Tetrahedron Letters 53 (2012) 5008–5011

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Studies on the acylation of 4-(2-aminoethylthio)-7-nitrobenzofurazan: the role of bases in promoting the formation of fluorescent *S*-acyl derivatives through S–N Smiles rearrangement

Daniele Castagnolo [a], Mafalda Pagano [a], Martina Bernardini [a], Maurizio Botta [a,b,*]

[a] *Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, via A. Moro, 53100 Siena, Italy*
[b] *Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, BioLife Science Bldg., Suite 333, 1900 N 12th Street, Philadelphia, PA 19122, USA*

ARTICLE INFO

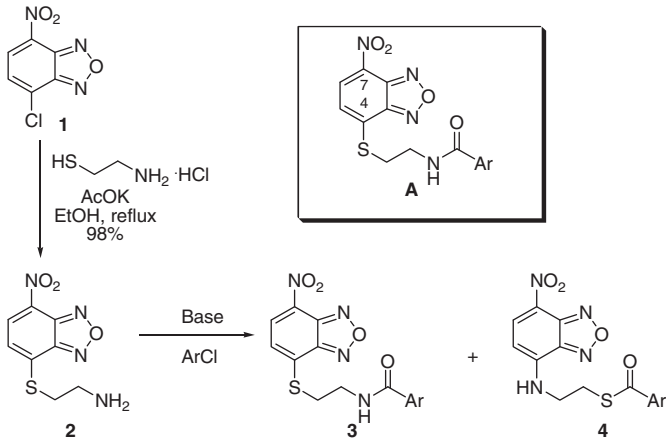
Article history:
Received 8 May 2012
Revised 29 June 2012
Accepted 9 July 2012
Available online 16 July 2012

Keywords:
S–N acylation
Smiles rearrangement
Fluorescent probe
Benzofurazan
Cysteamine

ABSTRACT

The acylation of 4-(2-aminoethylthio)-7-nitrobenzofurazan has been investigated. Depending on the use of the base, a competitive Smiles rearrangement occurs during the acylation step leading to the formation of *N*-acyl and/or fluorescent *S*-acyl derivatives. The acylating agent also affects the ratio of N/S acylated isomers.

Benzofurazans (Bf) represent an interesting class of organic compounds which is receiving increasing attention due to their use in different fields of chemistry.[1] Compounds containing a benzofurazan (benzo[1,2-*c*][1,2,5]oxadiazole) skeleton have been widely used in the field of bioscience as fluorogenic and fluorescent reagents due to their chemical and physical properties such as the large Φ_f values of the resultant derivatives, the high reactivity to analytes, and the long excitation and emission wavelengths.[2] In particular, the 4,7-disubstituted-benzofurazans found a broad application in bioanalytical chemistry[3–5] due to their unique character regarding fluorescent properties.[6] Experimental and theoretical works showed that the key factors in control of the fluorescence of 4,7-substituted benzofurazan dyes are the dipole moment across the benzene ring (from position 4 to 7) and the electron density found on the ring.[7] The commercially available 4-chloro-7-nitro-benzofurazan (NDBf-Cl) **1** (Scheme 1) and, more in general, the 7-nitro-4-substituted benzofurazans (NDBf), are 10π electron heteroaromatic substrates which exhibit an extremely high electrophilic character[8] and have been used as reactivity probes and as fluorescent labeling reagents in the study of a number of proteins.[9] In addition, the NDBf derivatives also proved to possess several biological properties finding growing application in medicinal chemistry as antileukemic/antiapoptotic agents and monoamine oxidase inhibitors.[10,11]



Scheme 1. Acylation of cysteamine derivative **2**.

In the course of our studies on antiviral compounds[12,13] we became interested in the synthesis of novel hits containing the NDBf nucleus. In particular we focused on the synthesis of NDBf derivatives with general structure A (Scheme 1) containing an acylated

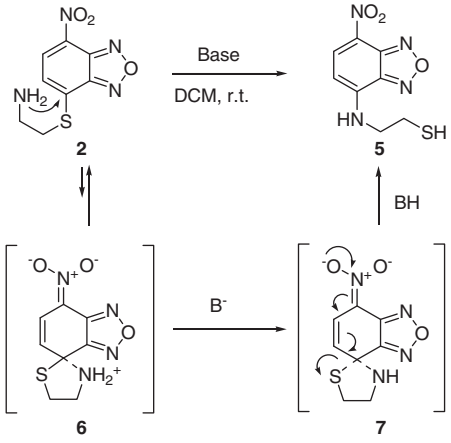
* Corresponding author.
E-mail address: botta.maurizio@gmail.com (M. Botta).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.07.033

cysteamine chain at C-4. Surprisingly we found that acylation of cysteamine derivative **2** led in the presence of Et_3N to the formation of desired compound **3** together with a fluorescent side product which, after NMR and MS analyses, revealed to be the *S*-acyl-derivative **4** (Scheme 1). The formation of this latter compound was assumed to be due to a S–N Smiles rearrangement of the cysteamine chain of **2** on the electrophilic benzofurazan core. The Smiles rearrangement is an intramolecular aromatic nucleophilic substitution of an appropriately placed nucleophile onto aromatic rings and it is usually catalyzed by bases, acids, or heat.[16] Few examples of O–O and S–O Smiles rearrangements on furazan, benzofurazan, and benzofurazan-N-oxide heterocycles have been described in the literature and generally they occur in the presence of strong bases such as NaOH or KOMe.[14] However, to the best of our knowledge, no examples of S–N Smiles rearrangement on benzofurazans have been reported so far.

Herein, we decided to investigate the influence of the Smiles rearrangement in the acylation of 7-(2-aminoethylthio)-4-nitrobenzofurazan. In particular, we demonstrated that the occurrence of the S–N Smiles rearrangement on benzofurazan **2** is strictly dependent on the base and acylating agents used and that an appropriate setting up of the reaction conditions and a careful use of reagents could allow the selective S- or N-acylation of **2**.

Cysteamine-NDBf **2** was prepared in almost quantitative yield through the reaction of cysteamine hydrochloride (1.1 equiv) with NDBf-Cl **1** (1.0 equiv) in refluxing ethanol and in the presence of a catalytic amount of KOAc (Scheme 1).[10–18] As expected the more nucleophilic sulfur of cysteamine reacts with NDBf-Cl **1** faster than nitrogen atom leading selectively to C4–S derivative **2** while no traces of the C4–N regioisomer were observed. We first decided to react **2** with different bases under mild conditions (DCM, 25 °C), in order to investigate its tendency to give the S–N-Smile rearrangement (Scheme 2, Table 1). Treatment of NDBf **2** with 1–2 equiv of pyridine did not lead to the formation of the rearranged NDBf **5** even after several hours and the starting product **2** was completely recovered from the reaction mixture (entries 1–2). On the other hand, when **2** was treated with the more basic Et_3N (1 equiv) partial conversion into **5** was observed by ^1H NMR (entry 3). Complete conversion was achieved by treatment of **2** with 2 equiv of Et_3N (entry 4). The ^1H NMR of compound **2** shows a typical H-5 peak at 7.74 ppm while the H-5 proton of 4-N-analog **5** falls at 6.35 ppm. Similarly, treatment of **2** with MeONa (2 equiv) led to complete conversion into **5** (entry 5), while in the presence of K_2CO_3 or KOH no rearrangement was observed even if higher amounts of bases were used (entries 6–7). According to the literature data [14] the Smiles rearrangement is supposed to proceed through a S_NAr at *ipso* position with the formation of a spirocyclic



Scheme 2. Smiles rearrangement: mechanism.

Table 1
Base promoted Smiles rearrangement on **2**

Entry	Base	Amount (equiv)	Ratio[a,b] (%)	
			2	**5**
1	Pyr	1	100	N.D.[c]
2	Pyr	2	100	N.D.[c]
3	Et_3N	1	70	30
4	Et_3N	2	N.D.	100
5	MeONa	2	N.D.	100
6	KOH	2	100	N.D.[c]
7	K_2CO_3	2	100	N.D.[c]

[a] Ratios were determined by GC–MS and ^1H NMR analysis of the crude mixtures.
[b] Reactions were completed after 1 h.
[c] Not detected.

Meisenheimer complex intermediate **6** (Scheme 2). The first step of the reaction is the formation of the five-membered intermediate **6** due to the attack of the nucleophilic amine at the *ipso* position. Deprotonation of **6** by a base leads to intermediate **7** which in turn is converted into rearranged compound **5**.[15]

Since Et_3N and MeONa favor the Smiles rearrangement of **2** leading to the formation of **5**, it was reasonable to assume that under appropriate acylation conditions the formation of *N*- or *S*-acyl derivatives could be selectively achieved. Hence, compound **2** was reacted with different electron rich/poor acylating agents (namely thienyl, benzoyl, and nicotinoyl chlorides) in the presence of different bases. Results are reported on Table 2.

Thienyl (ThCl), benzoyl, and nicotinoyl chorides (NicCl) were reacted with **2** in the presence of pyridine at rt leading to the slow formation of acyl derivatives **3a–c** in several hours. On the other hand, the addition of DMAP accelerated dramatically the rate of the reaction leading to **3a** and **3c** in a few hours (5.5 and 3 h respectively, entries 1 and 3). The formation of benzoyl derivative **3b** proved to be faster and was accomplished in only 30 min (entry 2). However, in all cases, only traces of the S-acylated rearranged products **4** could be detected by ^1H NMR. A similar behavior of **2** was observed when the same acylation reactions were carried out using KOH as a base (data not shown). Benzofurazan **2** was then reacted with ThCl in the presence of 1.1 equiv of Et_3N affording in 1 h a mixture of **3a/4a** in a 1:1 ratio (entry 4). Compound **4a** proved to be fluorescent as shown in Figure 1. Increasing the amount of Et_3N to 1.5 equiv led to the formation of **3a/4a** in a 7:3 ratio in only 30 min, while the further increase of the base to 2 equiv resulted in a drop of the isomeric ratio to 1:1 (entries 5–6).

These surprising results may be explained if a competition between the acylation step of **2** and the Et_3N promoted Smiles rearrangement is assumed. Hence, it is reasonable to think that the base promotes the rearrangement of the cysteamine chain and in the meantime favors the N-acylation of **2** and the S-acylation of the forming **5**.[16] To confirm this assumption, compound **2** was treated with 2 equiv of Et_3N and the reaction mixture was stirred 2 h before adding ThCl. Formation of **4a** as the only product was expected. Surprisingly, a 1:1 mixture of **3a** and **4a** was recovered from the reaction mixture (entry 7). A base mediated equilibration between *N*- and *S*-acyl derivates **3a** and **4a** was then hypothesized and, in order to explain these results, we decided to treat compound **4a** with 2 equiv of Et_3N. Quantitative conversion of **4a** into **3a** was observed by ^1H NMR within 24 h proving that compound **4a** is the kinetic product of the reaction and compound **3a** is the thermodynamic. According to Wieland and Bokelmann studies on cysteamine,[17] a plausible mechanism for the conversion of **4a** into **3a** is reported in Scheme 3. On the basis of this result we can assume that the treatment of **2** with Et_3N led to **5** and addition of ThCl after 2 h led to the formation of kinetic product **4a**. However, the base starts meanwhile to catalyze the conversion of **4a** into **3a**, thus affording a 1:1 mixture of the two products. As fur-