Synthesis of *N*-(protected)aminophthalimides: application to the synthesis of singly labelled isoniazid

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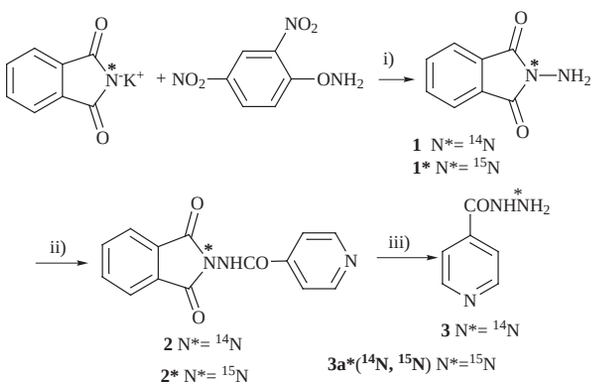
The synthesis of a series of *N*-(protected)aminophthalimides and the removal of their phthaloyl group leading to *N*-(protected) or *N*,*N*-bis(protected)hydrazines is described. As illustrated by the synthesis of monolabelled isoniazid **3b***, this strategy can be utilized for the preparation of monolabelled substituted hydrazines.

N-(Substituted)- or *N*,*N*-bis(substituted)hydrazines play an important role in organic chemistry because of their broad range of synthetic applications which include the preparation of azapeptides[1] and heterocyclic[2] and α-hydrazino acid compounds.[3] Several methods have been described in the literature for the preparation of these substituted hydrazines which usually require hydrazine or other commercially available substituted hydrazines as starting materials. One possible alternative starting material is *N*-aminophthalimide **1**. This compound contains a protected nitrogen atom within the phthalide moiety and a second nitrogen atom, capable of reacting with electrophilic reagents which should lead to regiospecific functionalization of **1**. However, there are certain synthetic limitations associated with **1** as well. For example, although benzylation is possible *via* formation and hydrogenation of the corresponding hydrazone,[4] direct alkylation has not been observed. In addition, acylation of **1** appears to be difficult to perform. Studies described in the literature indicated that **1** is inert towards even very reactive esters under conditions which normally allow acylation of simple hydrazines. Furthermore, when acyl chlorides are used this results in a mixture of products.[5]

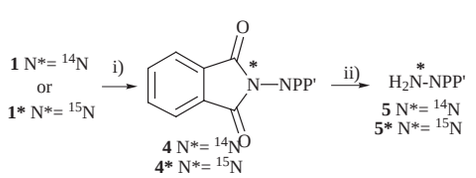
Perhaps the best result was obtained by M. J. Hearn and Prisch who succeeded in acylating **1** under extreme conditions by warming the neat compound in an excess of liquid acid anhydrides at 120 °C.[5] It is also worth noting that **1** undergoes skeletal rearrangement to yield phthalohydrazide under acidic or basic conditions.[6] Thus, the degree of this conversion must be taken into account when critically assessing the value of any synthesis based on **1**.

As part of our research dealing with the elucidation of the mechanism of action of isoniazid (INH), a front-line antituberculosis drug,[7] we showed for the first time that **1** can be easily acylated, under mild conditions without rearrangement and in good yield, with isonicotinic acid *via* a coupling reaction generally used for peptidic syntheses. Moreover, when *N*-amino[^{15}N]-phthalimide **1*** was used as a starting material (obtained *via* electrophilic amination of the commercially available potassium [^{15}N]phthalimide) we were able to synthesize *N*-isonicotinamido[^{15}N]phthalimide **2*** which was subsequently used as an intermediate in the preparation of singly labelled isoniazid **3a***(**^{14}N, ^{15}N**)[8] (Scheme 1).

As a result, we postulated that this pathway could also provide a convenient means for preparing *N*-(protected) or *N*,*N*-bis(protected)hydrazines and the corresponding singly labelled compounds. In this paper, we now report conditions which allow the synthesis of a series of *N*-(protected)aminophthalimides **4** and the removal of their phthaloyl group leading to *N*-(acyl)-, *N*-(alkyl)- and *N*,*N*-bis(alkoxycarbonyl)hydrazines **5** (Scheme 2).



Scheme 1 *Reagents and conditions*: i) DMF, rt, 1 h, 88%; ii) 4-C_6H_4N-COOH, $CO(Im)_2$, THF, reflux, 60 h, 78%; iii) N_2H_4, H_2O, 15 min, rt, 70%.



Scheme 2 *Reagents and conditions*: i) see conditions and yields in Table 1; ii) H_2NNH_2, H_2O, EtOH, rt, 15 min, see footnotes and yields in Table 1.

A synthetic application of this strategy is illustrated by the preparation of the singly labelled *N*,*N*-bis(protected)hydrazine **5g***, required for the synthesis of isonicotinoyl[^{15}N, ^{14}N′]hydrazine **3b***(**^{15}N, ^{14}N**), the isotopic isomer of **3a***(**^{14}N, ^{15}N**).

Results and discussion

The results obtained are summarized in Table 1 and in Scheme 2.

Preparation of *N*-(protected)aminophthalimide 4

Based upon our previous studies, we first performed acylation of **1** by acetic, benzoic and pyvaloic acid using 1,1′-carbonyldiimidazole [$CO(Im)_2$] as an activating agent. These reactions allowed the formation of the corresponding *N*-(acyl)amino-

Table 1

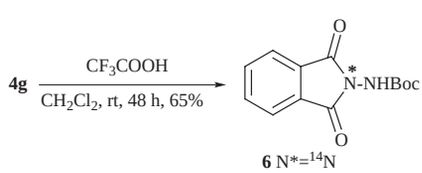
Item	P	P′	Conditions	*t*/h	Compounds obtained	Yield of **4** (%)[a]	Yield of **5** (%)[b]
1	CH_3CO	H	CH_3COOH, $CO(Im)_2$, THF, reflux	20	**4a**	82	**5a**, 75
2	C_6H_5CO	H	C_6H_5COOH, $CO(Im)_2$, THF, reflux	96	**4b**	65	**5b**, 68
3	^tBuCO	H	^tBuCOOH, $CO(Im)_2$, THF, reflux	96	**4c**	72	**5c**, 55
4	F_3CCO	H	$(F_3CCO)_2O$, neat, 0 °C	2	**4d**	87	**5d**, 70[c]
5	Cl_3CCO	H	Cl_3CCOCl, pyridine, CH_2Cl_2, rt	4	**4e**	90	0
6	$(C_6H_5)_3C$	H	$(C_6H_5)_3CCl$, DMAP, Et_3N, DMF, rt	20	**4f**	34	**5f**, 100
7	Boc	Boc	Boc_2O, DMAP, Et_3N, THF, rt	2	**4g**	65	**5g**, 87[d]

[a] Isolated yield calculated from **1**. [b] Isolated yield calculated from the corresponding compound **4**. [c] $C_6H_5NHNH_2$ was used instead of hydrazine hydrate. [d] Hydrazinolysis was performed at −20 °C during 2 hours.

phthalimides **4a**, **4b** and **4c** in good to excellent yields (items 1, 2 and 3). Unfortunately, using trihalogenoacetic acids under the same conditions led to the degradation of the reaction mixture. However, we did succeed in obtaining *N*-(trifluoromethylcarbonyl)aminophthalimide **4d** using neat trifluoroacetic acid anhydride (item 4). *N*-(Trichloromethylcarbonyl)aminophthalimide **4e** was also synthesized using trichloroacetyl chloride and pyridine in CH_2Cl_2 (item 5). In addition, monoalkylation of **1** by a triphenylmethyl group was achieved, although in poor yield, using triphenylmethyl chloride, DMAP as a catalyst and Et_3N in DMF at room temperature (item 6).

As carbamates are extensively used as protective groups for amines, we attempted to obtain suitable conditions for the introduction of a Boc or Z group to **1**. Unreported results obtained from our group showed that the use of BocCl,[9] $BocN_3$[10] and ZCl[11] led to a slow degradation of the reaction mixture. Furthermore, when using $PhCH_2OCOCCl_3$ in acetonitrile[12] only compound **4e** was obtained resulting from the splitting of the C–O bond of the starting ester.

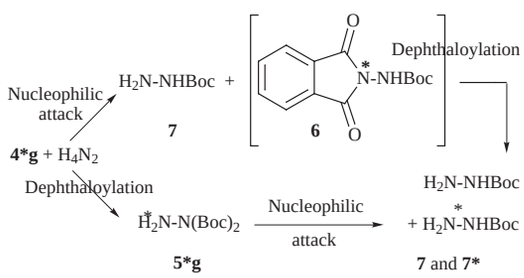
Successful introduction of Boc groups was eventually achieved using Boc_2O with DMAP as a catalyst and Et_3N in THF. Surprisingly under these conditions the unexpected *N*,*N*-bis(*tert*-butoxycarbonyl)aminophthalimide **4g** was isolated. It is important to note that we never observed the formation of the corresponding *N*-(*tert*-butoxycarbonyl)-aminophthalimide **6** even when one equivalent of Boc_2O was used. Furthermore, we observed from monitoring the progression of the reaction by TLC that yield of **4g** generally improved as the amount of Boc_2O was increased (from one to eight equivalents). However, the best yield of **4g** was obtained when six equivalents of Boc_2O were used as greater values tended to make subsequent purification of the product troublesome. Finally, it was possible to synthesize compound **6** in 65% yield from **4g** by using 1.5 equivalents of trifluoroacetic acid in CH_2Cl_2 (Scheme 3).



Scheme 3

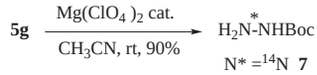
Preparation of protected hydrazines 5

In most cases removal of the phthaloyl group from compounds **4** was performed using hydrazine hydrate as a nucleophilic agent and gave the corresponding protected hydrazines **5** in good yields. However, dephthaloylation of compound **4d** necessitated the use of phenylhydrazine instead of hydrazine hydrate. This avoided the formation of a complex between the phthalohydrazide by-product and the protected hydrazine **4d**, described previously,[13] which would have complicated the purification (item 4). With regard to compound **4e** we observed that regardless of the reactant used (hydrazine hydrate, phenylhydrazine or methylhydrazine) we were unable to isolate the corresponding protected hydrazine as degradation of the reaction mixture occurred. The triphenylmethylhydrazine **5f** was obtained quantitatively but unfortunately this compound was unstable and so we only succeeded in performing its ^{13}C NMR spectrum. The hydrazinolysis of **4g** led to the formation of the new and interesting compound 1,1-bis(*tert*-butoxycarbonyl)hydrazine **5g** which has, to the best of our knowledge, not been described in the literature until now. Curiously, this reaction was impeded by the formation of the *N*-(*tert*-butoxycarbonyl)hydrazine **7** which can be explained by a nucleophilic attack of the hydrazine on one of the two Boc groups as illustrated in Scheme 4. Based upon this mechanism it appears that the hydrazine moiety of **7** arises equally from **4g** and hydrazine hydrate (Scheme 4).



Scheme 4

With regard to the ^{15}N labelled synthesis of isonicotinoyl[^{15}N,$^{14}N'$]hydrazine **3b***(^{15}N, ^{14}N), it is necessary to minimise the nucleophilic attack of hydrazine on the Boc group in order to avoid a decrease in the isotopic yield. From an earlier systematic study we determined that an optimal yield of **5g** could be obtained by using 1.5 equivalents of hydrazine hydrate in ethanol at −20 °C. It is also interesting to note that compound **7** may be produced in 90% yield by treating **5g** with a catalytic amount of $Mg(ClO_4)_2$.[14] As shown in Scheme 5, this

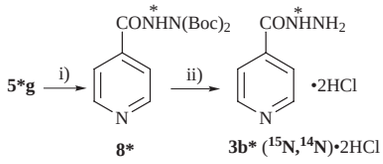


Scheme 5

condition could thus be used in the labelled series to selectively synthesize **7***.

Synthesis of isonicotinoyl[^{15}N, $^{14}N'$]hydrazine 3b*(^{15}N, ^{14}N)

Based upon our strategy to obtain protected hydrazines, the singly labelled *N*,*N*-bis(*tert*-butoxycarbonyl)hydrazine **5*g** was obtained using commercially avalaible potassium [^{15}N]phthalimide as starting material. Acylation with isonicotinic acid was performed using 1,1′-carbonyldiimidazole as a coupling reagent to produce compound **8*** in good yield. Deprotection of the amino group was easily performed using 3 M HCl and resulted in the formation of singly labelled isoniazid **3b***(^{15}N,



Scheme 6 *Reagents and conditions*: i) 4-C_6H_4N-COOH, $CO(Im)_2$, THF, reflux, 72 h, 75%; ii) 3 M HCl, AcOEt, quantitative yield.

^{14}N) as shown in Scheme 6 in an overall yield of 36% from potassium [^{15}N]phthalimide.

In conclusion, we have found appropriate conditions for the acylation and alkylation of *N*-aminophthalimide without allowing skeletal rearrangement to occur. A subsequent dephthaloylation leads to the formation of a series of 1-monoprotected hydrazines and, for the first time, the formation of *N*,*N*-bis(*tert*-butoxycarbonyl)hydrazine **5g**. As illustrated by the synthesis of compound **3b***(**^{15}N, ^{14}N**), we have demonstrated that this strategy can be effectively utilized for the preparation of hydrazine synthons which can then be used in other synthetic schemes.

Experimental

Melting points were obtained on a Kofler hot-stage apparatus and were uncorrected. The IR spectra were recorded on a FTIR ATI MATTSON Genesis Series. Elemental analyses were performed by the 'service de Microanalyse du CNRS, Division de Vernaison'. NMR spectra were recorded on a Bruker AM 400 MHz or 250 MHz. *J* Values are given in Hz. Mass spectra were run on a micromass AutoSpec-Q spectrometer in the chemistry department at Imperial College, London. Potassium [^{15}N]-phthalimide was purchased from Eurisotop CEA and *N*-aminophthalimide from Lancaster. Merck silica gel 60 was used for all chromatographic separations and thin layer chromatographic techniques used Merck silica gel 60 F_{254} TLC plates. Ether refers to diethyl ether. Tetrahydrofuran and dichloromethane were dried by distilling respectively over sodium–benzophenone and P_2O_5.

N-(Acetylamino)phthalimide 4a

To a solution of acetic acid (1.5 g, 24.6 mmol) in THF (20 ml) was added 1,1′-carbonyldiimidazole (4 g, 24.6 mmol). The mixture was refluxed for 10 min (corresponding to the end of CO_2 evolution). *N*-Aminophthalimide **1** (2 g, 12.3 mmol) was then added. The reflux was continued during the time indicated in Table 1. The medium was concentrated *in vacuo* and the residue was partitioned between ether and water; the water layer was extracted twice with ether. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The solid obtained by precipitation into hexane was recrystallized from AcOEt–hexane to give pure compound **4a** (82%), mp 226–228 °C; ^{1}H NMR (400 MHz, $CDCl_3$–DMSO) δ 2.15 (s, 3H, CH_3), 7.78–7.92 (m, 4H, arom.), 10.37 (m, 1H, NH); ^{13}C NMR ($CDCl_3$–DMSO) δ 21.0 (CH_3), 124.4 (arom. CH), 130.4 (arom. C), 135.1 (arom. CH), 165.7 (O=*C*-Pht), 169.6 (CH_3-*C*O); IR (KBr) ν_{max}/cm^{-1} 1672, 1745, 1798 (C=O). Calcd. for $C_{10}H_8N_2O_3$: C, 58.83; H, 3.94; N, 13.71. Found: C, 58.80; H, 4.07; N, 13.49%.

N-(Benzoylamino)phthalimide 4b

The procedure previously described for the preparation of **4a** was employed using 4 equivalents of benzoic acid and 4 equivalents of $CO(Im)_2$ (65%), mp 204–206 °C; ^{1}H NMR (400 MHz, DMSO) δ 7.50–7.67 (m, 3H, arom.), 7.86–8.05 (m, 6H, arom.), 11.30–11.44 (m, 1H, NH); ^{13}C NMR (DMSO) δ 124.7, 128.7, 129.7 (arom. CH), 130.4, 131.6 (arom. C), 133.7, 136.2 (arom. CH), 166.2, 166.3 (2 CO); IR (KBr) ν_{max}/cm^{-1} 1664, 1750, 1799 (C=O). Calcd. for $C_{15}H_{10}N_2O_3$: C, 67.67; H, 3.79; N, 10.52. Found: C, 67.54; H, 3.94; N, 10.71%.

N-(*tert*-Butylcarbonylamino)phthalimide 4c

The procedure previously described for the preparation of **4a** was employed using 4 equivalents of pivalic acid and 4 equivalents of $CO(Im)_2$ (72%), mp 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 9H, CH_3), 7.55–7.67 (m, 2H, arom.), 7.79–7.84 (m, 2H, arom.), 10.40–10.45 (m, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.7 (CH_3), 38.9 (CMe_3), 124.1 (arom. CH), 130.5 (arom. C), 134.9 (arom. CH), 166.0 (O=*C*-Pht), 179.0 (COtBu); IR (KBr) ν_{max}/cm^{-1} 1737, 1795 (C=O). HRMS Calcd. for $C_{10}H_5N_2F_3O_3$ *m*/*z* 246.1004, found 246.1004.

N-(Trifluoromethylcarbonylamino)phthalimide 4d

N-Aminophthalimide **1** (18.5 mmol, 3 g) was dissolved with stirring at 0 °C in 5 ml of trifluoroacetic anhydride. The solution was kept in an ice–water bath under stirring for 2 hours. The excess of trifluoroacetic anhydride was removed *in vacuo* and the remaining solid was washed with hexane to give **4d** (90%), mp 122–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–8.11 (m, 4H, arom.), 9.69–10.11 (m, 1H, NH); ^{13}C NMR (CD_3OD–DMSO) δ 90.6 (q, J_{CF} = 288 Hz, CF_3), 115.6 (arom. CH), 124.1 (arom. CH), 129.9 (arom. C), 135.7 (arom. CH), 156.7 (q, J_{CF} = 39 Hz, *C*OCF_3), 164.4 (O=*C*-Pht); IR (KBr) ν_{max}/cm^{-1} 1746, 1802 (C=O). HRMS Calcd. for $C_{10}H_5N_2F_3O_3$ *m*/*z* 258.0252, found 258.0255.

N-(Trichloromethylcarbonylamino)phthalimide 4e

To a cooled solution of *N*-aminophthalimide **1** (20 mmol, 3.24 g) and pyridine (30 mmol, 2.4 g) in CH_2Cl_2 (200 ml) was added dropwise 1 equivalent of trichloroacetyl chloride (20 mmol). Stirring was continued at room temperature for 4 hours. After the mixture was poured into ice–water, the organic layer was separated and aqueous layer was extracted with CH_2Cl_2. The combinated organic layer was washed with diluted HCl, dried over $MgSO_4$ and evaporated *in vacuo*. The solid residue was recrystallized with AcOEt–hexane to give pure **4e** (87%), mp 170–172 °C; ^{1}H NMR (400 MHz, CD_3OD–DMSO) δ 7.77–8.12 (m, 4H, arom.), 10.89–12.41 (m, 1H, NH); ^{13}C NMR (CD_3OD–DMSO) δ 90.6 (*C*Cl3), 124.1 (arom. CH), 129.9 (arom. C), 135.6 (arom. CH), 161.7 (*C*OCCl_3), 164.4 (O=*C*-Pht); IR (KBr) ν_{max}/cm^{-1} 1723, 1747, 1807 (C=O). HRMS Calcd. for $C_{10}H_5N_2Cl_3O_3$ *m*/*z* 305.9365, found 305.9363.

N-(Triphenylmethylamino)phthalimide 4f

A mixture of *N*-aminophthalimide **1** (6.2 mmol, 1 g), Et_3N (1.5 ml), triphenylmethyl chloride (10.8 mmol, 3 g) and a catalytic amount of DMAP in DMF (30 ml) was stirred during 20 hours at room temperature. The mixture was poured into ice–water and extracted with CH_2Cl_2. The combined organic layer was washed with diluted HCl, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel to give **4f** (34%), ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.16–7.64 (m, arom.); ^{13}C NMR ($CDCl_3$) δ 74.9 (CPh_3), 122.8, 127.0, 127.4, 129.6 (arom. CH), 129.6 (arom. C), 133.7 (arom. CH), 143.6 (arom. C), 166.1 (O=*C*-Pht); IR (KBr) ν_{max}/cm^{-1} 1725, 1783 (C=O), 3320 (NH_2). Because of the instability of **4f**, no mass spectrum was performed.

N,*N*-Bis(*tert*-butoxycarbonyl)aminophthalimide 4g

A suspension of *N*-aminophthalimide **1** (2.5 g, 15.4 mmol), Et_3N (90 mmol) and a catalytic amount of DMAP in THF (100 ml) was stirred and cooled in an ice–water bath. Di-*tert*-butyl dicarbonate (20 g, 90 mmol) was added and stirring was continued at room temperature for 2 hours. The solution was concentrated *in vacuo*. Water and ether were added, the aqueous layer was separated and extracted twice with ether. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed through silica gel with AcOEt–

hexane to give **4g** (65%), mp 175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 18H, CH_3), 7.68–7.74 (m, 2H, arom.), 7.77–7.87 (m, 2H, arom.); ^{13}C NMR ($CDCl_3$) δ 28.1 (CH_3), 85.5 (CMe_3), 124.3 (arom. CH), 130.2 (arom. C), 135.2 (arom. CH), 149.1 (*C*OOtBu), 164.2 (O=*C*-Pht); IR (KBr) ν_{max}/cm^{-1} 1751, 1778, 1801 (C=O), 3320 (NH_2). HRMS Calcd. for $C_{18}H_{23}N_2O_6$ $[M + H]^+$ *m/z* 363.1556, found 363.1548.

Dephthaloylation of compounds 4

To a suspension of compound **4** in EtOH (10 ml) was added at 0 °C (or in one case at −20 °C, see Table 1) 1.5 equivalents of H_2NNH_2, H_2O (or in one case $PhNHNH_2$, see Table 1) *via* syringe. The solution was then allowed to warm to room temperature. After 15 min a white precipitate was filtered off. The filtrate was then evaporated to give a solid which was recrystallized from EtOH to give pure compoud **5**. *Acetic hydrazide* **5a** (75%, mp 66–68 °C) and *benzoic hydrazide* **5b** (68%, mp 113–117 °C) were found to be identical to authentic samples commercially available (Lancaster). *Pyruvic hydrazide* **5c** (55%), mp 58–60 °C (lit.,[15] 56–57 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (s, 9H, CH_3), 4.00–4.30 (m, 3H, NH); ^{13}C NMR ($CDCl_3$) δ 27.6 (CH_3), 38.8 (CMe_3), 179.3 (*C*OtBu); IR (KBr) ν_{max}/cm^{-1} 1637 (C=O), 3300 (NH). *Trifluoroacetic hydrazide* **5d** (70%), mp 114 °C (lit.,[16] 110–113 °C). *Triphenylmethylhydrazine* **5f** (100%) (this compound being unstable, we only succeeded in performing its ^{13}C NMR spectrum) ^{13}C NMR ($CDCl_3$) δ 74.8 (CPh_3), 127.3, 128.6, 129.3 (arom. CH), 144.5 (arom. C).

1,1-Bis(*tert*-butoxycarbonyl)hydrazine 5g. (87%), mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, CH_3, 18H), 4.36 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$) δ 28.1 (CH_3), 83.6 (CMe_3), 150.3 (*C*OOtBu); IR (KBr) ν_{max}/cm^{-1} 1741 (C=O), 3353 (NH). HRMS Calcd. for $C_{10}H_{21}N_2O_4$ *m/z* 233.1501, found 233.1503.

N-(*tert*-Butoxycarbonyl)aminophthalimide 6

To a solution of **4g** (500 mg) in CH_2Cl_2 was added 1.5 equivalents of trifluoroacetic acid and the solution was stirred during 36 h. After removing the solvent *in vacuo*, the crude product was chromatographed to give **6** (65%), ^{1}H NMR (400 MHz, $CDCl_3$–DMSO) δ 1.50 (s, 9H, CH_3), 7.88–7.96 (m, 4H, arom.), 9.60 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 28.7 (CH_3), 81.9 (CMe_3), 124.1 (arom. CH), 130.4 (arom. C), 135.3 (arom. CH), 154.8 (*C*OOtBu), 166.0 (O=*C*-Pht); IR (KBr) ν_{max}/cm^{-1} 1706, 1740, 1783 (C=O), 3395 (NH). HRMS Calcd. for $C_{13}H_{14}N_2O_4$ $[M+H]^+$ *m/z* 263.1032, found 263.1034.

N′,*N′*-Bis(*tert*-butoxycarbonyl)isonicotinohydrazide 8†

The typical conditions previously described for the preparation of **4a** were used starting from **5g** (180 mg, 0.8 mmol), $CO(Im)_2$ (420 mg, 2.6 mmol) and isonicotinic acid (pyridine-4-carboxylic acid) (320 mg, 2.6 mmol) in THF at reflux during 72 hours (75%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, CH_3, 18H), 7.13 (d, J = 5.9 Hz, 2H, arom.), 8.59 (d, J = 5.9 Hz, 2H, arom.), 9.90 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 28.2 (CH_3), 84.7 (CMe_3), 121.8 (arom. CH), 139.9 (arom. C), 150.6 (arom. CH), 150.9 (*C*OOtBu), 164.5 (Pyr-*C*O); IR (KBr) ν_{max}/cm^{-1} 1698, 1756 (C=O). HRMS Calcd. for $C_{16}H_{24}N_3O_5$ *m/z* 338.1715, found 338.1708.

Deprotection of INH

The *N*,*N*-bis(*tert*-butoxycarbonyl)isonicotinic hydrazide **8**† (7.4 mmol) was dissolved in 1.5 ml of 3 M HCl and left to stand at room temperature for one hour. The solution was evaporated to dryness *in vacuo* to give **3b**·2HCl (quantitative yield), mp 225–235 °C; ^{1}H NMR (400 MHz, D_2O) δ 8.28 (d, J = 6.7 Hz, arom.), 8.85 (d, J = 6.7 Hz, arom.); ^{13}C NMR (D_2O) δ 126.5 (arom. CH), 143.2 (arom. CH), 146.8 (arom. C), 163.7 (CO). These data were found to be identical to the dihydrochloride salt of isoniazid prepared from commercially available isoniazid.

Spectroscopic data of ^{15}N labelled compounds

4g*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 18H, CH_3), 7.72–7.79 (m, 2H, arom.), 7.83–7.90 (m, 2H, arom.); ^{13}C NMR ($CDCl_3$) δ 28.3 (CH_3), 85.5 (CMe_3), 124.4 (arom. CH), 130.2 (d, $J_{CN^{15}}$ = 10.3 Hz, arom. C), 135.2 (arom. CH), 149.1 (*C*OOtBu), 164.2 (d, $J_{CN^{15}}$ = 13.3 Hz O=*C*-Pht).

6*. ^{1}H NMR (400 MHz, D_2O) δ 1.46 (s, CH_3, 18H), 7.13 (d, J_{HH} = 5.9 Hz, 2H, arom.), 8.59 (d, J_{HH} = 5.9 Hz, 2H, arom.), 10.07 (d, $J_{HN^{15}}$ = 102.8 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 28.2 (CH_3), 84.6 (CMe_3), 121.9 (arom. CH), 139.9 (d, $J_{CN^{15}}$ = 11.6 Hz, arom. C), 150.5 (arom. CH), 150.9 (*C*OOtBu), 164.5 (d, $J_{CN^{15}}$ = 14.9 Hz, Pyr-*C*O-); HRMS Calcd. for $C_{16}H_{24}N_2{}^{15}NO_5$ *m/z* 339.1686, found 339.1681.

3b*(^{15}N, ^{14}N). ^{1}H NMR (400 MHz, D_2O) δ 8.28 (d, J_{HH} = 6.5 Hz, 2H, arom.), 8.87 (d, J_{HH} = 6.5 Hz, 2H, arom.); ^{13}C NMR (D_2O) δ 126.1 (arom. CH), 143.2 (arom. C), 146.8 (arom. CH), 163.6 (d, $J_{CN^{15}}$ = 13.3 Hz, Pyr-*C*O-). HRMS Calcd. for $C_6H_7N_2{}^{15}NO$ *m/z* 138.0560, found 138.0563.

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References

1 A. Lecoq, G. Boussard and M. Marraud, *Tetrahedron Lett.*, 1992, **33**, 5209; D. Limal, V. Grand, R. Vanderesse and M. Marraud, *Tetrahedron Lett.*, 1994, **35**, 3711; C. J. Gray, M. Quibell, K. J. Jiang and N. Baggett, *Synthesis*, **1991**, 141.
2 (*a*) A. C. Church, M. U. Koller, M. A. Hines and C. F. Beam, *Synth. Commun.*, 1996, **26**, 3659; (*b*) V. V. Khau and M. J. Martinelli, *Tetrahedron Lett.*, 1996, **37**, 4323; (*c*) T. Chou and R. C. Chang, *J. Org. Chem.*, 1993, **58**, 493; (*d*) C. M. Pant, R. J. Stoodley and A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1989, 297.
3 R. V. Hoffman and H. O. Kim, *Tetrahedron Lett.*, 1990, **31**, 2953.
4 M. J. Hearn and E. R. Lucero, *J. Heterocycl. Chem.,* 1982, **19**, 1537.
5 M. J. Hearn and S. B. Prisch, *Org. Prep. Proced. Int.*, 1981, 421.
6 H. D. K. Drew and H. H. Hatt, *J. Chem. Soc.,* 1937, 16.
7 For a review on tuberculosis see J. S. Blanchard, *Annu. Rev. Biochem.*, 1996, **65**, 215; Y. Zhang, B. Heym, B. Allen, D. Young and S. T. Cole, *Nature*, 1992, **358**, 591; B. Heym, Y. Zhang, S. Poulet, D. Young and S. T. Cole, *J. Bacteriol.*, 1993, 4255; Y. Zhang, T. Garbe and D. Young, *Mol. Microbiol.*, 1993, **8**, 521; B. Heym, P. M. Alzari, N. Honore and S. T. Cole, *Mol. Microbiol.*, 1995, **15**, 235; K. Johnson and P. G. Schultz, *J. Am. Chem. Soc.*, 1994, **116**, 7425.
8 B. Jamart-Grégoire, N. Brosse and J. Bodiguel, *Synthesis*, 1998, 209.
9 S. A. Chaudhary and O. Hernandez, *Tetrahedron Lett.*, 1979, **2**, 95.
10 M. Bodansky and A. Bodansky, *The practice of peptide synthesis*, 1984, Springer Verlag, Berlin Heidelberg, New York, Tokyo.
11 A. C. Pierce and M. M. Joullié, *J. Org. Chem.*, 1963, **28**, 258.
12 M. S. Gibson and R. W. Bradshaw, *Angew. Chem.*, 1968, **80**, 986.
13 I. Schumann and R. A. Boissonnas, *Helv. Chim. Acta*, 1952, **35**, 2235.
14 J. A. Stafford, M. F. Brackeen, D. S. Karanewsky and N. L. Valvano, *Tetrahedron Lett.*, 1993, **34**, 7873.
15 A. Bühler and B. Fierz-David, *Helv. Chim. Acta*, 1943, **26**, 2131.
16 W. Ried and G. Franz, *Liebigs Ann. Chem.*, 1961, **644**, 141.

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† IUPAC name: *N′*,*N′*-bis(*tert*-butoxycarbonyl)pyridine-4-carbohydrazide.