JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 127–138.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.805

Research Article

Synthesis of carbon-14 and stable isotope labelled NN414: a potent potassium channel opener

Steen K. Johansen*, Jesper B. Kristensen, Lars K. Müller, Claus U. Jessen and Christian Foged
Novo Nordisk A/S, Novo Nordisk Park, Måløv DK-2760, Denmark

Summary

Currently, NN414, a potent β-cell selective potassium channel opener, is undergoing clinical trials for the treatment of type 2 diabetes. Here, we report the synthesis of carbon-14 and stable isotope labelled NN414 for use in metabolic studies and as an internal standard in pharmacokinetic assays, respectively. The carbon-14 labelling was performed in two steps starting from an advanced intermediate. This provided [^{14}C]NN414 in 60% overall radiochemical yield with a specific activity of 58 mCi/mmol. The stable isotope labelling was accomplished from benzyl *tert*-butyl malonate in eight steps using [^{13}C,$^{2}H_3$]iodomethane and [$^{2}H_2$]dibromomethane as the source of carbon-13/deuterium. The synthetic sequence, which included a Mannich reaction followed by deamination, a Simmons–Smith-type cyclopropanation and a modified Curtius reaction, provided [^{13}C,$^{2}H_5$]NN414 in 8.6% overall yield. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: carbon-13; carbon-14; deuterium; Mannich reaction; Simmons–Smith cyclopropanation; Curtius reaction

Introduction

Type 2 diabetes is the most prevalent type of diabetes mellitus, a complex metabolic disease affecting a significant proportion of people worldwide.[1] Among other things, type 2 diabetes is characterized by insulin resistance in the peripheral tissue. Owing to the lowered response to insulin, the pancreatic β-cells responsible for production of insulin are stressed in order to produce more of the hormone. This can ultimately lead to the death of the β-cells and the onset of type 1 diabetes.

It has been shown that potassium channel openers[2] such as diazoxide (Figure 1) induce a resting state in the β-cells, thereby prolonging the life of

*Correspondence to: S. K. Johansen, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.
E-mail: skjo@novonordisk.com

Received 2 October 2003
Revised 11 November 2003
Accepted 13 November 2003

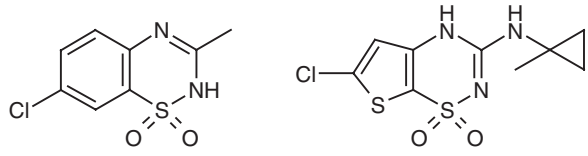


Figure 1. Structure of diazoxide and NN414

these cells and postponing the time by which treatment with insulin is necessary.[3]

NN414[4] (Figure 1) is a potent β-cell selective potassium channel opener, which is currently undergoing clinical trials for the treatment of type 2 diabetes. In order to study the metabolic properties of NN414, a carbon-14 radiolabelled form of the compound was required for use in absorption, distribution, metabolism and excretion (ADME) studies. A stable isotope labelled form of the compound was also desirable for use as an internal analytical standard in pharmacokinetic assays. Here we report the labelling of NN414 with carbon-14 and various stable isotopes.[5]

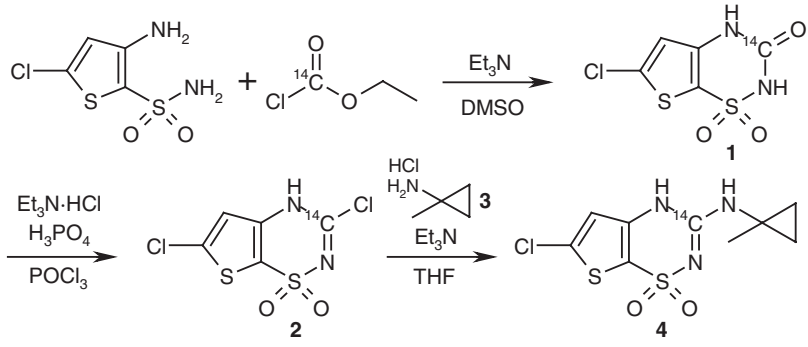
Results and discussion

Carbon-14 labelling

From a metabolic point of view, it is advantageous to have the carbon-14 label located in the heterocyclic part of NN414, as the methylcyclopropyl moiety is susceptible to elimination *in vivo*. Therefore, we started the synthesis of carbon-14 labelled NN414 from the thiadiazine derivate **1**, which can be prepared by coupling of 3-amino-5-chlorothiophene-2-sulfonamide and ethyl [^{14}C]chloroformate with triethylamine in DMSO. This was performed by the authors for the first preparation of [^{14}C]NN414 with low specific activity and subsequently performed at Amersham Biosciences, Cardiff, UK.

Direct amination of thiadiazine derivate **1** with alkylamines is possible but was not viewed as a suitable approach, as the yields are low.[4] Instead, we decided to introduce a more suitable leaving group at C-3 in order to facilitate coupling with the amine. This was accomplished by chlorination of **1** with triethylamine hydrochloride in activated phosphoryl chloride,[6] which provided the chloride **2** in quantitative yield with a radiochemical purity of 90% (Scheme 1).

Generally, the nucleophilic aromatic substitution of the chloride **2** gives the best yields with simple unbranched amines, whereas bulky amines give low yields due to incomplete conversion.[4] This was initially also true in our hands, as the reaction with 1-methylcyclopropyl-1-amine (**3**) consistently stopped at



Scheme 1. Synthesis of [^{14}C]NN414 (4)

30–40% conversion of starting material when running the reaction with triethylamine in ethanol even when using extended reaction times and high temperatures.

The reaction can be improved by switching to a better leaving group such as the fluoride. Thus, the reported yield of NN414 is increased from 26 to 54% using the fluoride instead of the chloride.[4] However, this would introduce an additional step to the synthesis and was not viewed as optimal.

Instead, we screened a number of solvents and bases hoping to optimize the reaction conditions. This proved successful, and we found that optimal conditions could be achieved by switching the solvent from ethanol to THF and keeping triethylamine as the base. These conditions gave complete conversion of starting material although extended reaction times and high temperatures were still necessary, and [^{14}C]NN414 (**4**) was obtained in 60% overall radiochemical yield after purification by HPLC. The chemical and radiochemical purities were found to be >98% and the specific activity was found to be 58 mCi/mmol by mass spectroscopy.

Stable isotope labelling

The use of an internal standard is important in the determination and quantification of analytical samples in pharmacokinetic studies, as it provides a simple and accurate analytical method. Consequently, the choice of the internal standard is equally important as properties such as solubility, extraction and chromatographic behaviour and stability can affect the assay. Generally, it is acknowledged that a stable isotope labelled version of the drug candidate provides the ideal standard for such an assay. This is due to the standard having identical properties in all these aspects, while at the same time, the difference in molecular weight makes it distinguishable in LC/MS assays. Normally, a three mass unit difference between the parent compound and the isotope labelled standard is sufficient to obtain good separation

between drug candidate and standard in the assay. However, in the case of NN414, a five mass unit difference is required due to the presence of a chlorine atom in the NN414 and the fact that naturally occurring chlorine is a mixture of chlorine-35 and chlorine-37.

Looking at the structure of NN414, it quickly became clear that introduction of five extra mass units in the heterocyclic part of the molecule would not be feasible. Therefore, we were left with the task of introducing these into the methylcyclopropylamine moiety. While alkylation of cyclopropanecarboxylic acid with carbon-13/deuterium labelled iodomethane could give access to 1-methyl-1-cyclopropylcarboxylic acid labelled with four extra mass units we dismissed this approach as no obvious way exists of introducing the last extra mass unit.

Instead, we decided to go back one step and synthesize the cyclopropyl moiety according to the retrosynthetic sequence shown in Figure 2. Starting from a suitable protected malonic acid **A**, the first four extra masses would be introduced by alkylation with [^{13}C,$^{2}H_3$]iodomethane. After removal of one of the protecting groups, a Mannich reaction with diethylamine and formaldehyde followed by elimination *in situ* would give access to the acrylic ester **B**. A standard copper/zinc catalysed Simmons–Smith reaction would then produce the cyclopropyl moiety and simultaneously introduce an additional two mass units (from [$^{2}H_2$]dibromomethane) to give **C**. Finally, removal of the second protecting group followed by a modified Curtius reaction would lead to the desired product, 1-methyl-1-cyclopropylamine **D**.

A number of reports exist in the literature on the preparation of deuterium labelled methacrylate esters starting from malonic esters.[7] However, we felt that there was still room for improvement concerning three important aspects. First of all, the reported yields for the three steps leading to the methacrylate ester could be improved (40–42%). Also, due to the lack of functional groups, these compounds are very difficult to visualize either by using UV-detection or by various TLC reagents, and therefore the introduction of a UV-absorbing

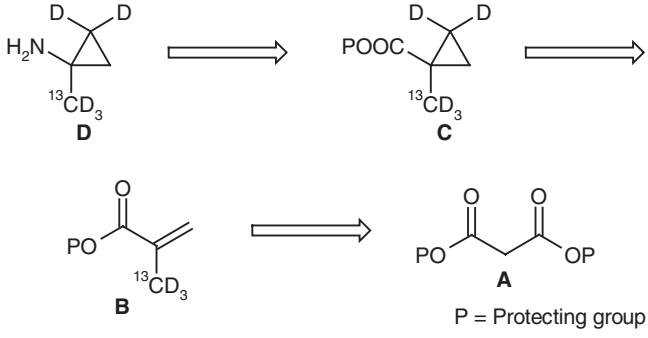
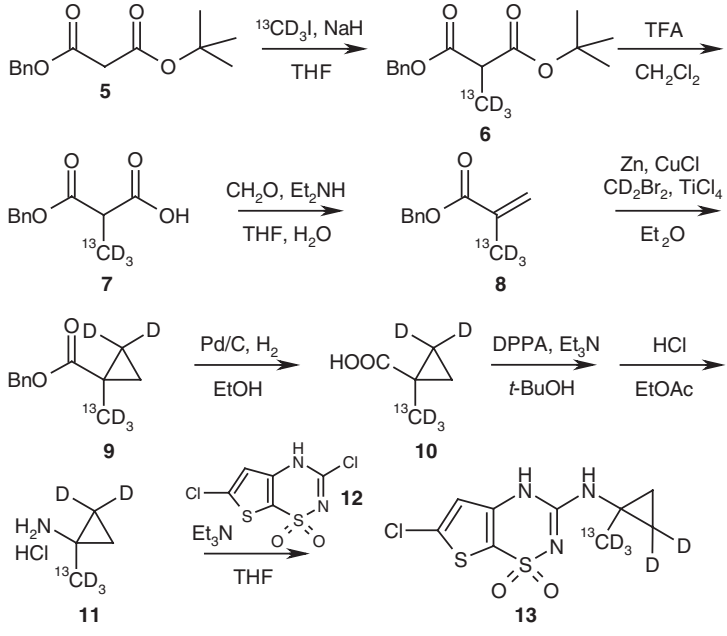


Figure 2. Retrosynthesis of stable isotope labelled NN414

functionality could be beneficial. Finally, by introducing an orthogonal protection scheme, sequential elimination of the protecting groups would be possible, thus avoiding the need to carefully monitor the partial hydrolysis of a symmetric diester.

With these aspects in mind, benzyl *tert*-butyl malonate (**5**) was selected as the starting material for the synthesis of stable isotope labelled 1-methyl-1-cyclopropylamine (Scheme 2). Alkylation with [^{13}C,$^{2}H_3$]iodomethane and NaH was carried out with a slight excess of **5** (1.1 eq.) in order to minimize formation of the dialkylated product. This provided the pure monomethylated malonate **6** in excellent yield (82% based on methyl iodide) after flash chromatography. The use of flash chromatography was instrumental in removing unreacted **5** as well as the small quantities of dimethyl malonate formed, something which has posed a problem for some of the reported syntheses.[7b,c] Removal of the *tert*-butyl protecting group was accomplished by treatment with trifluoroacetic acid in dichloromethane to give **7** in quantitative yield. Then compound **7** was reacted in a Mannich-type reaction with formaldehyde and diethylamine to give the methacrylate **8**, after an *in situ* deamination, in excellent purity and yield (82%) after flash chromatography. The overall yield for the three steps was 67%, which is a distinct improvement over the reported syntheses.



Scheme 2. Synthesis of stable isotope labelled NN414 (13)

Friedrich and co-workers have reported on a convenient version of the Simmons–Smith cyclopropanation reaction,[8] which avoids the use of specially prepared zinc–copper couples by using titanium(IV) chloride catalysis (the use of acetyl chloride as catalyst in the reaction has also been reported.[9] Reaction of methacrylate **8** with [$^2H_2$]dibromomethane using activated zinc dust, copper(I) chloride and titanium(IV) chloride in refluxing anhydrous diethyl ether provided the carboxylic ester **9** in moderate yield (49%) and excellent purity after flash chromatography. We found the use of properly activated zinc dust important for this reaction, as the use of partially or un-activated zinc dust gave incomplete conversion of the starting material.[10]

The benzyl protecting group was removed by hydrogenolysis to give the acid **10** in excellent yield (97%). Then, this was subjected to a modified Curtius reaction using diphenyl phosphorazidate (DPPA).[11] Reaction of **10** with Et_3N and DPPA in anhydrous *tert*-butanol provided the BOC-protected amine, which then was deprotected by treatment with acidic ethylacetate to provide 1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropyl-1-amine hydrochloride (**11**) in moderate yield (48%) after recrystallization. The use of anhydrous conditions in the Curtius reaction proved paramount to the success of the reaction. Initially, the reaction was run under normal conditions using standard analytical grade *tert*-butanol. However, this gave a mixture of **11** and a closely related unknown compound as seen by ^1H NMR. LC–MS revealed the unknown compound to have a MW of 181.3 (M + 1), which (combined with the ^1H NMR data, which suggested a symmetrical structure) lead us to believe that the compound is the sym-urea derivative of **11** (MW 180.2). The formation of this derivative is almost certainly caused by the presence of water in the solvent, something which is widely supported by the literature.[12] This was confirmed by the use of anhydrous conditions and freshly distilled *tert*-butanol, which avoided the formation of this derivative altogether.

It should be noted that the use of [$^2H_2$]formaldehyde in the Mannich-type reaction would replace the last two hydrogen atoms present and thereby give access to 1-methylcyclopropyl-1-amine labelled with deuterium at all positions. Furthermore, the use of carbon-13 labelled formaldehyde and/or malonic acid (which is available labelled in various positions) would give access to 1-methylcyclopropyl-1-amine labelled with carbon-13 in any combination of positions.

The final step in the synthesis of stable isotope labelled NN414 was the coupling of the amine **11** with the heterocyclic moiety **12**. This was performed using the optimized conditions developed for the carbon-14 labelled synthesis but employing 0.88 equivalents of **11** instead of the 3.2 equivalents used in the former synthesis. This provided [^{13}C,$^2H_5$]NN414 in 56% yield and >99% purity after purification by flash chromatography and recrystallization giving

an overall yield of 8.6% for the eight-step synthesis. The isotopic purity was confirmed to be >98% by mass spectroscopy.

Conclusion

In summary, we have developed synthetic routes for the labelling of the potent β-cell selective potassium channel opener NN414 with carbon-14 as well as carbon-13/deuterium. NN414 was labelled with carbon-14 in the thiadiazine moiety in 60% overall starting from the thiadiazine derivative **2**. Following HPLC purification, [^{14}C]NN414 was obtained in high radiochemical purity (>98%) and with high specific radioactivity (58 mCi/mmol).

Stable isotope labelled NN414 was synthesized from benzyl *tert*-butyl malonate (**5**) using [^{13}C,$^{2}H_3$]iodomethane and [$^{2}H_2$]dibromomethane as the source of carbon-13/deuterium. This provided [^{13}C,$^{2}H_5$]NN414 in an overall yield of 8.6% for the eight-step synthesis, which included a Mannich reaction, a Simmons–Smith-type cyclopropanation and a modified Curtius reaction. The methodology presented here can be applied to the synthesis of 1-methylcyclopropyl-1-amine labelled with deuterium in many different combinations including complete labelling. Likewise, the methodology can be applied to the synthesis of 1-methylcyclopropyl-1-amine labelled with carbon-13 in any combination of positions.

Experimental

General

[3-^{14}C]-6-Chloro-2,3-dihydro-3-oxo-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (specific activity: $\geqslant$54 mCi/mmol) was supplied by Amersham Biosciences, UK. [^{13}C,$^{2}H_3$]iodomethane and [$^{2}H_2$]dibromomethane were supplied by Aldrich. Benzyl *tert*-butyl malonate and diphenyl-phosphonic azide (DPPA) were supplied by Lancaster. 1-Methylcyclopropyl-1-amine hydrochloride and 3,6-dichloro-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide were supplied Chemical Development, Novo Nordisk A/S. THF was distilled from sodium/benzophenone and *t*-BuOH from calcium hydride under nitrogen. Dry diethyl ether was obtained from Riedel–deHaën and stored under nitrogen. All other reagents and solvents were of analytical grade and used without further purification. Reactions performed under a nitrogen atmosphere were performed in flame dried glassware.

HPLC was performed using a Merck Hitachi Intelligent Pump L6200A equipped with a Merck Hitachi Column Thermostat T5025 (set at 40°C) with a Rheodyne injector, and a Merck Hitachi UV Detector L4000A (detection at 220 nm). Detection of carbon-14 was performed on a Canbarra Packard flow detector A500.

Analytical HPLC was performed on an RP C18 column (4.6 × 250 mm, 5 μm, OdDMeSi 120 Å, Novo Nordisk) with a flow of 1.0 ml/min. using one of the following systems. System 1: 85% → 60% A 0–20 min (A: 10% acetonitrile in 0.1% aq. TFA; B: 90% acetonitrile in 0.1% aq. TFA). System 2: 100% A 0–40 min followed by 0% A for 10 min (A: 25% acetonitrile in 0.25% aq. TEA, pH 7; B: 90% acetonitrile in 0.1% aq. TFA). Purifications were performed out using system 2 employing a 10 × 250 mm column (5.0 ml/min.).

Radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyser using Ultima Flo^{TM} M (Packard Bioscience) as liquid scintillation cocktail. Specific activity was determined on a Sciex API 300 mass spectrometer equipped with an ionspray interface. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 300 spectrometer. Flash chromatography was performed with silica gel 60 Å (Merck, 40–63 μm). TLC was performed on Alugram SIL G/UV_{254} pre-coated silica plates and spots were detected by UV-detection and/or by dipping in a solution of potassium permanganate (1.5 g $KMnO_4$, 10 g K_2CO_3, 2.5 ml 5% aq. NaOH and 150 ml water). Concentrations were performed on a rotary evaporator at a temperature below 40°C. Melting points are uncorrected. Elemental analyses were performed by the Department of Structural Chemistry, Novo Nordisk.

*[3-^{14}C]-3,6-Dichloro-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (**2**)*

$Et_3N \cdot HCl$ (382 mg, 2.77 mmol) and H_3PO_4 (0.094 ml, 85% aq., 1.39 mmol) were added to a solution of [3-^{14}C]-6-chloro-2,3-dihydro-3-oxo-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (**1**) (49.5 mCi, 0.85 mmol, ⩾54 mCi/mmol) in $POCl_3$ (5 ml, 54.6 mmol) in a glass flask. A glass stopper was securely fastened, and the mixture was stirred at 100°C for 4 days, at which time HPLC analysis (System 1) showed 89% radiochemical conversion. The resulting black solution was carefully quenched by adding it to water (10 ml) (Caution–exothermic reaction!) followed by addition of acetonitrile (5 ml) and additional water (40 ml). This solution was applied to a pre-activated Sep-Pak® cartridge (Waters, RP-C18, 10 g) followed by washing with water (20 ml). Then the product was eluted with aqueous acetonitrile (90%, 40 ml) to give **2** (49.5 mCi, 100%) with a radiochemical purity of 90% (System 1).

*[3-^{14}C]-6-Chloro-3-(1-methylcyclopropyl)amino-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (NN414) (**4**)*

1-Methylcyclopropyl-1-amine hydrochloride (**3**) (242 mg, 2.25 mmol) and Et_3N (0.53 ml, 3.80 mmol) were added to a solution of [3-^{14}C]-3,6-dichloro-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (**2**) (40.6 mCi, 0.70 mmol) in dry THF (4 ml) in a pressure vial. The screw cap was securely fastened, and the mixture was stirred at 80°C for 6 days, at which time HPLC analysis (System 2) showed 82% radiochemical conversion. The reaction mixture was purified

by HPLC (System 2) and the fractions containing **4** were concentrated followed by addition of 10% aq. EtOH (50 ml). This mixture was applied to a pre-activated Sep-Pak® cartridge (Waters, RP-C18, 10 g) followed by washing with water (20 ml). Then the product was eluted with aqueous EtOH (90%, 40 ml), concentrated and re-dissolved in EtOH (100 ml). This provided **3** (24.2 mCi, 60%) with chemical and radiochemical purities >98% (System 2). The specific activity was calculated to be 58 mCi/mmol (MS).

*Benzyl tert-butyl [^{13}C,$^{2}H_3$]methylmalonate (**6**)*

A solution of benzyl *tert*-butyl malonate (**5**) (7.55 g, 30.2 mmol) in THF (7.5 ml) was slowly added over 5 min to a suspension of pentane-washed NaH (964 mg, 40.2 mmol) in THF (20 ml) under a nitrogen atmosphere. The mixture was stirred for 0.5 h resulting in a clear solution; to this was added a solution of [^{13}C,$^{2}H_3$]iodomethane (3.91 g, 26.8 mmol) in THF (3 ml). The solution was stirred for a further 0.75 h and then quenched with aqueous acetic acid (10%, 10 ml) followed by evaporation of THF. The residual aqueous phase was extracted with dichloromethane (3 × 40 ml) and the combined organic phases were washed with brine (10 ml), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (EtOAc/heptane 1:19) gave the title compound **6** as a colourless oil (5.86 g, 82% based on [^{13}C,$^{2}H_3$]iodomethane). R_F 0.42 (EtOAc/heptane 2:8).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (9 H, s), 3.35 (1 H, d, *J* 4.5), 5.09–5.19 (2 H, m,), 7.24–7.35 (5 H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.4 (septet, $^{13}CD_3$), 27.4, 46.6, 66.4, 81.2, 128.0, 128.2, 135.4, 169.7, 169.9.

$C_{14}{}^{13}CH_{17}D_3O_4$ (268.34): calculated C + ^{13}C as C 67.14, H + D as H 7.51; found C + ^{13}C as C 66.90, H + D as H 7.85.

*Benzyl [^{13}C,$^{2}H_3$]methylmalonate (**7**)*

TFA (4 ml) was added to a solution of benzyl *tert*-butyl [^{13}C,$^{2}H_3$]methylmalonate (**6**) (2.71 g, 10.1 mmol) in dichloromethane (20 ml), and the mixture was stirred for 6 h. Concentration gave the title compound **7** as a clear oil (2.28 g, 100%). R_F 0.14 (EtOAc/heptane 2:8).

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.55 (1 H, d, *J* 5.0), 5.18 (2 H, s), 7.25–7.35 (5 H, m), 11.30 (1 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.5 (septet, $^{13}CD_3$), 45.7, 67.7, 128.0, 128.5, 134.8, 170.5, 175.9.

*Benzyl [^{13}C,$^{2}H_3$]methacrylate (**8**)*

Formaldehyde (0.97 ml, 37% aq., 12.9 mmol) and Et_2NH (1.35 ml, 13.0 mmol) were added to a solution of benzyl [^{13}C,$^{2}H_3$]methylmalonate (**7**) (2.71 g, 12.8 mmol) in THF/water (10 ml/10 ml), and the reaction mixture was stirred at 50°C for 16 h. The mixture was concentrated and the residue dissolved in

water (10 ml) and extracted with dichloromethane (3 × 40 ml). The combined organic phases were washed with brine (10 ml), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (EtOAc/heptane 1:19) gave the title compound **8** as a colourless oil (1.89 g, 82%). R_F 0.52 (EtOAc/heptane 2:8).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (2 H, s), 5.54 (1 H, d, *J* 1.5, *J* 6.0), 6.14 (1 H, dd, *J* 2.0, *J* 10.0), 7.24–7.36 (5 H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.3 (septet, $^{13}CD_3$), 66.1, 125.8, 127.8, 127.9, 128.3, 136.0, 166.9.

$C_{10}{}^{13}CH_9D_3O_2$ (180.23): calculated C + ^{13}C as C 73.31, H + D as H 6.71; found C + ^{13}C as C 73.11, H + D as H 6.99.

*Benzyl 1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropane-1-carboxylate (**9**)*

A solution of benzyl [^{13}C,$^2H_3$]methacrylate (**8**) (1.68 g, 9.32 mmol) in Et_2O (2 ml) as well as [$^2H_2$]dibromomethane (2.00 ml, 5.00 g, 28.4 mmol) were added to a suspension of activated zinc dust† (2.20 g, 33.7 mmol) and CuCl (0.336 g, 3.39 mmol) in Et_2O (20 ml) under a nitrogen atmosphere. After stirring at room temperature for 0.5 h, $TiCl_4$ (0.08 ml, 0.73 mmol) was added, and the mixture was refluxed for 21 h, at which time further [$^2H_2$]dibromomethane (0.42 ml, 1.05 g, 5.97 mmol) was added and the mixture refluxed for an additional 2.5 h. After cooling to room temperature, the mixture was carefully quenched with aqueous HCl (2 N, 5 ml) followed by filtration and addition of water (5 ml). The phases were separated, and the aqueous phase was extracted with Et_2O (2 × 20 ml). The combined organic phases were washed with brine (10 ml), dried ($MgSO_4$) and concentrated. Purification by flash chromatography (EtOAc/heptane 1:19) gave the title compound **9** as a colourless oil (0.886 g, 49%). R_F 0.52 (EtOAc/heptane 2:8).

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.63 (1 H, t), 1.22 (1 H, s), 5.07 (2 H, s), 7.23–7.35 (5 H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.3, 18.3 (septet, $^{13}CD_3$), 65.9, 127.6, 127.8, 128.3, 136.2, 175.4.

$C_{11}{}^{13}CH_9D_5O_2$ (196.28): calculated C + ^{13}C as C 73.43, H + D as H 7.19; found C + ^{13}C as C 73.36, H + D as H 7.37.

*1-[^{13}C,$^2H_3$]Methyl-[2,2-$^2H_2$]cyclopropane-1-carboxylic acid (**10**)*

Pd/C (40 mg) was added to a solution of benzyl 1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropane-1-carboxylate (**9**) (0.853 g, 4.35 mmol) in EtOH (15 ml). The mixture was stirred under an H_2 atmosphere for 16 h and then filtered and concentrated to give the title compound **10** as a colourless oil (0.448 g, 97%).

†Zinc dust was activated by sequential washing with 1N NaOH (10 min), water, 1N NC1 (10 min), water, ethanol, acetone and diethyl ether followed by drying in *in vacuo* for 2 h.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (1 H, t, *J* 4.0), 1.27 (1 H, s), 11.89 (1 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.2, 17.9 (septet, $^{13}CD_3$), 183.3.

*1-[^{13}C,$^2H_3$]Methyl-[2,2-$^2H_2$]cyclopropyl-1-amine hydrochloride (**11**)*

Et_3N (0.65 ml, 4.66 mmol) and diphenylphosphonic azide (0.96 ml, 4.45 mmol) were added to a solution of 1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropane-1-carboxylic acid (**10**) (0.448 g, 4.22 mmol) in dry *t*-BuOH (10 ml) under a nitrogen atmosphere, and the reaction mixture was stirred at 50°C for 16 h. The mixture was concentrated to an oil, which was extracted with Et_2O (4 × 50 ml). The combined organic phases were washed with aqueous NaOH (1N, 2 × 20 ml), aq. H_2SO_4 (0.1N, 2 × 20 ml) and brine (20 ml), dried ($MgSO_4$), and concentrated to give the BOC protected amine as a yellow oil (0.591 g, 79%).

^{1}H NMR (300 MHz, MeOH-d_4): δ 0.50 (1 H, t, *J* 4.0), 0.65 (1 H, d, *J* 4.5), 1.42 (9 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5, 22.4 (septet, $^{13}CD_3$), 23.1, 28.8, 79.6, 158.2.

A solution of the BOC protected amine (0.591 g) in EtOAc (50 ml) was cooled to 0°C and HCl (g) was passed through the solution for 0.5 h followed by stirring at room temperature for 16 h. Concentration gave a crystalline product, which was purified by recrystallization (EtOAc/MeOH) to give the title compound **11** as colourless crystals (0.230 g, 48% for the two steps); Mp 206–209°C (an unlabelled sample had mp 196–197°C).

^{1}H NMR (300 MHz, MeOH-d_4): δ 0.73 (1 H, dd, *J* 4.0), 0.92 (1 H, dd, *J* 5.5). ^{13}C NMR (75 MHz, MeOH-d_4): δ 11.2, 20.2 (septet, $^{13}CD_3$), 30.3.

$C_3\ 13^C\ CH_5\ D_5\ NCl$ (113.62): calculated C + ^{13}C as C 42.28, H + D as H 8.87, N 12.33, Cl 31.20; found C + ^{13}C as C 42.37, H + D as H 9.20, N 12.28, Cl 30.93.

*6-Chloro-3-(1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropyl)amino-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (NN414) (**13**)*

A suspension of 1-[^{13}C,$^2H_3$]methyl-[2,2-$^2H_2$]cyclopropyl-1-amine hydrochloride (**11**) (0.100 g, 0.88 mmol) and 3,6-dichloro-4H-thieno[3,2-e]-1,2,4thiadiazine 1,1-dioxide (**12**) (0.257 g, 1.00 mmol) in dry THF (20 ml) was heated to 65°C. Then, Et_3N (0.20 ml, 1.44 mmol) was added and the mixture stirred at 65°C for 5 days. Concentration gave a dark oil, which was purified by flash chromatography (dichloromethane/MeOH, 19:1) to give a crude product (0.173 g, 66%). Final purification was performed by recrystallization (EtOH) to give the title compound **13** as off-white crystals (0.147 g, 56%), Mp 263–264°C (an unlabelled sample had mp 251–252°C). Purity >99% (HPLC System 2). R_F 0.17 (dichloromethane/MeOH 19:1).

^{1}H NMR (300 MHz, MeOH-d_4): δ 0.72 (1 H, t, J 4.5), 0.83 (1 H, d, J 5.0), 6.92 (1 H, s). ^{13}C NMR (75 MHz, MeOH-d_4): δ 15.1, 21.8 (septet, $^{13}CD_3$), 29.7, 113.3, 118.7, 137.1, 140.1, 152.0.

MS (ionspray): 298.2 (M + H) shows less than 1.1% of the unlabelled compound present (292.2).

Acknowledgements

We are grateful to Mr Ole Wassmann for mass spectra analyses, Mr Flemming Gundertofte for elemental analyses and Dr Flemming E. Nielsen for helpful discussions.

References

1. Johansen SK, Kristensen JB, Müller LK, Jessen CU, Foged C. *Presented at the 8th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds*, Boston, MA, USA, 1–5 June 2003.
2. WHO fact sheet No. 236 September 2002 (http://www.who.int/mediacentre/factsheets/fs236/en).
3. Coghlan MJ, Carroll WA, Gopalakrishnan. *J Med Chem* 2001; **44**: 1627–1653.
4. Tagmose TM, Mogensen JP, Agerholm PC, Arkhammar POG, Wahl P, Worsaae A, Hansen JB. *Bioorg Med Chem Lett* 2001; **11**: 1749–1752.
5. Nielsen FE, Bodvarsdottir TB, Worsaae A, MacKay P, Stidsen CE, Boonen HCM, Pridal L, Arkhammar POG, Wahl P, Ynddal L, Junager F, Dragsted N, Tagmose TM, Mogensen JP, Koch A, Treppendahl SP, Hansen JB. *J Med Chem* 2002; **45**: 4171–4187.
6. Andersen K, Begtrup M. *Acta Chem Scand* 1992; **46**: 1130–1132.
7. (a) Hosokawa T, Yamanaka T, Itotani M, Murahashi S. *J Org Chem* 1995; **60**: 6159–6167. (b) Keah HH, Rae ID, Hawthorne DG. *Aust J Chem* 1992; **45**: 659–669. (c) Nikoletić M, Borĉić S, Sunko DE. *Tetrahedron* 1967; **23**: 649–660.
8. Friedrich EC, Lunetta SE, Lewis EJ. *J Org Chem* 1989; **55**: 2388–2390.
9. Friedrich EC, Lewis EJ. *J Org Chem* 1990; **56**: 2491–2494.
10. For a review on the preparation of activated zinc metal, see Erdik E. *Tetrahedron* 1987; **43**: 2203–2212.
11. Ninomiya K, Shioiri T, Yamada S. *Tetrahedron* 1974; **30**: 2151–2157.
12. Smith PAS. *Org Reactions* 1946; **3**: 337–449.

