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Review Article

Carbon-14 labelling of acarbose – a challenge over more than 20 years†

D. SEIDEL[1],*, H. WEHLMANN[2] and J. LENZ[2]

[1] Drug Metabolism and Isotope Chemistry, Global Drug Discovery, Bayer HealthCare AG, D-42096 Wuppertal, Germany
[2] Biotechnology, Product Supply, Bayer HealthCare AG, D-42096 Wuppertal, Germany

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Abstract: Acarbose is a potent α-glucosidase inhibitor which is used successfully as a drug for the treatment of type II diabetes. The compound prevents enzymes of the α-glucosidase group from cleaving carbohydrates. For the investigation of pharmacokinetics and drug metabolism a first carbon-14 labelling synthesis was performed in 1981. According to the pharmaceutical production process, the radiosynthesis was performed by fermentation. Due to limited radiolytic stability, repeated radiosyntheses were necessary. Finally, carbon-14 labelling was optimized to prepare [^{14}C]acarbose on a gram scale with a significant increase of the radiochemical yield. The aspects of the fermentation over a period of about 20 years are described focussing the attention on the optimized radiosynthesis. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: acarbose; glucosidase inhibitor; carbon-14 synthesis

Introduction

Acarbose belongs to the so-called α-glucosidase inhibitors, a class of drugs that slow down the digestion of carbohydrates and lengthen the time needed for carbohydrates to convert to glucose.[1] Acarbose reversibly binds to pancreatic α-amylase and membrane-bound intestinal α-glucoside hydrolases. These enzymes inhibit the hydrolysis of complex starches to oligosaccharides in the lumen of the small intestine and hydrolysis of oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. Therefore, acarbose is indicated as an adjunct to diet to lower blood glucose in patients with type II diabetes mellitus whose hyperglycaemia cannot be managed on diet alone.[2]

The drug substance was introduced in 1990 into the therapy of type II diabetes[3] (brand names Glucobay®, Precose™, Glucor®, Prandase®). Acarbose is one of the safest anti-diabetes drugs. It has been shown to have a good safety profile in numerous clinical trials and over 15 years of patient treatment.[4]

A characteristic structural element of acarbose is the acarviosyl moiety (rings A and B, Figure 1). This part consists of valienamine that is linked *N*-glycosidically to 4,6-didesoxyglucose. This so-called 'core' of the molecule is essential for the α-glucosidase inhibitory action.[5] The core is coupled by an α(1 → 4)-linkage to a maltose unit.

The product is produced using biotechnology on an industrial scale.[6,7] In an optimized process, starch hydrolysate and other media compounds are fermented with the soil bacterium Actinoplanes spec. SE 50/110 or mutant strains therefrom. The mutant strains result from a strain development program and offer improved productivity.

During the drug development process carbon-14-labelled acarbose was required to study pharmacokinetics and drug metabolism. With respect to subsequent studies and a limited stability of [^{14}C]acarbose against radiolysis several radiosyntheses were performed over the years.

Carbon-14 labelling of acarbose

The first radiosynthesis was performed by Maul *et al.* at the beginning of the eighties and is described later.[8]

*Correspondence to: D. Seidel, Bayer HealthCare AG, Aprather Weg 18a, D-42096 Wuppertal, Germany. E-mail: dietrich.seidel@bayer healthcare.com
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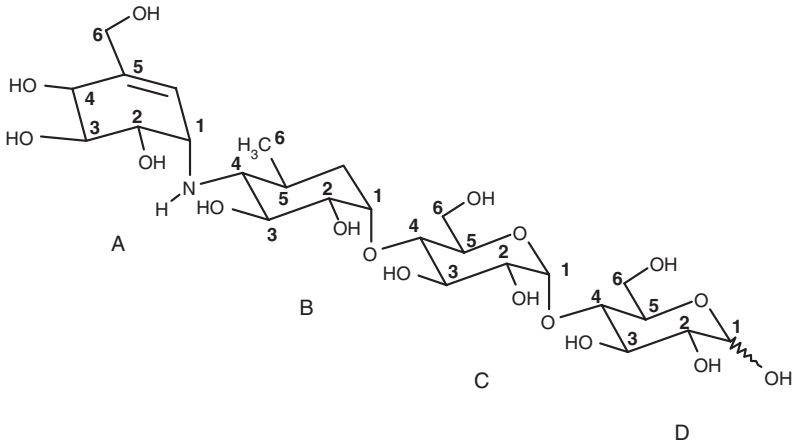

Figure 1 Structure of acarbose with carbon assignment.

Due to the experience with the manufacturing of acarbose, the labelling was also performed biochemically. Former studies had shown that fermentation with glucose or maltose instead of starch as carbon source led to higher amounts of the desired product.[9] Based on this experience, Maul and co-workers used uniformly carbon-14-labelled glucose as a suitable precursor. The fermentation was performed with Actinoplanes mutant SN 1667/47. This strain proved to be the best suited out of several mutants studied. The Actinoplanes culture was pre-incubated in a special medium containing glycerol, dextrin, soymeal, and calcium carbonate in tap water. An aliquot of this culture was used for inoculation of a second propagation step using a medium containing maltose, D-glucose, sodium pyruvate, yeast extract, calcium carbonate, and potassium dihydrogenphosphate. The Actinoplanes mycelium of this second cultivation step was isolated and submitted for the biosynthesis in a third medium containing maltose, D-[^{14}C]glucose, peptone, K_2HPO_4, and antifoaming agent in deionized water. All media were pre-sterilized. The labelling reaction was performed for 20 h at 27°C. The mycelium was killed by the addition of toluene and the culture liquor was centrifuged. Isolation of the carbon-14-labelled acarbose was performed by repeated chromatography. The concentrated supernatants were eluted over a CM-Sephadex® phase. The [^{14}C]acarbose containing fractions were pooled and concentrated. Threefold rechromatography on CM-Sephadex® gave pure product. A total of 217 MBq of [^{14}C]acarbose was obtained. From different fermentations with typically 7.4 GBq of D-[U-^{14}C]glucose, the authors obtained radiochemical yields of 1.58–2.56%. The range of the specific radioactivity of the carbon-14-labelled acarbose batches was 7.77 MBq/mg (5016 MBq/mmol) to 9.14 MBq/mg (5901 MBq/mmol). Radiochemical purities of >98% were achieved in all cases.

The location of the carbon-14 was determined by hydrolysing the labelled acarbose. Radio-TLC revealed at least 92% of the radioactivity in the core.[9]

In this and all the following [^{14}C]acarbose biosyntheses, the formed carbon-14-labelled carbon dioxide was absorbed by washing bottles filled with triethanolamine solution.

After this first radiosynthesis, it was found that the carbon-14-labelled acarbose was of limited stability. Storage was performed in water/ethanol mixtures (9:1) to prevent bacterial degradation. Nevertheless, radiolysis could not be stopped.

Therefore, additional labelling syntheses were necessary during the time of product development and product studies.

A total of four carbon-14-labelling syntheses of acarbose were performed additionally within the next 15 years. In spite of some optimization studies no sweeping success was achieved. All fermentations were started from 37 GBq of D-[U-^{14}C]glucose.

In the second and third radiosynthesis, the known mutant SN 1667/47 was used as a bio-agent due to no better alternative. The radiochemical yields were approximately 1.5%.

With nearly the same conditions and results, a third fermentation was carried out in the mid-eighties. As before, D-[U-^{14}C]glucose served as a radioactive precursor. Actinoplanes strain SN 1667/47 was used again.

A fourth radiosynthesis was performed in 1994. Four Actinoplanes mutants were compared in pre-experiments. Only the older mutants resulted in reasonable acarbose production from glucose. So it was decided to choose the known mutant SN 1667/47 again. Isolation

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of [^{14}C]acarbose was performed by repeated chromatography but under modified conditions. By elution of the supernatant concentrates over CM-Sephadex®, salts were separated at low temperature and [^{14}C]acarbose was eluted at higher temperature. Final purification was performed chromatographically on an amino phase with acetonitrile/phosphate buffer. Chromatography on CM-Sephadex® with demineralized water gave the desalted product.

The radiochemical yield was about 0.45%. The combined batches showed a specific radioactivity of 2846 MBq/mmol and a product purity >98%.

Within a pre-experiment with D-[U-^{13}C]glucose, carbon-13-labelled acarbose was produced and studied by ^{13}C NMR to verify the labelling positions. Carbon-13 was introduced with a statistical distribution over all carbon atoms in the core (Figure 2). Table 1 shows the chemical shifts of the ^{13}C signals of acarbose.

The separated triplets at 59 ppm (A1) and 68 ppm (B4) in the [^{13}C]acarbose spectrum revealed two neighbouring carbon-13 atoms. The other signals are overlapped partially. Analogous signal splitting was not detected for rings C and D. The accuracy of the method did not allow a complete exclusion of carbon-13 enrichment in rings C and D, but the results were in good accordance with the results of Maul *et al.* for the location of the carbon-14 isotopes in [^{14}C]acarbose.[8]

A fifth carbon-14 labelling of acarbose was performed two years later in 1996. As the known Actinoplanes mutant SN 1667/47 was no longer available another strain was selected, namely mutant SN 223/29. Within a pre-experiment with D-[U-^{13}C]glucose, carbon-13-labelled acarbose was produced and studied by ^{13}C NMR to verify the labelling positions (Figure 3). The spectrum was comparable to the [^{13}C] NMR spectrum of the previously synthesised [^{13}C]acarbose (Figure 2). Carbon-13 was introduced with a statistical distribution over all carbon atoms in the core. It could be concluded that no alteration in the labelling positions was resulted by the application of the different mutant.

Isolation of the carbon-14-labelled acarbose was performed analogously to the previous preparation. [^{14}C]Acarbose was obtained with a radiochemical yield of 1.2%.

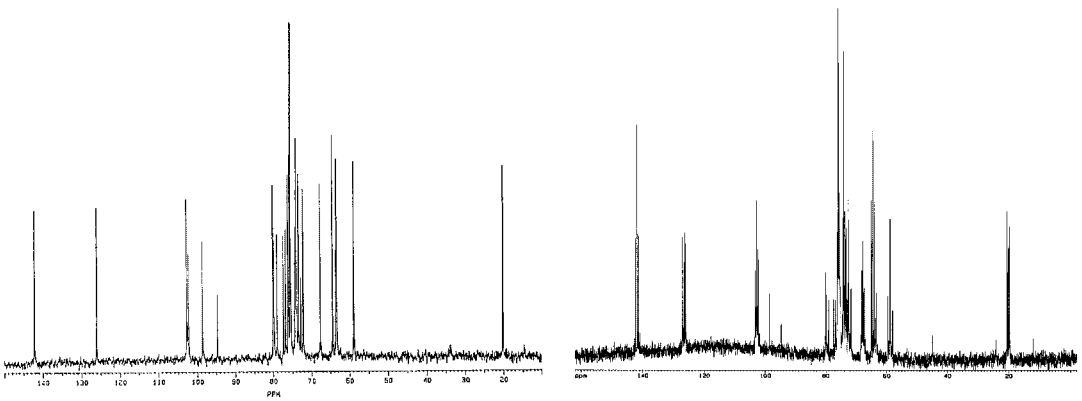

Figure 2 ^{13}C NMR spectrum of acarbose (left) and of [^{13}C]acarbose produced prior to the fourth radiosynthesis (right).

Table 1 Chemical shift of the carbon-13 signals of acarbose

Ring A	Ring B	Ring C	Ring D
A1 58.84 ppm	B1 102.77 ppm	C1α 102.39 ppm	D1α 94.70 ppm
A2 75.65 ppm	B2 73.92 ppm	C1β 102.39 ppm	D1β 98.58 ppm
A3 75.65 ppm	B3 75.51 ppm	C2α 74.26 ppm	D2α 74.08 ppm
A4 73.36 ppm	B4 67.64 ppm	B2β 74.36 ppm	D2β 76.78 ppm
A5 142.16 ppm	B5 72.15 ppm	C3 76.14 ppm	D3α 75.98 ppm
A6 126.06 ppm	B6 20.15 ppm	C4 80.03 ppm	D3β 78.97 ppm
A7 64.38 ppm		C5 74.02 ppm	D4α 80.03 ppm
		C6 63.31 ppm	D4β 79.79 ppm
			D5α 72.75 ppm
			D5β 77.36 ppm
			D6α 63.31 ppm
			D6β 63.49 ppm

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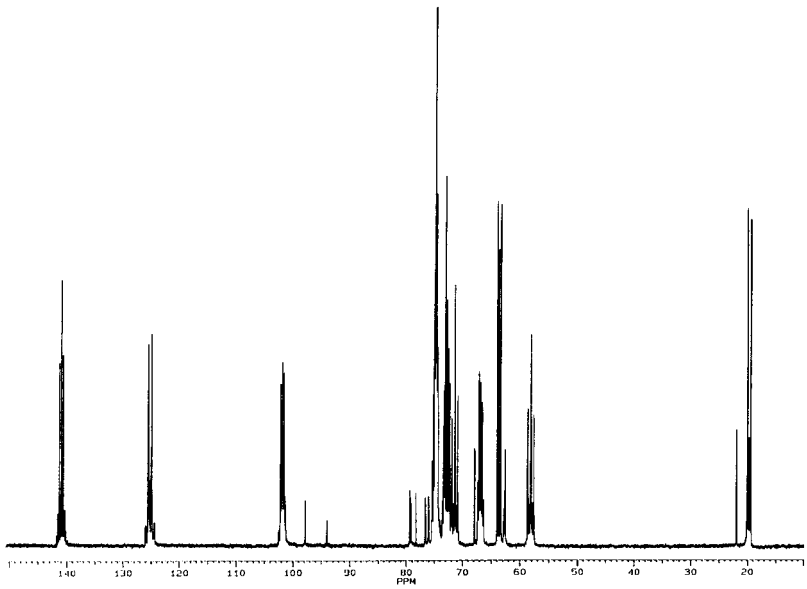

Figure 3 ^{13}C NMR spectrum of [^{13}C]acarbose produced prior to the fifth radiosynthesis.

The radiosyntheses described up to now are summarized in Table 2. No progress was achieved in the carbon-14 labelling of acarbose regarding the radiochemical yield. But in all cases the product demands were met.

In all preparations typically 50–70% of the starting radioactivity was found in the $^{14}CO_2$ absorption bottles.

A new and strong incentive for optimization of the biosynthesis was given by a request of a high amount of [^{14}C]acarbose in 2003. On the basis of fermentation results obtained so far the enormous amount of more than 370 GBq of starting [U-^{14}C]glucose would be necessary for this first demand for carbon-14-labelled acarbose in the gram scale. Very intensive studies of the whole fermentative process were started to optimize the radiochemical yield. Especially, new Actinoplanes mutants, fermentation conditions, and isolation procedures were checked.

Pre-experiments revealed that the mutant SN19910/37/21 was able to convert glucose to the desired acarbose with better results than the previously used mutants. The procedure is summarized in Scheme 1.

The induced mycelium was prepared as before by a three-step fermentation process. The third cultivation step was run in a 10-l-fermenter for 96 h. The biomass was harvested by spinning down 600 ml of aliquots of the culture broth.

The new mutant led to a much higher product content per volume. Consequently, work-up had to be optimized as well.

Especially, the use of a different Actinoplanes mutant implicated the check of an unchanged distribution of the carbon-14 labels of the newly produced [^{14}C]acarbose again. Therefore, a preliminary fermentation with uniformly ^{13}C-labelled glucose was performed with the same intention as described earlier.

Table 2 Summary of the first five acarbose labelling results

[^{14}C]Acarbose synthesis	Radiochemical yield (%)
1	1.58–2.56
2	1.5
3	1.5
4	0.45
5	1.2

The carbon-13 distribution (Figure 4) in the final product was identical to that of the [^{13}C]acarbose synthesized previously indicating that the new Actinoplanes mutant gave no alteration in the isotope pattern. From the ^{13}C satellites of the ^{1}H NMR spectrum, a content of carbon-13 in the range of 3–20% in the rings A and B was calculated. A mean carbon-13 enrichment of approximately 7% per carbon atom in the rings A and B was calculated therefrom. This was in accordance with the previous syntheses. The optimized process revealed higher radiochemical yield but no increased carbon isotope incorporation.

To produce about 7 GBq of [^{14}C]acarbose a starting amount of 92.5 GBq of [U-^{14}C]glucose was necessary. This was a reduction to about 25% compared with the first assumptions.

The optimization experiments revealed that scale-up of the fermentation bottles larger than 1 l gave poorer

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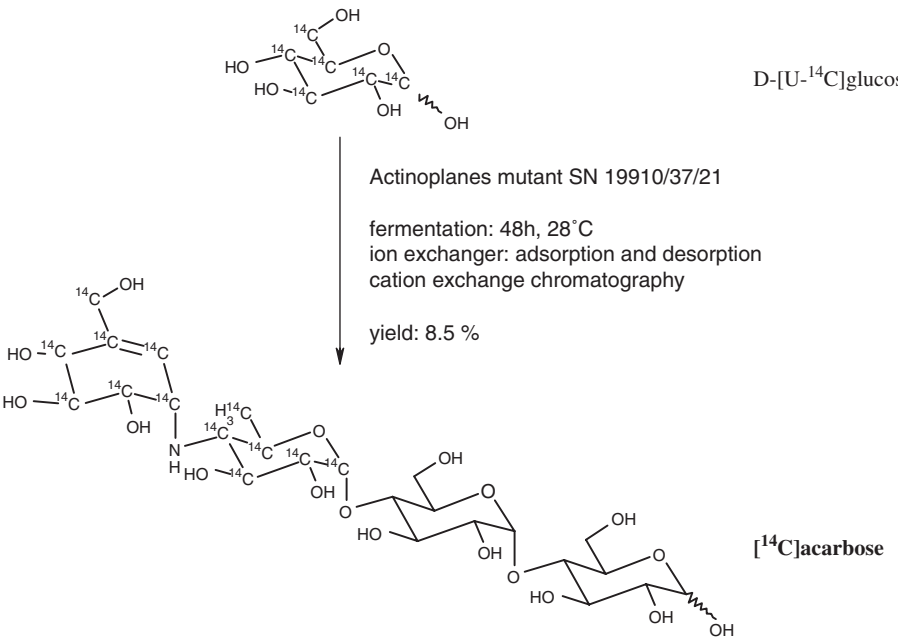

Scheme 1

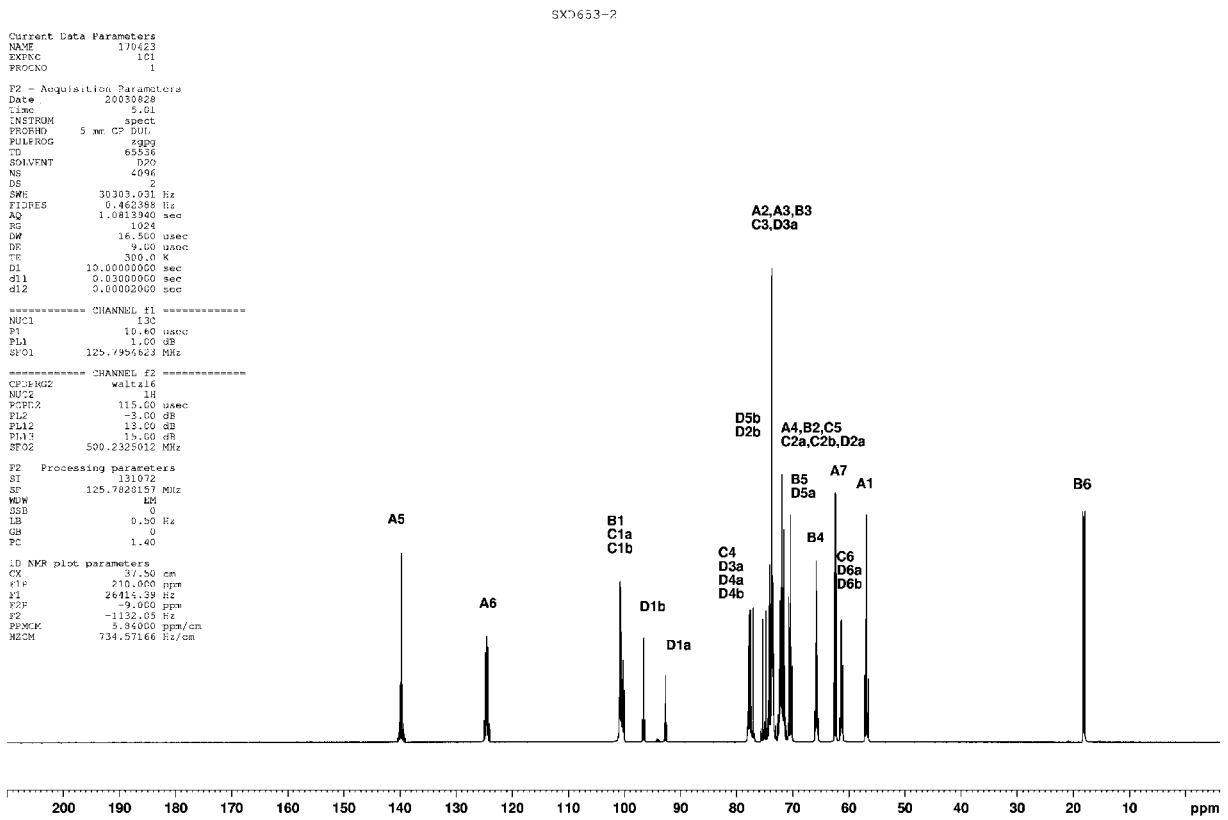

Figure 4 ^{13}C NMR spectrum of [^{13}C]acarbose produced prior to the optimized radiosynthesis.

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yields. Hence, the radio-fermentation was performed in five parallel bottles each with 18.5 GBq of carbon-14-labelled glucose. An example of the set-up is shown in Figure 5.

A marked yield optimization was achieved by the new work-up procedure. To handle the higher amount of [^{14}C]acarbose, isolation and purification were changed nearly completely. Ion exchanger adsorption and desorption led to an easier and improved product isolation.

In the first step, acarbose was absorbed by ion exchanger. The acarbose-loaded resin was filtered off and washed with water. Desorption of the labelled acarbose was performed using water and 1 M sodium hydroxide solution at pH 8.1. The radiochemical purity of [^{14}C]acarbose was 51.8% by radio-high-performance liquid chromatography (HPLC).

Further purification was performed by cation-exchange chromatography. The evaporated product solution was pumped onto the separation column filled with a special cation exchanger. After loading, the column was washed with water and elution was performed with a hydrochloric acid gradient. Detection was performed by polarimetry and subsequent conductometry. The radiochemical purity after this step was 91.7%. For acarbose recovery, the solution was adjusted with an anion exchanger MP 62 to a pH of 5.1 and desorbed subsequently with water.

Final purification was performed by a second cation-exchange chromatography. After loading, the column was washed with warm water (70°C) until the conductivity reached the zero value. The [^{14}C]acarbose-containing fraction was sampled by polarimetric detection as shown in Figure 6.

The work-up steps are summarized in Table 3. The advantage of the new isolation and purification procedure is shown by the good to excellent recovery in each step.

[^{14}C]Acarbose was obtained in a total radioactivity amount of 7936 MBq. This corresponded to 2.5 g of labelled product calculated with the specific radioactivity of 2028 MBq/mmol (determination is given in the experimental part). The radiochemical yield of carbon-14-labelled acarbose was 8.5% based on the starting D-[U-^{14}C]glucose.

A graphic comparison of all acarbose radiosyntheses reveals clearly the advantage achieved by the intensive optimization of the whole procedure (Figure 7).

Electrospray mass spectral analysis revealed the molecular ion of [^{12}C]acarbose as base peak with $m/z = 646[M + H]^+$. The molecular ions of different carbon-14-labelled acarbose molecules were found up to $m/z = 672$.

The identity of the labelled product was confirmed by ^{1}H (Figure 8) and ^{13}C NMR (Figure 9) spectroscopy. The spectra complied with the expected structure.

In comparison to all previously performed biosyntheses, the last one gave significantly higher yield. The specific radioactivity was in the region of the former syntheses.

The conditions of the gram-scale fermentation are given in the experimental part.

Experimental

Materials

D-[U-^{14}C]glucose was delivered by American Radiolabeled Chemicals, Inc. The total amount was 92.5 GBq

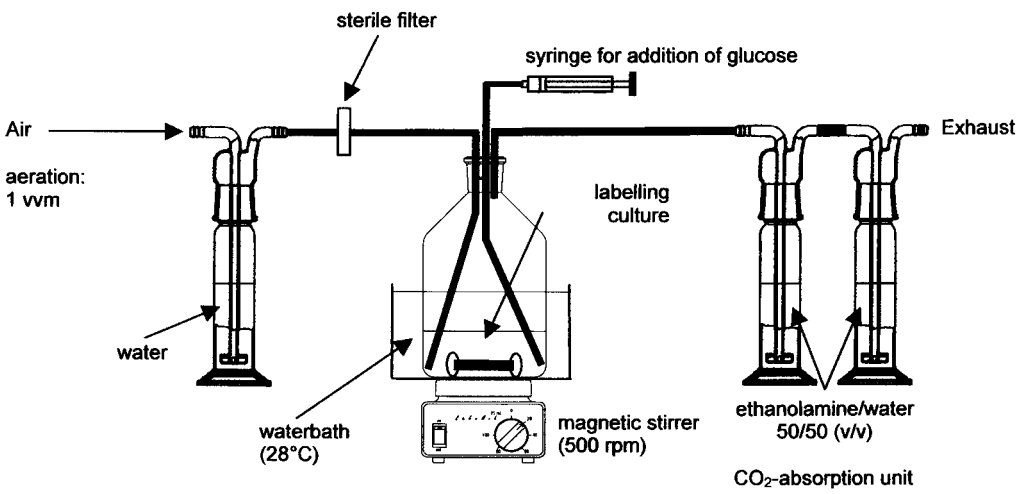

Figure 5 Set-up of the labelling culture system.

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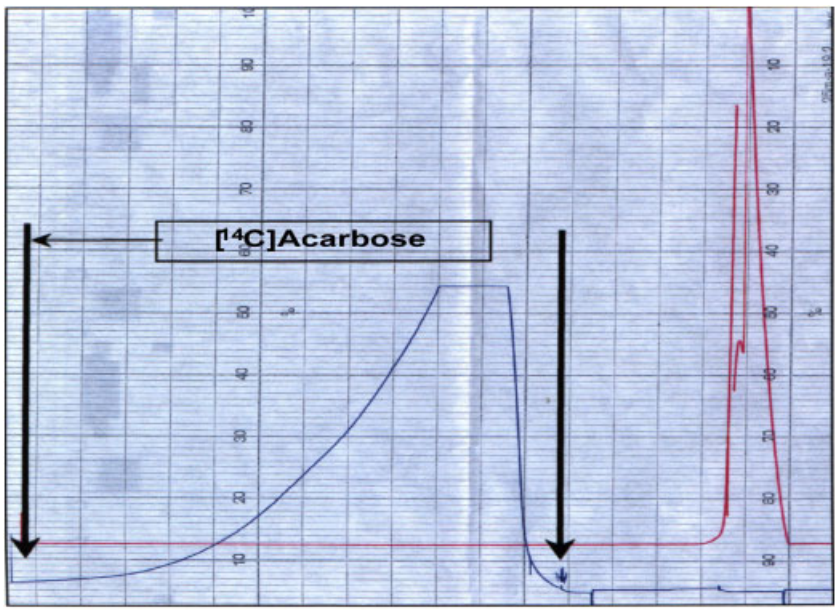

Figure 6 Polarimetry chromatogram of the second cation exchange chromatography.

Table 3 Overview recovery of the optimized [^{14}C]acarbose radiosynthesis

Step	Volume (ml)	Acarbose content (g/l)	Acarbose amounts (g)	Recovery (%)
Culture broth	1008	3.4	3.45	100
Combined desorbate	690	4.84	3.34	96.9
Pool first cation exchange chromatography	515	5.37	2.77	80.2
Pool second cation exchange chromatography	620	4.38	2.72	78.8
[^{14}C]Acarbose concentrate	49	50.7	2.484	72

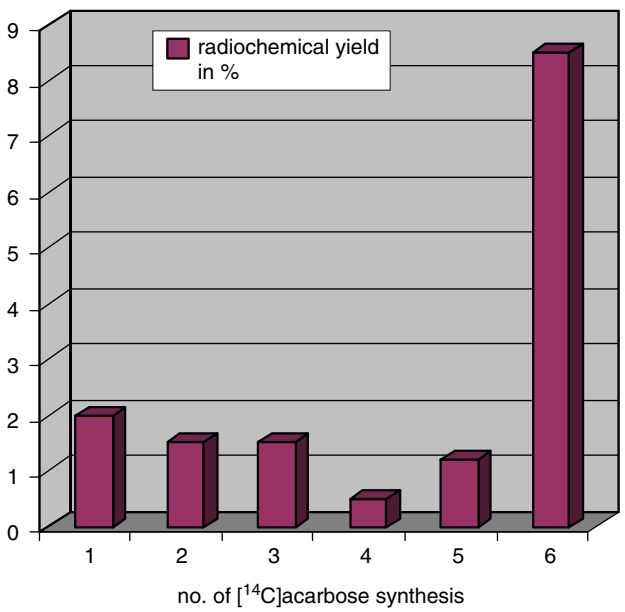

Figure 7 Summary of all acarbose labelling results.

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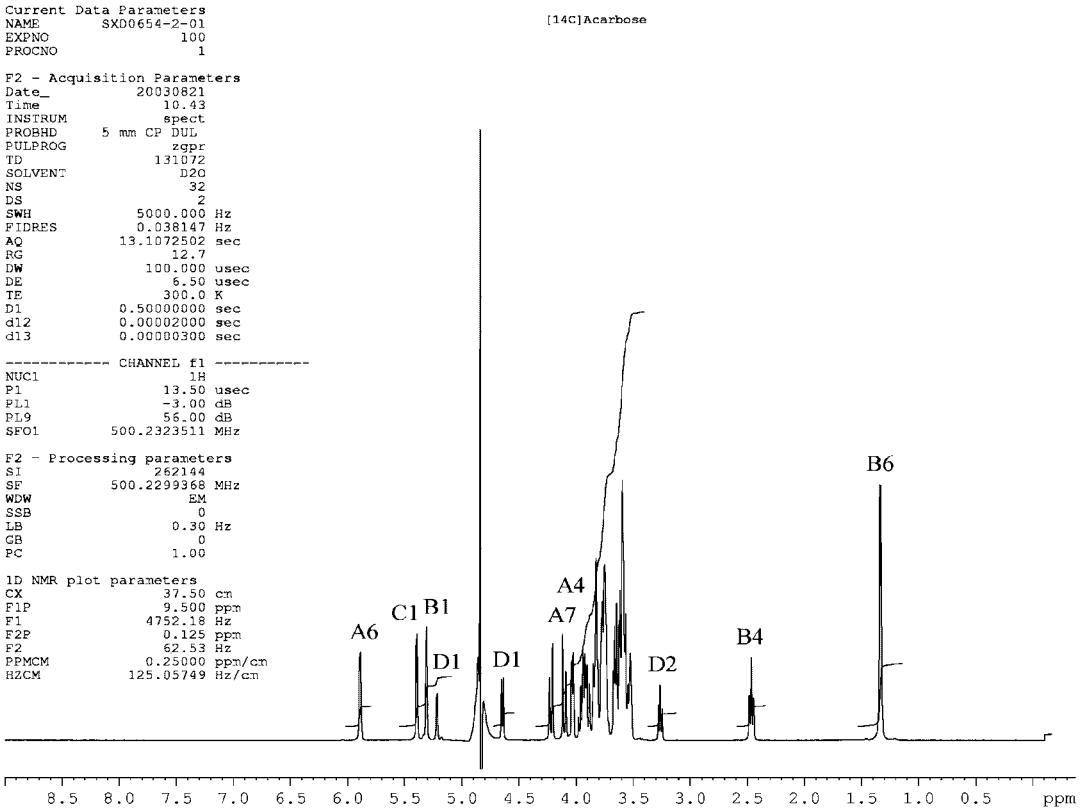

Figure 8 ^{1}H NMR spectrum of [^{14}C]acarbose produced by the optimized process, with water suppression, solvent D_2O.

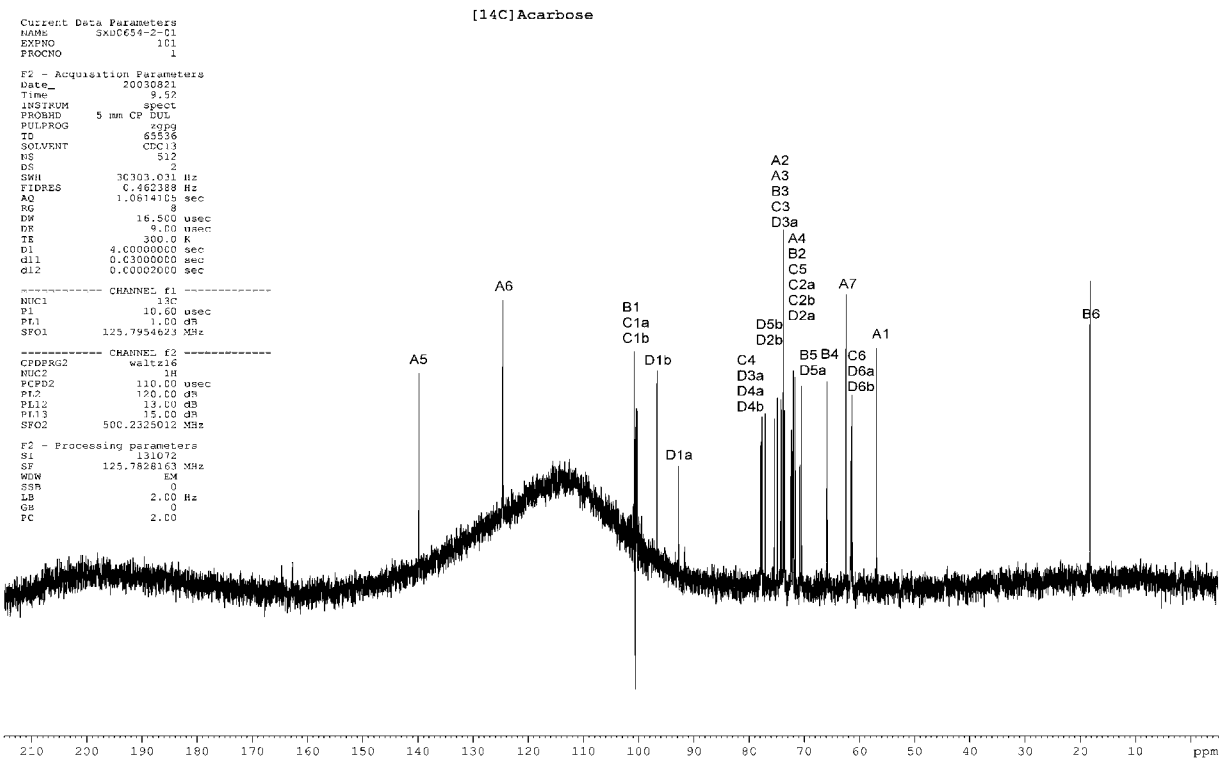

Figure 9 ^{13}C NMR spectrum of [^{14}C]acarbose produced by the optimized process, solvent D_2O.

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in five batches of 18.5 GBq each. Each batch was dissolved in 50 ml of ethanol/water (9:1). We determined a radiochemical purity of 95.49% by radio-HPLC.

Actinoplanes strain SN19910/37/21 was obtained from Biotechnology, Bayer HealthCare AG. All remaining solvents and reagents were obtained from commercial sources and used without further treatment unless indicated otherwise.

Liquid scintillation counting

Quantification of radioactivity was performed using a PerkinElmer TRI-CARB® 2500 TR liquid scintillation analyzer, with Ultima Gold™ cocktail used throughout.

High-performance liquid chromatography

The final compound was analysed by HPLC using a HP 1050 system Series II (Hewlett-Packard, Waldbronn, Germany) with a Ramona® Cell 5 (Raytest, Straubenhardt, Germany) for radioactivity detection. The LC–MS analytics were obtained at a Q-Star-Pulsar® with API source (Sciex, Toronto, Canada). The following HPLC and TLC systems were used for the purity check:

1. Hypersil® 120 APS-2 250-4 mm (Muder & Wochele, Berlin, Germany), column temperature: 35°C, flow rate: 1.8 ml/min, 28 phosphate buffer (0.61 g KH_2PO_4+0.78 g Na_2HPO_4/lH_2O), 72% acetonitrile, isocratic, detection: UV 210 nm.
2. Silica gel plate Si F254 20-5 cm (Merck, Darmstadt, Germany), eluent: methanol/8% ammonia 3:1 (v/v), detection: Raytest Rita®-3200 analyser (Raytest, Straubenhardt, Germany).

NMR spectra

The NMR spectra were recorded on a Bruker DRX 400 magnetic resonance spectrometer (Bruker, Rheinstetten, Germany).

Fermentation and work-up equipment

Water bath heating: immersion circulator UC-5B (Julabo Labortechnik, Seelbach, Germany), aeration: pressure pump WISA (ASF Thomas, Puchheim, Germany), stirring: magnetic stirrer Ikamag REO (IKA Labortechnik, Staufen, Germany), titrator: Mettler DL 21 (Mettler-Toledo, New York, USA), pH instrument: pH meter CG 822 (Schott Geräte, Mainz, Germany), polarimeter: Polar (IBZ Messtechnik, Hannover, Germany), conductometer: LP 42 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), pump: peristaltic pump Minipuls 2 (Gilson, Middleton, USA).

Table 4 Media recipe

	Medium 3	Medium 3a
Maltose hydrate	8.50 g/l	8.50 g/l
Bacto peptone	0.63 g/l	0.63 g/l
D(+)-glucose hydrate	2.31 g/l	
Deionized water		
pH	6.8	6.8
Autoclaving	20 min at 121°C	20 min at 121°C

Fermentation

Actinoplanes mutant SN19910/37/21 was used as the bio-agent. The induced mycelium was prepared by a three-step fermentation process with parameters similar to the manufacturing process. The third cultivation step was run in a 10-l fermenter for 96 h. The biomass was harvested by spinning down 600 ml of aliquots of the culture broth at $4100 \times g$ and 4°C for 10 min using sterilized 1-l centrifuge bottles. The supernatant was discarded and the pellet was re-suspended in each centrifuge bottle in 500 ml of sterile cold medium 3 (see recipe in Table 4). The biomass was spun down again at $4100 \times g$ for 10 min. The pellet in each bottle was re-suspended in cold medium 3a (Table 4) to a volume of 600 ml. The resulting suspensions were combined in a single sterile Erlenmeyer flask with stirring. The complete procedure of harvesting and washing the biomass was performed under sterile conditions. This pool of mycelium suspension offered a pH of 6.9 and a concentration of non-labelled acarbose of 1.7 g/l.

Radio-fermentation was performed in five parallel bottles.

Aliquots (150 ml) of the mycelium suspension were filled into sterile 1-l-glass bottles to form the labelling cultures. Each bottle was closed by a screw cap with an aperture containing a stainless steel lid with three connections, one for the inlet of sterile filtered air, one for the offgas and the third one for sample withdrawing and addition of liquids.

Five bottles were incubated side by side at a temperature of 18°C in a waterbath under agitation by magnetic stirrer at 500 rpm. The system was aerated by 1 vvm of moistened sterile air. An example of the set-up is shown in Figure 5.

After 1 h of incubation, the pH of each culture was measured. Pre-experiments had shown that the pH-value should be above 6.8.

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Labelling with carbon-14

The five D-[U-^{14}C]glucose solution batches, each of 18.5 GBq, were combined and evaporated to a final volume of approximately 10ml. After dilution with 8 ml of water the solution was filtered through a membrane filter (0.45 μm) to remove a small amount of insoluble colourless solid. The clear filtrate was filled up with water to 25 ml to prepare the administration solution.

The administration to the culture bottles was performed via syringe over a sterile filter in approximately 5-ml portions per incubation bottle as shown in Figure 5. In case of the last bottle, a smaller amount of labelled glucose remained.

The incubation was performed for 48 h under the following conditions: water bath temperature 27.8–28.0°C, magnetic stirring with 500 rpm, aeration with approximately 1 vvm (10 l/h). The incoming air was sterile filtered and moistened by passing through a washbottle filled with water. The outcoming air of each incubation bottle was passed through two washbottles filled with 200 ml (first bottle) and 150 ml (second bottle) of ethanolamine/water (1:1) (v/v) to absorb $^{14}CO_2$ formed during the incubation.

After 48 h, incubation was stopped by the addition of 10 ml of toluene to each incubation bottle via the administration tube without opening the system. Aeration and stirring were continued for 2 h to remove all $^{14}CO_2$.

Then the culture broths of all bottles were harvested and combined. The volume was determined as 1008 ml subtracting 10% as part of the contained mycelium. The content of acarbose was determined by HPLC/UV quantification using reference compound. The resulting product was 3.4 g of [^{14}C]acarbose.

Isolation and purification of [^{14}C]acarbose

Cation exchanger (45 g) and anion exchanger (15 g) were added to the above mixture to absorb the labelled acarbose, after careful stirring for 2 h and keeping for 16 h at 4°C without stirring. LS counting revealed more radioactivity in the solution than expected indicating that [^{14}C]acarbose adsorption was incomplete. Therefore, additional 13 g of cation exchanger and 4 g of anion exchanger were added. The mixture was stirred for 1.5 h. The remaining acarbose content was 0.11 g/l.

The resin was filtered off over a sieve nutsch filter that retained only the acarbose-loaded resin. The mycelium was removed completely. The resin was washed with water. Then it was suspended in 150 ml of water and 1 M sodium hydroxide solution (43 ml) was added by a titrator under stirring until a pH of 8.1 was reached. The mixture was filtered off from the resin which was washed with water giving a combined amount of 230 ml. LS counting revealed less radioactivity in the solution than expected indicating that [^{14}C]acarbose desorption was incomplete. So the resin was submitted in 100 ml of water and titrated with 1 M sodium hydroxide solution (6 ml) to pH 8.1. After suction and washing, 170 ml of filtrate was obtained with a content of 0.9 g of [^{14}C]acarbose. A third desorption (consumption of 3 ml of 1 M sodium hydroxide solution) gave 250 ml of filtrate with 0.35 g of [^{14}C]acarbose. Quantitative acarbose determination of the combined filtrates revealed 3.34 g. A total radioactivity of 16.5 GBq was determined. The radiochemical purity of [^{14}C]acarbose was 51.8% by radio-HPLC.

Further purification was performed by cation exchange chromatography. The solution was evaporated to a final volume of approximately 30 ml. This solution was pumped with a flow of 5 ml/min onto the separation column (16-mm diameter) filled with 185 ml of a special cation exchanger to a height of 840 mm. After loading, the column was washed with 550 ml of water. Elution was performed with a hydrochloric acid gradient. Detection was performed by polarimetry and subsequent conductometry. A pre-run fraction of 960 ml and product fractions of 17.5 ml each were sampled. The pure [^{14}C]acarbose-containing fractions 16–30 were combined. The radiochemical purity of the labelled acarbose was 91.7%. For acarbose recovery, the solution was adjusted with an anion exchanger MP 62 to a pH of 5.1. The resin was removed by filtration and washed with water giving a solution of 515 ml with 2.77 g of [^{14}C]acarbose. The radioactivity content was 8.399 GBq.

Final purification was performed by a second cation exchange chromatography. The above solution was concentrated to approximately 50 ml. This amount was pumped with a flow of 4 ml/min onto the separation column (26-mm diameter) filled with cation exchanger (122 ml). After loading, the column was washed with warm water (70°C) until the conductivity reached the zero value. The [^{14}C]acarbose-containing fraction was sampled by polarimetric detection.

A product fraction of 620 ml was sampled. The solution was concentrated to 15 ml and filled up with ethanol/water (1:9) to exactly 50 ml. This solution was used as the delivery form and all final analytics were made therefrom.

The total radioactivity was found to be 7936 MBq. The specific radioactivity was 2028 MBq/mmol (LS counting/UV quantification). This gave 2.5 g of [^{14}C] acarbose. The radiochemical purity was 99.09% (radio-HPLC) and the chemical purity was 97.68% (HPLC; 210 nm). Radio-TLC revealed a radiochemical purity of 98.77%.

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The radiochemical yield of carbon-14-labelled acarbose was 8.5% based on the starting D-[U-^{14}C]glucose.

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