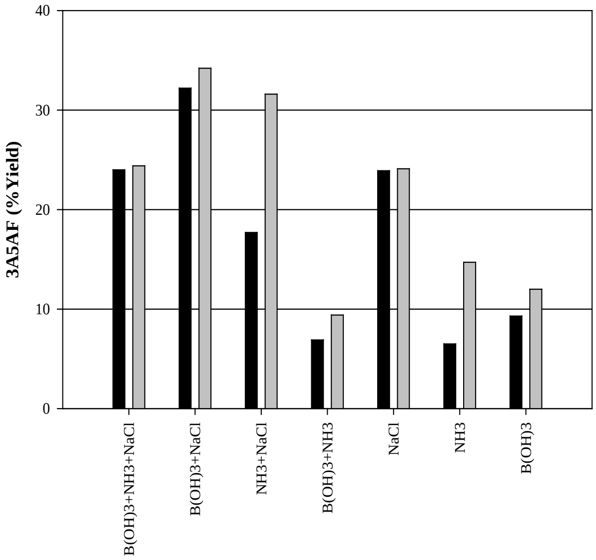


Figure 5



3A5AF (%Yield)
40
30
20
10
0
B(OH)3+NH3+NaCl
B(OH)3+NaCl
NH3+NaCl
B(OH)3+NH3
NaCl
NH3
B(OH)3

Figure 6

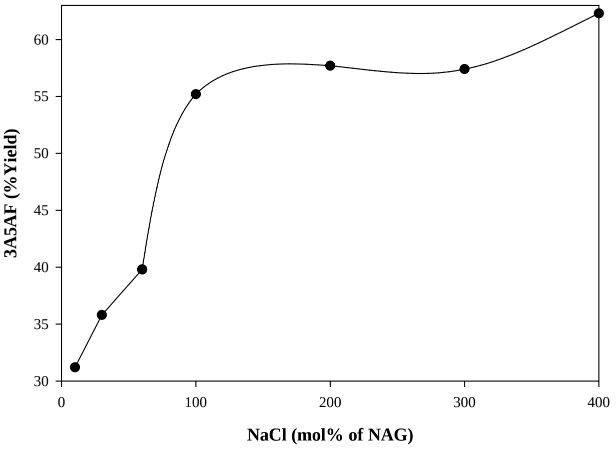


Figure 7

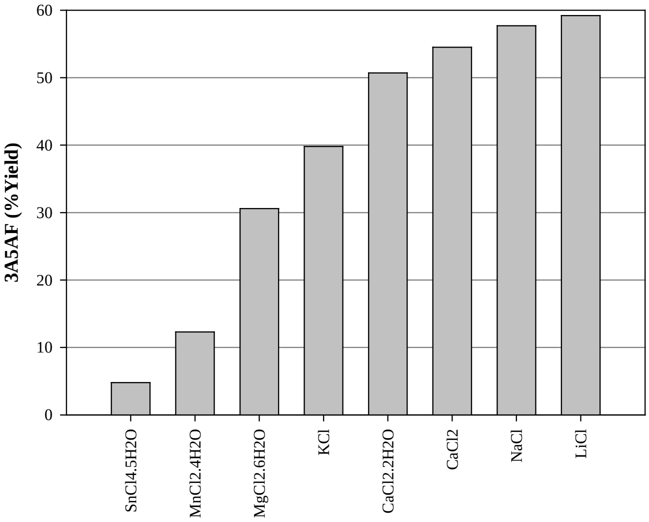

3A5AF (%Yield)
60
50
40
30
20
10
0
SnCl4.5H2O
MnCl2.4H2O
MgCl2.6H2O
KCl
CaCl2.2H2O
CaCl2
NaCl
LiCl

Figure 8

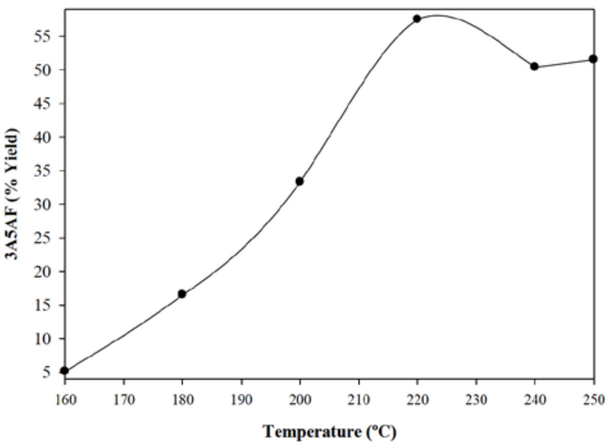

3A5AF (% Yield)
Temperature (°C)
5
10
15
20
25
30
35
40
45
50
55
160
170
180
190
200
210
220
230
240
250

Figure 9

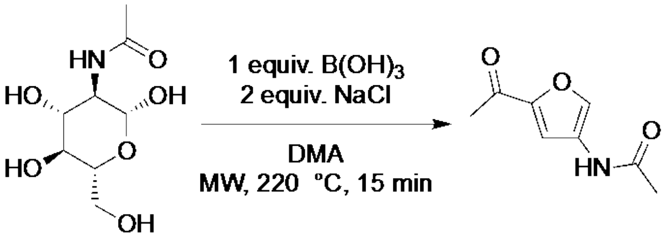
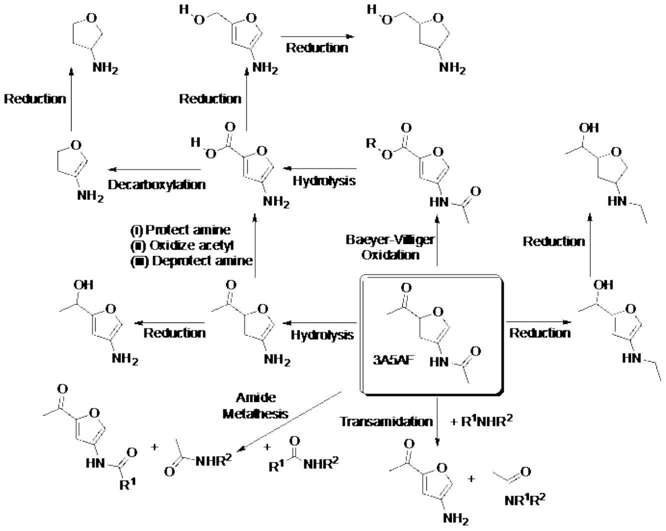

1 equiv. B(OH)3
2 equiv. NaCl
DMA
MW, 220 °C, 15 min
HN
O
HO
OH
HO
O
OH
O
O
O
HN

Figure 10


Reduction
Reduction
Reduction
Decarboxylation
Hydrolysis
(i) Protect amine
(ii) Oxidize acetyl
(iii) Deprotect amine
Baeyer-Villiger Oxidation
Reduction
Reduction
Hydrolysis
3A5AF
Reduction
Amide Metathesis
Transamidation
+ R¹NHR²
NH₂
HN
OH
O
R
H
R¹
NHR²
NR¹R²
+

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