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Improved synthesis of 4-amino-7-nitrobenz-2,1,3-oxadiazoles using NBD fluoride (NBD-F)

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ABSTRACT

The 7-nitrobenz-2,1,3-oxadiazole (NBD) unit is a highly useful fluorescent tag with wide application in biology. Installation of the NBD group typically proceeds via the S_NAr reaction between an amine and an NBD halide. Herein, we demonstrate that NBD-F **1** results in significantly higher yields than NBD-Cl **2**, and that triethylamine in dimethylformamide at 23 °C overnight is a broadly applicable set of conditions for this reaction. In particular, the highly useful fluorescent carbohydrate 2-NBD-glucosamine (2-NBDG, **3**) can now be prepared in 75% yield with NBD-F as compared to 12% with NBD-Cl.

First introduced in 1968,[1a] the 7-nitrobenz-2,1,3-oxadiazole (NBD) unit is a very useful fluorescent tag that has found wide application in biology,[1b] for example, in the labeling of amino acids,[2] lipids,[3] biotin,[4] sugars,[5] proteins,[6] and other molecules.[7] It is typically installed via an S_NAr reaction between an amine (either primary or secondary) and the corresponding halide (either the chloride **1** or fluoride **2**) to produce the highly fluorescent product. While both simple primary and secondary aliphatic amines generally react with NBD-Cl to afford the corresponding S_NAr products in good yield (50–100%),[8] other amines have been known to result in very low yields,[9] possibly due to steric hindrance or to the presence of competing hydroxyl functional groups (Fig. 1). In particular, 2-NBD-glucosamine (2-NBDG, **3**) is a small molecule that has been used extensively to study the uptake of glucose in a variety of organisms. Used in conjunction with flow cytometry, it has become a very useful method for measuring glucose uptake in real time in bacteria,[10] yeast,[11] and mammalian cells.[5a] During the course of a study, we needed a large quantity of this compound. Surprisingly, the best reported literature yield of 2-NBDG **3** was only 12%, and involved the reaction of glucosamine hydrochloride and NBD-Cl in an aqueous sodium bicarbonate solution.[5a] Similarly, a synthesis of the NBD-fructosamine **4** was also reported in low yield (<5%),[5b] as were several other amines of biological relevance.[9] There have been numerous studies in the literature (mainly involving the pre-column derivatization of amino acids for HPLC) that have demonstrated the increased reactivity of NBD-F compared to NBD-Cl.[12] However, many of these studies were carried out on extremely small scale (μmol) and in most cases, the reaction products were not isolated nor the yields reported. To the best of our knowledge, there has not yet been a systematic comparison of the relative reactivities of NBD-F **2** and NBD-Cl **1**. Thus, we sought to quantitatively demonstrate the utility of NBD-F by carrying out the synthesis of very low-yielding NBD-labeled amines, specifically with the aim of improving the synthesis of 2-NBDG **3**.

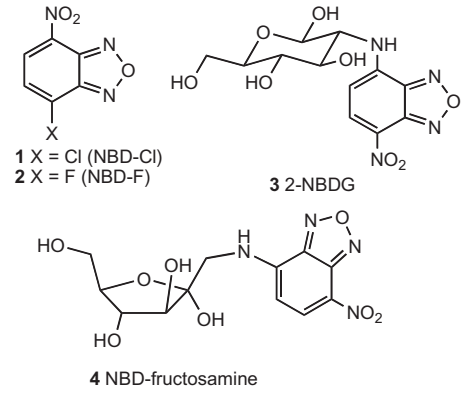
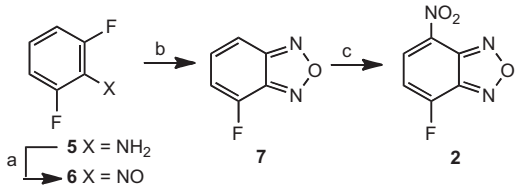


Figure 1.



Scheme 1. Reagents and conditions: (a) mCPBA, DCM, 0 °C to 23 °C, 2 h, 100%; (b) NaN_3, DMSO, 23 °C, 2 h; (c) $NaNO_3$, H_2SO_4 (aq), 0 °C, 3 min, 24% over two steps.

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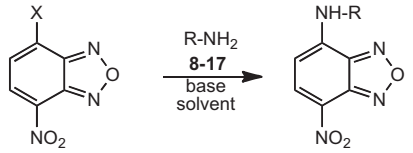
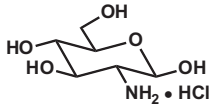
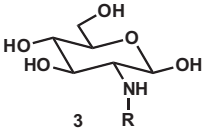
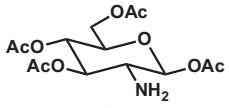
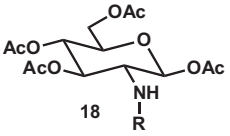
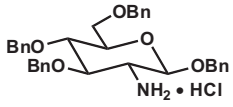
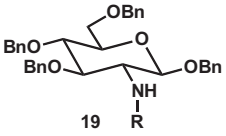
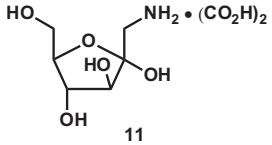
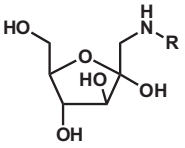
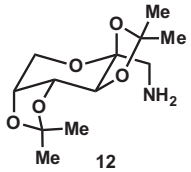
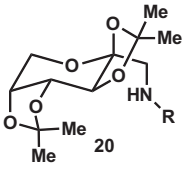
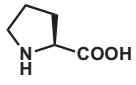
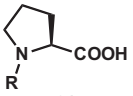
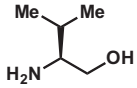
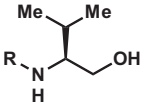
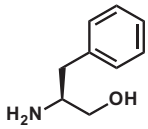
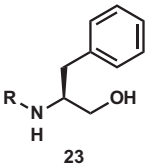
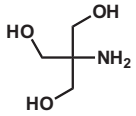
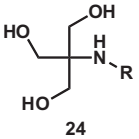
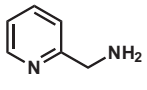
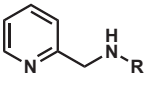
Table 1
Comparison of the yields of the synthesis of the NBD-amines using NBD-Cl **1** versus NBD-F **2**



1 X = Cl (NBD-Cl)
2 X = F (NBD-F)

3-4, 18-25

Entry	Amine	Conditions	Product (R = NBD)	Isolated yield (%)	
				Cl	F
1	 **8**	aq $NaHCO_3$ CH_3CN 23 °C/18 h	 **3**	12[5a]	75
2	 **9**	NEt_3 CH_3CN 23 °C/18 h	 **18**	Trace	61
3	 **10**	NEt_3 DMF 23 °C/49 h	 **19**	3	71
4	 **11**	aq $NaHCO_3$ CH_3CN 23 °C/14 h	 **4**	5[5b]	62
5	 **12**	NEt_3 DMF 23 °C/2.5 days	 **20**	46	85
6	 **13**	aq $Na_2B_4O_7$ CH_3CN 23 °C/21 h	 **21**	27[12b]	84
7	 **14**	NEt_3 DMF 23 °C/2.5 h	 **22**	18[9a]	95
8	 **15**	NEt_3 DMF 23 °C/2.5 h	 **23**	19[9a]	82
9	 **16**	EtOH 50 °C/2.5 h	 **24**	22[9b]	58
10	 **17**	$NaHCO_3$(s) MeOH 23 °C/18 h	 **25**	52[9b]	79

The synthesis of NBD-F **2** was first reported by Di Nunno et al. in 1970.[13] Starting from 2,6-difluoroaniline **5**, NBD-F is produced in three steps in 24% overall yield (Scheme 1). Oxidation of the aniline **5** gives the nitrosoarene **6** in quantitative yield. S_NAr reaction of **6** with sodium azide in DMSO and subsequent cyclization at ambient temperature affords the benzoxadiazole **7**. Final nitration completes the synthesis of **2**. The analogous three step sequence from 2,6-dichloroaniline yields NBD-Cl in 20% yield.[14] While 2,6-difluoroaniline is only four times more expensive than 2,6-dichloroaniline, it is surprising that NBD-F **2** is 300 times more expensive than NBD-Cl **1**.[15] Using Di Nunno's procedure, we were able to conveniently produce one gram of NBD-F **2** in a single synthetic sequence. Due to its susceptibility to hydrolysis, NBD-F **2** was stored in the cold under argon and allowed to warm to ambient temperature just prior to use.

We repeated each literature synthesis, using reaction conditions identical to those reported to prepare each NBD-labeled amine, except that NBD-F **2** was used in place of NBD-Cl **1**. As shown in Table 1, for a given set of reaction conditions, NBD-F afforded significantly higher yields of the desired NBD-labeled amines than NBD-Cl. This was true for highly water-soluble amines, for example, glucosamine **8** (entry 1), fructosamine **11** (entry 4), proline **13** (entry 6), and tris(hydroxymethyl)methylamine (Tris) **16** (entry 9), as well as for the highly organic-soluble amines, for example, the protected glucosamines **9** and **10** (entries 2–3), protected fructosamine **12** (entry 5) and the aminoalcohols, valinol **14** and phenylalininol **15** (entries 7–8). In particular, we found that 2-NBDG **3** and NBD-fructosamine **4** were produced in yields of 75% and 62%, respectively,––each a significant improvement over the previously reported literature yields. Since the NBD unit is used fairly often to analyze amino acids, the fact the NDB–F gave greatly improved yields of the NBD–proline **21** is likewise important. Finally it should be pointed out that hydrolysis of the bis(acetonide) **20** gave the NBD–fructosamine **4** in 77% yield.

Interestingly, while the reaction of NBD–Cl with unprotected fructosamine **11** gave low yield (5%), the bis(acetonide) protected fructosamine **12** gave the significantly higher yield of 46% (entry 4 and 5). Reaction of other protected sugars, for example, glucosamine tetraacetate **9** and tetra-*O*-benzyl glucosamine hydrochloride **10**, with NBD–Cl produced consistently low yields. As can be seen in Table 1, many different reaction conditions for the S_NAr reaction of NBD-halides have been reported in the literature. We wanted to find a single set of reaction conditions that could be applied to a wide range of amine substrates. Encouraged by the high yields obtained from the reaction conditions of triethylamine in DMF at ambient temperature overnight[9a] (entries 7 and 8) and also by the ability of DMF to dissolve both water-soluble and organic-soluble amines, we applied those same reaction conditions to the aforementioned substrates. The results are given in Table 2. In most cases, the reactions of the amines (1.1 equiv) using 2–18 equiv of triethylamine in DMF (0.6 M in amine) at 23 °C for 24 h worked well and led to comparable yields (entries 1–3, 5, and 7–9, Table 2). However, for unknown reasons, this set of conditions led to poor yields for the reaction of fructosamine **11** to give **4**, of proline **13** to give **21**, and of 2-pyridylmethylamine **17** to give **25** (entries 4, 6, and 10).

Table 2
Reaction of amines with NBD-F in DMF using triethylamine as base

Entry	Amine	Product	Yield (%)
1	**8**	**3**	65
2	**9**	**18**	86
3	**10**	**19**	71
4	**11**	**4**	0
5	**12**	**20**	85
6	**13**	**21**	0
7	**14**	**22**	95
8	**15**	**23**	82
9	**16**	**24**	51
10	**17**	**25**	0

Conditions: NBD-F (1.0 equiv), amine (1.1 equiv), NEt_3 (2–18 equiv), DMF (0.6 M in amine), 23 °C, overnight in dark.

In conclusion, we have found that the reaction of primary and secondary amines with NBD-F **2** yields significantly higher yields of the NBD-labeled amines than reaction with NBD-Cl **1**. These results confirm the qualitative results known for some time of the higher reactivity of NBD-F compared to that of NBD-Cl and are also in agreement with the large body of literature regarding the higher reactivity of aryl fluorides compared to aryl chlorides in the S_NAr reaction.[16] Using NBD-F **2**, we were able to produce the highly useful fluorophores 2-NBDG **3** and NBD-fructosamine **4** in good yields (72% and 62%, respectively).

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and spectral data for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.02.111.

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