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# Radiosynthesis of ML03, a novel positron emission tomography biomarker for targeting epidermal growth factor receptor via the labeling synthon: [<sup>11</sup>C]acryloyl chloride

Iris Ben-David, Yulia Rozen, Giuseppina Ortu, Eyal Mishani\*

*Department of Medical Biophysics and Nuclear Medicine, Hadassah Hebrew University Hospital, Jerusalem 91120, Israel*

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## Abstract

An automated procedure for the radiosynthesis of the labeling synthon [<sup>11</sup>C]acryloyl chloride was developed and applied for labeling several N-acryl amides with carbon-11. [<sup>11</sup>C]-6-acrylamido-4-(3,4-dichloro-6-fluoroanilino)quinazoline (ML03), a novel PET biomarker targeting the epidermal growth factor receptor tyrosine kinase (EGFr-TK) in cancer, was successfully prepared using this labeled synthon in a fully automated manner. Two other potential anticancer drugs were also labeled using the developed methodology. The potency of ML03 to inhibit autophosphorylation of EGFr-TK was evaluated by an ELISA assay indicating a low IC<sub>50</sub> of 0.037 nM.

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*Keywords:* PET; Carbon-11; Acid chloride; EGFr-TK

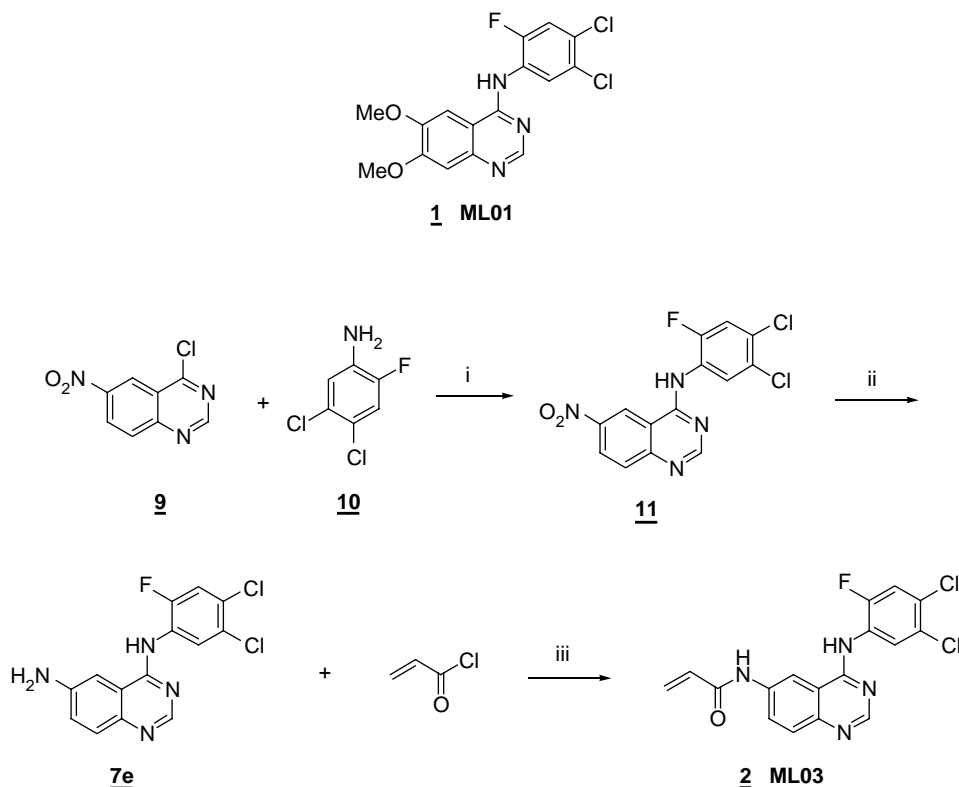
## 1. Introduction

Positron emission tomography (PET), a nuclear medicine imaging technology, allows the three-dimensional, quantitative determination of the distribution of radioactivity within the human body. Our research interest is associated with the development of PET tracers that would target the epidermal growth factor receptor tyrosine kinase (EGFr-TK) (Levitzki and Gazit, 1995), as a tool for the diagnosis and mapping of malignant tumors over-expressing these receptors (Mishani et al., 1999). During the course of this study, several reversible inhibitors were labeled with fluorine-18 ( $T_{1/2}$  109.6 min), and their potential as PET biomarkers was examined in vitro. The most promising compound, ML01 (**1**), was also tested by in vivo experiments, and by PET study in mice (Bonasera et al., 2001). The reversible inhibitors were proved to be

highly specific to the EGFr-TK, however, the presence of high cellular concentration of competitive ATP molecules affected fast washout of **1** from the target cells, and thus disqualified it for further advancement (Bonasera et al., 2001). In order to overcome the ATP competitive binding, we turned to label an irreversible analogue of **1**, 6-acrylamido-4-(3,4-dichloro-6-fluoroanilino)quinazoline, ML03 (**2**) (Scheme 1). Compounds with similar structure, demonstrating both reversible and irreversible inhibitory activities towards EGFr-TK, have been reported to enter clinical trials as potential anticancer drugs (Renhowe, 2001). The irreversible activity displayed by ML03 is a result of the high chemical reactivity of the  $\alpha,\beta$ -unsaturated amide moiety towards thiol groups, resulting in covalent binding between cysteine residues in the receptor's active site and the inhibitor molecule (Smaill et al., 1999). This reaction pattern made it especially attractive for labeling **2** with carbon-11 ( $T_{1/2}$  20.4 min) on the acryl group, which may assure the remaining of the radioactivity within the active site of the enzyme even after metabolic degradation (Bonasera et al., 2001).

\*Corresponding author. Tel.: +972-2-6777931; fax: 972-2-6421203.

E-mail address: [mishani@md.huji.ac.il](mailto:mishani@md.huji.ac.il) (E. Mishani).



Scheme 1. Cold synthesis of ML03. Reaction conditions: (i) isopropanol, reflux; (ii) Raney-Ni,  $(\text{NH}_2)_2$ , EtOH; and (iii) THF, 0°C.

Chemically reactive acid chlorides are widely used as acylation agents in organic transformations, and carbon-11-labeled acid derivatives and acid chlorides are correspondingly well-known labeling synthons (Banks et al., 1990; Luthra et al., 1985).  $\alpha,\beta$ -unsaturated  $[^{11}\text{C}]$ -acryloyl chloride (**5**) has also been described (Lasne et al., 1992), yet with limited use as labeling synthon.

We report here the development of an automatic routine procedure for the preparation of **5** in high specific activity, as a labeling synthon for the radiosynthesis of  $[^{11}\text{C}]$ ML03 (**2**), a novel potential PET biomarker targeting the EGFr-TK. The application of  $[^{11}\text{C}]$ acryloyl chloride synthon (**5**) for labeling several other *N*-acryl amides is described.

## 2. Results and discussion

We initially considered labeling ML03 (**2**) with fluorine-18. However, labeling **2** with fluorine-18 would involve five-step radiosynthesis, with the introduction of the labeled isotope at the beginning of the procedure. Both the long synthesis time and the difficulties associated with automation of the procedure disfavored labeling with fluorine-18. Labeling with carbon-11, on

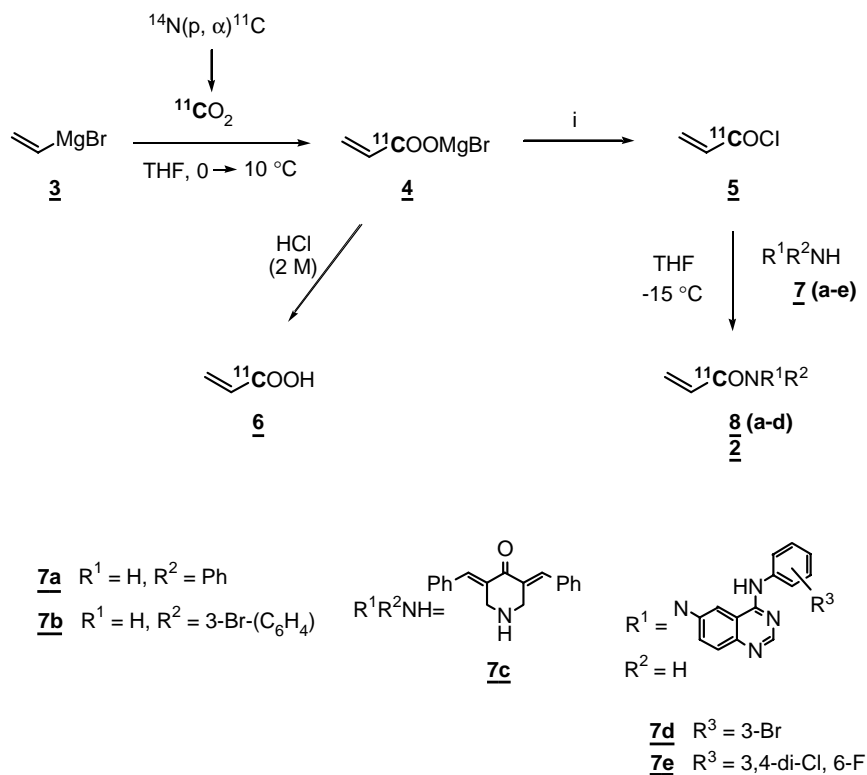
the other hand, would enable the desirable introduction of the radioisotope into the molecule at the last step of the synthesis. Using 1- $[^{11}\text{C}]$ acryloyl chloride (**5**) as the labeling synthon allows short-time synthesis and relatively simple automation.

Radiosynthesis of  $\alpha,\beta$ - $[^{11}\text{C}]$ -unsaturated acyl derivatives is known, although their use as labeling synthons is quite limited, despite the presence of these functionalities in many drugs. The low motivation is probably associated with the high chemical reactivity of the  $\alpha,\beta$ -unsaturated moiety, leading to undesirable polymerization side reactions and hence low yields. The need for a reliable and high specific activity procedure prompted us to develop an automated synthesis of **5**, allowing routine production of various compounds containing the labeled acryl moiety. The automation was aimed to make use of existing instrumentation, which has been approved by our Pharmacy Department for the synthesis of several biomarkers already in clinical use, such as  $[^{11}\text{C}]$ Choline and  $[^{11}\text{C}]$ Deprenil. A commercial module (Nuclear Interface, Munster, Germany), although dedicated for the synthesis of  $[^{11}\text{C}]$ MeI, was easily adapted by minor modifications to its new purpose: the synthesis of  $[^{11}\text{C}]$ acryloyl chloride labeling synthon and radiosynthesis of *N*- $[^{11}\text{C}]$ acrylamides. The higher boiling point of

acryloyl chloride (compared to MeI) entailed the shortage of the lines through which the synthon should be distilled. This was achieved by connecting V9, coming from the first reactor directly to the T-connector, connected to reactor-2, thus bypassing the distillation columns. In general,  $^{11}\text{C}$ -labeled acid chlorides are obtained by the carbonation of alkyl magnesium bromide (Grignard reagents) or the corresponding alkyl lithium with  $[^{11}\text{C}]\text{CO}_2$ .  $[^{11}\text{C}]$ Acrylic acid (**6**) and its derivatives have been synthesized in the past, both in a carrier-added level (Kutzmann et al., 1962) and no-carrier-added procedure (Lasne et al., 1992). The latter was performed using vinyl magnesium bromide (**3**), and provided the labeled acid in low specific radioactivity (500 MBq/ $\mu\text{mol}$ , at end of bombardment (EOB)). Thus, the conditions for trapping  $[^{11}\text{C}]\text{CO}_2$  in a solution of **3** were discussed in those publications, where temperature seemed to be of major importance (Lasne et al., 1992). Our own attempt to synthesize  $[^{11}\text{C}]$ carbonate (**4**), revealed the following optimal conditions: carbonylation reactor temperature kept between  $0^\circ\text{C}$  and  $10^\circ\text{C}$  (set to  $7^\circ\text{C}$ ), cooling trap warmed to  $-50^\circ\text{C}$  during the transfer of  $[^{11}\text{C}]\text{CO}_2$ , and argon flow rate of 20 ml/min. After 90 s, the temperature of the reactor was raised to

$30^\circ\text{C}$  for 1 additional minute. Lower initial temperature in the reactor was found to affect crystallization of the Grignard complex and caused insufficient trapping. As a result, slower release of the activity from the cooling trap (warmed to  $-50^\circ\text{C}$ ) was necessary. The Grignard reagent's quality and concentration were also qualitatively evaluated. Since commercially available vinyl magnesium bromide solution in THF failed to yield any labeled acrylic acid, freshly and carefully prepared Grignard reagent had to be used. The use of high reagent concentrations did not increase trapping efficiency. Instead, crystallization of the reagent and significant decrease in specific activity were observed. Solutions of 0.3 M were found to be useful and stable for a few days under argon at  $-20^\circ\text{C}$ .

$[^{11}\text{C}]$ Acrylic acid (**6**, Scheme 2) was prepared by trapping  $[^{11}\text{C}]\text{CO}_2$  in a solution of **3**, followed by hydrolysis of the salt **4** with 200  $\mu\text{l}$  of hydrochloric acid (2 M). The reaction mixture was then diluted and analyzed by HPLC. In addition to 55% of the expected acrylic acid, the radiochromatogram indicated the presence of ca. 40% polar by-product, with a retention time of 2.8 min. Performing the hydrolysis at a higher temperature did not affect product distribution.



Scheme 2. Radiosynthesis of  $[^{11}\text{C}]$ acryloyl chloride and its reactions with various amines. Reaction conditions: (i) Method I, 1,2- $\text{C}_6\text{H}_4(\text{COCl})_2/2,6\text{-di-}t\text{-BuPyridine}$ ; Method II,  $\text{SOCl}_2/\text{DMF}$ , polymer-bound 2,6-di- $t$ -BuPyridine column; and Method III, citric acid,  $\text{PCl}_5$ , polymer-bound 2,6-di- $t$ -BuPyridine column.

However, adding a larger volume of HCl (2 ml) and immersing the closed vessel reaction mixture in an ultrasonic bath for 10 min increased the ratio between 6 and the polar material from 50:40 to 60:30. These results may suggest that the polar material contains aggregated magnesium [ $^{11}\text{C}$ ]acrylate salts.

The [ $^{11}\text{C}$ ]acryloyl chloride synthon (**5**) was indirectly evaluated through its reaction with aniline (**7a**) as a model amine, and comparison of the product's chromatographic properties with those of unlabeled standard. Chlorination of the [ $^{11}\text{C}$ ]acrylic acid magnesium salt (**4**) was carried out by reaction with phthaloyl dichloride in the presence of 2,6-di-*tert*-butylpyridine, according to previously reported methods (Lasne et al., 1992). The reagents were prediluted with 150 ml dry THF in order to overcome their relatively high viscosity and allow the free introduction to the reactor. The [ $^{11}\text{C}$ ]acryloyl chloride synthon (**5**), warmed to 90°C, was carried by a flow of argon from first reactor to a second reactor, containing **7a** in dry THF at -15°C. In some cases, undesired bumping was observed during distillation followed by transfer of crude mixture droplets to the trapping reactor. These cases resulted in chemical and radiochemical unclean product mixtures and low yield of the desired anilide. The flow of argon was therefore maintained low at the beginning of distillation (25 ml/min) and increased after 2 min to 30 ml/min. The radiochemical yield of *N*-phenyl-1-[ $^{11}\text{C}$ ]acryl amide

(**8a**), 23–25% (EOB), obtained in the automated synthesis was similar to previously reported results, both with non-automated procedures and  $^{11}\text{C}$ -labeling of saturated analogues (Luthra et al., 1990). The attained specific activity, on the other hand, displayed great improvement compared to non-automated syntheses. Running the production under automatic carefully controlled conditions diminished isotopic dilution and enabled a specific activity as high as 92.5 GBq/ $\mu\text{mol}$ , average 55.5 GBq/ $\mu\text{mol}$  (2.5 Ci/ $\mu\text{mol}$ , average 1.5 Ci/ $\mu\text{mol}$ ). Other chlorination reagents for synthesizing the acryloyl chloride were examined. For example, **4** was treated with thionyl chloride and small amount of dry DMF and the mixture was distilled at 110°C through a column containing polymer-bound 2,6-di-*tert*-butylpyridine. Distillation through a column evidently gave cleaner reaction solutions in the second reactor. However, lower radiochemical yields and significant isotopic dilution were observed, probably due to  $\text{CO}_2$  contamination of the thionyl chloride reagent. In another experiment, **4** was hydrolyzed with citric acid dissolved in triethylene glycol, and **6** was distilled at 120°C through two columns; the first of which contained  $\text{PCl}_5$  to perform a solid-gas phase chlorination reaction, and the second one contained polymer-bound 2,6-di-*tert*-butylpyridine to trap vapors of HCl. The latter method yielded even poorer results (the results are summarized in Table 1).

Table 1  
Carbon-11-labeled *N*-acryl amides **2**, **8** (a-d)

| Precursor | Labeling method <sup>a</sup> | HPLC <sup>b</sup>  |                    |               |                      | No. of entries | Synthesis time <sup>c</sup> (min) | R.C.Y. <sup>d</sup> at EOB (%) | S.A. <sup>e</sup> at EOB (GBq/ $\mu\text{mol}$ ) |
|-----------|------------------------------|--------------------|--------------------|---------------|----------------------|----------------|-----------------------------------|--------------------------------|--|
|           |                              | A <sup>f</sup> (%) | B <sup>g</sup> (%) | Flow (ml/min) | Retention time (min) |                |                                   |                                |  |
| 7a        | I                            | 30                 | 70                 | 1.2           | 8.1                  | 3              | 25                                | 24 ± 3                         | 55.5 (1.5)                                       |
| 7a        | II                           | 30                 | 70                 | 1.2           | 8.1                  | 2              | 25                                | 5-7                            | 7.4 (0.2)  |
| 7a        | III                          | 30                 | 70                 | 1.2           | 8.1                  | 2              | 25                                | <1                             | <sup>h</sup>                                     |
| 7b        | I                            | 30                 | 70                 | 1.7           | 16.8                 | 3              | 35                                | 21 ± 3                         | 51.8 (1.4)                                       |
| 7c        | I                            | 52                 | 48                 | 1.5           | 10.6                 | 3              | 30                                | 13 ± 5                         | 62.9 (1.7)                                       |
| 7d        | I                            | 36                 | 64                 | 1.4           | 15.1                 | 5              | 35                                | 12 ± 5                         | 33.3 (0.9)                                       |
| 7e        | I                            | 40                 | 60                 | 1.7           | 11.7                 | 30             | 45 <sup>i</sup>                   | 13 ± 3                         | 66.6 (1.8)                                       |
|           |                              | (46)               | (54)               | (13)          | (22)                 |                |                                   |                                |  |

<sup>a</sup> For methods I, II, III see Scheme 2.

<sup>b</sup> Numbers in parentheses refer to preparative separation.

<sup>c</sup> Overall, including HPLC purification.

<sup>d</sup> Radiochemical yield, corrected to EOB, determined according to the activity measured after HPLC separation and initially trapped [ $^{11}\text{C}$ ]CO<sub>2</sub>.

<sup>e</sup> Specific radioactivity. Numbers in parentheses are in Ci/ $\mu\text{mol}$  units.

<sup>g</sup> Acetate buffer 0.1 M pH 3.8.

<sup>f</sup> Acetonitrile.

<sup>h</sup> Was not determined.

<sup>i</sup> Preparative HPLC separation.

3-Bromoaniline (**7b**) was treated with **5** in the same manner as aniline (**7a**). The yield and specific activity of *N*-(3-bromophenyl)-1- $^{11}\text{C}$ acryl amide (**8b**) were similar, in spite of the presence of electron-withdrawing substituent on the aromatic ring.

3,5-Bisphenylidene-4-piperidone (**7c**) and *N*-Acryl-3,5-bisphenylidene-4-piperidone (**8c**) were described as potential cytotoxic and anticancer agents (Dimmock et al., 2001). We labeled **8c** with carbon-11 on the acryl group, as another example for the application of **5**. Piperidone **7c** in dry THF was placed in a second reactor and allowed to react with **5** as described above. HPLC analysis of the crude mixture revealed a single radioactive compound, which was recovered in 13% radiochemical yield. **8c** was identified according to its retention time and by co-injection with cold standard. Despite the higher nucleophilicity of an aliphatic secondary amine, further reaction of the  $\alpha,\beta$ -unsaturated amide with large excess of the amine precursor, via Michael addition, was not observed (Lasne et al., 1992).

6-Acrylamido-4-(3-bromoanilino)-quinazoline (**8d**) is one of the first structures, based on a quinazoline building block, that was reported to have an irreversible inhibition activity towards EGFr-TK (Smaill et al., 1999; Vincent et al., 2000). Non-labeled **8d** demonstrates good therapeutic indices against A431 epidermoid and H125 human lung xenograft in mice (Smaill et al., 1999), and similar compounds are present in clinical trials against erb-B positive breast cancer (Renhowe, 2001). **5** was used to label **8d** in the same manner described for the anilines using 6-amino-4-(3-bromoanilino)-quinazoline (**7d**) (Rewcastle et al., 1995) as precursor. **8d** was produced in 12% recovered radiochemical yield in 35 min synthesis time (including purification).

The cold preparation of 6-acrylamido-4-(3,4-dichloro-6-fluoroanilino)quinazoline (ML03, **2**) is outlined in Scheme 1. 4-Chloro-6-nitroquinazoline (**9**) was coupled with 3,4-dichloro-6-fluoroaniline (**10**), and the nitro moiety reduced by means of Raney-Ni and hydrazine hydrate (Mishani et al., 1997; Yuste et al., 1982) to yield the corresponding amine **7e** (Mishani et al., 2001). Further reaction with acryloyl chloride gave **2** in overall 45% yield. The ability of ML03 to inhibit the autophosphorylation of EGFr-TK was screened by an ELISA assay. The dose-response curve shown in Fig. 1 was obtained from four assays, in which the concentrations of **2** ranged from  $6.1 \times 10^{-8}$  to 15 nM, for the first three, and between  $1.6 \times 10^{-9}$  and 10 nM for the fourth one. The curve ( $r^2=0.8403$ ) obtained from the combined data gave an  $\text{IC}_{50}$  of 0.037 nM with an interval of confidence ranging between 0.010 and 0.134 nM indicating a high potency of ML03 towards EGFr-TK. The potential PET biomarker was successfully labeled with carbon-11 as follows. The distillation and reaction of synthon **5** with **7e** were followed by HPLC purification, using the built-in HPLC system of the  $^{11}\text{C}$ MeI module.

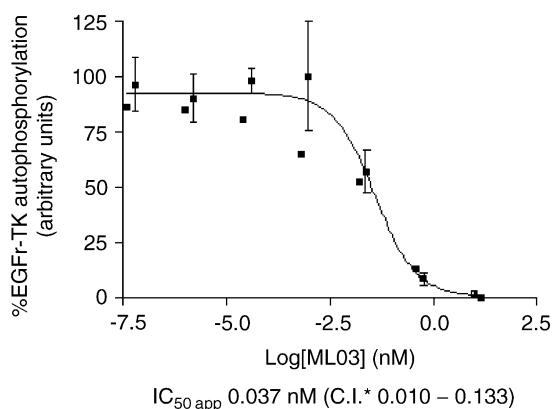


Fig. 1. ML03 inhibitory activity of the autophosphorylation in A431 cells lysate (ELISA assay). The function is a sigmoidal dose-response curve with variable slope ( $n=4$ ). \*C.I., 95% interval of confidence.

The diluted crude mixture (ca. 1.2 ml) was automatically loaded onto 2 ml loop, and injected to a C18 reverse phase preparative column. The desired fraction ( $R_t$  22 min) was collected into the solid-phase extraction flask. In order to dilute the acetonitrile in the mobile phase, the flask was preloaded with 70 ml of water and 2.5 mmol NaOH. Basic conditions were provided to obtain **2** as a free base. Finally the product was separated by a C18 cartridge and eluted with ethanol and saline. The automated procedure described enabled us to reliably and reproducibly ( $n > 35$ ) obtain ML03 in high yields (0.55–0.74 GBq, 15–20 mCi), sufficient for potential human use. HPLC analysis of the product mixture revealed high radiochemical (>99%) and chemical purities (the product solutions contained 0.5–3 ppm of remaining precursor which have moderate affinity to the receptor and therefore decrease the effective specific activity). The high specific radioactivity achieved in the automated radiosynthesis (66.6 GBq/ $\mu\text{mol}$ , 1.8 Ci/ $\mu\text{mol}$ , EOB) is an important factor for the developments of PET biomarkers in general, and for ML03, designed to target in vivo low capacity receptor systems in particular.

### 3. Conclusions

A reliable automated radiosynthesis for the preparation of  $^{11}\text{C}$ acryloyl chloride labeling synthon (**5**) was developed and used for the radiosynthesis of several *N*- $^{11}\text{C}$ acryl amides in good yields and high specific activity. ML03 (**2**), a novel potential PET biomarker was synthesized and found to have high potency towards EGFr-TK in cells study. ML03 (**2**) was successfully labeled with carbon-11 at the acryl group using synthon

5. The described methodology was also applied to label two other potential anticancer drugs: *N*-acryl-3,5-bisphenylidene-4-piperidone (**8c**) and 6-acrylamido-4-(3-bromoanilino)-quinazoline (**8d**), and can be used for the labeling of various other biologically reactive molecules, aimed for PET.

## 4. Experimental

### 4.1. General

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Merck or J.T. Baker. Chemicals were used as supplied, excluding THF which was refluxed over sodium and benzophenone and freshly distilled prior to use. Mass spectroscopy was performed in EI mode on an Thermo Quest—Finnigan Trace MS—mass spectrometer at the Hadassah-Hebrew University mass spectroscopy facility.  $^1\text{H}$ -NMR spectra were obtained on a Bruker AMX 400 MHz instrument, in  $d_8$ -THF. Elemental analysis was performed at the Hebrew University Microanalysis Laboratory. Melting points are reported without correction.

Radiosyntheses were carried out on a [ $^{11}\text{C}$ ]CH $_3$ I module (Nuclear-Interface, Munster, Germany). The module contains a built-in HPLC system: pump, Sykam S1021 (flow 0.5–30 ml/min); UV detector Linear UVIS 200 with preparative UV flow cell, path length 3 mm; and a radioactivity flow detector. Automated HPLC preparative purifications were performed on Bischoff Nucleosil 100-7-C18 reverse phase preparative column (7  $\mu\text{m}$ , 250  $\times$  16 mm $^2$ ).

Other HPLC separations were carried out on a Varian 9012Q pump, a Varian 9050 variable wavelength detector operating at 254 nm, and a Bioscan Flow-Count radioactivity detector with a NaI crystal. Analyses of the labeled and unlabeled compounds were performed on a reversed phase system using Waters  $\mu$ -Bondapak C18 analytical column (10  $\mu\text{m}$ , 300  $\times$  3.9 mm $^2$ ), with mobile phase systems composed of various ratios of acetonitrile and acetate buffer 0.1 M pH 3.8. Specific radioactivities were determined by HPLC, using cold mass calibration lines.

[ $^{11}\text{C}$ ]Carbon dioxide was produced by the  $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$  nuclear reaction on nitrogen containing 1% oxygen, using a 18/9 IBA-cyclotron. Bombardment was carried out for 15–30 min with a 26  $\mu\text{A}$  beam of 16 MeV protons. At the EOB, the target gas was delivered and trapped by a cryogenic trap in the [ $^{11}\text{C}$ ]CH $_3$ I module.

### 4.2. Preparation of vinyl magnesium bromide (**3**)

The Grignard reagent solution was prepared according to a previously known method (Normant, 1957): Access magnesium turnings (0.5–1 g, 20–40 mmol) and a

small crystal of iodine were introduced into a three-neck flask, equipped with a dry ice condenser and flushed with argon. Two to three milliliters solution of vinyl bromide (1 M, in THF) were added, and after initiation, additional 3–5 ml of the same solution were added dropwise during 10–15 min. The solution was stirred for 10 min at room temperature, then heated for 30 min at 70–80°C. The solution was diluted three times its volume with freshly distilled dry THF (to a final concentration of ca. 0.3 M) and was either used immediately or kept in sure/seal<sup>®</sup> bottles under argon at –20°C for a week.

### 4.3. Preparation of unlabeled acrylamides

Unlabeled amides were prepared as follows. Anilines **7a** or **7b** (1 mmol) were dissolved in dry THF (ca. 25–30 ml) and cooled in an ice bath. Acryloyl chloride (100  $\mu\text{l}$ , 1.1 mmol) was added and the mixture was stirred for 10 min at 0°C. The solvent was then evaporated, ca. 15 ml NaHCO $_3$  (5%) were added, and extracted with dichloromethane. The organic solution was dried (MgSO $_4$ ) and evaporated to quantitatively yield the corresponding *N*-aryl acrylamides in high purity (>96%). *Acrylanilide* (**8a**), mp 103–105°C. *N*-(3-Bromo-phenyl)acrylamide (**8b**) (Witek et al., 1992), mp 122–124°C.

3,5-Bisphenylidene-4-piperidone (**7c**) and *N*-Acryl-3,5-bisphenylidene-4-piperidone (**8c**) were prepared according to known procedures (Dimmock et al., 2001); mp (95% ethanol) 180–182°C, and (95% ethanol) 128–130°C, respectively.

6-Acrylamido-4-(3-bromoanilino)quinazoline (**8d**) (Smaill et al., 1999), mp 265–267°C, was prepared from **7d** (Rewcastle et al., 1995), mp 202–205°C, following the published procedures.

### 4.4. Preparation of unlabeled ML03 (**2**) (Mishani et al., 2001)

#### 4.4.1. 6-Amino-4-(3,4-dichloro-6-fluoroanilino)quinazoline (**7e**)

**9** (Morley and Simpson, 1948) (700 mg, 3.3 mmol) and **10** (Bonasera et al., 2001) (1.47 ml, 8.2 mmol) were dissolved in 10 ml *i*-PrOH and refluxed for 3–4 h. The precipitate was collected and washed with *i*-PrOH. The crude product was analyzed on HPLC C18 analytical column (eluent, 55% acetonitrile, 45% acetate buffer 0.1 M pH 3.8,  $R_t$  9.2 min). Small amounts of polar impurities were removed by heating with a few milliliters of water and filtration. The yield of 6-nitro-4-(3,4-dichloro-6-fluoroanilino)quinazoline (**11**) was 75%; mp 270–271°C; MS ( $m/z$ ) 353.2, 355.2 ( $\text{M}^+$ ).

6-Nitro-4-(3,4-dichloro-6-fluoroanilino)quinazoline (**11**) (710 mg, 2.2 mmol) was dissolved in 200 ml of EtOH:H $_2$ O (9:1) by heating to reflux. The temperature

was reduced to 60°C and hydrazine hydrate (250 µl, 5.15 mmol) and ca. 0.5 ml Raney-Ni suspension were added (Mishani et al., 1997; Yuste et al., 1982). Reflux was then maintained for 15 min. The cold mixture was filtered over Celite and the solvent evaporated. The crude product was purified by silica gel chromatography (eluent 4–7% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to yield pure **7e** in 83%; HPLC analysis (eluent, 55% acetonitrile, 45% acetate buffer 0.1 M pH 3.8, *R*<sub>t</sub> 6.6 min); mp 268–270°C; MS (*m/z*) 323.4, 325.4 (M<sup>+</sup>); Anal. calcd.: C, 52.9; H, 2.78; N, 17.33. Found: C, 52.19; H, 2.99; N, 17.14.

#### 4.4.2. 6-Acrylamido-4-(3,4-dichloro-6-fluoroanilino)quinazoline (**2**) (Mishani et al., 2001)

**7e** (146 mg, 0.44 mmol) dissolved in 80 ml dry THF was cooled in an ice bath. Sixty microliters of acryloyl chloride (0.74 mmol) were added in two portions and the mixture was stirred at 0°C for 20 min. Saturated NaHCO<sub>3</sub> was added, the mixture was extracted with ethyl acetate, and the combined organic layers were dried (MgSO<sub>4</sub>) and evaporated. The crude product was purified by silica gel chromatography (eluent 3–7% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) and analyzed on HPLC C18 analytical column (mobile phase, 40% acetonitrile, 60% acetate buffer 0.1 M pH 3.8, *R*<sub>t</sub> 11.7 min). The yield of pure **2** was 73%; mp 217–219°C; <sup>1</sup>H NMR 9.54 (1H, s), 8.92 (1H, s), 8.8 (1H, bs), 8.54 (1H, s), 8.45 (1H, d, *J* = 6 Hz), 7.78 (1H, d, *J* = 8 Hz), 7.69 (1H, d, *J* = 8 Hz), 7.49 (1H, d, *J* = 10 Hz), 6.42 (1H, dd, *J*<sub>1</sub> = 17 Hz, *J*<sub>2</sub> = 3 Hz), 6.36 (1H, dd, *J*<sub>1</sub> = 17 Hz, *J*<sub>2</sub> = 9 Hz), 5.71 ppm (1H, dd, *J*<sub>1</sub> = 9 Hz, *J*<sub>2</sub> = 3 Hz); <sup>19</sup>F NMR—126 ppm (bs); MS (*m/z*) 377.3, 379.2 (M<sup>+</sup>); Anal. calcd.: C, 54.13; H, 2.94; N, 14.85. Found: C, 54.31; H, 3.24; N, 14.19.

#### 4.5. Synthesis of [<sup>11</sup>C]acrylic acid (**6**)

[<sup>11</sup>C]CO<sub>2</sub> (22.2 GBq, 600 mCi) was trapped at –160°C. The temperature of the cooling trap was increased to –50°C and the activity was transferred by a stream of argon (20 ml/min) into reactor-1, containing 300 µl vinyl magnesium bromide (**3**) in THF (0.3 M) at 7°C. After 90 s, the temperature of the first reactor was increased to 30°C and the flow through the trap was maintained for one additional minute. In this manner, 90% of the activity was trapped.

For the synthesis of [<sup>11</sup>C]acrylic acid (**6**), after the [<sup>11</sup>C]CO<sub>2</sub> transfer, 200 µl of HCl 2 M were added through vial-1 and the temperature was maintained at 30°C for 2 min. The solution was then withdrawn from the reactor, diluted with 2 ml of acetonitrile, and analyzed by reverse phase HPLC (C18 analytical column; mobile phase, 96% acetonitrile/4% acetate buffer 0.1 M pH 3.8, flow 1.6 ml/min). **6** eluted after 6 min (55%) and was identified by comparing the absorbance retention time with that of authentic sample

and by co-injection. The acid was accompanied by 40% of unidentified polar high-boiling point by-product, with a retention time of 2.8 min. The recovery of radioactivity from the HPLC column was 85% (measured by collecting the radioactive fractions after purification). The overall synthesis time was 10–12 min, the radiochemical yield 47% (EOB), and radiochemical purity > 99%.

#### 4.6. Automated synthesis of [<sup>11</sup>C]acryloyl chloride (**5**) and [<sup>11</sup>C]-*N*-acrylamides

##### 4.6.1. Method I

The [<sup>11</sup>C]CH<sub>3</sub>I module was modified for the synthesis of [<sup>11</sup>C]acryloyl chloride synthon by connecting V9 to the T-connector before reactor-2, in order to bypass the drying columns and V10. At the end of the [<sup>11</sup>C]CO<sub>2</sub> transfer, phthaloyl dichloride (100 µl) and 2,6-di-*tert*-butylpyridine (200 µl) in 150 µl of dry THF were added through vial-1 to the first reactor, containing the solution of **4** at 30°C, and the temperature of the reactor was increased to 90°C. **5** was distilled with a flow of argon (first 25 ml/min for 2 min and then 30 ml/min) through V9 to the second reactor containing 3–4 mg of amine in 0.5 ml of dry THF at –15°C. After 7 min of distillation, the solution was withdrawn from the second reactor, diluted with 2 ml of acetonitrile, and analyzed by HPLC, on reverse phase C18 analytical column (the corresponding eluent systems are presented in Table 1). All *N*-acryl amides were identified by comparing their HPLC retention times (UV absorbance) with those of authentic samples, and corresponding co-injections.

##### 4.6.2. Method II

The [<sup>11</sup>C]CH<sub>3</sub>I module was used with the original configuration. A solution of **4** in reactor-1 was treated with thionyl chloride (50 µl) and dry DMF (2 µl) in 200 µl dry THF, drawn through vial-1. Reactor-1 was then heated to 110°C, and the mixture was distilled with a flow of argon (35 ml/min) through a column containing polymer-bound 2,6-di-*tert*-butylpyridine preheated to 110°C. **5** was trapped in reactor-2, containing 3 µl of **7a** in 0.5 ml dry THF at –15°C, and after 7 min of distillation, the reaction mixture was withdrawn from the reactor, diluted and analyzed as described above.

##### 4.6.3. Method III

The [<sup>11</sup>C]CH<sub>3</sub>I module was used with the original configuration. A solution of **4** in reactor-1 was treated with 100 µl citric acid in 350 µl triethylene glycol and 100 µl dry THF through vial-1. Reactor-1 was then heated to 120°C, and the mixture was distilled with a flow of argon (40 ml/min) through two columns, one of which contained PCl<sub>5</sub>, and the other one polymer-bound

2,6-di-*tert*-butylpyridine, that were preheated to 120°C. The produced [<sup>11</sup>C]acryloyl chloride (**5**) was trapped in reactor-2, equipped with 3 μl **7a** in 0.5 ml dry THF at –15°C. After 7 min of distillation, the reaction mixture was withdrawn from the reactor, diluted and analyzed as described above.

#### 4.7. Automated synthesis of [<sup>11</sup>C]ML0<sub>3</sub> (**2**) (Mishani et al., 2001)

Trapped [<sup>11</sup>C]CO<sub>2</sub> (37 GBq, 1 Ci) was transferred to the first reactor as described. After the addition of phthaloyl dichloride and 2,6-di-*tert*-butylpyridine, the first reactor was heated to 90°C, and **5** was distilled with a flow of argon (first at 25 ml/min for 2 min and then 30 ml/min) through V9 to the second reactor. Reactor-2 contained 3.5 mg of **7e** in 0.5 ml of dry THF at –5°C. After 7 min of distillation, the temperature in reactor-2 was increased to 30°C, 0.7 ml of HPLC solvent were added from vial-3 and the solution was injected through V11 onto the built in HPLC (mobile phase, 46% acetonitrile, 54% acetate buffer 0.1 M pH 3.8, flow 13 ml/min). The product (*R*<sub>t</sub> 22 min, the fraction was selected by the operator according to the radiochromatogram) was collected into the solid phase extraction vial containing 70 ml of water and 2.5 ml of NaOH (1 M). The solution was passed through a C-18 cartridge (Waters Sep-Pak<sup>®</sup> Plus, pre-activated with 10 ml EtOH and 10 ml of sterile water) and the cartridge washed with 10 ml of sterile water. The product was eluted with 0.7 ml of EtOH, followed by 4.3 ml of saline and collected into the product vial, containing 50 μl of HCl 0.1 M. The solution was then transferred through a sterile Millex<sup>®</sup> 0.22 μm filter to a 20 ml sterile vial placed in a lead container. The chemical and radiochemical purities were analyzed by reverse phase HPLC C18 analytical column. The final doses were approximately 0.74 GBq (20 mCi).

#### 4.8. Biological evaluation of ML0<sub>3</sub>

Measurement of the apparent IC<sub>50</sub> of **2** was carried out as described by Bonasera et al. (2001). Briefly, A431 lysate was used as EGFR source. Ninety-six-wells ELISA plate was coated with anti-EGFR antibody and the A431 lysate added. The lysate was then incubated with **2** in seven different concentrations, ranging from 1.6 × 10<sup>–9</sup> nM to 15 nM. Unbound EGFR-TK were phosphorylated by the addition of ATP and the reaction quenched by the addition of EDTA. Finally anti-phosphotyrosine from rabbit followed by anti-rabbit peroxidase conjugated antibody was added, and a colorimetric reaction initiated by ABTS-H<sub>2</sub>O<sub>2</sub> allowed the quantification of the phosphorylated EGFR-TK fraction.

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