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CAN WE PREDICT REACTIVITY FOR AROMATIC NUCLEOPHILIC SUBSTITUTION WITH [^{18}F]FLUORIDE ION?

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SUMMARY

The correlation between the ^{13}C-NMR chemical shift of the aromatic ring carbon bearing the leaving group and the yield of nucleophilic aromatic displacement with no-carrier-added [^{18}F]fluoride ion was evaluated. In comparison of structurally analogous compounds (fluoro, nitro and trimethylammonium substituted benzaldehydes, benzophenones and benzonitriles), the ^{13}C-NMR chemical shift of the reactive aryl ring carbon correlated quite well with the [^{18}F]fluorination yield ($r^2 = 0.87$) for most but not all ring structures. Compounds with trimethylammonium leaving groups or methyl ring substituents were found to not fit the proposed correlation. Kinetic studies indicated clearly different rates of reaction for these compounds, with much higher than expected reactivity for the ccompounds with the cationic leaving group. Competition experiments suggest that low reactivity of methyl-substituted rings may be due to conversion of [^{18}F[fluoride to an unreactive form. Our results indicate that the correlation between [^{18}F]fluorination yields for nucleophilic aromatic substitution reactions and the ^{13}C NMR chemical shift of the aryl ring carbon bearing the leaving group is applicable to numerous structurally analogous compounds, but cannot be simply generalized to aromatic rings with different leaving groups or ring substituents.

Key words: Nucleophilic Aromatic Substitution, Kinetics, Fluorine-18

INTRODUCTION

Fluorine-18 labelled compounds make up one of the most important classes of radiopharmaceuticals used for Positron Emission Tomography (PET). One excellent method for synthesis of such compounds utilizes [^{18}F]fluoride ion and aromatic nucleophilic substitution (1), which provides a good [^{18}F]fluorination yield in a short period of time. However, in many instances the incorporation of the radionuclide takes place early in a multi-step synthesis, and thus high yields

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are necessary for a good overall radiochemical yield. The ability to predict the reactivity of a particular aromatic substrate towards substitution with [^{18}F]fluoride ion could aid radiochemists in pursuing synthetic routes that would lead to a higher final yields of labelled products.

In a recent attempt to develop such predictive capabilities for nucleophilic aromatic substitution with [^{18}F]fluoride ion, Ding et al. reported a correlation between the ^{13}C-NMR chemical shift of the aryl ring carbon bearing the leaving group and the yield of the aryl [^{18}F]fluoride (2). Since ^{13}C-NMR chemical shifts have been shown to be sensitive to electron density (3,4), this correlation suggests a dependance of reaction yield on the electrophilicity of the reactive aryl ring carbon. As a part of our research into new methods for the synthesis of aryl [^{18}F]fluorides, we have attempted to extend this correlation to other aromatic rings bearing a variety of different activating groups, leaving groups or other ring substituents.

EXPERIMENTAL

Materials and Methods

The following compounds were obtained from Aldrich Chemicals Inc: dimethylsulfoxide (DMSO), acetonitrile, 4-nitrobenzaldehyde, 2-nitrobenzaldehyde, 4-nitrobenzonitrile, 4-nitrobenzophenone, 4-fluorobenzaldehyde, 6-nitropiperonal, 3-methoxy-2-nitrobenzaldehyde, and 3,4-dimethoxy-6-nitrobenzaldehyde. 3-Methyl-4-nitrobenzaldehyde, 2-nitro-5-methylbenzaldehyde, 4-trimethylammmoniumbenzonitrile triflate and 4-trimethylammoniumbenzaldehyde triflate were prepared by literature methods (5,6). 3-Methoxy-4-nitrobenzaldehyde was prepared by oxidation of 3-methoxy-4-nitrobenzyl alcohol (5).

Carbon-13 NMR. Chemical shift values for all compounds were calculated using published substituent additivity values (3,4,7). Experimental values were obtained either from the literature (2-nitrobenzaldehyde, 4-nitrobenzaldehyde, 6-nitroveratraldehyde, 6-nitropiperonal, and 3-methoxy-4-nitrobenzaldehyde (2,4,7)) or determined in this laboratory (3-methoxy-2-nitrobenzaldehyde, 2-methoxy-4-nitrobenzaldehyde, 4-trimethylammonium benzaldehyde triflate, 4-trimethylammonium benzophenone triflate, 3-methyl-4-nitrobenzaldehyde and 2-nitro-5-methylbenzaldehyde) as approx. 0.5 M solutions in DMSO.

Preparation of [^{18}F]Fluoride Ion

Fluorine-18 was produced by the ^{18}O(p,n)^{18}F (bombardment of $H_2{}^{18}O$ with protons) in a silver target fitted with a titanium foil (8). The aqueous [^{18}F]fluoride was combined with 10 mg of Kryptofix-222 and equimolar K_2CO_3. This mixture was dried under nitrogen, and the resulting residue was dissolved in dimethyl sulfoxide (DMSO). Resolubilization yields were about 65%-70% of the original [^{18}F]fluoride ion.

Reactions of Aromatic Substrates with [^{18}F]Fluoride Ion

A 100 µL portion of a DMSO solution containing 5 mg/ml of organic substrate was added to a reaction vial containing 200 µL of the [^{18}F]fluoride/DMSO solution. The resulting mixture was allowed to react without stirring at 114° C for 25 minutes. Reactions were quenched with 3 ml of water and the fluorine-18 labelled product was isolated by liquid-liquid extraction into diethyl ether. The ether layers were analyzed by radioanalytical TLC (silica gel, diethyl ether) . All aryl [^{18}F]fluorides were found to have R_f values in the range of 0.50 to 0.75. Radiochemical purities of the labelled products were always greater than 97%. The yield of fluorine-18-labelled product was determined as the following ratio:

$$\text{Reaction yield (\%)} = (^{18}\text{F in ether layer}) / (^{18}\text{F in ether and water layers})$$

All yields are reported corrected for radioactive decay.

Kinetics: Rate of [^{18}F]Fluoride Incorporation.

A 100 µL portion of a DMSO solution containing 5 mg/ml of organic substrate was added to a reaction vial containing 1500 µL of the [^{18}F]fluoride/DMSO solution (to minimize possible influence of varying fluoride ion concentrations, all studies were done using approximately 400 µCi of [^{18}F]fluoride ion of nearly the same specific activity). The resulting mixture was allowed to react at 114°C without stirring. A 100 µL aliquot of this reaction mixture was removed at 2 min, 5 min, 10 min, 25 min, 1 hr, and 2 hr time points and product was isolated by liquid-liquid extraction. The percentage of unreacted [^{18}F]fluoride ion was calculated as that remaining in the water layer (divided by total fluorine-18). A plot of ln[^{18}F] vs. time was made, and the slope of the linear portion of this graph was taken to be the apparent rate constant.

Competition Experiments: Simultaneous Addition.

In the first experiment, 50 µL of a 5 mg/ml DMSO solution of test substrate (compound **2**,**6** or **15**) and 50 µL of a 5 mg/ml solution of 4-trimethylammoniumbenzaldehyde in DMSO were added to a reaction vial containing 1500 µL of the [^{18}F]fluoride/DMSO mixture. The resulting solution was allowed to react without stirring for 25 mins at 114°C, and the product(s) were then isolated by liquid-liquid extraction.

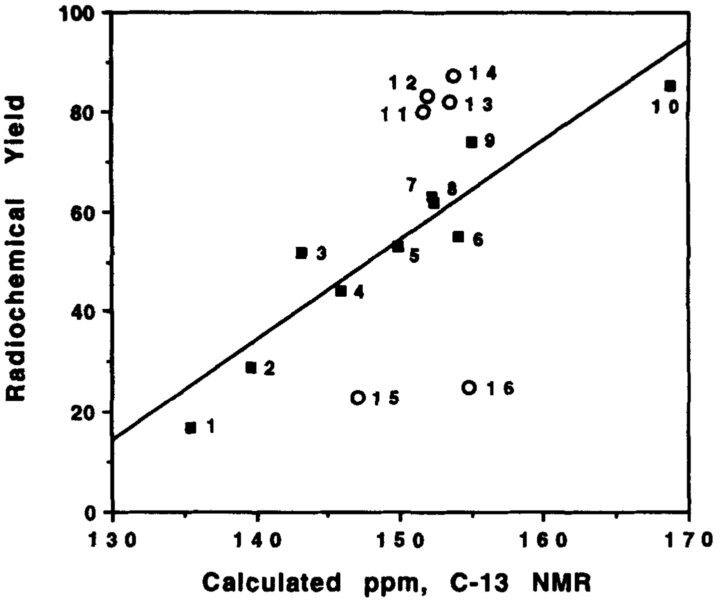
Competition Experiments: Sequential Addition.

In this second experiment, 50 µL of a solution of test substrate (**2**,**6** or **15**) in DMSO was added to a reaction vial containing 1500 µL of the [^{18}F]fluoride/DMSO mixture. This was allowed to react for 25 mins at 114°C without stirring. A 100 µL aliquot of this reaction mixture was removed and product yield determined by liquid-liquid extraction. To the remaining reaction mixture, 50 µL of a 5 mg/ml DMSO solution of 4-trimethylammoniumbenzaldehyde was added, and the reaction allowed to continue for an additional 25 mins at 114°C without stirring. The product(s) were then isolated by liquid-liquid extraction.

RESULTS

Radiochemical Yields

The radiochemical yields for the reaction of no-carrier-added (NCA) [^{18}F]fluoride ion with several substituted aromatic rings are shown in Fig. 1. Due to the very low concentrations of fluoride ions present in NCA [^{18}F]fluoride ion preparations, yields of [^{18}F]fluorinations of a given aromatic ring are subject to variability between days and even between target irradiations. To allow for these fluctuation in product yield, each reaction was conducted a minimum of five times with [^{18}F]fluoride ion obtained from the same target, and in all cases a "control" reaction using 4-nitrobenzaldehyde was performed to rule out low yields due to unreactive preparations of [^{18}F]fluoride ion. The compounds examined in this study included aromatic rings with aldehyde or nitrile activating groups; nitro, trimethylammonium and fluoro leaving groups; and methyl or alkoxy substituents. Also included in Table 1 are data obtained previously, under slightly different reaction conditions 5 mg, 114 ^{o}C (6)), for nitro and trimethylammonium substituted benzophenones; we have found that these reactions are not very sensitive to small changes in substrate concentration or temperature. For the correlation between [^{18}F]fluorination yield and ^{13}C NMR chemical shift of the aromatic ring carbon bearing the leaving group, the calculated ^{13}C-NMR chemical shifts were used except in the case of 6-nitropiperonal, as a substituent value for the methylenedioxy group is not available. With the exception of the methyl and trimethylammonium substituted rings, the reaction yields were well correlated with the ^{13}C-NMR chemical shift of the aryl ring carbon bearing the leaving group (r^2= 0.87), in general agreement with the earlier study of Ding et al. (2). Inclusion of all sixteen compounds gave a much poorer correlation between yields and chemical shifts (r^2 = 0.45). Of the compounds which fit the correlation line, 4-fluorobenzaldehyde gave the highest radiochemical yield and a relatively high ^{13}C-NMR shift of the carbon attached to the leaving group (168 ppm). The reaction of 3-methoxy-2-nitrobenzaldehyde with [^{18}F]fluoride ion gave the lowest yield (17%). This result is expected due to



	Compound	Reaction Yield
1	3-methoxy-2-nitrobenzaldehyde	16.8 ± 1.9
2	3-methoxy-4-nitrobenzaldehyde	29 ± 6.9
3	6-nitroveratraldehyde	52 ± 1.2
4	6-nitropiperonaldehyde	44 ± .35
5	2-nitrobenzaldehyde	53 ± 8.6
6	4-nitrobenzaldehyde	55 ± 1.2
7	4-nitrobenzophenone	63[a]
8	4-nitrobenzonitrile	62
9	2-methoxy-4-nitrobenzaldehyde	74 ± 4.1
10	4-fluorobenzaldehyde	85 ± 3.6
11	4-trimethylammoniumbenzophenone	80 ± 4.6[a]
12	4-trimethylammoniumbenzonitrile	83
13	4-trimethylammoniobenzaldehyde	82 ± 5.0
14	4-trimethylammoniumnitrobenzene	87
15	3-methyl-4-nitrobenzaldehyde	25 ± 2.8
16	2-nitro-5-methylbenzaldehyde	23 ± 4.8

[a] Data from reference 6

Fig. 1. Correlation of radiochemical yields with calculated carbon-13 NMR chemical shift values for various substituted benzaldehydes. Compounds **1-10** were used for the construction of the correlation line shown, with an r2 value of 0.87.

the ortho, para electron donating characteristics of the methoxy group, creating a higher electron density at the leaving group carbon, and a low ^{13}C-NMR shift of the aryl ring carbon bearing the leaving group (134 ppm).

Table 1. Relative kinetic rates for substitution of aromatic rings with NCA [^{18}F]fluoride ion.

Compound	k (min^{-1})	relative k	^{13}C-NMR (ppm)
3-methyl-4-nitrobenzaldehyde	0.004	0.333	155
3-methoxy-4-nitrobenzaldehyde	0.005	0.416	140
2-nitrobenzaldehyde	0.012	1.0	150
4-nitrobenzonitrile	0.012	1.0	152
4-trimethylammoniumbenzaldehyde	0.040	3.33	151
4-trimethylammoniumbenzonitrile	0.046	3.83	149

Kinetic Measurements

Relative kinetic rate constants were determined for the reaction of six of the substituted aromatic rings with [^{18}F]fluoride ion (Table 1). As the organic substrates were present in extremely large excess over the NCA [^{18}F]fluoride ion, the reactions were assumed to obey pseudo-first-order kinetics, so the slope of ln[^{18}F] vs. time was used to determine the rate constant. The rate constants for 4-trimethylammoniumbenzaldehyde (0.04 min^{-1}), and 4-trimethylammoniumbenzonitrile (0.05 min^{-1}) were the largest. 2-Nitrobenzaldehyde and 4-nitrobenzonitrile both had identical, intermediate rate constants of 0.01 min^{-1}, and 3-methyl-4-nitrobenzaldehyde exhibited a rate constant (0.004 min^{-1}), which was an order of magnitude lower than that of the trimethylammonium salt and one third that of the unsubstituted nitrobenzaldehyde. The rate constant for 3-methoxy-4-nitrobenzaldehyde was slightly larger (0.005 min^{-1}) than that of the corresponding methyl-substituted ring.

Competition Experiments with Substituted Nitrobenzaldehydes

To evaluate the possibility of an impurity in the methyl-substituted nitrobenzaldehyde, a mixture of 3-methyl-4-nitrobenzaldehyde and 4-trimethylammoniumbenzaldehyde triflate was allowed to react with NCA [^{18}F]fluoride ion. This reaction was then repeated using 4-nitrobenzaldehyde and 3-methoxy-4-nitrobenzaldehyde. In all cases, the yield was quite high (81%) and consistent with the yield to be expected using the 4-trimethylammoniumbenzaldehyde triflate, indicating that there was no rapid consumption of [^{18}F]fluoride by an impurity in any of these compounds. To determine whether there was any interaction between the [^{18}F]fluoride ion and the methyl-substituted nitrobenzaldehyde which did not produce aryl [^{18}F]fluoride, 4-trimethylammoniumbenzaldehyde triflate was introduced after 3-methyl-4-nitrobenzaldehyde was allowed to react with [^{18}F]fluoride ion for 25 min at 114°C. In this case, the yield (25%) did not increase upon addition of the trimethylammonium salt, in contrast with the other substituted nitrobenzaldehydes (compounds **2** and **8**) where a significant increase in yield (from 30 and 55%, respectively, to a final yield of 70% for each) was observed upon addition of the trimethylammonium salt.

DISCUSSION

The ability to predict radiochemical yields of aromatic nucleophilic substitution reactions using [^{18}F]fluoride ion would be of great value in the design of multistep syntheses of new radiopharmaceuticals. Consistent with the report by Ding et al (2), we have found that within a series of structurally similar nitro-substituted benzaldehydes (compounds **1**-**7**), [^{18}F]fluorination yields correlated quite well (r^2= 0.92) with ^{13}C-NMR chemical shifts of the aryl ring carbons bearing the leaving group. This correlation could even be extended to certain compounds with different leaving groups such as 4-fluorobenzaldehyde, and to compounds with different activating groups, as in the case of the benzonitrile and benzophenone (compounds **1**-**10** inclusive, r^2 = 0.87, see Fig. 1). However this correlation did not accommodate all the compounds tested, and in particular substrates with trimethylammonium leaving groups (compounds **11**-**14**) or methyl ring substituents (compounds **15** and **16**) reacted either better or worse than would have been predicted from the correlation line. 4-Trimethylammoniumbenzaldehyde triflate and 4-trimethylammoniumbenzonitrile triflate gave an approximately 30% higher yield of product than compounds with a similar ^{13}C-NMR chemical shift (e.g., 82% for 4-trimethylammoniumbenzaldehyde vs. 53% for 2-ntirobenzaldehyde). The two methyl substituted compounds had anomalously low reaction yields (25% for 3-methyl-4-nitrobenzaldehyde, 23% for 2-nitro-5-methylbenzaldedhyde) when compared with compounds having similar ^{13}C-NMR chemical shifts (e.g., 55% for 4-nitrobenzaldehyde).

In previous work using the trimethylammonium substituent as the leaving group in nucleophilic aromatic substitutions, we had noticed rapid reactions with high yields (6), and this has been the general observation of others who have adopted this method of [^{18}F]fluorination of aryl rings. This had been clearly evident in our original study comparing nitro- and trimethylammonium substituted benzonitriles and benzophenones (6), where the cationic leaving group had provided consistently higher yields, even at lower reaction temperatures. This was reinforced in the kinetic studies performed here, as the trimethylammonium salt clearly reacted faster then the corresponding nitro substituted aldehyde. Although kinetic studies performed with no-carrier-added [^{18}F]fluoride ion are difficult to interpret, due to the highly unusual stoichiometry and the inability to accurately measure fluoride ion concentrations, the relative rates of reaction remain informative as to the reactivities of different substrates. The higher mobility of trimethylammonium leaving group relative to nitro or fluoro substituents has been previously recognized (9), and has been ascribed to a rate enhancement by the electron-attracting power of the cationic group. Additionally, there can be the formation an ion pair between the positively charged 4-trimethylammoniumbenzaldehyde and [^{18}F]fluoride ion, which would bring both reacting species in close contact with one another and might increase the probability of nucleophilic attack by the [^{18}F]fluoride ion. Since the formation of the Meisenheimer complex is generally the rate determining step in nucleophilic aromatic substitution reactions, these and other less well understood effects may make the trimethylammonium substituents particularly reactive towards [^{18}F]fluoride ion.

The methyl-substituted compounds also did not fit with the proposed correlation, as they clearly gave lower yields than other substrates with similar ^{13}C-NMR chemical shifts. In the kinetic study, compound **15** reacted at only one third the rate of compounds with similar chemical shifts (155 ppm) at the reactive carbon (see Table 1). The slower reaction rate was not due to steric effects, since 2-nitro-5-methylbenzaldehyde also gave low reaction yields when compared with compounds of similar chemical shift (23% for 2-nitro-5-methylbenzaldehyde vs 44% for 6-nitropiperonal), and the methyl group is para to the nitro leaving group. Explanations of the apparent deleterious effects of a methyl group are not readily apparent. The unusually high electron releasing capabilities of alkyl groups has previously been explained by hyperconjugation, however, the data in support of this effect is ambiguous and it has been argued that the electron releasing properties of alkyl groups might be an artifact of the solvent medium (10). From the competition experiments performed here, the methyl substituted nitrobenzaldehyde appears to be somehow converting [^{18}F]fluoride ion to an unreactive form, such that it was not available for reaction with the trimethylammonium salt which was subsequently added. Other compounds tested did not exhibit such behavior. The possibility of a rapidly-reacting impurity which consumed [^{18}F]fluoride ion could be ruled out, as high yields were obtained in reaction mixtures containing trimethylammonium salt and equimolar methyl-substituted benzaldehyde. This suggests the slow formation of an unreactive complex of methylnitrobenzaldehyde with the fluoride ion, perhaps by formation of a relatively stable Meisenheimer complex. Unfortunately, at the NCA [^{18}F]fluoride level there is no possibility of isolating and characterizing this potential stable intermediate.

These studies demonstrated that methyl-substituted nitrobenzaldehydes and trimethylammonium substituted aryl rings did not fit with the proposed relationship between ^{13}C-NMR chemical shift and [^{18}F]fluorination yield. The results can not be explained on the basis of incorrectly calculated ^{13}C-NMR chemical shift values, as we obtained an excellent correlation between experimental ^{13}C-NMR values and calculated ^{13}C-NMR ($r^2 = 0.945$) for compounds **1**,**2**,**3**,**5**,**6**,**11**,**12**,and **15**, a subset which included both methyl and trimethylammonium substituted ring structures. Carbon-13 NMR chemical shifts are sensitive, to some extent, to solvent and concentration effects. To place the trimethylammonium or the methyl substituted compounds on the correlation line, large positive (for the $-NMe_3^+$) and negative (for $-CH_3$) changes, on the order of 10 ppm or more, would be necessary. Such large changes would not be expected simply due to concentration effects; although large shifts have been observed due to changes in solvents, this has been found for such substituents as anilines in strongly acidic solutions (3,7). Methyl groups are particularly insensitive to solvent effects (lack of ionizability or hydrogen bonding capabilities) (3,7), and it might be expected that the permanently charged trimethylammonium group would be similarly insensitive.

In conclusion, with the exception of trimethylammonium or methyl substituted aryl rings, a good correlation between reactivity towards [^{18}F]fluoride ion and the ^{13}C-NMR chemical shifts has been demonstrated for structurally analogous compounds. In practice, this might allow prediction of reactivity for new substrates, as there is a generally good agreement between experimental and calculated ^{13}C-NMR chemical shifts (this work and literature (4)), and there was a good correlation between radiochemical yields and the set of entirely calculated chemical shift values ($r^2 = 0.87$). However, the results obtained with the methyl and trimethylammonium substituted rings provides evidence that the correlation cannot be generalized to aryl rings with other leaving groups and different ring substituents.

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REFERENCES

1. Kilbourn, M.R. - Fluorine-18 Labelling of Radiopharmaceuticals, National Academy Press, Washington D.C., 1990.
2. Ding, Y-S., Shiue C-Y, Folwer J.S., Wolf, A.P., and Plenevaux A. - J. Fluorine Chem. 48:189 (1990).
3. Levy, G.C. and Nelson, G.L. - Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, Wiley-Interscience, New York, 1972.
4. Kalinowski, H.-O., Berger, S., and Braun, S. - Carbon-13 NMR Spectroscopy, Wiley and Sons, New York, 1988.
5. Chakraborty, P.K. and Kilbourn, M. R. - Appl. Radiot. Isot. 42:7:673 (1991)
6. Haka, M.S., Kilbourn, M.R., Watkins, G.L., and Toorongian, S.A. -J. Labelled Compounds and Radiopharmaceuticals, 28: 824, 1988).
7. Ewing, D.F. - Org. Magnetic Resonance, 12: 499 (1979).
8. Mulholland G.K., Kilbourn M.R., and Moskwa, J.J. - Appl. Radiat. Isot. 41: 1193 (1990).
9. Miller, J. - Aromatic Nucleophilic Substitution, Elsevier Publishing Company, London, 1968.
10. Shiner, V. J. -J. American Chemical Society, 76: 1603 (1976).