Synthesis of Isotopically Labelled L-Phenylalanine and L-Tyrosine

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A synthetic route to stable-isotope-substituted L-phenylalanine is presented, which allows the introduction of ^{13}C, ^{15}N, and deuterium labels at any position or combination of positions. For labelling of the aromatic ring, a synthetic route to ethyl benzoate (or benzonitrile) has been developed, based on the electrocyclic ring-closure of a 1,6-disubstituted hexatriene system, with in situ aromatization by elimination of one (amino) substituent. Several important (highly isotopically enriched) synthons have been prepared, namely benzonitrile, benzaldehyde, ethyl benzoate, and ethyl diphenyloxyacetate. Labelled L-phenylalanines have been synthesized from both aromatic precursors by initial conversion into sodium phenylpyruvate and subsequent transformation of this intermediate into the L-α-amino acid by an enzymatic reductive amination reaction. In this manner, highly enriched phenylalanines are obtained on the 10-gram scale and with high enantiomeric purities (≥ 99% *ee*). The method has been validated by the synthesis of [1′-^{13}C]-L-Phe and [2-D]-L-Phe. In addition, two methods are described for the introduction of isotopes into L-tyrosine starting from the isotopically enriched precursors benzonitrile and ethyl benzoate.

Introduction

Aromatic amino acids specifically labelled with stable isotopes (e.g. ^{13}C, ^{15}N, and ^{2}H) are valuable tools for the investigation of proteins at the atomic level. In order to study these systems without perturbation, the use of a combination of stable-isotope enrichment (99%) and non-invasive, isotope-sensitive techniques (for instance NMR, EPR, FTIR, and UV laser resonance Raman spectroscopy) is of enormous potential and value.[1] The availability of stable-isotope-labelled amino acids is a prerequisite for this approach.

For instance, only a few specifically labelled L-phenylalanines are commercially available and synthetic procedures for only two of these ring-labelled compounds have been published to date.[2] No method is known which allows the introduction of labels at any desired position or combination of positions. Therefore, a synthetic scheme meeting this requirement is needed. Several criteria must be met by such a synthesis: (i) the synthesis should be based on simple compounds that can be purchased in specifically and highly enriched (> 99%) form (e.g. acetonitrile, acetic acid, potassium cyanide); (ii) since these starting materials are quite expensive, an efficient and thoroughly optimized synthetic scheme is required; (iii) care should be taken that no scrambling or dilution of the label can occur at any stage in the course of the synthesis; (iv) the synthesis should be enantioselective, since only the L-isomer occurs in proteins. The challenge of synthesizing these stable-isotope-labelled aromatic amino acids is twofold. The first requirement is to develop a synthetic scheme for a mono-substituted benzene synthon with which each carbon and hydrogen position and combination of positions can be obtained in highly enriched form. Secondly, the aromatic precursor must be extended to the full carbon skeleton and the chirality at the amino acid group must be introduced.

In this paper, a synthesis of two members of the aromatic amino acids (L-Phe and L-Tyr) is described which focusses on the introduction of ^{13}C, but can be adapted for the introduction of ^{2}H and ^{15}N at any position or combination of positions.

Results

Our approach to the synthesis of a mono-substituted benzene synthon is based on the electrocyclic ring-closure of 7-(dimethylamino)hepta-2,4,6-trienenitrile (**3**), followed by in situ elimination of the amino substituent from the intermediate cyclohexadiene affording benzonitrile (**4**)[3] (see Scheme 1). For the introduction of the chiral side chain an approach was chosen in which an achiral precursor, incorporating the full carbon skeleton, was enzymatically converted into optically pure **1**. To realize this, benzonitrile was first converted into benzaldehyde (**5**), the key intermediate in the synthetic scheme. Benzaldehyde was subsequently converted into the achiral precursor sodium phenylpyruvate (**6**) in two steps. In the final step, sodium phenylpyruvate was transformed into **1** by an enzymatic reductive amination. This synthetic scheme was optimized using commercially available non-enriched starting materials. In this manner, [1′-^{13}C]-L-Phe (**1a**) and [2-D]-L-Phe (**1b**) have been synthesized, as will be described in the next section. A synthetic route to benzaldehyde allowing the introduction of ^{13}C-labels at the other atomic positions will be described in later sections.

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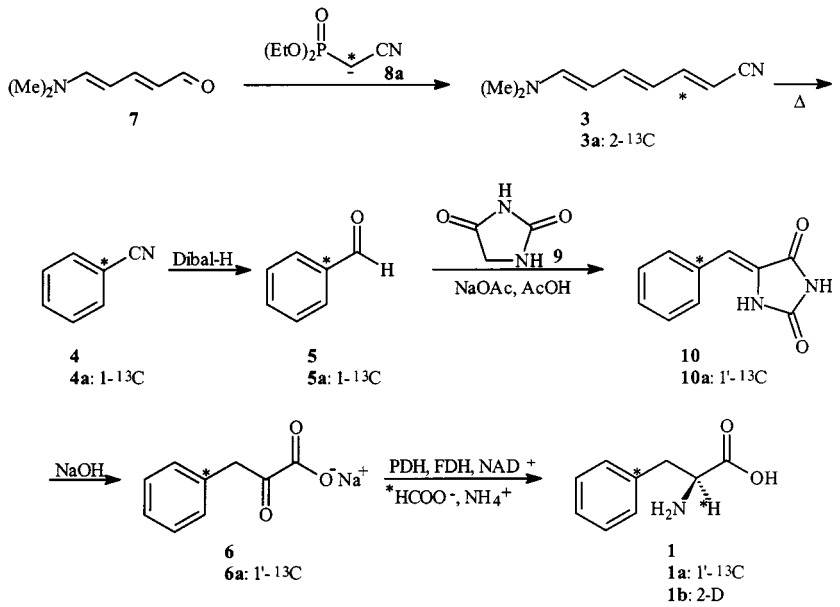


Scheme 1. Synthesis of isotopically labelled L-phenylalanines (**1a**,**b**) starting from the easily available synthon **7**; labels at the polar group can be introduced by using isotopically enriched hydantoin

Isotope Labelling of L-Phe at the 1′-Position as well as at the Polar Group Positions

For the synthesis of **1a**, 1.0 g of [2-^{13}C]acetonitrile was used as the starting material. This was first deprotonated at −70°C in THF using one equivalent of lithium diisopropylamide (LDA) in the presence of a second equivalent of LDA. In the subsequent reaction of the lithioacetonitrile with one equivalent of diethyl chlorophosphate, the initially formed diethyl cyanomethylphosphonate was immediately deprotonated by the second equivalent of LDA.[4] The resulting labelled anion of diethyl cyanomethylphosphonate (**8a**) was then treated with 5-(dimethylamino)pentadienal (**7**), affording [1-^{13}C]aminoheptatriene **3a** (41%) (see Scheme 1). Compound **7** is readily available by known procedures.[5] Benzonitrile was obtained from heptatriene **3** by heating a quinoline solution of compound **3** in a sealed Carius tube at 250°C for 4 h (0.5 g scale). Removal of the solvent and purification of the residue by column chromatography gave benzonitrile (**4**) in 66% yield. Reduction of the nitrile function with diisobutylaluminium hydride in dichloromethane at −70°C followed by mild acidic workup afforded a 100% yield of crude benzaldehyde, which was immediately reacted with hydantoin (**9**) to give 5-benzalhydantoin (**10**) in 76% yield; 13% of unchanged benzaldehyde was recovered from the filtrate. Compound **10** was hydrolysed with NaOH, affording a 70% yield of the sodium salt of phenylpyruvate (**6**). No organic side-products were formed during the hydrolysis; only phenylpyruvate, ammonia, and carbon dioxide were formed. Further purification of the product was not necessary; it could be converted into **1** using the commercially available enzyme L-phenylalanine dehydrogenase (PHEDH; E.C. 1.4.1.20).[6] For this reductive amination, one equivalent of the co-enzyme NADH was required. In situ regeneration of NADH was achieved by a second enzymatic reaction, namely the conversion of formate into carbon dioxide by the enzyme formate dehydrogenase (FDH, E.C. 1.2.1.2). In this manner, a high concentration of NAD^+, which has an inhibitory effect on PHEDH, is avoided. Moreover, only a catalytic amount of the expensive NADH was needed. A small scale reaction [using 158 mg (0.8 mmol) of **6a**] afforded **1a** in 35% yield. The product was found to be > 99% (chemically) pure by HPLC and its enantiomeric excess (*ee*) was found to be > 99% (see Experimental Section). The overall yield of **1a** based on [2-^{13}C]acetonitrile was 2.5%.

The enzymatic conversion described above was scaled-up to a multi-gram synthesis for the preparation of **1b**. In analogy to the multi-gram synthesis of ^{15}N-labelled L-glutamic acid,[7] the principle of this scale-up was the repeated use of the enzymes by ultrafiltration methods in a repetitive batch synthesis. Slightly modified reaction conditions were used compared to those for the synthesis of [1′-^{13}C]-L-Phe. Six consecutive batches afforded 8.93 g (57%) of **1b** with a chemical purity of 97.5% as established by HPLC. The enantiomeric purity of the product was, as for **1a**, > 99%.

Thus, by using the aforementioned method, **1** can be isotopically labelled at each position of the polar group (since it is possible to synthesize ^{13}C-labelled hydantoin[8] from commercially available starting materials and ^{15}N can be introduced by substituting $^{15}NH_4^+$ for NH_4^+ during the reductive amination of **6**) and at the carbon atom of the aromatic ring to which the side chain is attached (C-1′).

^{13}C-Labelling of L-Phe at the 2′-Position (as well as at the 1′- or 3-Position)

In order to extend the aforementioned method to all other aromatic carbon atoms, a synthetic scheme for benzaldehyde is required that would allow the introduction of

labels at any desired position. By elaborating on the successful synthesis of [1-^{13}C]benzaldehyde (**4a**), attempts were made to develop a synthesis of 5-dimethylaminopentadienal (**7**). To achieve this, commercially available 3-(dimethylamino)propenal (**11**) was first condensed with the anion of diethyl cyanomethylphosphonate (**8**), affording 5-dimethylaminopentadienenitrile (**12**). Attempted reduction of the nitrile with 1 equivalent of diisobutylaluminium hydride (DIBAL−H) at −78°C failed completely, probably due to the electron-donating effect of the dimethylamino group.[9]

Apparently, starting from the dimethylamino end of the molecule is not a viable approach for synthesizing 7-(dimethylamino)heptatrienenitrile (**3**) with labels at other ring positions. The electrocyclic ring-closure of **3** is, however, a very versatile way of synthesizing benzonitrile, and therefore a second synthetic approach to compound **3** was attempted, starting in this case from the cyano-end of the molecule. In this manner, the electron-rich dimethylamino group, which was most probably the cause of the lack of reactivity towards DIBAL-H (vide supra), could be introduced at the latest possible step in the synthesis of **3**. The approach is based on the introduction of carbon atom 7 as an enamine group, by a Horner−Wittig (HW) reaction of 5-cyano-2,4-pentadienal[10] (**18**) with an α-dimethylamino-substituted phosphane oxide (Scheme 2) (a wide variety of enamines have been prepared by such a HW coupling of α-amino-substituted phosphane oxides with the appropriate aldehydes[11,12a−12d]). The starting material for the preparation of **18** was the commercially available fumaraldehyde bis(dimethyl acetal) (**15**), which was mono-deprotected to give aldehyde **16** by heating with 5% H_3PO_4 in water and removing the methanol azeotropically.[13] HWE coupling of 4,4-dimethoxy-2-butenal (**16**) with the anion of diethyl cyanomethylphosphonate (**8**) afforded nitrile acetal **17**, which was easily deprotected with dilute hydrochloric acid in acetone to afford 5-cyano-2,4-pentadienal (**18**).

The synthesis of 7-(dimethylamino)heptatrienenitrile (**3**) was attempted by coupling of compound **18** with the anion of (dimethylaminomethyl)diphenylphosphane oxide (**19**). It has been reported that in reactions of this type the initial lithiated adduct **20** does not directly eliminate diphenylphosphinate.[12c] Neutralization with aqueous ammonium chloride should afford the alcohol, which then has to be converted into the enamine with KO*t*Bu. Although 7-(dimethylamino)heptatrienenitrile (**3**) was obtained in this manner, the yield did not exceed 13%.

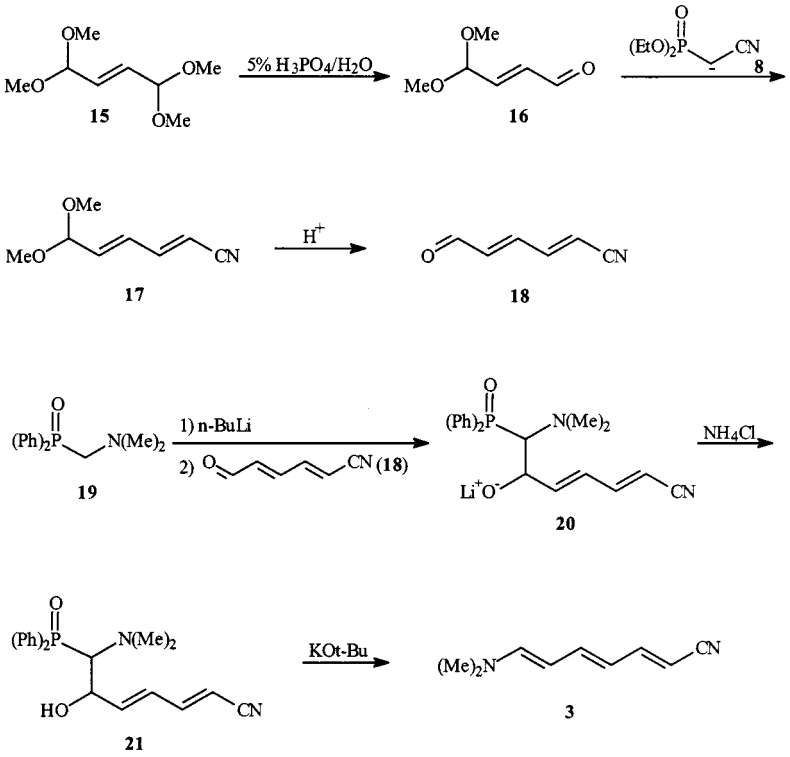
A possible explanation for the low yields of this HW coupling might be that the conjugated nitrile is too reactive under the reaction conditions employed. Since conjugated esters are less reactive towards nucleophiles than conjugated nitriles, our attention was turned from the nitrile group to an ethyl ester, in the hope of achieving better results in the HW coupling. Cyclization of the resulting 1,6-disubstituted heptatriene would then afford ethyl benzoate as a mono-substituted benzene synthon.

In order to test this approach, 5-carbethyloxy-2,4-pentadienal (**23**) was first prepared in analogy with the synthesis of 5-cyano-2,4-pentadienal[14] (**18**) (see Experimental Section). Fumaraldehyde mono-dimethyl acetal (**16**) was condensed with the anion of the commercially available triethyl phosphonoacetate, yielding 5-carbethyloxy-2,4-pentadienal (dimethyl acetal) (**22**). Compound **22** was then deprotected with dilute hydrochloric acid in acetone, affording 5-carbethyloxy-2,4-pentadienal (**23**). Fortunately, HW coupling of this aldehyde **23** with the anion of (dimethylaminomethyl)diphenylphosphane oxide (**19**) afforded, after treatment of the initial adduct with potassium *tert*-butyloxide (KO*t*Bu), ethyl 7-(dimethylamino)heptatrienoate (**24**) in reasonable yield (46%). Cyclization of compound **24** at 200°C for 4 h afforded ethyl benzoate (**25**) in 46% yield (as determined by gas chromatography; see Discussion).

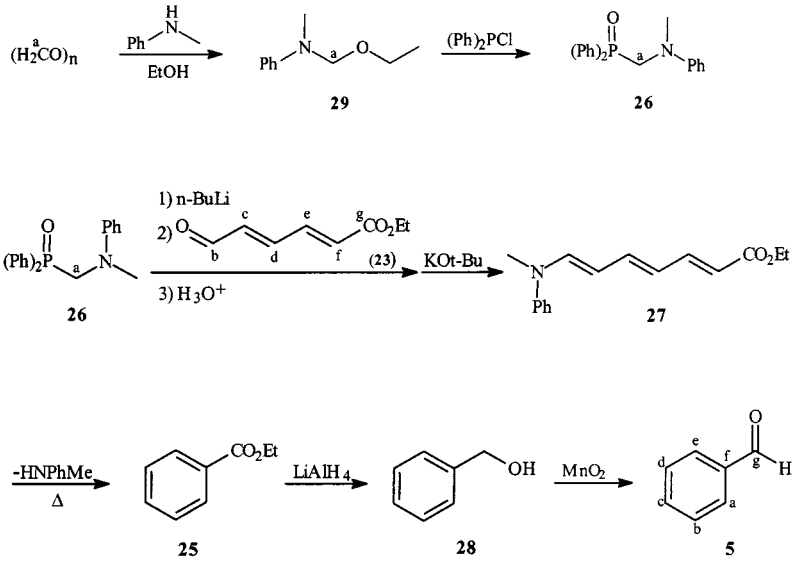
Although the HW coupling of 5-carbethyloxy-2,4-pentadienal (**23**) with the anion of phosphane oxide **19** was satisfactory and the resulting 1,6-disubstituted heptatriene **24** could be cyclized to ethyl benzoate, an alternative HW coupling of aldehyde **23** was attempted, namely with (*N*-methylanilinomethyl)diphenylphosphane oxide (**26**) (Scheme 3). This phosphane oxide has been reacted with a wide variety of aldehydes and ketones, affording the corresponding enamines in high yields.[12c] Due to the phenyl group, the basicity and nucleophilicity are expected to be lower for the anion of phosphane oxide **26** compared to that of **19**,[15] which might have a beneficial effect on the HW coupling by reducing possible side reactions. HW reaction of aldehyde **23** with **26** indeed afforded ethyl 7-(*N*-methylanilino)heptatrienoate (**27**), although only in slightly better yield (50%). Subsequently, compound **27** was cyclized at 200°C for 4 h to afford ethyl benzoate (**25**) in 40% yield after purification by column chromatography (50% as determined by gas chromatography). Since the yield of the HW coupling of aldehyde **23** was slightly better with phosphane oxide **26** than with phosphane oxide **19**, and the cyclization reaction of the resulting ethyl 7-(*N*-methylanilino)heptatrienoate (**27**) afforded a higher yield of ethyl benzoate than the cyclization of ethyl 7-(dimethylamino)heptatrienoate (**24**), it was decided to proceed with the *N*-methylanilino-substituted phosphane oxide **26**. Furthermore, phosphane oxide **26** is easier to synthesize with a ^{13}C label than compound **19**. Reduction of ethyl benzoate (**25**) with $LiAlH_4$ afforded benzyl alcohol (**28**) almost quantitatively (98% yield after purification; Scheme 3). Subsequent oxidation with MnO_2 in dichloromethane produced crude benzaldehyde (**5**) in 100% yield. This crude benzaldehyde could be used directly for the condensation with hydantoin (**9**) as described above (Scheme 1).

Using the aforementioned method, as depicted in Scheme 3, benzaldehyde was prepared from the starting compounds 5-carbethyloxy-2,4-pentadienal (**23**) and (*N*-methylanilinomethyl)diphenylphosphane oxide (**26**).

In order to introduce ^{13}C-labels at any position or combination of positions in benzaldehyde, it is imperative that these building blocks can be synthesized in isotopically enriched form. The synthesis of **23** described above is not suitable for this purpose since it starts from the symmetrical fumaraldehyde bis(dimethyl acetal). A synthetic route to **23**



Scheme 2. Synthesis of aminoheptatriene **3** by elongation of the C_6-synthon to dimethylaminoheptatrienenitrile **3**



Scheme 3. Synthetic scheme to benzaldehyde enabling introduction of ^{13}C labels at any position or combination of positions (symbolized by a–g)

and **26** allowing the introduction of ^{13}C at any position or combination of positions is described in the next section.

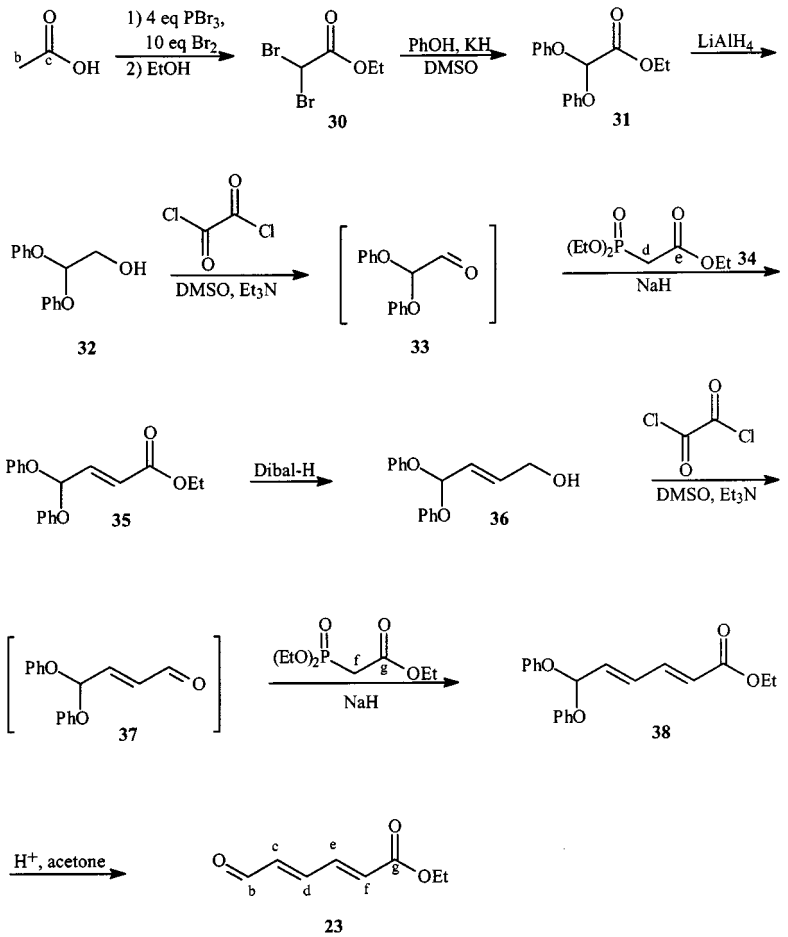
A Synthetic Route to Aldehyde 23 and Phosphane Oxide 26 Allowing the Introduction of ^{13}C-Labels at Any Position of Benzaldehyde 5

For the synthesis of **1** isotopically labelled at any possible carbon position or combination of positions, the building blocks need to be available in specifically enriched form. Since these compounds are not commercially available, it is necessary to develop methods for the isotopic labelling of 5-carbethyloxy-2,4-pentadienal (**23**) and (the methylene carbon of) (*N*-methylanilinomethyl)diphenylphosphane oxide **26**. Compound **26** was prepared from paraformaldehyde (commercially available with a ^{13}C-label) according to a literature procedure[16] (Scheme 3). Paraformaldehyde was condensed with *N*-methylaniline in ethanol to give *N*-ethyloxymethyl-*N*-methylaniline (**29**) in 72% yield after distil-

lation. Reaction of **29** with chlorodiphenylphosphane afforded phosphane oxide **26** in 70% yield after recrystallization.

For the synthesis of 5-carbethyloxy-2,4-pentadienal (**23**), a new C-2 synthon, namely an oxalic dialdehyde mono-acetal (**33**), was required (see Scheme 4). Using phosphonate chemistry, which has been extensively exploited in our group for the synthesis of conjugated unsaturated systems (e.g. in constructing the carbon skeletons of retinals, carotenoids, and astaxanthins[17–19]), this synthon can be extended to the full carbon skeleton of **23** with deprotection of the aldehyde in the final step.

As group R, the phenyl group was chosen; 2,2-diphenyloxy-1-ethanal (**33**) was prepared and converted into 5-carbethyloxy-2,4-pentadienal (**23**) as shown in Scheme 4. Starting materials in this scheme are acetic acid, which is commercially available with > 99% ^{13}C at either (or both) carbon atoms, and triethyl phosphonoacetate (**34**), which can be synthesized incorporating stable-isotope labels from acetic acid. To achieve this, acetic acid was first converted into ethyl bromoacetate in a Hell–Volhard–Zelinskii (HVZ) reaction by refluxing with one equivalent of phosphorus tribromide and 2.5 equivalents of bromine for 3 h and then treating the resulting bromoacetyl bromide with ethanol. In this manner, isotopically labelled ethyl bromoacetate was obtained in 86% yield.[20][21] Previously, this reaction has only been used for the introduction of one bromo substituent; the reaction conditions have now been optimized for the introduction of two bromine atoms. Rather severe conditions are needed for this conversion: acetic acid has to be refluxed at 170°C with 4 equivalents of phosphorus tribromide and 10 equivalents of bromine for 5 days. Subsequent reaction of the resulting dibromoacetyl bromide with ethanol afforded ethyl dibromoacetate (**30**) in 85% yield after column chromatography, which was found to contain a trace (1.5%) of the mono-brominated compound. This geminal dibromide, which is in fact a masked aldehyde, was converted into an acetal (protected aldehyde) by reaction with potassium phenolate in DMSO, affording ethyl diphenyloxyacetate (**31**). The ethyl ester of the crude **31** was reduced with $LiAlH_4$ to yield 2,2-diphenyloxyethanol (**32**) in 55% yield after purification (yield based on ethyl dibromoacetate). Compound **32** was transformed into ethyl 4,4-diphenyloxy-2-butenoate (**35**) in a two-step one-pot procedure.[23] First, the alcohol was converted into 2,2-diphenyloxy-1-ethanal (**33**) by Swern oxidation[24] in THF; then, two equivalents of the separately synthesized anion of triethyl phosphonoacetate (**34**; a subsequent Arbusov reaction with triethyl phosphite gave isotopically labelled triethyl phosphonoacetate) were added in



Scheme 4. Synthesis of 5-carbethyloxy-2,4-pentadienal (**23**), allowing introduction of ^{13}C labels at any position or any combination of positions

situ at −80°C. After stirring at room temperature overnight, workup and column chromatography afforded **35** in 94% yield. The unsaturated ester group in **35** was reduced with diisobutylaluminium hydride (DIBAL-H) in THF; basic workup gave the allylic alcohol **36** in 98% yield after column chromatography. A second Swern oxidation converted **36** into aldehyde **37**, which was subjected to in situ HWE coupling with five equivalents of the anion of triethyl phosphonoacetate (**34**), affording ethyl 6,6-diphenyloxyhexa-2,4-dienoate (**38**) in 91% yield after purification. Deprotection of **38** was effected with several drops of 1 N aqueous HCl solution in acetone,[19] affording the required 5-carbethyloxy-2,4-pentadienal (**23**) in 98% yield after purification by column chromatography. The overall yield of **23** based on acetic acid was 43%.

Conversion of Benzonitrile 4 (or Ethyl Benzoate 25) to Phenol and Preparation of Isotopically Labelled L-Tyrosine

The products of the cyclization reactions described above were used as starting materials for the synthesis of tyrosine. The route proceeding via **4** is preferred for the introduction of labels at position 1 of the mono-substituted aromatic compound, whereas for labelling of the other positions **25** is used. The key intermediate along this route is acetophenone (**54**), which was formed from **25** in two steps. First, compound **25** was converted into (*N*-methoxy-*N*-methyl)-benzamide (**53**) by attack of the anion of *N*-methoxy-*N*-methylamine (formed from the salt with 2 equivalents of *n*-butyllithium) on the ethyl ester. Compound **53** was obtained in 96% yield after purification by column chromatography. Treatment of **53** with 1.4 equivalents of methyllithium (MeLi) afforded, after purification, 89% of acetophenone (**54**). The same compound was also formed from **4** in a one-pot two-step procedure: reaction with methyllithium afforded the lithium salt of the intermediate imine, which was hydrolysed with sulfuric acid at 50°C to give a 70% yield of acetophenone after purification by column chromatography. Acetophenone was subsequently converted into phenyl acetate (**55**) by a Baeyer−Villiger reaction with *meta*-chloroperoxybenzoic acid (*m*-CPBA) in water. Compound **55** was isolated in 89% yield after extraction of the reaction mixture; further purification was not necessary. The ester was hydrolysed with sodium methanolate in methanol. After completion of the reaction, phenol (**56**) was isolated in 99% yield from the formed sodium phenolate. No further purification was needed for conversion of this phenol into L-tyrosine by the previously described method.[22]

Discussion

Synthesis of Two Precursors of Benzaldehyde 5: Benzonitrile 4 and Ethyl Benzoate 25

The main challenge of our schemes has been the formation of a mono-substituted six-membered aromatic ring based on the electrocyclic ring-closure of a hexatriene system. A great advantage of this method is that when using a di-substituted hexatriene with one good leaving group, the cyclization reaction is immediately followed by an elimination, leading directly to the required mono-substituted benzene synthon. A disadvantage, as discussed above, is the fact that the synthesis is linear. Especially when the label is introduced at the beginning of the synthetic scheme, it has to be preserved through many consecutive steps.

Four di-substituted hexatriene systems have been synthesized and cyclized. Upon heating, 7-(dimethylamino)heptatrienenitrile (**3**) and 7-(*N*-methylanilino)heptatrienenitrile (**40**) afforded benzonitrile (**4**), whereas ethyl 7-(dimethylamino)heptatrienoate (**24**) and ethyl 7-(*N*-methylanilino)heptatrienoate (**27**) analogously produced ethyl benzoate (**25**). The yields were determined by gas chromatography of the crude reaction mixtures. Only the cyclizations of **3** and **27** at the optimal temperatures (250°C and 200°C, respectively) were performed on a preparative scale. At 250°C, heptatrienenitriles **3** and **40** afford benzonitrile (**4**) in 85% and 77% yield, respectively, whereas at 200°C the yield of benzonitrile is lower with both compounds. For ethyl heptatrienoates **24** and **27**, however, the yield of ethyl benzoate (**25**) is highest at 200°C (46% and 50%, respectively) whereas on elevation of the temperature the yield diminishes; compounds **24** and **27** are apparently unstable at higher temperatures. Since the cyclization of 7-(dimethylamino)heptatrienenitrile (**3**) (at 250°C) gives the highest yield of mono-substituted benzene, it would be desirable to use **3** for the introduction of stable-isotope labels in benzaldehyde. However, it proved unfeasible to develop a synthesis for **3** by which each position could be labelled with ^{13}C. If labels are required at position 1 or at the carbon atom of the nitrile (ultimately, positions 1′ and 3 of **1**), the best method is to synthesize and cyclize compound **3**, as illustrated by the synthesis of **1a**. If labels are required at the other carbon atoms of the aromatic ring, the method of choice is to use **27**.

For the conversion of benzonitrile (**4**) and ethyl benzoate (**25**) to benzaldehyde, we used the reactions depicted in Schemes 1 and 3, respectively. Benzaldehyde (**5**) was prepared in excellent yields, in one step from **4** (100%) and in two steps from **25** (98%). Reduction of ethyl benzoate (**25**) to benzaldehyde in one step with 1.05 equivalents of DIBAL-H at low temperature was possible, but proceeded in much lower yield (70%) than the two-step procedure.

At this point, it is interesting to note that two new C-2 synthons have been developed, namely 2,2-diphenyloxy-1-ethanal (**33**) and ethyl 2,2-diphenyloxyacetate (**31**). These compounds are easily available from acetic acid, and can now be specifically labelled with ^{13}C at any position or combination of positions. Furthermore, compound **31** can, in principle, be converted in two steps to pyruvaldehyde diphenyl acetal (**39**), a very valuable C-3 synthon, in analogy with the synthesis of pyruvaldehyde dimethyl acetal developed previously by our group.[21]

The method described here is, in principle, suitable for the introduction of deuterium at all positions in L-Phe other

than the 3-position. At this position (the methylene group) deuterium exchange can occur in (sodium) phenylpyruvate (**6**). Enantioselective introduction of deuterium at the 3-position can be achieved by chiral reduction of α-benzoylaminocinnamic acid.[1][26]

Conclusions

An enantioselective total synthesis of **1** has been developed, based on simple starting materials that are commercially available in highly isotopically enriched form. For the synthesis of the required mono-substituted benzene synthon, the very versatile electrocyclic ring-closure of a disubstituted hexatriene system with elimination of one (amino) substituent has been employed. The use of an enzymatic conversion for the introduction of chirality at C-2 ensures a very high degree of enantioselectivity (> 99% *ee*) of the synthesized L-phenylalanines. The value of this method is illustrated by the synthesis of [1′-^{13}C]-L-Phe (**1a**) and [2-D]-L-Phe (**1b**). NMR data showed that these compounds were prepared without any scrambling of the labels. Furthermore, synthons such as benzaldehyde, ethyl benzoate, benzonitrile, and phenol are important precursors for the labelling of other molecules. For instance, L-tyrosine can be labelled by microbiological coupling of isotopically enriched phenol to L-serine, using the bacterium *Erwinia herbicola*. In conclusion, we have now developed methods for labelling all of the aromatic amino acids, i.e. L-phenylalanine, L-tyrosine, L-tryptophan,[27] and L-histidine,[28] at each atomic position or combination of positions.

Experimental Section

Experiments were performed in a dry nitrogen atmosphere except where aqueous conditions were used. Where appropriate, glassware was flame-dried under nitrogen prior to use. Organic solvents were dried either by distillation [MeOH from Mg, EtOH from CaO, ether (diethyl ether), light petroleum (refers to the 40−60 °C fractions), and dichloromethane from P_2O_5] or by storing over molecular sieves (4 Å) for at least 16 h [DMSO, THF, triethylamine (after distillation)]. Solvents were stored over sodium wire (ether and light petroleum) or molecular sieves (3 Å: MeOH; 4 Å: dichloromethane, EtOH, quinoline). Reactions were generally monitored using thin-layer chromatography (TLC, on Merck F_{254} silica gel 60 coated aluminium sheets, 0.2 mm); spots were visualized with UV light (λ = 254 nm). The enzymatic conversion of sodium phenylpyruvate to L-phenylalanine was monitored by measurement of the optical rotation at 436 nm with a Polarimeter 241 from Perkin−Elmer. All solvents were evaporated in vacuo. Column chromatography was performed on Merck silica gel 60 (0.040−0.063 mm, 230−400 mesh). Melting points were measured on a Büchi apparatus and are uncorrected.

$LiAlD_4$ (98% D) and deuterium gas (12 L container, 99.7% D) were obtained from Aldrich and Messer Griesheim, respectively. [2-^{13}C]Acetonitrile (99% ^{13}C) and DCOOH (98% D) were purchased from Cambridge Isotope Laboratories, U.S.A. (Dimethylaminomethyl)diphenylphosphane oxide (**19**) was a kind gift from the group of Prof. Dr. A. van der Gen, Leiden University.[29] Dowex 50 W-X2 (220−400 mesh, H^+ form) cation-exchange resin was obtained from BioRad. Phenylalanine dehydrogenase (PHEDH) was kindly provided by Dr. W. Hummel, Institut für Enzymtechnologie der Heinrich-Heine-Universität Düsseldorf. Formate dehydrogenase (FDH) was produced according to a literature procedure.[30] All other chemicals were purchased from Aldrich, Fluka, or Acros Chimica.

All reactions were optimized using non-labelled material; experimental conditions, and spectral data are described for the unlabelled compounds. For labelled compounds, only the changes relative to their unlabelled counterparts are given.

NMR: ^{1}H-NMR spectra were recorded on a Jeol FX-200, a Bruker WM-300, or a Bruker AM-600 spectrometer with tetramethylsilane (TMS; δ = 0.00) as internal standard for spectra recorded in $CDCl_3$, CD_3OD, and [D_6]DMSO. TSP [3-(trimethylsilyl)tetradeuteriopropionic acid, sodium salt] was used as an internal standard (δ = 0.00) for recording spectra in D_2O solution at pH 7.00 (meter reading). ^{1}H-noise-decoupled ^{13}C-NMR spectra were recorded using a Jeol FX-200 at 50 MHz, a Bruker WM-300 at 75 MHz, or a Bruker AM-600 spectrometer at 151 MHz, with chloroform (δ = 77.0), CD_3OD (δ = 49.0), [D_6]DMSO (δ = 39.5), or TSP (δ = 0.0) as internal standards. 2D-NMR spectra were recorded on a Bruker WM-300 or on a Bruker AM-600 spectrometer.

HPLC: The enantiomeric excesses (at C-2) of the amino acids were determined by HPLC. The apparatus consisted of a C-18 RP Spherisorb ODS-2 (5 μm) column (25 × 4 mm), a Pharmacia LKB gradient delivery system, a Rheodyne injection valve fitted with a 10 μL loop, and a SpectroVision FD-300 fluorescence detector. The amino acids were derivatized using *o*-phthalaldehyde (OPA) and (*R*)-*N*-acetylcysteine (NAC)[31] prior to chromatography. The excitation and emission wavelengths for detection of the OPA-NAC derivatives were 330 and 450 nm, respectively. Derivatization was carried out using a solution of 2 mg phenylalanine in H_2O (I), a 0.2 M borax buffer (pH 10.4) (II), a solution of 15 mg NAC in 0.75 mL of methanol (III), and a solution of 15 mg OPA in 0.75 mL of methanol (IV). 10 μL of I, 30 μL of II, 10 μL of III, and 10 μL of IV were mixed, vortexed for 1 min, and injected. Elution buffers: buffer A: 5% acetonitrile/95% 30 mM NaOAc (pH 4); buffer B: 75% acetonitrile/25% 30 mM NaOAc (pH 7.6); flow rate 0.4 mL/min. For the separation of the diastereomers of phenylalanine, an initial linear elution gradient of 100% A to 70% A/30% B in 10 min was followed by a second linear gradient to 50% A/50% B in 40 min. Retention times: 29 min (L-Phe) and 30 min (D-Phe).

MS: Mass spectra were recorded on a Finnigan MAT 900 mass spectrometer equipped with a direct insertion probe (EI-MS, 70 eV) or on a Finnigan MAT ITD 700 (EI, 70 eV) coupled to a Packard 438A gas chromatograph equipped with a Chrompack 25 m fused silica column (CP-Sil-5CB; 0.25 mm i.d.) (GC-MS). Prior to the measurements, the amino acids were derivatized as their *N*(*O*,*S*)-ethyloxycarbonyl ethyl esters by reaction with ethyl chloroformate (ECF).[32] The sample was dissolved in 200 μL of a mixture of water/ethanol/pyridine (60:32:8) and 10 μL of ECF was added. The tube was vortexed for 5 s, whereupon gas evolution occurred (CO_2 release). A solution of 1% ECF in chloroform (200 μL) was added and the derivatives were extracted into the organic phase by vortexing for 15 s; 10 μL of this organic layer was injected. The enrichments were determined from the experimental isotope patterns of the protonated molecules and are calculated from the contributions of the labelled versus the unlabelled peak intensities. This calculation was performed using a custom-made program that takes the [M − 1] peak into account.

[1′-^{13}C]-L-Phenylalanine (1a): In a small flask, **6a** (112 mg, 0.60 mmol; 0.1 M) was dissolved in 6 mL of a solution containing

1 M ammonium formate, 1 mM of NAD^+, 2 U/mL of formate dehydrogenase (FDH, E.C. 1.2.1.2), and 2 U/mL of phenylalanine dehydrogenase (PHEDH, E.C. 1.4.1.20).[33] The pH was adjusted to 8.5 with ammonium hydroxide and the mixture was stirred at room temperature. The progress of the reaction was monitored by measurement of the optical rotation at 436 nm with a Perkin–Elmer polarimeter 241. When the optical rotation reached a steady state (2 h), the mixture was applied to a 100 mL Duolite C26 H^+ column. The contaminants were removed by elution with 200 mL water and then the product was eluted with 1 M NH_4OH (250 mL). Evaporation of the solvent from the appropriate fractions followed by lyophilization afforded 37 mg of **1a**. Similar treatment of a second batch of 46 mg of **6a** afforded 12 mg of **1a**. Total yield: 49 mg (0.29 mmol, 35%); white powder. Enantiomeric excess (HPLC): > 99%. MS: 97% enrichment. – ^{1}H NMR (600 MHz, D_2O): δ = 3.13 (ddd, 1 H, $^2J_{H3R\text{-}3S}$ = 14.4, $^2J_{13C1'\text{-}H3R}$ = 5.7, $^3J_{H2\text{-}H3R}$ = 8.2 Hz, 3*R*-H), 3.29 (ddd, 1 H, $^2J_{H3R\text{-}3S}$ = 14.4, $^2J_{13C1'\text{-}H3S}$ = 5.6, $^3J_{H2\text{-}H3S}$ = 5.2 Hz, 3*S*-H), 4.00 (dd, 1 H, $^3J_{H2\text{-}H3R}$ = 8.2, $^3J_{H2\text{-}H3S}$ = 5.2 Hz, 2-H), 7.34 (m, 2 H, 2′-/6′-H), 7.39 (m, 1 H, 4′-H), 7.44 (m, 2 H, 3′-/5′-H). – ^{13}C NMR (151 MHz, D_2O): δ = 39.1 (d, $^1J_{C3\text{-}C1'}$ = 43.2 Hz, C-3), 58.8 (C-2), 130.5 (d, $^3J_{C1'\text{-}C4'}$ = 9.4 Hz, C-4′), 131.9 (C-3′/C-5′), 132.2 (d, $^1J_{C1'\text{-}C2'/C6'}$ = 56.6 Hz, C-2′/C-6′), 137.8 (strongly enhanced ^{13}C-signal, C-1′), 176.8 (C-1).

[2-D]-L-Phenylalanine (1b): An Amicon 202 ultrafiltration cell (200 mL) equipped with a YM 10 membrane, cut-off 10000 g/mol, was charged with 100 mL of a solution containing 0.2 M **6**, 0.16 M DCOOH, 1 M NH_4Cl, 1 mM NAD^+, 1 U/mL L-phenylalanine dehydrogenase (PHEDH), and 10 U/mL formate dehydrogenase (FDH). The pH was adjusted to 8.5 with ammonium hydroxide and the solution was incubated at room temperature. The reaction was monitored as described in the case of **1a**. After stirring at room temperature for 1 day, the product solution was removed by ultrafiltration, pressurizing the cell with argon. 100 mL of fresh substrate solution was then added to the remaining enzymes and the procedure was repeated. Processing of six consecutive batches in this manner afforded 600 mL of product solution. The volume was reduced to about 150 mL and the product was purified by cation-exchange column chromatography (800 mL Duolite C26, H^+ column; elution with 3 L water, followed by 3 L 1 M NH_4OH). The column was regenerated by elution with 2 L water, 1.5 L 1 M HCl solution, and 2 L water. The product fractions were collected and the solvent was evaporated under reduced pressure (60°C, 50 mBar). Drying in vacuo afforded 8.93 g (57%) of **1b** with a purity of 97.5% as determined by HPLC. Enantiomeric excess (HPLC): > 99%. MS: 90% enrichment. – ^{1}H NMR (600 MHz, D_2O): δ = 3.13 (dd, 1 H, $^2J_{H3R\text{-}3S}$ = 14.4, $^3J_{H2\text{-}H3R}$ = 8.2 Hz, 3*R*-H), 3.29 (dd, 1 H, $^2J_{H3R\text{-}3S}$ = 14.4, $^3J_{H2\text{-}H3S}$ = 5.2 Hz, 3*S*-H), 7.34 (m, 2 H, 2′-/6′-H), 7.39 (m, 1 H, 4′-H), 7.44 (m, 2 H, 3′-/5′-H). – ^{13}C NMR (151 MHz, D_2O): δ = 39.1 (C-3), 58.8 (t, $^1J_{C2\text{-}D}$ = 22.9 Hz, C-2), 130.5 (C-4′), 131.9 (C-3′/C-5′), 132.2 (C-2′/C-6′), 137.8 (C-1′), 176.8 (C-1).

(2*E*,4*E*,6*E*)-7-(Dimethylamino)heptatrienenitrile (3): A flame-dried three-necked flask fitted with a dropping funnel, a low-temperature thermometer, and a septum cap was charged with diisopropylamine (1.73 mL, 12.3 mmol) and THF (20 mL). The solution was cooled to −20°C, whereupon *n*BuLi solution (7.7 mL, 12.3 mmol) was added by means of a syringe. After stirring for 10 min, the solution was cooled to −80°C and a solution of acetonitrile (0.32 mL, 6.1 mmol) in THF (5 mL) was added dropwise. A white precipitate of lithioacetonitrile slowly formed. After stirring for 15 min at −80°C, a solution of diethyl chlorophosphate (0.89 mL, 6.1 mmol) in THF (5 mL) was slowly added and the mixture was allowed to warm to 0°C over a period of 1 h. 5-Dimethylaminopentadienal (1.0 g of **7**; 8.0 mmol) in THF (10 mL) was then added to the clear yellowish solution and stirring was continued overnight. Workup was accomplished by the addition of 30 mL of water. The THF was evaporated and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined dichloromethane layers were washed with brine, dried with Na_2SO_4, and concentrated to afford the crude triene as a dark oil. Purification was performed by column chromatography (5% triethylamine, 5% methanol in diethyl ether); the silica gel used was pretreated with 10% triethylamine in ether in order to prevent decomposition of the acid-labile product. Yield: 0.41 g (2.8 mmol, 46%) of **3** as a dark solid. Exact mass: 148.0991 (calcd. for $C_9H_{12}N_2$: 148.1001). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.86 (s, 6 H, 2 × NCH_3), 4.88 (d, 1 H, $^3J_{H1\text{-}H2}$ = 15.5 Hz, 2-H), 5.05 (dd, 1 H, $^3J_{H6\text{-}H7}$ = 12.7, $^3J_{H6\text{-}H5}$ = 11.3 Hz, 6-H), 5.89 (dd, 1 H, $^3J_{H4\text{-}H5}$ = 14.2, $^3J_{H4\text{-}H3}$ = 11.4 Hz, 4-H), 6.51 (dd, 1 H, $^3J_{H5\text{-}H4}$ = 14.2, $^3J_{H5\text{-}H6}$ = 11.3 Hz, 5-H), 6.55 (d, 1 H, $^3J_{H7\text{-}H6}$ = 12.7 Hz, 7-H), 6.95 (dd, 1 H, $^3J_{H3\text{-}H2}$ = 15.5, $^3J_{H3\text{-}H4}$ = 11.4 Hz, 3-H). – ^{13}C NMR (151 MHz, $CDCl_3$): δ = 40.4 (NCH_3), 87.4 (C-2), 97.6 (C-6), 117.0 (C-4), 120.8 (CN), 144.5 (C-5), 149.0 (C-7), 152.1 (C-3).

(2*E*,4*E*,6*E*)-[2-^{13}C]-7-(Dimethylamino)heptatrienenitrile (3a): This compound was prepared from [2-^{13}C]acetonitrile (1.00 g, 23.8 mmol) according to the procedure used for the synthesis of **3**; 1.44 g (9.7 mmol, 41%) of **3a** was obtained as a dark solid. Exact mass: 149.1028 (calcd. for $^{12}C_8{}^{13}C_1H_{12}N_2$: 149.1034). – ^{1}H NMR (600 MHz, $CDCl_3$): As for the unlabelled compound with additional signals at δ = 4.88 (dd, 1 H, $^1J_{C-H}$ = 171.0, $^3J_{H2\text{-}H3}$ = 15.5 Hz, 2-H), 5.89 (ddd, $^3J_{C-H}$ = 5.1, $^3J_{H4\text{-}H5}$ = 14.2, $^3J_{H4\text{-}H3}$ = 11.4 Hz, 4-H). – ^{13}C NMR (151 MHz, $CDCl_3$): As for the unlabelled compound with additional signals at δ = 87.4 (C-2), 120.8 ($^1J_{C-C}$ = 82.5 Hz, CN), 152.1 ($^1J_{C-C}$ = 72.1 Hz, C-3).

Benzonitrile (4): In a Carius tube, 0.41 g (2.8 mmol) of **3** was dissolved in 9.0 mL of quinoline. The tube was sealed at low temperature under vacuum and was then allowed to warm to room temperature. Cyclization was performed in a Carius oven at 240°C for 4 h. The tube was then opened at low temperature and the dark-brown solution was transferred to a separatory funnel with diethyl ether. The organic layer was washed with 2.5 M HCl solution (3 × 75 mL) to remove the quinoline. The combined HCl extracts were then extracted three times with diethyl ether, and the combined ethereal layers were washed with brine. Drying with $MgSO_4$, filtration, and evaporation of the solvent afforded a yellow oil, which was purified by column chromatography (30% diethyl ether in light petroleum). Yield: 0.19 g (1.85 mmol, 66%) of **4** as a colourless oil. Exact mass: 103.0423 (calculated for C_7H_5N: 103.0422). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.45–7.50 (m, 2 H, 3-/5-H), 7.59–7.63 (m, 1 H, 4-H), 7.64–7.67 (m, 2 H, 2-/6-H). – ^{13}C NMR (151 MHz, $CDCl_3$): δ = 112.4 (C-1), 118.8 (CN), 129.1 (C-3/C-5), 132.1 (C-2/C-6), 132.7 (C-4).

[1-^{13}C]Benzonitrile (4a): Similarly, **3a** (1.37 g, 9.2 mmol) was cyclized in two batches, affording 0.63 g (6.0 mmol, 66%) of **4a** as a colourless oil. Exact mass: 104.0452 (calcd. for $^{12}C_6{}^{13}C_1H_5N$: 104.0456). – ^{1}H NMR (600 MHz, $CDCl_3$): As for the unlabelled compound but with an additional coupling in the multiplet between δ = 7.45 and 7.50 (3-H/5-H). – ^{13}C NMR (151 MHz, $CDCl_3$): As for the unlabelled compound with additional signals at δ = 112.4 (C-1), 118.8 ($^1J_{C-C}$ = 81.0 Hz, CN), 132.1 ($^1J_{C-C}$ = 60.2 Hz, C-2/C-6), 132.7 ($^3J_{C-C}$ = 11.0 Hz, C-4).

Benzaldehyde (5): *Method (a):* In a 100 mL three-necked flask fitted with a septum inlet, a low-temperature thermometer, and a septum outlet, **4** (0.60 g, 5.8 mmol) was dissolved in dry dichlorometh-

ane (30 mL). The solution was cooled to −70°C, whereupon DIBAL-H (6.4 mL, 6.4 mmol, 1 M solution in hexanes) was added dropwise by means of a syringe. The solution was allowed to warm to −40°C over a period of 1 h. Hydrolysis was effected by slowly adding a homogeneous mixture of 8.6 g silica gel and 2.6 mL water. After stirring for 1 h at 0°C, K_2CO_3 and $MgSO_4$ were added, the solids were filtered off and rinsed thoroughly with dichloromethane and diethyl ether. The solvents were evaporated *carefully* (the product is volatile), yielding 0.68 g (6.4 mmol, 100%) of crude **5**, which was sufficiently pure for use in the next reaction. Exact mass: 106.0387 (calcd. for C_7H_6O: 106.0419). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.50−7.54 (m, 2 H, 3-/5-H), 7.60−7.64 (m, 1 H, 4-H), 7.85−7.89 (m, 2 H, 2-/6-H), 10.01 [s, 1 H, C(O)H]. − ^{13}C NMR (151 MHz, $CDCl_3$): δ = 128.9 (C-3/C-5), 129.6 (C-2/C-6), 134.3 (C-4), 136.3 (C-1), 192.2 [C(O)].

Method (b): To a suspension of MnO_2 (20.9 g, 0.24 mol) in dichloromethane (50 mL) was added a solution of **28** (2.00 mL, 2.09 g, 19.3 mmol) in dichloromethane (5 mL). The resulting mixture was stirred overnight at room temperature and then filtered through Celite. The solids were thoroughly washed with dichloromethane and then the solvent was evaporated from the combined filtrate and washings, affording 2.05 g (19.3 mmol, 100%) of crude **5**, which was sufficiently pure for use in the next step. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.7 (m, 5 H, Ph), 10.01 [s, 1 H, C(O)H]. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 128.5, 129.1, 133.9, 135.9 (Ph), 191.7 [C(O)].

[1-^{13}C]Benzaldehyde (5a): According to the same procedure, reaction of **4a** (0.59 g, 5.7 mmol) afforded 0.61 g (5.7 mmol, 100%) of **5a**. Exact mass: 107.0452 (calcd. for $^{12}C_6{}^{13}C_1H_6O$: 107.0452). − ^{1}H NMR (600 MHz, $CDCl_3$): As for the unlabelled compound, but with an additional signal at δ = 10.01 [d, $^2J_{C-H}$ = 23.8 Hz, C(O)H]. Also, an extra coupling in the multiplet between δ = 7.50 and 7.54 (3-/5-H) was seen. − ^{13}C NMR (151 MHz, $CDCl_3$): As for the unlabelled compound, but with additional signals at δ = 129.6 ($^1J_{C-C}$ = 58.2 Hz, C-2/C-6), 134.3 ($^3J_{C-C}$ = 8.7 Hz, C-4), 136.3 (C-1), 192.2 [$^1J_{C-C}$ = 53.6 Hz, C(O)].

Phenylpyruvate, Sodium Salt (6): A 100 mL three-necked round-bottomed flask fitted with a septum inlet and a dropping funnel was charged with 1.00 g (5.3 mmol) of **10**. A nitrogen atmosphere was introduced in the flask, and 25 mL of a 20% aq. NaOH solution was added. After refluxing in an oil bath at 140°C for 3 h, the resulting light-yellow solution was cooled in an ice bath and carefully neutralized with concentrated HCl (about 10 mL). The mixture was buffered with $NaHCO_3$ (0.53 g) and extracted with diethyl ether in a continuous extractor for 2 h in order to remove any organic impurities. The aqueous layer was acidified with 6.4 mL of 12 M HCl and phenylpyruvic acid was isolated by continuous extraction with diethyl ether for 4.5 h. After evaporation of the solvent, the product was redissolved in water and very carefully neutralized with 1 M aq. NaOH solution. Lyophilization afforded sodium phenylpyruvate as a white powder (0.76 g, 4.1 mmol, 77%), which could be used directly for the enzymatic conversion into L-phenylalanine.

^{1}H-NMR of phenylpyruvic acid (200 MHz, [D$_6$]DMSO): δ = 4.14 (s, CH_2 of keto form), 6.40 (s, CH of enol form), 7.1−7.8 (m, Ph). Integral values depend on the ratio of the keto/enol forms in the NMR sample and were consequently not reproducible.

[1′-^{13}C]Phenylpyruvate, Sodium Salt (6a): Similarly, **10a** (0.36 g, 1.90 mmol) afforded 172 mg of [1′-^{13}C]phenylpyruvate, sodium salt (0.92 mmol, 48%), which was used directly in the enzymatic conversion into [1′-^{13}C]-L-Phe.

(2*E*,4*E*)-5-Dimethylaminopentadienal (7): A suspension of *N*-(2′,4′-dinitrophenyl)pyridinium chloride (50.0 g, 0.178 mol; see below) in 96% ethanol (350 mL) was treated with dimethylamine (16.0 g, 0.356 mol, 40.0 mL of a 40% aq. solution). The resulting dark solution was stirred at 70°C for 30 min. The solvent was then evaporated and the residue was treated with 300 mL of cold water. The precipitated orange dinitroaniline was filtered off and the filtrate was treated with 50 mL of 5 N NaOH solution. The resulting solution was extracted with dichloromethane (3 × 100 mL) and the combined organic layers were dried with Na_2SO_4. Filtration and evaporation of the solvent afforded 16.82 g of crude product, which was purified by recrystallization from cyclohexane. Yield: 11.36 g (90.8 mmol, 51%) of **7** as yellow-brown crystals, m.p. 56−57°C. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.96 (s, 6 H, 2 × CH_3), 5.26 (dd, 1 H, $^3J_{H4-H5}$ = 12.5, $^3J_{H4-H3}$ = 11.7 Hz, 4-H), 5.81 (dd, 1 H, $^3J_{H2-H3}$ = 14.3, $^3J_{H2-H1}$ = 8.4 Hz, 2-H), 6.82 (d, 1 H, $^3J_{H5-H4}$ = 12.5 Hz, 5-H), 7.11 (dd, 1 H, $^3J_{H3-H2}$ = 14.3, $^3J_{H3-H4}$ = 11.7 Hz, 3-H), 9.26 (d, 1 H, $^3J_{H1-H2}$ = 8.4 Hz, 1-H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 40.3 (br, 2 × CH_3), 97.0 (C-4), 119.3 (C-2), 152.6 (C-5), 156.7 (C-3), 192.0 (C-1).

5-Benzalhydantoin (10): A solution of sodium acetate (3.76 g, 45.8 mmol), hydantoin (**9**; 2.90 g, 29.0 mmol), and crude benzaldehyde (2.05 g, 19.3 mmol) in glacial acetic acid (15 mL) was refluxed for 5 h. The hot solution was then poured into 80 mL cold water and allowed to cool to room temperature. The yellow crystals thus formed were filtered off and dried in vacuo at 75°C overnight. Yield: 2.75 g (14.6 mmol, 76%).

To recover unchanged benzaldehyde, the filtrate was neutralized with 10% NaOH and extracted three times with diethyl ether. The combined ether layers were washed with brine and dried with $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified by column chromatography (30% diethyl ether in light petroleum), affording 0.27 g of benzaldehyde (2.5 mmol, 13%). Exact mass: 188.0576 (calcd. for $C_{10}H_8N_2O_2$: 188.0586). − ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 6.40 (s, 1 H, olefinic H), 7.30−7.33 (m, 1 H, 4′-H), 7.36−7.40 (m, 2 H, 3′-/5′-H), 7.58−7.60 (m, 2 H, 2′-/6′-H). − ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 108.5 (olefinic C), 128.0 (C-5), 128.4 (C-4′), 128.8 (C-3′/C-5′), 129.4 (C-2′/C-6′), 133.0 (C-1′), 155.8 (C-2), 165.6 (C-4).

[1′-^{13}C]-5-Benzalhydantoin (10a): Similar treatment of crude **5a** (0.44 g, 4.1 mmol) afforded 0.42 g (2.2 mmol, 54%) of **10a**. 0.18 g of **5a** was recovered from the filtrate (1.7 mmol, 41%). Exact mass: 189.0618 (calcd. for $^{12}C_9{}^{13}C_1H_8N_2O_2$: 189.0619). − ^{1}H NMR (600 MHz, [D$_6$]DMSO): As for the unlabelled compound, but with an additional coupling in the multiplet between δ = 7.36 and 7.40 (3′-/5′-H). − ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 133.0 (C-1′).

(2*E*)-4,4-Dimethoxybutenal (16): A mixture of 15.0 mL (85.8 mmol) fumaraldehyde bis(dimethyl acetal) (**15**) and 1.0 mL of a 5% aqueous H_3PO_4 solution was heated in an oil bath at 90°C. Methanol and water were azeotropically removed by distillation. At 30-min intervals, 1.5 mL aliquots of water were added. After 2 h, the reaction mixture was diluted with dichloromethane and solid $NaHCO_3$ was added. The mixture was then dried with $MgSO_4$, filtered, and concentrated in vacuo. Column chromatography of the residue (20% diethyl ether in light petroleum) afforded 7.40 g (56.9 mmol, 66%) of **16** as a colourless liquid. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.37 (s, 6 H, 2 × OCH_3), 5.07 (d, 1 H, $^3J_{H-H}$ = 3.8 Hz, 4-H), 6.35 (dd, 1 H, $^3J_{H-H}$ = 15.8/7.9 Hz, 2-H), 6.67 (dd, 1 H, $^3J_{H-H}$ = 15.8/3.8 Hz, 3-H), 9.62 (d, 1 H, $^3J_{H-H}$ = 7.9 Hz, 1-H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 52.6 (2 × OCH_3), 100.2 (C-4), 133.7 (C-2), 150.1 (C-3), 192.8 (C-1).

(2*E*,4*E*)-6,6-Dimethoxyhexadienenitrile (17): In a dry three-necked round-bottomed flask fitted with a thermometer, a septum, and a dropping funnel, NaH (1.30 g, 60% dispersion in mineral oil, 32.5 mmol) was washed three times with dry light petroleum. THF (30 mL) was then added and the suspension was cooled to 0°C. Then, diethyl cyanomethylphosphonate (6.10 g, 34.4 mmol) in THF (10 mL) was added dropwise. After a clear solution had been obtained, a solution of of aldehyde **16** (3.00 g, 23.0 mmol) in THF (25 mL) was slowly added at 0°C. After stirring for 30 min, TLC (50% diethyl ether in light petroleum) indicated complete conversion of the aldehyde, and the reaction was quenched by adding 50 mL of saturated NH_4Cl solution. The resulting mixture was extracted with diethyl ether (2 × 100 mL) and the combined organic layers were washed with brine (2 × 100 mL), dried with $MgSO_4$, and filtered. Evaporation of the solvents followed by column chromatography of the residue (20% diethyl ether in light petroleum) yielded 3.05 g (19.9 mmol, 86%) of **17** as a slightly yellow liquid. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.33 (s, 6 H, 2 × OCH_3), 4.92 (d, 1 H, $^3J_{H\text{-}H}$ = 4.1 Hz, 6-H), 5.45 (d, 1 H, $^3J_{H\text{-}H}$ = 16.0 Hz, 2-H), 6.01 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.5, $^3J_{H\text{-}H}$ = 4.1 Hz, 5-H), 6.47 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.5, $^3J_{H\text{-}H}$ = 10.9 Hz, 4-H), 7.03 (dd, 1 H, $^3J_{H\text{-}H}$ = 16.0, $^3J_{H\text{-}H}$ = 10.9 Hz, 3-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 52.7 (OCH_3), 100.7 (C-2), 101.2 (C-6), 117.5 (CN), 130.3 (C-4), 138.6 (C-5), 148.9 (C-3).

(2*E*,4*E*)-5-Formylpentadienenitrile (18): To a solution of **17** (0.65 g, 4.2 mmol) in acetone (20 mL) were added two drops of 1 M aq. HCl. After stirring at room temperature for 70 h, TLC (20% diethyl ether in light petroleum) indicated complete conversion of the starting material. K_2CO_3 and $MgSO_4$ were added, the solids were filtered off and thoroughly rinsed with dichloromethane. Evaporation of the solvents afforded the crude product, which was purified by column chromatography (20% diethyl ether in light petroleum). Yield: 0.33 g (3.1 mmol, 73%) of **18** as a yellow oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.74 (d, 1 H, $^3J_{H\text{-}H}$ = 10.7 Hz, 2-H), 6.45 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.5, $^3J_{H\text{-}H}$ = 7.7 Hz, 5-H), 7.12 (dd, 1 H, $^3J_{H\text{-}H}$ = 11.4, $^3J_{H\text{-}H}$ = 10.7 Hz, 3-H), 7.52 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.5, $^3J_{H\text{-}H}$ = 11.4 Hz, 4-H), 9.77 [d, 1 H, $^3J_{H\text{-}H}$ = 7.7 Hz, C(O)H]. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 105.8 (C-2), 114.8 (CN), 137.5 (C-5), 143.3 (C-4), 145.5 (C-3), 192.8 [C(O)].

(2*E*,4*E*)-Ethyl 6,6-Dimethoxyhexadienoate (22): Compound **22** was synthesized according to the procedure used for the preparation of **17** on a 17.4 mmol scale. Yield: 3.20 g (16.0 mmol, 92%) of **22** as a light-yellow oil. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (t, 3 H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_3), 3.33 (s, 6 H, 2 × OCH_3), 4.21 (q, 2 H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_2), 4.92 (dd, 1 H, $^3J_{H\text{-}H}$ = 4.5, $^4J_{H\text{-}H}$ = 1.4 Hz, 6-H), 5.95 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.4, $^4J_{H\text{-}H}$ = 0.7 Hz, 2-H), 5.99 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.4, $^3J_{H\text{-}H}$ = 4.5 Hz, 5-H), 6.49 (dddd, 1 H, $^3J_{H\text{-}H}$ = 15.4, $^3J_{H\text{-}H}$ = 11.3, $^4J_{H\text{-}H}$ = 1.4, $^4J_{H\text{-}H}$ = 0.7 Hz, 4-H), 7.28 (dd, 1 H, $^3J_{H\text{-}H}$ = 11.3, $^3J_{H\text{-}H}$ = 15.4 Hz, 3-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (CH_3), 52.4 (OCH_3), 60.2 (CH_2), 101.2 (C-6), 123.0 (C-2), 130.8 (C-4), 137.2 (C-5), 142.7 (C-3), 166.3 (C-1).

(2*E*,4*E*)-5-Ethyloxycarbonylpentadienal (23): Compound **38** (0.32 g, 0.99 mmol) was dissolved in acetone (30 mL) and a few drops of 1 N HCl were added. After stirring the solution for 5 h at room temperature, all the starting material had been consumed (TLC: diethyl ether/light petroleum, 1:1). K_2CO_3 and $MgSO_4$ were then added, the solids were filtered off, and the solvent was evaporated. The product was purified by column chromatography (30% diethyl ether in light petroleum), affording 0.15 g (0.97 mmol, 98%) of ethyl 5-formyl-2,4-pentadienoate as a clear light-yellow oil, which crystallised at −20°C. Exact mass: 154.0659 (calcd. for $C_8H_{10}O_3$: 154.0630). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.33 (t, 3 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_3), 4.26 (q, 2 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_2), 6.33 (d, 1 H, $^3J_{H\text{-}H}$ = 15.3 Hz, 2-H), 6.43 (dd, 1 H, $^3J_{H\text{-}H}$ = 7.7/15.4 Hz, 5-H), 7.21 (dd, 1 H, $^3J_{H\text{-}H}$ = 11.3/15.4 Hz, 4-H), 7.44 (dd, 1 H, $^3J_{H\text{-}H}$ = 11.3/15.3 Hz, 3-H), 9.69 [d, 1 H, $^3J_{H\text{-}H}$ = 7.7 Hz, C(O)H]. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.0 (CH_3), 60.9 (CH_2), 129.8 (C-2), 136.8 (C-5), 140.1 (C-3), 147.1 (C-4), 165.2 (C-1), 192.8 (CO).

(2*E*,4*E*,6*E*)-Ethyl 7-(Dimethylamino)heptatrienoate (24): In a flame-dried three-necked round-bottomed flask fitted with a low-temperature thermometer, a septum cap, and a dropping funnel, (dimethylaminomethyl)diphenylphosphane oxide (1.20 g, 4.6 mmol) was dissolved in dry THF (25 mL). The solution was cooled to −40°C, whereupon *n*BuLi solution (2.70 mL, 1.6 M in hexane, 4.3 mmol) was slowly added by means of a syringe. The resulting clear orange-red solution was cooled to −78°C, and then a solution of **23** (0.55 g, 3.6 mmol) in THF (15 mL) was added dropwise. After stirring at −78°C for 3.5 h, TLC (50% diethyl ether in light petroleum) indicated complete conversion of the aldehyde. Workup was accomplished by the addition of 50 mL water and neutralization with saturated NH_4Cl solution. The THF was removed and the mixture was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The residual mixture was purified by column chromatography (eluent: diethyl ether) affording 1.48 g of the crude adduct, which was treated at room temperature for 3 h with KO*t*Bu (0.60 g, 5.3 mmol) in dry THF (50 mL). Brine (50 mL) was then added and the aqueous layer was extracted three times with diethyl ether. The combined organic layers were washed with brine (2 × 100 mL) and dried with Na_2SO_4. After filtration and evaporation of the solvent, the product was purified by column chromatography (eluent: 5% triethylamine in diethyl ether; the silica gel used was pretreated with 20% triethylamine in ether in order to prevent decomposition of the acid-labile product). Yield: 0.32 g (1.7 mmol, 46%) of **24** as a dark-yellow solid. Exact mass: 195.1270 (calcd. for $C_{11}H_{17}NO_2$: 195.1259). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (t, 3 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_3), 2.83 (s, 6 H, 2 × NCH_3), 4.16 (q, 2 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_2), 5.08 (dd, 1 H, $^3J_{H6\text{-}H7}$ = 12.8, $^3J_{H6\text{-}H5}$ = 11.2 Hz, 6-H), 5.57 (d, 1 H, $^3J_{H2\text{-}H3}$ = 15.0 Hz, 2-H), 5.96 (dd, 1 H, $^3J_{H4\text{-}H5}$ = 14.3, $^3J_{H4\text{-}H3}$ = 11.5 Hz, 4-H), 6.49 (d, 1 H, $^3J_{H7\text{-}H6}$ = 12.8 Hz, 7-H), 6.56 (dd, 1 H, $^3J_{H5\text{-}H4}$ = 14.3, $^3J_{H5\text{-}H6}$ = 11.2 Hz, 5-H), 7.32 (dd, 1 H, $^3J_{H3\text{-}H2}$ = 15.0, $^3J_{H3\text{-}H4}$ = 11.4 Hz, 3-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.3 (CH_3), 40.3 (NCH_3), 59.4 (CH_2), 98.2 (C-6), 112.3 (C-2), 118.0 (C-4), 143.8 (C-5), 147.1 (C-3), 147.8 (C-7), 168.2 (C-1).

Ethyl Benzoate (25): In a Carius tube, compound **27** (0.21 g, 0.82 mmol) was dissolved in 3.0 mL quinoline. The tube was sealed at low temperature under vacuum and was then allowed to warm to room temperature. Cyclization was performed in a Carius oven at 200°C for 4 h. After opening of the tube at low temperature, the dark-brown solution was transferred to a separatory funnel with diethyl ether. The organic layer was washed with 2.5 M HCl solution (3 × 25 mL) to remove the quinoline. The combined HCl layers were extracted three times with diethyl ether, and the combined ethereal layers were washed with brine. Drying with $MgSO_4$, filtration, and evaporation of the solvent afforded a yellow oil, which was purified by column chromatography (eluent: dichloromethane). Yield: 49 mg (0.33 mmol, 40%) of **25** as a colourless oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.40 (t, 3 H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_3), 4.38 (q, 2 H, $^3J_{H\text{-}H}$ = 7.2 Hz, CH_2), 7.3−8.1 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.9 (CH_3), 60.4 (CH_2), 127.9, 129.1, 130.1, 132.3 (Ph), 165.9 [C(O)].

(*N*-Methylanilinomethyl)diphenylphosphane Oxide (26): To a solution of **29** (8.28 g, 50.1 mmol) in dry THF (50 mL) chlorodiphenyl-

phosphane (9.00 mL, 50.1 mmol) was added dropwise with the temperature being maintained below 50°C. After stirring for 30 min, 5.0 g of K_2CO_3 was added and the mixture was stirred for a further 15 min. It was then poured into 500 mL of cyclohexane under stirring vigorously. The product was filtered off and dried overnight in vacuo. Recrystallization from ethyl acetate afforded 14.5 g (45.1 mmol, 90%) of **26** as white crystals, m.p. 118−119°C (ref.: 118−119°C[9]). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.93 (s, 3 H, NCH_3), 4.19 (d, 2 H, $^2J_{P-H}$ = 3.8 Hz, CH_2), 6.6−7.2 (m, 5 H, NPh), 7.4−7.9 (m, 10 H, 2 × PPh). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 39.9 (NCH_3), 55.3 (d, $^1J_{P-H}$ = 83.5 Hz, CH_2), 113.4, 117.8 (NPh), 128.6 (d, $^3J_{P-C}$ = 11.7 Hz, C-3″/C-5″), 128.9 (NPh), 131.3 (d, $^2J_{P-C}$ = 10.2 Hz, C-2″/C-6″), 131.6 (d, $^1J_{P-C}$ = 93.8 Hz, C-1″), 132.0 (C-4″), 149.9 (NPh).

(2*E*,4*E*,6*E*)-Ethyl 7-(*N*-Methylanilino)heptatrienoate (27): Similarly, compound **27** was synthesized on a 2.6 mmol scale from (*N*-methylanilinomethyl)diphenylphosphane oxide (**26**) and aldehyde **23**. Yield: 0.32 g (1.25 mmol, 48%) of **27** as an orange solid. Exact mass: 257.1435 (calcd. for $C_{16}H_{19}NO_2$: 257.1416). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (t, 3 H, $^3J_{H-H}$ = 7.1 Hz, CH_3), 3.18 (s, 3 H, NCH_3), 4.17 (q, 2 H, $^3J_{H-H}$ = 7.1 Hz, CH_2), 5.49 (dd, 1 H, $^3J_{H6-H7}$ = 13.1, $^3J_{H6-H5}$ = 11.1 Hz, 6-H), 5.68 (d, 1 H, $^3J_{H2-H3}$ = 15.0 Hz, 2-H), 6.10 (dd, 1 H, $^3J_{H4-H5}$ = 14.3, $^3J_{H4-H3}$ = 11.4 Hz, 4-H), 6.6−6.7 (m, 1 H, 5-H), 6.95−7.05 (m, 4 H, Ph and 7-H), 7.36 (dd, 1 H, $^3J_{H3-H2}$ = 15.0 Hz, $^3J_{H3-H4}$ = 11.4 Hz, 3-H), 7.25−7.35 (m, 2 H, Ph). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.3 (CH_3), 35.6 (NCH_3), 59.6 (CH_2), 103.6 (C-6), 114.7 (C-2), 118.4 (Ph), 121.4 (C-4), 122.6, 129.2 (Ph), 141.5 (C-7), 142.3 (C-5), 146.2 (C-3), 146.5 (Ph), 167.8 (C-1).

Benzyl Alcohol (28): To a cooled suspension of $LiAlH_4$ (0.80 g, 21.0 mmol) in dry diethyl ether (100 mL) a solution of **25** (2.10 g, 2.00 mL, 14.0 mmol) in diethyl ether (15 mL) was added dropwise. After stirring at room temperature for 5 min, TLC analysis (30% diethyl ether/light petroleum) showed complete conversion of the starting material. The excess $LiAlH_4$ was destroyed by the addition of 15 mL of ethyl acetate, followed by the dropwise addition of 15 mL of 10% sulfuric acid and 15 mL of 30% sulfuric acid. Stirring was continued until all the solids had dissolved, and then the layers were separated. The aqueous layer was extracted three times with diethyl ether, and the combined organic layers were washed with brine and dried with $MgSO_4$. Filtration and evaporation of the solvent afforded a crude oil, which was purified by column chromatography (50% diethyl ether in light petroleum). Yield: 1.48 g (13.7 mmol, 98%) of **28** as a colourless oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.45 (s, 2 H, CH_2), 7.2 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 64.5 (CH_2), 126.7, 127.1, 128.2, 140.6 (Ph).

***N*-Ethyloxymethyl-*N*-methylaniline (29):** A 100 mL round-bottomed flask fitted with a Dean−Stark trap and a condenser cooler was charged with *N*-methylaniline (10.0 mL, 92.2 mmol) and toluene (25 mL). Paraformaldehyde (2.77 g, 92.2 mmol) and dry ethanol (25 mL) were added and the mixture was refluxed for 6 h. The product was purified by distillation under reduced pressure. Yield: 11.0 g (66.6 mmol, 72%) of **29** as a colourless oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.19 (t, 3 H, $^3J_{H-H}$ = 7.2 Hz, CH_2CH_3), 3.04 (s, 3 H, NCH_3), 3.46 (q, 2 H, $^3J_{H-H}$ = 7.2 Hz, OCH_2CH_3), 4.76 (s, 2 H, NCH_2), 6.7−7.3 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 15.1 (CH_2CH_3), 38.3 (NCH_3), 62.6 (OCH_2CH_3), 83.6 (NCH_2), 113.1, 117.9, 128.9, 148.3 (Ph).

Ethyl Dibromoacetate (30): A dry 250 mL three-necked flask fitted with a condenser and a dropping funnel was charged with acetic acid (5.25 g, 5.0 mL, 87.3 mmol). Phosphorus tribromide (94.5 g, 0.349 mol) was then added and the mixture was cooled to 0°C, whereupon bromine (140 g, 0.876 mol) was added dropwise. The reaction mixture was then refluxed in an oil bath at 170°C for 120 h. After cooling to 0°C, 150 mL of dry ethanol was carefully added and the solution was stirred at room temperature for 1 h. The reaction mixture was quenched with water (200 mL) at 0°C and the aqueous layer was extracted with diethyl ether (3 × 250 mL). The combined organic layers were adjusted to pH 4 with solid $NaHCO_3$ and then washed with saturated $NaHCO_3$ solution (2×), saturated $Na_2S_2O_3$ solution (2×), water (2×), brine, and finally dried with $MgSO_4$. After filtration and evaporation of the solvent, the crude product was purified by column chromatography (eluent: light petroleum, then 30% diethyl ether in light petroleum), affording 18.0 g (73.2 mmol, 84%) of **30** as a colourless oil. Exact mass: 243.8750 (calcd. for $C_4H_6Br_2O_2$: 243.8735). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.35 (t, 3 H, $^3J_{H-H}$ = 7.2 Hz, CH_3), 4.33 (q, 2 H, $^3J_{H-H}$ = 7.2 Hz, CH_2), 5.84 (s, 1 H, 2-H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.7 (CH_3), 32.5 (C-2), 63.5 (CH_2), 164.5 (C-1).

Ethyl Diphenyloxyacetate (31): Potassium hydride (7.50 g, 35% suspension in mineral oil, 65.4 mmol) was washed three times with dry light petroleum. Then, 80 mL of dry DMSO was carefully added, and the resulting suspension was stirred at room temperature until the potassium hydride had completely dissolved. A solution of phenol (6.79 g, 72.1 mmol) in DMSO (40 mL) was then added and the resulting mixture was stirred for 15 min at 50°C. A solution of ethyl dibromoacetate (**30**) (8.01 g, 32.6 mmol) in DMSO (100 mL) was then added dropwise over a period of 2 h. TLC analysis (20% diethyl ether in light petroleum) showed complete conversion of the starting material. Water (150 mL) was then added, and the mixture was adjusted to pH 8 with saturated NH_4Cl solution. The aqueous layer was extracted with diethyl ether (3 × 200 mL), and the combined organic layers were washed with brine (2 × 75 mL) and dried with Na_2SO_4. Filtration and evaporation of the solvent afforded 8.13 g of crude material, of which 3.56 g was purified by column chromatography (20% diethyl ether in light petroleum). Yield: 1.98 g (7.4 mmol, 52%) of **31** as a clear oil. Exact mass: 272.1064 (calcd. for $C_{16}H_{16}O_4$: 272.1049). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.30 (t, 3 H, $^3J_{H-H}$ = 7.2 Hz, CH_3), 4.30 (q, 2 H, $^3J_{H-H}$ = 7.2 Hz, CH_2), 6.03 (s, 1 H, 2-H), 7.2 (m, 10 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.0 (CH_3), 62.2 (CH_2), 96.3 (C-2), 117.4 (C-2′/C-6′, C-3′/C-5′), 123.3 (C-4′), 129.5 (C-3′/C-5′, C-2′/C-6′), 155.6 (C-1′), 165.9 [C(O)].

2,2-Diphenyloxyethanol (32): To a cooled suspension of $LiAlH_4$ (0.85 g, 22.4 mmol) in dry diethyl ether (75 mL) a solution of crude **31** (4.54 g) in dry diethyl ether (20 mL) was added dropwise. After stirring at room temperature for 30 min, all the starting material had been consumed (TLC: 20% diethyl ether in light petroleum). Workup was accomplished by the subsequent addition of ethyl acetate (10 mL), diethyl ether (30 mL), and water (50 mL). The resulting mixture was neutralized with saturated NH_4Cl solution, the layers were separated, and the aqueous layer was extracted three times with diethyl ether. The combined organic layers were washed with brine and dried with Na_2SO_4. After filtration and evaporation of the solvent, column chromatography (40% diethyl ether in light petroleum) afforded 2.31 g (10.0 mmol, 55% yield based on ethyl dibromoacetate) of pure **32** as white crystals, m.p. 37−38°C. Exact mass: 230.0925 (calcd. for $C_{14}H_{14}O_3$: 230.0943). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.97 (d, 2 H, $^3J_{H-H}$ = 5.2 Hz, 1-H), 5.87 (t, 1 H, $^3J_{H-H}$ = 5.2 Hz, 2-H), 7.1 (m, 10 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 63.2 (C-1), 100.8 (C-2), 117.6 (C-2′/C-6′, C-3′/C-5′), 122.9 (C-4′), 129.6 (C-3′/C-5′, C-2′/C-6′), 156.1 (C-1′).

(2*E*)-Ethyl 4,4-Diphenyloxybutenoate (35): In a 250 mL three-necked flask fitted with a dropping funnel, a low-temperature thermometer and a septum, a solution of oxalyl chloride (1.13 mL, 1.64 g, 13.0 mmol) in dry THF (20 mL) was cooled to −60°C. DMSO (1 mL, 1.10 g, 14.0 mmol) in THF (5 mL) was then added over a period of 5 min. The solution was stirred at −30°C for 5 min and then cooled to −60°C once more. A solution of compound **32** (2.50 g, 10.9 mmol) in THF (20 mL) was then added over a period of 5 min affording a white suspension, which was slowly warmed to −30°C. After stirring at this temperature for 30 min, the mixture was cooled to −60°C and dry triethylamine (7.6 mL, 5.52 g, 54.5 mmol) was added. The resulting suspension was allowed to warm to room temperature and then stirred for 2 h. Meanwhile, in a second 100 mL three-necked flask connected to the first by a cannula, sodium hydride dispersion (0.868 g, 60% in mineral oil, 0.52 g NaH, 21.7 mmol) was washed three times with dry light petroleum. THF (20 mL) was added and the suspension was cooled to 0°C. A solution of triethyl phosphonoacetate (4.73 mL, 5.34 g, 23.8 mmol) in THF (5 mL) THF was then added dropwise at 0°C and the resulting solution was stirred at room temperature for at least 15 min.

After 2 h at room temperature, the oxidation mixture was cooled to −80°C. By applying nitrogen pressure and lowering the cannula into the solution, the phosphonate anion was transferred into the first flask. The resulting slightly yellow suspension was allowed to slowly warm to room temperature and then stirred overnight. Workup was accomplished by the addition of 100 mL of diethyl ether and 100 mL of water; the layers were separated and the aqueous layer was extracted with diethyl ether (2 × 75 mL). The combined organic layers were washed with brine (3 × 75 mL) and dried with Na_2SO_4. After filtration and removal of the solvent, purification of the residue by column chromatography (eluent: dichloromethane/light petroleum, 1:1) afforded 3.07 g (10.3 mmol, 94%) of **35** containing 6% of the (*Z*)-isomer. The (*E*)-isomer was further purified by column chromatography and was obtained as a white solid, m.p. 45−46°C. Exact mass: 298.1194 (calcd. for $C_{18}H_{18}O_4$: 298.1205). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, 3 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_3), 4.20 (q, 2 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_2), 6.26 (dd, 1 H, $^3J_{H\text{-}H}$ = 4.1, $^4J_{H\text{-}H}$ = 1.4 Hz, 4-H), 6.34 (dd, 1 H, $^3J_{H\text{-}H}$ = 15.75, $^4J_{H\text{-}H}$ = 1.4 Hz, 2-H), 7.0−7.3 (m, 11 H, Ph and 3-H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.2 (CH_3), 60.9 (CH_2), 98.1 (C-4), 117.7, 123.1 (Ph), 125.5 (C-2), 129.7 (Ph), 141.2 (C-3), 155.7 (Ph), 165.5 (C-1).

(2*E*)-4,4-Diphenyloxybuten-1-ol (36): A solution of of **35** (1.2 g, 4.0 mmol) in dry THF (80 mL) was cooled to −70°C. DIBAL-H (12 mL, 12.0 mmol, 1 M solution in hexanes) was then added by means of a syringe and the solution was allowed to warm to room temperature. TLC analysis (2% MeOH in dichloromethane) revealed complete conversion of the starting material. Hydrolysis was effected by the addition of 50 mL of wet diethyl ether, followed by 3 mL of water, 3 mL of 10% aq. NaOH solution, and 3 g of Na_2SO_4. The resulting suspension was stirred for 30 min, filtered through Celite and washed thoroughly with diethyl ether. The filtrate was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (2% MeOH in dichloromethane), affording 1.01 g (3.9 mmol, 98%) of **36** as a colourless oil. Exact mass: 256.1097 (calcd. for $C_{16}H_{16}O_3$: 256.1099). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.13 (m, 2 H, 1-H), 6.00 (m, 1 H, 2-H), 6.16 (m, 2 H, 3-/4-H), 6.9−7.3 (m, 10 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 61.9 (C-1), 99.2 (C-4), 117.4, 122.5 (Ph), 125.7 (C-2), 129.4 (Ph), 135.0 (C-3), 155.8 (Ph).

(2*E*,4*E*)-Ethyl 6,6-Diphenyloxyhexadienoate (38): This compound was prepared from **36** on a 2.9 mmol scale in analogy to the preparation of compound **35**; 5.3 equiv. triethyl phosphonoacetate and 5 equiv. NaH were used. After stirring overnight at room temperature, a red solution was obtained. The compound was purified by column chromatography (eluent: 2% triethylamine, 48% diethyl ether in light petroleum). Yield: 0.85 g (2.6 mmol, 91%) of **38** as a light-yellow oil. Exact mass: 324.1343 (calcd. for $C_{20}H_{20}O_4$: 324.1362). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.27 (t, 3 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_3), 4.19 (q, 2 H, $^3J_{H\text{-}H}$ = 7.1 Hz, CH_2), 5.97 (ddd, 1 H, $^3J_{H2\text{-}H3}$ = 15.4, $^4J_{H2\text{-}H4}$ = 0.9, $^5J_{H2\text{-}H5}$ = 0.8 Hz, 2-H), 6.21 (dd, 1 H, $^3J_{H6\text{-}H5}$ = 4.5, $^4J_{H6\text{-}H4}$ = 1.0 Hz, 6-H), 6.26 (dddd, 1 H, $^3J_{H5\text{-}H4}$ = 15.4, $^3J_{H5\text{-}H6}$ = 4.5, $^4J_{H5\text{-}H3}$ = 0.8, $^5J_{H5\text{-}H2}$ = 0.8 Hz, 5-H), 6.64 (dddd, 1 H, $^3J_{H4\text{-}H5}$ = 15.4, $^3J_{H4\text{-}H3}$ = 11.1, $^4J_{H4\text{-}H6}$ = 1.0, $^4J_{H4\text{-}H2}$ = 0.9 Hz, 4-H), 6.9−7.3 (m, 10 H, Ph), 7.27 (ddd, 1 H, $^3J_{H3\text{-}H4}$ = 11.1, $^3J_{H3\text{-}H2}$ = 15.4, $^4J_{H3\text{-}H5}$ = 0.8 Hz, 3-H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (CH_3), 60.4 (CH_2), 98.7 (C-6), 117.6, 122.8 (Ph), 124.1 (C-2), 129.5 (Ph), 131.7 (C-4), 135.6 (C-5), 142.2 (C-3), 155.6 (Ph), 166.3 (C-1).

***N*-Methoxy-*N*-methylbenzamide (53):** A suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (3.41 g, 35.0 mmol) in dry THF (100 mL) was cooled to −20°C. To this, *n*BuLi solution (43.8 mL, 70.0 mmol) was added dropwise by means of a syringe and the mixture was stirred until all the salt had dissolved. To the resulting yellow solution, ethyl benzoate (**25**; 1.00 mL, 7.0 mmol) dissolved in THF (5 mL) was slowly added and the mixture was allowed to warm to room temperature. After stirring for 3 h, TLC analysis (50% diethyl ether in light petroleum) indicated complete conversion of the starting material. Water (25 mL) was then added and the aqueous layer was extracted with diethyl ether (3 × 75 mL). The combined organic layers were washed with brine and dried with $MgSO_4$. After filtration and evaporation of the solvent, the crude product was purified by column chromatography (50% diethyl ether in light petroleum). Yield: 1.10 g (6.7 mmol, 96%) of **53** as a colourless oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.31 (s, 3 H, OCH_3), 3.51 (s, 3 H, NCH_3), 7.3−7.7 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 33.2 (NCH_3), 60.5 (OCH_3), 127.5, 130.0, 133.7 (Ph), 169.3 (CO).

Acetophenone (54): *From N-Methoxy-N-methylbenzamide:* A solution of **53** (0.75 g, 4.5 mmol) in dry THF (50 mL) was cooled to −20°C. MeLi (3.1 mL, 5.0 mmol, 1.6 M solution in diethyl ether) was then added dropwise by means of a syringe, resulting in a colour change (yellow to red). After stirring for 1 h at 0°C, a further 0.8 mL (1.4 mmol) of MeLi was added. Stirring was continued at 0°C for 30 min, after which TLC analysis (50% diethyl ether in light petroleum) indicated complete conversion of the starting material. A homogeneous suspension of 6.3 g silica gel and 2.6 mL water was added and the suspension was stirred at 0°C for 2 h. $MgSO_4$ was then added and the solids were filtered off and thoroughly rinsed with diethyl ether. After evaporation of the solvent, the product was purified by column chromatography (50% diethyl ether in light petroleum), affording 0.48 g (4.0 mmol, 89%) of pure **54** as a clear oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.59 (s, 3 H, CH_3), 7.4−8.0 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 26.1 (CH_3), 127.8, 128.1, 132.6, 136.6 (Ph), 197.4 (CO).

From Benzonitrile: A 100-mL flame-dried three-necked flask fitted with a dropping funnel, a septum inlet, and a condenser cooler was charged with MeLi (30.6 mL, 49.0 mmol, 1.6 M solution in diethyl ether) and dry THF (15 mL). The solution was cooled to 0°C, whereupon a solution of benzonitrile (1.00 mL, 9.8 mmol) in THF (10 mL) was added dropwise resulting in a red colouration. After 30 min, all of the starting material had been consumed, and then

12 mL of 3 M H_2SO_4 was carefully added. The mixture was stirred at 50°C for 3 h and subsequently extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with brine and dried with $MgSO_4$. Filtration and removal of the solvent afforded a light-yellow oil, which was purified by column chromatography (50% diethyl ether in light petroleum). Yield: 0.83 g (6.9 mmol, 70%) of acetophenone. ^{1}H- and ^{13}C-NMR spectra: As above.

Phenyl Acetate (55): A solution of **54** (1.0 mL, 8.6 mmol) and of *m*-chloroperbenzoic acid (3.17 g, 12.9 mmol; 70−75%) in water (25 mL) was stirred at 80°C for 2 h. The reaction mixture was allowed to cool and then extracted with dichloromethane (3 × 30 mL). The combined organic layers were subsequently washed with saturated Na_2SO_4 solution, saturated $NaHCO_3$ solution, and brine, and finally dried with $MgSO_4$. Filtration and evaporation of the solvent afforded 2.09 g (15.3 mmol, 89%) of **55** as a colourless oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.21 (s, 3 H, CH_3), 7.0−7.4 (m, 5 H, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.8 (CH_3), 121.3, 125.5, 129.2, 150.5 (Ph), 169.1 (CO).

Phenol (56): In a 250 mL flame-dried three-necked flask fitted with a dropping funnel, sodium (0.73 g, 31.2 mmol) was dissolved in dry methanol (100 mL). A solution of **55** (2.0 mL, 15.8 mmol) in dry methanol (10 mL) was then added dropwise and the resulting mixture was stirred at room temperature. After 15 min, TLC analysis indicated complete conversion of the starting material. The solvents were evaporated in vacuo and the remaining solid was dissolved in water. The aqueous layer was neutralized with 1 M HCl and extracted with dichloromethane (4 ×50 mL). The combined organic layers were dried with $MgSO_4$, filtered, and the solvent was evaporated, affording 1.48 g (15.7 mmol, 99%) of phenol. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.8−7.3 (m, Ph). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 115.3, 120.9, 129.7, 155.1 (Ph).

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