

The facile synthesis of a pyrimidinyl sulfonamide (*N,N,N,6-tetramethyl-2-(4-nitrophenylsulfonamido)pyrimidin-4-ammonium chloride*) as a PET tracer precursor

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Abstract

We synthesized the novel sulfonamide derivative, *N,N,N,6-tetramethyl-2-(4-nitrophenylsulfonamido)pyrimidin-4-ammonium chloride* (**7**), for use as a precursor in [¹⁸F]fluoride chemistry. For this, 4-nitrobenzenesulfonyl guanidine was used as the starting material for condensation with ethyl acetoacetate. The ¹⁹F-compound could be synthesized in four steps with moderate yields. The ¹⁸F-labelled analogue, which has high potential as an intermediate for a positron emission tomography tracer, was produced with a radiochemical yield of 58±12% (n = 4, decay corrected).

Keywords: Anti-infective, positron emission tomography, [¹⁸F]fluoride

Introduction

In order to develop an appropriate treatment for an infection, it is essential to identify the causing agent. Our aim is to develop PET tracers that can detect bacterial foci. An effective tracer should have appropriate pharmacokinetic properties (i.e, absorption, distribution, metabolism, and excretion) and a high target affinity.¹ Antibacterial sulfonamides, which are second-line antibiotics for the treatment of urinary tract infections, meningitis, and toxoplasmosis,² may be suitable for ¹⁸F-labeling experiments. Because of selective binding (i.e, to dihydropteroate synthetase during folic acid biosynthesis), the tracer would allow for the specific detection of bacteria.

Fluorine-18 is a commercially available isotope with ideal properties for PET experiments as it has a low maximum energy (yielding high resolution images) and an effective half-life of 109 minutes. In most cases, ¹⁸F-labelled tracers are synthesized via nucleophilic substitution. The precursors are therefore synthesized with highly effective leaving groups, such as triflate, tosylate, or trimethylammonium halides. We focused on the short-route synthesis of such sulfonamide

precursors and started with 2-amino-6-chloropyridine, isocytosine, and 2-amino-4-chloro-6-methylpyrimidine as substrates for reaction with 4-nitrobenzenesulfonylchloride. The desired products have either a chlorine substituted heteroaromatic system or, in the case of isocytosine, an easily transformable OH-group. There are some examples³⁻⁶ which describe the possibility of exchanging the chlorine on pyridine moieties or analogues with [¹⁸F]F⁻ in order to synthesize tracers for PET experiments (for a review of this chemistry, see Dollé⁷). The nitro group of the ¹⁸F-labelled compound will ultimately be reduced to an amino moiety to allow for binding to bacterial enzymes.

Results and Discussion

Synthesis from 4-nitrobenzenesulfonyl chloride and heteroaromatic compounds.

We initially considered compounds which may easily be synthesized from commercially available starting materials. The derivative **1**, which was synthesized from 2-amino-6-chloropyridine and 4-nitrobenzenesulfonyl chloride in pyridine, was the first candidate. Such α -chloropyridines have already been transformed into their respective fluoro compounds⁸ (for a review see the article of Gakh and the references herein⁹). When **1** was reacted with fluoride under conditions for nucleophilic substitution (dry, 2,2,2-Cryptand, 180 °C, 0.5 h), no ¹⁹F-substituted product was observed (Figure 1) and the starting material remained completely unconsumed (HPLC chromatogram not shown).

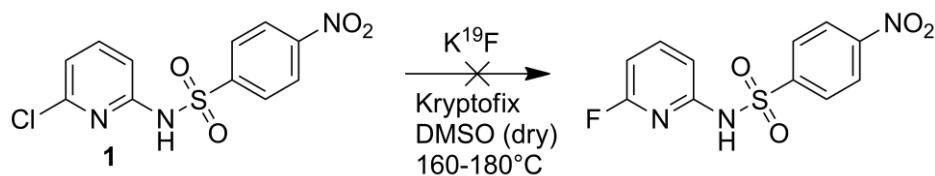
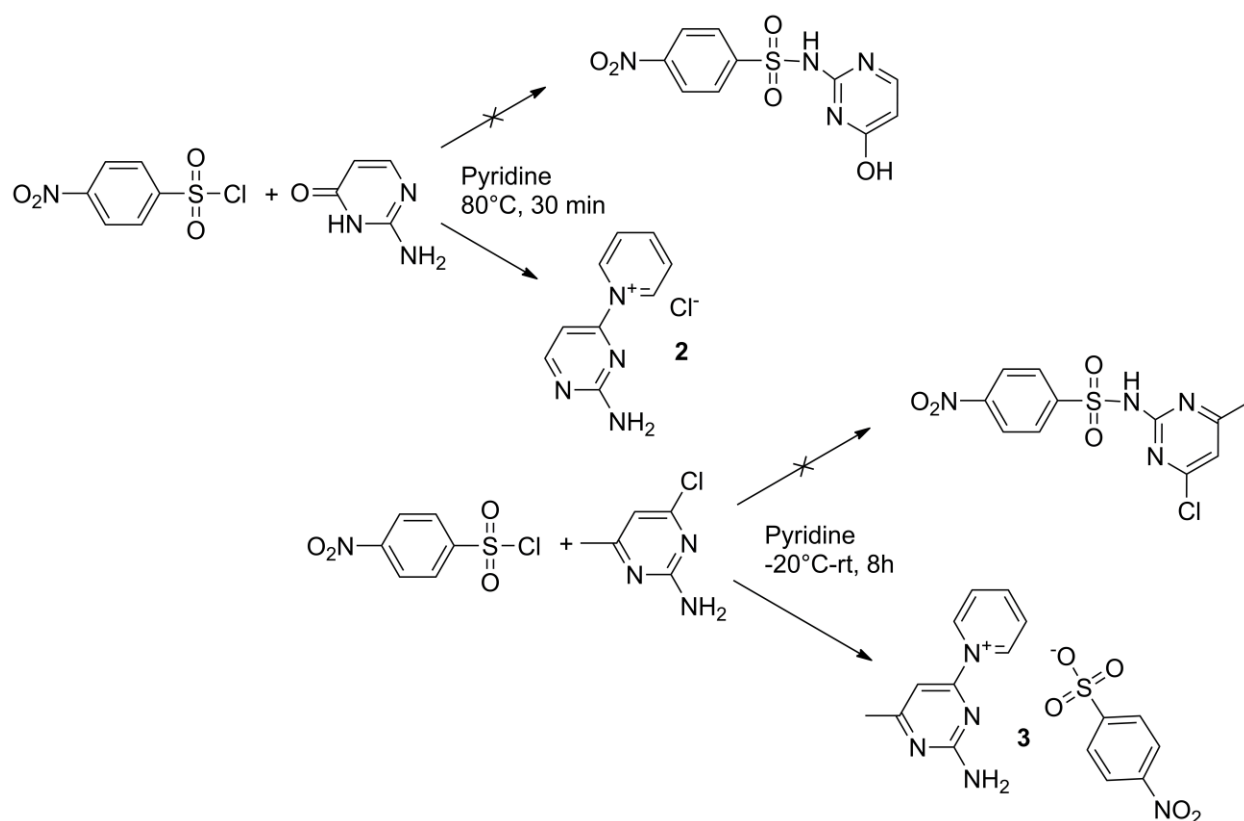


Figure 1. The pyridyl sulfonamide derivative is inert to nucleophilic fluorination.

The electron density of the pyridine ring may be too high to allow atom exchange under these conditions and therefore, heterocycles containing more than one nitrogen atom were considered because of the electron withdrawing effect.

We chose isocytosine as the pyrimidine derivative, as we thought it would allow chlorination in exchange of the hydroxy group once the starting material is coupled with the sulfonyl chloride. Instead of this, however, the isocytosine component reacted with pyridine to form the respective 1-(2-aminopyrimidin-4-yl)pyridinium chloride (**2**, Scheme 1). In analogy to this, attempts to couple 2-amino-4-chloro-6-methylpyrimidine with 4-nitrobenzenesulfonyl chloride in pyridine did not result in the desired sulfonamide, but rather the 1-(2-amino-6-methylpyrimidin-4-yl)-pyridinium salt **3**. To our knowledge, this phenomenon has only been reported by Price et al. who described bromopyrimidines that did not react to form the desired sulfanilyl amides.¹⁰



Scheme 1. Unexpected pyridinium products.

The structures of the unforeseen pyridinium products were confirmed by 2D NMR (Figure 2 and 3a-d). The atoms could be assigned, and all expected correlations were found. The C-H values for 4-nitrobenzenesulfonate were assigned via three-bond HMBC, and compared to values obtained by estimation software (ChemDraw, Cambridge/USA).

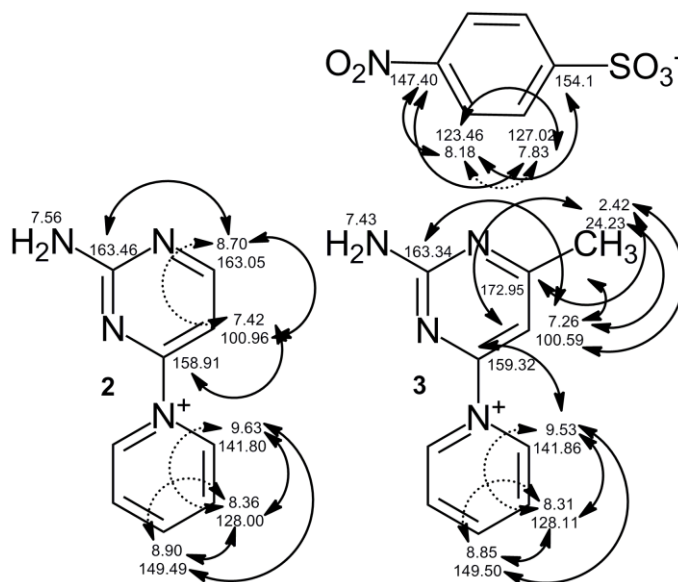


Figure 2. HMBC (solid arrow) and COSY (dotted arrow) analysis of pyridinium salts.

As pyridine is often the solvent of choice for such reactions, and the analogous synthesis was described,^{11,12} these products were unexpected.

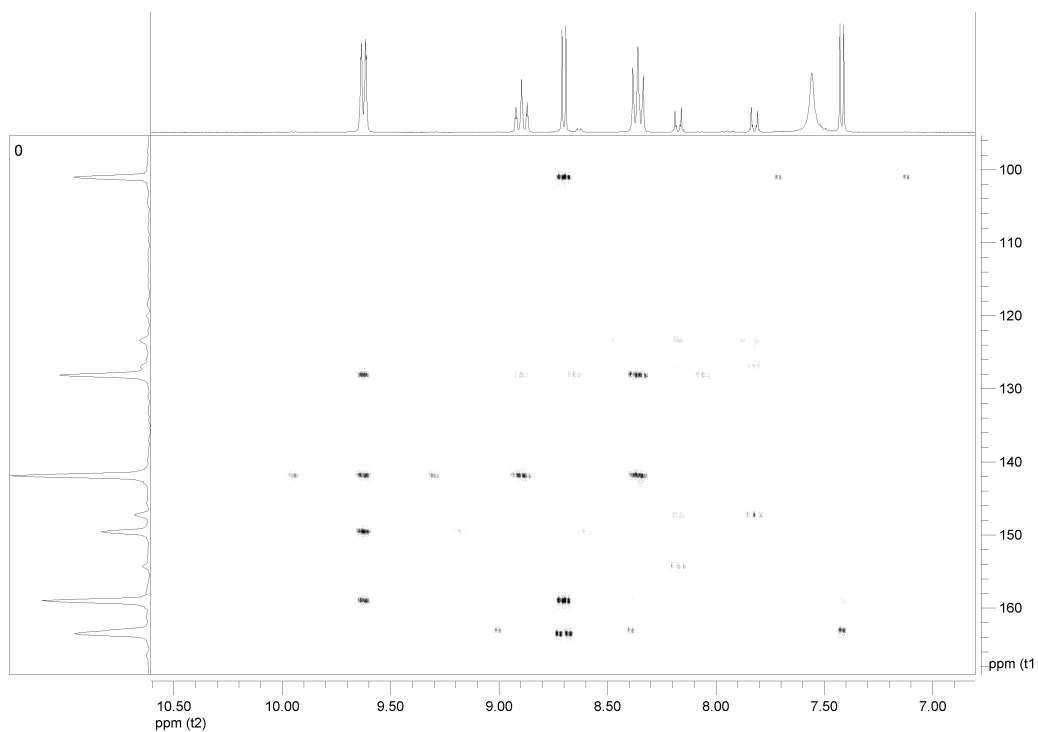


Figure 3a. HMBC spectrum of compound **2**.

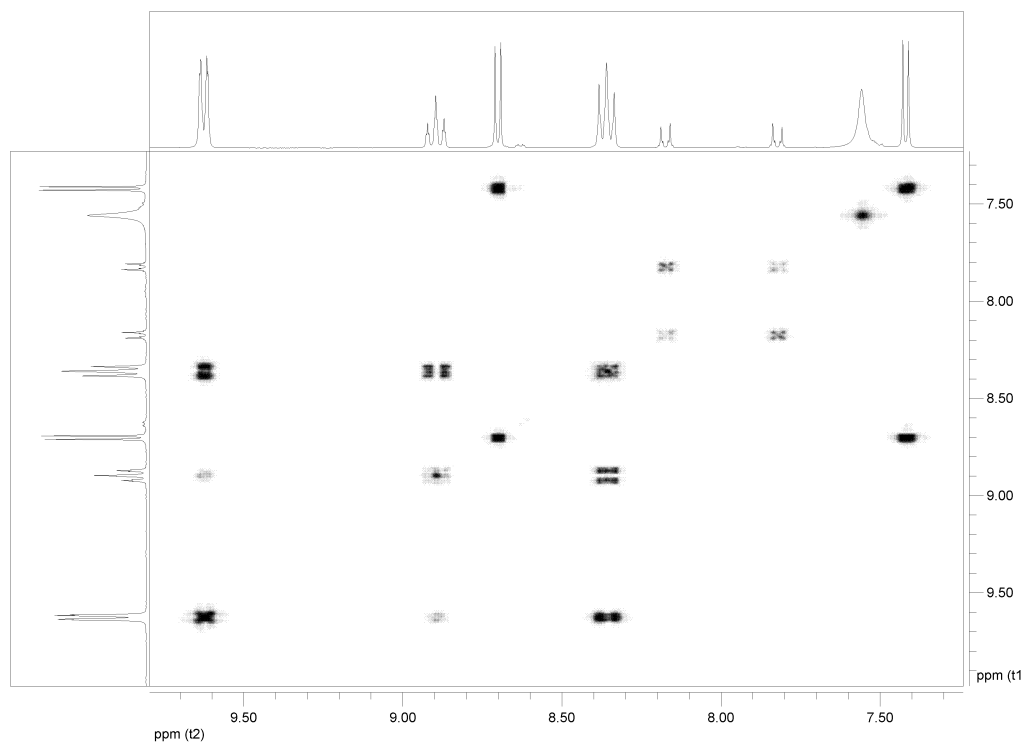


Figure 3b. COSY spectrum of compound **2**.

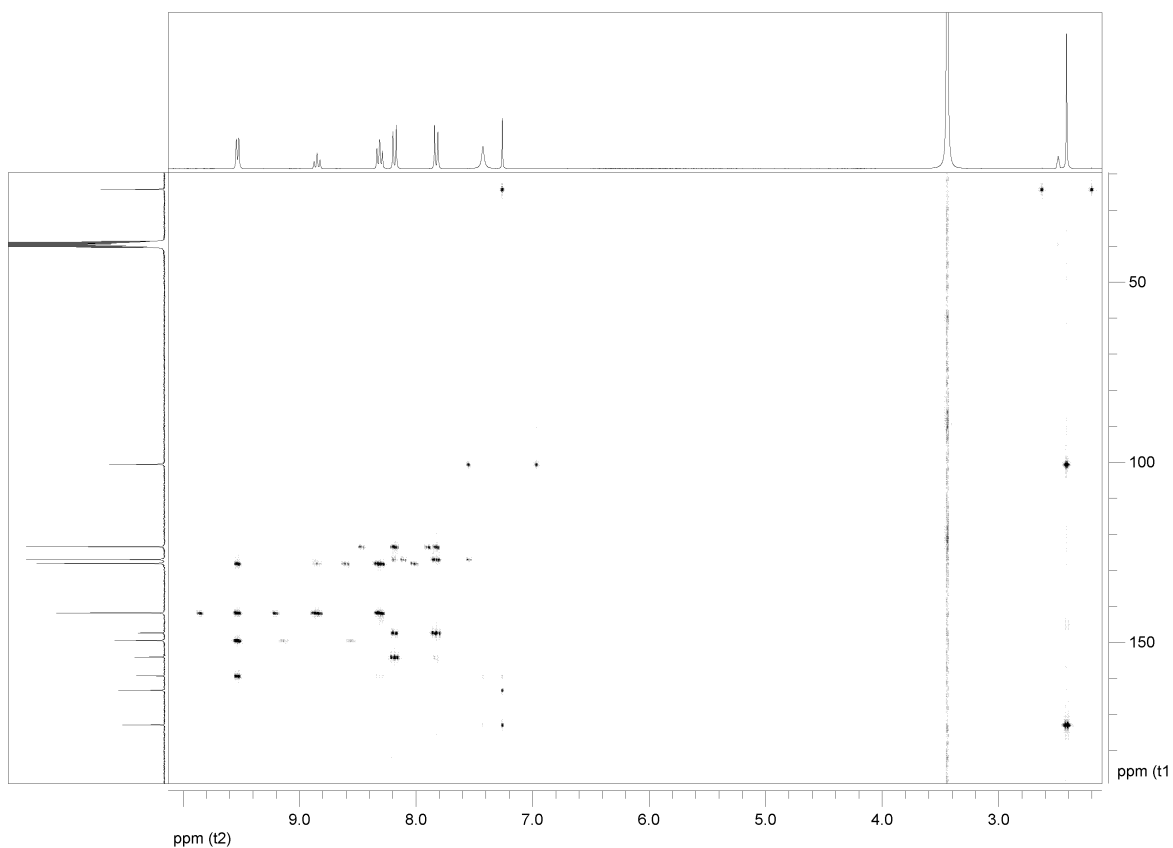


Figure 3c. HMBC spectrum of compound **3**.

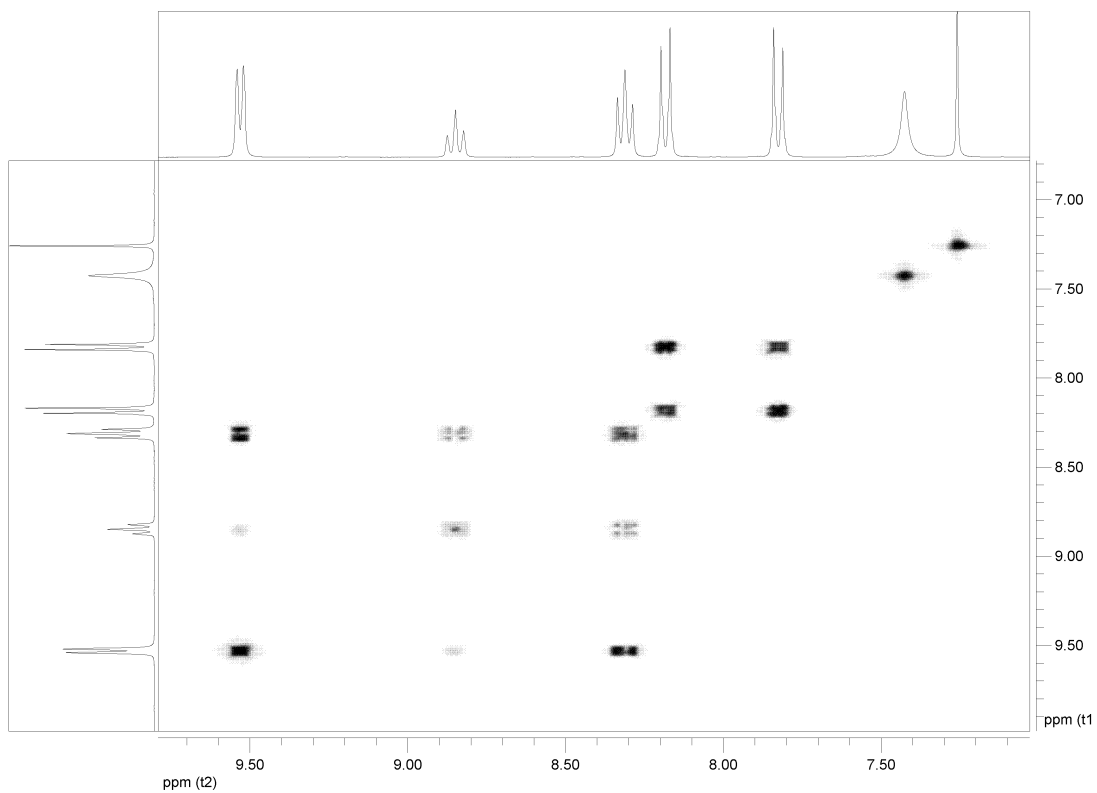
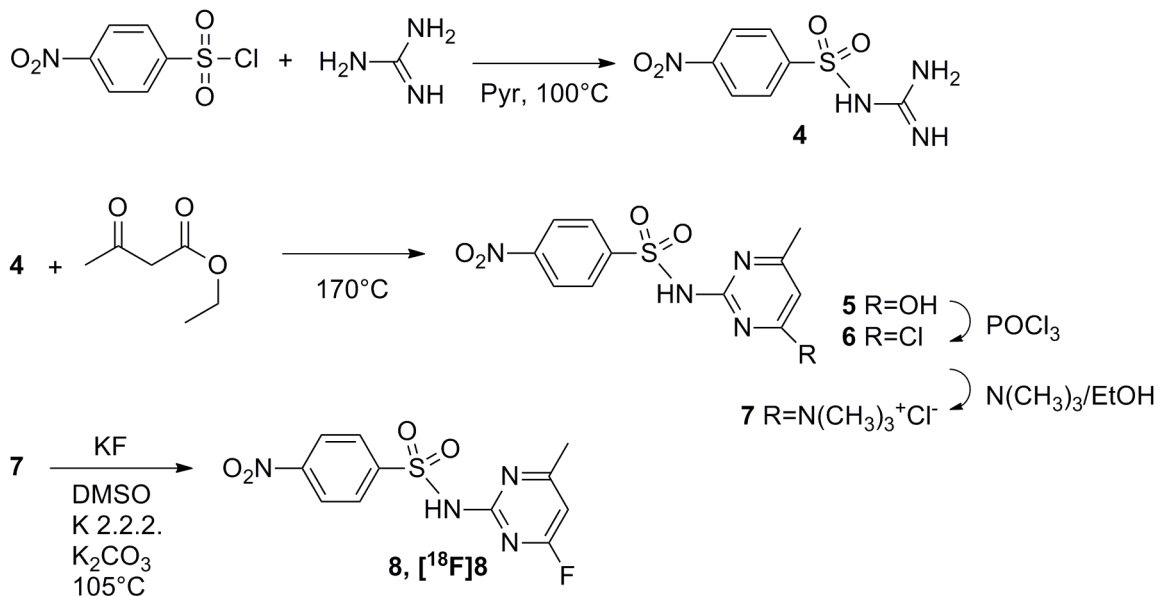


Figure 3d. COSY spectrum of compound **3**.

Synthesis via condensation of the guanidyl compound

We adopted a new strategy and decided to use 4-nitrobenzenesulfonyl guanidine (**4**, Scheme 2) as the starting material in a condensation reaction. The initial attempts to synthesize **4** yielded only the guanidinium salt. We succeeded using a fourfold excess of guanidinium chloride and rapid heating of the mixture to reflux after mixing the components. In this way, it was possible to obtain good yields of the desired products. Consecutive steps were performed according to a standard reaction protocol¹³ (Scheme 2). Using this precursor (**7**), **8** was synthesized in 100% yield as a “cold” reference



Scheme 2. Synthesis of **8**/¹⁸F]-**8**, a 4-fluorinepyrimidin-*N*-sulfonamide derivative.

Semi automated synthesis of *N*-(4-[¹⁸F]fluoro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide ([¹⁸F]-**8**)

For the radiosynthesis a Hotbox III remote controlled synthesis module was used. Reaction times and temperature were adapted to optimize yield and purity of the product.

The overall reaction time for this semiautomated synthesis (Figure 4) of ¹⁸F-labeled **8** was 45 min. The product was manually purified using RP18 cartridges. The average yield of [¹⁸F]-**8** was 58±12% (n = 4, RCY, decay corrected) in ethanol. The tracer can be dissolved in 0.9% NaCl and a small amount of PEG200 to avoid precipitation.

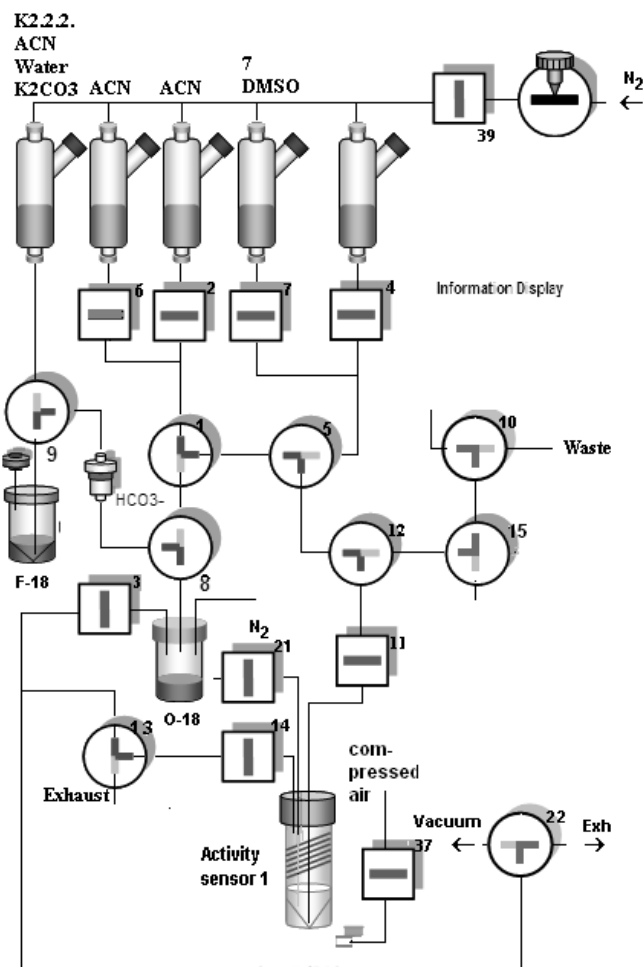


Figure 4. Setup of the Scintomics Hotbox III synthesizer.

Radiochemical purity and radiochemical identity were monitored via HPLC and TLC and the product was confirmed by overlapping the UV and radioactivity peaks, whereby the latter could be confirmed as slightly delayed due to the installation of detectors (Figure 5). TLC purity ($89.2 \pm 8.4\%$, $n = 4$) was in the same range of HPLC ($94.9 \pm 3.2\%$, $n = 3$).

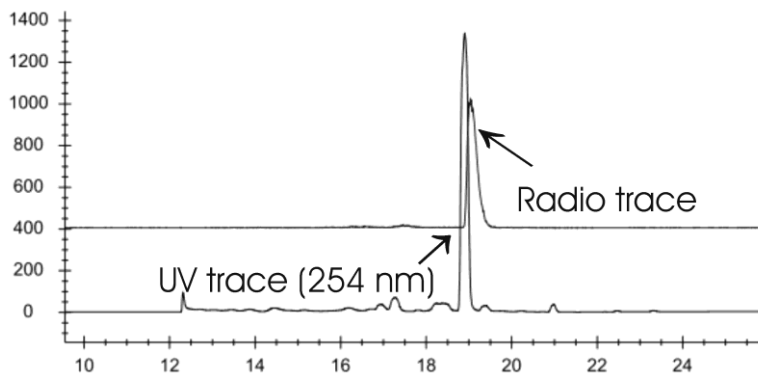


Figure 5. Comparison between UV (8) and radio trace [^{18}F]-8.

Conclusions

Although the successful synthesis of sulfonamides has been described a number of times in recent decades, there are still obstacles in synthesizing functionalized halopyrimidines. We found that in isocytosine and 2-amino-4-chloro-6-methylpyrimidine, the OH- or Cl-substituted carbons quaternize more easily than formation of sulfonamide bonds occurred. An alternative strategy involving the condensation of the *N*-guanidinium sulfonamide **4** with ethyl acetoacetate followed by chlorination was successful. Using this strategy, the precursor **7** for [¹⁹F] and [¹⁸F]fluoride nucleophilic substitution was synthesized with good yields. Both *in vitro* and *in vivo* studies of positron emitting compound [¹⁸F]-**8** are currently being investigated. To be used as a tracer for bacterial infection, the nitro group will need to be reduced.

Experimental Section

General. HR ESI-MS analysis was performed with a TSQ Quantum AM Ultra (Thermo Electron). NMR analysis was performed using Avance DPX 300, 500 and 600 MHz spectrometers (Bruker, Karlsruhe, Germany). The activimeter (Isomed 2000 and the Isomed 2000 well counter) was from MED Nuklear-Medizintechnik Dresden GmbH (Dresden, Germany). For radioanalytical TLC, a Rita Star apparatus with Rita Control 1.24 software was used (Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany). The analytical radio HPLC was from Sykam GmbH (Eresing, Germany) and was equipped with a DAD-Smartline UV detector 2600 and scintillation radio detector (γ -sensorPE Scintomics, Fürstenfeldbrück, Germany), connected in series. For separation, a RP18 column was used (Eurospher 250x4.6mm, 100-5, Knauer, Germany).

The mobile phase consisted of 0.05% TFA in water (A) and acetonitrile (B). The eluting conditions were: 0–5 min, 100% A isocratic; 5–15 min, gradient from 100 to 50% A, 15–18 min, gradient from 50 to 100% B, 18–25 min, 100% B isocratic, 25–30 min, 100% A isocratic to re-equilibrate the system.

All HPLC runs were performed under these conditions. Water was deionized and purified by a Purelab Ultra system (Elga Berkefeld GmbH, Celle, Germany). HPLC grade acetonitrile was from Merck (Darmstadt, Germany). Anhydrous pyridine was purchased from Sigma-Aldrich (Taufkirchen, Germany). Unless otherwise stated, chemicals and solvents were of analytical grade and used as received.

***N*-(6-Chloropyridin-2-yl)-4-nitrobenzenesulfonamide (1).** 2.22 g (1 mmol) 4-nitrobenzenesulfonyl chloride were added to a stirred solution of 1.28 g (1 mmol) 2-amino-6-chloropyridine in 50 ml pyridine. The mixture was stirred for 3 h at 85 °C and allowed to cool to ambient temperature. The pyridine was evaporated, and 50 ml water were added to remove residual pyridine. The residue was dissolved in 250 ml of dichloromethane and the organic phase was washed with sodium hydrogencarbonate (80 ml), brine (80 ml) and dried over sodium sulfate. TLC gave no evidence of starting material. The oily residue crystallized after 3 d. HRMS: 311.9847 [M-

H], calc. for $C_{11}H_7N_3O_4ClS$ 311.985, 1H (300.1 MHz, $DMSO-d_6$) δ ppm 11.76 (s, 1H), 8.40 (d, J 8.9 Hz, 2H), 8.19 (d, J 8.9 Hz, 2H), 7.74 (t, J 7.94, 7.94 Hz, 1H), 7.12 (d, J 7.7 Hz, 1H), 7.02 (d, J 8.1 Hz, 1H), ^{13}C (75.5 MHz, $DMSO-d_6$) ppm 150.75, 149.97, 148.18, 145.17, 141.84, 128.96, 124.36, 118.79, 110.61

(2-Aminopyrimidin-4-yl)pyridinium chloride (2). A slurry of 0.55 g isocytosine in 40 ml dry pyridine was heated to 80 °C with stirring. After 30 min, 1.11 g of 4-nitrobenzenesulfonyl chloride were added. After a short time, the mixture was nearly dissolved. A precipitate formed after a few minutes. Stirring continued for 2.5 h. The precipitate was then filtered and washed with pyridine. Drying under high vacuum yielded 0.745 g of 1-(2-aminopyrimidin-4-yl)pyridin-1-ium chloride (72.2%). HRESI-MS: m/z 173.0826 [M^+], 173.0822 $C_9H_9N_4^+$. 1H (300 MHz, $DMSO-d_6$) ppm 9.63 (dd, J 6.8, 1.2 Hz, 2H), 8.90 (tt, J 7.90, 1.24, Hz, 1H), 8.70 (d, J 5.3 Hz, 1H), 8.36 (m, 1H), 7.56 (s, 2H), 7.42 (d, J 5.3 Hz, 1H). ^{13}C (75.5 MHz, $DMSO-d_6$) δ ppm 163.46, 163.05, 158.91, 149.49, 141.80, 128.00, 100.96.

(2-Amino-6-methylpyrimidin-4-yl)pyridinium 4-nitrobenzenesulfonate (3). A stirred solution of 0.71 g (0.5 mmol) 2-amino-4-chloro-6-methylpyrimidine in 30 ml pyridine was cooled to -20 °C, and 1.11 g (0.5 mmol) of 4-nitrophenylsulfonyl chloride were added. The solution was then allowed to warm to ambient temperature over 2 h and stirred for another 6 h, after which analytical HPLC showed complete consumption of the starting material. The pyridine was evaporated, and residual pyridine was removed by the addition of water (30 ml) and evaporation. This was repeated twice. The residue was mixed with 3 ml n-propanol, warmed to ambient temperature, and cooled to 0 °C. The precipitate was collected on a glass frit, washed with n-propanol, and dried at 10 mBar. The yield was 1.05 g (54.0%). HRMS: 187.0977 M^+ , calc. for $C_{10}H_{11}N_4^+$ 187.0978, m/z : 187.098 (100.0%), 188.102 (10.8%), 188.095 (1.5%). 1H (300 MHz, $DMSO-d_6$) δ ppm 9.53 (d, J 5.74 Hz, 2H), 8.85 (t, J 7.74, 7.74 Hz, 1H), 8.31 (t, J 7.17, 7.17 Hz, 2H), 8.18 (d, J 8.72 Hz, 2H), 7.83 (d, J 8.71 Hz, 2H), 7.43 (s, 2H), 7.26 (s, 1H), 2.42 (s, 3H). ^{13}C (75.5 MHz, $DMSO-d_6$) δ ppm 172.95, 163.34, 159.32, 154.11, 149.50, 147.40, 141.86, 128.11, 127.02, 123.46, 100.59, 24.23.

4-Nitrobenzenesulfonyl guanidine (4). A suspension of 6.7 g (30 mmol) 4-nitrophenylsulfonyl chloride and 12.7 g guanidinium chloride (0.134 mol) in 30 ml pyridine was stirred and heated to 100 °C. The mixture became red, and after a short time a precipitate formed. The pyridine was evaporated, 50 ml water were added, and the mixture was distilled to remove pyridine, after which 50 ml water and 50 ml dichloromethane were added. A precipitate formed, which was collected on a frit, washed thoroughly with water, and dried in a dessicator. 5.02 g of a colourless product was obtained (64.0%). HRMS: 245.0342 [$M+H$] $^+$, calc. for $C_7H_9N_4O_4S$ 245,0339. 1H (300 MHz, $DMSO-d_6$) δ ppm 8.33 (d, J 8.79 Hz, 2H), 7.98 (d, J 8.77 Hz, 2H), 6.83 (s, br, 4H). ^{13}C (75.5 MHz, $DMSO-d_6$) δ ppm 158.32, 150.00, 148.92, 127.19, 124.28.

N-(4-Hydroxy-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide (5). A mixture of 3.12 g (12.8 mmol) 4-nitrobenzenesulfonyl guanidine (4) and 15 ml ethyl acetoacetate was refluxed at 170-180 °C for 1.5 h. The condenser was removed after 30 min to evaporate alcohol and water formed during the reaction. The resulting red solution was cooled to ambient temperature and allowed to crystallize overnight. The precipitate was collected on a glass frit. The product was

washed with a small amount of methanol and was evaporated to dryness. The reaction yielded 2.12 g (53.4%) of a white product. HRMS: 309.0296 [M-H]⁻, calc. for C₁₁H₉N₄O₅S 309.0299. ¹H (300 MHz, DMSO-*d*₆) δ ppm 11.74 (s, br, 1H), 9.33 (d, *J* 8.83 Hz, 2H), 9.11 (d, *J* 8.83 Hz, 2H), 5.73 (s, 1H), 2.11 (s, 3H). ¹³C (75.5 MHz, DMSO-*d*₆) δ ppm 160.52, 153.58, 150.47, 149.53, 148.14, 127.55, 124.57, 102.68, 18.61.

***N*-(4-Chloro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide (6).** A mixture of 2.05 g (6.61mmol) *N*-(4-hydroxy-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide and 10 ml phosphoryl chloride was refluxed for 30 min with stirring. After cooling, 100 g of ice was added in portions. The red mixture became colourless and a precipitate was formed, which was collected on a glass frit. The precipitate was dissolved in 30 ml of 0.05 M sodium hydroxide. The brown solution was then cooled on ice and acidified with diluted acetic acid (10% v/v) with stirring. The precipitate was separated on a glass frit, washed thoroughly with water, and dried in a desiccator over potassium hydroxide yielding 1.925 g (88.5%) of the brownish product. HRMS: 326.9956 [M-H]⁻, calc. for C₁₁H₈ClN₄O₄S 326.9960. ¹H (300 MHz, DMSO-*d*₆) δ ppm 8.37 (d, *J* 8.70 Hz, 2H), 8.19 (d, *J* 8.70 Hz, 2H), 7.08 (s, 1H), 2.29 (s, 3H), 2.10 (s, 1H). ¹³C (75.5 MHz, DMSO-*d*₆) δ ppm 169.13, 161.35, 155.75, 149.87, 145.83, 129.57, 124.15, 113.98, 22.18.

***N,N,N*,6-Tetramethyl-2-(4-nitrophenylsulfonamido)pyrimidin-4-ammonium chloride (7).** In a sealed flask, a solution of 580 mg *N*-(4-chloro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide in 20 ml tetrahydrofuran was stirred under argon and cooled to 0 °C. A solution of trimethylamine in ethanol (33 wt.%, Acros Organics, Nidderau/Germany) was added dropwise. After 10 min, a suspension formed. It was stirred overnight to allow the mixture to reach ambient temperature. The resulting precipitate was separated immediately on a glass frit, washed with anhydrous diethyl ether and dried, yielding 670 mg (98.0%) of a greyish to white product. HRMS: 352.1074 M⁺, calc. for C₁₄H₁₈N₅O₄S⁺ 352.1074; ¹H (600 MHz, DMSO-*d*₆) δ ppm 10.42 (s, br, 1H), 8.25 (d, *J* 8.83 Hz, 2H), 8.01 (d, *J* 8.83 Hz, 2H), 6.89 (s, 1H), 3.21 (s, 9H), 2.26 (s, 3H). ¹³C (150.9 MHz, DMSO-*d*₆) δ ppm 171.30, 164.35, 163.04, 152.31, 147.91, 128.18, 123.32, 97.41, 53.13, 24.14.

***N*-(4-Fluoro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide (8).** A suspension of potassium fluoride (anhydrous, 135 mg), Kryptofix 222 (33 mg), potassium carbonate (anhydrous, 0.3 mg), *N,N,N*,6-tetramethyl-2-(4-nitrophenylsulfonamido)pyrimidin-4-ammonium chloride (124 mg) in dry DMSO was warmed to 105 °C over 2.5 h. The product was applied to a short column (silica gel 60 0.04-0.06 mm) (l=80 mm, d=20 mm) and eluted with ethyl acetate, yielding 82 mg (73.8%) of the white product. HRMS: 311.0252 M⁺, calc. for C₁₁H₈FN₄O₄S 311.0256. ¹H NMR (500.3 MHz, DMSO-*d*₆) δ ppm 13.0 (s, br, 1H), 8.35 (d, *J* 8.9 Hz, 2H), 8.14 (d, *J* 8.8 Hz, 2H), 6.63 (s, 1H), 2.31(s, 3H). ¹³C (125.8 MHz, DMSO-*d*₆) δ ppm 169.78 (d, ¹*J*_{FC} 253.5 Hz), 156.14 (d, ³*J*_{FC} 21.0 Hz), 149.25, 147.47, 129.04, 123.84, 97.29 (d, ²*J*_{FC} 33.5 Hz), 21.46.

Semiautomated synthesis of *N*-(4-fluoro-6-methylpyrimidin-2-yl)-4-nitrobenzenesulfonamide ([¹⁸F]8). For labelling experiments, a Scintomics synthesis module was used. Drying and labelling were controlled by Scintomics software adapted for this application. [¹⁸F]fluoride in H₂¹⁸O was purchased from f-con (Holzhausen, Germany) and was produced in the PET center of the Central Clinic in Bad Berka Germany. The activity was trapped on a QMA cartridge (SPE cartridge Chromafix 30-PS-HCO₃, preconditioned with 5 ml of 0.5 M potassium carbonate and 2 x 5 ml of

water). The activity was eluted from the resin with a solution of 3 mg K_2CO_3 and 20 mg Kryptofix 222 (synthesis grade; Merck) in 1 ml water/acetonitrile 0.3/0.7 (v/v) into a 5 ml conical glass vial (Wheaton V-Vial, 20 x 65 mm, purchased from Neolab, Heidelberg), which was preheated to 200 °C. The solvent was removed under a stream of nitrogen. After 2 min, a portion of 0.7 ml acetonitrile (Sigma-Aldrich, anhydrous) was added to the residue, and it was dried for another 3 min. A second portion of 0.7 ml acetonitrile was added, and after complete evaporation the system was cooled to 80 °C. Next, a solution of 1-2 mg **7** in 0.5 ml DMSO was added. This mixture was then heated (preset of 80 °C) in a closed vial and in 3 min intervals, a nitrogen stream mixed the solution. After a few minutes, the solution became visibly red. The overall reaction time was 20 min, and the mixture became dark red. The product was purified manually with a RP18 cartridge. The DMSO solution (~0.5 ml) was diluted with 5 ml water and the mixture was applied to a RP18 cartridge (Waters Corporation), which was activated with 10 ml of DMSO and 10 ml of a 1:9 (v/v) DMSO-water mixture. The cartridge was washed with 5 ml water. The product was eluted with 1.5 ml of ethanol. The radiochemical yield was up to 74% with radiochemical purity of 91%-99% (see HPLC conditions above). Radio TLC (on unmodified silica gel 60 F₂₅₄, VWR, Germany) was performed using ethyl acetate/methanol 9:1 yielding a radioactive peak at $R_F=0.57$.

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