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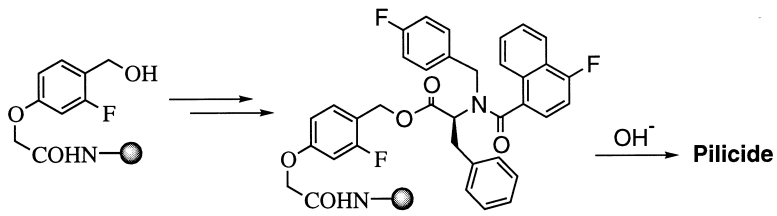

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Preparation of Fluorinated Linkers: Use of ^{19}F NMR Spectroscopy to Establish Conditions for Solid-Phase Synthesis of Pilicide Libraries

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Three fluorinated linkers which are analogues of linkers commonly used in solid-phase peptide synthesis have been prepared. One of the linkers was used in combination with gel-phase ^{19}F NMR spectroscopy to develop conditions for solid-phase synthesis of two libraries of pilicides, i.e. compounds designed to inhibit assembly of adhesive pili in uropathogenic *Escherichia coli*. Attachment to and cleavage from the linker could be monitored based on the chemical shift of the fluorine atom of the linker. In addition, use of the linker as internal standard allowed quantification and optimization of reactions occurring further away from the linker when fluorinated building blocks were employed. Importantly, high-quality ^{19}F NMR spectra were obtained for compounds linked to a TentaGel resin in a standard NMR tube using an ordinary NMR instrument.

Introduction

Solid-phase synthesis was developed into a fast and reliable technique for synthesis of peptides almost 40 years ago.[1] During the last 10 years interest in solid-phase *organic* synthesis has increased substantially due to the emergence of combinatorial and parallel synthesis strategies that are now being widely applied in pharmaceutical research.[2−6] This has brought about a need for adoption of synthetic organic methodology developed in solution so as to become compatible with various solid supports. In addition, methods are required that allow monitoring of reactions performed on solid phase as well as determination of the structures of the resulting products while still linked to the solid support.

NMR spectroscopy is a well-established technique in solution-phase organic chemistry and appears to be the analytical tool of choice also for solid-phase organic synthesis.[7−14] However, conventional ^{1}H and ^{13}C NMR spectra of substances attached to a solid support usually suffer from drawbacks such as inhomogeneous line broadening, prolonged spectral acquisition, and interference of signals from the solid support. To circumvent these problems, several techniques for structural elucidation have been developed including magic angle spinning,[7−9,11,15,16] use of selectively ^{13}C enriched building blocks,[12] presaturation of support signals,[13] and combinations of these techniques.[15] The drawbacks of these methods are associated with high costs and/or requirements for specialized instrumentation.

Problems with interference of signals from the solid support can be avoided by substituting ^{1}H or ^{13}C for another nucleus, such as ^{19}F, which is not part of commonly used solid supports. In addition, ^{19}F NMR spectroscopy is almost as sensitive as ^{1}H NMR spectroscopy since the natural abundance of ^{19}F is 100%. Another advantage of ^{19}F is that the large polarizability of the fluorine electron cloud makes it sensitive to remote changes in electron density, thereby spreading ^{19}F resonances over a large range of chemical shifts. These features render fluorine very well suited as a sensor for monitoring solid-phase chemical conversions using gel-phase ^{19}F NMR spectroscopy.

The choice of the linker is an important aspect in all solid-phase synthesis. The bond between the linker and the compound being synthesized should be orthogonal to the different reaction conditions employed for synthesis, but should then allow quantitative cleavage under mild conditions to release the final product. It is also an advantage if the linker can serve analytical purposes. We,[17] and later others,[18] have disclosed preliminary results concerning development of fluorine-derivatized solid supports and their use for quantitative monitoring of solid-phase organic synthesis. Other reports have also indicated the value of using ^{19}F NMR spectroscopy to study reactions performed on solid support[19−22] or for identification of members in libraries encoded with fluorinated tags.[23]

We now give a full account of the synthesis of three fluorinated linkers and demonstrate the use of one of them when establishing conditions for synthesis of compounds referred to as pilicides. The pilicides have been designed to bind to the active site of the periplasmic chaperones PapD and FimC,[24] using information obtained from the structures of complexes between PapD and peptides.[25,26] PapD and FimC play crucial roles in assembly of adhesive organelles called pili in *Escherichia coli* that cause urinary tract infections.[24] Blocking of the active site of the chaperones

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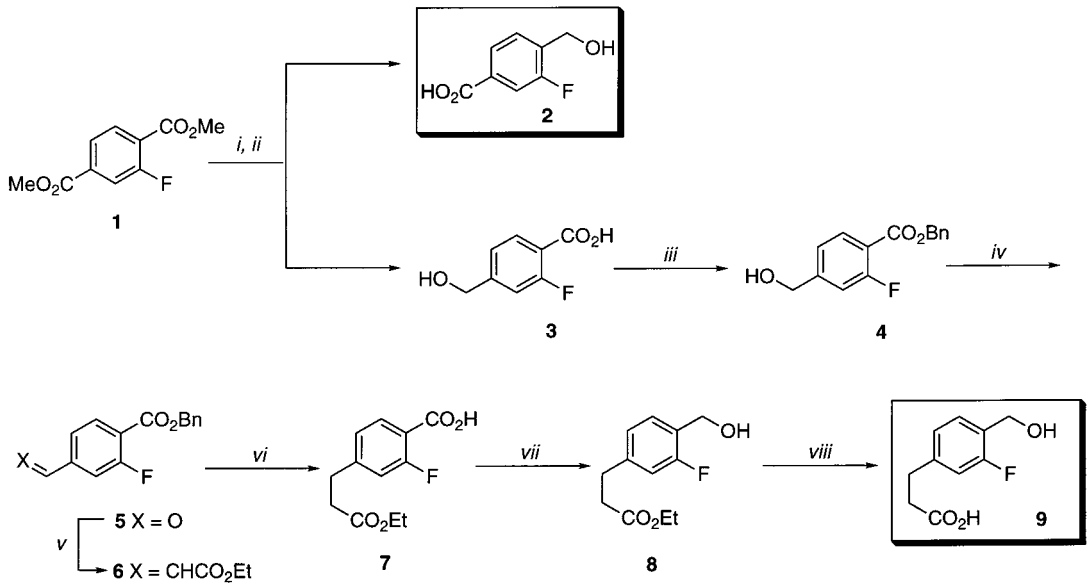
Scheme 1[a]



[a] Reaction conditions: (*i*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C → rt; (*ii*) $LiBH_4$, THF, **2** 41%, and **3** 31% over two steps; (*iii*) aqueous Cs_2CO_3, MeOH:H_2O (10:1), then BnBr, DMF, 79%; (*iv*) TPAP, *N*-methylmorpholine *N*-oxide, 4 Å molecular sieves, CH_2Cl_2, 74%; (*v*) NaH, $(EtO)_2P(O)CH_2CO_2C_2H_5$, THF, 0 °C → rt, 76%; (*vi*) Pd/C, H_2, EtOH:EtOAc (3:1), 88%; (*vii*) BH_3-DMS, $(CH_3O)_3B$, THF, 89%; (*viii*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C → rt, 93%.

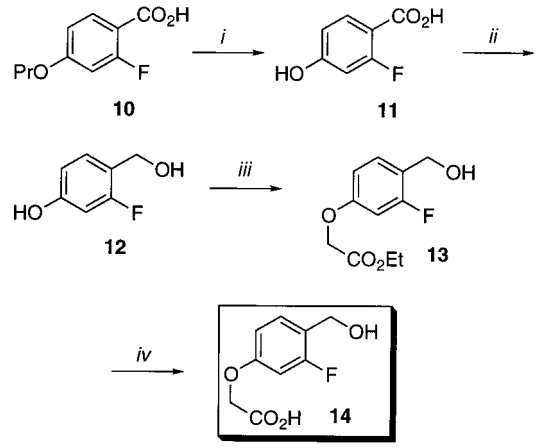
should prevent interactions with their natural ligands, i.e. pilus proteins targeted for incorporation in the growing pilus. A reduced assembly of functional, adhesive pili has been demonstrated to render the bacterium less pathogenic in animal models for urinary tract infection,[27] and pilicides therefore constitute potential novel antibiotics. Development of key steps in the routes to pilicides were facilitated by use of one of the fluorinated linkers alone or in combination with fluorinated building blocks.

Results and Discussion

Synthesis of Fluorinated Linkers. Solid-phase synthesis generally requires a linker that tethers the compound being synthesized to the solid support. Insertion of a fluorine atom in a key position on the linker could serve as a diagnostic marker for coupling to and cleavage from the linker, but also for reactions at some distance from the linker. We have therefore developed three fluorinated linkers suited for use in monitoring reactions by gel-phase ^{19}F NMR spectroscopy (cf. **2**, **9**, and **14** in Schemes 1 and 2). The linkers are analogues of linkers commonly used in solid-phase peptide synthesis, and were chosen so as to have different stability toward acidic and basic exposure.[28,29] Esters involving the hydroxymethyl group of *p*-hydroxymethyl benzoic acid (nonfluorinated **2**) are stable even under strongly acidic conditions (HF, RSO_3H), but they may be cleaved under basic conditions. The related esters derived from 3-[4-(hydroxymethyl)phenyl]propanoic acid (nonfluorinated **9**) require strongly acidic conditions for cleavage. In contrast, esters of 4-(hydroxymethyl)phenoxyacetic acid (nonfluorinated **14**) are significantly more acid labile and are cleaved by relatively mild acids such as trifluoroacetic acid (TFA).

Synthesis of linkers **2** and **9** started by unselective basic hydrolysis of one of the ester moieties in dimethyl 2-fluoroterephthalate (**1**, Scheme 1). Reduction of the resulting mixture of methyl esters with lithium borohydride and separation of the two regioisomers by flash column chromatography gave the linker 3-fluoro-4-(hydroxymethyl)-benzoic acid (**2**) and hydroxy acid **3**. Protection of **3** as a benzyl ester, followed by oxidation and olefination of the resulting aldehyde with triethyl phosphonoacetate, afforded alkene **6**. Deprotection of the benzylic ester and simultaneous hydrogenation of the double bond in **6** furnished **7**. Reduction of the carboxyl group of **7** with borane dimethyl sulfide complex, followed by basic hydrolysis of the ethyl ester, gave the linker 3-(3-fluoro-4-hydroxymethylphenyl)propanoic acid (**9**) in 32% overall yield from **3**.

Scheme 2[a]



[a] Reaction conditions: (*i*) BBr_3, CH_2Cl_2, −78 °C → rt, 89%; (*ii*) BH_3-DMS, $(CH_3O)_3B$, THF, 90%; (*iii*) $BrCH_2CO_2C_2H_5$, DBU, CH_3CN, reflux, 74%; (*iv*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C → rt, 87%.

Linker **14** was prepared in four steps from 2-fluoro-4-propoxybenzoic acid (**10**, Scheme 2). Dealkylation of **10** using boron tribromide followed by reduction of carboxylic acid **11** with borane dimethyl sulfide complex afforded compound **12**. Alkylation of the phenolic hydroxyl group of **12** with ethyl bromoacetate using DBU as base yielded **13**, which upon subsequent hydrolysis under basic conditions

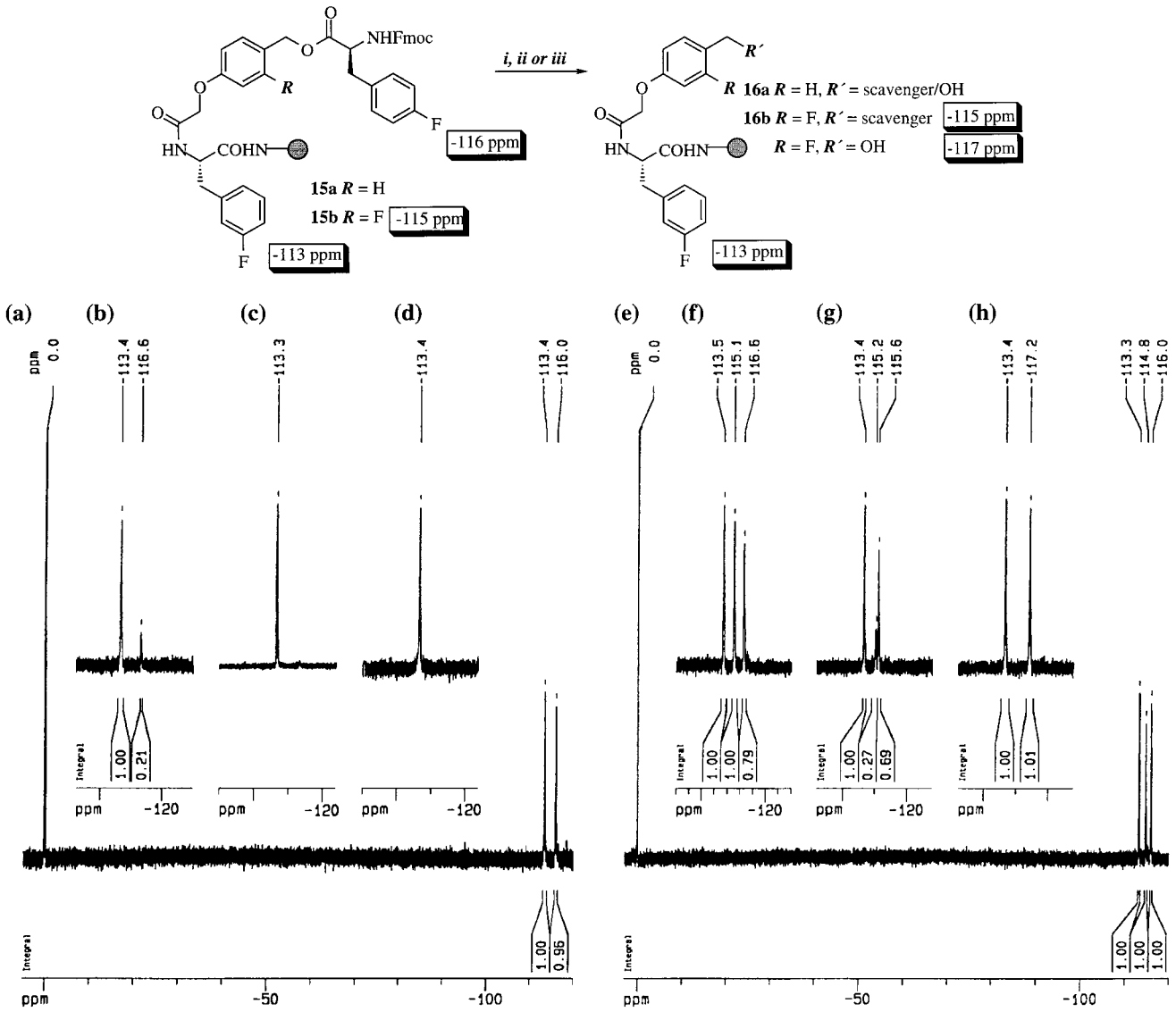


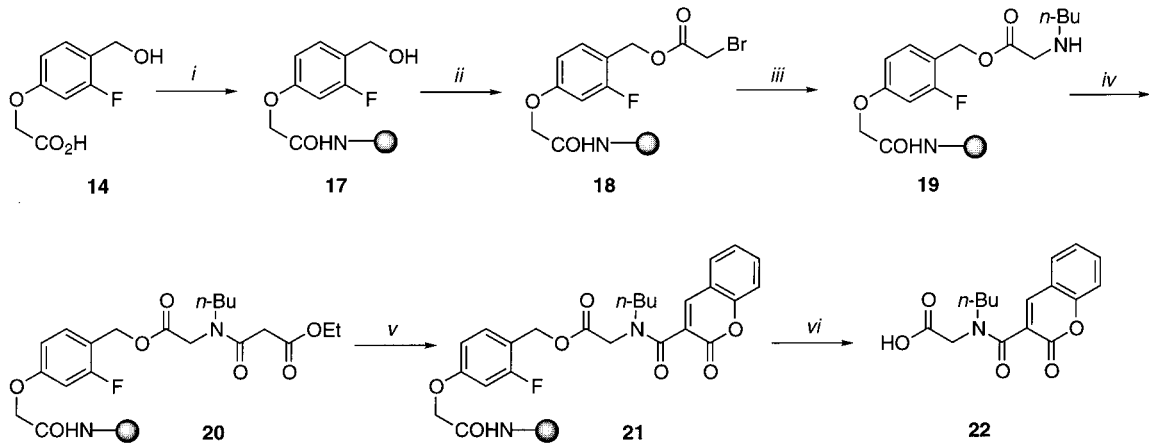
Figure 1. Use of gel-phase ^{19}F NMR spectroscopy to monitor cleavage of 4-(hydroxymethyl)phenoxyacetic acid derived linkers under acid- and base-catalyzed conditions. The *m*-fluorophenylalanine residue (^{19}F δ −113 ppm) linked directly to the resin served as an internal reference, and the *p*-fluorophenylalanine residue (^{19}F δ −116 ppm) allowed for monitoring of the cleavage. The ^{19}F resonance at 0.0 ppm originates from Cl_3CF used as internal standard. The spectra under (a) and (e) were obtained from **15a** and **15b** before cleavage. The following conditions were employed for cleavage: (*i*) TFA containing cationic scavengers (TFA:water:thioanisole:ethanedithiol, 87.5:5:5:2.5) at room temperature for 2 h; (*ii*) the same reagent at 60 °C for 2 h; (*iii*) aqueous 1 M LiOH in THF:MeOH:H_2O (3:1:1), 0 °C → room temperature. Integration over ^{19}F resonances in the ^{19}F NMR spectra revealed the following: (b) ∼80% cleavage from the nonfluorinated linker in **15a** to give **16a** under conditions (*i*), (c) and (d) complete cleavage of **15a** using conditions (*ii*) or (*iii*), respectively. For the fluorinated linker in **15b**, the ^{19}F NMR spectra showed the following: (f) ∼20% cleavage to give **16b** under condition (*i*), (g) and (h) complete cleavage under condition (*ii*) or (*iii*), respectively. The ^{19}F resonances at δ −115.2 and −115.6 ppm in (g) originate from the cleaved linker when trapped with the sulfur containing scavengers.

gave the linker 3-fluoro-4-(hydroxymethyl)phenoxyacetic acid (**14**) in 52% overall yield from **10**.

Development of Conditions for Cleavage of 4-(Hydroxymethyl)phenoxyacetic Acid Derived Linkers. Linkers based on benzylic esters are susceptible to cleavage under both acidic and basic conditions. The efficiency of base-catalyzed cleavage of this class of linkers relies upon steric factors. Under acidic conditions the lability of the linker increases with the number of electron-donating substituents on the aromatic ring which stabilize the benzylic cation formed upon cleavage of the ester linkage.[28] On the other hand, electron withdrawing substituents, such as the fluorine atom in linker **14**, would be expected to destabilize the benzylic cation.

We have previously employed gel-phase ^{19}F NMR spectroscopy for monitoring and optimization of acid-catalyzed cleavage of glycopeptides linked to a solid support via the Rink linker.[30] Complete cleavage required use of TFA and cationic scavengers,[31] as commonly used in peptide synthesis, but at elevated temperature. To explore conditions for cleavage of the fluorinated linker **14** versus the corresponding nonfluorinated linker, resin-bound **15a** and **15b** were prepared and subjected to cleavage under different conditions (Figure 1). The *m*-fluorophenylalanine residue linked directly to the resin in **15a** and **15b** served as an internal reference while the *p*-fluorophenylalanine residue was used to determine the amount of cleavage. Treatment of **15a** and **15b** with TFA containing cationic scavengers (TFA:water:thioanisole:

Scheme 3[a]



[a] Reaction conditions: (*i*) pentafluorophenol, DIC, TentaGel S NH_2, EtOAc; (*ii*) $BrCH_2CO_2H$, DIC, HOBt, DMAP, THF; (*iii*) *n*-butylamine, CH_3CN, 0 °C → rt; (*iv*) $ClCOCH_2CO_2C_2H_5$, DIPEA, CH_2Cl_2, 0 °C → rt; (*v*) salicylaldehyde, piperidine, CH_3CN, reflux; (*vi*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C → rt, 48%, or TFA containing water, thioanisole, and ethanedithiol (87.5:5:5:2.5), 60 °C, 48%. Yields are based on the capacity of the resin and were determined after purification of **22** by chromatography.

ethanedithiol, 87.5:5:5:2.5) at ambient temperature for 2 h effected cleavage of **15a** to an extent of ~80% (Figure 1b). However, for the fluorinated analogue **15b** a large part (~80%) remained unaffected under these conditions (Figure 1f). In contrast, using the same mixture of TFA and scavengers, but at 60 °C for 2 h, induced complete cleavage of both **15a** and **15b** (Figure 1c,g). Note that the ^{19}F resonances at −115 ppm (Figure 1g) originate from the linker trapped with cationic scavengers during cleavage. The ester linkage in both **15a** and **15b** can also be cleaved under mild, basic conditions. As judged by ^{19}F NMR spectroscopy, cleavage employing aqueous 1 M LiOH in a mixture of THF, MeOH, and H_2O (3:1:1) resulted in complete cleavage of both linkers (Figure 1d,h).

Use of Fluorinated Linkers To Monitor Solid-Phase Synthesis. We have previously shown that ^{19}F chemical shifts, as well as line widths for compounds attached to poly(ethylene glycol) grafted polystyrene resins (TentaGel resins), approach those in solution.[21] This makes gel-phase ^{19}F NMR spectroscopy well suited for adoption of solution-phase chemistry to various solid supports and for optimization of reaction conditions. In a preliminary study, linker **14** was attached to an amino functionalized TentaGel resin (TentaGel S NH_2) and to amino functionalized polystyrene, after which the primary hydroxyl group of the linker was acylated with *p*-fluorobenzoyl chloride. ^{19}F NMR spectroscopy of the two functionalized resins revealed that the ^{19}F line widths were 2−3 times larger for the polystyrene resin, as compared to the TentaGel resin, when $CDCl_3$, DMSO-d_6, benzene-d_6, or pyridine-d_5 were used to swell the resins. This result is in good agreement with previous studies of the influence of the resin on the quality of ^1H NMR spectra of solid-supported compounds.[13,14] Since the TentaGel resin was found to be superior, it was used in combination with linker **14** and gel-phase ^{19}F NMR spectroscopy when developing conditions to be used for solid-phase synthesis of a small library of pilicides. This library consisted of *N*-alkylated glycines acylated with substituted coumarine carboxylic acids and was assembled as shown for compound **22** in Scheme 3.

Efficient functionalization of the solid support with a linker is critical for the success of the subsequent synthesis. Attachment of **14** to a TentaGel S NH_2 resin using 1-hydroxy-7-azabenzotriazole (HOAt) and *N*,*N*′-diisopropylcarbodiimide (DIC) for activation was also found to result in some coupling of the activated linker to the hydroxyl group of **17**. This was indicated in the ^{19}F NMR spectrum which showed a peak for acylated linker at δ −115 ppm in addition to the peak at δ −117 ppm which originates from linker-resin **17** (peak ratio 1:3). Employing milder reaction conditions, i.e. coupling of the linker activated as the pentafluorophenyl ester, circumvented this *O*-acylation (Figure 2a) but still allowed complete coupling of **14** to the resin as revealed by monitoring with bromophenol blue.[32] Acylation of **17** with bromoacetic acid (3 equiv) in the presence of 1-hydroxybenzotriazole (HOBt), DIC, and a catalytic amount of 4-(dimethylamino)pyridine (DMAP)[33] did not give complete conversion into **18** (Figure 2b). As judged by ^{19}F NMR spectroscopy, the conversion of **17** into **18** was improved from 90% to 100% by repeating the acylation using 1.5 equiv of bromoacetic acid (Figure 2c). It should be pointed out that high-quality spectra were obtained within minutes for samples of resin (~100 mg) in an ordinary NMR tube using a standard NMR spectrometer.

Nucleophilic substitution of the α-brominated ester **18** with *n*-butylamine (Scheme 3), followed by amidation of **19** with ethyl malonyl chloride and *N*,*N*′-diisopropylethylamine (DIPEA), did not induce any change in the ^{19}F NMR chemical shift. The ^{19}F NMR spectrum of **21** showed that Knoevenagel condensation of **20** with salicylaldehyde in CH_3CN using piperidine as base was accompanied by a side reaction (cf. Figure 2d). An additional peak having a shift identical to that of **17** indicated that some cleavage (~20%) of **22** from the solid support had occurred. Different reaction conditions were explored, but did not lead to any improvement. Pilicide **22** was finally cleaved from the resin using either aqueous LiOH in THF:MeOH:H_2O or the optimized conditions based on TFA as described above. In both cases **22** was isolated in 48% yield based on the overall capacity of the resin.

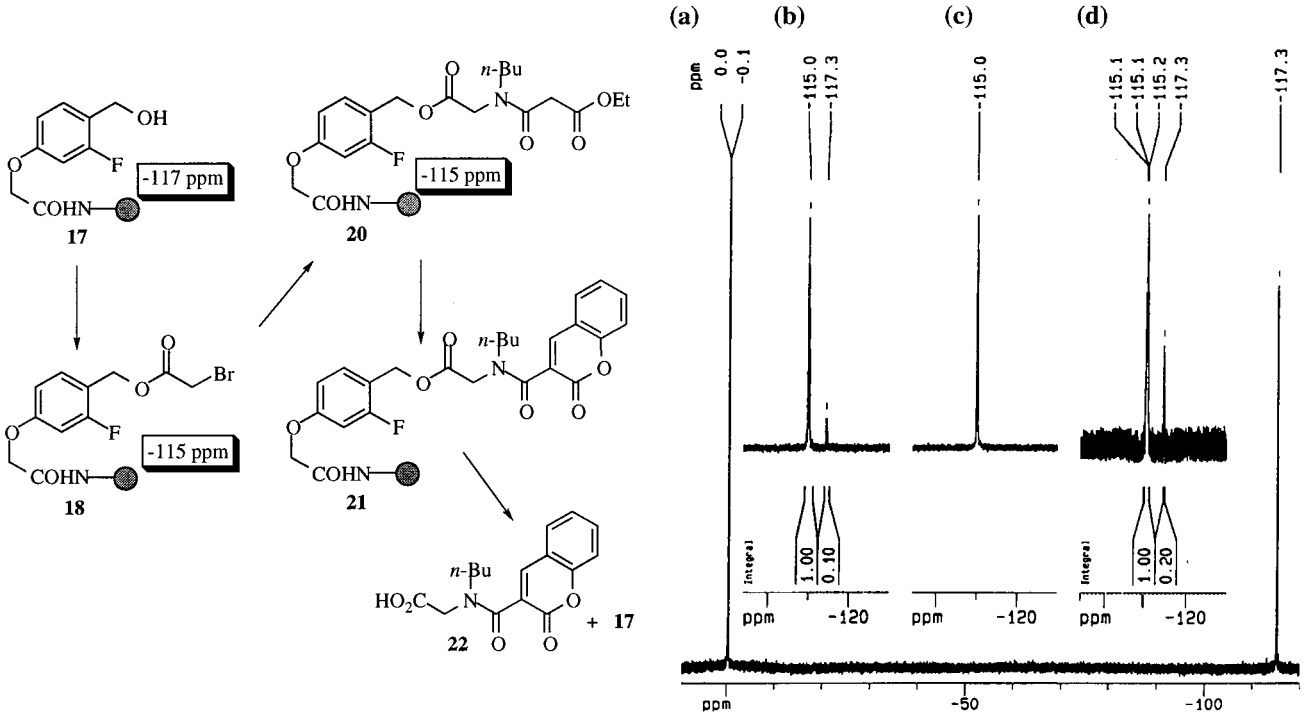
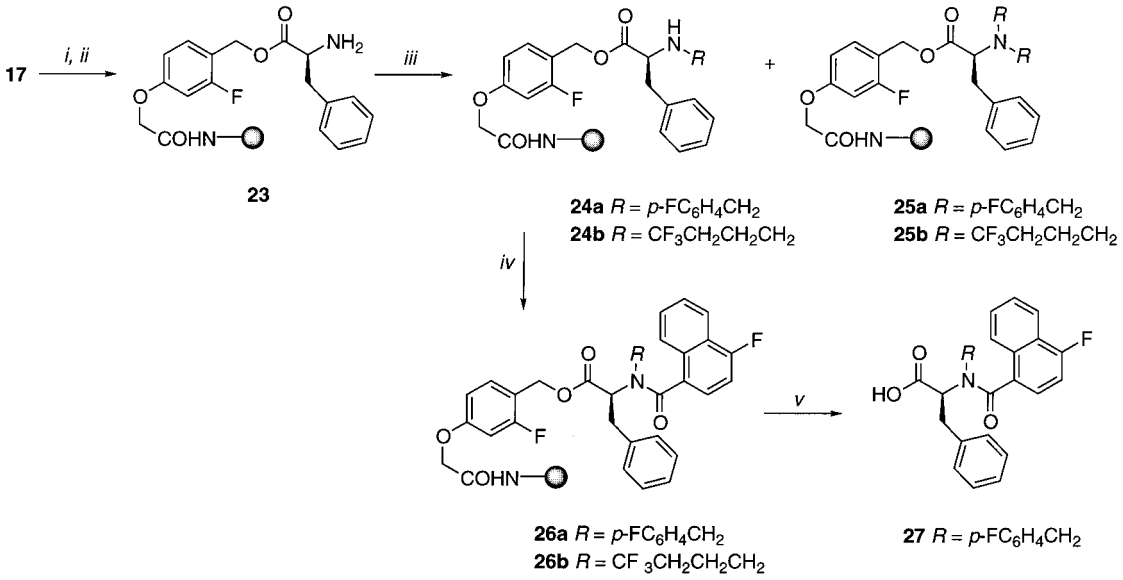


Figure 2. ^{19}F NMR spectra showing (a) resin-bound linker **17**, (b) that ~90% conversion of **17** into **18** was obtained by acylation using 3 equiv of bromoacetic acid, (c) complete transformation of **17** into **18** was obtained after repeating the acylation with 1.5 equiv of bromoacetic acid, and (d) **21** after Knoevenagel condensation of **20** with salicylaldehyde and piperidine, indicating that ~20% of the product was cleaved form the resin during the condensation.

Scheme 4[a]



[a] Reaction conditions: (*i*) N^{α}-Fmoc-Phe-OH, HOBt, DIC, DMAP, THF; (*ii*) 20% piperidine in DMF; (*iii*) *p*-F-C_6H_4CHO or $F_3CCH_2CH_2CHO$, MeOH containing 1% HOAc; then $NaBH_3CN$; (*iv*) 4-fluoronaphthoyl chloride, DIPEA, CH_2Cl_2, 0 °C → rt; (*v*) aqueous 1 M LiOH, THF:MeOH:H_2O (3:1:1). Compound **27** was obtained in 76% overall yield in addition to cleaved **25a** (<2%).

Reductive Alkylation of α-Amino Groups in Amino Acids. Another class of potential pilicides consisted of *N*-alkylated and *N*-acylated amino acids different from glycine (cf. **27** in Scheme 4). These pilicides were also to be synthesized on a TentaGel S NH_2 resin functionalized with linker **14**. In this case the fluorinated linker, in combination with fluorinated building blocks, was used to establish conditions for reductive alkylation of amino acids that could be applied to both aromatic and aliphatic aldehydes (cf. Figures 3−5). These studies were performed using phenylalanine resin **23**, prepared by coupling Fmoc-Phe-OH to **17** in the presence of HOBt, DIC, and DMAP, followed by removal of the N^{α}-Fmoc protecting group with 20% piperidine in DMF. The α-amino group in **23** was then alkylated with *p*-fluorobenzaldehyde using $NaBH_3CN$ as reducing agent under different conditions (cf. Table 1).[34−36] The ^{19}F resonance originating from the linker moiety of **23** served as internal reference, and integration over the ^{19}F resonance of the *N*-linked *p*-fluorobenzyl residue enabled evaluation of the outcome of the reactions (cf. Figures 3 and 4).

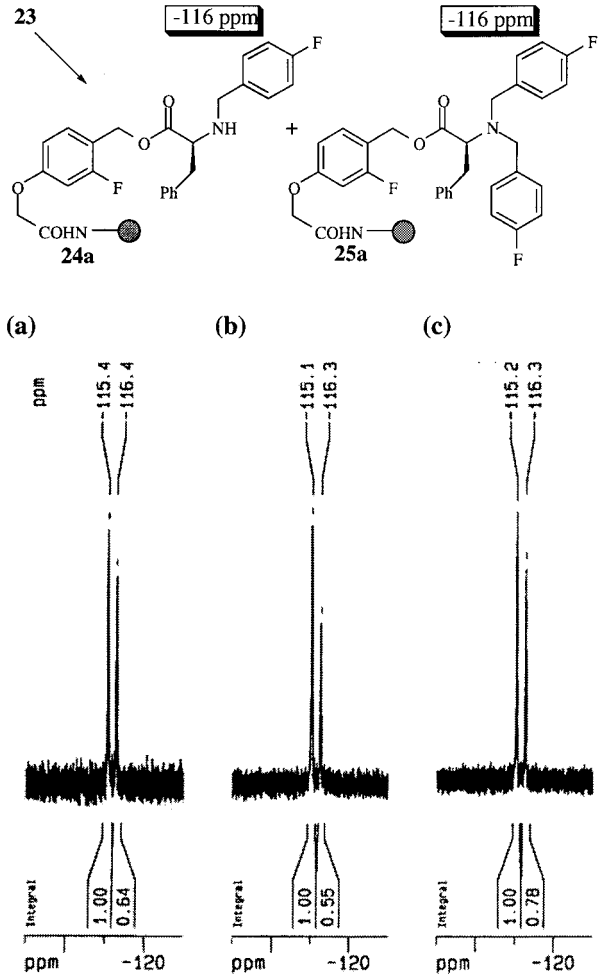


Figure 3. ^{19}F NMR spectra of resin-bound products obtained after reductive alkylation of **23** using the reaction conditions given in entries 1−3, Table 1. The ^{19}F resonance at δ −115 ppm originates from the linker and shows that coupling of Fmoc-Phe-OH to **17** was quantitative. This resonance was used as internal standard. Integration of the ^{19}F resonance originating from the *p*-fluorobenzyl residue (δ −116 ppm) showed that (a) the reaction conditions in entry 1 resulted in ∼64% alkylation of **23**, (b) the conditions in entry 2 led to ∼55% alkylation, and (c) those in entry 3 gave ∼78% alkylation of **23**.

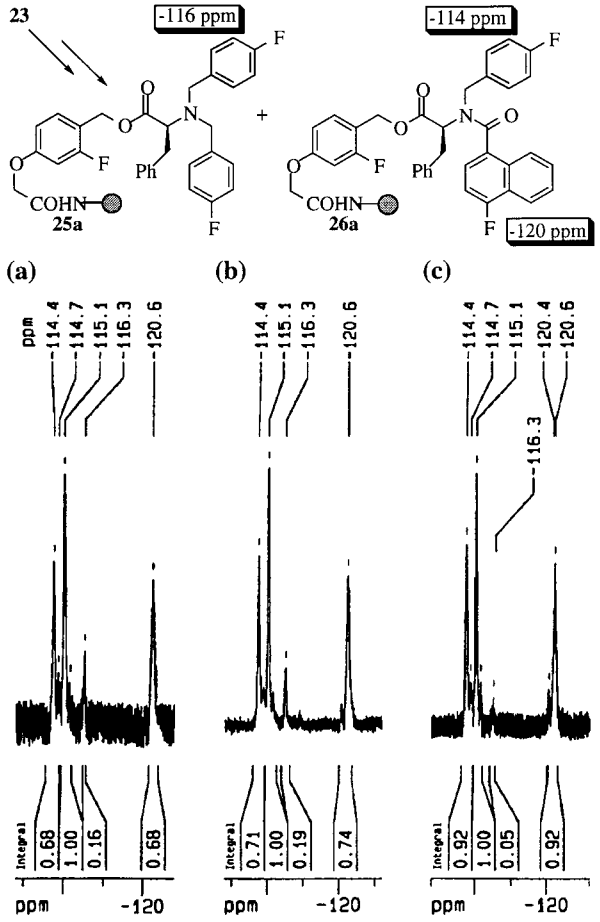


Figure 4. Gel-phase ^{19}F NMR spectroscopy of resin-bound products obtained after reductive alkylation of **23**, as described in entries 4−6, Table 1, and subsequent acylation with 4-fluoronaphthoyl chloride. The line-broadening in the spectra is due to rotamers about the amide bond in **26a**. Integration over the ^{19}F resonances revealed that (a) use of 1.5 equiv of *p*-F-benzaldehyde (entry 4) resulted in ∼8% dialkylation, (b) increasing the excess of *p*-F-benzaldehyde to 3 equiv (entry 5) gave slightly increased formation of dialkylated **25a** (∼10%), whereas (c) removing excess aldehyde prior to addition of $NaBH_3CN$ (entry 6) proved to be the most efficient method, which reduced the formation of **25a** to <2% and increased the overall yield of **26b** to ∼92%.

When an excess of *p*-fluorobenzaldehyde (10 equiv) and $NaBH_3CN$ (10 equiv) was employed (entry 1, Table 1), alkylation[34] of **23** was achieved to an extent of ∼64% as revealed by ^{19}F NMR spectroscopy (cf. Figure 3a). Use of a smaller excess of both *p*-fluorobenzaldehyde and $NaBH_3CN$ (entry 2)[35] resulted in reduced alkylation of **23** (∼55% conversion, cf. Figure 3b). On the other hand, exchanging the THF mixture for MeOH containing 1% HOAc as solvent[36] and increasing the amount of $NaBH_3CN$ in combination with a longer reaction time (entry 3) led to a substantially increased alkylation of **23** (∼78% conversion, cf. Figure 3c). Therefore, it was decided to optimize these reaction conditions further by variation of both the amounts of aldehyde and $NaBH_3CN$. As expected, when the amount of aldehyde was increased the ^{19}F resonance originating from the *N*-linked fluorobenzyl residue increased in intensity (data not shown). However, the higher intensity of this signal could also arise from formation of the unwanted dialkylated compound **25a**.

To find reaction conditions under which dialkylation of the α-amino group in **23** could be suppressed, resins resulting from alkylation of **23** were acylated with 4-fluoronaphthoyl chloride, thereby generating resin-linked pilicide **26a** (cf. Scheme 4 and Figure 4). Interestingly, *N*-acylation of **24a** induced a change of ∼2 ppm in the ^{19}F chemical shift of the N-linked *p*-fluorobenzyl residue to δ −114 ppm in **26a** (Figure 4). Since **25a** was unaffected by the acylation, the corresponding ^{19}F resonance remained at δ −116 ppm. This, together with the fluorine signal of the fluoronaphthoyl residue (δ −120 ppm), allowed for determination of the extent of dialkylation simultaneously with formation of the target pilicide-resin **26a**. Reductive alkylation of **23** using 1.5 equiv of *p*-fluorobenzaldehyde (entry 4, Table 1) resulted in formation of dialkylated **25a** in ∼8% yield (cf. Figure 4a). When twice as much *p*-fluorobenzaldehyde was used (entry 5), formation of **25a** increased to ∼10%, with little improvement in the yield of **26a** (cf. Figure 4b). However, when excess aldehyde was removed from the resin by

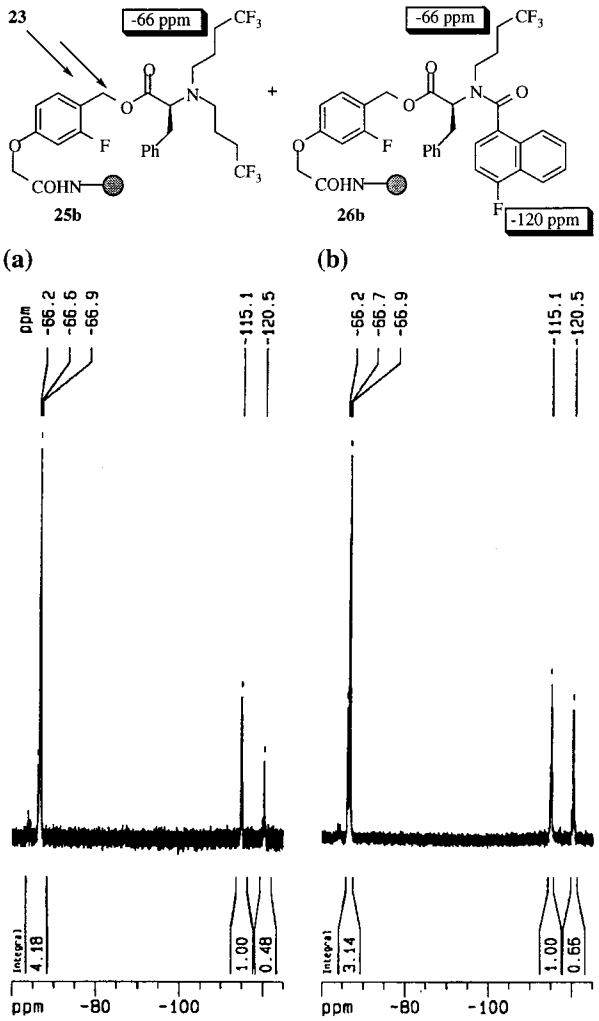


Figure 5. Gel-phase ^{19}F NMR spectra of **26b** obtained after reductive alkylation of **23** with 4,4,4-trifluorobutyraldehyde under the following conditions: (a) 4,4,4-trifluorobutyraldehyde (3 equiv) with direct addition of $NaBH_3CN$ gave **26b** in 48%, whereas (b) removal of excess aldehyde prior to the addition of $NaBH_3CN$ increased the yield of **26b** to 66%. The resins were acylated with 4-fluoronaphthoyl chloride after the reductive alkylation.

filtration prior to addition of $NaBH_3CN$ (entry 6), formation of **25a** dropped to 2−3%, which improved the yield of **26a** to ∼92% (cf. Figure 4c). Use of these reaction conditions for reductive alkylation, followed by acylation with 4-fluoronaphthoyl chloride and cleavage under basic conditions, furnished pilicide **27** in 76% isolated yield based on the capacity of the resin. In addition, <2% of the dialkylated compound originating from cleavage of **25a** was obtained.

This improved method also proved to work well for reductive alkylation of resin-linked **23** using aliphatic aldehydes (cf. Scheme 4 and Figure 5). Alkylation of **23** by treatment with 4,4,4-trifluorobutyraldehyde and $NaBH_3CN$ using the conditions of entry 5 in Table 1, followed by acylation with 4-fluoronaphthoyl chloride, resulted in formation of **26b** in 48% yield (Figure 5a). In contrast, removal of excess aldehyde prior to the addition of $NaBH_3CN$, i.e. using the conditions of entry 6 in Table 1, and subsequent acylation furnished **26b** in 66% yield (Figure 5b).

Conclusion

Techniques that allow monitoring and optimization of reactions performed on solid supports are important in solid-phase organic chemistry. In this work three fluorinated linkers which are analogues of linkers commonly used in solid-phase peptide synthesis have been prepared. The linker 3-fluoro-4-(hydroxymethyl)phenoxyacetic acid (**14**) was found to be somewhat more stable toward TFA than the nonfluorinated analogue, which is used routinely in Fmoc solid-phase peptide synthesis. It could, however, be cleaved completely with TFA by raising the temperature to 60 °C. Linker **14** was used in combination with gel-phase ^{19}F NMR spectroscopy to develop conditions for solid-phase synthesis of two focused libraries of pilicides, i.e. compounds designed to inhibit assembly of disease-associated pili in uropathogenic *E. coli*. It was found that reactions involving attachment to and cleavage from the linker could be monitored by ^{19}F NMR spectroscopy based on changes in the chemical shift of the fluorine atom of the linker. Moreover, use of the fluorine resonance of the linker as internal standard allowed optimization of reactions involving functionalites further away from the linker when fluorinated building blocks were employed. In this way suitable conditions for reductive alkylation of amines with aldehydes and acylation of the resulting secondary amines were found. It should be noted that high-quality ^{19}F NMR spectra were obtained on an ordinary NMR instrument in a couple of minutes for samples of resin in a standard NMR tube. The simplicity of the technique should allow use of the linkers described herein for optimization of a large variety of solid-supported reactions and lead to development of other types of fluorinated linkers. The reaction conditions developed in the present work are now being used for preparation of pilicide libraries which will be reported together with structure−activity studies once completed.

Table 1. Reductive Alkylation of **23** with *p*-Fluorobenzaldehyde Using $NaBH_3CN$ as Reducing Agent

	reaction conditions			yield (%)[a]		
entry	solvent	ratio **23**:*p*-F-PhCHO:$NaBH_3CN$	reaction time	**24a**/**25a**	**25a**	**26a**
1	TMOF[b]:HOAc (99:1)	1:10:10	30 min + 10 min	64		
2	THF:HOAc:H_2O (90:5:5)	1:1.2:0.9	5 min + 3 h	55		
3	MeOH:HOAc (99:1)	1:1.1:5	1 h + 3 h	78		
4	MeOH:HOAc (99:1)	1:1.5:5	1 h + 3 h		8	68
5	MeOH:HOAc (99:1)	1:3:7	1 h + 3 h		10	71
6[c]	MeOH:HOAc (99:1)	1:3:7	1 h + 3 h		<2	92

[a] Conversion of **23** as revealed by gel-phase ^{19}F NMR spectroscopy. [b] TMOF = trimethylorthoformate. [c] The aldehyde was allowed to react with **23** for 1 h; excess aldehyde was then removed by filtration prior to addition of $NaBH_3CN$.

Experimental Section

General Methods and Materials. TLC was performed on silica gel 60 F_{254} (Merck). Flash column chromatography employed Matrex normal-phase silica gel 60 Å (35−70 μm) with distilled solvents. CH_2Cl_2 and CH_3CN were distilled from calcium hydride immediately before use; THF was distilled from sodium-benzophenone ketyl, and ethanol was dried over 4 Å molecular sieves. Reactions in these solvents were performed under an atmosphere of nitrogen; solvents, reactant solutions, and liquid reagents being transferred via oven-dried syringes. DMF was distilled under reduced pressure prior to use. Solid-phase synthesis was performed on a TentaGel S NH_2 resin (130 μm, 0.22−0.26 mmol/g). ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-400 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27 ppm) and $CDCl_3$ (δ_C 77.23 ppm) as internal standard], or MeOH-d_4 [residual CH_2DOD (δ_H 3.31 ppm) and CD_3OD (δ_C 49.00 ppm) as internal standard] at 295 K. The ^{1}H NMR spectrum of pilicide **27** was recorded on a Bruker ARX-300 spectrometer for a solution in DMSO-d_6 [residual DMSO-d_5 (δ_H 2.50 ppm) as internal standard] at 420 K. Gel-phase ^{19}F NMR spectra were recorded with a Bruker ARX-400 or Bruker DRX-500 spectrometer operating at 376.5 or 470.6 MHz, respectively. Resins were suspended in $CDCl_3$ or MeOH-d_4 with Cl_3CF (δ_F 0.0 ppm) as internal standard. For accurate ^{19}F quantification, a T_1 measurement using the inversion recovery technique settled a T_1 of less than 400 ms for the signal with the longest relaxation time. The interpulse relaxation delay was set to 1.9 s and the acquisition to 100 ms, giving a repetition time of 2.0 s. High-resolution mass spectra [HRMS (EI$^+$)] were recorded on a JEOL JMS-SX 102 spectrometer.

3-Fluoro-4-hydroxymethylbenzoic Acid (2) and 2-Fluoro-4-hydroxymethylbenzoic Acid (3). Aqueous LiOH (1 M, 1.55 mL, 1.55 mmol) was added to a stirred solution of dimethyl 2-fluoroterephthalate (300 mg, 1.41 mmol) in THF:MeOH:H_2O (3:1:1, 4 mL) at 0 °C. The resultant solution was allowed to reach room temperature and was then stirred overnight. The solution was diluted with EtOAc (10 mL), and the water phase was separated and cooled to 0 °C, acidified with aqueous 1 M HCl, and extracted with EtOAc (4 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (heptane:EtOAc 7:1 → 6:1, containing 1% HOAc) gave a mixture of terephthalic acid monomethyl esters (215 mg, 77%), as well as terephthalic acid (70 mg). The mixture of terephthalic acid monomethyl esters (80 mg, 0.49 mmol) was dissolved in THF (0.8 mL) and added to a stirred solution of $LiBH_4$ (28 mg, 1.21 mmol) in THF (0.8 mL). The solution was stirred at ambient temperature for 30 min, then ethanol (3.2 mL) was added droppwise, and the resulting slurry was stirred overnight. The reaction mixture was cooled to 0 °C, and acetone (0.2 mL), followed by aqueous 1 M HCl (1.2 mL), was added to give a clear solution. The water phase was extracted with EtOAc (4 × 10 mL), and the combined organic phases were washed once with brine (10 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (heptane:EtOAc 4:1→1:1, containing 1% HOAc) of the residue gave **2** (36 mg, 53%) and **3** (28 mg, 40%). Compound **2**: ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.94 (dd, 1H, J = 7.9, 1.4 Hz, Ar-H), 7.64 (dd, 1H, J = 10.6, 1.4 Hz, Ar-H), 7.58 (t, 1H, J = 7.6 Hz, Ar-H), 4.73 (s, 2H, ArCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 168.6, 162.6, 160.1, 135.1, 130.2, 126.8, 117.2, 58.7; HRMS (EI$^+$) calcd for $C_8H_7O_3F$ 170.0379, found 170.0374. Compound **3**: ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.90 (t, 1H, J = 7.9, 1.4 Hz, Ar-H), 7.21 (m, 2H, Ar-H), 4.65 (s, 2H, ArCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 167.5, 165.05, 162.5, 151.5, 133.5, 122.9, 115.7, 64.0; HRMS (EI$^+$) calcd for $C_8H_7O_3F$ 170.0379, found 170.0374.

Benzyl 2-Fluoro-4-hydroxymethylbenzoate (4). A solution of **3** (492 mg, 2.89 mmol) in MeOH:H_2O (10:1, 13.2 mL) was titrated to pH 7 with aqueous Cs_2CO_3 (20%, ∼3.5 mL). The resulting solution was concentrated to dryness, and the residue was concentrated twice from freshly distilled DMF (2 × 8 mL). Benzyl bromide (0.41 mL, 3.47 mmol) was added to a slurry of the solid cesium salt in DMF (8 mL), and the solution was stirred overnight at ambient temperature. The mixture was concentrated, and H_2O was added to the residue. The water phase was extracted with EtOAc (4 × 50 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (heptane:EtOAc 4:1→1:1) of the residue gave **4** (588 mg, 79%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (t, 1H, J = 7.8 Hz, Ar-H), 7.45 (m, 2H, Ar-H), 7.38 (m, 3H, Ar-H), 7.17 (m, 2H, Ar-H), 5.35 (s, 2H, $PhCH_2O$), 4.76 (d, 2H, J = 6 Hz, ArCH_2OH); ^{13} C NMR ($CDCl_3$, 400 MHz) δ 164.3, 164.2, 163.8, 161.2, 148.9, 148.8, 135.9, 132.5, 128.8, 128.4, 128.3, 121.8, 121.7, 117.6, 117.5, 115.0, 114.8, 67.1, 64.1; HRMS (EI$^+$) calcd for $C_{15}H_{13}O_3F$ 260.0848, found 260.0846.

Benzyl 2-Fluoro-4-formylbenzoate (5). Tetrapropylammonium perruthenate (TPAP, 5 mg, 13 μmol, 5 mol %) was added in one portion to a stirred slurry of **4** (70 mg, 0.26 mmol), 4-methylmorpholine *N*-oxide (47 mg, 0.40 mmol), and 4 Å molecular sieves (134 mg) in CH_2Cl_2 (5 mL). The resulting slurry was stirred at ambient temperature for 30 min and then filtrated through a pad of silica gel eluted with CH_2Cl_2 (50 mL). Concentration of the solution and flash chromatography (heptane:EtOAc 7:1) of the residue gave the aldehyde **5** (51 mg, 74%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.05 (d, 1H, J = 1.5 Hz, ArCHO), 8.12 (t, 1H, J = 7.3 Hz, Ar-H), 7.74 (dd, 1H, J = 7.9, 1.3 Hz, Ar-H), 7.64 (dd, 1H, J = 10.1, 1.3 Hz, Ar-H), 7.48−7.33 (m, 4H, Ar-H), 5.45 (s, 2H $ArCH_2O$); HRMS (EI$^+$) calcd for $C_{15}H_{11}O_3F$ 258.0692, found 258.0693.

Benzyl 4-((*E*)-2-Ethoxycarbonylvinyl)-2-fluorobenzoate (6). $(EtO)_2P(O)CH_2CO_2C_2H_5$ (397 mg, 1.77 mmol) was added to a slurry of NaH (55−65%, 62 mg, 1.55 mmol) in THF (5 mL) at 0 °C. After a few minutes the mixture became clear and was allowed to reach room temperature after which **5** (286 mg, 1.10 mmol) dissolved in THF (5 mL) was added. After the mixture was stirred for 1 h, H_2O (20 mL) was added, and the solution was poured into Et_2O (30 mL). The water phase was extracted with Et_2O (4 × 30 mL), and the combined organic layers were washed with H_2O (15 mL) and brine (2 × 10 mL). The organic phase was dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (heptane:EtOAc 8:1) to give **6** (276 mg, 76%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (t, 1H, J =

7.7 Hz, Ar-H), 7.62 (d, 1H, J = 16.0 Hz, Ar-H), 7.46 (m, 2H, Ar-H), 7.41−7.35 (m, 4H, Ar-H), 7.27 (m, 1H, ArC*H*═CH), 6.49 (d, 1H, J = 16.0 Hz, CH═C*H*CO_2Et), 5.38 (s, 2H $PhCH_2O$), 4.28 (q, 2H, J = 7.1 Hz, $CO_2CH_2CH_3$), 1.34 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1, 163.6, 160.8, 141.7, 140.9, 135.6, 132.7, 128.6, 128.3, 128.1, 123.4, 119.6, 116.0, 115.8, 67.1, 60.9, 14.2; HRMS (EI^+) calcd for $C_{19}H_{17}O_4F$ 328.1110, found 328.1111.

4-(2-Ethoxycarbonylethyl)-2-fluorobenzoic Acid (7). A small amount of 10% Pd-C was added to a solution of **6** (254 mg; 0.77 mmol) in dry EtOH:EtOAc (3:1, 8 mL), and the resulting mixture was hydrogenated at 4 atm for 15 h. The mixture was filtered through a pad of Celite and concentrated, and the residue was purified by flash chromatography (heptane:EtOAc 3:1 → 1:1, containing 1% HOAc) to give **7** (164 mg, 88%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.94 (bs, 1H, CO_2H), 7.93 (bs, 1H, Ar-H), 7.05 (d, 1H, J = 7.7, Ar-H), 7.01 (d, 1H, J = 11.4 Hz, Ar-H), 4.15 (q, 2H, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.00 (t, 2H, J = 7.6 Hz, $ArCH_2CH_2$), 2.65 (t, 2H, J = 7.6 Hz, $ArCH_2CH_2$), 1.24 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 161.8, 159.4, 142.7, 129.5, 124. 1, 115.3, 60.5, 59.3, 35.5, 30.4, 14.1; HRMS (EI^+) calcd for $C_{12}H_{13}O_4F$) 240.0798, found 240.0801.

Ethyl 3-(3-Fluoro-4-hydroxymethylphenyl)-propionate (8). BH_3-DMS (92 mg, 1.21 mmol) was added to a solution of **7** (145 mg, 0.60 mmol), after which $(CH_3O)_3B$ (251 mg, 2.41 mmol) in THF (8 mL) was added. The resulting mixture was stirred at ambient temperature until **7** was consumed (TLC) and a clear solution was obtained. MeOH (1 mL) was added, and the mixture was concentrated to dryness. The residue was dissolved in Et_2O (10 mL) and washed with H_2O (5 mL) and saturated aqueous $NaHCO_3$ (2 × 3 mL). The organic solution was dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (heptane:EtOAc 4:1) to give **8** (106 mg, 89%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (t, 1H, J = 7.8 Hz, Ar-H), 6.99 (dd, 1H, J = 7.7, 1.6 Hz, Ar-H), 6.91 (dd, 1H, J = 11.0, 1.5 Hz, Ar-H), 4.72 (d, 2H, J = 6.0 Hz, $ArCH_2OH$), 4.12 (q, 2H, J = 7.1 Hz, $CO_2CH_2CH_3$), 2.93 (t, 2H, J = 7.7 Hz, $ArCH_2CH_2$), 2.60 (t, 2H, J = 7.7 Hz, $ArCH_2CH_2$), 1.80 (bt, 1H, J = 6.0 Hz, $ArCH_2OH$), 1.23 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 162.3, 159.8, 143.1, 129.9, 124.6, 115.7, 60.9, 59.7, 35.9, 30.8, 14.6; HRMS (EI^+) calcd for $C_{12}H_{15}O_3F$ 226.1005, found 226.1002.

3-(3-Fluoro-4-hydroxymethylphenyl)propionic Acid (9). Aqueous LiOH (1 M, 0.44 mL, 0.448 mmol) was added to a stirred solution of **8** (84.5 mg, 0.37 mmol) in THF:MeOH:H_2O (3:1:1, 1.5 mL) at 0 °C. After 30 min the solution was allowed to reach room temperature and was then stirred for 4.5 h. The solution was then recooled to 0 °C, acidified with aqueous 1 M HCl, and poured into EtOAc:H_2O (4:1, 10 mL). The water phase was extracted with EtOAc (2 × 5 mL), and the combined organic phases were washed once with brine (6 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (heptane:EtOAc 4:1 → 3:1, containing 1% HOAc) to give **9** (69 mg, 93%): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.35 (t, 1H, J = 7.8 Hz, Ar-H), 7.04 (dd, 1H, J = 7.8, 1.5 Hz, Ar-H), 6.96 (dd, 1H, J = 11.2, 1.5 Hz, Ar-H), 4.62 (s, 2H $ArCH_2OH$), 2.91 (t, 2H, J = 7.5 Hz, $ArCH_2CH_2$), 2.60 (t, 2H, J = 7.5 Hz, $ArCH_2CH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4, 162.0, 159.6, 143.2, 143.1, 129.6, 129.2, 129.1, 126.1, 124.2, 124.1, 115.0, 114.8, 109.0, 57.7, 35.3, 30.4; HRMS (EI^+) calcd for $C_{10}H_{11}O_3F$ 198.0692, found 198.0692.

2-Fluoro-4-hydroxybenzoic Acid (11). 2-Fluoro-4-propoxybenzoic acid (**10**, 1.21 g, 6.09 mmol) was dissolved in CH_2Cl_2 (16 mL), the solution was cooled to −78 °C, and BBr_3 (18.3 mL, 18.3 mmol, 1 M in CH_2Cl_2) was added. The solution was slowly allowed to reach room temperature and was then stirred at ambient temperature overnight. H_2O (35 mL) was added, and the resulting mixture was poured into Et_2O (20 mL). The water phase was extracted with Et_2O (4 × 40 mL), and the combined organic phases were dried ($MgSO_4$). Concentration and flash chromatography (heptane:EtOAc 2:1 → 1:2, containing 1% HOAc) of the residue gave **11** (850 mg, 89%): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.80 (t, 1H, J = 8.7 Hz, Ar-H), 6.54 (dd, 1H, J = 8.7, 2.3 Hz, Ar-H), 6.54 (dd, 1H, J = 12.9, 2.3 Hz, Ar-H); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 167.7, 166.7, 165.1, 164.1, 135.0, 112.6, 104.6; HRMS (EI^+) calcd for $C_7H_5O_3F$ 156.0222, found 156.0221.

3-Fluoro-4-hydroxymethylphenol (12). Compound **11** (679 mg, 4.35 mmol) in THF (20 mL) was added to a stirred solution of $(CH_3O)_3B$ (3.62 g, 34.8 mmol) and BH_3-DMS (1.32 g, 17.4 mmol) in THF (50 mL). The mixture was stirred at ambient temperature until **11** was consumed (TLC) and a clear solution was obtained. MeOH (26 mL) was added, the mixture was concentrated, and the residue was co-concentrated from MeOH (3 × 50 mL). The residue was purified by flash chromatography (heptane:EtOAc 2:1 → 1:1) to give **12** (558 mg, 90%): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.21 (t, 1H, J = 8.6 Hz, Ar-H), 6.57 (dd, 1H, J = 8.4, 2.4 Hz, Ar-H), 6.49 (dd, 1H, J = 11.8, 2.4 Hz, Ar-H), 4.53 (s, 2H, $ArCH_2OH$);^{13}C NMR (MeOH-d_4, 100 MHz) δ 164.1, 161.7, 160.1, 131.9, 120.1, 112.3, 103.5, 58.8; HRMS (EI^+) calcd for $C_7H_7O_2F$ 142.0430, found 142.0427.

Ethyl 3-Fluoro-4-(hydroxymethyl)-phenoxyacetate (13). DBU (777 mg, 5.10 mmol) was added to a stirred solution of **12** (558 mg, 3.93 mmol) and ethyl α-bromoacetate (1.18 g, 7.07 mmol) in CH_3CN (50 mL). The solution was refluxed overnight and then cooled to room temperature. The solution was poured into Et_2O (100 mL), washed with aqueous HCl (0.05 M, 2 × 50 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (toluene:EtOAc 6:1 → 3:1 → 1:1) to give **13** (108 mg, 74%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31 (t, 1H, J = 8.5 Hz, Ar-H), 6.68 (dd, 1H, J = 8.3, 2.8 Hz, Ar-H), 6.63 (dd, 1H, J = 11.5, 2.5 Hz, Ar-H), 4.66 (s, 2H, $ArCH_2OH$), 4.60 (s, 2H, OCH_2CO_2H), 4.27 (q, 2H, J = 7.1 Hz, OCH_2CH_3), 1.98 (bs, 1H, $ArCH_2OH$), 1.30 (t, 3H, J = 7.1 Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.7, 162.6, 160.1, 158.9, 130.5, 121.3, 110.3, 102.9, 65.7, 61.7, 59.1, 14.3; HRMS (EI^+) calcd for $C_{11}H_{13}O_4F$ 228.0797, found 228.0795.

3-Fluoro-4-(hydroxymethyl)-phenoxyacetic Acid (14). Aqueous LiOH (1 M, 1.03 mL, 1.03 mmol) was added to a

stirred solution of **13** (157 mg, 0.69 mmol) in THF:MeOH:H_2O (3:1:1, 10 mL) at 0 °C. After 30 min the solution was allowed to reach room temperature and was then stirred at ambient temperature for 1.5 h. The solution was recooled to 0 °C, acidified with aqueous 1 M HCl, and poured into EtOAc (30 mL). The water phase was extracted with EtOAc (3 × 10 mL), and the combined organic phases were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (heptane:EtOAc 1:2, containing 1% HOAc) to give **14** (119 mg, 87%): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.34 (t, 1H, J = 8.6 Hz, Ar-H), 6.76 (ddd, 1H, J = 8.3, 2.5, 0.8 Hz, Ar-H), 6.70 (dd, 1H, J = 11.8, 2.5 Hz, Ar-H), 4.66 (s, 2H, ArCH_2-OH), 4.58 (s 2H, OCH_2OH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 172.4, 163.8, 161.4, 160.4, 131.6, 122.5, 111.4, 103.5, 66.2, 58.6; HRMS (EI$^+$) calcd for $C_9H_9O_4F$ 200.0484, found 200.0486.

Preparation of Resin-Bound 15a and 15b. DIC (66 μL, 0.42 mmol) was added to a solution of Fmoc-*m*-F-Phe-OH (182 mg, 0.45 mmol) and HOBt (90 mg, 0.67 mmol) in DMF (4 mL). After 1 h 45 min, the activated amino acid was added to TentaGel S NH_2 resin (1.0 g, 0.22 mmol, preswollen in DMF), and the mixture was agitated at ambient temperature for 12 h. The resin was washed with portions of DMF (3 × 20 mL). A solution of 20% piperidine in DMF (4 mL) was then added to the resin followed by agitation at ambient temperature for 25 min. The resin was filtered and washed with portions (20 mL) of DMF, MeOH, and THF, and finally dried under vacuum. DIC (29 μL, 0.19 mmol) was added to an ice-cold solution of pentafluorophenol (55 mg, 0.30 mmol) in DMF (3 mL). After 30 min 4-(hydroxymethyl)phenoxyacetic acid (36 mg, 0.20 mmol) was added, and the solution was stirred at 0 °C for 1 h. The resulting mixture was then added to the resin from above (450 mg, 0.10 mmol, preswollen in DMF), and the mixture was agitated at ambient temperature overnight. The resin was washed with portions (20 mL each) of DMF, MeOH, and THF, dry THF (5 mL), and dried under vacuum. DIC (39 μL, 0.25 mmol) was added to a solution of Fmoc-*p*-F-Phe-OH (122 mg, 0.30 mmol) and HOBt (27 mg, 0.20 mmol) in dry THF (4 mL). After 1 h, DMAP (4 mg, 0.03 mmol) dissolved in dry THF (0.5 mL) and the activated amino acid were added to the resin from above (0.10 mmol, preswollen in dry THF), and the mixture was agitated at ambient temperature overnight. The resin was washed with THF, MeOH, and THF (2 × 20 mL each) and dried over vacuum. For preparation of resin **15b**, the synthesis was repeated as above using 3-fluoro-4-(hydroxymethyl)phenoxyacetic acid (**14**).

Evaluation of Conditions for Cleavage of Resin-Bound 15a and 15b. Resin-bound **15a** and **15b** were split into portions of 100 mg (26 μmol) each and treated with (*i*) TFA:H_2O:thioanisole:ethanedithiol (87.5:5:5:2.5, 5 mL) for 2 h at room temperature, (*ii*) the same cleavage mixture (5 mL) for 2 h at 60 °C, and (*iii*) aqueous LiOH (1 M, 200 μL) in THF:MeOH:H_2O (3:1:1, 1.5 mL) for 2 h at room temperature. After filtration, the resins were washed with HOAc and THF (2 × 20 mL portions of each solvent) and dried under vacuum. It was found that sharper resonances were obtained in the ^{19}F NMR spectra if the N^{α}-Fmoc protecting group was first removed. N^{α}-Fmoc cleavage was performed by treatment with 20% piperidine in DMF (3 mL) for 30 min. The resins were filtrated and washed with 2 × 10 mL portions each of DMF, MeOH, and THF and were then dried under vacuum. Gel-phase ^{19}F NMR spectroscopy of the resins was recorded as described in the General Methods and Materials section.

Resin-Bound 3-Fluoro-4-(hydroxymethyl)phenoxyacetic Acid (17). DIC (99 μL, 0.64 mmol) was added to an ice-cold solution of pentafluorophenol (187 mg, 1.00 mmol) in EtOAc (5 mL). After 30 min, 3-fluoro-4-(hydroxymethyl)-phenoxyacetic acid (**14**, 135 mg, 0.67 mmol) was added, and the solution was stirred at 0 °C for 1 h. The mixture was then added to TentaGel S NH_2 resin (1.3 g, 0.34 mmol, preswollen in EtOAc) and agitated at ambient temperature. Monitoring[32] using the aborbance of bromophenol blue at 600 nm revelaed complete *N*-acylation after reaction overnight (12 h). The resin was washed with EtOAc, MeOH, and THF (30 mL portions each) and dry THF (10 mL), and then dried under vacuum.

Resin-Bound Bromoacetic Acid (18). DIC (100 μL, 0.65 mmol) was added to a solution of bromoacetic acid (108 mg, 0.78 mmol) and HOBt (70 mg, 0.52 mmol) in THF (4 mL), and the solution was stirred at ambient temperature for 1 h. The mixture, and a catalytic amount of DMAP (10 mg, 90 μmol) dissolved in THF (1 mL), were then added to **17** (0.26 mmol, preswollen in dry THF), and the mixture was agitated overnight. The resin was washed with THF, MeOH, THF (each 60 mL), and dry THF (10 mL) and dried under vacuum. To obtain complete coupling of bromoacetic acid to **17**, the reaction was repeated as above using DIC (50 μL, 0.32 mmol), bromoacetic acid (54 mg, 0.39 mmol), and HOBt (35 mg, 0.26 mmol).

Resin-Bound *N*-*n*-Butylglycine (19). A solution of *n*-butylamine (77 μL, 0.78 mmol) in CH_3CN (5 mL) was added to **18** (0.26 mmol, preswollen in dry CH_3CN) at 0 °C. The resin was agitated at ambient temperature for 90 min and was then washed with CH_3CN, MeOH, THF (20 mL each), and dry THF (10 mL) and finally dried under vacuum.

Resin-Bound *N*-*n*-Butyl-*N*-(malonamic acid ethyl ester)-glycine (20). Ethyl malonyl chloride (99 μL, 0.78 mmol) dissolved in CH_2Cl_2 (1 mL) was added to a suspension of **19** (0.26 mmol, preswollen in dry CH_2Cl_2) and *N*,*N*′-diisopropylethylamine (DIPEA, 130 μL, 0.78 mmol) in CH_2Cl_2 (5 mL) at 0 °C, and the resin was agitated at ambient temperature for 1 h. The resin was washed with CH_2Cl_2, MeOH, THF (20 mL of each), and dry THF (10 mL) and then dried under vacuum.

Resin-Bound *N*-Butyl-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine (21). A solution of salicylaldehyde (83 μL, 0.78 mmol) in freshly distilled CH_3CN (4 mL) was added to **20** (0.26 mmol, preswollen in dry CH_3CN). Piperidine (30 μL, 0.31 mmol) in CH_3CN (1 mL) was added, and the mixture was heated at reflux overnight. The resin was allowed to reach room temperature before filtration and washing of the resin with CH_3CN, MeOH, THF (each 50 mL), and dry THF (10 mL). The resin was then dried under vacuum.

***N*-Butyl-*N*-(2-oxo-2*H*-1-benzopyran-3-carbonyl)-glycine (22).** Two portions of resin **21** (each 300 mg, 78 μmol)

were treated with (a) aqueous LiOH (1 M, 1 mL) in THF:MeOH:H_2O (3:1:1, 15 mL) for 2 h at room temperature or (b) TFA:H_2O:thioanisole:ethanedithiol (87.5:5:5:2.5, 20 mL) for 2 h at 60 °C. Each resin was filtered and washed with HOAc (20 mL) and MeOH (20 mL). Each filtrate was concentrated almost to dryness and then concentrated from toluene (1 × 50 mL and 2 × 25 mL), and then dissolved in a mixture of EtOAc (30 mL) and aqueous HCl (0.05 M, 10 mL). For the mixture from the basic cleavage, aqueous HCl (1 M, 5 mL) was added. For each mixture, the water phase was then separated and extracted with EtOAc (2 × 20 mL). The combined organic phases from each cleavage were washed with aqueous HCl (0.05 M, 2 × 15 mL), dried (Na_2SO_4), and concentrated. Flash column chromatography (toluene:EtOAc:HOAc 80:15:5 → 60:35:5) of the two crude products yielded **22** (11.4 mg, for each cleavage, 48% yield, based on the initial loading capacity of the resin): ^{1}H NMR ($CDCl_3$, 400 MHz) for a mixture of rotamers δ 7.97 (s, $1H_{maj+min}$, C═CH), 7.60 (m, $2H_{maj+min}$, Ar-H), 7.36 (m, $2H_{maj+min}$, Ar-H), 4.26 (s, $2H_{maj}$, (O)CCH_2N), 4.06 (s, $2H_{min}$, (O)CCH_2N), 3.35 (t, $2H_{min}$, J = 7.4 Hz, NCH_2CH_2), 3.33 (t, $2H_{maj}$, J = 7.7 Hz, NCH_2CH_2), 1.61 (m, $2H_{maj+min}$, NCH_2CH_2), 1.41 (m, $2H_{min}$, CH_2CH_3), 1.26 (m, $2H_{maj}$, CH_2CH_3), 0.96 (t, $3H_{min}$, J = 7.3 Hz, CH_2CH_3), 0.85 (t, $3H_{maj}$, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 172.5, 166.4, 166.0, 158.5, 158.1, 154.3, 144.2, 143.5, 133.3, 133.2, 129.2, 129.1, 125.3, 125.2, 124.7, 124.5, 118.4, 118.3, 117.1, 116.9, 50.7, 50.1, 47.5, 47.1, 30.3, 29.2, 20.2, 19.9, 13.9, 13.8; HRMS (EI^+) calcd for $C_{16}H_{17}NO_5$ 303.1106, found 303.1104.

Resin-Bound Phenylalanine (23). DIC (201 μL, 1.30 mmol) was added to a solution of Fmoc-Phe-OH (604 mg, 1.56 mmol) and HOBt (140 mg, 1.04 mmol) in dry THF (4 mL). After 1 h, the activated amino acid and DMAP (21 mg, 0.17 mmol, dissolved in dry THF, 1 mL) were added to **17** (2.0 g, 0.52 mmol, preswollen in dry THF), and the mixture was agitated at ambient temperature overnight. After filtration, the resin was washed with THF, MeOH, and THF (50 mL of each solvent) and dried over vacuum. A solution of 20% piperidine in DMF was added to the resin (preswollen in DMF), and the mixture was agitated for 30 min. After filtration, the resin was washed with portions of DMF, MeOH, THF, and dry THF and then dried under vacuum.

Solid-Phase Reductive Alkylation of 23. Resin **23** was split into portions and subjected to different conditions for reductive alkylation. These, and the results from the reductive alkylation, are summarized under entries 1−6 in Table 1a and described in detail below.

Entry 1. *p*-Fluorobenzaldehyde (42 μL, 390 μmol) was added to **23** [150 mg, 39 μmol, preswollen in dry trimethyl orthoformiate (TMOF)] in TMOF (2 mL), and the mixture was agitated for 40 min. $NaBH_3CN$ (25 mg, 390 μmol) dissolved in TMOF (1 mL) and HOAc (18 μL) was added, and the mixture was agitated for 10 min. After filtration the resin was washed with portions of TMOF, MeOH, and THF (2 × 10 mL of each solvent) and dried under vacuum.

Entry 2. *p*-Fluorobenzaldehyde (5 μL, 47 μmol) was added to **23** [150 mg, 39 μmol, preswollen in THF:HOAc:H_2O (90:5:5)] in THF:HOAc:H_2O (90:5:5, 1 mL), and the mixture was agitated for 10 min. A solution of $NaBH_3CN$ (35 μL, of a 1 M solution in THF, 35 μmol) was added, and the mixture was agitated for 3 h. After filtration, the resin was washed with portions of THF, H_2O, MeOH, and THF (10 mL of each solvent) and dried under vacuum.

Entry 3. *p*-Fluorobenzaldehyde (4.6 μL, 43 μmol) was added to a suspension of **23** (150 mg, 39 μmol, preswollen in MeOH containing 1% HOAc) in MeOH containing 1% HOAc (1 mL), and the mixture was agitated for 60 min. $NaBH_3CN$ (12 mg, 195 μmol) dissolved in MeOH (200 μL) was added, and the mixture was agitated for 3 h. After filtration, the resin was washed with MeOH, H_2O, MeOH, and THF (10 mL of each solvent) and dried under vacuum.

Entries 4 and 5. Reductive alkylation was performed as described for entry 3 using **23** (100 mg, 26 μmol), but with larger amounts of *p*-fluorobenzaldehyde (4.2 μL, 39 μmol for entry 4; 8.4 μL, 78 μmol for entry 5) and $NaBH_3CN$ (8 mg, 130 μmol for entry 4; 11 mg, 182 μmol for entry 5).

Entry 6. *p*-Fluorobenzaldehyde (8.4 μL, 78 μmol) was added to a suspension of **23** (100 mg, 26 μmol, preswollen in MeOH containing 1% HOAc) in MeOH containing 1% HOAc (1 mL), and the mixture was agitated for 60 min. The solution was removed by filtration and additional MeOH containing 1% HOAc (1 mL) was added followed by $NaBH_3CN$ (11 mg, 182 μmol) dissolved in MeOH (200 μL). The mixture was agitated for 3 h after which the resin was filtered and washed with MeOH, H_2O, MeOH, and THF (10 mL of each solvent) and dried under vacuum.

Gel-phase ^{19}F NMR spectroscopy of the resins was recorded as described in the General Methods and Materials section.

Acylation of Resins Obtained by Reductive Alkylation of 23. Resins obtained by reductive alkylation as described under entries 4−6 above (each 100 mg, 26 μmol) were suspended in dry CH_2Cl_2 (1 mL). DIPEA (13 μL, 78 μmol) was added, followed by 4-fluoronaphthoyl chloride (16 mg, 78 μmol) dissolved in dry CH_2Cl_2 (200 μL), after which the mixture was agitated at ambient temperature for 2 h. The solution was filtered off, and the resin was washed with CH_2Cl_2, MeOH, and THF (20 mL portions of each solvent). The resin was then dried under vacuum. Gel-phase ^{19}F NMR spectroscopy of the resins was recorded as described in the General Methods and Materials section.

***N*-(4-Fluorobenzyl)-*N*-(4-fluoronaphthoyl)-phenylalanine (27).** Solid-phase reductive alkylation of **23** was performed as described in entry 6 above by treatment of resin **23** (1.0 g, 0.26 mmol) with *p*-fluorobenzaldehyde (84 μL, 0.78 mmol) and $NaBH_3CN$ (114 mg, 1.82 mmol). Acylation of the resulting resin was accomplished using 4-fluoronaphthoyl chloride (162 mg, 0.78 mmol) and DIPEA (130 μL, 0.78 mmol) as described above. Compound **27** was cleaved from the resin using aqueous LiOH (1 M, 4 mL) in THF:MeOH:H_2O (3:1:1; 40 mL) at ambient temperature for 2 h. After filtration and subsequent washing of the resin with HOAc and THF (80 mL of each solvent), the filtrate was concentrated and finally co-concentrated from toluene (3 × 50 mL). The residue was dissolved in a mixture of EtOAc (30 mL) and aqueous HCl (0.05 M, 10 mL). The water phase was separated and acidified with aqueous HCl (1 M) and

extracted with EtOAc (2 × 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was purified by flash column chromatography (heptane:EtOAc 4:1 → 2:1, containing 1% HOAc) to give **27** (88 mg, 76% yield, based on the initial loading capacity of the resin): ^{1}H NMR (DMSO-d_6, 300 MHz, 420 K) δ 8.07 (d, 1H, J = 8.2 Hz, Ar-H), 7.84 (d, 1H, J = 8.0 Hz, Ar-H), 7.66−7.50 (dt, 2H, J = 7.6, 1.2 Hz, Ar-H), 7.26−7.05 (m, 9 H, Ar-H), 6.90 (t, 2 H, J = 8.7 Hz, Ar-H), 4.63 (dd, 1H, J = 8.3, 6.2 Hz, Phe-Hα), 4.55 (m, 1H, Phe-Hβ), 4.27 (bd, 1H, J = 15.6 Hz, Phe-Hβ), 3.43 (dd, 1H, J = 14.0, 6.1 Hz, $ArCH_2N$, 273 K), 3.26 (m, 1H, $ArCH_2N$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.8, 171.7, 163.8, 161.3, 160.7, 158.2, 138.0, 130.5, 129.6, 129.5, 129.2, 129.0, 128.7, 128.6, 128.4, 127.3, 127.2, 125.2, 121.1, 115.7, 115.5, 108.8, 60.6, 54.8, 54.8, 34.9; HRMS (EI$^+$) calcd for $C_{27}H_{22}NO_3F_2$ 446.1567, found 446.1573.

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Supporting Information Available. ^{1}H NMR spectra of compounds **2**, **9**, **14**, **22**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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