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Alkylation of Rink's Amide Linker on Polystyrene Resin: A Reductive Amination Approach to Modified Amine-Linkers for the Solid Phase Synthesis of N-Substituted Amide Derivatives.

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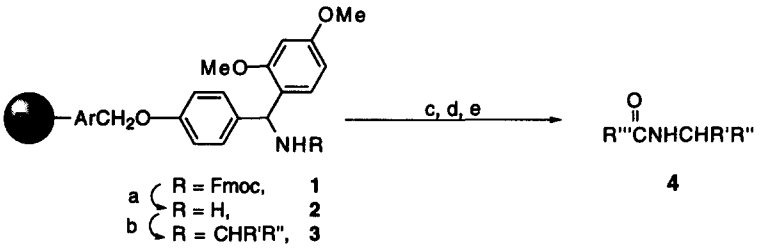
Abstract: Reductive amination of aldehydes and ketones using sodium cyanoborohydride and Rink's 4-(2',4'-dimethoxyphenyl-aminomethyl)-phenoxymethyl-linked polystyrene resin [Rink's amine linker on copoly-(styrene-1%-divinylbenzene)][1] **2** affords high yields of linker-bound, N-alkyl amines with excellent chemical selectivity. Subsequent coupling with acid derivatives gave derivatized N-substituted amides in excellent yields after cleavage from the solid-support.

The explosive development of combinatorial synthetic methods over the past several years has lead to a renaissance in the use and development of solid phase synthesis methods by organic chemists. Although many new developments in the field of linker-technology for solid-phase organic synthesis applications have been introduced[1,2a-g], perhaps the most widely used now for amide synthesis is the acid labile linker developed by Rink[1] over a decade ago, which affords C-terminal primary amides[3] upon cleavage from a substituted benzyhydryl amine linked to a polystyrene resin. While this linker is quite useful for the synthesis of primary amides, to our knowledge no methods have been reported for the direct alkylation of this commercially available amine resin to facilitate the incorporation of a secondary amide and concomitant diversity at the resin bound amide position. We envisioned that such a modification would allow one to perform Structure Activity Relationship (S.A.R.) studies on peptides or other derivatives having C-terminal substituted amides.

Recently, as part of our continuing efforts in the solid phase synthesis of pharmacologically active materials, we required such a class of modified acid-labile linkers which would yield N-alkylated amides upon cleavage from the solid support. We felt that modification of Rink's "amine linker" **2** by reductive alkylation would be the most straightforward strategy to follow for our particular needs (Scheme 1), given the generality of this method in solution and solid phase reactions with a variety of ketone and aldehyde components[4a-y]. Since the reductive alkylation of this hindered, resin bound benzhydrylamine group had not yet been reported, we attempted initially to utilize previously reported procedures for the reductive N-alkylation of amino-termini of resin-supported peptides[4f,4m,4o,4v]. We were surprised to find that our efforts to monoalkylate **2** gave unacceptable results under these conditions (1-10 eq. ArCHO/ $NaBH_3CN$/ 1% HOAc in DMF/ 20°C[4f,4m,4o,4v]). We therefore began to explore the parameters which governed this reaction's progress in an effort to make this conversion straightforward and amenable to library synthesis.

After considerable experimentation, the reductive alkylation of **2** was found to proceed smoothly simply by adjusting the solvent system used for the reaction; the most dramatic effect was found when aqueous THF was substituted for anhydrous DMF as the solvent. As is the case for DMF, THF has several useful properties as a solvent for solid-phase synthesis, namely it swells polystyrene resin adequately, it is an excellent co-solvent for many organic reactions including reductive aminations using sodium cyanoborohydride, and it is stable to a wide variety of reaction conditions. In contrast to DMF, however, there

are no amine-derived decomposition products from THF which might compete with resin-supported amine for aldehyde during the reduction/alkylation process[5]. We also found that the presence of a small amount of water was crucial for rapid reduction of the hindered *in situ* generated iminium intermediates using sodium cyanoborohydride. Further work is underway to explore the cause of this interesting dependency of the reductive amination process on the presence of water in the solvent mixture.



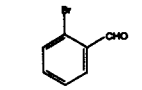
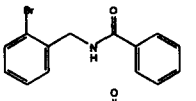
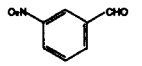
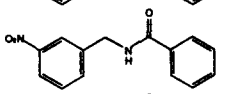
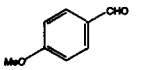
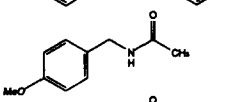
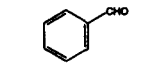
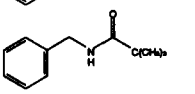
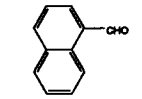
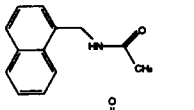
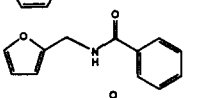
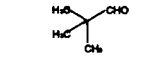
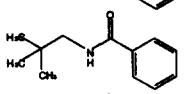
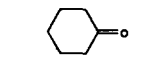
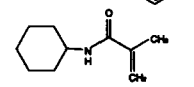
Scheme 1: *Reagents and Conditions:* (a) 20: 80 :: Piperidine: DMF, 3 x 20 min; (b) R'COR", NaCNBH3, 95: 5: 5 :: THF: HOAc: H_2O, 3 hr @ 23°C; (c) 0.2 eq CBZNHCH2CO2-(succinimide), Et_3N, THF, 30 min @ 23°C; (d) R'''COX, Et_3N, CH_2Cl_2, 23°C; (d) CH_2Cl_2: TFA: H_2O:: 95: 5: 1, 2 x 20 min @ 23°C.

Ultimately, we discovered that a mixture of THF/H_2O/HOAc :: 90:5:5 gave rapid reaction rates for the selective monoalkylation of **2** with a variety of carbonyl derivatives. The results of our studies are given in Table 1. Note that the ratios of starting materials are slightly different with an aliphatic aldehyde than with aromatic aldehydes and ketones, as larger amounts of aliphatic aldehyde led to reduced yields and impure products in most cases. These alkylation reactions proceed nearly to completion (>95%)[6] in a short time at room temperature, but the rates slow considerably after ca 95% of the primary amine groups are alkylated, presumably due to severe hindrance at the remaining 5% of sites. Since repeated reactions did not alkylate the remaining 2-5% of Resin-NH_2 groups to any significant extent, we capped the primary amine positions using the selective acylating agent CBZ-glycine NHS ester[4o] prior to acylation with anhydrides or acid chlorides.

As one can see in Table 1, a wide variety of carbonyl compounds are compatible with this process. The products are isolated as the amide derivatives following acylation and subsequent cleavage from the resin.

In summary, we have shown that Rink's amide linker **1** via the amine **2** can be employed directly as an alkylated diversity scaffold using a simple deprotection/reductive alkylation procedure. The method is useful not only for the formation of a variety of compounds for S.A.R. studies, but also provides a tool for the chemoselective, efficient installation of difunctional amines on polystyrene resin. The application of this method to the solid phase synthesis of libraries of peptidic and non-peptidic materials will be reported in due course.

Table 1. Solid Phase Synthesis of N-Alkylated Amides.

Entry	Starting Material	Acylating Agent	Product	Yield	Purity
1		A		95%	>98%
2		A		94%	>98%
3		B		97%	>94%
4		C		77%	>85%
5		B		85%	>97%
6		A		50%	>80%
7		A		88%	>70%
8		D		99%	>97%

Acylating agents: A = Benzoyl Chloride; B = Acetic Anhydride; C = Pivaloyl Chloride; D = Methacryloyl Chloride. Reactions performed as shown in Scheme 1. Purity determined by HPLC analysis; $\lambda = 214$ nm.

References and Notes:

Abbreviations used in this paper: THF = tetrahydrofuran; DMF = N,N-dimethylformamide; HOAc = acetic acid; $NaCNBH_3$ = sodium cyanoborohydride; TFA = trifluoroacetic acid; MeOH = methanol; FMOC = 9-fluorenylmethyloxycarbonyl-; GCMS = gas chromatography/mass spectrometry analysis. Rink amide resin = 1 = 4-[(2',4'-dimethoxyphenyl)-N-(9-fluorenylmethyl-oxycarbonyl)aminomethyl]-phenoxymethyl-linked polystyrene resin (0.48 mmol/g) was purchased from Novabiochem and was initially deprotected using 20%:80% :: piperidine : DMF before use. Products were characterized by comparison of their ^1H NMR, ^{13}C NMR, and GCMS data with literature values. Purities were determined by HPLC with 214 nm monitoring wavelength.

FMOC Deprotection: Rink's FMOC-protected amide resin 1 was deprotected in the usual manner[1] and was dried under vacuum at 70° C for 24 hr. Resin loading of "Rink amine resin" 2 was determined to be 0.55 meq/g by ninhydrin test[7].

Typical Reductive Amination, Acylation, and Cleavage Procedure: To a mixture of amine resin 2 (100 mg, 0.055 mmol), peroxide-free THF[6] (1.0 ml), and benzaldehyde (7.0 µl, 0.067 mmol) in a fritted reaction vessel was added 100 µl of 50:50 HOAC:water. After shaking 5 minutes at 23°C, a 1M THF solution of $NaCNBH_3$ (50 µl, 50 µmol) was added (with mild effervescence) and the mixture was shaken for 3 hours at room temperature. The reaction was filtered and washed in succession with THF, H_2O, MeOH, THF and CH_2Cl_2 (2 x 1 ml each) and was treated with a solution of CBZ-glycine N-hydroxysuccinimide ester (3.1 mg, 0.010 mmol) and triethylamine (4.0 µl, 0.033mmol) in THF (1.0 ml) for 30 min to cap unalkylated resin-bound

amine groups. The resin was filtered and washed as before, then was treated with a solution of triethylamine (400 µl, 2.9 mmol) and pivaloyl chloride (100 µl, 0.75 mmol) in CH_2Cl_2 (700 µl) for 3 hours at 23°C. The resin was filtered and washed in succession with CH_2Cl_2, THF, H_2O, MeOH, THF and CH_2Cl_2 (2 x 1 ml each) and air-dried briefly to give the resin as a tan solid. Cleavage of the amide material from the resin was performed using 94: 5: 1 :: CH_2Cl_2: TFA: H_2O (2 x 700 µl, 20 min) as for typical peptide cleavages. After combining the cleavage filtrates and CH_2Cl_2 washings (3 x 500 µl) the solution was concentrated under vacuum to give an amber oil (8.1 mg, 77% yield). Purity, as judged by HPLC using detection at 214 nm was 86%. ^1H NMR (300 MHz, $CDCl_3$): ∂ 7.42-7.25 (m, 5H), 6.05-5.87 (bs, 1H), 4.44 (d, 1H, J= 7.2 Hz), 1.23 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): ∂ 174, 138, 129, 128, 127.5, 43, 28, 27. ESMS: m/e= 191, 176, 149, 106, 91, 57.

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2. For examples of new linkers, see: a) Tietze, L.F.; Steinmetz, A. *Synlett* ., **1996**, 667-668; b) Beaver, K.A.; Siegmund, A.C.; Spear, K.L. *Tetrahedron Lett.* **1996**, *37*, 1145-1148, and references therein; c) Zhang, X.; Jones, R.A. *Tetrahedron Lett.* **1996**, *37*, 3789-3790; d) Han, Y.; Bontems, S.L.; Hegyes, P.; Munson, M.C.; Minor, C.A.; Kates, S.A.; Albericio, F.; Berany, G. *J. Org. Chem.* , **1996**, *61*, 6226-6339; e) Boehm, T.L.; Showalter, H.D.H. *J. Org. Chem.* , **1996**, *61*, 6498-6499; f) Kaljuste, K.; Undén, A. *Tetrahedron Lett.* , **1996**, *37*, 3031-3034; g) Hoffmann, S.; Frank, R. *Tetrahedron Lett.* , **1994**, *35*, 7763-7766. For reviews of combinatorial chemistry, see: h) Früchtel, J.S.; Jung, G. *Angew . Chem. Int. Ed. Engl.*, **1996**, *35*, 17-42; i) Terrett, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron*, **1995**, *51*, 8135-8173; j) Desai, M.C.; Zuckermann, R.N.; Moos, W.H. *Drug Development Research*, **1994**, *33*, 174-188.

3. The term "C-terminal amide" denotes the point of attachment to Rink's linker, in analogy to the peptide synthesis term.

4. For recent examples of solution-phase reductive aminations, see: a) Abdel-Magid, A.F.; Carson, K.G.; Harris, B.D.; Maryanoff, C.A.; Shah, R.D. *J. Org. Chem.* , **1996**, *61*, 3849-3862.

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5. Contamination will occur, however, if THF-derived oxidation products are present in the solvent. The THF we employed in these reactions was peroxide-free when tested with damp starch-iodine paper. The presence of an oxidation inhibitor (BHT) in the THF does not affect the reaction appreciably.

6. Determined from the ratios of NH_2Bz: NHRBz by benzoylation of aliquots of crude resins, cleavage, and GCMS analysis.

7. a) Sarin, V. K.; Kent, S.B.H.; Tam, J.P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147-157; b) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-601.

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