JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 543–556.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.838

Research Article

Simplified synthesis of 1,1′[^{14}C]-methylene-di (2-naphthol). A radiochemical and kinetic approach

Giancarlo Angelini[1,*], Ornella Ursini[1], Patrizia Minetti[2], Diana Celona[2] and Francesco De Angelis[3]

[1] *Istituto di Metodologie Chimiche C.N.R., Area della Ricerca di Roma (Montelibretti) C.P. 10, I-00016 Monterotondo Stazione, Roma, Italy*

[2] *Sigma Tau Industrie Farmaceutiche Riunite, Via Pontina km.30,400, I-00040 Pomezia, Roma, Italy*

[3] *Dipartimento di Chimica, Universita' dell'Aquila, Coppito, I-67010 L'Aquila, Italy*

Summary

The synthesis of the 1,1′[^{14}C]-methylene-di-(2-naphthol) **2**, as the radiolabeled probe of a possible interaction between the β-amyloid fibrils and the di-naphthol mojety in the Alzheimer's disease, is reported. Very simple radiochemical procedure, starting from [^{14}C]paraformaldehyde, produced 8.66 MBq of compound **2** at the specific radioactivity of 1.22 TBq/mol. A mechanistic and kinetic approach allowed the comprehension of the right experimental conditions. The stability of compound **2** in acetonitrile solution was investigated, denoting a significative decomposition process through the transient formation of the 1,2-naphthyne intermediate. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: Alzheimer's disease; 1,1′[^{14}C]-methylene-di-(2-naphthol); 2-naphthol hydroxymethylation; 1,2-naphthyne

Introduction

Alzheimer's disease (AD) is one of the most common progressive neurodegenerative diseases that affect elderly people. The presence of amyloid plaques and neuronal cytoskeleton alterations is the most common evidence of AD. The β-amyloid (βA) peptide is composed of 39–43 amino acids, and is able to form fibrils. Significant evidence suggests that fibrillary plaques are mainly

*Correspondence to: G. Angelini, Istituto di Metodologie Chimiche C.N.R., Area della Ricerca di Roma (Montelibretti) C.P. 10, I-00016 Monterotondo Stazione, Roma, Italy. E-mail: giancarlo.angelini@imc.cnr.it

Received 24 November 2003
Revised 27 April 2004
Accepted 30 April 2004

formed by aggregates of the βA peptide which, on its turn, is a metabolic product of a transmembrane glycoprotein present in nervous system tissues, the so-called amyloid precursor protein (APP). Proteolytic processing of the APP by the action of different secretes,[1] in fact, splits APP into several fragments, one of them being the βA peptide.

The pathologic properties of this peptide, such as neurotoxicity and resistance to proteolytic degradation, depend on the ability of βA to form β-sheet structures which rapidly collape into amyloid fibrils.[2] Recent etiopathogenesis studies demonstrate, in fact, the significant role of the insoluble β-amyloid fibrils and plaques[3] in AD affected patients. It is thus conceivable that, under the molecular point of view, inhibition of the β-sheet formation as well as of the βA peptide self aggregation, or even the possibility to degrade the amyloid plaques, could represent an important therapeutic target.[4]

As a part of our research in this field, we are involved in the study of molecules possibly able to prevent the βA aggregation process and fibrils formation: one of these, the 1,1′-methylene-di-(2-naphthol), is the object of a recently published patent.[5] The di-naphthol frame itself, in our project, is also used as a scaffold suitable for the assembly of new functionalities.[5]

Under a different point of view, the possibility of a binding between the di-naphthol molecule itself and the β-amyloid fibrils suggests the use of this molecule as a molecular label for the evaluation of amyloidic deposits in the cerebral tissue of AD patients or, even, for the AD diagnosis. In this context, the autoradiographic techniques appear to be the methods of choice. In this paper, the synthesis of the 1,1′[^{14}C]-methylene-di-(2-naphthol) as a radiolabeled probe is described, together with a comprehensive study of the cold reaction for the optimization of the radiolabeling conditions. Due to the ability of di-naphthol to bind to β 1-42 it could be also possible, by using the labeled molecule, to evaluate its binding in competition with other non labeled compounds, putative intercalators during the β-amyloid aggregation process.

As to the radiochemical synthesis, which is to be performed at high specific radioactivity, it has to be carried out in a simple way and its performance must fit with the best yield of the product.[6–9] In this context an important point of the radiosynthetic procedure is the isolation and purification of the product. The usual radioactivity of ^{14}C employed in a radiochemical process carried out at the maximum specific radioactivity, means massive amounts of products of only a few milligrams or less. Any purification process determines, as a negative consequence, the loss of consistent amounts of the product.

An efficient, fast and clean procedure moves through synthetic steps which allow to avoid any purification of the product. This means the complete consuming of the reagents and the absence of any by-products and

decomposition products as well. All these reaction features are not always easy to introduce in the same synthetic procedure but, in most of the cases, preliminary mechanistic and kinetic studies promote the design of simple, effective and clean procedures.

The synthesis of dimeric compounds such as methylene-di-naphthyl or phenyl derivatives is a well-known procedure which uses hydroxymethylation of the aromatic compound in the acidic medium as the first reaction step.[10–12] In particular, the presence of an extra hydroxyl group on the aromatic ring favors the reaction even if the formation of several naphthoquinone derivatives has also been described.[13] The 1,1′-methylene-di-(2-naphthol), whose anthelmintic activity and antinflammatory properties have been studied more than 20 years ago,[14–16] has been also recently employed as an intermediate compound for the preparation of heat-resistant polyesters.[17]

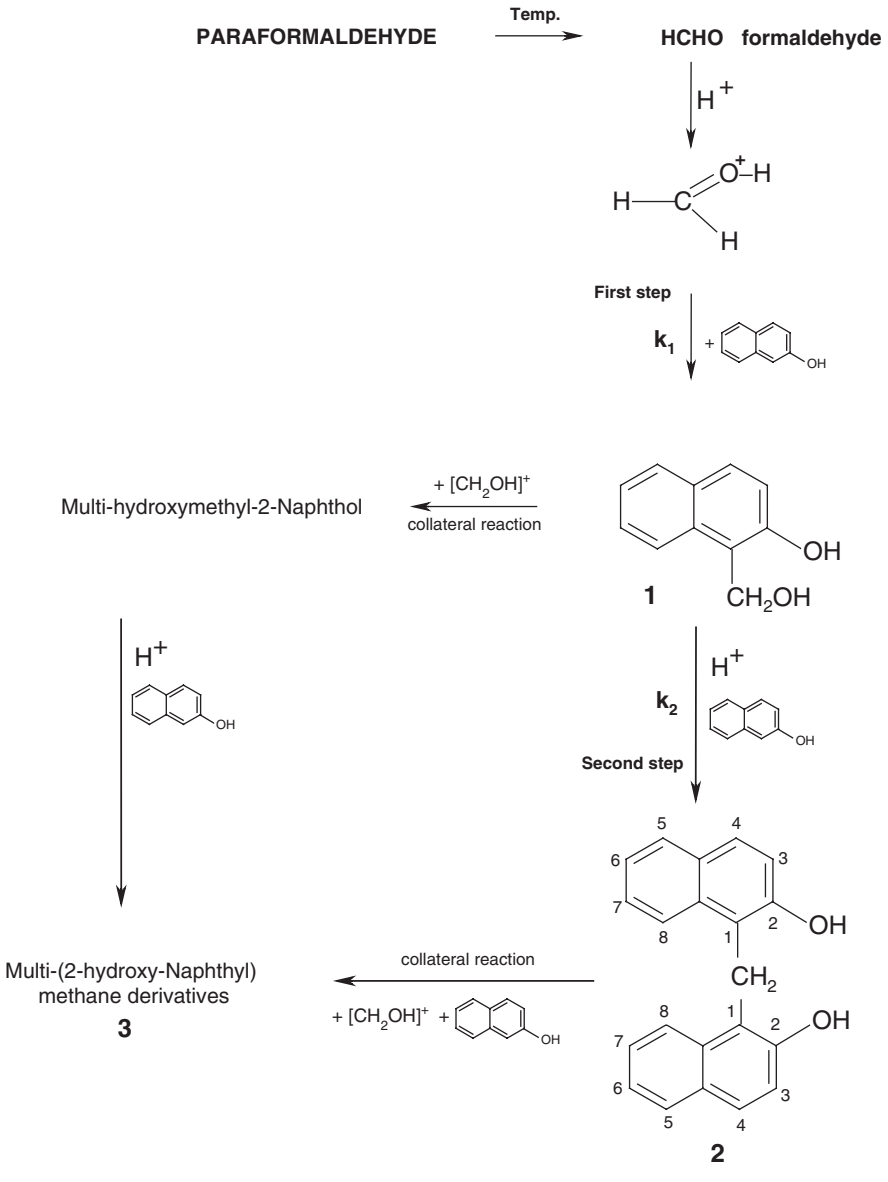
Its synthesis is reported in literature[17–19] but, despite the high production yields, the experimental procedures are characterized by drastic conditions (long reaction time and high temperature) which do not match our synthetic requirements. Oliver *et al.*[20] have also prepared 1,1′-methylene-di-(2-naphthol) labeled with ^{14}C at C-8 of both aromatic rings and, in a different synthesis, at the methylene carbon, starting from 2-[8-^{14}C]naphthol and [^{14}C]formaldehyde, respectively. However in the former case, they obtained the product labeled on the naphtalene moieties only with a limited yield, probably due to the high reaction temperature. In the latter case the product was labeled at the methylene position (in accordance with our own project) but the specific radioactivity is very low, due to the low radio labeling value of the starting [^{14}C]formaldehyde they used.

We propose, herein, a very simple radiochemical procedure for the synthesis of 1,1′[^{14}C]-methylene-di-(2-naphthol) **2**, starting from 2-naphthol and [^{14}C]paraformaldehyde at very high specific radioactivity. Indications for its preparation in a simply efficient way came from the study of the reaction mechanism and of the kinetic parameters.

Results and discussion

A reaction mechanism for the synthesis of 1,1′-methylene-di-(2-naphthol), which comprises two steps, can be conceivably depicted as follows (Scheme 1):

(i) thermal depolymerization of paraformaldehyde ($T > 90^{\circ}C$) to formaldehyde;
(ii) *in situ* protonation of formaldehyde;
(iii) *first step*: electrophilic aromatic substitution and formation of 1-hydromethyl-2-naphthol (intermediate **1**);
(iv) *second step*: *in situ* protonation of **1** and electrophilic aromatic substitution to produce 1,1′-methylene-di-(2-naphthol) **2**.



Scheme 1.

Due to the high propensity of intermediate **1** and product **2** as well to be further hydroxy-methylated, also collateral reactions have to be considered.[21] As a consequence, a mixture of multi (2-hydroxy-naphthyl)-methane derivatives **3** has been evidenced.

The importance of these collateral reactions depends on the following factors:

(i) the free formaldehyde concentration;

(ii) the concentration of 2-naphthol;
(iii) the reaction temperature;
(iv) the reaction time.

Considering that the 2-naphthol and formaldehyde concentrations must be linked by the stoichiometry ratio of 2:1, their absolute values have not any direct influence on the collateral reactions. Conversely, these reactions are kept to an acceptable level if prolonged reaction times and high reaction temperature are be avoided. The by-products of series **3** and their precursors are quite negligible when the reaction is carried out at $T < 100^{\circ}C$ and within 1 h time. These strict reaction conditions, the low reaction temperature in particular, determine the incomplete depolymerization of paraformaldehyde. Accordingly, the free formaldehyde which evolved was roughly estimated at about 50% of the starting paraformaldehyde. This defect of formaldehyde does limit the complete hydroxy-methylation 2-naphthol to form intermediate **1** and, as a consequence, a defect of 2-naphthol itself was employed, in order to ensure its complete consumption.

Accordingly, a thorough study was undertaken in order to find the best experimental conditions, as far as the correct ratio between the free formaldehyde and the 2-naphthol concentrations is concerned, at a reaction temperature which was accurately kept below 100°C (see Table 1).

Some kinetic considerations can facilitate the comprehension of the synthetic process. As reported in Table 1, intermediate **1** is never isolated in appreciable amounts, with

$$\frac{\mathrm{d}[\text{intermediate 1}]}{\mathrm{d}t} = 0 = k_1[CH_2OH^+][\text{2-naphthol}] - k_2[\text{intermediate 1}][\text{2-naphthol}] \quad (1)$$

the only exception of entry 5, where a large defect of 2-naphthol is employed. Its instantaneous concentration can be considered very low and constant during the reaction course. Under this condition the steady-state approximation is conceivable.[22] As a consequence, the reaction rate of the first step is quite similar to the second one. It follows that:

$$k_1[CH_2OH^+] = k_2[\text{intermediate 1}] \quad (2)$$

Clearly both the reaction rates depend on the concentration of 2-naphthol but, by considering the instantaneous concentration of intermediate **1** always lower than the protonated formaldehyde, it follows that k_2 is higher than k_1 . From a qualitative point of view, we can conclude that

(i) the second step can be sufficiently fast also if the 2-naphthol concentration is low;
(ii) under the same conditions the first step is very slow.

Table 1. Results of the micro-synthesis of 1,1′-methylene-di-(2-naphthol)

Entry n.	Reagents						Products							
	2-Naphthol (mmol)	Paraformaldehyde (mmol)	R[a]	T (°C)	Time (min)	Solvent AcOH (ml)	1,1′-Methylene-di-(2-naphthol)		2-Naphthol residual		1-Hydroxymethyl-2-hydroxy-naphthalene		Co-products[b]	
							(mmol)	(Yield%)[c]	(mmol)	(Yield%)	(mmol)	(Yield%)[c]	(mmol)	(Yield%)[c]
1	0.0799	0.0530	1.51	125	120	0.25	0.0335	83.9	0.0004	0.5	0.0000	0.0	0.0062	15.5
2	0.0743	0.0380	1.96	125	120	0.25	0.0223	60.2	0.0108	14.5	0.0000	0.0	0.0094	25.3
3	0.0972	0.0490	1.98	100	60	0.2	0.0370	76.2	0.0209	21.5	0.0000	0.0	0.0011	2.3
4	0.0304	0.0177	1.72	100	60	0.2	0.0123	80.9	0.0045	14.8	0.0012	3.8	0.0000	0.0
5	0.0149	0.0157	0.95	90	60	0.2	0.0064	86.2	0.0007	4.8	0.0013	8.9	0.0000	0.0
6	0.0178[d]	0.0157	1.14	90	120[e]	0.2	0.0082	91.8	0.0004	2.1	0.0011	6.0	0.0000	0.0
7	0.0351	0.0223	1.57	170W[f]	20	0.2	0.0153	87.0	0.0040	11.4	0.0006	1.6	0.0000	0.0
8	0.0351	0.0233	1.57	300W[f]	5	0.2	0.0152	86.6	0.0011	3.1	0.0024	6.8	0.0006	3.4
9	0.0894	0.0493	1.81	400W[f]	5	0.2	0.0390	87.2	0.0038	4.3	0.0028	3.1	0.0026	5.1
10	0.0369	0.0227	1.63	400W[f]	5	0.2	0.0163	88.3	0.0014	3.8	0.0008	2.2	0.0011	5.7
11	0.0369	0.0227	1.63	400W[f]	10	0.2	0.0162	87.8	0.0014	3.9	0.0004	1.1	0.0013	7.1
12[g]	0.0146	0.0118[h]	1.24	90	60	0.2	0.0049	66.7	0.0044	29.9	0.0005	3.4	0.0000	0.0
13[g]	0.0146	0.0151[i]	0.97	90	120[j]	0.2	0.0071	97.2	0.0000	0.0	0.0004	2.7	0.0000	0.0

[a] Ratio between 2-naphthol and paraformaldehyde concentrations.
[b] Multi-hydroxymethylated products and multiple naphthyl coupled products.
[c] Calculated yields with respect to 2-naphthol.
[d] 0.0029 mmol of 2-naphthol added to the test of entry n.5 and left to react for further 60 min.
[e] Total reaction time as sum of entries n.5 and n.6.
[f] Tests carried out in a microwave oven at temperature ranging from 100°C to 170°C, depending by the irradiation power.
[g] Radiochemical synthesis.
[h] Radioactive ^{14}C-paraformaldehyde at specific activity of 1.48 GBq/mmol.
[i] 0.0033 mmol of 'cold' paraformaldehyde added to test of entry n.12 and left to react for further 60 min.
[j] Total reaction time as sum of entries n.12 and n.13.

Accordingly, appreciable amounts of 2-naphthol remain unreacted during the scheduled reaction time. In order to ensure the complete consumption of 2-naphthol, this has to be present in the reaction mixture as a minor component with respect to the free formaldehyde. Following these requisites, the correct [2-naphthol]/[paraformaldehyde] ratio was approximately estimated as equal to 0.8, which turns out to be 1.6 [2-naphthol]/[paraformaldehyde] by considering the partial paraformaldehyde depolymerization: 50% at $T < 100^{\circ}$C. The absence of the 2-naphthol and of the by-products of series **3** in the products mixture allows to avoid any time-consuming chromatographic process for the purification of **2**; it is also worth noting that its chemical yield in entry 13 (Table 1) is very high (97.2%). In this way, the synthetic procedure is very fast and can be easily used also for the preparation of the [^{11}C]-analog of **2**. This study has been already undertaken and will represent our ultimate goal.

The negative drawback of the overall procedure is the incomplete consumption of paraformaldehyde (and formaldehyde as a consequence), which turns out in a low level of the total radioactivity transferred to the product. Conversely, the di-naphthol derivative **2** can be isolated without the intervention of any purification step. The chemical yield of **2** is calculated with respect to the starting phenol, which is the reagent present in defect.

Table 1 reports the results obtained in a number of experiments for the synthesis of 1,1′-methylene-di-(2-naphthol) **2**. Different reaction parameters have been taken into account, which showed to play important and specific roles. First of all, high reaction temperature (equal or higher than 120°C) and long reaction time (120 min) resulted in a massive paraformaldehyde decomposition (more than 80%) and quite high yields in the formation of **2**. The presence of consistent amounts of by-products, like multi-hydroxymethylated compounds and multi-2-hydroxynaphthyl coupling by-products, had to be considered (entries 1 and 2). The control of these side reactions leading to oligomerization compounds was achieved by lowering the temperature and shortening the reaction times to get, finally, their complete disappearance (entries 3–13).

Another important parameter is the *R*-value, which determines the distribution of the reagents. *R* is defined as the ratio between 2-naphthol and paraformaldehyde starting concentrations. The reaction stoichiometry should give an *R*-value equal to 2 but, by operating at this level (entries 2–4), the negative consequence is the presence of unreacted 2-naphthol in the reaction mixture. A valid explanation for this behavior can be found either in the kinetic development of the process (where $k_2 > k_1$) or in the applied relatively low temperature ($< 100^{\circ}$C) which is not sufficient for the complete paraformaldehyde decomposition. Conversely, 2-naphthol was totally consumed in entries 5 and 6, where *R* approaches the unit value. Under these last

conditions the effective concentration of free formaldehyde is about 50% with respect to the nominal paraformaldehyde amount used, thus implying that the operative value of R approximates 2, according to the stoichiometry necessary for the complete consumption of 2-naphthol.

As to 1-hydroxymethyl-2-hydroxynaphthalene (intermediate **1**), this is a minor product under all the experimental conditions, thus confirming its nature of transient, low concentration intermediate, as the kinetic treatment of the reaction suggests.

Different considerations can be drawn from the analyses of the tests carried out by using a microwave oven as the energy source, in the temperature range of 100–170°C, depending on the irradiation power. First of all, at very short reaction times (5–20 min) high yields of **2** were obtained (entries 7–11). This result could be of paramount importance if **2** is to be produced with a short life radioisotopic label (i.e. ^{11}C). Moreover under these conditions, paraformaldehyde depolymerization takes place very efficiently (more than 80% undergoes decomposition), thus generating a high free formaldehyde concentration.

However, the fast and rapid heating promoted by the microwave radiation takes to a partial loss of formaldehyde, which leads to the survival of appreciable amounts of 2-naphthol. Also, consistent amounts of by-products are produced. These events convinced us to carry out the radiochemical synthesis according to the conventional way, with a total reaction time of 120 min (entries 12 and 13), and using the experimental conditions of entry 5 as a reference. A second addition of a paraformaldehyde aliquot after the first 60 min of reaction allowed the complete consumption of 2-naphthol. This addition, performed with 'cold paraformaldehyde', induced further depolymerization of the radioactive residual paraformaldehyde, thus contributing to the increase of the yield of **2** without detriment to the specific radioactivity.

Following this procedure, an almost quantitative transformation of the starting 2-naphthol was obtained while the level of the impurities was not appreciable. The 1,1′[^{14}C]-methylene-di- (**2**-naphthol) **2** was isolated at the specific radioactivity of 1.22 TBq/mol (total radioactivity = 8.66 MBq). Only limited amount of the radioactive intermediate **1** was recovered, at the specific radioactivity of 0.74 TBq/mol and a total radioactivity of 296 KBq.

In Table 2 several tests are reported, very useful to elaborate some kinetic considerations. These tests have been carried out at 90°C, by employing a large excess of paraformaldehyde (164.3 μmol as the nominal starting value) with respect to 2-naphthol (42.2 μmol). The reaction mixtures have been checked after 5 and 12 min. Clearly, these reaction conditions do not appear to be useful for radiosynthetic purposes (the amount of radioactive starting paraformaldehyde is too high and the formation of **2** is too low), but they allow to follow the two steps reaction pathway, by the isolation of large quantities of intermediate **1**.

Table 2. Kinetic implications for the micro-synthesis of 1,1′-methylene-di-(2-naphthol)

	Reagents					Products			Average rate 1° step Intermed.1 (mmol/s)	Average rate 2° step Product 2 (mmol/s)
Entry n.	2-Naphthol (mmol)	Paraformaldehyde (mmol)	T (°C)	Time (s)	Solvent AcOH (ml)	1-Hydroxymethyl-2hydroxynaphthalene Intermed.1[a] (mmol)	1,1′Methylene-di-(2-naphthol) Product 2[b] (mmol)	Real Intermed.1[c] (mmol)		
1	0.0422	0.1643 0.0822[d]	90	300	0.2	0.0144	0.0116	0.0260	8.67E-05	3.87E-05
2	0.0422	0.0822	90	720	0.2	0.0147	0.0138	0.0285	3.96E-05	1.92E-05
3	0.0046[e]	0.0562[f]		420[g]		0.0003[h]	0.0022[h]	0.0025[h]	5.95E-06	5.24E-06

[a] Residual intermediate **1** determined from HPLC analyses.
[b] Formed product **2** determined from HPLC analyses.
[c] Sum of residual intermediate **1** and its part transformed in product **2.**
[d] Real free quantity of formaldehyde developed at 90°C.
[e] Residual quantity of 2-naphthol after 300 s of reaction.
[f] Residual quantity of free formaldehyde after 300 s of reaction.
[g] Time elapsed between the first (300 s) and the second (720 s) analytical examination.
[h] Differential values relevant to the second analytical examination.

On the grounds of the data reported in Table 2, the following considerations can be proposed:

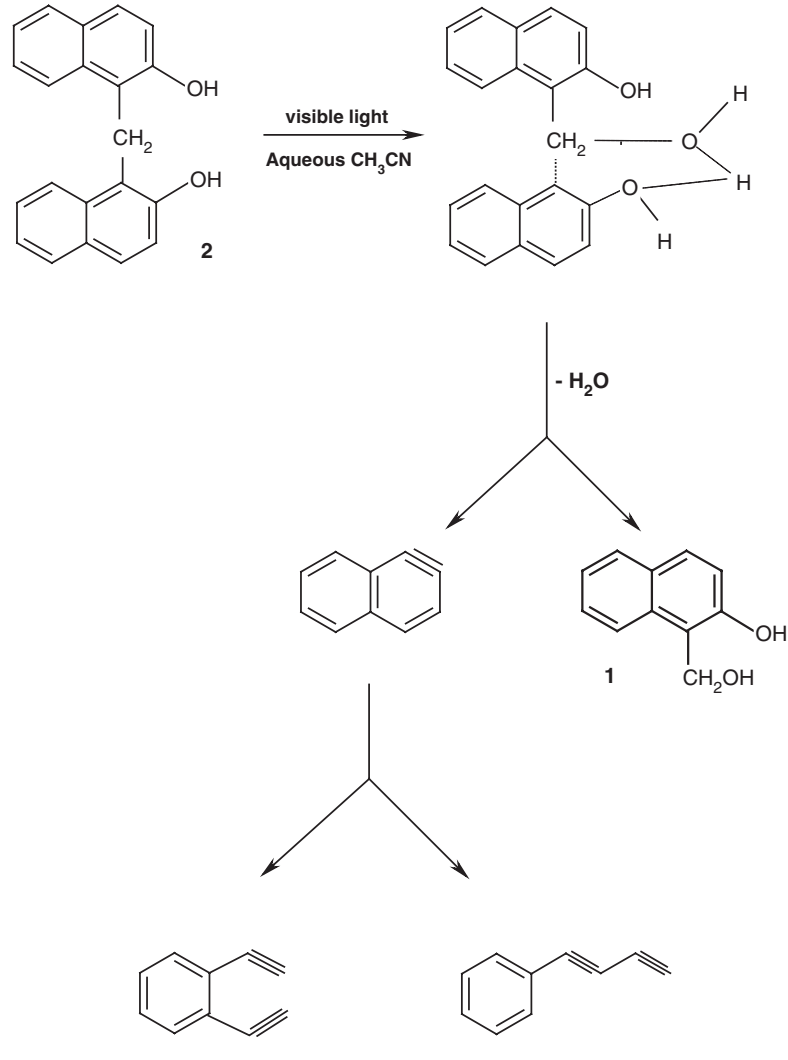
(i) After 300 s of reaction, the average formation rate of intermediate **1** (8.67×10^{-5} mmol/s) is about two times that of the product **2** (3.87×10^{-5} mmol/s). The same is the result after 720 s. Obviously, under these particular conditions it is not conceivable to expect similar reaction rates for both the first and the second steps, as the steady-state approximation for intermediate **1** would suggest. In fact, the large excess of free formaldehyde which is generated would guarantee a consistent formation of intermediate **1**, whose concentration clearly does not remain constant as the reaction goes on.

(ii) Considering the high concentration of free formaldehyde during the whole observation time, an average rate for the first step much greater than the second one should be expected. This suggests that k_2 is well greater than k_1.

(iii) A rough estimation of k_2 can be derived from the data relevant to the second part of the reaction, considering the elapsed time of further 420 s. Here, the residual quantity of 2-naphthol and free formaldehyde are, respectively, 0.0046 and 0.0562 mmol, and the differential average rate for the first and the second steps are both decreased of about one order of magnitude if compared with the relevant values determined after 300 s. The lowering of these values strictly follows the massive decrease observed for 2-naphthol concentration, denoting its first-order dependence for the first and the second step rates. However during the elapsed time of 420 s, the average rates for the first and the second reaction steps are so slow and similar to each other that we can consider them as constant along the observation time. At this condition, the steady-state approximation for intermediate **1** is reached. Considering that the free formaldehyde amount remains nearly constant (at around 0.054 mmol), and that the same can be assumed for the intermediate **1** (at around 0.0145 mmol), we can estimate, by applying Equation (2), a k_2/k_1 ratio greater than 2.

Belyanin *et al.*[23] reported an interesting decomposition reaction of 1,1′-methylene-di-(2-naphthol) **2** when heated in benzene with paraformaldehyde and boric acid. The final product is a characteristic spirodimer. Along the course of our experiments, we have never observed this particular reaction, probably because of to the soft reaction conditions we have employed. Another decomposition process, however, clearly emerged. Although the 1,1′-methylene-di-(2-naphthol) **2**, when isolated, is totally stable, as it is in completely anhydrous solution of acetonitrile, we observed that in diluted wet

acetonitrile solution, once exposed to visible light it thoroughly decomposes. Three decomposition products formed in this, reasonably radical, process (Scheme 2): the first one is represented by intermediate **1** itself, which contains all the radioactivity initially located in **2**; the other two, present in quite similar amounts, once isolated and analyzed by GC-MS as well as mono and bidimensional ^{1}H NMR, revealed to be the two alkynyl derivatives of benzene, 1,2-diethynylbenzene and 1,3-butadiynylbenzene, which do not contain any radioactivity at all. It is thus conceivable to suppose the transient formation of the 1,2-naphthyne intermediate.[24–27]



Scheme 2.

Experimental

Specific procedure for the synthesis of 1,1′[^{14}C]-methylene-di-(2-naphthol) **2**

(Entries 12 and 13 of the Table 1. The procedure is also valid for the other entries of the same table).

In a small, tight screw cap mini-vial (1.8 ml), equipped with a magnetic stirrer, 2.1 mg (0.0146 mmol) of 2-naphthol and 0.378 mg (0.0118 mmol) of [^{14}C]-paraformaldehyde (American Radiolabelled Chemical Inc., specific radioactivity 1.48 GBq/mmol) were weighted.

Acetic acid (0.2 ml) was added and the mini-vial was heated at 90°C for 60 min. A sample of 2 µl of the obtained light-red solution was added to 50 µl of a 1:1 mixture of ethyl acetate/acetonitrile. This solution was analyzed by a HPLC chromatographic system (5 µm C18 Phenomenex 'LUNA' 250 × 4.6 mm column, isocratic elution 50% water–acetonitrile at 1.0 ml/min) equipped with the UV-detector Rainin ($\lambda = 254$ nm) and the Berthold mod. 503 radiochemical flow monitor.

To complete the consuming of the 2-naphthol, a further addition of 'cold' paraformaldehyde (0.1 mg, 3.33 µmol) was done. The solution was heated for 60 min at 90°C. The HPLC analysis confirmed the nearly quantitative formation of 1,1′[^{14}C]-methylene-di-(2-naphthol) **2**.

Acetic acid was then evaporated by a gentle flux of nitrogen at room temperature, and the radioactive product **2** was recovered as a gray solid (7.1 µmol at the specific radioactivity of 1.22 TBq/mol, chemical yield: 97.2% with respect to 2-naphthol; radiochemical yield: 49.6% with respect to [^{14}C]-paraformaldehyde. The latter becomes close to 98% if the partial depolymerization of paraformaldehyde is considered).

^{1}H NMR spectrum was performed at 600.13 MHz on a Bruker Avance 600 spectrometer in the following conditions: room temperature, c. = 5 mg in 0.7 ml of $CDCl_3$.

1D spectrum (TD = 32K; Nscans = 64; Sweep = 16 ppm; 90° pulse = 10 µs; Relaxation delay = 6 s), COSY and COSYLR parameters used with gradients (TD in F2 1K; Nscans = 4; TD in F1 512K). The proton spectrum consists of 8 signals, all with the same intensity and attributed as follows (proton identification refers to the numeration of compound **2** in Scheme 1): 8.22 ppm, doublet, 2 protons (n. 5); 7.81 ppm, doublet, 2 protons (n. 8); 7.67 ppm, doublet, 2 protons (n. 4); 7.45 ppm, triplet, 2 protons (n. 6); 7.34 ppm, triplet, 2 protons (n. 7); 7.06 ppm, doublet, 2 protons (n. 3); 6.07 ppm, broad singlet, 2 protons (–OH); 4.82 ppm, singlet, 2 protons ($-CH_2$). Peak assignment was assisted by 2D COSY and 2D long range COSY maps. No other resonances were found.

Conclusion

It was possible to find the appropriate experimental conditions for a simple and soft synthesis of 1,1′[^{14}C]-methylene-di-(2-naphthol) **2** by using the indications deriving from the mechanism and from the reaction kinetic, as evidenced in Scheme 1. In particular, the reported procedure allows the complete consumption of the starting compound (2-naphthol) and also to eliminate the formation of any by-product. The recovery of product **2** turned out to be very easy. An extensive decomposition of product **2** was observed once diluted in acetonitrile solution and exposed to light. The intermediacy of 1,2-naphthyne was supposed along this last process.

Acknowledgements

The authors are thankful to Dr Annalaura Segre and Mrs Ida Bernabei for their involvement in NMR determinations and synthetic procedures, respectively.

References

1. Wolfe MS. *J Med Chem* 2001; **44** (13): 2039–2060.
   Thorsett ED, Latimer LH. *Curr Opin Chem Biol* 2000; **4**: 377–382.
2. Teplow DB. *Int J Exp Clin Invest* 1998; **5**: 121–142.
3. Soto C. *Mole Med Today* 1999; **5**: 343–350.
4. McKeon-O'Malley C, Saunders AJ, Bush AI, Tanzi RE. *Emerg Ther Targ* 1998; **2** (2):157–179.
   Sabbagh MN, Galasko D, Thal LJ. *Alzhheimer's Dis Rev* 1997; **3**: 1–19.
5. Sigma Tau Patent *PCT WO* 2002000603. Use of pamoic acid or one of its derivatives, or one of its analogues, for the preparation of a medicament for the treatment of diseases characterised by deposits of amyloid aggregates.
6. Iannone F, Ursini O. *J Radioanal Nucl Chem* 2002; **251**: 363–368.
7. Angelini G, Lilla G, Sparapani C, Ursini O, Rossi E, Segre AL, McAllister MA, Tidwell TT. *Appl Radial Isot* 1994; **45**: 386.
8. Angelini G, Lilla G, Sparapani C, Ursini O, Rossi E, Segre AL, McAllister MA, Tidwell TT. *Can J Chem* 1992; **70**: 1221–1228.
9. Angelini G, Carnevaletti F, Piccinini F. *J Label Compd Radiopharm* 1992; **31**: 289.
10. Hofmann JE, Schriesheim A. Alkylation of aromatic with aldehydes and ketones. In *Friedel-Crafts and related reactions*, Olah GA (ed). Wiley: New York, 1964; 597–640.
11. Okido S. *Patent Application: JP* 19680318, 1972.
12. Parulekar MH, Bhatt HA, Potnis SP. *J Indian Chem Soc* 1972; **49**: 1201–1207.
13. Wurm G. *Patent Application: DE* 80-3035279, 1982.
14. Tandan SK, Vijjan VK. *Indian Drugs* 1982; **19**: 156–157.
15. Poupelin JP, Saint-Ruf G, Foussard-Blanpin O, Narcisse G, Uchida-Ernouf G, Lacroix R. *Eur J Med Chem* 1978; **13**: 67–71.

16. Poupelin JP, Saint-Ruf G, Lacroix R, Narcisse G, Foussard-Blanpin O, Uchida-Ernouf G. *Eur J Med Chem* 1978; **13**: 381–385.
17. Mirci LE. *Patent Application : RO* 92-149221, 1992.
18. Casiraghi G, Casnati G, Pochini A, Puglia G, Ungaro R, Sartori G. *Synthesis* 1981; **2**: 143–146.
19. Casiraghi G, Casnati G, Cornia M, Pochini A, Puglia G, Sartori G, Ungaro R. *J Chem Soc, Perkin Trans* 1978; **4**: 318–321.
20. Oliver JE, Lamb CN, Smith RH. Jr., *J Agric Food Chem* 1983; **31**(6): 1178–1183.
21. Arduini A, Pavan F, Pochini A, Ungaro R, Corno C. *Tetrahedron* 1990; **46**: 3613–3620.
22. Laidler JK. *Chemical Kinetics* (3rd ed), Harper & Row Publishers: New York, 1987.
23. Belyanin ML, Filimonov VD, Raldugin VA, Krasnov EA. *Russ Chem Bull* 2001; **50**: 914–916.
24. Sato T, Niino H, Yabe A. *J Photochem PhotoBiol A: Chem* 2001; **145**: 3–10.
25. Sato T, Niino H, Yabe A. *J Phys Chem A* 2001; **105**: 7790.
26. Gribble GW, Perni RB, Onan KD. *J Org Chem* 1985; **50**: 2934.
27. Chen YL, Zhang HK, Wong WY, Lee WM. *Tetrahedron Lett* 2002; **43**: 2259–2262.

