

Total synthesis and radiolabelling of an efficient rt-PA inhibitor: $[^{11}\text{C}]$ (Z,Z)-BABCH. A first route to $[^{11}\text{C}]$ labelled amidines

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A rapid route for the synthesis of radiolabelled t-PA_{stop} **8**, an efficient serine protease inhibitor, is described. (*E,E*)-2,7-Bis(4-iodobenzylidene)cycloheptan-1-one **2a** was obtained in high yields (>90%) from cycloheptanone and 4-iodobenzaldehyde, with the unprecedented use of CsOH or by microwave irradiation using catalytic amounts (<20%) of bis(methoxyphenyl) telluroxide (BMPTO). These methods are general and have been successfully applied to the high yielding preparation of other aldol adducts such as **2b** and **2c**. The two step transformation of (*E,E*) **2a** into the asymmetric (*Z,Z*)-2-(4-cyanobenzylidene)-7-(4-iodobenzylidene)cycloheptan-1-one **5** has been optimized. The radiochemical yield for the radiolabelling of **5** with $\text{K}[^{11}\text{C}]\text{CN}$ followed by palladium catalysis to give the labelled bisnitrile **7** was 80–90%. A series of experiments with various methods is reported and the first procedure for the preparation of $[^{11}\text{C}]$ amidines from the corresponding $[^{11}\text{C}]$ bisnitriles with *N*-acetylcysteine is presented; the radiochemical yield, based on analytical liquid chromatography was 80% for the radioamidination. $[^{11}\text{C}]$ t-PA_{stop} was isolated in a radiochemical yield ranging from 50 to 60% in 55 min overall and with a radiochemical purity higher than 95%.

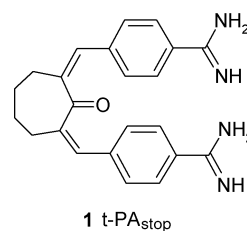
Introduction

The serine proteases are a family of proteins in which most members exert their proteolytic activity after arginine residue cleavage. These proteins have been studied extensively in relation to coagulation and thrombolysis (for a review see Carmeliet *et al.*¹). In addition to their existence in the blood, several of these proteases have been localized within the central nervous system (CNS).^{2,3} In the brain, the CNS serine proteases most studied are the plasminogen activators: tissue type-plasminogen activator (t-PA) and urokinase type-plasminogen activator (u-PA) which are thought to play a critical role in the homeostasis of the central nervous system. By its enzymatic activity, t-PA regulates the balance between the accumulation and the degradation of the extracellular matrix and it is involved in many physiological functions ranging from synaptic outgrowth during peri-natal development to plasticity in the adult brain.

t-PA_{stop} (Z,Z)-bis(amidinobenzylidene)cycloheptanone **1** ((Z,Z)-BABCH) is a synthetic antagonist of t-PA characterized by its ability to interact with one of t-PAs catalytic sites.⁴ Experimental evidence showed that t-PA_{stop} could link with t-PA by forming a salt bridge with some of the constituent amino acids of the protease such as aspartic or glutamic acid.⁵ The role of the very basic amidine in the catalytic pockets of the serine enzymes seems crucial and the formation of more than one salt bridge is likely to contribute significantly to the affinity and the potency of this family of inhibitors.⁶

To improve our understanding of the role of t-PA in the CNS *in vivo*, it seemed reasonable to develop a radioligand that could be used in positron emission tomography (PET).

In this paper, we propose a total synthesis of $[^{11}\text{C}]$ (Z,Z)-BABCH. The use of this labelled compound should be helpful to evaluate *in vivo* the amount of t-PA in the cerebral parenchyma following acute brain injury. Such data could represent an important new tool for the measurement of neuronal pain and an efficient strategy for the treatment of acute cerebral injury in humans.



Results and discussion

The (*E,E*)-BABCH symmetric molecule was first prepared in a one step procedure from 4-amidinobenzaldehyde and cycloheptanone in low yields (10%).⁷ This synthesis was recently studied by Shaw's group,⁸ who showed that (*E,E*), (*E,Z*) and (*Z,Z*)-BABCH isomers exhibited different affinity against human factor Xa (FXa); the K_i/nM values were 17000, 200 and 0.66 respectively.⁴ This experimental evidence is provided to support assignment of **1** as the bioactive olefin isomer in the BABCH series.

The synthesis of a labelled analogue of **1** requires the preparation of a precursor that can react rapidly with a selected carbon-11 reagent. With this aim, we have selected the $[^{11}\text{C}]$ cyanation route from (Z,Z)-2-(4-cyanobenzylidene)-7-(4-iodobenzylidene)cycloheptanone **5** prepared as shown in Scheme 1.

Chemistry

During the aldol reaction, cycloheptanone showed a weak reactivity compared to cyclopentanone and cyclohexanone; the relative rate of enol formation decreasing drastically with the increasing ring size.⁹ Classically, the aldol reaction may be catalyzed by acids or bases, the latter being more frequently employed in spite of the fact that a method has been described with the use of bis(4-methoxyphenyl) telluroxide (BMPTO)^{10,11} as catalyst.

Table 1 Preparation of **2** (*E,E*) (**a–c**) with various bases

Base ^a	MeONa	NaOH	KOH	CsOH
2a	29	49	77	81
2b	25	25	40	75
2c	—	59	70	85

^a In a boiling ethanolic aqueous solution, except for MeONa which was in methanol.

Table 2 Synthesis of **2** (*E,E*) (**a–c**) with BMPTO using microwave (MW) and classical heating

Compounds	Δ , 24 h, yield (%) ^a	MW, 15 min ^b yield (%)
2a	90	92
2b	79	78 ^c
2c	88	90

^a In toluene. ^b In acetonitrile—DMSO. ^c 20 min irradiation.

To investigate the role of various parameters (stoichiometry, nature of the base or catalyst) and to determine the nature of the products in the cross-aldol reaction with cycloheptanone and various substituted benzaldehydes, a series of experiments has been designed (Scheme 2). The reactions were conducted either in boiling aqueous ethanolic solution with an aldehyde–ketone–base ratio ranging from 3 to 3.2 : 1 : 2, with the use of MOH (M = Na, K, Cs) for one hour (Table 1) or with BMPTO using classical heating and microwave irradiation (Table 2).

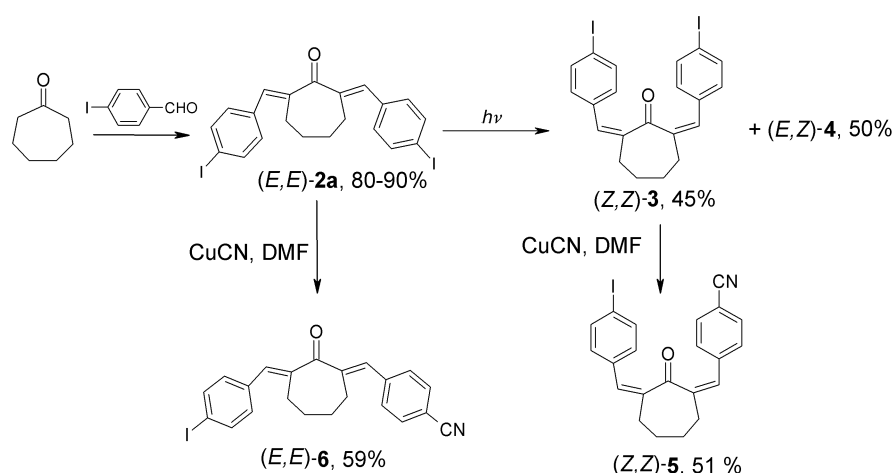
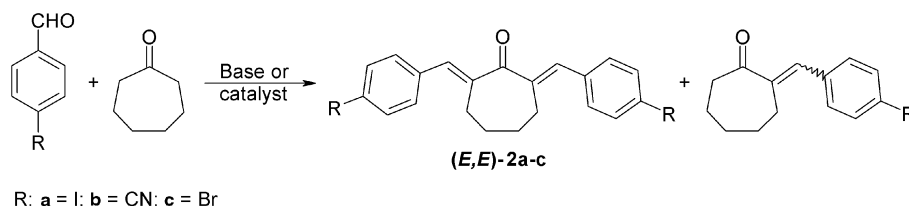
Our experiments showed that the nature of the basic agent is of essential importance. In fact, it appeared to us that the reaction of cycloheptanone and 4-iodobenzaldehyde with MeONa, NaOH or even KOH afforded mainly the expected (*E,E*)-diarylidene cycloheptanone accompanied by the (*Z,Z*) and (*E,Z*) analogues, and variable amounts of a complex mixture of the respective monoarylidene cycloheptanone isomers with other inextricable compounds. For example, with the use of KOH, in a complete study of the mother liquors, we have

observed that non-negligible amounts of (*Z,Z*) **3** and some (*E,Z*) **4** diarylidene cycloheptanones were present in solution.

These isomers were more soluble in the aqueous ethanolic solutions than the corresponding (*E,E*) **2a**. We have observed that the *E,E* : *E,Z* : *Z,Z* ratio during the preparation of **2a** was 8 : 0.5 : 1 (yield of *E,E* = 77%) and that an additional 7% of the monosubstituted derivative was isolated with an *E* : *Z* = 9.5 : 0.5 ratio. However, the optimum yields were reached with CsOH (81%) where only traces of isomers **3** and **4** could be detected (<2%) and 5% of the *E* monoarylidene compound.

Thus the use of the conditions described above, *i.e.* alkaline bases and polar solvents, made more favourable the formation of isomers and by-products even in the absence of light. The results obtained when using 20% BMPTO in refluxing toluene for 24 hours, were better than with any base (Table 2) and a major advantage of this procedure was the use of stoichiometric conditions (2.0 : 1 aldehyde : ketone ratio). The most significant improvement in the yields by this procedure was due essentially to a total conversion into (*E,E*)-diarylidene derivatives (*e.g.* **2a** = 90%) without any trace of monoarylidene (*E* or *Z*) nor other by-products. Finally, it appeared that the use of microwave irradiation¹² did not increase the yields notably, but the reaction times, for maximum completion, were from 15 to 20 min whereas the classical heating needed a 24 hour period. Similar improvement in the yields has been obtained in the cross-aldol reaction for the preparation of **2b** and **2c** from 4-cyano and 4-bromobenzaldehyde respectively either with CsOH or BMPTO (Table 1, Table 2).

Photoisomerisation conditions have been considered to produce (*Z,Z*) isomer **3** from (*E,E*) **2a** as starting material. Thus, short irradiation of a methanolic solution of **2a** with a high pressure mercury lamp produced isomers **3** and **4**, which were separated by chromatography and identified as the (*Z,Z*) and (*E,Z*) isomers respectively. The three bisiodobenzylidene isomers could be differentiated and assigned from their characteristic NMR spectra in such a way that **2a** and **3** exhibited symmetric signals in ¹H and ¹³C NMR while **4** appeared as an asymmetric structure. The upfield chemical shifts of the vinylic protons ($\delta_{(E,E)} = 7.29$ ppm and $\delta_{(Z,Z)} = 6.58$ ppm) were consistent

**Scheme 1****Scheme 2**

with related compounds containing the same double configuration.¹³ A kinetic study by HPLC showed that the photoisomerisation reached a 45% **3**, 50% **4** and 5% **2a** equilibrium after 20–30 min irradiation.

The preparation of **5** via a monocyanation reaction is a crucial step since the nitrilation of aromatic molecules containing more than one halogen atom usually leads to polynitriles.¹⁴ This kind of reaction has been well studied¹⁵ but there are only a few examples of syntheses which leave one out of several halogens unaltered.¹⁶ Various cyanation procedures with KCN using organometallic catalysts have been described in the literature.¹⁷ However, in our hands, any attempt to realise a monocyanation of the bisiodobenzylidene **3** with KCN and palladium catalyst (tetrakis(triphenylphosphine)-palladium(0) or palladium acetate) led essentially to bisnitriles as major products in addition to unreacted bishalogen compounds. In some cases, some intermolecular coupling product and deshalogenated compound have been detected in small quantities (<3%); no effort has been made to optimize these results. The very significant reactivity of palladium catalysts observed during the cyanation reaction, led us to consider this step in the classical Rosenmund reaction conditions. Thus, when copper cyanide was allowed to react with a two-fold ratio of bisiodobenzylidenes **3** in acetonitrile or hexamethylphosphorous triamide (HMPTA), the monocyanated derivative **5** was isolated in 35% yield; the use of refluxing degassed DMF, under nitrogen, raised the yields to 51%. The by-products detected under these conditions were the unreacted bisiodobenzylidene **5** (30%), and the biscyanated derivative (15%). No isomerisation was observed when the reaction was performed in the absence of light. A similar procedure applied to compound **2a**, led to **6** in 59% yield. Isomers **5** and **6** have been identified by ¹H, ¹³C and COSY NMR as asymmetric structures with two double bonds in the same configuration either *E* or *Z*. These configurations were ascertained by comparison with the corresponding structures of isomerically pure bisiodo and biscyanobenzylidenes.

Radiochemistry

Due to the very short half-life of many radioelements (for example, $t_{1/2}$ ¹¹C = 20.4 min), the preparation of radiopharmaceuticals labelled for use in PET (positron emission tomography) implies the tuning of very particular procedures. The number of radiochemical steps needs to be reduced as much as possible (one to two steps with [¹¹C]) and the delay for every process should not exceed one period ($t_{1/2}$) leaving time enough for a workup and the purification of the labelled compound.

Labelled hydrocyanic acid H¹¹CN, was produced according to classical conditions¹⁸ and trapped efficiently by freezing it in a glass vial at –20 °C. Cu¹¹CN could be obtained from H¹¹CN by heating with a solution of copper(II) sulfate and sodium metabisulfite. Application of the radiochemical equivalent procedure¹⁹ to the Rosenmund reaction led to **7** in 46% radiochemical yield and any attempt to optimize this result failed (Scheme 3). In our case and in view of the first results obtained

could be a more efficient method for cyanation. K¹¹CN was formed *in situ* from H¹¹CN by addition of KOH powder in dry THF and compound **7** was obtained in 80–90% radiochemical yield in 20 min overall (from the end of irradiation) using Pd(PPh₃)₄ as catalyst.

The labelling of t-PA_{stop} required the development of a new route leading to [¹¹C] labelled amidines. In classical chemistry, amidines are efficiently produced from nitriles *via* frequently multistep processes, that proceed in moderate to poor yields.^{21a,b} However, although a procedure for [¹⁴C] radioamidination has been described²² previously, to our knowledge, no previous author has until now described a [¹¹C] radioamidination procedure.

Taking into account the radiochemical constraints described previously for the preparation of [¹¹C] radiopharmaceuticals, we examined a series of methods for radioamidination. Three procedures have been selected and tested in radiochemistry: the Pinner²³ synthesis, the Garigipati²⁴ process and Lange's method.²⁵

First, we tried to adapt the Pinner reaction to [¹¹C]radiochemistry, however, such a two-step reaction cannot be efficiently simplified due to the inevitable passage from strong acid to strong basic conditions. Thus, the addition of **7** to 2 M HCl in absolute ethyl alcohol led, in 10 min at 0 °C, to the formation of only 30% of an intermediate. All attempts to purify this intermediate or to prepare the corresponding amidine by using a 2 M NH₃ solution failed. To overcome these difficulties we have focused on the one-pot processes suitable for direct amidination.

A one-step procedure for amidination has been reported by Garigipati using aluminium amide reagents such as AlMe₂(Cl)NH₂ in toluene. In our hands, application of similar conditions in the course of a radiochemical synthesis, led to the complete transformation of the radiolabelled **7** into an undetermined compound complexed in an inextricable white and bulky mass of aluminium salts. The expected amidine could not be liberated from that mixture in a short time.

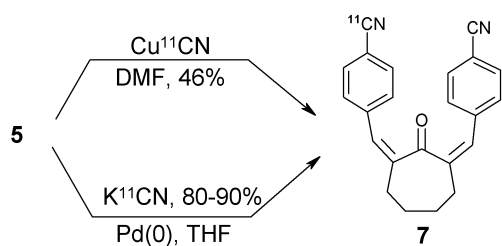
Recently, a catalytic one step process for direct amidination has been described by Lange and co-workers with *N*-acetylcysteine (NAC).²⁵ This synthesis is favoured by the ability of thiolate salts to react strongly with various nitriles²⁶ and give a reactive iminothioether intermediate.

We applied this procedure to our radiochemical process and, addition of NAC to **7** within a solution of anhydrous NH₄Cl in methanol, with 8 minutes at reflux, allowed us to obtain **8** in 51% radiochemical yield. The use of a concentrated NH₃ solution in methanol permitted us to optimize this reaction and the cysteine salt of **8** was isolated in 80% radiochemical yield (Scheme 4). The structure of the [¹¹C] t-PA_{stop} cysteine salt **8** was verified by radioHPLC with coinjection of non-radioactive reference material.

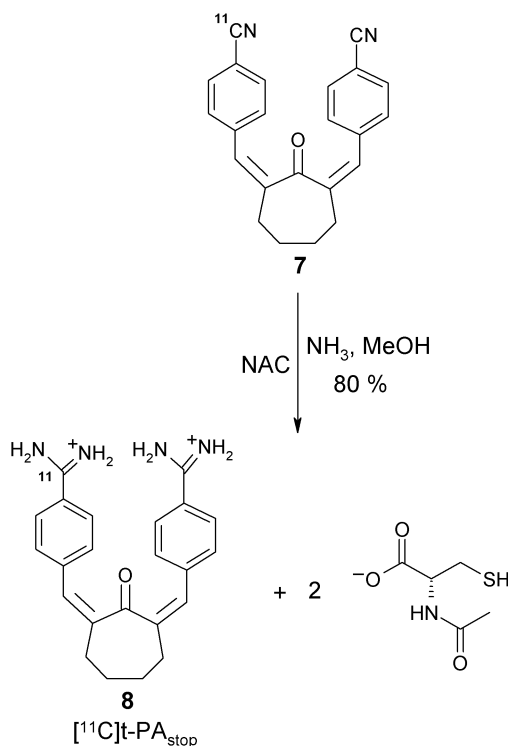
We are currently developing a fully automated version for the radioamidination of t-PA_{stop} or any other target molecule. Amidine groups, which can be conveniently obtained, from labelled nitriles are common among biologically active substances. Their [¹¹C] labelling would represent an interesting route for the study of various psychiatric or pathological disorders²⁷ because the amidine functional group is present in structures with important properties such as blood coagulation factors,²⁸ antidepressants,²⁹ antipsychotic³⁰ or even antiparasitic agents.³¹

Conclusions

In this paper, we describe some new high yielding procedures for the preparation of aldol adducts for a series of cycloheptanones; the unprecedented use of CsOH or the efficient catalyst BMPTO under microwaves are simple methods to produce the expected (*E,E*) bisaldol compounds with very high purity. With the aim of preparing new radiotracers, a first effi-



with Cu¹¹CN, we considered that the use of palladium catalysts with K¹¹CN, according to the recent published procedures,²⁰



Scheme 4

cient method for radioamidation has been described and the presented procedure is rapid, mild, general and conducted in a one-pot process suitable for automation. In this way, preparation of [¹¹C] t-PA_{stop} **8** has been successfully realized in 55 min with a radiochemical yield of 55% and with a radiochemical purity superior to 95%. Work is now in progress to evaluate these new tracers on animal models presenting an ischemia.

Experimental

¹H NMR and ¹³C NMR spectra were recorded with a "Bruker AC 250" spectrometer (at 250.13 MHz and 62.89 MHz respectively) in CDCl₃, with respect to tetramethylsilane as an internal standard, δ are given in ppm and J in Hz. Conventional abbreviations are used. The FT infra-red spectra were recorded with a "Perkin Elmer 16 PC" spectrometer as liquid films and ν are given in cm⁻¹. Mass spectra were obtained at 70 eV. Elemental analyses were performed at the Institut Supérieur de la Matière et du Rayonnement, UMR CNRS 6507, Université de Caen. Melting points were determined on a Kofler bank and are uncorrected. Flash chromatography was performed using 230–400 mesh silica gel. 4-Iodobenzaldehyde³² was prepared according to a previously described procedure. All commercial reagents were purchased from Aldrich Co. and used without further purification, except 4-cyanobenzaldehyde which was from Accros Chemicals. The ¹¹C was produced by the ¹⁴N(p, α)¹¹C nuclear reaction using a baby cyclotron (CGR Mev 325) at the CYCERON Center of Caen. Samples of pure tPA_{stop} have been prepared according to procedures previously described.^{5,7}

General procedure for the preparation of (*E,E*)-2,7-bis(4-iodobenzylidene)cycloheptan-1-one (**2a**) using CsOH

Cycloheptanone (0.48 g, 4.31 mmol) and 4-iodobenzaldehyde (3 g, 12.93 mmol) were suspended in 20 mL absolute ethanol. This was followed by the dropwise addition of CsOH (1.29 g, 8.62 mmol) in aqueous ethanolic solution (5 mL) with constant stirring. The solution was heated at reflux for one hour, then the precipitate was allowed to deposit during 12 hours at room temperature in the absence of light. The solid precipitate was

removed by filtration and rinsed thoroughly with 30 mL alcohol, 10 mL of 3% acetic acid and 20 mL water. The residue obtained was recrystallized in high volumes of boiling ethanol to give pure **2a** as white crystals (1.86 g, 81%), mp 138–140 °C (Found: C, 46.6; H, 3.5. Calc. for C₂₁H₁₈I₂O: C, 46.7; H, 3.4%); δ_{H} (250.13 MHz; CDCl₃; Me₄Si) 1.95 (m, 4H, CH₂CH₂C), 2.65 (m, 4H, CH₂CH₂C), 7.17 (d, 4H, J 8.28, CH_{ar}Cl), 7.29 (s, 2H), 7.73 (d, 4H, J 8.41, CH_{ar}C); δ_{C} (62.9 MHz; CDCl₃; Me₄Si) 28.4, 29.1, 94.6, 131.5, 135.1, 135.7, 138.1, 142.7, 199.3; m/z 540 (M⁺, 100%), 413 (21), 326 (27), 241 (24); ν_{max} /cm⁻¹ 2920, 1672 (C=O), 1620, 1098, 500 (C–I).

In similar conditions, the use of NaOH or KOH afforded after chromatography lower yields of **2a** (see Table 1) and two main by-products corresponding to the mono benzylidene derivatives (*E*)-2-(4-iodobenzylidene)cycloheptan-1-one³³ and (*Z*)-2-(4-iodobenzylidene)cycloheptan-1-one. The latter was recrystallized in methyl alcohol to give golden crystals, mp 98 °C (Found: C, 51.3; H, 4.7. Calc. for C₁₄H₁₅IO: C, 51.55; H, 4.6%); δ_{H} (250.13 MHz; CDCl₃; Me₄Si) 1.78 (m, 6H), 2.41 (d, 2H, J 9.19, CH₂CO), 2.56 (d, 2H, J 10.13, CH₂C=C), 6.39 (s, 1H), 6.98 (d, 2H, J 8.35, CH_{ar}Cl), 7.60 (d, 2H, J 8.45, CH_{ar}C); δ_{C} (62.9 MHz; CDCl₃; Me₄Si) 24.8, 30.2, 31.1, 35.7, 43.7, 93.6, 130.5, 135.5, 135.9, 137.8, 145.6, 204.9; m/z 326 (M⁺, 17%), 284 (100), 216 (15), 200 (12); ν_{max} /cm⁻¹ 2920, 1680 (C=O), 1606 (C=C), 1280, 1000, 560 (C–I), 530.

General procedure for the preparation of **2a** using BMPTO

By thermal heating: cycloheptanone (0.242 g, 2.154 mmol), 4-iodobenzaldehyde (1 g, 4.308 mmol) and bis(methoxyphenyl) telluroxide (0.154 g, 0.431 mmol) were placed in a 25 mL flask with 6 mL of dry toluene and refluxed for 24 h. The cold mixture was evaporated and chromatographed on silica gel (cyclohexane–AcOEt 95 : 5) to give 1.05 g **2a** (90%) as white crystals. By microwave heating: this procedure was performed in a similar manner using a Prolabo Synthwave 402, with 2 mL of CH₃CN–DMSO (9 : 1) as solvent and the reactants were irradiated at 150 W for 15 min. After cooling, the mixture was purified as described above to give **2a** in 92% yield.

(*Z,Z*)-2,7-Bis(4-iodobenzylidene)cycloheptan-1-one (**3**)

0.5 g of **2a** was suspended in 70 mL of 95% ethanol under dry nitrogen in a quartz flask. The mixture was stirred and irradiated at 150 W during 25 minutes. Isomerization was followed by HPLC and stopped when a 45–50% **3** \rightleftharpoons **4** equilibrium was reached. Evaporation of the solvent followed by reversed phase chromatography (MeOH–H₂O 75 : 25) gave 0.21 g of **3** as a pale yellow powder, mp 124–126 °C (Found: C, 46.7; H, 3.4. Calc. for C₂₁H₁₈I₂O: C, 46.7; H, 3.4%); δ_{H} (250.13 MHz; CDCl₃; Me₄Si) 1.91 (m, 4H, CH₂CH₂C), 2.53 (m, 4H, CH₂CH₂C), 6.58 (s, 2H), 7.03 (d, 4H, J 8.28, CH_{ar}Cl), 7.55 (d, 4H, J 8.45, CH_{ar}C); δ_{C} (62.9 MHz; CDCl₃; Me₄Si) 32.3, 36.8, 94.2, 131, 134.9, 135.7, 137.6, 143.6, 200.0; m/z 540 (M⁺, 46%), 413 (54), 288 (20), 242 (40), 191 (100); ν_{max} /cm⁻¹ 3036, 1672 (C=O), 1620, 1098, 1038, 566 (C–I). (*E,Z*)-2,7-Bis(4-iodobenzylidene)cycloheptan-1-one (**4**) was isolated in the same manner as **3** as pale yellow crystals, mp 126–128 °C (Found: C, 46.5; H, 3.3. Calc. for C₂₁H₁₈I₂O: C, 46.7; H, 3.4%); δ_{H} (250.13 MHz; CDCl₃; Me₄Si) 1.90 (m, 4H, CH₂CH₂C), 2.46 (m, 2H, CH₂CH₂C), 2.72 (m, 2H, CH₂CH₂C), 6.47 (s, 1H), 6.96 (d, 2H, J 8.32, CH_{ar}Cl), 7.13 (d, 2H, J 8.29, CH_{ar}Cl), 7.36 (s, 1H), 7.58 (d, 2H, J 8.17, CH_{ar}C), 7.73 (d, 2H, J 8.37, CH_{ar}C); δ_{C} (62.9 MHz; CDCl₃; Me₄Si) 28.3, 30.1, 32.4, 36.3, 93.6, 95.1, 130.6, 130.6, 131.6, 135.4, 135.8, 136.5, 137.9, 138.1, 139.8, 146.2, 200.3; m/z 540 (M⁺, 28%), 413 (26), 217 (100), 165 (73); ν_{max} /cm⁻¹ 2926, 2850, 1674 (C=O), 1032, 560 (C–I).

(*Z,Z*)-2-(4-Cyanobenzylidene)-7-(4-iodobenzylidene)-cycloheptan-1-one (**5**)

In a 25 mL two-necked round-bottomed flask, **3** (0.6 g, 1.11

mmol) and CuCN (0.04 g, 0.45 mmol) were mixed in 12 mL of degassed DMF. The solution was heated at reflux for 24 hours, then the mixture was cooled and the DMF was evaporated to dryness under vacuum with toluene. The residue was taken up in dichloromethane and filtered through Celite. The resulting brown solution was chromatographed on silica gel to give **5** (0.25 g, 51%) as a pale yellow powder (Found: C 60.1; H, 4.0; N, 3.3. Calc for $C_{21}H_{18}I_2O$: C, 60.15; H, 4.1; N, 3.2%); δ_H (250.13 MHz; $CDCl_3$; Me_4Si) 1.92 (m, 4H, CH_2CH_2C), 2.55 (m, 4H, CH_2CH_2C), 6.62 (s, 1H), 6.64 (s, 1H), 7.03 (d, 2H, J 8.28, $CH_{ar}Cl$), 7.33 (d, 2H, J 8.12, $CH_{ar}CN$), 7.49 (d, 2H, J 8.43, $CH_{ar}C$), 7.53 (d, 2H, J 8.43, $CH_{ar}C$); δ_C (62.9 MHz; $CDCl_3$; Me_4Si) 32.2, 32.4, 36.7, 36.8, 94.4, 111.5, 119.2, 129.7, 131.0, 132.3, 133.7, 135.5, 135.7, 137.6, 141.0, 143.0, 146.1, 199.3; m/z 440 (M^+ , 90.5%), 439 (22), 313 (100), 243 (69); ν_{max}/cm^{-1} 2922, 2220 ($C\equiv N$), 1728, 1666 ($C=O$), 1014, 560 ($C-I$).

(*E,E*)-2-(4-Cyanobenzylidene)-7-(4-iodobenzylidene)cycloheptan-1-one (6)

Compound **6** was obtained from **2a** by a similar procedure, yielding 59% of white crystals (Found: C, 60.2; H, 4.1; N, 3.1. Calc for $C_{21}H_{18}I_2O$: C, 60.15; H, 4.1; N, 3.2%); δ_H (250.13 MHz; $CDCl_3$; Me_4Si) 1.96 (m, 4H, CH_2CH_2C), 2.66 (m, 4H, CH_2CH_2C), 7.16 (d, 2H, J 8.24, $CH_{ar}Cl$), 7.33 (s, 1H), 7.34 (s, 1H), 7.51 (d, 2H, J 8.32, $CH_{ar}CN$), 7.68 (d, 2H, J 8.37, $CH_{ar}C$), 7.74 (d, 2H, J 8.43, $CH_{ar}C$); δ_C (62.9 MHz; $CDCl_3$; Me_4Si) 28.3, 28.5, 29.2, 30.1, 94.8, 112.0, 119.0, 130.2, 131.5, 132.6, 133.9, 135.5, 135.8, 138.1, 140.9, 142.0, 145.1, 199.3; m/z 439.8 (M^+ , 100%), 412 (5.5), 313 (81), 242.5 (46); ν_{max}/cm^{-1} 2922, 2220 ($C\equiv N$), 1728, 1666 ($C=O$), 1228, 562 ($C-I$).

(*Z,Z*)-2-(4-[^{13}C]Cyanobenzylidene)-7-(4-cyanobenzylidene)-cycloheptan-1-one (7)

$K[^{13}C]CN$ was produced *in situ* by adding a THF solution of 2 M KOH (100 μ L) to trapped $H[^{13}C]CN$. The mixture was heated to 90 °C and evaporated to dryness under a flux of helium. To this was added a solution of **5** (4 mg) with Kriptofix® 2.2.2 (2 mg) in THF (100 μ L). 1.2 mg of tetrakis(triphenylphosphine)palladium(0) (99%) was dissolved in THF (100 μ L) and added to the mixture of reagents and the substitution reaction was carried out at 90 °C for 10 min. Radio TLC was achieved with a sample of the mixture in cyclohexane–AcOEt 60 : 40 (R_f = 0.48). An aliquot was taken and analyzed by HPLC employing the following conditions: μ Bondapak C-18, MeOH– H_2O 85 : 15, flow 2 mL min^{-1} , wavelength 254 nm. The retention time for **7** was 9.1 min.

(*Z,Z*)-2-(4-[^{13}C]Amidinobenzylidene)-7-(4-amidinobenzylidene)-cycloheptan-1-one (8)

Compound **7** in THF was evaporated to dryness under a flux of helium. A methanolic solution of *N*-acetylcysteine (4 mg), NH_4OAc (4 mg) and NH_3 (7 M, 30 μ L) dissolved was added and the mixture was heated to 80 °C for 8 min. An aliquot was taken and analysed by HPLC employing the following conditions: Waters Symmetry C-18, acetonitrile : H_2O : TFA 20 : 80 : 0.1, flow 2 mL min^{-1} , wavelength 254 nm and the retention time for **8** was 5.5 min.

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