Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1007–1010

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

Solid-Phase/Solution-Phase Combinatorial Synthesis of Neuroimmunophilin Ligands

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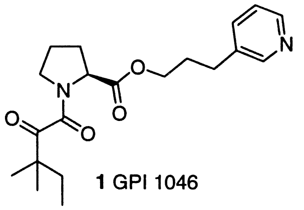
Received 29 November 1999; accepted 9 February 2000

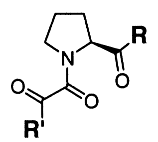
Abstract—A novel solid-phase/solution-phase strategy for the synthesis of neuroimmunophilin ligands based on GPI 1046 was developed. The synthesis employs a solid-phase esterification strategy followed by a solution-phase pyruvic amide formation to produce multi-milligram quantities of discrete compounds for assay. The protocol was applied to a production library of 880 discrete compounds. A highlight of the strategy is an aqueous extractive purification of the final compounds using a novel liquid/ice extraction system developed for high throughput. © 2000 Published by Elsevier Science Ltd.

Introduction and Background

Over the past few years a number of laboratories have disclosed novel compounds derived from the peptidyl-prolyl isomerase (PPIase) inhibition domains of the immunosuppressive drugs FK506 and rapamycin.[1] These nonimmunosuppressive small molecules ($M_r < 500$) were found to be potent neurotrophic agents in neurite outgrowth assays.[2] Certain analogues such as GPI 1046 (**1**)[3] have also been shown to be effective in models of Parkinson's and Alzheimer's diseases. Dosing paradigms have shown these compounds to produce both neuroprotective and neuroregenerative effects in models of CNS pathogenicity,[3] although these results have recently been questioned.[4]



1 GPI 1046



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The biological target for the exceptional neurotrophic activity of compounds such as **1** is not known. FKBP-12 is a likely candidate as it is upregulated in models of CNS injury,[5] but it has been shown that there is no clear correlation between rotamase inhibition activity and ED_{50} for neurite outgrowth.[6] For our purposes, it was necessary to obtain a large collection of compounds of general structure **2** to assess the SAR of the ester and pyruvate moieties, and to vary the α-amino acid scaffold in the hopes of separating the neuroprotective from neuroregenerative properties of these compounds. This letter reports the development of a chemical protocol for the synthesis of an 18-member rehearsal library based on general structure **2**, which was used as a model for the production of an 880 member neuroimmunophilin library.

Retrosynthetic Analysis

The solution-phase synthesis of compounds of general structure **2** is quite straightforward involving conventional techniques. They have been typically made by Grignard addition to methyl esters of oxalic semiamides.[1b] Our goal was the synthesis of up to 1000 discrete compounds on a multimilligram scale, and as such we were constrained by the general production techniques available as well as the variety of monomers at our disposal. With an eye toward maximizing the diversity of ester and pyruvic monomers, we decided that the most straightforward approach would be a two-tiered synthesis involving solid-phase esterification followed by solution-phase amidation. Thus, charging a solid support with *N*-bound α-amino acid, followed by esterification with alcohol monomers, cleavage, and

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PII: S0960-894X(00)00104-9

amidation with pyruvate monomers should readily provide access to a focused library of desired compounds **2**. Our greatest concern about this approach centered on solid-phase esterification of a resin-bound acid. There are relatively few examples of this transformation in the literature due to the undesirable kinetic pairing of the activated, resin-bound carboxylate and the weakly nucleophilic alcohol.

We envisioned purification of the final compounds produced by solution-phase reaction using a novel solid/liquid extraction system, developed in our laboratories (see Fig. 1).[7]

Chemistry

para-Nitrophenyl carbonate resin (Scheme 1) was prepared from Wang polystyrene resin (**3**, 0.90 meq/g) and *para*-nitrophenyl chloroformate. Nitrogen combustion analysis showed a 96% yield (0.77 meq/g). (L)-Proline was loaded onto this resin through transient protection as its TMS ester.[8]

Esterification of the resulting resin-bound carboxylic acid (**4**) was challenging. Conventional coupling with DIC and DMAP resulted in a mixture of desired ester, acyl urea, pyrrolidinohydantoin and isopropyl amine in a 50:26:10:10 ratio by crude ^{1}H NMR after TFA cleavage. Mitsunobu esterification has been reported to be particularly useful for resin-bound carboxylic acids, but incomplete reaction was observed using DEAD,[9] ADDP, and TMAD[10] (eq 1, Table 1). However, when esterification was attempted in the presence of 3 equivalents of the nucleotide coupling reagent 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole[11] (MSNT), the only identifiable product by crude ^{1}H NMR was found to be the desired ester, **9**.

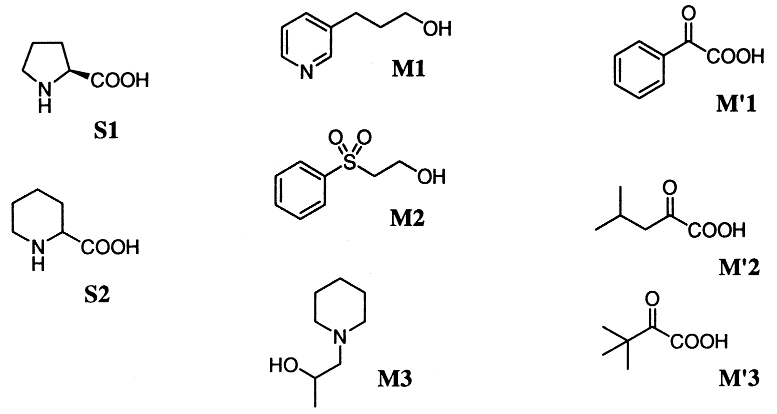
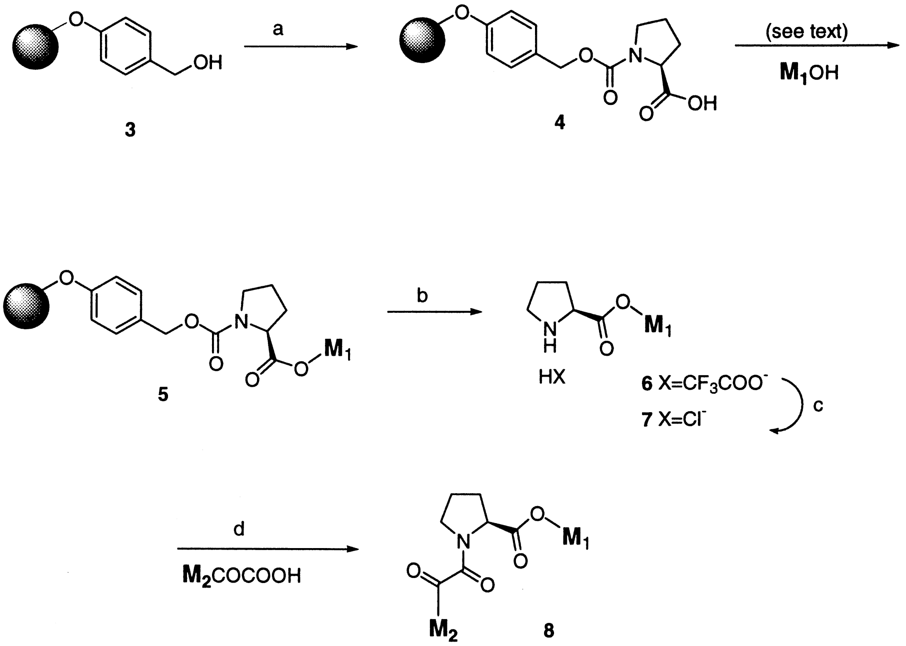
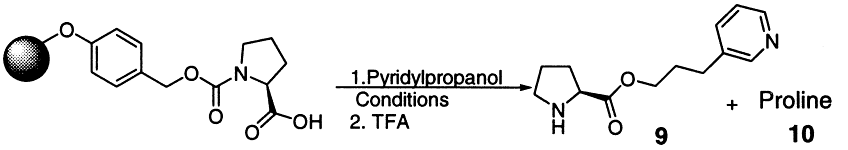


Figure 1.



Scheme 1. (a) (i) *p*-Nitrophenyl chloroformate, NMM; (ii) (L)-proline, BSA, resin, DMAP, 45 °C, MeOH; (b) TFA, CH_2Cl_2; (c) HCl; (d) TBTU, HOBt, DIEA, aq extraction.



(eq 1)

Attention was next turned to the solution-phase α-ketoamide formation. Initial attempts involving coupling of the cleaved proline ester with trimethylpyruvic acid chloride or with trimethylpyruvic imidazolide met with little success due to the unavoidable formation of contaminating quantities of pivalic amide. DCC coupling of **1** with trimethylpyruvic acid led to the formation of a stable, unreactive pyruvic acid/DCC adduct.

The successful reaction involved coupling of the cleaved proline ester (**7**) with a 10% excess of the desired α-ketoacid in the presence of *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-bis(tetramethylene)uronium tetrafluoroborate (TBTU) and Hunig's base in acetonitrile. Prior conversion of the TFA salt to the HCl salt was found to be necessary to circumvent undesired trifluoroacetamide formation. Aqueous work up with Li_2CO_3 afforded the desired immunophilin ligands in good purities.

A model library of 18 compounds (2×3×3) was then synthesized using these optimized conditions. Two resin-loaded amino acid scaffolds, (L)-proline (**S1**) and (±)-piperidine-2-carboxylic acid (**S2**), were coupled using MSNT to three alcohols (3-(pyridyl)-3-propanol (**M1**), 2-phenylsulfonylethanol (**M2**), and (±)-*N*-(2-hydroxypropyl)piperidine (**M3**)). After cleavage (TFA,

Table 1.

Conditions	Product ratios (9:10)[a]
DEAD/Ph_3P	< 5: >95
ADDP/Bu_3P	1:9
TMAD/Bu_3P	2.5:1
MSNT (2 equiv)	3:1
MSNT (3 equiv)	>200:1

[a] Ratios determined by 400 MHz NMR.

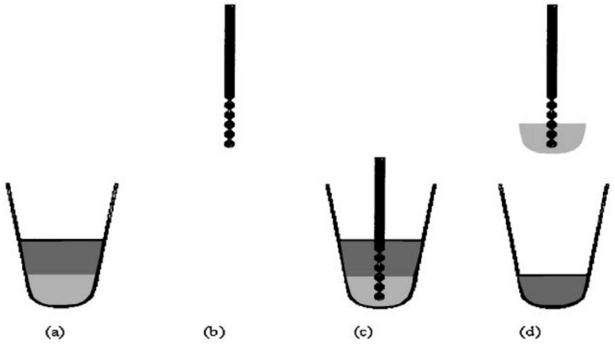


Figure 2.

Table 2.

Product[a]	M_r[b]	Rt (min)[b]	Yield%[c]	Purity%[d]	^{1}H NMR	FKBP12 SPA[g] pIC_{50}
S1M1M′1	366	4.8/5.1	30	89	[e]	< 5
S1M1M′2	346	5.60	35	78	[e]	< 5
S1M1M′3	346	5.38	30	80	[e]	< 5
S1M2M′1	415	6.28/6.61	30	76	[e]	< 5
S1M2M′2	395	6.70/6.90	59	64	[e]	< 5
S1M2M′3	395	6.19/6.63	36	49	[e]	< 5
S1M3M′1	372	4.21	53	92	[e,f]	< 5
S1M3M′2	352	4.37	70	87	[e,f]	< 5
S1M3M′3	352	3.90	50	75	[e,f]	< 5
S2M1M′1	380	6.63	28	61	[e]	ND[h]
S2M1M′2	360	6.88	27	65	[e]	ND[h]
S2M1M′3	360	6.69	38	69	[e]	ND[h]
S2M2M′1	429	7.15	12	52	[e]	ND[h]
S2M2M′2	409	7.41	33	42	[e]	ND[h]
S2M2M′3	409	7.20	30	35	[e]	ND[h]
S2M3M′1	386	5.92	30	84	[e,f]	ND[h]
S2M3M′2	366	6.52	39	74	[e,f]	ND[h]
S2M3M′3	366	6.27	37	67	[e,f]	ND[h]
GPI 1046	—	—	—	—	—	4.68 ± 0.11
FK 506	—	—	—	—	—	8.50 ± 0.13
Rapamycin	—	—	—	—	—	8.46 ± 0.13

[a] See text for identities of **S1**, **S2**, **M1**, **M2**, **M3**, **M′1**, **M′2**, **M′3**.
[b] Determined by LCMS and HPLC; HPLC conditions: HP1100, Supelcosil ABX + column, 1 mL/min, MeCN:H_2O (0.1% ammonium formate) 15:85 to 80:20 over 15 min. Diode array detection at 220 nM. Double entries refer to a separation of prolyl *cis* and *trans* rotamers.
[c] Isolated yields by weight of final product.
[d] Purity based on HPLC integration.
[e] Mixture of *cis* and *trans* prolyl rotamers.
[f] Mixture of diastereomers.
[g] See ref 12.
[h] Not determined.

Me_2S, CH_2Cl_2), counter ion exchange (i.e. **6**→**7**; HCl, ether), the cleaved amino esters were coupled with three α-ketoacids (phenylglycolic acid (**M′1**), 2-oxo-4-methylpentanoic acid (**M′2**), and trimethylpyruvic acid (**M′3**)) in the presence of TBTU. The crude products were purified by aqueous extraction using the solid/liquid extraction apparatus in 96-well format[7] (Fig. 2; single well shown for clarity). Thus, each compound (96-well format) was partitioned between with 1% Li_2CO_3 and ethyl acetate with vigorous mixing for 30 min. After the layers were allowed to separate (Fig. 2a), the extractor pin assembly (Fig. 2b and c) was inserted, and, following freezing, the aqueous plugs were removed, 96 simultaneously (Fig. 2d). Isolated yields were typically 30–40% with moderate to good purities. Table 2 shows the analytical results of the overall process

An 880 member production library was then synthesized in 96-well format using the techniques described above. The overall extractive purification process required 5 h to process 880 solution-phase reactions. The product wells were each examined by LCMS analysis and found to have purity profiles similar to those of the model library (i.e., Table 2). Assessment of the **S1** monomer-derived compounds in our FKBP12 SPA binding assay[12] shows these derivatives to have low affinity for this protein compared to FK506 and rapamycin (Table 2). The success of this library approach makes it possible to apply these reaction protocols to the synthesis of novel amino acid scaffolds, and such work will be reported in the future.

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