Synthesis of nonpolar peptide nucleic acid monomers containing fluoroaromatics

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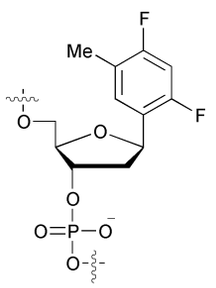
A general strategy for the synthesis of nonpolar peptide nucleic acid monomers containing fluoroaromatics (F-PNA) is described. These compounds have been designed as hybrid analogues of the difluorotoluene nucleoside, F (**1**) with PNA. Fluorophenylacetic acid derivatives **9** were coupled to the Boc-protected pseudopeptide backbone **8** by a standard peptide coupling reaction using DhbtOH and DCC in the presence of triethylamine to afford the doubly protected F-PNA monomers **14** in moderate to good yields. The ethyl esters **14a**, **14c** and **14e** underwent hydrolytic cleavage under basic conditions to generate *N*-protected F-PNA monomers **15** in good yields. The *tert*-butyl esters **14b**, **14d** were treated with TFA in dichloromethane to produce the free F-PNA monomers **16** in good to excellent yields. The β-F-PNA monomers designed based on the structure of 2′,5′-linked isoDNA were also synthesized in a similar fashion to the preparation of F-PNA monomers in moderate to good yields as both *N*-protected and free monomers.

Introduction

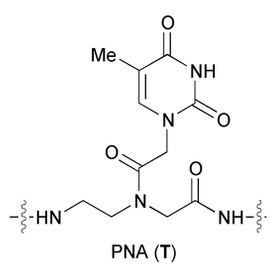
Aryl C-nucleosides have received considerable attention due to their ability for aromatic stacking while relinquishing their hydrogen bonding potentials.[1] DNA–duplex stability and sequence specificity are founded on the complementary Watson–Crick hydrogen bonding patterns of adenine with thymine and cytosine with guanine. However, recent investigations have demonstrated that the aromatic stacking and hydrophobicity are major driving forces in DNA structure and synthesis. Nonpolar DNA base analogues such as aryl C-nucleosides stabilize significantly the helices even when the bases in question do not undergo pairing.[2] Among the most important analogues in this field is difluorotoluene nucleoside F (**1**) which has been developed by Kool *et al*.[3] Compound F is designed as a nonpolar shape mimic for natural thymidine. It serves as a template for DNA synthesis even though it lacks standard hydrogen bonding (Fig. 1).



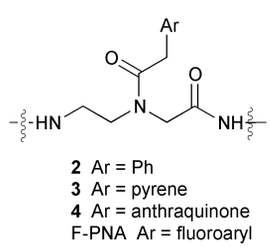
F (**1**)

Fig. 1

Another interesting class of substrates derived from DNA is peptide nucleic acids (PNAs). PNAs have a pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine where only nucleobases are left of the original DNA structure. PNAs are one of the most successful mimetics of nucleic acids, which exhibit a remarkable affinity for both complementary DNA and RNA, which is greater than their natural counterparts.[4] They hold the prestigious position of being potential replacements of oligonucleotides for use in antisense therapeutics,[5] molecular biology reagents and chemical diagnostics. With these points as a background, the design and synthesis of a new class of PNA analogues having aromatics in either their backbone[6] or side chain[7,8] have received much interest because of their ability to demonstrate π–π stacking interactions. Woski and Challa[7] recently demonstrated the synthesis of PNA analogues containing aromatic moieties as a replacement of natural nucleobases to highlight the roles of base–base stacking and hydrogen bonding and also to examine the sequence specificity to form a PNA–DNA double helical structure.[7,8] Oligomers containing phenyl residue **2** could form PNA–DNA double helices although this showed little sequence specificity. PNA monomers containing pyrene **3** and anthraquinone **4** have also been synthesized for their potential ability to intercalate into DNA to form PNA–DNA hybrid duplexes.[8] Although several analogues of PNA having aromatics have been synthesized,[7,8] to the best of our knowledge, there is no report published yet about the synthesis of nonpolar PNA analogues having fluoroaromatics (F-PNA) which could be much more interesting as a probe to study the role of hydrophobic and stacking interactions (Fig. 2).



PNA (**T**)



2 Ar = Ph
3 Ar = pyrene
4 Ar = anthraquinone
F-PNA Ar = fluoroaryl

Fig. 2

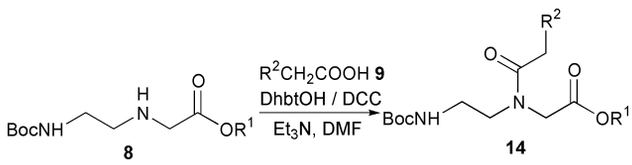
In this paper we describe the first synthesis of F-PNA monomers as well as the β-F-PNA monomers designed based on 2′,5′-linked isoDNA **5** (Fig. 3).

Results and discussion

Synthesis of F-PNA monomers

We recently reported the one-pot synthesis of *N*-fluoroarylalkyl pendented α,α- and α,β-dipeptides **6**, **7** by the Ugi four-

Table 1 Synthesis of protected F-PNA monomers **14**



Entry	8	R^1	9	R^2	14	Yield (%)[a]
1	**8a**	Et	**9a**	2,4-Difluorophenyl	**14a**	51
2	**8b**	Bu^t	**9a**	2,4-Difluorophenyl	**14b**	50
3	**8a**	Et	**9b**	Pentafluorophenyl	**14c**	58
4	**8b**	Bu^t	**9b**	Pentafluorophenyl	**14d**	55
5	**8a**	Et	**9c**	2,4-Difluoro-5-methylphenyl	**14e**	52

[a] Yields were determined based on **8** employed.

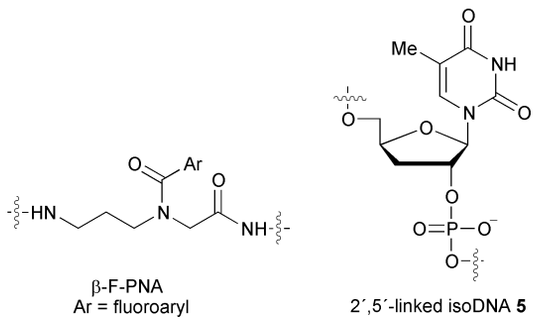


Fig. 3

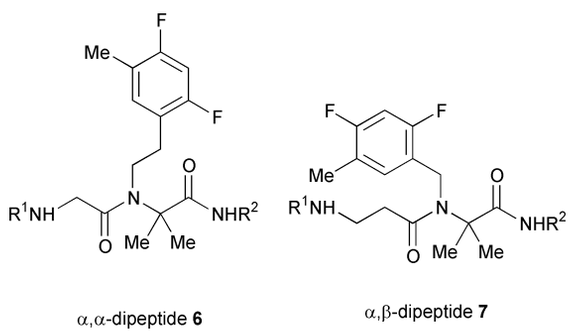
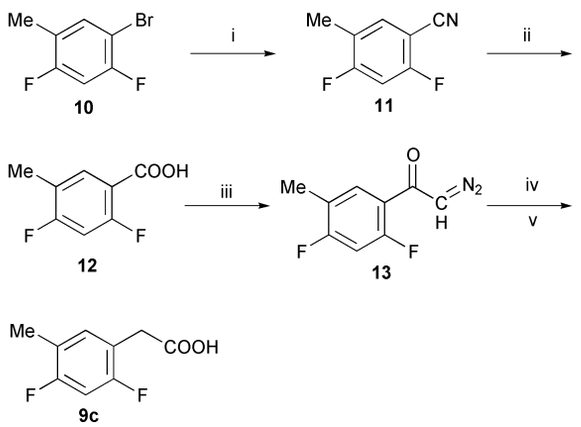


Fig. 4

component condensation.[9] This method, however, cannot be applied for the preparation of F-PNA because the backbone of the products obtained is not pseudopeptide but peptide (Fig. 4).

Therefore we synthesized F-PNA monomers using conventional stepwise methods. One of the key intermediates for the synthesis of F-PNA monomers is *N*-(2-Boc-aminoethyl)-glycinates **8** which have been very often used as the backbone for PNA monomer synthesis. The backbone **8** was prepared according to the literature procedure.[10] Other key moieties are the fluoroarylacetic acid derivatives **9**. 2,4-Difluorophenylacetic acid (**9a**) and pentafluorophenylacetic acid (**9b**) are commercially available and 2,4-difluoro-5-methylphenylacetic acid (**9c**) was prepared from 2,4-difluoro-5-methylbromobenzene (**10**)[3a] in four steps as follows. Compound **10** was first converted into the corresponding cyanide **11** by treatment with copper(I) cyanide[11] in DMF at 160 °C in 50% yield. The cyanide underwent acidic hydrolysis[12] to give the 2,4-difluoro-5-methylbenzoic acid (**12**) in quantitative yield. The acid **12** was then treated with ethyl chlorocarbonate and diazomethane to give the diazoketone **13** in 98% yield, which was then converted to the methyl ester through Arndt–Eistert rearrangement by treating with methanol catalyzed with silver benzoate in 60% yield. The methyl ester underwent basic hydrolysis with 1 M LiOH in THF[13] to give **9c** in 58% yield (Scheme 1).

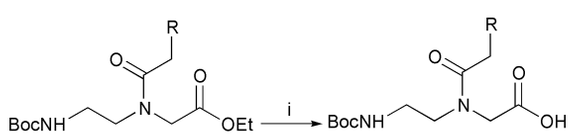


Scheme 1 *Reagents and conditions*: i, CuCN, DMF, 160 °C, 50%; ii, conc. H_2SO_4, H_2O, reflux, 100%; iii, $ClCO_2Et$, Et_3N, CH_2N_2, 98%; iv, MeOH, PhCOOAg, 60%; v, 1 M LiOH, THF, 58%.

The coupling of the acid **9** to the backbone **8** using peptide coupling agents 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (DhbtOH) and DCC in DMF in the presence of triethylamine proceeded nicely to afford the protected F-PNA monomers **14a–e** in 50–58% yields (Table 1).

Compound **14e** provides considerable interest as the 2,4-difluorotoluene moiety directly acts as an isostere of thymine which might mimic the natural PNA analogue having a thymine base moiety.

Selective removal of the C-terminus of ethyl esters **14a**, **14c** and **14e** was accomplished by basic hydrolysis with 1 M LiOH in THF to afford the *N*-protected F-PNA monomers **15a–c** in 54–70 yields (Scheme 2).



14a R = 2,4-difluorophenyl
14c R = pentafluorophenyl
14e R = 2,4-difluoro-5-methylphenyl

15a (70%)
15b (55%)
15c (54%)

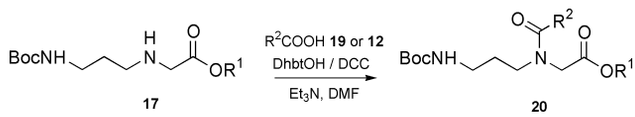
Scheme 2 *Reagents and conditions*: i, 1 M LiOH, THF, rt, 1 h.

The *tert*-butyl esters of **14b** and **14d** underwent hydrolytic cleavage by the action of TFA in dichloromethane to produce the free F-PNA monomers **16a** and **16b** respectively, in 77–95% yields (Scheme 3).

Synthesis of β-F-PNA monomers

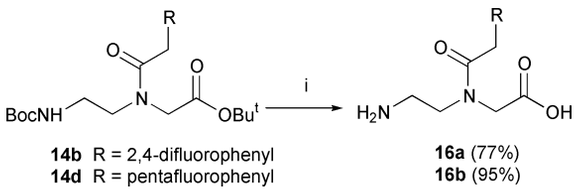
We next focused our attention on the synthesis of β-F-PNA monomers, that were designed based on the structure of PNA

Table 2 Synthesis of protected β-F-PNA monomers **20**



Entry	17	R^1	Acid	R^2	20	Yield (%)[a]
1	**17a**	Et	**19a**	2,4-Difluorophenyl	**20a**	60
2	**17b**	Bu^t	**19a**	2,4-Difluorophenyl	**20b**	50
3	**17a**	Et	**19b**	Pentafluorophenyl	**20c**	22 (51[b])
4	**17b**	Bu^t	**19b**	Pentafluorophenyl	**20d**	24 (50[b])
5	**17a**	Et	**12**	2,4-Difluoro-5-methylphenyl	**20e**	65

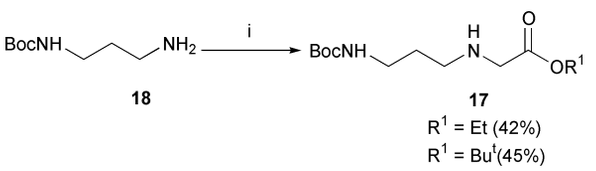
[a] Yields were determined based on **17** employed. [b] The reaction was carried out in the absence of Et_3N.



Scheme 3 *Reagents and conditions*: i, TFA, DCM, rt, 2 h.

and 2′,5′-linked isoDNA **5** (Fig. 3). 2′,5′-Linked isoDNA has recently drawn much interest due to its ability to form a heteroduplex with RNA that is as stable as the comparable normal DNA–RNA duplexes.[14] The β-F-PNA backbone **17** is composed of *N*-(3-aminopropyl)glycine, which is one carbon longer than the *N*-(2-aminoethyl)glycine backbone in PNA. Therefore, the β-F-PNA consists of a repeating unit of 7 atoms. Fluoroaromatics are attached to the new backbone through carbonyl linkers. This should be a suitable structural analogue of 2′,5′-linked isoDNA.

The new backbone **17** was easily prepared following the same procedure as applied to **8**[10] by direct alkylation of *N*-Boc-diaminopropane (**18**) instead of *N*-Boc-diaminoethane using ethyl chloroacetate or *tert*-butyl chloroacetate (Scheme 4).

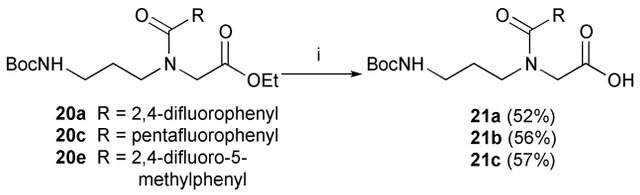


Scheme 4 *Reagents and conditions*: i, $ClCO_2R^1$ (R^1 = Et or Bu^t) Et_3N, KI, 50 h.

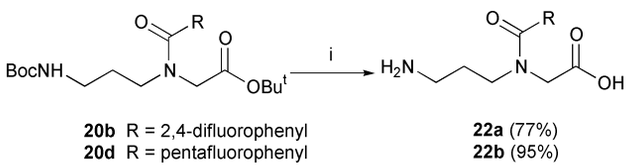
Another part of β-F-PNA is fluorobenzoic acids such as **19a**, **b** and **12**. 2,4-Difluorobenzoic acid (**19a**) and pentafluorobenzoic acid (**19b**) are commercially available and 2,4-difluoro-5-methylbenzoic acid (**12**) was already prepared in two steps as shown in Scheme 1. We first attempted the coupling of acids **19** and **12** to the backbone **17** using standard peptide coupling agents DhbtOH and DCC in DMF in the presence of triethylamine. The yields are moderate except in the case of coupling for **20c** and **20d** (22–24%) presumably due to the high acidity of **19b**. After optimization of the reaction conditions, the coupling reaction was performed nicely with the peptide coupling agents DhbtOH and DCC *in the absence of triethylamine* to furnish **20c** and **20d** in 51 and 50% yield, respectively (Table 2).

Completion of the synthetic studies was accomplished by deprotection of **20** to produce *N*-protected and free β-F-PNA monomers intended to be used for oligomerization. The ethyl esters **20a**, **20c** and **20e** were deprotected selectively by treating with 1 M LiOH in THF to give *N*-Boc-β-F-PNA monomers **21a**–**21c** in 52–57% yields (Scheme 5).

Likewise, the *tert*-butyl esters **20b** and **20d** underwent acidolysis with TFA in dichloromethane to afford the β-F-PNA monomers **22a** and **22b** respectively, in 77–97% yields (Scheme 6).



Scheme 5 *Reagents and conditions*: i, 1 M LiOH, THF, rt, 1 h.



Scheme 6 *Reagents and conditions*: i, TFA, DCM, rt, 2 h.

Conclusion

We have demonstrated the synthesis of a novel class of non-polar peptide nucleic acid monomers containing fluoroaromatics namely F-PNA and β-F-PNA by the standard peptide coupling reaction. Of particular interest to the synthesis, we have incorporated the 2,4-difluorotoluene moiety into the F-PNA analogues as an isosteric replacement for thymine which might confer unique properties to the compounds. Conversion of the F-PNA monomers into oligomers is under investigation.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected.

IR spectra (cm^{-1}) were recorded on a Perkin-Elmer 1600 spectrometer.

^{1}H-NMR spectra were measured as solutions in $CDCl_3$ or CD_3OD and chemical shifts are expressed in ppm relative to internal Me_4Si (0.00 ppm) and were recorded on a JEOL GX-270 (270 MHz) spectrometer. ^{19}F-NMR spectra were measured with $CFCl_3$ as an internal standard and were taken with a JEOL GX-270 (254 MHz) spectrometer. Upfield shifts are quoted as negative δ values. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. *J* Values are given in Hz.

Electron ionization (EI) mass spectra were taken with a JEOL JMS-D300 spectrometer. Column chromatography and preparative TLC were performed on BW-200 (Fuji Silysia) and Kieselgel 60 (Merck, art. 7748), respectively.

All reactions were carried out under a dry N_2 atmosphere. Unless otherwise noted, reagents were added by syringe. Commercially available dehydrated THF [stabilized with butylated

hydroxytoluene (BHT)] was used for reaction. DMF was distilled over CaH_2 immediately prior to use.

2,4-Difluoro-5-methylbenzonitrile 11

To a well-stirred solution of **10**[3a] (900 mg, 4.34 mmol) in dry DMF (40 ml), copper(I) cyanide (487 mg, 5.43 mmol) was added in one portion and the mixture was heated at 160 °C for 3 h. Then the reaction mixture was cooled and poured into water (20 ml), whereupon 20% aqueous $FeCl_3$ (9 ml) was added. The mixture was extracted with ether (2 × 100 ml) and the ether portion was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate 98 : 2) to afford **11** (335 mg, 50%) as a white crystalline solid; mp 55–56 °C (ethyl acetate–hexane); ν_{max} (KBr)/cm^{-1} 3054, 2932, 2232 (CN), 1535, 1089, 1004; δ_H (270 MHz, $CDCl_3$) 2.28 (3H, s, ArCH_3), 6.92 (1H, t, *J* 9.1, Ar*H*), 7.47 (1H, t, *J* 7.5, Ar*H*); *m*/*z* (EI) 153 (M^+), 126 (M^+ − HCN); HRMS found M^+ 153.0365. $C_8H_5F_2N$ requires 153.0342. Found: C, 62.80; N, 8.96; H, 3.11. Calc. for $C_8H_5F_2N$: C, 62.75; N, 9.15; H, 3.29%.

2,4-Difluoro-5-methylbenzoic acid 12

A solution of **11** (1.0 g, 6.53 mmol) in a mixture of concentrated H_2SO_4 (3.8 ml) and H_2O (2.8 ml) was stirred under reflux for 1 h. Then the mixture was poured into ice–water (20 ml). The precipitate obtained was filtered under reduced pressure and dried to yield **12** (1.74 g, 100%) as a white crystalline solid; mp 122–123 °C (dichloromethane–hexane); ν_{max} (KBr)/cm^{-1} 2981, 1699 (C=O); δ_H (270 MHz, $CDCl_3$) 2.26 (3H, s, ArCH_3), 6.82 (1H, t, *J* 9.9, Ar*H*), 7.83 (1H, t, *J* 8.2, Ar*H*); δ_F (254 MHz, $CDCl_3$) −109.3 (m), −105.7 (m); *m*/*z* (EI) 172 (M^+), 155 (M^+ − OH), 127 (M^+ − COOH); HRMS found M^+ 172.0371. $C_8H_6F_2O_2$ requires 172.0386. Found: C, 55.86; H, 3.52. Calc. for $C_8H_6F_2O_2$: C, 55.82; H, 3.51%.

2-Diazo-1-(2,4-difluoro-5-methylphenyl)ethanone 13

To a solution of **12** (500 mg, 2.92 mmol) in THF (15 ml) were added triethylamine (0.4 ml) and ethyl chlorocarbonate (0.27 ml) at −15 °C and stirred for 15 minutes. Then the mixture was allowed to warm to 0 °C and diazomethane in ether solution was added dropwise until a yellowish colour persisted for a long period. The whole mixture was stirred for 3 hours at room temperature. The reaction mixture was diluted with hexane–ethyl acetate (1 : 1; 100 ml) and washed successively with saturated $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtered and concentrated to obtain **13** (710 mg, 98%) as a yellowish solid mass which was used for the next step without further purification; ν_{max} (neat)/cm^{-1} 2986, 2109 (CH=N_2), 1735 (C=O); δ_H (270 MHz, $CDCl_3$) 2.28 (3H, s, ArCH_3), 4.34 (1H, s, CH=N_2), 6.87 (1H, t, *J* 9.9, Ar*H*), 7.85 (1H, t, *J* 8.2, Ar*H*); δ_F (254 MHz, $CDCl_3$) −106.6 (m), −101.8 (m); *m*/*z* (EI) 196 (M^+); HRMS found M^+ 196.0427. $C_9H_6N_2F_2O_2$ requires 196.0449.

(2,4-Difluoro-5-methylphenyl)acetic acid 9c

To a solution of **13** (668 mg, 3.40 mmol) in methanol (15 ml) under N_2 at −25 °C with the exclusion of light was added silver benzoate (85.6 mg, 0.373 mmol) dissolved in triethylamine (1.37 ml, 9.85 mmol) and the whole mixture was allowed to warm to room temperature within 3 h. The reaction mixture was diluted with ethyl acetate (200 ml) and successively washed with saturated $NaHCO_3$, NH_4Cl and brine, dried over Na_2SO_4, filtered and concentrated to obtain the methyl ester as a white solid (409 mg, 60%) which was characterized by NMR and mass spectral data; δ_H (270 MHz, $CDCl_3$) 2.26 (3H, s, ArCH_3), 3.71 (2H, s, ArCH_2), 3.91 (3H, s, OCH_3), 6.78 (1H, t, *J* 9.9, Ar*H*), 7.81 (1H, t, *J* 8.2, Ar*H*); δ_F (254 MHz, $CDCl_3$) −109.1 (m), −115.1 (m); *m*/*z* (EI) 200 (M^+), 185 (M^+ − Me); HRMS found M^+ 200.0638. $C_{10}H_{10}F_2O_2$ requires 200.0649. To a solution of the methyl ester (409 mg, 2.04 mmol) in THF (3 ml), 1 M LiOH (3 ml) was added and stirred overnight at room temperature. Then the reaction mixture was diluted with ether (20 ml). Water (10 ml) was added and extracted to take the aqueous portion. The aqueous portion was acidified with 1 M HCl up to pH 2. A solid precipitate was observed which was collected by filtration and on subsequent drying yielded **9c** (220 mg, 58%) as a white amorphous solid; mp 119–120 °C (dichloromethane–hexane); ν_{max} (KBr)/cm^{-1} 2980, 1705 (C=O), 1143, 1087; δ_H (270 MHz, $CDCl_3$) 2.28 (3H, s, ArCH_3), 3.67 (2H, s, ArCH_2), 6.86 (1H, t, *J* 9.9 Ar*H*), 7.88 (1H, t, *J* 8.2, Ar*H*); δ_F (254 MHz, $CDCl_3$) −107.6 (m), −103.7 (m); *m*/*z* (EI) 186 (M^+), 141 (M^+ − COOH); HRMS found M^+ 186.0470. $C_9H_8F_2O_2$ requires 186.0493.

General experimental procedure for the synthesis of protected F-PNA monomers 14: {*N*-[2-(*tert*-butoxycarbonylamino)ethyl]-*N*-[2-(2,4-difluorophenyl)acetyl]amino}acetic acid ethyl ester 14a

To a stirred solution of **8a** (200 mg, 0.813 mmol), DhbtOH (132 mg, 0.813 mmol) and **9a** (139 mg, 0.813 mmol) in DMF (5 ml) were added triethylamine (164 mg, 1.62 mmol) and DCC (168 mg, 0.813 mmol) at 0 °C. The ice-bath was removed after 1 h and stirring was continued for another 2–3 h at room temperature. The precipitated DCU was removed by filtration and washed twice with dichloromethane. To the combined filtrates was added dichloromethane (100 ml) and the resulting mixture was successively washed with dilute aqueous $NaHCO_3$, dilute aqueous $KHSO_4$, and brine. The organic phases were combined, dried over anhydrous Na_2SO_4, and concentrated to produce an oily residue which was chromatographed on silica gel (hexane–ethyl acetate 6 : 4) to give **14a** (165 mg, 51%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3429 (NH), 2981, 1741, 1704, 1651 (C=O), 1508, 1250, 1171; δ_H (270 MHz, $CDCl_3$) 1.29 (3H, t, *J* 7.2, OCH_2CH_3), 1.43 (9H, s, Bu^t), 3.28–3.57 (4H, m, >N(CH_2)$_2$N<), 3.72, 4.01 (total 2H, each s, ArCH_2), 3.74, 4.03 (total 2H, each s, >NCH_2CO), 4.21 (2H, q, *J* 7.2, 14.1, OCH_2CH_3), 4.95, 5.49 (total 2H, each br s, NH × 2), 6.83 (2H, m, Ar*H*), 7.27 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −111.9, −112.2 (each m), −113.2, −114.1 (each m); *m*/*z* (EI) 400 (M^+), 401 (M^+ + 1), 343 (M^+ − Bu^t); HRMS found M^+ 400.1780. $C_{19}H_{26}F_2N_2O_5$ requires 400.1804. Found: C, 56.66; N, 6.62; H, 6.21. Calc. for $C_{19}H_{26}F_2N_2O_5$: C, 56.99; N, 7.00; H, 6.54%.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(2,4-difluorophenyl)acetyl]amino}acetic acid *tert*-butyl ester 14b. This compound was prepared analogously to **14a** from **8b** (200 mg, 0.729 mmol), **9a** (125 mg, 0.728 mmol), DhbtOH (119 mg, 0.728 mmol), triethylamine (147 mg, 1.45 mmol) and DCC (150 mg, 0.728 mmol) in DMF (5 ml) to give **14b** (157 mg, 50%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3315, 3302 (NH), 2980, 1735, 1704, 1670 (C=O), 1508, 1247, 1160; δ_H (270 MHz, $CDCl_3$) 1.38, 1.39 (total 9H, each s, Bu^t), 1.41, 1.44 (total 9H, each s, Bu^t), 3.27–3.71 (6H, m, >N(CH_2)$_2$N< + ArCH_2), 3.88, 3.98 (total 2H, each s, >NCH_2CO), 5.04, 5.47 (total 2H, each br s, NH × 2), 6.83 (2H, m, Ar*H*), 7.27 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −112.2, −112.5 (each m), −113.3, −114 (each m); *m*/*z* (EI) 429 (M^+ + 1), 428 (M^+), 371 (M^+ − Bu^t); HRMS found M^+ + 1 429.2220. $C_{21}H_{31}F_2N_2O_5$ requires 429.2201. Found: C, 58.50; N, 6.55; H, 6.79. Calc. for $C_{21}H_{30}F_2N_2O_5$: C, 58.87: N, 6.54; H, 7.06%.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(pentafluorophenyl)acetyl]amino}acetic acid ethyl ester 14c. This compound was prepared from **8a** (200 mg, 0.813 mmol), **9b** (184 mg, 0.812 mmol), DhbtOH (133 mg, 0.812 mmol), triethylamine (164 mg, 1.62 mmol) and DCC (168 mg, 0.812 mmol) in DMF (5 ml) by a procedure analogous to **14a**. A similar purification procedure gave **14c** (214 mg, 58%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3434, 3321 (NH), 3006, 1743, 1703, 1661

(C=O), 1512, 1248, 1171, 1009; δ_H (270 MHz, $CDCl_3$) 1.24, 1.28 (total 3H, each t, *J* 7.2, OCH_2CH_3), 1.44 (9H, s, Bu^t), 3.33–3.67 (4H, m, >N(CH_2)$_2$N<), 3.74, 4.02 (total 2H, each s, $ArCH_2$), 3.83, 4.07 (total 2H, each s, >NCH_2CO), 4.21 (2H, q, *J* 7.2, 14.1, OCH_2CH_3), 5.02, 5.62 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −142.6, −143.2 (each m), −156.3, −156.8 (each m), −163.3, −163.6 (each m); *m*/*z* (EI) 454 (M^+), 398 ($M^+ - OC_2H_5$), 397 ($M^+ - Bu^t$), 381 ($M^+ - Bu^tO$); HRMS found ($M^+ - Bu^t$) 397.1156. $C_{15}H_{14}F_5N_2O_4$ requires 397.1177.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(pentafluorophenyl)acetyl]amino}acetic acid *tert*-butyl ester 14d. This compound was prepared from **8b** (200 mg, 0.729 mmol), **9b** (165 mg, 0.728 mmol), DhbtOH (119 mg, 0.728 mmol), triethylamine (147 mg, 1.45 mmol) and DCC (150 mg, 0.728 mmol) in DMF (5 ml) by a procedure analogous to **14a**. A similar chromatographic purification gave **14d** (193 mg, 55%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3340 (NH), 2981, 1703, 1657 (C=O), 1512, 1245, 1160, 1008; δ_H (270 MHz, $CDCl_3$) 1.43, 1.44 (total 9H, each s, Bu^t), 1.44, 1.45 (total 9H, each s, Bu^t), 3.29–3.72 (6H, m, >N(CH_2)$_2$N< + $ArCH_2$), 3.92, 4.13 (total 2H, each s, >NCH_2CO), 4.95, 5.53 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −139.1, −139.7 (each m), −152.7, −153.3 (each m), −159.6, −159.8 (each m); *m*/*z* (EI) 482 (M^+), 481($M^+ - 1$), 425 ($M^+ - Bu^t$), 368 ($M^+ - Bu^t \times 2$); HRMS found M^+ 482.1847. $C_{21}H_{27}F_5N_2O_5$ requires 482.1840. Found: C, 52.49; N, 5.66; H, 5.29. Calc. for $C_{21}H_{27}F_5N_2O_5$: C, 52.28; N, 5.81: H, 5.64%.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(2,4-difluoro-5-methylphenyl)acetyl]amino}acetic acid ethyl ester 14e. This compound was prepared from **8a** (200 mg, 0.813 mmol), **9c** (151 mg, 0.813 mmol), DhbtOH (133 mg, 0.813 mmol), triethylamine (164 mg, 1.62 mmol) and DCC (168 mg, 0.813 mmol) in DMF (5 ml). A similar work-up and purification procedure yielded compound **14e** (175 mg, 52%) as a mixture of rotamers; colourless oil; ν_{max} (neat)/cm^{-1} 3447 (NH), 2981, 1743, 1704, 1644 (C=O), 1507, 1247, 1174, 1109; δ_H (270 MHz, $CDCl_3$) 1.21, 1.33 (total 3H, each t, *J* 7.2, OCH_2CH_3), 1.44, 1.38 (total 9H, each s, Bu^t), 2.21, 2.24 (total 3H, each s, $ArCH_3$), 3.19–3.71 (4H, m, >N(CH_2)$_2$N< + $ArCH_2$), 3.98, 4.17 (total 2H, each s, >NCH_2CO), 4.26 (2H, q, *J* 7.2, 14.5, OCH_2CH_3), 5.02, 5.29 (2H, each br s, NH × 2), 6.78 (1H, t, *J* 9.2, Ar*H*), 7.22 (1H, m, Ar*H*); δ_F (254 MHz. $CDCl_3$) −110.2, −110.4 (each m), −115.1, −115.7 (each m); *m*/*z* (EI) 414 (M^+), 413 ($M^+ - 1$), 357 ($M^+ - Bu^t$); HRMS found M^+ 414.1967. $C_{20}H_{28}F_2N_2O_5$ requires 414.1974.

General procedure for the selective deprotection of 14: {*N*-[2-(*tert*-butoxycarbonylamino)ethyl]-*N*-[2-(2,4-difluorophenyl)acetyl]-amino}acetic acid 15a

Compound **14a** (50 mg, 0.125 mmol) was suspended in THF (2 ml) and 1 M LiOH (2 ml) was added. The mixture was stirred for 1 h at room temperature. Water (2 ml) was added to the solution and it was washed with dichloromethane (10 ml). Additional water (2 ml) was added and the solution was washed once more with dichloromethane (5 ml). The aqueous solution was cooled to 0 °C and the pH was adjusted to 2 by dropwise addition of 1 M HCl. A white precipitate was formed which was collected by decantation or filtration. The precipitate was dried to afford **15a** (33 mg, 70%) as a mixture of rotamers; amorphous solid; mp 134–135 °C; ν_{max} (KBr)/cm^{-1} 3441 (NH), 2931, 1705, 1602 (C=O), 1509, 1206, 1174; δ_H (270 MHz, $CDCl_3$) 1.43 (9H, s, Bu^t), 3.30–3.73 (6H, m, >N(CH_2)$_2$N< + $ArCH_2$), 3.73, 4.06 (total 2H, each s, >NCH_2CO), 4.14, 5.34 (total 2H, each br s, NH × 2), 6.83 (2H, m, Ar*H*), 7.26 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −112.0, −112.4 (each m), −113.2, −114.0 (each m); *m*/*z* (EI) 372 (M^+), 355 ($M^+ - OH$), 315 ($M^+ - Bu^t$); HRMS found M^+ 372.1518. $C_{17}H_{22}F_2N_2O_5$ requires 372.1497.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(pentafluorophenyl)acetyl]amino}acetic acid 15b. Compound **15b** (26 mg, 55%) was prepared analogously to **15a** from the selective basic hydrolysis of **14c** (50 mg, 0.11 mmol) as a mixture of rotamers; white solid; mp 123–125 °C; ν_{max} (KBr)/cm^{-1} 3415 (NH), 2990, 1740, 1654 (C=O), 1442, 1369, 1183, 1132; δ_H (270 MHz, $CDCl_3$) 1.42 (9H, s, Bu^t), 3.33–3.82 (6H, m, >N(CH_2)$_2$N< + $ArCH_2$), 3.82, 4.04 (total 2H, each s, >NCH_2CO), 4.18, 5.52 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −142.7 (m), −156.2, −156.6 (each m), −162.8, −163.3 (each m); *m*/*z* (EI) 426 (M^+), 425 ($M^+ - 1$), 409 ($M^+ - OH$), 369 ($M^+ - Bu^t$); HRMS found M^+ 426.1232. $C_{17}H_{19}F_5N_2O_5$ requires 426.1214. Found: C, 47.62; N, 6.54; H, 4.38. Calc. for $C_{17}H_{19}F_5N_2O_5$: C, 47.89; N, 6.57; H, 4.49%.

{*N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-*N*-[2-(2,4-difluoro-5-methylphenyl)acetyl]amino}acetic acid 15c. Compound **15c** (25 mg, 54%) was obtained analogously to **15a** from the selective basic hydrolysis of **14e** (59 mg, 0.12 mmol) as a mixture of rotamers; white powder; mp 68–69 °C; ν_{max} (KBr)/cm^{-1} 3388, 3158 (NH), 2980, 1715, 1664 (C=O), 1512, 1174; δ_H (270 MHz, $CDCl_3$) 1.43, 1.49 (total 9H, each s, Bu^t), 2.19, 2.28 (total 3H, each s, $ArCH_3$), 3.17–3.69 (6H, m, >N(CH_2)$_2$N< + $ArCH_2$), 4.01, 4.06 (total 2H, each s, >NCH_2CO), 4.23, 5.45 (total 2H, each br s, NH × 2), 6.71 (1H, m, Ar*H*), 7.52 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −114.8, −118.4 (each m), −120.1, −121.7 (each m); *m*/*z* (EI) 386 (M^+), 387 ($M^+ + 1$), 329 ($M^+ - Bu^t$); HRMS found M^+ 386.1672. $C_{18}H_{24}F_2N_2O_5$ requires 386.1653.

General procedure for the full deprotection of 14: {*N*-(2-aminoethyl)-*N*-[2-(2,4-difluorophenyl)acetyl]amino}acetic acid 16a

A clear solution of **14b** (50 mg, 0.116 mmol) in a mixture of dichloromethane (2 ml) and trifluoroacetic acid (1 ml) was stirred at room temperature for 2 h. The reaction mixture was dried *in vacuo* and the residual trifluoroacetic acid was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether, filtered and dried to yield **16a** (24 mg, 77%) as a mixture of rotamers; amorphous solid; mp 118–120 °C; ν_{max} (KBr)/cm^{-1} 3088 (NH_3^+), 1727 (C=O), 1512, 1325 (COO^-), 1194, 1142; δ_H (270 MHz, CD_3OD) 3.13–3.70 (4H, m, >N(CH_2)$_2$N<), 3.70, 3.87 (total 2H, each s, $ArCH_2$), 4.12, 4.31 (2H, each s, NCH_2CO), 6.89 (2H, m, Ar*H*), 7.26 (1H, m, Ar*H*); δ_F (254 MHz, CD_3OD) −111.6, −112.1 (each m), −112.7 (q, *J* 8.7, 17.9); *m*/*z* (EI) 272 (M^+), 271 ($M^+ - 1$), 255 ($M^+ - OH$), 254 [($M^+ - 1$) − OH]; HRMS found [($M^+ - 1$) − OH] 254.0867. $C_{12}H_{12}F_2N_2O_2$ requires 254.0855.

{*N*-(2-Aminoethyl)-*N*-[2-(pentafluorophenyl)acetyl]amino}-acetic acid 16b. Compound **16b** (32 mg, 95%) was obtained by a similar treatment of **14d** (50 mg, 0.103 mmol) with trifluoroacetic acid in dichloromethane as a mixture of rotamers; amorphous solid; mp 129–130 °C; ν_{max} (KBr)/cm^{-1} 3127 (NH_3^+), 1633 (C=O), 1514, 1334 (COO^-), 1193, 1138; δ_H (270 MHz, CD_3OD) 3.12 (2H, t, *J* 5.8, >N(CH_2CH_2N<), 3.66 (2H, t, *J* 5.8, >NCH_2CH_2N<), 3.79, 3.98 (total 2H, each s, $ArCH_2$), 4.02, 4.21 (2H, each s, >NCH_2CO); δ_F (254 MHz, CD_3OD) −142.6, −142.8 (each dd, *J* 8.4, 21.2), −157.4, −157.7 (each t, *J* 20.3), −164.3, −164.6 (each m); *m*/*z* (EI) 326 (M^+), 309 ($M^+ - OH$); HRMS found ($M^+ - OH$) 309.0659. $C_{12}H_{10}F_5N_2O_2$ requires 309.0662.

General procedure for the preparation of 17: {[3-(*tert*-butoxycarbonylamino)propyl]amino}acetic acid ethyl ester 17a

To a stirred solution of **18** (2.08 g, 11.4 mmol) in dichloromethane (10 ml) were added triethylamine (1.16 g, 11.4 mmol) and potassium iodide (1.9 g, 11.4 mmol). Ethyl chloroacetate (1.17 ml, 11.4 mmol) was then added dropwise with stirring and the mixture was stirred at 40 °C for 50 h. Water (50 ml) was added and the mixture was agitated vigorously. The organic phase was

separated and the aqueous phase was extracted with dichloromethane (2 × 100 ml). The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude product was chromatographically purified on silica gel (ethyl acetate–methanol 95 : 5) to yield **17a** (1.25 g, 42%) as a pale yellow oil; δ_H (270 MHz, $CDCl_3$) 1.29 (3H, t, *J* 7.1, OCH_2CH_3), 1.44 (9H, s, Bu^t), 1.67 (2H, m, $CH_2CH_2CH_2$) 2.66 (2H, t, *J* 6.7, CH_2CH_2N), 3.22 (2H, br d, *J* 4.3, NCH_2CH_2CO), 3.53 (2H, s, $>NCH_2CO$), 4.17 (2H, q, *J* 7.1, 14.2, OCH_2CH_3), 4.85 (1H, br s, NH), 5.05 (1H, br s, NH); *m*/*z* (EI) 260 (M^+), 261 ($M^+ + 1$), 204 ($M^+ - Bu^t$); HRMS found M^+ 260.1682. $C_{12}H_{24}N_2O_4$ requires 260.1706.

{[3-(*tert*-Butoxycarbonylamino)propyl]amino}acetic acid *tert*-butyl ester 17b. This compound was prepared analogously to **17a** from **18** (2.25 g, 12.9 mmol), triethylamine (1.29 g, 12.9 mmol), potassium iodide (2.1 g, 12.9 mmol), *tert*-butyl chloroacetate (1.95 g, 12.9 mmol) in dichloromethane (10 ml). A similar work-up and purification procedure yielded **17b** (1.7 g, 45%) as a pale yellow oil; δ_H (270 MHz, $CDCl_3$) 1.41 (9H, s, Bu^t), 1.44 (9H, s, Bu^t), 1.72 (2H, m, $CH_2CH_2CH_2$), 2.66–3.21 (4H, m, $NCH_2CH_2CH_2N$), 3.31 (2H, s, $>NCH_2CO$), 4.97 (1H, br s, NH), 5.02 (1H, br s, NH); *m*/*z* (EI) 288 (M^+), 289 ($M^+ + 1$), 215 ($M^+ - Bu^t \times 2$); HRMS found M^+ 288.2086. $C_{14}H_{28}N_2O_4$ requires 288.2065.

General procedure for the preparation of 20: {*N*-[3-(*tert*-butoxycarbonylamino)propyl]-*N*-(2,4-difluorobenzoyl)amino}acetic acid ethyl ester 20a

This compound was prepared from **17a** (200 mg, 0.769 mmol), **19a** (121 mg, 0.768 mmol), DhbtOH (125 mg, 0.768 mmol), triethylamine (156 mg, 1.53 mmol) and DCC (159 mg, 0.768 mmol) in DMF (5 ml) by a procedure analogous to **14a**. A similar work-up and purification yielded **20a** (185 mg, 60%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3356 (NH), 2981, 1744, 1704, 1642 (C=O), 1510, 1369, 1267, 1171, 1101; δ_H (270 MHz, $CDCl_3$) 1.21, 1.28 (total 3H, each t, *J* 7.2, OCH_2CH_3), 1.39, 1.44 (total 9H, each s, Bu^t), 1.78 (2H, m, $CH_2CH_2CH_2$), 2.88–3.64 (4H, m, $>NCH_2CH_2CH_2N<$), 3.93, 4.21 (total 2H, each s, $>NCH_2CO$), 4.21 (2H, q, *J* 7.2, 14.2, OCH_2CH_3), 4.95, 5.34 (total 2H, each br s, NH × 2), 6.88 (2H, m, Ar*H*), 7.37 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −106.9, −107.2 (each m), −110.7, −111.4 (each q, *J* 8.3, 16.5); *m*/*z* (EI) 400 (M^+), 401 ($M^+ + 1$), 343 ($M^+ - Bu^t$); HRMS found M^+ 400.1830. $C_{19}H_{26}F_2N_2O_5$ requires 400.1810. Found: C, 57.06; N, 7.09; H, 6.51. Calc. for $C_{19}H_{26}F_2N_2O_5$: C, 56.99; N, 7.00; H, 6.54%.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(2,4-difluorobenzoyl)amino}acetic acid *tert*-butyl ester 20b. This compound was prepared analogously to **14a** from **17b** (200 mg, 0.694 mmol), **19a** (110 mg, 0.693 mmol), DhbtOH (113 mg, 0.693 mmol), triethylamine (140 mg, 1.38 mmol) and DCC (143 mg. 0.693 mmol) in DMF (5 ml). A similar work-up and purification procedure yielded **20b** (150 mg, 50%) as a mixture of rotamers; colourless oil; ν_{max} (neat)/cm^{-1} 3344 (NH), 2980, 1739, 1704, 1642 (C=O), 1510, 1247, 1160; δ_H (270 MHz, $CDCl_3$) 1.38, 1.39 (total 18H, each s, $Bu^t \times 2$), 1.77 (2H, m, $CH_2CH_2CH_2$), 2.82–3.63 (4H, m, $>NCH_2CH_2CH_2N<$), 3.81, 4.13 (total 2H, each s, $>NCH_2CO$), 4.68, 5.31 (total 2H, each br s, NH × 2), 6.87 (2H, m, Ar*H*), 7.37 (1H, t, *J* 7.4, Ar*H*); δ_F (254 MHz, $CDCl_3$) −107.2 (m), −110.7, −111.3 (each m); *m*/*z* (EI) 428 (M^+), 427 ($M^+ - 1$), 371 ($M^+ - Bu^t$); HRMS found M^+ 428.2123. $C_{21}H_{30}F_2N_2O_5$ requires 428.2138. Found: C, 58.52; N, 6.48; H, 6.89. Calc. for $C_{21}H_{30}F_2N_2O_5$: C, 58.87; N, 6.54; H, 7.06%.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(pentafluorobenzoyl)amino}acetic acid ethyl ester 20c. This compound was prepared from **17a** (200 mg, 0.769 mmol), **19b** (163 mg, 0.768 mmol), DhbtOH (125 mg, 0.768 mmol), DCC (159 mg, 0.768 mmol) in DMF (3 ml) and dichloromethane (2 ml) by a procedure analogous to **14a**. A similar work-up and purification gave **20c** (189 mg, 51%) as a mixture of rotamers; colourless oil; ν_{max} (neat)/cm^{-1} 3364 (NH), 2981, 1744, 1703, 1660 (C=O), 1503, 1251, 1171; δ_H (270 MHz, $CDCl_3$) 1.81 (3H, t, *J* 7.2, OCH_2CH_3), 1.39, 1.44 (total 9H, each s, Bu^t), 1.76 (2H, m, $CH_2CH_2CH_2$), 3.03–3.68 (4H, m, $>NCH_2CH_2CH_2N<$), 3.94, 4.17 (total 2H, each s, $>NCH_2CO$), 4.16 (2H, q, *J* 7.2, 14.2, OCH_2CH_3), 4.47, 5.11 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −140.2, −140.5 (each br d, *J* 15.7), −151.3, −151.9 (each t, *J* 19.4), −159.7 (m); *m*/*z* (EI) 454 (M^+), 397 ($M^+ - Bu^t$), 409 ($M^+ - OC_2H_5$); HRMS found M^+ 454.1505. $C_{19}H_{23}F_5N_2O_5$ requires 454.1527.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(pentafluorobenzoyl)amino}acetic acid *tert*-butyl ester 20d. This compound was prepared from **17b** (200 mg, 0.694 mmol), **19b** (147 mg, 0.694 mmol), DhbtOH (113 mg, 0.694 mmol), DCC (143 mg, 0.698 mmol) in DMF (3 ml) and dichloromethane (2 ml) by a procedure analogous to **14a**. A similar work-up and purification procedure gave **20d** (158 mg, 50%) as a mixture of rotamers; pale yellow oil; ν_{max} (neat)/cm^{-1} 3451, 3340 (NH), 2980, 1706, 1659 (C=O), 1504, 1247, 1162; δ_H (270 MHz, $CDCl_3$) 1.40, 1.41 (total 9H, each s, Bu^t), 1.44, 1.46 (total 9H, s, Bu^t), 1.72 (2H, m, $CH_2CH_2CH_2$), 3.03–3.65 (4H, m, $>NCH_2CH_2CH_2N<$), 3.82, 4.14 (total 2H, each s, $>NCH_2CO$), 4.34, 5.13 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −139.9, −140.5 (each m), −151.6, −152.1 (each t, *J* 19.4), −159.9 (m); *m*/*z* (EI) 482 (M^+), 481 ($M^+ - 1$), 425 ($M^+ - Bu^t$); HRMS found M^+ 482.1805. $C_{21}H_{27}F_5N_2O_5$ requires 482.1830.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(2,4-difluoro-5-methylbenzoyl)amino}acetic acid ethyl ester 20e. This compound was prepared from **17a** (200 mg, 0.769 mmol), **12** (132 mg, 0.768 mmol), DhbtOH (125 mg, 0.768 mmol), triethylamine (156 mg, 1.53 mmol) and DCC (159 mg, 0.768 mmol) in DMF (5 ml). A similar work-up and purification yielded **20e** (209 mg, 65%) as a mixture of rotamers; colourless oil; ν_{max} (neat)/cm^{-1} 3453 (NH), 2982, 1744, 1704, 1641 (C=O), 1507, 1248, 1176, 1026; δ_H (270 MHz, $CDCl_3$) 1.21, 1.31 (total 3H, each t, *J* 7.1, OCH_2CH_3), 1.39, 1.44 (total 9H, each s, Bu^t), 1.78 (2H, m, $CH_2CH_2CH_2$), 2.22, 2.25 (total 3H, each s, $ArCH_3$), 2.99–3.64 (4H, m, $>NCH_2CH_2CH_2N<$), 3.94, 4.17 (2H, each s, $>NCH_2CO$), 4.27 (2H, q, *J* 7.1, 14.3, OCH_2CH_3), 4.43, 5.32 (total 2H, each br s, NH × 2), 6.82 (1H, m, Ar*H*), 7.22 (1H, m, Ar*H*); δ_H (254 MHz, $CDCl_3$) −110.2, −110.5 (each d, *J* 8.3), −115.1, −115.7 (each q, *J* 8.3, 15.6); *m*/*z* (EI) 414 (M^+), 415 ($M^+ + 1$), 358 [($M^+ + 1$) − Bu^t]; HRMS found M^+ 414.1961. $C_{20}H_{28}F_2N_2O_5$ requires 414.1967. Found: C, 57.94; N, 6.75; H, 6.54. Calc. for $C_{20}H_{28}F_2N_2O_5$: C, 57.96; N, 6.76; H, 6.81%.

Selective deprotection of 20: {*N*-[3-(*tert*-butoxycarbonylamino)-propyl]-*N*-(2,4-difluorobenzoyl)amino}acetic acid 21a

Compound **21a** (29 mg, 52%) was obtained by a procedure analogous to **15a** from the selective basic hydrolysis of **20a** (60 mg, 0.15 mmol) as a mixture of rotamers; white amorphous solid; mp 69–70 °C; ν_{max} (KBr)/cm^{-1} 3356 (NH), 2981, 2936, 1744, 1702, 1642 (C=O), 1269, 1170; δ_H (270 MHz, $CDCl_3$) 1.39, 1.44 (total 9H, each s, Bu^t), 1.72 (2H, m, $CH_2CH_2CH_2$), 3.01–3.74 (4H, m, $>NCH_2CH_2CH_2N<$), 3.95, 4.26 (total 2H, each s, $>NCH_2CO$), 4.48, 5.31 (total 2H, each br s, NH × 2), 6.91 (2H, m, ArH), 7.38 (1H, m, ArH); δ_F (254 MHz, $CDCl_3$) −106.6, −106.8 (each m), −110.5, −111.4 (each m); *m*/*z* (EI) 372 (M^+), 371 ($M^+ - 1$), 355 ($M^+ - OH$), 315 ($M^+ - Bu^t$); HRMS found M^+ 372.1498. $C_{17}H_{22}F_2N_2O_5$ requires 372.1497.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(pentafluorobenzoyl)amino}acetic acid 21b. Compound **21b** (30 mg, 56%)

was obtained by a procedure analogous to **15a** from the selective basic hydrolysis of **20c** (60 mg, 0.124 mmol) as a mixture of rotamers; white powder; mp 62–63 °C; ν_{max} (KBr)/cm^{-1} 3455 (NH), 2930, 1708, 1658 (C=O), 1503, 1368, 1246, 1166; δ_H (270 MHz, $CDCl_3$) 1.40, 1.44 (total 9H, each s, Bu^t), 1.73 (2H, m, $CH_2CH_2CH_2$), 3.06–3.67 (4H, m, >$NCH_2CH_2CH_2N$<), 3.97, 4.29 (total 2H, s × 2, >NCH_2CO), 4.56, 5.15 (total 2H, each br s, NH × 2); δ_F (254 MHz, $CDCl_3$) −140.1 (m), −151.6 (m), −159.8 (m); *m*/*z* (EI) 426 (M^+), 425 ($M^+ - 1$), 369 ($M^+ - Bu^t$); HRMS found M^+ 426.1263. $C_{17}H_{19}F_5N_2O_5$ requires 426.1245.

{*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-*N*-(2,4-difluoro-5-methylbenzoyl)amino}acetic acid 21c. Compound **21c** (32 mg, 57%) was obtained by a procedure analogous to **15a** from the selective basic hydrolysis of **20e** (60 mg, 0.144 mmol) as a mixture of rotamers; white solid; mp 81–82 °C; ν_{max} (KBr)/cm^{-1} 3355 (NH), 2979, 1703, 1639 (C=O), 1513, 1368, 1250, 1175, 1009; δ_H (270 MHz, $CDCl_3$) 1.39, 1.44 (total 9H, each s, Bu^t), 1.76 (2H, m, $CH_2CH_2CH_2$), 2.22, 2.26 (total 3H, each s, $ArCH_3$), 3.03–3.75 (4H, m, >$NCH_2CH_2CH_2N$<), 3.97, 4.26 (total 2H, each s, >NCH_2CO), 4.44, 5.31 (total 2H, each br s, NH × 2), 6.82 (1H, m, Ar*H*), 7.24 (1H, m, Ar*H*); δ_F (254 MHz, $CDCl_3$) −112.2, −112.4 (each m), −117.3, −118.6 (each m); *m*/*z* (EI) 386 (M^+), 387 ($M^+ + 1$), 329 ($M^+ - Bu^t$); HRMS found M^+ 386.1680. $C_{18}H_{24}F_2N_2O_5$ requires 386.1704. Found: C, 55.56; N, 7.26; H, 6.11. Calc. for $C_{18}H_{24}F_2N_2O_5$: C, 55.95; N, 7.25; H, 6.26%.

Full deprotection of 20: [*N*-(3-aminopropyl)-*N*-(2,4-difluorobenzoyl)amino]acetic acid 22a

Compound **22a** (37 mg, 97%) was obtained by a acid treatment of **20b** (60 mg, 0.14 mmol) similar to **16a** as a mixture of rotamers; sticky solid; ν_{max} (neat)/cm^{-1} 3066 (NH_3^+), 1634 (C=O), 1471, 1430 (COO^-), 1200, 1138; δ_H (270 MHz, CD_3OD) 1.88 (2H, m, $CH_2CH_2CH_2$), 3.04–3.71 (4H, m, >$NCH_2CH_2CH_2N$<), 3.98, 4.24 (total 2H, each s, >NCH_2CO), 7.08 (2H, m, Ar*H*), 7.44 (1H, m, Ar*H*); δ_F (254 MHz, CD_3OD) −106.6, −106.8 (each m), −110.9, −111.4 (each q, *J* 8.3, 17.6); *m*/*z* (EI) 272 (M^+), 255 ($M^+ - OH$); HRMS found M^+ 272.0973. $C_{12}H_{14}F_2N_2O_3$ requires 272.0995.

[*N*-(3-Aminopropyl)-*N*-(pentafluorobenzoyl)amino]acetic acid 22b. Compound **22b** (31 mg, 75%) was obtained by a similar acid treatment of **20d** (60 mg, 0.124 mmol) as a mixture of rotamers; amorphous solid; mp 94–95 °C; ν_{max} (KBr)/cm^{-1} 3116 (NH_3^+), 1658 (C=O), 1504, 1320 (COO^-), 1201, 1142; δ_H (270 MHz, CD_3OD) 1.98 (2H, m, $CH_2CH_2CH_2$), 3.04–3.72 (4H, m, >$NCH_2CH_2CH_2N$<), 4.08, 4.17 (total 2H, each s, >NCH_2CO); δ_F (254 MHz, CD_3OD) −140.6, −141.5 (each m), −152.9, −153.1 (each m), −160.8, −161.1 (each m); *m*/*z* (EI) 326 (M^+), 309 ($M^+ - OH$); HRMS found M^+ 326.0724. $C_{12}H_{11}F_5N_2O_3$ requires 326.0720.

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