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Synthesis and Scintillating Efficiencies of 4-Functionalised-2,5-Diphenyloxazoles

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Abstract: A series of 4-functionalised-2,5-diphenyloxazoles has been synthesised. Each member of the series has been assessed for the ability to scintillate in the presence of ionising radiation. The scintillation counting efficiency of each member of the series has been determined relative to 2,5-diphenyloxazole, a widely used scintillant molecule. © 1997 Published by Elsevier Science Ltd.

The study of molecular recognition phenomena is reliant upon the ability to detect non-covalent molecular interactions[1]. Commonly, such interactions are investigated using a variety of analytical techniques such as NMR spectroscopy[2], potentiometry[3], mass spectrometry[4], fluorescence spectroscopy[5] and UV-vis spectroscopy[6]. More recently, analytical methods such as the Scintillation Proximity Assay (SPA)[7], invented and marketed by Amersham International plc., have been developed. SPA allows intermolecular interactions to be detected and quantified directly, but requires the use of a solid support. A method which might be used to detect and quantify intermolecular interactions directly in a single solution phase and the preliminary steps towards it's development are described below.

Our proposed detection method, which may be likened to the SPA, will rely on tagging one half of a "host-guest" system with a molecule which scintillates in the presence of ionising radiation. The other half of the "host-guest" system will be radiolabeled with tritium. In analogous fashion to the SPA, formation of a "host-guest" complex will place the radiolabel in close proximity to the scintillant tag resulting in the emission of light. This response may be detected and quantified in a scintillation counter. If the "host-guest" complex is not formed the tritium remains too remote (mean pathlength of tritium in water is 1.5μm[7]) from the scintillant to elicit a significant response. Since detection of scintillation events is an extremely sensitive technique[8], the detection system proposed should be more sensitive than many other existing analytical methods and unlike the SPA there is no reliance upon the use of a solid support (in SPA technology, the host/guest is immobilised on scintillant filled fluomicrospheres). As a first step towards developing this novel analytical procedure, we report here the design and synthesis of a series of 4-functionalised-2,5-diphenyloxazoles and their subsequent evaluation for use as scintillant tags.

The design of the tag must fulfill a number of important criteria. The tag must i) function effectively as a scintillant molecule, ii) be readily incorporated into a variety of molecules and iii) be chemically inert.

To ensure that the tag is able to scintillate, it was based upon 2,5-diphenyloxazole (PPO) **1** which is a well known scintillating molecule and is a major component of most commercially available scintillation cocktails[9]. It was expected that molecules containing this oxazole moiety should retain the ability to scintillate. For attachment

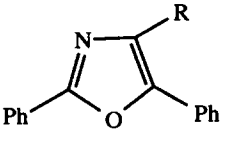
of the tag to other molecules it must possess a functional group which is capable of reacting further. To this end a series of 4-functionalised-2,5-diphenyloxazoles have been constructed. The relatively robust nature of the oxazole skeleton also ensures that it is an excellent tagging molecule since it is relatively stable and inert to many commonly encountered reaction conditions[10].

Ethyl 2,5-diphenyl-4-oxazolecarboxylate **2** was synthesised from the commercially available ethyl benzoylacetate according to literature precedent[11]. Ester **2** was hydrolysed to the corresponding carboxylic acid **3** using standard alkaline conditions. The acid functionality was activated as the acid chloride by reaction with oxalyl chloride in the presence of a catalytic amount of *N,N*-dimethylformamide. The acid chloride reacted smoothly to furnish amide **4** in 84% overall yield from acid **3**. Ester **2** was reduced to the corresponding alcohol **5** using an ethanolic solution of sodium borohydride. Alcohol **5** was readily alkylated under standard Williamson ether synthesis conditions to provide ether **6**, oxidised with pyridinium chlorochromate (PCC) to provide aldehyde **7**, esterified to give ester **8** and brominated using phosphorous tribromide to furnish bromide **9**. Bromide **9** was converted into the corresponding azide **10** by reaction with sodium azide in wet *N,N*-dimethylformamide. The azido function was reduced to amine **11** by hydrogenolysis using a palladium charcoal catalyst. Amine **11** was converted into amide **12** and imide **13** by reaction with acetic anhydride and acetyl chloride respectively. As scheme 1 shows, all of the reactions proceeded in high yield[12] with the exception of the formation of imide **13**. In every case, the relatively robust 2,5-diphenyloxazole ring system remained intact.

Having synthesised the 4-functionalised-2,5-diphenyloxazoles (**2-13**) they were evaluated for the ability to scintillate in the presence of ionising radiation. This was important for two reasons, firstly, to ascertain which functionalities the proposed scintillant based detection system will tolerate and secondly, to establish the optimum

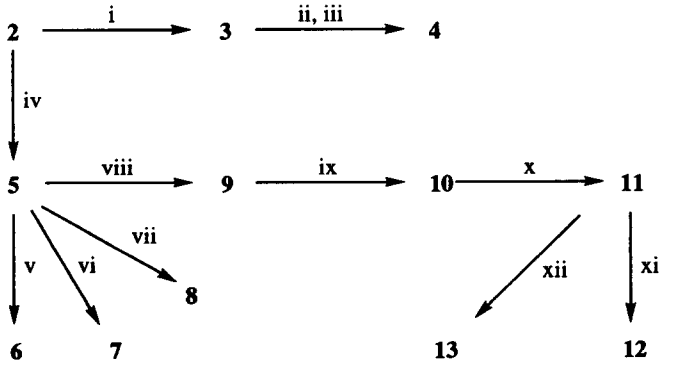
SCHEME 1

4-Functionalised-2,5-Diphenyloxazoles:



1 R = H
2 R = COOEt
3 R = COOH
4 R = $CONEt_2$
5 R = CH_2OH
6 R = CH_2OCH_3
7 R = CHO
8 R = CH_2OCOCH_3
9 R = CH_2Br
10 R = CH_2N_3
11 R = CH_2NH_2
12 R = $CH_2NHCOCH_3$
13 R = $CH_2N(COCH_3)_2$

Reaction Scheme:



Reagents:

i) NaOH, H_2O, MeOH, 99%
ii) $(COCl_2)_2$, DMF (cat), DCM
iii) Et_2NH, Et_3N, DCM, 81%
iv) $NaBH_4$, EtOH, 90%
v) NaH, MeI, DMF, 99%
vi) PCC, silica gel, DCM, 67%
vii) CH_3COCl, Et_3N, DCM, 76%
viii) PBr_3, DCM, 100%
ix) NaN_3, DMF, 97%
x) H_2, Pd/C, EtOAc, 77%
xi) $(CH_3CO)_2O$, Et_3N, DCM, 89%
xii) CH_3COCl, Et_3N, DCM, 47%

covalent linkage for attaching the scintillant tag to other molecules. These linkages are exemplified by esters **2** and **8** (-COOMe and -OCOMe respectively), amides **4** and **12** (-$CONR_2$ and -NHCOR respectively), ether **6** and imide **13**.

In evaluating the scintillating efficiency of each of the 2,5-diphenyloxazole derivatives, the following scintillation counting experiments were undertaken. The ionising radiation source chosen for this study was tritium, a low energy β-particle emitter[7], in the form of tritiated hexadecane (Amersham International plc.).

Tritiated stock solution. A tritiated stock solution was prepared by adding an aliquot of tritiated hexadecane to toluene. The activity of this stock solution was assessed by adding 200 µl, in triplicate, of the stock solution to vials containing 10 ml of the commercial scintillation fluid, Ultima Gold™ (Canberra Packard). Subsequent scintillation counting of the vials (2250CA Canberra Packard scintillation counter) gave an average counts per minute (cpm) of 3 666 which represents the optimum cpm per assay. The stock solution was used throughout as the tritium source.

Preparation of compounds for assay. Serial dilutions of each oxazole compound (**1-13**) were prepared in toluene. Concentrations of 200 mM, 20 mM, 2 mM and 200 µM were chosen so that in the assay procedure the final concentrations of each compound would be 100 mM, 10 mM, 1 mM and 0.1 mM.

Assay procedure. A 200 µl aliquot of the tritiated stock solution was added to 200 µl of each dilution of each compound in triplicate. Each sample was counted for half an hour in a scintillation counter. The averaged cpm results obtained, for each oxazole derivative at each concentration, are shown below in Table 1.

TABLE 1

Conc	Counts per minute (cpm) for 4-Functionalised-2,5-Diphenyloxazoles												
/ mM	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
100	2995	1858	*	*	2560	2656	1	1844	*	87	1716	*	*
10	3309	2155	87	1280	2960	2993	2	2690	2	119	3008	2636	2900
1	1227	688	53	360	1130	994	4	871	7	70	1163	944	909
0.1	172	114	47	82	161	153	15	165	25	49	180	161	167

* = insoluble in toluene at this concentration

Table 1 demonstrates that at the higher concentrations, the 4-functionalised-2,5-diphenyloxazoles which scintillated effectively, give similar results to the commercial scintillation cocktail, Ultima Gold™. The 4-functionalised-2,5-diphenyloxazoles are at sufficiently high concentrations to detect disintegrations from unbound tritiated molecules. As the concentration of each scintillant molecule is decreased this effect is diminished. It is these lower concentrations of scintillant molecules which we propose for use in studying scintillation proximity effects such as might be observed in "host-guest" complexation. In addition, Table 1 demonstrates that self quenching[13] occurs at concentrations of 100 mM for each compound which is soluble at this high concentration. For each compound the optimum cpm were produced at a concentrations of 10 mM. Thus to enable a comparison of the scintillating efficiencies of each derivative to be made, the cpm obtained for each compound at this concentration have been converted into a percentage of the cpm obtained for 10 mM PPO **1** and are shown in Table 2.

TABLE 2

	4-Functionalised-2,5-Diphenyloxazoles											
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
% Efficiency at 10 mM												
Relative to PPO 1	65	3	39	89	90	0	81	0	4	91	80	88

Table 2 demonstrates that the tag scintillates efficiently in the presence of a wide variety of functionalities with the exception of aldehydes, azides, bromides and carboxylic acids. This finding is in good agreement with studies undertaken previously where halogens, aldehydes and carboxylic acids have been shown to be effective quenching agents of scintillation[14]. The finding that amine **11** scintillates strongly is notable, since amines are well known to quench both scintillation[14] and fluorescence[5,15] - a closely related emissive phenomenon. Table 2 also demonstrates that each of the potential covalent linkages investigated allowed scintillation to occur, with the ether and imide linkages being the most favourable. Thus, in conclusion, we have established that the scintillant tag functions effectively in the presence of a wide range of functional groups and that the optimum method for attaching the tag to other molecules covalently is via either an ether or an imide linkage. In future studies, we intend to incorporate a scintillant tag into a "host" molecule via an ether linkage and to demonstrate that "host" molecules tagged in this way possess the ability to both bind and detect radiolabeled "guests".

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