

Selective acetylation of primary amino groups with phenyl acetate: simple conversion of polyamines into *N,N'*-diacetyl derivatives

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Abstract

Phenyl acetate was studied as a general reagent for highly selective *N*-acetylation of primary amino groups in the presence of alcohol and secondary amino groups. Two polyamines, namely 1,8-diamino-4-azaoctane (Spd) and 1,12-diamino-4,9-diazadodecane (Spm), were *N,N'*-diacetylated with an efficient and mild methodology.

Keywords: Acyl transfer; aminolysis; selective acetylation; diacetylpolyamines

Introduction

Naturally occurring polyamines spermidine (1,8-diamino-4-azaoctane, Spd) and spermine (1,12-diamino-4,9-diazadodecane, Spm) are required for normal cell growth. Their concentrations can reach millimolar levels in rapidly proliferating cells. Changes in the homeostasis of the natural polyamines Spd and Spm is linked to various pathological conditions, such as acute pancreatitis, diabetes and cancer.^{1,2} The main catabolic reaction of natural polyamines is *N*-acetylation by Spermidine/Spermine *N'*-Acetyltransferase (SSAT) and the mono- or di-acetylated polyamines, which are the natural products of this enzyme, can be subsequently exported from cells and excreted.³ Furthermore, the enzymatic activity of SSAT is up-regulated as a result of various stresses to cells and the acetylated metabolites of Spd and Spm are now considered as potential biomarkers of cancer. Our main research interests are in the analysis of peculiarities of polyamine metabolism with various types of polyamine analogues and the analysis of the concentrations of free and acetylated polyamines from urine to study differences in excretion between cancer patients and healthy humans. For these purposes we are continuously developing

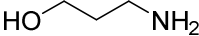
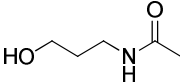
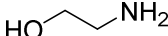
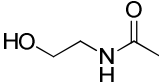
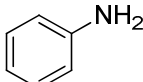
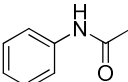
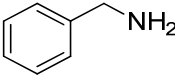
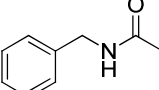
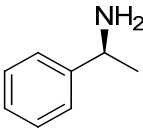
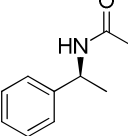
simpler methods for the synthesis of polyamines and their acetylated derivatives. In this context, direct acetylation of Spd and Spm to the corresponding N^1,N^8 -Ac₂-Spd and N^1,N^{12} -Ac₂-Spm without any additional protective steps has been one of our main goals.

N-Acylation of an amino group is usually performed using an acyl chloride or acid anhydride in the presence of base. Amino groups can also be acetylated by an acyl transfer reaction with esters. The pioneering work of Gordon, Miller and Day^{4,5} showed that aminolysis of various esters is relatively slow at room temperature and the reaction kinetics depend on the structure of the ester. The rate of aminolysis of esters is solvent dependent and slow in anhydrous solvents, but it can be increased in certain glycols and related compounds.⁵ Moreover, there is a plethora of reported catalysts for this type of acyl transfer reaction for aminolysis of different esters, such as indium triiodide,⁶ *N*-heterocyclic carbenes,⁷ 1,5,7-triazabicyclo[4.4.0]dec-5-ene,⁸ 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),⁹ 1,2,4-triazole-DBU,¹⁰ sodium methoxide,¹¹ potassium *tert*-butoxide¹² and a pincer-ruthenium complex.¹³ Ethyl trifluoroacetate has been successfully used for selective protection of primary amines of Spm¹⁴ and phenyl acetate is known to react efficiently with aliphatic diamines¹⁵ and has been studied for mono acylation of polyamines with in the presence of H₂O.¹⁶

Results and Discussion

Inspired by the success of other groups to use catalyst-free aminolysis for selective acylation of primary amino groups, we screened the reactivity of a series of aliphatic and aromatic acetates with 3-aminopropanol at elevated temperatures in solvent-free conditions (Table S1). 3-Aminopropanol was selected as a model compound because it can be used as a building block to synthesize the aminopropyl units of natural polyamines or their analogues.¹⁷ Based on these preliminary experiments, the reactivity of the aromatic benzyl and phenyl acetates best suited our purpose of developing an efficient synthetic method for the synthesis of diacetylated polyamines. Furthermore, phenyl acetate reacted quickly with 3-aminopropanol at room temperature (Table S2). A significant difference in the reactivity of phenyl acetate as compared to benzyl acetate was observed because the hydroxyl group of phenol is a stronger Brønsted acid due to the electron-withdrawing effect of the aromatic ring and the charge of the leaving anion can thus be delocalized into the aromatic ring. Furthermore, the reactivity of phenyl acetate could be modified by varying substituents on the aromatic ring which change the electrophilicity of the carbonyl group.¹⁸ Thus, electron-withdrawing substituents such as -Cl or -NO₂ at *para*- or *ortho*-positions lead to faster reaction rates and electron-donating substituents lower rates. However, the reaction rate of unsubstituted phenyl acetate was already high enough for our purposes, and thus, we decided to evaluate its reactivity with various primary amines in acetonitrile at room temperature (Table 1).

Table 1. Phenyl acetate as an acetylating agent of various primary amines^a

| Amine | Product | Time (h) | Conversion ^b (%) |
|--|--|----------|-----------------------------|
|  |  | 0.5 | 97 |
|  |  | 0.5 | 98 |
|  |  | 0.5 | 3.5 |
|  |  | 0.5 | 96 |
|  |  | 0.5 | 51 |

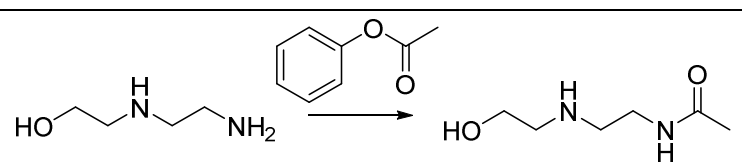
^a amine (1 equiv, 1.33 mmol), phenyl acetate (1.2 equiv, 1.59 mmol), were mixed at rt in MeCN under argon for 0.5 h. ^b Determined by ¹H NMR spectroscopic analysis without isolation of the product.

Similar high conversion rates were observed for 3-aminopropanol, 2-aminoethanol and benzylamine in MeCN, when 1.2 equiv of phenyl acetate was used as acetylating agent for 0.5 h at room temperature. The primary amino groups of these molecules are not as sterically hindered as in (*S*)- α -methylbenzylamine where 51% of the amino group was converted to the corresponding acetamide under the same reaction conditions. Aniline did not react under these conditions because it is much weaker nucleophile compared to the other amines. Based on these results, phenyl acetate can be considered to be a selective acetylating agent for aliphatic primary amino groups in the presence of primary hydroxyl groups in mild reaction conditions.

One of the main synthetic strategies that we use for the synthesis of polyamines is to start from commercially available amino alcohols that are first protected and activated with a mesyl group followed by chain elongation with the requisite amine (for example see reference 19). These building blocks usually contain a primary amino group or primary and secondary amino groups in addition to the hydroxyl group needed for the chain elongation. Synthesis of polyamines using this strategy requires many steps due to protection and deprotection steps, and thus, to simplify the synthesis of acetylated polyamines, the selectivity of phenyl acetate was studied using *N*-(2-aminoethyl)aminoethanol (Table 2). This building block has both primary and secondary amino groups as well as the hydroxyl group and selective acetylation of the primary

amino group can reduce the synthetic steps for acetylated polyamine analogues with ethylene linkers between the amino groups.

Table 2. Selective acetylation of the primary amino group of *N*-(2-aminoethyl)aminoethanol with phenyl acetate^a

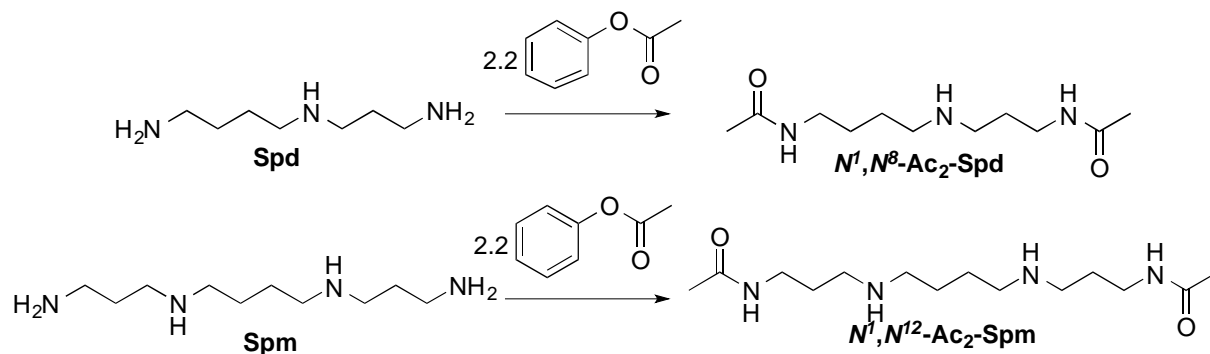


| Acetate | Time (h) | Conversion ^b (%) |
|---------|----------|-----------------------------|
| 1 eq | 0.5 | 91 |
| | 1 | 93 |
| 2 eq | 0.5 | 95 |
| | 1 | 99 |
| 10 eq | 0.5 | 96 |
| | 1 | 94 |

^a *N*-(2-aminoethyl)aminoethanol (1 equiv, 1.33 mmol) and phenyl acetate (1-2 equiv), were mixed at r.t. in MeCN under argon for 0.5-1 h or under solvent-free conditions with 10 equiv of phenyl acetate. ^b Determined by ¹H NMR spectroscopic analysis without isolation of the end product.

Phenyl acetate reacted selectively with the primary amino group of *N*-(2-aminoethyl)aminoethanol and high conversion to the corresponding acetamide was achieved within 0.5 h without detectable by-products. An additional solvent-free reaction using 10 equiv of phenyl acetate was tested to evaluate if higher amounts of acetate promote unwanted side reactions. However, only a slight increase in the formation of terminally *N,O*-diacetylated product was observed. Based on these results, phenyl acetate can be considered a versatile reagent for selective *N*-acetylation of primary amino groups in the presence of primary hydroxyl and secondary amino groups.

Diacetylated metabolites of Spd and Spm (*N*¹,*N*⁸-Ac₂-Spd and *N*¹,*N*¹²-Ac₂-Spm) have been described as promising metabolites for the detection of various cancers²⁰⁻²² and chemoselective acetylation is the most efficient way for the synthesis of these compounds. To the best of our knowledge, chemoselective acetylation of Spd has only been previously achieved by using *N,N*-diacetylaminoquinazolinone,²³ which is not commercially available. Therefore, we applied our acetylation strategy to the synthesis of both *N*¹,*N*⁸-Ac₂-Spd and *N*¹,*N*¹²-Ac₂-Spm in a single step (Scheme 1).



Scheme 1. Synthesis of diacetylated metabolites of Put, Spd and Spm, using phenyl acetate.

High conversion of both of the primary amino groups of Spd and Spm to the corresponding acetates using a 2.2 fold molar excess of phenyl acetate was achieved in 0.5 h. After acetylation, the MeCN was evaporated *in vacuo* and the synthesized polyamines were dissolved in EtOH and converted into their hydrochloride salts by passing HCl gas into the reaction mixture. In the initial trials to precipitate the polyamines we observed undesired cleavage of the acetyl groups. The cleavage was prevented by adding the HCl gas into the reaction mixture over 30 min with efficient cooling to 0 °C. Both of the products were isolated by simple filtration resulting in the target compounds with >95 % purity in reasonable yields (50% for *N*¹,*N*⁸-Ac₂-Spd and 41% *N*¹,*N*¹²-Ac₂-Spm) without any optimization of the acetylation reaction or isolation of the compounds. The high purity was achieved by washing the isolated precipitate with cold diethyl ether or EtOH, respectively. This method allows the preparation of terminally diacetylated polyamines with good purity and yields by a simple and direct protocol. These compounds are potential biomarkers of various cancers and can now be easily prepared with this method.

Conclusions

Based on these observations, acetylation can be achieved in the absence of catalyst using various acetates but the reaction times vary from 30 min to several days. Phenyl acetate reacted selectively and rapidly with primary amino groups in the presence of primary hydroxyl and secondary amino groups, and thus, was found to be the most versatile reagent for synthesis of diacetylated metabolites of polyamines and their analogues. This was demonstrated by the simple one-step syntheses of, *N*¹,*N*⁸-Ac₂-Spd and *N*¹,*N*¹²-Ac₂-Spm.

Experimental Section

General. All of the chemicals were purchased from commercial sources and used as obtained without further purification. All reactions were performed under argon. ^1H and ^{13}C NMR spectra were recorded using a Bruker AVANCE DRX spectrometer operating at 500.1 and 125.8 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to residual protic solvent peaks (7.26 and 77.16) in CDCl_3 or sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) for D_2O . Normal $^3J_{\text{HH}}$ coupling constants are indicated with the letter “*J*” and given in Hz. Positive ESI mass spectra were obtained on Applied Biosystems/MDS Sciex QSTAR XL spectrometer.

General procedure for acetylation using phenyl acetate described in Table 2. Amine (1.33 mmol) and phenyl acetate (1.2 eq, 1.60 mmol) were stirred for 0.5 or 1 h in MeCN (5 mL). The solvent was then evaporated *in vacuo* and the conversion assessed by ^1H NMR spectroscopic analysis.

***N*¹,*N*⁸-Ac₂-Spd hydrochloride.** Phenyl acetate (0.2062 g, 1.5 mmol) was added to a mixture of spermidine (0.100 g, 0.688 mmol) in MeCN (5 mL), and the solution stirred for 0.5 h at rt. The reaction mixture was cooled to 0 °C in an ice bath and the product was precipitated by slow addition of gaseous HCl (20-30 min) while keeping the temperature of the reaction mixture below +4 °C. The product was filtered off and washed with dry Et₂O to give *N*¹,*N*⁸-Ac₂-Spd monohydrochloride (0.079 g, 43%) as colorless crystals. ^1H NMR (D_2O) δ : 3.27 (2H, t, *J* 6.9 Hz), 3.18-3.00 (4H, m), 1.99 (3H, s, CH₃), 1.98 (3H, s, CH₃), 1.92-1.84 (2H, m), 1.73-1.65 (2H, m), 1.73-1.65 (2H, m), 1.61-1.54 (2H, m); ^{13}C NMR (D_2O) δ 177.58, 177.10, 50.18, 47.91, 41.54, 39.03, 28.49, 28.40, 25.91, 24.81, 24.81, 24.75 HRMS ($\text{C}_{11}\text{H}_{24}\text{N}_3\text{O}_2$) [$\text{M}+\text{H}$]⁺ calcd. 230.1869, obs. 230.1879

***N*¹,*N*¹²-Ac₂-Spm dihydrochloride.** Phenyl acetate (0.2062 g, 1.5 mmol) was added to a mixture of spermine (0.100 g, 0.494 mmol) in MeCN (5 mL), and the solution stirred for 0.5 h at room temperature. The reaction mixture was cooled to 0 °C in an ice bath and the product was precipitated by slow addition of gaseous HCl (20-30 min) while keeping the temperature of the reaction mixture below +4 °C. The product was filtered and washed with dry EtOH to give *N*¹,*N*¹²-Ac₂-Spm dihydrochloride (0.0585 g, 37%) as colorless crystals. ^1H NMR (500.0 MHz, D_2O) δ : 3.31-3.24 (4H, m), 3.12-3.02 (8H, m), 2.00 (3H, s, CH₃), 1.93-1.85 (4H, m), 1.81-1.74 (4H, m); ^{13}C NMR (125.75 MHz, D_2O) δ 177.60, 49.80, 47.99, 38.97, 28.50, 25.72, 24.73 HRMS ($\text{C}_{14}\text{H}_{31}\text{N}_4\text{O}_2$) [$\text{M}+\text{H}$]⁺ calcd. 287.2447, obs. 287.2456.

Supplementary Material Available

Table S1 and S2 for the reactivity of a series of aliphatic and aromatic esters acetates. ^1H and ^{13}C spectra of N^1, N^8 -Ac₂-Spd and N^1, N^{12} -Ac₂-Spm.

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