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SYNTHESIS OF CARBON-11 LABELLED CALCIUM CHANNEL ANTAGONISTS

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KEYWORDS

^{11}C-labelled 1,4-dihydropyridines, synthesis, calcium channel antagonists

SUMMARY

A useful synthetic approach to carbon-11 labelled 1,4-dihydropyridines is described. Carbon-11 labelled calcium channel antagonists ^{11}C-Nifedipine, ^{11}C-Nisoldipine, ^{11}C-Nitrendipine and ^{11}C-CF_3-Nifedipine were synthesized by a modified Hantzsch method using protected carboxy functions. Deprotection of the carboxylic acids by alkaline hydrolysis followed by conversion into the corresponding potassium salts and subsequent methylation with $^{11}CH_3I$ produced the labelled compounds in very good chemical and radiochemical yields (94%).

INTRODUCTION

The vasodilating character and the hypotensive structure-activity relationships of dihydropyridines have been described[1,2]. Continuing interest in these compounds led us to investigate the synthesis of carbon-11 labelled 1,4-dihydropyridines as potential imaging radiopharmaceuticals in Positron Emission Tomography (PET).

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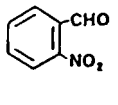
SYNTHESIS OF 1,4-DIHYDROPYRIDINES

There are numerous approaches in the literature to 1,4-dihydropyridine monocarboxylic acids which serve as precursors for our radiolabelling procedure[3-7], which either include various synthetic steps or result in poor yields of the desired products.

In our synthesis we used a modified Hantzsch-type cyclocondensation procedure[8] based on the extensive work of Meyer et al[9]. Condensation of the aromatic aldehydes **1-3** with the appropriate 3-aminocrotonic acid esters **4-6** and the acetoacetic acid ester **7** led in a one-pot synthesis to the methyl-ethylsulfonyl protected dihydropyridines **8-11** in yields varying from 41 to 74%, scheme 1



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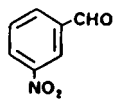

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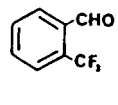

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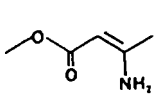

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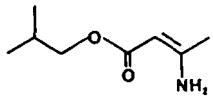

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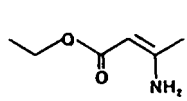

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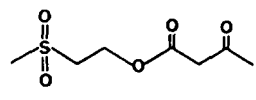

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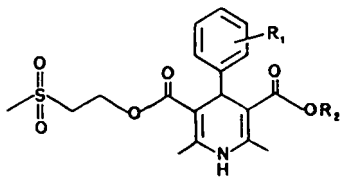
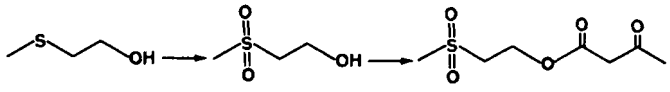


8-11

R_1	R_2	No.
o-NO_2	CH_3	**8**
o-NO_2	CH_2-CH(CH_3)$_2$	**9**
m-NO_2	CH_2-CH_3	**10**
o-CF_3	CH_3	**11**

scheme 1

The starting material **7** was obtained in almost quantitative yield by the reaction of 2,2,6-trimethyl-1,3-dioxin-4-one(2,2,4-1,3-6) with methylsulfonylethanol **7a** in refluxing xylene, scheme 2

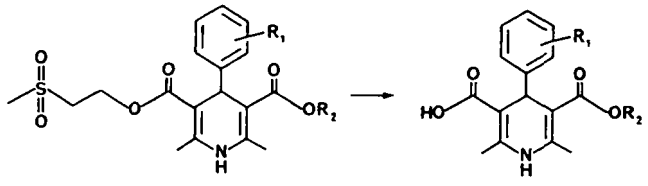


7a **7**

scheme 2

The methylsulfonylethyl moiety is a carboxylic acid protecting group which can selectively be removed under very mild alkaline conditions[10-13] and is stable under the reaction conditions during pyridine synthesis[7].

Cleavage of the protecting groups in compounds **8-11** with aqueous alkali yielded the dihydropyridine monocarboxylic acids **12-15** in yields ranging from 81 to 96%, scheme 3



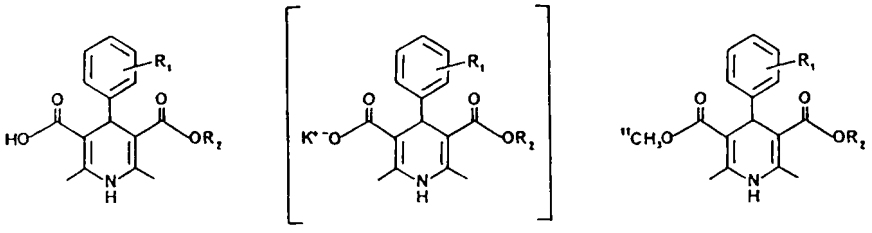
8-11 **12-15**

R_1	R_2	No.	R_1	R_2	No.
o-NO_2	CH_3	**8**	o-NO_2	CH_3	**12**
o-NO_2	CH_2-$CH(CH_3)_2$	**9**	o-NO_2	CH_2-$CH(CH_3)_2$	**13**
m-NO_2	CH_2-CH_3	**10**	m-NO_2	CH_2-CH_3	**14**
o-CF_3	CH_3	**11**	o-CF_3	CH_3	**15**

scheme 3

Until now carbon-11 labelling of 1,4-dihydropyridines has only been described twice in the literature: Syrota and his group[15] reacted the monocarboxylic acid derivative of PN 200-110 (Isradipine) with $^{11}CH_2N_2$ at a specific activity of approximately 7 x 10^5 Ci mol^{-1}. In an earlier synthesis Wilson and coworkers[16] labelled Nifedipine and Nicardipine by esterification of the monocarboxylic acids with $^{11}CH_3I$.

In our labelling procedure we used the readily available $^{11}CH_3I$ produced at the Compact Cyclotron CV 28 in Jülich.
Dihydropyridine monocarboxylic acids **12-15** were converted in-situ into their corresponding potassium salts **16a-19a** followed by methylation with $^{11}CH_3I$ under phase transfer conditions to yield the products **16-19** in high purity and good chemical and radiochemical yields, scheme 4



12-15 16a-19a 16-19

R_1	R_2	No.	No.	No.
o-NO_2	CH_3	**12**	**16a**	**16**
o-NO_2	CH_2-$CH(CH_3)_2$	**13**	**17a**	**17**
m-NO_2	CH_2-CH_3	**14**	**18a**	**18**
o-CF_3	CH_3	**15**	**19a**	**19**

scheme 4

MATERIALS AND METHODS

2-Nitrobenzaldehyde,**1**, 3-nitrobenzaldehyde,**2**, a,a,a-trifluoro-o-tolyladehyde,**3**, 3-aminocrotonic acid methyl-,**4**, and ethylester,**6**, 2,2,6-trimethyl-1,3-dioxin-4-one(2,2,4-1,3-6) (Diketene-acetone-addukt) and 2-(methylthio)ethanol were purchased from Aldrich/Steinheim,FRG.
The Diketene-acetone-addukt was distilled shortly before use under high vacuum, the bath temperature not exceeding 90 °C.
2-(methylsulphonyl)ethanol **7a** was prepared by oxidation of the corresponding thioalcohol by a modified procedure of Shim[7].

3-Aminocrotonic acid isobutyl ester **5** was synthesized by the method of Meyer et al[14].

^{1}H-NMR spectra were performed on a Varian EM 390 and a Varian VXRD 300 spectrometer.All values are in d with TMS as internal standard.IR-values (in cm^{-1}) were obtained on a Perkin-Elmer 1700 spectrophotometer.Melting points are taken in glass capillaries and are uncorrected.TLC was performed on silica plates Polygram^{R} Sil G/UV_{254}(Macherey-Nagel/Düren,FRG) with the solvent system toluene/methanol 98/2 (v/v).

$^{11}CH_3I$ was produced at the CV 28 Compact Cyclotron in Jülich via the $^{14}N(p,\alpha)^{11}C$-reaction, using a nitrogen gastarget. The radiochemical purity of $^{11}CH_3I$, checked by radio gas chromatography, was higher than 98%.

All carbon-11 labelled products were purified by HPLC on a RP-18 column with acetonitrile/water 80/20 (v/v) as an eluent.

3-Aminocrotonic acid isobutyl ester, (**5**)

In a three-necked flask, equipped with a distillation head an efficient stirrer and a thermometer, are mixed 74,12 g (1mol) iso-butanol and 100 ml dry xylene. Under vigorous stirring 142,15 g (1 mol) of freshly distilled 2,2,6-trimethyl-1,3-dioxin-4-one(2,2,4-1,3-6) are added all at once and the reaction flask is imediately immersed into an oilbath, preheated to 160°C. After 5 to 10 minutes the reaction starts and acetone begins to distill In the course of 45 minutes the reaction goes to completion and xylene is evaporated under reduced pressure (water aspirator). Vacuum distillation of the red residue yields 144,8 g (91%) of a colourless oil, bp_{10} 73-76°C.

144,8 g (0,91 mol)of the oil are dissolved in 300 ml of toluene. The flask is equipped with a gas inlet and a separatory funnel and 2 g of p-TsOH are added to the reaction mixture. Under reflux gaseous ammonia is bubbled through the mixture until the calcu-

lated amount of water has separated (6-9 hours). The organic solution is washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue is distilled under reduced pressure to yield **5** as a colourless liquid.

bp_{10}: 122-124°C colourless liquid, solidifies at 10°C

yield: 117 g = 76%

2-(Methylsulfonyl)ethanol, (**7a**)

To a well stirred solution of 46,1 g (0,5 mol) 2-(methylthio)-ethanol in 300 ml dry $CHCl_3$ are added under a nitrogen atmosphere at 0°C 2,2 eq. of MCPBA during 4 hours while MCBA precipitates. After 4 hours the reaction mixture is cooled to -10°C for 30 minutes and the precipitate is filtered off under Argon. The organic filtrate is dried over anhydrous Na_2SO_4 and the $CHCl_3$ is evaporated to leave a white, extremely hygroscopic distillable solid.

bp_{5}: 164-165°C mp:26-28°C, white, hygroscopic crystals

yield: 50,9 g = 82% ^{1}H-NMR ($CDCl_3$): 2.31(s, 3H, CH_3-SO_2-), 3,24(m, 2H, -CH_2OH), 3,61(s_{broad}, 1H, -OH), 4,57(m, 2H, -SO_2-CH_2-)

IR (KBr): 3510(OH), 1290(SO_2), 1143(SO_2)

2-(Methylsulfonyl)ethyl acetoacetate, (**7**)

In a flask, equipped with a distillation head a thermometer and a stirrer are placed 50 g (0,4 mol) 2-(methylsulfonyl)ethanol, 40 ml dry xylene and 63 g freshly distilled 2,2,6-trimethyl-1,3-dioxin-4-one(2,2,4-1,3-6). Under vigorous stirring the flask is immersed into an oil bath, preheated to 150°C. After 5 minutes the reaction starts and has gone to completion in 1 hour. Evaporation of xylene leaves a yellow, oily residue which gives one spot on TLC and is used without further purification (distillation leads to decomposition). After some days at 0°C (**7**) solidifies to give yellow crystals.

mp: 34-37°C, yellow crystals yield: 78,8 g = 94% ^{1}H-NMR ($CDCl_3$):

2,25(s, 3H, $-COCH_3$), 2,31(s, 3H, CH_3SO_2-),3.30(m, 2H, $-CH_2SO_2-$), 3,51(s, 2H, $-COCH_2-$), 4,63(m, 2H, $-OCH_2-$) IR (KBr): 1730(ester), 1695(C=O), 1285(SO_2), 1140(SO_2)

General procedure for the synthesis of 1,4-dihydropyridines (8-11)
0,1 mol benzaldehyde, 0,1 mol 3-aminocrotonate and 0,1 mol acetoacetate are dissolved in 100 ml of dry ethanol under a nitrogen atmosphere. The mixtures are refluxed for 9 to 12 hours and cooled to room temperature. Evaporation of the solvent gives oily residues which are crystallised from appropriate solvents or chromatographed on silica gel 60.

2,6-dimethyl-4-(2'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-methyl-5-(2'-methylsulfonyl)ethyl ester, (8) mp: 124-126°C (125-127°C)[6], yellow crystals (EtOH) yield: 17,4 g = 41% ^{1}H-NMR ($CDCl_3$): 2,2o(s, 3H, $-CH_3$), 2,27(s, 3H, $-CH_3$), 2,85(s, 3H, $-SO_2-CH_3$), 3,31(m,2H, $-CH_2-SO_2$), 3,42(s, 3H, $-OCH_3$), 4,28(m, 2H, $-OCH_2-$), 5,53(s, 1H, -CH), 7,45(m, 4H, phenyl), 8,96(s, 1H, -NH) IR (KBr): 3320(NH), 1713(C=O), 1282(SO_2), 1141(SO_2) $C_{19}H_{22}N_2O_8S$, 426,459 g x mol^{-1}

2,6-dimethyl-4-(2'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-isobutyl-5-(2'-methylsulfonyl)ethyl ester, (9) mp: tan coloured oil yield: 20,6 g = 44% 1H-NMR (CDCl3): 0.98-1,22(2xd, 6H, $-(CH_3)_2$), 2,37(s, 3H, $-CH_3$), 2,39(s, 3H, $-CH_3$), 2,91(s, 3H, $-SO_2-CH_3$), 3,36(m, 2H, $-CH_2-SO_2$), 3,39(d, 2H, $-O-CH_2-CH-$), 4,41(m, 2H, $-OCH_2-$), 4,91(m, 1H, $-CH-(CH_3)_2$), 5,82(s, 1H, -CH), 6,64(s, 1H, -NH), 7,41-7,96(m, 4H, phenyl) IR (cap): 3342(NH), 1694(C=O), 1294(SO_2), 1149(SO_2) $C_{22}H_{28}N_2O_8S$ 468,539 g x mol^{-1}

2,6-dimethyl-4-(3'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-ethyl-5-(2'-methylsulfonyl)ethyl ester, (10) mp: 108-111°C, yellow crystals (EtOH) yield: 33,4 g = 76%

^{1}H-NMR ($CDCl_3$): 1,13(d, 3H, $-CH_2-CH_3$), 2,31(s, 3H, $-CH_3$), 2,37(s, 3H, $-CH_3$), 2,89(s, 3H, $-SO_2-CH_3$), 3.14(m, 2H, $-CH_2-SO_2-$), 3,42(s, 3H, CH_3O-), 4,32(m, 2H, $-OCH_2-$), 5,71(s, 1H, -CH), 8,08(s, 1H, -NH), 7,49-8,20(m, 4H, phenyl) IR (KBr): 3335(NH), 1698(C=O), 1291(SO_2), 1145(SO_2) $C_{20}H_{24}N_2O_8S$ 440,486 g x mol^{-1}

2,6-dimethyl-4-(2'-trifluoromethylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-methyl-5-(2'methylsulfonyl)ethyl ester, **(11)**

mp: yellow oil yield: 25,6 g = 57% ^{1}H-NMR ($CDCl_3$): 2,23(s, 6H, 2xCH_3), 2,97(s, 3H, CH_3-SO_2-), 3,45(s, 3H, CH_3O-), 3,48(m, 2H, $-CH_2-SO_2-$), 4,39(m, 2H, $-OCH_2-$), 5,38(s, 1H, -CH), 7,30-7,87 (m, 4H, phenyl), 8,76(s, 1H, -NH) IR (cap): 3374(NH), 1691(C=O), 1300(SO_2), 1158(SO_2) $C_{20}H_{22}F_3NO_6S$ 449,459 g x mol^{-1}

General procedure for the alkaline hydrolysis of the protected compounds **(8-11)** and synthesis of the monocarboxylic acids **(12-15)**

To 10 mmol of the appropriate ester, suspended in 100 ml ethanol 50% (v/v) is added under vigorous stirring a solution of 1N-NaOH from a burette. After adjusting the pH of the solution to 9,5-10, the pH is maintained during the hydrolysis by the addition of the calculated amount of alkali. The progress of the reaction is monitored by clarification of the mixture. When all the alkali has been used, stirring is continued for a further 2 hours. The reaction mixture is then carefully poured into 250 ml of 4N HCl and the resulting slurry is stirred at 5°C for 6 hours. The product is filtered, washed with ice-cold diluted HCl and dried over P_2O_5 under high vacuum. On TLC the products show only one spot, no starting material is detectable (silica plates, light petroleum/diethylether/ acetic acid 80/20/1 (v/v/v)).

2,6-dimethyl-4-(2'nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-monomethyl ester, **(12)**

mp: 183-185°C (181-184°C)[6], yellow crystals yield: 2,7 g = 81%
^{1}H-NMR ($CDCl_3$): 2,25(s, 6H, 2x-CH_3), 3,51(s, 3H, -OCH_3),
5,72(s, 1H, -CH), 5,99(s_{broad}, 1H, -NH), 7,25-7,67(m, 4H, phenyl),
11,80(s_{broad}, 1H,-COOH) IR (KBr): 3384(NH), 1697(C=O)
$C_{16}H_{16}N_2O_6$ 332,312 g x mol^{-1}

2,6-dimethyl-4-(2'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-monoisobutyl ester, **(13)**
mp: 179-181°C dark-yellow crystals yield: 3,4 g = 91%
^{1}H-NMR ($CDCl_3$): 0,98-1,24(2xd, 6H, $(CH_3)_2$), 2,34(s, 3H, -CH_3),
2,38(s, 3H, -CH_3), 3,39(d, 2H, -OCH_2-), 4,93(m, 1H, -CH-$(CH_3)_2$),
5,80(s, 1H, -CH), 6,64(s, 1H, -NH), 7,42-7,91(m, 4H, phenyl),
11,74(s_{broad}, 1H, -COOH) IR (KBr): 3332(NH), 1672(C=O)
$C_{19}H_{22}N_2O_6$ 374,393 g x mol^{-1}

2,6-dimethyl-(3'-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-monoethyl ester, **(14)**
mp: 157-159°C, light brown crystals yield: 3,3 g = 96%
^{1}H-NMR ($CDCl_3$): 1,15(t, 3H, -OCH_2-CH_3), 2,29(2xs, 6H, 2x-CH_3),
4,00(m, 2H, -OCH_2-), 5,00(s, 1H, -CH) 7,54-8,04(m, 4H, phenyl),
8,95(s, 1H, -NH), 11,82(s_{broad}, 1H, -COOH) IR (KBr): 3360(NH),
1659(C=O) $C_{17}H_{18}N_2O_6$ 346,339 g x mol^{-1}

2,6-dimethyl-4-(2'-trifluoromethylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-monomethylester, **(15)**
mp: 143-146°C, yellow crystals yield: 3,3 g = 93%
^{1}H-NMR ($CDCl_3$): 2,23(s, 6H, 2x-CH_3), 3,45(s, 3H, -OCH_3),
5,38(s, 1H, -CH), 7,24-7,83(m, 4H, phenyl), 8,76(s, 1H, -NH),
11,78(s_{broad}, 1H, -COOH) IR (KBr): 3358(NH), 1679(C=O)
$C_{17}H_{16}F_3NO_4$ 355,312 g x mol^{-1}

Methylation reactions of monoacids **(12-15)** and synthesis of ^{11}C-labelled compounds **(16-19)** via potassium salts **(16a-19a)**

1 μmol monoacid is suspended in 0,98 eq. ethanolic KOH

(2 mg solid KOH per ml dry ethanol) and stirred for 2 minutes at room temperature or sonicated for 30 seconds. The cloudy solution thus obtained is used without further purification and may be kept for one week at -20°C under an inert gas atmosphere. The potassium salts may be isolated but decompose spontaneously if not handled under inert gas and absolute exclusion even of traces of moisture.
Isolation of the salts proceeds as follows:
Repeated addition and evaporation of dry ethanol leaves a residue which is taken up in dry, ice cold acetonitrile, filtered under argon, washed twice with ice cold acetonitrile and dried over P_2O_5. Yields range from 30 to 50%.

The following methylation procedure is described for compound (**18**) and has been used for all calcium antagonists.
1 ml (11 μmol) of the potassium salt suspension (4 mg (**18a**)) is placed in a reaction vessel. $^{11}CH_3I$, prepared by $LiALH_4$-reduction of $^{11}CO_2$ and subsequent iodination with HI, is bubbled through a cooled (-78°C) mixture of 500 μl anhydrous THF, 500 μl acetonitrile and 0,2 mg cis-Dicyclohexano-18-crown-6 until trapping is completed (1 min.). The mixture is transfered to the reaction vessel, heated to 80°C for 4 minutes, cooled to room temperature and transfered onto a RP 18 HPLC column (500x20 mm i.d.).
Purification is carried out by using acetonitrile/water 80/20 (v/v) as an eluent, the flow rate being 12 ml/min. On leaving the column the radioactivity (Herfurth, Monitor H 1359 A) and the mass (LKB, Uvicord S) are measured and recorded. Analysis (NMR, IR, MS) of the cold products showed no impurities and were identical with those of authentic specimen.
radiochemical purity: 99%
chemical yield: 94%
specific activity: 1,2 x 10^6 Ci mol^{-1}

DISCUSSION

A potential application of these labelled dihydropyridine derivatives is the in vivo study of Ca^{2+} in smooth and cardiac muscle cells.

In the hypertrophic cardiomyopathy of syrian hamsters a calcium overload with an about 10-fold increase in myocardial calcium has been observed[17,18]. Furthermore a significant increase in the density of calcium channels in hamsters as well as in humans with hypertrophic cardiomyopathy has been described[19,20]. Nevertheless the heterogeneity of myocardial tissue which contains cardiomyocytes as well as vascular smooth muscle cells has to be taken into account using ^{11}C-labelled calcium antagonists in vivo.

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