The Synthesis of the High-Potency Sweetener, NC-00637. Part 3: The Glutamyl Moiety and Coupling Reactions

ARTICLE *in* ORGANIC PROCESS RESEARCH & DEVELOPMENT · DECEMBER 2003

Impact Factor: 2.53 · DOI: 10.1021/op0341115

The Synthesis of the High-Potency Sweetener, NC-00637. Part 3: The Glutamyl Moiety and Coupling Reactions

David J. Ager,*,† Scott Babler, Robert A. Erickson, Diane E. Froen, Jeannine Kittleson, David P. Pantaleone, Indra Prakash,‡ and Ben Zhi

NutraSweet R&D, 601 East Kensington Road, Mount Prospect, Illinois 60056, U.S.A.

Abstract:

The synthesis of the high-potency sweetener, NC-00637 (1), required selective preparation of the γ-protected glutamic acid. Coupling of the three components could be performed in any order, but the final route involved *N*-acylation of the protected L-glutamic acid with the acid chloride derived from (*S*)-2-methylhexanoic acid. Activation of the α-carboxyl group allowed condensation with 5-amino-2-cyanopyridine (4). Saponification of the γ-ester 19 then provided the sweetener 1.

Introduction

In our studies on the synthesis of the new sweetener candidate, NC-00637 (**1**) the coupling of the components was of paramount importance as it provided the active material. The sweetener candidate (**1**) can be disconnected to the three components that require amide bond formation in the forward direction (Scheme 1). We have already described the synthesis of the acid (**2**).[1] The glutamic acid moiety (**3**) is the central linchpin. The synthesis of the pyridine moiety (**4**) has also been described.[2]

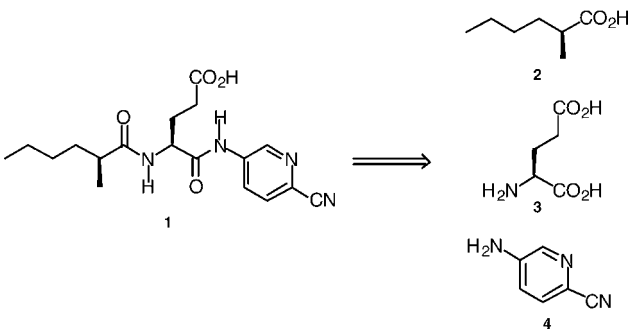
The major concern associated with the glutamic moiety was to ensure that coupling with the α-carboxylate group occurred. The two main strategies were to protect the γ-position as an ester or to use an enzymatic approach that would be regioselective.

With regard to assembly of the components, there are two possible ways of joining the three units (Scheme 2). Each variation was attempted using a chemical and biochemical approach. Obviously, activation of the appropriate functional groups also had to be performed.

Historical Perspective

The first coupling procedure was provided by the discoverers of the compound.[3] This can be considered the equivalent of the medicinal chemistry route for a pharmaceutical candidate. In this process, L-glutamic acid (**3**)was first treated with the acid chloride **7** derived from racemic 2-methylhexanoic acid (Scheme 3). The resultant diacid was then treated with DCC at room temperature for 3 h to yield

Scheme 1



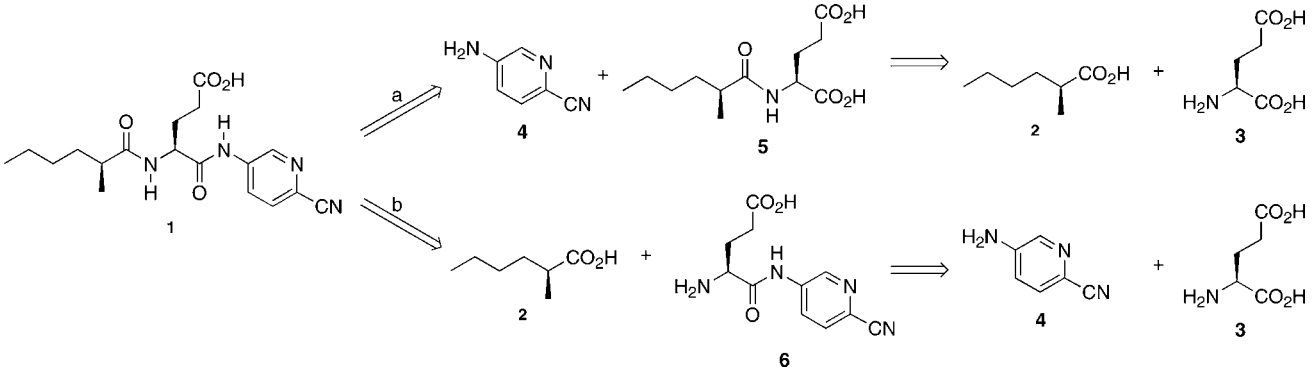
a cyclic anhydride **9** in quantitative yield. This anhydride **9** reacted with the amino pyridine (**4**) to give a mixture of two α-diastereoisomers that could be separated by preparative HPLC. The discoverers reported a sweetness of 7000 (2% sucrose) and $[\alpha]_D = +0.3°$ (*c* 6.6, MeOH) for the (*S*,*S*)-diastereoisomer **1** and a sweetness of 1000 (2% sucrose) and $[\alpha]_D = -0.3°$ (*c* 7.5, water) for the (*R*,*S*)-isomer **10**.[3] At this time, we were able to prepare optically pure (*S*)- and (*R*)-2-methylhexanoic acid.[1] We decided to use this approach to avoid the HPLC separation. However, when we tried to repeat this process using (*S*)-2-methylhexanoic acid (**2**), we discovered that this process had racemised the glutamic acid unit. Thus, the original claim to the (*S*,*S*)-diastereoisomer was actually a racemic mixture of (*S*,*S*)- and (*R*,*R*)-isomers and the so-called (*R*,*S*)-diastereoisomer was a mixture of *R*,*S* and *S*,*R*. We also found that the coupling of the anhydride **9** with **4** was not regiospecific; γ-coupling products were also obtained. It should be noted that formation of the acid chloride **11** from chiral 2-methylhexanoic acid is not trivial (vide infra), but fortuitously, conditions were employed in the conversion of the parent acid to the acid chloride that did not result in racemisation.

The second coupling procedure (Scheme 4) was also provided by the discoverers of **1**.[3] In this procedure, L-glutamic acid (**3**) was treated with trifluoroacetic anhydride to form a cyclic anhydride **11** in high yield. The amino group was protected as a trifluoroacetate amide at the same time. The anhydride **11** reacted with the pyridylamine **4** to give two regioisomers **12** and **13** (65:35 in favour of the desired product **12**) in 67% total yield. The two isomers were separated by preparative HPLC. After removal of the trifluoroacetate by ammonia solution (90% yield) to give **6**, acylation by (*S*)-2-methylhexanoic acid chloride (**11**) to produce the final product **1**. The drawback of this process

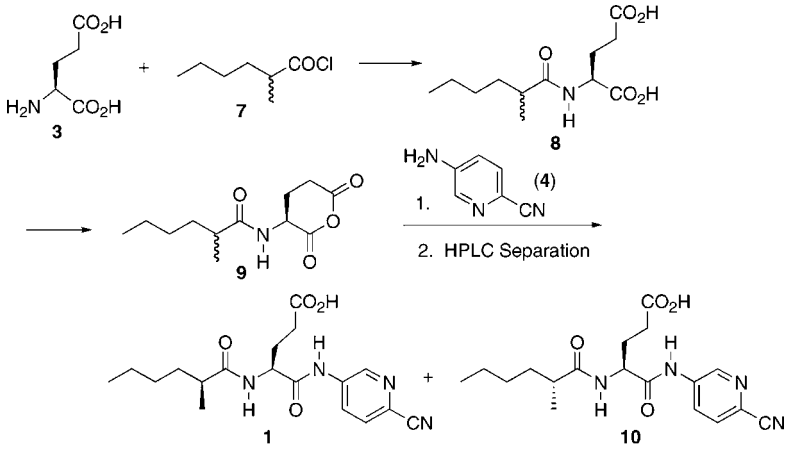
† Current address: RCCorp, 805 Darfield Drive, Raleigh, NC 27615.
‡ Current address: NutraSweet, 1801 Maple Ave, Evanston, IL 60201.

(1) Ager, D. J.; Babler, S.; Froen, D. E.; Laneman, S. A.; Pantaleone, D. P.; Prakash, I.; Zhi, B. *Org. Process. Res. Dev.* **2003**, *7*, 369.
(2) Ager, D. J.; Erickson, R. A.; Froen, D. E.; Prakash, I.; Zhi, B. *Org. Process. Res. Dev.* **2004**, *8*, 62−71.
(3) Nofre, C.; Tinti, J.-M. U.S. Patent 5,430,182, 1995; U.S. Patent 5,374,733, 1994; U.S. Patent 5,310,908, 1994; and U.S. Patent 5,196,540, 1993.

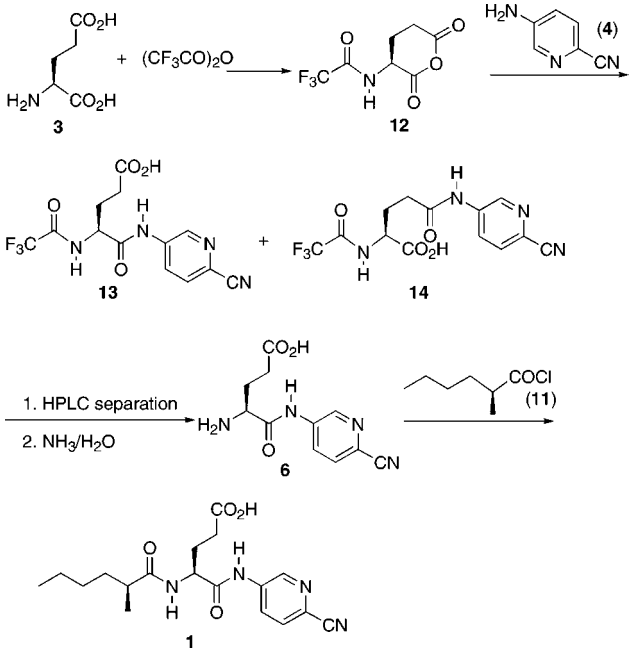
Scheme 2



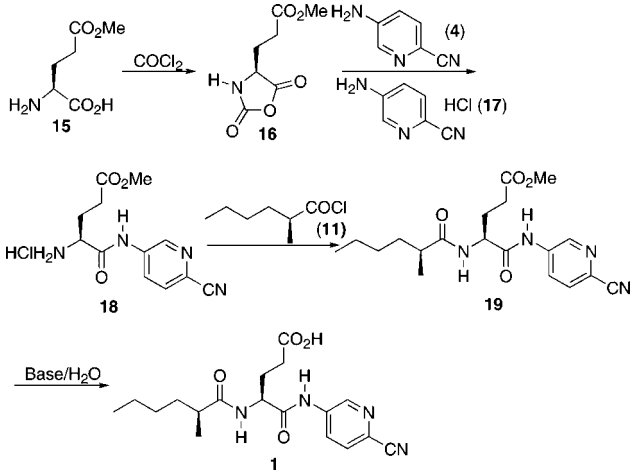
Scheme 3



Scheme 4



Scheme 5

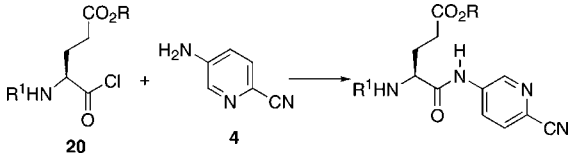


was the separation of the two regioisomers. It could not be used in a large-scale preparation.

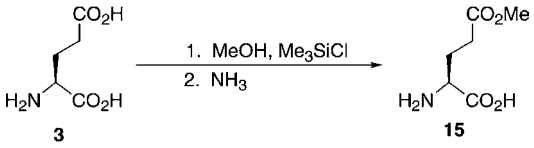
The third iteration at a coupling process gave some indication that it could be used for reasonable amounts of material. This process started with γ-methyl L-glutamate (**15**) (Scheme 5). It was first converted to the *N*-carboxylate anhydride (NCA) (**16**) using phosgene or triphosgene. The NCA was coupled with the hydrochloride **17** of the amine **4** in the presence of free amine to give **18**, followed by acylation with (*S*)-2-methylhexanoic acid chloride (**11**). Finally, the γ-methyl ester **19** was hydrolysed to give the desired product **1**. This method does not form undesired isomers, and thus no complicated separation was needed. The reagents are relatively cheap. All these factors make larger-scale preparation possible.

One unique feature of this process is the use of the hydrochloride salt **17** as well as the free amine **4**. At first,

Scheme 6



Scheme 7



the pyridylamine **4** was used in this process alone. It is a much weaker nucleophile than the amino group on glutamic acid. Once the reaction started, the amino group of the glutamyl moiety freed from the NCA would attack unreacted NCA **16** to form a polymer. This problem was solved by use of the hydrochloride salt of **4**.[4] As the amino group of glutamic acid is also a stronger base than the amine **4**, the HCl transfers from the salt **17** to the glutamylamine group once it is freed to give **18**. This leaves the amine **4** as the only nucleophile in the system. The drawback is that the reaction is relatively slow due to the low concentration of the free amine **4**.

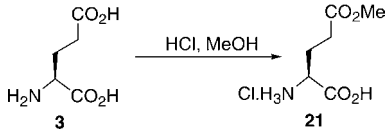
Many other coupling procedures were tried using reagents that activated the carboxylic moiety, such as DCC, as well as preparing activated esters. In some cases, the final product **18** or **6** was obtained, but none were as clean as the NCA approach. Surprisingly, one successful approach was to use the acid chloride **20** of *N*-protected glutamates (Scheme 6), but racemisation proved to be a problem on scale-up.[5]

Thus, for our first large-scale syntheses, we decided to use the NCA of the glutamic acid moiety and couple this with the amine **4** and follows the sequence of pathway b (Scheme 2).

NCA Component

The NCA **16** requires the preparation of γ-methyl glutamate (**15**). Two methods were used for the preparation of this compound in kilogram amounts; one used trimethylsilyl chloride (Scheme 7),[6] the other, just hydrogen chloride. The NCA was prepared by treatment of the γ-ester with phosgene. Triphosgene had been used at laboratory scale with success, but it was felt that phosgene would be simpler at scale. In addition, we had access to toll manufacturers who had experience in reactions of phosgene with amino acid derivatives; the aspartic anhydride derivative used in the Z-process for the preparation of aspartame uses phosgene.[7] In the initial studies, the zwitterion form of L-glutamic acid was used to prepare the NCA, but this was later changed to the hydrochloride salt **21** (Scheme 8). This change impacted the ester formation step, as the hydrochloride is much easier to isolate than the zwitterion.

Scheme 8



Analysis of the reaction mixtures showed that the ester formation is a complex series of equilibria involving the free acid, mono esters, of which the γ-ester predominates by a significant amount, the diester, and pyroglutamate, depending on whichever acid catalyst is used. Only trace amounts of the α-ester are produced. As long as the formation of pyroglutamate is minimised, as this is an irreversible side reaction, the isolation method only needs to preferentially crystallise the desired product as this pushes the equilibria in the desired direction and maximises yield. The action of trimethylsilyl chloride with methanol, of course, produces HCl in situ. The advantage of the silyl chloride approach is that it is easier to control the amount of acid used.

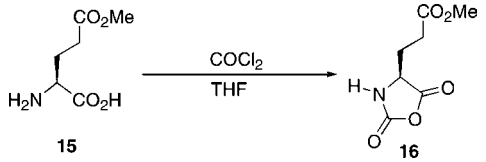
The reaction described in Scheme 6 is straightforward and proceeds in high yield for the first step. As hydrogen chloride is produced in this reaction, the hydrochloride salt of the amino acid is produced. Conversion to the zwitterion form was troublesome. Ammonia was found to be the best base as this reduced the amount of hydrolysis, but an equimolar amount of ammonium chloride is formed. Precipitation of the desired zwitterion of γ-methyl glutamate (**15**) was always accompanied by deposition of ammonium chloride. Although multiple washings of the solid product with water or methanol could remove most of the inorganic salt, the recovery of the ester was low. Thus, the mixture of the ester and ammonium chloride obtained was taken through to the phosgenation step without any further purification. This, however, resulted in an extra filtration at the end of the phosgenation to remove the ammonium chloride that caused some operational problems (vide infra).

We turned to the use of the hydrochloride salt **21** of γ-methyl glutamate as the substrate for the phosgenation as it is formed during the phosgenation reaction of **15** due to the production of hydrogen chloride. At the same time, we moved from the use of trimethylsilyl chloride as our acid source—this had been used to reduce the amount of diester formed. However, as noted above, equilibria exist, and it is the isolation of the desired product that is key. To isolate the monoester from the HCl reaction, a simple precipitation procedure was used—isopropyl acetate being the antisolvent to force the salt from methanol solution. This method has been scaled up successfully to over 200-kg-sized batches without any significant loss in yield.

Phosgenation. The phosgenation reaction is straightforward and well-known.[8] Tetrahydrofuran was our first solvent

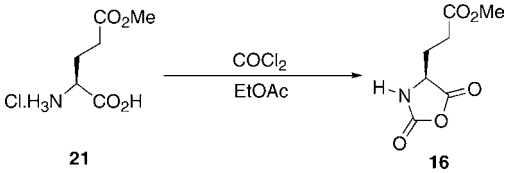
(4) Knobler, Y.; Bittner, S.; Virov, D.; Frankel, M. *J. Chem. Soc. C* **1969**, 1821; Knobler, Y.; Bittner, S.; Frankel, M. *J. Chem. Soc.* **1964**, 3941.
(5) Bodanszky M.; A. Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1984 and references therein.
(6) Webster, K. L.; Maude, A. B.; O'Donnell, M. E.; Mehrota, A. P.; Gani, D. *J. Chem. Soc., Perkin Trans. I* **2001**, 1673; Baldwin, J. E.; North, M.; Flinn, A.; Moloney, M. G. *Tetrahedron Lett.* **1989**, *45*, 1453; Baldwin, J. E.; North, M.; Flinn, A.; Moloney, M. G. *J. Chem. Soc., Chem. Commun.* **1988**, 828. See also Albert, R.; Danklmaier, J.; Hoenig, H.; Kandolf, H. *Synthesis* **1987**, 635.
(7) Ager, D. J.; Pantaleone, D. P.; Henderson, S. A.; Katritzky, A. R.; Prakash, I.; Walters, D. E. *Angew. Chem., Int. Ed.* **1998,** *37*, 1803.
(8) Kricheldorf, H. R. *α-Aminoacid-N-carboxy-anhydrides and Related Heterocycles*; Springer-Verlag: Berlin, 1987 and references therein.

Scheme 9



15 16

Scheme 10



21 16

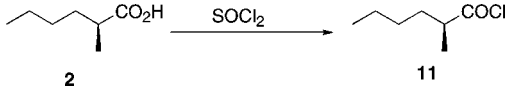
of choice for the transformation of the zwitterion of γ-methyl glutamate to the NCA as the product is soluble in this solvent (Scheme 9). The reaction proceeds in reasonable yield whether phosgene gas or phosgene as a solution in toluene is used. Isolation was by simple crystallisation after removal of the ammonium salts (vide supra) from the reaction mixture. Temperatures during the isolation procedure had to be kept down as the NCA is thermally unstable. It was also necessary to keep the material in a freezer to avoid unwanted degradation on storage.

For our needs, phosgene in toluene solution was simpler to use than phosgene gas. Some yield advantage was seen if the reaction was allowed to proceed at a slightly elevated temperature (45 °C). For this reaction, the scale required volumes that were too large for our equipment, and workup had to be done by splitting the batch (see Experimental Section). The second batch was recrystallised with the mother liquors from the first batch, and this decreased recovery. Obviously, fresh solvents were required. In addition, attempts to obtain second crops from the mother liquors resulted in impure material crystallising out.

Later in the development stage, the preparation of the NCA was tolled out. Shipment of the compound had to be at low temperature to ensure that degradation did not occur—this is an exothermic event. In addition, the degradation results in the evolution of carbon dioxide, and pressure buildup is a potential serious hazard. Problems associated with the stability of the NCA contributed to the decision to change the route.

When the hydrochloride salt of γ-methyl glutamate (**21**) was used as the substrate, THF proved inferior as a solvent—presumably because of the low solubility of the amino acid salt. In addition, side products were found that were derived from reaction of the THF and NCA under the acidic conditions. Analysis of the reaction mixture showed that not only were NCA and products derived from its subsequent reactions present, but isocyanates were also formed. Purification of the desired product was not simple, and often an oil resulted. However, ethyl acetate was found to be a good solvent for the desired transformation even though the mixture did require heating (Scheme 10). It should be noted that ethyl chloride is formed during the reaction and this has to be dealt with in the gaseous stream as well as phosgene. Degradation, however, could be minimised as long

Scheme 11



2 11

as phosgene was present in the reaction mixture. Isolation was also simplified as the NCA could be obtained by precipitation through the addition of hexane to the ethyl acetate solution.

When the reaction was scaled up, mechanical problems became evident due to the large amount of phosgene in the gaseous state, as well as evolved hydrogen chloride. This work was performed by an outside vendor, and they had to revert to use of the zwitterion which they prepared in situ by the addition of one mol equivalent of base such as ammonia or triethylamine to the hydrochloride salt in THF. Phosgenation was then performed on this whole mixture. The ammonium salts were removed prior to the isolation steps as described in our initial procedure. It was also found that if the reaction mixture was left for a prolonged period, the quality of the NCA could be improved by an additional charge of phosgene. This is very similar to a published finding on the purification of our NCA.[9]

Acid Chloride 11 from 2-Methylhexanoic Acid. As described in the coupling section (vide infra), the hexanoic acid has to be converted to the acid chloride **11** to allow reaction with the glutamyl amino group. During the early stages of the development, the acid chloride was prepared by the traditional method of reaction with thionyl chloride in an inert solvent, usually dichloromethane.[10] The acid chloride was distilled to ensure that excess thionyl chloride and acidic byproducts were removed prior to the coupling. On a small scale this did not cause problems due to the short heat history. Thus, in the initial preparations of **1**, racemisation of the acid chloride was not an issue. However, it was found that the acid chloride was prone to racemisation upon heating. A few hours at 60 °C could ensure that racemisation was complete. As this presented an obvious problem for scale-up, together with the use of a chlorinated solvent, we chose to eliminate both the solvent and the distillation step. Thus, the acid **2** was added to a large excess of thionyl chloride with the exotherm being controlled by external cooling and addition rate (Scheme 11). After the reaction was complete, the excess thionyl chloride was removed by vacuum distillation, and then the acid chloride was used without further purification in the next step. Any residual thionyl chloride (up to 10%) did not adversely impact the coupling yields as long as there was sufficient base present. At scale, the removal of the thionyl chloride was monitored by weight. For all operations the temperature was kept at or below 40 °C as this minimised racemisation problems.

With all the components in hand, we can now consider the coupling steps.

Formation of L-Glutamic Acid 5-Amino-2-cyanopyridine Amide γ-Methyl Ester (18). As noted above, the

(9) Dorman, L. C.; Shiang, W. R.; Meyers, P. A. *Synth. Commun.* **1992**, *22*, 3257.

(10) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; p 1158.

coupling of the pyridylamine **4**, with the NCA **16** of γ-methyl glutamic acid (Scheme 5), requires the presence of acid so that the product **18** does not react with the NCA once it is formed. The original reaction was found to take approximately 10 days as the amine **4** is such a poor nucleophile. Attempts to speed the reaction up by heating led to decomposition of the NCA; the NCA is known to be thermally unstable (vide supra). Formation of the desired product necessitates attack of the pyridine amine with a molecule of the NCA. An increase in the amounts of NCA or amine (as a mixture of free base and hydrochloride salt) led to a corresponding increase in the coupling rate. This was the expected observation for second-order reaction kinetics with both the NCA and amine playing active roles in the rate-determining step. Our optimisation of this step was to take the original procedure, modify it so that unreacted starting material could be removed by extraction, and then reduce volumes. As noted below, this made some of the extraction work obsolete.

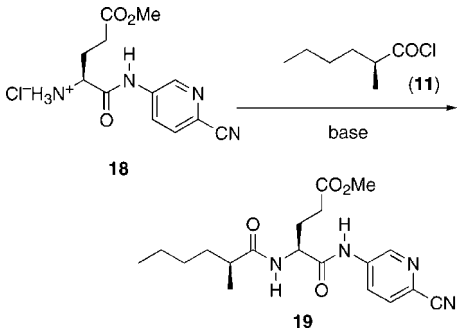
As the pyridylamine **4** is also playing a role as base (as evident from the observation that the addition of only the hydrochloride salt results in an extremely slow reaction), the addition of other non-nucleophilic bases to the reaction were evaluated. Examples of such bases were pyridine, 4-(*N*,*N*-dimethylamino)pyridine (DMAP), *N*,*N*-dimethylaniline, and *N*-methylmorpholine. All of these bases allowed the reaction to proceed, but none enhanced the rate compared to the addition of the free base **4**. As these "third-party" bases complicated the isolation procedure, they were not pursued.

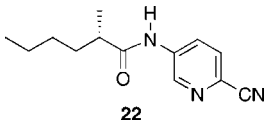
To speed up this coupling process a variety of reaction conditions were tried. DMF was found to be the solvent of choice as all of the reactants are soluble. Ethereal solvents, such as THF, and esters, such as ethyl acetate, did allow coupling, but the reaction rates were slow compared to DMF, presumably because of the low solubility of the amine hydrochloride salt in these solvents. The concentration of both reactants could be increased in DMF. An optimum concentration was found (see Experimental Section). Under these conditions the amine hydrochloride is not completely in solution at the start of the reaction and can take from 8 to 24 h (typically 12−16 h) for a clear solution to result. If the amount of solvent is reduced further, the coupling reaction slows down (again, presumably due to the solubility of the amine salt becoming important in the rate-limiting step). In addition, low solvent usage lead to a very viscous slurry.

The amine hydrochloride seems to be the best method for the introduction of the acid catalyst. Use of free base with the NCA, followed by the addition of hydrogen chloride gas, resulted in little coupling and degradation of the NCA.

Early reactions were plagued by the presence of unreacted amine **4**. This carried through to the next step with the resultant formation of an unwanted product—5-*N*-(2-methylhexanoyl)amino-2-cyanopyridine (**22**). In addition, the DMF solution of **18** was taken directly on to the next step without any purification or workup. This allowed for the acid chloride coupling step to be performed in the presence of an organic base (vide infra). It was found that a simple ethyl acetate extraction of the reaction mixture, after dilution of the DMF with water, removed most of this starting material. The amount of water used to dilute the reaction was then halved with respect to this modified procedure. This allowed a larger throughput. As more water is added in the next step (the coupling to form the ester **19**), further volume reductions were performed at this stage. Material balance studies showed that three ethyl acetate extractions were required to reduce the amount of the pyridylamine **4** to acceptable levels. However, when the reaction time was shortened by the increase in concentration, the amount of unreacted amine detected became so small that this extra step was considered unnecessary. The removal of three extraction steps also simplified the operation procedure significantly.

Scheme 12

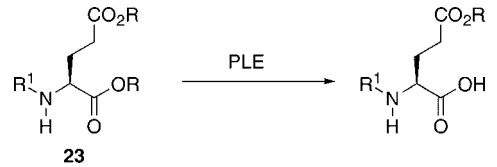




Ester 19 Preparation. As described above, DMF had become our solvent of choice for the first coupling reaction. To ease operational steps, our original method development concentrated on the addition of the acid chloride **11** to the amide **6** without any isolation procedures. To achieve this, a base had to be used in the reaction as the glutamyl unit was the hydrogen chloride salt **18** (Scheme 12). Thus, pyridine was added to the DMF solution of **18**, followed by the acid chloride **11**. Heat helped the reaction rate. In many reactions, a second nonpolar compound was formed. This was first assigned as the diastereoisomer of **19**. However, the ratio of this compound to **19** varied greatly from reaction to reaction although the ee of the acid chloride derived from **2** remained constant. In addition, when a racemic mixture of 2-methylhexanoic acid was used in the reaction, a 50:50 mixture of the ester **19** and the other nonpolar compound did not result. Isolation of this unknown showed that its structure was the *N*-acylated pyridine **22**. In addition to compound **22**, sometimes significant quantities of unreacted amine **4** contaminated the desired ester **19**. Separation of the amine could be achieved by simply dissolving the mixture in THF and then passing in hydrogen chloride gas to form the hydrochloride salt that was then removed by filtration.

The formation of the undesired compound **22** led us to investigate means to reduce the amount of unreacted amine **4** in the starting reaction mixture (vide supra). As a consequence of these studies, the amide **6** was no longer in DMF but was available for this reaction in aqueous DMF.

Scheme 13



23

A traditional Schotten−Baumann procedure[11] proved adequate for the conversion of **6** to **19**, although sodium bicarbonate was employed as the base to ensure that no hydrolysis of the resultant ester occurred. As described below, the isolation of the ester **19** was associated with other studies, and this compound became our key intermediate to which all other investigations were aimed.

The original procedure developed with the sodium bicarbonate method used an organic extraction procedure for product isolation. However, some problems were encountered due to DMF being present in the final product, which presumably inhibited crystallisation. As part of these studies, a material balance was performed during each of the separation and extraction steps. It was rapidly determined that the amount of water could be reduced in the acid chloride step with no detrimental effects to the reaction yield. Also, the ester product is insoluble in water. No extraction is necessary at the completion of the reaction; the product can be isolated, along with inorganic salts, by simple filtration. In a manner similar to the extraction procedure, it was found that removal of DMF was necessary to ensure crystallisation of the product. This could be achieved by simply stirring the salt−ester mixture with water to remove the DMF. Separation of the product **19** from the inorganic salts was achieved by dissolution of the desired ester in a solvent, such as ethyl acetate. The addition of a hydrocarbon antisolvent, such as hexane, to the ethyl acetate solution of the ester, then provided **19**. Simple trituration of this ester in ethyl acetate−hexane could then remove any impurities, but usually this was not necessary, as high-purity (>98%) material was typically obtained. Charcoal treatment of the ethyl acetate solution of **19** prior to hexane treatment was found to reduce the colour of the resultant solid product significantly.

Enzymatic Approaches. A considerable amount of effort was expended in looking at the resolution of 2-methylhexanoic esters.[1] The chemical approaches offered the best solutions (especially the asymmetric hydrogenation as it provided reasonable enantioselectivity from a cheap starting material). Enzymatic approaches were both expensive and had prolonged reaction times. There are alternative approaches that could employ the power of enzymatic reactions such as the selective hydrolysis of glutamic esters, either as the *N*-amide or amino acid itself (Scheme 13).[12] A number of enzymes were screened, but PLE was found to provide the best selectivity for hydrolysis of the α-ester and reaction rates (Table 1).

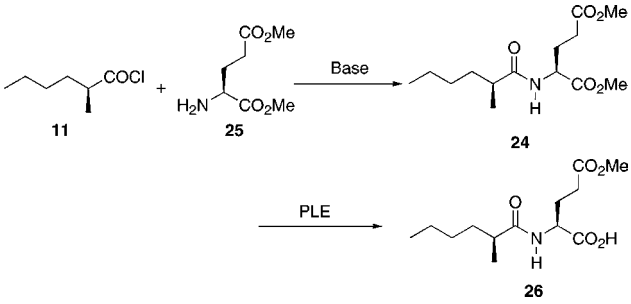
(11) March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 3rd ed.; Wiley: New York, 1985; p 346.
(12) Prakash, I.; Ager, D. J.; Pantaleone, D. P. U.S. Patent 5,773,261, 1998; U.S. Patent 5,928,909, 1999.

Table 1. **Hydrolysis of glutamyl diesters 23 with PLE (Scheme 13)**

R	R^1	concn (mmol)	enzyme units	time (h)	yield (%)
Me	H	5.02	150	1.5	60
Me	H	26.00	575	4.5	81
Et	H	4.96	300	17.7	76
t-Bu	H	0.66[a]	150	38.1	50
Me	EtCO	1.76	300	11.7	90

[a] 5% methanol was used as cosolvent.

Scheme 14



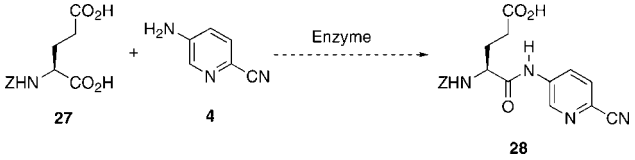
As the chemical synthesis is selective to give the γ-ester as well and the enzymatic method is a hydrolysis of the diester that would have to be prepared by chemical means, this approach was never a serious contender in terms of cost.

The PLE method could be considered for the selective hydrolysis of the α-ester group of **24** that could be formed by coupling of **11** with dimethyl glutamate **25** (Scheme 14). The product could then be activated by chemical means and coupled with the amine **4**. Although this approach is viable and the preparation of the diester is not difficult, it offers no economic advantages when the monoester **26** is available by a simple, cheap reaction. In addition, an alternative to PLE would be required as the final product **1** is a food additive and would have to be kosher (Many food additives, such as aspartame, are produced to kosher standards as they can then be used by religious faiths without any concern. The use of an animal-derived enzyme, especially a porcine-derived one, left us with many questions about whether this standard could be realized.). Although an alternative was found, it was not a cheap alternative, and the approach was abandoned.

Enzymatic hydrolysis of the methyl ester **19** could be implemented in one of two ways: (1) to clean up the problems associated with the presence and subsequent reaction of the (*R*)-isomer of 2-methylhexanoic acid in the coupling reactions to form the diastereoisomer of **19** (2) to use racemic 2-methylhexanoate in the coupling reactions and then rely on a selective hydrolysis of just the desired (*S*,*S*)-isomer to provide the target molecule **1**.

A screening approach with a wide variety of enzymes was used to see if the (*S*,*S*)-diastereoisomer **19** was preferentially cleaved over the (*R*,*S*)-isomer. Reaction was observed with PLE, Alcalase subtilisin, and papain. The major reaction with papain was amide bond cleavage to give the aminopyridine **4** and the diacid **5**. The enzyme seemed to become inactive after 3 h, indicating that either it had been inactivated

Scheme 15



or that equilibrium had been reached. Alcalase and PLE were found to selectively hydrolyse the undesired (*R*,*S*)-isomer. All these reactions were hindered by the low solubility of the substrate in aqueous media.

Similar reactions were performed with the desired product **1** to determine if the methyl ester of **19** had an effect on the enzymes. Similar results were obtained, that is, papain cleaved the amide bond at about the same rate, while PLE and Alcalase did not perform amide bond cleavage.

An alternative amide bond synthesis is to couple the glutamic acid unit, such as **27**, with the amine **4** (Scheme 15). This reaction would be analogous to the thermolysin synthesis of aspartame.[7]

In addition to thermolysin, other enzymes were screened. None provided the desired product **28**. As a control, *Z*-glutamic acid (**27**) was incubated with the methyl ester of L-phenylalanine in the presence of thermolysin. HPLC analysis of the reaction mixture showed that coupling occurred. For the reaction of **27** with the amine **4**, aqueous conditions have to be employed, and the amine is not soluble under the reaction conditions. This problem is no doubt exacerbated by the low nucleophilicity of the amine functionality of **4**. This approach, was, therefore, dropped.

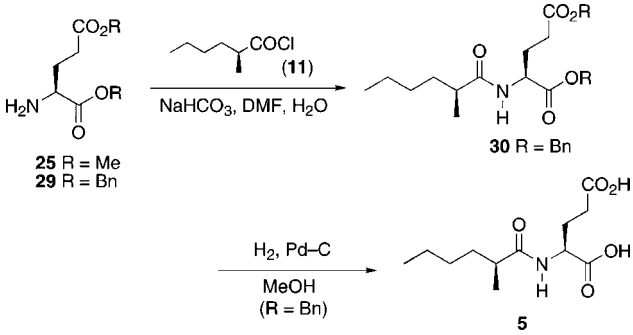
The final enzymatic approach was to react a derivative of 2-methylhexanoic acid with glutamic acid to make the other amide bond. This approach could also be used as a resolution of the 2-methylhexanoic acid. A variety of proteases were screened with the ethyl ester of (*S*)-2-methylhexanoic acid as failure to perform this coupling would not allow a resolution approach to even be considered. Under a wide variety of conditions with commercially available enzymes, none of the desired *N*-acyl product **5** was observed.

Despite the expenditure of considerable effort, none of our enzymatic approaches had shown promise for use in a commercial process during the screening phase. Thus, our attention returned to the chemical methods.

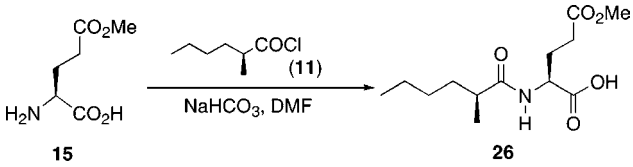
Alternative Chemical Routes. While the chemical route and biological studies were being performed, alternative chemical approaches were investigated. As summarised above, there were specific handling problems associated with the use of the NCA **16**, and these became more apparent at scale. Its inherent instability and the need to store it at −20 °C were both safety and cost issues.

It was found that diesters (e.g., **25** and **29**) of glutamic acid gave good yields when reacted with the acid chloride **11**. Some of these compounds were made as possible substrates for enzymatic reactions. Hydrogenolysis of the dibenzyl ester **30** resulted in the formation of the free diacid **5** (Scheme 16). However, no direct, selective α-coupling reactions of **5** with the amine **4** could be found.

Scheme 16



Scheme 17



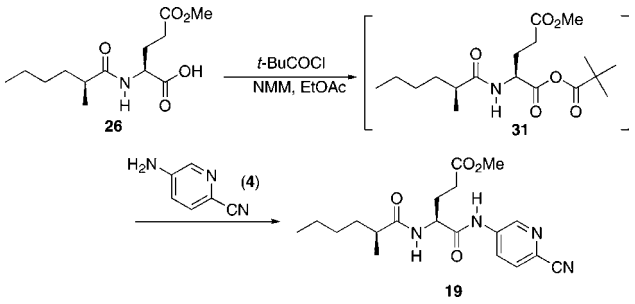
The new sequence that became the preferred method was the alternative sequence of coupling the three components (see Scheme 2, pathway a). It was also appreciated that the formation of the acid chloride, obtaining the NCA, and performing the coupling reactions could be a logistic nightmare on plant scale. A problem with any single component could result in a complete failure of the coupling reactions with little or no chance of recovering the expensive starting materials, with the possible exception of the amine **4**.

The γ-methyl ester of L-glutamic acid **15** was prepared as previously described, and the ammonium chloride was removed by washing. This was necessary as we did not want the coupling reaction complicated by the presence of ammonia. The initial coupling reactions of the glutamate and acid chloride **11** were performed in aqueous conditions, but isolation of the product was not simple, and hydrolysis of the ester **26** also occurred. With an eye on telescoping the steps, DMF was found to be an excellent solvent for the coupling reaction (Scheme 17).

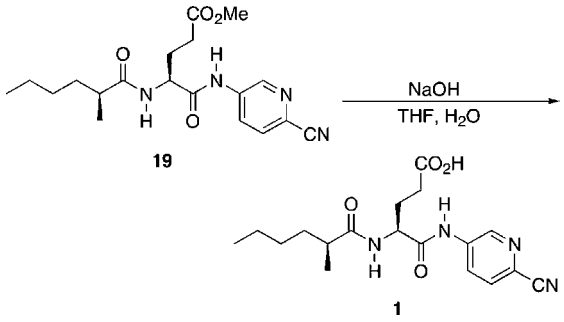
With the selectively protected *N*-alkylglutamate **26** in hand, the α-carboxylate group had to be activated for amide formation without loss of regioselectivity. Use of the standard procedure with isobutyl chloroformate followed by the amine **4** led to a good yield of **19**.[5] However, this activating agent is relatively expensive. The bulky alkyl group is used to promote attack at the amino acid carbonyl group in the intermediate anhydride. As a cheaper alternative, we found that pivaloyl chloride could be used and the anhydride **31** could be reacted with **4** in a "one-pot" sequence (Scheme 18). It was now not necessary to use the amine hydrochloride as the problems associated with the reactivity of the amine group of **4** compared to that of the glutamyl were removed.

This sequence provides **19**, so that the subsequent hydrolytic conversion to the product **1** remains unchanged. Once the sequence had been shown to be viable, we were delighted to find that an ethyl acetate extraction of the acid chloride−glutamate coupling reaction provided a solution that could be used in the pivaloyl activation step. Unfortu-

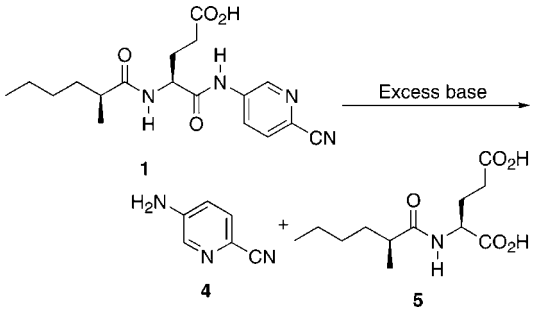
Scheme 18



Scheme 19



Scheme 20



nately, before this sequence could be performed at scale, the decision to develop Neotame as the sweetener candidate rather than NC-00637 was taken.

Hydrolysis of Ester 19 to NC-00637. This step is the last in the sequence (Scheme 19). It is a straightforward ester hydrolysis. As described below, this step was not as trivial to accomplish in a reproducible manner, and in high yield, as originally thought.

A large number of experiments have been run on this transformation to understand why it can cause trouble. Initially, we found that the methyl ester **19** was very slow to hydrolyse. The use of an organic cosolvent, such as THF, was found to accelerate the hydrolysis when aqueous base was used. On a small scale, the use of two equivalents of sodium hydroxide ensured that the saponification proceeded. Thus, the use of two equivalents of sodium hydroxide, as a 1 M solution, with THF as cosolvent became our "standard" method to provide NC-00637. A cursory study of the pH requirements for hydrolysis to occur showed that pH had to be at least 12 in the reaction medium.

The hydrolysis was also accompanied by a small amount of degradation of the product **1** from cleavage of the amide bond. The resultant products of this bond cleavage are the

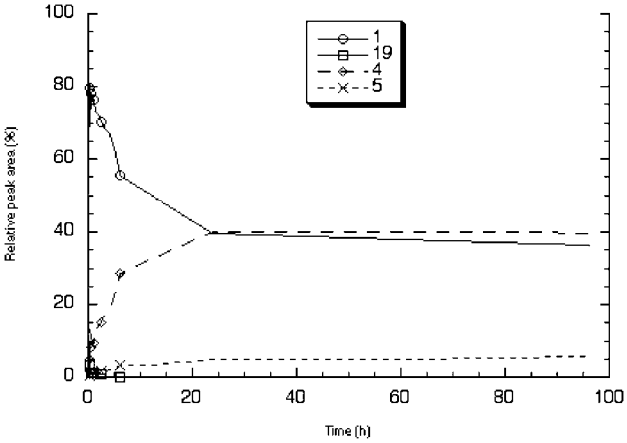


Figure 1. **Hydrolysis of ester 19 with 2 equiv of sodium hydroxide.**

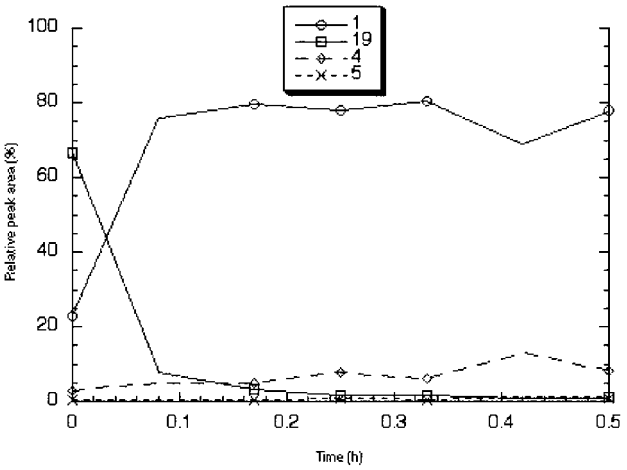


Figure 2. **Hydrolysis of ester 19 with 2 equiv of sodium hydroxide over the first 0.5 h.**

amine **4** and acid **5** (Scheme 20). As these impurity compounds could be removed by recrystallisation, the method was still acceptable. However, on scale-up, the amount of degradation increased to unacceptable levels. If too much amide bond cleavage occurred, crystallisation of the sweetener candidate was inhibited. We, therefore, returned to this hydrolysis step and looked at it in detail.

The reaction was followed by HPLC (Figure 1). The reaction times were extended to determine the effect of excess base over time on the sweetener **1**. The data are presented below in terms of relative peak area percent of the important components. In some reactions, additional byproducts were formed—these are not apparent from the data's presentation. In addition, the chromophores of the components are very different at the wavelength used. The pyridylamine **4** has a relatively high chromophore compared to **1** so that its concentration is less than would appear from the peak area percent. Conversely, the diacid **5** has a very weak chromophore, so that its concentration is higher than would appear. The results of two equivalents of base with the methyl ester **19** are summarised in Figure 1.

The ester disappears very rapidly; a close up of the first part of the reaction (Figure 2) shows that the reaction is over in a matter of minutes. The plot in Figure 1 shows that the

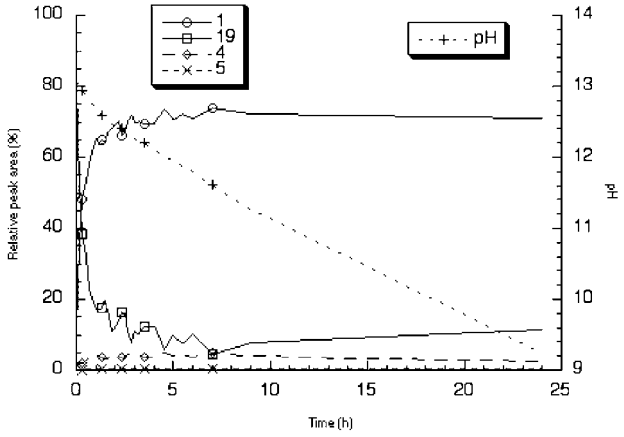


Figure 3. **Hydrolysis of ester 19 with 1 equiv of base.**

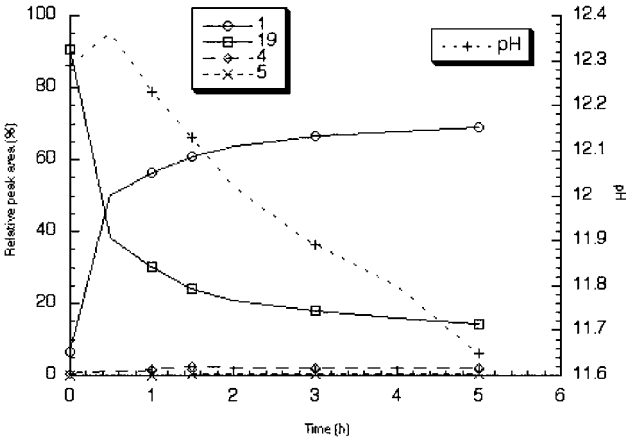


Figure 4. **Hydrolysis of ester 19 with 1 equiv of base.**

desired product **1** is degraded over time, but with an initial relatively rapid rate and then a slow degradation. The rate was shown to be pH dependent.

In the laboratory, 2-methylhexanoic acid (**2**) was found to be a contaminant in early samples of the ester **19**. As this is a carboxylic acid, this would form the sodium salt immediately upon treatment with base. This would then decrease the apparent amount of sodium hydroxide present in the hydrolysis reaction. Thus, in the later samples of **19**, where the presence of 2-methylhexanoic acid was circumvented, the base concentration was actually much higher. The very rapid reaction was too fast to handle when the reaction was scaled up. We, therefore, had to turn to slowing the reaction down. The use of sodium carbonate led to complete recovery of the ester—the pH of the reaction medium was between 9 and 10. In an effort to monitor the course of the hydrolysis reaction and to determine the exact amount of base needed (at least one equivalent is required as the sodium salt of the acid, NC-00637, is the initial product), the base was added in aliquots. The two equivalents of base were added in four equal aliquots at 30-min intervals. Degradation of **1** still occurred to a significant degree over time. However, hydrolysis of the ester had been slowed.

In addition to the slower hydrolysis rate, reaction was occurring even after the addition of only 0.5 mol equiv of base. After 1 h, when only one equivalent of base had been added, the reaction was nearing completion. We, therefore, turned our attention to the use of just one equivalent of base. Figure 3 shows that degradation of the desired product, **1**, has been circumvented by the use of only one equivalent of base. The pH of the reaction, however, does diminish over time. This was due to the formation of secondary degradants, not shown in this graphic presentation. Figure 3 also suggests that the hydrolysis of the ester **19** also stopped when the pH fell below 12.

Although the amount of base could be increased slightly to diminish the amount of unreacted **19**, the use of much more than 1 equiv of sodium hydroxide lead to an increase in the competing hydrolysis reaction of **1**. In the above reactions, it was necessary to determine the stability of **1** to the reaction conditions over an extended period of time. For preparative purposes, the reaction should provide the maximum amount of product in the shortest practical time. Five hours was determined as our optimum reaction time (Figure 4).

In practice, the pH was monitored either throughout the reaction or at specific time points. If acid had been introduced as an impurity from the starting material, the amount of base could be increased to compensate. We also considered continuous addition of base to keep the pH at 12, but this could not be achieved in our pilot plant at the time; therefore, only a few experiments were conducted along this theme.

The final workup was derived from performing a number of alternatives. To our surprise the evaporation of the THF from the reaction mixture, without acidification, led to very little decomposition of the sweetener **1**. The stability may be partially due to the sodium salts' relative insolubility in water—it precipitates out of the aqueous medium. The preferred method, however, was to acidify the reaction mixture and then extract the organic material. The desired product was then purified by basic extraction—potassium hydroxide was the base of choice as the potassium salt of **1** was much more water soluble than the sodium analogue. Re-acidification and extraction then provided the product. The material could be purified further, if necessary, by recrystallisation or trituration from an ester solvent, such as ethyl acetate, and an alkane antisolvent, such as hexane or heptane. However, other studies associated with the application of **1** as a sweetener suggested that a salt form was the preferred method and this would be prepared from the parent carboxylic acid. It was at this time that the sweetener candidate was surpassed by Neotame, and no further work was performed. However, many kilograms of NC-00637 (**1**) were prepared and tested by the above method.

Summary

A route to the new sweetener candidate, NC-00637 (**1**) was developed that activated a glutamic acid derivative by formation of the *N*-carboxyanhydride **16** to allow reaction with 5-amino-2-cyanopyridine (**4**). The crude product was then coupled with the acid chloride derived from (*S*)-2-methylhexanoic acid (**2**). The reaction conditions had to be carefully controlled to avoid polymerisation of the NCA as well as racemisation of the components. The final ester

hydrolysis was performed with base, and this reaction was investigated to minimise side reactions.

Alternative approaches were developed to circumvent the logistical problems associated with the first synthesis, but these were not scaled up as the opportunity did not present itself.

Experimental Section

The Experimental Section has been split into laboratory-scale runs and pilot and production runs under the appropriate headings.

The melting points are uncorrected. IR spectra (Nujol) were recorded on a Nicolet FT-IR spectrometer. ^{1}H NMR spectra were recorded on a GE 300 spectrometer in $CDCl_3$ using TMS as internal standard. Optical rotation was measured on a Perkin-Elmer 241 Polarimeter.

Analytical. The separation was achieved by HPLC using a Supelco LC-18 25 cm × 4.6 mm i.d. column eluting with 72% 0.02 M heptanesulfonic acid sodium salt and 28% mixture of acetonitrile and triethylamine (9:1 vol/vol) at a pH of 2.5. Flow rate was 2 mL/min with detection at 210 nm. Injection volume was 10 μL. Samples were prepared by weighing out 20 mg of solid and making up to 25 mL with the mobile phase. Retention times were **1** (12 min), **19** (25 min), **4** (2.5 min), and **18** (2 min).

Analysis of 2-methylhexanoic acid whose method was also used for the acid chloride **11** has already been described.[1]

*Small-Scale Preparations Of **1**. Through Glutamyl Anhydride (Scheme 3).* To a solution of L-glutamic acid (**3**) (5 g, 34 mmol) in 0.34 N sodium hydroxide solution (200 mL) was slowly added, at room temperature, racemic 2-methylhexanoyl chloride (**7**) (5.39 g, 37.4 mmol) in THF (50 mL). At the end of the addition, 1 N NaOH (34 mL) was added. The vigorous stirring was maintained for 15 min before removal of the THF under vacuum. The aqueous solution was washed with ether (3 × 50 mL) before acidification to pH 2 and extraction with ethyl acetate (3 × 100 mL). The extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give 6.7 g (75%) of an oily product **8**, that crystallised on standing; mp 92 °C.

Dicyclohexylcarbodiimide (4.77 g, 23 mmol) was added to a cold solution of *N*-(2-methylhexanoyl)-L-glutamic acid (**8**) (6 g, 23 mmol) in THF (60 mL). When the addition was complete, the mixture was stirred for 3 h at 20 °C. After removal of the white precipitate of dicyclohexylurea, the filtrate was concentrated to give **9** as an oil in essentially quantitative yield. The anhydride, thus obtained, was directly used for the next step.

A solution of the glutamyl anhydride **9** (3.8 g, 16 mmol) and the pyridylamine **4** (1.9 g, 16 mmol) in THF (20 mL) was stirred for 48 h at 20 °C. The solvent was then removed, and the residue was dissolved in a solution of 5% sodium carbonate. The resultant solution was washed with methylene chloride (3 × 50 mL) and acidified to pH 2−3, and the oily product was extracted with ether (3 × 30 mL). Removal of the ether gave 4.6 g (80%) of the amides **1** and **10**. The two diastereoisomers were separated by preparative HPLC.

Through N-Trifluoroacetyl L-Glutamic Acid Anhydride (Scheme 4). Trifluoroacetic acid anhydride (57 mL, 0.408 mol) was slowly added to L-glutamic acid (30 g, 0.204 mol). The mixture was kept over 2 h at 70 °C. After complete removal of trifluoroacetic acid, the oily residue was directly used for the next step.

A solution of the anhydride **12** (5.5 g, 24.4 mmol) and of amine **4** (2.6 g, 21.8 mmol) in THF (20 mL) was stirred for 12 h at 40 °C. The solvent was then removed, and the residue was dissolved in a solution of 5% sodium carbonate (15 mL) and water (70 mL). The solution was rapidly washed with methylene chloride (3 × 50 mL) and acidified to pH 2−3, and the resultant mixture was first extracted with ether (3 × 30 mL) to remove an impurity and then extracted with ethyl acetate (3 × 50 mL). This ethyl acetate solution was dried (Na_2SO_4) and concentrated to give an oil (5.1 g, 67%) of the α-**13** and γ-**14** amide isomers (65:35). This oily mixture was made solid after trituration in a diethyl ether−hexane mixture. Separation of these isomers was performed by preparative HPLC. Melting point of the α-isomer **13** was 157 °C.

A solution of **13** (2.4 g) in 12.7% aqueous ammonia solution (25 mL) was stirred for 4 h at 20 °C. After removal of the solvent (without heating), the residue was washed with ethyl acetate (2 × 10 mL) to give 1.5 g (90%) of L-glutamic acid (2-cyano-5-aminopyridyl) α-amide (**6**); mp 153 °C.

To a solution of **6** (1.39 g) and $NaHCO_3$ (4.62 g) in water (38 mL) was added a solution of (*S*)-2-methylhexanoyl chloride (**11**) (1.63 g) in THF (38 mL). After stirring at room temperature for 30 min, 6 N HCl was added until pH 2−3 was reached. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give an oil that was made solid (1.98 g, 98%) after trituration in hexane. An analytical sample of **1** was obtained by chromatography.

*Preparation of γ-Methyl L-Glutamate (**15**). Procedure 1.* To a stirred solution of L-glutamic acid (**3**) (7.36 g, 0.05 mol) in dry MeOH (166 mL) under nitrogen was added chlorotrimethylsilane (11.95 g, 0.11 mol). The reaction mixture was stirred at room temperature for 10 min. The methanol was removed under reduced pressure and the white residue was dried under vacuum. ^{1}H NMR indicated that the crude product was relatively pure and the dimethylation, if any, was not significant at all.

Procedure 2. To a stirred suspension of L-glutamic acid (**3**) (147 g, 1.0 mol) in dry methanol (2 L) was added chlorotrimethylsilane (254 mL, 2.0 mol) over a period of 20 min under nitrogen. During the addition, the flask was cooled with a cold water bath. The clear solution was then stirred at room temperature for another 20 min. The solution was concentrated to approximately half volume by vacuum distillation. Aqueous ammonia (28%) was added until the pH was about 4.5. The mixture was then heated to 50 °C and then allowed to cool overnight. After filtration, the product, glutamic acid γ-methyl ester (**15**), was dried under vacuum (85.2 g, 53%). The product contained variable amounts of ammonium chloride, which does not interfere with the next step other than requiring additional filtration.

*Preparation of γ-Methyl Glutamate Hydrochloride (**21**).* To a 12-L three-necked round-bottom flask equipped with a

thermometer, condenser, HCl inlet, and an overhead stirrer was charged methanol (8 L). The methanol was cooled to 0 °C with an ice bath, and gaseous HCl (536.6 g) was added by bubbling through a glass frit. To this methanolic HCl solution was added L-glutamic acid (**3**) (2067.9 g) over 15 min. After stirring for a further 10 min, all the solids were in solution, and the reaction mixture was allowed to stir for 6 h at ambient temperature. The methanol was then distilled off at 30−35 °C under house vacuum until a small amount of solid formed. To the thick solution at 30−35 °C was added isopropyl acetate (4 L) over 15 min. The resultant thick slurry was then cooled with an ice-bath to 0 °C and filtered. The resulting white solid was washed with ethyl acetate and dried overnight in the vacuum oven at 40 °C at 15 mmHg to afford 1732.0 g (62.4%) of γ-methyl glutamate salt (**21**). No attempt was made to recover product from the filtrate.

NCA (16) Preparation. *From Zwitterion.* Into a 3-L four-neck round-bottom flask fitted with a stirrer, thermometer, addition funnel, and condenser with a nitrogen inlet was charged γ-methyl glutamic acid (**15**) (100 g, 0.62 mol; with the weight based on ester as some samples contained NH_4Cl), and dry THF (1242 mL). To the slurry was charged 20% phosgene in toluene solution (644 mL, 1.24 mol) dropwise via addition funnel over 10 min. The mixture was then heated to 45 °C for 6 h. All solids dissolved after 2 h, if NH_4Cl was not present. The solution was purged with N_2 for 30 min to remove any excess phosgene. The mixture was filtered, if necessary, to remove any solids, and concentrated under reduced pressure to yield a syrup with some solids. This syrup was dissolved in hot ethyl acetate (600 mL), and then hexane (1.5 L) was added to precipitate the product which was isolated by vacuum filtration. The crude product was washed with hexane and and dried overnight in a vacuum desiccator to give the NCA **16** (102.12 g; 88%) as a white solid; mp 99−100 °C; ^{1}H NMR (DMSO-d_6/TMS) δ 9.1 (s, 1H), 4.48 (t, 1H), 3.6 (s, 3H), 2.45 (t, 2H), 2.0 (m, 2H); IR ν_{max}(KBr disk) 3320, 1870, 1850, 1780, 1720, 1250, 950 cm^{-1}.

Alternative Procedure. Into a 12-L four-neck round-bottom flask fitted with a stirrer, thermometer, addition funnel, and condenser with a nitrogen inlet was charged the γ-methyl glutamic acid (**15**) (375 g, 2.33 mol; the weight of ester present with no account for any NH_4Cl) and dry THF (2.5 L). To the slurry was added 25% phosgene in toluene solution (2.0 L, 4.81 mol) dropwise via addition funnel over about 50 min, at such a rate that the temperature did not exceed 45 °C. When the addition was complete, the mixture was kept at 45 °C for 6 h. The mixture was cooled to ambient temperature and then purged with N_2 for 30 min to remove any excess phosgene and then concentrated under reduced pressure to yield a solid suspended in a liquid. This residue was split in two; each portion was recrystallised from hot ethyl acetate (1500 mL) and hexane (4.5 L). A crystalline solid formed rapidly. This was isolated by vacuum filtration, washed with hexane (500 mL), and dried overnight in a vacuum oven at ambient temperature. The yield was about 88% of white solid.

*From Hydrochloride Salt (**21**). With THF as Solvent.* Into a 1-L three-necked round-bottom flask fitted with a stirrer, thermometer, addition funnel, and condenser with a nitrogen inlet was charged γ-methyl glutamic acid hydrochloride salt (**21**) (49.4 g, 0.25 mol) and dry THF (275 mL). To the slurry was charged 20% phosgene in toluene solution (220 mL) dropwise via addition funnel over 1 h. The mixture was heated to 45 °C for 6 h and then purged with N_2 for 30 min to remove any excess phosgene. The mixture was filtered, if necessary, to remove any solids, and concentrated under reduced pressure to yield a syrup containing solid. This residue was dissolved in hot ethyl acetate (225 mL), and then hexane (1 L) was added to precipitate the product, which was isolated by vacuum filtration. The NCA (**16**) was washed with hexane and dried overnight in a vacuum desiccator to give 29.3 g (66%) of white solid, identical to that described above.

With Ethyl Acetate as Solvent. To a slurry of γ-methyl glutamate hydrochloride (**21**) (49.40 g, 0.25 mol) in ethyl acetate (575 mL) at 70 °C was added gaseous phosgene (24.73 g, 1.0 equiv). An additional 4.0 g (0.4 equiv) of gaseous phosgene was required as the phosgene escaped through the coldfinger (−78 °C). The reaction mixture was stirred for 4.5 h at 70 °C and then cooled to room temperature and filtered to remove unreacted γ-methyl glutamate hydrochloride (1.3 g, 2.7%). The filtrate was then concentrated to 250 mL under house vacuum at 45 °C and then heated to 50 °C followed by slow addition of hexane (500 mL). The resultant slurry was filtered to afford 35.8 g (77%) of the NCA **16** of γ-methyl glutamate.

Acid Chloride (11). Racemic 2-methylhexanoic acid (13 g, 0.1 mol) was placed in a flask with dichloroethane (100 mL) followed by sodium sulphate (∼5 g). The mixture was stirred for 15 min and then filtered, washing the solid with dichloroethane (25 mL). The solution was put into a 250-mL three-neck flask equipped with an addition funnel, magnetic stir bar, a condenser, nitrogen inlet, and a thermometer. The mixture was heated to 40 °C. Thionyl chloride (8 mL, 0.11 mol) was added slowly. The mixture was then heated to 80 °C and stirred overnight. After cooling to room temperature, the solvent was removed under reduced pressure. Vacuum distillation of the residue under house vacuum (∼40 mmHg) collecting the fraction at 28−30 °C gave the acid chloride **7**. The yield was nearly quantitative.

Alternative Small-Scale Procedure. (*S*)-2-Methylhexanoic acid (**2**) (13 g, 0.1 mol) was placed in a flask with dichloromethane (100 mL) followed by sodium sulphate (∼5 g). The mixture was stirred for 15 min and then filtered, washing the solid with dichloromethane (25 mL). The solution was put into a 250-mL three-neck flask equipped with an addition funnel, magnetic stir bar, a condenser, nitrogen inlet, and a thermometer; pyridine (1 drop) was added. The mixture was heated to 40 °C, and thionyl chloride (8 mL 0.11 mol) was added slowly. The mixture was then heated at reflux for 1 h. After cooling to room temperature, the solvent was removed under reduced pressure. Vacuum distillation of the residue under house vacuum (∼40 mmHg)

collecting the fraction at 28−30 °C gave the acid chloride **11**. The yield was nearly quantitative.

Large-Scale Procedure. Thionyl chloride (578 mL) was placed in a 2-L flask equipped with an addition funnel, condenser, thermometer, and guard tube. The acid (185.3 g) was added dropwise while the flask was cooled with a dry ice−2-propanol bath (∼−10 °C). Once the addition was complete and the mixture started to warm towards room temperature, there was a large evolution of gases. The mixture was stirred overnight, and then the excess thionyl chloride was removed by vacuum distillation (up to 40 °C at house vacuum). The yield was 178.6 g. The product was used without further purification.

Preparation of L-Glutamic Acid 5-Amino-2-cyanopyridine Amide γ-Methyl Ester (18). *Without Purification.* A 500-mL one-neck flask containing a magnetic stir bar under nitrogen was used for this reaction. The NCA (**16**) of γ-methyl-L-glutamate (24.81 g, 0.13 mol) was dissolved in DMF (333 mL). 5-Amino-2-cyanopyridine hydrochloride (**17)** (18.52 g, 0.12 mol) and then free aminopyridine **4** (1.20 g, 0.01 mol) were added, and the mixture was stirred until the solids dissolved. The mixture was allowed to stand for 7−10 days (or longer if necessary). HPLC assay was used to determine completion. (The analysis seemed to show little conversion during the early stages of the reaction and rapid build up of product at the end. This is due to the large differences in extinction coefficients.) Peak area >90% product. The product was not isolated but used in situ for the next reaction.

With Removal of Unreacted Amine 4. The NCA **16** of γ-methyl glutamate (3.06 g) was added to DMF (19.0 g) and the mixture stirred for a few minutes to dissolve the solids. The pyridine hydrochloride **17** (2.28 g) and then the free amine **4** (148 mg) were added. The mixture was stirred under nitrogen at ambient temperature for 10 days. The reaction was poured into water (65 g), stirred for 30 min, and then extracted with EtOAc (25.8 g, then 2 × 12.9 g). The aqueous phase contained the required coupled product. The organic extracts contained unreacted amine **4**.

Scaled Method. The NCA (**16**) of γ-methyl glutamate (42.93 g) was added to DMF (75 mL) under nitrogen. The mixture was stirred until all solids had dissolved. The pyridine hydrochloride **17** (31.96 g) and then the free amine **4** (2.07 g) were added. The mixture was stirred under a nitrogen atmosphere at ambient temperature for 4−5 days. The reaction was monitored by HPLC; usually after 4 days, very little amine **4** could be detected. Prior to the next step, water (75 mL) was added slowly to the mixture—the decomposition of the NCA was accompanied by the evolution of carbon dioxide. Once the gas evolution had ceased, this solution was used for the next step without any purification.

Formation of Ester 19. *With Pyridine as Base.* The crude reaction mixture from the NCA coupling reaction (0.13 mol scale) was placed in a 500-mL three-neck flask fitted with a condenser, nitrogen inlet, mechanical stirrer, and thermometer. Pyridine (10.17 mL, 0.14 mol) was added, followed by the slow addition of the acid chloride **11** (23 g, 0.14 mol). The reaction mixture was heated to 40 °C for 5 h and then monitored by HPLC until acylation was complete; the exact reaction time depends on impurities present; it can be 24 h or longer. The mixture was poured into water (875 mL) and extracted with ethyl acetate (2 × 450 mL). The organic extracts were washed with 1.5 N HCl (350 mL) followed by 1 N aqueous sodium bicarbonate solution (350 mL). The solvent was removed under reduced pressure, and the ester **19** recrystallised from ethyl acetate with hexane added as the antisolvent.

With Sodium Bicarbonate as Base. The solution of **18** [from NCA (42.93 g), **17** (31.96 g), and **4** (2.07 g), in DMF (75 mL) as described above and then diluted with water (90 mL)] was added to a paste prepared from $NaHCO_3$ (220 g) and water (100 mL). This was followed by the addition of the acid chloride **11** (46 g) with THF (∼2 mL) as chaser to rinse lines and equipment. After 1 h the solid was filtered off, washing it with water (2 × 50 mL). The solid was then stirred overnight with water (250 mL). After filtration, the solid was heated to reflux in ethyl acetate (300 mL). After filtration, the remaining solid was washed further with ethyl acetate (100 mL). To this yellow solution was added charcoal, and the mixture was heated under reflux for 15 min and then filtered. Water (100−150 mL) was then added, and the organic solvent was removed under vacuum. After filtration, the solid was warmed to a gentle boil in ethyl acetate (50 mL) and hexane (50 mL)—just enough to make a reasonable slurry. Filtration then gave the ester **19** (43−53%); ^{1}H NMR ($CDCl_3$) δ 10.16 (1H, s), 8.66 (1H, m), 8.24 (1H, m), 7.59 (2H, d), 4.72 (1H, m), 3.70 (3H, s), 2.70−2.06 (5H, m), 1.64 (1H, m), 1.44 (1H, m), 1.27 (4H, m), 1.19 (3H, d), and 0.84 (3H, t).

Preparation of 1. *Hydrolysis with 2 equiv of Base.* The ester **19** (36.81 g) was placed in THF (500 mL) and water (716 mL). NaOH was added (1 N, 190 mL), and the mixture was stirred at ambient temperature for 30 min. HPLC showed almost complete reaction. HCl (1 N) was added to bring the pH to 2. The THF was removed under reduced pressure. The solid was filtered off, washed with water (25 mL), and dried in a vacuum oven at 40 °C overnight to give 32.2 g of NC-00637 (**1**) (87% purity by HPLC). This material was recrystallised from ethyl acetate (500 mL) and hexane (1.5 L) to give 25.71 g of product after drying at 40 °C in a vacuum oven overnight.

Hydrolysis with 1 equiv of base. The ester **19** (3.27 g) was added to THF (25 mL) and stirred to dissolve. NaOH (1 N, 8.7 mL) was added in one portion. The pH was monitored at 3 h to ensure that the pH had not fallen below 12 (if it did, 0.44 mL more sodium hydroxide solution was added). After 5 h, the mixture was made acidic (pH 1−2) with concentrated hydrochloric acid. The THF was removed by vacuum distillation (>30 °C). The resultant mixture was extracted with ethyl acetate (2 × 15 mL). The organic extracts were washed with 0.5 N potassium hydroxide (20 mL). The aqueous layer was then acidified with concentrated hydrochloric acid to pH ∼1 and extracted with ethyl acetate (25 mL). This organic extract was concentrated to half volume, then treated at reflux with an equal volume of

hexane, and stirred until the temperature dropped to ambient, when two more volumes of hexane were added. The product was isolated by filtration. Further purification (if necessary) was achieved by heating the solid under reflux with a 1:1 mixture of ethyl acetate and hexane (10 mL total). Mp 162−163 °C; ^{1}H NMR (d_6-DMSO) δ 10.80 (1H, s), 8.96 (1H, m), 8.24 (2H, m), 7.94 (1H, d), 4.37 (1H, m), 2.50 (1H, s), 2.40−1.80 (4H, m), 1.46 (1H, m), 1.30−1.05 (5H, m), 0.98 (3H, d), and 0.82 (3H, t);^{13}C (NMR) δ 176.1, 173.7, 171.8, 141.9, 138.8, 129.5, 125.9, 125.8, 117.6, 52.9, 33.4, 30.2, 28.9, 26.6, 22.1, 17.9, and 13.8; $[\alpha]_D$ −24.9° (50 mg in 2 mL of MeOH)

Alternative Methods. *Enzymatic Approach.* To a thin slurry of dimethyl glutamate (**25**) (8.46 g, 0.04 mol) and sodium bicarbonate (10.08 g, 0.12 mol) in water was added (*S*)-2-methylhexanoyl chloride (**11**) (5.94 g, 0.04 mol) in THF (10 mL) over a period of 15 min. The mixture was allowed to stir at room temperature for 1 h. The THF was evaporated on a rotary evaporator at room temperature, and the aqueous layer was extracted with ethyl acetate (30 mL). The organic layer was washed with aqueous saturated sodium bicarbonate (30 mL) and then with water (30 mL). The ethyl acetate layer was dried (MgSO4) and filtered, and the filtrate was concentrated to an oil. The oil solidified within 5 min. Yield 10.1 g (88%).

PLE (0.8 mL suspension, from Sigma) was added to a suspension of dimethyl *N*-((*S*)-2-methylhexanoyl)-L-glutamate (**24**) (7.4 g, 0.26 mol) in 0.01 M phosphate buffer (pH 7) at room temperature. The pH value was kept at about 7 by the addition of 1 N sodium hydroxide. After consumption of 0.95 mol equiv of base, the pH was adjusted to 9 by the addition of 1 N sodium hydroxide, and the mixture was extracted with ethyl acetate (20 mL). The aqueous layer was separated and acidified to pH 2−3 by the addition of 1 N hydrochloric acid and extracted with ethyl acetate (2 × 20 mL). The organic layer was washed with water (20 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated to an oil. The oil solidified on standing. Yield 5.68 g (80%).

Chemical Method to Ester 19. To a cold (0 °C) solution of **27** (2.73 g, 0.01 mol) in ethyl acetate (20 mL) was added *N*-methylmorpholine (1.01 g, 1.1 mL, 0.01 mol), followed by trimethylacetyl chloride (1.2 g, 1.23 mL, 0.01 mol). After 10 min, the amine **4** (1.19 g, 0.01 mol) was added, and the mixture was warmed to room temperature. The mixture was stirred at room temperature for 24 h and then diluted with 10 mL of 1 N HCl. The organic layer was separated, washed successively with saturated aqueous ammonium chloride solution (10 mL), saturated sodium bicarbonate solution (10 mL), and water (10 mL), and dried ($MgSO_4$). Filtration and concentration of the filtrate gave a pale solid, which was recrystallised from a mixture of ethyl acetate and hexane (35 mL/35 mL) to give a white solid (2.1 g, 54%).

Large-Scale Runs. Many of these procedures were limited by the size of equipment available to us. In many cases, multiple runs had to be performed to obtain sufficient material. In the case of the NCA **16** this was run at a toll manufacturer using our procedure. The pyridine amine **4** was also transferred to a toll manufacturer, but quality suffered, and subsequent clean up had to be performed before use.[2]

*Purification of Amine **4**.* As noted above impure samples were obtained from tollers (assays ranged from 78 to 98% on a wt/wt basis by HPLC and titration), and these were cleaned up as follows:

EtOAc (32 kg) was placed in a 30-gal reactor followed by the impure amine **4** (7.0 kg). The mixture was heated under reflux for 30 min and then filtered hot through a Nutsche filter. The procedure was repeated on the filter cake. Charcoal (Darco, 280 g) was added to the combined filtrates which was then heated under reflux for 30 min. The mixture was filtered hot through cartridge filters. The solution was concentrated to one-third of its original volume by vacuum distillation and then cooled to ambient temperature. The solid was collected on a Nutsche filter and allowed to dry under a stream of dry nitrogen. A second crop was obtained from the filtrate by heating it to boiling and then setting the jacket temperature at 60 °C. Hexane (2× the weight of EtOAc present) was then added. The mixture was cooled to ambient temperature over 1 h and then filtered through a Nutsche, drying in a nitrogen stream. Both crops were within specifications (>98% wt/wt by HPLC and titration, and the solid was off-white to light yellow in colour).

*Preparation of Amine Hydrochloride **17**.* THF (96 kg) was charged to a glass-lined 100-gal reactor. The amine **4** (12.0 kg) was added, and the mixture was heated to boiling and then cooled to ambient temperature. The solution was then flitered through a sparkler Nutsche filter, returning the clear mother liquors to the reactor. The solution was cooled to 0 °C, and then HCl gas was bubbled through at 60 psig for 15 min. Nitrogen was then bubbled through the mixture for 15 min. The HCl addition was repeated twice more. After the final addition, the mixture was stirred for 1 h at 0 °C. The solid was collected on a Nutsche filter. The reactor, line, and then cake were washed with THF (11 kg). The solid **17** was dried overnight at ambient temperature on the filter bed under a stream of dry nitrogen.

*Coupling of NCA **16**.* DMF (40 kg) was added to a 30-gal reactor followed by the NCA **16** (24 kg). The mixture was stirred at ambient temperature for 30 min to ensure dissolution. The pyridine hydrochloride **17** (17.86 kg) was then added followed by the free amine **4** (1.16 kg). The reaction was stirred at ambient temperature for 3 days. The mixture was then filtered, rinsing the reactor and lines with DMF (10 kg). The filtrate was stirred for a further 2 days. After this time the free amine concentration should be below 5% by peak area. It was then used in the subsequent coupling step as is.

*Acid Chloride **11**.* This procedure had to performed so that the acid chloride was ready for the coupling reactions without any undue delay.

Thionyl chloride (12.3 L, 20.1 kg) was charged to a 5-gal reactor under nitrogen and then cooled to 0 °C. 2-Methylhexanoic acid **2** (3.00 kg) was then added, ensuring that foaming did not occur and the internal temperature did not exceed 35°. The transfer lines were rinsed with THF (1 L). The reaction was stirred at 0 °C for 30 min and brought to

25° over 1 h. Vacuum was then slowly applied to the system, ensuring that excessive foaming did not occur. Once full vacuum had been applied, the mixture was slowly heated to 40 °C, ensuring that the jacket temperature also did not exceed 40 °C. Once at 40° this temperature was maintained until the contents of the reactor were between 3.6 and 4 kg. The acid chloride **11** was used directly in the next step.

*Ester **19***. To the aqueous solution of **18**, in a 100-gal reactor from the previous step, was added water (42 kg) at such a rate that excessive foaming did not occur. DMF (5 kg) was used to rinse transfer lines. In a separate 100-gal reactor was charged water (47 kg) and sodium bicarbonate (102.5 kg). This mixture was stirred to form a slurry. The solution of **18** was then added slowly to the bicarbonate slurry so that there was no excessive foaming. When the addition was complete, including a 25-kg water rinse of the reactor that had contained the amide **18** and the transfer lines, 2-methylhexanoyl chloride (**11**) (22 kg) was added at a rate so that excessive foaming did not occur. The mixture was stirred for 1 h and then filtered through a Nutsche filter, washing with water (20 kg). The wet cake was added back to the reactor that had been charged with water (116 kg), and the mixture stirred at ambient temperature overnight before being refiltered through the Nutsche filter. The solid was then placed back in the reactor that now had been charged with ethyl acetate (126 kg). This mixture was heated to boiling and kept there for 15 min when it was filtered through a preheated Nutsche filter. The cake was washed with warm ethyl acetate (∼50 °C). To the warm filtrate was added ADP carbon (200 g), and the mixture was stirred as it cooled to ambient temperature. After 30 min, the mixture was filtered through line filters to remove the carbon, rinsing the reactor and lines with ethyl acetate (5 kg). To the filtrate was added water (100 kg), and the ethyl acetate was distilled off under vacuum (∼140 kg) with good agitation to ensure formation of small particles. The mixture was filtered through a Nutsche filter. The solid was placed in a 30-gal reactor, and ethyl acetate (21 kg) was added followed by hexane (16 kg). The mixture was heated under reflux for 1 h and then cooled to ambient temperature over another 1 h, followed by cooling to 5 °C over 30 min. The solid was removed by filtration through a Rosenmund filter using the mother liquors to rinse reactor and lines. The cake was then washed with a mixture of ethyl acetate (14 kg) and hexane (9.5 kg). The washed product was then dried in the Rosenmund under vacuum with a jacket temperature of 50 °C.

*Sweetener **1***. A sodium hydroxide solution was prepared by the slow addition of 50% NaOH (1 kg) to water (12 kg) with stirring. When the solution had cooled to ambient temperature, it was titrated to determine molarity.

THF (29 kg) was charged to a 30-gal reactor. The ester **19** (4.25 kg) was added as a solid and the mixture stirred for 15 min to dissolve. The 1 M NaOH solution (11.5 kg) prepared above was added (correcting the volume if the molarity was not 1 M). The mixture was stirred for 3 h while monitoring the reaction by HPLC. After 3 h, if the pH was below 12, more 1 M NaOH (0.58 kg) was added, and the reaction was stirred for an additional 2 h. If the pH was above 12, or after the additional charge and reaction time, the reaction was quenched by the addition of concentrated HCl until the pH was 1−2. The THF was removed by vacuum distillation with a jacket temperature of 30 °C. When 29 kg of liquid had been removed, the vacuum was released and ethyl acetate (18 kg) added. The mixture was stirred for 15 min and then allowed to stand for the layers to separate. The layers were split. The aqueous layer was extracted with more ethyl acetate (18 kg). The organic extracts were combined and then stirred with a solution of KOH [(0.75 kg) in water (27 kg)]. The layers were allowed to stand and then separated (could be slow). The aqueous layer was run off to another 30-gal reactor. The pH was adjusted to between 1 and 2 by the addition of concentrated HCl. Ethyl acetate (30 kg) was then used to extract the mixture. The extract was concentrated to half weight under vacuum. The vacuum was then released, and the mixture was heated to reflux when an equal volume of hexane was added. The mixture was then cooled to 20 °C over 1 h when more hexane (2 volumes) was added. After stirring overnight, the crude product was collected in a Rosenmund filter. Ethyl acetate (20 kg) and hexane (15 kg) were charged to the reactor, and the crude product was added. The mixture was heated to reflux for 15 min and then cooled to ambient temperature overnight when it was filtered through the Rosenmund. Water (20 kg) was added to the reactor and the solid added to it. The mixture was stirred at ambient temperature overnight and then filtered through the Rosenmund filter, before drying under vacuum below 60 °C jacket temperature. Yield was 3.6 kg (88%).

Acknowledgment

We thank the members of the NutraSweet discovery group, the analytical support, and especially Dalpat Brahmbhatt, of the Discovery and Process groups without whom this work would not have been possible.

Received for review August 5, 2003.

OP0341115